Hybrid Semiconducting Polymer Dot-Quantum Dot with Narrow-Band Emission, Near-Infrared Fluorescence, and High Brightness

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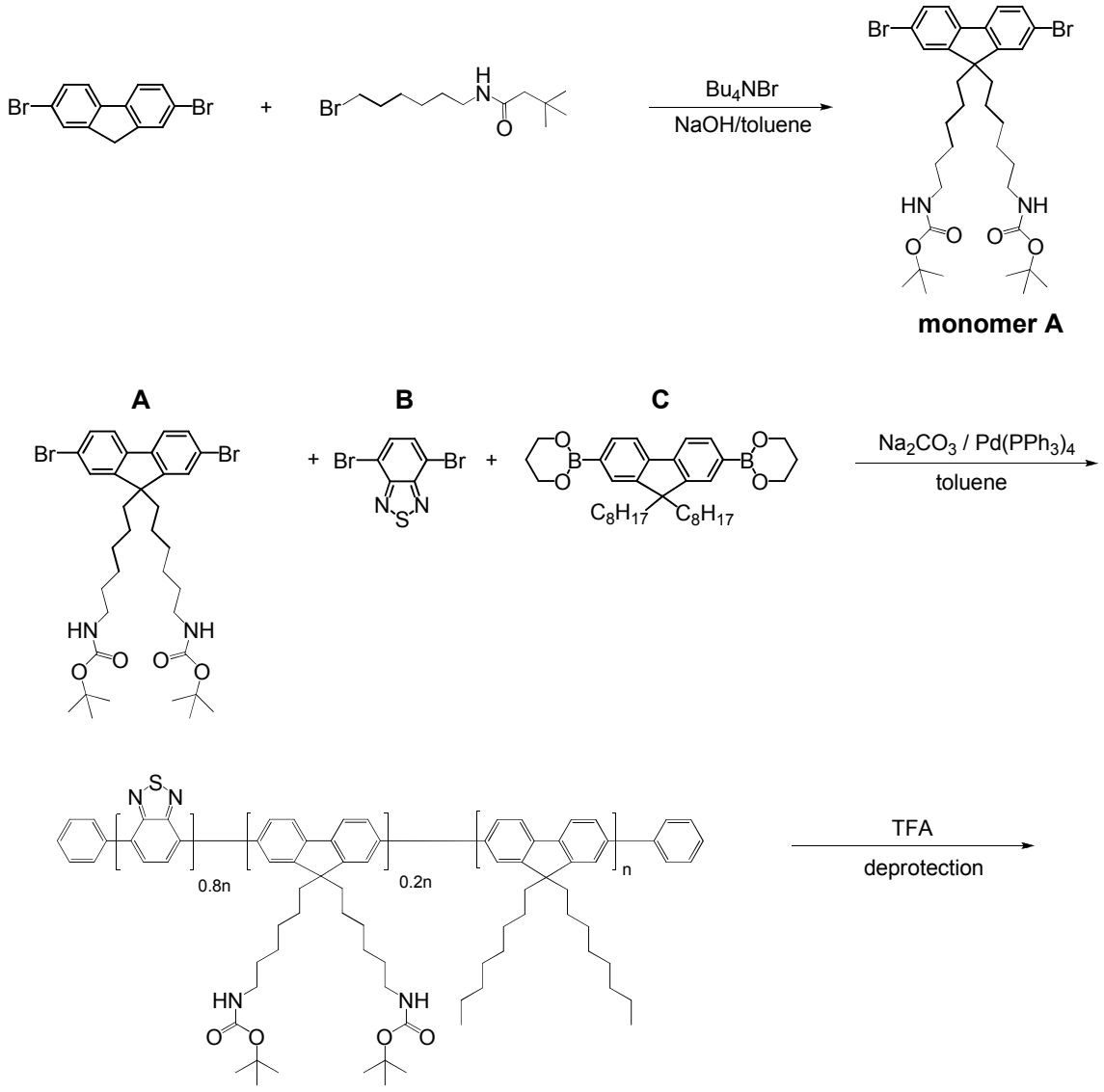
Supporting Information

Materials. The following chemicals were purchased from Sigma-Aldrich and used as received: 2,7-dibromofluorene, sodium hydroxide, tert-butyl 6-bromohexylcarbamate, tetrahydrofuran (THF; anhydrous, ≥ 99.9%, inhibitor-free), toluene, tetrabutylammonium bromide (Bu_4NBr), phenylboronic acid, bromobenzene, methanol, tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$), dichloromethane (CH_2Cl_2), 4,7-Dibromobenzo[c]-1,2,5-thiadiazole, 9,9-Dihexylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester, and sodium carbonate. A comb-like polymer, polystyrene grafted with ethylene oxide functionalized with carboxyl groups (PS-PEG-COOH, main chain MW 8,500, graft chain MW 1,200, total chain MW 21,700, polydispersity 1.25), was

purchased from Polymer Source Inc. (Quebec, Canada) and used as received without further purification. Quantum dots were purchased from Invitrogen (Carlsbad, CA) and used as received. High purity water (18.2 MΩ•cm) was used throughout the experiment.

Synthesis of Amino-Functionalized PFBT.

Scheme S1.



Monomer A: To a round-bottomed single-necked flask was added 2,7-dibromofluorene (1.62 g, 5 mmol), *tert*-butyl 6-bromohexylcarbamate (3.05 g, 11 mmol), sodium hydroxide solution (40%, 25 mL), Bu_4NBr (0.32 g, 1 mmol), and toluene (40

mL). The reaction mixture was stirred at 85 ^{o}C for 12 h and worked up. The reaction mixture was then extracted with water. The organic layer was separated, dried over $MgSO_4$, and concentrated for column chromatography. The crude product was purified on a silica gel column by eluting with CH_2Cl_2. The desired fractions were collected and concentrated to afford 2.19 g (61%) of **monomer A** (white solid). ^{1}H NMR (δ, $CDCl_3$): 0.80-1.10 (m, 16H), 1.38 (s, 18H), 1.96 (t, 4H), 2.89 (t, 4H), 7.40-7.60 (m, 6H).

PFBT Polymer: Polymers were synthesized by copolymerization of Monomers **A**, **B**, and **C** by Suzuki coupling. In a 100 mL flask, monomer **A** (0.4 mmol, 287.2 mg), **B** (0.6 mmol, 176.4 mg), and **C** (1 mmol, 558.4 mg) were dissolved in toluene (20 mL), and then Bu_4NBr (0.04 mmol, 12.5 mg) and Na_2CO_3 (2M, 12 mL) was added. The mixture was degassed and refilled with N_2 (repeated 4 times) before and after addition of $Pd(PPh_3)_4$ (0.035 mmol, 40 mg). The reactants were stirred at 90°C for 40 hours and phenylboronic acid (100 mg) dissolved in THF (1 mL) was added. After two hours, bromobenzene (1 mL) was added and further stirred for 3 hours. The mixture was poured into methanol (200 mL). The precipitate was filtered, washed with methanol, water, and acetone to remove monomers, small oligomers, and inorganic salts. The crude product was dissolved in CH_2Cl_2 (15 mL), filtered through 0.2μm membrane, and re-precipitated in methanol (150 mL). The powder was then stirred in acetone (200 mL) for 4 hours and then collected by filtration, and dried in vacuum. Yield: 420 mg (76%). Figure S1 shows the ^{1}H NMR (δ, $CDCl_3$) data.

The protecting *tert*-butyl ester groups were removed by Trifluoroacetic acid (TFA) at room temperature. The polymer (100 mg) was dissolved in 20 mL of CH_2Cl_2 and then 1.5 mL of TFA was added. The mixture was stirred overnight in darkness and

then washed with 10% NaOH water solution three times. The organic phase was separated, concentrated to ca. 5mL, and then added into methanol (50 mL) to obtain the polymer product.

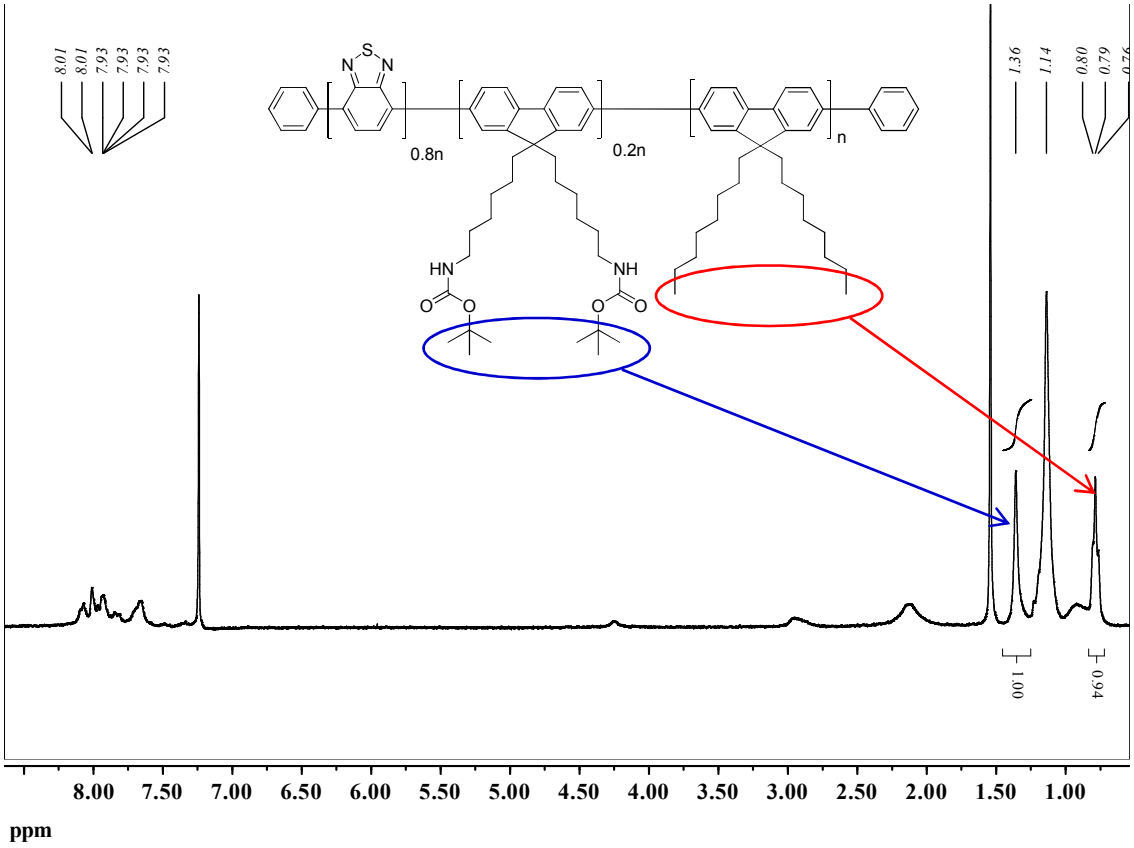


Figure S1. ^{1}H NMR of amino-functionalized PFBT polymer.

Preparation of Pdot-Qdot Nanoparticles (NPs). First, Qdots in decane (1 μM) were transferred into THF solutions. Here, to 0.3 mL of Qdots in decane was added 0.3 mL of isopropanol and 0.9 mL of methanol, sequentially. The mixture was thoroughly mixed and then centrifuged for 5 mins at 5000 rpm. The supernatant was discarded and then the pellet was washed by methanol twice. After that, the Qdots were re-dispersed in 0.3 mL THF under sonication. Typically, 20 μL of PFBT (2 mg/mL in THF), 5 μL of 2-iminothiolane (1 mg/mL in DMSO), and 120 μL of freshly prepared Qdots were added

into 2 mL of THF and then stirred for at least 48 h in darkness. After reaction, to this mixture was added 10 μL of PS-PEG-COOH (2 mg/mL in THF) and then quickly injected into 4 mL of water under vigorous sonication. The THF was then removed by purging with nitrogen on a 96 ºC hotplate for one hour. The resulting Pdot-Qdot solution was filtered through a 0.2 μm cellulose acetate membrane filter to remove any aggregates formed during preparation.

Bioconjugation and Characterization of Pdot-Qdot NPs. Bioconjugation was performed by utilizing the EDC-catalyzed reaction between carboxyl Pdot-Qdot NPs and the respective amine-containing streptavidin. Typically, 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 Pdot-Qdot solution, resulting in a Pdot-Qdot solution in 20 mM HEPES buffer with a pH of 7.3. Then, 240 μL of streptavidin (1 mg/mL) was added to the solution and mixed on a vortex. After that, 80 μL of freshly-prepared EDC solution (5 mg/mL in MilliQ water) was added to the solution, and the mixture was stirred for 4 hours at room temperature. After bionconjugation, 80 μL of BSA (10 wt%) was added to the resulting Pdot-Qdot solution and the reaction was continued for another 20 minutes. To the Pdot-streptavidin mixture was added 80 μL of Triton X-100 in MilliQ water (2.5 wt%) and subsequently transferred to a centrifugal ultrafiltration tube (Amicon® Ultra-4, MWCO: 100kDa), and then concentrated to 0.5 mL by centrifugation. Finally, the Pdot-Qdot-streptavidin bioconjugates were purified by gel filtration using Sephacryl HR-300 gel media.

The average particle size was determined by DLS (dynamic light scattering) and TEM (transmission electron microscopy) to be 25 nm in diameter for Pdot-QD655,

26 nm in diameter for Pdot-QD705, and 28 nm in diameter for Pdot-QD800. TEM images of the synthesized Pdot-Qdot NPs were acquired using a FEI Tecnai F20 transmission electron microscope at an acceleration voltage of 200 kV. For TEM, a drop of Pdot-Qdot aqueous solution was placed onto a carbon-coated grid and allowed to evaporate at room temperature. The absorption spectra of Pdot-Qdot NPs were measured using UV-visible spectroscopy (DU 720, Beckman Coulter, Inc., CA USA). The fluorescence spectra were collected using a Fluorolog-3 fluorometer (HORIBA Jobin Yvon, NJ USA). Fluorescence quantum yield of Pdot-Qdot NPs was determined by an integrating sphere (Model C9920-02, Hamamatsu Photonics) under 450 nm excitation from a 150W CW Xenon lamp.

Cell Culture. The cervical cancer cell line HeLa and breast cancer cell line MCF-7 were ordered from American Type Culture Collection (ATCC, Manassas, VA, USA). Primary cultured cells were grown in Dulbecco's Modified Eagle Medium (cat. no. 11885, Invitrogen) supplemented with 10% Fetal Bovine Serum and 1% penicillin-streptomycin solution at 37 °C with 5% CO_2 humidified atmosphere. The cells were pre-cultured in a T-75 flask and allowed to grow for 5-7 days prior to experiments until ~80% confluence was reached. To prepare cell suspensions, the adherent cancer cells were quickly rinsed with media and then incubated in 5 mL trypsin-ethylenediaminetetraacetic (EDTA) solution (0.25 w/v % trypsin, 2.5 g/L EDTA) at 37°C for 5 min. After complete detachment, cells were rinsed, centrifuged, and re-suspended in the culture media. Their concentration was determined by microscopy using a hemacytometer.

Cellular and Subcellular Labeling. For cell labeling experiments, BlockAid™ blocking buffer were purchased from Invitrogen (Eugene, OR, USA). Pdot

bioconjugates were generated in our laboratories by using methods described above. For labeling cell-surface markers with IgG, a million MCF-7 cells in 100-μL labeling buffer (1× PBS, 2 mM EDTA, 1% BSA) was incubated with 0.3 μL of 0.5 mg/mL primary biotin anti-human CD326 EpCAM antibody (eBioScience, San Diego, CA, USA) on a rotary shaker in the dark and at room temperature for 30 minutes, followed by a washing step using labeling buffer. Then the cells were incubated with 1.5 nM Pdot-Qdot-streptavidin conjugates in BlockAid^{TM} buffer for 30 minutes on a shaker in the dark and at room temperature, followed by two washing steps with labeling buffer. Prior to cell incubation, Pdot-Qdot solutions were sonicated for 3 minutes in order to disperse any potential aggregates. Negative controls were obtained by incubating cells with Pdot-Qdot-streptavidin conjugates in the absence of primary biotinylated-antibody. Cell fixation was performed by dissolving the cell pellet obtained by centrifugation in 500 μL of fixing buffer (1× PBS, 2 mM EDTA, 1% BSA, 1% PFA).

Flow cytometry measurements were performed on fresh samples with 10^6 cells/0.5 ml and prepared following the procedure previously described. The flow cytometer BD FacScan II (BD Bioscience, San Jose, CA USA) was used: cells flowing by the detection region were illuminated by a 488-nm laser, and side and forward scattered light and fluorescence emission were collected. The fluorescence emission was filtered by a 650 long-pass filter, and then detected by a PMT (photomultiplier tube). Representative populations of detected cells were chosen by selection of an appropriate gate. Detection of cell fluorescence was continued until at least 10^4 events had been collected in the active gate.

For subcellular microtubule-labeling experiments, ten thousands HeLa cells were plated on a 22 × 22-mm glass coverslip and cultured until the density reached 60-70% confluence. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.25% Triton-X 100 in PBS buffer for 15 minutes, and then blocked in BlockAid^{TM} blocking buffer for another 30 minutes. The fixed and blocked HeLa cells were subsequently incubated with 5 μg/mL biotinylated monoclonal anti-α-tubulin antibody (Biolegend, San Diego, CA, USA) for 60 minutes, and then Pdot-Qdot-streptavidin conjugates for 30 minutes. The Pdot-Qdot labeled cells were then counterstained with Hoechst 34580 and imaged immediately on a fluorescence confocal microscope (Zeiss LSM 510).

Cellular Imaging. The fluorescence spectra of Pdot-tagged cells were acquired with a fluorescence confocal microscope (Zeiss LSM 510) under ambient conditions (24 ± 2 °C). The confocal fluorescence images were collected using a diode laser at 405 nm (~15 mW) as the excitation source and an integration time of 1.6 μs/pixel. A Carl Zeiss 63× ("C-Apochromat" 63×/1.2 W Corr) objective was utilized for imaging and spectral data acquisition; the laser was focused to a spot size of ~5 μm^2.

For single-particle brightness measurements, PFBT-DBT Pdots or Pdot-QD655 nanoparticles were diluted in Milli-Q water, dried under vacuum on cleaned glass coverslips, and imaged with a home-built Total Internal Reflection Fluorescence (TIRF) microscope. The 488-nm laser beam from a sapphire laser (Coherent, Santa Clara, CA USA) was directed into an inverted microscope (Nikon TE2000U, Melville, NY, USA) using home-built steering optics. Laser excitation power was measured at the nosepiece before the objective. The objective used for illumination and light collection was a 1.45

NA 60× TIRF objective (Nikon, Melville, NY, USA). Fluorescence signal was filtered by a 510-nm long pass filter (HQ510LP; Chroma, Rockingham, VT, USA) and imaged onto an EMCCD camera (Photometrics Cascade: 512B, Tucson, AZ USA).

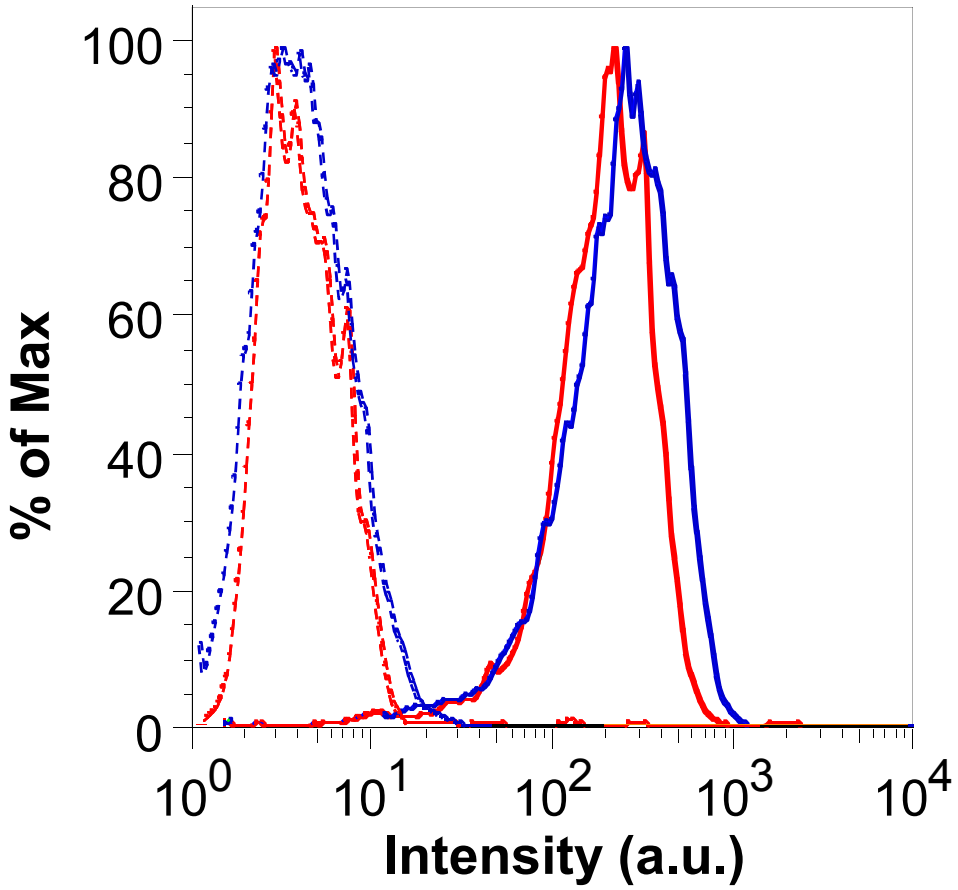


Figure S2. Evaluation of stability of Pdot-QD800 NPs by flow cytometry. The blue and red lines represent the fluorescence intensity distribution of MCF-7 cells labeled with freshly prepared and two-month-old Pdot-QD800-streptavidin, respectively. The results of their corresponding negative control samples (no primary biotin anti-human CD326 EpCAM antibody) were displayed in dashed lines.

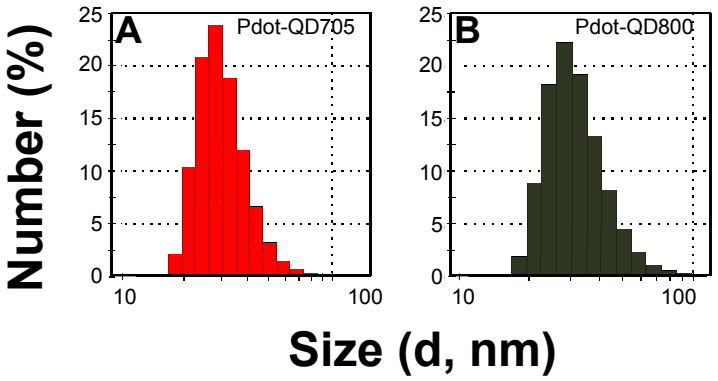


Figure S3. Dynamic light scattering measurements of Pdot-Qdot nanoparticles. Hydrodynamic diameters of (A) Pdot-QD705, and (B) Pdot-QD800.

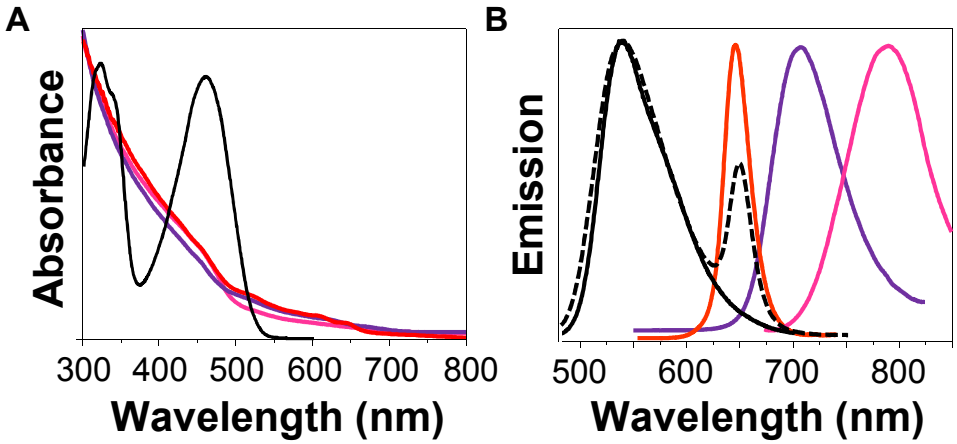


Figure S4. (A) UV-visible spectra of neat PFBT Pdots in water (black line), QD655 in decane (red line), QD705 in decane (purple line), and QD800 in decane (pink line). (B) Emission spectra of neat PFBT Pdots in water (black line), QD655 in decane (red line), QD705 in decane (purple line), and QD800 in decane (pink line). The dash black line shows the emission spectrum of Pdot-Qdot after ligand exchange (before nanoprecipitation) in THF.

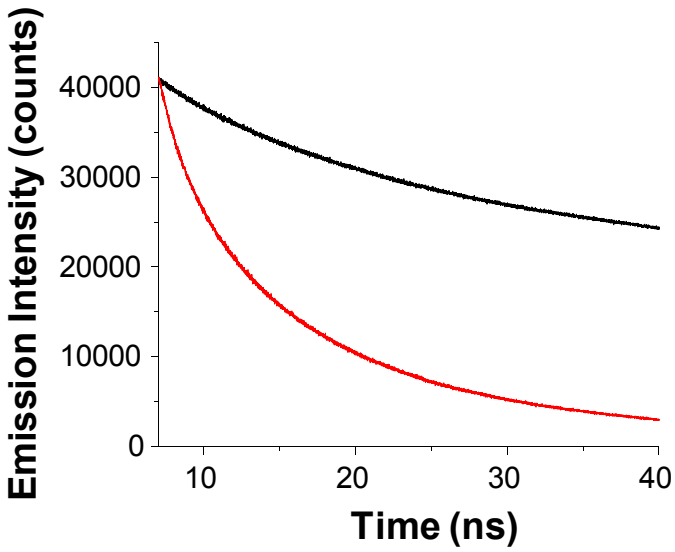


Figure S5. Time resolved fluorescence decay of QD655 (black line) and Pdot-QD655 (red line). The fluorescence lifetimes for QD655 and Pdot-QD655 are 20 ns and 9 ns, respectively.