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Highly Selective and Sensitive Near-Infrared Fluorescent Sensors for Cadmium in Aqueous Solution

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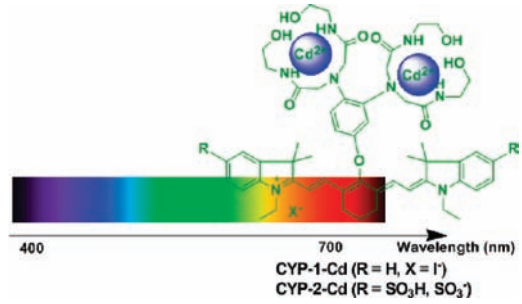
ABSTRACT



On the basis of tricabocyanine, two near-infrared fluorescent sensors CYP-1 and CYP-2 have been designed and synthesized. Both of them can selectively and sensitively recognize Cd^{2+} from other metal ions, especially the CYP-2, which can distinguish Cd^{2+} in neutral buffer solution.

Wide use of cadmium, such as in fertilizers and batteries,[1] makes it a severe environmental hazard but also a serious health threat to humans.[2] Either short-term or long-term human exposures to environmental cadmium may promote the chances of getting lung, prostate, breast, or endometrial cancer.[3] Therefore developing excellent methods to monitor cadmium both in the environment and in vivo settings is in urgent demand.

Fluorescent sensing technology displays paramount sensitivity and affords quick responses in a high spatial and temporal resolution, which makes it a most popular method to detect the analytes of interest. Recently, many fluorescent sensors for cadmium have been reported in the literature.[4] Most of them exhibited good selectivity against zinc and rendered soluble in aqueous conditions. But some limitations existed for in vivo detection of cadmium. Most of these sensors have to be excited by UV−vis light (<600 nm) which would cause damage to the living cells and does not penetrate biological tissues very well. Additionally, short wavelength excitation induces relatively strong autofluorescence, which limits the detection sensitivity.[5] For in vivo applications, it is desirable to have both the excitation and emission wavelengths of the probe in the range of 650−900 nm.[6] Compared with other short-wavelength fluorophores, tricarbocyanine dyes are superior due to its strong near-infrared (NIR) absorption and emission,[7] and are commonly utilized in designing NIR fluorescent probes and in vivo imaging.[8] To the best of our knowledge, there is no report of fluorescent

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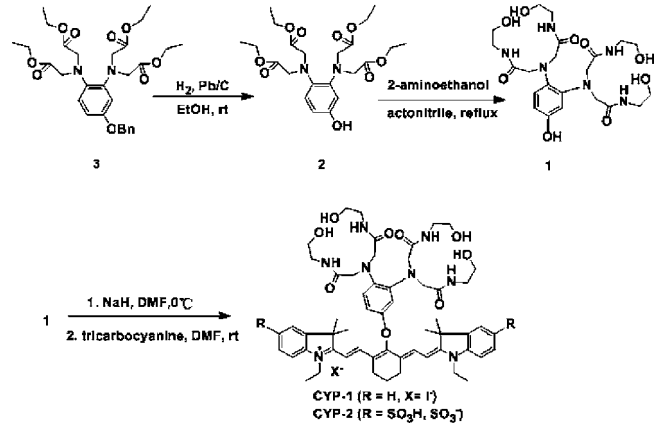
sensors for cadmium with the excitation and emission wavelengths beyond 650 nm.

For the metal ion fluorescent sensors, one of the most commonly employed signaling mechanisms is photoinduced electron transfer (PET).[9] These sensors usually consist of three moieties: an ion selective receptor and a fluorophore tethered by a covalent linker.[10] Herein, we report two novel NIR fluorescent sensors based on the PET mechanism for cadmium, **CYP-1** and **CYP-2**, which display high sensitivity and selectivity in aqueous solution and have the potential to be applied for in vivo cadmium imaging in biological systems.

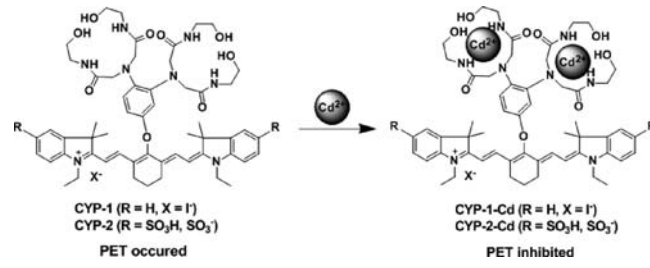
The sensors (**CYP-1** and **CYP-2**) were synthesized according to Scheme 1. The water-soluble tetraamide **1** has been shown to be an excellent receptor for Cd^{2+}.[4m] The **CYP-1** was designed as a cell-permeable probe and the **CYP-2** was functioalized with two sulfonate groups to increase the solubility and to reduce the self-aggregation in water.

Scheme 1. Synthesis of **CYP-1** and **CYP-2**



Nagano et al. reported an NIR fluorescent probe for NO, whose fluorescence was largely quenched by the electron-rich *o*-phenylenediamine moiety through PET.[9e] Our Cd^{2+} probes were expected to behave in a similar fashion. Upon addition of Cd^{2+}, metal−ligand coordination will inhibit the PET process and the NIR fluorescence of the tricabocyanine fluorophore was recovered (Scheme 2).

Scheme 2. The Predicted Coordination between Sensors and Cd^{2+}



We then examined the spectral properties of our sensors. We first evaluated the effect of pH on the fluorescence properties of **CYPs** (Figures S1 and S2, Supporting Information) and find that the **CYPs** are inert to pH in the range of 6.5−8.0. Therefore **CYPs** work well under physiological pH condition. So the spectroscopic properties of **CYP-2** were studied in neutral buffer solution (12.5 mM Tris-HCl solution

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containing 0.05 mM sodium phosphate, pH 7.2). The UV−vis and fluorescent spectra of the **CYP-2** (see Figure 1

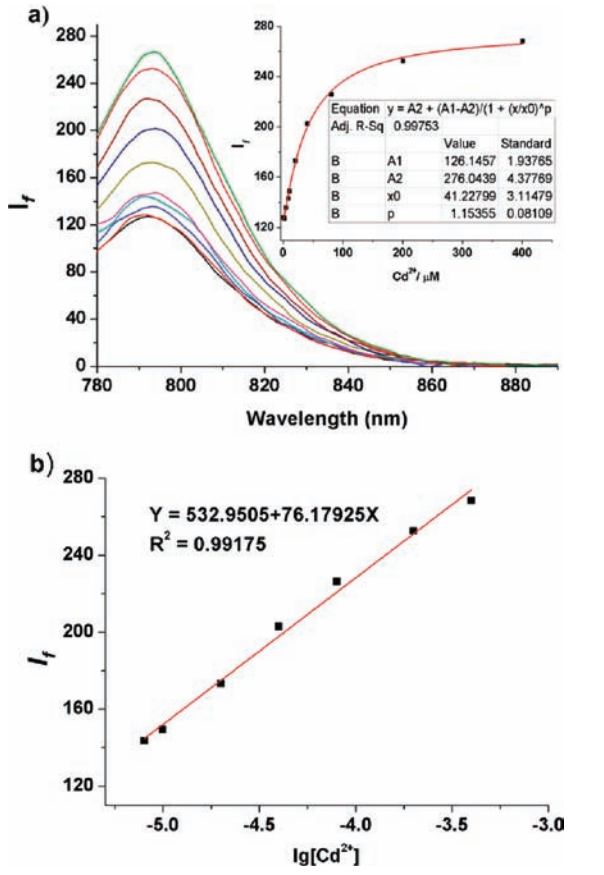

Figure 1. (a) Fluorescence emission spectra (λ_{ex} = 771 nm) of **CYP-2** (3 μM) in Tris-HCl (12.5 mM) solution (containing 0.05 mM sodium phosphate, pH 7.2) upon addition of Cd^{2+} (0 − 400 μM). Inset: Binding isotherm between **CYP-2** and Cd^{2+} with emission intensity at 793 nm. (b) Fluorescence intensity of **CYP-2** at 793 nm as a function of lg[Cd^{2+}] (8 − 400 μM) in the same condition of the Cd^{2+} titration.

and Table S1, Supporting Information) exhibited a maximum absorption at 771 nm and an emission at 793 nm.

The cadmium titration experiment was then carried out with **CYP-2**. As shown in Figure 1a, the fluorescent intensity increased as the Cd^{2+} concentration increased. There is a only minor spectra shift (<5 nm) that occurred, which indicated that the lone pair of two aniline nitrogen atoms becomes involved in Cd^{2+} coordination and the PET process from nitrogen atoms to tricarbocyanine was removed. The fluorescence quantum yield of **CYP-2** increased from 0.0065 to 0.0145 in this process (see Table S1, Supporting Information).[11] The enhancement of fluorescence intensity of **CYP-2** and the corresponding lg[Cd^{2+}] (8−400 μM) (Figure 1b) showed good linear relationship (R^2 = 0.99175). The association constants K_{11} and K_{21} were determined by a nonlinear least-squares analysis of fluorescence intensity versus Cd^{2+} ion concentration to be 8.8 × 10^3 and 1.9 × 10^5 (Table S2, Supporting Information). And at the same time, the **CYP-1** yielded similar results (Table S1 and Figures S3 and S4, Supporting Information), which could selectively recognize cadmium from other metal ions in Tris-HCl (12.5 mM) solution (acetonitrile/water = 9/1, v/v, containing 0.05 mM sodium phosphate, pH 7.2), with some interference of Zn^{2+} and Pb^{2+}. The fluorescence emission spectra were reversed by adding an excess of EDTA when the sensors were saturated with Cd^{2+}, indicating that the sensing action was reversible (Figure S5, Supporting Information). And at the same time the detection limit of **CYP**s was found to be 3.1 μM for **CYP-1** and 2.3 μM for **CYP-2** (Figure S6, Supporting Information).

The selectivity of **CYP-2** toward different metal ions was then examined. As shown in Figure 2a, there is no obvious

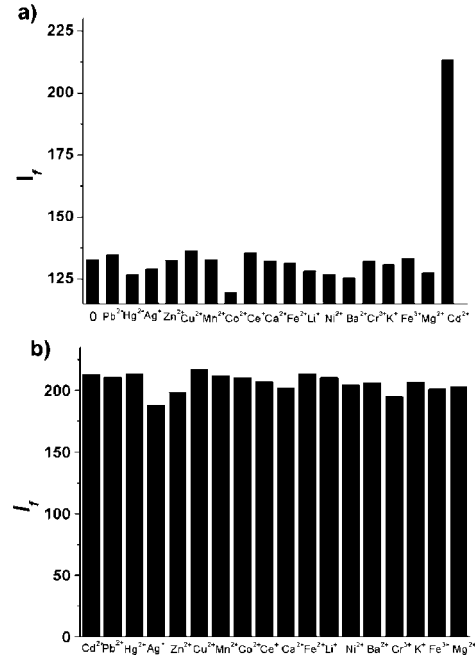

Figure 2. (a) Fluorescence intensity of **CYP-2** (3 μM) at 793 nm in the presence of different metal ions (40 μM) in Tris-HCl (12.5 mM) solution (containing 0.05 mM sodium phosphate, pH 7.2). (b) The fluorescence intensity of **CYP-2** at 793 nm with 40 μM M^{n+}, followed by 40 μM Cd^{2+}.

change of fluorescent emission with Pb^{2+}, Hg^{2+}, Ag^{+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Co^{2+}, Ca^{2+}, Fe^{3+}, Li^{+}, Ni^{2+}, Ba^{2+}, Cr^{3+}, K^{+}, Fe^{2+}, and Mg^{2+} while an obvious fluorescence enhancement was observed when adding the same number of equivalents of Cd^{2+} in the same condition, which demonstrated that **CYP-2** can distinguish Cd^{2+} from other metal ions in neutral buffer solution. The results of competition experiment were shown in Figure 2b. The fluorescence was increased when Cd^{2+} was added into the solution that already contained the different metal ions and **CYP-2** (3 μM). The stoichiometry of Cd^{2+}:**CYP-2** was determined by the Job's plot (Figure 3). An abrupt discontinuity indicated a 2:1 species.

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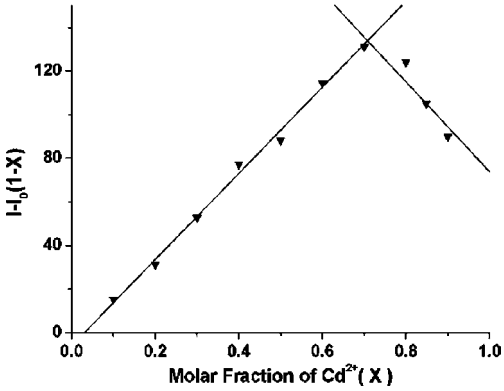

Figure 3. Job's plot showing the 1:2 binding of **CYP-2** with Cd^{2+}. The total concentration of the sensor and Cd^{2+} is 10 μM in Tris-HCl (12.5 mM) solution (containing 0.05 mM sodium phosphate, pH 7.2).

To further demonstrate the practical application of the probe, we carried out experiments in living cells. The HeLa cells was incubated with **CYP-1** (10 μM) for 0.5 h at 37 °C and washed once. And then Cd^{2+} (500 μM) was added for another 30 min, which was washed three times. The fluorescence images were taken, as shown in Figure 4. The results suggest that **CYP-1** can penetrate the cell membrane and can be used for imaging of Cd^{2+} in living cells and in vivo potentially. However, we did not get the fluorescence images of **CYP-2** since it was hard to penetrate into the cell membrane.

In conclusion, we have designed and synthesized two selective, sensitive NIR fluorescent sensors for cadmium based on a PET mechanism. The **CYP-1** can distinguish Cd^{2+} in Tris-HCl (12.5 mM,) solution (acetonitrile/water = 9/1, v/v, containing 0.05 mM sodium phosphate, pH 7.2), and

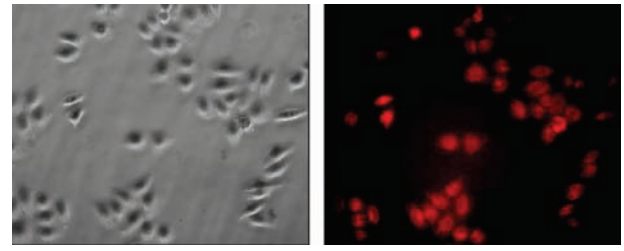

Figure 4. Fluorescent images of Cd^{2+} in HeLa cells for sensor **CYP-1** (3 μM): (a) bright-field transmission image of HeLa cells incubated with **CYP-1** after adding Cd^{2+}(500 μM); (b) fluorescence image of HeLa cells incubated with **CYP-1** after adding Cd^{2+}.

CYP-2 can also discriminate Cd^{2+} from other metal ions in Tris-HCl (12.5 mM) solution (containing 0.05 mM sodium phosphate, pH 7.2). Both the excitation and emission wavelengths are in the NIR region. The living cell image experiments further demonstrate its value in the practical applications of biological systems.

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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.

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