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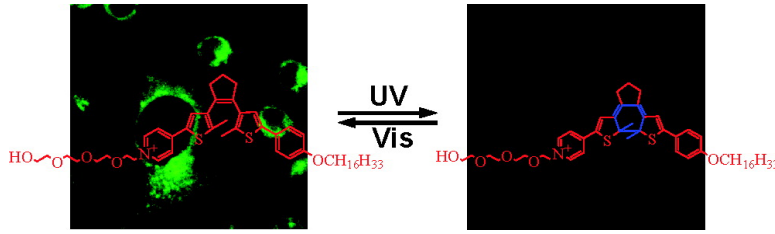

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Amphiphilic Diarylethene as a Photoswitchable Probe for Imaging Living Cells

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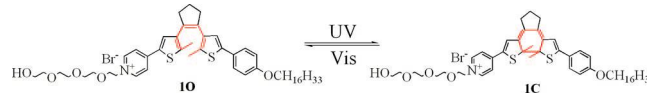
Fueled by advancements in imaging technology, examination of cells by fluorescence techniques has been widely used to study cellular processes.[1,2] Conventional fluorescent probes for fluorescent imaging, such as rhodamine[3] and cyanine dyes,[4] are useful for cellular studies but can respond only irreversibly to one event.[5] In contrast, fluorophores that can respond reversibly provide a simple and powerful technique for regional optical marking and thus would be much more valuable for cellular fluorescent labels.[6] Though several photoactivatable fluorescent proteins have been developed to make possible precise photo labeling and tracking of the target protein,[6c] the large size of fluorescent proteins often results in improper protein functioning. Therefore, the development of a novel small photocontrollable fluorescent labeling molecule would be a powerful tool to elucidate the protein dynamics in living cells.[7]

Very recently, Li et al. reported a dual-color fluorescent probe based on spiropyran loaded nanoparticles;[6b] nevertheless, the irradiation source provides shorter than 365 nm UV light and the thermal instability of the open-ring form of spiropyran limited its application in bioimages. It is well-known that diarylethene is the most promising switchable unit within those classical photochromic systems owing to its excellent fatigue resistance and thermal stability.[8] However, most of these studies are carried out in organic systems, including the newly developed fluorescent photochromic diarylethene for labeling biomolecules by M. Irie,[7] which is not suitable for biological application. These problems may be resolved by inducing amphiphilic molecules, which is a widely used technique to construct highly organized nanostructures in aqueous systems with the driving force of hydrophobic interaction.[9] Herein an amphiphilic molecule (**1**, Scheme 1) was designed with hydrophilic and hydrophobic chains at two ends of a rigid diarylethene core. **1** forms stable vesicles in water and exhibits obviously different fluorescence in open and closed states under alternating irradiation of UV and visible light. Thus, this molecule would serve well as a photoswitchable probe for imaging living cells.

The formation of the aggregate state of **1** in aqueous solution was proved by the plot of the conductivity of **1** (open isomer, **1O**) as a function of the concentration with the critical aggregation concentration at $\sim 1.2 \times 10^{-6}$ M (Supporting Information, SI). A scanning electron microscope (SEM) image shows a spherical morphology with a diameter of 50−200 nm for the freeze-dried sample from a low-concentration aqueous solution (1.0×10^{-5} M, Figure 1A). On the other hand, column-shaped single crystals and aggregated crystals were obtained from the mixed solvent of water and methanol 1:1 (v/v, 1.0×10^{-5} M) (Figure 1B).

Similar to the previously studied diarylethene pyridinium moieties,[10] **1O** exhibited greenish fluorescent emission centered at 510 nm in water (1.0×10^{-5} M), with the fluorescent quantum yield (QY) being 0.0044 by using Rhodamine B as reference. A slightly increased QY was found with increasing concentration (For example, QY = 0.0073 for 5.0×10^{-5} M of **1O**). The emissions

Scheme 1. Structure and Photochromic Process of **1**



were obviously blue-shifted (40 nm) with enhanced intensity when the concentration increased from 2.5×10^{-6} to 1.0×10^{-5} M (Figure 2A). However, in regular organic solvents such as methanol, the fluorescence was completely quenched for the breaking of aggregation (Figure S2, SI) and became intense and blue-shifted as we added water. This finding determines the strongly aggregation-dependent fluorescence of **1** in water. The confocal laser scanning microscopy (CLSM) images for one drop of **1O** water solution (1.0×10^{-5} M) on a glass substrate showed well-dispersed micrometer-sized fluorescent circles (Figure 1C), indicating that the hydrophobic interaction and the hydrophilic repulsion between the molecules governed the arrangement of the molecules to vesicles.[9] Those vesicles can disperse in aqueous solution and remain unchanged for more than a month in a concentration range

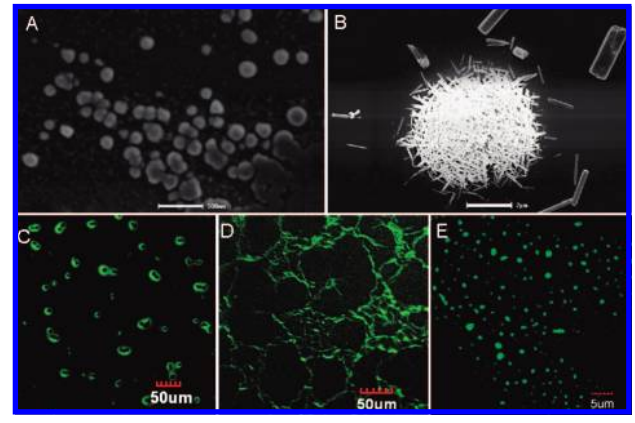


Figure 1. SEM images of **1O** (A) from water solution (scale bar, 0.5 μm); (B) from the mixed solvent of H_2O/MeOH = 1:1 (v/v) (scale bar, 2 μm). CLSM images of **1O** (C) in water (1.0×10^{-5} M); (D) in mixed solvent of H_2O/MeOH = 1:5 (v/v, 1.0×10^{-5} M); (E) in PBS solution (2.0×10^{-5} M). λ_{ex} = 405 nm, λ_{em} = 480−580 nm.

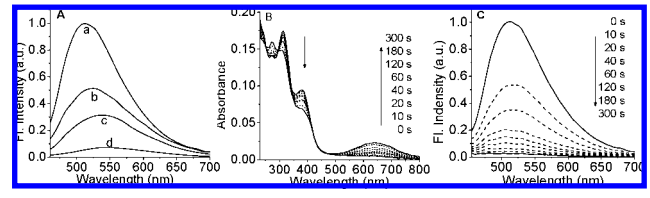


Figure 2. (A) Concentration-dependent fluorescent emission spectra of **1O** (a: 1.0×10^{-5} M, b: 7.5×10^{-6} M, c: 5.0×10^{-6} M, d: 2.5×10^{-6} M in water). (B) Absorption and (C) emission spectral changes of **1O** under 365 nm light irradiation (1.0×10^{-5} M, λ_{ex} = 385 nm).

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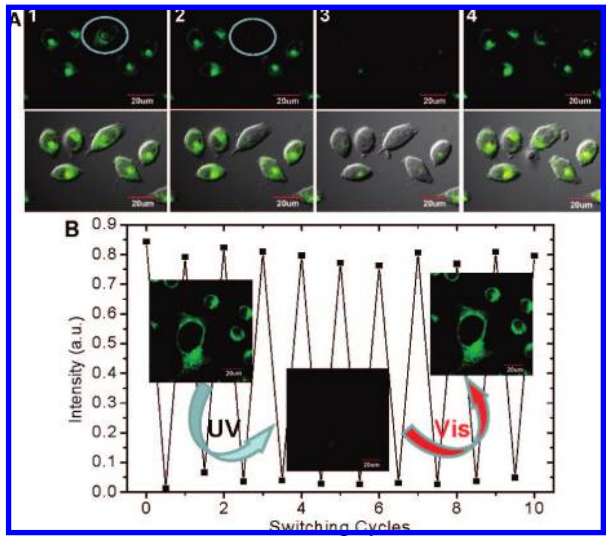

Figure 3. (A) CLSM image (above) and the overlay image (bottom) of KB living cells incubated with **1O** for 20 min at 25 °C (1) in original state, (2) irradiated by 405 nm light (2 mW) for a single cell, (3) all cells, and (4) recovered by 633 nm light (0.7 mW) (λ_{ex} = 405 nm, 0.15 mW). (B) Fluorescence switching of fixed KB cells by alternating UV (405 nm, 2 mW, 10 s/time) and visible (633 nm, 0.7 mW, 20 min/time) light illumination. Inset: images of one cycle.

of 1.2×10^{-6}−7.5×10^{-5} M. With addition of methanol to the aqueous solution, the vesicles changed to entangled fibers (Figure 1D).

1O has a maximum absorption at 385 nm (extinction coefficient, $\epsilon_{385} = 6700\ L \cdot mol^{-1} \cdot cm^{-1}$) and performs obvious photochromism in aqueous solution. Irradiation of colorless **1O** with 365 nm light led to a new absorption at 640 nm with a photocyclic quantum yield of 33.3% (Figure 2B), accompanied by quenching of most of the fluorescence (98% quenched within 30 s in photo stationary state [PSS]) because of the enlargement of π-electron delocalization (Figure 2C).[8] Under visible light irradiation, both absorption and fluorescence were completely converted into the open isomer according to the spectral change, showing the classical switchable property of diarylethene. This water-"soluble" fluorescent switchable material thus provides a nice platform for application in bioimaging owing to the high-ratio signal change in water.

With CLSM, we then developed a practical application of **1** as a fluorescence switch in a biological system (Figures S4, S5). After KB cells (human nasopharyngeal epidermal carcinoma cell) were incubated with a PBS solution (pH = 7) of 20 μM **1O** (0.6−1.0 μm in size from DLS and CLMS, Figures 1E, S3) for 20 min at 25 °C, we observed a marked fluorescence increase in the cytoplasm of the cells (Figure 3A1). Intense intracellular luminescence with a high signal-to-noise ratio ($I_1/I_2 > 50$) was detected between the cytoplasm and background (Figure S6). Further quantization by line plots revealed large signal ratios ($I_1/I_3 = 14$) between the cytoplasm and nucleus, implying that weak nuclear uptake occurred for **1**. The luminescence was still clearly observed when the KB cell was loaded with **1** at 4 °C, indicating that **1** is unlikely to enter the cells by endocytosis (Figure S7), which may be beneficial for intracellular target recognition. The cytotoxic characteristic is important for **1** as a bioprobe; therefore, the effect of **1** on cell proliferation was determined by means of an MTT assay (SI). The cellular viabilities were estimated to be greater than 85% in 24 h in the presence of 0.2−20 μM **1** (Figure S8). Meanwhile, a Trypan blue staining cell viability experiment gave a cell viability of more than 99.5 (%) incubated with 20 μM **1** for 20 min, which shows that **1** has low cytotoxicity. A selected cell was then irradiated with a 405 nm light for 3 min, and the fluorescence of the irradiated cell was turned off (Figure 3A2), with the denotation of **1** in the cells changing from an open state to a closed state. All cells can also be lamped off upon 405 nm irradiation for 3 min (Figure 3A3). It is amazing that the fluorescence of the cells could be recovered by 633 nm light irradiation from the CLSM lamp for 1 h (Figure 3A4).

The reversibility and stability of the cell images by the utility of **1** was demonstrated in fixed KB cells. The microfluorescence switching of fixed KB cells was achieved while alternating between UV and visible light illumination. We were excited that, as evident in Figure 3B and Figures S9, S10, the optical switching of fluorescence can be repeated many times without any apparent "fatigue" effects, or photobleaching, that were thought to be the fateful disadvantage of the general fluorophore. Thus, we concluded that **1** was a novel and superior fluorescence dye for use as a cell marker or fluorescence switch in a cell system.

In conclusion, we developed a new amphiphilic diarylethene, which forms stable vesicle nanostructures in aqueous solution and exhibits switchable fluorescence between open and closed states. These fluorescent vesicles can enter the living cells with low cytotoxicity and have potential utility as a cellular marker or fluorescence switch in living cells with high-ratio signal change and excellent resistance to fatigue. We expect that such materials will be of great benefit to biomedical researchers for further study.

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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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