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# Red-Emission Fluorescent Probe Sensing Cadmium and Pyrophosphate Selectively in Aqueous Solution

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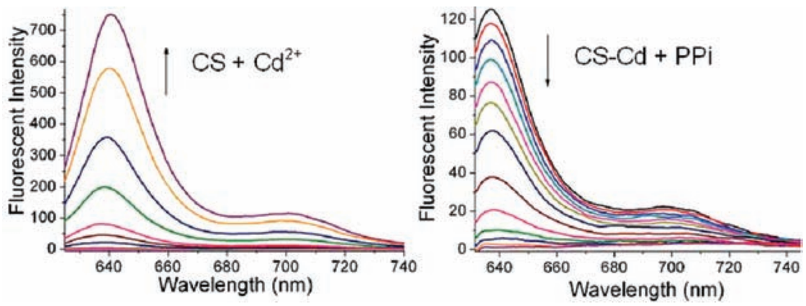
## ABSTRACT




**A fluorescent sensor for cadmium (CS) based on the BODIPY fluorophore exploiting the PET (Photoinduced Electron Transfer) mechanism was prepared. CS exhibited high selectivity and sensitivity for detecting cadmium in aqueous buffer solution. In addition, the complex of CS with cadmium could detect pyrophosphate (PPi) selectively and sensitively.**

Heavy metal contamination has become an increasingly serious threat to human health and ecology.[1] Cadmium is an important heavy metal and widely used in industry and agriculture including the production of various metal alloys, batteries, and also phosphate fertilizers.[2] Cadmium is a very toxic element and easily absorbed and accumulated by plants and other organisms.[3] It causes many serious diseases such as lung, prostatic, and renal cancers, even at very low concentrations.[4]

Fluorescence based techniques are robust yet convenient methods for sensing a wide range of analytes[5] including cations, anions, and neutral molecules. The photoinduced electron transfer (PET) mechanism is a practical control over the signaling of a fluorophore and has yielded numerous sensitive OFF-ON type probes.[6]

Due to the toxicity of cadmium, it drew the close attention of scientists. Many fluorescent sensors for cadmium have been reported in past years. However, only a few probes[7] displayed high selectivity to cadmium over zinc due to their similarity in physical and chemical properties.

Pyrophosphates (PPi) play very important roles in biological processes. For example, the products of ATP hydrolysis are AMP and PPi.[8] The level of PPi is related to various diseases, such as arthritis and Mönckeberg's

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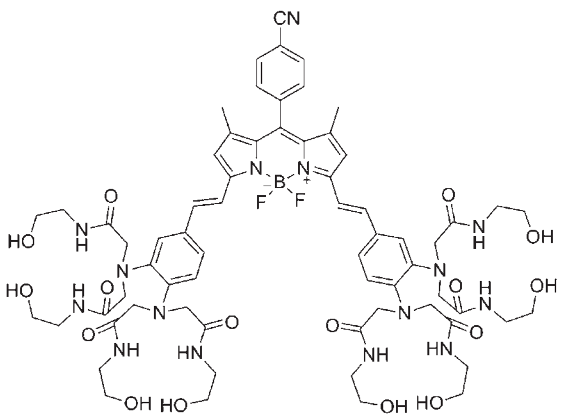
arteriosclerosis (MA).[9] Therefore, PPi sensing has received attention in recent years, and many PPi fluorescent probes have been reported. Many receptors for PPi were found, and most of them are based on metal cations.[10] Only a few probes had good selectivity to distinguish PPi from analogues such as ATP and AMP.[10c,e,l,m,11]

In this work, a long-wavelength and water-soluble cadmium sensor (CS, Figure 1) was designed and synthesized (Supporting Information). The sensor is based on the BODIPY fluorophore, which has excellent spectral properties, such as sharp absorption and emission bands, high stability against photobleaching, and high molar absorptivity and fluorescence quantum yield. To attain NIR and sensitive sensors, a water dissoluble polyamide receptor which selectively binds $Cd^{2+}$ was chosen and two of them were conjugated to BODIPY. CS showed good selectivity for detecting cadmium in a buffer solution, and the complex of CS with $Cd^{2+}$ provided excellent selectivity toward PPi in water.



**CS**

**Figure 1.** Structure of fluorescent sensor of CS.

The effect of pH on the fluorescence properties of probe CS was determined first. As shown in Figure S1, the spectra of absorption (Figure S1a) and emission (Figure S1b) remained stable with the pH above 2. This indicates that CS could be used for sensing $Cd^{2+}$ in a wide range of pH. We chose a neutral Tris-HCl (0.02 M) solution (10% DMSO, containing 0.1 mM sodium phosphate, pH 7.5) as a testing system.

Cadmium titration was conducted by addition of an aliquot of $Cd^{2+}$ stock solutions to the aforementioned buffer solution containing 5 $\mu$M of CS. As shown in Figure 2, free CS had an absorption maximum at 665 nm with a shoulder peak at 614 nm. And it showed very weak fluorescence when it was excited at 620 nm, because of the efficient PET quenching from two diaminobenzene moieties to the BODIPY fluorophore. Upon addition of $Cd^{2+}$, two blue-shifted peaks appeared around 627 and 580 nm and the peak at 665 nm diminished gradually. The blue shift indicated the coordination of four anilinal nitrogen atoms to $Cd^{2+}$ and hence the intermolecular charge transfer (ICT) process of the sensor was affected. Two isoabsorptic points at 654 and 640 nm were shown. This may be attributed to the successive association of $Cd^{2+}$ ions to the two binding cavities presented on the probe. The appearance of two peaks was presumably caused by fluorophore aggregation in aqueous media.[12] With the addition of $Cd^{2+}$, the fluorescence intensity at 638 nm increased significantly. And the quantum yield increased from 0 (< 0.001) to 0.3. A Job plot indicated that CS chelated a $Cd^{2+}$ ion with 1:4 stoichiometry (Figure S2).

As shown in Figure S4, CS showed no fluorescence in the buffer solution. Only $Pb^{2+}$, $Ag^{+}$, $Zn^{2+}$, and $Mn^{2+}$ caused minimal fluorescence intensity increase with representative 5 equiv metal ion ($Ni^{2+}$, $Pb^{2+}$, $Ca^{2+}$, $Li^{+}$, $Ba^{2+}$, $Ag^{+}$, $Hg^{2+}$, $Cr^{3+}$, $Co^{2+}$, $Zn^{2+}$, $K^{+}$, $Cs^{+}$, $Na^{+}$, $Cu^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Fe^{3+}$) addition. However, when 5 equiv of $Cd^{2+}$ were added into the CS solution, the fluorescence intensity enhanced significantly under the same conditions. This indicated that CS had good selectivity in the buffer solution. Competition experiments were also conducted. When 3 equiv of $Cd^{2+}$ were added into the solution of CS

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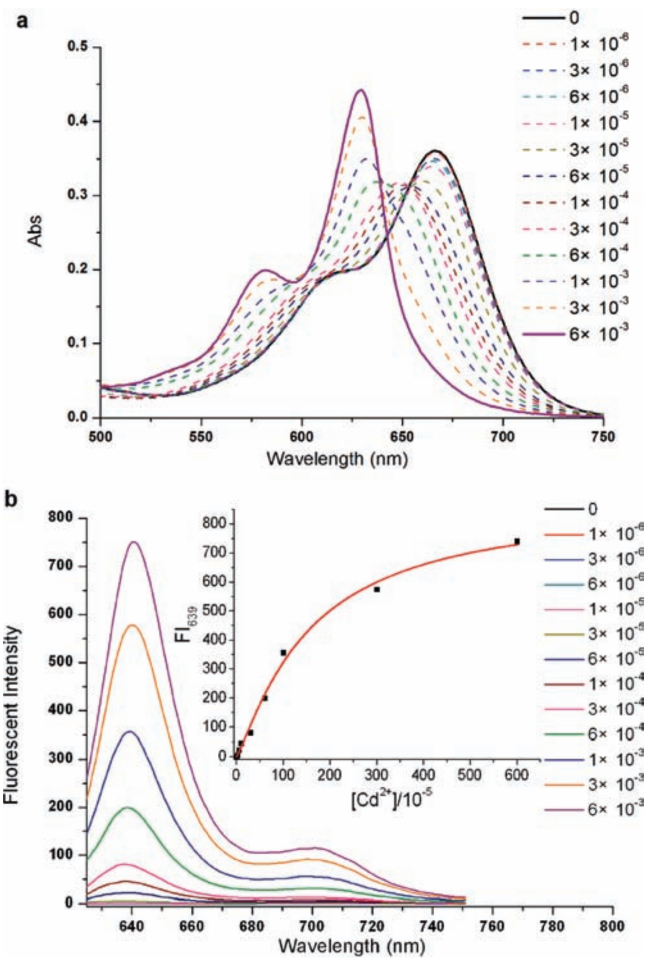


**Figure 2.** Absorption (a) and emission (b) spectra of CS in Tris-HCl (20 mM) solution (10% DMSO, pH = 7.5, containing 0.1 mM sodium phosphate) in the presence of increasing concentration of $Cd^{2+}$. The concentration of CS was 5 $\mu$M. The samples were excited at 620 nm with excitation and emission slit widths set at 5 and 2.5 nm respectively. Inset: The curve of fluorescence intensity at 639 nm versus increasing $Cd^{2+}$ concentration in Tris-HCl (20 mM) solution (10% DMSO, pH = 7.5, containing 0.1 mM sodium phosphate) (Figure S3).

in presence of 5 equiv of other metals, the presence of most other metal ions except $Hg^{2+}$ had not induced any noticeable spectral change compared to $Cd^{2+}$ alone. The fluorescence intensity with $Hg^{2+}$ presence was much stronger than that with $Cd^{2+}$.

Because $Hg^{2+}$ caused additional enhancement of the fluorescence intensity, we investigated the change of emission with both $Cd^{2+}$ and $Hg^{2+}$ present. The time course of the fluorescence of CS (5 $\mu$M) in the presence of $Hg^{2+}$, $Cd^{2+}$, and $Hg^{2+}$ and $Cd^{2+}$ is shown in Figure 3. A very slight change of fluorescence intensity was observed in the presence of 10 equiv of $Hg^{2+}$ in 10 min, and the fluorescence intensity at 639 nm did not change after 20 s in the presence of 10 equiv of $Cd^{2+}$. However, the fluorescence intensity greatly enhances within 10 min in the presence of 10 equiv of both $Hg^{2+}$ and $Cd^{2+}$ compared to that

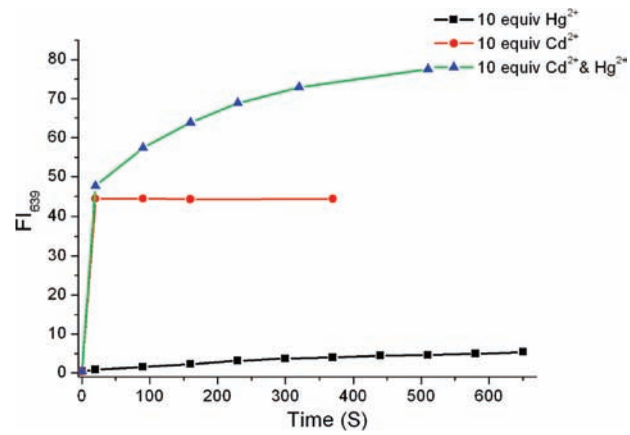


**Figure 3.** Time course of the response of CS to $Hg^{2+}$ and $Cd^{2+}$. The increasing of fluorescent intensity at 639 nm was monitored at time intervals after $Hg^{2+}$ or $Cd^{2+}$ addition. Experiments were conducted in Tris-HCl (0.02 M) solution (10% DMSO, containing 0.1 mM sodium phosphate, pH 7.5). The concentration of CS was 5 $\mu$M. Slit widths were 5 nm.

with $Cd^{2+}$ only. This may be because the structure of the receptor changed somewhat in the presence of $Hg^{2+}$, and that change was good for $Cd^{2+}$ binding. Thus we could confirm if there was $Hg^{2+}$ present with the time element taken into consideration.

The complex of CS with $Cd^{2+}$ (CS-Cd) was chosen as a fluorescent probe for PPi. Free probe of CS-Cd showed strong fluorescence in water with 0.5% $CH_3CN$ (v/v). As shown in Figure S5, the fluorescence intensity of CS-Cd remained stable when the pH was in the range of 5.5 to 8.5. The fluorescence intensity of CS-Cd increased gradually when the pH went below 5.5. This was likely because CS-Cd was protonated, and the PET process was blocked. On the other hand, when the solution was very alkaline, the fluorescence intensity of CS-Cd decreased. This may be because excess hydroxyl bound with $Cd^{2+}$ and the PET process recovered.

Experiments focused toward sensing PPi were conducted in water with 0.5% $CH_3CN$ (v/v). As shown in Figure 4, the absorption wavelength shifted to red with PPi added into the solution. However, the absorption spectra did not recover to the original intensity of CS, which may be because PPi did not lead to the dissociation of CS-Cd. Meanwhile, the fluorescence intensity around 637 nm decreased obviously and stabilized gradually after 60 $\mu$M PPi addition.

Then the selectivity of CS-Cd was determined, as shown in Figure 5. Nearly no fluorescence changes were observed with the addition of other anions (such as (2) AMP, (3) ADP, (4) ATP, (5) $HCO_3^-$, (6) $F^-$, (7) $I^-$, (8) $Br^-$, (9) $SO_4^{2-}$, (10) $CF_3SO_3^-$, (11) $NO_3^-$, (12) $AcO^-$, (13) $H_2PO_4^-$, (14) $HSO_4^-$, (15) $Cl^-$) added into the solution. Therefore, CS-Cd could distinguish ADP and ATP from PPi.

In conclusion, a red emitting fluorescent probe (CS) for cadmium based on the BODIPY fluorophore was designed

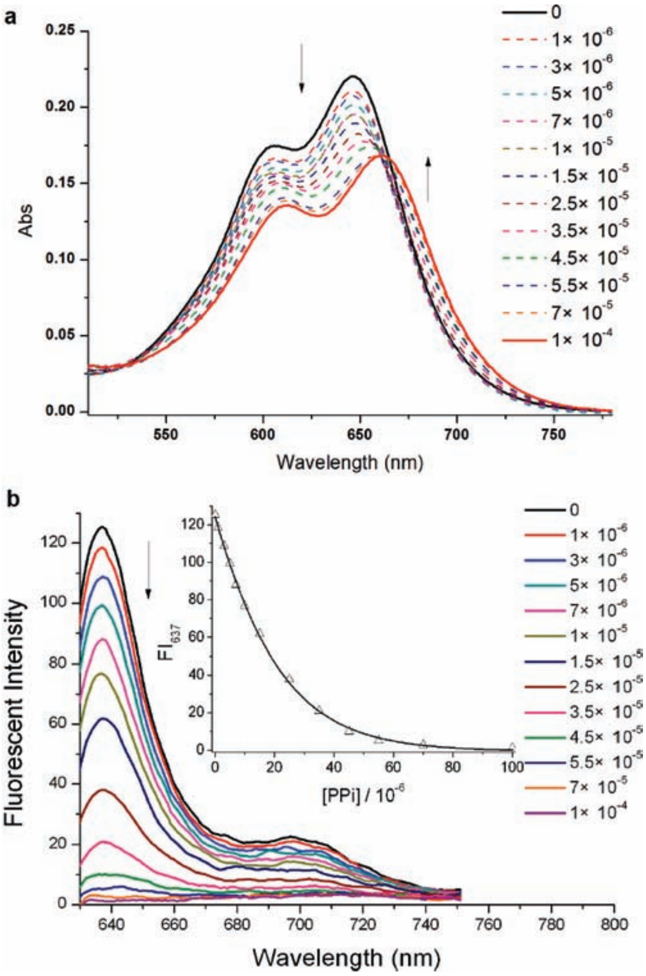


**Figure 4.** Absorption (a) and emission (b) spectra of CS-$Cd^{2+}$ in water (0.5% $CH_3CN$) in the presence of increasing concentration of PPi (0−100 $\mu$M). The concentration of CS-Cd was 10 $\mu$M. The samples were excited at 620 nm. Inset: Curve of fluorescent intensity at 637 nm versus increasing PPi concentration (Figure S6).

and synthesized and showed good selectivity toward $Cd^{2+}$. $Cd^{2+}$ induced a blue shift of the absorption and a dramatic enhancement of the emission intensity. The complex of CS with $Cd^{2+}$ (CS-Cd) could sense PPi selectively and

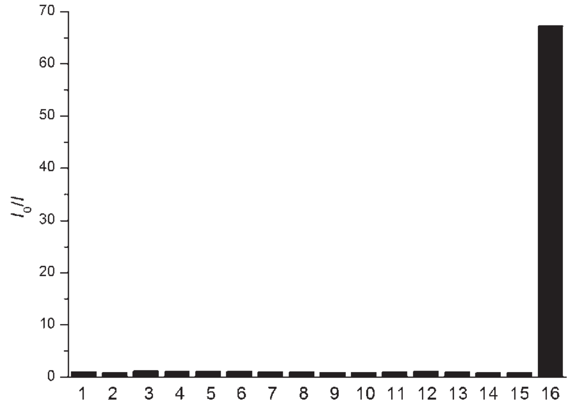


**Figure 5.** Fluorescence intensity change at 637 nm in water (0.5% $CH_3CN$) with 10 equiv of anions presence. The concentration of CS-Cd was 10 $\mu$M, excitation wavelength was 620 nm. (1) CS-Cd, (2) AMP, (3) ADP, (4) ATP, (5) $HCO_3^-$, (6) $F^-$, (7) $I^-$, (8) $Br^-$, (9) $SO_4^{2-}$, (10) $CF_3SO_3^-$, (11) $NO_3^-$, (12) $AcO^-$, (13) $H_2PO_4^-$, (14) $HSO_4^-$, (15) $Cl^-$, (16) PPi.

sensitively in water. Both ATP and ADP did not cause the spectra change of CS-Cd. When PPi was added into the solution of CS-Cd, a red shift of the absorption wavelength was observed and the fluorescence intensity around 637 nm diminished.

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**Supporting Information Available.** Synthesis, experimental details, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.