Published in final edited form as:

ACS Nano. 2013 January 22; 7(1): 376–384. doi:10.1021/nn304376z.

Multicolor Fluorescent Semiconducting Polymer Dots with Narrow Emissions and High Brightness

Yu Rong[†], Changfeng Wu[§], Jiangbo Yu[†], Xuanjun Zhang[‡], Fangmao Ye[†], Maxwell Zeigler[†], Maria Elena Gallina[†], I-Che Wu[†], Yong Zhang[†], Yang-Hsiang Chan[†], Wei Sun[†], Kajsa Uvdal[‡], and Daniel T. Chiu^{†,*}

[†]Department of Chemistry, University of Washington, Seattle, Washington 98195, United States

[§]State Key Laboratory on Integrated Optoelectronics, College of Electronic Science and Engineering, Jilin University, Changchun 130012, China

[‡]Division of Molecular Surface Physics & Nanoscience, Department of Physics, Chemistry, and Biology, Linköping University, Linköping 58183, Sweden

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 intra-particle energy transfer led to highly bright, narrow emissions. The emission full-width at half maximum (FWHM) of the resulting Pdots varies from 40 nm to 55 nm, which is 1.5–2 times narrower than those of conventional semiconducting polymer dots. BODIPY520 Pdots was about an order of magnitude brighter than commercial Qdot 525 under identical laser excitation conditions. Fluorescence imaging and flow cytometry experiments indicate 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

Corresponding Author: Prof. Daniel T. Chiu, Department of Chemistry, University of Washington, Seattle, Washington 98195, United States, Fax: (+1) 206-685-8665, chiu@chem.washington.edu.

Present address

Yang-Hsiang Chan, Currently at National Sun Yatsen University at Taiwan.

Supporting Information. 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>.

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.

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 nanoparticles 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 co-precipitation 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, 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 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-dipyromethene (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 intra-particle 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 semiconducting polymer in nanoparticle form have not been reported in the literature so far.

First, we synthesized a BODIPY-based monomer (Monomer a, Scheme 2) that exhibit narrow emission with a FWHM of 36 nm in its molecular form (Fig. S1, Table S1). 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. Fig 1a shows the fluorescence spectra of the BODIPY-fluorene copolymers in tetrahydrofuran (THF) solution. While the BODIPY Monomer **1a** 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 the copolymers in THF exhibit narrow emissions with FWHMs in the range of 45–49 nm, indicating 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 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 BODIPY-fluorene polymer shown in Scheme 2, BODIPY monomers were incorporated into 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 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-position *via* Yamamoto polymerization (Scheme S2). Fig S2A and S2B (green curves) show 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 dominant absorption feature only in the deep blue region (for 405nm laser excitation), which is a drawback for many biological applications that require 488nm excitation. To overcome this issue, we incorporated benzothiadiazole (BT) donor into the backbone of the BODIPY-fluorene copolymer to extend the 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) have very good overlap with the absorption of the BODIPY chromophore (546 nm), thus increasing the intra-particle energy transfer efficiency to completely quench the donor fluorescence. Based on 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 (Fig. S2A and S2B, 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. Based on this strategy, we synthesized another monomer (Monomer 3a) and used it to obtain 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 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 (Fig S2B).

Previously, we have shown successful surface functionalization of Pdots by co-precipitating 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 co-precipitation strategy, we successfully prepared BODIPY520 Pdots (polymer 1b) and BODIPY600 Pdots (Polymer 2b), which were blended with PS-PEG-COOH amphiphilic polymers, respectively. The BODIPY520 Pdots and BODIPY600 Pdots showed identical emission spectra as compared with the Polymer 1b and 2b Pdots without PS-PEG-COOH (Fig 1c–d, Fig 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). BODIPY690 Pdots (Polymer 3c) show comparable emission spectra as compared with the Polymer 3b Pdots without side-chain functionalization (Fig 1c–d, Fig 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 previously⁹— photobleaching for 1.5 hours using the 488 nm excitation of Xenon lamp did not result in observable decrease in fluorescence intensity (Fig. S5 b). Qdot 525 and BODIPY 520 Pdots exhibit single exponential photobleaching decays under identical conditions (405 nm excitation of Xenon lamp) (Fig. S5 a), 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, 18 nm, as characterized by TEM and DLS (Fig. 2), while commercial available Qdots 525 (Invitrogen, Eugene, OR, USA) was dispersed in MilliQ 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 BODIPY600 Pdots.

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 firstly measured, fluorescence signal was filtered by a 500-nm long pass filter. The measured average per-particle brightness of BODIPY 520 Pdots (Fig. 3b) is ~9 times that of Qdots 525 (Fig. 3a). With the same particle size, the measured average per-particle brightness of BODIPY 520 Pdots (Fig. 3d) is 2 times that of PFBT Pdots (Fig. 3c),

which is 3 times that of BODIPY 590 Pdots (Fig. 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 7.6 times of that of Qdots 525, while the calculated brightness of BODIPY 520 Pdots is 1.7 times of 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 the above results indicate that the single-particle brightness of the new Pdots reported in this work have the same order of magnitude brightness as the PFBT dots.⁴

Specific Labeling of Cellular Targets with Pdots

To apply these multi-color narrow emissive Pdots for fluorescence imaging, bioconjugation was successfully performed using these Pdots *via* EDC-catalyzed coupling. The Pdot-streptavidin bioconjugates (Pdot-SA) were 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. Fig 4a, 4b and 4c show the flow cytometry results, which proved that all three Pdot-SA probes and Qdots 525 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 BODIPY520 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. Fig 4a shows BODIPY520 Pdots was 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 (Fig 5 and Fig S4). From Fig 5a, 5b and 5c, 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 (Fig S4), which showed there was highly specific cellular labeling with no non-specific binding.

Conclusion

We have successfully synthesized multi-color 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 FWHMs of these Pdots varies from 40 nm to 55 nm, which are 1.5~2 times narrower than those of conventional Pdots. BODIPY520 Pdots was 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—¹H (500 MHz), ¹³C (125 MHz) NMR spectra were recorded on Bruker AV500 spectrometers. ¹H NMR and ¹³C NMR spectra used tetramethylsilane (TMS) as an internal standard in CDCl₃. 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 was 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 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 the chemicals were purchased from Sigma-Aldrich and TCI America company. Qdot525 was purchased from life technologies company.

Synthesis of BODIPY monomer a (8-Mesityl-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene).¹¹

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-1H-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 $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (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%. ^1H 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 (125MHz, 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^+ , $\text{C}_{22}\text{H}_{25}\text{BF}_2\text{N}_2$) 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 4.57 g (18 mmol) of powdered I_2 was added and allowed to dissolve. 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 $^\circ\text{C}$ 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. ^1H NMR (500MHz, 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^+ , $\text{C}_{22}\text{H}_{23}\text{BF}_2\text{I}_2\text{N}_2$) calcd 618.0051; found 618.0039.

Synthesis of BODIPY fluorene copolymer series—BODIPY fluorene copolymer series with different BODIPY monomer molar ratio (2%, 5%, 10%, 25%, 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, 10ml of 2M Na_2CO_3 aqueous solution, and 15ml of toluene were placed in a 50ml flask. The flask was evacuated and refilled with N_2 four times by using the freeze/thaw method and $\text{Pd}(\text{PPh}_3)_4$ (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, 0.2ml of bromobenzene and 15mg 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 0.2M of HCl. The precipitate was stirred in 50ml of acetone at room temperature for 24h and dried in vacuum oven to obtain dark pink to dark red solid. Yield: 73–81%. NMR results for PFO-BODIPY10: ^1H 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 (125MHz, 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, 132.17, 130.63, 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 Mn: 23048, Mw: 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 re-crystallized from ethyl acetate to give light yellow solid. Yield: 2.13g, 40.9%. ^1H NMR (CDCl_3 , 500MHz): δ = 9.823 (s, 1H), 8.306 (d, 2H), 2.842 (s, 3H). ^{13}C NMR (CDCl_3 , 125MHz): 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-1H-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 $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (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 an 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. ^1H NMR (CDCl_3 , 500MHz): 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 , 125MHz): 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^+ , $\text{C}_{20}\text{H}_{19}\text{BF}_2\text{I}_2\text{N}_2$) 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 bipyridine 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. 4 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 MgSO₄, 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 green solid. Yield: 75 mg, 64.5 %. ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃, 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 Mn: 57512, Mw: 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 2M Na₂CO₃ aqueous solution, and 15 mL of toluene were placed in a 50 mL round bottom flask. The flask was evacuated and refilled with N₂ four times by using the freeze/thaw method and Pd(PPh₃)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 N₂. 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 0.2M 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%. ¹H NMR (500 MHz, CDCl₃): 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, 6H), 1.20 (s, 12 H), 0.85 (m, 6H). ¹³C NMR (125 MHz, CDCl₃): 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 Mn: 14480, Mw: 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 ethyl acetate/hexane 1:7. 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. ¹H NMR (500 MHz, CDCl₃): 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^+ , $\text{C}_{38}\text{H}_{35}\text{BF}_2\text{I}_2\text{N}_2$) calcd 822.0990; found 822.0983.

Synthesis of BODIPY copolymer 3b—4,7-Bis(2-bromo-5-thienyl)-2,1,3-benzothiadiazole (5.5mg, 0.012mmol), 4,7-Dibromobenzo[c]-1,2,5-thiadiazole (52.9mg, 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 (7.3mg, 0.012mmol), 2 drops of aliquat 336, 10ml of 2M Na_2CO_3 aqueous solution, 15ml of toluene were placed in a 50ml round bottom flask. The flask was evacuated and refilled with N_2 four times by using the freeze/thaw method and $\text{Pd}(\text{PPh}_3)_4$ (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, 0.2ml of bromobenzene and 15mg 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 0.2M of HCl. The dried precipitate was stirred in 50ml of acetone at room temperature for 24h. Polymer 1b was obtained as a dark brown powder. Yield: 83 mg, 78.3 %. ^1H NMR (500MHz, 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 Mn: 11330, Mw: 29933, PDI: 2.64.

Synthesis of monomer 4¹⁶—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%. ^1H 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): 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 (5.5mg, 0.012mmol), 4,7-Dibromobenzo[c]-1,2,5-thiadiazole (49.4mg, 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 (7.3mg, 0.012mmol), 2 drops of aliquat 336, 10ml of 2M Na_2CO_3 aqueous solution, and 15ml of toluene were placed in a 50ml round bottom flask. The flask was evacuated and refilled with N_2 four times by using the freeze/thaw method and $\text{Pd}(\text{PPh}_3)_4$ (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, 0.2ml of bromobenzene and 15mg 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 0.2M of HCl. The dried precipitate was stirred in 50ml of acetone at room temperature for 24hr. 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 (150ml $\times$ 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 vacuum oven to obtain a dark brown solid. Yield: 70 mg, 62.1%. ^1H NMR (500MHz, 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). ¹³C NMR (125 MHz, CDCl₃): 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 Mn: 12606, Mw: 26054, PDI: 2.067.

Preparation of Pdots and bioconjugation

A polymer solution of polymer 1b, 2b, 3b in THF (4 mL, 50 ppm) was injected into water (10 mL) under ultrasonication, respectively. THF was evaporated by N₂ flow at 70°C and the solution was concentrated to 4–5 mL, followed by filtration through a 0.2 micron 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 MilliQ 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. 80 μL of freshly-prepared EDC solution (10 mg/mL in MilliQ water) was added to the solution, and the above mixture was left on a rotary shaker. After 4 hours 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 one hour. 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 nosepiece 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 N.A (Nikon, Melville, NY, USA). Fluorescence signal was filtered by a 500nm 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% CO₂ 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 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.

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 $\mu\text{g/mL}$ (based on Pdots) Pdot-SA for 30 minutes each, followed by two washing steps using labeling buffer. Finally, the specifically labeled cells were fixed in 0.6 mL 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, 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 488nm laser and that of FACS Canto II are 405nm and 488nm 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 minutes 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 BODIPY520 labeled MCF-7 cells were excited by 405 nm diode laser, while the BODIPY600 labeled MCF-7 cells and the BODIPY690 labeled MCF-7 cells were excited by 488 nm Argon laser. A Plan-Apochromat 63 $\times$ /1.40 Oil DIC objective lens was utilized for imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Keck Imaging Center and the Center of Nanotechnology at the University of Washington for use of their facilities. The work was supported by the National Institutes of Health (CA147831 and NS062725) and the University of Washington.

ABBREVIATIONS

BODIPY	4-difluoro-4-bora-3a, 4a-diaza-s-indacene
Pdots	Polymer dots
FWHM	full-width at half maximum
Qdots	quantum dots
PFBT	poly[9,9-dioctylfluorenyl-2,7-diyl-co-1,4-benzo-{2,1'-3}-thiadiazole)]
Pdot-SA	Pdot-streptavidin bioconjugates

REFERENCE

1. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP. Semiconductor Nanocrystals as Fluorescent Biological Labels. *Science*. 1998; 281:2013–2016. [PubMed: 9748157]
2. Chan WCW, Nie S. Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection. *Science*. 1998; 281:2016–2018. [PubMed: 9748158]
3. Wang L, Wang K, Santra S, Zhao X, Hilliard LR, Smith JE, Wu Y, Tan W. Watching Silica Nanoparticles Glow in the Biological World. *Anal. Chem*. 2006; 78:646–654.
4. Wu C, Schneider T, Zeigler M, Yu J, Schiro P, Burnham D, McNeill JD, Chiu DT. Bioconjugation of Ultrabright Semiconducting Polymer Dots for Specific Cellular Targeting. *J. Am Chem. Soc*. 2010; 132:15410–15417. [PubMed: 20929226]
5. Pu K, Li K, Shi J, Liu B. Fluorescent Single-Molecular Core–Shell Nanospheres of Hyperbranched Conjugated Polyelectrolyte for Live-Cell Imaging. *Chem. Mater*. 2009; 21:3816–3822.
6. Fernando LP, Kandel PK, Yu J, McNeill J, Ackroyd PC, Christensen KA. Mechanism of Cellular Uptake of Highly Fluorescent Conjugated Polymer Nanoparticles. *Biomacromolecules*. 2010; 11:2675–2682. [PubMed: 20863132]
7. Wu C, Jin Y, Schneider T, Burnham DR, Smith PB, Chiu DT. Ultrabright and Bioorthogonal Labeling of Cellular Targets Using Semiconducting Polymer Dots and Click Chemistry. *Angew. Chem. Int. Ed*. 2010; 49:9436–9440.
8. Wu C, Hansen S, Hou Q, Yu J, Zeigler M, Jin Y, Burnham D, McNeill J, Olson J, Chiu DT. Design of Highly Emissive Polymer Dot Bioconjugates for *In Vivo* Tumor Targeting. *Angew. Chem. Int. Ed*. 2011; 50:3430–3434.
9. Wu C, Bull B, Szymanski C, Christensen K, McNeill, Multicolor Conjugated Polymer Dots for Biological Fluorescence Imaging. *ACS Nano*. 2008; 2:2415–2423. [PubMed: 19206410]
10. Loudet A, Burgess K. BODIPY Dyes and Their Derivatives: Syntheses and Spectroscopic Properties. *Chem. Rev*. 2007; 107:4891–4932. [PubMed: 17924696]
11. Nepomnyashchii AB, Bröring M, Ahrens J, Bard AJ. Synthesis, Photophysical, Electrochemical, and Electrogenenerated Chemiluminescence Studies. Multiple Sequential Electron Transfers in BODIPY Monomers, Dimers, Trimers, and Polymer. *J. Am Chem. Soc*. 2011; 133:8633–8645. [PubMed: 21563824]
12. Alemdaroglu FE, Alexander SC, Ji D, Prusty DK, Börsch M, Herrmann A. Poly(BODIPY)s: A New Class of Tunable Polymeric Dyes. *Macromolecules*. 2009; 42:6529–6536.
13. Nagai A, Chujo Y. Aromatic Ring-Fused BODIPY-Based Conjugated Polymers Exhibiting Narrow Near-Infrared Emission Bands. *Macromolecules*. 2010; 43:193–200.
14. Thivierge C, Loudet A, Burgess K. Brilliant BODIPYFluorene Copolymers with Dispersed Absorption and Emission Maxima. *Macromolecules*. 2011; 44:4012–4015. [PubMed: 21804648]
15. Ye F, Wu C, Jin Y, Wang M, Chan Y-H, Yu J, Sun W, Hayden S, Chiu DT. A compact and highly fluorescent orange-emitting polymer dot for specific subcellular imaging. *Chem. Comm*. 2012; 48:1778–1780. [PubMed: 22218705]
16. Zhang X, Yu J, Wu C, Jin Y, Rong Y, Ye F, Chiu DT. Importance of having low-density functional groups for generating high-performance semiconducting polymer dots. *ACS Nano*. 2012; 6:5429–5439. [PubMed: 22607220]
17. Kraszkiewicz L, Sosnowski M, Skulski L. Oxidative iodination of deactivated arenes in concentrated sulfuric acid with I₂/NaIO₄ and KI/NaIO₄ iodinating systems. *Synthesis*. 2006; 7:1195–1199.

SYNOPSIS

Novel BODIPY containing semiconducting polymers emitting at different wavelengths were synthesized and used as precursors for preparing the Polymer dots, where intra-particle energy transfer led to highly bright, narrow emissions. BODIPY520 Pdots was about an order of magnitude brighter than commercial Qdot 525 under identical 405 nm laser excitation. Fluorescence imaging and flow cytometry indicate that these new narrow emissive Pdots are promising probes for multiplexed biological detections.

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1.

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

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

Figure 3.

Histograms of the distributions of single-particle brightness of (a) Qdots 525 ($\lambda_{\text{ex}} = 405 \text{ nm}$), (b) BODIPY520 Pdots ($\lambda_{\text{ex}} = 405 \text{ nm}$), (c) PFBT/PS-PEG Pdots ($\lambda_{\text{ex}} = 405 \text{ nm}$), (d) BODIPY 520 Pdots ($\lambda_{\text{ex}} = 405 \text{ nm}$), (e) PFBT/PS-PEG Pdots ($\lambda_{\text{ex}} = 488 \text{ nm}$), (f) BODIPY 600 Pdots ($\lambda_{\text{ex}} = 488 \text{ nm}$), respectively. The red curves were obtained by fitting a lognormal distribution to the histogram, and gave 6,200, 55,000, 23,000, 33,000, 21,000, and 7,000 mean CCD counts for the two histograms, respectively. Insets: single-particle brightness images. (a) and (b), (c) and (d), (e) and (f) were obtained under identical excitation and detection conditions. All scale bars represent $10 \mu\text{m}$.

Figure 4.

Flow-cytometry measurements of the intensity distributions of MCF-7 breast-cancer cells labeled *via* non-specific binding (N: negative control) and positive specific targeting (P: positive control) using Qdots 525 (QN: Qdot negative control; QP: Qdot positive control), BODIPY520 Pdots (520N: BODIPY520 negative control; 520P: BODIPY520 positive control) (a), BODIPY600 Pdots (600N, 600P) (b), and BODIPY690 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 μ m.

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

Scheme 2.

Chemical structures of BODIPY monomers and BODIPY-containing polymers

\$watermark-text

\$watermark-text

\$watermark-text

Table 1
Size, zeta potential, and photophysical properties of BODIPY 520 Pdots and Qdots 525.

Probe	Size ^[a]	ξ ^[b]	Abs(10 ⁻¹³ cm ²) ^[c]	Φ ^[d]	B (CCD counts)
BODIPY520	16	-48.9	2.50 (405nm)	35	55,000 (405nm)
Qdots525	13	-56.4	1.72 (405nm)	13	6,200 (405nm)

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