Supporting Information

Construction of Polysiloxane-Based Fluorescent Probe for Visualizing The pH Down-regulation

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1. Instruments

MTT was obtained from Sigma-Aldrich. The ^{1}H NMR and ^{13}C NMR spectra were measured on an AVANCE III 400 MHz Digital NMR Spectrometer. High-resolution mass spectra (HRMS) for the characterization of structures were collected using a Bruker apex-Ultra mass spectrometer (Bruker Daltonics Corp., USA) in electrospray ionization (ESI) mode. Fluorescence spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell. Absorption spectra were obtained on a Shimadzu UV-2700 Power spectrometer. The molecular weight and molecular weight distribution of polymer were determined by Waters gel permeation chromatograph. Fluorescence imaging of HeLa cells and HepG2 cells were performed with Nikon A1MP confocal microscopy. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter.

2. MTT assay

Cytotoxicity studies were performed using MTT assay. HeLa cells (1×10^{6} cells / mL) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL well. Plates were maintained at 37℃ in a 5% CO_2 / 95% air incubator for 24 h. **PN-1** was diluted to different concentrations of solution with medium and added to each well after the original medium has been removed. HeLa cells were incubated with probe concentrations (0, 1, 5, 10, 20, 30 μM) for 24 h. 200 μL MTT solution (5.0 mg / mL, HEPES) was added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. Finally, the absorbance was measured at 520 nm using a microplate reader (Infinite M 200 Pro).

$$\text{Survival rate (\%)} = \frac{A_{\text{sample}} - A_b}{A_c - A_b} \times 100$$

Where A_{sample} is the absorbance of the wells with cells treated with the probes, A_b is the absorbance of wells pretreated with only culture medium, and A_c is the absorbance of wells with cells treated with no reagents.

3. Synthesis of aminopropyl–functional polysiloxanes (P0)

Aminopropyl–functional polysiloxanes (**P0**) was synthesized according to the classical procedure.[1-2] As shown in **Scheme S1**, a mixture of (aminopropyl) methyldimethoxylsilane (3.98 g, 0.02 mol) and dimethyldimethoxysilane (7.31 g, 0.06 mol) 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 ℃ 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 %.

4. Synthesis of N0 and N-1

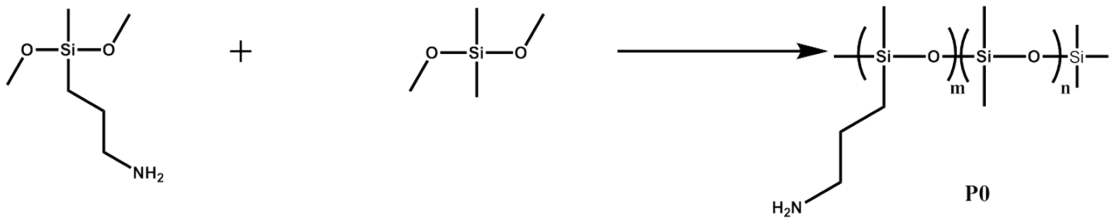
As shown in Scheme S2, 4-(diphenylamino) benzaldehyde (1.4 g, 0.5 mmol) and N-bromosuccinimide (1.01 g, 0.6 mmol) were dissolved in glacial acetic acid (25 mL) and chloroform (25 mL) and refluxed at 45℃ for 8 h (Scheme 1). The solvent was cooled to room temperature, after then ice water was added to the reaction flask. Extracted with a mixture of dichloromethane and deionized water for 3 times. The crude product could be obtained by filtration, and purified by column chromatography with CH_2Cl_2/CH_3OH (v/v= 50:1) as eluent, to afford powder product with a yield of 69%.

Product **N0** (0.524 g 2 mmol) and 4-Vinylpyridine (0.249 g 1 mmol) were weighed and dissolved in DMF under reflux at 100℃ for 12 h. The catalysts were K_2CO_3 (3eq.), Tris (2-methylphenyl) phosphine (0.3 eq) and Palladiumacetate (0.1 eq). After the reaction is completed then ice water was added to the reaction flask, and a pale red precipitate was precipitated. The excess solution was removed by suction filtration, and the solid on the filter paper was dried to obtain a pale red product **N-1**. Prior to the use of Compound **N-1**, the product need further purified by column chromatography. The eluent is dichloromethane and methanol

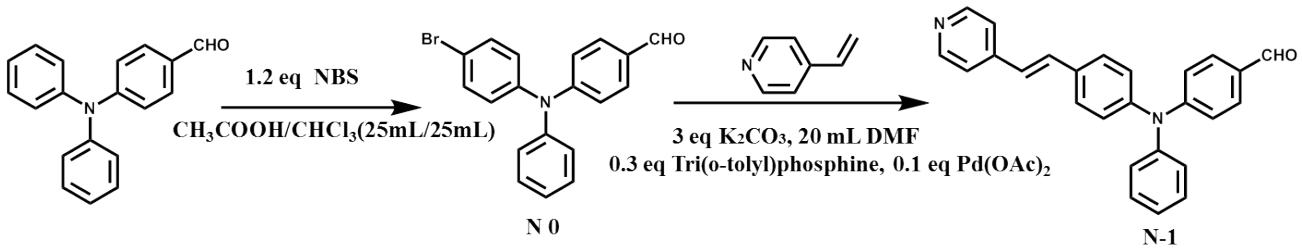
(v/v = 30:1) to afford target product. The product need further purified by column chromatography. The eluent is Petroleum ether and Ethyl acetate (v/v = 10:1) to afford target product. (yield: 63 %).^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.84 (s, 1H), 8.55 (d, J = 5.8 Hz, 3H), 7.81 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.6 Hz, 4H), 7.58–7.55 (m, 4H), 7.20 (d, J = 8.4 Hz, 4H), 7.09 (d, J = 8.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 191.17, 150.46, 144.82, 132.67, 131.76, 130.58, 129.07, 126.93, 126.08, 121.27, 119.87. HRMS m/z: $(M+H)^+$ calculated for $C_{26}H_{20}N_2O$: 377.1648 Found: 377.1643.

5. Gel Permeation Chromatography (GPC) assay of P0 and PN-1

The molecular weight and molecular weight distribution of **P0** and **PN-1** were determined by Waters gel permeation chromatograph, equipped with 1525 HPLC pump and 2414 type RI refractometer. The test conditions were as follows: column temperature 32℃, flow rate 1 mL/ min, polystyrene (PS) as standard sample, THF as mobile phase, the concentration of **P0** and **PN-1** polymer solution were 2 mg/mL, and manual injection was 0.2 mL. Then the test results date are shown in **Table S1**.



Scheme S1. Synthesis of probe **P0**



Scheme S2. Synthesis of probe **N-1**

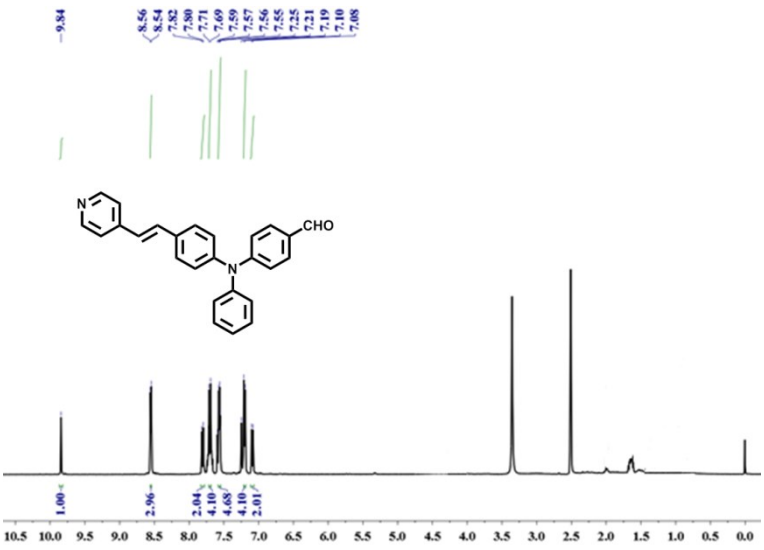


Figure S1. ^{1}H NMR spectrums of **N-1** in DMSO-*d6*

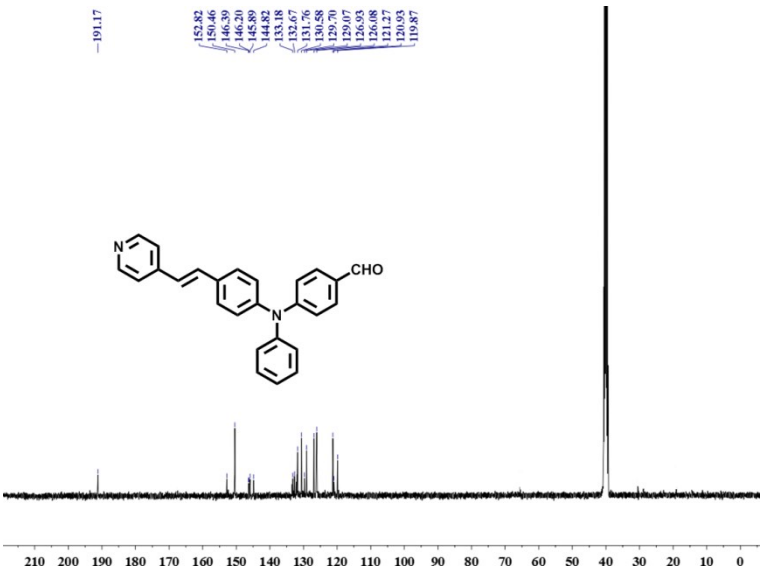


Figure S2. ^{13}C NMR spectrums of **N-1** in DMSO-*d6*

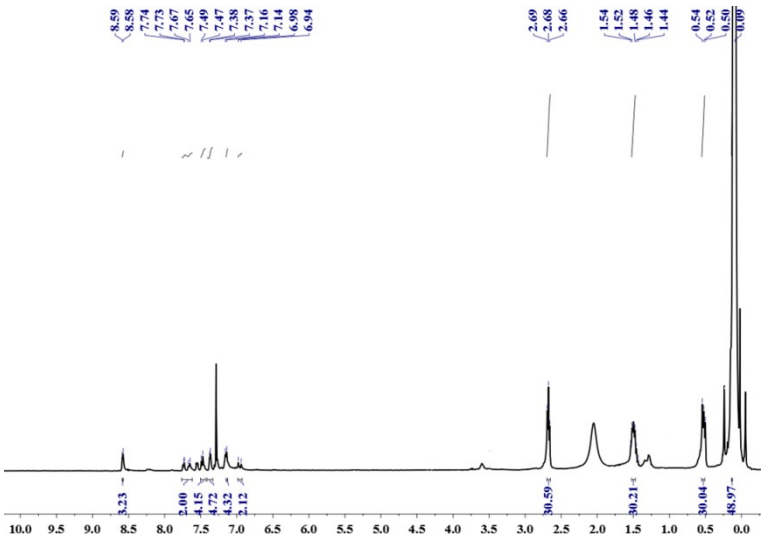


Figure S3. ^{1}H NMR spectrums of **PN-1** in $CDCl_3$

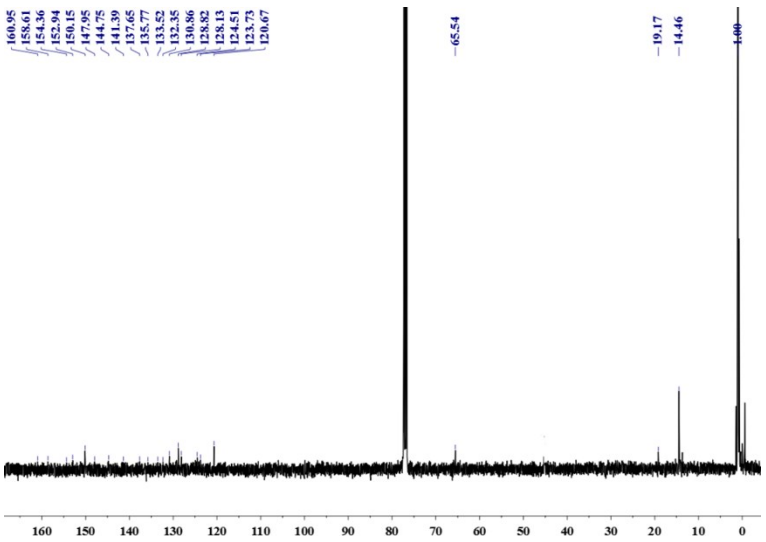


Figure S4. ^{13}C NMR spectrums of **PN-1** in $CDCl_3$

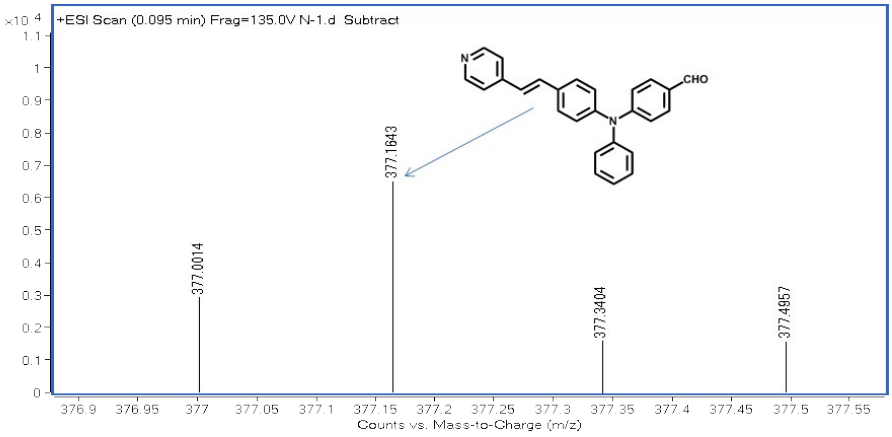


Figure S5. MS spectrum of **N-1** in methanol

Table S1. Molecular weights of **P0** and the probe **PN-1**.

Sample	Mn g/mol	Mw g/mol	PDI (Mw/Mn)
P0	3543	4198	1.18
PN-1	5263	5473	1.04

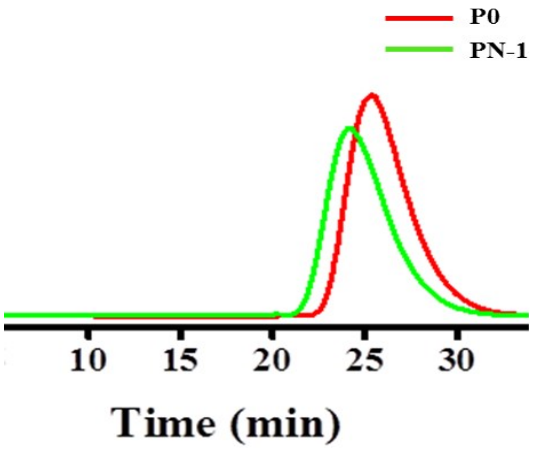


Figure S6. GPC trace of **P0** and **PN-1** in THF on the basis of a linear PS calibration

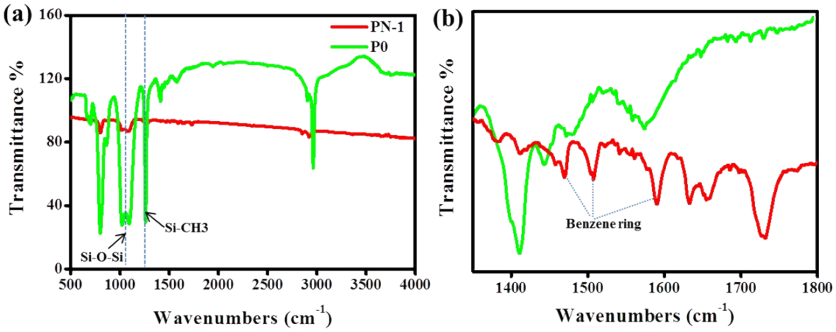


Figure S7. (a) The Infrared spectra of **P0** and **PN-1**; Figure (b) is a partial amplied view of Figure (a). (1360-1800 cm^{-1}).

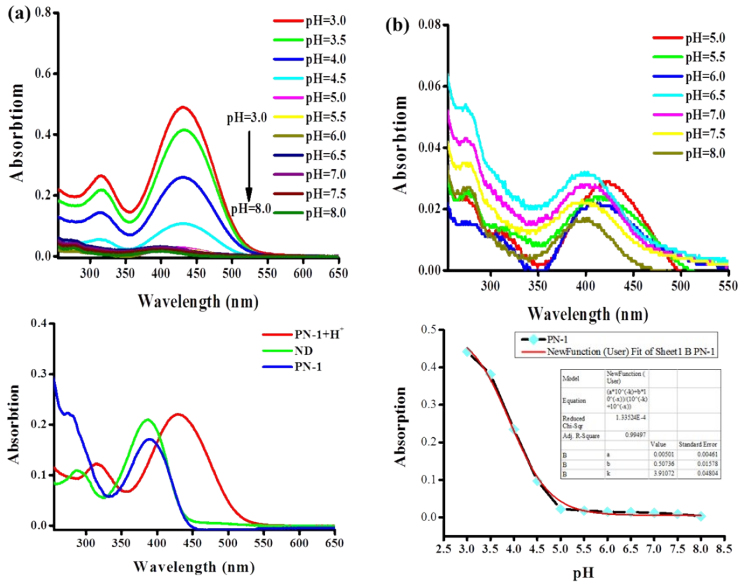


Figure S8. (a) Absorption spectra of **PN-1** (10 μM) in different pH (3.0-8.0); (b) Figure b is the amplify view of Figure 1(a) pH 5.0-8.0; (c) blue line is the **PN-1** (10 μM) in Ethanol solution, green line is the **N-1** (10 μM) in Ethanol solution, red line is the **PN-1** (10 μM) in pH =3 buffer solution. (d) The linear relationship between absorption intensity (450 nm) and various pH values.

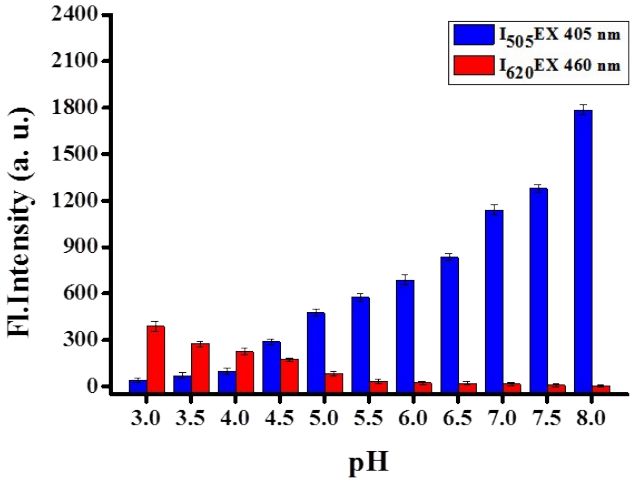


Figure S9. The fluorescence spectra of the probe **PN-1** (10 μM) column data at different pH values with the excitation of 405 nm and 460 nm.

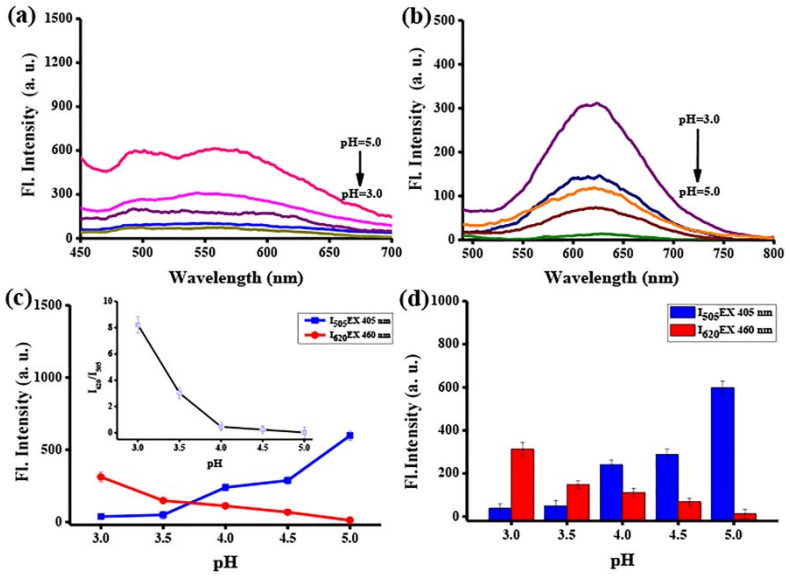


Figure S10. The fluorescence spectra of the probe **PN-1** (10 μM) at different pH values (3.0-5.0) with the excitation of 405 nm (a) and 460 nm (b) in BR buffer solution; (c) the dependent fluorescence intensity at 505 nm (excited by 405 nm) and 620 nm (excited by 460 nm), Inset: the pH dependent intensity ratio of 620 nm to 505 nm; (d) The probe **PN-1** (10 μM) column data at different pH values with the excitation of 405 nm and 460 nm.

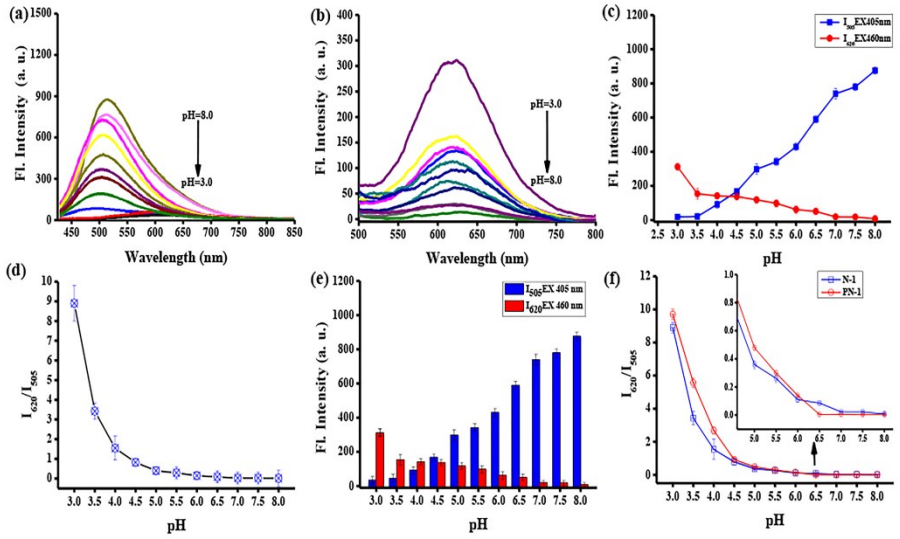


Figure S11. The fluorescence spectra of the probe **N-1** (10 μM) at different pH values in BR buffer solution with the excitation of 405 nm (a) and 460 nm (b); (c) the dependent fluorescence intensity at 505 nm (excited by 405 nm) and 620 nm (excited by 460 nm) curve graph; (d) the pH dependent intensity ratio of 620 nm to 505 nm curve graph; (e) The probe **N-1** (10 μM) column data at different pH values with the excitation of 405 nm and 460 nm; (f) The probe P**N-1** and **N-1** pH dependent intensity ratio of 620 nm to 505 nm curve graph.

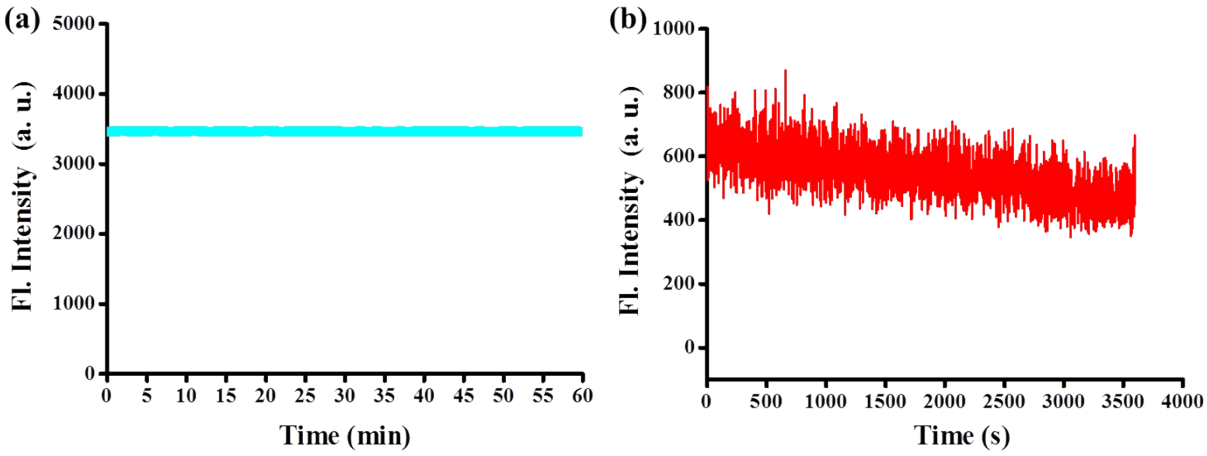


Figure S12. Photo-stability testing result for **PN-1** (10 μM) and **N-1** (10 μM) in aqueous solution of Ethanol /PBS (v/v = 2:8). λ_{ex} = 405 nm.

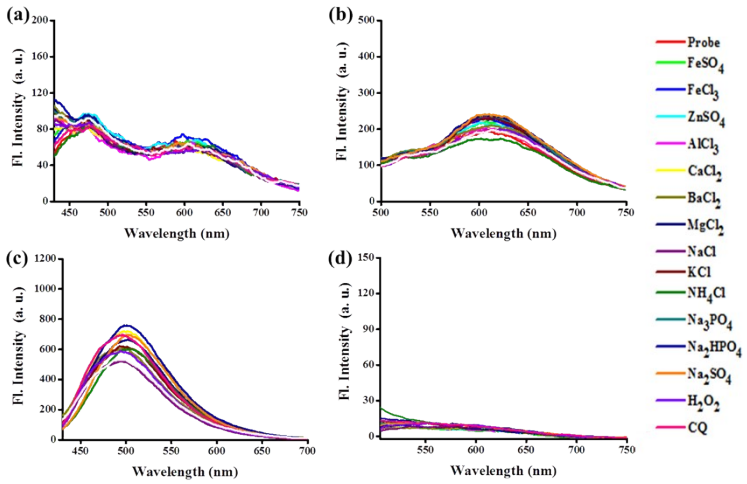


Figure S13. The fluorescence spectra of the probe **PN-1** (10 μM) in the absence and presence of various reagents in PBS buffer with pH of 4.0 (a,b) and 7.4 (c,d). (a,c) λ_{ex}= 405 nm ; (b,d) λ_{ex} = 460 nm; concentration of FeSO4,$FeCl_3$, $ZnSO_4$, $AlCl_3$, $CaCl_2$, $BaCl_2$, $MgCl_2$, NaCl, KCl, NH_4Cl, Na_3PO_4, Na_2HPO_4, Na_2SO_4, H_2O_2, CQ 100 mM.

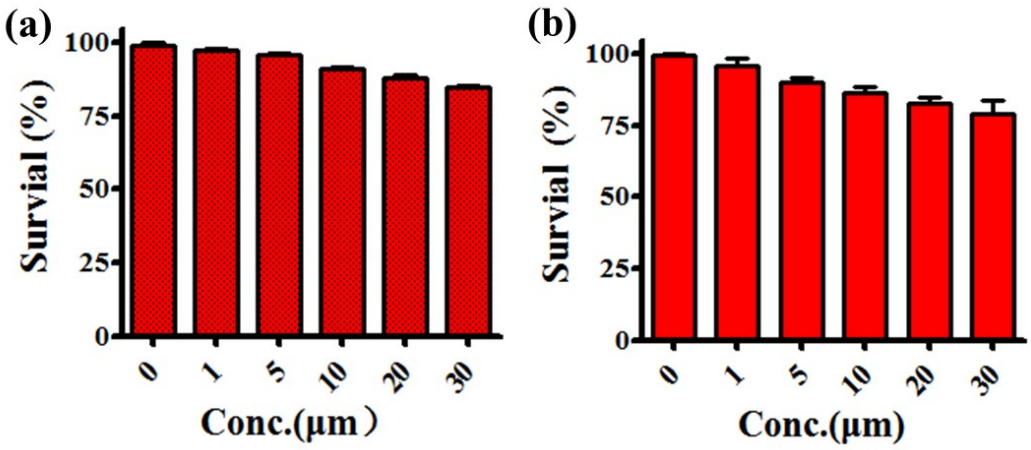


Figure S14. Cytotoxicity of the probe **PN-1** and **N-1** in HeLa cells determined by MTT.

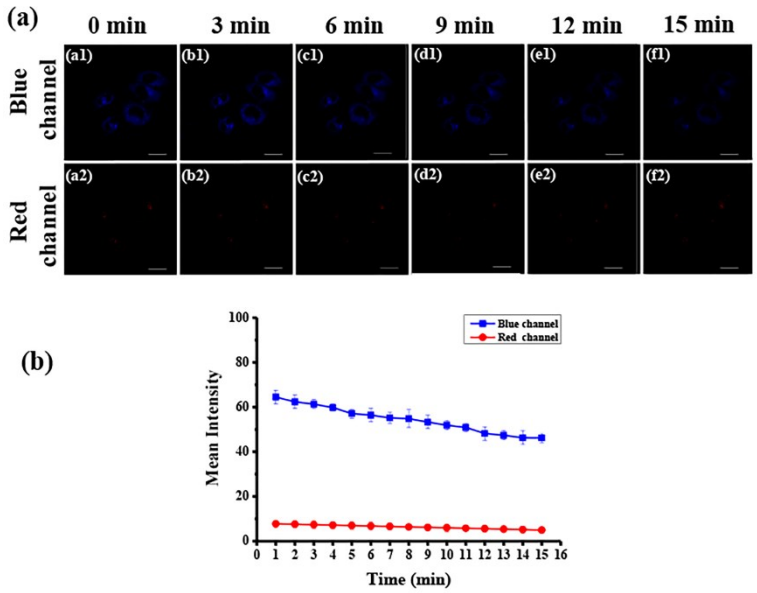


Figure S15. (a) Fluorescence images of HepG2 cells incubated with **N-1** (10 μM) acquired under successive excitation with 405 nm and 561 nm laser (a1-f2) at different times; (b) Analysis of the quantitative fluorescence intensity in (a) using Image software. The error bars represent the standard deviation (± S.D.), n= 3. Scale bar= 20 μm.

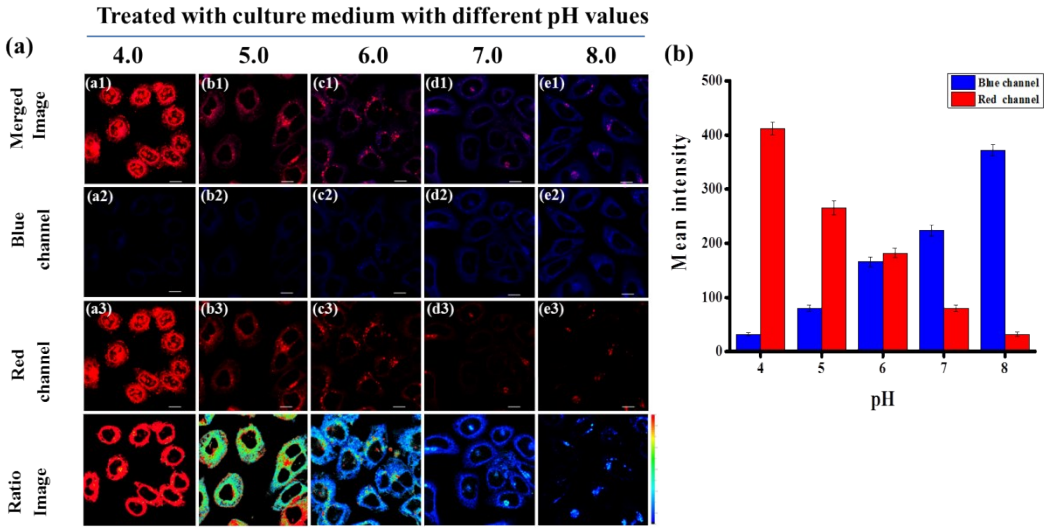


Figure S16. (a)The fluorescent and ratiometric images of HepG2 cells pre-treated with cultural medium with different pH values for 30 min then stained with **PN-1** (10 μM) for 30 min. Bar = 10 μm; (b) Analysis of the quantitative fluorescence intensity in (a) using Image software. The error bars represent the standard deviation (± S.D.), n= 3. Scale bar= 10 μm.

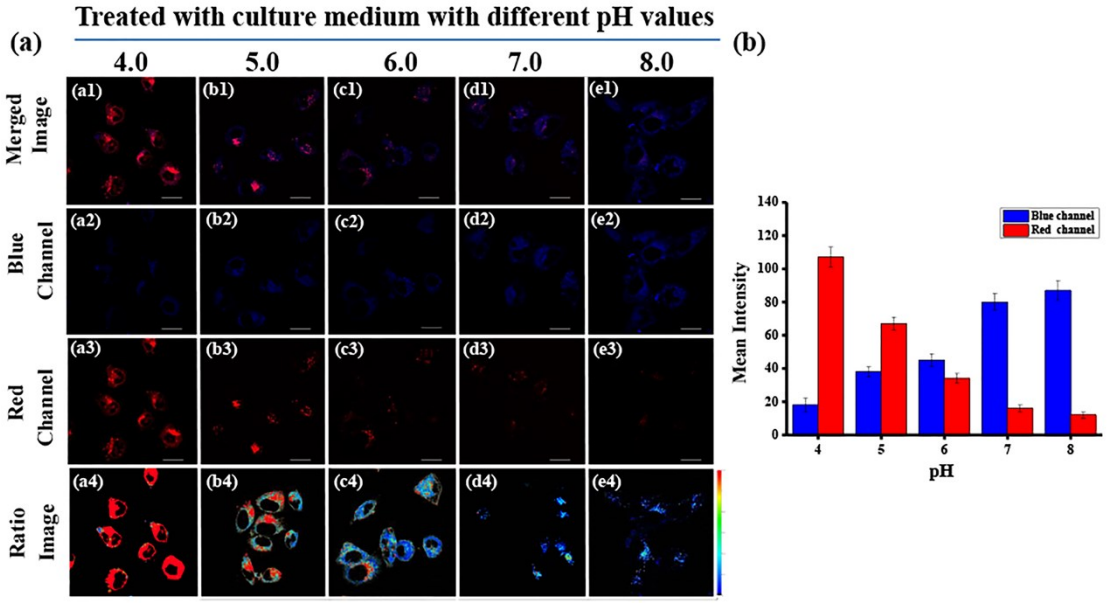


Figure S17. (a)The fluorescent and ratiometric images of HepG2 cells pre-treated with cultural medium with different pH values for 30 min then stained with **N-1** (10 μM) for 30 min. Bar = 10 μm; (b) Analysis of the quantitative fluorescence intensity in (a) using Image software. The error bars represent the standard deviation (± S.D.), n= 3. Scale bar= 20 μm.

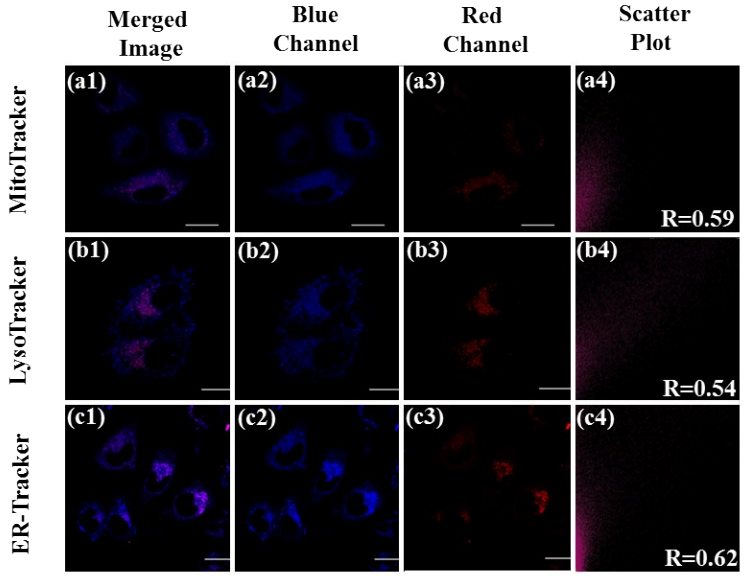


Figure S18. Images of HepG2 cells costained with **PN-1** and then treated with MitoTracker deep red and LysoTracker deep Red and ER-tracker red, respectively. Scalebar = 20 μm.

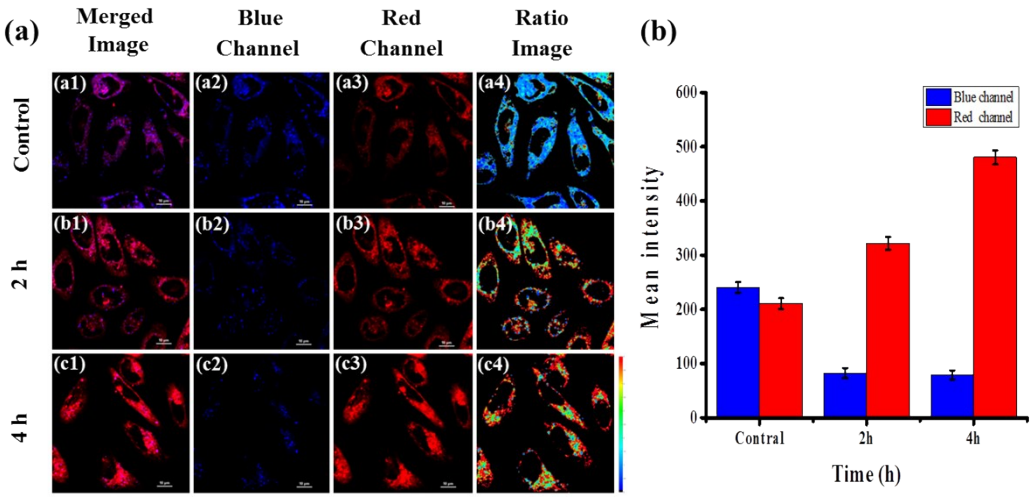


Figure S19. The fluorescent images of HepG2 cells pre-stained with **PN-1** (10 μM). (a) then incubated with culture medium for 4h (Control) and PBS buffer for 2h and 4h; (b) The mean intensity in blue channel, red channel, and the intensity ratio of red to blue channel of the three groups of cells. Bar = 10 μm.

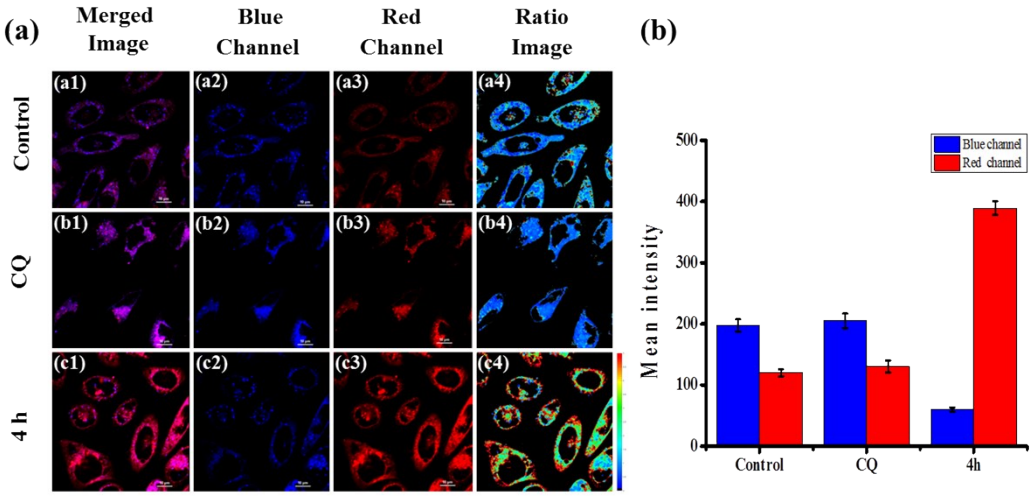


Figure S20. (a) The fluorescence images of HepG2 cells pre-stained with **PN-1** (10 μM) in culture medium for 4h; (b) HeLa cells incubated in PBS containing CQ for 4 h. (c) HeLa cells incubated in PBS containing **PN-1** (10 μM) for 4 h Bar = 10 μm.

References

1. Y. Zuo, Y. Zhang, B.Dong, Z. Gou, T.Yang, W. Lin, Anal. Chem., 2019, **91**, 1719−1723.
2. T. Yang, Y. Zuo, Y. Zhang, Z. Gou, X. Wang, W. Lin, Analytica Chimica Acta, 2019, **1091**, 88-94.