Electronic Supplementary Material (ESI) for Chemical Communications

Highly Luminescent, Fluorinated Semiconducting Polymer Dots for Cellular Imaging and Analysis

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Experimental Section

Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on Bruker AV 300 or 500 spectrometers. ^{1}H NMR and ^{13}C NMR spectra used tetramethylsilane (TMS) as an internal standard in $CDCl_3$. The molecular weight of polymers was measured by the GPC method (Viscotek TDA305 GPC), and polystyrene was used as the standard (THF as eluent). ESI-MS spectra were obtained using a Bruker APEX Qe 47e Fourier transform (ion cyclotron resonance) mass spectrometer. The particle size and zeta-potentials of Pdots in bulk solution were characterized by DLS (Malvern Zetasizer NanoS). TEM measurements were recorded on a transmission electron microscope (FEI Tecnai F20). UV–vis absorption spectra were recorded with a DU 720 scanning spectrophotometer (Beckman Coulter, Inc., CA, USA) using 1-cm quartz cuvettes. Fluorescence spectra of Pdots in aqueous solution were obtained using a Perkin Elmer LS-50B Luminescence Spectrophotometer. The fluorescence quantum yields (QYs) of Pdot samples were measured with an absolute photoluminescence quantum yield measurement system (Hamamatsu photonic multichannel analyzer C10027). This system consists of a Xe arc lamp, a monochromator, an integrating sphere, and a multichannel detector. A monochromatic light source was used as the excitation light source. The excitation light was introduced into the integrating sphere by an optical fiber. A CCD camera was used as the multichannel detector.

The fluorescence quantum yield $\emptyset_f$ is given by

$$\emptyset_f = \frac{PN(Em)}{PN(Abs)} = \frac{\int \frac{\lambda}{hc}\left[I_{em}^{sample}(\lambda) - I_{em}^{reference}(\lambda)\right]d\lambda}{\int \frac{\lambda}{hc}\left[I_{ex}^{reference}(\lambda) - I_{ex}^{sample}(\lambda)\right]d\lambda}$$

where PN(Abs) is the number of photons absorbed by a sample and PN(Em) is the number of photons emitted from a sample, λ is the wavelength, h is Planck's constant, c is the velocity of light, $I_{ex}^{reference}$ and $I_{ex}^{sample}(\lambda)$ are the integrated intensities of the excitation light with and

without a sample, respectively, while I_{em}^{sample} and $I_{em}^{reference}$ are the photoluminescence intensities with and without a sample, respectively.

Materials

All chemicals were purchased from Sigma-Aldrich or TCI America, and used directly without further purifications. Compound 3 in scheme 1 was synthesized by following the method found in the literature.[S1].

Synthesis of monomers and polymers

4,4-diphenyl-5-fluoro-[2,1,3]benzothiadiazole (S2 in Scheme 1). Solutions of 4,7-dibromo-5-fluoro-[2,1,3]benzothiadiazole (S1) (0.35 g, 1.1 mmol) and tributylstannyl benzene (1.0 g, 2.7 mmol) in toluene (5 mL) were added to $Pd_2(dba)_3$ (50 mg) and $P(o\text{-}tol)_3$ (100 mg). The resulting mixture was degassed twice and was heated to reflux overnight. After cooling to room temperature, the organic solvent was removed. The crude product was then purified in a silica column to give compound S2 (0.4 g, 96%). ^{1}H NMR ($CDCl_3$, δ, ppm) 8.01 (d, J =7.0 Hz, 2H), 7.85 (d, J = 8.20 Hz, 2H), 7.71 (d, J = 11.05 Hz, 1H), 7.61-7.57 (m, 4H), 7.54-7.49 (m, 2H). ESI-MS: $C_{18}H_{11}FN_2S$ calcld. 306.0627, found. 306.0658.

4,4-di(4'-bromophenyl)-5-fluoro-[2,1,3]benzothiadiazole (2). To a solution of compound S2 (0.4 g, 1.3 mmol) in $CHCl_3$ (20 mL), bromine (1.2 mL) and a small amount of iodine (50 mg) were added in the dark. The solution was stirred at room temperature for 24 hours before adding saturated Na_2CO_3 aqueous solution. The precipitate was filtered out and washed with methanol and hexane, and dried overnight under vacuum (0.5 g, 83%). ^{1}H NMR ($CDCl_3$, δ, ppm) 7.87 (d, J = 8.49 Hz, 2H), 7.71-7.66 (m, 7H). ESI-MS: $C_{18}H_9Br_2FN_2S$ calcld.; 461.8837, found; 461.8855.

Polymer PFDPFBT. 2,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-di(2-ethylhexyl) fluorene (1) (120 mg, 0.19 mmol) and 2 (88 mg, 0.19 mmol) were dissolved in a mixture of toluene (4 mL) and aqueous Na_2CO_3 (2M, 2 mL). The mixture was degassed twice after adding tetrakis(triphenylphosphine) palladium ($Pd(PPh_3)_4$ (6 mg). Then, the mixture was heated to reflux with vigorous stirring for two days under an argon atmosphere. After the mixture was cooled to room temperature, the solution was poured into methanol. The precipitated polymer was recovered by filtration. The crude polymer was further purified by washing with water, methanol and acetone to remove oligomers and catalyst residues. Yield: 76%. ^{1}H NMR ($CDCl_3$, ppm) 8.17-8.12 (m, 4H), 7.89-7.74 (m, 11H), 2.15 (br, 4H), 0.92 (br, 15H), 0.69-0.61 (m, 15H). M_n (GPC): 24.5 k, PDI: 1.8.

Polymer PFDPBT. PFDPBT was synthesized by a similar procedure for PFDPFBT using monomers 1 and 3. ^{1}H NMR ($CDCl_3$, ppm) 8.16 (m, 4H), 7.93-7.83 (m, 8H), 7.76-7.71 (m, 4H), 2.15 (br, 4H), 0.92 (br, 15H), 0.69-0.55 (m, 15H). M_n (GPC): 19.8 k, PDI: 2.1.

Preparation of Pdots

Various amounts of PS-PEG-COOH (10 µL, 30 µL, 50 µL, 100 µL or 150 µL, 1000 ppm) was added to the polymer solution of PFDPFBT or PFDPBT in THF (4 mL, 50 ppm). The mixture solution was injected into DI water (10 mL) under ultrasonication. THF in the aqueous solution was then evaporated under nitrogen flow at 90 °C, and the solution was concentrated to 4–5 mL. The solution was then filtered through a 0.2-µm filter. The Pdot solutions were stored at 4 °C until further use.

Pdots Bioconjugation

Pdot bionconjugation was performed via the EDC-catalyzed reaction between carboxyl groups on the Pdots' surface and the amine groups on the 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 Pdot solution (~50 mg/mL in water), resulting in a Pdot solution in 20 mM HEPES buffer with a pH of 7.3. Then, 240 µL of streptavidin (Invitrogen, Eugene, OR, USA) was added to the solution and mixed well on a vortex. 8 µL of a freshly prepared EDC solution (10 mg/mL in water) was added to the solution, and the mixture was left on a rotary shaker. After stirring for 4 h at room temperature, Triton-X 100 (0.25% (w/v), 80 µL) and bovine serum albumin (BSA; 2% (w/v), 80 µL) were added. The mixture was left on rotary shaker for 1 h. 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 was purchased from American Type Culture Collection (Manassas, VA, USA). Cells were cultured at 37 °C, 5% CO_2 in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (FBS), 50 U/mL penicillin, and 50 µg/mL streptomycin. The cells were 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 ^{o}C for 5–15 min. 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.

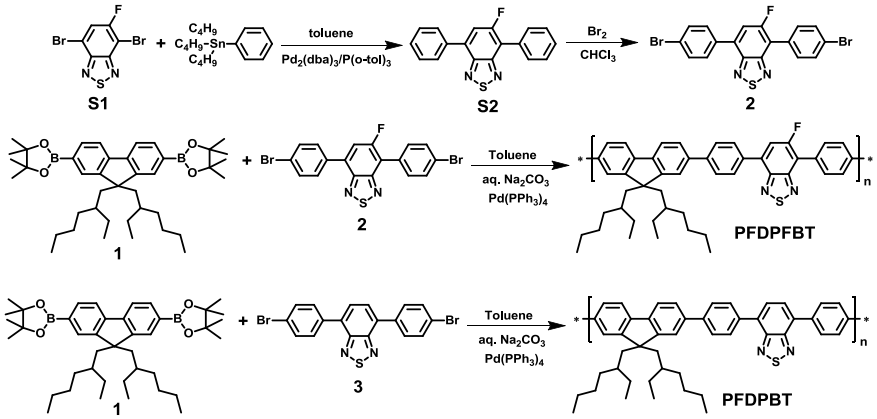
Flow Cytometry Measurements

For specific cell labeling with the Pdot-streptavidin conjugates, a million cells were blocked with BlockAid blocking buffer (Invitrogen, Eugene, OR, USA). The cells were then incubated sequentially with biotinylated primary anti-EpCAM antibody (used to label the cell-surface EpCAM receptors on MCF-7 cells) and 10 μg/mL (based on Pdots) Pdot-streptavidin for 30 min each, followed by two washing steps using labeling buffer. Finally, the specifically labeled cells were fixed in 0.6 mL of 4% (v/v) paraformaldehyde solution. For the control labeling, biotinylated primary anti-EpCAM antibody was not added. Flow cytometry measurements were performed on fresh samples with 10^6 cells/0.5 mL, prepared following the procedure described previously.[S2] The flow cytometer FACS Canto II (BD Bioscience, San Jose, CA, USA) was used for both Pdots with a 405-nm laser. Corresponding detection channels for fluorescence emission were filtered by a 502 nm long-pass followed by a 510/50 nm band-pass filter. Scattered light and fluorescence emission were detected by photomultiplier tube arrays. Representative populations of cells were chosen by selection of appropriate gates. Detection of cell scattered and fluorescent light was continued until at least 10^4 events had been collected in the active gate. Data were analyzed using FlowJo Software (Tree Star, Inc., Ashland, OR, USA).

Cellular Surface Labeling and Imaging

For labeling cell-surface proteins with the Pdot-streptavidin conjugates, live MCF-7 cells in the glass-bottomed culture dish were blocked with BlockAid blocking buffer (Invitrogen, Eugene, OR, USA). Then, the MCF-7 cells were incubated sequentially with biotinylated primary anti-EpCAM antibody (used to label the cell-surface EpCAM receptors on MCF-7 cells) and 5 nM Pdot-streptavidin for 30 min each, followed by two washing steps after each incubation. Biotinylated primary anti-EpCAM antibody was not added to the control sample. The Pdot-tagged cells were then counterstained with Hoechst 34580 and imaged immediately on a

fluorescence confocal microscope (Zeiss LSM 510). Both types of Pdot-labeled MCF-7 cells were excited by a 405-nm diode laser or a 488-nm argon laser. A Plan-Apochromat 63×/1.40 oil DIC objective lens was utilized for imaging.



Scheme S1. The synthetic route of monomers and polymers.

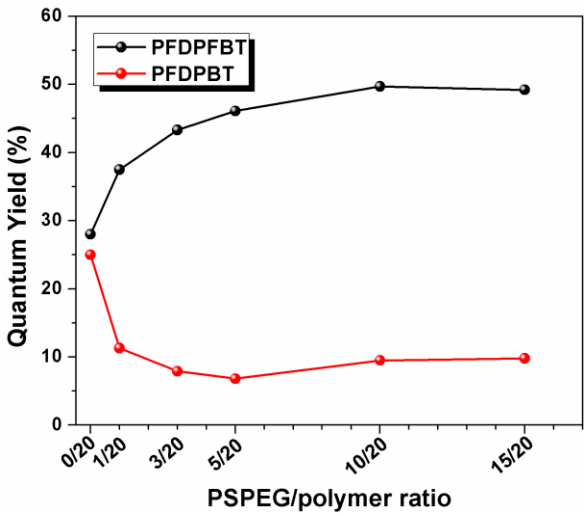


Fig. S1. The quantum yield changes of different PS-PEG-COOH concentration at 20 ppm polymer.

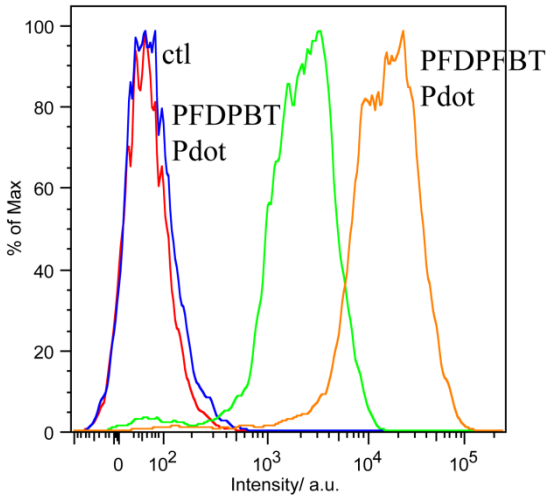


Fig. S2. The intensity distributions of flow cytometry (measured with 530nm/30 nm band-pass filter) of MCF-7 breast cancer cells labelled via non-specific binding (negative control) and positive specific targeting (positive) using PFDPFBT/PSPEGCOOH and PFDPBT/PSPEGCOOH Pdots conjugated with streptavidin.

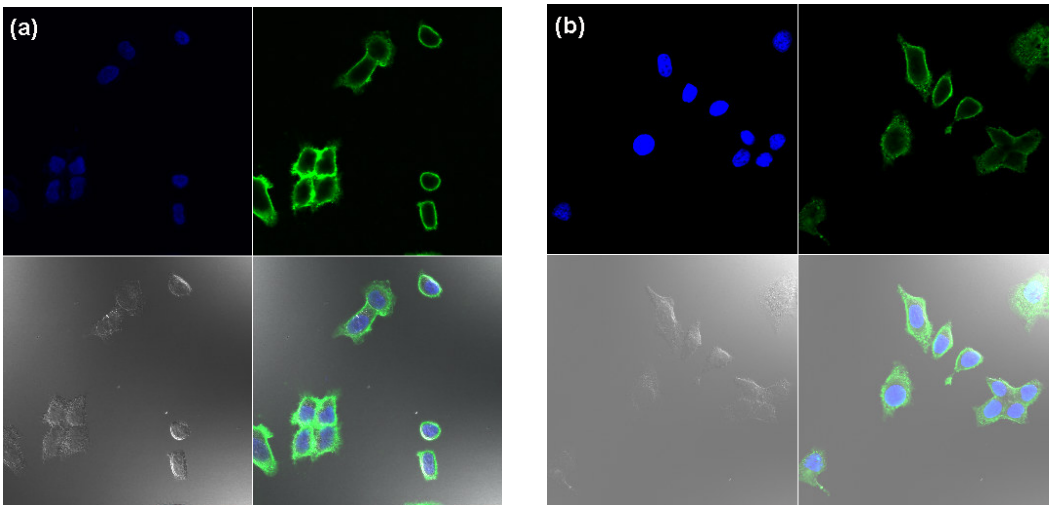


Fig. S3. Confocal fluorescence images of MCF-7 breast cancer cells labeled with (a) PFDPFBT/PS-PEG-COOH/streptavidin probes and (b) PFDPFBT/PS-PEG-COOH/streptavidin probes under 488-nm excitation.

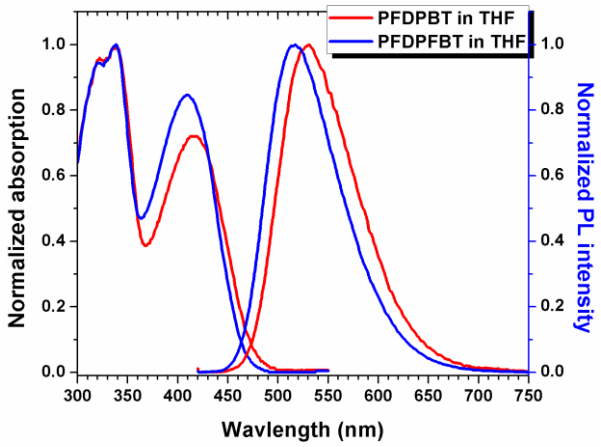


Fig. S4. The UV-Vis absorption and fluorescence spectra of PFDPBT and PFDPFBT in THF solution.

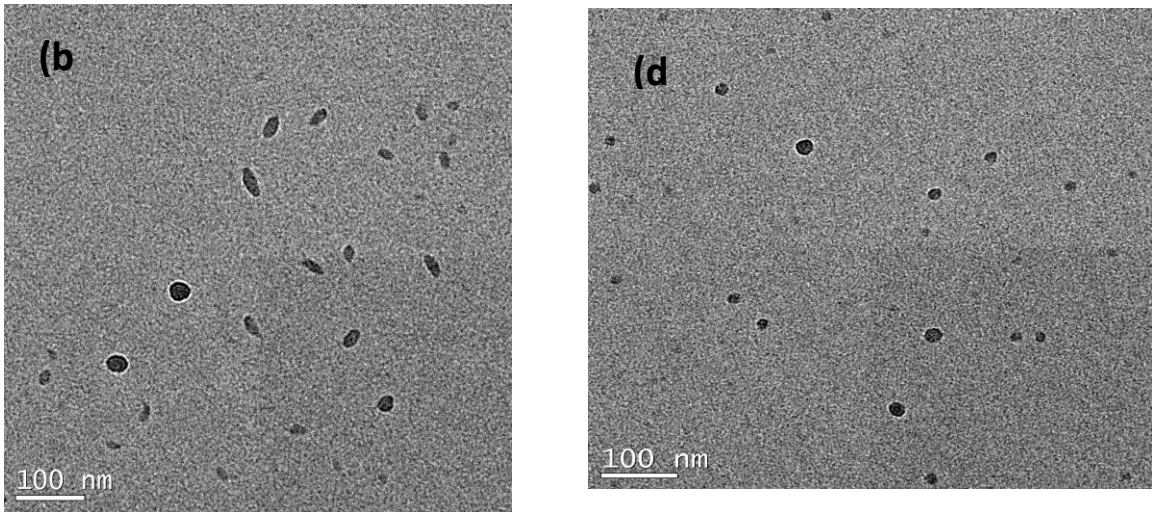


Fig. S5 The enlarged TEM images from Fig. 2.

S1. Liu, J.; Bu, L. J.; Dong, J. P.; Zhou, Q. G.; Geng, Y. H.; Ma, D. G.; Wang, L. X.; Jing, X. B.; Wang, F. S. *J. Mater. Chem.* 2007, *17*, 2832-2838

S2. (a) Yu, J.; Wu, C.; Zhang, X.; Ye, F.; Gallina, M. E.; Rong, Y.; Wu, I. C.; Sun, W.; Chan, Y.-H.; Chiu, D. T. *Adv. Mater.* **2012,** *24*, 3498-3504. (b) Wu, C.; Hansen, S. J.; Hou, Q.; Yu, J.; Zeigler, M.; Jin, Y.;

Burnham, D. R.; McNeill, J. D.; Olson, J. M.; Chiu, D. T. *Angew. Chem. Int. Ed.* **2011,** *50,* 3430-3434.