Materials and Methods: All solvents were reagent grade. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.062mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-13 NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported relative to chloroform (δ 7.26) for 1H NMR and chloroform (δ 77.0) for 13C NMR. Absorption spectra were recorded on a Lambda 20 UV/VIS spectrophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on Cary Eclipse fluorescence spectrophotometer.

Chemical Synthesis

Probe SSP2: to a solution of compound S1 (110.0 mg, 0.186 mmol) in THF/H2O (6.0 mL/3.0 mL) was added PPh3 (121.9 mg, 0.47 mmol) slowly at 0 °C. The mixture was allowed to warmed to r.t. and stirred for 0.5 h. THF was removed under reduced pressure and 10 mL of HCl (1N) was added to acidify the solution. Then the mixture was extracted with CH2Cl2 (20 mL). The organic layer was seperated and washed with brine. After dried by MgSO4, the solvent was removed under reduced pressure and the resulted residue was purified by fals column chromatography. SSP2 was obtained as a white solid (72.6 mg, 81 % yield). 1H NMR (300 MHz, CD3Cl) δ 3.89 (s, 3H), 4.62 (s, 1 H), 6.16-6.31 (m, 5 H), 7.40-7.71 (m, 5 H), 7.66 (m, 2 H), 8.03 (dd, J = 6.3, 0.9 Hz, 1 H), 8.24 (d, J = 7.8 Hz, 1 H) ; 13C NMR (75 MHz, CD3Cl) δ 169.6, 164.9, 161.7, 153.3, 152.5, 152.2, 152.0, 140.0, 135.5, 133.7, 132.5, 131.4, 130.1, 129.4, 129.3, 126.7, 125.7, 125.3, 124.7, 124.3, 117.8, 117.2, 112.2, 111.1, 110.8, 101.1, 82.7, 55.8; MS (ESI+) m/z 505.0 (M+Na+); IR 3063.
Probe **SSP1** was prepared using the same method as for **SSP2**. $^1$H NMR (300 MHz, CD$_3$Cl) δ 4.61 (s, 1H), 6.44 (d, $J = 9.6$ Hz, 1 H), 7.17-7.28 (m, 3 H), 7.41 (m, 2 H), 7.56 (d, $J = 8.4$ Hz, 1 H), 7.73 (d, $J = 9.6$ Hz, 1 H), 8.26 (d, $J = 8.4$, 1 H). $^{13}$C NMR (75 MHz, CD$_3$Cl) δ 164.4, 160.2, 154.6, 153.0, 142.8, 139.9, 133.5, 132.2, 128.6, 124.9, 124.2, 118.6, 116.8, 116.1, 110.6; MS (ESI$^+$) m/z 321.0 (M+Na$^+$); IR 3094, 2922, 2530, 1731, 1618, 1583, 1461, 1395. mp 141-142 °C.

Compound 2 was prepared using the same method as for **SSP2**. NMR data is the same as literature data.$^2$

**Preparation of Sulfane Sulfur Species**

Na$_2$S$_2$ was prepared using a known procedure.$^3$

**Polysulfides:**

![SSP1](image)

To a stirred solution of $N$-Ac-cysteine methyl ester (106 mg, 0.6 mmol) and triethylamine (60.6 mg, 0.6 mmol) in 10 mL of dry dichloromethane at -20 °C was added dropwise a solution of sulfur monochloride (40 mg, 0.3 mmol) in 2 mL of dichloromethane. The reaction was brought to room temperature after the addition was completed. Stirring was continued for about 1 h. The reaction was quenched by the addition of ice-cold water. Organic layer was separated and washed with water and brine. After concentration, the product was purified by silica gel chromatography. The polysulfide product was obtained in 72% yield as a 1:1 mixture of trisulfide and tetrasulfide. $^1$H NMR (300 MHz, CD$_3$Cl) δ 2.07 (s, 3 H), 2.08 (s, 3 H), 3.20 (d, $J = 4.8$ Hz, 2 H), 3.42 (d, $J = 6.8$ Hz, 2 H), 3.77 (s, 3 H), 3.79 (s, 3 H), 4.84-4.97 (m, 2 H), 6.82-7.02 (m, 2 H); $^{13}$C NMR (75 MHz, CD$_3$Cl) δ 170.8, 170.6, 170.1, 170.0, 52.7, 52.6, 51.6, 51.5, 41.1, 40.4, 22.92, 22.92; MS (ESI$^+$) m/z 439.0 (tetrasulfide$+$Na$^+$), 407.2 (trisulfide$+$Na$^+$).

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Polysulfide S3 was obtained using the same procedure as for S2 in 78% yield (trisulfide/tetrasulfide = 4/1), NMR data matches literature data.4

S8 was purchased from Alfa Aesar (Lot# G21X028).

Potassium tetrathionate was purchased from Sigma-Aldrich (Lot# SLBF3363V)

Model reactions of the probe with Sulfane Sulfur Species

To the solution of 2 (23.0 mg, 0.1 mmol) in CH3CN (2.5 mL) and PBS buffer (2.5 mL, 100 mM, pH 7.4) was added Na2S2 (55 mg, 0.5 mmol). The mixture was stirred for 1 hour at rt and then diluted with CH2Cl2. The organic layer was separated and dried by MgSO4, and concentrated. Purification by flash column chromatography afforded compound 1 as light yellow solid (15.2 mg, 91% yield).

To the solution of 2 (23.0 mg, 0.1 mmol) in CH3CN (2.0 mL), CCl4 (0.5 mL) and PBS buffer (2.5 mL, 100 mM, pH 7.4) was added elemental sulfur (16 mg, 0.5 mmol). The mixture was stirred for 1 hour at rt and then diluted with CH2Cl2. The organic layer was separated and dried by MgSO4, and concentrated. Purification by flash column chromatography afforded compound 1 as light yellow solid (14.8 mg, 88% yield).

To the solution of 2 (23.0 mg, 0.1 mmol) in CH3CN (2.5 mL), and PBS buffer (2.5 mL, 100 mM, pH 7.4) was added S3 (105 mg, 0.5 mmol). The mixture was stirred for 1 hour at rt and then diluted with CH2Cl2. The organic layer was separated and dried by MgSO4,


and concentrated. Purification by flash column chromatography afforded compound 1 as light yellow solid (14.5 mg, 86% yield).

**Quantum Yields**

The quantum yield was calculated according to the equation:\(^6\)

\[
\Phi_{\text{sample}} = \Phi_{\text{standard}} \times \left( \frac{I_{\text{sample}}}{I_{\text{standard}}} \right) \times \left( \frac{A_{\text{standard}}}{A_{\text{sample}}} \right) \times \left( \frac{n_{\text{sample}}}{n_{\text{standard}}} \right)^2
\]

\(\Phi\) denotes the quantum yield; \(I\) denotes the area under the fluorescence band; \(A\) denotes the absorbance at the excitation wavelength; \(n\) denotes the refractive index of the solvent.

For quantum yield of **SSP1**, it was determined using 7-hydroxycoumarin as a standard by comparing the area under the corrected emission spectrum of the test sample with that of a solution of 7-hydroxycoumarin excited at 330 nm in sodium phosphate buffer (0.1 M; pH 7.4), which has a quantum efficiency of 0.76 according to the literature.\(^7\)

For quantum yield of **SSP2**, quantum yield was determined using fluorescein as a standard by comparing the area under the corrected emission spectrum of the test sample with that of a solution of fluorescein excited at 490 nm in 0.1 N NaOH, which has a quantum efficiency of 0.85 according to the literature.\(^6\)

**Preparation of the solutions and fluorescence measurements**

The stock solution of **SSP1** (1 mM) and **SSP2** (1 mM) were prepared in CH\(_3\)CN, respectively. The solutions of various testing species were prepared from Cysteine (Cys), GSH, Homocysteine (Hcy), Glutathione disulfide (GSSG), Na\(_2\)S·9H\(_2\)O, Na\(_2\)S\(_2\)O\(_3\), Na\(_2\)SO\(_3\), Na\(_2\)SO\(_4\), Na\(_2\)S\(_2\) in 50 mM PBS buffer. The stock solution of Cetrimonium bromide (CTAB, 100 mM) and S\(_8\) (10 mM) were prepared in EtOH, respectively. The stock solution of Cys-polysulfide (10 mM) was prepared in CH\(_3\)CN. All the test solution need to be freshly prepared.

Unless otherwise noted, all the measurements were carried out for 10 min at room temperature in 50 mM PBS buffer (pH 7.4) with 1 mM CTAB according to the following procedure. In a test tube, 3.5 mL of 50 mM PBS buffer (pH 7.4) and 40 \(\mu\)L of the stock solution of CTAB were mixed, and then added 20 \(\mu\)L of the stock solution of **SSP1** or **SSP2**. The resulting solution was mixed well, followed by addition of a requisite volume of testing species sample solution. The final volume of the reaction solution was adjusted to 4 mL with 50 mM PBS buffer (pH 7.4). After mixing and then standing for 10 min at room temperature, a 4-mL portion of the reaction solution was transferred into a 1-cm quartz cell to measure fluorescence with \(\lambda_{ex} = 380\) nm (for **SSP1**) or 482 nm (**SSP2**). PMT detector voltage = 600V. In the meantime, a blank solution containing no testing species sample was prepared and measured under the same conditions for comparison. All the measurements were repeated three times and data reported were averages.

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Figure S1. Fluorescence intensity changes of (A) 5.0 μM SSP1 (PMT detector voltage = 400V) and (B) 5.0 μM SSP2 at different pH values in the absence (■) or presence (●) of Na₂S₂ (25 μM). The reactions were carried out for 10 min at room temperature in 50 mM PBS buffer with 1 mM CTAB. Data (A) were acquired at 458 nm and excited at 380 nm. Data (B) were acquired at 518 nm and excited at 482 nm.

Figure S2. The plot of fluorescence intensity change of (A) 5.0 μM SSP1 and (B) 5.0 μM SSP2 against varied concentration of Na₂S₂ from 0.5 to 25 μM. The reactions were carried out for 10 min at room temperature in 50 mM PBS buffer with 1 mM CTAB. Data (A) were acquired at 458 nm and excited at 380 nm. Data (B) were acquired at 518 nm and excited at 482 nm.
Figure S3. Fluorescence enhancement (F/F₀) of SSP2 (5.0 μM) to Na₂S₂ (25 μM) in the presence of RSS or aldehydes. (Ex/Em = 482/518 nm). The reactions were carried out for 10 min at room temperature in PBS buffer (50 mM, pH 7.4) with 1 mM CTAB. (1) 500 μM Cys; (2) 1 mM GSH; (3) 100 μM Hcy; (4) 100 μM GSSG; (5) 100 μM Na₂S; (6) 100 μM Na₂S₂O₃; (7) 100 μM Na₂SO₃; (8) 100 μM Na₂SO₄; (9) 50 μM formaldehyde; (10) 50 μM acetaldehyde; (11) only 25 μM Na₂S₂.

Cell culture and fluorescence imaging

H9c2 cells and HeLa cells were grown on glass-bottom culture dishes (Corning Inc.) in DMEM supplemented with 10% (v/v) FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C under a humidified atmosphere containing 5% CO₂. Before use, the adherent cells were washed one time with FBS-free DMEM. For intracellular H₂S₂ imaging, the cells were incubated with 50 μM SSP2 in FBS-free DMEM (containing 200 μM CTAB) at 37 °C for 20 min. After removal of excess probe and washed with PBS (pH 7.4), the cells were incubated with 50 or 100 μM Na₂S₂ for 30 min in PBS buffer (pH 7.4, containing 500 μM CTAB). Cell imaging was carried out after washing the cells three times with PBS (pH 7.4). All microscopy images were taken on an EVOS fl fluorescence microscope from Advanced Microscopy Group (AMG).
SSP1

$^1$H NMR (300 M, CD$_3$Cl)
$^{13}$C NMR (75 M, CD$_3$Cl)
$^1$H NMR (300 M, CD$_2$Cl)
$^{13}$C NMR (75 M, CD$_3$Cl)
$^1$H NMR (300 M, CD$_3$Cl)
$^{13}$C NMR (75 M, CD$_3$Cl)