Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary content

A unique cysteine selective water soluble fluorescent probe operable in multiple sensing cycles for detection of biogenic cysteine in multicellular living species

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Table S1: Crystal data and structure refinement parameters of 1

Formula	**$C_{32}H_{32}Cl_2Cu_3N_4O_{12}$**
CCDC	1474805
M_r	926.17
T/K	296(2)
λ /Å	0.71073
Crystal system	Monoclinic
Space group	P (21)/n
a, b, c /Å	12.979(4), 8.793(2), 16.272(5)
α, β, γ / °	90.00, 111.84, 90.00
V / $Å^3$	1723.7(9)
$_{Dc}$ / gcm^{-3}	1.785
Z	2
M_u / mm^{-1}	2.060
F(000)	938
θ min, max / °	1.7, 25.025
Data set, *hkl*	15, 10, 19
Total, Unique data, R_{int}	19960, 3048, 0.024
Completeness to 2θ (%)	99.9
Goodness-of-fit on F^2	1.149
N_{ref}, N_{par}	3048, 241
R, wR_2, S	0.0365, 0.1208, 1.15

Table S2: Selected bond lengths (Å) and angles (°) for 1

Cu(1) – O(8)	1.952(2)
Cu(1) – N(2)	1.962(3)
Cu(1) – O(1)	1.964(2)
Cu(1) – N(1)	2.001(3)
Cu(2) – O(1)	1.903(2)
Cu(2) – O(8)	1.904(2)
O(1) – C(12)	1.429(4)
N(1) – C(13)	1.340(5)
N(1) – C(1)	1.357(5)
N(2) – C(4)	1.280(5)
N(2) – C(3)	1.470(5)
O(8) – C(11)	1.335(4)
O(8) – Cu(1) – N(2)	90.93(12)
O(8) – Cu(1) – O(1)	79.22(10)
N(2) – Cu(1) – O(1)	169.94(12)
O(8) – Cu(1) – N(1)	161.40(12)
N(2) – Cu(1) – N(1)	95.32(13)
O(1) – Cu(1) – N(1)	94.69(12)
N(2) – Cu(1) – Cu(2)	129.49(10)
O(1) – Cu(2) – O(8)	98.06(10)
Cu(2) – O(1) – Cu(1)	97.52(10)
O(8) – Cu(2) –Cu(1)	138.34(7)

Table S3: Electronic excitation wavelength (λ), oscillator strengths (f_{cal}) and extinction coefficient (ε) for **1** obtained by the TD-DFT/B3LYP/6-31+G(d,p) calculation on ground state geometries with CPCM solvation model in water. The experimentally obtained UV-Vis absorption (Obs) parameters for **1** are depicted for comparison.

Complex(1)	**λ(nm)**	**f_{cal}**	**$\varepsilon\times10^{-4}(M^{-1}cm^{-1})$**
Cal	365.33	0.019	0.20
Obs	373.00	-	0.23

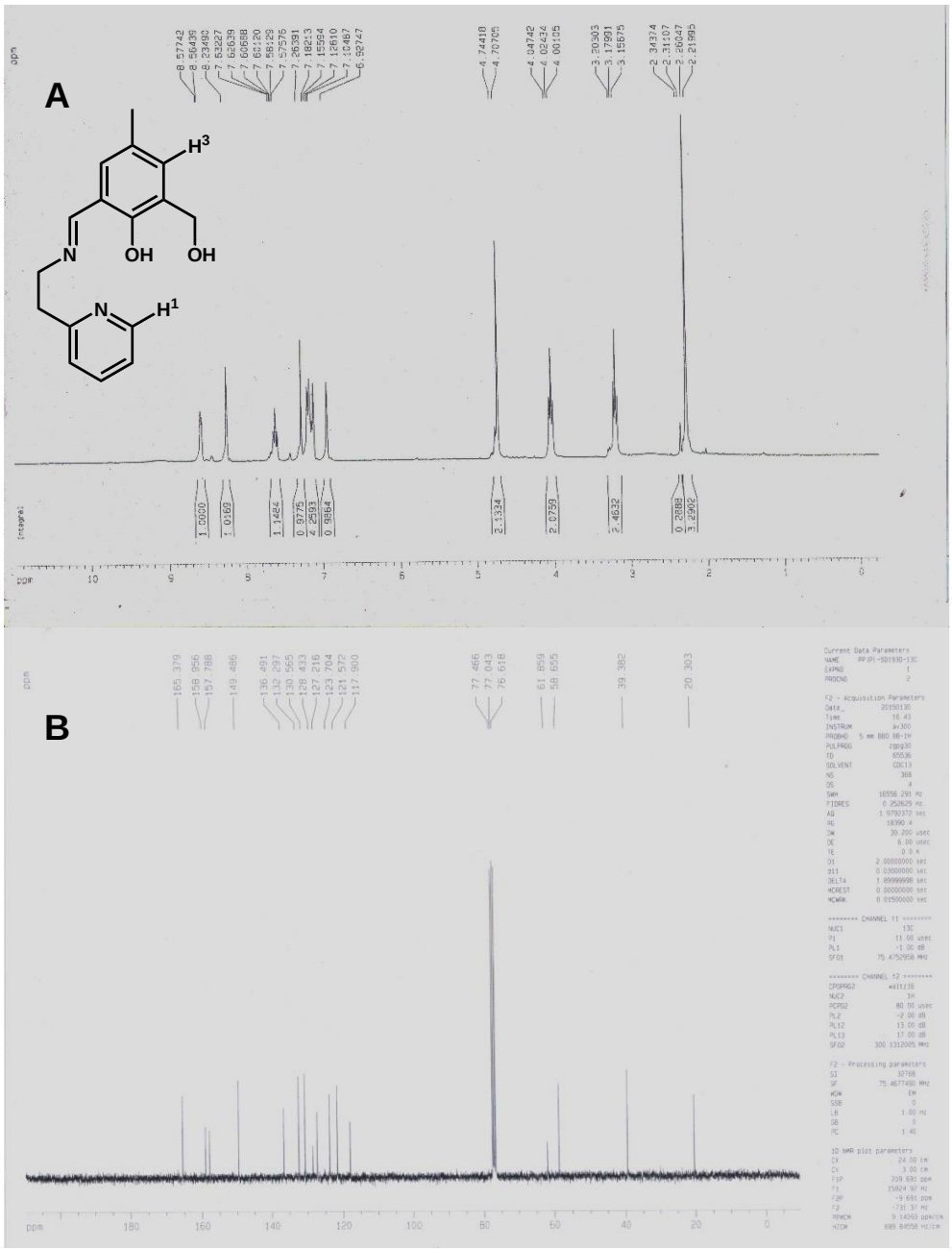

Fig. S1. (A) ^{1}H-NMR and (B) ^{13}C-NMR spectra of L_1 in $CDCl_3$.

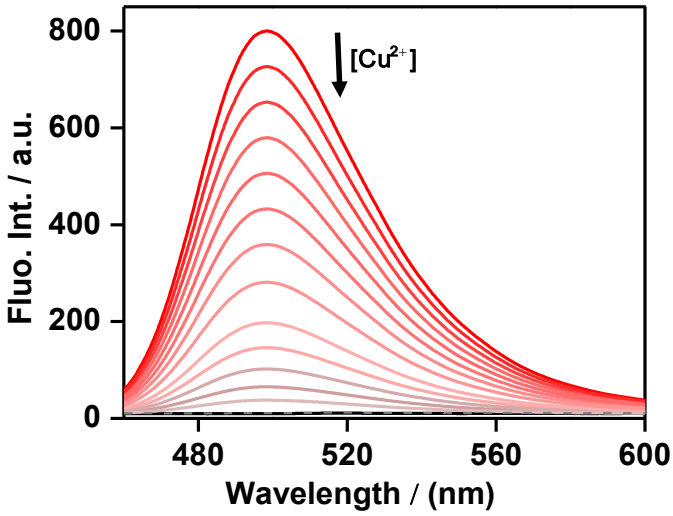

Fig. S2. Fluorescence spectra of L_1 (5μM) in presence of gradual increase of $Cu(ClO_4)_2$ (0–20 μM) in 20 mM HEPES-NaOH, pH 7.4 (excitation wavelength: 440 nm). The decrease in intensity with increase of Cu^{2+} ion concentrations is shown by arrow.

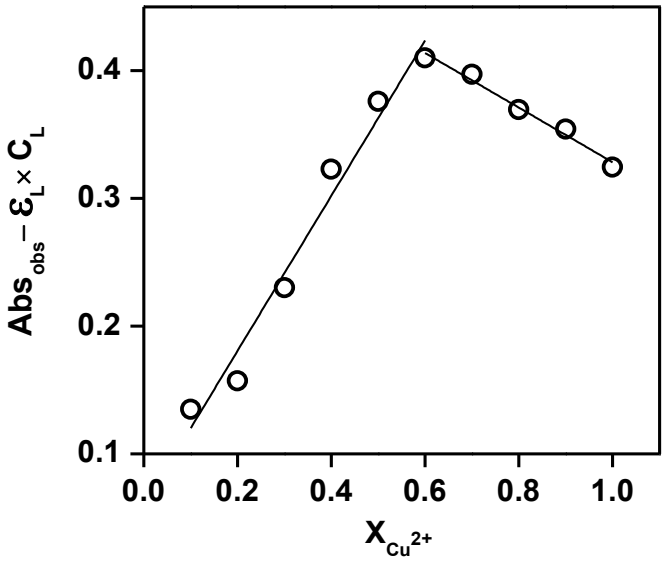

Fig. S3. Job's plot for determining the stoichiometry of the complex between L_1 and Cu^{2+}. The difference between the observed and L_1 absorbance at 370 nm were plotted with mole fraction of Cu^{2+} in the mixture of L_1 and $Cu(ClO_4)_2$ with various compositions (ε_L and C_L are the extinction coefficient and concentration of L_1, respectively).

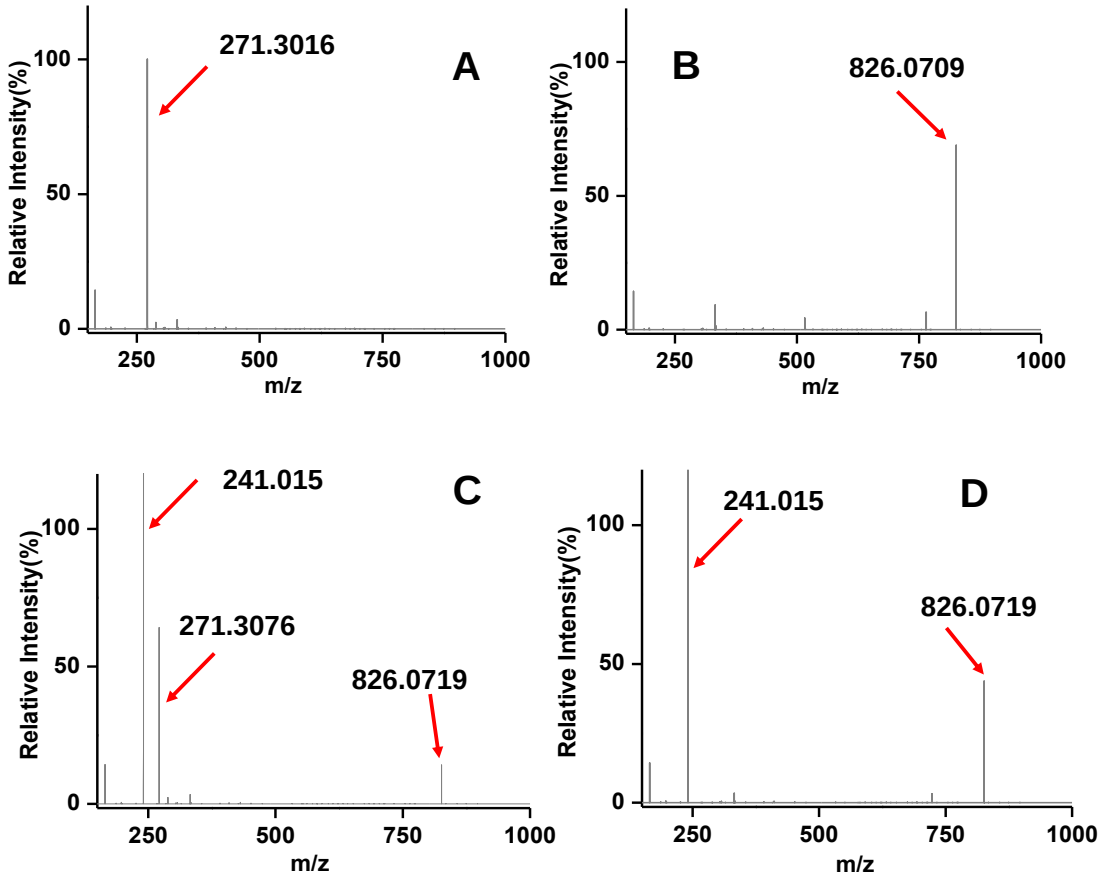

Fig. S4. ESI-MS$^+$ of (A) L_1 (*m/z*: found 271.3076; calcd. 271.3412) for $[L_1+H]^+$), (B) **1** (*m/z*: found 826.0719; calcd. 826.7112 for $[C_{32}H_{32}Cu_3N_4O_4+ClO_4]^+$), (C) **1**+Cys (*m/z* for $[C_{32}H_{32}Cu_3N_4O_4+ClO_4]^+$: found 826.0719; calcd. 826.7112, *m/z* for $[L_1+H]^+$: found 271.3076; calcd. 271.34, *m/z* for [Cystine+H]$^+$): found 241.0150; calcd. 241.3412) and (D) **1**+Cys after 60 min incubation (*m/z* for $[C_{32}H_{32}Cu_3N_4O_4+ClO_4]^+$: found 826.0719 and 826.7112, *m/z* for [Cystine+H]$^+$): found 241.0150; calcd. 241.3412) in water.

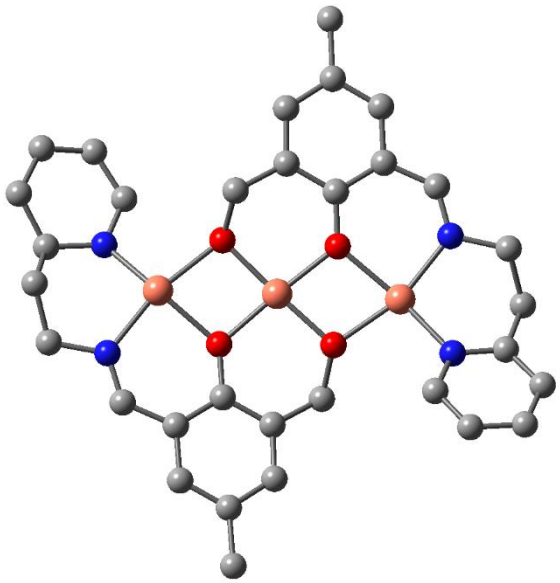

Fig. S5. DFT optimized structure of **1**. All H's-atoms are excluded for clarity. Color code: gray, C; blue, N; red, O; brown, Cu.

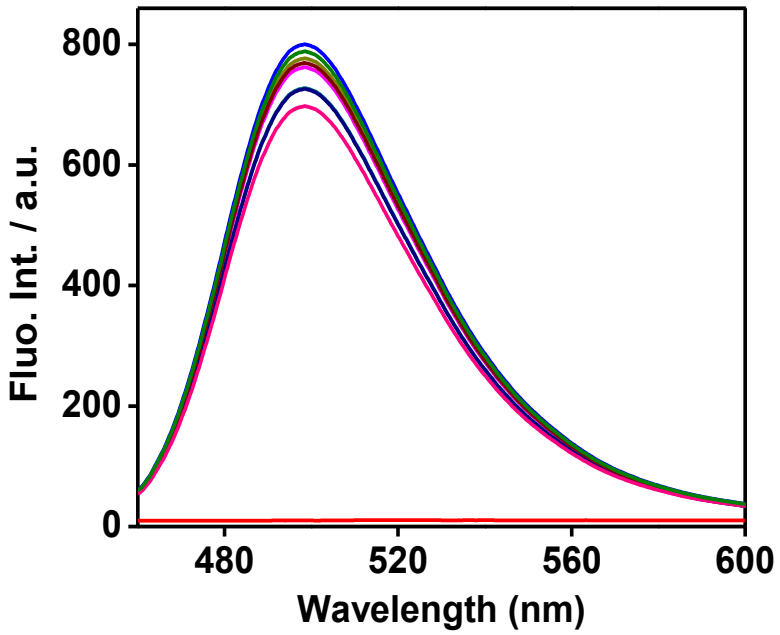

Fig. S6. Fluorescence spectra of L_1 (5 μM) in various competing metal ions (Mn^{2+},Co^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, $Fe^{2+/3+}$ etc, 20 μM each) in 20 mM HEPES-NaOH, pH 7.4 (excitation wavelength: 440 nm). The spectrum in presence of Cu^{2+} (20 μM) is depicted by red.

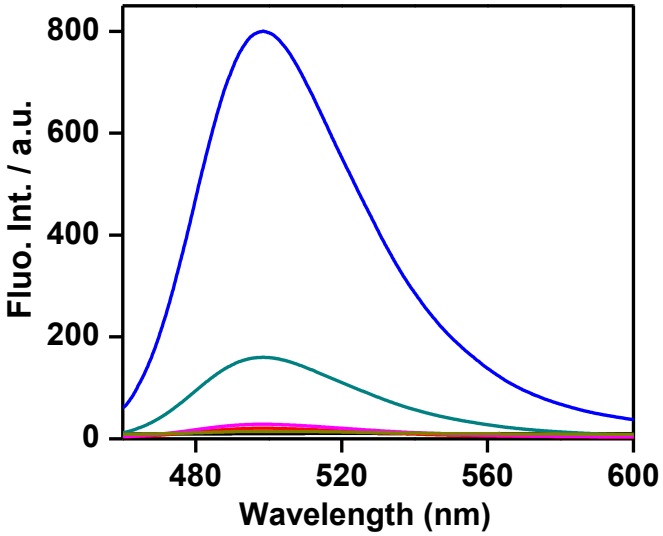

Fig. S7. Fluorescence spectra of **1** (5 μM with respect to L_1) in presence of various anions (CN^-, N_3^-, S^{2-}, OAc^-, $H_2PO_4^-$ and HPO_4^{2-}etc.: 100 equiv. each) in 20 mM HEPES, pH 7.4 (excitation wavelength: 440 nm). The spectrum in presence of Cys (60 equiv.) and S^{2-} ion (300 equiv.) are depicted by blue and dark cyan, respectively. All spectra recorded after 30 s with different anion/molecule.

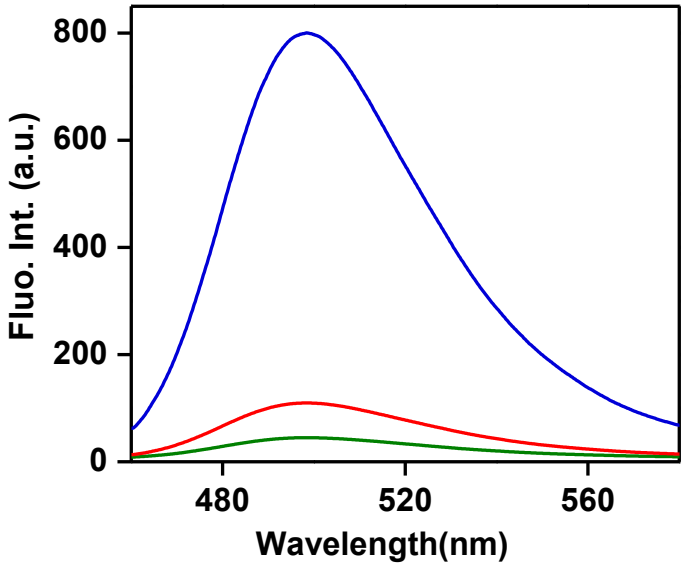

Fig. S8. The fluorescence spectra for **1** (5 μM with respect to L_1) in presence of different biothiols (60 equiv.) (Cys, blue; GSH, red; green, Hcy) in 20 mM HEPES-NaOH, pH 7.4 (excitation wavelength: 440 nm). All spectra recorded after 30 s with biothiol.

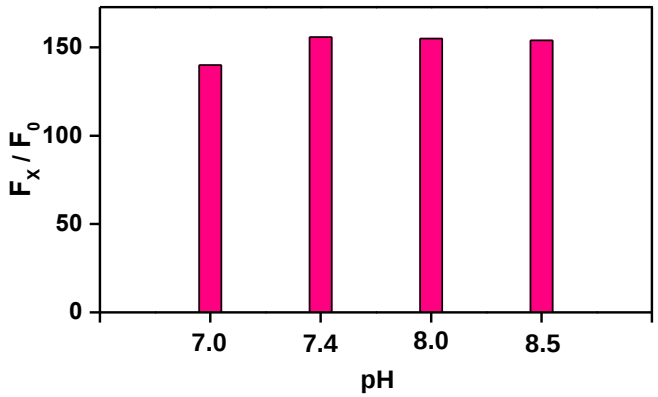

Fig. S9. The extent of 500-nm fluorescence intensity enhancement (F_X/F_0) for **1** in presence of Cys (60 equiv. each) at 30 s in 20 mM HEPES-NaOH, at various pH of the medium (7.0–8.5) are depicted by bar-diagram.

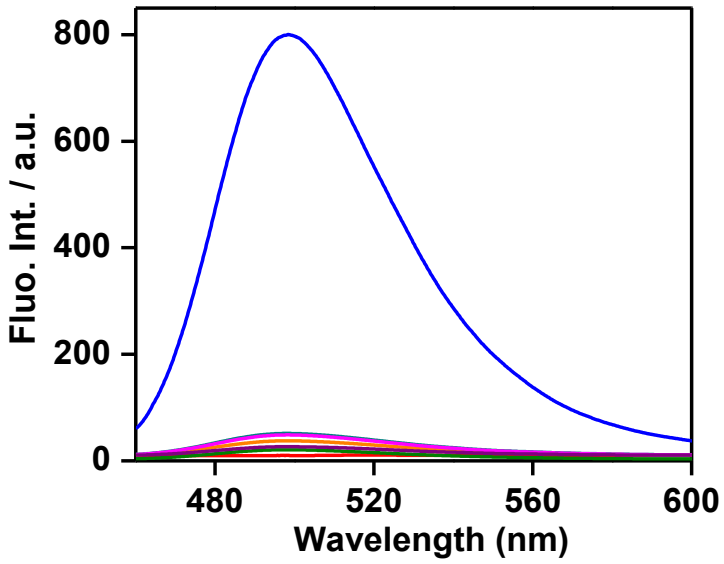

Fig. S10. Fluorescence spectra of **1** (5 μM with respect to L_1) in presence of low Cys concentration (0.5–5.0 equiv.) in 20 mM HEPES-NaOH, pH 7.4 (excitation wavelength: 440 nm). The spectrum in presence of 60 equiv. of Cys is depicted by blue for comparison. All spectra recorded after 30 s with Cys.

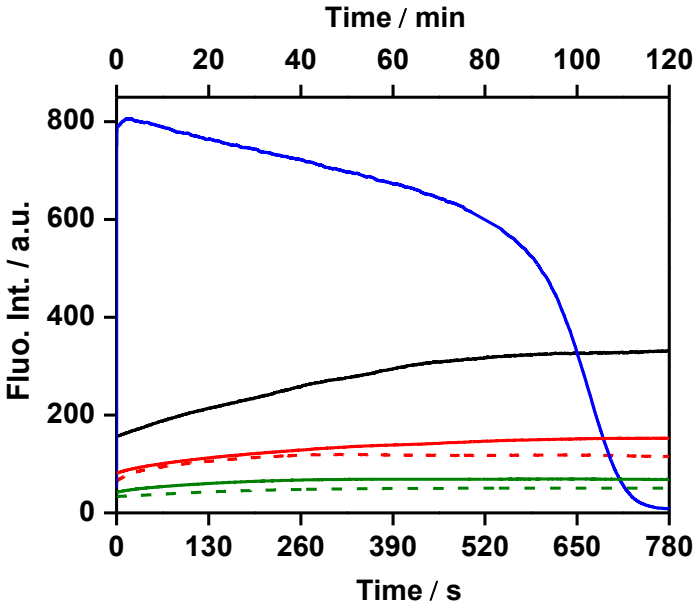

Fig. S11. Different biothiols (Cys, blue; GSH, red; green, Hcy) (60 equiv.) induced time dependent fluorescence intensity at 500-nm for (solid curve) **1** (5 μM with respect to L_1) and (broken curve) **1**+Cys (60 equiv.) at intensity quenched condition in 20 mM HEPES-NaOH, pH 7.4 (excitation wavelength: 440 nm). The black curve (upper x-axis) represents time dependent fluorescence intensity for **1** at higher GSH concentration (200 equiv.).

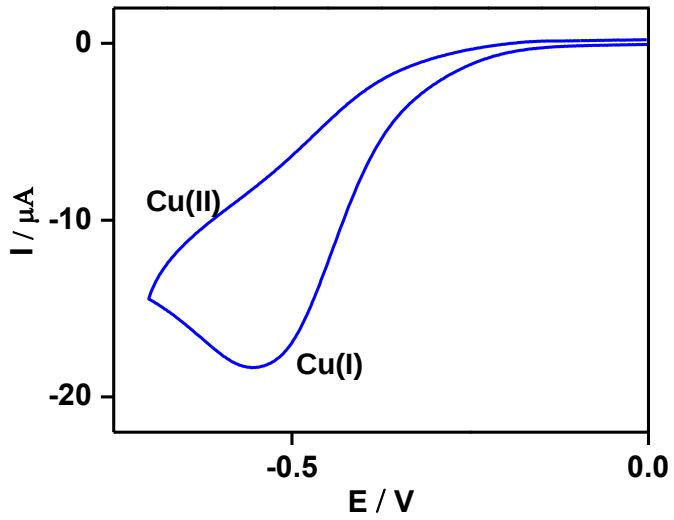

Fig. S12. Cyclic Voltammograms of **1** (130 µM) in 10 mM HEPES-NaOH, pH 7.4 at 100 $mV.s^{-1}$ scan rate.

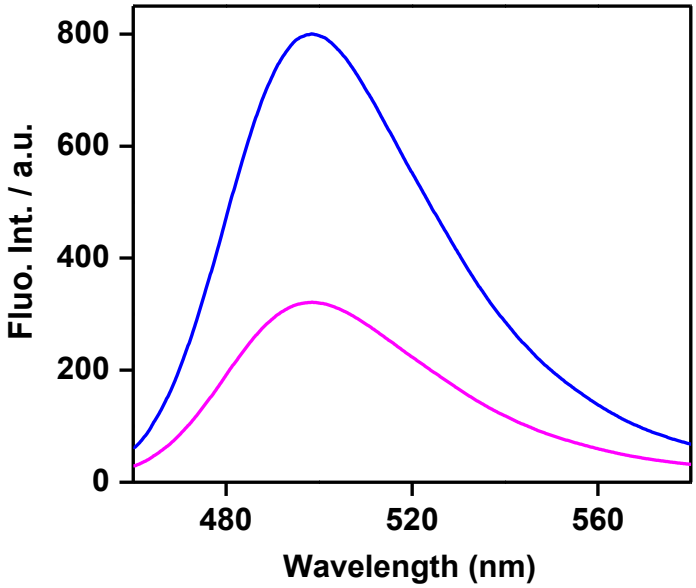

Fig. S13. pH dependent fluorescence spectra of **1** (5 μM with respect to L_1) in presence of Cys (60 equiv.) in 20 mM HEPES, pH 7.4 (excitation wavelength: 440 nm): pink, pH 6.0 and blue, pH 7.4. Spectra recorded after 30 s with Cys.

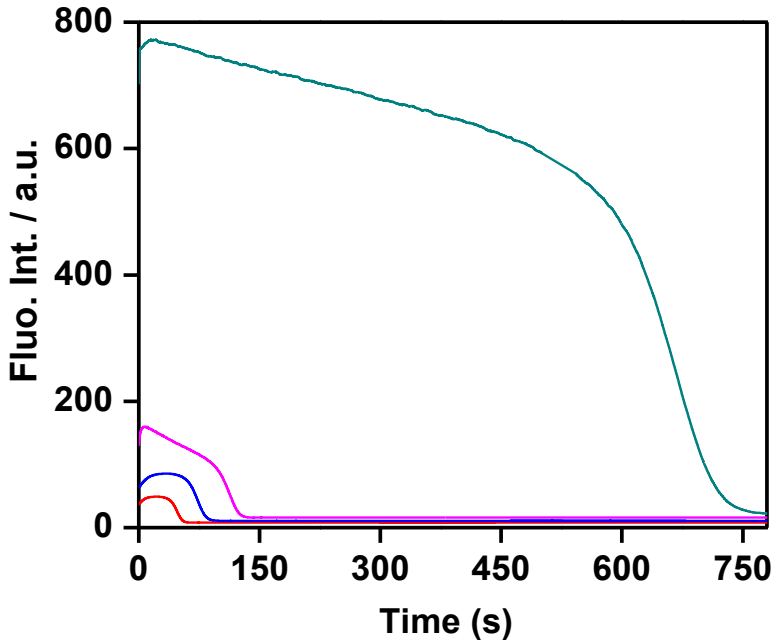

Fig. S14. The time dependent fluorescence intensity at 500-nm for **1** (5 μM with respect to L_1) in presence of low Cys concentration (red, 1.0; blue, 3.0 and pink, 5.0 equiv.) in 20 mM HEPES-NaOH, pH 7.4 (excitation wavelength: 440 nm). The time dependent intensity profile in presence of 60 equiv. Cys is depicted by dark cyan for comparison.

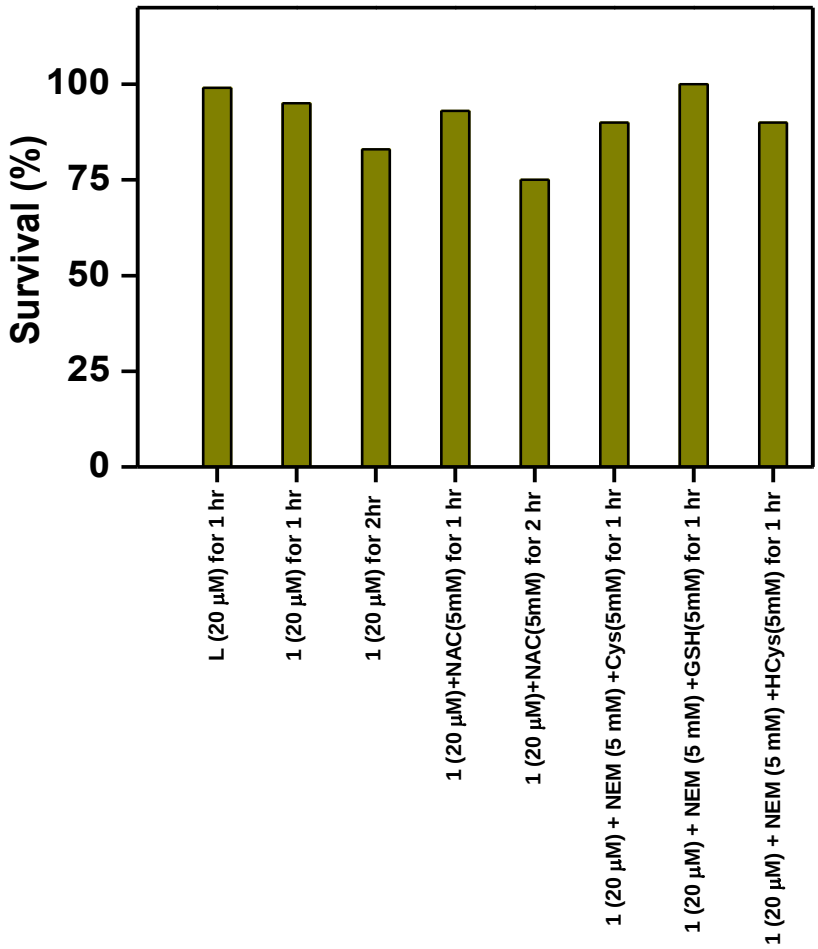

Fig. S15. Assessment of toxicity of the **1** in presence and absence of various analytes after different interval of times by survival assays in *C. elegans* are depicted by bar-diagram.

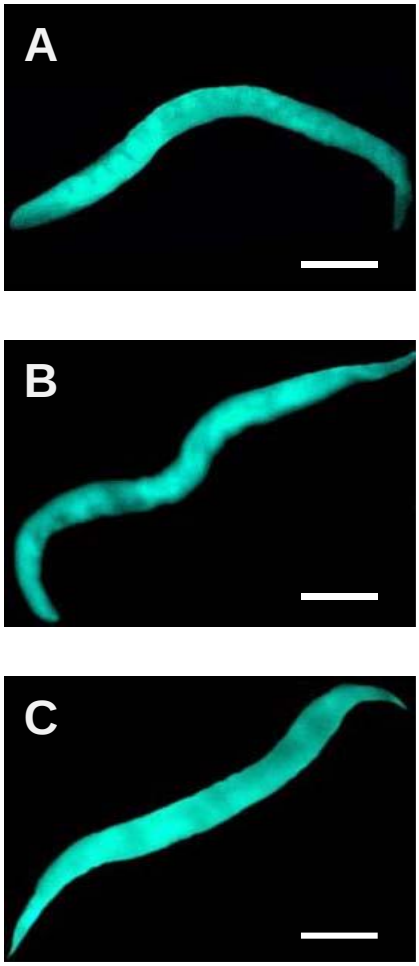

Fig. S16. Fluorescence images of the *C. elegans* exposed to L_1 (20 μM) for different interval of time: (A) 15 min, (B) 45 min and (D) 120 min. The scale bars: 40 μm.