Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Electronic Supporting Information

Stimuli-Controlled Peptide Self-assembly with Secondary Structure Transitions and the Application of Drug Release

Liu Yang [a,c], Shenglong Gan [a,c], Qiang Guo [a,c], Huatang Zhang [b*], Qingxin Chen [a,c], Huangxu Li [a,c], Jiahai Shi [d], Hongyan Sun [a,c*]

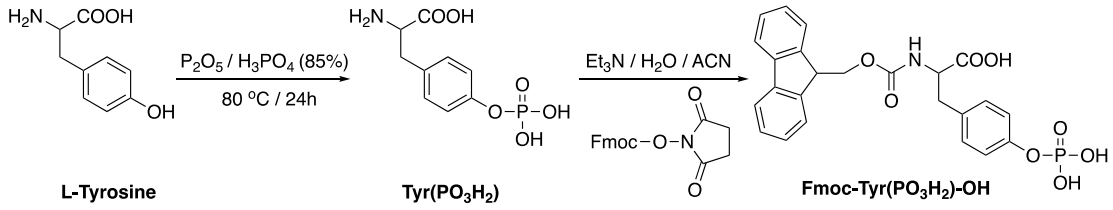
[a] City University of Hong Kong Shenzhen Research Institute, Shenzhen, 518057, China. Email: hongysun@cityu.edu.hk

[b] School of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou, Guangdong 510006, China. Email: htzhang@gdut.edu.cn

[c] Department of Chemistry and COSADAF (Centre of Super-Diamond and Advanced Films), City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China.

[d] Department of Biomedical Sciences, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China

1. Synthetic route of Fmoc-Tyr (PO_3H_2)-OH



Scheme S1. Synthetic route of Fmoc-Tyr(PO_3H_2)-OH

1.1 Synthesis of Tyr(PO_3H_2)

Phosphorous pentoxide (80 mmol, 11.36g) was added to 7.74 mL of 85% phosphoric acid and stirred until the reaction mixture reached room temperature. L-tyrosine (20 mmol, 3.6g) was then added to the reaction mixture and stirred at 80 °C under N_2 atmosphere. After reacting for 24h, 30 mL of distilled water was added to the reaction solution and the reaction was continuously heated for another 30 min. Subsequently, the mixture was cooled to room temperature, diluted with 650 mL of n-butanol and incubated in a refrigerator overnight. The fine white product was collected by filtration, followed by washing with water (20 mL × 2), ethanol (20 mL × 2) and ether (20 mL × 4). After drying under vacuum, 4.28 g of white product could be obtained (yield 82%).

1.2 Synthesis of Fmoc-Tyr (PO_3H_2)-OH

N-(9-Fluorenylmethoxycarbonyloxy) succinimide (Fmoc-OSu, 2.4 mmol, 812 mg) and Tyr (PO_3H_2) (2 mmol, 520 mg) were dissolved in a solution of 5 mL of water and 5 mL of acetonitrile. The solution was stirred at 25 °C for 30 min. Triethylamine (2 mmol, 285 μL) was then added dropwise to the reaction mixture over 20 min. The resulting homogeneous solution was concentrated under vacuum, followed by addition of 50 mL of ethyl acetate (EA) and 50 mL of distilled water. The mixture was then acidified to *p*H 2 with 12 M HCl (aq). Next, the EA layer was separated, and the aqueous phase was further extracted with EA (50 mL × 2). The organic phases were combined and washed with 1 M HCl (aq) (40 mL × 2), water (40 mL × 2) and saturated aqueous NaCl

(40 mL × 2). The organic phase was then filtrated and dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure, and the solid product (396 mg, yield 41%) was collected without further purification.

1.3 Synthesis of P1 (Fmoc-KKY$_p$Y$_p$-COOH) and P2 (Fmoc-KKYY-COOH)

P1 and **P2** were synthesized by standard solid phase peptide synthesis (SPPS) method with 2-Cl-trityl chloride resin.

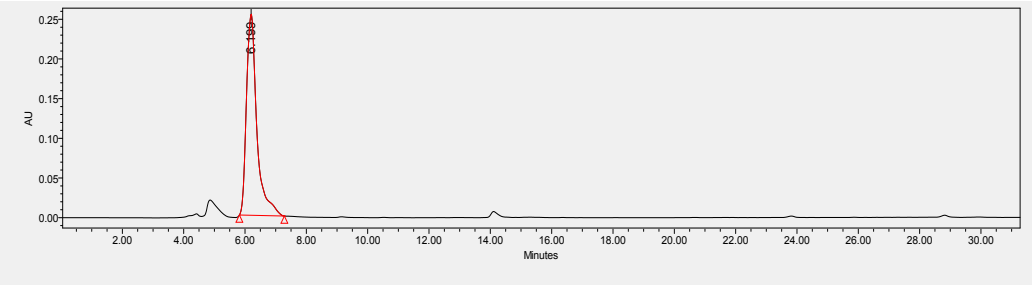


Fig. S1 HPLC spectrum of peptide **P1** (Fmoc-KKYpYp-COOH), 254 nm channel.

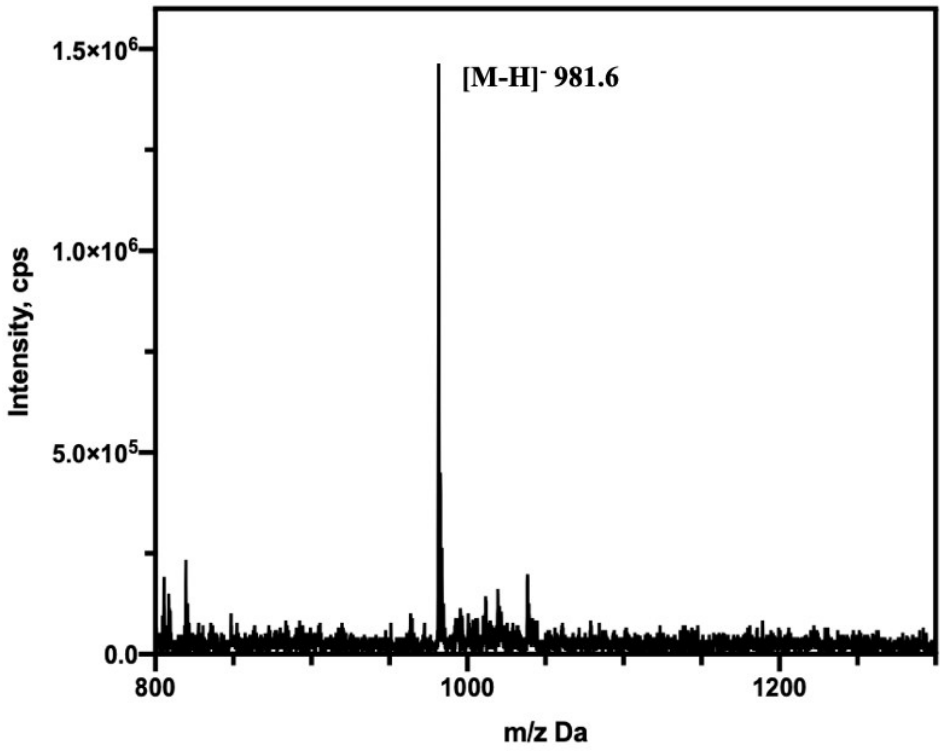


Fig. S2 ESI-MS spectrum of **P1** (Fmoc-KKYpYp-COOH). $[M-H]^-$ calculated for

$C_{45}H_{56}N_6O_{15}P_2$: 981.33; Found 981.6.

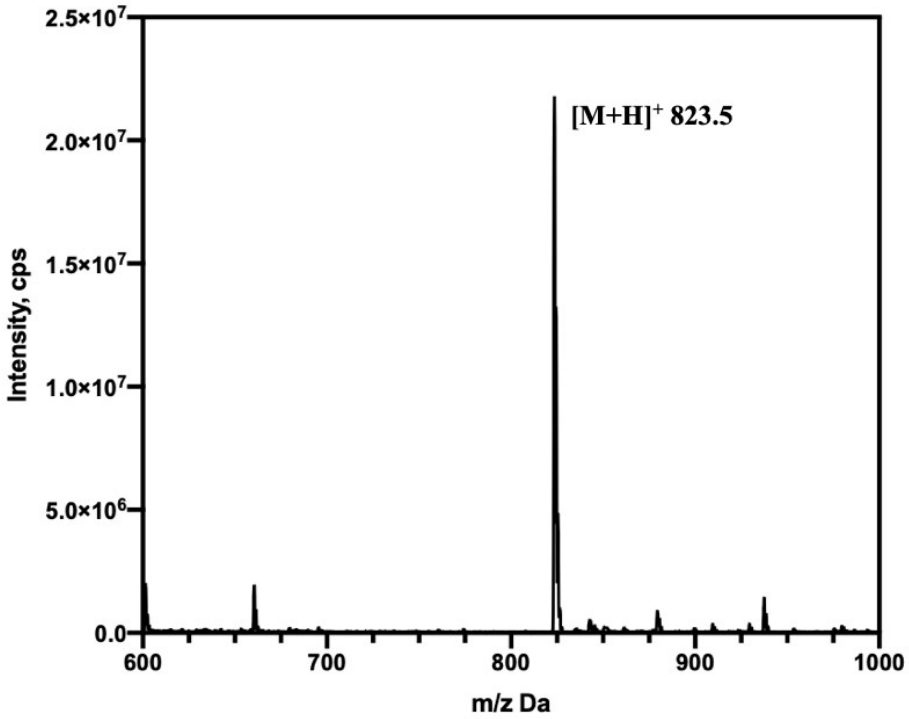


Fig. S3 ESI-MS spectrum of **P2** (Fmoc-KKYY-COOH). $[M+H]^+$ calculated for $C_{45}H_{54}N_6O_9$: 823.40; Found 823.5.

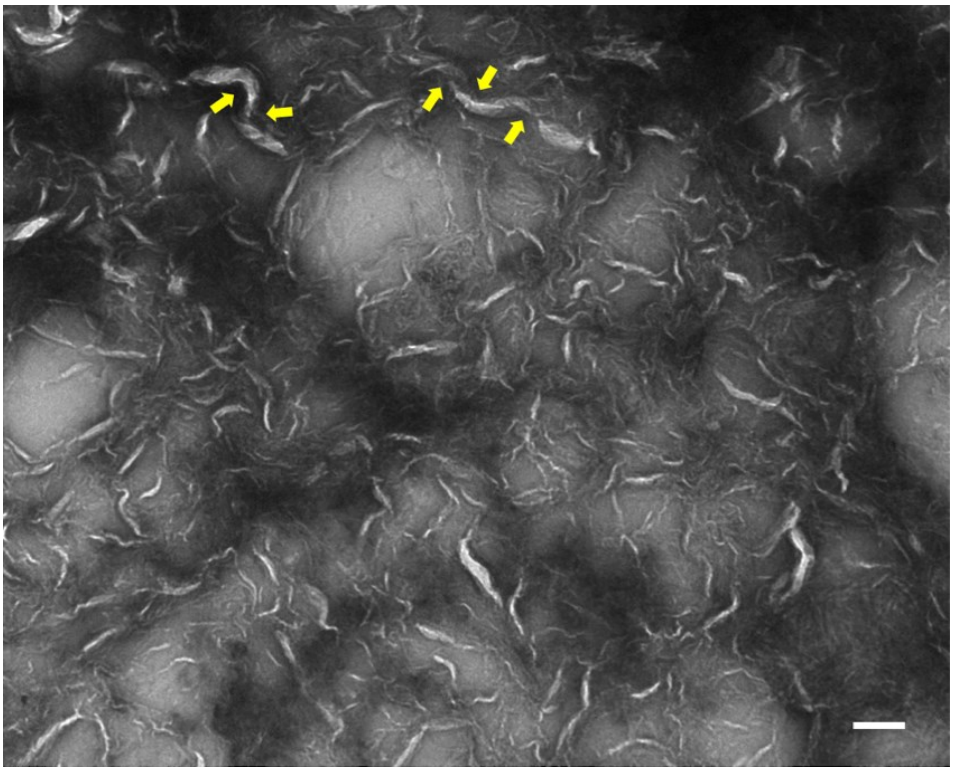


Fig. S4 The zoomed in TEM imaging of the hydrogel of 1% wt (10 mM) P1 triggered by 1U/mL ALP in Fig. 2a (scale bar = 100 nm).

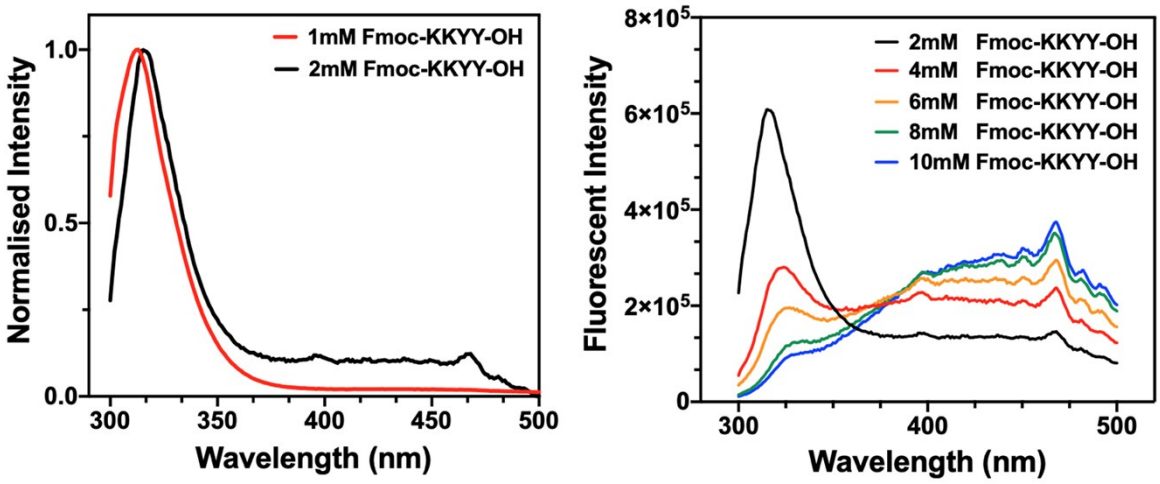


Fig.S5 Fluorescent emission spectra of different concentrations of **P2** in water.

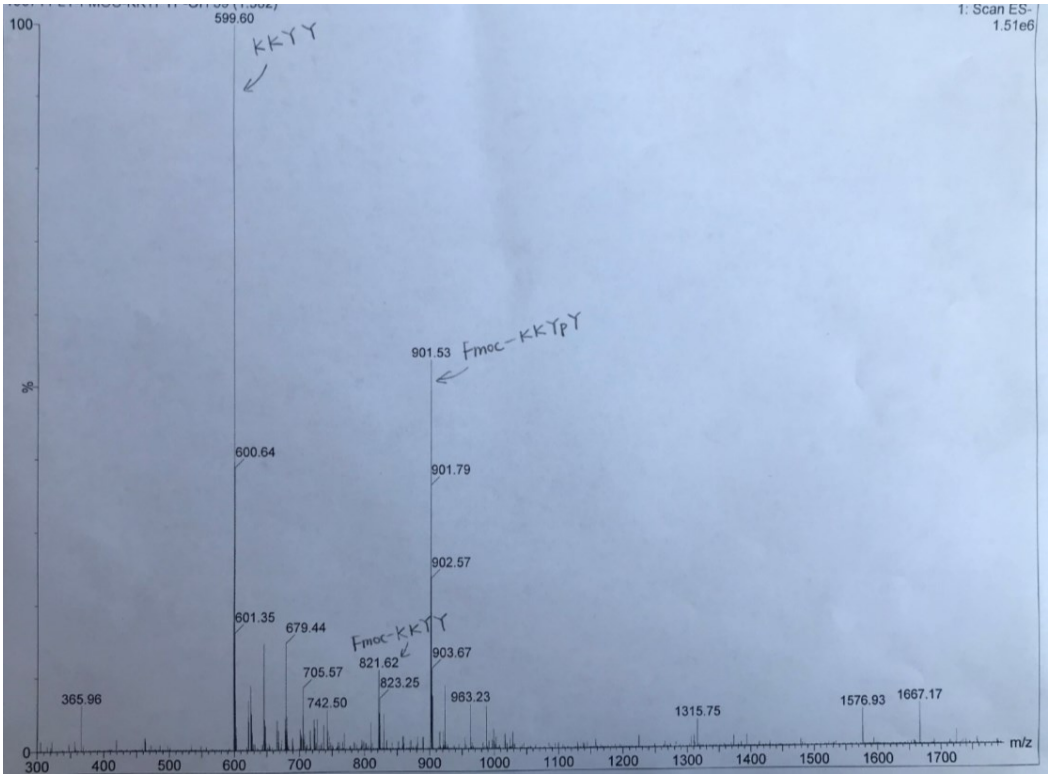


Fig. S6 LC-MS analysis of the reaction of **P1** (1 wt%) with ALP (1 U/mL) in distilled water for 30 min.

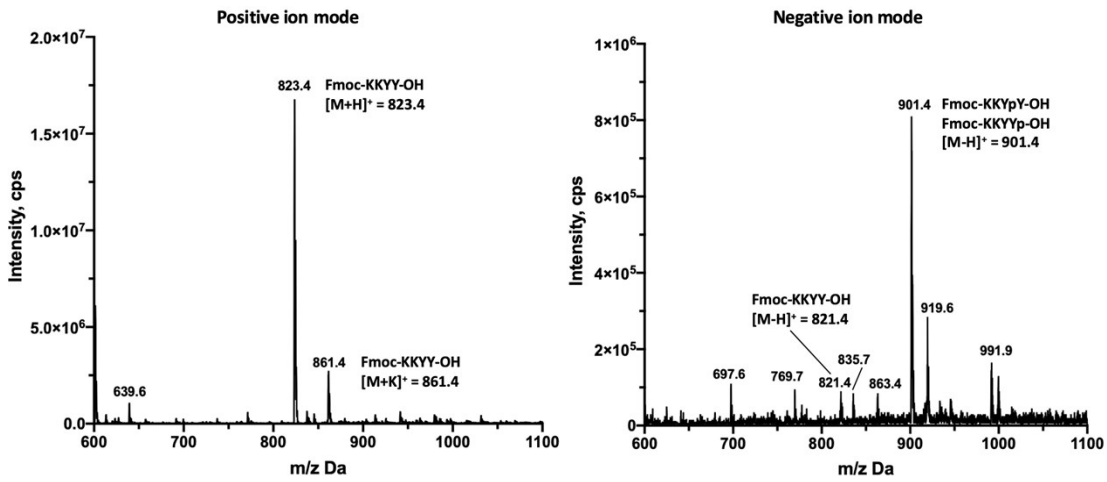


Fig. S7 The mass spectra of the reaction solution of **P1** (1.0% wt, 10 mM) with ALP (1U/mL) for 30 min at room temperature.

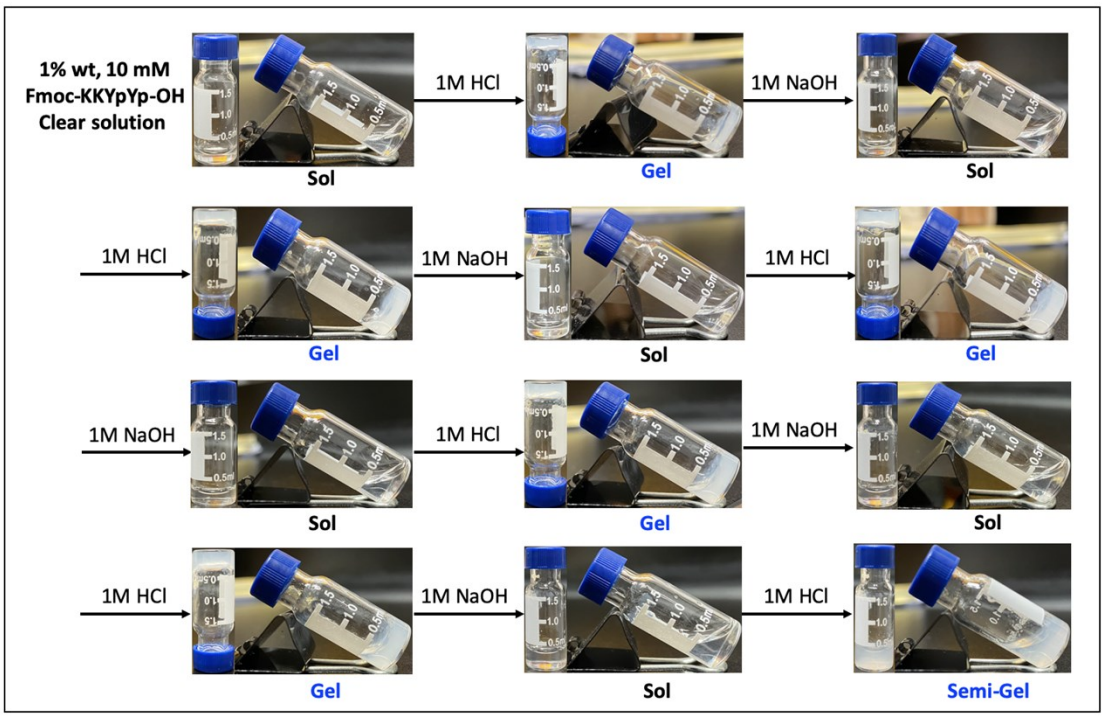


Fig. S8 The transition between the clear solution and solid-like hydrogel of peptide **P1** (1.0% wt, 10 mM) modulated by pH values.

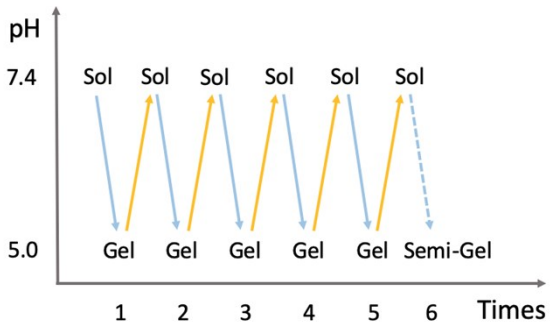


Fig. S9 The illustration of the reversibility of the pH-Gel modulated by pH.

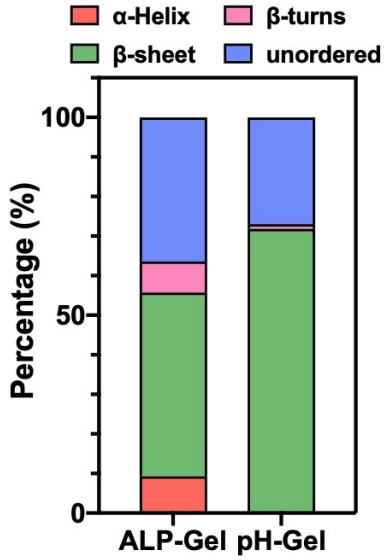


Fig. S10 The percentage of secondary structures in the two gels analyzed via CD.

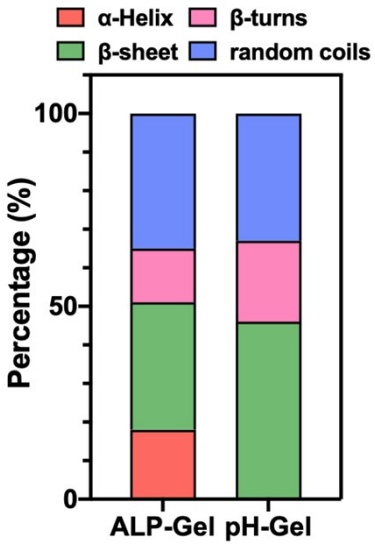


Fig. S11 The percentage of secondary structures in the two gels analyzed via FT-IR.

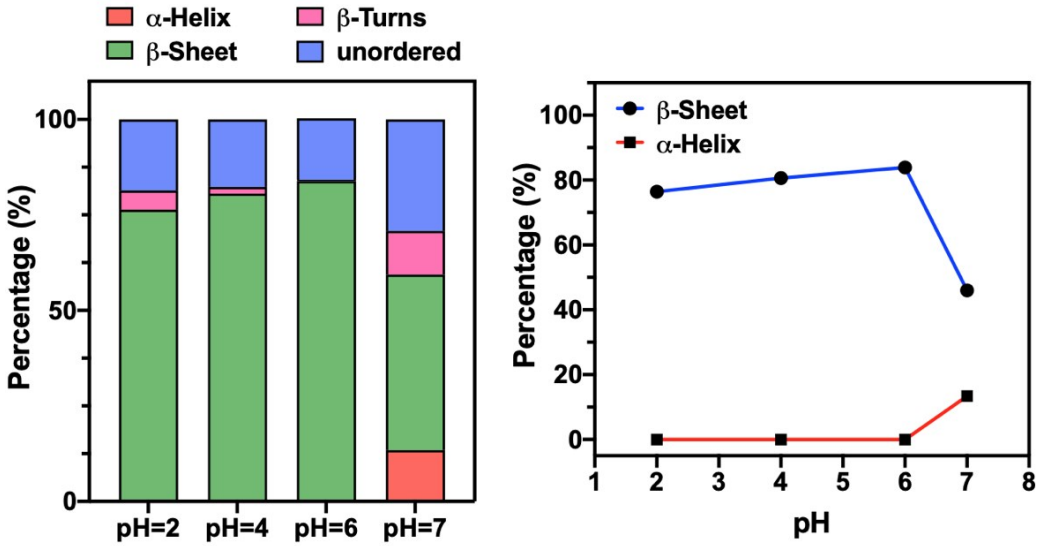


Fig. S12 (a) The percentage of secondary structures of 5 mM **P2** (Fmoc-KKYY-COOH) under different pH; (b) Corresponding percentage of α-helix and β-sheet structure under different pH.

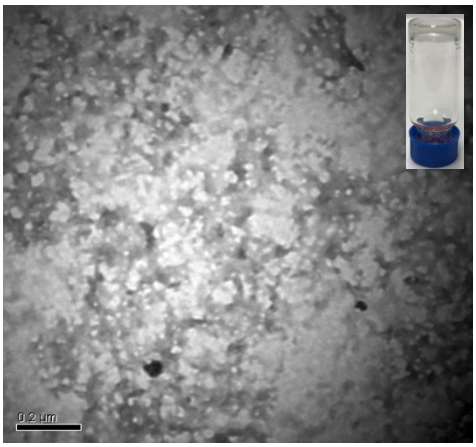


Fig. S13 TEM imaging of hydrogel formed by 1 wt% P1 co-assembled with 100 μM Ep (inset is the optical image) under the treatment with 1 U/mL ALP. Scale bar is 200 nm.

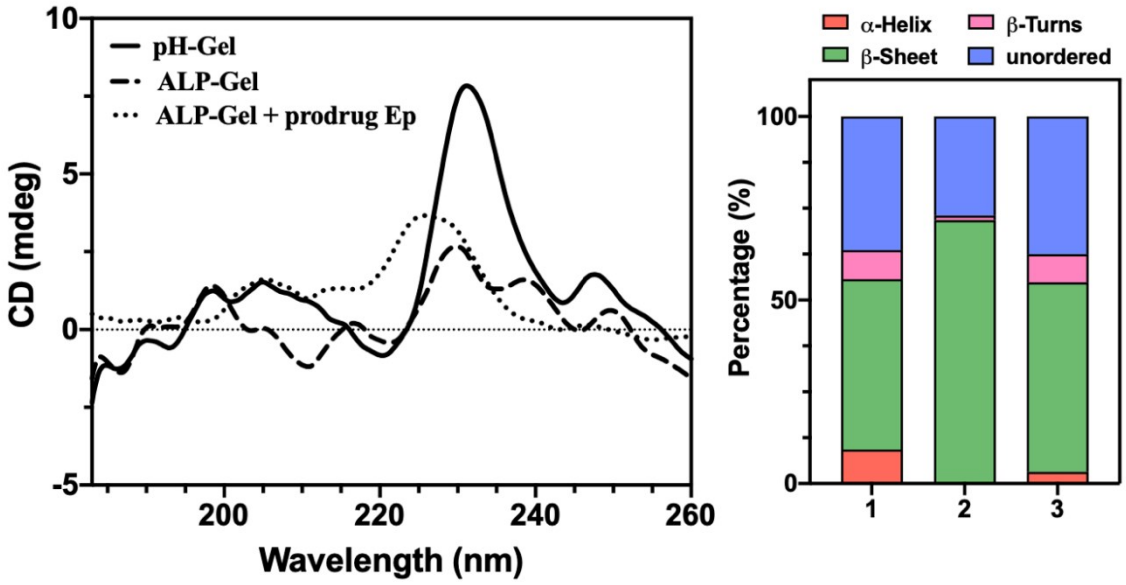


Fig. S14 (a) CD spectra of the solution of pH-Gel (1 wt%), ALP-Gel (1 wt%, with 1 U/mL ALP) and ALP-Gel (1 wt%, with 1 U/mL ALP) co-incubated with prodrug Ep (100 μM) in distilled water. (b) The corresponding secondary structure composition analysis of the three gels (1 for ALP-Gel, 2 for pH-Gel and 3 for ALP-Gel with Ep).

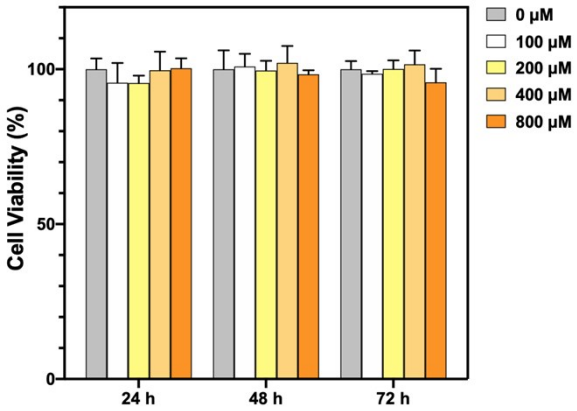


Fig. S15 CCK8 viability assay of HeLa cells incubated with different concentrations (0/ 100/ 200/ 400/ 800 μM) of Fmoc-KKYpYp-COOH (**P1**) for 24/ 48/ 72 h, respectively.

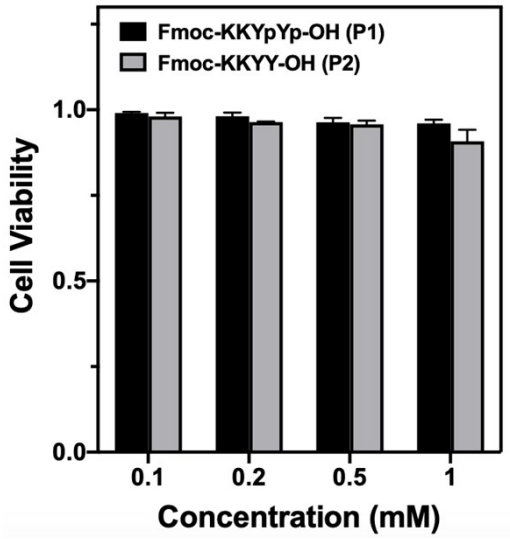


Fig. S16 CCK8 viability assay of HeLa cells incubated with different concentrations of Fmoc-KKYpYp-COOH (**P1**) and Fmoc-KKYY-COOH (**P2**) for 24 h. The concentrations of **P1** and **P2** are 0.1, 0.2, 0.5 and 1 mM, respectively.

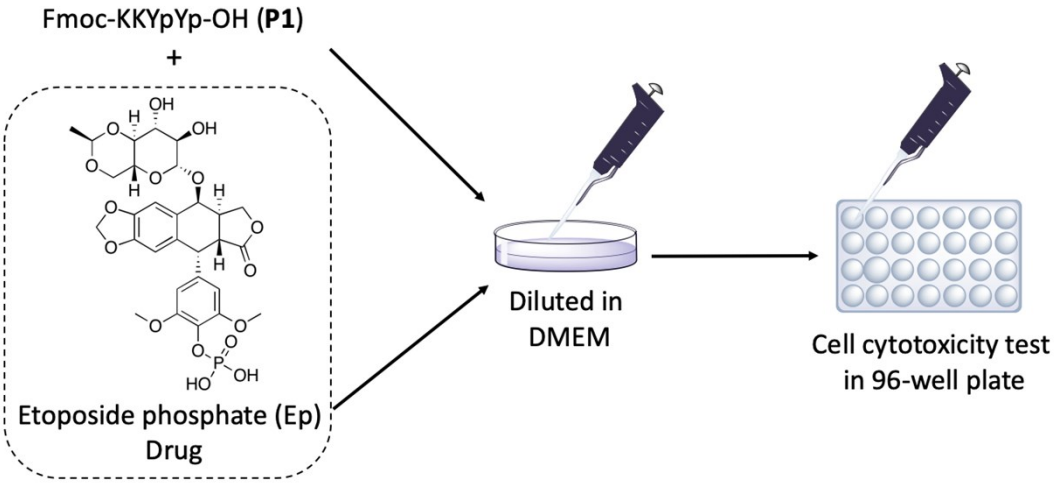


Fig. S17 Illustration of the processes for cell cytotoxicity test of **P1** co-incubated with Ep.

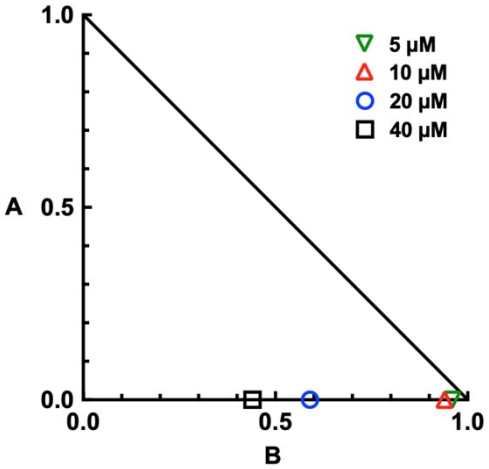


Fig. S18 Normalized isobologram for the non-constant combination of **P1** (400 μM) and Ep (5/ 10/ 20/ 40 μM, respectively).

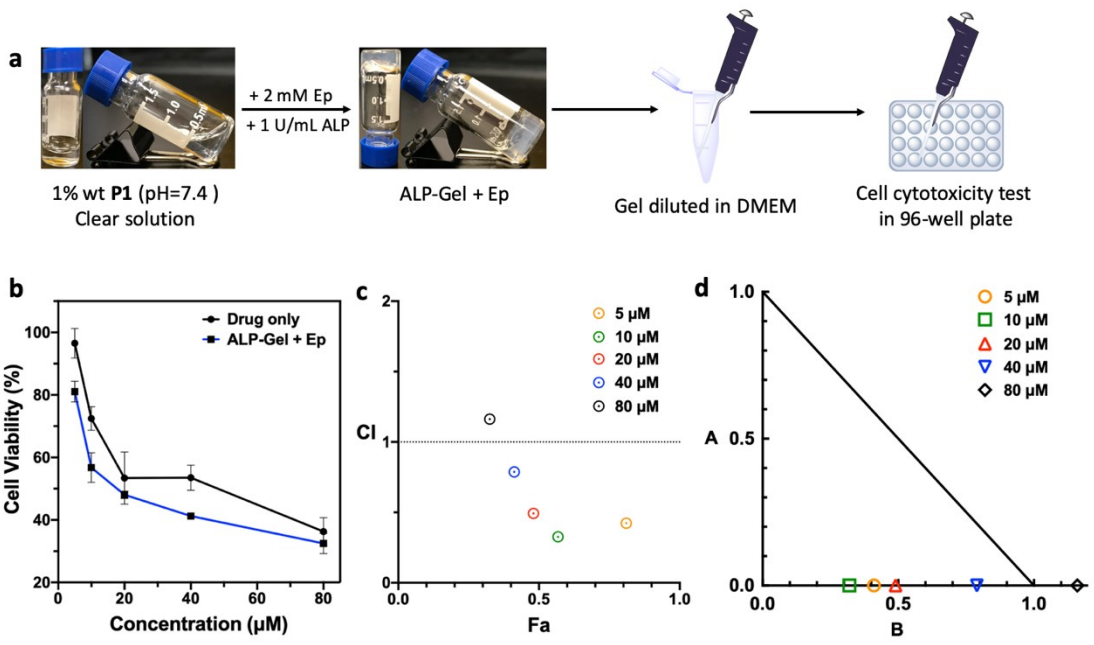


Fig. S19 (a) Illustration of the processes for cell cytotoxicity test of ALP-Gel + Ep (co-assembly of 1 wt% **P1** with 2 mM Ep under the ALP treatment); (b) cell viability of Ep (5-80 μM) only or ALP-Gel + Ep after 72 h incubation; (c) combination index plot of **P1** with Ep for 72 h; (d) Normalized isobologram for the constant combination of **P1** (25.5/ 51/ 102/ 204/ 408 μM) and Ep (5/ 10/ 20/ 40/ 80 μM, respectively).

Table S1. CI values for non-constant combination of P1 and Ep

Dose (P1/μM)	Dose (Ep/μM)	Effect	CI
400.0	5.0	0.703	0.96
400.0	10.0	0.633	0.93
400.0	20.0	0.511	0.59
400.0	40.0	0.402	0.43

Table S2. CI values for constant combination of **P1** and Ep

Dose (P1/μM)	Dose (Ep/μM)	Effect	CI
25.5	5.0	0.812	0.42
51.0	10.0	0.567	0.32
102.0	20.0	0.481	0.49
204.0	40.0	0.412	0.78
408.0	80.0	0.325	1.16