Supporting Information

Construction of a fluorescence turn-on probe for highly discriminating detection of cysteine

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1. Materials and instruments

All chemical reagents and solvents for synthesis were purchased from commercial suppliers and were used without further purification. 1H NMR and 13C NMR spectra were recorded on a Bruker AV-400 spectrometer with chemical shifts reported in ppm at room temperature. Mass spectra were measured on a HP 1100 LC-MS spectrometer.

UV-vis absorption spectra were recorded on a Varian Cary 100 spectrophotometer. Fluorescence spectra were measured with a Varian Cary Eclipse Fluorescence spectrophotometer. Spectral-grade solvents were used for measurements of UV-vis absorption and fluorescence. For absorption or fluorescence measurements, compounds were dissolved in CH3CN to obtain stock solutions (5.0 mM). These stock solutions were diluted with aqueous solutions to the desired

Cells culture and imaging. HeLa cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI-1640) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5/95 CO2/air incubator for 24 h. For fluorescence imaging, cells were incubated in glass bottom dishes for 24 h.

In a control experiment, the cells were treated with 500 μM N-methylmaleimide in culture media for 20 min in incubator at 37 °C, and then washed with D-Hanks. The cells were further incubated with 10 μM **HO-BODIPY-Cl** for 20 min, washed with D-Hanks. For imaging of Cys, HeLa cells pretreated with 500 μM N-methylmaleimide for 20 min, further incubated with 100 μM Cys for 20 min, then loaded with **HO-BODIPY-Cl** (10 μM) for 20 min. The samples were excited with 561 nm.

2. The fluorescence color changes.

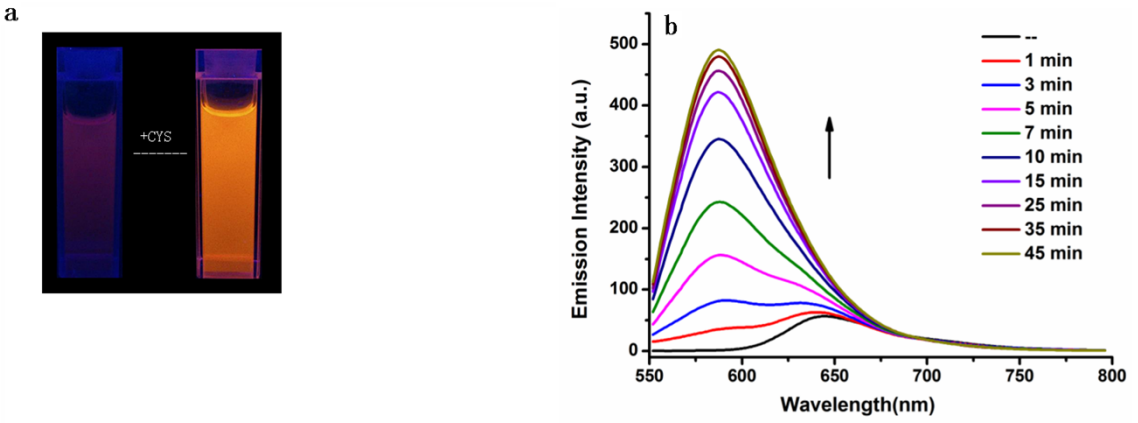


Figure S1. (a) The fluorescence color changes of **HO-BODIPY-Cl** in the absence and presence of Cys. (b) Time-dependent fluorescence spectra changes of **HO-BODIPY-Cl** (5μM) in the presence of 5 mM Cys in acetonitrile / HEPES buffer (1:1, v/v , 20 mM, pH 7.4) at 37°C.

3. Pseudo first-order kinetic plots.

Time-course kinetic measurements of **HO-BODIPY-Cl** with Cys were performed using the fluorescence intensity at 587 nm. Data were collected under pseudo-first-order conditions. Spectra were acquired in acetonitrile / HEPES buffer (1:1, v/v, 20 mM, pH 7.4) at 37 °C. The pseudo-first-order rate constant for the reaction was determined by fitting the fluorescence intensity changes of the samples to the pseudo first-order equation: $Ln((I_{max}-I_t)/I_{max})) = - k_{obs}\ t$

Where I_t and I_{max} represent the fluorescence intensities at times *t* and the maximum value obtained after the reaction was complete. k_{obs} is the observed rate constant.

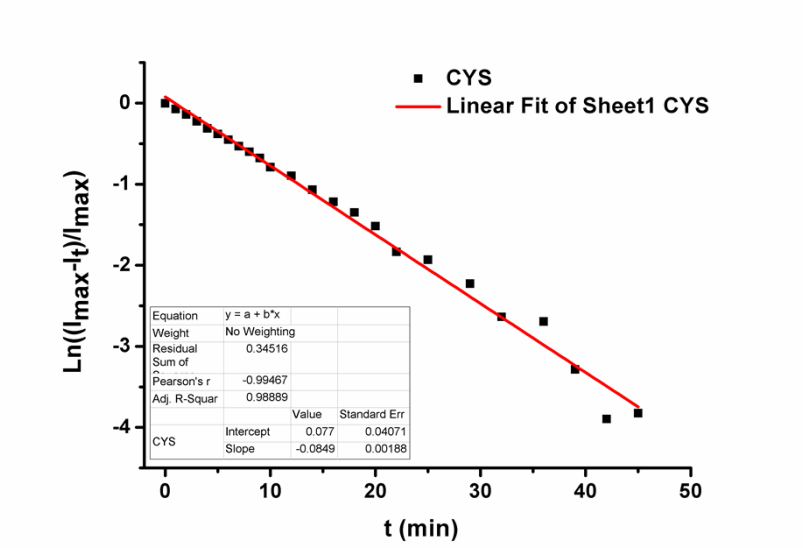
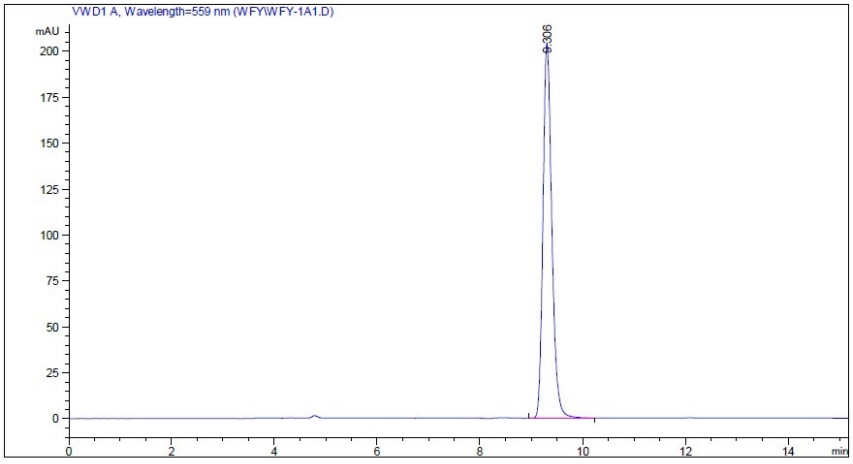


Figure S2. Pseudo first-order kinetic plots of the reaction of **HO-BODIPY-Cl** (5 μM) with Cys (5 mM).

4. HPLC and MS spectra of the products of HO-BODIPY-Cl with Cys, Hcy and GSH.



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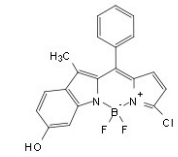

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
337 formula(e) evaluated with 35 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-20 H: 0-20 N: 0-5 O: 0-4 Cl: 0-1 F: 0-2 B: 0-1

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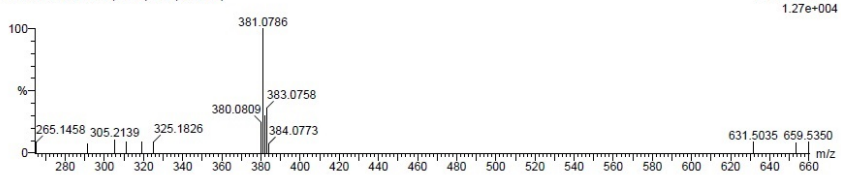

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Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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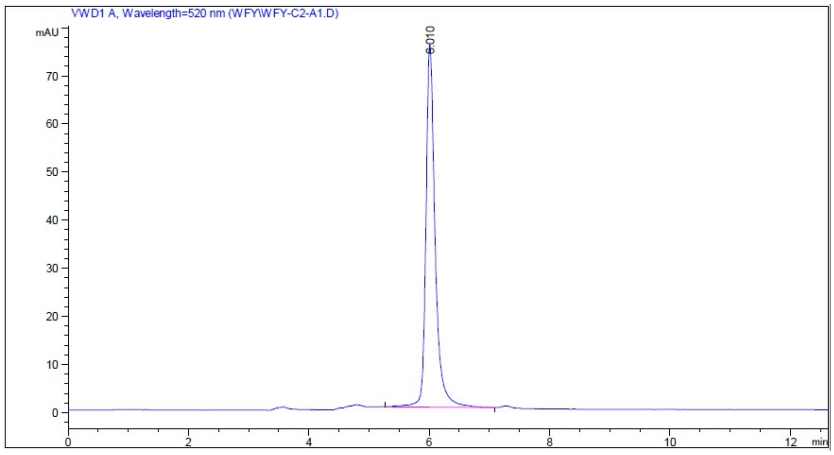

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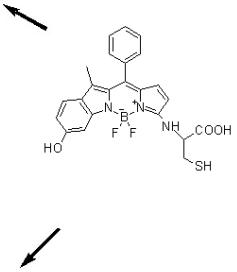

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Single Mass Analysis
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Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
200 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-23 H: 0-150 11B: 0-1 N: 0-3 O: 0-3 F: 0-2 S: 0-1

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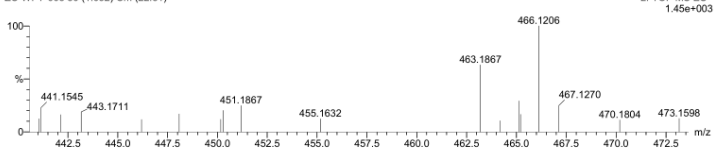

Minimum:				-1.5								
Maximum:		30.0	50.0	100.0								
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula					
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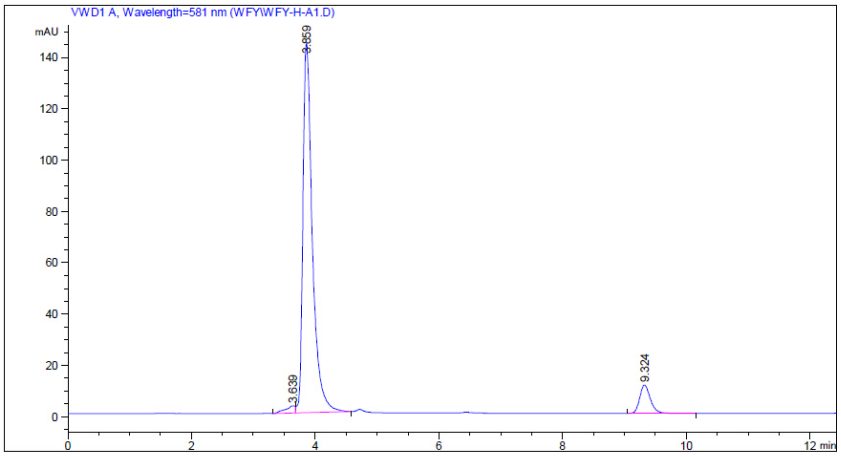

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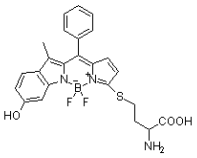

Elemental Composition Report

Single Mass Analysis

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Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
279 formula(e) evaluated with 37 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-31 H: 0-33 11B: 0-1 N: 0-3 O: 0-3 F: 0-2 S: 0-1

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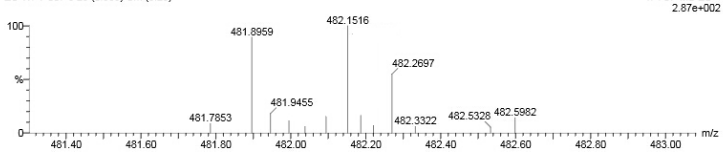
2.87e+002



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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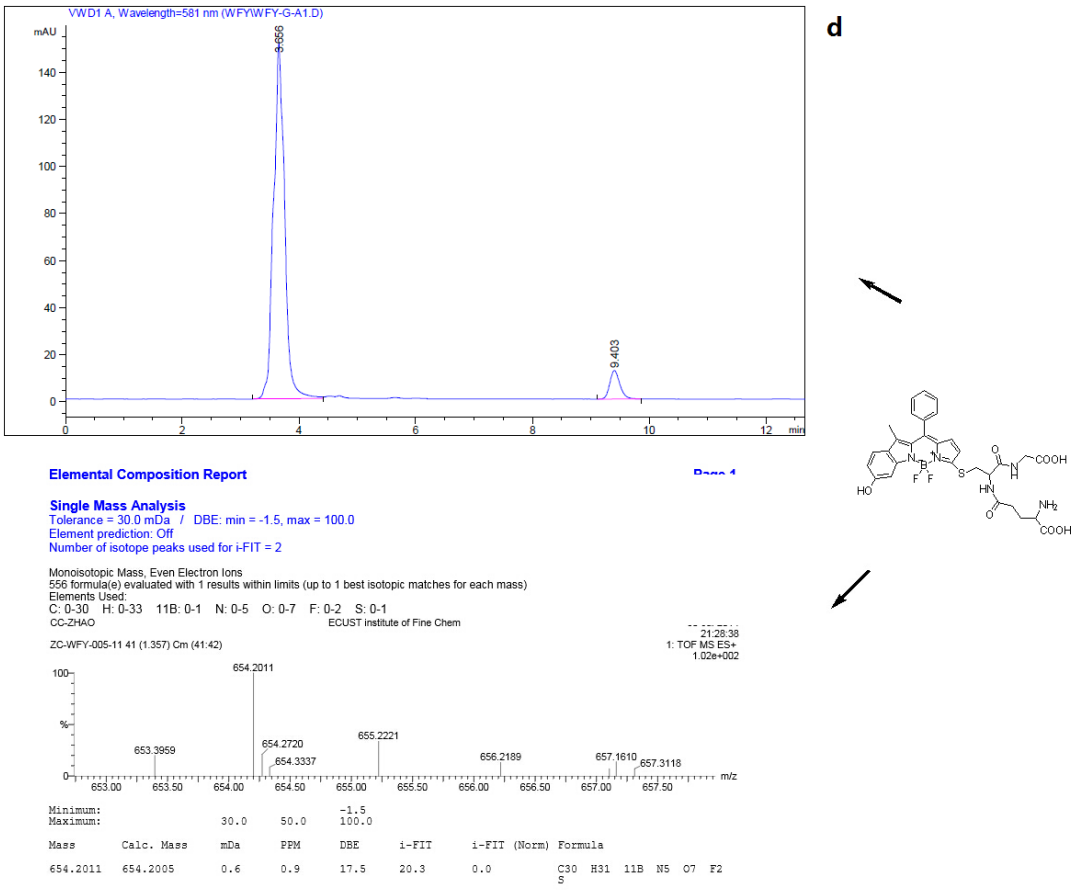


Figure S3. HRMS and HPLC spectra of (a) **HO-BODIPY-Cl**, (b) **HO-BODIPY-Cl** + Cys, (c) **HO-BODIPY-Cl** + Hcy and (d) **HO-BODIPY-Cl** + GSH.

5. The absorption and emission spectra of HO-BODIPY-Cl in the presence of N-acetyl-cysteine.

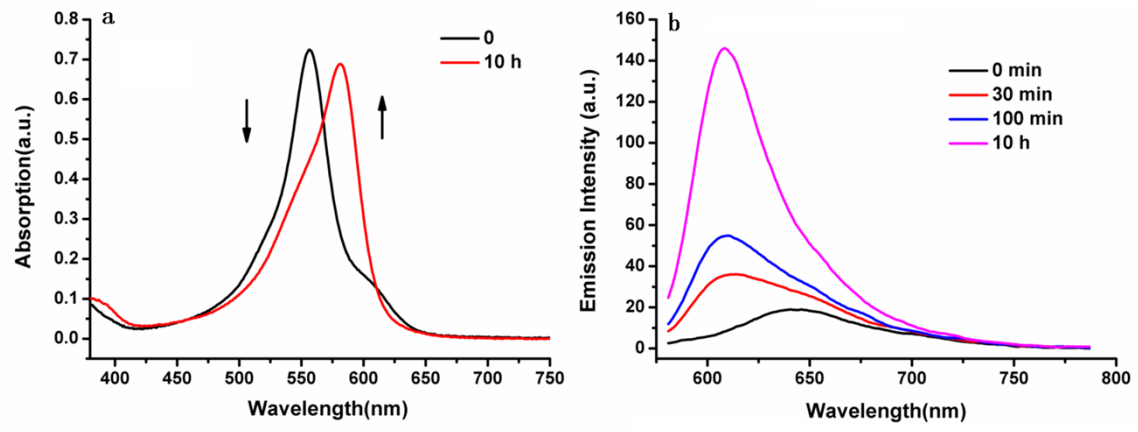


Figure S4. Changes of absorption and emission spectra of HO-BODIPY-Cl (5 μM) before and after the addition of N-acetyl-cysteine in acetonitrile/HEPES buffer(1:1, v/v, 20 mM, pH 7.4) at 37 °C (λex = 557 nm).

6. Time dependent fluorescence changes of HO-BODIPY-Cl upon incubation with CGL.

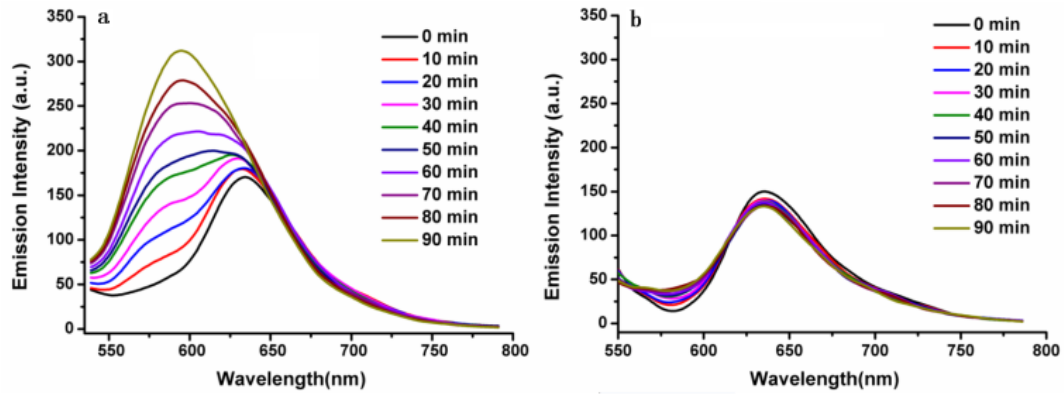
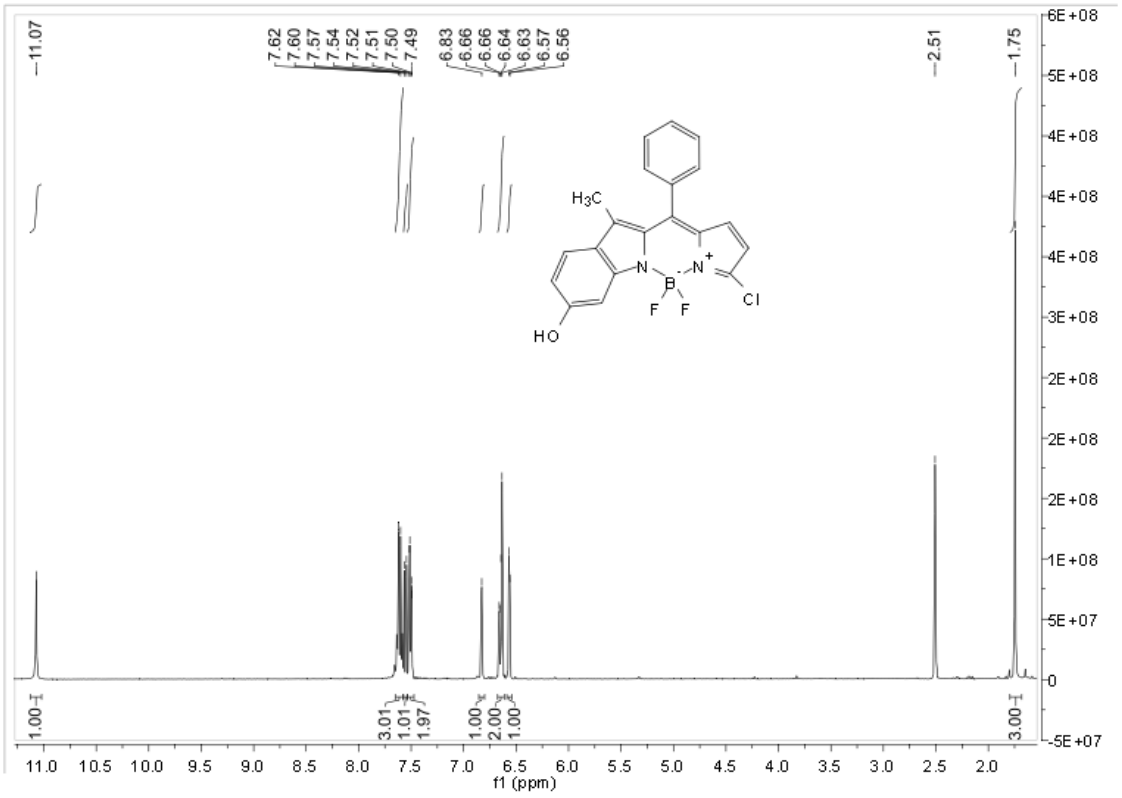
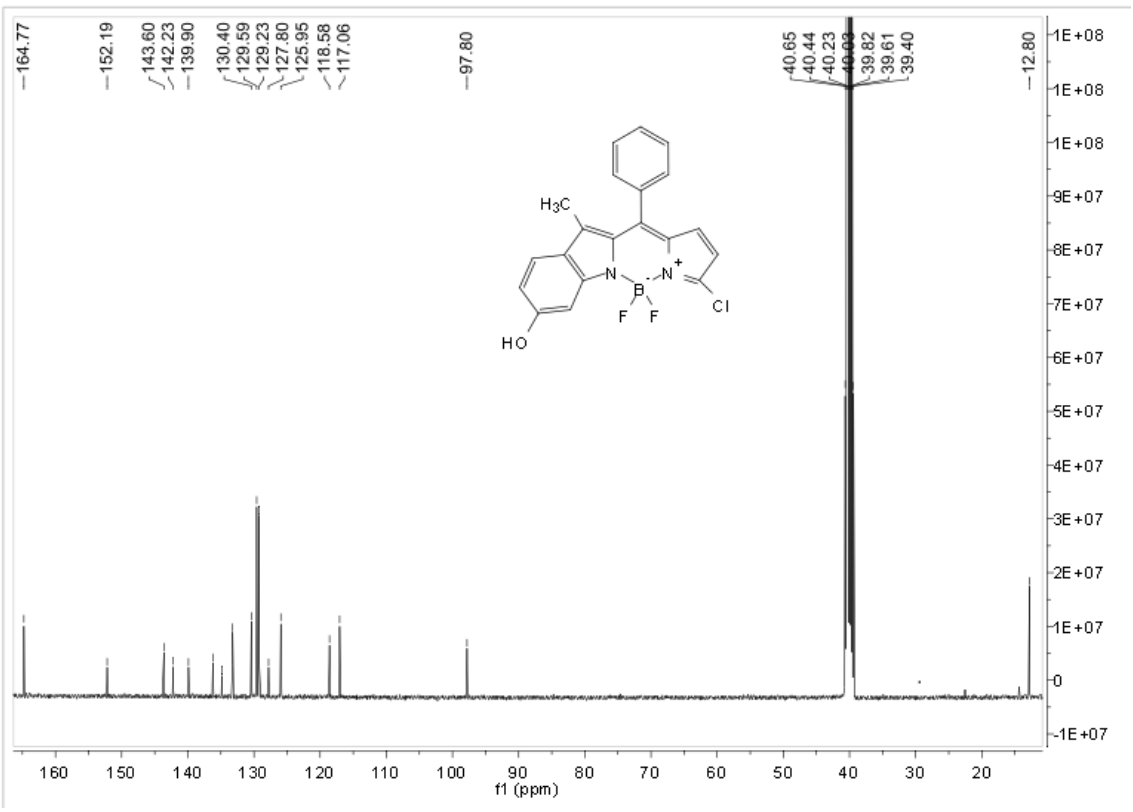


Figure S5. Time dependent fluorescence changes of **HO-BODIPY-Cl** (10 μM) upon incubation with CGL and its substrate cystathionine (3mM), (a) in the presence and (b) absence of PAG (CGL inhibitor).

7. NMR and HRMS Spectra





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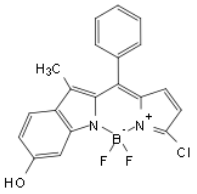

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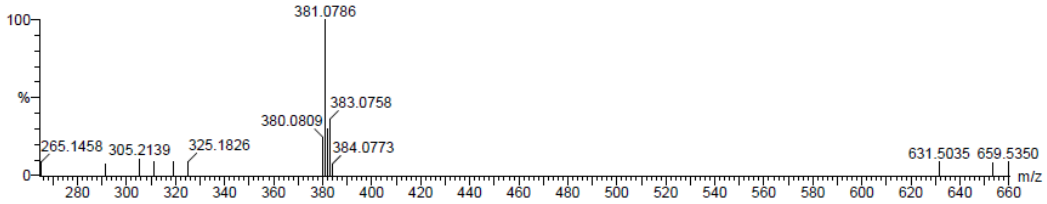

Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
381.0786	381.0778	0.8	2.1	14.5	24.1	0.0	C20 H13 N2 O Cl F2 B