Supporting Information

Photoreversible cellular imaging using photochrome-conjugated fullerene-silica nanoparticle

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Experimental details

1. Preparation of photochrome (dithienylethene)

Compounds 4 were prepared following the literature procedures.\textsuperscript{1-4}

1,2-Bis-(5-[4-(2-[2-(2-[methoxycarbomethoxy]-ethoxy)-ethoxy]-ethoxy]-ethoxy)-phenyl]-2-methyl-thiophene-3-yl]-perfluorocyclopentene (5)

To a mixture of compound 4 (0.5 g, 0.9 mmol) and 2-[2-[2-[methoxycarbomethoxy]-ethoxy]-ethoxy]-ethoxy]yl p-toluenesulfonate (0.67 g, 1.9 mmol) in dry CH\textsubscript{3}CN (40 mL) were added anhydrous K\textsubscript{2}CO\textsubscript{3} (0.37 g, 2.7 mmol) under nitrogen atmosphere. The reaction mixture was refluxed for 16 h. After removal of the solvent under reduced pressure, the residual was extracted with dichloromethane (100 mL), and the organic layer was washed 3 times with 50 mL of water, dried over anhydrous MgSO\textsubscript{4} and concentrated. The crude product was purified by a silica gel column chromatography (ethyl acetate) to yield 5 (0.75 g, 80\% yield) of 5 obtained in 80\% yield.

\textsuperscript{1}H-NMR (300 MHz, CDCl\textsubscript{3}): δ 1.94 (s, 6H: S-C-\textsuperscript{CH\textsubscript{3}}), 3.66-3.77 (m, 30H: 24H: -CH\textsubscript{2}[-CH\textsubscript{2}-O-CH\textsubscript{2}], -CH\textsubscript{2}-O-CH\textsubscript{2}-COOH, 6H: -CO-O-CH\textsubscript{2}), 3.87 (t, J = 5.1 Hz 4H: Ar-O-CH\textsubscript{2} - CH\textsubscript{2}-O), 4.11-4.17 (m, 8H: 4H for Ar-O-CH\textsubscript{2}-CH\textsubscript{2}-O, 4H for -CH\textsubscript{2}-CH\textsubscript{2}-O-CH\textsubscript{2}-CO), 6.92 (d, J = 5.1 Hz 4H: -S-C-C-CH\textsubscript{2}-C-O-), 7.14 (s, 2H: -S-C-CH-C-), 7.45 (d, J = 8.9 Hz 4H: -S-C-C-CH\textsubscript{2}-CH\textsubscript{2}-CO-)

1,2-Bis-(5-[4-(2-[2-(2-[hydrazidocarbomethoxy]-ethoxy)-ethoxy]-ethoxy)-ethoxy]-ethoxy)-phenyl]-2-methyl-thiophene-3-yl]-perfluorocyclopentene (6)
To a solution of 5 (0.248 g, 0.236 mmol) in MeOH (20 mL) was added hydrazine hydroxide solution (0.019 g, 0.38 mmol). After being stirred at 70 °C for 14 h, the volatiles were evaporated under reduced pressure. The solution was washed with 0.2M sodium bicarbonate and extracted with ethyl acetate (3 times with 30 mL) and washed with distilled water. The combined extracts were dried over anhydrous MgSO₄ and filtered. The filtrate was evaporated to dryness in vacuo. The crude compound was purified by a silica gel column chromatography (EA/MeOH = 1/1) to yield 6 (0.1042 g, 42% yield).

¹H-NMR (300 MHz, CDCl₃): δ 1.94 (s, 6H: S-C-CH₃), 3.66-3.77 (m, 24H: -CH₂[-CH₂-O-CH₂]₂- CH₂-O-CH₂-COOH), 3.87 (t, J = 5.1 Hz 4H: Ar-O-CH₂ CH₂-O), 4.11-4.17 (m, 8H: 4H for Ar-O-CH₂ CH₂-O, 4H for -CH₂-CH₂-O-CH₂-CNH), 7.14 (s, 2H: -S-C-CH-C-), 7.45 (d, J = 8.7 Hz, 4H: -S-C-C-CH₂-CH₂-C-O-), 8.4 (s, 2H: --C-O-NH-NH₂)

Fig. S1. (A) Synthetic scheme of amine-functionalized photochrome (PC), (B) photoswitchable interconversion of dithienylethene photochrome, (C) LC-MS spectrum of PC.
2. Preparation and characterization of PC-FSNP

**Materials.** Octafluorocyclopentene was purchased from Tokyo Chemical Industry Co., LTD (Tokyo, Japan). C$_{60}$ fullerene (99%) was obtained from SES Research (Houston, TX, USA) and toluene was purchased from Samchun Pure Chemical Co., LTD (Pyeongtaek, Gyeonggi, Korea). Ammonium hydroxide (28 wt %) purchased from Daejung Chemicals and Metals (Siheung, Gyeonggi, Korea) and ethanol (99.9%) was obtained from Merck (Darmstadt, Germany). Sodium hydroxide (NaOH) was purchased from Junsei chemical Co., Ltd., (Tokyo, Japan). Cyclohexane, hexyl alcohol, Triton X-100, tetraethyl orthosilicate (TEOS, 98%) and all chemical reagents were from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Milli-Q water was used.

**Preparation of PC-FSNP.** Monodisperse, fluorescent photochromic fullerene-silica nanoparticles were synthesized by the modified reverse micelle microemulsion method efficiently. In the experimental procedure, first, C$_{60}$ fullerene was dissolved in toluene at a concentration of 2 mg / mL. Then a solution was prepared by mixing C$_{60}$ solution (2 mL), cyclohexane (5.5 mL), hexyl alcohol (2 mL), Triton X-100 (1.7 mL) and Milli-Q water in 50 ml vial. Triton X-100 and hexyl alcohol were used as a surfactant and a cosurfactant respectively. After a transparent blue solution was obtained (about 5 min of stirring), ammonium hydroxide (0.6 mL) and mixture of the photochrome (5 mg in 0.05 mL toluene) and tetraethyl orthosilicate (TEOS) (0.1 mL) were subsequently added under stirring at 600 rpm. Ammonium hydroxide is a catalyst for TEOS hydrolysis. The reaction mixture was stirred for 20 hr in the darkness to prevent optical contamination of photochrome at room temperature. To finish the reaction, 25 ml of ethanol was added then the mixture was stirred continuously for 6 hr. The solution containing fullerene-silica nanoparticles incorporated photochrome was centrifuged at 3000 rpm for 30 min. After removing the supernatant, precipitate was re-dissolved in ethanol as a washing step. To remove the materials which did not react, this washing step was repeated twice more. After washing the nanoparticles were resuspended in distilled water.

**Characterization of PC-FSNP.** The particle size and morphology were analyzed with a Field-emission Scanning Electron Microscope (FE-SEM) (FEI, Sirion, Netherlands). The chemical composition was measured by using Fourier transform infrared spectrometer (Jascow, FTIR-4100, Japan) and thermogravimetric analyzers (Netzsch, TG209F3, Germany) with N$_2$ gas flow. The absorbance was measured by UV/VIS spectrophotometer (Beckman culter, Du 800, USA) and the photoluminescence was obtained using Ar ion laser. The UV and visible light irradiation were supplied by the portable UV lamp (VILBER LOURNAT, VL-4LC, France; 4 W, 365 nm) and visible light lamp (Changchun New Industries Optoelectronics Tech, MGL-III-532 nm, China) respectively.

3. Bioimaging using PC-FSNPs

**Cell lines and Cell culture.** The HeLa cells (American Type Culture Collection, [ATCC] Manassas, VA) were maintained at 37 °C with 5 % CO$_2$ in a water saturated atmosphere in Dulbecco’s Modified Eagle Medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS;GIBCO), 50 U/ml penicillin and 50 mg/ml streptomycin(GIBCO).
Live cell uptake and imaging. HeLa cells was cultured in an 8-well microscopy chamber (ibidi GmbH, Munich, Germany) with a density of 1×10⁴ cells per well. FSNP and PC-FSNP (50 µg/ml) solutions were prepared by diluting the each stock (2 mg/ml) solution with serum free DMEM. The cells were treated with 50 µg/ml each solution for the 1h. The images observed at ex 490 nm and ex 617 nm using a DeltaVision RT microscope (applied Precision, BMS Korea). (Scale bar 20 µm)

Cytotoxicity of PC-FSNPs. HeLa cells were seeded in flat bottomed 96-well plate (Corning Costar, Cambridge, MA) at a density of 1×10³ cells per well and incubated 24 h total low cell attachment. The cells were treated with 10, 50, 100, 500 and 1000 g/ml FSNPs and PC-FSNPs for 48 h. After incubation, the formulations were replaced with DMEM containing MTT (5 mg/ml) and cells were then incubated for additional 4 h. MTT assay was aspirated off and DMSO was added to dissolve the formazan crystals²². Absorbance was measured at 570 nm using a BioRad microplate reader. Assays were carried out as quintuplicates.
**Fig. S2. FTIR spectrum of NaOH treated PC-FSNPs.** PC-FSNPs were prepared by adding to the 1M sodium hydroxide solution and reacted at room temperature for 12 h to remove the silica moieties. The NaOH-treated PC-FSNP was washed by distilled water for three times. After dried, the samples were mixed and ground with KBr then pressed into pellets using a disk-forming Handi-Press suitable for FT-IR analysis. (Inset: FTIR spectrum of the conjugate of PC-C$_{60}$)
Fig. S3. TGA profiles of FSNP and PC-FSNP. Figure S4 shows the thermal gravimetric analysis profiles measured under N₂ gas with a heating rate of 10 °C/min. The elimination pattern of both nanoparticles were similar to each other, however, the weight losses in 512 °C corresponding to the silanol (Si-OH) sublimation were significantly different about 20 %. The PC-FSNP was less lost in that region than FSNP, indicating that an amount of silica conjugated to C₆₀ was smaller than that of FSNP maybe because of the conjugation of PC in a nanoparticle.
Fig. S4. Time-dependent fluorescence changes of PC-FSNP after UV and Visible light irradiation.
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