Supporting Information

Materials: 2-Cl-trityl chloride resin (1.1 mmol/g) was obtain from Nankai University resin Co., Ltd. Fmoc-amino acids and o-benzotriazol-1-yl-N,N,N',N'-tetramehtyluronium hexafluorophosphate (HBTU) were bought from GL Biochem(Shanghai). Chemical regents and solvents were used as received from commercial sources. Commercially available reagents were used without further purification, unless noted otherwise. Alkaline phosphatase was purchased from TaKaRa. Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) was purchased from Gibco.

General methods: ^{1}H NMR (Bruker ARS 400) and HR-MS (Agilent 6520 Q-TOF LC/MS) was used to character the compounds. TEM (JEM100CXII) was performed at the Tecnai G2 F20 system, operating at 100 kV. Reology (TA instrument) test was done on an AR 2000ex system, 25 mm parallel plates was used during the experiment at the gap of 500 μm. Circular dichroism (CD) spectrum was obtained by a BioLogic (MOS-450) system. Dynamic light scattering was measured by ZETAPALS/BI-200SM (BROOKHAVEN).

Synthesis of peptides: All peptides were prepared by standard solid phase peptide synthesis (SPPS) by using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected. Firstly the C-terminal of the first amino acid was conjugated on the resin. Anhydrous N,N'-dimethyl formamide (DMF)

containing 20% piperidine was used to remove Fmoc protected group. To couple the next amino acid to the free amino group, O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) was used as coupling reagent. Peptides chain was entended according the standard SPPS protocol. Lastly, 95% TFA containing 2.5% H_2O and 2.5% TIS was used to cleave peptides derivative from resin and the mixture was filtered. Ice cold diethylether was poured into filtrate concentrated by rotary evaporation. The precipitate was centrifuged for 5 min at 5000 rpm speed. The solid was dried by vaccum pump and then purified by HPLC to obtain the pure compounds.

Characterization of the compounds:

Compound Nap-FFGGpYGSSSRRAPQT: ^{1}H NMR (300 MHz, DMSO) δ 8.61 – 8.49 (m, 1H), 8.46 – 7.87 (m, 12H), 7.85 – 6.57 (m, 30H), 4.63 – 4.38 (m, 5H), 4.35 – 4.11 (m, 7H), 3.62 (ddd, *J* = 42.9, 22.6, 12.5 Hz, 35H), 3.12 – 2.91 (m, 7H), 2.86 – 2.69 (m, 3H), 2.27 (s, 1H), 2.12 (t, *J* = 7.9 Hz, 1H), 1.45 (d, *J* = 21.7 Hz, 4H), 1.20 (d, *J* = 6.3 Hz, 2H), 1.04 (d, *J* = 6.1 Hz, 2H). MS: calc. M = 1865.89, obsvd. $(1/2\ M + H)^+$ = 933.9037.

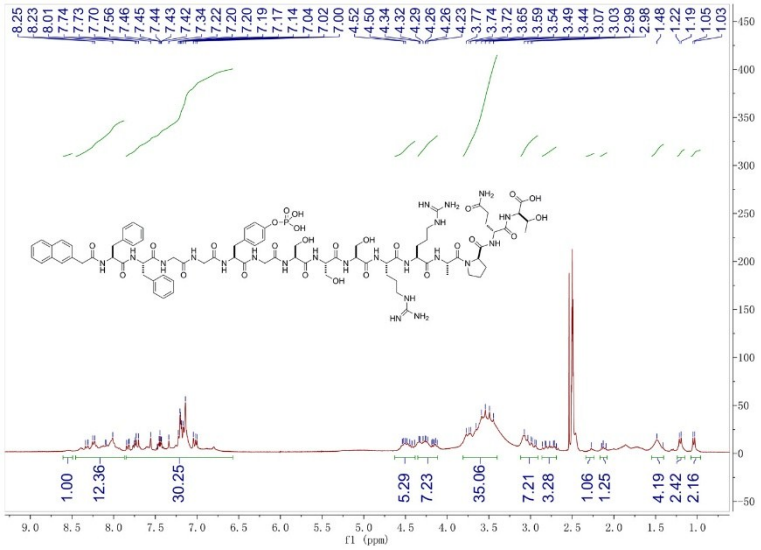

Fig. S1. ^{1}H NMR spectrum of Nap-FFGGpYGSSSRRAPQT

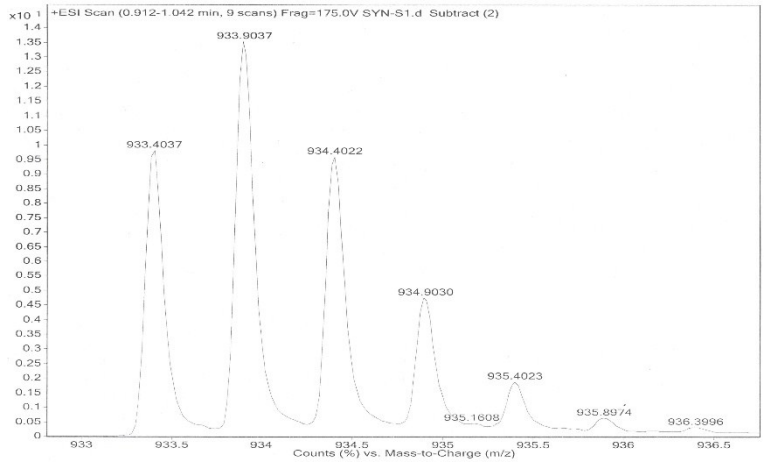

Fig. S2. HR-MS spectrum of Nap-FFGGpYGSSSRRAPQT

Compound NBD-GFFpYGAVPIAQK: ^{1}H NMR (300 MHz, DMSO) δ 8.49 (d, *J* = 9.1 Hz, 1H), 8.31 – 7.28 (m, 10H), 7.25 – 6.52 (m, 11H), 6.40 (d, *J* = 10.0 Hz, 2H), 4.77 – 3.57 (m, 27H), 2.84 – 2.48 (m, 33H), 2.46 – 1.31 (m, 4H), 1.25 – 1.04 (m, 2H), 0.92 – 0.72 (m, 4H). MS: calc. M = 1612.63, obsvd. $(1/2\ M + H)^{+}$ = 806.3528, M= 1612.7016.

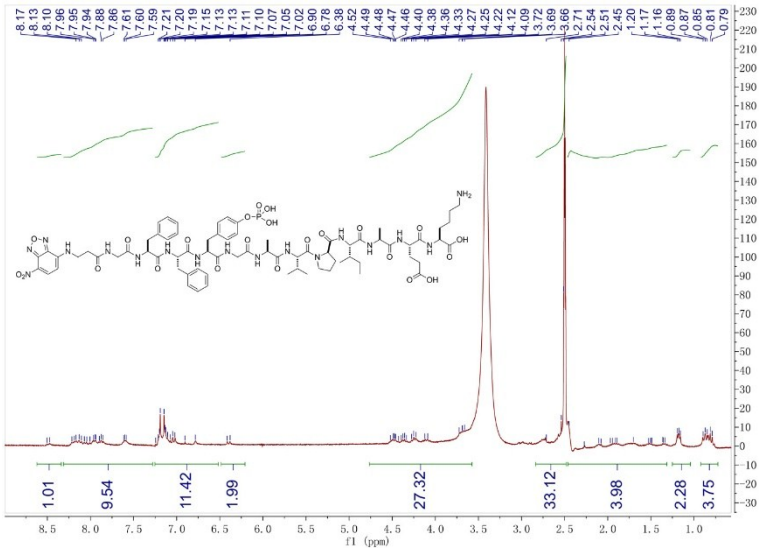

Fig. S3. ^{1}H NMR spectrum of NBD-GFFpYGAVPIAQK

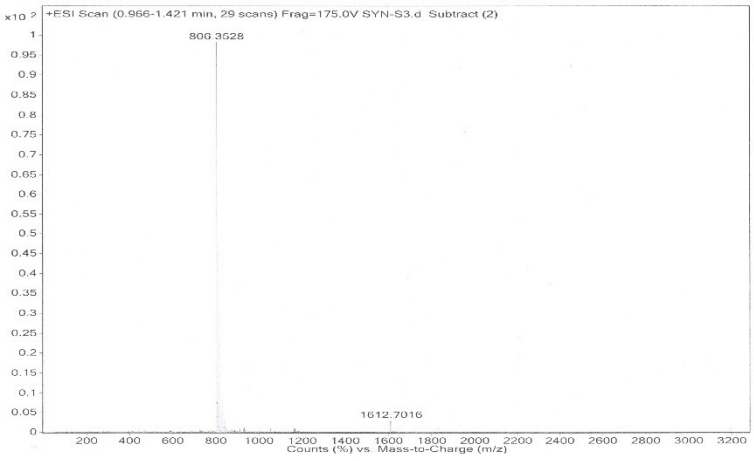

Fig. S4. HR-MS spectrum of NBD-GFFpYGAVPIAQK

Conversion Ratio: 1 mL of the samples (0.4 wt%) were used to determine the conversion ratio of the compounds. After adding 1 U/mL phosphatase into the sample, 100 μL of each sample was taken out at each time point and then 100 μL of methanol was added to terminate the reaction. The conversion ratio was then analyzed by the LC-MS. We detected the peaks of precursor and hydrogelator at each time point, and the conversion ratio was calculated by integrating the peaks area.

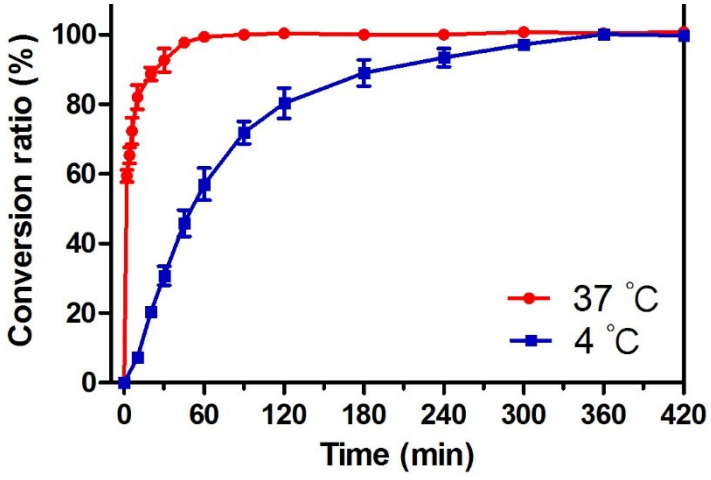

Fig. S5. Conversion ratio of compound ***1*** to ***2*** by adding ALP at 4 ^{0}C and 37 ^{0}C

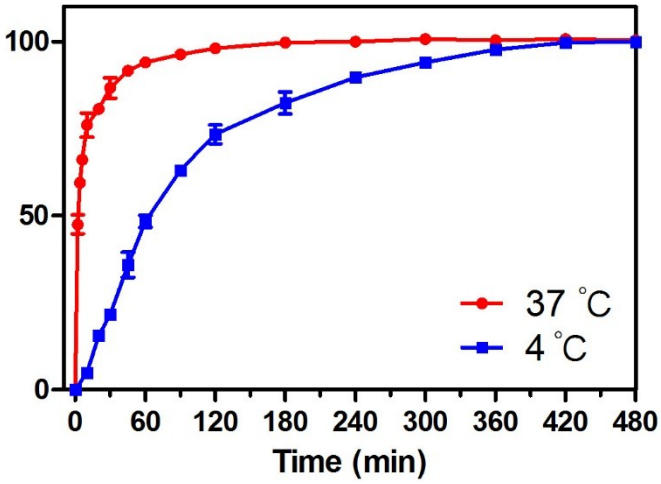

Fig. S6. Conversion ratio of compound ***3*** to ***4*** by adding ALP at 4 ^{0}C and 37 ^{0}C

TEM: Negative staining technique was used to observe the TEM images of micro structure in samples. Firstly 10 μL hydrogels were loaded on the 400 mesh copper grids coated with continuous thick carbon film, dd-water was then used to rinse the grid for twice. After rinsing, the grids containing samples were stained with 2 % uranyl acetate. Finally the grids were allowed to dry in a desiccator before observation.

Rheology: Rheology test was carried out on an AR 2000ex (TA instrument) system, 25 mm parallel plates was used during the experiment at the gap of 500 μm. For the dynamic temperature sweep, the solution of compounds at 4 ^{0}C were directly transferred to the rheometer and it was conducted at the frequency of 1 rad/s and the strain of 1% immediately. The samples were also characterized by the mode of dynamic temperature sweep in the region from 4 ^{0}C to 60 ^{0}C.

Circular dichroism (CD) spectrum: CD spectrum were measured by a BioLogic (MOS-450) system. All samples were placed in 0.1 cm quartz spectrophotometer cell (20-C/Q/0.1). The wavelength range was from 185 to 250 nm. The acquisition period was 0.5 s and the step was 0.5 nm. The resultant CD spectra was acquired after subtracting the solvent background.

Critical aggregation concentration (CAC) value: The CAC values of all samples were determined by dynamic light scattering (DLS). Solutions containing different concentration of compounds were tested and the light scattering intensity was recorded for each concentration analyzed. The lower CAC values represented better assembly capacity.

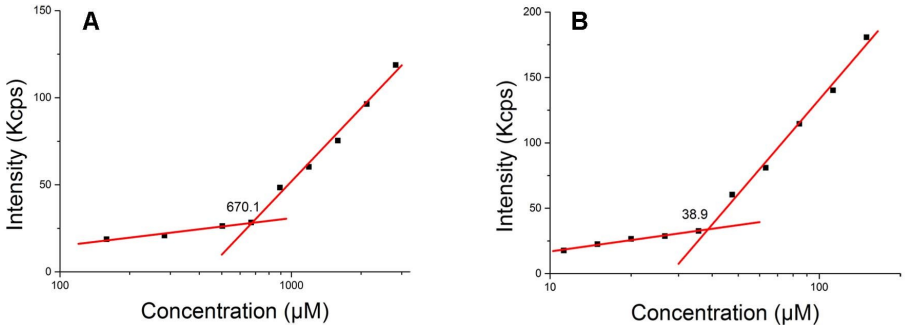

Fig. S7. Critical aggregation concentration (CAC) value of compound ***3*** at A) 4 ℃ and B) 37 ℃

Determination of gelation time: Different concentrations of compounds were dispersed in 0.9 mL of PBS buffer solution (pH = 7.4). 1 eq Na_2CO_3 was added to the above solution to adjust the final pH = 7.4, and PBS was then pipetted into the solution to make the volume of solution 1.0 mL. The solution was incubated at 4 °C for 30 min in fridge. The

phosphatase (1 U/mL) was then added to the above solution for 12 h at 4 °C. We then incubated the solution at the higher temperature (37 °C).

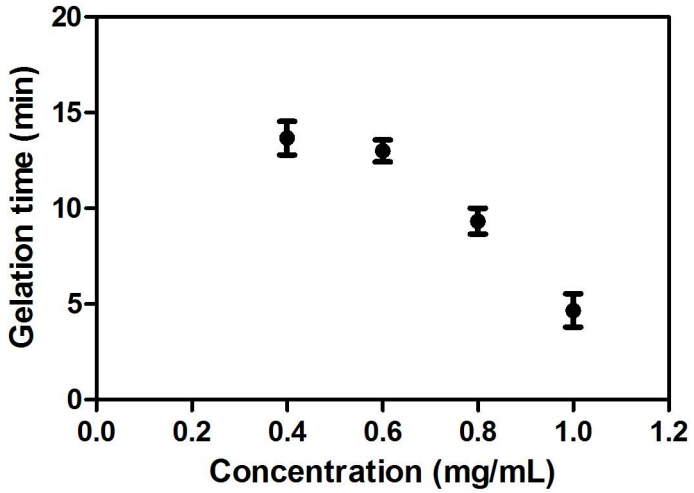

Fig. S8. Gelation time of compound ***1*** by ESAI at different concentration from 4 ^{0}C to 37 ^{0}C

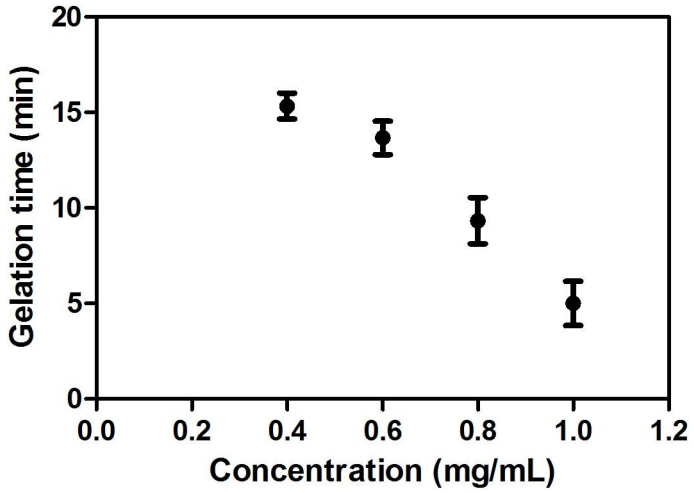

Fig. S9. Gelation time of compound ***3*** by ESAI at different concentration from 4 ^{0}C to 37 ^{0}C

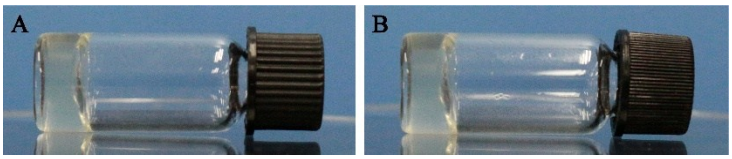

Fig. S10. Optical images of thermogel of ***2*** after being put back to 4 oC for A) 15 minutes and B) 12 hours.

Fig. S11. Optical images of A) suspension of ***2*** in PBS (0.4 wt%) formed by sonication after being kept at 4 ^{o}C for 12 hours, and suspension of ***2*** in A) after being kept at 37 ^{o}C for B) 15 minutes and C) 12 hours.

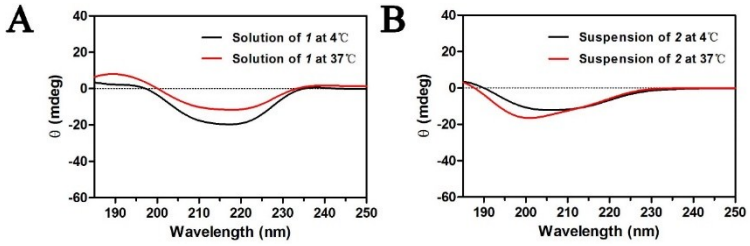

Fig. S12. A) The CD spectra of solution of ***1*** at 4 and 37 ^{o}C, respectively. B) The CD spectra of suspension of ***2*** at 4 and 37 ^{o}C, respectively.

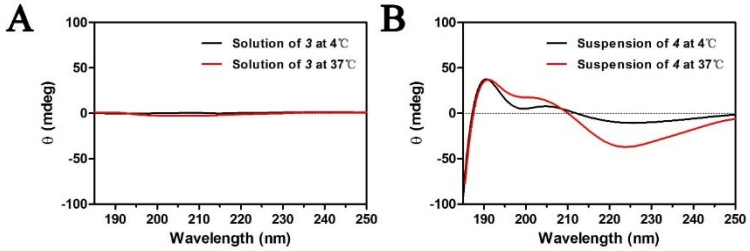

Fig. S13. A) The CD spectra of solution of 3 at 4 and 37 ^{o}C, respectively. B) The CD spectra of suspension of ***4*** at 4 and 37 oC, respectively.

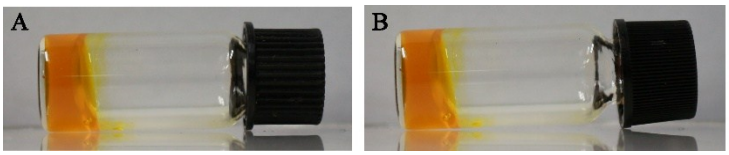

Fig. S14. Optical images of thermogel of ***4*** after being put back to 4 oC for A) 15 minutes and B) 12 hours.

Fig. S15. Optical images of A) suspension of ***4*** in PBS (0.4 wt%) formed by sonication after being kept at 4 ^{o}C for 12 hours, and suspension of ***4*** in A) after being kept at 37 ^{o}C for B) 15 minutes and C) 12 hours.

Cell culture: We firstly prepared a PBS solution from 4.0 wt% of ***1*** by EISA at 4 ^{0}C, which was kept as a stock solution and stored in a fridge at 4 ^{0}C. Subsequently, 1 volume of the peptide stock solution was mixed with 9 volume of DMEM/F12 culture medium containing Mesenchymal stem cells (MSCs, from GFP rat) at the final density of 10^5 cells/mL in a 96 well plate, which was then kept in the cell culture incubator at 37 ^{0}C. The hydrogel-cell construct would form within 15 minutes. We measured the cell proliferation in hydrogel at day 1, 3 and 5 by a quantitative CCK-8 assay.