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A New Highly Selective, Ratiometric and Colorimetric Fluorescence Sensor for Cu^{2+} with a Remarkable Red Shift in Absorption and Emission Spectra Based on Internal Charge Transfer

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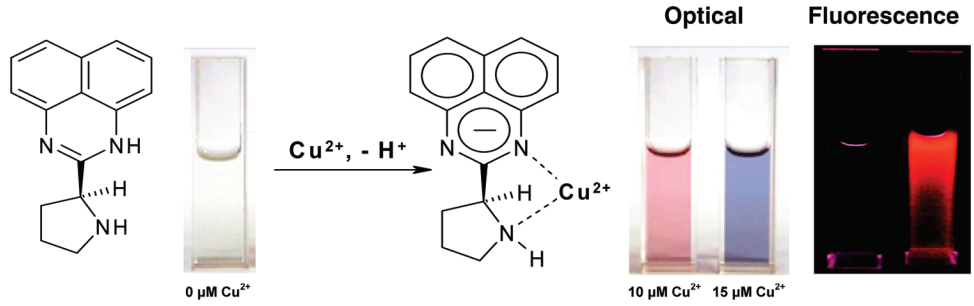
ABSTRACT



A new 1,8-diaminonaphthalene based ratiometric and highly selective colorimetric "*off-on*" type of fluorescent probe, receptor 2 has been designed and synthesized that senses only Cu^{2+} among the other heavy and transition metal ions examined on the basis of internal charge transfer (ICT). The visual sensitivity of the receptor 2 is remarkable, showing dual color changes from colorless (receptor) to purple followed by blue and a large red shift in emission upon Cu^{2+} complexation.

In recent years, there has been great emphasis placed on the development of new highly selective colorimetric fluorescent sensors of biologically active metal ions because of their potential applications in clinical biochemistry and in environmental research.[1−3] Development of fluorescent molecular sensors directed specifically toward the detection and estimation of divalent copper either in vitro or in vivo under physiological relevant conditions always constitutes an active area of research.[4] The third most abundant (after Fe^{2+} and Zn^{2+}) soft transition metal ion Cu^{2+} is an essential trace element present in the human body and plays a vital role in

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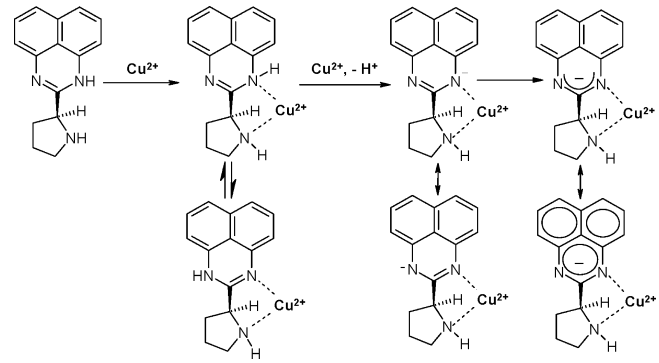
a variety of fundamental physiological processes in organisms ranging from bacteria to mammals but can often be toxic to certain biological systems when the levels of Cu^{2+} exceed cellular needs. It is also associated with neurodegenerative diseases such as Alzheimer's and Parkinson's and is also suspected to cause amyloidal precipitation and toxicity.[5]

For most of the reported fluorescent sensors of Cu^{2+}, binding of the metal ion causes a quenching of the fluorescence emission[6] due to its paramagnetic nature.[7] Only a few sensors in which the binding of Cu^{2+} ion causes an increase in the fluorescence intensity have been reported.[8] In this regard, therefore to improve the selectivity and sensitivity of a measurement upon binding of Cu^{2+} to the receptor, ratiometric and colorimetric measurements are often utilized as chemosensors exhibiting both of the properties often combined to raise the sensitivity of fluorescence, and therefore they have an aesthetic appeal in colorimetric assay. In particular, ratiometric measurements have important features that permit signal rationing when receptors interact with analytes; a dual emission system can minimize the measurement errors because of factors such as phototransformation, receptor concentrations, and environmental effects.[9] However, up to now, few colorimetric and ratiometric fluorescent chemosensors for Cu^{2+} have been found in the literature.[10]

In this communication, we report our successful development of a 1,8-diaminonaphthalene based ratiometric and colorimetric fluorescent chemosensor selective for Cu^{2+} based on the mechanism of internal charge transfer (ICT) that shows a large red shift in UV−vis as well as in fluorescence emission spectra. Such a large red shift in absorption spectroscopy in the case of receptor **2** is an easy assay for the colorimetric detection of the receptor upon guest binding, and again the long-wavelength shift in emission intensity will certainly be useful in applications as it will overcome the disturbances induced by environmental fluorescence. An ICT mechanism for our system can be explained on the basis of deprotonation of the secondary amine of the pyrimidine ring as pyrrolidinium nitrogen has less ability to coordinate Cu^{2+} compared to a pyrimidine group, and thereby the negative charge resides on the nitrogen atoms that undergo delocalization with the conjugated naphthalene ring leading to an increased interaction with Cu^{2+} (Scheme 1). Electron-donating nitrogen atoms facilitate easy removal of the proton from the pyrimidine core, and as a result red shift in both absorption and fluorescence spectra would be expected. Interestingly the second stage color variation from purple to blue upon Cu^{2+} complexation with receptor **2** is remarkable and rare. This does not happen with the *N-tert*-Boc protected receptor **1** in which only change from colorless to purple is observed. This may be due to the presence of hindered carbamate and absence of free pyrrolidinium nitrogen compared to receptor **2**, suggesting the role of the free pyrrolidinium nitrogen in the second stage of color formation (purple to blue).

Scheme 1. Proposed Cu^{2+} Sensing Process



Synthesis of the receptors[11] **1** and **2** (Scheme 2) is achieved by coupling of 1,8-diaminonaphthalene with Boc-L-proline

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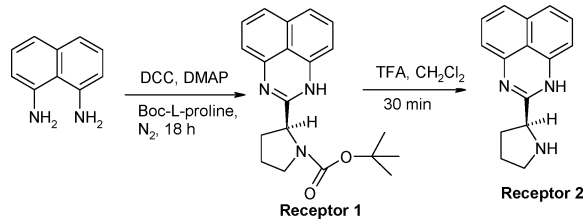
Scheme 2. Synthesis of Colorimetric Fluorescent Sensors



using DCC in the presence of DMAP (catalyst) under nitrogen atmosphere (receptor **1**) followed by deamidation of the *N*-*tert*-Boc group using TFA/CH_2Cl_2 (1:1) leading to a 40% yield of the receptor **2**. The synthesized receptors composed of an ionophore for selective recognition of metal ion are constituted by pyrimidine and chiral pyrrolidine moieties as cation binding sites with a fused naphthalene fluorophore unit which is responsible for signal transduction during spectroscopic studies.

The photophysical properties of the receptor **2** with several metal cations (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, and Ag^+) using their perchlorate salts in CH_3CN/H_2O (80:20, v/v) are investigated by UV−vis and fluorescence measurements and titration studies that are conducted at pH 7.2 (50 mM HEPES buffer).

The absorption spectrum of the receptor **2** ($c = 4.2 \times 10^{-5}$ M) in CH_3CN/H_2O (80:20, v/v) exhibits λ_{max} at 328 nm. Titration experiments are carried out by using the above-mentioned set of metal cations ($c = 2.5 \times 10^{-4}$ M) and demonstrate that only Cu^{2+} promotes remarkable response (Figure 1a). On addition of 5 μM Cu^{2+} to the receptor

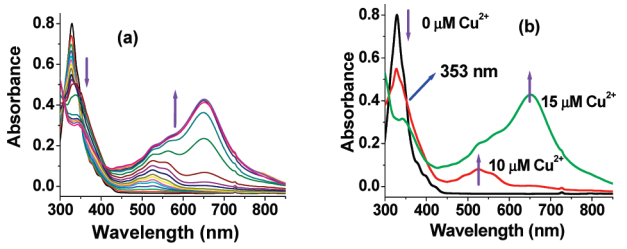


Figure 1. (a) UV−vis absorption spectra of the chemosensor with Cu^{2+}. (b) Dependence of the UV−vis spectra of the sensor on the different concentrations of Cu^{2+}.

solution, the absorbance at 328 nm gradually disappears and a new low energy (LE) broadband appears (around 540 nm) that increases progressively with an isosbestic point at 352 nm, confirming the deprotonation process, thereby inducing a promising color change from colorless to purple. However, with addition of solutions up to 15 μM Cu^{2+}, the peak at 328 almost disappears, and again a significant red shift of the peak from 540 to 650 nm is observed whose intensity increases very sharply (Figure 1b), confirming intermediate complex formation. The red shift is observed for the new LE absorption band resulting in a naked eye color change from purple to blue. It is noteworthy to mention that addition of excess Cu^{2+} produced no significant changes in UV−vis spectra. The resulting titration isotherm fits nicely with a 1:1 binding model as suggested by a Job plot diagram (Figure 2). The association constant (K_a) determined by the UV−vis

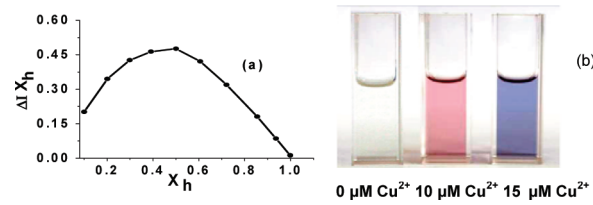


Figure 2. (a) Job plot diagram of receptor for Cu^{2+} (where X_h is the mole fraction of the host and ΔI indicates the change of absorbance). (b) Variation of dual colors upon Cu^{2+} complexation with receptor **2**.

method[12] is found to be 2.58×10^4 M^{-1} (error <10%). The addition of other metal ions such as Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+} and Ag^+ produces insignificant changes in absorption spectra.

Appearance of the blue color in the second stage of the titration experiment for receptor **2** is supposed to be due to participation of the free pyrrolidinium nitrogen atom, which is again confirmed very nicely when we examined our system, i.e., for receptor **1**, having a sterically hindered protected *N*-*tert*-Boc group, only purple color appears initially (Figure 3a) just as in the case of receptor **2**.

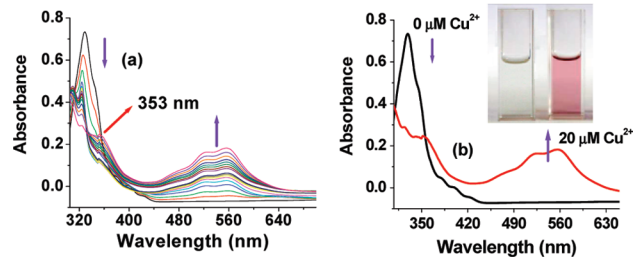


Figure 3. (a) UV−vis absorption spectra of the receptor **1** with Cu^{2+}. (b) Dependence of the UV−vis spectra of the sensor on the different concentrations of Cu^{2+}; inset, color changes upon addition of Cu^{2+}.

Since the bulky carbamate group restricts the metal ion coordination with pyrrolidinium nitrogen, no blue color is

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observed upon addition of excess Cu^{2+} and just the purple color only deepens (inset, Figure 3b).

Fluorescence emission spectra of the receptor **2** are recorded upon excitation at 328 nm to understand the nature of interactions in the excited state. A sharp decrease in the fluorescence intensity at 496 nm is observed reaching its limit value after adding 5 μM Cu^{2+}. Initially the metal−ligand charge transfer between Cu^{2+} and pyrimidine nitrogen quenched the fluorescence emission. Addition of 10 μM Cu^{2+} to the receptor solution as expected leads to a large red-shifted broad emission band centered at 620 nm whose intensity increases very sharply up to addition of 15 μM Cu^{2+} showing an isoemissive point at 550 nm (Figure 4a) attributed

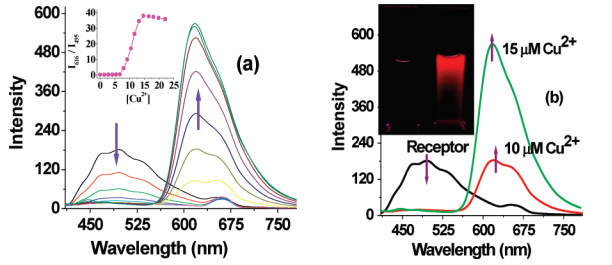

Figure 4. (a) Fluorescence emission spectra of the receptor **2** in the presence of different concentrations of Cu^{2+}; inset, ratiometric calibration curve I_{616}/I_{495} as a function of Cu^{2+} concentration. (b) Dependence of the emission intensity of the sensor on the different concentrations of Cu^{2+}; inset, red emission as obtained by visual naked eye during titration experiment with Cu^{2+}.

to the host−guest complex formation via coordination of the free pyrrolidinium nitrogen with Cu^{2+} in the second stage. Then further addition of Cu^{2+} produces only a nominal decrease in fluorescence intensity. For other tested alkali, alkaline earth, and transition metal ions, only slight quenching of fluorescence intensity is observed due to their low affinity with the sensor. The large red-shifted ICT emission from 495 to 616 nm of the receptor in the presence of Cu^{2+} in the second stage is actually pointed to an enlarged energy gap between the emissive ICT state and its corresponding ground state, and hence a decreased radiationless rate constant results.[13] Again coordination of Cu^{2+} to the receptor probably induces a conformational restriction, thereby preventing Cu^{2+} from quenching the fluorescence of the fluorophore. Thus the chemosensor **2** behaves as an "*off-on*" type of fluorescence probe toward Cu^{2+}. Since the sufficiently large red shift in the emission band from 495 to 618 nm is in the visible region, this visible emission allows the sensor to readily detect Cu^{2+} simply by naked eye experiment, and an almost colorless to promising red fluorescence is clearly observed (Figure 4b).

For the receptor **1** similar quenching of emission intensity around 496 nm takes place (Figure 5a) initially, but shifting

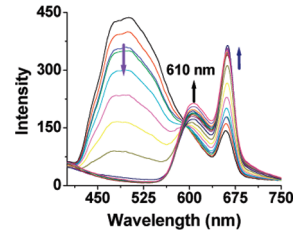

Figure 5. (a) Fluorescence emission spectra of the receptor **1** in the presence of different concentrations of Cu^{2+}.

of the emission band with receptor **1** is less prominent compared to receptor **2**, and only a slight increase in the intensity of peak at 610 nm is observed at a much higher concentration of Cu^{2+} (up to addition of 25 μM). Some sort of interactions with the pyrrolidinium nitrogen probably occurs, but no naked eye red emission is observed with **1** like that with receptor **2**.

In summary, we have reported here a simple yet highly selective ratiometric and colorimetric fluoroionophore (receptor **2**) for Cu^{2+} based on ICT in which the metal ionophore has been incorporated in the electron donor moiety of the fluorophore via deprotonation. A remarkable red shift in both the UV−vis and emission spectra of the chemosensor in CH_3CN/H_2O (80:20, v/v) solution has been observed in the presence of Cu^{2+}.

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Supporting Information Available: Synthetic procedures, spectroscopic data and supplementary spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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