

Supplementary information for

## **One-colour control of activation, excitation, and deactivation of a fluorescent diarylethene derivative in super-resolution microscopy.**

Yuhei Arai<sup>a</sup>, Syoji Ito<sup>a\*</sup>, Hajime Fujita<sup>a</sup>, Yusuke Yoneda<sup>a</sup>, Takahiro Kaji<sup>b</sup>, Satoshi Takei<sup>c</sup>, Ryota Kashihara<sup>d</sup>, Masakazu Morimoto<sup>d</sup>, Masahiro Irie<sup>d\*</sup> and Hiroshi Miyasaka<sup>a\*</sup>

<sup>a</sup> *Division of Frontier Materials Science and Center for Advanced Interdisciplinary Research, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan.*

<sup>b</sup> *Advanced ICT Research Institute, National Institute of Information and Communications Technology, 588-2 Iwaoka, Nishi-ku, Kobe 651-2492, Japan.*

<sup>c</sup> *Eco-material Engineering, Toyama Prefectural University, Imizu, Toyama 939-0398, Japan.*

<sup>d</sup> *Department of Chemistry and Research Center for Smart Molecules, Rikkyo University, 3-34-1 Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501, Japan.*

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## S1. Details on the experimental setup

### S1.1. Sample preparation

As a sample for single-molecule tracking with the diarylethene derivative, poly(2-hydroxyethyl acrylate) (polyHEA,  $M_n = 2670$ ,  $M_w/M_n = 1.49$ ,  $T_g = 290$  K) was used as a host polymer. Thin film (with a thickness of ca. 80 nm) was prepared on a well-cleaned glass substrate by spin-casting (at 2000 rpm for 120 s) with an ethyl lactate solution of polyHEA (1.0 wt%) and 1,2-Bis(2-ethyl-6-(5-methylthiophen-2-yl)-1-benzothiophen-1,1-dioxide-3-yl)perfluorocyclopentene (**1o**) in Fig. 1 (a), at a 0.5 nM concentration in the mother solution [S1].

For one-colour PALM, we prepared microstructures of dried ionic polymer gel in the following manner. 100 mg of poly(dimethylaminoethyl methacrylate) (PDMEMA, Kanto Kagaku, Japan) and 100 mg of N,N,N',N'-tetra(trifluoromethanesulfonyl)-dodecane-1,12-diamine (C12TFSA, Kanto Kagaku, Japan) were dissolved with 1 mL of toluene including small amount of **1o** ( $\sim 10^{-13}$  M) in a vial. The mixed solution was heated up at 353K for 3 hours using a water bath. This thermal treatment efficiently led to the formation of crosslinked polymer gel including the toluene solution of **1o**. Then the polymer gel in the vial was kept in a light-shielding container in an experimental room for at least one night to cool down to a room temperature. Small volume of the gel was spread onto a well-cleaned coverslip and the specimen was dried in a constant-temperature oven at 353K for 3 hours.

Microstructures of PMMA (ALDRICH,  $M_w$  996000) was also prepared for PALM by using electron beam lithography. PMMA films with 60-nm thickness were produced on Si substrate by spin-casting of toluene solution of the PMMA (1 wt%) including small amount of **1o** ( $\sim 10^{-7}$  M). The films were exposed by high-energy (at 75 kV accelerating voltage) electron beam using the electron beam lithography system (ELS-7700, Elionix) and developed with isopropyl alcohol : methyl isobutyl ketone = 3:1 solution.

### S1.2. Wide-field fluorescence imaging

Single-molecule fluorescence imaging was carried out with a home-built wide-field system consisting of an inverted optical microscope (IX70, Olympus), an objective lens (UPlanApo Oil Iris [100x/NA1.35], Olympus), a continuous wave laser at 532 nm (Exelsior 532, Spectra-Physics) and an electron-multiplying charge-coupled device (EM-CCD) camera (ImagEM C9100-13, Hamamatsu Photonics). Details were reported previously [S2]. The illumination area of the green (532 nm) light at the sample plane was adjusted by using two external lenses with a focal length of 200 mm and a beam expander. The spot size of the laser at 532 nm was ca. 20  $\mu\text{m}$  in diameter at the sample position. The fluorescence from individual DEs in the samples was detected by the EM-CCD camera as digital videos. All the measurements were performed at  $295 \pm 0.5$  K., unless otherwise specified.

## S2. Excitation via Urbach tail in the absorption spectrum

Molecules in the vibrationally excited state can allow the optical transition into the electronically excited state with the energy lower than that of the 0-0 transition,  $E_{00}$ . The absorption intensity of the hot band scales with the Boltzmann factor of the vibrationally excited state in the electronically ground state [S3]. Even in the case where the vibrational progression in absorption spectra is obscure, an exponential tail as represented by eq. S1 has been observed as Urbach tails [S4-6].

$$A(E_{exc}) \propto A(E_{00}) \cdot \exp\left[-\frac{\sigma \cdot (E_{00} - E_{exc})}{k_B T}\right] \quad (\text{S1})$$

Here,  $k_B$ ,  $T$  and  $\sigma$  are Boltzmann constant, temperature and the steepness parameter relating to the coupling between the solute and the environment and the difference of the minimum positions in the ground- and excited states. This  $\sigma$  is dependent on the temperature and the values of 0.5 – 1.5 in the temperature range of 50 -330 K were reported for organic dyes in solution and in the crystalline phase [S5, S6].

### Absorption spectra of the open-ring isomer, **1o**.

To investigate the absorption tail of the open-ring isomer, **1o**, we measured absorption spectra in three kinds of solutions, n-hexane, carbon tetrachloride and ethyl acetate (Figure S1). Although an absorption spectrum in the polymer film provides the direct information, the small value of the absorbance due to the low concentration of **1o** and the thin thickness of the film inhibit the reliable measurement with rather wide range of the absorbance. The ordinate of Figure S1 is given as the absorbance divided by the frequency corresponding to the wavelength. In addition, this value was normalized around 370 nm ( $27000 \text{ cm}^{-1}$ ,  $\epsilon = 26000 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ). The spectra in these solutions clearly show the exponential tails of  $A(\nu)/\nu$  in the frequency region  $< \text{ca. } 24000 \text{ cm}^{-1}$  ( $> \text{ca. } 415 \text{ nm}$ ). The slope of the exponential tail,  $\sigma$ , in eq.1 is ca. 0.4 – 0.5 for these three solutions. Because the absorption spectra and their tails were independent of the concentration, these tails are not attributable to the aggregation.

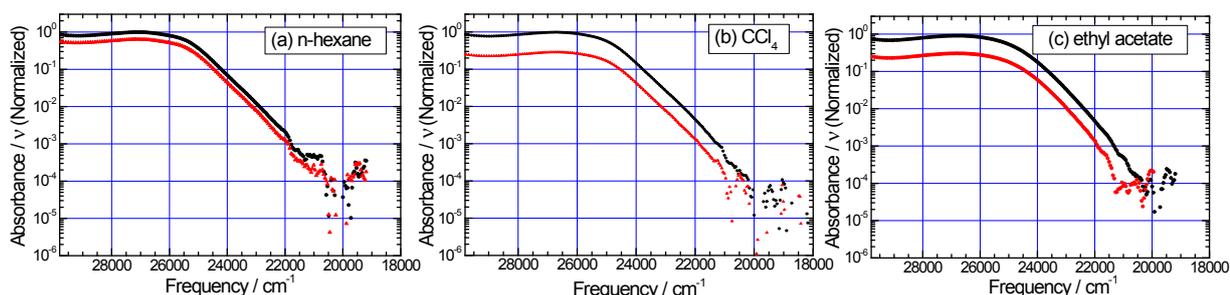


Figure S1. Steady-state absorption spectra of **1o** in (a) n-hexane, (b) carbon tetrachloride and (c) ethyl acetate solutions. The ordinate is given as the absorbance divided by the frequency and normalized at ca. 370 nm. Concentrations of **1o** in n-hexane are  $4.7 \times 10^{-5} \text{ M}$  for the black line and  $3.0 \times 10^{-5} \text{ M}$  for the red line. Those for carbon tetra chloride are  $1.0 \times 10^{-4}$  and  $3.0 \times 10^{-5} \text{ M}$  and those for ethyl acetate are  $9.3 \times 10^{-5}$  and  $3.1 \times 10^{-5} \text{ M}$ .

### Estimation of the absorption probability of **1o** at 532 nm.

As was shown in the previous section, the present diarylethene derivative, **1o**, has small  $\sigma$  values. With the  $\sigma$  value of 0.5, we estimated the excitation probability of **1o** at 532 nm in the polymer film under the excitation conditions shown in table S1.

Table S1. Excitation conditions under the microscope.

Excitation Intensity at 532 nm ( $I_0$ )	2 mW (0.88 kW/cm <sup>2</sup> )	5.358×10 <sup>15</sup> photons
Concentration of <b>1o</b> ( $C$ )	3×10 <sup>-8</sup> mol/L	Concentration of the mother solution was 5 x 10 <sup>-10</sup> mol/L.
Molar absorption coefficient at 532 nm ( $\epsilon$ )	1×10 <sup>-2</sup> M <sup>-1</sup> cm <sup>-1</sup>	Estimated from the extrapolated value of the absorption spectrum of ethyl acetate solution.
Thickness of the polymer film ( $d$ )	80 nm	8×10 <sup>-6</sup> cm

The absorbance of **1o** in the film was estimated to be  $4.0 \times 10^{-15}$  with the values in Table S1.

$$\begin{aligned} A(532) &= \epsilon \times C \times d \\ &= (1 \times 10^{-2}) \times (3 \times 10^{-8}) \times (8 \times 10^{-6}) = 2.4 \times 10^{-15} \end{aligned} \quad (\text{S2})$$

The absorbed photon (the number of the molecule excited) is represented by eq. S3.

$$I_0 - I = I_0(1 - 10^{-\epsilon cd}) \approx I_0(\ln 10 \epsilon cd) = (5.358 \times 10^{15}) \times 2.303 \times (2.4 \times 10^{-15}) \approx 29.6 \quad (\text{S3})$$

The number of molecules excited in the exposed area is estimated to be ca. 30 in 1 sec. (1776 molecules in 1 min.). By taking the cyclization quantum yield (0.23) into account, the number of the fluorescence spots is 408 in 1 min.

As was shown in Figure 2a in the main text, the number of fluorescent molecules newly appearing under the 532-nm irradiation was 100 in 1 min. (The number of molecules excited could be 435 molecules in 1 min. or 7.2 molecules in one sec., by taking the cyclization into account.) Although the value estimated above (eq.S3) is several times larger than that experimentally obtained, the exposed area is larger than the observation one. In addition, some conditions such as molar absorption coefficient and concentration in the film may involve ambiguity to some extent. The value thus estimated is, however, in the same order of the number of the fluorescence spot experimentally obtained. This result strongly supports that the excitation of the hot band (Urbach tail) leads to the OFF  $\rightarrow$  ON switching at 532 nm.

### S3. Fluorescence spectra the diarylethene derivative in PMMA film

To assign the species of the fluorescent spots turned on by the irradiation with the 532-nm laser light, we measured fluorescence spectra of the PMMA film including **1o**. The film was prepared on well-cleaned cover slips by spin-casting of the toluene solution of PMMA (Sigma Aldrich,  $M_w$ : 15000,  $T_g$ : 355K, 5 wt%) and the open form (**1o**) of the DE ( $1.0 \times 10^{-6}$  M). The CW 532-nm laser light was focused onto the PMMA films by using a microscope objective (UPLFLN 100XO12,  $\times 100$ , NA 1.30, Olympus). The intensity of the laser light at the sample was estimated to be  $7.0 \times 10^2$  W/cm<sup>2</sup>. The emission spectrum of the photo-excited area of the PMMA films was measured by using a spectrometer (SP2358, Princeton Instruments) and an EM-CCD detector (PIXIS-100B, Princeton Instruments). The fluorescence spectrum thus obtained is shown as a red curve in figure S2 (a). To compare the fluorescence spectrum with that of **1c**, we measured a fluorescence spectrum of the PMMA film under simultaneous

exposure to ultraviolet (355 nm, pulse width 0.5 ns, intensity 0.1 W/cm<sup>2</sup>) and 532-nm (intensity 11 W/cm<sup>2</sup>) laser light. This UV irradiation induced efficient photoisomerization from the open form (**1o**) to the closed form (**1c**) and the fluorescence spectrum of **1c** could be measured at very weak excitation intensity at 532 nm. The spectrum of **1c** in the PMMA film thus obtained is shown in Fig. S2 (a) as a blue curve, indicating that **1c** is the emissive species under the 532-nm irradiation (without 355-nm laser).

The time course of fluorescence intensity from the beginning of the irradiation at 532 nm is shown in Fig. S2 (b). The sample of this measurement was the same as used in Fig. S2 (a). For this measurement, we employed the wide-field microscope and fluorescence signals of all the pixels were accumulated. The intensity of the 532-nm laser light was  $1.1 \times 10^3$  W/cm<sup>2</sup>, the value of which is almost comparable with that for the single molecule tracking measurement. Figure S2 (b) shows that the fluorescence intensity of **1c** gradually decreases in the initial time region of a few tens of seconds under the 532-nm irradiation. This decrease of the fluorescence intensity can be ascribed to the decrease of the **1c** being involved as a trace of the sample, as observed in Fig. 1(c) in the main text. At and after ca. 25-30 sec after the time origin of the irradiation, almost constant intensity of the fluorescence was observed. Because this emission is due to **1c** as was shown in Fig. S2 (a), the constant intensity of the emission is ascribable to the photostationary state where the cyclization and cycloreversion reactions are balanced under the irradiation at 532 nm. This result also ensures the weak absorption of **1o** at 532 nm leading to the production of **1c** by the cyclization reaction.

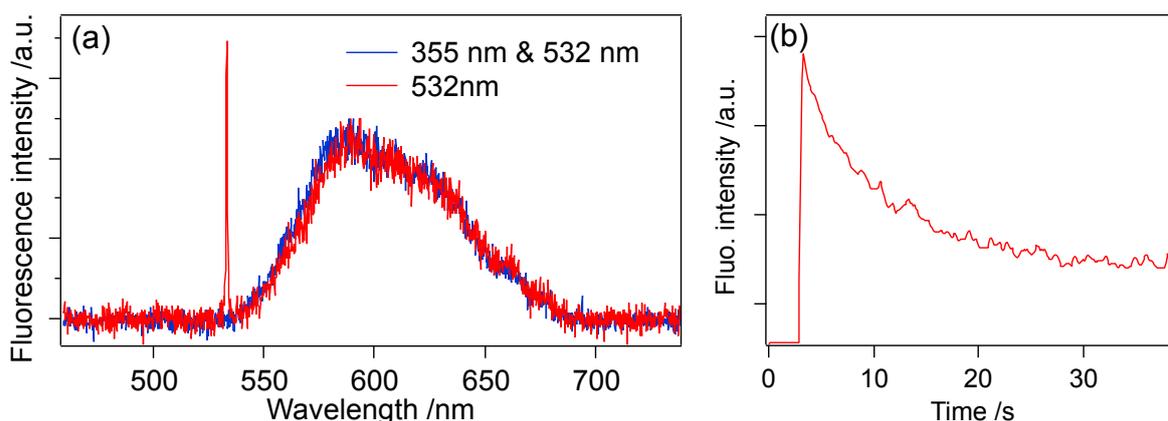


Fig. S2. (a) Fluorescence spectra of the DE in a PMMA film under photo-exposure with the 532-nm laser (red solid curve) and simultaneous photo-exposure with a 355-nm and the 532-nm lasers (blue solid curve). For the measurement of the red spectrum, the intensity of the 532-nm light at the sample was  $7.0 \times 10^2$  W/cm<sup>2</sup>, while the intensities of the 355-nm and 532-nm light at the sample were 0.1 and 11 W/cm<sup>2</sup>, respectively for the measurement of the blue spectrum. A sharp peak at ca. 530 nm is ascribed to the scattered 532-nm light. (b) The time course of fluorescence intensity from the beginning of the irradiation with the 532-nm light. The intensity of the 532-nm laser light was  $1.1 \times 10^3$  W/cm<sup>2</sup>.

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