## **Supporting Information**

## A FRET-based ratiometric fluorescent probe for highly selective detection of cysteine based on a coumarin-rhodol derivative

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Figure S1. The effect of CH<sub>3</sub>CN volume fraction on fluorescence intensity ratio ( $I_{543nm}/I_{470nm}$ ) of probe 1 (10  $\mu$ M) in the absence (filled circles) and presence of 100  $\mu$ M cysteine (clear circles).



Figure S2. Fluorescence emission spectra of compound **5** (10.0  $\mu$ M) and the reaction mixture of fluorescent probe **1** (10.0  $\mu$ M) with cysteine (100  $\mu$ M) in 0.01 M PBS buffer (CH<sub>3</sub>CN/water = 6:4, V/V, pH=7.40). The solid line (—) and medium dash (– ) represent compound **5** and the reaction mixture of fluorescent probe **1** with cysteine, respectively.  $\lambda_{ex} = 418$  nm.



Figure S3. The ESI-MS data of reaction mixture of probe 1 with Cys for 10 min



Figure S4. Time course of fluorescence intensity ratio ( $I_{543nm}/I_{470nm}$ ) of probe **1** (10 µM) in the absence (filled circles) and presence of 100 µM cysteine (clear circles).Time points represent 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 and 80 min. The inset show the visual fluorescence color of probe **1** (10 µM) before (left) and after (right) incubation with cysteine for 60 min (UV lamp, 365 nm).

NMR and MS data for compounds



Figure S5. <sup>1</sup>H NMR spectrum of compound **2** in CD<sub>3</sub>OD.



**Figure S6**. <sup>1</sup>H NMR spectrum of compound 3 in DMSO- $d_6$ .



Figure S7. <sup>1</sup>H NMR spectrum of compound 4 in CDCl<sub>3</sub>.



Figure S8. <sup>1</sup>H NMR spectrum of compound 5 in CDCl<sub>3</sub>.



**Figure S9**. <sup>1</sup>H NMR spectrum of compound 6 in DMSO- $d_6$ .



Figure S10. ESI-MS spectrum of compound 6.



Figure S11. <sup>1</sup>H NMR spectrum of compound 1 in CDCl<sub>3</sub>.



Figure S12. <sup>13</sup>C NMR spectrum of compound 1 in CDCl<sub>3</sub>.



Figure S13. DEPT135 spectrum of compound 1 in  $CDCl_3$ .



Figure S14. ESI-MS spectrum of compound 1.