Supporting Information

A long lifetime switch-on iridium(III) chemosensor for the visualization of cysteine in live zebrafish

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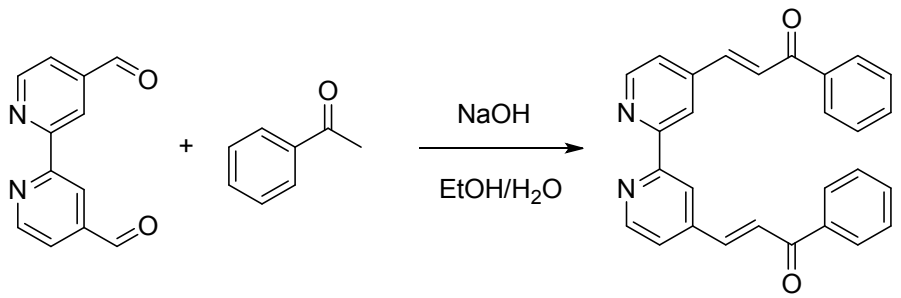
Materials. Reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO) and used as received. Iridium chloride hydrate ($IrCl_3.xH_2O$) was purchased from Precious Metals Online (Australia).

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). 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.

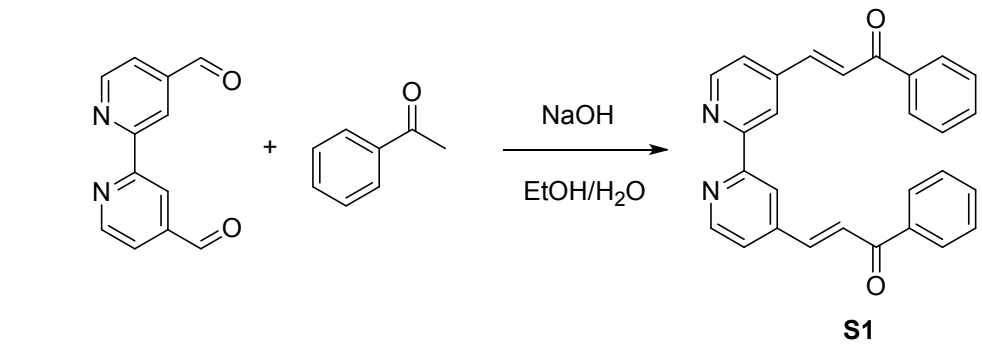
Synthesis



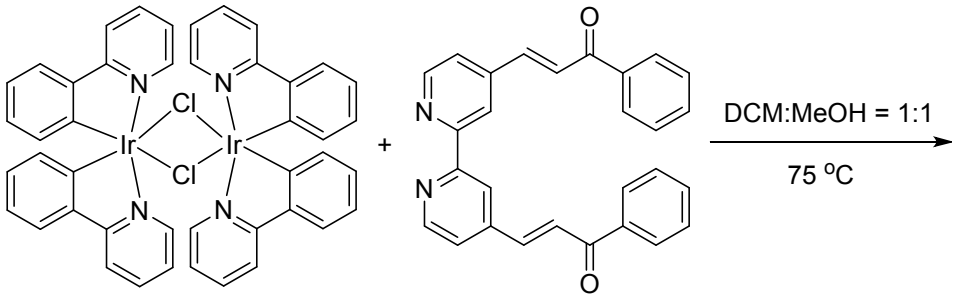
S1

To an ethanol solution (8 mL) of acetophenone (144 mg, 1.2 mmol) and [2,2'-bipyridine]-4,4'-dicarbaldehyde (106 mg, 0.5 mmol) was added a 10% aqueous solution of sodium hydroxide (2 mL) with stirring at room temperature overnight. The solvents were then evaporated *in vacuo* and the residue was purified by

chromatography on a silica gel column using dichloromethane–methanol (v/v = 10 : 1) as eluent. Yield: 68%.



S1



S1

A solution of ligand **S1** (12.8 mg, 0.03078 mmol) and the dichloro-bridged $[Ir(ppy)_2Cl]_2$ (15 mg, 0.014 mmol) in dichloromethane (3 mL) and methanol (3 mL) was stirred at 75 °C overnight. After the reaction completed, an excess of solid NH_4PF_6 was added and stirred for another 0.5 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluent, methanol/ dichloromethane, 1/20, v/v) to yield **1** as a brown powder. Yield: 65%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 8.25 – 8.05 (m, 5H), 7.94 (dd, *J* = 16.5, 6.8 Hz, 6H), 7.80 – 7.66 (m, 6H), 7.65 – 7.38 (m, 12H), 7.05 (ddt, *J* = 16.5, 13.0, 6.6 Hz, 3H), 6.98 – 6.87 (m, 2H), 6.31 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.98, 166.57, 155.21, 149.40, 149.07, 147.80, 145.07, 142.44, 137.21, 137.06, 135.87, 132.63, 130.72, 129.85, 129.63, 128.27, 127.88, 126.31, 123.77, 122.57, 122.25, 121.78, 118.58. MALDI-TOF-HRMS: Calcd. for $C_{50}H_{36}F_6IrN_4O_2P$ $[M–PF_6]^+$: 917.2455, found: 917.2465.

Cysteine detection. 10 mM of complex stock solution was prepared by dissolving complex **1** in DMSO. The complex was then added into DMSO to a final concentration of 10 μM. Different concentrations of Cys (or other amino acids) were then added to 800 μL of DMSO containing complex **1** (20 μM) and 200 μL 10 mM HEPES, pH = 7 in a cuvette. Emission spectra was recorded. Luminescence emission

spectra were recorded on a PTI QM-4 spectrofluorometer (Photo Technology International, Birmingham, NJ) at 25 °C, with the slits for both excitation and emission set at 2.5 nm. UV-Vis absorption spectra were recorded on a Cary UV-300 spectrophotometer (double beam).

Time-resolved emission spectra (TRES) measurement

2 μL of 1 mM competitive organic dye was added into 30 μM complex **1** in 2 mL DMSO–HEPES (10 mM, pH 7.0, 4:1 v/v). Cys was added and incubate 10 min. Steady state emission spectra of mixture were recorded by QM-4 Photon Technology International while TRES was measured by a Horiba Fluorolog TCSPC spectrophotometer.

Imaging of zebrafish

Zebrafish was kept at 28 °C and maintained at optimal breeding conditions. For mating, male and female zebrafish was maintained in one tank at 28 °C on a 12 h light/12 h dark cycle and then the spawning of eggs were triggered by giving light stimulation in the morning.[1] Almost all the eggs were fertilized immediately. The 3-day old zebrafish was maintained in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM MgSO4, 1 mM $CaCl_2$, 0.15 mM, KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10^{-5}% methylene blue; pH 7.5). The zebrafish was incubated with or without 200 μM of NEM for 15 min in E3 media at 28 °C. After washing with E3 media to remove the remaining NEM, the zebrafish was further incubated with 10 μM of **1** in E3 media for 30 min at 28 °C. After washing with E3 media, the zebrafish was imaged by fluorescence microscopy.

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

Quantum yield	λ_{em}/ nm	Lifetime/ μs	UV/vis absorption λ_{abs} / nm (ε/ dm^3 mol^{-1} cm^{-1})
0.0053	580	4.297	259 (7.06× 10^4), 383 (2.18× 10^4)

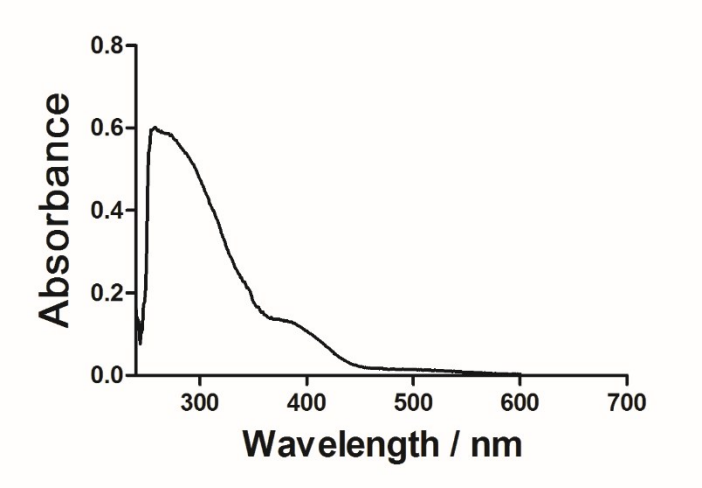


Fig. S1 UV-Vis absorption spectra of **1** (10 μM) in DMSO.

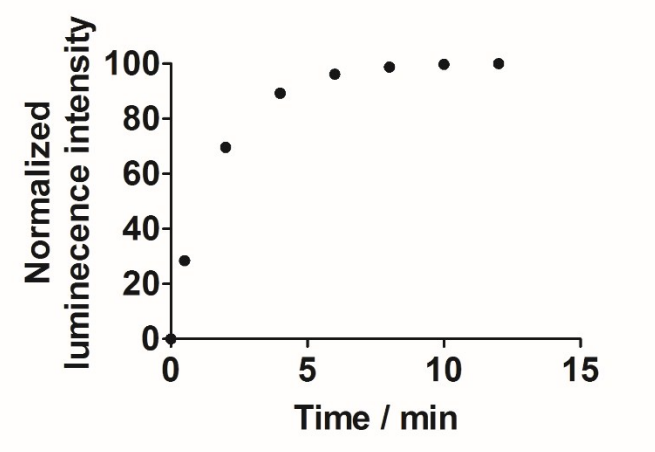


Fig. S2 Normalized luminescence intensity of **1** upon addition of 40 μM Cys in DMSO–HEPES (10 mM, pH 7.0, 4:1 v/v).

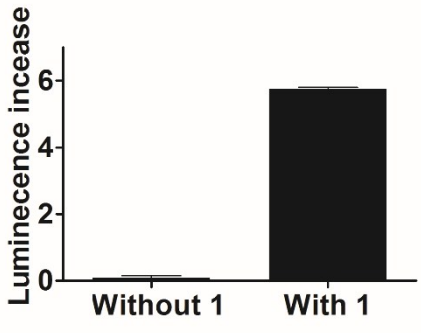


Fig. S3 Luminescence enhancement of system in response to 40 μM Cys in the presence or absence of 10 μM **1** in DMSO–HEPES (10 mM, pH 7.0, 4:1 v/v).

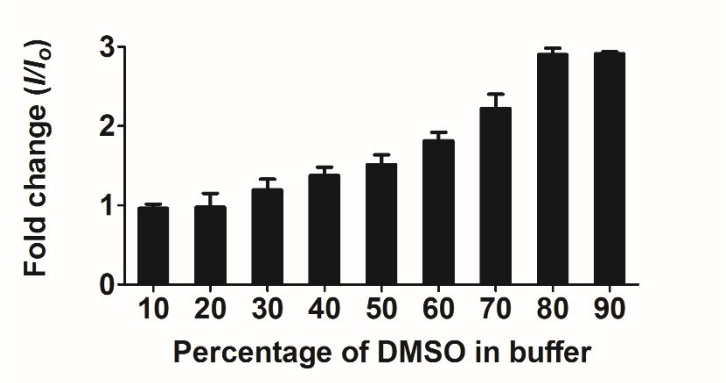


Fig. S4 Luminescence enhancement of 10 μM **1** with 40 μM Cys in various percentage of DMSO in HEPES (10 mM, pH 7.0).

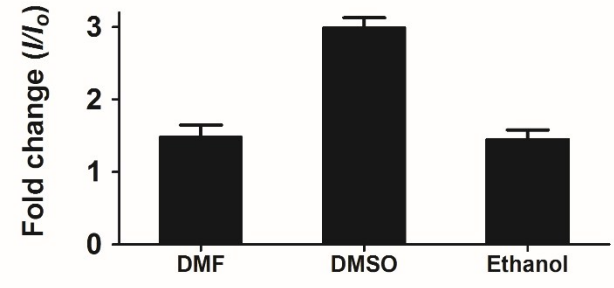


Fig. S5 Luminescence enhancement of 10 μM **1** with 40 μM Cys in various types of organic solvents with 20% HEPES (10 mM, pH 7.0).

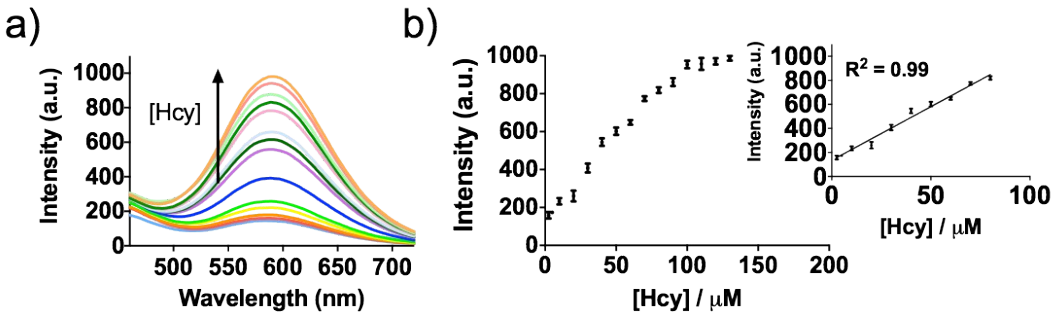


Fig. S6 (a) Luminescence spectra of **1** (10 μM) upon addition of increasing concentrations of Hcy (0–130 μM) in DMSO–HEPES (10 mM, pH 7.0, 4:1 *v/v*). (b) The relationship between luminescence intensity and Hcy concentration.

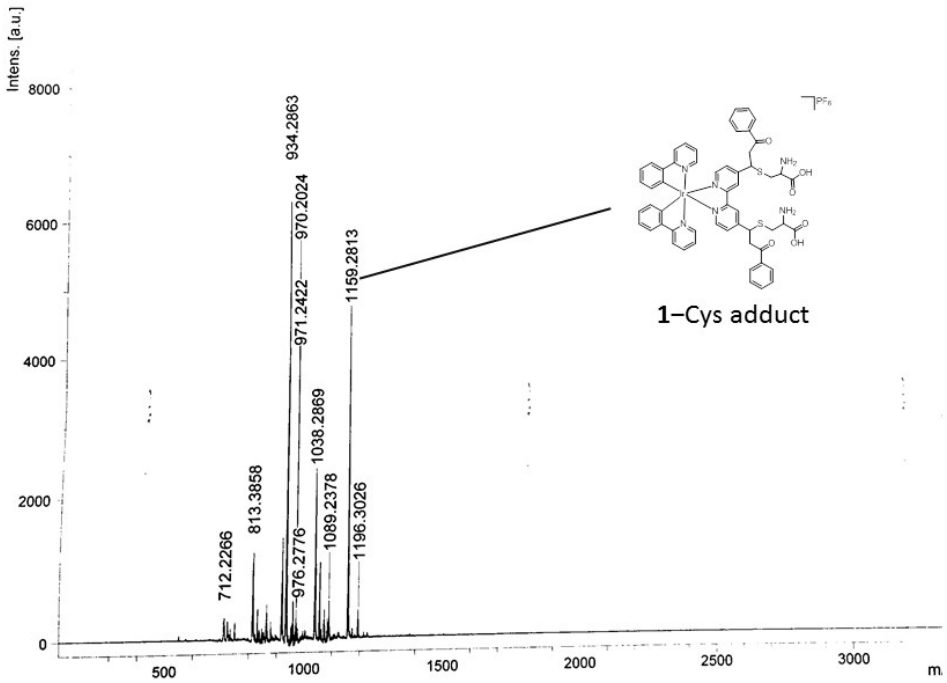


Fig. S7 High-resolution mass spectrum of the reaction product of **1** and Cys.

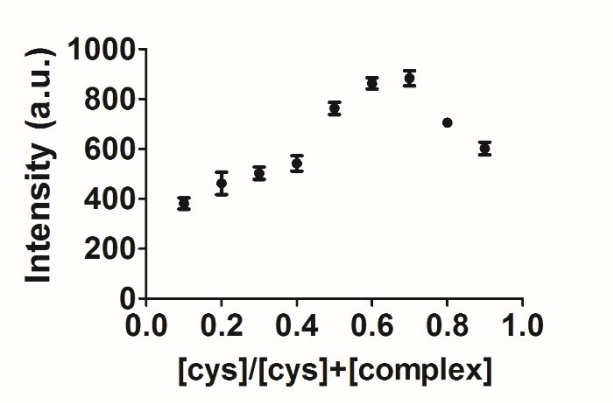


Fig. S8 Job's plot of both **1** and Cys in DMSO–HEPES (10 mM, pH 7.0, 4:1 v/v).

Reference

1. Y.-K. Yang, S.-K. Ko, I. Shin and J. Tae, *Nat. Protocols*, 2007, **2**, 1740-1745.