AN INDOCYANINE-BASED TURN-ON FLUORESCENT PROBE FOR SPECIFIC DETECTION OF BIOTHIOLS

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Experimental

Reagents and apparatus

All reagents and solvents were purchased from commercial sources and used without further purification. Deionized water was used throughout this study. NMR was taken on a Bruker Avance Ⅲ 400 MHz spectrometer where δ values are in ppm relative to TMS. High-resolution mass spectra (HRMS) were measured with a Bruker Daltonics microTOF-Q Ⅱ instrument (ESI). UV-vis absorption spectra were recorded on L8 spectrophotometer. Fluorescence emission spectra were measured on Hitachi F-2500 fluorescence spectrophotometer. All measurements were carried out at room temperature under ambient atmosphere, and the excitation and emission slit widths were both 2.5 nm. The fluorescence quantum yield was measured by Edinburgh company FLS 920P spectrophotometer.

Sample preparation

The stock solution of probe Cy-DNBS (1.0×10^{-3} M) was prepared by dissolving an appropriate amount of probe (6.9 mg) in ethanol and diluting it to the mark in a 10 mL volumetric flask. The working probe solution (1×10^{-5} M) was obtained by diluting 2.5 mL stock solution to 250 mL with ethanol-PBS solution (10 mM, pH 7.4, 3:7, v/v). Stock analyte solutions (1×10^{-2} M) were prepared by dissolving proper amount of each analytes in deionized water. In test experiments, working solution (9 mL) was added to a 10 mL volumetric flask, to which different amounts of analyte stock solutions were added via a microsyringe, and then diluted to the mark with ethanol-PBS solution (10 mM, pH 7.4, 3:7, v/v).

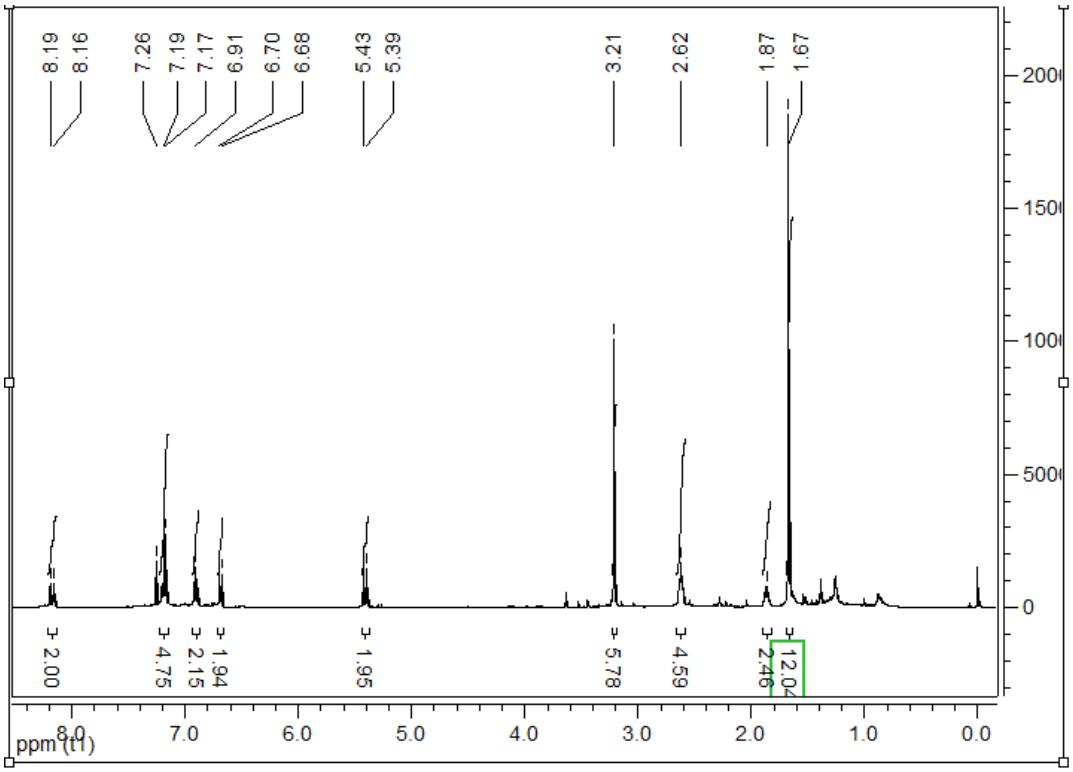

Figure S1. ^{1}H NMR spectrum of compound CyK in $CDCl_3$

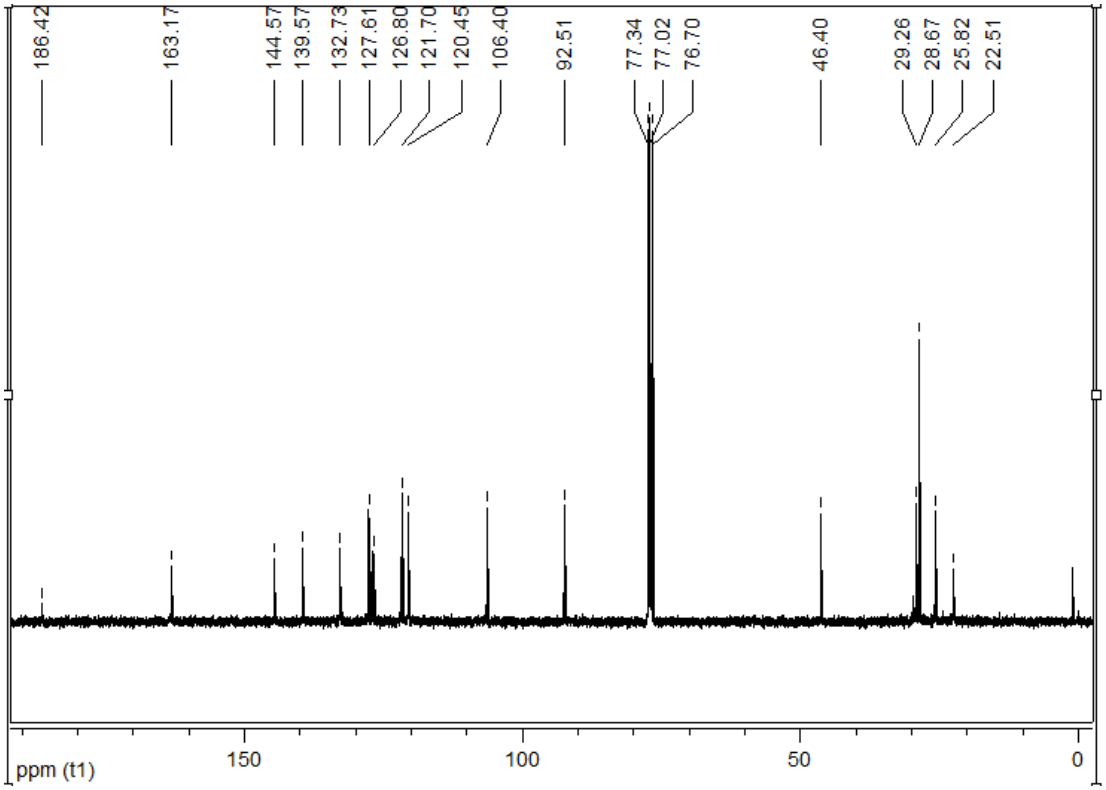

Figure S2. ^{13}C NMR spectrum of compound CyK in $CDCl_3$

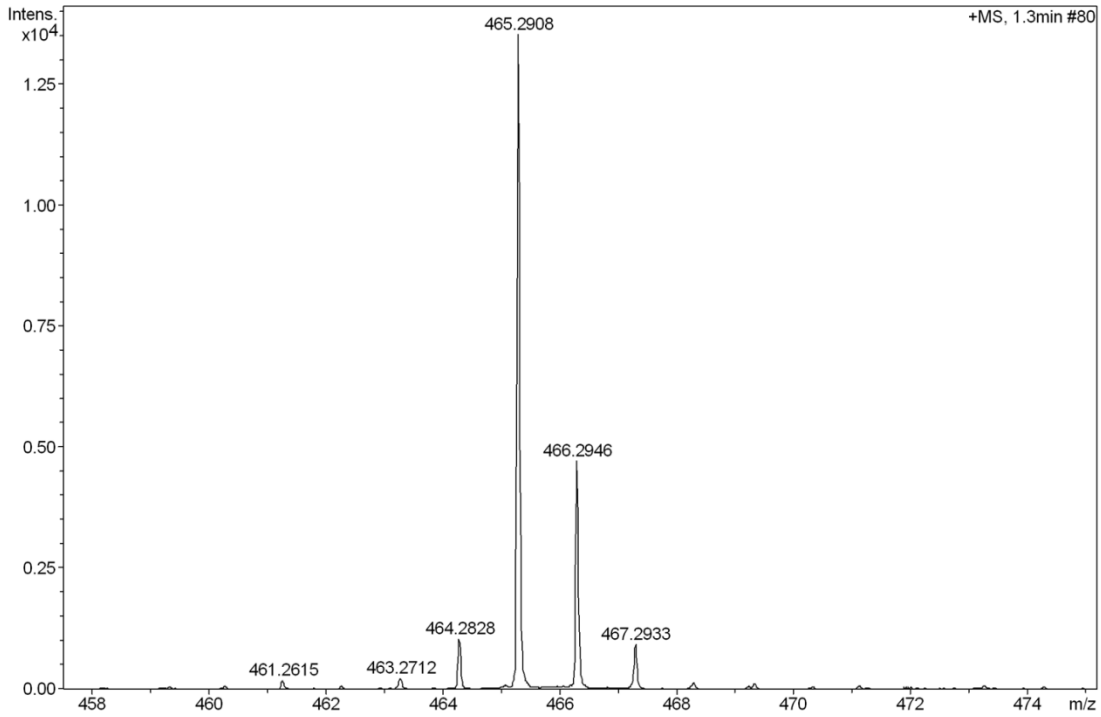

Figure S3. HRMS spectrum of compound CyK

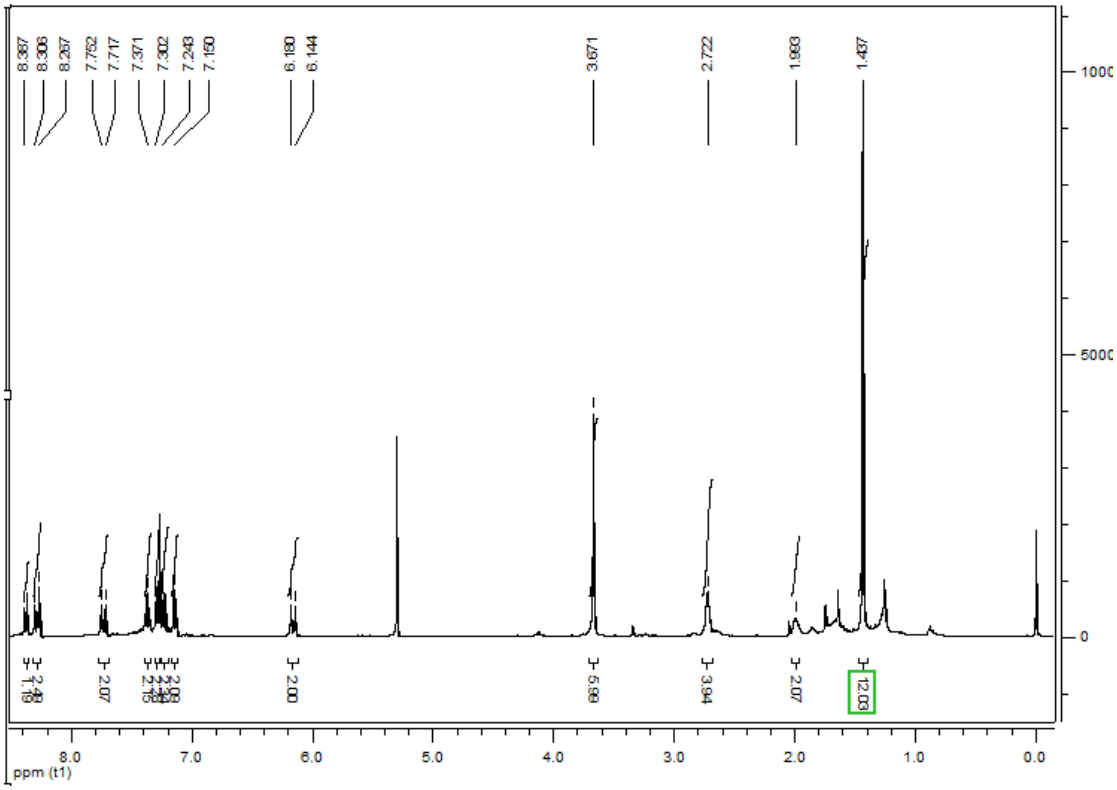

Figure S4. ^{1}H NMR spectrum of probe Cy-DNBS in $CDCl_3$

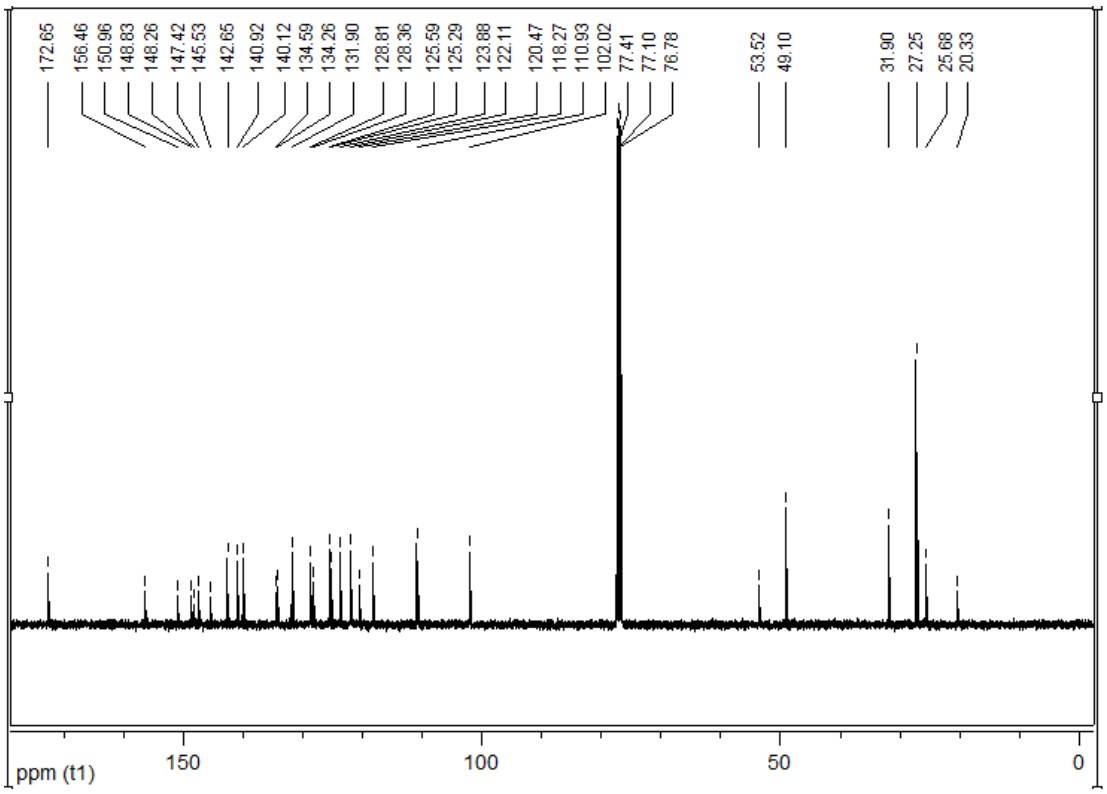

Figure S5. ^{13}C NMR spectrum of probe Cy-DNBS in $CDCl_3$

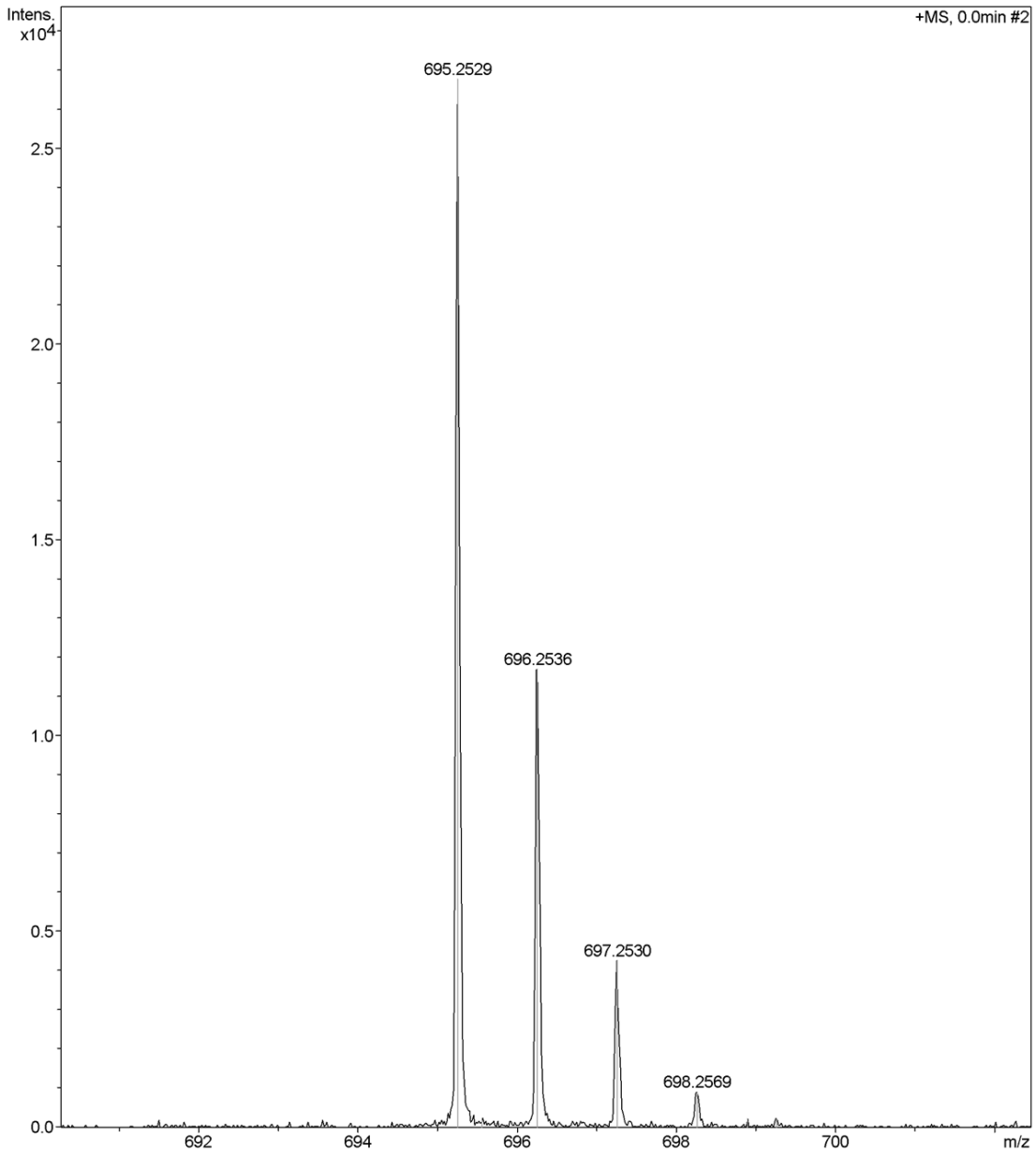

Figure S6. HRMS spectrum of probe Cy-DNBS

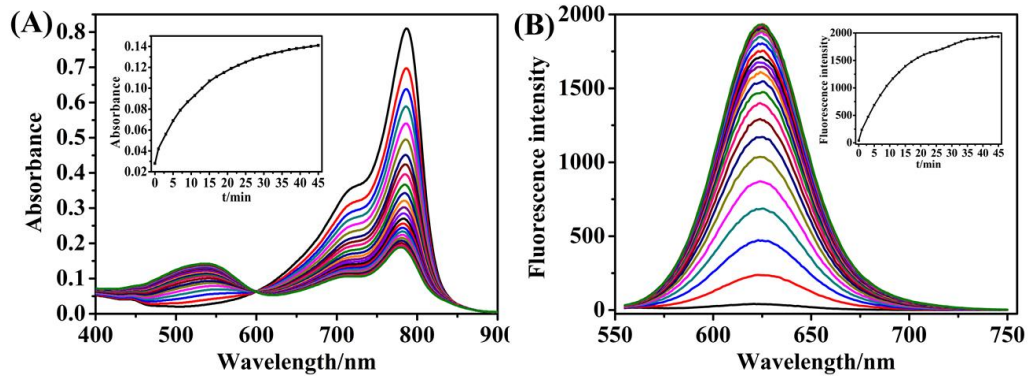

Figure S7. (A) Time-dependent absorption spectra of probe Cy-DNBS (10μM) after adding 1 equiv. GSH. Inset: the absorbance at 544 nm along with time. (B) Time-dependent

fluorescence spectra of probe Cy-DNBS (10μM) after adding 1 equiv. GSH. Inset: the fluorescence intensity at 625 nm along with time.

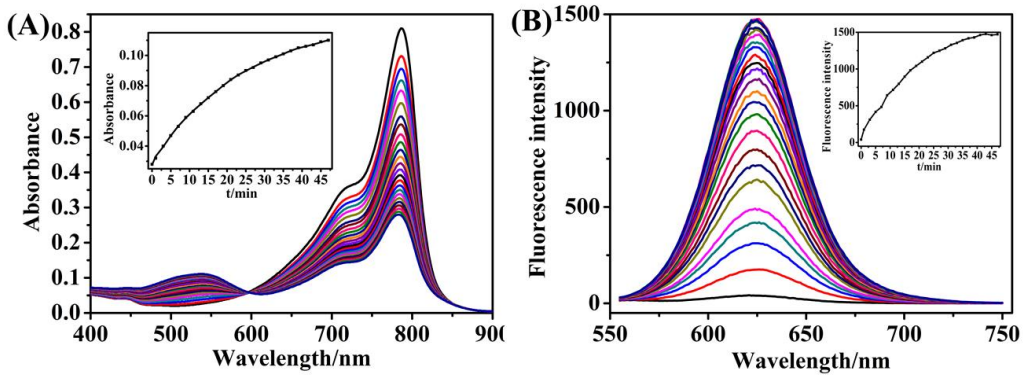

Figure S8. (A) Time-dependent absorption spectra of probe Cy-DNBS (10μM) after adding 1 equiv. Hcy. Inset: the absorbance at 544 nm along with time. (B) Time-dependent fluorescence spectra of probe Cy-DNBS (10μM) after adding 1 equiv. Hcy. Inset: the fluorescence intensity at 625 nm along with time.

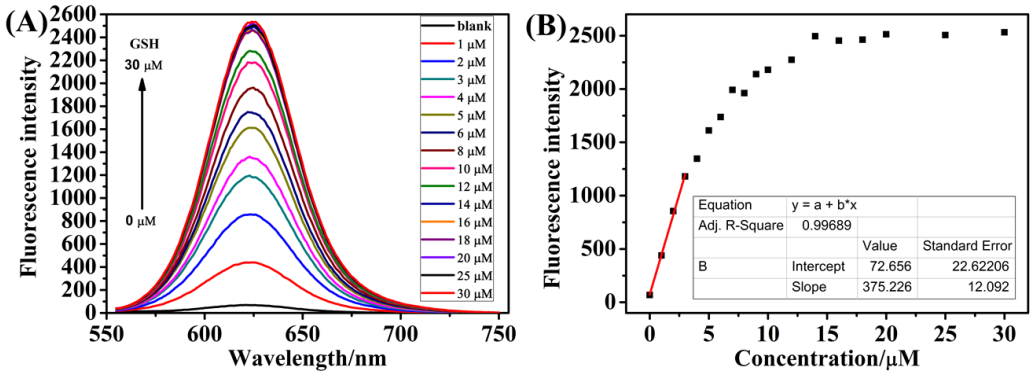

Figure S9. (A) Fluorescence spectra of probe Cy-DNBS treated with various concentrations of GSH, (B) Fluorescence intensity at 625 nm for probe Cy-DNBS as a function of the concentration of GSH.

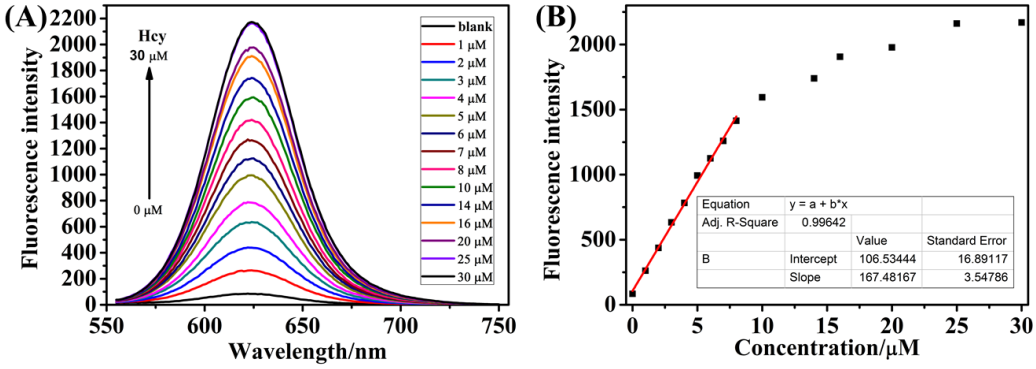

Figure S10. (A) Fluorescence spectra of probe Cy-DNBS treated with various concentrations of Hcy, (B) Fluorescence intensity at 625 nm for probe Cy-DNBS as a function of the

concentration of Hcy.

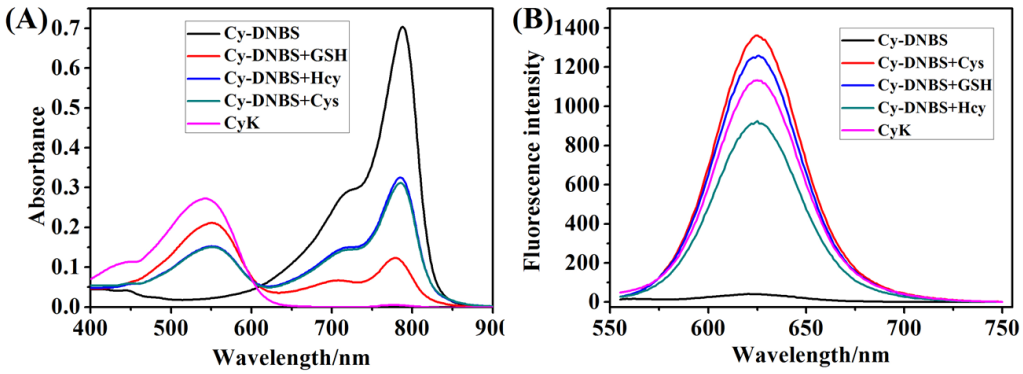

Figure S11. (A) Absorption spectra and (B) fluorescence emission spectra of CyK and the probe Cy-DNBS in the absence and presence of 1 equiv. biothiol

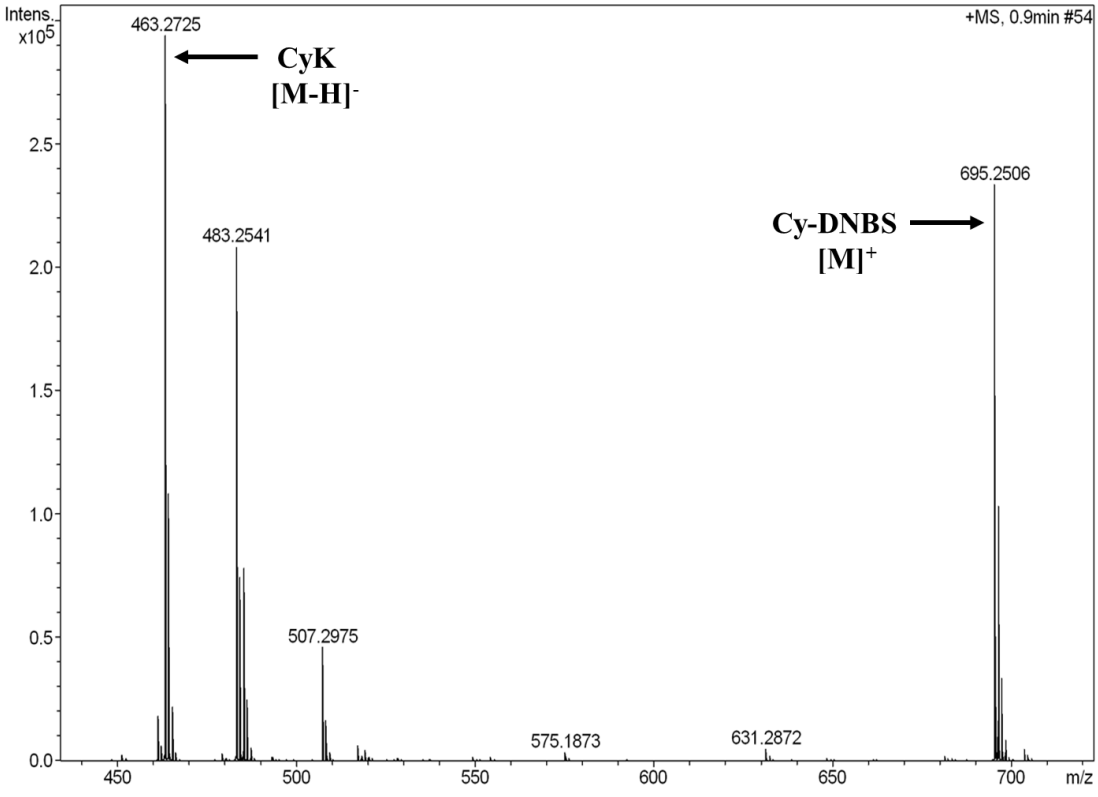

Figure S12. HRMS of probe of the reaction product of probe Cy-DNBS and 1 equiv. Cys