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Ratiometric Zn^{2+} Fluorescent Sensor and New Approach for Sensing Cd^{2+} by Ratiometric Displacement

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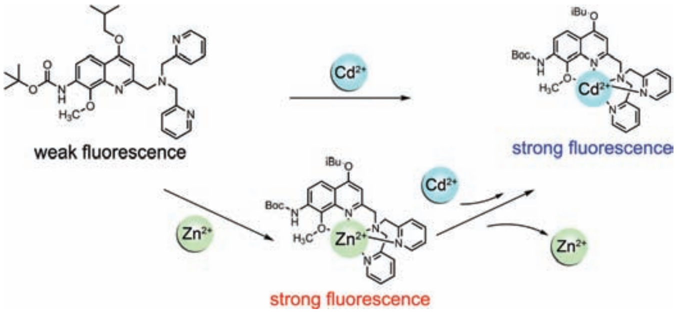
ABSTRACT



A fluorescent sensor QB, based on quinoline with DPA as receptor, is designed as a ratiometric sensor for Zn^{2+} and CHEF (chalation enhanced fluorescence) sensor for Cd^{2+}. Moreover, another ratiometric signal output for Cd^{2+} can be observed when the bound Zn^{2+} in the QB−Zn^{2+} complex is displaced by Cd^{2+}. These results demonstrate that QB can act not only as a ratiometirc sensor for Zn^{2+} but also as a dual-mode Cd^{2+}-selective sensor via the CHEF mechanism and ratiometric displacement.

The development of fluorescent chemosensors for sensing and reporting heavy transition-metal ions has been receiving considerable attention in recent years.[1] Such sensors offer many advantages such as high sensitivity and simplicity, especially for real-time and online analysis.[2] So far, a number of excellent metal ion sensors based on metal−ligand coordination or chemical reaction have been designed and reported;[3] however, it is still a challenge to develop sensors that can selectively recognize multiple analytes, especially species with similar chemical properties such as Mg^{2+}/Ca^{2+},[4] Zn^{2+}/Cd^{2+},[5] etc.

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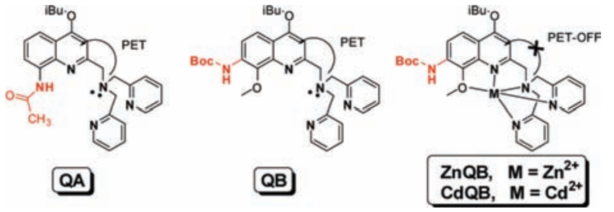
Zn and Cd, group IIb elements of the periodic table, exhibit highly similar chemical properties. Consequently, they often induce similar spectral changes while coordinated with fluorescent sensors. However, they play totally different roles in biochemical processes. Zn^{2+} is the second most abundant transition-metal ion in the human body and is involved in biological activities such as structural and catalytic cofactors, neural signal transmitters or modulators, and regulators of gene expression and apoptosis.[6] On the contrary, Cd^{2+}, known as a toxic metal ion, can cause serious diseases, such as renal dysfunction, calcium metabolism disorders, prostate cancer, etc.[7] Thus, it is desirable to develop a new method for monitoring and distinguishing Zn^{2+} and Cd^{2+}, which would offer a promising approach to study their different actions in biological, toxicological, and environmental circumstances.

Until now, a variety of fluorescent Zn^{2+} sensors[8] have been documented, based on photoinduced electron transfer (PET), internal charge transfer (ICT), excimer/exciplex formation and extinction, chelation-enhanced fluorescence (CHEF), and fluorescence resonance energy transfer (FRET) mechanisms. However, the reports on Cd^{2+} sensors are still less common.[9] We recently reported that sensor **QA** (Scheme 1) can successfully distinguish Cd^{2+} from Zn^{2+} via two different sensing mechanisms, i.e., PET for Cd^{2+} and ICT for Zn^{2+}.[10] In spite of its ratiometric response to Zn^{2+}, the quantum yield of **QA** is really low in the presence of Zn^{2+}. This is presumably due to the strong interaction between the deprotonated amide group and Zn^{2+}, which indicates that the PET process of the tertiary amine was not fully shut down. We rationalized that this disadvantage could be avoided by moving the amide group from the 8 to the 7 position at the quinoline ring (Scheme 1). On the other hand, in order to maintain its selectivity and binding conformation, a methoxy group was introduced at the 8 position on the basis of our previous study.[11] In this case, the Boc-protected amino group does not get involved into the binding processes and only acts as an electron-donor group to regulate the electronic push−pull system, which consequently leads to two different sensing mechanisms for Cd^{2+} and Zn^{2+}. Accordingly, we designed and synthesized fluorescent sensor **QB** for Zn^{2+} and Cd^{2+}. As expected, **QB** exhibits a ratiometric signal response to Zn^{2+} and a CHEF signal response to Cd^{2+}. Moreover, the fluorescent emission maximum of **ZnQB** is blue-shifted with the addition of Cd^{2+}, demonstrating that **ZnQB** could be a good ratiometric Cd^{2+} sensor candidate. Sensor **QB** was synthesized from 2-methoxy-1,3-dinitrobenzene according to the strategy shown in Scheme S1 and was characterized by ^1H NMR, ^{13}C NMR, and ESI-mass spectra (see the Supporting Information).

Scheme 1. Design of Sensor **QB**



As shown in Figure S1 (Supporting Information), UV spectra of **QB** exhibit a maximum absorption at 255 nm ($\varepsilon = 8.0 \times 10^4\ M^{-1}\ cm^{-1}$). Upon increasing the amount of Zn^{2+} or Cd^{2+} (0−2.0 equiv), the absorbance at 255 nm decreased gradually, accompanied by slight red shifts (4.5 nm for Zn^{2+}; 2 nm for Cd^{2+}). Meanwhile, a red-shift peak appeared around 320 nm with three isosbestic points as observed for **HQs** sensors.[11] When **QB** was continuously titrated with Zn^{2+} or Cd^{2+}, the absorbance remained constant up to a mole ratio (**QB**/M^{2+}) of 1:1. This indicates the 1:1 binding model between Zn^{2+}, Cd^{2+}, and **QB**, which was further confirmed by Job's plot, ^1H NMR, and ESI-MS analysis (Supporting Information).

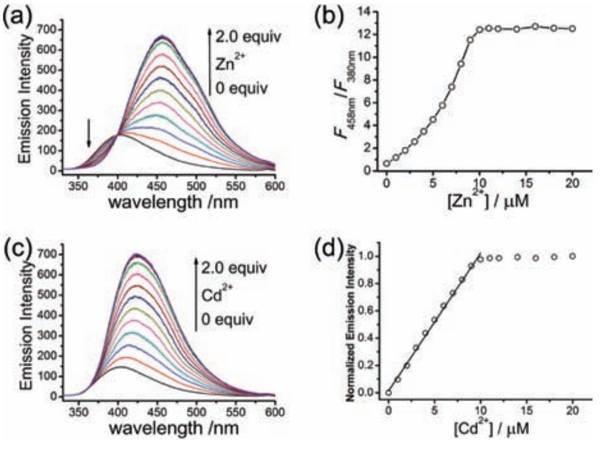


Figure 1. Fluorescence spectra ($\lambda_{ex} = 295$ nm) of 10 μM **QB** upon the titration of M^{2+} (0−2.0 equiv) in buffer solution (10 mM Tris−HCl, 0.1 M KNO_3, 50% CH_3CN, pH = 7.4): (a) **QB** + Zn^{2+}; (b) ratiometric calibration curve F_{458nm}/F_{380nm} as a function of Zn^{2+} concentration; (c) **QB** + Cd^{2+}; (d) fluorescence intensity at 425 nm as a function of Cd^{2+} concentration.

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Figure 1 displays the emission spectra changes of **QB** upon the addition of an increasing amount of Zn^{2+} and Cd^{2+}. Free **QB** showed weak fluorescence emission at 405 nm upon excitation at 295 nm ($\varepsilon = 0.87 \times 10^4$ M^{-1} cm^{-1}, $\Phi_0 = 0.057$) in buffer solution because of the efficient PET quenching from the DPA moiety to the quinoline fluorophore. Remarkable fluorescence enhancements were detected upon the addition of Zn^{2+} and Cd^{2+}, respectively. Upon binding Zn^{2+}, **QB** showed a larger emission red-shift of 53 nm from 405 to 458 nm with an isoemissive point at 400 nm (Figure 1a), indicating that a promoted ICT process occurred with consequent ratiometric fluorescent signals. The ratio of emission intensities ($F_{458\ nm}/F_{380\ nm}$) varies from 0.6 to 12 and is saturated up to a molar ratio (**QB**/Zn^{2+}) of 1:1 (Figure 1b). In contrast, upon binding Cd^{2+}, only enhanced fluorescent intensity ($\Phi_{Cd} = 0.22$) with a smaller wavelength shift (20 nm), but without an isoemissive point, were observed (Figure 1c), suggesting that sensor **QB** recognizes Cd^{2+} principally based on the CHEF mechanism. The titration curves as shown in Figure 1b,d demonstrate the high binding affinities[12] and 1:1 complexes between Zn^{2+}, Cd^{2+}, and **QB**.

To obtain an insight into the sensing properties of **QB** toward metal ions, we investigated the fluorescence titration of different ions in CH_3CN/H_2O buffer solution (Figure 2). Quenching effects were observed when heavy metal ions of Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}, and Pb^{2+} were added, as observed in other quinoline−DPA-based sensors. Addition of Mg^{2+}, Ca^{2+}, and Na^+ exerted no or little effect on the emission of **QB**, even at high concentration (1 mM). As expected, remarkable fluorescence changes were detected upon the addition of either Zn^{2+} or Cd^{2+}. The Zn^{2+}-selective ratiometric response (F_{458nm}/F_{380nm} up to 12) of **QB** was clearly observed (Figure 2b) and was not interfered by the presence of Mn^{2+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, K^+, and Na^+ (Figures S4 and S5, Supporting Information). Considering the distinct fluorescent enhancement upon binding Cd^{2+}, **QB** can also be used as a CHEF Cd^{2+}-selective sensor. In this case, other metals behave similarly as in the case of Zn^{2+} (Figure S3, Supporting Information). These results suggest that **QB** had good selectivity for Zn^{2+} and Cd^{2+}.

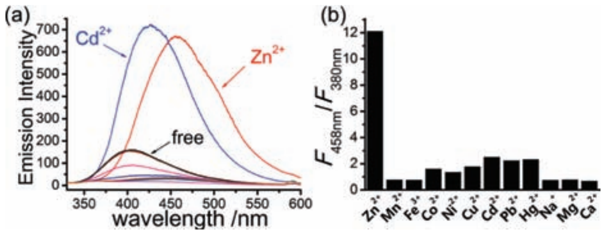

Figure 2. (a) Fluorescence spectra ($\lambda_{ex} = 295$ nm) of **QB** (10 μM) and **QB** in the presence of various metal ions (1 equiv of Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} and 100 equiv of Mg^{2+}, Ca^{2+}, and Na^+) in buffer solution. (b) Ratio $F_{458}/F_{380\ nm}$ of **QB** (10 μM) in the presence of indicated metal ions.

Moreover, pH effects on the fluorescence of the free **QB**, **ZnQB**, and **CdQB** are shown in Figures S7 and S8, (Supporting Information). Fluorescence emission of **QB** exhibits a weak emission maximum at 455 nm at low pH and then rapidly blue shifts to 405 nm as the pH increases up to pH 7; emission intensity remains unaffected up to pH 11. A pK_a value was fitted to be 6.1, which is attributed to the protonation of tertiary amine. As for **CdQB**, fluorescence intensity is silent to pH in the range from 3.25 to 11.89, indicating little dependence on pH in sensing Cd^{2+}. On the other hand, the pH profiles of **ZnQB** are analyzed with two-component pK_a, suggesting two deprotonation events. The fluorescence enhancement showed a ∼1.5-fold enhancement as the pH was increased from 3.48 to 8.36, with p$K_{a1} = 7.1$, assigned to the deprotonation of tertiary amine. A further increase of the pH caused fluorescence quenching, with p$K_{a2} = 10.3$, implying the second deprotonation of the amino group, presumably, being the *NH*Boc group in **ZnQB**. These results indicate that Zn^{2+}, possessing the stronger Lewis acidity, affects the acidity of the *NH*Boc group and promotes the ICT process. Similar phenomena were also described by Aoki et al.[13]

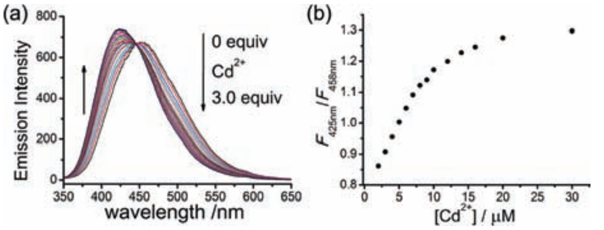

Figure 3. (a) Fluorescence spectra ($\lambda_{ex} = 295$ nm) of 10 μM **QB** + 1 equiv of Zn^{2+} upon the titration of Cd^{2+} (0−3.0 equiv) in buffer solution. (b) Ratiometric calibration curve F_{425nm}/F_{458nm} as a function of Cd^{2+} concentration.

Based on the fluorimetric experiments (vide supra), we realized that **QB** would have higher binding affinity for Cd^{2+} than for Zn^{2+}. As expected, the addition of Cd^{2+} into **ZnQB** solution resulted in quick fluorescence changes (<2 min). The emission maximum of **ZnQB** ($\Phi_{Zn} = 0.23$) underwent a gradual blue-shift from 458 to 425 nm, with an obvious isoemissive point at 445 nm (Figure 3 and S6, Supporting Information), indicating that Cd^{2+} can displace Zn^{2+} to form the **CdQB** complex. The displacement was further confirmed by NMR titration (vide infra). The ratio of **CdQB** to **ZnQB** emission intensities (F_{425nm}/F_{458nm}) varied from 0.8 to 1.3, and the detection limit for the analysis of Cd^{2+} was calculated to be 2.38×10^{-6} M. These data indicate that **ZnQB** could be used for sensing Cd^{2+} in micromolar range. Thus, **QB** could be a dual-mode Cd^{2+}-selective sensor via the CHEF mechanism and ratiometric displacement approach (Figure 4). Although the displacement assays have been used in

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(12) Compared with those sensors bearing a quinoline−DPA moiety, the binding affinities were estimated down to the subnanomolar range or lower. However, herein, the K_d values were not quantitated due to the large proportion of organic solvent.

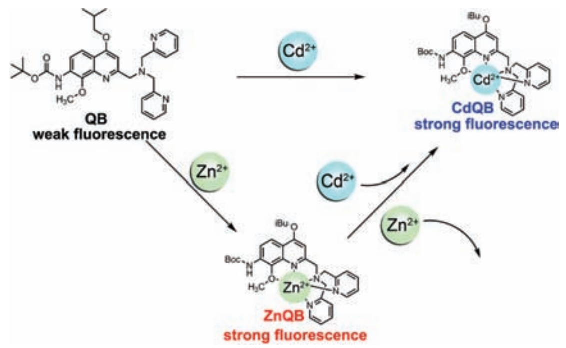

Figure 4. Process of **QB** in sensing of Zn^{2+}/Cd^{2+}.

cation recognition,[14] there are only a few successful systems realized by a ratiometric displacement approach,[15] including the one described herein.

For studying the nature of the interaction between sensor **QB** and Zn^{2+}/Cd^{2+}, ^1H NMR titrations were also carried out (Figure 5 and Table S2, Supporting Information). Upon interaction with Zn^{2+} and Cd^{2+}, the protons of the methoxy CH_3 experienced significant upfield shifts ($\Delta\delta_{Zn} = 0.97$; $\Delta\delta_{Cd} = 0.41$ ppm), indicating that the oxygen atom of the methoxy group strongly bonded with Zn^{2+}/Cd^{2+}. The protons at ortho position of pyridines downfield shifted from 8.49 to 8.66 (Zn^{2+}) and to 8.80 (Cd^{2+}), respectively, by *N*-metal coordination effect. Meanwhile, similar upfield shifts were also observed for the proton at the 3-position of quinoline, suggesting the direct interaction between quinoline platform and metal ions. When 4 equiv of Cd^{2+} was further introduced into the **ZnQB** solution, compare Figure 5d with 5b, chemical shifts of all protons changed significantly and are almost identical with these of the sole **CdQB** (Figure 5c). The finding indicates that **QB** has higher affinity for Cd^{2+} than for Zn^{2+}, which consequently incurs the ion displacement process.

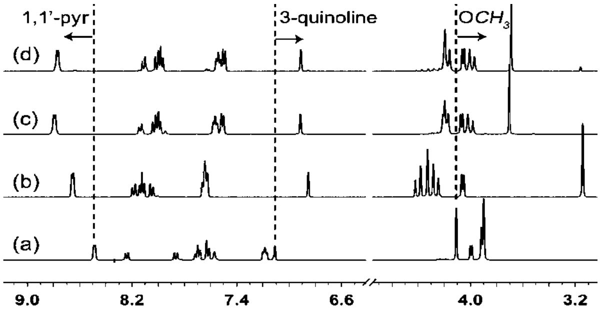

Figure 5. Partial ^1H NMR spectra (400 MHz) of **QB** (10 mM) in CD_3CN: (a) free **QB**; (b) **QB** + 1 equiv of Zn^{2+}; (c) **QB** + 1 equiv of Cd^{2+}; (d) (b) + 4 equiv of Cd^{2+}.

In conclusion, we have developed a novel fluorescent sensor, **QB**. It shows a ratiometric response to Zn^{2+} based on both CHEF and ICT mechanisms but a CHEF response to Cd^{2+}. Moreover, we show that the central ion of **ZnQB** can be displaced by Cd^{2+}, resulting in another ratiometric sensing signal output. Thus, **QB** can be a dual mode Cd^{2+}-selective sensor via the CHEF mechanism and ratiometric displacement approach.

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

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