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Highly fluorescent and water soluble turn-on type diarylethene for super-resolution bioimaging over a broad pH range

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ABSTRACT

We report a water-soluble turn-on type diarylethene SO3-BPDBTEO that reversibly switches its fluorescence on and off upon light stimuli. We note that the two sodium sulfonate groups enable SO3-BPDBTEO to exhibit excellent photo-switching properties in aqueous solution over a wide range of pH between 3 and 10. We demonstrated that SO3-BPDBTEO could be applied to conventional fluorescence imaging of mammalian cell lines such as HeLa cell at various pH conditions with low cytotoxicity. We also examine its feasibility for super-resolution fluorescence imaging as a photoswitching probe.

1. Introduction

Recent advances in far-field fluorescence microscopy opened a way to investigate cellular structure and dynamics with low sample damage and high imaging contrast [1,2]. Particularly, super-resolution microscopy is drawing much attention because it can reveal sub-nanostructure of soft-matter materials and biological samples at a single molecule level [3,4]. In the super-resolution microscopy, the photophysical and chemical properties of photoswitchable fluorophore used as an imaging probe are a key factor that determines the quality of imaging [5]. However, conventional imaging probes for the super-resolution microscopy have intrinsic limitations. For example, cyanine dyes have low chemical stability while photoswitchable fluorescent proteins are difficult to trace cell dynamics and have limited resolution due to their own size [6–9].

On the other hand, diarylethenes (DAEs) have high thermal stability, reversibility, fatigue resistance, and fast switching rate, which make them an ideal candidate for the super-resolution imaging [10–16]. Particularly, the turn-on type DAEs have been successfully applied to the photoactivation localization microscopy (PALM)-based super-resolution microscopy to reveal soft-matter structures with an advantage of high fluorescence on/off contrast [17,18]. Recently, Hell group developed water-soluble DAEs bearing multiple carboxylic acid groups and applied them to super-resolution bioimaging on the RESOLFT and STORM platforms [19]. These DAEs showed suitable fluorescence modulation and sufficient solubility in aqueous buffers at physiological pH, but the high pK_a value of the carboxylic acid group (pK_a ~4.2) [20] limits their application in wider biological environments. For example, lysosomes, tumor cells, and certain micro-organisms that can experience highly acidic conditions (e.g., eukaryotic cells and enteric pathogens) would be beyond their reach [21]. Therefore, developing a new family of DAEs that are highly water-soluble and applicable as a super-resolution imaging probe over a broad pH range is highly desirable and indispensable [22,23].

In this paper, we present a water-soluble turn-on type DAE that can be used as a probe in the PALM-based super-resolution microscopy for imaging biological samples over a broad pH range. To this end, we prepared SO3-BPDBTEO (**1**) bearing two sodium sulfonate groups, whose pK_a value of −2.8 is significantly lower than that of the carboxylate group [18], in the phenyl ring at the C6,6′-position of 1,2-bis(2-ethyl-6-phenyl-1-benzothiophene-1,1-dioxide-3-yl)perfluorocyclopentene core (see Scheme 1). SO3-BPDBTEO was synthesized according to the synthetic route which was originally developed by Irie group and us independently [24,25]. The chemical structure of **1**

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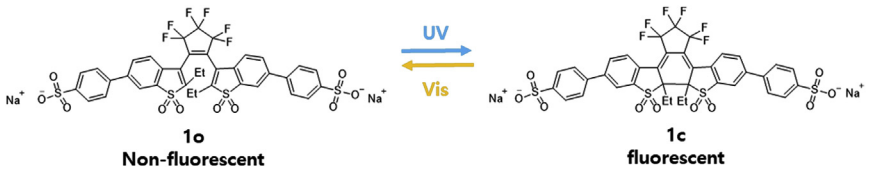

Scheme 1. Chemical structure and photochromic reaction scheme between open (**1o**) and closed (**1c**) form of SO3-BPDBTEO (**1**).

is fully characterized by ^{1}H NMR, ^{13}C NMR, 2D NMR (HSQC), and MALDI-TOF (see the experimental section in supporting information).

2. Material and methods

2.1. General

All reagents, metal catalysts and solvents were obtained from commercial sources (Sigma Aldrich, Acros, Alfa Aesar and others) and used without further purification. ^{1}H-NMR spectra were obtained by a Bruker Avance-300 in $CDCl_3$ and methanol-d_4 solutions. ^{13}C-NMR and 2D-NMR (HSQC) spectra were obtained by a Bruker Avance-500 in methanol-d_4 solutions. In NMR data, (ap) and (p) mean proton signals of antiparallel and parallel conformations, respectively. Mass spectra (MALDI-TOF) were measured using a Voyager-DE STR Biospectrometry Workstation.

2.2. Synthesis and characterization

SO3-BPDBTEO was synthesized according to the literature method shown in Scheme S1 and was obtained as white powder (Yield: 50%) [24–26]. ap:p = 70:30. ^{1}H NMR($MeOD_4$, 300 MHz): δ 1.01(t, J = 7.5 Hz, 4.2H(ap)), 1.40(t, J = 7.5 Hz, 1.85H(p)), 2.47(m, 1.39H (p)), 2.68(m, 2.45H(ap)), 7.54(d, J = 7.9 Hz, 1.18H(ap)), 7.57(d, J = 7.6 Hz, 0.67H(p)), 7.72(d, J = 8.5 Hz, 0.99H(p)), 7.82(d, J = 8.4 Hz, 2.51H(ap)), 7.95(m, 1.48H(p)), 7.98(d, J = 8.4, 2.59H (ap)), 8.06(d, J = 1.7 Hz, 0.66H(p)), 8.08(m, 1.14H(ap)), 8.19(d, J = 1.6 Hz, 1.17H(ap)). ^{13}C NMR ($MeOD_4$, 125 MHz): δ = 12.228, 12.499, 20.208, 20.446, 121.973, 124.529, 124.591, 125.413, 128.010, 128.095, 128.341, 128.385, 129.494, 129.786, 133.918, 134.250, 137.947,138.164, 141.181, 141.252, 144.926, 144.953, 146.889, 146.994, 150.277, 150.367. HRMS *m/z*: $[M+H]^+$ Calcd for $C_{37}H_{25}F_6Na_2O_{10}S_4$ 917.0030, Found 917.0027.

2.3. Photophysical properties of SO3-BPDBTEO

UV–visible absorption spectra were obtained by a Shimazu UV-1650 PC spectrometer. Photoluminescence emission spectra were obtained using a Varian Cary Eclipse fluorescence spectrophotometer. The relative photoluminescence quantum efficiency was measured using Fluorescein in 0.1 M NaOH (1×10^{-5} M) as a reference. The pH variation test was performed using KCl buffer solutions ranging from pH 3 to 10. Time-resolved fluorescence lifetime were measured using the TCSPC technique with a PicoQuant Fluotime200 spectrometer including a PicoHarp300 TCSPC board and a PMA182 photomultiplier. The excitation source was a 377 nm ps pulsed diode laser (PicoQuant, LDH375) with a PDL800-D driver.

2.4. Photoswitching properties of SO3-BPDBTEO

The light source of the photocyclization was 365 nm of a handheld UV lamp, and the light source of the photocycloreversion was a 300 W Xe lamp equipped with a 450 nm monochromator. All experiments were carried out in 1×10^{-5} M solutions and the samples of each experiments were irradiated at a distance of 50 cm from the light source. BPDBTEO in dioxane solution was used as a reference material [26]. In the cyclization measurement, SO3-BPDBTEO (MeOH and H_2O solution) and BPDBTEO (dioxane solution) were irradiated with 365 nm light source at intervals of 20 s. In the case of cycloreversion, SO3-BPDBTEO (MeOH and H_2O solution) and BPDBTEO (dioxane solution) were irradiated with 450 nm light source at 5 min and 15 min intervals, respectively. The absorption peaks collected at each time interval were converted by using the following equation and plotted against the time [27,28].

$$I_{corrected\ absorption\ intensity} = \frac{A_{\text{Maximum peak in visible region}}}{1 - 10^{-A_{\text{Peak at irradation light wavelength}}}} \quad (1)$$

The slopes of the fitted lines from the plots were substituted into the following equation to find the cyclization quantum yield and the cycloreversion quantum yield.

$$\Phi_{1o\rightarrow 1c} = \frac{\Phi_{\text{slope, O3-BPDBTEO}}}{\Phi_{\text{slope, PDBTEO}}} \times 0.420 \quad (2)$$

$$\Phi_{1c\rightarrow 1o} = \frac{\Phi_{\text{slope, SO3-BPDBTEO}}}{\Phi_{\text{slope, BPDBTEO}}} \times 4 \times 10^{-4} \quad (3)$$

2.5. Cell preparation for imaging

HeLa cells were grown on the bottom of a cover glass-bottom dish (SPL Life sciences, 100350) in Dulbecco's Modified Eagle's Medium (DMEM) with 10% FBS at 37 °C and 5% CO_2 concentration. After we removed the medium, the cells were incubated with 50 μM SO3-BPDBTEO for 24 h. The cells on the dish were washed with PBS and fixed in 4% paraformaldehyde in PBS. For confocal imaging, we filled up the dish with PBS.

2.6. Trypan blue exclusion for cell viability

The viability test of HeLa cells was examined by trypan blue exclusion assay referring to reference. [29] HeLa cells were seeded into 6-well plates at 2×10^4 per a well. After the cells were grown for 24 h at 37 °C under 5% CO_2, SO3-BPDBTEO at concentrations of 2, 10, 20, 50, and 70 μM were added to each well and incubated for 24 h under the identical cell culture condition. After then, they were washed with 1x PBS three times and collected by trypsinization. The collected cells were centrifuged and suspended with 0.4% trypan blue solution. We counted the unstained and stained cells separately using hemocytometer and calculated the percentage of viable cells as follows.

$$\text{viable cells (\%)} = \frac{\text{total number of viable cells per mL of aliquot}}{\text{total number of cells per mL of aliquot}} \times 100 \quad (4)$$

2.7. Confocal fluorescence imaging

A confocal microscope (TCS SP8 X, Leica) was used with a HCX PL APO CS 10x/0.40 dry objective and a HC PL APO CS2 63x/1.40 oil immersion objective to observe the fixed HeLa cell. To turn on the fluorescence, 365 nm light from a UV lamp was irradiated for 3 min at a distance of 5 cm from the sample. To turn off the fluorescence, a 458 nm argon laser was exposed with 100% intensity for 5 min. The 50% intensity of 458 nm excitation source from the argon laser was used for fluorescence imaging. The fluorescence from 499 nm to 610 nm was collected by a prism dispersion-based spectral detector. All of images were optically magnified twice by LAS X software.