Electronic Supplementary Information (ESI)

Hydroxypropyl-β-Cyclodextrin versus Its α- Homologue for Tetra-PEG Based Three-Dimensional Modified Polyrotaxane Network Formation and Properties: the Threading Mechanism and the Relationship between Modified CD and Polymer Revealed through the Comparison

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S1. Experimental Section

S1.1 Materials

Tetrahydroxyl-Terminated PEG (THPEG) (M_w = 20000) was purchased from Sinopeg Biotech Co., Ltd., and dried in vacuum with magnetic stirring at 85 ^{0}C for 2 h before use. Tetra-Azido-Terminated PEG (TAPEG) and Tetra-Propargyl-Terminated PEG (TPPEG) were synthesized through terminal modification of THPEG according to the method reported previously.[1] (2-Hydroxypropyl)-β-CD (Hy-β-CD) (M_w ～ 1542) was purchased from Aladdin Industrial Co., Ltd. and dried in vacuum at 85 ^{0}C overnight before use. (2-Hydroxypropyl)-α-CD (Hy-α-CD) (M_w ～ 1180) was obtained from Sigma-Aldrich Corp. and dried in vacuum at 85 ^{0}C before use. Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium ascorbic acid, deuterium oxide (D_2O) and tetramethylammonium (TMA) chloride of analytical grade were purchased from Sigma-Aldrich Corp. and used as-received. Chloroform were obtained from Sigma-Aldrich Corp. and dried with 4A molecular sieve.

S1.2 Complexation between Hy-β-CD and Tetra-PEG Macromonomers in Pre-Gel Solution

THPEG was used as the model of tetra-PEG macromonomers (TAPEG and TPPEG) to complex with Hy-β-CD. Nuclear magnetic resonance (NMR) spectroscopy was used to characterize the complexation between Hy-β-CD and model tetra-PEG macromonomer in the pre-gel solution. ^{1}H-, ^{13}C- and 2D NOESY NMR spectroscopies were involved in the

experiment. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Varian MERCURY plus-400 spectrometer at 25.0 ± 0.5 ^{0}C with 64 and 1024 scans respectively for every sample. 2D NOESY NMR (400MHz) spectra were obtained using a Bruker AVANCEIII 400 spectrometer with a mixing time of 300 ms at 25.0 ± 0.5 ^{0}C. The 2048 experiments were performed with four scans per experiment. Deuterium oxide was used as solvent with chloroform sealed in a glass capillary of 1.0 mm diameter as an external reference.

It was reported that the chemical shifts of all protons of CDs and internal reference compounds decreased with the increase of CD concentration.[2-3] During this work, it was found that the protons of THPEG, the carbons of THPEG, Hy-β-CD and internal references all showed CD concentration dependence. So data from the ^{1}H- and ^{13}C-NMR spectra were corrected for this effect.[1]

Monitoring the Complexation Process between Hy-β-CD and THPEG. Hy-β-CD and THPEG were dissolved in D_2O and incubated at 37 ^{0}C. The mass ratio of D_2O to THPEG (m_{D_2O} : m_{THPEG}) was kept at 9, whereas the molar ratio of Hy-β-CD to THPEG ($n_{Hy\text{-}\beta\text{-}CD}$: n_{THPEG}) changed from 5 to 75. ^{1}H-NMR spectra were taken every a certain period of time. Sample **0b** was THPEG's D_2O solution without Hy-β-CD and it was the reference to calculate the chemical shift variation ($\Delta\delta$) of THPEG of samples **1b** to **5b**. The formulations of NMR samples **1b** to **5b** of Hy-β-CD/THPEG system are identical to those of samples **1a** to **5a** of Hy-α-CD/THPEG system except for replacing Hy-α-CD with Hy-β-CD (Table 3 in the main text).

Determination of Structures of Poly-Pseudo-Rotaxanes Formed by Hy-β-CD and THPEG. ^{1}H-, ^{13}C- and 2D NOESY NMR were performed to samples **1b** to **5b** after they reached dynamic equilibrium. The time for these samples to reach the equilibrium was obtained from the ^{1}H-NMR monitoring experiment above. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian MERCURY plus-400 spectrometer at 25.0 ± 0.5 ^{0}C.

S1.3 Preparation of βSSS Hydrogel

For the preparation of a certain βSSS hydrogel sample, typically, TAPEG (0.1000 g, 4.975 μmol) and TPPEG (0.1011 g, 4.975 μmol) were dissolved in DI water (1.7599 g), followed by addition of a certain amount of Hy-β-CD. This pre-gel solution was incubated in an oven at 37 ^{0}C until it reached the equilibrium. Then copper sulfate (1.592 mg, 9.950 μmol) in DI water (48.41 mg) and sodium ascorbate (9.8 mg, 49.5 μmol) were added. The hydrogel formed within minutes and stood for another 48 h at 37 ^{0}C. The hydrogel was treated with 0.5 M aqueous ethylenediamine tetraacetic acid (EDTA) solution followed by plenty of water to remove the copper catalysis and unthreaded Hy-β-CD. The hydrogel was incubated in DI water to fully swell before characterizations.

S1.4 Characterizations of the βSSS Hydrogel

ATR-FTIR Measurement. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra were recorded on a Paragon 1000 instrument equipped with ATR accessory. The scan range was 4000

to 660 cm^{-1} and seven scans were collected for each sample with a resolution of 2 cm^{-1}. The hydrogel was frozen in liquid nitrogen and broken. Then it was freeze dried. The sectioned surface was characterized by ATR. Peak intensities of samples **1bg** to **5bg** were normalized to the C=O in carbamate group of sample **0bg** in ATR spectra.

XPS Measurement and Calculation of the Number of Hy-β-CD Introduced into the Network of the βSSS Hydrogel. A Shimadzu-Kratos (AXIS Ultra) X-ray photoelectron spectrometer (XPS) equipped with monochromatic Al Kα X-rays was used to characterize the hydrogel's elemental composition of carbon, oxygen and nitrogen. A takeoff angle of 90^0 was employed for the sectioned surface of freeze dried sample. The scan area is 300 × 700 μm. At least three random positions on the sectioned surface of every hydrogel sample were chosen to be executed with XPS measurement. Initial representative survey scans were acquired from 0 to 1200 eV for three sweeps (sw). For further elemental analysis, high-resolution scans were acquired for the C 1s (~ 276 to 298 eV, 3 sw), O 1s (~ 524 to 543 eV, 2 sw) and N 1s (~ 392 to 412 eV, 12 sw) regions. For all spectra, the backgrounds of all regions were subtracted using the Shirley background.[4] Peak positions were normalized to the C 1s peak at 285.0 eV. The data analysis was carried out by using XPSPeak 4.1 software.

Based on the XPS high-resolution spectra of C 1s of βSSS hydrogel samples **1bg** to **5bg** and the blank reference **0bg** (Fig. S2), the numbers of Hy-β-CD introduced into the networks can be calculated according to the following equation:[3]

$$N_{Hy-\beta-CD} = \frac{A_{288.1eV} \times 130.6}{A_{286.6eV} - A_{288.1eV} \times 7.0} \qquad S(1)$$

In the equation, $N_{\text{Hy-β-CD}}$ represents the number of Hy-β-CD corresponding to every tetra-PEG macromonomer (TAPEG or TPPEG) and was used to evaluate the amount of Hy-β-CD introduced into the βSSS hydrogel. $A_{286.6\text{eV}}$ and $A_{288.1\text{eV}}$ stand for areas of peaks at 286.6 and 288.1 eV in the XPS high-resolution spectra of C 1s.

The threading ratio [SR(β)] of Hy-β-CD in the βSSS hydrogel is defined as:

$$SR(\beta) = \frac{N_{Hy-\beta-CD}}{N_{(\beta)Initial}} \times 100\% \qquad S(2)$$

Here $N_{(\beta)\text{Initial}}$ is the feed ratio of Hy-β-CD to the tetra-PEG macromonomers in the pre-gel solution, i.e. the number of Hy-β-CD corresponding to every tetra-PEG macromonomer fed initially in the pre-gel solution. With the XPS high-resolution spectra of C 1s of the βSSS hydrogel samples, the values of $N_{\text{Hy-β-CD}}$ and SR(β) are calculated according to the equation S(1) and S(2) and the results are listed in Table 1 in the main text.

Compression Test. A MTS Criterion 43 universal texting machine was used to measure mechanical performance of the hydrogel. Compression mode was used. A cylindrical sample about 7 mm in diameter and 5 mm in initial thickness was placed on a metal plate coated with silicon oil to decrease the friction.[5] The maximal displacement of crosshead is 95% of the height of sample to prevent the damagement of sensor. The cross-head speed was 1.0 mm min^{-1}. At least five parallel samples were recorded for each specimen in both tests.

Swelling Measurement. Hydrogel samples were freeze-dried and then immersed in DI water for 3 days at room temperature to fully swell. The equilibrium water

contents (EWCs) of the hydrogels were calculated according to the following equation:

$$EWC = \frac{m_{wet} - m_{dry}}{m_{wet}} \times 10 \qquad S(3)$$

At least three parallel samples were recorded for each hydrogel specimen. The results of EWC of the hydrogel samples **0bg** to **5bg** are listed in Table 1 in the main text.

Internal Morphology. The sectioned surface of the freeze-dried hydrogel sample was observed using a Philips Sirion 200 instrument scanning electron microscope (SEM). Photographs were taken with a Canon IXUS 800IS digital camera. The dried hydrogel samples were mounted on metal holder and vacuum coated with a gold layer prior to SEM examination.

S1.5 Differential Scanning Calorimetry (DSC) Measurements of Dried Clickable Tetra-PEG, βSSS and αSSS Gels

Samples of clickable tetra-PEG hydrogel **0bg**, the βSSS hydrogel **5bg**, the αSSS hydrogel **4ag**[3] and THPEG were dried at 37 ^{0}C for 48 h in air and heated in vacuum at 70 ^{0}C until the samples were drain dried. DSC measurements were conducted using a Q2000 Modulated DSC with a RSC90 mechanical cooling system (TA Instruments). Each sample about 20 mg was encapsulated in an aluminum pan. The sample was first heated to 90 ^{0}C at a heating rate of 10 ^{0}C min^{-1}, and was held at 90 ^{0}C for 10 min to remove thermal history of it, followed by cooling at a rate of 10 ^{0}C min^{-1} to -80 ^{0}C and was held at -80 ^{0}C for 10 min. A second scan was carried out from -80 to 90 ^{0}C at

a heating rate of 5 ^{0}C min^{-1}. The data were collected during the second scan.[6] The glass transition temperature (T_g) was taken as the midpoint of the heat capacity change by the TA Universal Analysis software. The temperature of the peak and the area of the endothermic curves were taken as the crystalline melting temperature (T_m) and the heat of fusion (ΔH_m) respectively by the TA Universal Analysis software. Values of T_g, T_m and ΔH_m are listed in Table 2 in the main text.

S1.6 Preparation of βSSS, αSSS Hydrogels with the Concentration of Tetra-PEG Macromonomers being 0.5 *wt*% and Preparation of the Pristine Clickable Tetra-PEG hydrogels with the Concentration of Tetra-PEG Macromonomers being 0.5 and 1.0 *wt*%

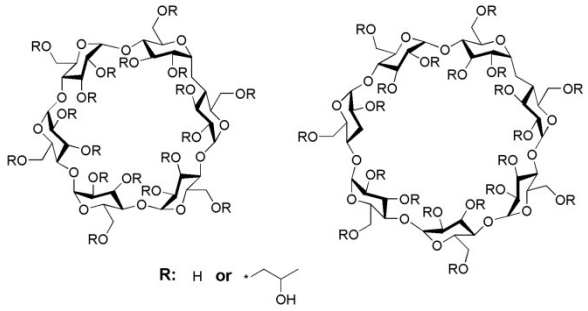
A βSSS, an αSSS and a pristine clickable tetra-PEG hydrogel sample with a constant concentration of tetra-PEG macromonomers (TAPEG and TPPEG) being 0.5 *wt*% in water, i.e. the value of $[m_{Macromonomers}/(m_{DI\ Water} + m_{Macromonomers})] \times 100$ is constant at 0.5 %, were prepared and named as **0.5%-βSSS**, **0.5%-αSSS** and **0.5%-Tetra-PEG** respectively. In addition, a clickable tetra-PEG hydrogel sample with the concentration of tetra-PEG macromonomers being 1.0 % was prepared and named as **1.0%-Tetra-PEG**.

For the preparation of the **0.5%-βSSS** sample, TAPEG (0.0500 g, 2.488 μmol) and TPPEG (0.0506 g, 2.488 μmol) were dissolved in DI water (19.9468 g). Then 0.5772 g (0.3743 mmol) of Hy-β-CD was added and dissolved ($n_{Hy\text{-}\beta\text{-}CD} : n_{Macromonomers} = 75$), and the resulted pre-gel solution was incubated at 37 ^{0}C for 3.5 days. Afterwards

copper sulfate (2.388 mg, 14.92 μmol) in DI water (72.612 mg) and sodium ascorbate (14.9 mg, 75.2 μmol) were added. The solution was incubated at 37 ^{0}C for three weeks. The **0.5%-αSSS** sample was prepared under feed ratios and conditions identical to the **0.5%-βSSS** sample except for replacing Hy-β-CD with Hy-α-CD. The **0.5%-Tetra-PEG** sample was prepared under feed ratios and conditions identical to the **0.5%-βSSS** sample without Hy-β-CD.

For the preparation of the **1.0%-Tetra-PEG** sample, TAPEG (0.0500 g, 2.488 μmol) and TPPEG (0.0506 g, 2.488 μmol) were dissolved in DI water (9.9110 g). This pre-gel solution was incubated at 37 ^{0}C for 3.5 days. Then copper sulfate (1.592 mg, 9.950 μmol) in DI water (48.408 mg) and sodium ascorbate (9.8 mg, 49.5 μmol) were added. The solution was incubated at 37 ^{0}C for at least three weeks.

S2. Further Results and Discussion



Scheme S1 Chemical structures of Hy-α-CD (left) and Hy-β-CD (right).

S2.1 Chemical Composition of the βSSS Hydrogel

ATR-FTIR Result. ATR-FTIR spectroscopy was used to determine the chemical composition and semiquantify the amount of Hy-β-CD introduced into the βSSS hydrogel. Samples of the βSSS hydrogel were freeze-dried and the sectional surfaces of them were undergone ATR-FTIR characterization. Fig. S1 shows the spectra of samples **0bg** to **5bg**. Sample **0bg** is a reference and provides a baseline to the others. In the spectrum of **0bg**, the reflection peak of stretching vibration of C-O-C group of poly(ethylene oxide) (PEO) of the hydrogel's network appears at 1100 cm^{-1}. The peak of stretching vibration of C=O in carbamate group of the network locates at 1721 cm^{-1}, and this signal was used as internal reference to normalize all the hydrogel samples due to its invariance through all of them. Spectra of **0bg** to **5bg** in Fig. S1 are normalized spectra. With the introduction of Hy-β-CD, in spectra of **1bg** to **5bg**, a new broad peak around 3370 cm^{-1} appears which is corresponded to stretching vibration of OH group on Hy-β-CD. There is also a peak at 1030 cm^{-1} appearing in spectra of **4bg** and **5bg** compared with that of **0bg**, and this peak is corresponded to

stretching vibration of C-O-C group on Hy-β-CD. Samples **1bg** to **3bg** should also have the peak of Hy-β-CD's C-O-C group at 1030 cm^{-1}. This peak is difficult to be observed because it is sheltered by the strong peak of C-O-C group on PEO. The appearance of OH and C-O-C groups on Hy-β-CD in spectra of **1bg** to **5bg** indicate that Hy-β-CD is confined within the network of the βSSS hydrogel. The intensity of the stretching vibration of the OH group on Hy-β-CD increases gradually from **1bg** to **4bg**, and then decreases from **4bg** to **5bg**. This indicates that the amount of introduced Hy-β-CD increases from **1bg** to **4bg**, and decreases from **4bg** to **5bg**, with **4bg** owning the maximum.

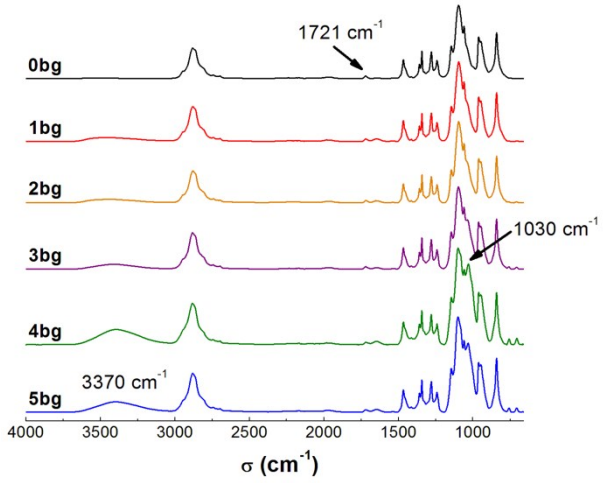


Fig. S1 ATR-FTIR spectra of the sectioned surfaces of freeze-dried hydrogel samples **0bg** to **5bg**.

XPS Result. ATR-FTIR spectroscopy is just a semiquantitative characterization for determination of the chemical composition of the βSSS hydrogel. To precisely determine the amount of Hy-β-CD introduced into the βSSS hydrogel, XPS was used. Fig. S2 shows the high-resolution narrow scans of the C 1s regions of hydrogel

samples **0bg** to **5bg**. After the spectral fitting, in the spectrum of reference **0bg**, the peak at 285.0 eV is attributed to contaminate carbon and the peak at 286.6 eV is C(C-O/C-N) region of PEO network. With the introduction of Hy-β-CD, new peaks at 288.1 eV appear which are attributed to anomeric C(O-C-O) of Hy-β-CD in spectra of 1bg to **5bg**. These indicate that Hy-β-CDs are confined within the networks of the βSSS hydrogel samples. Using the equations S(1) and S(2), the number of Hy-β-CD introduced into the network of the βSSS hydrogel ($N_{\text{Hy-β-CD}}$) and the threading ratio of Hy-β-CD [SR(β)] can be calculated, and the results were listed in Table 1 in the main text.

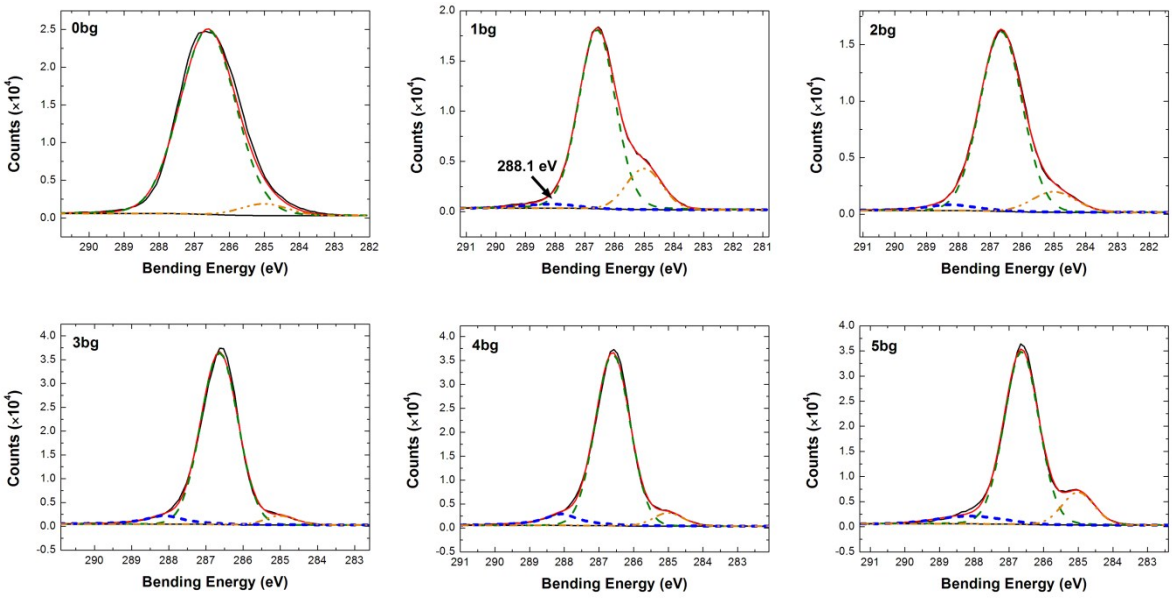


Fig. S2 XPS high-resolution (C 1s) spectra of the sectioned surfaces of freeze-dried hydrogel samples **0bg** to **5bg**.

S2.2 Swelling Properties of SSS Hydrogels

The equilibrium water content (EWC) the βSSS hydrogel was calculated by the equation S(3). EWCs of βSSS and αSSS hydrogels are depicted to a bar diagram in Fig. S3. The EWC of pristine clickable tetra-PEG is 95.4% and is represented with a dotted gray line in Fig. S3. It can be found in the Fig. that EWSs of all samples of the βSSS hydrogel system slightly surpass the dotted line, whereas EWSs of all samples of the αSSS hydrogel system are slightly below the line. Introduction of Hy-α-CD increases the rigidity of the network chain in tetra-PEG hydrogel and reduces the ability to accommodate water of the hydrogel. On the other hand, because loosely threaded Hy-β-CD has little influence on the rigidity of the network chain and Hy-β-CD carries more hydrophilic hydroxyl groups, the hydrophilicity of the βSSS hydrogel increases. Therefore with the value of N_{Initial} in the range from 5 to 75, the EWC of the βSSS hydrogel is higher than that of the αSSS hydrogel.

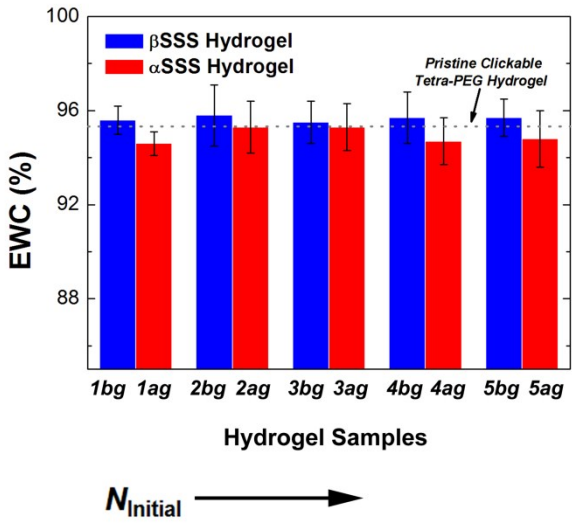


Fig. S3 Equilibrium water contents (EWCs) of βSSS and αSSS hydrogel systems.

S2.3 Internal Morphologies of Freeze-Dried Hydrogels

Fig. S4 shows SEM photographs of freeze-dried βSSS hydrogel **5bg**, αSSS hydrogel **5ag** and their blank reference of tetra-PEG hydrogel **0bg**. **0bg** is the same as **0ag**. Method of freeze-drying retains the network structure of fully swollen hydrogel to some extent. In the network of freeze-dried tetra-PEG hydrogel **0bg**, network chains of PEO have strong tendency to gather and crystallize, which leads to a thick texture. With the introduction of Hy-CDs, the threaded Hy-CDs disturb the aggregation of PEO chains, suppress the crystallization of PEO and raise the rigidity of the network. As a result, the texture of hydrogel network turns finer and slimmer. Comparing βSSS hydrogel with αSSS one, the former has finer network texture than the latter. The cavity size of Hy-β-CD is larger than that of Hy-α-CD. Impact brought from Hy-β-CD to the aggregation of PEO chains is stronger than that from Hy-α-CD. The existence of Hy-β-CD in the network hinders the PEO chains to gather and rearrange conformations more seriously. This leads to the homogeneous and anisotropic network structure with slimmer texture of the βSSS hydrogel.

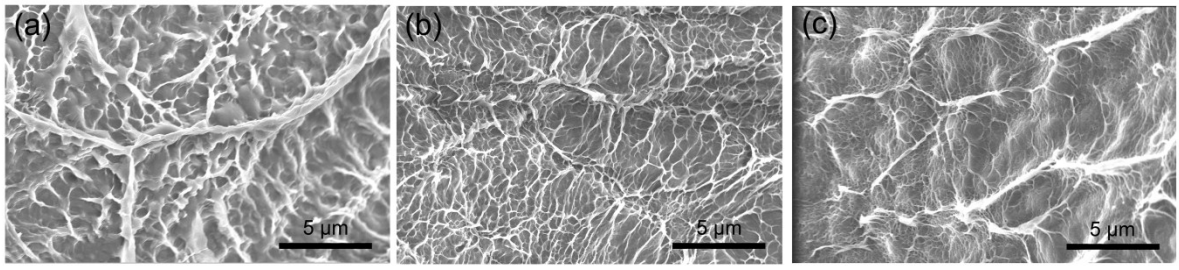


Fig. S4 SEM images of the sectional surfaces of freeze-dried hydrogel samples (a) **0bg**, (b) **5bg** and (c) **5ag**.

S2.4 Critical Gelation Concentration of Clickable Tetra-PEG Hydrogel

The critical gelation concentration (CGC) of gelator (TAPEG and TPPEG) of clickable tetra-PEG hydrogel is 1.0 *wt*%. Fig. S5 is the photograph of clickable tetra-PEG hydrogel sample **1.0%-Tetra-PEG** with gelator concentration of 1.0 *wt*%.

Fig. S5 Photograph of sample **1.0%-Tetra-PEG**. The mass of gelator (tetra-PEG macromonomers TAPEG and TPPEG) in the vial is about 0.1 g.

S2.5 Nuclear Magnetic Resonance Spectra of Samples 0b – 5b and 1b(Blank) – 5b(Blank)

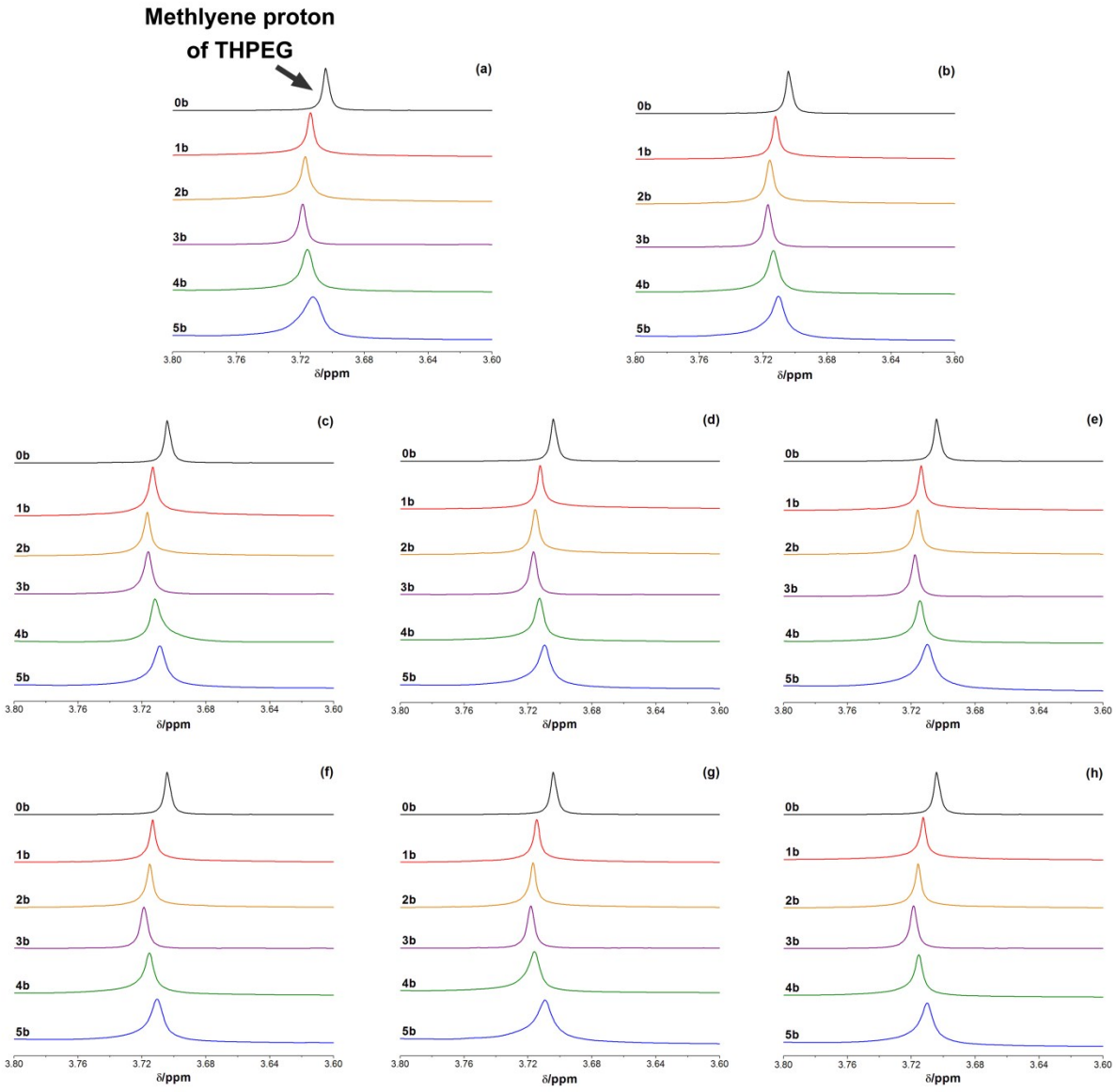


Fig. S6 Partial ^{1}H-NMR spectra of samples **0b** to **5b** with different incubation time: (a), 11.5 h; (b), 37.0 h; (c), 63.0 h; (d), 86.0 h; (e), 110.5 h; (f), 134.5 h; (g), 158.0 h; (h), 254.0 h. The scan range is 3.80 – 3.60 ppm.

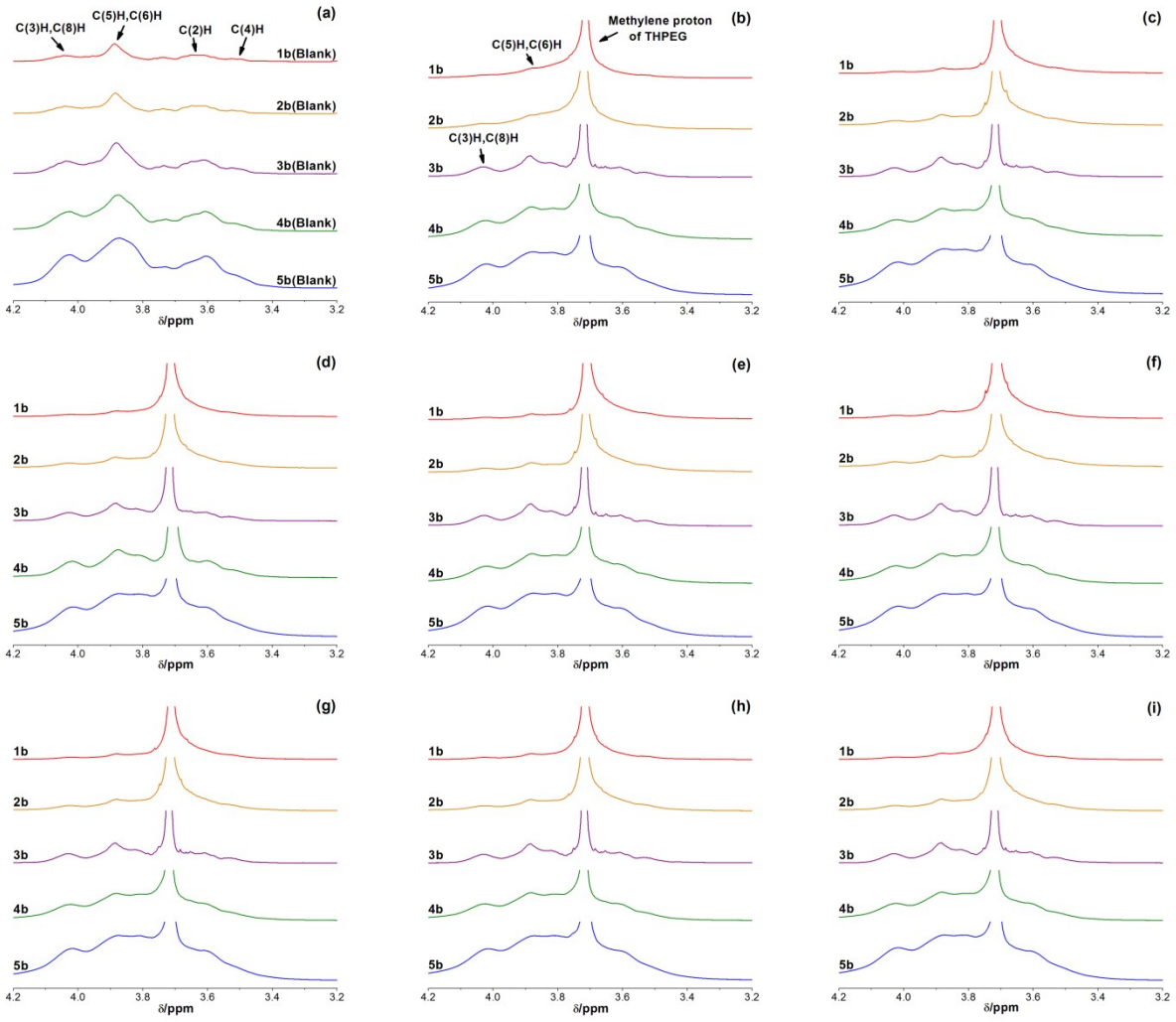


Fig. S7 (a), Partial ^{1}H-NMR spectra of samples **1b(Blank)** to **5b(Blank)**; Partial ^{1}H-NMR spectra of samples **1b** to **5b** with different incubation time: (b), 11.5 h; (c), 37.0 h; (d), 63.0 h; (e), 86.0 h; (f), 110.5 h; (g), 134.5 h; (h), 158.0 h; (i), 254.0 h. The scan range is 4.20 – 3.20 ppm.

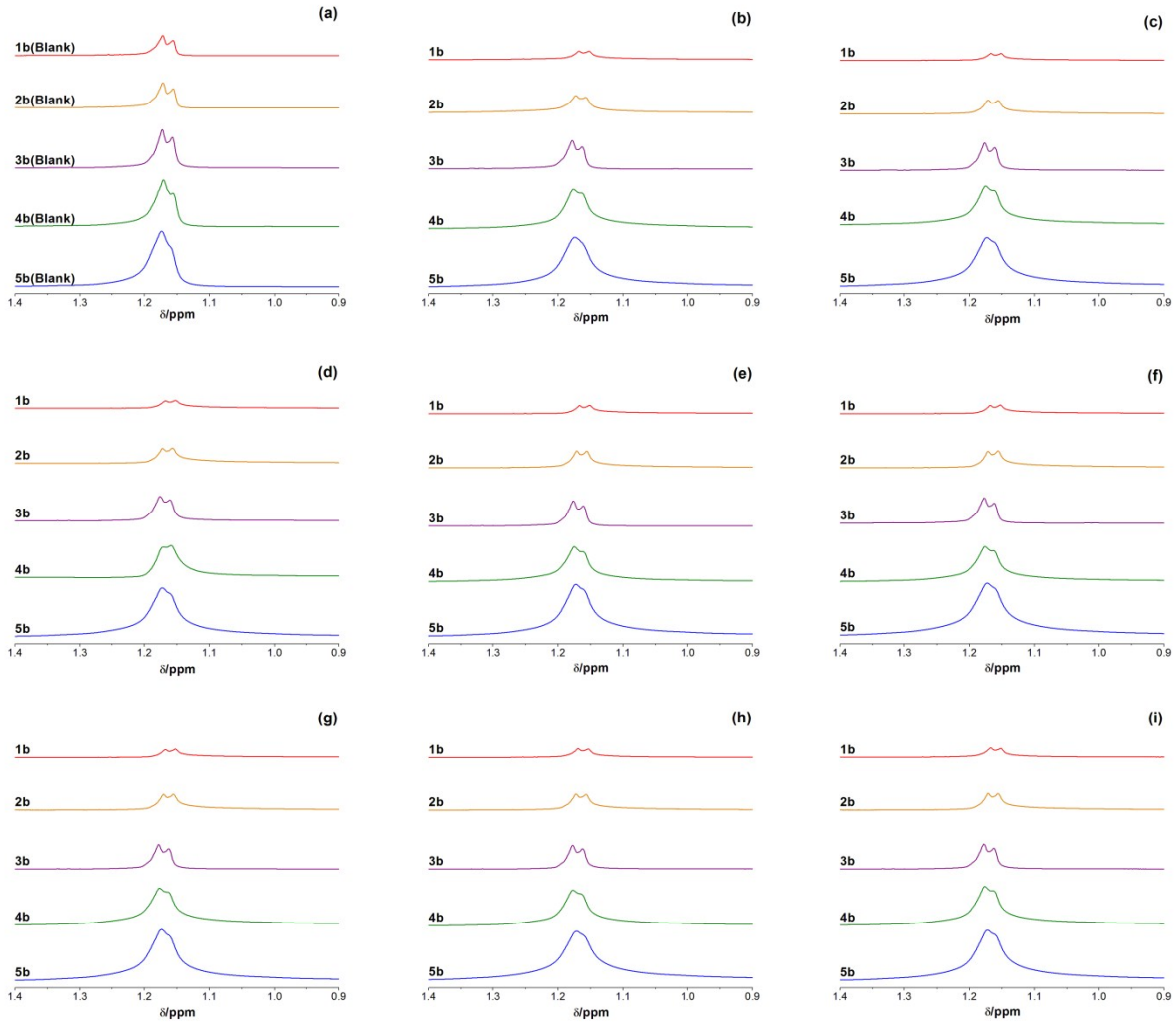


Fig. S8 (a), Partial ^{1}H-NMR spectra of references **1b(Blank)** to **5b(Blank)**; Partial ^{1}H-NMR spectra of samples **1b** to **5b** with different incubation time: (b), 11.5 h; (c), 37.0 h; (d), 63.0 h; (e), 86.0 h; (f), 110.5 h; (g), 134.5 h; (h), 158.0 h; (i), 254.0 h. The scan range is 1.40 – 0.90 ppm.

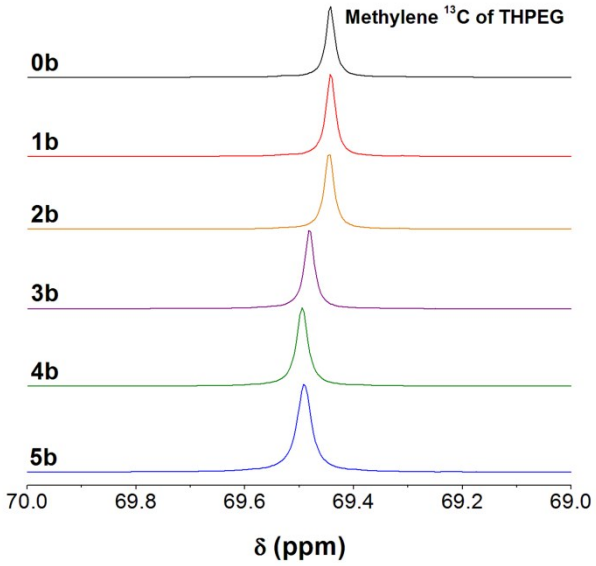


Fig. S9 ^{13}C-NMR spectra of samples **0b** to **5b** at the dynamic equilibrium with scan range of 70.0 – 69.0 ppm.

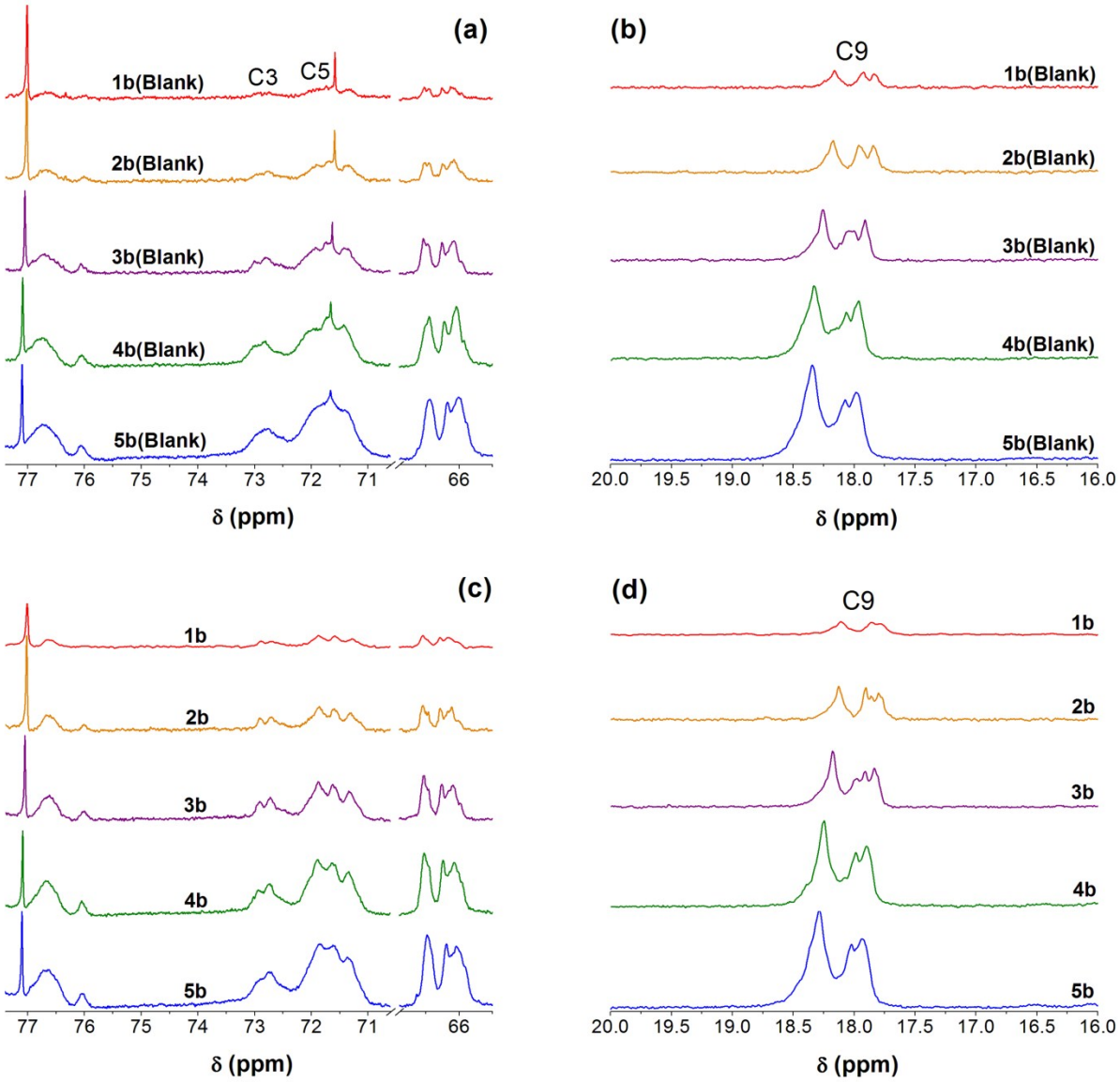


Fig. S10 ^{13}C-NMR spectra of references **1b(Blank)** to **5b(Blank)** with scan range of (a) 77.4 – 65.5 ppm and (b) 20.0 – 16.0 ppm; ^{13}C-NMR spectra of samples **1b** to **5b** at the dynamic equilibrium with scan range of (c) 77.4 – 65.5 ppm and (d) 20.0 – 16.0 ppm.

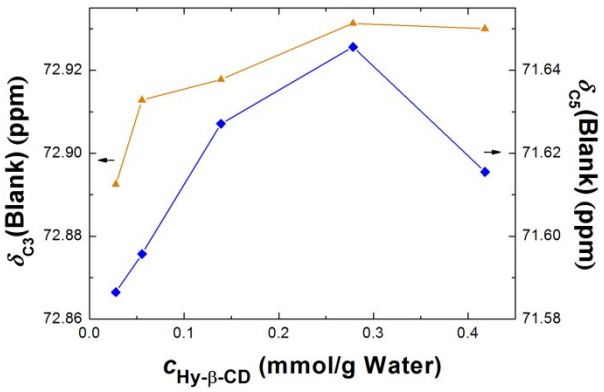


Fig. S11 Chemical shifts of carbons C3 [δ_{C3}(Blank)] and C5 [δ_{C5}(Blank)] of Hy-β-CD without THPEG as a function of mass concentration of Hy-β-CD ($c_{Hy\text{-}\beta\text{-}CD}$).

S3. References

1 Z. Li, Z. Zheng, S. Su, L. Yu and X. L. Wang, *Macromolecules*, 2016, **49**, 373-386.

2 D. R. Alston, T. H. Lilley and J. F. Stoddart, *J . Chem. Soc., Chem. Commun.*, 1985, 1600-1602.

3 N. Funasaki, M. Nomura, H. Yamaguchi, S. Ishikawa and S. Neya, *Bull. Chem. Soc. Jpn*, 2000, **73**, 2727-2728.

4 J. H. Ennist, E. A. Gobrogge, K. H. Schlick, R. A. Walker and M. J. Cloninger, *ACS Appl. Mater. Interfaces*, 2014, **6**, 18087-18097.

5 T. L. Sun, T. Kurokawa, S. Kuroda, A. Bin Ihsan, T. Akasaki, K. Sato, M. A. Haque, T. Nakajima and J. P. Gong, *Nat. Mater.*, 2013, **12**, 932-937.

6 Y. Nomoto, T. Matsunaga, T. Sakai, M. Tosaka and M. Shibayama, *Polymer*, 2011, **52**, 4123-4128.