Supporting Information for

Yellow Fluorescent Semiconducting Polymer Dots with High Brightness, Small Size and Narrow Emission for Biological Applications

Yu Rong[†], Jiangbo Yu[†], Xuanjun Zhang[‡], Wei Sun[†], Fangmao Ye[†], I-Che Wu[†], Yong Zhang[†], Sarah Hayden[†], Yue Zhang[†], Changfeng Wu[§], Daniel T. Chiu*

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

‡ Division of Molecular Surface Physics & Nanosciecne, Department of Physics, Chemistry, and Biology, Linköping University, Linköping 58183, Sweden.

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

KEYWORDS: Polymer dots, fluorescence, semiconducting polymer, bioimaging, narrow emission.

Materials and Synthesis

Instrumentation. ^{1}H (500 MHz), ^{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 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. 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. Qdot565 was purchased from life technologies company.

Synthesis of 4-methyl-3,5-diiodobenzaldehyde. [1] 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%. ^{1}H 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 1. (Scheme S1) 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 BF_3. 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 red solid. Yield: 0.48 g, 19.5%. ^{1}H NMR ($CDCl_3$, 500MHz): δ =7.94 (d, 2 H), 6.40 (s, 2H), 3.07 (t, 4H), 2.83 (t, 3H), 2.54 (t, 4H), 1.86 (m, 4H), 1.76 (m, 4H). ^{13}C NMR ($CDCl_3$, 125MHz): δ =158.3, 144.7, 140.7, 136.4, 135.5, 133.6, 129.9, 126.3, 98.7, 35.3, 24.9, 23.4, 23.0, 22.5. HRMS (ESI): (M^+, $C_{24}H_{23}BF_2I_2N_2$) calcd 643.0090; found 643.0095.

Synthesis of monomer 2,7-dibromo-9,9-bis(3-(tert-butyl hexylcarbamate) fluorene. [2]

A mixture of 2, 7-dibromofluorene (5 mmol, 1.62 g), tert- butyl 6-bromohexyl carbamate (11 mmol, 3.05 g), sodium hydroxide solution (40%, 25 mL), Bu_4NBr (1 mmol, 0.32 g), toluene (40 mL) was stirred at 85℃ 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). ^{1}H-NMR (500 MHz, Acetone-d6): δ= 7.72 (d, 2H), 7.62 (s, 2H), 7.48 (d, 2H), 5.74 (s, 2H), 2.88 (q, 4H), 2.76 (d, 2H), 2.02-2.08 (m, 4H), 1.31 (s, 18H), 1.20-1.27 (m, 4H), 1.00-1.09 (m, 8H), 0.50-0.59 (m, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ= 155.81, 152.20, 138.97, 130.17, 126.00, 121.43, 121.17, 55.50, 40.36, 40.01, 29.83, 29.41, 28.34, 26.32, 23.49.

Synthesis of PF5BODIPY565-4NH2. (Scheme S1) In a glovebox under nitrogen atmosphere, a dry three neck 25 mL round-bottom flask with stir bar was charged with 316 mg (1.15 mmol) of bis(1,5-cyclooctadiene) nickel(0), 128 mg (1.15 mmol) of cyclooctadiene, and 178 mg (1.15 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 70 ℃. In the glovebox, a dry 20 mL flask was charged with 16.1 mg (0.025 mmol) of BODIPY monomer 1, 250 mg (0.455 mmol) of 9,9-Dioctyl-2,7-dibromofluorene and 12.8 mg (0.02 mmol) of 2,7-dibromo-9,9-bis (3-(tert-butyl hexylcarbamate) fluorene 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 3 days. 9 drops of iodobenzene was added to end-cap the polymer chain and the reaction was stirred for an additional 6 h at 70 ºC. The product was precipitated in 50 mL of a mixture of methanol and 10% concentrated hydrochloric acid, filtered and dried. 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. Polymer was obtained as red solid. De-protection of amine group from the polymer was finished as the following procedure, polymer was dissolved in chloroform (50 mL) and TFA (0.8 mL) was added to remove protecting groups and generate amine groups. The mixture was stirred at room temperature overnight (in dark) and then rotary evaporated to remove solvent and TFA. Polymer was dissolved in 10ml of chloroform and then added into methanol (100 mL) to precipitate the final polymer. Yield: 155 mg, 82 %. ^{1}H NMR ($CDCl_3$, 500 MHz): δ=7.86-7.85 (m, 4H), 7.69 (m, 2H), 2.13 (s, 4H), 1.54 (s, 4H), , 1.22-1.15 (s, 24H), 0.83 (s, 6H). GPC Mn: 33419, Mw: 56966, PDI: 1.70.

Synthesis of PF46BT4NH2. (Scheme S2).

Polymer is synthesized by palladium-catalyzed Suzuki coupling reaction from 9,9-dioctylfluorene and 4,7-Dibromobenzo[c]-1,2,5-thiadiazole. 335.05 mg (0.6 mmol) of 9,9-Dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester, 162.27 mg (0.552 mmol) of 4,7-Dibromobenzo[c]-1,2,5-thiadiazole, 30.65 mg (0.048 mmol) of 2,7-dibromo-9,9-bis(3-(tert-butyl hexylcarbamate) fluorene, 3 drops of aliquot 336, 15ml of 2M Na_2CO_3 aqueous solution, 25ml 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 $Pd(PPh_3)_4$ (1.5 mol%) was added. The flask was further degassed four times, then reaction was heated to 110 ºC and stirred under N_2. After 70 h 0.3ml of bromobenzene and 25mg of phenylboronic acid in toluene were added to end-cap the polymer chain and the reaction was stirred for an additional 2 h at 110 ºC respectively. The whole mixture was poured into 100 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 yellow solid. De-protection of amine group from the polymer was finished as the following procedure, polymer was dissolved in chloroform (50 mL) and TFA (0.8 mL) was added to remove protecting groups and generate amine groups. The mixture was stirred at room temperature overnight (in dark) and then washed with 10% NaOH aqueous solution three times. Organic phase was separated, concentrated to ca. 3mL, and then added into methanol (60 mL) to precipitate the final polymer. Yield: 242 mg, 73 %. ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.13 - 8.05 (m, 2 H), 7.99 - 7.70 (m, 6 H), 2.16 (m, 4H), 1.55 (s, 4H), 1.17 (m, 40 H), 0.83-0.80 (m, 6H). GPC: Mn: 18900, Mw: 48025, PDI: 2.54.

Crosslinking reaction of polymers. 1.4 ml of 1000 ppm PF5BODIPY 565-4NH2 THF solution, 2.6 ml of 1000 ppm PF46BT-4NH2 THF solution and 0.25 ml of 4000 ppm PSMA-8000 THF solution were added into a 25 ml flask, and then was stirred at room temperature at the dark state for 48 hrs. The polymer solution was ready to use for the preparation of Pdots.

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),

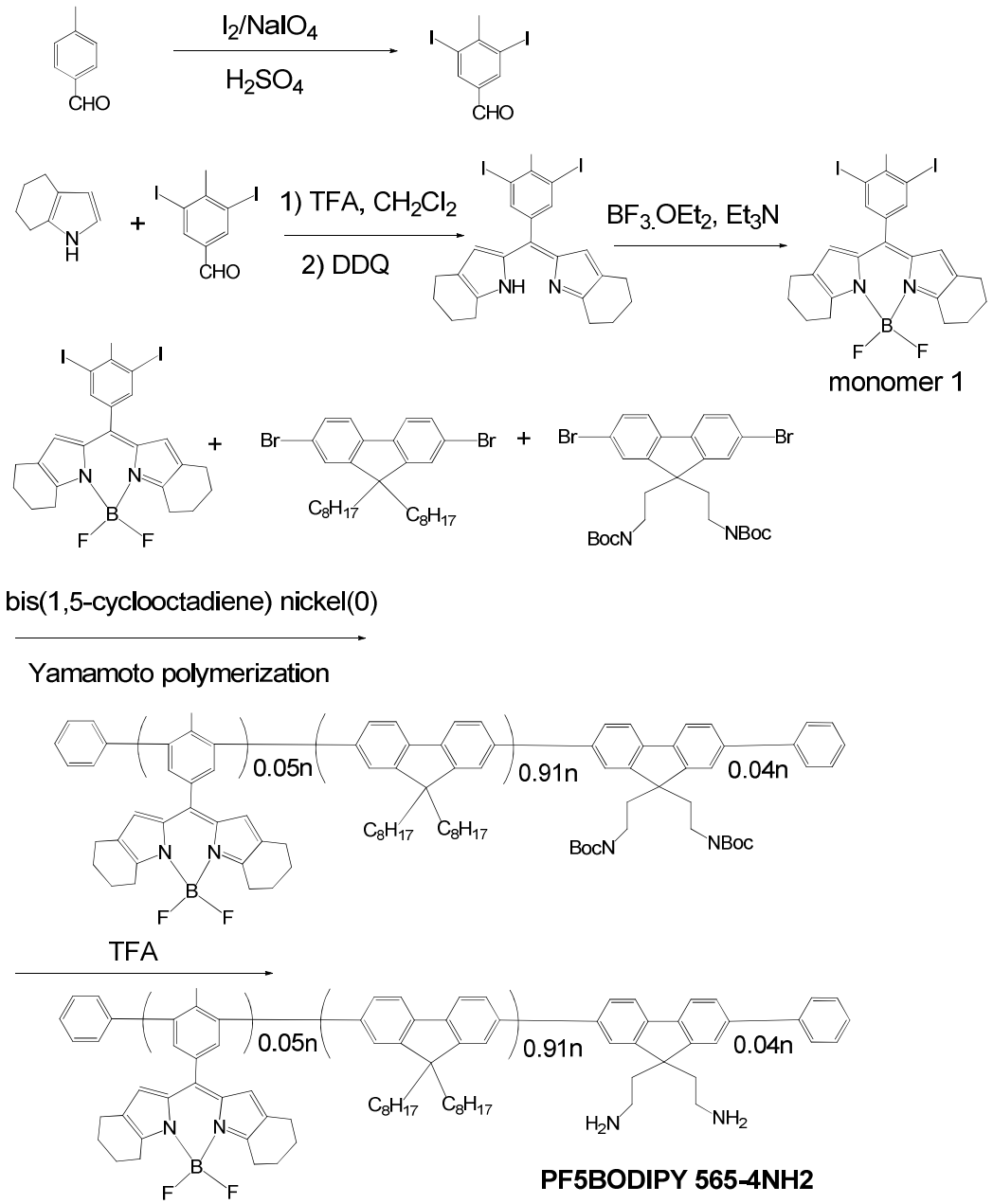
80 μL) were added. The mixture was then left on rotary shaker for one hour. Finally, the resulting Pdot bioconjugates were separated from free biomolecules by gel filtration using Sephacryl HR-300 gel media.

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 ℃, 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 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.

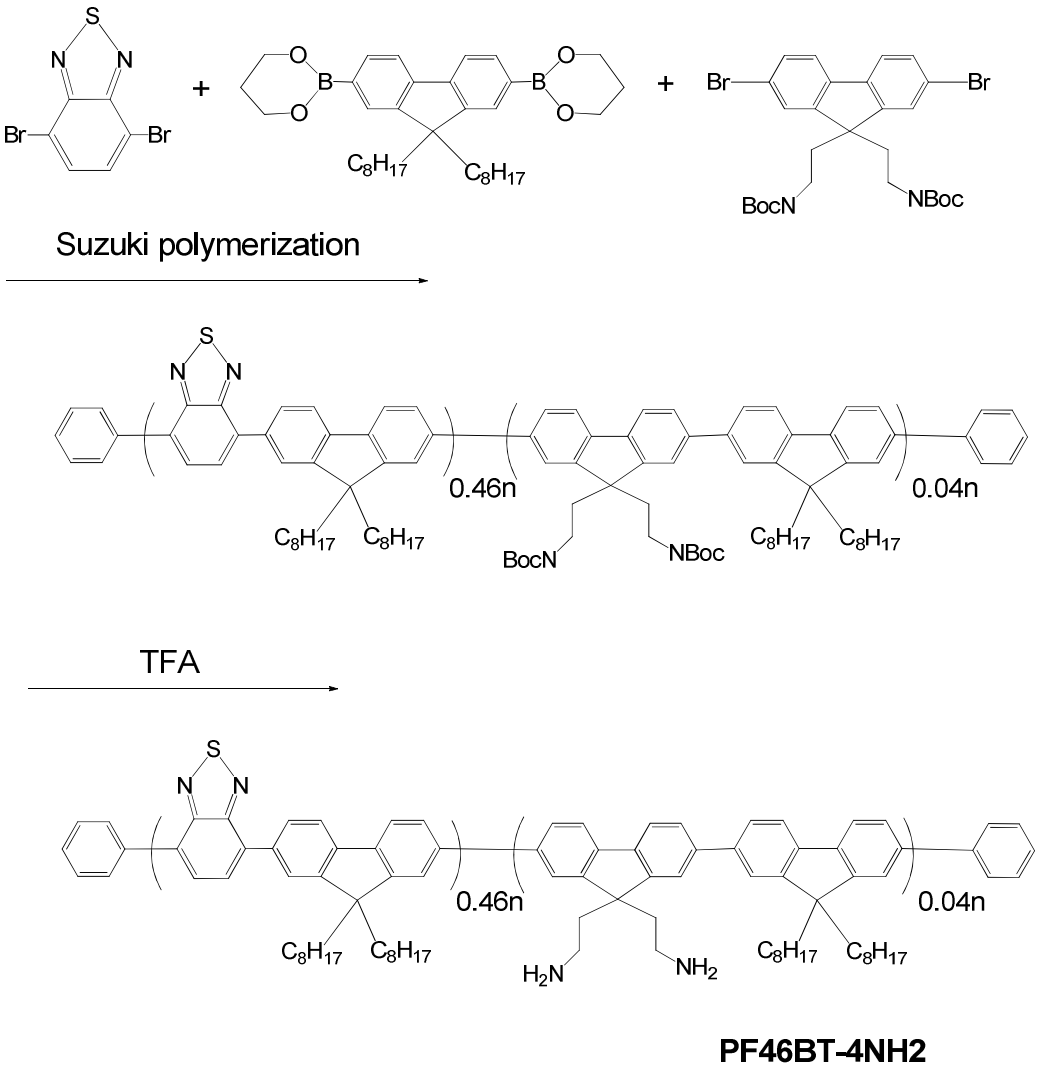
Flow cytometry measurement. For specific cell labeling with the narrow emissive Pdot-streptavidin (Pdot-SA), a million cells were blocked with BlockAid blocking buffer (Invitrogen, Eugene, OR, USA) and then were incubated sequentially with biotinylated primary anti-EpCAM antibody (used to label the cell-surface EpCAM receptors on MCF-7 cells) and 10 μg/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 CL-BODIPY 565 and Qdots 565. Excitation source of BD FACScan is a 488nm laser. Corresponding detection channels for fluorescence

emission were filtered by a 585/42 band-pass (BD FACScan). 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 CL-BODIPY565 labeled MCF-7 cells was excited by 488 nm Argon laser. A Plan-Apochromat 63×/1.40 Oil DIC objective lens was utilized for imaging..



Scheme S1. Synthesis of BODIPY monomer 1 and PF5BODIPY 565-4NH2 copolymer.



Scheme S2. Synthesis of PF46BT-4NH2 copolymer.

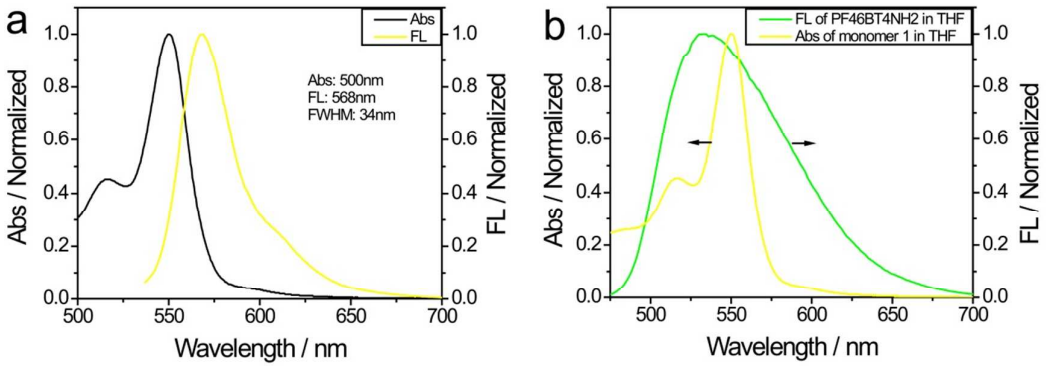


Figure S1. a. absorption and fluorescence spectra of BODIPY monomer1 in THF solution; b. overlap of fluorescence spectra of PF46BT-4NH2 and absorption spectra of BODIPY monomer 1.

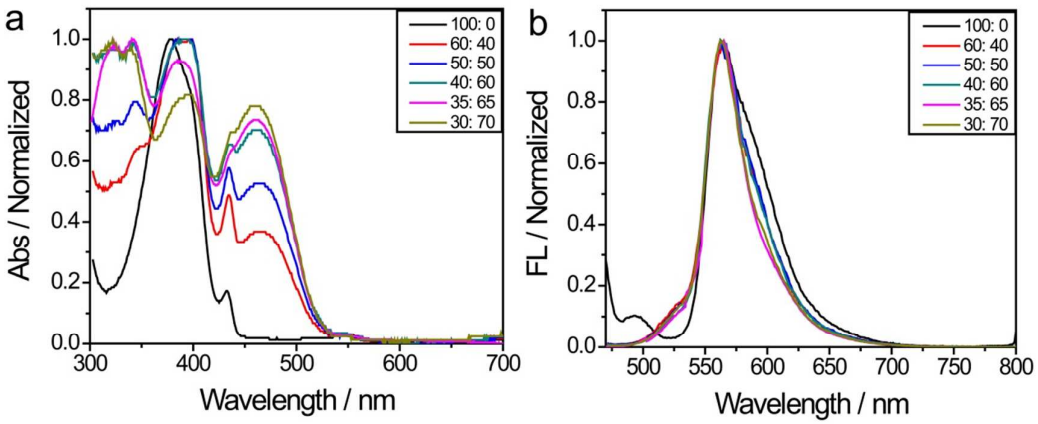


Figure S2. Concentration-dependent absorption spectra (a) and fluorescence spectra (b) of polymer-blend dots (PBdots) containing a narrow emissive PF5BODIPY 565-4NH2 acceptor polymer and PF46BT-4NH2 donor polymer.

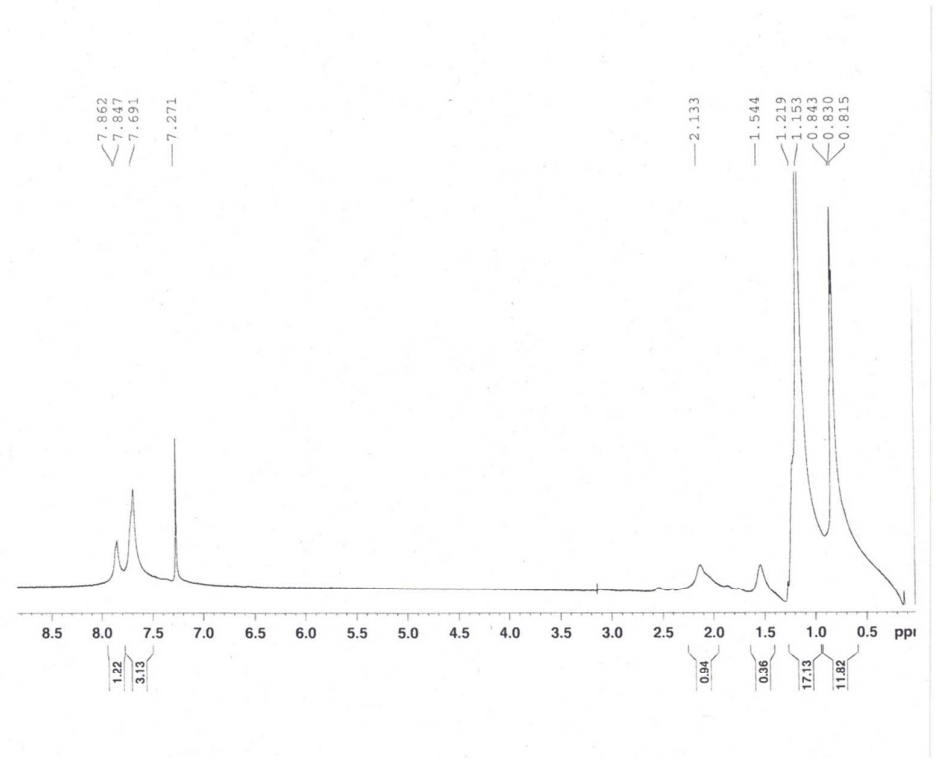


Figure S3. ^{1}H-NMR of PF5BODIPY565-4NH2.

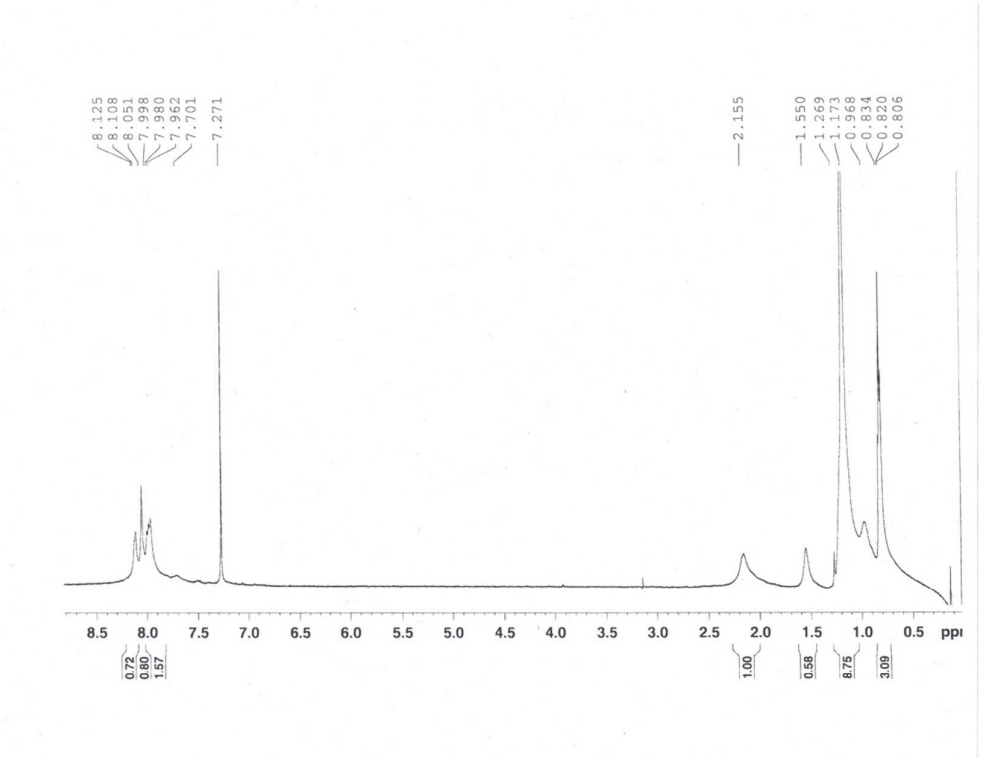


Figure S4. ^{1}H-NMR of PF46BT-4NH2.

---

[1] 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.

[2] Jiangbo Yu , Changfeng Wu , Xuanjun Zhang , Fangmao Ye , Maria Elena Gallina , Yu Rong , I-Che Wu , Wei Sun , Yang-Hsiang Chan , and Daniel T. Chiu, Stable Functionalization of Small Semiconducting PolymerDots via Covalent Cross-Linking and Their Application for Specific Cellular Imaging, Adv. Mater. 2012, 24, 3498–3504