Supplementary Information for

A mitochondria-targeted fluorescence probe with viscosity sensitivity to distinguish between normal and cancer cells

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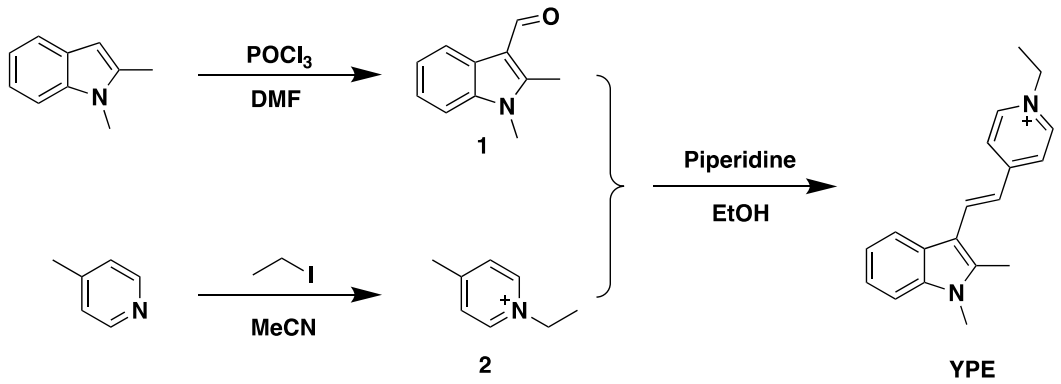
Experimental

Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. All experiments used ultra-pure water. Solvents were purified by standard methods prior. Ultra-pure water (18.2 MΩ·cm) is used by ULPURE. TLC analysis was carried out on silica gel plates, and column chromatography was conducted over silica gel (mesh 200-300); both of them were purchased from Qingdao Ocean Chemicals. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance III HD 600 MHz NMR spectrometer (United States of America). High-resolution mass spectrometric (HRMS) analyses were measured on Brooke solanX 70 FT-MS, Agilent 6540T. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer, and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer. The fluorescence imaging of cells was performed with a Leica TCS SP8 CARS confocal microscope.

Steps of synthesis

Synthesis routine of YPE.



Scheme S1 Synthetic of probe **YPE**

Optical research and analysis

A stock solution (1 mM) of probe **YPE** was initially prepared in dimethyl sulfoxide (DMSO). All spectrometric probes were used at a concentration of 10 μM. The adjunction of 20 μL of stock solution was added to 2.0 mL of different solvent systems to obtain the probe diluent. The solutions of various interfering substances (cations, anions, amino acids and active small molecules) were prepared with twice-distilled water. The providing solutions were mixed well before texting the spectra. Unless otherwise specified, the required fluorescence spectral measurement is generally an excitation wavelength of 445 nm, an excitation slit width of 5.0 nm, and an emission slit width of 5.0 nm.

Fluorescence quantum yield

The fluorescence quantum yields of **YPE** were evaluated by using Fluorescein as a reference standard. The fluorescence quantum yield Φ_s is calculated by the following formula:

$$\Phi_u = \Phi_s(F_u/F_s)(A_s/A_u)$$

where Φ_s is the quantum yield of the sample, F is the area integral value of the corrected fluorescence spectrum, and A and n represent the absorbance and the refractive index of the solvent, respectively. The subscript "u" stands for the unknown to be tested and "s" is the standard.

Culture and preparation of cells

HeLa, HepG 2, L-O2, Cos 7 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Before the experiments, the HeLa cells in 35-mm glass-bottomed dishes were cultured to a density of 2×105 cells per dish. Incubate the cells for 24 h. Cells will attach to the glass surface during this time.

Cytotoxicity assay

HeLa, HepG 2, L-O2, Cos 7 cells were respectively seeded into 96-well plates, and 0, 1, 2, 5, 10, 20, 30, 40, and 50 μM (final concentration) of the probe **YPE** were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 hours. Next, MTT (10 μL, 5 mg/mL) was injected into every well and incubated for 4 h. Then, violet formazan was dissolved with DMSO (100 μL). The absorbance of the solution was measured at 492 nm by way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **YPE**.

The cell viability (%) = $(OD_{sample} - OD_{blank}) / (OD_{control} - OD_{blank}) \times 100\ \%$.

Co-location experimental imaging.

The cells were incubated with **YPE** (10 μM) for 15 min in an incubator of 95% air and 5% CO_2 at 37 °C. Then HeLa cells were incubated with Mito-Tracker Deep Red (500 nM, MTDR, a commercial dye targeting mitochondria) for 10 min. Afterward, wash thrice with PBS (10 mM, pH 7.4) prior to co-staining fluorescence imaging. The mitochondria localization ability of **YPE** was analyzed by the Pearson coefficient. laser confocal imaging. **YPE**: λ_{ex} = 405 nm, λ_{em} =520 - 580 nm. Mito-Tracker Red: λ_{ex} = 665 nm, λ_{em} =660 - 720 nm.

Fluorescence imaging of distinguishing normal cells and cancer cells.

Normal cells (L-02, Cos 7) and cancer cells (HeLa, HepG 2) were respectively incubated with 10 μM probe for 15 min, and then washed with PBS three times for confocal imaging.

Confocal imaging of intracellular viscosity.

For cellular viscosity change, the HeLa cells were respectively incubated with monensin (10 μM), nystatin (10 μM), LPS (10 μM), and CCCP (10 μM) for 30 min. **YPE** incubated cells were rinsed three times with PBS before performing laser confocal imaging. λ_{ex} = 405 nm, λ_{em} =520 - 580 nm.

Confocal imaging of viscosity sensibility in cells.

For verifying cellular viscosity sensibility, HeLa cells were respectively incubated with 0, 5, 10, 20, and 30 μM monensin for 30 min. **YPE** incubated cells were rinsed three times with PBS prior to performing laser confocal imaging. λ_{ex} = 405 nm, λ_{em} =520 - 580 nm.

Glycerol (%)	Fluorescence quantum yield (%)
0	1.20
10	1.92
20	3.33
30	3.72
40	5.97
50	8.59
60	12.52
70	20.27
80	30.61
90	45.05
95	62.34
99	79.07

Table S1 Fluorescence quantum yield of **YPE** in different viscosity systems

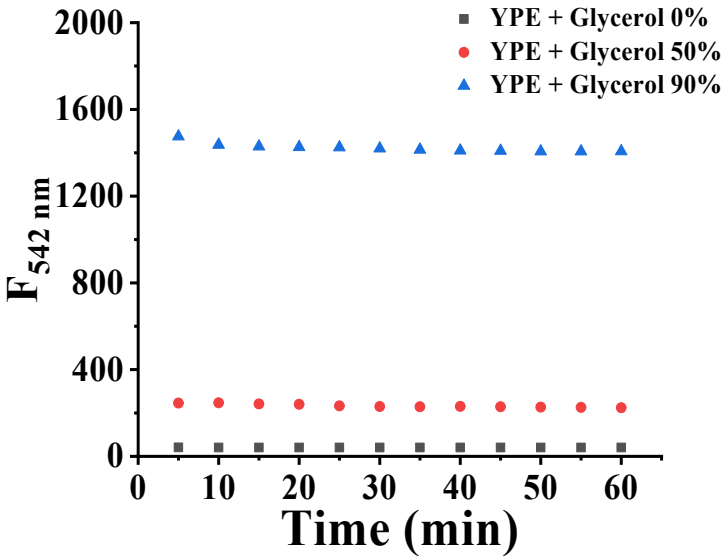


Fig. S1 The photostability of **YPE** (10 μM) in solvents of different glycerol ratios with continuous laser scanning during 60 min, and the emission peak was collected at the time interval of 1 min.

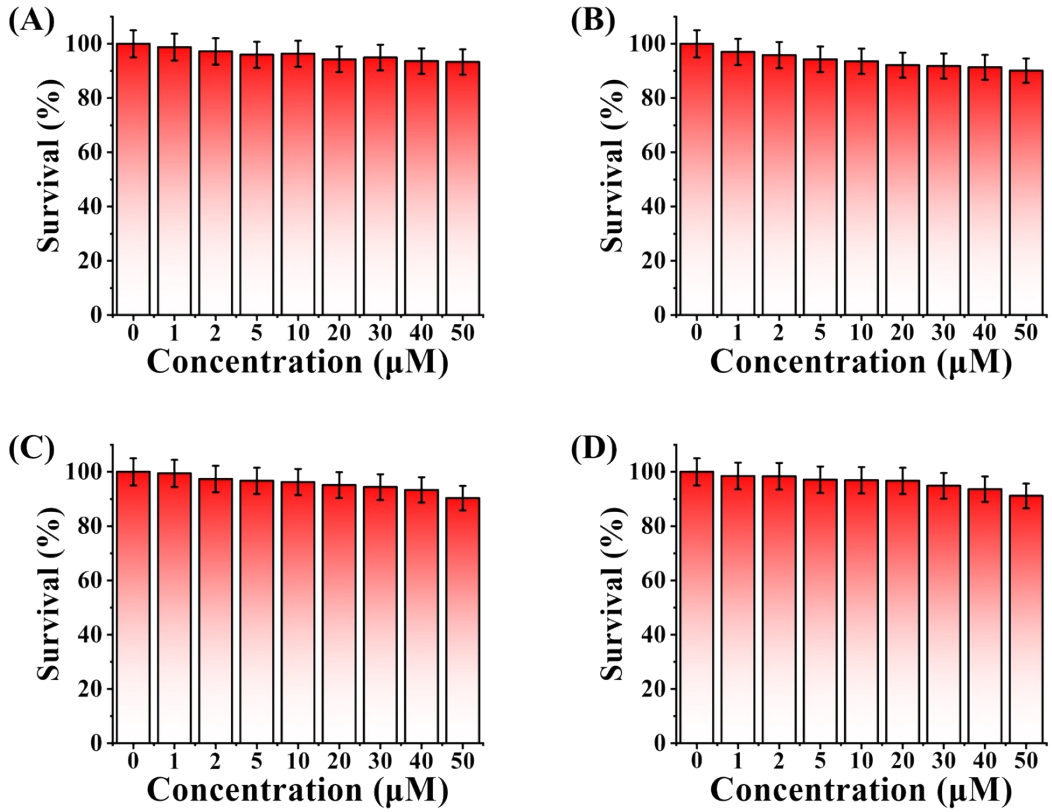


Fig. S2 Cytotoxicity assays of **YPE** at different concentrations for (A)HeLa, (B)HepG2, (C)LO-2, (D)Cos 7 cells with MTT.

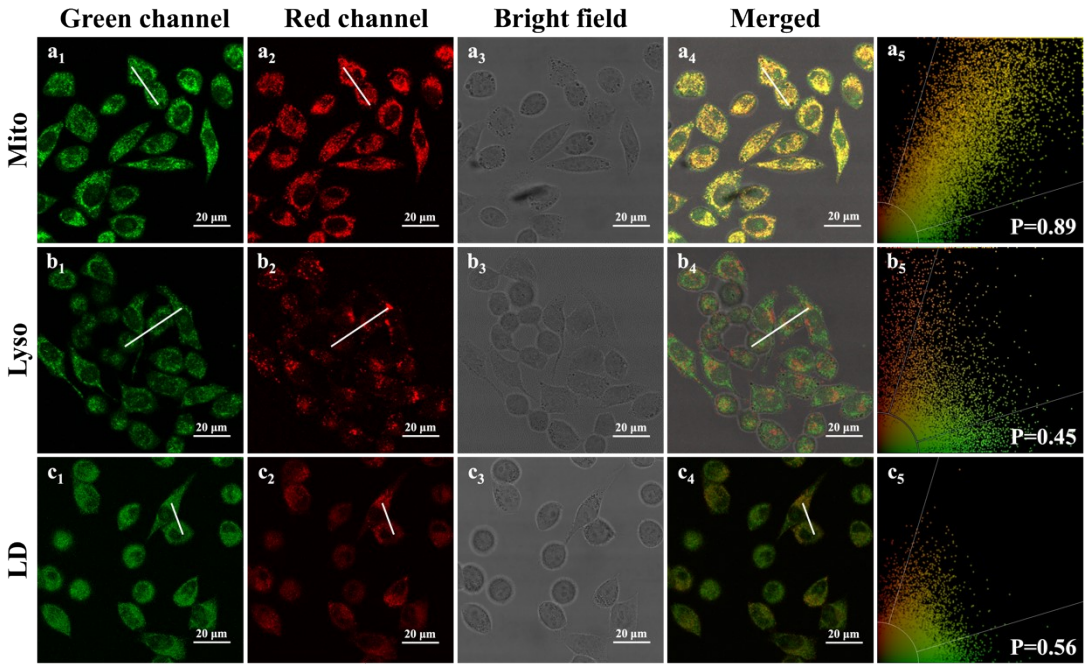


Fig. S3 The fluorescence colocalization imaging of HeLa cells staining with **YPE** and different Tracker. (a_1 - a_5) The cells were stained with **YPE** (10 μM) for 15 min and Mito-Tracker Deep Red for 10 min, λ_{ex} = 570 nm, λ_{em} =660 - 680 nm; (b_1 - b_5) The cells were stained with **YPE** (10 μM) for 15 min and Lyso-Tracker Deep Red for 10 min, λ_{ex} = 647 nm, λ_{em} =660 - 680 nm; (c_1, - c_5) The cells were stained with **YPE** (10 μM) for 15 min and Nile Red for 10 min, λ_{ex} = 559 nm, λ_{em} =620 - 650 nm. Scale bar: 20 μm.

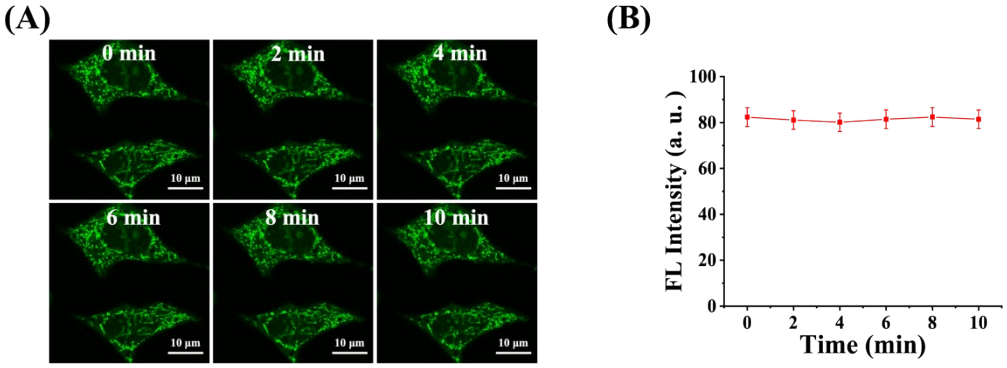


Fig. S4 (A) Photostability of **YPE** in living HeLa cells. HeLa cells were incubated with 10 μM **YPE** for 15 min at 37 ℃, and then were irradiated continuously by scanning using a confocal microscope; (B) the mean fluorescence intensity at different times. Irradiation time: 60 s per scan. λ_{ex} = 405 nm, λ_{em} = 520 - 560 nm.

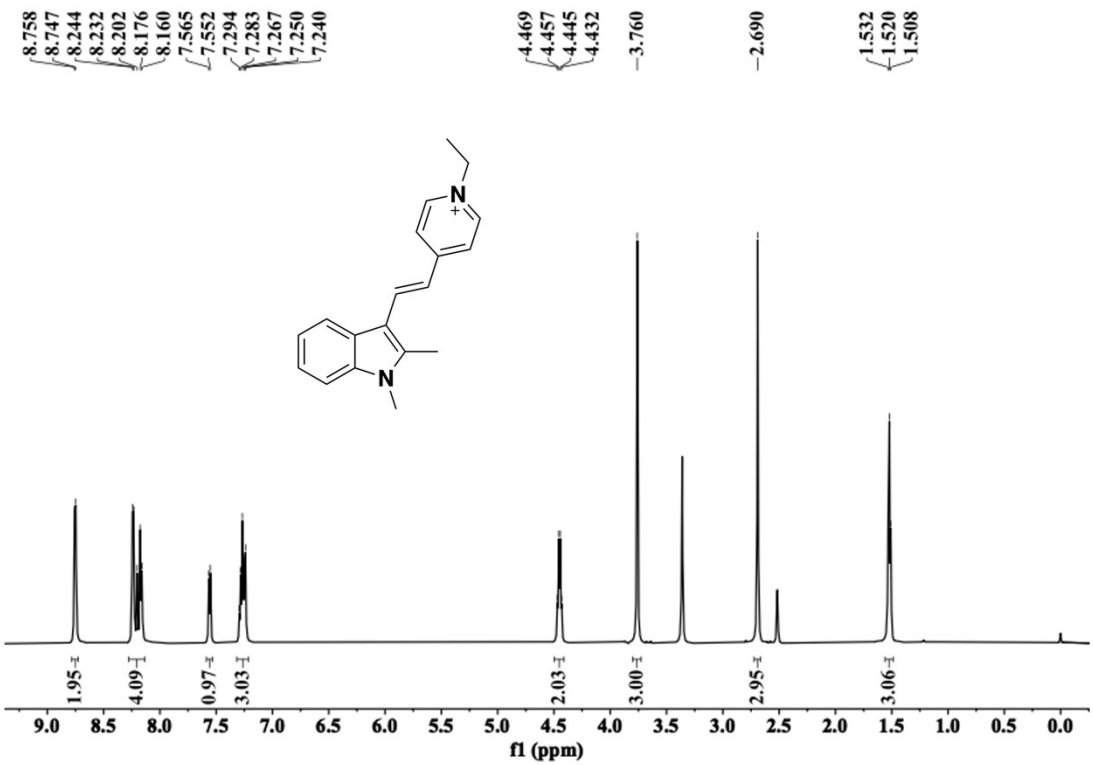


Fig. S5 The ^{1}H NMR spectrum of **YPE** in DMSO-*d6.*

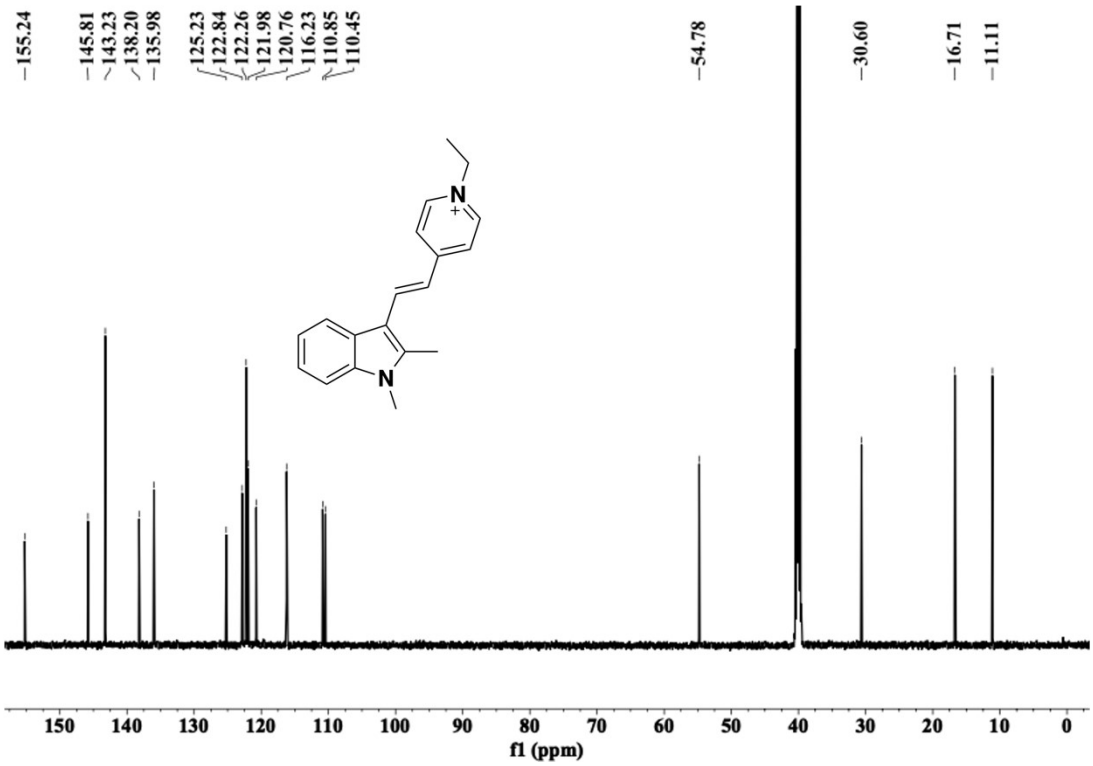


Fig. S6 The ^{13}C NMR spectrum of **YPE** in DMSO-*d6*.

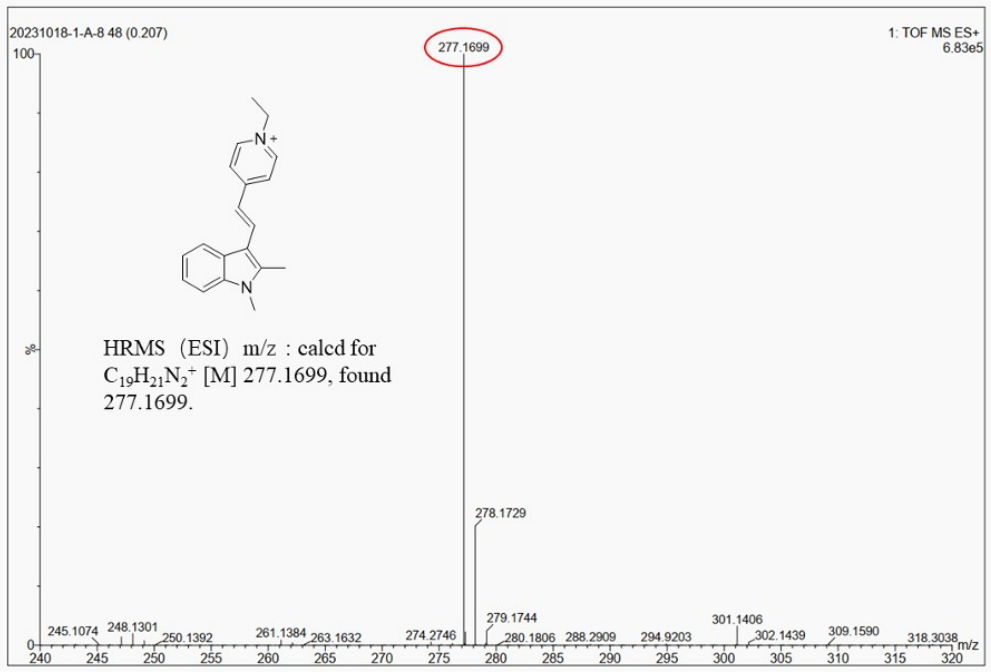


Fig. S7 The HRMS spectrum of **YPE**.

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