Electronic Supplementary Information

A naphthalimide-rhodamine based fluorescent logic gate for detecting H₂S and NO in aqueous media and inside live cells

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Experimental

Reagents and Materials: 4-Bromo-1,8-naphthalic anhydride, p-phenylenediamine, thiocarbonyl chloride, 3-(dimethylamino)phenol, phthalic anhydride were purchased from Sigma Aldrich and used as received. 3-(1-piperazinyl) phenol and o-phenylenediamine were purchased from Alfa Aesar. N,N-Dimethyl-formamide (DMF) was dried with CaH₂ and vacuum distilled. 1,2-dichloroethane and acetonitrile were analytically pure solvents and distilled before use. The water used throughout the experiments was the double-distilled water which was further treated by ion exchange columns and then by a Milli-Q water purification system. Sodium hydrogen sulfide (NaHS), DEA/NONate (diethylamine NONate), tert-butylhydroperoxide (TBHP) and KO₂ were obtained from commercial sources and used without additional purification.

Synthesis of 1: A solution of 4-bromo-1,8-naphthalic anhydride (277 mg, 1 mmol) and p-phenylenediamine (216 mg, 2 mmol) in ethanol (10 mL) was heated under reflux for 24 h. After left to cool, the precipitated crystals were filtered and washed with ethanol to give 1 as gray solid (294 mg, 80%). ¹H NMR (DMSO-d₆, 400 MHz, ppm): 8.54-8.56 (d, 2H), 8.30-8.32 (d, 1H), 8.20-8.22 (d, 1H), 7.98-8.02 (t, 1H), 6.95-6.97 (d, 2H), 6.64-6.67 (d, 2H), 5.26-5.30 (m, 2H).

Synthesis of 2: A solution of 1 (440 mg, 1.2 mmol) in CH₂Cl₂ (20 mL) was added slowly to a solution of thiocarbonyl chloride (115 μL, 1.5 mmol) in CH₂Cl₂ (15 mL) and NEt₃ (0.21 mL), and the mixture was stirred for 5 h at room temperature. Then the reaction mixture was washed with water, dried over Na₂SO₄, and evaporated under reduced pressure to give gray solid (450 mg, 90%). This crude product was used for next step without further purification.

Synthesis of 4: First, compound 3 was synthesized according to the reported method. A solution of compound 1 (0.359 g, 1 mmol) in dry 1,2-dichloroethane (10 mL) was added to a solution of thiocarbonyl chloride (115 μL, 1.5 mmol) in CH₂Cl₂ (15 mL) and NEt₃ (0.21 mL), and the mixture was stirred for 5 h at room temperature. Then the reaction mixture was washed with water, dried over Na₂SO₄, and evaporated under reduced pressure to give gray solid (450 mg, 90%). This crude product was used for next step without further purification.

Synthesis of 5: A solution of 2 (550 mg, 1.34 mmol) and 4 (612 mg, 1.34 mmol) in DCM was stirred for 10 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was purified on flash gel using CH₂Cl₂/MeOH (10:1,v/v) as eluent to give purple solid (600 mg, 50%). ¹H NMR (CDCl₃, 400 MHz, ppm): 8.52-8.57 (t, 2H), 8.28-8.32 (d, 1H), 8.06-8.10 (d, 1H), 7.96-8.00 (d, 1H), 7.77-7.81 (d, 1H), 7.55-7.64 (m, 4H), 7.17-7.20 (d, 2H), 7.13-7.16 (d, 1H), 6.68-6.74 (t, 2H), 6.64-6.67 (s, 1H), 6.54-6.58 (d, 1H), 6.49-6.51 (s, 1H), 6.42-6.46 (d, 1H), 4.00-4.30 (d, 4H), 3.30-3.45 (t, 8H), 1.10-1.20 (t, 6H). MS(ESI): m/z 866.59 [M+H]⁺.

Synthesis of 6: A solution of 5 (60 mg, 0.07 mmol) in dry 1,2-dichloroethane (10 mL)
was stirred until the solid dissolved completely, and phosphorus oxychloride (1 mL) was added with vigorous stirring at room temperature for 5 min. Then the solution was refluxed for 4 h. The reaction mixture was cooled and evaporated in vacuo to give acid chloride which was used without further purification. The crude acid chloride was dissolved in acetonitrile (5 mL) and added dropwise to a solution of o-phenylenediamine (15 mg, 0.14 mmol) in CH₃CN (5 mL) and NEt₃ (1 mL). Column chromatography produced 26 mg of 6 (yield 40%). ¹H NMR (CDCl₃, 400 MHz, ppm): 8.65-8.67 (d, 1H), 8.60-8.63 (d, 1H), 8.40-8.43 (d, 1H), 8.04-8.06 (d, 1H), 7.99-8.02 (t, 1H), 7.82-7.88 (q, 2H), 7.54-7.58 (m, 2H), 7.36-7.39 (d, 2H), 7.23-7.25 (d, 2H), 7.19-7.22 (m, 1H), 6.90-6.94 (t, 1H), 6.71-6.74 (d, 1H), 6.62-6.66 (d, 1H), 6.47-6.51 (d, 2H), 6.43-6.45 (m, 1H), 6.37-6.41 (t, 1H), 6.25-6.28 (m, 1H), 6.04-6.07 (d, 1H), 3.96-4.00 (t, 4H), 3.26-3.35 (m, 8H), 1.12-1.17 (t, 6H). MS(ESI): m/z 954.27 [M+H]^+.

Synthesis of Naph-RhB: The mixture of Compound 6 (40 mg, 0.04 mmol) and sodium azide (27 mg, 0.4 mmol) in 2 mL of dry N,N-dimethylformide (DMF) was heated to 110 °C for 4 h. After cooling to room temperature, the reaction mixture was poured into 40 mL of water and then extracted with DCM. The organic phase was collected, washed with brine, and dried with anhydrous Na₂SO₄. The solvent in the filtrate was removed under reduced pressure and the solid residue was purified by flash chromatography column using methanol/dichloromethane (v/v 1:5) to afford a light yellow solid (35 mg, 90%). ¹H NMR (DMSO-d₆, 400 MHz, ppm): 8.64-8.72 (m, 2H), 8.59-8.60 (d, 1H), 8.58-8.59 (d, 1H), 8.54-8.55 (s, 1H), 8.53-8.54 (d, 1H), 8.51-8.53 (d, 1H), 8.10-8.13 (dd, 3H), 7.94-7.96 (d, 1H), 7.91-7.94 (d, 1H), 7.88-7.91 (m, 1H), 7.83-7.84 (s, 1H), 7.82-7.83 (s, 1H), 7.58-7.61 (dd, 4H), 7.08-7.11 (m, 1H), 6.82-6.86 (m, 1H), 6.60-6.64 (m, 1H), 6.53-6.56 (dd, 1H), 6.19-6.22 (m, 1H), 6.15-6.19 (t, 1H), 3.19-3.23 (m, 4H), 1.23-1.28 (m, 8H), 1.05-1.09 (t, 6H). MS(ESI): m/z 917.35 [M+H]^+.

General Procedure for H₂S and NO Detection. Double-distilled water and spectroscopic grade CH₃CN were used for spectroscopic studies. A stock solution of NaHS was prepared in double-distilled water; and a stock solution of DEA/NONOate was prepared in 0.01M NaOH solution. Unless otherwise stated, all the fluorescence measurements were performed in 10 mM PBS buffered (pH 7.4) water solution (containing 20% CH₃CN as a cosolvent), according to the following procedure. In a 5 mL cuvette, 2 mL of PBS, 570 μL of CH₃CN and 30 μL of 1 mM Naph-RhB (final concentration, 10 μM) were mixed, followed by addition of an appropriate volume of stock solution. The final volume of liquid in the cuvette was adjusted to 3.0 mL with PBS. After incubation at 37 °C for a certain period of time in a thermostat, the solution was transferred to a quartz cell of 1 cm optical length to measure the absorbance or fluorescence. Also, a solution containing neither NaHS (H₂S donor) nor DEA/NONOate (NO donor) was prepared as the control and its spectra were measured under the same conditions. For the detection of two analytes, three different analyte addition methods were adopted: (a) incubating the probe with NaHS for 35 min followed by addition of DEA/NONOate; (b) incubating the probe with DEA/NONOate for 30 min followed by addition of NaHS; (c) addition of both NaHS and DEA/NONOate at the same time.
Selectivity evaluation of the probe: Superoxide (O$_2^•$) was added as solid KO$_2$. Hydroxyl radicals (OH•) and tert-butoxy radicals (•OtBu) were generated by reaction of Fe$^{2+}$ with H$_2$O$_2$ or TBHP, respectively. Singlet oxygen (¹O$_2$) was generated from ClO• and H$_2$O$_2$. Various analytes (20 μM H$_2$S, 20 μM NO, 100 μM for ROS and RNS, 5.0 mM for GSH, 1.0 mM for others) include K$^+$, Ca$^{2+}$, Na$^+$, Fe$^{3+}$, Zn$^{2+}$, GSH, Cys, H$_2$O$_2$, O$_2^•$, •OtBu, OH•, ascorbic acid (AA), NO$^2•$, NO$^3•$, SCN•, HClO, ¹O$_2$. Unless otherwise stated, all the fluorescence measurements were performed in pH 7.4 PBS buffered water solution (containing 20% CH$_3$CN as a cosolvent), according to the following procedure. In a 5 mL tube, 2 mL of PBS, 570 μL of CH$_3$CN and 30 μL of 1 mM Naph-RhB (final concentration, 10 μM) were mixed, followed by addition an appropriate volume of analyte solution. The final volume of the solution was adjusted to 3.0 mL with PBS, and the solution was mixed rapidly. After incubation at 37 °C for 35 min in a thermostat, the solution was transferred into a quartz cell of 1 cm optical length to measure fluorescence.

Cell viability assay: To examine the toxicity of the probe in living cells, L929 cells (murine aneuploid fibrosarcoma cells) were incubated in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and allowed to grow for 24 h at 37 °C with 5% CO$_2$. After removal of the medium, cells were treated with the probe and incubated for an additional 24 h. The cytotoxicity of the probe against L929 cells was assessed by MTT assay according to ISO 10993-5.

Cell imaging: L929 cells (murine aneuploid fibrosarcoma cell) were incubated in RPMI1640 medium supplemented with 10% Fetal Bovine Serum (FBS, Invitrogen). One day before imaging, cells were passaged and plated on polylysine-coated cell culture glass slides inside 30-mm glass culture dishes and allowed to grow to 50-70% confluence. Afterwards, cells (on glass slides) were washed with RPMI1640 and re-incubated in RPMI1640 medium containing with the probe Naph-RhB (10 μM) for 1.0 h at 37 °C and then washed with phosphate-buffered saline (PBS) three times. After incubating with 100 μM NaHS (H$_2$S donor) or DEA/NONOate (NO donor) for another 1.0 h at 37 °C. After that, the culture dishes were washed with PBS and then imaged on Confocal Laser Scanning Microscopy (CLSM) images were collected using a Leica TCS-SP5 confocal microscope.

Measurements. ¹H NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. Mass spectra were obtained through a Bruker Esquire HCT Plus mass spectrometer. UV-vis spectra were recorded on a Hitachi U-3010 UV-vis spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer. Confocal laser scanning microscopy (CLSM) images were collected using a Zeiss LSM710 confocal microscope.

Reference:

Scheme S1. Synthetic route of the probe (Naph-RhB).
Figure S1. $^1$H-NMR spectrum (in DMSO-$d_6$) of 1.
**Figure S2.** $^1$H-NMR spectrum (in CDCl$_3$) of 4.

**Figure S3.** Mass spectrum of 4. MS(ESI): m/z 456.56 [M+H]$^+$. 
**Figure S4.** $^1$H-NMR spectrum (in CDCl$_3$) of 5.

**Figure S5.** Mass spectrum of 5. MS(ESI): m/z 866.59 [M+H]$^+$. 
Figure S6. $^1$H-NMR spectrum (in CDCl$_3$) of 6.

Figure S7. Mass spectrum of 6. MS(ESI): m/z 954.27 [M+H]$^+$. 
Figure S8. $^1$H-NMR spectrum (in DMSO-$d_6$) of Naph-RhB.

Figure S9. Mass spectrum of Naph-RhB. MS(ESI): m/z 917.35 [M+H]$^+$. 
**Figure S10.** Normalized absorption and emission spectra of naphthalimide-amine and rhodamine, the spectral overlap between the emission of the donor (the green solid) and the absorption of the acceptor (the red dash) can be observed.
Figure S11. Absorption and Emission spectra of Naph-RhB (10 μM) in pH 7.4 PBS buffered water solution (containing 20% CH$_3$CN as a cosolvent) before and after addition of 100 μM of NaHS (H2S donor) or DEA/NONOate (NO donor).
Figure S12. Fluorescence spectra for Naph-RhB (10 μM) in pH 7.4 PBS buffered water (containing 20% CH₃CN as a cosolvent) at different time periods upon addition of 100 μM NaHS when excited at 425 nm (A) or NO donor when excited at 550 nm (B). The insets display the plot of emission intensity vs. incubation time.
Figure S13. Emission spectra of Naph-RhB (10 μM) in the presence of different concentrations of NaHS when excited at 550 nm (A) or in the presence of NO donor when excited at 425 nm.
Figure S14. Fluorescence spectra of Naph-RhB (20 μM) in PBS buffered aqueous solution (pH 7.4, containing 20% CH₃CN as a cosolvent). The probe was pretreated with 20 μM of NaHS (H₂S donor) for 35 min and then incubated with 100 μM DEA/NOOate (NO donor) for different time periods. Excitation wavelength: 425 nm. The inset displays the plot of emission ratio ($I_{570}/I_{539}$) vs. incubation time.

Note: for this experiment, H₂S was added before NO, thus the addition of NO could induce the formation of “open-ring” structure of rhodamine and accept the excited state energy of naphthalimide moiety, thereby gradually reducing the fluorescent intensity at 539 nm. And this fluorescence variation behavior is different from that shown in Figure S15 for which H₂S was added after NO.
Figure S15. (A) Time-dependent fluorescence spectra (The probe was treated with NO donor for 30 min and then incubated with H₂S donor for different time periods); (B) concentration-dependent fluorescence spectra (The probe was treated with NO donor for 30 min and then incubated with H₂S donor for 35 min before fluorescence measurement); Naph-RhB (20 μM) in pH 7.4 PBS buffered water (containing 20% CH₃CN as a cosolvent).

Note: For this experiment, H₂S was added after addition of NO, the H₂S-induced fluorescence variation (at 539 nm) could not be observed since the excited energy of naphthalimide moiety (FRET donor) had been transferred to rhodamine moiety (FRET acceptor, emission at 570 nm).
Figure S16. (A) Time-dependent fluorescence spectra (H$_2$S donor and NO donor were added simultaneously, and the solution was incubated for different time periods); (B) concentration-dependent fluorescence spectra (upon simultaneous addition of H$_2$S donor and NO donor, the solution was incubated for 35 min); Naph-RhB (20 μM) in pH 7.4 PBS buffered water (containing 20% CH$_3$CN as a cosolvent).

Note: For this experiment, when the probe was simultaneously treated with NO and H$_2$S, the excited state energy of the FRET donor (naphthalimide moiety) could be efficiently transferred to the FRET acceptor (rhodamine moiety), hence the fluorescent intensity variation of the donor could not be observed.
**Figure S17.** Time-dependent fluorescence spectra for Naph-RhB (20 μM) in pH 7.4 PBS buffered water (containing 20% CH₃CN as a cosolvent). The NO donor and NaHS were added into probe’s solution simultaneously before subject to fluorescence measurement.

Note: The difference between this figure and Figure S16(A) is that the excitation wavelength for this one is 550 nm, which can only excite the rhodamine moiety (FRET acceptor) to generate a fluorescent band at 570 nm.
Figure S18. Cell viability for L929 cells in the presence of the probe at varied concentrations. The results are the mean standard deviation of eight separate measurements.