Electronic Supplementary Information

FRET-Based Supramolecular Nanoprobe with Switch on Red Fluorescence to Detect SO_2 Derivatives in Living Cells

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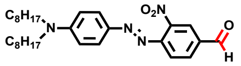
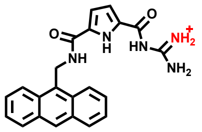
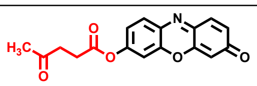
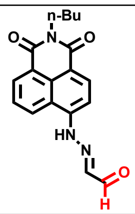
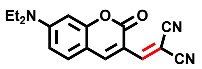
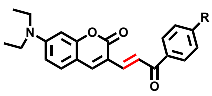
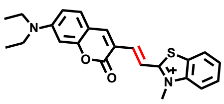
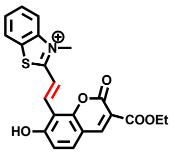
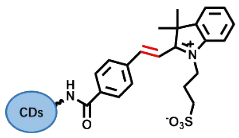
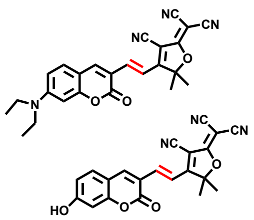
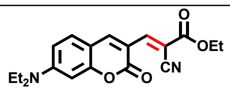
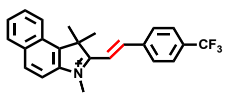
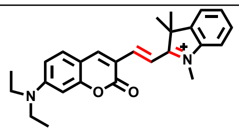
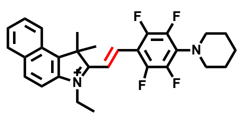
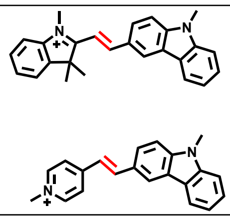
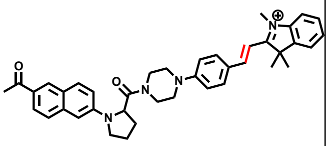
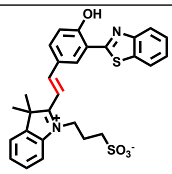
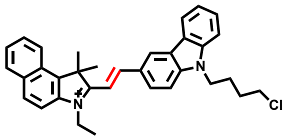
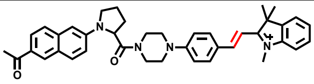
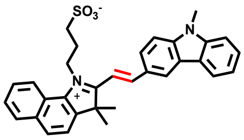
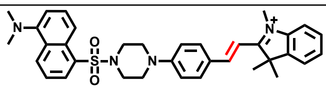
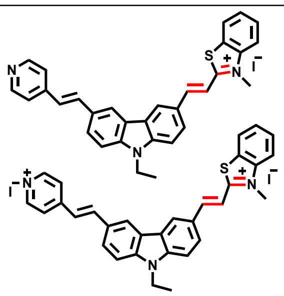
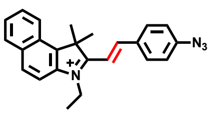
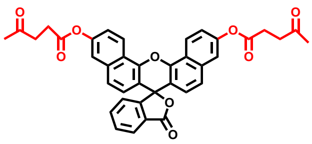
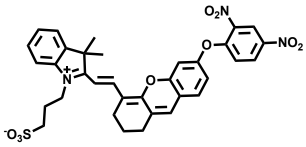
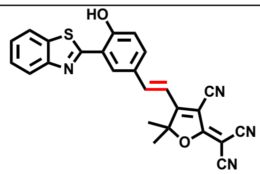
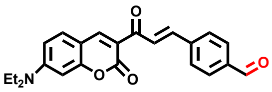
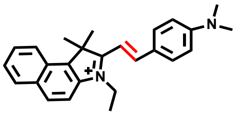
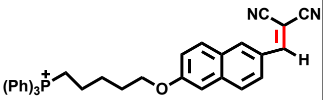
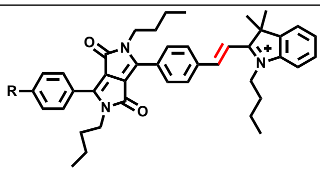
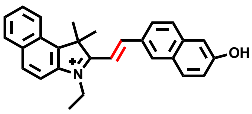
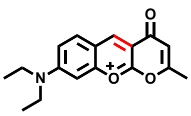
Contents

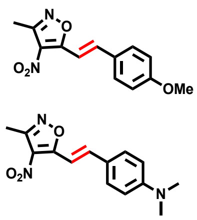
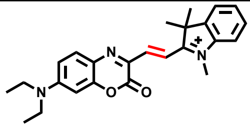
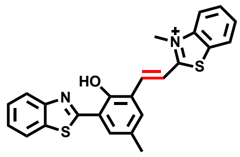
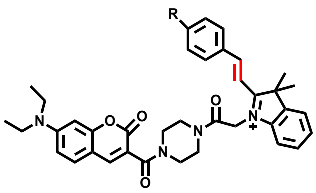
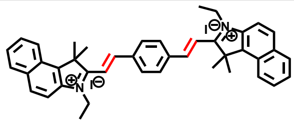
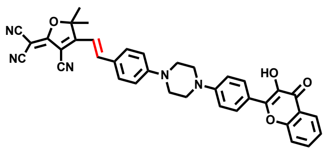
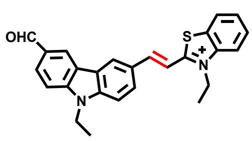
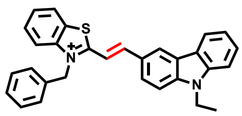
1. Previous work to detect sulfite

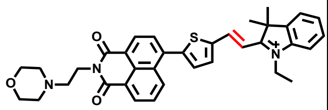
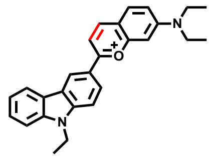
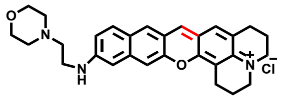
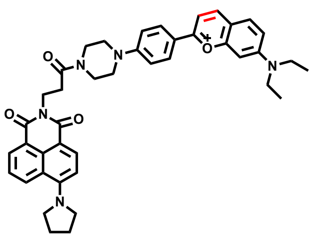
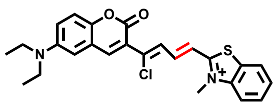
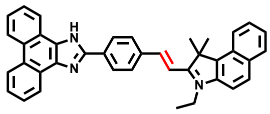
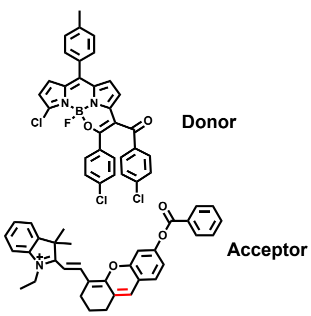
number	Probe Structure	Detection Medium	type	year
1		within the polymer layer in contact with plain citrate buffer and buffered solutions of bisulfite, all at pH = 4.9.	Absorption blue-shift from 524 nm to 484 nm	2002[1]
2		90% water/DMSO solution (pH = 7.2, 10 mM Tris-HCl buffer)	Ratiometric (Absorption blue-shift from 348nm, 366nm, 386nm to 290nm and fluorescence blue-shift from 417 nm to 353 nm)	2009[2]
3		CH_3CN-H_2O (98:2, v/v) HEPES buffer (pH = 7.0, 10 mM)	Turn-on (absorption red-shift from 359nm to 571 nm and fluorescence at 588 nm)	2010[3]
4		DMSO-acetate buffer (0.1 M, pH = 5.0, 1 : 1, v/v)	Turn-on (absorption at 425 nm and fluorescence at 535 nm)	2012[4]
5		20% DMF buffer solution. (pH = 7.4)	Ratiometric (Absorption blue-shift from 529nm to 411nm and 368 nm and fluorescence blue-shift from 578 nm to 480 nm)	2013[5]
6		PBS (20 mM, pH = 7.4) containing 1 mM CTAB.	Ratiometric (Absorption blue-shift from 470nm to 390nm and fluorescence blue-shift from 592 nm to 465 nm)	2013[6]
7		PBS buffer (pH = 7.4, 10 mM, containing 30% DMF)	Ratiometric (Absorption blue-shift from 545nm to 463nm to 410nm and fluorescence blue-shift from 633 nm to 478 nm)	2013[7]

8		MeOH buffer (Na_2HPO_4/ citric acid, pH = 7.4, 30.0 mM, 1:1 v/v)	Ratiometric (Absorption blue-shift from 500 nm to 400 nm and fluorescence blue-shift from 600 nm to 460 nm)	2014[8]
9		PBS	Turn-on (absorption blue-shift from 392nm to 234 nm, 278 nm and fluorescence at 450 nm increase)	2014[9]
10		HEPES buffer (20 mM pH = 7.4)	Ratiometric (Absorption blue-shift from 570nm to 473nm and 330nm and fluorescence blue-shift from 663 nm to 523 nm)	2014[10]
11		PBS buffer (pH = 7.4, 10mM, containing 30% DMSO)	Ratiometric (Absorption blue-shift from 500nm to 400nm and fluorescence blue-shift from 575 nm to 477 nm)	2014[11]
12		PBS buffer (pH = 7.4, 10 mM)	Ratiometric (absorption at 310nm and 360nm decrease and fluorescence blue-shift from 571 nm to 465 nm)	2014[12]
13		PBS (pH = 7.4, containing 30% EtOH)	Ratiometric (Absorption blue-shift from 570 nm to 420 nm and fluorescence blue-shift from 650 nm to 480 nm)	2015[13]
14		glycerol/PBS = 4/6, pH=7.40	Ratiometric (Absorption blue-shift from 499nm to 322nm and fluorescence blue-shift from 592 nm to 465 nm)	2015[14]
15		pH = 7.4 (10mM, containing 30% DMF)	Ratiometric (absorption blue-shift from 487nm to 290nm and fluorescence blue-shift from 590 nm to 490 nm)	2015[15]

16		aqueous buffer (10 mM PBS, pH = 7.4) containing 10% DMF	Turn-on (absorption at 514 nm decrease and fluorescence at 500 nm increase)	2016[16]
17		PBS buffer solution (pH = 7.0)	Ratiometric (Absorption 520nm and 390nm decrease and fluorescence blue-shift from 590 nm to 450 nm)	2016[17]
18		EtOH/PBS solution (v/v = 1/9, pH = 7.4)	Ratiometric (Absorption blue-shift from 500nm to 290nm and fluorescence blue-shift from 600 nm to 450 nm)	2016[18]
19		PBS solution (pH = 7.4, containing 20% EtOH)	Ratiometric (Absorption 510nm and 380 nm decrease and fluorescence blue-shift from 590 nm to 500 nm)	2016[19]
20		PBS buffer (pH = 7.4, 10 mM)	Ratiometric (absorption blue-shift from 506nm to 266 nm, 320nm and fluorescence blue-shift to 470 nm)	2016[20]
21		PBS (10mM, pH = 7.4, containing 30% DMF)	Ratiometric (absorption at 508nm decrease and fluorescence blue-shift from 582 nm to 530 nm)	2016[21]
22		PBS buffer (20 mM, pH = 7.4, containing 20% DMF)	Turn on (Absorption blue-shift from 482nm to 420 nm and fluorescence at 469 nm\554 nm)	2016[22]
23		PBS buffer (10 mM, pH = 7.4, containing 25 µM CTAB)	Ratiometric (Absorption blue-shift from 443 nm to 325 nm and fluorescence blue-shift from 580 nm to 467 nm)	2016[23]

24		DMSO-PBS buffer (20 mM, pH = 7.0, 1: 1, v/v)	Turn-on (fluorescence at 695 nm)	2016[24]
25		PBS (20 mM, pH = 7.4 with 10% DMSO v/v)	Turn off (absorption blue-shift from 595nm to 375 nm and fluorescence turn off)	2016[25]
26		PBS buffer (10 mM, pH = 7.4, containing CTAB 1 mM)	Ratiometric (Absorption blue-shift from 628nm to 338nm and fluorescence blue-shift from 665 nm to 465 nm)	2017[26]
27		PBS/DMSO (1/1, v/v, pH = 7.4)	Turn-on (fluorescence at 576 nm)	2017[27]
28		PBS buffer (DMSO:H_2O = 3:7, 50 mM, pH = 7.4)	Ratiometric (Absorption blue-shift from 550nm to 323nm and fluorescence blue-shift from 611 nm to 467 nm)	2017[28]
29		PBS buffer (10% DMSO v/v, pH = 7.4, 20 mM)	Ratiometric (absorption blue-shift from 425nm to 350nm and fluorescence blue-shift from 600 nm to 425 nm)	2017[29]
30		PBS: DMSO = 7:3, v/v, pH = 7.4	Ratiometric (Absorption blue-shift from 520 nm to 480 nm and fluorescence blue-shift from 720 nm to 545 nm)	2017[30]
31		PBS solution (50 mM, pH = 7.4)	Ratiometric (Absorption blue-shift from 475nm to 322nm and fluorescence blue-shift from 593 nm to 467 nm)	2017[31]
32		Tris buffer solution (pH = 7.4, containing 70% DMSO v/v)	Ratiometric (Absorption blue-shift from 510nm to 300-375nm and fluorescence blue-shift from 560 nm to 510 nm)	2017[32]

33		Britton-Robinson buffer solution (20 mM, pH = 7, 1% DMSO)	Turn-off	2017[33]
34		PBS buffer (10 mM, pH = 7.4, with 10% DMF, v/v)	Ratiometric (Absorption blue-shift from 632nm to 454nm and fluorescence blue-shift from 717nm to 560 nm)	2017[34]
35		CH_3CN/H_2O (1/1, v/v, HEPES 10 mM, pH = 7.4)	Ratiometric (absorption at 373nm and 575nm decrease and fluorescence blue-shift from 695nm to 508 nm)	2017[35]
36		PBS buffer (pH = 7.4, 0.01 M, containing 40% DMF)	Ratiometric (absorption at 560nm decrease and fluorescence blue-shift from 589 nm to 476 nm)	2017[36]
37		phosphate buffer solution (1%DMSO, pH = 7.4)	Ratiometric (Absorption blue-shift from 330-570nm to 250-280 nm and fluorescence blue-shift from 550-680 nm to 450-550 nm)	2018[37]
38		PBS (pH = 7.4, 10 mM, containing 40% EtOH)	Ratiometric (Absorption blue-shift from 560nm to 335nm and fluorescence blue-shift from 641nm to 530 nm)	2018[38]
39		EtOH/PBS (v/v = 1/4, pH = 7.4)	Ratiometric (Absorption blue-shift from 455 nm to 280 nm and fluorescence 552 nm decrease, 460 nm increase)	2018[39]
40		PBS solution (0.01 M, pH = 7.4, 50% DMSO)	Ratiometric (Absorption 475nm decrease and fluorescence blue-shift from 600 nm to 483 nm)	2018[40]

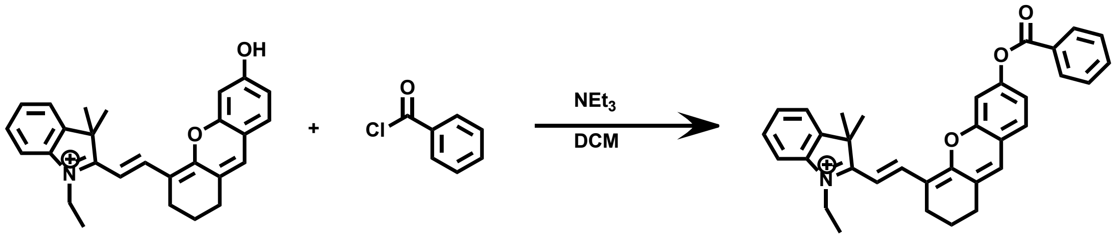
41		PBS solution (10 mM, 5% DMSO, pH = 5.5)	Turn-on (absorption at 475 nm decrease and fluorescence at 524 nm increase)	2019[41]
42		HEPES buffer (10 mM, 20% CH_3CN)	Ratiometric (absorption blue-shift from 570 nm to 270 nm and fluorescence from 635 nm blue-shift to 425 nm)	2019[42]
43		PBS buffer (pH = 5.0, containing 30% EtOH)	Ratiometric (Absorption blue-shift from 613 nm to 426 nm and fluorescence blue-shift from 704 nm to 512 nm)	2019[43]
44		PBS buffer.	Ratiometric (absorption blue-shift from 590 nm to 400-500 nm and fluorescence blue-shift from 645 nm to 540 nm)	2019[44]
45		HEPES buffer (10 mM, pH = 7.4, with 10% DMF, v/v)	Ratiometric (Absorption blue-shift from 544 nm to 424 nm and fluorescence blue-shift from 749 nm to 490 nm)	2019[45]
46		EtOH-PBS buffer (10 mM, pH = 7.4, v/v, 4:6)	Dual-emission (the absorption blue-shift from 493 nm to 319 nm and fluorescence at 450 nm and 605 nm increase)	2019[46]
47 (This work)		PBS (pH = 7.4) No organic solvent	Turn-on (absorption blue-shift from 590 nm to 390 nm and fluorescence at 615 nm is turned on)	

The reaction sites to sulfite are labeled by the red line.

Table S1. Comparison of Fluorescent Probes for SO_2 Derivatives

2. Experimental Section.

2.1 Synthesis of Acceptor HEM-CO-Ph.



Scheme S1: The synthesis of acceptor of HEM-CO-Ph.

3. Spectra Response.

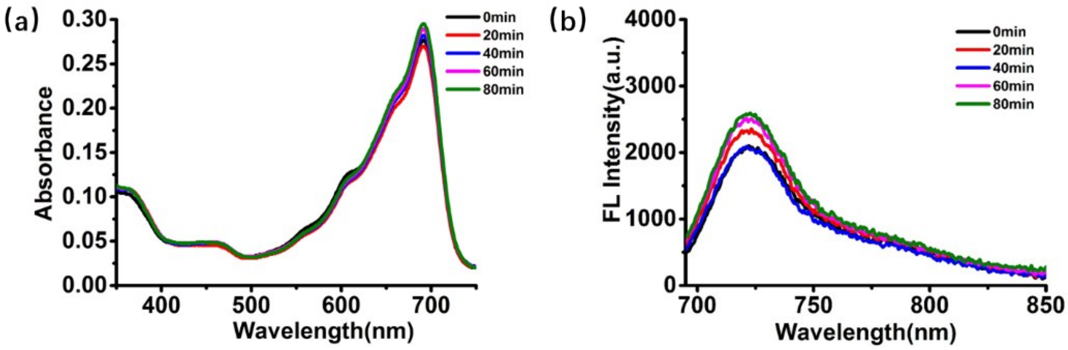


Fig. S1. (a) The absorption spectra of hemicyanine-OH (5 μM) reacted with Na_2SO_3 (250 μM) in PBS (10 mM PBS, PH = 7.4, containing 50 % acetonitrile, 37°C). (b) The emission spectra of hemicyanine-OH (5 μM) reacted with Na_2SO_3 (250 μM) in PBS (10 mM PBS, PH = 7.4, containing 50 % acetonitrile, 37°C).

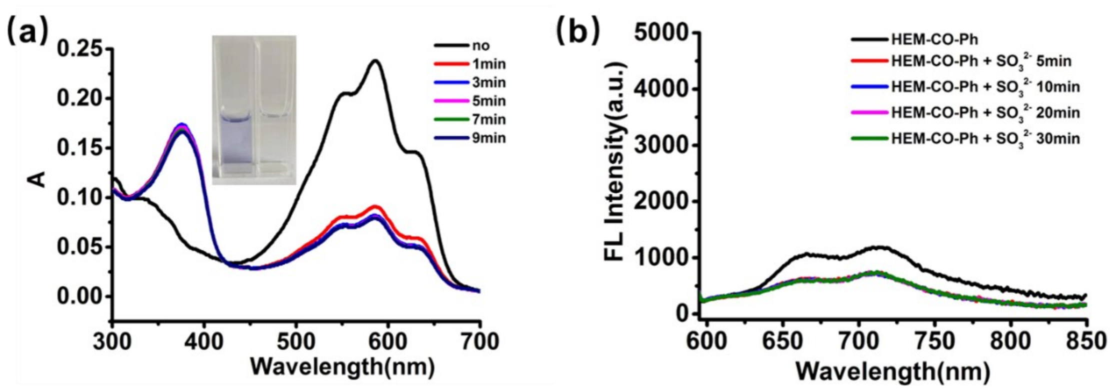


Fig. S2. (a) The absorption spectra of HEM-CO-Ph (5 μM) react with Na_2SO_3 (100 μM) in PBS (10 mM PBS, PH = 7.4, containing 20 % acetonitrile, 37°C). Inset: the color change of HEM-CO-Ph before and after the addition Na_2SO_3 under day light. (b) The emission spectra of HEM-CO-Ph (5 μM) react with Na_2SO_3 (250 μM) in PBS (10 mM PBS, PH = 7.4, containing 20 % acetonitrile, 37°C). (Ex = 580 nm slit: 5 nm/10 nm).

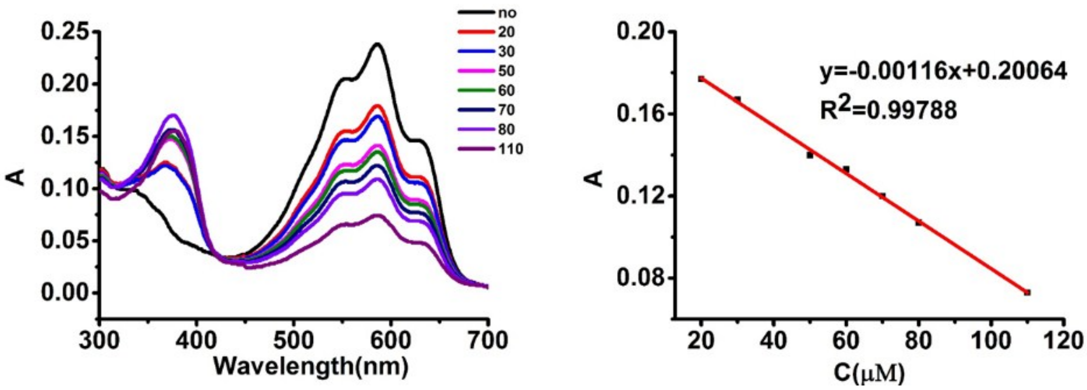


Fig. S3. The absorption spectra of HEM-CO-Ph (5 μM) upon addition of different concentrations of Na_2SO_3(0-110 μM) in PBS (10 mM PBS, PH = 7.4, containing 20 % acetonitrile, 37 °C) and the liner relationship between absorption intensity and different concentration of Na_2SO_3.

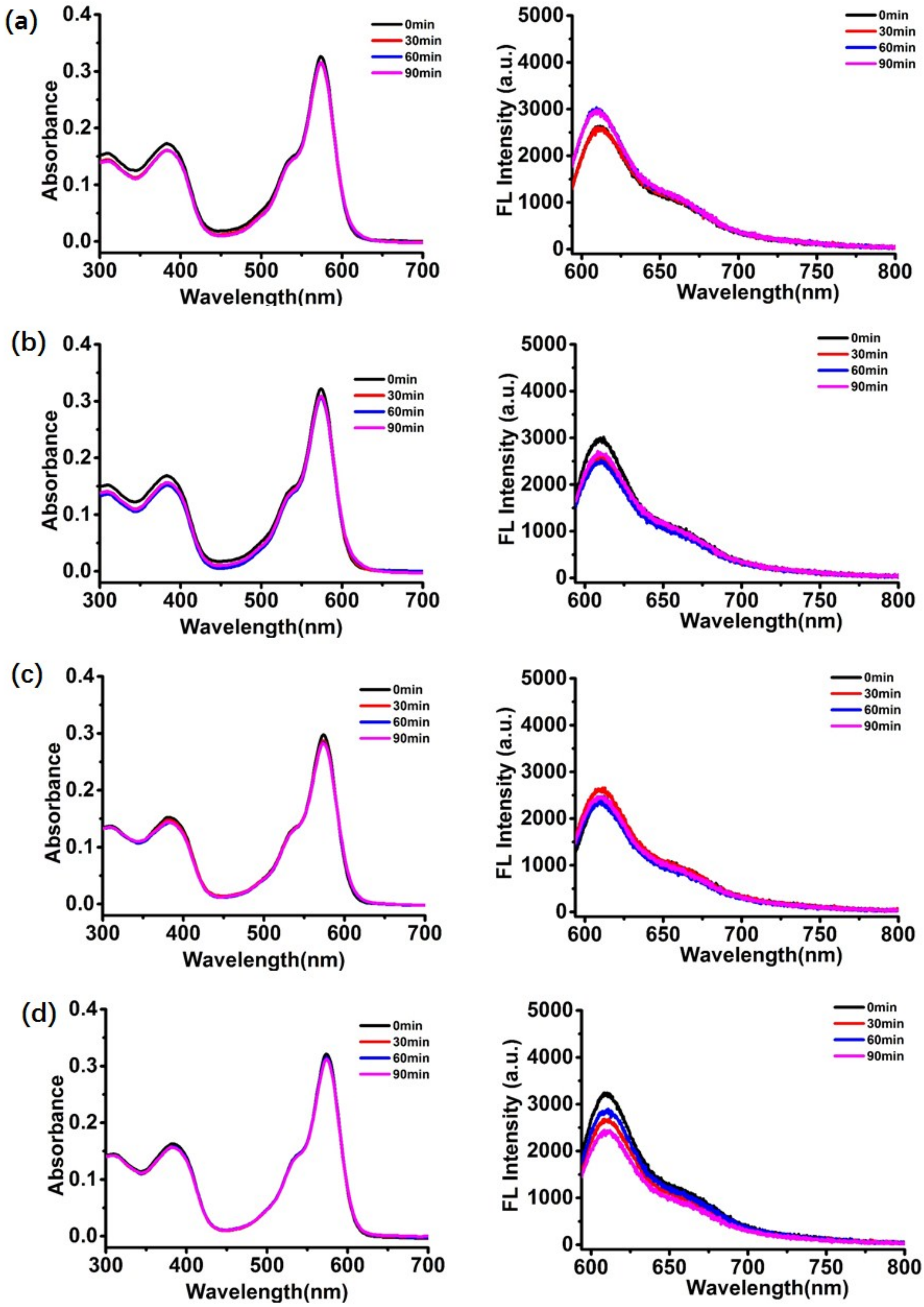


Fig. S4. Time-dependent absorption (left) and emission (right) spectra of BDP (5 µM) in the presence of (a) GSH (500 µM), (b) Hcy (500 µM), (c) Cys (500 µM) and (d) Na_2SO_3 (250 µM) in PBS (10 mM PBS, PH = 7.4, containing 50% acetonitrile, 37 °C) (Ex = 580 nm slit: 2.5 nm/2.5 nm).

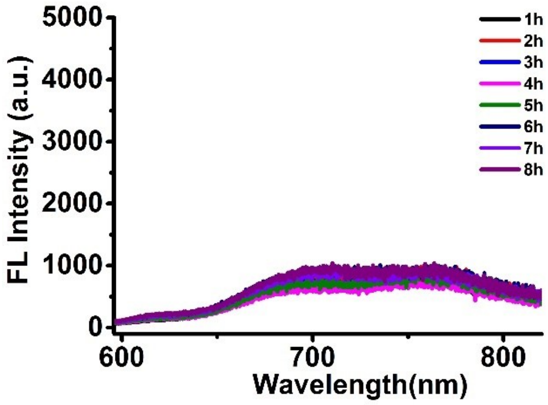


Figure S5. The emission spectrum of nanoprobe at room temperature. (10 mM PBS, pH = 7.4).

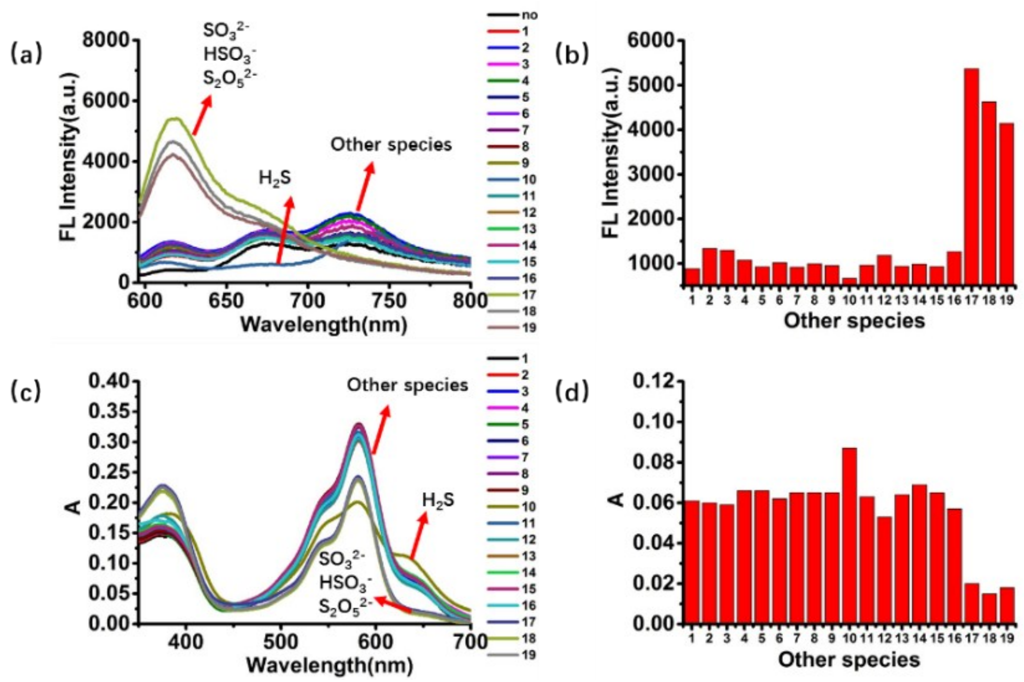


Fig. S6. (a) The emission spectrum of nanoprobe (5 μM) in the presence of respective species (250 μM) in PBS (10 mM PBS, PH = 7.4) within 60 min. (b). The emission spectrum at 615 nm of nanoprobe (5 μM) in the presence of respective species (250 μM) in PBS (10 mM PBS, PH = 7.4) within 60 min. (c).The absorption of nanoprobe (5 μM) in the presence of respective species (250 μM) in PBS (10 mM PBS, PH = 7.4, 37 °C) within 60 min. (d) The absorption in the channel of 650 nm. 1-19: 1-none, 2-GSH, 3-Hcy, 4-Cys, 5-KBr, 6-NaCl, 7-NH_4Cl, 8-$NaNO_2$, 9-$Na_2S_2O_3$, 10-NaHS, 11-$NaHSO_4.H_2O$, 12-L-Asp, 13-L-Ala, 14-L-Leu, 15-Glu, 16-L-His, 17-$Na_2S_2O_5$, 18-$NaHSO_3$, 19-Na_2SO_3.

4. Mechanism:

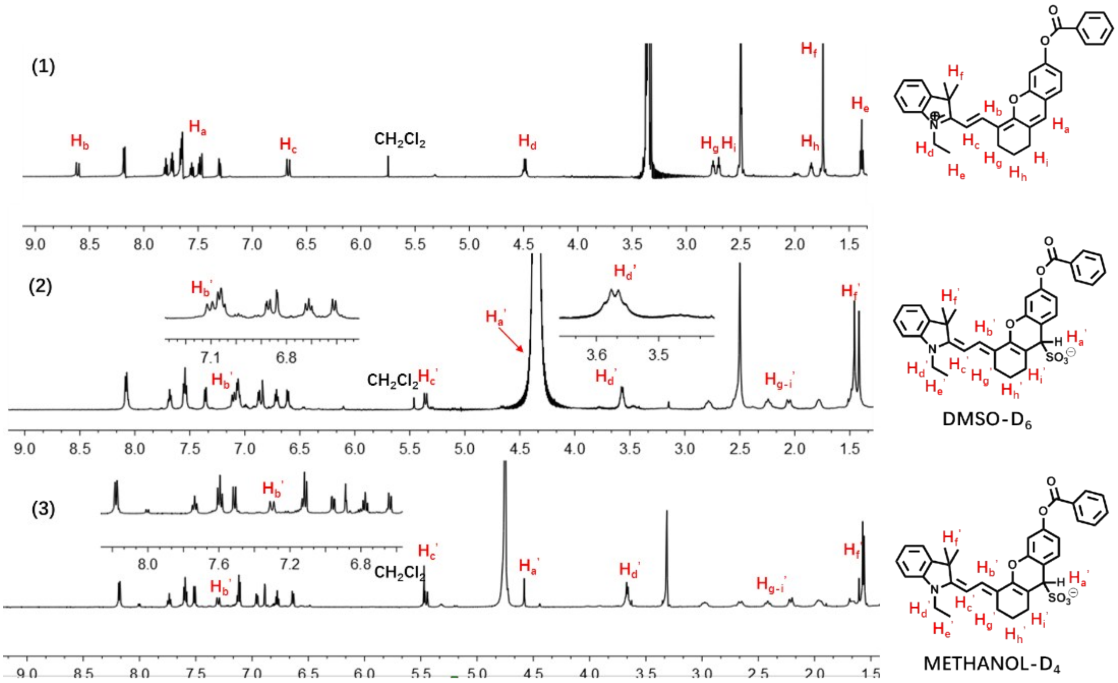


Fig. S7. (1)^{1}H NMR of HEM-CO-Ph (DMSO-D$_6$, 600 MHz). (2)^{1}H NMR of the HEM-CO-Ph-SO$_3$ (DMSO-D$_6$: D$_2$O = 1:3, 600 MHz). The sulfite is excessive. (3)^{1}H NMR comparison of the adduct-SO$_3$ (METHANOL-D$_4$: D$_2$O = 1:3, 600MHz). The sulfite is 4 eq. excessive.

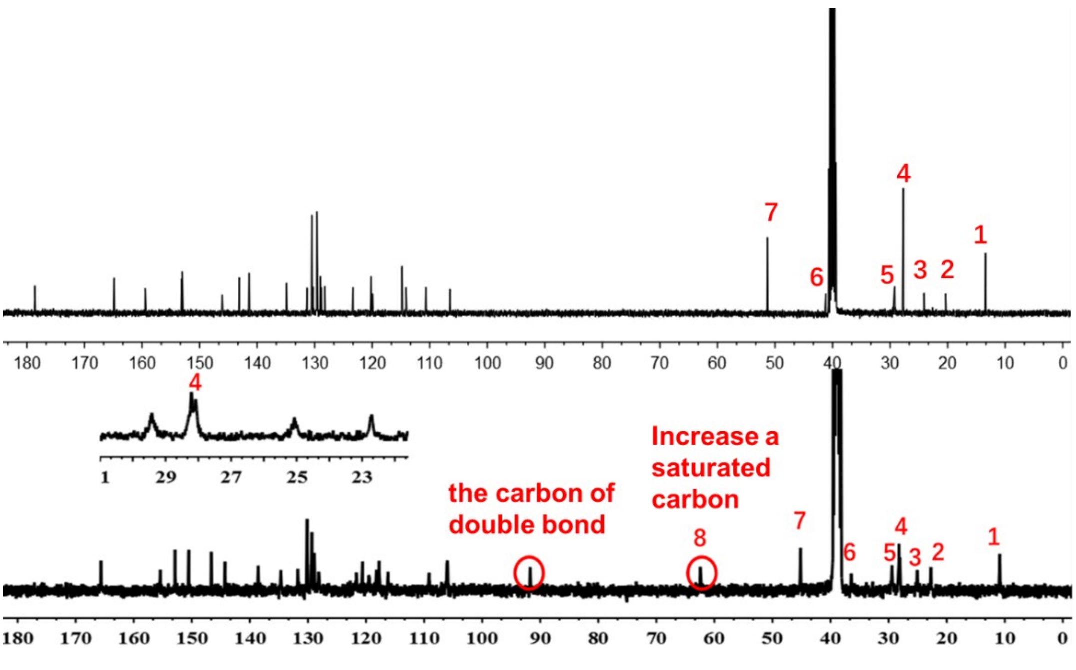


Fig. S8. ^{13}C NMR comparison of acceptor HEM-CO-Ph and the HEM-CO-Ph-SO$_3$ (DMSO-D$_6$: D$_2$O = 1:3, 600 MHz). The sulfite is 4 eq. excessive.

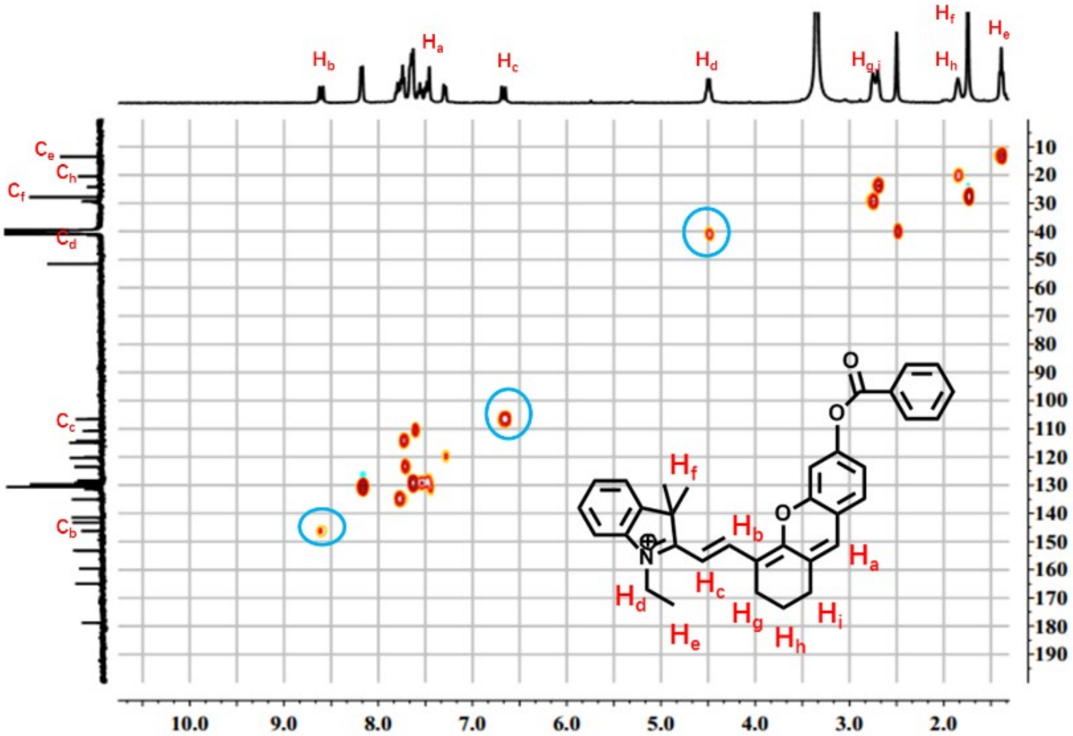


Fig. S9. ^{1}H-^{13}C HSQC spectrum of HEM-CO-Ph (DMSO-D_6, 400MHz).

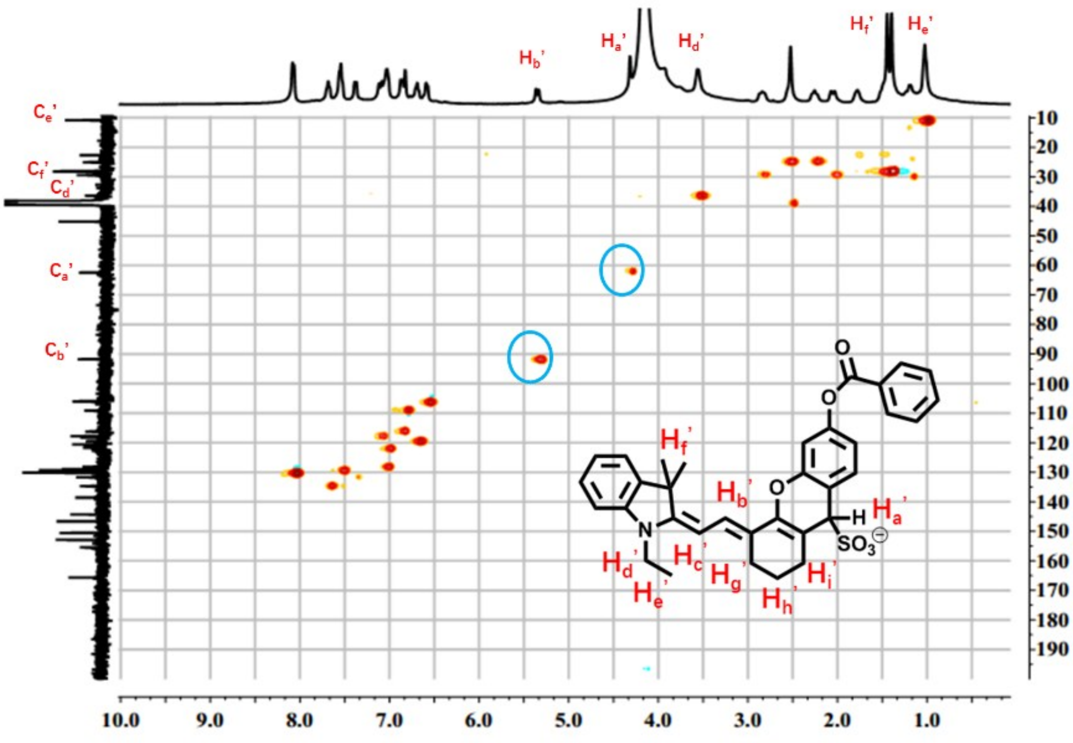


Fig. S10. ^{1}H-^{13}C HSQC spectrum of HEM-CO-Ph-SO_3 (DMSO-D_6: D_2O = 1:3, 400MHz). The sulfite is 4 eq. excessive.

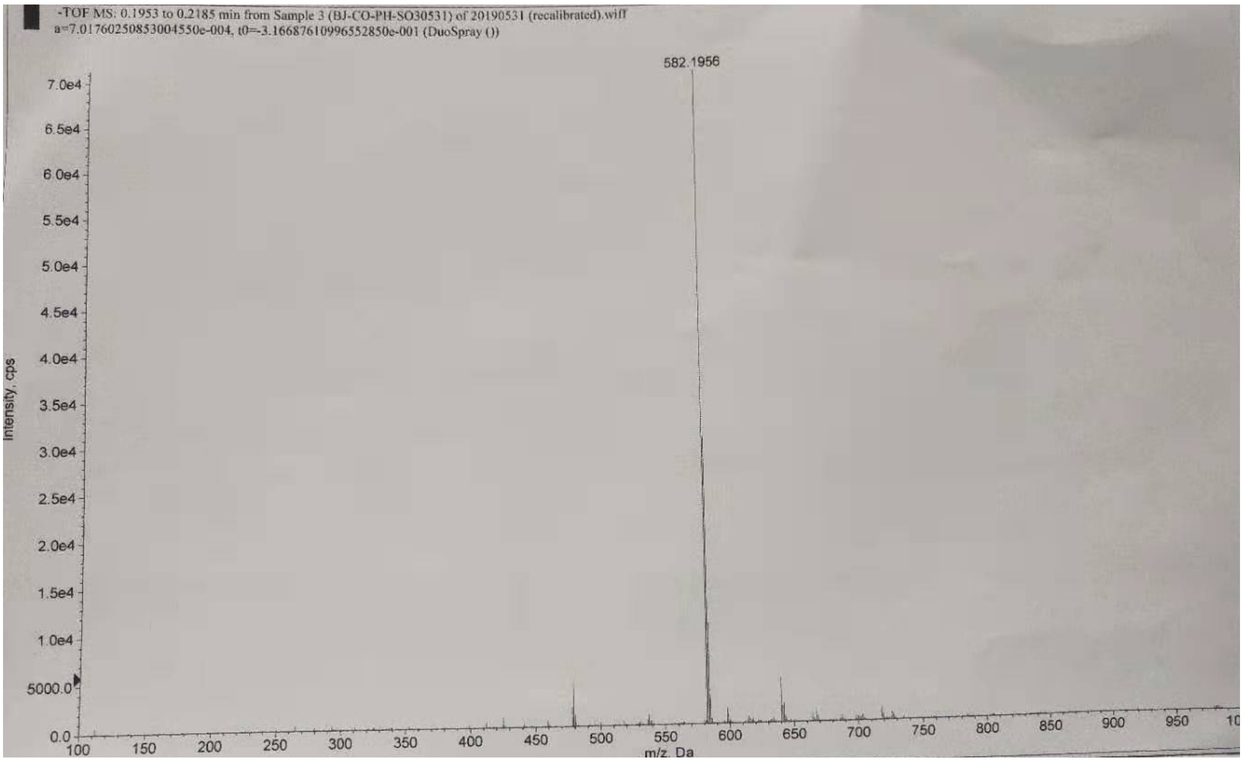


Fig. S 11. HR-Mass spectrum of the HEM-CO-Ph-SO_3. MS (ESI): Found: m/z = 582.1956. (calculated for: 582.1956)

5. Cell Viability and Cellular Imaging

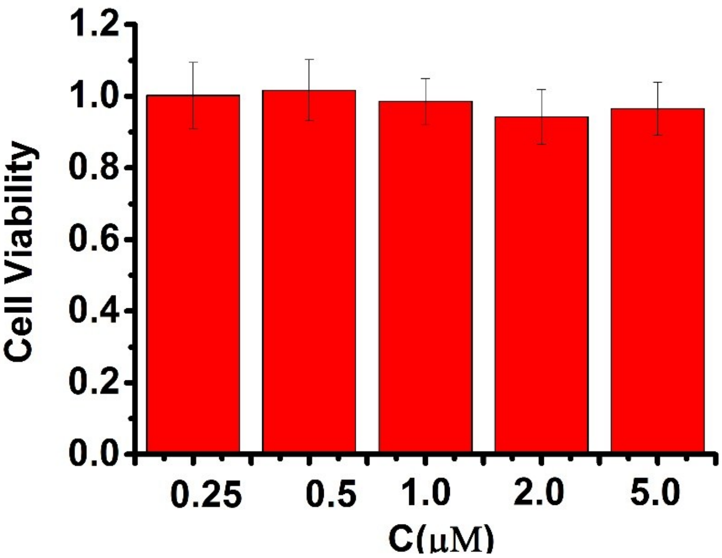


Fig. S 12. Cell viability by a standard CCK-8 assay. HepG-2 cells were incubated with different concentrations of nanoprobe for 12 h.

5.1 Exogenous Imaging of Nanoprobe and Sulfite. In the imaging experiments, the imaging divided into two groups, the first group was that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with probe (5 μM, 30 min), and then imaged; the second group was that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with Na_2SO_3 (250 μM, 30 min) and nanoprobe (5 μM, 30 min), and then imaged. (red channel of 570-620 nm)

5.2 Endogenous Imaging of Nanoprobe and Sulfite. The first group is that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with probe (5 μM, 30 min), and then imaged; The second group is that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with GSH (500 μM, 30 min) and nanoprobe (5 μM, 30 min), and then imaged. The third group is that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with $Na_2S_2O_3$ (250 μM, 30 min) and nanoprobe (5 μM, 30 min), and then imaged. The fourth group is that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with GSH/ $Na_2S_2O_3$ (500 μM/250 μM, 30 min) and incubated with probe (5 μM, 30 min), and then imaged. The fifth group is that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with SNAP (60 min), then incubated GSH/ $Na_2S_2O_3$ (500 μM/250 μM, 30 min) and incubated with probe (5 μM, 30 min), and then imaged. The sixth group is that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with SO_3^{2-}(250 μM, 30 min) and nanoprobe (5 μM, 30 min), and then imaged.

5.3 Subcellular Localization of the Nanoprobe. The first group is that cells were only incubated with probe (5 μM, 30 min), and then imaged; the second group is that cells were pre-treated with NEM (0.5 mM, 30 min), subsequently incubated with Na_2SO_3 (250 μM, 30 min) and nanoprobe (5 μM, 30 min), and subsequently treated with commercially available mitochondrial dye, Mito-Tracker Green and common dye, Hoechst 33342 for a co-localization experiment and then imaged.

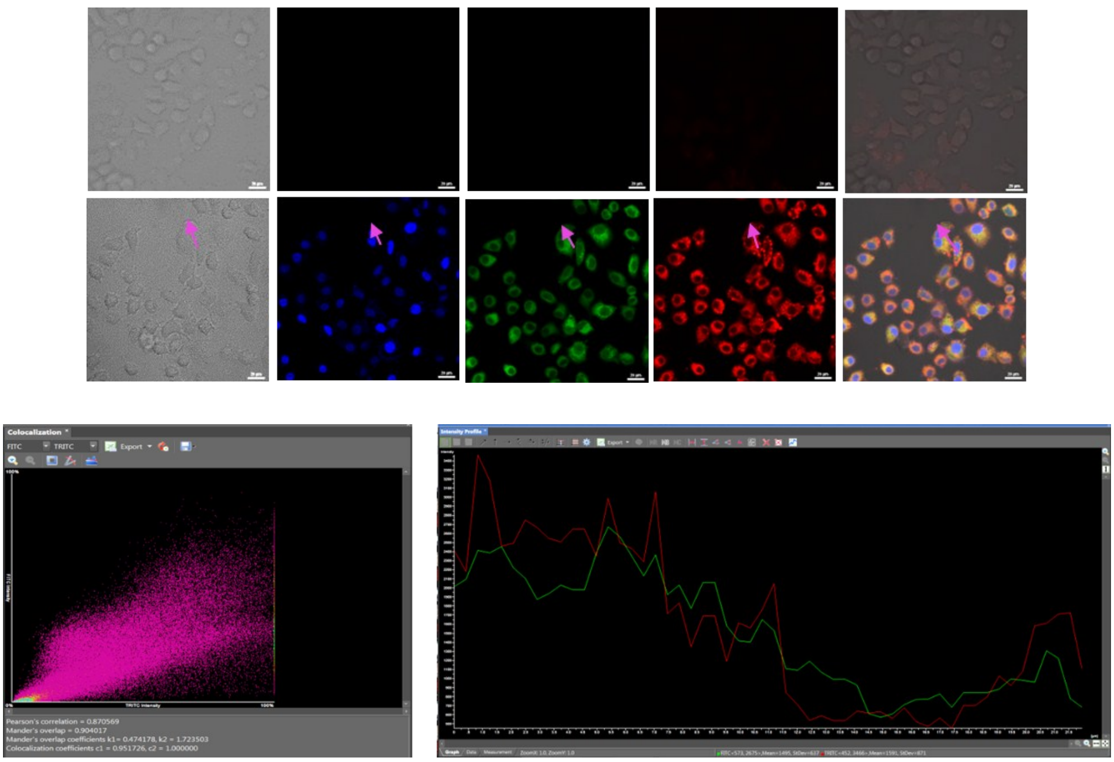


Fig. S13.The subcellular co-localization of the nanoprobe (5 μM) with commercially available mitochondrial dye.

6. Appendix Information

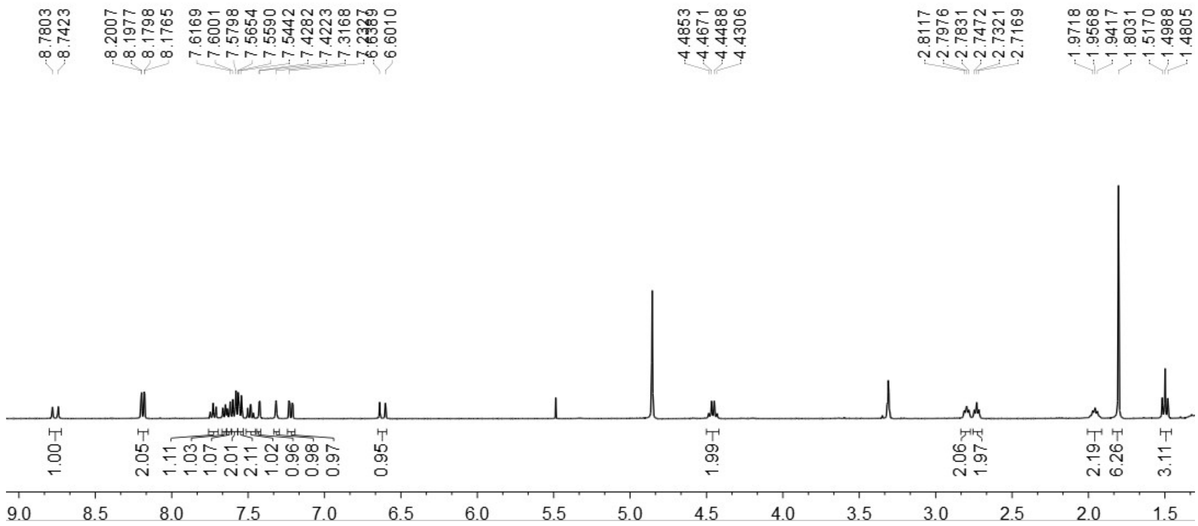


Fig. S14. ^{1}H NMR spectrum of HEM-CO-Ph (methanol-D_4, 400 MHz).

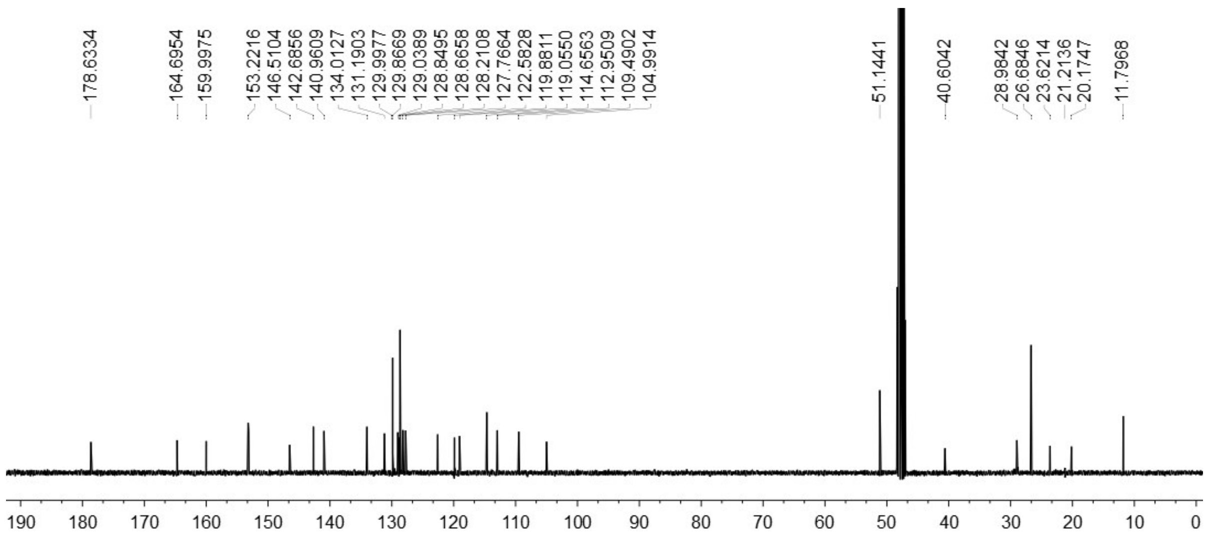


Fig. S15. ^{13}C NMR spectrum of HEM-CO-Ph (methanol-D_4, 400 MHz).

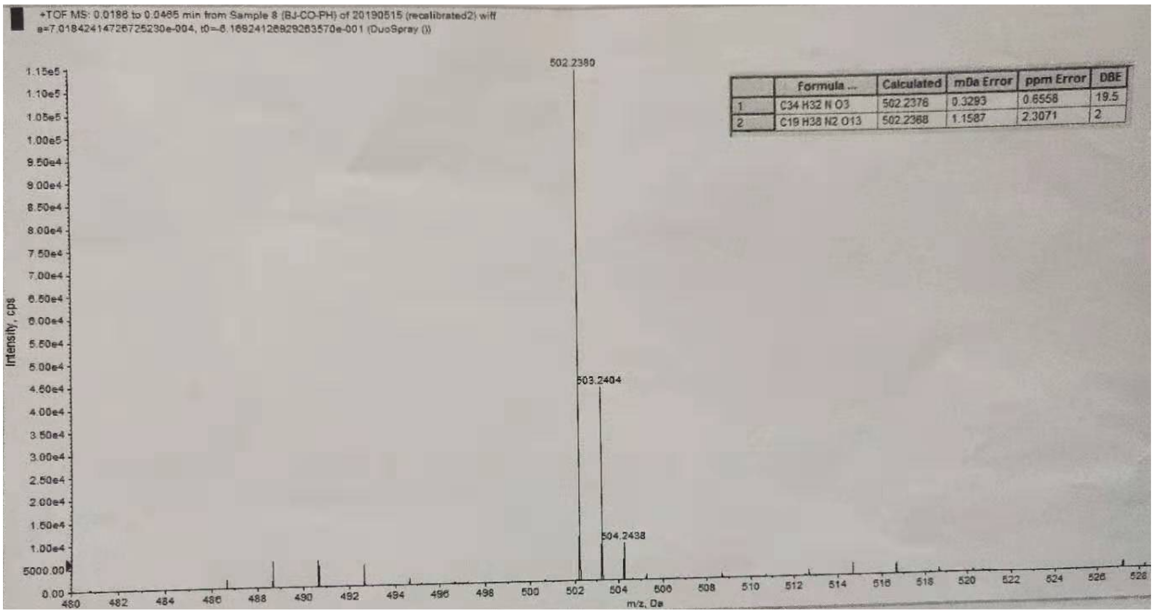


Fig. S16. HR-Mass spectrum of HEM-CO-Ph. MS (ESI): m/z = 502.2380.

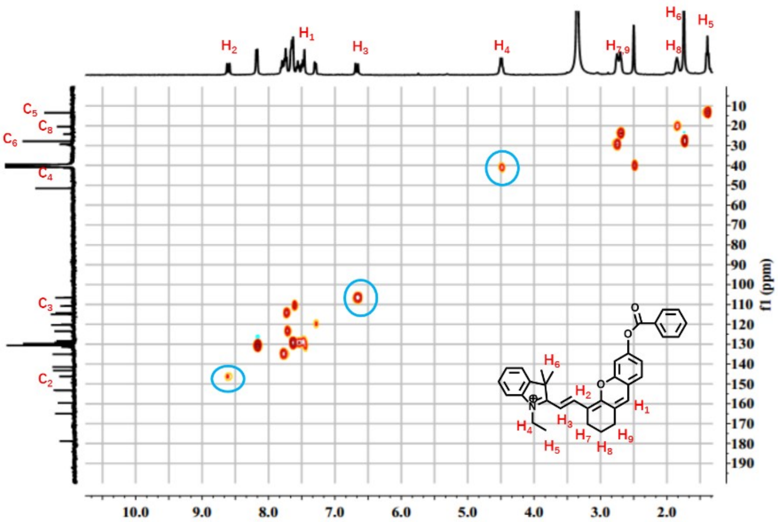


Fig. S17. ^{1}H-^{13}C HSQC spectrum of HEM-CO-Ph.

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