Multicolor Fluorescent Semiconducting Polymer Dots with Narrow Emissions and High Brightness

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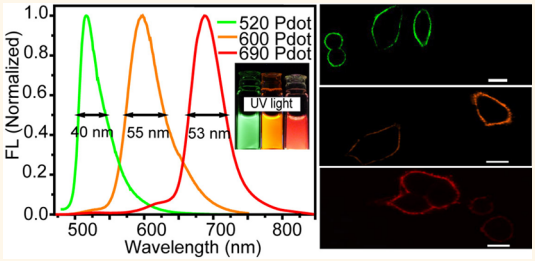
ABSTRACT Fluorescent semiconducting polymer dots (Pdots) have attracted great interest because of their superior characteristics as fluorescent probes, such as high fluorescence brightness, fast radiative rates, and excellent photostability. However, currently available Pdots generally exhibit broad emission spectra, which significantly limit their usefulness in many biological applications involving multiplex detections. Here, we describe the design and development of multicolor narrow emissive Pdots based on different boron dipyrromethene (BODIPY) units. BODIPY-containing semiconducting polymers emitting at multiple wavelengths were synthesized and used as precursors for preparing the Pdots, where intraparticle energy transfer led to highly bright, narrow emissions. The emission full width at half-maximum of the resulting Pdots varies from 40 to 55 nm, which is 1.5−2 times narrower than those of conventional semiconducting polymer dots. BODIPY 520 Pdots were about an order of magnitude brighter than commercial Qdot 525 under identical laser excitation conditions. Fluorescence imaging and flow cytometry experiments indicate that the narrow emissions from these bright Pdots are promising for multiplexed biological detections.



KEYWORDS: polymer dots · fluorescence · semiconducting polymer · bioimaging · narrow emission

Fluorescent probes coupled with bioconjugation techniques have been used extensively for advanced fluorescence detection in chemistry and the life sciences, such as fluorescence microscopy, flow cytometry, versatile biological assays, and biosensors. Because conventional organic dyes show limited brightness and poor photostability, a number of strategies for developing brighter fluorescent probes have been pursued. For example, luminescent nanocrystals, such as inorganic semiconductor quantum dots (Qdots) are under active development and now commercially available from Life Technologies (Invitrogen).[1,2] Another type of fluorescent nanoparticle is dye-doped latex spheres, which exhibit improved brightness and photostability as compared to single fluorescent molecules because of multiple dye molecules per particle and the protective latex matrix.[3]

Recently, fluorescent semiconducting polymer dots (Pdots) have attracted great interest because of their extraordinary fluorescence brightness and photostability.[4] The use of fluorescent polymer dots as fluorescent probes also confers other useful advantages, such as the lack of heavy metal ions that could leach out into solution and which would be toxic for living organisms or biological cells. Previous studies have also shown that Pdots have good biocompatibility.[5,6] Very recently, surface functionalization has been achieved by a coprecipitation scheme where amphiphilic polymer molecules bearing functional groups were blended with semiconducting polymers to form Pdots with surface reactive groups. The Pdot bioconjugates can specifically and effectively label biomolecules for cellular imaging, bioorthogonal labeling, and *in vivo* tumor targeting.[4,7,8]

Despite this progress, a severe drawback to the use of Pdots in practical applications is that currently available Pdot species exhibit very broad emission spectra. For example,

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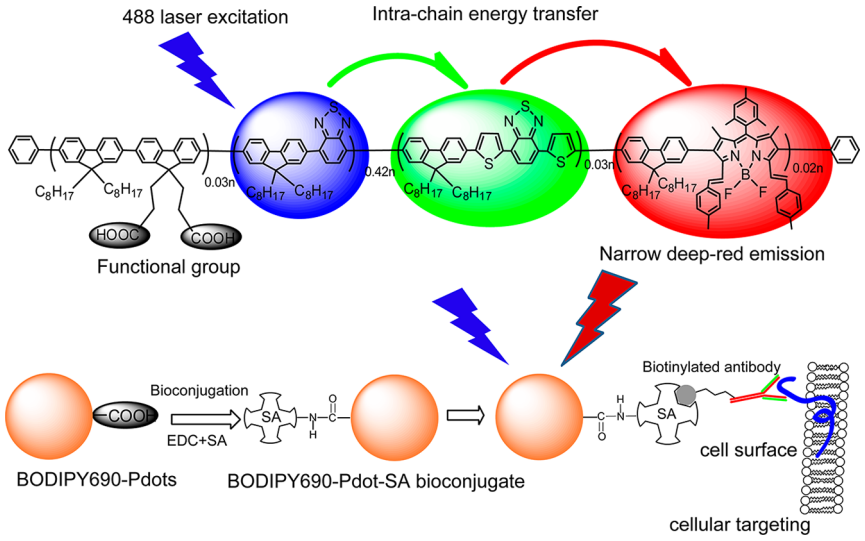


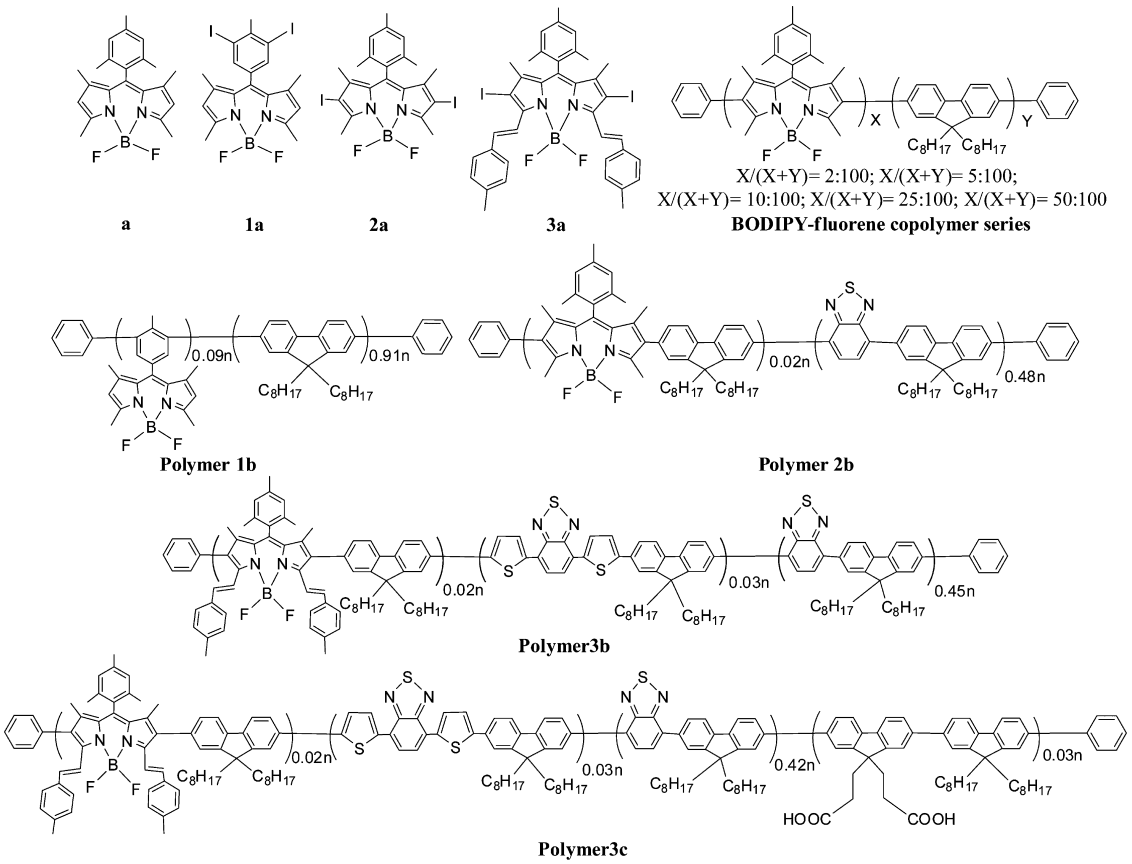
Scheme 1. Schematic illustration of narrow emissive semiconducting polymer and Pdot bioconjugates for specific cellular targeting.

poly[9,9-dioctylfluorenyl-2,7-diyl)-*co*-1,4-benzo-{2,1′-3}-thiadiazole)] (PFBT), a widely studied Pdot, exhibits fwhm of about 75 nm,[9] even though PFBT dots are ~30 times brighter than commercial Qdots 565.[4] Most biological applications demand that multiple targets be detected simultaneously, thus spectral multiplexing requires that the probes possess narrow emissions. The broad emission spectra from conventional Pdot species significantly limit their usefulness in practical applications. Therefore, there is an urgent need to develop new types of Pdots that can emit at different wavelengths with narrow spectral width. Here we describe the design and synthesis of semiconducting polymers containing boron dipyrromethene (BODIPY) units. The polymer structure and composition were systematically tuned to obtain multicolor, highly bright, and narrow emissive Pdots. We performed biomolecular conjugation and demonstrated specific cellular labeling by fluorescence imaging and flow cytometry experiments. Our results indicate that these new Pdot probes are promising for practical biological applications.

RESULTS AND DISCUSSION

Synthesis of Multicolor BODIPY-Containing Copolymer with Narrow Emission. To design narrow emissive Pdots, we employed a donor−acceptor strategy that comprises BODIPY units as narrow emissive species. BODIPY units as energy acceptors were incorporated into the polymer backbone, and after Pdot formation, efficient intraparticle energy transfer led to narrow emissions (Scheme 1). BODIPY dyes were selected because they emit sharp fluorescence peaks and possess good photostability, high absorption coefficients and quantum yields.[10,11] Although BODIPY-containing fluorescent conjugated polymers have been the subject of an increasing number of literature reports in recent years,[12−14] narrow emissions from a semiconducting polymer in a nanoparticle form have not been reported in the literature so far.

First, we synthesized a BODIPY-based monomer (monomer a, Scheme 2) that exhibits narrow emission with a fwhm of 36 nm in its molecular form (Figure S1 and Table S1 in the Supporting Information). We used this monomer as a model to copolymerize with fluorene monomer at different BODIPY molar ratios in order to investigate the influence of copolymer composition on the emission properties of the resulting Pdots. Scheme 2 shows BODIPY-fluorene copolymers at different molar ratios (2−50%) of BODIPY monomers. These copolymers have good solubility in THF, which made it possible to prepare Pdots using the reprecipitation method. Figure 1a shows the fluorescence spectra of the BODIPY-fluorene copolymers in tetrahydrofuran (THF) solution. While the BODIPY monomer a exhibits narrow emission centered at 515 nm, the corresponding polymers show red-shifted emissions around 586 nm because of the increased conjugation length. However, all of the copolymers in THF exhibit narrow emissions with fwhm values in the range of 45−49 nm, indicating that the polymers in their molecular form maintained the narrow emissions from the BODIPY units. When these polymers were prepared into Pdots, the nanoparticles in aqueous solution showed quite different emission bandwidths, which became broader with increasing molar ratios of BODIPY monomers. We attribute this phenomenon to the aggregation of the BODIPY chromophores in Pdots, which can give rise to shoulder peaks in the longer wavelength region compared to that of the free polymer molecules in THF solution. This aggregation also caused self-quenching of the BODIPY fluorescence in Pdots. As a result, the fluorescence quantum yields of the BODIPY-fluorene Pdots were decreased from 13 to 2% when the molar ratios of the BODIPY chromophore



Scheme 2. Chemical structures of BODIPY monomers and BODIPY-containing polymers.

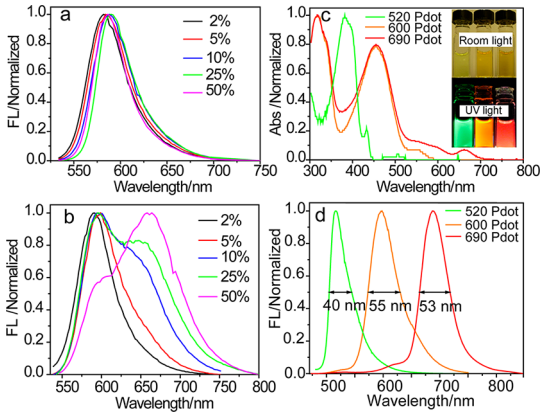


Figure 1. (a) Fluorescence spectra of BODIPY-fluorene copolymer series in THF. (b) Fluorescence spectra of BODIPY copolymer series in Pdot form in water. (c) Absorption spectra of BODIPY 520 Pdots (polymer 1b), BODIPY 600 Pdots (polymer 2b), and BODIPY 690 Pdots (polymer 3c) in water. (d) Fluorescence spectra of BODIPY 520 Pdots, BODIPY 600 Pdots, and BODIPY 690 Pdots in water.

were increased from 2 to 50%. The BODIPY-fluorene Pdots containing 2% molar ratio of BODIPY chromophore exhibited much narrower emission band (53 nm of fwhm) as compared with conventional Pdots such as PFBT nanoparticles (75 nm of fwhm).

We employed a synthetic strategy to obtain green emissive Pdots with narrow spectral bandwidth. In the series of the BODIPY-fluorene polymer shown in Scheme 2, BODIPY monomers were incorporated into the polyfluorene backbone by reacting the BODIPY units at the meso- and 2,6-positions, and the resulting polymers showed red-shifted emissions. As indicated previously,[10] functionalization of the benzene ring at the meso-position has little effect on the absorption and emission wavelengths of BODIPY cores. Therefore, we synthesized a BODIPY monomer with iodine groups in the benzene ring (monomer 1a, Scheme 2) and further synthesized the corresponding polymer by reacting fluorene with BODIPY through its benzene ring at the meso-positon *via* Yamamoto polymerization (Scheme S2). Figure S2A,B (green curves) shows the absorption and emission spectra of the resulting green Pdots, respectively. As expected, the Pdots exhibit narrow green emission centered at 516 nm with a fwhm as narrow as 40 nm. To the best of our knowledge, this is the narrowest emission bandwidth among various Pdot species reported so far.

The BODIPY-fluorene polymers shown in Scheme 2 have a dominant absorption feature only in the ultraviolet region (for 405 nm laser excitation), which is a drawback for many biological applications that require 488 nm excitation. To overcome this issue, we incorporated a benzothiadiazole (BT) donor into the backbone of the BODIPY-fluorene copolymer to extend the

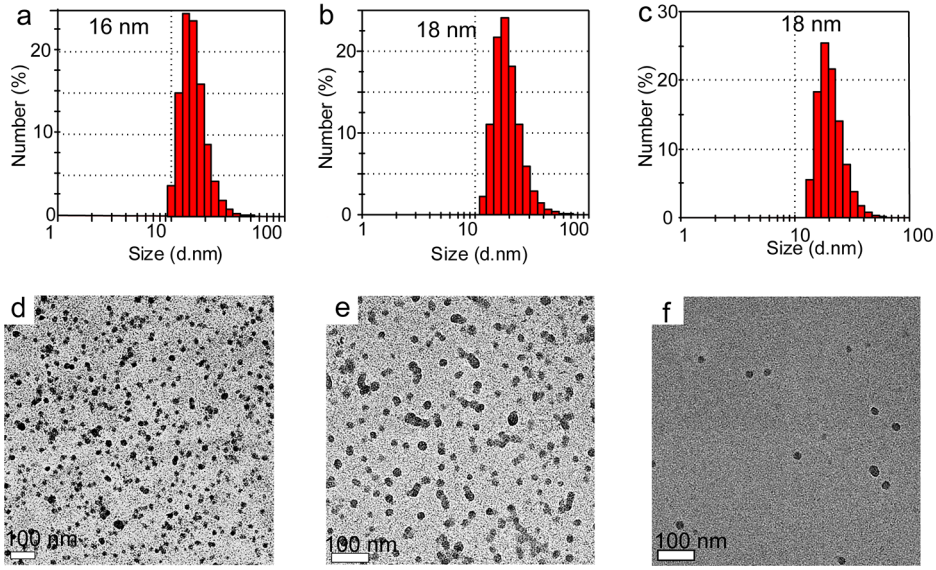


Figure 2. (a–c) Histograms of the distribution of the sizes of BODIPY 520 Pdots, BODIPY 600 Pdots, and BODIPY 690 Pdots, respectively, measured by DLS (the mean size is (a) 16 nm, (b) 18 nm, and (c) 18 nm). (d–f) TEM images of BODIPY 520 Pdots, BODIPY 600 Pdots, and BODIPY 690 Pdots, respectively.

polymer absorption to the visible region. We have previously demonstrated that PFBT is an excellent polymer for preparing Pdots with high absorption cross section, single-particle brightness, and excellent photostability.[4] Besides the excellent photophysical properties of PFBT polymer, its absorption peak is around 450–460 nm, which is very close to the generally used excitation wavelength (488 nm) in biological applications. Furthermore, the PFBT emission (~540 nm) has very good overlap with the absorption of the BODIPY chromophore (546 nm), thus increasing the intraparticle energy transfer efficiency to completely quench the donor fluorescence. On the basis of this strategy, a copolymer (polymer 2b) was synthesized *via* Suzuki polycondensation by introducing the benzothiadiazole (BT) donor into the backbone of the BODIPY-fluorene copolymer (Scheme S3). Pdots prepared from this polymer precursor exhibited strong absorption in the blue region, while maintaining the narrow emission centered at 597 nm with a fwhm of 55 nm (Figure S2A, B, orange curves).

We further modified the structure of BODIPY unit to tune the Pdot emission color to the deep red region, while maintaining their narrow emission bandwidth. Electronic conjugation can be increased with unsaturated linkers at the 3,5-positions of the BODIPY core, thereby causing a red shift in both absorption and emission spectra. On the basis of this strategy, we synthesized another monomer (monomer 3a) and used it to obtain a narrow emissive polymer in the deep red region (polymer 3b, Scheme S4). BT monomer was also introduced as donor for 4,7-bisthienyl-2,1,3-benzothiadiazole (TBT), which in turn served as the donor for BODIPY monomer 3a to get efficient cascade energy transfer. Similarly, excited at the absorption peak wavelength of BT monomer, the Pdots exhibited a pure deep red emission peak at 688 nm with a fwhm of 53 nm (Figure S2B).

Previously, we have shown successful surface functionalization of Pdots by coprecipitating semiconducting polymers with amphiphilic polymers bearing functional groups, which was further used for bioconjugation and finally applied to label biomolecules for fluorescence imaging.[4,8,15] With this coprecipitation strategy, we successfully prepared BODIPY 520 Pdots (polymer 1b) and BODIPY 600 Pdots (polymer 2b), which were blended with PS-PEG-COOH amphiphilic polymers. The BODIPY 520 Pdots and BODIPY 600 Pdots showed identical emission spectra as compared with the polymer 1b and 2b Pdots without PS-PEG-COOH (Figure 1c,d and Figure S2).

However, an elegant and more robust approach for Pdot functionalization is to covalently introduce a small number of carboxylate groups into the side chain of the copolymer.[16] We demonstrate this strategy using polymer 3c as an example. Carboxylate functional groups were covalently linked to the fluorene monomer and then were incorporated into the backbone of polymer 3c (Scheme S5). BODIPY 690 Pdots (polymer 3c) show comparable emission spectra as compared with the polymer 3b Pdots without side-chain functionalization (Figure 1c,d and Figure S2), indicating that a small amount of carboxylate group on the side chain did not affect emission bandwidth. The BODIPY 600 and BODIPY 690 Pdots appear to be remarkably photostable, similar to the PFBT Pdots that we characterized previously[9]—photobleaching for 1.5 h using the 488 nm excitation of xenon lamp did not result in observable decrease in fluorescence intensity (Figure S5b). Qdot 525 and BODIPY 520 Pdots exhibit single exponential photobleaching decays under identical conditions (405 nm excitation of xenon lamp) (Figure S5a),

with BODIPY 520 Pdots showing slightly better photostability than Qdot 525.

Single-Particle Fluorescence Brightness. In order to evaluate their photophysical properties, BODIPY 520 Pdots, BODIPY 600 Pdots, and BODIPY 690 Pdots were prepared with the same particle size of 16, 18, and 18 nm, as characterized by TEM and DLS (Figure 2), while commercial available Qdot 525 with carboxyl groups were dispersed in Milli-Q water with the particle size of ~13 nm (measured by DLS). Also, the previously reported PFBT Pdots were prepared to have the same size,[4] so we could use them as reference in the evaluation of BODIPY 520 Pdots and BODIPY 600 Pdots.

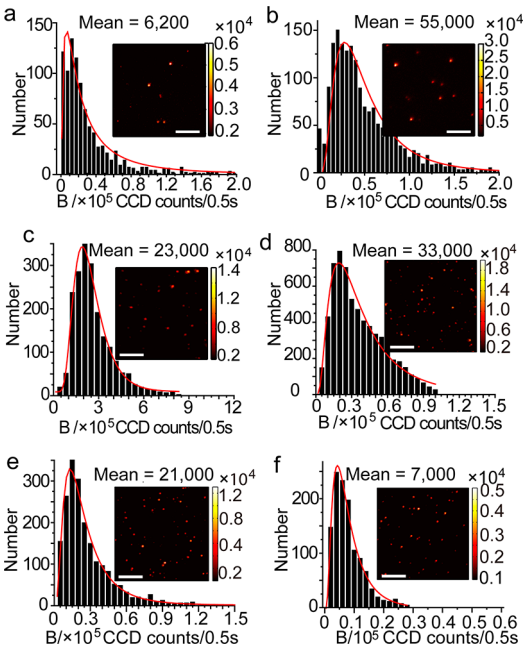


Figure 3. Histograms of the distributions of single-particle brightness of (a) Qdots 525 (λ_{ex} = 405 nm), (b) BODIPY 520 Pdots (λ_{ex} = 405 nm), (c) PFBT/PS-PEG Pdots (λ_{ex} = 405 nm), (d) BODIPY 520 Pdots (λ_{ex} = 405 nm), (e) PFBT/PS-PEG Pdots (λ_{ex} = 488 nm), (f) BODIPY 600 Pdots (λ_{ex} = 488 nm). The red curves were obtained by fitting a log-normal distribution to the histogram and gave 6200, 55 000, 23 000, 33 000, 21 000, and 7000 mean CCD counts for the six histograms, respectively. Insets: single-particle brightness images: (a) and (b), (c) and (d), and (e) and (f) were obtained under identical excitation and detection conditions. All scale bars represent 10 μm.

TABLE 1. Size, Zeta-Potential, and Photophysical Properties of BODIPY 520 Pdots and Qdots 525

probe	size[a]	ζ[b]	abs (10^{-13} cm^2)[c]	Φ[d]	B (CCD counts)[e]
BODIPY 520	16	−48.9	2.50 (405 nm)	35	55000 (405 nm)
Qdots 525	13	−56.4	0.0172 (405 nm)	13	6200 (405 nm)

[a] Size was measured by DLS. [b] Zeta-potential. [c] Absorption cross section per single Pdot. [d] Absolute photoluminescence quantum yield. [e] Single particle brightness.

Single-particle brightness is one of the important characteristics for Pdots when they are applied in fluorescence imaging. BODIPY 520 Pdots, Qdots 525, and PFBT Pdots were measured under identical conditions (405 nm laser excitation), whereas BODIPY 590 Pdots and PFBT Pdots were measured under the same conditions (488 nm laser excitation). Comparison of single-particle brightness between BODIPY 520 Pdots and the commercial Qdots 525 was first measured, and the fluorescence signal was filtered by a 500 nm long-pass filter. The measured average per-particle brightness of BODIPY 520 Pdots (Figure 3b) is ~9 times that of Qdots 525 (Figure 3a). With the same particle size, the measured average per-particle brightness of BODIPY 520 Pdots (Figure 3d) is 2 times that of PFBT Pdots (Figure 3c), which is 3 times that of BODIPY 590 Pdots (Figure 3f). The fluorescence brightness of a nanoparticle is determined by the per-particle absorption cross section and the quantum yield. The per-particle brightness was calculated based on the photophysical parameters shown in Table 1 and Table S3. The calculated brightness of BODIPY 520 Pdots is 1.7 times that of PFBT Pdots. The calculated brightness of PFBT dots is 3.2 times that of BODIPY 600 Pdots, consistent with the experimentally measured results of single-particle brightness. All of the above results indicate that the single-particle brightness of the new Pdots reported in this work has the same order of magnitude brightness as the PFBT dots.[4]

Specific Labeling of Cellular Targets with Pdots. To apply these multicolor narrow emissive Pdots for fluorescence imaging, bioconjugation was successfully performed using these Pdots *via* EDC-catalyzed coupling. The Pdot-streptavidin bioconjugates (Pdot-SA) were

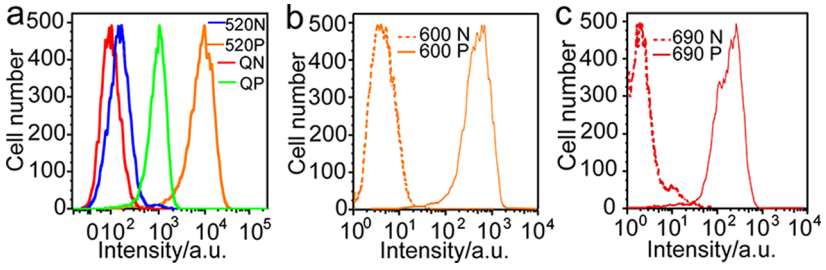


Figure 4. Flow cytometry measurements of the intensity distributions of MCF-7 breast cancer cells labeled *via* nonspecific binding (N, negative control) and positive specific targeting (P, positive) using Qdots 525 (QN, Qdot negative control; QP, Qdot positive), BODIPY 520 Pdots (520N, BODIPY 520 negative control; 520P, BODIPY 520 positive) (a), BODIPY 600 Pdots (600N, 600P) (b), and BODIPY 690 Pdots (690N, 690P) (c). All Qdots and Pdots were conjugated with streptavidin.

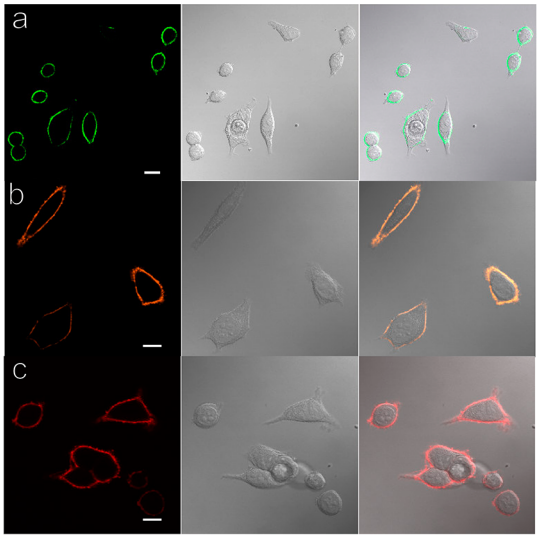


Figure 5. Confocal fluorescence microscopy images of MCF-7 cells labeled with BODIPY Pdot-SA probes: (a) BODIPY 520 Pdot-SA, (b) BODIPY 600 Pdot-SA, and (c) BODIPY 690 Pdot-SA. Images from left to right: green, orange, and deep red fluorescence images from BODIPY Pdot-SA probes; Nomarski (DIC) images; combined fluorescence images. Scale bars: 20 mm.

used to label cell-surface markers in MCF-7 breast cancer cells. The cells were sequentially incubated with biotinylated primary anti-EpCAM antibody and Pdot-SA probes. Figure 4a–c shows the flow cytometry results, which proved that all three Pdot-SA probes and Qdot 525-SA probes effectively labeled EpCAM receptors on the cell surface, while the negative control samples (identical conditions but no incubation with primary biotinylated antibody) could not label the cell surface. We compared our BODIPY 520 Pdots with commercial Qdots 525 at the same labeling concentration and under identical experimental conditions to provide a further comparison of their brightness, cell labeling efficiency, and overall performance. Figure 4a shows BODIPY 520 Pdots were about an order of magnitude brighter than commercial Qdot 525. The result is consistent with the single-particle brightness measurements. The specific cellular labeling with these BODIPY Pdot-SA probes was further confirmed by confocal fluorescence imaging (Figure 5 and Figure S4). From Figure 5a–c, all three BODIPY Pdot-SA probes effectively labeled EpCAM receptors on the MCF-7 cell surface; however, no fluorescence was detected in the negative control experiments, carried out in the absence of the biotinylated primary antibody (Figure S4), which showed there was highly specific cellular labeling with no nonspecific binding.

CONCLUSION

We have successfully synthesized multicolor BODIPY-containing fluorescent semiconducting copolymers by introducing donors for energy transfer *via* Suzuki or Yamamoto polymerization. The corresponding Pdots were shown to be excellent fluorescent probes because they exhibit narrow emission in addition to possessing high absorption cross section, photoluminescence quantum yield, and high fluorescence brightness. The emission fwhm values of these Pdots vary from 40 to 55 nm, which are 1.5–2 times narrower than those of conventional Pdots. BODIPY 520 Pdots were about an order of magnitude brighter than commercial Qdot 525 under identical 405 nm laser excitation. We performed bioconjugation and demonstrated specific cellular targeting using the new Pdot bioconjugates by fluorescence imaging and flow cytometry experiments, which indicated that these bright narrow emissive Pdots are promising probes for many multiplexed biological detections.

EXPERIMENTAL SECTION

Materials and Synthesis. *Instrumentation.* ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on Bruker AV500 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). The particle size and zeta-potentials of Pdots in bulk solution were characterized by dynamic light scattering (Malvern Zetasizer NanoS). TEM measurements were recorded on a transmission electron microscope (FEI Tecnai F20). UV–vis absorption spectra were recorded with DU 720 scanning spectrophotometer (Beckman Coulter, Inc., CA, USA) using 1 cm quartz cuvettes. Fluorescence spectra and photostability of Pdots and Qdots in bulk aqueous solution were obtained using a commercial Fluorolog-3 fluorometer (HORIBA Jobin Yvon, NJ, USA). Fluorescence quantum yields were measured using a Hamamatsu photonic multichannel analyzer C10027 equipped with a CCD integrating sphere. The FTIR spectra were recorded on Bruker Vector 33 infrared spectrometer. Potassium bromide (KBr) was used as an inert background material to get the spectra of the monomers. The analysis was done in the region of 500–4000 cm^{-1}. ESI-MS spectra were obtained using a Bruker APEX Qe 47e Fourier transform (ion cyclotron resonance) mass spectrometer.

Materials. All chemicals were purchased from Sigma-Aldrich and TCI America. Qdots [Qdot 525 with carboxyl groups (Qdot 525 Carboxyl Quantum Dots) and Qdot 525-SA (Qdot 525 streptavidin conjugate)] were purchased from Life Technologies.

Synthesis of BODIPY Monomer a (8-Mesityl-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene)[11]. First, 110 μL of trifluoroacetic acid in dry CH_2Cl_2 (10 mL) was added slowly to a solution of 2,4,6-trimethylbenzaldehyde (1.482g, 10 mmol) and 2,4-dimethyl-1*H*-pyrrole (2.38g, 25 mmol) in dry CH_2Cl_2 (250 mL) at room temperature. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (2.27 g, 10 mmol) was added after 3 h stirring under ice bath cooling and stirred for 20 min. The solution was stirred for an additional 1 h at room temperature. NEt_3 (20 mL, 144 mmol) was added, followed by slow addition of $BF_3 \cdot Et_2O$ (23 mL, 170 mmol). The reaction mixture was washed after 12 h of stirring at room temperature with saturated aqueous Na_2CO_3 solution (2 × 150 mL), dried over Na_2SO_4, and concentrated on a rotary evaporator. The brown, oily residue was purified by column chromatography on silica with hexane/CH_2Cl_2 = 3:1. The product fraction with greenish fluorescence was dried to yield a red-brown solid. Yield: 2.3 g, 62.8%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.979 (s, 2H), 5.993 (s, 2H), 2.592 (s, 6H), 2.368 (s, 3H), 2.128 (s, 6H), 1.417 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 155.09, 142.31, 141.68, 138.57, 134.92, 131.13, 130.62, 129.0, 120.79, 21.22,

19.51, 14.64, 13.41. HRMS (ESI): (M^+, $C_{22}H_{25}BF_2N_2$) calcd 367.2155; found 367.2157.

Synthesis of Monomer 2a. A 250 mL round-bottom flask was first charged with 2.2 g (6 mmol) of BODIPY monomer dissolved in 80 mL of ethanol. To this solution was added and allowed to dissolve 4.57 g (18 mmol) of powdered I_2. Then, 2.15 g (12.2 mmol) of HIO_3 was dissolved in 0.7 g of water, and this solution was added dropwise by a syringe over 20 min. After the addition was complete, the solution was heated to 60 °C and refluxed for 5 h. Ethanol was removed on a rotary evaporator. The residue was purified by column chromatography with a silica with hexane/CH_2Cl_2 = 3:1. The product 2a was dried to obtain a metallic dark red solid. Yield: 2.5g, 68%. FTIR (KBr, cm^{-1}): 3432.2, 3015.1, 2954.9, 2918.8, 2852.7, 2734.4, 1609.6, 1526.8, 1483.4, 1456.7, 1400.6, 1343.8, 1309.1, 1248.3, 1182.9, 1121.9, 1095.4, 1056.7, 999.9, 931.1, 886.4, 851.7, 777.6, 704.1, 681.1, 665.5, 647.9, 627.6, 590.6, 560.3, 525.2. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.008 (d, 2H), 2.682 (s, 6H), 2.391 (s, 3H), 2.096 (s, 6H), 1.437 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 156.42, 144.57, 141.72, 139.29, 134.81, 130.86, 130.52, 129.31, 85.30, 21.28, 19.55, 16.06, 15.80. HRMS (ESI): (M^+, $C_{22}H_{23}BF_2I_2N_2$) calcd 618.0051; found 618.0039.

Synthesis of BODIPY-Fluorene Copolymer Series. BODIPY-fluorene copolymer series with different BODIPY monomer molar ratios (2, 5, 10, 25, and 50%) were synthesized by palladium-catalyzed Suzuki coupling reaction from 9,9-dioctylfluorene and BODIPY monomer 2a. 9,9-Dioctyl-2,7-dibromofluorene, 9,9-dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester, BODIPY monomer 2a, 2 drops of Aliquat 336, 10 mL of 2 M Na_2CO_3 aqueous solution, and 15 mL of toluene were placed in a 50 mL flask. The flask was evacuated and refilled with N_2 four times by using the freeze/thaw method, and $Pd(PPh_3)_4$ (1−1.5 mol %) was added. The flask was further degassed four times, then the reaction was heated to 80 °C and stirred under N_2. After 70 h, 0.2 mL of bromobenzene and 15 mg of phenylboronic acid were added to end-cap the polymer chain, and the reaction was stirred for an additional 2 h at 80 °C. The whole mixture was poured into 200 mL of MeOH, filtered, and washed with 0.2 M of HCl. The precipitate was stirred in 50 mL of acetone at room temperature for 24 h and dried in a vacuum oven to obtain dark pink to dark red solid. Yield: 73−81%. NMR results for PFO-BODIPY 10: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.89−7.61 (m), 7.53 (m), 7.42 (m, 6H), 7.25 (m, 5H), 7.05 (m, 2H), 2.69 (s, 6H), 2.39 (s, 3H), 2.32 (s, 6H), 2.09−2.17 (s, 4H), 1.31 (s, 6H), 1.19 (s, 24), 0.87 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 154.07, 151.85, 151.74, 151.08, 141.94, 140.55, 140.08, 138.16, 135.05, 133.96, 132.24, 132.17130.63, 129.15, 128.96, 128.83, 128.57, 128.47, 127.25, 126.82, 126.19, 124.86, 121.53, 120.01, 119.55, 55.39, 55.29, 40.44, 30.08, 29.76, 29.26, 29.19, 23.95, 22.64, 21.31, 19.91, 14.11, 13.65, 11.74. GPC M_n = 23048, M_w = 43610, PDI = 1.89.

Synthesis of 4-Methyl-3,5-diiodobenzaldehyde[17]. Powdered I_2 (3.04 g, 12 mmol) and $NaIO_4$ (0.86 g, 4 mmol) were added slowly to stirred 98% H_2SO_4 (50 mL). Stirring was continued for 30 min at room temperature to give a dark brown iodinating solution. *p*-Tolualdehyde (1.5 g, 14 mmol) was added in one portion to the iodinating solution, and the resulting solution was stirred for 5 h at room temperature. Then the reaction mixture was slowly poured into stirred ice water. The crude solid products were collected by filtration, washed with water until the filtrates were neutral, vacuum-dried in the dark to get light brown powder, and recrystallized from ethyl acetate to give light yellow solid. Yield: 2.13g, 40.9%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 9.823 (s, 1H), 8.306 (d, 2H), 2.842 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 188.63, 149.91, 140.42, 136.62, 99.54, 35.54.

Synthesis of Monomer 1a. To a solution of 4-methyl-3,5-diiodobenzaldehyde (1.5g, 4.2 mmol) and 2,4-dimethyl-1*H*-pyrrole (1g, 10.5 mmol) in dry CH_2Cl_2 (120 mL) was added a solution of 110 μL trifluoroacetic acid in dry CH_2Cl_2 (5 mL) slowly at room temperature. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.95 g, 4.2 mmol) was added after 3 h stirring under ice bath cooling and stirred for 10 min. The solution was stirred for an additional 1 h at room temperature. NEt_3 (10 mL, 72 mmol) was added, followed by slow addition of $BF_3 \cdot Et_2O$ (12 mL, 81 mmol). The reaction mixture was washed after 10 h of stirring at room temperature with saturated aqueous Na_2CO_3 solution (2 × 100 mL), dried over Na_2SO_4, and concentrated on a rotary evaporator. The brown, oily residue was purified by column chromatography on silica with hexane/CH_2Cl_2 = 3:1. The product fraction with greenish fluorescence was dried to yield a orange solid. Yield: 0.48 g, 19.5%. FTIR (KBr, cm^{-1}): 3417.2, 3099.7, 2975.2, 2955.9, 2925.2, 2857.3, 1542.7, 1514.7, 1470.9, 1412.1, 1384.1, 1359.1, 1309.4, 1256.4, 1194.2, 1154.1, 1122.8, 1110.3, 1076.1, 976.7, 908.5, 865.6, 838.3, 816.8, 807.1, 765.9, 756.6, 702.1, 688.5, 667.1, 612.5, 583.8, 562.6. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.831 (s, 2H), 6.042 (s, 2H), 2.874 (s, 3H), 2.581 (s, 6H), 1.544 (s, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 156.25, 144.12, 142.83, 138.94, 135.89, 131.11, 121.67, 99.09, 34.93, 15.14, 14.61. HRMS (ESI): (M^+, $C_{20}H_{19}BF_2I_2N_2$) calcd 590.9777; found 590.9787.

Synthesis of BODIPY Copolymer 1b. In a glovebox under nitrogen atmosphere, a dry three-neck 50 mL round-bottom flask with stir bar was charged with 205.8 mg (0.75 mmol) of bis(1,5-cyclooctadiene) nickel(0), 80.6 mg (0.75 mmol) of cyclooctadiene, and 116.7 mg (0.75 mmol) of bypyridine in 7.0 mL of a 1:1 mixture of toluene and dimethylformamide (DMF). A dark purple color then developed. The solution was heated to 60 °C. In the glovebox, a dry 20 mL flask was charged with 15.9 mg (0.027 mmol) of BODIPY monomer 1a, 149.7 mg (0.273 mmol) of 9,9-dioctyl-2,7-dibromofluorene in 4.0 mL of a 1:1 mixture of toluene and DMF, then they were added dropwise into the above catalyst mixture. The flask containing this solution was covered with foil to protect it from light, and the reaction mixture was refluxed for 4 days. Four drops of iodobenzene was added to end-cap the polymer chain, and the reaction was stirred for an additional 6 h at 60 °C. The product was precipitated in 30 mL of a 1:1 mixture of methanol and concentrated hydrochloric acid. The polymer was dissolved in dichloromethane and washed with aqueous 15 wt % of sodium thiosulfate solution (3 × 30 mL) followed by washing with Milli-Q water and drying over $MgSO_4$ for the removal of residual iodine from polymer. The concentrated polymer solution in dichloromethane was poured into 100 mL of MeOH and filtered. The precipitate was stirred in 50 mL of acetone at room temperature for 24 h and filtered. Polymer was obtained as a green solid. Yield: 75 mg, 64.5%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.90−7.75 (m), 7.53 (m), 7.42−7.43 (m, 6H), 6.08 (m, 2H), 2.64 (s, 6H), 2.18 (s, 4H), 1.63 (s, 6H), 1.21 (s, 24H), 0.88 (s, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 151.87, 140.57, 140.07, 126.21,121,53, 120.02, 55.40, 40.46, 31.86, 30.1, 29.78, 29.3, 23.99, 22.66, 14.15. GPC M_n = 57512, M_w = 90491, PDI = 1.573.

Synthesis of BODIPY Copolymer 2b. BODIPY copolymers were synthesized by palladium-catalyzed Suzuki coupling reaction from 9,9-dioctylfluorene, benzo[*c*]-1,2,5-thiadiazole, and BODIPY monomer 2a. 4,7-Dibromobenzo[*c*]-1,2,5-thiadiazole (56.4 mg, 0.192 mmol), 9,9-dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester (111.68 mg, 0.20 mmol), BODIPY monomer 2a (5.03 mg, 0.008 mmol), 2 drops of Aliquat 336, 10 mL of 2 M Na_2CO_3 aqueous solution, and 15 mL of toluene were placed in a 50 mL round-bottom flask. The flask was evacuated and refilled with N_2 four times by using the freeze/thaw method, and $Pd(PPh_3)_4$ (10 mg, 0.0086 mmol) was added. The flask was further degassed four times, then reaction was heated to 80 °C and stirred under N_2. After 70 h, 0.2 mL of bromobenzene and 15 mg of phenylboronic acid were added to end-cap the polymer chain and, the reaction was stirred for an additional 2 h at 80 °C. The whole mixture was poured into 200 mL of MeOH, filtered, and washed with 0.2 M of HCl. The dried polymer was stirred in 50 mL of acetone at room temperature for 24 h. Polymer was obtained as a dark red solid. Yield: 112 mg, 73.2%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.15−8.06 (m, 2 H), 8.03−8.00 (m), 7.85−7.84 (m), 7.78−7.75 (m, 6H), 7.45−7.41 (m, 5H), 7.07 (m, 2H), 6.93 (m,4H), 6.87−6.89 (m, 4H), 3.95 (s,4H), 2.71 (s, 6H), 2.40 (s, 3H), 2.19 (s, 10H), 1.51 (m, 6 H), 1.20 (s, 12 H), 0.85 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 155.77, 155.67, 154.42, 153.78, 153.72, 151.83, 151.78, 150.6, 141.36, 141.0, 140.95, 140.9, 136.53, 136.34, 135.11, 133.88, 133.82, 133.67, 133.4, 129.73, 128.38, 128.22, 128.03, 127.79, 127.55, 124.08, 123.78, 120.94, 120.34, 120.12, 119.82, 118.9, 114.19, 110.27, 55.5, 55.35, 55.24,40.51, 40.28, 31.9, 31.88, 31.8, 30.18, 30.15, 29.34, 29.31, 24.12, 24.08, 23.91, 22.67, 14.14. GPC M_n = 14480, M_w = 28396, PDI = 1.96.

Synthesis of Monomer 3a. *p*-Tolualdehyde (392 mg, 4.24 mmol), monomer 2a (500 mg, 0.81 mmol), *p*-toluene sulfonic acid (90 mg), 3 mL of acetic acid, and piperidine (3 mL) were dissolved in 100 mL

of benzene refluxed for 12 h by using a Dean−Stark apparatus. The mixture was cooled to room temperature, the solvents were removed under vacuum, and the crude product was purified by column chromatography on silica gel eluted with 1:7 ethyl acetate/hexane. The crude was recrystallized from chloroform/methanol to give the product as a metallic shiny solid. Yield: 320 mg, 48%. FTIR (KBr, cm^{-1}): 3420.3, 3079.6, 3021.1, 2966.1, 2917.6, 2852.1, 1619.9, 1603.5, 1568.9, 1515.5, 1462.0, 1427.1, 1408.6, 1382.9, 1352.6, 1310.9, 1213.4, 1176.2, 1095.0, 1034.5, 1007.4, 960.0, 934.5, 850.5, 801.1, 781.0, 770.0, 707.9, 654.9, 574.8, 510.7. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.157−8.191 (s, 2H), 7.689−7.722 (s, 2H), 7.589−7.605 (s, 4H), 7.258−7.274 (s, 4H), 7.029 (s, 2H), 2.435 (s, 6H), 2.409 (s, 3H), 2.127 (s, 6H), 1.512 (s 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 150.41, 145.17, 139.50, 139.48, 139.35, 139.32, 135.27, 134.05, 132.11, 131.32, 129.57, 129.33, 127.71, 117.98, 82.62, 21.53, 21.31, 19.73, 16.28. HRMS (ESI): (M^+, $C_{38}H_{35}BF_2I_2N_2$) calcd 822.0990; found 822.0983.

Synthesis of BODIPY Copolymer 3b. 4,7-Bis(2-bromo-5-thienyl)-2,1,3-benzothiadiazole (5.5 mg, 0.012 mmol), 4,7-dibromobenzo[*c*]-1,2,5-thiadiazole (52.9 mg, 0.18 mmol), 9,9-dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester (111.68 mg, 0.20 mmol), BODIPY monomer 3a (6.58 mg, 0.008 mmol), monomer 4 (7.3 mg, 0.012 mmol), 2 drops of Aliquat 336, 10 mL of 2 M Na_2CO_3 aqueous solution, and 15 mL of toluene were placed in a 50 mL round-bottom flask. The flask was evacuated and refilled with N_2 four times by using the freeze/thaw method, and $Pd(PPh_3)_4$ (10 mg, 0.0086 mmol) was added. The flask was further degassed four times, then the reaction was heated to 80 °C and stirred under N_2. After 70 h, 0.2 mL of bromobenzene and 15 mg of phenylboronic acid were added to end-cap the polymer chain, and the reaction was stirred for an additional 2 h at 80 °C. The whole mixture was poured into 200 mL of MeOH, filtered, and washed with 0.2 M of HCl. The dried precipitate was stirred in 50 mL of acetone at room temperature for 24 h. Polymer 1b was obtained as a dark brown powder. Yield: 83 mg, 78.3%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.11 (m, 2H), 8.04 (m, 2H), 7.99−7.96 (m, 2H), 7.99−7.75 (m, 2H), 7.56 (m, 2H), 7.58, 7.18−7.06 (m, 4H), 7.00 (m, 2H), 2.32 (s, 3H), 2.15 (s, 6H), 1.57 (s, 6H), 1.17 (s, 24H), 0.82 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 154.62, 152.0, 141.12, 136.73, 133.85, 128.56, 128.25, 124.28, 120.28, 55.67, 53.64, 40.44, 32.07, 30.35, 29.95, 29.52, 29.49, 24.29, 22.85, 14.32. GPC M_n = 11330, M_w = 29933, PDI = 2.64.

Synthesis of Monomer 4[16]. A mixture of 2,7-dibromofluorene (15 mmol, 4.86 g), *tert*-butyl 3-bromopropanoate (33 mmol, 6.86 g), sodium hydroxide solution (40%, 35 mL), Bu_4NBr (1.5 mmol, 0.48 g), and toluene (70 mL) was stirred at 85 °C overnight. The organic phase was separated, washed with water, and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography (DCM). The product was obtained as a white solid. Yield: 4.81 g, 83%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.47−7.54 (m, 6H), 2.30 (t, 4H), 1.47 (t, 4H), 1.33 (s, 18H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.71, 150.47, 139.60, 131.56, 126.99, 122.57, 121.93, 80.97, 54.58, 34.92, 30.36, 28.52.

Synthesis of BODIPY Copolymer 3c. 4,7-Bis(2-bromo-5-thienyl)-2,1,3-benzothiadiazole (5.5 mg, 0.012 mmol), 4,7-dibromobenzo[*c*]-1,2,5-thiadiazole (49.4 mg, 0.168 mmol), 9,9-dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester (111.68 mg, 0.20 mmol), BODIPY monomer 3a (6.58 mg, 0.008 mmol), monomer 4 (7.3 mg, 0.012 mmol), 2 drops of Aliquat 336, 10 mL of 2 M Na_2CO_3 aqueous solution, and 15 mL of toluene were placed in a 50 mL round-bottom flask. The flask was evacuated and refilled with N_2 four times by using the freeze/thaw method, and $Pd(PPh_3)_4$ (10 mg, 0.0086 mmol) was added. The flask was further degassed four times, then reaction was heated to 80 °C and stirred under N_2. After 70 h, 0.2 mL of bromobenzene and 15 mg of phenylboronic acid were added to end-cap the polymer chain, and the reaction was stirred for an additional 2 h at 80 °C. The whole mixture was poured into 300 mL of MeOH, filtered, and washed with 0.2 M of HCl. The dried precipitate was stirred in 50 mL of acetone at room temperature for 24 h. Polymer 1b was obtained as a dark brown powder. Deprotection of the *tert*-butyl esters was then followed by adding 1 mL of trifluoroacetic acid into a solution of polymer in DCM (40 mL) and stirred overnight. The organic layer was washed with water (150 mL × 5) and concentrated to 10 mL and precipitated in methanol (100 mL). The final powder was collected by filtration, washed with acetone, and dried in a vacuum oven to obtain a dark brown solid. Yield: 70 mg, 62.1%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.23 (m, 2H), 8.08−8.14 (m, 2H), 8.02−7.98 (m, 2H), 7.85−7.83 (m, 2H), 7.78 (m, 2H), 7.58 (m, 4H), 7.44−7.38 (m, 4H), 7.21 (m, 4H), 7.08 (m, 2H), 6.97 (m, 2H), 2.38 (s, 3H), 2.33 (s, 6H), 1.48 (s, 6H), 1.20 (s, 24 H), 0.85 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 154.42, 151.82, 140.95, 136.53, 133.66, 128.37, 128.03, 124.08, 120.11, 55.49, 55.26, 40.28, 31.88, 30.17, 30.12, 29.33, 29.30, 24.11, 22.66, 14.11. GPC M_n = 12606, M_w = 26054, PDI = 2.067.

Preparation of Pdots and Bioconjugation. A polymer solution of polymers 1b, 2b, and 3b in THF (4 mL, 50 ppm) was injected into water (10 mL) under ultrasonication. THF was evaporated by N_2 flow at 70 °C, and the solution was concentrated to 4−5 mL, followed by filtration through a 0.2 μm filter.

Bioconjugation. Bionconjugation was performed by utilizing the EDC-catalyzed reaction between carboxyl groups on Pdots' surface and amine groups on 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 functionalized Pdot solution (50 mg/mL in Milli-Q water), resulting in a Pdot solution in 20 mM HEPES buffer with a pH of 7.3. Then, 240 μL of streptavidin (purchased from Invitrogen, Eugene, OR, USA) was added to the solution and mixed well on a vortex. Eighty microliters of freshly prepared EDC solution (10 mg/mL in Milli-Q water) was added to the solution, and the above mixture was left on a rotary shaker. After 4 h at room temperature, Triton-X 100 (0.25% (w/v), 80 μL) and BSA (2% (w/v), 80 μL) were added. The mixture was then 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.

Single-Particle Brightness Measurement. For the measurement of single-particle fluorescence brightness, fluorescent samples were diluted in Milli-Q water, dried on cleaned glass coverslips (previously functionalized with (3-aminopropyl)trimethoxysilane (APTMS)), and imaged on a customized wide-field epifluorescence microscope described as follows. The 488 nm laser beam from a sapphire laser (Coherent, Santa Clara, CA, USA) or 405 nm laser beam from a diode laser (World Star Technologies, Toronto, Canada) was directed into an inverted microscope (Nikon TE2000U, Melville, NY, USA) using home-built steering optics. Laser excitation power was measured at the nose piece before the objective. The objective used for illumination and light collection was a Nikon CFI Plan Fluor 100XS oil (with iris) objective with 100× magnification and 0.5−1.3 NA (Nikon, Melville, NY, USA). Fluorescence signal was filtered by a 500 nm long-pass filter (HQ500LP; Chroma, Rockingham, VT, USA) and imaged onto an EMCCD camera (Photometrics Cascade: 512B, Tucson, AZ, USA). Fluorescence intensity emitted per frame for a given particle was estimated by integrating the CCD signal over the fluorescent spot.

Cell Culture. The breast cancer cell line MCF-7 was 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) supplemented with 10% fetal bovine serum (FBS), 50 U/mL penicillin, and 50 μg/mL streptomycin. The cells were precultured 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 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 Measurement. For specific cell labeling with the narrow emissive Pdot-streptavidin (Pdot-SA), a million cells were blocked with BlockAid blocking buffer (Invitrogen, Eugene, OR, USA) and then were 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-SA 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, no biotinylated primary anti-EpCAM antibody was added. Flow cytometry measurements were performed on fresh samples with 106 cells/0.5 mL, prepared following the procedure described previously. Flow cytometers BD FACScan was used for BODIPY 600 and BODIPY 690, and FACS Canto II (BD Bioscience, San Jose, CA, USA) was used for BODIPY 520 Pdots and Qdot 525, respectively. Excitation source of BD FACScan is a 488 nm laser and that of FACS Canto II are 405 and 488 nm lasers. Corresponding detection channels for fluorescence emission were filtered by a 585/42 band-pass (BD FACScan) and by a 502 long-pass followed by a 510/50 band-pass (FACS Canto II). Scattered light and fluorescence emission were detected by PMT arrays. Representative populations of cells were chosen by selection of appropriate gates. Detection of cell scattered and fluorescent light was continued until at least 104 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 narrow emissive Pdot-SA 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-SA for 30 min each, followed by two washing steps after each incubation. For the control, no biotinylated primary anti-EpCAM antibody was added. The Pdot-tagged cells were then counterstained with Hoechst 34580 and imaged immediately on a fluorescence confocal microscope (Zeiss LSM 510). The BODIPY 520 labeled MCF-7 cells were excited by the 405 nm diode laser, while the BODIPY 600 labeled MCF-7 cells and the BODIPY 690 labeled MCF-7 cells were excited by the 488 nm argon laser. A Plan-Apochromat 63×/1.40 oil DIC objective lens was utilized for imaging.

Conflict of Interest: The authors declare the following competing financial interest(s): D.T.C. has financial interest in Lamprogen, which has licensed the described technology from the University of Washington.

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Supporting Information Available: Synthesis scheme of BODIPY monomers and polymers, DLS data, and spectroscopic properties of BODIPY monomers and Pdots. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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