Supporting Information for

Synthesis of a Cationic Supramolecular Block Copolymer with Covalent and Noncovalent Polymer Blocks for Gene Delivery

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1. Synthesis Details

1.1 Synthesis of *β*-CD-monosubstituted poly(ethylene glycol) (PEG-CD)

1.1.1 Synthesis of Monoalkynyl-terminated PEG (PEG-Alkyne)

The synthesis procedure of PEG-Alkyne was as follows:[1] PEG_{113}-OH (7.5 g, 1.5 mmol) was dissolved in toluene (90 mL) at 60 ^{o}C. After azeotropic distillation of 15-20 mL of toluene under vacuum to remove traces of water, sodium hydride (0.108 g, 4.5 mmol) was added to the solution under stirring. After H_2 evolution for about 15 min, propargyl bromide (0.665 mL, 7.5 mmol) in 10 mL of dry toluene was added dropwise to the above mixture. The resulting solution was then stirred at 60 ^{o}C for 18 h. The insoluble salts were filtered before the filtrates were evaporated to dryness. Then the obtained solid was dissolved in 50 mL CH_2Cl_2. After that the above solution was extracted with saturated aqueous $NaHCO_3$ solution (15 mL) for three times, the organic phase was dried over anhydrous Na_2SO_4 and treated with activated charcoal. After filtration, the solution was precipitated into a large number of *n*-hexane. The dissolution and precipitation cycle was repeated three times. After drying in a vacuum oven overnight at room temperature, PEG-Alkyne was obtained as a white solid (6.24 g, yield: 83%).

^{1}H NMR ($CDCl_3$, 400 MHz) (***Figure S1***): δ_H (ppm) = 4.2 (2H, -O*C*H_2C≡CH), 3.7 (450H, -O*C*H_2*C*H_2O-), 3.4 (3H, *C*H_3O-), and 2.4 (1H, -OCH_2C≡*CH*). **^{13}C NMR** ($CDCl_3$, 100 MHz) (***Figure S2*)**: δ_C (ppm) = 58.33, 58,97, 69.03, 70.52, 71.88, 74.62, 79.61. **FTIR** (KBr) (***Figure S7***): ν (cm^{-1}): 3256 (C≡C). **GPC** (***Figure S8*)**: 5.025 kDa with PDI of 1.02, which is consistent with the value of 4.963 kDa determined by NMR.

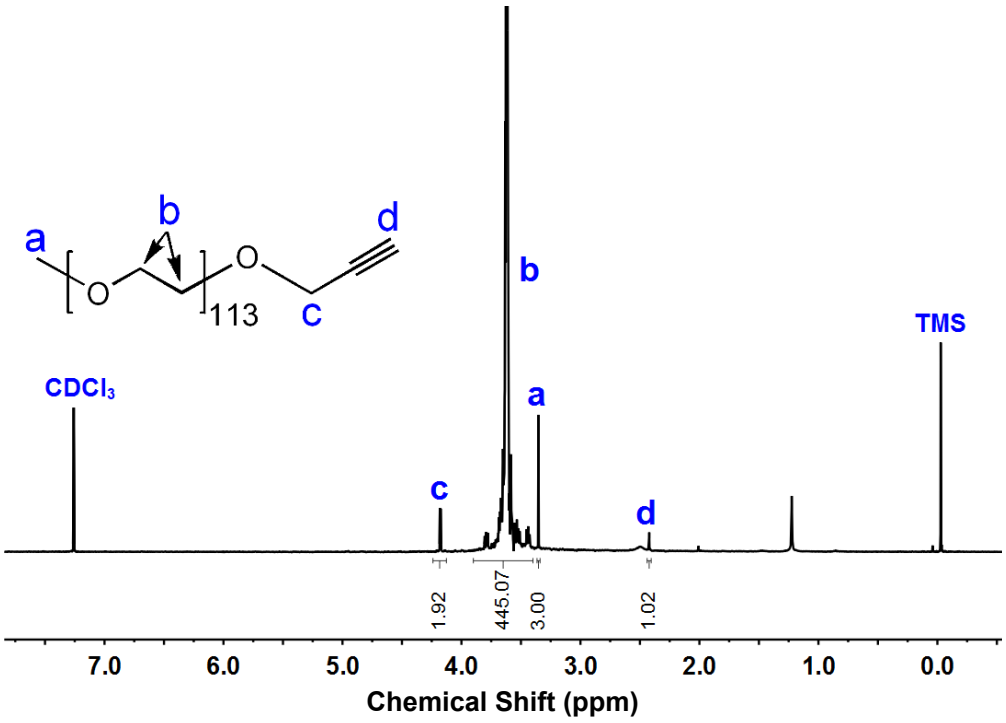

Figure S1. ^{1}H NMR spectrum of PEG-Alkyne in $CDCl_3$.

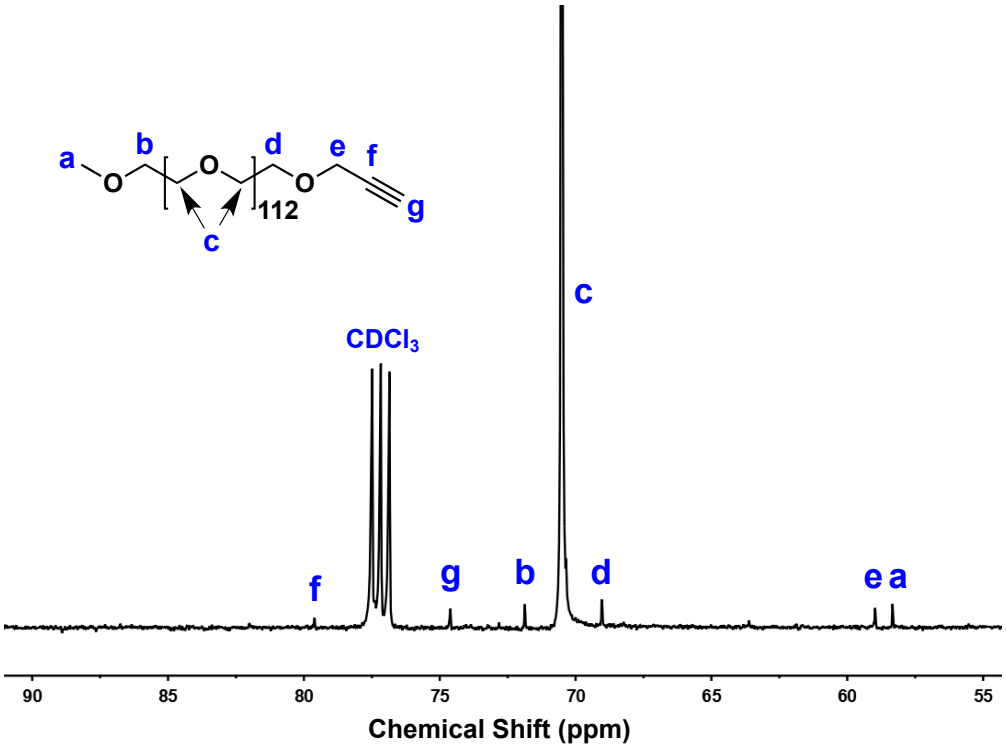

Figure S2. ^{13}C NMR spectrum of PEG-Alkyne in $CDCl_3$.

1.1.2 Synthesis of mono-(6-*O*-(*p*-tolylsulfonyl))-*β*-CD (*β*-CD-OTs)

Mono-(6-*O*-(*p*-tolylsulfonyl))-*β*-CD (*β*-CD-OTs) was prepared according to our previous report.[2] Anhydrous *β*-CD (50.0 g, 44.0 mmol) was dissolved in 500 mL of 0.4 M aqueous sodium hydroxide and cooled down to 0 ^{o}C. Subsequently, *p*-toluenesulfonyl chloride (35.0 g, 184 mmol) was slowly added under vigorous stirring over 10 min to the solution. The resulting suspension was stirred for another 30 min at below 5 ^{o}C, and then filtered quickly. The filtrate was neutralized to pH 8.5 with 1 M HCl solution and stirred for another 1 hr. The resultant precipitate was filtered off, washed three times with deionized water and dried at 60 ^{o}C for 48 h. Yield: 20.0 g (15.5 mmol, 35%).

^{1}H NMR (DMSO-d_6, 400 MHz) (***Figure S3***): δ_H (ppm) = 2.41 (s, Ph-CH_3, 3H), 3.16-3.75 (m, H-2,3,4,5,6, 42H), 4.11-4.55 (m, OH-6, 6H), 4.72-4.90 (m, H-1, 7H), 5.25-6.25 (br, OH-2,3, 14H), 7.40 (d, *J*= 8.6 Hz, H-*Ph*-CH_3, 2H), 7.75 (d, *J* = 7.8 Hz, H-*Ph*-SO_3, 2H). **^{13}C NMR** (DMSO-d_6, 100 MHz): δ_C (ppm) = 21.90, 60.57, 72.70, 73.07, 73.37, 73.73, 82.15, 102.59, 128.25, 130.57, 133.28, 145.52.

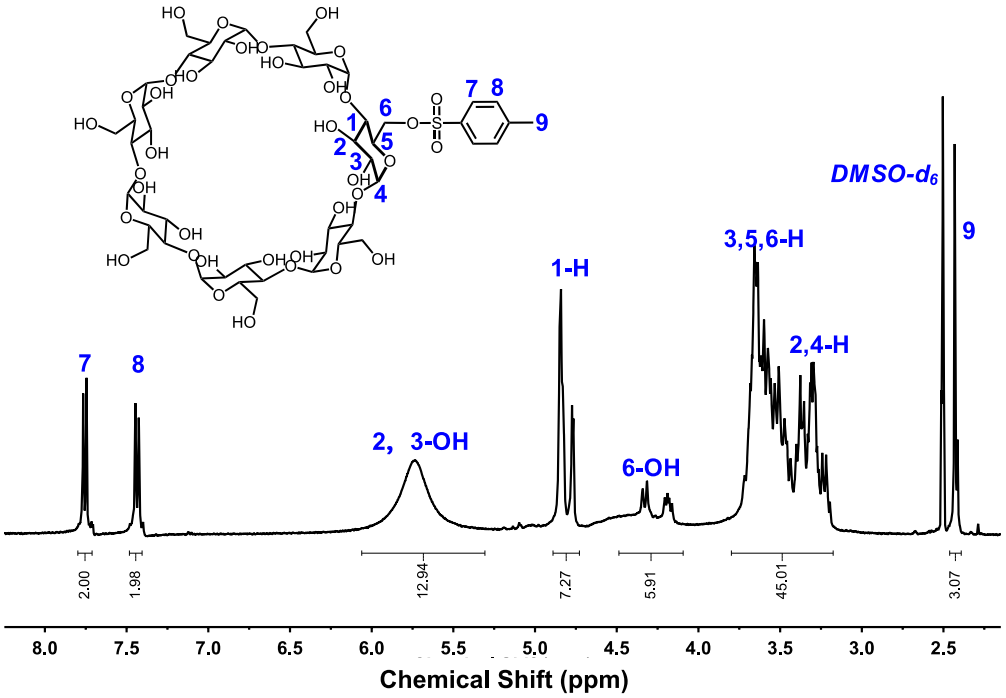

Figure S3. ^{1}H NMR spectrum of *β*-CD-OTs in DMSO-d_6.

1.1.3 Synthesis of Mono-(6-azido-6-desoxy)-*β*-CD (*β*-CD-N_3)

Mono-(6-azido-6-desoxy)-*β*-CD (*β*-CD-N_3) was prepared according to our previous literature.[2] Mono-(6-*O*-(*p*-tolylsulfonyl))-*β*-CD (10.0 g, 7.76 mmol) was dispersed in 100 mL of deionized water followed by heating up to 80 ^{o}C. Subsequently, sodium azide (2.53 g, 38.5 mmol) was added to the suspension, and stirred for 12 h until the reaction mixture became transparent. The solution was poured into 800 mL of acetone and the resulting white solid was filtered, and then dissolved in 50 mL of water and precipitated in 400 mL of acetone once again. The collected white powder was then dried in vacuum oven at 60 ^{o}C for 48 h yielding 8.19 g (7.06 mmol, 91.1%).

^{1}H NMR (DMSO-d_6, 400 MHz) (***Figure S4***): δ_H (ppm) = 3.12-3.42 (m, H-2,4, 14H),3.49-3.82 (m, H-3,5,6, 28H), 4.40-4.58 (m, OH-6, 6H), 4.75-4.92 (m, H-1, 7H), 5.52-5.92 (m, OH-2,3, 14H). **^{13}C NMR** (DMSO-d_6, 100 MHz): δ_c (ppm) = 51.73, 60.57, 70.85, 72.68, 73.03, 73.71, 82.16, 83.62, 102.58. **FTIR** (KBr) (***Figure S7***): ν (cm^{-1}) = 2106 (N_3 unit).

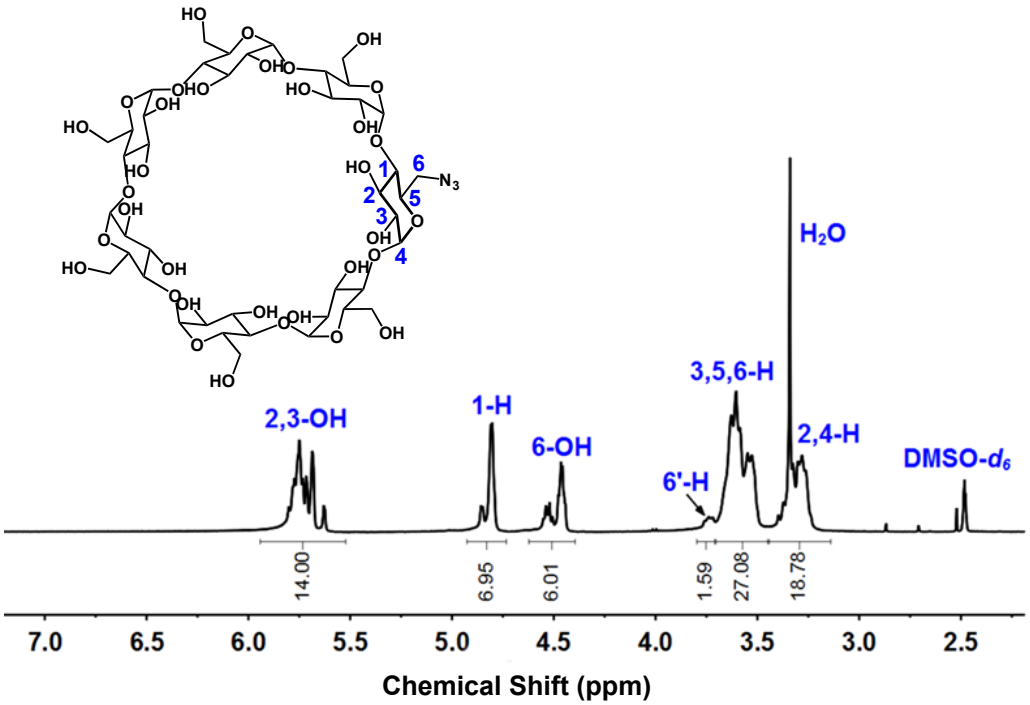

Figure S4. ^{1}H NMR spectrum of *β*-CD-N_3 in DMSO-d_6.

1.1.4 Synthesis of PEG-CD by click chemistry

The synthesis procedure of PEG-CD is as follows: PEG-Alkyne (2.50 g, 0.5mmol, 1.0 *equiv.*) and *β*-CD-N_3 (0.70 g, 0.6mmol, 1.2 *equiv.*) were firstly dissolved in 60 mL of anhydrous DMF, followed by adding catalyst CuBr (72mg, 4.31 mmol, 1.0 *equiv.*) and PMDETA (87 mg, 4.31 mmol, *1.0 equiv.*). Under nitrogen atmosphere, the mixture was heated up to 70 ^{o}C with vigorous stirring for 48 h. The resulting system was exposed to air under stirring for 30 min to terminate the reaction, and the excess DMF was removed in vacuum, followed by dilution with 200 mL of chloroform and passing through neutral alumina to get rid of the copper catalyst. The filtrate was concentrated and then purified by dialyzing against deionized water for 48 h using a dialysis tube with molecular-weight cut-off (MWCO) of 2 kDa, to remove excess *β*-CD-N_3 and other impurities. After removal of the water by freeze-drying, a white powder was obtained (2.23 g, yield: 72%).

^{1}H NMR (DMSO-d_6, 400 MHz) (***Figure S5***): δ_H (ppm) = 3.2 (m, CH_3O-,3H), 3.40-3.75(m, H-2,3,4,5,6, -OCH_2CH_2O-, 492H), 4.43-4.62 (m, 6-OH, OCH_2CN=C, 8H), 4.75-4.92 (m, H-1, 7H), 5.52-5.92 (m, OH-2,3, 14H), 8.05(s, H on triazole, 1H). **^{13}C NMR** (DMSO-d_6, 100 MHz) (***Figure S6***): δ_C (ppm) = 58.51, 60.42, 60.69, 63.80, 70.26, 71.75, 72.53, 72.85, 73.51, 81.99, 102.43, 124.43, 145.21. **FTIR**(KBr) (***Figure S7***): ν (cm^{-1}) = 1601 (C=C), 1518/1499 (N=N). **GPC** (***Figure S8***): 6.725 kDa with PDI of 1.20, in good agreement of the value of 6.163 kDa calculated by NMR.

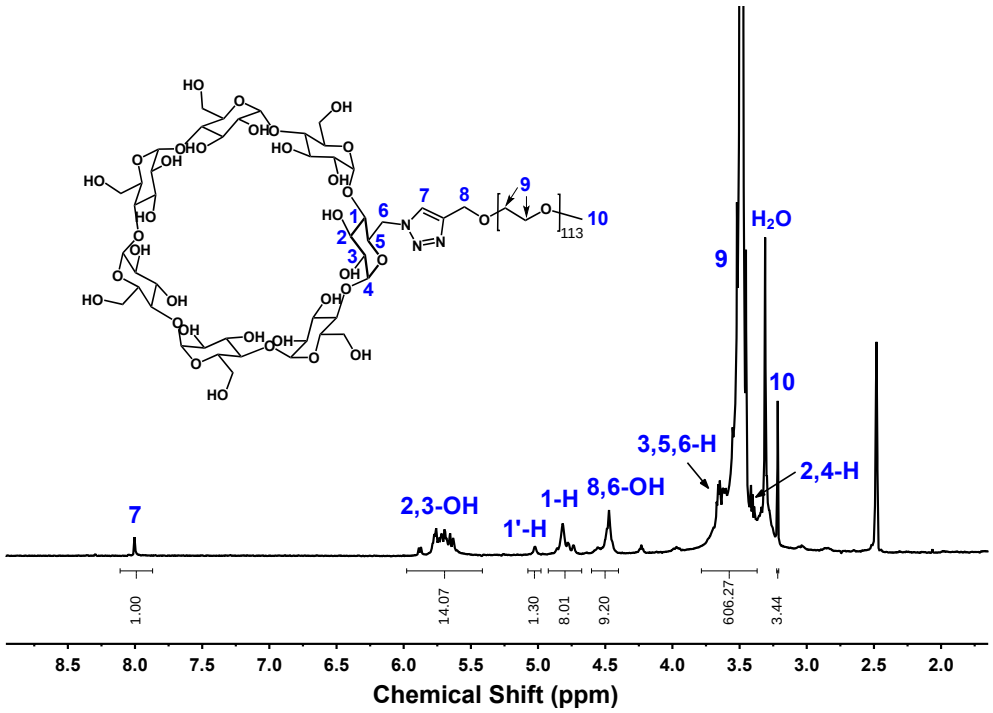

Figure S5. ^{1}H NMR spectrum of PEG-CD in DMSO-d_6.

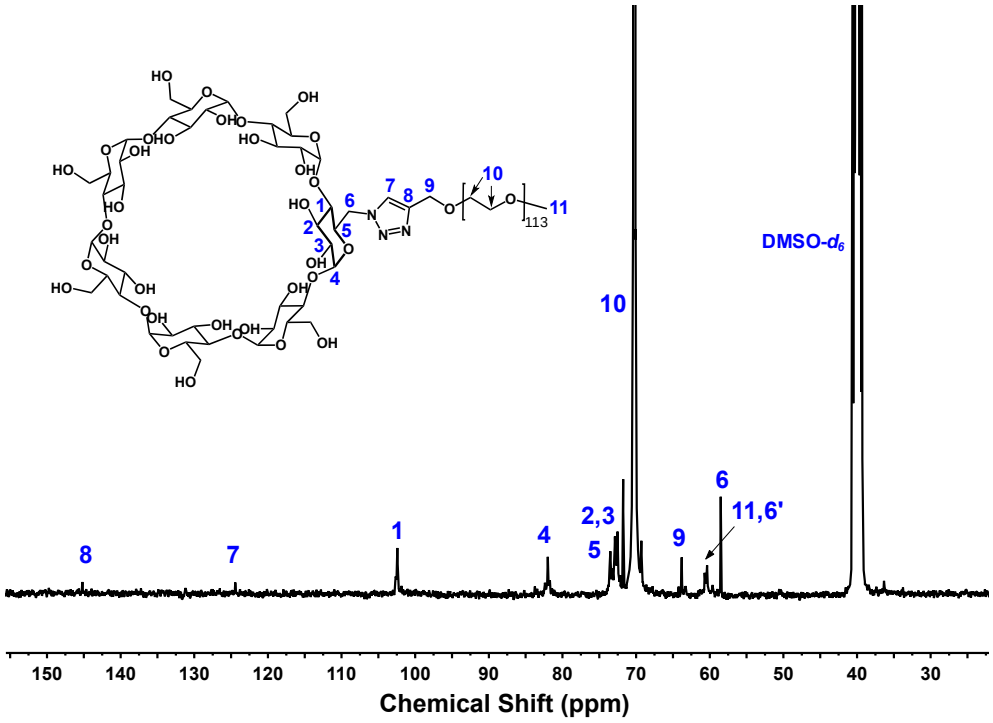

Figure S6. ^{13}C NMR spectrum of PEG-CD in DMSO-d_6.

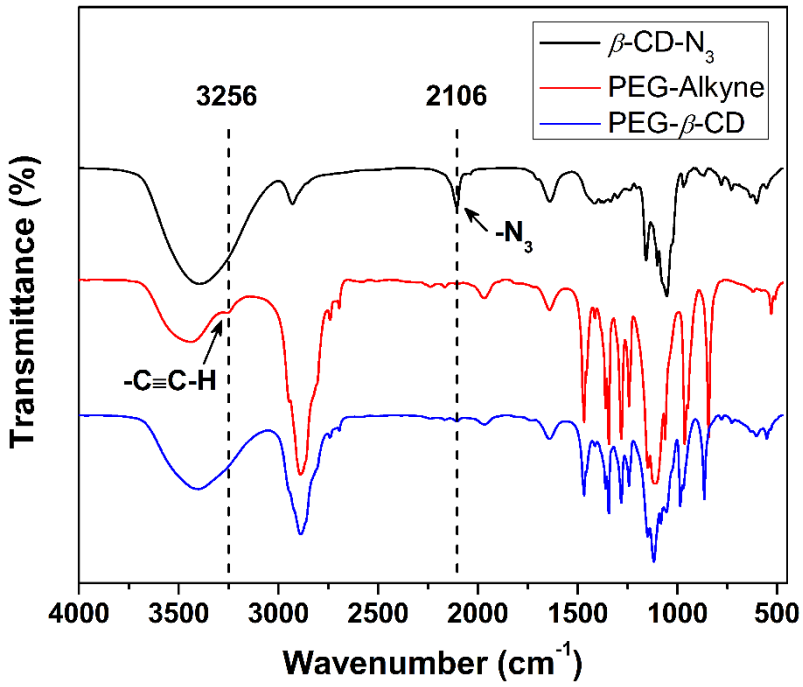

Figure S7. FTIR spectrum of β-CD-N_3, PEG-Alkyne and PEG-CD.

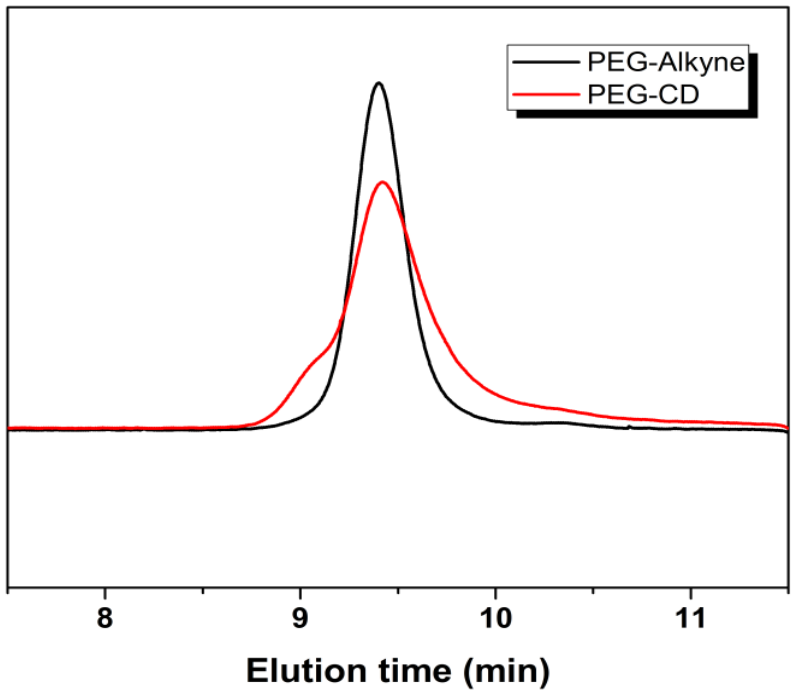

Figure S8. GPC profiles of PEG-Alkyne and PEG-CD.

1.2 Synthesis of *β*-CD and ferrocene-terminated PEHA (Fc-PEHA-CD)

1.2.1 Synthesis of *β*-CD-monosubstituted PEHA (*β*-CD-PEHA)

β-CD-PEHA was synthesized according to the literature.[3] Under a nitrogen atmosphere, 5.8 g of mono-(6-*O*-(*p*-tolylsulfonyl))-*β*-CD (*β*-CD-OTs) was suspended in an excess amount of PEHA (33 mL), and the reaction system was heating up to 75 ^{o}C under stirring for 8 h. Then the mixture was cooled down to room temperature naturally and 40 mL cold acetone was subsequently added into this mixture. The precipitate was filtrated and purified by dissolving in 50 mL of a water-methanol mixture and reprecipitating in 50 mL acetone several times to eliminate the unreacted PEHA. After drying at 60 ^{o}C for 48 h in a vacuum oven, a white solid (*β*-CD-PEHA) was obtained. The yield was 66.3%.

^{1}H NMR (D_2O, 400 MHz) (***Figure S9***): δ_H (ppm) = 2.74 (m, -NHCH_2CH_2NH-, 20H), 3.36-3.95 (m, H-2,3,4,5,6, 42H), 4.72-4.90 (m, H-1, 7H). **UPLC & Q-TOF-MS** of *β*-CD-PEHA: calculated for m/z = 1348.60, found m/z: 1349.6096 $[M + H]^+$.

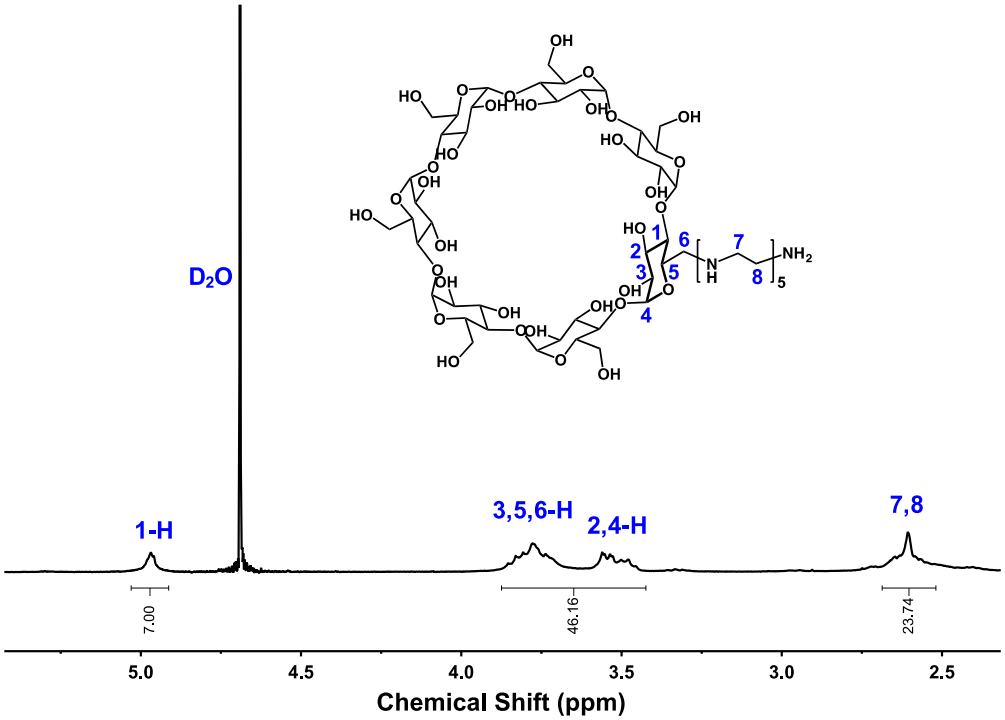

Figure S9. ^{1}H NMR spectrum of *β*-CD-PEHA.

1.2.2 Synthesis of *β*-CD/Fc-terminated PEHA (Fc-PEHA-CD)

The Fc-PEHA-CD was prepared according to the following procedure: *β*-CD-PEHA (1.35 g, 1.0 mmol) and ferrocenecarboxaldehyde (0.428 g, 2 mmol) were dissolved in a mixture of 30 mL of anhydrous methanol and 20 mL of anhydrous DMF, and then degassed and filled with nitrogen. The reaction mixture was then placed in an oil bath preheated at 70 ^{o}C for 24 h. After the reaction solution was cooled down to 0 ^{o}C in an ice-water bath, 0.23 g (6 mmol) of $NaBH_4$ was slowly added to the above solution within 15 min. Subsequently, the reaction mixture was left to react for 12 h at room temperature under stirring. With the addition of 2 mL of 3 M HCl aqueous solution, the reaction system was continuously stirred for another 2 h. After evaporation of methanol and DMF under vacuum, the crude product was purified with silica gel column chromatography (CH_2Cl_2-methanol). Finally, the product was collected as red-brown oil. Yield: 1.24 g (80%).

^{1}H NMR (D_2O, 400 MHz) (***Figure S10***): δ_H (ppm) = 2.00-3.05 (m, -NHC*H*$_2$C*H*$_2$NH-, 20H), 3.48 (m, Ferrocene-C*H*$_2$-NH-, 2H), 3.16-3.75 (m, H-2,3,4,5,6, 42H), 4.04-4.35 (m, *H* on Ferrocene, 9H), 4.92-5.15 (s, H-1, 7H). **UPLC & Q-TOF-MS** of Fc-PEHA-CD: calculated for m/z = 1546.61, found m/z: 1547.6245 $[M + H]^+$.

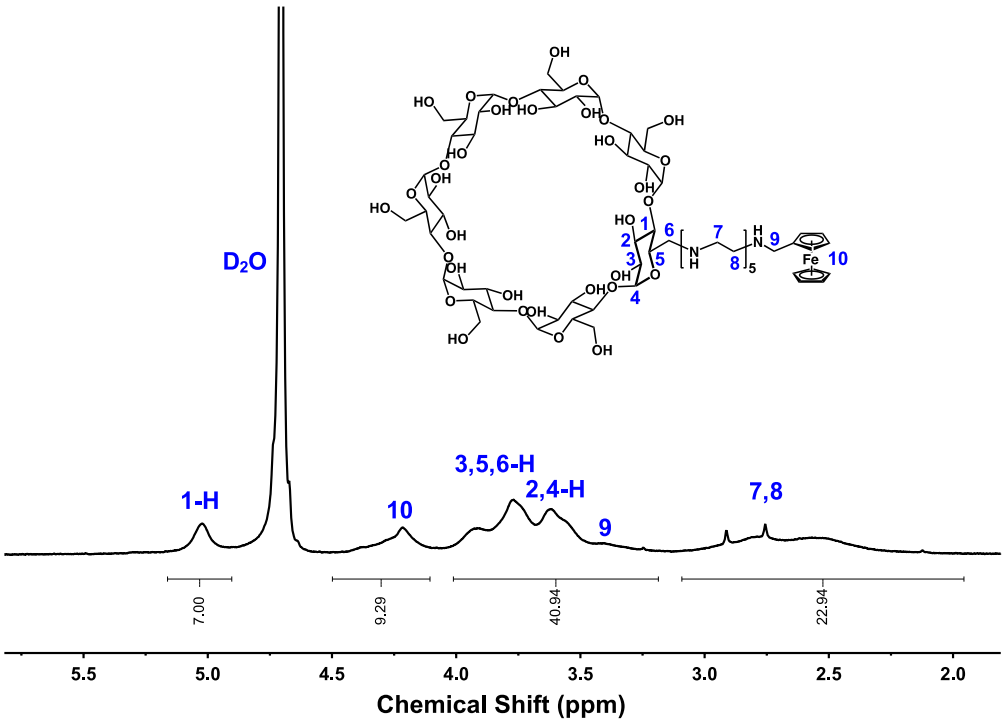

Figure S10. ^{1}H NMR spectrum of Fc-PEHA-CD in D_2O.

2. Supplemented Figures

2.1 2D-NOESY spectrum of supramolecular homopolymer

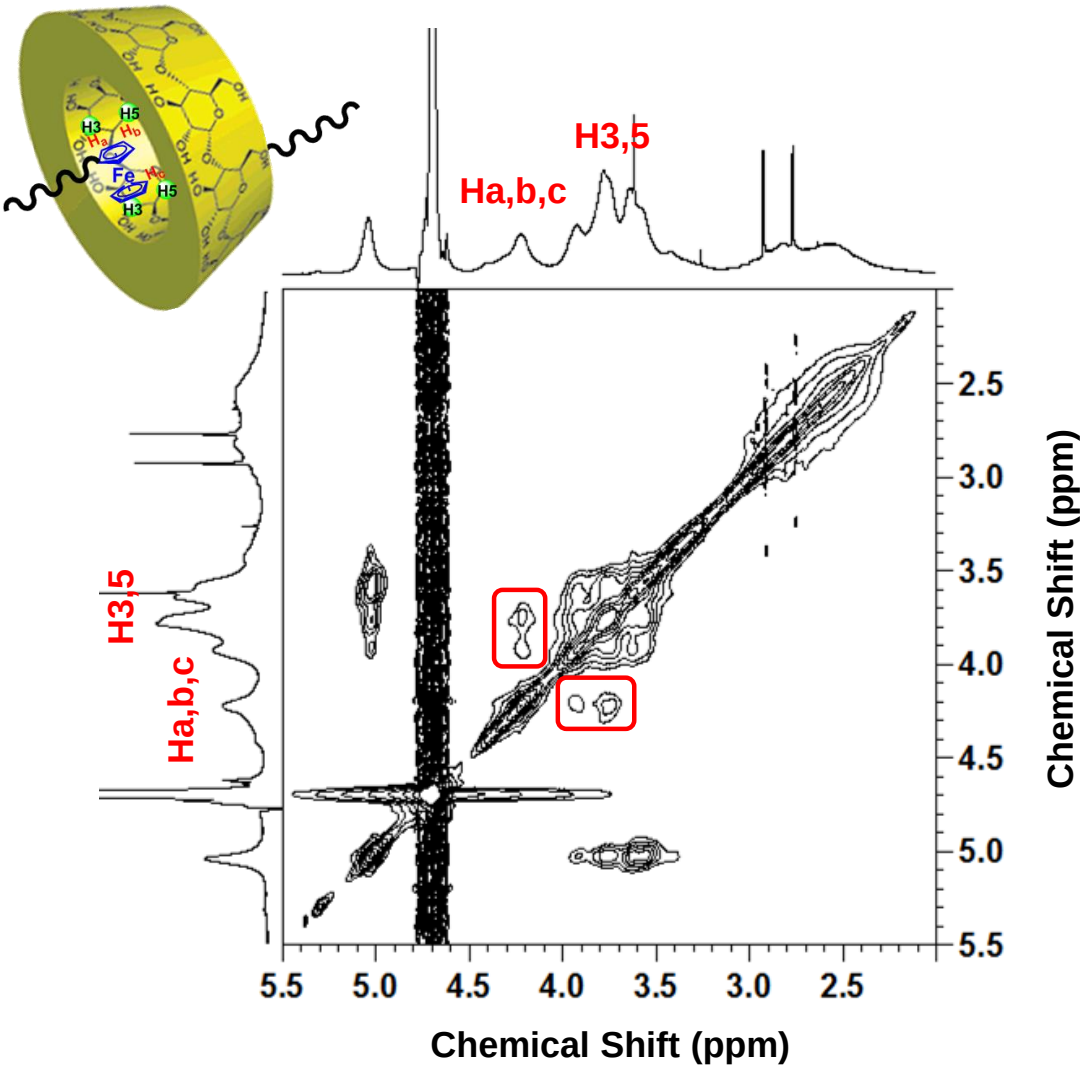

Figure S11. 2D-NOESY ^{1}H NMR spectrum of supramolecular homopolymer at 10 mM in D_2O.

2.2 Concentration-dependent ^{1}H NMR spectra of the supramolecular homopolymer

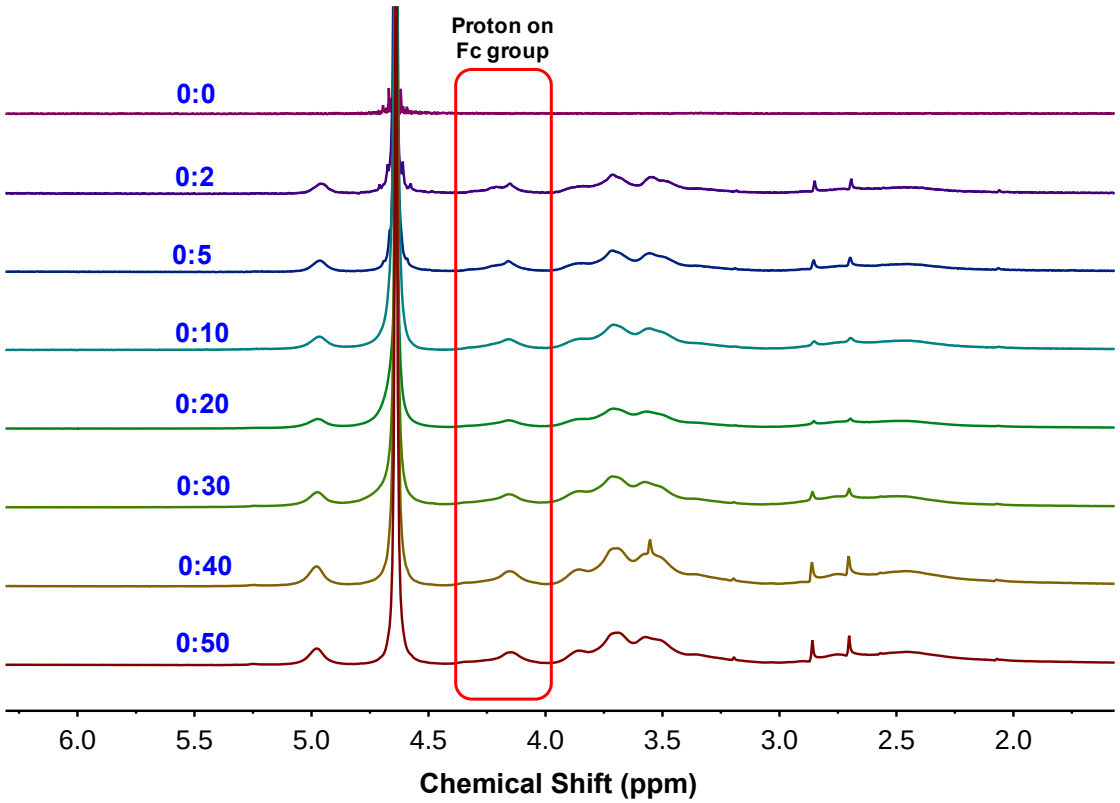

Figure S12. ^{1}H NMR spectrum of the supramolecular homopolymer at different concentrations of Fc-PEHA-CD in D_2O.

As shown in ***Figure S12***, upon increasing the solution concentration from 0 mM to 50 mM, the proton signals of Fc group gradually become broad, demonstrating the formation of high-molecular-weight supramolecular homopolymer in water.

2.3 Determination of zeta potential, mobility and conductivity of supramolecular block copolymer and supramolecular homopolymer

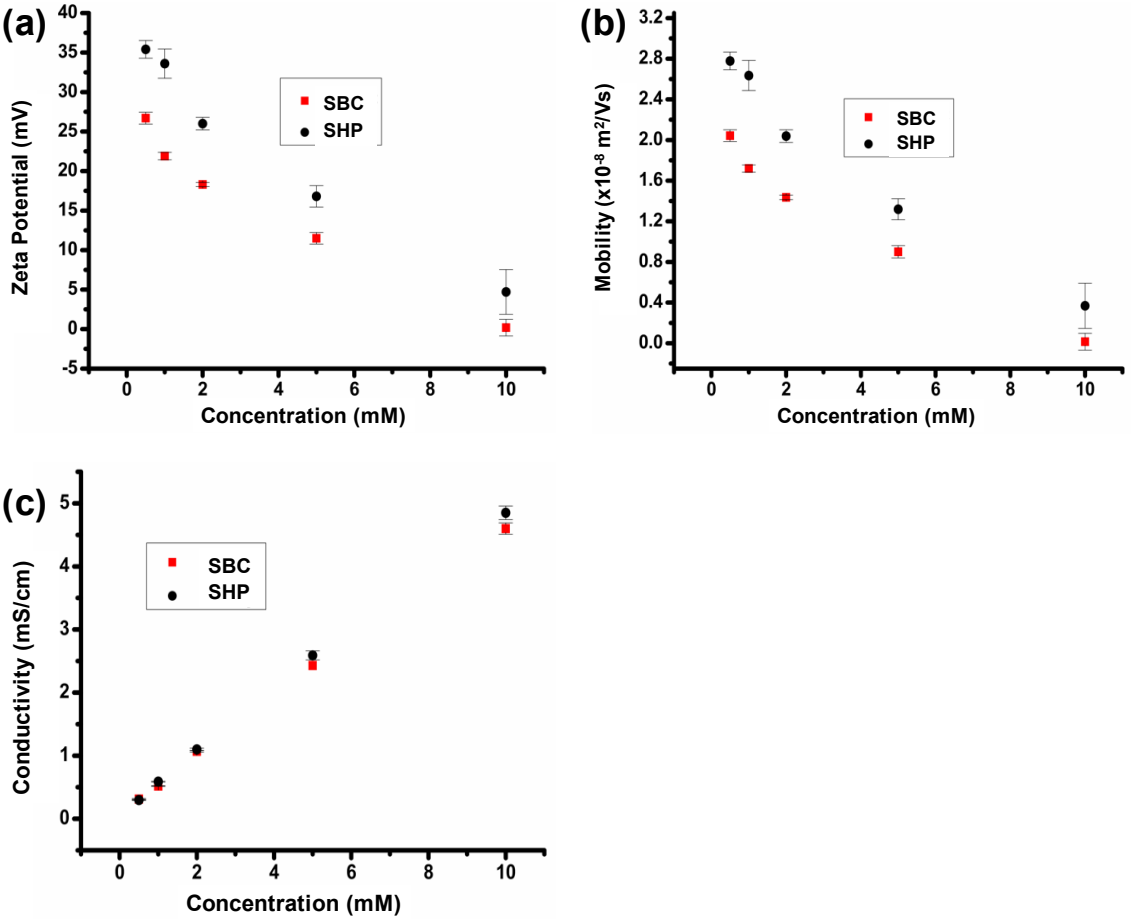

Figure S13. (a) Zeta potential, (b) mobility and (c) conductivity of supramolecular block copolymer (SBC) and supramolecular homopolymer (SHP) in PBS buffer (pH 7.4) as a function of molar concentration.

2.4 Degree of polymerization and molecular weight of supramolecular block copolymer

Table S1. The degree of polymerization (DP) and molecular weight of supramolecular block copolymers with different concentrations in water.

Molar ratio	Concentration (mM)	DP	M_n (kDa) [a]
1 : 0	0	0	6.20
1 : 0.5	0. 5	4	12.39
1 : 1	1	5	13.93
1 : 2	2	8	18.57
1 : 5	5	12	24.76
1 : 10	10	17	32.50
1 : 20	20	24	43.33
1 : 30	30	30	52.61
1 : 40	40	34	58.80
1 : 50	50	38	64.98

[a] $M_n = M_{PEG\text{-}CD} + DP \times M_{FC\text{-}PEAH\text{-}CD}$ $(DP = (K_a[Conc.])^{1/2}; K_a = 2.95 \times 10^4\ M^{-1})$

2.5 Gel permeation chromatography (GPC) profiles of SBC and SHP

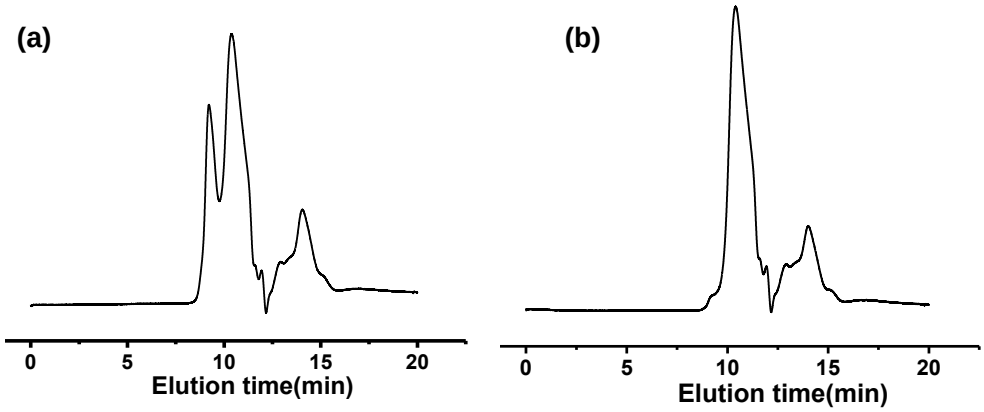

Figure S14. GPC profiles of SBC (a) and SHP (b) at 10mM.

2.6 Agarose gel electrophoresis retardation by PEHA and SHP

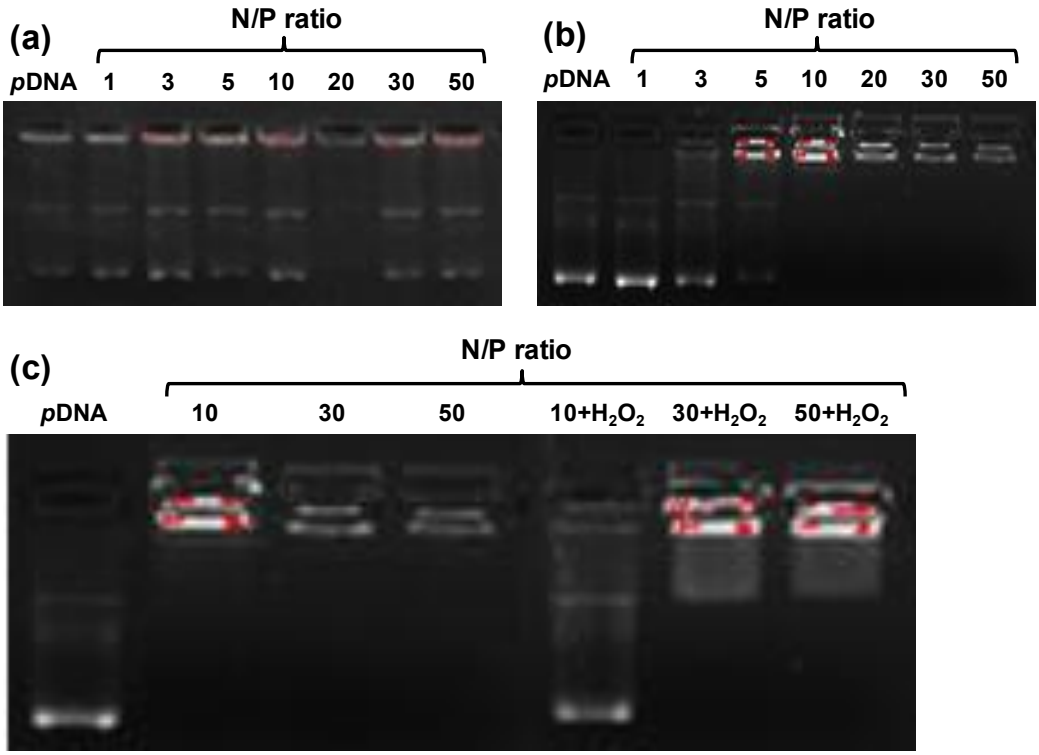

Figure S15. Agarose gel electrophoresis retardation of *p*DNA by (a) PEHA and (b) supramolecular homopolymer (SHP) at various N/P ratios of 1, 3, 5, 10, 20, 30 and 50. (c) Agarose gel electrophoresis retardation of *p*DNA by SHP at N/P ratios of 10, 30 and 50 before and after addition of H_2O_2.

In ***Figure S15a***, even though the N/P ratio of PEHA/*p*DNA is up to 20, *p*DNA can migrate through agarose gel at roughly the same rate as naked *p*DNA. This result indicates that *p*DNA cannot be retarded by the small molecule of PEHA. In contrast, *p*DNA can be effectively condensed by supramolecular homopolymer while the molar ratio of SHP/*p*DNA is above 5 (***Figure S15b***). With addition of H_2O_2, supramolecular homopolymer will readily depolymerize into small chain pieces, further leading to a rapid release of *p*DNA from the SHP/*p*DNA polyplex as shown in ***Figure S15c***.

2.7 Luciferase expression of SBC with 10% FBS

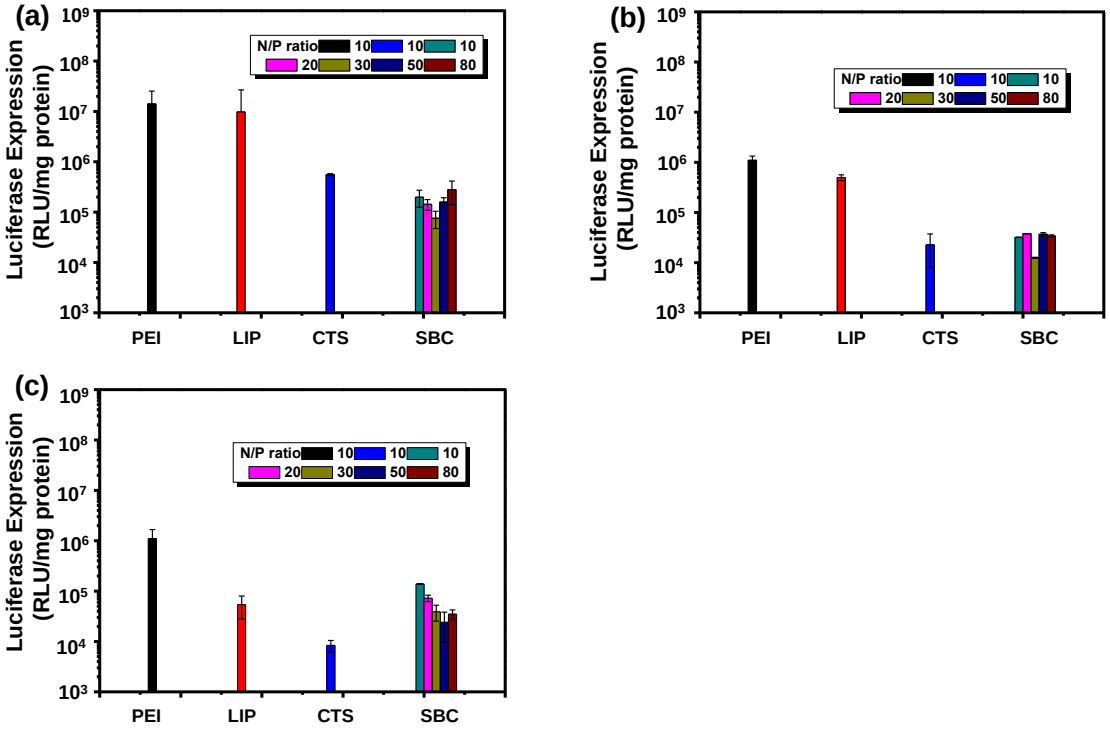

Figure S16. Luciferase expression of SBC/pDNA polyplexes at the N/P ratios of 10, 20, 30, 50 and 80, and branched PEI, LIP or CTS/pDNA polyplexes at a N/P ratio of 10 after 48 h in (a) COS-7 cells, (b) HeLa cells and (c) MCF-7 cells. The gene delivery experiment was performed in fresh pre-warmed DMEM with 10% FBS.

3. References

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