# -- Supporting Material --

## Bioconjugation of Ultrabright Semiconducting Polymer Dots for Specific Cellular Targeting

Changfeng Wu[1], Thomas Schneider[1], Maxwell Zeigler[1], Jiangbo Yu[2], Perry Schiro[1], Daniel R. Burnham[1], Jason D. McNeill[2], & Daniel T. Chiu[1, *]

[1]Department of Chemistry, University of Washington, Seattle, WA, USA.
[2]Department of Chemistry, Clemson University, Clemson, SC, USA.

## Materials and Methods

**Functionalization of semiconducting polymer dots.** Fluorescent semiconducting polymer Poly[(9,9-dioctylfluorenyl-2,7-diyl)-co-(1,4-benzo-{2,1',3}-thiadiazole)] (PFBT, MW 157,000, polydispersity 3.0) was purchased from ADS Dyes, Inc. (Quebec, Canada). 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). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), and all experiments were performed at room temperature unless indicated otherwise. Functionalized Pdots in aqueous solution are prepared by using a modified nano-precipitation method. First, PFBT was dissolved in tetrahydrofuran (THF) to make a stock solution with a concentration of 1 mg/mL. PS-PEG-COOH was also dissolved in THF and mixed with a diluted solution of PFBT to produce a solution mixture with a PFBT concentration of 50 μg/mL and a PS-PEG-COOH concentration

ranging from 0 to 10 μg/mL. The mixture was sonicated to form homogeneous solutions. A 5-mL quantity of the solution mixture was added quickly to 10 mL of MilliQ water in a bath sonicator. The THF was removed by nitrogen stripping, and the solution was concentrated by continuous nitrogen stripping to 5 mL on a 90 ºC hotplate, followed by filtration through a 0.2 micron filter. The resulting functionalized Pdot dispersions are clear and stable for months without signs of aggregation.

**Biomolecular conjugation to functionalized Pdots.** Streptavidin and goat anti-mouse IgG antibodies were purchased from Invitrogen (Eugene, OR, USA). In this paper, we performed bionconjugation by utilizing the EDC-catalyzed reaction between carboxyl groups on Pdots and amine groups on biomolecules. In a typical bioconjugation reaction, 20 μL of polyethylene glycol (5% w/v PEG, MW 3350) and 20 μL of concentrated HEPES buffer (1 M) were added to 1 mL of functionalized Pdot solution (50 μg/mL in MilliQ water), resulting in a Pdot solution in 20 mM HEPES buffer with a pH of 7.3. Then, 40 μL of streptavidin or IgG antibody (1 mg/mL) was added to the solution and mixed well on a vortex. Last, 20 μL of freshly-prepared EDC solution (5 mg/mL in MilliQ water) was added to the solution, and the above mixture was left on a rotary shaker for 4 hours at room temperature. Finally, the resulting Pdot bioconjugates were separated from free biomolecules by gel filtration using Sephacryl HR-300 gel media.

**Cell culture.** The breast cancer cell line MCF-7 and SK-BR-3 were ordered from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured at 37 °C, 5% $CO_2$ in Eagles minimum essential medium (for MCF-7) or McCoy's 5A medium (for SK-BR-3) supplemented with 10% Fetal Bovine Serum (FBS), 50 U/mL

penicillin, and 50 μg/mL 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 minutes. After complete detachment, the cells were rinsed, centrifuged, and resuspended in labeling buffer (1× PBS, 2 mM EDTA, 1% BSA). The cell concentration was determined by microscopy using a hemacytometer.

**Immunofluorescent labeling and imaging.** For cell labeling experiment, Qdot 565-streptavidin, Alexa 488-IgG, and BlockAid™ blocking buffer were purchased from Invitrogen (Eugene, OR, USA). Pdot bioconjugates were synthesized in our lab by using the methods as described above. For labeling cell-surface marker with IgG conjugates, a million cells in 100-μL labeling buffer was incubated with 5 μg/mL primary anti-human CD326 antibody (anti-EpCAM, Biolegend, San Diego, CA, USA) for MCF-7 cells, or 5 μg/mL primary anti-human CD340 (anti-Her2, Biolegend, San Diego, CA, USA) on a rotary shaker for 30 minutes in the dark and at room temperature, followed by a washing step using labeling buffer. Then the cells were incubated with 5 nM Pdot-IgG or Alexa 488-IgG conjugates for 30 minutes on a shaker in the dark and at room temperature, followed by another two washing steps. For labeling cell-surface marker with streptavidin conjugates, a million MCF-7 cells in 100 μL labeling buffer was incubated sequentially with 5 μg/mL primary anti-human CD326 antibody, 5 μg/mL biotinylated secondary anti-mouse IgG (Biolegend, San Diego, CA, USA), and 5 nM Pdot-streptavidin or Qdot 565-steptavidin (Invitrogen, Eugene, OR, USA) for 30 minutes each, followed by another two washing steps. A drop of cell suspension was placed on a

coverslip, covered with a glass slide, and imaged immediately under a fluorescence confocal microscope (Zeiss LSM 510).

For microtubule labeling, ten thousands of MCF-7 cells were plated on a 22×22 mm glass coverslip, cultured until the density reach 60-70% confluence. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton-X 100 in PBS for 15 minutes, and blocked in 2% BSA (w/v) for 30 minutes. The fixed and BSA-blocked MCF-7 cells were incubated sequentially with 5 μg/mL biotinylated monoclonal anti-α-tubulin antibody (Biolegend, San Diego, CA, USA) for 60 minutes, and 10 nM Pdot-streptavidin conjugates for 30 minutes. The stained cells were mounted on a glass slide and imaged with the fluorescence confocal microscope (Zeiss LSM 510).

**Cell-labeling brightness quantification.** The cell-labeling brightness of Pdot bioconjugates with those of commercially available Qdot 565-streptavidin and Alexa-IgG probes was first quantified using a microfluidic flow cytometer. The flow-through experiments were conducted with a microfluidic chip with 200-μm wide and 50-μm high straight channels on an inverted light microscope equipped with a 20× NA 0.4 objective (Nikon Eclipse TE 2000-U, Melville, NY, USA). The as-labeled cell suspensions (10,000 per ml) were introduced into the rectangular channel using a syringe pump at 50 μl $min^{-1}$ for up to 5 minutes. A 488-nm sapphire laser (Coherent, Santa Clara, CA) was guided into the microscope to excite the sample. Before each sample acquisition, the laser power was measured in the path of light before entering the microscope using a power meter. Fluorescence signal was filtered by a 500-nm long pass filter (HQ500LP; Chroma, Rockingham, VT, USA), and collected by a Single Photon Counting Module (APD, PerkinElmer SPCM-QC9-QTY2, Salem, MA, USA). A personal computer and a

LabView coded program (National Instruments Corporation, Austin, TX, USA) were used to read out the signals of the SPCM at a sampling frequency of 10 kHz. The raw APD counts for each sample were stored in text files and converted in to frequency plots using a custom-coded Maple 5.1 program (MapleSoft, Waterloo, ON, Canada).

The cell-labeling brightness of Pdot-streptavidin and Qdot 565-streptavidin probes was also quantified by analyzing fluorescence images of the labeled MCF-7 cells. A drop of cell suspension was placed on a coverslip, covered with a glass slide, and viewed on an upright microscope with an AZ-Plan Apo 4× NA 0.4 objective (Nikon AZ100, Melville, NY, USA). The excitation light was provided with a fiber illuminator (130 W mercury lamp), and filtered by a band pass filter (Semrock FF01-482/35-25, Rochester, NY USA). Fluorescence signal was filtered by a 520-nm long pass filter (HQ520LP; Chroma, Rockingham, VT, USA), and imaged on a CCD camera (Prosilica GC1380, Newburyport, MA). Fluorescence images were processed with a custom-coded Labview program, and intensity distributions of single-cell labeling brightness were obtained (Supplementary Figure 4).

**AFM and fluorescence measurements.** For the AFM measurements, one drop of the nanoparticle dispersion was placed on a freshly cleaved mica substrate. After evaporation of the water, the surface topography was imaged with a Dimension 3100 multimode AFM in tapping mode. UV-Vis absorption spectra were recorded with a DU 720 scanning spectrophotometer (Beckman Coulter, Inc., CA USA) using 1 cm quartz cuvettes. Fluorescence spectra were obtained using a commercial Fluorolog-3 fluorometer (HORIBA Jobin Yvon, NJ USA). Fluorescence quantum yield of PFBT dots was determined by using a dilute solution of coumarin 6 in ethanol as standard.

Fluorescence decay lifetime of the PFBT dots was measured using a custom-built time-correlated single-photon counting instrument (TCSPC) (Supplementary Figure 5). The sample was excited by the second harmonic (400 nm, ~100 fs pulses) of a mode-locked femtosecond Ti:Sapphire laser (Coherent Mira 9000). The output of a fast PIN diode (Thorlabs, DET210) monitoring the laser pulse was used as the start pulse for a time-to-amplitude converter (TAC, Canberra Model 2145). Fluorescence signal from the aqueous nanoparticle dispersion was collected in perpendicular to the excitation, passed through a 500-nm long pass filter (HQ500LP; Chroma, Rockingham, VT, USA), and detected by a single photon counting module (id Quantique, ID100-50). The output of the detector was used as the stop pulse for the TAC. The laser was attenuated to maintain the count rate below 20 kHz. The signal from the TAC was digitized using a multichannel analyzer (FastComTec, MCA-3A). The instrument response function was measured before and after each fluorescence lifetime measurement using the scattered laser light from a dilute suspension of polystyrene beads. The combination of the detector and electronics results in an instrument response function with a width of ~50 ps (FWHM). Custom software written for the MATLAB environment (Mathworks) employing standard fitting methods was employed to determine the fluorescence lifetime. Statistical analyses yielded an estimated uncertainty in lifetime of 15 ps or better.

**Single-particle imaging.** Fluorescent samples were diluted in Milli-Q water, dried under vacuum on cleaned glass coverslips, and imaged on a 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 lab-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 500-nm long pass filter (HQ500LP; Chroma, Rockingham, VT, USA) and imaged on an EMCCD camera (Photometrics Cascade: 512B, Tucson, AZ USA). Because saturation of the detector was observed for some Pdot particles in Figure 2, a neutral density filter (optical density of 1.5) was placed together with the emission filter when imaging Pdot samples. Fluorescence intensity of Pdot particles was back-calculated according to the attenuation factor. Single-particle photobleaching measurements were obtained by acquiring a series of consecutive frames. Fluorescence intensity emitted per frame for a given particle was estimated by integrating the CCD signal over the fluorescence spot.

## Supporting Figures

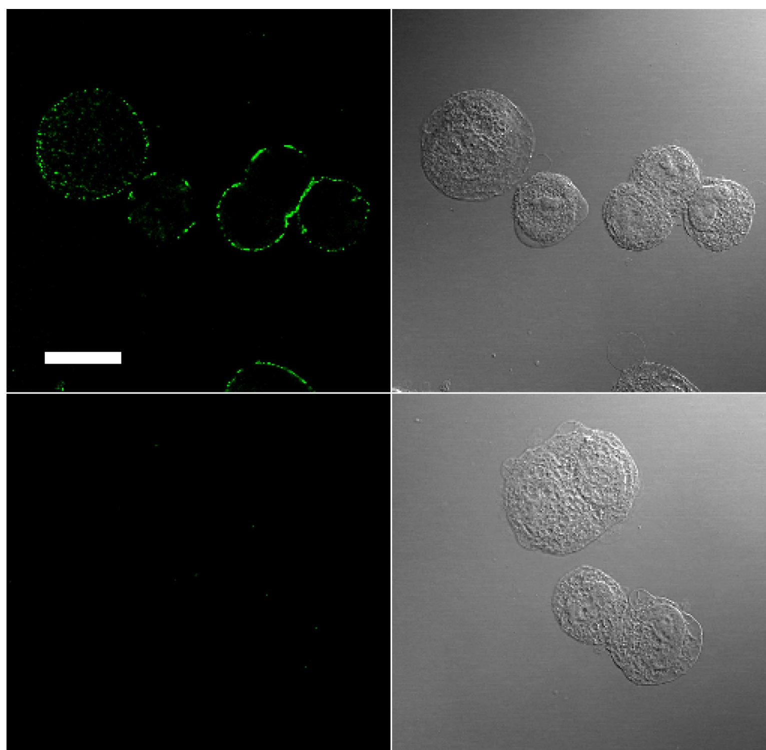

**Figure S1.** Confocal fluorescence imaging of cell-surface marker Her2 labeled with Pdot bioconjugates. The top two panels show the fluorescence imaging of live human breast cancer cells (SK-BR-3) incubated sequentially with anti-Her2 primary antibody and Pdot-IgG conjugates. The bottom two panels show control samples in which the cells were incubated with Pdot-IgG alone (no primary antibody). The Nomarski (DIC) images are shown to the right of the confocal fluorescence images. Scale bar represents 20 μm.

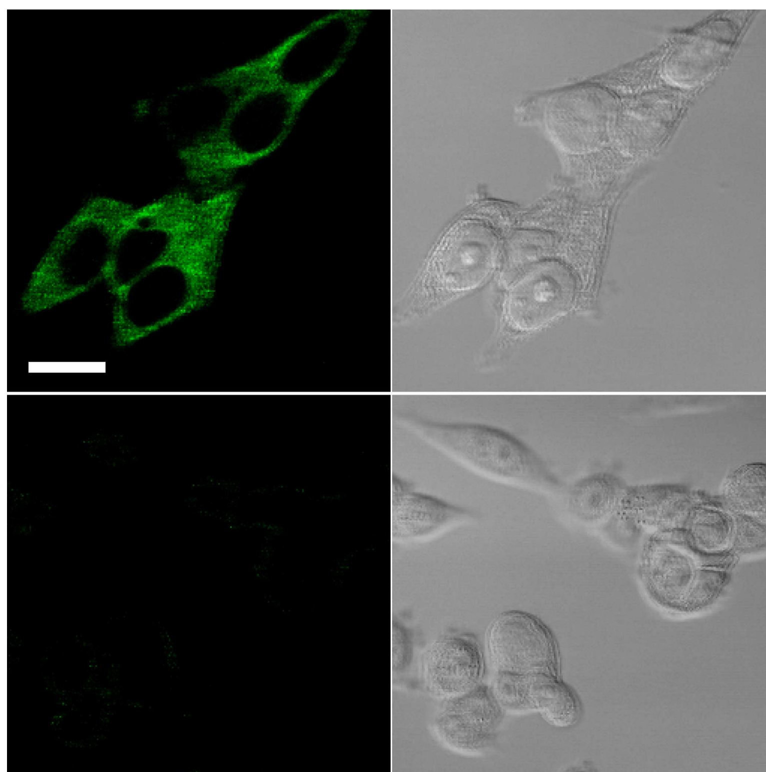

**Figure S2.** Confocal fluorescence imaging of microtubules labeled with Pdot bioconjugates. The top two panels show the fluorescence imaging of fixed MCF-7 cells incubated sequentially with biotinylated anti-tubulin primary antibody and Pdot-streptavidin conjugates. The bottom two panels show control samples in which the cells were incubated with Pdot-streptavidin alone (no primary antibody). The Nomarski (DIC) images are shown in the right sides of the confocal fluorescence images. Scale bar represents 20 μm.

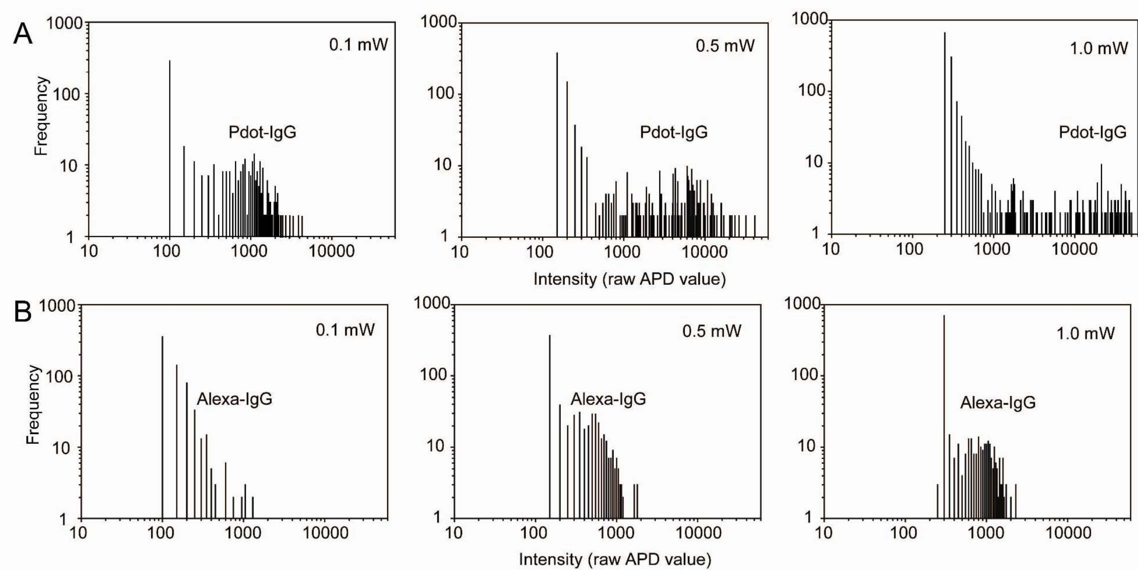


**Figure S3.** (A) Fluorescence intensity distributions obtained by flowing Pdot-IgG-labeled MCF-7 cells through a microfluidic flow cytometer; laser excitation was varied from 0.1 to 0.5 to 1 mW. (B) Fluorescence intensity distributions for Alexa 488-IgG-labeled MCF-7 cells obtained under identical experimental conditions as those used in (A).

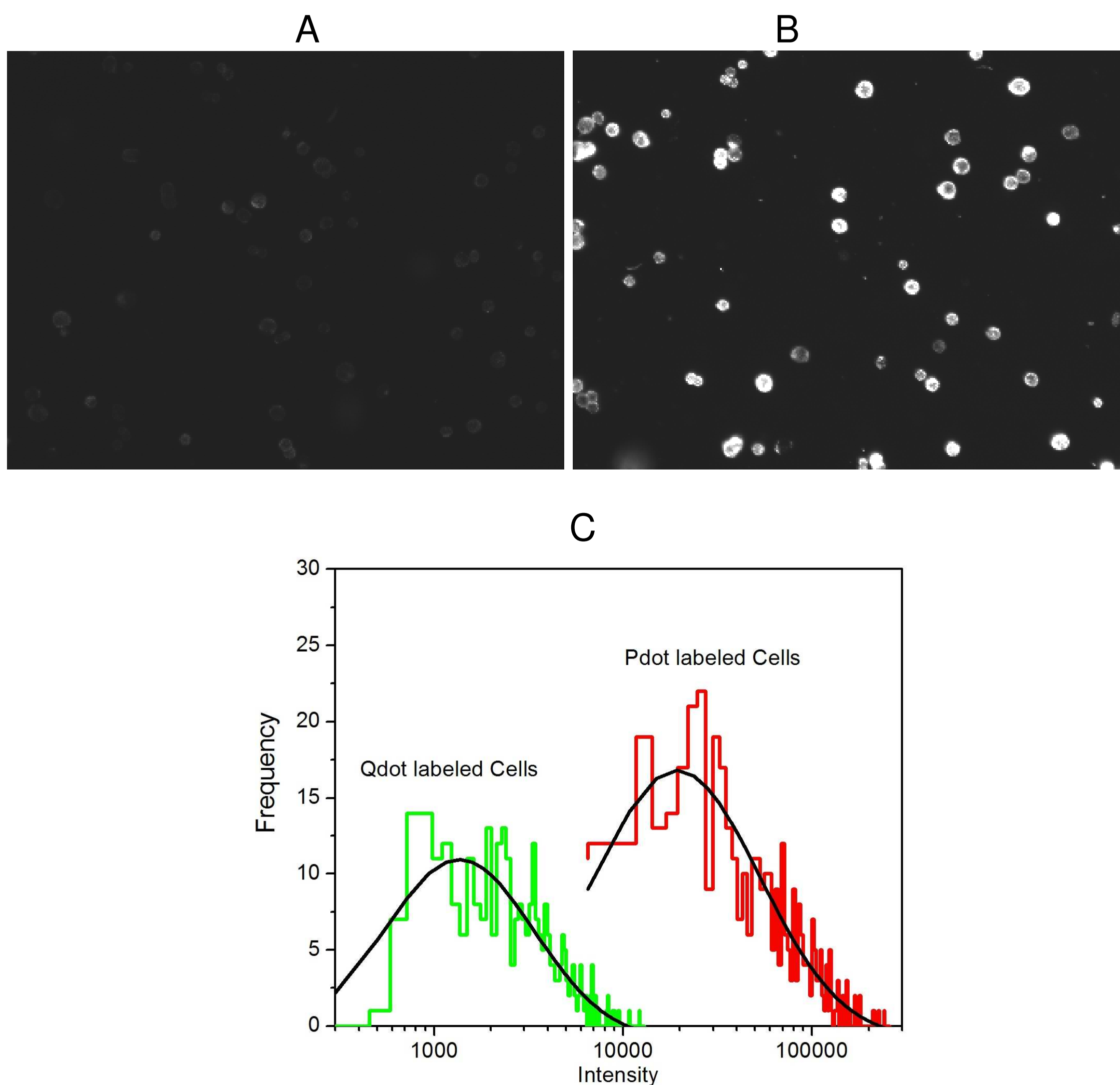


**Figure S4.** (A) Fluorescence images for Qdot 565-streptavidin-labeled MCF-7 cells obtained on a low numerical aperture wide-field microscope. (B) Fluorescence images for Pdot-streptavidin-labeled MCF-7 cells obtained under identical conditions as those used in (A). (C) Fluorescence intensity distributions of Pdot-labeled cells compared to Qdot-labeled ones.

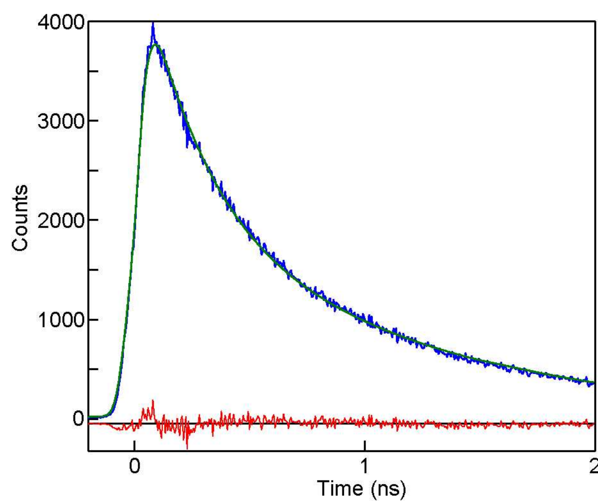


**Figure S5.** Fluorescence decay lifetime (0.6 ns) of PFBT dots measured by a TCSPC setup. The blue line represents experimental data, and the green line is fitting curve obtained employing an iterative deconvolution method. Residual is shown below the curves (red line).