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Ayyappanpillai Ajayaghosh, Priya Carol, and Sivaramapanicker Sreejith

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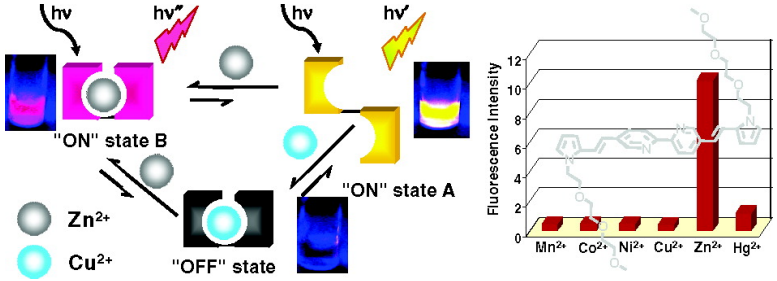

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A Ratiometric Fluorescence Probe for Selective Visual Sensing of Zn^{2+}

Ayyappanpillai Ajayaghosh,* Priya Carol, and Sivaramapanicker Sreejith

Photosciences and Photonics Group, Chemical Sciences Division, Regional Research Laboratory, CSIR, Trivandrum, India

Received June 23, 2005; E-mail: aajayaghosh@rediffmail.com

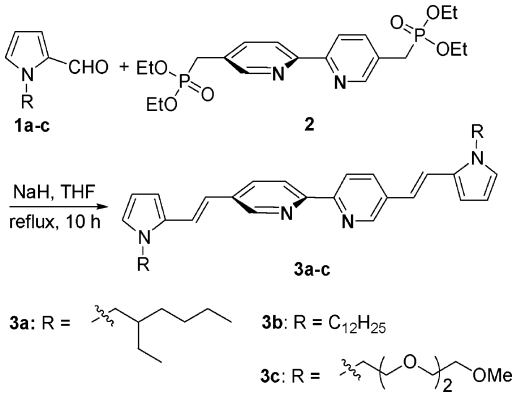
Zinc is the second most abundant transition metal in the human body, which is vital for biological functions, such as gene expression, apoptosis, enzyme regulation, and neurotransmission.[1] Zn^{2+} is also known to be responsible for the formation of amyloid plaques during the onset of Alzheimer's disease.[2] The estimation of the extent of zinc deficiency which prevails upon the young children in many parts of Africa and Asia is a challenging problem because of the lack of suitable biochemical probes, which are specific for Zn^{2+}. This has been a concern of chemists which resulted in the emergence of considerable activities in the development of Zn^{2+}-specific molecular probes.[3]

Recent progress in the area of fluorophore-based chemosensors[4] has contributed significantly to the development of a variety of probes based on quinoline,[5] dansyl,[6] anthracene,[7] and fluorescein[8] for the sensing of Zn^{2+}. Among these, fluorescein-based probes are the widely studied systems. Though these second-generation probes have higher affinity for Zn^{2+} and are brighter than the first-generation UV-based probes, several issues, such as easy synthesis, sensitivity, selectivity, optical compatibility with biological tissues, and the mode of signaling, need further attention. Therefore, it is necessary to design easily accessible probes that selectively bind Zn^{2+} under biological conditions, thus facilitating visual sensing of the binding event at a red-shifted emission, which is from that of the virgin fluorophore. In this context, fluorophores that facilitate ratiometric sensing[9] at biological absorption wavelength region are of extreme importance.

The demand for Zn^{2+}-specific visual ratiometric sensors when coupled with our expertise in the design of specific cation probes[10,11] has prompted us to investigate, on a simple fluorophore, the structure of which is shown in Scheme 1. Pyrrole end-capped divinyl aromatic systems[12] are known to be strongly fluorescent building blocks for the synthesis of electrochromic[13] and low band gap[14] polymers. Since bipyridyl moiety is known to be a good ligand for transition metal ions,[15] we designed a few pyrrole end-capped 5,5′-divinyl-2,2′-bipyridyl derivatives (**3a**−**c**) using Wittig−Horner reaction of the corresponding pyrrole carbaldehyde (**1**) and the 2,2′-bipyridyldiphosphonate (**2**) in 25−40% yields. **3a**−**c** showed absorption at 407 nm and a strong emission at 537 nm ($\Phi_f = 0.4$) in acetonitrile with a large Stoke shift of 130 nm. In buffered aqueous acetonitrile (1:9) solution, **3c** showed a 10 nm red-shift in emission ($\lambda_{max} = 547$ nm) with slight quenching when compared to that in acetonitrile, whereas in aqueous buffer (HEPES, pH 7.2), the emission is red-shifted to 600 nm with significant quenching. However, under these conditions, **3c** is sufficiently fluorescent, thus allowing visual sensing by the naked eye.

Titration of alkali and alkaline earth metal perchlorates did not show much change to the optical properties of **3c**. However, Cu^{2+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Mn^{2+}, and Zn^{2+} showed significant decrease in the absorption band at 407 nm with the concomitant formation of a new red-shifted absorption band at 450 nm through two isosbestic points (see Supporting Information), indicating the planarization

Scheme 1



of the metal ion bound fluorophore. Most importantly, the emission of **3c** at 537 nm was significantly quenched by Cu^{2+}, Ni^{2+}, Hg^{2+}, Co^{2+}, and Mn^{2+}, whereas the titration of Zn^{2+} resulted in a strong orange−red emission at 635 nm ($\Delta E_{em} = 98$ nm) through an isoemissive point at 605 nm. A comparison of the emission behavior of **3c** against Cu^{2+} and Zn^{2+} is shown in Figure 1. The Job plot

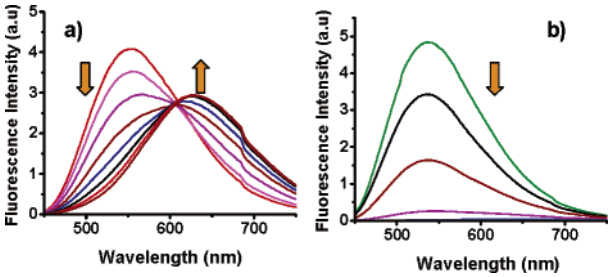


Figure 1. Changes in the emission spectra of **3c** (6 μM in acetonitrile−water, 9:1 v/v) upon addition of (a) Zn $(ClO_4)_2$ (0−6 μM) and (b) Cu$(ClO_4)_2$ (0−6 μM) in HEPES buffer (pH 7.2).

and the ratiometric plot revealed a 1:1 complexation between the fluorophore and the metal ion. Benesi−Hildebrand plots gave binding constants of 1.23×10^5 and 3.8×10^5 M^{-1} for Zn^{2+} in acetonitrile and in aqueous acetonitrile (1:1), respectively (see Supporting Information). Change of perchlorate counterions with sulfate or chloride did not make much change to the optical properties of **3c**.

The individual emission response of **3c** against different transition metal ions revealed a remarkable selectivity of Zn^{2+} binding (Figure 2a). However, the most important criterion for a selective cation probe is the ability to detect a specific cation in the vicinity of other competing ions under biological pH. To clarify this point, we have investigated the effect of pH on the optical properties of **3c** in the absence and in the presence of Zn^{2+} under aqueous conditions (see Supporting Information). Under the biological pH window of 6.8−7.4, addition of Zn^{2+} to **3c** showed fairly intense emission at around 624 nm, though below and above these values

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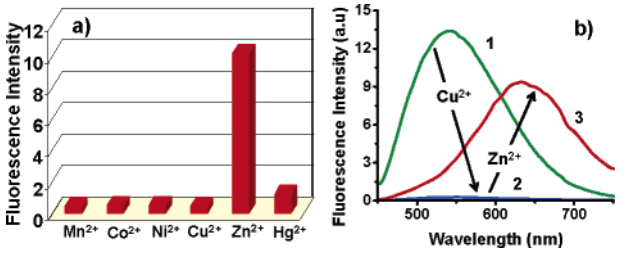

Figure 2. (a) Plot of fluorescence intensity of **3c** (6 μM) monitored at 650 nm with different metal ions. (b) Emission spectra of (1) **3c** (blank), (2) **3c** + Cu^{2+}, and (3) **3c** + Cu^{2+} + Zn^{2+}.

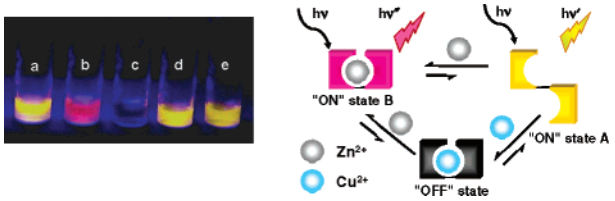

Figure 3. Visual emission color changes of **3c** (30 μM) in water (HEPES buffer, pH 7.2) with different metal ions (5×10^{-5} M). (a) **3c** (blank), (b) **3c** + Zn^{2+}, (c) **3c** + Cu^{2+}, (d) **3c** + Na^{2+} and K^{2+}, and (e) **3c** + Ca^{2+} and Mg^{2+} (left). A schematic representation of the binding and the signaling events (right).

considerable quenching is observed. Furthermore, addition of Zn^{2+} into the nonfluorescent solution of the **3c**−Cu^{2+} complex showed the red fluorescence of **3c**−Zn^{2+} (Figure 2b). These observations unambiguously prove that **3c** competes with Cu^{2+} in the selective sensing of Zn^{2+} under biological pH and the nonfluorescent **3c**−Cu^{2+} can be used as a latent fluorescent sensor for Zn^{2+}.

The visual emission changes of **3c** with different biologically significant cations are shown in Figure 3. Alkali and alkaline earth metal ions did not considerably change the emission of **3c**. Cu^{2+} significantly quenched the fluorescence, whereas Zn^{2+} showed a unique red emission. Even in a mixture of alkali, alkaline earth, and transition metal cations, the red emission was fairly intense, revealing the high selectivity of **3c** for Zn^{2+}. Since the concentration of Zn^{2+} in synaptic vesicles are reported to be in micro- to millimolar range, which often is released into synaptic space to achieve a peak level of 10−30 μM, it is necessary to have probes that detect micromolar concentrations of Zn^{2+} under physiological conditions.[16] In this context, **3c** is an ideal probe for the imaging of Zn^{2+}, particularly at micromolar concentrations.

From a mechanistic viewpoint, the diamagnetic Zn^{2+} with d^{10} electronic configuration having flexible coordination geometry is advantageous for the specific binding. Upon binding of Zn^{2+}, the absorption and emission of **3c** are red-shifted due to a decrease in the twist angle between the two pyridyl moieties, leading to a near planar conformation of the **3c**−Zn^{2+} complex, thus changing the initial fluorescence "ON" state A to the "ON" state B. In contrast, binding of the paramagnetic Cu^{2+} quenches the emission to form the fluorescence "OFF" state. Thus the fluorescence technique becomes an efficient visual tool in distinguishing Zn^{2+} from other competing cations at an emission wavelength that is compatible for biological samples.

In conclusion, the reported fluorophores facilitate selective ratiometric and visual sensing of Zn^{2+} in the presence of other competing metal ions with a red-shifted emission color in the visible region, under physiological pH window. This will allow differential fluorescence imaging of Zn^{2+} in biological specimens. In addition, the reported fluorophores can be electropolymerized on electrode surfaces and are amenable for the synthesis of fluorescent polymers, useful for the fabrication of ion responsive devices. These studies are in progress.

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Supporting Information Available: Synthetic details, spectral changes and pH effect of **3a**. Job, ratiometric, and Benesi−Hildebrand plots of **3a** and **3c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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