Supporting information for

Visualizing cellular sodium hydrosulfite ($Na_2S_2O_4$) by azo-based fluorescent probes with high signal-to-noise ratio

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Materials and Instruments

Unless otherwise stated, all the reagents were commercially available and used without further purification. Twice-distilled water was used in all the experiments. HeLa cells and calf bovine were obtained from College of Life Science, Nankai University (Tianjin, China). UV/vis spectra were acquired with a Shimadzu UV-2700 spectrophotometer. Fluorescence spectra were measured with a Hitachi F-4600 spectrofluorimeter with a 10 mm quartz cuvette. High-resolution mass spectra (HRMS) were collected using a Bruker apex-Ultra mass spectrometer (BrukerDaltonics Corp., USA) in electrospray ionization (ESI) mode. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE III 400 MHz Digital NMR Spectrometer, using tetramethylsilane (TMS) as internal reference. LC-MS data were collected using Agilent 1200 series HPLC instrument. Cells imaging was performed with a Nikon A1R MP confocal microscope.

Synthesis of the probe NS1

To a solution of 4-aminobenzenesulfonic acid (519 mg, 3 mmol) in 2.5 mL 5% NaOH aqueous solution was added $NaNO_2$ (344 mg, 5 mmol). After the mixture was stirred vigorously at 0 ℃ for 30 min, 1.5 mL concentrated hydrochloric acid was added dropwise to provide diazonium salt solution. A solution of naphthalen-2-ol (432 mg, 3 mmol), NaOH (120 mg, 3 mmol) and Na_2CO_3 (210 mg, 2 mmol) in 20 mL water was stirred vigorously for 30 min under ice-salt bath. Then, diazonium salt solution was added dropwise to this solution under 0~5℃. After the mixture was stirred for 15 min at room temperature, the residue was filtered to provide red solid which was purified by silica column (CH_2Cl_2: MeOH = 5:1) to afford the probe **NS1** as red solid (423 mg, 43%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 15.85 (s, 1 H), 8.56 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 9.6 Hz, 1H), 7.81 (m, 5 H), 7.62 (t, 1 H), 7.49 (d, J = 7.2 Hz, 1H), 6.92 (d, J = 9.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): 170.19, 148.07, 145.14, 140.74, 133.19, 129.86, 129.65, 129.41, 128.33, 127.63, 126.45, 124.58, 121.90, 118.59. HRMS (ESI): m/z calculated for $C_{16}H_{12}N_2O_4S$ $[M-H]^-$ 327.0445, found: 327.0442.

Synthesis of the probe NS2

To a solution of 4-aminobenzenesulfonic acid (519 mg, 3 mmol) in 2.5 mL 5% NaOH aqueous solution was added $NaNO_2$ (344 mg, 5 mmol). After the mixture was stirred vigorously at 0 ℃ for 30 min, 1.5 mL concentrated hydrochloric acid was added dropwise to provide diazonium salt solution. A solution of 6-hydroxy-2-naphthoic acid (564 mg, 3 mmol), NaOH (120 mg, 3 mmol) and Na2CO3 (210 mg, 2 mmol) in 20 mL water was stirred vigorously for 30 min under ice-salt bath. Then, diazonium salt solution was added dropwise to this solution under 0~5℃. After the mixture was stirred for 15 min at room temperature, the residue was filtered to provide red solid which was purified by silica column (CH_2Cl_2: MeOH = 5:1) to afford the probe **NS2** as red solid (401 mg, 36%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.59 (d, J = 8.4 Hz, 1H), 8.39 (s, 1 H), 8.15 (m, 2 H), 7.86 (m, 4 H), 6.97 (d, J = 9.2 Hz, 1H), 3.17 (s, 1 H). ^{13}C NMR (DMSO-d_6, 100 MHz): 170.40, 148.24, 145.14, 141.09, 135.14, 130.95, 129.92, 129.80, 127.71, 127.64, 124.88, 121.56, 118.85. HRMS (ESI): m/z

calculated for $C_{17}H_{12}N_2O_6S$ $[M-H]^-$ 371.0343, found: 371.0347.

Cytotoxicity experiment

Cytotoxicity of the probes **NS1** and **NS2** was evaluated using the MTT assay. HeLa cells were seeded into 96 well-plates at the density of about 8000 cells/well. After 24 h, various concentrations of **NS1** or **NS2** were added into wells for the further cultured of 24 h. Then, 10 μL MTT (5 μg/mL) was mixed into cells for incubated another 4 h. After that, 100 μL of DMSO were used to resolve the formazan, and then the plate was shaken for 30 min. Finally, the absorbance was determined at 570 nm by a microplate reader (Thermo Fisher Scientific). Cell viability was expressed as a percentage of the control culture value.

Cells culture and imaging.

HeLa cells were cultured in modified Eagle's medium supplemented with 10 % calf bovine serum in an atmosphere of 5 % CO_2 and 95 % air at 37 °C. The cells were seeded into the glass-bottom culture dishes and cultured for 24 h. The HeLa cells were incubated with 5 μM **NS1** or **NS2** for 20 min at 37 °C, then the media was replaced with PBS buffer and the cells were treated with 200 μM $Na_2S_2O_4$ for 30 min. The imaging was acquired using a Nikon A1 MP confocal microscope.

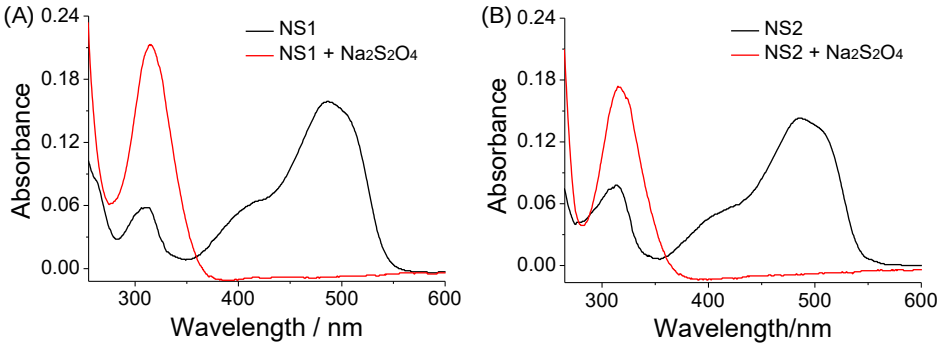


Fig. S1 Absorption spectra of 5 μM **NS1** (A) and **NS2** (B) in absence or presence of 250 μM $Na_2S_2O_4$ in PBS (pH = 7.4, 20% EtOH, 20 mM).

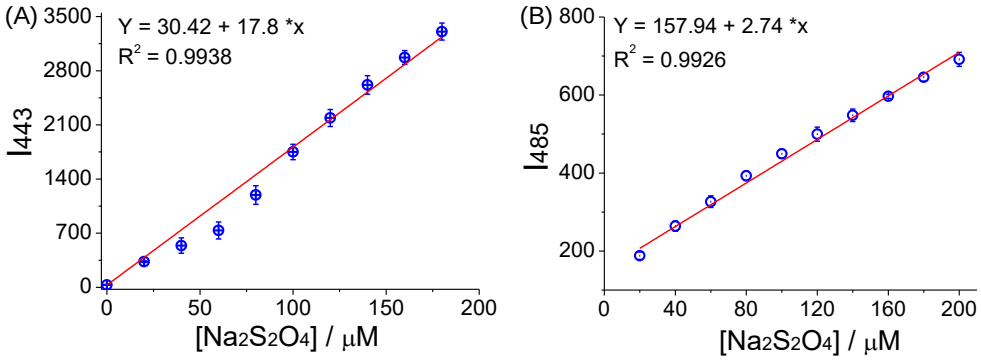


Fig. S2 Linear relationship between fluorescence intensity and $Na_2S_2O_4$ concentration for **NS1** (A) and **NS2** (B).

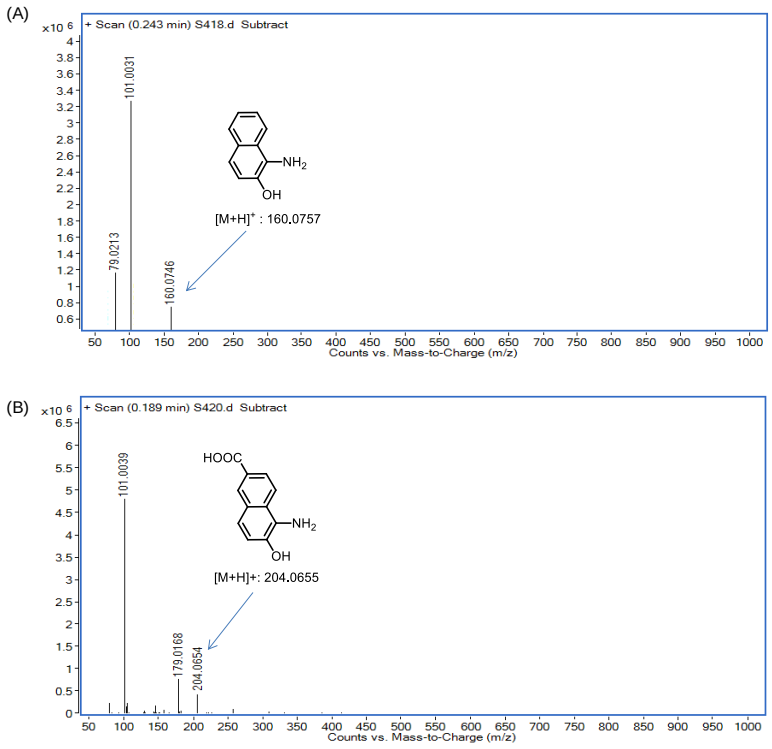


Fig. S3 HRMS data of the **NS1** (A) and **NS2** (B) after the treatment with $Na_2S_2O_4$.

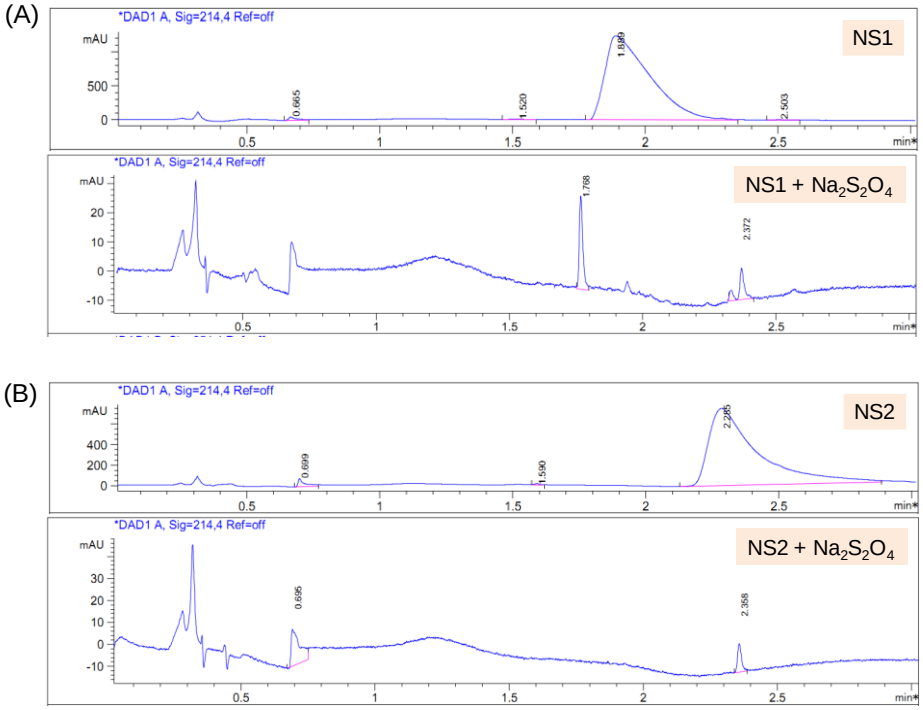


Fig. S4 HPLC data of **NS1** (A) and **NS2** (B) in absence and presence of $Na_2S_2O_4$.

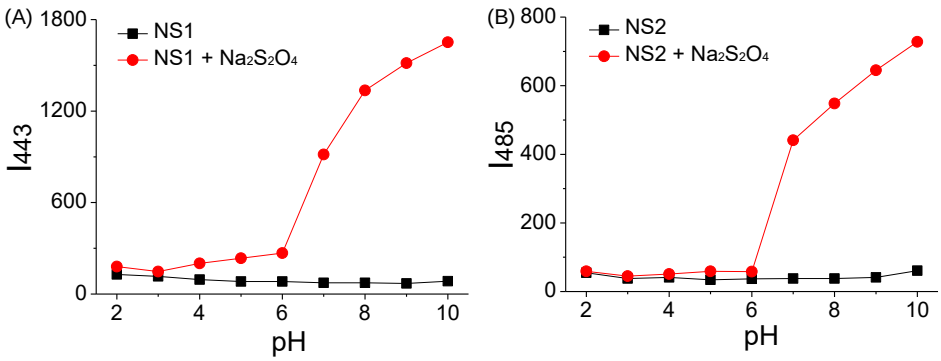


Fig. S5 Fluorescence intensity at 443 nm of 5 μM **NS1** (A) and **NS2** (B) in absence and presence of 100 μM $Na_2S_2O_4$ at various pH. λ_{ex} = 360 nm.

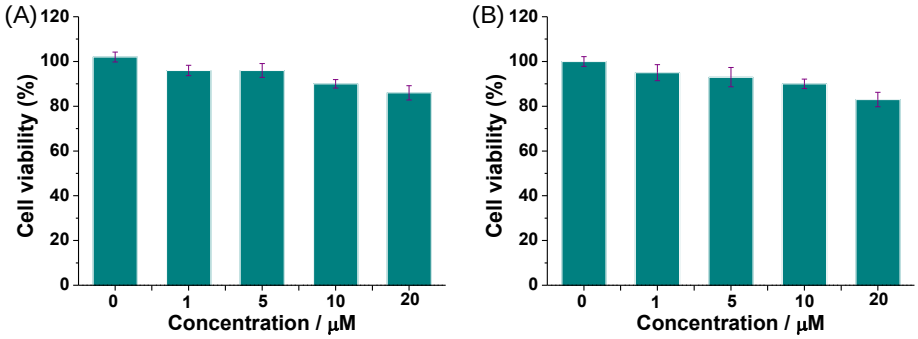


Fig. S6 Survial of HeLa cells in the presence of **NS1** (A) and **NS2** (B) at various concentrations measured using MTT assay.

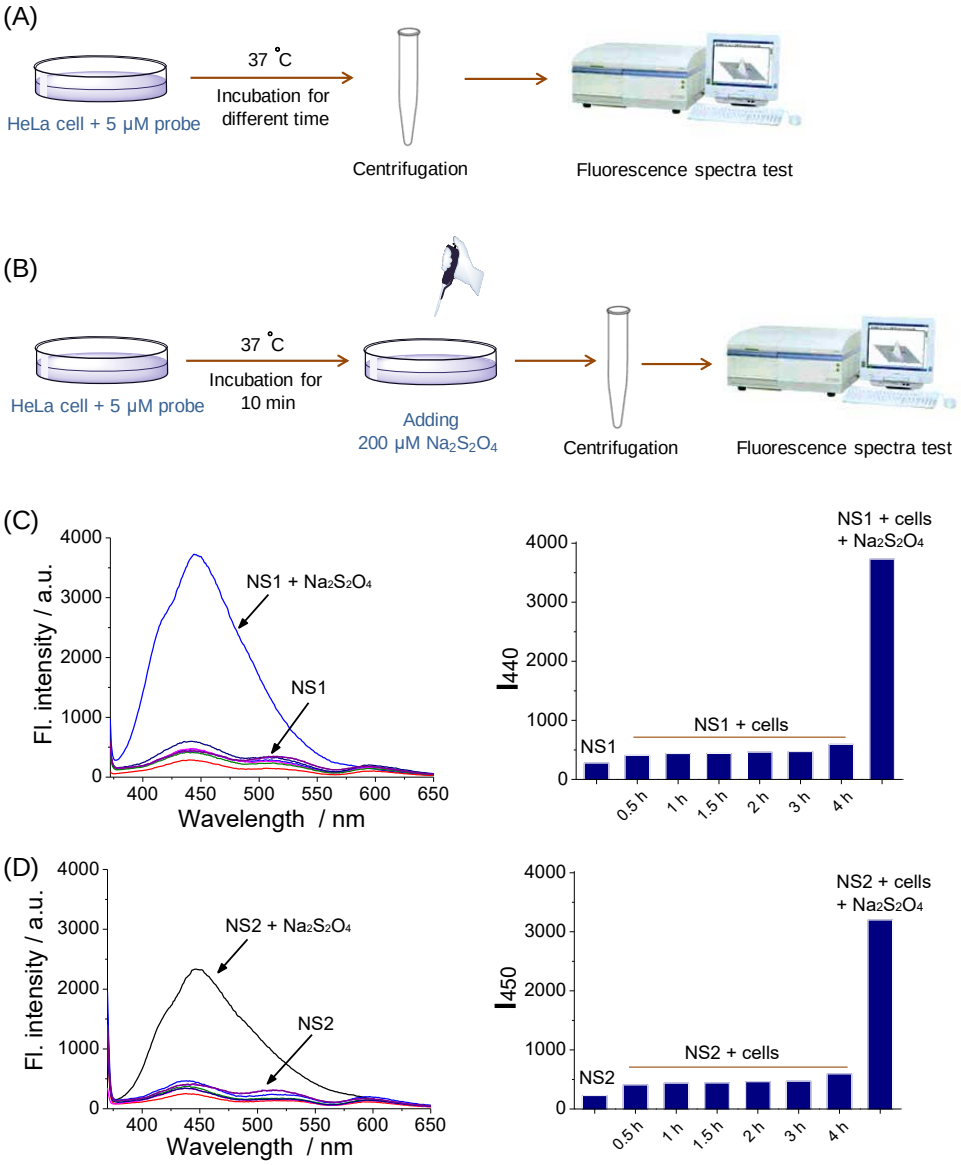


Fig. S7 (A) Experimental procedure for the evaluation of the stability of the probes in living HeLa cells. (B) Experimental procedure for the evaluation of the fluorescence response of the probes to $Na_2S_2O_4$ in living HeLa cells. (C) Fluorescence spectra of **NS1** in dulbecco's modified eagle medium (DMEM), **NS1** in living cells after different incubation time, and **NS1** in living cells after the treatment of 200 μM $Na_2S_2O_4$. (D) Fluorescence spectra of **NS2** in dulbecco's modified eagle medium (DMEM), **NS2** in living cells after different incubation time, and **NS2** in living cells after the treatment of 200 μM $Na_2S_2O_4$. λ_{ex} = 360 nm.

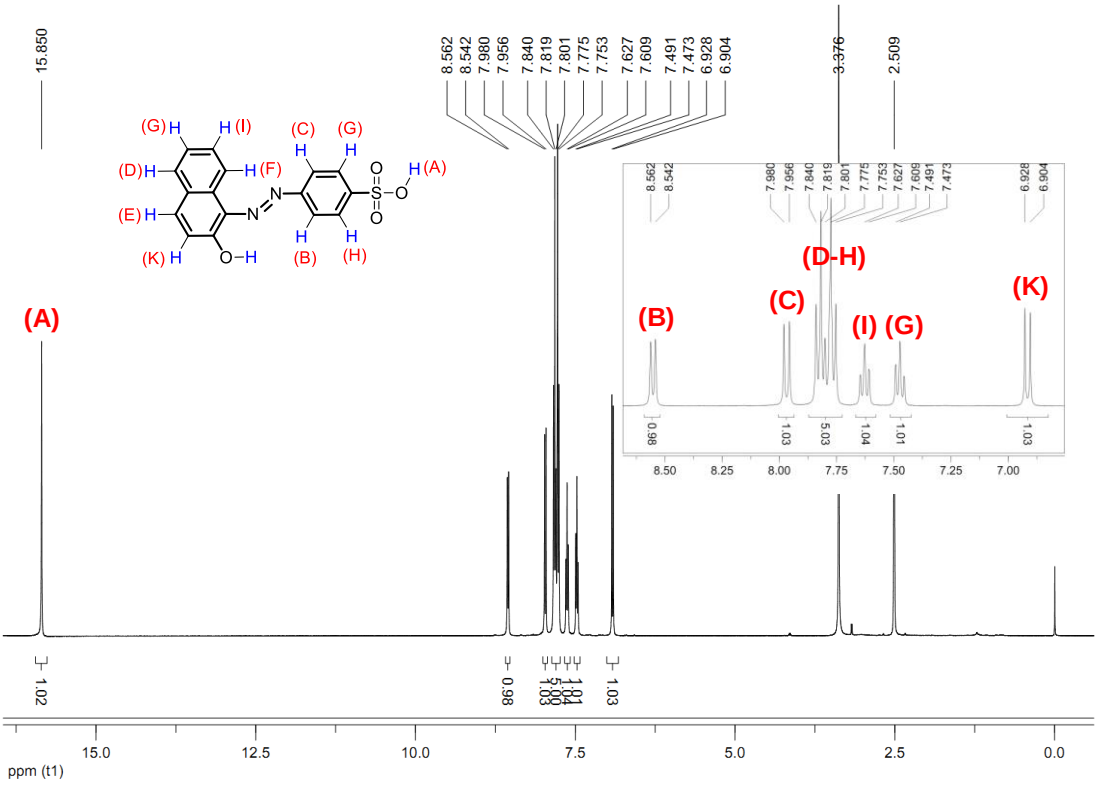


Fig. S8 ^{1}H NMR spectrum (DMSO-d_6) of the probe **NS1**.

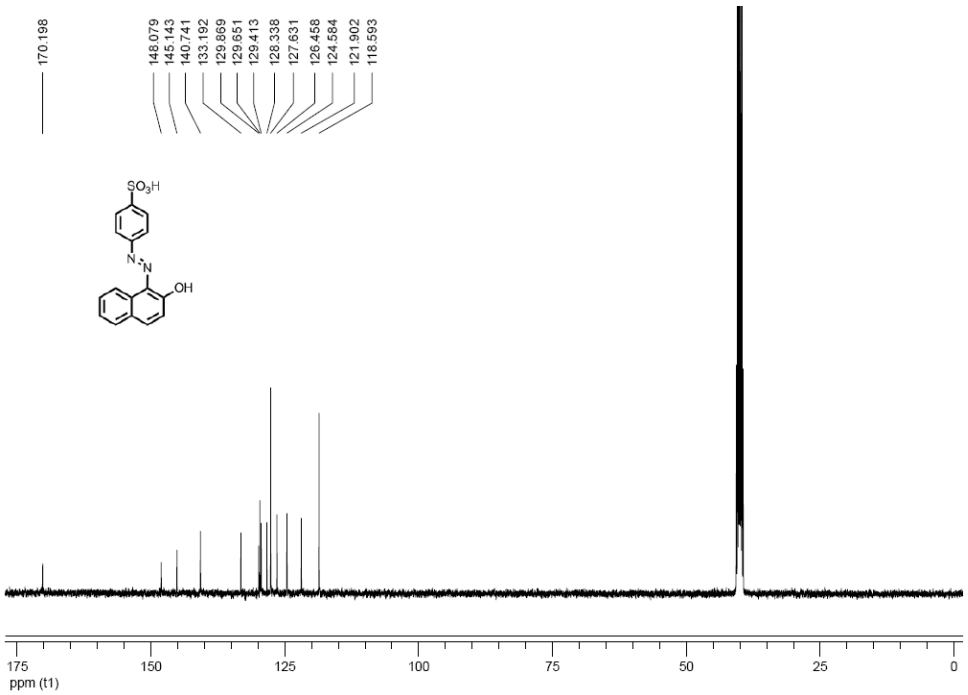


Fig. S9 ^{13}C NMR spectrum (DMSO-d_6) of the probe **NS1**.

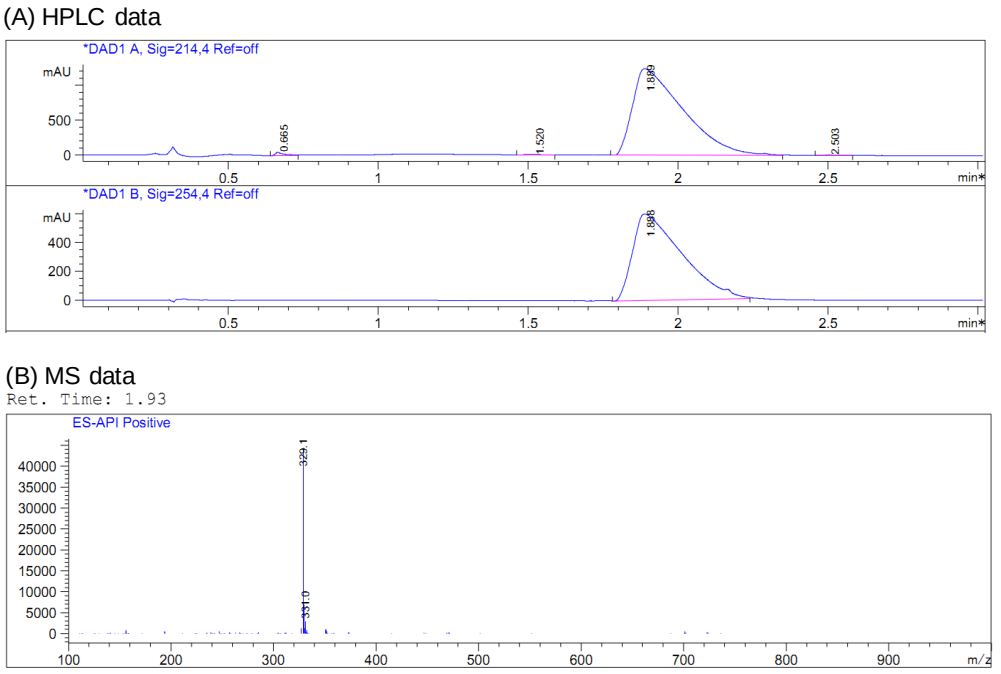


Fig. S10 LC-MS data of the probe **NS1**.

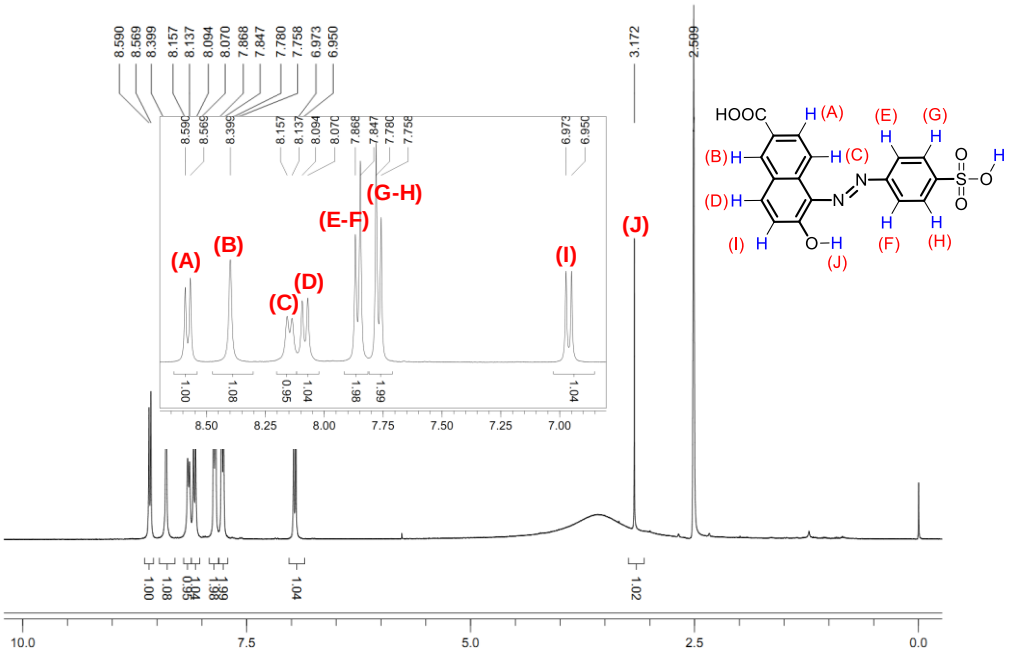


Fig. S11 ^{1}H NMR spectrum (DMSO-d_6) of the probe **NS2**.

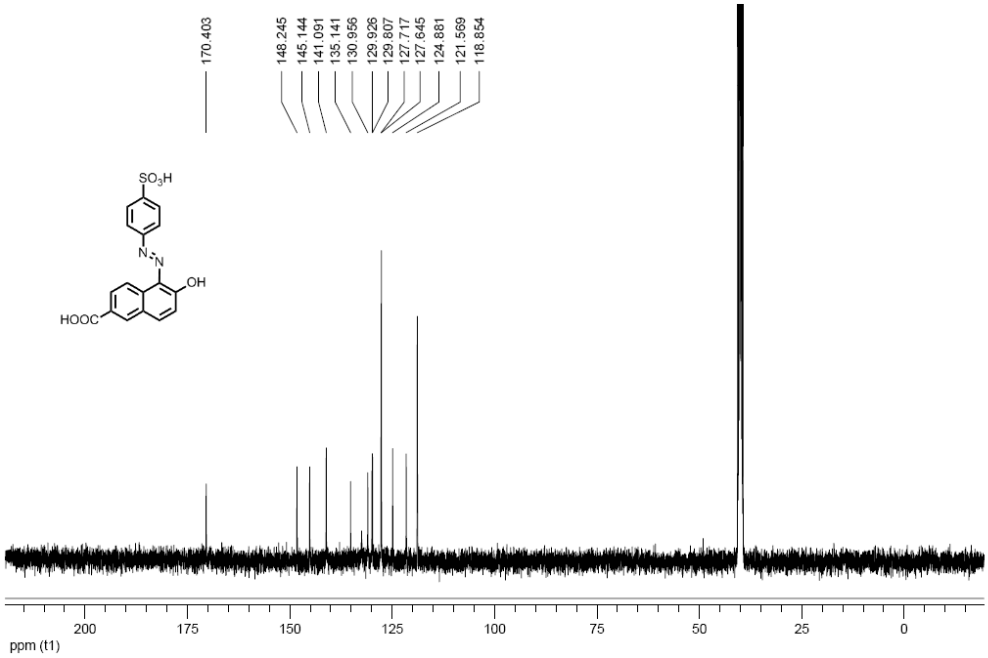
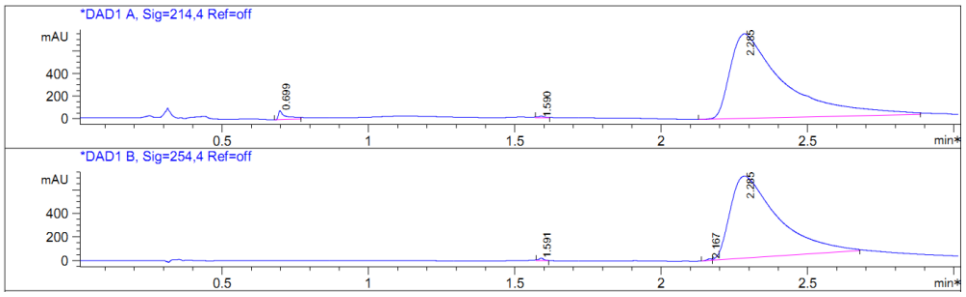


Fig. S12 ^{13}C NMR spectrum (DMSO-d_6) of the probe **NS2**.

(A) HPLC data



(B) MS data

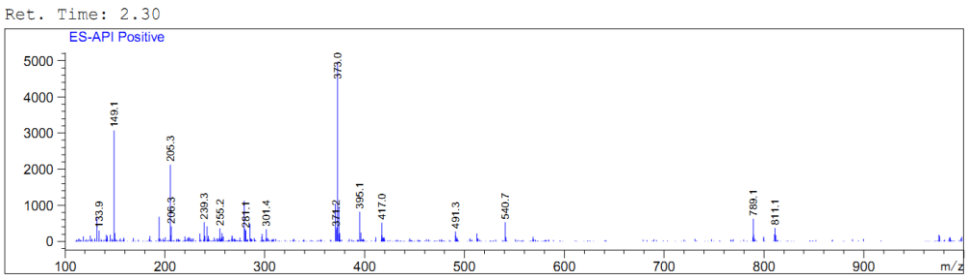


Fig. S13 LC-MS data of the probe **NS2**.