SUPPORTING INFORMATION

BODIPY-Based Near-Infrared Semiconducting Polymer Dot for Selective Yellow Laser Excited Cell Imaging

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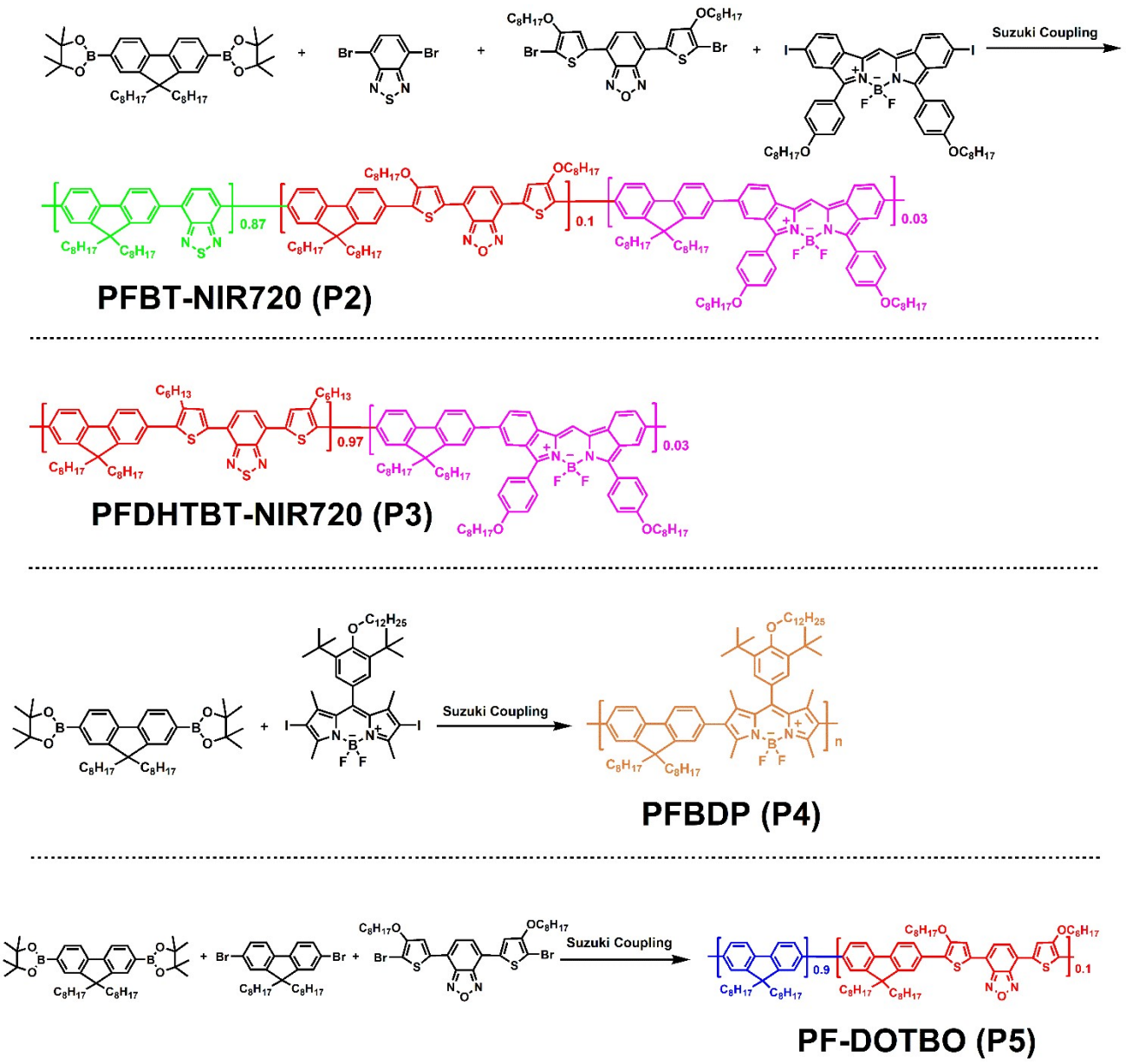
1. Materials and Synthesis



Scheme S1. The chemical structure and synthetic routes of P2-P5 polymers.

Materials

The amphiphilic polymer PS-PEG-COOH was purchased from Polymer Source Inc. (Quebec, Canada). The molecular weights of PS-PEG-COOH, the PS backbone, and the PEG-COOH side chain were 36500, 6500 and 4600 Da, respectively. 3,5-di-tertbutyl-4-hydroxybenzaldehyde (1) was obtained from TCI America (Portland, OR, USA) and was used directly. 9,9-dioctylfluorene-2,7-diboronic acid pinacol ester monomer (5) was purchased from Sigma-Aldrich and recrystallized twice in ethanol for

polymerizations. 4,7-dibromobenzothiadiazole monomer was purchased from Sigma-Aldrich and recrystallized twice in chloroform for polymerizations. 4,7-bis(5-bromo-4-octyloxy-2-thienyl)-2,1,3-benzooxadiazole monomer was synthesized from 3-methoxythiophene as described previously.[1] NIR-720 monomer (7) and PFDHTBT-NIR720 (P3) have been described in our previous work.[2]

Synthesis of compound 2

3,5-di-tertbutyl-4-hydroxybenzaldehyde (50 mmol, 11.7 g), 1-bromododecane (100 mmol, 24.9 g), and K_2CO_3 (200 mmol, 27.0 g) were dissolved in degassed acetonitrile (250 mL) and stirred at 80 °C for 24 h. The reaction was cooled and the mixture was filtered. The filtrate was concentrated then purified by silica gel column chromatography (hexane:CH_2Cl_2, 4:1) to produce 3,5-di-tertbutyl-4-(dodecyloxy)benzaldehyde (2) as a white solid product (12.3 g, yield: 61.1%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 9.96 (s, 1H), 7.84 (s, 2H), 3.77 (t, J = 7.5 Hz, 2H), 1.96 (m, 2H), 1.51 (s, 18H), 1.41-1.33 (m, 22H), 0.94 (t, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 192.07, 164.11, 145.05, 131.21, 128.68, 35.99, 31.84, 29.56, 25.69, 22.68, 14.10; ESI-MS (m/z): 402 (M^+).

Synthesis of compound 3

Compound 2 (7.3 mmol, 2.52 g), 2,4-dimethylpyrrole (17.4 mmol, 1.66 g), and trifluoroacetic acid (0.3 mL) were dissolved in degassed dichloromethane (500 mL), and the mixture was stirred for 3 h. 2,3-dichloro-5,6-dicyano-l,4-benzoquinone (DDQ) (7.3 mmol, 1.67 g) was added over four portions and the mixture was stirred for 1 h. After cooling the mixture to 4 °C, triethylamine (Et_3N) (15 mL) was injected, then $BF_3{\cdot}Et_2O$ (20 mL) was added dropwise. The mixture was stirred overnight and the resulting solution was washed twice with saturated aqueous K_2CO_3, dried over Na_2SO_4, and filtered. The filtrate was concentrated and purified by silica gel column chromatography (hexane:CH_2Cl_2, 1:2) to produce BODIPY compound 3 as an orange solid (1.91 g, yield: 42.0%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.12 (s, 2H), 3.72 (t, J = 7.5 Hz, 2H), 2.70 (s, 6H), 1.95 (m, 2H), 1.46 (s, 18H), 1.41-1.33 (m, 22H), 0.95 (t, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 158.71, 155.08, 145.01, 143.08, 131.70, 128.70, 126.01, 53.40, 36.08, 32.12, 29.59, 25.80, 22.70, 14.55, 14.10; ESI-MS (m/z): 620 (M^+).

Synthesis of compound 4

BODIPY compound 3 (1.0 mmol, 0.62 g) and N-iodosuccinimide (2.4 mmol, 0.54 g) were added to degassed dichloromethane (30 mL), and the mixture was stirred at room temperature in darkness for 24 h. The resulting mixture was concentrated under vacuum then purified by silica gel column chromatography (hexane:CH_2Cl_2, 1:1) to produce BODIPY monomer (4) as a red product (0.69 g, yield: 78.8%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.16 (s, 2H), 6.04 (s, 2H), 3.72 (t, J = 7.5 Hz, 2H), 2.62 (s, 6H), 1.94 (m, 2H), 1.46 (s, 18H), 1.41-1.33 (m, 22H), 0.95 (t, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 159.21, 156.39, 145.56, 142.73, 131.56, 128.45, 125.80, 53.40, 36.15, 32.09, 29.64, 25.77, 22.70, 16.63, 15.97, 14.13; ESI-MS (m/z): 872 (M^+).

General procedure for the synthesis of polymers

A mixture of 9,9-dioctylfluorene-2,7-diboronic acid pinacol ester (0.5 mmol, 321.3 mg), and totally 0.5 mmol dihalogen monomers with corresponding feed ratios, $Pd(PPh_3)_4$ (10 mg), toluene (6 mL), 10 wt% Et_4NOH (3 mL), was added and degassed 5 times under nitrogen gas. The resulting mixture was stirred at 100 °C for 48 h, then end-capped with 0.1 M phenylboronic acid (1 mL) and bromobenzene (1 mL). After cooling, the reaction mixture was poured into methanol and filtered. The precipitate was collected and dissolved in CH_2Cl_2, washed with water, and dried with anhydrous Na_2SO_4. After evaporating most of the solvent, the residue was precipitated in stirred methanol solvent to give a fiber-like solid. The final product was obtained after drying in vacuum with a yield of 52%−87%.

P1-1 $M_n = 1.8 \times 10^4$ Da; PDI = 2.1

P1-2 $M_n = 2.5 \times 10^4$ Da; PDI = 2.2

P1-3 $M_n = 2.6 \times 10^4$ Da; PDI = 2.1

P2 $M_n = 2.1 \times 10^4$ Da; PDI = 2.3

P4 $M_n = 1.6 \times 10^4$ Da; PDI = 1.8

P5 $M_n = 2.0 \times 10^4$ Da; PDI = 2.1

2. Preparation of Pdots

Pdots were prepared by using a nanoprecipitation method as described in our previous work.[3] Generally, the fluorescent semiconducting polymers were first dissolved in THF to make a 1.0 g L^{-1} stock solution. The stock polymer solution was diluted with the corresponding weight ratio of fluorescent polymers (total 0.08 g L^{-1}) and 0.02 g L^{-1} PS-PEG-COOH copolymer to produce a 10 mL THF solution mixture with a final polymer concentration of 0.1 g L^{-1}. A 1-5 mL aliquot of the mixture was quickly injected into 10 mL of Milli-Q water under sonication. THF was removed by blowing nitrogen gas into the solution at 70 ºC for 30 min. The resulting Pdot solution was sonicated for 1-2 min and filtered through a 0.2-μm cellulose membrane filter to remove any aggregates to obtain a Pdot solution at ~0.01-0.05 mg mL^{-1}. For cell imaging experiments, the Pdot solution was diluted to ~0.02 mg mL^{-1}.

3. Apparatus for Pdot characterization

^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AV500 spectrometer. Mass spectra were obtained using a Bruker Esquire-LC ion trap mass spectrometer (ESI-MS). The molecular weights of polymers were determined by using gel permeation chromatography using a SHIMADZU LC-20AD liquid chromatograph instrument with polystyrene as standard. The particle size of Pdots in aqueous solution was characterized by dynamic light scattering (Malvern Zetasizer NanoS). UV-Vis absorption spectra were recorded using a DU 720 scanning spectrophotometer (Beckman Coulter, Inc., CA USA). Fluorescence spectra were obtained and calibrated using a commercial fluorimeter (Fluorolog-3, HORIBA Jobin Yvon, NJ USA) and a 1×1 cm quartz cuvette. NIR emission spectra were collected using a corrected SOP. Fluorescence quantum yields were obtained using a Hamamatsu photonic multichannel analyzer (C10027) equipped with a CCD and integrating sphere using DI water solvent as the reference.

4. Single-particle brightness analysis using TIRF microscopy

To remove background issue, we cleaned No 1-1.5 glass with acetone, 1 M KOH, and methanol sequentially with sonication for 10 min, and washed the glass with DI water twice immediately after each cleaning step. The cleaned glass was assembled with a PDMS mold containing six wells (~5 mm diameter) after oxygen plasma cleaning. We then coated the glass surface in the wells with 0.3% 3-aminopropyltriethoxysilane (APTES). Pdots (0.01 mg mL^{-1}) were immobilized by drop-casting onto an APTES-coated cover glass for TIRF microscopy analysis. The background signal was negligible compared to that of the fluorophores. Single-molecule TIRF images were acquired using a custom-built microscope based on a Nikon Eclipse TE2000 microscope with an APO TIRF 100×/1.49 N.A. objective. Images were collected using an EMCCD ixon897 camera (Andor) and a custom controller written in LabVIEW as described previously.[4] Pdot probes were excited using a 561 nm laser with minimized power (10 mW) to reduce potential photobleaching, and photons were collected by using an Alexa 647 filter set (700/75 nm band-pass filter). 10 images were averaged to increase the signal-to-noise ratio for single-particle intensity measurements. To obtain intensities of individual fluorophores, we used a MATLAB-based program designed for single-particle tracking.

5. Single-particle brightness analysis using multi-excitation flow instrument

Single-particle brightness was also obtained using a custom-built flow analyzer with multiple laser sources. The setup was modified from an instrumental setup that we have described previously.[5] A Nikon TE2000 inverted microscope equipped with a 60× N.A. 1.45 objective was used. Three lasers (405, 488, and 561 nm) were used for fluorescence illumination, with a typical excitation power density of 800 W/cm^2. Cylindrical optics was used to shape the laser beam prior to entering the microscope. The same objective was used to collect fluorescence emission. A piezo stage (APB302, Thorlabs) was used to position and focus the sample. A custom-made rectangular pinhole was placed in the image plane. D mirrors were placed after the pinhole to split fluorescence to separate avalanche photodiodes (APDs; SPCM-AQR-14, PerkinElmer, Fremont, CA). An aspherical lens and bandpass filter (660 nm long pass) were inserted in front of each APD to collect fluorescence signals.

Details of the channel microfabrication have been described previously.[6] Briefly, the microfluidic channel was designed in AutoCAD, then written onto a chrome blank to generate the photomask (HTA Photomask, San Jose, CA). The pattern on the photomask was transferred onto a silicon wafer using photolithography. The wafer was silanized in a desiccator containing tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (Sigma−Aldrich, St. Louis, MO) then used as a master to replicate features in PDMS. Reservoirs were created by punching holes in PDMS at the ends of the straight channels. The PDMS chip was then bonded to a clean cover glass after oxidation of both the PDMS chip and the cover glass in oxygen plasma. The resulting channel has a 2×2 μm cross-section, suitable for single-molecule detection.

In experiments, diluted Pdot stock solution (~5×10^{-5} mg mL^{-1}) was injected into the inlet reservoir of the chip. Due to the height difference between the inlet and outlet reservoir (~5 mm), the solution flowed through the channel toward the outlet reservoir. When the Pdots passed through the detection laser lines, signals were detected in the corresponding APD. Fluorescence intensity trajectories were analyzed using a custom MATLAB (Mathworks) script to find peaks and generate fluorescence intensity histograms.

6. Intracellular fluorescence analysis using confocal microscopy

1×10^5 MCF-7 cells in Dulbecco's modified Eagle's medium (DMEM) medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin mix, were seeded into a glass-bottomed culture dish and allowed to adhere for 24 h with 20 ppm Pdots in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Prior to fluorescence imaging, the stained cells were rinsed with PBS to remove any Pdots that were nonspecifically absorbed on the cell membrane. Fluorescence imaging experiments were performed using a confocal microscope (Nikon A1) with excitation at 561, 488, and 405 nm through a 20X objective. Cells incubated with Pdots were excited by 561 nm, 488 nm and 405 nm lasers at the same power (24 mW). Fluorescent images were collected through one channel with bandpass at 663−

738 nm. 20 regions of interest were selected to compare the emission intensity of Pdots under excitation at different wavelengths (561, 488, and 405 nm). Image J was used to calculate the mean intensity of Pdot-labeled cells, and the mean intensity of background signals for each image were subtracted from the fluorescent intensity of the labeled cells.

7. Absorption and emission spectra of P1 Pdots

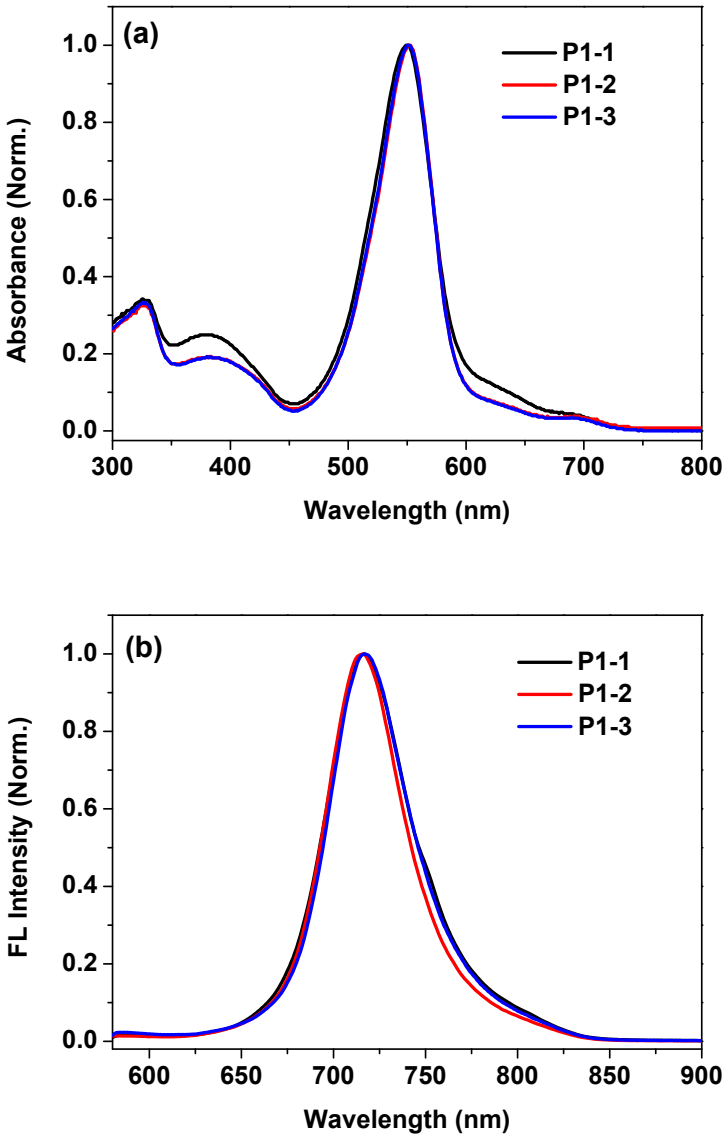


Figure S1. Absorption **(a)** and emission **(b)** spectra of P1 Pdot variants.

8. Comparison of PFBT (P2), PFDHTBT (P3), and PFBDP (P1-2) based NIR Pdots

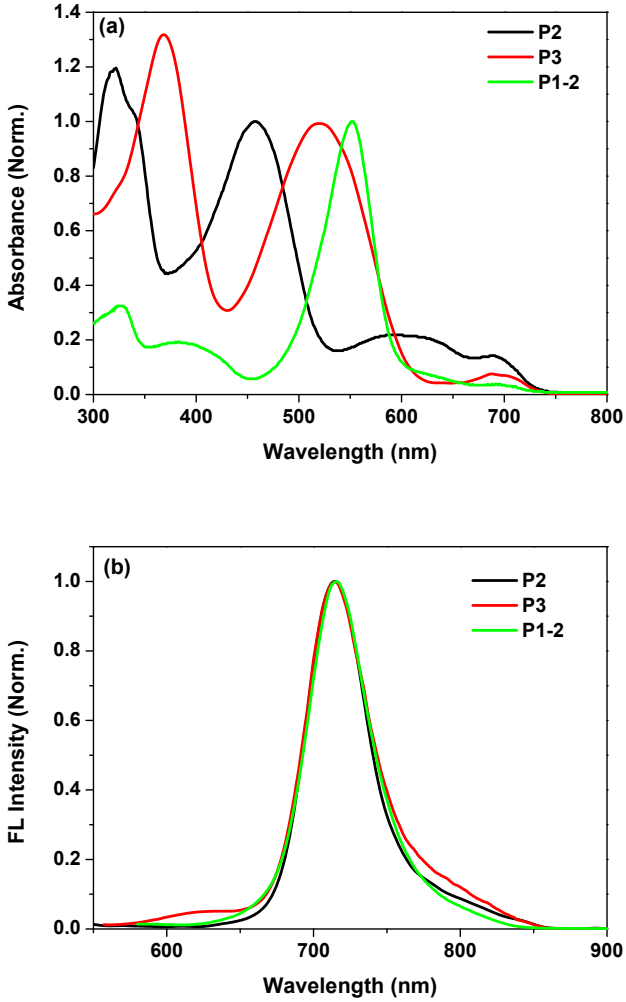


Figure S2. Absorption (a) and emission spectra of P2, P3, and P1-2 Pdots.

Table S1. Photophysical properties and diameters by DLS of P2, P3, and P1-2 Pdots

Pdot	$\lambda_{abs,CT}$ [nm]	λ_{PL} [nm]	$FWHM_{abs}$ [nm]	A_{max}[b]	Φ_f [%]	*Size* [nm]
P2	458	715	104	0.16	16.8	24.6
P3[a]	520	715	112	0.11	17.7	32.6
P1-2	551	715	56	0.30	23.2	21.8

[a] Reported in Ref. S2. [b] The absorbance was measured using 0.005 g L^{-1} Pdot aqueous solution.

9. The photophysical properties of P1-2 Pdot and PE-Cy5.5

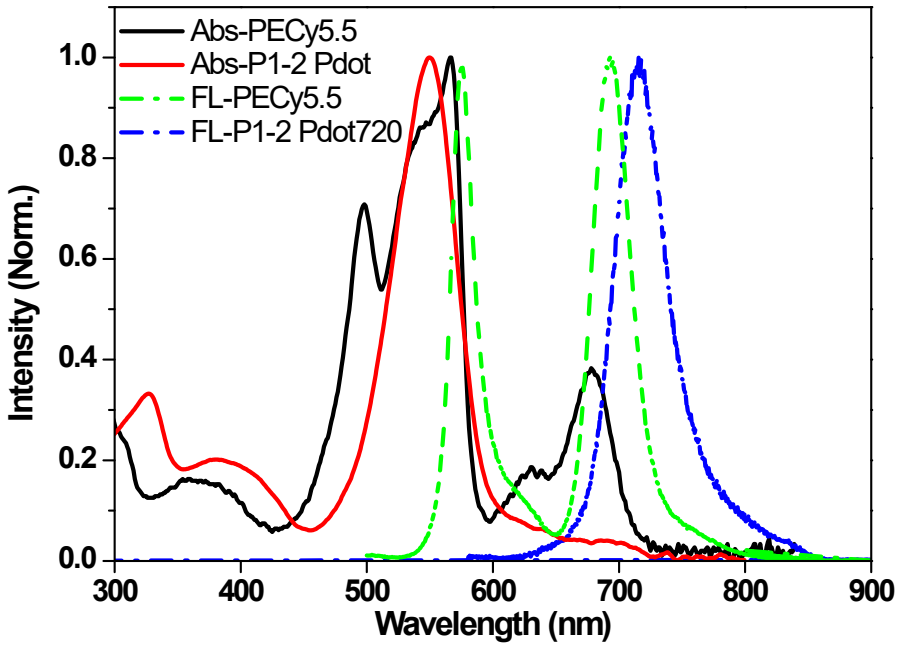


Figure S3. Absorption and FL spectra of P1-2 Pdot and PE-Cy5.5 probes.

10. Calculation of cell brightness ratios at different excitation wavelengths

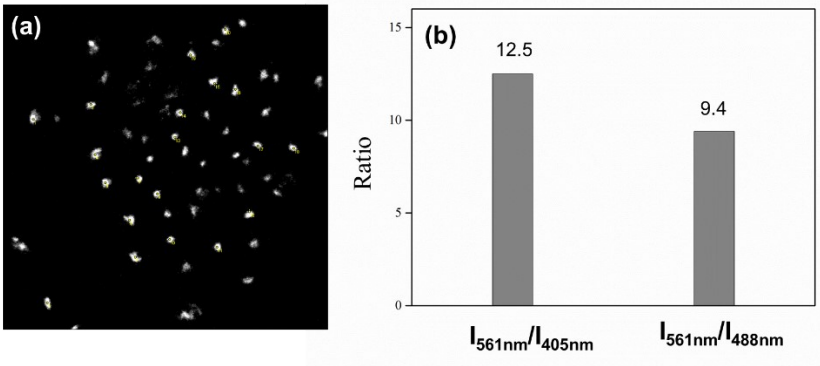


Figure S4. (a) 20 ROIs were selected for cell brightness calculations. **(b)** Cell brightness ratios at different excitation wavelengths.

11. GPC elution traces of the synthesized polymers

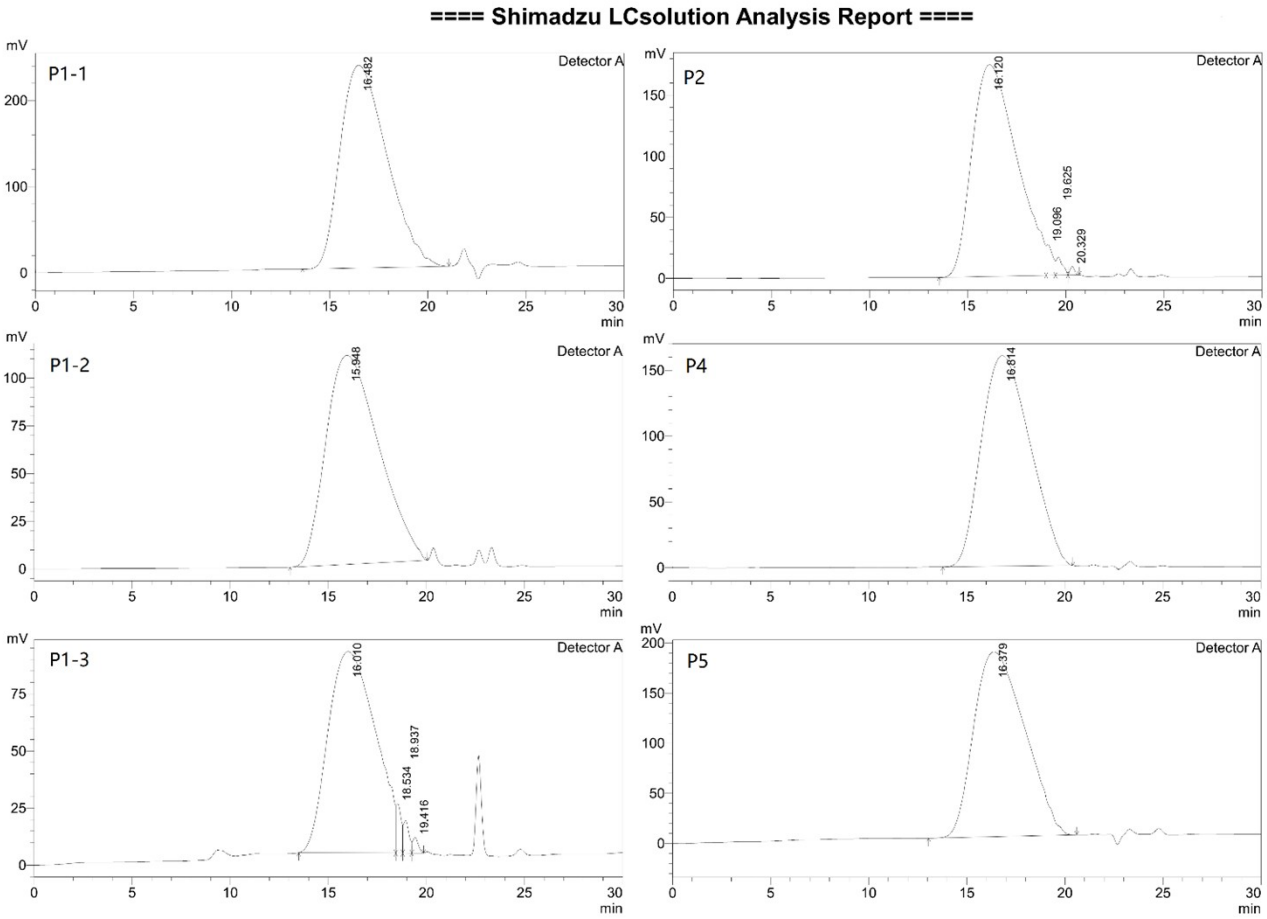


Figure S5. The GPC elution traces of the synthesized polymers.

References

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