A highly sensitive ratiometric fluorescent probe for Cd^{2+} detection in aqueous solution and living cells†

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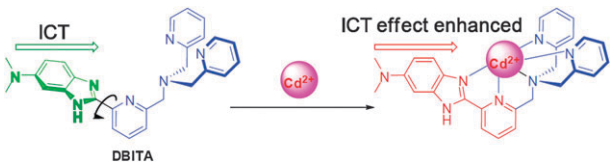
A ratiometric fluorescent Cd^{2+} sensor DBITA which featured the Cd^{2+}-induced red-shift of emission (53 nm) and picomolar sensitivity in both aqueous media and living cells was developed.

Cadmium, which is widely used in many fields, such as industry and agriculture, and then can be found in air and water,[1] has been recognized as a highly toxic heavy metal and listed as high as the seventh on the *Top 20 Hazardous Substances Priority List* by the Agency for Toxic Substances and Disease Registry and US Environmental Protection Agency (EPA).[2] According to US EPA and World Health Organization (WHO) standards,[2,3] the maximum limit for bottled water is about 4 and 40 nM. Chronic exposure to Cd^{2+} sources can cause serious health disorders and even certain cancers owing to the increased Cd^{2+} accumulation in the human body.[4] However, the mechanisms involved in the Cd^{2+}-uptake and carcinogenesis remain undefined.[4] On the other hand, the low Cd^{2+} concentration down to 100 pM in living cells was reported to stimulate cell growth and DNA synthesis significantly.[5] Therefore, developing reliable methods for Cd^{2+} quantification in environmental samples and in cell/tissue is of great significance for clarifying the Cd^{2+}-carcinogenesis and other biological effects.

Fluorescent sensing *via* suitable sensors is an attractive alternative for this goal due to the simple instruments, high sensitivity and selectivity.[6] However, few fluorescent Cd^{2+} sensors have been explored up to now,[7] and their practical application is still restrained due to their poor water solubility, UV-excitation and pH-dependent fluorescence in physiological environments. Moreover, the Cd^{2+} level in normal living systems and the required level in water established by US EPA and WHO are beyond the detection limits of most Cd^{2+} sensors reported so far.[7a–j] Due to the similarity of Cd^{2+} and Zn^{2+}, it is difficult to reduce the Zn^{2+}-induced interference in Cd^{2+} detection by most of the reported sensors. In addition, the quantitative Cd^{2+} detection in living systems demands a ratiometric sensor for Cd^{2+} other than the intensity-based sensor, to reduce the interference induced by the unknown local sensor concentration and the deviations in detecting conditions/microenvironments. Therefore, the development of a ratiometric fluorescent sensor with high sensitivity and selectivity for quantitative Cd^{2+} detection is currently attracting the attention of scientists from many disciplines. Herein, we describe a new ratiometric fluorescent sensor **DBITA** (Scheme 1) with picomolar sensitivity and selectivity for Cd^{2+} in aqueous media and at cellular level.

In this work, a ratiometric Cd^{2+} sensor, **DBITA**, was developed from an internal charge transfer (ICT) fluorophore, DBI (5-dimethylamino-2-(2-pyridinyl)-benzoimidazole), displaying large Stokes shift and bright signal. This fluorophore has been frequently used as ICT fluorophore for the construction of sensors for different ions.[8] Kool and coworkers have reported a DBI-based fluorescent sensor of *N*-deoxyriboside motif for metal ion sensing.[9] However, its "turn-on" response in organic solvent and low specificity to Cd^{2+} restricted its application in quantitative detection, especially in living systems. Its Cd^{2+}-induced emission red shift (59 nm) in methanol suggests that DBI is a valuable platform to construct ratiometric fluorescent sensors for Cd^{2+}. In fact, the 2,2′-N chelation to the metal center has been proposed to induce the co-planation of pyridine and benzoimidazole in DBI and the resulting ICT deviation should be the origin for emission shift.[10] To achieve the goals of ratiometric Cd^{2+} sensing and higher Cd^{2+} affinity, an ion chelator, bis(pyridin-2-ylmethyl)amine (BPA),[11] was incorporated with DBI at its 3′-position as the synergic Cd^{2+} coordination motif of its 2,2′-N atoms. Besides the enhanced Cd^{2+} affinity, the synergic coordination to Cd^{2+} by BPA and DBI motif may enhance the quantum efficiencies, since the photo-induced energy transfer *via* aryl rotation of the biaryl fluorophore in the excited state may be more effectively blocked. Therefore, the enhanced sensitivity of the new sensor is expected.

The UV-vis spectrum of **DBITA** in HEPES buffer (pH = 7.2) exhibits an absorption maximum at 340 nm ($\varepsilon = 1.3 \times 10^4$ M^{-1} cm^{-1}), which can be assigned to the



Scheme 1 Proposed Cd^{2+} binding mode of **DBITA**.

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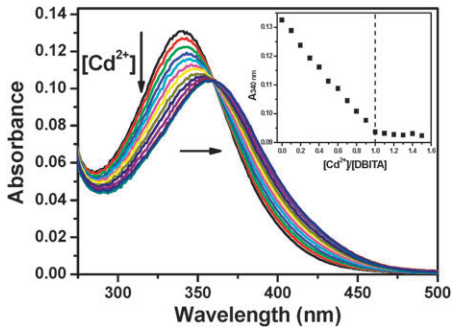

Fig. 1 Absorption spectra of **DBITA** (10 μM) in HEPES buffer (50 mM, 0.1 M KNO_3, pH 7.2) obtained by adding aliquots of 12.5 μL $CdCl_2$ (1.2 mM) solution. The $[Cd^{2+}]_{total}$ increases from 0.0 to 15.0 μM along the direction of the arrow. Inset, the titration profile based on the absorbance at 340 nm.

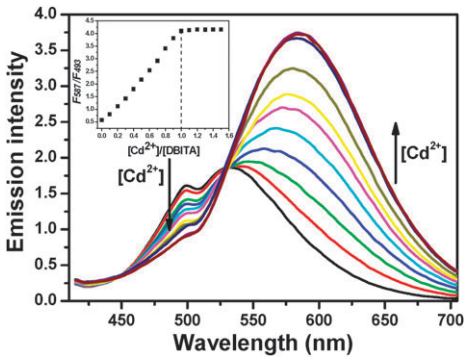

Fig. 2 Emission spectra of **DBITA** (10 μM) in HEPES buffer (50 mM, 0.1 M KNO_3, pH 7.2) obtained by adding aliquots of 12.5 μL $CdCl_2$ (1.2 mM) solution. Inset, the titration profile based on the emission ratio at 587 and 493 nm, F_{587}/F_{493}. Excitation was at 362 nm.

π–π* transition band (Fig. 1). When titrated by Cd^{2+} (0–1.5 eq.), this band is decreased gradually, accompanied by the red-shift to 359 nm. The increased ICT effect due to the Cd^{2+} coordination-induced enhancement of acceptor electron-withdrawing ability is the origin for it. The linear decrease of absorbance at 340 with $[Cd^{2+}]_{total}$ up to a molar ratio ($[Cd^{2+}]_{total}$/[**DBITA**]) of 1 : 1 and the unchangeable spectrum at even higher $[Cd^{2+}]_{total}$ imply the 1 : 1 binding stoichiometry.

Free **DBITA** in HEPES buffer displays two excitation bands centered at 362 and 393 nm, and two emission bands centered at 493 and 534 nm, respectively (Fig. S4). The Stokes shift of 172 nm (from 362 to 534 nm) should help to reduce the excitation interference. Its quantum yield was determined as 0.18 with quinine sulfate in 0.5 M H_2SO_4 as the reference.[12] Cd^{2+} titration leads to the distinct emission red-shift from 534 to 587 nm with a clear isoemission point at 530 nm. The emission ratio at 587 and 493 nm (F_{587}/F_{493}) increases linearly with $[Cd^{2+}]_{total}$ from 0.57 to 4.16 till the $[Cd^{2+}]_{total}$/[**DBITA**] ratio reaches 1 : 1, which is consistent with 1 : 1 Cd^{2+} binding stoichiometry disclosed by UV-vis titration (Fig. 2). The quantum yield of Cd^{2+}/**DBITA** complex is 0.42. The K_d value of Cd^{2+}/**DBITA** complex was determined to be ~ 25 pM by a competitive binding experiment, suggesting the extremely high affinity of **DBITA** toward Cd^{2+} (Fig. S6). The distinct fluorescent ratiometric response of **DBITA** to the EGTA-buffered Zn^{2+} solutions demonstrates the Cd^{2+} sensing ability of **DBITA** at $[Cd^{2+}]_{free}$ being lowered to the level of 0.3 pM, which is far below the EPA and WHO standards for drinking water (See supporting information S4). This property makes **DBITA** a practical sensor for Cd^{2+} detection.

Fluorescent screening against other metal ions indicates that 1 equiv of Hg^{2+}, Cd^{2+}, Pd^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, and Fe^{2+} does not induce any emission red shift and enhancement (Fig. 3). It should be noted that Zn^{2+} addition induces a red-shift of emission from 534 to 609 nm, but it only has little interference with F_{587}/F_{493}. Moreover, the presence of all the abovementioned cations does not alter the Cd^{2+}-induced F_{587}/F_{493} distinctly. Moreover, Na^+, K^+, Ca^{2+} and Mg^{2+}, which are abundant in water and living cells, even though the concentration is 1000 times higher, do not result in distinct change in F_{587}/F_{493} of **DBITA**. Fluorescent pH titration of **DBITA** and Cd^{2+}/**DBITA** complex showed the stable F_{587}/F_{493} ratio from pH 6.0 to 8.0, which warrants its application in physiological detection (Fig. 4).

The application of **DBITA** to track intracellular cadmium levels was tested on HeLa cells *via* dual-channel ratiometric imaging. To reduce the irradiation damage, a laser of 405 nm was selected as the excitation source considering the excitation wavelength of the confocal fluorescence instrument. The fluorescent titration experiments demonstrated that the ratiometric response and selectivity of **DBITA** towards Cd^{2+} were almost unaffected upon irradiation at 405 nm (Fig. S5). The ratiometric imaging of cells loaded with **DBITA** show very low levels of background intracellular emission ratio, indicating the membrane permeability of **DBITA** (Fig. 5b). When exogenous Cd^{2+} was introduced *via* incubation with $CdCl_2$ solution, intensive blue to yellow was observed inside the cell, displaying the enhanced Cd^{2+} level compared to the cells not treated with cadmium salts (Fig. 5c). Treatment with the metal ion chelator TPEN (*N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)-ethylenediamine) for 1 min at 25 °C reduces the emission ratio enhancement distinctly (Fig. 5d), implying **DBITA** can

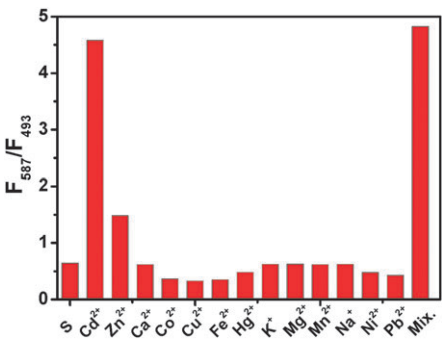

Fig. 3 Emission ratio at 587 and 493 nm (F_{587}/F_{493}) of **DBITA** (10 μM) in HEPES buffer induced by indicated metal ions. The final concentration for Cd^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, and Pd^{2+} is 10 μM, for Na^+, K^+, Ca^{2+}, and Mg^{2+} is 10 mM. Excitation was at 362 nm. S = free sensor. Mix. = a mixed solution containing all the tested cations.

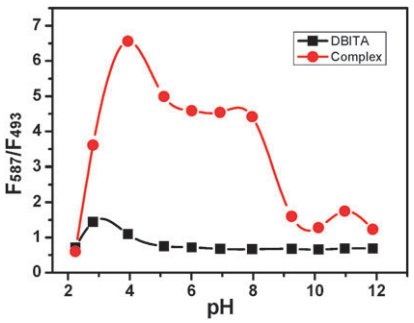

Fig. 4 Emission ratio F_{587}/F_{493} of **DBITA** and **DBITA/Cd^{2+}** complex (10 μM) in aqueous solutions at different pH. λ_{ex}, 362 nm.

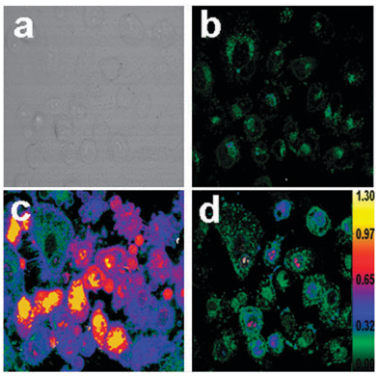

Fig. 5 Confocal fluorescent images of HeLa cells when stained by **DBITA** solution (10 μM in PBS) at 25 °C; λ_{ex}, 405 nm. (a) Brightfield transmission image; (b) ratio image according to the images collected at 460–510 and 560–610 nm, respectively; (c) ratio image of cells in (b) with further treatment with 50 μM $CdCl_2$ solution (2 h), followed by washing with **DBITA** stock; (d) ratio image of cells in (c) followed by treatment with 25 μM TPEN (10 min).

monitor the change of [Cd^{2+}] in cells reversibly. Similar imaging results were obtained on macrophage cells (Fig. S8).

In conclusion, the ratiometric sensor for Cd^{2+}, **DBITA**, is able to discriminate Cd^{2+} from Zn^{2+} and features a large Cd^{2+}-induced red emission shift (53 nm). It possesses large Stokes shift and high quantum yield and can detect Cd^{2+} at picomolar level. Its cell membrane permeability is favorable for monitoring Cd^{2+} levels in living cells. Besides the ratiometric sensing mechanism *via* the metal chelation-induced co-planation of 2,2′-azo-1,1′-biaryl fluorophore, the more practical ratiometric sensing behavior of **DBITA** than **PBITA** suggests that the promoted ICT effect in **DBITA** provides this metal chelation-effect more distinctly.

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