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Coumarin-derived transformable fluorescent sensor for Zn^{2+}†

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We report a coumarin-derived fluorescent sensor for Zn^{2+} termed CTS. CTS shows excellent binding selectivity for Zn^{2+} over competing metal ions due to the transformable ability of CTS, that is the displacement of other metal ions by Zn^{2+}, which induces transformation of chelation from an amide to an imidic acid tautomeric form.

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 mammalian reproduction.[1] Zinc imbalance is connected to severe neurological disorders, including Alzheimer's and Parkinson's diseases.[2] Up to now, a variety of fluorescent sensors for Zn^{2+} have been developed with some successful applications to image Zn^{2+} in living cells,[3] hippocampus slices,[4] and zebrafish,[5] perhaps most notably by Lippard[3c,4c] and Nagano.[3b,4a,b] In order to understand the details of zinc homeostasis, particularly the distribution and concentration of Zn^{2+} in living cells, in the past few years there have been some efforts to develop small molecule fluorescent sensors[6] and genetically encoded biosensors[7] to monitor Zn^{2+} in targeted subcellular domains. However, specific binding selectivity for Zn^{2+} over biologically abundant transition metal ions like Fe^{2+}/Fe^{3+} and Cu^{2+}, as well as exogenetically toxic metal ions such as Cd^{2+} and Hg^{2+}, is still a challenge for the design of a fluorescent sensor.[8] To address this problem, we present here a coumarin-derived fluorescent sensor for Zn^{2+} with a transformable receptor.

The lower affinity of most reported sensors for Zn^{2+} over some heavy and transition metal (HTM) ions results from the moderate coordination nature of Zn^{2+} and the use of di-2-picolylamine (DPA), acyclic and cyclic polyamines, iminodiacetic acid, bipyridine, quinoline and Schiff-bases as Zn^{2+}-chelators,[9] which have a defined coordination pattern and show larger affinities to these HTM ions than Zn^{2+}. Most receptors in existing host–guest chemistry have a confined binding 'cavity'. The selectivity can be improved through imposing a conformational restraint to the receptor, but due to a single binding pattern, a high level of specificity to an analyte is extremely difficult to achieve. In order to make analyte binding specific and more favorable, it would be beneficial if the binding pattern of the receptor with the analyte is different from that with other competitors. In other words, the receptor is transformable to bind to the analyte of choice. To set up a transformable receptor, it is necessary for a transformable factor to be involved. In our previous study, we found tautomerization may play the role of a transformable factor.[5b] The naphthalimide-derived fluorescent sensor (**ZTRS**) with an amide-containing DPA receptor shows extreme selectivity for Zn^{2+}.[5b] The specificity of **ZTRS** for Zn^{2+} comes from its different binding modes to metal ions, *i.e.* it binds Zn^{2+} in an imidic acid tautomeric form of the receptor in aqueous solution but most other HTM ions are bound in an amide tautomeric form.[5b] However, the fluorophore naphthalimide has some intrinsic drawbacks. It is fairly prone to photobleaching and is toxic to biological systems. In order to improve biocompatibility in biological imaging, a much more biologically-friendly fluorophore coumarin is connected with this amide-containing DPA receptor to make a new coumarin-derived transformable sensor (**CTS**).

CTS was synthesized easily as described in the ESI†. Similar to **ZTRS**, **CTS** also showed excellent binding selectivity for Zn^{2+} over competing metal ions (Fig. 1). In HEPES buffer at pH 7.4 (5% DMSO), **CTS** displays two emission maxima of near equal intensity centered at 435 nm (Φ = 0.012) and 485 nm (Φ = 0.014) upon excitation at 340 nm. Addition of 1 equiv. of Zn^{2+} induces a bathochromic shift of the dominant emission band to 505 nm with a concomitant 9-fold fluorescence increase (Φ = 0.09). Interestingly, the **CTS**/Cd^{2+}, **CTS**/Hg^{2+}, and **CTS**/Pb^{2+} complexes showed an enhanced blue-shift in emission to 425 nm. We notice there is a shoulder around 505 nm in the profile of **CTS**/Hg^{2+}. This may be due to the existence of **CTS**/Hg^{2+} in imidic acid form. The addition of other metal ions, such as Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Ag^{+}, produced a negligible change in the fluorescence spectra of **CTS** (Fig. 1a). More importantly, competition experiments showed that the emission profile of the **CTS**/Zn^{2+} complex is unperturbed in the presence of alkali and alkaline earth cations. Of the transition metal ions we tested, only Ni^{2+} and Cu^{2+} limit the turn-on response of CTS, indicating the strongest affinity and selectivity for Zn^{2+} (Fig. 1b) over these metal ions. We believe the specificity for Zn^{2+} and unique fluorescence responses result from the transformable ability of **CTS**, that is the displacement of other metal ions by Zn^{2+} induces transformation of chelation from an amide to an imidic acid tautomeric form (Scheme 1).

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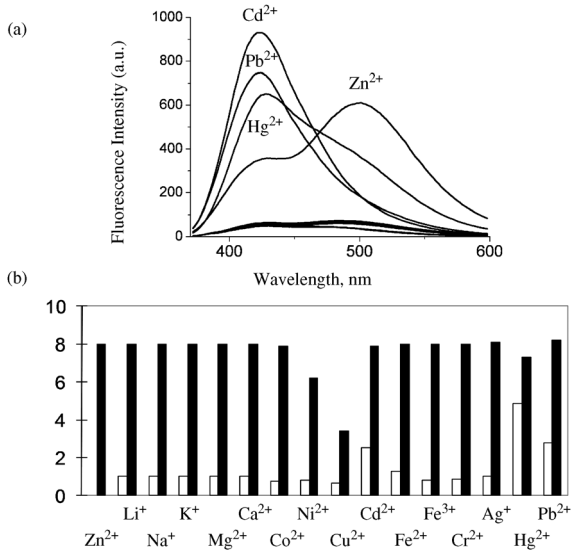
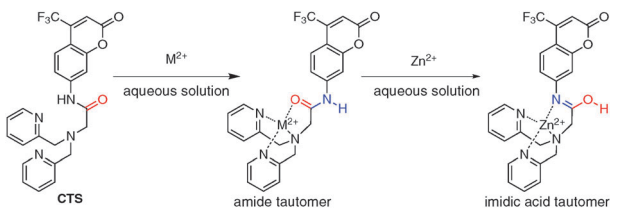


Fig. 1 (a) Fluorescence spectra of 10 μM **CTS** in the presence of various metal ions in aqueous solution (DMSO : 0.5 M HEPES (pH 7.4) = 5 : 95). Excitation at 340 nm. (b) Fluorescence responses of **CTS** to various metal ions in aqueous solution (DMSO : 0.5 M HEPES (pH 7.4) = 5 : 95). Bars represent the final fluorescence intensity at 505 nm (I_f) over the original emission at 505 nm (I_o). White bars represent the addition of 3 equiv. of metal ions (for Na^+, K^+, Mg^{2+} and Ca^{2+}, 300 equiv.) to a 10 μM solution of **CTS**. Black bars represent the subsequent addition of 1 equiv. of Zn^{2+} to the solution.



Scheme 1 Different binding modes of **CTS** with metal ions in aqueous solutions

That means **CTS** works in the same way as **ZTRS** but with better selectivity, because in **ZTRS** the addition of Hg^{2+} induced a slight red-shifted fluorescence enhancement. The blue shifts in emission of **CTS** with HTM ions are attributed to the coordination of the amide oxygen with metal ions which increases the electron-withdrawing ability of the amide group *via* the intramolecular charge transfer (ICT) mechanism, whilst the red shift with Zn^{2+} is attributed to the coordination with the imidic acid nitrogen.

The chemical shift of the amide NH can be used to distinguish between whether Zn^{2+} (or other metal ions) is bound to the carbonyl oxygen or imidic acid nitrogen.[5b] In CD_3CN the Zn–O bond formation results in large upfield shifts of the resonance of the adjacent NH proton from 11.48 to 9.66 (Fig. 2a). With the electron-withdrawing nature of the carbonyl group, the lone pair of electrons on the amide nitrogen is delocalized by resonance, thus forming a partial double bond with the carbonyl carbon and putting a partial negative charge on the oxygen. The complexation of the carbonyl oxygen with Zn^{2+} in CD_3CN blocks the resonance structure and then shifts the NH resonance upfield. In contrast,

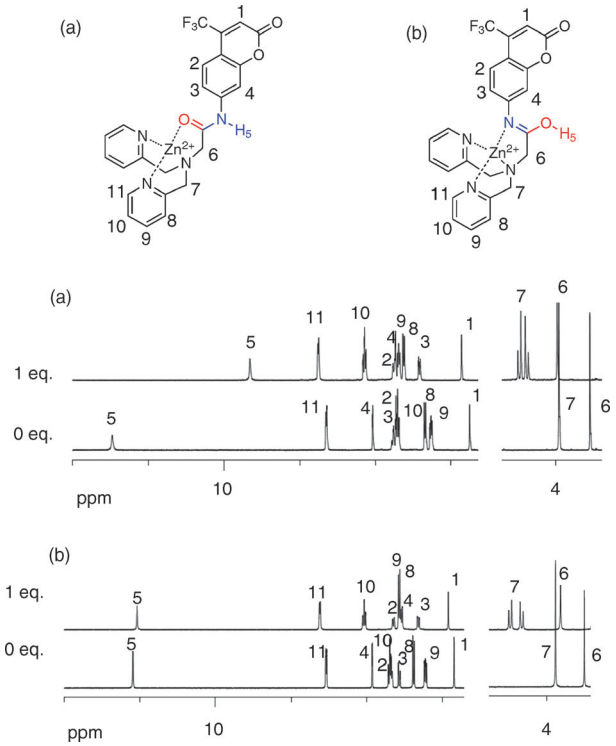


Fig. 2 ^{1}H-NMR spectra of **CTS** in the presence of Zn^{2+} in (a) CD_3CN and (b) DMSO-d_6.

the same proton in **CTS** in DMSO-d_6 with the addition of 1 equiv. of Zn^{2+} undergoes a much smaller upfield shift from 11.09 to 11.04 (Fig. 2b). The binding of the amide nitrogen with Zn^{2+} in DMSO acts as an electron-withdrawing group to shift the OH resonance downfield. 2D NOESY studies of **CTS**/Zn^{2+} (1 : 1) either in CD_3CN in an amide tautomeric form or in DMSO-d_6 in an imidic acid tautomeric form shows cross peaks between H3–H5 and H4–H5 because they are spatially close (Fig. S1–S2, see ESI†).

IR spectra also confirms the imidic acid binding mode. As shown in Fig. S12 (see ESI†), the typical acidic O–H (3000 cm^{-1}, broad) and C–O (1102 cm^{-1}) stretching absorptions further verify the **CTS**/Zn^{2+} (1 : 1) complex in DMSO has the imidic acid binding pattern.

Some available Zn^{2+} sensors have difficulty in distinguishing Zn^{2+} and Cd^{2+}, since Cd^{2+} is in the same group of the periodic table and has similar properties to Zn^{2+}. But with our sensor the different fluorescence responses allow **CTS** to easily distinguish between Cd^{2+} and Zn^{2+} in aqueous solution. Titration experiments indicate the **CTS**/Zn^{2+} and **CTS**/Cd^{2+} complexes all have 1 : 1 stoichiometry (Fig. S3–S4, see ESI†). The apparent dissociation constants (K_d) of **CTS** with Zn^{2+} and Cd^{2+} were determined by fluorescence spectroscopy to be 18 nM and 0.34 μM, respectively. Since the affinity of **CTS** with Zn^{2+} is much stronger than that with Cd^{2+}, when Zn^{2+} was added to the solution of **CTS**/Cd^{2+} complex, Cd^{2+} was displaced by Zn^{2+}, resulting in a significant decrease in the 425 nm emission and an increase of a red-shifted emission band centered at 505 nm (attributed to the formation of a **CTS**/Zn^{2+} complex) with a clear isoemission point at 462 nm (Fig. 3). The inset in Fig. 3 exhibits the dependence of the intensity ratios of emission at 505 nm to that at 425 nm (I_{505}/I_{425}) on Zn^{2+}. Therefore, the **CTS**/Cd^{2+}

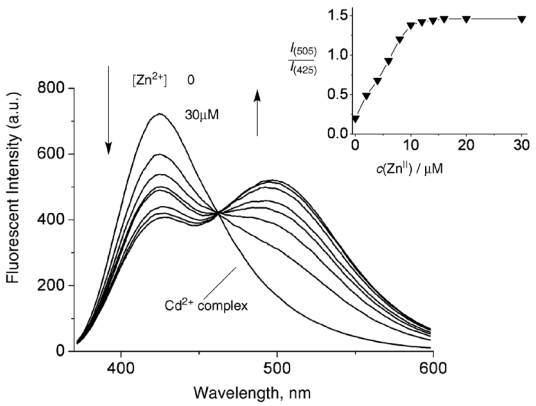


Fig. 3 Fluorescence spectra of 10 μM **CTS**/Cd^{2+} in the presence of different concentrations of Zn^{2+} in aqueous solution (DMSO : 0.5 M HEPES (pH 7.4) = 5 : 95).

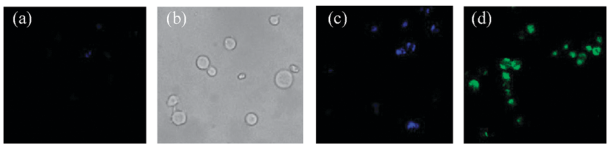


Fig. 4 Fluorescence images of Jurkat cells incubated with 10 μM **CTS** and ions. Cells treated with **CTS** (a) in the absence and (b) bright field image, and (c) presence of 20 μM of $Cd(ClO_4)_2$, and (d) after treatment with **CTS** and 20 μM $Cd(ClO_4)_2$ and subsequent treatment of the cells with 20 μM $Zn(ClO_4)_2$.

complex can be used as a ratiometric fluorescent sensor for Zn^{2+} with a large emission wavelength shift from 425 nm to 505 nm *via* a Cd^{2+} displacement approach. This exceptional property of **CTS** is similar to **ZTRS**. But notably, unlike **ZTRS**, the **CTS**/Hg^{2+} and **CTS**/Pb^{2+} complexes can also act as ratiometric fluorescent sensors for Zn^{2+} *via* a displacement approach (Fig. S7–S8, see ESI†).

We then sought to examine in the context of living cells whether **CTS** can discriminate Zn^{2+} and Cd^{2+}, and whether the **CTS**/Cd^{2+} complex can sense Zn^{2+} ratiometrically. Jurkat cells treated with 10 μM **CTS** alone exhibited very weak background fluorescence (Fig. 4a). The cells incubated with 20 μM $Cd(ClO_4)_2$ and **CTS** displayed enhanced blue fluorescence (Fig. 4c). Interestingly, the fluorescent blue cells (treated with $Cd(ClO_4)_2$ and **CTS**) changed to green fluorescence on exposure of the cells to 20 μM $Zn(ClO_4)_2$ (Fig. 4d). These experiments indicate **CTS** can discriminate intracellularly between Zn^{2+} and Cd^{2+} with green and blue fluorescence, respectively. More attractively, Zn^{2+} could be ratiometrically detected in cells with a large fluorescence color change from blue to green *via* the Cd^{2+} displacement approach. The cytotoxicity of **CTS** was examined towards jurkat cells by a MTT assay. The results showed an IC_{50} value of 300 μM, demonstrating that **CTS** was of low toxicity toward cultured cell lines at a concentration of 10.0 μM.

In conclusion, we have synthesized a new coumarin-based fluorescent sensor **CTS** for ratiometric Zn^{2+} sensing which contains a transformable amide–DPA receptor. In comparison with the sensor **ZTRS**, **CTS** has better photostability and biocompatibility. **CTS** has the strongest affinity with Zn^{2+} among competitive metal ions and displays an excellent fluorescent selectivity for Zn^{2+} with an enhanced red-shift in emission resulting from the Zn^{2+} triggered amide tautomerization. Although **CTS** can bind to both Zn^{2+} and Cd^{2+}, these metal ions can be differentiated by this sensor; upon binding to Zn^{2+} and Cd^{2+} to the sensor, green and blue fluorescence was observed, respectively. Also, the ratiometric detection of Zn^{2+} with a large emission wavelength shift from 425 nm to 505 nm can be achieved *via* a Cd^{2+} displacement approach. Furthermore, this sensor is cell-permeable and can be applied to discriminate Zn^{2+} and Cd^{2+} in cells.

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