A Versatile Method for Generating Semiconducting Polymer Dot Nanocomposites

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Supporting Information

Experimental Section

1. Materials

Poly(9,9-dioctylfluorenyl-2,7-diyl) end capped with dimethyl phenyl (**PFO**, MW 120000 Da), and poly[(9,9-dioctylfluorenyl-2,7-diyl)-co-(1,4-benzo-(2,10,3)-thiadiazole)] (**PFBT**, MW 157000 Da) were purchased from American Dye Source Inc (Quebec, Canada). Thiol-terminated polystyrene (PSSH, MW 1000 Da) was purchased from Polymer Source Inc. (Quebec, Canada). Tetrachloroauric acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), potassium carbonate (K_2CO_3), 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), and magnetic nanoparticles (10 nm, 20 nm average size, 5 mg/mL in toluene) were obtained from Sigma (St. Louis, MO). Magnetic nanoparticles (40 nm average size, oleic acid coating, 25 mg/mL in chloroform) were purchased from Ocean NanoTech (Springdale, Arkansas).

Polystyrene-grafted ethylene oxide functionalized with carboxyl groups (PS-PEG-COOH; MW 21,700 Da of PS moiety; 1200 Da of PEG-COOH; polydispersity, 1.25) was from Polymer Source Inc. (Quebec, Canada). Sucrose was ordered from Avantor Performance Materials (Phillipsburg, NJ).

2. Synthesis of gold nanoparticles

4 mL of 1.0% tetrachlorauric acid (HAuCl4) aqueous solution and 1.3 mL of 0.2 M K_2CO_3 were added into 100 mL MilliQ water in an ice bath. The solution was vigorously stirred, after which 1.0 mL of 1.54 mg/mL sodium borohydride solution (kept in ice bath) was added. This procedure was repeated 5 times (5 mL in total). The solution was then stirred overnight at 4 °C.

3. Preparation of Au-NP-Pdot

2 mL of the Au NPs solution was mixed with 4-mL toluene containing 1mg/mL PSSH. After being vigorously shaken, the mixture was placed on the bench and allowed to phase-separate. After the Au nanoparticles were phase-transferred to the upper toluene solution, the bottom water solution was discarded. Anhydrous sodium sulfate was added to dry the toluene. Then the Au nanoparticles containing toluene solution was centrifuged at 14,000 rpm for 40 min. The pellet was re-dispersed in 2 mL tetrahydrofuran (THF). 500 μL of 1 mg/mL PFO and 50 μL of 1 mg/mL PS-PEG-COOH solution were added to the THF solution containing Au nanoparticles. An additional amount of THF was added to adjust the final volume to 5 mL. The 5-mL mixture was then quickly injected into 10 mL of MilliQ water under vigorous sonication. The THF was removed by blowing

nitrogen gas into the solution at 90 °C. The THF-free Pdot solution was subsequently sonicated for 1-2 minutes and filtrated through a 0.2-μm cellulose membrane filter.

4. Purification of Au-NP-Pdot

1.5 mL of 1.5 M sucrose-water solution was added in a centrifuge tube, after which 2 mL of the Au NP-Pdot solution as prepared above was placed on top of the sucrose layer. The centrifuge tube was put into a Beckman Optima^{TM} Max-E Ultracentrifuge machine and spun at 45000 rpm for 45 min. The solution above the sucrose layer was collected and labeled as "pure Pdots". The pellet that formed at the bottom of the sucrose layer was re-dispersed in 2 mL of water with sonication and was labeled as "Au-NP-Pdot". The hydrodynamic diameter of Au-NP-Pdots was measured with a dynamic light scattering (DLS) spectrometer (Malvern Zetasizer Nano ZS, Worcestershire, United Kingdom). Fluorescence quantum yields were collected using an integrating sphere (model C9920-02, Hamamatsu Photonics) with excitation light from a 150 W CW xenon lamp. The absorption and fluorescence spectra of Pdots were measured with a DU 720 scanning spectrophotometer (Beckman Coulter, Inc., CA) and a Fluorolog-3 fluorospectrometer (HORIBA JobinYvon, NJ), respectively.

5. Preparation of magnetic nanoparticles embedded inside Pdots (FeO_x-NP-Pdots)

2 mL of magnetic nanoparticles in an organic solvent was spun at 14,000 rpm for an appropriate amount of time (1 hour for 10-nm and 20-nm magnetic nanoparticles, 15 minutes for 40-nm magnetic nanoparticles). The pellet was re-dispersed in THF containing PFBT and PS-PEG-COOH under sonication. The solution was subsequently

processed in the same way as that for Au nanoparticles. Pdots containing magnetic nanoparticles were separated from pure Pdots by sucrose gradient centrifugation.

6. Streptavidin conjugation to FeO_x-NP-Pdots

In a typical conjugation reaction, 80 μL of polyethylene glycol (5% w/v PEG, MW 3350) and 80 μL of HEPES buffer (1M, PH 7.3) were added to 4 mL of Fe_3O_4-NP-Pdots solution. Streptavidin (1 mg/mL, 30 μL) was then added to the solution and mixed well. Next, 80 μL of freshly prepared EDC solution (5 mg/mL in MilliQ water) was added to the solution. The above mixture was magnetically stirred for 4 hr at room temperature. The resulting Pdot conjugates were concentrated with a (100K MW) spin column and purified with a Bio-Rad Econo-Pac 10DG column (Hercules, CA). After purification, the proper amount of bovine serum albumin (BSA) was added to reach a final concentration of 1% (w/w).

6. Cell culture

The human cervical cancer cell line (HeLa) and breast cancer cell line (MCF-7) were ordered from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured at 37 °C in 5% CO_2 in Eagles Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS) and 1% Pen Strep (5000 units/mL penicillin G, 50 μg/mL streptomycin sulfate in 0.85% NaCl). 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 3 mL of Trypsin-EDTA solution (0.25 % w/v Trypsin, 0.53 mM 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.

7. Dark field and fluorescence microscopy imaging of Au-NP-PFO Pdots

To image Au-NP-Pdots on a coverslip, a drop of the solution containing Au NP-Pdots was placed on a clear coverslip which was surface modified with (3-aminopropyl)triethoxysilane (APTES). To image Au-NP-Pdots inside cells, the procedure was as follows: When the confluence of HeLa cells in culture flask reached the appropriate level, the cells were harvested from the culture flask by briefly rinsing with culture media followed by incubation with 3 mL of Trypsin-EDTA solution (0.25 % w/v Trypsin, 0.53 mM EDTA) at 37°C for 5 min. After complete detachment, cells were rinsed, centrifuged, and re-suspended in the culture media. About 100 μL of the suspended cell solution was placed on top of a clean glass coverslip inside a Petri dish. The coverslip was left in cell incubator for 1 h to let the cells attach to the glass surface. Then 1.5 mL of cell culture medium was added to the Petri dish to immerse the coverslip. The Petri dish was left in the cell incubator for 1 day. The proper amount of Au-NP-Pdots solution was next added to the Petri dish, and the cells were incubated for approximately 4 hours. Then the glass coverslip was placed into paraldehyde solution for 15 min to fix the cells before the microscopy experiments were carried out. A Nikon TE2000 inverted microscope was used for imaging. When the microscope was operated in dark-field mode, a numerical aperture (NA) 1.3 oil condenser and a 100× objective with adjustable NA (0.7-1.3) were installed. A 100 W halogen lamp was used as the light source. When the

microscope was operated in epi-fluorescence mode, a 405-nm laser was used as the excitation light source and a 410-nm dichroic mirror and 460/80 nm bandpass filter were used to filter the fluorescence emission from the Pdots. Both dark-field and fluorescence images were taken with prosilica cameras (GC1380 and GC660, Newburyport, MA).

8. Cell labeling with FeO_x-NP-PFBT Pdots

For labeling a cell-surface marker with IgG conjugates, a million MCF-7 cells in 100-μL labeling buffer (1×PBS, 2 mM EDTA, 1% BSA) were incubated with 0.3 μL of 0.5 mg/mL biotinylated primary anti-human CD326 EpCAM antibody (eBioScience, San Diego, CA) 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 streptavidin-conjugated Pdots in BlockAid^{TM} blocking buffer (Invitrogen, Eugene, OR) for 30 minutes on a shaker in the dark and at room temperature, followed by two washing steps with labeling buffer. Cells were later fixed by dissolving the cell pellet obtained by centrifugation in 500 μL of fixing buffer (1× PBS, 2 mM EDTA, 1% BSA, 1% paraformaldehyde).

9. Imaging cells labeled with FeO_x-NP-PFBT Pdots

A Nikon TE2000 inverted microscope was used for imaging. When the microscope was operated in differential interference contrast (DIC) mode, a numerical aperture (NA) 1.3 oil condenser, a 100× objective with NA 1.4, two polarizers and Nomarski prisms were installed. A 100 W halogen lamp was used as the light source. When the microscope was operated in epi-fluorescence mode, a 488-nm laser was used as the

excitation light source, and a 505-nm dichroic mirror and 550/60 nm bandpass filter were used to filter the fluorescence emission from the Pdots. Both DIC and fluorescence images were taken with prosilica cameras (GC1380 and GC660, Newburyport, MA).

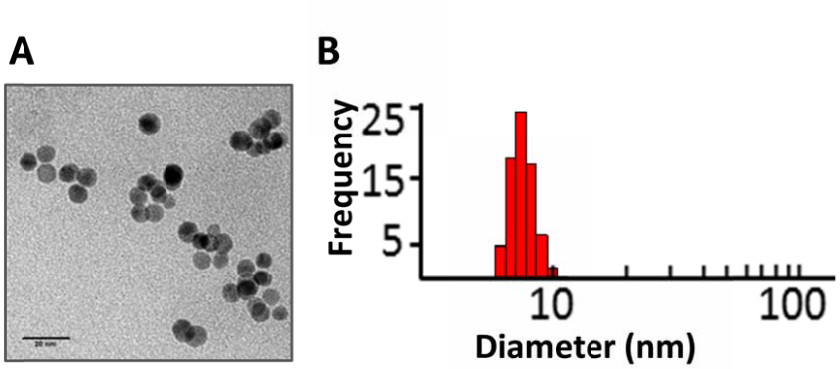

Figure S1. Synthesized Au nanopaticles. **A)** TEM image of Au naoparticles. **B)** Size histogram of Au nanoparticles. The average size was 6.5 nm. Scale bar represents 20 nm.

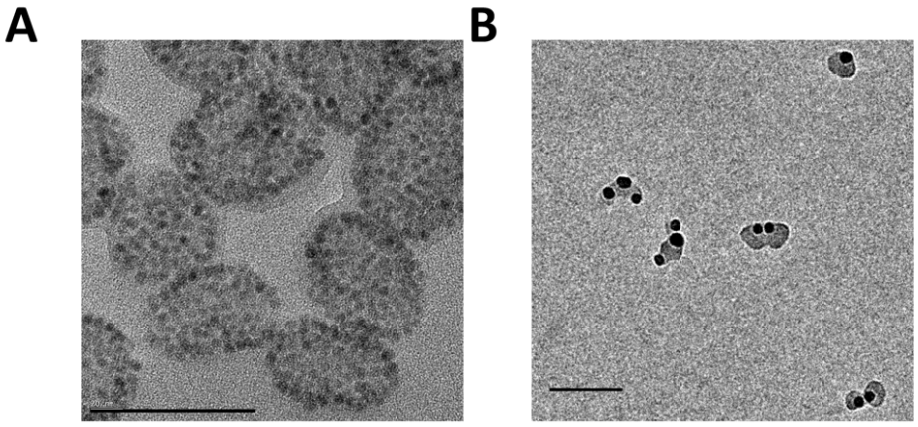

Figure S2. TEM image of magnetic nanoparticles embedded in PFO Pdots (FeO_x-NP-Pdot). **A)** FeO_x-NP-Pdots containing 10 nm magnetic nanoparticles. **B)** FeO_x-NP-Pdots containing 20 nm magnetic nanoparticles. Scale bar represents 100 nm.