Supplementary Information

A Novel Fluorescence Probe for Localization of Nucleoli Developed via Chain Reaction of Endogenous Cysteine in Cells

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Experimental Section

Instrumentation and Reagents

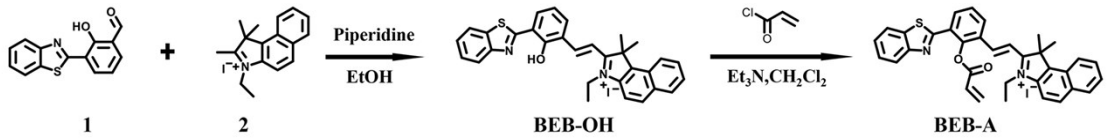
^{1}H-NMR spectra were recorded on Avance III500 NMR spectrometer (Bruker Inc., Germany). Mass spectra (MS) were recorded on a MALDI-TOF-MS (Bruker Inc., Germany). pH was measured on an INESA Scientific PHS-3C pH meter (Sartorius AG, Germany). Fluorescence spectra and Quantum Yield were recorded on an F-7000 fluorescence spectrometer (Hitachi Co., Ltd. Japan) with a 1-cm quartz cell. Absorption spectra were recorded on a Cary 60 UV-spectrophotometer (Agilent Technologies, USA) with a 1-cm quartz cell. Cell imaging experiments were carried out on an LSM 710 laser scanning confocal microscope (Carl Zeiss, Oberkochen, Germany).

Cysteine (Cys) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (China). 2-(2-Hydroxyphenyl)benzothiazole, 1,1,2-trimethyl-1H-benz[e]indole, and iodoethane were purchased from Sun Chemical Technology (Shanghai) Co., Ltd (China). Leucine (Leu), isoleucine (Ile), serine (Ser), threonine (Thr), threonine (Trp), tyrosine (Tyr), glycine (Gly), lysine (Lys), glutamic acid (Glu), phenylalanine (Phe), alanine (Ala), asparagine (Asn), histidine (His), valine (Val), arginine (Arg) were of analytical reagent grade and used without further purification or treatment. DNase and RNase were purchased from Beijing Solarbio Science & Technology Co., Ltd. Other reagents were of analytical reagent grade and used without further purification or treatment.

All aqueous solutions were prepared with ultrapure water obtained by a Milli-Q water purification system (18.2 MΩ cm). Hela cells, HepG2 cells, HUVEC cells and human serum were obtained from the Life Sciences College of Jilin University (Jilin, China).

Synthesis

The synthetic route of BEB-A is shown below. Compounds 1 and 2 were synthesized according to the previously published methods.[1, 2]



1 2 BEB-OH BEB-A

Scheme S1 The synthesis and the characterization of BEB-A

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Synthesis of BEB-OH. Compound 1 (250 mg, 0.98 mmol) and compound 2 (365 mg, 0.98 mmol) were dissolved in ethanol (30 mL) and 3.98 mmol of piperidine was then slowly added. The mixture was stirred at 80°C for 12 h. After cooling down to room temperature, the mixture was concentrated under reduced pressure. The obtained crude product was purified by silica gel chromatography using CH_2Cl_2/CH_3OH (100/1 to 30/1, v/v) as the eluent, from which the target blue solid (136 mg) product was obtained. ^{1}H NMR (500 MHz, DMSO-d_6) 1H NMR (500 MHz, DMSO) δ 8.82 (s, 1H), 8.48 (d, J = 13.0 Hz, 1H), 8.27 (t, J = 11.1 Hz, 1H), 8.22 (s, 1H), 8.11 (dd, J = 16.0, 8.5 Hz, 2H), 8.05 (d, J = 7.8 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.8 Hz, 1H), 7.70 (t, J = 7.3 Hz, 1H), 7.55 (t, J = 7.4 Hz, 1H), 7.51 – 7.43 (m, 1H), 7.34 (t, J = 7.4 Hz, 1H), 6.90 (d, J = 14.8 Hz, 1H), 6.57 (d, J = 9.0 Hz, 1H), 4.48 (d, J = 6.9 Hz, 2H), 2.02 (s, 6H), 1.40 (t, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 179.59, 176.44, 164.00, 152.59, 152.07, 139.53, 136.19, 134.69, 132.07, 131.59, 130.84, 130.40, 128.31, 128.10, 126.07, 125.62, 124.02, 122.99, 122.17, 122.17, 121.56, 119.68, 112.15, 99.33, 51.71, 27.13, 13.29, 0.58. MS (MALDI-TOF-MS, m/z) Calcd for $[C_{31}H_{27}N_2OS]^+$: 475.1839, found: 475.1840.

Theoretical Calculation and Analysis

Calculations of optimal geometry and electronic structure of the probes were carried out by density functional theory (DFT) and time-dependent DFT (TDDFT) at

the CAM-B3LYP/6-31G(d) level using Gaussian 16 software.[3] The obtained data were further analyzed by Multiwfn 3.7 software.[4]

Cell Culture

HeLa (cervical cancer), HepG2 (liver cancer), HUVEC (human umbilical vein endothelial) cells were cultured in a Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37 °C and in a 5% CO_2 atmosphere. The cells were then plated on a 35-mm culture dish and allowed to attach for 24 h.

Cytotoxicity Assay

Cell cytotoxicity was evaluated using CCK-8 assay. Cells were cultivated in a 96-well plate until 50–70% confluence and then incubated with different concentrations of probe for 10 h; after that, they were subjected to CCK-8 assay (n = 5). Cells treated with CMEM (control; n=5) were also prepared in parallel under the same conditions.

Fluorescence Imaging

Fluorescence imaging experiments were carried out using cells treated with four different conditions: (1) cells were incubated with PBS only; (2) cells were incubated with the probe BEB-A (5 μM) at 37°C for 10 min; (3) cells were treated with NEM (a sulfhydryl-containing trapping agent; 1 mM) for 20 min, followed by BEB-A (5 μM) for another 3 min. Following incubation, the cells were subjected to fluorescence imaging using a confocal laser scanning microscope at an excitation wavelength of 543 nm (a laser diode) and emission wavelengths between 580 and 730 nm.

Co-staining Experiments

Co-staining of Mitochondria: HeLa cells were first treated with MitoTracker Green FM (200 nM) for 30 min and were then rinsed twice with PBS buffer. Subsequently, the cells were treated with BEB-A (5 μM) for 3 min.

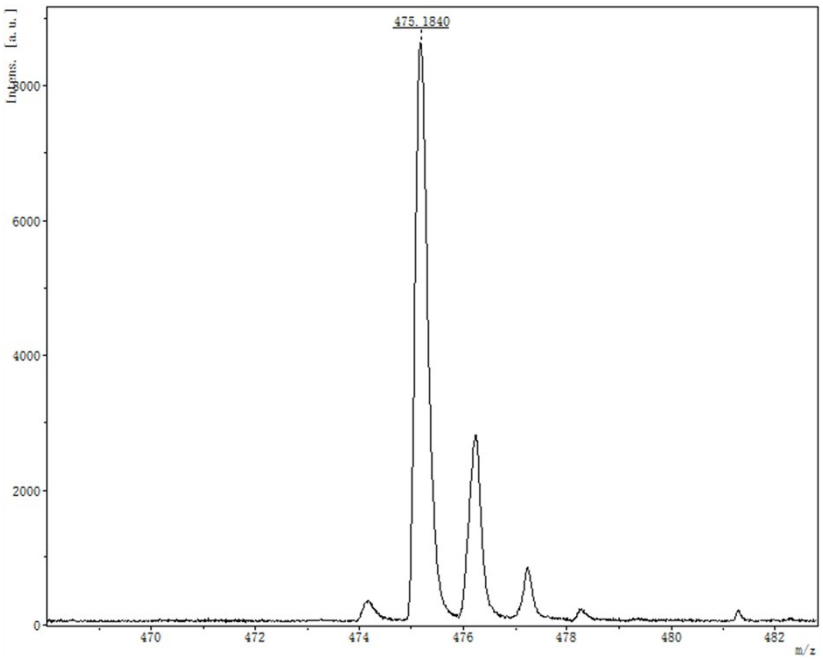
Co-staining of Nucleus*:* HeLa cells were first treated with DAPI for 30 min and

then rinsed twice with PBS buffer. After that, the cells were treated with BEB-A (5 μM) for 3 min.

DNase and RNase Digestion Experiments. Cells were fixed in prechilled methanol at −20 °C for 1 min. After that, the cells were permeabilized with 1% Triton X-100 in PBS solution for 2 min at room temperature. After rinsing twice with PBS, the cells were incubated with 5 μM BEB-A dissolved in PBS/CH_3CN (v/v=8:2, pH 7.4) for 20 min at 37 °C under a 5% CO_2 atmosphere. Subsequently, the cells were washed twice with PBS buffer and were thereafter treated with DNase I (100 U/mL), RNase A (20 μg/mL), or PBS buffer at 37 °C under a 5% CO_2 atmosphere for 3 h. Before imaging, the cells were rinsed twice with PBS buffer.

Theoretical Calculations of the interactions between BEB-OH and RNA The molecular docking was carried out using a crystal structure of RNA (PDB ID: 1ASY) on AutoDock 4.0 software. In the calculation of Gasteiger charge, the BEB-OH was set as AD4 atomic mode, and all rotating keys were set to have twisting force. The docking cycles and the parameters between ligand and RNA were set according to the default values. PyMOL software was used to display the docking model.

Figures



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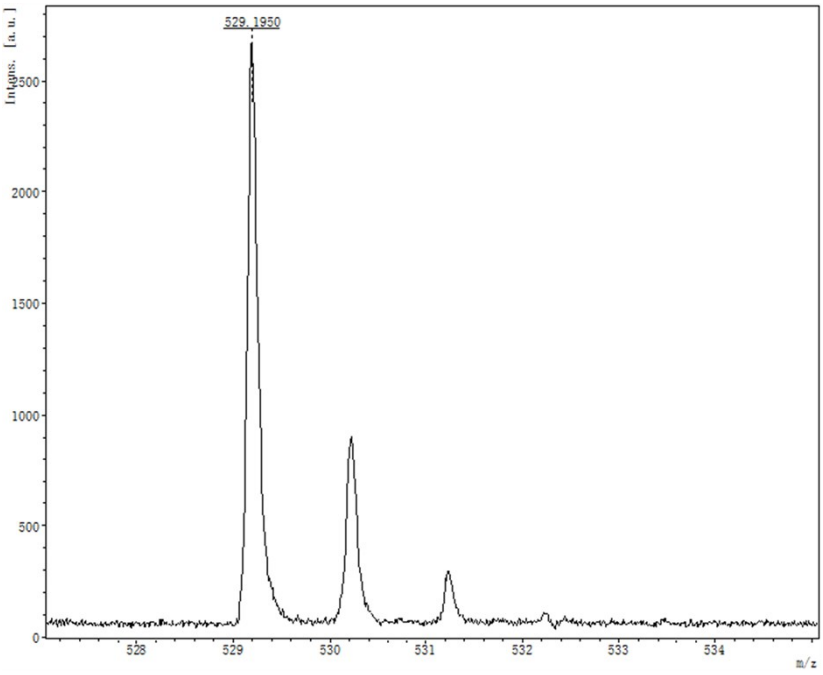
108 **Figure S1** MALDI-TOF-MS spectrum of BEB-OH.



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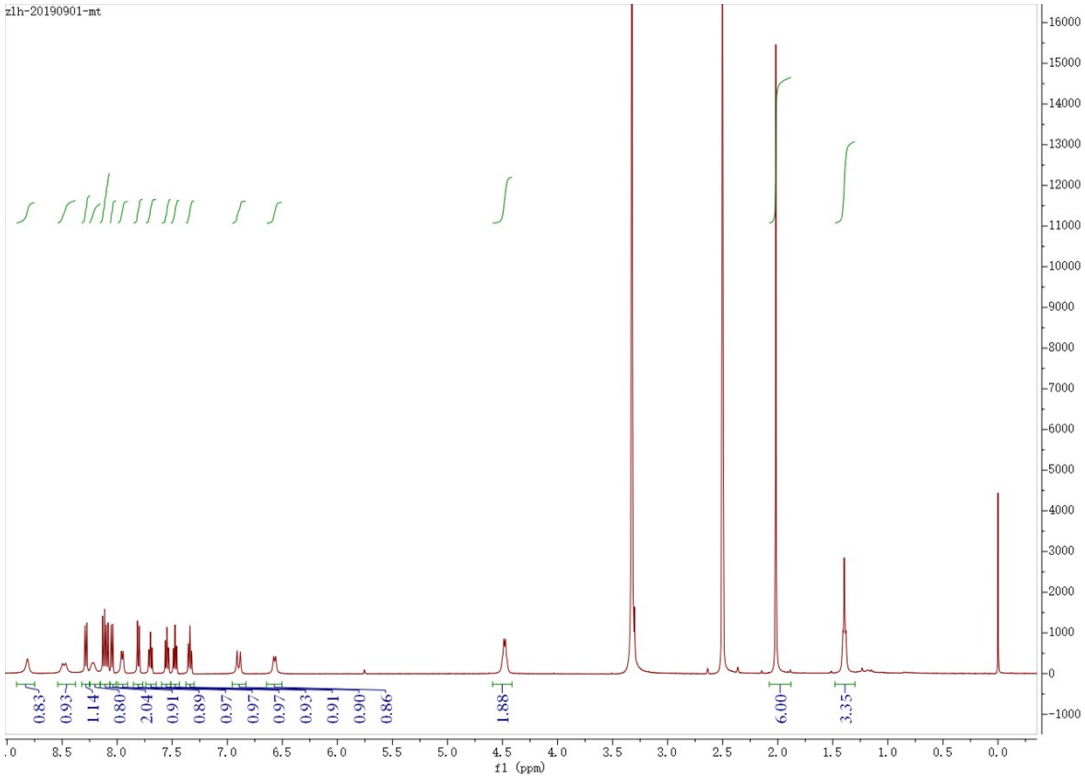
110 **Figure S2** MALDI-TOF-MS spectrum of BEB-A.



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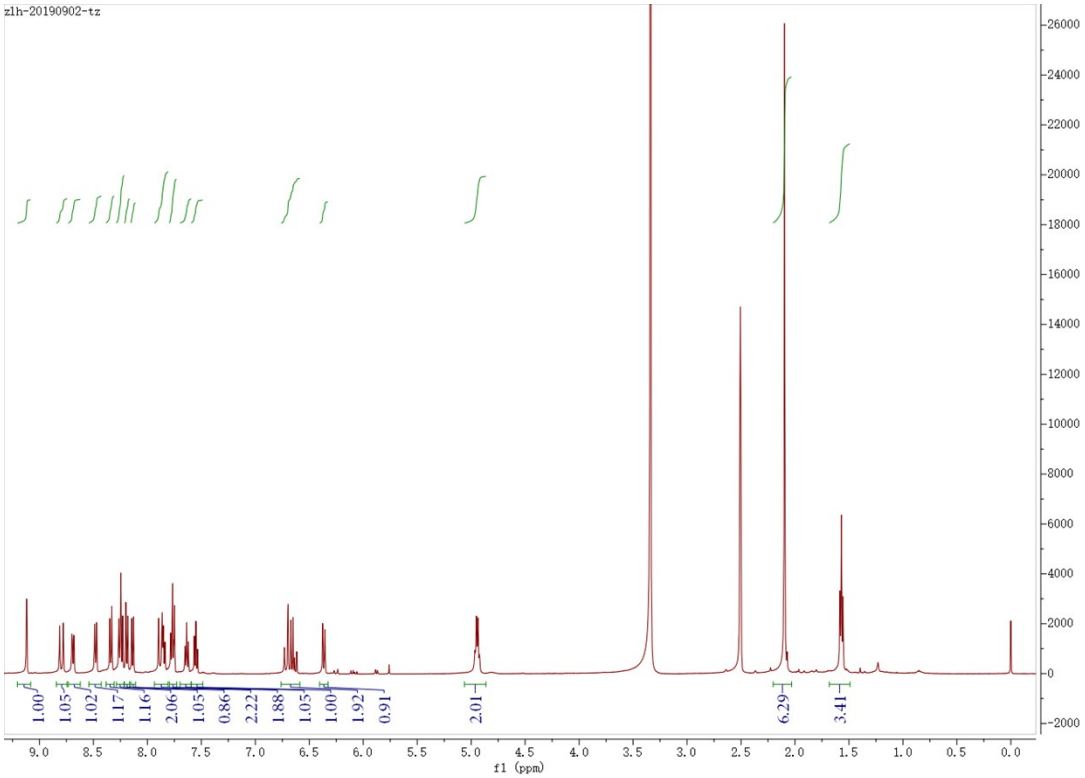
112 **Figure S3** [1]H-NMR spectrum of BEB-OH in DMSO-d_6.



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114 **Figure S4** [1]H-NMR spectrum of BEB-A in DMSO-d_6.

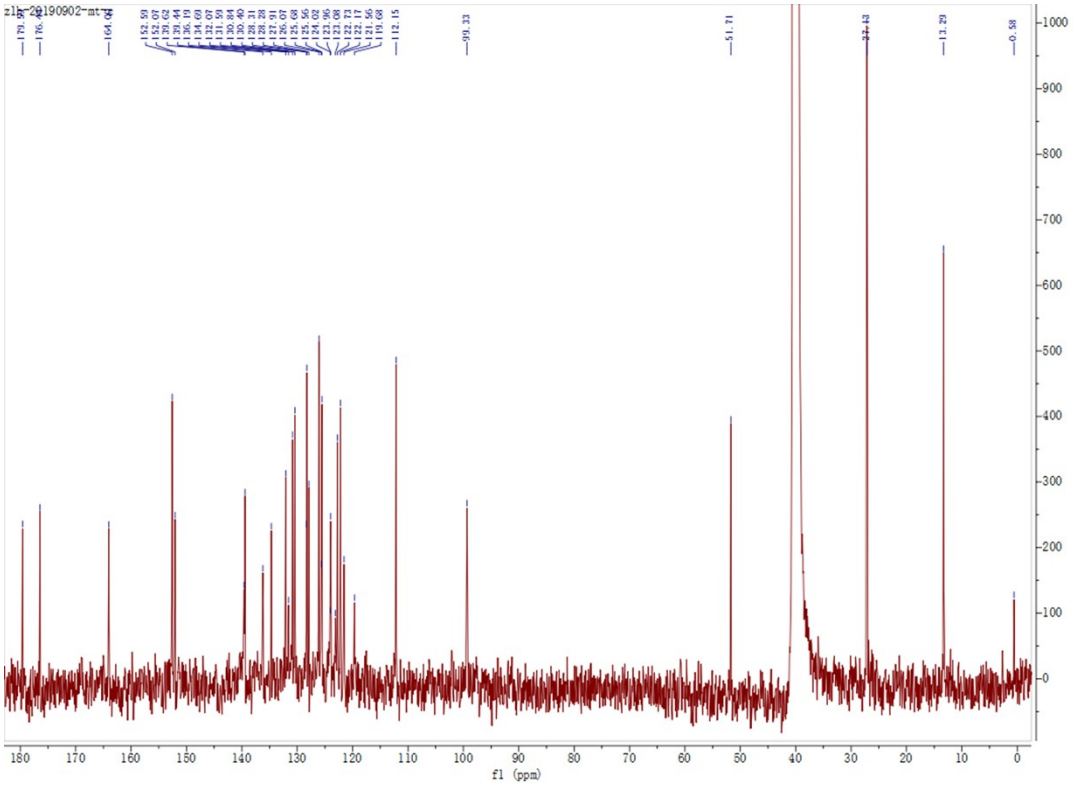


Figure S5 ^{13}C-NMR spectrum of BEB-OH in DMSO-d_6.

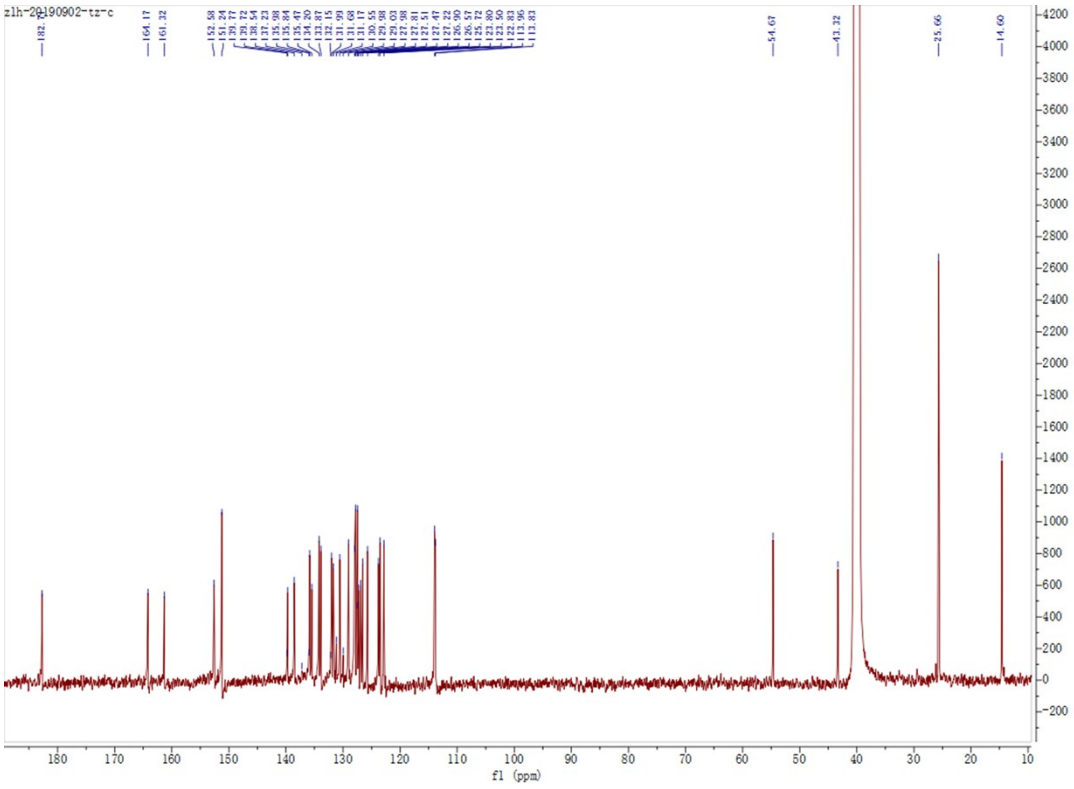
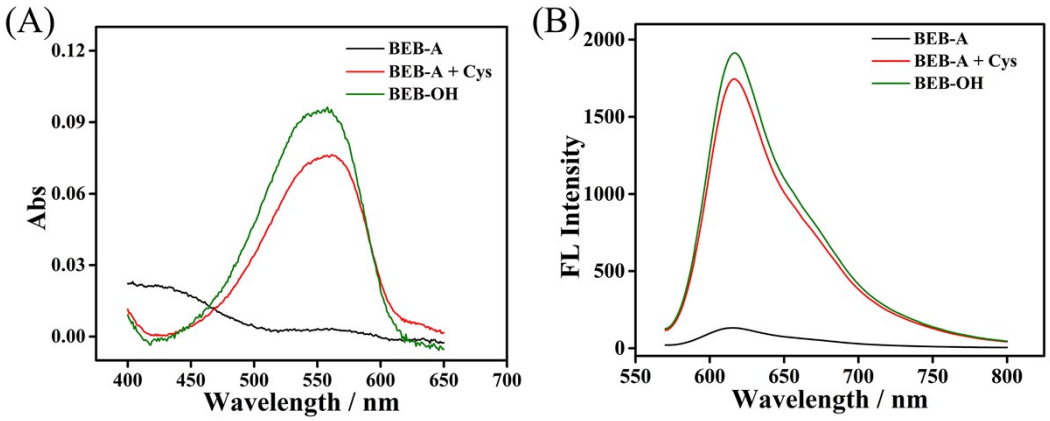


Figure S6 ^{13}C-NMR spectrum of BEB-A in DMSO-d_6.



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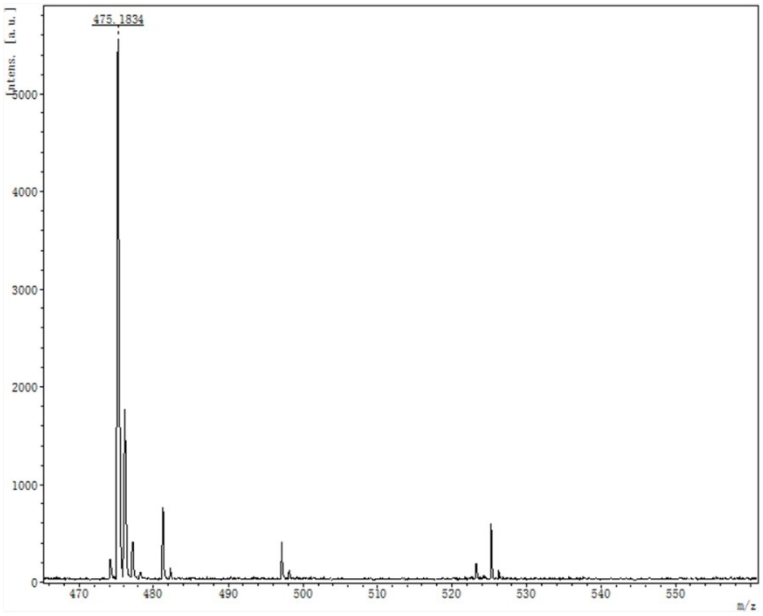
121 **Figure S7** (A) Absorption spectra of BEB-A (5 μM), BEB-A with Cys (10 μM) and BEB-OH

122 (5 μM) in PBS/CH_3CN (v/v=8:2, pH 7.4). (B) Fluorescence spectra of BEB-A (5 μM), BEB-A with

123 Cys (10 μM) and BEB-OH (5 μM) in PBS/CH_3CN (v/v=8:2, pH 7.4).



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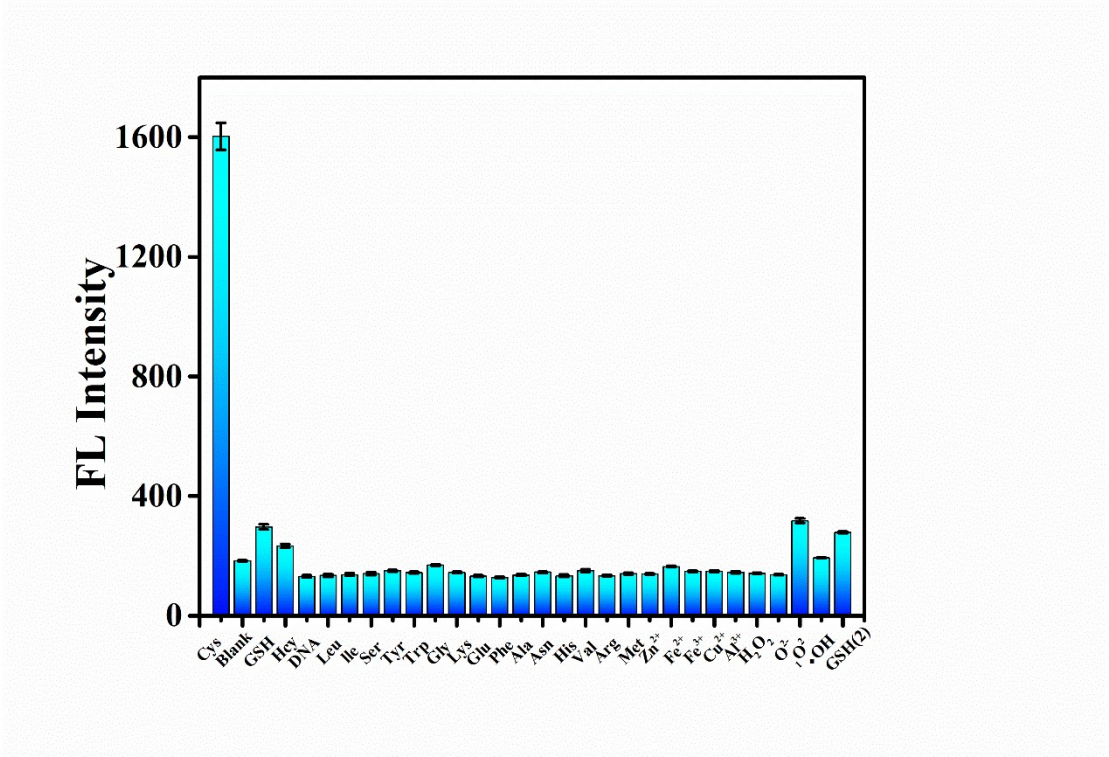
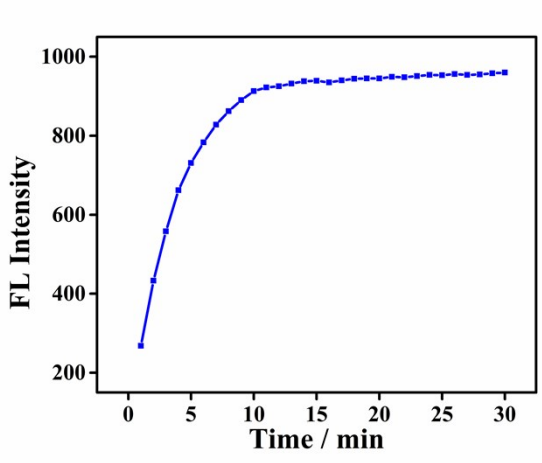
125 **Figure S8** MALDI-TOF-MS spectrum of BEB-A treated with Cys (30 μM).



126

127 **Figure S9** Fluorescence intensity of BEB-A (5 μM) in PBS/CH_3CN (v/v=8:2, pH 7.4) to
128 various analytes . Each spectra were recorded at 10 min after addition of the analytes (Cys(10 μM),
129 GSH(10 μM), Hcy(10 μM), Leu(10 μM), lle(10 μM), Ser(10 μM), Tyr(10 μM), Trp(10 μM), Gly(10
130 μM), Lys(10 μM), Glu(10 μM), Phe(10 μM), Ala(10 μM), Asn(10 μM), His(10 μM), Val(10 μM),
131 Arg(10 μM), Met(10 μM), Zn^{2+}(10 μM), Fe^{2+}(10 μM), Fe^{3+}(10 μM), Cu^{2+}(10 μM), Al^{3+}(10 μM),
132 H_2O_2(50 uM), O^{2-}(50 uM), $^1O_2$(50 uM),·OH(50 uM), GSH(2)(100uM)).



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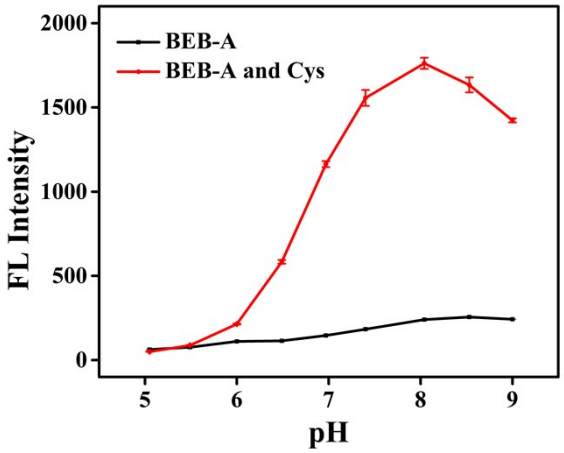
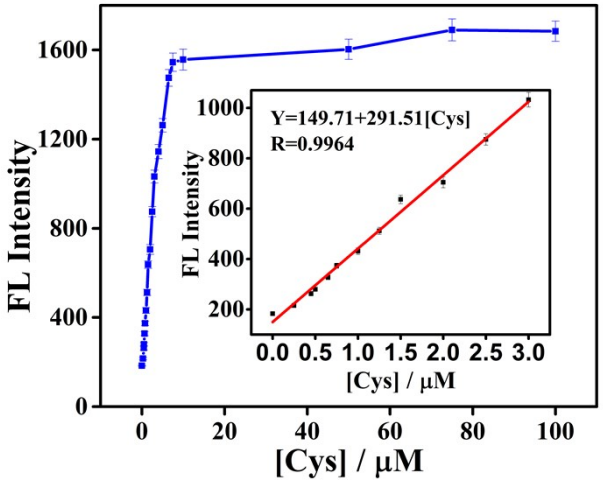
134 **Figure S10** Time-dependent fluorescence intensity changes of BEB-A upon addition of Cys
135 in PBS/CH_3CN (v/v=8:2, pH 7.4).



136

137 **Figure S11** Fluorescence intensity of BEB-A and BEB-A (5 μM) with Cys (10 μM) in 10

138 min at various pH values, respectively, λ_{ex} = 550 nm, λ_{em} = 616 nm in PBS/CH_3CN (v/v=8:2).



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140 **Figure S12** Fluorescence intensity of BEB-A (5 μM) the presences of various concentrations

141 of Cys in PBS/CH_3CN (v/v=8:2, pH 7.4).

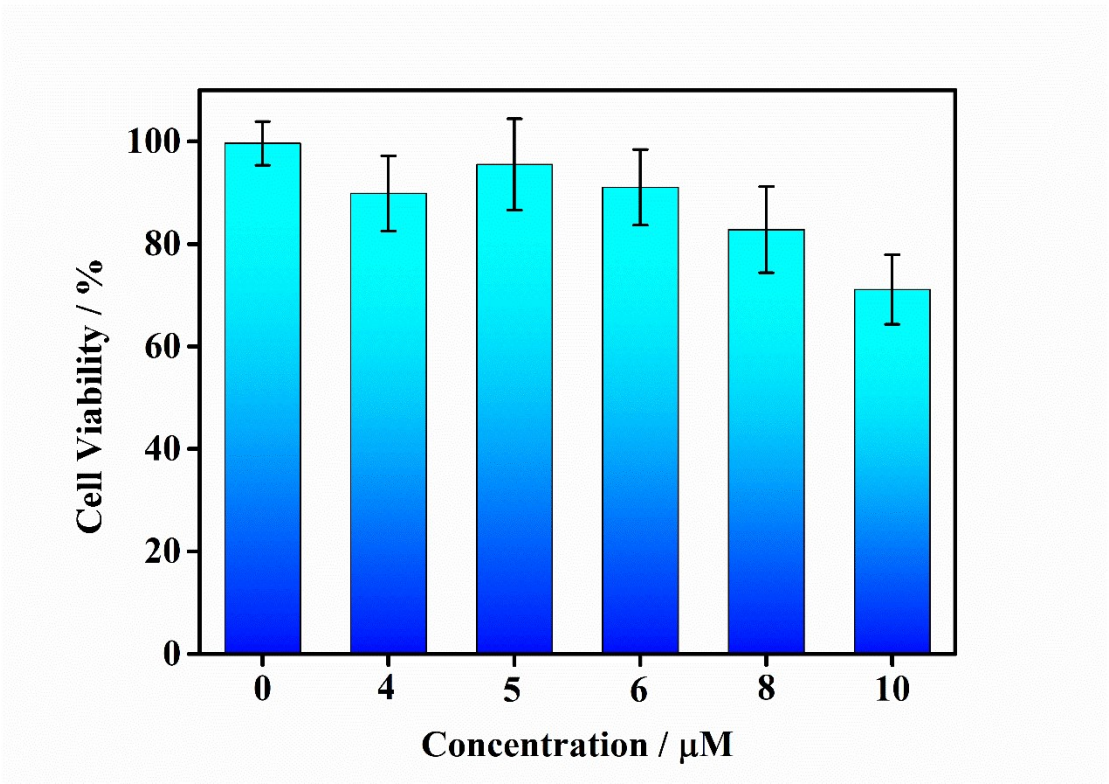
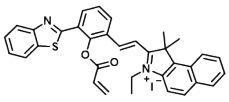
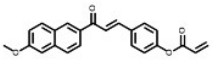
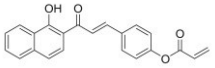
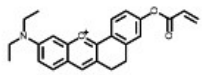
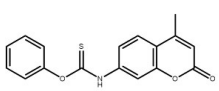
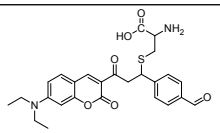
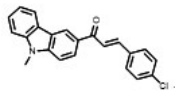
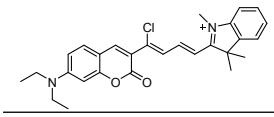


Figure S13 CCK8 assay for the survival rate of Hela cells treated with various concentrations of BEB-A for 24 h.

Tables

Table S1 Comparison of the present method with other reported Cys selective fluorescence probe.

Probe structure	Reaction medium	$\lambda_{ex}/\lambda_{em}$ (nm)	Time (min)	LOD (nM)	Apply	Ref.
	PBS/CH_3CN (*v*/*v*=8.5:1.5, pH 7.4)	550/616	10	27	Cys detection Nucleolus imaging	This work
	PBS, pH 7.4	325/440	10	22	Cys detection	5
	PBS/DMSO (*v*/*v*=1:1, pH 7.4)	425/620	3	91	Cys detection	6
	PBS/EtOH (*v*/*v*=1:1, pH 7.4)	570/615	15	120	Cys detection	7
	Tris-HCl, pH 7.4	340/443	15	160	Cys detection	8

	PBS/DMSO (*v*/*v*=1:1, pH 7.4)	463/514	2	460	Cys detection	9
	PBS/DMSO (*v*/*v*=4:6, pH 7.4)	342/470	10	1489	Cys detection	10
	PBS/MeOH (*v*/*v*=4:1, pH 7.4)	417/489	30	2965	Cys detection	11

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149 **Table S2** Quantitative analysis of Cys in human serum samples (n = 5).

Sample	Spiked (μM)	Recovered (μM)	Recovery (%)
A	2.00	1.94±0.07	96.9
	3.00	2.75±0.05	91.8
B	2.00	1.96±0.02	97.9
	3.00	2.67±0.05	89.1

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151 ## References

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