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Aqueous Fluorescence Turn-on Sensor for Zn^{2+} with a Tetraphenylethylene Compound

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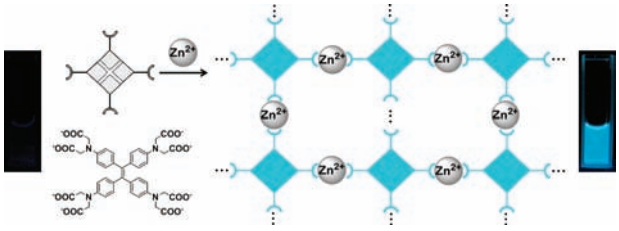
ABSTRACT



A new sensitive and selective fluorescence turn-on sensor for Zn^{2+} (1) was developed by taking advantage of the aggregation-induced emission of the tetraphenylethylene framework. In addition, the corresponding ester precursor of 1 was successfully used for intracellular Zn^{2+} imaging.

It is known that the zinc ion (Zn^{2+}) is the second most abundant transition metal ion in the human body after iron. Many studies reveal that Zn^{2+} is involved in a number of biological processes, such as the brain function and pathology, gene transcription, immune function, and mammalian reproduction.[1] Zn^{2+} is believed to be an essential factor in some pathological processes, including Alzheimer's disease, epilepsy, and ischemic stroke.[2] It is also recognized to be one of the most important cations as catalytic cofactors and structural centers of Zn^{2+}-containing enzymes and DNA-binding proteins.[3] In addition, free zinc pools exist in some tissues, such as the brain, intestines, pancreas, and retina.[4] However, in comparison with other cations such as Na^+, K^+, and Ca^{2+}, the biological functional mechanisms of Zn^{2+} are not completely understood.[5] Therefore, sensitive and selective chemosensors for Zn^{2+} are highly desirable to monitor Zn^{2+} in biological systems.[6,7]

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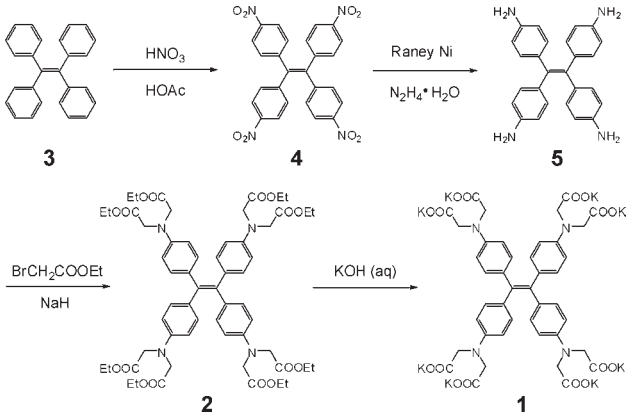
Up to now, a variety of Zn^{2+}-selective fluorescent sensors have been reported. Most of them are based on photoinduced electron transfer (PET),[8] internal charge transfer (ICT),[9] and fluorescence resonance energy transfer (FRET),[10] respectively. Nevertheless, most of these chemosensors for Zn^{2+} have some limitations. For instance, some exhibit low water-solubility, and thus, organic solvents are needed for Zn^{2+} detection;[11] interferences from other metal ions still exist, especially from Cd^{2+}.[8d,12]

Herein we report a new fluorescence turn-on sensor with compound **1** for Zn^{2+} by taking advantage of the aggregation-induced emission (AIE) feature of tetraphenylethylene compounds (Scheme 1).[13,14] As will be discussed below, the sensing mechanism of **1** toward Zn^{2+} is attributed to two facts: (1) the coordination between Zn^{2+} and $-N(CH_2COO^-)_2$ groups in **1** should inhibit the intramolecular PET; (2) the intermolecular coordination of **1** with Zn^{2+} will lead to aggregation and fluorescence enhancement. Additionally, compound **2**, the ester precursor of **1**, is potentially applicable for intracellular Zn^{2+} imaging.

The syntheis of **1** started from tetraphenylethylene **3** as shown in Scheme 1. In the presence of HNO_3 and HOAc, **3** was nitrated into tetra-*p*-nitrophenylethylene **4**, which was reduced to tetra-*p*-aminophenylethylene **5** with Raney Ni and $N_2H_4 \cdot H_2O$.[15] Compound **5** was allowed to react with excess ethyl bromoacetate in the presence of NaH, leading to **2** in 38% yield. Hydrolysis of **2** with KOH afforded **1** in 90% yield. The synthetic details and characterization data are provided in the Supporting Information.

Scheme 1. Chemical Structure of **1** and the Synthetic Approach



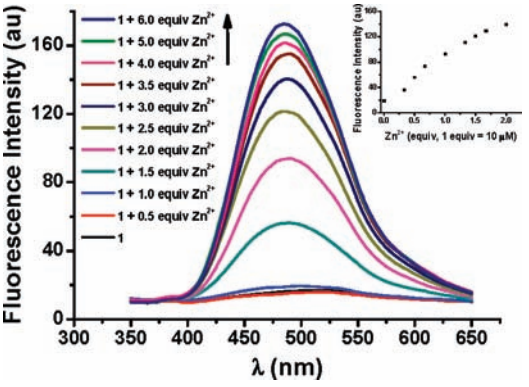


Figure 1. Fluorescence spectra (λ_{ex} = 340 nm) of **1** (10 μM) upon addition of 0−6.0 equiv of Zn^{2+} [$ZnSO_4$] in HEPES buffer (50 mM, pH = 7.4, 0.1 M NaCl); inset shows the linear increase of the fluorescence intensity at 485 nm for the ensemble of **1** and 1.0 equiv of Zn^{2+} upon further addition of Zn^{2+}.

Compound **1** can be well dissolved in aqueous solution, and the HEPES buffer solution (pH = 7.4, HEPES = *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) of **1** was prepared for the absoprtion and fluorescence spectral studies. To maintain the ionic strength, NaCl (0.1 M) was added to each solution. As depicted in Figure 1, the solution of **1** was almost nonemissive in the absence of Zn^{2+} [$ZnSO_4$] as expected.[13,14] However, the fluorescence started to increase gradually after addition of Zn^{2+}. Figure S1a (Supporting Information) shows the variation of the fluorescence intensity at 485 nm of **1** vs the amount of Zn^{2+} added to the solution. Clearly, the fluorescence enhancement was rather minor when less than 1.0 equiv of Zn^{2+} was present, but it became much more obvious when more than 1.0 equiv of Zn^{2+} was added. Compound **1** can be regarded as a new *off-on* fluorescent sensor for Zn^{2+}. In fact, the fluorescence intensity at 485 nm for the ensemble

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of **1** and 1.0 equiv of Zn^{2+} increased almost linearly with the amount of Zn^{2+} further added to the ensemble as depicted in the inset of Figure 1 and Figure S1b (Supporting Information).

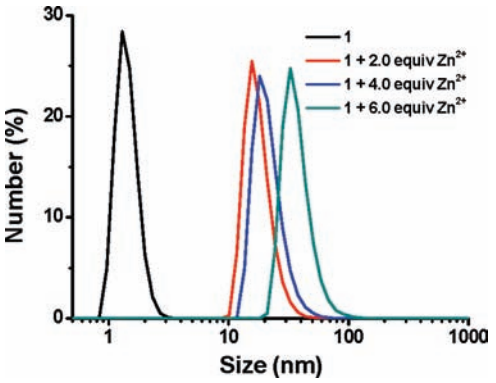
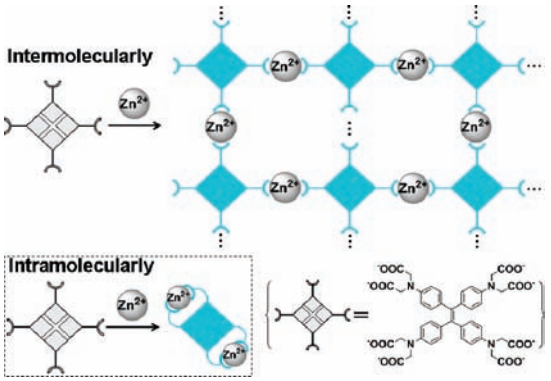


Figure 2. DLS profiles (from left to right) of **1** (50 μM in aqueous solution) and in the presence of 2.0 equiv of Zn^{2+}, 4.0 equiv of Zn^{2+}, and 6.0 equiv of Zn^{2+}, respectively.

The absorption spectra of **1** were also recorded upon addition of Zn^{2+} (see Figure S2, Supporting Information). The absorptions around 293 and 370 nm decreased gradually in the presence of increasing amounts of Zn^{2+}, and a long absorption tail above 450 nm emerged simultaneously. These spectral changes should be attributed to the coordination of Zn^{2+} with $-N(CH_2COO^-)_2$ groups. Such coordination could inhibit the PET within **1**, but this alone still cannot turn on the fluorescence of **1**. Two possible coordination modes of **1** with Zn^{2+} may exist (Scheme 2): (1) intramolecular coordination of two $-N(CH_2COO^-)_2$ groups in **1** with Zn^{2+}, which can restrict the molecular rotation and as a result the fluorescence of **1** can be enhanced;[13] (2) intermolecular coordination of the $-N(CH_2COO^-)_2$ groups (of neighboring molecules of **1**) with Zn^{2+}, which may lead to coordination oligomers and even polymers. These coordination oligomers and polymers may exhibit lower solubility in aqueous solution and accordingly the fluorescence from the tetraphenylethylene core in **1** will emerge. The intramolecular coordination may be effective when the concentration of Zn^{2+} is low in the solution. However, the intermolecular coordination may become dominant at high concentration of Zn^{2+} as indicated by the dynamic light scattering (DLS) results. As depicted in Figure 2, the initial DLS signal corresponds to species of 1.3 nm approximately, being in line with the molecular size of **1**. This also indicates that **1** can be well dissolved and dispersed in aqueous solution. However, aggregates of about 16 nm were observed in the presence of 2.0 equiv of Zn^{2+}. Moreover, the aggregates became larger when more Zn^{2+} were added. Such aggregates should be owing to the aggregation of the coordination oligomers and polymers. In fact, the formation of aggregates is consistent with the observation of long absorption tail above 450 nm (see Figure S2, Supporting Information).

Scheme 2. Schematic Illustration of the Coordination Modes between **1** and Zn^{2+}



The fluorescence response of **1** toward Zn^{2+} was also investigated in solutions of different pH values. In the absence of Zn^{2+}, the fluorescence of **1** was rather weak in either acidic or basic solutions (see Figure S3, Supporting Information). In acidic solutions, the intramolecular photoinduced electron transfer within **1** can be inhibited. But, as reported previously,[13,14] tetraphenylethylene compounds are nonemissive in solutions. Figure S4 (Supporting Information) displays the fluorescence spectra of **1** and 4.0 equiv of Zn^{2+} in solutions at variable pH values. Obviously, the fluorescence enhancement of **1** after addition of Zn^{2+} was dependent on the pH values of the solutions. The fluorescence of **1** increased more significantly in solutions of pH $\cong$ 7.0, and it became much weaker in either acidic or basic solutions. This could be interpreted: on one hand, in acidic solutions, the $-N(CH_2COO^-)_2$ groups are protonated and thus the coordination ability will be weakened; in basic solutions, on the other hand, Zn^{2+} can be bound with OH^-.

The selectivity of **1** toward Zn^{2+} was examined by recording the fluorescence spectra of **1** in the presence of competing metal ions. As shown in Figure S5(Supporting Information), the fluorescence enhancement was not observed for **1** after addition of 1000 equiv of K^+, Mg^{2+} or Ca^{2+} (these ions exist in high concentrations in biological systems), 100 equiv of Cs^+ or Ba^{2+}, or 4.0 equiv of transition or heavy metal ions (including Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Hg^{2+}). Even in the presence of Cd^{2+}, only slight fluorescence enhancement was detected. Moreover, further addition of Zn^{2+} to the solution of **1** which contains one of these ions led to similar fluorescence enhancement (Figure 3), except for Co^{2+}, Ni^{2+}, and Cu^{2+}, which are known as fluorescence quenchers. Since Co^{2+}, Ni^{2+}, or Cu^{2+} are in rather low concentrations in vivo,[16] the interferences of these ions for the detection of Zn^{2+} with **1** can be neglected. These results manifest **1** exhibits good selectivity toward Zn^{2+} over most competing metal ions.

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Additionally, the fluorescence spectra of **1** in the presence of Zn^{2+} with various counter-anions (SO_4^{2-}, NO_3^-, Cl^-, Br^-, ClO_4^-, and CH_3COO^-) were measured (see Figure S6, Supporting Information). The results reveal the influence of counter-anions on the detection of Zn^{2+} with **1** is rather minor. Therefore, it can be concluded **1** can be practically useful for the fluorescence turn-on detection of Zn^{2+} in aqueous solution.

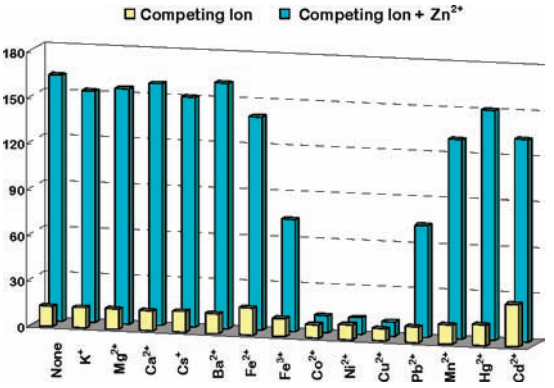

Figure 3. Variation of the fluorescence intensity at 485 nm ($\lambda_{ex.}$ = 340 nm) of **1** (10 μM) in HEPES buffer (50 mM, pH = 7.4, 0.1 M NaCl) after addition of various metal cations: 1000 equiv of K^+, Mg^{2+}, and Ca^{2+}, 100 equiv of Cs^+ and Ba^{2+}, and 4.0 equiv of Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Hg^{2+}, and Cd^{2+} (yellow bars) and those after further addition of 4.0 equiv of Zn^{2+} (cyan bars).

Finally, the application of compound **2** (see Scheme 1), the ester precursor of **1**, in intracellular Zn^{2+} imaging is demonstrated. Compound **2** is more hydrophobic than **1** and thus it possesses better cell membrane permeability. Besides, it can be hydrolyzed into **1** in intracellular environment.[17] Figure 4 shows the cofocal fluorescence images of NIH 3T3 cells (mouse embryonic fibroblast cell line) after incubation with **2**/Zn^{2+}. Faint intracellular fluorescence was observed after incubation only with **2**, indicating that molecules of **2** were penetrated into the cells.[18] However, intensive fluorescence was detected when the cells were incubated with $ZnSO_4$ and pyrithione. Furthermore, the intensive fiuorescence was greatly depressed after treatment of the cells with membrane permeable metal ion chelator, *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN). These results manifest that **2** is potentially applicable for intracellular Zn^{2+} imaging.

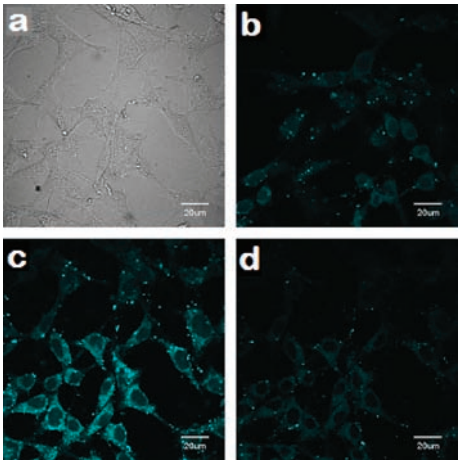

Figure 4. Cofocal fluorescence images of NIH 3T3 cells in PBS buffer (λ_{ex} = 375 nm): (a) bright-field transmission image of cells after incubation with **2** (10 μM) at 37.0 °C for 30 min; (b) the corresponding fluorescence image of (a); (c) fluorescence image after incubation with $ZnSO_4$ (50 μM) and pyrithione (20 μM, Zn^{2+} ionophore) for 15 min; (d) fluorescence image after further incubation with TPEN (50 μM) for 8 min; the scale bar is 20 μm.

In summary, a new fluorescent sensor for Zn^{2+} has been developed by taking advantage of the AIE feature of tetraphenylethylene compounds. This sensor for Zn^{2+} is unique in terms of the following points: (1) the sensing mechanism is different from those reported previously;[8−12] (2) the detection of Zn^{2+} with **1** can be carried out in aqueous solution without any organic solvents; and (3) compound **1** exhibits good selectivity toward Zn^{2+}. Moreover, compound **2**, the ester precursor of **1**, is successfully demonstrated for intracellular Zn^{2+} imaging.

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Supporting Information Available. Synthesis and characterization of **1** and **2** and supplementary absorption and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Intramolecular PET exists within **2** in the absence of Zn^{2+}, and it is understandable that only weak fluorescence was observed.