

Electronic Supplementary Information for

# **A Supramolecular Approach to the Preparation of Charge-Tunable Dendritic Polycations for Efficient Gene Delivery**

Ruijiao Dong,<sup>a</sup> Linzhu Zhou,<sup>a</sup> Jieli Wu,<sup>b</sup> Chunlai Tu,<sup>a</sup> Yue Su,<sup>a</sup> Bangshang Zhu,<sup>b</sup> Hongchen Gu,<sup>c</sup> Deyue Yan<sup>a</sup> and Xinyuan Zhu<sup>\*a,b</sup>

<sup>a</sup> School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, People's Republic of China; <sup>b</sup> Instrumental Analysis Center, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, People's Republic of China; <sup>c</sup> Med-X Research Institute, Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200030, People's Republic of China

\* To whom correspondence may be addressed. E-mail: [xyzhu@sjtu.edu.cn](mailto:xyzhu@sjtu.edu.cn)

## **Table of Contents**

### **1. Experimental Section**

#### **1.1. Materials**

#### **1.2. Synthesis Details**

#### **1.3. Characterization**

### **2. Supplementary Tables and Figures**

Table S1 The molecular weights and polydispersities of HPG and HPG-AD

Table S2  $\zeta$ -Potential of various  $\beta$ -CD derivatives and SDP solutions

Scheme S1 Synthesis of HPG-AD, per-6-amino- $\beta$ -CD and per-6-DMAE- $\beta$ -CD

Fig. S1 NMR spectra of HPG

Fig. S2 NMR spectra of HPG-AD

Fig. S3 NMR spectra of per-6-amino- $\beta$ -CD (4) and per-6-DMAE- $\beta$ -CD (5)

Fig. S4 GPC profiles of HPG and HPG-AD

Fig. S5 Temperature-variable UV-vis spectra of HPG-AD, IC1 and IC5 solutions

Fig. S6 DLS plots of aqueous solutions of HPG-AD, IC1 and IC5

Fig. S7 Acid-base titration curves of IC1, IC2, IC3, IC4, IC5 and PEI

Fig. S8 Cell viability assay in COS-7 cells

### **3. References**

## 1. Experimental Section

### 1.1. Materials

Glycerol (97%, Aldrich), 1-adamantanecarbonyl chloride (97%, Alfa Aesar), sodium azide ( $\text{NaN}_3$ ) (99%, Aldrich), *N,N*-dimethylethylenediamine (DMAE) (99%, Shanghai Wokai Chemical Industry Co. Ltd.), 1,1'-carbonyldiimidazole (CDI) (98%, Shanghai Wokai Chemical Industry Co. Ltd.), triphenylphosphine ( $\text{PPh}_3$ ) (99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.),  $\text{I}_2$  (99.8%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), Na (98%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), and ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) (25%, Shanghai Sinopharm Chemical Reagent Co. Ltd.) were used as received.  $\beta$ -Cyclodextrin ( $\beta$ -CD) (Shanghai Sinopharm Chemical Reagent Co. Ltd.) was dried for 48 h in vacuum oven before use.  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ , methylene dichloride ( $\text{CH}_2\text{Cl}_2$ ), dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), pyridine and toluene from Shanghai Sinopharm Chemical Reagent Co. Ltd., were treated with calcium hydride and distilled before use. Tetrahydrofuran (THF), methanol and ethanol were obtained from Shanghai Sinopharm Chemical Reagent Co., Ltd.

### 1.2. Synthesis Details

#### Synthesis of Hyperbranched Polyglycerol (HPG)

According to the literature,<sup>1-3</sup> the cationic polymerization of glycidol was conducted under an atmosphere of nitrogen at  $-20 \pm 5$  °C. Prior to polymerization, a 500 mL four-necked round-bottomed flask equipped with a magnetic stirrer, a tap funnel and a thermometer, was degassed for at least three times by vacuum-pumping and back-filling with nitrogen. Subsequently,  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (5.35 g, 37.7 mmol) with  $\text{CH}_2\text{Cl}_2$  (250 mL) was added by a syringe, and then glycidol (22.34 g, 302 mmol) was introduced dropwise by the tap funnel under stirring for about 1 h. After the completion of glycidol addition, the reaction solution gradually turned heterogeneous accompanying with the precipitation of resulting product. After 24 h, the polymerization was quenched with deionized water. The resulting solution was evaporated under reduced pressure to remove the  $\text{CH}_2\text{Cl}_2$ . The raw product was dissolved in 50 mL deionized water and purified by precipitation in THF for three times. 16.67 g of a clear, viscous liquid was obtained with a 74.6% yield.

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta_{\text{H}}$  (ppm) = 3.4-4.0 (m, -CH, -CH<sub>2</sub>), 4.85 (s, -OH);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta_{\text{C}}$  (ppm) = 61.20, 61.42, 61.89, 63.10, 69.30, 69.60, 70.84, 71.07, 71.49, 72.58, 78.47, 78.77, 80.02, 80.22, 81.80; IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) = 584, 679, 865, 934, 1095, 1254, 1337, 1411, 1461, 1648, 1718, 2095, 2885, 3403.

### Synthesis of Adamantane-Modified HPG (HPG-AD)

The procedure for the preparation of HPG-AD with different grafting ratios of adamantane (AD) was follows. HPG was azeotropically dried using toluene in a three-necked flask under nitrogen, and anhydrous pyridine was added subsequently to dissolve the HPG. Adamantanecarbonyl chloride was rapidly added into the system by syringe under vigorous stirring at 75 °C. After 24 h, the reaction solution was condensed, and then washed with cyclohexane twice. The resulting solution was enclosed in dialysis membrane (MWCO 1.0 kDa), and purified by dialysis against deionized water for 48 h to remove the impurities. After removal of the water by freeze drying, pale yellow and viscous products were obtained with 60-70% yields.

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta_{\text{H}}$  (ppm) = 1.7-2.1 (m, adamantyl-H), 3.4-4.4 (m, -CH, -CH<sub>2</sub>), 4.82 (s, -OH);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta_{\text{C}}$  (ppm) = 28.24, 36.43, 38.87, 40.89, 61.42, 62.12, 63.34, 65.23, 66.67, 68.42, 68.72, 69.50, 69.88, 71.05, 71.28, 71.76, 72.84, 77.90, 78.72, 78.95, 79.17, 80.24, 82.04, 177.81; IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) = 586, 666, 752, 865, 924, 1077, 1186, 1235, 1324, 1454, 1578, 1647, 1728, 2103, 2685, 2907, 3428.

### Synthesis of Per-6-Iodo- $\beta$ -Cyclodextrin (2)

The following reaction workup is analogous to the literature procedure.<sup>4</sup>  $\text{Ph}_3\text{P}$  (40.1 g, 153 mmol) was dissolved in anhydrous DMF (160 mL), and then  $\text{I}_2$  (40.5 g, 160 mmol) was slowly added into this solution over 10 min under vigorous stirring with the evolution of heat. Dry  $\beta$ -cyclodextrin (1) (11.6 g, 10.2 mmol) was then added to this dark brown solution, and the temperature was raised to 70 °C. The resulting system was carried out under nitrogen for 20 h. The solution was concentrated under reduced pressure to approximately 60 mL. The NaOMe solution (3 M, 60 mL) was then added to the reaction flask with efficient cooling, and the resulting mixture was continually stirred for another 1 h. The raw product was obtained by precipitating the reaction mixture into 800 mL MeOH, which was washed with MeOH twice, superficially dried, and Soxhlet extracted with MeOH for 20 h. The product was

removed from the Soxhlet extractor and allowed to air dry before being dried at 60 °C under high vacuum. The compound was collected as a white powder (17.9 g, 92%).

$^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz):  $\delta_{\text{H}}$  (ppm) = 3.27 (t,  $J$  = 8.97 Hz, 7H), 3.34-3.46 (m, 14H), 3.54-3.66 (m, 14H), 3.79 (bd,  $J$  = 9 Hz, 7H), 4.97 (d,  $J$  = 3.43 Hz, 7H), 5.91 (d,  $J$  = 1.81 Hz, 7H), 6.01 (d,  $J$  = 6.78 Hz, 7H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 400 MHz):  $\delta_{\text{C}}$  (ppm) = 10.20, 71.64, 72.58, 72.83, 86.61, 102.78; IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) = 587, 756, 941, 1044, 1101, 1155, 1371, 1413, 1566, 1637, 2304, 2918, 3392.

### Synthesis of Per-6-Azido- $\beta$ -Cyclodextrin (3)

Per-6-iodo- $\beta$ -cyclodextrin **2** (4.5 g, 2.36 mmol) was dissolved in DMF (60 mL), and sodium azide ( $\text{NaN}_3$ ) (1.5 g, 23.1 mmol) was added subsequently. The reaction solution was stirred at 60 °C under nitrogen atmosphere for 20 h. The resulting suspension was then condensed under reduced pressure to 10 mL, then precipitated in a large excess of deionized water. A fine white precipitate was formed and filtered off carefully, which was washed with deionized water three times and dried at 60 °C under high vacuum to yield a stable white powder (2.78 g, 92.6%).

$^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz):  $\delta_{\text{H}}$  (ppm) = 3.28-3.40 (m, 14H), 3.54-3.64 (m, 14H), 3.66-3.80 (m, 14H), 4.89 (d,  $J$  = 3.46 Hz, 7H), 5.74 (d,  $J$  = 2 Hz, 7H), 5.88 (d,  $J$  = 6.78 Hz, 7H);  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 400 MHz):  $\delta_{\text{C}}$  (ppm) = 51.98, 70.99, 72.65, 73.26, 83.86, 102.71; IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) = 598, 758, 845, 941, 1041, 1102, 1155, 1371, 1417, 1565, 1637, 2304, 2916, 3393.

### Synthesis of Per-6-Amino- $\beta$ -Cyclodextrin (4)

The heptaazide **3** (2.60 g, 1.98 mmol) was dissolved in DMF (50 mL), and  $\text{Ph}_3\text{P}$  (8.27 g, 31.5 mmol) was slowly added with the evolution of nitrogen. After 1 h, the concentrated aqueous ammonia (11 mL, approximately 25%) was added dropwise to the solution. After the addition of the aqueous ammonia was complete, the reaction mixture gradually turned into an off-white suspension. The reaction was carried out at room temperature for 18 h before the resulting suspension was concentrated under reduced pressure to approximately 15 mL. The product was then precipitated in 200 mL of EtOH. The precipitate was washed with EtOH three times and dried at 60 °C under high vacuum to yield a white solid (2.1 g, 94.2%). To allow characterization by NMR spectroscopy, the HCl salt of **4** was formed by suspending compound **4** in a small volume of deionized water followed by the addition of a dilute

solution of hydrochloric acid (HCl) until the pH reached 6. At this pH, a clear solution formed which gave a yellow glass when evaporated under reduced pressure.

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 400 MHz):  $\delta_{\text{H}}$  (ppm) = 3.12-3.20 (m, 7H), 3.35 (dd,  $J = 3.08, 13.50$  Hz, 7H), 3.48 (t,  $J = 9.30$  Hz, 7H), 3.58 (dd,  $J = 3.43, 10.06$  Hz, 7H), 3.86 (t,  $J = 9.6$  Hz, 7H), 4.04-4.14 (m, 7H), 5.07 (d,  $J = 3.45$  Hz, 7H);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 400 MHz):  $\delta_{\text{C}}$  (ppm) = 40.38, 67.92, 71.76, 72.31, 82.36, 101.56; IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) = 577, 752, 818, 945, 1032, 1086, 1155, 1339, 1413, 1506, 1619, 2015, 2303, 2924, 3419.

### Synthesis of $\beta$ -Cyclodextrin-(DMAE) $_7$ (5)

The dry  $\beta$ -cyclodextrin (4.0 g, 3.52 mmol) was dissolved in anhydrous DMSO (30 mL), and then added dropwise to a solution of CDI (4.4 g, 27.14 mmol) in anhydrous DMSO (40 mL) under stirring for about 2 h. After the addition was completed, the reaction mixture was stirred for 16 h under nitrogen at room temperature. The DMAE (4.35 g, 49.34 mmol) was slowly added via a syringe to continuous reacting for another 20 h with stirring. The resulting solution was concentrated to 30 mL volume, and precipitated in 500 mL of diethyl ether and then filtrated off. The precipitate was redissolved in 30 mL of methanol and then reprecipitated in diethyl ether (500 mL). This reprecipitation and filtration steps were repeated four times before the polymer was isolated, collected and dried at 60 °C in vacuum to yield a white powder (4.68 g, 69%).

$^1\text{H}$  NMR ( $\text{DMSO-}d_6$ , 400 MHz):  $\delta_{\text{H}}$  (ppm) = 2.13 (br, 42H); 2.28 (br, 14H); 3.05 (br, 14H); 3.40 (br, 7H); 3.61 (m, 14H); 3.76 (br, 7H); 3.98 (br, 7H); 4.23 (br, 7H); 4.85 (bd,  $J = 21.72$  Hz, 7H); 5.6-6.0 (m, 14H); 6.94 (br, 7H);  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ , 400 MHz):  $\delta_{\text{C}}$  (ppm) = 38.94, 45.70, 58.95, 63.86, 69.88, 72.96, 73.57, 82.56, 102.61, 156.90; IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) = 584, 778, 856, 944, 1038, 1086, 1155, 1257, 1336, 1462, 1546, 1704, 2305, 2778, 2828, 2947, 3337.

### 1.3. Characterization

$^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, quantitative  $^{13}\text{C}$  NMR and 2D  $^1\text{H}$  ROESY NMR spectra were recorded on Varian Mercury plus 400 NMR spectrometer (400 MHz) with dimethyl sulfoxide- $d_6$  ( $\text{DMSO-}d_6$ ),  $\text{CD}_3\text{OD}$  and  $\text{D}_2\text{O}$  as solvents. Gel permeation chromatography (GPC) was performed on a Perkin-Elmer series 200 system (10  $\mu\text{m}$  PL gel 300  $\times$  7.5 mm mixed-B and mixed-C column, polystyrene calibration) equipped

with a refractive index (RI) detector. DMF containing 0.01 mol/L lithium bromide was used as the mobile phase at a flow rate of 1 mL/min at 70 °C. Fourier transform infrared (FTIR) spectra were recorded on a Paragon 1000 instrument by using the KBr sample holder method.

The temperature-variable UV-vis measurements were performed on a Perkin-Elmer Lambda 20/2.0 UV-vis spectrometer equipped with a thermal cell. Measurements were made at 500 nm with a heating rate of 1 °C/min. The dynamic lighting scattering (DLS) measurements were carried out at 25 °C by using a Malvern Zetasizer NanoS apparatus equipped with a 4.0 mW laser operating at  $\lambda = 633$  nm. All samples were measured at a scattering angle of 173° with polymer concentration of 0.2 mg/mL.

The  $\zeta$ -potentials of various SDPs in PBS buffer were measured using a Malvern Zetasizer NanoS at 25 °C. The cuvettes were filled with the SDP solution, and the measurements were performed in the  $\zeta$ -model for a minimum of 10 cycles and a maximum of 100 cycles.

The polyplex morphology was visualized using an atomic force microscopy (AFM) system with the Dimension 3100 model with a Nanoscope IIIa controller (Veeco, Santa Barbara, CA). 20  $\mu$ L of SDPs/pDNA complexes in Hepes buffer solution (4 mM Hepes, 10 mM NaCl, and 2 mM MgCl<sub>2</sub> (pH 7.4)) containing 0.08  $\mu$ g of pDNA at various N/P ratios were dropped onto freshly cleaved mica sheets for 5 min, then rinsed with distilled water several times and dried naturally in air overnight. The samples were imaged using the tapping mode with setting of 256  $\times$  256 pixels. Image analysis was performed using Nanoscope software after removing the background slope by flattening images.

### **Agarose Gel Electrophoresis**

The SDP/pDNA complexes with various N/P ratios were prepared by adding different volumes of SDP solutions to pDNA solutions in PBS containing 0.4  $\mu$ g pDNA, followed by vortexing for 6 s and incubated for 30 min at room temperature. After mixing 5  $\mu$ L of 0.5  $\times$  loading buffer with polyplex solutions, the resulting polyplex solution was analyzed on 1% agarose gel containing 0.5  $\mu$ g/mL ethidium bromide. Gel electrophoresis was carried out in 0.5  $\times$  Tris-Borate-EDTA (TBE) buffer at 100 V for 1 h in a Sub-Cell system (Bio-Rad Laboratories, CA). DNA bands were visualized by a UV lamp using a Gel Doc system (Synoptics Ltd., UK).

### **Acid-Base Titration**

The buffering capacity of SDPs was determined by acid-base titration. SDPs with 0.5 mmol of amine groups were dissolved in 10 mL of 0.15 M NaCl aqueous solution. The pH of SDP solution was set at 2.0 with 1 M HCl, and the solution was titrated with 0.1 M NaOH solution by adding 100  $\mu$ L 0.1 M NaOH solution. For comparison, branched PEI (25 kDa) dissolved in 0.15 M NaCl aqueous solution adjusted to pH 2.0, was also titrated using the same method. The buffering capacity was defined as the percentage of amine groups becoming protonated from pH 5.1 to 7.4.<sup>5</sup>

### **Cell Cultures**

COS-7 cells were cultured in DMEM (4.5 g/L glucose) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100  $\mu$ g/mL) at 37 °C under a 5% CO<sub>2</sub> humidified atmosphere. Confluent cells were subcultured every 3 days using standard procedure.

### **Cell Viability**

For MTT assay, COS-7 cells were seeded into 96-well plates at a seeding density of 5000 cells/well in 200  $\mu$ L medium. After 24 h incubation, the culture medium was removed and replaced with 200  $\mu$ L of medium containing 50  $\mu$ L serial concentrations of SDPs. The cells were grown for 24 h. Then, 20  $\mu$ L of 5 mg/mL MTT assays stock solution in PBS was added to each well. After the cells were incubated for 4 h, the medium containing unreacted dye was carefully removed. The obtained blue formazan crystals were dissolved in 200  $\mu$ L of DMSO, and the absorbance was measured in a Perkin-Elmer 1420 Multi-label counter at a wavelength of 490 nm.

### **In Vitro Transfection Assay**

For luciferase expression studies, cells were seeded at a density of 10<sup>4</sup> cells per well in 96-well plates and incubated for 16-24 h until 60-70% confluent at 37 °C and 5% CO<sub>2</sub>. Immediately prior to transfection, the medium was removed. Then, cells were washed and replaced with fresh and prewarmed DMEM in the absence of 10% FBS. Polyplexes were added to each well, and the cells were incubated at 37 °C for 4 h. The sample concentration added to the cells was about 7  $\mu$ g/mL at N/P ratio of 10 and 42  $\mu$ g/mL at N/P ratio of 60, respectively. The medium was then replaced with fresh DMEM supplemented with 10% FBS and incubated for an additional 48 h. The luciferase assay was carried out according to manufacturer's protocol (Promega, Madison, WI). Relative light units (RLUs) were measured with GloMax<sup>TM</sup> 96 microplate luminometer (Promega). The obtained RLUs were

normalized with respect to protein concentration in the cell extract determined using the BCA protein assay kit (Beyotime, China).

For GFP expression studies, plasmid pEGFP-C1 encoding GFP was also used to examine the GFP expression. In brief, COS-7 cells were seeded into a 12-well plate at a density of  $1 \times 10^4$  cells per well in 1 mL of complete DMEM. After 24 h, 500  $\mu$ L of serum-free DMEM, in which 200  $\mu$ L polyplexes containing 1.5  $\mu$ g of pDNA were added, was used to replace the complete DMEM. After 4 h, the transfection media was removed and the cells were incubated in serum-containing media for another 20 h. At the end of transfection, the cells were washed with warm phosphate-buffered saline (PBS) twice and imaged under a laser scanning confocal microscope (LSM 410, Carl Zeiss, USA). Fluorescence was excited at 488 nm and emission was collected using a 515 nm filter.

## 2. Supplementary Tables and Figures

**Table S1** The Molecular Weights and Polydispersities of HPG and HPG-ADs.

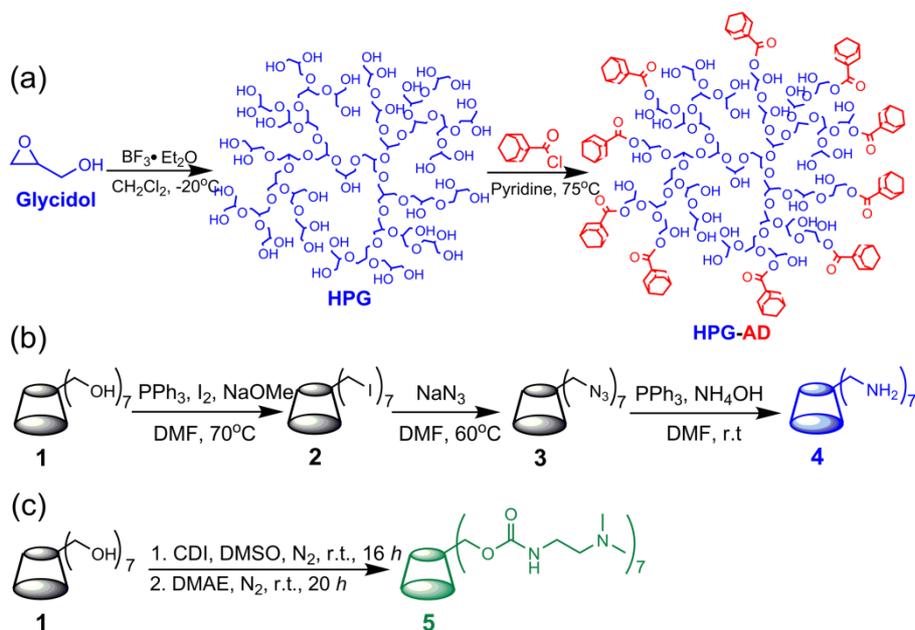
Samples	$M_n$ ( $\times 10^4$ g/mol)	$M_w/M_n^c$	DB
HPG	0.80 <sup>a</sup>	1.80	0.59 <sup>d</sup>
HPG-AD-18%	1.11 <sup>b</sup>	1.86	—
HPG-AD-28.2%	1.29 <sup>b</sup>	1.90	—

<sup>a,c</sup> Determined by GPC; <sup>b,d</sup> Determined by NMR.

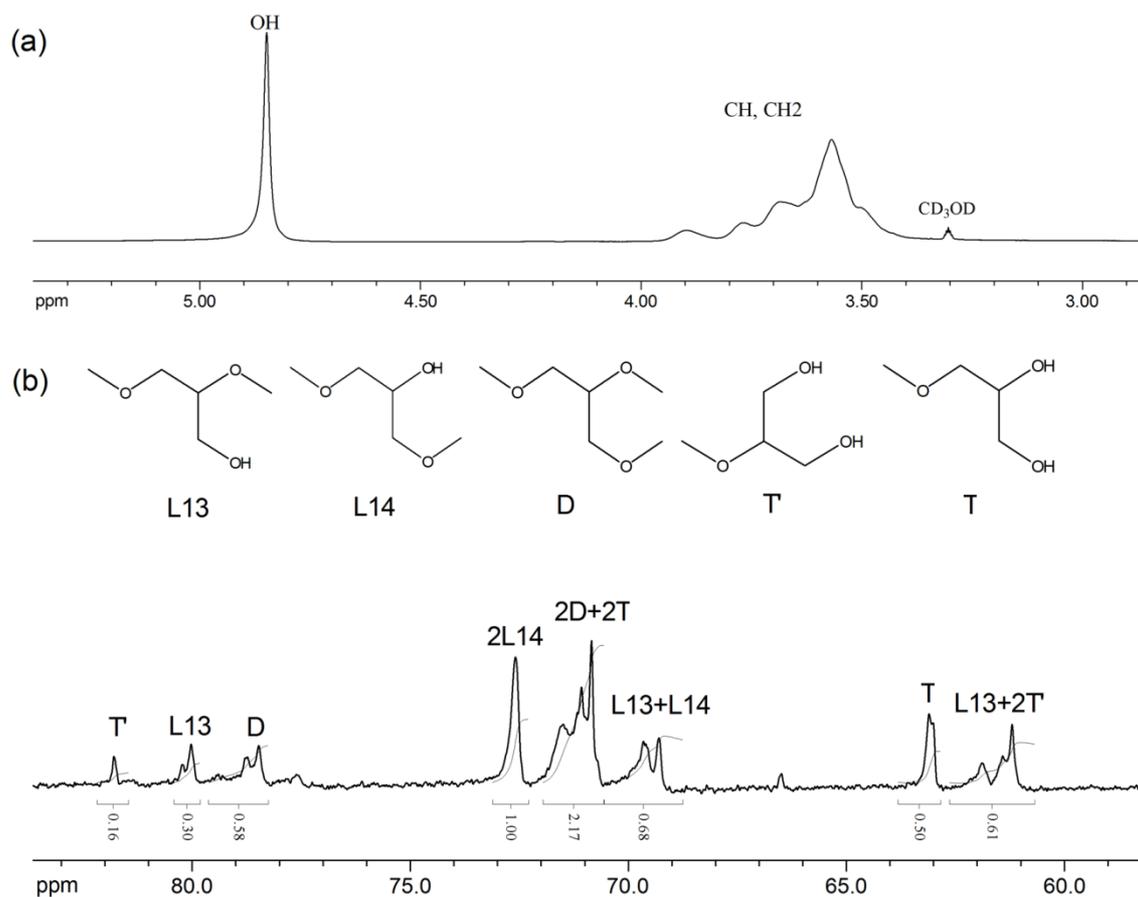
**Table S2**  $\zeta$ -Potential of various  $\beta$ -CD derivatives and SDP solutions

Sample	$\zeta$ -Potential (mV) <sup>a</sup>
4	9.8 $\pm$ 0.9
5	15.1 $\pm$ 0.7
IC1	9.1 $\pm$ 1.9
IC2	11.0 $\pm$ 0.4
IC3	12.1 $\pm$ 0.5
IC4	13.6 $\pm$ 0.3
IC5	15.0 $\pm$ 0.6

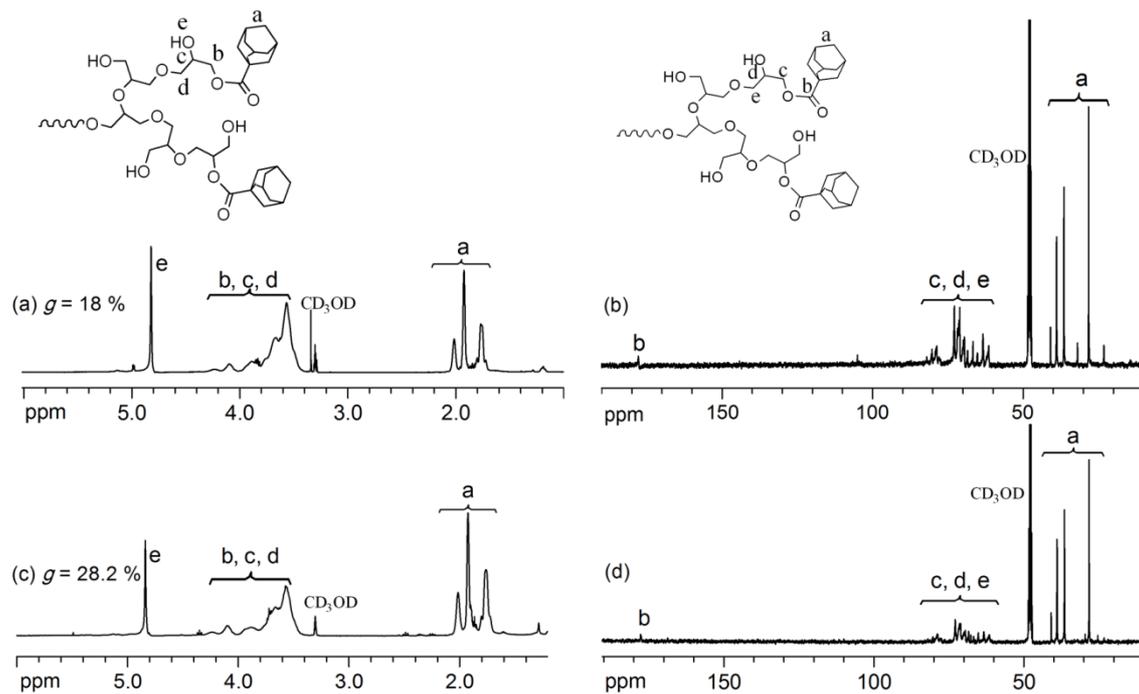
<sup>a</sup> Data represent mean standard deviation (n = 3)



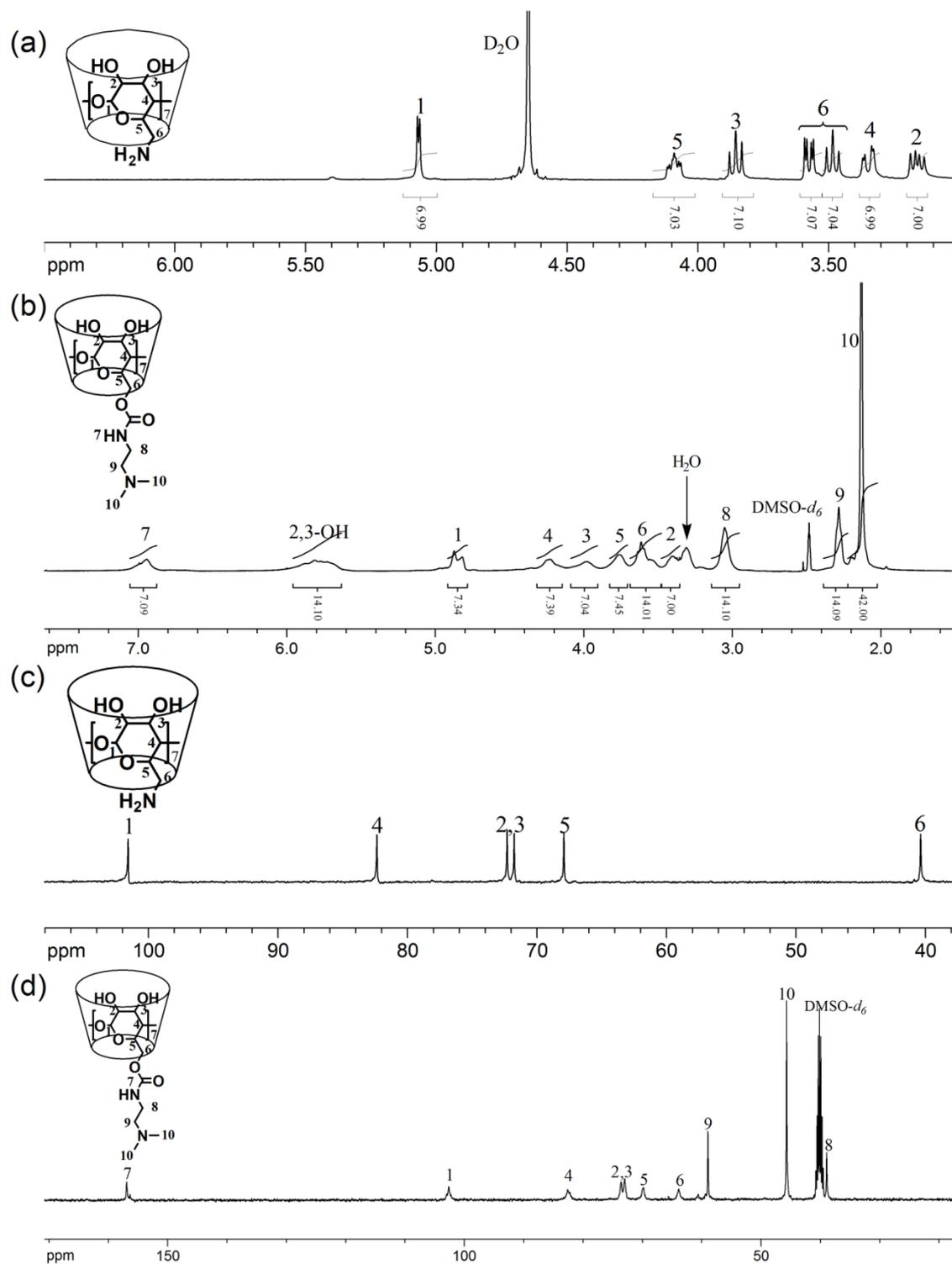
**Scheme S1** Synthesis of HPG-AD (a), per-6-amino- $\beta$ -CD (b) and per-6-DMAE- $\beta$ -CD (c).



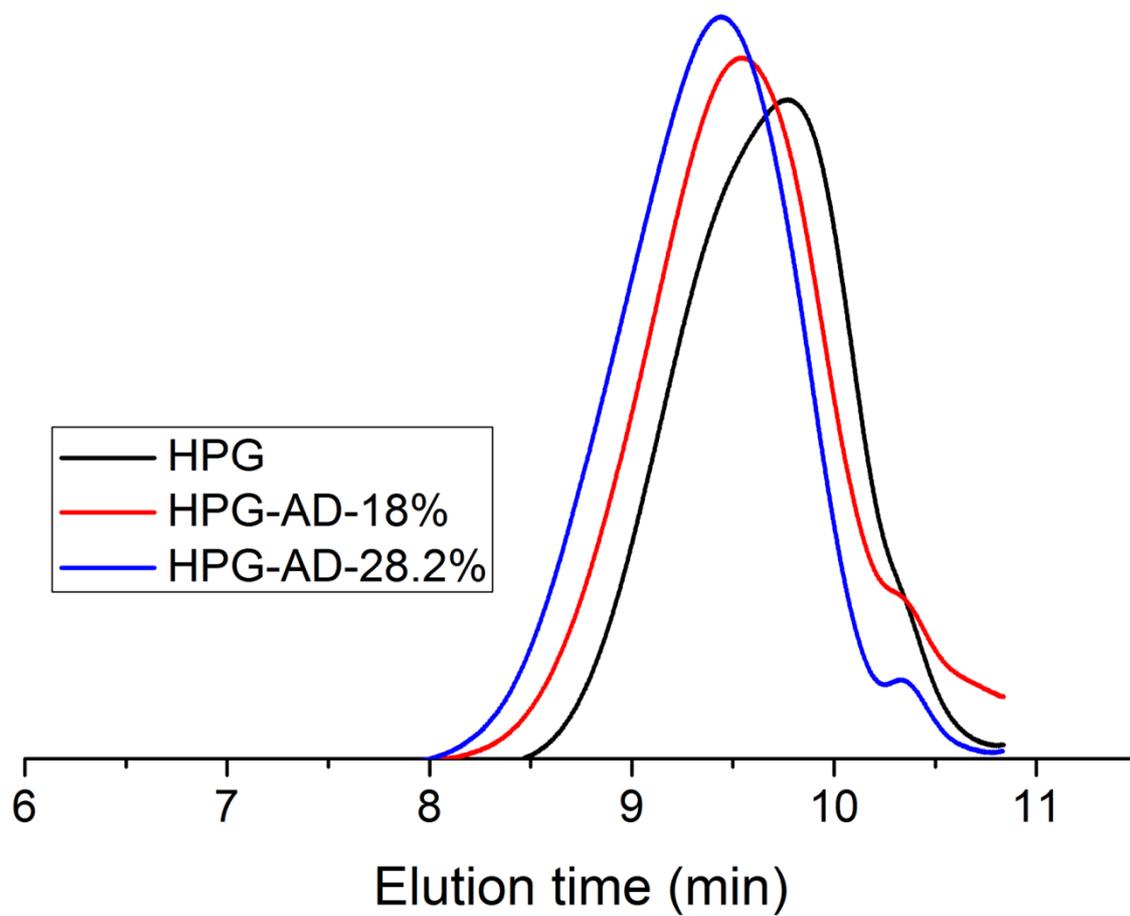
**Fig. S1**  $^1\text{H}$  NMR spectrum (a) and quantitative  $^{13}\text{C}$  NMR spectrum (b) of HPG in  $\text{CD}_3\text{OD}$ .



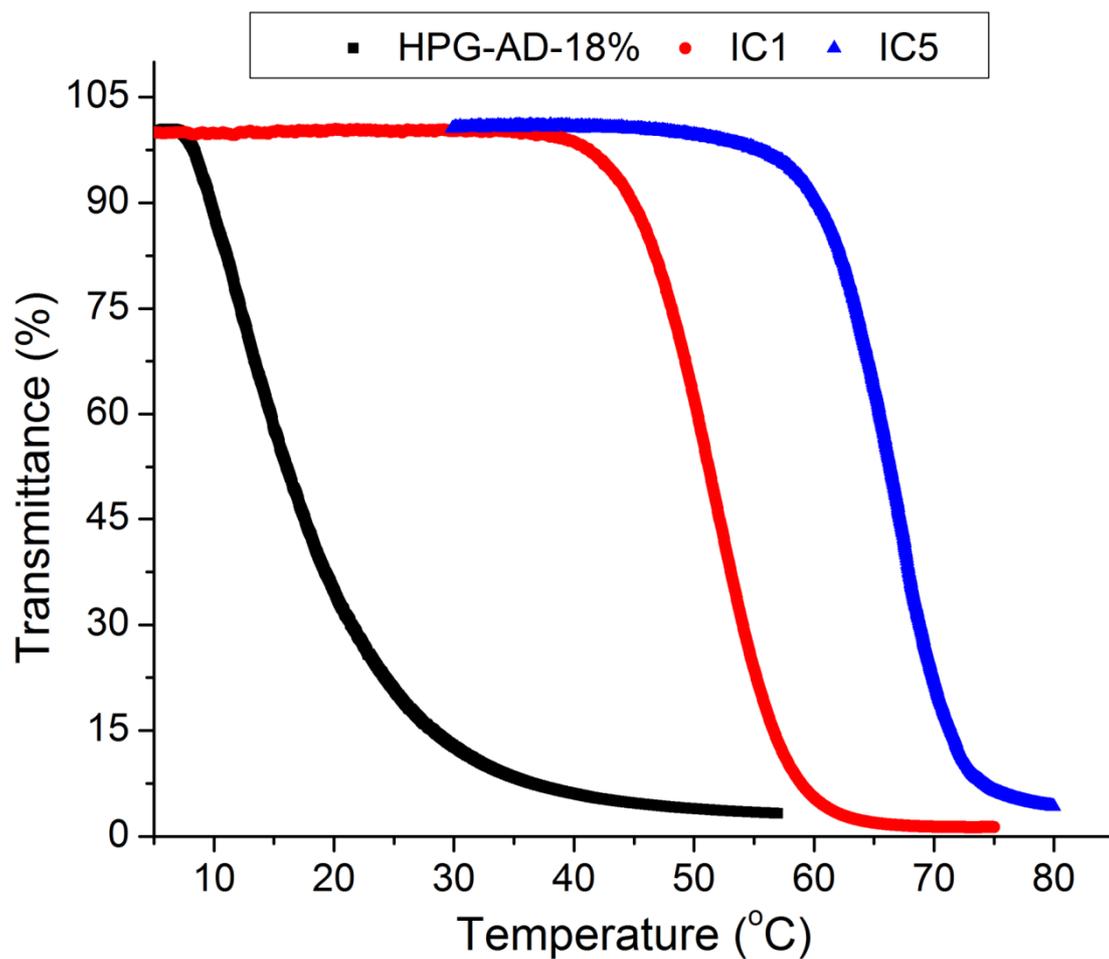
**Fig. S2**  $^1\text{H}$  NMR spectra and  $^{13}\text{C}$  NMR spectra of HPG-AD-18% (a, b) and HPG-AD-28.2% (c, d) in  $\text{CD}_3\text{OD}$ .



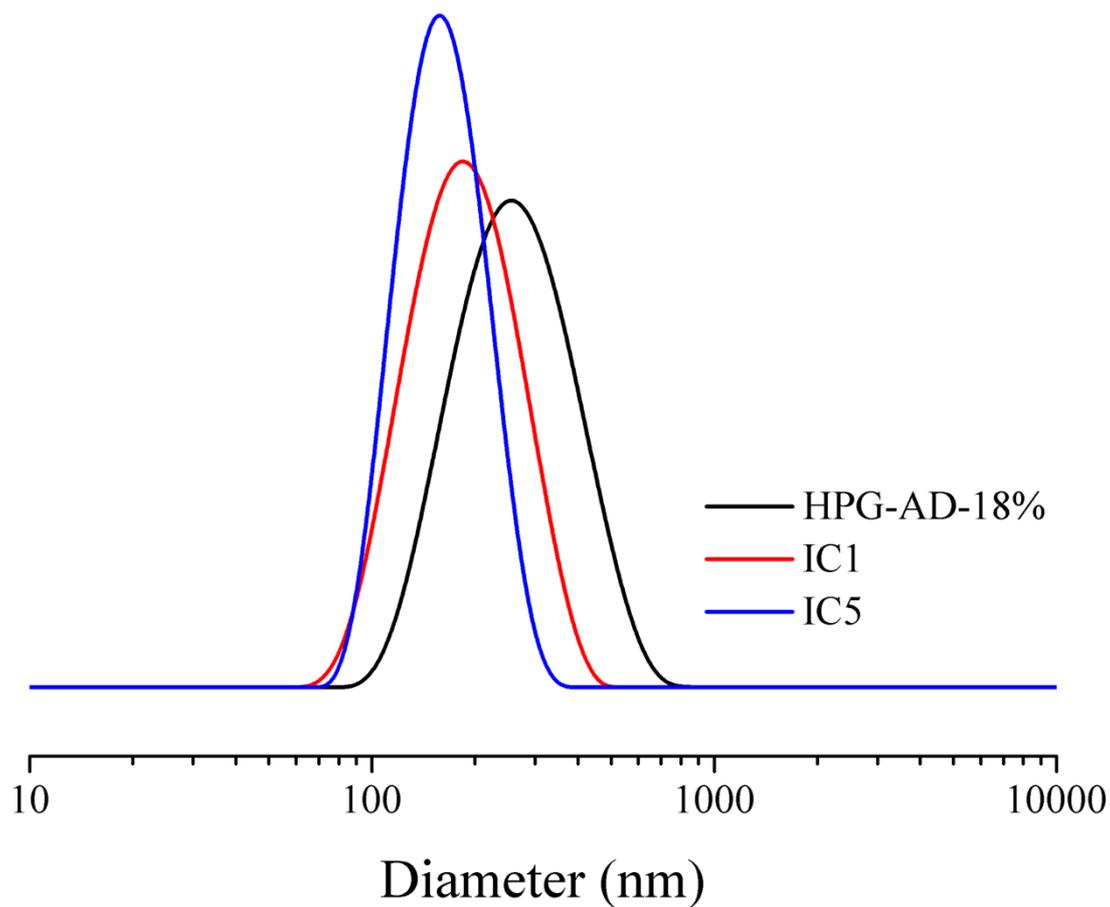
**Fig. S3** <sup>1</sup>H NMR spectrum (a) and <sup>13</sup>C NMR spectrum (c) of per-6-amino-β-CD (4) in D<sub>2</sub>O, and <sup>1</sup>H NMR spectrum (b) and <sup>13</sup>C NMR spectrum (d) of per-6-DMAE-β-CD (5) in DMSO-*d*<sub>6</sub>.



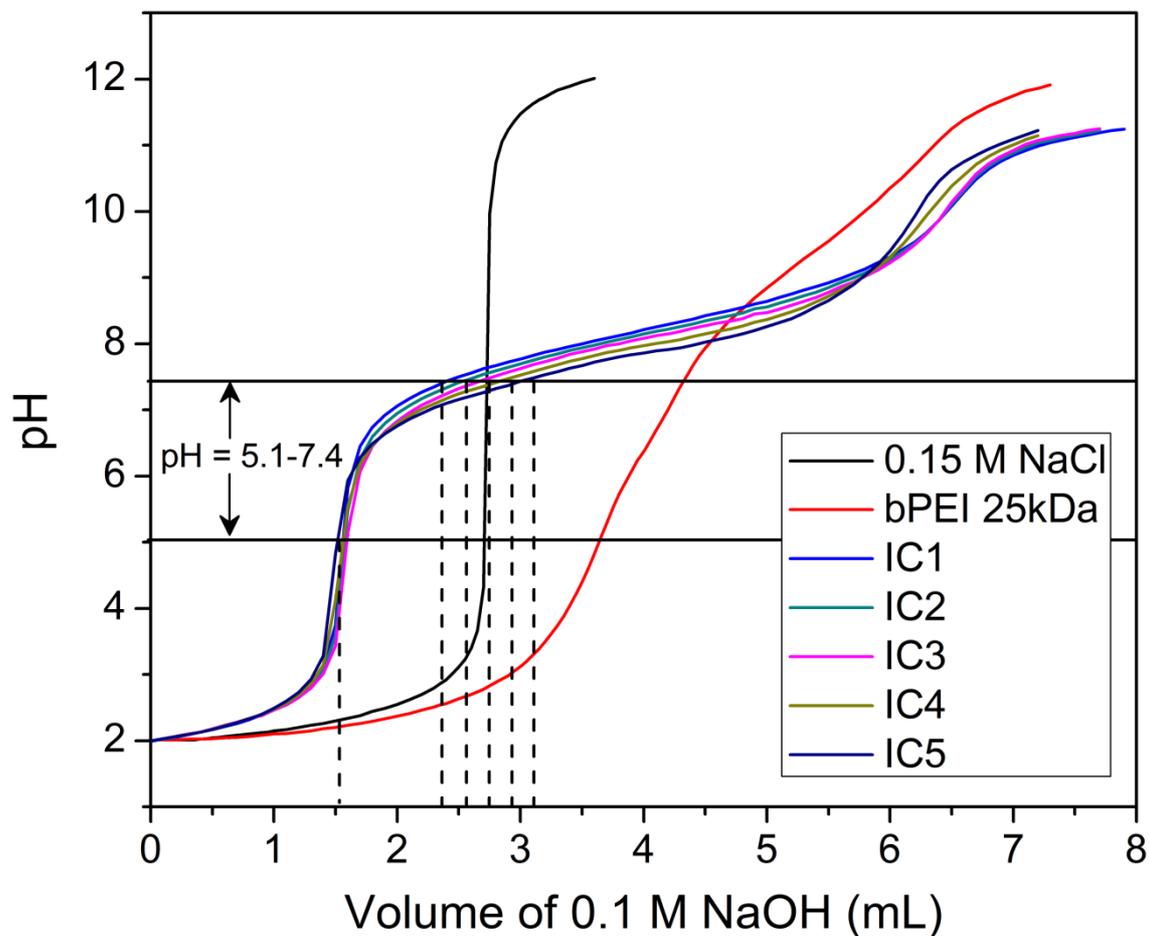
**Fig. S4** GPC profiles of HPG, HPG-AD-18% and HPG-AD-28.2%.



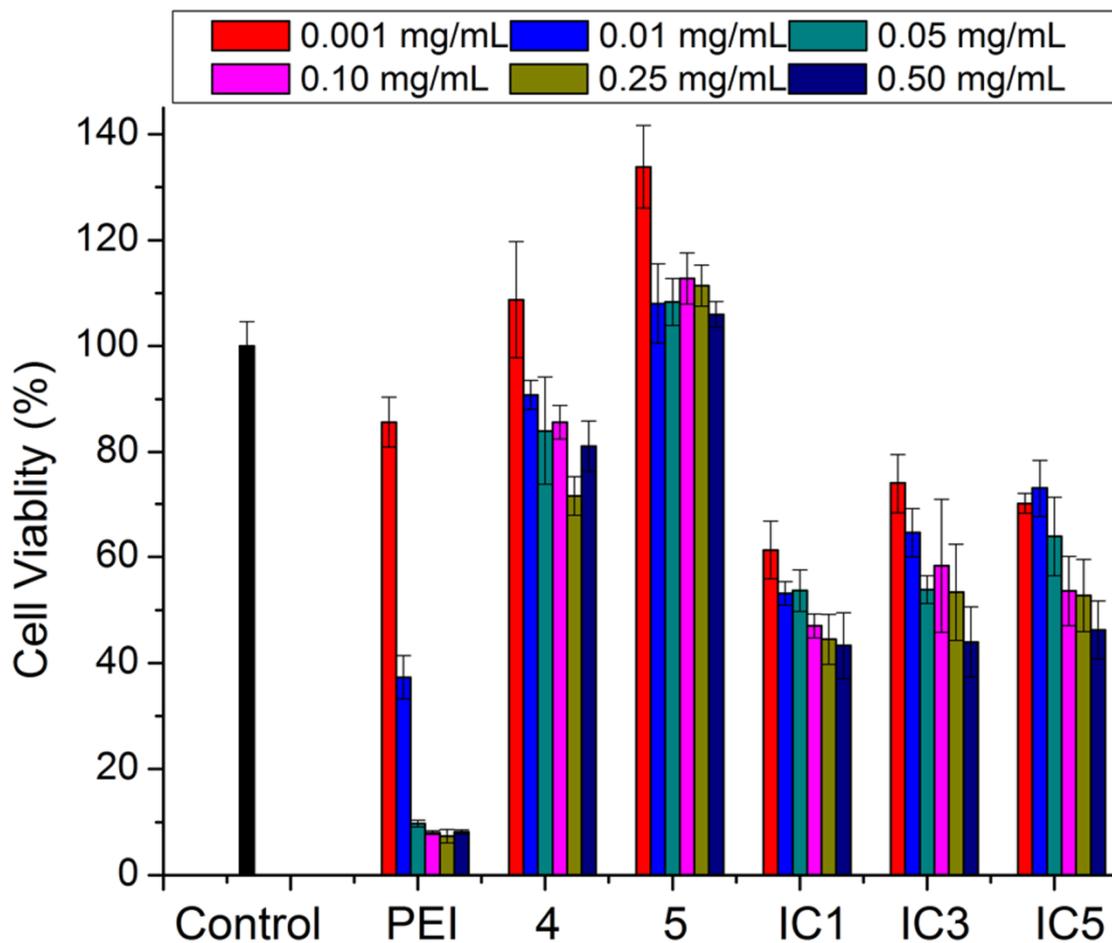
**Fig. S5** Temperature dependence of light transmittance for aqueous solutions of HPG-AD, HPG-AD/4 (IC1) and HPG-AD/5 (IC5). The polymer concentration was 1.0 mg/mL.



**Fig. 6** DLS plots of aqueous solutions of HPG-AD, HPG-AD/4 (IC1) and HPG-AD/5 (IC5) at 25 °C. The polymer concentration was 0.2 mg/mL.



**Fig. S7** Acid-base titration curves for SDPs (IC1, IC2, IC3, IC4 and IC5), branched PEI (25 kDa) and blank (0.15 M NaCl) solution from pH 2 to 11.



**Fig. S8** Cell viability assay of per-6-amino- $\beta$ -CD (**4**), per-6-DMAE- $\beta$ -CD (**5**), IC1, IC3, IC5, and branched PEI (25 kDa) in COS-7 cells for 24 h. Data represent mean standard deviation ( $n = 4$ ).

### 3. References

- (1) Tokar, R.; Kubisa, P.; Penczek, S. *Macromolecules* **1994**, *27*, 320.
- (2) Sunder, A.; Hanselmann, R.; Frey, H.; Mülhaupt, R. *Macromolecules* **1999**, *32*, 4240.
- (3) Royappa, A. T.; Vogt, M. L.; Sharma, V. *J. Appl. Polym. Sci.* **2004**, *91*, 1344.
- (4) Alker, D.; Harding, V. D. *J. Org. Chem.* **1996**, *61*, 903.
- (5) Zhong, Z.; Feijen, J.; Lok, M. C.; Hennink, W. E.; Christensen, L. V.; Yockman, J. W.; Kim, Y. H.; Kim, S. W. *Biomacromolecules* **2005**, *6*, 3440.