Electronic Supplementary Material (ESI) for Chemical Science
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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

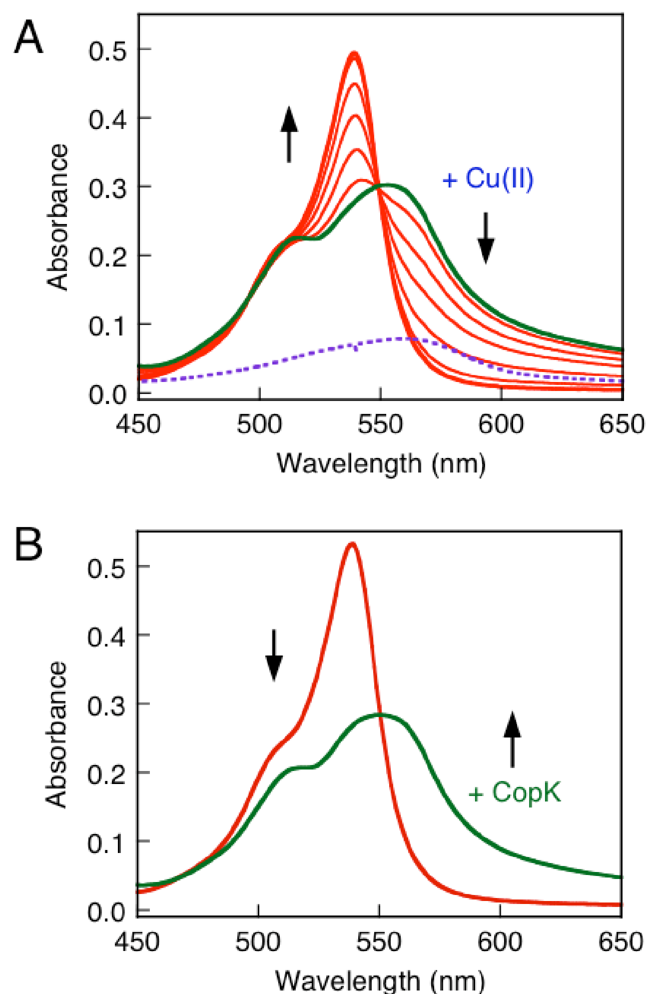

Figure S1. A: Change in solution spectrum of *apo*-CS1 (10 μM; green trace) in MOPS buffer (50 mM, pH 7.4) containing NH_2OH (0.4 mM) upon titration with $CuSO_4$ (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 equivalents; red traces). The purple dashed line is the spectrum of a 10 μM $[Cu^I(Bca)_2]^{3-}$ solution. **B**: 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 NH_2OH, 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).

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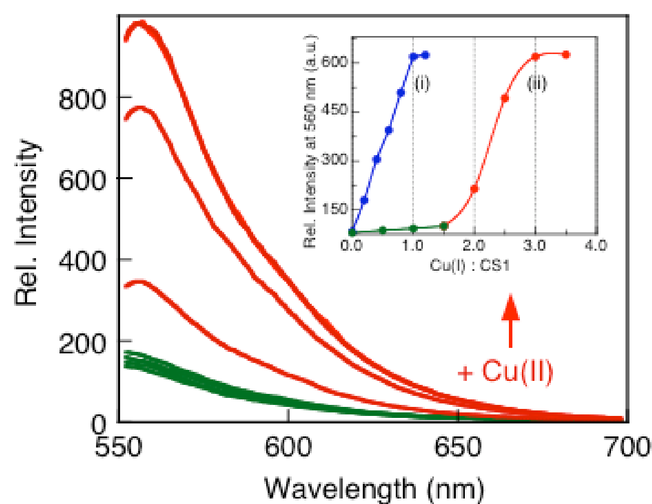

Figure S2. Change in fluorescence spectrum of *apo*-CS1 (2.0 μM; green trace) in MOPS buffer (50 mM, pH 7.4) containing CopK (2.0 μM) and NH_2OH (0.4 mM) upon titration with $CuSO_4$. The inset (ii) is the plot of F_{560} versus Cu:CS1 for the titration and the insert (i) is the plot for the control 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 Cu^{I} and Cu^{II} to produce the stable species $Cu^{I}Cu^{II}$-CopK.

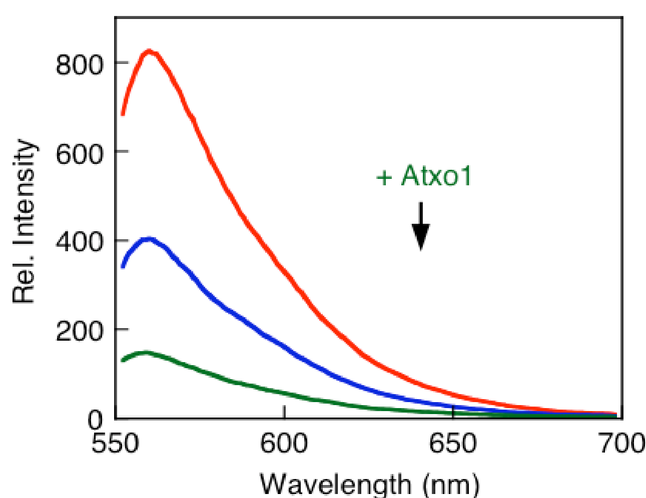

Figure S3. Change in fluorescence spectrum of Cu^{I}-CS1 (2.0 μM; red trace) in MOPS buffer (50 mM, pH 7.4) containing ascorbate (1.0 mM) upon titration with *apo*-Atox1 (0.5 and 1.0 equivalent; blue and green trace, respectively). The Cu^{I} in Cu^{I}-CS1 was removed quantitatively by a stoichiometric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

References

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