Supplementary Information

A phase-transfer catalyst-based nanoreactor for accelerated

hydrogen sulfide bio-imaging

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Supplementary Methods

Arrhenius equation

The Arrhenius equation is:

$$k = Ae^{-E_a/RT}$$

Taking the natural logarithm of the Arrhenius equation yields:

$$Ink = InA + \left(-\frac{E_a}{R}\right)\left(\frac{1}{T}\right)$$

Where *R* is molar gas constant (8.314 J·K⁻¹·M⁻¹), *k* is rate constant, *T* is thermodynamic temperature, *A* is the frequency factor, and E_a is the activation energy.

Cell Culture

RAW 264.7 cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) Fetal Bovine Serum (FBS, Gibco), 100 U/mL penicillin, and 100 μ g/mL streptomycin at 37 °C with 5% CO2. Then cells were transferred to culture dishes. Cell imaging was carried out after washing the cells with PBS (10 mM, pH 7.4).

Synthesis of the compounds

2,2'-Dipyridyl disulfide benzoic acid was synthesized by the literature methods.¹⁻² Synthesis of **DS-Blue**



4-Methylumbelliferone (88 mg, 0.5 mmol), DMAP (6.1 mg, 0.05 mmol), 2,2'-Dipyridyl disulfide benzoic acid (158 mg, 0.6 mmol), and EDCI (115 mg, 0.6 mmol) were dissolved in CH₂Cl₂ (5 mL). The reaction mixture was stirred at room temperature for overnight. The solvent was evaporated, and the crude product was purified by silica gel column chromatography to afford **DS-Blue** as a white solid (26% yield). ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.49 (dd, *J* = 7.8 Hz, 1H), 8.34 (dd, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 7.8 Hz, 2H), 7.79 (td, *J* = 7.8 Hz, 1H), 7.76 – 7.73 (m, 1H),

7.55 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.51 – 7.47 (m, 1H), 7.42 (dd, J = 7.8 Hz, 1H), 7.30 – 7.27 (m, 1H), 6.43 (d, J = 7.8 Hz, 1H), 2.47 (s, 3H). ¹³C NMR (150 MHz, DMSO- d_6) δ 163.84, 159.60, 156.96, 153.59, 152.95, 152.66, 149.89, 140.24, 138.13, 134.60, 132.32, 126.57, 126.55, 125.58, 125.55, 121.86, 119.82, 118.53, 117.92, 113.97, 110.39, 18.22. HRMS (ESI): m/z calculated for C₂₂H₁₄NO₄S₂⁻, 420.0364. Found 420.0365.



Synthesis of 7-Hydroxycoumarin-Azido

7-Hydroxycoumarin-4-acetic acid (220 mg, 1.0 mmol), DMAP (12.2 mg, 0.1 mmol), 3-Azido-1-propanamine (300 mg, 3.0 mmol), and EDCI (575 mg, 3.0 mmol) were dissolved in CH₂Cl₂ (5 mL). The reaction mixture was stirred at room temperature for overnight. The solvent was evaporated, and the crude product was purified by silica gel column chromatography to afford 7-Hydroxycoumarin-Azido as a white solid (62% yield). ¹H NMR (600 MHz, DMSO- d_6) δ 10.56 (s, 1H), 7.59 (d, J = 8.7 Hz, 1H), 6.79 (dd, J = 8.7 Hz, 1H), 6.72 (d, J = 8.7 Hz, 1H), 6.16 (s, 1H), 3.63 (s, 2H), 3.12 (dd, J = 8.6 Hz, 2H), 1.65 (p, J = 8.7 Hz, 2H), 1.23 (s, 1H).

Synthesis of DS-Blue-Azido

To a solution of 7-Hydroxycoumarin-Azido (120 mg, 0.4 mmol), DMAP (4.9 mg, 0.04 mmol), 2,2'-dipyridyl disulfide benzoic acid (126 mg, 0.48 mmol), and EDCI (92 mg, 0.48 mmol) were dissolved in CH₂Cl₂ (5 mL). The reaction mixture was stirred at room temperature for overnight. The solvent was evaporated, and the crude product was purified by silica gel column chromatography to afford **DS-Blue-Azido** as a white solid (28% yield). ¹H NMR (600 MHz, DMSO- d_6) δ 8.50 – 8.47 (m, 1H), 8.34 (dd, J = 7.8 Hz, 1H), 8.29 (t, J = 7.6 Hz, 1H), 7.89 (t, J = 7.8 Hz, 2H), 7.79 (td, J = 7.9 Hz, 1H), 7.77 – 7.73 (m, 1H), 7.55 (dd, J = 7.8 Hz, 2H), 7.51 – 7.48 (m, 1H), 7.42 (dd, J = 7.8 Hz, 2H), 1.67 (p, J = 7.8 Hz, 2H). ¹³C NMR (150 MHz, DMSO- d_6) δ 167.53, 163.85, 159.55, 156.96, 153.76, 152.65, 150.47, 149.90, 140.26, 138.14, 134.63,

132.34, 126.60, 126.57, 125.59, 125.52, 121.87, 119.83, 118.54, 117.42, 115.79, 110.59, 48.36,
38.82, 36.24, 28.25. HRMS (ESI): m/z calculated for C₂₆H₂₀N₅O₅S₂⁻, 546.0906. Found 546.0911.
Synthesis of **DN-Green**



Fluorescein (332 mg, 1.0 mmol), 1-bromo-2,4-dinitrobenzene (247 mg, 1.00 mmol) and potassium carbonate (138 mg, 1.0 mmol) were dissolved in DMF (5 mL). The mixture was stirred for overnight at 50 °C. The reaction mixture was evaporated, and the crude compound was purified by silica gel column chromatography to afford **DN-Green** as a yellow solid (57% yield). ¹H NMR (600 MHz, DMSO- d_6) δ 10.22 (s, 1H), 8.91 (d, J = 7.8 Hz, 1H), 8.48 (dd, J = 7.8 Hz, 1H), 8.03 (dd, J = 7.8 Hz, 1H), 7.83 (td, J = 7.6 Hz, 1H), 7.75 (td, J = 7.6 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.32 (d, J = 7.8 Hz, 1H), 7.00 (dd, J = 7.7 Hz, 1H), 6.92 (d, J = 7.7 Hz, 1H), 6.71 (d, J = 7.8 Hz, 1H), 6.65 – 6.59 (m, 2H). ¹³C NMR (150 MHz, DMSO- d_6) δ 169.00, 160.22, 156.09, 154.20, 152.73, 152.38, 151.92, 142.72, 140.45, 136.32, 130.87, 130.21, 129.64, 126.28, 125.33, 124.55, 122.42, 121.42, 116.99, 116.18, 113.68, 109.57, 108.48, 102.71, 82.35. HRMS (ESI): m/z calculated for C₂₆H₁₃N₂O₉⁻, 497.0621. Found 497.0629.

Synthesis of 5-azidofluorescein



5-Aminofluorescein (694 mg, 2.0 mmol) was dissolved in a solution of 8 mL acetic acid and 4 mL water. The solution was stirred at 0 °C for 30 min, then sodium nitrite (207 mg, 3.0 mmol) was added. The mixture was kept at 0 °C for another 1 h. Next, sodium azide (260 mg, 4.0 mmol) was added gradually, the solution turned to be a yellow slurry, and the mixture was stirred for another 2 h at 0 °C. The yellow slurry was filtered and purified by silica gel column chromatography to afford 5-azidofluorescein as an orange solid (71% yield). ¹H NMR (600 MHz, DMSO) δ 7.12 (s, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.14 – 6.07 (m, 4H), 5.99 (d, *J* = 8.6 Hz, 2H).

Synthesis of DN-Green-Azido

5-azidofluorescein (373 mg, 1.0 mmol), 1-bromo-2,4-dinitrobenzene (247 mg, 1.00 mmol) and potassium carbonate (138 mg, 1.0 mmol) were dissolved in DMF (5 mL). The mixture was stirred for overnight at 50 °C. The reaction mixture was evaporated, and the crude compound was purified by silica gel column chromatography to afford **DN-Green-Azido** as a yellow solid (32% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.85 (s, 1H), 8.37 (dd, *J* = 8.2 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.32 (dd, *J* = 8.2 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 7.04 – 6.98 (m, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.80 (dd, *J* = 8.2 Hz, 1H), 6.73 (d, *J* = 8.2 Hz, 1H), 6.63 (d, *J* = 8.2 Hz, 1H), 6.60 (dd, *J* = 8.2 Hz, 1H). ¹³C NMR (150 MHz, CDCl₃) δ 168.52, 163.05, 159.12, 155.61, 155.54, 154.95, 152.88, 152.16, 148.86, 143.04, 142.37, 140.16, 130.45, 129.23, 129.14, 128.51, 126.65, 125.54, 122.30, 122.27, 119.97, 117.01, 115.65, 114.77, 113.30, 109.89, 108.70, 108.59, 103.31. HRMS (ESI): m/z calculated for C₂₆H₁₂N₅O₉⁻, 538.0635. Found 538.0625.

Synthesis of 4-azido-1,8-naphthalic anhydride

4-bromo-1,8-naphthalic anhydride (277 mg, 1.0 mmol) and sodium azide (98 mg, 1.5 mmol) were dissolved in DMF (5 mL). The mixture was stirred vigorously for overnight at 50 °C. Then the solution was poured into ice water (90 mL). The precipitate was filtered and redissolved with DCM to obtain the final product, which was used in the next step without further purification.



Synthesis of AZ-Green and AZ-Green-COOH

4-Azido-1,8-naphthalic anhydride (239 mg, 1.0 mmol), DMAP (12 mg, 0.1 mmol), and corresponding amine (2 mmol) were dissolved in methanol (20 mL). The mixture was refluxed for overnight. After cooling, the precipitate was filtered and further purified by silica gel column chromatography to afford **AZ-Green** and **AZ-Green-COOH** (75% and 68% yield, respectively). Characterization data are in agreement with literature report.³⁻⁶ **AZ-Green** ¹H NMR (600 MHz, CDCl₃) δ 8.61 (dd, J = 8.3 Hz, 1H), 8.56 (d, J = 8.3 Hz, 1H), 8.41 (dd, J = 8.4, 1.0 Hz, 1H), 7.73 (dd, J = 8.3 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 4.16 (t, 2H), 1.74 – 1.68 (m, 1H), 1.48 – 1.41 (m, 1H), 0.97 (t, J = 8.4 Hz, 1H). ¹³C NMR (151 MHz, CDCl₃) δ 164.00, 163.59, 143.36, 132.17, 131.66, 129.15, 128.70, 126.85, 124.35, 122.69, 118.99, 114.67, 40.28, 30.23, 20.40, 13.86. **AZ-**

Green-COOH ¹H NMR (600 MHz, DMSO- d_6) δ 12.02 (s, 1H), 8.49 (dd, J = 8.3 Hz, 1H), 8.42 (d, J = 8.3 Hz, 1H), 8.37 (dd, J = 8.4 Hz, 1H), 7.83 (dd, J = 8.3 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 4.06 (t, J = 8.0 Hz, 2H), 2.30 (t, J = 8.3 Hz, 2H), 1.88 (p, J = 8.2 Hz, 2H). ¹³C NMR (150 MHz, DMSO- d_6) δ 173.99, 163.33, 162.88, 142.73, 131.53, 131.44, 128.33, 128.25, 127.25, 123.49, 122.19, 118.22, 115.90, 31.32, 30.70, 22.99.

Synthesis of probe-polymer conjugations



Synthesis of DBCO-PEG-PPG-PEG-DBCO and AZ-Green-polymer conjugation

Carbonyl compounds (0.1 mmol), DMAP (12.2 mg, 0.1 mmol), NHS (12 mg, 0.1 mmol), EDCI (57.5 mg, 0.3 mmol), and PEG-PPG-PEG (378 mg, 0.03 mmol) were dissolved in DMF (5 mL). The reaction mixture was stirred at room temperature for overnight. The final product was purified by dialysis, followed by lyophilization.

Synthesis DS-Blue-polymer and DN-Green-polymer conjugation

Azide compounds of **DS-Blue** and **DN-Green** (0.1 mmol) and DBCO-PEG-PPG-PEG-DBCO (0.03 mmol) were dissolved in DMF (5 mL). The reaction mixture was stirred at room

temperature for 1 h. The final product was purified by dialysis, followed by lyophilization.

Supplemental figures



Fig. S1 Absorbance of **DS-Blue** (0-50 μ M) in DMSO and the standard curve of absorbance at 313 nm for the determination of the concentration of probe in the **DS-Blue**-polymer conjugation.



Fig. S2 Absorbance of **DN-Green** (0-50 μ M) in DMSO and the standard curve of absorbance at 275 nm for the determination of the concentration of probe in the **DN-Green**-polymer conjugation.



Fig. S3 Absorbance of **AZ-Green** (0-40 μ M) in DMSO and the standard curve of absorbance at 370 nm for the determination of the concentration of probe in the **AZ-Green**-polymer conjugation.



Fig. S4 SEM images (a) and number weighted distributions (b) by nanoparticle tracking analysis (NTA) of **DS-Blue-Nano**, **DN-Green-Nano**, and **AZ-Green-Nano**. Scale bar was 500 nm.



Fig. S5 Linear relationship between different concentration of H_2S (0-75 μ M) and the emission of probe **DS-Blue-nano** (10 μ M) at 450 nm in PBS (10 mM, pH 7.4).



Fig. S6 Linear relationship between different concentration of H_2S (0-100 μ M) and the emission of probe **DN-Green-nano** (10 μ M) at 525 nm in PBS (10 mM, pH 7.4).



Fig. S7 Linear relationship between different concentration of H_2S (0-100 μ M) and the emission of probe **AZ-Green-nano** (10 μ M) at 540 nm in PBS (10 mM, pH 7.4).



Fig. S8 Competitive experiments for H_2S selectivity. Fluorescence responses of **DS-Blue-nano**, **DN-Green-nano**, and **AZ-Green-nano** (10 μ M for each) to H_2S (20 μ M) and other analytes (200 μ M for each) in PBS (10 mM, pH 7.4) at room temperature. F_0 and F are the fluorescence intensity at the maximum emission wavelength without and with the presence of analyte, respectively.



Fig. S9 Stability of **DS-Blue-nano** and **DN-Green-nano** in different pH. a) The shape stability of **DS-Blue-nano** and **DN-Green-nano** at pH 4.0, 6.0, 7.4, 9.0, and 11.0; b) The detection stability of **DS-Blue-nano** and **DN-Green-nano** for H₂S at pH 4.0, 6.0, 7.4, 9.0, and 11.0. F₀ and F are the fluorescence intensity at the maximum emission wavelength without and with the presence of 20 μ M H₂S, respectively.



Fig. S10 Concentration dependent cytotoxicity of **DS-Blue-nano** and **DN-Green-nano** to RAW 264.7 (a and b) and MCF-7cells (c and d) at 24 h determined by CCK-8 assay.



Fig. S11 Detection of H_2S by **DS-Blue-Nano** and **DN-Green-Nano** in living MCF-7 cells. Cells were treated with Na_2S (100 μ M) to generate exogenous H_2S , with no addition as control. The cells were then incubated with **DS-Blue-Nano** (10 μ M, as a) or **DN-Green-Nano** (10 μ M, as b). The photos were taken under a fluorescent microscope. Scale bar: 50 μ m.







Fig. S13 ¹³C NMR of **DS-Blue** in DMSO- d_6 .



Fig. S15 ¹H NMR of 7-Hydroxycoumarin-Azido in DMSO-d₆.



Fig. S16 ¹H NMR of **DS-Blue-Azido** in DMSO-*d*₆.







Fig. S18 HRMS of **DS-Blue-Azido**.

-10.22 -10.22-10.



Fig. S19¹H NMR of **DN-Green** in DMSO-*d*₆.



Fig. S20 ¹³C NMR of **DN-Green** in DMSO-*d*₆.



Fig. S21 HRMS of **DN-Green**.

$\begin{array}{c} 7.12\\ 6.94\\ 6.92\\ 6.75\\ 6.75\\ 6.76\\ 6.09\\ 6.09\\ 5.98 \end{array}$



Fig. S22 ¹H NMR of 5-azidofluorescein in DMSO-*d*₆.





Fig. S23 ¹H NMR of **DN-Green-Azido** in CDCl₃.

168.52 155.61 155.61 155.61 155.61 155.61 155.64 155.64 155.64 155.64 152.88 152.88 152.88 142.04















Fig. S27 ¹³C NMR of AZ-Green in CDCl₃.







Fig. S29 ¹³C NMR of **AZ-Green-COOH** in DMSO-*d*₆.



Fig. S30 FT-IR spectra of PEG-PPG-PEG, DBCO-PEG-PPG-PEG-DBCO, **DS-Blue-polymer**, **DN-Green-polymer**, and **AZ-Green-polymer**.

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