Electronic Supplementary Information

New method for effectively and quantitatively labeling cysteine residues on chicken eggshell membrane

Xiaojing Wang,[a] Qian Li,[a] Yue Yuan,[a] Bin Mei,[a] Rui Huang,[a] Ying Tian,[b] Jing Sun,[b] Chunyan Cao,[a] Guang-Ming Lu[b] and Gaolin Liang*[a]

[a]CAS Key Laboratory of Soft Matter Chemistry, Department of Chemistry, University of Science and Technology of China, 96 Jinzhai Road, Hefei, Anhui 230026, China

[b]Laboratory of Laboratory of Molecular Pathology and Molecular Imaging, Department of Radiology, Nanjing Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu 210002, China

***Corresponding author:**

Gaolin Liang, Ph. D.,

Department of Chemistry

University of Science and Technology of China

96 Jinzhai Road, Hefei, Anhui 230026

P. R. China

Tel.: +86-551-3607935

Fax: +86-551-3600730

E-mail: gliang@ustc.edu.cn

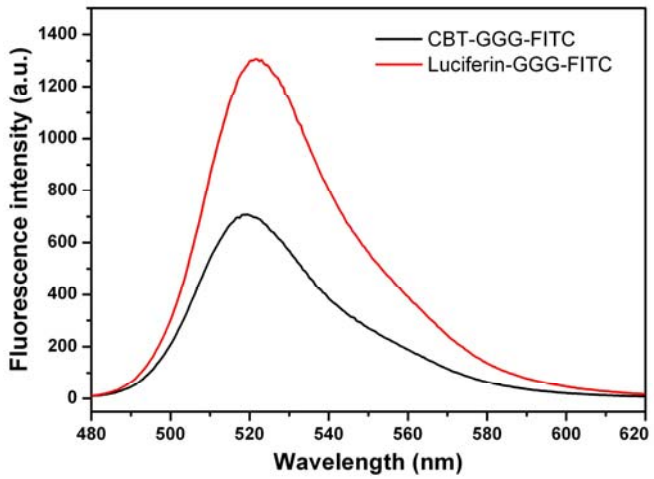


Figure S1. Fluorescent spectra of CBT-GGG-FITC (**2**, black) and Luciferin-GGG-FITC (**3**, red) at 15 μM in PBS (pH 7.4). Excitation: 465 nm.

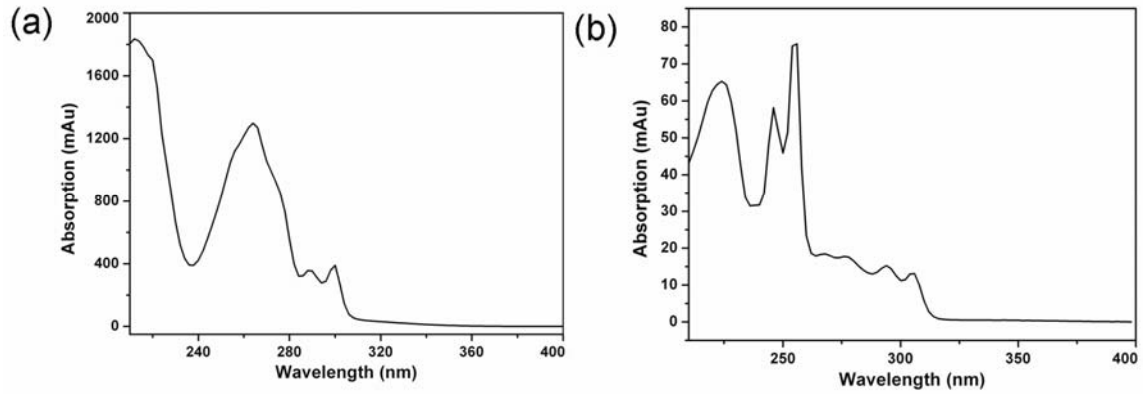


Figure S2. (a) The UV-Vis spectrum of the peak at 8 min in Fig. 2a. (d) The UV-Vis spectrum of the peak at 22 min in Fig. 2b.

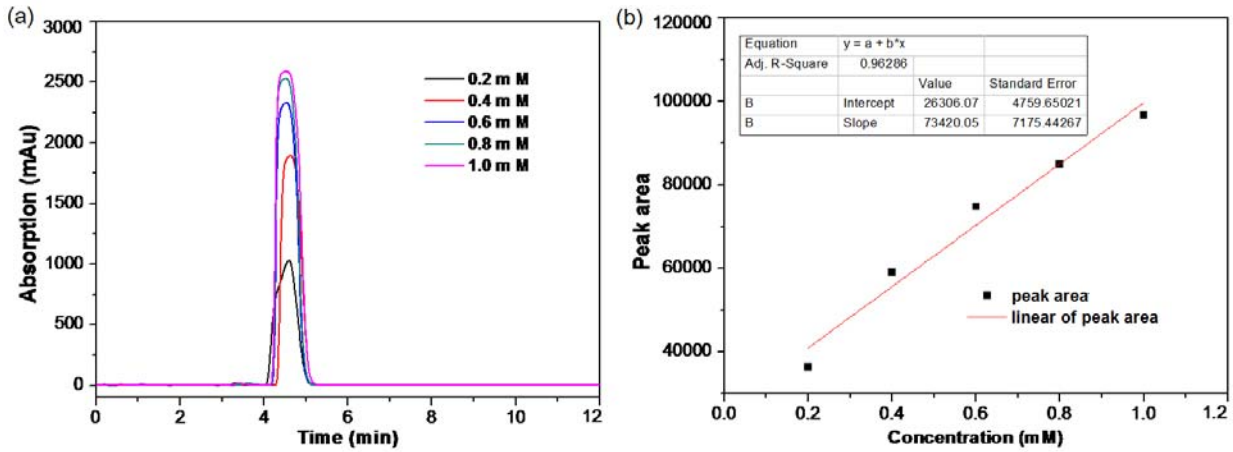


Figure S3. (a) HPLC traces of **1** at different concentrations. (b) Profiles of peak area and concentration of **1** in a and their linearity plot.

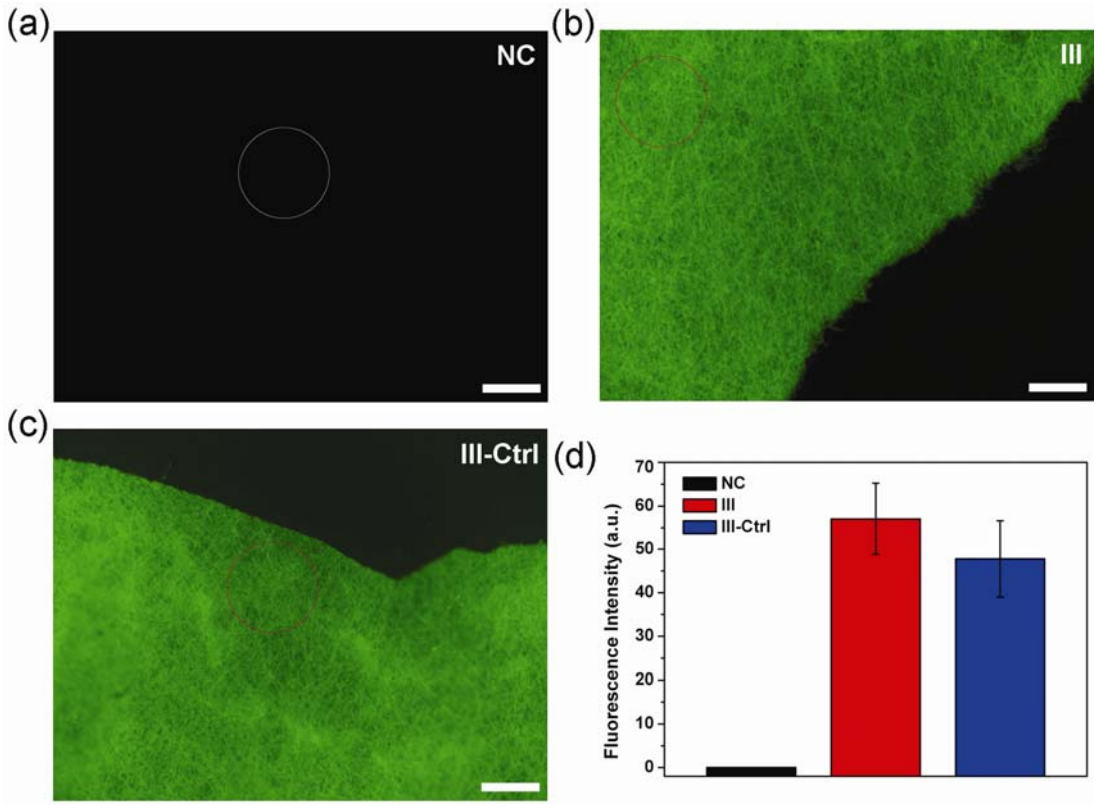


Figure S4. Microscopic fluorescence images of chicken ESMs. At step 5, chicken ESMs incubated with different concentrations of **2** for 6 h at RT after the sequential treatments from step 1 to step 4: (a) 0 mM (Group **NC**); (b) 4 mM (Group **III**). (c) Chicken ESMs incubated with 4 mM of **2** for 6 h

at RT at step 5 after the sequential treatments from step 1 to step 3 but without step 4 (Group **III-Ctrl**). Scale bar: 100 μm. Excitation: 465 nm, emission: EGFP channel. (e) Total fluorescence intensity of the region of interest (ROI, circular shape) in a-c.

Supplementary Table S1. HPLC condition for the purification of compound MMA-Cys(Fmoc)(StBu) (**1**).

Time (minute)	Flow (ml/min.)	H_2O %	CH_3OH %
0	7.0	30	70
3	7.0	30	70
35	7.0	0	100
37	7.0	0	100
38	7.0	30	70
40	7.0	30	70

Supplementary Table S2. HPLC condition for the analysis of compound CBT-GGG-FITC (**2**) and Luciferin-GGG-FITC (**3**)**.**

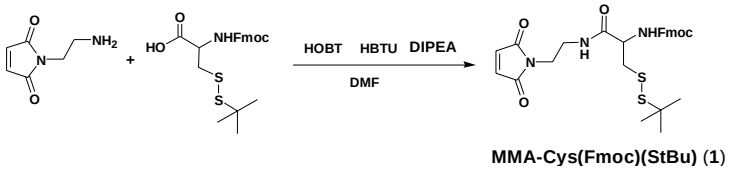
Time (minute)	Flow (ml/min.)	H_2O %	CH_3OH %
0	3.0	50	50
3	3.0	50	50
35	3.0	5	95
37	3.0	5	95
38	3.0	50	50
40	3.0	50	50

Supplementary Methods

2-cyano-6-aminobenzothiazole (CBT) was synthesized following the literature method (White, E. H., Worther, H., Seliger, H. H., McElroy, W. D. Amino analogs of firefly luciferin and biological activity thereof. J. Am. Chem. Soc. 1966, 88, 2015-2019).

*Preparation of MMA-Cys (Fmoc) (StBu) (**1**)*:

Scheme S1. Synthetic route for compound MMA- Cys(Fmoc)(StBu) (**1**) .



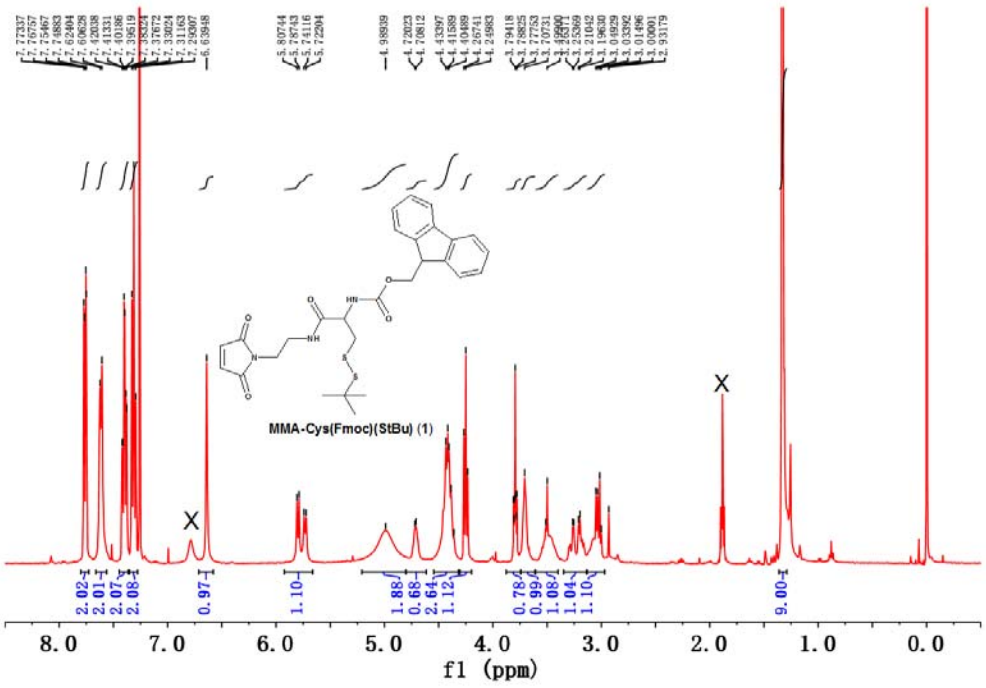


Figure S5. ^{1}H NMR spectrum of compound MMA-Cys (Fmoc) (StBu) (**1**).

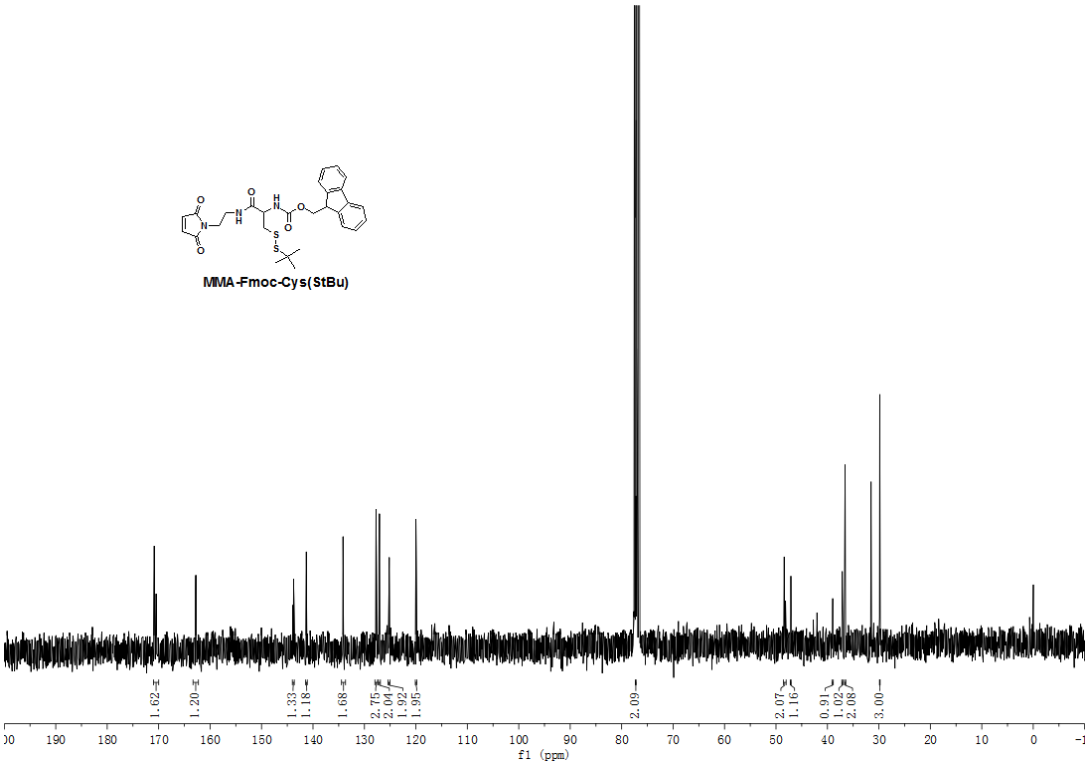


Figure S6. ^{13}C NMR spectrum of compound MMA-Cys (Fmoc) (StBu) (**1**).

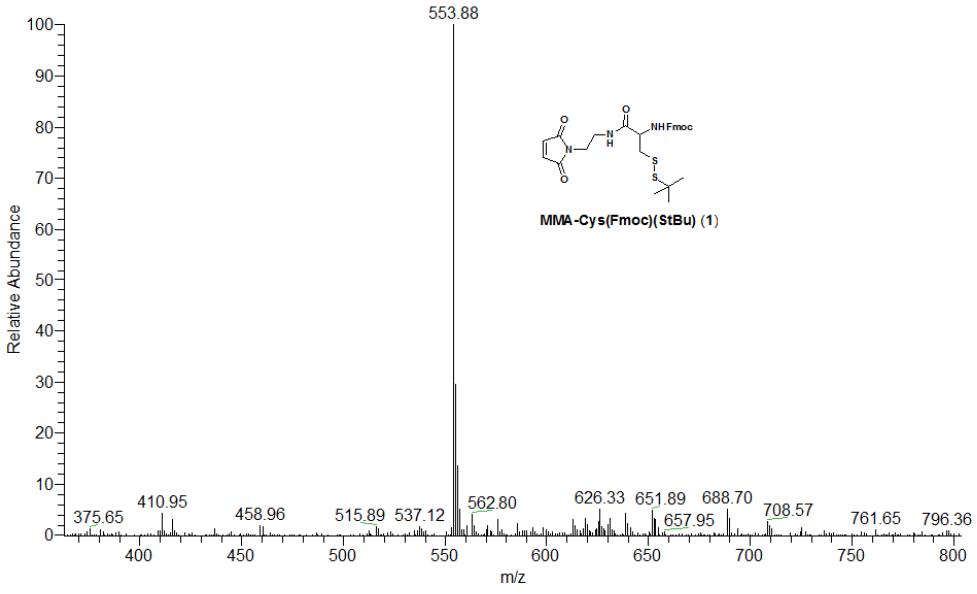
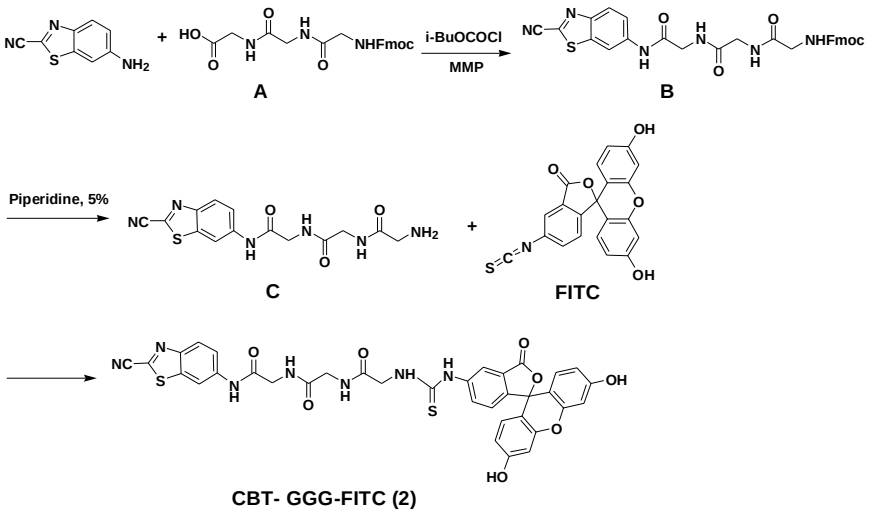


Figure S7. ESI-MS spectrum of compound MMA-Cys (Fmoc) (StBu) (**1**).

Scheme S2. Synthetic route of CBT-GGG-FITC (**2**).



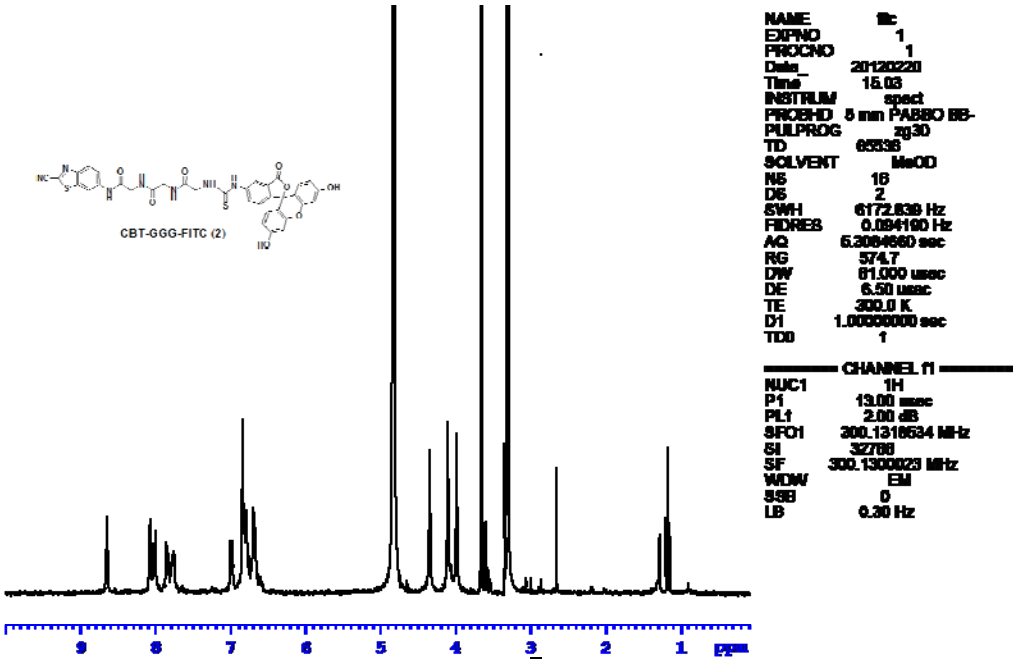


Figure S8. [1]HNMR spectrum of compound CBT-GGG-FITC (**2**).

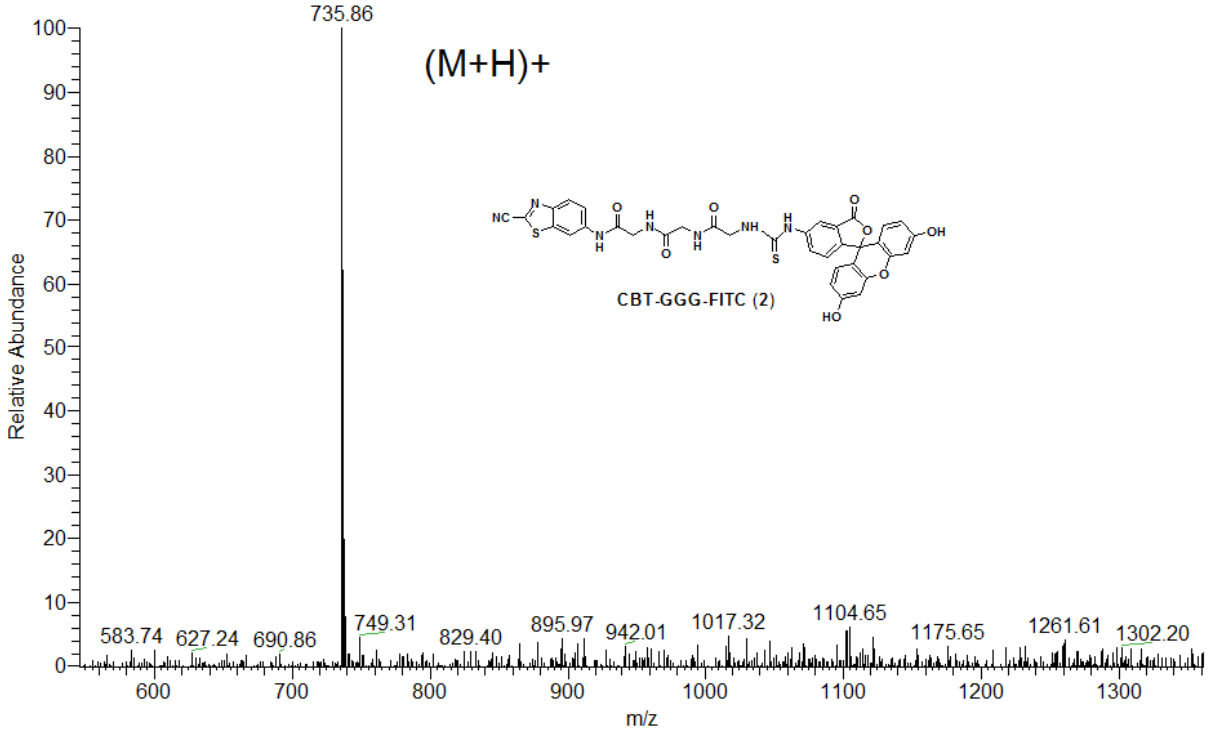
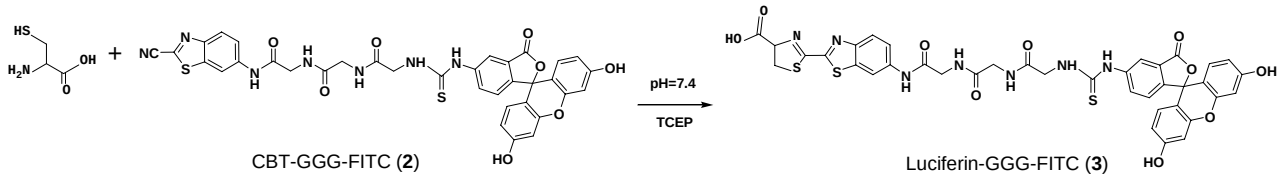


Figure S9. ESI-MS spectrum of compound CBT-GGG-FITC (**2**).

Scheme S3. Synthetic route for Luciferin-GGG-FITC (**3**).



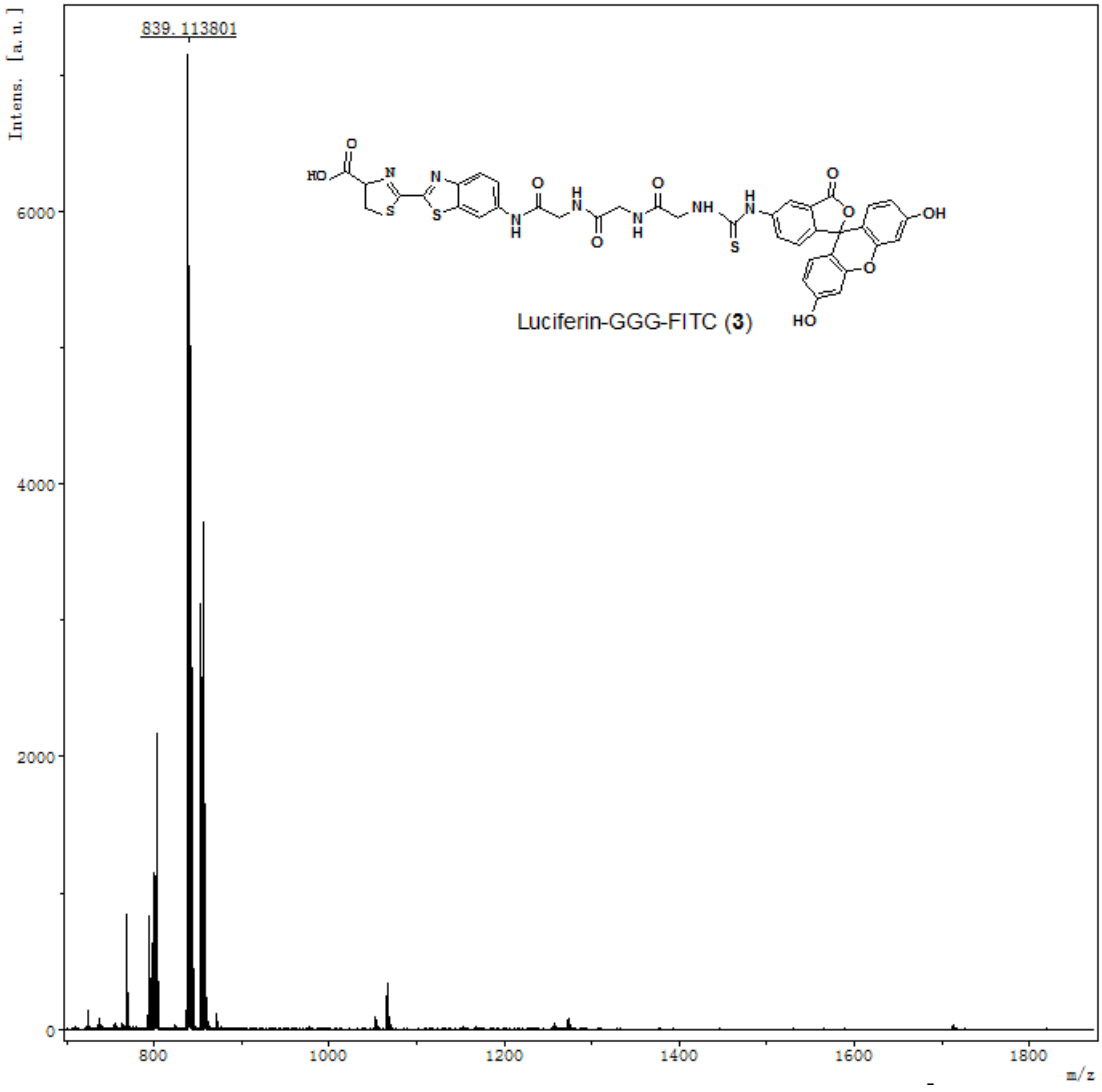


Figure S10. High resolution MALDI-Mass spectrum of Luciferin-GGG-FITC (**3**).