

## Shear-assisted hydrogels based on self-assembly of cyclic dipeptide derivatives

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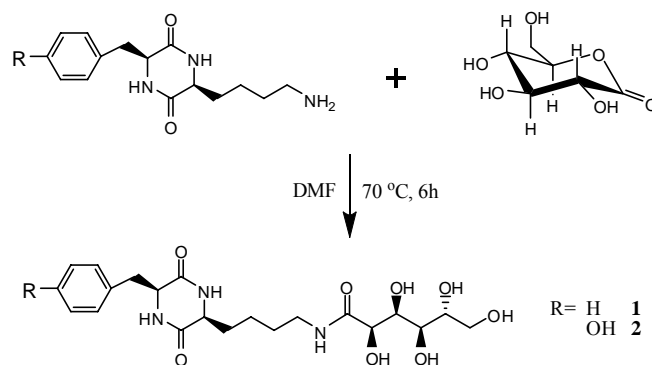
### 1. Materials

Cyclo(L-Phe-L-Lys) and cyclo(L-Tyr-L-Lys) were prepared according to our previous report.<sup>1</sup>  $\delta$ -Gluconolactone was purchased from Alfa Aesar China (Tianjin) Co., Ltd. All other reagents were supplied by VAS Chemical Reagent Company, Tianjin, China. All substances were used without further treatment. TEM images were taken on a JEM-2010 (JEOL) microscope. Rheological measurements were performed on an Anton-Paar Physica MCR300 rheometer using concentric cylinder geometry (CC27) in a concentric cylinder geometry of bob radius at 13.33 mm and cup radius at 14.46 mm. <sup>1</sup>H NMR analysis was performed on a Bruker AV-400 NMR. Mass measurement was carried out with an Apex II-ESI-FT-ICR mass spectrometer from Bruker Daltonik. Dynamic Light scattering measurement was performed with an ALV 5022 laser light-scattering (LLS) instrument equipped with a cylindrical He-Ne laser (output power = 22 mW at  $\lambda = 632.8$  nm) in combination with an ALV SP-86 digital correlator (ALV/DLS/SLS-5022F, ALV Co.). Elemental analysis was carried out on an Elementar Vario EL Elemental Analyzer. X-ray diffraction (XRD) patterns were obtained from a Philips X'Pert Pro MPD (Panalytical, The Netherlands).

### 2. Methods

#### 2.1 Synthesis of 1 and 2

Both Gelator **1** and **2** were synthesized according to Scheme S1.



Scheme S1. Synthetic description of **1** and **2**.

(1) (a) Xie, Z.; Zhang, A.; Ye, L.; Feng, Z. *Acta Chimica Sinica* **2008**, *66*, 2620. (b) Xie, Z.; Zhang, A.; Ye, L.; Feng, Z. *Soft Matter* **2009**, *5*, 1474.

In brief, 550 mg cyclo(L-Phe-L-Lys) (2 mmol) or 582 mg cyclo(L-Tyr-L-Lys) (2 mmol) was added in 60 ml DMF containing 712 mg  $\delta$ -Gluconolactone (4 mmol). The reaction mixture was stirred vigorously at 70 °C for 6 hours. The resulting solution was then precipitated in 500 ml chloroform at ambient temperature, the filtered residue was further washed with 50 ml chloroform two times and then dried under vacuum at 60 °C. Afterward, the solid was dissolved in water and filtrated to removed unsolvable impurity. The filtrate was then freeze-dried to gain product as white powder.

**1:** Yield: 76 %; m. p. 168-170 °C (decomp.);  $[\alpha]_D^{20}=+32.1$  (C=1.0, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, 25 °C):  $\delta=0.24-0.36$  (m, 1H), 0.68-0.84 (m, 2H), 0.98-1.06 (m, 1H), 1.10-1.24 (m, 2H), 2.95 (dd,  $J=14.0$  4.8 Hz, 1H), 3.07 (t,  $J=7.2$  Hz, 2H), 3.25 (dd,  $J=14.0$  3.6 Hz, 1H), 3.52-3.60 (m, 1H), 3.62-3.76 (m, 4H), 3.98 (t,  $J=3.2$  Hz, 1H), 4.20 (d,  $J=3.6$  Hz, 1H), 4.35 (t,  $J=4.0$  Hz, 1H), 7.14-7.20 (m, 2H), 7.29-7.39 (m, 3H); <sup>1</sup>H NMR (400 MHz, [D<sub>6</sub>]DMSO, 25 °C):  $\delta=0.58-0.804$  (m, 3H), 0.964-1.18 (m, 3H), 2.82 (dd,  $J=4.8$  13.6 Hz, 1H), 3.00 (dd,  $J=3.6$  13.2 Hz, 1H), 2.90 (q,  $J=6.4$  Hz, 2H), 3.40-3.50 (s, br, 1H), 3.52-3.60 (s, br, 3H), 3.84-3.92 (s, br, 1H), 3.94-4.00 (s, br, 1H), 4.14-4.22 (s, br, 1H), 4.28-4.60 (m, 4H), 5.37 (s, br, 1H), 7.10-7.30 (m, 5H), 7.51 (t,  $J=5.6$  Hz, 1H), 8.02 (s, br, 1H), 8.13 (s, br, 1H); ESI-MS: m/z 454.3 (M+H<sup>+</sup>), 476.3 (M+Na<sup>+</sup>); elemental analysis calcd (%) for C<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>8</sub>: C 55.63, H 6.84, N 9.27; found: C 55.41, H 6.75, N 9.12.

**2:** Yield: 78 %; m.p.: 174-176 °C (decomp.);  $[\alpha]_D^{20}=19.9$  (C=1.0, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, 25 °C):  $\delta=0.26-0.36$  (m, 1H), 0.72-0.88 (m, 2H), 1.00-1.12 (m, 1H), 1.15-1.28 (m, 2H), 2.81 (dd,  $J=14.0$  4.8 Hz, 1H), 3.02 (t,  $J=6.8$  Hz, 2H), 3.12 (dd,  $J=10.4$  3.6 Hz, 1H), 3.54-3.60 (m, 1H), 3.62-3.78 (m, 4H), 3.99 (t,  $J=3.2$  Hz, 1H), 4.21 (d,  $J=4.0$  Hz, 1H), 4.32 (t,  $J=3.6$  Hz, 1H), 6.78 (d,  $J=8.4$  Hz, 2H), 6.99 (d,  $J=8.8$  Hz, 2H); <sup>1</sup>H NMR (400 MHz, [D<sub>6</sub>]DMSO, 25 °C):  $\delta=0.62-0.74$  (m, 1H), 0.74-0.90 (m, 2H), 1.04-1.12 (m, 1H), 1.12-1.22 (m, 2H), 2.72 (dd,  $J=4.8$  12.8 Hz, 1H), 3.00 (dd,  $J=3.6$  13.6 Hz, 1H) [ABX spin system], 2.92 (q,  $J=6.4$  Hz, 2H), 3.37 (s, br, 1H), 3.39 (s, br, 1H), 3.55 (s, 1H), 3.56 (s, br, 1H), 3.59 (s, br, 1H), 3.91 (s, br, 1H), 3.97 (s, br, 1H), 4.08 (s, br, 1H), 4.20-4.70 (m, 4H), 5.39 (s, br, 1H), 6.64 (d,  $J=8.4$  Hz, 2H), 6.92 (d,  $J=8.4$  Hz, 2H), 7.61 (t,  $J=5.6$  Hz, 1H), 8.00 (s, br, 1H), 8.04 (s, br, 1H), 9.20 (s, br, 1H); ESI-MS: m/z 470.3 (M+H<sup>+</sup>), 492.3 (M+Na<sup>+</sup>); elemental analysis calcd (%) for C<sub>21</sub>H<sub>31</sub>N<sub>3</sub>O<sub>9</sub>: C 53.70, H 6.61, N 8.95; found: C 53.58, H 6.52, N 8.69.

## 2.2 Determination of $T_{sol-gel}$ :

The two curves with sol-gel phase transition temperature ( $T_{sol-gel}$ ) were determined by visually observation of gelation process with or without stirring.

For the  $T_{sol-gel}$  without stirring, 2 ml aqueous solution of gelator was introduced to a tube with diameter of 18 mm. The tube was then placed in a water bath with a temperature decreasing rate of 2 °C/h. The  $T_{sol-gel}$  was recorded when a homogenous clear hydrogel was formed exhibiting no gravitational flow upon inversion.

For the  $T_{sol-gel}$  with stirring, 2 ml aqueous solution of gelator was introduced to a tube in diameter of 18 mm containing a magnetic bar of 8 mm in length. The tube was then placed in a water bath with a temperature decreasing rate of 10 °C/h. During the cooling process, the bar was allowed to stir at an approximate rate of 1000 r/min. The  $T_{sol-gel}$  was then recorded when the solution turned to be somewhat turbid with increased viscosity. Usually, this viscous fluid can form a hydrogel after 10 minutes aging.

### 2.3 Transmission electron microscopy (TEM)

A carbon-coated Cu grid was inserted in the hydrogel sample and took out soon with some liquid, which was removed by filter paper. The grid was then allowed to dry in air atmosphere overnight and another 12 h under vacuum. The grid was further negative stained by placing the grid in a drop of 1 wt% uranyl acetate in ethanol, blotting off excess liquid after 1 minute, and then drying in air.

### 2.4 Dynamic light scattering (DLS)

The light scattering measurement was carried out at 25 °C. The hot aqueous solutions of **1** at 3.0 wt% and **2** at 2.5 wt% were purified by passing through a hydrophilic filter (Millipore, 0.45 μm) to remove dust, and allowed to cool to 25 °C in the spectrometer. However, the intensity of scattered light from the sample was found to be too low to calculate, even after half an hour of incubation. This result indicates no aggregation to occur in this study, suggesting the solution samples remained in a supersaturated state during the incubation.

### 2.4 Powder X-ray diffraction of xerogels

XRD patterns were obtained using Cu-Kα ( $\lambda = 1.5405 \text{ \AA}$ ) radiation and Ni filter at 40 kV and 40 mA, with a step size of 0.33 ° ( $2\theta$ ) from 1-30 °. The hydrogel samples were deposited on a glass substrate and dried by slow evaporation under atmosphere to form xerogel, and then submitted to XRD analysis.

## 3. Results and discussion

### 3.1 <sup>1</sup>H NMR analysis

To determine the aggregation of gelator molecules, <sup>1</sup>H NMR technique was used to trace the alteration of integrated intensity ratio  $I_{\text{solvent}}/I_{\text{gelator}}$ . As known,<sup>2</sup> when free gelator molecules self-assemble into solid NMR-unobservable aggregates, they would never contribute to the total signal intensity. Since  $I_{\text{solvent}}$  is constant, the ratio change of  $I_{\text{solvent}}/I_{\text{gelator}}$  can reflect the aggregation status within sample.

As shown in Fig. S1, the <sup>1</sup>H NMR spectra of 5.0 wt% solution of **1** in D<sub>2</sub>O were recorded at 50, 35 and 20 °C, respectively, while the temperature was controlled to decrease from 50 to 20 °C. The sample was allowed to stay at each temperature for 15 minutes, prior to the NMR scan. Herein,  $I_{\text{solvent}}/I_{\text{gelator}}$  was calculated by  $I_{D_2O}/I_2$ . As can be seen,  $I_{D_2O}/I_2$  presents similar value at 50 and 35 °C, while sharply increases at 20 °C, indicating the formation of hydrogel. Moreover, a nearly fixed value at 35 °C suggests that all the gelator molecules stay in supersaturated solution without aggregation at this temperature, in accordance with its gelation testing result.

The similar results were also obtained for the sample of **2** (3.0 wt%, D<sub>2</sub>O) as shown in Fig. S2.

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(2) (a) Menger, F.; Yamasaki, Y.; Catlin, K. K.; Nishimi, T. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 585. (b) Duncan, D. C.; Whitten, D. G. *Langmuir* **2000**, *16*, 6445.

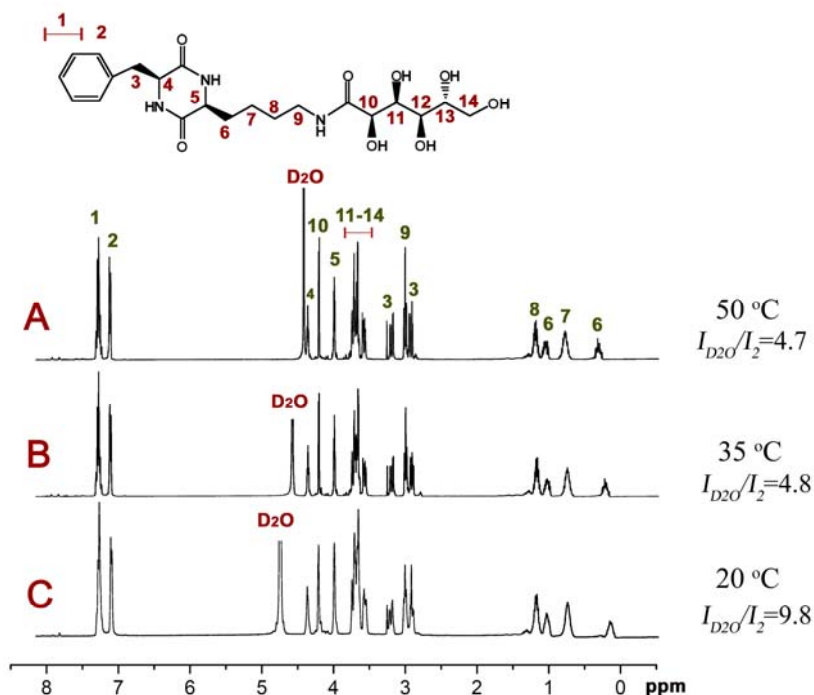


Figure S1.  $^1\text{H}$  NMR spectra of solution of **1** ( $\text{D}_2\text{O}$ , 5.0 wt%) at different temperatures.

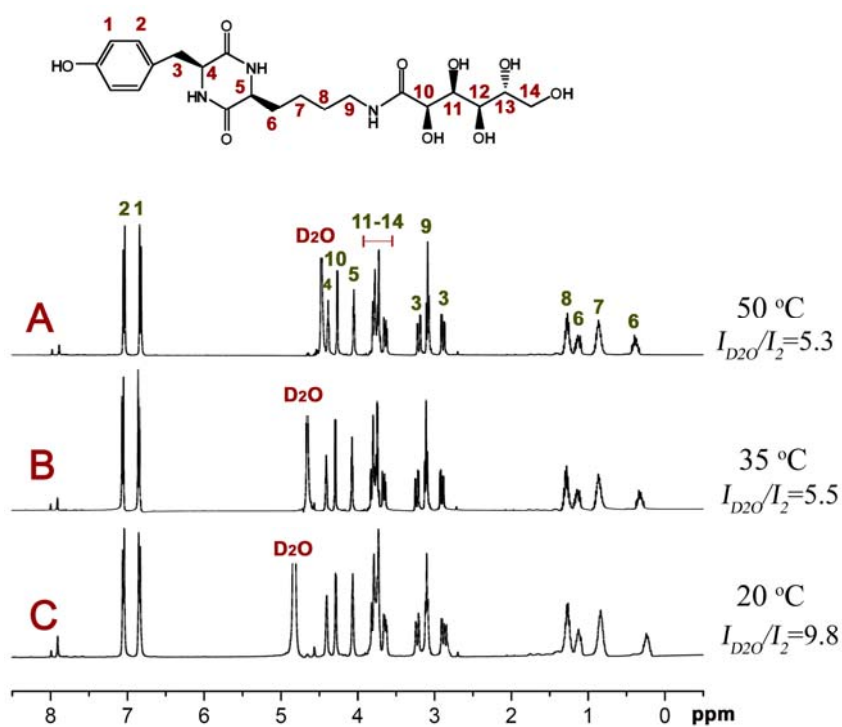


Figure S2.  $^1\text{H}$  NMR spectra of solution of **2** ( $\text{D}_2\text{O}$ , 3.0 wt%) at different temperatures.

### 3.2 Rheological properties of hydrogel

Hydrogel samples of **1** and **2** at 5.0 wt% were produced by *in situ* cooling their solutions in the bob-cup from 80 °C to 20 °C followed by another 1 hour of ageing. As shown in Figure S3, An increase of  $G'$  during its cooling course demonstrated that the aqueous solutions at 5.0 wt% can spontaneously undergo gelation, well in consistence to their phase curves.

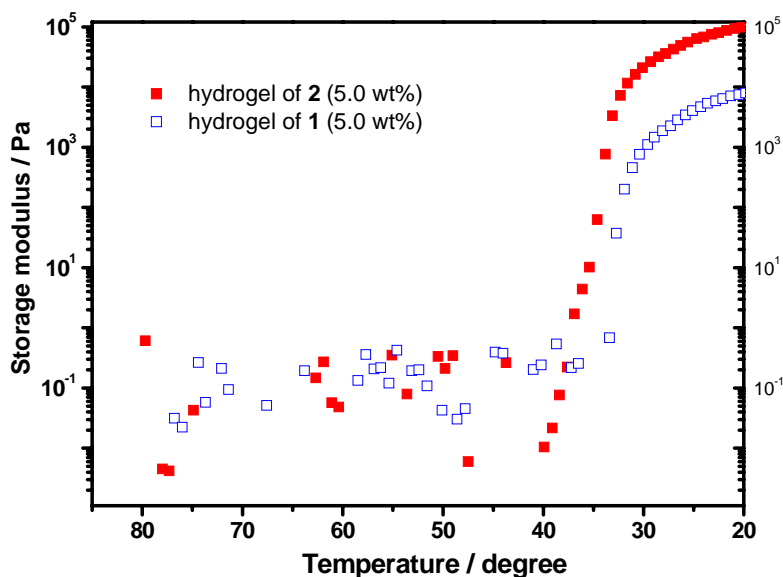


Figure S3. Temperature sweep oscillation from 80 °C to 20 °C over 40 minutes ( $f=1$  Hz,  $\gamma_0=0.1$  %). Solid squares: 5.0 wt% hydrogel of **2**; Hollow squares: 5.0 wt% hydrogel of **1**

A strain-sweep experiment was first conducted to determine the linear viscoelastic regime of deformations and yield strain of hydrogel. In this linear regime, both dynamic modulus are independent of the strain amplitude displaying the mechanical properties of the unperturbed network. As shown in the inset of Figure S4, the flowing response of hydrogels (5.0 wt%) to an ascending strain ramp is presented by the curves of the dependence of  $G'$  with the applied oscillatory strain ( $f=1$  Hz). As can be seen, for both hydrogels of **1** and **2**,  $G'$  value exhibits a neat plateau at a low strain range, and starts to sharply decrease at  $\gamma_0=6-7$  % and to 50 % at  $\gamma_0=8-10$  %, attributed to a partial breakup of hydrogel network.

Afterwards, the hydrogel was subjected to a non-destructive frequency sweep experiment with a constant strain of 0.1 % (within linear viscoelastic regime as indicated by the arrow in the inset of Figure S4). The dynamic storage modulus  $G'$  is always higher or even stands 1 order greater than the loss modulus  $G''$  among all testing frequencies (Figure S4), showing the dominant elastic character of these hydrogels resulting from cyclic dipeptide derivatives. Moreover, a frequency sweep gave a slightly sloped  $G'$  vs.  $f$ , suggesting a slow rearrangement in hydrogel. The complex viscosity  $|\eta^*|$  does not show any Newtonian plateau at low frequencies and decays with a slope of -1. Such mechanical behaviour is characteristic of soft viscoelastic solids, implied that the sample can be qualified as a “true” hydrogel.

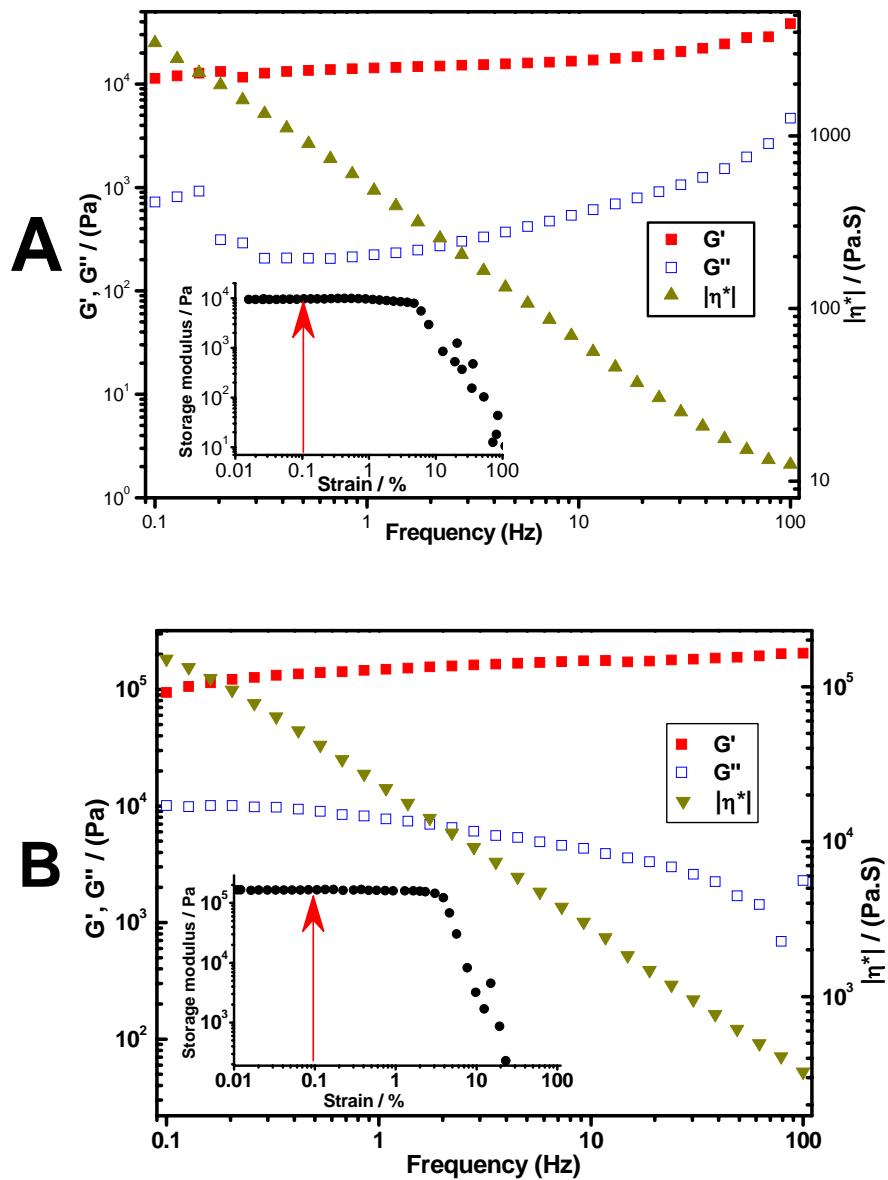


Figure S4. Viscoelastic properties of the hydrogel of **1** (5.0 wt%, A) and **2** (5.0 wt%, B). Frequency sweep using a constant strain of 0.1% from 0.1 to 100 Hz. Inset: Variation of the elastic modulus of the hydrogel as a function of the applied strain at 20 °C. ( $f = 1$  Hz).

### 3.3 TEM images of nanofibers

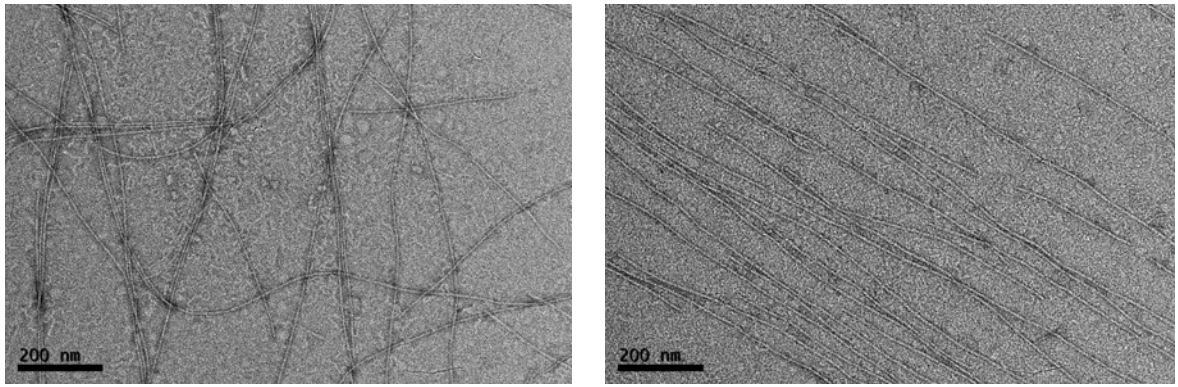


Figure S5. Low magnification TEM images of nanofibers. Left: hydrogel of **1** at 3.0 wt%. Right: hydrogel of **2** at 2.0 wt%.

### 3.4 Powder X-ray diffraction (XRD) results

As seen in Fig. S6, the low peak intensity/background ratio found in the XRD results generally suggested the low crystallinity of xerogel of **1** and **2**, as well as the self-assembled fibers as shown in Fig. 4.

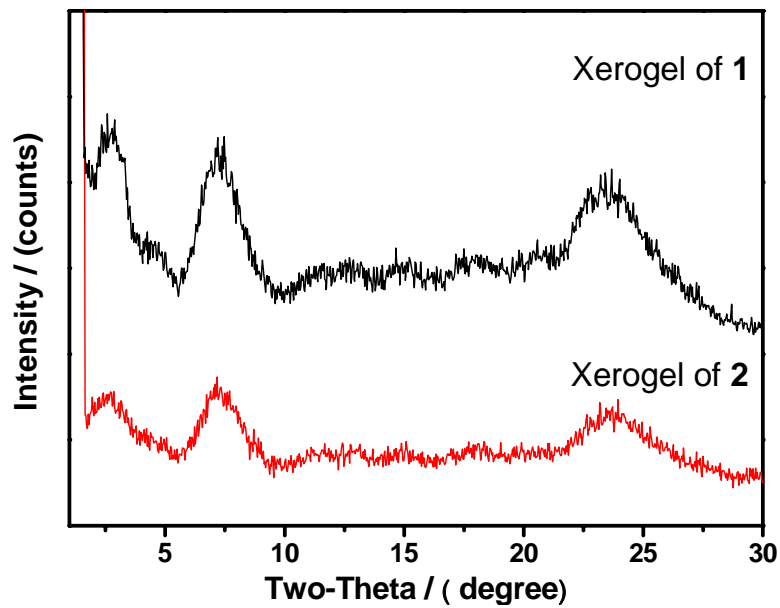


Figure S6. XRD patterns of xerogel of **1** and **2** in the region of 1-30°.