The Challenges of Using a Copper Fluorescent Sensor (CS1) to Track Intracellular Distributions of Copper in Neuronal and Glial Cells

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Supporting Information
Figure S1. A: Change in solution spectrum of apo-CS1 (10 µM; green trace) in MOPS buffer (50 mM, pH 7.4) containing NH₂OH (0.4 mM) upon titration with CuSO₄ (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 equivalents; red traces). The purple dashed line is the spectrum of a 10 µM [Cu^(I)(Bca)_2]³⁻ solution.

B: Change in solution spectrum of Cu^(I)-CS1 as generated in A (10 µM; red trace) upon titration with one equivalent of apo-CopK (green trace).

Note: Binding of Cu(I) to CS1 is characterized by an increase in the absorbance around 540 nm in the electronic spectrum (Zeng, 2006; Miller, 2006). The data in Figure A showed that, in the presence of NH₂OH, the added Cu(II) ions are reductively trapped by CS1 quantitatively to produce Cu(I)-CS1. The data in Figure B indicated that CopK was able to remove Cu(I) quantitatively from Cu(I)-CS1. CopK has no absorbance in the visible spectral range and under the experimental condition, CopK binds Cu(I) and Cu(II) cooperatively with sub-picomolar affinity for Cu(I) (K_D, ~10⁻¹³ M) (Chong, 2009).
Figure S2. Change in fluorescence spectrum of apo-CS1 (2.0 µM; green trace) in MOPS buffer (50 mM, pH 7.4) containing CopK (2.0 µM) and NH₂OH (0.4 mM) upon titration with CuSO₄. The inset (ii) is the plot of F₅₆₀ versus Cu:CS1 for the titration and the insert (i) is the plot for the control titration without CopK. As noted in Figure S1B, under the experimental condition, CS1 was not able to sense the added Cu until the competing protein CopK was almost saturated with Cu¹ and Cu² to produce the stable species Cu¹Cu²-CopK.

Figure S3. Change in fluorescence spectrum of Cu¹-CS1 (2.0 µM; red trace) in MOPS buffer (50 mM, pH 7.4) containing ascorbate (1.0 mM) upon titration with apo-Atox1 (0.5 and 1.0 equivalent; blue and green trace, respectively). The Cu¹ in Cu¹-CS1 was removed quantitatively by a stoichiometric amount of apo-Atox1. Atox1 binds one equivalent of Cu¹ with sub-femtomolar affinity (Xiao, 2010; 2011).
References


