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Supporting Information for

Simultaneous Near-Infrared and Green Fluorescence from Single Conjugated Polymer Dots with Aggregation-Induced Emission Fluorogen for Cell Imaging

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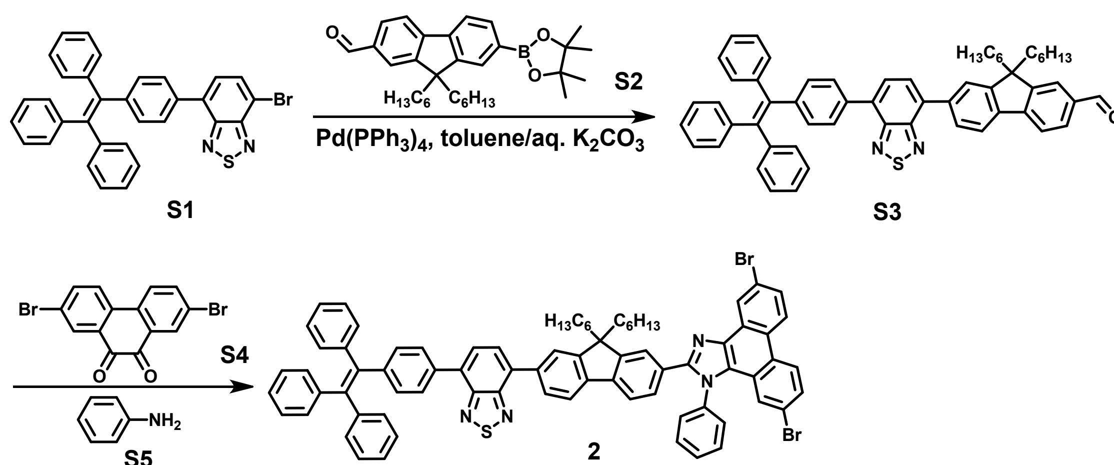
Experimental Section



Scheme S1. The synthesis of compound 2.

Materials. Compounds S1, S2, S4 and 3 were prepared according to previously reported literature.[1-4] All chemicals and solvents were purchased from the commercial sources (such as JK Chemical Co., Energy Chemical Co, Sigma-Aldrich, TCI, Thermo Scientific) without further purifications unless otherwise stated.

Synthesis of compound S3. A solution of S1 (3.0 g, 5.51 mmol) and S2 (3.22 g, 6.61 mmol) in toluene (60 mL) /EtOH (10 mL)/2M K_2CO_3 (10 mL) was deoxygenated with the nitrogen gas for about 10 min. Then, $Pd(PPh_3)_4$ (636 mg, 0.55 mmol) was added quickly and the mixture was stirred at 90 °C overnight. After cooling down to room

temperature, the mixture was poured into 100 mL of water and the organic layer was collected and washed with distilled water (100 mL×2). The organic layer was dried with anhydrous magnesium sulfate, concentrated in reduced pressure and the crude product was purified by column chromatography (silica gel, hexane:dichloromethane=2:1) to yield S3 as yellow solid (4.01 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 10.09 (s, 1H), 8.03 (dd, 1H), 7.98 (s, 1H), 7.94-7.90 (m, 4H), 7.85-7.79 (m, 4H), 7.22 (d, 2H), 7.15-7.11 (m, 13H), 7.07-7.05 (m, 2H), 2.14-2.03 (m, 4H), 1.14-1.03 (m, 12H), 0.77-0.70 (m, 10H).

Synthesis of compound 2. To a solution of S3 (2 g, 2.42 mmol), S4 (868 mg, 2.37 mmol), S5 (226 mg, 2.42 mmol) in AcOH (20 mL) was added ammonium acetate (2.24 g, 29.04 mmol) and the mixture was refluxed for 48 h under nitrogen. After cooling down to room temperature, the mixture was poured into 30 mL of water and extracted with dichloromethane (50 mL×3). The combined organic layers were washed with distilled water (150 mL×2), dried with anhydrous magnesium sulfate and then concentrated in reduced pressure. The crude product was purified by column chromatography (silica gel, hexane:dichloromethane=2:1) to yield pure 2 as yellow solid (1.51 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 9.06 (d, 1H), 8.54 (d, 1H), 8.49 (d, 1H), 7.99 (dd, 1H), 7.93-7.89 (m, 2H), 7.85 (d, 1H), 7.82-7.77 (m, 5H), 7.74 (dd, 1H), 7.69-7.64 (m, 3H), 7.61-7.56 (m, 3H), 7.27 (d, 2H), 7.21 (d, 2H), 7.14-7.11 (m, 13H), 7.07-7.05 (m, 2H), 1.98-1.91 (m, 2H), 1.75-1.67 (m, 2H), 1.15-1.02 (m, 12H), 0.78 (t, 6H), 0.59-0.52 (m, 4H).

Synthesis of PTPE-DTNT4. A mixture of compound 1 (120.7 mg, 0.188 mmol), compound 2 (212.2 mg, 0.17 mmol), compound 3 (7.5 mg, 0.015 mmol), Aliquat 336 (5 mg) in toluene (4 mL)/2M K_2CO_3 (2 mL) was deoxygenated with the nitrogen gas for about 20 min, and then $Pd(PPh_3)_4$ (5 mg, 0.55 mmol) was added quickly. After

stirring at 110 °C for 48 h, 0.3 mL of bromobenzene was added and the mixture was refluxed for another 12 h. The reaction mixture was added drop-wise to 150 mL of methanol and stirred for 2 h. Then, the solid was collected by filtration, washed with a lot of water and dried in vacuum. The crude product was dissolved in hot toluene and the insoluble residue was removed by filtration. The filtrate was concentrated in reduced pressure and then precipitated in 150 mL of methanol. The final product was collected by filtration and dried in vacuum to afford the polymer as blackish green solid (253 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 9.29 (s), 8.84 (br), 7.99-7.79 (m), 7.34 (s), 7.22-6.99 (m), 1.98 (s), 1.74 (s), 1.13 (br), 0.80-0.78 (br).

Synthesis of PTPE-DTNT2. PTPE-DTNT4 was synthesized by following the method of PTPE-DTNT2 with a 2 mol% of compound 3. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 9.31 (s), 8.85 (d), 7.99-7.79 (m), 7.34 (s), 7.22-6.99 (m), 1.98 (s), 1.76 (s), 1.12 (br), 0.80 (br).

Preparation of Pdots. The standard nanoprecipiation method was applied to prepare the Pdots. The THF solution of PTPE-DTNT2 (or PTPE-DTNT4) (250 ppm) and PSMA (1000 ppm) at the ratio of 1:4 was rapidly injected into DI water (10 mL) under ultrasonication condition. THF was evaporated under nitrogen flow at 70 ^{o}C until the solution was concentrated to 4-5mL. The prepared Pdots solution was then filtered through a 0.22 μm filter and stored at 4 ^{o}C for further use.

Cell Culture. The human cervical adenocarcinoma HeLa cell and the human breast cancer cell line MCF-7 was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). HeLa cells were cultured at 37 ^{o}C, 5% CO_2 in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (5000 units/mL penicillin G, 50 μg/mL streptomycin sulfate in 0.85% NaCl). MCF-7 cells were cultured at the same condition

except that 1% penicillin-streptomycin solution was changed as 50 U/mL penicillin and 50 μg/mL streptomycin. The cells were cultured prior to experiments until confluence was reached. The cells were harvested from the culture flask by briefly rinsing with culture media followed by incubation with 5 mL of trypsin-EDTA solution (0.25 w/v % trypsin, 0.53 mM EDTA) at 37 ^{o}C for 5-15 min. After complete detachment, the cells were rinsed, centrifuged, and resuspended in labeling buffer (1× PBS, 2 mM EDTA, 1% BSA).

Cell Uptake. First, put a rounded coverslip on the well of cell culture dishes. After incubating, remove the cell culture with pipette from the well. Then, the coverslip is washed with 200μL PBS buffer (1x) for 3 times. Take out the solution again and 200μL paraformaldehyde is added dropwise to fix the cell. Then place the circular coverslip in dark place for 15min before washing with 200μL PBS buffer (1x) for 2 times. At last, the coverslip drawn out from the culture dishes is prepared to image on a confocal microscope (Zeiss LSM 510 Meta). The Pdots uptaken Hela cells were excited by a 405 nm argon laser. A Plan-Apochromat 63x/1.40 oil DIC objective lens was utilized for imaging. The green color imaging was collected using the band pass (BP505-570) filter, and the deep red color imaging was obatined using a long pass (LP615).

Pdots Bioconjugation. Pdots bioconjugation was performed via the EDC-catalyzed reaction between carboxyl groups on the Pdots' surface and the 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 Pdot solution (~50 mg/mL in DI water), resulting in a Pdot solution in 20 mM HEPES buffer with a pH of 7.3. Then, 60 μL of streptavidin (Invitrogen, Eugene, OR, USA) was added to the solution and mixed well on a vortex. 20 μL of a freshly prepared EDC solution (5 mg/mL in MiliQ water) was added to the solution, and the mixture

was left on a rotary shaker. After stirring for 4 hours at room temperature, Triton-X 100 (0.25% (w/v), 80 μL) and bovine serum albumin (BSA; 2% (w/v), 80 μL) were added. The mixture was left on rotary shaker for 1 h. Finally, the resulting Pdot bioconjugates were separated from free biomolecules by gel filtration using Sephacryl HR-300 gel media.

Cellular Surface Labeling and Imaging. For labeling cell-surface proteins with the Pdot-streptavidin (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 antiEpCAM antibody (used to label the cell-surface EpCAM receptors on MCF-7 cells) and 5 nM Pdotstreptavidin for 30 min each, followed by two washing steps after each incubation. As for the control sample, biotinylated primary anti-EpCAM antibody was not added. The Pdot-tagged cells were then fixed with 4% para-formaldehyde for 15 min and imaged immediately on a confocal microscope (Zeiss LSM 510 Meta). The Pdots uptaken Hela cells were excited by a 405 nm argon laser. A Plan-Apochromat 63x/1.40 oil DIC objective lens was utilized for imaging. The green color imaging was collected using the band pass (BP505-570) filter, and the deep red color imaging was obatined using a long pass (LP615).

In Vivo Imaging on Zebrafish. The transgenic zebrafish, Tg(kdrl:eGFP)la116 expressing eGFP in the endothelial cells, were kept at 28 ^{o}C and bred under standard conditions. The experimental procedures for Zebra fishes in this study were approved by the National SunYat-sen University Animal Care Committee following the guideline and requirement from National Act for use of laboratory animals (ROC). For angiography imaging, 37 nL of Pdots (125 nM) in 15 mM HEPES buffer was injected into the sinus venosus of the anaesthetized zebrafish embryos 3 day post fertilization

with 5% (v/v) tricaine. After recovered for 30 min, the injected embryos were immobilized in 1.5% low melting point agarose (Life Technologies) and then imaged immediately on a fluorescence confocal microscope (Zeiss LSM 700). The green fluorescence was acquired by filtering through a 515/30 band-pass (excitation laser at 405 nm), while the red emission was acquired by using a 570 long-pass filter (excitation laser at 405 nm).

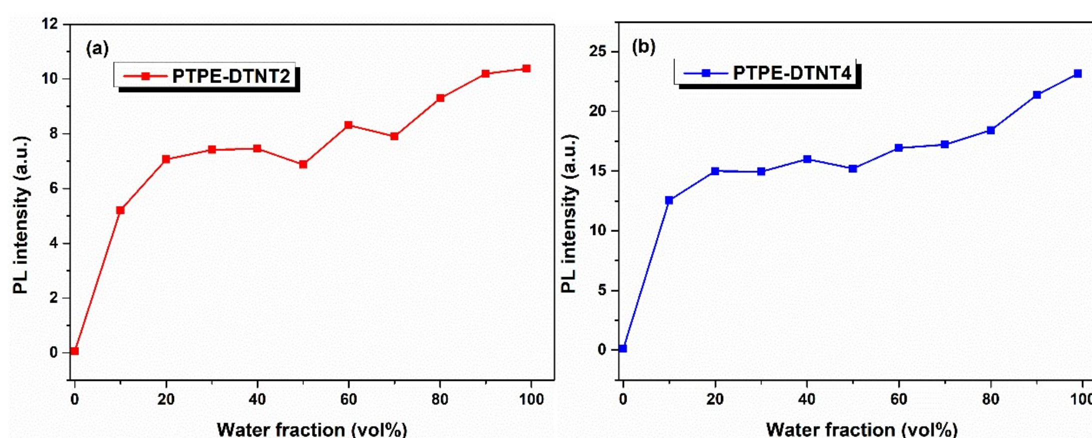


Figure S1. The corresponding plots of fluorescent intensity of PTPE-DTNT2 (a) and PTPE-DTNT4 (b) versus water fractions. The water fractions are 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 99%.

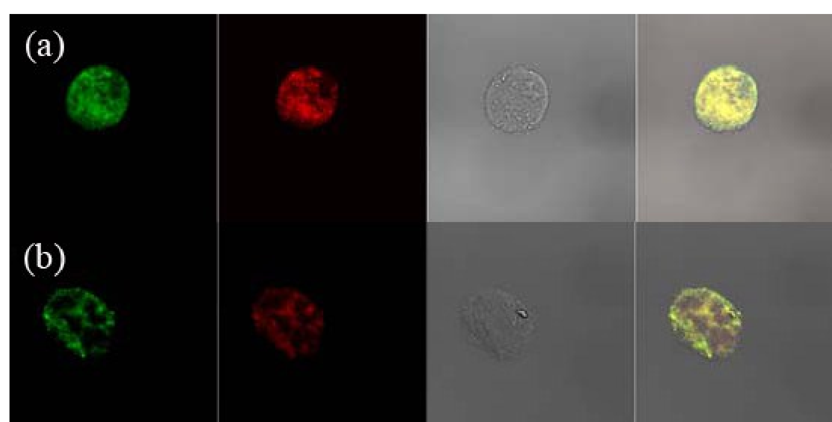


Figure S2. Confocal fluorescence images of HeLa cells incubated with PTPE-DTNT2/PSMA Pdots (a) and PTPE-DTNT4 Pdots (b). From left to right: with BP505-570, LP615 filter, birght field and the merged images

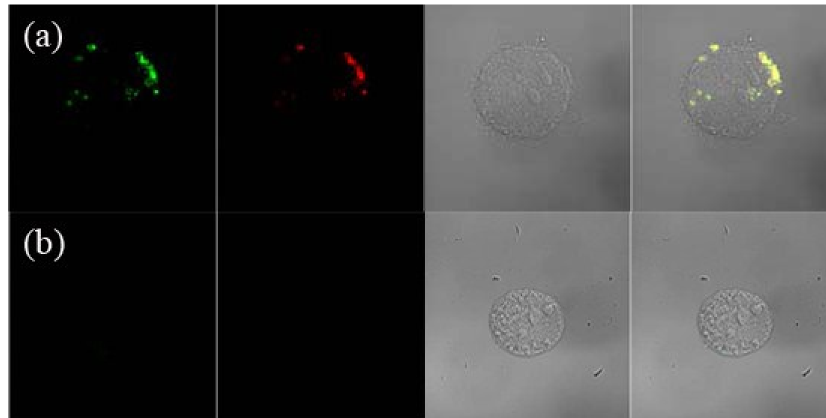


Figure S3. Confocal fluorescence images of MCF-7 cells labelled with PTPE-DTNT2/PSMA/ Streptavidin Pdots (a) and the negative control (b). From left to right, with BP505-570 filter, LP615 filter, bright field and merged images.

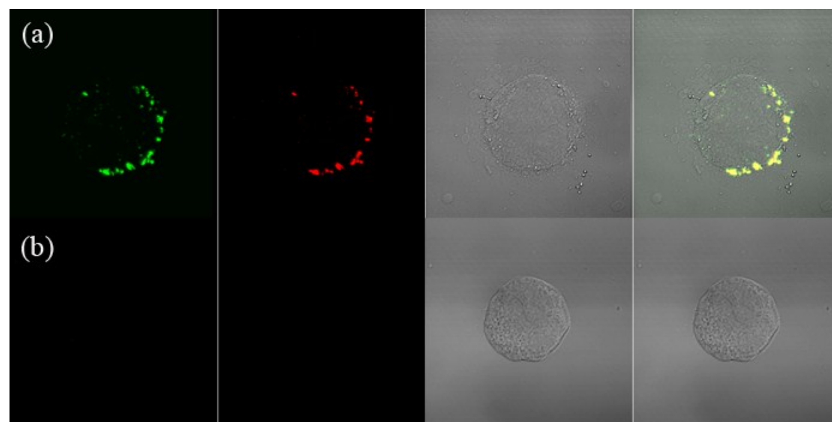


Figure S4. Confocal fluorescence images of MCF-7 cells labelled with PTPE-DTNT4/PSMA/ Streptavidin Pdots (a) and the negative control (b). From left to right, with BP505-570 filter, LP615 filter, bright field and merged images.

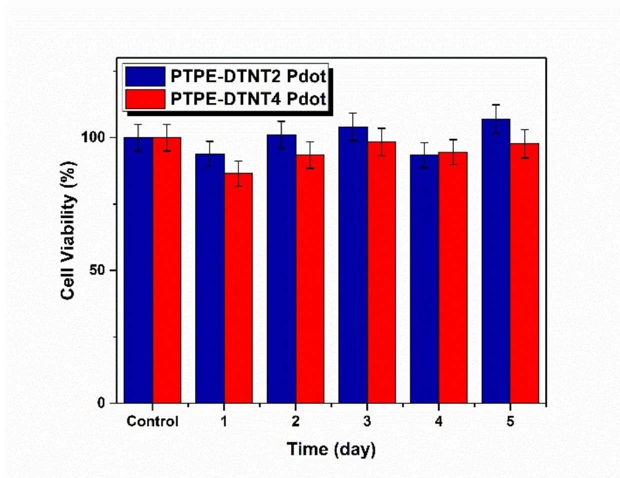


Figure S5. The cytotoxicity test of PTPE-DTNT2 and PTPE-DTNT4 Pdots.

Figure S5 shows the cell viability of the PTPE-DTNT2 Pdot and PTPE-DTNT4 in 5 days, which evaluated by the MTT assay, an experiment rely on the enzyme reduction activated in live cells rather than dead cells. Each well were cultured with the concentrations of Pdots with 200 pM in the culture plate. For further assay, the cells were incubated in the well with 20 μL (5 mg/mL) of MTT aqueous solution for 24h, 48h, 72h, 96h and 120h at 37 °C. As the Figure show, the cell viability of the PTPE-DTNT2 Pdot remains over 90%, and the PTPE-DTNT4 maintain over 80%. The viability increases from the second day, all of these reveal the cytotoxicity of the Pdots are minimal.

Table S1. The optical properties of PTPE-DTNT2/PSMA and PTPE-DTNT4/PSMA Pdots.

Pdots	λ_{abs} (nm)	λ_{em}(nm)	QY-Green	QY-NIR

PTPE-DTNT2/PSMA	391, 450	540, 710	6.2%	31.8%
PTPE-DTNT4/PSMA	391, 450, 572	540, 716	2.4%	26.3%

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