Supporting Information

A Near-infrared Ratiometric Fluorescent Probe for Rapid and Highly Sensitive Imaging of Endogenous Hydrogen Sulfide in Living Cells

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1. General Experimental Section

Materials. The solution of the probe HS-Cy (acetonitrile, 1.0 mM) could be maintained in refrigerator at 4 °C. Unless stated otherwise, solvents were dried by distillation. All reagents were of commercial quality and used without further purification. 2-[4-Chloro-7-(1-ethyl-3,3-dimethyl(indolin-2-ylidene)]-3,5-(propane-1,3-diyl)-1,3,5-heptatrien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium (Cy.7.Cl) was synthesized in our laboratory.[1] Oxalyl chloride, 2-carboxybenzaldehyde, glutathione (GSH), L-cysteine (L-cys), ascorbic acid (Vc), *tert*-butylhydroperoxide (*t*-BuOOH, 70%) were obtained from Aladdin Chemical Company (Shanghai, China). Phorbol myristate acetate (PMA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), SIN-1 (3-morpholinosydnonimine, a model compound for the continuous release of $ONOO^-$), S-nitroso-N-acetyl-dl-penicillamine (SNP), H_2O_2, xanthine (1 mM) in 10 mM NaOH solution, and xanthine oxidase (5 UmL^{-1}) were purchased from Sigma Chemical Company. Mito-Tracker Green and Hoechst 33342 were obtained from Beyotime Institute of Biotechnology (Shanghai, China). The solvents were used after appropriate distillation or purification. HepG2 (Human hepatocellular liver carcinoma cell line) and A549 cells (Human lung carcinoma cell line) were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Sartorius ultrapure water (18.2 MΩ cm) water was used throughout.

$^1O_2$ was generated by the reaction of H_2O_2 with NaClO.[2] $O_2^{\cdot-}$ was created by the enzymatic reaction of xanthine/xanthine oxidase (XA/XO) at 25 °C for 5 min. ·OH was generated by Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\cdot}OH + OH^-$).[3] *t*-BuOOH could also be used to induce ROS in biological systems.[4] NaClO was used as the source of HClO. The $ONOO^-$ source was the donor 3-morpholinosydnonimine hydrochloride (SIN-1).[5] NO was generated from SNP. SNP was added into degassed deionized water under Ar atmosphere then stirred for 30 min at room temperature. The probe solution was also degassed before the reaction with NO.

Instruments. Fluorescence spectra were obtained by FLS-920 Edinburgh Fluorescence Spectrometer (Edinburgh Instruments Ltd, England) with a Xenon lamp and 1.0-cm quartz cells at the slits of 2.0/2.0 nm. Fluorescence kinetics were recorded on a Cary Eclipse spectrofluorometer (Varian, Australia) with a Xenon lamp and 1.0-cm quartz cells at the slits

of 5.0/5.0 nm. Absorption spectra were measured on a pharmaspect UV-1700 UV-visible spectrophotometer (Shimadzu, Japan). High-resolution mass spectral analyses were carried out on a Bruker maxis UHR-TOF Ultra High Resolution Quadrupole-time of flight mass spectrometer (Bruker Co., Ltd., Germany) and an Agilent 1100 LC/MSD Trap system (Agilent Co., Ltd., USA). ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Varian Advance 600-MHz spectrometer (Varian, USA), δ values are in *ppm* relative to TMS. The fluorescence images of cells were taken using a TCS SP5 confocal laser scanning microscopy (Leica Co., Ltd. Germany) with an objective lens (×20, ×40). All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode.

Fluorescence analysis. Fluorescence spectra were obtained with a Xenon lamp and 1.0-cm quartz cell. The probe HS-Cy (acetonitrile, 1.0 mM) was added to a 10.0-mL color comparison tube. After dilution to 5.0 μM with 20 mM HEPES buffer solution, various amounts of NaHS were added. The fluorescence intensity was measured at λex/em = 700 nm/780 nm and λex/em = 510 nm/625 nm, respectively.

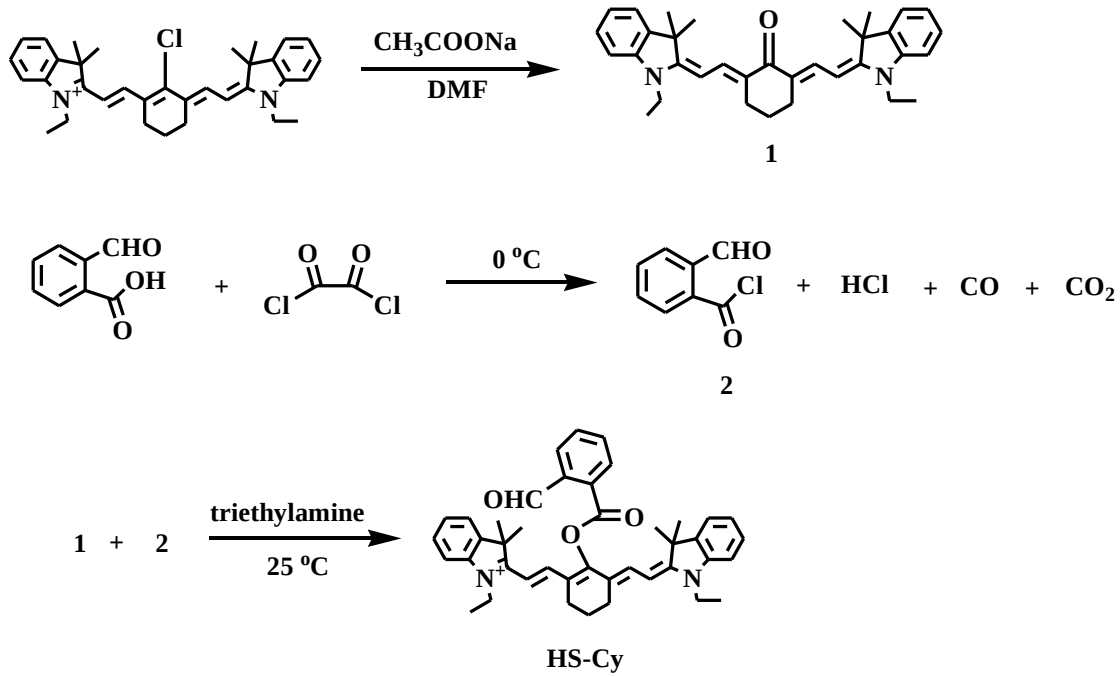
Absorption analysis. Absorption spectra were obtained with a 1.0-cm cuvette cell. The probe HS-Cy (acetonitrile, 1.0 mM) was added to a 10.0-mL color comparison tube. After dilution to 5.0 μM with 20 mM HEPES buffer solution, various amounts of NaHS were added. Then the absorption spectra were scanned and recorded.

Cell culture. HepG2 cells (Human hepatocellular liver carcinoma cell line) were maintained following protocols provided by the American Type Tissue Culture Collection. Cells were seeded at a density of 1×10^{6} cells mL^{-1} in high glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2 g/L) and 1% antibiotics (penicillin/streptomycin, 100 U/mL). A549 cells (Human lung carcinoma cell line) were seeded at a density of 1×10^{6} cells mL^{-1} in 1640 Medium supplemented with 10% FBS, $NaHCO_3$ (2 g/L) and 1% antibiotics (penicillin/streptomycin, 100 U/mL). Cultures were maintained in a humidified incubator at 37 °C, in 5% CO_2/95% air. Cells were maintained one day before imaging by being passed and plated on 18-mm glass coverslips in culture dish.

Confocal imaging. HepG2 cells were rinsed with HEPES (pH 7.4, 0.10 M), incubated with

DMEM containing 10% FBS, 5 μM HS-Cy, and 0.1% CH_3CN as a cosolvent for different times at 37 °C, then washed with HEPES three times, and mounted on the microscope stage. As to A549 cells, the procedures were the same except replacing DMEM with 1640 medium. Excitation of probe-loaded cells at 633 nm and 543 nm were carried out with a HeNe laser and the emission was collected between 640-800 nm and 550-600 nm, respectively. In the case of mitochondria staining and nucleus staining, 488 nm and 405 nm lasers were used and the emission was collected between 490-550 nm and 410-480 nm, respectively.

2. Synthesis and Characterization of Compounds



Scheme S1. Synthetic route for HS-Cy.

Synthesis of compound 1

A solution of Cy7.Cl (230 mg, 0.45 mmol) and sodium acetate (100 mg, 1.2 mmol) in anhydrous N,N-dimethylformamide (15 mL) was heated at 90 °C for 5h under Ar atmosphere. After the mixture cooled to room temperature, it was first filtered. Then the obtained solution was concentrated by rotary evaporation to obtain red oil product. Finally, **1** (ketone cyanine, 150 mg, 67 % yield) was isolated by silica chromatography eluted with CH_2Cl_2/CH_3OH (8:2, v/v). ^{1}HNMR (600 MHz, $CDCl_3$): δ = 1.240 (s, 6H), 1.659 (s, 12H),

1.781 (s, 2H), 2.586 (s, 4H), 3.770 (s, 4H), 5.479-5.499 (d, *J*=12.0 Hz, 2H), 6.700-6.712 (d, *J*=7.2 Hz, 2H), 6.920-6.944 (t, *J*=7.2 Hz, 2H), 7.189 (s, 4H), 8.213-8.234 (d, *J*=7.2 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 161.785, 143.716, 139.795, 132.803, 127.623, 126.478, 121.798, 120.402, 110.050, 110.027, 110.008, 109.996, 106.369, 92.095, 46.533, 37.016, 28.677, 25.836, 22.551, 11.123. ESI-MS: calculated for $[M+H]^+$ = 493.3, found 493.3.

Synthesis of HS-Cy

To a suspension of 2-carboxybenzaldehyde (750 mg, 5 mmol) in dichloromethane (50 mL) was added oxalyl chloride (0.95 mL, 10 mmol) and DMF (7.5 μL). The reaction mixture was stirred at room temperature for 4 h and then the volatiles were evaporated under reduced pressure and subsequently dried with vacuum pumping. The residue was dissolved in a small amount of dry dichloromethane. The solution was slowly added into the dispersed dichloromethane solution (50 mL) containing ketone cyanine (100 mg, 0.19 mmol) and triethylamine 0.278 mL (0.6 mmol) and kept stirring at 0 ^{o}C for 30 min. Then the mixture was warmed to room temperature and stirred overnight. The obtained solution was diluted with CH_2Cl_2 (30 mL) for extraction. The solvent was removed in vacuo to obtain a crude mixture of deep green solid. Finally, HS-Cy was isolated by silica chromatography eluting with CH_2Cl_2/CH_3OH, from 100/1 to 20/1 as a deep blue solid (40 mg, 38 % yield). ^{1}H-NMR (600 MHz, CD_3OD): δ= 0.936-0.961 (t, *J*=7.2, 7.8 Hz, 6H), 1.157-1.180 (t, *J*=6.6, 7.2 Hz, 1H), 1.249 (s, 4H), 1.391-1.416 (q, *J*=7.8 Hz, 5H), 1.631 (s, 1H), 1.666-1.712 (t, *J*=14.4, 13.2 Hz, 5H), 1.934 (s, 3H), 3.293 (s, 2H), 3.331 (s, 1H), 3.826-3.886 (m, 1H), 4.249-4.270 (t, *J*=6.0, 6.6 Hz, 4H), 4.762 (s, 8H), 7.614 (s, 2H), 7.691 (s, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ (ppm): 172.426 171.705 144.242 141.755 141.732 141.312 141.140 141.102 135.114 131.616 130.914 128.511 128.431 126.447 125.414 125.135 124.815 123.770 122.568 122.137 122.023 110.603 110.324 110.088 110.015 110.000 109.988 109.969 100.487 99.476 65.215 49.229 48.958 48.134 38.873 38.679 30.283 29.322 26.839 26.778 26,728 ,26.678 25.885 24.413 18.825 12.615 11.028 10.921. ESI-MS: calculated for $[M^+]$ =625.3, found 625.3.

3. Effect of Buffer Solution and pH Value

Because the type of buffer solution plays an important role in the detection, we investigated the effect of three types of buffer solutions: HEPES, PBS and Tris-HCl. The results are

obtained and shown in Figure S1. As can be seen in Fig. S1, the fluorescence response was the best in the case of 20 mM HEPES. Moreover, over the pH range of 6.4-8.2, HS-Cy showed relatively stable response toward H_2S. Therefore, the probe was expected to work well under physiological conditions (20 mM HEPES, pH 7.4).

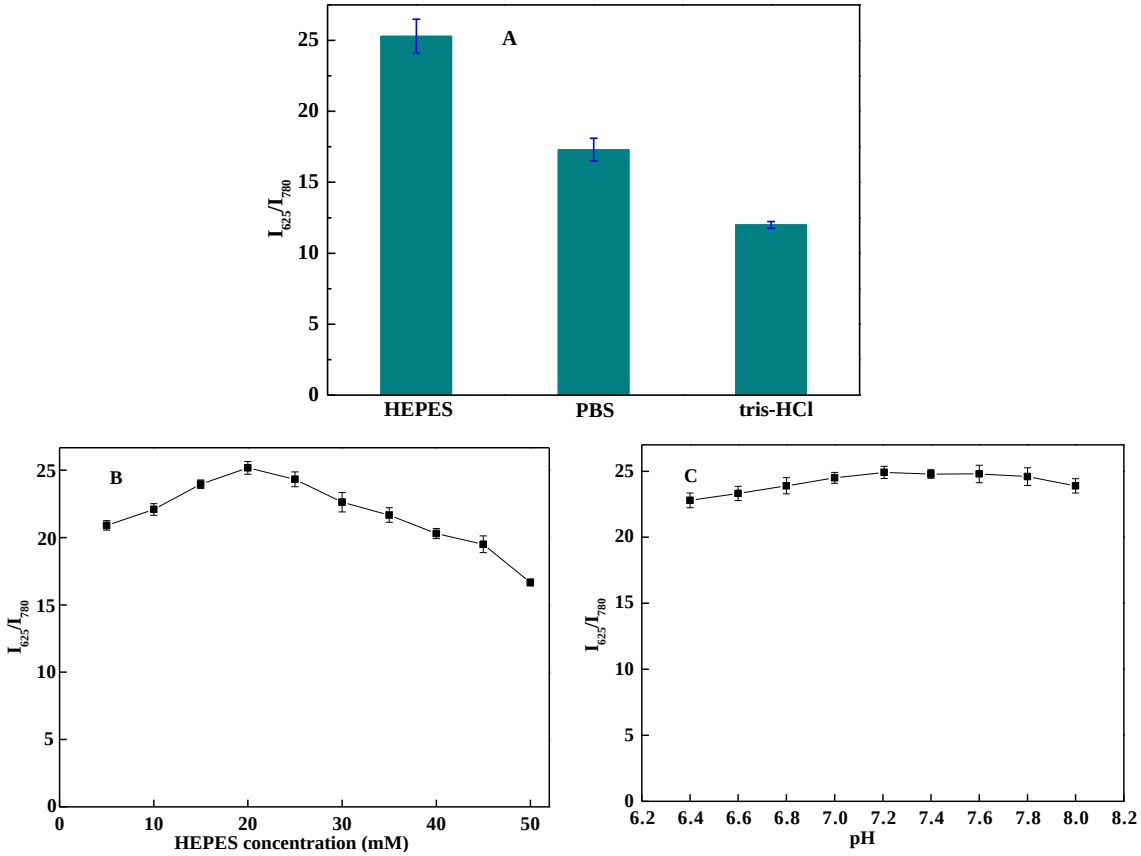


Fig. S1. Effect of of buffer solution and pH on the reaction system of HS-Cy (5 μM) plus NaHS (70 μM) at 37 ^{o}C. (A) Effect of HEPES (20 mM), PBS (20 mM) and Tris-HCl (20 mM). (B) Effect of HEPES concentration. (C) Effect of pH. The experiment was repeated three times and the data are shown as mean (±S.D.).

4. Fluorescence Kinetics of HS-Cy in Physiological Buffer

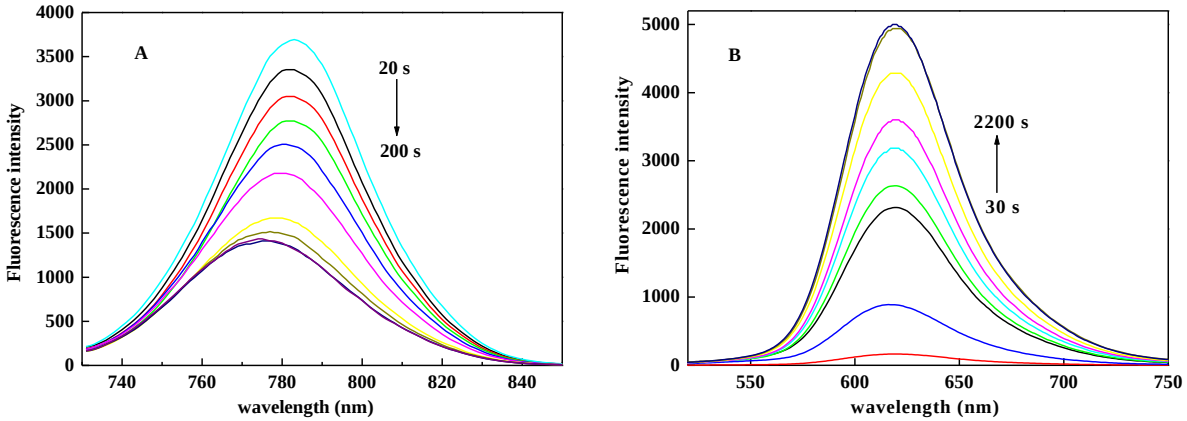


Fig. S2. Fluorescence kinetics of HS-Cy (5 μM) upon reaction with NaHS (20 μM) at pH 7.4 (HEPES buffer, 20 mM, 0.5% CH_3CN) at 37 ^oC. (A) Fluorescence quenching kinetic recorded at λex/em = 700 nm/780 nm corresponding to 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 s, respectively. (B) Fluorescence kinetic recorded at λex/em = 510 nm/625 nm corresponding to 30, 300, 600, 900, 1200, 1500, 1800, 2100, and 2200 s, respectively.

5. Linear Equation and Detection Limit

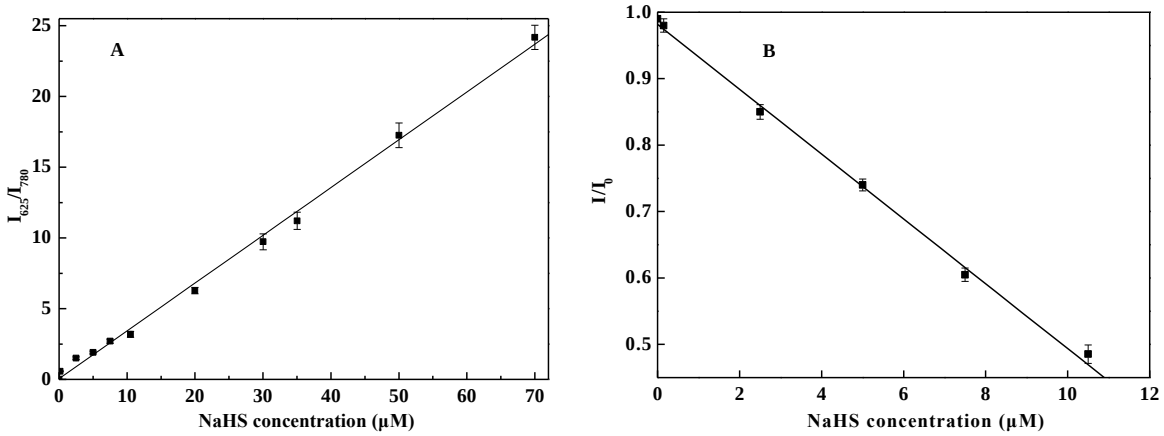


Figure S3. The working curves obtained from (A) the ratio signal of I_{625}/I_{780} and (B) the fluorescence quenching at λex/em = 700 nm/780 nm. The linear equations were: (A) $F_{(I625/I780)}$= 0.00867+0.34049 [H_2S] (μM) with R=0.9962; (B) F/F_0=0.9817-0.04881[H_2S] (μM) with R=0.9976. F_0 denotes the fluorescence intensity in the absence of NaHS and F corresponds to the fluorescence intensity at a certain NaHS concentration. The experiment was repeated three times and the data are shown as mean (±S.D.).

The detection limits of HS-Cy were determined as the concentration of H_2S that resulted in a statistically significant increase in $F_{(I625/I780)}$ (ratiometric detection) and decrease in F_{780} (quenching detection) with a p-value < 0.01 when compared with a blank control.[6]

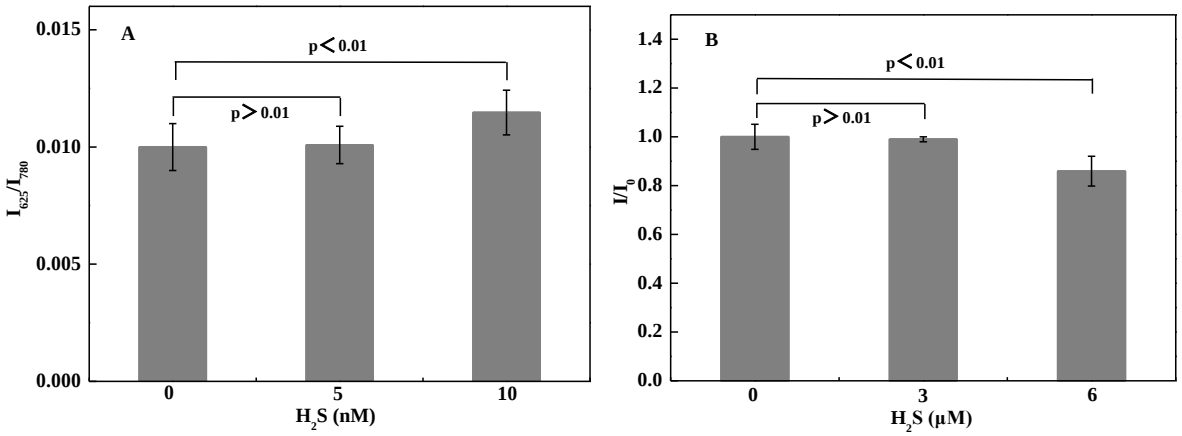


Figure S4. Fluorescence responses of 5 μM HS-Cy to H_2S. (A) The responses of ratiometric signal at I_{625}/I_{780} to 0, 5, and 10 nM H_2S. (B) The responses of I/I_0 at 780 nm to 0, 3, and 6 μM H_2S. Data were acquired at 37 ºC in 20 mM HEPES (pH 7.4, 0.5% CH_3CN). Statistical analyses were performed with a two-tailed Student's *t*-test (n = 3). Error bars are standard deviation.

6. Mass Spectra of the Reaction Solution of HS-Cy plus H_2S

We testified the reaction mechanism shown in Scheme 1 by HRMS. The probe HS-Cy was dissolved in acetonitrile and then diluted to 5 μM with 20 mM HEPES buffer of pH 7.4. Then 80 μM NaHS was injected into the probe solution. The mixture was stirred at 37 °C for 20 min. Then the solution was concentrated by evaporating the organic solvent. The reaction product was analyzed by HRMS. The mass spectra of the reaction solution exhibited characteristic peaks at m/z 659.6406 corresponding to $[M_{qHS\text{-}Cy}]^+$, at m/z 493.3208 corresponding to $[M_{ketone\text{-}Cy}+H]^+$, and at m/z 167.1694 corresponding to $[M_1+H]^+$, respectively. The characteristic peak of HS-Cy was not found because the nucleophilic addition of H_2S towards HS-Cy was so fast that no HS-Cy was remained. As the second nucleophilic addition proceeded with a slower kinetic, qHS-Cy can be found in the solution. The results agreed well with the conclusions drawn from the absorption spectra (Figure 1) and the fluorescence spectra (Figure 2).

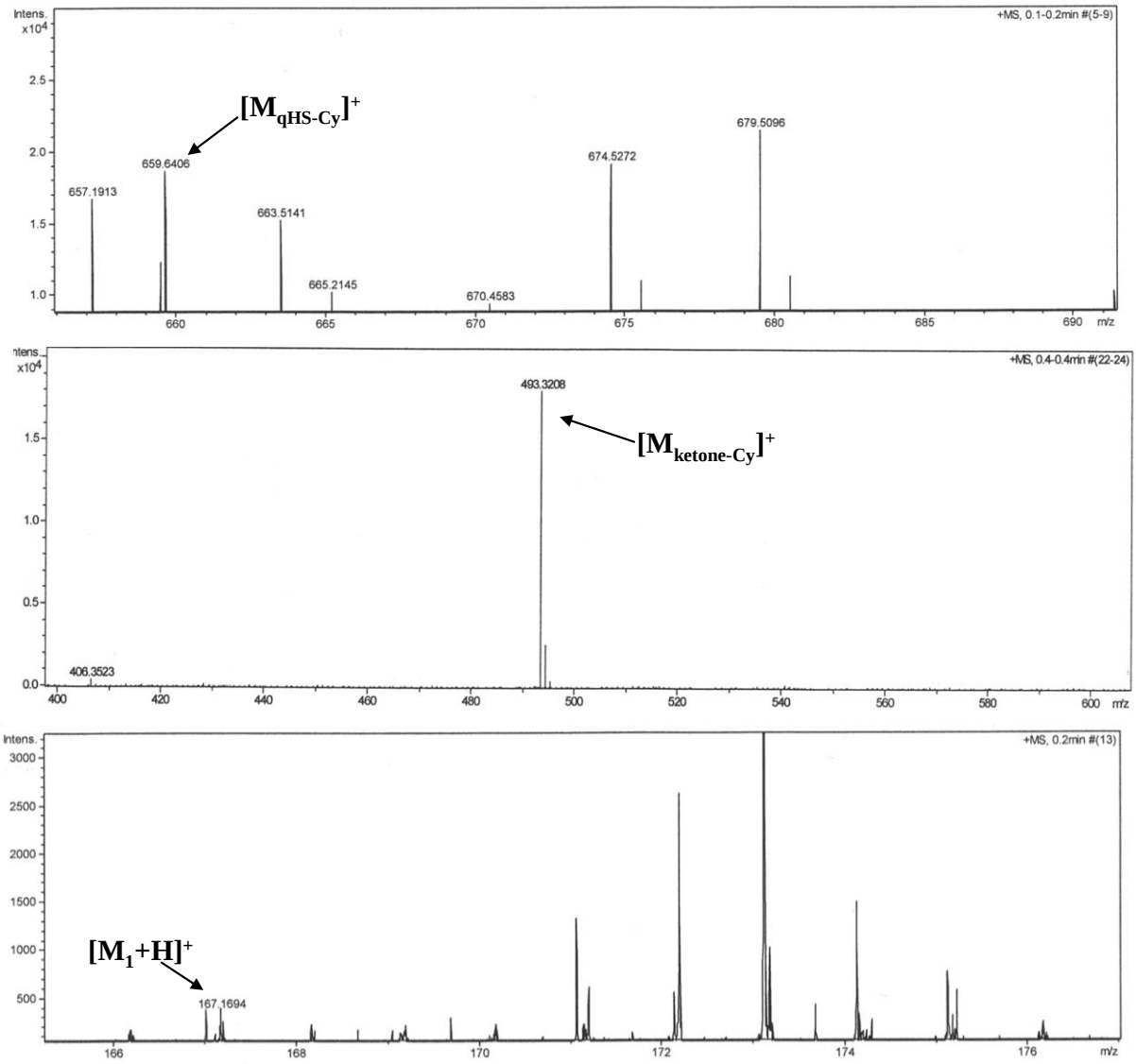


Figure S5. Mass spectra of the reaction solution of HS-Cy plus H_2S.

7. Selectivity Investigation of HS-Cy in the Presence of Amino-containing Substances

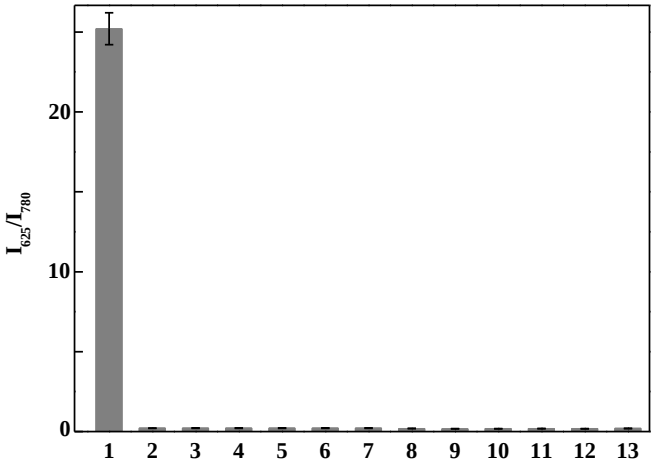


Figure S6. Fluorescence ratio (I_{625}/I_{780}) of 5.0 μM HS-Cy in 20 mM HEPES buffer (pH 7.4, 0.5% CH_3CN) to diverse analytes. Bars represent fluorescence ratio (I_{625}/I_{780}) to each compound. (1) NaHS (70 μM); (2) glycine (100 μM); (3) methionine (100 μM); (4) tyrosine (100 μM); (5) tryptophan (100 μM); (6) glutamic acid (100 μM); (7) histidine (100 μM); (8) arginine (100 μM); (9) serine (100 μM); (10) phenylalanine (100 μM); (11) lysine (100 μM); (12) NH_4Cl (100 μM); (13) dopamine (100 μM). The solution was incubated for 35 min at 37 ^{o}C. The experiment was repeated three times and the data were shown as mean (±S.D.).

8. Stability Investigation of HS-Cy in Physiological Buffer

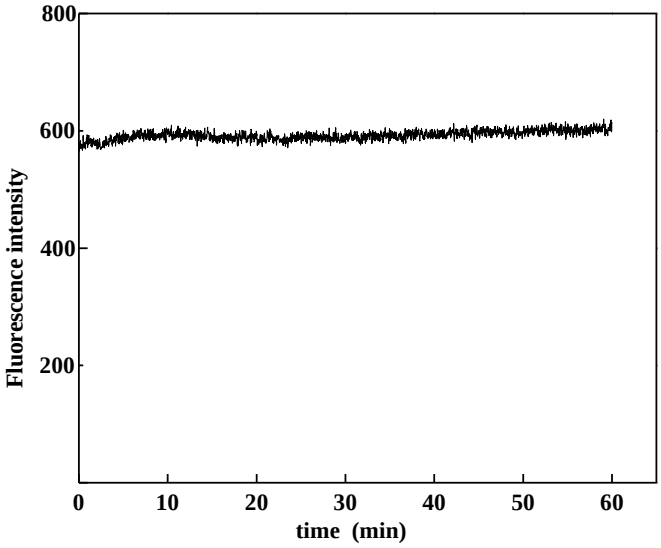


Figure S7. The stability investigation of HS-Cy (5 μM) in physiological buffer (HEPES, 20 mM, pH 7.4, 0.5% CH_3CN) with 60 μM NaHS. λex/em = 700 nm/780 nm, 37 ^{o}C.

9. MTT Assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. HepG2 cells (10^6 cell mL^{-1}) were dispersed within replicate 96-well microtiter plates to a total volume of 200 mL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO_2/95% air incubator for 5 hours. Macrophages were then incubated for 24 hours with different probe concentrations of 5×10^{-4}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M, respectively. MTT (Sigma) solution (5.0 mg mL^{-1} in PBS) was then added to each well. After 4 h, the remaining MTT solution was removed and DMSO (150 μL) was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm in a TRITURUS microplate reader. The calculation of IC50 values was done according to the method of Huber and Koella.[7]

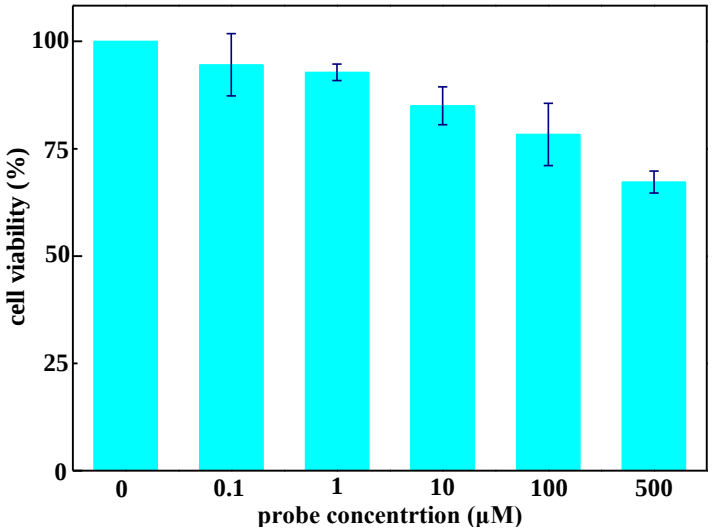


Figure S8. MTT assay of HepG2 cells in the presence of different concentrations of HS-Cy. The experiment was repeated three times and the data are shown as mean (±S.D.).

10. Confocal Microscopy Images

Confocal fluorescence images of exogenous H_2S in HepG2 cells. The confocal images of HepG2 cells obtained from individual band path of 550-600 nm and 640-800 nm were shown in Figure S9. After HepG2 cells were incubated with 5 μM HS-Cy for 30 min, strong red fluorescence was observed with band path of 640-800 nm upon excitation by a 633 nm laser (Figure S9d), while the yellow fluorescence (pseudo color) obtained from band path of 550-600 nm by a 543 nm laser excitation was faint (Figure S9g), indicating good cell membrane permeability of the probe. Next, a gradual fluorescence decrease appeared with band path of 640-800 nm after incubating the probe-loaded cells with 50 μM NaHS for 10

min (Figure S9e) and 20 min (Figure S9f), respectively. Meanwhile, the fluorescence with band path of 550-600 nm gradually increased (Figure S9h, 10 min; Figure S9i, 20 min). This resulted in the ratiometric images shown in Figure 4 in the main manuscript. The bright-field image (Figure S9j) confirmed that the cells were viable throughout the imaging experiments. The pixel intensity of selected ROI in Figure S9 was shown in Table S1.

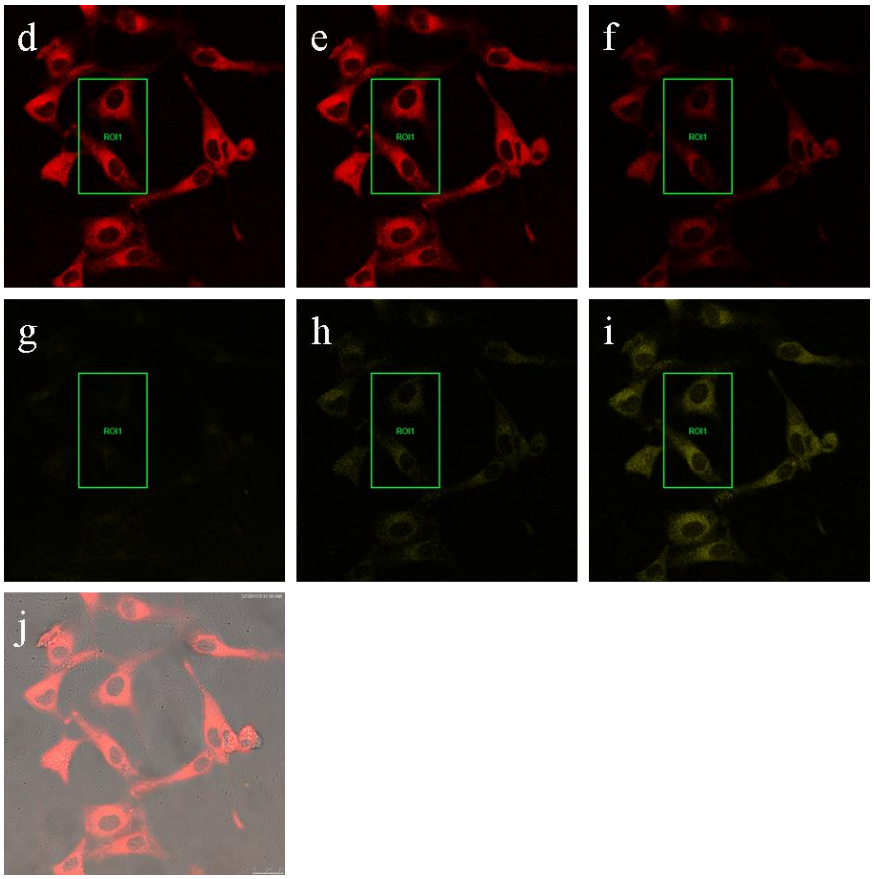


Figure S9. Confocal fluorescence images of exogenous H_2S in living HepG2 cells by HS-Cy. (d) and (g), HepG2 cells loaded with 5 μM probe for 30 m in. (e) and (h), Dye-loaded cells treated with 50 μM NaHS for 10 min. (f) and (i), Dye-loaded cells treated with 50 μM NaHS for 20 min. (j), merging of bright field image and dark field images. (d)-(f), images obtained with band path of 640-800 nm upon 633 nm excitation. (g)-(i), images obtained with band path of 550-600 nm upon 543 nm excitation. 37 ^{o}C, scale bar = 50 μm.

Table S1. The pixel intensity of selected ROI in Figure S9 (*n*=3).

data images / regions	d	e	f	g	h	i
ROI1	162.36	100.14	39.94	12.47	18.48	28.32

Confocal fluorescence images of endogenous H_2S in A549 cells. The confocal images of A549 cells from individual band path of 550-600 nm and 640-800 nm were shown in Figure S10. After A549 cells were incubated with 5 μM HS-Cy for 30 min, strong red fluorescence was observed with band path of 640-800 nm (Figure S10d), while the yellow fluorescence (pseudo color) obtained from band path of 550-600 nm was faint (Figure S10g), indicating good cell membrane permeability of the probe. When the cells were first stimulated by 100 μM SNP for 1h and then imaged with 5 μM HS-Cy, a gradual fluorescence decrease appeared with band path of 640-800 nm for 10 min (Figure S10e) and 20 min (Figure S10f), respectively. Meanwhile, the fluorescence with band path of 550-600 nm gradually increased (Figure S10h, 10 min; Figure S10i, 20 min). This indicated that the levels of endogenous H_2S were increased after stimulation. The bright-field image (Figure S10j) confirmed that the cells were viable throughout the imaging experiments. The pixel intensity of selected ROI in Figure S10 was shown in Table S2.

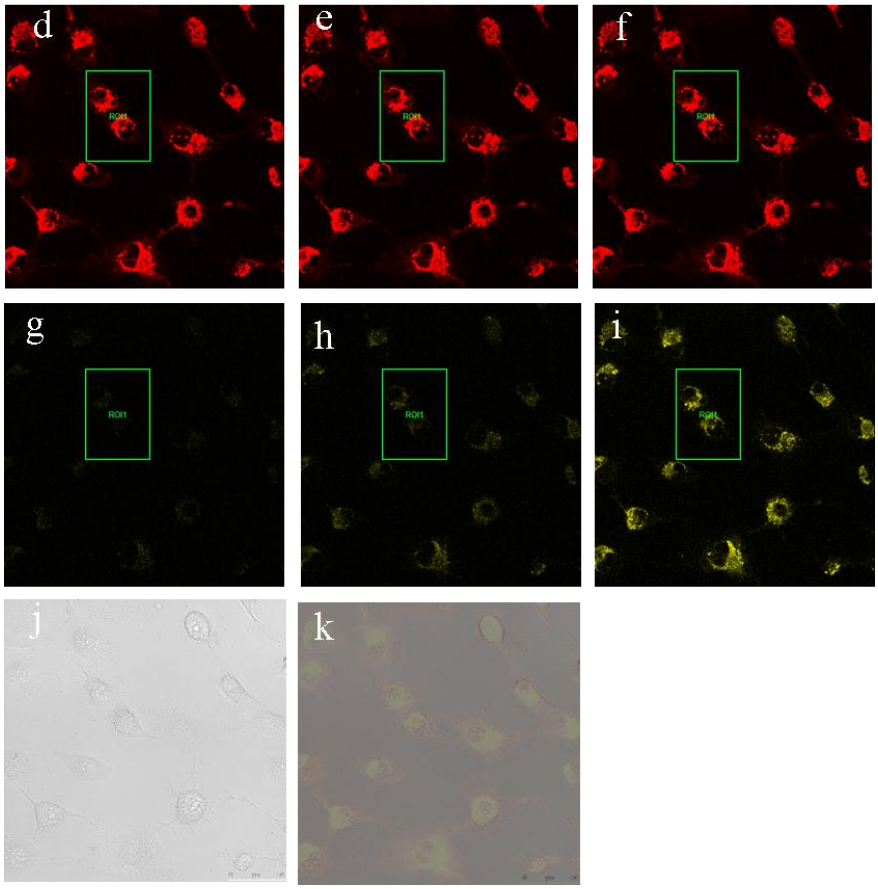


Figure S10. Confocal fluorescence images of endogenous H_2S in living A549 cells. (d) and (g), A549 cells incubated with 5 μM probe for 30 min. (e) and (h), cells were prestimulated with 100 μM SNP for 1h, then

incubated with 5 μM probe for 10 min. (f) and (i), cells were prestimulated with 100 μM SNP for 1h, then incubated with 5 μM probe for 20 min. (j), Brightfield image of living A549 cells in panels (d)-(i). (k), merging of bright field image and dark field images. (d)-(f), images obtained with band path of 640-800 nm upon 633 nm excitation. (g)-(i), images obtained with band path of 550-600 nm upon 543 nm excitation. 37 ^{o}C, scale bar = 50 μm.

Table S2. The pixel intensity of selected ROI in Figure S10 (*n*=3).

data / images / regions	d	e	f	g	h	i
ROI1	46.47	45.23	44.13	3.97	13.97	17.59

To further verify that the variation of images from Figure 5 and Figure S10 was indeed resulted from the changes of endogenous H_2S, a parallel experiment was performed by adding DL-propargylglycine (PPG, 50 mg/L), an inhibitor for CBS and CSE, to A549 cells and incubated for 20 min. Then the cells were stimulated by 100 μM SNP for 1 h and subsequently imaged with 5 μM HS-Cy for the same time period as no inhibitor. The results in Figure S11 showed obvious difference from that of Figure 5 and Figure S10 that almost no fluorescence increment and decrease were observed with band path of 550-600 nm and 640-800 nm, respectively. The variation of ratiometric signal intensity was also neglectable (Table S3). The results confirmed the suppression of endogenous H_2S production, which was resulted from enzyme inactivation.

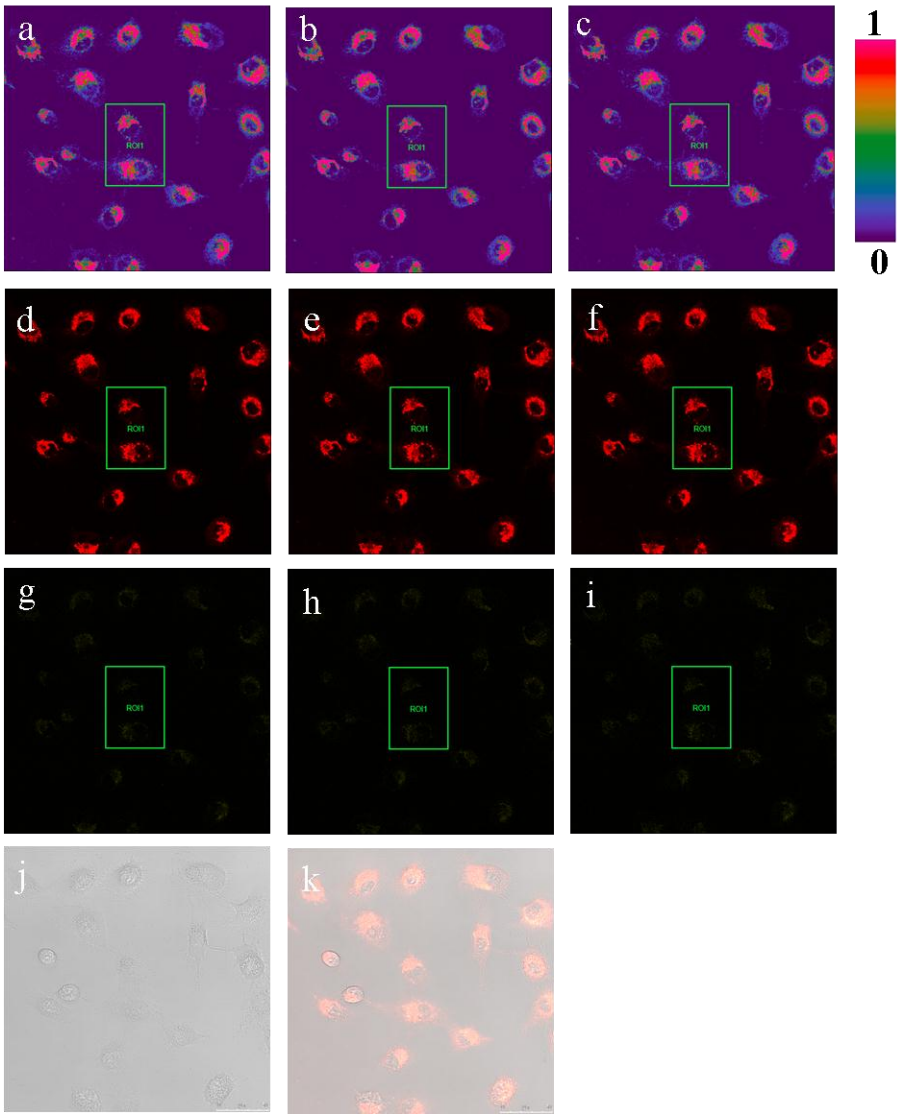


Figure S11. Confocal fluorescence images of endogenous H_2S in living A549 cells in the presence of PPG. The cells were preincubated with 50 mg/L PPG for 20 min, and then were stimulated with 100 μM SNP for 1h. After that, the cells were incubated with 5 μM probe for 10 min (a, d, g), 20 min (b, e, h), and 30 min (c, f, i), respectively. (a)-(c), the ratiometric images. (d)-(f), images obtained with band path of 640-800 nm upon 633 nm excitation. (g)-(i), images obtained with band path of 550-600 nm upon 543 nm excitation. (j), Brightfield image of living A549 cells in panels (a)-(i). (k), merging of bright field image and dark field images. 37 ^{o}C, scale bar = 50 μm.

Table S3. The pixel intensity of selected ROI in Figure S11 (*n*=3).

data / images / regions	a	b	c	d	e	f	g	h	i
ROI1	0.1032	0.1030	0.1033	31.79	31.76	31.76	3.28	3.27	3.28

The stability of HS-Cy in A549 cells. A549 cells were preincubated with 5 μM HS-Cy for 20 min at 37°C, then the stability of HS-Cy were investigated by continuous scanning a selected ROI for 1h and the mean pixel intensity was recorded (Figure S12), showing good anti-photobleaching ability of HS-Cy.

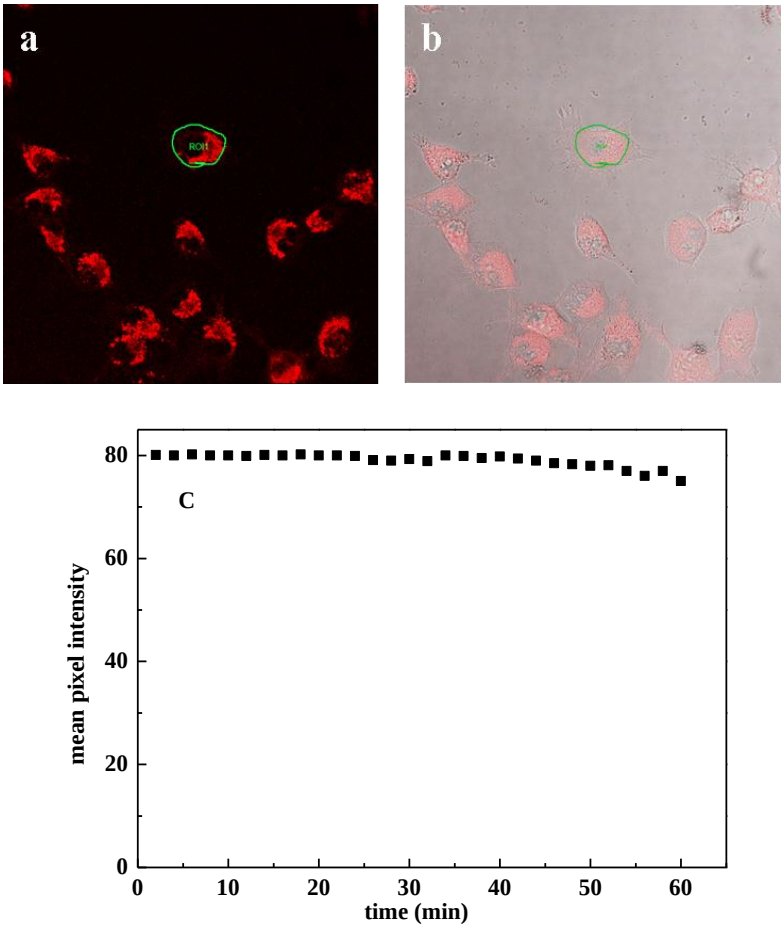


Figure S12. Time dependent stability of HS-Cy in A549 cells under 633 nm laser excitation. (a) Confocal fluorescence images of HS-Cy-incubated A549 cells with labeled ROI. (b) The brightfield image of (a). (c) Pixel intensity of ROI recorded for 60 min. 37 °C, scale bar = 25 μm.

Cell viability of A549 cells. The good morphology of A549 cell obtained by co-staining A549 cell with HS-Cy and a nucleus specific dye Hoechst 33342 suggested satisfing biocompatibility of the probe (Figure S13).

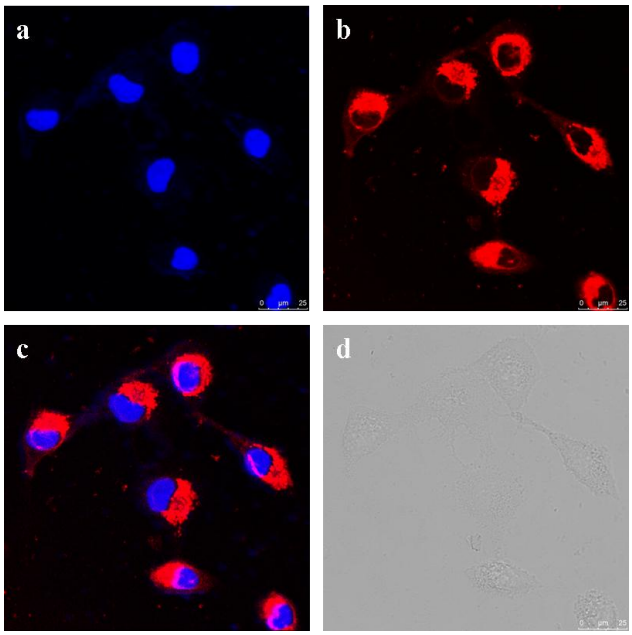


Figure S13. Confocal fluorescence imaging of cell viability in A549 cells using HS-Cy and Hoechst 33342. The cells were costained by HS-Cy (5 μM) and Hoechst 33342 (1 μM) for 20 min at 37^{o}C, then the images were obtained. (a) Images obtained by a 405 laser excitation with emission from 410-480 nm. (b) Images obtained by a 633 laser excitation with emission from 640-800 nm. (c) The merged images of blue (a) and red (b) channels. (d) The brightfield image of (a-c). 37 ^{o}C, scale bar = 25 μm.

Mitochondria targeting of HS-Cy.

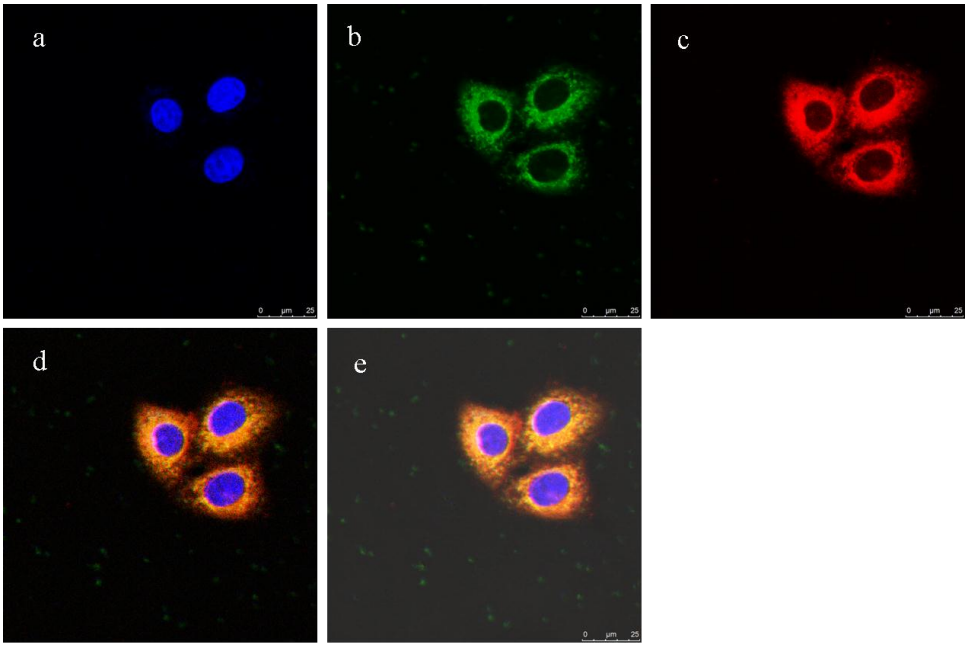


Figure S14. Co-staining experiments of probe with Mito-Tracker Green FM. (a) and (b), HepG2 Cells incubated with 1 μM Hoechst 33342 (excited by a 405 nm Argon laser) and 50 nM Mito-Tracker Green FM (excited by a 488 nm Argon laser) for 10 min, respectively. (c) HepG2 Cells incubated with 5 μM HS-Cy

for 10 min with a 633 nm He-Ne laser excitation. (d) Overlay image of (a-c). (e) Overlay image of brightfield image and (d). 37 ^{o}C, scale bar = 25 μm.

11. The Analytical Characteristics of Fluorescent Probes for H_2S Based on Nucleophilic Addition Trapping (Table S4)

Table S4. Summary of fluorescent probes for H_2S based on nucleophilic addition trapping

Probe	Fluorophore	λex/λem (nm)	Ratiometric or not	Response time	Detection limit	Samples (endogenous/exogenous)	Organelle targeting	Ref. (in the main manuscript)
HS-Cy	cyanine	700/780,510/625	yes	3 min (pH 7.4) 30 s (pH 8.0)	5.0 nM	human A549 cell (endogenous) human HepG2 (exogenous)	mitochondria	the present work
E1	2-(2'-Hydroxyphenyl)benzothiazole	295/356,483	yes	2 min	0.12 μM	HeLa cell (exogenous)	/	16a
SFP-3	BODIPY	500/515	no	2-3 min	/	mice blood plasma (endogenous) C57BL6/J mice brain (endogenous)	/	16b
SFP-2	BODIPY	465/515	no	60 min	5.0 μM	HeLa cell (endogenous)	/	16c
SFP-1	3-(3,5-difluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol	300/388	no	60 min	/	HeLa cell (endogenous)	/	16c
Probe 1	Fluorescein	465/515	no	60 min	1-10 μM	COS7 cell (exogenous)	/	16d
Probe 5	Fluorescein	465/513	no	30 min	1.0 μM	/	/	16e
Probe 6	Fluorescein	465/513	no	30 min	1.0 μM	COS7 cell (exogenous)	/	16e

"/" not mentioned

12. Additional Spectroscopic Data

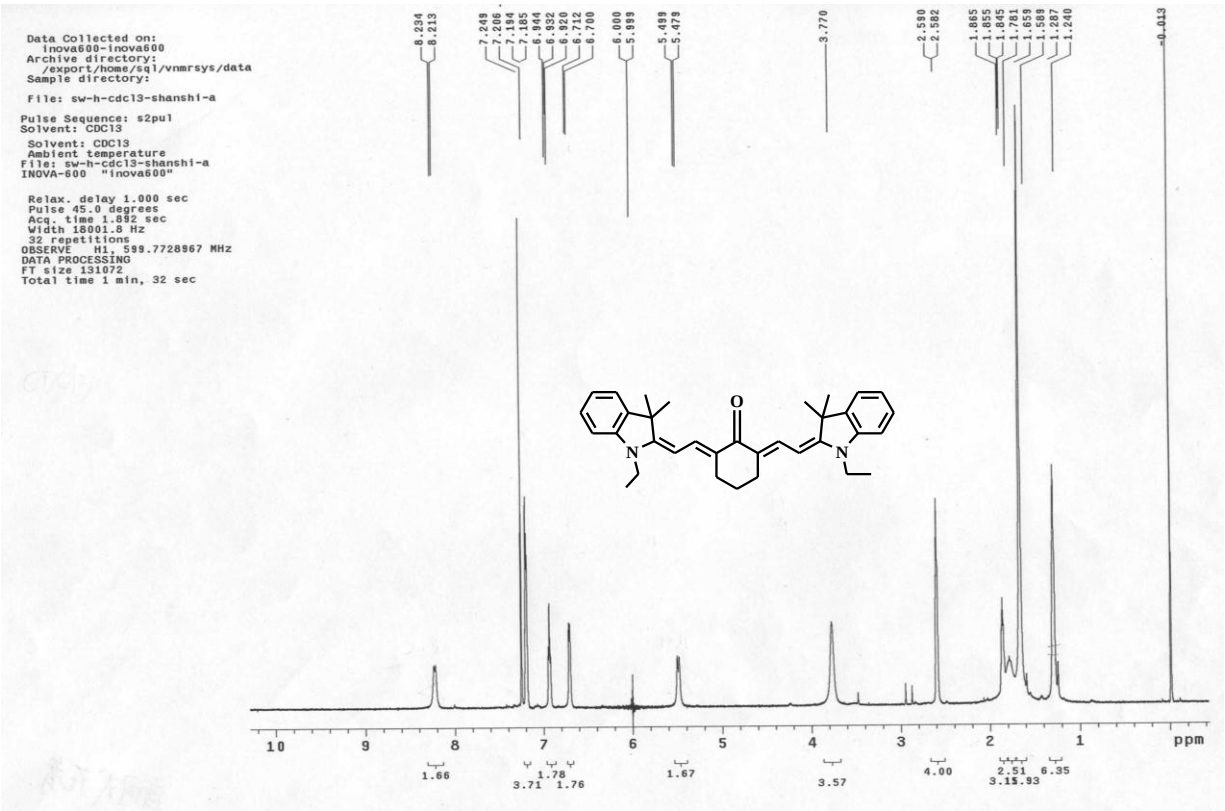


Figure S15. ^{1}H-NMR spectrum of ketone-Cy

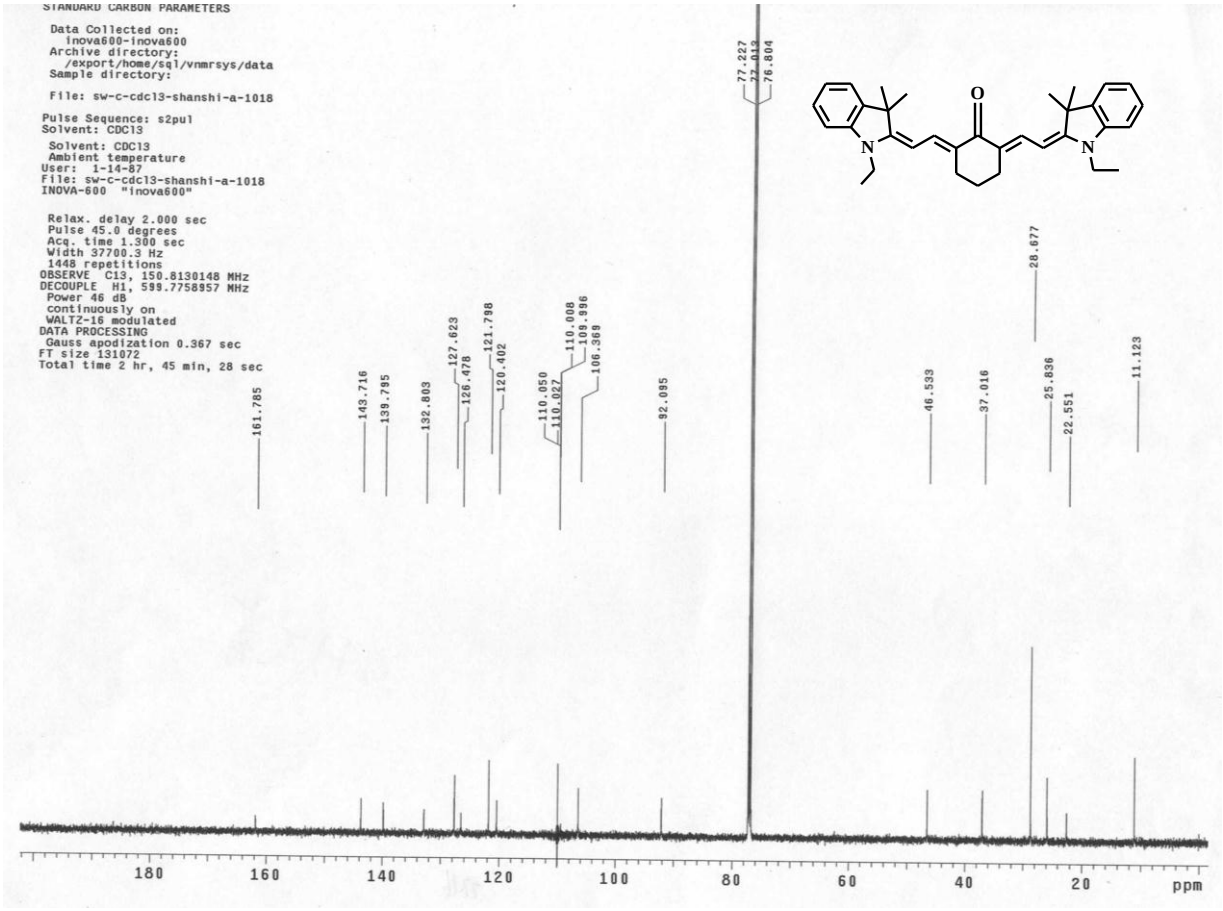


Figure S16. ^{13}C-NMR spectrum of ketone-Cy

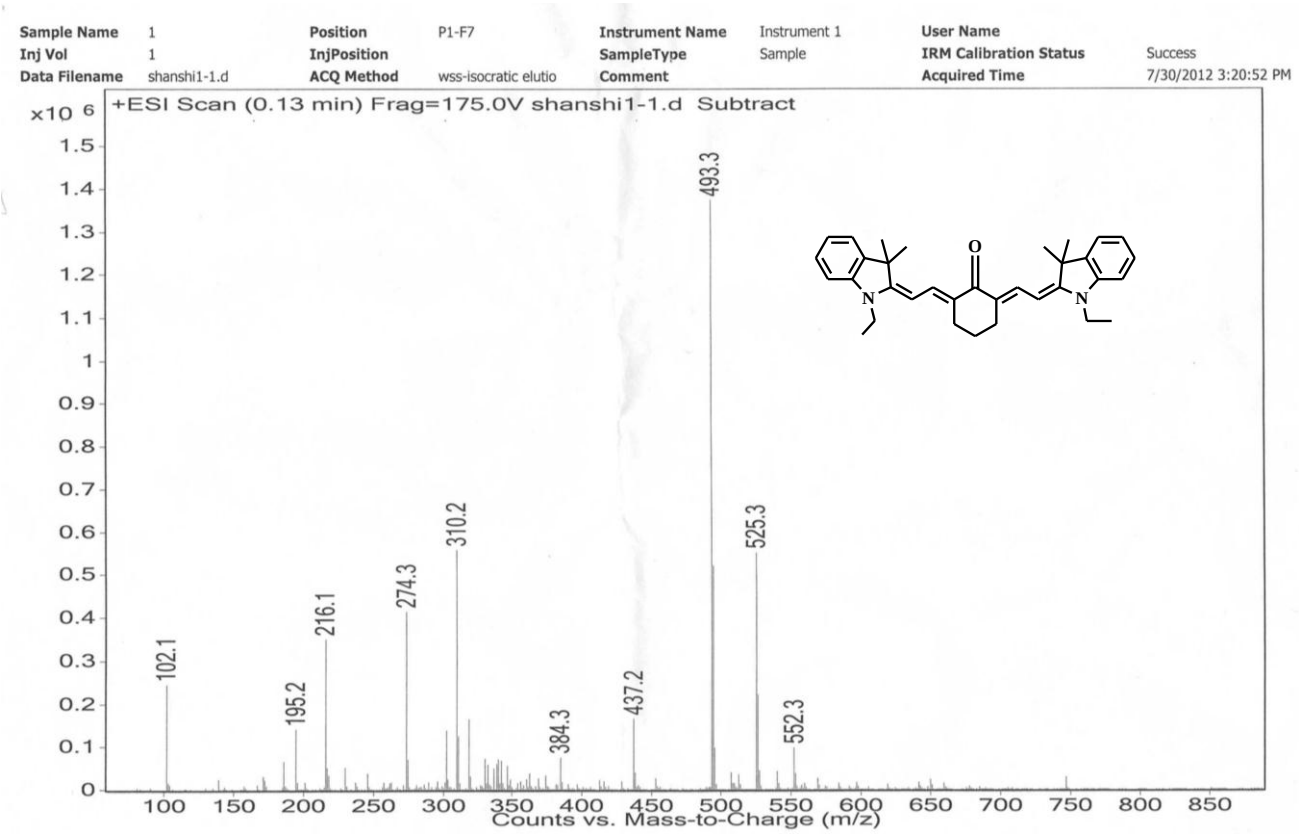


Figure S17. ESI-MS spectrum of ketone-Cy

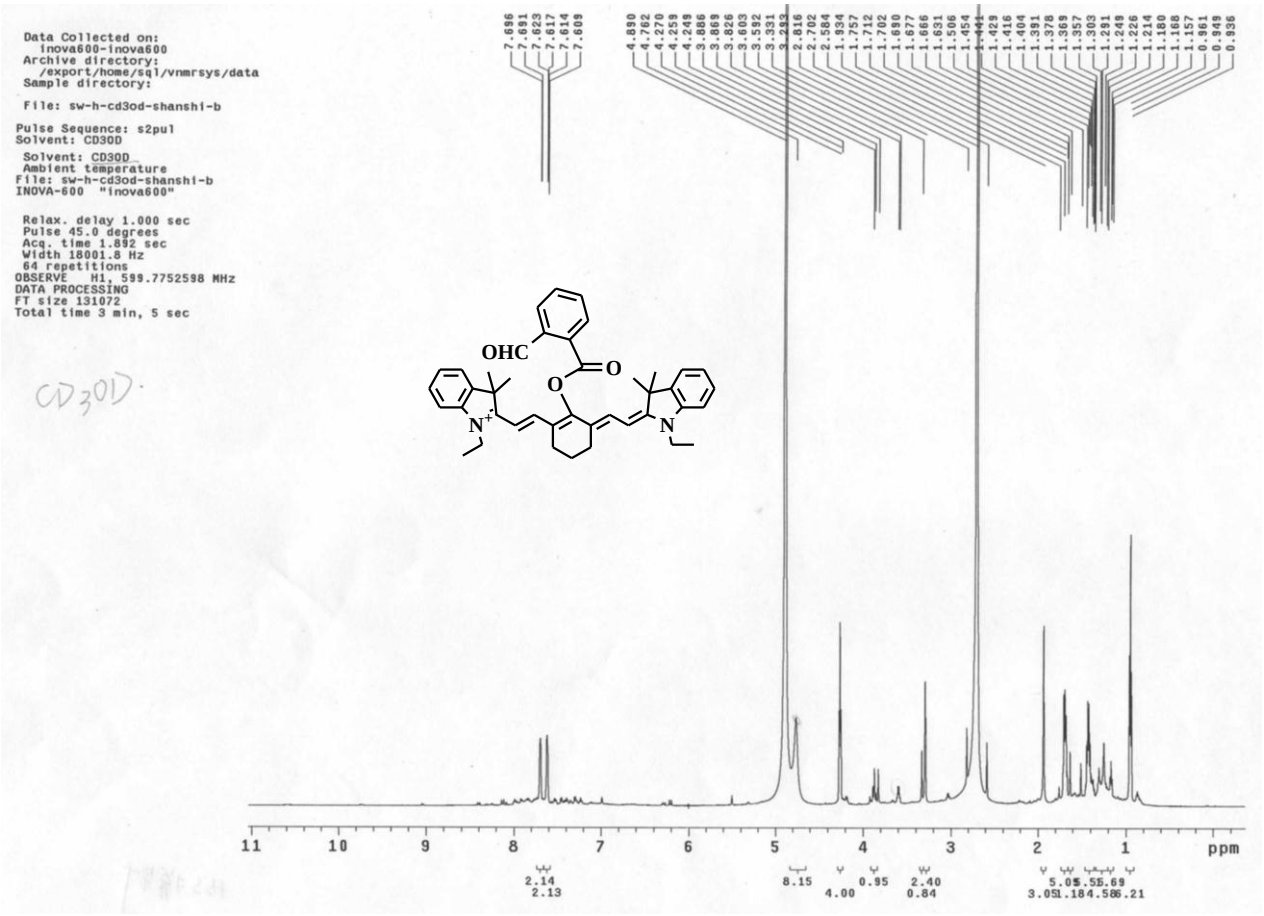


Figure S18. ^{1}H-NMR spectrum of HS-Cy

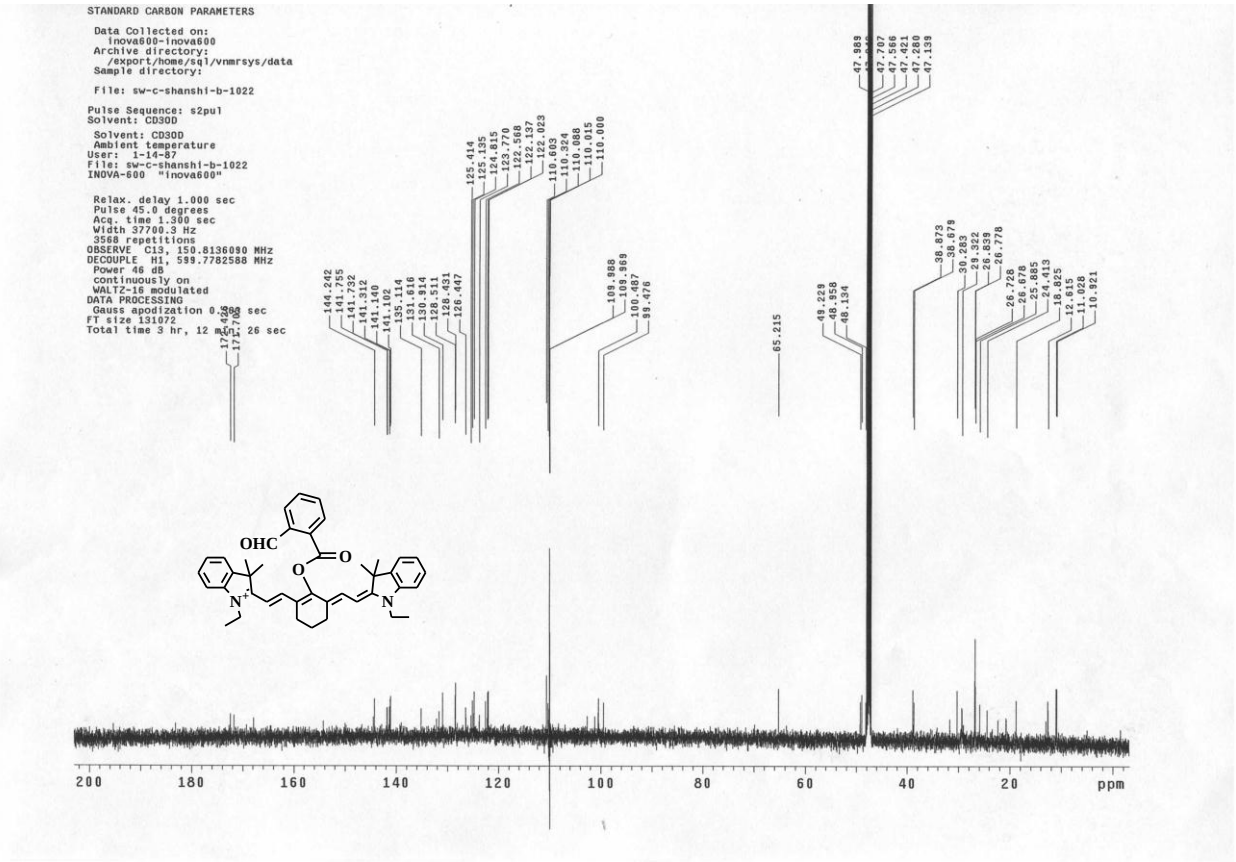


Figure S19. ^{13}C-NMR spectrum of HS-Cy

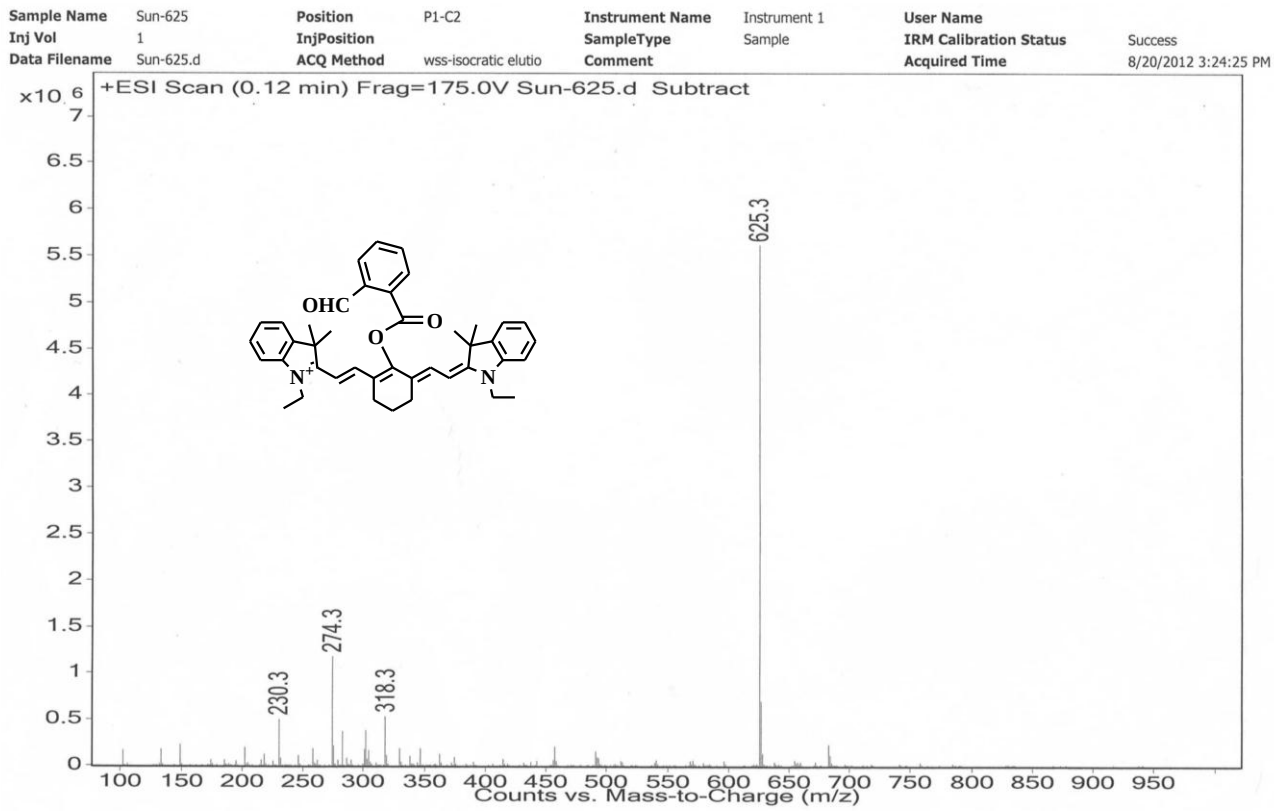


Figure S20. ESI-MS spectrum of HS-Cy

13. References

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