Supporting Information

**Enzyme-instructed assembly of the core of yeast prion Sup35 to form supramolecular hydrogels**

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1. Materials and instruments

   **Materials**

   All of the solvents and chemical reagents were used as received from the commercial sources without further purification unless otherwise noted. Amino acids and HBTU were purchased form GL Biochem (Shanghai) Ltd. Proteinase K was purchased from Sigma (> 800 unit/mL). The HeLa cell line (CCL-2) was purchased from American Type Culture Collection. All of the media were purchased from Invitrogen. Other materials and solvents were purchased from Fisher Scientific.

   **Instruments**

   We purified all the products with a Water Delta600 HPLC system, equipped with an XTerra C18 RP column and an in-line diode array UV detector. We obtained \(^1\)H-NMR spectra on Varian Unity Inova 400, LC-MS spectra on a Waters Acouity ultra performance LC with Waters MICRO-MASS detector, TEM images on Morgagni 268 transmission electron microscope, rheological data on TA ARES G2 rheometer with 25 mm cone plate, MTT assay for cell viability test on DTX 880 multimode detector.

2. Synthesis and characterization

   Synthesis of L-3P: L-3P was prepared via solid phase peptide synthesis (SPPS).\(^{[1]}\) We used 2-chlorotrityl chloride resin and N-Fmoc amino acids with side chain trityl protecting groups (Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH). Fmoc-Tyr(PO\(_2\)H\(_2\))-OH was obtained
according to reported literatures in two steps. For SPPS, all the steps are done under N\textsubscript{2} atmosphere. First, we swelled 0.8 g 2-Chlorotrityl chloride resin in dry methylene chloride (DCM) for 30 min and washed by dimethylformamide (DMF) for 3 times. Second, we loaded Fmoc-Tyr(PO\textsubscript{3}H\textsubscript{2})-OH onto resin at C-terminal in a DMF solution of Fmoc-Tyr(PO\textsubscript{3}H\textsubscript{2})-OH (2 mmol, 966 mg) and N,N-Diisopropylethylamine (DIEA) (5 mmol, 0.87 mL) for 30 min and washed by DMF for 5 times. Then, we blocked the unreacted resin sites by methanol/DCM/DIEA (80/15/5) for 10 min \times 2 and washed by DMF 3 times. After the resin was treated by 20\% piperidine in DMF for 5min \times 3 to deprotect Fmoc group and washed by DMF for 5 times, the next Fmoc-Asn(Trt)-OH was coupled to the free amino group by coupling reagent HBTU/DIEA and washed by DMF for 5 times. To elongate the peptide chain, we repeated the deprotecting and coupling steps. After the final 2-naphthylacetic acid was connected to the resin, we washed the resin by DMF, DCM, methanol, and hexane respectively, each 5 times. Finally L-\textbf{3P} was released by 95\% TFA cleavage method. The resulted crude products were purified by reverse phase HPLC. \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}\textsubscript{6}, \textit{\delta}): 8.31-8.24 (m, 3H), 8.22-8.16 (m, 2H), 8.05-7.96 (m, 4H), 7.85 (d, 1H), 7.78-7.72 (m, 2H), 7.57 (s, 1H), 7.49-7.41 (m, 2H), 7.22-7.16 (m, 12H), 7.08 (d, 1H), 6.91 (s, 1H), 6.79 (s, 1H), 4.49-4.96 (m, 4H), 4.16 (s, 1H), 4.26-4.16 (m, 2H), 3.77 (s, 2H), 3.66 (s, 2H), 3.32 (br s, 4H), 3.07-2.57 (m, 8H), 2.12 (t, 4H), 1.89-1.76 (m, 4H); \textsuperscript{31}P NMR (400 MHz, DMSO-\textit{d}\textsubscript{6}, \textit{\delta}): -9.25; LC-MS (ESI) (m/z): C\textsubscript{63}H\textsubscript{75}N\textsubscript{14}O\textsubscript{20}P, calcd. 1378.50; found 1379.44 [M+1]\textsuperscript{+}, 1377.43 [M-1]-.
We followed the SPPS procedure described above to obtain compound L-4P, L-5P, D-3P, D-4P, and D-5P.

3. Hydrogel preparation

First, we added precursor (1.5 mg) in phosphate buffered saline (PBS, 295 µL) at pH = 7.4, which gave a clear solution. Then, we added 5 µL ALP (1U/µL) to the solution, which resulted in a hydrogel in 10 minutes. The final concentration of ALP should be 17 U/mL.

4. MTT assay

After confirming the enzymatic responses of precursors to phosphatases, we incubated HeLa cell, a common used cervical cancer cell line, with them. We used MTT assay\(^4\) to evaluate the cell viability. At first, we seeded HeLa cells (100 µL of \(1 \times 10^5\) cell/mL) in each well of a 96-well plate. After 4 hours' incubation for cell attachment, we replaced the medium with 100 µL of fresh medium that contained 100 to 500 µM of precursors (serial diluted from 10 mM stock solution in deionized water). During the viability measurement of HeLa cells,
which were incubated for three days. We added 10 µL MTT reagent into the assigned wells every 24 h. Following the MTT protocol, we added sodium dodecyl sulfate (SDS) solution to the wells and measured the absorbance by a plate reader. We did three times MTT assays for L-3P and D-4P, and twice for other precursors. We obtained of the IC₅₀ values (at 48h) of L-3P, L-4P, L-5P, D-3P, D-4P, and D-5P are 476.1 ± 21.4, >500, >500, >500, 264.5 ± 28.4, and >500 µM respectively.

![Figure S1.](image)

Figure S1. The first try: Cell viability of HeLa cells incubated with (A) L-3P; (B) L-4P; (C) L-5P; (D) D-3P; (E) D-4P; (F) D-5P for three days.
Figure S2. The second try: cell viability of HeLa cells incubated with (A) L-3P; (B) L-4P; (C) L-5P; (D) D-3P; (E) D-4P; (F) D-5P for three days.

Figure S3. The third try: cell viability of HeLa cells incubated with (A) L-3P; (B) D-4P for three days.

5. TEM

The fiber width measurement: Choosing three fibers randomly, and using the function of ruler tool in Photoshop to measure the width of three times, we calculated the average and standard deviation of the fiber widths.
Figure S4. TEM images of gels (1.1 wt %) formed by (A) L-3P, pH = 5; (B) L-4P, pH = 4; (C) L-5P, pH = 2; (D) D-3P, pH = 5; (E) D-4P, pH = 4; (F) D-5P, pH = 2. Inserts are the corresponding optical images. Scale bar represents 100 nm.

6. Rheology

Figure S5. Strain dependence of the dynamic storage modulus (G’) and the loss modulus (G’’) of gels shown in Figure 4S: (A) L-3P and D-3P, pH =5; (2) L-4P and D-4P, pH = 4; (C) L-4P and D-5P, pH = 2.

