Electronic Supplementary Information for:

Diarylethene doped biocompatible polymer dots for fluorescence switching

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Experimental section.

Materials.

Poly[9,9-dioctylfluorenyl-2,7-diyl]-co-(1,4-benzo-{2,1',3}-thiadiazole)] (PFBT, cat. number; ADS133YE) was purchased from ADS Dyes, Inc. (Quebec, Canada). Polystyrene graft ethylene oxide functionalized with carboxylic end group (PEG-COOH, Mn total 36,500, Mn of each branch; 4600) was purchased from Polymer Source Inc. (Quebec, Canada). 1,2-Bis(2,4-dimethl-5-phenyl-3-thienyl)-3,3,4,4,5,5-hexafluoro-1-cycl opentene was purchased from TCI (diarylethene, Tokyo, Japan). Streptavidin (SA) was purchased from New England Biolabs Inc. (MA, USA). All the other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Characterization. UV, FL and TEM.

UV-vis absorption spectra were measured with a Perkin Elmer Lambda35 UV-vis spectrometer. Fluorescence spectra were recorded using a Perkin Elmer LS 55 fluorescence spectrometer. Transmission electron microscopy (TEM) was carried out on an FEI Tecnai G2 F20 transmission electron microscope.

Photoswitching.

For the light illumination of bulk sample, transilluminator (325 nm for taking picture), black light (Spectroline, 365 nm long wave ultraviolet for UV and FL measurements, 15
s) and halogen lamp (JH technologies, SCHOTT dissection lamp equipped with 520 nm long path filter, 3 min) were used. For cellular switching, NIKON intensilight (equipped with 330 nm WB 80 nm band path filter, 30 s) and light passed through GFP filter cube of microscope (leica, CTR6000) were used for UV and visible light illumination, respectively.

**Synthesis of photoswitchable p-dot.**

Photoswitchable p-dots in aqueous solution were prepared by using a modified precipitation method. In a typical preparation, the PFBT, PEG-COOH and diarylethene were dissolved in tetrahydrofuran (THF) to make a 1 mg/ml stock solution, respectively. The two polymers and diarylethene were diluted in THF to produce a solution mixture with a PFBT concentration of 50 μg/ml, a PEG-COOH concentration of 400 μg/ml and diarylethene concentration of 0 to 1000 μg/ml. The mixture was sonicated to form a homogeneous solution. A 300 μl of the solution mixture was pipetted to 1 ml of milliQ water in a bath sonicator. The THF was removed with speed vac and the solution was filtrated though a 0.2 micron filter. Water was added to make 1 ml solution of p-dot. The p-dot solutions were clear and stable for months without aggregation.

**Surface modification with streptavidin.**

Surface modification was conducted based on Wu et al.’s method. In a typical
modification, 60 ml of polyethylene glycol (5% w/v PEG, MW 3350) and 60 µl of HEPES buffer (1 M) were added to 250 µl of filtered p-dot solution. Then, 10 µl of SA solution (1 mg/ml) was added to the solution. Lastly, 5 ul of freshly prepared EDC solution (5 mg/ml in milliQ water) was added to the solution and reacted at room temperature overnight. For purification from P-dot-streptavidin, Sephacyl HR-300 gel media was packed into 1ml syringe and rinsed thoroughly with 20 mM HEPES containing 0.1 wt% PEG.

**Cell culture and imaging.**

BHK21 cells were cultured in DMEM (high glucose formulation) containing 10% fetal bovine serum. Cells were plated in 48 wells plates at the density of 0.02 M/well. To visualize microtubule, we fixed the cultures with 4% paraformaldehyde in PBS buffer for 30 min at room temperature. Then, the cultures were rinsed two times with PBS. Cultures were permialized for 2 h with 0.5% triton and blocked overnight with 3% bovine serum albumin, respectively and then, incubated in PBS containing 1:50 dilution of anti tubulin-biotin (10 µg/µl) overnight at 4°C. Cells were rinsed three times and incubated with 10 nM P-dots. After three times washing, cells are imaged under Leica microscope.
Fig. S1 Energy diagram of photoswitchable biocompatible P-dots.
Fig. S2 TEM characterization of the P-dot. (a, b) TEM images and size distributions of non-doped P-dots (a) and diarylethene doped P-dot (1000 µg/ml), respectively. Sample was mounted on copper grids and stained with OsO₄ to increase the contrast.
**Fig. S3** Fluorescence images of cells stained with non-doped P-dots in the fixed BHK cells. Photoswitching upon UV irradiation was not observed. Scale bar is 50 µm.
Fig. S4 Photoswitching of P-dots doped with diarylethene in the fixed BHK cells. Top three images are same as shown in the Fig. 3. UV and visible light illumination toggled fluorescence back and forth in the fixed BHK cells. Scale bar is 50 μm.