Supporting Information

For

A lysosome-targetable fluorescent probe for imaging hydrogen sulfide in living cells

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Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. 1H-NMR and 13C-NMR spectra were recorded on a VARIAN INOVA-400 spectrometer, using TMS as an internal standard. Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer. UV-visible spectra were collected on a Perkin Elmer Lambda 35 UV/VIS spectrophotometer. Fluorescence measurements were performed on a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018).

Synthesis
N-(Morpholinoethylamino)-4-Bromo-1,8-Naphthalimide (4).

4-bromo-1,8-naphthalic anhydride (2) (5 g, 0.018 mol) and 4-(2-aminoethyl)-morpholine (3) (5 g, 0.036 mol) were dissolved in 100 mL ethanol, 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 4 (6.2 g, yield: 89.9%). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.65 (d, $J = 8.0$ Hz, 1H), 8.58 (d, $J = 8.0$ Hz, 1H), 8.11 (d, $J = 8.0$ Hz, 1H), 8.05 (d, $J = 8.0$ Hz, 1H), 7.86 (t, $J = 8.0$ Hz, 1H), 4.34 (t, $J = 7.2$ Hz, 2H), 3.68 (br, 4H), 2.72 (br, 2H), 2.60 (br, 4H).

N-(Morpholinoethylamino)-4-methoxy-1,8-naphthalimide (5).

A mixture of compound 4 (2.5 g, 6.4 mmol) and K$_2$CO$_3$ (3.5 g, 25.3 mmol) in 30 mL CH$_3$OH was refluxed for 24 h. The precipitate was filtered and washed with water (30 mL × 3). Compound 5 was obtained as yellow needles (1.6 g, yield: 76%). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.61–8.55 (m, 3H), 7.71 (t, $J = 8.0$, 1H), 7.06 (d, $J = 8.0$ Hz, 1H), 4.35 (t, $J = 6.6$ Hz, 2H), 3.71 (br, 4H), 2.73 (br, 2H), 2.63 (br, 4H).

N-(Morpholinoethylamino)-4-hydroxy-1,8-Naphthalimide (1).

A mixture of compound 5 (1 g, 3 mmol) and 50 mL concentrated HI (57%) was refluxed for 6 h. After cooling and adjusting pH to neutral, the precipitate was filtered to give compound 1 as yellow needles (0.82 g, yield: 86.3%). $^1$H NMR (400 MHz, DMSO) δ 12.03 (s, 1H), 8.60 (d, $J = 8.0$, 1H), 8.54 (d, $J = 8.0$, 1H), 8.42 (d, $J = 8.0$ Hz, 1H), 7.82 (t, $J = 8.0$ Hz, 1H), 7.21 (d, $J = 8.0$ Hz, 1H), 4.40 (t, $J = 5.8$ Hz, 2H), 4.03 (m, 2H), 3.52–3.75 (m, 6H), 3.20 (m, 2H).

Lyso-NHS

Compound 1 (1 g, 3.07 mmol), 1-bromine-2,4- dinitrobenzene (1.5 g, 6.14 mmol) and K$_2$CO$_3$ (0.848 g, 6.14 mmol) were dissolved in anhydrous DMF (10 mL). The reaction mixture was then heated at 90˚C for 4 hours under N$_2$ atmosphere. Cooling to room temperature, the reaction mixture was poured into ice water (100 mL). The crude product was extracted with ethyl acetate (3 x 25 mL) and dried over MgSO$_4$, and purified by flash column chromatography (ethyl acetate/CH$_2$Cl$_2$ = 1/1) to obtain the compound Lyso-NHS as a white solid (0.8 g, yield: 53%). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.98 (s, 1H), 8.70 (s, 1H), 8.57 (d, $J = 8.0$ Hz, 1H), 8.45–8.51 (m, 2H), 7.86 (t, $J = 8.0$ Hz, 1H), 7.22 – 7.28 (m, 2H), 4.36 (t, $J = 7.2$ Hz, 2H), 3.69 (br, 4H), 2.73 (br, 2H), 2.62 (br, 4H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 163.82, 163.19, 155.51, 153.83, 143.33, 140.73, 132.52, 131.97, 129.90, 129.32, 127.96, 127.77, 124.18, 123.03, 122.47, 121.32, 120.15, 114.23, 66.99, 58.44, 56.14, 53.81, 37.28, 18.45. HRMS (ESI) calcd for C$_{24}$H$_{21}$N$_4$O$_8$ [MH$^+$] 493.1359, found 493.1357.

The linear range and detection limit

The physiological relevant H$_2$S concentration is estimated ranging from nano- to millimolar levels. $^1$ The limit of detection of Lyso-NHS for H$_2$S is 0.48µM, which falls well within this range. The detection limit was calculated based on the method reported in the previous literature.$^2$ The
fluorescence emission spectrum of Lyso-NHS was measured by three times and the standard deviation of blank measurement was achieved. The fluorescence intensity at 555 nm was plotted as a concentration of H2S. The detection limit was calculated by using detection limit 3σ/k: Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus NO concentration.

**Culture of MCF-7 cells and fluorescent imaging**

MCF-7(human breast carcinoma) was cultured in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO2 and 95% air at 37 °C. The cells were seeded in 24-well flat-bottomed plates and then incubated for 24 h at 37 °C under 5% CO2. Lyso-NHS (5 µM) was then added to the cells and incubation for another 30 min followed. Neutral Red (NR) (5 µM) was used to co-stain the cells. The cells were washed three times with phosphate-buffered saline (PBS). Fluorescence imaging was observed under a confocal microscopy (Olympus FV1000) with a 60×objective lens.

**Reference**


Figure S1. MS spectra of 10 µM compound Lyso-NHS in the presence of 10 equiv of H2S in aqueous solution (CH3CN:50 mM HEPES = 1:9, pH = 7.4). MS: calcd for C18H17N2O4 [M-H]− 325.1, found 325.1.
Figure S2. HPLC chromatogram in the reaction of **Lyso-NHS** (5 µmol) with NaHS (5 equiv.) in PBS (pH 7.4) at 37°C.
Figure S3. Influence of pH on the fluorescence of Lyso-NHS with the addition of H$_2$S in aqueous solution (10% CH$_3$CN). Excitation wavelength is 450 nm. [Lyso-NHS] = 10 µM.

Figure S4. Fluorescence intensity of Lyso-NHS versus increasing concentrations of H$_2$S. Excitation wavelength is 450 nm. [Lyso-NHS] = 10 µM.
**Figure S5.** Colour change of the solution of Lyso-NHS with the addition of H$_2$S.

**Figure S6** (a) Fluorescence intensity of the probe Lyso-NHS (10 µM) incubated with 100 µM NaHS after 0, 1, 2, 3, 4, 5, 6, 8, 11, 15, & 20 min in bovine serum at 25°C. (b) Lyso-NHS probe (10 µM) incubated with 0, 40, 60, 80, 100, 130, 150 µM NaHS after 25 min in bovine serum at 25°C. The data represents the average of three independent experiments.
Figure S7. Fluorescence responses of 10 µM Lyso-NHS in bovine serum to various analytes in aqueous solution (CH$_3$CN:PBS = 1:9, pH = 7.4, 37 °C). Excitation at 450 nm. Bars represent the final fluorescence intensity of Lyso-NHS with 1 mM analytes over the original emission of free Lyso-NHS. 1) free Lyso-NHS; 2) Zn$^{2+}$; 3) Na$^+$; 4) Ca$^{2+}$; 5) K$^+$; 6) Mg$^{2+}$; 7) HCO$_3^-$; 8) F$^-$; 9) Cl$^-$; 10) Br$^-$; 11) I$^-$; 12) NO$_3^-$; 13) S$_2$O$_3^{2-}$; 14) S$_2$O$_4^{2-}$; 15) S$_2$O$_5^{2-}$; 16) SO$_3^{2-}$; 17) N$_3^-$ 18) CO$_3^{2-}$; 19) CH$_3$COO$^-$; 20) SO$_4^{2-}$; 21) H$_2$O$_2$; 22) HSO$_4^-$; 23) homocysteine; 24) ascorbic acid; 25) cysteine; 26) glutathione; 27) NaHS.

Figure S8. Cell viability of Lyso-NHS (5.0 µM) at different times in MCF-7 cell.
Fig S9. $^1$H-NMR spectra of compound 4 in CDCl$_3$.

Fig S10. $^1$H-NMR spectra of compound 5 in CDCl$_3$. 
Fig S11. $^1$H-NMR spectra of compound 1 in DMSO-$d_6$.

Fig S12. $^1$H-NMR spectra of compound Lyso-NHS in CDCl$_3$. 
Fig S13. $^{13}$C-NMR spectra of compound Lyso-NHS in CDCl$_3$. 

Lyso-NHS