

## Supporting Information

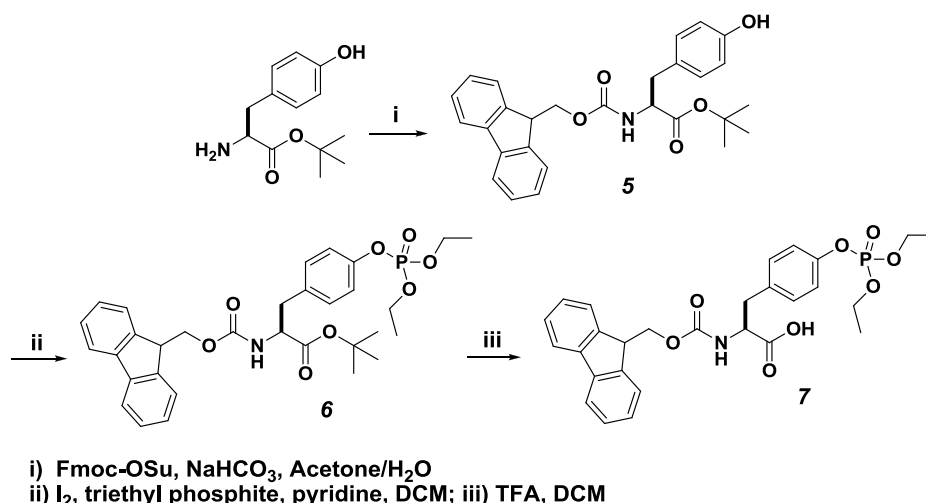
### Materials and general methods:

**Chemicals:** Fmoc-OSu, L-tyr- O<sup>t</sup>Bu and other Fmoc-amino acid were obtained from GL Biochem (Shanghai). Chemical reagents and solvents were used as received from commercial sources. Alkali phosphatase was purchased from Takara (Dalian, China) Bio. Commercially available reagents were used without further purification, unless noted otherwise. All the reagents such as DMF and DCM were used as received unless noted

**General methods:** <sup>1</sup>H NMR and <sup>31</sup>P NMR (Bruker ARX 400) were used to characterize the synthesized compounds. ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. Conversion of compound **1** was carried out by LCMS-2020 (Shimadzu). Scanning electron micrograph (SEM) was done on a Hitachi X650 system (Japan) operating at 15 kV. The SEM sample was prepared as following: a thin layer of the gel was firstly coated on the surface of a silica wafer and then the silica wafer was put into a small grass vial. The small vial was plugged into liquid nitrogen and then the gel sample was freeze-dried. Emission spectra were detected on a Perkin-Elmer LS-55 luminance spectrometer at excitation wavelength of 272 nm. CD spectra were conducted on a JASCO J-820 Spectropolarimeter. AFM was measured on a Veeco multimode V system (tip: Veeco RTESP), Rheology test was done on an AR 2000ex (TA instrument) system, 40 mm parallel plates was used during the experiment at the gap of 500 μm. The FT-IR was measured on a Bio-Rad FTS 6000 Fourier Transform Infrared Spectrometer. The gel samples were freeze-dried to get powders for FT-IR analysis.

**Peptide synthesis.** All the peptide derivatives were prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group. The first amino acid (**5**) was loaded on the resin at the C-terminal with the loading efficiency about 0.6 mmol/g. 20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used during the deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, the N-terminus of the peptides was coupled with 2-naphthalene acetic acid to attach the aromatic group on the tetrapeptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 ml per gram of resin), followed by five steps of washing using dichloromethane (DCM) for 2 min (5 ml per gram of resin). The peptide derivatives were cleaved from the resin by ice-cold reagent B and the mixture was stirred at room temperature, filtered, and poured into ice-cold diethylether. The resulting precipitate was centrifuged for 10 min at 2 °C at 10,000 rpm. Afterward the

supernatant was decanted and dissolved in double-distilled (dd) water and lyophilized.



*Scheme S-1.* Synthetic route for compound **7**

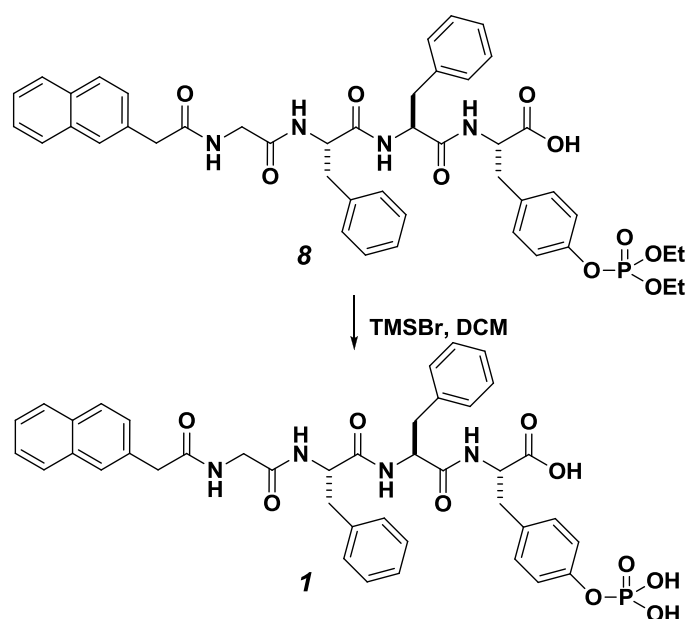
The synthesis of compound **1** was according the method we reported before<sup>1</sup>.

#### Hydrogelator synthesis and characterizations:

**Synthesis of Fmoc-L-Tyr-OtBu (5):** L-Tyr-OtBu (2.37 g, 10 mmol) and DIPEA (1.74 ml, 10 mmol) were firstly dissolved in 75 mL of acetone with stirring. And then the solution of Fmoc-OSu (3.3 g, 9.8 mmol, dissolved in 75 mL acetone) was added dropwise. The reaction mixture was stirred at room temperature overnight. After the reaction mixture being concentrated, the residue was purified by flash chromatography on silica gel (eluent: Hexane/Ethyl acetate = 3/1) to afford 4.4 g of title compound **5** (95.8%). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 8.03-8.05 (d, 2H), 7.81-7.85 (t, 2H), 7.56-7.59 (t, 2H), 7.46-7.48 (m, 2H), 7.19-7.21 (d, 2H), 6.81-6.83 (d, 2H), 4.30-4.40 (m, 3H), 4.2-4.24 (m, 1H), 3.30-3.36 (m, 2H), 1.50 (s, 9H). MS: calc. M<sup>+</sup> = 459.2, obsvd. (M+1)<sup>+</sup> = 459.78.

**Synthesis of Fmoc-L-Tyr(PO(OEt)<sub>2</sub>)-OtBu (6):** I<sub>2</sub> (7.5 mmol, 1.5 equivalents) was added to a solution of the Triethyl phosphate (8 mmol, 1.6 equivalents) in 80 mL CH<sub>2</sub>Cl<sub>2</sub> at 0 °C. 20 minutes after the addition, the clear, colorless solution was allowed to warm to room temperature (25°C). Then the solution was added dropwise, over a period of 30 minutes, to a flask containing **5** (5.0 mmol, 1.0 equivalent) and pyridine (20.0 mmol, 4.0 equivalents) in 50 mL CH<sub>2</sub>Cl<sub>2</sub> at 0°C. After an additional 2 hours, the reaction mixture was diluted with ethyl acetate (200 ml). The organic layer was washed with NaHSO<sub>4</sub> (5 wt%) (100 ml\*2), brine (100 ml), dried over anhydrous MgSO<sub>4</sub>, successively. The residue was purified by flash chromatography on silica gel (eluent: Hexane/Ethyl acetate = 3/2) to afford 2.55 g of title compound **6** (85.5%). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 8.03-8.05 (d, 2H), 7.82-7.84 (d, 2H), 7.57-7.59 (t, 2H), 7.42-7.48 (m, 4H), 7.25-7.28 (d, 2H), 4.10-4.40 (m, 8H), 3.11-3.13 (m, 1H), 3.03-3.07 (m, 1H), 1.49 (s, 9H), 1.37-1.41 (t, 6H). <sup>31</sup>P NMR (δ -6.366 ppm). MS: calc. M<sup>+</sup> = 595.2, obsvd. (M+1)<sup>+</sup> = 596.01.

**Synthesis of Fmoc-L-Tyr(PO(OEt)<sub>2</sub>-OH (7):** To a solution of **6** (600 mg, 1.0 mmol) in 5 mL CH<sub>2</sub>Cl<sub>2</sub>, TFA (10 ml) was added at 0 °C. After the reaction mixture being stirred at 0 °C for 4 hours, the solvent was removed by rotary evaporator. The solid obtained was co-evaporated with toluene twice. 530 mg of title compound **7** was obtained after removing the solvent in vacuum (98.3%). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 8.23-8.25 (d, 2H), 8.01-8.05 (t, 2H), 7.76-7.79 (t, 2H), 7.59-7.62 (m, 4H), 7.47-7.52 (d, 2H), 4.30-4.60 (m, 8H), 3.45-3.48 (m, 1H), 3.21-3.27 (m, 1H), 1.58-1.61 (t, 6H). <sup>31</sup>P NMR (δ -6.348 ppm). MS: calc. M<sup>+</sup> = 539.2, obsvd. (M+H)<sup>+</sup> = 540.14.



*Scheme S-2.* Chemical structure and synthetic route for compound **1**

**Synthesis of hydrogelator (compound 1):** After obtaining compound **7**, we used solid phase peptide synthesis (SPPS) to synthesis compound **8**, then 25 equiv. of TMSBr in 10 ml of dry DCM was added. The solvent was removed and 10 ml of dd water was added with stirring for 30 min, the resulting solution was freeze-dried and purified by HPLC to afford title compound **1**.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.29 (d, J=7.605, 1H), 8.16 (d, J=7.176, 2H), 8.01 (d, J=8.375, 1H), 7.80-7.88 (m, 3H), 7.75 (s, 1H), 7.41-7.48 (m, 3H), 7.16-7.24 (m, 12H), 7.07-7.10 (m, 2H), 4.43-4.58 (m, 3H), 3.62-3.74 (m, 4H), 3.01-3.05 (m, 2H), 2.89-2.93 (m, 2H), 2.73-2.82 (m, 1H), 2.62-2.69 (m, 1H). <sup>31</sup>P NMR (δ -6.201 ppm). MS: calc. M<sup>+</sup> = 781.29, obsvd. (M+1)<sup>+</sup> = 782.30.

#### **Formation of small molecular hydrogels:**

**SM(1+2) gel:** 12 mg of **1**, 1.6 mg of Na<sub>2</sub>CO<sub>3</sub> (to neutralize the phosphoric acid group on **1**) were dissolved in 3 mL of Tris-HCl buffer (pH = 8.0). The hydrogel was formed by treating the above solution with alkaline phosphatase (90 U/mL).

**SM(3+4) gel:** 12 mg of Nap-GFFYE-ss-EE (**3**), and 2.9 mg of Na<sub>2</sub>CO<sub>3</sub> were dissolved in 3 mL

of Tris-HCl buffer (pH = 7.4). The hydrogel was formed by treating the above solution with Tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 5.2mg).

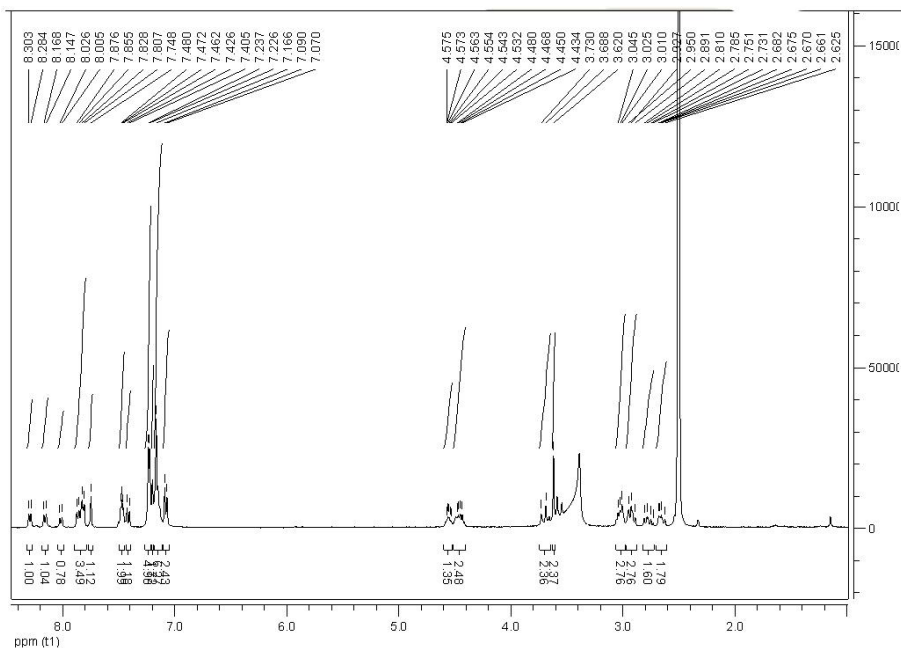


Figure S-1.  $^1\text{H}$  NMR of compound 1

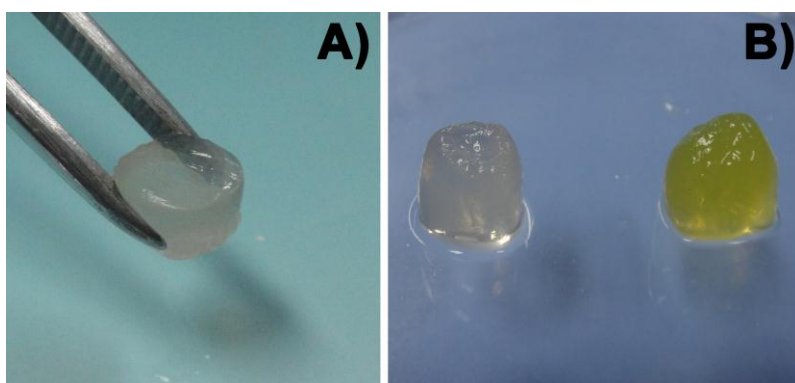
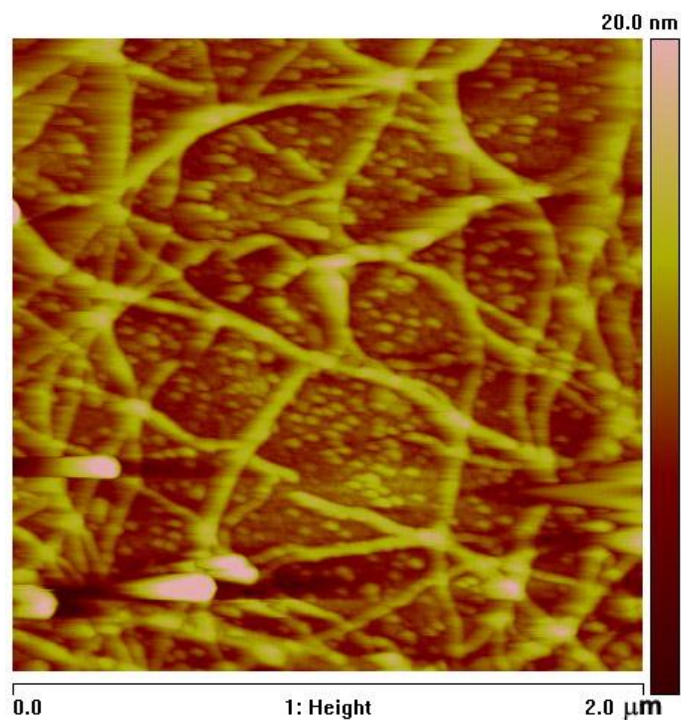
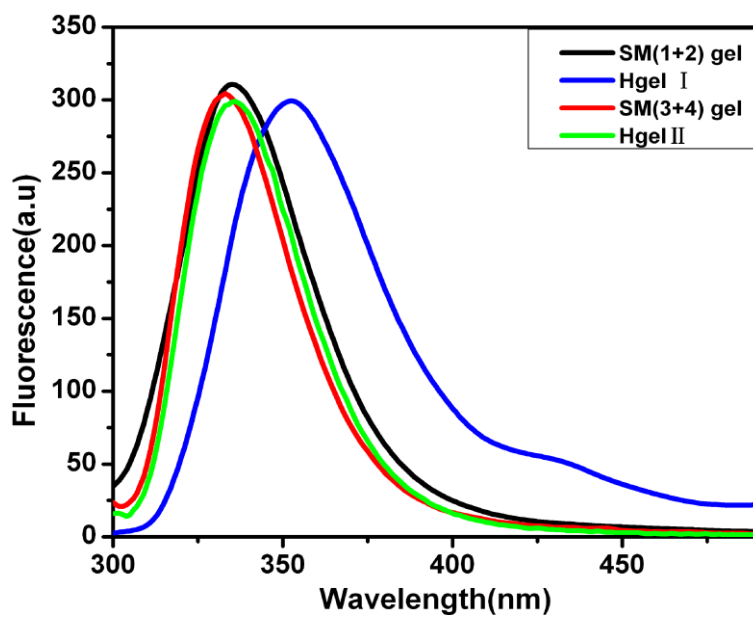


Figure S-2. A) Schematic illustration for Hgel II held by a forceps, B) optical images of 0.6 mL of Hgel II before and after enzymatic reactions for 30 times (fresh reaction mixtures were used for each test)



*Figure S-3.* The AFM image of SM(1+2) gel.



*Figure S-4.* The fluorescence spectra of SM(1+2) gel, Hgel I, SM(3+4) gel and Hgel II.

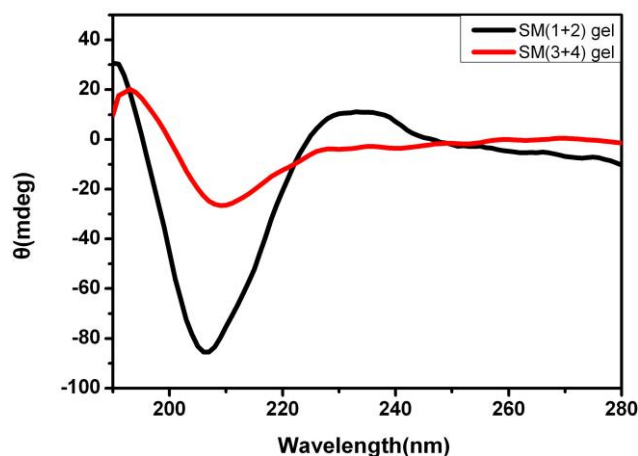


Figure S-5. The CD spectra of SM(1+2) gel and SM(3+4) gel.

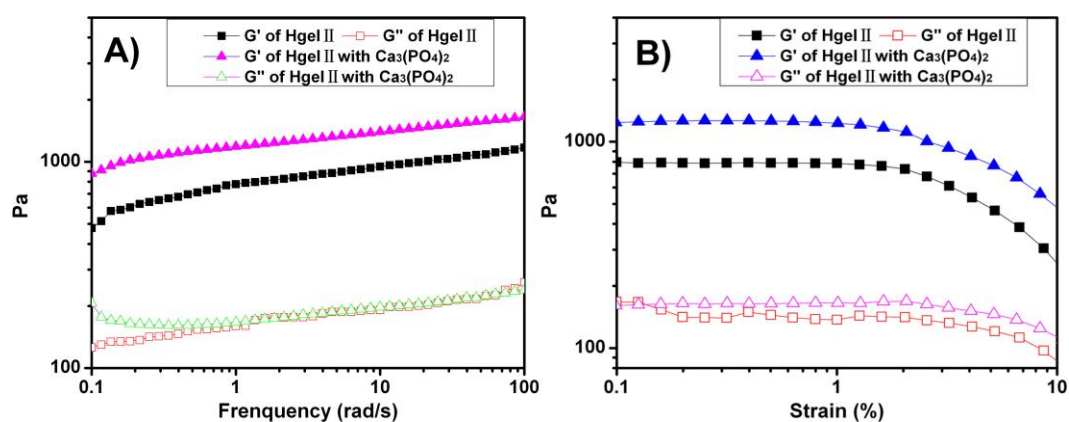


Figure S-6. A) The dynamic frequency sweep (strain of 1%) and B) dynamic strain sweep (frequency of 1 rad/s) of Hgel II with or without  $\text{Ca}_3(\text{PO}_4)_2$ .

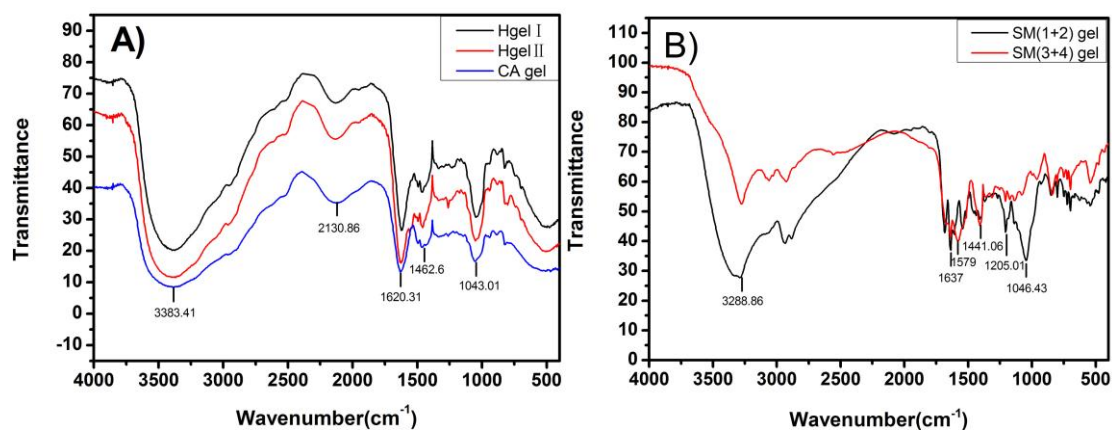


Figure S-7. The FT-IR spectra of A) Hgel I, Hgel II, and CA gel, and B) SM(1+2) gel and SM(3+4) gel.

## References

- (1) Wang, H. M.; Wang, Z. H.; Song, D. H.; Wang, J. Y.; Gao, J.; Wang, L.; Kong, D. L.; Yang, Z. M. *Nanotechnology*, **2010**, 225606.