Supporting Information

An Endoplasmic Reticulum Targeted Ratiometric Fluorescent Probe for Sensing of Hydrogen Sulfide in Living Cells and Zebrafish

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Contents

1. Experimental reagents and instruments

All other chemicals were obtained from commercial suppliers and used without further purification. Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance at 400MHz or at 100 MHz, δ values are in parts per million relatives to TMS in DMSO-d_6. Mass spectra (MS) were measured with Bruker Apex IV FTMS using electrospray ionization (ESI). Absorption spectra were recorded on a Purkinje TU-1901 spectrophotometer. Fluorescence measurements were taken on a Hitachi F-7000 fluorescence spectrometer with a 10mm quartz cuvette. pH measurements were carried out with a pH acidometer (Mettler Toledo FE-30). Fluorescence imaging was observed under an Olympus IX81 confocal fluorescence microscope.

HeLa cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin with 5% CO_2 at 37°C.

4-day-old zebrafish were obtained from Shanghai Feixi Biotechnology Co., Ltd.

2. Normalized spectra of probe M-H_2S to H_2S

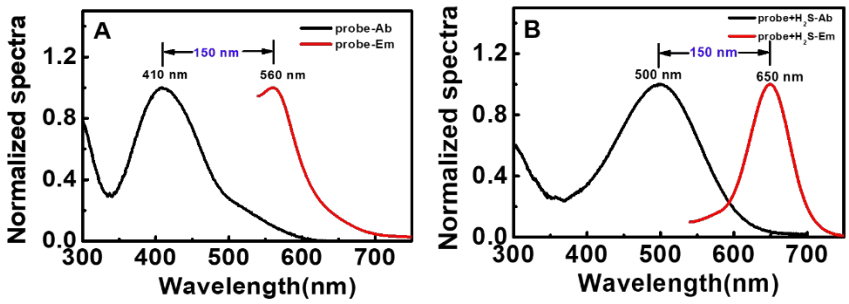

Fig. S1. (A) Normalized UV–*vis* absorption spectra and fluorescence spectra of probe **M-H_2S**. (B) Normalized UV–*vis* absorption spectra and fluorescence spectra of **M-H_2S** recognized after H_2S. λ_{ex} = 480 nm, slit width: d_{ex} = d_{em} = 10 nm, 10 mM PBS, pH 7.4, containing 10% DMSO, v/v at

room temperature.

3. The stability of M-H_2S at different pH values

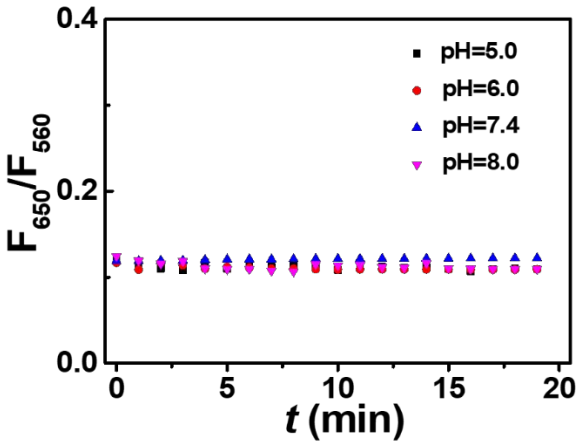

Fig. S2. Fluorescent intensity (F_{650}/F_{560}) responses of **M-H_2S** (10 μM) at different pH values (pH=5.0, 6.0, 7.4, 8.0). Spacing interval is 1 min.

4. Time response spectra of probe M-H_2S to H_2S

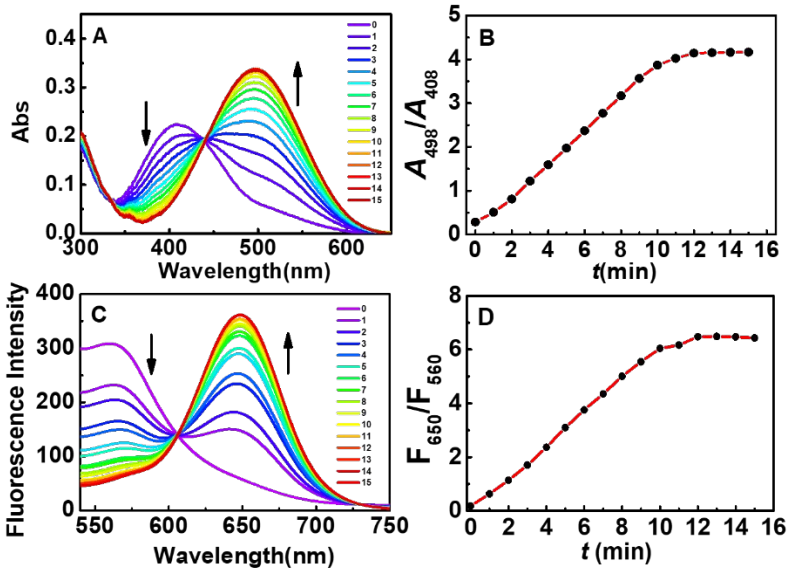

Fig. S3. UV–*vis* absorption A) and ratio intensity changes B) of **M-H_2S** (10 μM) upon addition of H_2S (50 μM). Fluorescence spectra C) and ratio intensity changes D) of **M-H_2S** (10 μM) upon addition of H_2S (50 μM).

5. Determination of the Detection Limit

According to IUPAC, the detection limits were determined based on the fluorescence titrations, carried out in PBS / DMSO (9:1, v/v), pH 7.4, using the following equation:

$$\text{Detection limit} = 3\sigma/k$$

where σ is the standard deviation of blank measurements and k is the slope of the plot of fluorescence intensity vs H_2S concentration. The standard deviations $\sigma = 0.0011$.

6. The capabilities of M-H_2S for detecting H_2S at different pH

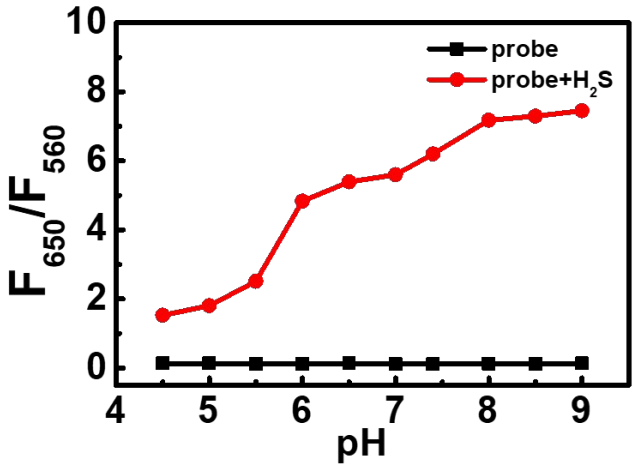

Fig. S4. Fluorescence responses of **M-H_2S** (10 μM) in the absence and presence of H_2S (50 μM) at different pH.

7. High-resolution mass spectra of reaction system

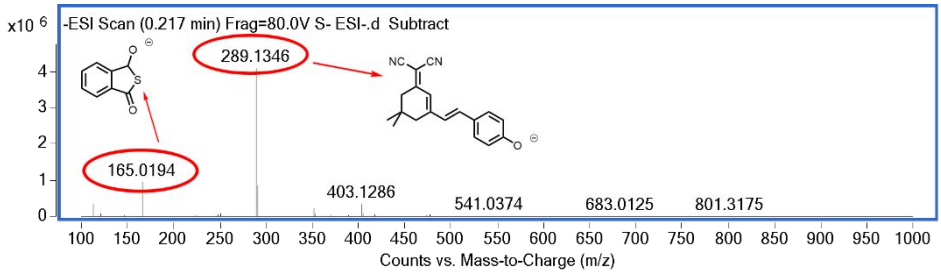

Fig. S5. The HRMS of the product after the reaction between **M-H_2S** and **H_2S**.

8. Cytotoxicity assay

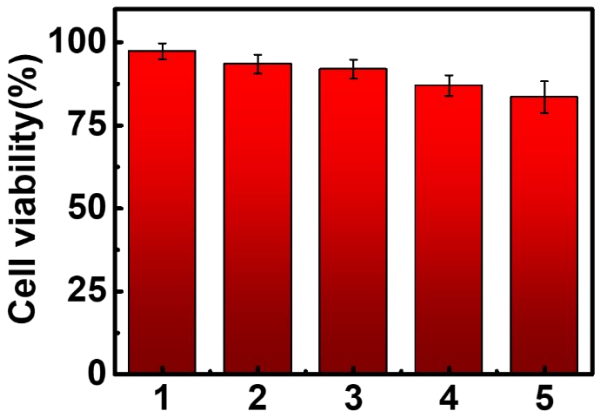

Fig. S6. MTT assay for estimating cell viability (%) of HeLa cells. The concentrations of probe **M-H_2S** were used: 1: blank, 2: 5 μM, 3: 10μM, 4: 15 μM, 5: 20 μM, respectively.

9. Characterization data

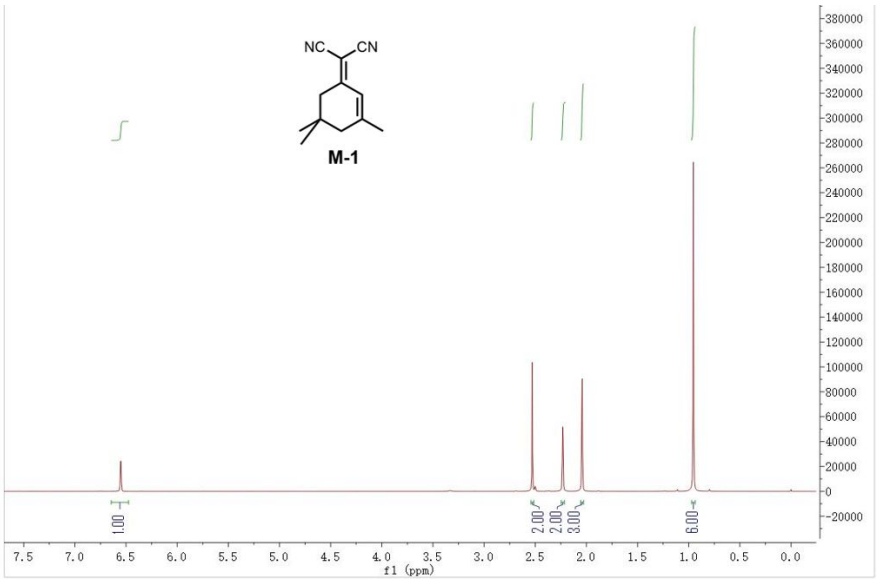

Fig. S7. ^{1}H NMR spectral of compound **M-1**.

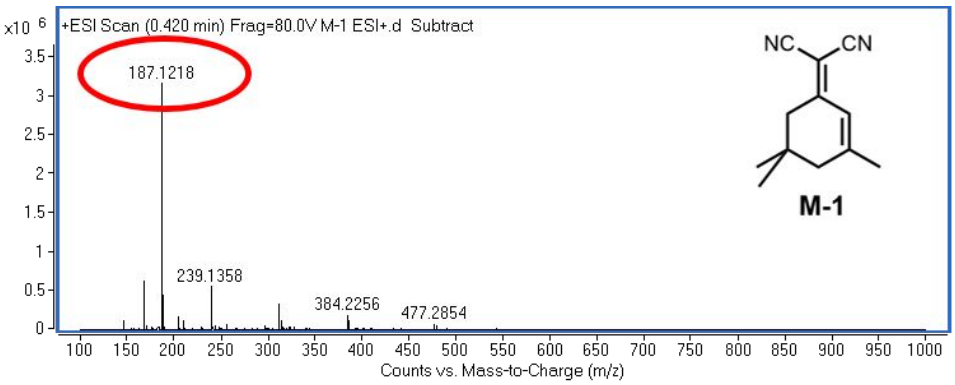

Fig. S8. HRMS spectral of compound **M-1**.

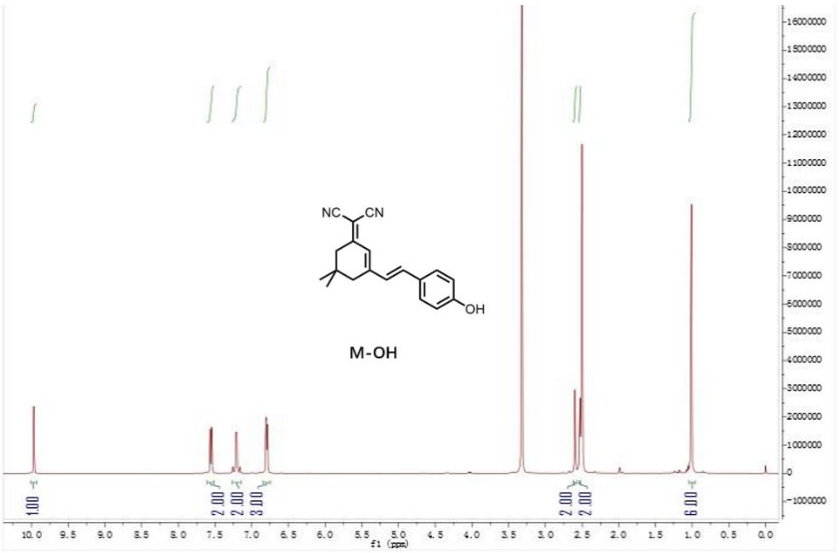

Fig. S9. ^{1}H NMR spectral of compound **M-OH**.

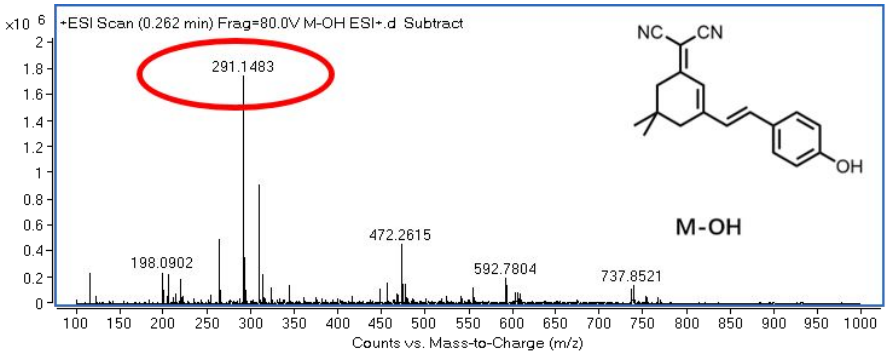

Fig. S10. HRMS spectral of compound **M-OH**.

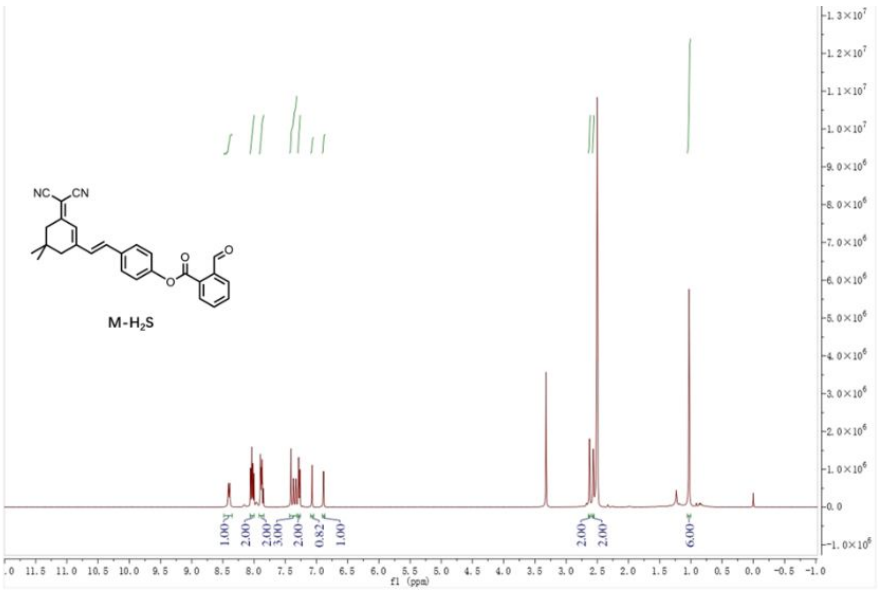

Fig. S11. ^{1}H NMR spectral of compound **M-H_2S**.

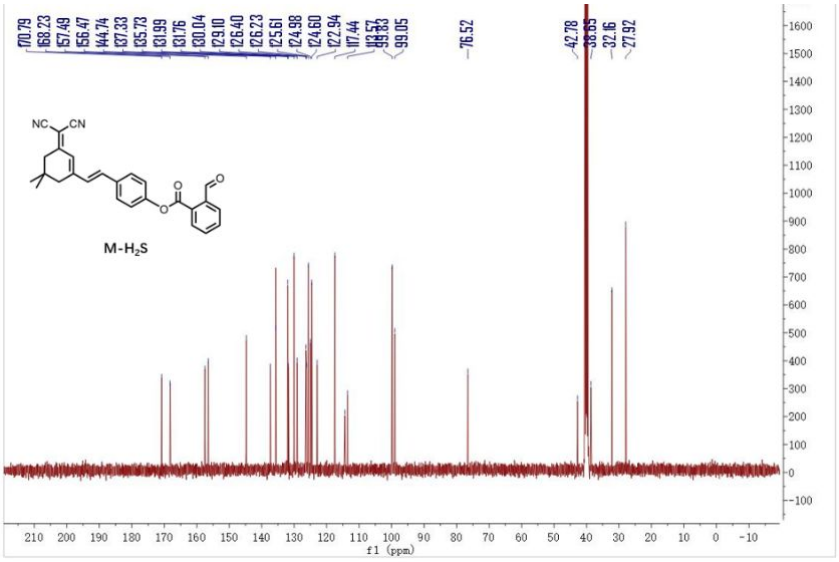

Figure. S12 ^{13}C NMR spectral of compound **M-H_2S**.

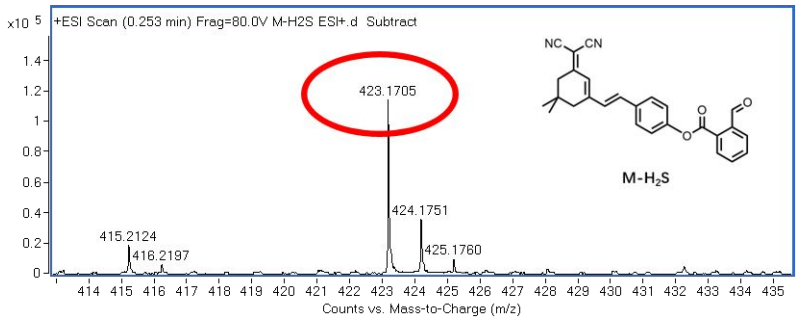

Fig. S13. HRMS spectral of compound **M-H_2S**.