

Electronic Supporting Information

A G-quadruplex-based platform for the detection of Hg²⁺ ions using a luminescent 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 (IrCl₃.xH₂O) was purchased from Precious Metals Online (Australia). 96-well plates for amined DNA-binding were purchased from Corning (USA). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China). DNA sequences used in this project:

ssDNA: 5'-C₂AGT₂CGTAGTA₂C₃-3', ds17: 5'-C₂AGT₂CGTAGTA₂C₃-3' and 5'-G₃T₂ACTACGA₂CTG₂-3', ckit87: 5'-AG₃AG₃CGCTG₃AG₂AG₃-3', PS2.M: 5'-GTG₃TAG₃CG₃T₂G₂-3', Amine-Hg-DNA (probe B): H₂N-5'-A₅CT₃CT₂CT₂C₄T₂GT₂GT₃GC₂A₂C-3', Complex-Hg-DNA Ps (probe A): 5'-GTG₃TAG₃CG₃T₂G₂CA₃CA₂CA₂G₄A₂GA₂GA₃G-3', Complex-Hg-DNA Ps M (probe A mutant): 5'-GTGTGTA₂GTC₂AGT₂G₂CA₃CA₂CA₂G₄A₂GA₂GA₃G-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 ¹³C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (¹H) and 100 MHz (¹³C). ¹H and ¹³C chemical shifts were referenced internally to solvent shift (CD₃CN: ¹H, δ 1.94, ¹³C δ 118.7; d₆-DMSO: ¹H δ 2.50, ¹³C 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 ¹³C. Coupling constants are typically ± 0.1 Hz for ¹H-¹H and ±0.5 Hz for ¹H-¹³C 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 400 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][PF₆]₂ in degassed acetonitrile as a standard reference solution (Φ_r = 0.062) and calculated according to the following equation: Φ_s = Φ_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

Luminescence response of complexes towards different forms of DNA. The G-quadruplex DNA-forming sequences were annealed in Tris-HCl buffer (10 mM Tris, 20 mM KCl, pH 7.2) and were stored at $-20\text{ }^{\circ}\text{C}$ before use. Complexes (1 μM) was added to 5 μM of ssDNA, dsDNA or G-quadruplex DNA in Tris-HCl buffer (10 mM Tris, pH 7.2).

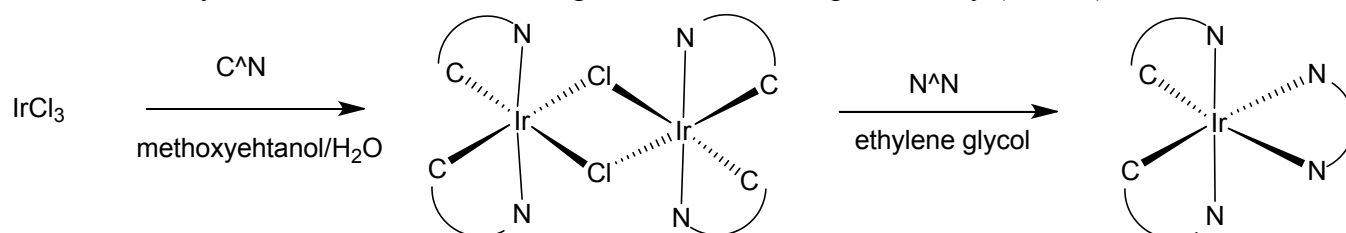
FRET melting assay. The ability of **1** to stabilize G-quadruplex DNA was investigated using a fluorescence resonance energy transfer (FRET) melting assay. The labelled G-quadruplex-forming oligonucleotide F21T (5'-FAM-d(G₃[T₂AG₃]₃)-TAMRA-3'; donor fluorophore FAM: 6-carboxyfluorescein; acceptor fluorophore TAMRA: 6-carboxytetramethylrhodamine) was diluted to 200 nM in a potassium cacodylate buffer (100 mM KCl, pH 7.0), and then heated to 95 $^{\circ}\text{C}$ in the presence of the indicated concentrations of **11**. The labeled duplex-forming oligonucleotide F10T (5'-FAM-dTATAGCTA-HEG-TATAGCTATAT-TAMRA-3') (HEG linker: [(-CH₂-CH₂-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 $^{\circ}\text{C}$ over the range of 25 to 95 $^{\circ}\text{C}$.

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 PS2.M and ds17) in Tris-HCl buffer (10 mM, pH 7.2) 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).²

Hg²⁺ ion detection. The DNA substrate (50 μM) was dissolved in Tris-acetate buffer (10 mM, pH 7.2). The solution was heated to 95 $^{\circ}\text{C}$ for 10 min and then cooled at 0.1 $^{\circ}\text{C}/\text{s}$ to room temperature to allow the formation of the duplex structure. The annealed product was stored at $-20\text{ }^{\circ}\text{C}$ before use. For Hg²⁺ detection, 5 μL of the DNA substrate in Tris-acetate buffer (10 mM, pH 7.2) was diluted to 100 μL by phosphate buffer (0.5 mM K₂HPO₄, pH 8.0), and was added to each well of the binding plate to start the binding reaction. After incubation at room temperature for 15 min, the wells were washed three times with Tris-acetate buffer (10 mM, pH 7.9), and then the indicated concentrations of Hg²⁺ ions were added. After incubation at room temperature for 30 min, the mixture was eluted, re-annealed and diluted using Tris buffer (10 mM Tris, 20 mM KCl, pH 7.2) to a final volume of 500 μL . Finally, 2 μM of complex **1** was added to the mixture. Emission spectra were recorded in the 500–750 nm range using an excitation wavelength of 300 nm.

Synthesis

The complexes **2–5**³ were prepared according to (modified) literature methods. All complexes are characterized by ¹H-NMR, ¹³C-NMR and high resolution mass spectrometry (HRMS).



Complex 1. ^1H NMR (400 MHz, Acetone) δ 8.93 (dd, $J = 8.5, 1.3$ Hz, 1H), 8.73 (dd, $J = 8.3, 1.3$ Hz, 1H), 8.51 (s, 1H), 8.43 (dd, $J = 5.1, 1.3$ Hz, 1H), 8.36 (dd, $J = 5.1, 1.4$ Hz, 1H), 8.07 (dd, $J = 8.5, 5.1$ Hz, 1H), 7.96 (dd, $J = 8.3, 5.1$ Hz, 1H), 7.32 (dd, $J = 7.6, 1.3$ Hz, 2H), 7.18 (dd, $J = 7.1, 2.7$ Hz, 2H), 7.09–7.04 (m, 2H), 6.95 (tt, $J = 7.4, 1.3$ Hz, 2H), 4.89 (dtd, $J = 10.6, 8.6, 2.3$ Hz, 2H), 4.49 (dtd, $J = 10.6, 8.6, 5.2$ Hz, 2H), 3.65 (dddd, $J = 12.0, 10.7, 8.5, 3.4$ Hz, 2H), 2.97 (dddd, $J = 11.7, 10.7, 7.9, 3.8$ Hz, 2H). ^{13}C NMR (101 MHz, Acetone) δ 181.36, 181.34, 153.95, 153.44, 150.55, 150.30, 149.61, 148.14, 138.33, 135.80, 133.87, 133.86, 133.32, 133.30, 132.20, 131.68, 131.63, 131.21, 130.07, 128.22, 128.09, 128.04, 127.65, 127.63, 122.89, 122.88, 72.40, 50.40, 50.32. MALDI-TOF-HRMS: Calcd. For $\text{C}_{30}\text{H}_{23}\text{ClIrN}_4\text{O}_2$ $[\text{M}]^+$: 699.1139. Found: 698.9120. Elemental Analysis: C, 42.68; H, 2.75; N, 6.64. Found C, 42.78; H, 2.85; N, 6.54.

Complex 2. Reported.³

Complex 3. Reported.³

Complex 4. Reported.³

Complex 5. Reported.³

Table S1. 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 PS2.M
Quantum yield Φ	0.031	0.033	0.030	0.43

Table S2. The Hg^{2+} concentration in the natural water samples.

	Spiked sample	After sample loading
$[\text{Hg}^{2+}] / \mu\text{M}$	1.00	0.23

Fig. S1 Diagrammatic bar array representation of the luminescence enhancement selectivity ratio of complexes 1–5 for PS2.M G-quadruplex DNA over ds17.

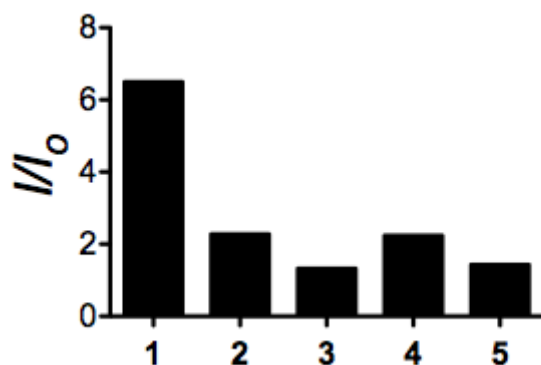


Fig. S2 UV/Vis spectrophotometric titration of complex 1 with increasing concentrations of PS2.M.

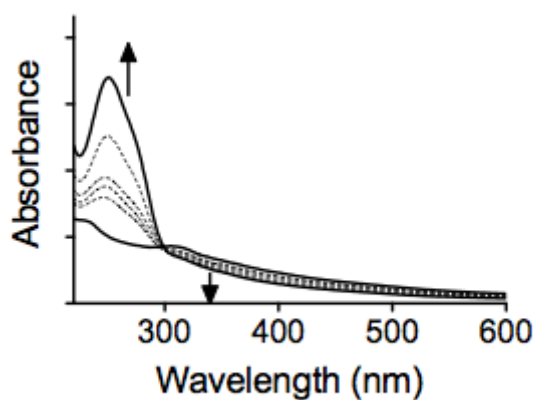
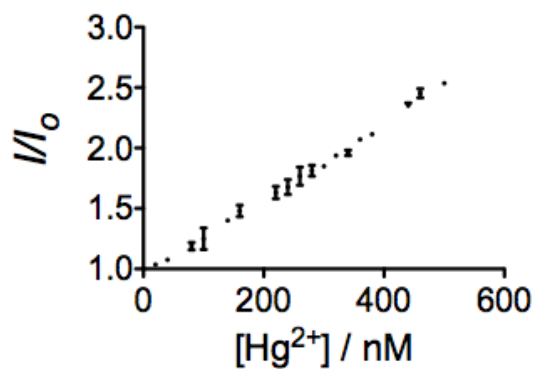


Fig. S3 Luminescence response of the system at $\lambda = 604$ nm vs. Hg^{2+} ion concentration in 50-fold diluted river water sample.



Reference

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