One-Pot Synthesis of Monodispersed Silica Nanoparticles for Diarylethene-Based Reversible Fluorescence Photoswitching in Living Cells

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Table of Contents

<table>
<thead>
<tr>
<th>Schemes</th>
<th>Synthetic schemes to prepare compounds 3a, 3b, 4, and 6.</th>
<th>p. S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme S5</td>
<td>Synthetic scheme to prepare silica nanoparticle 1a.</td>
<td>p. S3</td>
</tr>
<tr>
<td>Text</td>
<td>Additional Descriptions</td>
<td>pp. S4-S6</td>
</tr>
<tr>
<td>Text</td>
<td>Experimental Section</td>
<td>pp. S7-S19</td>
</tr>
<tr>
<td>Text</td>
<td>References</td>
<td>p. S20</td>
</tr>
<tr>
<td>Table S1</td>
<td>Characterization of silica nanoparticles 1a, 1b, 2a, and 2b.</td>
<td>p. S21</td>
</tr>
<tr>
<td>Table S2</td>
<td>Photoswitching efficiency of silica nanoparticle 1a.</td>
<td>p. S21</td>
</tr>
<tr>
<td>Figure S1</td>
<td>ESI HRMS spectra of silane derivatives 3a, 3b, and 4.</td>
<td>p. S22</td>
</tr>
<tr>
<td>Figure S2</td>
<td>Photochromic conversion efficiency of 10 under photoswitching conditions as determined by 1H NMR integrals in D2O.</td>
<td>p. S23</td>
</tr>
<tr>
<td>Figure S3</td>
<td>Energy-minimized structure of 10.</td>
<td>p. S24</td>
</tr>
<tr>
<td>Figure S4</td>
<td>Determination of response time of silica nanoparticle 1a.</td>
<td>p. S25</td>
</tr>
<tr>
<td>Figure S5</td>
<td>UV-Vis and fluorescence spectra of compounds 10 and Cy3 during photoswitching experiments in PBS.</td>
<td>p. S26</td>
</tr>
<tr>
<td>Figure S6</td>
<td>UV-Vis and fluorescence spectra of silica nanoparticles 1a and 1b during photoswitching experiments in PBS.</td>
<td>p. S27</td>
</tr>
<tr>
<td>Figure S7</td>
<td>Cytotoxicity assay of silica nanoparticle 1a.</td>
<td>p. S28</td>
</tr>
<tr>
<td>Figure S8</td>
<td>Flow cytometry histograms of HeLa and hADS cells incubated with silica nanoparticle 1a for different time periods.</td>
<td>p. S29</td>
</tr>
<tr>
<td>Figure S9</td>
<td>Arrangement of optical equipment for photoswitching experiments on living cells.</td>
<td>p. S30</td>
</tr>
<tr>
<td>Figure S10</td>
<td>Effect of UV light irradiation on cell viability under photoswitching conditions.</td>
<td>p. S31</td>
</tr>
</tbody>
</table>

Scheme S2. Synthesis of DAE-TES (3a).\(^a\)

\(^a\) All of the diarylethene derivatives are shown as ring-open isomers.

Scheme S3. Synthesis of Cy3-TES (4).

Scheme S4. Synthesis of mTEG-TES (3b).
Scheme S5. Preparation of silica nanoparticle (NP) 1a.\textsuperscript{a} The subscript “o” in the compound numbers of silica NPs 1a and 2a denote the ring-open isomers of diarylethene derivatives. The non-switchable silica NP 1b (control) and its amine-terminated precursor 2b were made similarly using 3b instead of 3a (see Experimental Section for details).
ADDITIONAL DESCRIPTIONS

Degree of Photochromic Conversion of Compound 10 by $^1$H NMR

The conversion extent of diarelethene (DAE) 10—a water-soluble derivative of DAE 3a—was estimated by the analysis of $^1$H NMR integration (Figure S2). Here, the deuterium oxide (D$_2$O) was used as the NMR solvent for 10 to conduct photoswitching under a similar condition to that for the silica nanoparticle (NP) 1a (in phosphate buffered saline (PBS)). When the aqueous solution of 10 was irradiated with UV light (365 nm, 2 min), a broad singlet emerged at ca. 1.80 ppm, which presumably corresponded to the methyl protons of the ring-closed isomer substituted at the 2-position of thiophene. Indeed the sum of the integrals of the methyl peaks for ring-closed and ring-open (at ca. 1.74 ppm) isomers matched the theoretical values (ca. 6H) when normalized against the integrals of the peaks in the aromatic region (ca. 4H, 2H, and 4H for broad singlets centered at ca. 7.10, 6.95, and 6.61 ppm, respectively). Also, this peak at 1.80 ppm disappeared upon irradiation with visible light (590 nm, 30 min), suggesting its correlation with the ring-closed isomer. Because the thiophene methyl peaks of the ring-closed and ring-open isomers overlapped significantly, the conversion extent upon irradiation with UV light was determined by obtaining the relative integral of each peak through a line-fitting method. The photochromic conversion of 10 estimated as such was 0.1104 for cyclization reaction. For cycloreversion reaction, the conversion extent of 10 appeared to be nearly 100% when analyzed similarly by $^1$H NMR integration. Furthermore, the effect of solvent on photocyclization efficiency was shown by estimating conversion extent of 10 in chloroform-$d$ (CDCl$_3$) by $^1$H NMR (data not shown): the increased population (2-3 folds) of ring-closed isomer was noticed upon irradiation with UV light (365 nm, 2 min) relative to that obtained in D$_2$O.

Energy-Minimized Structures of Compound 10

The structural information of DAE derivatives such as the heat of formation$^2$ and the distances between reacting carbon atoms (C2 and C2$'$ of thiophene rings)$^3$ in the antiparallel conformer may aid in estimating the relative population of the antiparallel and parallel conformers and the cyclization quantum yield$^4$ (i.e., conversion extent). When energy-minimized structures of the antiparallel conformer of DAE 10 was obtained
by a semi-empirical AM1 method (Figure S3), the distance between the reacting carbon atoms (C2-C2') was found to be ca. 3.88 Å. This value falls into a favorable range (< 4 Å) for photocyclization in the crystalline state. Also, while the energy-minimized structures were obtained with the ethylene oxide linkers (side arms) in all-anti conformations, the difference in energy (heat of formation) between the antiparallel and parallel conformers of 10 was relatively large ($\Delta E \approx 24$ kJ/mol), suggesting the antiparallel conformer as a predominant species of the ring-open isomer. The high content of antiparallel conformation is known to lead to high cyclization quantum yield.

**Calculation of Cyclization Quantum Yield of Compound 10**

The photocyclization quantum yield ($\Phi_{o\rightarrow c}$) of DAE 10 was determined based on the photoswitching results monitored by $^1$H NMR spectroscopy in D$_2$O and UV-Vis spectroscopy in deionized water. The following formula was used for the calculation:

$$\Phi_{o\rightarrow c} = N_c / N_{\text{photons}}$$

where $N_c$ is the number of ring-closed isomer formed upon irradiation with 365 nm light (2 min, 2.60 mW/cm$^2$), and $N_{\text{photons}}$ is the number of photons absorbed. $N_c$ can be calculated as

$$N_c = [10] \cdot V \cdot N_A \cdot f$$

where $[10]$ is the initial concentration of compound 10 as a ring-open isomer in a cuvette (= 10 $\mu$M, assumed no ring-closed isomer present before the irradiation with UV light), $V$ the volume of the sample in a cuvette (= 1.0 mL), $N_A$ the Avogadro’s number, $f$ the degree of conversion for cyclization reaction determined by $^1$H NMR integration (= 0.1104). $N_{\text{photons}}$ can be calculated as

$$N_{\text{photons}} = (\lambda/hc) \cdot (1-10^{-A}) \cdot I_0 \cdot t$$

where $\lambda$ is the irradiation wavelength, $h$ the Planck’s constant, $c$ the speed of light, $A$ the
absorbance at 365 nm of the ring-open isomer at \( t_0 = 0.0289 \), assumed no ring-closed isomer present before the irradiation with UV light), \( I_0 \) the irradiation intensity \( = (2.60 \text{ mW/cm}^2)\cdot(0.95 \text{ cm})^2 \), and \( t \) the exposure time \( = 120 \text{ s} \).

The photocyclization quantum yield \( (\Phi_{o\rightarrow c}) \) of \( 10 \) in deionized water calculated as such was 0.020, which was lower than that of a 5-(\( p \)-methoxyphenyl)thienyl analogue (0.56, irradiated with 293 nm light) determined in hexane.\(^7\)

**Determination of Response/Switching Time of Silica NP 1a**

The response time of silica NP 1a upon photoswitching was determined by measuring the fluorescence intensities at selected time points during the irradiation with UV and visible light (Figure S4). Here, the irradiation time was extended to fully reach the equilibrium state for both visible (Figure S4a,c) and UV (Figure S4b,d) light irradiations. Specifically, the rate constants for cycloreversion reaction (fluorescence activation) and cyclization reaction (fluorescence quenching) were determined by curve-fitting (exponential decay) each plot of the fluorescence intensity at the emission maximum (586.5 nm) of silica NP 1a against time. The corresponding rate constant \( (k) \) of 0.002084 s\(^{-1}\) for cycloreversion reaction (irradiation with 590 nm light) and 0.07245 s\(^{-1}\) for cyclization (irradiation with 365 nm light) reaction appeared to be similar to those of other DAE-based fluorescence photoswitching systems.\(^1,8\)
EXPERIMENTAL SECTION

Materials

Glassware was oven-dried and cooled to room temperature in a desiccator before use. All reactions were carried out under a dry argon atmosphere. Solvents were purchased as anhydrous grade and used without further purification. Suppliers of the commercial compounds are listed as follows: Cy3 mono N-hydroxysuccinimide (NHS) ester was purchased from GE Healthcare (Amersham); m-dPEG®4-NHS ester (NHS ester of tetra(ethylene glycol) methyl ether; mTEG-NHS) was purchased from Quanta Biodesign; chloroform-d (CDCl₃) and dimethyl sulfoxide (DMSO)-d₆ were purchased from Cambridge Isotope Laboratories. All other commercial compounds were purchased from Sigma-Aldrich unless otherwise mentioned. Compounds 5⁹ and 7¹⁰ were synthesized following the literature procedure.

General Methods

Analytical thin layer chromatography (TLC) was performed on 0.2 mm silica glass coated sheets (E. Merck) with F-254 indicator. Visualization of the products on TLC plate was performed by UV light, iodine (I₂), potassium permanganate (KMnO₄), and ninhydrin. Flash column chromatography was performed on Merck 40-63 μm silica gel.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian INOVA 400 (for ¹³C NMR), a Varian Unity 500, or a Varian INOVA 600 spectrometer at 25.0 °C under an optimized parameter setting for each sample. ¹H NMR chemical shifts were measured relative to the residual solvent peak at 7.26 ppm in CDCl₃. ¹³C NMR chemical shifts were measured relative to the residual solvent peak at 77.23 ppm in CDCl₃.

Electrospray ionization (ESI) mass spectrometry (MS) experiments were performed either on a Quadrupole time-of-flight (Q-TOF) Ultima mass spectrometer at the Mass Spectrometry Laboratory of the University of Illinois or on a Shimadzu hybrid ion-trap (IT) TOF mass spectrometer by the Mass Spectrometry Research Team of the Korea Basic Science Institute.

UV-Vis spectroscopy was performed (250-800 nm scan range, 0.5 nm interval, 600 nm/min scan speed) on an Agilent Cary 60 UV-Vis spectrophotometer, a Beckman Coulter DU 800 spectrophotometer, or a Shimadzu UV-3600 UV-VIS-NIR
spectrophotometer (high resolution) using a semi-micro disposable cuvette (10 mm path length, 1.6 mL nominal capacity, polymethylmethacrylate) from Sarstedt. Fluorescence spectroscopy was performed (irradiation at 510 nm) on either an Agilent Cary Eclipse fluorescence spectrophotometer or a Perkin Elmer LS 55 fluorescence spectrometer using a quartz Suprasil macro/semi-micro cell (4 mm path length, 0.5 mL nominal capacity) from Perkin Elmer.

Zeta potentials were measured using a Zetasizer Nano ZS from Malvern Instruments at 25.0 °C. Samples of silica nanoparticles (NPs) 1a, 1b, 2a, and 2b dispersed in deionized water at 100 μg/mL were prepared by sonication and vortexing. Five measurements were made and the average values were calculated after excluding the highest and lowest data points (n = 3). Results are summarized in Table S1.

A JEM-2100F field-emission (FE) transmission electron microscopy (TEM) from JEOL was used to obtain TEM images at 200 kV. A drop (2 μL) of each suspension of silica NP 1a, 1b, 2a, or 2b in deionized water (1 mg/mL) was placed on a 200 mesh carbon coated copper grid (Ted Pella) and air-dried, which was repeated for up to 10 times. Excess liquid was removed with a filter paper. TEM images shown in Figure 1d (main text) were obtained without staining. TEM-estimated sizes of silica NPs 1a, 1b, 2a, and 2b are listed in Table S1.

**Synthesis of Compound 6**

At 0 °C, an aqueous solution of ammonium hydroxide (NH₄OH, 28.0-30.0% NH₃ basis, 5 mL) was slowly added to a solution of compound 5₁ (680 mg, 1.62 mmol) in methanol (MeOH, 5 mL). The mixture was stirred for 12 h while it warmed slowly to room temperature. The reaction was quenched at 0 °C by adding a 1 N aqueous solution of HCl. Subsequently, the crude product was extracted with methylene chloride (CH₂Cl₂), and the combined organic extracts were dried over MgSO₄, concentrated under reduced pressure, and chromatographed on a silica gel column (10:1 CH₂Cl₂/MeOH) to give 450 mg of 6 (1.11 mmol, 69%). $R_f = 0.30$ [silica gel, 10:1 CH₂Cl₂/MeOH]; $^1$H NMR (600
MHz, CDCl₃) δ 7.81 (d, 2H, J = 8.5 Hz, H₃), 7.34 (d, 2H, J = 8.0 Hz, H₂), 7.23 (br s, 2H, H₁₃), 4.19 (t, 2H, J = 4.9 Hz, H₄), 3.98 (s, 2H, H₁₂), 3.72-3.57 (m, 14H, H₅, H₆, H₇, H₈, H₉, H₁₀, and H₁₁), 2.45 (s, 3H, H₁); ¹³C NMR (150 MHz, CDCl₃) δ 173.2, 145.0, 140.0, 130.1, 128.2, 71.4, 71.1, 70.7, 70.6 (70.635), 70.6 (70.625), 70.6 (70.596), 70.3, 69.5, 69.0, 21.9; HRMS (ESI) Calcd for C₁₇H₂₇NO₈SNa (M + Na)⁺: 428.1350, Found: 428.1351.

Synthesis of Compound 8

[Chemical structure of Compound 8]

To a solution of compound 7 (518 mg, 0.937 mmol) and 5 (472 mg, 1.12 mmol) in acetonitrile (20 mL) was added the oven-dried K₂CO₃ (389 mg, 2.81 mmol). The reaction mixture was stirred at 80 °C for 12 h, cooled to room temperature, and concentrated under reduced pressure. The crude product was chromatographed on a silica gel column (4:1:1 hexane/CH₂Cl₂/acetone) to give 117 mg of 8 (0.146 mmol, 16%). Rᵢ = 0.22 [silica gel, 3:1:1 hexane/CH₂Cl₂/acetone]; ¹H NMR (600 MHz, CDCl₃, ring-open isomer) δ 7.43 (d, 2H, J = 8.8 Hz, H₃o), 7.39 (d, 2H, J = 8.7 Hz, H₃o'), 7.13 (7.134) (s, 1H, H₂o or H₂o'), 6.90 (d, 2H, J = 9.1 Hz, H₄o), 6.85 (d, 2H, J = 8.6 Hz, H₄o'), 4.16 (s, 2H, H₁₃), 4.13 (t, 2H, J = 4.7 Hz, H₅), 3.86 (t, 2H, J = 4.9 Hz, H₆), 3.74 (s, 3H, H₁₄), 3.73-3.66 (m, 12H, H₇, H₈, H₉, H₁₀, H₁₁, and H₁₂), 1.95 (s, 3H, H₁₀ or H₁₀'), 1.94 (s, 3H, H₁₀ or H₁₀'); ¹³C NMR (150 MHz, CDCl₃, ring-open isomer) δ 171.2, 158.9, 156.1, 142.3, 142.2, 140.5, 140.4, 139.2, 136.3, 127.3, 127.1, 126.6, 126.3, 126.0, 125.9, 121.6, 121.5, 116.1, 115.3, 110.8, 71.1, 71.0, 70.9, 70.8 (70.836), 70.8 (70.812), 69.9, 68.8, 67.8, 52.0, 14.7; HRMS (ESI) Calcd for C₃₈H₃₈F₆O₈S₂Na (M + Na)⁺: 823.1805, Found: 823.1805.

Synthesis of Compound 9

[Chemical structure of Compound 9]
To a solution of compound 8 (117 mg, 0.146 mmol) and 6 (118 mg, 0.291 mmol) in DMF (10 mL) was added the oven-dried K$_2$CO$_3$ (81 mg, 0.59 mmol). The reaction mixture was stirred at 100 °C for 12 h, cooled to room temperature, and concentrated under reduced pressure. The crude product was chromatographed on a silica gel column (1:1 CH$_2$Cl$_2$/acetone) to give 126 mg of 9 (0.122 mmol, 84%). $R_f = 0.27$ [silica gel, 2:3 CH$_2$Cl$_2$/acetone]; $^1$H NMR (600 MHz, CDCl$_3$, ring-open isomer) $\delta$ 7.44 (7.444) (d, 2H, J = 8.7 Hz, H$_{30}$ or H$_{30}'$), 7.44 (7.437) (d, 2H, J = 8.8 Hz, H$_{30}$ or H$_{30}'$), 7.14, 7.13 (s each, 2H, H$_{20}$ and H$_{20}'$), 6.96 (d, 2H, J = 8.8 Hz, H$_{40}$ or H$_{40}'$), 6.95 (d, 2H, J = 9.0 Hz, H$_{40}$ or H$_{40}'$), 4.24, 4.21 (m each, 4H, H$_5$ and H$_5'$), 4.20 (s, 2H, H$_{13}$), 3.99 (s, 2H, H$_{13}'$), 3.89 (m each, 4H, H$_6$ and H$_6'$), 3.85-3.64 (m, 24H, H$_7$, H$_7'$, H$_8$, H$_8'$, H$_9$, H$_9'$, H$_{10}$, H$_{10}'$, H$_{11}$, H$_{11}'$, H$_{12}$, and H$_{12}'$), 3.77 (s, 3H, H$_{14}$), 1.96, 1.95 (s each, 6H, H$_1$ and H$_1'$); HRMS (ESI) Calcd for C$_{49}$H$_{57}$F$_6$NO$_{13}$S$_2$Na (M + Na)$^+$: 1056.3068, Found: 1056.3068.

**Synthesis of Compound 10**

At 0 °C, a 1 N aqueous solution of LiOH (0.58 mL, 0.58 mmol) was slowly added to a solution of compound 9 (119 mg, 0.115 mmol) in a mixture of tetrahydrofuran (THF, 4 mL) and MeOH (1 mL). The mixture was stirred at 50 °C for 1 h and allowed to cool to room temperature. The reaction was quenched by adding a 1 N aqueous solution of HCl at 0 °C. Subsequently, the crude product was extracted with ethyl acetate (EtOAc), and the combined organic extracts were dried over MgSO$_4$, concentrated under reduced pressure, and loaded on a preparative size exclusion chromatography (SEC) column (Sephadex LH-20, exclusion limit 4000-5000 Da, 18-111 μm, GE Healthcare, H 10 cm × O.D. 3.0 cm) for purification in methanol. The bluish column fractions were combined, concentrated under reduced pressure, and dried in vacuo to give 102 mg of 10 (0.100 mmol, 87%). $R_f = 0.80$ [C-18, MeOH]; $^1$H NMR (600 MHz, CDCl$_3$, ring-open isomer) $\delta$ 7.44 (d, 2H, J = 8.8 Hz, H$_{30}$ or H$_{30}'$), 7.43 (d, 2H, J = 9.1 Hz, H$_{30}$ or H$_{30}'$), 7.13 (s, 2H, H$_{20}$
and H$_{2o}$), 6.94 (d, 2H, J = 9.0 Hz, H$_{4o}$ or H$_{4o}'$), 6.92 (d, 2H, J = 8.8 Hz, H$_{4o}$ or H$_{4o}'$), 4.19 (t, 2H, J = 4.8 Hz, H$_{5}$ or H$_{5}'$), 4.15 (m, 2H, H$_{5}$ or H$_{5}'$), 4.08 (s, 2H, H$_{13}$), 4.15 (m, 2H, H$_{5}$ or H$_{5}'$), 4.08 (s, 2H, H$_{13}$), 4.19 (t, 2H, J = 4.8 Hz, H$_{5}$ or H$_{5}'$), 4.15 (m, 2H, H$_{5}$ or H$_{5}'$), 4.08 (s, 2H, H$_{13}$), 4.15 (m, 2H, H$_{5}$ or H$_{5}'$), 4.08 (s, 2H, H$_{13}$)

$^{13}$C NMR (150 MHz, CDCl$_3$, ring-open isomer) $\delta$ 174.0, 173.1, 158.9, 158.8, 142.3, 140.5, 138.5, 136.3, 127.1, 126.6, 125.9, 121.8, 121.6, 115.3, 71.5, 71.2, 71.1, 71.0, 70.9, 70.7 (70.748), 70.7 (70.670), 70.6 (70.588), 70.6 (70.562), 70.5 (70.508), 70.5 (70.470), 70.5 (70.454), 70.4, 70.2, 69.9 (69.899), 69.9 (69.852), 67.8, 67.7, 14.7; HRMS (ESI) Calcd for C$_{47}$H$_{54}$F$_6$NO$_{13}$S$_2$ (M − H)$^-$: 1018.2946, Found: 1018.2948.

**Synthesis of DAE-TES (3a)**

To a solution of compound 10 (16 mg, 16 $\mu$mol) in DMF (2 mL) were added N-hydroxysuccinimide (NHS, 1.8 mg, 15 $\mu$mol) and 1-ethyl-3-(3-dimethylamino)propylcarbodiimide hydrochloride (EDC, 3.6 mg, 19 $\mu$mol). The reaction mixture was stirred at room temperature for 5 h. Subsequently, 3-aminopropyl)triethoxysilane (APTES, 4.4 $\mu$L, 19 $\mu$mol) and triethylamine (7.9 $\mu$L, 57 $\mu$mol) were added to this mixture, the reaction was stirred at room temperature for 12 h, and dried in vacuo. The reaction mixture containing 3a was used for next step to prepare silica NP 2a without purification. HRMS (ESI) Calcd for C$_{56}$H$_{77}$F$_6$N$_2$O$_{15}$S$_2$Si (M + H)$^+$: 1223.4439, Found: 1223.4427.

**Synthesis of mTEG-TES (3b)**

To a solution of mTEG-NHS 12 (5.2 mg, 16 $\mu$mol) in DMF (966 $\mu$L) were added APTES (4.5 $\mu$L, 19 $\mu$mol) and triethylamine (4.5 $\mu$L, 32 $\mu$mol). The reaction was stirred at room temperature for 4 h and dried in vacuo. The reaction mixture containing 3b was used for next step to prepare silica NP 2b without purification. HRMS (ESI) Calcd for C$_{19}$H$_{41}$NO$_8$Si (M + H)$^+$: 440.2680, Found: 440.2680.

**Synthesis of Cy3-TES (4)**

Compound 4 was freshly made in two separate batches before its use to make silica NP 2a or 2b. For 2a, to a solution of Cy3 mono NHS ester 11 (2.0 mg, 80.43% reactive...
chromophore content, 2.1 µmol) in DMSO-d₆ (100 µL) were added APTES (0.60 µL, 2.6 µmol) and triethylamine (1.0 µL, 7.2 µmol). The reaction was stirred at room temperature for 17 h and dried in vacuo. For 2b, to a solution of Cy3 mono NHS ester 11 (2.0 mg, 73.70% reactive chromophore content, 1.9 µmol) in DMSO-d₆ (100 µL) were added APTES (0.60 µL, 2.6 µmol) and triethylamine (0.54 µL, 3.9 µmol). The reaction was stirred at room temperature for 8 h and dried in vacuo. The reaction mixture containing 4 was used for next step to prepare silica NP 2a or 2b without purification. HRMS (ESI) Calcd for C₄₀H₆₀N₃O₁₀S₂Si (M + H)+: 834.3489, Found: 834.3480.

Preparation of Silica NP with Cationic Surface (2a)¹¹

An oil-in-water microemulsion was formed by adding Tween-80 (3.76 mL), 1-butanol (6.0 mL), and DMSO (2.0 mL) to deionized water (200 mL) with stirring. Next, the vacuum-dried reaction mixtures containing 3a and 4 were dissolved in 400 µL and 100 µL of DMSO-d₆, respectively. Out of these solutions, 200 µL of crude 3a (7.7 µmol if 100% yield is assumed for the reaction to prepare 3a) and 50 µL of crude 4 (1.1 µmol if 100% yield is assumed for the reaction to prepare 4) were combined in a single vial and the mixture was further diluted to a total volume of 2.0 mL by adding 1.75 mL of additional DMSO-d₆. Subsequently, this mixture of 3a and 4 (2.0 mL) and vinyltriethoxysilane (VTES, 2.0 mL, 9.2 mmol) were added to the above microemulsion and the mixture was stirred for 1 h. To initiate the polymerization reaction, an aqueous solution of NH₄OH (200 µL, 28.0-30.0% NH₃ basis, Sigma-Aldrich) was added to the microemulsion followed by N′-[3-(trimethoxysilyl)propyl]diethylenetriamine (DETA, 200 µL, 0.78 mmol). The reaction was protected from light and stirred overnight at room temperature. The crude mixture was then dialyzed (Spectra/Por Biotech Regenerated Cellulose (RC) membrane, MWCO 15000, Spectrum Laboratories) extensively against DMSO (× 2, for 2 h each), methanol (2 h), and deionized water (× 2, for 2 h and overnight) with stirring to disassemble the Tween-80 micelles and remove the unassociated small reagents. The crude product was dried by lyophilization and stored at 4 °C. A portion of the crude product was redispersed in deionized water and centrifuged in a Nanosep centrifugal device (100K, Omega™ membrane, Pall corporation) at 13200
rpm for 10 min (× 4) to remove remaining Tween-80 micelles from silica NP 2a. The NP 2a captured in the membrane was redispersed in deionized water by sonication and vortexing, sterile-filtered (pore size: 0.45 μm), and lyophilized to afford 2a as a solid.

Preparation of Silica NP with Anionic Surface (1a)\textsuperscript{11,12}

To a suspension of silica NP 2a (229 mg) free of Tween-80 micelles in DMSO (10 mL) was added succinic anhydride (201 mg, 2.00 mmol) and triethylamine (10.0 μL, 71.7 μmol). The reaction was protected from light and stirred for 17 h at room temperature. The mixture was then dialyzed (Spectra/Por Biotech RC membrane, MWCO 15000, Spectrum Laboratories) extensively against DMSO (× 2, overnight and for 2 h), methanol (2 h), and deionized water (× 2, for 2 h each) with stirring. The dialyzed suspension was sterile-filtered (pore size: 0.45 μm) and the filtrate was lyophilized to collect the silica NP 1a as a solid.

Preparation of Silica NP with Cationic Surface (2b)\textsuperscript{11}

Silica nanoparticles 1b and 2b were made following the procedures similar to those for 1a and 2a, respectively. An oil-in-water microemulsion was formed by adding Tween-80 (0.94 mL), 1-butanol (1.5 mL), and DMSO (0.5 mL) to deionized water (50 mL) with stirring. Next, the vacuum-dried reaction mixtures containing 3b and 4 were dissolved in 200 μL and 100 μL of DMSO-d\textsubscript{6}, respectively. Out of these solutions, 25 μL of crude 3b (1.9 μmol if 100% yield is assumed for the reaction to prepare 3a) and 15 μL of crude 4 (0.28 μmol if 100% yield is assumed for the reaction to prepare 4) were combined in a single vial and the mixture was further diluted to a total volume of 500 μL by adding 460 μL of additional DMSO-d\textsubscript{6}. Subsequently, this mixture of 3b and 4 (500 μL) and VTES (500 μL, 2.3 mmol) were added to the above microemulsion and the mixture was stirred for 1 h. To initiate the polymerization reaction, an aqueous solution of NH\textsubscript{4}OH (200 μL, 28.0-30.0% NH\textsubscript{3} basis, Sigma-Aldrich) was added to the microemulsion followed by DETA (50 μL, 0.19 mmol). The reaction was protected from light and stirred overnight at room temperature. The crude mixture was then dialyzed (Spectra/Por Biotech RC membrane, MWCO 15000, Spectrum Laboratories) extensively.
against DMSO (× 2, for 2 h each), methanol (2 h), and deionized water (× 2, for 2 h and overnight) with stirring to disassemble the Tween-80 micelles and remove the unassociated small reagents. Water was removed in vacuo and a portion of the crude product was redispersed in deionized water and centrifuged in a Nanosep centrifugal device (100K, Omega™ membrane, Pall corporation) at 13200 rpm for 10 min (× 4) to remove remaining Tween-80 micelles from silica NP 2b. The NP 2b captured in the membrane was redispersed in deionized water by sonication and vortexing, and dried in vacuo to afford 2b as a solid.

Preparation of Silica NP with Anionic Surface (1b)\textsuperscript{11,12}

To a suspension of silica NP 2b (50 mg) free of Tween-80 micelles in DMSO (2.2 mL) was added succinic anhydride (50 mg, 0.50 mmol) and triethylamine (2.2 μL, 15.8 μmol). The reaction was protected from light and stirred for 21 h at room temperature. The mixture was then dialyzed (Spectra/Por Biotech RC membrane, MWCO 15000, Spectrum Laboratories) extensively against DMSO (× 2, overnight and for 2 h), methanol (2 h), and deionized water (× 2, for 2 h each) with stirring. Water was removed in vacuo to afford 1b as a solid.

Photoswitching Experiments using Solution Samples

The arrangement of the optical equipment for reversible photoswitching of solution samples in cuvettes was similar to that shown elsewhere.\textsuperscript{13} Each 1.0 mL solution of diarylethene 10 (10 μM; Figure S5a,b), Cy3 (1.0 μM, as a carboxylic acid form of 11, structure not shown; Figures 2a and S5c,d), silica NP 1a (500 μg/mL for UV-Vis spectra and 100 μg/mL for fluorescence spectra; Figures 2 and S6a,b), and silica NP 1b (250 μg/mL for UV-Vis spectra and 100 μg/mL for fluorescence spectra; Figures 2a and S6c,d) in Dulbecco’s phosphate buffered saline (PBS; 1X, pH 7.4, Gibco) was added to a standard disposable cuvette (10 mm path length, 4.5 mL nominal capacity, 4 optical sides, polymethylmethacrylate, Kartell). Then, the cuvette containing the sample solution was fixed in a sample holder, and the solution was irradiated for 2 min with a UV lamp (365 nm, 2.60 mW/cm\textsuperscript{2} at 2 cm; catalog no. ENF-280C, Spectroline) which was placed 2 cm
apart from the center of the sample holder. To obtain absorption spectra, the UV-irradiated solution was immediately transferred into a semi-micro disposable cuvette (10 mm path length, 1.6 mL nominal capacity, polymethylmethacrylate, Sarstedt) at dark and scanned (250-800 nm scan range, 0.5 nm interval, 600 nm/min scan speed) using an Agilent Cary 60 UV-Vis spectrophotometer, a Beckman Coulter DU 800 spectrophotometer, or a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. Before each UV scan, a blank PBS solution (1.0 mL) was scanned as a reference. Next, the solution was transferred back to a standard disposable cuvette (Kartell) at dark and irradiated for 30 min with a 590 nm laser beam (90 ± 5 mW at 590 nm, Shanghai Dream Lasers Technologies) which was placed 4 cm apart from the center of the sample holder. For the irradiation with 590 nm light, a magnifier lens was placed in the middle of the sample holder and laser beam (i.e., 2 cm apart from each), in order to simultaneously irradiate the entire area of the sample solution. The absorption spectra of the solution irradiated with 590 nm light was obtained in the same manner. To obtain emission spectra, the sample solution was irradiated alternately with UV (365 nm, 2 min) and visible (590 nm, 30 min) light in the same manner in a quartz Suprasil macro/semi-micro cell (4 mm × 4 mm light path, 0.5 mL cell volume, Perkin Elmer) at dark and scanned (500-700 nm scan range, irradiation at 510 nm) immediately after each irradiation using either an Agilent Cary Eclipse fluorescence spectrophotometer or a Perkin Elmer LS 55 fluorescence spectrometer. A single photoswitching cycle consisting of the sequential irradiation with UV (365 nm, 2 min) and visible (590 nm, 30 min) light was repeated for up to three or five times. All cuvettes were covered with the top lids in order to prevent evaporation during the experiments.

Changes of emission spectra of 1a in PBS (pH 7.4, 100 μg/mL) during the irradiation with visible (590 nm, 30 min) and UV (365 nm, 2 min) light were monitored using an Agilent Cary Eclipse fluorescence spectrophotometer (irradiation at 510 nm). The solution samples in a quartz Suprasil macro/semi-micro cell (Perkin Elmer) were irradiated in the same manner as described before. The emission spectra were collected starting from the off-state (immediately after the irradiation with UV light for 2 min) during the third photoswitching cycle for visible light irradiation (Figure 2b, main text), and during the subsequent fourth cycle for UV light irradiation (Figure 2c, main text).
The irradiation was interrupted briefly to obtain the spectra at the selected time points.

Cell Cultures

Human epithelial cervical cancer (HeLa) cells and human adipose-derived stem (hADS) cells were obtained from ATCC and Hanson biotech, respectively. Cells were maintained at 37 °C under a 5% carbon dioxide atmosphere in Dulbecco’s modified eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS), 50 U/mL penicillin, and 50 μg/mL streptomycin.

Cytotoxicity Assays

A suspension of silica NP 1a in deionized water (5 mg/mL) was prepared. Serial dilutions were carried out starting from suspension 1a at 5 mg/mL immediately after each vigorous shaking using serum-free DMEM to prepare samples of the following concentrations: 0.1, 0.5, 1, 5, 10, 50, and 100 μg/mL. HeLa or hADS cells were seeded in a flat bottomed 96-well microplate (Falcon) at a density of $1 \times 10^3$ cells per well and incubated for 14 h at 37 °C to allow cell attachment. Cells were treated with 100 μL of each dilution or 100 μL of serum-free DMEM (as a control) per well and incubated at 37 °C for 24, 48, and 72 h. The formulations were replaced with serum-free DMEM containing 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and cells were incubated for additional 4 h. Subsequently, MTT was removed carefully and DMSO (100 μL) was added to each well to dissolve the formazan crystals. Absorbance was measured at 570 nm using a BioRad microplate reader. Assays were carried out in quadruplicates.

Cellular Uptake Studies by Flow Cytometry

HeLa or hADS cells were seeded in a flat bottomed 6-well plate (Falcon) at a density of $1 \times 10^5$ cells per well and incubated for 24 h at 37 °C to allow cell attachment. A suspension of silica NP 1a (5 mg/mL) was diluted using serum-free DMEM immediately after vigorous shaking to prepare 1.0 mL of sample at 2 mg/mL. To ensure maximal fluorescence activation and avoid deviations caused by varying degree of partial fluorescence quenching among different sample preparations, the 1.0 mL suspension of
**Photoswitching Experiments on Living Cells**

The arrangement of the optical equipment for reversible photoswitching on living cells is shown in Figure S9. HeLa or hADS cells were seeded in a μ-Slide 8-well microscope sample chamber (ibidi) at a density of $1 \times 10^5$ cells per well. A suspension of silica NP 1a (5 mg/mL) was diluted using serum-free DMEM (Gibco) immediately after vigorous shaking to prepare sample at 50 μg/mL. Cells were washed with PBS three times, treated with 50 μg/mL suspension of silica NP 1a (200 μL per well), and incubated for 30 min at 37 °C under a 5% CO$_2$ atmosphere. The formulations were removed by washing with PBS three times, and the cells were treated with 200 μL of serum-free DMEM. The fluorescence image of living cells was taken using a DeltaVision RT imaging system (Applied Precision) with a filter set of RD-TR-PE ($\lambda_{ex}$ 555 ± 14 nm, $\lambda_{em}$ 617 ± 36.5 nm). Subsequently, the entire area of a selected well containing cells treated...
as such was irradiated with a UV lamp (365 nm) for 2 min. Here, the UV lamp was placed at the top (facing downward) of the well-chamber so that the distance between the samples and the UV light source (i.e., 2 cm) matched that used for the optical experiments carried out in a cuvette (Figure S9b). The fluorescence image of the UV-irradiated living cells was obtained immediately using a DeltaVision RT imaging system. Next, the entire area of the same selected well was irradiated for 30 min with a 590 nm laser beam through a magnifier lens. Here, the 590 nm laser was mounted at the top of the sample securely (facing downward) and a magnifier lens was fixed in between the sample and the laser beam (2 cm apart from each) ensuring simultaneous irradiation of the entire area of the sample well (Figure S9c). The fluorescence image of the living cells irradiated with a 590 nm light was obtained immediately using a DeltaVision RT imaging system. A single photoswitching cycle consisting of irradiation of living cells with UV light (365 nm) for 2 min followed by irradiation with visible light (590 nm) for 30 min was repeated for up to 10 times. The entire sequence of the photoswitching experiments using living cells was performed in a darkroom. The average fluorescence intensity values (mean ± SD) at the selected regions of interest (ROIs) (dotted circles in Figure 3a,b; 36 data points each) after each light application were measured using the softWoRx software.

Effect of UV Light Irradiation on Cell Viability

In order to examine the effect of UV light (365 nm) irradiation on cell viability under the photoswitching conditions, three sets (UV1, UV3, and UV5; see Figure S10) of cytotoxicity assays were performed using HeLa cell cultures, each set consisting of a test group and two control groups. Briefly, for each test group, cells treated with silica nanoparticle 1a underwent a different number of photoswitching cycles ending with UV light irradiation. For control groups, cells without (“control A”) or with (“control B”) internalized 1a were placed alongside the corresponding test group with complete protection from light during the entire photoswitching period (i.e., similar temperature and delay time as those for the test group until treated with MTT). Both controls were used for each set of the assay to more accurately assess only the effect of light—particularly UV—irradiation on cell viability. Namely, “control A” was intended to
exclude the effect of natural cell death during the photoswitching period (delay time) when cells were left at the experimental area (ca. 25 ± 5 °C) instead of the incubator (37 °C), and “control B” was intended to exclude the effect of internalized 1a under the same conditions. Furthermore, a set of the cytotoxicity assay was added which was composed of only two control groups without the delay time (i.e., no photoswitching; see “before irradiation” in Figure S10). The specific irradiation scheme for each test group is the following: UV (365 nm, 2 min) → visible (590 nm, 30 min) for “UV1”; UV → visible → UV → visible → UV for “UV3”; UV → visible → UV → visible → UV → visible → UV → visible → UV for “UV5”.

A suspension of silica NP 1a (5 mg/mL) was diluted using serum-free DMEM immediately after vigorous shaking to prepare 1a at 50 μg/mL. HeLa cells were seeded in four adjacent central wells (2 × 2 block) of each flat bottomed 96-well microplate (Falcon) at a density of 1 × 10³ cells per well and incubated for 16 h at 37 °C to allow cell attachment. Usually, three plates were used for a single set of experiment to prepare samples for a test group, control A, and control B. The incubated medium was removed, cells were quickly rinsed with PBS (× 3), and each well was either filled with 100 μL of 1a at 50 μg/mL (for the test group and control B) or 100 μL of serum-free DMEM (for control A). These three plates were incubated at 37 °C for 30 min, the formulations were removed, and cells were quickly rinsed with PBS (× 3). Each well was then filled with 100 μL of serum-free DMEM, and only the test group underwent specific number of photoswitching cycles. Meanwhile, controls A and B were placed in the same experimental area as that of the test group for the entire photoswitching period with complete protection from light. Photoswitching experiment was conducted in the same manner as described in the previous section (Figure S9). Here, simultaneous irradiation of cells with visible light in four central wells of the test group was ensured by carefully adjusting the magnifier lens. Subsequently, 10 μL of MTT in PBS (5 mg/mL) was added to four sample wells of all three plates within 5 min, and the cells were incubated at 37 °C for 4 h. MTT was removed carefully and DMSO (100 μL) was added to each well to dissolve the formazan crystals. Absorbance was measured at 570 nm using a BioRad microplate reader. Assays were carried out in quadruplicates.
REFERENCES

Table S1. Characterization of silica nanoparticles 1a, 1b, 2a, and 2b.\textsuperscript{a}

<table>
<thead>
<tr>
<th>cmpd</th>
<th>zeta potential\textsuperscript{b} (mV)</th>
<th>size by TEM\textsuperscript{c} (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>-29.1 ± 0.1</td>
<td>28.6 ± 2.9</td>
</tr>
<tr>
<td>1b</td>
<td>-30.5 ± 0.5</td>
<td>23.1 ± 2.8</td>
</tr>
<tr>
<td>2a</td>
<td>33.6 ± 2.1</td>
<td>26.6 ± 2.2</td>
</tr>
<tr>
<td>2b</td>
<td>30.0 ± 2.9</td>
<td>21.4 ± 2.4</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Results are listed as mean ± SD. \textsuperscript{b} Samples were prepared in deionized water at 100 µg/mL. Five independent measurements were made, and the average values were calculated after excluding the highest and lowest values (\(n = 3\)). \textsuperscript{c} The number of nanoparticles analyzed (\(n\)) was 55 for 1a, 35 for 1b, 35 for 2a, and 37 for 2b. TEM images are shown in Figure 1d of the main text.
Table S2. Comparison of photoswitching efficiency of silica nanoparticle 1a in different environments.$^a$

<table>
<thead>
<tr>
<th>photoswitching cycles</th>
<th>in a cuvette (PBS)</th>
<th>in living cells$^b$</th>
<th>HeLa</th>
<th>hADS</th>
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<tr>
<td>after 1st UV</td>
<td>6.15</td>
<td>0.191</td>
<td>0.200</td>
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<tr>
<td>after 2nd UV</td>
<td>13.48</td>
<td>1.125</td>
<td>1.028</td>
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<tr>
<td>after 2nd Vis</td>
<td>6.06</td>
<td>0.186</td>
<td>0.202</td>
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<tr>
<td>after 3rd Vis</td>
<td>12.99</td>
<td>1.135</td>
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<td>after 3rd UV</td>
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<td>1.139</td>
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<tr>
<td>after 6th UV</td>
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<tr>
<td>after 6th Vis</td>
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<td>0.187</td>
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<td>after 7th UV</td>
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<tr>
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<tr>
<td>after 8th Vis</td>
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<tr>
<td>after 9th UV</td>
<td>—</td>
<td>1.169</td>
<td>1.053</td>
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<tr>
<td>after 9th Vis</td>
<td>—</td>
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<tr>
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<td>1.052</td>
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<tr>
<td>after 10th Vis</td>
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<tr>
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<td>81.38</td>
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<tr>
<td>contrast (ON/OFF)</td>
<td>2.19</td>
<td>6.22</td>
<td>5.37</td>
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</table>

$^a$ The fluorescence intensity was obtained using an Agilent Cary Eclipse fluorescence spectrophotometer for the solution sample in a cuvette ($n$ (number of cycles) = 5, 100 μg/mL each, at 568.5 nm) or a DeltaVision RT imaging system (Applied Precision) for cells ($n$ = 10; see Figure 3, main text) as an average value within each selected region of interest (ROI) as described in the Experimental Section. For all photoswitching experiments, samples were irradiated alternately with UV (365 nm, 2 min) and visible (590 nm, 30 min) light. $^b$ Cells were treated with 50 μg/mL suspensions of 1a in DMEM and incubated for 30 min at 37 °C. $^c$ Determined as (ON-OFF)/ON.
Figure S1. ESI HRMS spectra of silane derivatives, (a) DAE-TES (3a), (b) mTEG-TES (3b), and (c) Cy3-TES (4), used for copolymerization reactions to prepare silica nanoparticles 1a and 1b.
Figure S2. (a) Photochromic conversion efficiency of diarylethene derivative 10 (1.0 mM) under the photoswitching conditions as determined by $^1$H NMR integrals in D$_2$O (Varian INOVA 600, 25 °C). (b) The spectral range showing the 2-methyl peak of thiophene ring, which exhibits the most drastic change upon alternate irradiation with UV (365 nm, 2 min, 2.60 mW/cm$^2$) and visible (590 nm, 30 min, 90 ± 5 mW) light, is expanded. The sample solution of 10 (600 μL) in a standard 5 mm NMR tube (Wilmad, 535-PP-7) was irradiated in a similar manner as that for the experiments in cuvette before running each NMR experiment. (c) Peaks corresponding to H$_c$ and H$_{oa}$/H$_{op}$ were identified through a line fitting method using the MestReNova 8.1.1 software.
**Figure S3.** Energy-minimized structures of compound 10 as (a) an antiparallel conformation of the ring-open isomer, (b) a parallel conformation of the ring-open isomer, and (c) the ring-closed isomer. Energy-minimized structures and the heat of formation were calculated for 10 with their ethylene oxide linkers (side arms) in all-anti conformations using the HyperChem7.5.2 software through a semi-empirical AM1 method. The bond length shown in green for the antiparallel conformer corresponds to the C2–C2’ distance (Å) of two thiophene units which will be connected upon cyclization.
Figure S4. Determination of response time of silica nanoparticle 1a. Fluorescence spectra (irradiation at 510 nm) obtained during the extended irradiation of 1a with (a) visible (590 nm, 90 min, 41 mW) and (b) UV (365 nm, 4 min, 2.60 mW/cm²) light in PBS (pH 7.4, 100 μg/mL). Fluorescence spectra were collected (a) starting from the off-state for visible light irradiation, and (b) for the subsequent UV light irradiation, interrupting briefly to obtain the spectra at the selected time points. Fluorescence intensity at 568.5 nm (λ_{max}) was plotted from the corresponding spectra (a or b) for (c) visible and (d) UV light irradiation. Each plot was curve-fitted (exponential decay) to find the equilibrium state. The irradiation time used for visible (30 min) or UV (2 min) light for all other photoswitching experiments herein is marked in red.
Figure S5. Reversible photoswitching experiments using (a,b) compound 10 (10 μM) and (c,d) Cy3 (1 μM) as monitored by (a,c) UV-Vis and (b,d) fluorescence (irradiation at 510 nm; 5 nm excitation slit and 2.5 nm emission slit) spectroscopy in PBS (pH 7.4; total volume: 1.0 mL).
**Figure S6.** Reversible photoswitching experiments using silica nanoparticle (a,b) 1a (500 μg/mL for (a) and 100 μg/mL for (b)) and (c,d) its non-switchable control 1b (250 μg/mL for (c) and 100 μg/mL for (d)) as monitored by (a,c) UV-Vis and (b,d) fluorescence (irradiation both at 510 nm; 5 nm excitation slit and 2.5 nm emission slit for (b); 10 nm excitation slit and 10 nm emission slit for (d)) spectroscopy in PBS (pH 7.4; total volume: 1.0 mL).
Figure S7. Cytotoxicity assay results of the silica nanoparticle **1a** at (a) HeLa or (b) hADS cell cultures. See Experimental Section for details.
Figure S8. Flow cytometry histograms of (a,b) HeLa and (c,d) hADS cells each incubated at 37 °C with the silica nanoparticle 1a for 5, 15, 30, 45, 60, 120, or 180 min (control: no 1a added). Final concentration of 1a in the medium was (a,c) 5 μg/mL or (b,d) 50 μg/mL. See Experimental Section for details.
Figure S9. (a) Arrangement of optical equipment for photoswitching experiments on living cells. An 8-well microscope sample chamber containing the living HeLa or hADS cells in a selected well (seeded at a density of $1 \times 10^4$ cells per well) with internalized silica NP 1a (50 μg/mL) was placed directly underneath each light source (365 nm or 590 nm). The entire area of the selected well containing cell samples was irradiated alternately with (b) UV (365 nm, 2 min) and (c) visible (590 nm, 30 min) light. Usage of a magnifier lens was necessary when the 590 nm laser beam was used in order to simultaneously irradiate the entire area of a well. Immediately after the completion of each irradiation with either UV or visible light, fluorescence images of living cells (Figure 3a,b in the main text) were taken using a DeltaVision RT imaging system. These photoswitching experiments on living cells including fluorescence imaging were carried out in a darkroom. See Experimental Section for details.
Figure S10. Effect of UV light irradiation on cell viability during photoswitching experiments. Three sets (UV1, UV3, and UV5) of cytotoxicity assays were performed using HeLa cell cultures, each set consisting of a test group and two control groups. For the test group, cells treated with silica nanoparticle 1a underwent a different number of photoswitching cycles ending with UV light irradiation. For control groups, cells without (“control A”) or with (“control B”) internalized 1a were placed alongside the corresponding test group with complete protection from light during the entire photoswitching period (i.e., similar temperature and delay time as those for the test group until treated with MTT). An additional set of the assay composed of only two control groups without the delay time was added (“before irradiation”). The specific irradiation scheme for each test group is the following: UV (365 nm, 2 min, 2.60 mW/cm²) → visible (590 nm, 30 min, 90 ± 5 mW) for “UV1”; UV → visible → UV → visible → UV for “UV3”; UV → visible → UV → visible → UV → visible → UV → visible → UV for “UV5”. See Experimental Section for details.