Supplementary information

Glutathione selective “off-on” fluorescence response by probe displaced modified ligand for its detection in biological domain

Partha Pratim Parui,*a Ambarish Ray,*a,b Sanju Das,a,b Yeasmin Sarkar,a Tanaya Paul,c Snigdha Roy,a Rini Majumder,a and Jaya Bandyopadhyayc

aDepartment of Chemistry, Jadavpur University, Kolkata 700032, India. Fax: +91-33-24146223, Phone:+91-9433490492, E-mail: parthaparui@yahoo.com
bDepartment of Chemistry, Maulana Azad College, Kolkata 700013, India. Fax: +91-33-22268111, Phone:+91-9836650180, E-mail: r_ambarish@yahoo.co.in
cDepartment of Biotechnology, Maulana Abul Kalam Azad University of Technology, Kolkata 700064, India

Contents

Fig. S1 ¹H and ¹³C NMR of L₁ ... p S2
Fig. S2 ¹H and ¹³C NMR of L₁’ ... p S3
Fig. S3 ESI-MS⁺ studies ... p S4
Fig. S4 pH dependent UV-vis spectra of 1 ... p S5
Fig. S5 Time-response UV-vis curve for complex (1) formation ... p S6
Fig. S6 UV-vis spectra for complex formation for various metal ions ... p S7
Fig. S7 Job’s plot for complexation ... p S8
Fig. S8 TD-DFT calculated UV-vis spectrum of 1 ... p S9
Fig. S9 Biothiol induced decomplexation kinetics by UV-vis studies ... p S10
Fig. S10 FT-IR studies ... p S11
Fig. S11 pH metric titration of L₁’ ... p S12
Fig. S12 GSH concentration dependent decomplexation kinetics by UV-vis studies ... p S13
Fig. S13 Fluorescence studies of 1 in presence of biothiols ... p S14
Fig. S14 UV-vis studies for biothiols-induced decomplexation of 1 ... p S15
Fig. S15 EPR spectra of 1 with and without GSH ... p S16
Fig. S16 Solvent dependent GSH induced fluorescence increase for 1 ... p S17
Fig. S17 GSH induced decomplexation of 1 in solvents with and without oxygen ... p S18
Fig. S18 Fluorescence studies of 1 with various anions, cations, and amino acids ... p S19
Fig. S19 Toxicity in the form of survival assays of C. elegans ... p S20
Fig. S20 Bio-imaging studies for L₁’ ... p S21
Fig. S21 Cell viability assays of SH-SY5Y cells ... p S22
Fig. S1. (A) $^1$H-NMR and (B) $^{13}$C-NMR spectra of L₁ in DMSO-d₆.
Fig. S2. (A) $^1$H-NMR and (B) $^{13}$C-NMR spectra of L$_1'$ in DMSO-d$_6$. 
**Fig. S3.** ESI-MS$^+$ of (A) $L_1$ ($m/z$: found 233.216; calcd. 233.251) for [L$_1$+H]$^+$, (B) $L_1$' ($m/z$: found 231.088; calcd. 231.235, $m/z$ for [L$_1$'+H]$^+$, (C) 1 ($m/z$: found 725.109; calcd. 725.038 for [C$_{22}$H$_{22}$N$_8$O$_4$Cu$_2$+ClO$_4$]$^+$, (D) 1+GSH ($m/z$: found 231.305 for [L$_1$'+H]$^+$, and 613.183 for [GSSG+H]$^+$: calcd. 613.355), and (E) 1+Cys ($m/z$: found 233.581 for [L$_1$+H]$^+$; and 241.617 for [Cystine+H]$^+$) calcd. 241.341) in water.
**Fig. S4.** UV-vis absorption studies of 1 (5 µM with respect to L1) in buffer medium at different pH: cyan, pH 6.0; red, pH 7.0; blue, pH 8.0; orange, pH 9.0.
Fig. S5. Time response UV-vis absorption intensity at 405 nm by addition of Cu(ClO$_4$)$_2$ (40 µM) in 20 mM HEPES-NaOH buffer, pH 7.3 containing L$_1$ (5 µM) at 25°C.
Fig. S6. UV-vis absorption spectra of L₁ (5 µM) in 20 mM HEPES-NaOH buffer, pH 7.3, containing different metal ions (0–40 µM) at 25°C: red, Cu²⁺; dark yellow, Ni²⁺; cyan, Co²⁺; orange, Fe²⁺ and dark blue, Mn²⁺; The spectrum in absence of metal ions is shown by green for comparison.
Fig. S7. Job’s plot for determining the stoichiometry of the complex between L₁ and Cu²⁺. The difference between the observed and L₁ absorbance at 405 nm were plotted with mole fraction of Cu²⁺ ($X_{\text{Cu}^{2+}}$) in the various mixture of L₁ and Cu(ClO₄)$_2$ ($\varepsilon_L$ and C$_L$ are the extinction coefficient and concentration of L₁, respectively).
**Fig. S8.** Electronic excitation wavelength ($\lambda$) and extinction coefficient ($\varepsilon$) 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 and TD-DFT calculated spectra depicted by red and black respectively.
**Fig. S9.** Biothiols induced time response UV-vis absorption intensities at (blue) 440 nm for GSH (100 µM), (purple) 405 nm for Cys (250 µM) and (violet) 405 nm for Hcy (250 µM each) in 20 mM HEPES-NaOH buffer, pH 7.3 containing 1 (5 µM with respect to L₁) at 25°C.
Fig. S10. FT-IR spectrum of (A) 1+GSH and (B) synthesized L₁′.
**Fig. S11.** (A) pH dependent UV-vis absorption spectra of synthesized L₁: black, pH 5.0; dark blue, pH 5.7; blue, pH 6.2; purple, pH 6.8; dark cyan, pH 7.5; magenta, pH 8.0; dark yellow, pH 8.5 and red, pH 9.0. (B) Extinction coefficients at 440 are plotted with pH, fitted the data points with sigmoidal-Boltzmann equation.

Note: Transition mid-point of the fitted curve represents the pKₐ ~7.0.
**Fig. S12.** Time response fluorescence intensities at 555 nm by addition of various concentration of GSH (black, 12 equiv.; dark cyan, 15 equiv. and blue, 20 equiv.) in 20 mM HEPES-NaOH buffer, pH 7.3 containing 1 (5 µM with respect to L1) at 25°C. Excitation wavelength were 440 nm.
Fig. S13. Fluorescence spectra of I (5 μM with respect to L₁) in presence of GSH (dark yellow), Cys (purple) and Hcy (violet) (20 equiv. each) in 20 mM HEPES, pH 7.3 for the excitation at 365 nm. The spectrum in presence of GSH (broken blue) (20 equiv.) for excitation at 440 nm is depicted for comparison.
Fig. S14. UV-vis absorption studies of 1 (5 μM with respect to L₁) in absence (red) and presence of Cys (20 equiv., purple) or Hcy (20 equiv., orange). The spectrum of L₁ (green) is depicted for comparison.
**Fig. S15.** EPR spectrum of I (red) before and (blue) after addition of GSH addition in H$_2$O-MeCN (1:1) mixture at 77K. (blue) The spectrum was recorded in 40 s after addition of GSH.
**Fig. S16.** The extent of 555-nm fluorescence intensity enhancement ($F/F_0$) due to 440-nm excitation for 1 in presence of GSH (20 equiv.) for different solvent medium are depicted by bar-diagram.
**Fig. S17.** UV-vis absorption spectrum of 1 (5 µM with respect to L₁) in presence of GSH (20 equiv.) in different solvents (A) without and (B) with saturated oxygenated conditions: black, THF; red, MeCN; blue, DMSO; green, MeOH and dark yellow, DMF.
**Fig. S18.** The extent of 555-nm fluorescence intensity enhancement ($F/F_0$) due to 440-nm excitation for I in presence of various (A) anions (50 equiv. each), (B) biologically important metal ions (50 equiv. each) and (C) amino acids (20 equiv. each) in 20 mM HEPES-NaOH, pH 7.3.
Fig. S19. Assessment of toxicity of the 1 in presence and absence of various analytes after incubation of 30 min. by survival assays in *C. elegans* are depicted by bar-diagram.
**Fig. S20.** Fluorescence images of the *C. elegans* exposed to L1/ (40 µM) for 30 min. The scale bars: 40 µm.
Fig. S21. Percent (%) cell viability of SH-SY5Y cells treated with different analytic concentrations for 12 h determined by MTT assay.