A Colorimetric and Fluorescent Dual Probe for Specific Detection of Cysteine Based on Intramolecular Nucleophilic Aromatic Substitution

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Figure S1 Absorption (A) and emission (B) spectra of **NNA** in the presence of different thiols in DMF. (a) none; (b) GSH; (c) N-acetyl-L-cysteine; (d) Mercapto-propionic acid; (e) Mercaptoethylamine; (f) Hcy; [**NNA**] = 20 μ M, [thiols] = 200 μ M, the spectra were recorded after equilibrated at 50 °C for 2 hours, λ_{ex} = 390 nm.



Figure S2 ESI-MS spectrum of the substituent product of **NNA** reacted with Cys (Anal. Calcd: 317.04).



Figure S3 Time-dependent absorption (A) and emission (B) spectra of NNA-Cys in the presence of 1 equiv of acid in DMF, and those of the further addition of 1 equiv of base (C and D). [NNA] = 20 μ M, [Cys] = 200 μ M, the spectra were recorded at 50 °C, $\lambda_{ex} = 390$ nm.



Figure S4 Time-dependent absorption (A, B) and emission (C, D) spectra of **NNA** in the presence of Hcy in DMF; [**NNA**] = 20 μ M, [Hcy] = 200 μ M, reacted at 50°C, $\lambda_{ex} = 390$ nm (C); $\lambda_{ex} = 435$ nm (D).



Figure S5 Time-dependent absorption (A, B) and emission (C, D) spectra of **NNA** in the presence of MEA in DMF; [**NNA**] = 20 μ M, [MEA] = 200 μ M, reacted at 50°C, $\lambda_{ex} = 390$ nm.



Figure S6 Time-dependent HPLC spectra of **NNA** in the presence of Cys. [**NNA**] = 250 μ M, [Cys] = 2.5 mM, UV detector with λ = 390 nm; Injection volume: 20 μ L; Mobile phase: A-water, B-acetonitrile; Gradient elution: 0-5min 35-75%B; 5-15min, 75-95%B; Flow rate: 1.0 mL min⁻¹.



Figure S7 Effect of water content on the absorption (A) and emission (B) spectra of NNA in the presence of Cys. [NNA] = 20 μ M, [Cys] = 200 μ M, incubated at 50°C for 3 hours, $\lambda_{ex} = 390$ nm.



Figure S8 Time-dependent absorption (A, B) and emission (C, D) spectra of NNA reacted with Cys in DMF at 25°C; [NNA] = 20 μ M, [Cys] = 200 μ M, λ_{ex} = 390 nm.



Figure S9 The color change of **NNA** in the absence and presence of 10 equiv different additives (A) and their corresponding emission spectra (B, $\lambda_{ex} = 435$ nm). [**NNA**] = 20 μ M, the samples were equilibrated at 50°C for 2 hours. (a) none; (b) Cys; (c) Hcy; (d) MEA; (e) GSH; (f) ME; (g) MPA; (h) NAC; (i) *n*-Butylamine; (j) Ala.