Supporting information:

Binding Reaction Sites to Polysiloxanes: Unique Fluorescent Probe for Reversible Detection of ClO^-/GSH Pair and The in situ Imaging in Live Cells and Zebrafishes

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Experimental

1. Materials

2-Bromo-1,8-naphthalic anhydride and Sodium thiomethoxide solution were purchased from Aladdin Co. (China) and used as received. (aminopropyl)methyldimethoxylsilane, dimethyldimethoxylsilane, and were obtained as commercial products and used directly.

2. Characterization and measurements

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AVANCE 400 spectrometer at 25 °C using $CDCl_3$ as solvent and without tetramethylsilane as an interior label. Ultraviolet absorption (UV) spectra in THF solution were detected using a Beijing TU–1901 double beam UV–Vis spectrophotometer. The luminescence (excitation and emission) spectra of the samples were determined with a Hitachi F–4500 fluorescence spectrophotometer. Excitation and emission slits measured were 5 mm and 5 mm, respectively.

3. Synthesis of 6-(methylthio)-1H,3H-benzo[de]isochromene-1,3-dione (N1)

2-Bromo-1,8-naphthalic anhydride (0.388 g, 2 mmol) was dissolved in 10 mL of DMF. K_2CO_3 (282 mg) and CH_3SNa (280 mg) were added into the solution in sequence. Then the solution was heated to 80 °C for 4 h under Ar atomosphere. After the reaction was completed, the mixture was poured into iced-cold water to afford yellow solid. The solid was obtained after filtration. The crude product was purified by silica gel chromatography to obtain the product as light yellow solid (280 mg, yield 47 %).

^{1}H NMR (400 MHz, DMSO) δ 8.59 (dd, J = 13.3, 7.9 Hz, 2H), 8.44 (d, J = 8.0 Hz, 1H), 7.95 (dd, J = 8.4, 7.4 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 2.89 – 2.67 (m, 3H).

4. Synthesis of aminopropyl–functional polysiloxanes(P0)

Aminopropyl–functional polysiloxanes (**P0**) was synthesized according to the classical procedure. A mixture of (aminopropyl)methyldimethoxylsilane(3.98 g, 0.02 mol) and dimethyldimethoxysilane (7.31 g, 0.6mol) was added dropwise to the distilled water (200 mL), then KOH (3.00 g) was added to the solution. The mixture was then stirred at ambient temperature for 2 h, and then heated to 70 °C for 3 h. Then cooled to room temperature, water layer was removed. The solution was washed by distilled water (200 mL) for three times to remove the residual KOH. Then, the product was dried over vacuum drying for 24 h and P0 was obtained as a colorless viscous liquid. Yield: 90 %.^{1}H NMR (400 MHz, $CDCl_3$):2.68 (2H), 1.52 (2H), 1.41 (2H), 0.55 (2H), 0.10 (40 H). ^{13}C NMR (100 MHz, $CDCl_3$): 44.96, 27.10, 14.06, 0.56, -0.83. PDI=1.33.

5. Synthesis of naphthalimides–functional polysiloxanes (P1)

The synthetic route and the structure were shown in Scheme 1. P0 (2 g) and 6-(methylthio)-1H,3H-benzo[de]isochromene-1,3-dione (0.2 g) were mixed in ethanol (50 mL). The reaction mixture was refluxed for 8 hours. After cooled to room temperature, the solvent was evaporated, P1 was obtained after precipitation using water as light yellow viscous liquid. Yield: 71 %. ^{1}H NMR (400 MHz, CDCl3) δ 8.61 (d, 2H), 8.50 (t, J = 7.4 Hz, 1H), 7.72 (dd, 1H), 7.45 (d, 1H), 2.67 (dt, 19H), 2.04 (d, J = 69.9 Hz, 21 H), 1.64 – 1.37 (m, 19H), 0.55 (dd, 22H), 0.08 (d, 66 H).^{13}C NMR (100

MHz, $CDCl_3$): δ 164.75, 164.35, 148.69, 132.09, 130.94, 130.81, 129.28, 128.24, 126.62, 126.50, 125.03, 121.09, 45.20, 42.99, 26.89, 14.10, 0.40. PDI=1.24.

6. Fluorescence imaging in HeLa cells

HeLa cells were inoculated on a confocal plate for 24 hours, and washed three times with PBS buffer. HeLa cells were then incubated with P1 (concentration 20 μM) in an incubator for 20 min prior to cell imaging. The fluorescence cell images were achieved by a Nikon A1MP confocal microscopy. The fluorescence emission was obtained at 550-600 nm under excitation of 488 nm respectively.

7. Zebrafish pretreatment and fluorescence imaging

2 days old zebrafish was incubated with 20 μM RB-1 for 0.5 h. At the same time, another group was incubated with 10 μM RB-1 for 0.5 h, and then treated with 400 μM NaClO, and finally the fluorescence images were acquired. The fluorescence emission was obtained at 500 to 550 nm under the excitation of 405 nm laser excitation.

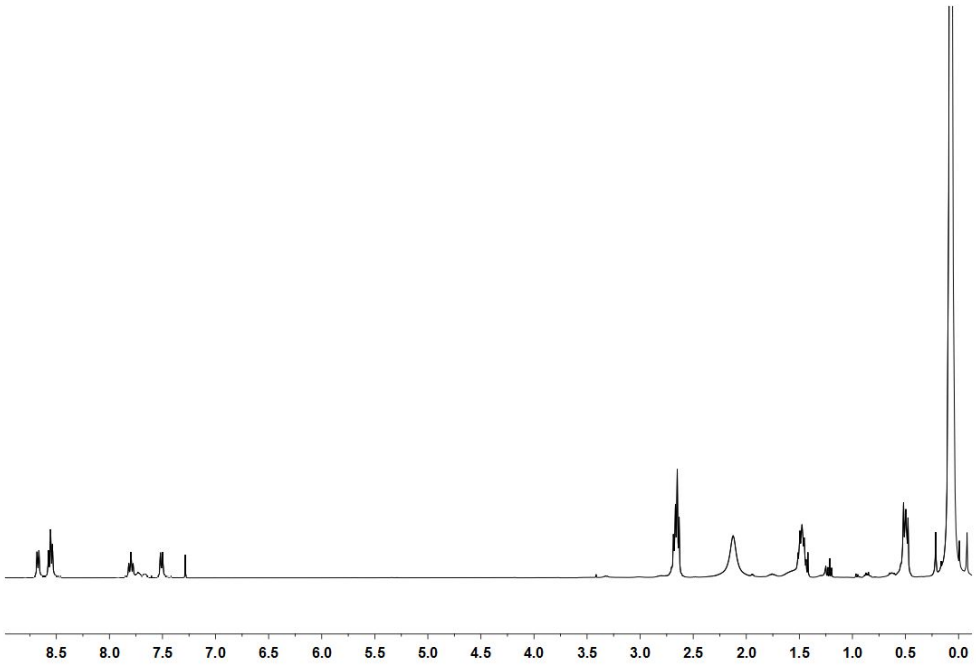


Figure S1. ^{1}H-NMR spectral of **P1**.

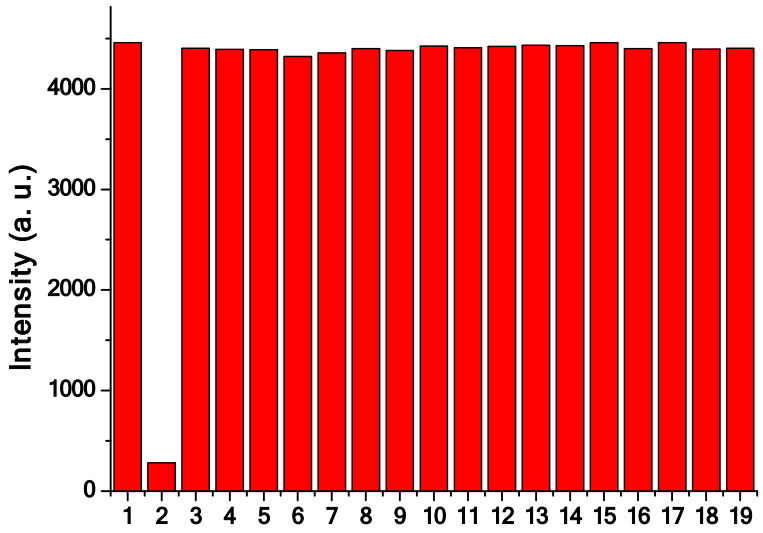


Figure S2. Fluorescence responses of the probe **P1** (10 μM) to various relevant species in aqueous solution (25 mM PBS buffer, pH 7.4, containing 10 % ethanol as cosolvent). 1, blank; 2, NaClO; 3, Cd^{2+}; 4, Al^{3+}; 5, Ag^{+}; 6, Ni^{2+}; 7, Ca^{2+}; 8, Co^{2+}; 9, Mg^{2+}; 10, Sn^{2+}; 11, SO_4^{2-}; 12, SCN^{-}; 13, $S_2O_3^{2-}$; 14, S^{2-}; 15, PO_4^{3-}; 16, OH^{-}; 17, NO^{3-}; 18, HSO^{3-}; 19, HCO_3^{-}.

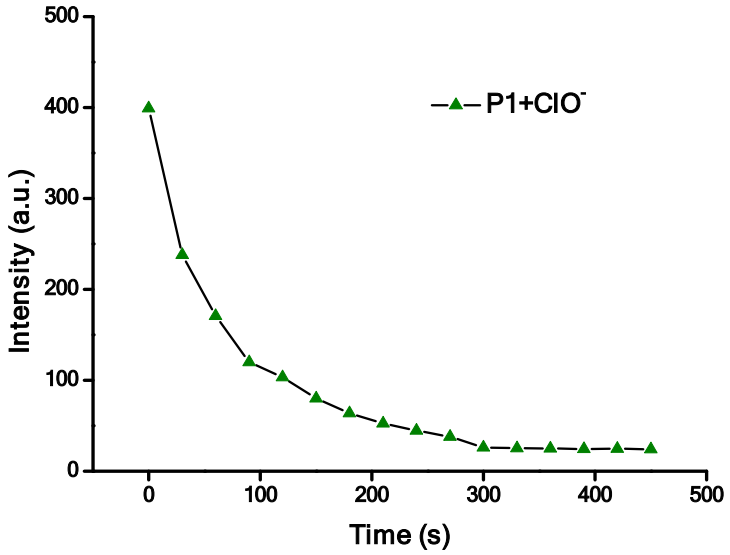


Figure S3. Time course study for the reaction between **P1** with NaClO.

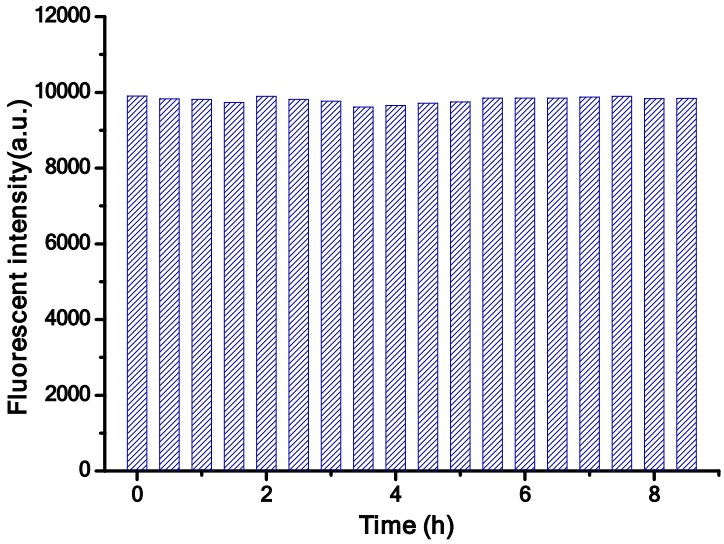


Figure S4. Photostability testing result for **P1** in THF solution. Fluorescence was measured at $\lambda_{ex}/_{em}$=405/489 nm.

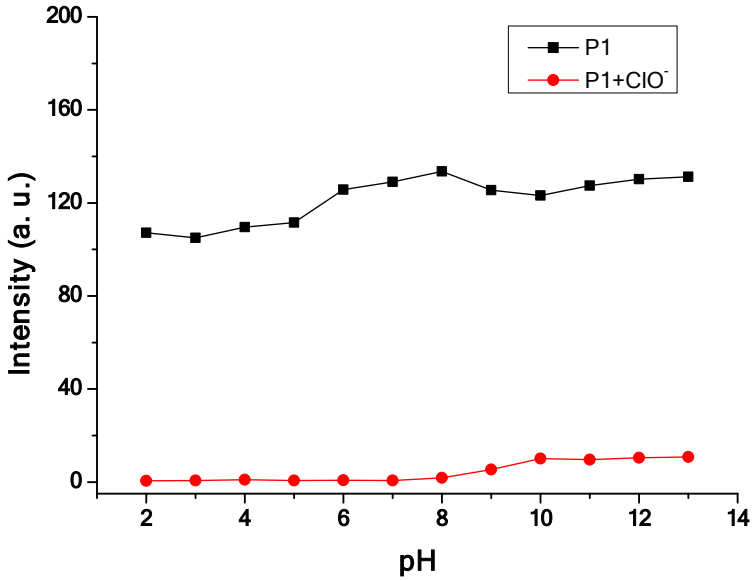
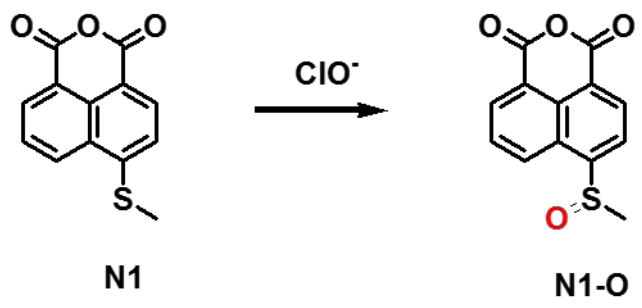
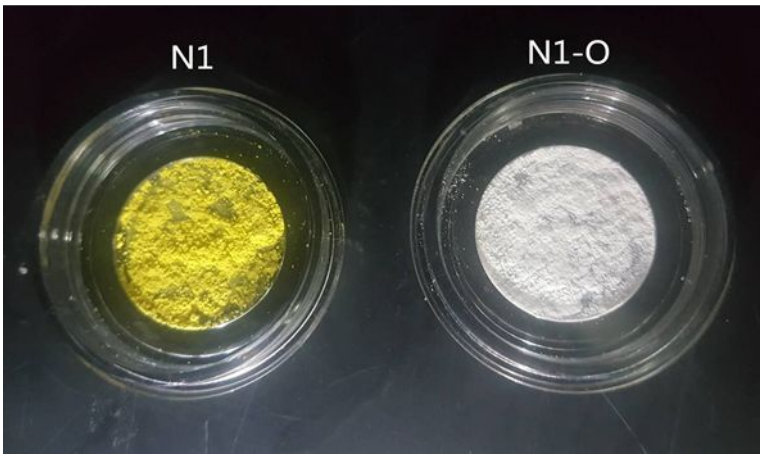


Figure S5. Effect of pH on the reaction of **P1** (5 uM) and **P1** treated with NaClO (25 uM).

Model reaction:



N1 N1-O



Scheme S1. Illustration of the model oxidation reaction from N1 to N1-O.

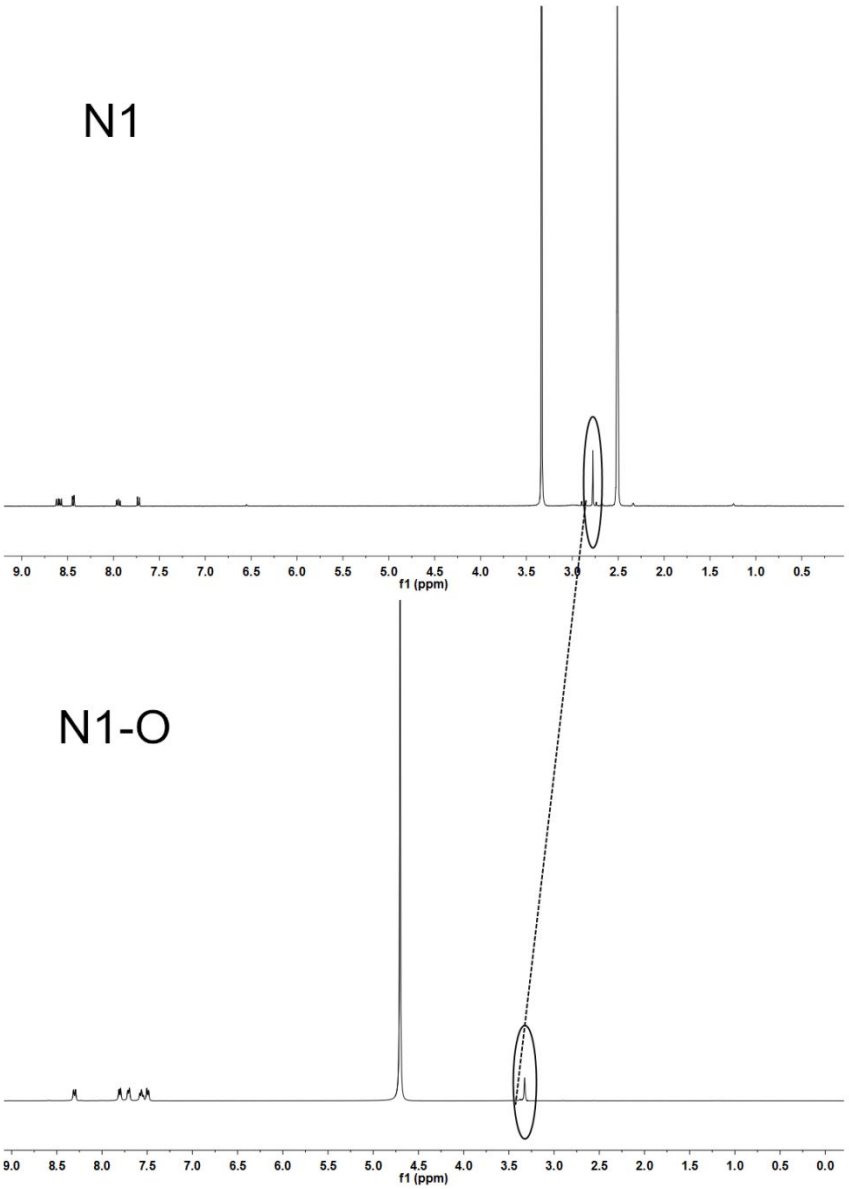


Figure S6. ^{1}H-NMR spectra of **N1** and **N1-O**.

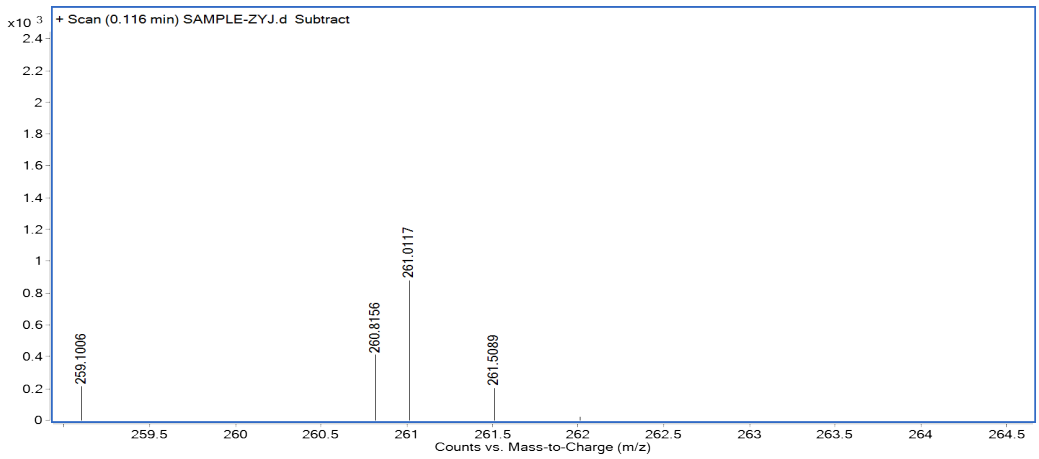


Figure S7. HR-MS spectra of 6-(methylsulfinyl)-1H,3H-benzo [de]isochromene-1,3 -dione (**N1-O**).

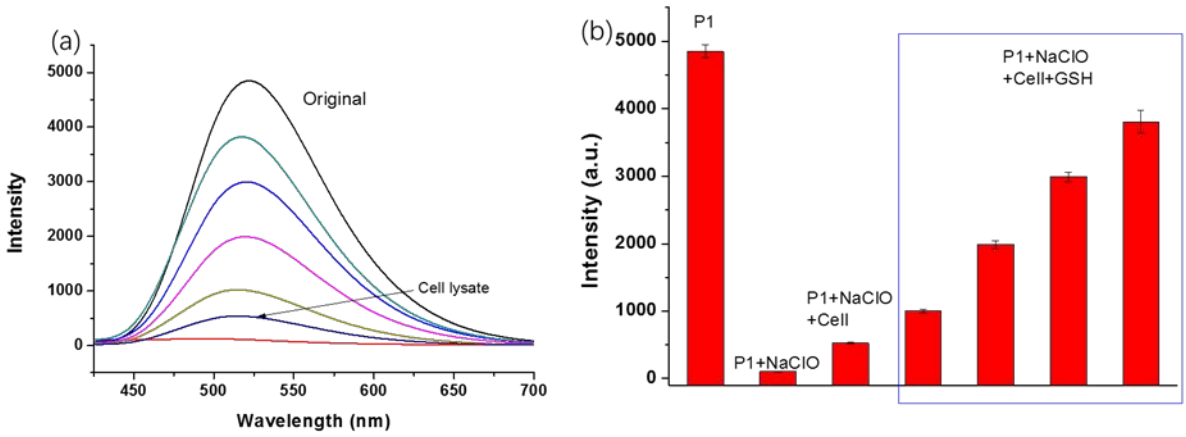


Figure S8. (a) Fluorescence spectra increasing of NaClO treated **P1** (10 μM) toward HeLa cell lysate and GSH with various concentrations (using ethanol and distilled water as the solvent, 1：3), (b) fluorescence intensity response of NaClO treated **P1** (10 μM) towards HeLa cell lysate and GSH. Excited by 405 nm.

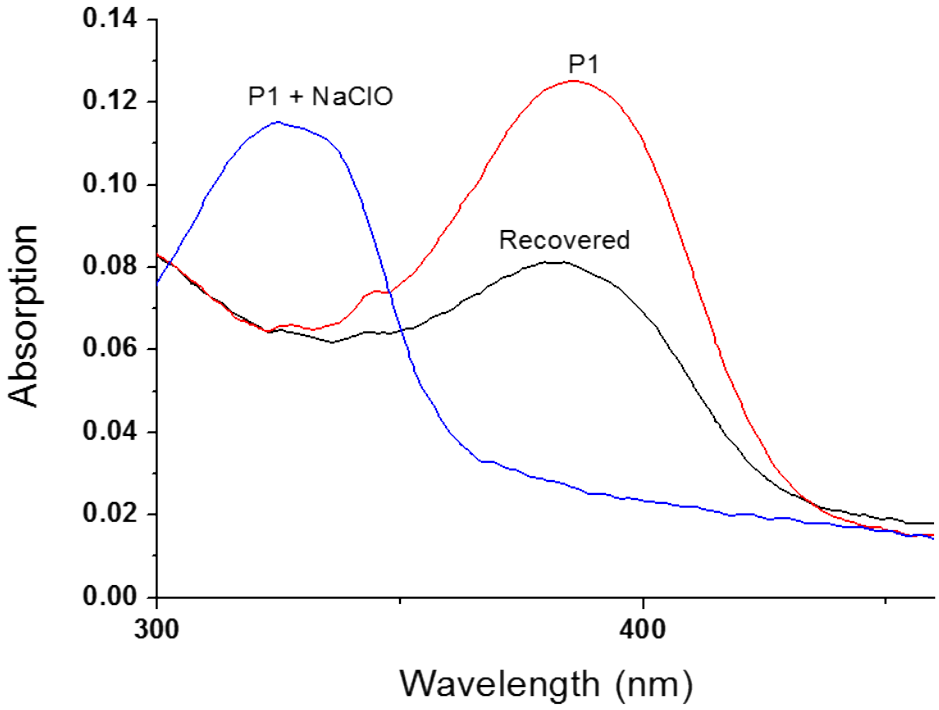


Figure S9. The absorption cycle of **P1**, treated by NaClO, and after GSH added.

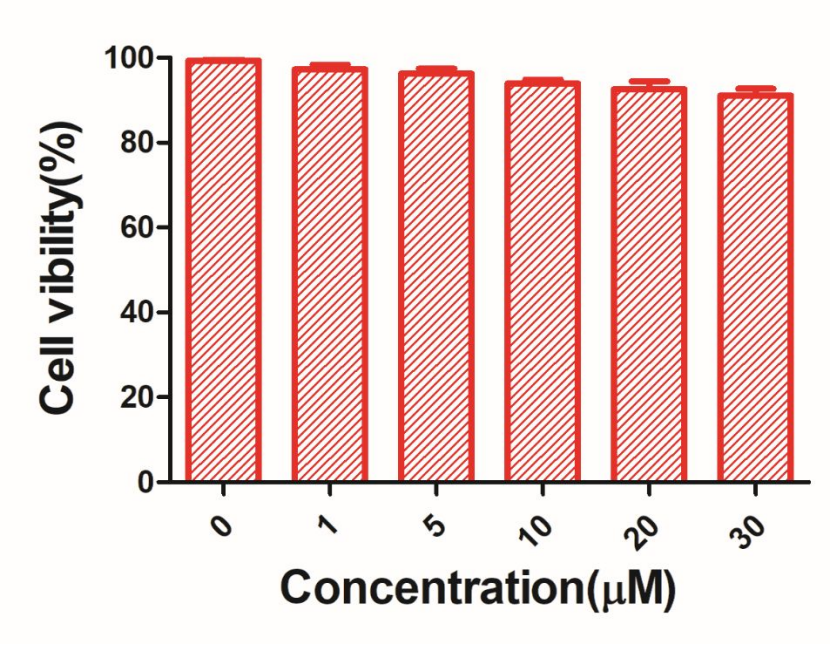


Figure S10. Cytotoxicity of **P1** on HeLa cells determined by MTT.

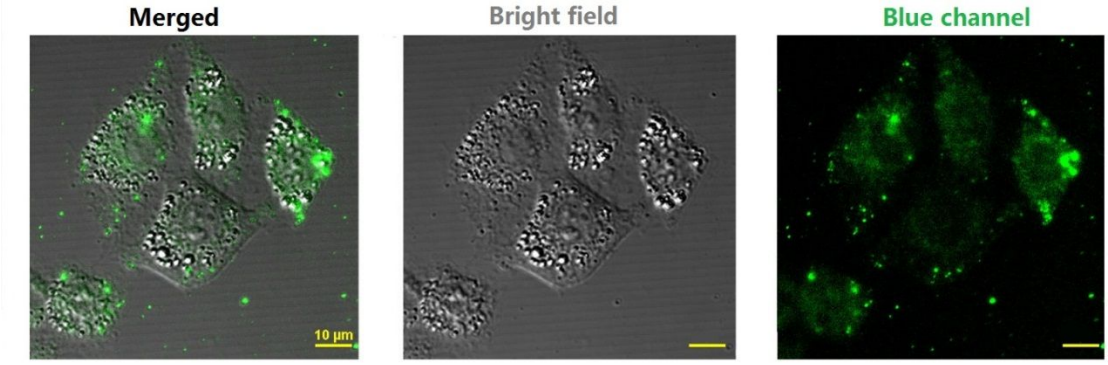


Figure S11. Laser scanning confocal microscope photographs of HeLa cells treated with **P1**. Magnification: 40×, scale bar: 10 μm.

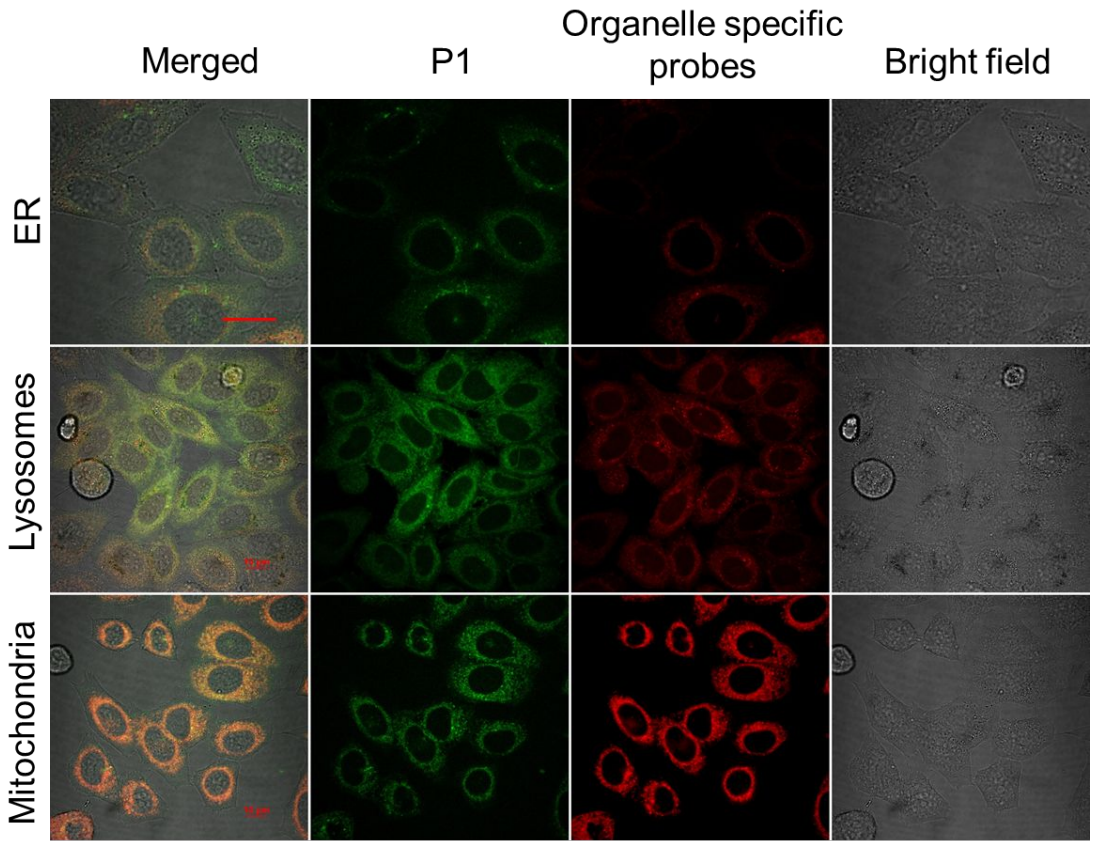


Figure S12. Subcellular distribution of P1. HeLa cells were costained with P1 (in the green channel) and different organelle specific probes, including, ER-Tracker Red, LysoTracker Red, and MitoTracker Red (in the red channel). Magnification: ×40 , scale bar :10 um.

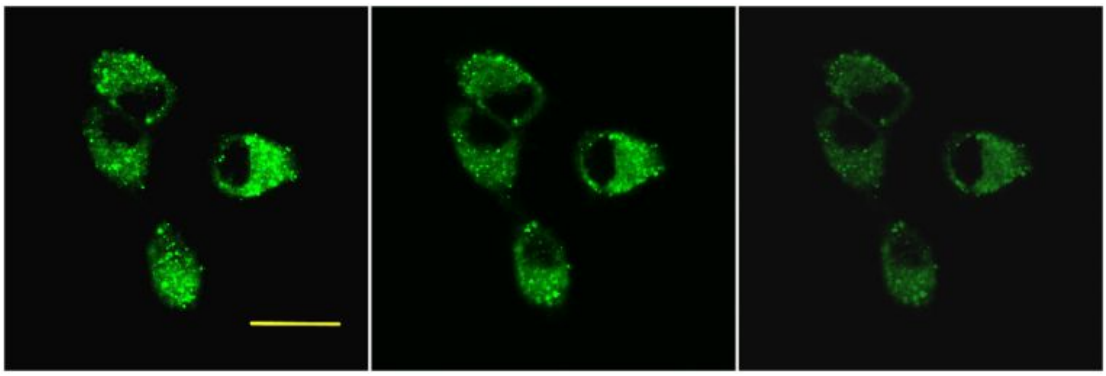


Figure S13. Two-photon fluorescent images of HeLa cells stained with **P1** and NaClO with different incubation times from green channel. Incubation time from 0 to 30 s, λex = 800 nm, λem = 425−475 nm.

Table S1. Comparation between the probe **P1** and the reported works (Macromolecules).

	Previous work (Macromolecules):	**This work**
Probe type	Colormetric	Fluorescent
Application for bioimaging	Not capable	Capable
Detecting signal	Color (Absorption)	Absorption and emission
Kind of polymer	Azobenzene	Polysiloxane

Table S2. Molecular weights of **P0** and **P1**.

	M_n (g/mol)	**M_w (g/mol)**	**PDI (M_w/M_n)**
P0	2700	3600	1.33
P1	4500	5600	1.24

Table S3. Comparation between the probe **P1** and the reported works.

	Previous work (Guo et al)	**Previous work (Peng et. al)**	**This work**
Detection limit	10.6 nM	0.56 nM	920 nM
Kinetics ($t_{1/2}$)	6 s	2 s	40 s
Mechanism	N-chlorination hydrolysis	Imidazole oxidation	Sulfur oxidation
Reversibility	No	No	Yes
Endogenous	Yes	No	Yes