Supporting Information for

An Unique Near-infrared Fluorescent Probe based on Dual-DNP Binding Sites with Highly Selectivity Sensing of Hydrogen Sulfide in Food Samples and Living Cells

Lili Ma [a], Yinliang Yang [c], Gulziba Anwar [b], Minqi Xie [a], Jie Yang [a], Jinwu Yan [b] *, Jingjing Wu [a] * and Chuanxiang Liu [a] *

School of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai 201418, P. R. China

b MOE International Joint Research Laboratory on Synthetic Biology and Medicines, School of Biology and Biological Engineering, South China University of Technology, Guangzhou 510006, P.R. China

c State Key Laboratory of Macromolecular Drugs and Large-scale Manufacturing, School of Pharmaceutical Sciences, Liaocheng University, Liaocheng 252059, China

* Email: cxliu@sit.edu.cn; yjw@scut.edu.cn; wujj@sit.edu.cn

Email: cxliu@sit.edu.cn; yjw@scut.edu.cn; wujj@sit.edu.cn

Contents of Supporting Information

1. Experimental Section

Instruments and Chemicals

Unless otherwise specified, solvents and reagents were purchased from commercial suppliers and used directly. The solvents were analytical-grade reagents and did not require purification. Column chromatography using silica gel (300-400 mesh) was performed with an eluent mixture of ethyl acetate and petroleum ether. NMR spectra (^{1}H and ^{13}C) were acquired using Bruker AVANCE and Quantum-I^{plus} spectrometers, with chemical shifts (reported in ppm, δ) referenced to tetramethylsilane. High-resolution mass spectra (HRMS) were obtained using a FT-MS spectrometer (solanX 70). UV-visible spectra were recorded using a spectrophotometer (SHIMADZU UV-1800). Fluorescence spectra were analyzed using a fluorescence apparatus (Hitach F-4600).

General Analytical Procedure for Spectral Experiment

A total of 38.76 mg of **DCIQ-2NDP** was dissolved in DMSO to prepare a 5 mM solution, which was placed in an amber bottle for subsequent experiments. This stock solution was diluted to 20 μM for testing. Analytes were prepared using deionized water. Given the instability of NaHS, it was selected as the donor of H_2S to facilitate the examinations. To prepare a 5 mM H_2S solution, 27.96 mg of NaHS was dissolved in 10 mL of deionized water. All spectroscopic experiments were conducted in phosphate-buffered saline (PBS)(10 mM, pH 7.4, 70% DMSO) with an λ_{ex} = 612 nm.

Detects H_2S in Food Spoilage Samples

The pork and eggs were purchased from the local supermarket. The pork examples were cleaned thoroughly by distilled water, and then one part was kept in the Erlenmeyer flask at 25 ℃ and the other parts as control samples were kept in the refrigerator at −4 ℃ for 5 days. Filter paper strips (1 × 3 cm) were soaked in a PBS/DMSO system (*v/v* = 3:7, pH 7.4) containing the **DCIQ-2NDP** (0.2 mM) for 60 minutes. After air drying, **DCIQ-2NDP**-loaded test strips were obtained. All pictures were taken after the colors of test strips were changed.

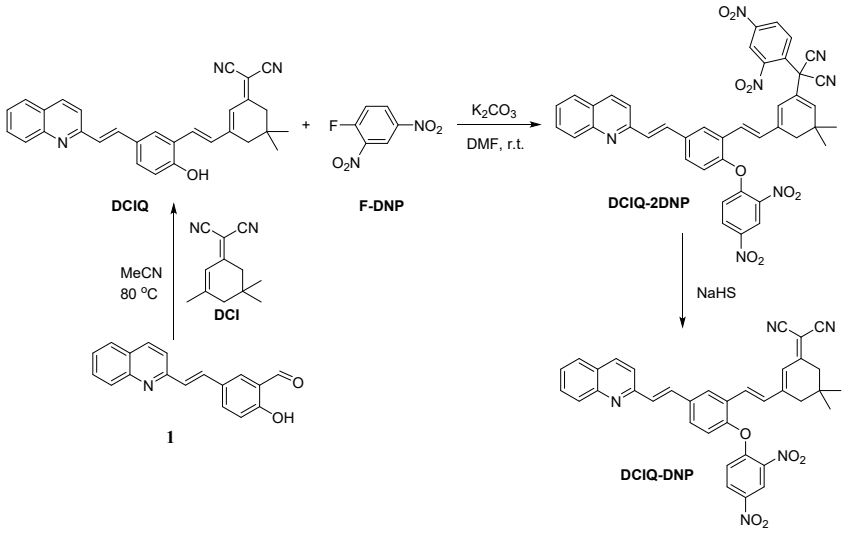
Detects H_2S in Beer Samples

The beer samples were diluted 100 times with deionized water and then employed for the preparation of a solution of beer/DMSO (3:7 = *v/v*). The **DCIQ-2DNP** solutions (20 μM) were prepared with the mixed solution of beer/DMSO (3:7 = *v/v*), and then different concentrations of H_2S were added. The absorbance at 440 nm was recorded.

Fluorescence Cell Imaging

HeLa cells were incubated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% CO_2 at 37 °C for 48 hours. For confocal imaging, cells at a density of 5 × 10^4 cells/mL were seeded into 20 mm confocal dishes. For subcellular imaging, two sets of HeLa cells were treated with different concentrations of NaHS (5, 10 μM) using probe **DCIQ-2DNP** for 30 minutes. To validate endogenous H_2S imaging, the HeLa cells were pre-incubated with cysteine (200 μM) for 1 h then treated with **DCIQ-2DNP** (10 μM) for another 30 min. Further, the HeLa cells were treated with 1 mM DL-propargylglycine (PAG) for 1 h and incubated with cysteine for another 1h (200 μM) before co-incubation with the **DCIQ-2DNP**. Post-incubation, cells were visualized using a Leica TCS SP8 confocal microscope equipped with a 40× oil-immersion objective lens. For imaging with probe **DCIQ-2DNP**, excitation was at 488 nm, and emission was captured between 640-750 nm, corresponding to the red channel. Images of the merged red and bright field views were also produced.

2. Synthesis of compound DCIQ, DICQ-DNP and DICQ-2DNP



Scheme S1 Synthesis of **DCIQ, DCIQ-DNP** and **DCIQ-2DNP**

Synthesis of DCIQ: In a 50 mL three-necked flask, **1**[1] (1.00 g, 3.6 mmol) was dissolved in 20 mL of acetonitrile, and **DCI** (1.00 g, 5.4 mmol) along with three drops of piperidine were added under a N_2 atmosphere. The reaction was carried out at 80°C for 24h. The progress of the reaction was monitored by TLC. Upon completion, the mixture was cooled, resulting in the precipitation of a red solid, which was then filtered. The solid was washed with 20 mL of ethanol to yield a red solid of **DCIQ** (1.20 g, 75.2% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ [ppm]: 8.4 (d, J = 8.5 Hz, 1H), 8.15 (s, 1H), 8.05-7.99 (m, 2H), 7.95 (d, J = 6.5 Hz, 1H), 7.8 (d, J = 9 Hz, 1H), 7.7-7. 4 (m, 1H), 7.62-7.52 (m, 3H), 7.33 (dd, J = 36.5 Hz, J = 16 Hz, 2H), 6.96 (d, J = 8.5 Hz, 1H), 6.858 (s, 1H), 2.616 (s, 2H), 2.561 (s, 2H), 1.031 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ [ppm]：170.80, 158.01, 157.15, 156.44, 148.21, 138.59, 137.02, 130.79, 130.32, 129.37, 129.13, 128.30, 128.09, 127.48, 127.44, 127.35, 126.57, 124.14, 122.08, 120.51, 117.00, 114.61, 113.83, 75.50, 32.16, 27.96. HRMS-ESI (m/z): $[M + H]^+$ Calcd. for ($C_{30}H_{25}N_3O$):444.2070; Found:444.2068.

The Synthesis of DCIQ-2DNP: To a solution of **DCIQ** (1.00 g 2.25 mmol) dissolve in DMF (20 mL), K_2CO_3 (0.78 g 5.64 mmol) was added. The mixture was stirred for 30 minutes, and then 2-dinitrofluorobenzene (**F-DNP**, 1.05 g 5.64 mmol) was added slowly in 3 batches at room temperature. The solution was stirred overnight and then quenched with water (200 mL), and extracted with CH_2Cl_2 (20 mL×3) to obtain the organic residue, which was further washed with water, then dried with anhydrous Na_2SO_4 and filtered. The crude product was concentrated in vacuum and purified by a silica gel chromatographic column (PE: EA = 6:1) to afford the probe **DCIQ-2DNP** (0.87 g, 50.68 % yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm]：8.86 (dd, J = 8.0, 2.5 Hz, 2H), 8.68 (dd, J = 8.5, 2.0 Hz, 1H), 8.29 (dd, J = 9.0, 2.5 Hz, 1H), 8.26 (d, J = 8.5 Hz, 1H), 8.11 (d, J = 9.0 Hz, 1H), 8.01 (m, 2H), 7.78 (d, J = 6.5 Hz, 1H), 7.76-7.69 (m, 2H), 7.63 (d, J = 8.5 Hz, 1H), 7.54-7.46 (m, 2H), 7.40 (dd, J = 8.5, 2.5 Hz, 1H), 7.08 (d, J = 8.5 Hz, 1H), 6.97 (d, J = 9.5 Hz, 1H), 6.88-6.74 (m, 2H), 6.18 (d, J = 1.5 Hz, 1H), 5.72 – 5.69 (m, 1H), 2.47 (s, 2H), 1.21 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ [ppm]：155.43, 155.30, 151.22, 149.56, 148.71, 148.01, 142.07, 140.49, 140.12, 139.76, 137.22, 135.93, 133.32, 132.39, 131.06,

130.47, 130.30, 129.56, 129.23, 128.57, 128.30, 127.64, 126.31, 122.87, 122.50, 121.95, 120.47, 119.63, 112.75, 44.22, 36.30, 33.41, 27.34. HRMS-ESI (m/z): $[M + H]^+$ Calcd. for ($C_{42}H_{29}N_7O_9$): 776.2105; Found:776.2057.

The Synthesis of DCIQ-DNP: In a 25 mL round-bottom flask, **DCIQ-2NDP** (0.20 g, 0.26 mmol) was dissolved in 8 mL of DMF at room temperature, followed by the addition of NaHS aqueous solution (0.50 mol/L, 0.60 mL). The mixture was stirred at room temperature for 30 minutes. After the reactants were fully consumed (monitored by TLC), the mixture was poured into water (100 mL) and then extracted with CH_2Cl_2 (10 mL × 3). The organic layer was washed with water, dried over anhydrous Na_2SO_4 and filtered. The residues was concentrated and purified by silica gel column chromatography (DCM: PE = 1:1) to obtain a pale yellow solid of **DCIQ-DNP** (0.10g, 63.69% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]：8.90 (s, 1H), 8.35 (d, J = 9.6 Hz, 1H), 8.22-8.15 (m, 2H), 8.09 (s, 1H), 7.92-7.83 (m, 2H), 7.79-7.75 (m, 1H), 7.70-7.65 (m, 2H), 7.61-7.56 (m, 2H), 7.17 (d, J = 8.4 Hz, 1H), 7.11 (s, 2H), 7.07 (d, J = 8.4 Hz, 1H), 6.96 (s, 1H), 2.67 (s, 2H), 2.54 (s, 2H), 1.16 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]：162.64, 148.07, 148.07, 137.71, 136.75, 136.37, 134.85, 134.77, 133.09, 130.01, 129.77, 129.50, 129.09, 128.85, 127.72, 127.58, 127.30, 127.20, 126.18, 125.83, 124.34, 122.15, 121.59, 121.29, 119.40, 118.66, 42.99, 39.20, 36.52, 31.46, 29.69, 28.03, 22.65, 22.63, 14.12. HRMS-ESI (m/z): $[M + Na]^+$ Calcd. for ($C_{36}H_{27}N_5O_5$): 632.1904; Found:632.1908.

3. The ^{1}H-NMR, ^{13}C-NMR, HRMS spectra of DCIQ (Fig. S1-3)

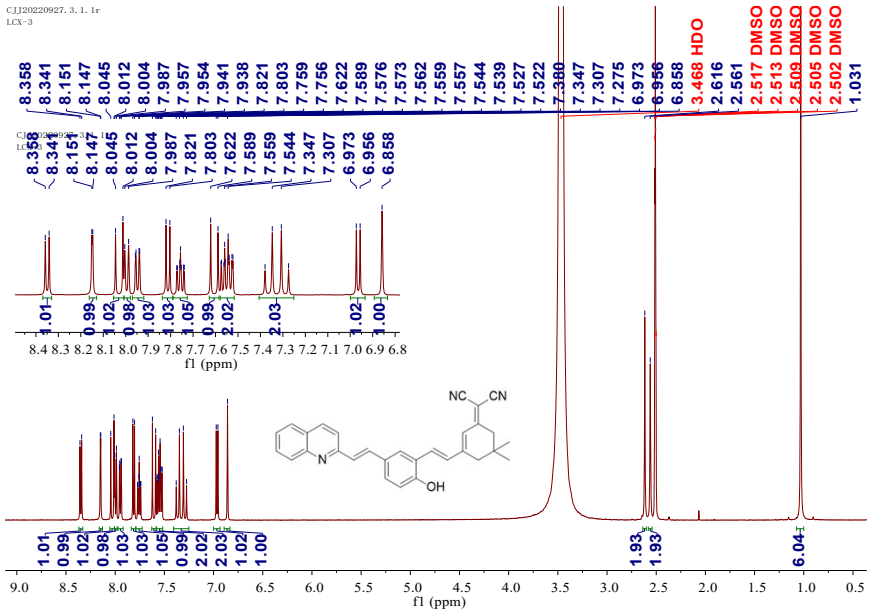


Figure S1 ^{1}H-NMR (500 MHz, DMSO-d_6) spectra of **DCIQ**.

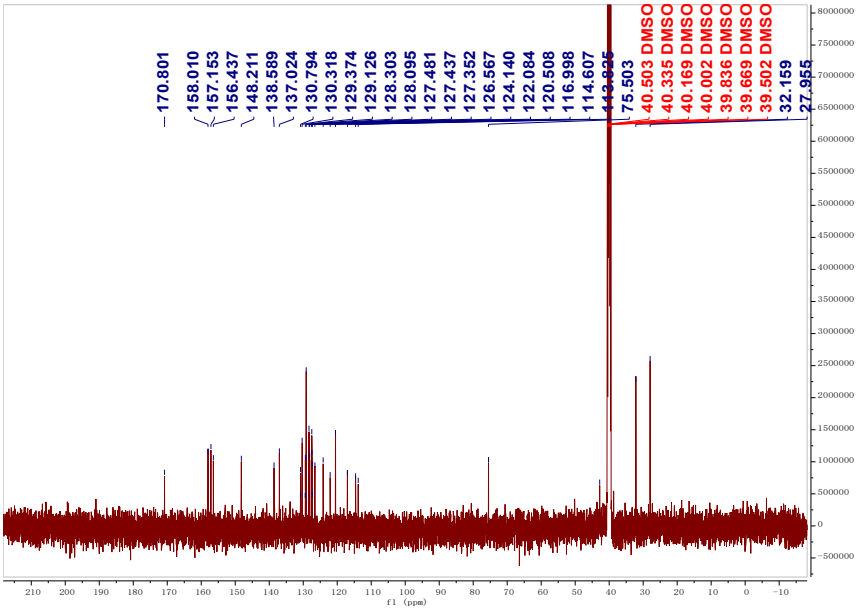


Figure S2 ^{13}C-NMR (125 MHz, DMSO-d_6) spectra of **DCIQ**.

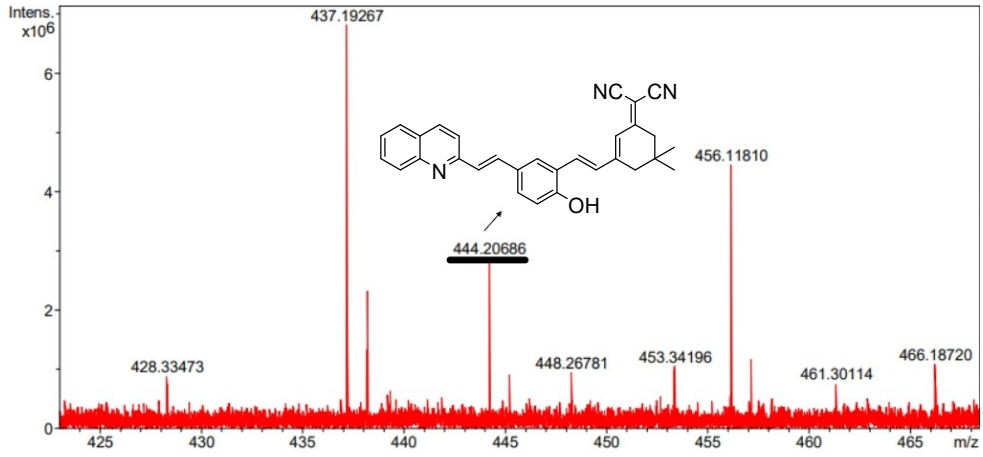


Figure S3 HRMS-ESI mass spectra of **DCIQ**.

4. The ^{1}H-NMR, ^{13}C-NMR, HRMS spectra of DCIQ-2DNP (Fig. S4-6)

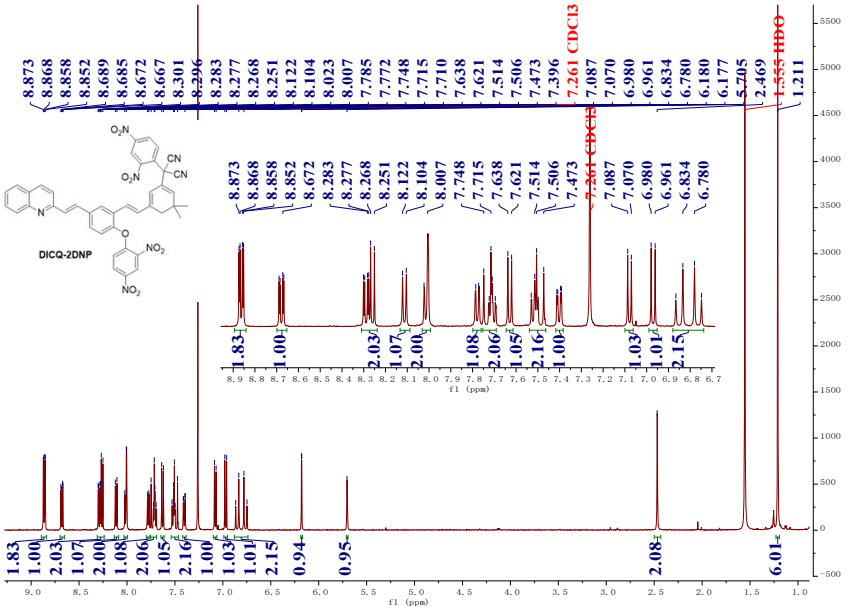


Figure S4 ^{1}H-NMR (500 MHz, $CDCl_3$) sectra of **DCIQ-2DNP**.

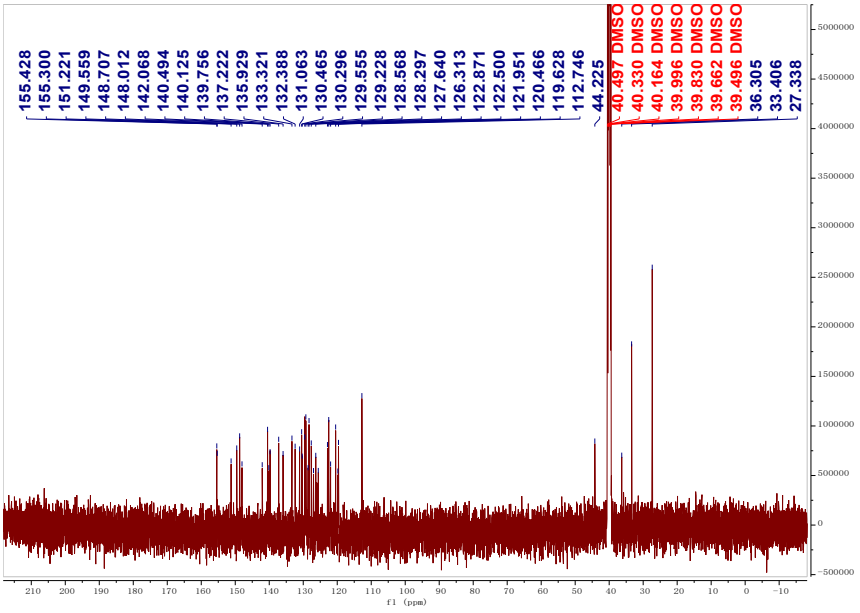


Figure S5 ^{13}C-NMR (125 MHz, DMSO-d_6) spectra of **DCIQ-2DNP**.

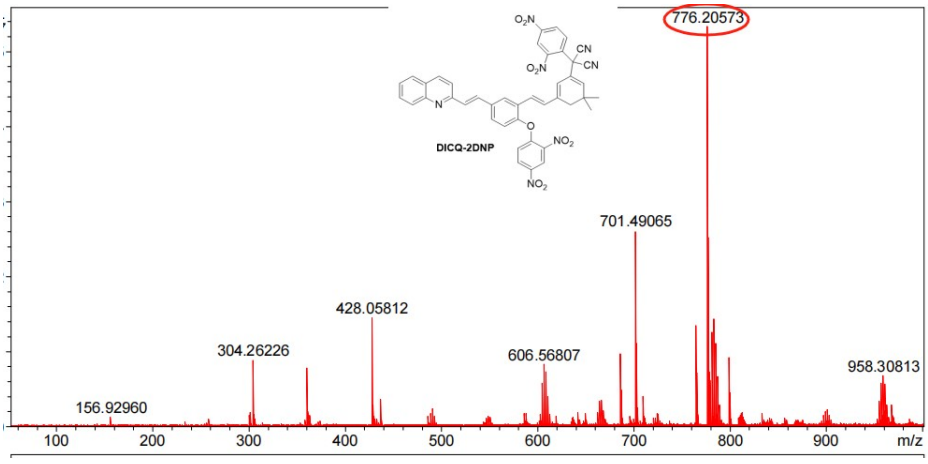


Figure S6 HRMS-ESI mass spectra of **DCIQ-2DNP**.

5. The ^{1}H-NMR, ^{13}C-NMR, HRMS spectra of DCIQ-DNP (Fig. S7-9)

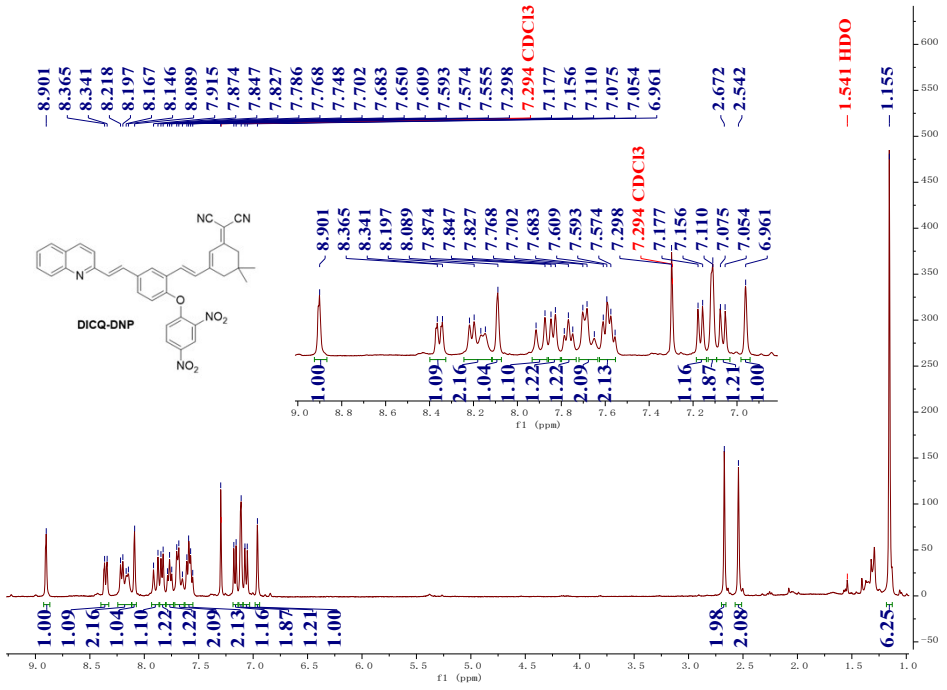


Figure S7 ^{1}H-NMR (400 MHz, $CDCl_3$) sectra of **DCIQ-DNP**.

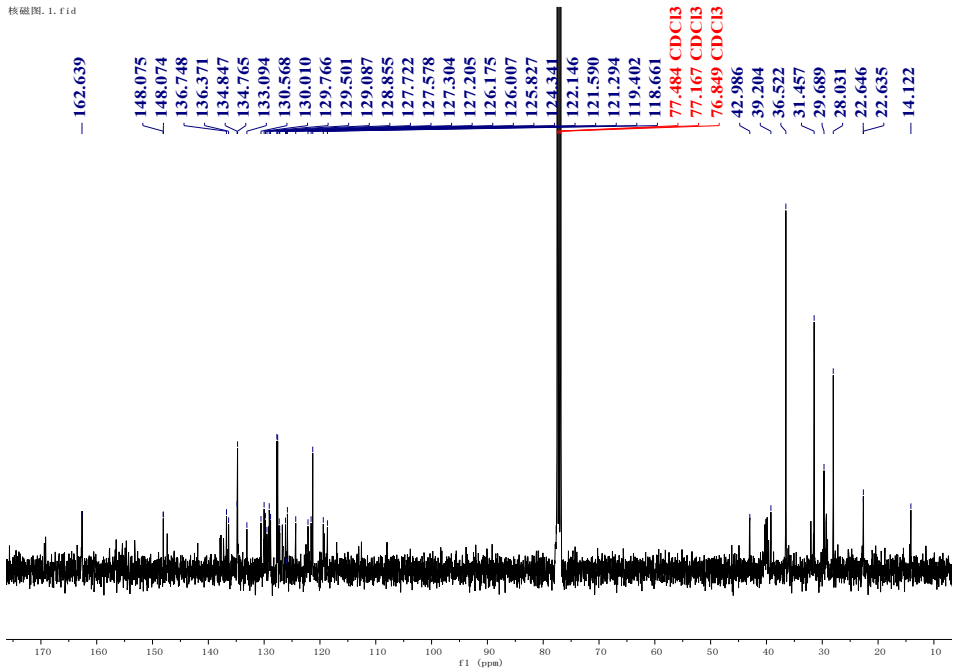


Figure S8 ^{13}C-NMR (100 MHz, $CDCl_3$) spectra of **DCIQ-DNP**.

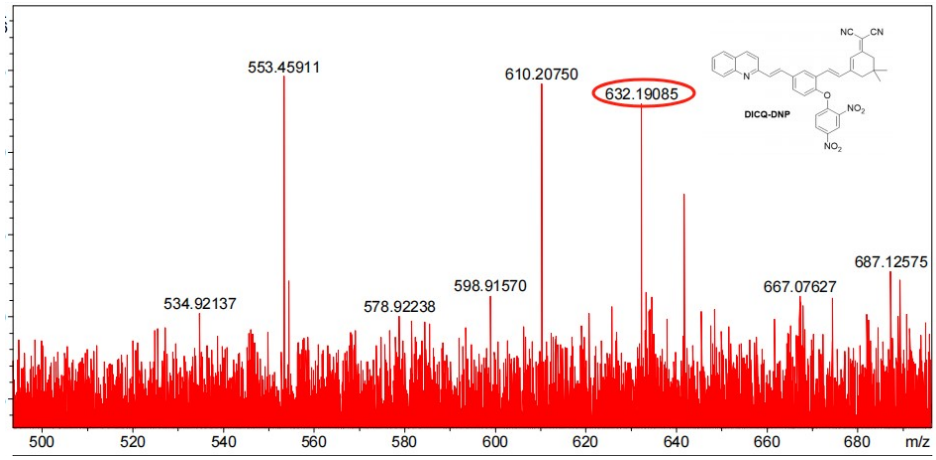


Figure S9 HRMS-ESI mass spectra of **DCIQ-DNP**

6. The UV−vis detection limit of DCIQ-2DNP with H_2S

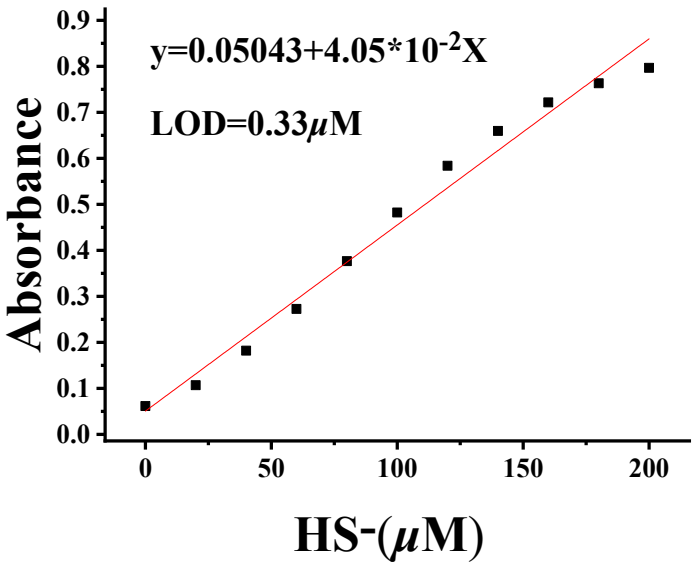


Figure S10 The linear relationship of **DCIQ-2DNP** against the concentration of H_2S from 20 to 200 μM in the DMSO/PBS (7:3, v/v, pH 7.4) aqueous environments

7. Interference experiments of the probe DCIQ-2DNP

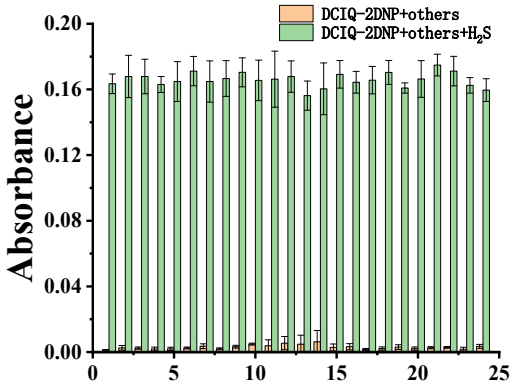


Figure S11 Interference experiments of probe **DCIQ-2DNP** (20 μM) for HS^- in the presence of other anions (20 equiv.) before (green bars) and after (yellow bars) incubation with HS^- (20 equiv.)The number (1-16) represent probe with analytes: (1) F^-, (2) Cl^-, (3) Br^-, (4) I^-, (5) NO_3^-, (6) ClO_4^-, (7) $H_2PO_4^-$, (8) OH^-, (9) AcO^-, (10) SCN^-, (11) CN^-, (12) BF_4^-, (13) S^{2-}, (14) HSO_4^-, (15) HSO_3^-, (16) $S_2O_3^{2-}$, (17) 2-ME, (18) DTT, (19) His, (20) Gly, (21) Leu, (22) Met, (23) GSH, (24) Cys.

8. Fluorescence changes of the probe DCIQ-2DNP towards NaHS

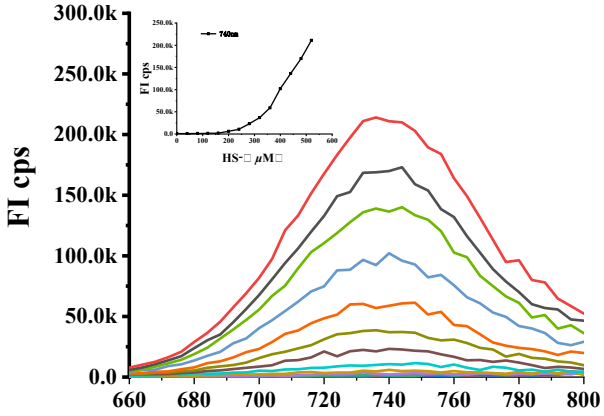


Figure S12 Fluorescence changes of the probe **DCIQ-2DNP** (20 μM) towards NaHS (0 to 26 equiv.) in DMSO/PBS (*v/v*, 1:1, PBS buffer; pH 7.4), λ_{ex} = 612 nm

9. The solvent effect of the probe DCIQ-2DNP

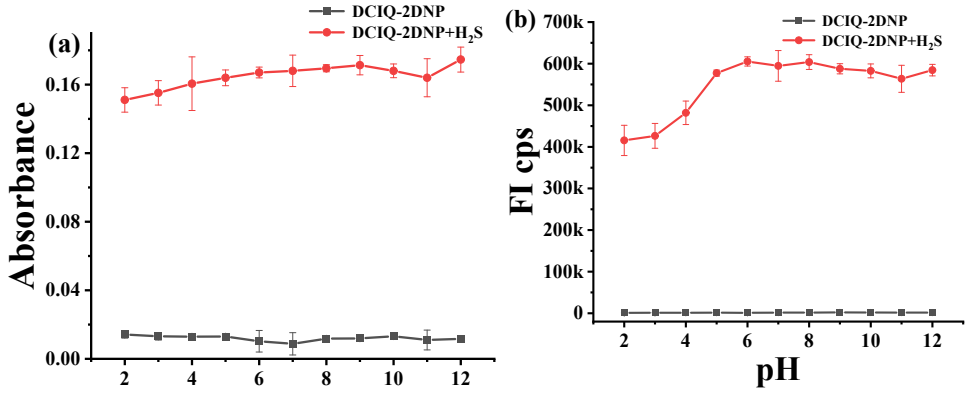


Figure S13 (a) Changes in the absorption spectral (at 612 nm) of **DCIQ-2DNP** (20 μM) at different pH with (●) and without (■) HS^- (20 equiv.) in a DMSO/PBS (7:3, *v/v*, pH 7.4) buffer solution. (b) Changes in the fluorescence intensity (at 740 nm) of **DCIQ-2DNP** (20 μM) at different pH with (●) and without (■) NaHS (26 equiv.)

10. Reaction time of the probe DCIQ-2DNP and DCIQ-DNP towards HS^-

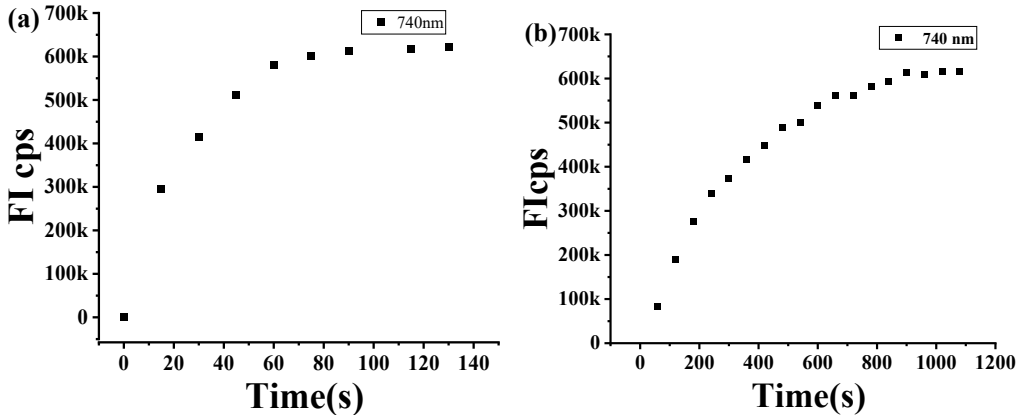


Figure S14 (a) Effect of reaction time on the fluorescence intensity of the probe **DCIQ-2DNP** (20 μM) towards NaHS in DMSO/PBS (*v/v*, 7:3, pH 7.4, λ_{ex}=612 nm). (b) Reaction time on the

fluorescence intensity of the probe **DCIQ-DNP** (20 μM) towards NaHS in DMSO/PBS (v/v, 7:3, PBS buffer; pH 7.4), λ_{ex}=612 nm

11. Fluorescence stability test of probe DCIQ-2DNP

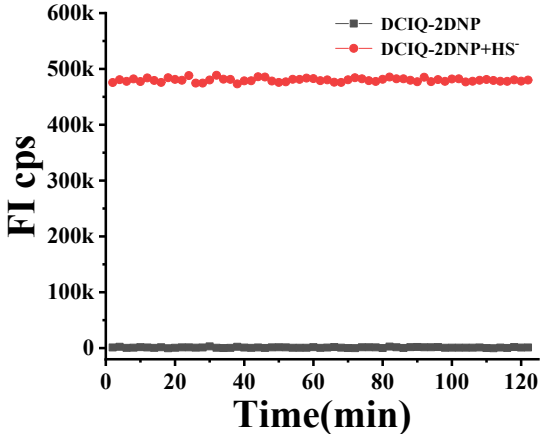


Figure S15 Changes in the fluorescence emission spectra (at 740 nm) of the **DCIQ-2DNP** (20 μM) in DMSO/PBS (7:3, v/v, pH 7.4) buffer solution with (●) and without (■) HS^- (20 equiv.) within 2 h.

12. Spectral comparison of probe DCIQ-2DNP and DCIQ-DNP

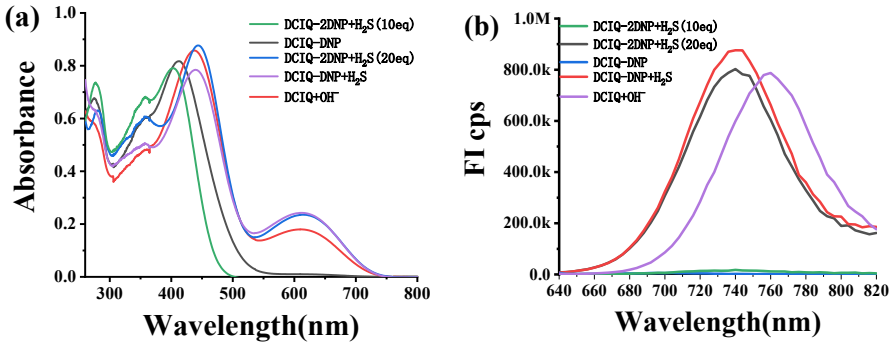


Figure S16 (a) Ultraviolet spectra of **DCIQ-DNP** and **DCIQ** after the interaction of probe **DCIQ-2DNP** and **DCIQ-DNP** with NaHS; (b) Fluorescence spectra of and **DCIQ-DNP** and **DCIQ** after the interaction of probe **DCIQ-2DNP** and **DCIQ-DNP** with NaHS.

13. ^{1}H NMR titration experiments

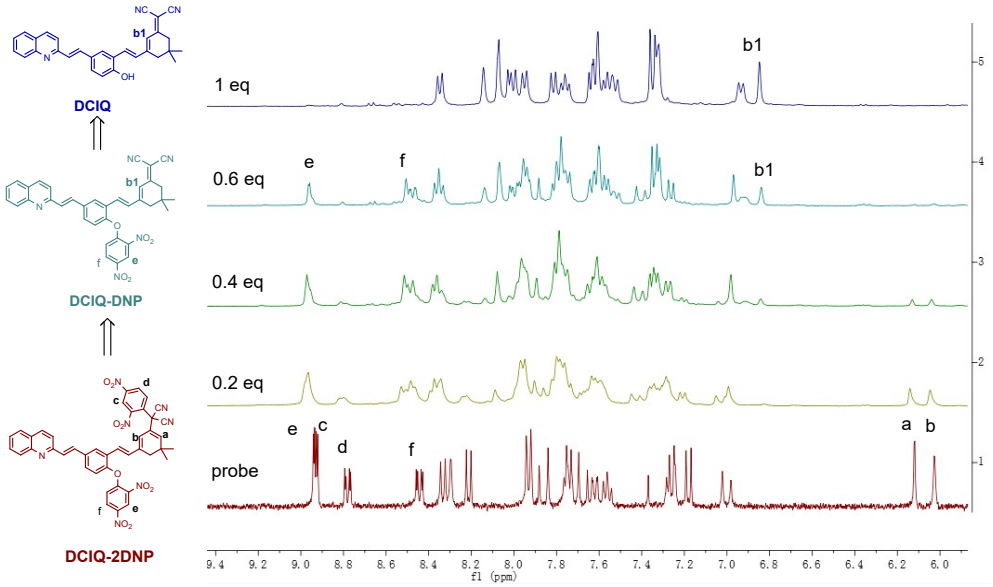


Figure S17 ^{1}H NMR titration spectra of **DCIQ-2DNP** (30 mM) upon addition of various equivalents NaHS in DMSO-d_6, from bottom to top: **DCIQ-2DNP**, 0.2, 0.4, 0.6, 1.0 equiv.

14. The LC-MS analysis of the DCIQ-2DNP solution with HS^-

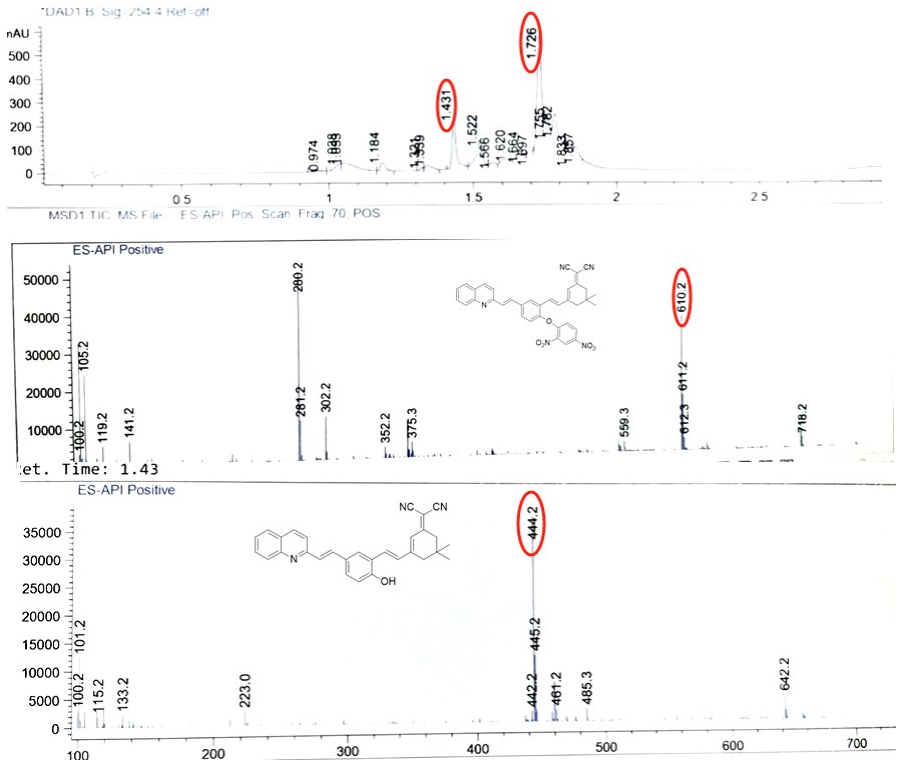


Figure S18 LC-MS spectrum of the **DCIQ-2DNP** and HS^-.

15. The test strips experiments of DCIQ-2DNP for H_2S

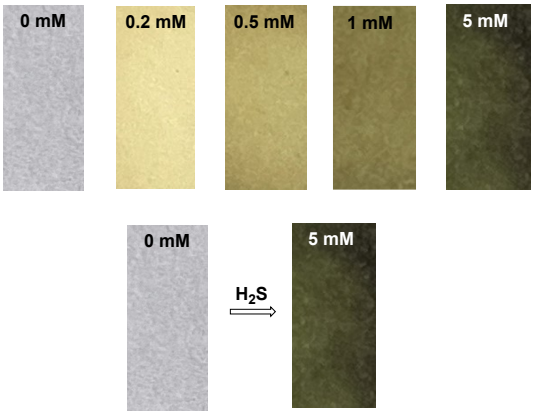


Figure S19 Color changes of **DCIQ-2DNP**-loaded test strips with different concentrations of NaHS under ambient light.

16. The experiment of DCIQ-2DNP with H_2S gas

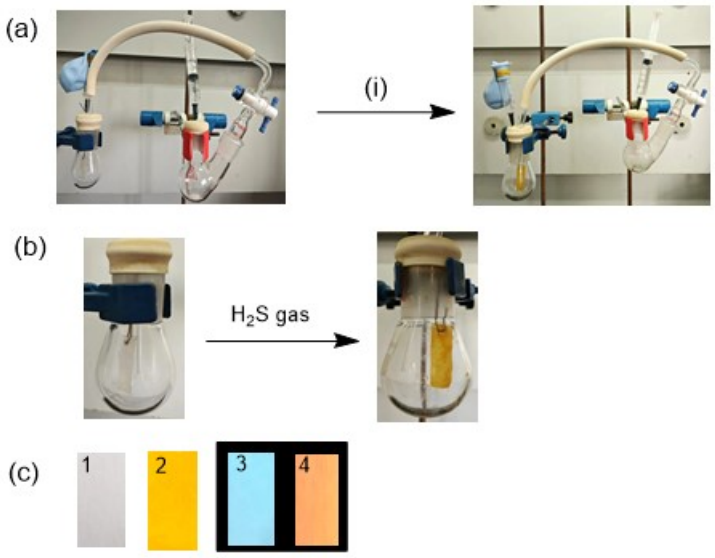


Figure S20 A diagram depicting the detection of H_2S gas by the bare eyes. (a) The H_2S gas produced by HClaq. and Na_2S. (i) few drops of HClaq. was added. (b) The enlarge photo of flask. (c) Color change of **DCIQ-2DNP**-loaded test strips with H_2S gas under ambient light (1: blank and 2: **DCIQ-2DNP** + H_2S gas) as well as under 365 nm lamp (3: blank and 4: **DCIQ-2DNP** + H_2S gas).

17. DCIQ-2DNP-loaded test strip for H2S detection in food samples

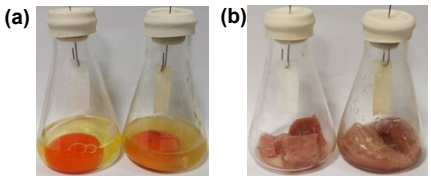


Figure S21 DCIQ-2DNP-loaded test strips for detection of H_2S gas generated in the process of (a) eggs; (b) pork. Each group was stored at −4 °C (left) and 25 °C (right).

18. The cytotoxicity test of the DCIQ-2DNP

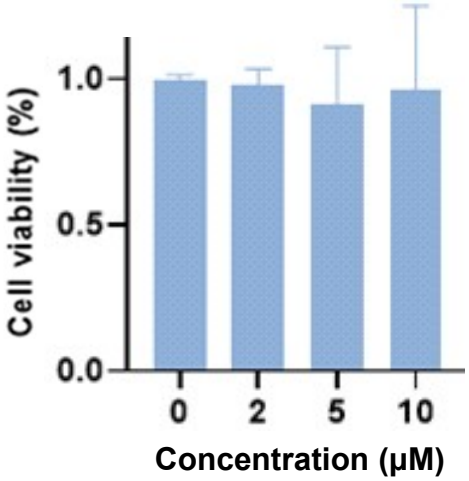


Figure S22 The cytotoxicity of the probe for HeLa cells.

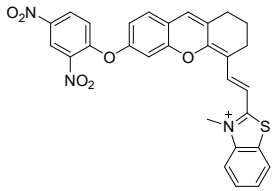
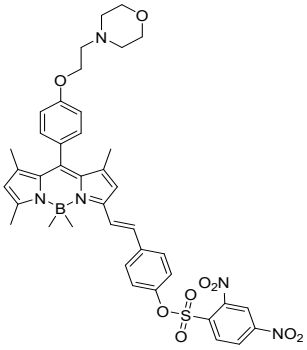
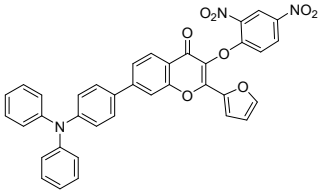
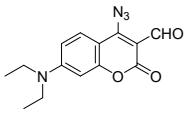
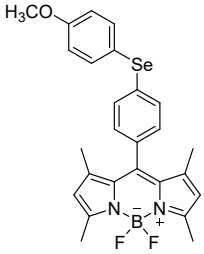
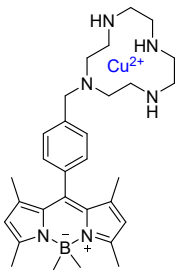
19. Table S1 Determination of H2S Concentrations in Beer

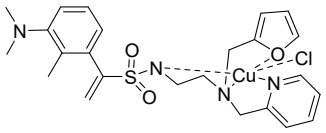
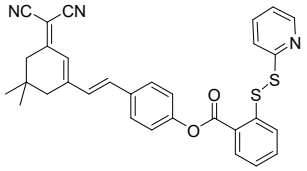
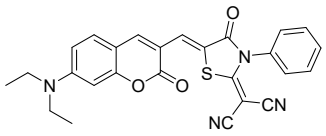
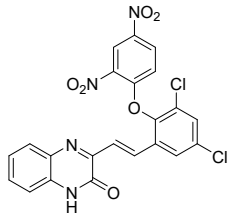
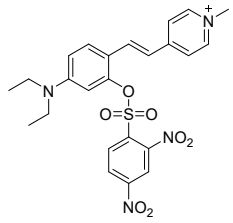
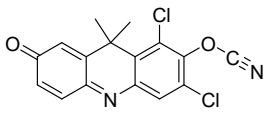
Sample name	H_2S level (μM)	H_2S added (μM)	H_2S found (μM)	Recovery (%)	SD, n=3
Beer I	1.61	20	20.87	96.30	0.94
		40	42.12	101.20	0.87
		80	82.52	101.13	0.24
Beer II	1.31	20	20.05	93.68	1.18
		40	40.77	98.65	0.48
		80	81.92	100.76	0.71

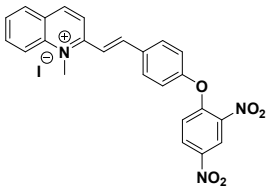
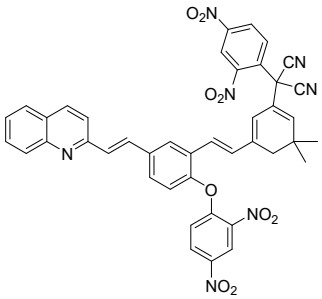
20. Fluorescent probes for HS^-

Table S2 Fluorescent probes for H_2S.

Probe	$\lambda_{ex}/\lambda_{em}$	Solvent	Time	LOD	Application

Probe	$\lambda_{ex}/\lambda_{em}$	Solvent	Time	LOD	Application
 R1[2]	680/720	MeOH/H_2O (3:7, *v/v*)	20 min	104 nM	Water samples, cells, zebrafish, rice
 R2[3]	574/592	DMSO/HEPES (1:1, *v/v*)	60 s	51 nM	Cells
 R3[4]	530/562,643	1,4-dioxane/ PBS(4:6, *v/v*)	4 min	192 nM	Cells
 R4[5]	360/422	MeCN/H_2O (3:7, *v/v*)	3 s	3.5 nM	Cells, zebrafish
 R5[6]	460/650	MeCN/PBS (3:7, *v/v*)	5 min	——	Cells
	497/510	HEPES	10 s	1.1 nM	Cells

Probe	$\lambda_{ex}/\lambda_{em}$	Solvent	Time	LOD	Application
R6[7] 	338/534	EtOH/H_2O (1:1, *v/v*)	——	11 nM	Water
R7[8] 	425/580	DMSO/PBS (4: 1, v/v)	20 min	86 nM	Cells
R8[9] 	535/611	DMSO/Tris-HCl (7: 3, *v/v*)	12 min	0.98 μM	Water, beer, food, cells, mice
R9[10] 	375/520	PBS (1% DMSO)	10 min	0.02 μM	Cells, mice
R10[11] 	497/608	DMF/H_2O (1:1, *v/v*)	4 s	356 nM	Water, beer, cells
R11[12] 	600/658	PBS	10 s	4.3 nM	Food, cells
R12[13]					

Probe	$\lambda_{ex}/\lambda_{em}$	Solvent	Time	LOD	Application
 R15[14]	380/490	DMSO/PBS (9: 1, *v/v*)	15 min	54 nM	Food
 This work	612/740	DMSO/PBS (7: 3, *v/v*)	1 min	14.7 nM	Food, beer, cells

References

(1) Y. Hu, L. Ma, Z. Zhang, Y. Huang, H. Zhang, J. Wu, C. Liu, *J Photoch Photobio A. Chem.* 2023**, 445,** 115082.

(2)Y. M. Lin, Q. He, X. Y.Wang, F. F. Hua, X. Y. Liu, Y. L. Fu, *J. Agric. Food Chem.* 2023, **71**, 5154-5161.

(3) J. Yue, ;Y. Tao, J. Zhang, H. Wang, N. Wang, W. Zhao, *Chem Asian J* 2021, **16** , 850.

(4) B. Y. Wei, X. M. Yan, Y. C. Yuan, J. Y. Miao, B. X Zhao, Z. M. Lin, *J. Photochem.* 2024, **450**, 115450.

(5) Q. Fang, H. Xiong, L. Yang, B. Wang, X. Song, *New J. Chem.* 2019, **43** , 13594-13599.

(6) W. Wang, P. Li, P. Yu, ;P. Song, X. Sun, S. Yang, Z. Lou, K. Han, *Chem. Commun.* 2013, **49** , 1014.

(7) X. Fang, S. Wang, Q. Wang, J. Gong, L. Li, H. Lu, P. Xue, Z.Ren, X. Wang, *Talanta* 2024, **268,** 125339.

(8) Y. Yan, L.Chen, R. Liu, A. Zheng, S. Wang, *RSC Adv.* 2019, **9** , 27652.

(9) Wu, Q. Huo, F. Wang, J. Yin, *Spectrochim Acta A* 2020, **238** , 118437.

(10) Z. Shang, Q. Meng, D. Tian, Y. Wang, Z. Zhang, R. Zhang, *Food Chem.* 2023, **427**, 136701.

(11) K.Wang, Guo, X. Y. Chen, X. L. Li, Z. G. Hu, X. Wang, C. Y. Wang, Y. J. Qin, K.Yao, Y. S. Yang, *Chem. Eng. J.* 2023, **468**, 143611.

(12) K. Zhong, X. Hu, S. Zhou, X. Liu, X. Gao, L. Tang, X. *J.* Yan, *Agric. Food Chem*. 2021, **69** , 4628-4634.

(13) Y. Ma, X. Wang, Z. Wang, G. Zhang, X. Chen, Y. Zhang, Y. Luo, G. Gao, X. Zhou, *Talanta* 2023, **256**, 124303.

(14) Y. Hu, Z. H. Chen, L. L. Ma, Z. Y. Zhang, H. Zhang, F.-P.Yi, C. X. Liu, *Tetrahedron.* 2022, **117-118**, 132837.