Supporting Information

Flash nanoprecipitation of ultra-small semiconducting polymer dots with size tunability

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1 Experimental section

1.1 Chemicals and instrument

Poly[{9,9-dioctyl-2,7-divinylene-fluorenylene}gd-alt-co-{2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylene}] (PFPV, MW 270 000, polydispersity 2.7), Poly[(9,9-dioctylfluorenyl-2,7-diyl)-co-(1,4-benzo-{2,1=,3}-thiadiazole)] (PFBT, MW 10 000, polydispersity 1.7) were purchased from ADS Dyes, Inc. (Quebec, Canada). Poly(styrene-comaleic anhydride) (PSMA, cumene terminated, average Mn ~1,600,) and Poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV, average Mn 40,000-70,000, polydispersity 4.0) were purchased from Sigma-Aldrich (Milwaukee,WI). Poly(ethylene glycol) methyl ether-*block*-poly(lactide-co-glycolide) (PEG average Mn 2,000, PLGA average Mn 15000) was purchased from Jinan

Daigang Biomaterial Co., Ltd (Shandong, China). Lipoic acid (ALA), N-ethylmaleimide (NEM), 4',6-Diamidino-2-phenylindole (DAPI), Dimethyl sulfoxide (DMSO), 3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromid (MTT), Dulbecco's modified Eagle medium (DMEM), bovine serum albumin (BSA), Trypsin-EDTAPenicillin-Streptomycin and dimethyl sulfoxide (DMSO)were bought from Sangon (Shanghai, China). Tetrahydrofuran (THF) was distilled before use. Other chemicals were used as received from commercial suppliers without further purification. Ultrapure water with a resistivity greater than 18.25 MΩ cm was used throughout this study.

The fluorescence spectra were recorded by a Lumina Fluorescence Spectrometer (Thermo Fisher Scientific, Korea). UV−vis absorption spectra were obtained by a Hitachi UV-2910 spectrophotometer (Hitachi, Japan). Luminescence lifetime and quantum yield measurements were characterized with an FLS 920 spectrofluorometer (Edinburgh Instruments, U.K.). Size distribution was measured by Dynamic light scattering (DLS) (ALV/CGS-8F, Germany). The morphology of semiconductiong polymer nanoparticles was carried out on a HT-7700 transmission electron microscope (Hitachi, Japan) operating at an accelerating voltage of 100 kV. MTT assay was assessed by measuring the absorbance at 570 nm on a Varioskan Flash instrument (TECAN, Switzerland). The Confocal laser scanning microscopy (CLSM) images were obtained on a Fluoview FV1000 (Olympus, Japan).

1.2 Nanoparticle preparation

Pdots were prepared by either the reprecipitation method or the FNP technology. Semiconducting polymer as precursor was dissolved in anhydrous THF to obtain 1.0 mg mL^{-1} solutions under vigorous sonication. In a typical experiment via the reprecipitation method[1], a 2 mL THF solution containing semiconducting polymer (20 μg mL^{-1}) was quickly added to 8 mL of ultrapure water under ultrasonic. Then the THF was removed by reduced pressure distillation, and a stable colloidal solution of Pdots was formed. A confined impinging jet (CIJ) includes two inlets, which a syringe containing 2 mL of semiconducting polymer (20 μg mL^{-1}, dissolved in THF) is placed at the inlet of stream 1, and a syringe containing the same volume of water was placed at the inlet of Stream 2. Two loaded syringes are propelled simultaneously, and the fluid was injected into the chamber. Then mixed exit stream falls into a reservoir containing 6 mL of ultrapure water. The THF is removed by reduced pressure distillation, and a stable colloidal solution of Pdots was formed.

1.3 Functionalization of Pdots

Functionalized Pdots were prepared by using a modified the FNP method. MEH-PPV and PSMA (Or PLGA-PEG) were dissolved in THF to produce a mixture solution with a MEH-PPV concentration of 20 μg mL^{-1} and a PSMA (Or PLGA-PEG) concentration of 50 μg mL^{-1}. The mixture was sonicated to form a homogeneous solution. A syringe containing 2 mL of s mixture solution is placed at the inlet of stream 1, and a syringe containing the same volume of water was placed at the inlet of Stream 2. Two loaded syringes are propelled simultaneously, and the fluid was injected into

the chamber. Then mixed exit stream falls into a reservoir containing 6 mL of ultrapure water. The THF is removed by reduced pressure distillation, and a stable colloidal solution of Pdots was formed.

1.4 Reynolds number

The Reynolds number (*Re)*, a ratio of inertial force to viscous force, was calculated using the relation references [2, 3] and was used to quantify the mixing.

$$\mathrm{Re} = \sum_{i=1}^{n} \mathrm{Re}_i = \frac{d}{A} \sum_{i=1}^{n} \frac{\rho_i Q_i}{\eta_i} = \frac{4}{\pi d} \sum_{i=1}^{n} \frac{\rho_i Q_i}{\eta_i} \qquad \text{Eq S1}$$

Where ρ_i is the density of the fluid (kg m^{-3}), Q_i is the volumetric flow rate (m^3 s^{-1}) of stream i, η_i is the viscosity of the fluid (Pa s), and d is a diameter of an inlet nozzle of the mixer. For the water stream, ρ is 1.0×10^3 kg·m^{-3}, and $\eta = 1.0 \times 10^{-3}$ Pa·s at room temperature. For the THF stream, ρ is 8.9×10^2 kg·m^{-3} and η is 4.8×10^{-4} Pa·s at room temperature. In CIJ-D mixing, n is 2, and d is 5.0×10^{-4} m. Q is about 1.0×10^{-6} m^3 s^{-1}.

1.5 Agarose gel electrophoresis

Agarose gel electrophoresis of the functionalized Pdots was carried out using a DYY-4C electrophoresis system (Liuyi Biology, Beijing). Pdots (in 30% glycerol) were loaded onto a 0.7% agarose gel. The bare Pdots, PLGA-PEG-functionalized Pdots, and PSMA-functionalized Pdots were loaded in each well of the gel. Then Pdot-loaded gel was run for 20 min at 135 V in tris-acetate-EDTA (TAE) buffer, and imaged under a 365 nm UV lamp.

1.6 Product yield of nanoparticles

Product yield of Pdots was calculated by using the following equation:

$$Product\ yield\ (\%) = \frac{C_1}{C_2} \times 100 \qquad \text{Eq S2}$$

C_1 is the measured concentration of semiconducting polymer. For determination of semiconducting polymer contained within Pdots. 1 mL of aqoeous solution of each Pdots was dried in an oven, and solubilized in 1 mL of THF. The concentration of semiconducting polymers in THF was determined by using UV−vis absorption spectra. MEH-PPV, PFBT and PFPV calibration curves were prepared in THF in the concentration range of 0.5–50.0 μg mL^{-1} and absorbance assessed at 475/480/455 nm, respectively. C_2 is the theoretical concentration of semiconducting polymer assuming no loss.

1.7 Cell Culture and cytotoxicity Assay

Under an humidified atmosphere of 5% CO_2 at 37 °C incubator, HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing bovine serum albumin (BSA; 10%), penicillin (100 units/mL), and streptomycin (50 units/mL).

The cytotoxicity of Pdots was tested with HeLa cells by a MTT assay. Hela cells with a density of 5×10^3 cell/well were seeded in 96-well culture plates in DMEM culture medium for 24 h. Then the medium was aspirated, and 100 μL of DMEM culture medium with a series of concentrations of Pdots (0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 μg mL^{-1}) was added to each well and continuing incubated with the cells for 24 h. Next,

MTT solution (10 μL, 5 mg mL^{-1}) were added to each well, followed by incubation for an additional 4 h. Then the medium was aspirated, and 100 μL of DMSO was added to each well. After vibrating for 15 min, their absorbance at 570 nm was measured to obtain the relative cell viability (%) by $(A_{test}/A_{control}) \times 100\%$ using a Varioskan Flash instrument (TECAN, Switzerland).

1.8 Cell imaging

HeLa cells with a density of 5×10^5 cells/well were seeded into Laser confocal special dishes, and incubated overnight for adhesion under 5% CO_2 at 37 °C. Then the cells were incubated with Pdots (5.0 μg mL^{-1}) for 3 h. The cells were washed three times with PBS to remove extracellular Pdots. The cells fixed with 4% paraformaldehyde solution at room temperature for 10 min, and their nuclei were stained with 1 μg mL^{-1} DAPI in PBS for 10 min. Cell images were acquired with confocal spectral microscopy imaging system. The imaging objective was a UPLSAPO 40.0 oil-immersion objective.

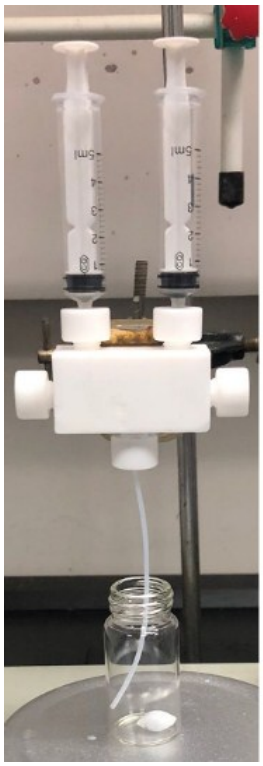

Fig.S1 The laboratory setup of the FNP mixing process to generate Pdots. The dimensions of the CIJ-D mixer was described in Ref [2].

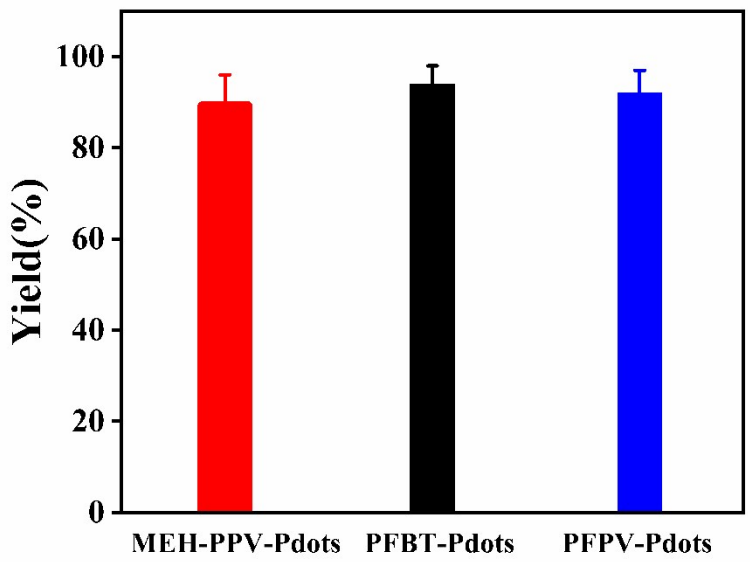

Fig.S2 Product yield (%) of the polymers precipitated out as particles

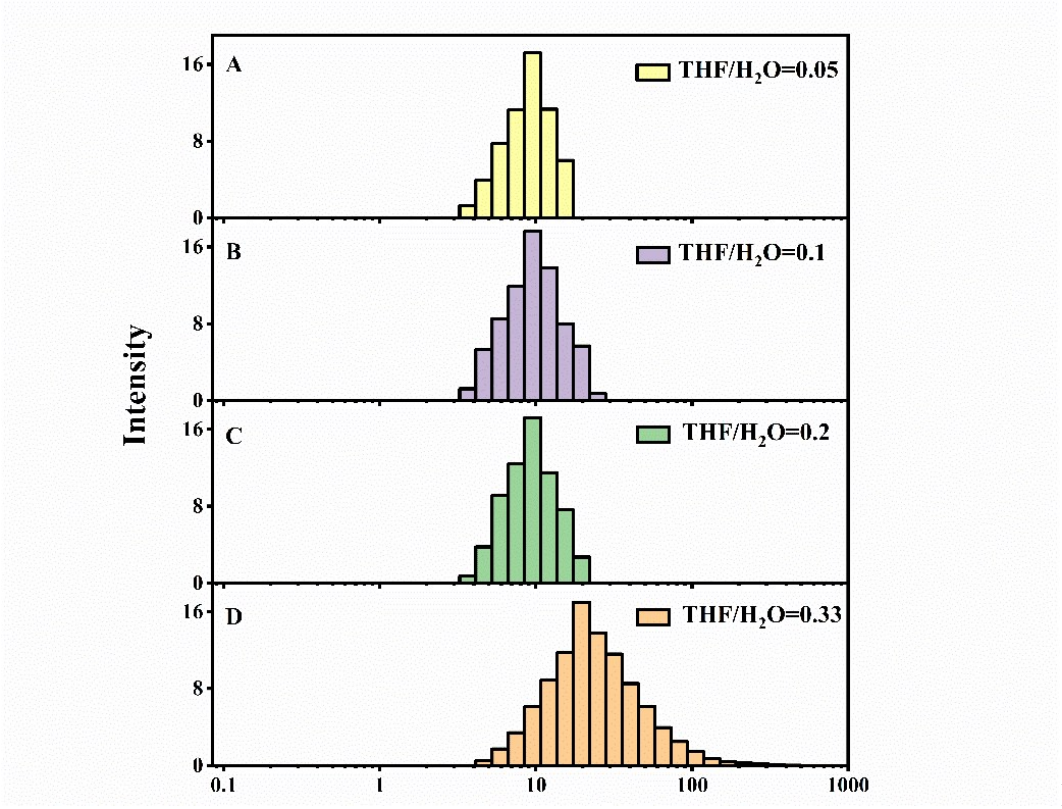

Fig.S 3 Hydrodynamic diameter of PFBT particles at different THF/water ration measured by DLS: (A) 0.05; (B) 0.1; (C) 0.2; and (D) 0.33.

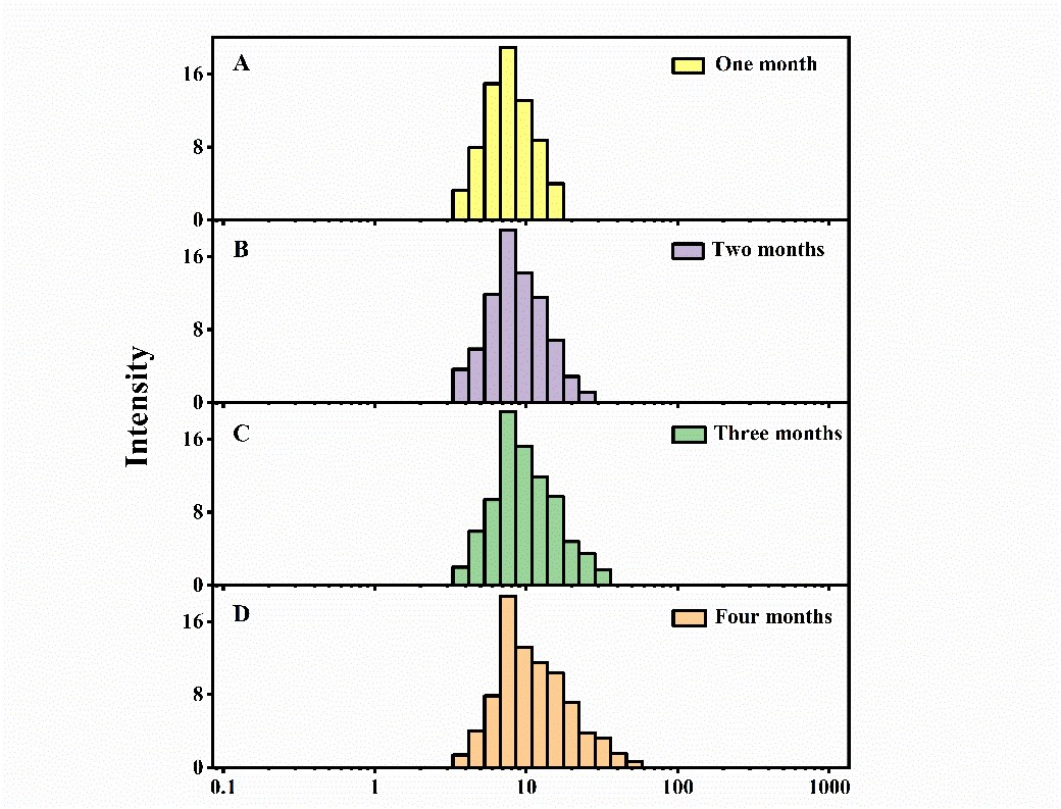

Fig.S4 Hydrodynamic diameter of PFBT Pdots in water solution measured by DLS: (A) One month; (B) Two months; (C) Three months; and (D) Four months.

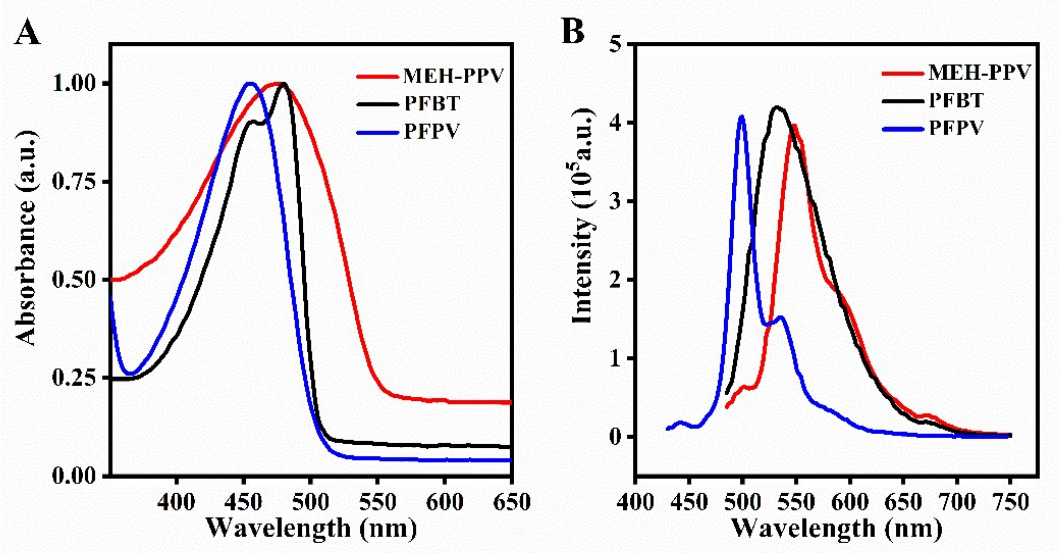

Fig.S5 (A) Absorption spectra of MEH-PPV, PFBT, and PFPV in THF, and their corresponding (B) fluorescence spectra

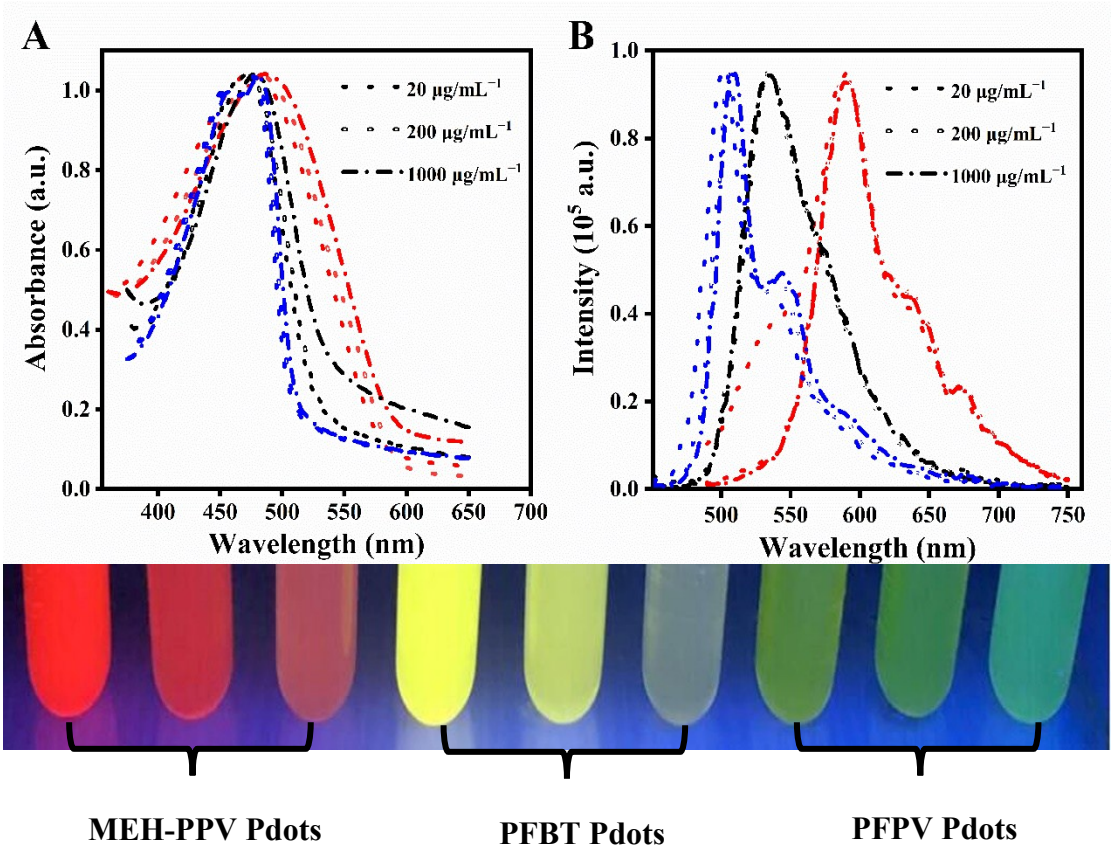

Fig.S6 Fluorescence spectra of Pdots prepared with different precursor concentration of MEH-PPV (Red), PFBT (Black), and PFPV (Blue); Insert: photographs of aqueous Pdot suspensions with different precursor concentrations (from left to right: 20, 200, and 1000 μg mL^{-1}) under UV light (right) illumination.

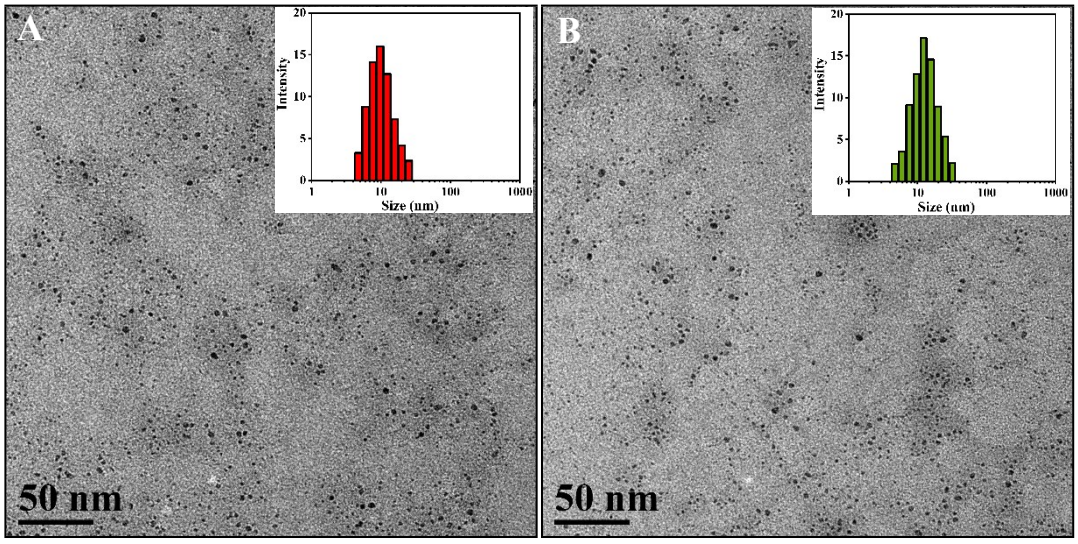

Fig.S7 TEM image: (A) MEH-PPV@PSMA Pdots, and (B) MEH-PPV@ PLGA-PEG Pdots; Insert: Dynamic light scattering MEH-PPV@PSMA Pdots and MEH-PPV@ PLGA-PEG Pdots.

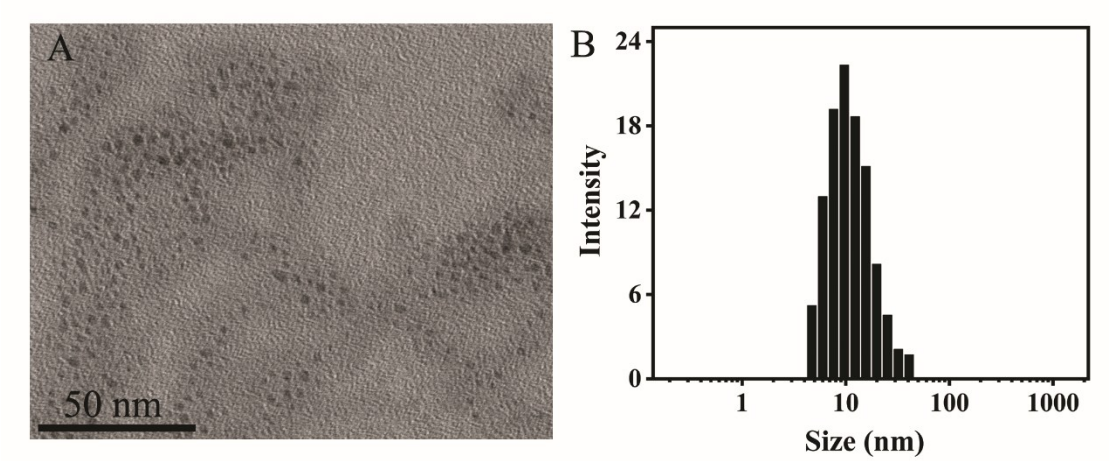

Fig.S8 (A) TEM image of bare PLGA-PEG nanoparticles, and (B) their dynamic light scattering.

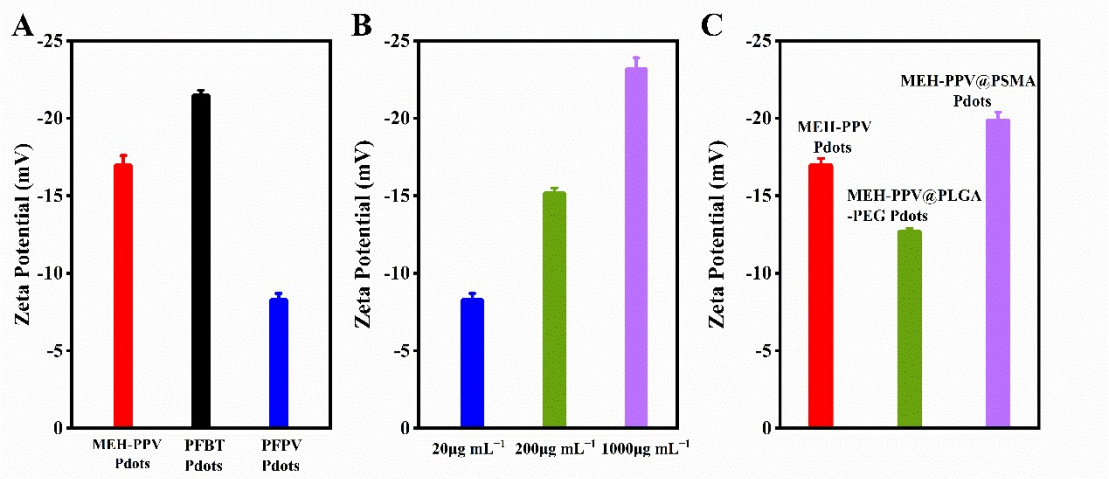

Fig.S9 (A) ζ-Potentials of MEH-PPV, PFBT, and PFPV Pdots; (B) ζ-Potentials of PFPV Pdots with different precursor concentration; (C) ζ-Potentials of MEH-PPV, MEH-PPV@ PLGA-PEG, and MEH-PPV@PSMA Pdots.

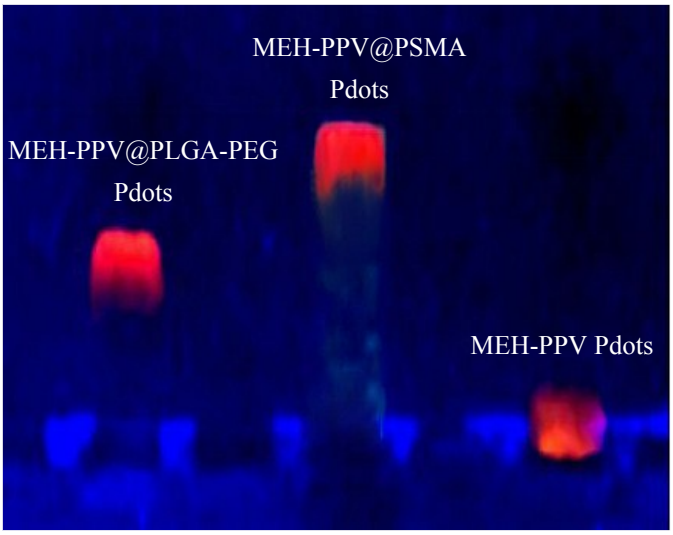

Fig.S10 Agarose gel electrophoresis assays of MEH-PPV@ PLGA-PEG Pdots, MEH-PPV@PSMA Pdots and MEH-PPV@PSMA Pdots. These Pdots in the gel were visualized under a 365 nm UV lamp.

Table S1 Hydrodynamic diameters of Pdots prepared by FNP method

Polymer species	MEH-PPV (c, μg mL^{-1})			PFBT (c, μg mL^{-1})			PFPV (c, μg mL^{-1})		
	20	200	1000	20	200	1000	20	200	1000
Hydrodynamic diameter (nm)	9.5	15.4	24.9	7.6	12.3	19.8	9.6	18.9	32.0

Table S2 Comparison of different methods to manufacture semiconductiong polymer nanoparticles

No.	Semiconducting polymer	Method	Diameters[a,b]	Ref.
1	MEH-PPV	By reprecipitation	5–50 nm	[4]
2	PFBT		10–35 nm	[1]
3	PFPV		50–70 nm	[1]
4	PFBT	By microfluidic method	100–260 nm	[5]
5	CN-PPV		100–260 nm	[5]
6	MEH-PPV	By FNP method	9.5–24.9nm	This study
7	PFBT		7.6–19.8 nm	
8	PFPV		9.6–32.0 nm	

[a] No 1–3: Data come from the atomic force microscope (AFM).

[b] No 4–8: Data come from dynamic light scattering.

References

1. C. Wu, B. Bull, C. Szymanski, K. Christensen and J. McNeill, *ACS Nano*, 2008, **2**, 2415-2423.
2. J. Han, Z. X. Zhu, H. T. Qian, A. R. Wohl, C. J. Beaman, T. R. Hoye and C. W. Macosko, *J. Pharm. Sci.*, 2012, **101**, 4018-4023.
3. Z. X. Zhu, *Mol. Pharmaceut.*, 2014, **11**, 776-786.
4. C. Wu, C. Szymanski, Z. Cain and J. McNeill, *J. Am. Chem. Soc.*, 2007, **129**, 12904-12905.
5. T. F. Abelha, T. W. Phillips, J. H. Bannock, A. M. Nightingale, C. A. Dreiss, E. Kemal, L. Urbano, J. C. deMello, M. Green and L. A. Dailey, *Nanoscale*, 2017, **9**, 2009-2019.