Supporting Information

Time-resolved analysis of photoluminescence at a single wavelength for ratiometric and multiplex biosensing and bioimaging

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Materials and Measurements. All solvents were of analytical grade and purified according to standard procedures. All chemical reagents used were purchased form Energy Chemical without further purification. HeLa cells were obtained from Jiangsu KeyGEN BioTECH Corp. Ltd. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ACF400 spectrometer at 298 K. Mass spectra were recorded on Bruker autoflex matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer (MS). The number-average molecular weight was measured via gel permeation chromatography (GPC) in tetrahydrofuran (THF) by using the calibration curve of polystyrene standards. UV–Vis absorption spectra were recorded using a Shimadzu UV-3600 UV–VIS–NIR spectrophotometer. Fluorescence spectra and lifetime decay curves were measured on Edinburgh FL 980 spectrophotometer. Cellular imaging was performed using an Olympus IX81 laser-scanning confocal microscope and time-resolved analysis was performed on a PLIM setup with professional software provided by PicoQuant Company.

Cell Viability Assay. The MTT assay was used to evaluate the cytotoxic effect of **P1**. HeLa cells were seeded in a 96-well flat-bottomed microplate (10,000 cells/well) in medium (100 μL) and incubated at 37°C under a 5% CO_2 atmosphere for 24 h. **P1** was then added into the wells with concentrations ranging from 0 – 600 μg/mL in a mixture of medium/DMSO (99:1, v/v). Wells containing the medium without cells were used as blank controls. The cells was incubated at 37°C under a 5% CO_2 atmosphere for 24 h. Then MTT (10 μL, 5 mg/mL) in PBS was added to each well and the microplate was

incubated for another 4 h. After that, the medium was replaced with DMSO (150 μL) and the cells was further incubated for 1 h. The absorbance at 570 nm was measured with a Microplate Spectrophotometer (TECAN SUNRISE).

Cellular imaging. Hela cells were grown in a 35-mm glass bottom tissue culture dish and incubated at 37 °C under a 5% CO_2 atmosphere for 48 h. The medium was removed and replaced with medium containing **P1** (200 μg/mL). After incubation for 1 h, the medium was removed and the cells were washed with PBS (1 mL × 3). Imaging was performed with an excitation wavelength at 405 nm. The emission was measured using a band-pass filter at 600±25 nm. For imaging of hypochlorite, sodium hypochlorite of different concentrations was added after washing with PBS. After incubation for 30 min, the cells were wash with PBS and imaging was performed. For imaging of endogenous hypochlorite, cells were preincubated with elesclomol (125 nM) for 2 h before incubation with **P1**. For imaging of oxygen contents, after incubation with **P1** and washed with PBS, the cells were incubated under an atmosphere containing 2% and 95% O_2 for 30 min. After imaging, sodium hypochlorite (5 μM) was added. The cells were incubated under air for 30 min, washed with PBS, and imaging was performed under air and 2% and 95% O_2 conditions.

Synthesis and characterization

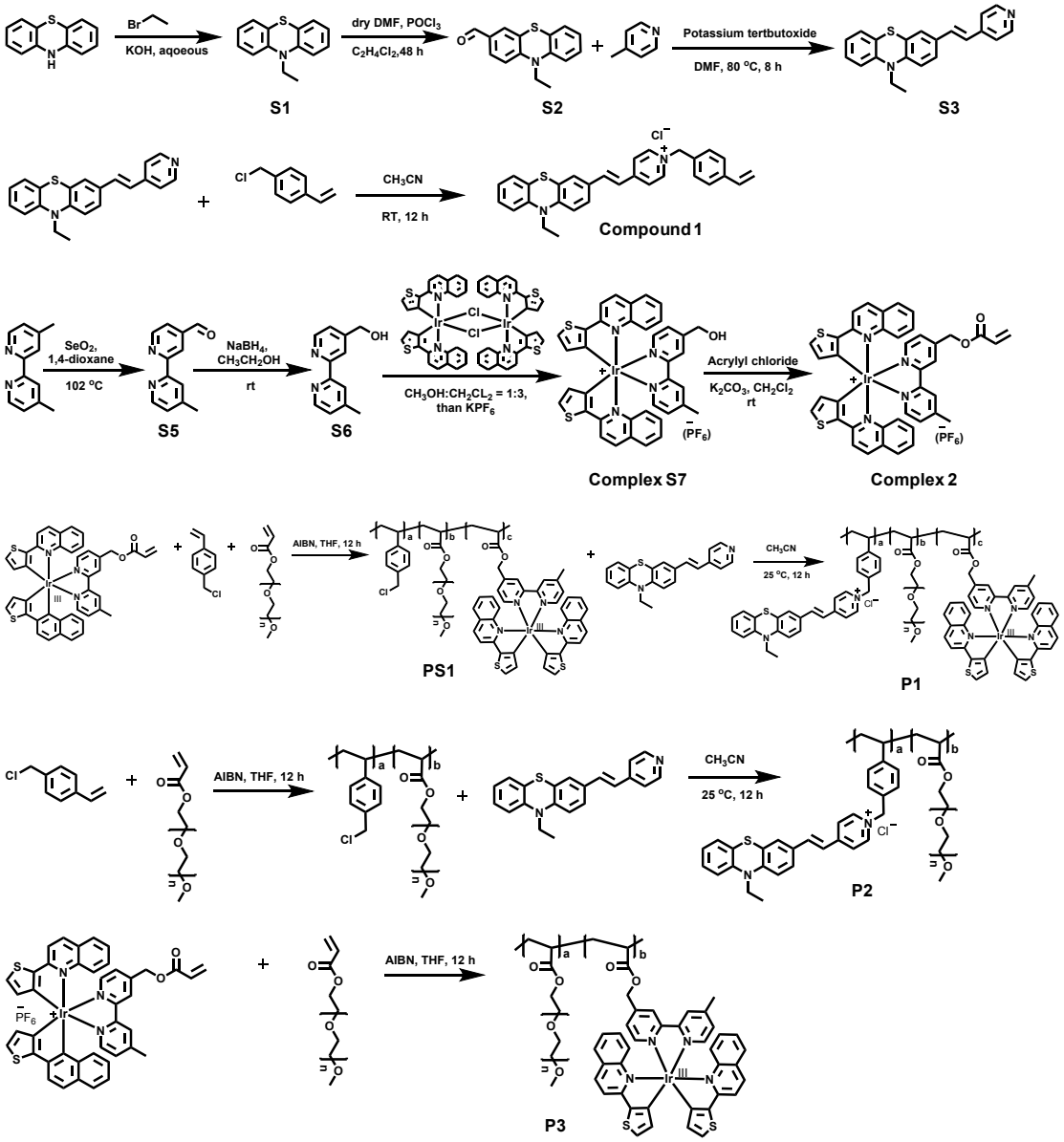
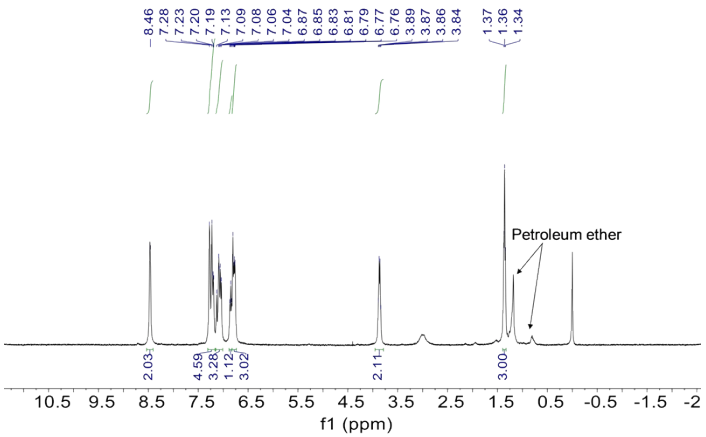


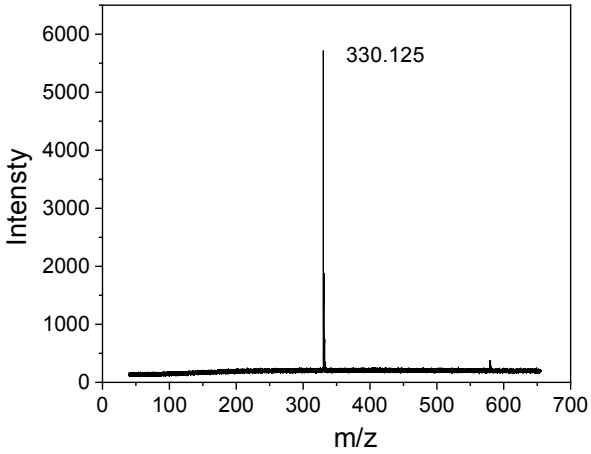
Fig. S1 The synthetic route of compound **1**, complex **2**, and **P1 – P3**.

Compound **S1**, **S2**, **S5** and **S6** were synthesized according to previous reports (*Chem. Commun.* **51**, 1442–1445 (2015); *Ind. Eng. Chem. Res.* **57**, 7735−7741 (2018)).

Compound S3. A mixture of 4-methylpyridine (204 mg, 2.2 mmol), compound **S2** (510 mg, 2 mmol), and potassium tert-butanolate (280 mg, 2.5 mmol) in DMF (10 mL) was stirred at 80 °C for 8 h. Then, the mixture was evaporated to dryness. The solid was dissolved in CH_2Cl_2 (50 mL) and washed with water (20 mL × 3). The organic layer dried with $MgSO_4$. The mixture was then evaporated to dryness and purified by column chromatography on silica gel. Compound **S3** was eluted with n-hexane/ethyl acetate (50:1, v/v) and isolated as a yellow powder (614 mg, 1.9 mmol, 93%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ = 8.46 (s, 2H), 7.28 – 7.19 (m, 4H), 7.13 – 7.03 (m, 3H), 6.85 – 6.76 (m, 4H), 3.88 – 3.84 (m, 2H), 1.36 (t, *J* = 6.8 Hz, 3H). MALDI-TOF MS: m/z = 330.1 $[M]^+$.

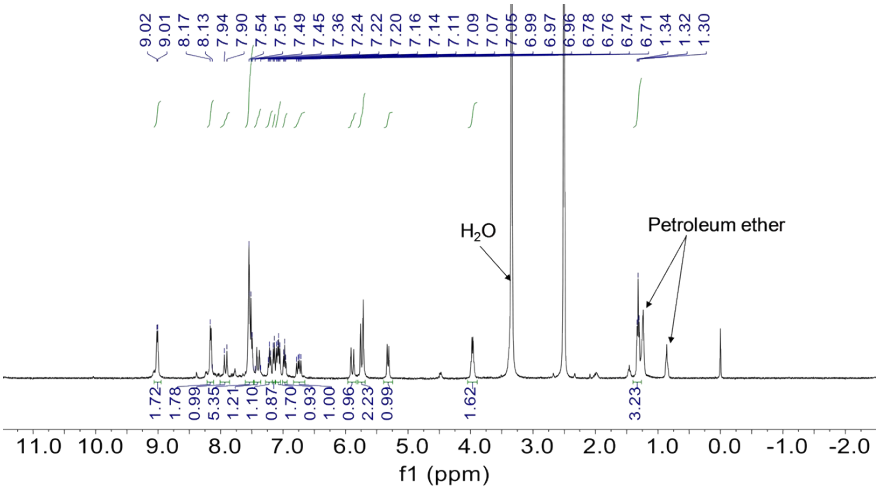


^{1}H NMR spectrum of compound **S3** in $CDCl_3$

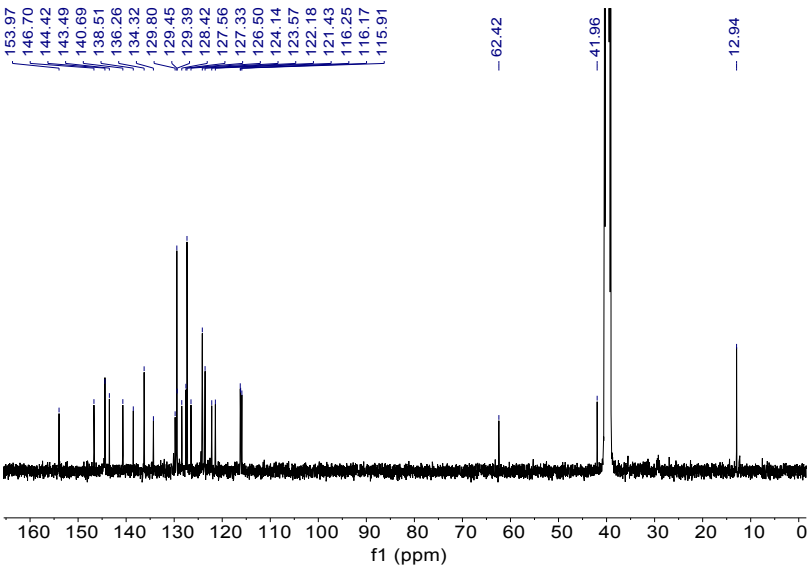


MALDI-TOF MS spectrum of compound **S3**

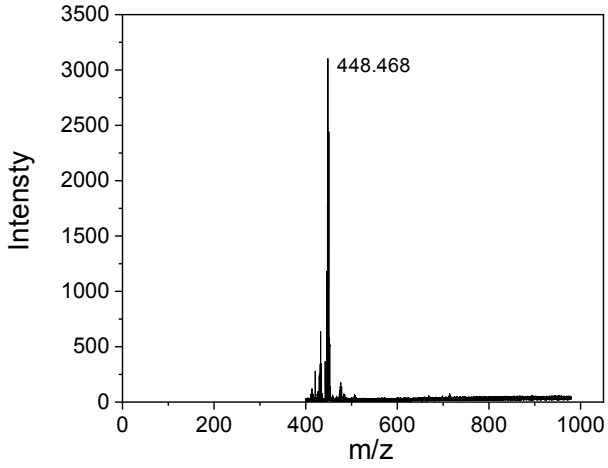
Compound 1. A mixture of compound **S3** (330 mg, 1 mmol) and 4-vinylbenzyl chloride (168 mg, 1.1 mmol) in CH_3CN (10 ml) was stirred at room temperature for 12 h. After that, the solution was evaporated to dryness and the crude product was purified by flash column chromatography on silica gel using dichloromethane/methanol (75:1, v/v) as an eluent. Compound **1** was isolated as a red powder (415 mg, 0.86 mmol, 86%). ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ = 9.01 (d, J = 6.4 Hz, 2H), 8.16 (d, J = 6.3 Hz, 2H), 7.92 (d, J = 16.2 Hz, 1H), 7.54 – 7.49 (m, 6H), 7.40 (d, J = 16.2 Hz, 1H), 7.22 (t, J = 8 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.10 – 7.05 (m, 2H), 6.98 (t, J = 6.6 Hz, 1H), 6.75 (m, 1H) 5.89 (d, J = 17.7 Hz, 1H), 5.74 (d, J = 17.4 Hz, 2H), 5.32 (d, J = 10.7 Hz, 1H), 3.99 – 3.94 (m, 2H), 1.32 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, 298 K, TMS) δ = 153.97, 146.70, 144.42, 143.49, 140.69, 138.51, 136.26, 134.32, 130.12, 129.80, 129.45, 129.39, 128.42, 127.56, 127.33, 126.50, 124.14, 123.57, 122.18, 121.43, 116.25, 116.17, 115.91, 62.42, 41.96, 12.94. MALDI-TOF MS: m/z = 448.5 $[M - Cl^-]^+$.



^{1}H NMR spectrum of compound **1** in DMSO-d_6



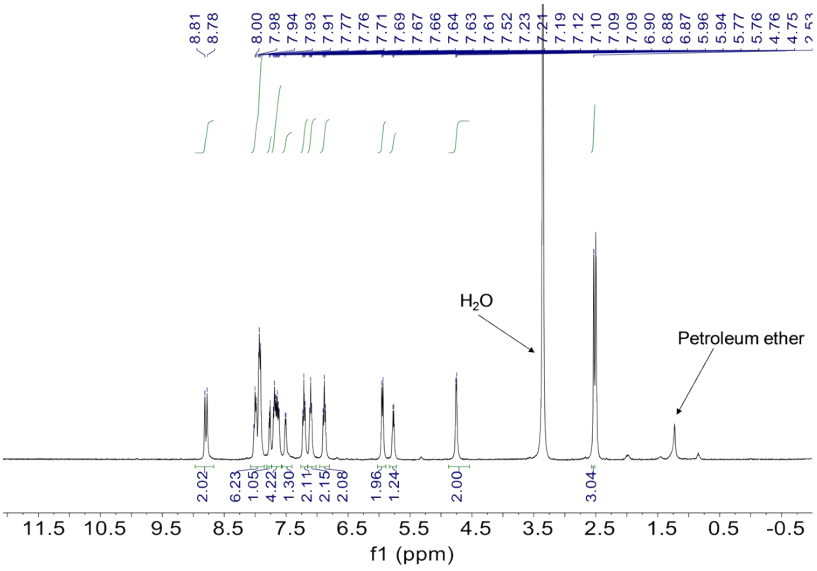
^{13}C NMR spectrum of compound **1** in DMSO-d_6



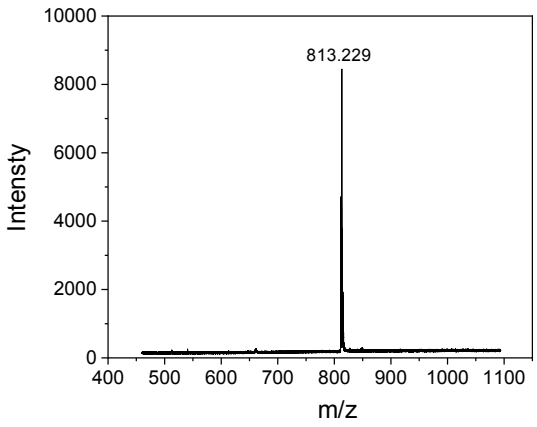
MALDI-TOF MS spectrum of compound **1**

Complex S7. A mixture of 4-hydroxymethyl-4'-methyl-2,2'-bipyridyl (**S6**) (200 mg, 1 mmol) and iridium(III) 2-thiophen-2-ylquinoline dichloro-bridged dimer (648 mg, 0.5 mmol) in CH_3OH/CH_2Cl_2 (15 mL, 1:3, v/v) was refluxed under an inert atmosphere of nitrogen in the dark for 8 h. The solution was then cooled to room temperature and KPF_6 (276 mg, 1.5 mmol) was added. After stirring at room temperature for 2 h, the solution was evaporated and the cured product was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (40:1, v/v) as an eluent to afford **S7** as a yellow powder (795 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.79 (d, J = 13.17 Hz, 2H),

8.02 – 7.91 (m, 6H), 7.77 (d, J = 5.6 Hz, 1H), 7.71 –7.61 (m, 4 H), 7.51 (d, J = 5.8 Hz, 1H), 7.21 (t, J = 6.45 Hz, 2H), 7.10 (t, J = 6.5 Hz, 2H), 6.88 (t, J = 7.7 Hz, 2H), 5.95 (d, J = 8.2 Hz, 2H), 5.76(d, J = 6.0, 1H), 4.75 (d, J = 5.3 Hz, 2H), 2.53 (s, 3H). MALDI-TOF MS: m/z = 813.2 $[M - PF_6^-]^+$.



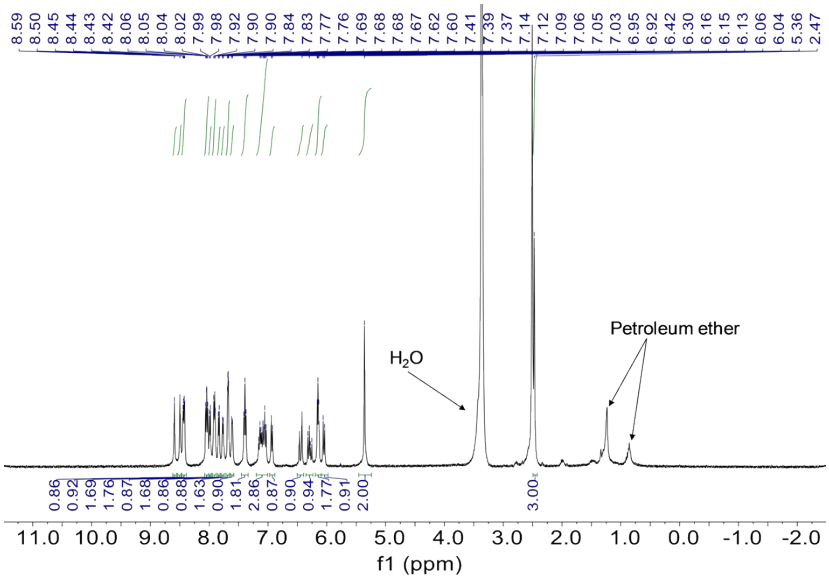
^{1}H NMR spectrum of complex **S7** in DMSO-d_6



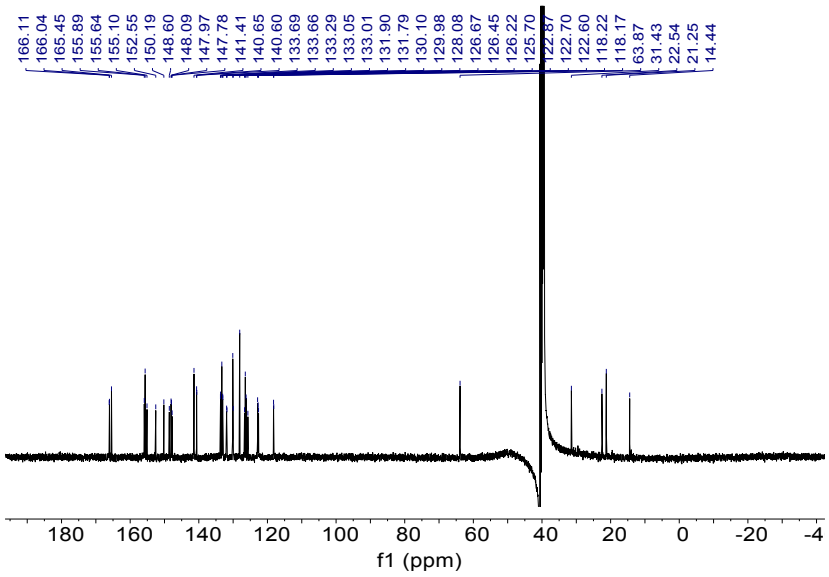
MALDI-TOF MS spectrum of complex **S7**

Complex 2. K_2CO_3 (160 mg, 1.5 mmol) was added to a solution of the complex **S7** (575 mg, 0.6 mmol) in CH_2Cl_2 (10 ml), and then acrylyl chloride (90 mg, 1 mmol) in CH_2Cl_2 (5 ml) was added dropwise slowly with stirring in an ice bath under an inert atmosphere of nitrogen in 2 h. After that, the mixture was stirred at room temperature

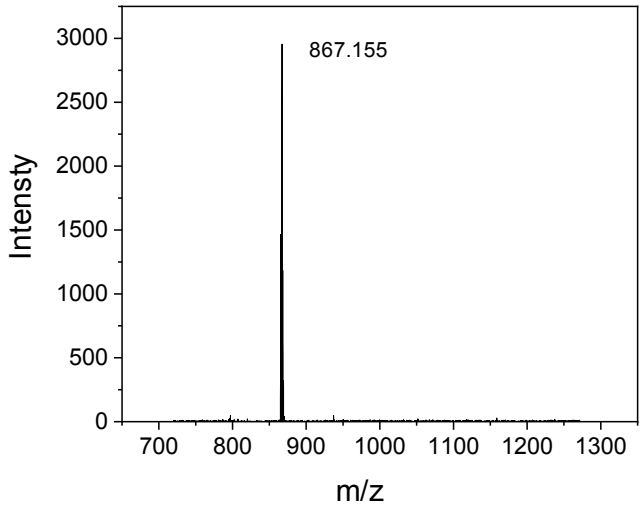
for 10 h. The solution was evaporated and the cured product was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (80:1, v/v) as an eluent to afford complex **2** as a yellow powder (582 mg, 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.59 (s, 1H), 8.50 (s, 1H), 8.43 (dd, J = 8.7, 4.0 Hz, 2H), 8.06 – 8.02 (m, 2H), 7.98 (d, J =5.8 Hz, 1H), 7.91 (d, J =7.5 Hz, 2H), 7.84 (d, J = 5.7 Hz, 1H), 7.77 (d, J = 5.9 Hz, 1H), 7.69 – 7.66 (m, 2H), 7.61 (d, J = 5.8 Hz, 1H), 7.39 (t, J = 7.5 Hz, 2H), 7.16 – 7.03 (m, 3H), 6.93 (d, J = 8.9 Hz, 1H), 6.45 (d, J = 17.3 Hz, 1H), 6.29 (dd, J = 17.3, 10.4 Hz, 1H), 6.15 (t, J = 5.5 Hz, 2H), 6.05 (d, J = 10.3 Hz, 1H), 5.36 (s, 2H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 166.11, 166.04, 165.45, 155.89, 155.64, 155.10, 152.55, 150.19, 148.60, 148.09, 147.97, 147.78, 141.41, 140.65, 140.60, 133.69, 133.66, 133.29, 133.05, 133.01, 131.90, 131.79, 130.10, 129.98, 128.08, 126.67, 126.45, 126.22, 125.70, 122.87, 122.70, 122.60, 118.22, 118.17, 63.87, 31.43, 22.54, 21.25, 14.44. MALDI-TOF MS: m/z = 867.2 $[M - PF_6^-]^+$.



^{1}H NMR spectrum of complex **2** in DMSO-d_6



^{13}C NMR spectrum of complex **2** in DMSO-d_6



MALDI-TOF MS spectrum of complex **2**

PS1. A mixture of 4-vinylbenzyl chloride (30 mg, 0.2 mmol), mPEG950 (950 mg, 1 mmol), complex **2** (51 mg, 0.05 mmol), and 2,2-azobisisobutyronitrile (AIBN, 3 mg, 0.02 mmol) in anhydrous tetrahydrofuran (THF, 2.0 mL) was stirred under nitrogen atmosphere at 80 °C for 48 h. After the mixture was cooled to room temperature, the solution was evaporated and the cured product was purified by dialysis in CH_2Cl_2, methanol and water for 12 h, respectively. The obtained yellow oil was used directly in

the next step.

P1. A mixture of **PS1** (600 mg) and compound **S3** (63 mg, 0.2 mmol) in CH_3CN (10 mL) was stirred at room temperature for 12 h. After that, the solution was evaporated and the cured product was purified by dialysis in methanol for 24 h. **P1** was isolated as a red oil (566 mg). GPC (THF, polystyrene standard): Mn = 117556, PDI = 1.13.

P2. A mixture of mPEG950 (950 mg, 1 mmol), complex **2** (51 mg, 0.05 mmol), and 2,2-azobisisobutyronitrile (AIBN, 3 mg, 0.02 mmol) in anhydrous tetrahydrofuran (THF, 2.0 mL) was stirred under nitrogen atmosphere at 80 °C for 12 h. After the mixture was cooled to room temperature, the solution was evaporated and the cured product was purified by dialysis in CH_2Cl_2, methanol and water for 12 h, respectively. **P2** was isolated as a red oil (826 mg). GPC (THF, polystyrene standard): Mn = 73396, PDI = 1.42.

P3. A mixture of 4-vinylbenzyl chloride (30 mg, 0.2 mmol), mPEG950 (950 mg, 1 mmol) and 2,2-azobisisobutyronitrile (AIBN, 3 mg, 0.02 mmol) in anhydrous tetrahydrofuran (THF, 2.0 mL) was stirred under nitrogen atmosphere at 80 °C for 12 h. After the mixture was cooled down, the solution was evaporated and the cured product was purified by dialysis in CH_2Cl_2, methanol and water for 12 h, respectively. Then the product was dissolved in $CNCH_3$ (5 mL), compound **S3** (63 mg, 0.2 mmol) was added and stirred at room temperature for 24 h. The solution was evaporated and

the cured product was purified by dialysis in methanol for 24 h. **P3** was isolated as a red oil (566 mg). GPC (THF, polystyrene standard): Mn = 92578, PDI = 1.09.

Fig. S2 (a) Luminescence spectra of compound **1** in MeOH/PBS (1:1, v/v) in the presence of 0–10 μM NaClO. (b) Luminescence spectra of complex **2** in MeOH/PBS(1:9, v/v) under an O_2/N_2 mixed atmosphere with 0 – 100% O_2 contents.

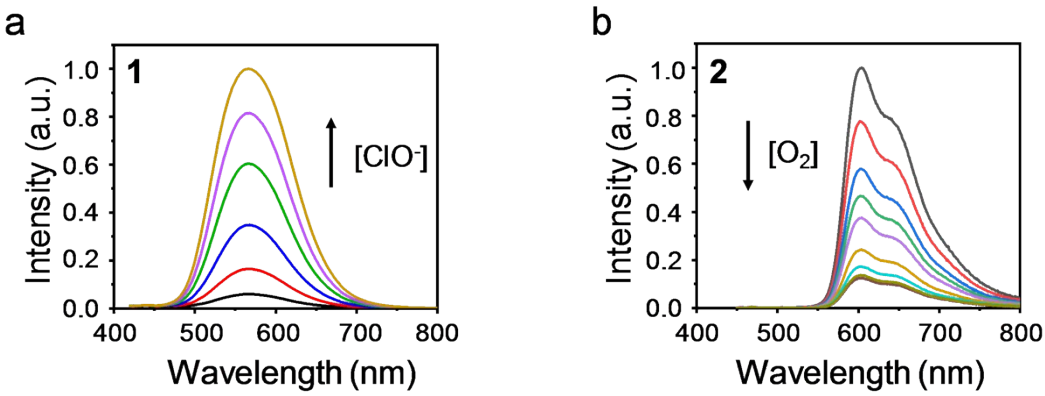


Fig. S3 Luminescence spectral traces of **P2** (a) and **P3** (b) in PBS upon addition NaClO.

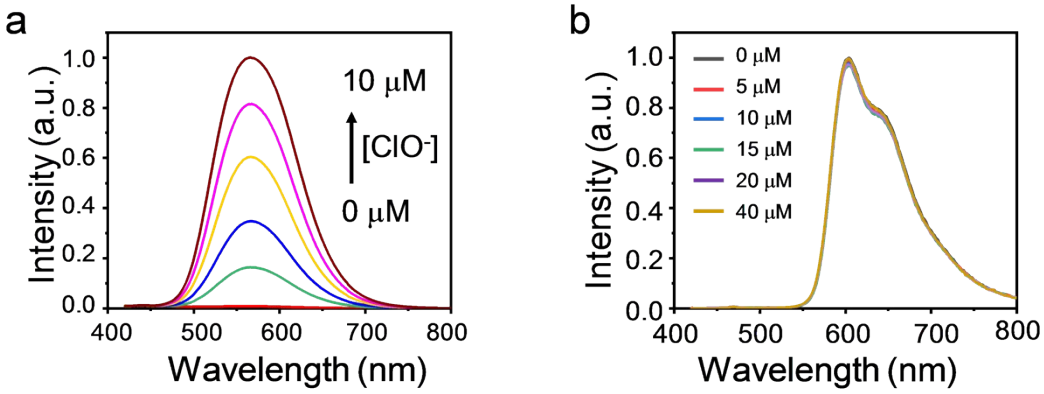


Fig. S4 Relative total luminescence intensity of **P1** in the presence and absence of different ROS (10 μM) and biothiols.

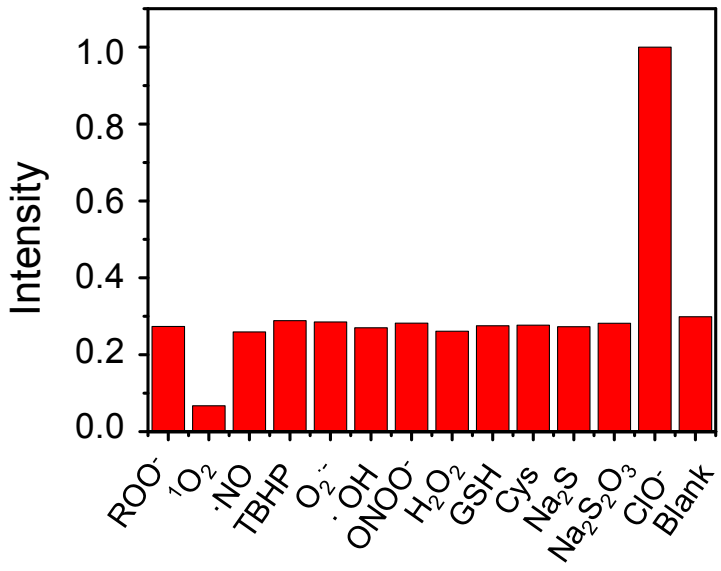


Fig. S5 Luminescence spectral traces of hypochlorite-oxidized **P2** (a) and **P3** (b) in PBS under an O_2/N_2 mixed atmosphere with 0 – 100% O_2 contents.

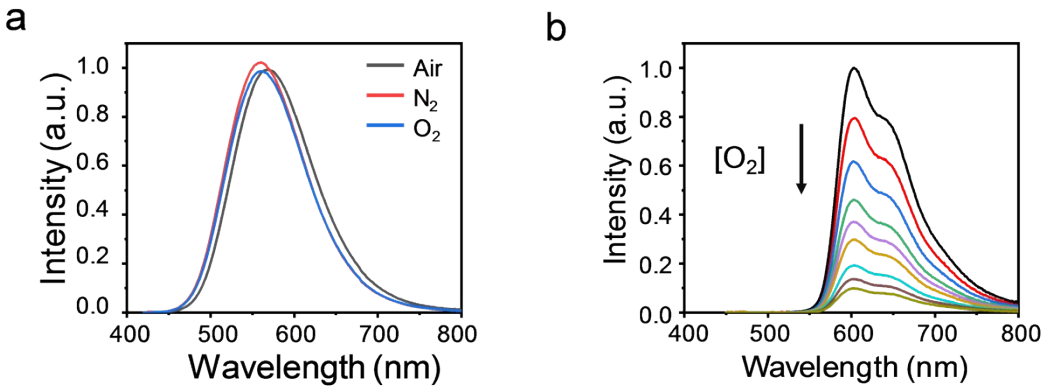


Fig. S6 Percentage of surviving HeLa cells after exposure to different concentrations of **P1** (0, 50, 150, 300, 400, 500, 600 μg/mL) at 37 °C under 5% CO_2 for 24 h.