Electronic Supplementary Material (ESI) for Analyst.

A resorufin-based fluorescent probe for imaging hydrogen polysulfides in living cells

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Experimental Section

Material and Instruments

General methods were used unless otherwise noted; Materials were obtained from J&K China Chemical Ltd and were used without further purification (The purity of chemicals were no less than 95%). The solvents were purchased from General-Reagents and used without further purification (The purity of solvents were no less than 99.9%). Column chromatography was performed using silica gel (300–400 mesh). NMR spectra were measured using Bruker AV-400 Nuclear Magnetic Resonance spectroscopy. Chemical shifts were expressed in ppm and coupling constants (J) in Hz. The high resolution mass spectrometry were measured using Thermo Q Exactive hybrid quadrupole-Orbitrap mass spectrometer equipped with Thermo Scientific™Dionex™UltiMate™ 3000U HPLC system. UV–vis spectra were recorded on an UV–vis spectrophotometer at 25 °C which was controlled by the thermo stated compartment of the spectrophotometer. Fluorescent data were measured using Hitachi F-7000 Fluorescence spectrophotometer. Standard quartz cuvettes with a 10 mm light path were used for all UV–vis spectra and fluorescent spectra measurements. All the fluorescent and UV–vis experiments were repeated three times. The slit widths for fluorescent data recording: ex= 5 nm, em= 5 nm. Confocal images were measured using Zeiss LSM710META. Spectral-grade solvents were used for measurements of UV-vis absorption and fluorescence.

Generation of RSS and ROS

Stock solution of Re-SS (1 mM) was prepared in MeCN. Stock solutions of 10 mM of $NaNO_2$, $NaNO_3$, Cys, GSH, Hcy, Na_2S, $Na_2S_2O_3$,Na_2SO_3, Na_2SO_4, Na_2S_2, Na_2S_4 were all prepared in deionized water.

S_8: The stock solution of S_8 (20 mM) was prepared in CH_2Cl_2, and then diluted to 10 mM with EtOH.

ClO^-: commercial available NaClO solution was used as the source of hypochlorite. The concentration of hypochlorite was determined from the absorption at 292 nm(ε=350$M^{-1}cm^{-1}$)

H_2O_2: commercial available H_2O_2 solution was used as the source of hypochlorite. The concentration of H_2O_2 was determined from the absorption at 240 nm(ε=43.6$M^{-1}cm^{-1}$)

ROO•: ROO• was generated from AAPH(2,2-azobis(2-amidino-propane) dihydrochloride), AAPH was added to deionized water and then stirred at room temperature for 1 h.

•OH: Hydroxyl radical was generated by the Fenton reaction. To prepare •OH solution, hydrogen peroxide(H_2O_2, 10 eq) was added to $FeSO_4$ in deionized water.

^{t}BuOOH: commerical available tBuOOH (75% aqueous solution) was used and diluted to the experiment concentration.

NO: Nitric oxide radical was generated from SNP (sodium nitroferricyanide (III) dihydrate). The deionized water was degassed by argon, then SNP was added to deionized water and then stirred for 1 h at room temperature. The probe solution should also degassed by argon.

$ONOO^-$: Simultaneously,0.6M $NaNO_2$, 0.6M HCl, 0.7M H_2O_2 was added to a 3M NaOH solution at 0 ºC. The concentration of peroxynitrite was estimated by using extinction co-

efficient of 1670 $cm^{-1}M^{-1}$ at 302 nm in 0.1M sodium hydroxide aqueous solutions.

General procedure for analysis

Unless otherwise specified, all fluorescence measurements were conducted in 10 mM PBS (pH 7.4, containing 30% MeCN) according to the following procedure: for H2Sn detection, in a 5 mL cuvette, 2 mL PBS (containing 30% MeCN) and 25 μL of 1 mM Re-SS (prepared with MeCN) were mixed; this was followed by addition of a proper volume of Na_2S_4 sample solution to obtain different concentrations. The final volume was adjusted to 5 mL with PBS (containing 30% MeCN), and the solution was mixed rapidly. After the reaction at 25 °C was completed in a thermostat, a 2 mL portion of the reaction solution was transferred to a quartz cell of 1 cm optical length to measure UV-Vis absorbance or fluorescence with $\lambda_{ex}/_{em}$ = 550/589 nm, and both excitation and emission slits had widths of 5 nm.

MTT assay

The study of cellular toxicity of probe Re-SS was carried out by using methylthiazolyldiphenyl-tetrazolium bromide(MTT) assay. RAW 264.7 cells growing in log phase were seeded into 96-well plates at density of 2×10^5 for 24 h. The cells were stained with Re-ss of different concentrations (5, 10, 20, 30 μM) for another 24 h at 37 °C in 95% air 5% CO_2. Subsequently, cells were treated with 0.5 mg/mL MTT (200 μL/well)away from light and incubated for an additional 4 h (37 °C, 5% CO_2). Then the cells were dissolved in DMSO (150 μL/well), and the absorbance at 570 nm was recorded. Each individual cytotoxic experiment was repeated for three times. The cell viability (%) was calculated according to equation.

Cellviability(%) = OD(sample)/OD(blank) X 100

Where OD (sample) is the optical density of the wells treated with various concentration of Re-ss and OD (blank) is that of the wells treated with RPMI-1640 containing 10% FBS.

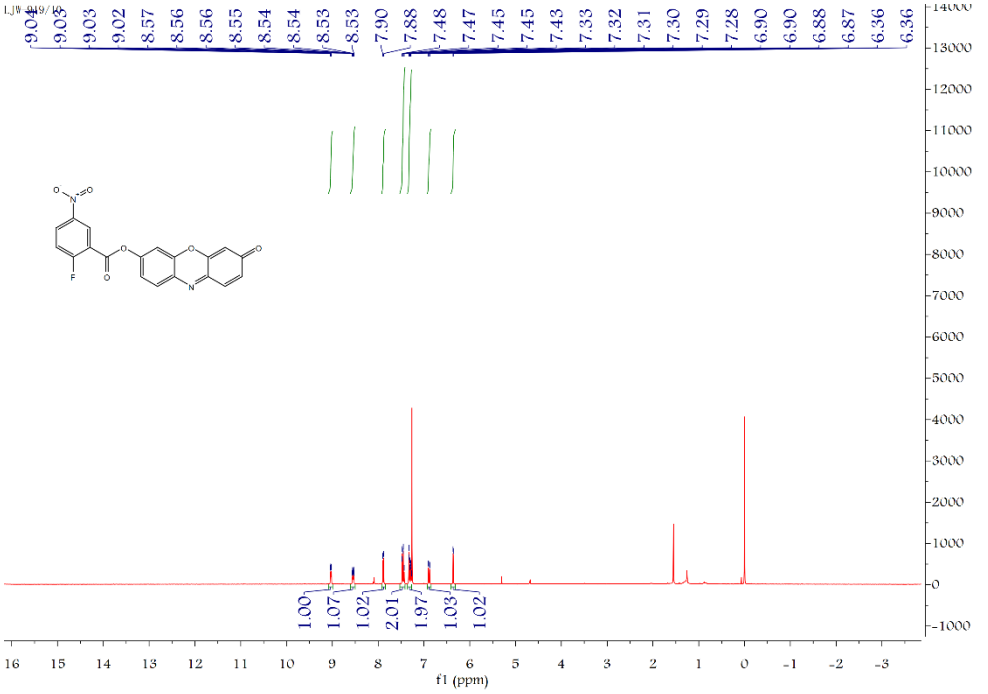
Cell culture and confocal imaging

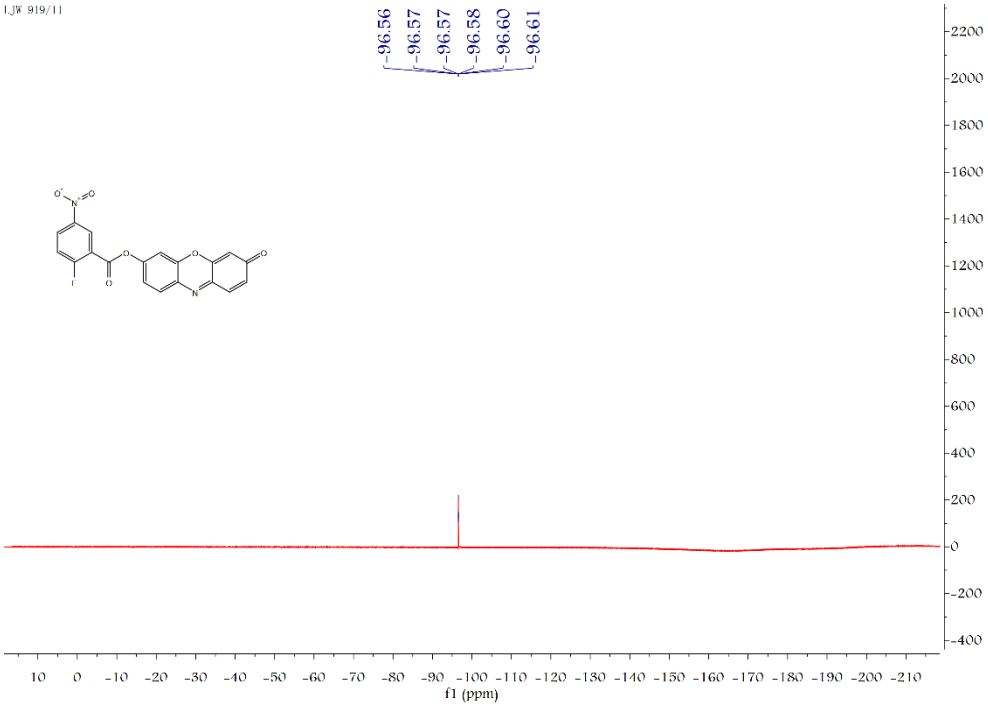
Mice macrophages (RAW 264.7) were cultured in RPMI-1640 supplemented with 10% fetal bovineserum (FBS), 1% penicillin, and 1% streptomycin at 37 °C in a 5% CO_2/ 95% air incubator. Subsequently, the cells were plated on a 35 mm confocal dishes and allowed to adhere for 24 h. Then, the cells were stained with reagents. After staining finished, washed the cell culture with PBS for 3 times, then fixing the cell culture with 4% HCHO for 20 min. Washed the cell culture with PBS for 3 times and confocal imaging.

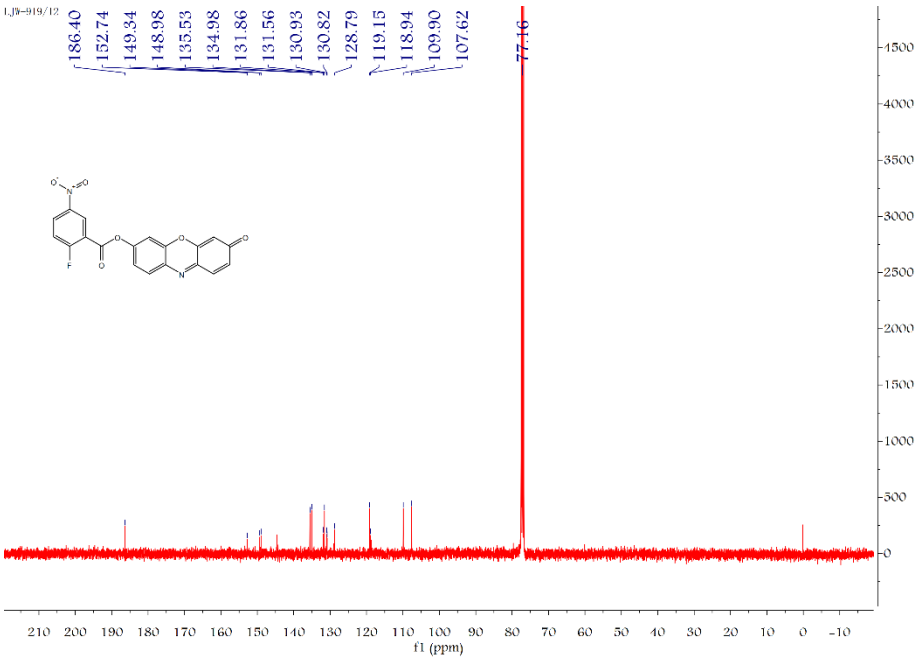
Synthesize of probe Re-SS

Resorufin (213 mg, 1 mmol), 2-fluoro- 5-nitrobenzoic acid (220 mg, 1.2 mmol) and 4-dimethylaminopyridine (DMAP, 12 mg, 0.1 mmol) were dissolved in dry dichloromethane (20 mL) and stirred at 0 8C. Then N,N-dicyclohexylcarbodiimide (DCC, 309 mg, 1.5 mmol) dissolved in dichloromethane (5 mL) were added to the solution dropwise and stirred at room temperature for 16 h. The precipitate of urea was removed by filtration and the filtrate was concentrated in high vacuum to give an oily residue. This residue was purified by silica gel column chromatography with eluent dichloromethane/ethyl acetate (v/v 20:1) to give

RTP-1 (130 mg, 34%).







^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (dd, J = 6.0, 2.9 Hz, 1H), 8.55 (ddd, J = 9.0, 3.9, 2.8 Hz, 1H), 7.89 (d, J = 8.7 Hz, 1H), 7.51 – 7.39 (m, 2H), 7.34 – 7.28 (m, 2H), 6.89 (dd, J = 9.9, 2.0 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H).

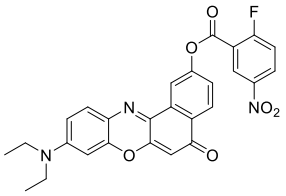
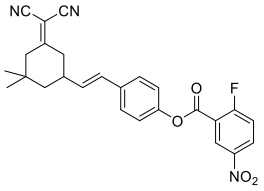
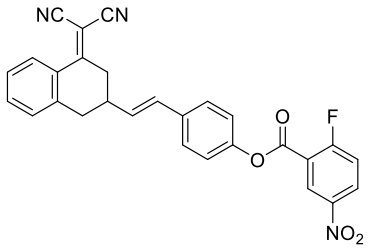
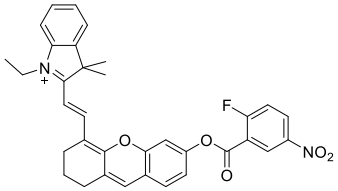
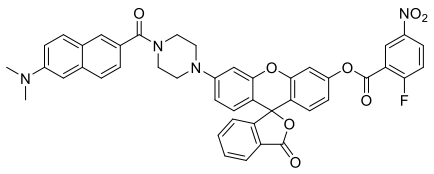
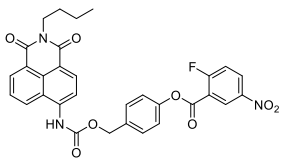
^{19}F NMR (376 MHz, CDCl3) δ -96.60

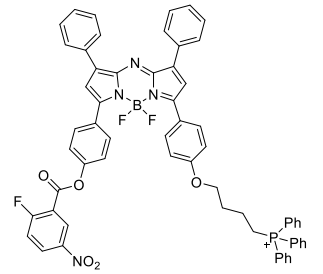
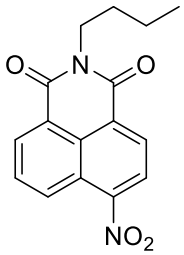
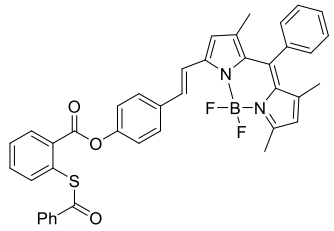
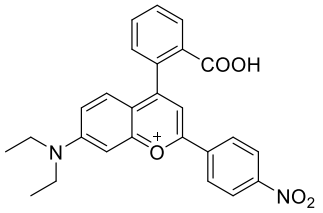
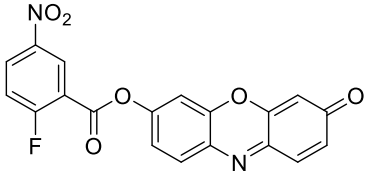
^{13}C NMR (101 MHz, CDCl3) δ 186.40, 152.74, 149.34, 148.98, 135.53, 134.98, 131.86, 131.56, 130.93, 130.82, 128.79, 119.15, 118.94, 109.90, 107.62.

HRMS-positive mode

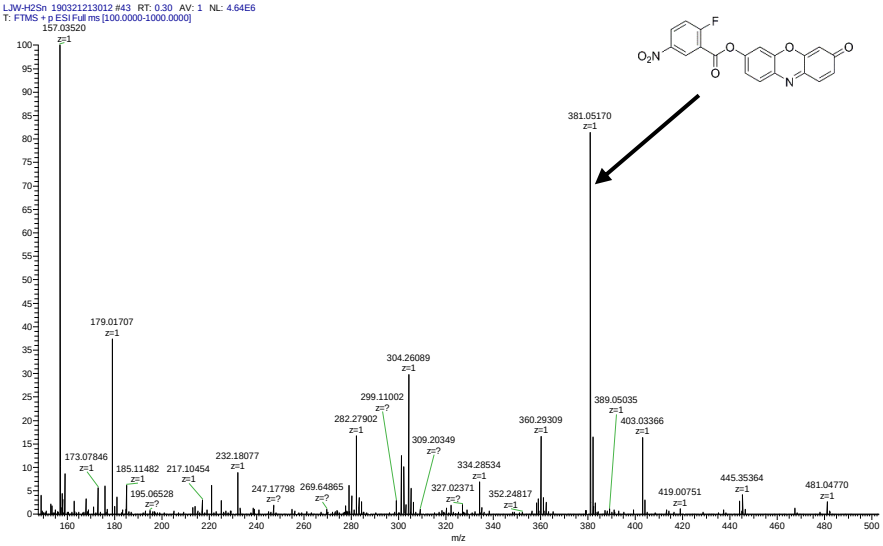
Calculated : 381.0517

Experitmental : 381.0517

Probe for H_2S_n	$\lambda_{ex}/\lambda_{em}$	Time	LOD	Linear Range
 Anal. Methods, 2017, 9, 6443–6447	550 nm/ 655 nm	5 min	3.9 nM	0-18 μM
 J. Mater. Chem. B, 2018, 6, 7015--7020	395 nm/ 482 nm, 655 nm	24 min	43 nM	0-50 μM
 Sensors and Actuators B 254 (2018) 222–226	535 nm/ 682 nm	5 min	8.2 nM	0-20 μM
 J. Mater. Chem. B, 2017, 5, 2574--2579	680 nm/ 720 nm	5 min	22 nM	0-10 μM
 Anal. Chem. 2016, 88, 11892−11899	370 nm/ 448 nm, 541 nm	30 min	700 nM	0-16 μM
 Anal. Chem., 2016, 88 (14), 7206–7212	405 nm/ 460 nm, 518 nm	5 min	100 μM	0-8 μM

 Anal. Chem. 2015, 87, 3631−3638	675 nm/ 730 nm	2 min	25 nM	0-10 μM
 Sensors & Actuators: B. Chemical 278 (2019) 64–72	435 nm/ 540 nm	20 min	26 nM	0.1-100 μM
 Chem. Commun., 2018, 54, 3735--3738	530 nm/ 584 nm	10 min	26 nM	0-10 μM
 RSC Adv., 2016, 6, 88519–88525	560 nm/ 620 nm	40 min	75 nM	0-60 μM
 This work	550 nm/ 589 nm	30 min	24 nM	0-50 μM

(A)



(B)

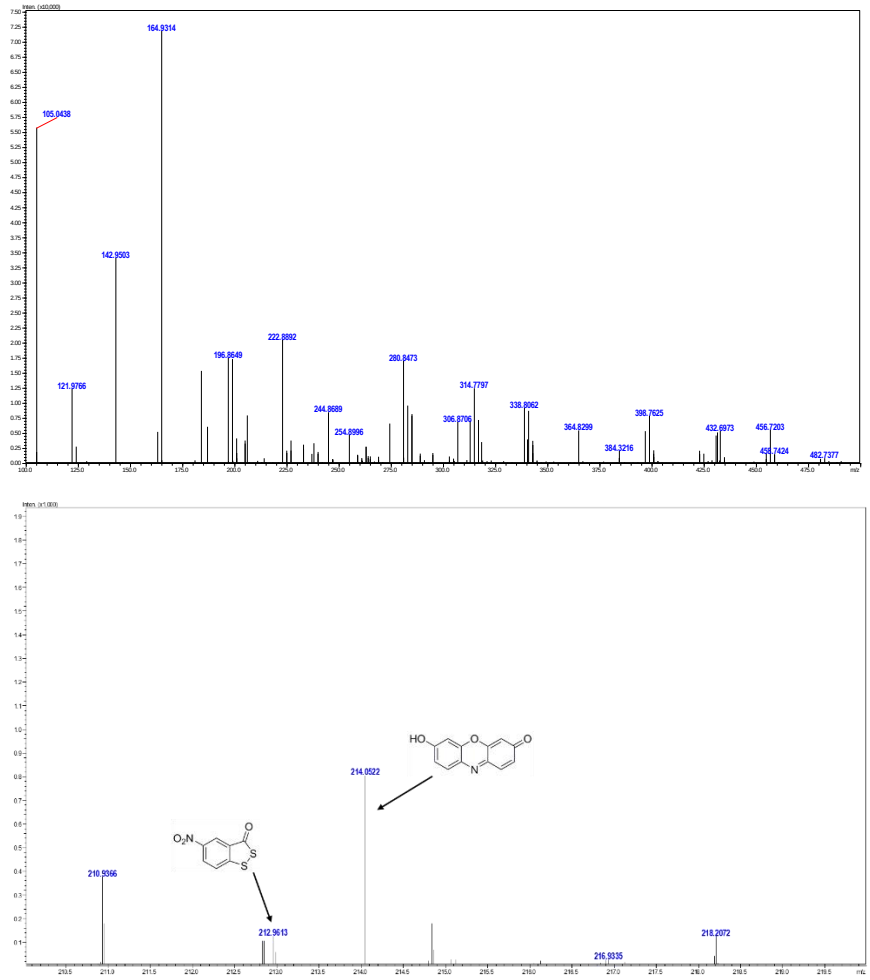


Fig S1. The mass spectrum of Probe Re-SS (A) and Re-SS+Na_2S_4 (B). In figure (A), we can see the obvious mass peak of probe Re-SS. In figure (B), After adding Na_2S_4, the mass peak of Re-SS is disappeared, and the mass peak of resorufin and cyclization product was observed, which means the postulated mechanism is verified.

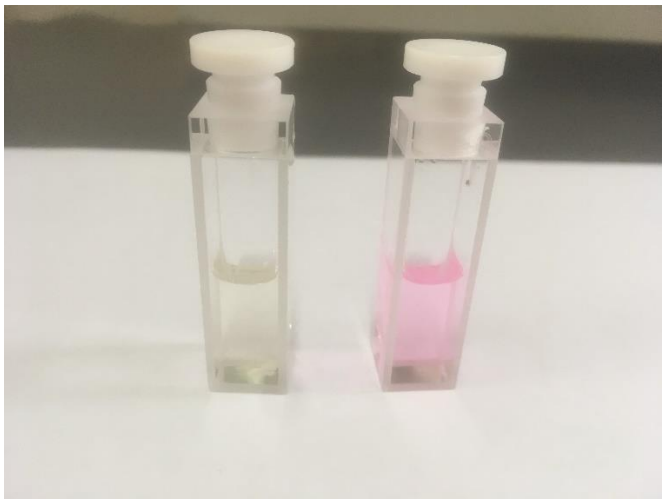


Fig S2. The color of probe Re-SS and Re-SS+Na_2S_4. The color of probe Re-SS is pale yeloow, after reaction with Na_2S_4, the color clearly changed to pink, which means this process could be monitered by naked eye.

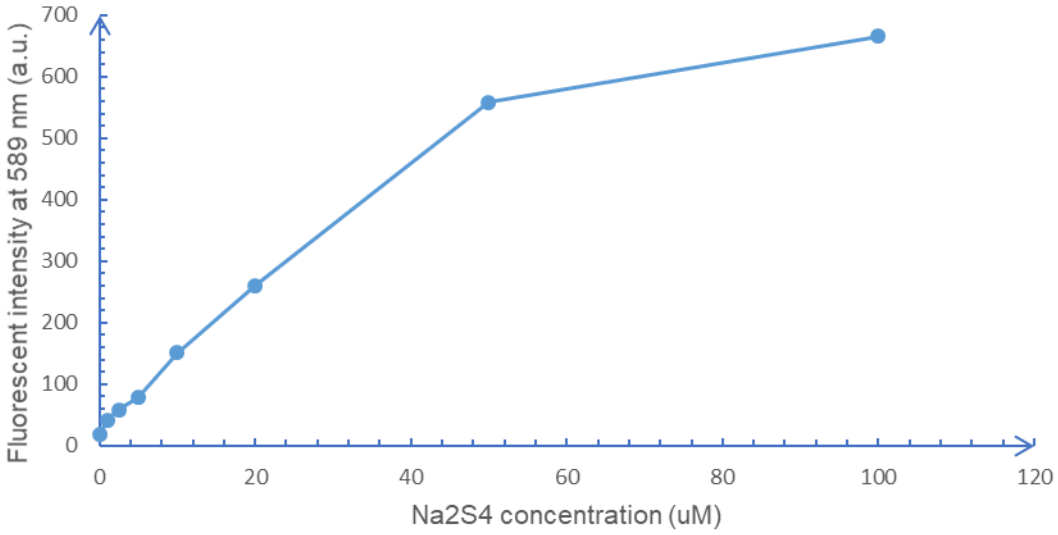


Fig S3. Fluorescent intensity in 589 nm of Re-SS (5 μM) in PBS buffer (pH = 7.4, 10 mM) containing 30% of MeCN upon addition of 0-100 μM Na_2S_4 for 60 mins at 25°C. Excitation wavelength: 550 nm.

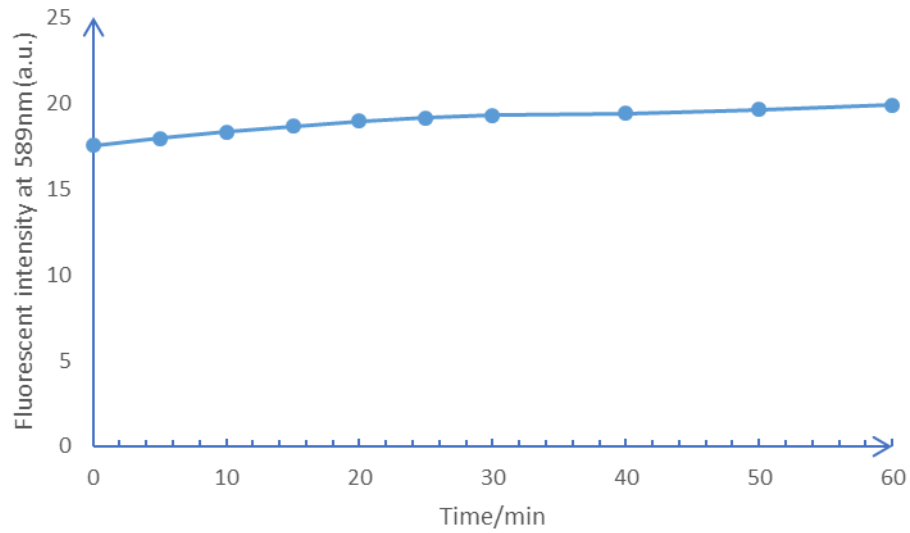


Fig S4. Stability of probe Re-SS. Fluorescent intensity of probe in 589 nm (5 μM) in PBS buffer (pH = 7.4, 10mM) containing 30% of MeCN for 60 min at 25°C was recorded. Excitation wavelength: 550 nm. The data showed the probe Re-SS was stable under experimental condition.

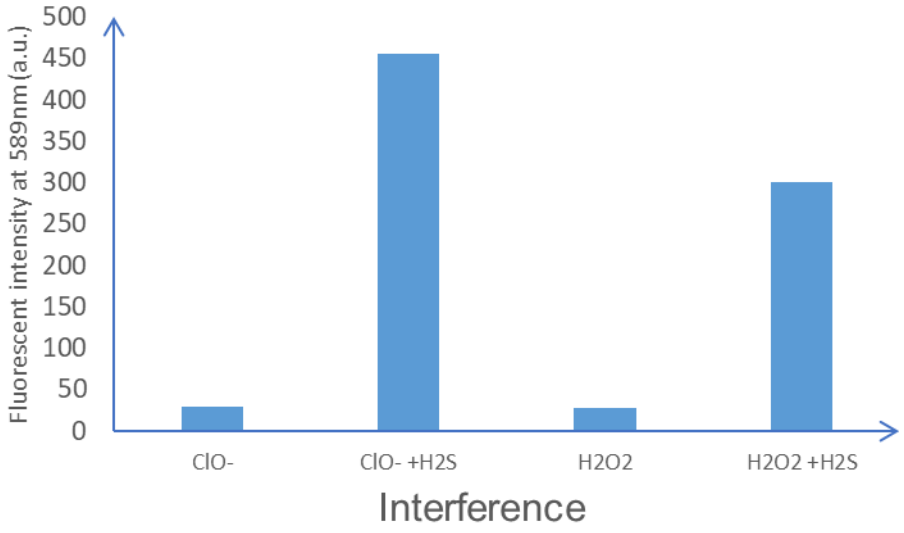


Fig S5. The response of probe Re-SS to in-situ generated H_2S_n. The concentration of probe was 5 μM and the concentration of hypochlorite. hydrogen peroxide and H_2S were 50,50 and 100 μM. This result showed probe Re-SS could detect in-situ generated H_2S_n well and hypochlorite is a better oxidizing agent than hydrogen peroxide.

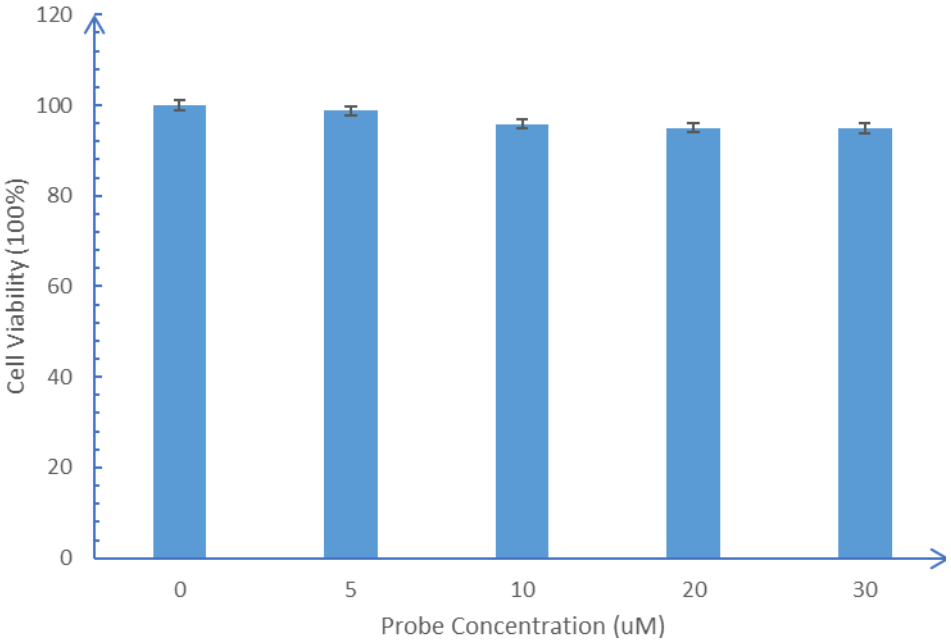


Fig S6. Viability assay for RAW 264.7 cells with probe Re-SS in various concentration in dark.