Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Content

Colorimetric and ON-OFF-ON fluorescent chemosensor for the sequential detection of Cu(II) and Cysteine and its application in imaging of living cells

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Equation for calculation of binding constant for 1:1 stoichiometry using UV-Visible spectral data

$$\frac{\Delta A}{b} = \frac{Q_t K_a \Delta\varepsilon[L]}{1 + K[L]} \qquad \text{(eq 1)}$$

where, ΔA refers to the change in absorbance from initial value at the required wavelength, b is cuvette path length (in cm), Q_t is total concentration of sensor, K_a is the apparent binding constant, $\Delta\varepsilon$ is the change in extinction coefficient between free and bound sensor and $[L]$ is the concentration of Cu^{2+} ion or Cysteine.

Equation for calculation of binding constant for 1:1 stoichiometry using Fluorescence spectral data

$$\frac{I_0}{I} = 1 + K[L] \qquad \text{(eq 2)}$$

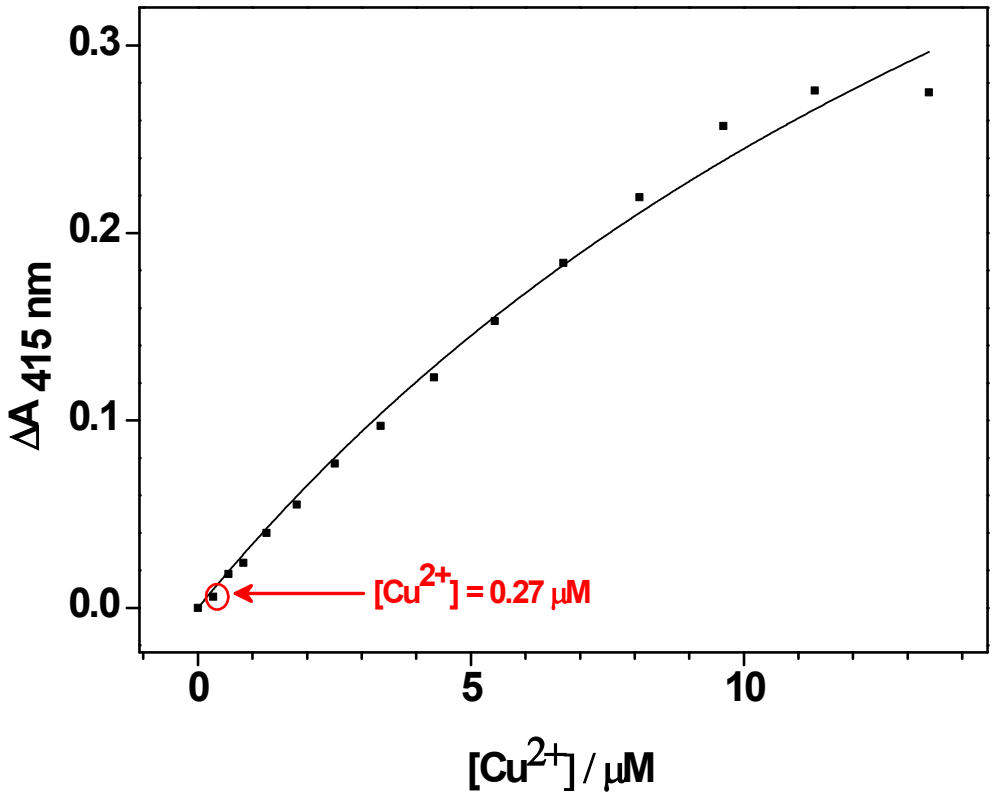
where, I_0 is the fluorescence intensity of the sensor **1** in the absence of Cu^{2+} ion, I is fluorescence intensity due to **1**-Cu(II) upon addition of Cu^{2+} ion, K is the equilibrium constant and [L] is the concentration of Cu^{2+} ion.

$$\frac{I}{I_0} = \frac{1 + \left(\frac{k_f}{k_s}\right) K[L]}{1 + K[L]} \qquad \text{(eq 3)}$$

where, I_0 is the fluorescence intensity of the **1**-Cu(II) in the absence of Cysteine, I is fluorescence intensity due to free **1** upon addition of Cysteine, k_f is proportionality constant of the free sensor **1**, k_s is proportionality constant of the **1**-Cu(II), K is the equilibrium constant and [L] is the concentration of Cysteine.

Calculation method for detection limit:

To determine the detection limit, UV-Visible titration of **1** with Cu^{2+} is carried out by adding aliquots of micromolar concentration of Cu^{2+}. The lowest concentration of Cu^{2+} that caused a sharp change in the absorbance is recorded as experimental and real detection limit.



Inset of Fig. 1. Scatter plot of the experimental data (change in absorbance for **1** at 415 nm ($\Delta A_{415\ nm}$) vs. [Cu^{2+}]), obtained from the UV-Visible spectral change for **1** upon addition of Cu^{2+} in DMSO/H_2O (3:7 v/v) at 298 K. [**1**] = 1.3×10^{-5} M, [Cu^{2+}] = $(0 - 1.33) \times 10^{-5}$ M.

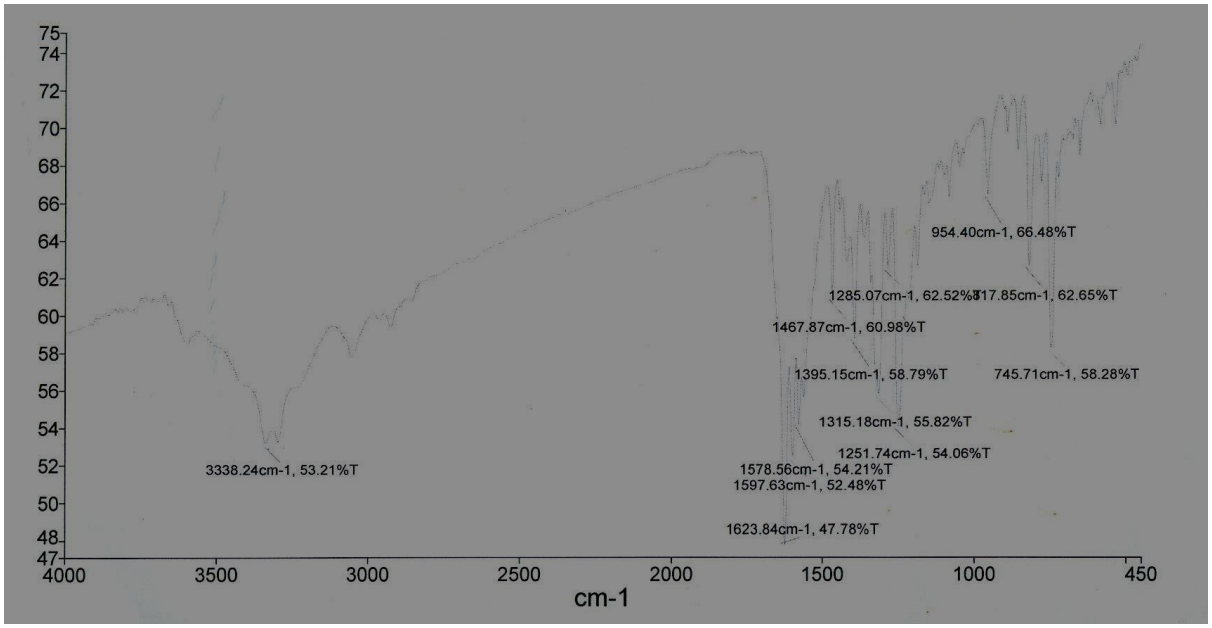


Fig. S1. FTIR Spectrum of **1**.

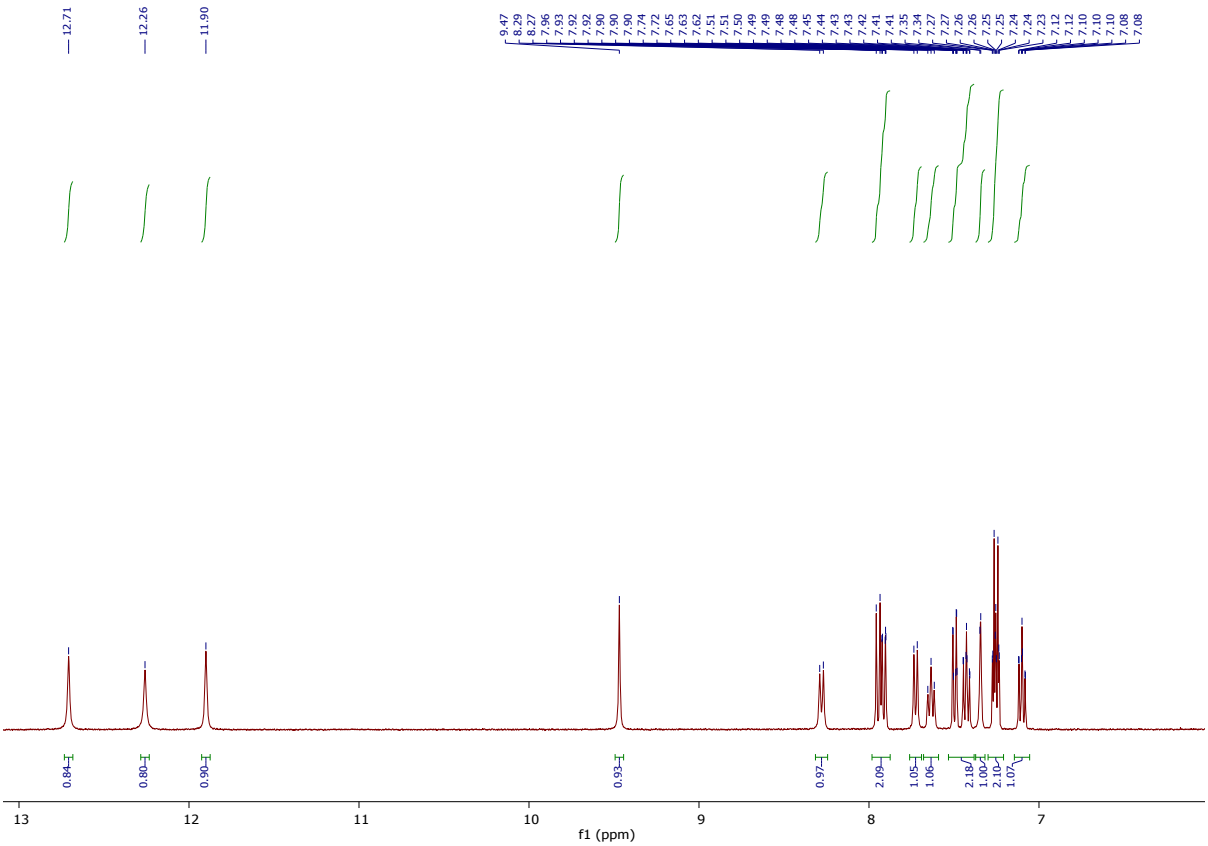


Fig. S2. ^{1}H NMR of **1** (400 MHz, DMSO-d_6).

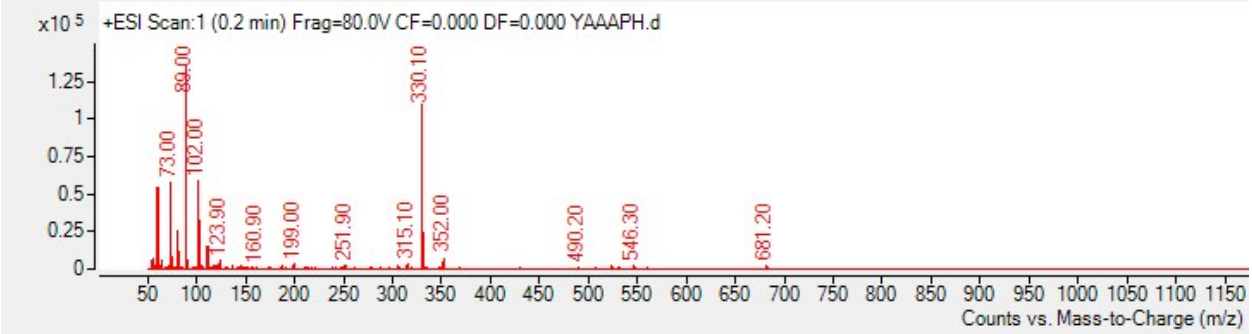


Fig. S3. ESI-MS of **1**.

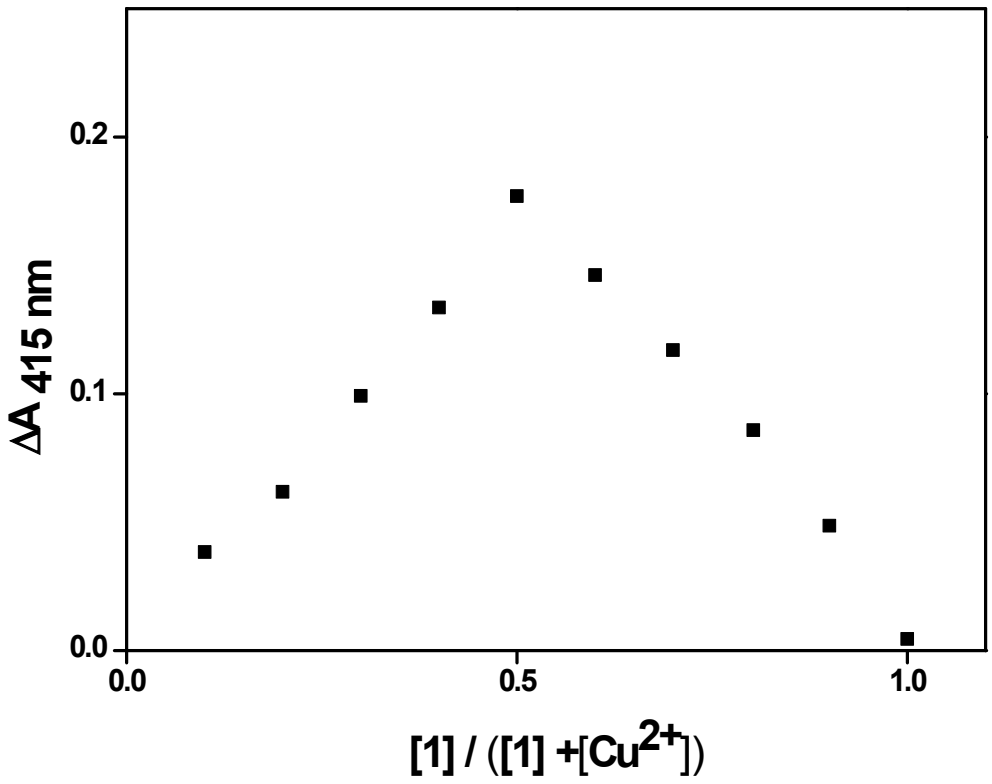


Fig. S4. The stoichiometric analysis of **1** with Cu^{2+} by Job′s plot; ([**1**] + [Cu^{2+}]) = 2×10^{-5} M in DMSO/H_2O (3:7 v/v).

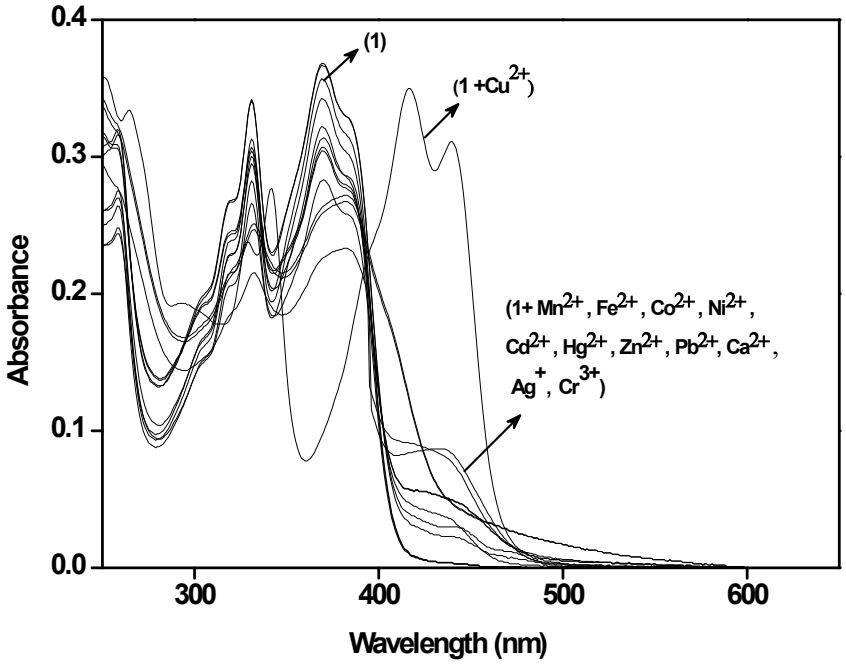
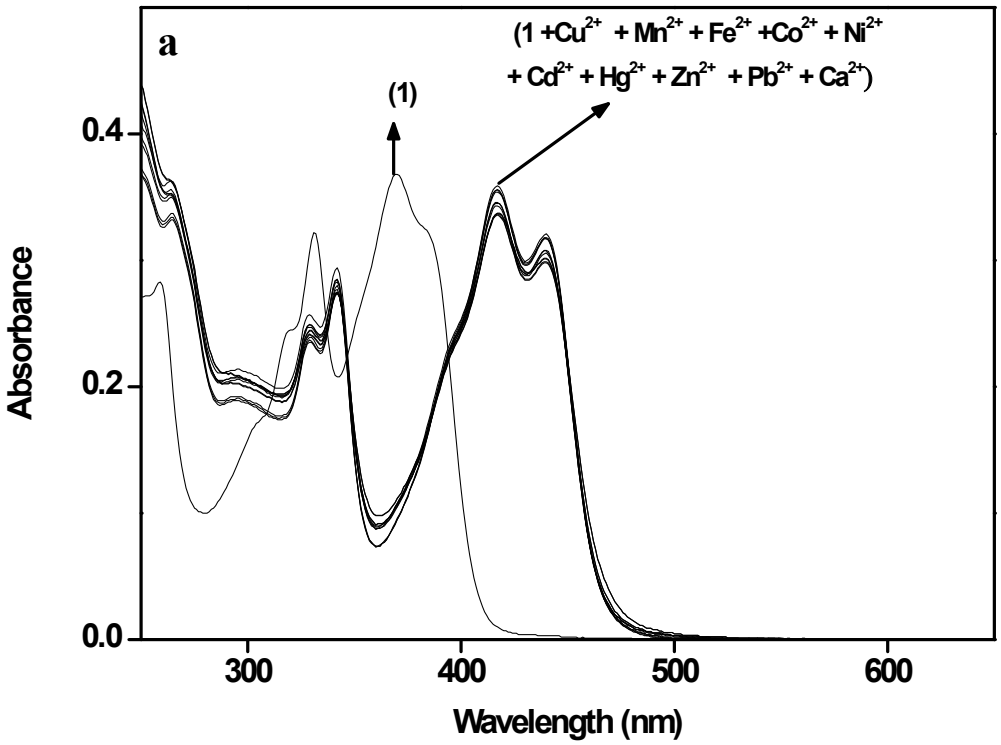


Fig. S5. UV-Visible spectrum of **1** (1.9×10^{-5} M) upon addition of different cations (2 equivalents) in DMSO/H_2O (3:7 v/v).



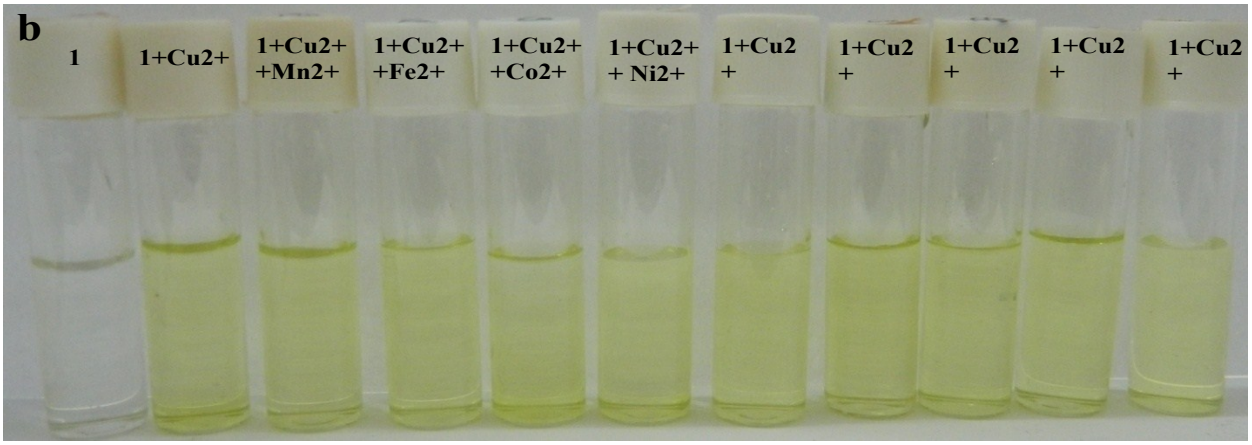


Fig. S6. (**a**) Absorption spectral changes observed for **1** (1.9×10^{-5} M) with Cu^{2+} (2 equivalents) in the presence of 2 equivalents of other cations in DMSO/H_2O (3:7 v/v). (**b**) Color changes observed for **1** (1.9×10^{-5} M) with Cu^{2+} (2 equivalents) in the presence of 2 equivalents of other cations in DMSO/H_2O (3:7 v/v).

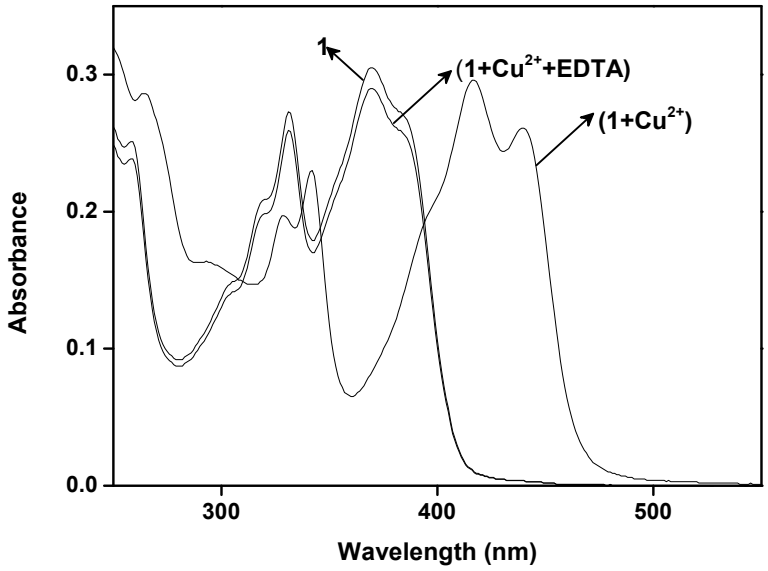


Fig. S7. UV-Visible spectral changes observed for **1** (1.3×10^{-5} M) with Cu^{2+} (2 equivalents) in the presence of 2 equivalents of EDTA in DMSO/H_2O (3:7 v/v).

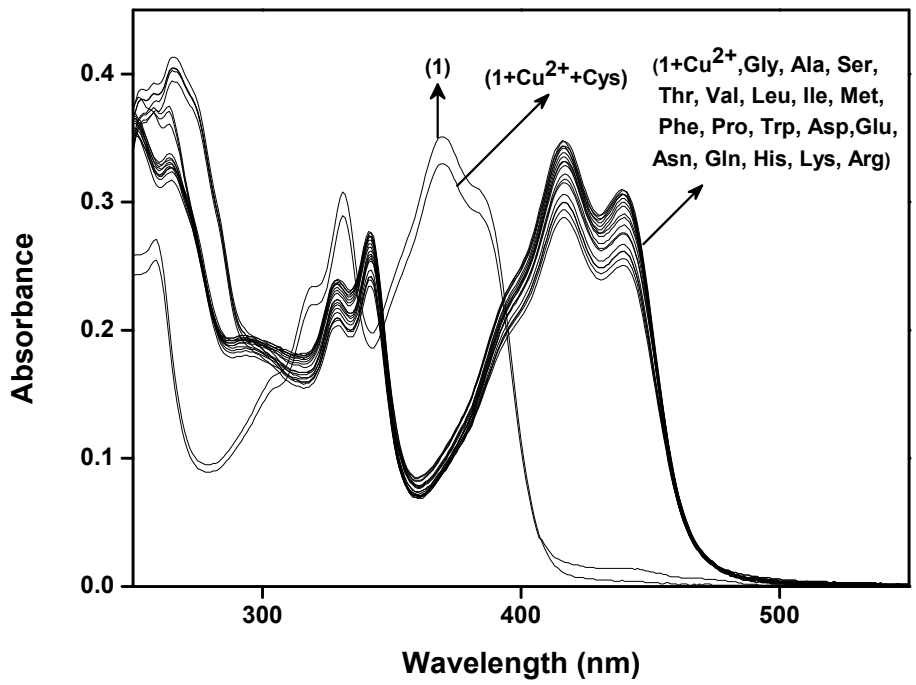


Fig. S8. Absorption spectral changes of **1**-Cu(II) upon addition of 30 equivalents of different amino acids in DMSO/H_2O (3:7 v/v) solution. [**1**-Cu(II)] = 6.2×10^{-6} M.

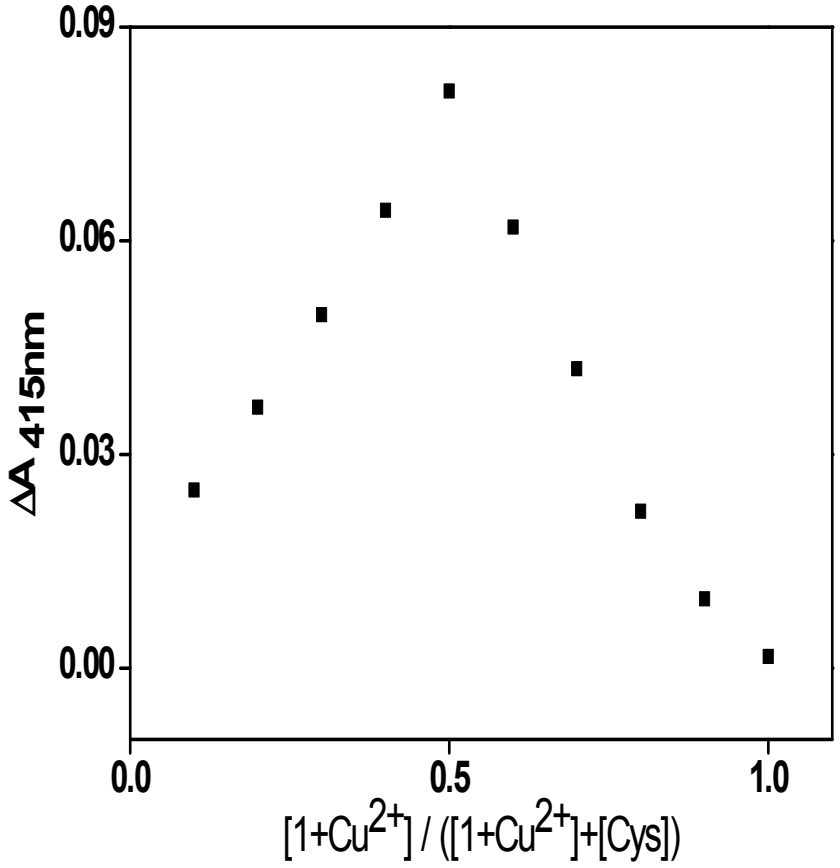


Fig. S9. The stoichiometric analysis of **1**-Cu(II) with Cys by Job′s plot; ([**1**-Cu(II)] + [Cys]) = 2×10^{-5} M in DMSO/H_2O (3:7 v/v).

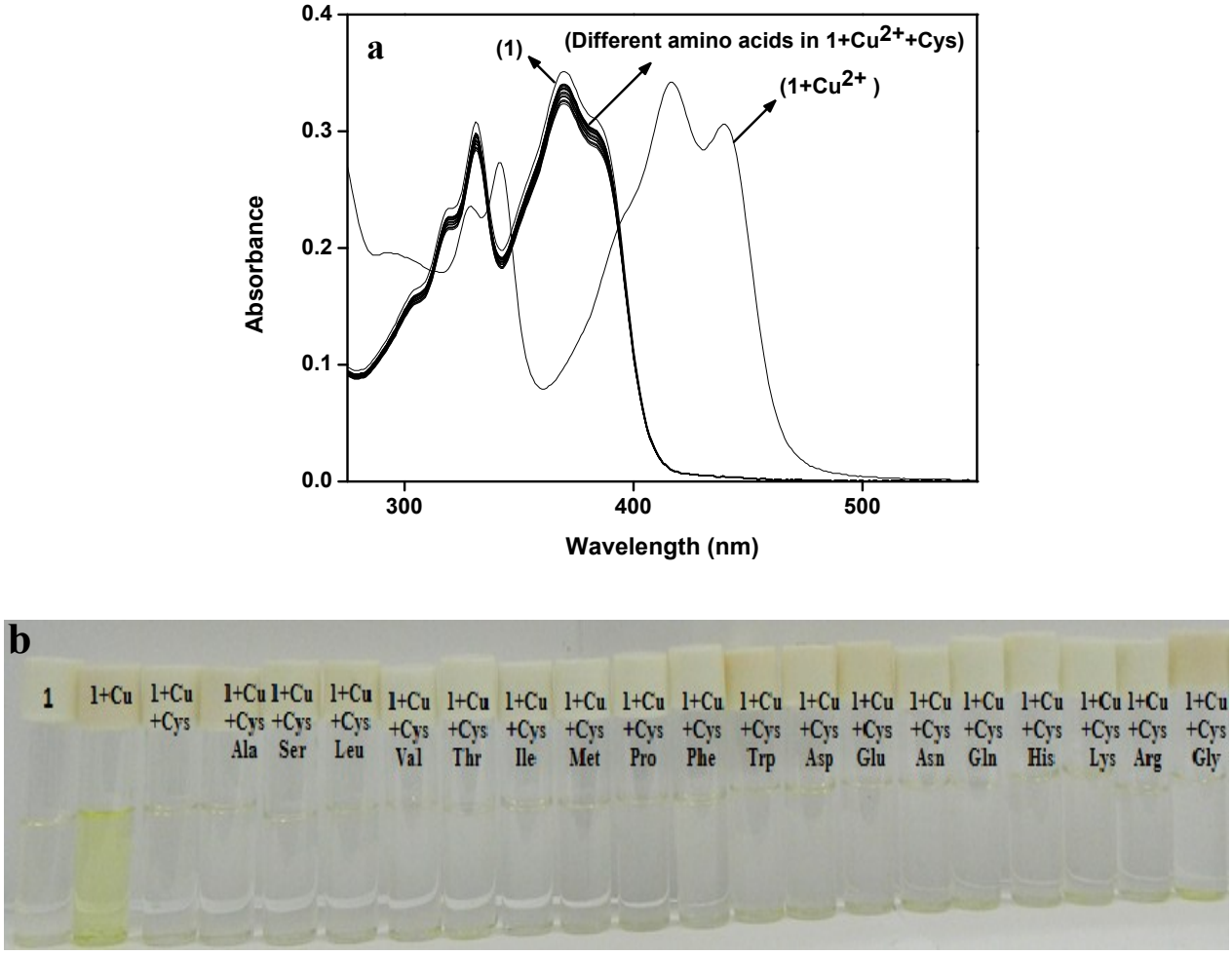


Fig. S10. (**a**) Absorption spectral changes of competitive selectivity of **1**-Cu(II), [**1**-Cu(II)] = 6.2×10^{-6} M, towards Cys (30 equivalents) in the presence of other amino acids (30 equivalents) in DMSO/H_2O (3:7 v/v) solution. (**b**) Colour changes of competitive selectivity of **1**-Cu(II), [**1**-Cu(II)] = 6.2×10^{-6} M, towards Cys (30 equivalents) in the presence of other amino acids (30 equivalents) in DMSO/H_2O (3:7 v/v) solution.