

A Novel Benzimidazole- π -Bridge-Dicyanoisophorone-Type Near-Infrared Fluorescent Probe for Rapid Monitoring of Hydrogen Sulfide Based on a Cascade Thiolytic Strategy

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Research Article

Keywords: Fluorescent probe, D- π -A, cascade thiolytic, near-infrared, hydrogen sulfide

Posted Date: August 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10661995/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Journal of Fluorescence on September 30th, 2026. See the published version at <https://doi.org/10.1007/s10895-026-04959-2>.

Abstract

Herein, we designed a dual DNP-caged near-infrared probe, **N-Bu-DCIB-2DNP**, by conjugating dicyanoisophorone (DCI) with an N-alkylated benzimidazole (B) through double vinylene linkers. This probe features two responsive sites—the DNP ether on the donor and the DNP-functionalized electron-deficient quaternary carbon on the DCI unit—to enable a cascade thiolysis strategy. The probe exhibits distinct dual-emission fluorescence signals at 610 nm and 745 nm with large Stokes shifts, and achieves a rapid response to H₂S within 6 min, with a detection limit as low as 51.48 nM. Furthermore, test strips loaded with **N-Bu-DCIB-2DNP** enable convenient visual detection of H₂S in spoiled food samples, and its applicability in real water samples has also been validated, demonstrating its promising practical utility.

Introduction

Hydrogen sulfide (H₂S) is a gas characterized by its typical rotten-egg odor and plays an indispensable regulatory role in a range of physiological and pathological processes^[1–5]. At the physiological level, H₂S participates in key biological events including neural regulation, maintenance of redox homeostasis, anti-inflammatory responses, and metabolic modulation^[6–10]; conversely, aberrant fluctuations in its concentration—whether elevation or reduction—can induce pronounced physiological disturbances and are closely associated with the pathogenesis of various diseases, such as hypertension, diabetes mellitus, and liver cirrhosis^[11–15]. Moreover, H₂S is one of the major volatile sulfur-containing compounds released during the spoilage of protein-rich foods, posing potential hazards to human health^[16–21]. Therefore, accurate and real-time monitoring of H₂S concentrations is not only of great significance for safeguarding daily life quality but also offers a feasible approach for the early diagnosis of related disorders^[22–26].

Compared with conventional detection methods (e.g., titrimetry, electrochemistry, ion chromatography, and biosensing), fluorescent probe technology has emerged as a widely adopted and effective tool for H₂S detection, owing to its distinct advantages including operational simplicity, high sensitivity, excellent selectivity, and capability for in situ visualization in living systems^[27–31]. To date, numerous small-molecule fluorescent probes have been reported for H₂S recognition, most of which are designed on the basis of specific chemical reactions with H₂S, including nitro/azide reduction^[32, 33], nucleophilic addition^[34, 35], metal precipitation/coordination^[36, 37], and thiolysis reactions^[38–41]. As shown in Scheme 1a, conventional probes based on DNP ether thiolysis are typically designed by capping the hydroxyl group of the fluorophore donor with a DNP unit. This modification effectively disrupts the intramolecular charge transfer (ICT) process, resulting in a quenched ("locked") fluorescent state. The DNP ether strategy is particularly compelling, as it enables the straightforward incorporation of the quenching moiety into hydroxyl-containing fluorophores, thereby providing a robust and efficient means of modulating the ICT pathway. In the presence of hydrogen sulfide, the DNP ether undergoes thiolysis to liberate the hydroxyl-containing fluorophore, concomitantly restoring the ICT and activating the fluorescence signal. However,

this conventional thiolysis approach predominantly relies on mono-DNP caging at the donor site, which still shows insufficient efficiency in the thiolysis reaction, resulting in limited sensitivity, sluggish response kinetics, and weak optical signals^[42, 43].

To address the limitations of thiolysis of mono-DNP ether, we developed a dual-DNP-locked cascade thiolysis strategy, in which the donor hydroxyl is modified with a DNP group and a second DNP unit is incorporated into the acceptor moiety. The simultaneous quenching of both donor and acceptor effectively disrupts the ICT process, leading to a completely quenched ("locked") fluorescence signal (Scheme 1b).

Herein, we designed a dual DNP-caged near-infrared probe, **N-Bu-DCIB-2DNP**, by conjugating dicyanoisophorone (DCI) with an N-alkylated benzimidazole (B) through double vinylene linkers. This probe features two responsive sites—the DNP ether on the donor and the DNP-functionalized electron-deficient quaternary carbon on the DCI unit—to enable a cascade thiolysis strategy. The probe **N-Bu-DCIB-2DNP** displays distinct dual-emission fluorescence with large Stokes shifts and responds rapidly to H₂S, featuring a low detection limit. The cascade thiolysis mechanism was further corroborated by LC-MS and DFT analyses. Its applicability for visual H₂S detection was evaluated using test strips, real water, and spoiled food samples, confirming its considerable practical potential.

Experimental section

Materials and Instruments

All reagents and solvents were obtained from commercial sources and used without further purification. Column chromatography was carried out on silica gel (300–400 mesh). Fluorescence spectra were measured on a Hitachi F-4600 spectrofluorometer, while UV-vis absorption spectra were recorded on a Shimadzu UV-1800 spectrophotometer. ¹H and ¹³C NMR spectra were acquired on a Bruker AVANCE III spectrometer. High-resolution mass spectra (HRMS) were acquired using a Bruker Solanx 70 FT-MS system.

Synthesis of Compound N-Bu-B

Compound **1** (0.50 g, 2.66 mmol) and compound **2** (0.48 g, 3.19 mmol) were mixed and stirred at 120°C for 12 h. After the disappearance of the starting materials (monitored by TLC), a yellow solid precipitated from the reaction mixture. The mixture was cooled to room temperature, and the precipitate was washed with anhydrous ethanol, collected by filtration to afford compound **N-Bu-B** as a yellow solid (0.95 g, 81% yield, Fig. S1-S2). ¹H NMR (400 MHz, DMSO-*d*₆) δ [ppm]: 11.33 (s, 1H), 9.89 (s, 1H), 8.37 (d, *J* = 2.20 Hz, 1H), 8.14 (d, *J* = 16.00 Hz, 1H), 7.76 (d, *J* = 2.00 Hz, 1H), 7.74 (d, *J* = 2.10 Hz, 1H), 7.36 (d, *J* = 16.0 Hz, 1H), 7.59 (m, 2H), 7.20 (m, 2H), 7.10 (d, *J* = 8.30 Hz, 1H), 4.42 (t, *J* = 7.00 Hz, 2H), 1.72 (m, 2H), 1.28 (m, 2H), 0.88 (t, *J* = 7.20 Hz, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ [ppm]: 191.59, 161.81, 151.35, 143.37, 135.59,

131.59, 130.96, 129.21, 123.87, 122.54, 122.50, 119.08, 117.02, 115.45, 110.77, 42.80, 32.67, 19.96, 14.12.

Synthesis of Compound N-Bu-DCIB

Under a nitrogen atmosphere, a mixture of compound **N-Bu-B** (0.50 g, 1.56 mmol) and compound **3** (0.44 g, 2.34 mmol) was dissolved in anhydrous acetonitrile (15 mL). The reaction mixture was refluxed for 24 hours, and the reaction progress was monitored by thin-layer chromatography until the starting materials were completely consumed. After cooling, the product gradually precipitated from the solution as a red solid. The solid was filtered off and washed with ethanol (10 mL), yielding the target compound **N-Bu-DCIB** (0.65 g, 80% yield, Fig. S3-S5), a red solid. ^1H NMR (400 MHz, DMSO- d_6) δ [ppm]: 10.71 (s, 1H), 8.14 (d, J = 16.0 Hz, 1H), 8.12 (s, 2H), 7.87 (d, J = 16.0 Hz, 1H), 7.57 (m, 5H), 7.29 (s, 2H), 7.21 (s, 2H), 6.83 (s, 1H), 4.43 (t, J = 7.0 Hz, 2H), 2.62 (s, 2H), 2.56 (s, 1H), 1.73 (m, 2H), 1.28 (m, 2H), 1.03 (s, 6H), 0.89 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO- d_6) δ [ppm]: 170.74, 158.17, 156.90, 151.56, 143.37, 138.43, 135.88, 131.44, 130.35, 128.37, 128.01, 127.45, 123.74, 122.49, 122.41, 122.14, 118.99, 117.09, 114.68, 114.56, 113.79, 110.71, 75.65, 42.80, 38.74, 32.59, 32.17, 27.93, 19.93, 14.10. HRMS-ESI (m/z): $[\text{M} + \text{H}]^+$ Calculated for $\text{C}_{32}\text{H}_{33}\text{N}_4\text{O}^+$, 489.2654; Found: 489.2654.

Synthesis of probe N-Bu-DCIB-2DNP

A 50 mL round-bottom flask was charged with **N-Bu-DCIB** (1.00 g, 2.05 mmol) and DMF (20 mL). After complete dissolution at room temperature, K_2CO_3 (0.99 g, 7.16 mmol) was added, and the resulting suspension was stirred for 30 minutes. Subsequently, 2,4-dinitrofluorobenzene (1.33 g, 7.16 mmol) was introduced in three batches, and the reaction mixture was stirred overnight at ambient temperature. The progress of the reaction was monitored by TLC. Upon complete consumption of the starting material, the mixture was poured into water (200 mL) and extracted with CH_2Cl_2 (3 $\times$ 20 mL). The combined organic layers were washed with saturated brine, dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (PE: EA = 6:1), to afford the desired product as a yellow solid, **N-Bu-DCIB-2DNP** (0.85 g, 50.5% yield, Fig. S6-S8). ^1H NMR (400 MHz, DMSO- d_6) δ [ppm]: 8.93 (d, J = 5.92 Hz, 2H), 8.77 (d, J = 6.16 Hz, 1H), 8.44 (d, J = 6.28 Hz, 2H), 8.29 (s, 1H), 8.21 (d, J = 8.72 Hz, 1H), 7.90 (d, J = 15.88 Hz, 1H), 7.64 (m, 2H), 7.55 (m, 2H), 7.20 (m, 2H), 6.97 (d, J = 16.20 Hz, 1H), 6.11 (s, 1H), 6.01 (s, 1H), 4.39 (t, J = 7.28 Hz, 2H), 1.64 (m, 2H), 1.20 (m, 2H), 1.15 (s, 6H), 0.80 (t, J = 7.28 Hz, 3H); ^{13}C NMR (100 MHz, DMSO- d_6) δ [ppm]: 155.19, 151.51, 150.51, 149.60, 148.71, 143.19, 142.24, 140.39, 139.84, 135.94, 135.86, 133.42, 130.42, 129.59, 129.41, 128.91, 128.70, 125.68, 122.80, 122.66, 121.78, 120.00, 119.30, 112.83, 111.00, 44.33, 42.97, 33.50, 32.77, 27.42, 19.96, 14.10. HRMS-ESI (m/z): $[\text{M} + \text{H}]^+$ Calculated for $\text{C}_{44}\text{H}_{37}\text{N}_8\text{O}_9^+$, 821.2683; Found: 821.2680.

Results and discussion

Optical Responses of N-Bu-DCIB-2DNP toward H₂S

To evaluate the sensing performance of **N-Bu-DCIB-2DNP** toward H₂S, the optical responses were systematically investigated. As shown in Fig. 1a, upon gradual addition of NaHS (0–30.0 equiv.), the characteristic absorption peak of the probe at 340 nm progressively diminished and red-shifted to 450 nm, indicating the formation of the intermediate **N-Bu-DCIB-DNP**. Upon further increasing the NaHS concentration (30.0–60.0 equiv.), a new long-wavelength absorption band emerged at 690 nm, accompanied by a visible color change of the solution from colorless to yellowish-green (inset of Fig. 1a). Corresponding fluorescence spectra (Fig. 1b) revealed that the probe itself was almost non-fluorescent; with increasing H₂S concentration, two distinct fluorescence emission peaks appeared at 610 nm and 745 nm, along with a change in solution fluorescence from non-emissive to bright orange-yellow (inset of Fig. 1b). Furthermore, to evaluate the specific response and anti-interference capability of the probe toward H₂S, its spectral responses were examined in the presence of various interfering species, including F[−], Cl[−], Br[−], I[−], NO₃[−], ClO₄[−], H₂PO₄[−], OH[−], AcO[−], SCN[−], CN[−], BF₄[−], S^{2−}, HSO₄[−], HSO₃[−], S₂O₃^{2−}, 2-ME, DTT, and GSH. The experimental results (Fig. 1c, d) demonstrated that the probe exhibited excellent selectivity for H₂S, with negligible interference from other analytes.

In addition, further analysis (Fig. 1e) showed that the absorbance at 690 nm was linearly correlated with H₂S concentration in the range of 150–300 μM, with a UV-vis limit of detection (LOD = 3δ/S) of 22.12 μM. Similarly, from Fig. 1f, a good linear relationship was observed between the fluorescence intensity at 745 nm and H₂S concentration over the range of 25–275 μM, with a detection limit of 51.48 nM. Collectively, these results indicate that the probe **N-Bu-DCIB-2DNP** exhibits excellent sensitivity, high selectivity, and good anti-interference ability for the detection of H₂S.

Analytical Performance of N-Bu-DCIB-2DNP toward HS

The analytical performance of **N-Bu-DCIB-2DNP** for H₂S detection was systematically evaluated, including response kinetics and pH tolerance. As shown in Figures S9 and 2a, the probe exhibited stable UV-vis absorption and fluorescence signals over a wide pH range (3–11). Upon addition of the analyte, significant changes in UV absorption intensity and remarkable fluorescence emission were observed across the entire pH range tested. These results indicate that the probe possesses good stability and is suitable for H₂S detection under various acidic and basic conditions. Furthermore, the response kinetics were investigated by monitoring the time-dependent change in fluorescence intensity after the addition of saturating equivalents of H₂S (60.0 equiv.). As depicted in Fig. 2b, upon addition of the analyte, the fluorescence intensity at the characteristic emission wavelength gradually increased with reaction time and reached a stable plateau within 6 min, after which it remained constant. This result demonstrates

that the probe **N-Bu-DCIB-2DNP** can respond to H₂S within a relatively short time, holding promise as a fluorescent sensor for hydrogen sulfide.

Response Mechanism

To elucidate the recognition mechanism of **N-Bu-DCIB-2DNP** toward H₂S, we combined liquid chromatography-mass spectrometry (LC-MS) with density functional theory (DFT) calculations to identify the reaction sites and pathway. For **N-Bu-DCIB-2DNP**, the highest occupied molecular orbital (HOMO) is predominantly distributed over the benzimidazole scaffold, whereas the lowest unoccupied molecular orbital (LUMO) is localized on the dinitrophenyl (DNP) moiety (Fig. 3b). This spatial separation facilitates photoinduced electron transfer (PET), leading to fluorescence quenching. Upon treatment with hydrogen sulfide, the DNP group at the dicyano-substituted electron-deficient quaternary carbon is removed, generating the intermediate **N-Bu-DCIB-DNP**. The orbital distribution of this intermediate shows that the HOMO remains mainly on the benzimidazole framework, while the LUMO reverts to the DNP ether linkage, confirming that the initial reaction with H₂S involves cleavage of the DNP group from the dicyano quaternary carbon. Further reaction with H₂S results in ether bond breakage and release of **N-Bu-DCIB**, with concomitant activation of the intramolecular charge transfer (ICT) effect, thereby producing a strong fluorescence signal. This substantiates the two-step thiolysis-based fluorescence “turn-on” response of probe **N-Bu-DCIB-2DNP** toward H₂S. Moreover, further LC-MS analysis provided direct evidence for the reaction pathway: characteristic peaks at m/z 655.2 [M + H]⁺ and m/z 489.2 [M + H]⁺ were detected in the [**N-Bu-DCIB-2DNP** + H₂S] reaction system, consistent with the molecular masses of the intermediate **N-Bu-DCIB-DNP** and the final product **N-Bu-DCIB**, respectively (Figure S10). Collectively, these results confirm the cascade thiolysis mechanism of **N-Bu-DCIB-2DNP** with H₂S.

Practical applications of probe N-Bu-DCIB-2DNP for H₂S sensing

Application of portable test strips

Based on the favorable colorimetric and fluorescence response characteristics of the probe **N-Bu-DCIB-2DNP** toward H₂S, this study further investigated its immobilized sensing performance to evaluate its potential for practical detection. The probe solution was loaded onto filter paper strips, which were then dried to obtain functionalized test strips. As shown in Fig. 4a upon exposure of the test strips to varying concentrations of H₂S, a distinct “turn-on” fluorescence response was observed under 365 nm UV light, with the strips changing from non-fluorescent to bright orange-yellow. Concurrently, under ambient light, the strips exhibited a visible color change from colorless to yellowish-green. These results demonstrate that the probe **N-Bu-DCIB-2DNP** enables rapid and simple detection of the analyte by naked-eye observation, holding great promise for on-site and point-of-care analysis.

To further evaluate the practical application performance of the **N-Bu-DCIB-2DNP**-loaded test strips, this study employed them for the detection of H₂S gas released from chicken and pork during spoilage. As illustrated in Fig. 4b, the samples were placed in sealed containers, and the probe-loaded test strips were suspended in the headspace of the containers. The experimental results showed that upon exposure to the H₂S gas generated from the spoiling samples, the test strips exhibited a significant fluorescence turn-on response, with the fluorescence color change consistent with that observed in the portable test strip experiments. These results indicate that the probe-loaded test strips enable visual detection of H₂S produced during food spoilage, demonstrating their potential as portable solid-state sensors for real-time monitoring of food freshness.

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Table 1
Recovery and statistical summary of H₂S spiked in tea samples (n = 3)

Sample Group	Parallel Samples	Theoretical Spike (μM)	Spike Found (μM)	Recovery (%)	RSD(n = 3) (%)
A1	A1-1	250	293.2	117.3	10.3
	A1-2	250	239.0	95.6	
	A1-3	250	259.8	103.9	
A2	A2-1	2500	2145.0	85.8	7.0
	A2-2	2500	2326.9	93.1	
	A2-3	2500	2466.3	98.7	

To further evaluate the practical applicability of probe **N-Bu-DCIB-2DNP** in complex matrices, it was employed for the spike recovery determination of H₂S in tea infusion samples. H₂S standard solutions at two concentration levels, low (250 μM) and high (2500 μM), were spiked into the tea samples, with three parallel replicates (n = 3) for each concentration. The spike recoveries and relevant statistical parameters were then calculated. As depicted in Table 1, the recoveries were 95.6%-117.3% (mean 105.6%, RSD = 10.3%) for the low-spike group and 85.8%-98.7% (mean 92.5%, RSD = 7.0%) for the high-spike group,

demonstrating the probe's promising applicability for H₂S quantification in complex aqueous environments.

Conclusion

In summary, we have designed and synthesized a novel dual-DNP-caged NIR fluorescent probe, **N-Bu-DCIB-2DNP**, for H₂S detection, and successfully demonstrated the cascade thiolysis of both the DNP ether on the donor and the DNP-functionalized electron-deficient quaternary carbon on the DCI acceptor. The probe displays dual emission peaks at 610 nm and 745 nm, together with excellent selectivity, high sensitivity, and rapid response kinetics. Furthermore, it was loaded onto test strips for visual detection of H₂S in food samples and applied to quantitative analysis in water samples, collectively illustrating its considerable practical potential. Further studies are currently underway to optimize the probe structure for improved optical properties and biocompatibility, thereby enabling more efficient detection and investigation of H₂S in biological settings.

Declarations

Supplementary Information

¹H NMR, ¹³C NMR, and HR-MS spectra of intermediates and the target compound; pH-dependent absorbance profiles of the probe; LC-MS analysis of the probe upon reaction with H₂S.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Jiali Zhao: writing—original draft, and conceptualization; Ruyu Liu: formal analysis; Minqi Xie: data curation; Shuang Wang: investigation; Shumin Jiang: methodology; Hua Zhang: formal analysis and methodology; Chuanxiang Liu: writing—review and editing, supervision, and methodology. All authors read and approved the final manuscript.

Data Availability

All data are available in the main text or the ESI, with additional data provided upon request.

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Figures

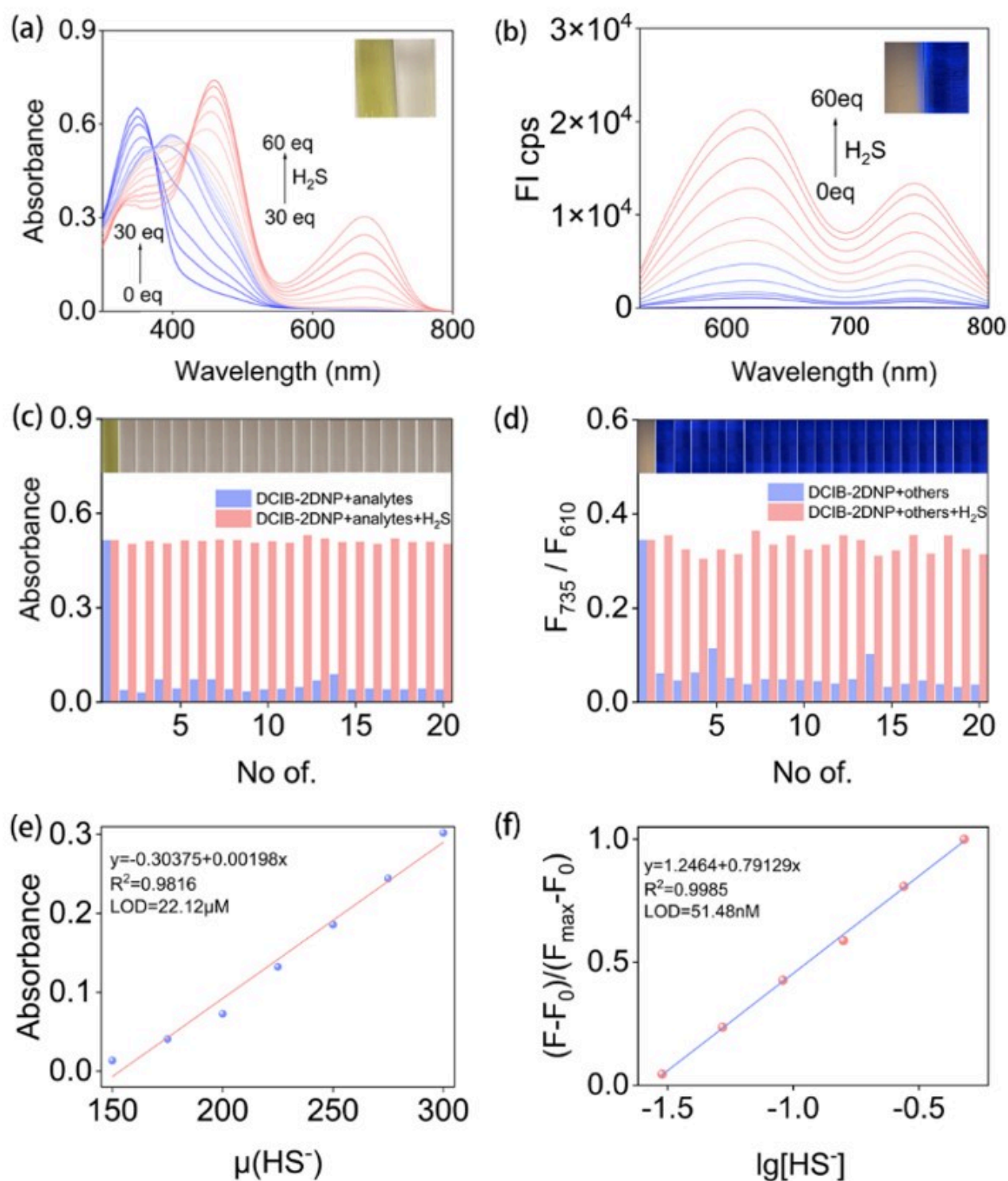


Figure 1

Spectral responses of probe **N-Bu-DCIB-2DNP** (20 μM) to H_2S in DMSO/PBS (9/1, v/v, pH 7.4). (a) UV-vis absorption spectra and (b) fluorescence emission spectra ($\lambda_{\text{exc}} = 480 \text{ nm}$) of **N-Bu-DCIB-2DNP** upon addition of different concentrations of H_2S (0-60 equiv.); insets show the color changes under ambient light and a 365 nm UV lamp; (c, d) Absorption spectral responses, fluorescence spectral responses, and anti-interference performance of **N-Bu-DCIB-2DNP** toward various analytes (60 equiv.), where 1-20

correspond to HS^- , F^- , Cl^- , Br^- , I^- , NO_3^- , ClO_4^- , H_2PO_4^- , OH^- , AcO^- , SCN^- , CN^- , BF_4^- , S^{2-} , HSO_4^- , HSO_3^- , $\text{S}_2\text{O}_3^{2-}$, 2-ME, DTT, and GSH, respectively; insets show the color changes; (e) Linear relationship between the absorbance at 670 nm and the concentration of HS^- (150-300 μM); (f) Linear relationship between the fluorescence intensity at 745 nm and $\log[\text{HS}^-]$

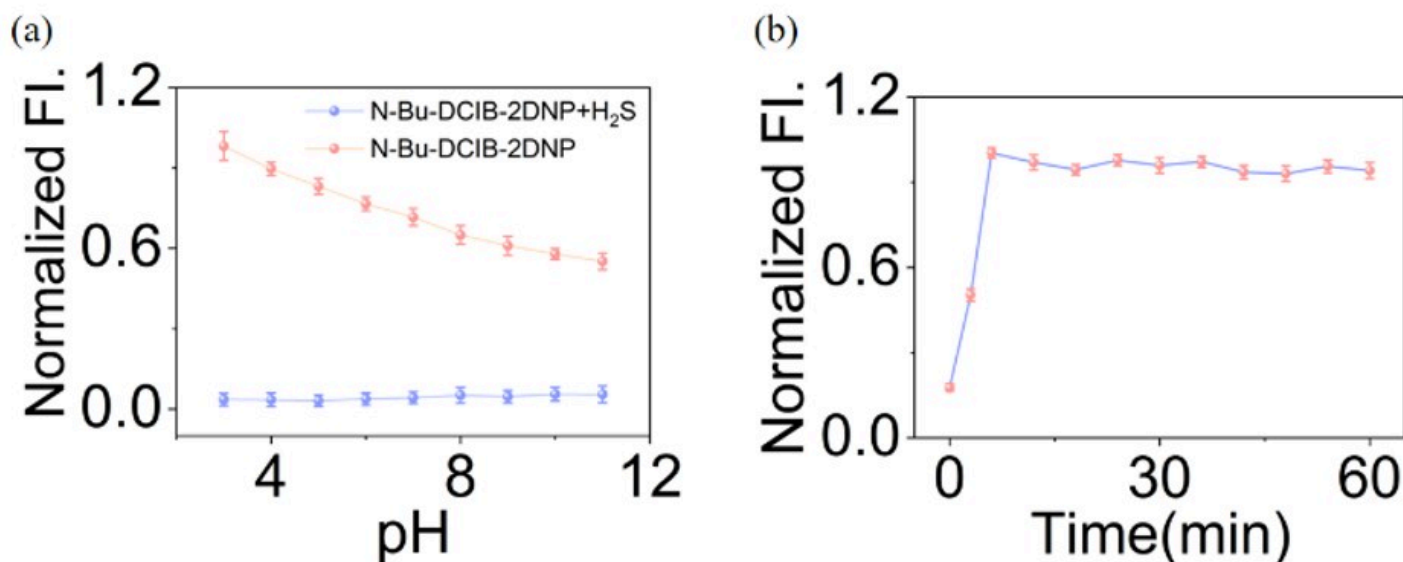


Figure 2

Analytical performance of probe **N-Bu-DCIB-2DNP** (20 μM , DMSO: PBS = 9:1, v/v, pH 7.4) toward H_2S . (a) Fluorescence intensity at 745 nm of **N-Bu-DCIB-2DNP** before and after treatment with H_2S (60.0 equiv.) as a function of pH; (b) time-dependent fluorescence response of the probe to H_2S (60.0 equiv.)

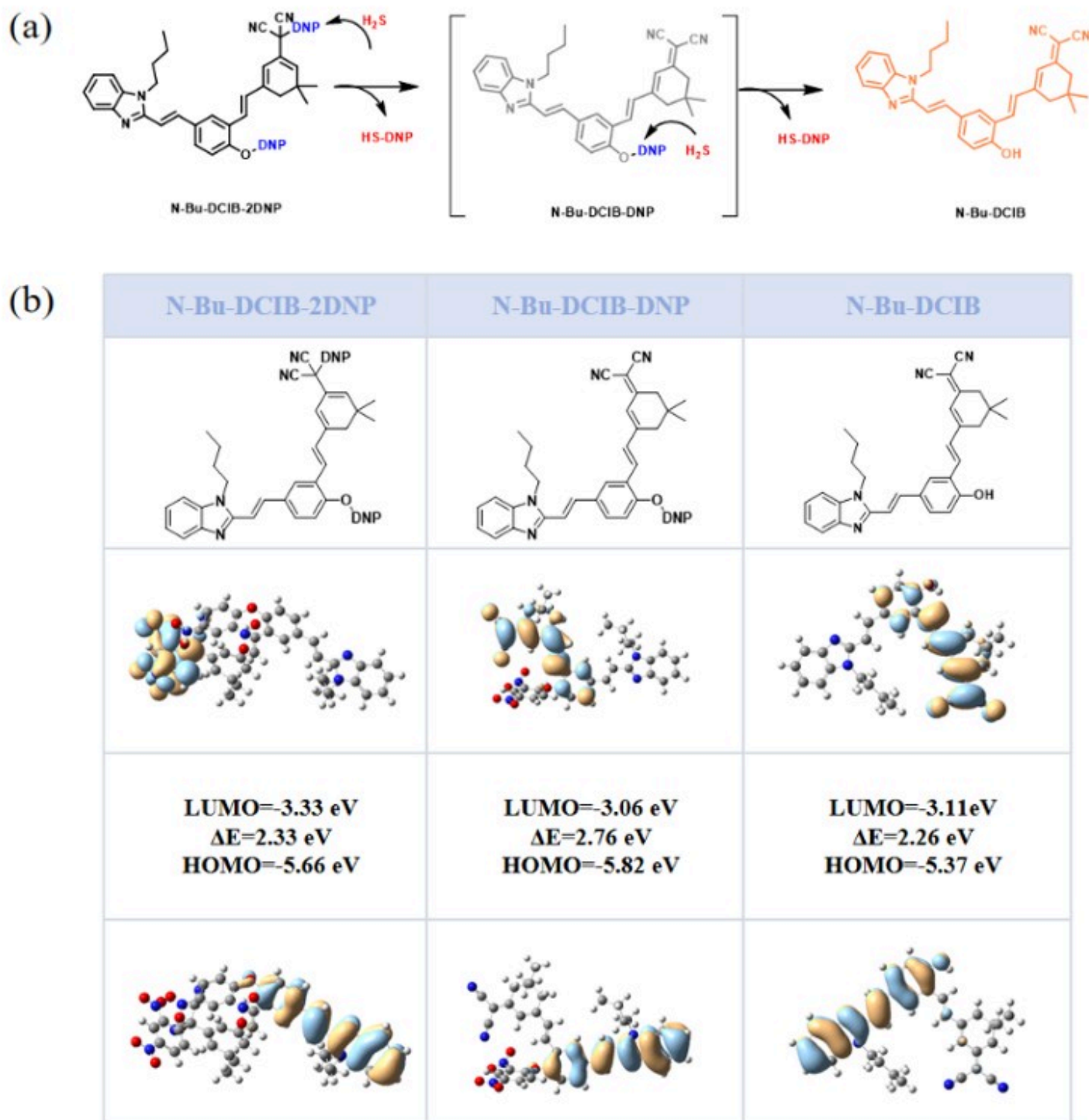


Figure 3

(a) Response mechanism of probe **N-Bu-DCIB-2DNP** toward H_2S ; (b) Frontier molecular orbital diagrams of **N-Bu-DCIB-2DNP**, **N-Bu-DCIB-DNP**, and **N-Bu-DCIB** obtained by density functional theory (DFT) calculations

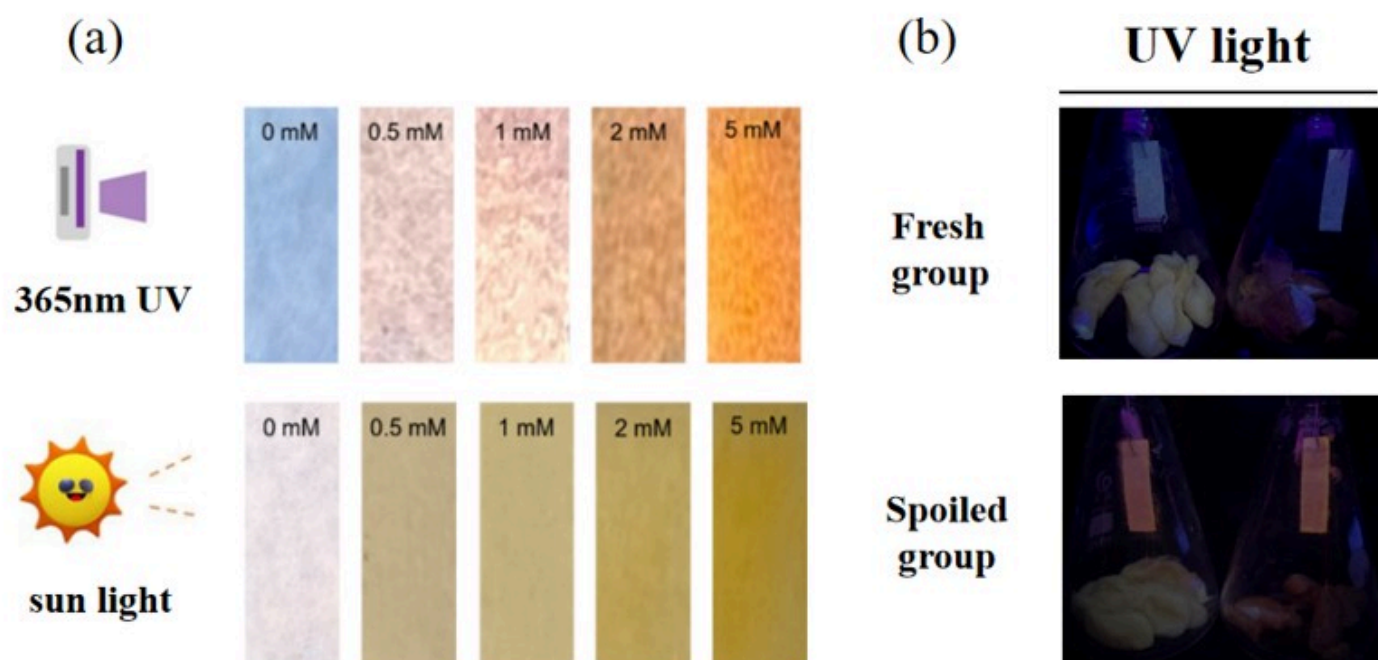


Figure 4

(a) Color changes of the test strip loaded with probe **N-Bu-DCIB-2DNP** upon exposure to different concentrations of H_2S , observed under 365 nm UV light and natural light; (b) Fluorescence monitoring of H_2S released during the spoilage process of fresh and spoiled chicken (left) and pork (right) using the test strip loaded with probe **N-Bu-DCIB-2DNP**

Supplementary Files

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