Electronic supplementary information

In situ quantification and evaluation of ClO^-/H_2S homeostasis in inflammatory gastric tissue with a rationally designed dual-response fluorescence probe featuring novel H^+-activated mechanism

Qiang Fu[a,b‡] Guang Chen[a,c‡*], Yuxia Liu[a], Ziping Cao[a], Xianen Zhao[a], Guoliang Li[a,], Fabiao Yu[c], Lingxin Chen[c], Hua Wang[a*] and Jinmao You[a,b*]

* Corresponding authors:

Qufu Normal University, Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, Qufu, China; Tel: +86 537 4456305; Fax: +86 537 4456305; E-mail addresses: chenandguang@163.com (Guang Chen), huawangqfnu@126.com (Hua Wang), jmyou6304@163.com (Jinmao You).

[a.] *The Key Laboratory of Life-Organic Analysis; Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, Qufu Normal University, Qufu 273165, Shandong, China chenandguang@163.com, huawangqfnu@126.com, jmyou6304@163.com*

[b.] *Qinghai Normal University, Xining, China*

[c.] *Key Laboratory of Coastal Environmental Processes and Ecological Remediation, The Research Centre for Coastal Environmental Engineering and Technology, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China*

†These authors contributed equally to the present work.

CONTENTS

Materials and Instruments

All solvents used were of analytical grade without further purification. UV-visible spectra were collected on Cary 300 Bio UV-vis spectrophotometer (VARIAN, USA). Fluorescence measurements were performed on Hitachi F-7000 fluorescence spectrophotometer (HITACHI, Japan), with the excitation (495 nm) and emission (560 nm) slit widths at 10.0 and 10.0nm respectively. Fluorescent confocal images of cells and tissues were acquired on an Olympus Fluo View FV1000 laser-scanning microscope with an objective lens (× 40) (OLYMPUS, Japan). TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 300-400), both of which were obtained from the company JianYou (Yan Tai, China). NMR were measured in the given solvent at RT on Bruker Ascend 500 (500.1 MHz, 1H; 125.8 MHz, 13C) instrument operating at the denoted spectrometer frequency given in mega Hertz (MHz) for the specified nucleus. Chemical shifts are given in parts per million (pp m) relative to tetramethylsilane (TMS) as an external standard for 1H- and 13C-NMR spectra and calibrated against the solvent residual peak. HPLC–MS/MS analysis was carried out on an Agilent 1100 Series HPLC coupled with the mass spectrometer 1100 Series LC-MSD Trap-SL (ion trap) from Bruker Daltonik (Bremen, Germany). All pH measurements were performed with a Mettler pH-meter FE20 (Shanghai, China) with a combined Mettler electrode of LE438. A H_2S survey meter (Beijing Yaou, DP-GDYS-103SN) was used to further validate the developed method.

Amino acid standards were purchased from Sigma Chemical Co. (St. Louis, MO), including alanine, histidine, methionine, arginine, glutamine, isoleucine, phenylalanine, asparaginate, leucine, proline, aspartic acid, glycine, lysine, sarcosine, serine, threonine, tryptophan, valine, glutamic acid and cysteine. Some enzymes including lactate dehydrogenase, aspartate aminotransferase, glutamic-pyruvic

transaminase, alkaline phosphatase and pepsin were purchased from Aladdin Chemical Company (Shanghai, China). S-nitroso-N-acetyl-dl-penicillamine (SNP) and 3-morpholinosydnonimine hydrochloride (SIN-1) were purchased from Sigma Company. Other compounds were purchased Qi Guang technical company (Jinan, China), which include the Cu^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ca^{2+}, F^-, SO_4^{2-}, HCO^{3-}, NO_2^-, NO_3^-, SO_3^{2-}, Cl^-, PO_4^{3-}, I^-, Mg^{2+}, $O_2^{-}\bullet$, H_2O_2, HSO_3^-, SO_3^{2-}, $S_2O_3^-$, $S_2O_4^{2-}$, $S_2O_5^{2-}$, SCN^-, Cl^-, Br^-, I^-, N_3^-, SO_4^{2-}, HPO_4^{2-}, OAc^-, citrate. ·OH was generated by a Fenton reaction with Fe^{3+} and H_2O_2, and the Fe II(EDTA) concentrations represented ·OH concentration[1]. NO was generated from the decomposition of SNP and the probe solution was also degassed with NO before the reaction[2]. The $ONOO^-$ was obtained with the donor of 3-morpholinosydnonimine hydrochloride (SIN-1) [3]. Superoxide ($O_2^{-}\bullet$) was prepared from KO_2[4]. Unless stated otherwise, solvents used in this work were dried by distillation and all reagents were of commercial quality and used without further purification.

Characterization of probe BNBD

1H NMR (500 M, d_6-DMSO): δ 8.09 (br, 1H), 6.99 (d, J =10.5 Hz, 2H), 5.83 (d, J =10.1 Hz, 2H). Note: the peak of amino H (3.39 (s, 1H)) was overlapped by the residual water in solvent. **13C NMR** (125.8 MHz, d_6-DMSO): δ 147.99, 145.21, 129.10, 121.38, 113.39, 79.65. MS ($BNBD^+$): m/z calcd for [C18H15N3O3+H+]: 282.1, found: 283.2.

Animals and Tissue Samples

Rabbits (3 month old) were divided into three groups, as control group (intact rabbits), chlorinated group (oral administration of highly chlorinated water (100 μM) for 14 days), and rehabilitation group (rabbits from chlorinated group after oral administration of L-Cys (50 mg/kg/day) for 14 days). Gastric mucosa was scraped off from the ether anesthetic rabbits, washed by simulated gastric fluid and flattened. The obtained gastric mucosa was incubated with the mixed solutions consisting of 30 μL of

BNBD stock solution, 100 μL of simulated /real gastric juice, 100 μL of Britton-Robison buffer (aqueous), 100 μL distilled water and 670 μL of ACN in 1 mL solution (pH 1.98). After 2 min, the tissue was washed by simulated gastric juice for three times.

All the animals were treated following the guideline approved by the institutional animal care and use committee of the National Health Research Institutes.

Detection Limits

The fluorescence emission spectra were measured (n=3) and the standard deviation σ was obtained. Together with the slope k between the fluorescence intensity versus target concentration, detection limit was calculated as: detection limit = 3σ/k.

Probe Photostability

Photostability of BNBD probe in tissues was evaluated by recording the fluorescence intensity with time in the BNBD (5 μM)-labeled gastric mucosa (at the depths of 60 μm).

Computational Details

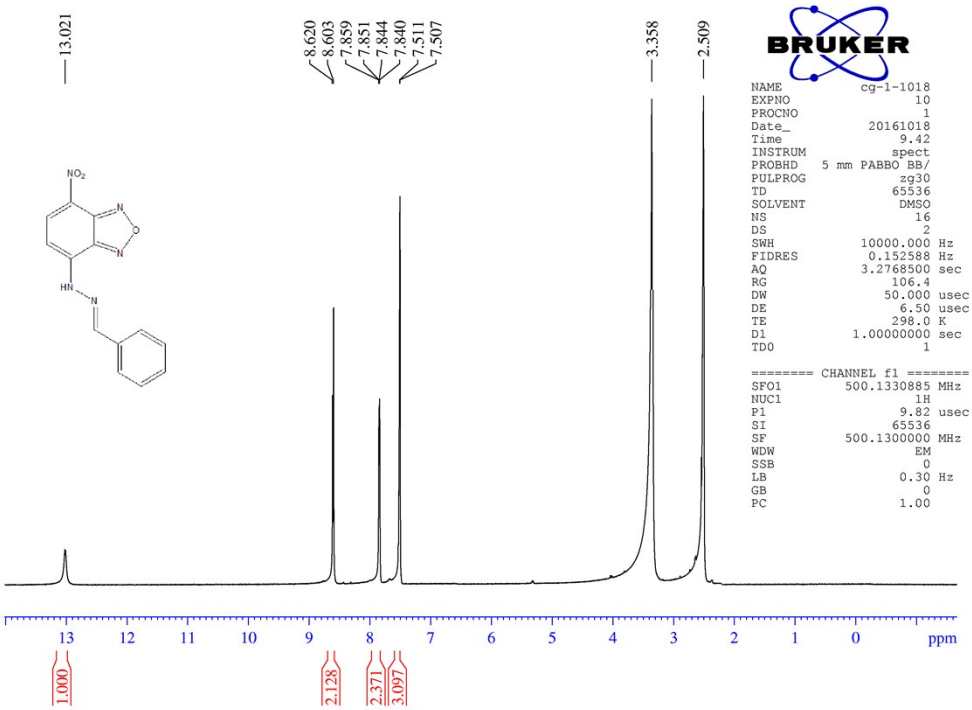
The geometry of the excited states were optimized with density functional theory (DFT) at B3LYP level with the standard 6-31g(d,p) basis set, which are based on the geometries of the ground states at the same DFT level. To obtain more reliable results, the single-point energy calculations of the excited states were carried out using B3LYP level with the larger 6-311+g(d,p) basis set for all atoms. All the energies were obtained with Gaussian 09 program.

Assessments of Gastric Mucosal Damage

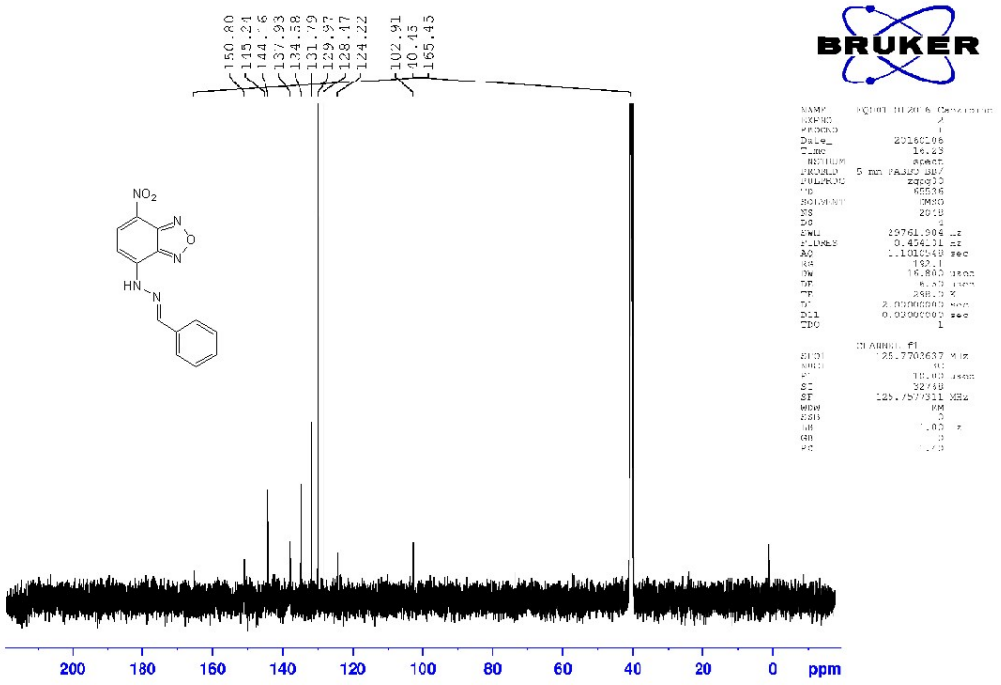
The mucosal injury was assessed under a light microscope by a pathologist. The degree of damage was identified as three levels: normal; surface epithelium injury; congestion and edema in upper mucosa (from replicate experiments n = 5).

Figures

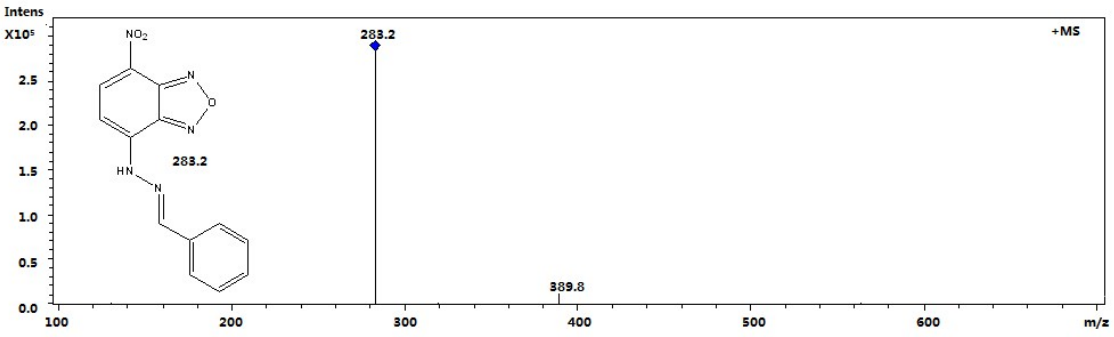
1.1 Figure S1 H-NMR for BNBD



1.2 **Figure S2 C-NMR for BNBD**



1.3 **Figure S3 MS for BNBD**



1.4 Figure S4 relationship between pH and fluorescence intensity of BNBD

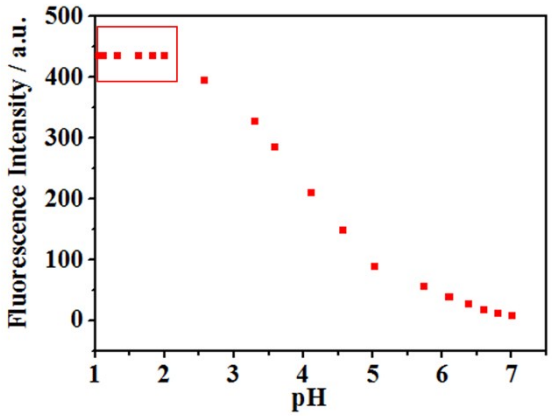


Fig. S4 The dependence of fluorescence intensity of BNBD probe (3 μM) to pH of 1.10 to 7.21. Red box: constant fluorescence intensity with pH from 1.98 to 1.10.

1.5 Figure S5 Investigation of H2S by the oxidation effect of ClO^-

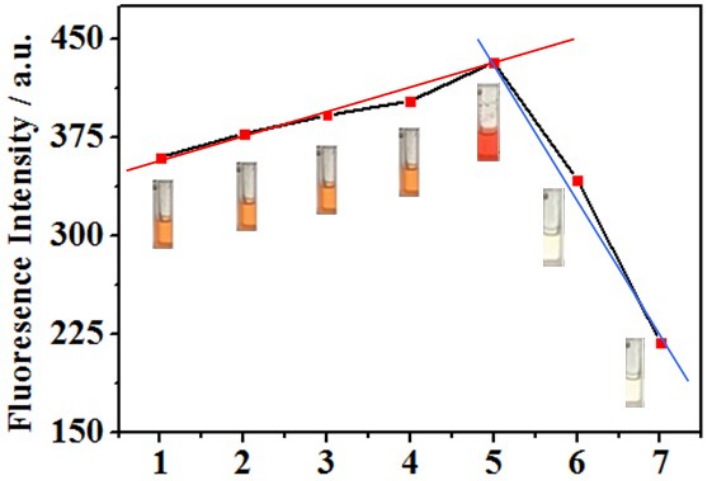
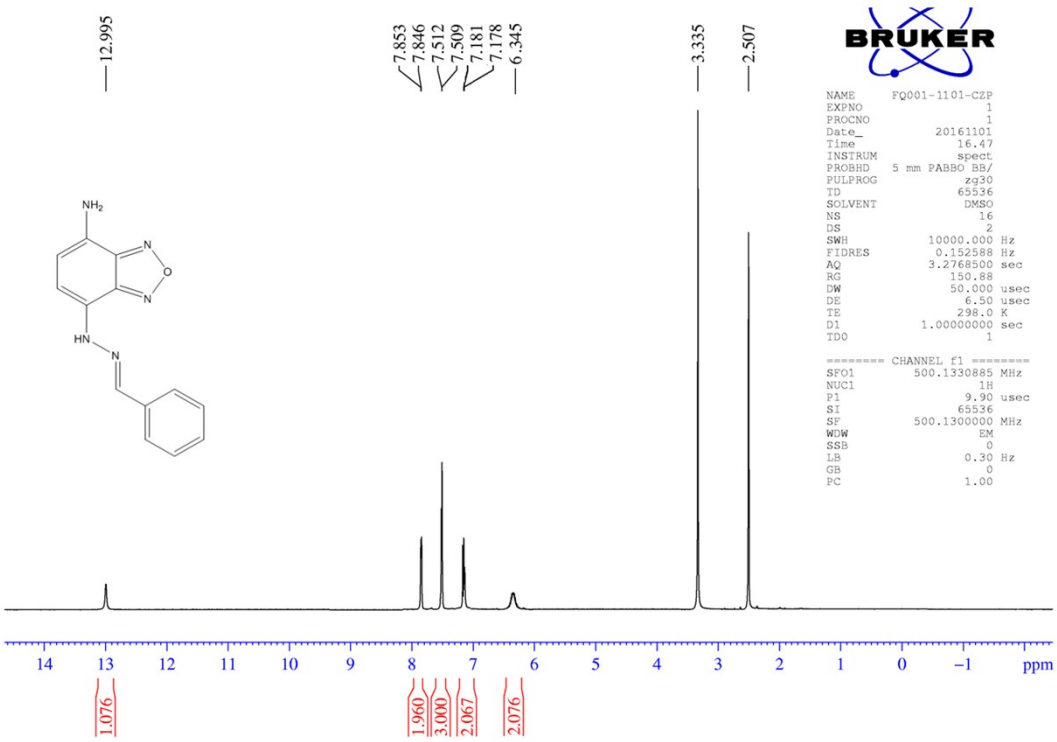
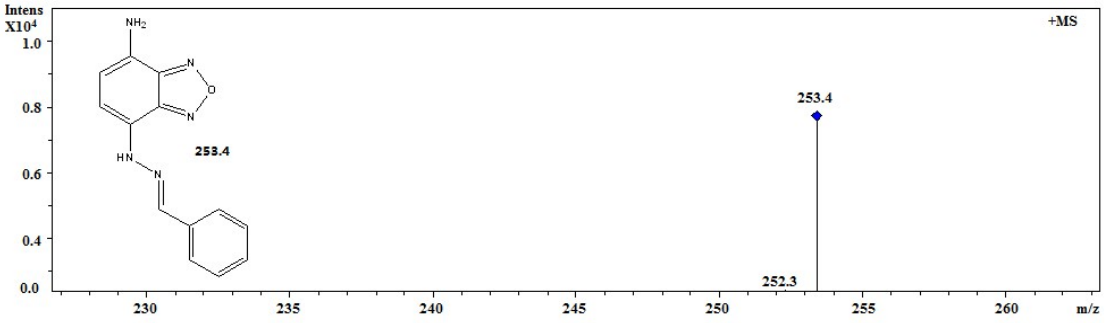


Fig. S5 Fluorescence investigation of H_2S by the oxidation effect of ClO^- in the simulated gastric juice (pH 1.98). 1: probe+40 μM H_2S; 2: probe+40 μM H_2S+20 μM ClO^-; 3: probe+40 μM H_2S+40 μM ClO^-; 4: probe+40 μM H_2S+60 μM ClO^-; 5: probe+40 μM H_2S+80 μM ClO^-; 6: probe+40 μM H_2S+100 μM ClO^-; 6: probe+40 μM H_2S+120 μM ClO^- (probe: 3 μM)..

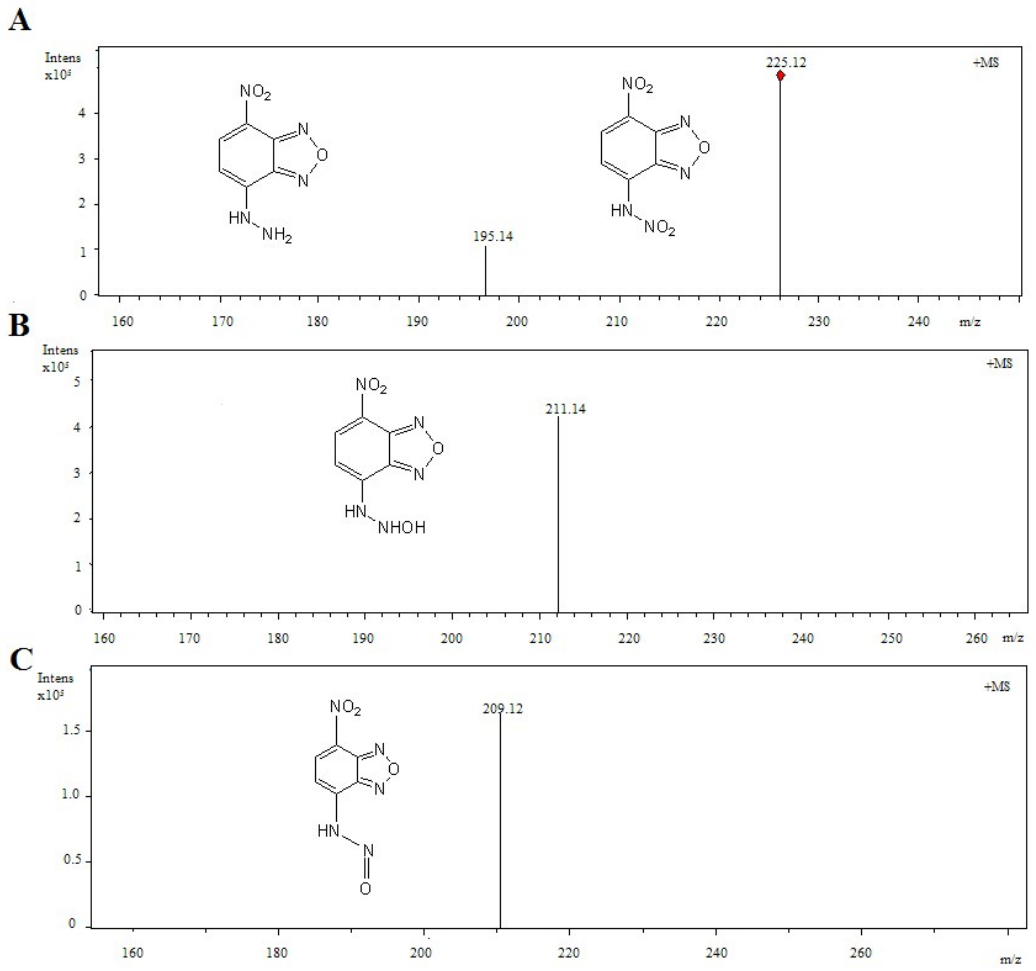
1.6 **Figure S6 H-NMR for product of nitro reduction of BNBD**



1.7 **Figure S7 MS for product of nitro reduction of BNBD**



1.8 Figure S8 Oxidation products of C=N oxidation by ClO^-



1.9 Figure S9 Amounts of probe

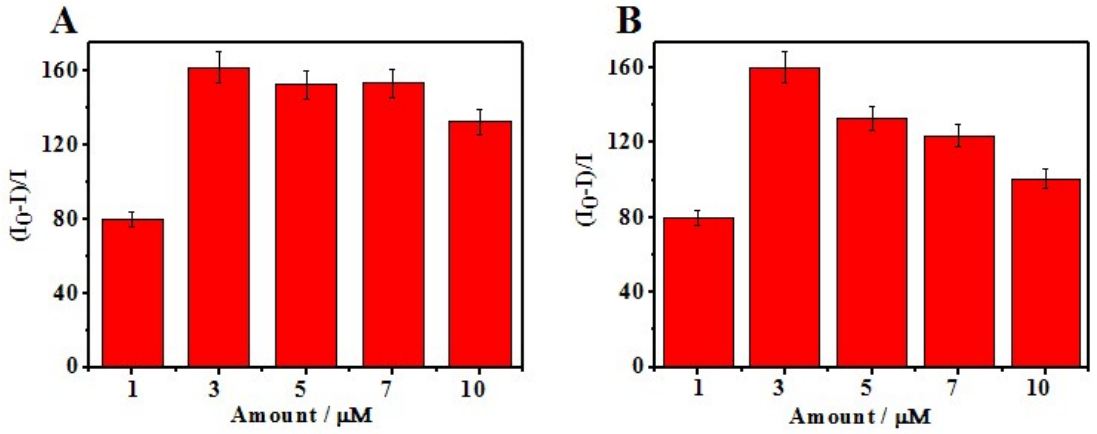


Fig. S9 Effects of probe mounts on the fluorescence intensities toward 3 μM of ClO^-(A) /H_2S (B).

1.10 Figure S10 Time-dependent responses

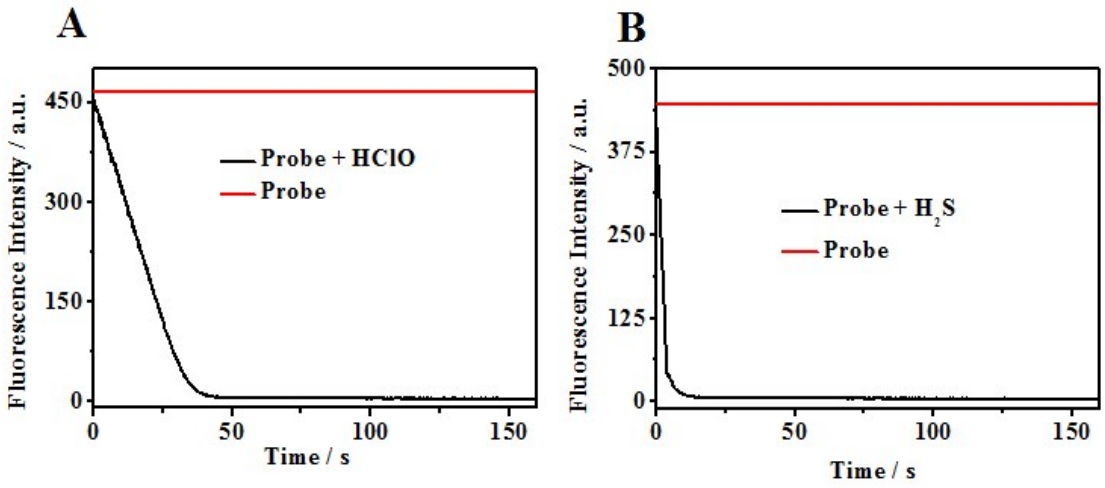


Fig. S4 Fluorescence kinetics of probe BNBD (3 μM) upon the addition of 3μM ClO^-(A) /H_2S (B).

1.11 Figure S11 Temperature-dependent responses

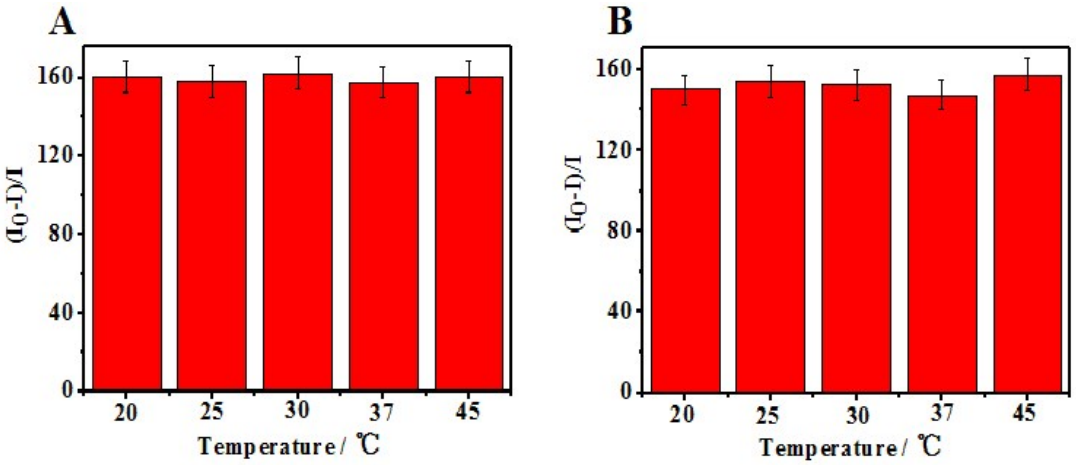


Fig. S11 Temperature-dependent fluorescence responses of probe BNBD (3 μM) to 3 μM of ClO^-(A) /H_2S (B).

1.12 Figure S12 UV absorbance investigations in simulated gastric juice

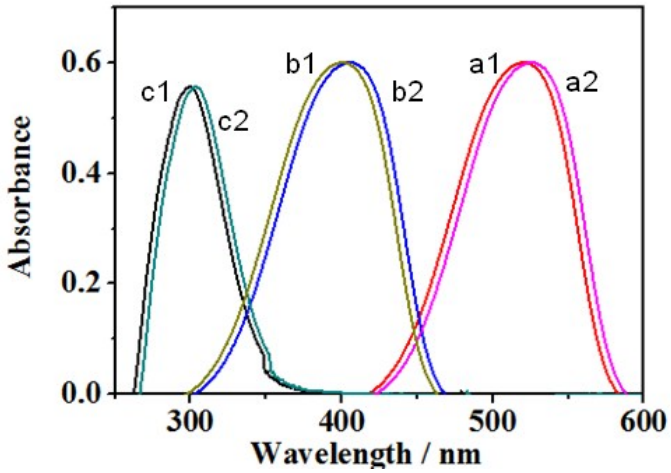


Fig. S12 UV absorbance investigations: a1, b1, c1 refers respectively to the probe, probe+H_2S, probe+ClO^- in BR buffer (pH 1.98); a2, b2, c2 refers respectively to the probe, probe+H_2S, probe+ClO^- in simulated gastric juice(pH 1.98).

1.13 Figure S13 Evaluation of quantification strategy with quality control solutions

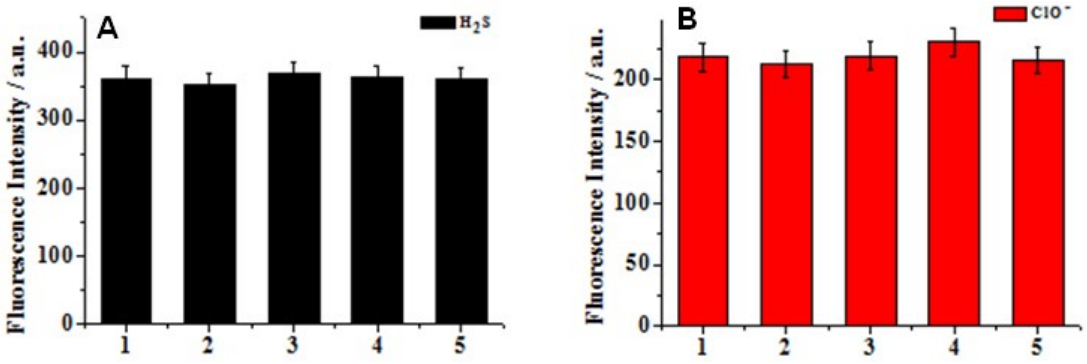


Fig. S13 Evaluation of quantification strategy with quality control solutions (QC): A for H_2S (40 μM), B for ClO^-(40 μM); 1: pH=2.1; 2: pH=2.9; 3: pH=3.4; 3: pH=4.1; 4: pH=4.8; 3: pH=5.1 (probe=3μM) (*n*=7).

1.14 **Figure S14 Linearity**

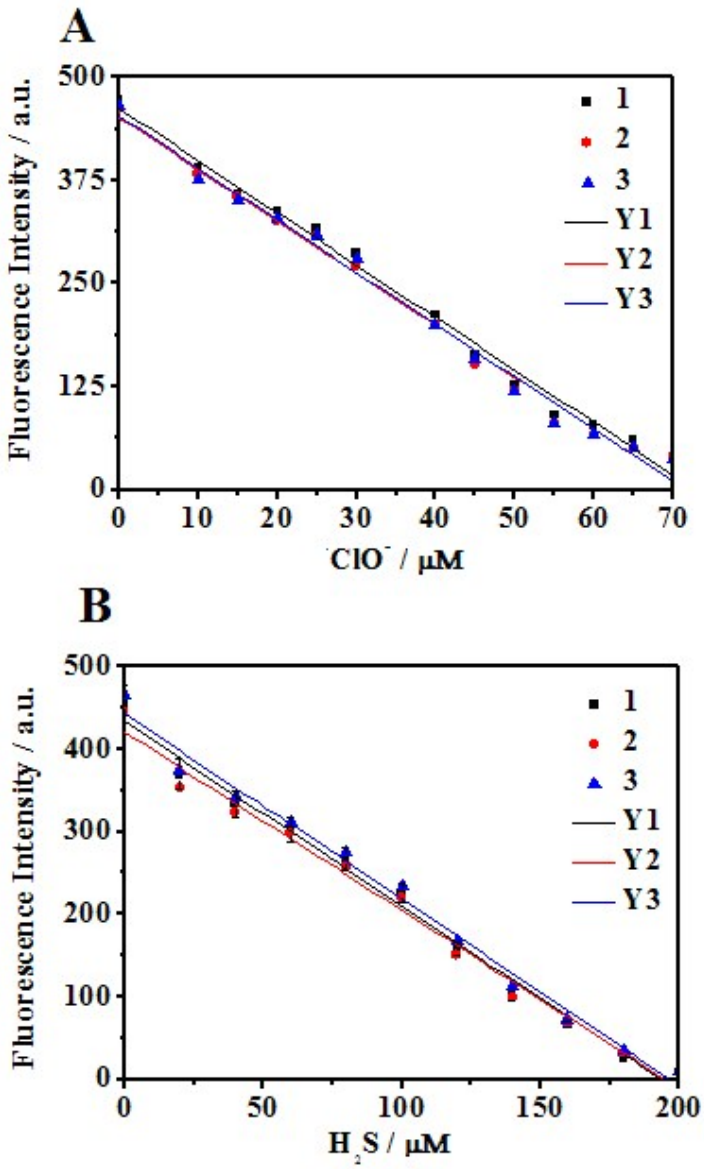


Fig. S14 Linear relationships established in Britton-Robison buffer (A1, B1), simulated gastric juice (A2, B2) and real gastric juice (A3, B3).

1.15 Figure S15 Probe photostability

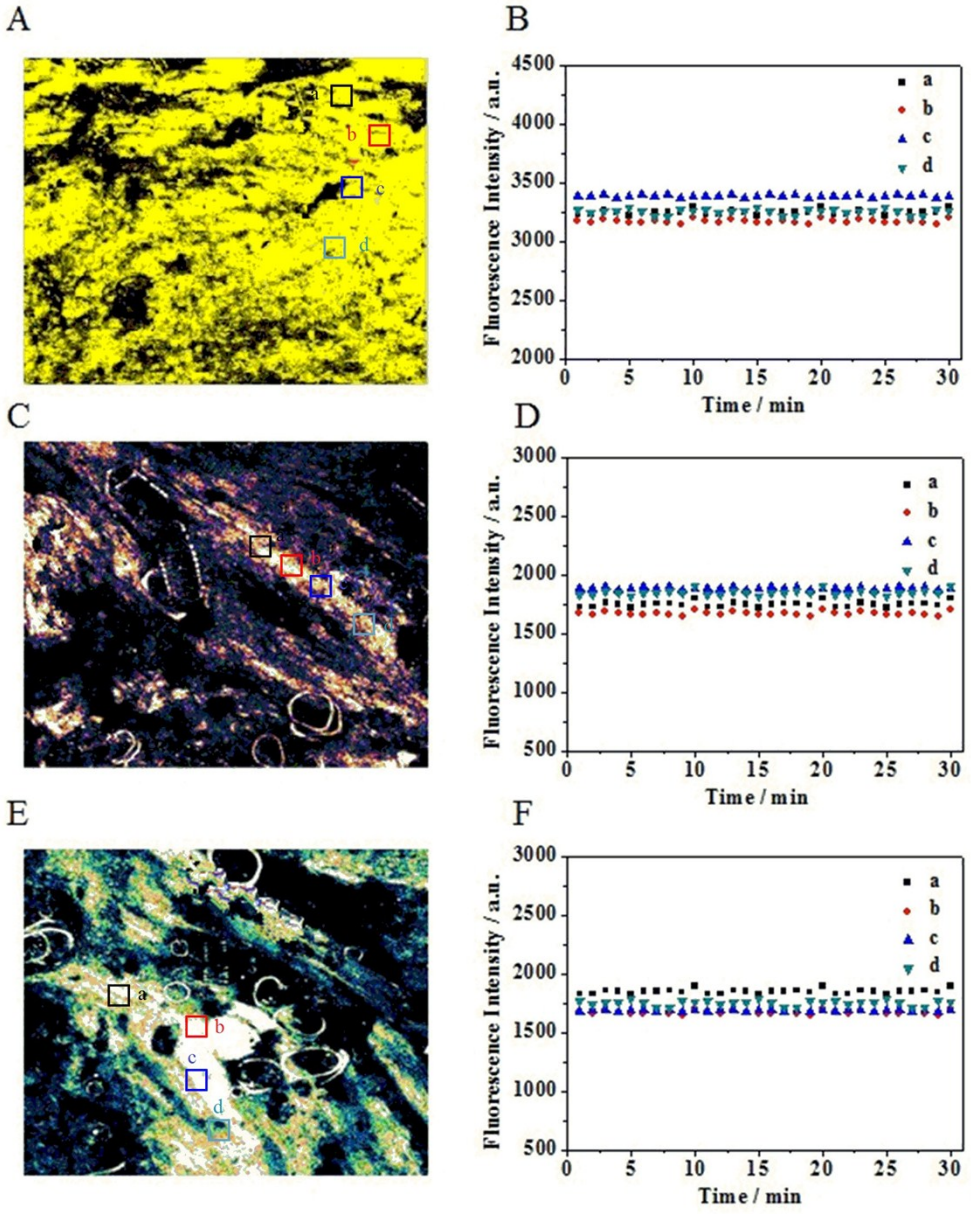


Fig. S15 Fluorescence confocal images of gastric mucosa: A (3 μM BNBD), C (3 μM BNBD + 3 μM ClO^-), E (3 μM BNBD + 3 μM H_2S), and fluorescence intensity from region of interest a~d from left images as a function of time. The fluorescence intensity was collected at the depth of 50 μm in gastric mucosa samples from replicate experiments (n = 5), with the excitation wavelength of 488 nm and the collection wavelength of 500–600 nm.

1.16 **Figure S16 H-NMR titration of BNBD with H_2S**

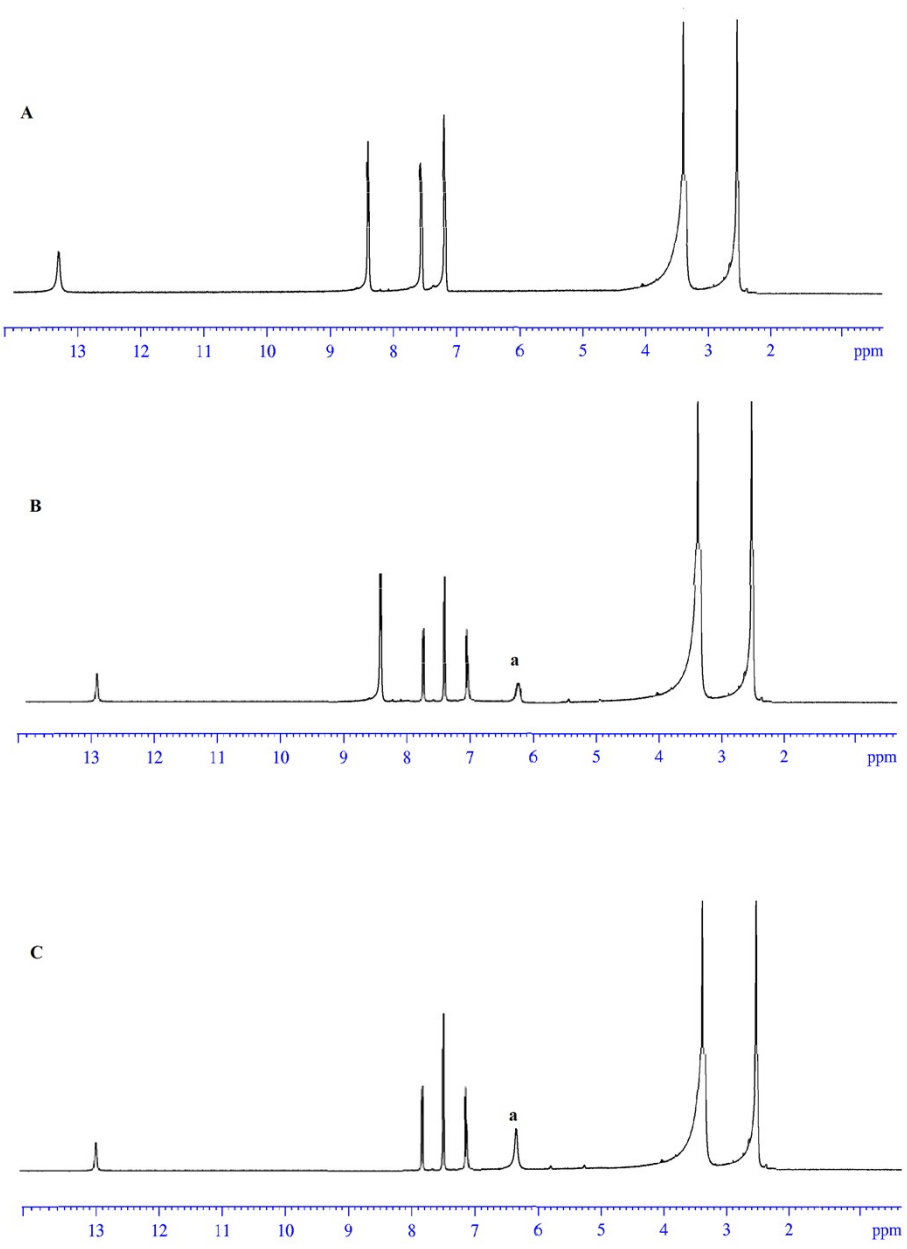


Fig. S16 ^{1}H NMR titration of the probe BNBD by H_2S, A: BNBD; B: BNBD+ H_2S; C: product, where a denoted the shift peak for -NH_2 group.

1.17 **Figure S17 Method validation with H_2S analysis instrument**

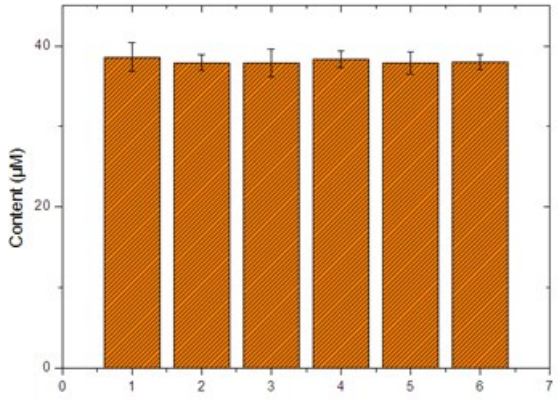


Fig. S17 Comparison of the detected H_2S in gastric juice, where the data 1, 3, and 5 were obtained from this method with probe BNBD, and the data 2, 4, and 6 were obtained from a H_2S analysis instrument

References

(1) Setsukinai, K.; Urano, Y.; Kakinuma, K.; Majima, H. J.; Nagano, T. *J Biol Chem* **2003**, *278*, 3170.

(2) Zhang, P.; Li, J.; Li, B.; Xu, J.; Zeng, F.; Lv, J.; Wu, S. *Chem Commun* **2015**, *51*, 4414.

(3) Rao, M. U.; Pryor, W. A. *Anal Biochem* **1996**, *236*, 242.

(4) Huang, Y.; Zhang, P.; Gao, M.; Zeng, F.; Qin, A.; Wu, S.; Tang, B. Z. *Chem Commun* **2016**, *52*, 7288.