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Highly sensitive and selective fluorescent sensor for Zn^{2+}/Cu^{2+} and new approach for sensing Cu^{2+} by central metal displacement†

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An "off-on" Zn^{2+} and "on-off" Cu^{2+} fluorescent chemosensor C was designed and synthesized. The binding of C and Zn^{2+}/Cu^{2+} is chemically reversible by the addition of EDTA disodium solution; moreover, the fluorescence emission signal of ZnC decreased with the addition of Cu^{2+}, demonstrating that ZnC could detect Cu^{2+} *via* metal displacement.

The development of fluorescent chemosensors with high selectivity, sensitivity and low detection limit has been receiving considerable attention because of their fundamental role in medical, environmental and biological applications.[1] Zinc is the second most abundant transition metal ion in the human body after iron, and is an essential cofactor in many biological processes such as brain function and pathology, gene transcription, immune function, and so on.[2] Thus, a variety of fluorescent sensors for Zn^{2+} has been developed with some successful applications to image Zn^{2+} *in vitro* and/or *in vivo*.[3] On the other hand, copper is a significant metal pollutant due to its widespread use, but is also an essential trace element in biological systems.[4] In this connection, considerable efforts have been made to synthesize Cu^{2+}-selective fluorescent chemosensors, some of which have been used successfully in biological applications.[5] There have been great achievements in the development of fluorescent sensors singly for zinc or copper, but reports about fluorescent chemosensors for both zinc and copper are rare.[6] Therefore, it is still a challenge to develop sensors that can selectively recognize multiple analytes.

Herein, we designed and synthesized a fluorescent chemosensor **C** (see ESI,† Scheme S1) for Zn^{2+} and Cu^{2+}, which possesses multiple benzene and pyridine rings. The rotation of C–C bonds between each two aromatic rings will be frozen if the nitrogen atom of the pyridine ring coordinates metal ions, bringing about an increase in the fluorescence emission intensity of **C**. As expected, compound **C** is a fluorescent molecule which displays a high selectivity for Zn^{2+} and Cu^{2+} ions among relevant metal ions. Furthermore, the fluorescence signals of **C** can be restored by the addition of ethylenediaminetetraacetic acid (EDTA) disodium solution into **ZnC** and **CuC** (Fig. 1) solutions respectively. Moreover, the fluorescence emission intensity of **ZnC** is decreased with the addition of Cu^{2+}, demonstrating that **ZnC** could be a good "on-off" Cu^{2+} sensor candidate.

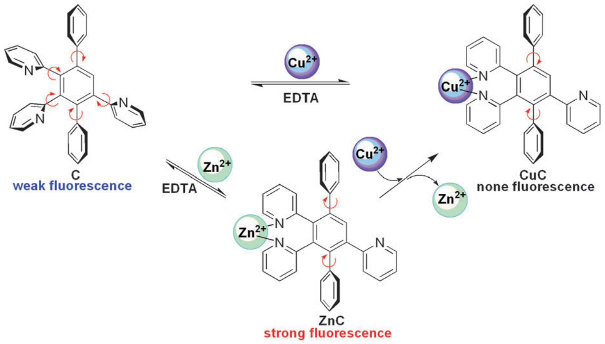

Fig. 1 Process of **C** in sensing of Zn^{2+}/Cu^{2+}.

Using Diels–Alder reaction, compound **C** was easily synthesized according to Scheme S1† and the reported method.[7] The synthetic procedure and detailed characterization of **C** are shown in the Supporting Information.

The absorption spectrum of compound **C** (2.0×10^{-5} M) in ethanol reveals three bands with λ_{max} at 205, 244, and 263 nm (molar extinction coefficient $\varepsilon = 4.46 \times 10^4\ M^{-1}\ cm^{-1}$, $3.46 \times 10^4\ M^{-1}\ cm^{-1}$, and $2.82 \times 10^4\ M^{-1}\ cm^{-1}$ respectively). The addition of 4.0 equiv. of $ZnCl_2$ (Zn^{2+}, 8.0×10^{-5} M) led to a slight increase of the absorbance with no absorption wavelength change (see ESI†, Fig. S5). The addition of 1.0 equiv. of $CuCl_2$ (Cu^{2+}, 2.0×10^{-5} M) (see ESI†, Fig. S6) was similar to that of Zn^{2+} with no change of the λ_{max}, but the absorbance increased greatly ($\varepsilon_{205} = 8.46 \times 10^4\ M^{-1}\ cm^{-1}$, $\varepsilon_{244} = 9.88 \times 10^4\ M^{-1}\ cm^{-1}$, and $\varepsilon_{263} = 12.33 \times 10^4\ M^{-1}\ cm^{-1}$). Such an absorbance change indicates that reformed new excited electronic states in **ZnC** and **CuC** are similar to those of compound **C** except for the higher ε.

^1H NMR analysis provides further evidence to support the M–N bond formation (M = Zn^{2+}/Cu^{2+}, N = nitrogen atom of the two adjacent pyridine rings). ^1H NMR spectra of compound **C** in DMSO-d_6, **C** and Zn^{2+} in DMSO-d_6, and **C** with Cu^{2+} in DMSO-d_6 were measured as shown in Fig. 2.

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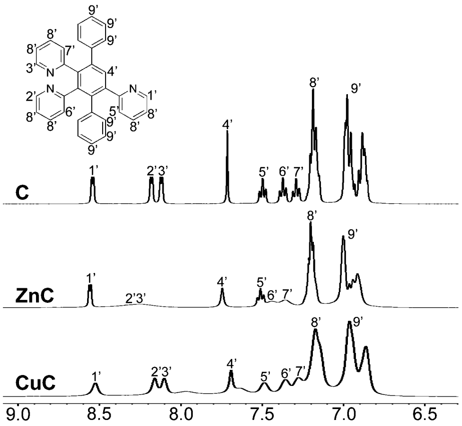

Fig. 2 ^{1}H NMR spectra of chemosensor **C** upon addition of 1.0 equiv. of Zn^{2+} and Cu^{2+} in DMSO-d_6.

The difference between **C** and **ZnC** is the shift to downfield of signal intensity at around 8.17 (from 8.17 to 8.25) and the decrease of signal intensity at 8.17, which was assigned to the formation of a Zn^{2+}–N bond. There is no obvious difference between **C** and **CuC** except the slight changes of shift peaks, resulting from the paramagnetic property of Cu^{2+}.

Compound **C** exhibits relatively weak fluorescence emission at about 375 nm in ethanol upon excitation at 246 nm (Fig. 3). Upon titration of Zn^{2+}, the emission intensity was dramatically enhanced (I/I_0 = 6.4) without change in emission wavelength (Fig. 3), indicating a Zn^{2+}-selective "off-on" fluorescent signaling behavior. However, when Cu^{2+} was added, the fluorescence of **C** was significantly quenched (I/I_0 = 0.23) with no fluorescence emission wavelength change (see ESI†, Fig. S7), indicating an efficient Cu^{2+}-selective "on-off" behavior. The increased emission intensity is probably due to the complex of Zn^{2+} and **C** (**ZnC**), in which the rotation of C–C bonds between each two aromatic rings is inhibited. As for Cu^{2+}, because of its paramagnetic property and unfilled d shell, Cu^{2+} could strongly quench the fluorescence of the fluorophore **C** through electron and/or energy transfer processes.[8] A Job's plot indicated that **C** chelated each of Zn^{2+} and Cu^{2+} with 1 : 1 stoichiometry (see ESI†, Fig. S8 and S9). The detection limits on fluorescence responses of the sensor to Zn^{2+} and Cu^{2+} are 1.7×10^{-5} M and 8.0×10^{-6} M respectively. The process of **C** in sensing of Zn^{2+}/Cu^{2+} is depicted in Fig. 1.

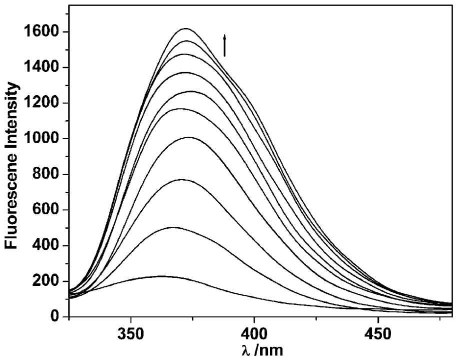

Fig. 3 Changes in fluorescence emission spectra of **C** (2.0×10^{-5} M) in ethanol upon addition of Zn^{2+}. The final ratio of Zn^{2+} to **C** is 4 equiv.

Experiments on the counterion effect on the selectivity of Zn^{2+} (see ESI†, Fig. S10) and Cu^{2+} (see ESI†, Fig. S11) were also performed. As for Zn^{2+}, addition of sulfate, nitrate and acetate anions enhanced the fluorescence of compound **C** very little. While for Cu^{2+}, those anions as above have little influence on the selective effect of compound **C**, indicating that anions didn't coordinate Cu^{2+} when Cu^{2+} reacted with **C**.

The specificity of the chemosensor **C** toward Zn^{2+} and Cu^{2+} was determined next, as shown in Fig. 4; upon addition of the same amount of the various metal ions, respectively, only Zn^{2+} enhanced the emission of **C** and Cu^{2+} decreased that of **C**. However, under identical conditions, nearly no fluorescence intensity changes were observed in emission spectra with Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, and Ni^{2+}, except that Cd^{2+} slightly increased and Hg^{2+} decreased the emission intensity of **C**. That is, sensor **C** can readily distinguish Zn^{2+} and Cu^{2+} from environmentally and biologically relevant metal ions by fluorescent spectra (see ESI†, Fig. S12).

Competition experiments were also conducted for **C** (Fig. 4A and B). When 4 equiv. of Zn^{2+} was added into the solution of **C** in the presence of 16 equiv. of other metal ions, a similar fluorescent spectra change was displayed to that with Zn^{2+} ion only, except those of Cu^{2+} and Hg^{2+}. Hg^{2+} induced very slight fluorescence decrease, whereas Cu^{2+} greatly quenched the fluorescence even if the amount of Zn^{2+} was larger than that of Cu^{2+}, showing the stronger binding ability of Cu^{2+} than that of Zn^{2+} to **C**. As for the competition experiments on Cu^{2+}, under the same conditions as those of Zn^{2+},

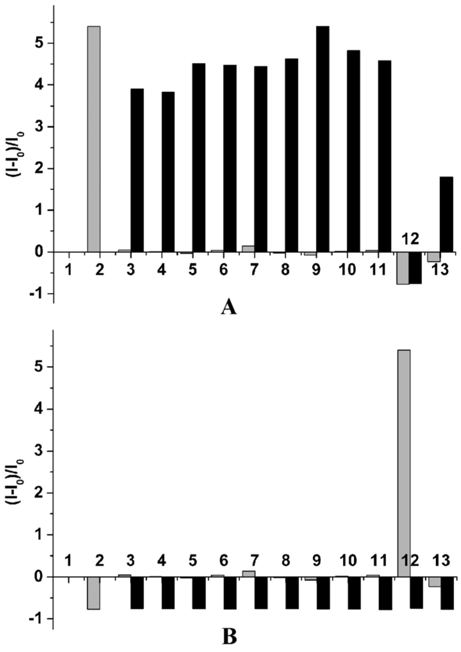

Fig. 4 The light bars represent the fluorescence emission of a solution of 4 equiv. of Zn^{2+} (Fig. 4A)/Cu^{2+} (Fig. 4B) and **C**. The dark bars show the fluorescence change that occurs upon addition of Zn^{2+} (Fig. 4A)/Cu^{2+} (Fig. 4B) to the solution containing **C** and other cation (the ratio of other cation to Zn^{2+}/Cu^{2+} is 4 : 1). 1, **C**; 2, Zn^{2+}/Cu^{2+}; 3, Na^{+}; 4, Mg^{2+}; 5, K^{+}; 6, Ca^{2+}; 7, Cr^{3+}; 8, Cd^{2+}; 9, Mn^{2+}; 10, Fe^{3+}; 11, Co^{2+}; 12, Ni^{2+}; 13, Cu^{2+}/Zn^{2+}; 14, Hg^{2+}. Bars represent the ratio of $I - I_0$ to I_0. I and I_0 represent the emission intensity at 375 nm. The overall emission spectra were measured at excitation of 246 nm.

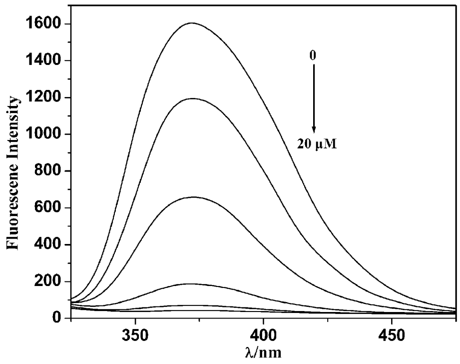

Fig. 5 Fluorescence spectra of **C** (2.0×10^{-5} M) + 4 equiv. of Zn^{2+} upon the titration of Cu^{2+} (0–1 equiv.) in ethanol solution with excitation at 246 nm.

the fluorescence emission spectra displayed a similar pattern at near 375 nm to that with Cu^{2+} only, indicating the stronger binding ability of Cu^{2+} than that of other ions. Because the amount of Na^+, Mg^{2+}, K^+, and Ca^{2+} is much more than Zn^{2+} and Cu^{2+} biologically, it is necessary to measure the disturbance when the amount of these metal ions is much higher than that of Zn^{2+} and Cu^{2+}. The measurement experiments show that no influorescence change was found even when the amount of these alkali and alkaline earth metal ions was at a high concentration of 6 mM.

To examine the binding reversibility of chemosensor **C** to the Zn^{2+}/Cu^{2+}, aqueous solutions of EDTA disodium were added to the complexed solution of **C** (2.0×10^{-5} M) and Zn^{2+} (8.0×10^{-5} M)/Cu^{2+} (2.0×10^{-5} M) in ethanol. As expected, fluorescence signals the same as those of **C** are completely restored (see ESI† Fig. S13 and S14), demonstrating that the binding of **C** and Zn^{2+}/Cu^{2+} is really chemically reversible.

Based on the fluorimetric experiments (Fig. 5), we realized that **C** would have higher binding affinity for Cu^{2+} than for Zn^{2+}. As expected, the addition of Cu^{2+} into **ZnC** solution resulted in quick fluorescence changes (<2 min). The emission intensity at about 375 nm of **ZnC** underwent a gradual decrease, indicating that Cu^{2+} can displace Zn^{2+} to form the **CuC** complex and **ZnC** could be a good "on-off" Cu^{2+} sensor candidate. Further experiments demonstrate that anions have no influence on metal displacement from Zn^{2+} to Cu^{2+} (see ESI†, Fig. S15).[9]

In summary, a Zn^{2+}-selective "off-on" and Cu^{2+}-selective "on-off" fluorescent chemosensor was designed and synthesized, which displays a high selectivity for the Zn^{2+} and Cu^{2+} among relevant metal ions. The mechanism of fluorescence change may be explained as follows: the addition of Zn^{2+} made the rotation of C–C among the aromatic rings inhibited while the paramagnetic property and partially filled d shell of Cu^{2+} led to the possibility of electron and/or energy transfer. Further experiments indicate that the fluorescent signals of **C** can be restored by the addition of EDTA disodium solution into **ZnC** and **CuC** solutions respectively. Moreover, the addition of Cu^{2+} can lead to the fluorescent emission intensity of **ZnC** being decreased, demonstrating that **ZnC** could be a good "on-off" Cu^{2+} sensor candidate.

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