Electronic Supplementary Information

Fluorine substitution enhances the self-assembling ability of hydrogelators

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1. Methods

Experimental materials and instruments

All the starting materials were obtained from Adamas, Sangon Biotech or Bachem. Commercially available reagents were used without further purification, unless noted otherwise. All other chemicals were reagent grade or better. Recombinant Intestinal Alkaline Phosphatase (ALP), was obtained from BaoMan Inc. (Shanghai, China) (one unit is the enzyme activity that cleaves 1 µmol of the standard substrate per minute at 37 °C). The phosphatase inhibitor complex II was bought from Sangong Biotech Inc. (Shanghai, China) (every 10 µL ALP inhibitor complex II in culture medium containing 1×10^7 cells). LoVo cells were kindly supplied by Prof. Yangzhong Liu's lab at USTC, routinely cultured in Dul-becco's modified Eagle's medium at 37 °C, 5% CO₂, and humid atmosphere. HeLa cells and NIH3T3 cells were routinely cultured in Dul-becco's modified Eagle's medium at 37 °C, 5% CO₂, and humid atmosphere. HPLC analyses were performed on an Agilent 1200 HPLC system equipped with a G1322A pump and in-line diode array UV detector using an Agilent Zorbax 300SB-C18 RP column with CH₃CN (0.1% of trifluoroacetic acid (TFA)) and water (0.1% of TFA) as the eluent. The spectra of electrospray ionization-mass spectrometry (ESI-MS) were operated on a LTQ Advantage MAX ion trap mass spectrometer (Thermo Fisher). ¹H NMR and ¹³C NMR spectra were obtained on a 300 MHz Bruker AV 300. Rheological measurements were performed on a Haake RheoStress 6000 (Thermo Scientific), with cone-and-plate geometry (1 deg/20 mm) at the gap of 50 µm. Cryo transmission electron microscopy (cryo-TEM) images were obtained on a Tecnai F20 transmission electron microscope from FEI company, operating at 200 kV. Negative staining TEM images were obtained on a Tecnai F12 transmission electron microscope from FEI company, operating at 120 kV. Fluorescence spectra were obtained on a Hitachi FL-4600 fluorescence spectrophotometer. MTT results were recorded on an IEMS Analyzer (Lab-system, Type 1401). Ultrapure water (18.2 M Ω •cm) was used throughout the experiment.

General procedures for hydrogel preparation

Hydrogelators 1, 2, or 3 (4 mg for each) was dissolved in 400 μ L phosphate buffer (0.2 M, pH 7.4) and heated to the certain temperature, cooled down to room temperature to form the physical hydrogel. Precursors 1P, 2P, or 3P (4 mg for each) was dissolved in 400 μ L phosphate buffer (0.2 M, pH 7.4), added with 80 units of ALP and incubated at 37 °C for 12 h to form the enzymatic hydrogel.

Cell culture

All cell lines were routinely culcured in Dul-becco's modified Eagle's medium(DMEM) (HyClone, Thermo Fisher Scientific, United States) supplemented with 10% fetal bovine serum (FBS) at 37 °C, 5% CO₂ and humid atmosphere. The medium was changed every other day.

MTT assay

The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay with all cell lines (LoVo, HeLa, and NIH3T3 cells) was used to measure the cytotoxicity. Cells growing in log phase were seeded into 96-well cell-culture plate at 2.5×10^3 /well. The cells were incubated at 37 °C under 5% CO₂. The solutions of compounds diluted by DMEM (100 µL/well) at concentrations of 62.5, 125, 250 or 500 µM in 100 µL medium were added to the wells, respectively. For inhibition test, LoVo cells were pretreated with ALP inhibitor complex II (every 10 µL ALP inhibitor complex II in culture medium containing 10^7 cells) for 1 h before incubation with the hydrogelator precursors (**1P**, **2P**, **3P**). The cells were incubated for 24, 48, or 72 h at 37 °C under 5% CO₂. A solution of 5 mg/mL MTT dissolved in phosphate buffered saline (PBS) (pH 7.4) (10 µL/well) was added to each well of the 96-well plate. A solution of DMSO (100 µL/well) was added to dissolve the formazan after an additional 10-minute shaking. The data were obtained using an ELISA reader (VARIOSKAN FLASH) to detect its absorption at 490 nm. Each of the experiments was performed at least three times.

Cell experiment for negative staining

LoVo cells were routinely cultured in Dul-becco's modified Eagle's medium (DMEM, Hycolon) supplemented with 10% fetal bovine serum at 37 °C, 5% CO₂, and humid atmosphere. Cells growing in log phase were seeded into 24-well cell-culture plate at 2×10^5 /well. LoVo cells were pretreated with ALP inhibitor complex II (every 10 µL ALP inhibitor complex II in culture medium containing 10^7 cells) for 1 h before incubation with the hydrogelator precursor **2P** for the control experiment.

Pericellular hydrogelation

 2×10^5 of the cells in exponential growth phase were seeded in a 24-well cell-culture plate with 1 mL complete culture medium. After overnight incubation, the medium was replaced by 800 µL of medium containing hydrogelator precursors (**1P**, **2P**, or **3P**) diluted by DMEM at concentration of 500 µM. After incubation at 37 °C for 24 h, the 24 hole plate was taken out from incubator and tilted

on bench to visualize the pericellular hydrogel. In the co-incubation of phosphatase inhibitors, the cells were pretreated with ALP inhibitor complex II (10 μ L ALP inhibitor complex II in culture medium containing 10⁷ cells) for 1 h before incubation with the hydrogelator precursor.

Negative staining TEM

Carbon coated copper grids were glow-discharged to increase their hydrophilicity before use. After obtain pericellular hydrogel/nanofibrils by incubating the hydrogelator precursor (**1P**, **2P**, or **3P**) at 500 μ M with LoVo cells for 24 h, the medium was removed to expose the pericellular hydrogel. The carbon-coated side of the grid was gently pressed onto the pericellular hydrogel for 1s, and then the sample-loaded surface was washed by touching 3 μ L ddH₂O. The edge of the grid was immediately leaned to touch a filter paper for three times to remove water from the grid. The grid was stained by letting the grid touch a drop of 2.0 % (w/v) uranyl acetate with the sample-loaded surface. Excessive stain solution was removed by gently touching the grid with a drop of ddH₂O then dried by touching the edge of the grid with a filter paper for 3 times. The grid was air dried for a few minutes and was then examined immediately.

2. Syntheses and characterizations of compounds

Solid phase peptide synthesis (SPPS) was used to prepare the compounds and their tBu groups were deprotected with dichloromethane (DCM, 300 μ L) and triisopropylsilane (TIPS, 200 μ L) in TFA (9.5 mL) for 3 h, purified with HPLC using water-acetonitrile added with 0.1% TFA as the eluent. Compounds **2**, **2P**, **3**, and **3P** were synthesized following the literatures.^{1,2} The following scheme illustrates the synthetic procedure for **1** and **1P**.



Scheme S1. The synthetic route for 1 and 1P.

Charecterization of 1. ¹H NMR of compound **1** (d_6 -DMSO, 300 MHz, Figure S1) δ (ppm) : 12.37 (s, 2 H), 9.16 (s, 1 H), 8.26 (d, J = 8.1 Hz, 1 H), 8.25 (d, J = 8.3 Hz, 1 H), 8.17 (d, J = 8.1 Hz, 1 H), 8.09 (d, J = 7.9 Hz, 1 H), 7.85 (d, J = 7.9 Hz, 1 H), 7.75 (s, 1 H), 7.72 (s, 1 H), 7.58 (s, 1 H), 7.52 – 7.30 (m, 2 H), 7.22 – 7.14 (m, 11 H), 7.04 (s, 1 H), 7.02 (s, 1 H), 6.62 (s, 1 H), 6.60 (s, 1 H), 4.61-4.48 (m, 3 H), 4.29 (m, 1 H), 4.18 (m, 1 H), 3.52 (m, 2 H), 2.89 (m, 2 H), 2.68 (m, 2 H), 2.21 (m, 2 H), 1.90-1.68 (m, 3 H), 1.44-1.13 (m, 2 H), 0.86-0.80 (m, 6 H). ¹³C NMR of **1** (75 MHz, d_6 -DMSO, Figure S2) δ (ppm) : 174.0, 172.7, 171.1, 171.1, 170.6, 169.7, 155.7, 137.7, 137.6, 133.8, 132.8, 131.6, 130.0, 129.2, 129.1, 127.9, 127.8, 127.5, 127.3, 127.3, 127.1, 126.1, 126.0, 125.9, 125.3, 114.7, 66.54, 56.23, 53.69, 53.60, 51.64, 42.15, 37.45, 37.20, 36.52, 36.36, 30.66, 27.87, 24.60, 23.34, 15.45, 11.22. MS (Figure S3): calculated for **1** (C₅₀H₅₄N₅O₁₀) [(M-H)⁻]: 884.3871; obsvd. HR-ESI/MS: m/z 884.3886.

Charecterization of 1P. ¹H NMR of compound **1P** (d_6 -DMSO, 300 MHz, Figure S4) δ (ppm) :

8.29 (S, 1 H), 8.26 (S, 1 H), 8.17 (d, J = 3.2 Hz, 1 H), 8.14 (d, J = 4.1 Hz, 1 H), 8.05 (d, J = 7.7 Hz, 1 H), 7.84 (d, J = 8.8 Hz, 1 H), 7.74 (d, J = 8.7 Hz, 1 H), 7.58 (s, 1 H), 7.48 (m, 1 H), 7.38-7.14 (m, 13 H), 7.04 (d, J = 5.1 Hz, 1 H), 7.02 (s, 1 H), 4.66-4.50 (m, 3 H), 4.35-4.27 (m, 1 H), 4.22-4.16 (m, 1 H), 3.62-3.42 (m, 2 H), 3.09-2.68 (m, 6 H), 2.22 (m, 2 H), 1.90-1.68 (m, 3 H), 1.44-1.15 (m, 2 H), 0.86-0.80 (m, 6 H). ¹³C NMR of **1P** (75 MHz, d_6 -DMSO, Figure S5) δ (ppm) : 174.0, 172.7, 171.2, 170.8, 170.8, 170.4, 169.7, 150.0, 137.7, 133.8, 133.0, 132.9, 131.9, 130.7, 130.1, 129.2, 128.2, 126.9, 126.6, 126.4, 126.3, 126.2, 125.1, 125.0, 124.4, 132.9, 131.7, 130.2, 130.0, 129.1, 127.8, 127.5, 127.4, 127.3, 127.2, 126.1, 125.9, 125.4, 124.7, 119.6, 57.95, 55.37, 54.83, 52.72, 52.55, 51.84, 51.25, 41.23, 35.41, 30.94, 29.51, 28.46, 24.07, 14.53, 10.28. MS (Figure S6): calculated for **1P** ($C_{50}H_{55}N_5O_{12}P$) [(M-H)⁻]: 964.3534; obsvd. HR-ESI/MS: *m/z* 964.3551.



Figure S1. ¹H NMR spectrum of compound 1 in d_6 -DMSO.



Figure S2. ¹³C NMR spectrum of compound **1** in d_6 -DMSO.



Figure S3. HR-ESI-TOF/MS spectrum of **1**.



Figure S4. ¹H NMR spectrum of compound **1P** in d_6 -DMSO.



Figure S5. ¹³C NMR spectrum of compound **1P** in d_6 -DMSO.



Figure S6. HR-ESI-TOF/MS spectrum of 1P.

3. Supporting Figures and Tables



Figure S7. HPLC traces for Gel **1** at 1.0 wt% (black), solution **1P** at 1.0 wt% treated with 200 U/mL ALP at 37 °C for 12 h (red), solution **1P** at 1.0 wt% (blue). Wavelength for detection: 268 nm.



Figure S8. HPLC traces for Gel 2 at 1.0 wt% (black), solution 2P at 1.0 wt% treated with 200 U/mL

ALP at 37 °C for 12 h (red), solution 2P at 1.0 wt% (blue). Wavelength for detection: 268 nm.



Figure S9. HPLC traces for Gel **3** at 1.0 wt% (blue), solution **3P** at 1.0 wt% treated with 200 U/mL ALP at 37 °C for 12 h (black), solution **3P** at 1.0 wt% (red). Wavelength for detection: 268 nm.



Figure S10. Strain dependence of the dynamic storage moduli (G') and the loss moduli (G'') of the hydrogels at 1 wt% for Gel 1, Gel 1P, Gel 2, Gel 2P, Gel 3, and Gel 3P. All rheological measurements were conducted at 25 °C and frequency of 1.0 Hz.



Figure S11. Gelator concentration-dependent fluorescence emission maximum of dilutions of Gel **1** (A), Gel **2** (B), and Gel **3** (C). Excitation: 265 nm.



Figure S12. MTT assay of the six compounds (1, 1P, 2, 2P, 3, and 3P) on LoVo cells.



Figure S13. MTT assay of the six compounds (1, 1P, 2, 2P, 3, and 3P) on NIH3T3 cells.



Figure S14. MTT assay of the six compounds (1, 1P, 2, 2P, 3, and 3P) on HeLa cells.



Figure S15. IC₅₀ values of the six compounds on NIH3T3 cells for 72 h in Figure S13.



Figure S16. IC₅₀ values of the six compounds on HeLa cells for 72 h in Figure S14.



Figure S17. Enzymatic formation of the hydrogels on the cells. Optical images of the LoVo cells incubated with 500 μ M hydrogelator precursors **1P** (a), **2P** (b), **3P** (c), or without hydrogelator precursor (d) for 24 h. White arrows point at the hydrogel.



Figure S18. Negative stained TEM images of the pericellular hydrogels on the LoVo cells treated by hydrogelator precursors (**1P**, **2P**, or **3P**) at 500 μ M. Scale bar, 200 nm.



Figure S19. MTT assay of the three hydrogelator precursors (**1P**, **2P**, and **3P**) on LoVo cells in the presence/absence of ALP inhibitor complex II for 72 h.

Table S1. Conditions for the hydrogelators (1, 2, and 3) and their corresponding precursors (1P, 2P, and 3P) to form supramolecular hydrogels.

Compound	Conc.(wt%)	Temp.(°C)	рН	Enzyme (amount)	Fibers' $\phi(nm)$	Incubation time
1	1.0	60 ^{<i>a</i>}	7.4	_	2.85±0.59	-
1P→1	1.0	37	7.4	ALP (200U/mL)	3.14±0.37	12 h
2	1.0	50 ^{<i>a</i>}	7.4	_	2.88±0.38	_
2P→2	1.0	37	7.4	ALP (200U/mL)	2.74±0.16	12 h
3	1.0	80^a	7.4	_	2.80±0.25	_
3P→3	1.0	37	7.4	ALP (200U/mL)	2.94±0.30	12 h

^aThe temperatures at which the hydrogelators dissolve at concentration of 1.0 wt%. The hydrogels form after the solutions being cooled to room temperature.

compound	1	2	3	1P	2P	3P
LoVo	213	126	159	302	167	187
HeLa	192	156	161	192	145	157
NIH3T3	264	213	221	260	119	245

Table S2. IC_{50} (72 h) values (μ M) of the six compounds on three different cancer cell lines (LoVo, HeLa and NIH3T3).

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