

Cu²⁺-Selective Ratiometric and “Off-On” Sensor Based on the Rhodamine Derivative Bearing Pyrene Group

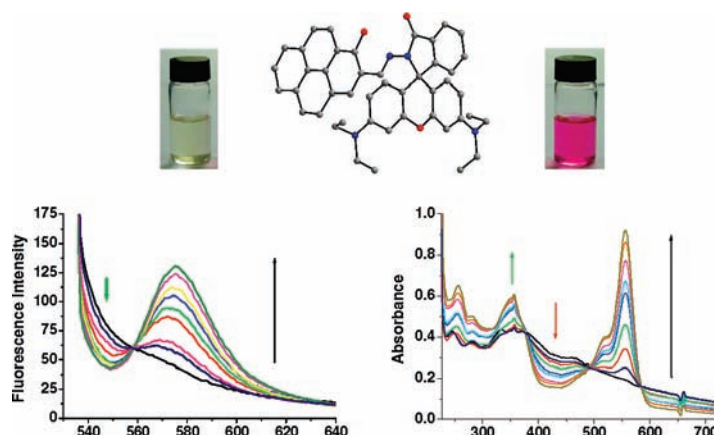
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ABSTRACT



A new rhodamine-based derivative bearing a pyrene group (PRC) was synthesized as a ratiometric and “off–on” chemosensor for Cu²⁺. PRC displayed a selective and chelation enhanced ratiometric fluorescence change and colorimetric change with Cu²⁺ among the metal ions examined.

Various transition-metal ions are crucial for the life of organisms.¹ Among these is the copper ion, which plays a critical role as a catalytic cofactor for a variety of metalloenzymes, including superoxide dismutase, cytochrome c oxidase, and tyrosinase. However, under overloading conditions, copper exhibits toxicity in that it causes neurodegenerative diseases (e.g., Alzheimer’s and Wilson’s diseases) probably by its involvement in the production of reactive oxygen species.² Consequently, organisms tightly regulate internal concentrations of copper. Owing to the Janus-faced

properties of copper in organisms, numerous efforts have been undertaken to develop efficient and selective methods to assess copper ions in cells and organisms. In addition,

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copper is a significant metal pollutant due to its widespread use. The toxicity of copper ions for humans is rather low compared to other heavy metals, but certain microorganisms are affected by even submicromolar concentrations of Cu^{2+} .³

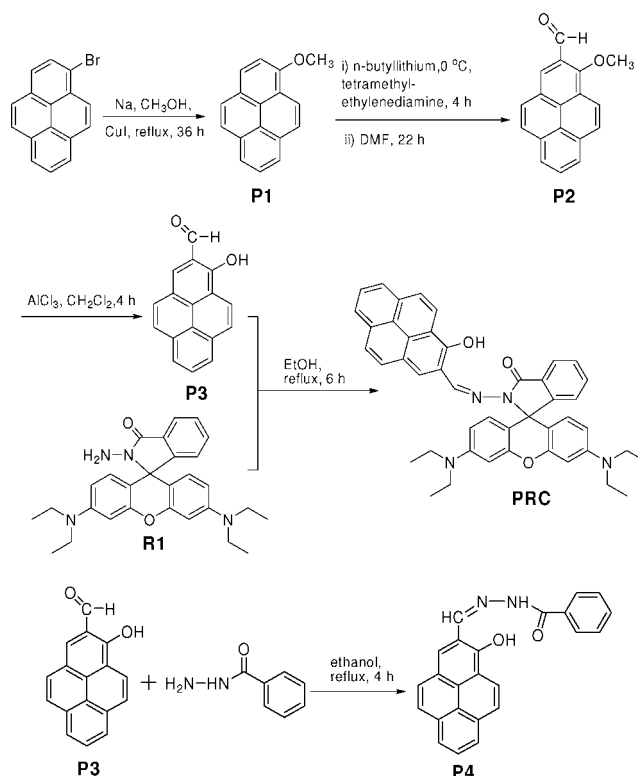
Even though fluorescent probes for copper ion have been extensively explored owing to biological significance of this metal ion,⁴ there are still only a few examples of “off–on” type sensors available in aqueous systems.⁵ Furthermore, only a few ratiometric fluorescent probes for Cu^{2+} have been found in the literature due the fluorescence quenching nature of paramagnetic Cu^{2+} ,⁶ and most of them were working only in pure organic solvents. Ratiometric fluorescent measurements observe changes in the ratio of the intensities of the emission at two wavelengths. Thus, ratiometric fluorescent sensors have an important feature that they can be used to evaluate the analyte concentration and provide built-in correction for environmental effects.

On the other hand, rhodamine derivatives are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission and a pink color. Recently, a spirolactam (nonfluorescent) to ring-opened amide (fluorescent) process was utilized for the detection of metal ions.^{5a,b,d,e,7}

We introduced rhodamine fluorophore onto the pyrene moiety, which was utilized as a selective fluorescent and colorimetric sensor for Cu^{2+} in aqueous solution. Among the various metal ions, the chemosensor **PRC** displayed highly selective ratiometric changes upon the addition of Cu^{2+} . As expected, the pyrene moiety served successfully as a source of these ratiometric changes. As far as we are aware, **PRC** is the first ratiometric sensor based on rhodamine derivative. For comparison, pyrene-based compound **P4** was synthesized to test the fluorescence change with Cu^{2+} .

As shown in Scheme 1, **P3** was first synthesized by modifying the reported procedure⁸ with an improved yield of 45%. **P3** was then reacted with rhodamine-based derivative **R1**^{5a,9} to give **PRC** in 46% yield. The detailed

Scheme 1. Synthesis of Chemosensors **PRC** and **P4**



experimental procedures and ^1H and ^{13}C NMR spectra are explained in the Supporting Information. Sensor **PRC** and **P4** were further confirmed by X-ray analysis (Figure 1).

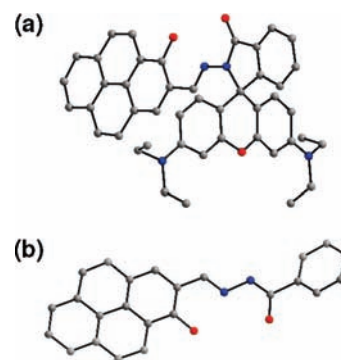


Figure 1. X-ray crystal structures of **PRC** (a) and **P4** (b). Blue: N atom. Red: O atom.

Single crystals of **PRC** and **P4** were grown in dichloromethane, and unique spirolactam structures were observed.

The nitrate salts of Cu^{2+} , Hg^{2+} , Zn^{2+} , Mg^{2+} , Pb^{2+} , Ca^{2+} , Cd^{2+} , Ag^{+} , and Fe^{3+} ions were used to evaluate the metal ion binding property and selectivity of compound **PRC** (20 μM) in CH_3CN –HEPES buffer (0.02 M, pH 7.4) (4:6, v/v). Among these metal ions (10 equiv), **PRC** showed a selective fluorescence enhancement only with Cu^{2+} among the various

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metal ions examined in aqueous solution, indicating that PRC displayed a high Cu^{2+} selectivity (Figure 2). The fluorescence

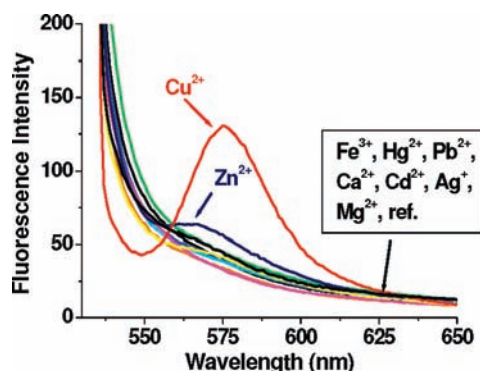


Figure 2. Fluorescent spectra of **PRC** ($20\ \mu\text{M}$) in CH_3CN –HEPES buffer ($0.02\ \text{M}$, $\text{pH} = 7.4$) ($4:6$, v/v) with $200\ \mu\text{M}$ of Cu^{2+} , Hg^{2+} , Zn^{2+} , Pb^{2+} , Fe^{3+} , Mg^{2+} , Cd^{2+} , Ca^{2+} , and Ag^{+} . Excitation wavelength was $520\ \text{nm}$.

spectra were obtained by excitation of the rhodamine fluorophore at $520\ \text{nm}$.

From the fluorescence titration experiments (Figure 3), clear ratiometric and “off–on” fluorescence changes of PRC

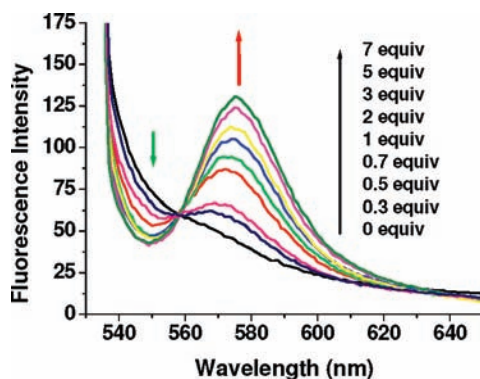


Figure 3. Fluorescent emission spectra of **PRC** ($20\ \mu\text{M}$) in the presence of different concentrations of Cu^{2+} in CH_3CN –HEPES buffer ($0.02\ \text{M}$, $\text{pH} = 7.4$) ($4:6$, v/v). Excitation wavelength was $520\ \text{nm}$.

to Cu^{2+} were observed. When Cu^{2+} was added to the solution, a significant decrease of the fluorescence intensity of $424\ \text{nm}$ and a new fluorescence emission band centered at $575\ \text{nm}$, which was attributed to the Cu^{2+} induced ring-opening of the spirolactam moiety, were observed with a clear isoemission point at $558\ \text{nm}$ (Figure 3).

We examined whether fluorescence energy transfer (FRET) occurs in our system by exciting the pyrene moiety at $360\ \text{nm}$. For rhodamine derivative, FRET can be observed only when the spirolactam ring was opened upon binding with analytes. In our case, an efficient FRET was not observed since the fluorescence of pyrene was quenched upon addition of Cu^{2+} .

As shown in Figure 4, upon addition of up to 7 equiv of Cu^{2+} to the solution of **PRC**, the absorbance at $424\ \text{nm}$

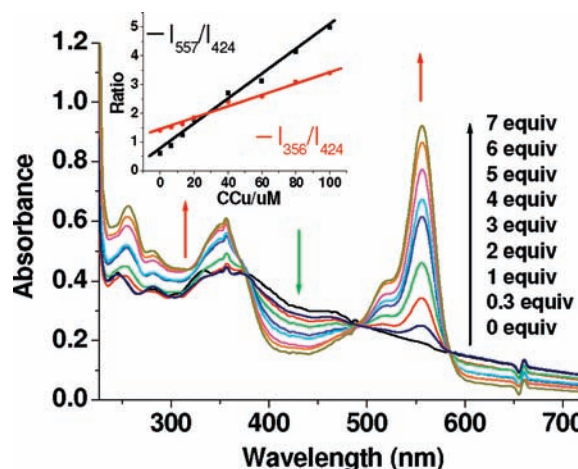


Figure 4. Absorbance spectra of **PRC** ($20\ \mu\text{M}$) in CH_3CN –HEPES buffer ($0.02\ \text{M}$, $\text{pH} = 7.4$) ($4:6$, v/v) in the presence of different amounts of Cu^{2+} . Inset: the ratio of absorbance at $557\ \text{nm}$ and absorbance at $424\ \text{nm}$ (black line) and the ratio of absorbance at $356\ \text{nm}$ and absorbance at $424\ \text{nm}$ (red line) as a function of Cu^{2+} concentration.

decreased sharply, while the ones at 356 and $557\ \text{nm}$ increased significantly, which induced a color change from primrose yellow to pink. Three isosbestic points at 377 , 491 , and $584\ \text{nm}$ were observed. A linear dependence of the ratio of absorbance at $557\ \text{nm}$ and absorbance at $424\ \text{nm}$ (black line) and the ratio of absorbance at $356\ \text{nm}$ and absorbance at $424\ \text{nm}$ (red line) as a function of Cu^{2+} concentration were observed.

All of the above absorption spectra responses were reversible, which was confirmed by the reversible titration using $\text{EDTA}/\text{Cu}^{2+}$ (Figure S10, Supporting Information). These results suggested that the spirolactam ring-opening induced by the complexation of Cu^{2+} meant PRC could serve as a ratiometric “naked-eye” chemosensor, which is selective for Cu^{2+} in neutral buffered media.

The nonlinear fitting of the titration curve and the data of Job’s plots from absorption spectra (Figures S8 and S9, Supporting Information) assumed a $1:1$ stoichiometry for the PRC – Cu^{2+} complex with an association constant of $2.5 \times 10^4\ \text{M}^{-1}$.

For comparison, compound **P4** was synthesized to check the fluorescence properties of pyrene moiety. In the UV–vis absorption spectra of **P4**, the increasing bands at 375 and $480\ \text{nm}$ were found with the addition of Cu^{2+} (Figure 5). The fluorescence titration of **P4** with Cu^{2+} showed fluorescence quenching effects of the peak at $550\ \text{nm}$. This result indicates that the decreasing portion at $550\ \text{nm}$ in the spectra of PRC could be caused by the quenching effects of the pyrene group in the presence of Cu^{2+} .

The proposed mechanism for these fluorescent changes was explained in Figure 6. Upon addition of Cu^{2+} to a

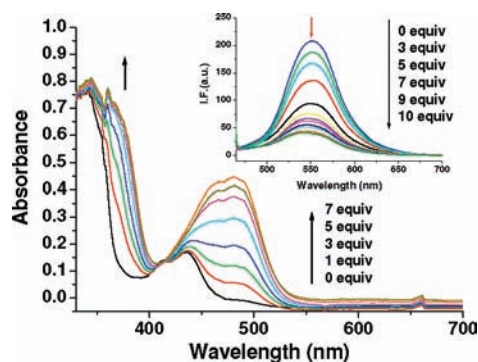


Figure 5. Absorbance spectra of **P4** (20 μ M) in CH_3CN –HEPES buffer (0.02 M, pH = 7.4) (4:6, v/v) in the presence of different amounts of Cu^{2+} . Inset: the fluorescent changes of **P4** as a function of Cu^{2+} concentration. Excitation wavelength was 450 nm.

primrose yellow solution of compound **PRC**, a red color and the fluorescence characteristics of rhodamine B appeared. The large fluorescence enhancement as well as the colorimetric change can be attributed to the spirolactam ring-opening, which was induced by the complexation of Cu^{2+} . As shown in Figure 6, oxygen on the pyrene, oxygen on the carbonyl group, as well as nitrogen on the hydrazone moiety can cooperatively participate in the binding with Cu^{2+} . It is believed that this process is reversible, which has been proved by the test using EDTA/ Cu^{2+} (Figure S10, Supporting Information).

In conclusion, a new rhodamine–pyrene derivative has been synthesized as a ratiometric and “off–on” sensor for the detection of copper ion in aqueous solution. **PRC** displayed a highly selective and ratiometric fluorescence change and a colorimetric change upon the addition of Cu^{2+} , in which the spirolactam (nonfluorescent) to ring opened

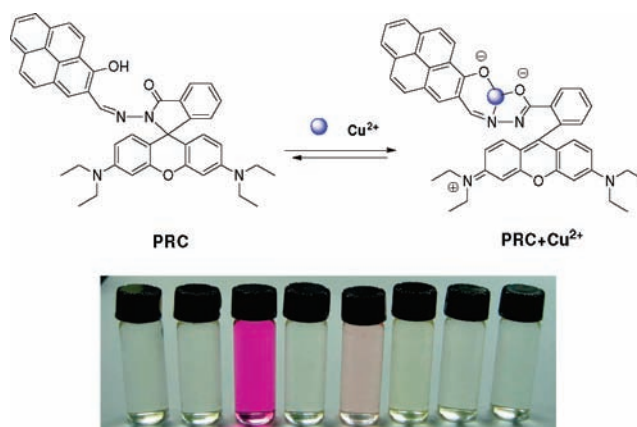


Figure 6. Proposed binding mechanism for **PRC** with Cu^{2+} and a photo of **PRC** (20 μ M) as a selective naked-eye chemosensor for Cu^{2+} in CH_3CN –HEPES buffer (0.02 M, pH = 7.4) (4:6, v/v). Bottom: 20 μ M **PRC**, from second one added with 200 μ M of Ca^{2+} , Cu^{2+} , Hg^{2+} , Zn^{2+} , Fe^{3+} , Ag^+ , Pb^{2+} (from left to right).

amide (fluorescent) process was utilized. Furthermore, pyrene moiety served successfully as a source of ratiometric changes of **PRC** with Cu^{2+} .

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Supporting Information Available: Experimental procedures and characterization data of compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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