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Ultraviolet-Visible Light (UV−Vis)-Reversible but Fluorescence-Irreversible Chemosensor for Copper in Water and Its Application in Living Cells

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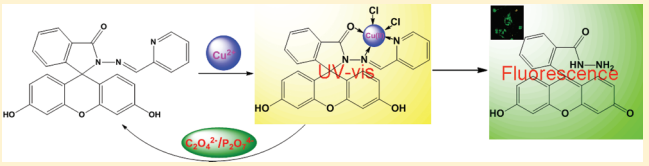
Supporting Information

ABSTRACT: An ultraviolet−visible light (UV−Vis)-reversible but fluorescence-irreversible chemosensor was developed for the detection of copper. Coordination between the probe, 2-pyridylaldehyde fluorescein hydrazone (FHP), and Cu^{2+} gave a reversible UV−Vis response, Storage of the probe−Cu complex resulted in hydrolytic cleavage of the N═C bond, which released the fluorophore (ring-opened fluorescein hydrazine) and gave irreversible fluorescence. Thus, FHP becomes a multifunctional chemosensor, and its reversibility can be controlled by the reaction time. Cu^{2+} in living cells could be detected using FHP and general fluorescence methods.



Anions are ubiquitous in nature and in biological processes, and they are also responsible for industrial and agricultural pollution.[1−9] While transition-metal ions are important in many different fields, including catalysis, organometallic reactions, and biochemistry, they can be toxic at high concentrations and disrupt normal cell function.[9−14] These ions can be detected using several instrumental techniques.[15,16] However, these methods are time-consuming and require expensive instrumentation. Chemosensors are powerful molecular tools that can be used to detect many target molecules, such as biological markers and environmental pollutants.[17−23] Chemosensors that use color and/or fluorescence intensity have been developed to detect various analytes.[24−28] To design new chemosensors, mechanisms for recognizing target analytes and the signal reporting units must be investigated.[29] Fluorescein derivatives can be produced in short synthetic routes, and they have several and useful properties for chemosensors, such as high water solubility, long excitation and emission wavelengths, and high fluorescence quantum efficiencies. Chemosensors[30−45] using fluorescein derivatives have been designed based on ion-induced changes in the fluorescence intensity. These sensors are simple to produce and have high detection sensitivities.[46−52] In this study, we investigated 2-pyridylaldehyde fluorescein hydrazone (FHP) as a novel ultraviolet−visible light (UV−Vis)-reversible but fluorescence-irreversible chemosensor for Cu^{2+}, as well as its application in living cells.

The FHP probe (Figure 1) was synthesized according to the literature[10] by a reaction between fluorescein hydrazine and picolinaldehyde in methanol containing acetic acid (see Figure S1 in the Supporting Information). The product was characterized by electrospray ionization mass spectrometry (ESI-MS), nuclear magnetic resonance (NMR) spectroscopy, and X-ray crystallography (see Figures S2 and S3 in the Supporting Information). The FHP molecule has the following

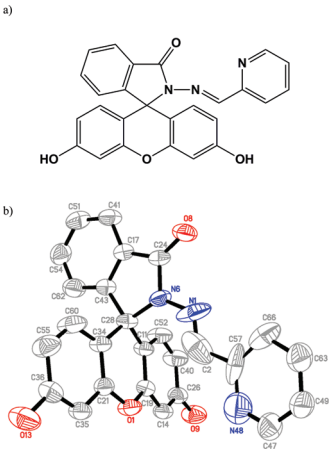


Figure 1. Structure and thermal ellipsoids of the probe, drawn at the 50% probability level.

data associated with it: ^{1}H NMR (300 MHz, 25 °C, DMSO-d_6): δ 10.0 (bs, 2H), 8.53 (s, N═C−H, 1H), 8.49 (d, Ar−H, 1H), 7.97 (t, pyridine-H, 1H), 7.79 (t, pyridine-H, 1H), 7.60−7.68 (m, Ar−H, 3H), 7.34 (t, pyridine-H, 1H), 7.13 (d, pyridine-H, 1H), 6.69 (d, xanthene-H, 2H, J = 2.4 Hz), 6.55 (d, xanthene-H, 2H, J = 8.8 Hz), 6.47 (dd, xanthene-H, 2H, J = 8.8 Hz, J = 2.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.22, 158.84, 153.17, 151.90, 151.21, 149.54, 146.49, 137.00, 134.56, 129. 25, 127.93, 127.80, 124.59, 123.52, 119.21, 112.65, 109.50, 102.73, 64.95; ESI-MS m/z 436[FHP + H]$^+$, 458[FHP + Na]$^+$; Elemental analysis (calcd %) for $C_{26}H_{17}N_3O_4$: C, 71.72; H,

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3.94; N, 9.65; Found: C, 71.70; N, 9.68; H, 4.01. Crystal data for $C_{26}H_{17}N_3O_4 \cdot {}^1/_2(CH_3OH)$: crystal size: 0.20 × 0.05 × 0.05, monoclinic, space group $P21/c$ (No. 14). a = 9.7947(8) Å, b = 26.813(2) Å, c = 16.8152(14) Å, β = 100.685°, V = 4339.5(6) Å^3, Z = 4, T = 296 K, θ_{max} = 26.0°, 24570 reflections measured, 8501 unique (R_{int} = 0.0633). Final residual for 614 parameters and 8501 reflections with $I > 2\sigma(I)$: R_1 = 0.0686, wR_2 = 0.1738, and goodness of fit (GOF) = 0.999. The ability of the probe to detect metal ions was investigated by UV−Vis and fluorescence spectroscopy.

The effects of a wide range of environmentally and physiologically active metal ions (Cu^+, Cu^{2+}, Ca^{2+}, Fe^{2+}, Zn^{2+}, Ni^{2+}, Bi^{3+}, Co^{2+}, VO^{2+}, Mn^{2+}, Ru^{3+}, Cd^{2+}, Pb^{2+}, Ag^+, La^{3+}, Ce^{4+}, Yb^{3+}, Cr^{2+}, Er^{3+}, Mg^{2+}, Sn^{2+}, Al^{3+}, Nd^{3+}, Zr^{4+}, K^+, Sm^{3+}, Fe^{3+}, and Eu^{3+}) on FHP were investigated. UV−Vis spectra were recorded of solutions containing FHP (36 μmol/L) and each metal (360 μmol/L) in the HEPES buffer (10 mmol/L) pH 7.0 aqueous buffer. Among the metal ions, only Cu^{2+} caused any changes in the UV−Vis spectra. When Cu^{2+} was added to the FHP solution, a strong absorption peaks appeared at 500 nm (Figure 2), and the solution changed color from colorless to yellow. (See Figure S4 in the Supporting Information.)

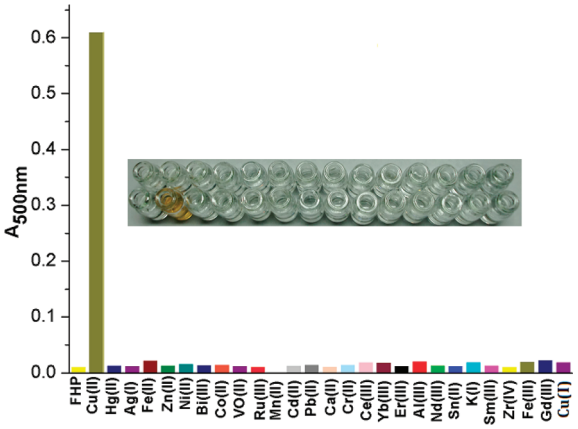

Figure 2. Optical density three-dimensional graph of the FHP probe (36 μM) at 500 nm upon the addition of several metal ions. Inset shows a color change photograph for Cu^{2+} and the other metal ions.

A detailed investigation on the FHP recognition of Cu^{2+} was performed. Figure 3a shows the change in the UV−Vis spectrum when the Cu^{2+} solution was added to the HEPES buffer (10 mmol/L, pH 7.0) containing the probe (36 μmol/L). As the concentration of Cu^{2+} increased, so did the absorbance at 500 nm. In the presence of several metal ions, we investigated the ability of FHP to detect Cu^{2+} in the presence of other metal ions. The other ions did not interfere with the detection of Cu^{2+} (see Figure S5 in the Supporting Information. It should be noted that FHP and FHP−Cu^{2+} did not fluoresce during the UV−VIS detection process. The probe could be used to detect 1.5−120 μmol/L of Cu^{2+} by the UV−Vis spectra changes, and the detection limit is 1.5 μmol/L. (See Figure S6 in the Supporting Information.)

It is well-known that chemosensor reversibility is required for reuse. When $P_2O_7^{4-}$ (PPi) or $C_2O_4^{2-}$ (XO) is added to the FHP−Cu^{2+} complex in HEPES buffer (10 mmol/L, pH 7.0) the absorbance at 500 nm decreased (Figure 3b). As the PPi or XO concentrations increased, $A_{500\ nm}$ gradually decreased until the original spectrum of free FHP was obtained. Simultaneously, the solution changed color from yellow to colorless. (See Figure S7 in the Supporting Information.)

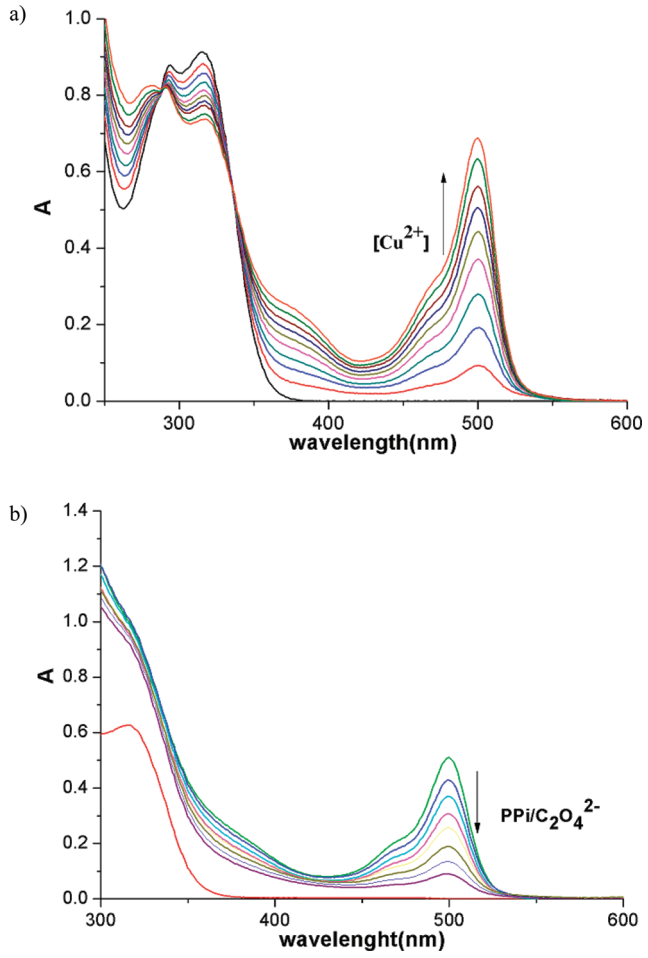

Figure 3. (a) Absorption spectral changes of FHP (36 μM) in 10 mM HEPES at pH 7.0 as an aqueous buffer upon the addition of Cu^{2+}; Cu^{2+} was added gradually, with the divalent copper species range being [Cu^{2+}] = 0−108 μM. Each spectrum was recorded 10 s after the addition of Cu^{2+}. (b) Absorption spectral changes of FHP−Cu^{2+} in 10 mM HEPES at pH 7.0 as an aqueous buffer upon the addition of PPi/$C_2O_4^{2-}$; PPi/$C_2O_4^{2-}$ was added gradually with [PPi/$C_2O_4^{2-}$] = 0−216 μM. Each spectrum was recorded 10 s after the addition of PPi/$C_2O_4^{2-}$.

Anions with a physiological function, including fluoride (F^-), chloride (Cl^-), bromide (Br^-), iodide (I^-), acetate (AcO^-), thiocyanate (SCN^-), nitrate (NO_3^-), sulfate (SO_4^{2-}), carbonate (CO_3^{2-}), oxalate ($^-OOCCOO^-$), phosphate (PO_4^{3-}), and CN^-, were also investigated; however, their effect on the UV−vis spectra was not as good as that for PPi/XO.

A series of FHP−Cu^{2+} HEPES solutions with different amount-of-substance ratios were stored for a long time at room temperature. Interestingly, an emission peak was observed at 518 nm (λ_{ex} = 325 nm) for all these solutions, and their fluorescence intensities increased rapidly as the concentration of FHP−Cu^{2+} increased (Figure 4a) from 6 × 10^{-7} μmol/L to 9 × 10^{-6} μmol/L. This results indicate that FHP can detect Cu^{2+} at low micromolar levels and produce a fluorescence signal after the complex has been stored for some time.

The emission peak of FHP−Cu^{2+} at 518 nm (λ_{ex} = 325 nm) did not decrease when large quantities of PPi or XO were

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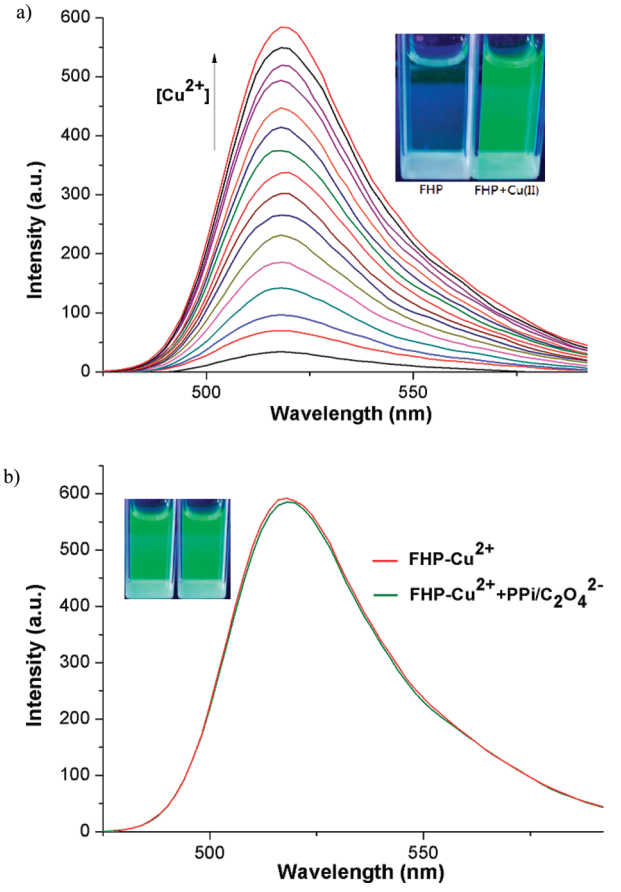


Figure 4. (a) Fluorescence spectral changes for FHP (3 μM) upon the addition of Cu^{2+} (0–9 μM) (λ_{ex} = 325 nm, λ_{em} = 517 nm, slit: 5 nm/5 nm) in 10 mM HEPES at pH 7.0 as an aqueous buffer. Each spectrum was recorded 48 h after Cu^{2+} addition. Inset: color (left) and visual fluorescence (right) change photographs for FHP (10 μM) upon the addition of Cu^{2+} in a HEPES (pH 7.0) buffer solution under UV illumination (365 nm). (b) Fluorescence spectral changes of FHP (10 μM)–Cu^{2+} upon the addition of plenty of $PPi/C_2O_4^{2-}$ (λ_{ex} = 325 nm, λ_{em} = 518 nm, slit: 5 nm/5 nm) in 10 mM HEPES at pH 7.0 as an aqueous buffer. The spectrum was recorded 60 h after $PPi/C_2O_4^{2-}$ addition. Inset: color (left) and visual fluorescence (right) change photographs for FHP (10 μM)–Cu^{2+} upon the addition of $PPi/C_2O_4^{2-}$ in a HEPES (pH 7.0) buffer solution under UV illumination (365 nm).

added (Figure 4b), and no changes were observed even after a few days.

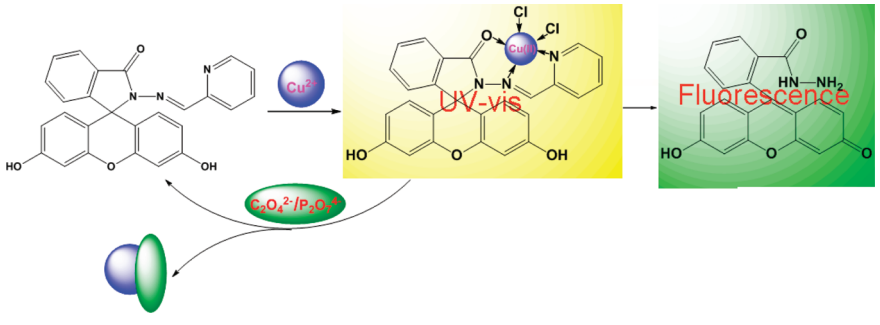
The UV–Vis absorption spectra of the free probe under different pH conditions were recorded (pH 2.0–13.0) (see Figure S12a in the Supporting Information). The free-probe absorption peaks at 500 nm were greatly affected by strong acidity, or strongly alkaline solutions, which produced new absorption peaks at 500 nm. This would affect the detection of Cu^{2+}. No peak was observed for the free probe at 500 nm when pH was between 2.0 and 7.0. However, the absorption peaks at 500 nm for the FHP–Cu^{2+} complex was best at pH 7.0 (see Figure S12b in the Supporting Information), so this pH was selected as being optimal for recognition.

No fluorescence is detected from FHP when it is in its free form with a closed ring. When Cu^{2+} is added to the FHP solution, the Cu^{2+} coordinates to FHP and forms a complex with a new absorption peak at 500 nm without fluorescence. (See Figure S13 in the Supporting Information.) After the addition of PPi/XO to this system, FHP is released as PPi/XO coordinates to Cu^{2+} and the peak at 500 nm disappears. Therefore, the probe is UV–Vis-reversible. However, after the FHP–Cu^{2+} solution is stored for a few hours, the system shows strong fluorescence. If PPi/XO is added to this system, neither the UV–Vis spectra nor the fluorescence spectra change if the solutions are allowed to sit for a few days. This is because Cu causes hydrolytic cleavage of the N=C bond and the release of the fluorophore, which is fluorescein hydrazine with an open ring. Therefore, the probe is fluorescence-irreversible (see Scheme 1, as well as Figures S14–S16 in the Supporting Information). The property has been observed with some other types of chemosensor.[53]

The ability of FHP to detect Cu^{2+} within living cells was also evaluated by laser confocal fluorescence imaging using an Olympus Model FV1000 laser scanning microscope. The optical window at the green channel (490–550 nm) was chosen as a signal output. Under selective excitation at 405 nm, HepG2 cells incubated with 50 μmol/L FHP for 48 h at 37 °C showed green fluorescence. (See Figure 5b.) When the cells were pretreated with a membrane-permeable copper chelator (ethylene diamine tetra(methylene phosphonic acid), EDTMPA), incubation with FHP showed no emission. Cells pretreated with EDTMPA and subsequently incubated with $CuCl_2$ and FHP displayed enhanced green fluorescence (see Figure 5d). This indicates that the green fluorescence is caused by the FHP responding to external copper ions. These cell experiments show FHP can permeate through cell membranes. Therefore, it could be used to detect Cu^{2+} within living cells.

In summary, in this study, a controllable, regenerating, and multifunctional chemosensor was developed. 2-Pyridylaldehyde fluorescein hydrazone (FHP) can recognize Cu^{2+} with very high selectivity, both UV–Vis spectrophotometrically and visually, via a simple coordination action between FHP and Cu^{2+}. PPi/XO can coordinate with Cu^{2+} and remove it from the

Scheme 1. The Proposed Determination Mechanism



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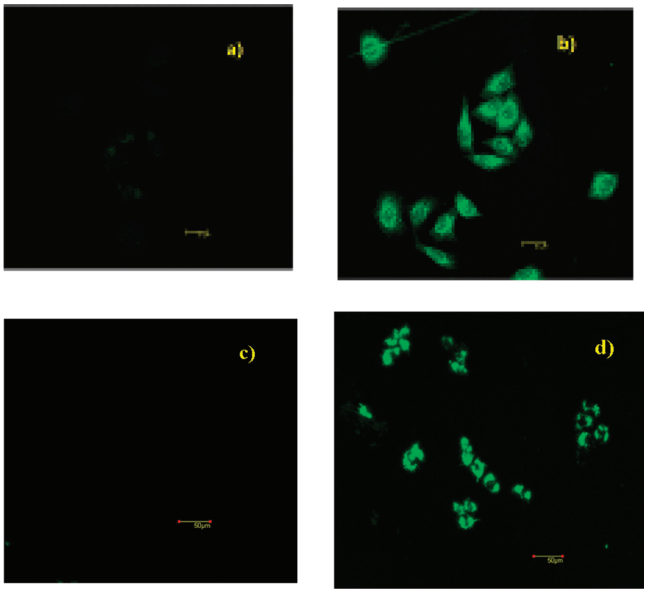

Figure 5. Confocal fluorescence images of HepG2 cells in the (a) absence and (b–d) presence of 50 μM FHP ((b) cells treated with FHP in the absence of 8 μM EDTMPA; (c) cells treated with FHP in the presence of 8 μM EDTMPA; and (d) after treatment with EDTMPA, 10 μM $CuCl_2$, and a subsequent treatment of the cells with 50 μM FHP).

FHP–Cu^{2+} complex, which makes the probe response-reversible and it can be reused. However, after FHP–Cu^{2+} was stored for a few hours, the Cu^{2+} caused FHP to hydrolyze and the release of the fluorophore (ring-opened fluorescein hydrazine). This made the probe an irreversible chemosensor. The reaction time can be controlled, so the probe is reversible and multifunctional. Moreover, confocal fluorescence microscopy confirmed that FHP can be used to monitor Cu^{2+} in living cells using general fluorescence methods. These results are significant and interesting, because this is a new type of chemosensor that may be applied in living cells.[54,55]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, spectroscopic data, kinetic study, ^{1}H NMR, ^{13}C NMR, ESI-MS data, and crystal data (CIF) are available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

§Contributed equally to this work.

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