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Multiresponsive Switchable Diarylethene and Its Application in Bioimaging

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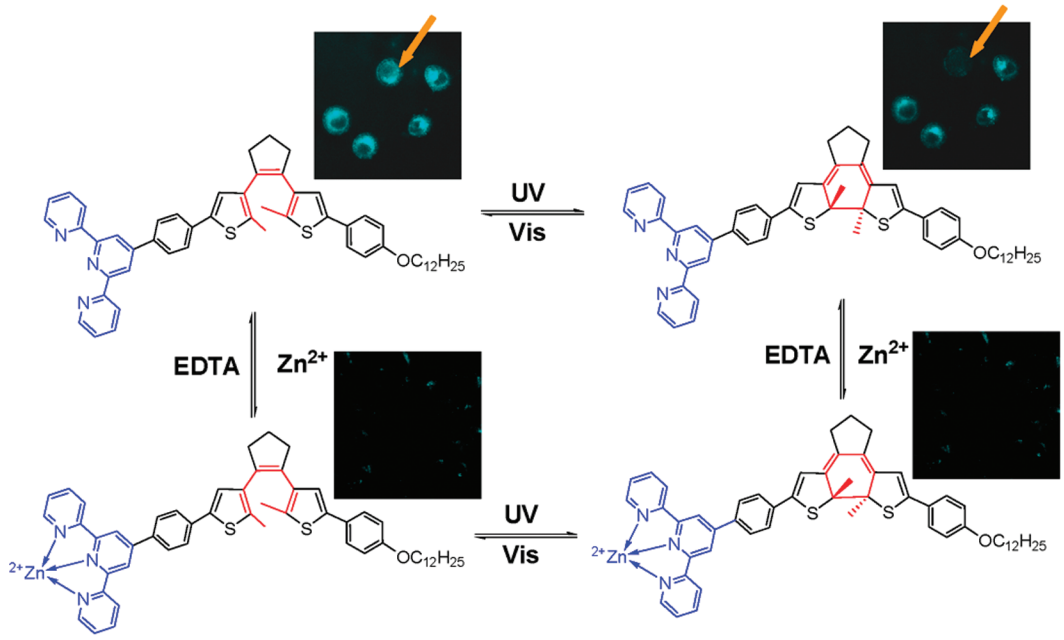
ABSTRACT



A multiresponsive fluorescent switch based on diarylethene and terpyridine units was developed. It exhibits effective switchable fluorescence which can be controlled by UV/visible light or metal ion/EDTA in solution. More importantly, having low toxicity, it can enter live cells as a fluorescent probe and can also serve as a detector for the biological process of metal ion transmembrane transport.

Fluorescence labeling technology is important to the advancement of biological imaging, which is widely used for the monitoring of internal cellular processes.[1] In the past few decades, much progress has been made in designing fluorescent probes for bioimaging[2] and some materials, such as organic dyes,[3] fluorescent proteins,[4] quantum dots,[5] metallic complexes,[6] and up-conversion nanoparticles[7] are now standard and widely used. While very important to

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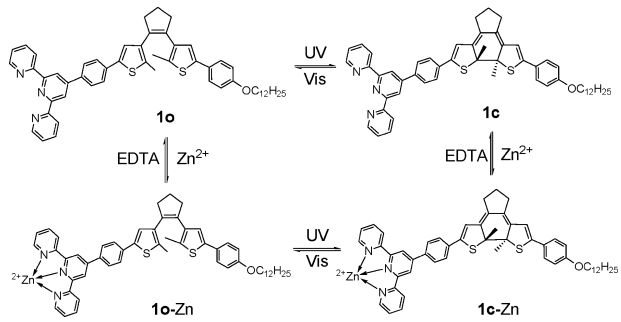
cellular imaging, most of these conventional probes respond irreversibly to a certain event[8] or nondynamically to environmental stimuli, so that the development of a novel photocontrollable and multiresponsive fluorescent labeling molecule would be a powerful tool in elucidating the physiological dynamics in living cells.[9]

During the past decade, much effort has been focused on designing and synthesizing fluorescence switches, since they have potential applications in information storage and light-controlled molecular data processing.[10] Our previous work focused on diarylethene-based switches.[11] Diarylethene is one of the most promising photoswitchable units within the photochromic system because of its high fatigue resistance and thermal stability.[12] We have designed and synthesized an amphiphilic diarylethene as a photoswitchable probe for imaging living cells.[13] However, static imaging may not meet the need to have probes that possess more functions, not only labeling the cells or components inside but also showing the biological processes of the metal ion transmembrane transport.

Scheme 1. Chemical Structure Changes of Compound **1o**



It is known that the terpyridine unit is able to coordinate with several kinds of metal ions,[14] especially Zn^{2+} and Cu^{2+}, causing fluorescence changes in the original ligands.[15] These metal ions are essential elements for life, but changes in their bioconcentration are associated with serious diseases.[16] For example, the disruption of the Zn^{2+} accumulation pattern is relevant to some types of prostate cancer, diabetes, and neurodegenerative disorders;[17] alterations in the cellular homeostasis of Cu^{2+} may cause serious neurodegenerative diseases such as Menkes and Wilson diseases and Alzheimer's disease.[18]

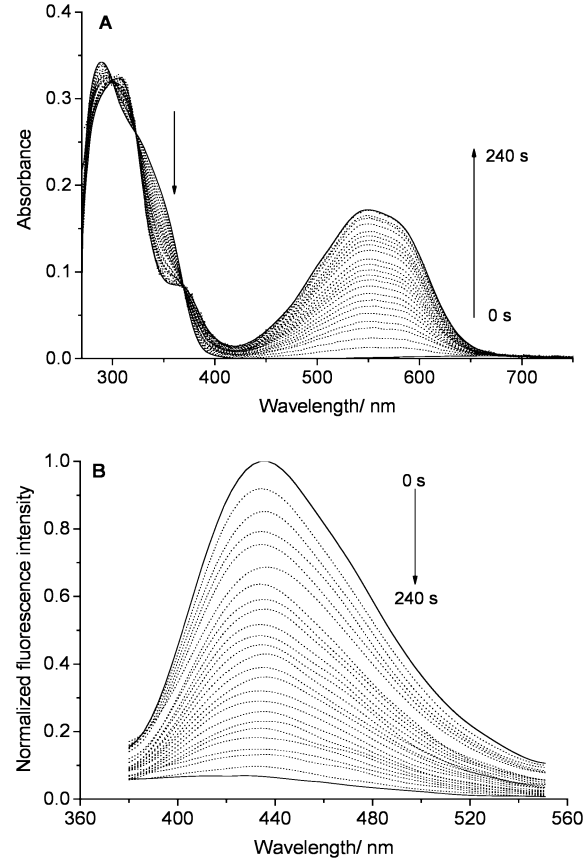


Figure 1. (A) Absorption and (B) fluorescence emission changes of **1o** in THF (1×10^{-5} M) upon irradiation with 365 nm light (λ_{ex} = 330 nm).

Herein, we designed a novel fluorescence switch combining diarylethene and terpyridine functional units (**1o**, Scheme 1). This compound **1o** exhibits several clearly different and

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reversible fluorescence states that can be controlled by varying light frequency and metal ion concentration. Besides functioning well in solution, it also has potential for effective application in biological systems. More importantly, it exhibits an easily detectable change in fluorescence when stimulated by metal ions and thus would serve well as a photoswitchable probe for imaging live cells and previously nonvisualizable biological processes.

1o was synthesized by a coupling of 1-(5-chloro-2-methylthien-3-yl)-2-[2-methyl-5-(4-(dodecyloxy) benzene-4-yl) thien-3-yl] cyclopentene (**2**) with 4′-(4-bromophenyl)-2, 2′:6′, 2″-terpyridine (**3**) (Scheme S1 in the Supporting Information). To 6.0 mL anhydrous THF solution of **2** (0.3 g, 0.54 mmol), *n*-BuLi (0.4 mL of 1.6 M solution in hexane) was added under Ar atmosphere at −5 °C. After stirred for 45 min at room temperature, $B(OBu)_3$ (0.40 mL, 1.15 mmol) was added in one portion to the mixture. The resulting reddish solution was then stirred for 6 h at room temperature and was added to a flask containing **3** (0.23 g, 0.59 mmol), $Pd(PPh_3)_4$ and 3 mL Na_2CO_3 solution (20 wt %) at 50 °C. The reaction was refluxed under Ar atmosphere for 19 h. The pure product was obtained as a white solid by column chromatograph (petroleum ether: triethylamine = 18:1) and washing with petroleum ether/ CH_3OH in a yield around 35%. The molecular structure of **1o** was confirmed by ^1H and ^{13}C NMR spectroscopy, MALDI-TOF mass spectrometry and elemental analysis (Supporting Information). Compound **1o** is easily dissolved in a variety of organic solvents, such as THF, DMSO, CH_2Cl_2, and $CHCl_3$.

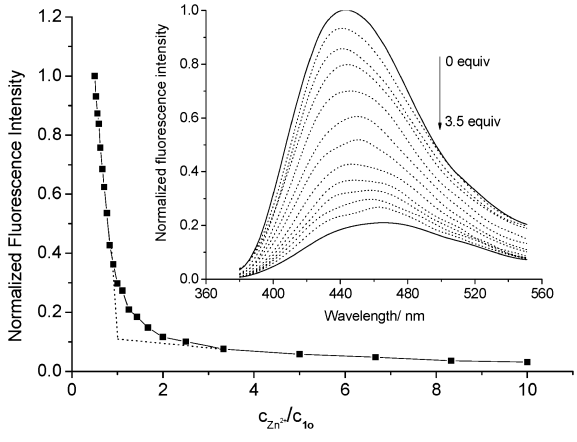

Figure 2. Fluorescence intensity changes at 440 nm with different molar ratio of Zn^{2+} and **1o** in THF/ water solution (100:1, v/v) (**1o** from 2×10^{-5} to 1×10^{-6} M, Zn^{2+} with the fixed concentration of 1×10^{-5} M). (Inset) Fluorescence spectral changes of **1o** (1×10^{-5} M) by the addition of Zn^{2+} from 0 to 3.5 equiv.

The photochromic behavior of **1** was studied in THF solution. As expected, compound **1** showed reversible absorption and fluorescence intensity changes with the alternate irradiation with ultraviolet and visible light. The UV−vis absorption spectrum of **1o** showed a single absorption band in the ultraviolet range ascribed to $\pi-\pi^*$ transition (300 nm for **1o**, Figure 1A). **1o** exhibited blue fluorescence centered at 440 nm with a fluorescent quantum yield of 0.041 when using Rhodamine B as reference (Figure 1B). Upon irradiation with ultraviolet light ($\lambda = 365$ nm), the colorless solution turned slightly purple within 4 min and a new absorption maximum appeared at 550 nm ($\varepsilon = 1.7 \times 10^4$ $dm^3mol^{-1}cm^{-1}$). This was ascribed to the closed isomer form with photocyclic quantum yield ($\Phi_{o\text{-}c}$) of 23% (365 nm), accompanied by quenching of 95% of the fluorescence in the photostationary state (PSS) caused by an increase of π-electron delocalization.[12,19] The photocyclic quantum yield was dependent on the irradiation wavelength, for example $\Phi_{o\text{-}c} = 21\%$ upon irradiation with 380 nm (Table S1 in the Supporting Information).

Under visible light irradiation (549 nm) in the PSS, absorption in the visible range ceased and fluorescence intensity was almost restored to the original state (before irradiation with UV), demonstrating diarylethene's classical switching characteristic. The quantum yield of photocycloreversion ($\Phi_{c\text{-}o}$) was 22% upon irradiation with 549 nm and decreased to 2% upon irradiation with 650 nm. The difference in absorbance at 550 nm for the open isomer and the PSS was less than 2% and 8%, respectively, after the open-close cycle was repeated 5 times by alternating irradiation with UV and visible light (Supporting Information). The low rate of fatigue apparent in the cycle repetition indicated that **1** possessed good fatigue-resistance. Thus, compound **1**, having changeable states that can be reversed multiple times with little fatigue, could be used in a repeated "write-erase" process.

1o Can coordinate with both Zn^{2+} and Cu^{2+}, resulting in the decrease of the fluorescent intensity. Alkali and alkaline-earth metal cations such as Na^+, K^+, Mg^{2+}, Ca^{2+} gave no interference at a 3.5-fold excess concentration, and transition-metal and heavy-metal ions such as Fe^{2+}, Cd^{2+}, Hg^{2+} gave a weak response (Supporting Information). Here, Zn^{2+} was chosen as the metal ion in the investigations into the quenching of the fluorescence of **1o** because aqueous solutions of Zn^{2+} are usually colorless and it is the strongest Lewis acid among divalent metal ions.[20] As shown in Figure 2 inset, fluorescence of **1o** could be gradually quenched by the addition of aqueous solution of $Zn(NO_3)_2$, which may be attributed to the formation of a **1o**−Zn complex, changing the charge density of the terpyridine unit. Nonlinear fitting of the fluorescence titration curve exhibited a 1:1 stoichiometry for **1o** and Zn^{2+}, with the association constant (K) of 1.76×10^6 M^{-1} (Figure 2).[21] When EDTA solution (1.0 eq to Zn^{2+}) was added, the fluorescence intensity was

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restored (Supporting Information), which can be explained by the difference in the association constant between EDTA-Zn and **1o**−Zn. The $K_{EDTA\text{-}Zn}$ (3.16×10^{16} M^{-1})[22] is much larger than that of **1o**−Zn. Therefore, Zn^{2+} acts as a trigger for fluorescence switch-OFF, while EDTA acts as a trigger for fluorescence switch-ON. The fluorescence intensity of **1o** can be reversibly controlled by UV/visible light or Zn/EDTA, and **1o** acts as a double-controlled molecular fluorescence switch reacting to light and chemical stimuli.

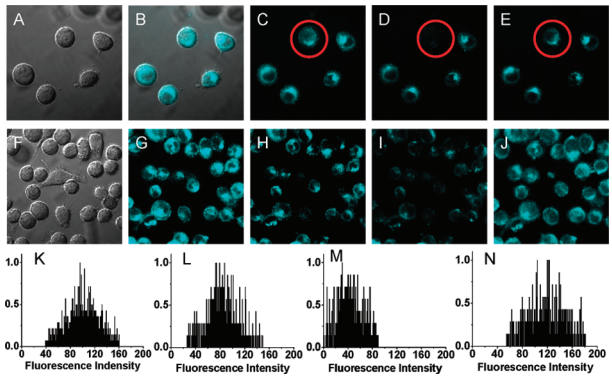

Figure 3. CLSM images of KB cells incubated with **1o** for 20 min at 25 °C (1×10^{-5} M in PBS/ DMSO, 100:2, v/v). (A, F) Bright-field transmission image of KB cells. (B) Overlay image of A and C. Confocal fluorescence image of (C) original state, (D) irradiated by 405 nm light (2 mW, 3 min) for one selected cell and (E) recovered by 633 nm light (0.7 mW, 40 min). Confocal fluorescence image of (G) original state of F, and incubation by Zn^{2+} solution with the concentrations of (H) 5×10^{-5} M, (I) 1×10^{-4} M. (J) Recovered by 5×10^{-4} M EDTA solution. (K), (L), (M), (N) were the distribution of fluorescence indensity of (G), (H), (I), (J), respectively.

Most diarylethene fluorescence switches have only been studied in solution[23] and little research has been carried out using a biological system. Our research shows that **1o** can be effectively applied as a fluorescent probe in living cells. Using confocal laser scanning microscopy (CLSM), we observed a blue luminescence in the cytoplasm of KB cells (human nasopharyngeal epidermal carcinoma cell) after incubation with a PBS/ DMSO (100:2, v/v) solution of **1o** (1×10^{-5} M) for 20 min at 25 °C (Figure 3A, B, F, and G).

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The luminescence of **1o** could be controlled as readily in living cells as in solution by using UV/ visible light as the switching trigger, and this photoswitchable labeling was demonstrated in one selected cell (shown in red circle, Figure 3C). After irradiation with 405 nm light (2 mW) for 3 min, the brightness of the selected cell noticeably decreased compared to the brightness of surrounding cells which remained almost unchanged (Figure 3D). Upon irradiation with 633 nm light (0.7 mW) the brightness of the selected cell was recovered within 40 min (Figure 3E). In contrast, when the **1o** labeled cells were treated with different concentrations of Zn^{2+}, an obvious change in fluorescence was observed with CLSM (Figure 3G-I). Luminescence intensity decreased with increasing Zn^{2+} concentration (Figure 3K−M). This effect provides a good means for visualizing the process of M^{2+} (M = Zn, Cu) uptake from the outside into the cell. As expected, the fluorescence was almost restored to the original state upon the addition of EDTA solution (Figure 3J and N). The cytotoxicity of **1o** is important for its use as a bioprobe, so the effect of **1** on cell proliferation was determined by means of an MTT assay (Suppporting Information). The cellular viabilities were estimated to be greater than 85% after 24 h in the presence of 1−100 μM **1** (Supporting Information). This indicates that **1** has low cytotoxicity.

In conclusion, we have developed a new multiresponsive fluorescence switch based on diarylethene and terpyridine units. It exhibits effective switchable fluorescence controlled by UV/visible light or metal ions/EDTA in solution. More importantly, having low cytotoxicity, it can enter live cells as a fluorescence probe and can act as a detector for the biological process of the metal ion transmembrane transport. We expect that this material will be of great benefit to biomedical research.

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Supporting Information Available: Synthetic and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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