Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Thiol-specific phosphorescent imaging in living cells with an azobis(2,2′-bipyridine)-bridged dinuclear iridium(III) complex

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Experimental Procedures

Instruments

Microanalysis (C, H and N) was carried out with a Perkin-Elmer 240Q elemental analyzer. Infrared spectra were recorder on a Bruker VECTOR22 spectrometer in KBr pellets over a range of 400-4000 cm^{-1}. ^1H NMR spectra were recorded on Varian-500 spectrometer. All chemical shifts were given relative to tetramethylsilane (TMS). Electrospray mass spectra were recorded on a LCQ system (Finnigan MAT, USA). The electronic absorption spectra were recorded using Pekin Elmer Lambda850 spectrometer. Emission spectra were recorded on a Perkin-Elmer L55 spectrofluorophotometer. Cyclic voltammetry measurements were performed on a CHI 660A electrochemical workstation. All samples dissolved in CH_3CN were purged with Ar prior and 0.1 M tetrabutylammonium perchlorate (TBAP) was used as a supporting electrolyte. A standard three-electrode system comprising a glassy carbon working electrode, Pt-wire auxiliary electrode and a saturated calomel reference electrode (SCE) was used. The scan rate was 100mV/s. The ESR spectra were measured using a Bruker A300 spectrometer with a 9.86GHz microwave frequency. The conductivity measurements were carried out in a DDS-12A digital conductivity meter, which was standardized using 1.0 mM KCl solution before all measurements.

Materials

Unless otherwise mentioned, all the amino acids and other reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments.

Stock solutions of amino acids (30 mM) were prepared in HEPES buffer (10 mM, pH 7.5). Stock solution of **Ir_2-azo** (1 mM) was prepared in acetonitrile. In a typical experiment, test solution were prepared by placing **Ir_2-azo** stock solution into 1.5 mL acetonitrile in test tube, diluting the solution to 3 mL with HEPES buffer, and adding an appropriate buffer of each amino acids stock solution. Both absorption spectra and emission spectra were measured after addition of amino acids for 5 min. Excitation was normally 430 nm in the emission spectra.

Syntheses

The compounds $[Ir_2(pq)_4Cl_2]$[1] and 4,4''-azobis(2,2'-bipyridine) (**azobpy**) ligand[2] were synthesized according to literature methods.

Synthesis of [Ir(pq)$_2$(azobpy)]Cl (**Ir$_1$-azo**)

A mixture of $[Ir_2(pq)_4Cl_2]$ (64 mg, 0.05 mmol) and **azobpy** ligand (100 mg, 0.3 mmol) were suspended in 15 mL MeOH/$CHCl_3$ (1:1, v/v) and heated at 65 ^oC under Ar. After 4 h reflux, the mixture was evaporated under reduced pressure, and the crude product was purified by column chromatography on silica with CH_2Cl_2-acetone (3:1, v/v) as eluent. Yield: 39 mg, 41%. Anal. Calcd. for $C_{50}H_{34}ClN_8Ir$ (%):C, 61.62; H, 3.52; N, 11.50. Found (%): C, 62.09; H, 3.46; N, 11.66. ES-MS (CH_3OH) m/z: 939.2 $[M\text{-}Cl^-]^+$. ^{1}H NMR (300 MHz, CD_3CN-d_3): 9.96 (1 H, d, J = 6.3), 9.68 (1 H, d, J = 5.7), 9.22 (1 H, d, J = 6.3), 8.98 (2 H, d, J = 5.1), 8.94(1 H, t, J = 4.5), 8.90 (2 H, d, J = 4.8), 8.84 (2 H, d, J = 7.5), 8.75 (2 H, d, J = 4.8), 8.54 (4 H, t, $J_1 = J_2$ = 6), 8.49 (2 H, d, J = 4.5), 8.39 (1 H, t, J = 6.0), 8.17 (1 H, d, J = 6.3), 8.10 (1 H, t, J = 7.8), 8.04 (1 H, d, J = 7.8), 7.97 (2 H, t, J = 9.0), 7.91 (2 H, t, J = 4.8), 7.87 – 7.77 (2 H, m), 7.69 (2 H, t, J = 6.6), 7.52 – 7.44 (4 H, m).

Synthesis of [Ir(pq)$_2$(azobpy)Ir(pq)$_2$]Cl$_2$ (**Ir$_2$-azo**)

A mixture of $[Ir_2(pq)_4Cl_2]$ (128 mg, 0.1 mmol) and **azobpy** ligand (34 mg, 0.1 mmol) were suspended in 20 mL MeOH/$CHCl_3$ (1:1, v/v) and heated at 65 ^oC under Ar. After 6 h reflux, the mixture was evaporated under reduced pressure, and the crude product was purified by column chromatography on silica with CH_2Cl_2-MeOH (20:1, v/v) as eluent. Yield: 109 mg, 67%. Anal.

Calcd. for $C_{80}H_{54}Cl_2N_{10}Ir_2$ (%):C, 59.65; H, 3.38; N, 8.70. Found (%): C, 60.09; H, 3.48; N, 8.60. ES-MS (CH_3OH) m/z: 770 $[M\text{-}2Cl^-]^{2+}$, 1574 $[M\text{-}Cl^-]^+$. ^1H NMR (300 MHz, CD_3CN-d_3): 8.63 (2H, s), 8.54 (2H, d, J = 6.0), 8.47 (8H, d, J = 5.1), 8.36 (4H, t, J = 4.5), 8.27 (4H, d, J = 7.8), 8.09 (2H, t, J = 7.8), 7.91 (6H, d, J = 8.7), 7.68 – 7.60 (2H, m), 7.49 (4H, t, J = 6.0), 7.42 (4H, t, J = 9.0), 7.30 (4H, t, J = 6.0), 7.19 – 7.12 (4H, m), 6.94 (4H, t, J_1 = 3.3, J_2 = 1.5), 6.63 (4H, dd, J_1 = 10.8, J_2 = 7.8).

Cell viability assay

HeLa cells were maintained in DMEM media with 10% FBS and 1% antibiotic solution at 37 °C at 5% CO_2 in the steri-cycle CO_2 incubator with HEPA Class 100 filters, Thermo Electron Corporation.

The cytotoxicity of **Ir_2-azo** was evaluated in HeLa cells by MTT assay[3] (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetraazolium bromide). At 2-3 days after seeding, the cells were counted by hemocytometer and seeded into a 96-well cell-culture plate at a cell density of 1×10^4 cells per well and then incubated for 24 h at 37 °C under 5% CO2. The complex was then added at indicated concentrations to quadruplicate wells. After 48 hours, the medium was replaced with fresh DMEM medium and MTT was added to each well at a final volume of 0.5 mg mL^{-1}. The microplates were incubated at 37 °C for 4h. The medium was then removed and 100 μL of DMSO solution was added to the plates and shaken to dissolve the formazan products. Tecan Infinite M200 monochromator-based multifunction microplate reader was used to measure the OD490 of each well. The cell survival rate in the control wells without **Ir_2-azo** solutions was considered as 100% cell survival.

Confocal luminescence imaging

The Hela cells were seeded at a density of 2×10^6 cells per mL in culture media. After 24 h, the cells were treated without or with 100 μM NEM in DMEM media for 30 min. After removing the DMEM media and washing with PBS to remove the remaining NEM, the Hela cells were further incubated with 5.0 μM **Ir_2-azo** solutions in PBS for 30 min. Cell images were captured with a monochromatic CoolSNAP FX camera (Roper Scientific, USA) and analyzed by usingAxioVision

4.2 software (Carl Zeiss).

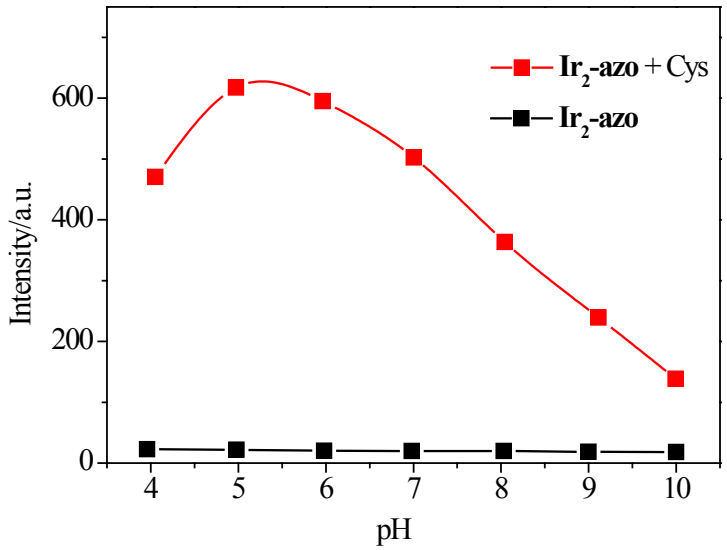


Fig. S1 The emission intensity of 5.0 μM **Ir_2-azo** at 564 nm in the absence (black square) and presence of 10.0 μM Cys (red square) under different pH in Britton-Robinson buffer.

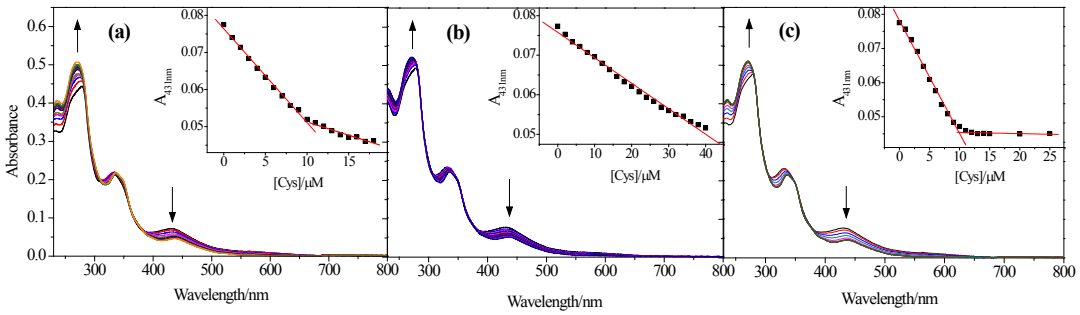


Fig. S2 Absorption spectra of **Ir_2-azo** (5.0 μM) upon addition of different concentrations of (**a**) Cys (0- 20 μM), (**b**) Hcy (0- 40 μM) and (**c**) GSH (0- 25 μM) in 1:1 CH_3CN/HEPES (10mM, pH = 7.5).

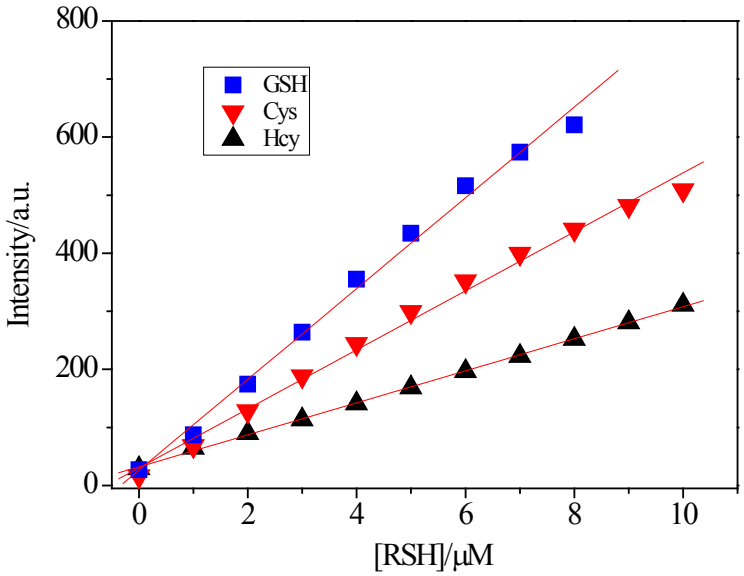


Fig. S3 The linearity relationship of intensity *vs* thiol concentrations.

Table S1 Calculation of detection limit of Cys, Hcy and GSH with **Ir_2-azo**.

Thiol	σ	M	R^2	S/N	DL
Cys	0.6258	5.080×10^7 M^{-1}	0.9963	3	3.69×10^{-8} M
Hcy	0.6258	2.775×10^7 M^{-1}	0.9996	3	6.76×10^{-8} M
GSH	0.6258	7.816×10^7 M^{-1}	0.9969	3	2.40×10^{-8} M

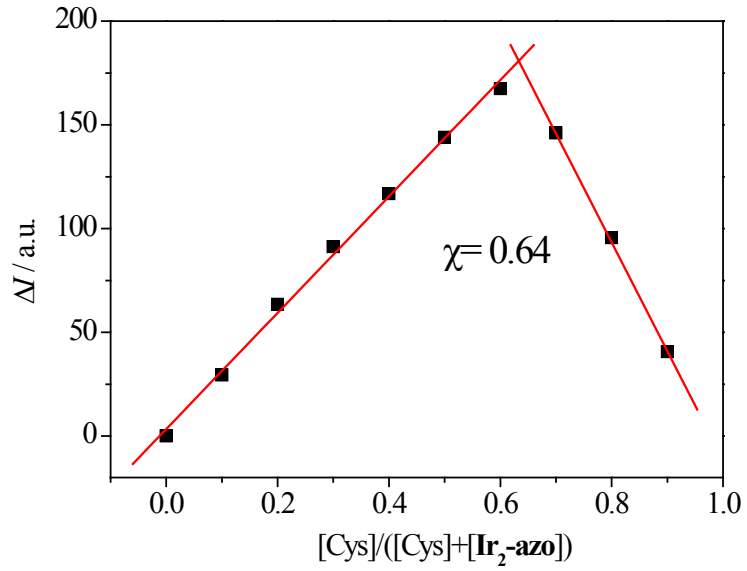


Fig. S4 Job's plot of the reaction between **Ir_2-azo** and Cys in CH_3CN/HEPES (1:1, v/v) solution. Total concentration of **Ir_2-azo** and Cys was kept constant at 20.0 μM.

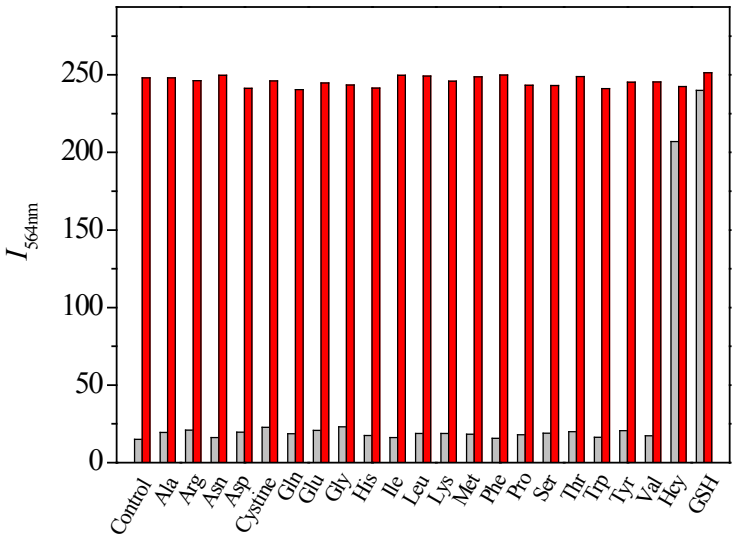


Fig. S5 The emission intensity of **Ir_2-azo** (2.5 μM) reacted with 20 equiv. different amino acids (gray bars) and then 2 equiv. Cys were added into the mixture (red bars).

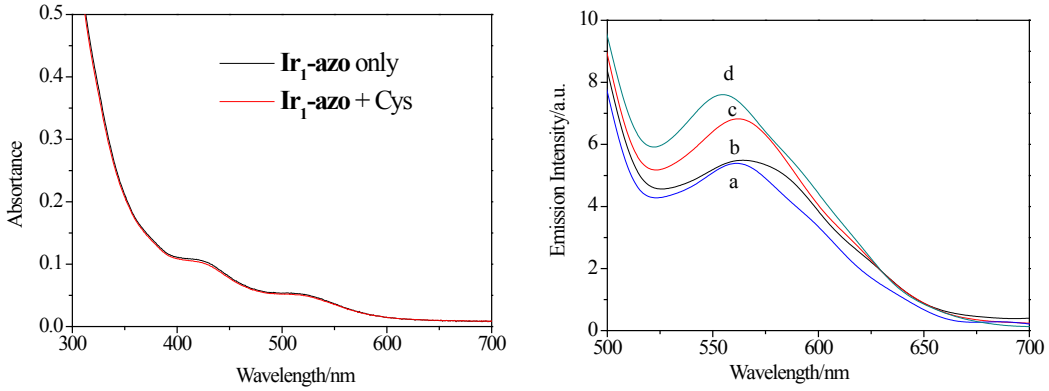


Fig. S6 Left: Absorption spectra of **Ir_1-azo** (10.0 μM) in the absence (black line) or presence of 100 μM Cys (red line) in 1:1 CH_3CN/HEPES (10 mM, pH = 7.5). Right: Emission spectra of **Ir_1-azo** (10.0 μM) in the absence (**a**) or presence of 100 μM Hcy (**b**), Cys (**c**) and GSH (**d**) in 1:1 CH_3CN/HEPES (10 mM, pH = 7.5) solution. λ_{ex} = 430 nm.

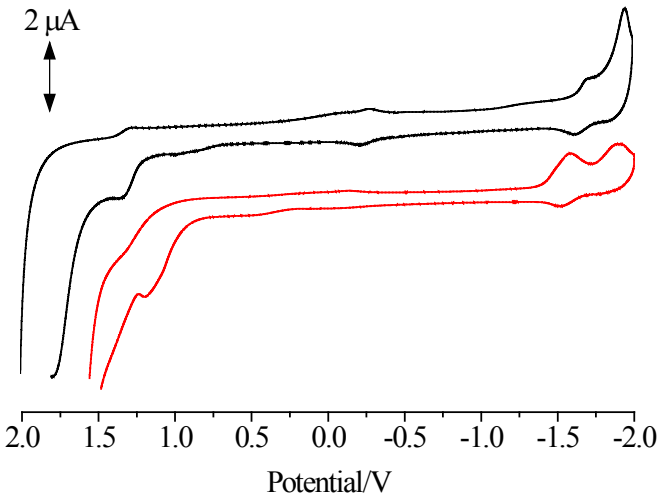


Fig. S7 Cyclic voltammograms for 100 μM **Ir_2-azo** in the absence (black line) or presence of 200 μM Cys (red line) in 0.1 M TABP CH_3CN solutions containing 2% HEPES buffer. Scan rate was 0.1 V/s.

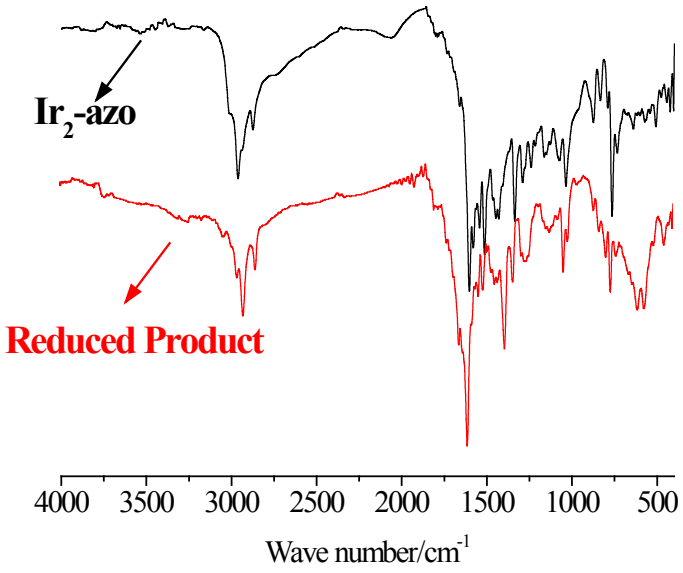


Fig. S8 Infrared spectra of **Ir_2-azo** (black line) and isolated reduced product (red line).

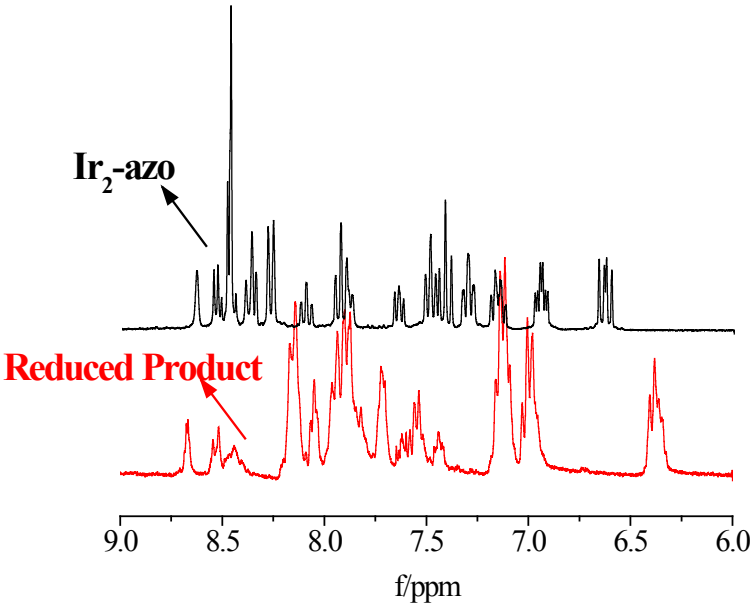


Fig. S9 ^{1}H NMR spectra of **Ir_2-azo** (black line) and isolated reduced product (red line) in CD_3CN-d_3, 300 MHz.

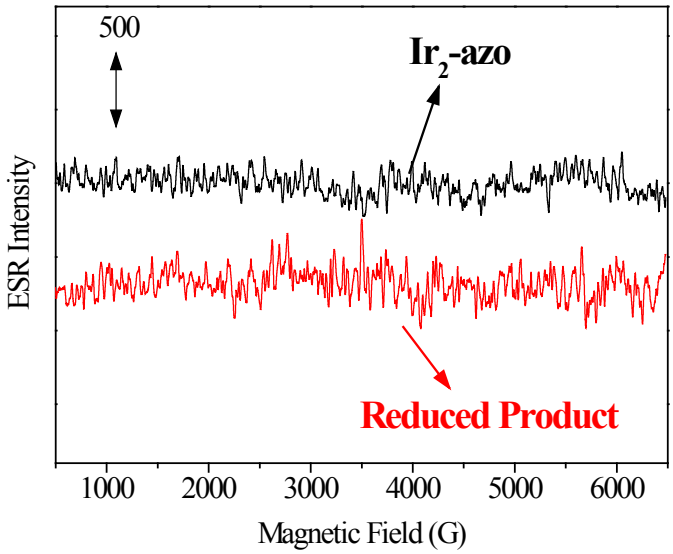
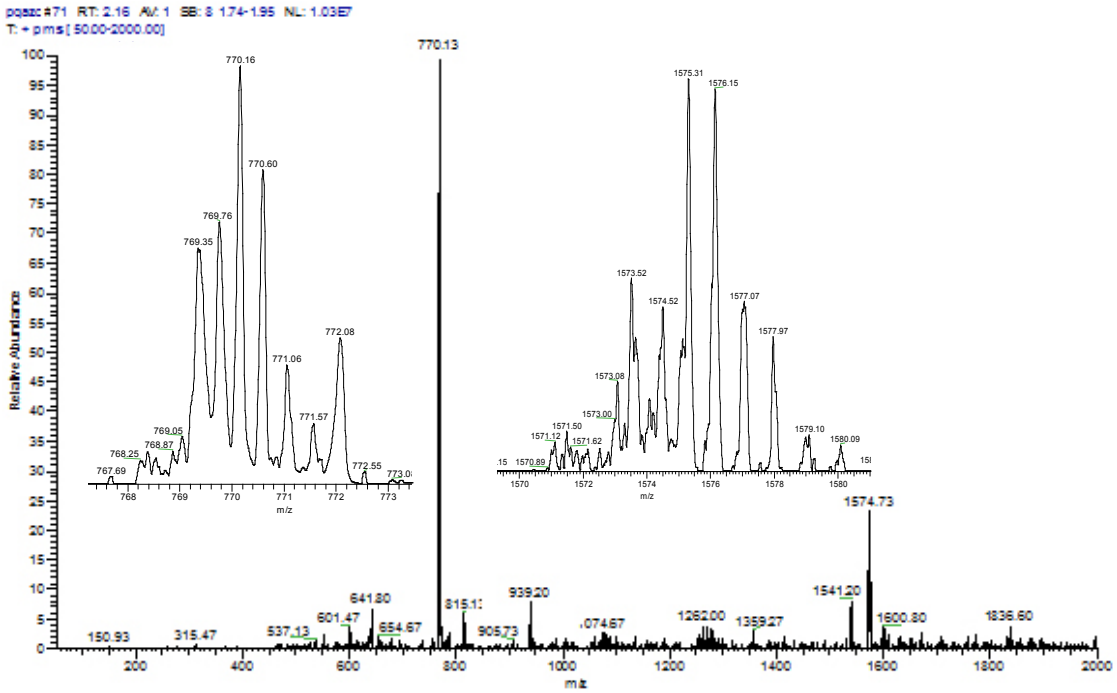


Fig. S10 EPR trace for **Ir_2-azo** (black line) and reduced product (red line) in deaerated CH_3CN solution.

(a)



(b)

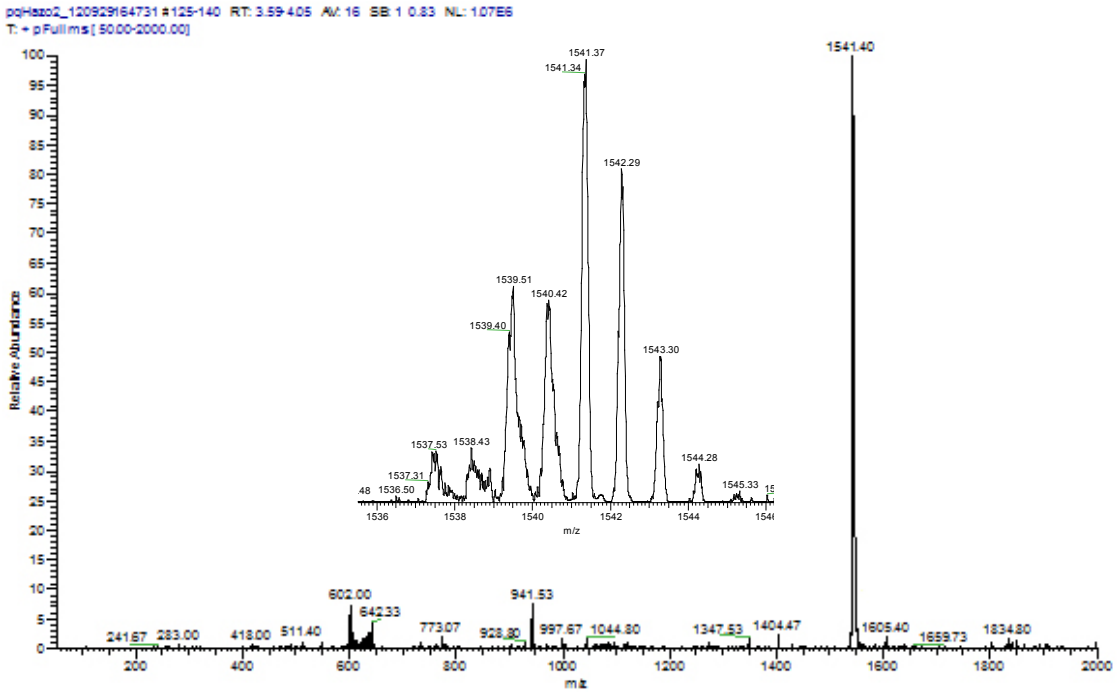


Fig. S11 ES-MS spectra of **Ir_2-azo** (**a**) and reduced product (**b**) in CH_3CN solutions.

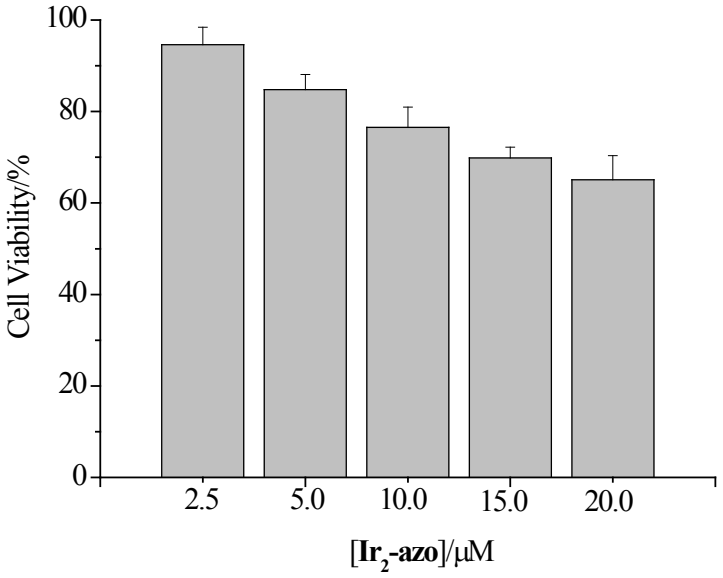


Fig. S12 Viabilities of Hela cells incubated with different concentration of **Ir_2-azo** for 48 h.

References

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