Supplemental Information

The application of G-quadruplex based assay with an iridium(III) complex to arsenic ion detection and its utilization in a microfluidic chip

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Experimental section

Materials

DNA sequences used in this project:

ssDNA: $5'-C_2AGT_2CGTAGTA_2C_3-3'$, dsDNA: $5'-CA_2TCG_2ATCGA_2T_2CGATC_2GAT_2G-3'$, PW17: $5'-G_3TAG_3CG_3T_2G_3-3'$, Pu22: $5'-TGAG_3TG_4AG_3TG_4A_4-3'$, c-kit87: $5'-AG_3AG_3CGCTG_3AG_2AG_3-3'$, TBA: $5'-G_2T_2G_2TGTG_2T_2G_2-3'$, Pu27: $5'-TG_4AG_3TG_4AG_3TG_4A_2G_2-3'$, c-myc: $5'-TGAG_3TG_3TAG_3TG_3TA_2-3'$, PS2.M: $5'-GTG_3TAG_3CG_3T_2G_2-3'$, As-3: $5'-GCTACT_2CT_2CATG_3C_2TCGCTGCA_2C_2A_2CA_2GACAT_2-3'$, G4-5: $5'-A_2TG_3CG_3CGCGAG_3AG_4AGC-3'$,

As-3-mA: 5'- GCTACT₂CTACATG₃C₂TCGCTGCACCTACCA₂GACAT₂-3', G4-5-mG: 5'- A_2 TGAGCGTGCGCGAG₃AGTAGAGC-3'.

General experimental

Mass spectrometry was performed at 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).

Synthesis

The complex was prepared according to (modified) literature methods.^{1, 2} All complexes are characterized by ¹H-NMR, ¹³C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis. The precursor iridium(III) complex dimer [Ir₂(C^N)₄Cl₂] was prepared as reported method.³ Then, a suspension of [Ir₂(C^N)₄Cl₂] (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 the 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 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 Yield: 71.3%. ¹H NMR (400 MHz, DMSO- d_6) δ 8.38 (s, 2H), 8.05 (d, J = 8.0 Hz, 2H), 8.00-7.98 (m, 4H), 7.27 (t, J = 8.0 Hz, 2H), 7.15-7.12 (m, 2H), 6.92 (t, J = 8.0 Hz, 4H), 6.33 (d, J = 7.2 Hz, 2H), 5.75 (d, J = 8.4 Hz, 2H), 2.78 (s, 6H), 2.31 (s, 6H); ¹³C NMR (100 MHz, DMSO- d_6) δ 181.7, 151.1, 151.0, 149.1, 148.1, 146.2, 140.8, 136.2, 133.3, 132.6, 131.6, 129.7, 128.6, 127.5, 126.5, 125.1, 124.9, 123.8, 117.2, 18.3, 15.4; MALDI-TOF-HRMS: Calcd. for C₄₂H₃₂IrN₄[M–PF₆]⁺: 849.1698 Found: 849.1722; Elem. Anal. (C₄₂H₃₂N₄IrS₂PF₆+H₂O) C, H, N: calcd 49.84, 3.39, 5.54; found 49.94, 3.39, 5.68.

Photophysical measurement

Emission spectra and lifetime measurements for complex was performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 395 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,⁴ [Ru(bpy)₃][PF₆]₂ in degassed acetonitrile as a standard reference solution ($\Phi_r = 0.062$) and calculated according to the following equation:

$$\Phi_{\rm s} = \Phi_{\rm r} (B_{\rm r}/B_{\rm s}) (n_{\rm s}/n_{\rm r})^2 (D_{\rm s}/D_{\rm r}) (1)$$

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.

Complex	Quantum yield	λ _{em} / nm	Lifetime/ μs	UV/vis absorption λ _{abs} / nm (ε/ dm ³ ·mol ⁻¹ ·cm ⁻¹)
1	0.204	530	$4.261 \pm 3.374 \times 10^{-3}$	227 (7.15 × 10 ⁴), 270 (4.76 × 10 ⁴)

Table S1. Photophysical properties of iridium(III) complex 1.

Figure S1. UV/Vis spectrophotometric titration of complex 1 with increasing concentrations of c-kit87.



Figure S2. Relative luminescence intensity of the system with (a) different concentrations of duplex DNA template (0.5, 1.0, 1.5, and 2.0 μ M); (b) different concentrations of complex 1 (0.5, 1.0, 1.5, and 2.0 μ M); (c) different concentrations of K⁺ ions (10, 20, 50, and 100 mM). (d) Relative luminescence enhancement of 1.0 μ M complex 1 at $\lambda = 530$ nm, dissolved with different organic solvents (at 0.2 % *v*/*v*) in the absence or presence of 0.4 μ M c-kit87.



Figure S3. The diagrammatic drawing and pictures for the one-step fabricated chip. The chip could be fast heated and cooled down by a portable electronic heater (below, left) with temperature inductor (below, long strip above the chip) and an iron block (below, right).



References

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