Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Rational Design of Water-dispersible and Biocompatible Nanoprobes with H_2S-Triggered NIR Emission for Cancer Cells Imaging

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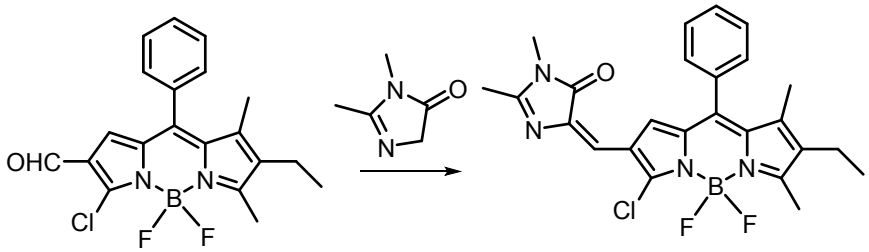
1. Synthesis.



Synthesis of compound BODVA-Cl. To an anhydrous EtOH solution of compound A (401 mg, 1 mmol) was added 1,2-dimethyl-1H-imidazol-5(4H)-one (560 mg, 5 mmol), and the resultant reaction mixture was refluxed for 12 h under argon. After removing the solvent under vacuum,

the crude residue was purified by column chromatography to afford compound BODVA-Cl (240 mg, yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ7.55-7.50 (m, 3H), 7.38(d, 2H), 7.33(s, 1H), 7.13(s, 1H), 3.14(s, 3H), 2.66(s, 3H), 2.40-2.34(q, 2H), 2.26(s, 3H), 1.44(s, 3H), 1.03(t, 3H); ^{13}C NMR ($CDCl_3$, 100MHz) δ169.87, 165.34, 160.91, 142.94, 140.56, 139.69, 137.61, 134.63, 134.12, 133.08, 129.60, 129.03, 128.65, 125.80, 121.91, 117.42, 26.56, 17.19, 15.60, 14.08, 13.54, 12.49. HRMS (ESI, m/z): calculated for $C_{25}H_{25}BClF_2N_4O$ $[M+H]^+$: 481.1178, found 481.1177.

Compound BPAB was synthesized according to S. Y. Zhang, Y. Zhao, *Macromolecules*, **2010**, *43*, 4020.

2. Preparation of NanoBOD-SCM.

In a typical procedure, NanoBOD-SCM were fabricated in three steps: 1) trapping the hydrophobic BODVA-Cl into the micellar core based on 4-(dodecyloxy) benzyltripropargylammonium bromide BPAB in water: BPAB (14.6 mg, 0.03 mmol) was rapidly poured into 3 mL pure water under sonication for 20 min. Then 10 μL BODVA-Cl (25 mM in DMSO) was added and the resultant solution was kept sonication for another 20 min; 2) Cu(I) catalyzed click reaction: cross linker 2 (0.03 mmol), 0.04 mg $CuCl_2$, and 1.5 mg sodium ascorbate were added to the above micelle solution and was stirred at room temperature for 12 h to afford SCMs with a covalently cross-linked shell; 3) N_3-PEG-2000 (60 mg，0.03 mmol) in 0.1 mL H_2O was finally added and was stirred at room temperature for 12 h. Dialyzing against deionized water with 0.22 μm PVDF film produced nanoprobe NanoBOD-SCM with good water-solubility and excellent biocompatibility.

3. Cells culture and imaging.

HCT116/HepG2 cells in Dulbecco's Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) were cultured at 37 °C in a humidified atmosphere of 5/95 CO_2/air incubator for 24 h.

For the imaging, cells were treated with NanoBOD-SCM and incubated in DMEM for 30 min. For inhibitor assay: cells were pretreated with 1 mM AOAA for 1 h, followed by incubation with NanoBOD-SCM for 30 min. For CBS activator assay: cells were pretreated with 3 mM SAM for 1 h, followed by incubation with NanoBOD-SCM for 30 min. The confocal imaging was recorded using Nikor AIR with a 60 × oil objective.488 nm was explored as the excitation wavelength and the emission collected at 580-650 nm as green channel; emission collected at 680-750 nm upon excitation at 561 nm as red channel, ratio image generated from red to green channel.

4. HRMS characterization.

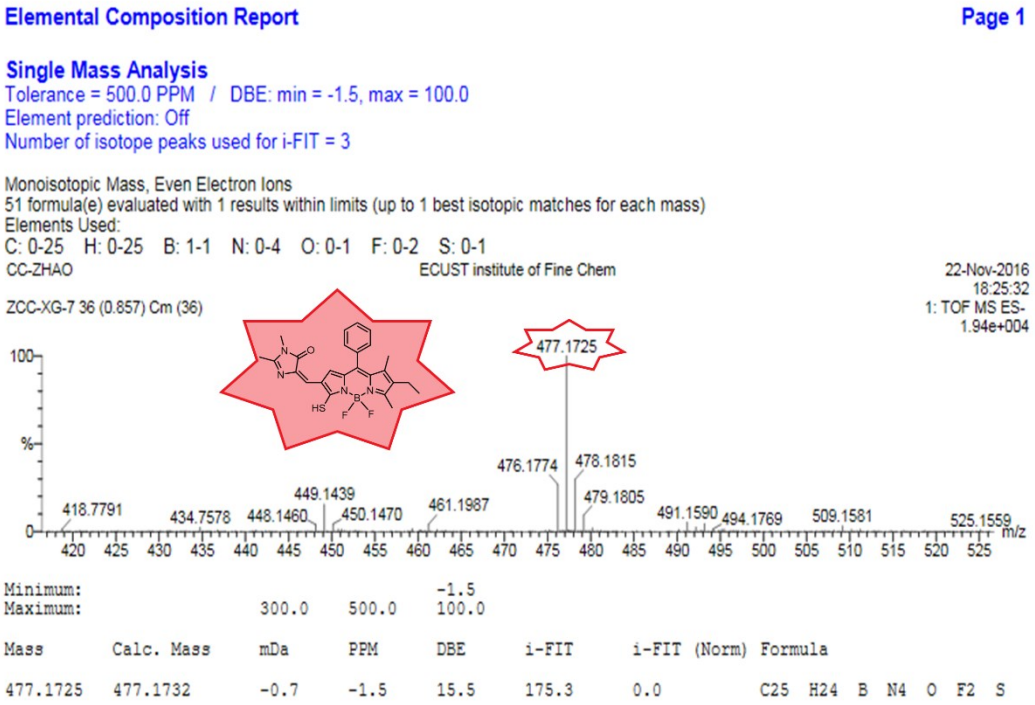


Fig. S1. HRMS demonstration of the incubation of BODVA-Cl with H_2S to transform into BODVA-SH in a buffer solution (pH = 7.4, 1 mM CTAB).

5. TEM and DLS.

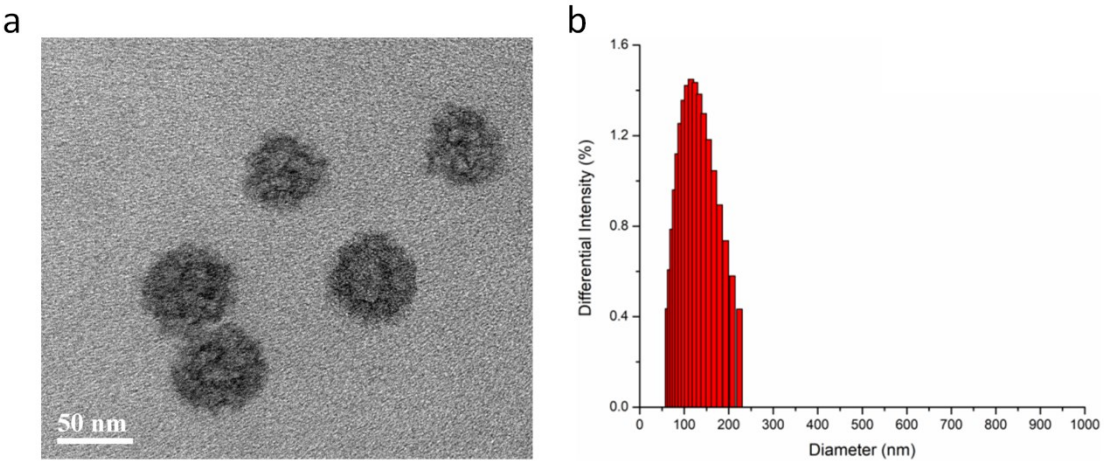


Fig. S2. Uniform spherical morphology with a diameter of 50 nm determined by TEM (a) and the hydrodynamic diameter by DLS (b).

6. Detection limit.

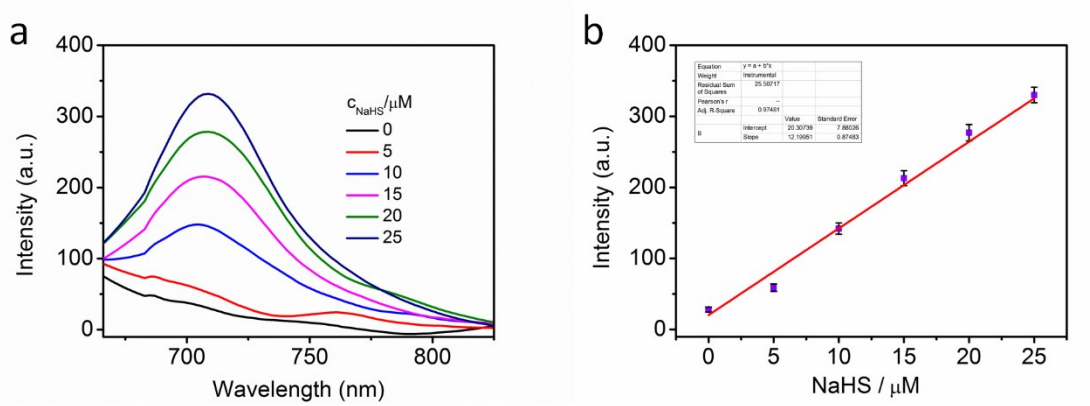


Fig. S3. (a) The fluorescence changes of NanoBOD-SCM (BODVA-Cl 5 μM) in the presence of various concentration of NaHS (1, 5, 10, 15, 20, 25 μM). (b) The linear relationship between NIR fluorescence intensity at 710 nm and H_2S concentration (0-25 μM), which afforded a valuable detection limit (DL) of 198 nM by using DL=3σ/k.

7. pH effect on the response.

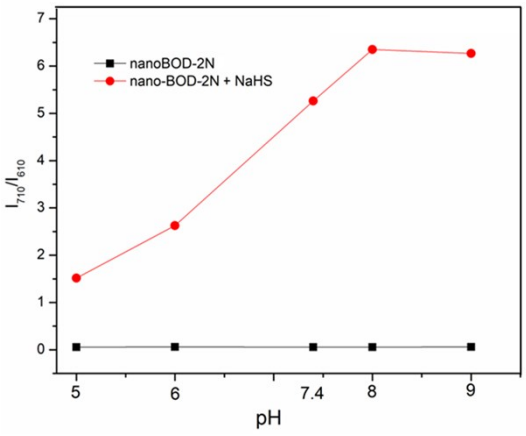


Fig. S4. NanoBOD-SCM showed obvious fluorescent turn-on response to H_2S within a physiological range from pH 9 to approximately 5, while inactivation in the absence of H_2S was observed within such testing conditions.

8. Selctivity.

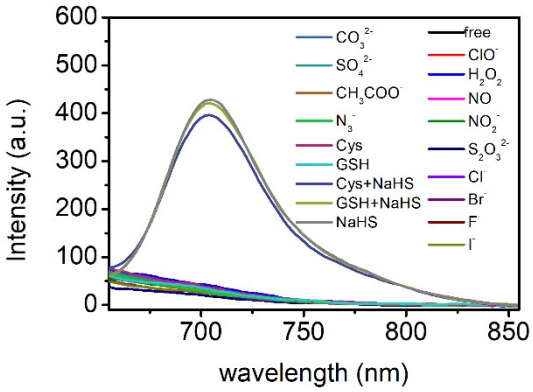


Fig. S5. NanoBOD-SCM undoubtedly showed highly selective fluorescence response to H_2S when compared to a panel of potentially interfering species.

9. The viability of HCT116 cells after treatment with NanoBOD-SCM for 12 h.

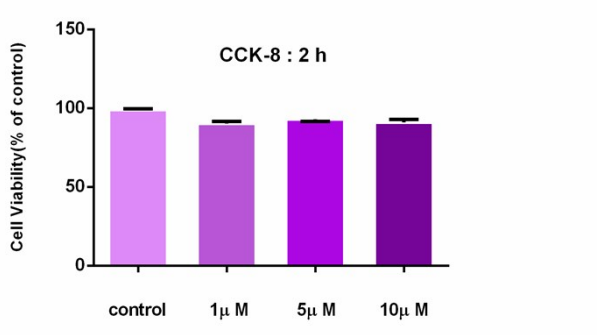


Fig. S6. The cytotoxicity of NanoBOD-SCM evaluated by cell counting kit-8 (CCK-8) assay treated with HCT116 cells in 12 h. Our experiments evidenced that no significant cytotoxicity was noted in the presence of CCK-8 for 2 h.

10. HepG2 cells imaging by confocal microscopy images.

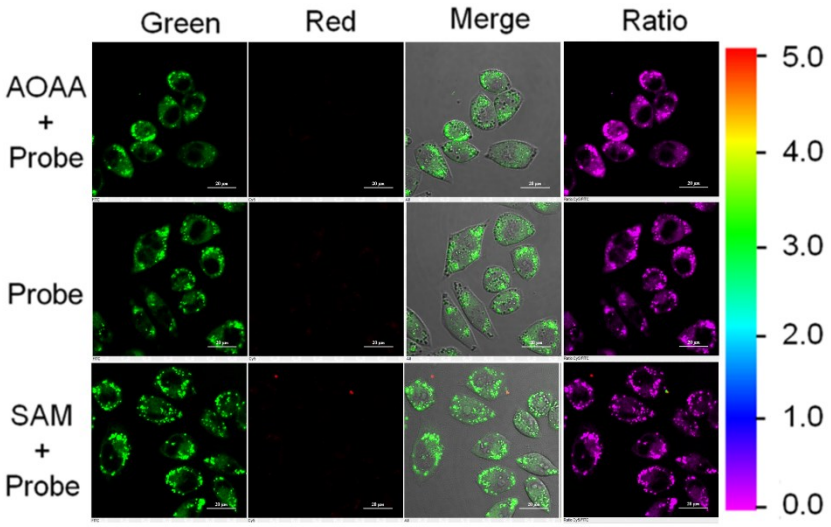
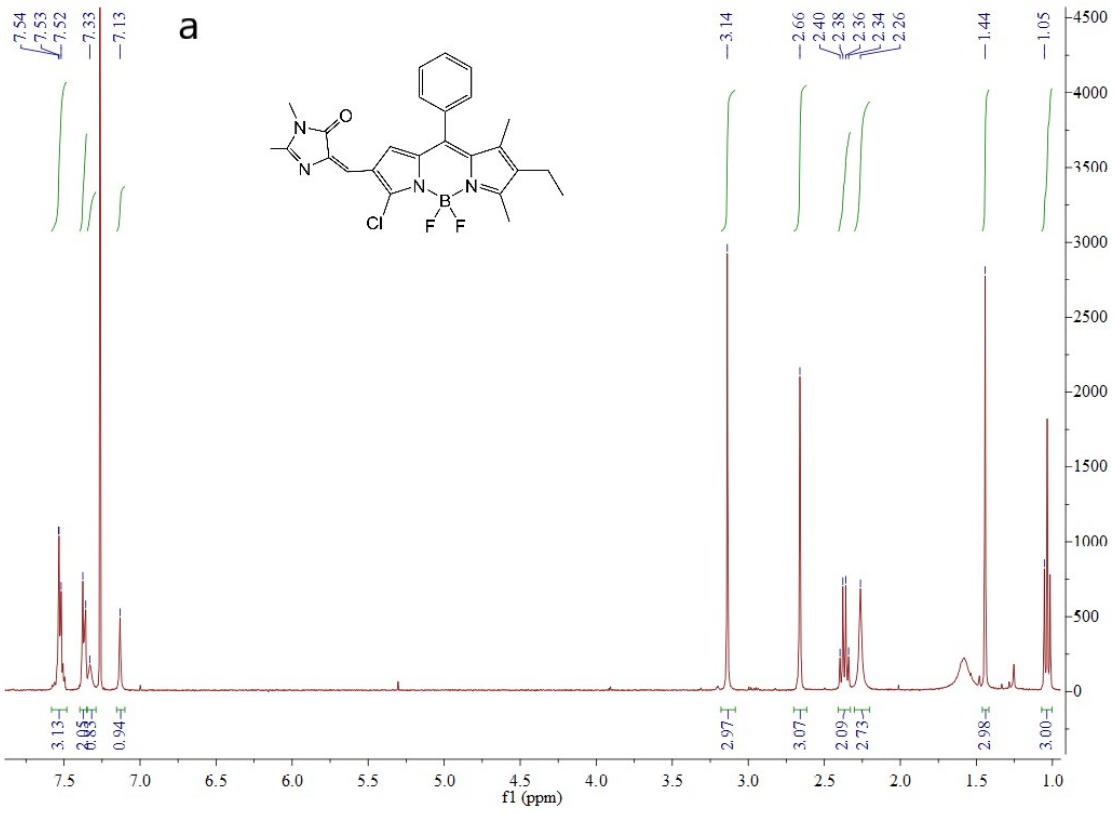
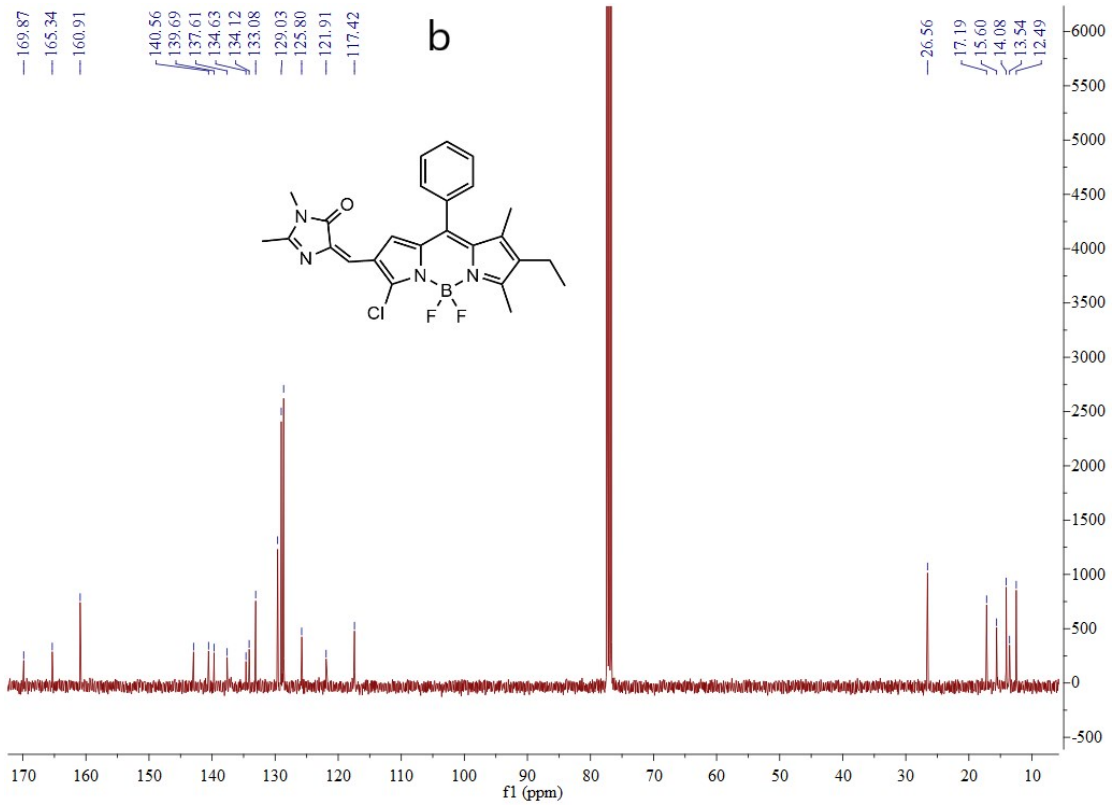


Fig. S7. The imaging of H_2S-deficient cancer cells in dual-color imaging modality with NanoBOD-SCM. a) The incubation of HepG2 cells with NanoBOD-SCM (BODVA-Cl 10 μM) for 30 min. b) HepG2 cells pretreated with AOAA(1 mM) for 1 h, followed by loading with NanoBOD-SCM for 30 min. c) HepG2 cells pretreated with SAM (3 mM) for 1 h were stained with NanoBOD-SCM for 30 min. Scale bar = 20 μm.

11. NMR and HRMS.





Single Mass Analysis
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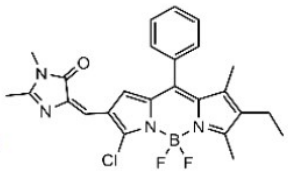
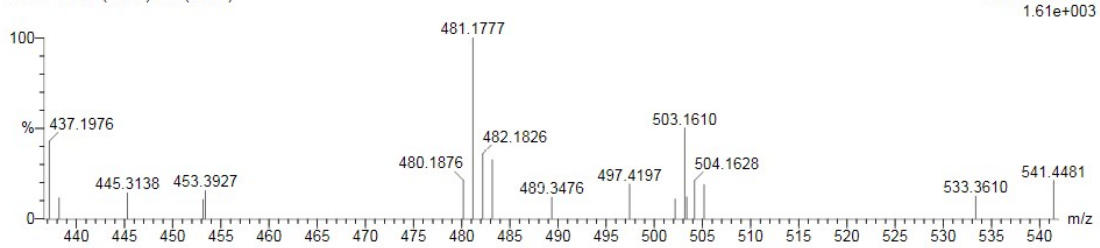
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

C



Monoisotopic Mass, Even Electron Ions
96 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-26 H: 0-30 B: 0-1 N: 0-4 O: 0-1 F: 0-2 Cl: 0-1
CC-ZHAO
ZC-HY-02 83 (0.942) Cm (82:85)

1: TOF MS ES+
1.61e+003



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
481.1777	481.1778	-0.1	-0.2	14.5	20.5	0.0	C25 H25 B N4 O F2 Cl