Electronic Supplementary Information

of

Switch on/off microcapsules for controllable photosensitive drug release in a 'release-cease-recommence' mode

Hang Lin,† Wang Xiao,† Si-Yong Qin, Si-Xue Cheng and Xian-Zheng Zhang*

Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry, Wuhan University, Wuhan 430072, P. R. China

†Hang Lin and Wang Xiao contributed equally to this work.

*Corresponding author. Tel./fax: +86-27-68754509.

E-mail address: xz-zhang@whu.edu.cn (X. Z. Zhang).

1. Materials

Poly(acrylic acid) (PAA) Mw=100,000 and *ρ*-aminoazobenzene were purchased from Sigma-Aldrich Co. Ltd. Poly(allylamine hydrochloride) (PAH) Mw=56,000, adamantane-1-carbonyl chloride, 1,12-dodecanediamine was obtained from Aladdin Co. Ltd. Calcium chloride was provided by Tianjin Kermel Chemical Reagent Co. Ltd. Fluorescein isothiocyanate was obtained from Alfa Aesar China (Tianjin). 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-Hydroxybenzotriazole (HOBT) and $_{L}$-aspartic acid were acquired form GL Biochem (Shanghai) Ltd. *β*-Cyclodextrin (*β*-CD), succinic anhydride and other reagents in this article were purchased from Sinopharm Chemical Reagent Co. Ltd. All solid reagents were used directly.

2. Experimental section

The brief synthesis steps of the microcapsule were shown in Scheme S1.

Synthesis of poly(aspartic acid-*graft*-*β*-cyclodextrin) (PASP-*g*-*β*-CD)

3.2 g NaOH was dissolved in 200 mL de-ionized water, and then 13.4 g *β*-CD was added. The mixture solution was cooled in icy salt water and followed by adding 10 g 4-toluene sulfonyl chloride (TsCl). The reaction lasted for 1 hour, resulting in the formation of *β*-CD-OTs. The product mixture solution was filtered and HCl was added into the liquid phase to neutralise the residual NaOH until the pH value dropped to 6. The *β*-CD-OTs was formed as white precipitate and was purified by recrystalliztion in water.

3.2 g *β*-CD-OTs was dissolved in 20 mL distilled ethylenediamine (EDA). The solution was heated to 80^{o}C for 24 hours. After cooled to room temperature, the solution was dripped into 350 mL acetone and white precipitate was obtained. For further purification, the precipitate was dissolved in 4 mL water and then added

dropwise into 350 mL acetone once again to obtain the precipitated *β*-CD-EDA.

Poly-$_L$-succinimide (PSI) was obtained according to our previous work.[1] Briefly, 15.0 g $_L$-aspartic acid and 7.5 g H_3PO_4 were mixed and reacted at 180^{o}C under reduced pressure for 2.5 hours. Then 100 mL N,N-Dimethylformamide (DMF) was added to dissolve the product. The solution was added dropwise into de-ionized water to obtain white PSI precipitate. The precipitate was purified by washing it with de-ionized water and then dried it in vacuum at 110 ^{o}C for 24 hours.

1.0 g PSI was dissolved in 20 mL DMF and then cooled in icy salt water bath. 0.41 g NaOH was dissolved in 20 mL de-ionized water and added dropwise into the DMF solution over 1 hour. The reaction was continued at room temperature for 24 hours. The product solution was dialyzed against de-ionized water (MWCO: 8,000-14,000) for 4 days and PASP (Mw: ~20,000) was obtained after lyophilised.

1.34 g *β*-CD-EDA and 0.44 g PASP were dissolved in 8 mL de-ionized water and the pH value of solution was adjusted to 5 by HCl. 0.33 g (N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride) (EDC·HCl) was added and the solution was stirred for 24 hours at room temprature. The solution was dialyzed against de-ionized water (MWCO: 8,000-14,000) for 4 days and the PASP-*g*-*β*-CD was obtained after lyophilisation.

Synthesis of poly(acrylic acid-*graft*-adamantane-*graft*-azobenzene) (PAA-*g*-AD-*g*-Azo)

2.18 g succinic anhydride (SA) and 3.57 g *ρ*-aminoazobenzene were dissolved in 30 mL distilled acetone. 1.43 g distilled pyridine was added after heated to 60^{o}C. The solution was stirred for 6 hours with reflux and thereafter the Azo-SA was obtained as red precipitate by centrifugation.

0.3 g Azo-SA was dissolved in 8 mL DMF/tetrahydrofuran (THF) (v:v=1:1) with

0.48 g HBTU, 0.17 g HOBT and 0.3 mL N,N-Diisopropylethylamine (DIEA). The solution was added dropwise in 4 mL EDA for over 20 minutes and then stirred for 24 hours. The product solution was concentrated by rotatory vaporization under reduced pressure and then dropped into $CHCl_3$. Indissolvable precipitate was filtered out and the solution was concentrated by rotatory vaporiz ation again. The concentrated solution was precipitated with diethyl ether to obtain Azo-SA-EDA.

0.30 g 1,12-dodecanediamine was dissolved in 7 mL distilled THF and then 1 mL distilled triethylamine (TEA) was added under icy salt water bath. 0.10 g adamantane-1-carbonyl chloride was dissolved in 10 mL distilled THF and then added dropwise into 1,12-dodecanediamine for 20 minutes. The reaction continued for 24 hours after 1 hour icy salt water bath. The precipitate was removed by centrifugation and the solvent was then removed by rotatory vaporization. The solute was dissolved in 4 mL DMF and added dropwise into 1 mol/L HCl. AD-C_{12}-NH_2 was precipitated and washed with de-ionized water then dried in vacuum.

100 mg PAA was dissolved in 6 mL distilled DMF, 110 mg HBTU, 45 mg HOBT and 1 mL DIEA and 45 mg Azo-SA-EDA were added. After stirred for 6 hours, 100 mg HBTU and 40 mg AD-C_{12}-NH_2 were added. The reaction continued for 24 hours. The resulting mixture was first dialyzed against DMF for 2 days and then against de-ionized water for 4 days. PAA-*g*-AD-*g*-Azo was obtained after lyophilised.

LbL assembly on quartz slide

Rectangular quartz slide was immersed in H_2O/H_2SO_4 (v:v=1:3) solution at 80^oC for 90 minutes and then immersed in $H_2O/H_2O_2/NH_3 \cdot H_2O$ (v:v:v=5:1:1) at 70^oC for 1 hour to acquired a negative charge surface. The slide was then dried in air. The 241 nm absorption of the slide was monitored by Lambda Bio40 UV/vis spectrometer. The slide was dipped in PAH solution (1 mg/mL) for 15 minutes at first to change the

surface charge into positive, then washed with de-ionized water and dried in air. The slide was then dipped into PAA-*g*-AD-*g*-Azo PBS solution (pH= 7.24) or PASP-*β*-CD PBS solution (pH=7.24) alternatingly to accomplish the LbL procedure. Control experiment using non-modified PAA PBS solution (pH= 7.24) and PASP-*g*-*β*-CD PBS solution (pH= 7.24) was also carried out.

Synthesis of fluorescent labeled calcium carbonate particles

300 mg PEG_{5000} was dissolved in 12 mL distilled DMF, 35 mg FITC was added. After stirring for 48 hours, the product solution was dialysis (MWCO: 3500) against dimethyl sulfoxide for 1 day then against de-ionized water for 4 days. Orange PEG_{5000}-FITC was obtained after lyophilised.

The $CaCO_3$ particles with model drug loaded inside were fabricated similar to literature.[2,3] In brief, 6 mg PEG_{5000}-FITC was dissolved in 10 mL de-ionized water. 5 mL of this solution was used to dissolved 230 mg K_2CO_3 and 10 mg PAH, the rest 5 mL to dissolve 185 mg $CaCl_2$. The two kinds of solutions were mixed up and stirred intensively for 30 seconds then kept still for over 2 minutes. $CaCO_3$ particles was precipitated and filtered off, then washed them with de-ionized water. PEG_{5000}-FITC was coprecipitated with the $CaCO_3$ particles as a model drug and fluorescent label.

Layer-by-layer assembly on $CaCO_3$ particles

PASP-*g*-*β*-CD and PAA-*g*-AD-*g*-Azo were dissolved separately to form 6 mL 1.0 mg/mL solutions (A and B). 50 mg $CaCO_3$ particles were dispersed in 1 mL B solution, and then the suspension was shaken for 20 minutes to complete the absorption. The particles were separated by centrifugation and then washed with de-ionized water for 3 times. Thus the microspheres were coated with a layer of PAA-*g*-AD-*g*-Azo and then dispersed in 1 mL A solution. Followed by the same procedure, the second layer, PASP-*g*-*β*-CD was absorbed on the surface of the particles. By

repeating this, totally 12 layers were attached to the particles. To obtain the hollow microcapsules, the $CaCO_3$ cores of the coated particles were dissolved by EDTA(0.4 mol/L, pH=7.07).

Transmission electron microscopy (TEM) characterization

The suspension of $CaCO_3$ particles coated with 12 layers was shaken intensively for dispersion. Then a small drop of suspension was dripped onto a copper grid and kept still for 10 minutes to let the $CaCO_3$ particles deposit on the grid. The copper grid was immersed in EDTA(0.4 mol/L, pH=7.07) for 3 hours, then in de-ionized water for 1 hour and renewed the water 3 times to remove the $CaCO_3$ cores. After drying, the copper grid was ready for TEM observation. TEM image was taken by JEM-2100 instrument.

Confocal laser scanning microscopy characterization

Several dishes were prepared and a drop of suspension containing coated $CaCO_3$ particles was dripped into each, then several drops of EDTA (0.4 mol/L pH=7.24) were added and waited 20 minutes for core removal. CFLS image was taken right after the cores were removed. One of the dishes was kept in dark for 30 minutes then taken CFLM images; another one was taken image after exposed to 365 nm UV ray for 30 minutes then retaken after 30 minute in dark. The images were taken by Nikon C1-si, BD Laser, and the excitation wavelength was 488 nm.

UV/visible light stimulated release-cease-recommence properties

100 μL coated $CaCO_3$ particles suspension was added to several dialysis bags (MWCO: 8,000-14,000), 300 μL 0.4 mol/L EDTA was added in the bags to remove the $CaCO_3$ cores. The bags were wrapped up and dialyzed against 15 mL de-ionized water for 1 day then against 15 mL PBS (pH=7.24) for 1 hour for pretreatment. Then the microcapsules were dialyzed against another 15 mL PBS in a 25 mL sample bottle.

After 1 hour, the liquid in the bottle was collected and renewed. According to Fig. S1, three different methods were used to study the following release behavior of the microcapsules.

Method a, every 3 hours, the bottles were exposed to 365 nm UV ray for 20 minutes and then to visible light for 10 minutes. The rest of the time they were kept in dark. Samples were collected every 1 hour.

Method b, the bottles were exposed to UV ray every 1 hour for a period of 4 hours and kept in dark for the rest hours. Samples were collected every 1 hour for the first 6 hours then every 2 hours for the rest.

Method c, the bottles were kept in dark for the entire procedure. Samples were collected with certain interval.

The UV ray source was provided by Spectronics FC-100/F UV lamp. The fluorescent intensities were measured by SHIMADZU RF-530/PC spectrofluorophotometer. The excitation wavelength is 488 nm, and the emission wavelength is 516 nm.

^{1}H NMR Characterization

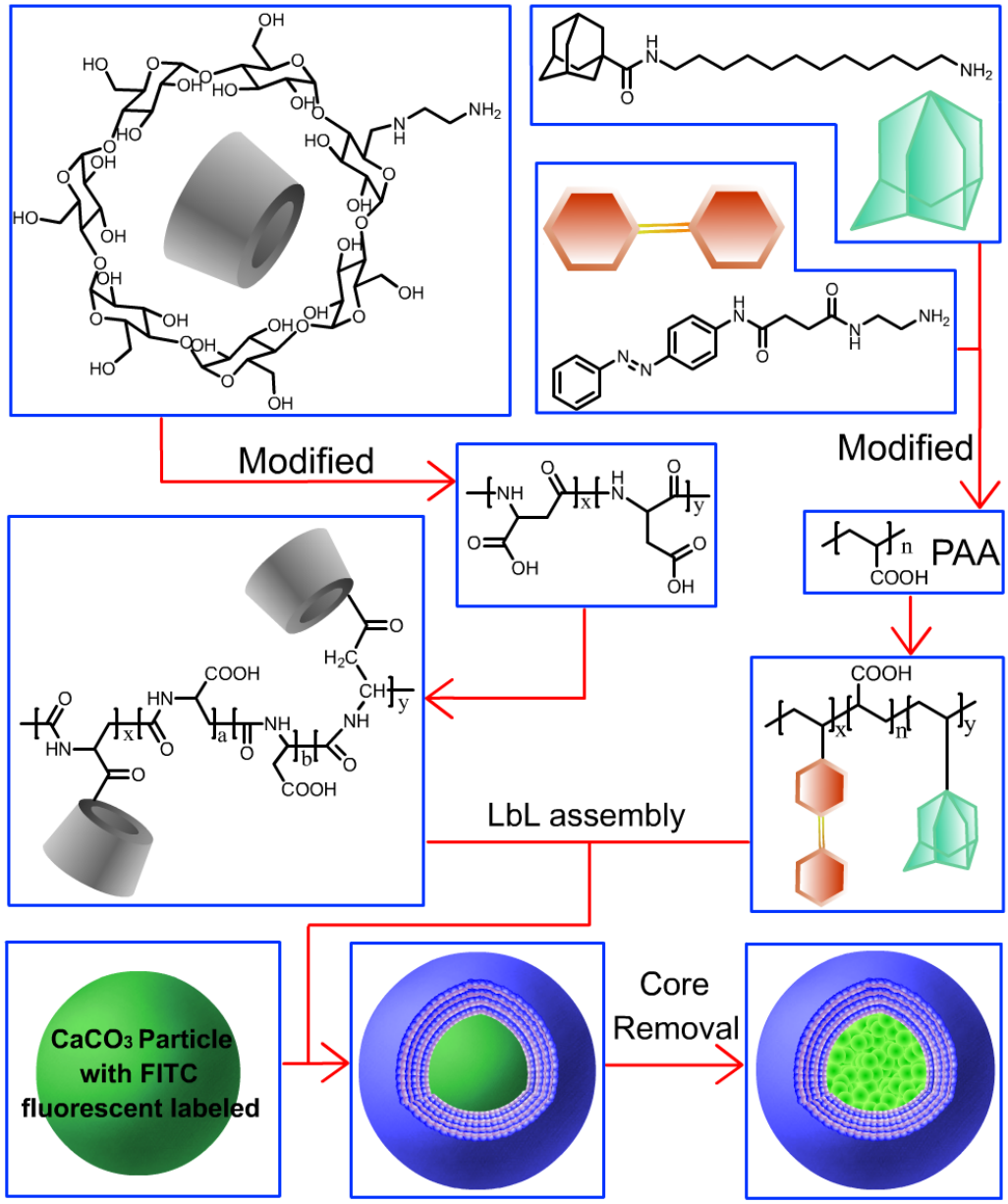
The ^{1}H NMR spectra of the products and modified polymer chains were recorded on a Mercury VX-300 spectrometer at 300 MHz (Varian, U.S.), which are shown in the Fig. S2-S6.

Supplementary References

S1 C. Li, J. Zhang, S. Yang, B. L. Li, Y. Y. Li, X. Z. Zhang, and R. X. Zhuo, *Phys. Chem. Chem. Phys*., 2009, **11**, 8835.

S2 D. V. Volodkin, A. I. Petrov, M. Prevot and G. B. Sukhorukov, *Langmuir*, 2004, **20**, 3398.

S3 G. B. Sukhorukov, D. V. Volodkin, A. M. Günther, A. I. Petrov, D. V. Shenoy and H. Möhwald, *J. Mater. Chem*., 2004, **14**, 2073.



Scheme S1. The synthesis step of the hollow microcapsule.

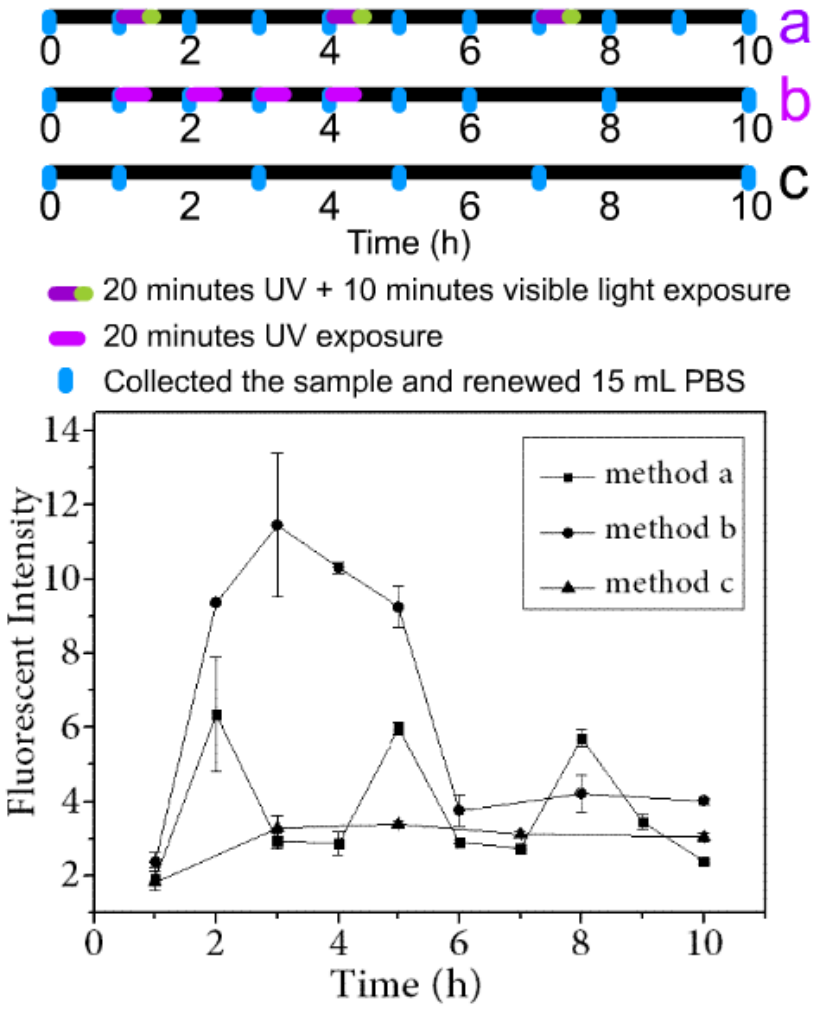


Fig. S1. Cumulative release amount of the model drug in three different methods.

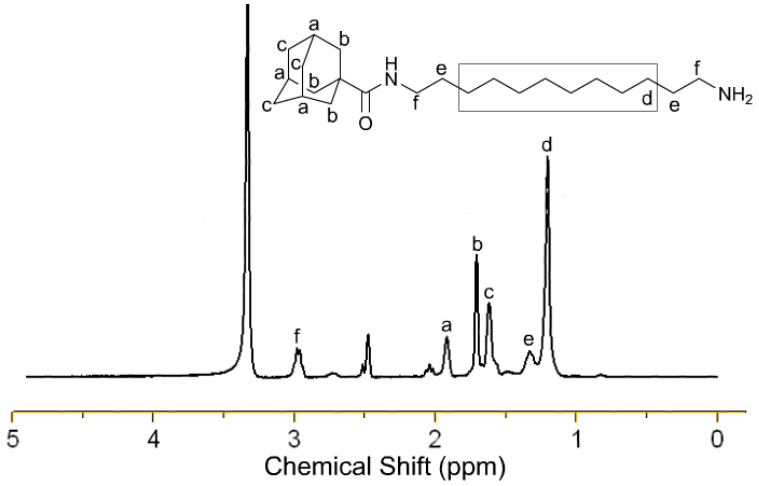


Fig. S2. ^{1}H NMR spectrum of AD-C_{12}-NH_2, solvent: DMSO-d_6.

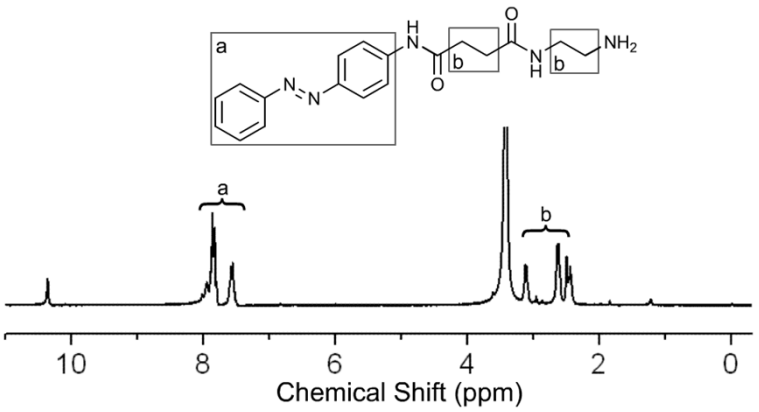


Fig. S3. ^{1}H NMR spectrum of Azo-SA-EDA, solvent: DMSO-d_6.

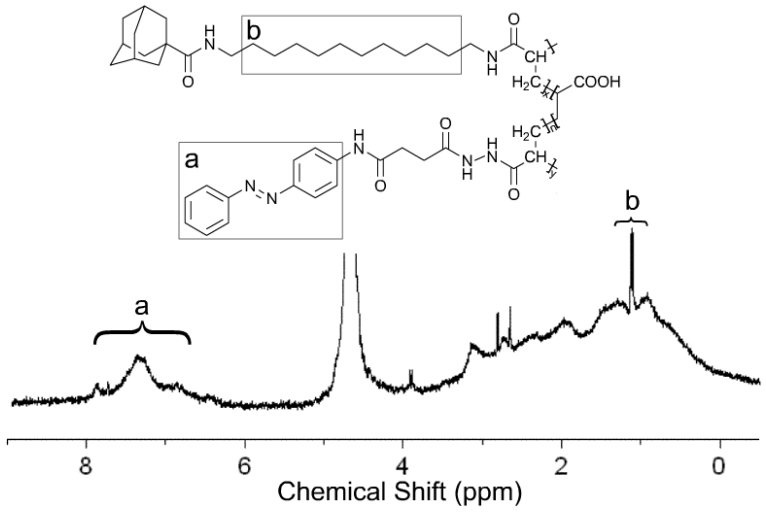


Fig. S4. ^{1}H NMR spectrum of PAA-*g*-Azo-*g*-AD, solvent: D_2O. The substitution degree of Azo is 7.3%, AD is approximate 1.5%.

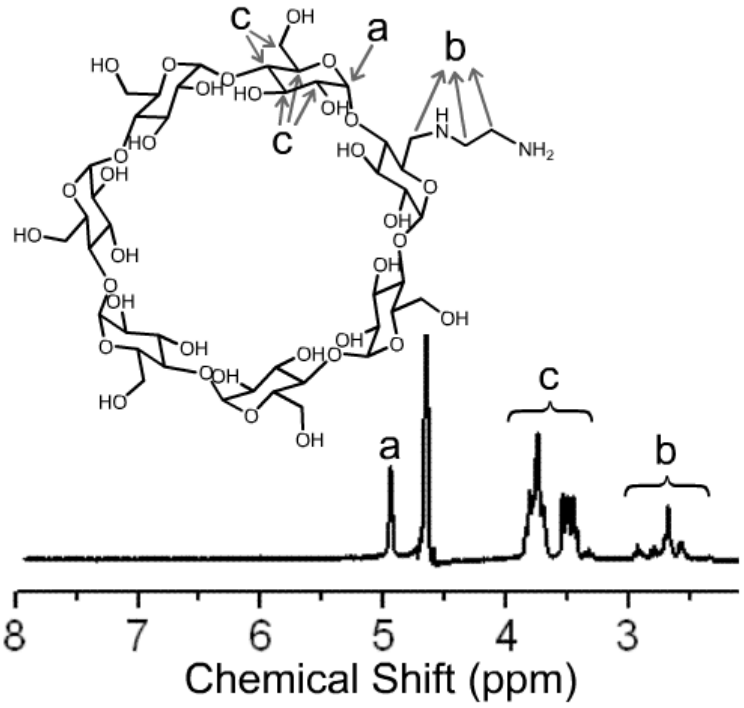


Fig. S5. ^{1}H NMR spectrum of *β*-CD-EDA, solvent: D_2O.

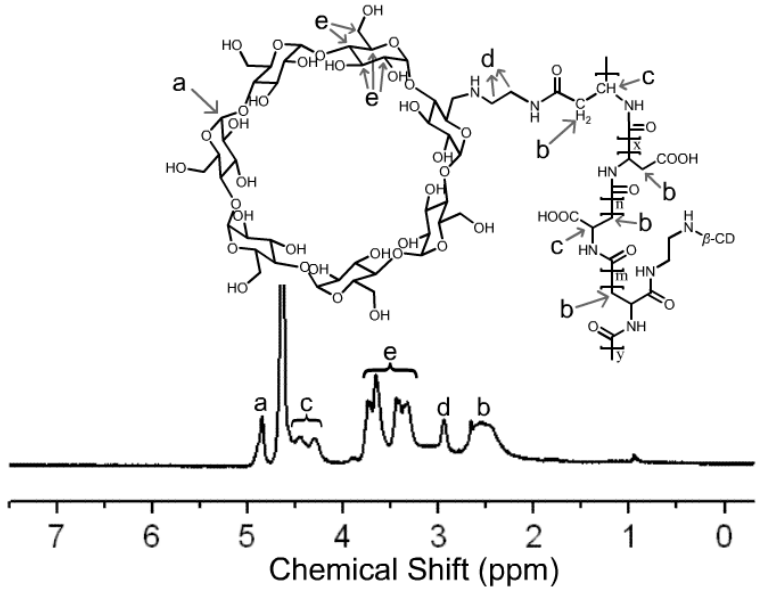


Fig. S6. ^{1}H NMR spectrum of PASP-*g*-*β*-CD, solvent: D_2O. The substitution degree of PASP-*g*-*β*-CD is 14.4%.