Supporting information

Monofunctional Polymer Nanoparticles Prepared through Intramolecularly Cross-linking the Polymer Chains Sparsely Grafted on the Surface of Sacrificial Silica Spheres

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S1. Experimental section

(1) Materials

Poly(ethylene oxide) monomethyl ether (PEO_{113}-OH, M_n = 5000), 2-bromoisobutyryl bromide (98%), cinnamoyl chloride (98%), (3-Aminopropyl) triethoxysilane (APTES, ≥98%), *N*,*N*′,*N*″,*N*‴-pentamethyldiethylenetriamine (PMDETA, 99%), 2, 2'-bipyridine (bpy, ≥99%) were purchased from Aldrich and used as received. 4, 4'-Azobis(4-cyanovaleric acid) (ACVA, 98%), 2-bromoethanol (97%) from J&K Scientific and hexylamine (99%) from Aladdin were used as received. 2-Hydroxyethyl methacrylate (HEMA, 98%, Aldrich) was purified twice by passing the monomer through a column filled with basic alumina to remove the inhibitor. 2-(Dimethylamino) ethyl methacrylate (DMAEMA, 99%, Aladdin) was purified by distillation under reduced pressure. Propargyl bromide (80 wt % in toluene, Aldrich) was dried with CaH_2 overnight and then distilled just before use. The RAFT agent, 4-cyanopentanoic acid dithiobenzoate (CPADB), was synthesized according to the method of McCormick.[1] CuCl, CuBr were washed with acetic acid, followed by washing with methanol to remove impurities. Pyridine was dried by refluxing with CaH_2 for a prolonged time prior to use. DMF was dried with $CaSO_4$ overnight and then distilled under reduced pressure. Tetraethyl orthosilicate (TEOS, 98%), Hydrofluoric acid (≥40%), sodium azide and other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received.

(2) Synthesis of (N, N-dipropargyl-3-aminopropyl) triethoxysilane (DPAPS)

DPAPS was prepared according to the procedure reported by Choi et al.[2] For its preparation, APTES (2.336 mL, 0.01 mol) and K_2CO_3 (3.04 g, 0.022 mol) were mixed in 30 mL of dry DMF. 5 min after the mixing, propargyl bromide (1.7 mL, 0.022 mol) was added slowly. The mixture was refluxed for 4 h. Afterwards, 30 mL of water was added. The reaction solution was extracted with diethyl ether until the ether layer was colorless. Then, the extract was dried over anhydrous $MgSO_4$ overnight, filtered and concentrated. The impurities and solvent residue was further removed by vacuum distillation.

The product is DPAPS, according to ^{1}H NMR and ^{13}C NMR characterizations (Figure S1).

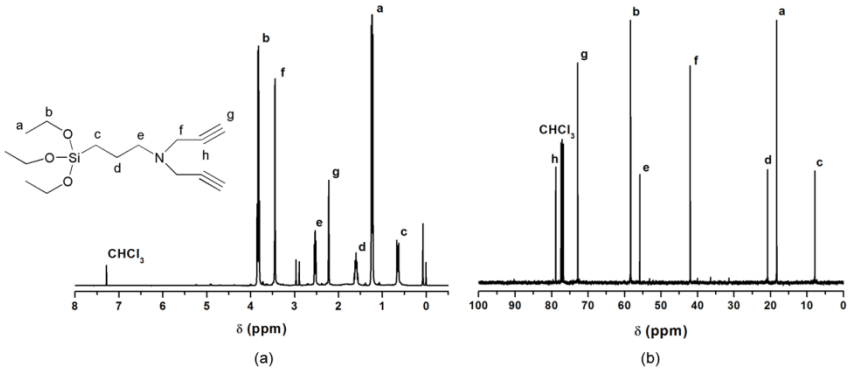

Figure S1 ^{1}H NMR (a) and ^{13}C NMR (b) of DPAPS in $CDCl_3$.

(3) Polymer synthesis

Synthesis of poly(ethylene oxide)-block-poly(2-cinnamoyloxyethyl methacrylate)-N_3 (PEO-*b*-PCEMA-N_3)

PEO-Br macro-initiator was synthesized by esterification of PEO_{113}-OH with 2-bromoisobutyryl bromide.[3] Poly(ethylene oxide)-block-poly(2-hydroxyethyl methacrylate) (PEO-*b*-PHEMA), the precursor of PEO-*b*-PCEMA, was synthesized via ATRP of HMEA in methanol using the PEO-Br (M_n = 5000, M_w/M_n = 1.05) as macro-initiator, and CuCl/2, 2'-bipyridine (bpy) as catalyst.[4] Typically, for preparing PEO-b-PHEMA, a clean and dry ampoule charged with CuCl (10 mg, 0.101 mmol) was treated by three cycles of evacuation and back-filled with argon. Then, 5 mL HEMA (0.041 mol), 3 mL methanol containing 300 mg of the PEO-Br macroinitiator (0.06 mmol) and 30 mg bpy (0.167 mmol), and 2 mL methanol were added sequentially into the ampoule. The mixture was deoxygenated by five freeze-pump-thaw cycles, and then the flask was sealed and immersed in a preheated oil bath at 40 ^{o}C. The polymerization was allowed to proceed for 9 h, and the reaction was quenched via exposure to air and dilution with methanol. The resultant polymer solution was filtered through a column filled with neutral alumina to remove the copper complex. The polymer was precipitated twice from a methanol solution into cold diethyl ether and dried under vacuum.

To the resultant bromo-terminated PEO-*b*-PHEMA (100 mg) dissolved in 2 mL dry DMF, excess sodium azide (10 mg) was added. The mixture was stirred at 50 ^{o}C for 24 h. Then, after the reaction mixture was cooled to 25 ^{o}C, excess cinnamoyl chloride (200 mg) and 5 mL dry pyridine were added into the reaction mixture. The resultant mixture was stirred for 2 days at 25 ^{o}C and subsequently filtered. The supernatant was added to excess diethyl ether to precipitate PEO-*b*-PCEMA-N_3. The block copolymer was dried at room temperature under vacuum. Figure S2 shows the ^{1}H NMR spectra of PEO-*b*-PHEMA-Br in d_6-DMSO (a) and the resultant PEO-*b*-PCEMA-N_3 in $CDCl_3$ (b). The ethylene signals of HEMA appearing in spectrum (a) at 3.89 and 3.57 ppm disappear completely in spectrum (b), accompanied by the appearance of the ethylene signal of CEMA at 4.22 ppm. This demonstrates complete cinnamoylation of PHEMA. The number averaged molecular weight of PEO-*b*-PCEMA-N_3 was determined by ^{1}H NMR to be 87.2 kg/mol (Figure S2(b)) according to the process previously reported.[5] According to the relative signal intensities of CEMA and EO

(repeating units of the PCEMA and PEO blocks, respectively) in spectrum (b), CEMA/EO number ratio of the obtained PEO-*b*-PCEMA was calculated to be 2.80. Therefore, the polymerization degree of PCEMA in PEO-*b*-PCEMA is 316 (2.80*113 (polymerization degree of the PEO block is 113)), and the number-averaged molecular weight of the block copolymer ($M_{n,\ NMR}$) is 87.2 kg/mol. The successful preparation of PEO-*b*-PCEMA-N_3 is confirmed by the successful grafting of the block copolymer onto surface of the silica spheres.

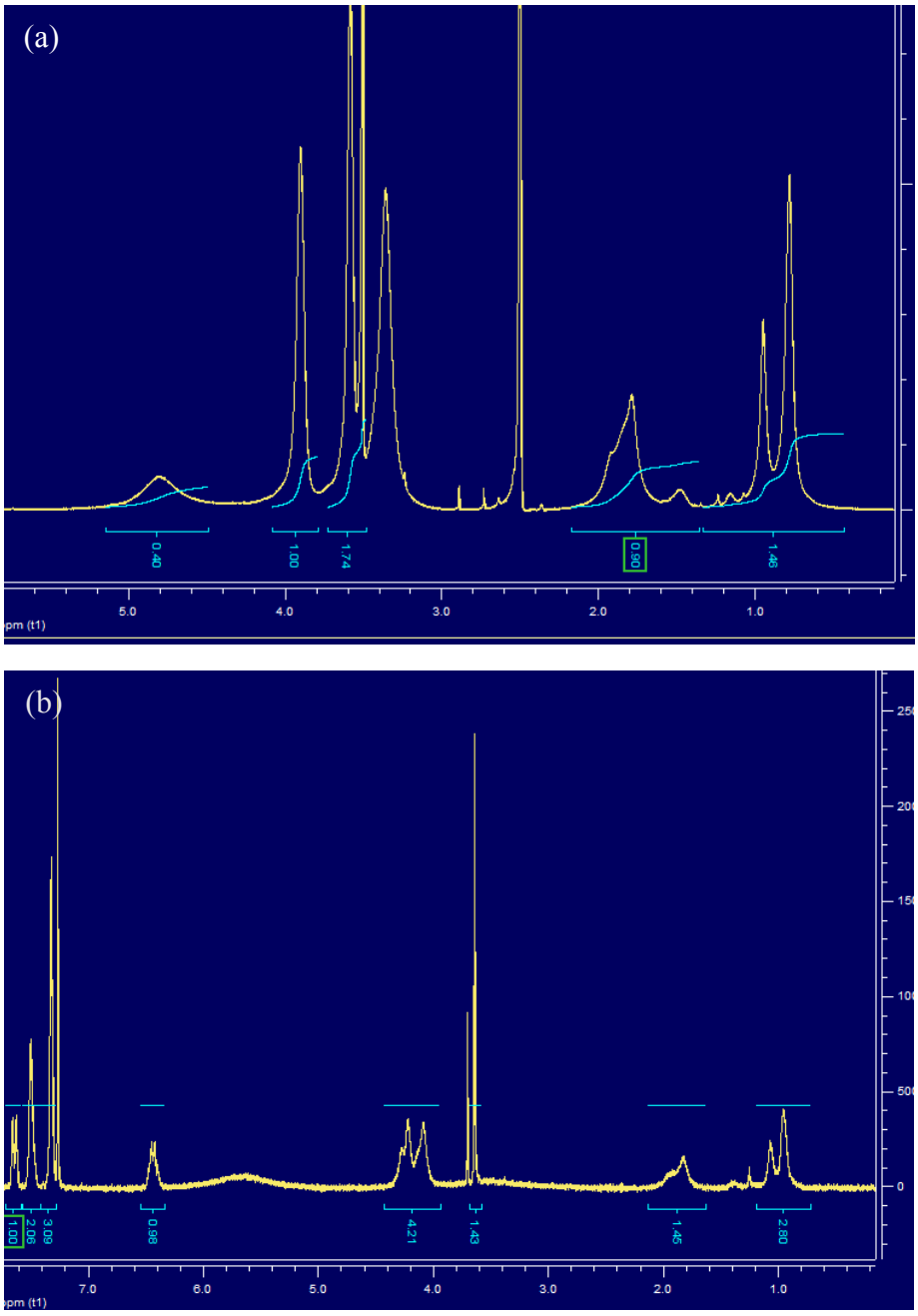

Figure S2. ^{1}H NMR spectra of PEO-*b*-PHEMA in d_6-DMSO (a) and PEO-*b*-PCEMA-N_3 in $CDCl_3$ (b).

Synthesis of poly(2-(dimethylamino) ethyl methacrylate)-N_3 (PDMAEMA -N_3)

PDMAEMA-N_3 was synthesized via CPADB initiated RAFT polymerization of

DMAEMA, followed by esterification of the α-end carboxyl group with 2-azidoethanol (Figures S3 and S4).

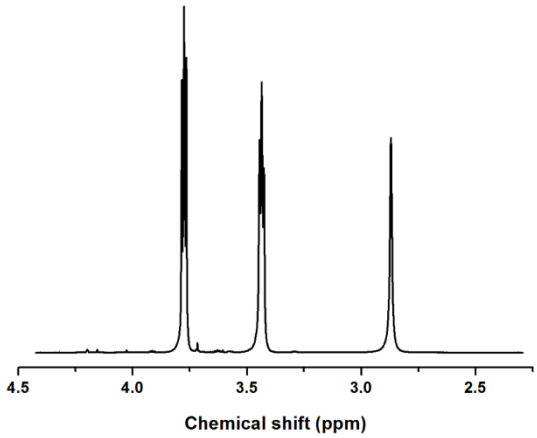

Figure S3 ^{1}H NMR spectrum of 2-azidoethanol in $CDCl_3$.

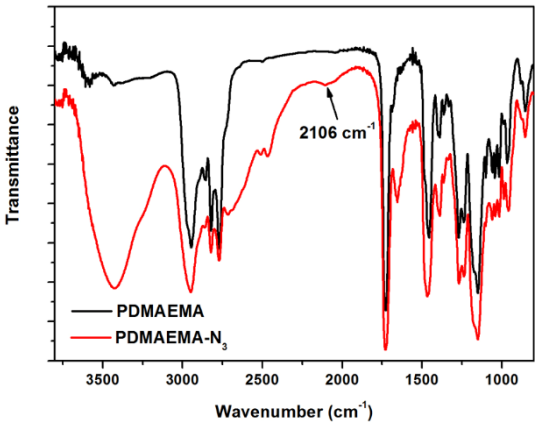

Figure S4 FTIR spectra of PDMAEMA before and after esterification of the α-end carboxyl group with 2-azidoethanol.

Typically, for the RAFT polymerization of DMAEMA, a solution of CPADB (35.5 mg, 0.1268 mmol), DMAEMA (4.00 g, 25.4 mmol), and ACVA (7.1 mg, 0.0254 mmol) in 13 mL of dioxane were added to a 50 mL ampoule. The solution was purged with argon for approximately 1 h, and then the ampoule containing the solution was sealed and placed in a preheated oil bath at 70 °C. The reaction was lasted for 24 h. Afterwards, it was stopped by placing the ampoule in liquid nitrogen followed by exposure to air. The product was purified by precipitation into hexane and dried under vacuum.

The as-prepared PDMAEMA (500 mg, $M_{n,\ GPC}$ = 5.5 kg/mol, PDI=1.23), 2-azidoethanol (23.7 mg, prepared by nucleophilic substitution of 2-bromoethanol with sodium azide[6]), and dicyclohexylcarbodiimide (DCC, 56.2 mg) were dissolved in 15 mL dry CH_2Cl_2. The mixture was cooled to 0 °C in an ice bath, and added with 4-dimethylamiopryidine (DMAP, 10 mg). Then, the reaction system was warmed to 25 °C and stirred at that temperature for 3 days. Followed by removal of most CH_2Cl_2 in vacuo, dialysis against methanol, precipitation into petroleum ether, and dried under vacuum, PDMAEMA-N_3 was prepared. The FTIR spectrum of as-purified polymer is

presented in Figure S4, in which the signal at 2106 cm^{-1} confirm the successful preparation of the PDMAEMA-N_3. It is noteworthy that after esterification with 2-azidoethanol, the polymer color remains pink, implying that the dithioester group at the ω-end is undisturbed (Figure S5). As described below, after "click" reaction with PMNs, the dithioester groups were converted to thiol groups, which can be used for coupling of PMNs. The molecular weight of PDMAEMA-N_3 is 5.5 kg/mol, according to GPC measurement (Figure S6).

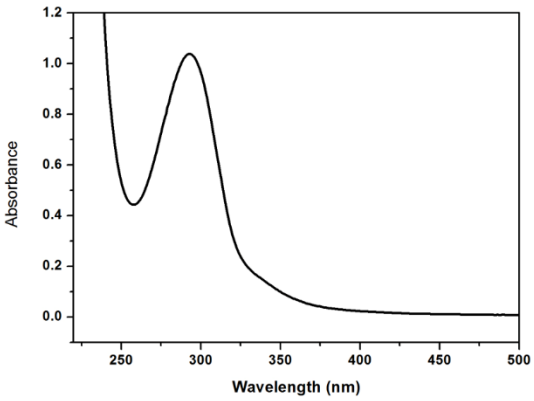

Figure S5. UV spectra of PDMAEMA after the esterification reaction.

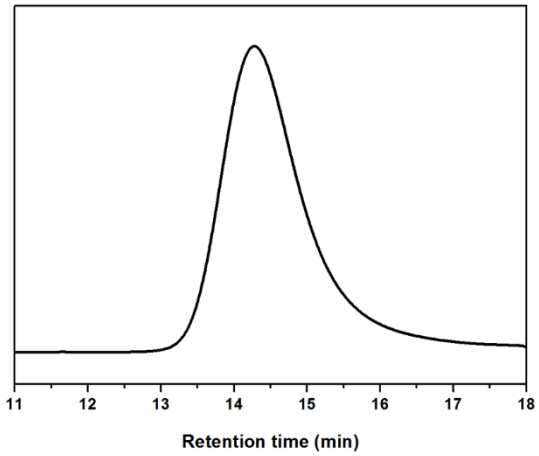

Figure S6 GPC curve of PDMAEMA.

(4) Sparse grafting of PEO-*b*-PCEMA-N_3 on silica spheres

The synthesis of silica spheres and their surface modification with the Y-shaped functional molecule containing two divergent propargyl groups were conducted by following processes: To the mixture of 10 mL 25-28% aqueous solution of ammonium hydroxide and 200 mL ethanol, tetraethyl orthosilicate (6 mL) was added dropwise, and then the system was stirred for 24 h. Then, DPAPS (400 μL) and APTES (400 μL) were added and the resultant mixture was stirred for an additional 24 h. Subsequently, the mixture was heated to reflux and kept at the reflux temperature for 1 h, and then cooled. The cooled mixture was purified by washing and centrifugation cycles (washed twice with ethanol, and then twice with DMF) to give the functionalized silica spheres dispersed in DMF; APTES of the same volume as DPAPS was added to increase dispersibility of the spheres in DMF. The concentration of the dispersed silica spheres in DMF was determined by gravimetry of the dried

sample from a certain volume of DMF suspension. The size of the silica sphere is about 85 nm.

Then, PEO-*b*-PCEMA-N_3 was grafted to the surface of the as-functionalized silica spheres via Cu (I) catalyzed azide-alkyne cycloaddition reaction. At first, the functionalized silica spheres (about 200 mg) in 20 mL DMF was ultrasonicated for 15 min. Then, 20 mg PEO-*b*-PCEMA-N_3 and 8 mg CuBr were added. The mixture was purged with argon for 20 min, added with PMDETA (13 μL), and purged with argon again for another 30 min. Then, the flask was sealed and immersed to a preheated oil bath at 50 ^{o}C. The reaction mixture was stirred vigorously to avoid sedimentation of the silica spheres. After 3 days, the reaction was terminated via exposure to air. The resultant mixture was purified by three cycles of washing with DMF and centrifugation to remove the copper complex and the unreacted polymer. According to UV-vis measurement, in the supernatant obtained after the last centrifugation, absorbance of CEMA units at 275 nm is negligible, indicating that the unreacted polymer has been completely removed from the silica spheres. The polymer grafted silica were finally dispersed in DMF at a silica concentration of about 10 mg/mL (the silica concentration is measured by the gravimetry method, as described above). For the FTIR and TGA characterizations, the grafted spheres were separated from the DMF suspension by centrifugation, thoroughly washed with ethanol, and then dried under vacuum.

(5) Acquirement of polymeric monofunctional nanoparticles (PMNs)

20 mL DMF suspension of the polymer grafted silica (about 10 mg/mL) was directly exposed to UV light that had passed through a 254 nm cutoff filter from a 500W mercury lamp (CHF-XM-500W). The irradiation was continued for 3 h under vigorous stirring to achieve sufficient cross-linking of the PCEMA block and meanwhile avoid sedimentation of the silica spheres. Then, to the vigorously stirred suspension of the UV-irradiated spheres (20 mL), 10 mL 40% HF aqueous solution was added to etch the silica spheres for 12 h to remove them. Subsequently, the resultant mixture was dialyzed against THF/H_2O (1/1, v/v), and then against pure THF to get rid of the impurities and switch the solvent to THF, in which the PMNs can be dispersed individually.

(6) Evaluating the stability of the polymer during the template removal process

As mentioned before, the PEO-Br macro-initiator for preparing PEO-*b*-PHEMA was synthesized by esterification of PEO_{113}-OH with 2-bromoisobutyryl bromide. Besides, PEO-*b*-PCEMA was prepared by esterification between the HEMA units and cinnamoyl chloride. There are ester bonds in the PEO-*b*-PCEMA, which is the precursor of the PMNs. Therefore, the PMNs have the ester bonds. The stability of the ester bonds during the etching reaction to remove the silica spheres is important for the stability of the PMNs. Obviously, the stability of PMNs can be evaluated by measuring the stability of the ester bonds in the precursor PEO-*b*-PCEMA, and the stability of the ester bonds in the single chain nanoparticles (SCNPs) prepared from the PEO-*b*-PCEMA precursor during the template removal process. For preparing the SCNPs, 20 mL DMF solution of PEO-*b*-PCEMA at a concentration of 1.0 mg/mL

was irradiated by UV (254 nm) for 3 h, which led to the SCNPs, as confirmed by our previous study.[5] The block copolymer precursor and the SCNPs were treated, respectively, with the same processes under the same conditions as those for the etching and the dialysis (20 mL DMF solution of the SCNPs or the precursor PEO-*b*-PCEMA at a concentration of 1 mg/mL was mixed with 10 mL 40% HF aqueous solution. After the mixture was stirred at room temperature for 12 h, it was dialyzed against THF/H_2O (1/1, v/v), and then against pure THF). After concentration, the solvent residue was removed by distillation under vacuum and the as-treated SCNPs and PEO-*b*-PCEMA were dissolved in $CDCl_3$, respectively, for ^1H NMR measurements.

Figure S7 shows the ^1H NMR spectra of the SCNPs (b) and the precursor PEO-*b*-PCEMA (c) after the treatments, compared with the spectrum of the precursor PEO-*b*-PCEMA without any treatment (a). Polymer concentrations of the three samples are the same, and CH_2Cl_2 with the same concentration was added as internal standard for quantitative analysis. The intensity ratio of PEO signal at 3.65 ppm to CH_2Cl_2 signal at 5.3 ppm is 0.275 in spectrum (b), which is very close to that in spectrum (a) (0.277). This indicates that after the stability experiments, the ester bonds connecting the PEO block and the cross-linked PCEMA block of the SCNPs are unaffected; if the PEO chains had been cleaved, the cleaved PEO chains should have been removed during the dialysis processes. The intensity ratio of PEO signal to PCEMA signal is 0.34 in spectrum (c), close to that in spectrum (a) (0.33), indicating that the ester bonds of the PCEMA side groups are unaffected during the stability experiments. Therefore, we can conclude that the PMNs are stable during the template removal process.

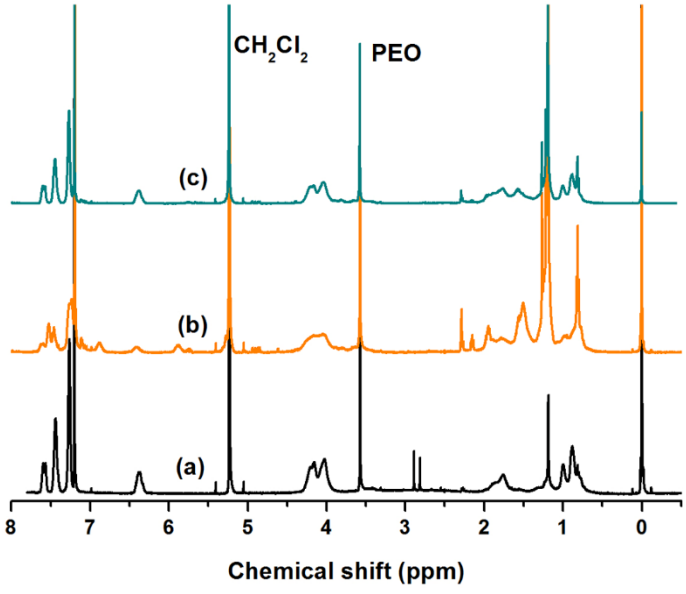

Figure S7 ^1H NMR spectra of the precursor PEO-*b*-PCEMA without any treatment (a), the as-treated SCNPs (b) and the as-treated PEO-*b*-PCEMA precursor (c) in $CDCl_3$. CH_2Cl_2 was used as the internal standard, the concentration of CH_2Cl_2 in $CDCl_3$ is 1.25 μL/0.6 mL.

(7) "Click" reaction of PMNs with PDMAEMA-N_3 to form PMN-Ps, and the

coupling of PMN-Ps

For forming PMN-Ps, PDMAEMA-N_3 (2 mg, $M_{n,\ GPC}$ =5.5 kg/mol, PDI = 1.23) dissolved in 100 μL methanol was added to 3 mL THF solution of PMNs, and the mixture was then transferred to an ampoule charged with 1 mg CuBr and 5 mL THF. Subsequently, PMDETA (1.5 μL) was added. The mixture was purged with argon for 30 min, and then the ampoule was sealed and placed in an oil bath at 30 ^{o}C. The reaction lasted for 3 days and then terminated via exposure to air. The mixture solution was concentrated and filtered through a mini column filled with neutral aluminum to remove the copper complex, using $CHCl_3$ as eluent. The excess PDMAEMA-N_3 was separated from the PDMAEMA modified PMNs (PMN-Ps) by chromatographic fractionation. The as-obtained PMN-Ps were finally dispersed in $CHCl_3$ after concentration.

The ω-end dithioester group of the PDMAEMA in PMN-Ps was used for the coupling of the PMN-Ps. Specifically, excess hexylamine (7.26 μmol in 147 μL $CHCl_3$) was added to 2 mL $CHCl_3$ solution of the PMN-Ps. The reaction system was stirred in air at room temperature for 24 h. Then, DMSO (300 μL) was added to 2 mL $CHCl_3$ solution of PMN-Ps, and the reaction mixture was stirred in air for additional 2 weeks. The thiols from the aminolysis of dithioesters by hexylamine were oxidized by the oxygen in air and dimethylsulfoxide (DMSO) to form disulfide linkage to realize the coupling.[7]

(8) Characterization Methods and Instruments

^{1}H and ^{13}C NMR measurements were recorded with a Bruker Advance 400 spectrometer and a Bruker DMX 500 spectrometer. Gel permeation chromatography (GPC) analysis was carried out with a Waters Breeze 1525 GPC analysis system with two PL mix-D columns, using DMF with 0.5 M LiBr as eluent at the flow rate of 1 mL/min at 80^{o}C, and PEO calibration kit (purchased from TOSOH) as the calibration standard. Fourier transform infrared spectra (FTIR) were recorded on a NEXUS-470 FTIR spectrometer. Thermal gravimetric analysis (TGA) was conducted with a PerkinElmer Pyris 1 TGA. Transmission electron microscopy (TEM) experiments were carried out on a Philips CM120 microscope operated at an accelerating voltage of 80 kV. Generally, for preparing TEM specimen, 4 μL of the sample solution was deposited onto a carbon-coated copper grid, followed by drying at room temperature. The specimen were stained by RuO_4 for 40 min. Dynamic laser light scattering (DLS) measurement was conducted on an ALV-5000 laser light scattering spectrometer at a fixed scattering angle of 90°. Before the measurements, the samples were all filtered through 450 nm PTFE filters to remove dust.

S2.

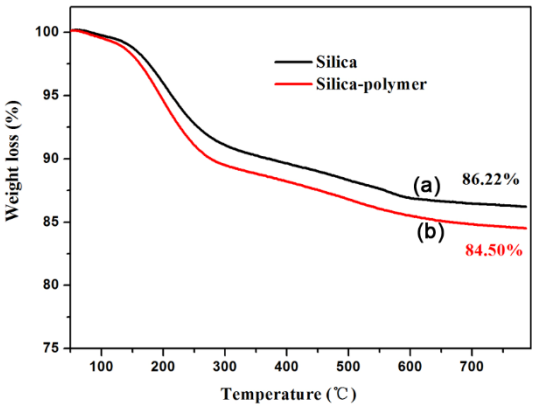

Figure S8 TGA curves of the silica spheres (a) before and (b) after grafting of PEO-*b*-PCEMA chains.

For the TGA measurements, the silica spheres of the same batch were divided into two parts: one part was used as the control, and the other part was used for the grafting. Before the TGA measurements, both the sample and the control were dried thoroughly. Therefore, the weight percentage of the grafted polymer could be estimated by the difference in the weight losses detected by TGA. Nevertheless, the amount estimated by TGA may have some uncertainty since the TGA curves are complicated: the weight losses continue to increase when the temperature is higher than 500 °C, which may lead to the uncertainty.

S3. Calculation of the graft density of PEO-*b*-PCEMA on the silica nanoparticles.

For the silica nanoparticles with a total weight of M_s, the total surface area of the silica is: $\frac{6M_s}{\rho D}$

ρ is the density of the silica nanoparticles, and D is the average diameter of the silica nanoparticles. On the silica nanoparticles with a total weight of M_s, if the grafted polymer has a total weight of M_p, the total chain number of the grafted polymer is:

$$\frac{M_p N_A}{M_{n,NMR}}$$

N_A is Avogadro constant, $M_{n,NMR}$ is the molecular weight of PEO-*b*-PCEMA. According to the two formulas above, the average graft density of the polymer can be calculated:

$$\frac{6M_s M_{n,NMR}}{\rho D M_p N_A}$$

S4. Calculation of the area one PEO-*b*-PCEMA chain occupied on the surface of the silica spheres.

The average hydrodynamic radius $<R_h>$ of the PEO-*b*-PCEMA chains in DMF is measured to be ~ 5 nm by DLS (Figure S9). Assuming that the grafted polymer chain adopts a random coil conformation in the good solvent DMF, the radius of gyration (R_g) of the PEO-b-PCEMA chain is calculated to be about 7.5 nm, as for random coils of linear polymer chains in the good solvents, R_g/R_h = ~ 1.5.[8] Therefore, a PEO-*b*-PCEMA chain on the silica surface would occupy an area of no more than 176 nm^2 (i.e., $\pi*R_g^2$).

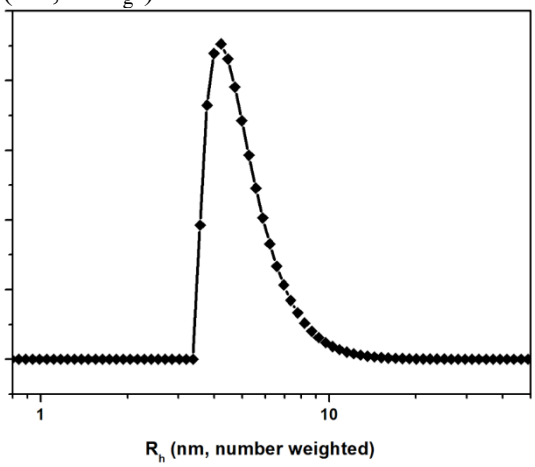

Figure S9 DLS profile of the precursor PEO-*b*-PCEMA in DMF.

S5.

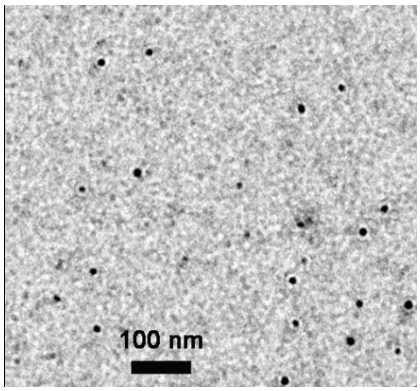

Figure S10 TEM image of the PMN-Ps in $CHCl_3$, stained by RuO_4 for 40 min.

Reference

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