

Supporting Information

Self-Assembly of All-Conjugated Block Copolymer Nanoparticles with Tailoring Size and fluorescence for Live Cell Imaging

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Materials

2,5-dibromo-3-thiophenemethanol, triethylene glycol monomethyl ether, Phosphorus tribromide, n-butyllithium, 3-bromothiophene, i-PrMgCl, 2,5-dibromo-3-hexylthiophene, [1,3-bis(diphenylphosphino)propane]-dichloronickel (II) and N-bromosuccinimide were purchased from Alfa Aesar and used as received without any further purification. Other chemicals were obtained from Sigma-Aldrich and used as received. Tetrahydrofuran (THF) and hexane were dried over sodium/benzophenone under nitrogen and freshly distilled before use. Indium tin oxide (ITO) glass was purchased from Delta Technologies Limited, whereas PEDOT:PSS (Baytron PA14083) was obtained from Bayer Inc.

Techniques

The nuclear magnetic resonance (NMR) spectra were collected on a Bruker ARX 400 NMR spectrometer with deuterated chloroform as the solvent and with tetramethylsilane ($\delta=0$) as the internal standard. The ultraviolet-visible (UV) spectra of the samples were recorded on a PerkinElmer Lambda 750 spectrophotometer. Fluorescence measurement for photoluminescence (PL) of the polymers was carried out on a Hitachi F-7000 PC spectrofluorophotometer with a xenon lamp as the light source. The gel permeation chromatography (GPC) was conducted with a Breeze Waters system equipped with a Rheodyne injector, a 1515 Isocratic pump and a Waters 2414 differential refractometer using polystyrenes as the standard and tetrahydrofuran (THF) as the eluent at a flow rate of 1.0 mL/min and 40 °C through a Styragel column set, Styragel HT3 and HT4 (19 mm×300 mm, 10^3+10^4 Å) to separate molecular weight

(MW) ranging from 10^2 to 10^6 . The X-ray diffraction (XRD) study of the samples was carried out on a Bruker D8 Focus X-ray diffractometer operating at 30 kV and 20 mA with a copper target ($\lambda = 1.54 \text{ \AA}$) and at a scanning rate of $1^\circ/\text{min}$. TEM images were recorded using a JEOL-2100F transmission electron microscope and an internal charge-coupled device (CCD) camera. Dynamic light scattering (DLS) was performed using a Nano ZS-90 apparatus utilizing 633 nm red laser (at 90° angle) from Malvern Instruments. Cellular fluorescent images were recorded on a Zeiss LSM510 Laser Scanning Confocal Microscope.

Synthesis

Synthesis of 2,5-dibromo-3-{2-[2-(2-methoxyethoxy)ethoxy]ethoxymethyl}thiophene (1)

The 2,5-dibromo-3-thiophenemethanol (3.0 g, 1.1×10^{-2} mol) in dry methylene chloride (100 mL) was added to a 250 mL flask under a nitrogen atmosphere. The flask was placed in an ice water bath, and the mixture stirred for 20 min. Phosphorus tribromide (1.73 mL, 1.84×10^{-2} mol) was added dropwise to the solution over a 15 min period. The reaction mixture was stirred at room temperature for 5 hours, then quenched by the addition of a 10% sodium bicarbonate solution. The mixture was passed through a plug of Celite, washed with water, and dried over anhydrous MgSO_4 . The solution was filtered and the removal of solvent gave the the product (3.53 g, 96% yield) as a light yellow solid. Then triethylene glycol monomethyl ether (3.0 g, 1.8×10^{-2} mol) was dissolved in THF (80 mL) in a 2-neck 250 mL flask. Sodium hydride (0.5 g, 2.1×10^{-2} mol) was added slowly under a nitrogen atmosphere, and after hydrogen gas evolution had finished, the flask was sealed under a nitrogen atmosphere. The product compound (3.0 g, 0.9×10^{-2} mol) was dissolved in THF (25 mL) and added dropwise to the reaction mixture at room temperature over the course of 10 minutes. Stirring was continued for 4 hours. The mixture was run through a plug of Celite and the solvent was removed by rotary evaporation. The crude product was eluted over silica gel with a hexane:ethyl acetate mixture (7:3). The second of two UV active spots, seen by TLC, was collected and dried to provide the product (2.84 g, 78% yield) as a yellow liquid. $^1\text{H NMR}$ (400

MHz, Chloroform-d) δ 6.99 (s, 1H), 4.42 (s, 2H), 3.5-3.7(m, 12H), 3.36(s, 3H).

Synthesis of P3HT-b-P3EGT (2)

The molar feed ratio of 2,5-dibromo-3-hexylthiophene and 2,5-dibromo-3-{2-[2-(2-methoxyethoxy)ethoxy]ethoxymethyl}thiophene was 60:40, 40:60 and 20:80. Diblock copolymer syntheses involved preparation of the Grignard derivative of each monomer and polymerization. The typical synthesis procedure of diblock copolymer (feed molar ratio of 40:60) was as follows: Three 2-necks round-bottomed flasks in the order were marked as A, B, C. Ni(dppp)Cl₂ catalys (24.9 mg, 4.59×10⁻⁵ mol), 2,5-dibromo-3-hexylthiophene(0.5 g, 1.53 mmol) , 2,5-dibromo-3-{2-[2-(2-methoxyethoxy)ethoxy]ethoxymethyl}thiophene(0.96 g, 2.30 mmol) was added into the A, B, C, respectively. Then three flasks were connected with rubber tubes were dried by heating under reduced pressure and pass through nitrogen for removing any water and oxygen inside. After adding dry THF (5ml, 10ml, 10ml) into the A, B, C via a syringe respectively, the solution was stirred at room temperature. 2 M solution of i-PrMgCl (0.76ml) in THF into the B via a syringe and i-PrMgCl (1.14 ml) into the C a syringe, the mixture was stirred at room temperature for 1 hour. Solution B was heated up to 70 °C, solution A was poured into solution B in one portion. After the reaction mixture was stirred at 70 °C until atropurpureus was observed (about 5 min), solution C also was poured into solution B in one portion for overnight. The reaction mixture was precipitated into hexane. The crude polymer was filtered and purified by sequential soxhlet extractions using acetone, hexane and chloroform. The solvent was removed by evaporation to give a purple solid.

Aqueous Self-Assembly of P3HT-b-P3EGT

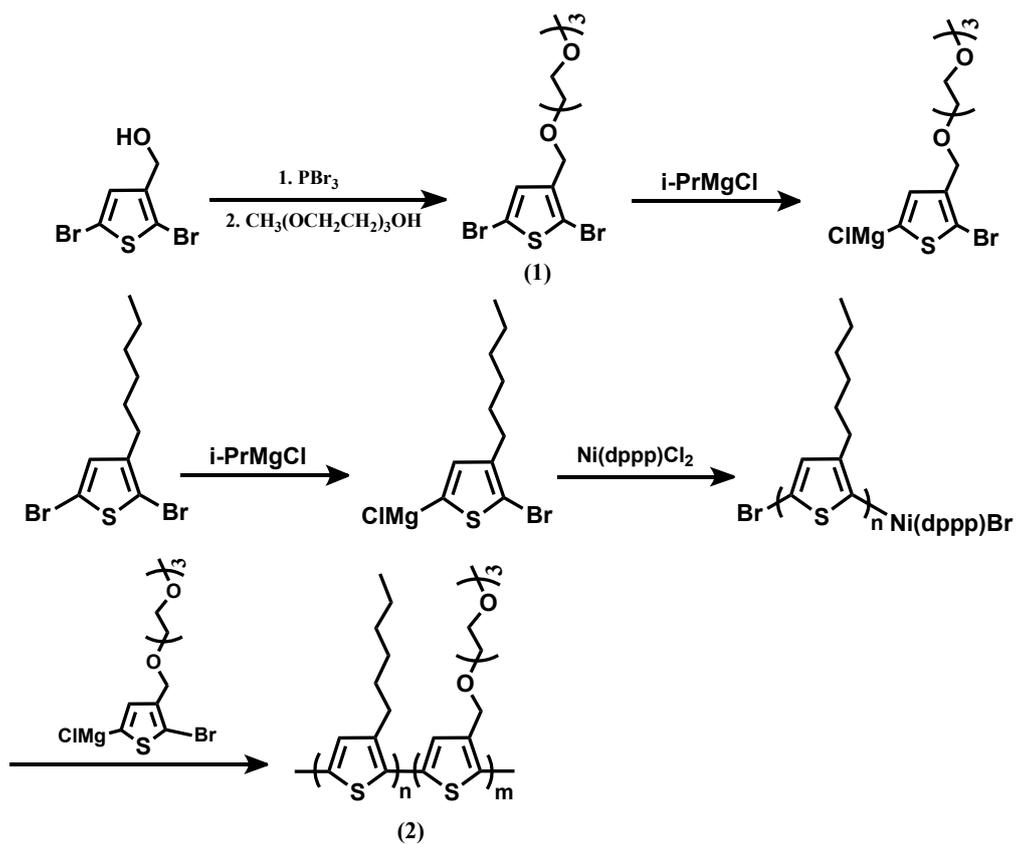
In a typical experiment, 5.0 mg of the polymer was dissolved in and THF (20 mL). The resulting solution was stirred at 25 °C under dark conditions for 4 h. The solution was transferred to the semi-permeable membrane having MWCO 2000 and then dialyzed against a large amount of deionized water for 48 h. Fresh water was replenished

periodically to ensure the removal of THF from the dialysis membrane. The dialyzed solution was filtered, lyophilized, and stored at 4 °C for further usage.

Cell Imaging and Cytotoxicity of Copolymer Nanospheres

For the cellular toxicity test, HeLa cells were seeded into a 96-well cell culture plate in DEME at a density of 10^4 cells per ml with 5% fetal bovine serum at 37°C and with 5% CO₂ for 24 h. Afterwards, the culture medium was replaced with 100mL of DMEM containing the copolymer nanospheres at different doses and cultured for various periods. Then, 20 mL of 5 mg mL⁻¹ MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) solution was added to every cell well. The cells were further incubated for 4 h, followed by removing the culture medium with MTT, and then 100mL of DMSO was added. The resulting mixture was shaken for 10 min at room temperature.

Cellular fluorescent images were recorded on a Zeiss LSM510 Laser Scanning Confocal Microscope. HEK 293 cells and Hela cells were seeded on cover slips in the cell culture DMEM for 1 day. Chemical treatment was performed in different dose for imaging at various times. Twenty four hours or longer after treatment, cells were fixed with 4% paraformaldehyde at room temperature for 20 min and then stained with DAPI, a nuclear marker. After incubation, cells were washed three times with 1×PBS buffer and then fixed by 75% ethanol for 20 min and further washed twice with 1×PBS buffer. 405 nm and 555 nm laser were used to check the fluorescence of DAPI and P3HT-b-P3TEGT, respectively.



Scheme S1. Synthesis of monomers and copolymers P3HT-b-P3EGT

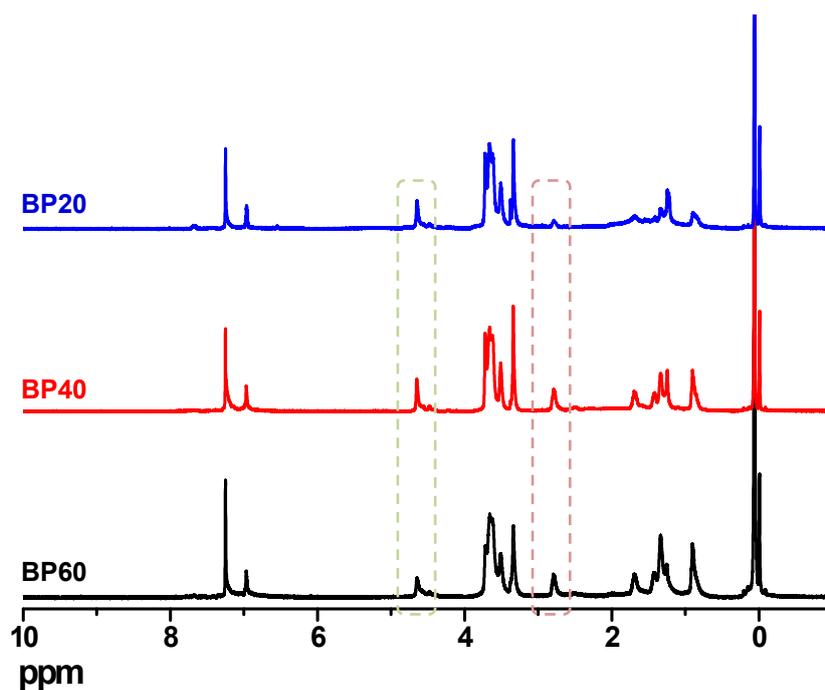


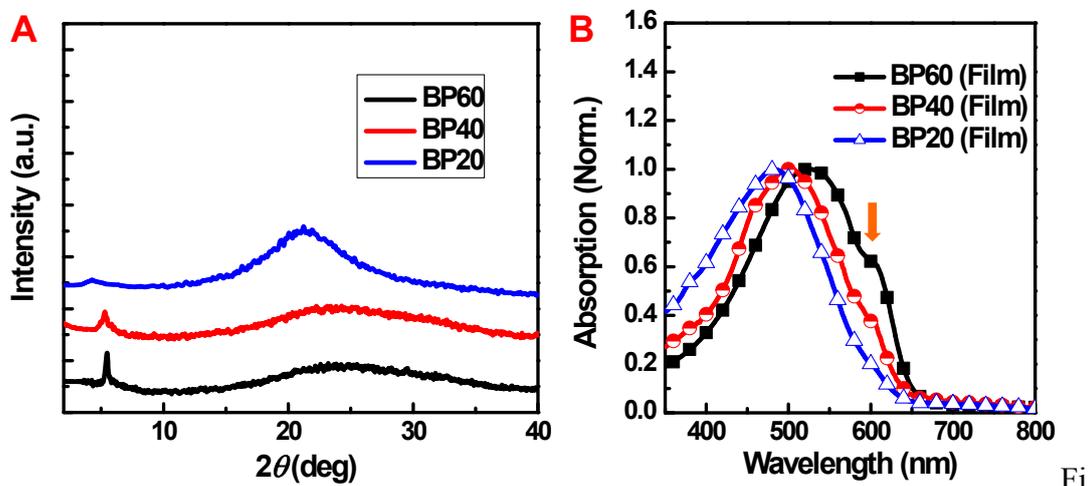
Fig. S1 ^1H NMR spectra of P3HT-b-P3TEGT with various block ratios of P3HT: P3TEGT at 60:40, 40:60 and 20:80.

Table S1 Molecular weights of the homopolymers and copolymers, and ratios of the blocks

Polymer	Molecular Weights ^a		Ratio (P3HT: P3TEGT)	
	$k M_n$ (g mol^{-1})	PDI	Monomer feed	Film ^b
P3HT	21.2	1.3	—	—
BP60	11.5	1.9	60:40	58:52
BP40	9.1	2.3	40:60	36:64
BP20	7.7	2.5	20:80	17:83
P3TEGT	5.4	2.9	—	—

^a Determined by GPC in chloroform using polystyrene standards.

^b Estimated from the NMR peaks for the proton at the thiophene 4-position of the copolymer



g. S2 (A) XRD spectra of copolymer in the out-of-plane direction; (B) UV-vis absorption spectra in thin films.

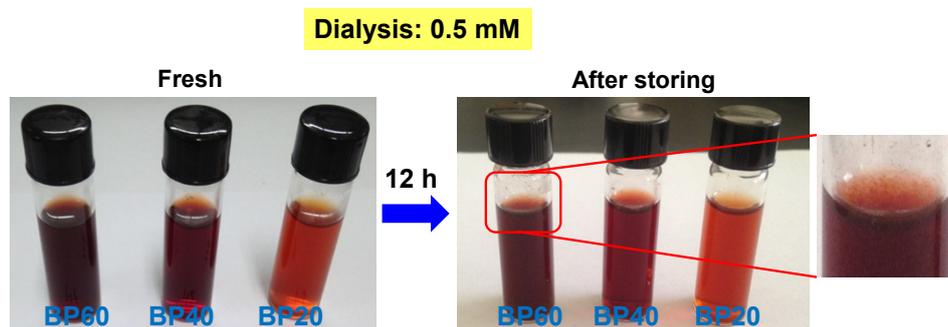


Fig. S3 Stability observation of various copolymers micelles in water at 5×10^{-4} M after storing for 12h at room temperature.

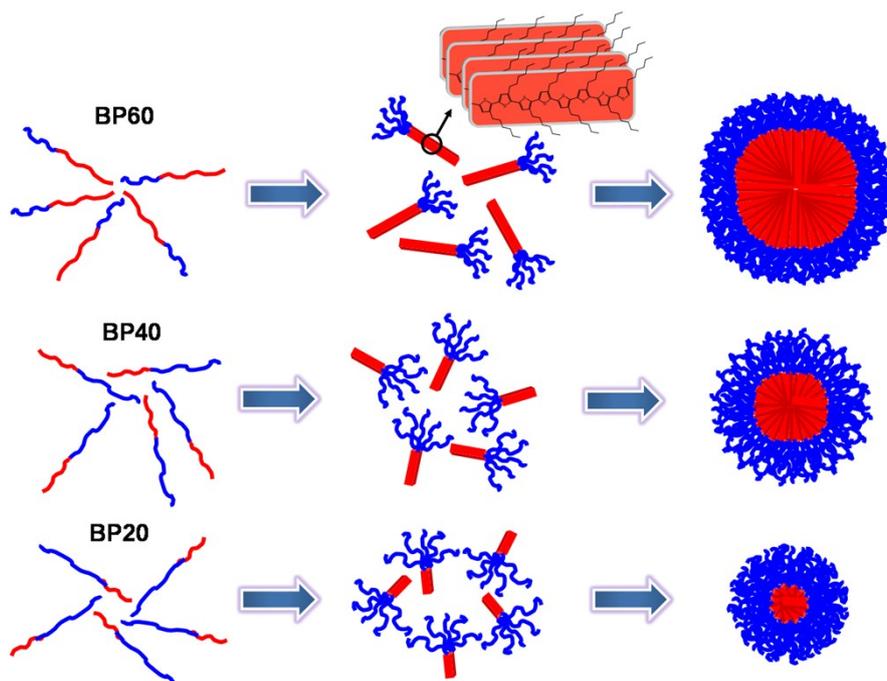


Fig. S4 Plausible models of different copolymers for the formation of self-assembled nanoparticles in water.

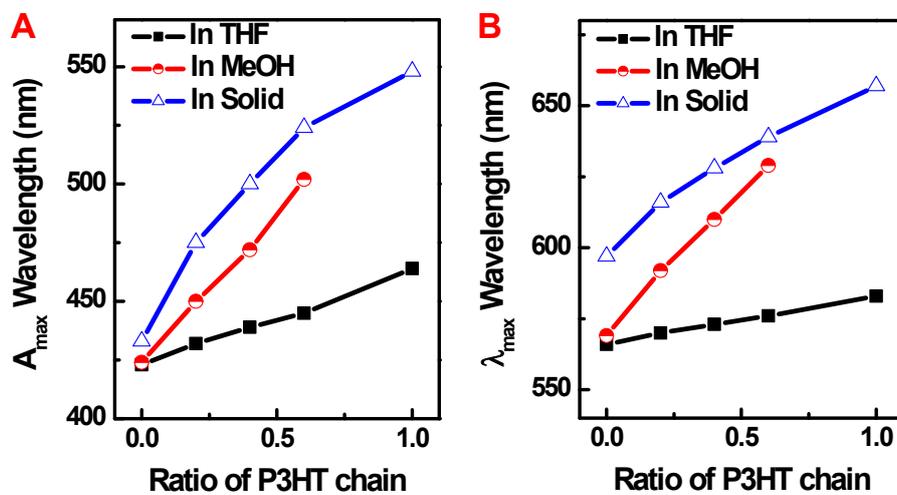


Fig. S5 (A) Absorption maxima of polymers depended on the ratio of P3HT in various state; (B) Emission maxima of polymers depended on the ratio of P3HT in various state.

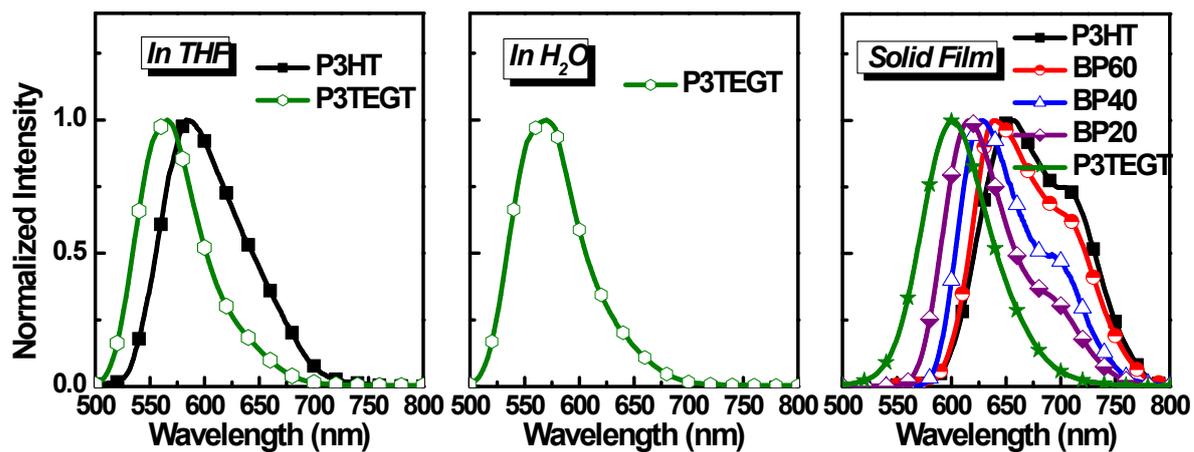


Fig. S6 Normalized photoluminescence (PL) spectra of homopolymers and copolymers in THF solution, H₂O solution and solid state.

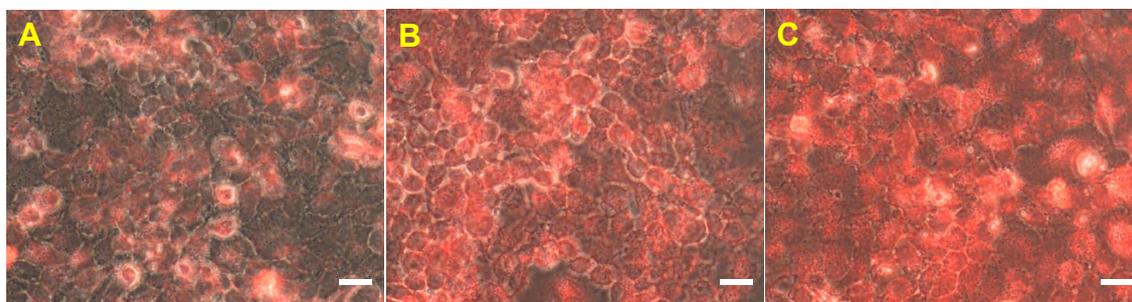


Fig. S7 Fluorescence/transmission overlapped image of HeLa cells after 48 h incubation ($\lambda_{\text{ex}} = 543 \text{ nm}$) treated with BP40 in different concentrations: (A) 2 μM , (B) 5 μM and (C) 10 μM .