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Development of a mitochondria-targeted fluorescent probe for ratiometric visualization of sulfur dioxide in living cells and zebrafish

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Instruments

Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan; High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP spectrometer; NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a LabTech UV Power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer; The optical density was measured by a Thermo Scientific Multiskan FC microplate reader in cytotoxicity assay; The fluorescence imaging of cells was performed with OLYMPUS FV1000 (TY1318) confocal microscopy; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the detection limit

The detection limit was determined from the fluorescence titration data based on a reported method.[1] **Cou-PCL** (10.0 μM) was titrated with different concentrations of SO_2, the linear relationship between the ratio values of emission intensity at 485 nm to 650 nm (I_{485}/I_{650}) and the concentration of SO_2 was fitted based on the fluorescence titration.

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of the blank sample and 'k' is the slope of the linear regression equation.

Quantum yields

The fluorescence quantum yields can be calculated by means of equation (1):

$$\Phi_s = \Phi_r \left(\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left(\frac{n_s^2}{n_r^2} \right) \frac{F_s}{F_r} \qquad (1)$$

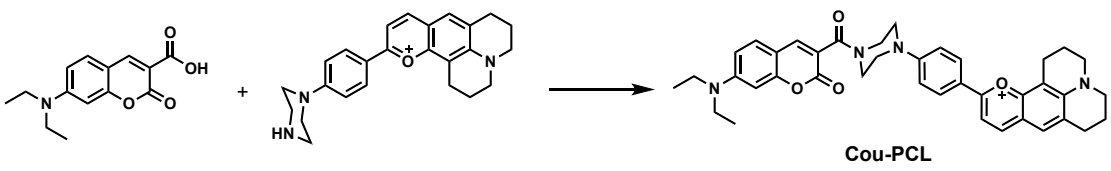
Where the subscripts *s* and *r* refer to the sample and the reference, respectively. Φ,

F, A and n stands for is quantum yield, the integrated emission intensity, the absorbance and refractive index, respectively. Quinine Sulfate (Φ=0.58) in 0.1 M H_2SO_4 solution and Rhodamine 101 (Φ=1) in ethanol were used as the standard for calculating fluorescence quantum yields of donor Cou and acceptor PCL in **Cou-PCL**, respectively.

Fluorescence imaging of SO_2 in living cells. The cell experiments were divided into control and experimental groups. As the control group, HeLa cells were incubated with **Cou-PCL** (5 μM) for 30 min. As the experimental groups, HeLa cells were incubated with $NaHSO_3$ (50, 100, 200 μM) for 60 min, followed by treating with **Cou-PCL** (5 μM) for another 30 min, and then washed by PBS buffer before imaging. We use Nikon A1MP confocal microscope to image, the excitation filter at 405 nm and 561nm. The ratiometric images were obtained by the images of blue channel dividing to the images of red channel.

Fluorescence imaging of SO_2 in living zebrafish. The zebrafish imaging experiments were divided into control and experimental groups, 5-day-old zebrafish was chosen as the imaging sample. The processing steps of zebrafish imaging experiment are the same as cell imaging. The ratiometric images were obtained by the images of blue channel dividing to the images of red channel.

Synthesis of Probe Cou-PCL.



Scheme S1. Synthesis route of the probe **Cou-PCL**

PCL (38.6 mg, 0.1 mmol), EDCI (28.8mg, 0.15 mmol), 7-diethylaminocoumarin carboxylic acid (26.1 mg, 0.1mmol) and HOBT (33.8 mg, 0.25 mmol) were dissolved in CH_2Cl_2 (3ml) and stirred for overnight at room temperature. The crude product obtained by distillation under reduced pressure. The crude product was purified by

column chromatography on silica gel using CH_2Cl_2/MeOH (v/v 10:1) as eluent to afford a dark purple solid (46 mg, yield 72%). Melting point: 237 ^oC. ^1H NMR (400 MHz, DMSO), δ (ppm): 1.121-1.155 (t, *J* = 6.8 Hz, 6H), 1.939-2.008 (d, *J* = 27.6 Hz, 4H), 2.845-2.873 (t, *J* = 5.2 Hz, 2H), 2.990-3.020 (t, *J* = 6.0 Hz, 2H), 3.440-3.748 (m, 16H), 6.573-6.577 (d, *J* = 1.6 Hz, 1H), 6.757-6.774 (d, *J* = 6.8 Hz, 1H), 7.142-7.165 (d, *J* = 9.2 Hz, 2H), 7.508-7.531 (d, *J* = 9.2 Hz, 2H), 7.825-7.846 (d, *J* = 8.4 Hz, 1H), 8.045 (s, 1H), 8.107-8.130 (d, *J* = 9.2 Hz, 2H), 8.409-8.430 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO): δ (ppm): 19.30, 19.49, 20.32, 27.47, 44.66, 46.17, 50.38, 50.87, 96.75, 99.99, 105.00, 107.30, 107.62, 109.93, 114.46, 116.03, 117.41, 118.04, 127.38, 130.20, 130.67, 144.77, 146.31, 151.82, 153.17, 154.25, 157.17, 158.96, 164.79, 165.39. HRMS (ESI) m/z calcd for $C_{39}H_{41}N_4O_4^+$ $[M + H]^+$: 629.3124. Found 629.3122.

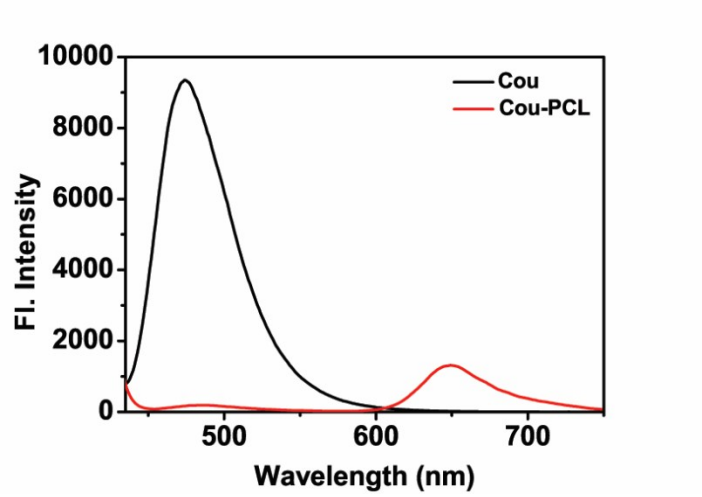


Fig. S1 Fluorescence spectra (λ_{ex} = 445nm) of 10 μM Cou (black) and Cou-PCL (red) in 25 mM phosphate buffer (pH 7.4, containing 25% DMF).

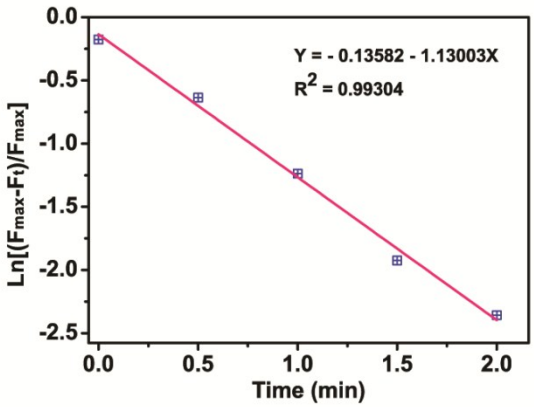


Fig. S2 Pseudo first-order kinetic plot of the reaction of **Cou-PCL** (10 μM) with $NaHSO_3$ (500 μM) in aqueous solution (25 mM PBS buffer, pH 7.4, mixed with 20% MeOH). Slope = 1.13003 min^{-1}.

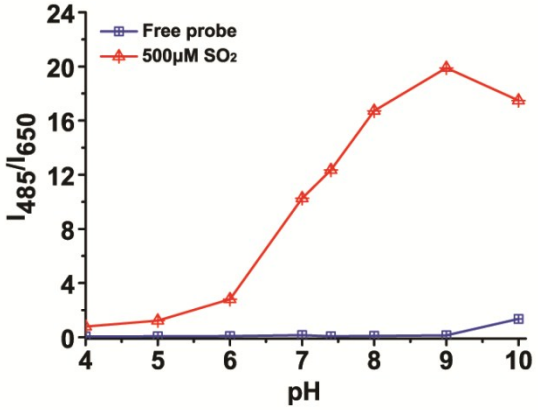


Fig. S3 The pH influence on the ratio values of fluorescence intensity (I_{485}/I_{650}) of **Cou-PCL** (10 μM) in the absence (square) or presence (triangle) of $NaHSO_3$ (500 μM).

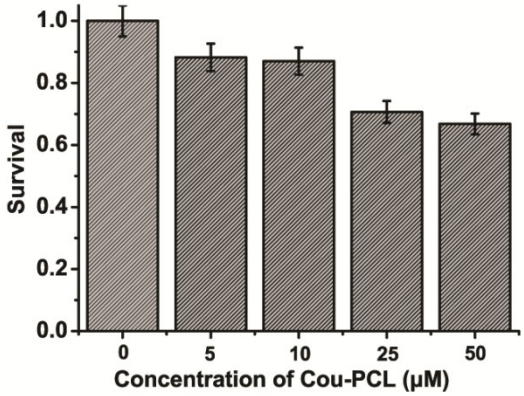


Fig. S4 Cell viability of HeLa cells incubated with chemosensor **Cou-PCL** of different concentration (0, 5, 10, 25, or 50 μM) for 24 h.

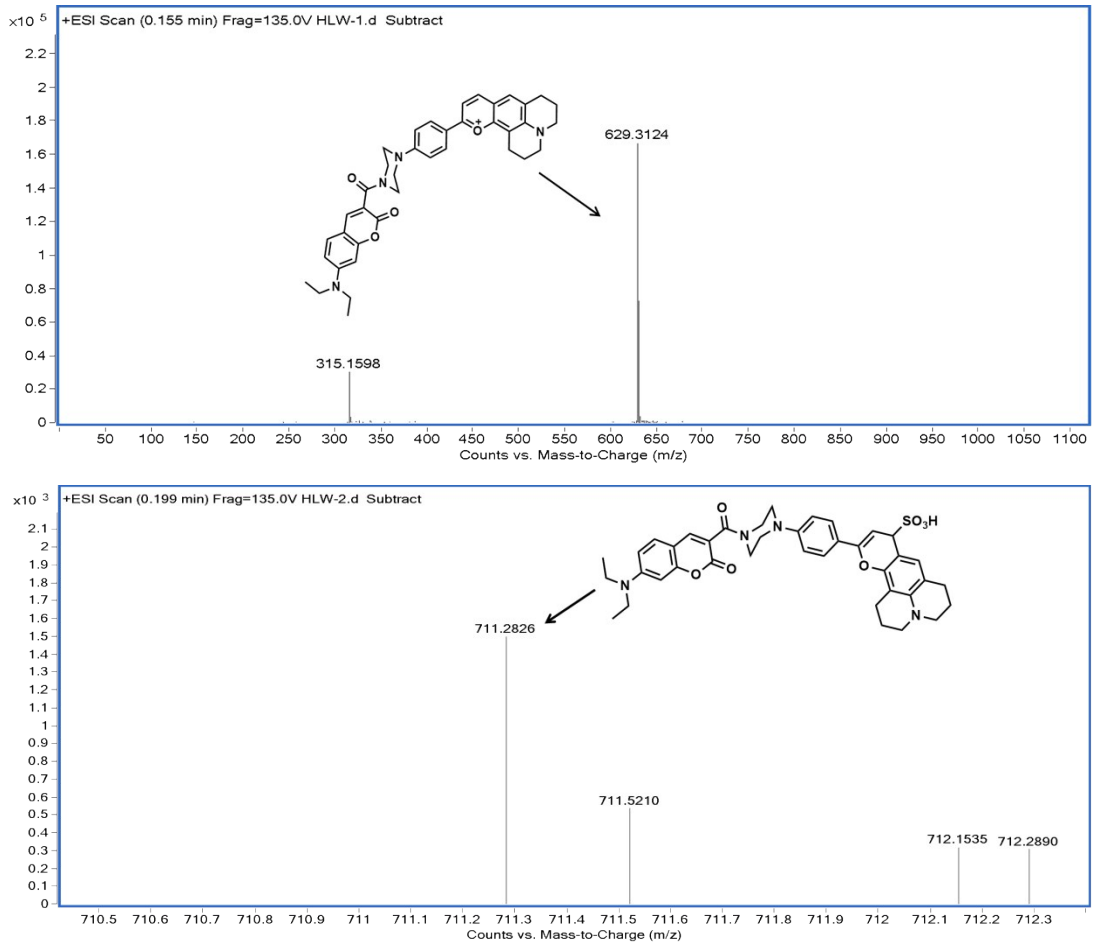


Fig. S5 Mass spectra (ESI) of **Cou-PCL** in the absence (above) or presence of 500 μM $NaHSO_3$ (bottom) in aqueous solution.

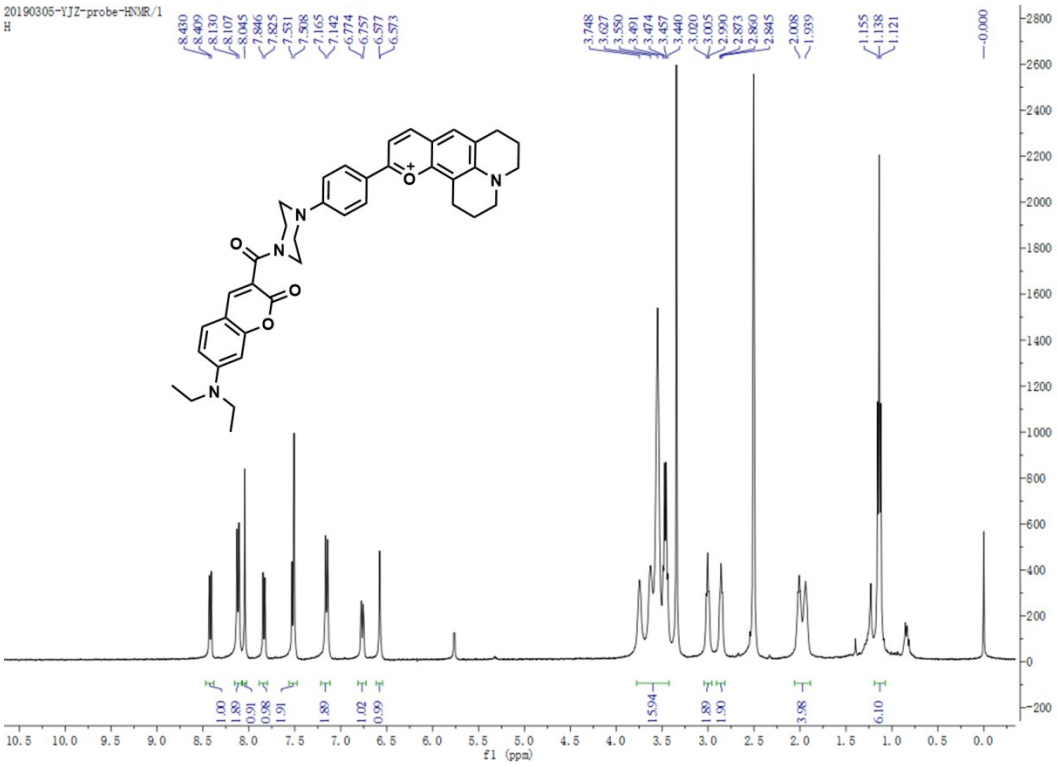


Fig. S6 ^{1}H NMR spectrum of compound **Cou-PCL** in DMSO-d_6.

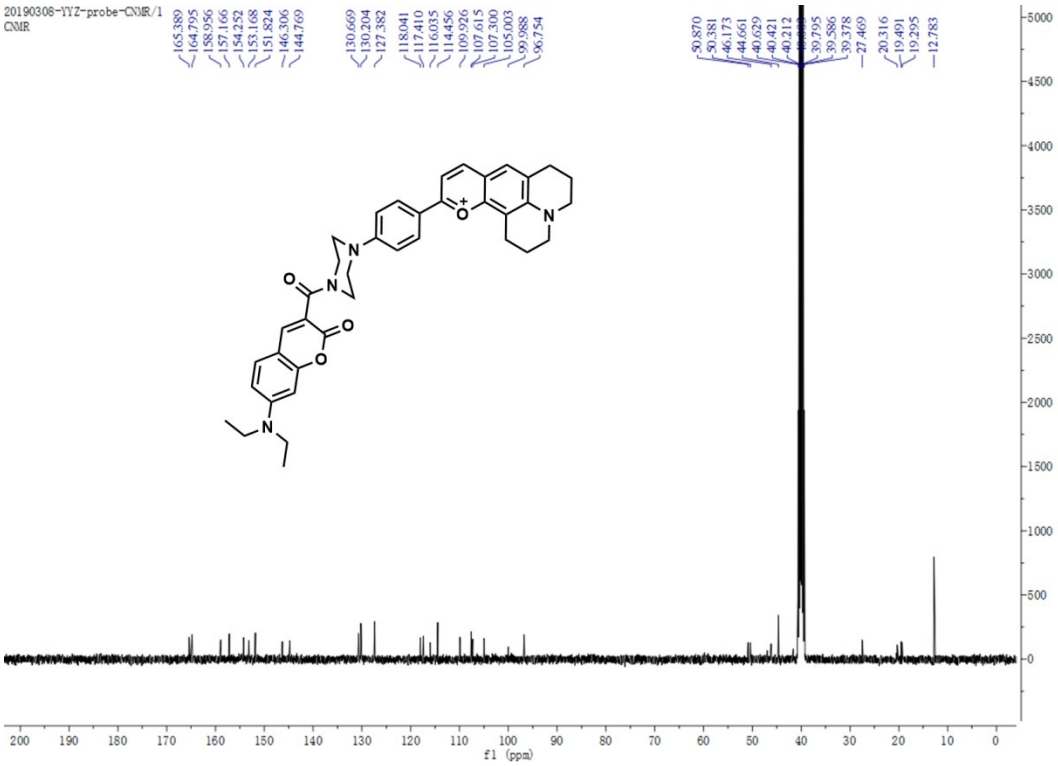


Fig. S7 ^{13}C NMR spectrum of compound **Cou-PCL** in DMSO-d_6.