Electronic Supplementary Information for

Construction of a polymer-based fluorescent probe with dual responsive sites for monitoring the changes of lysosomal viscosity

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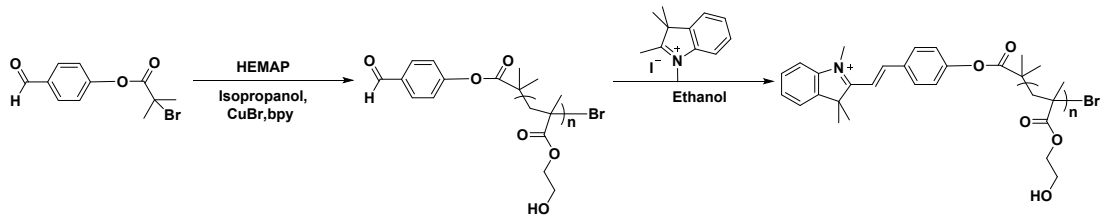
Materials and instruments

All chemical reagents were of analytical grade and used without further purification unless otherwise stated. Solvents were purified by freshly distilled before using. Ultrapure water was used throughout all the experiments. TLC analysis obtained on silica gel plates and column chromatography, which was conducted on silica gel (mesh 200-300) and purchased from the Qingdao Ocean Chemicals. ^{1}H and $^{13}CNMR$ spectra were recorded on an AVANCE III 400 MHz digital NMR spectrometer, with TMS as internal standard. High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer. UV-Vis absorption spectra were recorded on a Shimadzu UV-2700 spectrophotometer; fluorescence spectra were obtained on a HITACHI F4600 fluorescence spectrophotometer using a 1 cm standard quartz cell.

Synthesis of polymer-based fluorescent probe In-PHEM



Scheme S1. Synthesis routes and chemical structures of polymer-based fluorescent probe **In-PHEM**.

Spectrum measurement of probe In-PHEM

The stock solution (6 mg/mL) of probe **In-PHEM** was prepared by dissolving them in

methanol. 60 μg/mL of **In-PHEM** was used throughout all the photophysical experiments by adding 20 μL stock solution to 2.0 mL methanol and glycerol mixed solvents or PBS (pH 7.4). The solutions of the various interferents (100 mM) were prepared in the ultrapure water. The excitation wavelength is 350 nm and 440 nm, both of the excitation and emission slit widths are 5 nm.

Cells culture and cytotoxicity assays

Living HeLa cells were used in this work and they were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in an atmosphere of 5% CO_2 and 37 °C. After incubation with culture medium for 24 h, the cells will adhere to the glass surface during this period.

Colorimetric methyl thiazolyl tetrazolium (MTT) assays was applied to measure *in vitro* cytotoxicity of the probe **In-PHEM** to living HeLa cells. 2×10^4 cells/mL cells were seeded in 96-well plates and then incubated with various concentrations of **In-PHEM** (0, 6, 12, 30, 60 and 120 μg/mL) for 24 h. Subsequently, 10 μL MTT (5 mg/mL) was added to each well and incubated for another 3 h. At last, the media was removed and 100 μL of DMSO was added to dissolve the formazan crystals. The plate was shaken for about 10 min and the absorbance of the solution was measured at 570 nm with a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **In-PHEM**.

Cell imaging and co-localization experiments

For cell imaging, the cells were incubated with 60 μg/mL **In-PHEM** for 30 min and then the medium was removed. To remove residual probe, the cells was washed three times using PBS before imaging. Finally, confocal fluorescence cell imaging was carried out using Nikon fluorescence microscope equipped with 405 and 488 nm excitation. The fluorescent signals were collected at 425-475 nm (blue channel) and 500-550 nm (green channel), respectively.

The co-localization experiments were carried out using Nile Red as the lipid droplets staining dye. HeLa cells were stained with 2 μM Nile Red and 60 μg/mL **In-PHEM** simultaneously. Blue channel was collected at 425-475 nm with excitation of 405 nm and green channel was collected at 500-550 nm with excitation of 488 nm. Red channel was collected at 570-620 nm with excitation of 488 nm.

Fluorescent monitoring the viscosity changes in zebrafish

All procedures were according to the Guidelines for Care and Use of Laboratory Animals of Shandong University and approved by the Animal Ethics Committee of Shandong University. Before imaging, zebrafish was incubated with LPS, monensin or nystatin (10 μM) for 30 min and **In-PHEM** (60 μg/mL) for another 30 min. To remove residual probe, zebrafish was rinsed with water three times before imaging. Confocal microscopic imaging was performed under excitation at 405 and 488 nm and the emission wavelength was collected at 425-475 nm (blue channel) and 500-550 nm (green channel), respectively.

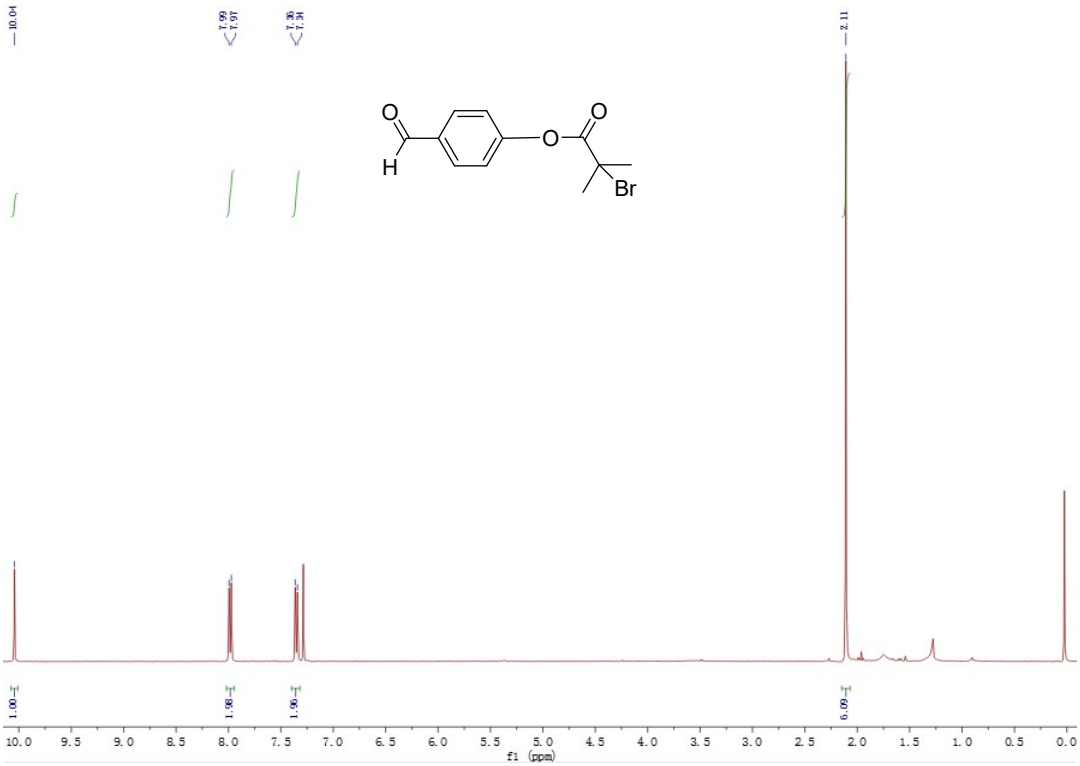


Fig. S1. The ^{1}H NMR spectrum of initiator CHOBr in $CDCl_3$.

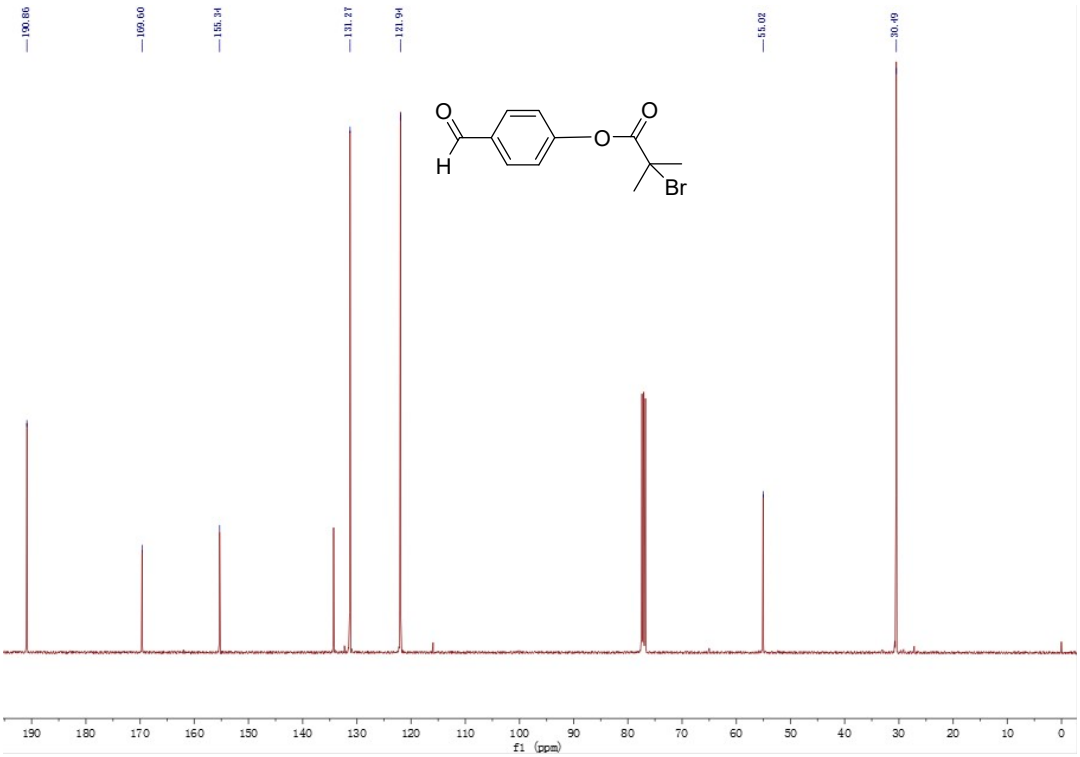


Fig. S2. The ^{13}C NMR spectrum of initiator CHOBr in $CDCl_3$.

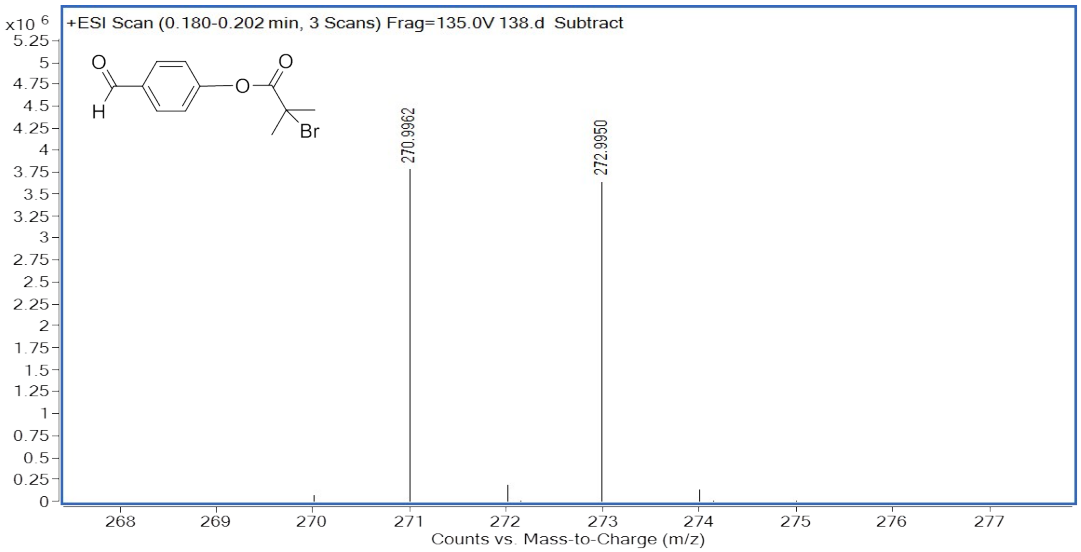


Fig. S3. The HRMS spectrum of initiator CHOBr.

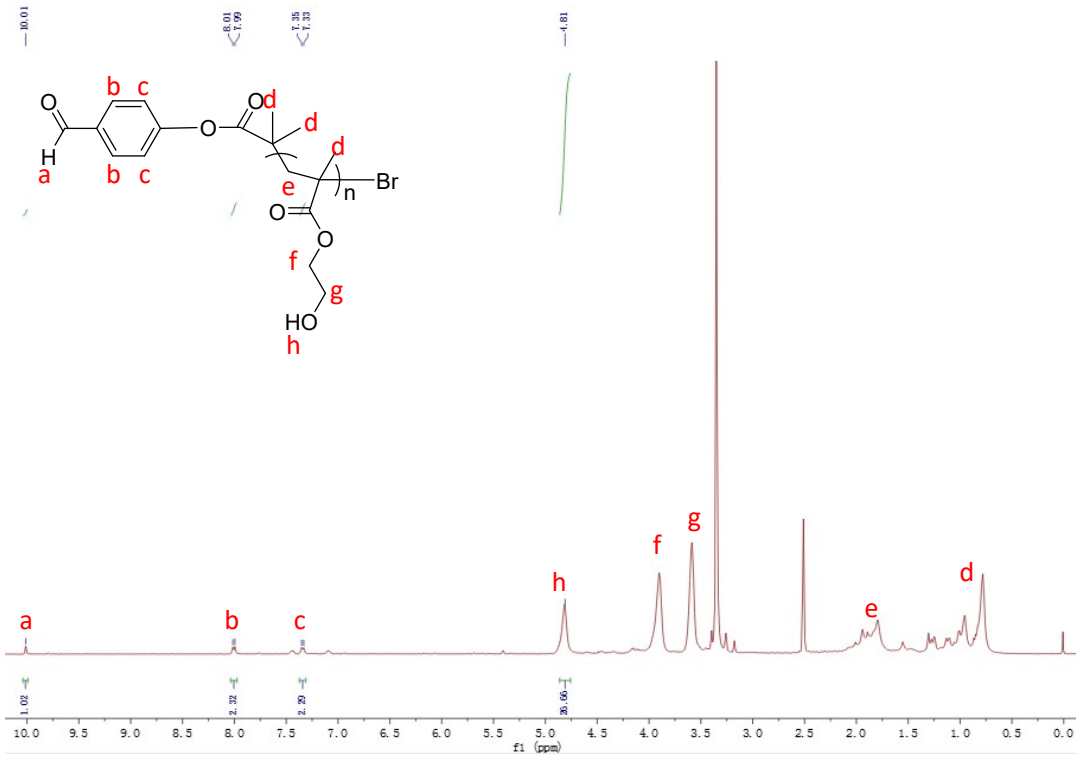


Fig. S4. The ^{1}H NMR spectrum of **CHO-PHEM** in DMSO-*d6*.

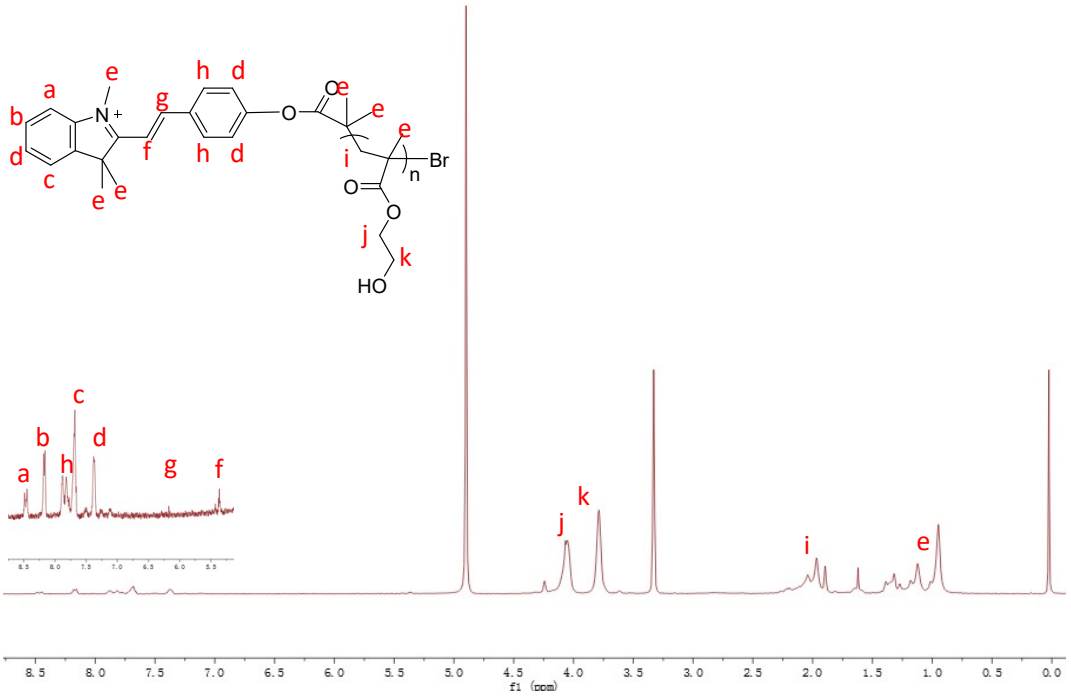


Fig. S5. The ^{1}H NMR spectrum of **In-PHEM** in CD_3OD.

Table S1. GPC data of polymer In-PHEM.

Polymer Name	M_n	M_w	M_z	M_z/M_w	PDI
In-PHEM	4906	5977	7443	1.26	1.22

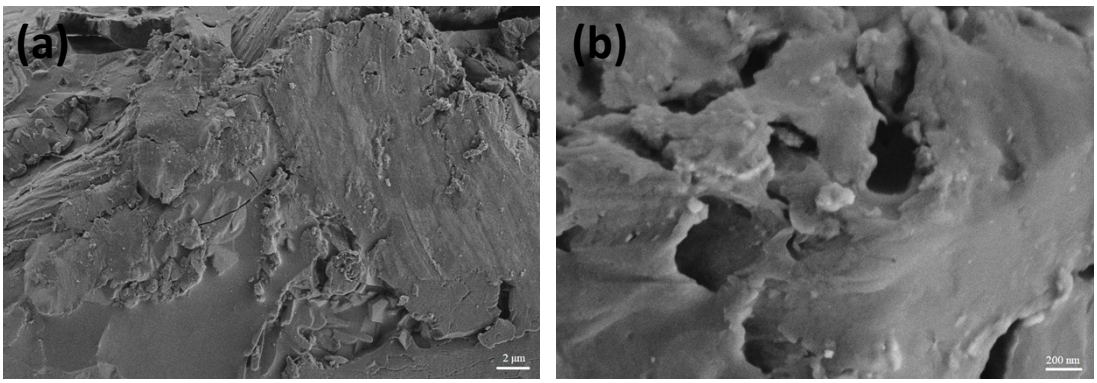


Fig. S6. (a, b) SEM images of polymer In-PHEM.

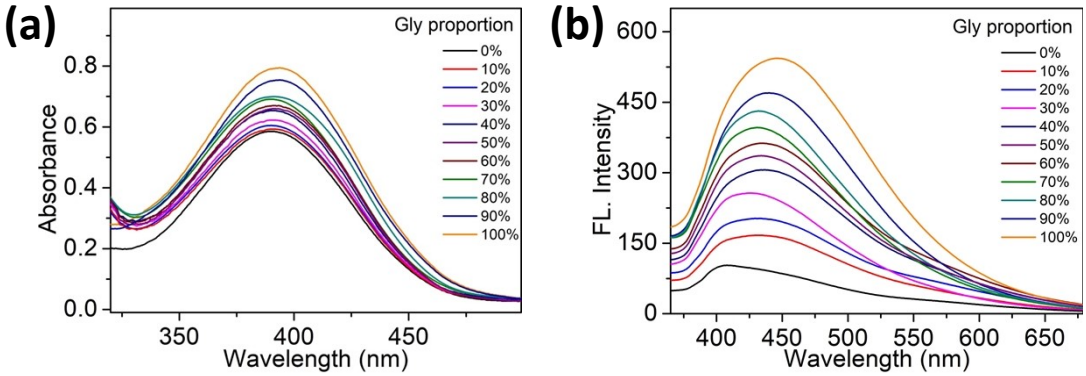


Fig. S7. (a) UV-Vis absorption spectra of **In-PHEM** (60 μg/mL) in methanol and glycerol with different volume fraction. (b) Fluorescence spectra of polymer CHO-PHEM (60 μg/mL) in methanol and glycerol mixture with different volume fraction (λ_{ex} = 350 nm).

Table S2. The viscosity and intensity properties of **In-PHEM** in methanol and glycerol mixture with different volume fraction.

glycerol / methanol (v: v)	η/cp	I_{440}	I_{540}
0:10	0.9	40.6	9.0
1:9	1.3	43.1	11.7
2:8	2.8	49.7	14.1
3:7	8.7	62.2	19.5
4:6	12.9	76.8	23.0
5:5	25.7	83.9	26.9
6:4	46.3	101.2	49.8
7:3	82	139.8	68.0
8:2	198.3	213.7	79.1
9:1	501.3	242.5	98.8
10:0	740	269.4	132.8

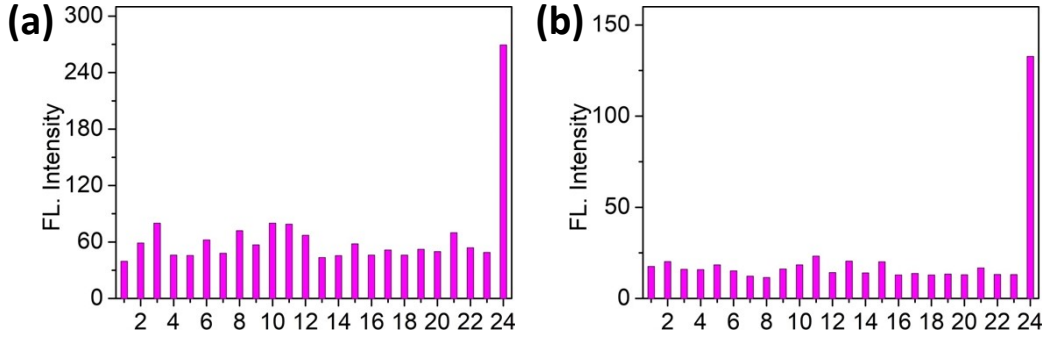


Fig. S8. (a) The fluorescence intensity of **In-PHEM** (60 μg/mL) to various relevant analytes in PBS (pH 7.4) under excitation at 350 nm. (b) The fluorescence intensity of **In-PHEM** (60 μg/mL) to various relevant analytes in PBS (pH 7.4) under excitation at 440 nm. 1, Only PBS; 2, $CaCl_2$; 3, $CoCl_2$; 4, $CuCl_2$; 5, $FeCl_3$; 6, $HgCl_2$; 7, KCl; 8, $MgCl_2$; 9, $NiCl_2$; 10, $SnCl_2$; 11, glucose; 12, Gly; 13, GSH; 14, Cys; 15, $Na_2S_2O_3$; 16, Na_2SO_3; 17, NaClO; 18 $NaHSO_3$; 19, $NaNO_2$; 20, NaOAc; 21, OH^-; 22, $ONOO^-$; 23, H_2O_2; 24, glycerol.

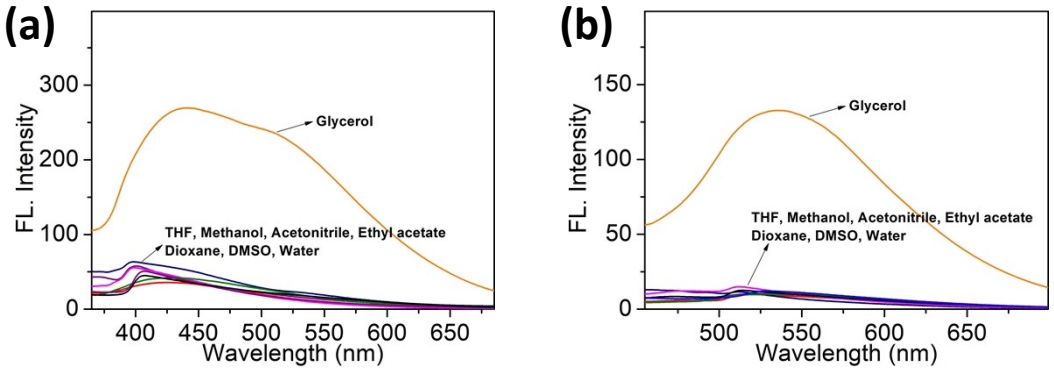


Fig. S9. (a) The fluorescence spectra of **In-PHEM** in solvents with different polarity values under excitation at 350 nm. (b) The fluorescence spectra of **In-PHEM** in solvents with different polarity values under excitation at 440 nm.

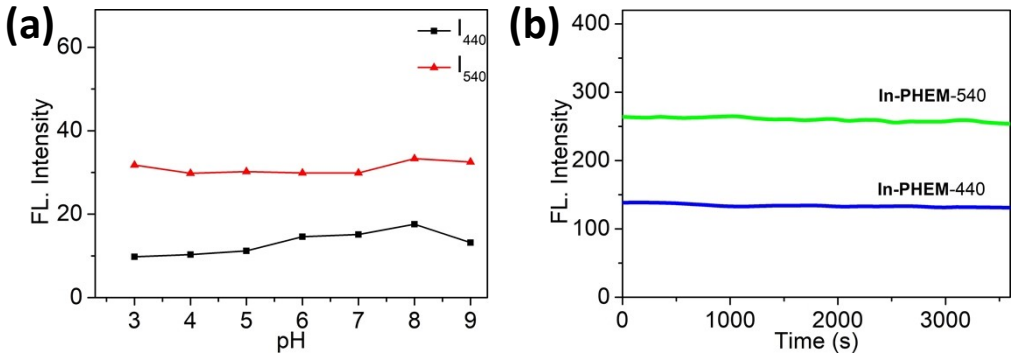


Fig. S10. (a) Fluorescent intensities (I_{542} and I_{457}) of probe **In-PHEM** with excitation at 350 and 440 nm in different pH values. (b) The photostability tests of 60 μg/mL of **In-PHEM** with ceaseless irradiation by laser light for 60 min.

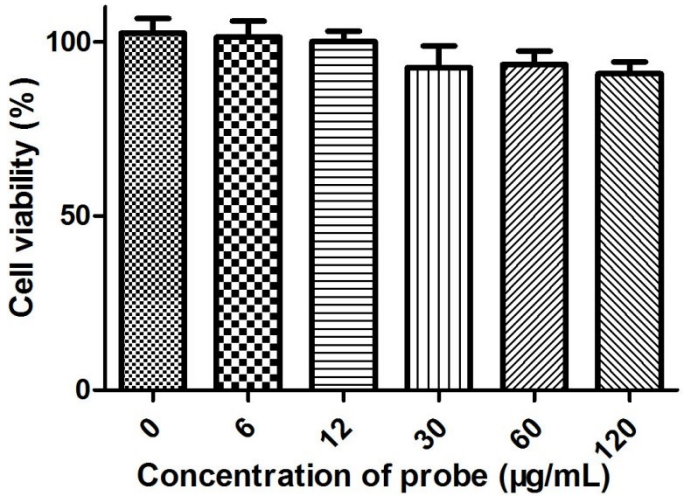


Fig. S11. The cell viability of living HeLa cells treated with 0, 6, 12, 30, 60 and 120 μg/mL **In-PHEM** for 24 h and measured by standard MTT assay.

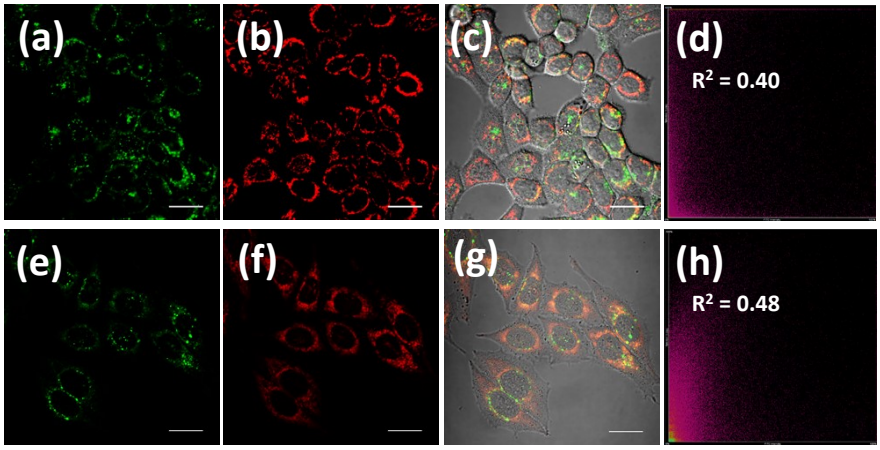


Fig. S12. Colocalization fluorescence images of the probe **In-PHEM** (60 μg/mL) with (a-d) 1 μM Mito-Tracker Red and (e-h)1 μM ER-tracker Red. Scale bar: 20 μm.

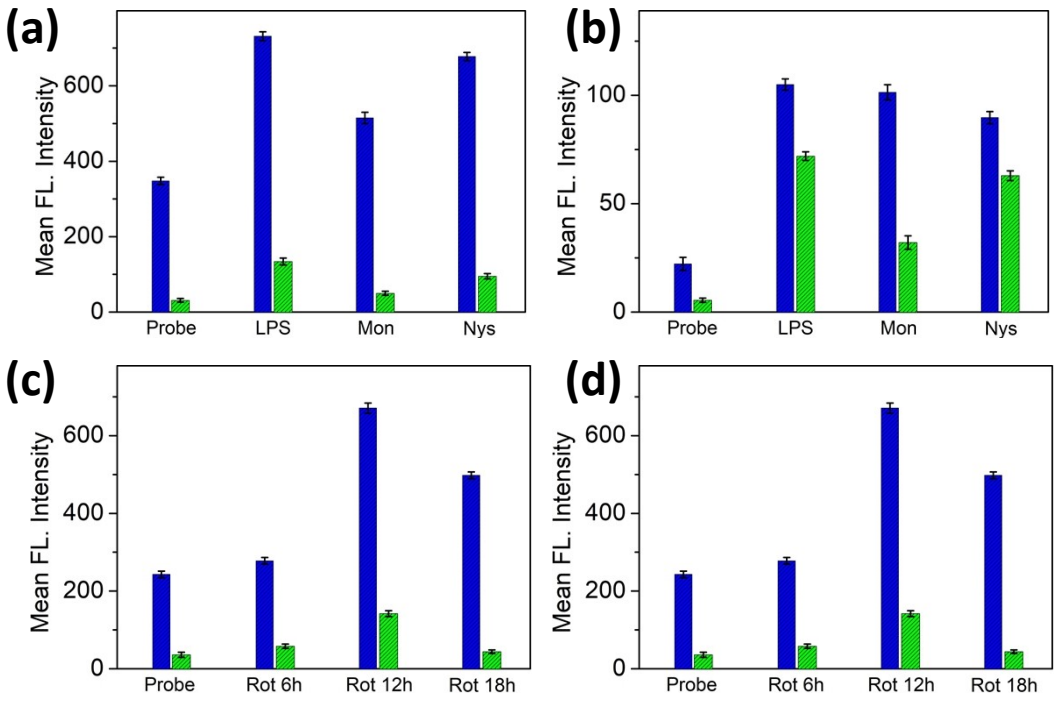


Fig. S13. The mean fluorescent intensities of blue and green channel shown in (a) Fig. 3, (b) Fig. 4, (c) Fig. 5 and (d) Fig. 6.