Supporting Information

*N*-annulated Perylene-Based Colorimetric and Ratiometric Near-Infrared Fluorescent Probes for the Selective Detection of Hydrogen Sulfide in Mitochondria, Lysosomes, and Serum

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1. Linear correction between the absorbance ratio and concentrations of NaHS

Figure S1. Linear correction between the absorbance ratio ($A_{444 \text{ nm}}/A_{514 \text{ nm}}$) of NPNM and concentrations of NaHS (0-80 μM). The absorbance was measured in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 °C.

2. Time-dependent absorption spectra of NPNM towards NaHS

Figure S2. (A) Time-dependent (0-10 min) absorption spectra of NPNM (10 μM) towards NaHS (100 μM) in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 °C. (B) The curve of time-dependent absorbance ratio ($I_{444 \text{ nm}}/I_{514 \text{ nm}}$) responses towards NaHS.
3. Time-dependent fluorescence spectra of NPNM towards NaHS

**Figure S3.** (A) Time-dependent (0-10 min) fluorescence emission spectra of NPNM (10 μM) towards NaHS in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 °C. (B) The variation curve of fluorescence intensity at 481 nm with the reaction time (0-10 min). $\lambda_{ex} = 435$ nm.

**Figure S4.** (A) Time-dependent (0-10 min) fluorescence emission spectra of NPNM (10 μM) towards NaHS in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 °C. (B) The variation curve of fluorescence intensity at 681 nm with the reaction time (0-10 min). $\lambda_{ex} = 510$ nm.
4. Absorption and fluorescence response of Mito-NPNM towards NaHS

Figure S5. The absorption (A) and fluorescence emission (B and C) changes of Mito-NPNM (10 μM) with the various concentration of NaHS (0-150 μM) in PBS buffer solution (pH = 7.4, 37 °C). (B) $\lambda_{ex} = 435$ nm. (C) $\lambda_{ex} = 510$ nm.

5. Absorption and fluorescence response of Lyso-NPNM towards NaHS

Figure S6. The absorption (A) and fluorescence emission (B and C) changes of Lyso-NPNM (10 μM) with the various concentration of NaHS (0-150 μM) in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 °C. (B) $\lambda_{ex} = 435$ nm. (C) $\lambda_{ex} = 510$ nm.
6. Selectivity

Figure S7. The selectivity of Mito-NPNM (10 μM) towards different molecules (1: blank; 2: NaHS; 3: HSO₃⁻; 4: SO₃²⁻; 5: SO₄³⁻; 6: S₂O₅²⁻; 8: S₂O₄²⁻; 9: F⁻; 10: Cl⁻; 11: Br⁻; 12: I⁻; 13: AcO⁻; 14: SCN⁻; 15: CO₃²⁻; 16: HCO₃⁻; 17: H₂PO₄⁻; 18: NO₂⁻; 19: OH⁻; 20: ClO⁻; 21: H₂O₂; 22: t-BuOOH; 23: Cys; 24: Hcy and 25: GSH) in PBS buffer solution at 37 °C. The concentrations of biological thiols (Cys, Hcy and GSH) were 5 mM, and the concentrations of other molecules were 200 μM.

Figure S8. The selectivity of Lyso-NPNM (10 μM) towards different molecules (1: blank; 2: NaHS; 3: HSO₃⁻; 4: SO₃⁻⁻; 5: SO₄⁻⁻; 6: S₂O₅²⁻; 8: S₂O₄²⁻; 9: F⁻; 10: Cl⁻; 11: Br⁻; 12: I⁻; 13: AcO⁻; 14: SCN⁻; 15: CO₃²⁻; 16: HCO₃⁻; 17: H₂PO₄⁻; 18: NO₂⁻; 19: OH⁻; 20: ClO⁻; 21: H₂O₂; 22: t-BuOOH; 23: Cys; 24: Hcy and 25: GSH) in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH=7.4) at 37 °C. The concentrations of biological thiols (Cys, Hcy and GSH) were 5 mM, and the
concentrations of other molecules were 200 μM.

7. The fluorescent emission of the core of N-annulated perylene

![Image of fluorescence emission spectra](image)

**Figure S9.** The fluorescence emission spectra of the core of N-annulated perylene in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 °C. λ<sub>ex</sub> = 400 nm.
8. The stability of NPNM

**Figure S10.** The absorption (A) and fluorescence intensity (B) of NPNM (10 μM) before and after reacting with NaHS (100 μM) for 7 minutes at different pH values. The maximum absorption wavelength at 514 nm and 444 nm were measured respectively before and after the reaction. The fluorescence emissions at 681 nm and 481 nm were also measured before and after the reaction. (C) Photostability of NPNM (10 μM) and the product after reacting with NaHS (100 μM) compared with fluorescein isothiocyanate were studied under 1 kW/m² light irradiation for 0-60 minutes. (D) Chemostability of NPNM (10 μM) and the product after reacting with NaHS (100 μM) compared with cyanine were also studied after the addition of different concentrations (0-40 equivalents) of NaClO. $I_0$ represented the initial fluorescence intensity and $I$ represented the intensity after the treatment.
9. The pH stability of Mito-NPNM and Lyso-NPNM

Figure S11. The absorption (A) and fluorescence intensity (B) of Mito-NPNM (10 μM) before and after reacting with NaHS (100 μM) for at different pH values. The maximum absorption wavelength at 514 nm and 444 nm were measured respectively before and after the reaction. The fluorescence emissions at 681 nm and 481 nm were also measured before and after the reaction.

Figure S12. The absorption (A) and fluorescence intensity (B) of Lyso-NPNM (10 μM) before and after reacting with NaHS (100 μM) at different pH values. The maximum absorption wavelength at 514 nm and 444 nm were measured respectively before and after the reaction. The fluorescence emissions at 681 nm and 481 nm were also measured before and after the reaction.
10. Photostability of Mito-NPNM and Lyso-NPNM

Figure S13. (A) Photostability of 10 μM Mito-NPNM (A), Lyso-NPNM (B) and their products after reacting with NaHS (100 μM) compared with fluorescein isothiocyanate were studied under 1 kW/m² light irradiation for 0-60 minutes. \( I_0 \) represented the initial fluorescence intensity at 481 nm and \( I \) represented the intensity after the treatment at 681 nm.

11. Chemostability of Mito-NPNM and Lyso-NPNM

Figure S14. Chemostability of 10 μM Mito-NPNM (A), Lyso-NPNM (B) and their products after reacting with NaHS (100 μM) compared with cyanine were also studied after the addition of different concentrations of NaClO (0-40 equivalents). \( I_0 \) represented the initial fluorescence intensity and \( I \) represented the intensity after the treatment.
12. The cytotoxicity of Lyso-NPNM and Mito-NPNM

![Figure S15](image_url)

Figure S15. Cell viability (%) estimated by MTT proliferation tests versus incubation concentration of Lyso-NPNM (A) and Mito-NPNM (B). Hela cells were incubated with 0-10 μM Lyso-NPNM or Mito-NPNM at 37 °C for 24 hours.

13. Detecting H₂S in fetal bovine serum

![Figure S16](image_url)

Figure S16. The changes of fluorescence spectra of NPNM in the presence of different concentration (0.0, 5.0, 10.0, 17.0, 20.0, 40.0, and 50.0 μM) of NaHS in the FBS serum. (A) $\lambda_{ex} = 435$ nm; (B) $\lambda_{ex} = 510$ nm.
Figure S17. The changes of fluorescence spectra of Mito-NPNM in the presence of different concentration (0.0, 5.0, 10.0, 17.0, 20.0, 40.0, and 50.0 μM) of NaHS in the FBS serum. (A) $\lambda_{ex} = 435$ nm; (B) $\lambda_{ex} = 510$ nm.

Figure S18. The changes of fluorescence spectra of Lyso-NPNM in the presence of different concentration (0.0, 5.0, 10.0, 17.0, 20.0, 40.0, and 50.0 μM) of NaHS in the FBS serum. (A) $\lambda_{ex} = 435$ nm; (B) $\lambda_{ex} = 510$ nm.
Figure S19. (A) Plotting the ratiometric fluorescence intensity (I$_{481\text{ nm}}$/I$_{681\text{ nm}}$) as a function of low NaHS concentration (0-50 μM) for NPNM (10 μM). (B) The spiked (7.0, 15.0, 25.0 and 35.0 μM) and measured concentrations (7.55, 14.76, 27.63 and 32.70 μM) of NaHS in diluted serum solution.

Figure S20. (A) Plotting the ratiometric fluorescence intensity (I$_{481\text{ nm}}$/I$_{681\text{ nm}}$) as a function of low NaHS concentration (0-50 μM) for Lyso-NPNM (10 μM). (B) The spiked (7.0, 15.0, 25.0 and 35.0 μM) and measured concentrations (6.62, 15.86, 27.03 and 34.02 μM) of NaHS in diluted serum solution.
14. Characterization of new compounds

**Figure S21.** $^1$H NMR spectrum of intermediate compound 2 in CDCl$_3$

**Figure S22.** $^{13}$C NMR spectrum of intermediate compound 2 in CDCl$_3$
Figure S23. HRMS of intermediate compound 2

Figure S24. $^1$H NMR spectrum of intermediate compound 3 in CDCl$_3$
Figure S25. $^{13}$C NMR spectrum of intermediate compound 3 in CDCl$_3$.

Figure S26. HRMS of intermediate compound 3.
Figure S27. $^1$H NMR spectrum of NPNM in CDCl$_3$

Figure S28. $^{13}$C NMR spectrum of NPNM in CDCl$_3$
Figure S29. HRMS of NPNM

Figure S30. $^1$H NMR spectrum of intermediate compound 4 in CDCl$_3$
Figure S31. \(^{13}\)C NMR spectrum of intermediate compound 4 in CDCl\(_3\)

Figure S32. HRMS of intermediate compound 4
Figure S33. $^1$H NMR spectrum of intermediate compound 5 in CDCl$_3$

Figure S34. $^1$H NMR spectrum of intermediate compound 5 in CDCl$_3$
Figure S35. HRMS of intermediate compound 5

Figure S36. $^1$H NMR spectrum of intermediate compound 6 in CDCl$_3$
Figure S37. $^{13}$C NMR spectrum of intermediate compound 6 in CDCl$_3$.

Figure S38. HRMS of intermediate compound 6.
Figure S39. $^1$H NMR spectrum of Mito-NPNM in CDCl$_3$

Figure S40. $^{13}$C NMR spectrum of Mito-NPNM in CDCl$_3$
Figure S41. HRMS of Mito-NPNM

Figure S42. $^1$H NMR spectrum of Lyso-NPNM in CDCl$_3$
Figure S43. $^{13}$C NMR spectrum of Lyso-NPNM in CDCl$_3$

Figure S44. HRMS of Lyso-NPNM