Supporting Information

4-(4,5-Diphenyl-1*H*-imidazol-2-yl)-*N*,*N*-dimethylaniline-Cu(II) complex, a highly selective probe for glutathione sensing in water-acetonitrile mixtures

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Titration experiments: UV/vis and fluorescence titration experiments were carried out with a freshly prepared solution of probe **1** (5.0×10^{-5} mol L^{-1}) dissolved in water (pH 7.4)-acetonitrile 1:1 (v/v) and stored in a dry atmosphere. Moreover, a solution of copper(II) perchlorate hexahydrate salt (7.5×10^{-3} mol L^{-1}) in acetonitrile was also prepared. For the titrations we used a fluorimeter cuvette containing probe **1** (3 mL) and then we added, subsequently, 5-200 μL of the Cu(II) acetonitrile solution. After each addition, the UV-visible and the fluorescence were measured. The same procedure was used for the titration of **1**·Cu(II) with GSH but, in this case the complex (6.2×10^{-6} mol L^{-1}) was prepared in water (pH 7.4)-acetonitrile 1:1 (v/v) and the biothiol was dissolved in water (pH 7.4).

Stability constant determination: The apparent binding constant for the formation of the respective complexes were evaluated using the Benesi–Hildebrand (B–H) plot (equation 1) [1-3]:

$$1/(A - A_0) = 1/\{K(A_{max} - A_0)C\} + 1/(A_{max} - A_0) \qquad (1)$$

A_0 is the absorbance of **1**·Cu(II) complex at the absorbance maximum (λ = 490 nm), A is the observed absorbance at that particular wavelength in the presence of a certain concentration of Cu(II) (C), A_{max} is the maximum absorbance value that was obtained at λ = 490 nm during titration with varying Cu(II) concentrations, K is the apparent binding constant, which was determined from the slope of the linear plot, and C is the concentration of the Cu(II) added during titration studies.

GSH limit of detection evaluation: The limit of detection of GSH using **1**·Cu(II) complex was calculated using the emission titration profiles obtained (see Figure S6) Detection limit was calculated with equation 2:

$$LOD = 3.3\ \sigma/k \quad (2)$$

where σ is the standard deviation of the blank measurement, and k is the slope between the ratio of UV–vis absorbance versus GSH concentration.

Selectivity assays of 1·Cu(II) complex for GSH in the presence of Cys and Hcy: In order to evaluate the selectivity of **1**·Cu(II) complex for GSH, competitive assays in the presence of other biothiols (such as Cys and Hcy) were carried out. For this purpose, the emission intensity at 404 nm (excitation at 320 nm) of a water (pH 7.4)-acetonitrile 1:1 (v/v) solution of **1**·Cu(II) complex was measured alone, in the presence of GSH (2.0 eq.) and in the presence of a mixture of GSH+Cys+Hcy (2.0 eq. of each biothiol). The obtained results are shown in Figure S7. As could be seen, nearly the same emission intensity was observed when **1**·Cu(II) complex was treated with GSH and with the mixture of the three biothiols. These results clearly demonstrated the selectivity of **1**·Cu(II) complex toward GSH because the presence of the other biothiols (Cys and Hcy) did not induce any emission enhancement.

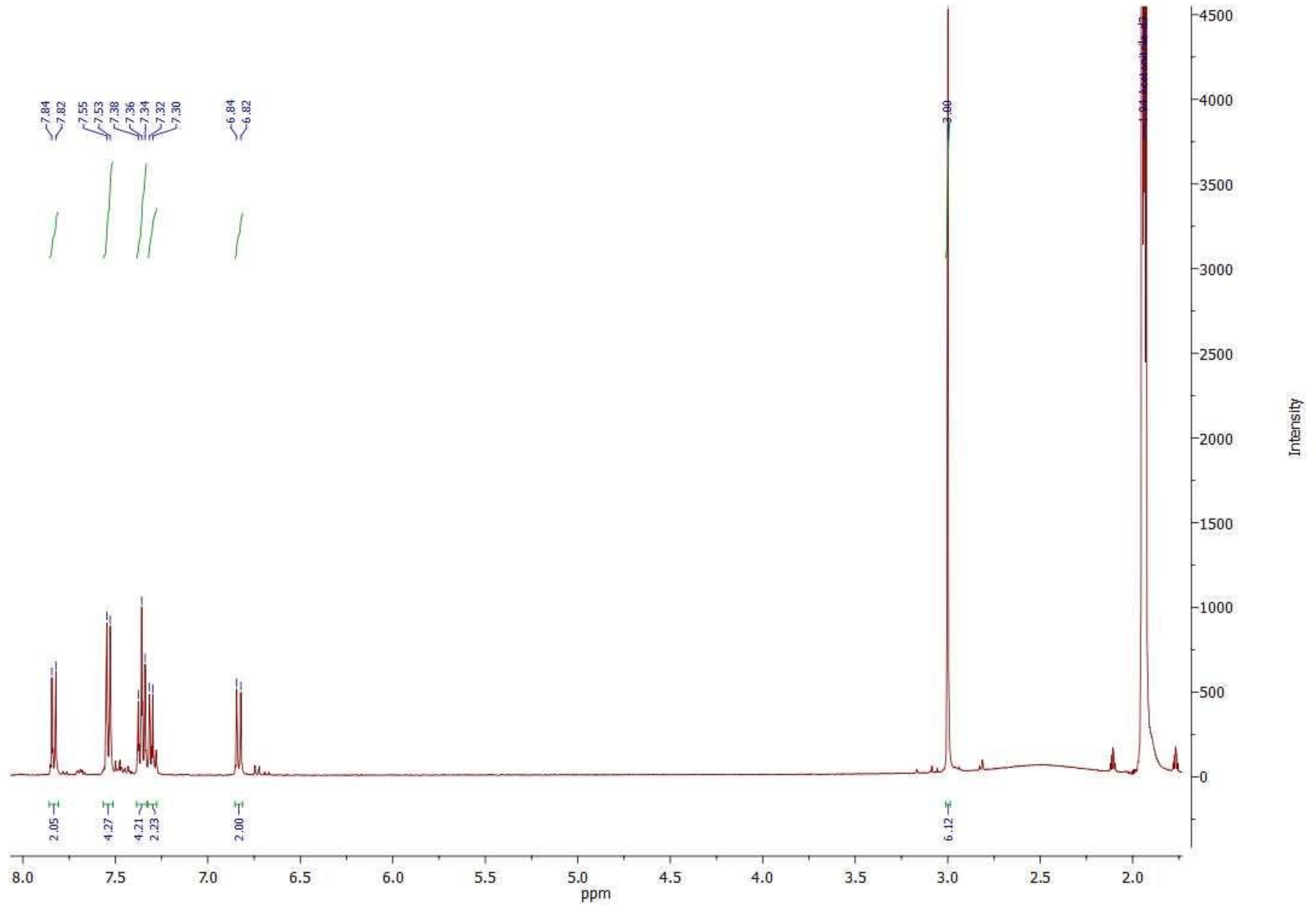

Figure S1. ^{1}H NMR spectra of probe **1** in DMSO-d_6.

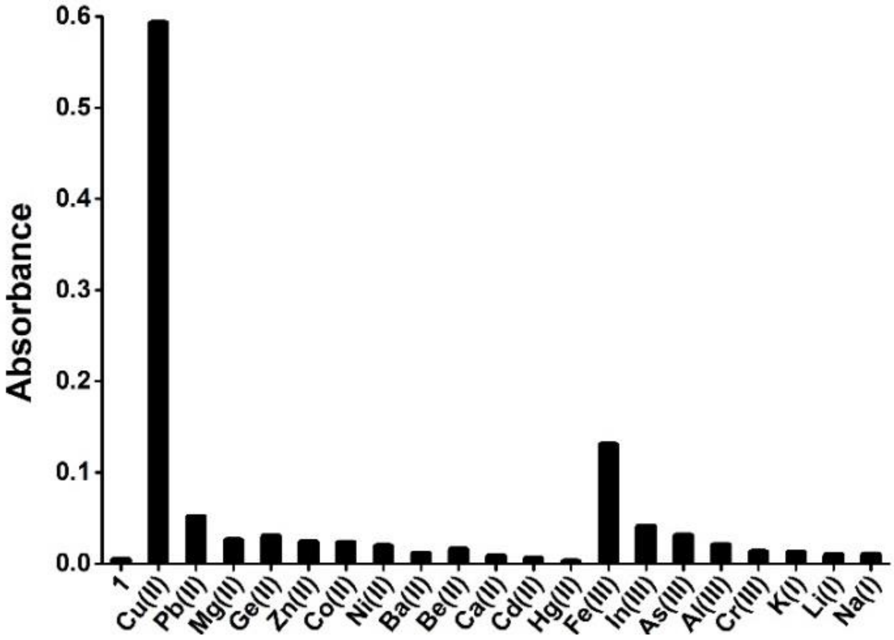

Figure S2. Absorbance at 489 nm of water (pH 7.4)-acetonitrile 1:1 (v/v) (5.0×10^{-5} mol L^{-1}) solutions of **1** alone and in the presence of selected metal cations (10 eq.).

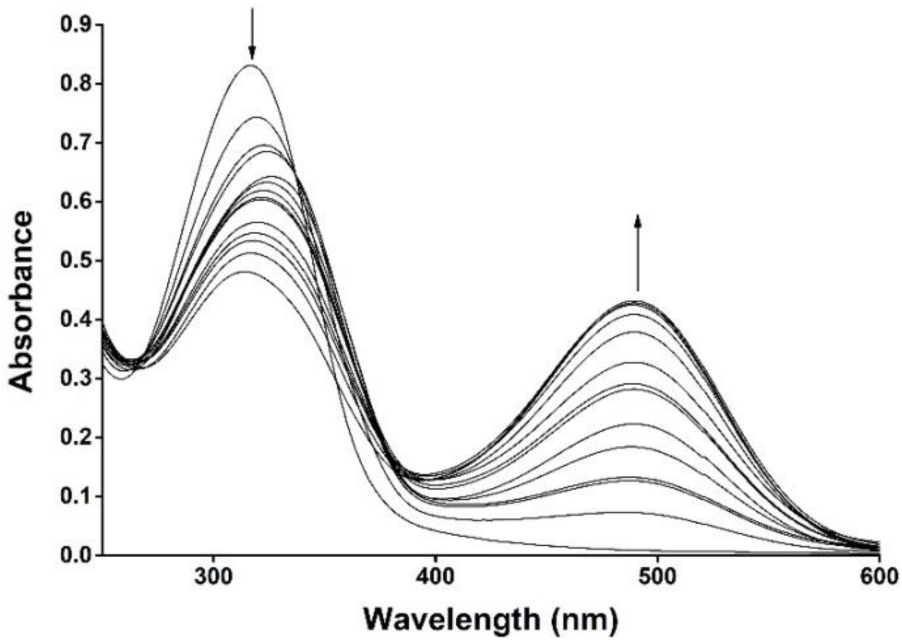

Figure S3. UV-visible titration profile of **1** in water (pH 7.4)-acetonitrile 1:1 (v/v) (5.0×10^{-5} mol L^{-1}) upon addition of increasing amounts of Cu(II) (from 0 to 10 eq.).

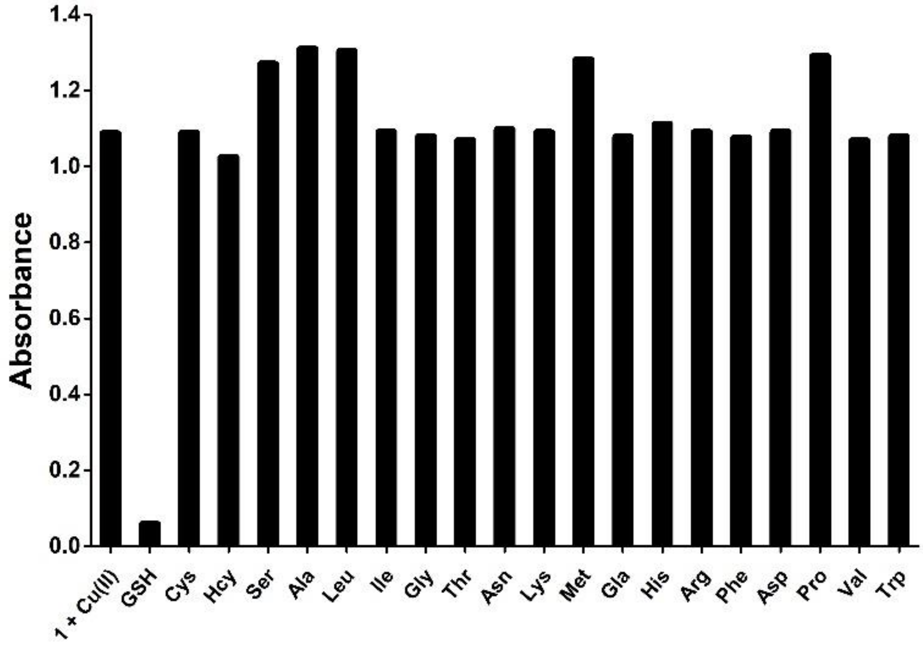

Figure S4. Absorbance at 489 nm of water (pH 7.4)-acetonitrile 1:1 (v/v) solutions of **1**·Cu(II) complex in the presence of GSH (2.0 eq.) and selected amino acids (2.0 eq.).

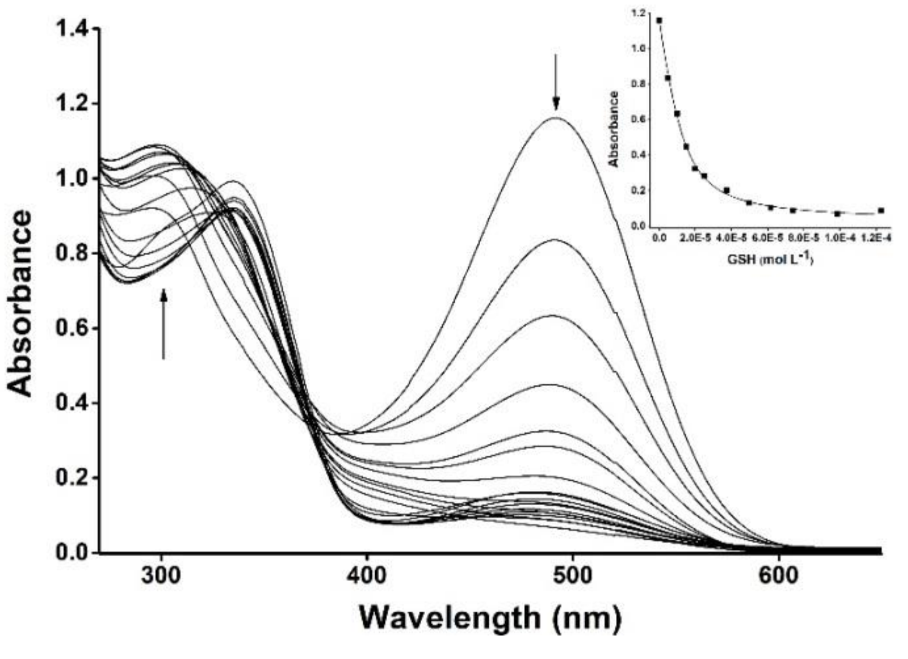

Figure S5. UV/Vis. titration profile of **1**·Cu(II) complex (6.2×10^{-6} mol L^{-1}) in water (pH 7.4)-acetonitrile 1:1 (v/v) upon addition of GSH (0-2.0 equivalents). Inset: plot of absorbance at 489 nm *vs* GSH concentration.

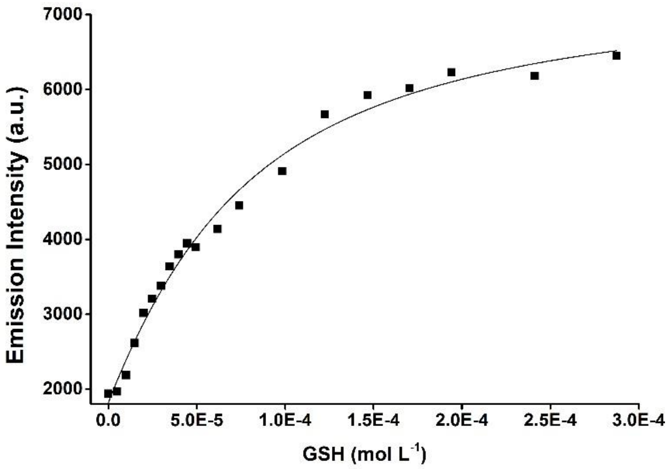

Figure S6. Emission intensity at 411 nm (excitation at 320 nm) of water (pH 7.4)-acetonitrile 1:1 v/v solutions of **1**·Cu(II) (5.0×10^{-5} mol L^{-1}) upon addition of increasing quantities GSH.

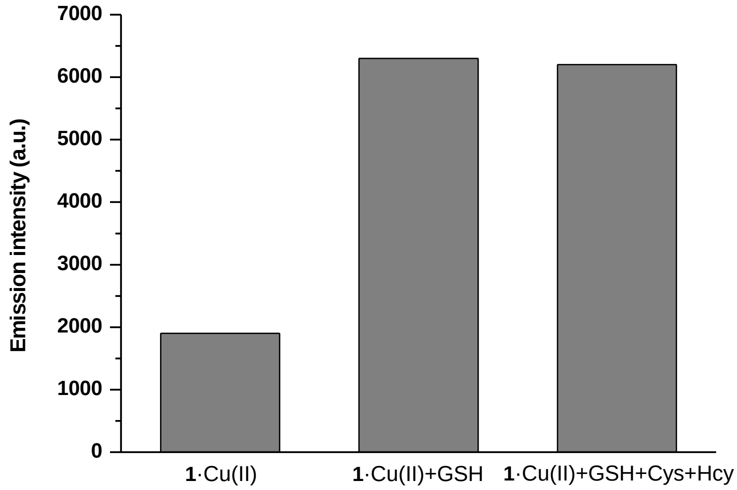

Figure S7. Emission intensity at 411 nm (excitation at 320 nm) of water (pH 7.4)-acetonitrile 1:1 v/v solutions of **1**·Cu(II) (5.0×10^{-5} mol L^{-1}) alone and in the presence of GSH (2.0 eq.) and a mixture of GSH+Cys+Hcy (2.0 eq. of each biothiol).

Table S1. Experimental features of the analytical methods used for GSH detection.

Method	Limit of detection (μM)	Interferences	Time (min)	Reference
Displacement assay of a Cu(II) complex	2	-	5	This paper
Gold nanoparticles assisted laser desorption/ionization mass spectrometry	Not reported	Not reported	Not reported	Anal. Chem. 2007; 79: 4852
HPLC with UV detection	5	-	10	Anal. Methods 2014; 6: 8039
HPLC-mass spectrometry	0.4	-	5	J. Chromatogr. B 2013; 929: 51
Chitosan membrane with glutathione reductase and sulfhydryl oxidase integrated onto the surface of graphite rods	200	-	5	Talanta 2008; 74: 1492
Voltammetric electronic tongue	3	Cys, Hcy	Not reported	Electroanalysis 2014; 26: 581
Surface enhanced Raman scattering	Not reported	-	60	Anal. Chem. 2013; 85: 9221
Surface enhanced Raman scattering	Not reported	Not reported	Not reported	J. Mol. Struct. 2012; 1029: 75
Surface enhanced Raman scattering	0.04	-	Not reported	Anal. Chim. Acta 2014; 816: 41
Dynamic combinatorial libraries of Cu(II) and Ni(II) complexes	Not reported	Not reported	Not reported	J. Comb. Chem. 2006; 8: 540
Dynamic combinatorial libraries of fluorophores	Not reported	-	30	J. Am. Chem. Soc. 2007; 129: 4510