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Photoinduced cytotoxicity of a photochromic diarylethene *via* caspase cascade activation

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Experimental

General

Light irradiation onto the photo-responsive culture substrates was carried out by using a PC-controlled microprojection system (DESM-01, Engineering System Co.) installed in an inverted research microscope (IX70, Olympus Co.). [S1-S4] Blue light with wavelength of 436 nm or near UV light with wavelength of 365 nm was irradiated onto arbitrary areas of the sample as observed through the same objective lens. ESR measurements were carried out by using an in situ quartz cell with an X-band EPR spectrometer (JEOL JES-FR30EX). The g value and the relative amount of radical species were determined using a Mn marker. Fluorescence measurements for liposome disruption were carried out by using a Jasco FP-6200 spectrophotometer.

Copolymerization of 3o with MMA to form poly(3o-MMA)

In a sealed tube, 110 mg (0.16 mmol) of **3o** in toluene 2 mL, and 110 mg of methyl methacrylate (MMA) 110 mg (1.10 mmol) disolved in toluene 2 mL, and 4.1 mg (0.02 eq.) of 2,2'-azobisisobutyronitrile (AIBN) in 1 mL of toluene were added successively. The mixed solution was bubbled with nitrogen gas for 5 min. The glass tube was sealed by melting. And the tube was heated for 18 h at 60 °C in the dark. After the polymerization was over, the tube was opened and the content was poured into 0.5 L of methanol and obtained the 64.1 mg of copolymer **poly(3o-MMA)** in 29% yield.

Examination of the influence on cell culture

Diarylethene **1c** was spincoated on a surface of tissue culture polystyrene dish (353001, BD Falcon), at the density of 0.6 μg/cm^2, and MDCK cells were disseminated and cultivated for 1 day until they grew confluent. Then blue light with the wavelength of 436 nm and the intensity of 140 mW/cm^2 was irradiated to the culture surface by using PC-controlled micropattern projection system (DESM-01, ESCO) in patterned area ("436") for 2 min. Two hours later, the cells in the irradiated area were observed.

In order to examine the effect of immobilization of diarylethene at the side chain of water-insoluble polymer, **poly(3o-MMA)** was spincoated on a surface of tissue culture polystyrene dish at the density of 0.4 mg/cm^2. UV light (254 nm) was irradiated to induce photocyclization to form **poly(3c-MMA)** (Fig. S1).[S5] On the polymer thin layer, MDCK cells were disseminated and cultivated for 1 day until they grew confluent. Then blue light with the wavelength of 436 nm and the intensity of 280 mW/cm^2 was irradiated on the rectangular area locally for 1 min. Two hours later, the cells in the irradiated area were observed.

Examination of interaction of diarylethene with DNA

Aqueous DNA solution was prepared by dissolving deoxyribonucleic acid sodium salt from salmon testes (D1626, Sigma-Aldrich) into pure water at 0.20 wt%. To obtain the denatured DNA, some portion of the solution was irradiated with UV light (main wavelength: 254 nm) from sterilizing lamp (GL-15, Panasonic) for 2 hours. Then three aqueous solutions were

prepared by adding pure water, 0.20 wt% aqueous solutions of native and denatured DNA by 10 wt%, respectively, to Dulbecco's phosphate-buffered saline (PBS) with pH 7.4, Finally, 0.20 wt% solution of **1c** in ethanol was added by 2.0 wt% to the three PBSs containing no, active and denatured DNA, respectively. The solutions were incubated for 15 hours at 40 ˚C and their absorbance spectra were measured by using a UV-Vis spectrometer (V-560, JASCO). Further, the absorbance spectra were measured for the solutions after reaching PSS under 436 nm light irradiation.

Fluorescence spectroscopy[S6]

Relative binding affinities of **1o, 1c, 2o**, and **2c** to sodium salts of DNA from salmon testes were investigated with EB-bound DNA (EB = ethidium bromide) in 1 mM sodium cacodylate, 20 mM NaCl buffer pH=7.2, containing 5v/v% aqueous solution (DMSO was used to dissolve diarylethenes). The experiments were carried out at constant concentrations of DNA and EB, 15 and 75 μM, respectively. And increased amounts of the diarylethenes were added from 5 to 25 μM. The fluorescence spectra of all samples were recorded at room temperature applying an excitation wavelength, $\lambda_{exc.}$ of 486 nm.

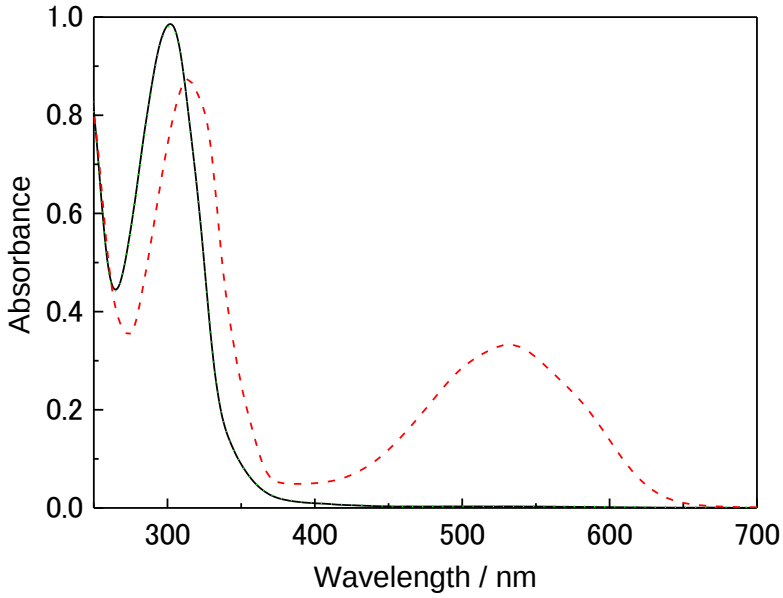

Fig. S1 Absorption spectral changes of **poly(3o-MMA)** in acetonitrile solution; solid line: absorption spectrum before UV irradaition, dotted red line: photostationary state upon 313 nm light irradiation.

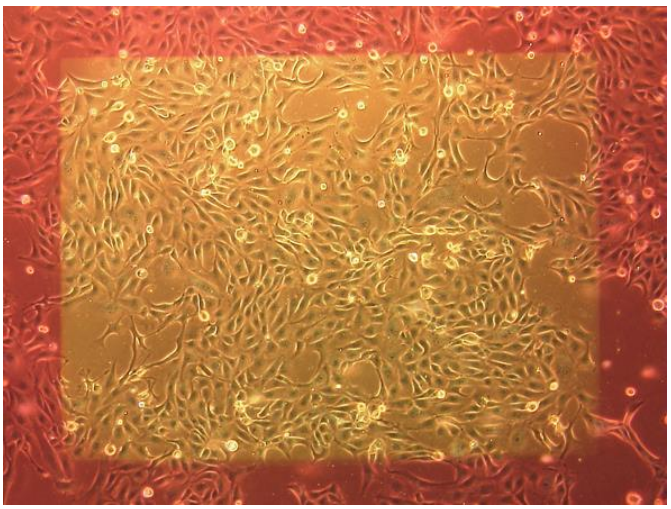

Fig. S2 MDCK cells on copolymer **poly(3c-MMA)** after blue light irradiation (wavelength: 436 nm, intensity: 140 mW/cm^2). No damage on cultured cells was observed.

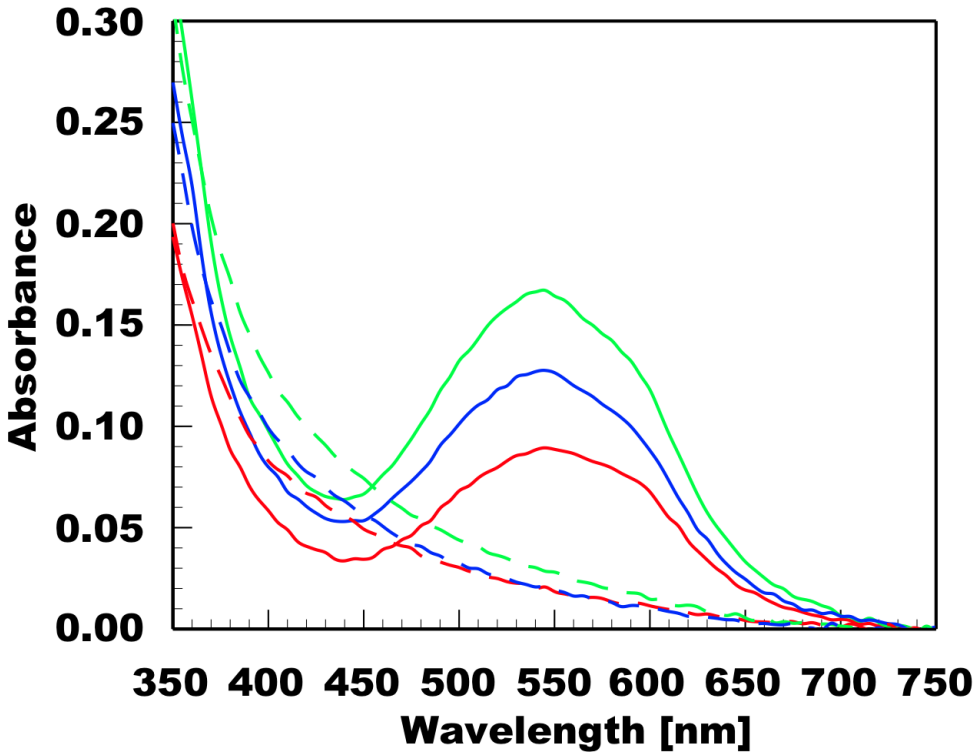

Fig. S3 Absorption spectra of **1c** in PBS under several conditions of DNA coexistence after the dissolution of **1c** and 15 hour incubation at 40 ˚C (solid lines: before 436 nm irradiation, dashed lines: after reaching PSS under 436 nm irradiation). Green lines: in the presence of 0.02% DNA, Blue lines: 0.02% in the presence of DNA decomposed by deep UV (254 nm) light irradiation, Red lines: without DNA. Each solution contains 0.0020% of **1c** or **1o** in pH 7.4 PBS. The broken lines extend wide range of wavelengths due to the scattering.

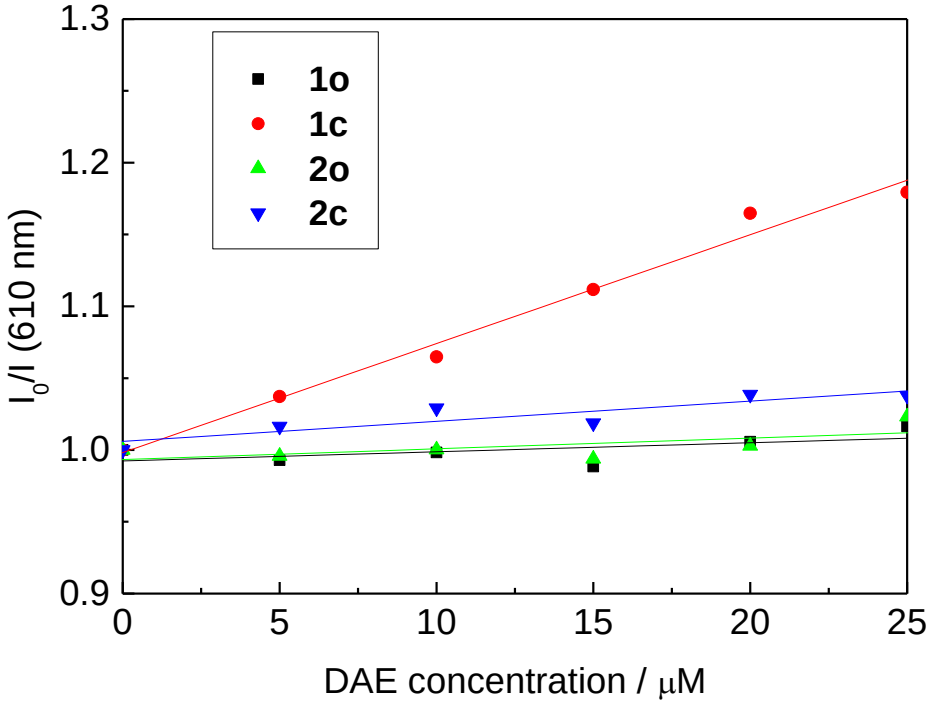

Fig. S4 Plots of I_0 / I vs. concentrations of diarylethenes for the titration of DNA-EB with diarylethenes **1** and **2** (open- and closed-ring isomers) at λ_{exc} =486 nm and λ_{em}=610 nm: experimental data points and linear fitting of the data. Concentration of **1o, 1c, 2o,** and **2c**: 5-25 μM; [DNA]: 15 μM; [EB]: 75 μM. The decrease of the fluorescence intensity was observed only for the closed-ring isomer **1c**.)[S6]

Table 1. Stern-Volmer quenching constant, K_{SV} and log K_{SV} obtained from Equation (1) and **Fig. S4.**

$$\frac{I_0}{I} = 1 + K_{SV}[\text{complex}] \qquad (1)$$

	K_{SV} / μM	Log K_{SV}
1o	$6.0 \times 10^2\ M^{-1}$	2.78
1c	$7.6 \times 10^3\ M^{-1}$	3.88
2o	$7.0 \times 10^2\ M^{-1}$	2.84
2c	$1.4 \times 10^3\ M^{-1}$	3.15

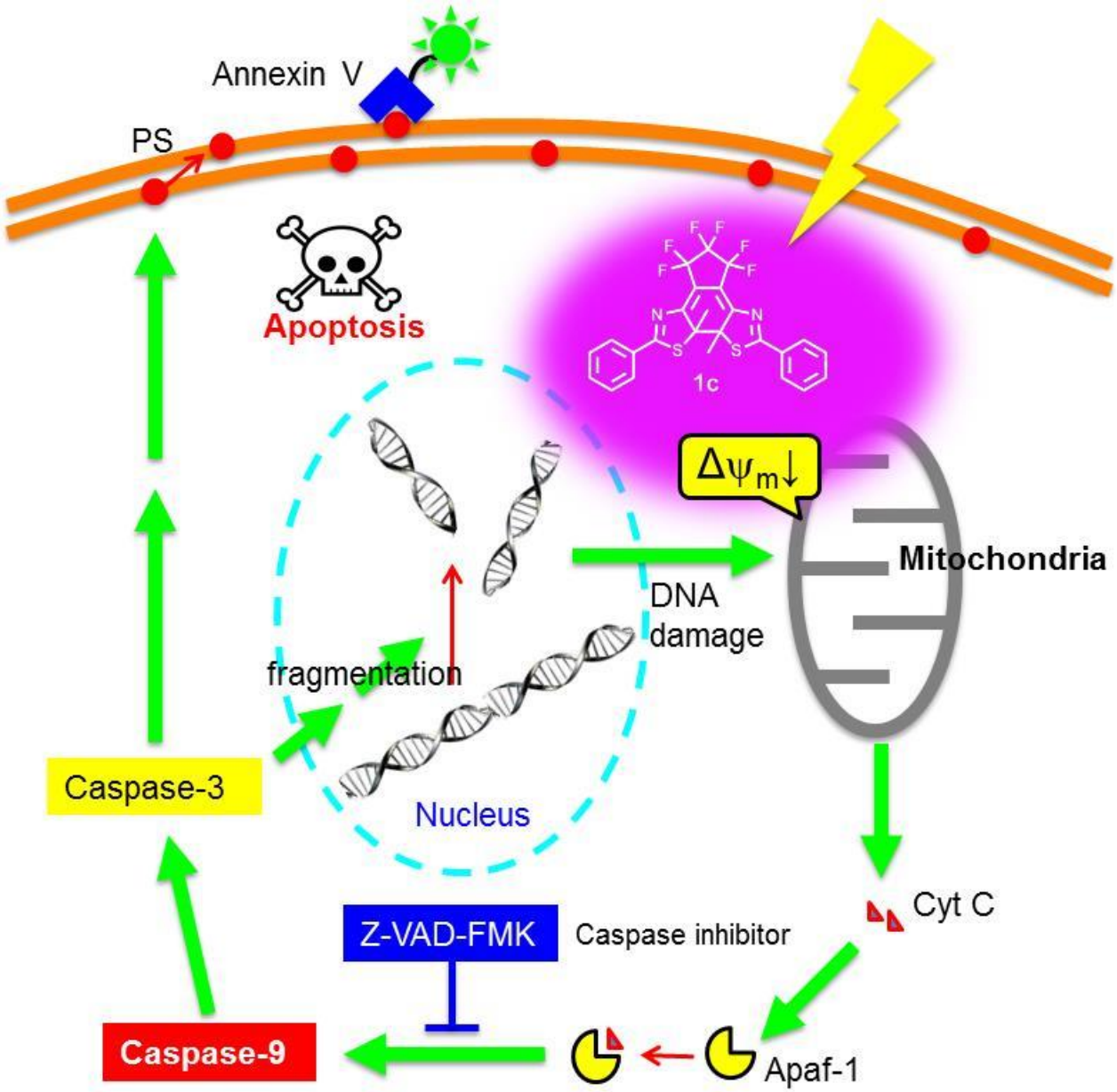

Fig. S5. Schematic diagram of programmed cell death: apoptosis.[S7-S10] Caspase inhibitor Z-VAD-FMK inhibits the activation of caspase-9.

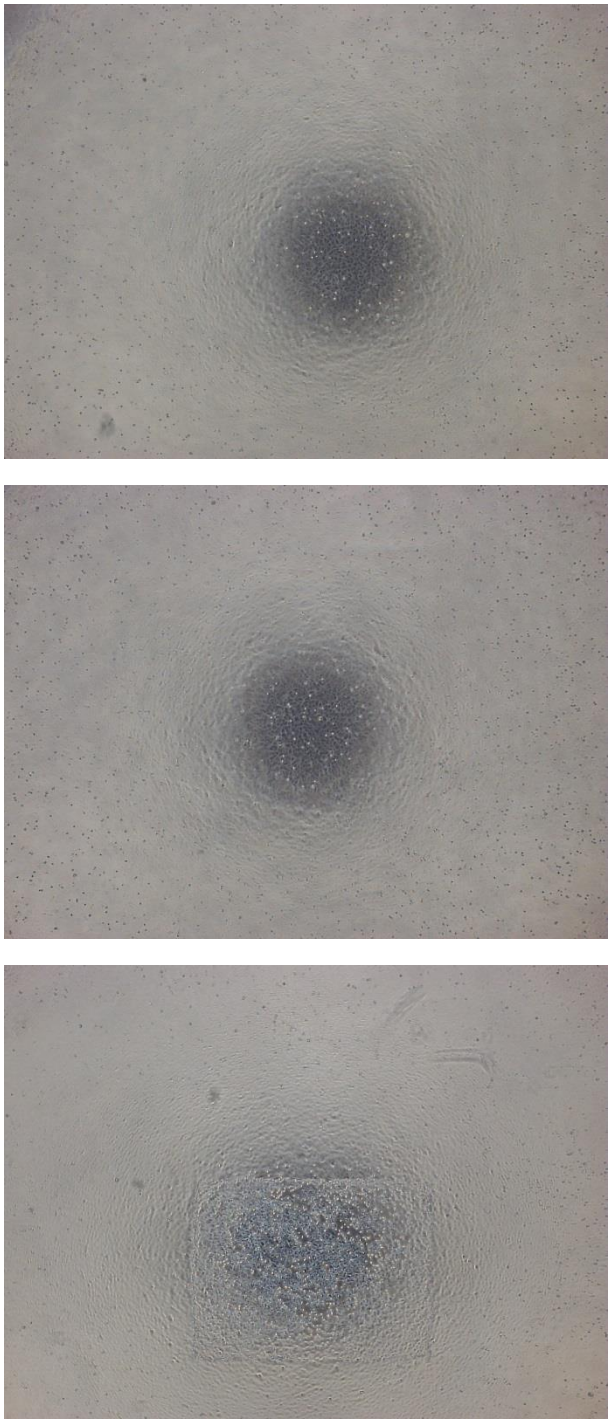

Fig. S6 MDCK cells cultured overnight in the medium containing 0.2 ppm of **2c** (top), **2o** (middle) or **1c** (bottom) were irradiated locally (in the rectangular irradiated areas) with blue light (436 nm) and then observed two hours after irradiation. No detectable influence was observed in the cells cultured with **2c** and **2o** while apparent damage was observed in the rectangular irradiated areas in the condition cultured with **1c**.

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