Supporting Information:

Colorimetric sensors with different reactivity for the quantitative determination of cysteine, homocysteine and glutathione in a mixture

Li-Ya Niu,[‡a] Ming-Yan Jia,[‡b] Peng-Zhong Chen,[a] Yu-Zhe Chen,[a] Yuan Zhang,[c] Li-Zhu Wu,[a] Chun-Feng Duan,[b] Chen-Ho Tung,[a,d] Ya-Feng Guan,[b] Liang Feng[*b] and Qing-Zheng Yang[*a,c]

[a] Key Lab of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China
[b] Key Lab of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, Liaoning 116023, P. R. China
[c] College of Chemistry, Beijing Normal University, Beijing 100875, P. R. China
[d] College of Chemistry, Shandong Normal University, Jinan 250014, P. R. China

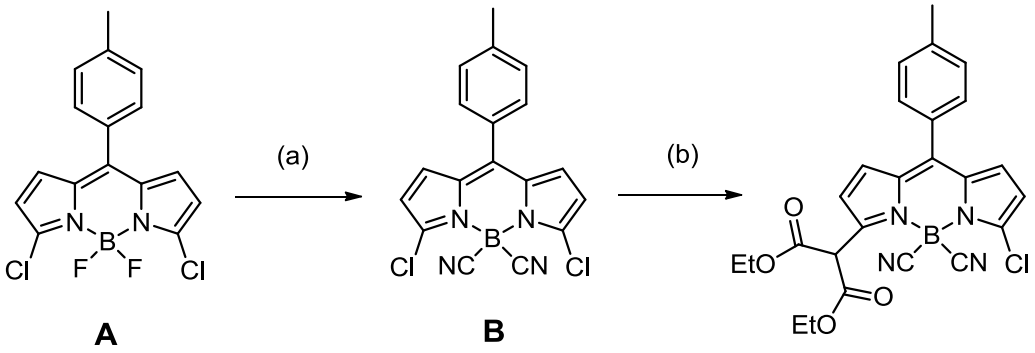
‡ These authors contributed equally to this work.

Materials and instruments

All the reagents and solvents are of commercial quality and without further purification. ^{1}H and ^{13}C NMR spectra were recorded on an Advance Bruker 400M spectrometer and referenced to solvent signals. Mass spectra were obtained on Bruker Apex Ⅳ Fourier Transform Mass Spectrometer. Absorption spectra were determined on a TU-1901 UV-Visible spectrophotometer (Beijing Perkinje General Instrument Co., Ltd, P. R. China).

Synthesis

Scheme S1. Synthesis of sensor **2**.



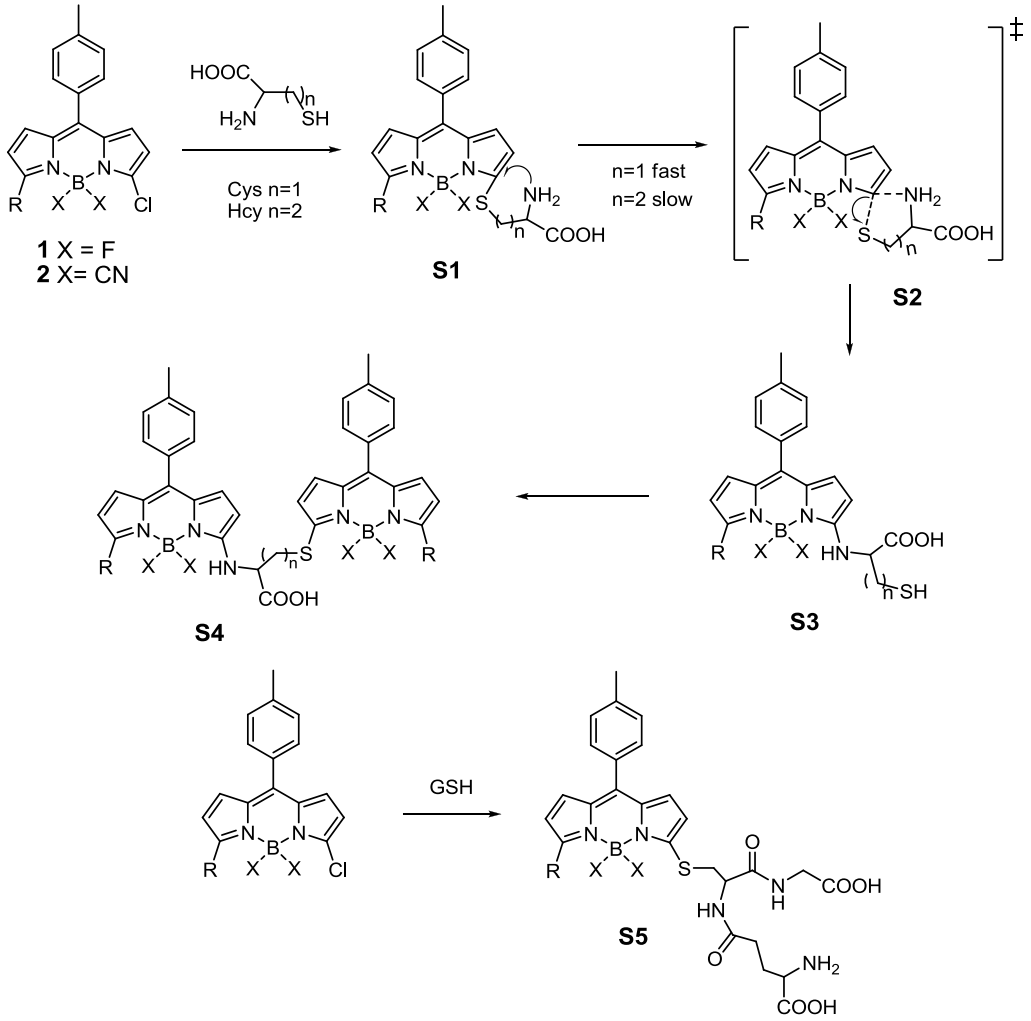
(a) trimethylsilyl cyanide, CH_2Cl_2; (b) diethyl malonate, NaH, CH_3CN.

Boron trifluoride etherate (0.1 ml) was added to a solution of Compound **A** (100 mg, 0.24 mmol) and trimethylsilyl cyanide (0.48 ml) in 10 ml dry dichloromethane. The reaction was stirred at room temperature for 2 h and then quenched with water (5 ml). The residue was extracted with dichloromethane (2 × 10 ml) and washed with sat. aqueous sodium bicarbonate (2 × 10 ml) and water (2 × 10 ml). Then the combined organic layers were dried over anhydrous sodium sulfate and purified through column chromatography over silica (dichloromethane / petroleum ether = 2/1 as eluent) to give Compound **B** (44 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (d, 2H, J = 8.0 Hz), 7.38 (d, 2H, J = 8.0 Hz), 7.07 (d, 2H, J = 4.8Hz), 6.65 (d, 2H, J = 4.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 146.48, 142.48, 132.34, 132.03, 130.80, 132.50, 129.61, 128.70, 119.71, 21.45 (one *C*-obscured). ESI-HRMS: calculated for $[M+K]^+$ 403.00909, found 403.00931.

Compound **2** (50 mg, 0.13 mmol) was dissolved in 20 mL of dry acetonitrile and sodium hydride of 57-63% oil dispersion (12 mg, 0.3 mmol) was added. Diethyl malonate (43 μL, 0.28 mmol) in dry acetonitrile (10 mL) was added dropwise with stirring. After stirring at room temperature for 2 h, the reaction was quenched with water and extracted with dichloromethane. The organic layer was dried over $MgSO_4$, concentrated and purified through column chromatography over silica

(dichloromethane / petroleum ether = 3/1 as eluent) to give **2** (44 mg, 69%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, 2H, *J* = 8.0 Hz), 7.37 (d, 2H, *J* = 8.0 Hz), 7.09 (d, 1H, *J* = 4.4 Hz), 7.03 (d, 1H, *J* = 4.4 Hz), 6.93 (d, 1H, *J* = 4.4 Hz), 6.61 (d, 1H, *J* = 4.4 Hz), 5.54 (s, 1H), 4.31 (q, 4H, *J* = 7.2 Hz), 2.49 (s, 3H), 1.33 (t, 6H, *J* = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.27, 152.34, 146.45, 144.75, 142.30, 133.44, 132.62, 132.07, 130.88, 130.60, 129.55, 129.42, 128.91, 122.08, 119.35, 62.99, 52.55, 21.50, 13.96.ESI-HRMS: calculated for $[M+Na]^+$ 511.13203, found 511.13195.

Scheme S2. The proposed reaction mechanism with excessive BODIPY sensors.



Scheme S3. The deduction for additivity.

Based on the reaction mechanism，suppose there exist absorptions for BODIPY sensors (***I***) and products (***S₁***, ***S₃***, ***S₄***, ***S₅***) at certain wavelength. The molar absorptivity (molar absorption coefficient) for ***I*** is ε_I; the molar absorptivity of product ***S₁***, ***S₃*** and ***S₄*** for Cys is $\varepsilon_{S_{1C}}$, $\varepsilon_{S_{3C}}$, and $\varepsilon_{S_{4C}}$, respectively; meanwhile, the molar absorptivity of S_1,S_3 and S_4 for Hcy is $\varepsilon_{S_{1H}}$, $\varepsilon_{S_{3H}}$, and $\varepsilon_{S_{4H}}$, respectively; the molar absorptivity of S_5 is ε_{S_5}.

We define that the initial concentration of BODIPY sensor (***I***) is $[I]_0$. After 1h reaction of ***I*** with Cys, the concentration of ***S₁***, ***S₃*** and ***S₄*** is defined as $[S_{1C}]$, $[S_{3C}]$ and $[S_{4C}]$, respectively. In a same way, with Hcy, the concentration of ***S₁***, ***S₃*** and ***S₄*** is defined as $[S_{1H}]$, $[S_{3H}]$ and $[S_{4H}]$ respectively, and with GSH, the concentration of S_5 is $[S_5]$.

After 1 h reaction, the original absorbance of ***I*** was subtracted from the final absorbance of reaction mixture to get the change of absorbance, ΔAbs. The absorbance change after the reaction of thiols with ***I*** is as follow(ΔA_C for Cys, ΔA_H for Hcy, ΔA_G for GSH; b is the optical path of cell):

$$\Delta A_C=A_c-A_0= \varepsilon_{S_{1C}} b [S_{1C}] + \varepsilon_{S_{3C}} b [S_{3C}] + \varepsilon_{S_{4C}} b [S_{4C}] + \varepsilon_I b ([I]_0 - [S_{1C}] - [S_{3C}] - [S_{4C}]) - \varepsilon_I b [I]_0$$

$$\Delta A_H=A_H-A_0= \varepsilon_{S_{1H}} b [S_{1H}] + \varepsilon_{S_{3H}} b [S_{3H}] + \varepsilon_{S_{4H}} b [S_{4H}] + \varepsilon_I b ([I]_0 - [S_{1H}] - [S_{3H}] - [S_{4H}]) - \varepsilon_I b [I]_0$$

$$\Delta A_G=A_G-A_0= \varepsilon_{S_{5H}} b [S_{5H}] + \varepsilon_I b ([I]_0 - [S_5]) - \varepsilon_I b [I]_0$$

In the mixture, three individual biothiol reacts with BODIPY sensors competitively. To keep the reaction in the mixture as close as possible to those in the single analyte-sensor system, the consumption of BODIPY sensor during the reaction should be small enough to keep the reaction equilibrium with biothiols unaffected. In the presence of largely excessive ***I***, the concentration change of the sensor could be neglected during the reaction due to its large amount, so that the reaction rate of each thiol in their mixture is equal to that in the single thiol-***I*** system**.** After 1 h reaction, the absorbance change of the thiols mixture is as follow.

$$\Delta A_m=A_m-A_0= \varepsilon_I b ([I]_0- [S_{1C}] - [S_{1H}] - [S_{3C}]- [S_{3H}]- [S_{4C}]- [S_{4H}]- [S_5]) + \varepsilon_{S_{1C}} b [S_{1C}] + \varepsilon_{S_{1H}} b [S_{1H}] + \varepsilon_{S_{3C}} b [S_{3C}] + \varepsilon_{S_{3H}} b [S_{3H}] + \varepsilon_{S_{4C}} b [S_{4C}] + \varepsilon_{S_{4H}} b [S_{4H}] + \varepsilon_{S_{5H}} b [S_{5H}]- \varepsilon_{I_1} b [I]_0$$

$$=\{ \varepsilon_{S_{1C}} b [S_{1C}] + \varepsilon_{S_{3C}} b [S_{3C}] + \varepsilon_{S_{4C}} b [S_{4C}] + \varepsilon_I b ([I]_0 - [S_{1C}] - [S_{3C}] - [S_{4C}]) - \varepsilon_I b [I]_0\} +$$

$$\{ \varepsilon_{S_{1H}} b [S_{1H}] + \varepsilon_{S_{3H}} b [S_{3H}] + \varepsilon_{S_{4H}} b [S_{4H}] + \varepsilon_I b ([I]_0 - [S_{1H}] - [S_{3H}] - [S_{4H}]) - \varepsilon_I b [I]_0\} +$$

$$\{ \varepsilon_{S_{5H}} b [S_{5H}] + \varepsilon_I b ([I]_0 - [S_5]) - \varepsilon_I b [I]_0\}$$

$$= \Delta A_C + \Delta A_H + \Delta A_G$$

It indicates that, in the condition of largely excessive ***I***, the normalized spectra thiols mixture is the summary of normalized spectrum of each single thiol. That is, the additivity is valid.

Scheme S4. The deduction of final concentration of each product of the reaction between BODIPY sensors and Cys, Hcy and GSH.

The reactions of biothiols with ***I*** are as follow: (the k_1, k_2, k_3, and k_4 is corresponding rate constant)

$$I + A_{C/H} \xrightarrow{k_1} S_1 + HCl$$

$$S_1 \xrightarrow{k_2} S_2 \rightarrow S_3$$

$$S_3 + I \xrightarrow{k_3} S_4 + HCl$$

$$I + A_G \xrightarrow{k_4} S_5 + HCl$$

Since the reactions are carried out in HEPES buffer solution, the effect of HCl is ignorable. The concentration change of excessive ***I*** is also neglectable.

For the reactions of Cys/Hcy with ***I***, the rate equations of reactions could be expressed as follow:

$$\frac{d[A_C]}{dt} = -k_1[A_C]$$

$$\frac{d[S_1]}{dt} = k_1[A_C] - k_2[S_1]$$

$$\frac{d[S_3]}{dt} = k_2[S_1] - k_3[S_3]$$

$$\frac{d[S_4]}{dt} = k_3[S_3]$$

When t=0，$[A_C]= [A_C]_0$，$[S_1]= [S_3]= [S_4]=0$.

Based on the principle of mass conservation, the follow equation is valid at any time.

$$[A_C]_0 = [A_C] + [S_1] + [S_3] + [S_4]$$

The differential equation of it is as follow.

$$\frac{d[A_C]}{dt} + \frac{d[S_1]}{dt} + \frac{d[S_3]}{dt} + \frac{d[S_4]}{dt} = 0$$

It indicates that, in the four differential rate equations of reaction, three are independent. Solving the equations, the results are as follows:

$$[A_C] = [A_C]_0 e^{-k_1 t}$$

$$[S_1] = \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t})[A_C]_0$$

$$[S_3] = [\frac{k_1 k_2}{(k_2 - k_1)(k_3 - k_1)}(e^{-k_1 t} - e^{-k_3 t}) + \frac{k_1 k_2}{(k_2 - k_1)(k_2 - k_3)}(e^{-k_2 t} - e^{-k_3 t})][A_C]_0$$

$$[S_4] = [A_C]_0 - [A_C] - [S_1] - [S_3] = [1 - e^{-k_1 t} - \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) - \frac{k_1 k_2}{(k_2 - k_1)(k_3 - k_1)}(e^{-k_1 t} - e^{-k_3 t})$$

$$- \frac{k_1 k_2}{(k_2 - k_1)(k_2 - k_3)}(e^{-k_2 t} - e^{-k_3 t})][A_C]_0$$

Since the reaction time (“t”) is constant (1h) in our case, then:

$$[A_C] = K_{A_C}[A_C]_0; \qquad [S_1] = K_{S_1}[A_C]_0; \qquad [S_3] = K_{S_3}[A_C]_0; \qquad [S_4] = K_{S_4}[A_C]_0$$

In the same way, for the reactions of GSH with ***I***, the rate equations of reactions could be expressed as follow:

$$\frac{d[A_G]}{dt} = -k_4[A_G]$$

When t=0，$[A_G]= [A_G]_0$，$[S_5] =0$.

Solving the equations, the results are as follows:

$$[A_G] = [A_G]_0 e^{-k_4 t} = K_{A_G}[A_G]_0$$

$$[S_5] = [A_G]_0 - [A_G] = [1 - e^{-k_4 t}][A_G]_0 = K_{S_5}[A_G]_0$$

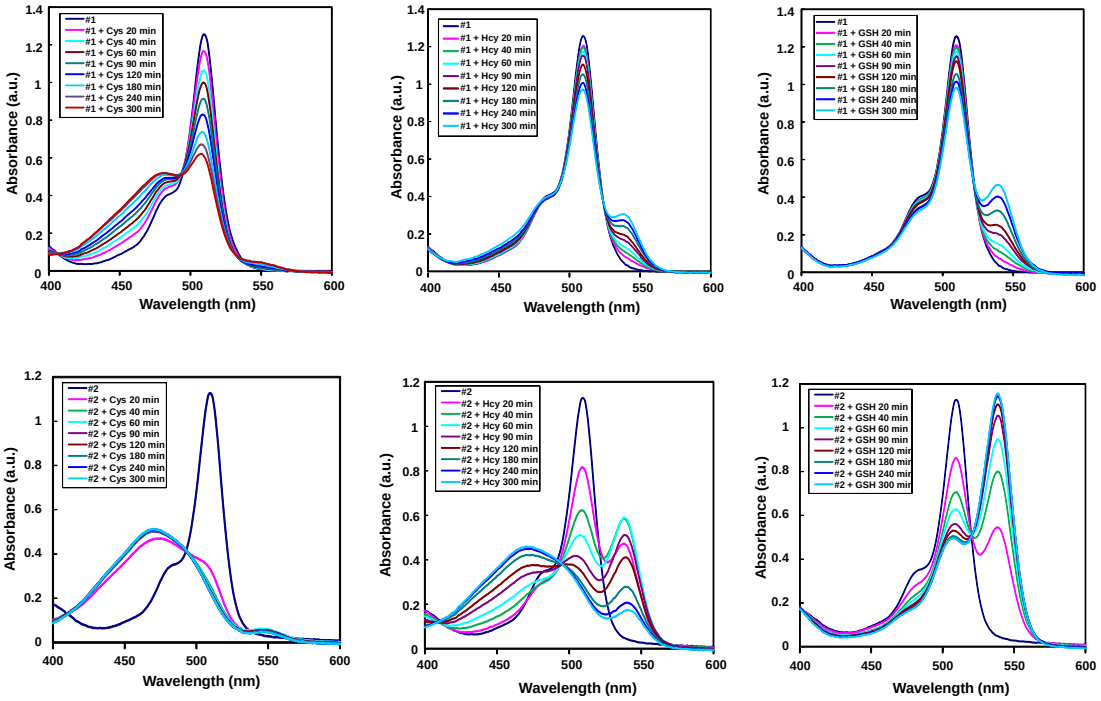


Fig. S1. The absorption spectra of BODIPY sensor **1** (20 μM) and **2** (20 μM) after addition of 100 μM Cys, Hcy, or GSH in acetonitrile/HEPES buffer (v:v=2:3, 20 mM, pH=7.4) at 25 ℃.

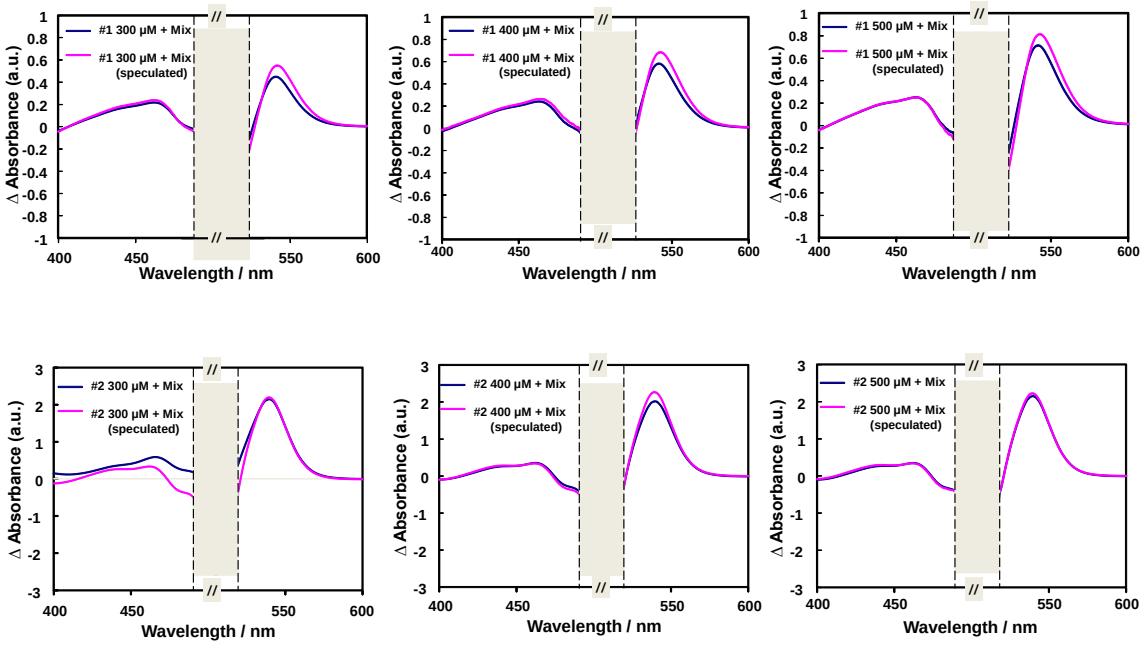


Fig. S2. The experimental and speculated normalized absorbance spectra for the biothiols mixture. Each single spectrum of reaction mixture of individual biothiol (Cys, Hcy, or GSH) at 80 μM with ***I*** at gradually increasing concentrations were collected, and a mixed spectrum was speculated. At the same time, the spectrum of biothiols mixture after reacting with BODIPY sensors was also collected as experimental spectrum.

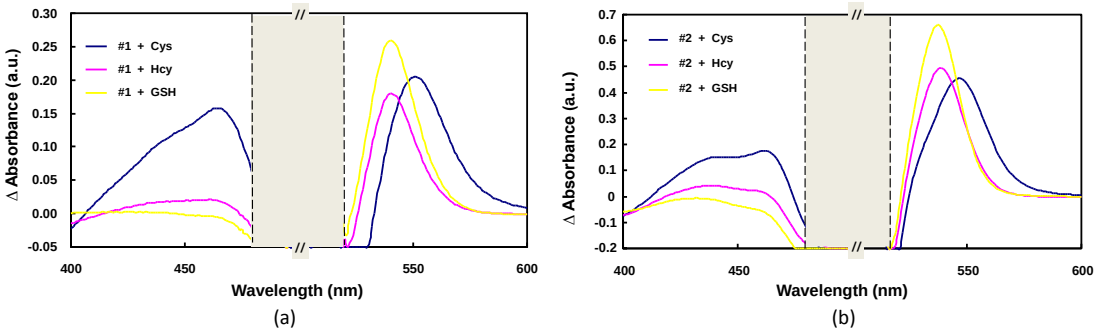


Fig. S3. The normalized absorption spectra of BODIPY sensor **1** (a) and **2** (b) (500 μM) after addition of 50 μM Cys, Hcy or GSH in acetonitrile/HEPES buffer (v:v=2:3, 20 mM, pH=7.4) at 25 ℃.

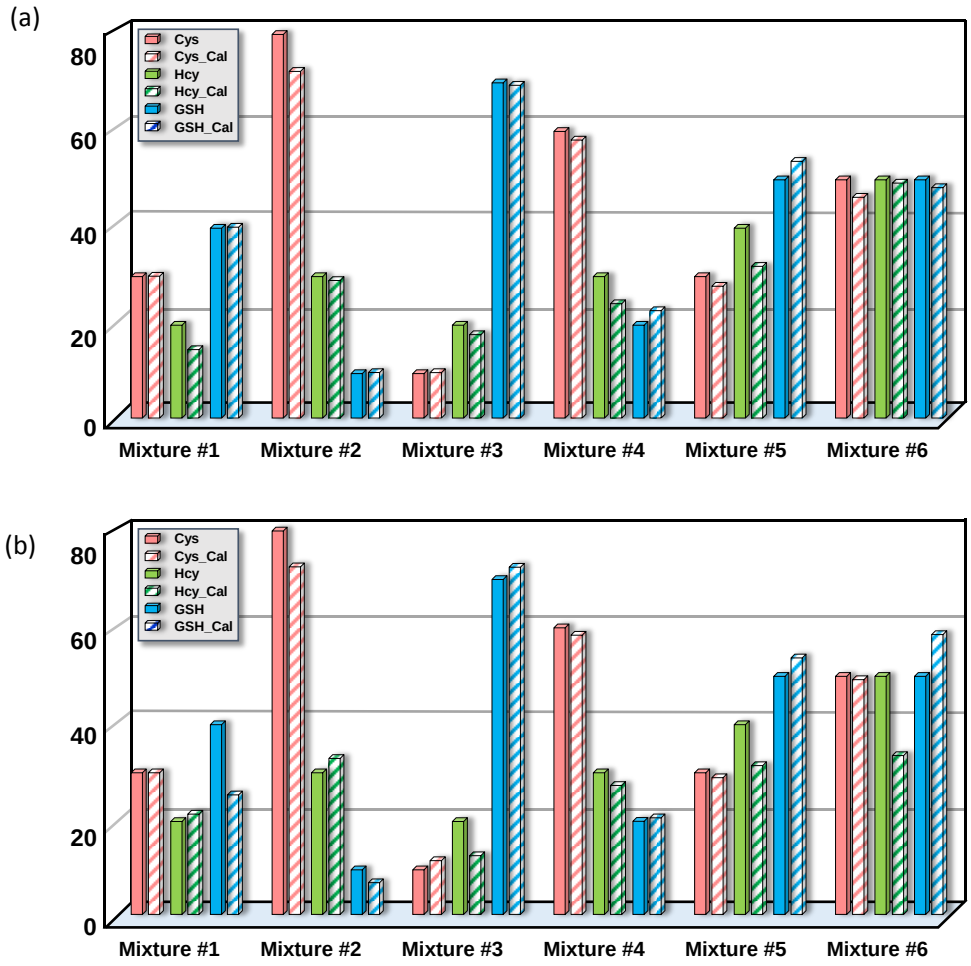


Fig. S4. The experimental and calculated concentrations of Cys/Hcy/GSH in the mixture under different wavelength combination (a) 445nm and 540nm of sensor **1**, 530nm of sensor **2**; (b) 440nm and 543nm of sensor **1**, 530nm of sensor **2**.

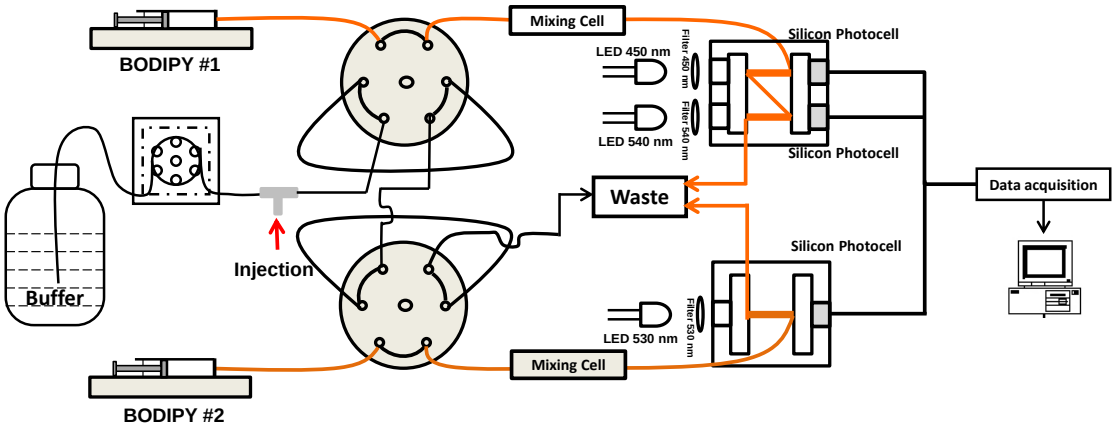


Fig. S5. The schematic for home-made prototype machine.