Supporting information

Coordination Geometry Induced Optical Imaging of L-Cysteine in Cancer Cells using Imidazopyridine-Based Copper(II) Complexes

Selvarasu Priyanga,[a] Themmila Khamrang,[b] Marappan Velusamy,[b] Sellamuthu Karthi,[c] Balasubramaniem Ashokkumar,[c] and Ramasamy Mayilmurugan[a]*

[a]Bioinorganic Chemistry Laboratory/Physical Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai, 625021. *Email;mayilmurugan.chem@mkuniversity.org.*
[b]Department of Chemistry, North-Eastern Hill University, Shillong, 793022.
[c]School of Biotechnology, Madurai Kamaraj University, Madurai, 625 021.

(a)

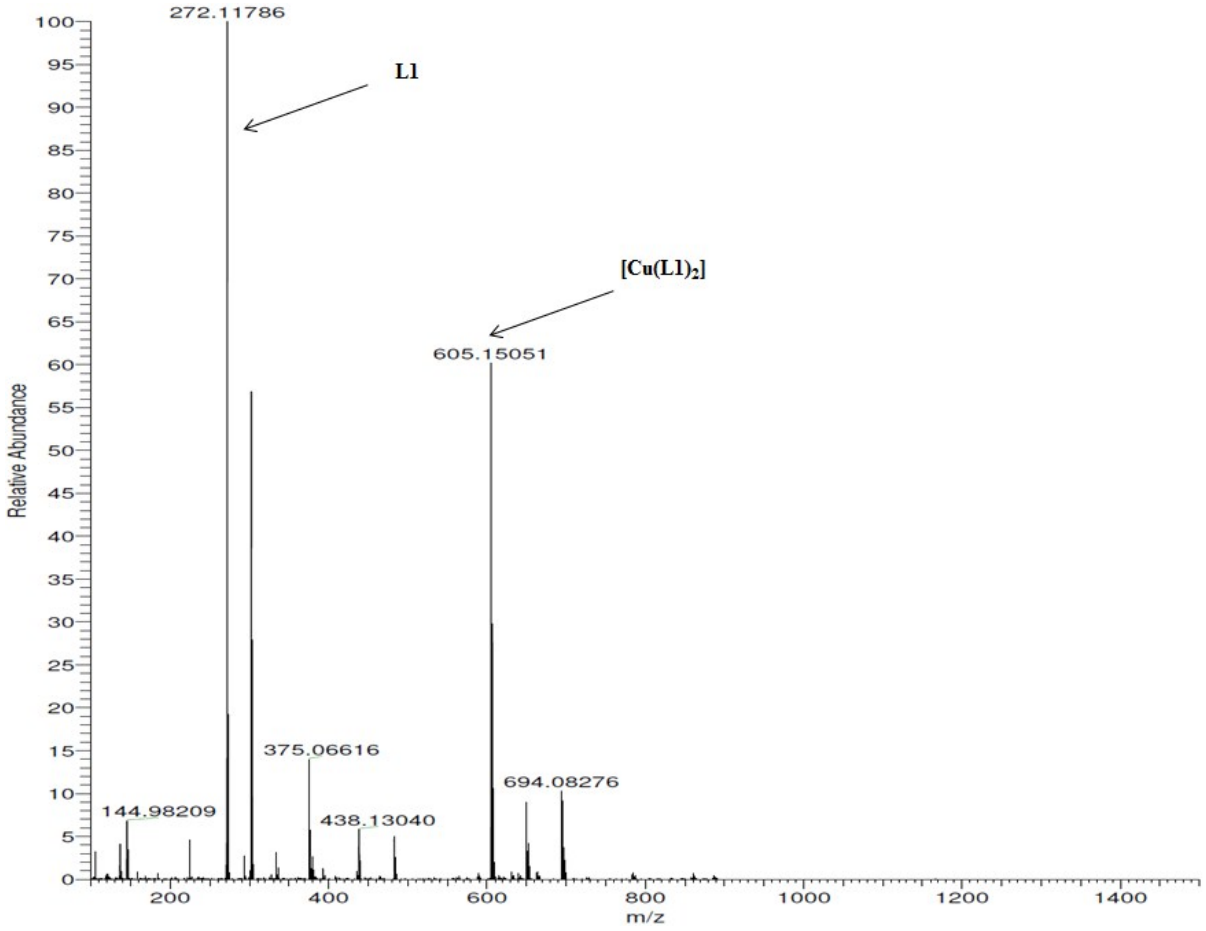


Figure S1. HR-ESI Mass spectra of **1** in CH_3CN.

(b)

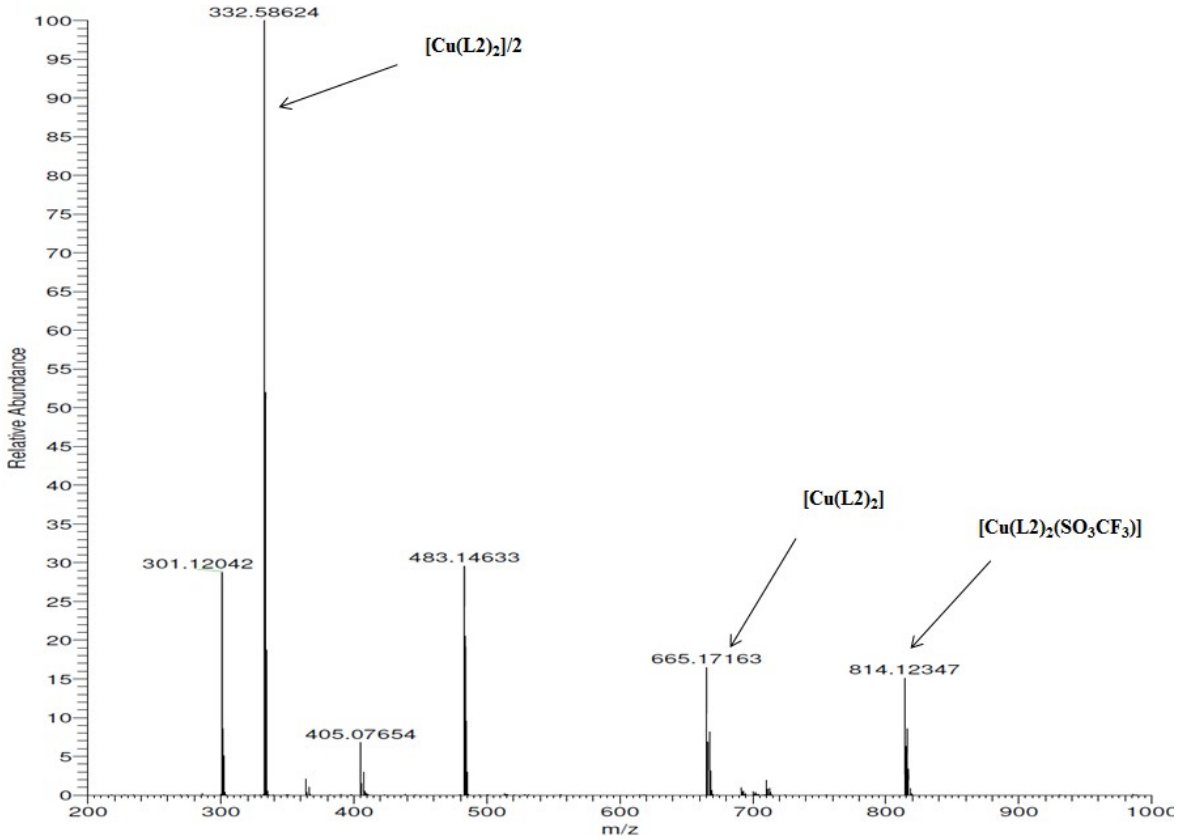


Figure S2. HR-ESI Mass spectra of **2** in CH_3CN.

(c)

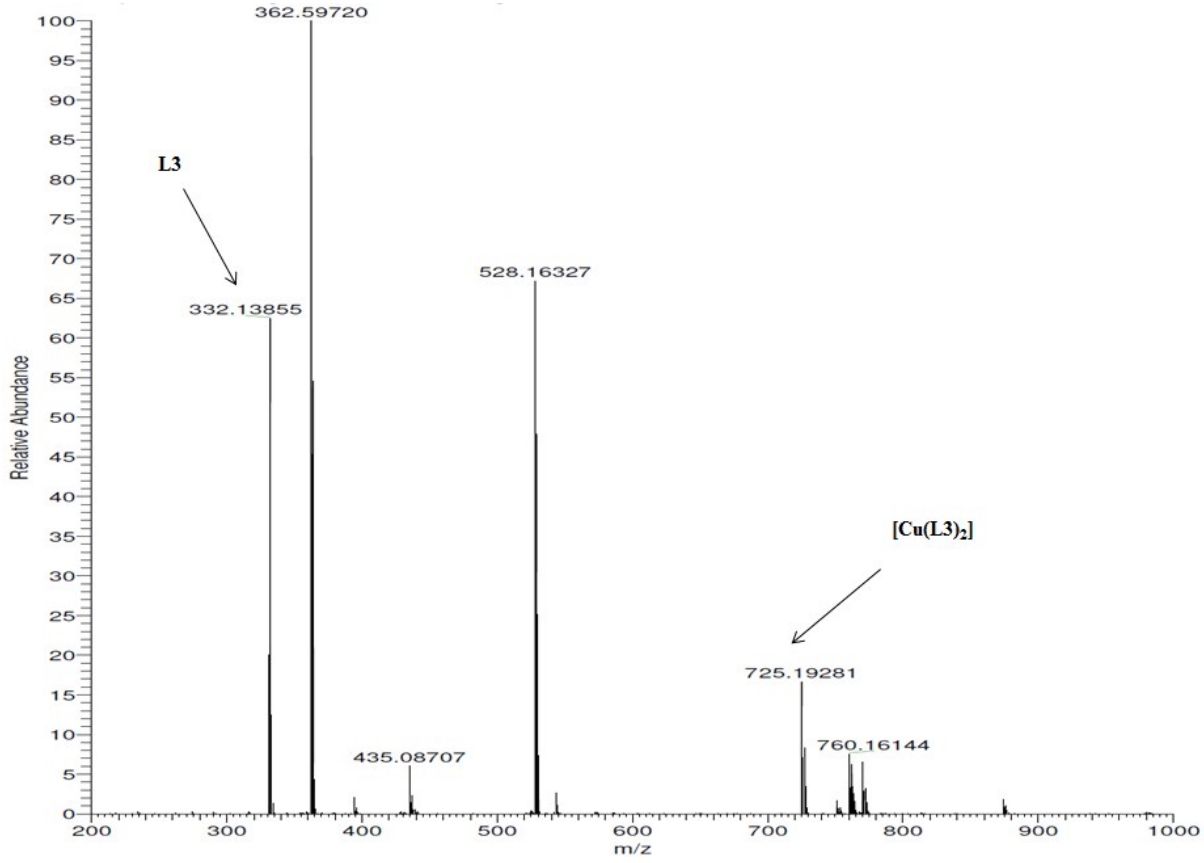


Figure S3. HR-ESI Mass spectra of **3** in CH_3CN

(d)

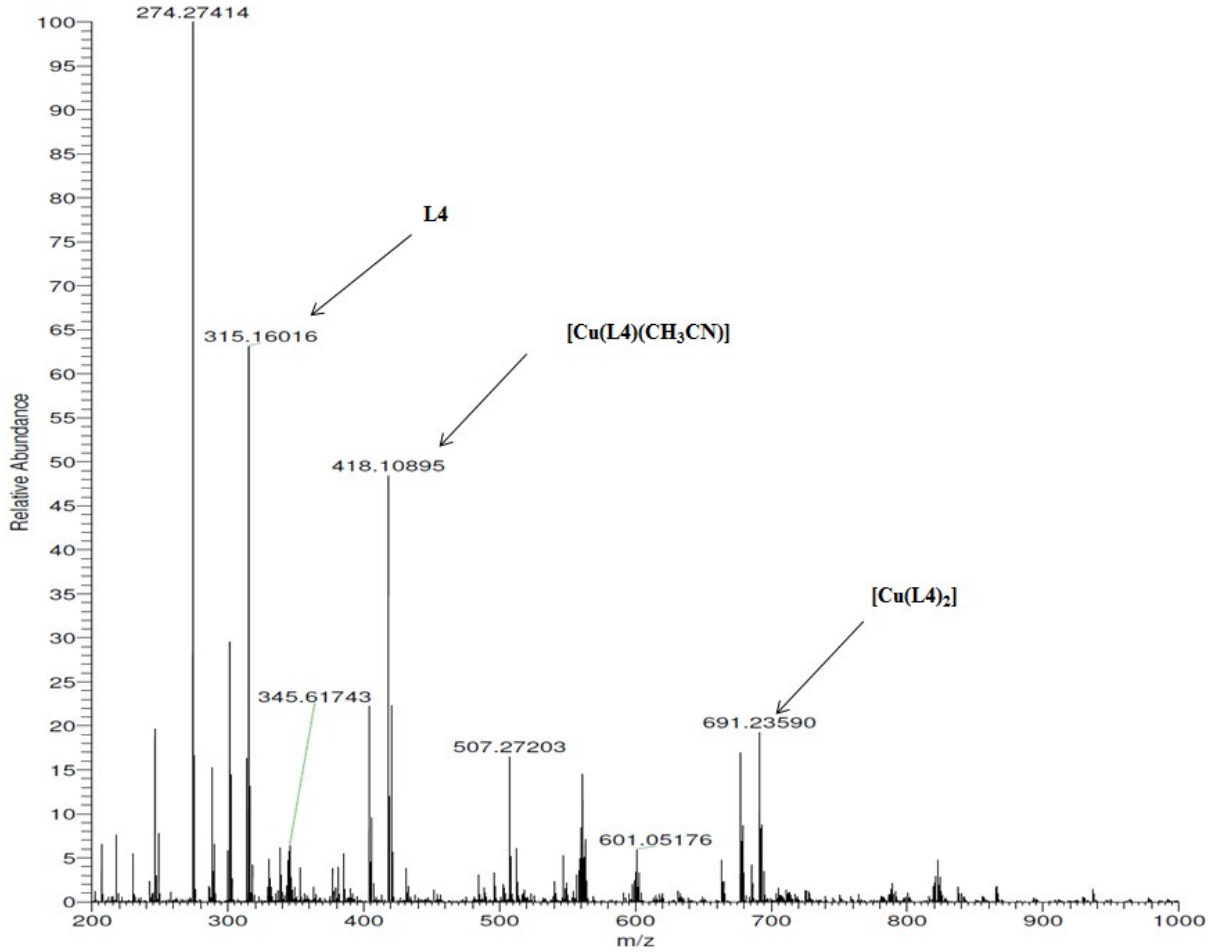


Figure S4. HR-ESI Mass spectra of **4** in CH_3CN.

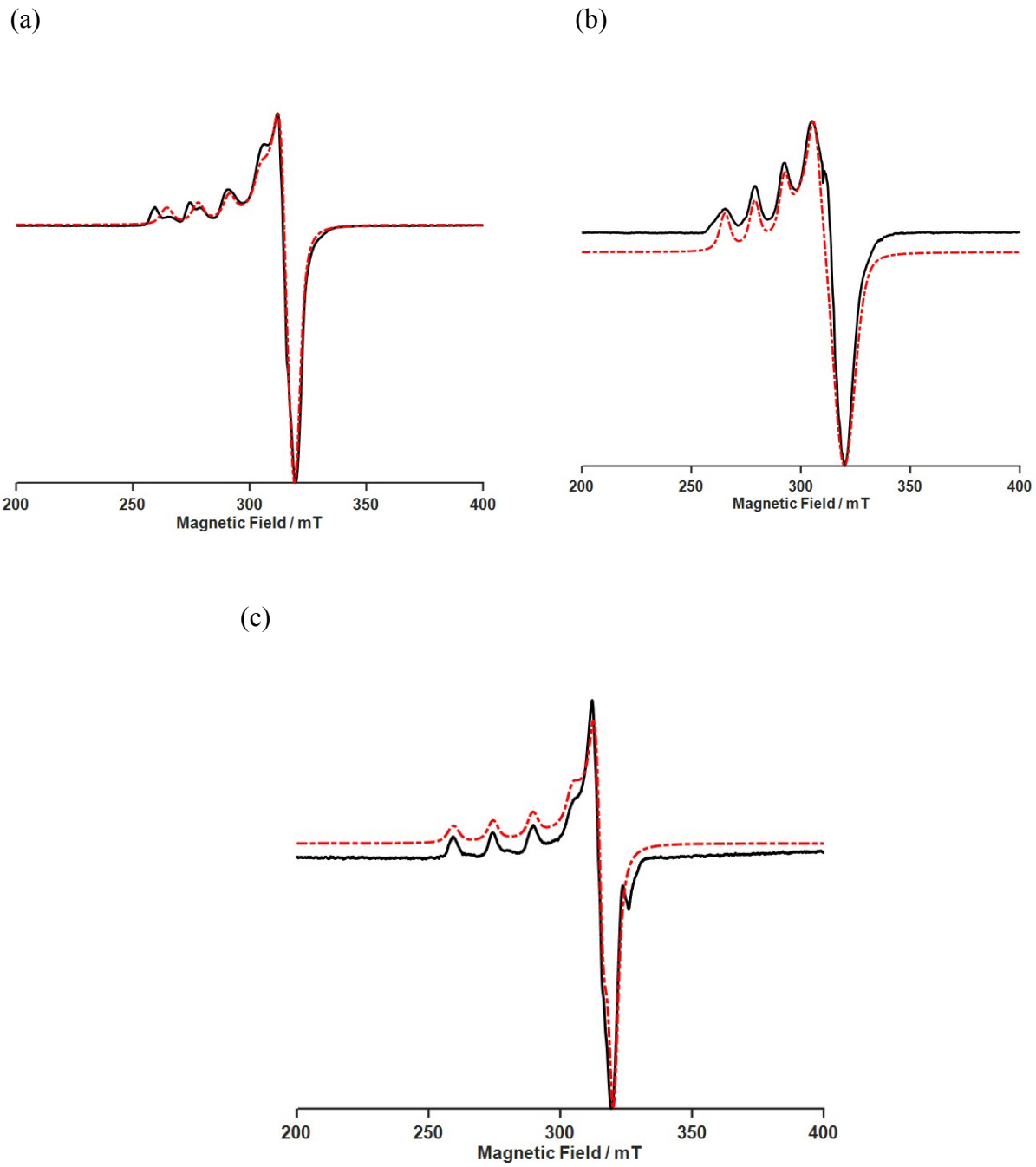


Figure S5. EPR spectra of **1** (a), **2** (b), **3** (c) in methanol/DMF. Experimental (black) and simulated spectra (red) are overlaid in each case.

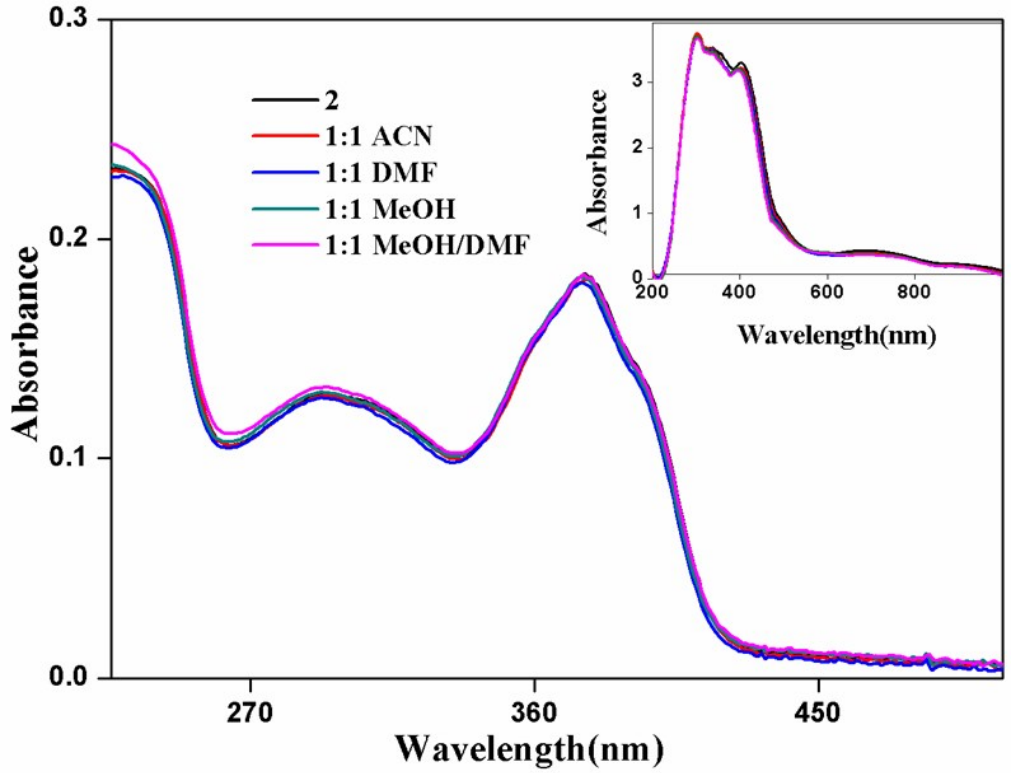


Figure S6. Absorption spectra of complex **2**, 1:1 equivalence of ligand and complex **2** in acetonitrile, methanol, DMF or in methanol-DMF mixture. Inset: d-d band changes.

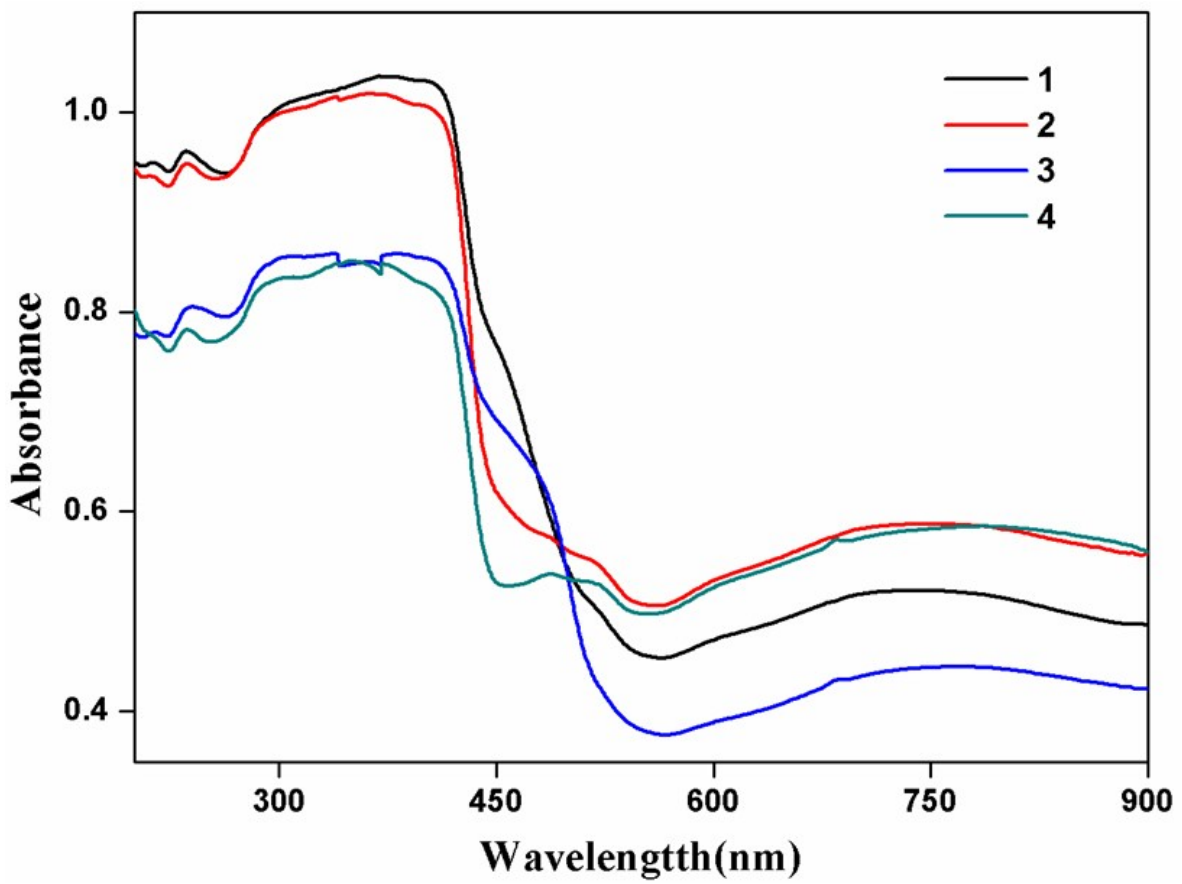


Figure S7. Solid state absorbance spectra for **1** - **4**.

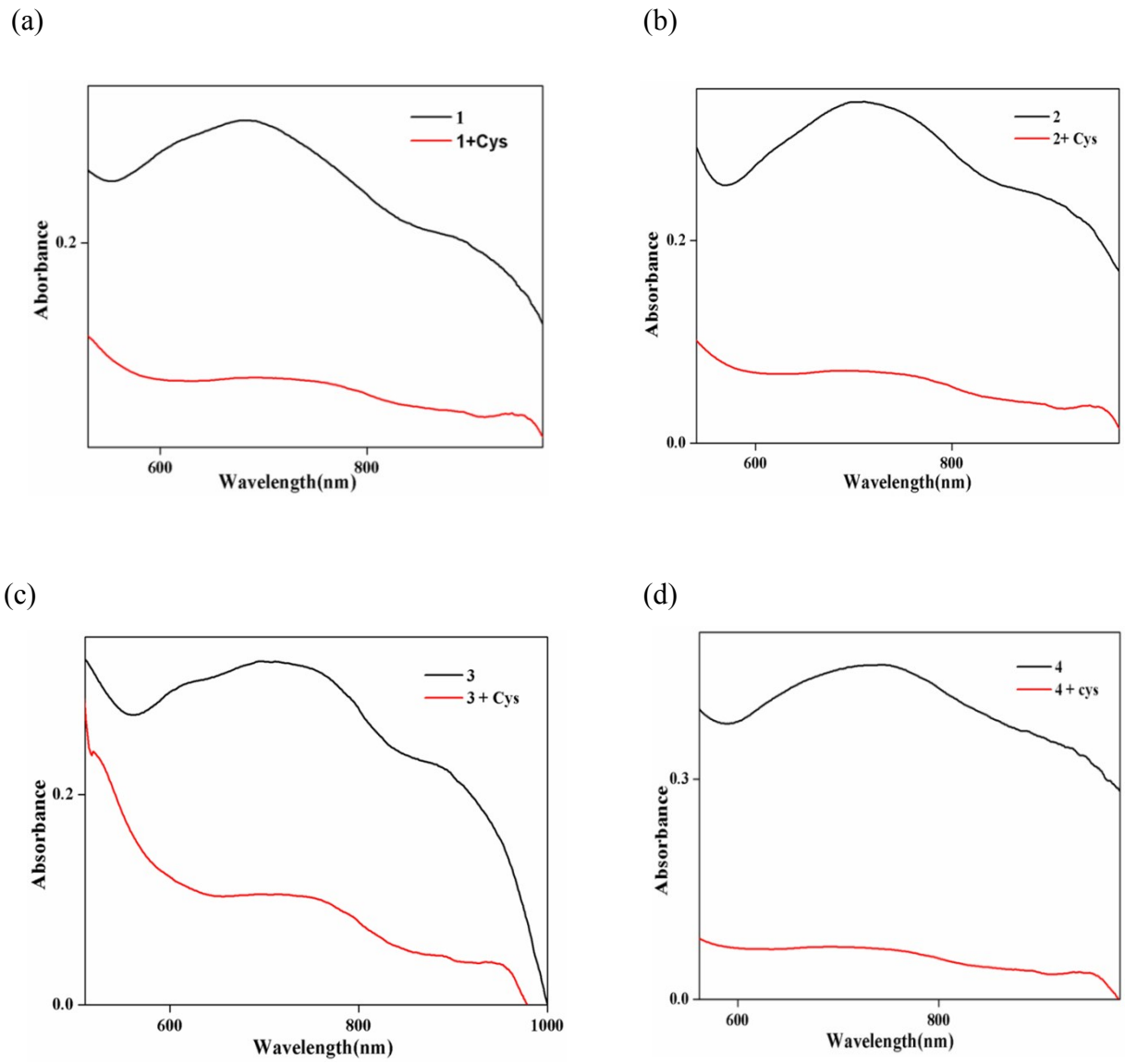


Figure S8. The changes in the d-d band of **1** (a), **2** (b), **3** (c) and **4** (d) with Cys in the concentration of 1×10^{-2} M (acetonitrile/HEPES buffer solution, pH 7.34) at 25°C.

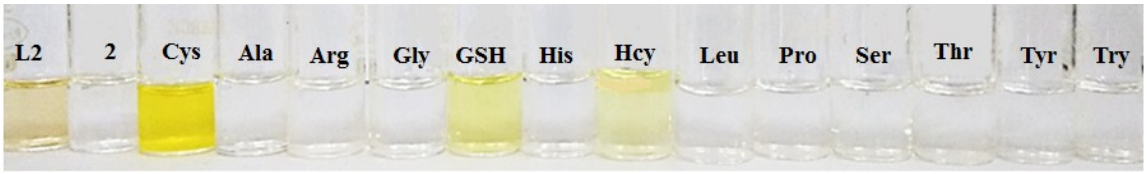


Figure S9. Color changes observed for **2** (40μM) upon the addition of 10 equivalents of various amino acid in HEPES buffer solution (pH 7.34) at 25°C.

a)

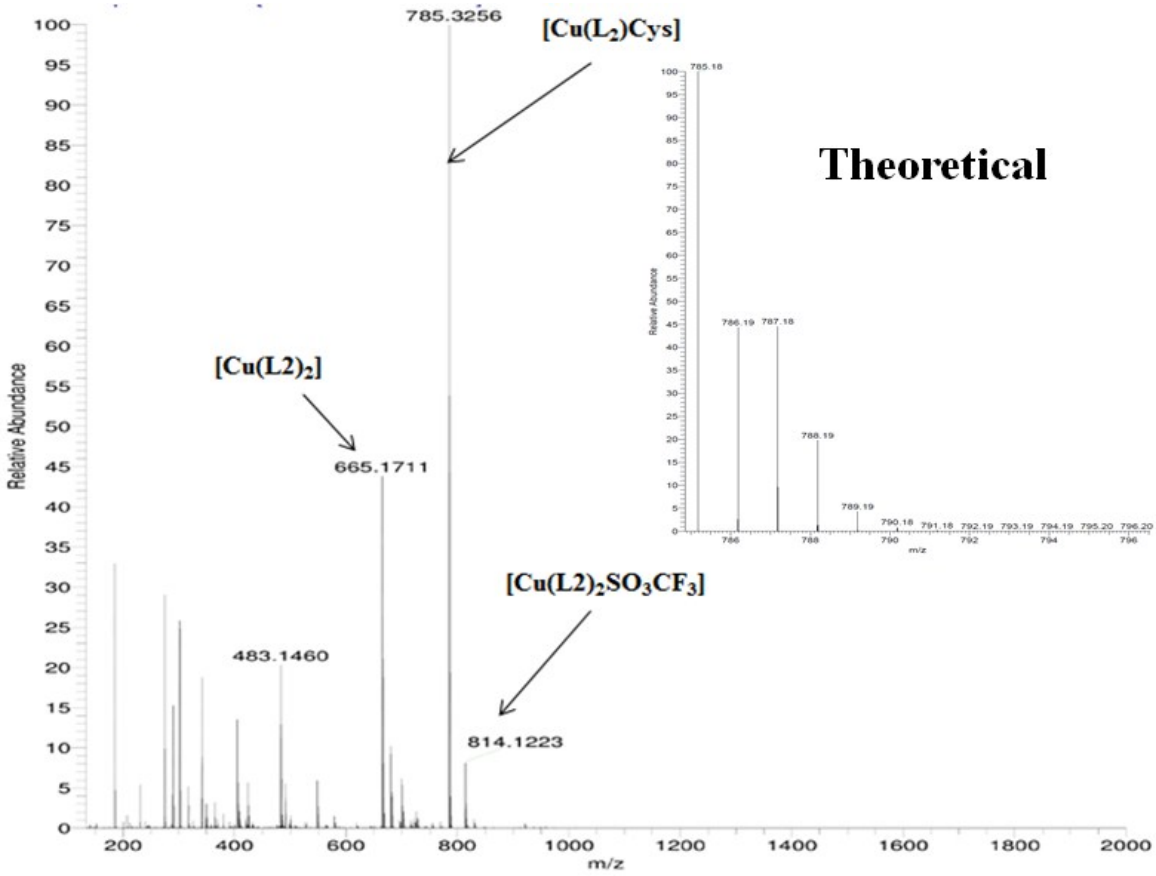


Figure S10. HR-ESI Mass spectra of **2** + Cys in CH_3CN, Inset: Theoretical.

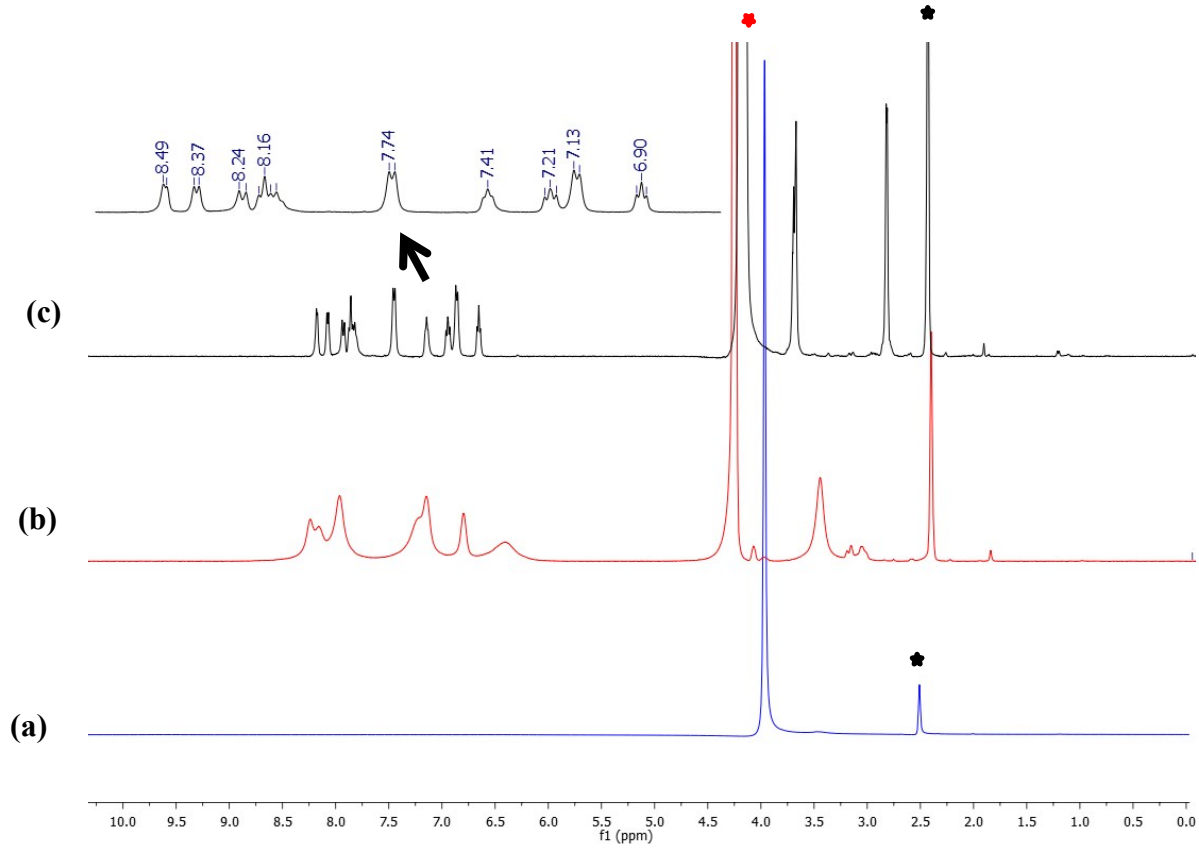


Figure S11. The ^{1}HNMR spectra of **2** (a), **2** + Cys (1 equivalent) (b), **2** + 5equivalents of Cys (c). Peaks corresponds to ✶ D_2O and ✶ DMSO.

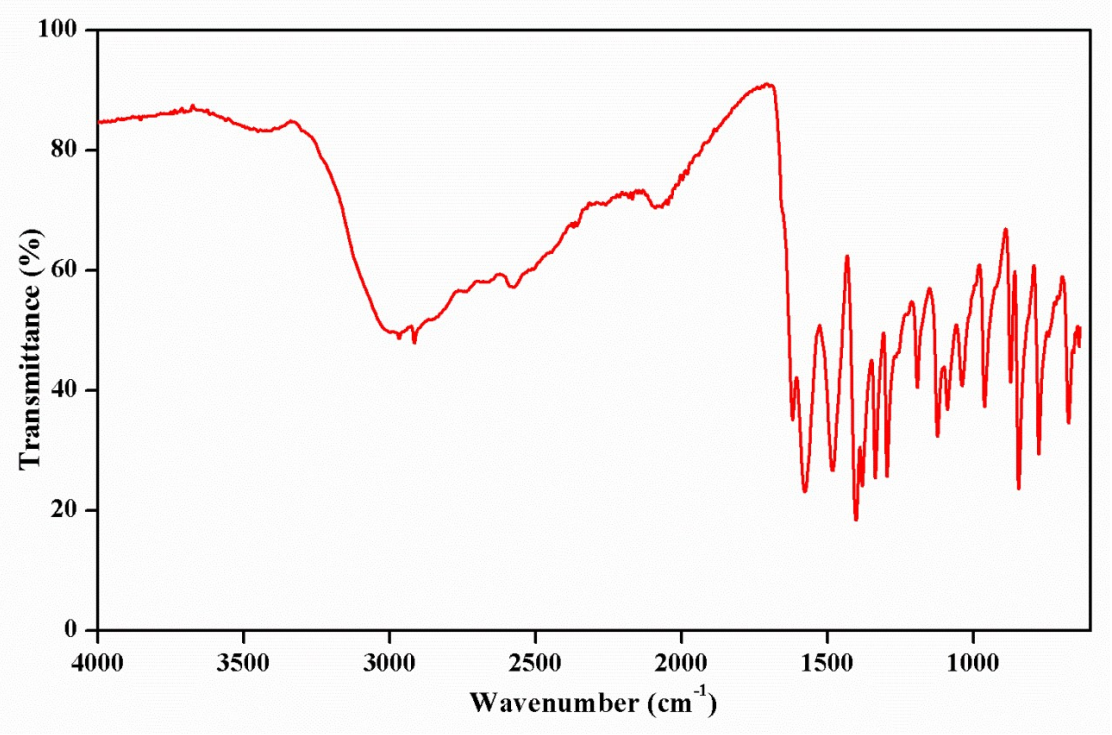


Figure S12. FT-IR spectra for $[Cu(L2)_2(Cys)]$ adduct.

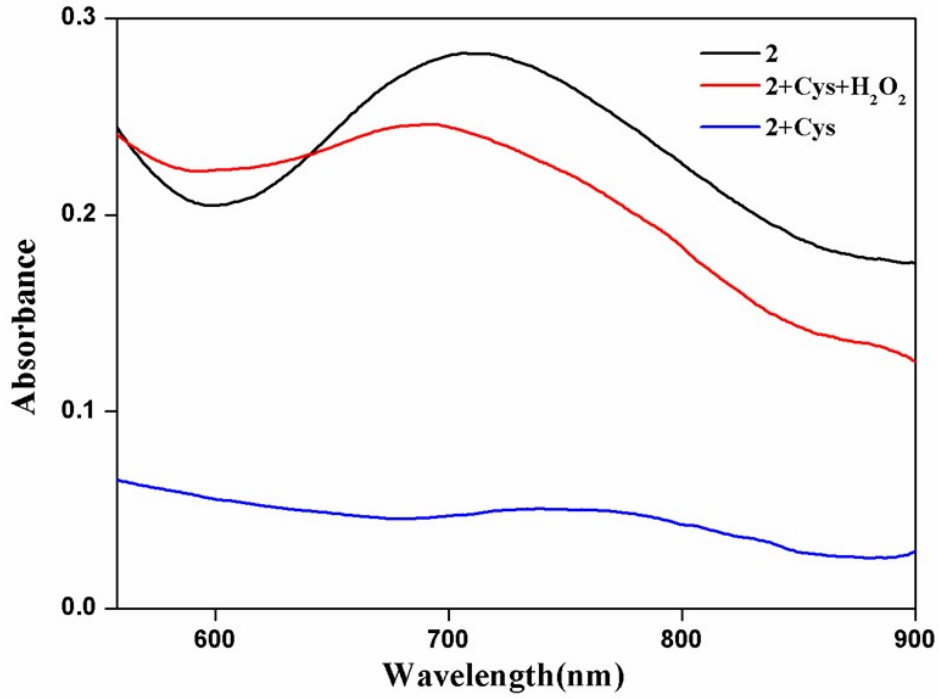


Figure S13. The changes in the d-d band of **2**, **2** + Cys and Cys adduct of **2** + H_2O_2 (1×10^{-2} M) in DMF/HEPES buffer solution, pH 7.34 at 25°C.

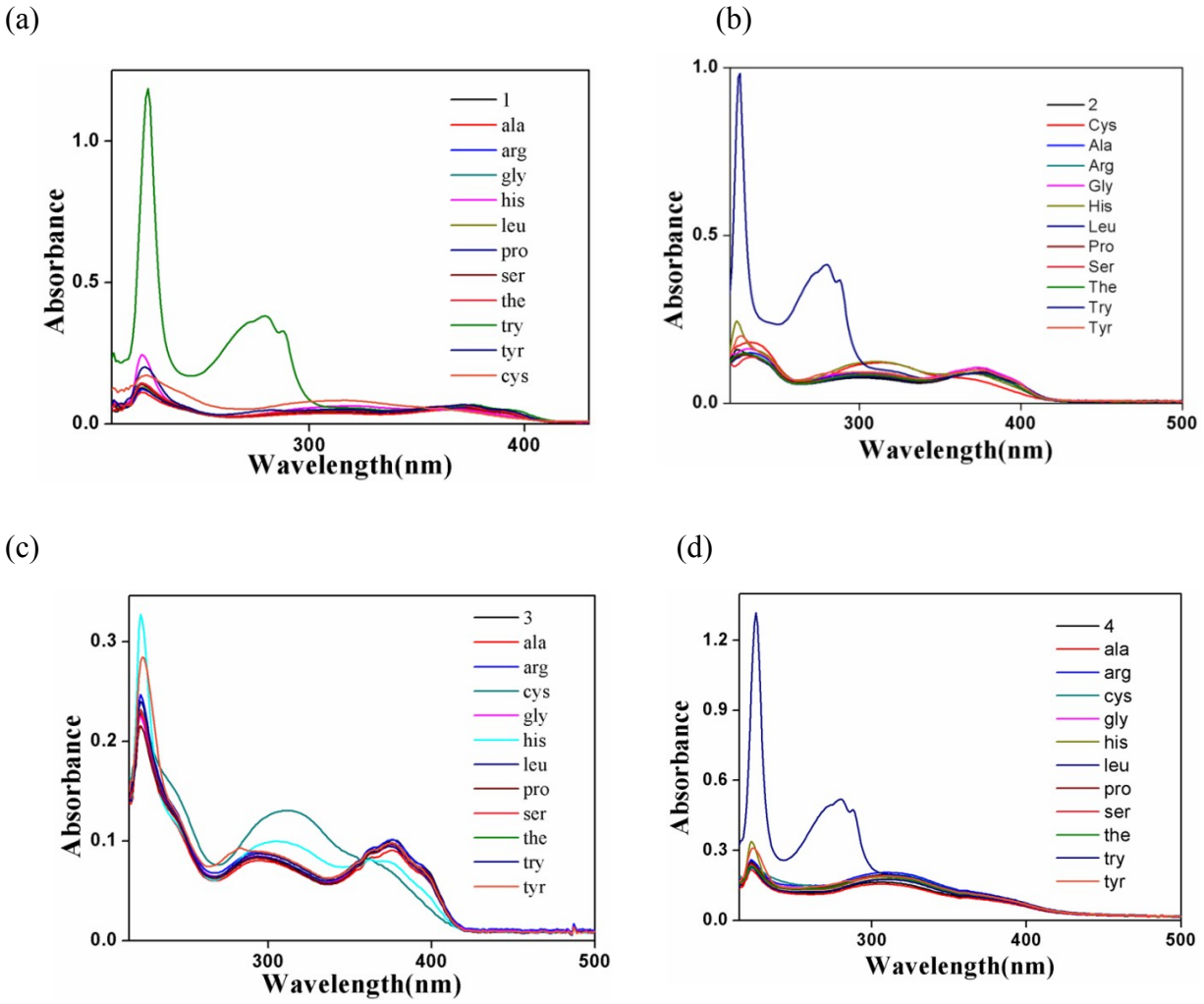


Figure S14. UV-Vis spectral changes for **1** (a), **2** (b) **3** (c) and **4** (d) (5×10^{-6} M) with selected amino acids in HEPES buffer (pH 7.34) at 25°C.

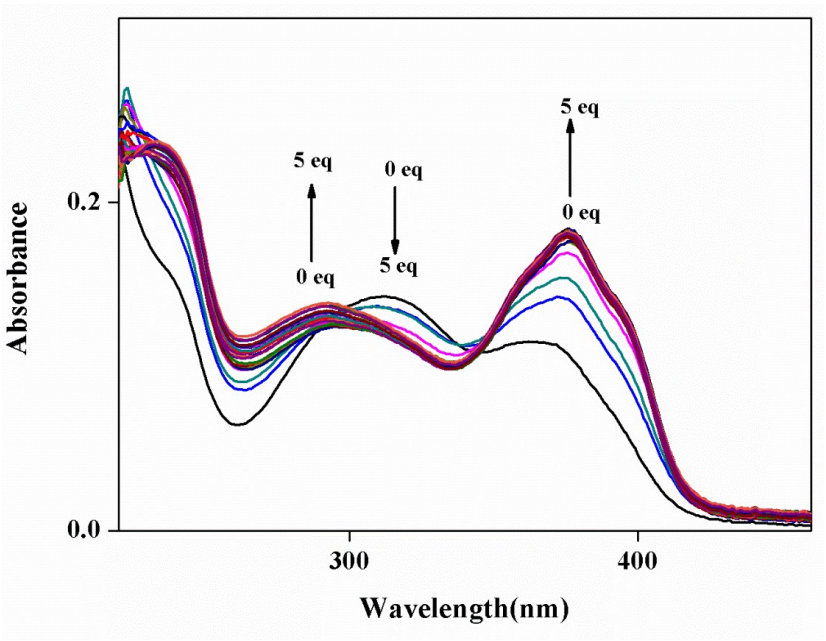


Figure S15. UV-Vis spectral changes for the addition of $Cu(SO_3CF_3)_2$ to L2 (5×10^{-6} M) in HEPES buffer (pH 7.34) at 25°C.

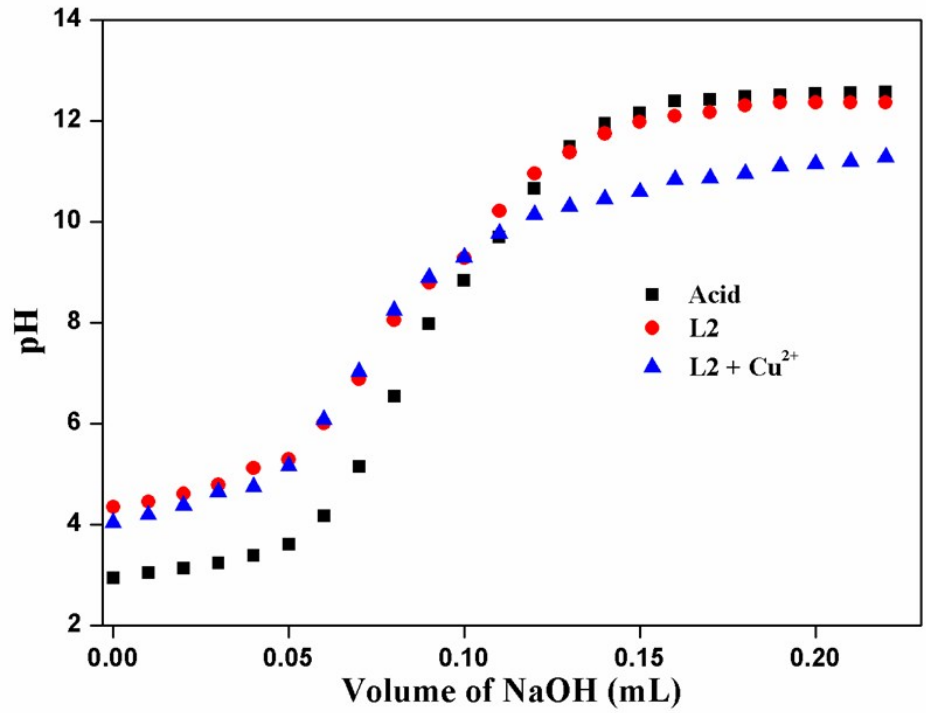


Figure S16. The plot of pH against 0.1 M of NaOH at 30˚C acid (nitric acid), L2, L2 + $Cu(SO_3CF_3)$ in dioxane water mixture.

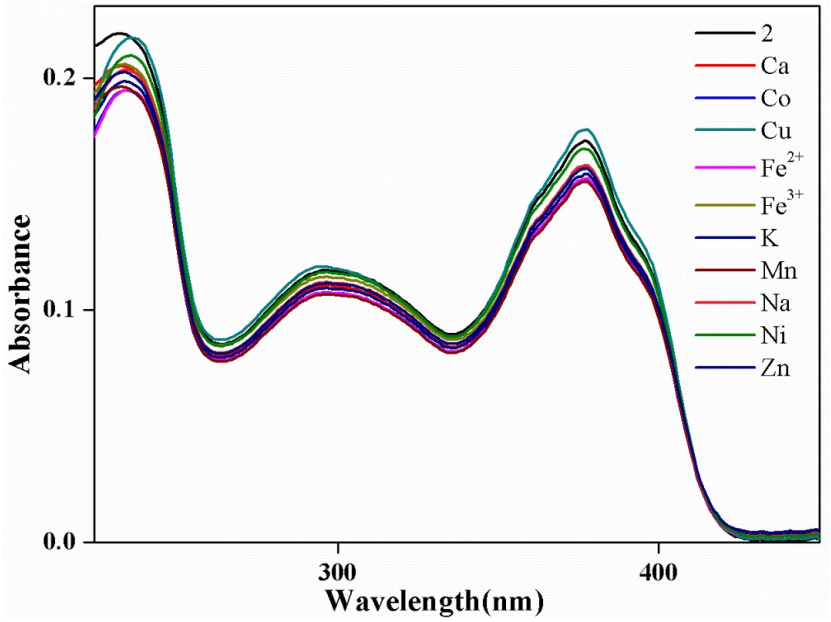
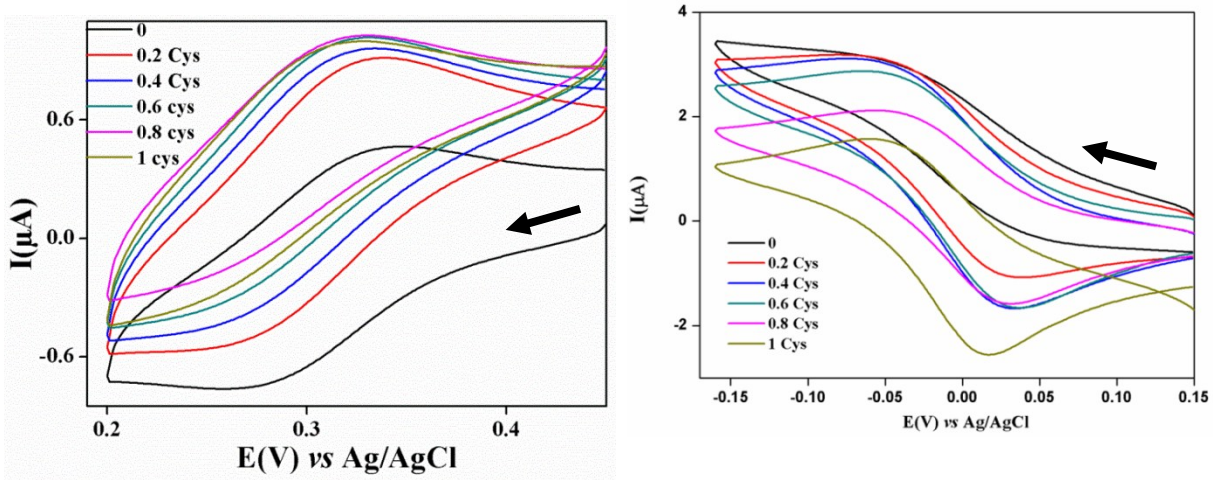


Figure S17. UV-Vis spectral changes of **2** with biologically relevant cations at 25°C in HEPES buffer pH 7.3

(a) (b)



(c)

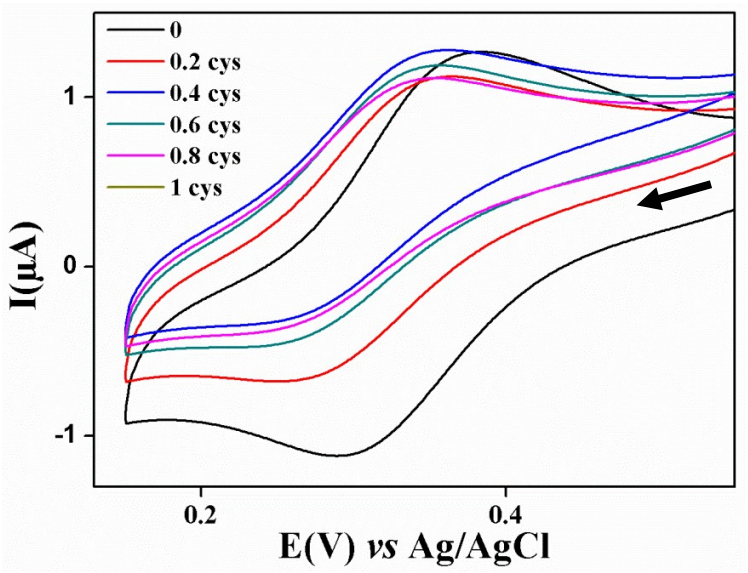
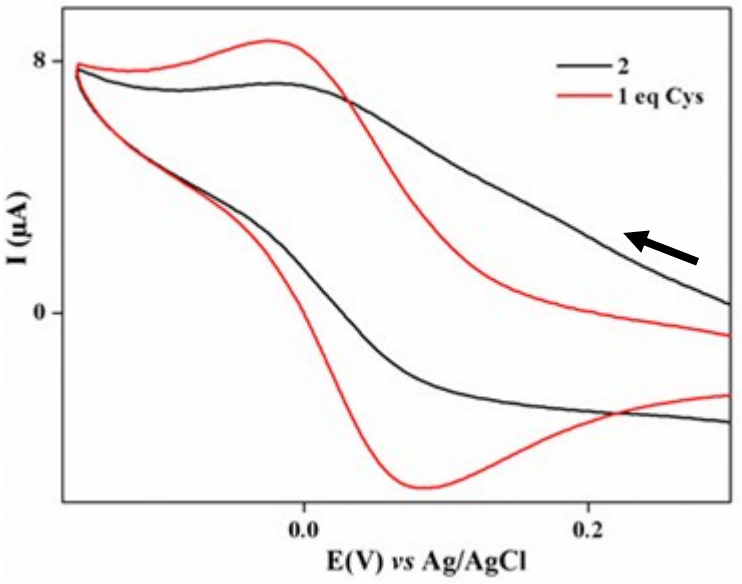


Figure S18. Electrochemical titration of complexes **1** (a), **3** (b) and **4** (c) (1×10^{-3}) with varying amount of Cys (0 to 1 equivalent,). [Reference: saturated Ag/Ag^{+}; counter electrode: Pt and supporting electrolyte: 0.1M NaCl solution; scan rate: = 50 mV s^{-1}].

(a)



(b)

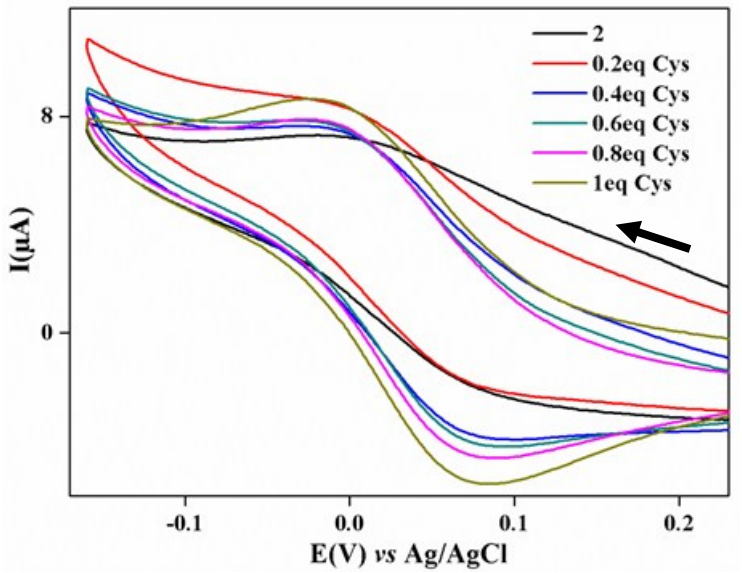


Figure S19. Cyclic voltammograms (CV) of complex $[Cu(L2)_2(SO_3CF_3)](SO_3CF_3)$ **2** (1×10^{-3}) with one equivalent of Cys (a). Complex **2** with different Cys concentrations from 0 to 1 equivalent (b). [Reference: saturated Ag/Ag^+; counter electrode: Pt and supporting electrolyte: 0.1M NaCl solution; scan rate: = 50 mV s^{-1}].

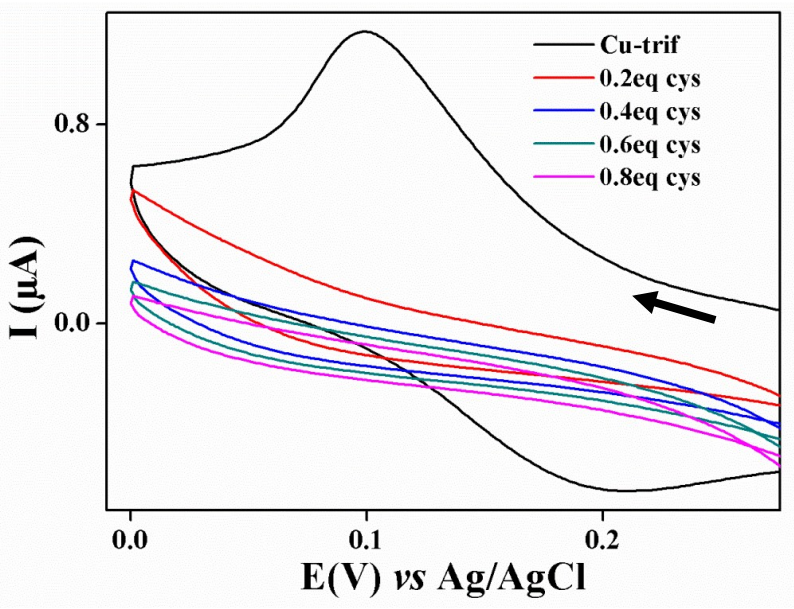
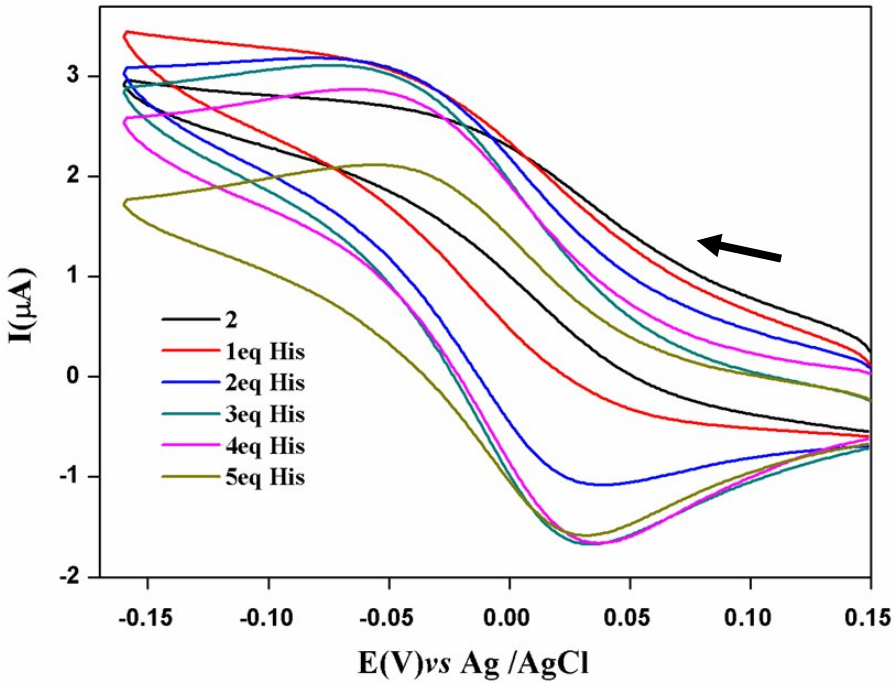


Figure S20. Electrochemical titration of Cys with Cu^{2+} ion solution in HEPES buffer pH = 7.34 at 25°C (concentration 1×10^{-3} M) [reference: saturated Ag/Ag^{+}; counter electrode: Pt and supporting electrolyte: 0.1M NaCl solution; scan rate = $50mVs^{-1}$].

a)



b)

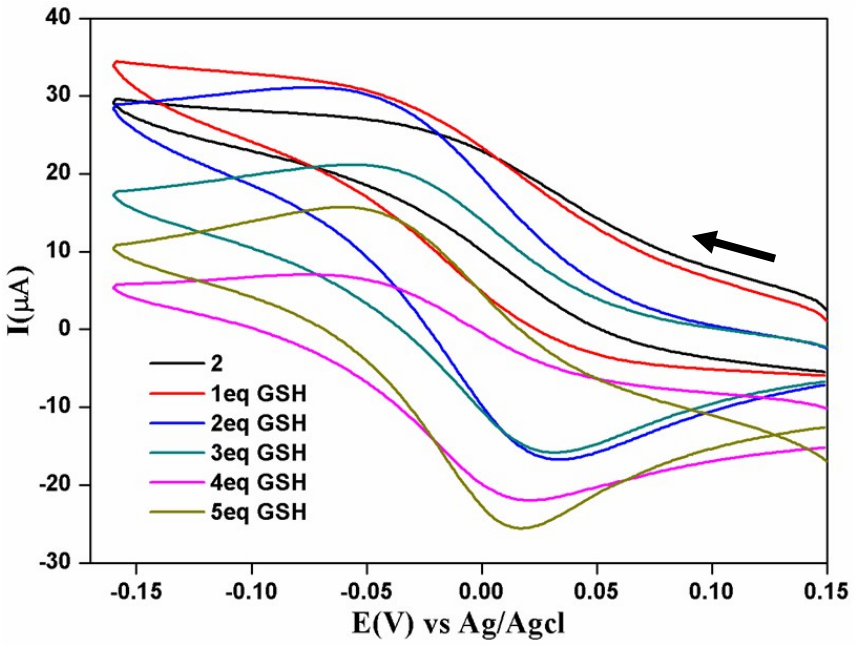


Figure S21. Cyclic voltammograms (CV) of complex $[Cu(L2)_2(SO_3CF_3)](SO_3CF_3)$ **2** (a) (1×10^{-3}) with 1 to 5 equivalents of His. (b) Complex **2** with different GSH concentrations from 1 to 5 equivalents. [Reference: saturated Ag/Ag^+; counter electrode: Pt and supporting electrolyte: 0.1M NaCl solution; scan rate: = 50 mV s^{-1}].

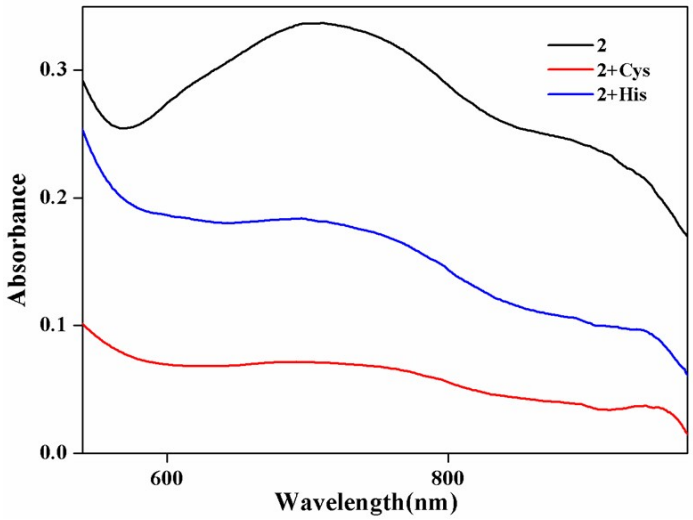


Figure S22. The changes in the d-d band of **2, 2** + Cys**, 2** + His (1×10^{-2} M) in acetonitrile/HEPES buffer solution, pH 7.34 at 25°C.

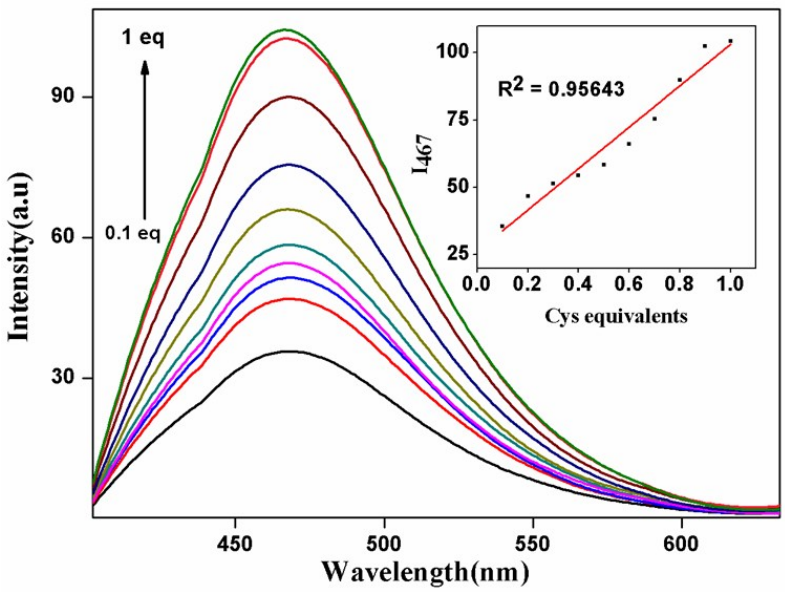


Figure S23. Changes in fluorescence intensity of **2** by addition of various concentrations of His (5×10^{-7} M to 5×10^{-6} M) in HEPES buffer pH, 7.34 at 25°C, [λ_{ex} = 367 nm, slits: 5nm/5nm]. Inset: Plot of fluorescence intensity vs [His].

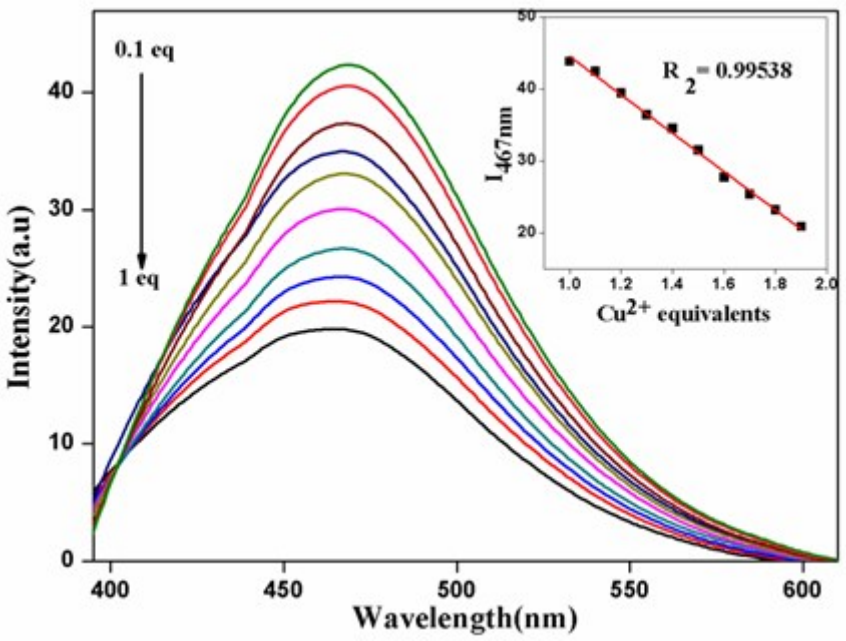


Figure S24. The changes in fluorescence intensity of L2 by addition of various concentrations of Cu^{2+} (5×10^{-7} M to 5×10^{-6} M) in HEPES buffer pH, 7.34 at 25°C, [λ_{ex} = 367 nm, slits: 5nm/5nm]. Inset: Plot of fluorescence intensity vs [Cys].

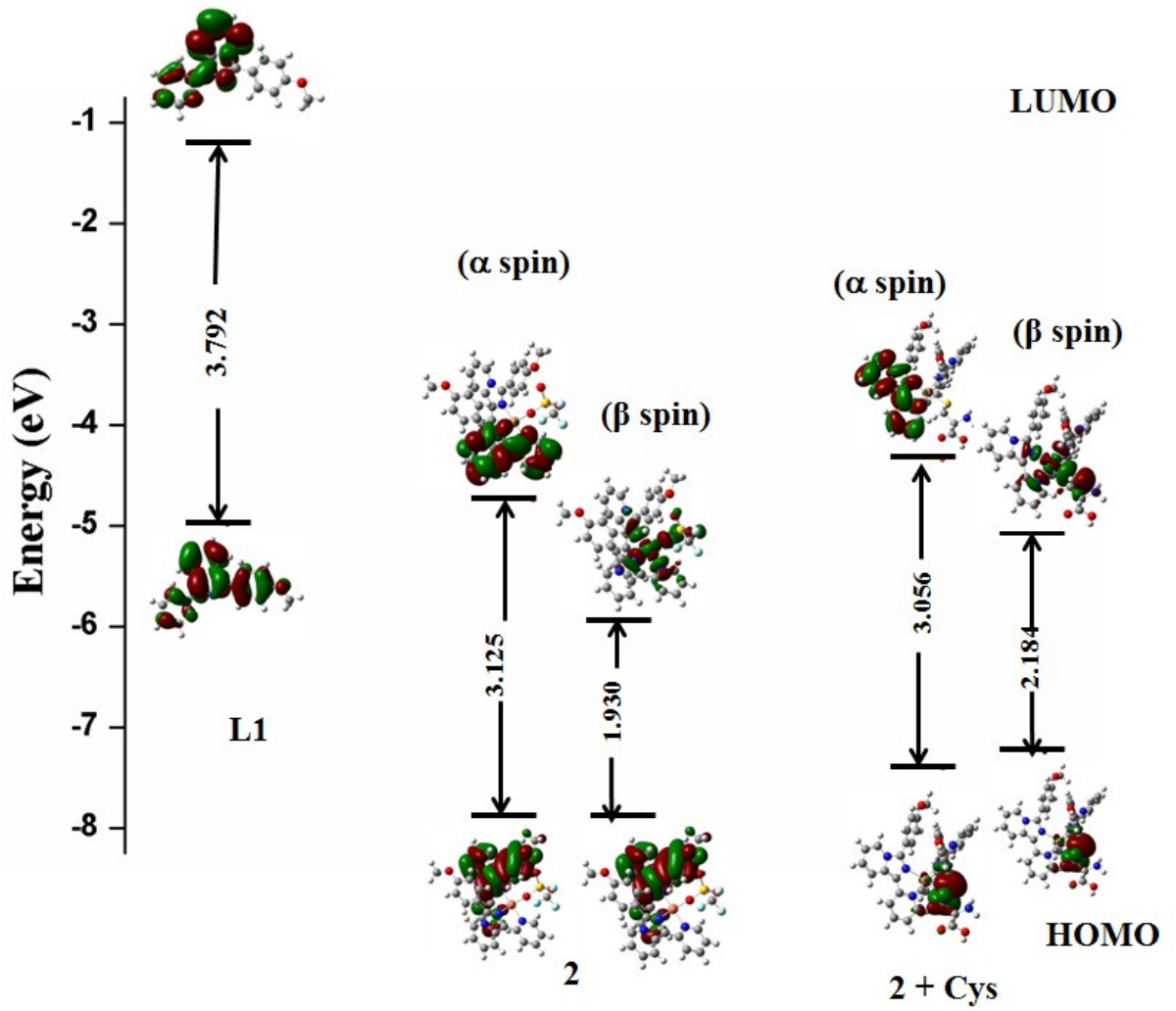


Figure S25. Energy profile diagram for L2, **2** and **2** + Cys; HOMO and LUMO are calculated by TD-DFT using B3LYP 6-31G/LANL2DZ level.

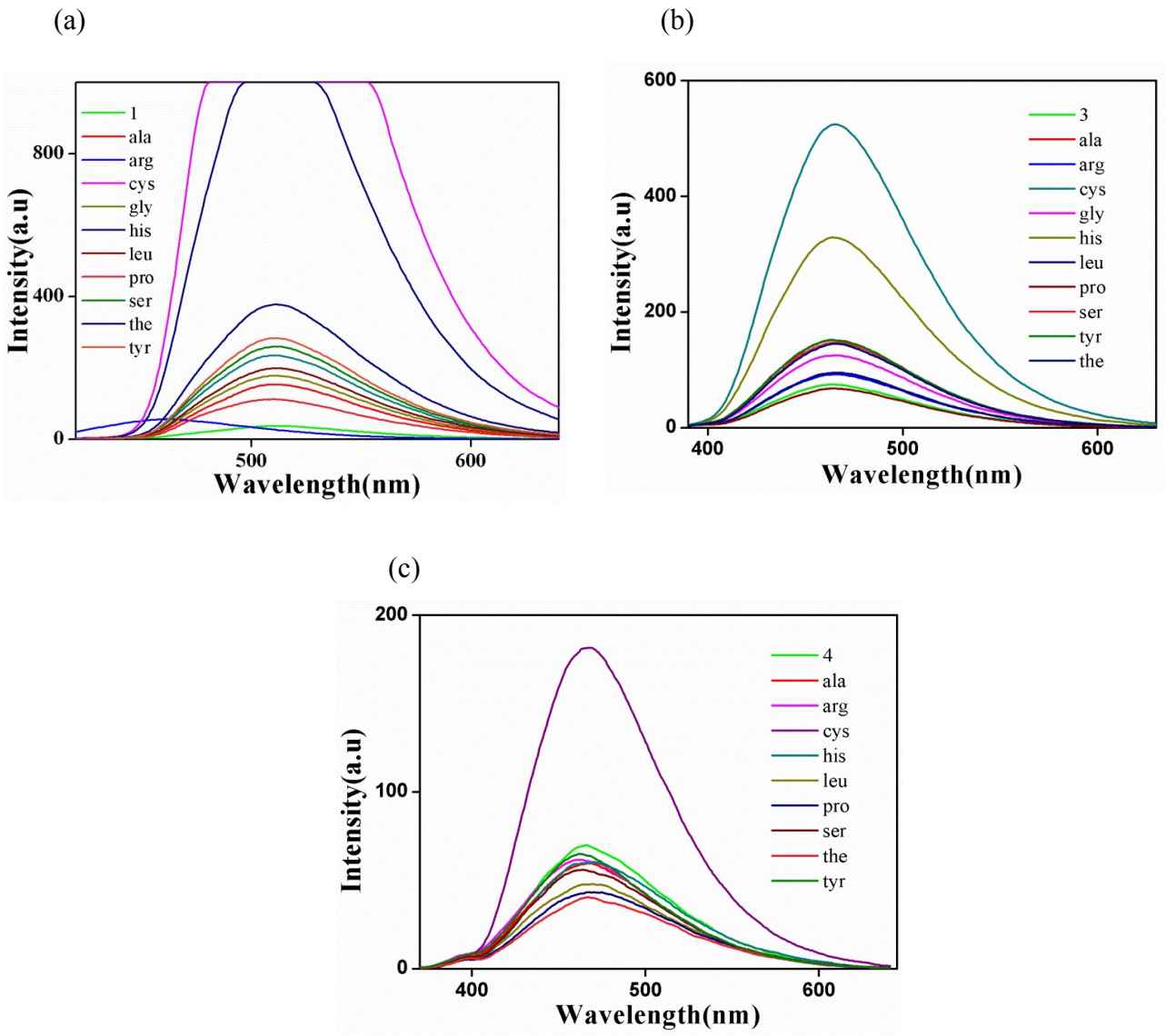


Figure S26. Fluorescence spectra of complexes **1** (a), **3** (b) and **4** (c) (5×10^{-6} M) and after adding various amino acids (5×10^{-5} M) in HEPES buffer pH, 7.34 at 25°.

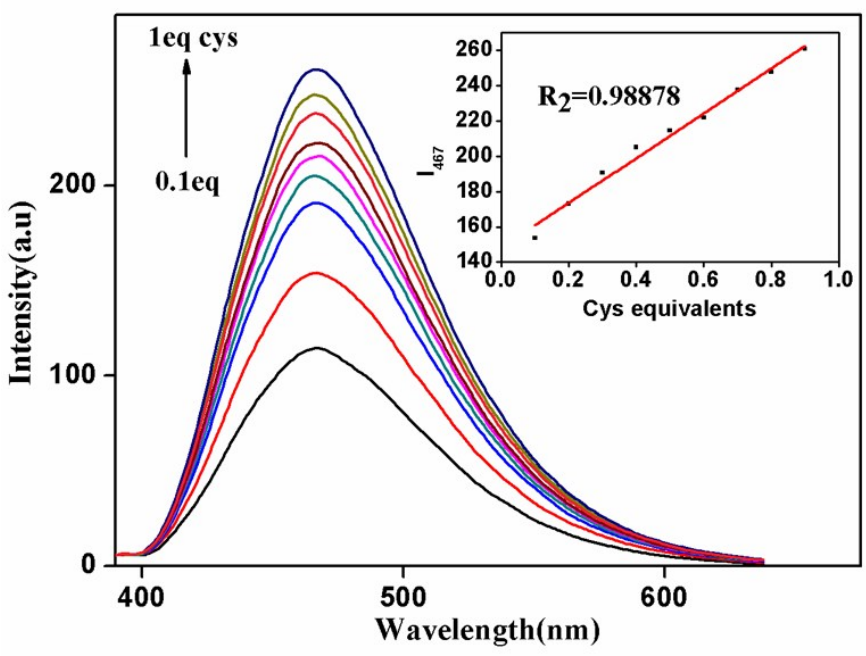


Figure S27. Changes in fluorescence intensity of **2** by addition of various concentrations of Cys (5×10^{-7} M to 5×10^{-6} M) in HEPES buffer pH, 7.34 at 25°C, [λ_{ex} = 367 nm, slits: 5nm/5nm]. Inset: Plot of fluorescence intensity vs [Cys].

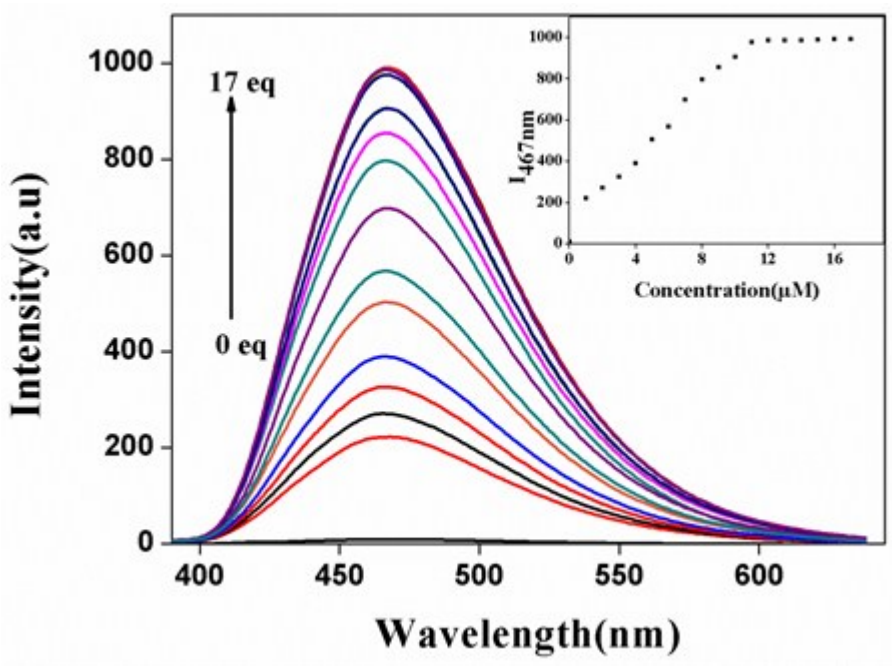


Figure S28. Fluorescence intensity changes of **2** on adding Cys in HEPES buffer, pH 7.34; λ_{exc} = 367 nm, slits: 5nm/5nm, time interval = 5 min].

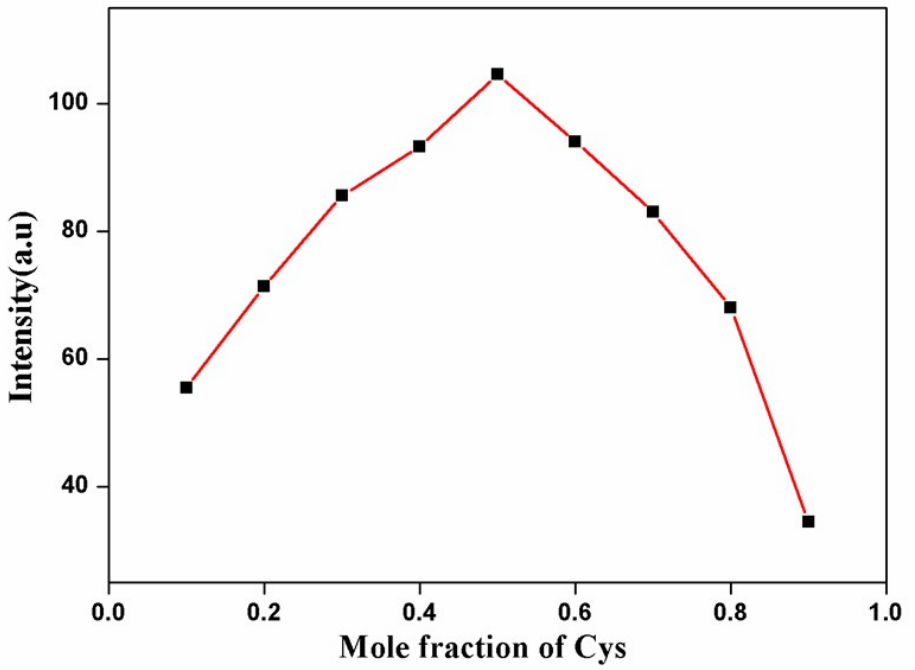


Figure S29. Job's plot, Fluorescence intensity at 467 nm was plotted as a function of the molar ratio of **2** and Cys.

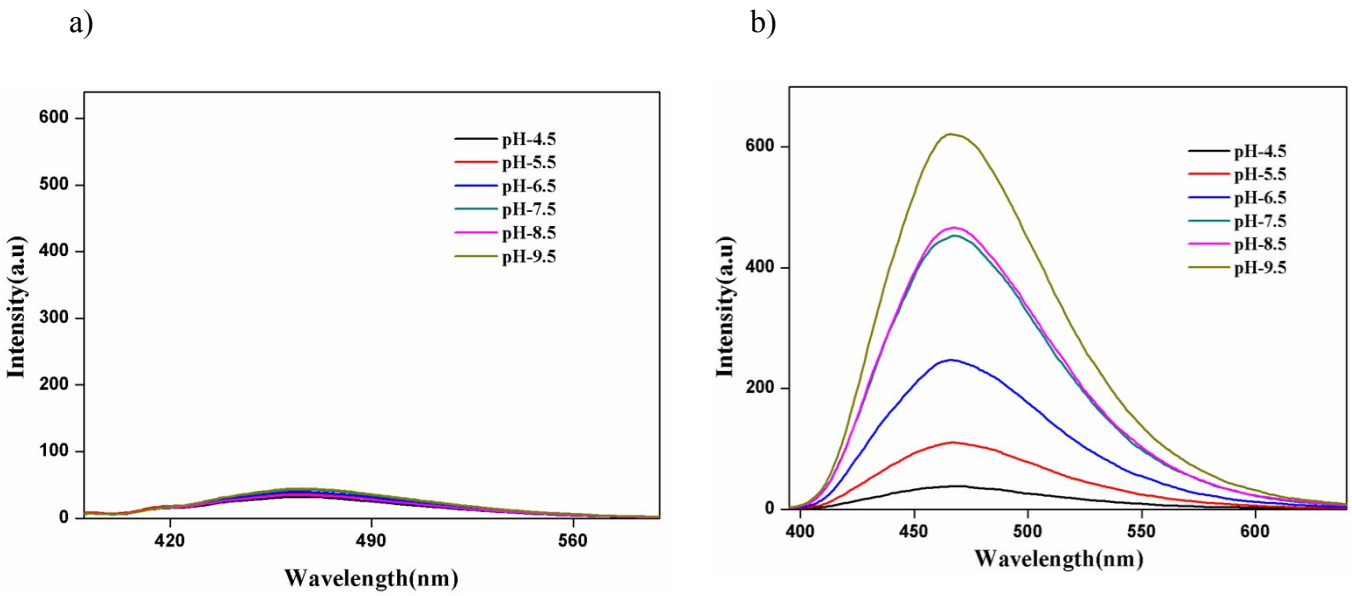


Figure S30. Emission spectral changes for **2** (a) and **2** + Cys (b) in various pH (5×10^{-6}M) at 25˚C.

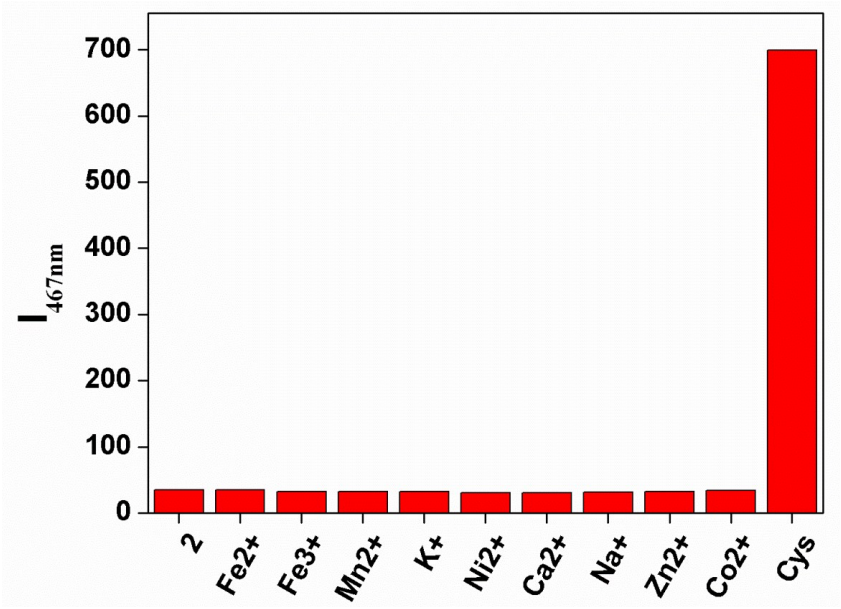


Figure S31. Bar diagram for fluorescence intensity of **2** with biologically relevant cations in HEPES buffer pH 7.34 (λ_{ex} = 467nm) at 25 °C.

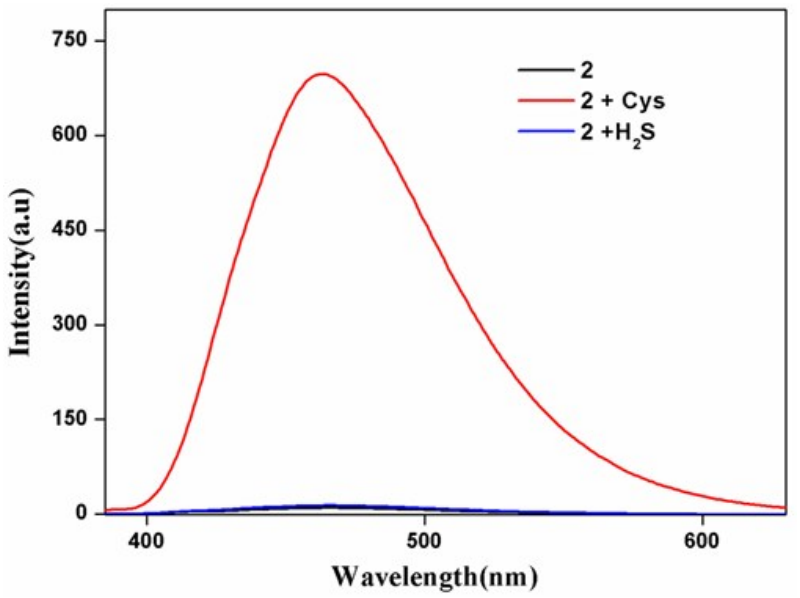


Figure S32. (a) Fluorescence spectra of **2, 2**+Cys and **2**+ H_2S (5×10^{-6} M) in HEPES buffer pH, 7.34 at 25 °C.

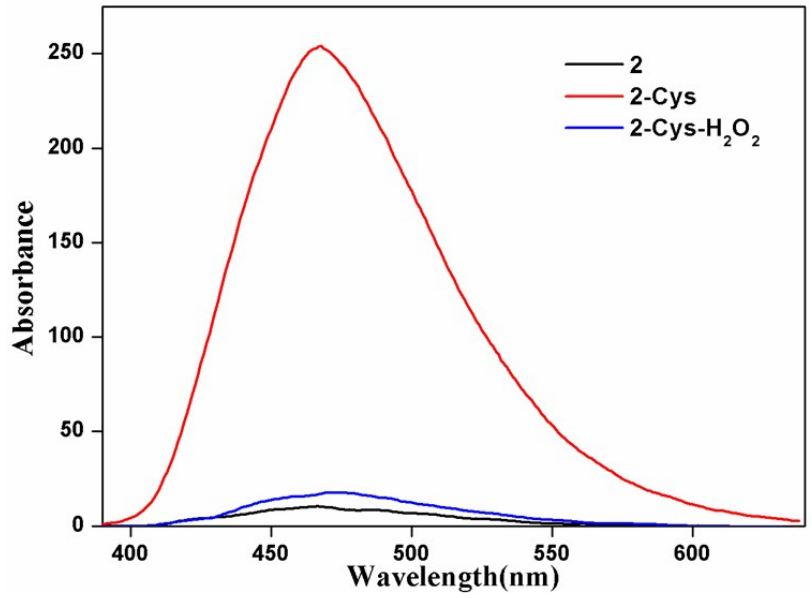


Figure S33. (a) Fluorescence spectra of **2** (5×10^{-6} M) with H_2O_2 in HEPES buffer pH, 7.34 at 25 °C.

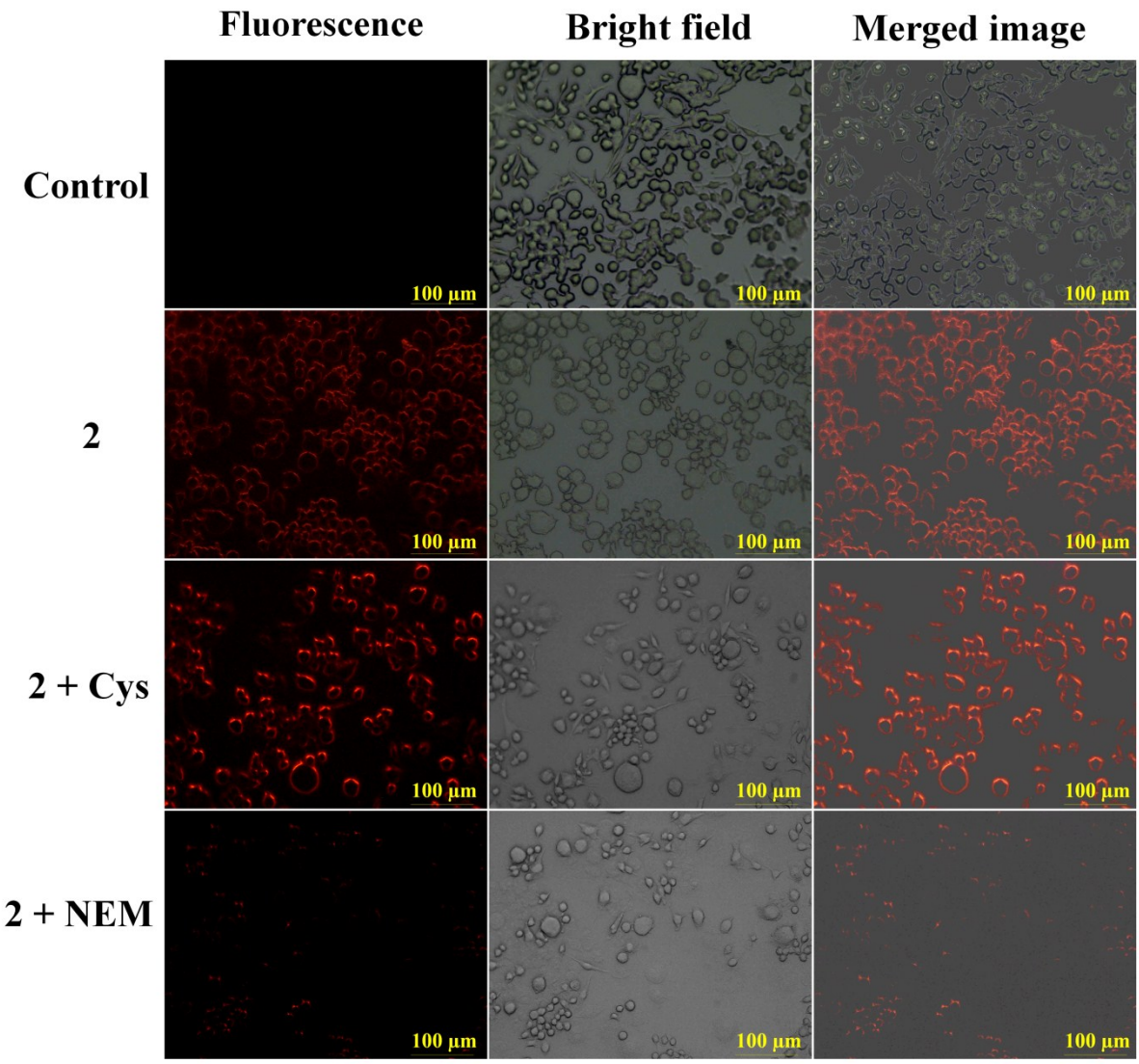


Figure S34. Fluorescence and bright-field images of Macrophage cells: (a) cells in the absence of **2**, (b) cells incubated with **2** (5 µM) for 30 minutes, (c) cells pre-treated with 100 µM Cys and incubated with **2** (5 µM) for 30 minutes, and (d) cells pre-treated with 200 µM NEM and incubated with **2** (5 µM) for 30 minutes

Table S1. Fluorescence quantum yield of L2 (2×10^{-6}M) and 2 + 20eq Cys (2×10^{-6}M) Fluorescence reference material: Quinine sulfate (2×10^{-6}M)

$$\mathbf{Y_u = Y_s \times (F_u/F_s) \times (A_s/A_u)}$$

	λ_{exe} (slits;5/5nm)	F (Integral fluorescence intensity)	A (Absorbance at 366nm)	Integration Range	Y (Fluorescence Quantum yield)
Quinine sulfate	362	76655.47673	0.021168	381- 641	-
L1	362	3076.67930	0.011683	386-641	4%
1 + Cys	362	5820.24758	0.059496	386-641	1.6%
Quinine sulfate	367	60288.75532	0.017108	386-641	-
L2	367	20021.7859	0.083895	381-641	3.8%
2 + Cys	367	32804.3097	0.035156	381-641	14%
Quinine sulfate	367	60288.75532	0.017108	386-641	-
L3	367	23155.4069	0.048026	386-641	7.6%
3 + Cys	367	5403.9150	0.077878	386-641	1.8%
Quinine sulfate	318	80456.1335	0.024580	337-641	-
L4	318	8182.14125	0.132585	337-641	1.1%
4 + Cys	318	7518.9157	0.093554	337-641	1.4%

Table S2. Computational data

Computational details[a] [eV]	L	**2**	**2**+ Cys
Optimized energy	-2.6456×10^4	-7.3850×10^4	-6.7320×10^4
HOMO	-4.986	-7.829 (α spin)	-7.401(α spin)
		-7.835 (β spin)	-7.252 (β spin)
LUMO	-1.194	-4.823(α spin)	-4.346 (α spin)
		-6.035 (β spin)	-5.068 (β spin)
Energy gap	3.792	3.006 (α spin)	3.0555 (α spin)
		1.800 (β spin)	2.184 (β spin)
TD-DFT			
Optimized energy	-2.6453×10^4	-7.3849×10^4	-6.7320×10^4
HOMO	-4.985	-7.870 (α spin)	-7.401(α spin)
		-7.869 (β spin)	-7.252 (β spin)
LUMO	-1.194	-4.745 (α spin)	-4.346 (α spin)
		-5.939 (β spin)	-5.064 (β spin)
Energy gap	3.792	3.125(α spin)	3.056 (α spin)
		1.930 (β spin)	2.184 (β spin)

[a] DFT method with B3LYP 6-31G (for C, H and N) and LANL2DZ (for Cu) basis sets in the Gaussian 09 program.

Reply for B-level Check CIF alerts for each compound

Complex 1

PLAT910_ALERT_3_B Missing # of FCF Reflection(s) Below Theta(Min) 19 Note

Authors Response: Some reflections had to be omitted in the refinement for technical reasons.

Complex 2

PLAT230_ALERT_2_B Hirshfeld Test Diff for S2 -- O1W_a .. 12.5 s.u.

PLAT230_ALERT_2_B Hirshfeld Test Diff for O1W -- O24_a .. 20.0 s.u.

PLAT230_ALERT_2_B Hirshfeld Test Diff for O24 -- O1W_a .. 20.0 s.u.

Authors Response: These alerts were appeared due to RIGU restraints. They were applied to atoms in the disordered chains. Several of the atoms were still not ideally shaped, however, this does not indicate an incorrect atom-type assignment.

PLAT415_ALERT_2_B Short Inter D-H..H-X H34 .. H77 .. 2.04 Ang

Authors Response: This alert is related to the hydrogen atoms of lattice water molecules. It is very difficult to locate hydrogen atoms accurately using X-ray data because these atoms have low scattering power. In addition, the severe disorder in the complex makes the situation even worse.

PLAT780_ALERT_1_B Coordinates do not Form a Properly Connected Set Please Do !

Author Response: These alerts are related to the disorder of solvent molecules and anions also data collection done in room temperature. These alerts are acceptable in crystallographic point of view.

PLAT910_ALERT_3_B Missing # of FCF Reflection(s) Below Theta(Min) 14 Note

Authors Response: Some reflections had to be omitted in the refinement for technical reasons.

Complex 3

THETM01_ALERT_3_B The value of sine(theta_max)/wavelength is less than 0.575

Calculated sin(theta_max)/wavelength = 0.5555

Author Response: A full set of data was collected, however, the very high angle data was dominated by noise [I/sigma(I) < 1.0] and was omitted. This arbitrary theta limit is inappropriate for highly disordered structures. It would rule out all macromolecular

structures. A limit on data / parameter ratio's that properly takes into account the number of restraints / constraints and the redundancy of the measurements would be more appropriate. Unfortunately the cifcheck routine does not do this.

PLAT231_ALERT_4_B Hirshfeld Test (Solvent) F4 --C42 . 13.7 s.u.

Author Response: These alerts are related to the disorder of solvent molecules and anions also data collection done in room temperature. These alerts are acceptable in crystallographic point of view.

PLAT234_ALERT_4_B Large Hirshfeld Difference S2 --C42 0.30 Ang.

Authors Response: This is due to RIGU restraints were applied to atoms in the disordered chains. Several of the atoms were still not ideally shaped, however, this does not indicate an incorrect atom-type assignment**.**

PLAT601_ALERT_2_B Structure Contains Solvent Accessible VOIDS of . 137 Ang**3

Authors Response: The minor part of the disordered guest molecule was not assigned, which resulted in the voids.

PLAT910_ALERT_3_B Missing # of FCF Reflection(s) Below Theta(Min). 20 Note.

Authors Response: Some reflections had to be omitted in the refinement for technical reasons.

Complex 4

PLAT214_ALERT_2_B Atom C42 (Anion/Solvent) ADP max/min Ratio 5.7 prolat

Authors Response: This alert generated because there is a large amount of disorder in the structure

PLAT231_ALERT_4_B Hirshfeld Test (Solvent) S2 -- C42 .. 12.3 s.u.

Author Response: These alerts are related to the disorder of solvent molecules and anions also data collection done in room temperature. These alerts are acceptable in crystallographic point of view.

PLAT231_ALERT_4_B Hirshfeld Test (Solvent) F5 -- C42 .. 20.0 s.u.

Author Response: These alerts are related to the disorder of solvent molecules and anions also data collection done in room temperature. These alerts are acceptable in crystallographic point of view.

PLAT234_ALERT_4_B Large Hirshfeld Difference F4 -- C42 .. 0.28 Ang.

Authors Response: RIGU restraints were applied to atoms in the disordered chains. Several of the atoms were still not ideally shaped, however, this does not indicate an incorrect atom-type assignment.

PLAT910_ALERT_3_B Missing # of FCF Reflection(s) Below Theta(Min) 20 Note

Authors Response: Some reflections had to be omitted in the refinement for technical reasons.