Supporting Information for

An Improved Aromatic Substitution-rearrangement-based Ratiometric Fluorescent Cysteine-specific Probe and Its Application of Real-time Imaging under Oxidative Stress in Living Zebrafish

Longwei He, Xueling Yang, Kaixin Xu and Weiying Lin*

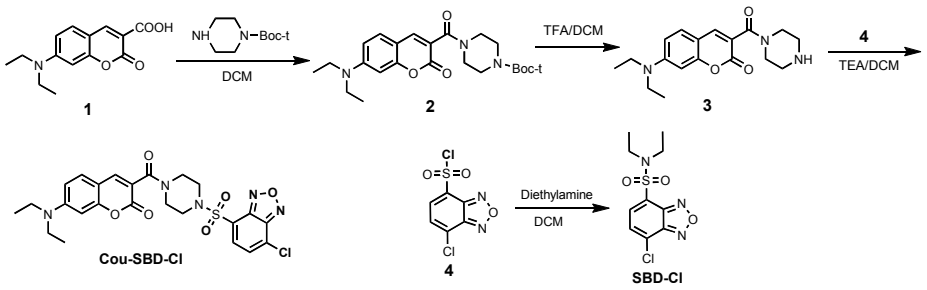
[a]Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Shandong 250022, P.R. China

E-mail: weiyinglin2013@163.com

Table of contents

Instruments

Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan; High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP spectrometer; NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a LabTech UV Power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer; The optical density was measured by a Thermo Scientific Multiskan FC microplate reader in cytotoxicity assay; The fluorescence imaging of cells was performed with OLYMPUS FV1000 (TY1318) confocal microscopy; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.



Scheme S1 Synthetic route of probe **Cou-SBD-Cl** and intermediate **SBD-Cl**.

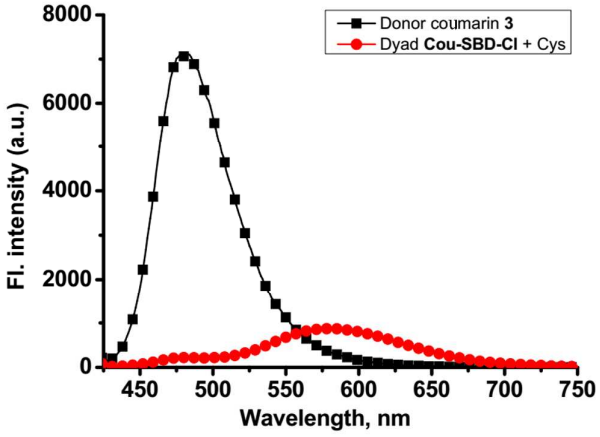


Figure S1 Emission spectra of donor coumarin **3** (10 μM, blacks quare) and dyad **Cou-SBD-Cl** (10 μM) with 100 equiv. of Cys (red circle) in aqueous solution (pH 7.4, 25 mM PBS buffer solution containing 20% DMF).

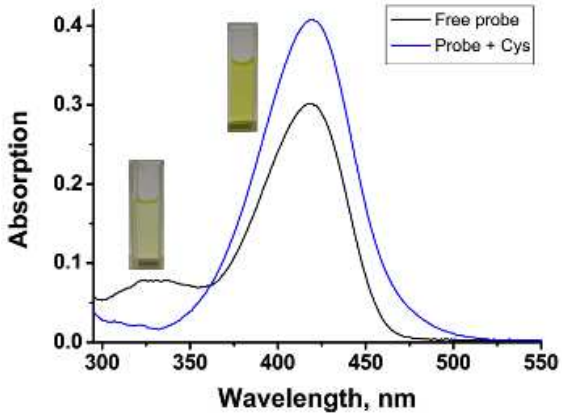


Figure S2 Absorption spectra of **Cou-SBD-Cl** (10 μM) with 100 equiv. of Cys in aqueous solution (pH 7.4, 25 mM PBS buffer solution containing 20% DMF). Inset: the visual colour of probe **Cou-SBD-Cl** in the absence (left) or presence (right) of Cys.

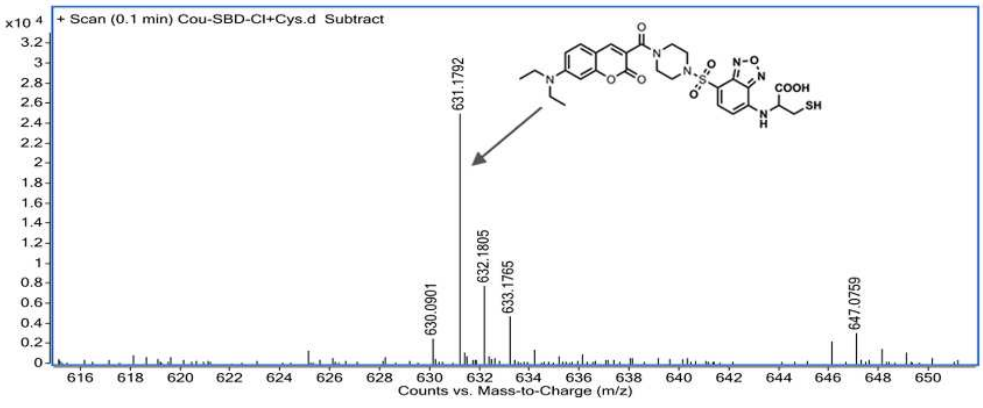


Figure S3 The high resolution mass spectrum of **Cou-SBD-Cl** in the presence of Cys in aqueous PBS solution.

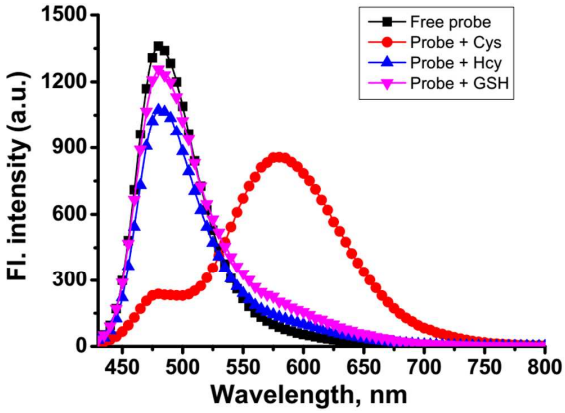


Figure S4 The emission spectra of **Cou-SBD-Cl** in the absence or presence of 1 mM for Cys, Hcy, or GSH in aqueous PBS solution (pH 7.4, 25 mM PBS buffer solution containing 20% DMF). Incubation time: 60 min. Excitation: 450 nm.

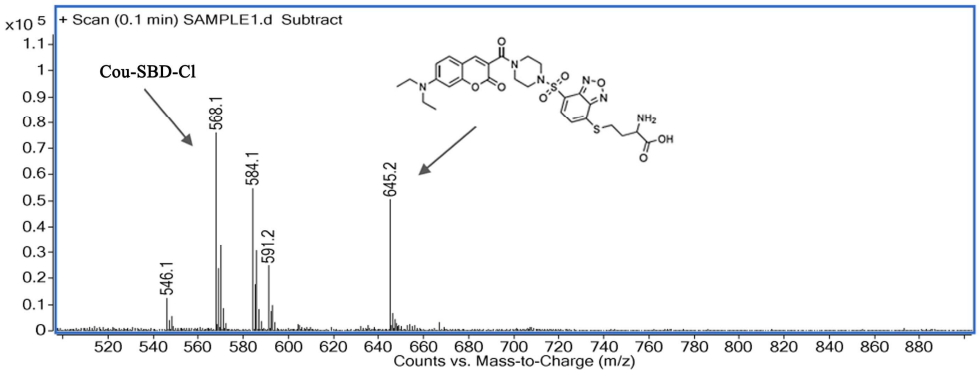


Figure S5 MS (ESI) of **Cou-SBD-Cl** in the presence of Hcy in aqueous PBS solution.

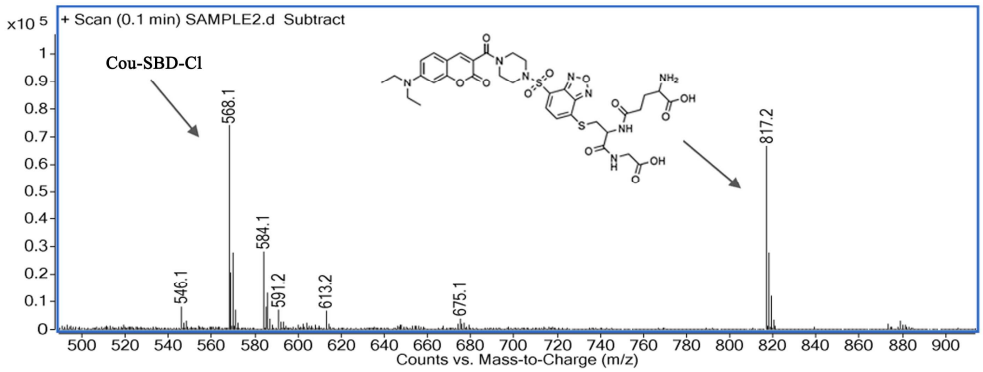


Figure S6 MS (ESI) of **Cou-SBD-Cl** in the presence of GSH in aqueous PBS solution.

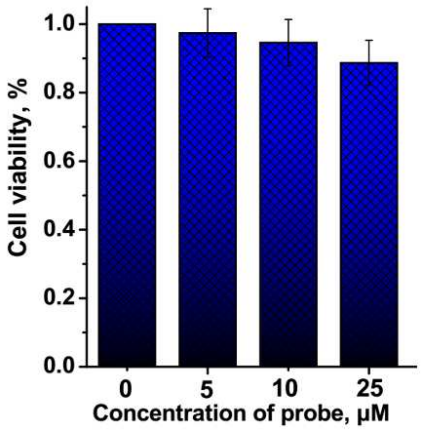


Figure S7. Cytotoxicity of probe **Cou-SBD-Cl** (5, 10, 25 μM) evaluated by the standard MTT assay. The cells were incubated with the probe for 24 h.

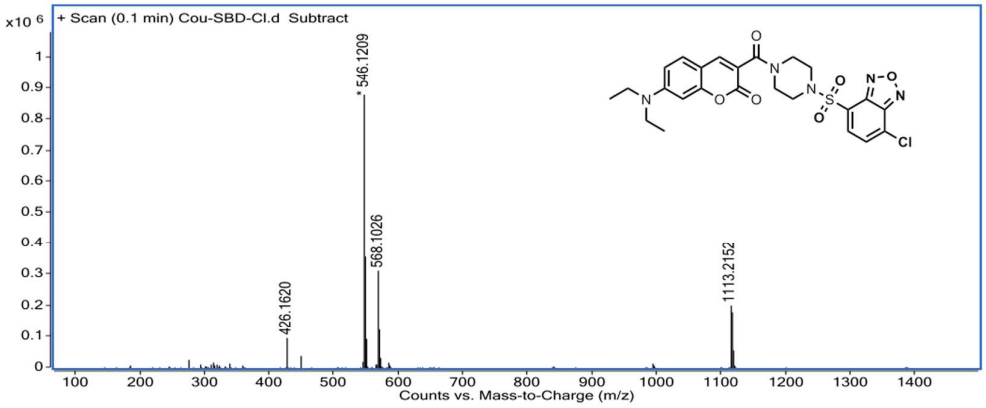


Figure S8 HRMS (ESI) of probe **Cou-SBD-Cl**, calcd for $C_{24}H_{24}ClN_5O_6S$ ($[M+1]^+$): 546.1209. Found 546.1209.

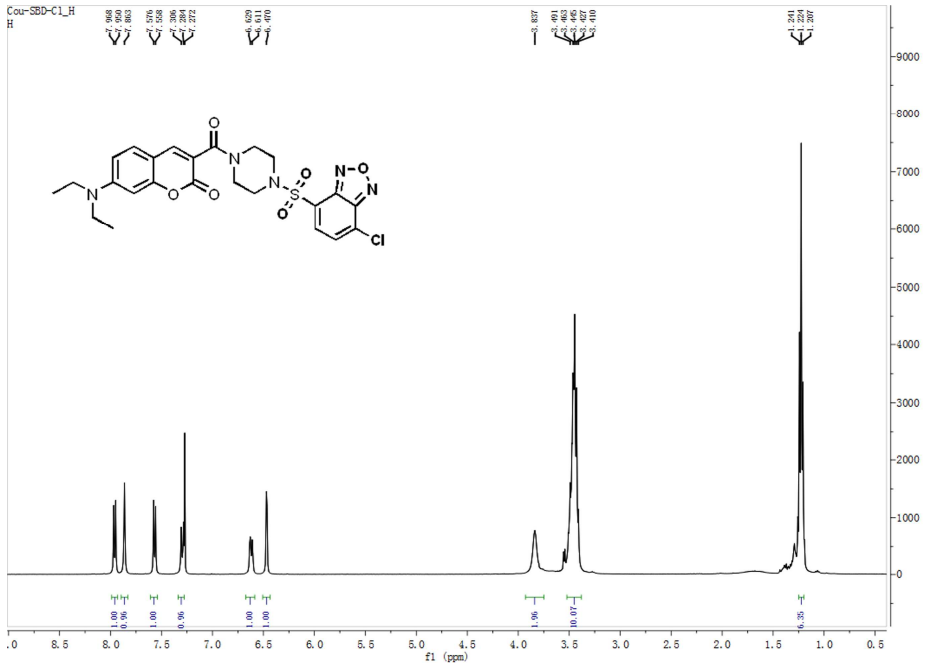


Figure S9 ^{1}H NMR spectrum of probe **Cou-SBD-Cl** in $CDCl_3$.

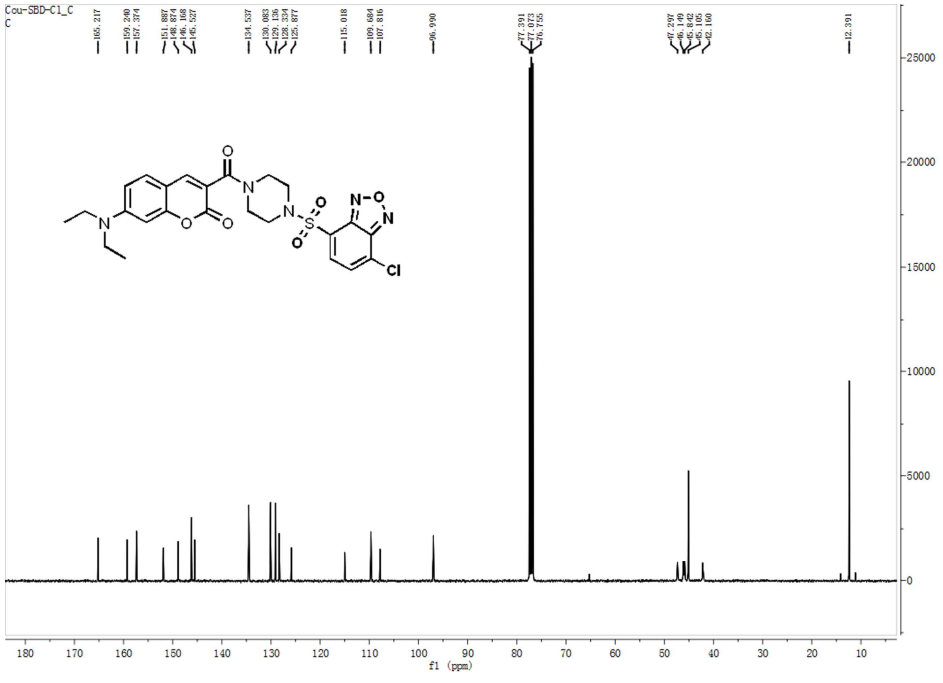


Figure S10 ^{13}C NMR spectrum of probe **Cou-SBD-Cl** in $CDCl_3$.

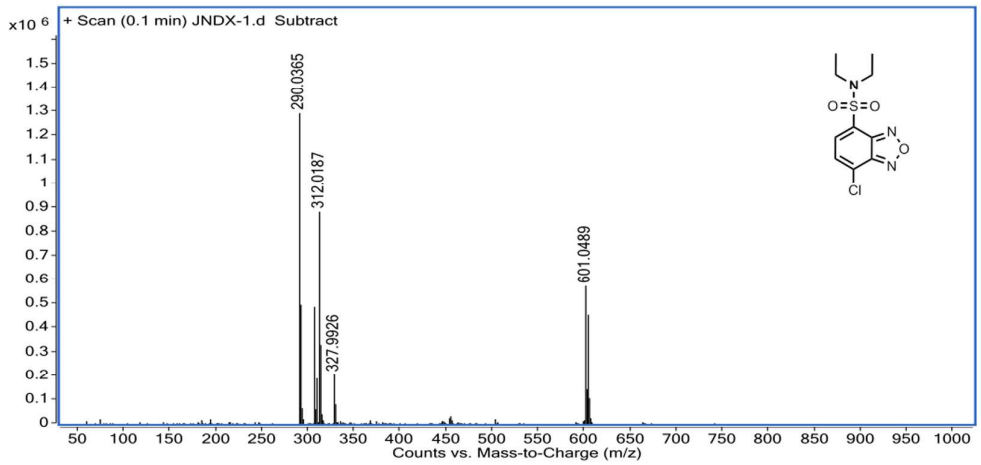


Figure S11 HRMS (ESI) of intermediate **SBD-Cl**, calcd for $C_{10}H_{13}ClN_3O_3S$ ($[M+1]^+$): 290.0361. Found 290.0365.

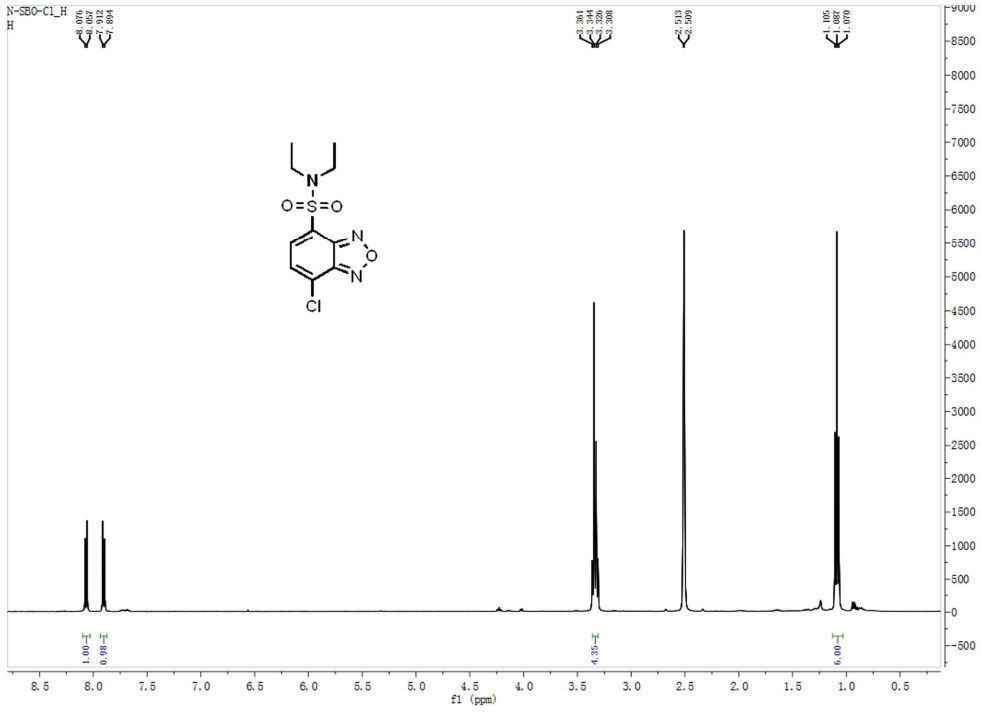


Figure S12 ^{1}H NMR spectrum of intermediate **SBD-Cl** in d_6-DMSO.

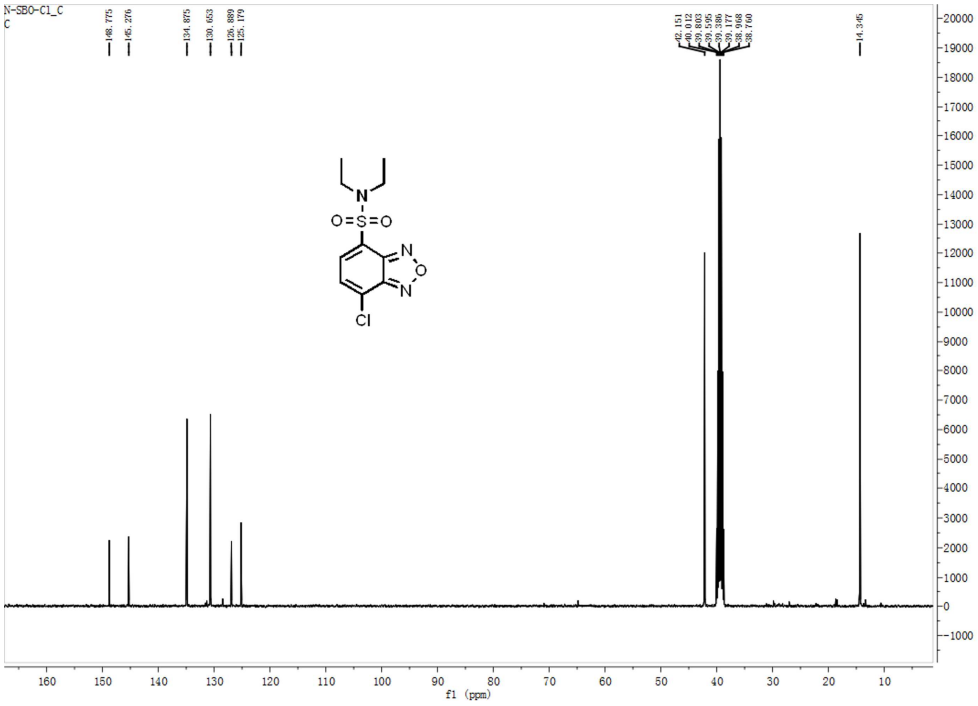


Figure S13 ^{13}C NMR spectrum of intermediate **SBD-Cl** in d_6-DMSO.