# Electronic Supplementary Information (ESI)

## *for*

# A highly sensitive and reductant-resistant fluorescent probe for nitroxyl in aqueous solution and serum

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## 1. Reagents and apparatus

All chemicals were obtained from commercial suppliers and used without further purification. Thin layer chromatography (TLC) was carried out using silica gel 60 F254, and column chromatography was conducted over silica gel (100-200 mesh), both of them were obtained from Qingdao Ocean Chemicals (Qingdao, China). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, USA). LC-MS analyses were performed using an Agilent 1100 HPLC/MSD spectrometer. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer (Thermo Finnigan). NMR spectra were recorded on a Bruker DRX-400 spectrometer using TMS as an internal standard. All chemical shifts are reported in the standard δ notation of parts per million. Fluorescence measurements were carried out on a Hitachi-F4500 fluorescence spectrometer with excitation and emission slits set at 5.0 nm and 2.5 nm, respectively. The pH was measured with a Mettler-Toledo Delta 320 pH meter.

## 2. Spectrophotometric experiments

Due to the weak water-solubility of **P-CM**, the fluorescence measurement experiments were measured in buffered (100 mM PBS, pH 7.4) DMF/$H_2O$ solution (1:99, v/v). The fluorescence emission spectra were recorded at excitation wavelength of 370 nm with emission wavelength range from 380 to 650 nm. A $1\times10^{-3}$ M stock solution of **P-CM** was prepared by dissolving **P-CM** in DMF. The solutions of various testing species were prepared from Angeli's salt (AS, a nitroxyl source), KCl, NaCl, $CaCl_2$, $MgCl_2$, $ZnCl_2$, $FeCl_3$, $NaNO_2$, $NaNO_3$, NaClO, $H_2O_2$, glutathione (GSH), ascorbic acid (AA), and $Na_2S$ (a hydrogen sulfide source) using twice-distilled water. Nitric oxide (NO) was generated from DEA/NONOate (1 mM stock solution in 0.01M NaOH). $ONOO^-$ in NaOH solution was obtained from Cayman. S-nitrosoglutathione (GSNO) was obtained from Sigma Aldrich. The test solution of the **P-CM** (10μM) was prepared by adding 20 μL of the **P-CM** stock solution into 1.98 mL of the buffered

solution of various analytes. The test solution was kept at 37 °C for 30 min and then the fluorescence intensities were recorded.

**3. HPLC analysis**

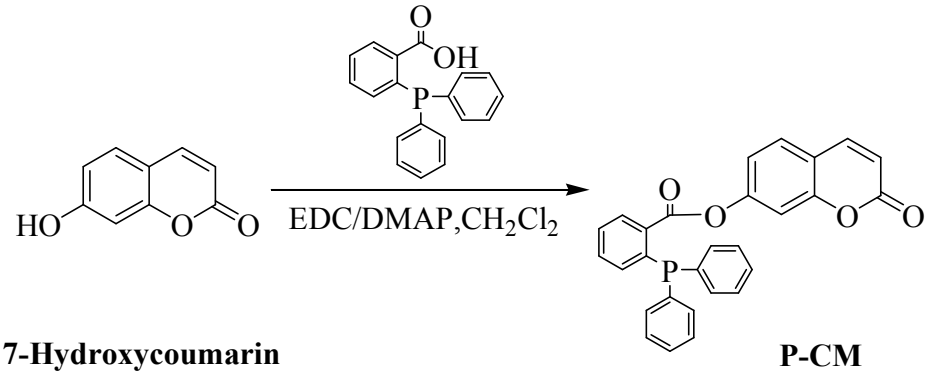
100 μM of **P-CM** in DMF (2.0 mL) and 100 μM of 7-hydroxycoumarin in buffered (100 mM PBS, pH 7.4) DMF/ $H_2O$ solution (1:99, v/v) were prepared as the control solution. The reaction solution (2.0 mL) was prepared with 100 μM **P-CM** in buffered DMF/ $H_2O$ solution incubated for 30 min at 37°C after addition of 2000 μM of AS. An aliquot of each solution (100 μL) was loaded onto an Inertsil ODS-3 (250 mm×Φ 4.6 mm) C18 column (GL Sciences, Inc.) fitted on an Agilent 1260 Infinity HPLC system, and the eluates were monitored with a photodiode array detector. Detection wavelength was kept at 330 nm, and flow rate was set at 1.0 mL/min. Milli-Q water containing 0.1% TFA (A) and MeCN (B) were used as developing solvents. Gradient conditions were chosen as follows: 10% A and 90% B for 20 min.

**4. Synthesis of probe P-CM**

2-(diphenylphosphino) benzoic acid (306 mg, 1 mmol) was dissolved in 50 mL of anhydrous $CH_2Cl_2$ under $N_2$, 4-(dimethylamino) pyridine (6.1 mg, 0.05 mmol) and 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide hydrochloride (191.7 mg, 1mmol) were added, and the reaction mixture was stirred at room temperature for 30 min. 7-hydroxycoumarin (194.6 mg, 1.2 mmol) was then added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified by the silica gel chromatography (EtOAc/hexane =1:2, v/v) to afford compound **P-CM** as a faint yellow solid (141.0 mg, 0.31mmol). $^1$HNMR($CDCl_3$, 400MHz) δ(ppm): 8.278-8.245 (m, 1H), 7.681-7.657 (d, $J$ = 9.6 Hz, 1H), 7.502-7.474 (m, 2H), 7.440-7.418 (m, 1H), 7.377-7.277 (m, 10H), 7.022-6.989 (m, 1H), 6.930-6.902 (m, 2H), 6.396-

6.372 (d, $J$ = 9.6 Hz, 1H). MS (ESI) m/z 451.0 $(M+H)^+$.

## 5. Supplementary figures and schemes




**7-Hydroxycoumarin** **P-CM**

**Scheme S1** The synthetic route of the probe **P-CM**.

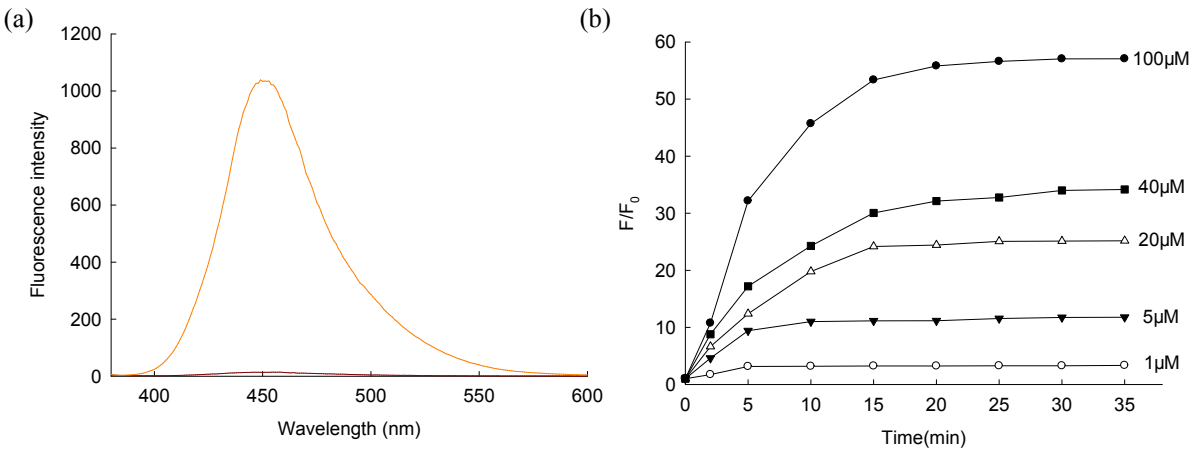



**Fig. S1** (a) Fluorescence spectra of 10 μM 7-Hydroxycoumarin (top line) and 10 μM **P-CM** (bottom line) in buffered (pH 7.4) aqueous DMF solution.(b)Time-dependent fluorescence spectral changes of **P-CM** with AS (probe 10 μM, AS =1, 5, 20, 40 or 100 μM ) in buffered (pH 7.4) aqueous DMF solution. Time points represent 0, 2, 5, 10, 15, 20, 25, 30 and 35 min.

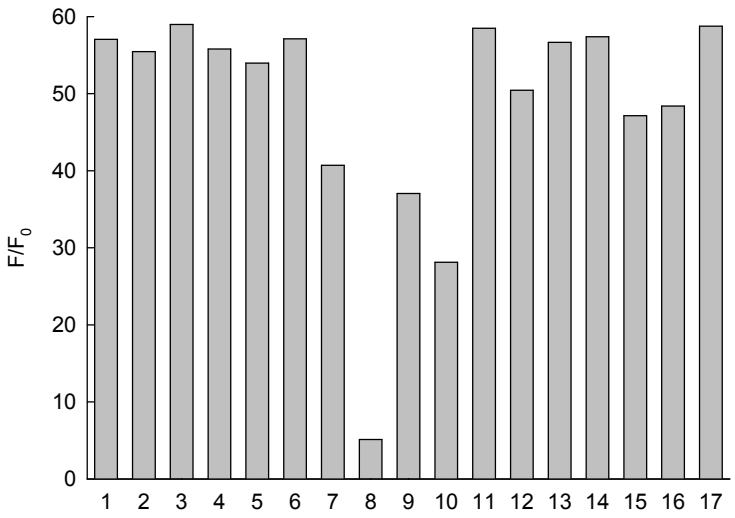



**Fig. S2** Fluorescence responses of the **P-CM** ( 10 μM ) to testing species in the presence of AS (100 μM): 1, 100 μM AS; 2, 1 mM $K^+$; 3, 1 mM $Na^+$; 4, 1 mM $Ca^{2+}$; 5, 1 mM $Mg^{2+}$; 6, 1 mM $Zn^{2+}$; 7, 1 mM $Fe^{3+}$; 8, 50 μM GSH; 9, 1 mM AA; 10, 1 mM $Na_2S$; 11, 20 μM $H_2O_2$; 12, 20 μM $ClO^-$; 13, 1 mM $NO_2^-$;14, 1 mM $NO_3^-$; 15, 20 μM $ONOO^-$; 16, 200 μM NO; 17, 200 μM GSNO.

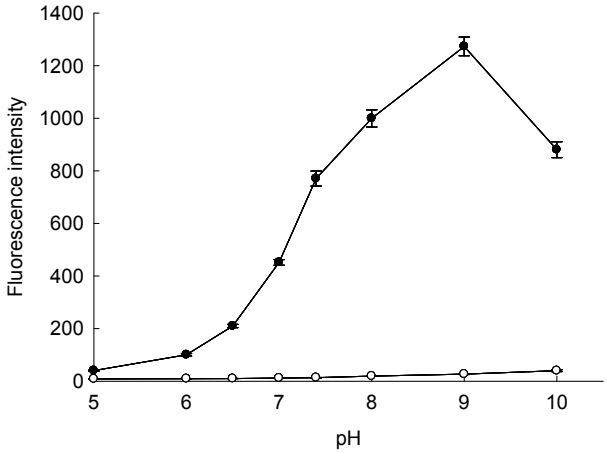


**Fig. S3** Effect of the pH on the fluorescence intensity of **P-CM** (10 μM, ○) and the reaction product between **P-CM** (10 μM, ●) and AS (100 μM) in buffered (pH 7.4) aqueous DMF solution. The excitation wavelength was 370nm.

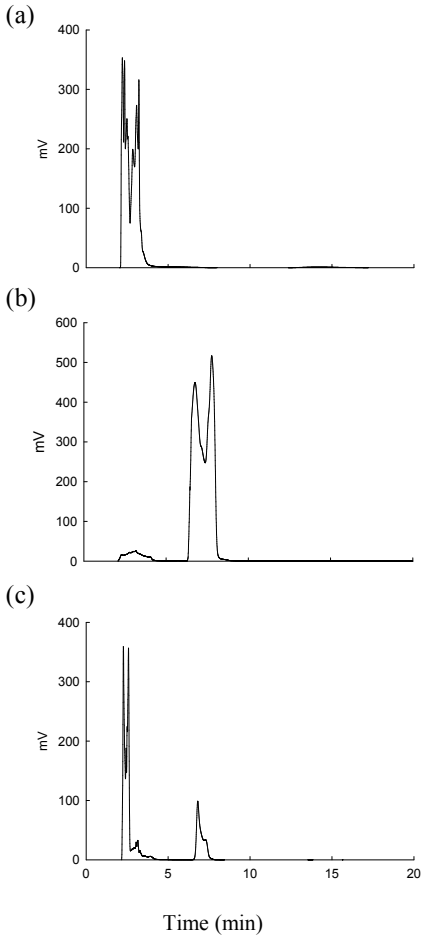



**Fig. S4** Reversed-phase HPLC chromatograms with absorption detection. (a) A solution of 100 μM 7-hydroxycoumarin. (b) A solution of 100 μM **P-CM**. (c) A sample solution of 100 μM **P-CM** with 2000 μM AS in 100 mM PBS (pH 7.4, 1%DMF) after incubation for 30 min at 37 ° C.

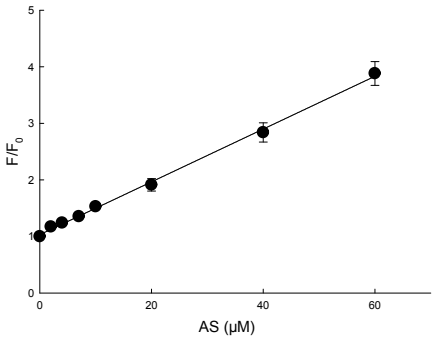



**Fig. S5** The linear response of **P-CM** (10 μM) at low AS concentrations (0, 2, 4, 7, 10, 20, 40, 60 μM) in buffered (pH 7.4) aqueous 20% bovine serum solution.

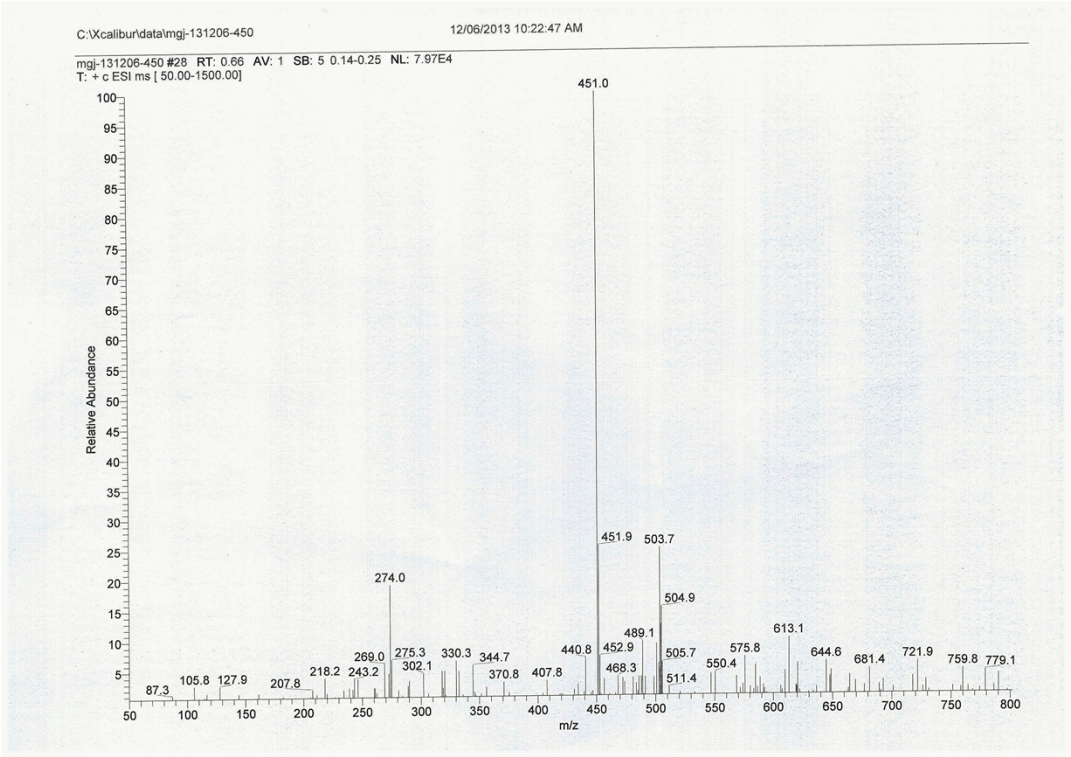



**Fig. S6** ESI mass spectrum of probe **P-CM.**

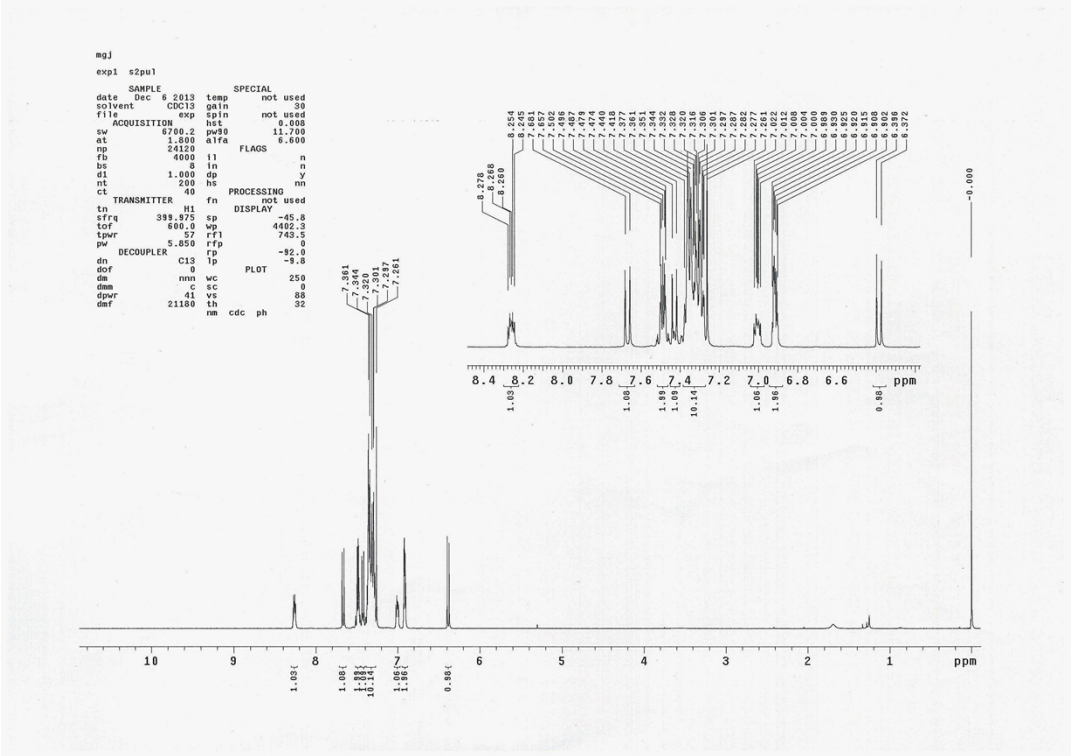



**Fig. S7** $^{1}$H NMR spectrum of of probe **P-CM.**