Copper is an essential element for life, but alterations in its cellular homeostasis are connected to serious neurodegenerative diseases, including Menkes and Wilson diseases, familial amyotrophic lateral sclerosis, Alzheimer’s disease, and prion diseases. Because of its essential yet toxic nature, cells exert strict control over intracellular copper distributions, and the thermodynamically estimated level of free copper in the cytosol of bacterial model systems is less than one ion per cell. Nevertheless, cellular copper uptake and release events are kinetically rapid; intracellular copper concentrations can increase by up to 20-fold within 1 h upon incubation in growth medium supplemented with micromolar copper, with facile exchange between cytosolic and mitochondrial stores. Many cytosolic, mitochondrial, and vesicular oxygen-processing enzymes require copper as a redox cofactor, but uncontrolled reactions of copper ions with oxygen and reactive oxygen species (ROS) can also trigger oxidative damage to proteins, nucleic acids, and lipids.

We are creating new chemical tools to help elucidate the complex physiological and pathological roles of copper and its oxidation biology. In this regard, live-cell optical imaging with copper-selective fluorescent sensors offers a potentially powerful approach for interrogating aspects of labile copper accumulation, speciation, and membrane permeability, long-wavelength excitation and emission profiles of apo or Cu-bound CS1 are unchanged in the presence of various biologically relevant metal ions. The emission spectrum of Cu-bound CS1 displays a single major visible absorption band at 540 nm (ε = 4.0 × 10^4 M⁻¹ cm⁻¹) with a shoulder at 530 nm. Binding analysis using the method of continuous variations (Job’s plot) establishes that a 1:1 Cu²⁺:CS1 complex is responsible for the observed fluorescence enhancement, and the apparent Kₐ for Cu²⁺ binding to CS1 is 3.6(0.3) × 10⁻¹² M (Supporting Information).

The emission response of CS1 is highly Cu²⁺ selective. Figure 1b displays the fluorescent responses of a 2 μM CS1 to the presence of various biologically relevant metal ions. The emission profiles of apo or Cu²⁺-bound CS1 are unchanged in the presence of 2 mM Cu²⁺, Mg²⁺, or Zn²⁺, indicating excellent selectivities for Cu²⁺ over these abundant cellular cations. Other first-row transition metal ions, including 50 μM Mn²⁺, Fe²⁺, Co²⁺, Ni²⁺, or Cu²⁺, produce no discernible changes in emission intensities for the apo probe and do not interfere with its Cu²⁺ response. Especially notable is the observed selectivity for Cu²⁺ over Cu⁺, rendering reporter having favorable optical properties and biological compatiblity with a thioether-rich receptor for selective and stable binding of soft Cu⁺ in water. A related phenyl-bridged BODIPY with a thioether macrocycle has been reported for detecting Cu²⁺ in acetonitrile solution. The synthesis of CS1 is outlined in Scheme 1. BODIPY-I is obtained in a one-pot, two-step procedure via condensation of 2,4-dimethyl-3-ethylpyrrole with chloroacetyl chloride followed by treatment with BF₃·OEt₂. The overall yield is 16% for two steps. The tetrathia receptor is also delivered in two steps. Conversion of ethyl 2-hydroxyethyl sulfide with thiourea and HBr proceeds smoothly to generate thiol in 84% yield. Treatment of 2 with bis(2-chloroethyl)amine hydrochloride under basic conditions furnishes the azatetrathia receptor in 79% yield. Coupling of 1 and 3 in refluxing acetonitrile affords CS1 (4) in 22% yield after workup and purification.

Scheme 1.

Synthesis of Coppersensor-1 (CS1)
Figure 1. (A) Fluorescence response of 2 μM CS1 to Cu²⁺. Spectra shown are for buffered [Cu⁺] of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 μM. Spectra were acquired in 20 mM HEPES, pH 7, with excitation at 540 nm. (B) Fluorescence responses of CS1 to various metal ions. Bars represent the final integrated fluorescence response (FI) over the initial integrated emission (FI). Initial spectra were acquired in 20 mM HEPES, pH 7. White bars represent the addition of an excess of the appropriate metal ion (2 mM for Cu²⁺, Mg²⁺, and Zn²⁺, and 50 μM for all other cations) to a 2 μM solution of CS1. Black bars represent the subsequent addition of 10 μM Cu²⁺ to the solution. Excitation was provided at 540 nm, and the emission was integrated over 550–650 nm.

Figure 2. Confocal fluorescence images of live HEK 293 cells. (A) Cells incubated with 5 μM CS1 for 5 min at 25 °C. (B) Cells supplemented with 100 μM CuCl₂ in the growth media for 2 h at 37 °C and stained with 5 μM CS1 for 5 min at 25 °C. (C) CuCl₂-supplemented cells pretreated with 50 μM of the competing Cu²⁺ chelator, 3,6,12,15-tetraethia-9-monooazaheptadecane, for 5 min at 25 °C before staining with 5 μM CS1 for 5 min at 25 °C. (D) Brightfield image of live HEK 293 cells shown in panel B, confirming their viability. Scale bar = 25 μm.

CS1 potentially valuable for studying specific copper-mediated redox events in biological systems.

We next sought to evaluate the ability of CS1 to operate within living cells. HEK 293 cells incubated with 5 μM CS1 for 5 min at 25 °C show faint intracellular fluorescence (Figure 2a). Supplementing cells with 100 μM CuCl₂ in the growth medium for 2 h at 37 °C and then staining with CS1 under the same loading conditions results in a marked increase in observed intracellular fluorescence, as determined from scanning confocal microscopy on live samples (Figure 2b). Control experiments without dye give no background fluorescence, and pretreatment of copper-supplemented cells with the membrane-permeable Cu²⁺ chelator, 3,6,12,15-tetraethia-9-monooazaheptadecane (500 μM for 5 min at 25 °C), before CS1 staining also shows faint intracellular fluorescence (Figure 2c). Brightfield measurements after copper and CS1 treatment confirm that the cells are viable throughout the imaging experiments (Figure 2d). Taken together, these experiments show that CS1 is cell-permeable and can respond to changes in intracellular [Cu²⁺] within living cells.

To close, we have described the synthesis, properties, and cellular applications of CS1, a new fluorescent chemosensor for imaging labile Cu²⁺ in living biological samples. CS1 is the first Cu²⁺-selective fluorescent probe with visible excitation and emission profiles, giving a 10-fold turn-on response for detecting this ion, and features excellent selectivity for Cu²⁺ over biologically relevant metal ions, including Cu²⁺. Moreover, confocal microscopy experiments establish that CS1 can be used for detecting Cu²⁺ levels within living cells. Applications of CS1 and related chemical tools to study copper oxidation biology in living systems are underway.

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Supporting Information Available: Synthetic and experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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