Supporting Information

2,4,5-Triaryl imidazole probes for the selective chromo-fluorogenic detection of Cu(II). Prospective use of the Cu(II) complexes for the optical recognition of biothiols

Hazem Essam Okda,[a,b,c] Sameh El Sayed,[a,b,c] Ismael Otri,[a,b,c] R. Cristina M. Ferreira,[d] Susana P. G. Costa,[d] M. Manuela M. Raposo,*[d] Ramón Martínez-Máñez,**[a,b,c] and Félix Sancenón[a,b,c]

[a] Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Universitat de València. Spain.

[b] Departamento de Química, Universitat Politècnica de València. Camino de Vera s/n, 46022, València, Spain.

[c] CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN).

[d] Centro de Química, Univesidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal.

1. Fluorescence measurements in acetonitrile.

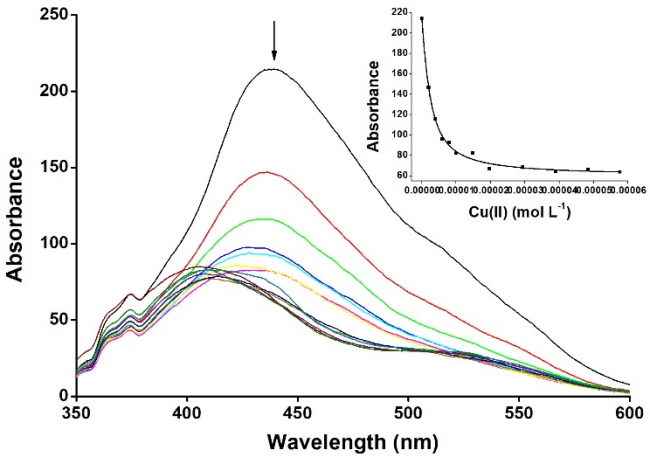

Figure SI-1. Changes in the emission intensity of probe **3b** in acetonitrile at 437 nm upon addition of increasing quantities of Cu(II) cation (0 -10 equiv.). Inset: Changes in the emission intensity of the emission band centred at 437 nm *vs.* Cu(II) concentration.

2. Fluorescence quantum yield measurements.

The fluorescence quantum yield of pyrene in cyclohexane (φ_f = 0.32) was used as a reference to determine fluorescence quantum yields of probes **3a** and **3b**. Equation 1 was used to calculate the fluorescence quantum yield:

$$\varphi_s = \varphi_f \frac{I_s A_f \eta_s^2}{I_f A_s \eta_r^2} \qquad (1)$$

Here φ_f is the fluorescence quantum yield of reference. I stand for the integrated area under the emission curves. The subscripts s and r stand for sample and reference, respectively. A is the absorbance at a particular excitation wavelength. η is the refractive index of the medium. The absorbance of the dye at the excitation wavelength was always kept ~0.1. The steady state absorption and emission spectra were fitted by the log normal line shape function. Consequently the fluorescence quantum yield for probe **3a** is φ = 0.41 and for **3b** is φ = 0.31.

3. Stability constant determination.

The apparent binding constant for the formation of the respective complexes were evaluated using the Benesi−Hildebrand plot (equation 2):

$$1/(A - A_0) = 1/\{K(A_{max} - A_0)C\} + 1/(A_{max} - A_0) \qquad (2)$$

Where A_0 is the absorbance of **3a** and **3b** at 321 and 339 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 555 (for **3a**) and 466 nm (for **3b**) 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.

4. Limit of detection evaluation for Cu(II) and biothiols.

The limit of detection for Cu(II) and biothiols using probes **3a and 3b** was calculated using the spectrophotometric/spectrofluorometric titration profiles obtained. The limits of detection were calculated using equation 3:

$$LOD = 3.3\,\sigma/k \qquad (3)$$

Where σ is the standard deviation of the blank measurement, and k is the slope between the ratios of UV−vis absorbance vs. Cu(II) or biothiols concentration.