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Ratiometric and Selective Fluorescent Sensor for Cu^{II} Based on Internal Charge Transfer (ICT)

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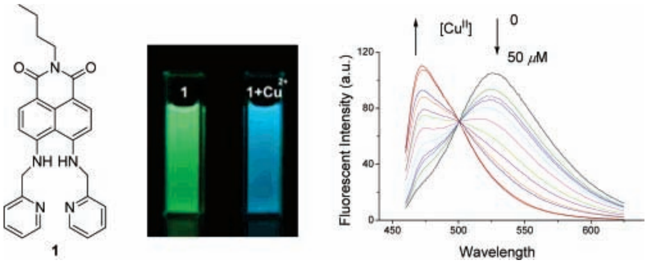
ABSTRACT



A Cu^{II}-sensing, ratiometric, and selective fluorescent sensor 1, *N*-butyl-4,5-di[(pyridin-2-ylmethyl)amino]-1,8-naphthalimide, was designed and synthesized on the basis of the mechanism of internal charge transfer (ICT). In aqueous ethanol solutions of 1, the presence of Cu^{II} induces the formation of a 1:1 metal–ligand complex, which exhibits a strong, increasing fluorescent emission centered at 475 nm at the expense of the fluorescent emission of 1 centered at 525 nm.

Fluorescent sensors for the detection and measurement of copper ions are actively investigated, as this metal ion is a significant environmental pollutant and an essential trace element in biological systems.[1] For most of the reported Cu^{2+} fluorescent sensors, the binding of the metal ion causes a quenching of the fluorescence emission.[2] Only a few sensors in which the binding of a Cu^{2+} ion causes an increase in the fluorescence have been reported.[3] However, in most practical applications, changes in fluorescence intensity (fluorescence quenching or enhancement) can also be caused by many other poorly quantified or variable factors such as photobleaching, sensor molecule concentration, the environment around the sensor molecule (pH, polarity, temperature, and so forth), and stability under illumination. To increase the selectivity and sensitivity of a measurement, ratiometric measurements are utilized. Ratiometric measurements involve the observation of changes in the ratio of the intensities of the absorption or the emission at two wavelengths. Ratiometric fluorescent probes have the important feature in that they permit signal rationing and thus increase the dynamic range and provide built-in correction for environmental effects.[4] The perceived color change would be useful not only for the ratiometric

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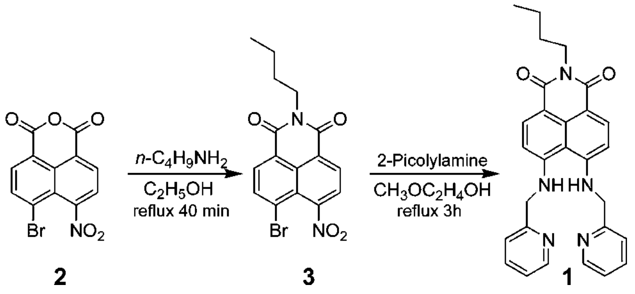
method of detection but also for rapid visual sensing.[4e] Up to now, many investigations have been conducted to make ratiometric fluorescent probes for Zn^{2+},[5] Ag^{+},[6] and Pb^{2+}.[7] In contrast, as Cu^{2+} is a notorious fluorescence quencher,[2] very few ratiometric fluorescent sensors for Cu^{2+} have been found in the literature. K. A. Mitchell et al. once reported two PET sensors that exhibit fluorescence enhancement and a hypsochromic shift of less than 20 nm in the presence of Cu^{2+}, Mn^{2+}, and Ni^{2+}.[8] However, those experiments were carried out in unbuffered nonaqueous solution, and therefore protonation worries exist regarding Mitchell's sensors.[9] Actually, we also observed that Cu^{2+} can strongly quench the fluorescence of a PET sensor similar to Mitchell's in buffered aqueous solution.[10] Thus, realization of ratiometric measurement for Cu^{2+}, especially in aqueous solution, is still a challenge.

The internal charge transfer (ICT) mechanism has been widely exploited for cation sensing.[11] The interaction between receptor and cation would blue shift both the absorption and fluorescence spectra. Recently, some ratiometric fluorescent sensors based on ICT such as the Ca^{2+} sensor fura-2[12] and the Zn^{2+} sensor ZnAF-R2[5b] have been developed. A tetradentate receptor site with the amide and pyridyl donors has been found to be suitable for strainless binding of the Cu^{2+} ion.[13] Bearing this in mind, we designed and synthesized the fluorescent sensor **1**, *N*-butyl-4,5-di[(pyridin-2-ylmethyl)amino]-1,8-naphthalimide, which contains a similar receptor site and shows ratiometric measurement for Cu^{2+}. The capture of Cu^{2+} by the receptor resulted in the reduction of the electron-donating ability of the two amino groups conjugated to the naphthalene ring; thus, the receptor showed a 50 nm blue shift of fluorescence emission and provided high selectivity for Cu^{2+} over other heavy and transition metal (HTM) ions.

Sensor **1** was easily synthesized by conjugating 2-(aminomethyl)pyridine and *N*-butyl-4-bromo-5-nitro-1,8-naphthalimide **3** (Scheme 1). Compounds **2** and **3** were prepared according to reported procedures.[14]

Scheme 1. Preparation of Sensor **1**



Fluoroionophores are usually disturbed by a proton in the detection of metal ions. Thus, the influence of pH on the fluorescence of **1** was first determined by fluorescence titration. The fluorescence of **1** at 525 nm remains unaffected between pH 13−4.7 and then gradually decreases from pH 4.7 to 2.1 with a 10 nm blue shift; below pH 2.1, no change in fluorescence is obtained, leading to a sigmoid curve. The fluorescence quenching was most likely caused by the photoinduced electron transfer (PET) from the fluorophore to protonated pyridine.[15] de Silva had found a similar phenomenon in the design of an "off−on−off" fluorescent PET sensor.[16] Therefore, further fluorescence studies were carried out at pH 7.2 maintained with HEPES buffer (50 mM).

The emission spectra of **1** and its fluorescence titration with Cu^{2+} were recorded in an ethanol−water solution (40:60, v/v) (Figure 1), and the emission spectrum of free **1** displays a broad band with a maximum at 525 nm. When

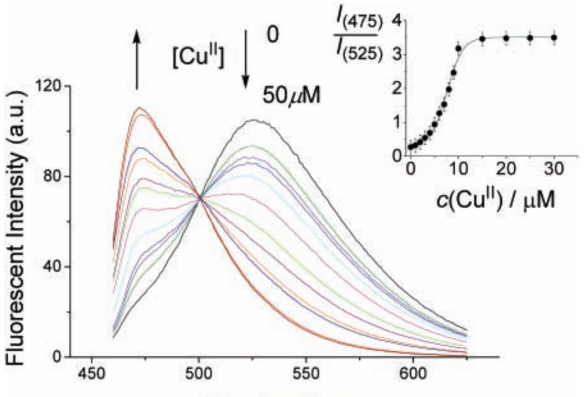


Figure 1. Fluorescent emission spectra of **1** in the presence of different concentrations of Cu^{2+} (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 μM) in an ethanol−water solution (40:60, v/v, 50 mM HEPES buffer, pH 7.2). Excitation wavelength was 451 nm, and emission was at 475 and 525 nm. The concentration of **1** was 10 μM. Inset: Ratiometric calibration curve I_{475}/I_{525} as a function of Cu^{2+} concentration.

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Cu^{2+} was added to the solution of **1**, a significant decrease in the 525 nm emission and a blue-shifted emission band centered at 475 nm, which was attributed to the formation of a **1**/Cu^{2+} complex and increased in intensity, were observed with a clear isoemission point at 501 nm. The inset in Figure 1 exhibits the dependence of the intensity ratios of emission at 475 nm to that at 525 nm (I_{475}/I_{525}) on Cu^{2+}, which indicates the formation of a **1**/Cu^{2+} adduct of 1:1 stoichiometry. In the UV−vis absorption spectra of **1** (Figure S3, Supporting Information), an increasing band around 500 nm was found with the addition of Cu^{2+}. This would indicate that the blue shift of fluorescence spectra was caused by a change of the charge-transfer character of the emissive species. In Figure 2, α, defined as the ratio between the free ligand concentration and the initial concentration of ligand, is given as a function of the logarithm of Cu^{2+} ion concentration.[5d] The curve fitting for the experimental data points was calculated from Li's equations with $\log K = 6.13$.[5d] The curve can serve as the calibration curve for the detection of Cu^{2+}. It was noted that there was no change except for the intensity of the excitation spectra of **1** with the addition of Cu^{2+}, and the two emission bands were all excited by 451 nm light, which would facilitate the optical detection. The Φ_F values of free **1** and **1**/Cu^{2+} adduct (1:1) are 0.112 and 0.114, respectively.[17]

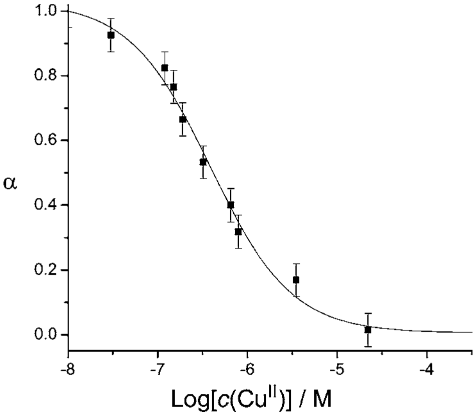

Figure 2. Response parameter values (α) as a function of the logarithm of Cu^{2+} concentration. α is defined as the ratio between the free ligand concentration and the initial concentration of ligand.

Furthermore, Cu^{2+} could be detected at least down to 1.0×10^{-8} M when **1** was employed at 1.0×10^{-7} M, and I_{475}/I_{525} also increased linearly with the concentration of Cu^{2+} ($(0.0-1.0) \times 10^{-7}$ M) up to a mole ratio (**1**/Cu^{2+}) of 1:1, and there it remained well (Figure S6, Supporting Information).

The fluorescence titration of **1** with various metal ions was conducted to examine the selectivity. Ag^+ can also react with **1**, and a gradually increasing emission band at 475 nm was observed. However, the I_{475}/I_{525} in the presence of Ag^+ is lower than that in the presence of Cu^{2+}, Fe^{2+}, Co^{2+}, and Ni^{2+} have a slight disturbance compared to Ag^+. The addition of other metal ions such as Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} produced a nominal change in the fluorescence spectra of **1** due to their low affinity with sensor **1**. Figure 3a shows the dependence of the intensity ratios (I_{475}/I_{525}) on the metal ions. The competition experiments were conducted in the presence of Cu^{2+} at 10 μM mixed with Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^+, and Pb^{2+} at 50 μM, as well as in a mixture of the metal ions, respectively; no significant variation in the intensity ratios (I_{475}/I_{525}) was found by comparison with that without the other metal ions besides Cu^{2+} (Figure 3b).

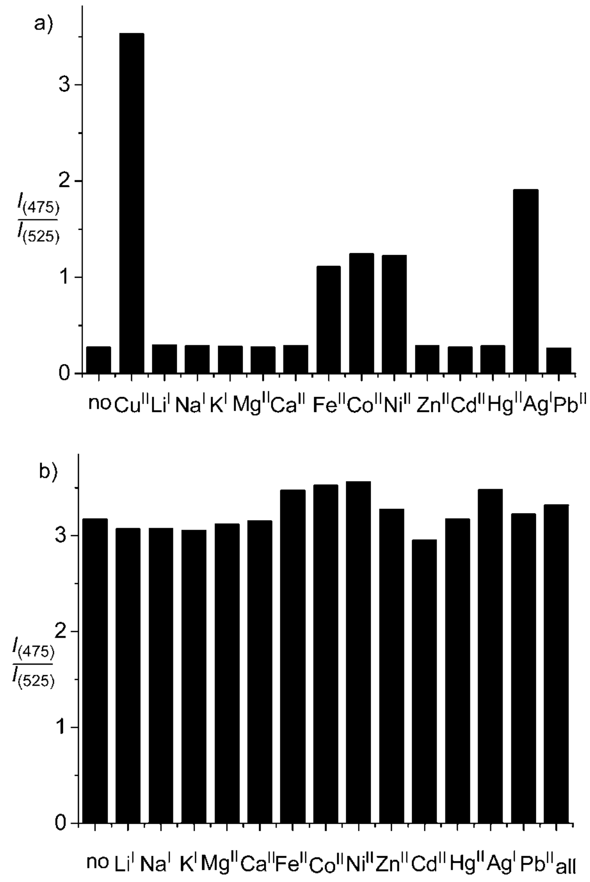

Figure 3. (a) Responses of sensor **1** to metal ions (50 μM, χ-axis markers). (b) Fluorescent response of sensor **1** containing 10 μM Cu^{2+} to the selected metal ions (50 μM). Excitation was at 451 nm, and emission was at 475 and 525 nm. The concentration of **1** was 10 μM.

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(17) Fluorescence quantum yields (Φ_F) were estimated in each case by calculating the integrated fluorescence intensity in a 10 nm band about the two emission maxima of 474 and 525 nm with *N*-butyl-4-butylamino-1,8-naphthalimide in absolute ethanol as a standard ($\Phi_F = 0.81$). Guo, X.; Qian, X.; Jia, L. *J. Am. Chem. Soc.* **2004**, *126*, 2272−2273.

This means that compound **1** has a high selectivity for Cu^{2+}. Additionally, to explore the effects of anionic counterions on the sensing behavior of **1** to metal ions, fluoescence reponses of **1** to perchlorate, chloride, and nitrate salts with differing cations were examined in ethanol−water solution (40:60, v/v) at pH 7.2 maintained with HEPES buffer (50 mM). The results were similar to those shown in Figure 3. There were no obvious changes in the fluorescence responses of **1** to $Cu(ClO_4)_2$, $CuCl_2$, and $Cu(NO_3)_2$ (Figure S7, Supporting Information).

Addition of Cu^{2+} to the solution of **1** sufficiently blue shifts the emission from 525 to 475 nm that the two emission bands are visible. This visible emission allows **1**/Cu^{2+} to be readily distinguished by the naked eye (Figure S8, Supporting Information), and sensor **1** thus combines the sensitivity of fluorescence with the convenience and aesthetic appeal of a colorimetric assay.[4e]

Cu^{2+} is a well-known paramagnetic ion with an unfilled d shell and could strongly quench the fluorescence of the fluorophore near it via electron or energy transfer.[11d] Thus, it is of interest to us that the capture of Cu^{2+} by **1** does not quench the fluorescence, especially when the Cu^{2+} is so close to the fluorophore after binding. For transition metal ions, the metal (M)−fluorophore (F) communication is too strong compared with the other interactions for commonly used receptors (R), which is believed to be the main reason for the quenching of the fluorescence. Whereas the quenching effects are predominantly connected to the nature of the metal ion, a fluorescence enhancement can result from ion-induced changes in the geometry or flexibility of the ligand as well as from a different availability of certain functional groups involved in fluorescence quenching for the ligand in the unbound state.[11d] A good case in point is Kumar's sensor displaying fluorescence enhancement with Cu^{2+}.[3b] Samanta and co-workers have also achieved fluorescence enhancement with Cu^{2+} using an electron-deficient fluorophore component to reduce the M−F interaction.[3c,d] The recent success of Bharadwaj et al. has been ascribed to the special topology of the cryptand receptor that binds M more strongly within the cavity, making it unavailable for fluorescence quenching. In other words, the M−R interaction has been increased to reduce indirectly the communication between M and F.[3d] In our case, the receptor also forms a proper cavity and has a strong binding with Cu^{2+}. The Cu^{2+}−F interaction might be suppressed in a similar way, and as a result fluorescence is maintained. Another cause might be the short fluorescence lifetime of naphthalimides, which was attributed as a major factor in similar systems by Samanta et al.[3c]

In conclusion, we have developed a new fluorescent sensor for Cu^{2+} on the basis of ICT with high sensitivity and selectivity. Moreover, this molecule makes it possible to detect the Cu^{2+} ratiometrically. The design strategy and remarkable photophysical properties of the sensor would help to extend the development of fluorescent sensors for HTM ions.

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Supporting Information Available: Synthesis, characteristics, and spectroscopic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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