Supporting information

# Single-Chain Tethered Nanoparticles with Tunable Softness: Scalable Synthesis and Unique Self-Assembly Behavior

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## 1. Materials

All the reagents and solvents were commercially available and used as received unless otherwise stated. *β*-Cyclodextrin *(β*-CD, 98%, Macklin) was purified by recrystallization in deionized water for three times prior to use. Poly(ethylene glycol) methyl ether ($PEG_{225}$-OH, $M_n$ = 10 kDa, Sigma Aldrich) was dried over anhydrous toluene by azeotropic distillation. *p*-toluenesulfonyl chloride (TsCl, 99%, TCI), sodium azide ($NaN_3$, 99%, Zhengzhou Paini Chemical Reagent Factory), 2-bromoisobutyryl bromide (98%, TCI), 4-dimethylaminopyridine (DMAP, 99%, Macklin), sodium hydride (NaH, 60% in oil, Aladdin), propargyl bromide (99%, Adamas) and *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, 99%, Sigma Aldrich) were used as received. Copper(I) bromide (CuBr, 99%, Aladdin) was purified by washing consecutively with acetic acid, ethanol, and diethyl ether, and then dried under reduced pressure. *tert*-Butyl acrylate (*t*BA, 99%, Aladdin) was distilled over $CaH_2$ before using. *N,N'*-Dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), triethylamine (TEA) and dichloromethane (DCM) were dried over $CaH_2$ and distilled prior to use.

## 2. Instrumentation and Methods

$^{1}H$ NMR and $^{13}C$ NMR spectra were conducted on a Bruker AVANCE III HD 600 MHz spectrometer. The samples were dissolved with deuterated DMSO or chloroform ($CDCl_3$). Chemical shifts (d) were given in *ppm* relative to tetramethylsilane (TMS) as internal reference.

Conversion of the monomer was determined by FULI 9790 Ⅱ gas chromatography equipped with a Collect AS-2912 auto sampler, FID detector and KB-5 GC capillary column (30 m, 0.320 mm, and 0.25 μm). Injector, column and detector temperatures were set at 180, 110 and 210 $^{o}$C, respectively. The conversion of monomer was determined based on the integral of monomer peak using MEK as internal standard.

Size-exclusion chromatography (SEC) characterization was performed on an Agilent 1260-series HPLC system equipped with a 1260 Infinity II automatic liquid sampler (ALS), a 1260 column compartment (TCC) at 50°C equipped with a Polargel-M guard column (50 x 7.5 mm) and two Polargel-M (300 x 7.5 mm) columns and a 1260 refractive index detector (RID). The used eluent was DMAc containing 50mM of LiBr at a flow rate of 0.6 mL/min. The spectra were analyzed using Agilent GPC/SEC software. Number averaged molecular weight ($M_n$) and dispersities (*Đ)* were calculated against PMMA standards.

The morphologies of the SCTSUNPs (PEG(P*t*BA)$_{20}$) and the polymeric assemblies were characterized with a JEM-2100Plus transmission electron microscope (TEM) at an acceleration voltage of 200 kV. The samples were prepared by dropping a 5 μL droplet of sample solution on an ultrathin copper grid (200 mesh) coated with carbon film, followed by drying at room temperature. Cryogenic transmission electron microscopy (*Cryo*-TEM) measurements were performed on a FEI Talos F200C operating at an acceleration voltage of 120 KV. Samples were prepared by dropping a drop of concentrated solution on a copper grid coated with carbon; after absorbing the excess solution with a piece of filter paper, the copper grid was quickly dipped into liquid ethane to be frozen. The vitrified samples were then stored in liquid nitrogen. Laser confocal microscopy (LSCM) images were taken with a Leica TCS SP8 STED 3X super-resolution confocal microscope. Rhodamine B-loaded vesicle solutions were dropped on confocal dishes respectively, and then dried at room temperature.

Then, the samples were observed under 458 nm laser excitation source.

Dynamic light scattering (DLS) were carried out on a Malvern Zetasizer Nano ZS instrument using disposable cuvettes. Each sample was equilibrated at 25 $^{o}$C for 5 min before measurement. Vertically polarized light by He-Ne laser (633 nm) was used as incident light, while the scattering light was collected at an angle of 173$^{o}$ and analyzed by an autocorrelator. The size of the particles is determined by Stokes-Einstein equation:

$$d(H) = \frac{kT}{3\pi\eta D} \qquad \text{Equation S1}$$

where $d$(H) is the mean hydrodynamic diameter, $k$ is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the dispersing medium, and D is the apparent diffusion coefficient determined by the autocorrelator. Before starting the measurements, samples were incubated for specific time depending on the temperature. All samples were filtered through membranes with pore sizes of 0.45 μm prior to measurement.

## 3. EXPERIMENTAL SECTION

**Synthesis of Mono-6-(*p*-tolylsulfonyl)-6-deoxy-*β*-cyclodextrin (*β*-CD-OTs).** *β*-CD (60.0 g, 53 mmol) was dissolved in 0.4 M NaOH aqueous solution in a 1000 mL round-bottom flask equipped with a stirring bar, which was then transferred to an ice-water bath to be cooled down to 0 °C. TsCl (15.1 g, 79 mmol, in 40 ml $CH_3CN$) was added dropwise over 60 min to the *β*-CD solution. Then the reaction was allowed to last for 2h at room temperature. After removing the unreacted TsCl by filtration, diluted HCl was added into the filtrate to adjust solution pH to 8. The solution was then kept at 4 °C overnight to allow the precipitation. Crude product was collected by filtration and washed by distilled water. Pure *β*-CD-OTs was obtained by recrystallization of the filtrated solid from hot water, followed by drying under reduced pressure at 50 $^{o}$C for 2 days, yielding a white power (9.2 g, 13%).

**Synthesis of Mono-6-azide-6-deoxy-*β*-cyclodextrin (*β*-CD-$N_3$).** To a solution of *β*-CD-OTs (5.0 g, 3.9 mmol) in DMF (20 mL), $NaN_3$ (2.6 g, 39 mmol) and KI (0.31 g,

1.9 mmol) were added during stirring. The mixture was allowed to react at 60 °C for 24 h, after which the solid was removed by centrifugation. The supernatant was precipitated into a mixture of acetone/water (4/1, v/v). The precipitate was then isolated and washed with cold water and dried under reduced pressure at 50 °C for 2 days (3.8g, 85%).

**Synthesis of Monoalkynyl-Terminated PEG (PEG-*yne*).** To a solution of PEG-OH (5.0 g, 0.5 mmol) in anhydrous toluene (40 mL), NaH (0.12, 5.0 mmol) was added during stirring. After stirring for 30 min, propargyl bromide (0.3 g, 2.5 mmol) was added into the mixture slowly. The reaction vessel was then stirred for 24 h at 60 °C. After removing insoluble salts via filtration, the solution was concentrated and precipitated into cold diethyl ether. The obtained solid was dissolved again in 100 ml DCM. After extraction with deionized water (3 times), the organic phase was dried over anhydrous $MgSO_4$. The dried organic solution was filtrated and concentrated. Precipitation of the solution in DCM was then performed with excess cold diethyl ether for three times. The purified product was dried under reduced pressure overnight at 40 °C, yielding 4.5 g white powder (90 %).

**Synthesis of Mono PEG Functionalized *β*-Cyclodextrin (PEG-CD).** The synthesis of PEG-CD was performed by click reaction of PEG-*yne* and *β*-CD-$N_3$. Typically, PEG-*yne* (3.0 g, 0.3 mmol), *β*-CD-$N_3$ (1.04 g, 0.9 mmol) and PMDETA (62 μL, 0.3 mmol) were first dissolved in anhydrous DMF (30 mL) in a Schlenk flask equipped with a stirring bar. After three freeze-pump-thaw cycles, the reaction mixture was added with CuBr (14.4 mg, 0.1 mmol) under the protection of $N_2$ flow. The Schlenk flask was then placed in a preheated oil bath at 80 °C. After reaction for 48 h, the solvent was removed via rotary evaporation. The residues were diluted with THF, and then passed through a neutral alumina column to remove the copper catalysts. The eluent was concentrated and dialyzed (molecular weight cut off: 3.5 kDa) against deionized water for 5 days to remove the unreacted *β*-CD-$N_3$. The final product was obtained by freeze-drying (2.8 g, 85%).

**Synthesis of PEG-$Br_{20}$.** PEG-$Br_{20}$ was prepared via a two-step esterification, as only ~70% of the hydroxyls were esterified from the first esterification in NMP, which

may be ascribed to the high steric effect close to the hydroxyl group. PEG-CD (2.0g, 0.18 mmol) dissolved in dried NMP (25 mL) in a flask placed in ice-water bath. 2-bromoisobutyryl bromide (2.48 g, 10.8 mmol, in 10 ml NMP) was added dropwise to the flask over 60 min during stirring. The mixture was stirred for 48 h at room temperature. The mixture was then diluted with DCM (100 mL) and washed successively with $NaHCO_3$ aqueous solution (3 times) and deionized water (3 times). The organic phase was dried over anhydrous $MgSO_4$, concentrated and precipitated into an excess of cold diethyl ether to yield crude product with the hydroxyl group partially coupled. The crude product was dissolved together with TEA (0.73 g, 7.2 mmol) and DMAP (0.1 g, 0.8 mmol) in fresh-distilled DCM (50 ml) in a round-bottom flask in ice-water bath, to which 2-bromoisobutyryl bromide (1.66 g, 7.2 mmol, in 10 ml DCM) was added dropwise. The reaction under stirring was performed at room temperature for 24 h. The work-up of the second step esterification is the same as the first step. The crude product was then dried under reduced pressure overnight at room temperature to yield finally product, PEG-$(Br)_{20}$ (1.8 g, 70%).

**Synthesis of PEG(P*t*BA)$_{20}$.** PEG(P*t*BA)$_{20}$ was synthesized by ATRP of *t*BA using PEG-$(Br)_{20}$ as macroinitiator. In a typical procedure, PEG-$(Br)_{20}$ (0.1 g, 0.007 mmol), *t*BA (1.82g, 14.2 mmol), PMDETA (30 μL, 0.14 mmol) and MEK (6 mL) were introduced into a Schlenk flask. After degassing of the reaction mixture by freeze-pump-thaw cycles for three times, CuBr (20 mg, 0.14 mmol) was added under the protection of $N_2$ flow. The polymerization was carried out at 50 $^oC$, during which monomer conversion was monitored by gas chromatography (GC) at certain time intervals. It should be noted that all the polymerizations were terminated at relatively low monomer conversions (< 30%) in order to avoid the intermolecular/intramolecular coupling between the P*t*BA chains. After terminating by dipping the flask into liquid nitrogen, the reaction mixture was diluted with THF and passed through a neutral alumina column to remove copper catalysts. Then the solution was concentrated and precipitated into the mixture of methanol/$H_2O$ (1/1, v/v) solvent for three times. The final product was dried under reduced pressure at 40 $^oC$ for 2 days.

**Synthesis of PEG-Br macroinitiator.** PEG-OH (2.0g, 0.2 mmol), TEA (0.1 g, 1.0 mmol), and DMAP (61 mg, 0.5 mmol) were added into a 100 mL round-bottom flask, to which freshly distilled DCM (20 mL) was added. The flask was then equipped with a stirring bar and placed in an ice-water bath. 2-Bromoisobutyryl bromide (0.23 g, 1.0 mmol, in 5 mL DCM) was added dropwise to the flask over 30 min. The reaction mixture was stirred at room temperature for 24 h and then diluted with DCM (100 mL), washed successively with $NaHCO_3$ aqueous solution (3 times) and deionized water (3 times). The organic phase was dried over anhydrous $MgSO_4$ and concentrated, precipitated into excess of cold diethyl ether. After drying under reduced pressure overnight at room temperature, PEG-Br was obtained (1.7 g, 84%).

**Synthesis of Linear PEG-*b*-P*t*BA.** PEG-*b*-P*t*BA was synthesized by ATRP of *t*BA using PEG-Br as the macroinitiator. In a typical procedure, PEG-Br (0.2 g, 0.02 mmol), *t*BA (1.28g, 10 mmol), PMDETA (5 μL, 0.02 mmol) and MEK (4 mL) were introduced into a Schlenk tube. After degassing by freeze-pump-thaw cycles for three times, CuBr (3 mg, 0.02 mmol) was added under the protection of $N_2$ flow. The polymerization was carried out at 60 $^oC$, during which monomer conversion was monitored by GC at a certain time intervals. After terminating by dipping the flask into the liquid nitrogen, the mixture was diluted with THF and passed through a neutral alumina column for removal of the copper catalysts. Then the eluent was concentrated and precipitated into methanol/$H_2O$ (1/1, v/v) solvent mixture. The precipitate was then dissolved again in THF and precipitated twice before being dried under reduced pressure for 2 days.

**4. Self-Assembly of PEG(P*t*BA)$_{20}$ and Linear PEG-*b*-P*t*BA.**

A certain volume of deionized water was added at 1 μL/min into a vial containing 4 mL PEG(P*t*BA)$_{20}$ (or PEG-*b*-P*t*BA) solution in DMF at 0.5 mg/mL. When the water content reached to 30% v/v, the mixture was transferred to a dialysis bag and dialyzed against deionized water for three days to remove the organic solvent.

**5. Conversion of hydroxyl groups to brome were calculated based on the following equation:**

$$C_{OH} = \frac{6 \times 20}{225 \times 4} \times \frac{Ib}{Ia}$$ Equation S2

Where $C_{OH}$ is the conversion of hydroxyl groups on terminal *β*-CD; $I_b/I_a$ are the integral ratio of methylene protons of PEG backbone and methyl protons of 2-bromoisobutyrnate residue.

**6. Discussion on the mono-modification of β-CD.**

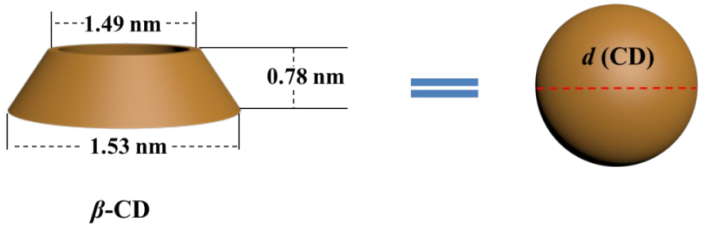
In this report, to confirm the single chain tether on the NP, mono-modification of *β*-CD by the above chemistry is necessary. We here claim that a single PEG has been introduced on NP based on the following reasons:

1) The protocol we employed have been well studied already and have been proved to be success in the mono-modification of CD. References are: *Angew. Chem. Int. Ed.* 2008, 47, 3435; Macromolecules 2006, 39, 2460; Macromolecules 2010, 43, 10221 and *J. Am. Chem. Soc.* 2006, 128, 3703.

2) $^1$H NMR has been done for β-CD-OTs by (Figure S2a, ESI). The signals of 7, 8 and 9 on Figure S2a are attributed to the *p*-toluenesulfonyl group, indicating the success of the tosylation of CD. Moreover, the integral ratio between aromatic ring and 2,3-OH is close to 4:14, indicating the ratio of toluenesulfonyl group to CD is 1:1. Note that β-CD-OTs was purified by recrystallization and hence is pure. With this, we can prove the mono-tosylation of CD.

3) With the mono-modified CD, β-CD-OTs, the rest success mono-modification of CD by N3 or PEG can be easily confirmed by complete conversion of OTs to azido groups or azido group to PEG, see Figures S2 and 1.

**7. Supporting Figures**




*β*-CD

$$d(\mathrm{CD}) = \frac{\frac{1.49+1.53}{2}+0.78}{2} \approx 1.15 \text{ nm}$$

**Scheme S1** Schematic illustration of the estimated diameter of *β*-CD when treated as sphere.

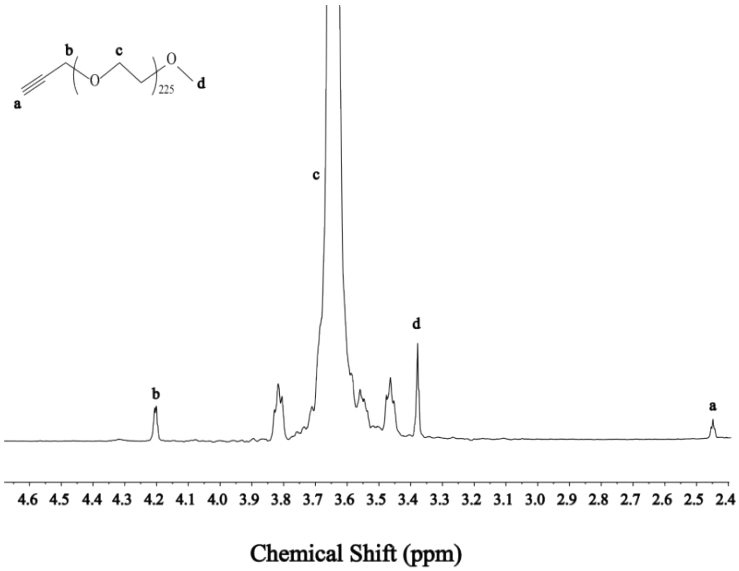



**Fig. S1** $^{1}$H NMR spectrum of PEG-*yne* in $CDCl_3$.

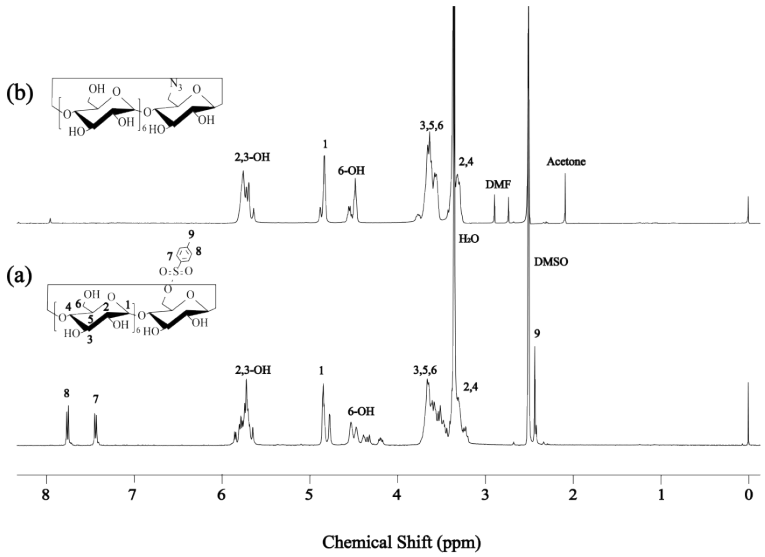



**Fig. S2** $^{1}$H NMR spectra of (a) *β*-CD-OTs and (b) *β*-CD-$N_3$ in deuteration DMSO.

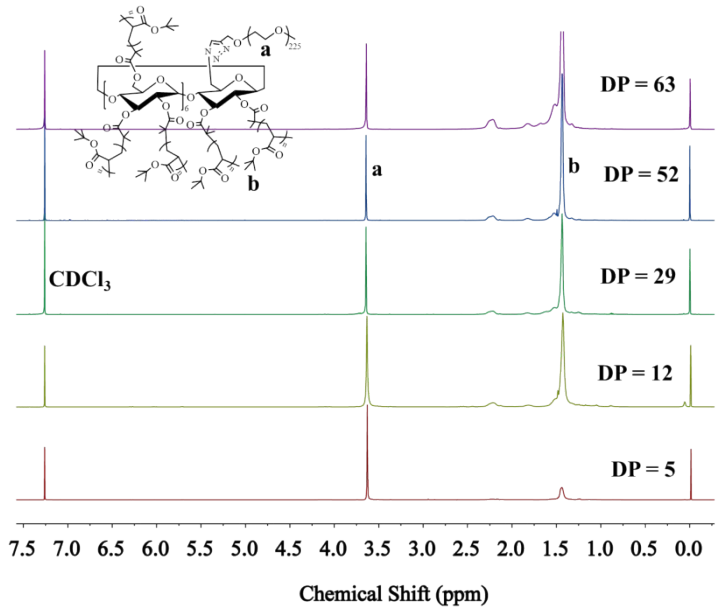



**Fig. S3** $^{1}$H NMR spectra of PEG(P*t*BA)$_{20}$ with different DP of P*t*BA in each arm in $CDCl_3$.

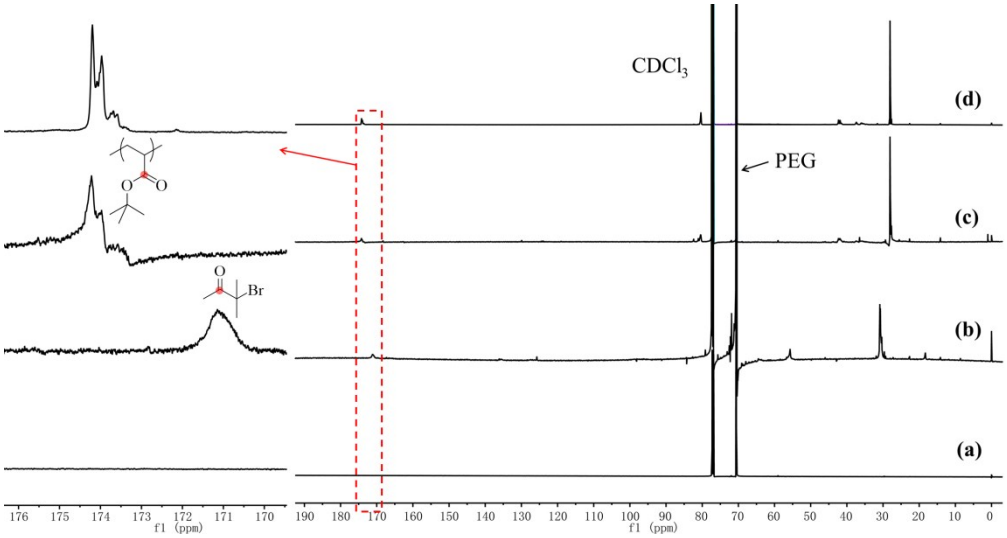



**Fig. S4**. $^{13}C$ NMR spectra of (a) PEG-CD, (b) PEG-$Br_{20}$, (c) PEG(P*t*$BA_5$)$_{20}$ and (d) PEG(P*t*$BA_{12}$)$_{20}$.

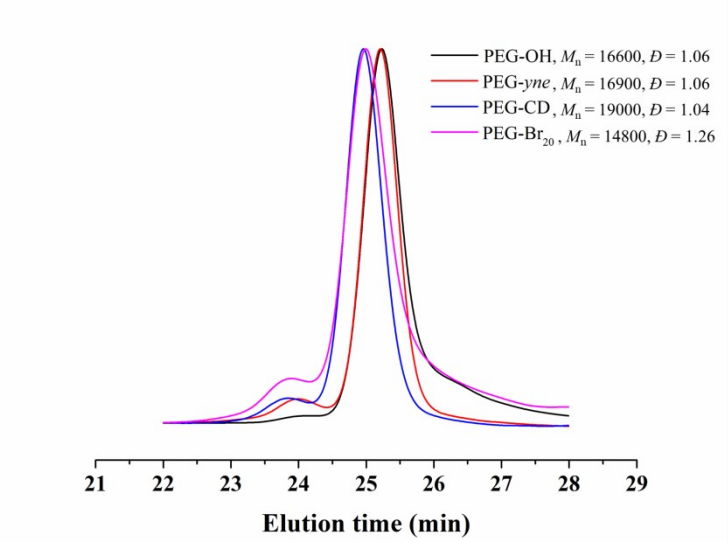



**Fig. S5** SEC traces of PEG-OH, PEG-*yne*, PEG-CD and PEG-$Br_{20}$.

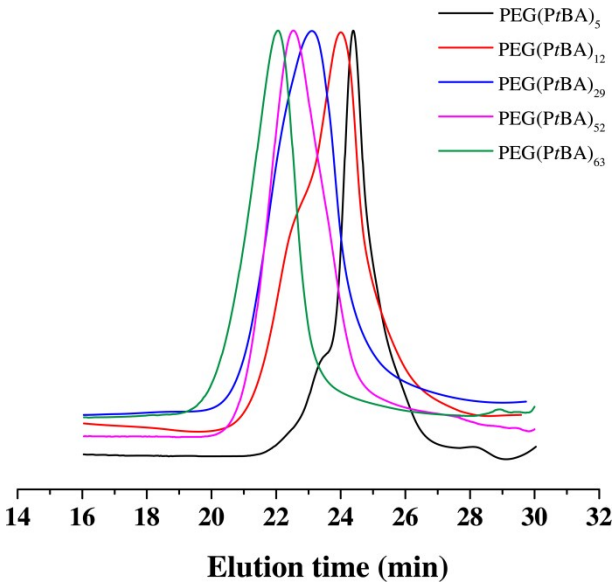



**Fig. S6** SEC traces of SCTSUNPs PEG(P*t*BA)$_{20}$.

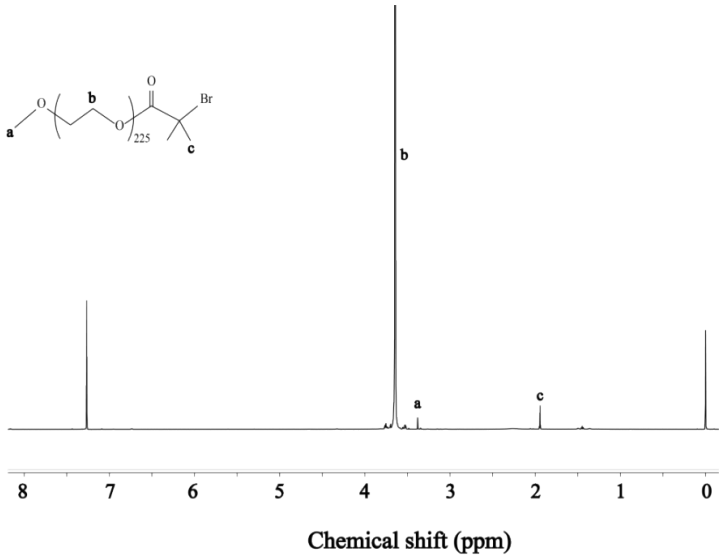



**Fig. S7** $^{1}$H NMR spectrum of PEG$_{225}$-Br in $CDCl_3$.

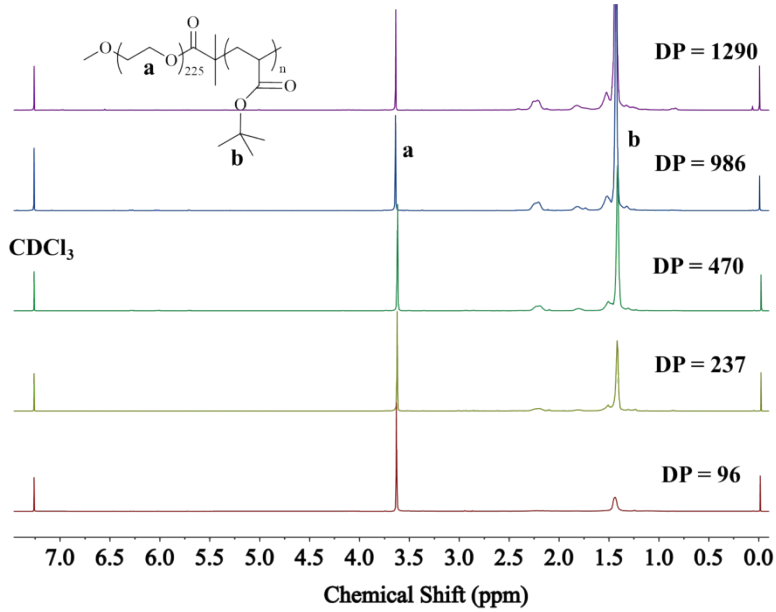



**Fig. S8** $^{1}$H NMR spectra of PEG-*b*-P*t*BA copolymers with different DPs of P*t*BA in $CDCl_3$.

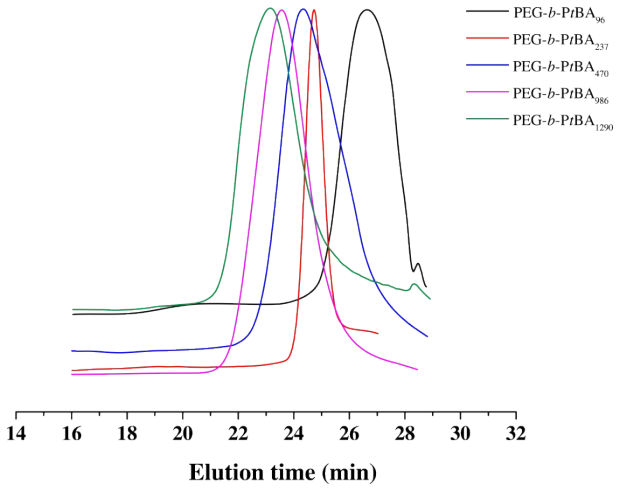



**Fig. S9** SEC traces of linear PEG-*b*-P*t*BA copolymers.

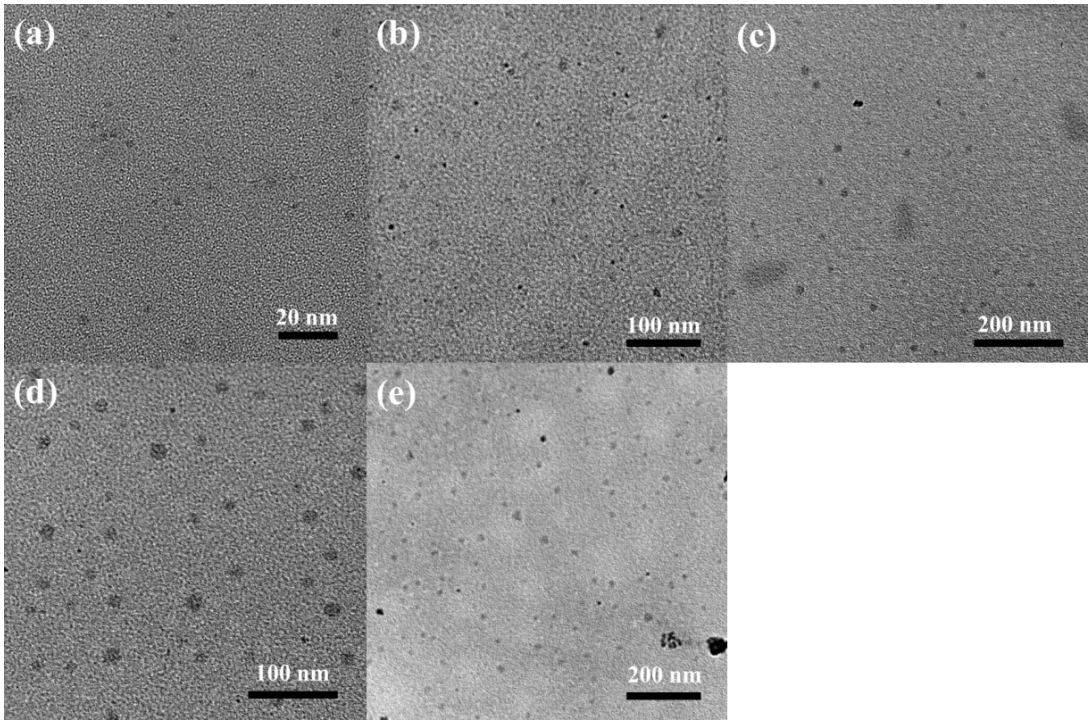



**Fig. S10** TEM images of PEG(P*t*BA)$_{20}$ with different P*t*BA chain length: (a) PEG(P*t*BA$_5$)$_{20}$; (b) PEG(P*t*BA$_{12}$)$_{20}$; (c) PEG(P*t*BA$_{29}$)$_{20}$; (d) PEG(P*t*BA$_{52}$)$_{20}$; (e) PEG(P*t*BA$_{63}$)$_{20}$.

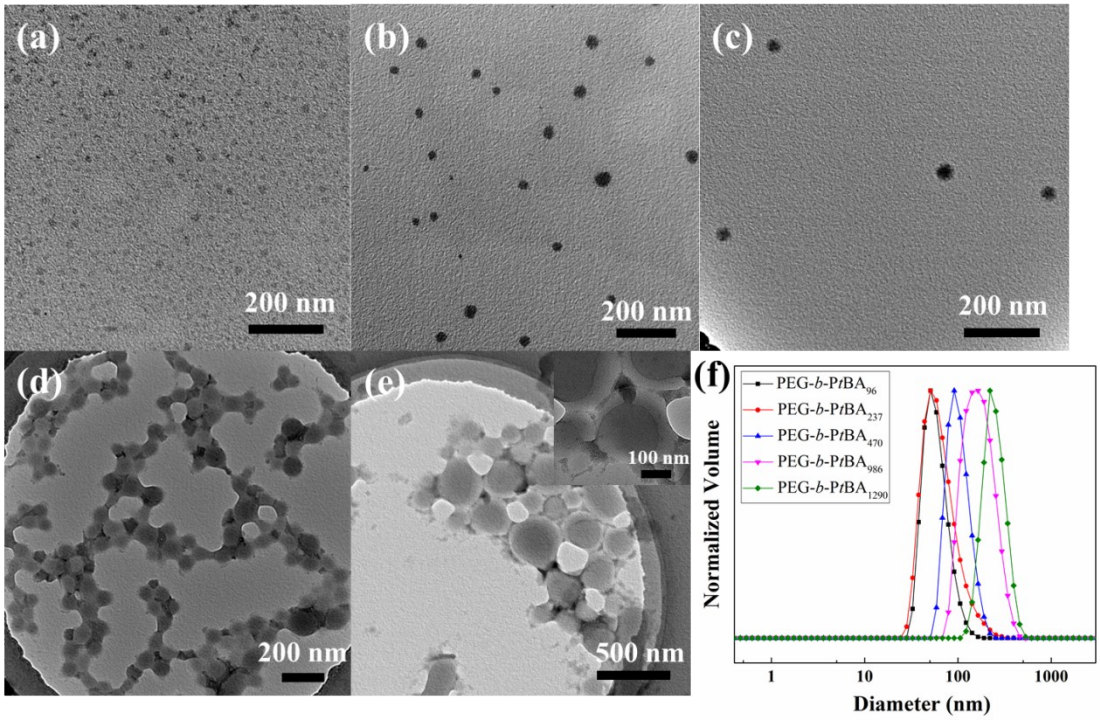



**Fig. S11** TEM images of self-assembly morphologies of PEG-*b*-P*t*BA with different P*t*BA chain length: (a) PEG-*b*-P*t*BA$_{96}$; (b) PEG-*b*-P*t*BA$_{237}$; (c) PEG-*b*-P*t*BA$_{470}$; (d) PEG-*b*-P*t*BA$_{986}$; (e) PEG-*b*-P*t*BA$_{1290}$; (f) Normalized volume distribution of the PEG-*b*-P*t*BA assemblies.

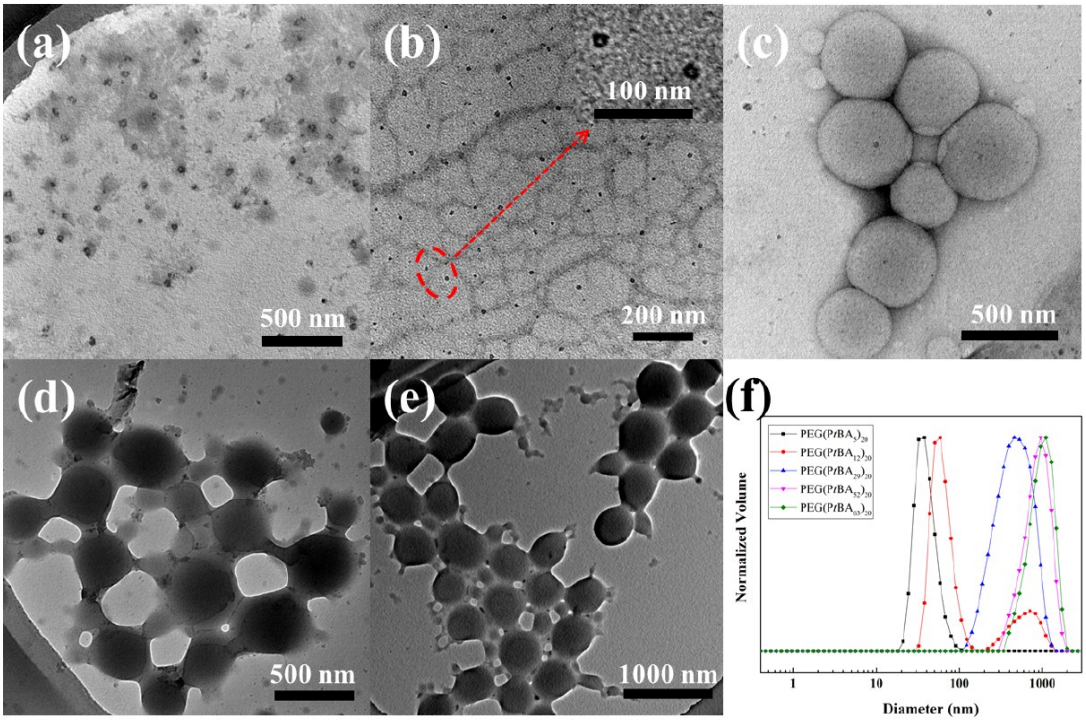



**Fig. S12** TEM images of self-assembly morphologies of SCTSUNPs: (a) PEG(P*t*BA$_5$)$_{20}$; (b) PEG(P*t*BA$_{12}$)$_{20}$; (c) PEG(P*t*BA$_{29}$)$_{20}$; (d) PEG(P*t*BA$_{52}$)$_{20}$; (e) PEG(P*t*BA$_{63}$)$_{20}$; (f) Normalized volume distribution of the PEG(P*t*BA)$_{20}$ assemblies.

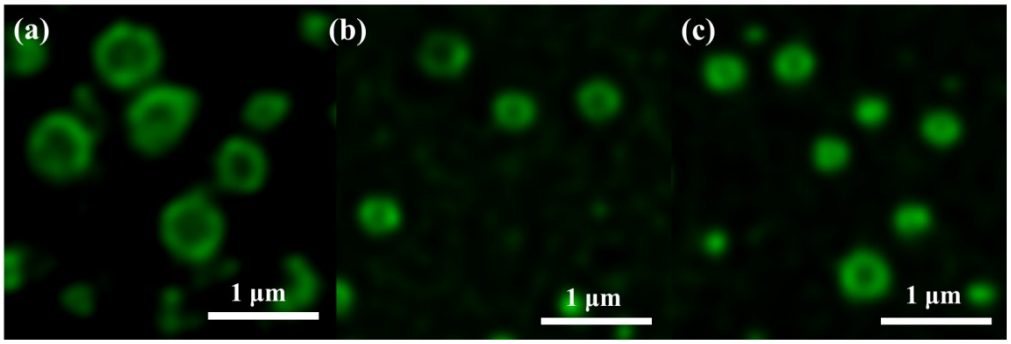



**Fig. S13** CLSM images of self-assembly morphologies of (a) PEG(P*t*BA$_{63}$)$_{20}$, (b) PEG(P*t*BA$_{52}$)$_{20}$ and (c) PEG-*b*-P*t*BA$_{1290}$.

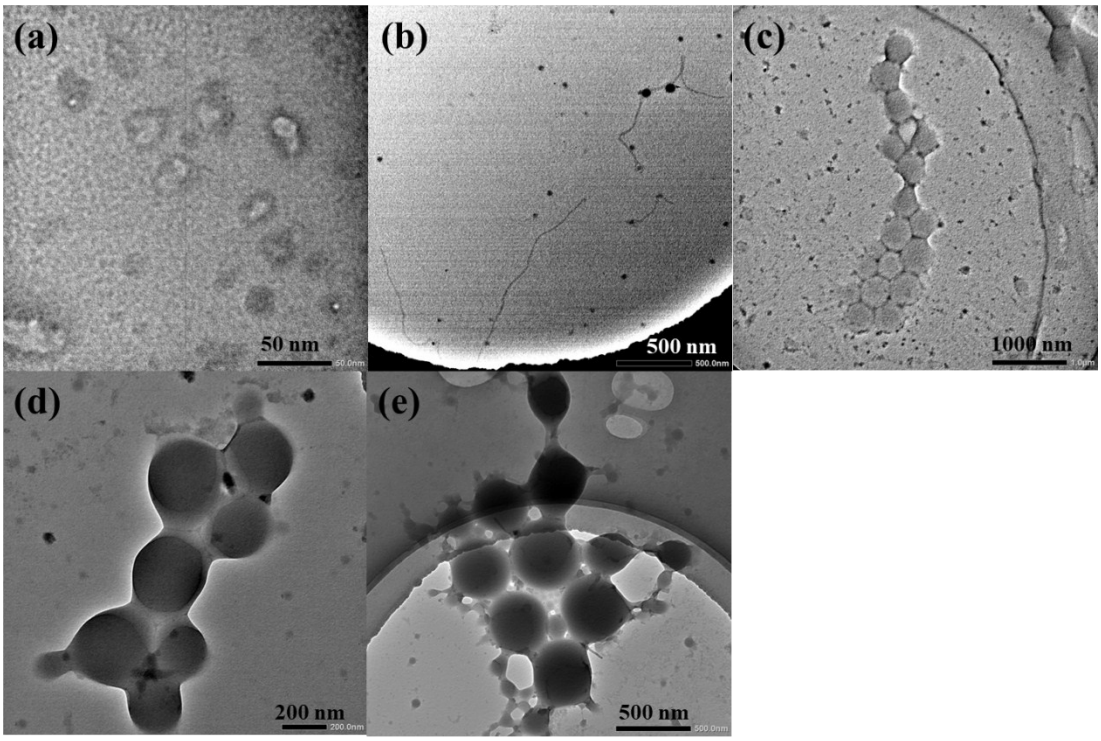



**Fig. S14** TEM images of self-assembly morphologies of SCTSUNPs after incubation at 4 °C for 6 months: (a) PEG(P*t*BA$_5$)$_{20}$; (b) PEG(P*t*BA$_{12}$)$_{20}$; (c) PEG(P*t*BA$_{29}$)$_{20}$; (d) PEG(P*t*BA$_{52}$)$_{20}$; (e) PEG(P*t*BA$_{63}$)$_{20}$.

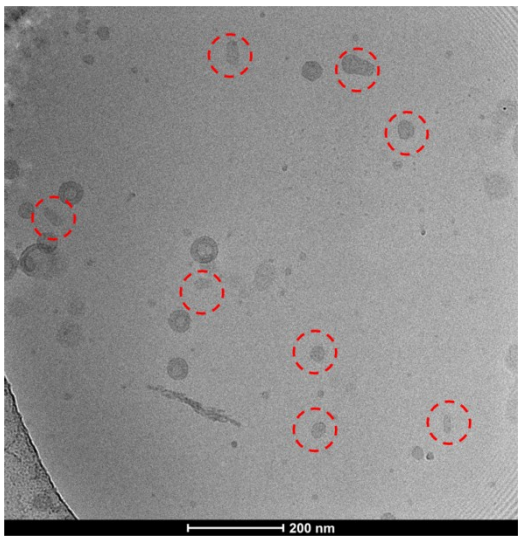



**Fig. S15** *Cryo*-TEM images of the self-assembly morphologies of PEG(P*t*BA$_5$)$_{20}$. Inside the red coil should be the non-vertical view images of torodial micelles.

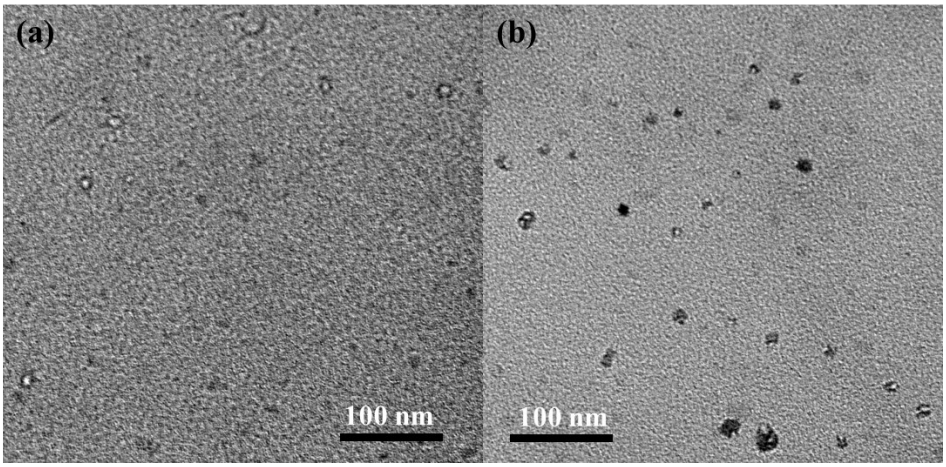



**Fig. S16** TEM images of assemblies of PEG(P*t*BA$_5$)$_{20}$ formed at 20% water content. The sample was prepared as following: One droplet of aggregate solution of PEG(P*t*BA$_5$)$_{20}$ formed in DMF/$H_2O$ (8/2; v/v) was dropped on a copper grid coated with carbon film, then the copper grid was transferred to liquid nitrogen and froze the sample immediately. The vitrified sample was dried by lyophilization and then observed on TEM directly.