A highly selective multi-responsive fluorescence sensor for Zn$^{2+}$ based on a diarylethene with a 4,6-dimethylpyrimidine unit†

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A novel turn-on mode fluorescent diarylethene containing a 4,6-dimethylpyrimidine unit was developed to fluorescently sense Zn$^{2+}$. Its multiple-responsive properties induced by Zn$^{2+}$/EDTA and ultraviolet/visible light have been systematically studied. The fluorescence sensor could efficiently detect Zn$^{2+}$ with a 10 times enhancement of emission intensity and fluorescence color change (dark-green). In addition, the sensor showed clear discrimination from Cd$^{2+}$. The limit of detection of the sensor was measured to be 8.48 × 10$^{-8}$ mol L$^{-1}$ for Zn$^{2+}$. Finally, a molecular logic circuit was fabricated with the emission at 528 nm as the output signal and light and chemical stimuli as input signals.

Introduction

Chemosensors are a promising research field due to the simplicity of assays, low cost and high sensitivity for metal ions in industry, medicine, biology and the environment. In biological systems, metal ions play essential roles in different ways. Among all the metal ions, Zn$^{2+}$ is the second most abundant transition metal ion in biological systems and is of utmost importance for maintaining normal cellular functions, such as cell division, repair of DNA, protein synthesis, mammalian reproduction, and muscle contraction. However, the abnormal accumulation of Zn$^{2+}$ within the body could create serious health issues, such as myopathy, Alzheimer’s disease, epileptic seizures, encephalopathy, and various cancers. Consequently, it is highly desirable for developing effective ways for monitoring Zn$^{2+}$, which would provide an effective and promising approach to study its physiological and pathological processes.

The usual detection procedures include traditional techniques, such as, chromatography, voltammetric and ion selective electrodes. However, many of these methods have their own disadvantages such as expensive instrument, insufficient sensitivity and analytes interference. To date, much effort has been made to seek ways for detecting and tracing Zn$^{2+}$. Among the various available techniques for sensing Zn$^{2+}$, fluorescence detection was much more preferable, because fluorescent probes have shown many promising advantages, including high sensitivity, real-time detection, quantitative capabilities, low cost, and easy operation. In general, since Cd$^{2+}$ and Zn$^{2+}$ are in the same group of the periodic table and have similar spectral response, how to eliminate the interference of Cd$^{2+}$ during Zn$^{2+}$ detection is a thorny problem. Undoubtedly, it is very important to design fluorescent sensor for the selective quantification of Zn$^{2+}$ without interference from Cd$^{2+}$ and other metal ions.

In order to deeply understand the crucial role of Zn$^{2+}$ in important life processes, large amounts of Zn$^{2+}$ fluorescent sensors have been accepted and effectively applied in vivo. Among the reported fluorescent chemosensors for detecting Zn$^{2+}$, diarylenethene are considered one of the most potential candidates due to their remarkable fatigue resistance, excellent thermal stability, rapid response and multi-responsively photoswitchable properties under various stimulations. To date, numerous diarylethene-based fluorescent sensors with various functional groups have been developed for the detection of Zn$^{2+}$. Pyrimidine derivatives have gained much interest in the field of drugs, agriculture chemicals, and many biological processes. However, diarylethene-based fluorescent sensors with pyrimidine unit for detecting Zn$^{2+}$ are relatively less known.

In this paper, we reported a novel photochromic diarylethene-based fluorescence sensor for Zn$^{2+}$ with a 4,6-dimethylpyrimidine structure (Scheme 1). It could recognize...
Zn$^{2+}$ with high selectivity and sensitivity through a strong blue-shift together with a remarkable fluorescence enhancement in acetonitrile solution. Furthermore, it could clearly discriminate Zn$^{2+}$ from Cd$^{2+}$ and other metal ions.

Experimental

General methods

All chemical reagents were provided by chemical reagent companies and used as received without further purification. The metal ions (Al$^{3+}$, Ca$^{2+}$, Pb$^{2+}$, Mg$^{2+}$, Cr$^{3+}$, Cd$^{2+}$, Mn$^{2+}$, Fe$^{3+}$, Sr$^{2+}$, Ag$^{+}$, Ba$^{2+}$, Ni$^{2+}$, Co$^{2+}$ and Cu$^{2+}$ as nitrates, and K$^+$, Ba$^{2+}$, Hg$^{2+}$, and Sn$^{2+}$ as chlorides) used in metal ion selectivity experiments were dissolved in double-distilled water. EDTA solution (0.1 mol L$^{-1}$) was prepared with Na$_2$EDTA in double-distilled water. Melting point determinations were conducted using an Agilent 8453 UV/vis spectrophotometer on a Bruker AmaZon SL spectrometer. The UV-vis experiments were performed using an MUL-165 UV lamp and a MVL-210 visible lamp. The fluorescence spectra measurements were performed on a Hitachi F-4600 spectrofluorometer. Photoirradiation experiments were measured using an MUL-165 UV lamp and a MVL-210 visible lamp.

Synthesis of compound 10

Scheme 2 shows the synthesis route of the diarylethene, 1-(2,5-dimethyl-3-thienyl)-2-{2-methyl-5-[4-hydroxyl-3-(2-thienyl)perchlorocyclopentene (3)) was prepared by adaptation of literature procedures.\textsuperscript{38-40}

Compound 2 (ref. 39) (3.10 g, 6.0 mmol) was cooled in dry CH$_2$Cl$_2$ at 195 K under nitrogen with vigorous stirring, followed by addition of BBr$_3$ (12.0 mL). The reaction mixture was stirred for 0.5 h at 195 K, and then warmed to room temperature for another 48 h. The resulting mixture was extracted with CH$_2$Cl$_2$, and then washed with water. The resulting solution was dried over Na$_2$SO$_4$. After filtration and solvent evaporation, compound 3 (0.75 g, 1.5 mmol) was well-separated via silica gel chromatography (eluent: petroleum ether/ethyl acetate (40:1)) in 35% yield. Mp 401–402 K. \textsuperscript{1}H NMR (DMSO-d$_6$, 400 MHz), $\delta$ (ppm): 1.85 (s, 3H), 1.91 (s, 3H), 2.41 (s, 3H), 6.84 (s, 1H), 7.07 (d, 1H), 7.39 (s, 1H), 7.81 (d, 2H), 10.30 (s, 1H), 11.00 (s, 1H)

RESULTS AND DISCUSSION

Photochromism properties of 10

The UV-vis spectroscopy and fluorescence changes of 10 induced by photoirradiation have been studied in CH$_3$CN (2.0 \times 10^{-5} \text{ mol L}^{-1}).\textsuperscript{41} The open-ring isomer 10 exhibited an absorption band at 299 nm attributed to $\pi-\pi^*$ transitions at room temperature (Fig. 1A).\textsuperscript{42} Upon ultraviolet light irradiation ($\lambda = 297$ nm), the absorption bands at 298 nm decreased and a new visible absorption band at 555 nm ($\epsilon = 1.45 \times 10^4 \text{ mol}^{-1} \text{L cm}^{-1}$) increased gradually, indicating the corresponding closed-ring isomer 1C was generated. At the same time, the photo-induced color of 10 changed from colorless to purple (Fig. 1A). A stable isosbestic point occurred at 311 nm when the photostationary state was reached, demonstrating the occurrence of a two-component photochromic reaction.\textsuperscript{43-44}

Conversely, upon visible light irradiation ($\lambda > 500$ nm), the purple closed-ring isomer 1C reverted to the colorless open-ring isomer 10 by cycloreversion reaction, and its absorption peak shifted back to 298 nm. Similar to most reported diarylethenes,\textsuperscript{45-47} the fluorescence emission of 10 in CH$_3$CN also revealed the photochromic reaction occurred (Fig. 1B). The open-ring isomer 10 displayed a dark yellow emission with an emission peak at 595 nm under excitation at 365 nm. Under ultraviolet light irradiation ($\lambda = 297$ nm), the emission intensity at 595 nm was gradually quenched to ca. 18.0% in the photostationary state due to the photocyclization product 1C generated.\textsuperscript{44} Upon back visible light irradiation ($\lambda > 500$ nm), the emission spectra of 1C was gradually increased and recovered to the original state of 10. In addition, the fatigue resistance of 10 was also determined by alternating irradiation of ultraviolet and visible lights at room temperature. Undergo 18 times of the coloration–decoloration cycle, the emission intensity of 10 was degraded by only 10%, as illustrated in Fig. 1C.

Spectral response of Zn$^{2+}$

As shown in Fig. 2, the fluorescence selectivity of sensor 10 toward 18 different various metal ions (Zn$^{2+}$, Al$^{3+}$, Ca$^{2+}$, Sn$^{2+}$, K$^+$, Mg$^{2+}$, Co$^{2+}$, Ba$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Sr$^{2+}$, Hg$^{2+}$, Cd$^{2+}$, Pb$^{2+}$, Fe$^{3+}$, Cr$^{3+}$, Mn$^{2+}$, Na$^+$, Zn$^{2+}$, Ca$^{2+}$, K$^+$),...
Pb²⁺, Mg²⁺, Cr³⁺, Cd²⁺, Hg²⁺, Mn²⁺, Fe³⁺, Sr²⁺, Ag⁺, Ba²⁺, Ni²⁺, Co²⁺ and Cu²⁺) was studied in CH₃CN (2.0 × 10⁻⁵ mol L⁻¹). As illustrated in Fig. 2A, 1O exhibited a weak fluorescence emission at 595 nm. Upon addition 1 equiv. of Zn²⁺, the fluorescence emission peak of 1O showed a dramatic enhancement by 10 times and blue-shifted to shorter wavelength region at 528 nm due to the complex 1O–Zn²⁺ (1O⁻Zn²⁺) form (Fig. 2A and B). The apparent fluorescence emission color varied from dark yellow to bright green (Fig. 2C). However, upon individual addition of other metal ions, they showed no obvious fluorescence variations except Al³⁺. Although a weak fluorescence response of 1O toward Al³⁺ was also observed, the emission intensity was increased less than 3 folds and the dark green fluorescence color of 1O–Al³⁺ (540 nm) was distinguishable from 1O–Zn²⁺. Moreover, it is noteworthy that there was no interference of Cd²⁺ (Fig. 2B), indicating high selectivity of Zn²⁺. These data demonstrated the high selectivity of sensor 1O toward Zn²⁺.

The absorption spectra response of sensor 1O induced by Zn²⁺ and ultraviolet/visible lights in CH₃CN was also investigated (Fig. 3). As shown in Fig. 3A, the absorption peak of 1O at 299 nm decreased when increasing concentrations of Zn²⁺, and a new absorption peak at 412 nm gradually increased due to the formation of complex 1O⁻ (Fig. 3A). With the addition of Zn²⁺, the solution color varied from colorless to light yellow. As shown in Fig. 3B, a dramatic absorption enhancement at 412 nm was found upon gradual addition of Zn²⁺ (0 to 9 equiv.) to 1O solution. And the absorption intensity reached a plateau when 2 equiv. of Zn²⁺ was added. Upon ultraviolet light irradiation (λ = 297 nm), a new absorption peak of complex 1O⁻ at 570 nm (ε = 2.33 × 10⁴ mol⁻¹ L cm⁻¹) appeared and increased due to the formation of the closed-ring isomer 1C⁻ (1C⁻–Zn²⁺ complex). At the same time, a visual color varied from light yellow to dark purple was observed (Fig. 3C). Upon the addition of Zn²⁺, the absorption intensity of 1C at 555 nm enhanced gradually with a moderate red shift (15 nm), indicating complex 1C⁻ formation (Fig. 3D). However, as shown in Fig. 3A and D, on addition excess EDTA to 1O⁻ and 1C⁻ solution, the absorption spectra of 1O and 1C could not be restored, respectively. The result elucidated that two isomers of 1 had large affinity for Zn²⁺ and readily competes with EDTA for Zn²⁺ resulting in the irreversible response of 1O and 1C to Zn²⁺.

In general, in order to detect metal ions, chemosensors bind with the target metal ions through non-covalent interactions to yield stable coordination complexes. The Zn²⁺ ion should have a stronger binding
energy to the sensor 1 than EDTA. The stronger affinity of sensor 1 to Zn^{2+} can not be reversed by EDTA.

To further elucidate the sensing behavior of 1O for Zn^{2+} detection, we carried out the fluorescence titration test with Zn^{2+} (Fig. 4). When increasing concentrations of Zn^{2+} (0 to 1.5 equiv.), the fluorescence intensity of 1O at 595 nm increased gradually accompanied by an obvious red-shift of 67 nm (Fig. 4A). And the fluorescence intensity reached a plateau until the concentration of Zn^{2+} reached 1 equiv. The fluorescence enhancement displayed good linearity in the Zn^{2+} concentration range 0–12 μM (Fig. 4B). Upon ultraviolet light irradiation (λ = 297 nm), the fluorescent intensity of complex 1O' nm was gradually quenched to ca. 18.0% in the photostationary state due to the closed-ring isomer 1C generated, and a concomitant color varied from bright green to dark green (Fig. 4C). As shown in Fig. S7,† the fluorometric titration of 1C by Zn^{2+} was also tested. Sensor 1C displayed emission peak at 595 nm, a blue-shift by 77 nm and increased fluorescence intensity (~10 folds) were observed with increasing concentrations of Zn^{2+}. At the same time, the fluorescent color varied from dark to dark green.

Job's plot analysis was used to estimate the binding parameters of 1O with Zn^{2+}. When the mole ratio of ([1O])/([1O] + [Zn^{2+}]) appeared at 0.5, the maximum emission intensity of 1O' at 528 nm was observed, indicating a 1 : 1 complexation stoichiometry of 1O with Zn^{2+} (Fig. 5A). The association constant (K_a) of the 1O-Zn^{2+} complex was measured as 1.72 × 10^{5} L mol^{-1} using Benesi–Hildebrand analysis (Fig. 5B). Additionally, the fluorescence sensor 1O provided a low Zn^{2+}
fluorescence detection limit of 8.48 \times 10^{-8} \text{ mol L}^{-1} \) (Fig. 5C), which was much lower than the maximum contaminant level (76 \mu\text{mol L}^{-1}) for Zn^{2+} in drinking water set by the WHO. To further confirm the preferential selectivity of the fluorescence sensor 1O for Zn^{2+} detection, we investigated the fluorescence responses of sensor 1O to Zn^{2+} in the presence of various competing metal ions (Fig. S8†). In the presence of Al^{3+}, Ca^{2+}, Sn^{2+}, K^{+}, Mg^{2+}, Cr^{3+}, Pb^{2+}, Hg^{2+}, Mn^{2+}, Fe^{3+}, Ag^{+}, Ba^{2+}, Cd^{2+}, and Sr^{2+}, there was small or no interference for Zn^{2+} detection, while Co^{2+}, Cu^{2+} and Ni^{2+} partially inhibited the emission intensity of the 1O-Zn^{2+} complex. These experiments indicated that 1O had high selectivity for Zn^{2+} detection as a fluorescence chemosensor.

The 1H NMR analysis was initiated to demonstrate the binding interaction between sensor 1O and Zn^{2+} in CD_{3}CN (Fig. 6). On addition of Zn^{2+}, the resonance signal of the \(-\text{OH}\) proton (H_a, 12.04) completely disappeared ultimately, suggesting that the O atom of \(-\text{OH}\) might coordinate to Zn^{2+}. Besides, the \(-\text{NH}\) (H_b, 9.48) signal of 1O showed a significant downfield shift and declined gradually, which corresponded to the protonation of \(-\text{NH}\), suggesting the N–Zn^{2+} coordinate bond was formed. Meantime, the signal of hydrogen (H_c) on the pyrimidine shifted from 6.70 ppm to 6.72 ppm, suggesting that the N atom of the pyrimidine unit might coordinate to Zn^{2+}. These results indicated that the O atom on \(-\text{OH}\) and the N atoms on \(-\text{NH}\)– and the pyrimidine unit were the most probably binding sites. To further confirm the formation of the 1O-Zn^{2+} complex, the ESI mass spectral analysis was also performed. As shown in Fig. S9†, the major peak at m/z 685.0577 was assigned to [1O + Zn^{2+} + H^+] (calcd 685.0492), advocating a 1:1 bonding mode of 1O with Zn^{2+}.

**Application in logic circuit**

The multi-responsive behaviors of sensor 1O have been investigated under stimuli by Zn^{2+}/EDTA and ultraviolet/visible lights.
Table 1  Truth table for all possible strings of four binary-input data and the corresponding output digit\(^a\)

<table>
<thead>
<tr>
<th>Inputs</th>
<th>Output (\lambda\text{em} = 528\text{ nm})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{In2 (vis)})</td>
<td>0</td>
</tr>
<tr>
<td>(\text{In3 (Zn}^{2+}\text{)})</td>
<td>1</td>
</tr>
<tr>
<td>(\text{In4 (EDTA)})</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) When the emission intensity at 528 nm more than 5-folds of the original state, the output signal is defined as ‘1’, otherwise defined as ‘0’.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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Notes and references


Conclusions

In this work, a new type of multi-responsive fluorescence sensor based on a diarylethene derivative with a 4,6-dimethylpyrimidine unit was developed. The sensor was highly selective and sensitive toward Zn\(^{2+}\) with a lower limit of detection \((8.48 \times 10^{-8} \text{ mol L}^{-1})\). Furthermore, an integrated circuit with multiple control switches was successfully constructed based on its multiple stimuli-responsive fluorescence switching behavior. All results provided a useful strategy for the construction of new fluorescent chemosensors based on diarylenes for the recognition of specific metal ions in the future.