Supplementary Material (ESI) for Chemical Communications

Fluorescence Probes for Thiol-Containing Amino Acids and Peptides in Aqueous Solution

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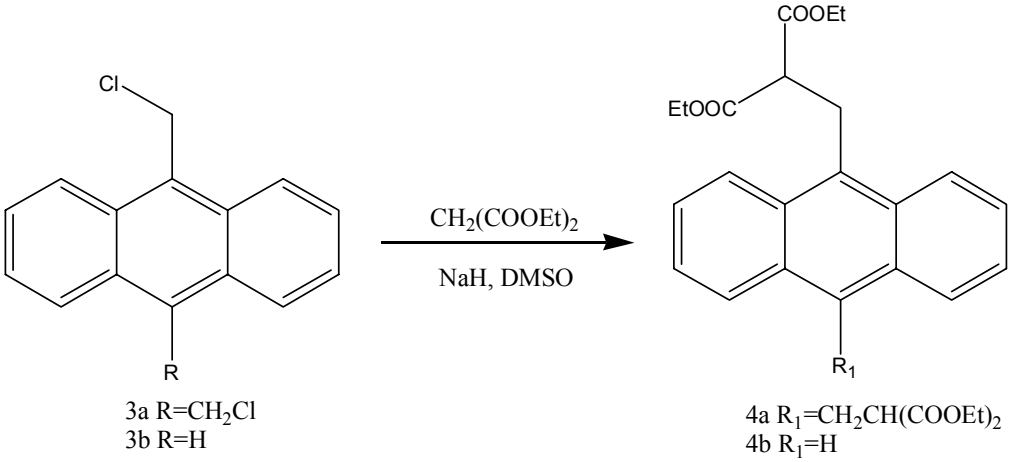
Experimental section



3a $R=CH_2Cl$
3b R=H

4a $R_1=CH_2CH(COOEt)_2$
4b $R_1=H$

1 $R_2=CH_2CH(COOK)_2$
2 $R_2=H$

Synthesis of **4a**

A suspended solution of sodium hydride (60% in mineral oil, 0.16g, 4.16 mmol) in 30 mL DMSO was added diethyl malonate (1.6g, 10.6mmol). After the solution was clear, **3a** (0.4g, 1.76 mmol) was added. The reaction mixture was refluxed for 5 hours, then cooled to room temperature. The reaction mixture was poured into 50 mL ice-cold HCl (0.04 M) aqueous solution, and the solid was collected. The solid was purified by flash chromatography (hexane/dichloromethane =1:9 v/v) to give **4a** as yellow solid (0.57 g, 62.5%). M.p.: 177□. ^{1}H-NMR (300 Hz, $CDCl_3$): δ 8.32 (d, 4H), 7.51 (d, 4H), 4.30(d, 4H), 4.02 (m, 8H), 3.84 (t, 2H), 1.05 (m, 12H). MS(EI): 522 (M+).

Synthesis of **4b**

4b was synthesized in the similar procedure as **4a** and obtained as yellow solid .Yield 63%. M.p.: 81□. ^{1}H-NMR (300 Hz, $CDCl_3$): δ 8.39(s, 1H), 8.26(d, 2H), 8.02 (d, 2H), 7.55-7.43 (m, 4H), 4.31 (d, 2H), 4.13-3.96 (m, 4H), 3.87 (t, 1H), 1.05 (t, 6H). MS(EI): 350 (M+).

Synthesis of **1**

A solution of **4a** (0.45g, 0.857 mmol) in 10 mL CH_3OH and 10 mL H_2O was added KOH (0.2 g, 3.58 mmol).The reaction mixture was refluxed overnight, then solvent was removed under vacuum. The residue was washed with 20

mL CH_2Cl_2 and 3×5 mL CH_3OH to give **1** as light yellow solid (0.46g, 95%). M.p>300□. ^{1}H-NMR (300Hz, D_2O): δ 8.31(m, 4H), 7.47(m, 4H), 4.00(d, 4H), 3.41(t, 2H). HRMS(FAB, negative) Calc. for $C_{22}H_{14}O_8K_3$, [M-H peak] m/z=522.9605; Found: 522.9600.

Synthesis of **2**

2 was synthesized in similar procedure as **1** and obtained as light yellow solid. Yield, 90% . M.p >300□. ^{1}H-NMR (300 Hz, D_2O): δ 8.32 (s, 1H), 8.23 (d, 2H), 7.94 (d, 2H), 7.36-7.46 (m, 4H), 3.95 (d, 2H), 3.38 (t, 1H). HRMS (FAB, negative) Calc. for $C_{18}H_{12}O_4K$, [M-H peak] m/z=331.0378; Found: 331.0376.

The UV-Visible absorption and fluorescence emission responses of **1** and **2** upon addition of Cu^{2+}

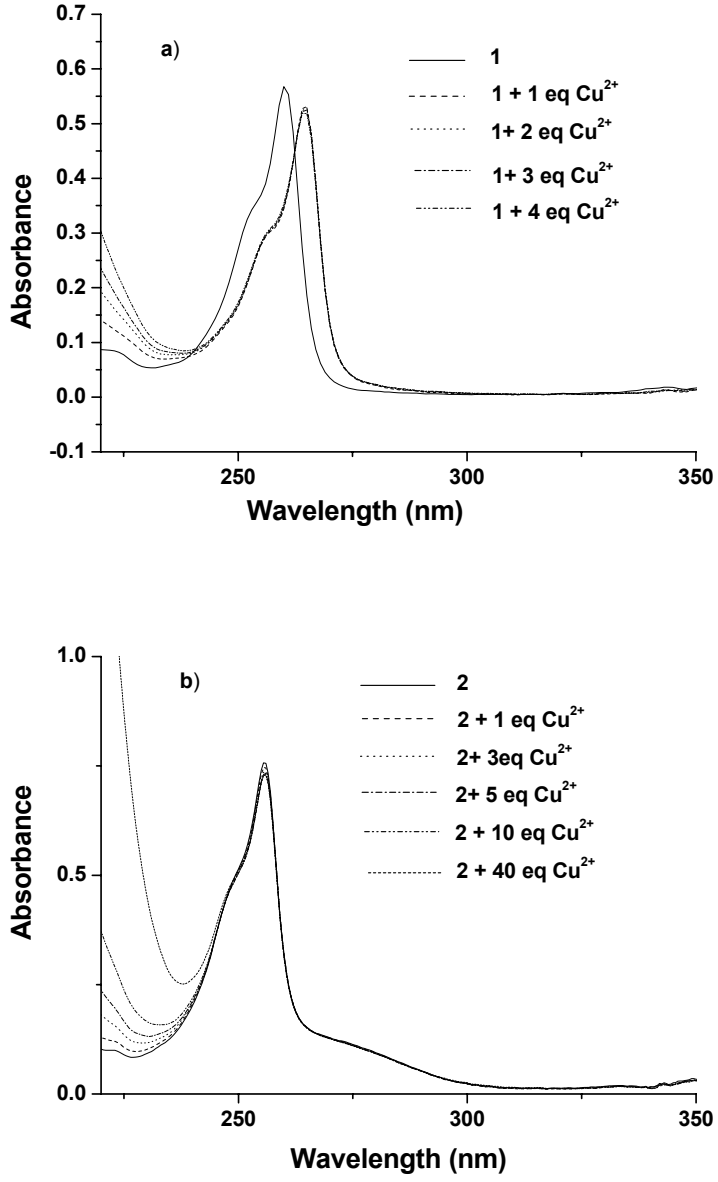


Figure s1. The UV-vis responses of **1** and **2** upon addtion of Cu^{2+} a) **1** (5×10^{-6} M); b) **2** (5×10^{-6} M).

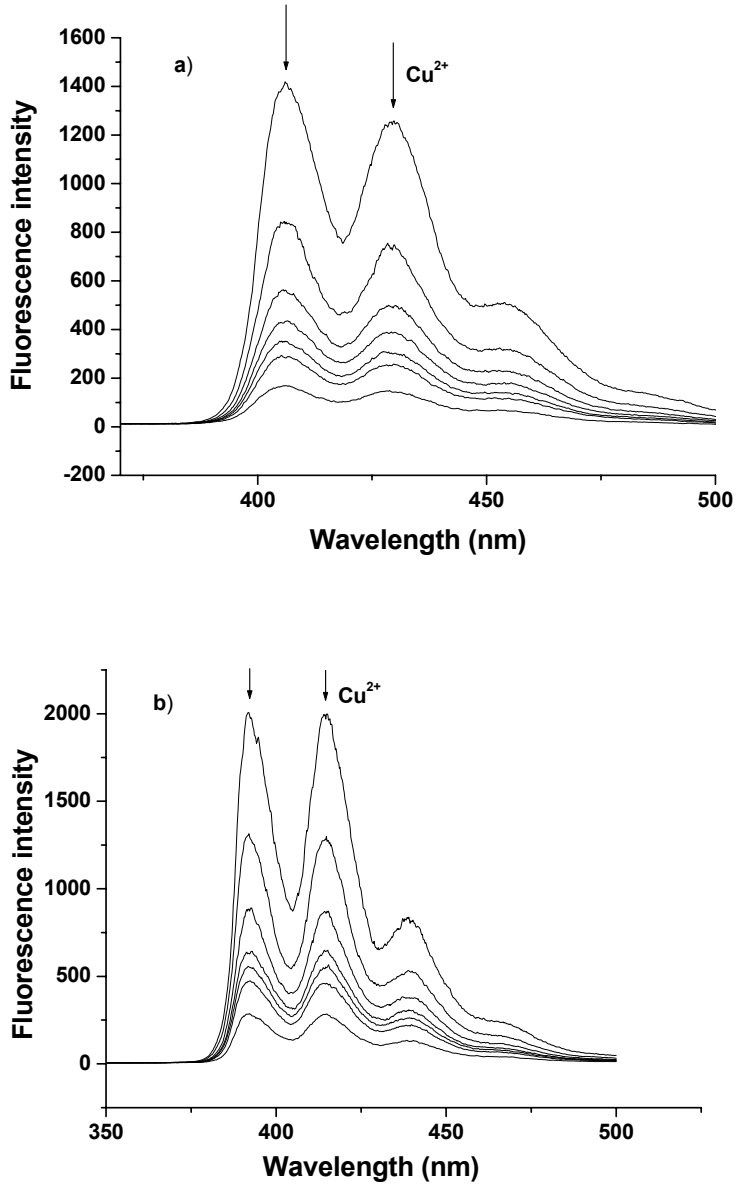


Figure s2 The fluorescence emission responses of **1** and **2** upon addition of Cu^{2+} a) **1** (5×10^{-7} M) with Cu^{2+} (0eq, 0.2eq, 0.4eq, 0.6eq, 0.8eq, 1eq, 2eq); b) **2**. (5×10^{-6} M) with Cu^{2+} (0eq, 1eq, 2eq, 3eq, 4eq, 5eq, 10eq)

The fluorescence emission responses of **2*Cu** before and after addition of amino acids and peptides

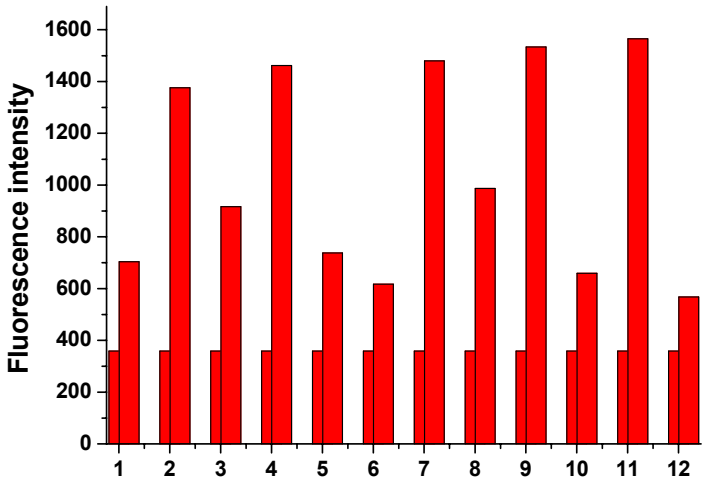


Figure s3 Fluorescene intensity changes of **2*Cu**$^{2+}$ (10^{-5} M, aqueous solution) before and after the addition of 10 eq of amino acids and peptides: 1. L-glycine; 2. L-glutamic acid; 3. methionine; 4. L-arginine; 5. L-tyrosine; 6. L-tryptophan; 7. L-glutathione; 8. L-serine; 9. L-cysteine; 10. L-ornithine; 11. homocysteine; 12. L-histidine

The relationship of time *vs* fluorescence intensity of the solution after analytes are added to **1*Cu**

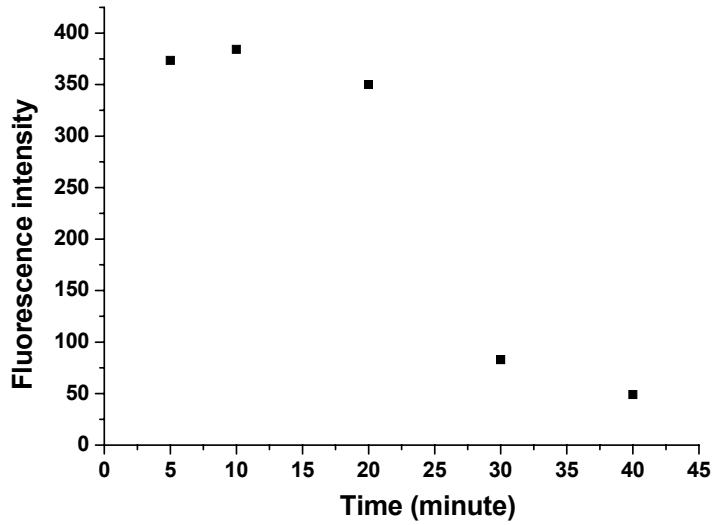


Figure s4 The relationship of time *vs* fluorescence intensity of the solution upon addition of cysteine (**1*Cu**: 5×10^{-7} M, cysteine: 5×10^{-6} M)

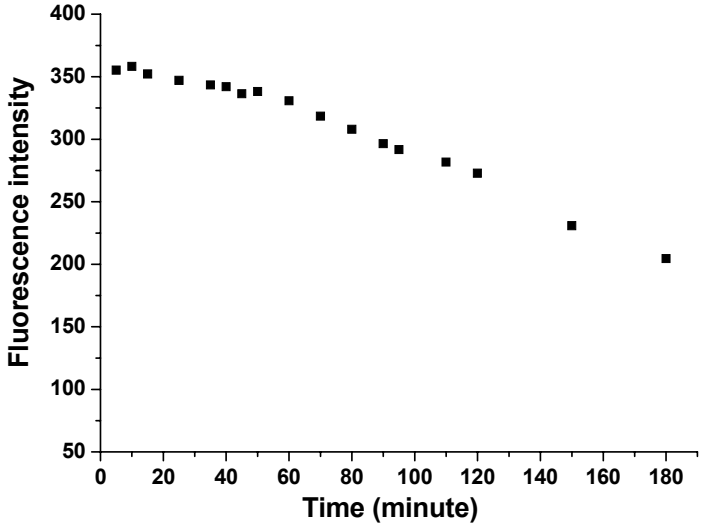


Figure s5 The relationship of time *vs* fluorescence intensity of the solution upon addition of homocysteine (**1*Cu**: 5×10^{-7} M, homocysteine: 5×10^{-6} M)

Absorption spectra of **1*Cu** with the addition of cysteine.

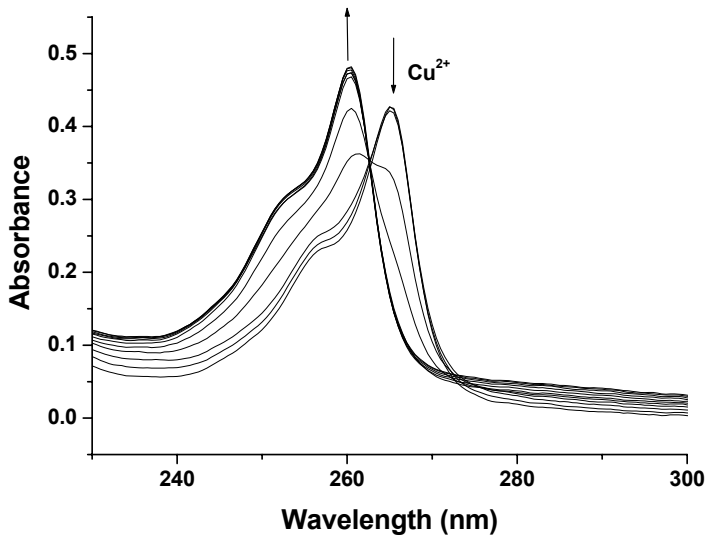


Figure s6 The absorption response of **1*Cu** (5×10^{-6} M)with the addition of cysteine (0eq, 1eq, 2eq, 3eq, 4eq, 5eq, 6eq, 7eq, 8eq, 9eq, 10eq).

Titration of 1*Cu with thiol-containing amino acids

Thiol-containing amino acids (cysteine and homocysteine) are used in the spectrofluorimetric titrations titration. The probe is formed *in situ* by adding 2 eq of Cu^{2+} to a aqueous solution of **1** (5×10^{-7} M), then thiol-containing amino acids are added to the probe until a constant value of I_{max} is reached. The apparent binding constants are defined by the equations $K=[Cu^*S]_{tot}/[1^*Cu]_{tot}[S]_{tot}$, where $[Cu^*S]_{tot}$, $[1^*Cu]_{tot}$ and $[S]_{tot}$(substrate: amino acid) are the total concentration of **Cu*S**, **1*Cu** and **S** in all their protonated and deprotonated forms at a given pH in water solution, and 1:1 probe/substrate interaction are assumed.

The apparent binding constants are obtained from the following equation:[1]

$F_0/(F-F_0)=a/(K[S])+a$

Where F_0 and F are the luminescence intensity of **1*Cu** solution in the absence and presence of amino acid/peptide substrate; a is constant; K is the apparent binding constants.

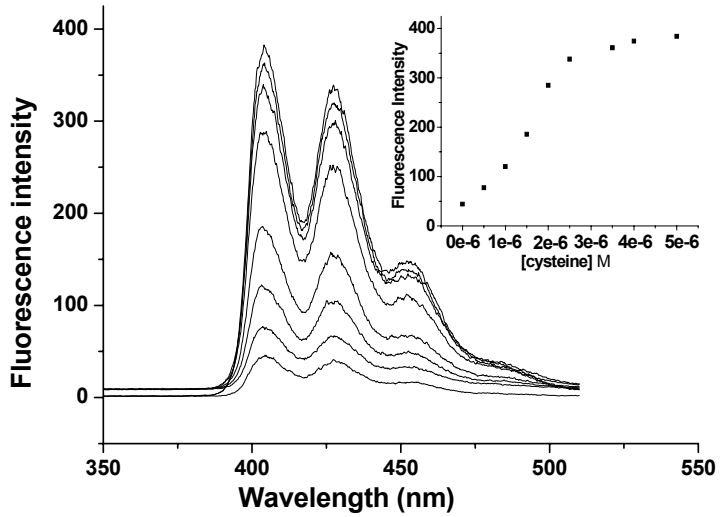


Figure S7 Titration of **1*Cu** (5×10^{-7} M) with cysteine in aqueous solution (pH=7). Inset: fluorescence intensity changes with the addition of cysteine.

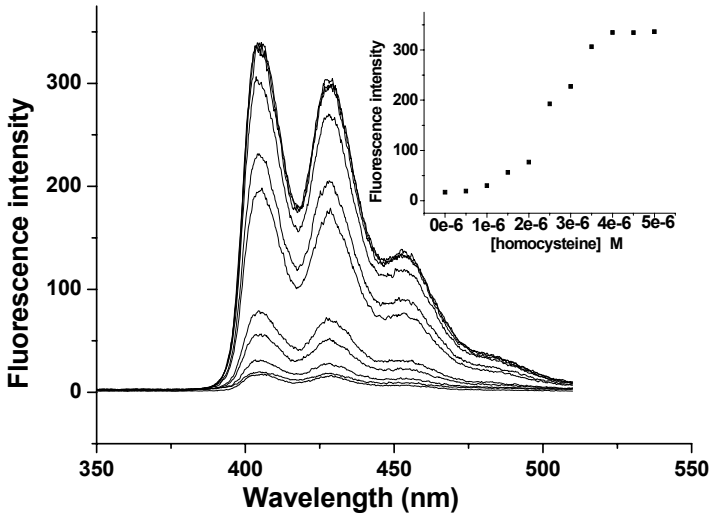


Figure s8 Titration of **1*Cu** (5×10^{-7} M) with homocysteine in aqueous solution (pH=7). Inset: fluorescence intensity changes with the addition of homocysteine.

Reference:

1. "*Comprehensive Supramolecular Chemistry*" vol. 8, H.Tsukube ed. Chap. 10, p 425-482, Pergamon Press (1996)