Environment-sensitive fluorescent supramolecular nanofibers for imaging applications

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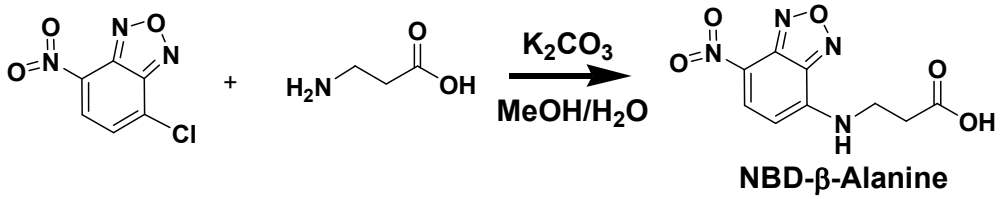
Experimental supporting information

Preparation of NBD-β-Alanine:

The preparation of NBD-β-Alanine was according to Scheme S-1. To a 15 mL water solution of 980 mg of β-Alanine (1.1 equiv.) and 4.14 g of K_2CO_3 (3 equiv.), 2 g of NBD-Cl (1 equiv., 10 mmol) in 20 mL of MeOH was added dropwisely in the N_2 atmosphere (low yields if without nitrogen protection). The reaction mixture was stirred at room temperature for about 3h (LC-MS detection). After the 3h reaction, MeOH was removed by a rotary evaporator. The obtained aqueous solution was acidified to around pH 3 by conc. HCl. The acidic aqueous solution was then extracted by ether for 3 times. The combined organic solution was dried over $MgSO_4$ and then concentrated by a rotary evaporator. The resulting yellowish solid (NBD-β-Alanine) was directly used for solid phase peptide synthesis.

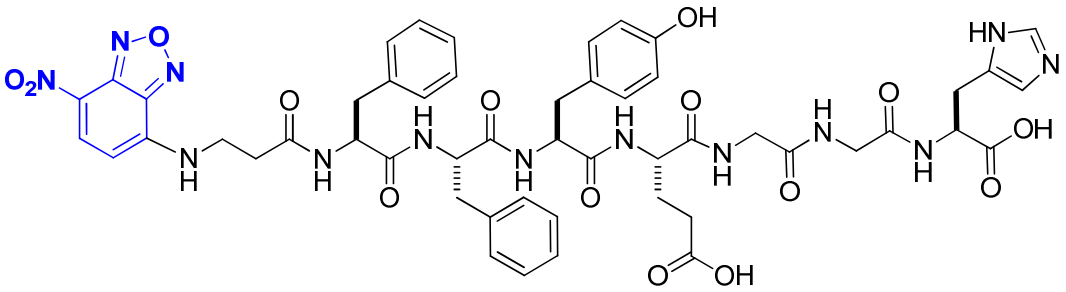


Scheme S-1. The synthetic route for NBD-β-Alanine

Peptide synthesis: The peptide derivative was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group or Pbf group. 20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used during deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. In the last coupling step, NBD-β-Alanine was used to produce NBD-peptides. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 mL per gram of resin), followed by five steps of washing using DCM for 2 min (5 mL per gram of resin). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of trimethylsilane

(TMS) and 2.5% of H_2O for 30 minutes. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was centrifuged for 10 min at 4 ^{0}C at 10,000 rpm. Afterward the supernatant was decanted and the resulting solid was dissolved in DMSO for HPLC separation using MeOH and H_2O containing 0.05% of TFA as eluents.

Characterization of the peptides



***Compound1:*NBD-FFYEGGH**

Scheme S-2. Chemical structure of NBD-FFYEGGH

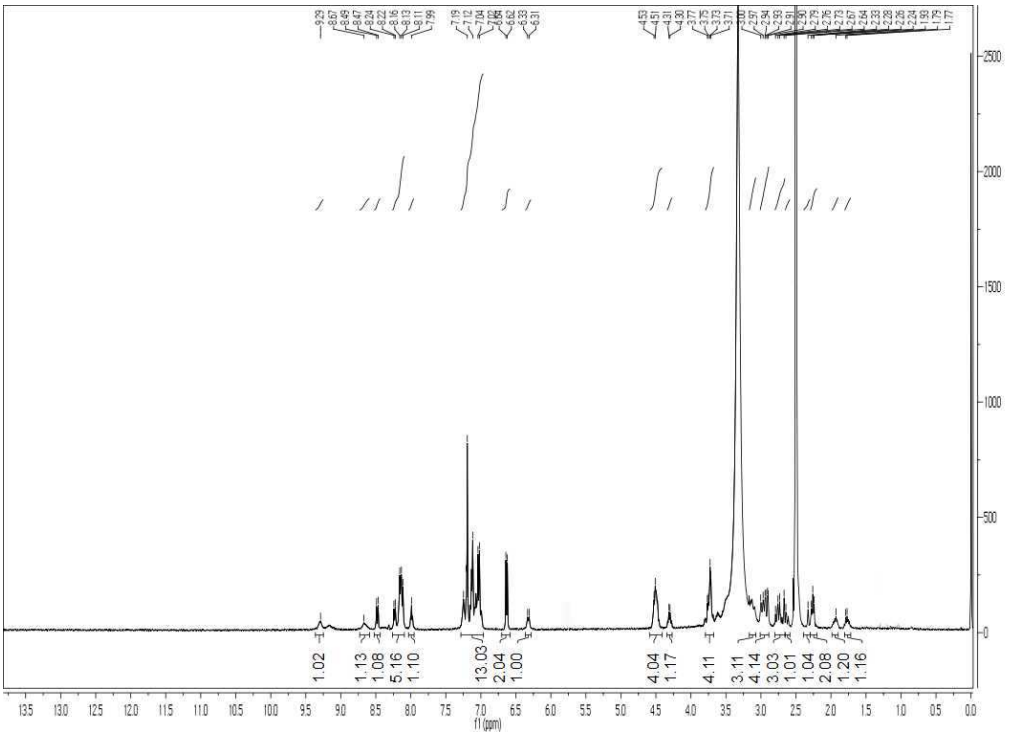


Figure S-1.[1]H-NMR of NBD-FFYEGGH

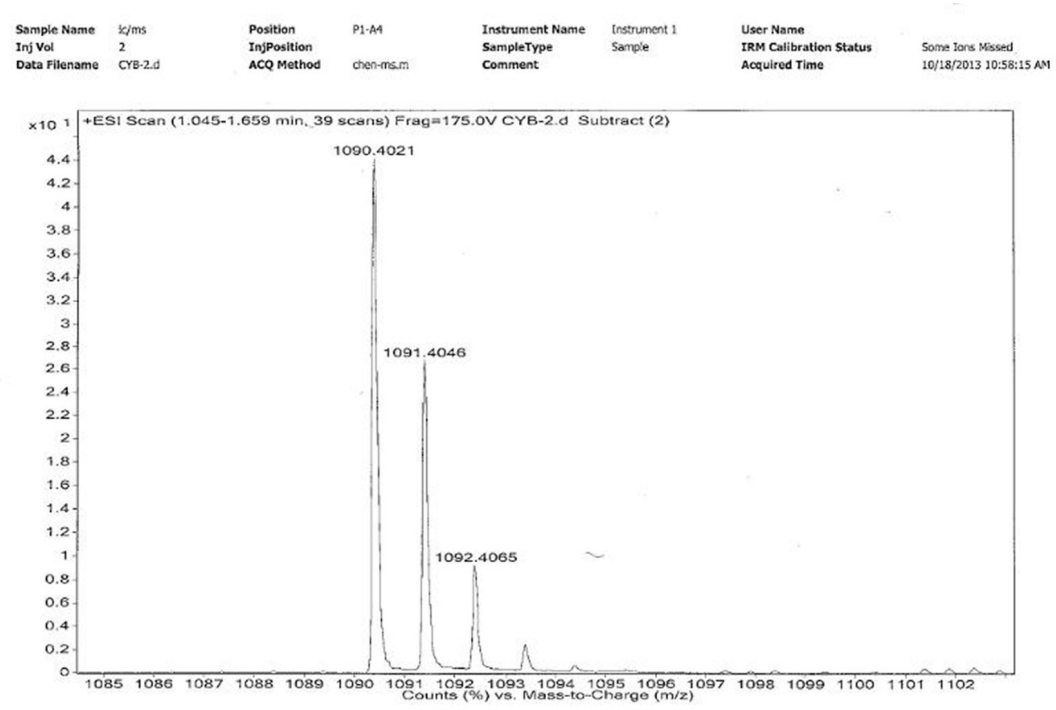
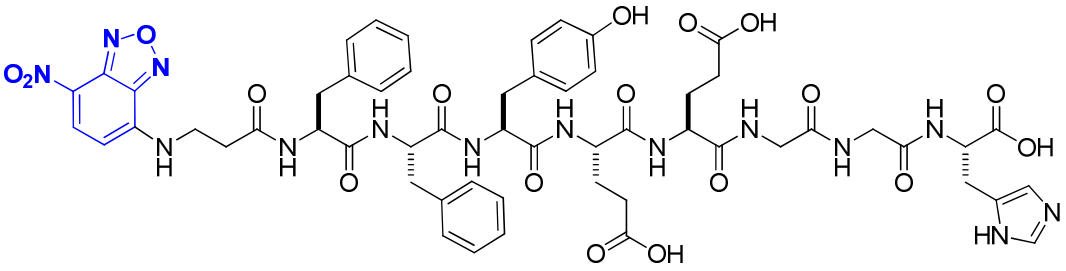


Figure S-2.HR-MS of NBD-FFYEGGH



*Compound 2:*NBD-FFYEEGGH

Scheme S-3. Chemical structure of NBD-FFYEEGGH

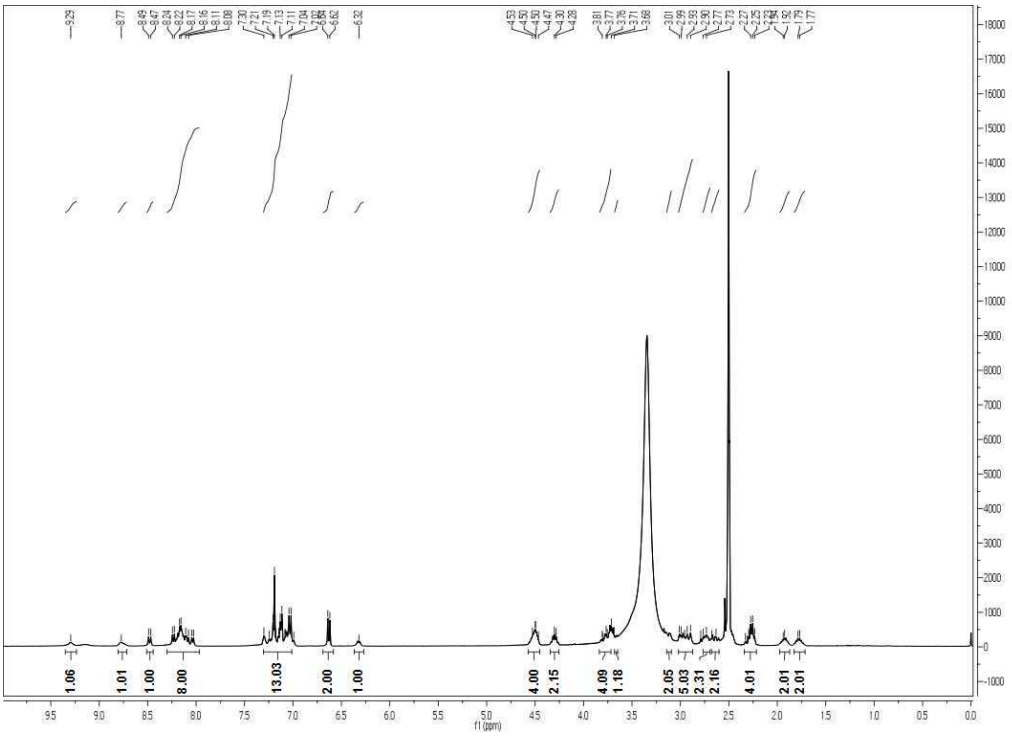


Figure S-3. [1]H-NMR of NBD-FFYEEGGH

Sample Name	lq/ms	**Position**	P1-A2	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	3	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Some Ions Missed
Data Filename	NBD-B-AFFYEEGGH.d	**ACQ Method**	chen-ms.m	**Comment**		**Acquired Time**	7/4/2013 10:03:10 AM

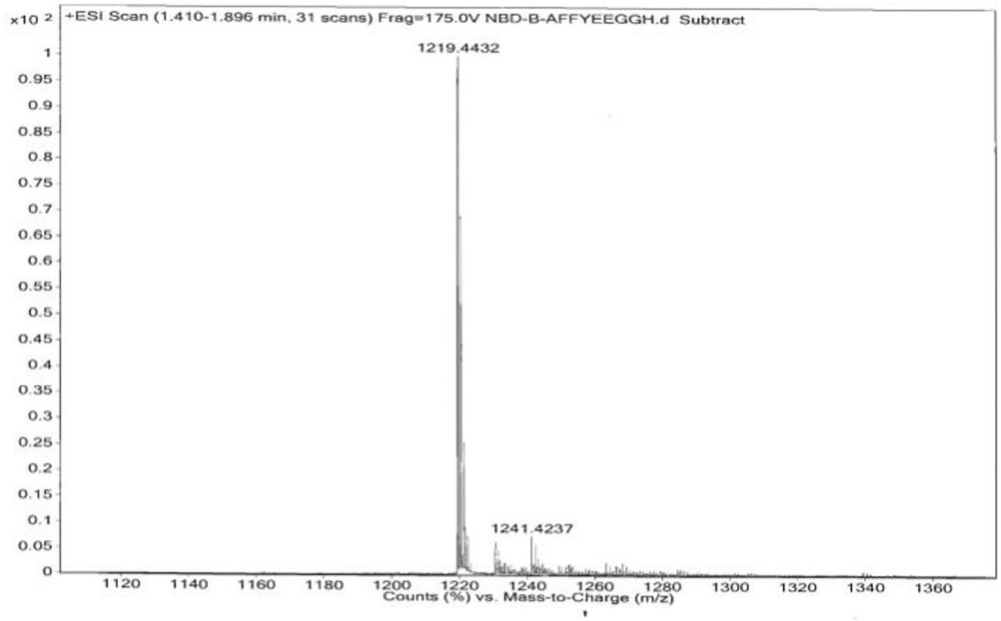
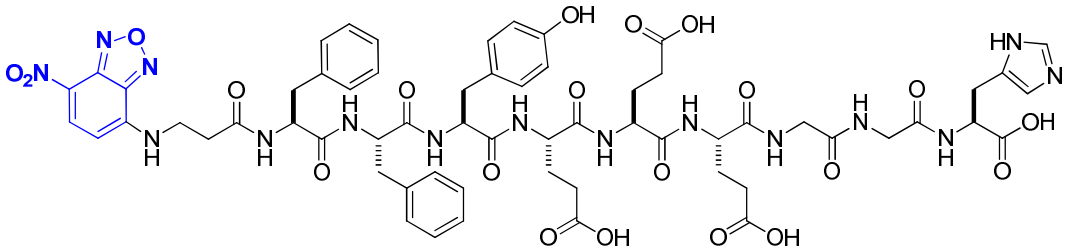


Figure S-4. HR-MS of NBD-FFYEEGGH



***Compound3:*NBD-FFYEEEGGH**

Scheme S-4. Chemical structure of NBD-FFYEEEGGH

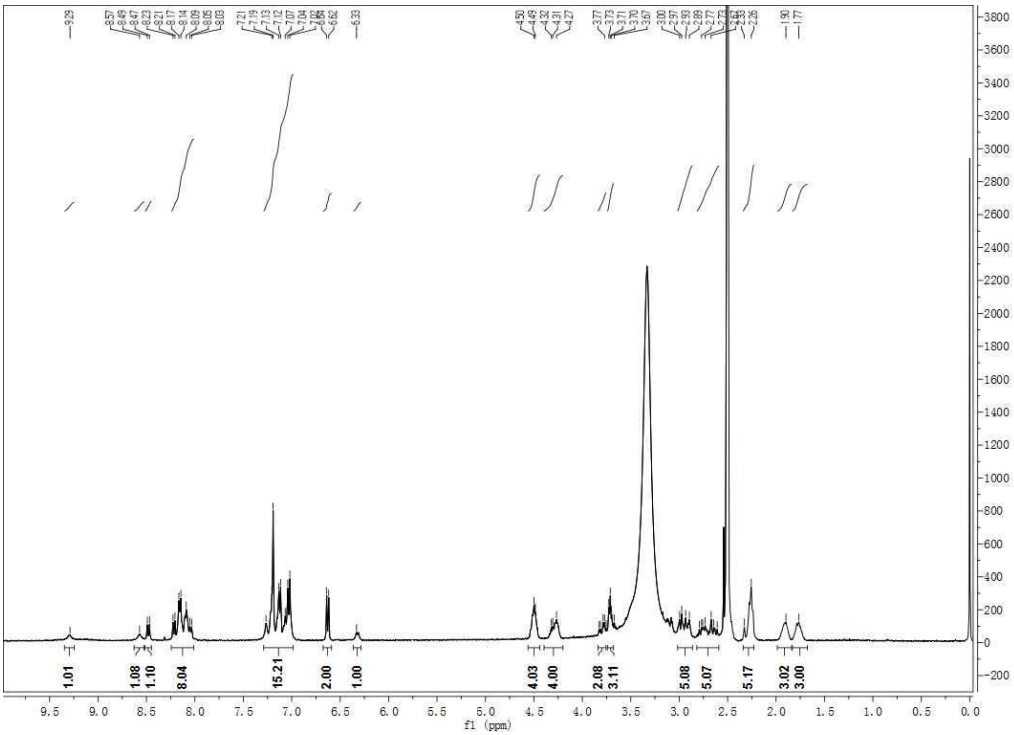


Figure S-5.^{1}H-NMR of NBD-FFYEEEGGH

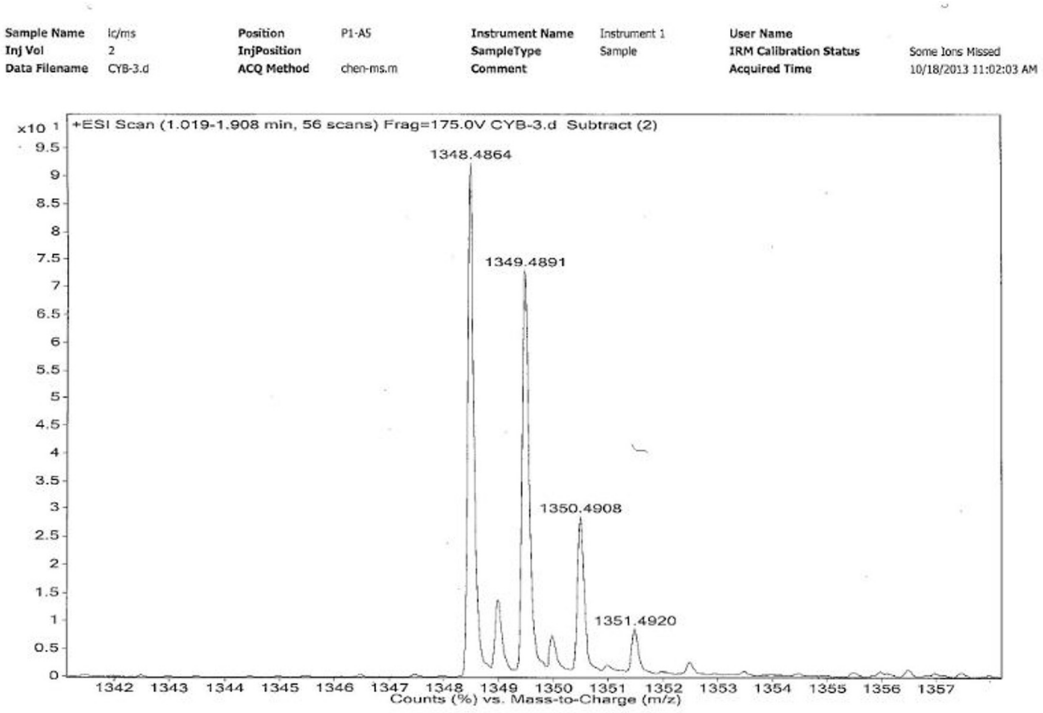
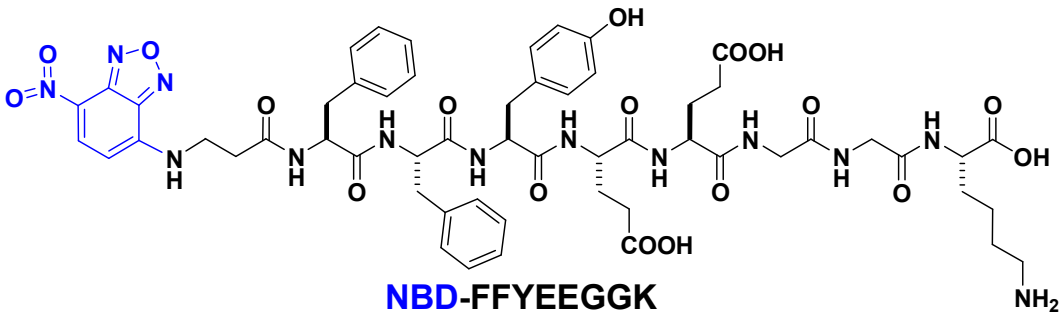


Figure S-6.HR-MS of NBD-FFYEEEGGH



Scheme S-5. Chemical structure of NBD-FFYEEGGK

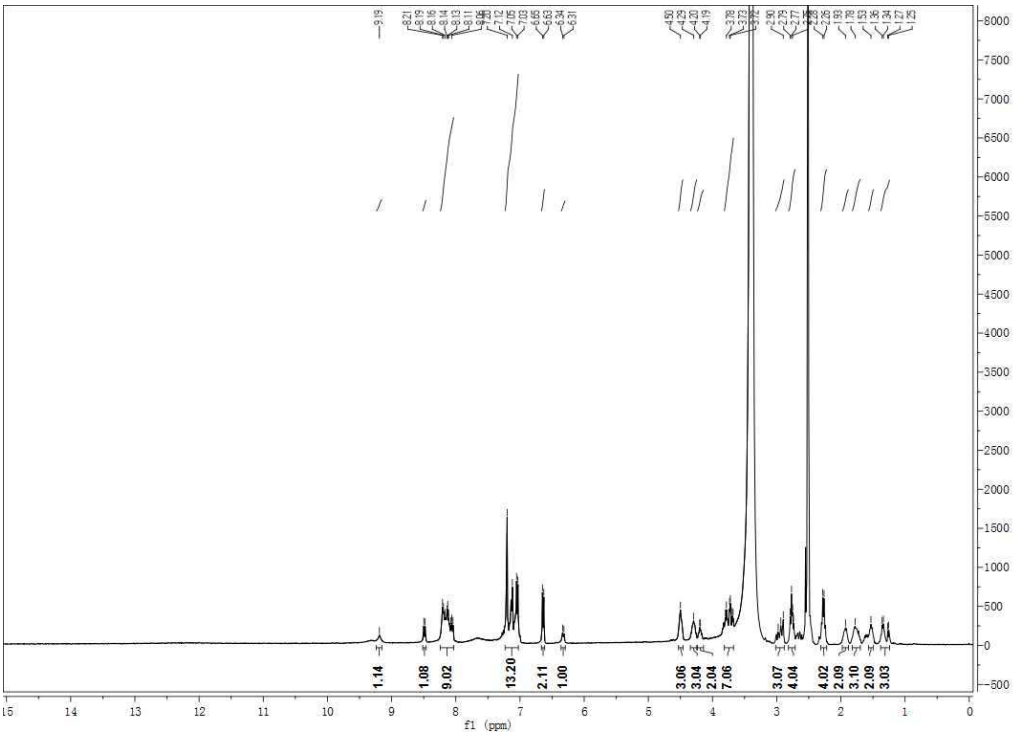


Figure S-7. ^{1}H-NMR of NBD-FFYEEGGK

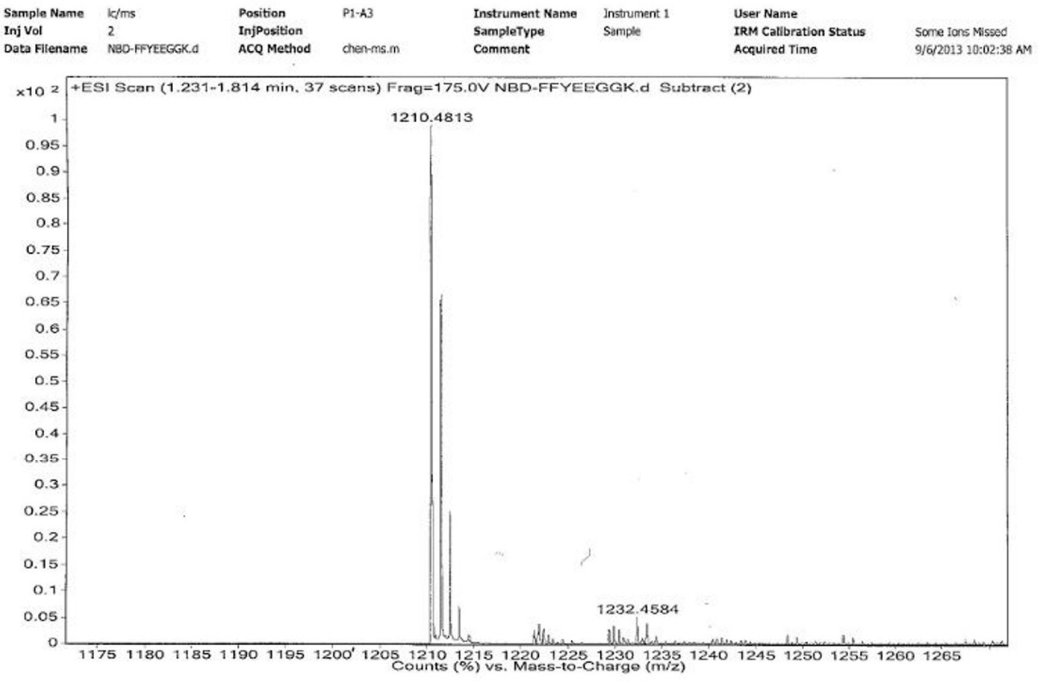
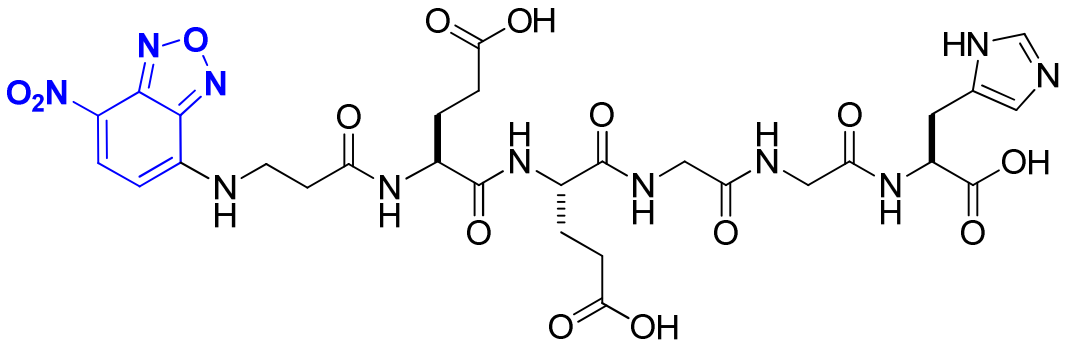


Figure S-8. HR-MS of NBD-FFYEEGGK



***Compound 4* :NBD-EEGGH**

Scheme S-6. Chemical structure of NBD-EEGGH

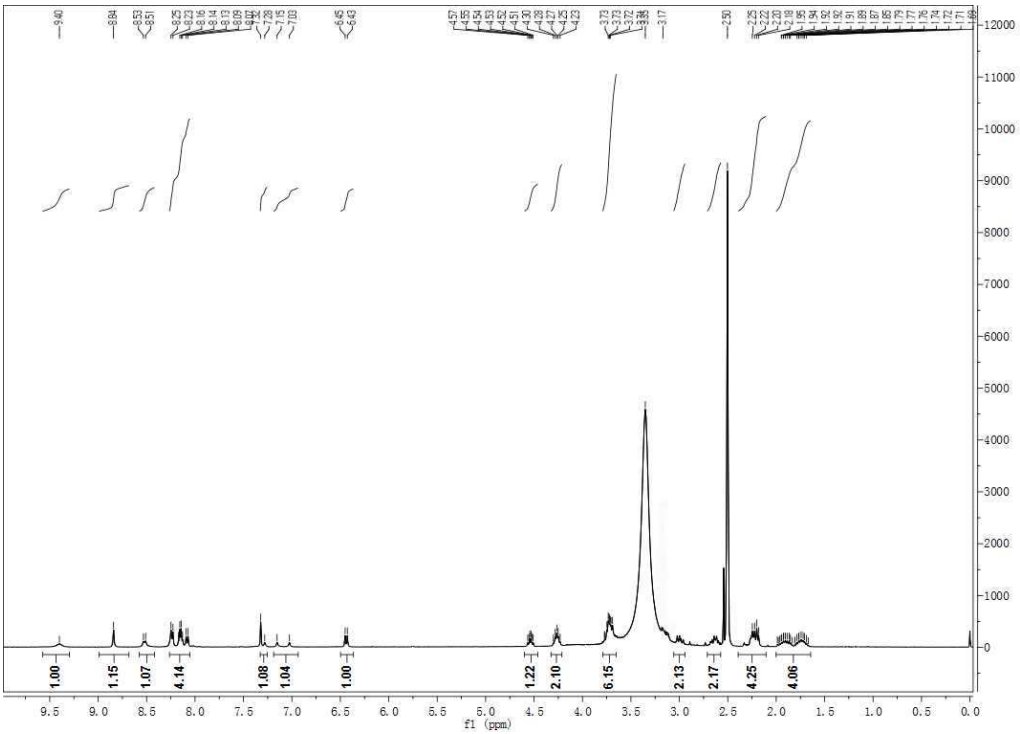


Figure S-9. ^{1}H-NMR of NBD-EEGGH

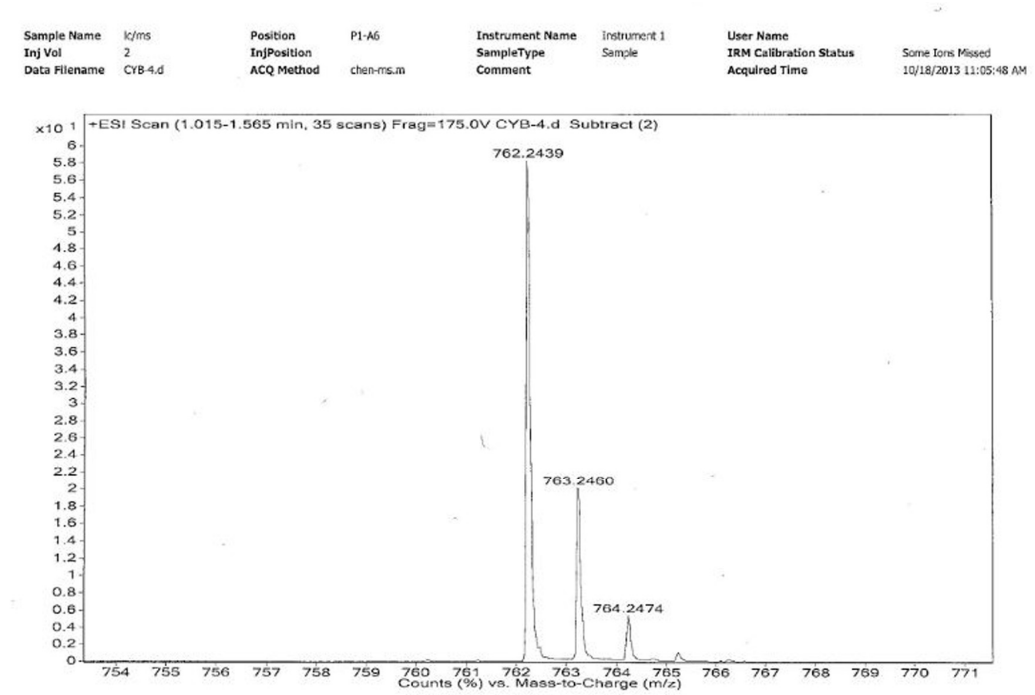
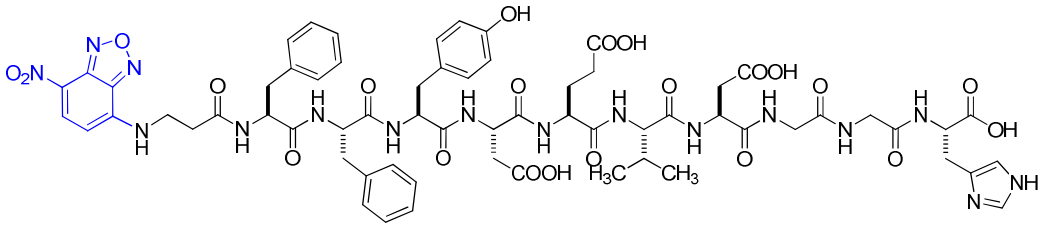


Figure S-10. HR-MS of NBD-EEGGH



***Compound 5:*NBD-FFFDEVDGGH**

Scheme S-7. Chemical structure of NBD-FFFDEVDGGH

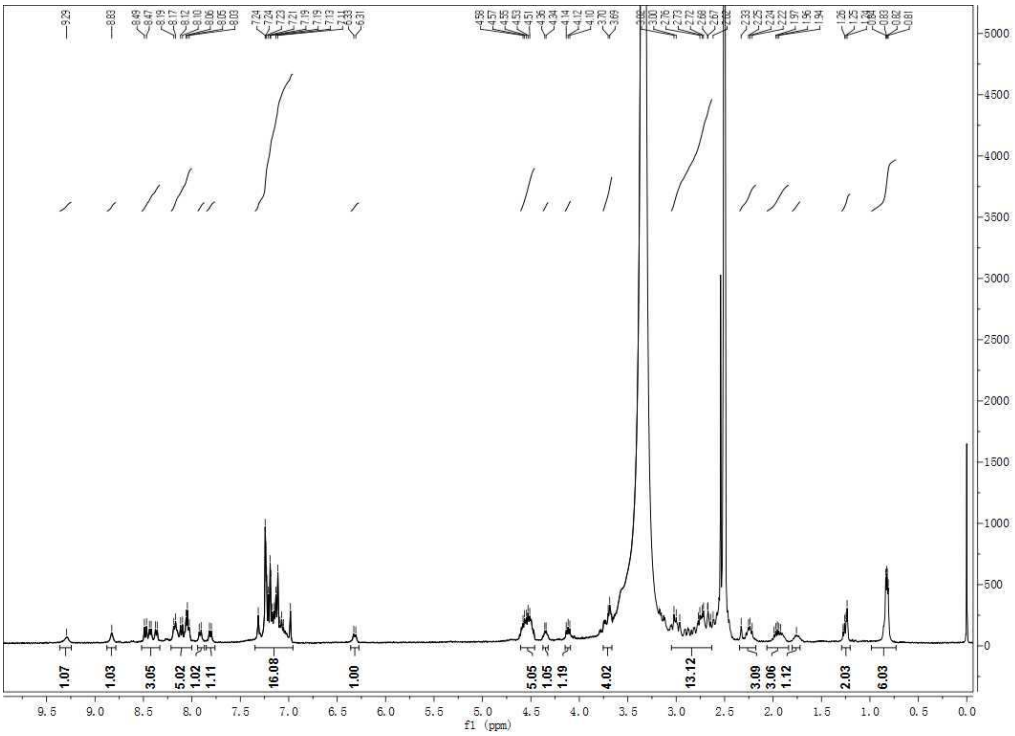


Figure S-11.[1]H-NMR of NBD-FFFDEVDGGH

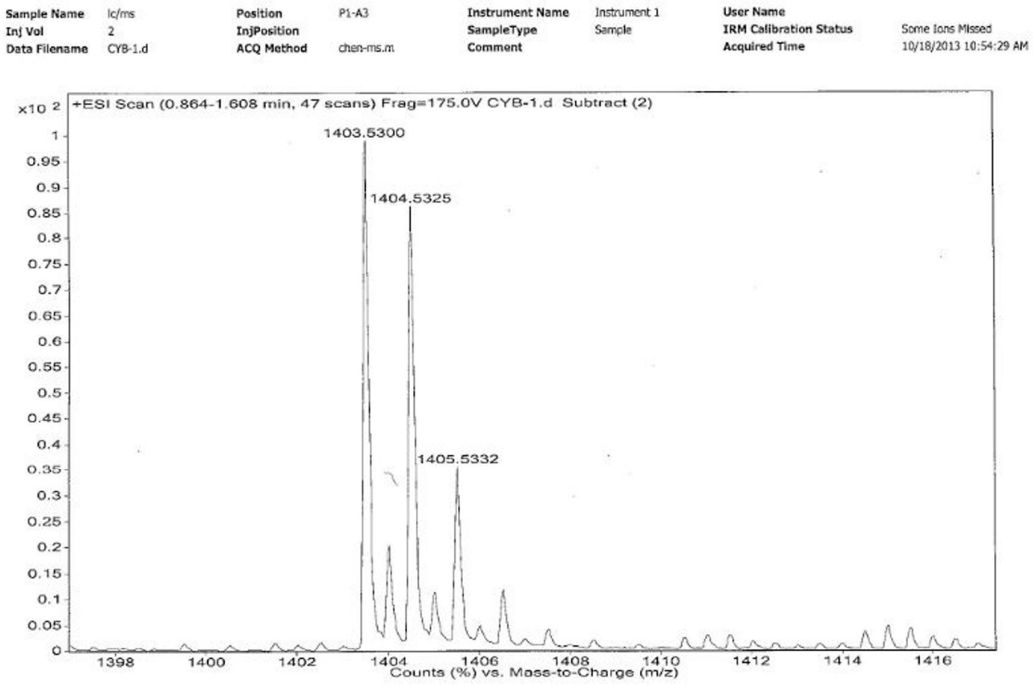


Figure S-12. HR-MS of NBD-FFFDEVDGGH

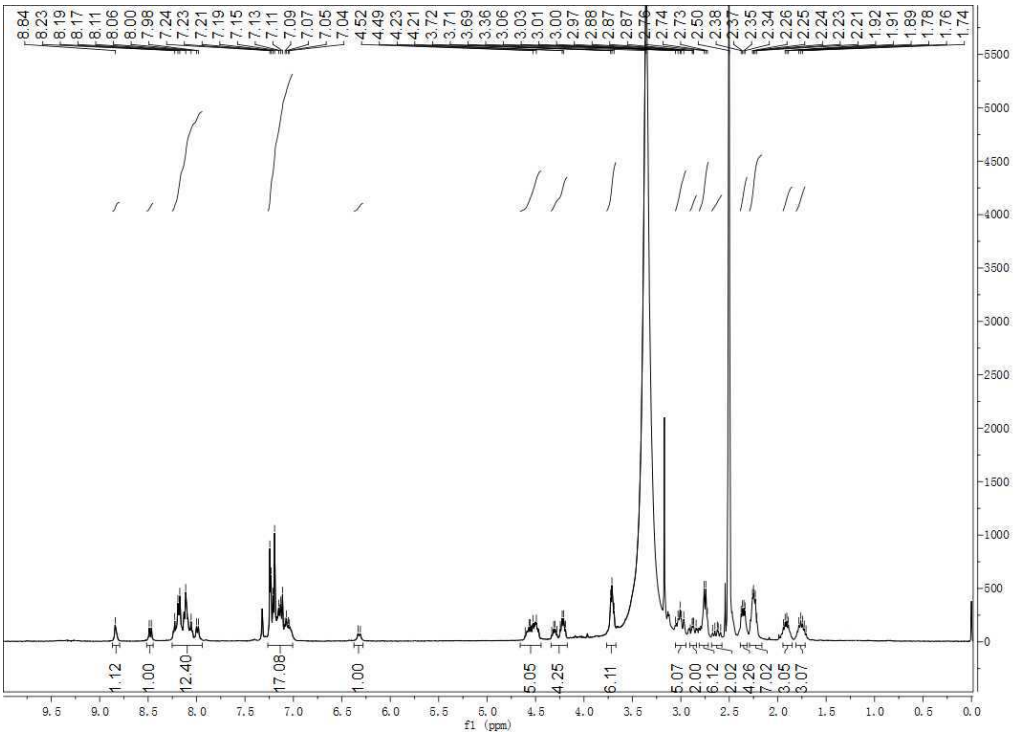


Figure S-13. ^{1}H-NMR of NBD-FFFEE-ss-EGGH

Sample Name	lc/ms	**Position**	P1-A2	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	2	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Some Ions Missed
Data Filename	NBD-FFFEE-SS-EGGH.d	**ACQ Method**	chen-ms.m	**Comment**		**Acquired Time**	9/6/2013 9:58:51 AM

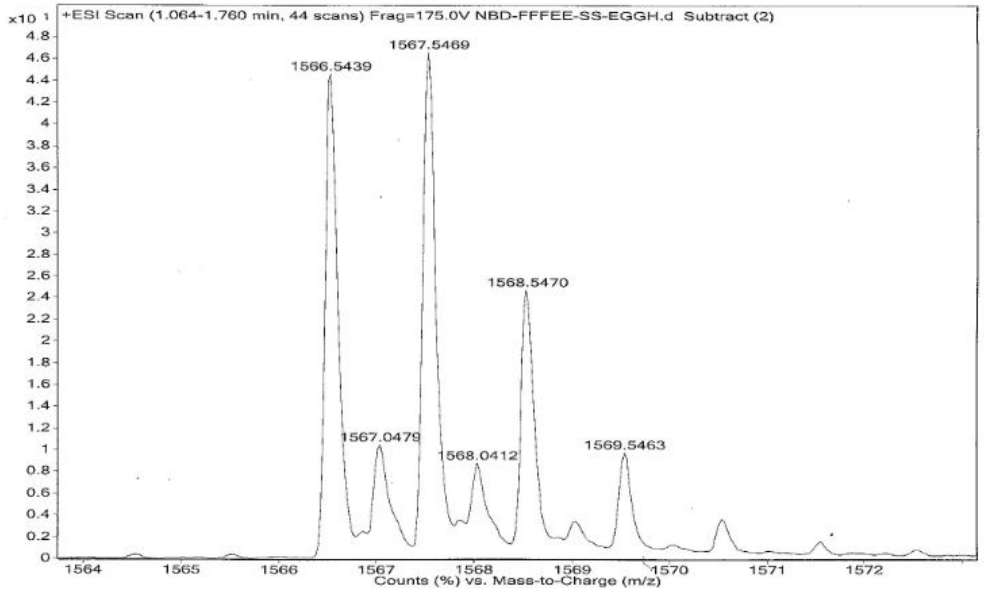


Figure S-14. HR-MS of NBD-FFFEE-ss-EGGH

Figure S-15. An optical image of water solution of NBD-FFYEEGGK with 1 equiv. of Cu^{2+}

Hydrogelation test of *2* by adding different metal ions: To a water solution of ***2*** (1.0 wt%, pH = 7.4, adjusted by Na_2CO_3), an equal volume of water solution containing 1 equiv. of different kinds of metal ions (Cu^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Zn^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Na^{+}, and K^{+}) was added (pH = 7.4). The hydrogelation could only be observed for Cu^{2+}.

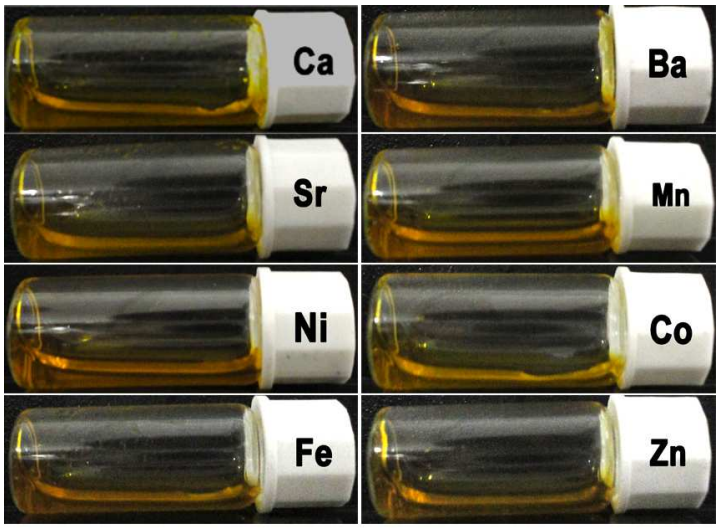


Figure S-16. Optical images of water solution of ***2*** with 1 equiv. of different metal ions (pH = 7.4)

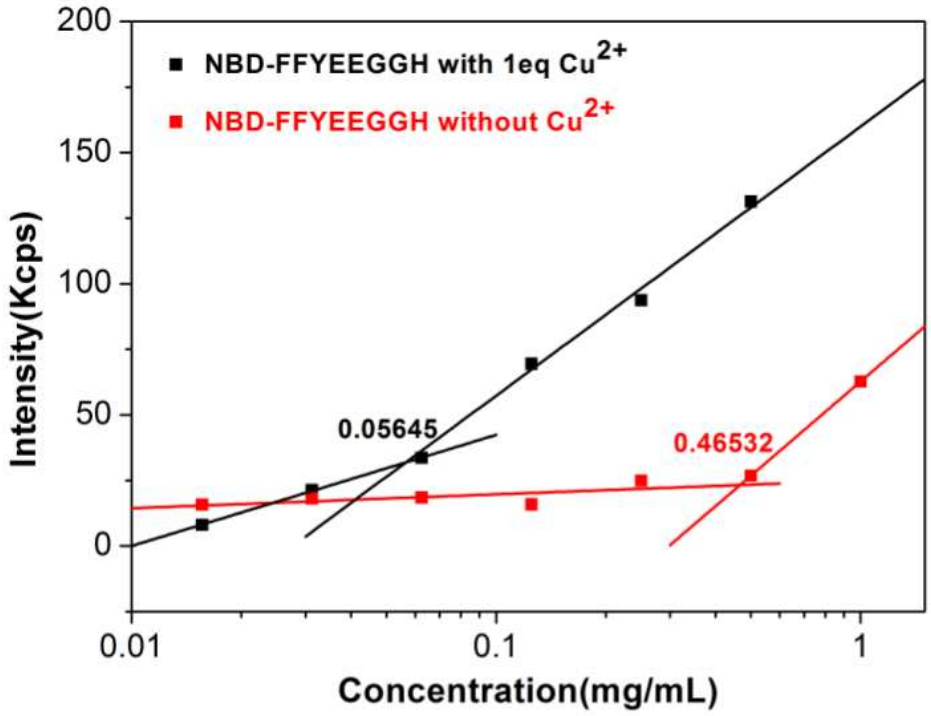


Figure S-17. CMC of compound ***2*** in the absence and presence of 1 equiv. of Cu^{2+}

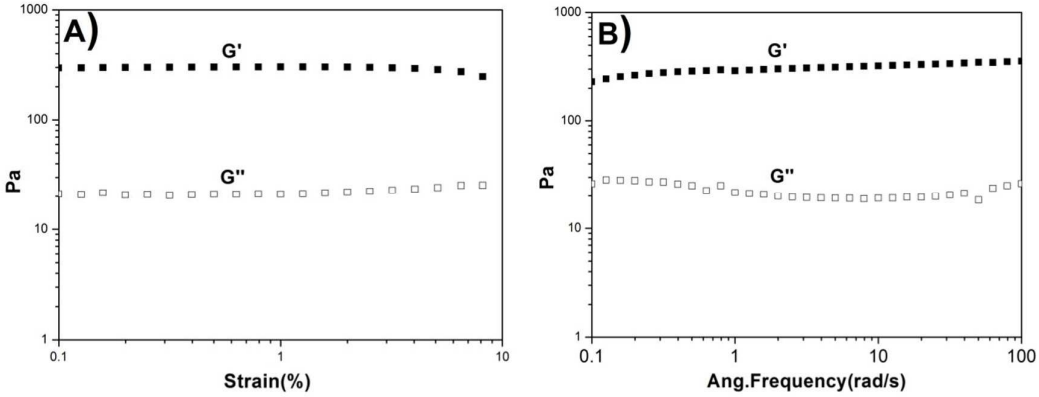


Figure S-18. Rheological measurements A) in dynamic strain sweep mode at the frequency of 1 rad/s and B) in dynamic frequency sweep at the strain of 1% for hydrogel containing 0.5% compound ***2*** and 1 equiv. of Cu^{2+}

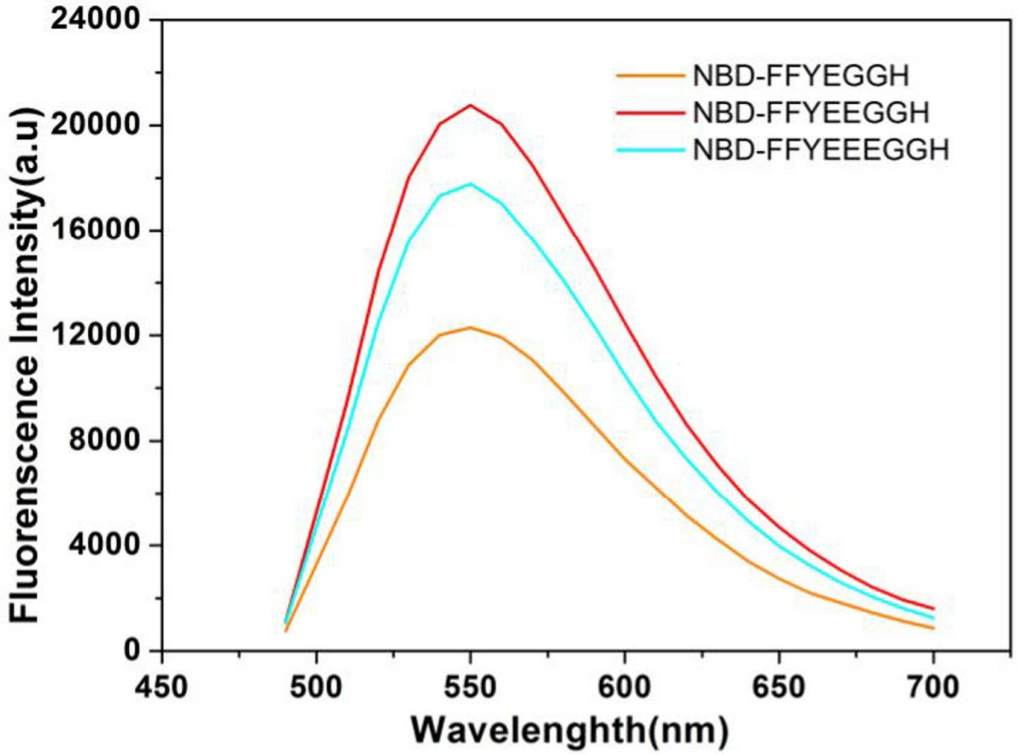


Figure S-19. Fluorescence spectra of aqueous solution containing 0.05 wt% of ***1-3***.

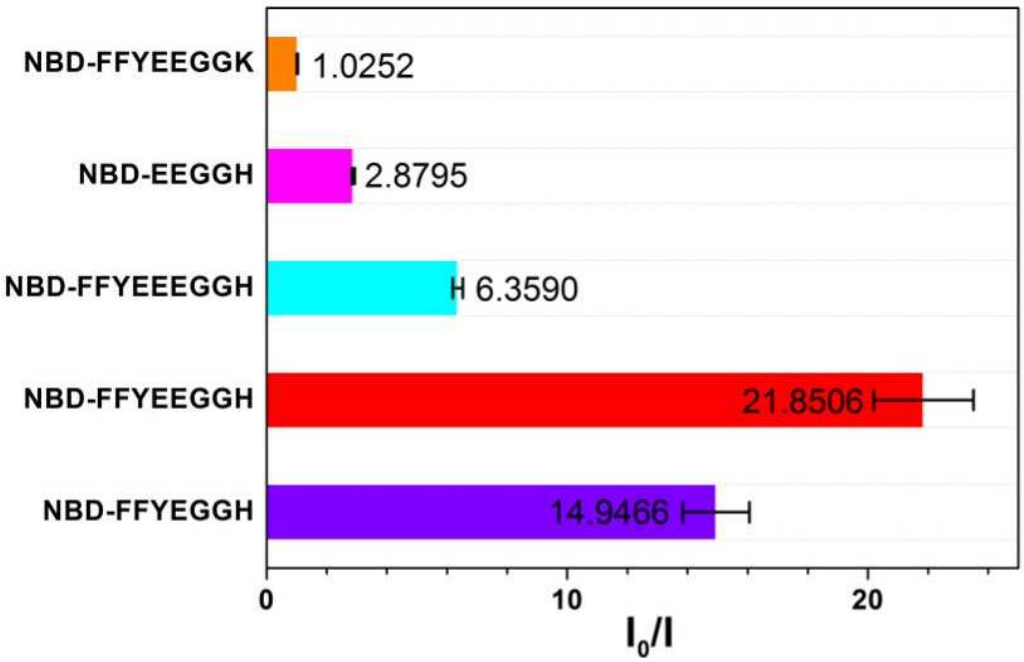


Figure S-20. **I_0/I** value of different compounds (the data were expressed as the mean ± standard error of the mean (SEM, N = 3))

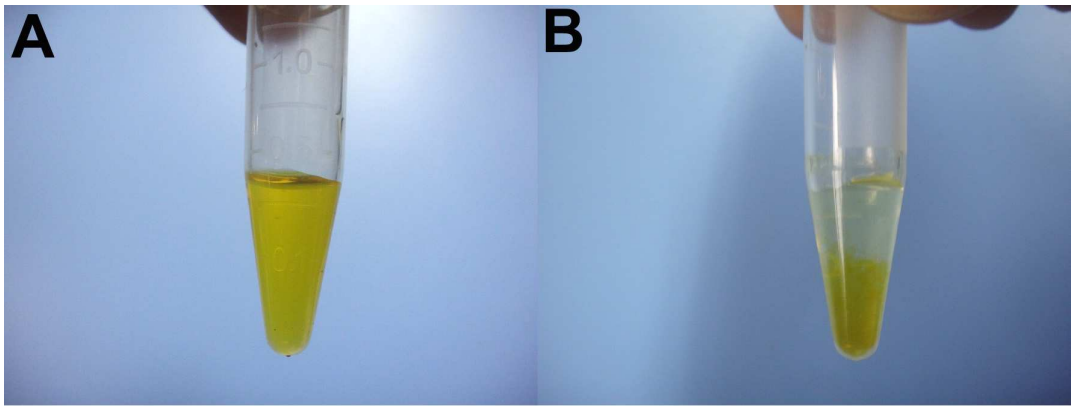


Figure S-21. Optical images of solutions of compound ***1*** with or without Cu^{2+}: A) 0.5 wt% of compound ***1*** without Cu^{2+}; B) 0.5 wt% of compound ***1*** with one equiv. of Cu^{2+}

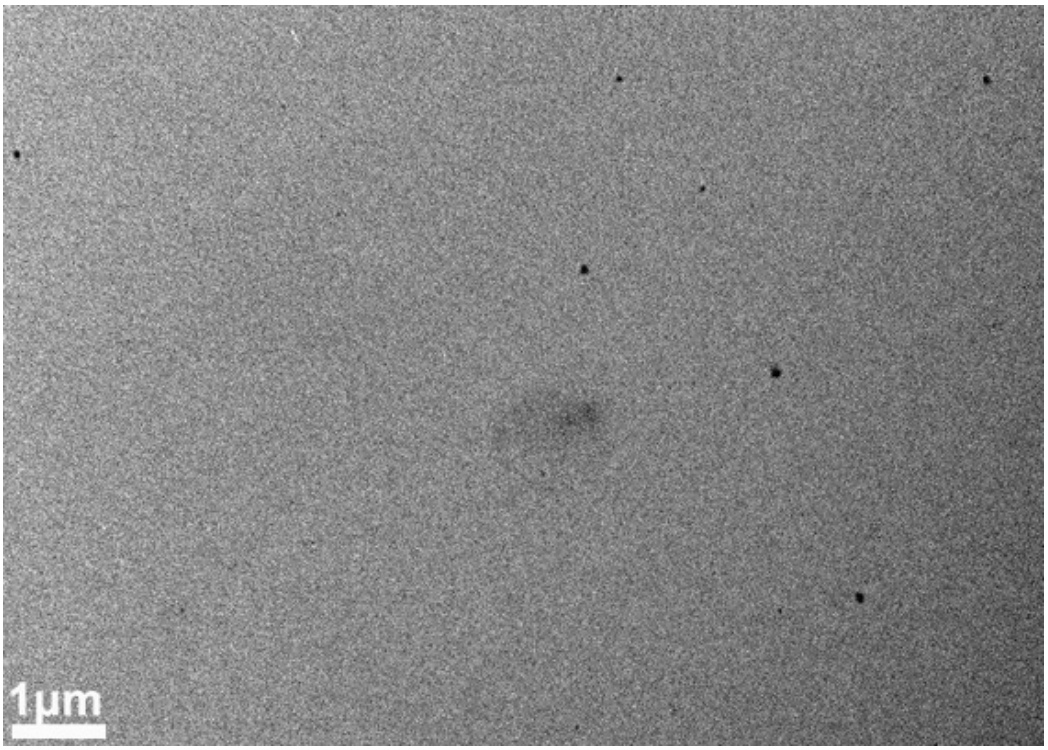


Figure S-22. Transmission electron microscopy (TEM) images of solution of ***4*** with one equiv. of Cu^{2+}

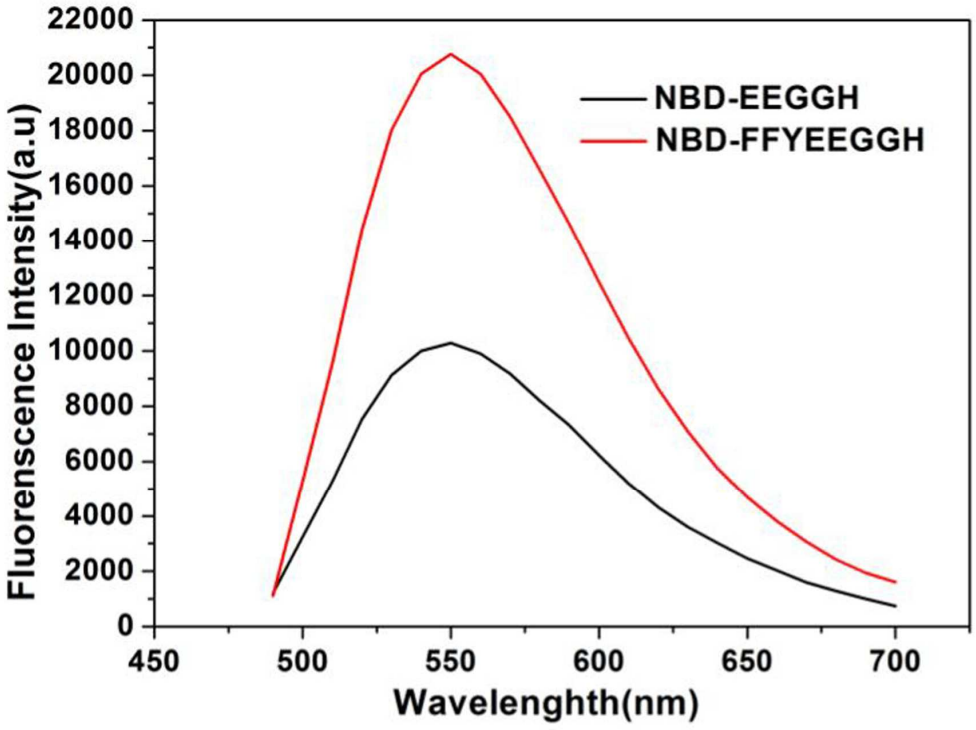


Figure S-23. Fluorescence spectra of aqueous solution containing compound ***2*** or compound ***4*** (410 μM, pH = 7.4)

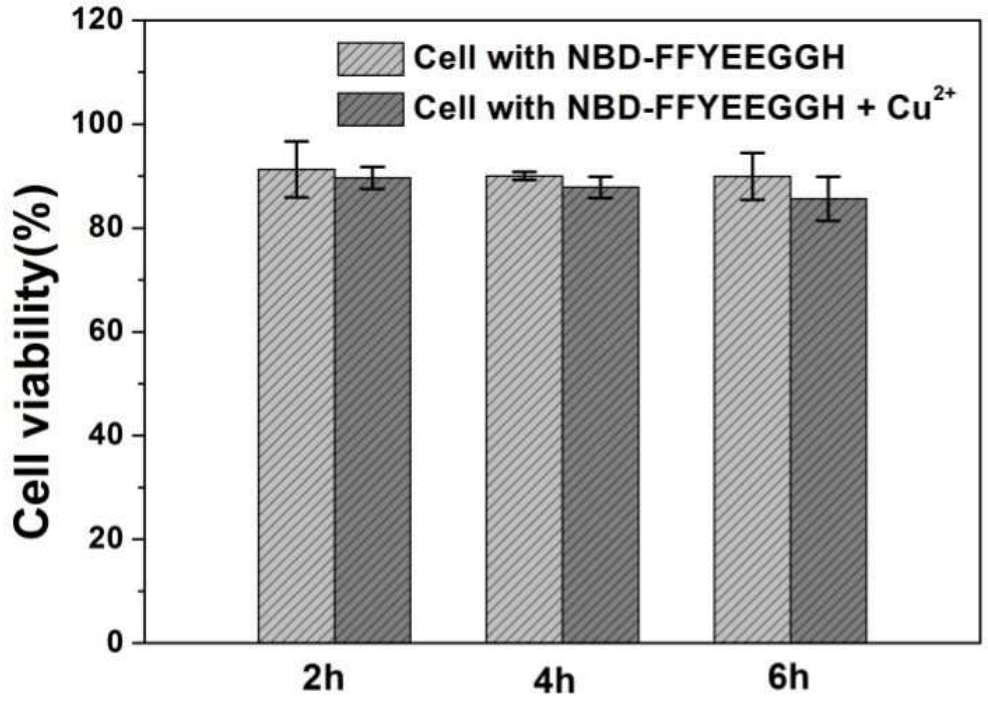


Figure S-24. Cell viability of Compounds ***2*** with 100 μM Cu^{2+} by MTT assay for 2 to 6 h (the data were expressed as the mean ± standard error of the mean (SEM, N = 3))

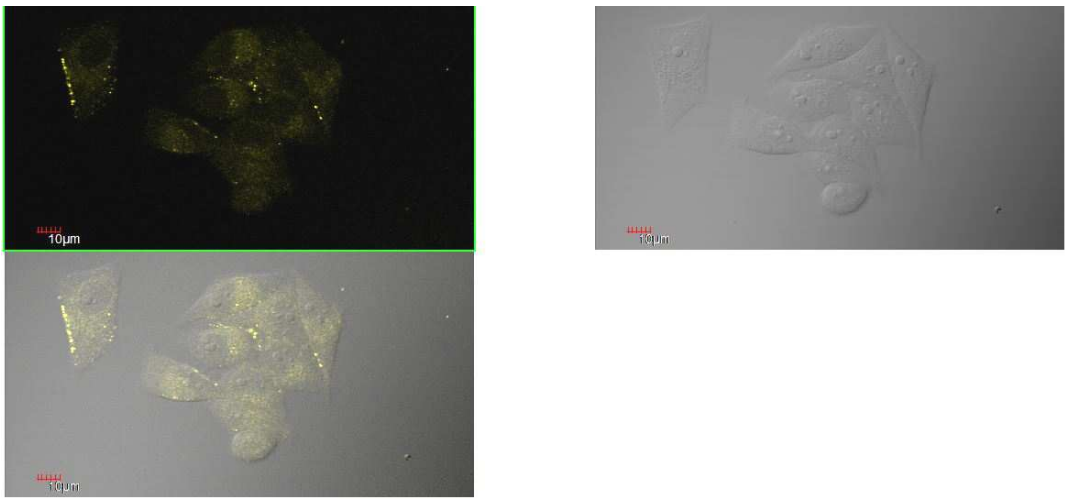


Figure S-25. Confocal images of HeLa cells treated with ***2*** (0.05 wt%) at 2 h time point (top left: fluorescence image, top right: bright field image, and down: overlay image)

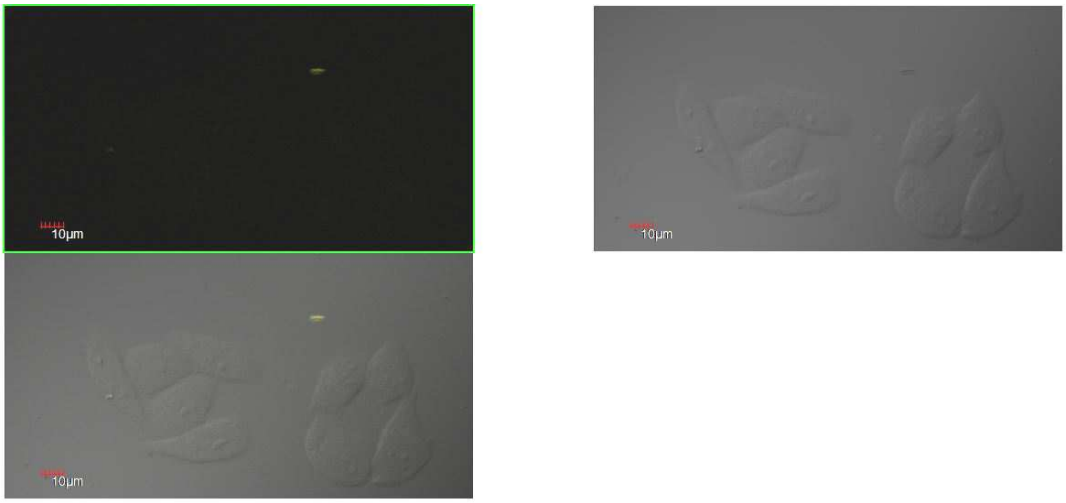


Figure S-26. Confocal images of HeLa cells pre-treated with 100 µM of Cu^{2+} and then treated with ***2*** (0.05 wt%) at 6 h time point (top left: fluorescence image, top right: bright field image, and down: overlay image)

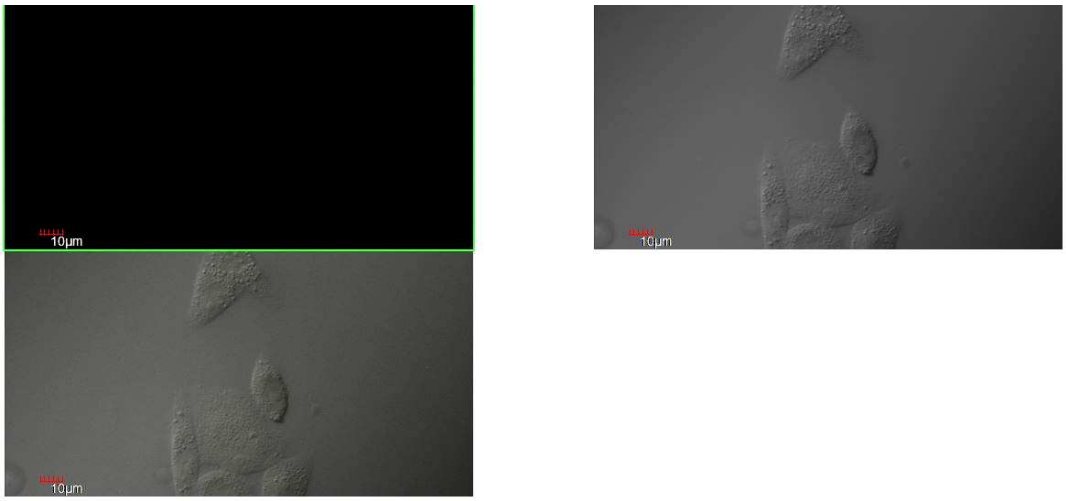


Figure S-27. Confocal images of HeLa cells treated with ***2*** (0.05 wt%) for 2h and then treated with 100 μM of Cu^{2+} for another 2h (top left: fluorescence image, top right: bright field image, and down: overlay image)

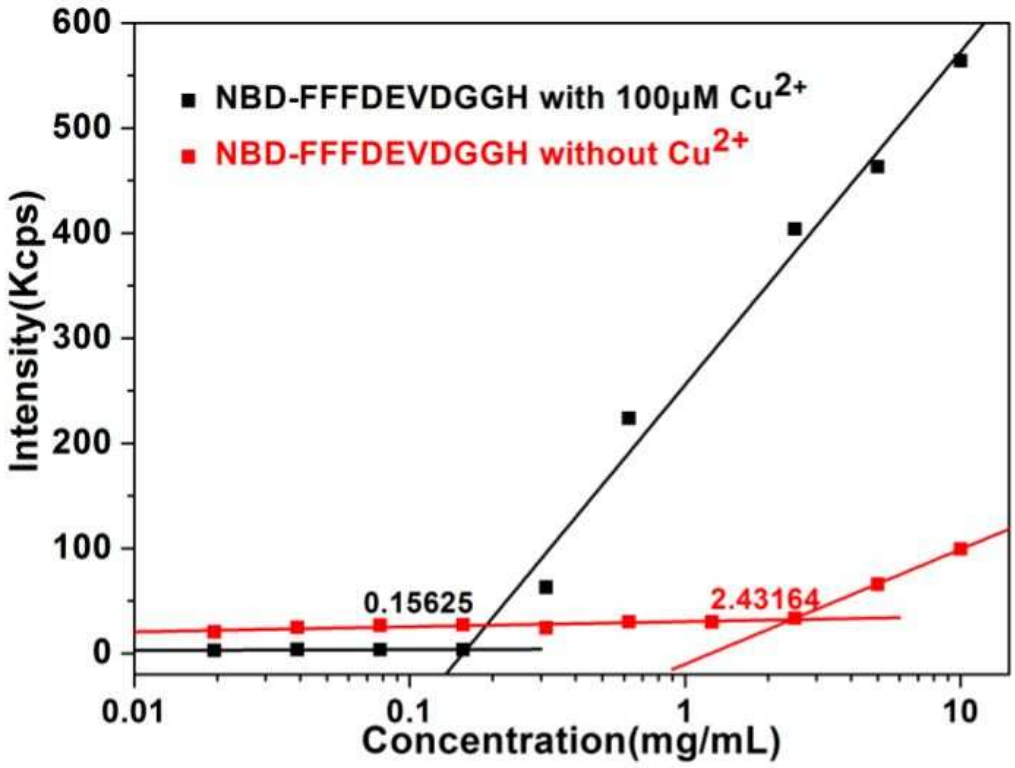


Figure S-28. CMC of compound ***4*** in the absence and presence of 100 μM of Cu^{2+}

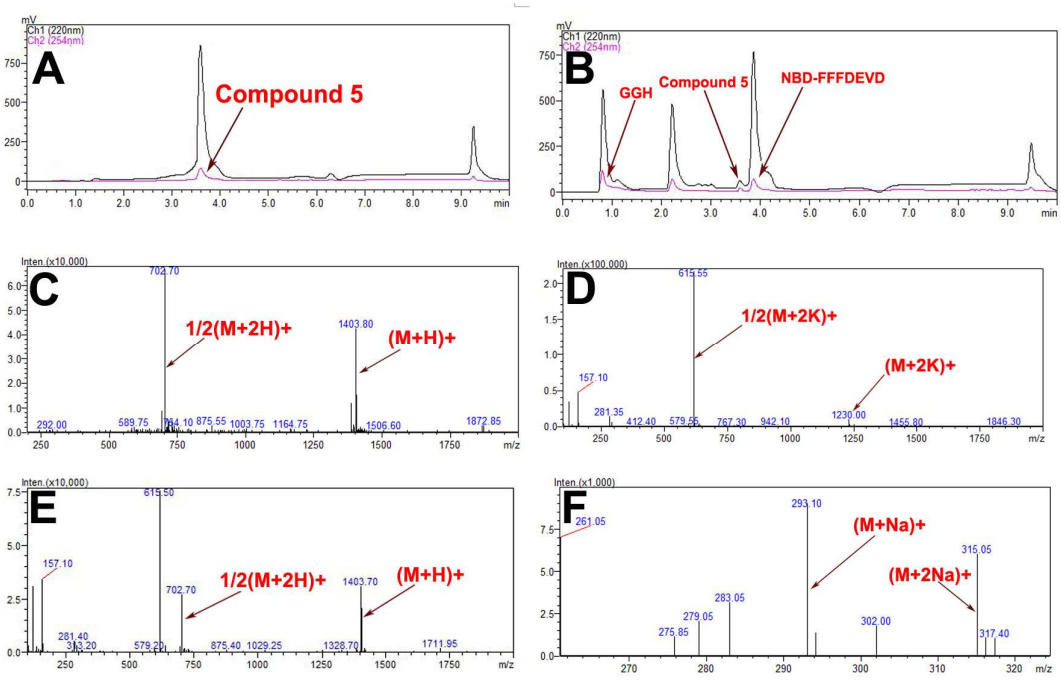


Figure S-29. A) HPLC of compound ***5*** ; B) HPLC of compound ***5*** with 1 U/mL caspase-3 for 1 h; C) MS of compound ***5*** in A); D) MS of NBD-FFFDEVD cleaved from compound ***5*** by caspase-3 in B); E) MS of compound ***5*** uncleaved by caspase-3 in B); F) MS of GGH cleaved from compound ***5*** by caspase-3 in B).

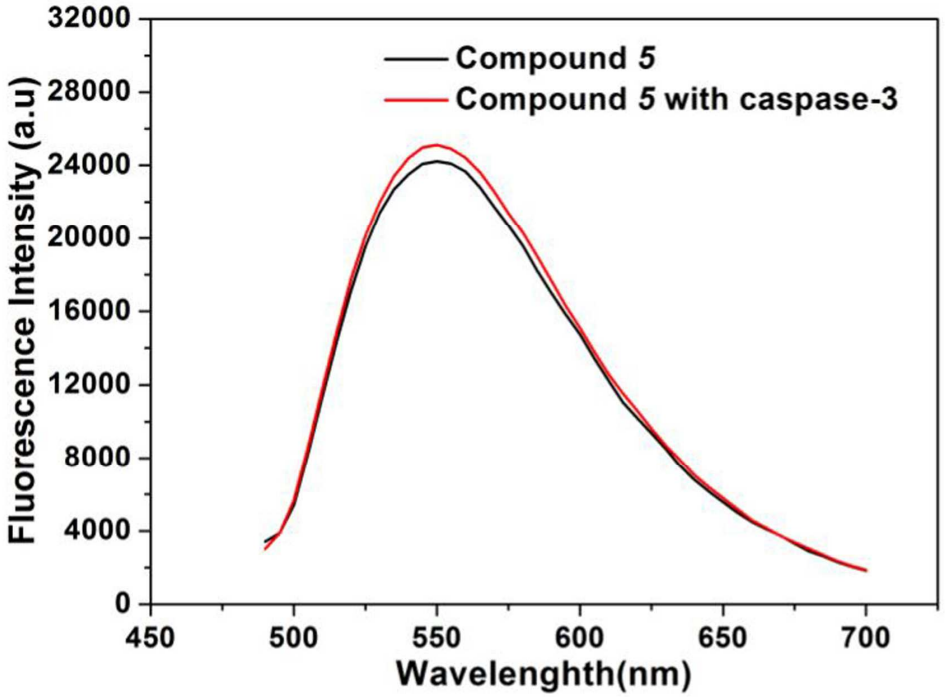


Figure S-30 Fluorescence spectra of aqueous solution containing compound ***5*** (0.05 wt%) before and after adding Caspase-3 in the absence of Cu^{2+}

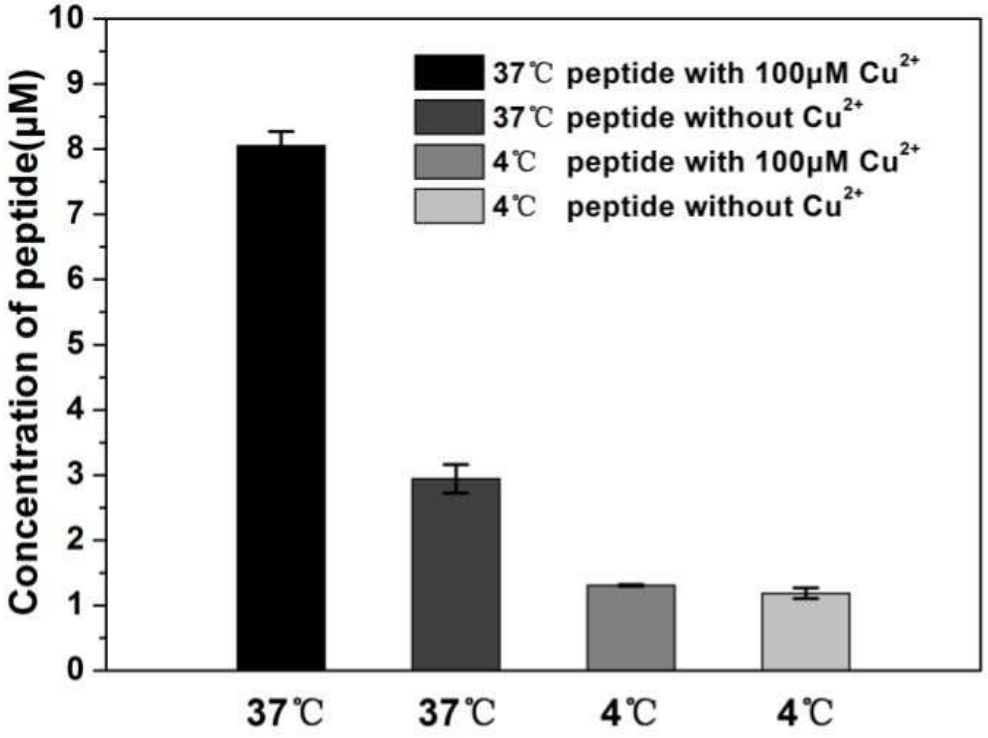


Figure S-31. The amount of compound ***4*** with Cu^{2+} and without Cu^{2+} within HeLa cells at 4℃ and 37 ℃ determined by LC-MS (the data were expressed as the mean ± standard error of the mean (SEM, N = 3))

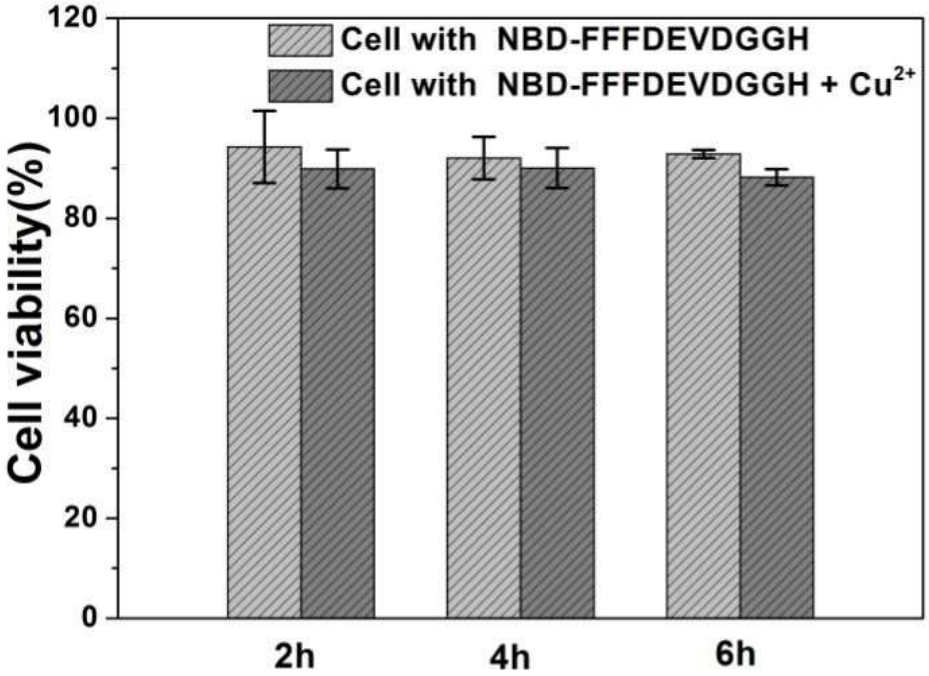


Figure S-32. Cell viability of Compound ***4*** with 100 μM Cu^{2+} by MTT assay for 2 to 6h (the data were expressed as the mean ± standard error of the mean (SEM, N = 3))

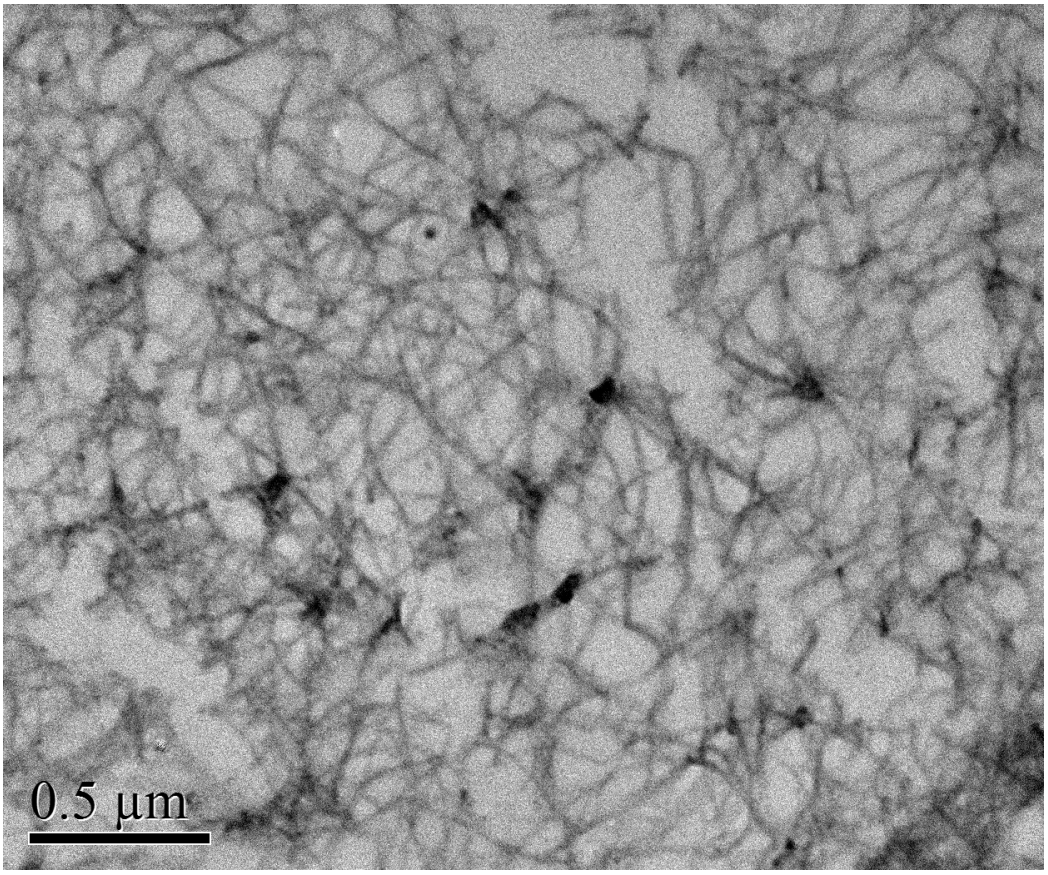


Figure S-33. Transmission electron microscopy (TEM) images of solution of ***6*** with one equiv. of Cu^{2+} and 4 equiv. of GSH

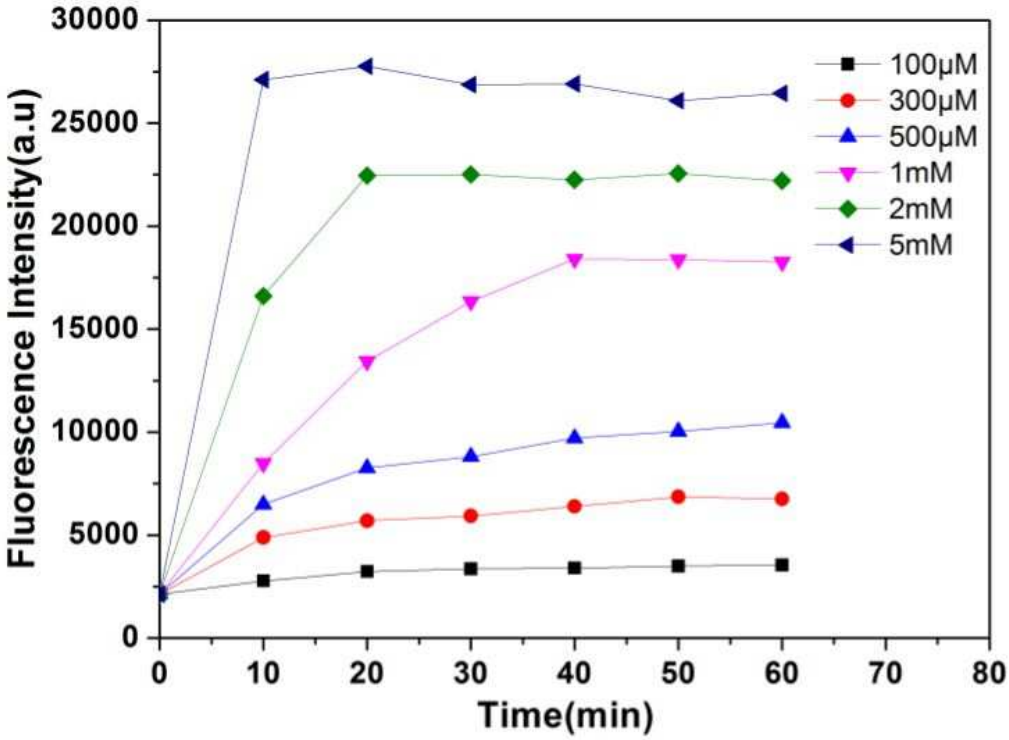
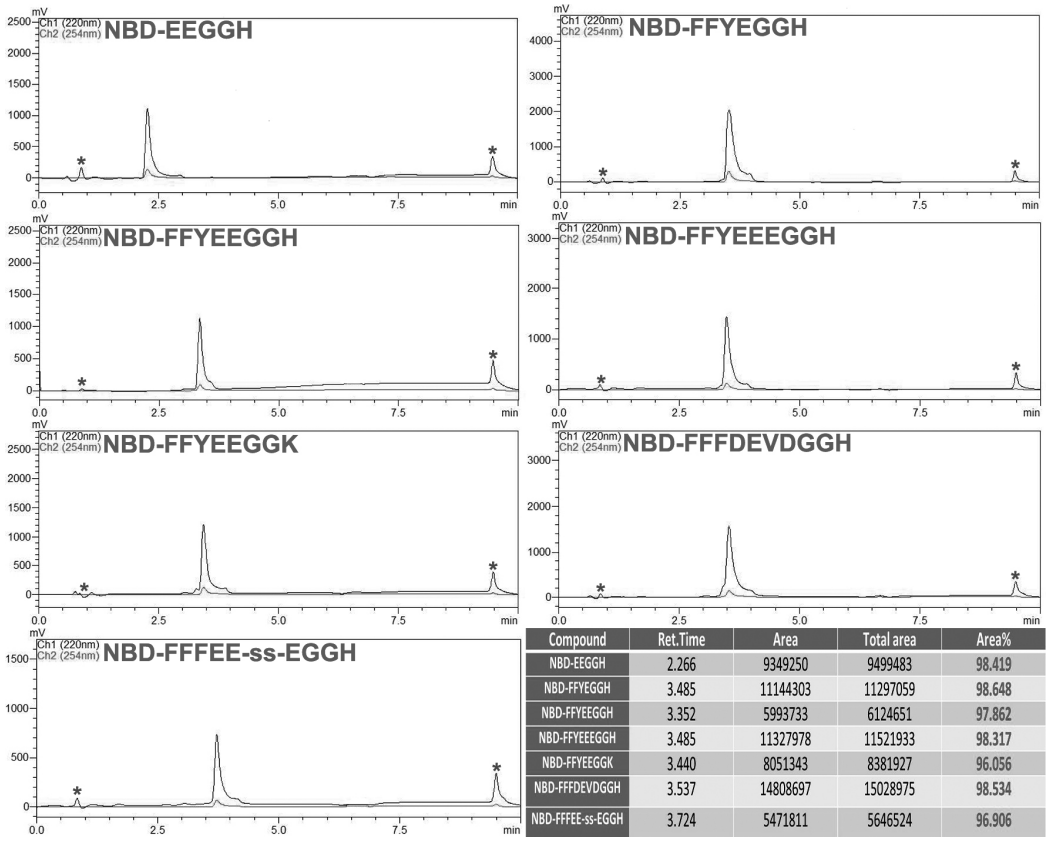


Figure S-34. Fluorescence values of ***6***:Cu^{2+} (0.05 wt% ***6*** with one equiv. of Cu^{2+}) treated with different concentrations of GSH at 0-60mins.



Compound	Ret.Time	Area	Total area	Area%
NBD-EEGGH	2.266	9349250	9499483	98.419
NBD-FFYEGGH	3.485	11144303	11297059	98.648
NBD-FFYEEGGH	3.352	5993733	6124651	97.862
NBD-FFYEEEGGH	3.485	11327978	11521933	98.317
NBD-FFYEEGGK	3.440	8051343	8381927	96.056
NBD-FFFDEVDGGH	3.537	14808697	15028975	98.534
NBD-FFFEE-ss-EGGH	3.724	5471811	5646524	96.906

Figure S-35.The purity of peptides obtained from LC-MS data (the peaks assigned by * in every figure are the system peaks).

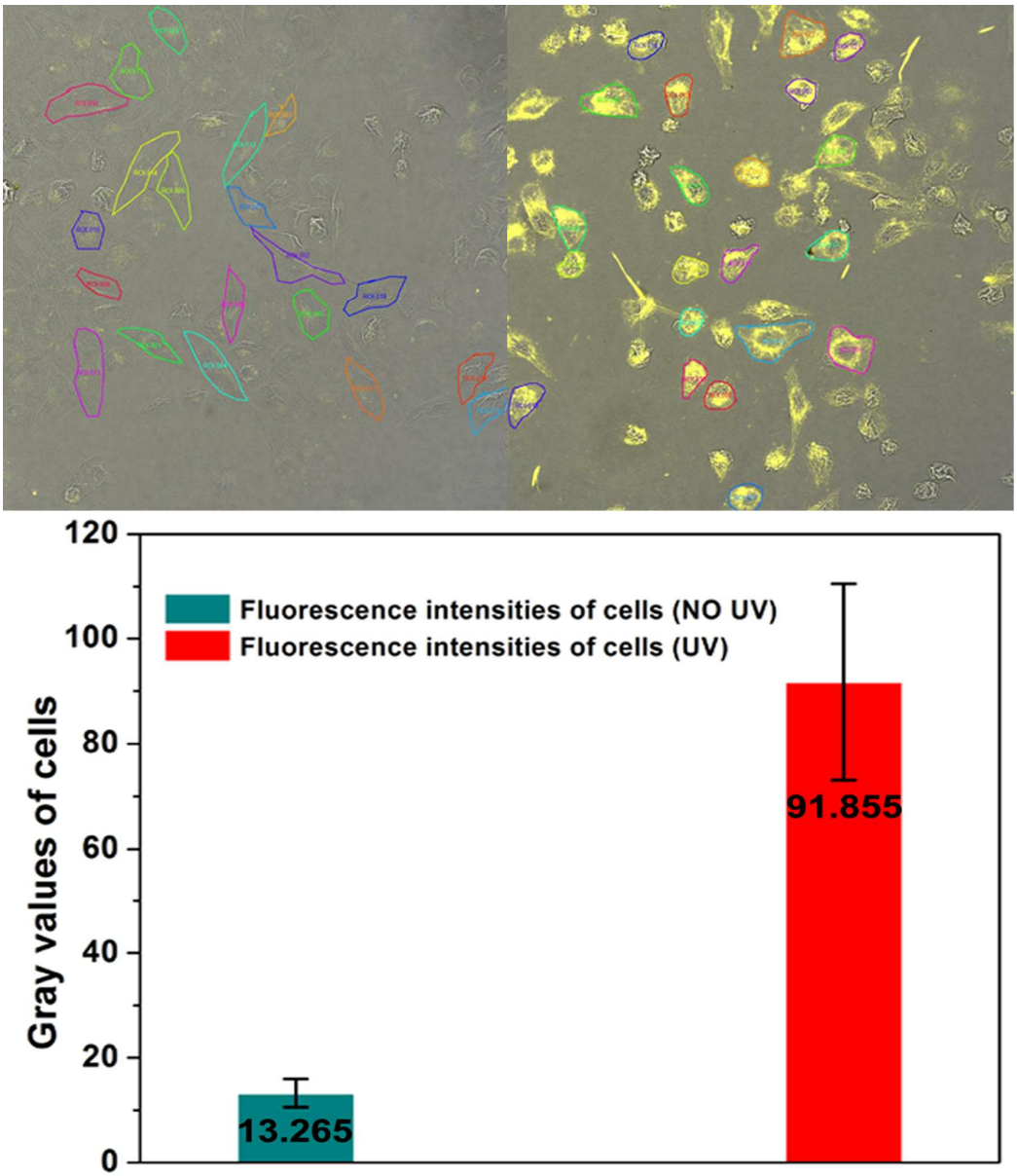


Figure S-36. The mean value of fluorescence intensities of cells from CLSM images (the data were expressed as the mean ± standard error of the mean (SEM, N = 20)).

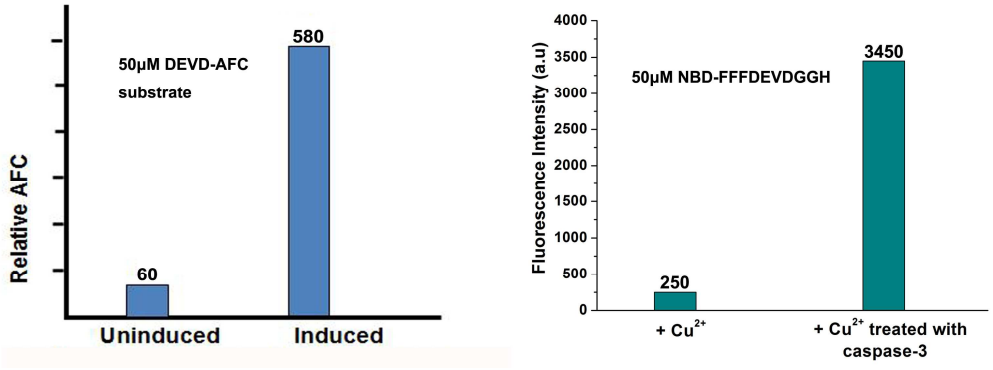


Figure S-36. The comparative data of the commercial probe with our probe (left: the change of fluorescence intensity of the commercial probe, the ratio of two histograms= 9.67; right: the change of fluorescence intensity of our probe, the ratio of two histograms=13.8).