Photoswitchable Semiconducting Polymer Dots for Pattern Encoding and Superresolution Imaging

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Materials

Acetone, diethyl ether, dichloromethane (DCM), Tetrahydrofuran (THF), toluene, and Na_2CO_3 were purchased from Beijing Chemical Plant. $Pd(PPh_3)_4$, n-BuLi (2.5 M, Hexane solution), trimethylsilyl chloride, hydrochloric acid, and CDCl3 were purchased from J&K Chemical Ltd. (Beijing, China). Octafluorocyclopentene, iodine monochloride, and tetrabutylammonium bromide were purchased from TCI. 9,9-dioctyl-9H-fluorene-2,7-diboronicacid bis(pinacol)ester, 4,7-Dibromo-2,1,3-benzothiadiazole and 4,7-Bis(5-bromo-4-hexylthiophen-2-yl)benzo[c][2,1,3]thiadiazole were purchased from Derthon Optoelectronic Materials Science & Technology Co., Ltd. All the above chemicals were of analytical grade and used as received without further purification. THF was distilled in the presence of sodium benzophenone under protection of dry nitrogen prior to use.

Optical setup

An N-STORM system (Nikon, Japan) equipped with a 100× TIRF objective (NA=1.45) and perfect focus system (PFS) was used for STORM imaging. Four laser sources,405-nm laser (OBIS405LX-100 mW, Coherent, America), 488-nm laser (OBIS488LS-100 mW, Coherent, America), 561-nm laser (Sapphire 561 LP-200 mW, Coherent, America) and 642-nm laser (2RU-VFL-P-500-642-B1R, MPB, MPB Communications Inc., Canada) were coupled with an optical fiber and introduced to an angle adjustable TIRF illuminator.

Synthesis of DTE-I

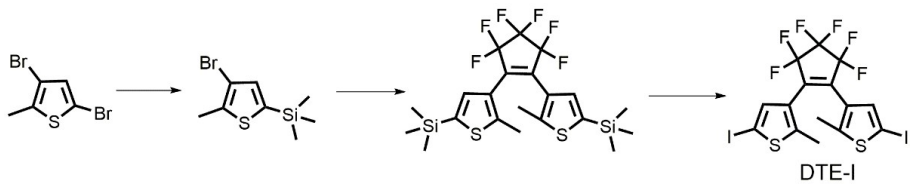

Figure S1. Synthetic route of DTE-I.

3,5-dibromo-2-methylthiophene (1.0 equiv, 3.13 g) and dry diethyl ether (130 mL) were added into a flame-dried Schlenk flask under Ar flow. The mixture was stirred and cooled to −78°C and dropwise added n-BuLi (1.1 equiv, 5.8 mL). The mixture was stirred for 90 min at −78°C, and then slowly added trimethylsilyl chloride (2.5 equiv, 3.88 mL). After stirring for another 90 min at −78°C, the solution returned to room temperature and stirred for 6 hours. The reaction mixture was poured into water, the organic layer was separated and the water layer was extracted with DCM for three times. The combined organic solution was washed with water several times. After evaporation of the DCM, the residual liquid reagent was removed under vacuum. The crude product was simply purified by column chromatography over silica gel with hexane as the eluent to yield a crude product as a primrose yellow liquid. The liquid (1 equiv, 4.39 g) was directly used in the next step and added into a Schlenk flask with 100 mL dried THF. The solution was cooled to -78°C and dropwise added n-BuLi (1.1 equiv, 7.7 mL) in 30 minutes. The mixture was stirred at this temperature for another 2 h. Then, octafluorocyclopentene (0.5 equiv, 1.18 mL) was added at -78°C quickly. The mixture was stirred at -78°C for 4 h and at room temperature for 18 h. Diluted hydrochloric acid was added to quench the reaction. The organic layer was separated and the water layer was extracted with DCM for three times. The combined organic solution was washed with water several times and dried with anhydrous Na_2SO_4. After evaporation of the DCM, the residual liquid reagent was removed under vacuum. The crude product was purified by column chromatography over silica gel with hexane as the eluent to yield a primrose yellow liquid, which changed into white solids at 0°C. ^{1}H NMR (400 MHz, CDCl3) δ 7.05 (s, 2 H), 1.90 (s, 6 H), 0.27 (s, 18 H).

In a two-necked round bottom flask, the product of above reaction (1.0 equiv, 4.99 g) was dissolved in dry chloroform (200 mL). The solution was cooled to 0°C in ice

bath before iodine monochloride (4.0 equiv, 2 mL) was added under Ar flow, the solution quickly turned red color. The solution was stirred at 0°C for 6 h and room temperature for 6 h. The reaction was quenched by adding into sodium thiosulfate aqueous solution. The organic layer was separated and the water layer was extracted with DCM for three times. The combined organic solution was dried with anhydrous Na_2SO_4 and solvents were evaporated under vacuum. The crude product was purified by column chromatography with hexane as the eluent to yield a red oily liquid (4.95 g, 82%). ^{1}H NMR (400 MHz, CDCl3) δ 7.18 (s, 2H), 1.89 (s, 6H). ^{13}C NMR (101 MHz, CDCl3) δ 147.71, 136.03, 126.59, 70.78, 14.33. MS: m/z calcd 619.81; found 620.81.

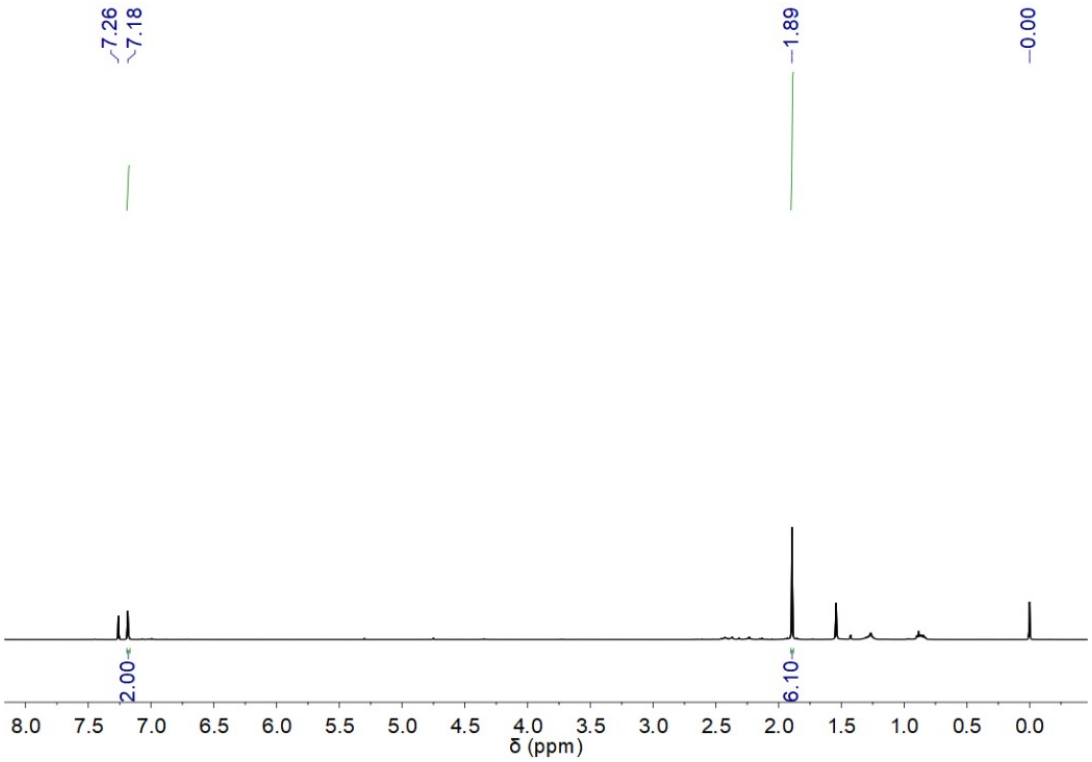

Figure S2. ^{1}H NMR of DTE-I.

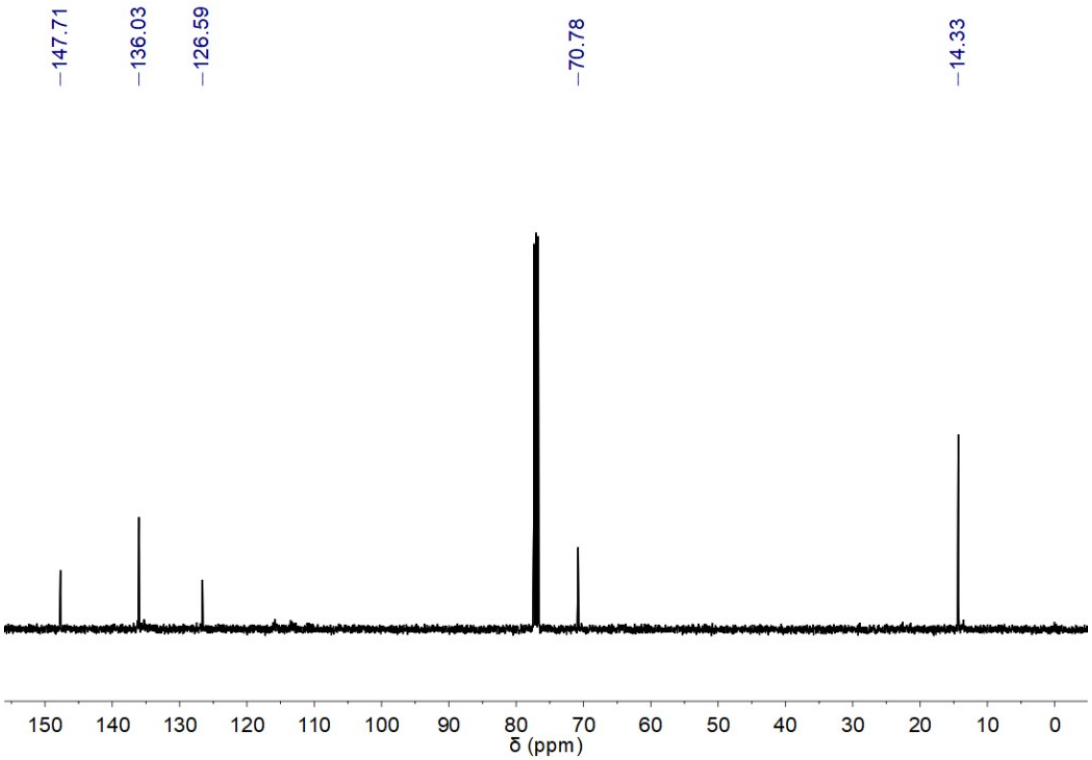

Figure S3. ^{13}C NMR of DTE-I.

Synthesis of DTE-PFBT and DTE-PFDTBT

The two photoswitchable polymers are synthesized by Suzuki coupling reaction, the general procedures are as follow:

DTE-PFBT: DTE-I (0.23 mmol, 0.1443 g), 9,9-dioctyl-9H-fluorene-2,7-diboronicacid bis(pinacol)ester (0.39 mmol, 0.2492 g), 4,7-Dibromo-2,1,3-benzothiadiazole (0.16 mmol, 0.0456 g) and tetrabutylammonium bromide (10 mg) were added into round-bottom flask. Then 15 mL toluene and Na_2CO_3 aqueous solution (10 mL, 2.0 M) mixture were added. The mixture was degassed and recharged with argon for three times, and added into tetrakis(triphenylphosphine) palladium (10 mg). The mixture was heated at 90^{o}C for 48 h. Before the polymerization completed, (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzene (10 mg) was added and stirred for 3 h. Then bromobenzene (0.1 mL) was added and stirred for another three hours to finish the polymerization reaction. The mixture was extracted by chloroform and the organic layer was washed with water and then dried over Na_2SO_4. The solvent was evaporated under vacuum and then dropwise added into methanol to yield solids. The solids had been future purified by Soxhlet extraction with acetone for 24 h and finally extracted

by THF. The final product was dried in vacuum at 40°C for 24 h (yellow solid 156 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 – 7.83 (m, 1H), 7.69 (s, 1H), 7.67 – 7.45 (m, 1H), 7.38 (d, J = 9.8 Hz, 1H), 6.98 (s, 1H), 2.27 (s, 1H), 2.05 (d, J = 5.3 Hz, 1H), 1.43 (s, 1H), 0.80 (t, J = 6.0 Hz, 1H). The DTE content in copolymer estimated via ^{1}H NMR is about 12%. Molecular weights were measured by gel-permeation chromatography (GPC) with polystyrene as a standard using THF as the eluent at 35°C: Mw = 30212, Mn = 12870, PDI = 2.34.

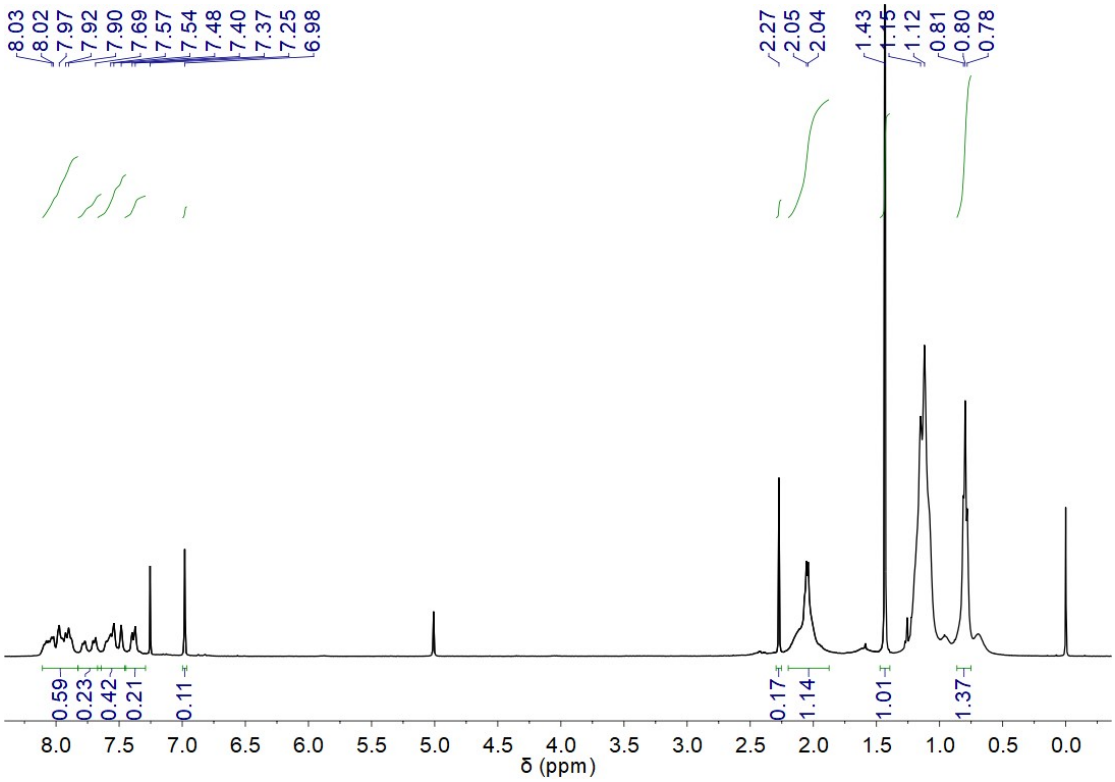

Figure S4. ^{1}H NMR of DTE-PFBT.

DTE-PFDTBT: DTE-I (0.22 mmol, 0.1343 g), 9,9-dioctyl-9H-fluorene-2,7-diboronicacid bis(pinacol)ester (0.36 mmol, 0.2319 g), and 4,7-Bis(5-bromo-4-hexylthiophen-2-yl)benzo[c][2,1,3]thiadiazole (0.14 mmol, 0.0905 g) were added and followed the same procedure as the synthesis of DTE-PFBT. Product: red solid 214 mg.

^{1}H NMR (400 MHz, $CDCl_3$) 8.07 (1 H, s), 7.91 (1 H, s), 7.75 (1 H, ddd, J 23.2, 14.4, 8.1), 7.53 (1 H, dt, J 24.3, 8.5), 7.38 (1 H, d, J 7.1), 6.98 (1 H, s), 2.81 (1 H, s), 2.27 (1 H, s), 2.05 (1 H, d, J 5.0), 1.75 (1 H, s), 1.47 – 1.27 (3 H, m), 1.28 – 1.00 (3 H, m), 0.89

(1 H, d, J 3.0), 0.85 – 0.75 (1 H, m). The DTE content in copolymer estimated via ^{1}H NMR is about 21%. Molecular weights were measured by gel-permeation chromatography (GPC) with polystyrene as a standard using THF as the eluent at 35°C: Mw = 14296, Mn = 6200, PDI = 2.31.

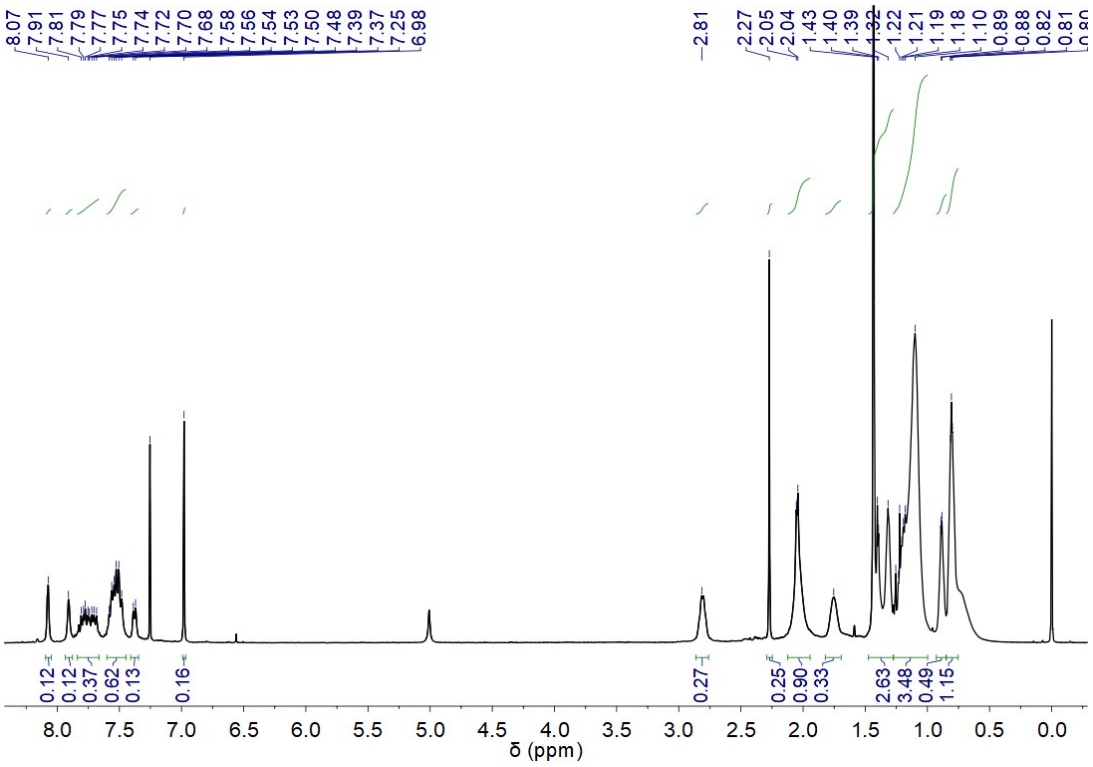

Figure S5. ^{1}H NMR of DTE-PFDTBT.

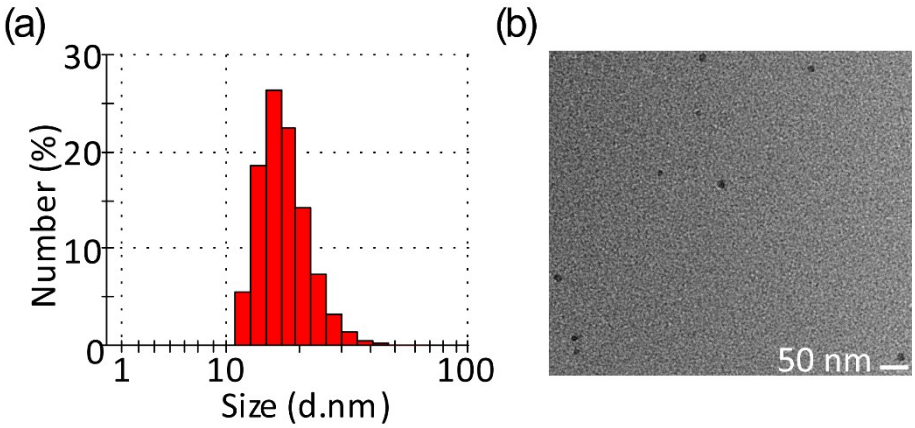

Figure S6. (a) Particle size of DTE-PFDTBT measured by DLS. (b) Representative TEM image of DTE-PFDTBT.

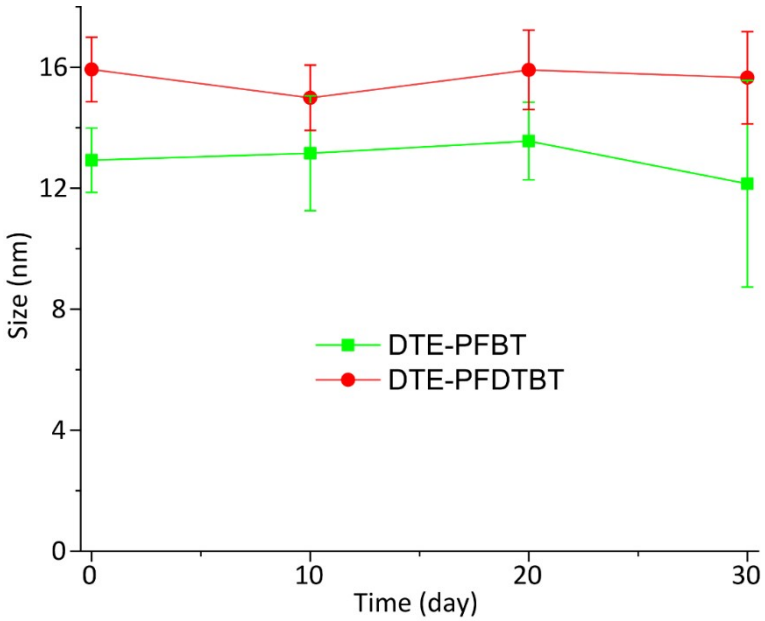

Figure S7. Change of average size of the DTE-PFBT and DTE-PFDTBTP dots with time.

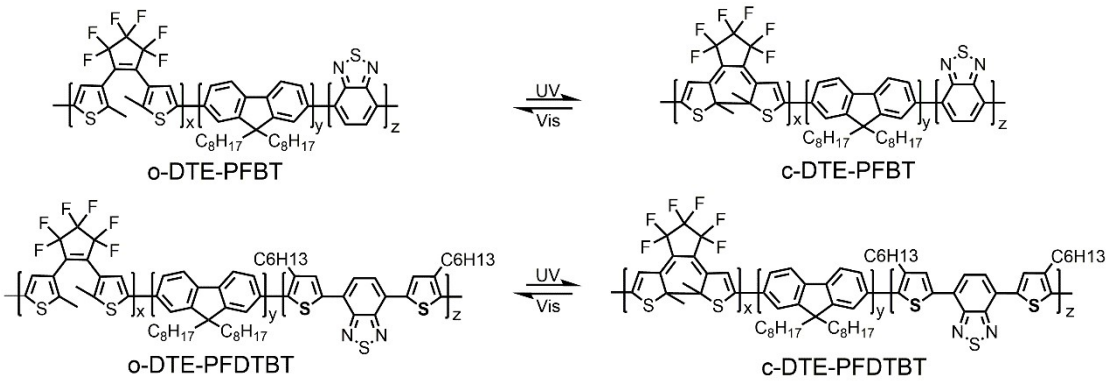

Figure S8. Open (o) and closed (c) isomers of DTE-PFBT and DTE-PFDTBT.

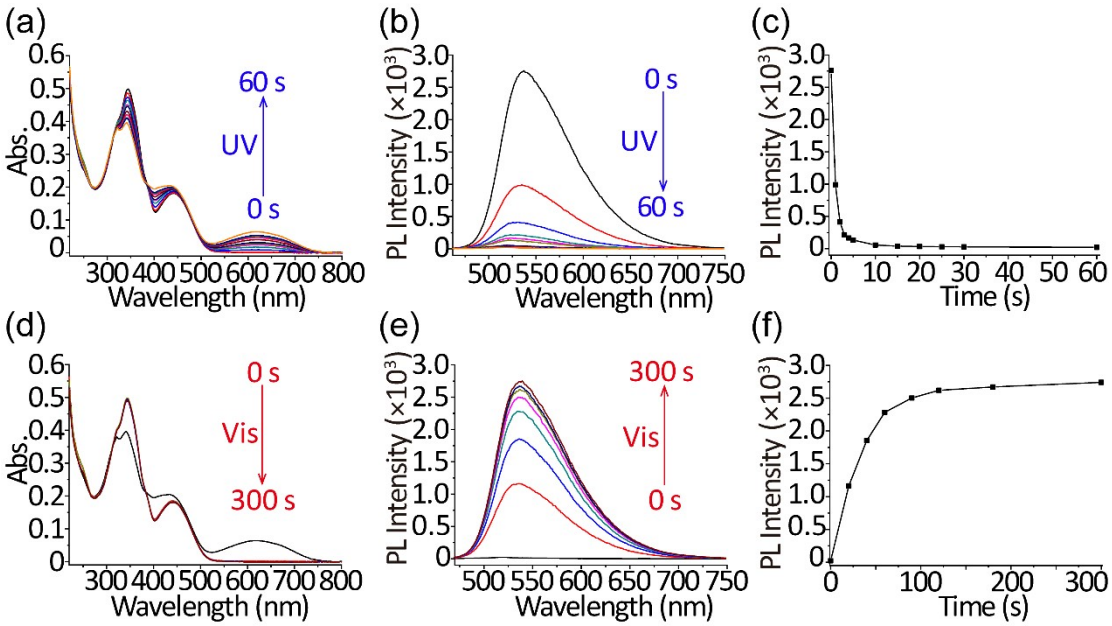

Figure S9. Changes of absorption spectra (a), fluorescence spectra (b) and fluorescence peak intensity (c) of DTE-PFBT under continuous UV (365 nm, 2 mW/cm^2) irradiation. Changes of absorption spectra (d), fluorescence spectra (e) and recovery of fluorescence peak intensity (f) of DTE-PFBT under continuous visible light (620 nm, 50 mW/cm^2) irradiation.

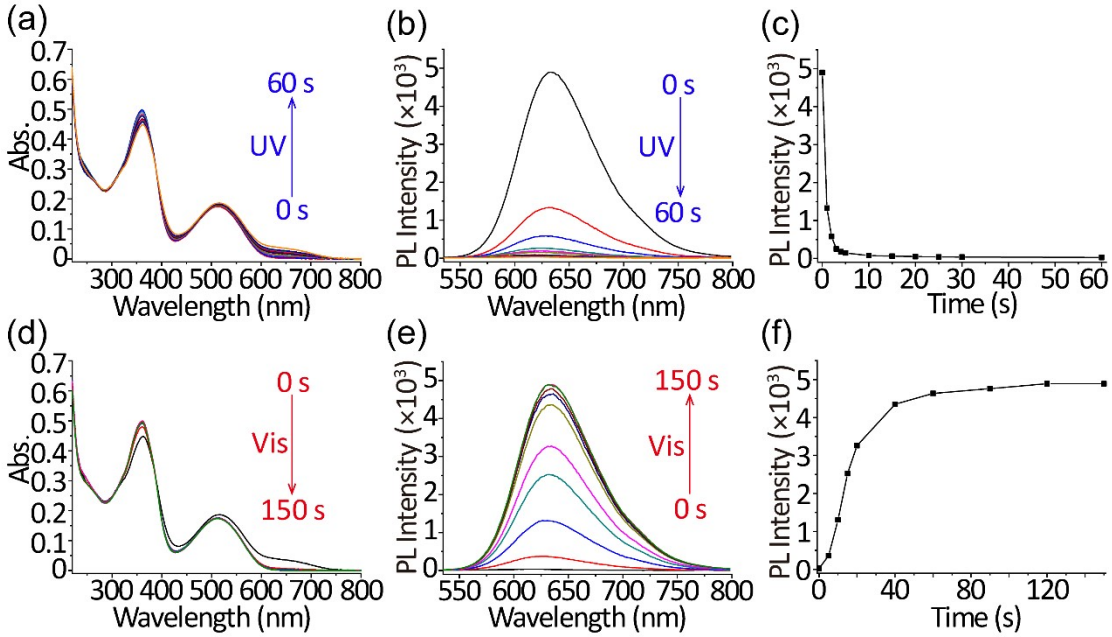

Figure S10. Changes of absorption spectra (a), fluorescence spectra (b) and fluorescence peak intensity (c) of DTE-PFDTBT under continuous UV (365 nm, 2 mW/cm^2) irradiation. Changes of absorption spectra (d), fluorescence spectra (e) and recovery of fluorescence peak intensity (f) of DTE-PFDTBT under continuous visible light (620 nm, 50 mW/cm^2) irradiation.

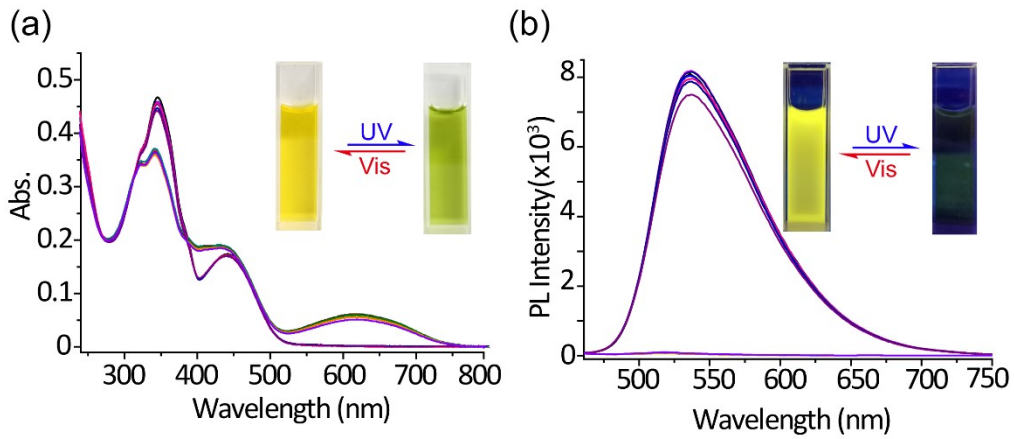

Figure S11. Absorption (a) and fluorescence (b) spectra of DTE-PFBT under alternative 365 nm (2 mW/cm^2) and 620 nm (50 mW/cm^2) irradiation. Insets: photographs of color and fluorescence changes upon irradiation with different light.

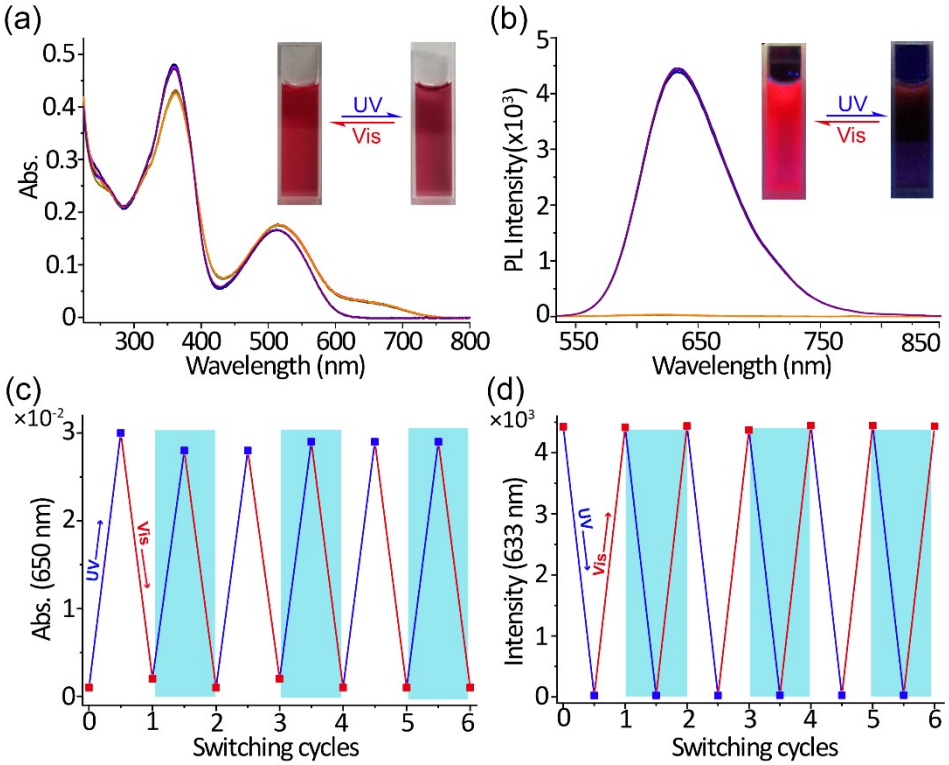

Figure S12. Absorption (a) and fluorescence (b) spectra of DTE-PFDTBT under alternative 365 nm (2 mW/cm^2) and 620 nm (50 mW/cm^2)irradiation. Insets: photographs of colour and fluorescence changes upon irradiation with different light. Absorbance at 650 nm (c) and emission at 633 nm (d) of DTE-PFDTBT during repetitive switching cycles upon alternative irradiation with 365 nm and 620 nm.

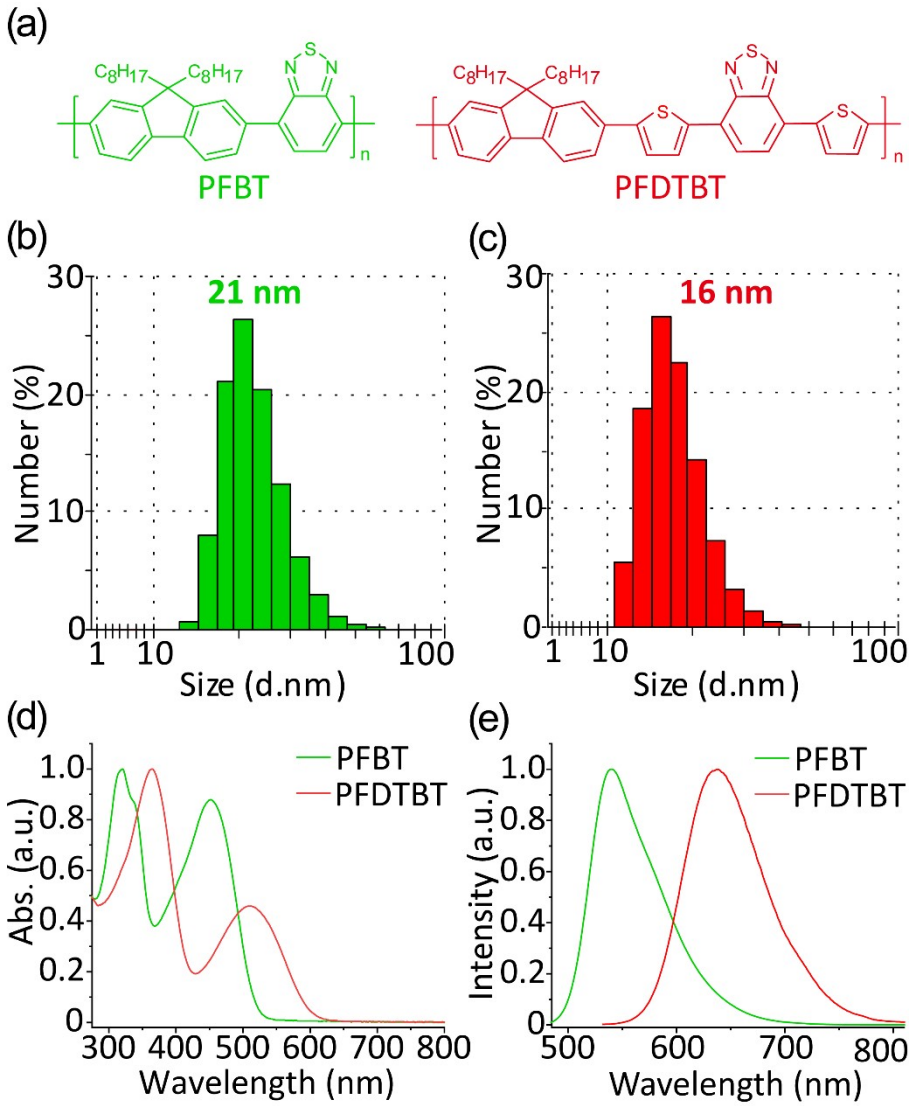

Figure S13. (a) Structure of PFBT and PFBTDT polymers. (b), (c) Size distribution of PFBT and PFBTDT Pdots measured by DLS, respectively. Absorption (d) and fluorescence (e) spectra of PFBT and PFBTDT.

Combination of different Pdots solutions for pattern encoding

Four types of Pdots are prepared by modified nanoprecipitation with the same concentrations of 50 ppm. Then the Pdots solution are mixed with certain ratio. The ratio of DTE-PFBT to DTE-PFBT is 1:1, the ratio of PFBT to DTE-PFDTBT IS 1:2, the ratio of DTE-PFBT to PFDTBT is 5:4, and the ratio of PFBT to PFDTBT is 2:3.

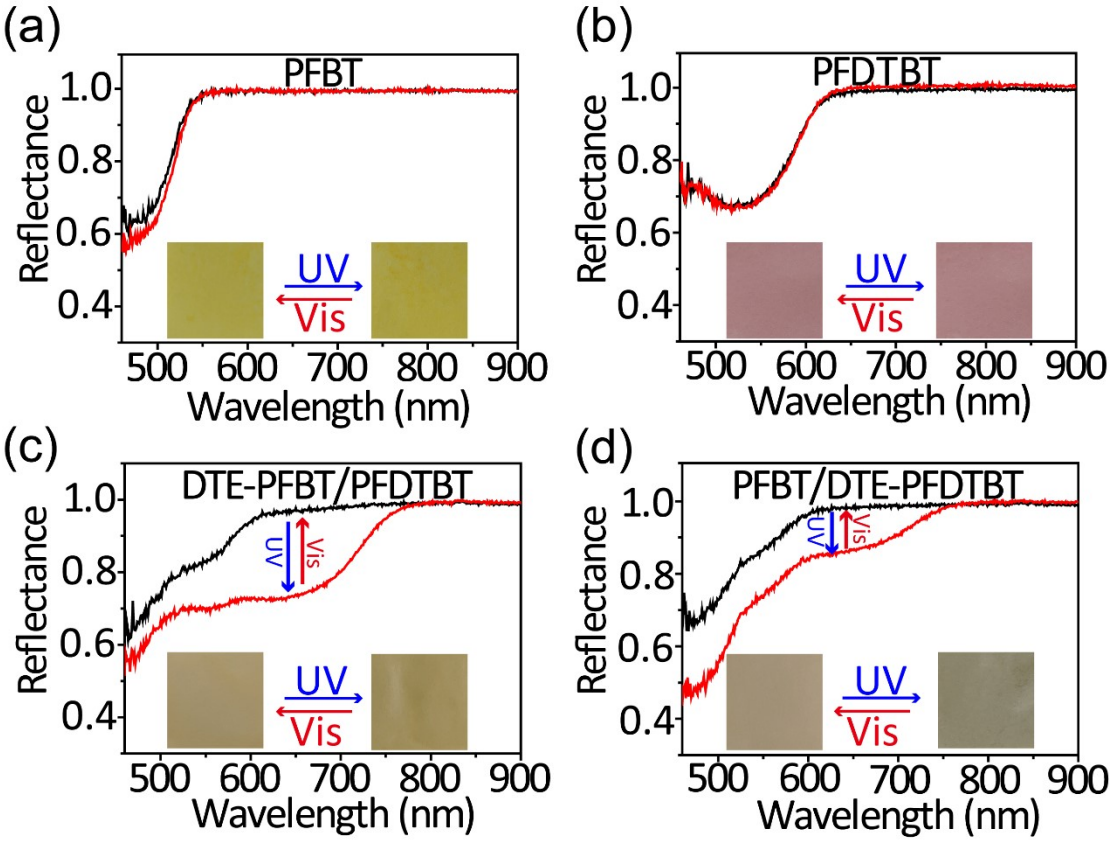

Figure S14. Reflectance spectra of PFBT, PFDTBT, DTE-PFBT and PFDTBT mixture, and PFBT and DTE-PFDTBT mixture under UV (365 nm, 2 mW/cm^2) and visible light (620 nm, 50 mW/cm^2) irradiation, respectively.

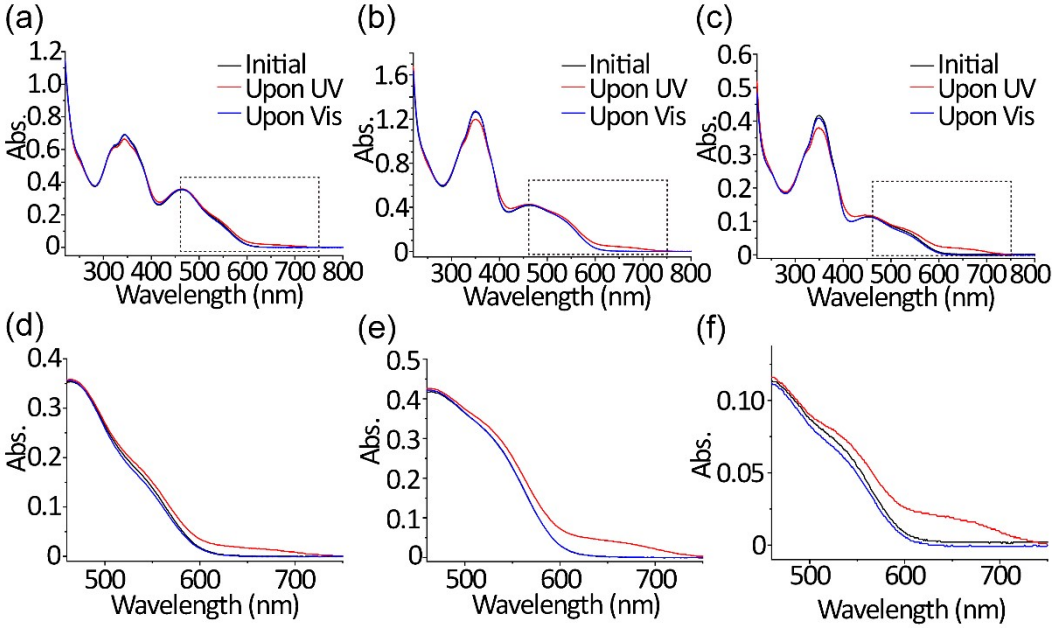

Figure S15. Absorption spectra of mixture solution of PFBT and DTE-PFDTBT (a), PFDTBT and DTE-PFBT (b), DTE-PFBT and DTE-PFDTBT (c) upon UV (365 nm, 2 mW/cm^2) or visible light (620 nm, 50 mW/cm^2) irradiation, respectively. (d)-(f) are

zoomed spectral range (460 nm-750 nm) marked with dotted box of (a)-(c).

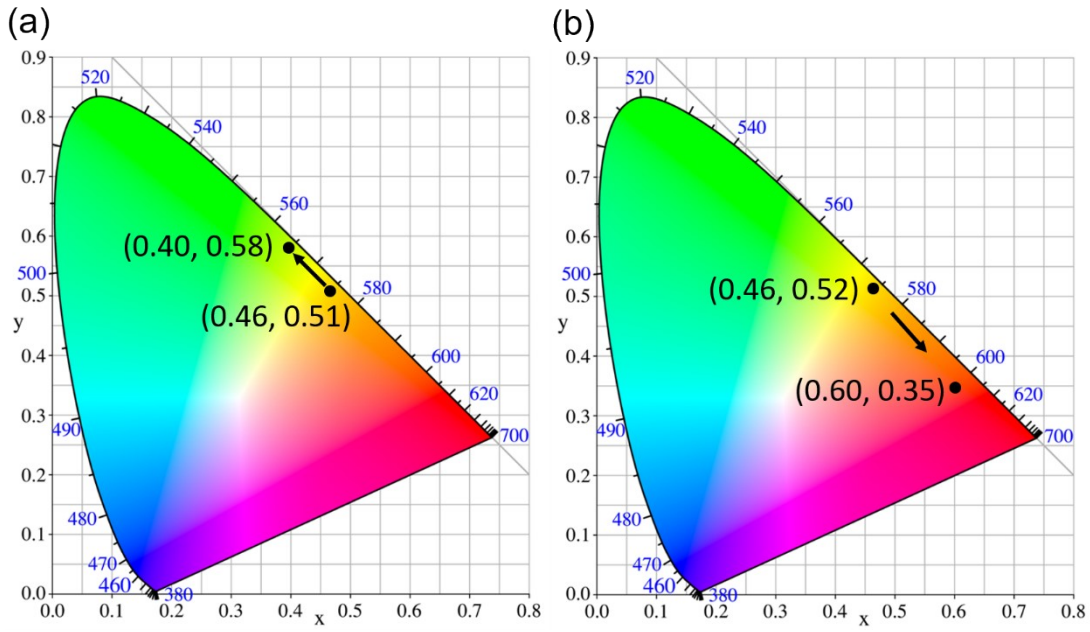

Figure S16. CIE chromaticity diagrams of the fluorescence changes under UV (365 nm, 2 mW/cm^2) irradiation of (a) PFBT and DTE-PFDTBT mixture, (b) PFDTBT and DTE-PFBT mixture, respectively.

Bioconjugation and specific subcellular labelling

Streptavidin bioconjugation of DTE-PFBT and DTE-PFDTBT Pdots for subcellular labeling were performed according to our previous work[1][2]. Except that before STORM imaging, an GLOX buffer containing 10% w/v glucose, 0.1% v/v β-mercaptoethanol, and 500 μg/ml glucose oxidase was introduced in order to reduce the influence of oxygen on the switch characteristics of the polymer dots.

Superresolution imaging of microtubules

Periodic 405 nm,488 nm and 640 nm laser pulses were used for STORM imaging. Specifically every 30 sections is a set of imaging cycles, in which only the 405 nm laser works in the first 20 sections, and the 488 nm laser and 640 nm laser are turned on at the same time in the last 10 sections. A perfect focus system (PFS) was used to minimize axial focus drift. The exposure time was set to 20 ms, 12000–27000 image

frames were collected for reconstruction of superresolution images. The activation/excitation/deactivation power typically used for imaging was 0.1-2 kW/cm^2 at the center of the laser spot. The images were subsequently analyzed using ThunderSTORM plugin in image J as follows.

In general, the wavelet filter is used to filter the original image sequence before a threshold to search for Pdots was set to 0.5 times the standard deviation. By means of centroid detection, the position of the pixel where the single particle was located was initially determined. The sub-pixel localization of single Pdot was determined by fitting the PSF to a Gaussian function with a fitting radius of 3 pixels. To correct for lateral drift during the experiment, we employed the cross-correlation drift-correction method supplied by ThunderSTORM.

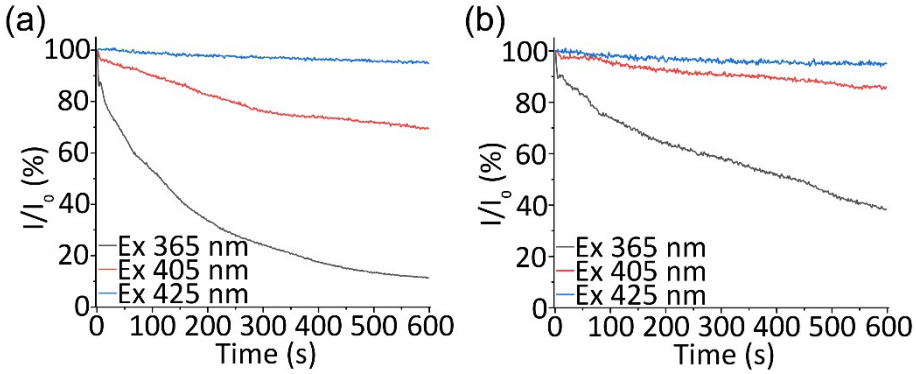

Figure S17. Normalized fluorescence intensity of DTE-PFBT (a) and DTE-PFDTBT (b) Pdots under 365 nm, 405 nm, and 425 nm continuous irradiation. The continuous irradiation are directly operated in F4600 spectrometer, the power density is very low and hard to measure accurately.

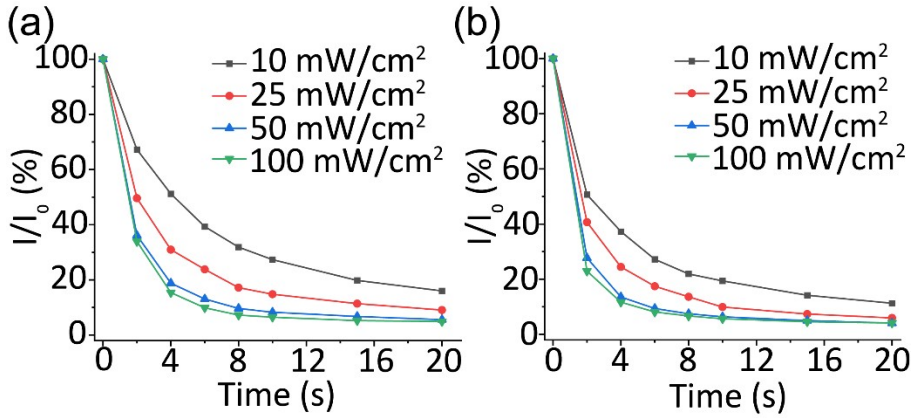

Figure S18. Normalized fluorescence intensity of DTE-PFBT (a) and DTE-PFDTBT

(b) Pdots under 10 mW/cm^2, 25 mW/cm^2, 50 mW/cm^2 and 100 mW/cm^2 405 nm laser irradiation.

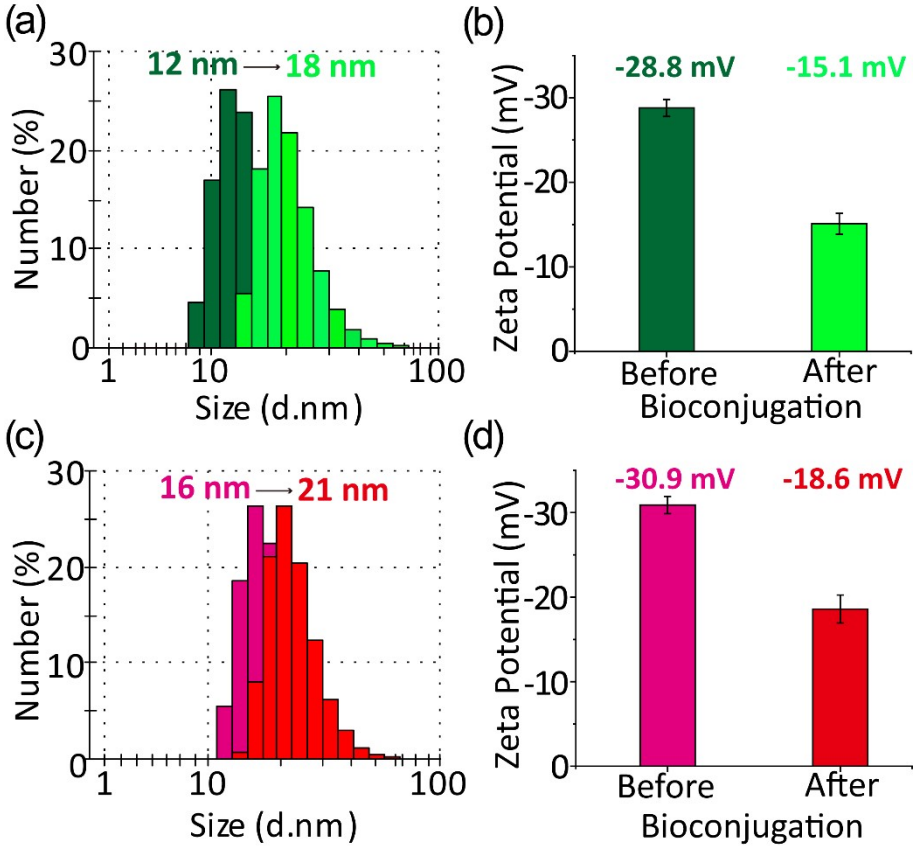

Figure S19. Particle size distribution (a), (c) and zeta potential (b), (d) of DTE-PFBT and DTE-PFDTBT Pdots before and after bioconjugation, respectively.

1. X. Chen, R. Li, Z. Liu, K. Sun, Z. Sun, D. Chen, G. Xu, P. Xi, C. Wu and Y. Sun, Adv. Mater., 2017, 29, 1-7.

2. X. Chen, Z. Liu, R. Li, C. Shan, Z. Zeng, B. Xue, W. Yuan, C. Mo, P. Xi and C. Wu, ACS Nano, 2017, 56, 8084–8091