Supplementary information

Sequential detection of H₂S and HClO fluorescence probes and their applications in real sample detection, smartphone

sensing development, and bioimaging

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1. Experimental part

1.1 Materials and instruments

All commercially available reagents were used without further depuration. The UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan) was determined on UV-Vis absorption spectra. Fluorescence emission was used for measurement of the F4500 fluorescence spectrometer (Hitachi, Tokyo, Japan). The 600 MHz spectrometer (Bruker Avance III) was used to record ¹H NMR and ¹³C NMR spectra. High-resolution mass spectral detection was recorded on the mass spectrometer (Bruker Autoflex). Cell imaging was performed with confocal laser scanning microscopy (Zeiss LSM-880).

1.2 Spectral response

The stock solution (2 mM) was prepared by dissolving BPCN-SSPy of dimethyl sulfoxide (DMSO). HSO_3^- , $S_2O_3^{2-}$, AC^- , Cl^- , Br^- , CO_3^{2-} , ClO_4^- , HCO_3^- , NO_3^- , SCN^- , SO_4^{2-} , SO_3^{2-} , HPO_4^- , $C_2O_4^{2-}$, CN^- , GSH, Cys, Hcy were dissolved in ultrapure water to prepare the interference stock solution with concentration of 10 mM. H₂S was provided by Na₂S and prepared immediately before use.

 $S_2O_3^{2-}$, HSO_3^{-} , Br^- , F^- , NO_2^{-} , SCN^- , CO_3^{2-} , Cl^- , SO_4^{2-} , PO_4^{3-} , K^+ , Mg^{2+} , Mn^{2+} , Na^+ , H_2O_2 , ONOO-, O_2^{-} , OH were dissolved in ultrapure water to prepare the interference stock solution with concentration of 200 mM. HClO was provided by NaClO and prepared immediately before use.

H₂S detection: Dissolve 10 μ L of BPCN-SSPy stock solution in 2 mL of HEPES (20 mM, pH 7.4, 50% DMF) to prepare 10 μ M test sample solutions. Let it stand for 5 minutes before conducting spectral analysis, $\lambda ex = 455$ nm, $\lambda em = 611$ nm.

HClO detection: Dissolve 10 μ L of BPCN-SSPy stock solution in 2 mL of HEPES (20 mM, pH 7.4, 50% DMF) to prepare 10 μ M test sample solutions. Let it stand for 3 minutes before conducting spectral analysis, $\lambda ex = 395$ nm, $\lambda em = 520$ nm.

1.3 Cell imaging

Detection of exogenous H₂S: HeLa cells were pre-incubated with 10 μ M BPCN-SSPy for 1 h, followed by three washes with PBS. Then, the cells were treated with different concentrations of H₂S (40 μ M and 60 μ M) for 20 min. Finally, confocal

imaging was performed by the confocal laser scanning (Zeiss LSM 880) equipped with corresponding excitation channel.

Imaging of endogenous H_2S : HeLa cells were pre-incubated with SNP for 1 h, followed by incubation with BPCN-SSPy for 1h. Finally, confocal imaging was performed by the confocal laser scanning (Zeiss LSM 880) equipped with corresponding excitation channel.

2. The ¹HNMR, ¹³CNMR and MS of BPCN-SSPy



Fig. S2. ¹³C NMR spectra of BPCN-SSPy



Fig. S3. Mass spectra of BPCN-SSPy





Fig. S4. Mass spectra of BPCN-SSPy upon addition of H₂S

4. MS of the interaction mechanism between BPCN-OH and HClO



Fig. S5. After reached equilibrium with H_2S , Mass spectra of BPCN-SSPy upon addition of HClO

5. The Selectivity of BPCN-SSPy to H₂S and HClO



Fig. S6. a) Fluorescence spectral response of BPCN-SSPy to other analytes (HSO_3^- , $S_2O_3^{2-}$, AC^- , CI^- , Br^- , CO_3^{2-} , CIO_4^- , HCO_3^- , NO_3^- , SCN^- , SO_4^{2-} , SO_3^{2-} , HPO_4^- , $C_2O_4^{2-}$, CN^- , GSH, Cys, Hcy, H_2S). b) After reached equilibrium with H_2S , fluorescence spectral response of BPCN-SSPy (10 μ M) to other analytes ($S_2O_3^{2-}$, HSO_3^- , Br, F^- , NO_2^- , SCN^- , CO_3^{2-} , CI^- , SO_4^{2-} , PO_4^{3-} , K^+ , Mg^{2+} , Mn^{2+} , Na^+ , H_2O_2 , ONOO-, O_2^- , OH, HCIO).

6. The photostability of BPCN-SSPy itself and after interaction with H₂S/HClO



Fig. S7. The photostability of BPCN-SSPy itself and after interaction with H₂S/HClO within 0.5 h in the HEPES : DMF = 1:1 (pH 7.4, 20 mM, V/V) solution.

7. Cytotoxicity of BPCN-SSPy



Fig. S8. Cytotoxicity of BPCN-SSPy: Cell survival after 24 h incubation by adding different concentrations of BPCN-SSPy (0-50 µM) using MTT.

8. The image capture and RGB analysis about the smartphone-based detection

Table ST KGB values extracted from test solution images with H_2S				
R	G	В	R/G+B	
51	66	87	0.33	
86	68	82	0.57	
98	72	85	0.62	
133	83	89	0.77	
138	79	84	0.85	
175	81	82	1.07	
184	81	84	1.12	

Table S1 RGR values extracted from test solution

190	73	84	1.21	
Table S2 RGB values extracted from test solution images with HClO				
R	G	В	R/G	
158	55	52	2.87	
154	63	44	2.46	
137	64	49	2.14	
119	69	58	1.72	
100	75	71	1.33	
63	64	66	0.98	
43	65	76	0.66	
31	59	83	0.53	