Supporting Information

Light-induced Crosslinkable Semiconducting Polymer Dots

*Yue Zhang, Fangmao Ye, Wei Sun, Jiangbo Yu, I-che Wu, Yu Rong, Yong Zhang, and Daniel T. Chiu**

Department of Chemistry, University of Washington, Seattle, Washington 98195, United States

E-mail: chiu@chem.washington.edu

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1. Experimental Section

All reagents were used as received, unless otherwise stated. Fluorene, *n*-BuLi in hexane solution (2.5 mol/L), 1-bromo-2-methoxyethane, 1-bromohexane, bromine, (2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dioctylfluorene), boron tribromide, acryloyl chloride, potassium iodide, 1, 6-dibromohexane, sodium carbonate, potassium carbonate, potassium *tert*-butoxide, tetrabutyl ammonium bromide, tetrahydrofuran, chloroform, toluene, 2,3-Naphthalocyaninato-bis(trihexylsiloxy)silane, Bis(trihexylsiloxy)silicon 2,3-naphthalocyanine (NIR775) were purchased from Sigma-Aldrich. Tetrakis(triphenylphosphine)palladium(0) was also purchased from Sigma-Aldrich, and the fresh chemical has shiny bright color. Polydioctylfluorene (c8-PFO) (ADS-229BE) were purchased from American Dye Sources, Inc. Monomer synthesis and polymerization were conducted following previous reference, with synthetic scheme shown in Scheme S1 and S2 [1].

1.1. Synthesis of Monomers

Preparation of 9-(2-methoxyethyl-9*H*-fluorene) (2)

To a mixed solution of fluorene **1** (5 g, 30.1 mmol) in tetrahydrofuran (50 mL) at -78°C, 13.2 mL (33.1 mmol) *n*-butyllithium (2.5 mol/L) in hexane was added by syringe. The mixture was stirred at -78°C for 1 h, and then warmed to room temperature and stirred for another 1 h. After cooling down again at -78°C, 2-Bromoethyl methyl ether was added dropwise to the mixed solution. The resulting mixed solution was warmed to room temperature again and stirred for 3 h to finish chemical reaction. The crude solution was extracted 3 times with ether. The collected organic layer was washed with brine and dried over anhydrous magnesium sulfate. After evaporation of the solvent, the crude product

was purified by hand-packed column chromatography using silica gel with hexane/ether (1:1) mixed solvents as eluents to yield white powders (3.5 g, 52%). 1H NMR (500 MHz, $CDCl_3$, δ(ppm)): 7.79 (2H, d, J = 7.42 Hz), 7.56 (2H, d, J = 7.35 Hz), 7.40 (2H, q, J = 7.27 Hz), 7.35 (2H, t, J = 7.37 Hz), 4.14 (1H, t, J = 6.32 Hz), 3.40 (2H, t, J = 6.80 Hz), 3.35 (3H, s), 2.28 (2H, q, J = 6.80 Hz).

Preparation of 9-hexyl-9-(2-methoxyethyl)-9*H*-fluorene (3)

To a solution of **2** (3.5 g, 15.6 mmol) in tetrahydrofuran (50 mL) at -78°C, 9.4 mL (23.4 mmol) *n*-butyllithium (2.5 mol/L in tetrahydrofuran) was added via syringe. The mixture was then warmed to room temperature and stirred for 1 h. After cooling down again at -78°C, 1-Bromohexane was added dropwise to the solution, and the resulting mixture was warmed to room temperature and stirred for 3 h to finish chemical reaction. The mixture was extracted 3 times with ether. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After evaporation of the solvent, the crude product was purified by hand-packed column chromatography using silica gel with hexane/EtOAc (9:1) mixed solvents as eluents to yield white powders (4.73 g, 98%). 1H NMR (500 MHz, $CDCl_3$, δ (ppm)): 7.71 (2H, t, J = 7.65 Hz), 7.41–7.31 (6H, m), 3.02 (3H, s), 2.65 (2H, t, J = 5.84 Hz), 2.35 (2H, t, J = 7.70 Hz), 1.99 (2H, t, J = 7.60 Hz), 1.13–1.01 (6H, m), 0.76 (3H, t, J = 6.94 Hz), 0.64–0.60 (2H, m).

Preparation of 2,7-dibromo-9-hexyl-9-(2-methoxyethyl)-9*H*-fluorene (4)

To a solution of 3 (4.73 g, 15.3 mmol) in chloroform at 0°C were added catalytic amount of iodine and 5 mL of bromine. The solution was warmed to room temperature and stirred for 3 h. An aqueous solution of saturated sodium thiosulfate was added to the solution to remove I_2. The mixture was poured into water and extracted with methylene

chloride. The organic layer was washed with brine and dried over hydrous sodium sulfate. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel using a mixture of ethyl acetate and petroleum ether (1:10) as the eluent, yielding 2.28 g (32%) of the pale-yellow solid. 1H NMR (500 Hz, $CDCl_3$, δ (ppm)): 7.53–7.45 (6H, q), 3.02 (3H, s), 2.64 (2H, t, J = 7.65 Hz), 2.27 (2H, t, J = 7.65 Hz), 1.94 (2H, t, J = 7.71 Hz), 1.13–1.05 (6H, m), 0.77 (3H, t, J = 6.51 Hz), 0.59–0.57 (2H, m).

1.2. Polymerization

Preparation of poly[2,7-(9,9-dioctylfluorene)-co-2,7-(9-hexyl-9-(2-methoxyethyl)-9*H*-fluorene)] (P1)

2,2'-(9,9-dioctyl-9H-fluorene-2,7-diyl)bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (873 mg, 1.6 mmol) and 2,7-dibromo-9-hexyl-9-(2-methoxyethyl)-9*H*-fluorene (728 mg, 1.6 mmol) were dissolved in a mixture of toluene (20 mL) and aqueous solution of 2 mol/L Na_2CO_3 (6 mL). All solvents and solutions were degassed in advance. The mixed solution was then refluxed with vigorous stirring for 3 days under nitrogen atmosphere. Then, phenyl boronic acid was added to remove bromine end groups and 1-bromobenzene was added to remove boronic ester end groups. After the mixture was cooled to room temperature, it was poured into methanol, and then the precipitated solid was filtered and washed by methanol, acetone and water. Then the precipitated solid was re-dispersed in acetone and stirred overnight; the resulting polymer was filtered, carefully washed and obtained as a pale-yellow solid (750 mg, 65%). 1H NMR (300 MHz, $CDCl_3$, δ (ppm)): 7.96 –7.36 (12H, m), 3.22 (3H, s), 2.95 (2H, m), 2.65 (2H, m), 2.27–2.21 (6H, m), 1.35– 1.34 (32H, m), 0.91– 0.89 (9H, m).

Preparation of poly[2,7-(9,9-dioctylfluorene)-co-2,7-(9-hexyl-9-(2-hydroxyethyl)-9*H*-fluorene)] (P2)

To a solution of P1 (750 mg, 1.05 mmol) in methylene chloride at -78°C was added 1.2 mL of boron tribromide (1.0 mol/L solution in methylene chloride). Then the mixture was stirred at room temperature for 24 h. The mixture was poured into water and extracted with methylene chloride. The organic layer was washed with brine and dried over sodium sulfate. After evaporation of the solvent, the crude product was precipitated from methanol/methylene chloride (20:1) to obtain pale-yellow solid (730 mg, 99%).

Preparation of poly[2,7-(9,9-dioctylfluorene)-co-2,7-(9-hexyl-9-(2-acrylate ethyl)-9h-fluorene)] (pc-PFO)

To a solution of P2 (500 mg, 0.73 mmol) in methylene chloride at room temperature was added 0.2 mL of triethylamine. The mixture was stirred for 5 min and 0.09 mL of acryloyl chloride was added to the solution. The resulting mixture was stirred at room temperature for 24 h. Then, the mixture was washed with 1M hydrochloric acid, aqueous solution of saturated $NaHCO_3$ and water, and extracted with methylene chloride. The organic layer was washed with brine and dried over anhydrous sodium sulfate. After evaporation of the solvent, the crude product was precipitated from methanol/methylene chloride (20:1) to yield pale-yellow solid (470 mg, 87.2%). 1H NMR (500 MHz, $CDCl_3$, δ(ppm)): 7.86 –7.50 (12H, m), 6.14–6.08 (1H, d), 5.86 –5.77 (1H, d), 5.62–5.59 (1H, d), 3.73 (O-CH3, s), 3.43 (2H, m), 2.14 (6H, m), 1.26 –1.15 (32H, m), 0.82– 0.81 (9H, m).

1.3. Thin film preparation and characterization

pc-PFO and c8-PFO thin films were prepared by spin-coating 5 mg mL^{-1} chloroform solution of semiconducting polymers on pre-washed glass substrates, followed by UV-

light irradiation with different irradiation time ranging from 10 s to 120 mins for photocrosslinking reaction. The irradiation was conducted by using a 365 nm UV lamp light source with a power of 0.35 mW cm^{-2}. UV-vis spectra of thin films were measured before and after photocrosslinking reactions. After measurements, the thin films were then immersed into THF solvents for 30 mins at 50 ºC.

1.4. Preparation and characterization of Pdots

1.4.1. Preparation of Pdots

Note that pc-PFO and c8-PFO polymers are hard to dissolve completely in THF solution at room temperature. Heating above 50 ºC to improve solubility and filtration through PVDF filters to remove large aggregations are necessary before Pdot preparation. The semiconducting pc-FPO Pdots were prepared using a nanoprecipitation method [2]. Well-mixed THF solution containing 50 μg mL^{-1} semiconducting polymer and 10 μg mL^{-1} PS-PEG-COOH were filtered through sterile PVDF membrane filter (pore size: 0.2 μm) before being quickly injected into 8 mL of water under vigorous sonication. THF was then removed by blowing nitrogen gas into the solution at 90 ºC for 1 h. After confirming no THF was left in solution, the concentrated Pdot aqueous solution was adjusted to 8 mL again, sonicated for few minutes and filtered through a cellulose membrane filter for future use (pore size: 0.2 μm). The sizes of both pc-PFO and c8-PFO Pdots were characterized as 18-19 nm by DLS analysis and TEM images. The two Pdot aqueous solutions were then set for photocrosslinking reactions.

1.4.2. Light irradiation experiment

The aqueous solutions of Pdots were irradiated under a 0.35 mW cm^{-2} UV light (365 nm). Note that nitrogen gas was blown to maintain an oxygen-free environment in aqueous

solution during light irradiation. After light irradiation, the concentrated Pdot solution was diluted to initial concentration by adding water, and 1-1.5 mL of Pdot solution was taken out for test and the rest was irradiated for longer times.

1.4.3. Bioconjugation

We performed bioconjugation by utilizing the EDC-catalyzed reaction between carboxyl groups on Pdots' surface and amine groups on biomolecules. In a typical bioconjugation reaction, 80 μL of polyethylene glycol (5% w/v PEG, Mw 3350) and 80 μL of concentrated HEPES buffer (1 M) were added to 4 mL of functionalized Pdot solution (50 μg mL^{-1} in MilliQ water), resulting in a Pdot solution in 20 mM HEPES buffer with a pH of 7.4. Then, 240 μL of 1 mg mL^{-1} streptavidin (from Invitrogen) was added to the solution and mixed well on a vortex. 80 μL of freshly prepared EDC solution (5 mg mL^{-1} in MilliQ water) was added to the solution, and the above mixture was kept under stirring. After 3 h at room temperature, 80 μL 10 wt% BSA solution was added, and the mixed solution was stirred for 20 min, then 80 μL of 2.5 wt% Triton-X 100 (0.25% (w/v), 20 μL) was added. Finally, the resulting Pdot bioconjugates were transferred to a centrifugal ultrafiltration tube (Amicon® Ultra-4, MWCO: 100 kDa), and concentrated to 0.5 mL by using the centrifuge, and then were separated from free biomolecules by gel filtration using Sephacryl HR-300 gel media.

1.4.4. Cell Culture

The breast cancer cell line MCF-7 was from American Type Culture Collection. Cells were cultured at 37 °C, 5% CO_2 in Eagles minimum essential medium supplemented with 10% Fetal Bovine Serum (FBS), 50 IU mL^{-1} penicillin, and 50 μg mL^{-1} streptomycin. The cells were pre-cultured prior to experiments until confluence was reached. The cells

were harvested from the culture flask by briefly rinsing with culture media followed by incubation with 5 mL of trypsin–EDTA solution (0.25 w/v% trypsin, 0.53 mM EDTA) at 37 °C for 5–15 min. After complete detachment, the cells were rinsed, centrifuged and resuspended in 1× PBS buffer. The cell concentration was determined by microscopy using a hemocytometer.

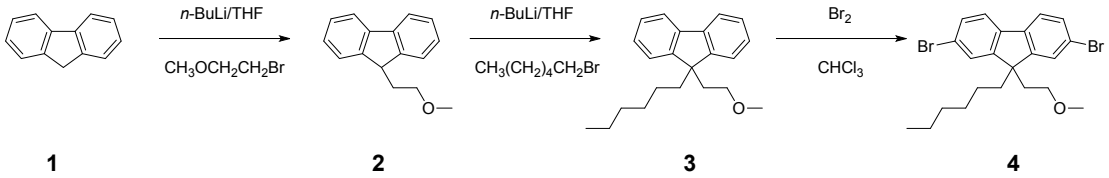
1.4.5. Flow Cytometry

For specific cell labeling with pc-PFO and c8-PFO Pdots, a million cells were blocked with BlockAid blocking buffer from Invitrogen and then were incubated sequentially with biotinylated primary anti-EpCAM antibody (used to label the cell-surface EpCAM receptors on MCF-7 cells) and 2 nM pc-PFO or c8-PFO for 30 min each, followed by two washing steps using labeling buffer. Finally, the specifically labeled cells were fixed in 0.5 mL 1% (v/v) paraformaldehyde solution. For the negative control labeling, no biotinylated primary anti-EpCAM antibody was added. Flow cytometry measurements were performed on fresh samples with 10^6 cells per 0.5 mL, prepared following the procedure described previously. Flow cytometers BD FACScan and FACS Canto II were used for cells labeled with pc-PFO or c8-PFO Pdots–SA, respectively. Excitation source was a 405nm laser. The fluorescence emission was collected with a bandpass 450/50nm filter. Scattered light and fluorescence emission were detected by PMT arrays. Representative populations of cells were chosen by selection of appropriate gates. Cell measurements were continued until at least 10,000 events had been collected in the active gate. Data were analyzed using the FlowJo Software from Tree Star.

1.5. Measurement

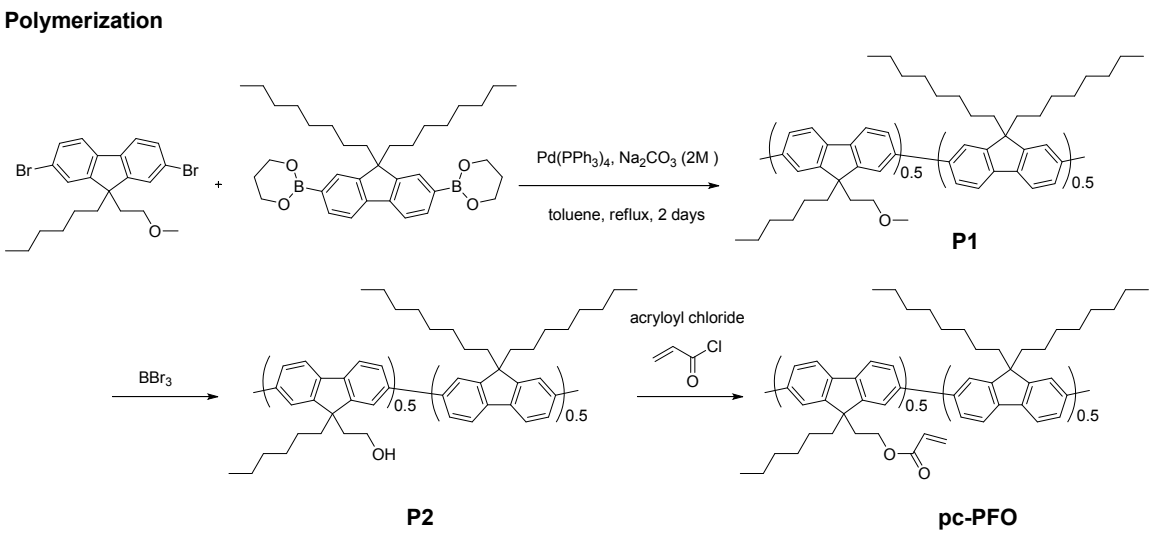
Gel permeation chromatography (GPC) was performed with a Shimadzu Prominence system equipped with a UV detector using THF as the eluent at 40 °C. The THF solution was filtered using a PVDF filter (pore size: 0.2 μm) before sample injection. ^{1}H NMR spectra in $CDCl_3$ were measured using a Bruker Advance III HD FT-NMR spectrometer equipped with an Oxford superconducting magnet system (500 MHz). UV−vis spectra were recorded using a DU 720 scanning spectrophotometer from Beckman Coulter. Fluorescence spectra were acquired using a Fluorolog-3 fluorospectrometer from HORIBA JobinYvon. Fluorescence Quantum Yields were collected using an integrating sphere (model C9920-02 from Hamamatsu Photonics). DLS experiments were conducted using a Malvern Zetasizer NanoZS. Fluorescence images of Pdots were obtained using an epi-fluorescence microscope with a 60x, 1.3 oil immersion objective and with a 405 nm diode laser. The fluorescence emission was filtered by a 410nm long pass filter and a 440/25nm bandpass filter.

Monomer Synthesis



1 **2** **3** **4**

Scheme 1. Synthetic route of pc-PFO monomer.



Scheme 2. Synthetic route of pc-PFO polymer.

Table S1. Size, zeta potential, and photophysical properties of c8-PFO Pdots formed under different durations of UV-light irradiation for photocrosslinking.

	Uncrosslinked (not irradiated)	30 mins	60 mins
Size (nm)[a]	19	19	19
ζ (mV)[b]	-41	-43	-40
Φ (%) [c]	50	35	28

[a] Hydrodynamic size. [b] Zeta potential. [c] Fluorescence quantum yield.

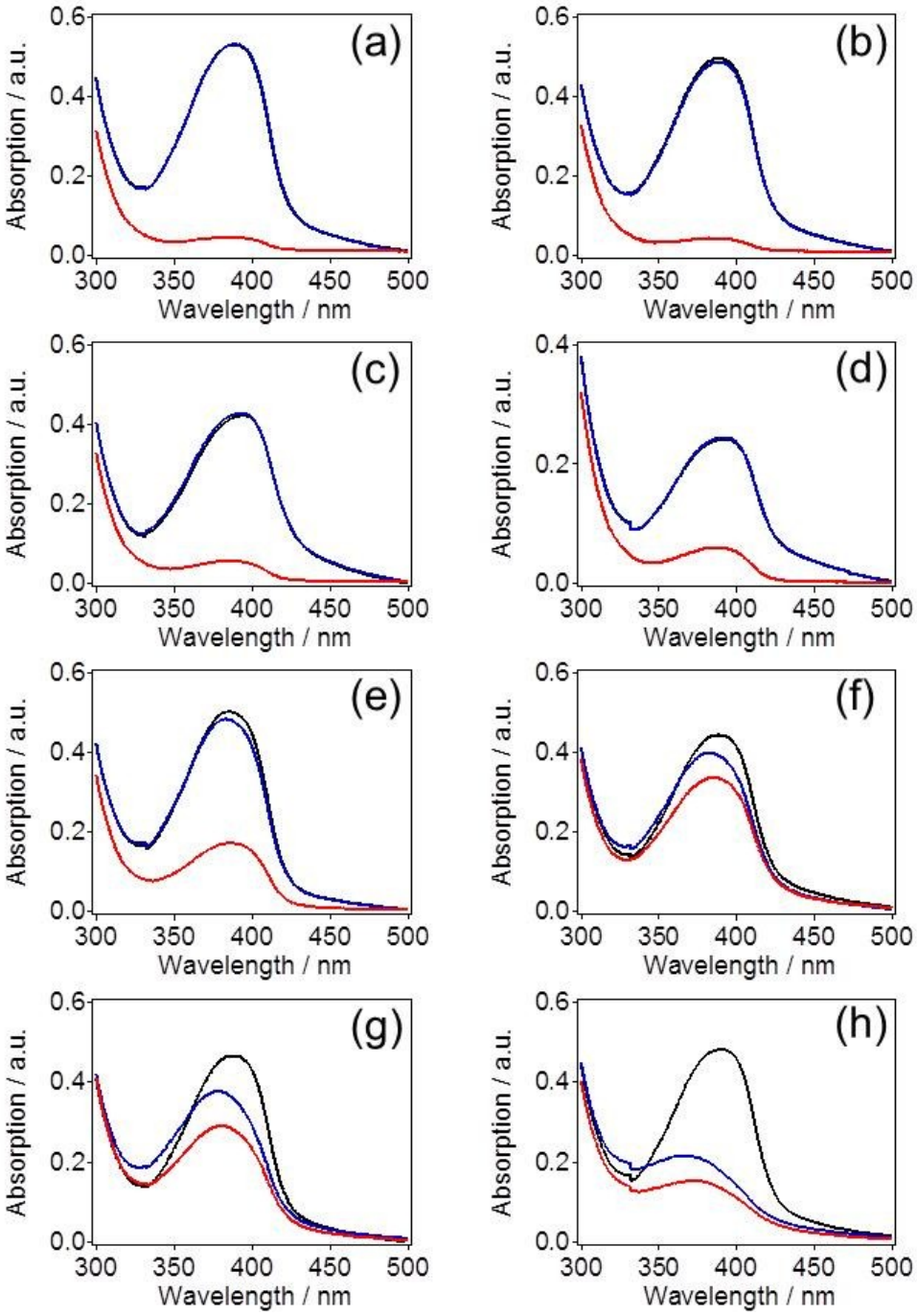


Figure S1. UV-vis spectra of photocrosslinked pc-PFO thin films after (a) 10 s, (b) 30 s, (c) 1 min, (d) 5 mins, (e) 10 mins, (f) 10 mins, (g) 60 mins, and (h) 120 mins of UV-light irradiation. Black, blue, and red solid lines are UV-vis spectra before (black) and after (blue) photocrosslinking, and after (red) dipping in THF solvents.

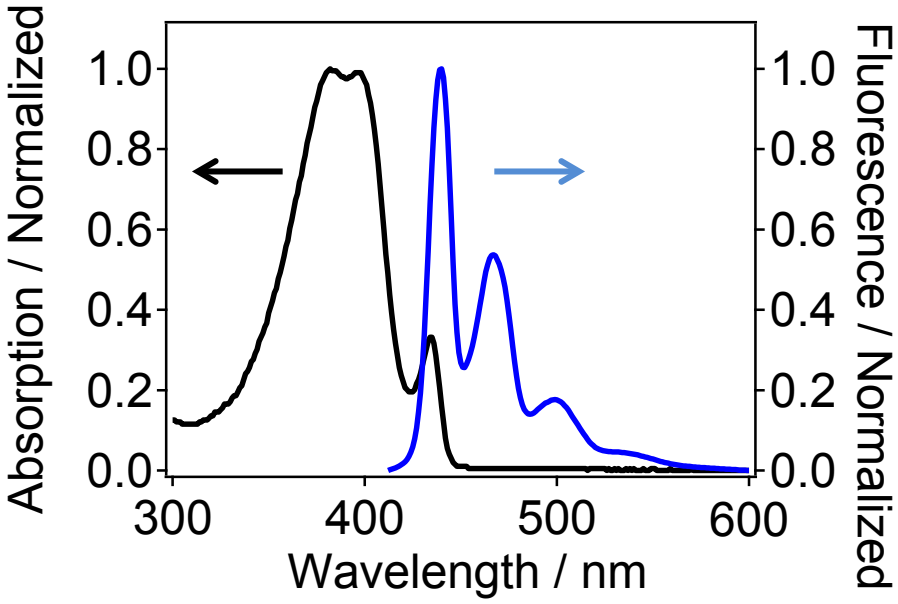


Figure S2. Absorption (black solid line) and fluorescence (blue solid line) spectra of uncrosslinked c8-PFO Pdots.

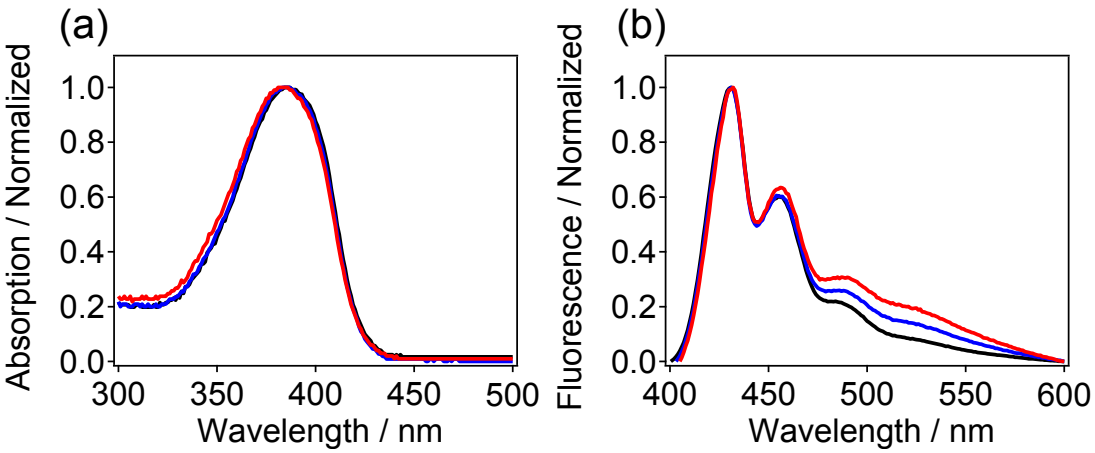


Figure S3. (a) Absorption and (b) fluorescence spectra of uncrosslinked (black solid lines) and photocrosslinked pc-PFO Pdots for 30 mins (blue solid lines) and 60 mins (red solid lines).

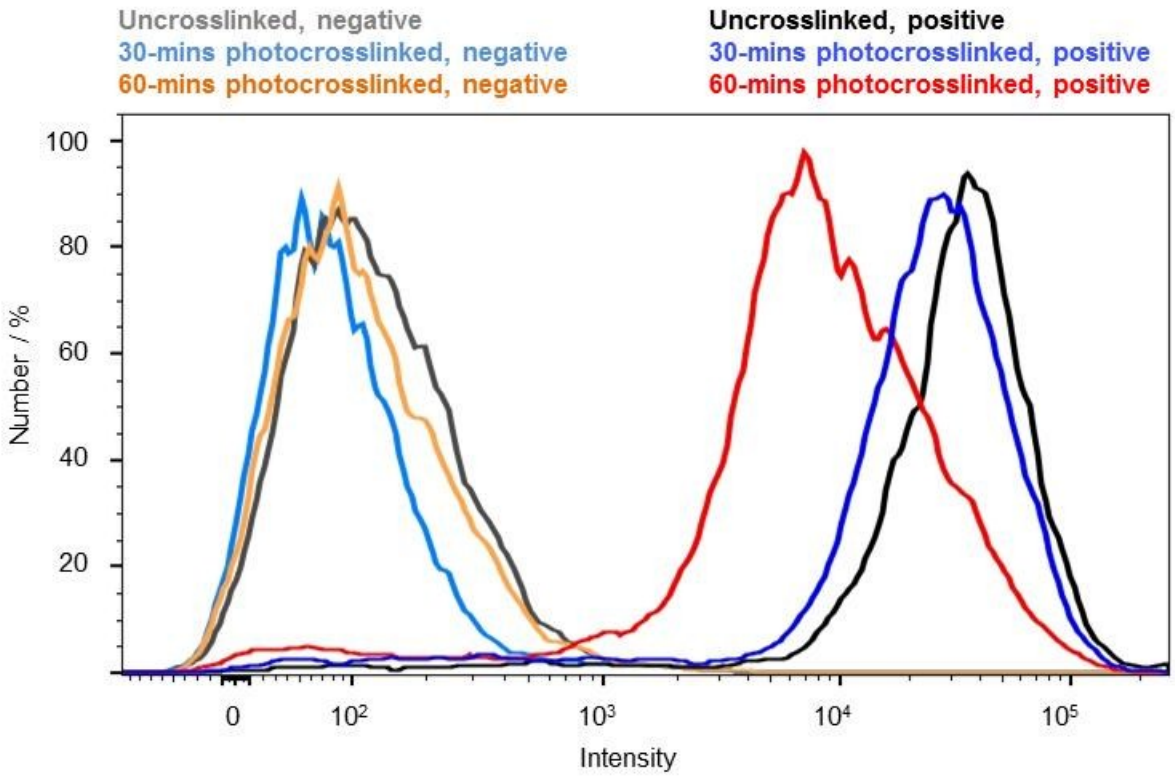


Figure S4. Flow cytometry measurements of the intensity distributions of MCF-7 breast cancer cells labeled via nonspecific binding (negative control, solid lines on the left) and positive specific cellular labeling (positive, solid lines on the right) of c8-PFO Pdots for uncrosslinked (black for positive and grey for negative), 30-mins photocrosslinked (blue for positive and light blue for negative), and 60-mins photocrosslinked (red for positive and orange for negative).

Reference

1. Wu, G.L., et al., *Synthesis and characterization of photo-crosslinkable polyfluorene with acrylate side-chains.* Journal of Applied Polymer Science, 2006. **100**(3): p. 2336-2342.
2. Wu, C., et al., *Energy transfer mediated fluorescence from blended conjugated polymer nanoparticles.* Journal of Physical Chemistry B, 2006. **110**(29): p. 14148-14154.