Electronic Supplementary Information

Carbon-dot-based fluorescent ratiometric sensor for detecting hydrogen sulfide in aqueous media and inside live cells

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Experimental Section

Materials and reagents: 4-Bromo-1,8-naphthalic anhydride, ethanolamine, ethylenediamine, glycerol, sodium salts of anions (HS^-, F^-, Cl^-, Br^-, I^-, CO_3^{2-}, NO_3^-, N_3^-, HPO_4^{2-}, SCN^-, SO_4^{2-}, SO_3^{2-}, HSO_3^-, $S_2O_3^{2-}$), glutathione, cysteine and succinic anhydride, dimethylsulfoxide (DMSO for HPLC) and citric acid were purchased from Sigma-Aldrich and used as received. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysulfosuccinimide sodium salt, 4-dimethylaminopyridine (DMAP) was purchased from Alfa Aesar. Fetal bovine serum was supplied by Hangzhou Sijiqing Biological Engineering Materials Co. Ltd. N,N-Dimethyl-formamide (DMF) was dried with CaH_2 and vacuum distilled. Ethanol and tetrahydrofuran (THF) were analytically pure solvents and distilled before use. The water used throughout the experiments was the triple-distilled water which was further treated by ion exchange columns and then by a Milli-Q water purification system.

Synthesis of the probe (naphthalimide-azide): Ethanolamine (0.183 g, 3.0 mmol) was added to the solution of 4-bromo-1,8-naphthalic anhydride (0.69 g, 2.5 mmol) in ethanol (40 mL). The mixture was heated to reflux and stirred for 4 h. After cooling to room temperature, the reaction mixture was filtered. The precipitate was collected and then purified by recrystallization from ethanol to give a pale solid as 6-bromo-2-(2-hydroxyethyl)-*1H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione (Compound **1)** (0.69 g, yield: 86.3%). ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 8.48-8.50 (d, 1H), 8.44-8.46 (d, 1H), 8.24-8.26 (d, 1H), 8.13-8.15 (d, 1H), 7.92-7.96 (t, 1H), 4.80-4.83 (t, 1H), 4.11-4.13 (t, 2H), 3.62-3.65 (t, 2H).

Next, the mixture of Compound **1** (0.32 g, 1.0 mmol) and sodium azide (0.65 g, 10 mmol) in 8 mL of dry N,N-dimethylformide (DMF) was heated to 105 °C for 10 h. After cooling to room temperature, the reaction mixture was poured into 40 mL of water and then extracted with ethyl acetate. The organic phase was collected, washed with brine, and dried with anhydrous $MgSO_4$. The solvent in the filtrate was removed under reduced pressure and the solid residue was purified by flash chromatography column using methanol/dichloromethane (v/v 1:20, R_f=0.50) to afford a light yellow solid as Compound **2** (6-azido-2-(2-hydroxyethyl)- *1H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione) (0.233 g, yield: 82.7%). ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 8.46-8.48 (d, 1H), 8.38-8.40 (d, 1H), 8.33-8.35 (d, 1H), 7.79-7.83 (t, 1H), 7.67-7.69 (d, 1H), 4.79-4.82 (t, 1H), 4.11-4.12 (t, 2H), 3.61-3.64 (t, 2H). MS (ESI): m/z 305.2 $[M+Na]^+$.

Compound **2** (0.12 g, 0.43 mmol) and 4-dimethylaminopyridine (2.9 mg, 0.023 mmol) were dissolved in 8 mL of anhydrous THF. The solution was cooled to 0 °C in an ice-water bath. Then the solution of succinic anhydride (0.43 g, 0.43 mmol) in 2 mL of anhydrous THF was added dropwise to the mixture and the resulting solution was stirred for 24 h at room temperature in the dark. The reaction mixture was then diluted with 1,2-dichloroethane (25 mL) and washed with 5% cold aqueous citric acid, brine and dried over anhydrous $MgSO_4$, The solvent was removed under reduced pressure and dried in vacuo at 45 °C to give the desired product as the probe (0.51 g, yield: 92.5%). ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 12.24 (s, 1H), 8.46-8.47 (d, 1H), 8.39-8.41 (d, 1H), 8.33-8.35 (d, 1H), 7.79-7.83 (t, 1H), 7.67-7.69 (d, 1H), 4.11-4.14 (t, 2H), 3.60-3.63 (t, 2H), 2.42 (m, 4H). MS (ESI): m/z 405.5 $[M+Na]^+$.

Preparation of amino-coated carbon dots (CDs): Amino-coated carbon nanodots were synthesized according to the literature (Xiaohui Wang, Konggang Qu, Bailu Xu, Jinsong Ren and Xiaogang Qu. *J. Mater. Chem.*, 2011, **21**, 2445-2450) with slight modification. Briefly, ethylenediamine (10 mL) and H_2O (6 mL) were added into 4 mL of glycerol/H_2O (2:1, wt) to form a clear solution which was sonicated for 5 min. Then the resulting solution was placed in a 750 W microwave oven for 6 min to obtain a yellow CDs solution. Finally, the CDs were dialyzed against water overnight and then stored at 4 °C. The concentration of carbon dots was about 7.5 mg/mL measured by the dry weight analysis method.

Preparation of the CD-based sensor: The CD-based sensor (the amount of probe conjugated onto CDs is 11.37 mg/g) was prepared as follows: The probe (38.2 mg, 0.1 mmol) was dissolved in DMSO (3 mL) under a N_2 atmosphere. 1-(3-Dimethylaminopropyl)-3 -ethylcarbodiimide hydrochloride (19.2 mg, 0.1 mmol) and N-hydroxysulfosuccinimide sodium salt (21.7 mg, 0.1 mmol) were added into the solution. After stirring for 1 h at room temperature, 2 mL solution of CDs, which was concentrated from the stored solution, was added dropwise to the mixture and the resulting solution was stirred for 36 h at room temperature in the dark. The reaction solution was dialyzed against water for 4 h and then against ethanol for 4 h, finally, diluted with ethanol and stored at 4 °C. The final concentration of the sensor solution was about 4.5 mg/mL measured by the dry weight analysis method.

The CD-based sensor (the amount of probe conjugated onto CDs is 4.48 mg/g) was prepared as follows: The probe (12.7 mg, 0.033 mmol) was dissolved in 3 mL of DMSO under a N_2 atmosphere. 1-(3-Dimethylaminopropyl)-3 -ethylcarbodiimide hydrochloride (6.4

mg, 0.033 mmol) and N-hydroxysulfosuccinimide sodium salt (7.2 mg, 0.033 mmol) were added into the solution. After stirring for 1 h at room temperature, 2 mL solution of CDs (concentrated from the stored solution) was added dropwise to the mixture and stirred for 36 h at room temperature in the dark. The reaction solution was dialyzed against water for 4 h and then against ethanol for 4 h, finally, diluted ethanol and stored at 4 °C.

The fluorescence responses of the sensor to H_2S: For the fluorescence responses of the sensing system to H_2S, the stock solution (4.5 mg/mL) of the sensor was diluted with HEPES buffer (50 mM, pH 7.4). Sodium hydrogen sulfide (NaHS) was used as the exogenous source of H_2S. Stock solutions of NaHS and various other ions (10^{-3} M) were prepared by dissolving their salts in pH 7.4 HEPES buffer. The test solution was prepared by adding the requisite amounts of stock solutions together, and then diluting with pH 7.4 HEPES buffer, the final solvent was HEPES buffered (pH 7.4) water-ethanol (3:1, v/v) and the final concentration of the CD-based sensor is 4.5 mg/mL. The test solution was stirred for 15 minutes, and then the fluorescence spectra were recorded.

Effect of pH Values: Fluorescence pH titrations were performed in buffer solutions at a sensor concentration of 0.45 mg/mL in different buffers from pH 4.0 to 9.0 containing ethanol (water: ethanol 3:1, v/v). Respectively, the buffers include Na_2HPO_4-citric acid buffer (pH 4.0), Na_2HPO_4-citric acid buffer (pH 5.0), PBS buffer (pH 6.0), HEPES buffer (pH 7.0), HEPES buffer (pH 8.0) and $NaHCO_3$-Na_2CO_3 (pH 9.0).

Determination of H_2S in Fetal Bovine Serum: To exclude the influence of the endogenous hydrogen sulfide in the serum, $ZnCl_2$ (1 μL in 100 μL blood, final concentration 1 mM) was used to trap any endogenous H_2S in serum according to literature reported method (Angew.

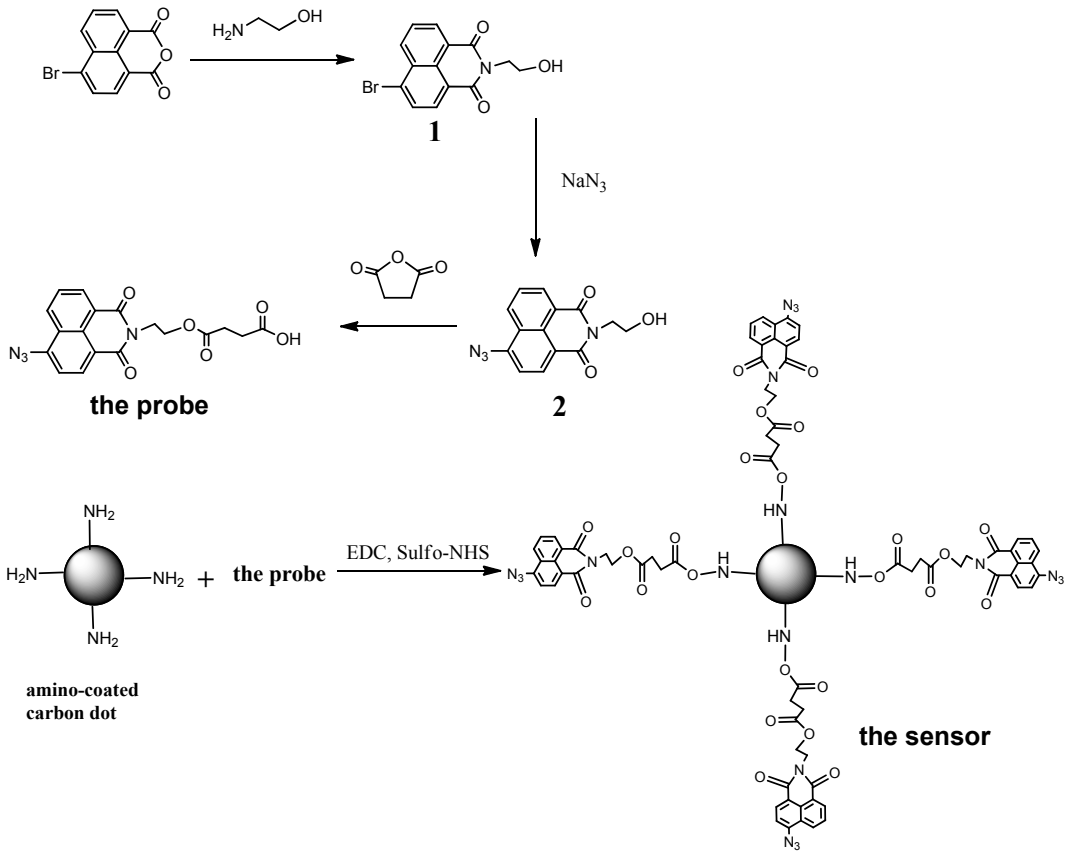
Chem. Int. Ed. 2011, 50, 9672-9675), then the sensor was added into the serum, and NaHS was added into the serum as the exogenous source of H_2S. The final concentration of the sensor was maintained at 0.45 mg/mL, while the concentrations of H_2S varied from 0 to 10 μM. The final concentration of fetal bovine serum is 10-fold diluted.

Cell Viability Assay: To examine the toxicity of the sensor in living cells, L929 cells (murine aneuploid fibrosarcoma cells) were incubated in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and allowed to grow for 24 h at 37°C with 5% CO_2. After removal of the medium, cells were treated with the sensor or plain CDs and incubated for an additional 24 h. The cytotoxicity of the sensor against L929 cells was assessed by MTT assay according to ISO 10993-5.

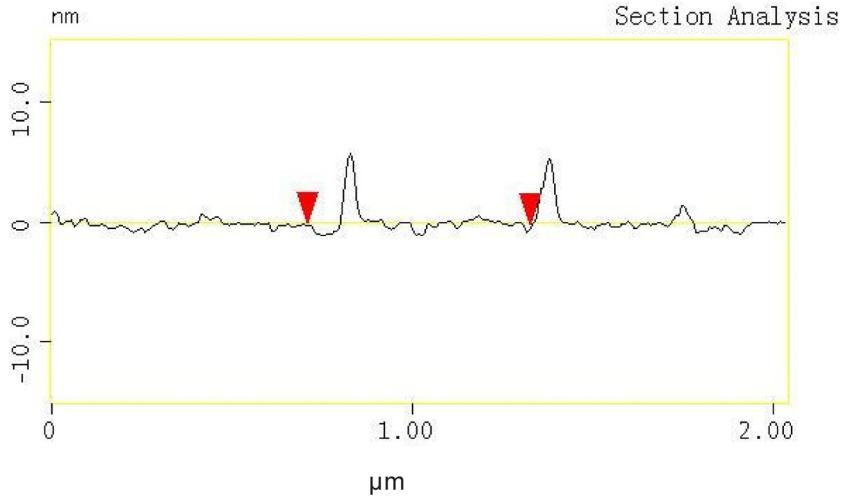
Cell incubation and imaging: Two cell lines, HeLa (human cervical cancer cell) and L929 (murine aneuploid fibrosarcoma cell), were incubated in RPMI1640 medium supplemented with 10% Fetal Bovine Serum (FBS, Invitrogen). One day before imaging, cells were passed and plated on polylysine-coated cell culture glass slides inside the 30-mm glass culture dishes and allowed to grow to 50-70% confluence. For the experiments, cells were washed with RPMI1640, incubated in RPMI1640 medium containing the sensor by use of the concentrated sensor stock solution (final sensor concentration 0.45 mg/mL containing 2% biology-grade DMSO) at 37°C under 5% CO_2 for 1.5 hour, and then treated with NaHS (30 μM and 100 μM respectively) for the 30 minutes. After that, the culture dishes were washed with PBS, then glass slides were taken out, the cells were washed with PBS for three times and then imaged on an Olympus IX71 inverted fluorescence microscope equipped with a DP72 color CCD (excitation filter: 330 – 385 nm, emission filter: 420 nm, dichromatic mirror: 400 nm).

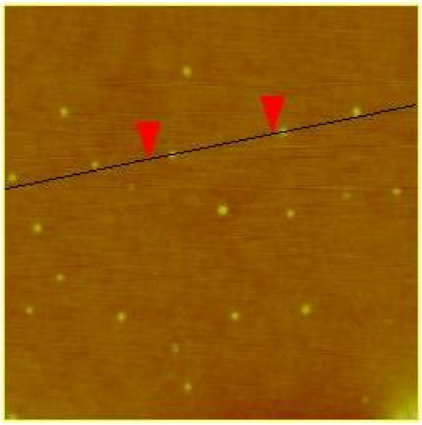
For the control sample (cells without being treated with the sensor or NaHS), cells were incubated for 2 h before being imaged on fluorescence microscope. For the cells being treated with only the plain CDs, one day before imaging, cells were passed and plated on polylysine-coated cell culture glass slides inside the 30-mm glass culture dishes and allowed to grow to 50-70% confluence. Afterwards, cells were washed with RPMI1640, incubated in RPMI1640 medium containing the plain CDs by use of the concentrated CD stock solution (final CD concentration 0.45 mg/mL containing 2% biology-grade DMSO) at 37°C under 5% CO_2 for 2 hour, then glass slides were taken out, the cells were washed with PBS for three times and then imaged on an Olympus IX71 inverted fluorescence microscope.

Measurements. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. Mass spectra were obtained through a Bruker Esquire HCT Plus mass spectrometer. UV-vis spectra were recorded on a Hitachi U-3010 UV-vis spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer. FT-IR spectra were measured using a MAGNA 760 (USA, Nicolet Instrument). The particle size and distribution was determined by dynamic light scattering (DLS) on a Malvern Nano-ZS90 particle size analyzer. Fluorescence images were obtained using an Olympus IX 71 with a DP72 color CCD. Transmission electronic microscopy (TEM) observation was carried out on a JEM-2010HR transmission electron microscopy (Japan). Atomic-force microscopy (AFM) was performed on a DI Veeko Multimode V atomic force microscope operated in the tapping mode.

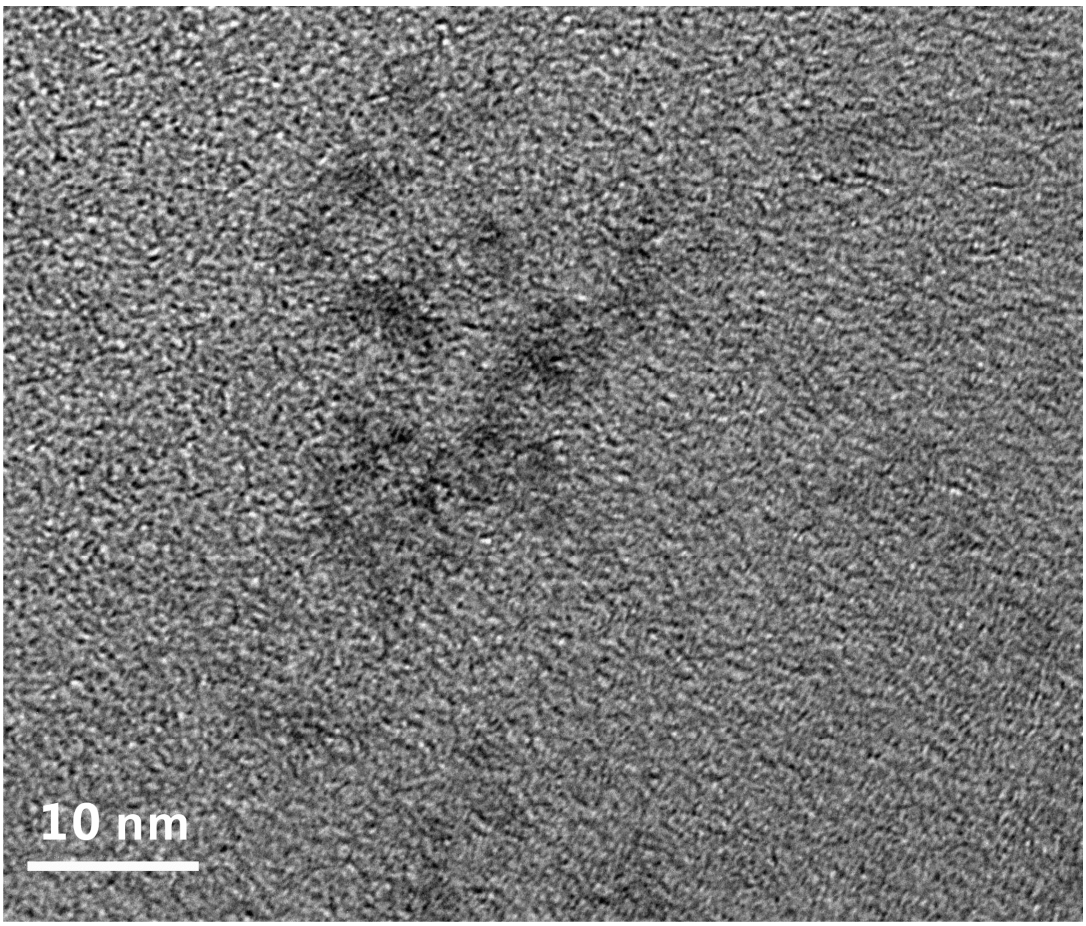


Scheme S1. Synthesis route for the carbon-dot-based sensor. In this scheme, the number of the probes on the CD does not reflect the actual number and is for illustration only.





(A)



(B)

Fig. S1. (A) AFM topography image of carbon dots on silicon substrate with the height profile along the line in the topographic image; (B) HR-TEM image of carbon dots on carbon-coated copper grid.

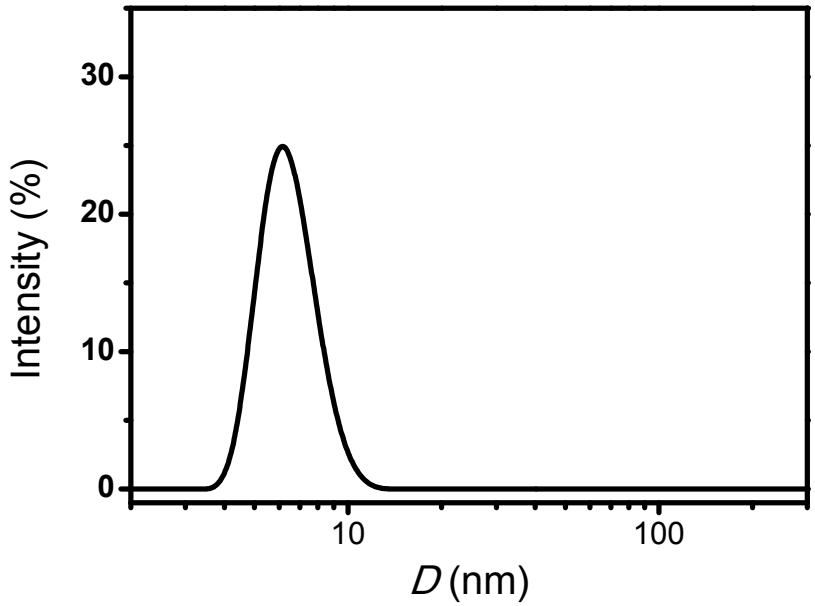


Fig. S2. Size distribution graph of carbon dots by dynamic light scattering.

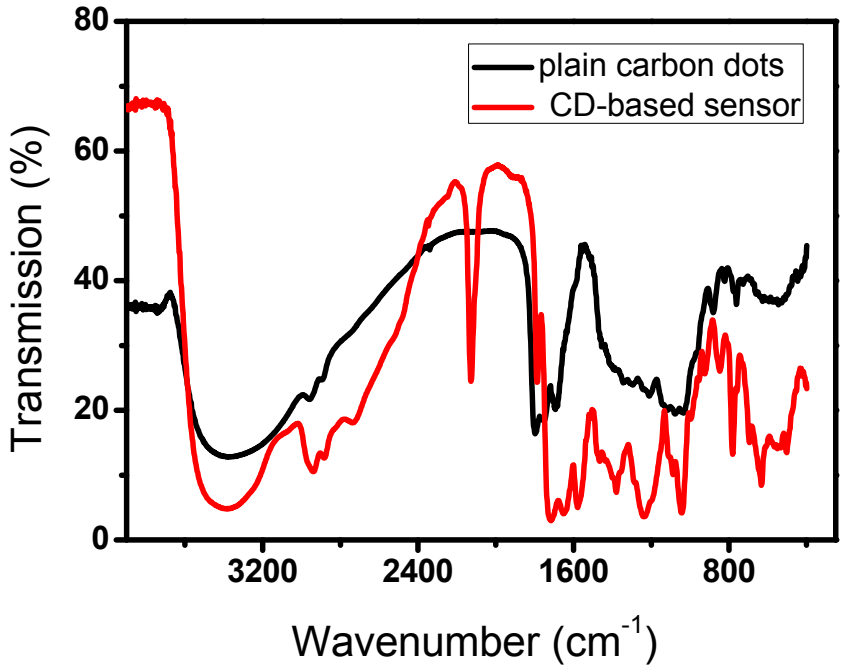
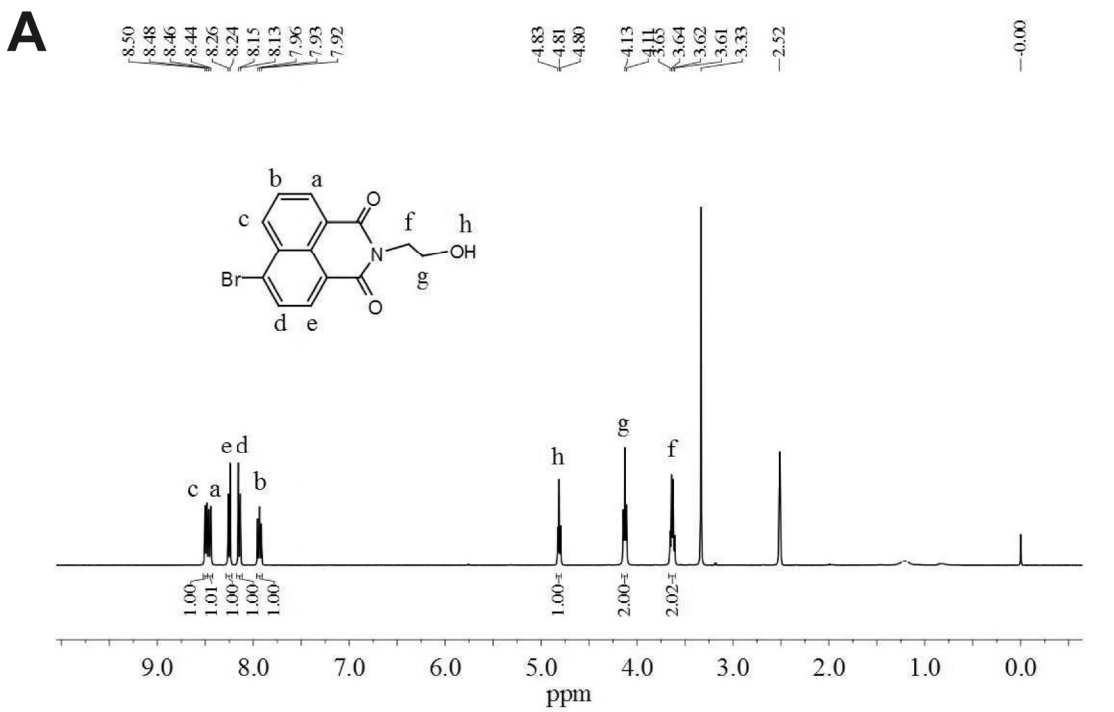
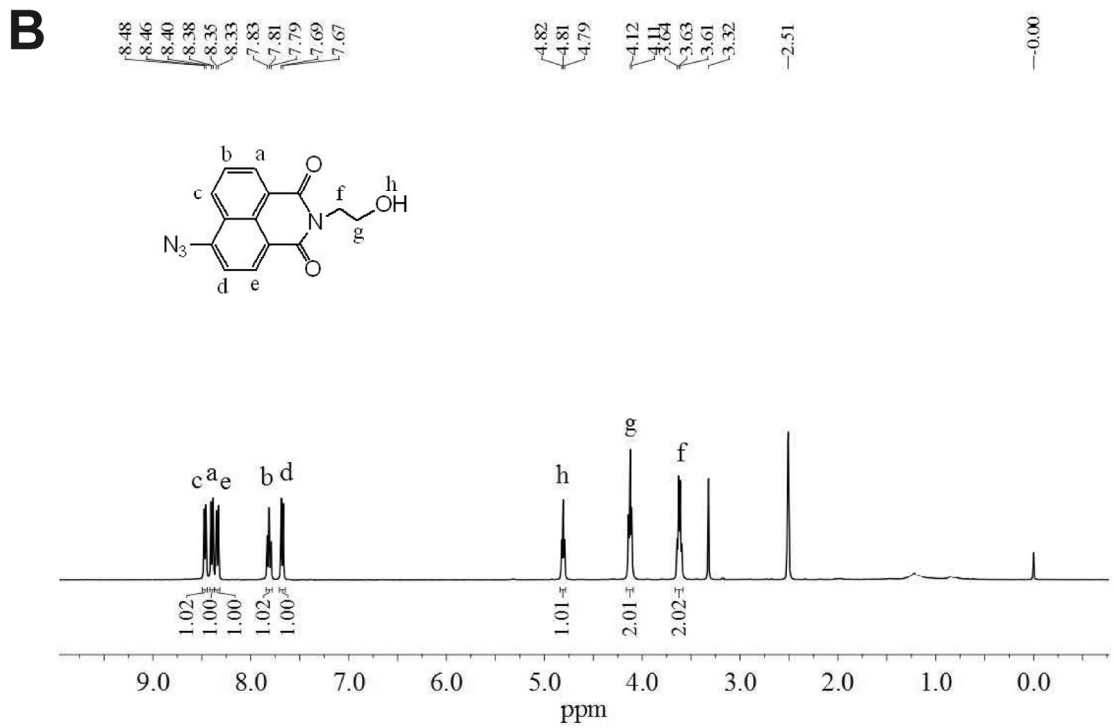


Fig. S3. FTIR spectra of plain carbon dots (black) and the CD-based sensor (red).

The successful azidation was evidenced by the appearance of azide stretching band at around 2120 cm^{-1}.


A
8.50
8.48
8.46
8.44
8.26
8.24
8.15
8.13
7.96
7.93
7.92
4.83
4.81
4.80
4.13
4.11
3.65
3.64
3.62
3.61
3.33
2.52
0.00
b a
c
f h
OH
N
Br
g
O
O
d e
e d
c a
b
h
g
f
1.00
1.01
1.00
1.00
1.00
1.00
2.00
2.02
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm


B
8.48
8.46
8.40
8.38
8.35
8.33
7.83
7.81
7.79
7.69
7.67
4.82
4.81
4.79
4.12
4.11
4.04
3.63
3.61
3.32
2.51
0.00
b a
c
f h
OH
N
N3
g
O
O
d e
c a e
b d
h
g
f
1.02
1.00
1.00
1.02
1.00
1.01
2.01
2.02
9.0
8.0
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6.0
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3.0
2.0
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0.0
ppm

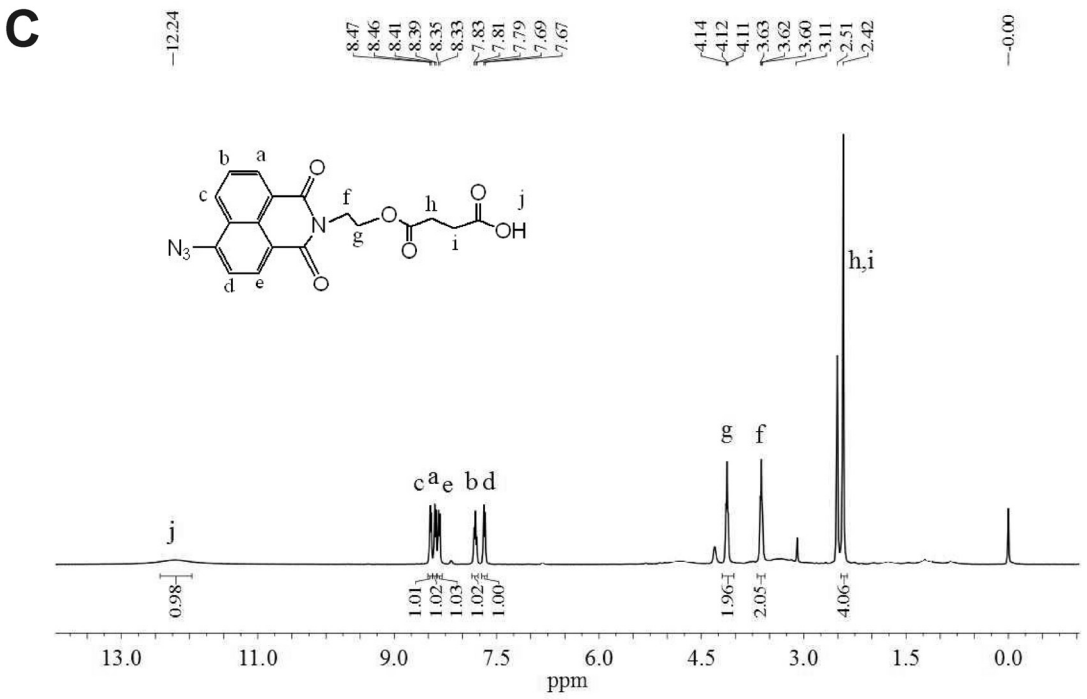


Fig. S4. ^{1}H NMR spectra (in DMSO-d6), A: 6-bromo-2-(2-hydroxyethyl)-*1H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione; B: 6-azido-2-(2-hydroxyethyl)-*1H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione; C: the probe --- naphthalimide-azide.

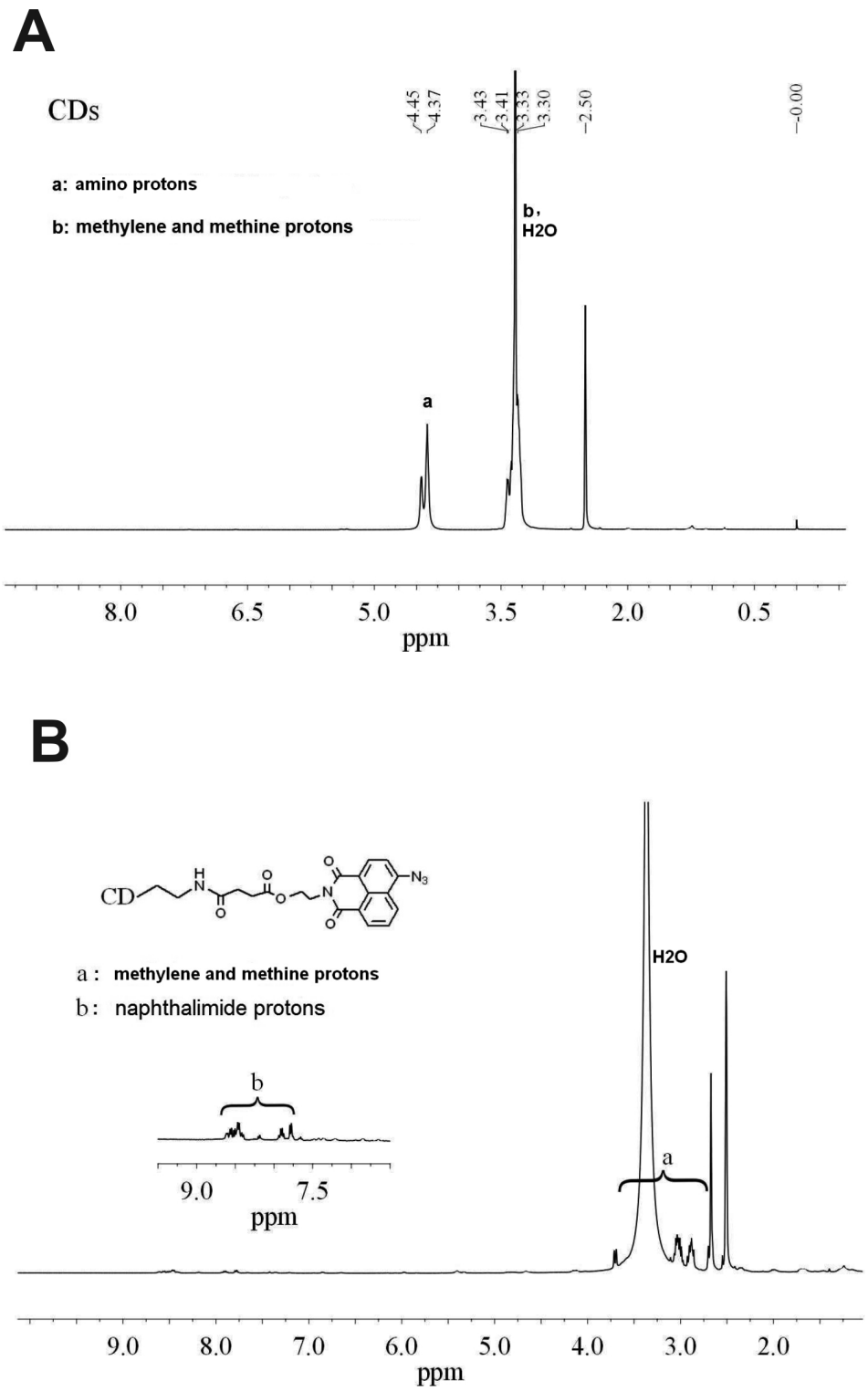
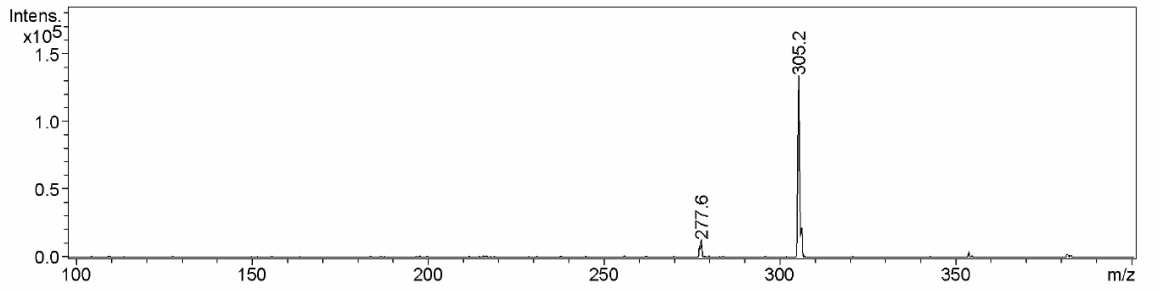
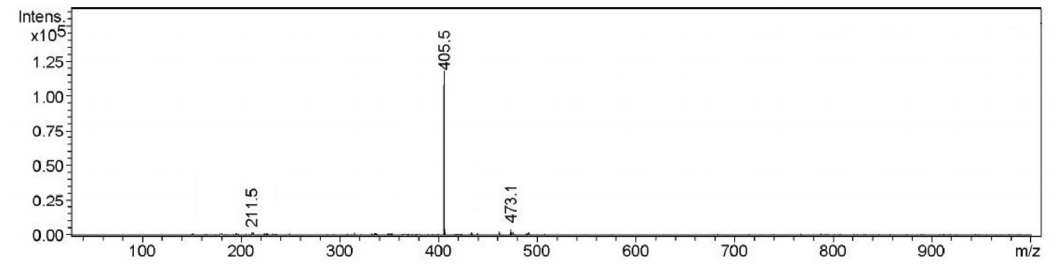


Fig. S5. ^{1}H NMR spectra (in DMSO-d6), A: plain carbon dots; B: the carbon-dot-based sensor.



#	m/z	I	I %
1	277.6	12612	9.4
2	279.8	925	0.7
3	305.2	133766	100.0
4	306.1	21484	16.1
5	354.5	837	0.6

(A)



#	m/z	I	I %	I %
1	405.5	117872	100.0	100.0
2	418.8	11357	9.6	9.6
3	421.5	10133	8.6	8.6
4	473.1	16004	3.6	3.6

(B)

Fig. S6. Mass spectra. (A) 6-azido-2-(2-hydroxyethyl)-*1H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione ESI MS m/z $[M+Na]^+$ 305.2; (B) the probe ESI MS m/z $[M+Na]^+$ 405.5.

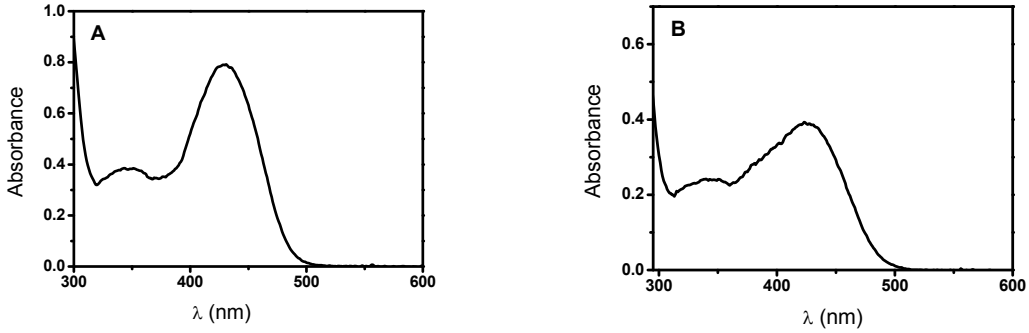


Fig. S7. Absorption spectra of the sensor (in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v)). A: the amount of probe conjugated onto CDs is 11.37 mg/g; B: the amount of probe conjugated onto CDs is 4.48 mg/g.

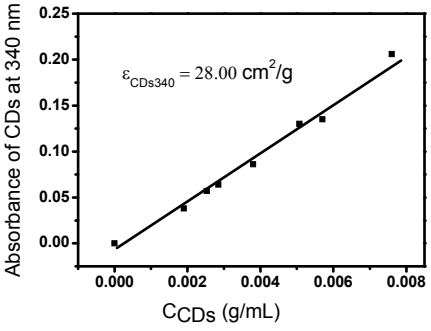
Calculation of the amount of the probe conjugated to CDs

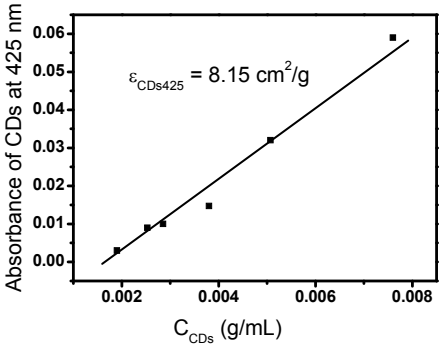
The amount of the probe conjugated to per gram of the CDs are calculated based on the additivity of absorbance of CDs and the probe. CDs produce the characteristic absorption band at 340 nm. Since the probe --- naphthalimide-azide exhibits characteristic absorption band at 370 nm which overlaps with the absorption of CDs; while upon reacting with NaHS, the probe turns into naphthalimide-amine and displays characteristic absorption at 425 nm (see Fig. S9) which will not overlap with the absorption of CDs; hence to more accurately determine the amount of probe conjugated to CDs, into the sensor solution or the probe solution was added excessive amount of NaHS, so as to transform the naphthalimide-azide into naphthalimide-amine. And the following equation set is used to calculate the conjugated amount of the probe:

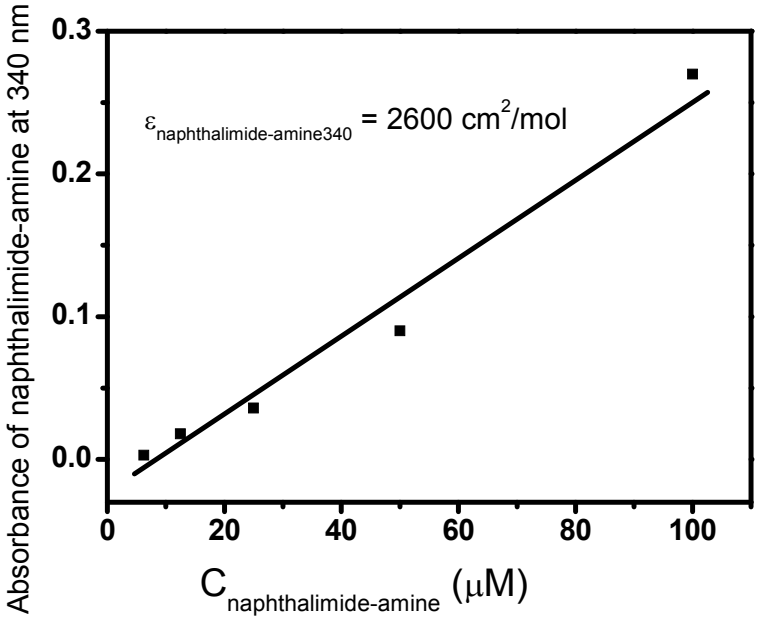
$$A_{340nm} = \varepsilon_{CDs340} \times C_{CDs} \times b + \varepsilon_{naphthalimide\text{-}amine340} \times C_{naphthalimide\text{-}amine} \times b$$
$$A_{425nm} = \varepsilon_{CDs425} \times C_{CDs} \times b + \varepsilon_{naphthalimide\text{-}amine425} \times C_{naphthalimide\text{-}amine} \times b$$

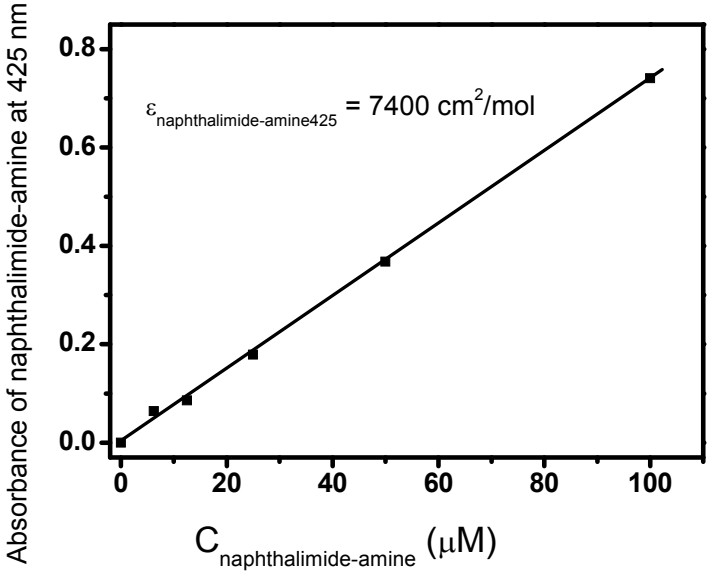
where A is the absorbance of the sensor at different wavelengths; ε and C are the absorptivity and the concentration of a given substance respectively; **b** represents the optical length 1 cm.

By measuring the absorption of the CDs at different concentrations and the absorption of naphthalimide-amine solution at different concentrations, ε_{CDs340}, ε_{CDs425}, $\varepsilon_{naphthalimide\text{-}amine340}$ and $\varepsilon_{naphthalimide\text{-}amine425}$ can be obtained. Then the absorption of the sensor in the presence of excessive amount of NaHS was measured, and by using the above equation, the concentrations of CDs and naphthalimide-amine can be calculated, and finally the amount of naphthalimide-amine on per gram of CDs can be determined. All absorbance measurements were performed in a quartz cell of 1 cm optical length.


Absorbance of CDs at 340 nm
ε_{CDs340} = 28.00 cm^2/g
C_{CDs} (g/mL)


Absorbance of CDs at 425 nm
ε_{CDs425} = 8.15 cm^2/g
C_{CDs} (g/mL)


Absorbance of naphthalimide-amine at 340 nm
$\varepsilon_{naphthalimide\text{-}amine340}$ = 2600 cm^2/mol
$C_{naphthalimide\text{-}amine}$ (μM)


Absorbance of naphthalimide-amine at 425 nm
$\varepsilon_{naphthalimide\text{-}amine425}$ = 7400 cm^2/mol
$C_{naphthalimide\text{-}amine}$ (μM)

For Fig. S7A:

$0.37 = 28.00 \times C_{CDs} + 2600 \times C_{naphthalimide\text{-}amine}$

$0.80 = 8.15 \times C_{CDs} + 7400 \times C_{naphthalimide\text{-}amine}$

Therefore, $C_{CDs} = 0.0035$ g/mL, $C_{naphthalimide\text{-}amine} = C_{naphthalimide\text{-}azide} = 104.21$ μM

Hence, the amount of the probe conjugated onto CDs is:

$104.21 \times 10^{-6} \times 1 \times 10^{-3} \times 382 \quad / 0.0035 = 11.37$ mg/g (382 is the molecular weight of naphthalimide-azide)

The amount of the probe conjugated to per gram of the CDs is calculated to be about 11.37 mg.

Similarly, for Fig. S7B, the amount of probe conjugated to per gram of the CDs is：

$0.24 = 28.00 \times C_{CDs} + 2600 \times C_{naphthalimide\text{-}amine}$

$0.39 = 8.15 \times C_{CDs} + 7400 \times C_{naphthalimide\text{-}amine}$

Therefore, $C_{CDs} = 0.0041$ g/mL, $C_{naphthalimide\text{-}amine} = C_{naphthalimide\text{-}azide} = 48.19$ μM

Hence, the amount of the probe conjugated onto CDs is:

$48.19 \times 10^{-6} \times 1 \times 10^{-3} \times 382 \quad / 0.0041 = 11.37$ mg/g (382 is the molecular weight of naphthalimide-azide)

The amount of the probe conjugated to per gram of the CDs is calculated to be about 4.48 mg.

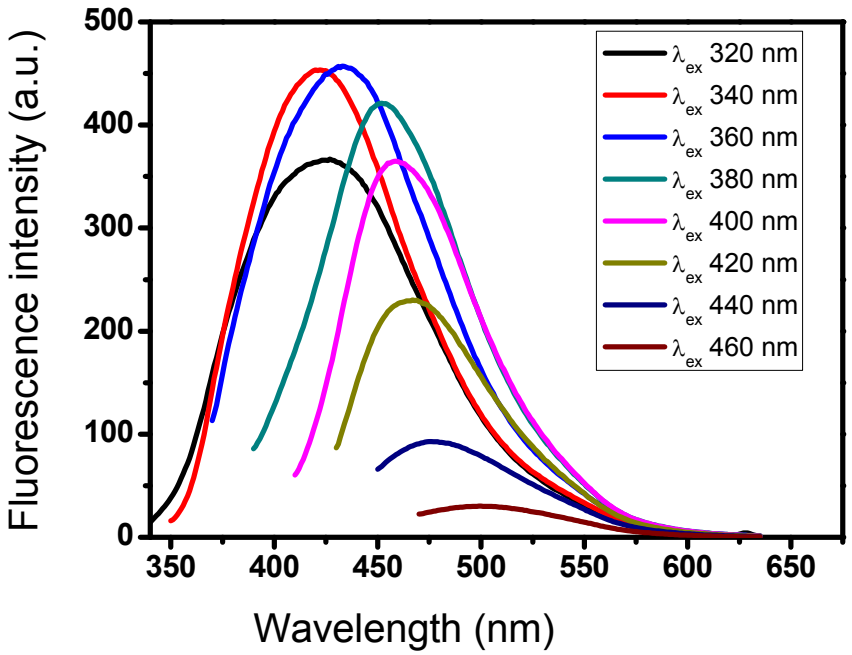


Fig. S8. Fluorescence emission spectra of carbon dots in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v) (0.40 mg/mL) with different excitation wavelengths.

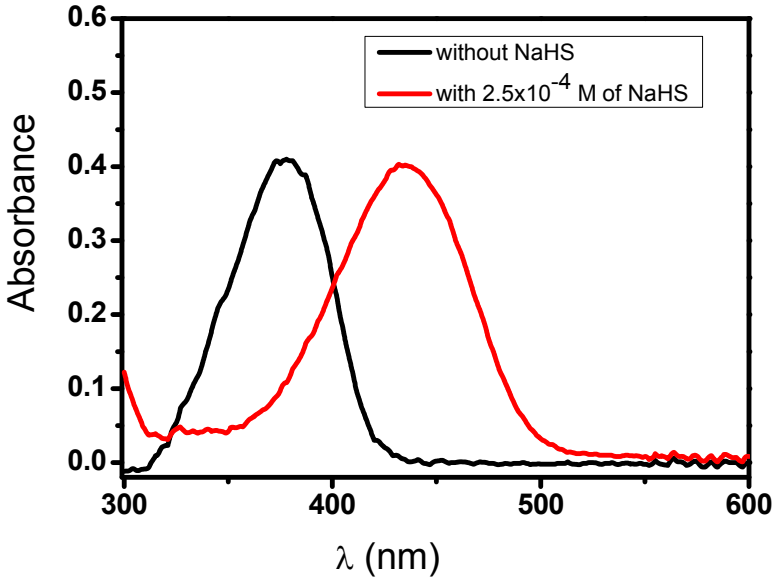


Fig. S9. Absorption spectra of the probe compound in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v) (5×10^{-5} M) before and after addition of NaHS (2.5×10^{-4} M).

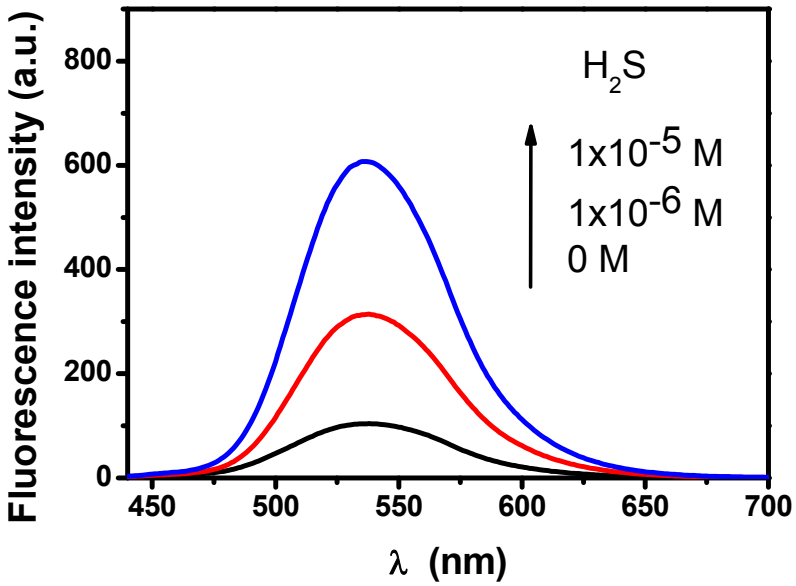


Fig. S10. Fluorescence emission spectra of the probe compound in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v) (1.5×10^{-5} M) before and after addition of H_2S. (λ_{ex} = 425 nm)

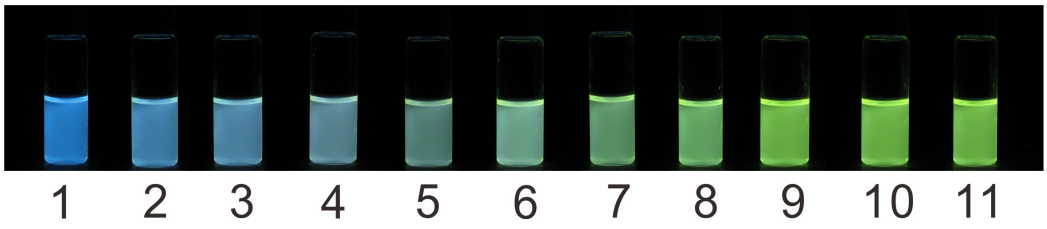


Fig. S11. Fluorescence change of the carbon-dot-based sensor in the absence of H_2S and upon addition of H_2S (the sensor: 0.45 mg/mL in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v)). 1: 0 μM H_2S; 2: 0.2 μM; 3: 0.5 μM; 4: 1 μM; 5: 2 μM; 6: 5 μM; 7: 10 μM; 8: 20 μM; 9: 50 μM; 10: 100 μM; 11: 200 μM. Photographs were taken under a hand-held 365 nm UV lamp.

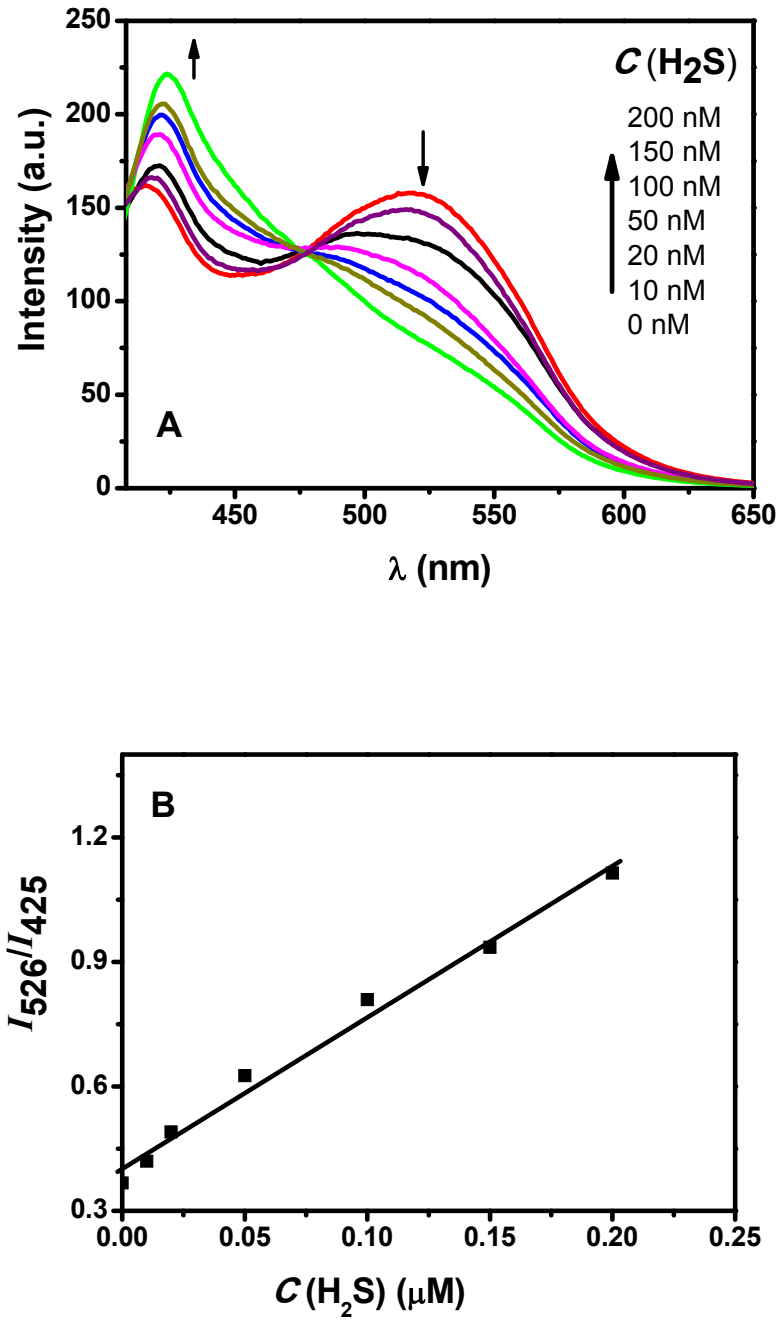


Fig. S12. (A) Fluorescence spectra of CD-based sensor (concentration: 0.15 mg/mL, the amount of probe conjugated onto CDs: 4.48 mg/g) in the presence of different amounts of H_2S in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v); (B) Fluorescence intensity ratio of CD-based sensor (concentration: 0.15 mg/mL, the amount of probe conjugated onto CDs: 4.48 mg/g) as a function of H_2S concentration in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v). (λexc = 340 nm)

The method for determining the detection limit:

First the calibration curve was obtained from the plot of fluorescence intensity ratio, I_{526}/I_{425}, as a function of the analyte concentration (H_2S). The regression curve equation was then obtained for the lower concentration part.
The detection limit = 3 × S.D./ k
where k is the slope of the curve equation, and S.D. represents the standard deviation for the CD-based sensor solution's fluorescence intensity ratio in the absence of H_2S.

I_{526}/I_{425} = 0.40 + 3.6 E6 [H_2S] (R = 0.993)
LOD = 3 × 0.012 / 3.6 E6 = 10 nM

References:
V. Thomsen, D. Schatzlein, D. Mercuro, *Spectroscopy* **2003**, *18,* 112-114.
A. D. McNaught, A. Wilkinson, *IUPAC Compendium of Chemical Terminology,* **1997**.

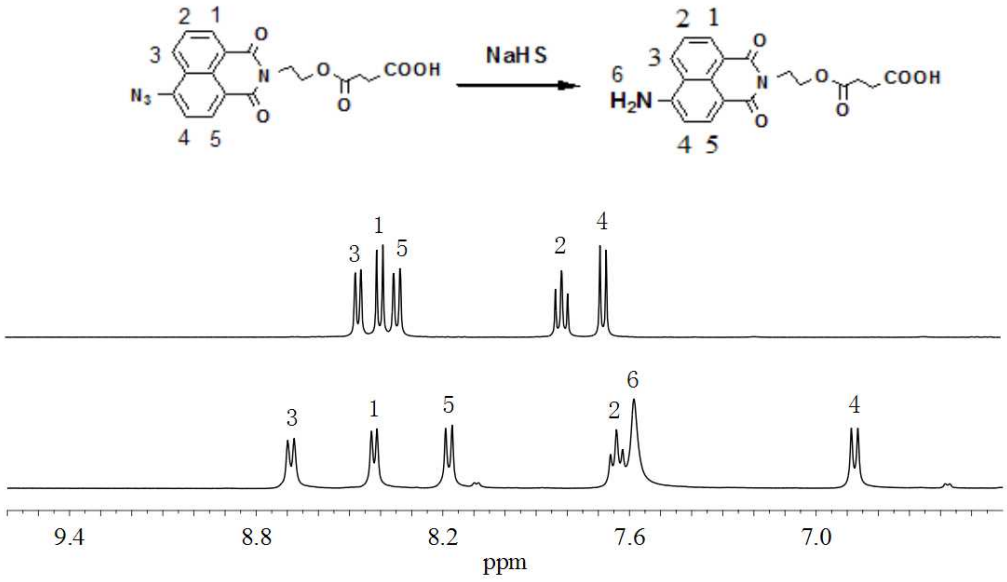


Fig. S13. Partial ^{1}H NMR spectra of the probe compound before and after addition of H_2S (5 equiv.) in DMSO-d6.

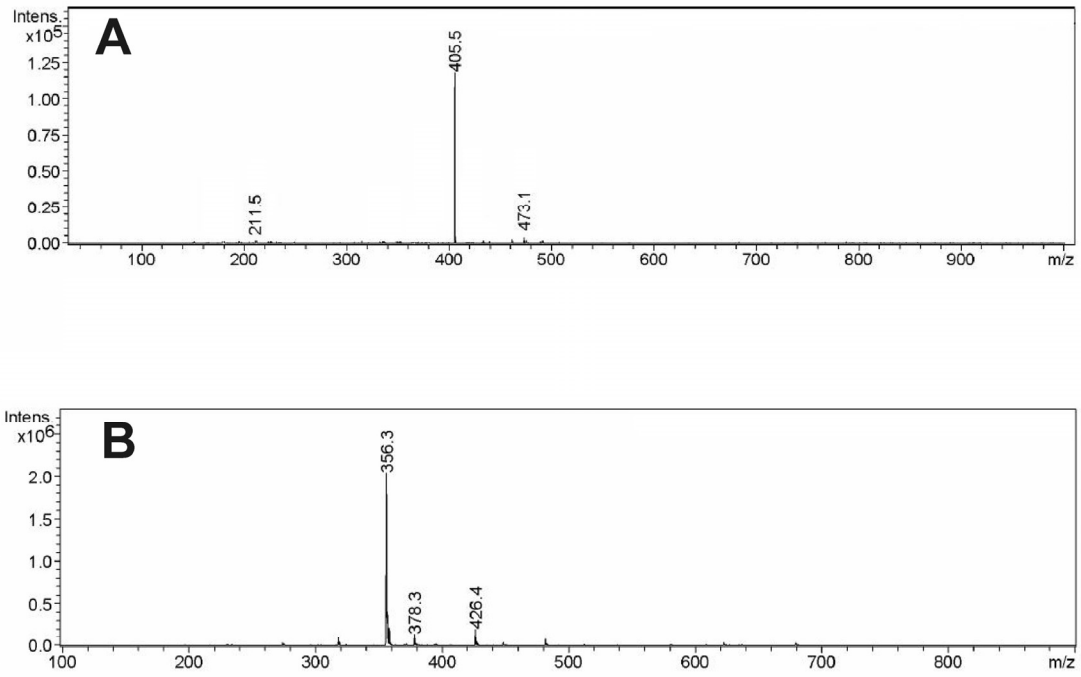


Fig. S14. Mass spectra of the as-prepared probe (A) and the resultant chemical after the probe being reduced upon addition of H_2S (B). For mass spectrum B, H_2S (5 equiv.) was added into the probe in ethanol under stirring for 20 min, then the solution was used for measurement. The signals at m/z 405.5 and m/z 356.3 are $[probe + Na]^+$ and $[naphthalimide\text{-}amine]^+$ respectively.

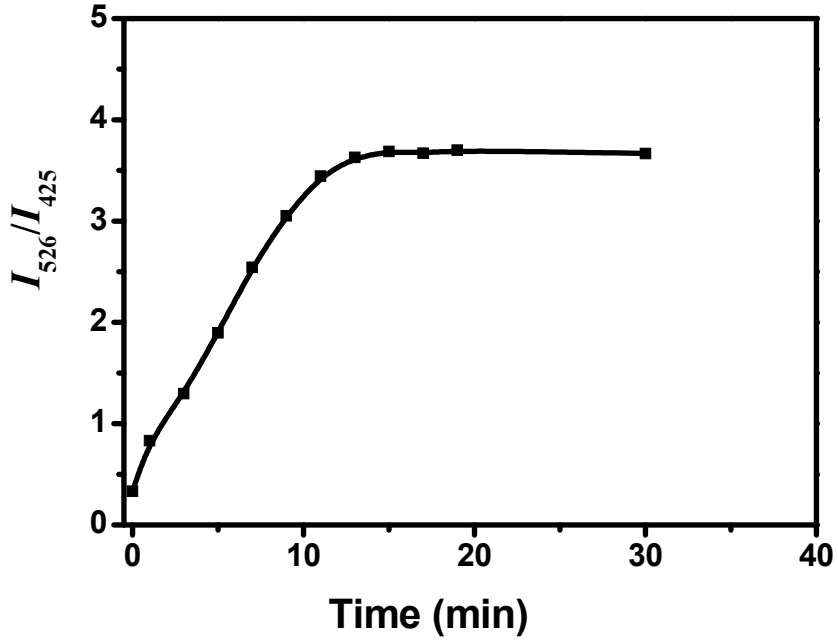


Fig. S15. Fluorescence intensity ratio (I_{526}/I_{425}) of the sensor (0.45 mg/mL) at different times after addition of H_2S (1×10^{-4} M) in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v). (λexc = 340 nm)

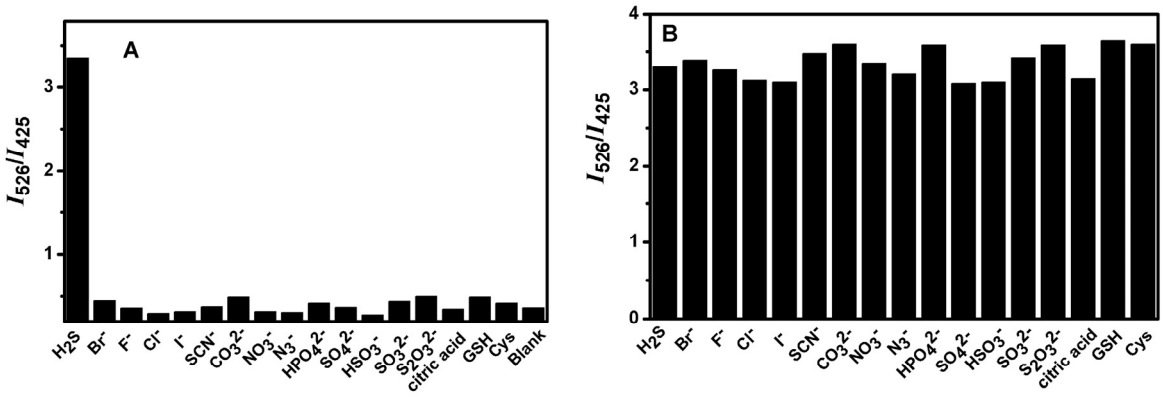


Fig. S16 (A) Fluorescence intensity ratio for the sensor (concentration: 0.45 mg/mL) in the presence of 100 μM H_2S and different anions and some thiols respectively. (B) Fluorescence intensity ratio for the sensor in the presence of 100 μM of H_2S, and simultaneously with the addition of 100 μM of different anions and some thiols respectively. (Measured in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v)). (λexc = 340 nm)

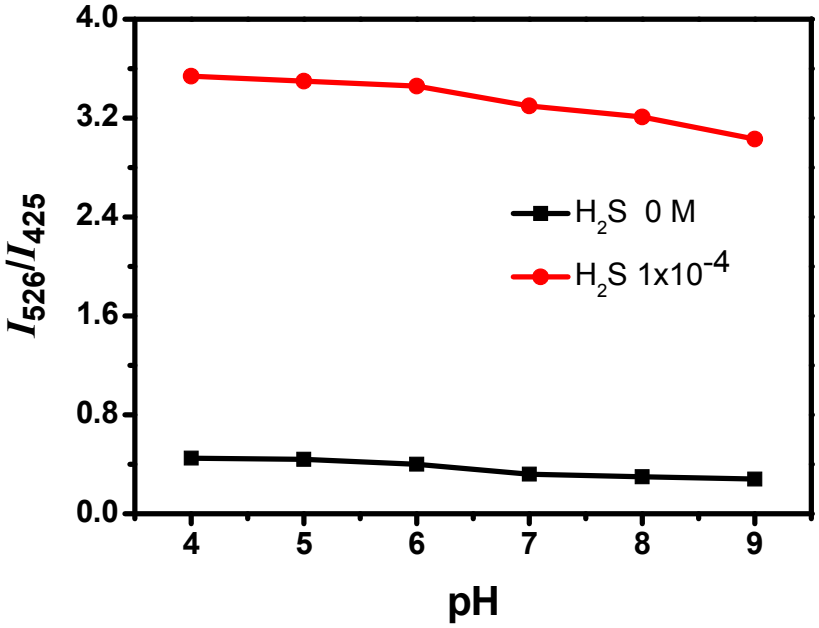
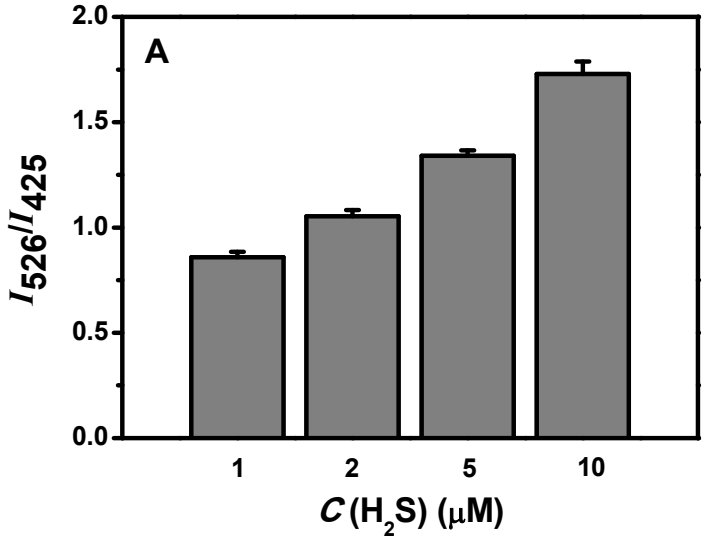
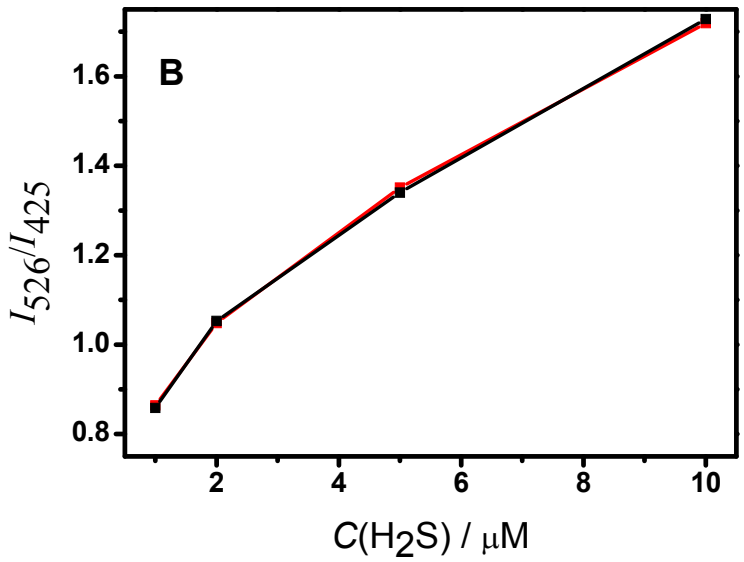


Fig. S17. Fluorescence intensity ratio of the sensor (concentration: 0.45 mg/mL) in the absence and in the presence of H_2S (1×10^{-4} M) as a function of pH (λexc: 340 nm).


A
2.0
1.5
1.0
0.5
0.0
I_{526}/I_{425}
1
2
5
10
$C(H_2S)$ (μM)


B
1.6
1.4
1.2
1.0
0.8
I_{526}/I_{425}
2
4
6
8
10
$C(H_2S)$ / μM

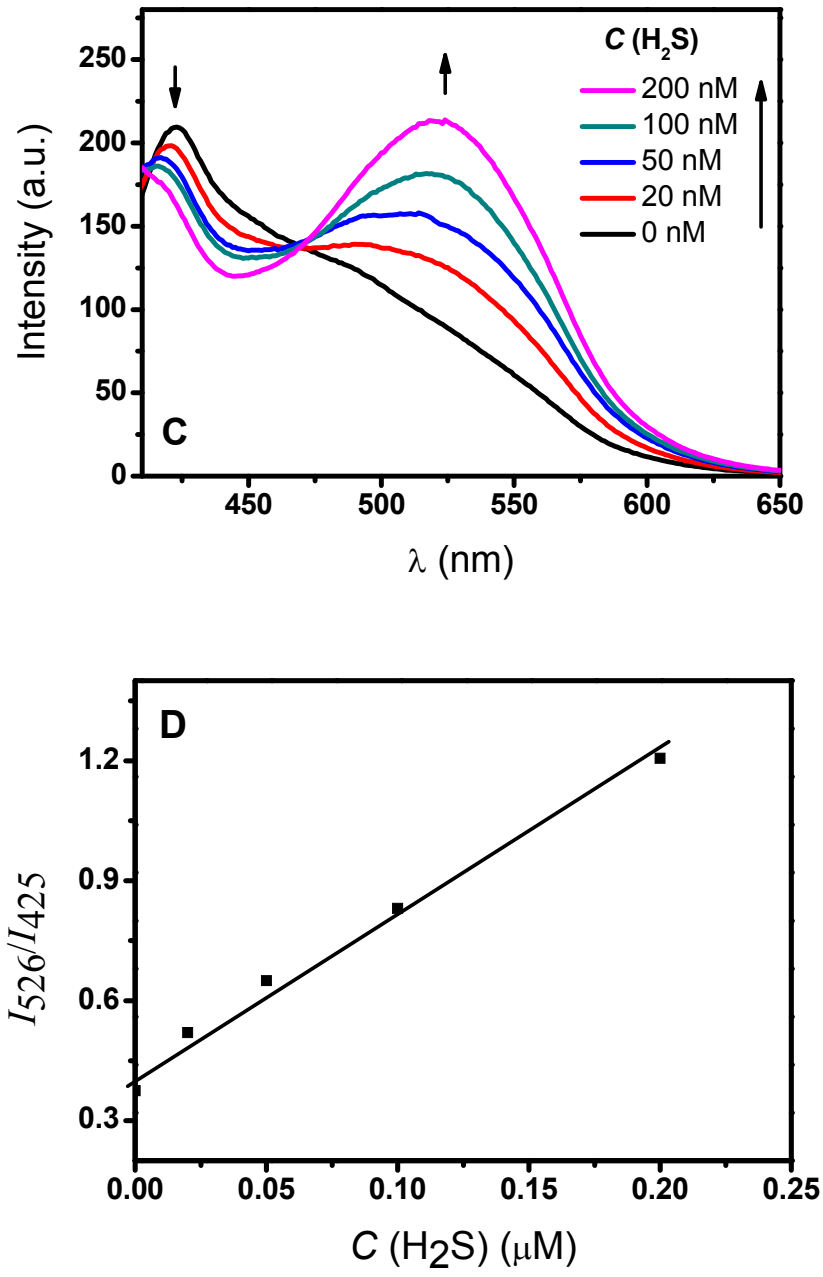


Figure S18. (A) Fluorescence intensity ratio of the sensor (concentration: 0.45 mg/mL) in serum; (B) Comparison of ffluorescence intensity ratio of the sensor (concentration: 0.45 mg/mL) at different H_2S concentrations in serum (black curve) and in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v) (red curve).
(C) Fluorescence spectra of CD-based sensor (concentration: 0.15 mg/mL, the amount of probe conjugated onto CDs: 4.48 mg/g) in the presence of different amounts of H_2S in serum sample; (D) Fluorescence intensity ratio of CD-based sensor (concentration: 0.15 mg/mL, the amount of probe conjugated onto CDs: 4.48 mg/g) as a function of H_2S concentration in serum sample. (λexc = 340 nm)

The detection limit in serum sample (final concentration: 10-fold diluted) is as follows:

I_{526}/I_{425} = 0.42 + 4.0 E6 [H_2S] (R = 0.993)

LOD = 3 × 0.026 / 4.0 E6 = 19.5 nM

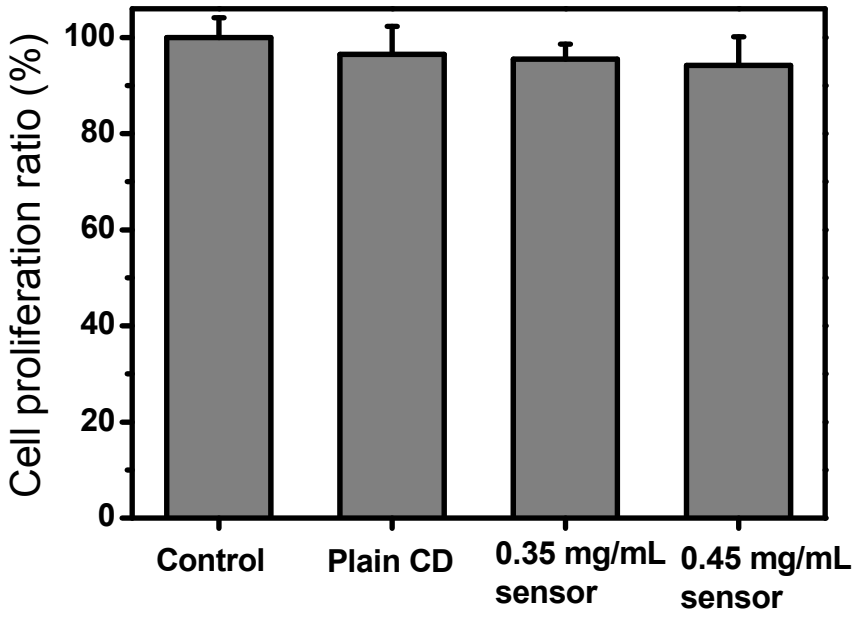


Fig. S19. Cytotoxic effects against L929 cells upon 24 hours of incubation. Control: L929 cells in the absence of the sensor; Plain CD: L929 cells in the presence of plain carbon dots at the concentration of 0.45 mg/mL; 0.35 mg/mL Sensor: L929 cells in the presence of the sensor at the concentration of 0.35 mg/mL; 0.45 mg/mL Sensor: L929 cells in the presence of the sensor at the concentration of 0.45 mg/mL.

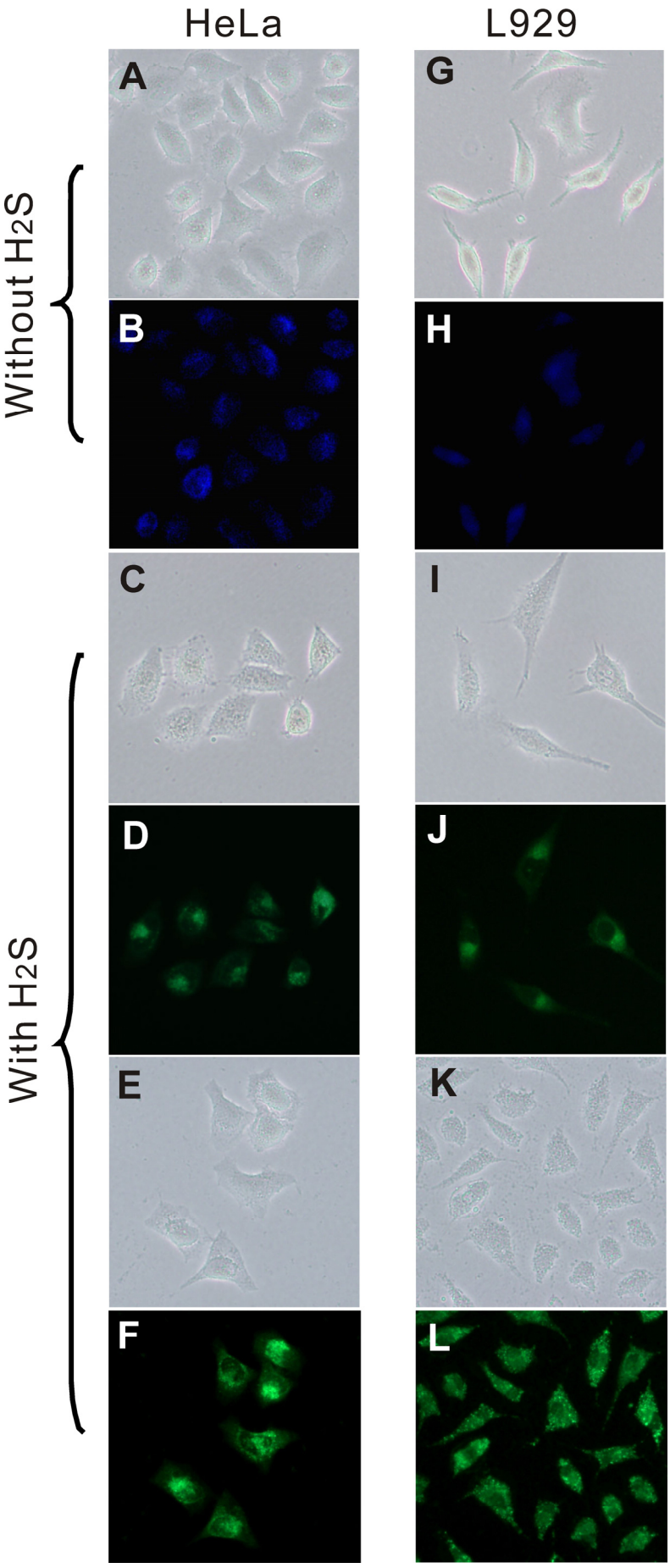

HeLa
L929
Without H2S
With H2S
A
B
C
D
E
F
G
H
I
J
K
L

Fig. S20. Microscope images for HeLa (A, B, C, D, E, F), and L929 (G, H, I, J, K, L) cells incubated with the sensor before (A, B, G, H) and after (C, D, E, F, I, J, K, L) being treated with H_2S (C, D, I and J: 30 μM H_2S; E, F, K and L: 100 μM H_2S). A, C, E, G, I and K are bright field images; B, D, F, H, J and L are fluorescence images. The photographs were taken under identical exposure condition.

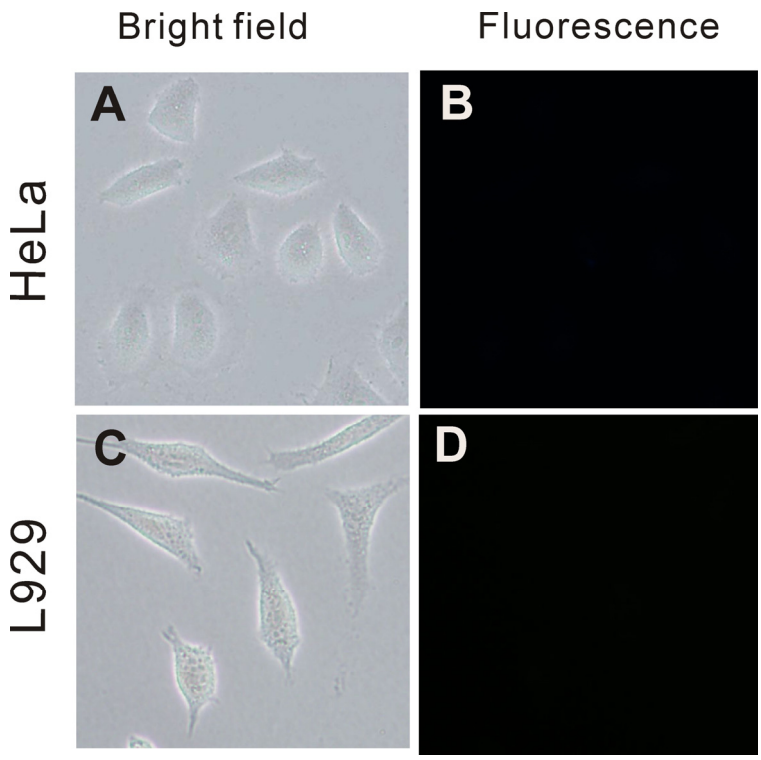


Fig. S21. Microscope images for HeLa (A, B), and L929 (C, D) cells incubated in the absence of the sensor or plain CDs as the control samples. A and C are bright field images; B and D are fluorescence images. The photographs were taken under identical exposure condition.

The control experiment on cells without the sensor gives no blue fluorescence at the same exposure condition.

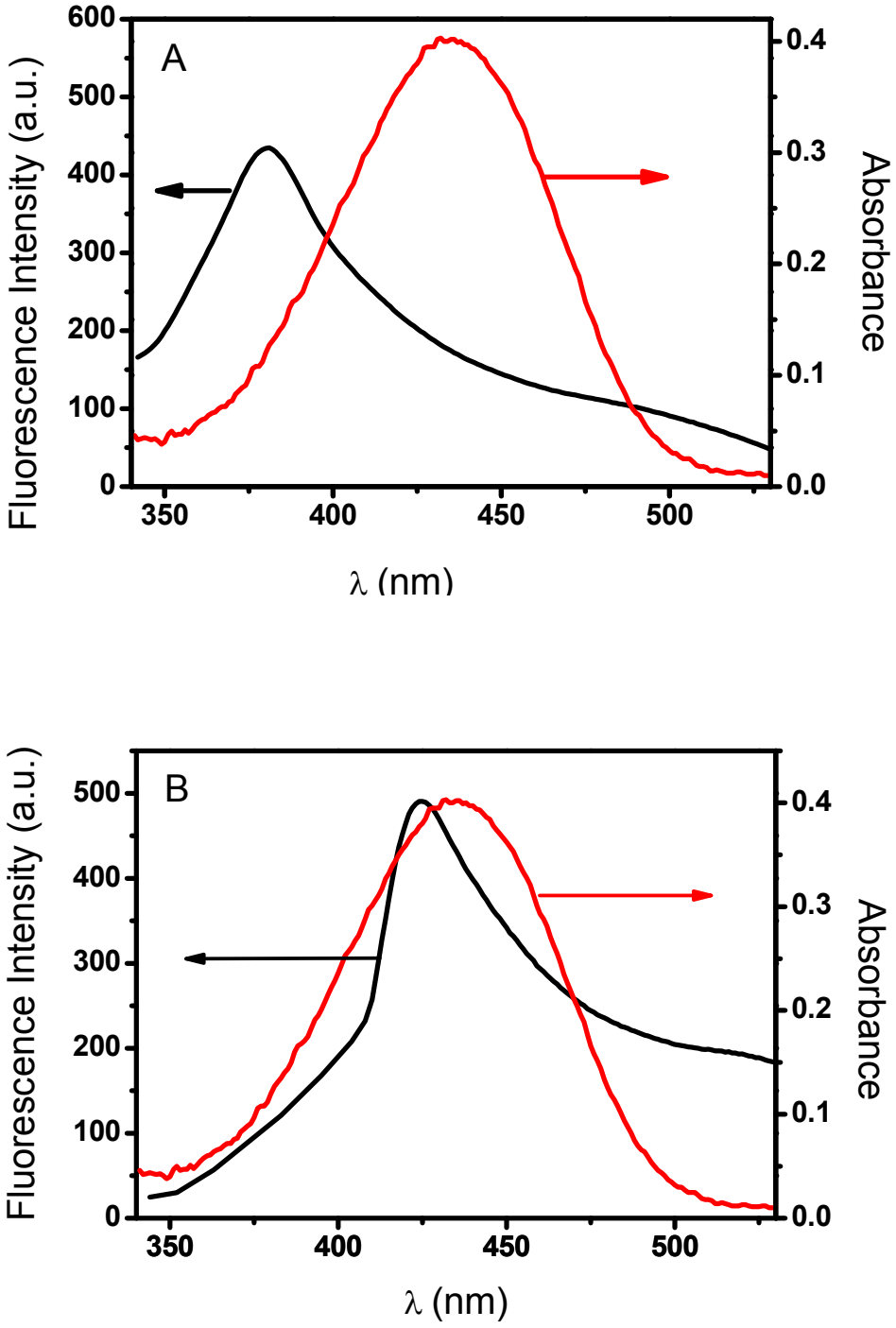


Fig. S22. Spectral overlap between the fluorescence emission band of the CD moiety (energy donor) of the CD-based sensor and the absorption band of the naphthalimide-amine (energy acceptor). (A) in in HEPES buffered (pH 7.4) water; (B) in HEPES buffered (pH 7.4) water-ethanol (3:1, v/v). (λ exc = 340 nm).

In this study, we performed quantitative analysis in buffer-ethanol solution, because in this solution there is better energy match between the donor's emission band and acceptor's

absorption band. Generally, FRET process requires that the emission band of the donor well overlap the absorption band of the acceptor; during FRET process, the adoption of the excitation wavelength for the donor should avoid direct excitation of the energy acceptor.

In HEPES buffered (pH 7.4) water-ethanol (3:1, v/v), the emission of CD exhibits better spectral overlap with the absorption of naphthalimide-amine; while in buffered water without ethanol, spectral overlap is not so satisfactory, as shown in this figure.

The possible reason is that, the conjugation of the probe (naphthalimide-azide) onto the CD's surface may lower the water solubility of the system to some extent, hence in buffered pure water the CD-based sensor does not have a very good spectral match with the energy acceptor (naphthalimide-amine). Thus, the quantitative analysis for H_2S using the sensor was conducted in buffer-ethanol solution.

In addition, for the sensing system in this study, the energy acceptor --- naphthalimide-amine exhibits minimum absorption at around 340 nm, and it has absorption at longer wavelength (e.g. ≥ 360 nm) (as shown in Fig. S9), hence the adoption of the excitation wavelength of 340 nm can ensure the excitation of the energy donor (CD), and at the same time avoid direct excitation of the energy acceptor; thus ensure the emission of the energy acceptor is the result of the FRET process.