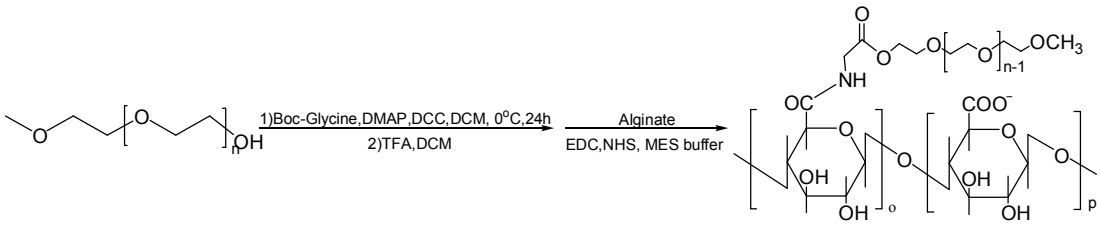
Supporting Information

Experimental

Materials: Methoxypolyethylene glycol (CH_3OPEG) (M_n = 2,000), *N-tert*-Butoxycarbonylglycine (N-*t*-Boc-glycine) were purchased from Aldrich Chemical Co. and dried in a vacuum at 40 °C for 24 h before use. L-asparaginase from *E. coli.* with absolute activity 1271.3 U/mg (the absolute activity of the enzyme was determined as literature (Shirfrin et al., 1974)) was purchased from Changzhou Bio-Pharma Qianhong Co., China. 4-(dimethylamino) pyridine (DMAP), dicyclohexylcarbodiimide (DCC), morpholinoethanesulfonic acid (MES), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS) were local commercial products and used as received.

Synthesis: the syntheses procedures of the products were listed in **Scheme 1**.



Scheme 1 The synthesis procedure of the Alg-g-PEG

CH_3OPEG-CH_2CH_2NH-Boc: CH_3OPEG-CH_2CH_2NH-Boc was synthesized according to the method of Eiselt et al [9a]. CH_3OPEG (2.5 mmol), N-*t*-Boc-glycine (5.5 mmol) and DMAP were added to a CH_2Cl_2 solution (30 mL). Subsequently, DCC (6 mmol) was added, followed by stirring for 24 h at 0 °C. After removing dicyclohexylurea (DCU) by filtration, the filtrate was concentrated in a vacuum at room temperature. The resultant was dissolved in minimal amount of acetone and cooled overnight, and

the precipitated DCU was filtered off. Evaporated the solvent, the product was obtained by dried under vacuum at room temperature for 24 h. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 1.45 (s, 9H, CH_3), 3.38 (s, 3H, CH_3), 3.64 (s, 50H, CH_2), 3.82 (t, 2H, CH_2), 3.94 (d, 2H, CH_2), 4.30 (t, 2H, CH_2), 5.12 (s. 1H, NH). ^{1}H NMR spectrum of $CH_3OPEG\text{-}CH_2CH_2NH\text{-}Boc$ was shown in Figure S1.

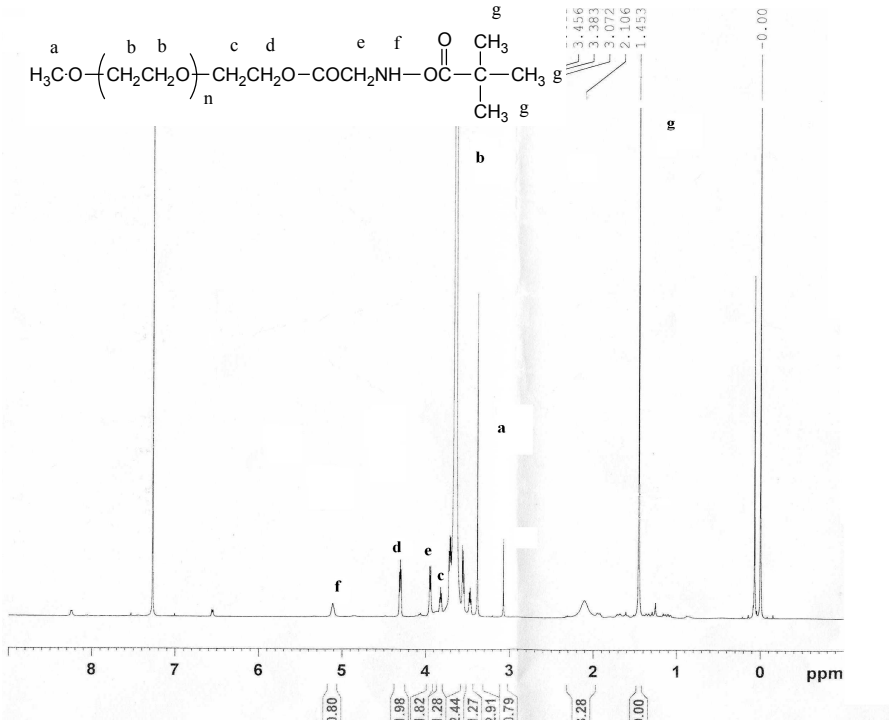


Figure S1 ^{1}H NMR spectrum of $CH_3OPEG\text{-}CH_2CH_2NH\text{-}Boc$ in $CDCl_3$

$CH_3OPEG\text{-}NH_2$: For the removal of *t*-Boc group, $CH_3OPEG\text{-}CH_2CH_2NH\text{-}Boc$ was dissolved in a mixture of CH_2Cl_2/THF (1/1, v/v). The reaction mixture was stirred for 2 h at 0 °C and then evaporated to dryness. The deprotected derivative ($CH_3OPEG\text{-}NH_2$) was dissolved in a NaCl solution (15%), and the pH was adjusted to 5. The filtrate was extracted three times with chloroform; the organic phases were combined and dried over Na_2SO_4. Filtered the Na_2SO_4 and evaporated the solvent, the oil residue was dried under vacuum over P_2O_5 at room temperature for 24 h. ^{1}H NMR

(600 MHz, $CDCl_3$, δ): 3.33 (s, 3H, CH_3), 2.47-3.72 (m, 52H, CH_2), 3.90 (s, 2H, CH_2), 4.32 (t, 2H, CH_2). ^{1}H NMR spectrum of $CH_3OPEG\text{-}NH_2$ was shown in Figure S2.

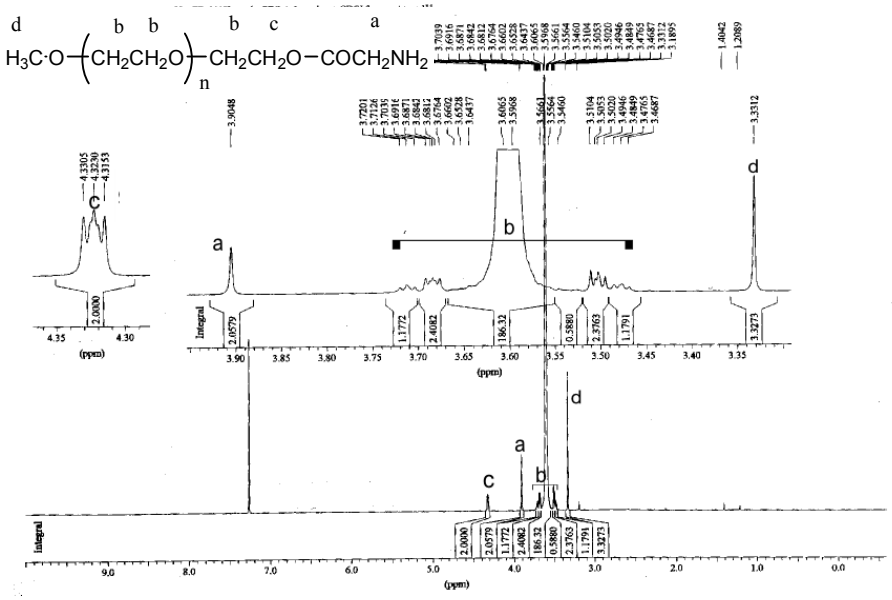


Figure S2 ^{1}H NMR spectrum of $CH_3OPEG\text{-}NH_2$ in $CDCl_3$

Alg-g-PEG: $CH_3OPEG\text{-}NH_2$ was grafted on the sodium alginate according to the procedure of Eiselt et al. [1] A sodium alginate solution (1% (w/v)) was prepared in a buffer solution of MES (0.1 M) and NaCl (0.5 M), and the pH was adjusted to 6. A sample of NHS (292.2 mg) and of EDC (98.8 mg, molar ration of EDC: NHS: COO^- = 1: 0.5: 1) were added to of alginate solution (100 mL) to activate the carboxylic acid groups on the polymer backbone. The solution was agitated to obtain a homogeneous solution followed by the addition of PEG solution (2% (w/v)). The reaction was carried out at room temperature for 10 h. The resulting mixture was dialyzed against pure water for 3 days.

^{1}H NMR (600 MHz, D_2O, δ): 3.36 (s, 3H, CH_3), 3.59-3.70 (m, 50H, CH_2, 2H, CH_2, H(3) of alginate, and H(5) of alginate), 3.86 (s, H(2) and H(4) of alginate), 4.31 (s,

2H, CH_2). ^{1}H NMR spectrum of Alg-*g*-PEG was shown in Figure S3. The PEG content was calculated from the peak integration of by ^{1}H NMR.

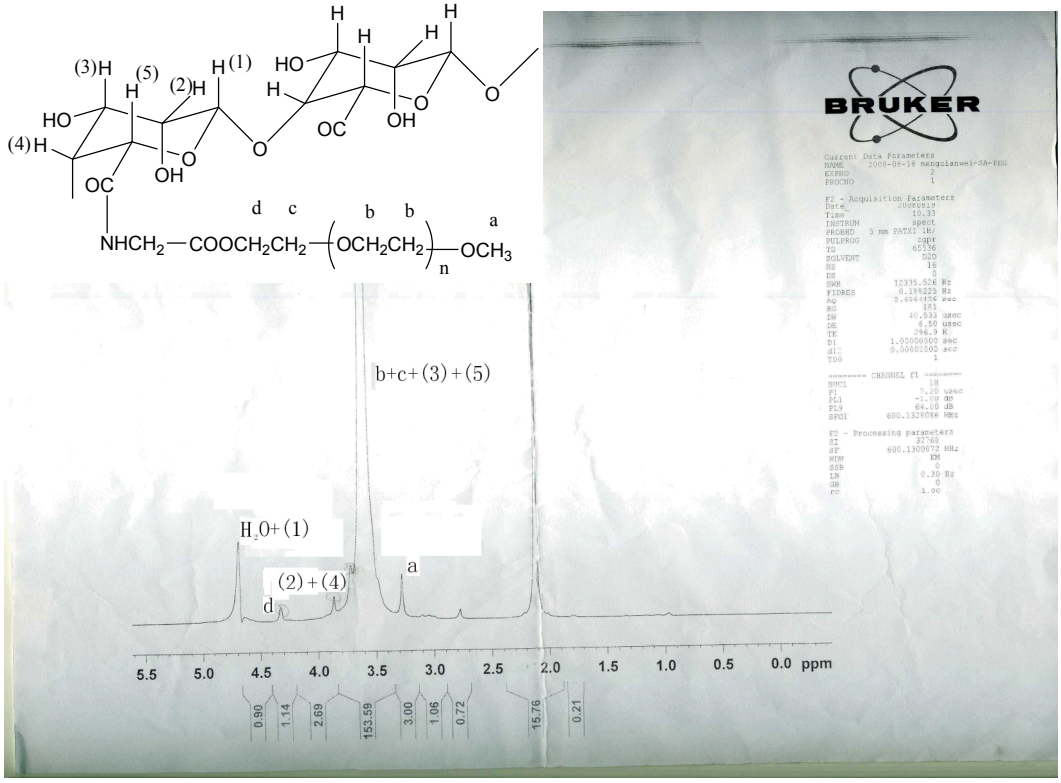


Figure S3 ^{1}H NMR spectrum of Alg-*g*-PEG in D_2O

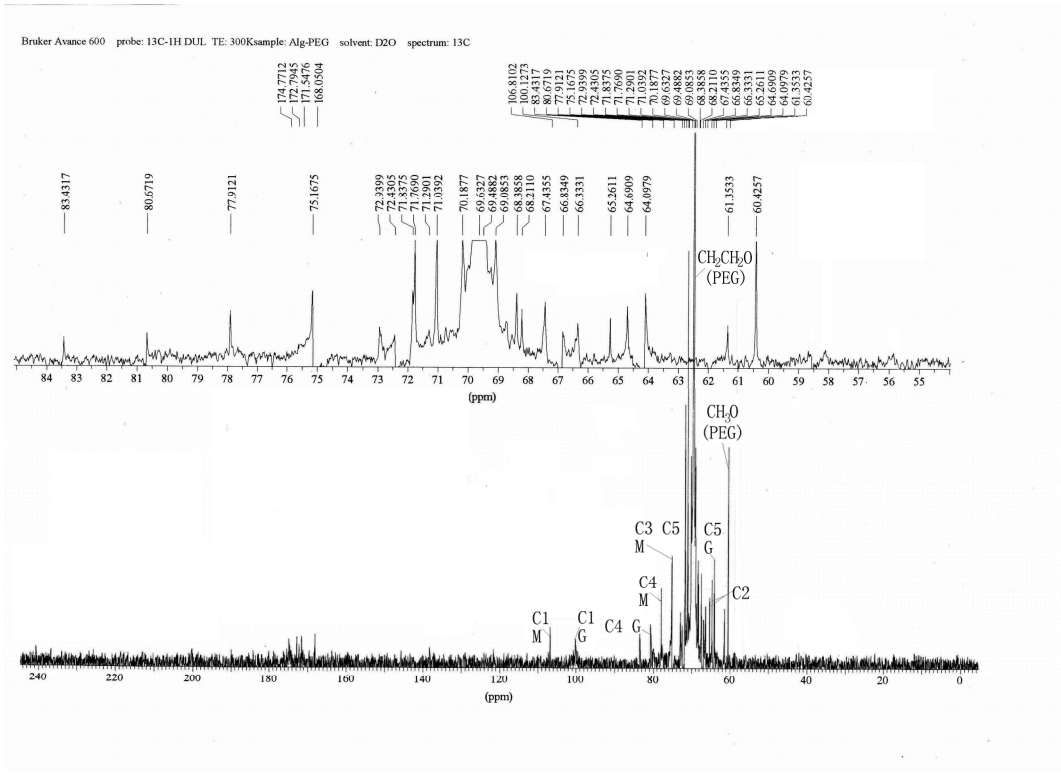


Figure S4 ^{13}C NMR spectrum of Alg-*g*-PEG in D_2O

^{13}C NMR (600 MHz, D_2O, δ): 70.0 ± 3.0 (s, CH_2 of PEG, and C(3) of G residues of alginate),), 60.4 (CH_3 of PEG), 106.0 (C(1) of M residues of alginate), 100.1 (C(1) of G residues of alginate), 80.6 (C(4) of G residues of alginate), 77.9 (C(4) of M residues of alginate), 75.1 (C(3) of M residues of alginate, and C(5) of M residues of alginate), 66.3 (C(5) of G residues of alginate), 65.2 and 65.7 (C(2) of alginate). ^{13}C NMR spectrum of Alg-*g*-PEG was shown in Figure S4.

Preparation of hollow nanospheres: Alg-*g*-PEG aqueous solution (pH = 8) (1 % (w/v), 2.5 mL) was added dropwise to α-CD aqueous solution (10 % (w/v) 3.5 mL) under stirring at room temperature. With the addition of Alg-g-PEG, the solution turned to turbid which indicated the formation of nanospheres. The resulting solution was dialyzed against pure water for 2 days to remove the excess α-CD. To produce hollow sphere with more robust structure, calcium chloride (5 %) solution was added to hollow nanospheres solution as cross-linker for alginate backbone.

Preparation of nanospheres encapsulated L-asparaginase: 2 mL Alg-*g*-PEG solution (1 % w/v) with 1 mg L-asparaginase were added dropwise into 6 mL α-CD (5% w/v) solutions at room temperature, stirred for 2 h, the solution turned to muddy and slightly blue, the hollow spheres were collected by centrifugation. The supernatants and the deposits of the centrifugation were collected for measurements.

Biodegradability Test: Degradability of the Alg-*g*-PEG/α-CD nanospheres was evaluated at 37 °C. The degradation was expressed by the weight loss. Nanospheres (20 mg) were added to pH 7.4 PBS medium. The test bottle was shaken at regular intervals. After predetermined periods of times, the samples were dialyzed against

pure water for 40 h to remove PBS and degraded small molecules. Evaporated the water, the residue was dried to constant weight in vacuum at 40 °C. The average weight loss of three dependent specimens was calculated and taken as the weight loss value for each sample.

Measurement:

The ^{1}H NMR and ^{13}C NMR spectra were recorded on an Advance Bruker 600 NMR spectrometer at 600 MHz at room temperature.

Transmission electron microscopy (TEM) observations were performed on a Jeol JEM-100CX electron microscope at an accelerating voltage of 80 kV.

Scanning electron microscopy (SEM) observations were performed on a Jeol JSM-5900LV electron microscope at an accelerating voltage of 20 kV. The specimens were coated with gold before SEM observations.

Dynamic light scattering (DLS) measurements were carried out on a Brookhaven BI-200SM equipment at awavelength 532 nm and a scattering angle 90 º.

The crystalline changes in the hollow nanospheres were confirmed by X-ray diffraction measurements, which were performed by using Cu-Kα irradiation with PHILP X'Pert MPD (20 kV; 35 mA; 2 º/min). Crystal size for α-CD-PEG inclusion was calculated using Sherer formula ($D = 57.3 \times 0.89\lambda / B_{1/2}\cos\theta$, $\lambda = 0.154$ nm, $B_{1/2}$ denotes the full wide half-maximum)

Zeta potential of Alg-*g*-PEG/α-CD hollow spheres was measured using a Zetasizer model Nano-ZS, Malvern Instruments, England.

The phase transition of sol (flow)-gel (no flow) of the Alg-*g*-PEG/α-CD complexes in water was recording using a vial inversion method. Briefly, predetermined amount of were added to glass vials and then a saturated aqueous solution of α-CD. The gel-sol transition was determined by inverting the vial horizontally after keeping the sample at a constant temperature for 10 min. Figure S5 showed the phase diagram of the Alg-*g*-PEG/α-CD complexes with different Alg-*g*-PEG concentration.

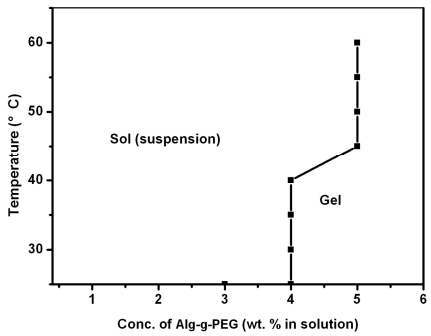


Figure S5. Phase diagram of the Alg-*g*-PEG/α-CD complexes with different Alg-*g*-PEG concentration

Reference

1. P. Eiselt, K. Y. Lee, D. J. Mooney, *Macromolecules*, 1999, **32**, 5561