

## Digital Photoswitching of Fluorescence Based on the Photochromism of Diarylethene Derivatives at a Single-Molecule Level

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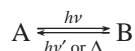
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**Abstract:** Photochromic reactions of diarylethene derivatives were detected at a single-molecule level by using a fluorescence technique. Fluorescent photoswitching molecules in which photochromic diarylethene and fluorescent bis(phenylethynyl)anthracene units are linked through an adamantyl spacer were synthesized, and switching of fluorescence upon irradiation with UV and visible light was followed in solution as well as on polymer films at the single-molecule level. Although in solution the fluorescence intensity gradually changed upon irradiation with UV and visible light, digital on/off switching between two discrete states was observed at the single-molecule level. The “on”- and “off”-times were dependent on the power of UV and visible light. When the power of UV and visible light was increased, the average on- and off-times became short in proportion to the reciprocal power of the light. The response-times were found to show distribution. The distribution of the on- and off-times is considered to reflect the difference in the micro-environment as well as conformation of the molecules.

### Introduction

Photochromism is defined as a reversible transformation of a chemical species induced in one or both directions by absorption of electromagnetic radiation between two forms, A and B, having different absorption spectra.<sup>1</sup> Therefore, the reaction is represented by the following scheme.



According to this reaction scheme, bulk molecular properties are expected to switch between two discrete states upon photoirradiation.<sup>2</sup> In other words, the observed properties or data, such as absorption intensity or refractive index, digitally switch on and off between the two states. Yet, in bulk systems, where average behavior is observed, this effect is masked by the inherent averaging process. The experimental data gradually change upon photoirradiation, because under normal conditions there exists a vast number of molecules in the system and only

the A/B ratio changes upon photoirradiation. Although such analogue behavior is so far named “switching”, the performance is not “switching” in the literal sense of the word.

If we can detect the photochromic reaction at a single-molecule level, real digital photoswitching is anticipated. Recent advances in fluorescence microscopy have allowed the detection, imaging, and spectroscopy of single molecules at room temperature.<sup>3–21</sup> Although the single-molecule detection tech-

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nique is successfully applied to characterize the properties of individual molecules in various micro-environments,<sup>22–31</sup> the direct observation of the photochromic reaction at the single-molecule level has not yet been accomplished and single-molecule photocontrol is limited.<sup>32–35</sup> For the observation, it is indispensable to prepare a photochromic molecule, which changes the fluorescence intensity along with the photochromic reaction and has high photostability. Green fluorescence protein is the only example that exhibits the optically induced switching of fluorescence at room temperature.<sup>19</sup> There are no artificial fluorescent photochromic molecules that can be applied to the single-molecule experiment. It is required to newly design and synthesize robust fluorescent photochromic or photoswitching molecules. As the photoswitching unit, diarylethene derivatives with heterocyclic aryl groups are the most promising candidates because of their fatigue resistant photochromic performance.<sup>36</sup> The derivatives can repeat photoinduced coloration/decoloration cycles more than 10<sup>4</sup> times, and the photocolored isomers are stable for more than a thousand years at 30 °C.<sup>37</sup> When an appropriate fluorescent unit is connected to the chromophores, the photochromic reaction can be detected by the fluorescence intensity change even at the single-molecule level.<sup>38</sup>

In this study, a new robust fluorescent diarylethene derivative with one methoxy group at the reactive carbon has been synthesized in addition to the previous derivative<sup>38a</sup> with two methoxy groups, and their photoswitching performance was studied in detail in solution (an ensemble system) as well as on polymer films at the single-molecule level. At the single-molecule level, digital switching between two distinct states was observed, and the different behavior of the two derivatives indicated that the switching of fluorescence is unambiguously based on the photochromic reaction.

## Results and Discussion

### Molecular Design of Fluorescent Photochromic Molecules.

The molecules that we synthesized are shown in Scheme 1. Photochromic diarylethene and fluorescent bis(phenylethynyl)-anthracene units are covalently linked through a rigid adamantyl spacer group. Methoxy substituents are introduced at the reactive carbons to decrease the cycloreversion quantum yield.<sup>39</sup> Two derivatives having one or two methoxy groups were prepared. Their photochromic performances are similar to each other except the cycloreversion quantum yield. The effectiveness of the methoxy substituents for the observation of the single-molecule photoswitching will be discussed in detail in the last part. The bis(phenylethynyl)anthracene unit with methoxy substituents was employed as the fluorescent unit, because of its high fluorescence quantum yield, appropriate fluorescence spectral region, and fairly good fatigue resistant property.

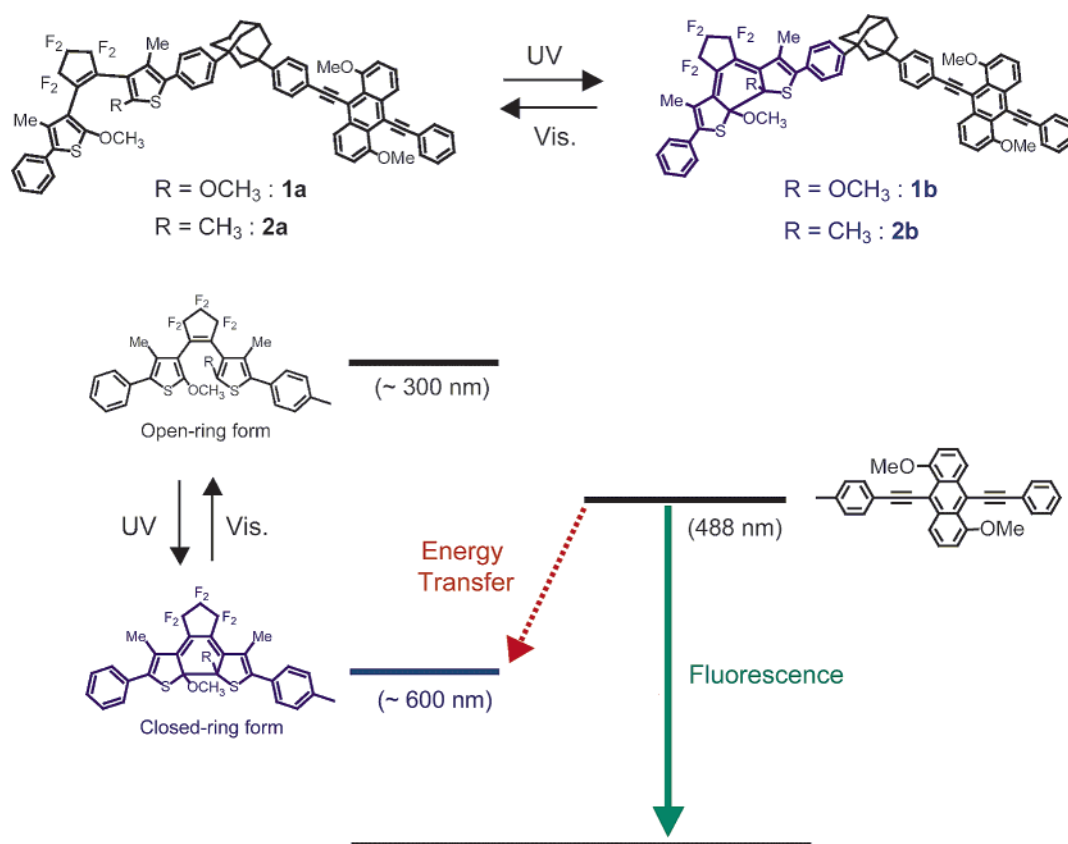
**1a** converts to **1b** upon irradiation with UV (300–350 nm) light, and **1b** returns to **1a** upon irradiation with visible (>450 nm) light.<sup>39,40</sup> The relative energy levels of the component chromophores are also shown in Scheme 1. The bis(phenylethynyl)anthracene unit has characteristic absorption and fluorescent bands at 488 and 503 nm, respectively.<sup>41</sup> The fluorescence spectrum well overlaps the absorption spectrum of the closed-ring form of the diarylethene unit. Therefore, the fluorescence is efficiently quenched when the diarylethene unit converts from the open- to the closed-ring forms. On the other hand, when the diarylethene is in the open-ring form, its energy level is higher than the level of the bis(phenylethynyl)anthracene unit and the fluorescence quenching does not take place. The detailed quantitative data are shown in the following section.

**Fluorescence Change in Ensemble Solution upon Photoirradiation.** Figure 1a shows the absorption spectral change of **1a** in a toluene solution (an ensemble system) upon irradiation with 313 nm light. A visible absorption band at around 630 nm gradually increased and reached a photostationary state. The absorption band is due to the closed-ring isomer **1b**, and the gradual increase is due to the photocyclization of the diarylethene unit.<sup>39,40</sup> Figure 1b shows the fluorescence spectral change along with the photocyclization. The initial fluorescence quantum yield of **1a** was 73%. The fluorescence intensity gradually decreased in proportion to the conversion from **1a** to

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Scheme 1

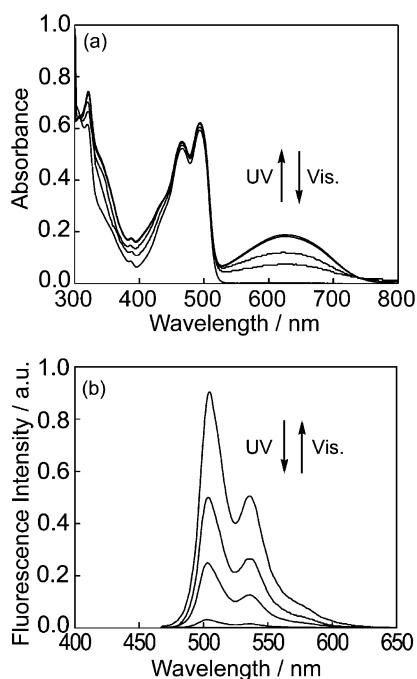


**1b**. At the conversion of 66%, the fluorescence decreased to 34% of the initial intensity. **1b** was almost nonfluorescent. Upon irradiation with  $>450$  nm light, the fluorescence again gradually returned to the initial intensity.

The fluorescence lifetimes and the quantum yields of **1a** and **1b** were measured and are shown in Table 1. The lifetime of

**1a** was 4.9 ns, while it decreased to 4.5 ps for **1b**. The lifetime as well as the fluorescence quantum yield of **1a** are similar to those of the bis(phenylethynyl)anthracene molecule.<sup>41</sup> This result confirms that the fluorescence is scarcely perturbed by the opening form of the diarylethene unit. On the other hand, the very short lifetime of **1b** indicates that the excited-state energy of the bis(phenylethynyl)anthracene unit is effectively intramolecularly transferred to the closed-ring form of the diarylethene unit. From the fluorescence lifetimes, the energy-transfer rate was estimated to be  $2.2 \times 10^{11} \text{ s}^{-1}$ .

The photocyclization quantum yield of **1a** (0.12) is smaller than that of the diarylethene molecule (0.52).<sup>42</sup> This decrease is ascribed to two factors. One factor is the overlapping of the absorption band of the bis(phenylethynyl)anthracene and diarylethene units, indicates that 47% of the absorption of **1a** at 313 nm is due to the bis(phenylethynyl)anthracene unit. Only a half of the absorbed photons by **1a** can be used for the photocyclization reaction. Another factor is the excited-state energy transfer from the diarylethene unit to the bis(phenylethynyl)anthracene unit through the adamantyl spacer, because the energy level of the bis(phenylethynyl)anthracene unit is lower than that of the open-ring form of the diarylethene unit (Scheme 1). The energy transfer is, however, much lower than the efficiency from the excited bis(phenylethynyl)anthracene unit to the closed-ring form of the diarylethene unit. This is ascribed to the difference in the lifetimes of the two excited states. The lifetime of the open-ring form of the diarylethene is around 1 ps,<sup>43</sup> while the lifetime of the bis(phenylethynyl)anthracene is



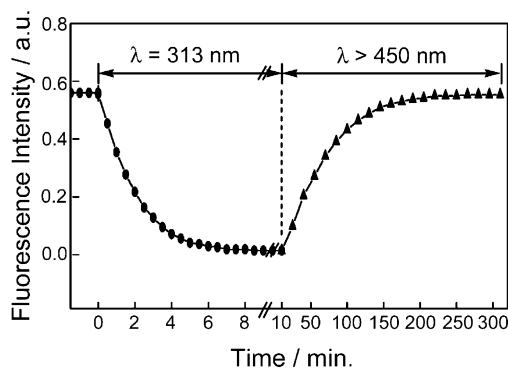
**Figure 1.** (a) Absorption and (b) fluorescence spectral changes of **1** in toluene upon irradiation with 313 nm light. The concentration of **1** in toluene was  $1.2 \times 10^{-5}$  M.

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**Table 1.** Photocyclization ( $\Phi_{o\rightarrow c}$ ), Photocycloreversion ( $\Phi_{c\rightarrow o}$ ) Quantum Yields of **1** and **2**, and Fluorescence Properties of Compounds **1**, **2**, and 1,5-Dimethoxy-9,10-bis(phenylethynyl)anthracene in Toluene

compound	$\Phi_{o\rightarrow c}^a$	$\Phi_{c\rightarrow o}$	$\lambda_{f,max}/nm$	$\Phi_f$	$\tau/ns$
<b>1a</b>	0.12		503, 539	0.73	4.9
<b>1b</b>		$<8 \times 10^{-5}^b$		$<0.001$	$0.0045 \pm 0.0015$
<b>2a</b>	0.12		503, 537	0.73	4.9
<b>2b</b>		$1.5 \times 10^{-3}^c$			
1,5-dimethoxy-9,10-bis(phenylethynyl)anthracene <sup>41</sup>			501, 537	0.72	5.0

<sup>a</sup> Measured by irradiation with 313 nm light. <sup>b</sup> Measured with 630 nm light. <sup>c</sup> Measured with 600 nm light.

**Figure 2.** Fluorescence intensity changes of **1** upon alternate irradiation with 313 and  $>450$  nm light in toluene. The concentration of **1** was  $5 \times 10^{-7}$  M.

4.9 ns. The short lifetime scarcely suppresses the photocyclization reaction, and the long lifetime results in efficient fluorescence quenching. The photocycloreversion quantum yield of **1b** was very low, less than  $10^{-4}$ . The very low quantum yield is a necessary condition to clearly detect the photoswitching performance at the single-molecule level.

Figure 2 shows the typical analogue-type photoresponse of **1** in a toluene solution (an ensemble system). Upon irradiation with 313 nm light, the fluorescence intensity gradually decreased in 10 min, while it increased upon irradiation with  $>450$  nm light. It took about 5 h for **1b** to return to **1a** under the present experimental conditions.

**Photoswitching of Fluorescence Based on Photochromism at the Single-Molecule Level.** The photoresponse behavior of **1** dramatically changed at the single-molecule level. Compound **1** was dispersed on a Zeonex polymer film (thickness:  $\sim 100$  nm) by spin-coating a toluene solution containing  $2 \times 10^{-11}$  M of **1a**, and the fluorescence change upon photoirradiation was followed at the single-molecule level. The polymer film was placed on the sample stage of a confocal microscope with an oil immersion lens (100 $\times$ , N.A. = 1.4) equipped with an avalanche photodiode detector (APD).

Figure 3 shows the images of the single molecules. All bright spots are due to fluorescence from single **1a** molecules. Upon UV (325 nm, 0.27 mW) light irradiation for 5 s, some of the spots, indicated by circles 1–4, disappeared, as shown in image (b). The lost spots again appeared, as shown in images (c) and (d), upon continuous irradiation with 488 nm light, which excite

both the bis(phenylethynyl)anthracene unit and the closed-ring form of the diarylethene unit. Interestingly, some of the spots (2 and 3) appeared after irradiation with visible light for 5 s, but others (1 and 4) appeared only after 10 s of irradiation. Different molecules showed different response-times.

Among 11 bright spots, 7 spots did not show any response to UV and visible light. These stable spots are considered to be in a nonphotoactive state. Diarylethenes are well known to take two kinds of conformations, parallel (mirror symmetry) and antiparallel ( $C_2$  symmetry), and only the antiparallel conformer can undergo the photocyclization reaction.<sup>44</sup> The parallel conformer is photochemically inactive. In the molecules that give stable bright spots, the diarylethene unit is in a parallel conformation or less probably in a photoinactive nonflexible antiparallel conformation. The photochemical response can give us information concerning the conformation of the diarylethene unit.

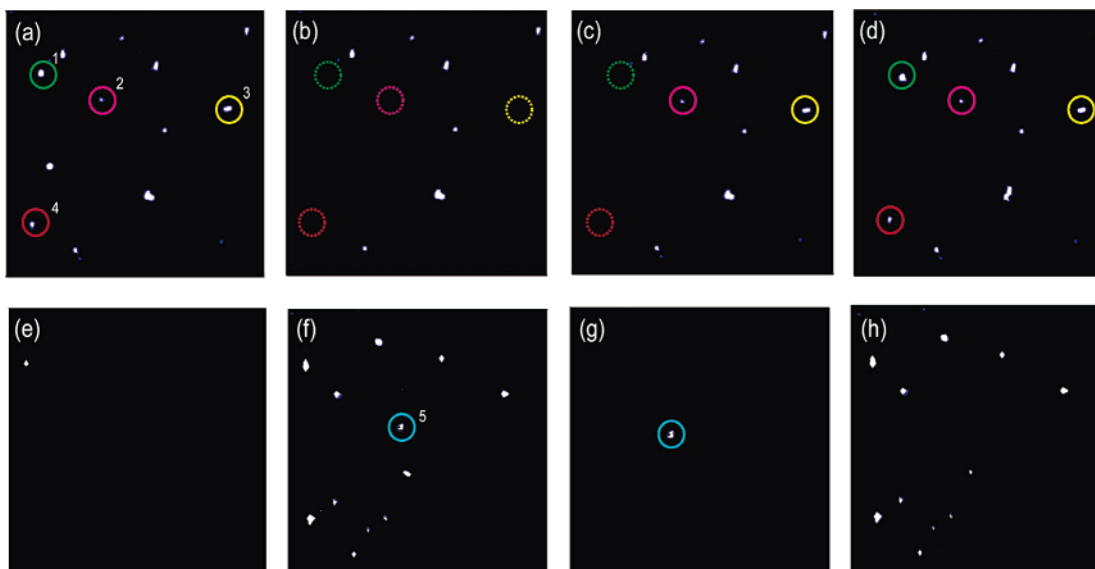
This interpretation was confirmed by dispersing nonfluorescent closed-ring isomers **1b** on the Zeonex film. Images (e–h) of Figure 3 show the photoresponse of the polymer film. Initially, the film is nonfluorescent, because all molecules are in the nonfluorescent closed-ring form.<sup>45</sup> Upon irradiation with visible (488 nm, 200 W/cm<sup>2</sup>) light for 10 s, 12 molecules became bright, as shown in image (f). These bright spots except one spot (shown by a circle) disappeared upon irradiation with UV light (325 nm, 0.27 mW/cm<sup>2</sup>) for 5 s as shown in image (g). In this system, 11 molecules are in the photoactive antiparallel conformation. Therefore, the molecules can undergo reversible photocyclization reactions. The bright spots again appeared upon irradiation with visible light. The dark/bright cycles could be repeated upon alternate irradiation with visible and UV light. The bright spots in image (g) disappeared possibly due to photodecomposition.

To reveal the detailed switching characteristics of the molecules, the time trace of single-molecule **1b** was measured under irradiation with both visible (488 nm, 100 W/cm<sup>2</sup>) and UV (0.027 mW/cm<sup>2</sup>) light, as shown in Figure 4a. Initially, the molecule was in the nonfluorescent off-state. At 5.6 and 21.0 s, the nonfluorescent molecule was abruptly switched to the fluorescent on-state. These digital responses are due to photoisomerization from **1b** to **1a** by the visible light irradiation. At 15.7 and 30.1 s, the fluorescence molecule was abruptly switched to the nonfluorescent off-state. These digital responses are due to photoisomerization from **1a** to **1b** by UV light irradiation. The fluorescence intensity switched on and off

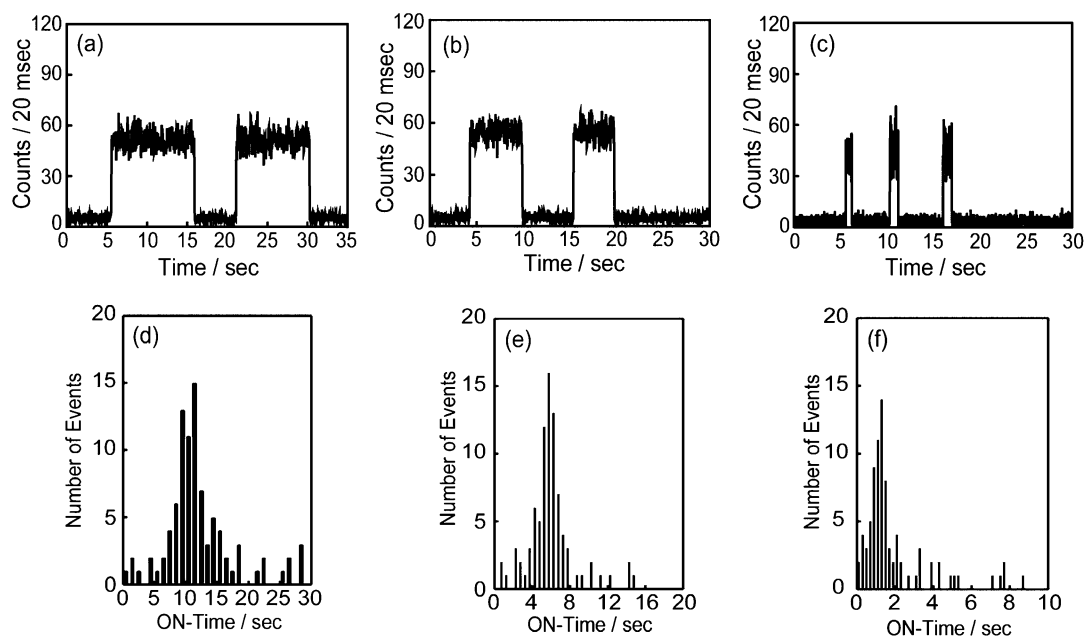
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(45) A bright spot in the image (e) is assigned to the **1a** molecule, which was photogenerated during the adjustment of the measuring system from the nonfluorescent **1b** molecule.



**Figure 3.** (a–d) Single-molecule fluorescence imaging of **1a** embedded on a Zeonex thin polymer film. (a) Before irradiation. (b) After irradiation with UV light (325 nm, 0.27 mW/cm<sup>2</sup>) for 5 s. After irradiation with visible light (488 nm, 200 W/cm<sup>2</sup>) for (c) 5 s, and (d) an additional 5 s. (e–h) Single-molecule fluorescence imaging of **1b** embedded on a Zeonex thin polymer film. (e) Before irradiation. (f) After irradiation with visible light (488 nm, 200 W/cm<sup>2</sup>) for 10 s. (g) After irradiation with UV light (325 nm, 0.27 mW/cm<sup>2</sup>) for 5 s. (h) After irradiation with visible light (488 nm, 200 W/cm<sup>2</sup>) for 10 s: imaging size, 10 μm × 10 μm (64 pixels × 64 pixels); integration time, 100 ms/pixel, N<sub>2</sub> atmosphere. The distorted images are due to the low resolution of the measuring system. The number of pixels is limited to 64 × 64.



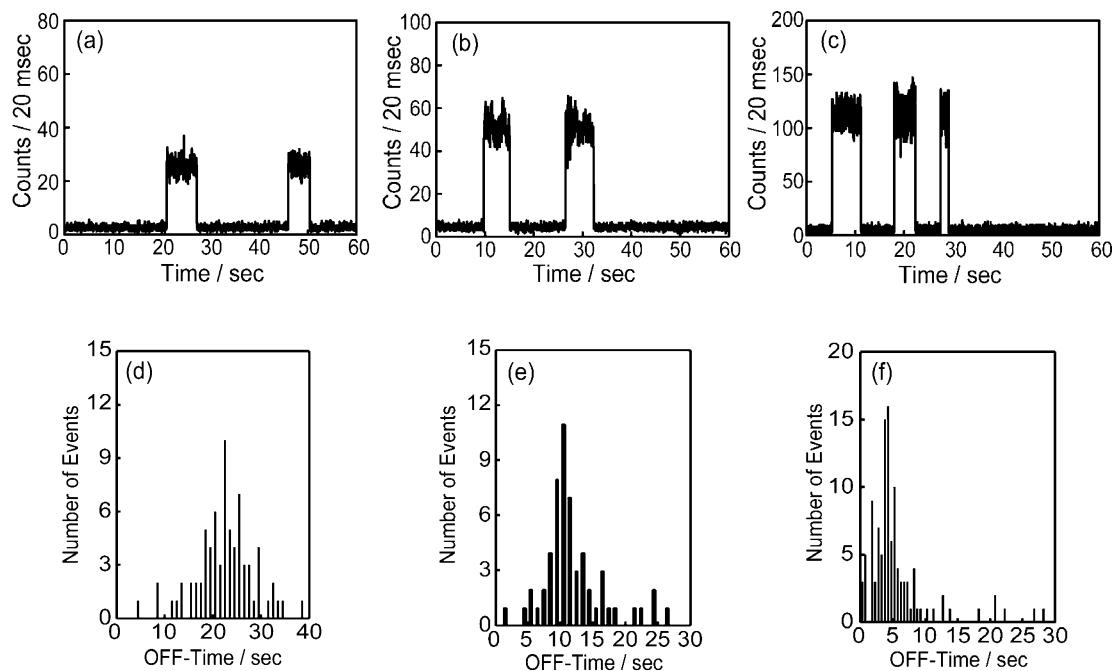
**Figure 4.** (a–c) Time traces of fluorescence intensity of **1** embedded on Zeonex thin films irradiated with both strong 488 nm light (fixed power, 100 W/cm<sup>2</sup>) and weak 325 nm light (variable power). (d–f) Histograms of on-time. The histograms were constructed by collecting on-times of 1–3 events for each molecule. Power of 325 nm light: (a,d) 0.027 mW/cm<sup>2</sup>; (b,e) 0.054 mW/cm<sup>2</sup>; (c,f) 0.27 mW/cm<sup>2</sup>. Average on-time: (d) 11 s (73 molecules); (e) 5.7 s (94 molecules); (f) 1.2 s (80 molecules).

between two discrete states based on the photochromic reaction of the diarylethene unit.

The on-time was dependent on the power of the UV light. When the power was increased, the duration of the on-time became short. As can be seen in Figure 4a–c, the on-time decreased in proportion to the power of the UV light. In this experiment, UV light is irradiated from the beginning. When the diarylethene unit is in the closed-ring form or the molecule is in the off-state, UV light scarcely affects the photocycloreversion reaction, because the UV power is very weak in comparison with the power of visible light (around 1/10<sup>6</sup>). The

photocycloreversion reaction is mainly controlled by the visible light. On the other hand, when the unit is in the open-ring form or the molecule is in the on-state, the weak UV light is effective at inducing the photocyclization reaction. The photocyclization quantum yield is around 10%, which means that only 10 photons are enough to induce the photocyclization reaction or switch from the on-state to the off-state. The single-molecule switch is very sensitive to UV light.

To obtain the details of the digital photoresponsive behavior of individual molecules, we measured the on-time for 50–100 molecules by changing the power of the UV light, as shown in



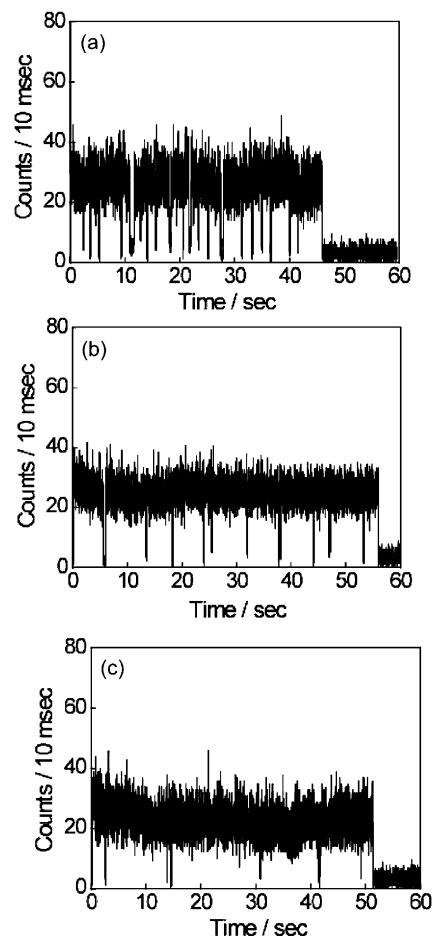
**Figure 5.** (a–c) Time traces of fluorescence intensity of **1** embedded in Zeonex thin films irradiated with both strong 488 nm light (variable power) and weak 325 nm light (fixed power, 0.054 mW/cm<sup>2</sup>). (d–f) Histograms of off-time. The histograms were constructed by collecting off-times of 1–3 events for each molecule. Power of 488 nm light: (a,d) 50 W/cm<sup>2</sup>; (b,e) 100 W/cm<sup>2</sup>; (c,f) 300 W/cm<sup>2</sup>. Average off-time: (d) 23 s (81 molecules); (e) 11 s (57 molecules); (f) 4.0 s (107 molecules).

Figure 4d–f. The average on-time correlated to the reciprocal power of the UV light. When the power was increased from 0.027 to 0.27 mW/cm<sup>2</sup>, the average on-time decreased from 11 to 1.2 s. The distribution of the on-time is considered to reflect the difference in micro-environments as well as the conformation of the molecules.

The off-time was also examined by changing the power of visible (488 nm) light, as shown in Figure 5. The off-time, or the interval between adjacent on-states, decreased with an increase in the power. It decreased from 23 to 4.0 s by increasing the power from 50 to 300 W/cm<sup>2</sup>.

**Photoswitching of Fluorescence of a Molecule with One Methoxy Substituent at the Reaction Site of the Diarylethene Unit.** Figure 6 shows the fluorescence switching of compound **2** dispersed on the Zeonex film, just as the previous experiment used for compound **1**, at the single-molecule level. The triplet blinking-like decrease of fluorescence<sup>10,17,46</sup> is due to the photochromic reaction from fluorescent **2a** to nonfluorescent **2b**. This was confirmed by changing the power of the UV light. When the power was decreased from 0.27 to 0.027 mW/cm<sup>2</sup>, the frequency of the fluorescence switching dramatically decreased. The decrease in the frequency indicates that the switching is not due to the triplet blinking but due to the photochromic reaction.

The molecule **2b** has a photocycloreversion quantum yield of  $1.5 \times 10^{-3}$ , which is more than 20–50 times larger than that of **1b**. The average off-time of the fluorescence for compound **2** is 220 ms under irradiation with visible light (488 nm, 100 W/cm<sup>2</sup>), while it is around 11 s for compound **1** under irradiation with the same light intensity. The decrease of the off-time is ascribable to the change of the cycloreversion



**Figure 6.** Time traces of fluorescence intensity of **2** embedded on a Zeonex thin film irradiated with both strong 488 nm light (fixed power, 100 W/cm<sup>2</sup>) and weak 325 nm light (variable power): (a) 0.27, (b) 0.054, (c) 0.027 mW/cm<sup>2</sup>.

(46) Vosch, T.; Hofkens, J.; Cotlet, M.; Köhn, F.; Fujiwara, H.; Gronheid, R.; Van Der Biest, K.; Weil, T.; Herrmann, A.; Müllen, K.; Mukamel, S.; Van der Auweraer, M.; De Schryver, F. C. *Angew. Chem. Int. Ed.* **2001**, *40*, 4643–4648.

quantum yield. In other words, the fluorescence switching is unambiguously based on the photochromic reaction. When the bleaching quantum yield is larger than  $10^{-2}$ , as observed in normal photochromic compounds, the switching phenomena are hardly detected. The very low photocycloreversion quantum yield is a necessary condition to clearly detect the digital fluorescence switching between two discrete states due to the photochromic reaction.

## Conclusions

Fluorescent photoswitching molecules in which photochromic diarylethene and fluorescent bis(phenylethynyl)anthracene units are covalently linked through an adamantyl spacer were synthesized, and the fluorescence switching of the diarylethene derivatives was observed by irradiation with UV and visible light at the single-molecule level. Digital on/off switching between two discrete states was observed on polymer films at the single-molecule level. This provides unequivocal evidence that the photochromic reaction takes place between two discrete states. The fluorescent diarylethene derivatives can be potentially utilized for ultrahigh-density erasable optical data storage (1 bit per molecule,  $\sim$ peta bit/in<sup>2</sup>), when novel techniques to address each molecule by photon are developed.

## Experimental Section

**General.** Solvents used in the photochemical measurements were spectroscopic grade and were purified by distillation before use.

Steady-state absorption and fluorescence spectra in solution were measured with an absorption spectrophotometer (Hitachi U-3500) and a fluorescence spectrophotometer (Hitachi F-3010), respectively. Photoirradiation was carried out using a 1000 W high-pressure mercury lamp or a 500 W xenon lamp as the light source. Monochromic light was obtained by passing the light through a monochromator (Ritsu MV-10N) or a band-pass filter ( $\Delta\lambda_{1/2} = 15$  nm).

The cyclization quantum yield was determined by comparing the photocyclization rates of samples and furyl-fulgide with the standard procedure.<sup>47</sup> The cycloreversion quantum yield was also measured using furyl-fulgide in toluene as a reference.<sup>47</sup> The fluorescence quantum yield ( $\Phi_f$ ) was evaluated utilizing 9,10-bis(phenylethynyl)anthracene as the standard of  $\Phi_f = 1.0$ .<sup>48</sup>

Fluorescence decay curves with the picosecond time resolution were measured by the time-correlated single-photon counting spectroscopy. A second harmonic (420 nm, pulse width  $\sim$ 200 fs) of a hybrid mode-locked dispersion compensated femtosecond dye laser (Coherent Satori 774) pumped by a CW mode-locked Nd:YAG laser (Coherent Antares 76S) was used as an excitation source. A repetition rate of the excitation pulse was reduced to 3.8 MHz with an external pulse picker (Conoptics,

model 360-80, 25D, and 305). A microchannel-plate photomultiplier (Hamamatsu, MCP R2809U) was used as a detector, which gave an instrument response-time constant of  $\sim$ 30 ps (fwhm). The emission wavelength of 510 nm was selected by a monochromator. The fluorescence decay curves were analyzed by the nonlinear least-squares iterative convolution method based on a Marquardt algorithm. For the nanosecond resolution measurements, a pulsed N<sub>2</sub> laser (Hamamatsu Photonics, LN-203, 337.1 nm, 600 ps) was used as a light source, and the emission was detected by a gated streak-scope system (Hamamatsu Photonics, C4334-1, C5094, and C-4792) in the single-photon counting mode.

**Materials.** Detailed synthetic procedures of compounds **1a** and **2a** are described in the Supporting Information. The molecules were carefully purified by GPC and HPLC. The molecular structure was confirmed via <sup>1</sup>H NMR, elemental analysis, and mass spectroscopy, and the purity was evaluated by HPLC. The closed-ring isomers **1b** and **2b** were prepared by irradiating the hexane or toluene solutions with UV light and were isolated by HPLC.

**Single-Molecule Detection.** Samples for single-molecule measurements were prepared by spin-coating a  $2 \times 10^{-11}$  M toluene solution of open-ring isomers (**1a** and **2a**) and closed-ring isomers (**1b** and **2b**) isolated by HPLC on a quartz cover glass coating with a Zeonex film (50–100 nm) at 4000 rpm. Zeonex is a kind of amorphous polyolefin of Nippon Zeon Co. Ltd.

The single-molecule fluorescence was detected using a confocal microscope (TCS-NT, Leica) with an oil immersion lens (Leica PL APO CS, NA 1.4) equipped with an avalanche photodiode (APD) in single-photon counting mode (EG&G, SPCM AQR-14) as the detector. Appropriate notch (Kaiser Optical) and long-pass (Chroma) filters were placed in the detection path to suppress remaining excitation light and detect the fluorescence longer than 500 nm. The fluorescence intensity transients were measured with a bin time of 20 ms or 10 ms. The excitation source was an argon ion laser at 488 nm (Spectra Physics Stabilite 2017). A He–Cd laser at 325 nm (KINMON ELCTRIC, IK3151R-E) was used for the photocyclization. Circular polarized light was used as the excitation and reaction light for equally exciting molecules. All single-molecule experiments were carried out under nitrogen atmosphere.

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**Supporting Information Available:** Detailed synthetic procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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