Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information for

The Ratio of Hydrogelator and Precursor Controls Enzymatic Hydrogelation of a Branched Peptide

Jiaqi Guo[a], Hongjian He[a], Beom Jin Kim[a], Jiaqing Wang[a], Meihui Yi[a], Cheng Lin[b,c], and Bing Xu[a]*

[a.] Department of Chemistry, Brandeis University, 415 South Street, Waltham, MA 02453, USA. E-mail: bxu@brandeis.edu

[b.] Center for Biomedical Mass Spectrometry, Boston University School of Medicine, Boston, MA 02118, USA.

[c.] Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118, USA.

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Materials and Instruments

Materials

2-Cl-trityl chloride resin, amino acid derivatives, and O-benzotriazole-N,N,N',N',-tetramethyluronium-hexafluorophosphate (HBTU) were purchased from GL Biochem (Shanghai, China). Piperidine, trifluoroacetic acid (TFA), and all other reagents and solvents were purchased from Fisher Scientific. All the chemical reagents and solvents were used without further purification. The intermediates and crude product were purified by HPLC to obtain the final product with > 95% purity.

Instruments

The purification was done by Agilent 1100 Series HPLC system equipped with a reverse phase C18 column. LC-MS was conducted on a Waters Acquity Ultra Performance LC with Waters MICRO-MASS detector. Fluorescence-based CMC determination was operated on a Shimadzu RF-5301 fluorescence spectrophotometer. Rheology tests were obtained by a TA ARES-G2 rheometer at 25 ℃. TEM images were taken on a Morgagni 268 transmission electron microscope. CD spectra were obtained by Jasco J-810 spectropolarimeter.

Solid phase peptide synthesis

a. 2-chlorotrityl chloride resin was weighed and dipped in methylene chloride (DCM) for 15 min.

b. The amino acids were weighed according to 1.2 mmol/g of resin. Dissolve the amino acid in DCM with 2.5 equivalent of N,N-Diisopropylethylamine (DIEA) addition. Mix resin with solution well on a rocker for 1 h. Wash with DCM.

c. Add capping solution (DCM: MeOH: DIEA= 17: 2: 1) and react for 15 min. Wash with DCM first and then dimethylformamide (DMF).

d. Use 20% piperidine in DMF for 30 min to remove Fmoc group. Wash with DMF.

e. Load amino acid (1 equivalent), HBTU (1 equivalent), and DIEA (2.5 equivalent) in DMF for 40 min. Wash with DMF.

f. Use DCM to wash out DMF. Use either i) 20% tetrafluoroethylene (TFE) in DCM for 1 h to collect side chain protected peptides or ii) 95% TFA, 2.5% triisopropyl silane (TIPS), 2.5% H_2O for 30 min for peptide cleavage and side chain deprotection.

The peptides were directly used for liquid phase coupling.

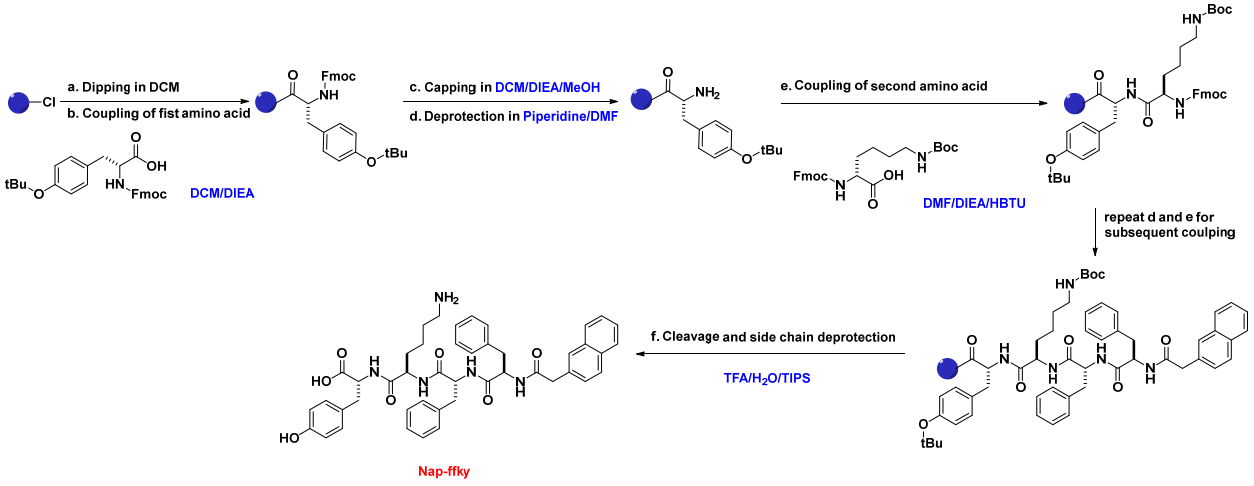

Fig. S1. Synthesis of Nap-ffky.

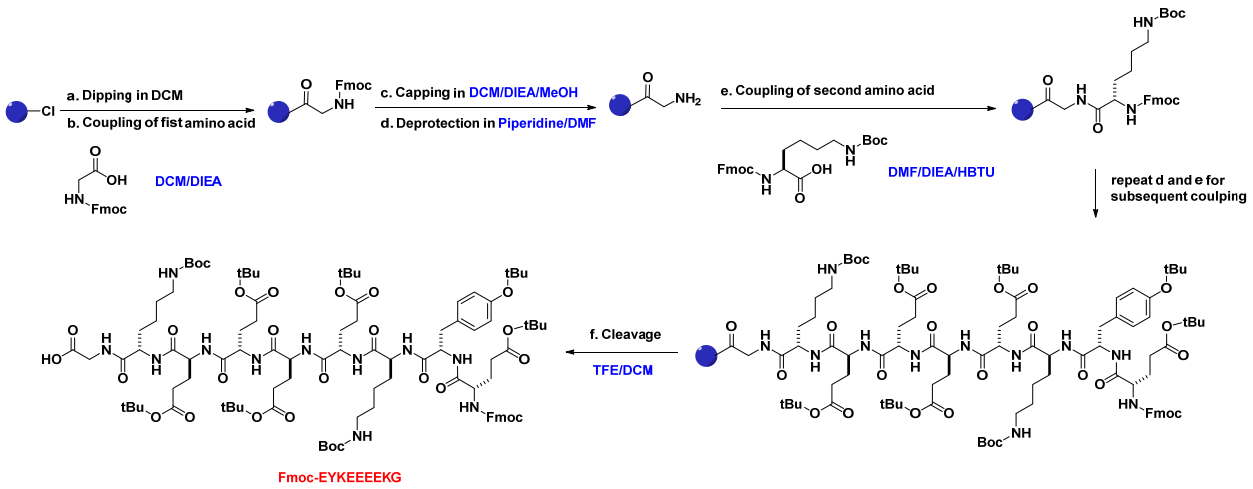

Fig. S2. Synthesis of Fmoc-EYKEEEEKG.

Synthesis of 1

a. Nap-ffky (77 mg, 0.1 mmol), Fmoc-EYKEEEEKG (200 mg, 0.1 mmol), HBTU (38 mg, 0.1 mmol) were dissolved in DMF. Add DIEA (32 mg, 0.25 mmol) and stir for 7 h. Air dry the solvent.

b. Add 20% piperidine in DMF and stir for 30 min. Air dry the solvent.

c. Add 95% TFA, 5% H_2O and stir for 1 h. Air dry the solvent.

The crude product of **1** was purified by reverse phase HPLC, using the following eluent gradient. 0 min: 80% H_2O, 20% CH_3CN, 15 min: 30% H_2O, 70% CH_3CN, 16 min: 100% CH_3CN, 21 min: 100% CH_3CN, 22 min: 80% H_2O, 20% CH_3CN, 24 min: 80% H_2O, 20% CH_3CN. Pure **1** was lyophilized with the yield of 72%.

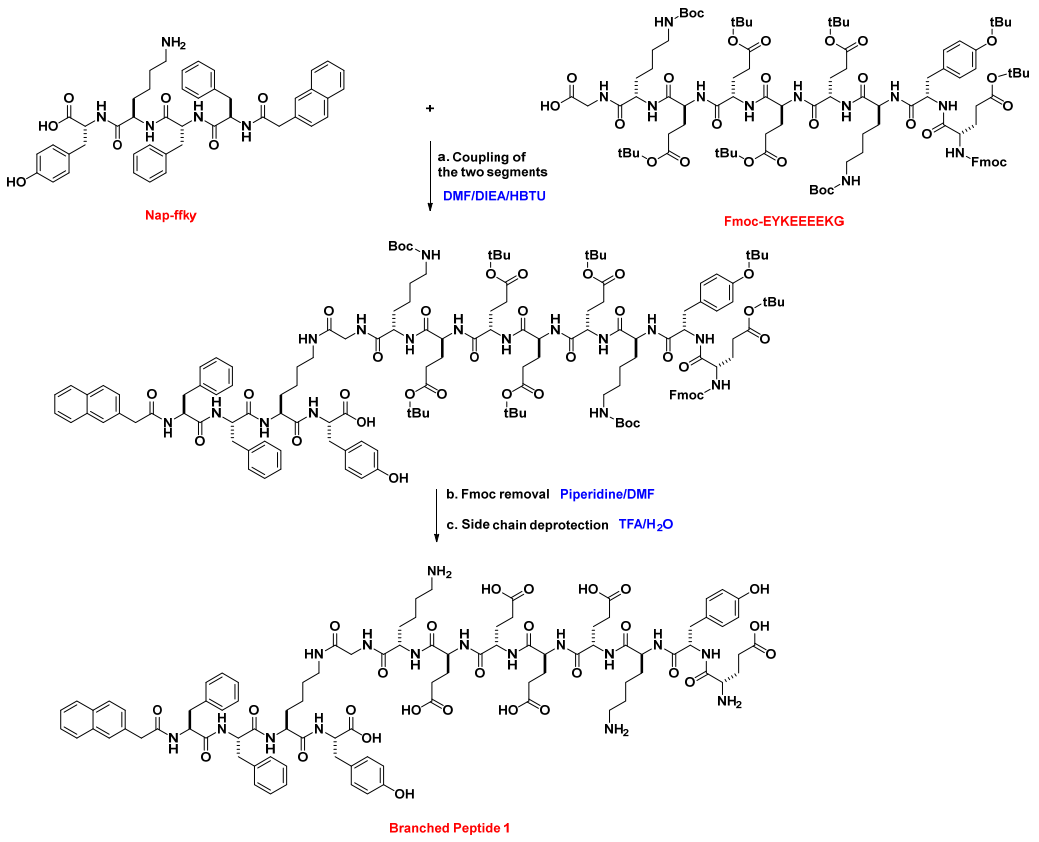

Fig. S3. Synthesis of **1**.

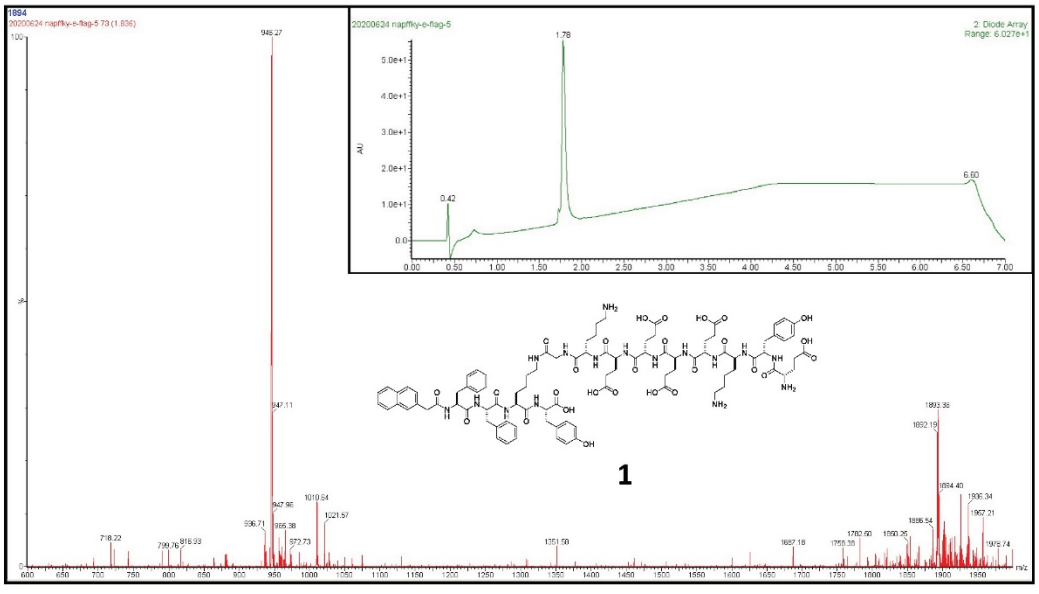

Fig. S4. The LC spectrum of **1** (inset) and its corresponding mass spectrum.

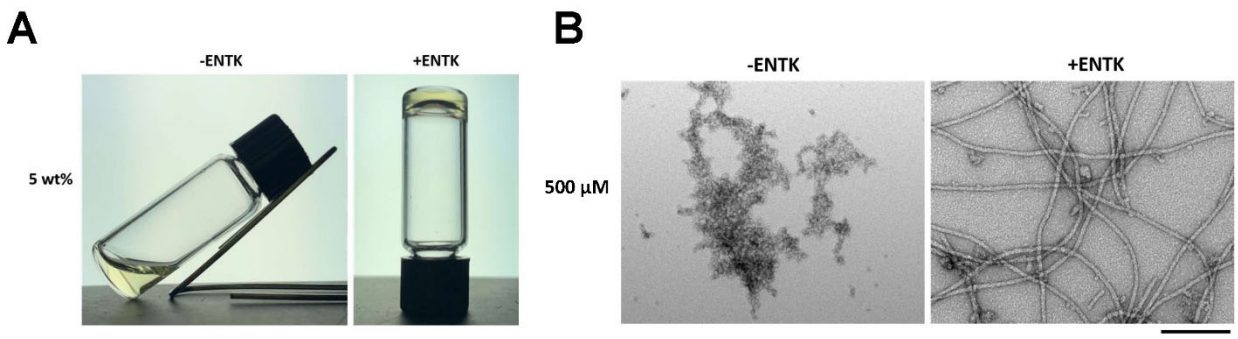

Fig. S5. (A) Gelation of **1** (5 wt%) treated with ENTK (5 U/mL) after 7 days in PBS. (B) TEM images of **1** (500 μM) treated with ENTK (5 U/mL) for 24 hours in PBS. Scale bar: 100 nm.

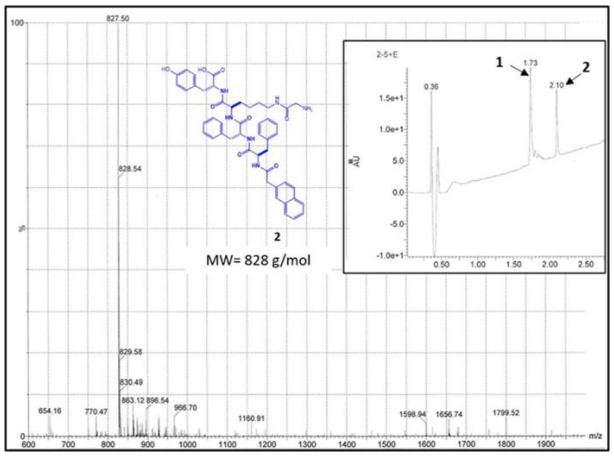

Fig. S6. LC-MS evidence of the cleavage product (**2**, M=828 g/mol) of **1** by ENTK (5 U/ml) after 48 hours at ambient condition.

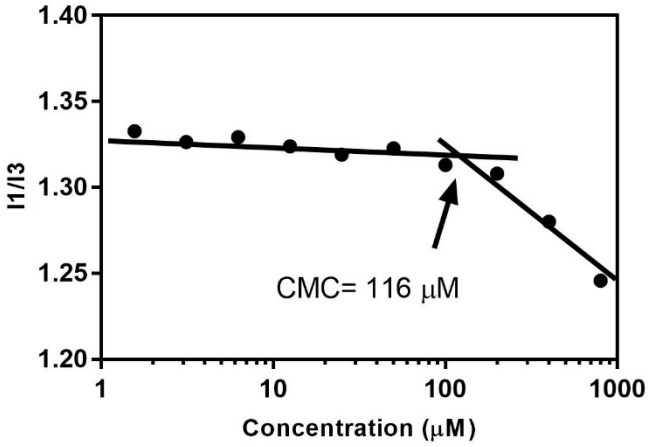

Fig. S7. CMC determination of **1** in PBS (I1 and I3 are the fluorescence intensity ratio of the first (370.4 nm) and the third peaks (380.6 nm) in the emission spectra of pyrene).

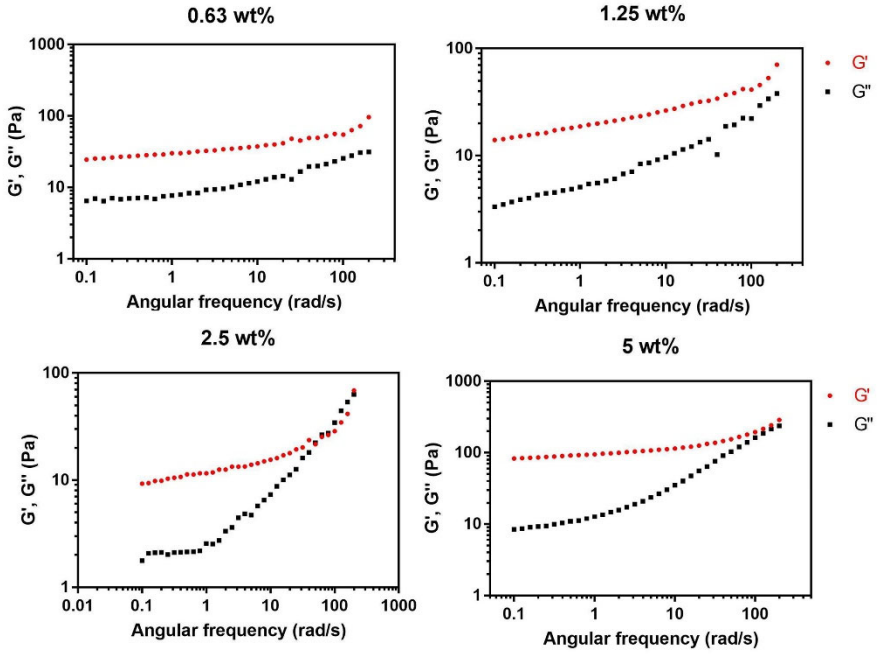

Fig. S8. Frequency sweeps of **1** (0.63 wt%-5 wt%) treated with ENTK (5 U/mL) after 48 h in PBS at the strain of 0.8%.

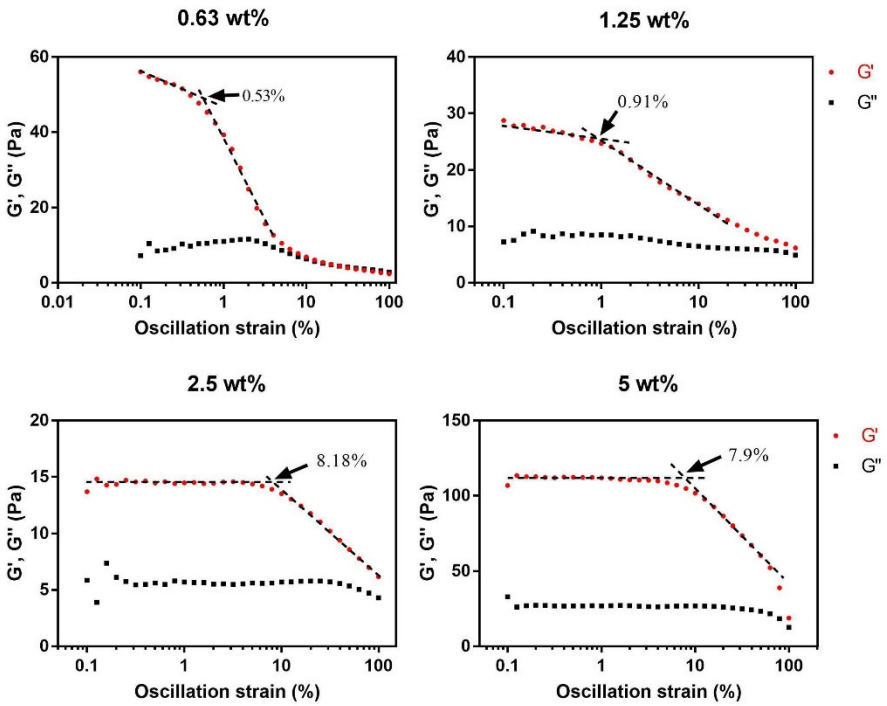

Fig. S9. Strain sweeps of **1** (0.63 wt%-5 wt%) treated with ENTK (5 U/mL) after 48 h in PBS at the frequency of 1 rad/s, critical strains are labelled with arrows.

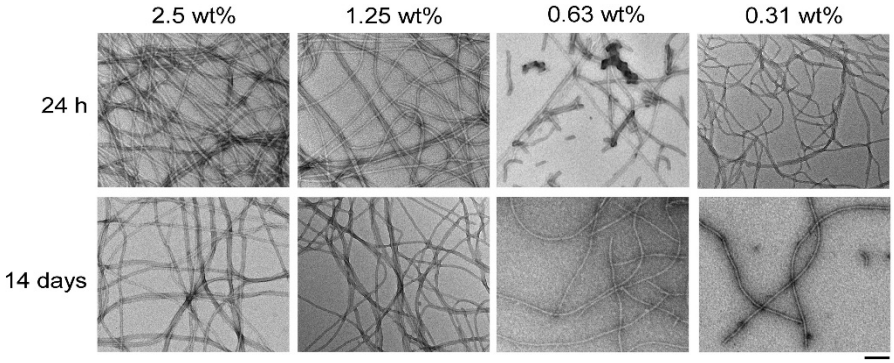

Fig. S10. TEM images of **1** (0.31 wt%-2.5 wt%) after 24 h or 14 days of incubation with ENTK (5 U/mL) in PBS. Scale bar: 100 nm.

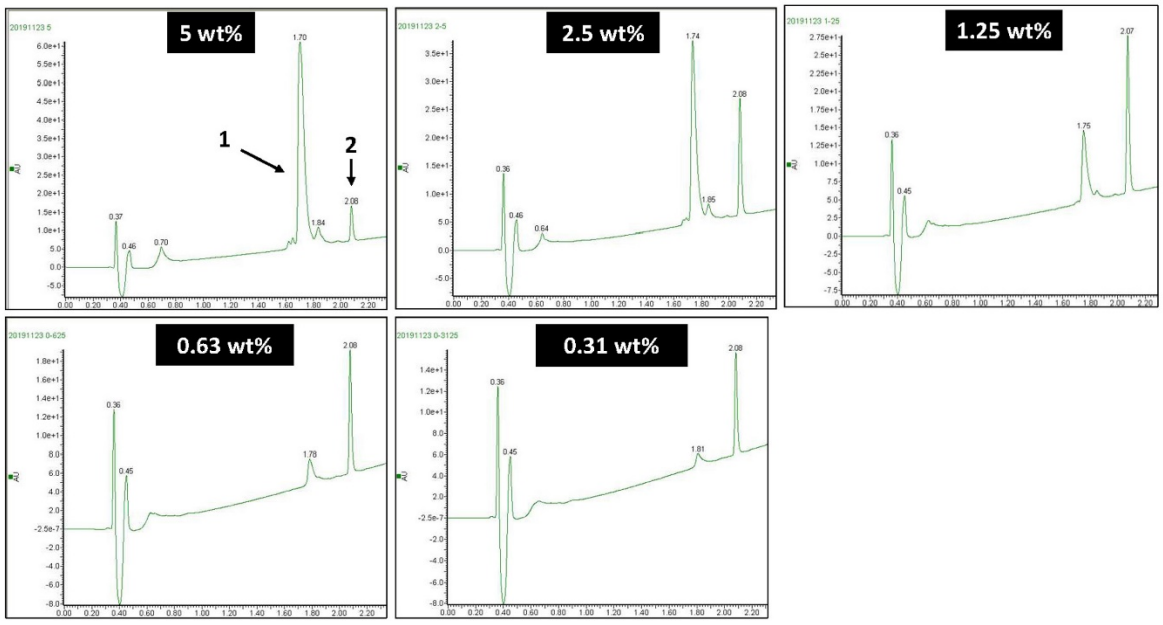

Fig. S11. LC spectra of **1** (0.31 wt%-5 wt%) treated with ENTK (5 U/mL) after 24 hours in PBS.

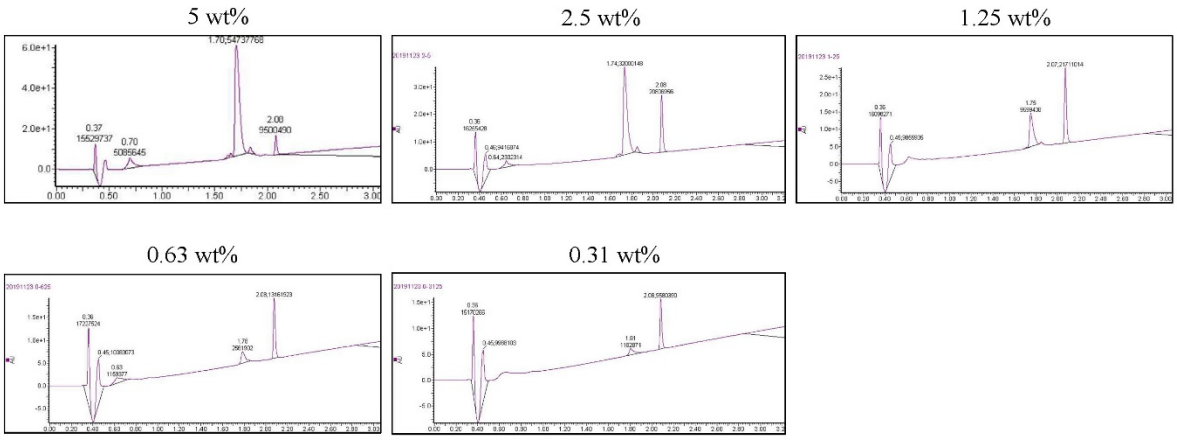

Fig. S12. Peak areas of precursor **1** and hydrogelator **2** (0.31 wt%-5 wt%) treated with ENTK (5 U/mL) after 24 hours in PBS.