Supporting Information

G-quadruplex-based logic gates for Hg^{II} and Ag^{I} ions employing a luminescent iridium(III) complex and extension of metal-mediated base pairs by polymerase

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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 ($IrCl_3 \cdot xH_2O$) was purchased from Precious Metals Online (Australia). Klenow Fragment (3′ → 5′ exo$^-$), dTTP were purchased from NEB Inc. (England). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China). DNA sequences used in this project:

ssDNA: 5′-$C_2AGT_2CGTAGTA_2C_3$-3′, ds17: 5′-$C_2AGT_2CGTAGTA_2C_3$-3′ and 5′-$G_3T_2ACTACGA_2CTG_2$-3′, Pu27: 5′-$TG_4AG_3TG_4AG_3TG_4A_2G_2$-3′, PS2.M: 5′-$GTG_3TAG_3CG_3T_2G_2$-3′, TBA: 5′-$G_2T_2G_2TGTG_2T_2G_2$-3′, DNA **S**: 5′-TACGACTCACTATAG-3′, DNA **D**: 5′-$G_3TAG_3T_{20}G_3T_2G_3$-3′, DNA **L1**: 5′-A_{20}AATAAACTATAGTGAGTCGTA-3′, DNA **L2**: 5′-A_{20}AACAAACTATAGTGAGTCGTA-3′, DNA **L3**: 5′-A_{20}AATAAACCTATAGTGAGTCGTA-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 ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C). ^1H and ^{13}C chemical shifts were referenced internally to solvent shift (CD_3CN: ^1H, δ 1.94, ^{13}C, δ 118.7; d_6-DMSO: ^1H, 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 ^1H and ±0.05 for ^{13}C. Coupling constants are typically ± 0.1 Hz for ^1H-^1H and ±0.5 Hz for ^1H-^{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 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 $[Ru(bpy)_3][PF_6]_2$ 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.[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 °C before use. Complex (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 **7** 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 **7**. 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. 0.25 μM pre-folded DNA target is mixed with thiazole orange (0.50 μM for Pu27 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]

DNA preparation and extension reaction: The DNA substrate (50 μM) was dissolved in Tris-acetate buffer (10 mM, pH 7.2). 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 structure. The annealed product was stored at –20 °C before use. For extension reaction, 0.8 μL of the DNA substrate in Tris-acetate buffer (10 mM, pH 7.2) was diluted into 20 μL reaction buffer (100 mM AcONa, 10

mM Tris-AcOH (pH 7.9), 10 mM $Mg(OAc)_2$, 5 mM NH4Cl and 8 μM dithiothreitol (DTT)) by enzyme free water with certain concentration of dTTP and Hg^{2+} or/and Ag^{+} ions. After incubation at 37 °C temperature for 30 min, the samples were diluted to 100 μL with Tris-acetate buffer (10 mM, 20 mM KCl, pH 7.2) and then incubated at room temperature for 30 min. The mixture was diluted using Tris-acetate buffer (10 mM, 20 mM KCl, pH 7.2) to a final volume of 500 μL. Finally, 1 μM of complex **7** was added to the mixture. Emission spectra were recorded in the 520−750 nm range using an excitation wavelength of 300 nm.

Synthesis

The complexes was prepared according to (modified) literature methods.[3] All complexes are characterized by ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis.The precursor iridium(III) complex dimer $[Ir_2(C\hat{}N)_4Cl_2]$ is prepared as reported method.[4] Then, a suspension of $[Ir_2(C\hat{}N)_4Cl_2]$ (0.2 mmol) and corresponding N^N ligands (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.

Complex **1**. (Reported)[5]

Complex **2**. (Reported)[3]

Complex **3**. (Reported)[3]

Complex **4**. (Reported)[3]

Complex **5**. (Reported)[3]

Complex **6**. (Reported)[6]

Complex **7**. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.88 (d, J = 8.3 Hz, 2H), 8.34 (s, 2H), 7.81 (d, J = 8.3 Hz, 2H), 7.59 (d, J = 8.1 Hz, 2H), 7.28–7.17 (m, 4H), 7.04–6.98 (m, 2H), 6.92 (dd, J = 11.4, 4.1 Hz, 2H), 6.74–6.61 (m, 8H), 6.46 (td, J = 7.5, 0.9 Hz, 2H), 6.12 (td, J = 7.6, 1.3 Hz, 2H), 6.02 (d, J = 8.2 Hz, 2H), 5.49 (d, J = 7.4 Hz, 2H), 3.56 (s, 2H). ^{13}C NMR (101 MHz, acetone-d_6) δ 167.05, 166.17 , 150.97 , 148.33 , 140.64 , 140.19 , 139.30 , 134.01 , 133.19 , 131.24 , 130.03 , 129.81 , 129.09 , 128.75 , 128.30 , 128.12 , 124.17 , 124.10 , 123.95 , 121.01 , 114.92 , 113.53. HRMS: calcd. for $C_{50}H_{34}IrN_6$ $[M]^{+}$: 911.2474, found: 911.0455. Anal. Calcd for C, 56.87; H, 3.25; N, 7.96, found: C, 56.86; H, 3.43; N, 7.92.

Complex **8**. (Reported)[7]

Complex **9**. (Reported)[8]

Table S1 Quantum yield and lifetime of complex **7** in ACN (12.5 μM at room temperature).

Quantum yield Φ	Lifetime / μs
0.0732	1.72

Scheme S1 Schematic representation of the G-quadruplex-based luminescence sensing logic gate for Hg^{II} and Ag^{I} ions detection.

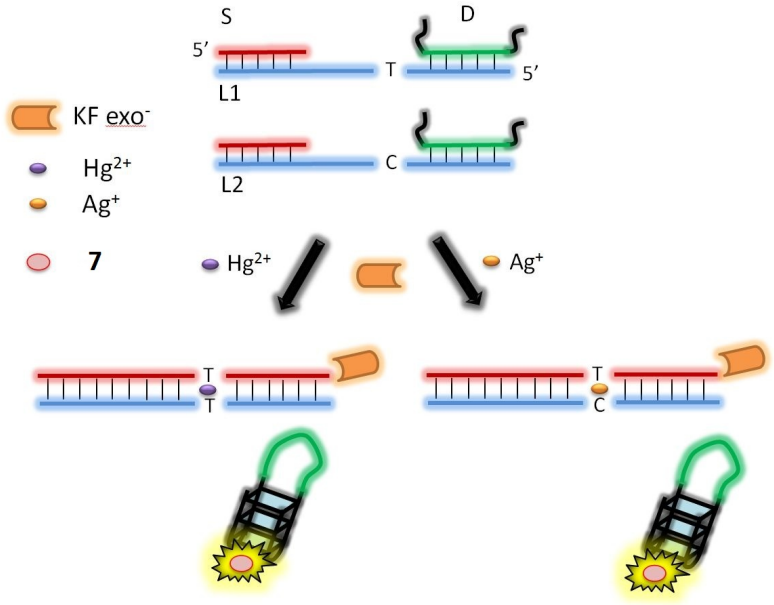


Fig. S1a Diagrammatic bar array representation of the luminescence enhancement selectivity ratio of complexes **1**–**5** for Pu27 G-quadruplex DNA over dsDNA (ds17) and ssDNA.

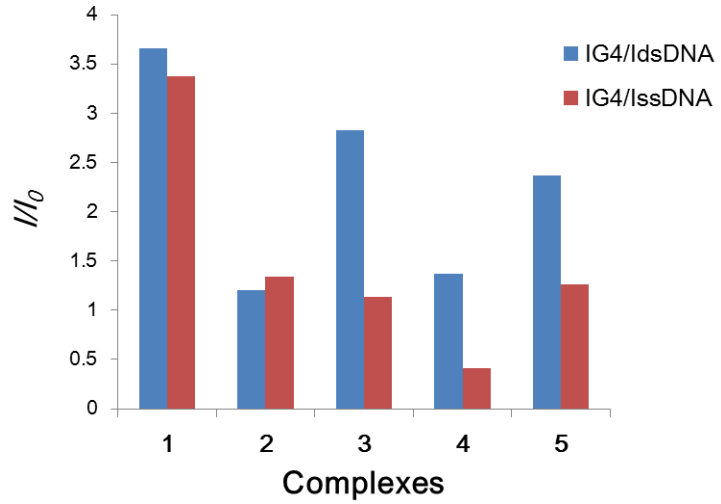


Fig. S1b Diagrammatic bar array representation of the luminescence enhancement selectivity ratio of complexes **6**–**9** for Pu27 G-quadruplex DNA over dsDNA (ds17) and ssDNA.

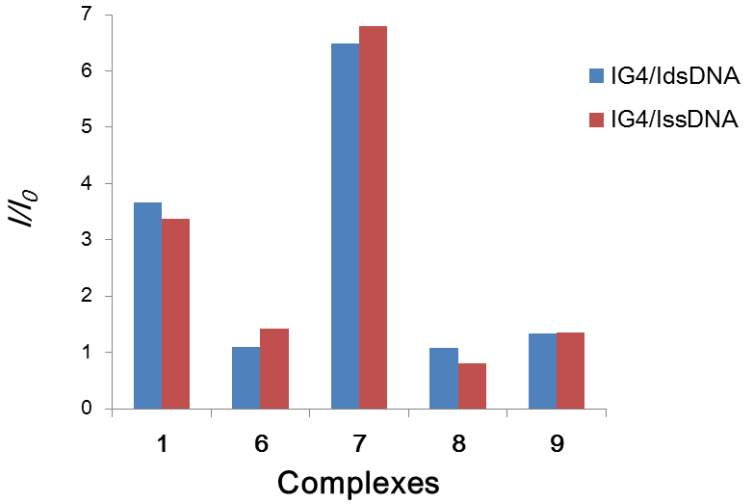


Fig. S2 UV/Vis spectrophotometric titration of complex **7** with increasing concentrations of Pu27.

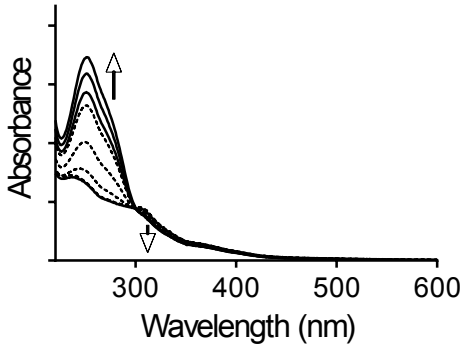


Fig. S3 Emission spectrum of complex **7** (1 μM) for the Boolean logic functions based on eight combinations of three inputs: In1 = 25 nM Hg^{2+}, In2 = 25 nM Ag^{+}, In3 = 1 μM GSH, (a) InhibAND logic gate, [(A AND B) INHIBIT C], (b) InhibOR logic gate, [(A OR B) INHIBIT C].

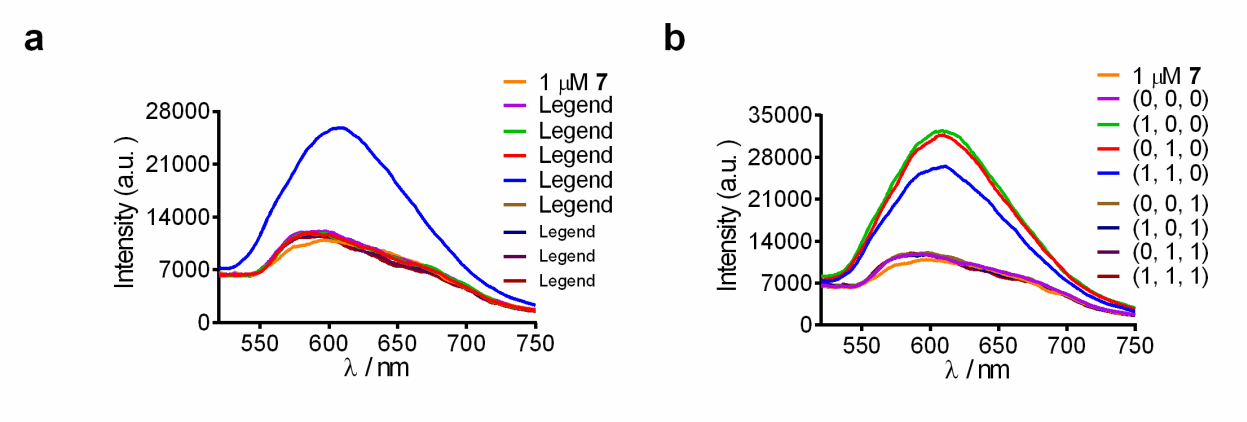
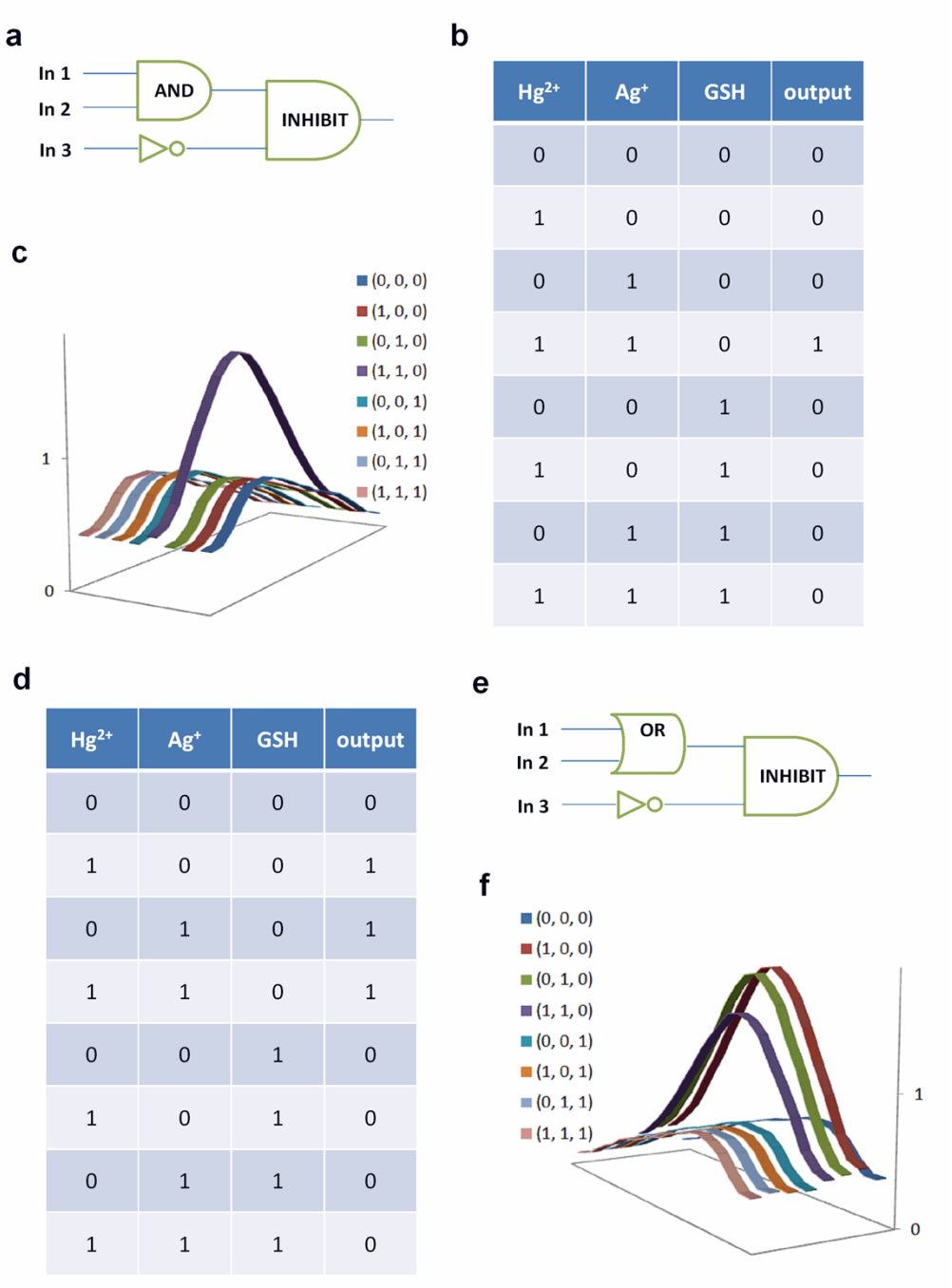


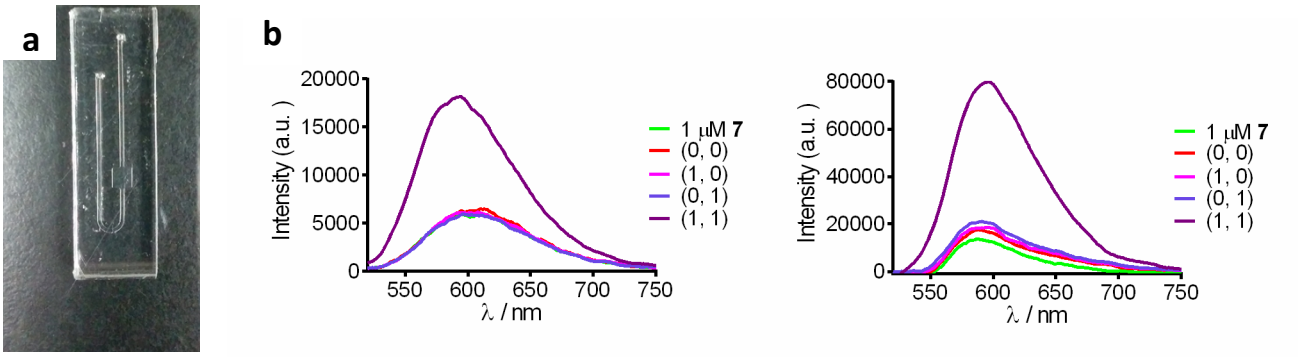
Fig. S4 Output values for the Boolean logic functions based on eight combinations of three inputs: In1 = 25 nM Hg^{2+}, In2 = 25 nM Ag^{+}, In3 = 1 μM GSH, (c) [Output = (A AND B) INHIBIT C], (f) [Output = (A OR B) INHIBIT C]. Truth table (b) and (d) and logic circuitry for the integrated logic system (a) and (e).



Hg^{2+}	Ag^{+}	GSH	output
0	0	0	0
1	0	0	0
0	1	0	0
1	1	0	1
0	0	1	0
1	0	1	0
0	1	1	0
1	1	1	0

Hg^{2+}	Ag^{+}	GSH	output
0	0	0	0
1	0	0	1
0	1	0	1
1	1	0	1
0	0	1	0
1	0	1	0
0	1	1	0
1	1	1	0

Fig. S5 Reproducibility performance of AND logic gate ion detection in (a) a microfluidic chip and (b) the comparison of the results (left, quartz cell; right, chip) at the same concentration of inputs (In1 = 25 nM Hg^{2+}, In2 = 25 nM Ag^{+}).



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