Supporting Information

Generation of Functionalized and Robust Semiconducting Polymer Dots with Polyelectrolytes

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MATERIALS AND METHODS.

Materials. Polymers: Poly(9,9-dioctylfluorene)-co-(4,7-di-2-thienyl-2,1,3-benzothiadiazole) (PFTBT, red fluorescent polymer) was synthesized in our lab based on the previous reports.[1,2] Poly(9,9-dioctylfluorenyl-2,7-diyl) (PFO, blue fluorescent polymer) and poly[(9,9-dioctylfluorenyl-2,7-diyl)-co-(1,4-benzo-(2,1',3)-thiadiazole)] (PFBT, green fluorescent polymer, Mw, 157,000Da; polydispersity, 3.0) were purchased from ADS Dyes Source, Inc. (Quebec, Canada). Polystyrene (PS, Mw, 3000Da), and poly(sodium methacrylate) (PMANa, Mw, 7400Da) were purchased from Polymer Source Inc. (Quebec, Canada). Poly(styrene sulphonate) (PSS, Mw, 70,000Da), poly(styrene-co-maleic anhydride) (PSMA, cumene terminated, average Mn ~1,700, styrene content 68%), and Poly(ethylene glycol) (PEG, Mw, 3350Da) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). 10× PBS stock solution was purchased from EMD chemicals (Darmstadt, Germany). Biotin anti-human CD326(Ep-CAM) was purchased from BioLegend Inc. (San Diego, CA, U.S.A.). Streptavidin was purchased from Invitrogen (Eugene, OR, U.S.A.). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Thermo Fisher Scientific Inc. (Rockford, IL, U.S.A.). All the other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Bare Pdot Preparation. Pdots of blue, green, and red fluorescence were prepared using the nanoprecipitation method. In a typical preparation, the semiconducting polymer (or the mixture of polymers) was first dissolved in anhydrous tetrahydrofuran (THF) at the concentration of 0.01% (w/v). A 1-mL aliquot of the polymer solution was then quickly injected into 10 mL of DI water under vigorous sonication for 1 minute. THF was evaporated from the Pdot suspension with nitrogen purging at 80 °C. The Pdot solution went through a 200-nm filter and the concentration was adjusted to 10 ppm. The green

fluorescent Pdots were prepared using the THF solution that only contained PFBT. The blue fluorescent Pdots were made using a THF solution with a mixture of PFO and PS (4:6 by weight). Red fluorescent Pdots were made using a THF solution that had a mixture of PFBT and PFTBT (7:3 by weight).

PSMA-Pdot Preparation. The carboxyl-functionalized Pdots were made using the co-precipitation method that we described previously.[3] Briefly, 250 µg of semiconducting polymer and 50 µg of amphiphilic polymer, PSMA, were mixed together in 5 mL of THF. The mixture was quickly dispensed into 10 mL of DI water under vigorous sonication. After removing THF, the Pdot solution was filtrated and the concentration was adjusted to 50 ppm.

Determination of Pdot Concentration. A calibration curve was built up using the maximum peak absorbance (380nm for PFO, 450nm for PFBT and PFBT-PFTBT) from Pdot solutions that contained a series of known mass concentrations (from 1ppm to 100ppm). The as-prepared Pdots' mass concentration was then determined by the absorbance. We converted the mass concentration (ppm) to molar concentration by taking account of the particle size and density.

Pdot Functionalization Using Polyelectrolyte. A 4-mL aliquot of a 10 ppm solution of bare Pdots was mixed with 20 µL of 10% (w/v) negatively charged polyelectrolyte(s), such as PSS for preparing PSS-Pdot or a mixture of polyelectrolytes composed of PSS and PMANa for preparing PSS/PMANa-Pdot. Sodium chloride was then added to the mixture (final concentration is 10 mM). The mixture was slowly stirred in the dark for 1 hour. The polyelectrolyte-functionalized Pdots were collected by centrifuging the Pdots at 80,000 rpm for 1 hour using a Beckman Optima™ Max-E Ultracentrifuge.

Conjugating Streptavidin to PSS/PMANa-Pdots. A 1-mL aliquot of a 10 ppm solution of PSS/PMANa-Pdots was mixed with 80 µL of 1 mg/mL streptavidin in the reaction buffer containing 20 mM HEPES (pH = 7.4) and 0.1% (w/v) PEG. After the addition of 20 µL of 5 mg/mL EDC to the mixture, the solution was gently stirred for 4 hours in the dark. It should be noted that excess amounts of streptavidin (typically, the feeding ratio of the number of streptavidin to the number of carboxyl groups from PMANa on Pdot is 20:1) was used to ensure the Pdot surface was fully covered with streptavidin. Finally, the unreacted streptavidin was removed through the Sephacryl 300HR column that was pre-flushed with a solution of 20 mM HEPES and 0.1% PEG.

Pdot Characterization. The size of Pdots was determined using a dynamic light scattering (DLS) instrument (Malvern Zetasizer Nano ZS), and a FEI Tecnai F20 transmitting electron microscope (TEM) at 200kV. The fluorescence spectra were measured using a HORIBA Jobin Yvon fluorospectrometer. The UV-Vis absorption spectra of Pdots were recorded with a DU 720 spectrophotometer. The zeta potential of

the Pdots was studied by Malvern Zetasizer and gel electrophoresis experiment. The gel was prepared using 0.7% of normal melting agarose, 0.2% of PEG (MW 3350), and 20mM HEPES buffer. The Pdot samples were loaded into the electrophoresis channels with the aid of 30% glycerol and ran in 20 mM HEPES buffer (pH 7.4) under an applied field strength of 10 V/cm for 10 min using a Mupid®-exU submarine electrophoresis system. The gel was then developed using a Kodak image station 440CF system.

Evaluating Pdot Stability. The colloidal stabilities of bare Pdots, PSMA-Pdots, PSS-Pdots and PSS/PMANa-Pdots were investigated and compared at the same concentration in phosphate buffered saline (1× PBS), 1 mM of Cu^{2+} ion ($CuSO_4$), and 100 μM of Fe^{2+} ion ($FeSO_4$). The size of the Pdots was periodically tracked after the addition of PBS or metal ions using DLS. The fluorescence intensities of Pdots were also compared before and after the addition of saline and metal ion solutions.

Cell culture. The breast cancer cell line MCF-7 was ordered from American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). Cells were cultured at 37 °C with 5% CO_2 in Eagle's minimum essential medium (EMEM) supplemented with 10% Fetal Bovine Serum (FBS), 50 U/mL penicillin, and 50 μg/mL streptomycin. Ten thousands of MCF-7 cells were plated on a 15-mm-diameter glass-bottomed culture dish and cultured until the density reached confluence for Pdot labelling and fluorescence imaging.

Labeling Cells Using Streptavidin-Functionalized Pdots. For labeling cell-surface markers with PSS/PMANa-Pdot-streptavidin conjugates, live MCF-7 cells in the glass-bottomed culture dish were incubated first with 5 μg/mL biotin anti-human CD326 antibody and then with 5 nM PSS/PMANa-Pdot-streptavidin. Each incubation lasted for 1 hour followed by two washing steps. The confocal fluorescence image of the Pdot-labeled MCF-7 cells was then acquired using a fluorescence confocal microscope (Zeiss LSM 510).

Table S1. Quantum yield of bare and polyelectrolyte coated Pdots

	Bare	PSS coated	PSS/PMANa coated
PFO	40%	38%	37%
PFBT	32%	32%	34%
PFBT-PFTBT	56%	50%	52%

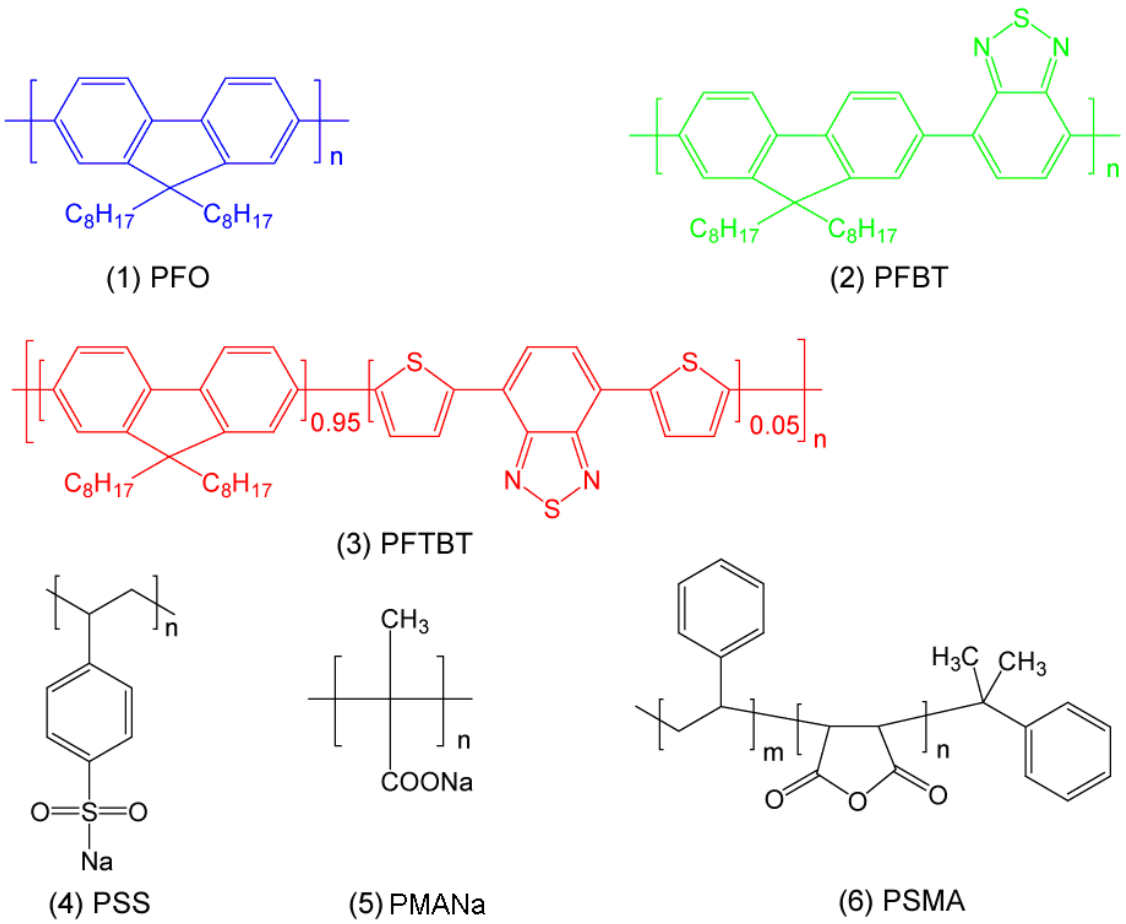

Figure S1. Chemical structures of the polymers used in this study. Semiconducting Polymers: (1) PFO (blue fluorescence); (2) PFBT (green fluorescence); (3) PFTBT (red fluorescence). Polyelectrolytes: (4) PSS; (5) PMANa. Amphiphilic polymer: (6) PSMA.

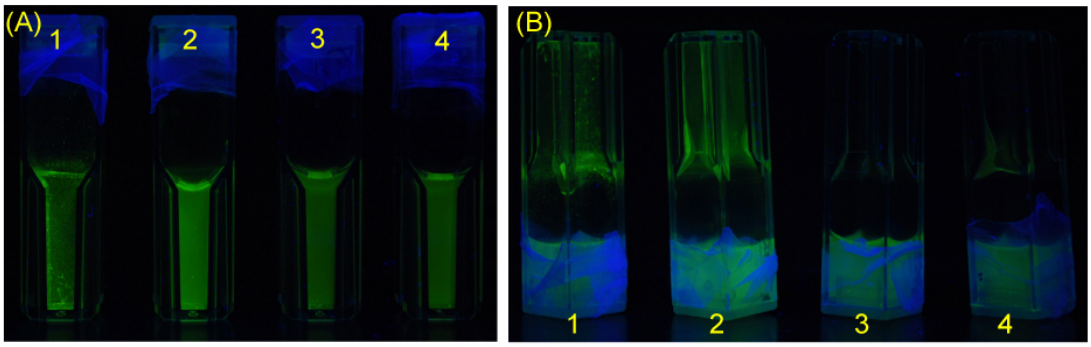

Figure S2. Fluorescence Images of Pdots under UV light after the Pdots were treated with 1× PBS for 24 hours. Samples: (1) bare Pdots, (2) PSMA-Pdots, (3) PSS-Pdots, (4)

PSS/PMANa-Pdots. (A) The aggregates were observed in the solution of bare Pdots (Sample (1)). (B) The aggregates of (1) bare Pdots and (2) PSMA-Pdots were stuck on the cuvettes (green fluorescence on the walls of the top half of the cuvette). PSS-Pdots and PSS/PMANa-Pdots were free of aggregation.

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