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Self-healing supramolecular hydrogels through host–guest interaction between cyclodextrin and carborane

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Experimental section

Materials. Dextran (average 7.0×10^4 Da) was used directly from J&K Scientific. Poly(acrylic acid) (PAA, average M.W. 25×10^4 Da, 35wt% in H_2O) purchased from Sigma-Aldrich Co. and used after lyophilization. Decaborane (B10H14) was purchased from Changchun Randall technology Co., Ltd and used without further purification. 1-(hydroxymethyl)-1,2-dicarba-carbrane (HMCB) was synthesized according to our previous work.[1] β-Cyclodextrin, ethylenediamine, succinic anhydride, dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), triethylamine (Et_3N) and amantadine hydrochloride was purchased from Aladdin Chemistry Co. Ltd. Shanghai, China. All other chemicals were purchased from Sigma-Aldrich and used as received.

Methods. ^{1}H, ^{13}C, and ^{11}B NMR spectra were measured by a Unity-400 NMR spectrometer (Bruker) at room temperature. ^{1}H and ^{13}C chemical shifts are reported in ppm with tetramethylsilane as an internal reference. ^{11}B chemical shifts are reported in ppm relative to an external standard of $BF_3{\cdot}Et_2O$. 2D-NOESY spectrum was measured to determine the host-guest interaction by a Unity-600 NMR spectrometer (Bruker) at room temperature. Fourier Transform Infrared (FTIR) spectra were recorded on a Bruker Vertex 70 spectrometer. Mass Spectroscopy (ESI-MS) measurements were performed on a Quattro Premier XE system (Waters) equipped with an electrospray interface (ESI). Inductively coupled plasma mass spectrometry and inductively coupled plasma optical emission spectroscopy (ICP-OES) were used to determine the boron contents. Gel permeation chromatography (GPC) measurements were operated on a TOSOH HLC-8220 SEC instrument using 0.1 M $NaNO_3$ as eluent with a flowing rate of 0.5 mL/min at 30 ^{o}C and hydroxyl poly (methyl methacrylate) was used as standard substance. The instrument was carefully calibration and all samples (1μg/mL) were filtrated on a 0.2μm pore membrane before injection. Rheological experiments were carried out at 25 ^{o}C on a strain-controlled ARES G2 rheometer (TA Instruments) with Peltier temperature control system, and cone−plate geometry (diameter of 20 mm, cone angle of 2°, truncation height 49 μm) was used. Morphology of hydrogel was observed using environmental scanning electron microscope (SEM, XL-30 ESEM FEG Scanning Electron Microscope FEI COMPANYTM) at an acceleration voltage of 5 kV. The tensile test of the different hydrogels was evaluated by a WSN-10 kN universal tension machine (Changchun Smart Instrument Co., Ltd, China) at room temperature.

Synthesis of mono- [2-(1,2-dicarba-closo-dodecaborane)methylene] succinate (CB-COOH).

1-(Hydroxymethyl)-1,2-dicarba-*closo*-carbrane (HMCB) was firstly synthesized according to our previous work.[1] As shown in Fig. S1, CB-COOH was synthesized by ring-opening reaction of succinic anhydride from the HMCB initiator. In brief, a dried flask containing HMCB (200 mg, 1.14mmol), succinic anhydride (170 mg, 1.70 mmol), DMAP (69 mg, 0.57 mmol) and dried DMF (10 mL) was warmed at 65 ^{o}C for 24 h. After cooling, the solvent was evaporated under reduced pressure. The crude product was redissolved in CH_2Cl_2, washed with saturated potassium hydrogen sulfate solution, dried over magnesium sulfate, filtered and concentrated under vacuum. The white solid was obtained after purified by chromatography (ethyl acetate−petroleum ether 1:5, Rf = 0.3). Yield: 180 mg, 58%. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) (Fig. S2): 5.24 (s, 1H, $C_{cage}H$), 4.65 (s, 2H, C_{cage}-CH_2O-), 2.60 (t, 2H, -$OCCH_2$-), 2.52 (m, 2H, -

CH_2COOH), 12.25 (s, 1H, -COOH), 1.5-3.0 (br, 10H, BH). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm) (Fig. S3):173.54, 170.91, 72.87, 63.47, 61.98, 28.40. ^{11}B NMR (400 MHz, $CDCl_3$, δ ppm) (Fig. S4): -1.99， -4.46， -9.40， -11.62，-13.08. FT-IR (KBr, cm^{-1}) (Fig. S5): ν 3046, 2965, 2929, 2600, 1747, 1710. MS (ESI^-) (Fig. S6): [M-H^+] calculated for $C_7B_{10}H_{17}O_4$: 273.2, found: 273.4.

Synthesis of carborane-modified dextran (DEX-CB)

Carborane was conjugated to dextran (average M.W. 7.0×10^4 Da) by a condensation reaction. A typically synthetic procedure of DEX-CB was given: Dextran (950 mg, 13.6 μmol), CB-COOH (133 mg, 0.49 mmol) and DMSO (20 mL) were added into a dried flask. After dissolution, DCC (254 mg, 0.97 mmol) and DMAP (60 mg, 0.49 mmol) were added. The molar ratio of D-glucose in dextran to CB-COOH was set at 12:1. After reaction for 48 h at room temperature, the mixture was precipitated in ethyl ether. The crude product was dissolved in water, and dialyzed for a week in a dialytic bag (MWCO = 3500 Da). After lyophilization, white spongy DEX-CB was obtained. Yield: 780 mg, 72%. ^1H NMR (400 MHz, DMSO-d_6, δ ppm) (Fig. S2): 5.12-5.21 ($C_{cage}H$ + C_2H(dextran)), 4.88 (C_4OH(dextran)), 4.82 (C_3OH(dextran)), 4.66 (C_1H(dextran) + C_{cage}-CH_2(CB)), 4.45 (C_2OH(dextran)), 3.73, 3.47 (C_6H(dextran)), 3.61 (C_5H(dextran)), 3.41 (C_3H(dextran)), 3.11-3.22 ($C_{2,4}H$(dextran)), 2.52-2.64 (-CH_2CH_2(CB)), 1.02-1.84 (BH(CB)). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm) (Fig. S3): 172.13, 171.30, 98.67, 73.19, 72.29, 70.82, 70.57, 66.52, 28.95. FT-IR (KBr, cm^{-1}) (Fig. S5): ν 3693-3095, 2951, 2558, 1750, 1693. To acquire the degree of side chain modification accurately, ICP-OES was used to determine the boron concentration. The concentration of carborane group was 0.33 mmol/g, and the degree of substitution was calculated as 5.8%.

Synthesis of β-CD-modified poly(acrylic acid) (PAA-CD)

β-Cyclodextrin-modified poly(acrylic acid) (PAA-CD) was obtained by a condensation reaction between a mono-amino-mono-deoxy-β-CD (β-CD-NH_2) and PAA. Firstly, β-CD-NH_2 was synthesized according to the reference.[2] PAA (650 mg, 2.6 μmol) was dissolved in 30 mL dried DMF. To this solution, PyBOP (389 mg, 0.88 mmol) and Et_3N (89 mg, 0.88 mmol) were added. After stirring for 2 h, β-CD-NH_2 (690 mg, 0.58 mmol) was added, and the solution was stirred for another 48 h at room temperature. The molar ratio of acrylic acid unit in PAA to β-CD-NH_2 was set at 15:1. The crude product was reprecipitated from ethanol (300 mL) and washed with ethanol. After redissolved in water, the polymer was dialyzed for a week in a dialytic bag (MWCO = 14000 Da). After lyophilization, faint yellow spongy PAA-CD was obtained. Yield: 1.14 g, 85%. ^1H NMR (400 MHz, D_2O, δ ppm) (Fig. S7): 1.53-1.76 (CH_2(PAA)), 2.20 (CH(PAA)), 3.51-3.56 ($C_{2,4}H$(CD)), 3.80-3.89 ($C_{3,5,6}H$(CD)), 5.00-5.07 (C_1H(CD)). ^{13}C NMR (100 MHz, D_2O, δ ppm) (Fig. S8): 35.07, 42.89, 60.64, 71.80, 71.04, 81.30, 101.84, 180.73. FT-IR (KBr, cm^{-1}) (Fig. S9): ν 3654-3103, 2972, 1730, 1560. MS (ESI^+) of β-CD-NH_2 (Fig. S10): [M+H^+] calculated for $C_{44}H_{77}N_2O_{34}$: 1177.4, found: 1177.7. Degree of side chain modification was calculated as 5.5% from the integral between PAA protons and C_1H(CD) protons in the ^1H NMR spectrum. The concentration of CD group was calculated as 0.41 mmol/g.

Isothermal titration calorimetry measurements

The host-guest interaction between PAA-CD and DEX-CB in water was characterized by using a NanoITC apparatus (TA Instruments) at room temperature at a stirring speed of 250 rpm. The sample cell (1.0 mL) was filled with degassed DEX-CB aqueous solution (0.2 mM). The syringe was

filled with PAA-CD aqueous solution (4.48 mM). The titration experiment was set to 25 injections of 10 μL each with 600 s intervals. Duplicate titrations were performed to ensure reproducibility. The heats of dilution were determined in blank experiments in which the PAA-CD solutions were injected into the sample cell containing dextran. The dilution heats were then subtracted to obtain the binding heats. The data was processed using ITCAnalyze software and fitting in an "independent" binding model.

Preparation of supramolecular hydrogels

Hydrogels were obtained by mixing PAA-CD aqueous solution and DEX-CB aqueous water. The typical procedure was as follows: a certain amount of DEX-CB and PAA-CD were dissolved in deionized water, respectively. The two solutions were mixed to form hydrogels with different polymer concentrations (7, 10, 15 wt%). The β-CD/CB molar ratio was kept at 1:1. To investigate the effect of molar ratio (n[β‑CD]/n[CB]) in the hydrogels on the viscoelastic behavior, the β-CD/CB molar ratio (2:1, 1:1, and 1:2) was changed to prepare the hydrogels at the same polymer concentration (10 wt%). As a control group, a competing guest molecule, amantadine hydrochloride (Ad) was added to DEX-CB solution and then mixed with the PAA-CD solution. The molar ratio of Ad to CB was set as 2:1 and 5:1.

Characterization of supramolecular hydrogels

Rheological characterization of the hydrogels was done with a ARES G2 rheometer (TA Instruments) equipped with a 2° steel cone geometry of 20 mm diameter and solvent trap. To test the viscoelastic behaviors of the supramolecular hydrogels (20 mm in diameter), the frequency sweep tests were operated, which covered angular frequencies from 0.1 to 100 rad/s at controlled regular strain of γ = 0.1.

The self-healing of hydrogels was operated in strain amplitude sweep (1−1500%) at a fixed angular frequency of 1.0 rad/s. The hydrogels disks were measured with the alternate step strain test (strain = 1 and 300%, angular frequency = 1.0 rad/s). The continuous step strains were switched with 300 s for every strain interval. In addition, we performed a break test, and the self-healing phenomenon could be observed optically. Cylindrical hydrogel was cut in half. The two semicylindrical supramolecular hydrogels leaned on each other at the site of fresh cuts. The healing process of the hydrogels was recorded on an optical microscopy at diverse time intervals. As a control group, Ad (1 mM) aqueous solution was spread on the cut surface.

The swelling property of hydrogels was estimated by the swelling ratio (Q). The prepared hydrogels were recorded their weight (W_0) and submersed in water at room temperature. At a predetermined time interval, the remaining hydrogel was weighed (W_t) after removing the water, and replaced with isometric fresh media. Swelling ratio was calculated by the following equation:

$$Q = (W_t - W_0)/ W_0$$

***In vitro* cytotoxicity study**

A mouse fibroblast cell line (L929) was used to evaluate the cell compability of polymers. The cell line was cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplied with 10% heat-inactivated fetal bovine serum (FBS, GIBCO), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma). L929 cells were seeded at a density of 4×10^3 cells per well in 96-well plates and incubated for 24 h, then treated with different concentrations of PAA-CD/

DEX-CB from 0 to 20 mg/mL at 37 °C for another 24 h. For cell imaging, cells were stained with live/dead cell assay (calcein-AM and propidium iodide, Nanjing KeyGen Biotech. Co. Ltd, China) and imaged with a fluorescence microscope (Nikon ECLIPSE/Ti Series, Japan). For cell viability, MTT assay was performed and the absorbance at 450 nm was measured using microplate reader (ELx 680, BioTek Instrument Inc).

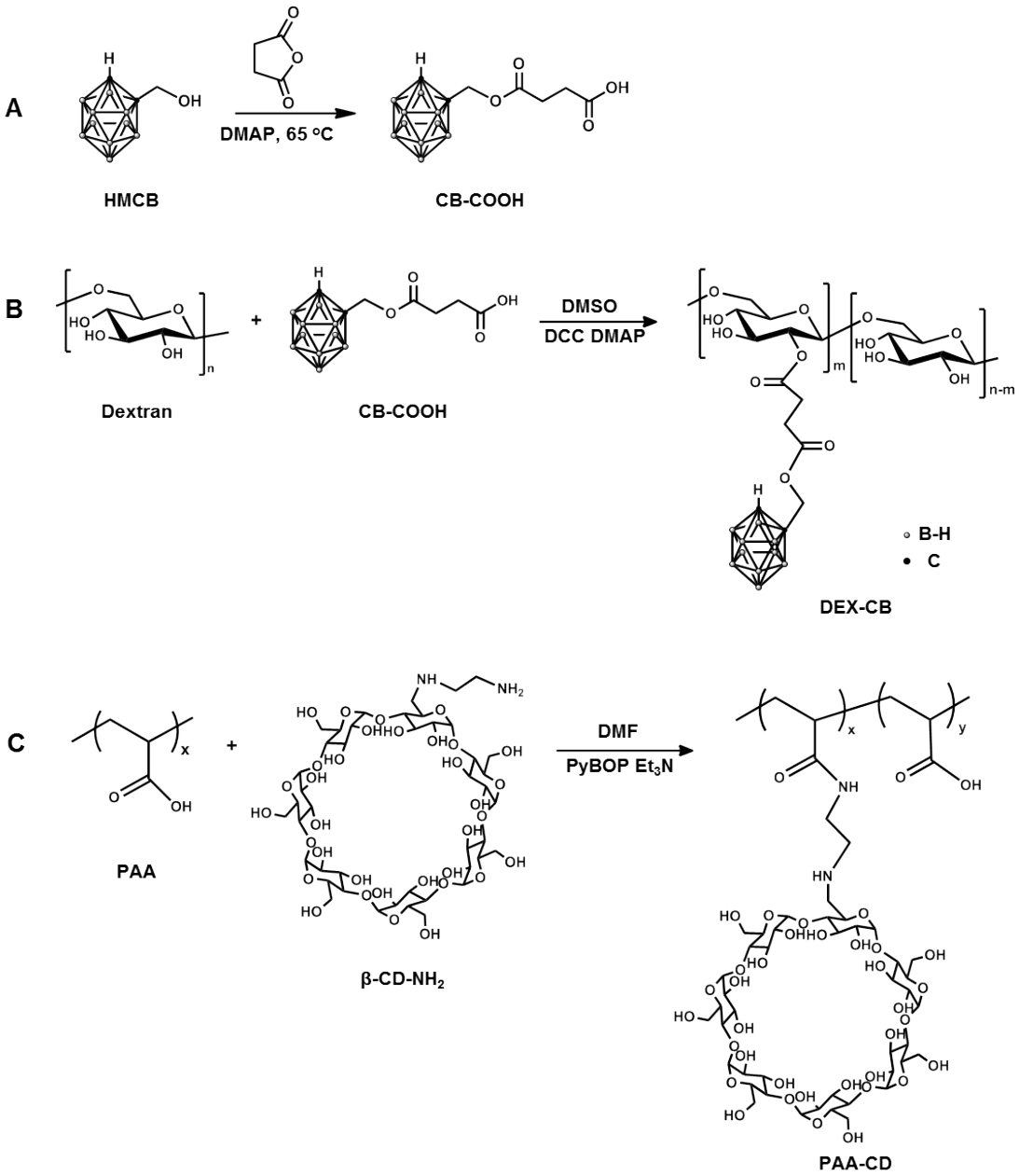

Fig. S1. Synthetic procedure of (A) CB-COOH, (B) DEX-CB and (C) PAA-CD.

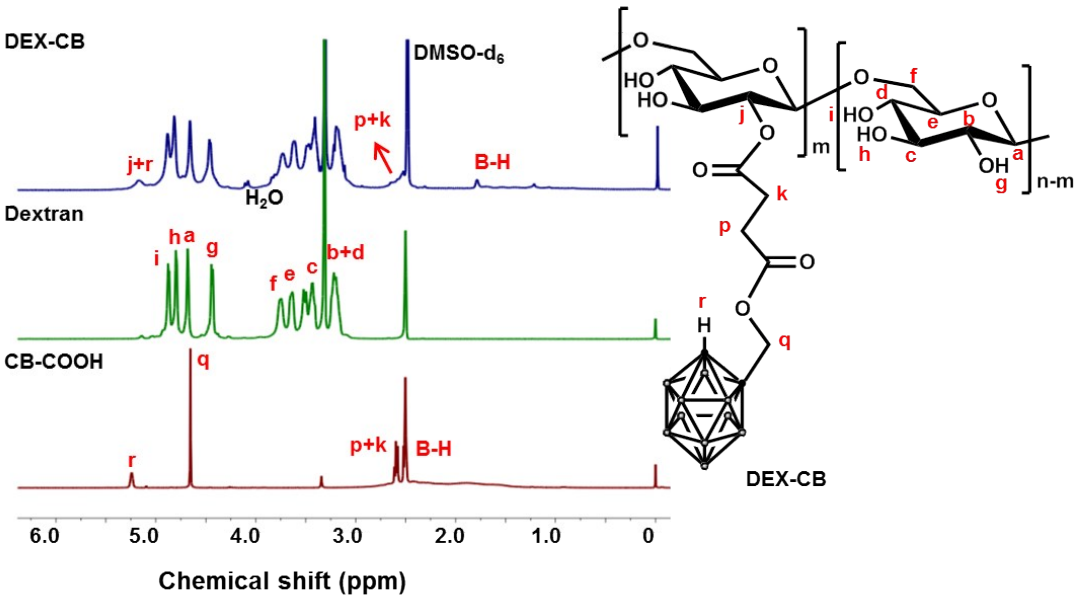

Fig. S2. ^{1}H NMR spectra of DEX-CB, dextran and CB-COOH in DMSO-d_6 (400 MHz).

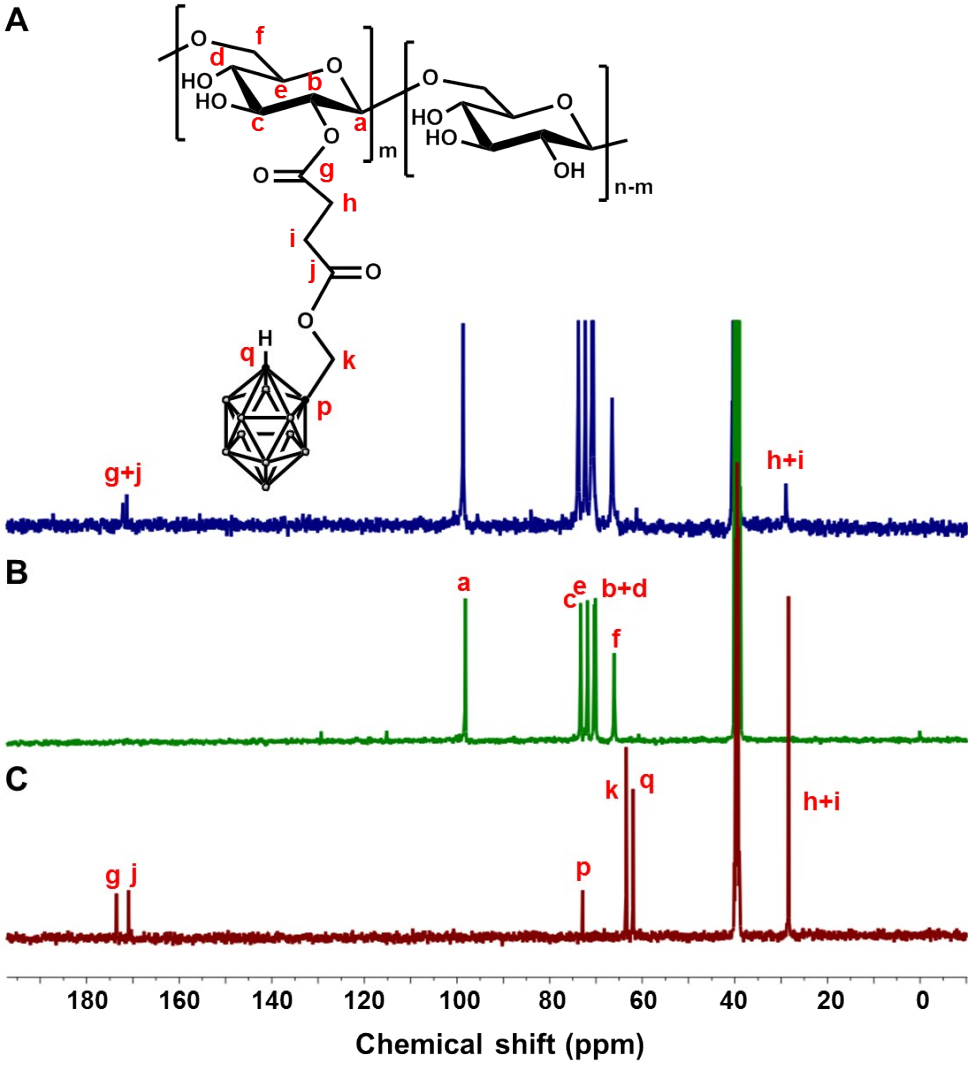

Fig. S3. ^{13}C NMR spectra of (A) DEX-CB, (B) Dextran and (C) CB-COOH in DMSO-d_6 (100 MHz).

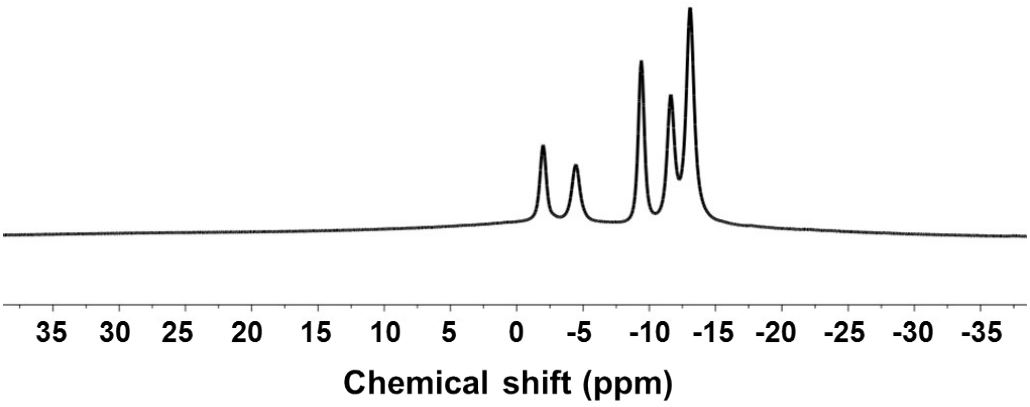

Fig. S4. ^{11}B NMR spectrum of CB-COOH in $CDCl_3$ (400 MHz).

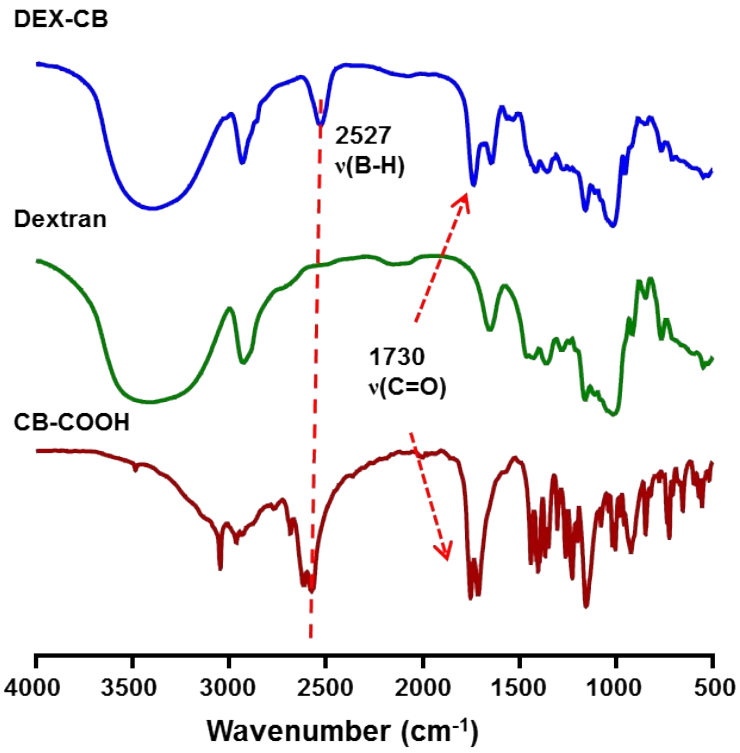

Fig. S5. FT-IR spectra of DEX-CB, dextran and CB-COOH.

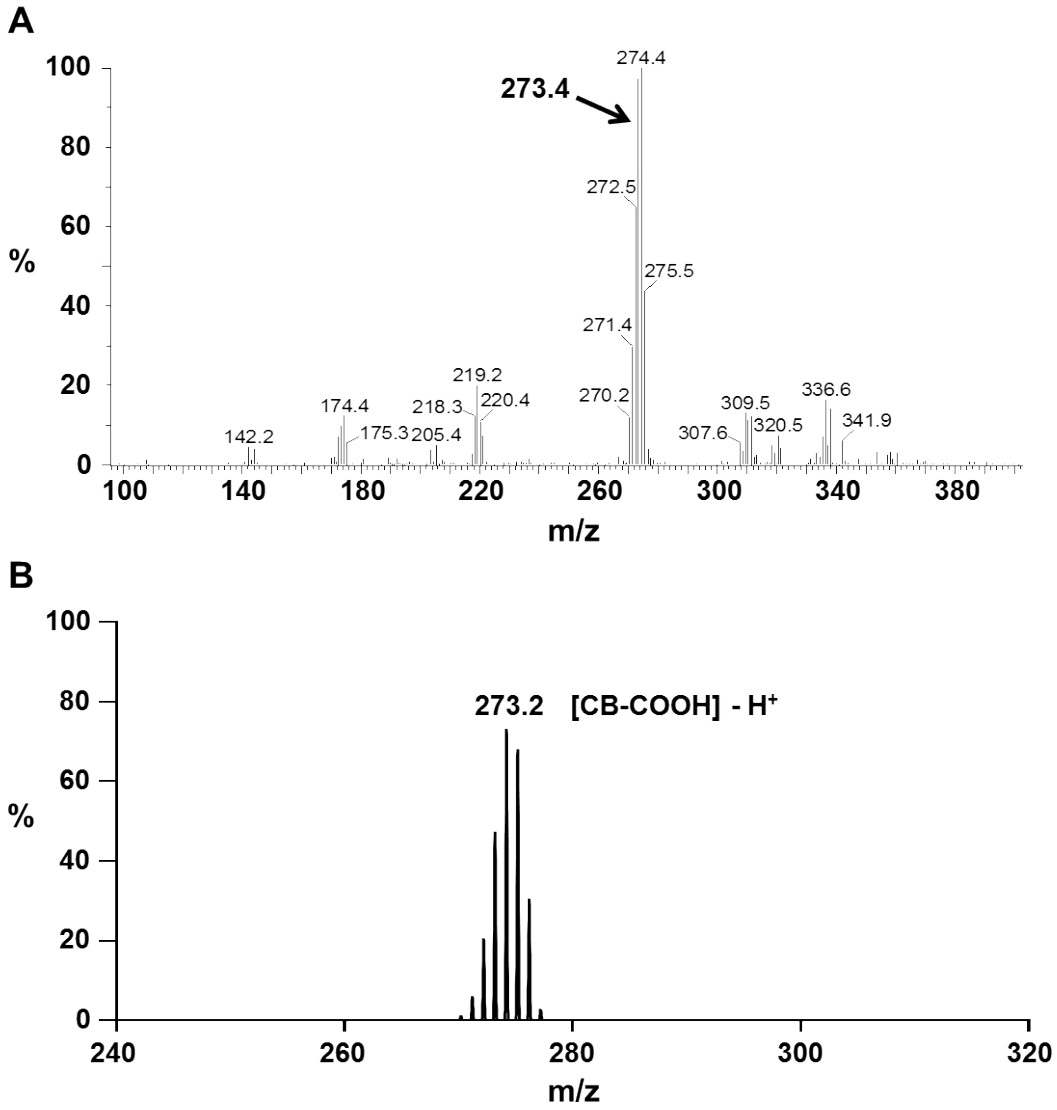

Fig. S6. (A) Measured and (B) theoretical ESI-MS spectra of CB-COOH in negative mode.

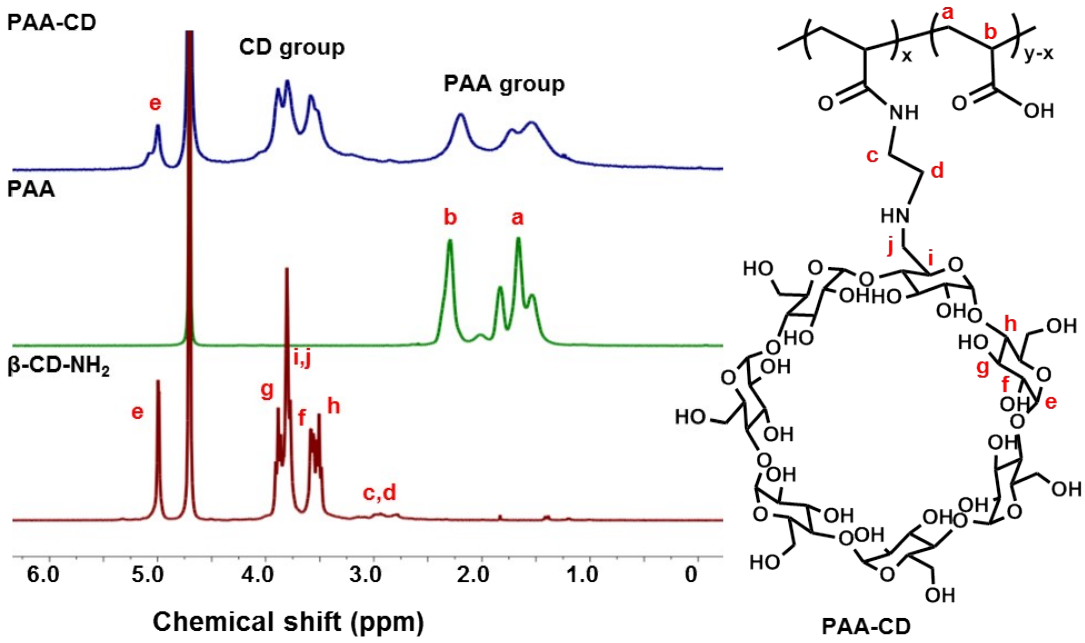

Fig. S7. ^{1}H NMR spectra of PAA-CD, PAA and β-CD-NH_2 in D_2O (400 MHz).

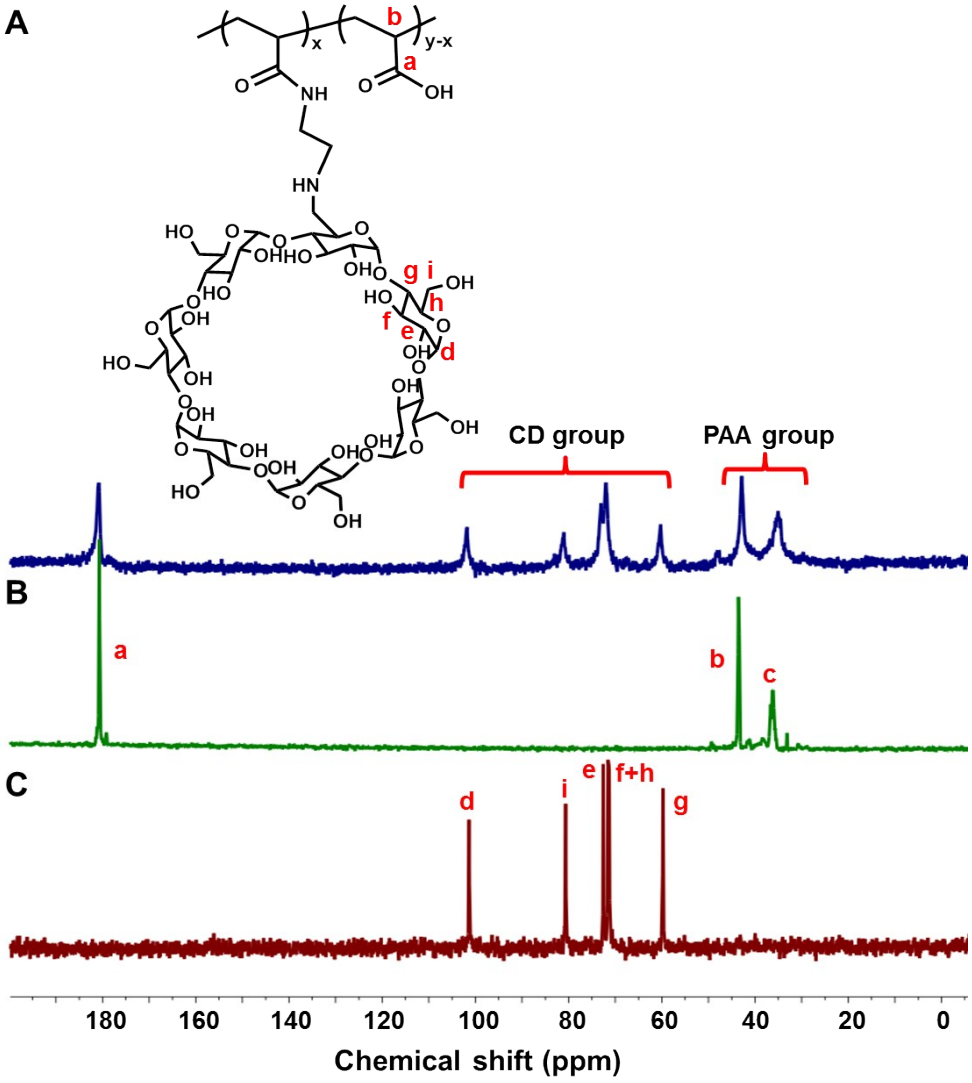

Fig. S8. ^{13}C NMR spectra of (A) PAA-CD, (B) PAA and (C) β-CD-NH_2 in D_2O (100 MHz).

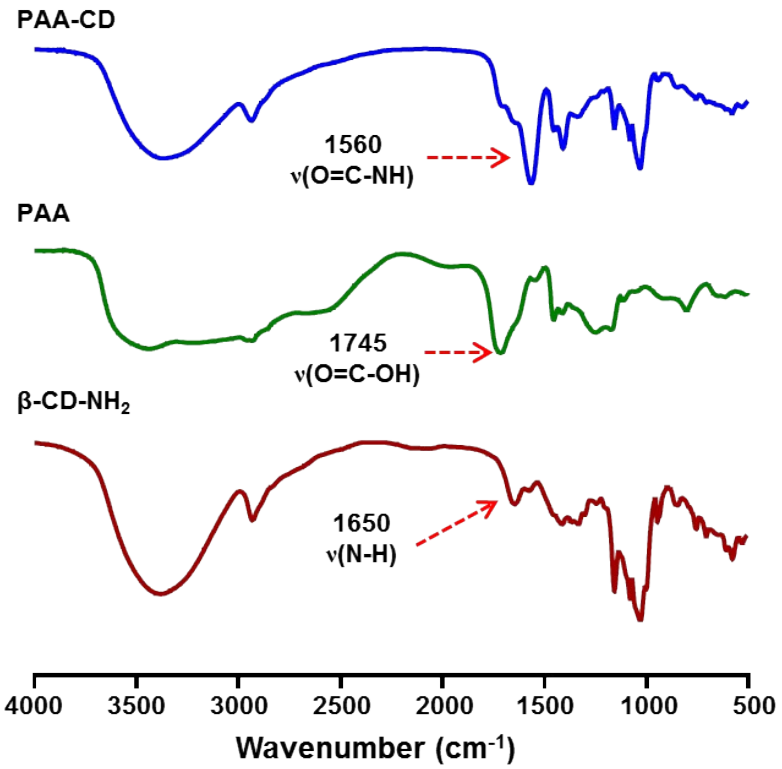

Fig. S9. FT-IR spectra of PAA-CD, PAA and β-CD-NH_2.

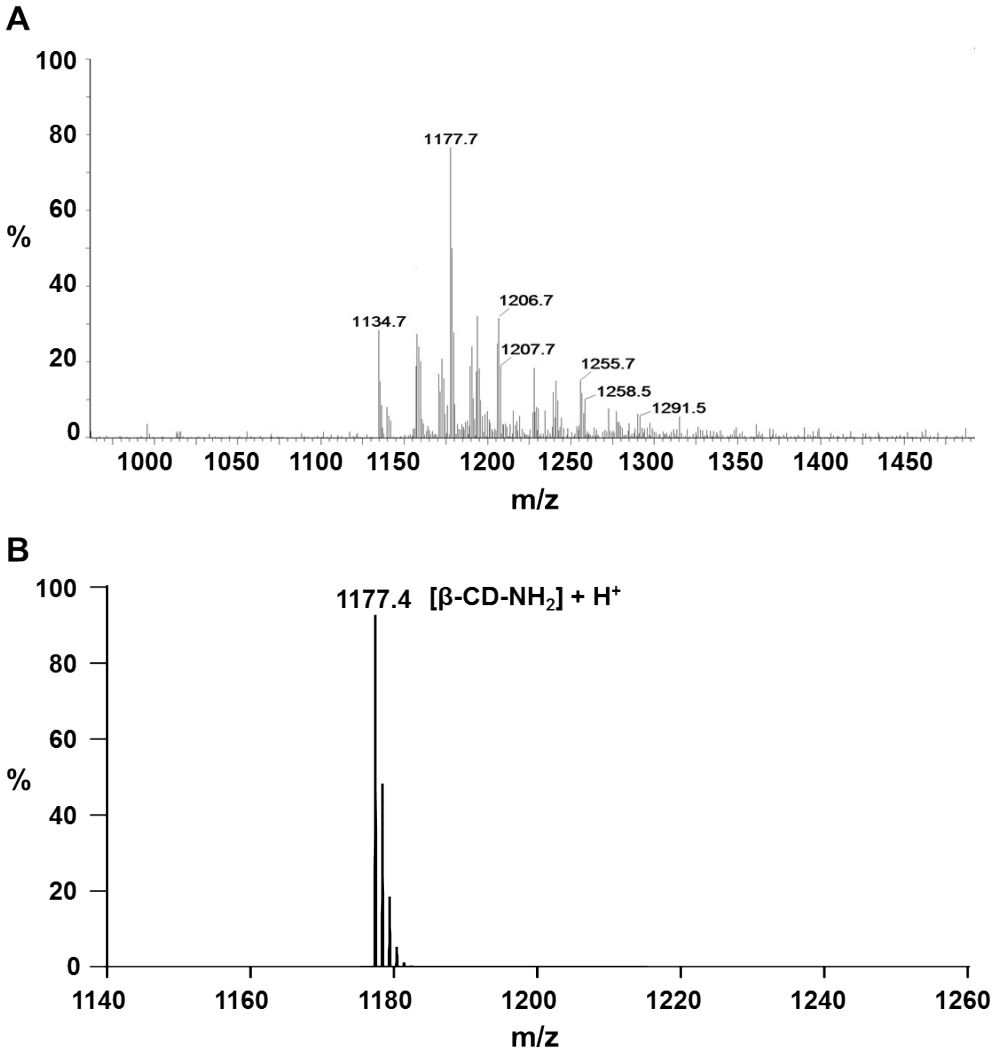

Fig. S10. (A) Measured and (B) theoretical ESI-MS spectra of β-CD-NH_2 in positive mode.

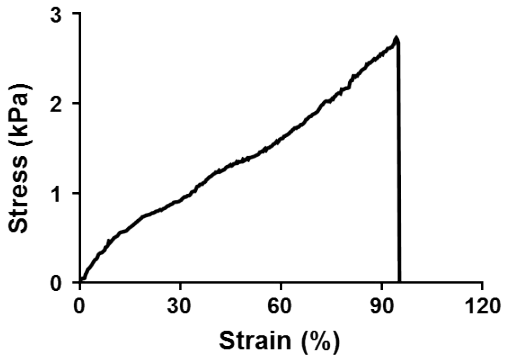

Fig. S11. Mechanical tensile curves of PAA-CD/DEX-CB hydrogel (10 wt%) after water balance.

Table S1. Characteristics of DEX-CB and PAA-CD

Polymer	Feed ratio[a]	$Mn^{b}/(\times 10^{4})$	Yield (%)	Side chain modification ratio[c] (%)
DEX-CB	12:1	7.84	70	5.8
PAA-CD	15:1	0.79	85	5.5

[a]The molar ratio of repetitive unit to carobrane or cyclodextrin in the feed; [b]Acquried form GPC measurement; [c]Degree of side chain modification in PAA-CD was calculated from the integral between PAA protons and C1H(CD) protons in the ^{1}H NMR spectrum. In the case of DEX-CB, ICP-OES was used to determine the boron concentration and calculate the carborane substition degree.

References

1. H. Xiong, D. Zhou, Y. Qi, Z. Zhang, Z. Xie, X. Chen, X. Jing, F. Meng and Y. Huang, *Biomacromolecules*, 2015, **16**, 3980.

2. M. Nakahata, Y. Takashima, H. Yamaguchi and A. Harada, *Nat. Commun.*, 2011, **2**, 511.