Supporting Information

A lysosome-targeting viscosity-sensitive fluorescent probe based on a novel functionalised near-infrared xanthene-indolium dye and its application in living cells

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Materials and equipment

All chemicals and solvents used for synthesis were purchased from commercial suppliers and applied directly in the experiment without further purification. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), Lyso-Tracker Green DND 26 and Mito-Tracker Green were purchased from Beyotime Institute of Biotechnology. $^1$H NMR and $^{13}$C NMR were measured on a Bruker AVANCE III HD 400MHz spectrometer. Chemical shifts (d values) were reported in ppm down field from internal Me$_4$Si. High resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) source. Melting points were recorded on a melting point apparatus (RY-2, Tianjin, China). UV-vis absorption spectra were obtained with UV-2550 (Shimadzu, Japan) spectrophotometer. A Hitachi F-4600 spectrophotometer (Tokyo, Japan) was used for fluorescence measurements with a 700 V PMT voltage. The pH values were reported by a Mettler Toledo Seven Excellence PH meter (Shanghai, China). The absorbance for MTT analysis was recorded on a microplate reader (PL-9602). The confocal microscopy imaging was used Olympus FV1000-IX81 inverted fluorescence microscope. Image processing was analyzed with Olympus software (FV1000-ASW) and Image J software.

Detection of fluorescence quantum yield

Rhodamine B was used as a standard to calculate the relative fluorescence quantum yields according to the following equation:

$$\Phi_B = \Phi_1 \left( \frac{F_B}{F_1} \right) \left( \frac{A_1}{A_B} \right) \left( \frac{\lambda_{ex1}}{\lambda_{exB}} \right) \left( \frac{\eta_B}{\eta_1} \right)^2$$

Here, $\Phi$ represents quantum yield; $F$ stands for integrated area under the corrected emission spectrum; $A$ is absorbance at the excitation wavelength; $\lambda_{ex}$ is the excitation wavelength; $\eta$ is the refractive index of the solution; and the subscripts 1 and B refer to the unknown and the standard, respectively.

Colocalization experiment in HeLa cells

Group 1: HeLa cells were pre-treated with the probe (0.2 μM) for 30 min and exposed to Rapamycin (2 μM) for another 30 min, then cells were treated with Lyso-Tracker Green (200 nM) for another 30 min.

Group 2: HeLa cells were pre-treated with the probe (0.2 μM) for 30 min and exposed to Nystatin (2 μM) for another 30 min, then cells were treated with Mito-Tracker Green (200 nM) for another 30 min.
**Table S1** Fluorescent probes for viscosity reported in the literatures.

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Fig. S1 Absorption and fluorescence spectra of Lyso-cy. a) Absorption spectra of Lyso-cy (10 μM) and b) fluorescence spectra of Lyso-cy (5 μM) in 95% glycerol and water, respectively. c) Absorption spectra of Lyso-cy (10 μM) and d) fluorescence spectra of Lyso-cy (5 μM) in solvents with different polarity. e) fluorescence spectra of Lyso-cy (5 μM) in PBS buffer and water. λ<sub>ex</sub> = 600 nm, slit: 10 nm.
**Fig. S2** a) The pH-dependence of absorption spectra of *Lyso-cy* (10 μM). b) pH-dependence of fluorescence spectra of *Lyso-cy* (5 μM). c) Curve of absorbance at 647 nm of the probe versus increasing pH from 1.3 to 7.4. The pKa was deduced to be 3.59 (with correlation coefficient $R^2 = 0.992$). d) Curve of absorbance at 720 nm of the probe versus increasing pH from 8.3 to 12.3. The pKa was deduced to be 10.39 (with correlation coefficient $R^2 = 0.999$). $\lambda_{ex} = 600$ nm, slit: 10 nm.

**Fig. S3** The pH-dependence of the fluorescence intensity of *Lyso-cy* (5 μM) with 95% glycerol. $\lambda_{ex} = 600$ nm, slit: 10 nm.
Fig. S4 Fluorescence intensity changes \([\frac{(F_i - F_{\text{Probe}})}{(F_{95\%\text{Gly}} - F_{\text{probe}})}]\) of the probe Lyso-cy (5 μM) at 710 nm in the presence of other relevant species (10 equiv.) in water. 1: Lyso-cy in 95% glycerol, 2: Lyso-cy + NaNO₂, 3: Lyso-cy + AgNO₃, 4: Lyso-cy + MgCl₂, 5: Lyso-cy + CaCl₂, 6: Lyso-cy + Fe(NO₃)₂, 7: Lyso-cy + Ni(NO₃)₂, 8: Lyso-cy + Hg(NO₃)₂, 9: Lyso-cy + Zn(NO₃)₂, 10: Lyso-cy + Cu(NO₃)₂, 11: Lyso-cy + GSH, 12: Lyso-cy + Hcy, 13: Lyso-cy + Cys, 14: Lyso-cy + ClO⁻, 15: Lyso-cy + ONOO⁻, 16: Lyso-cy + H₂O₂ and 17: Lyso-cy + TBHP; λₑₓ = 600 nm; slit: 10 nm.

Fig. S5 Cytotoxicity of Lyso-cy in HeLa cells. The cells were incubated with Lyso-cy at corresponding concentrations (0 μM, 0.5 μM, 1 μM, 3 μM, 5 μM, 10 μM) for 24 h.
**Fig. S6** Rapamycin stimulated fluorescence increase of the probe **Lyso-cy**. A) Fluorescence imaging of **Lyso-cy** in HeLa cells. The cells were treated with **Lyso-cy** (1 μM) for 30 min, washed with PBS, and then incubated with different concentrations of Rapamycin (0-10 μM) for 20 min, respectively. Concentrations of Rapamycin, a): 0 μM, b): 0.5 μM, c): 1.0 μM, d): 3.0 μM, d): 5.0 μM, f): 10.0 μM. Red channel: λ<sub>ex</sub> = 635 nm, λ<sub>em</sub> = 670-770 nm. Scale bar: 80 μm. B) Relative fluorescence intensities of HeLa in panels (a)-(f).

**Fig. S7** HRMS (LC/MS) spectra of **Lyso-cy**. The peak at m/z = 540.1467 was assigned to the mass of [**Lyso-cy - BF<sub>4</sub>**]⁺.
Fig. S8 $^1$H NMR of Lyso-cy (400 MHz, DMSO-$d_6$).

Fig. S9 $^{13}$C NMR of Lyso-cy (100 MHz, DMSO-$d_6$).
Reference