Electronic Supplementary Information

A fluorescent probe for specific lysosomes imaging in cells

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All chemicals and solvents were purchased from commercial suppliers and used without further purification. Distilled water was used after passing through a water ultrapurification system. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Varian Mercury 400 spectrometer. HR–MS data were obtained with a LC–MS spectrometer. UV–Vis and fluorescence spectra were recorded on a UV–Vis spectrophotometer and a fluorescence spectrophotometer with a temperature controller, respectively. Standard quartz cuvettes with a 1.0 cm lightpath were used for all optical spectra measurements.

Preparation of solutions of Lyso–NA and analytes. Stock solution of Lyso–NA (1.0 mM) was prepared in HPLC grade DMSO. Analytes cysteine (Cys), homocysteine (Hcy), glutathione (GSH), Na$^+$, K$^+$, Ca$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Ba$^{2+}$, NH$_4^+$, S$^{2-}$, F$^-$, Br$^-$, I$^-$, NO$_3^-$, PO$_4^{3-}$, ClO$_4^-$, SO$_3^{2-}$, HSO$_3^-$, S$_2$O$_3^{2-}$, S$_2$O$_8^{2-}$, CH$_3$COO$^-$ were dissolved in distilled water (10 mL) to afford 10 mM aqueous solution.

Measurements of fluorescence intensity of $I_{540}$ of Lyso–NA upon addition of various analytes. A solution of Lyso–NA (10 μM) was prepared in CH$_3$CN–PBS solution (1:1, v/v, 10 mM PBS buffer). Then 3.0 mL of Lyso–NA solution was placed in a quartz cell until the temperature reached at 37 °C over five minutes. The fluorescence spectra were then recorded upon addition of various analytes.

Photodegradation experiments. The photodegradation test was carried out in square cross-section quartz cells (1 × 1 cm) and solutions of the samples were irradiated with a 1000 W iodine-tungsten lamp at room temperature. To eliminate the heat and absorb short wavelength light, a cold trap (3 L solution of 50 g/L NaNO$_2$ in
10 cm (width) × 30 cm (length) ×20 cm (height) was set up between the cells and the lamp. The distance between the cells and the lamp was 25 cm. The irreversible bleaching of the dyes at the absorption peak was monitored as a function of time. Samples were tightly sealed, but not deoxygenated with nitrogen, before the test.

**Culture of cells and fluorescence imaging.** MCF–7 and HeLa cells were cultured in DEME medium supplemented with 10% fetal bovine serum at 37 ºC in an atmosphere containing 5% CO₂. For subcellular localization analysis of Lyso–NA staining, commercial fluorescent dyes were used. MCF–7 or HeLa cells were first stained with 2.0 μM of Lyso–NA at 37 ºC in an atmosphere of 5% CO₂ for 15 min, and then washed with PBS three times. Cells were then incubated with Lyso–Tracker Red (50 nM) at 37 ºC in an atmosphere of 5% CO₂ for 15 minutes, and then washed with PBS three times. After replacement of medium, cells were imaged using OLYMPUS FV100 confocal fluorescence microscope with a 200×objective lens.

**The synthesis of Lyso–NA.**

![Synthetic procedure for Lyso–NA](image)

**The synthesis of compound 2.** 4-bromo-1, 8-naphthalic anhydride (1) (1 g, 3.6 mmol) and 3-
Amino-1-propanol (0.28 g, 3.6 mmol) were dissolved in 20 mL acetic acid, and the solution was refluxed for 8 hours. After cooling to room temperature, the yellowish sediments were collected by filtration and then dried overnight at room temperature in a vacuum oven to give 2 (1.09 g, yield: 85.2%).

**The synthesis of compound 3.** A mixture of compound 2 (0.8 g, 2.4 mmol) and morpholine (0.32 g, 3.6 mmol) in 15 mL MeOCH₂CH₂OH was refluxed for 12 h. The precipitate was filtered and then dried overnight at room temperature in a vacuum oven to give 3. Compound 3 was obtained as yellow needles (0.82 g, yield: 81.6%).

**The synthesis of Lyso–NA.** Compound 3 (0.5 g, 1.5 mmol) and excess dess-martin periodinane were dissolved in 20 mL CH₃CN. The mixture was stirred in room temperature for 24 h. Then a solution of NaHCO₃ and Na₂S₂O₃ was added, the precipitate was filtered and then dried overnight at room temperature in a vacuum oven to give Lyso–NA as a yellow power (0.23 g, yield: 46.3%).

¹H NMR (400 MHz, DMSO) δ 9.74 (s, 1H), 8.45 (t, J = 8.3 Hz, 3H), 8.37 (d, J = 7.8 Hz, 1H), 7.79 (t, J = 7.7 Hz, 1H), 7.32 (d, J = 7.9 Hz, 1H), 4.30 (t, J = 6.7 Hz, 2H), 3.91 (s, 4H), 3.21 (s, 4H), 2.78 (t, J = 6.5 Hz, 2H).

¹³C NMR (101 MHz, DMSO) δ 202.30, 164.01, 163.47, 155.99, 132.67, 131.15, 131.09, 129.56, 126.53, 125.67, 122.96, 116.15, 115.49, 66.65, 53.49, 42.41, 34.26.

HRMS (ESI, m/z): calculated for C₁₉H₁₈N₂O₄, 338.1267, found 339.1340 (M + 1)

![Fig. S2 Synthetic procedure for NA.](image_url)

**The synthesis of compound b.** 4-bromo-1, 8-naphthalic anhydride (a) (1 g, 3.6 mmol) and Propylamine (2.13 g, 36 mmol) were dissolved in 20 mL EtOH, and the solution was refluxed for
8 hours. After cooling to room temperature, the yellowish sediments were collected by filtration and dried to give \( \text{b} \) (0.95 g, yield: 83.1%).

**The synthesis of NA.** A mixture of compound \( \text{b} \) (0.76 g, 2.4 mmol) and morpholine (2.09 g, 24 mmol) in 15 mL \( \text{MeOCH}_2\text{CH}_2\text{OH} \) was refluxed for 12 h. The precipitate was filtered and dried to give \( \text{NA} \). Compound \( \text{NA} \) was obtained as yellow needles (0.58 g, yield: 74.4%). \( ^1\text{H} \) NMR (400 MHz, DMSO) \( \delta \) 8.54 – 8.45 (m, 2H), 8.42 (d, \( J = 8.1 \) Hz, 1H), 7.86 – 7.78 (m, 1H), 7.36 (d, \( J = 8.1 \) Hz, 1H), 4.05 – 3.95 (m, 2H), 3.95 – 3.87 (m, 4H), 3.27 – 3.18 (m, 4H), 1.71 – 1.58 (m, 2H), 0.91 (t, \( J = 7.4 \) Hz, 3H).
Fig. S3  (a) Normalized UV–vis spectra of Lyso–NA (10 μM) in various solvents; (b) Normalized fluorescence spectra of Lyso–NA (10 μM) in various solvents. $\lambda_{ex} = 400$ nm, slit: 10, 10 nm.

Table S1. Spectral properties of Lyso–NA in various solvents.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>$\lambda_{abs}^{a}$</th>
<th>$\lambda_{em}^{b}$</th>
<th>$\Delta\lambda^{c}$</th>
<th>$\epsilon^{d}$</th>
<th>$\Phi_f^{e}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>396</td>
<td>544</td>
<td>148</td>
<td>9900</td>
<td>0.017</td>
</tr>
<tr>
<td>CH$_3$OH</td>
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<td>535</td>
<td>141</td>
<td>12400</td>
<td>0.031</td>
</tr>
<tr>
<td>CH$_3$CH$_2$OH</td>
<td>394</td>
<td>534</td>
<td>140</td>
<td>11000</td>
<td>0.072</td>
</tr>
<tr>
<td>CH$_3$CN</td>
<td>393</td>
<td>527</td>
<td>134</td>
<td>10200</td>
<td>0.250</td>
</tr>
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<td>DMSO</td>
<td>400</td>
<td>535</td>
<td>135</td>
<td>11000</td>
<td>0.036</td>
</tr>
</tbody>
</table>

$^{a}$ The two absorption peaks of Lyso–NA (nm). $^{b}$ The two emission peaks of Lyso–NA (nm), excited at 400 nm. $^{c}$ Stokes shift of Lyso–NA (nm). $^{d}$ × mol$^{-1}$ cm$^{-1}$ L. $^{e}$ Fluorescence quantum yields of Lyso–NA.
Fig. S4 Changes in fluorescence spectra of **Lyso-NA** in aqueous solution vs different pH values. 
$\lambda_{ex}$: 400 nm, slit: 10, 10 nm.

Fig. S5 MCF-7 cells are costained with (a) 2 µM **Lyso−NA** ($\lambda_{ex}$ =400 nm, $\lambda_{em}$ =490–540 nm) and (b) 50 nM Lyso−Tracker Red ($\lambda_{ex}$ =400 nm, $\lambda_{em}$ =570–600 nm) in PBS. (c) the brightfield image. (d) Overlay of (a), (b).

Fig. S6 The solubility test of **Lyso-NA** (a) and **NA** (b) in H$_2$O.
$^1$H–NMR spectra of Lyso–NA in DMSO.

$^{13}$C–NMR spectra of Lyso–NA in DMSO.

ESI–MS spectra of Lyso–NA in DMSO.
$^1$H NMR spectra of NA in DMSO.