

Electronic Supplementary Information

Disulfide Crosslinking and Helical Coiling of Peptide Micelles Facilitate the Formation of a Printable Hydrogel

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Experimental Section

Chemical and materials

The lyophilized form of Fmoc-Phe-Phe-Cys-COOH (Fmoc-FFC) and Fmoc-Phe-Phe-Ser-COOH (Fmoc-FFS) were purchased from GL Biochem Ltd. (Shanghai, China). The lyophilized form of Fmoc-diphenylalanine (Fmoc-FF) was purchased from Bachem (Switzerland). NaOH was commercially purchased from Fengchuan Co. (Tianjin, China). DTNB (5,5'-Dithio-bis-(2-nitrobenzoic acid)) was purchased from Gentihold Inc. (Beijing, China). Na₂HPO₄ and 2-amino-2-(hydroxymethyl)-3-propanediol were purchased from the Aladdin Reagent Corporation (Shanghai, China). L-cysteine was purchased from Heowns Inc. (Tianjin, China).

Synthesis of Peptide Hydrogels and Solutions

29.3 mg Fmoc-FFC was ultrasonically dispersed into 1 mL water and dissolved by the addition of 0.5 M NaOH with stirring slowly, giving rise to a transparent solution at pH 11. Then the solution was aged at 25 °C for about 4 hours without disturbance. Fmoc-FFS was treated in the same way. 14.76 mg Fmoc-FF was dispersed in 100 mM Tris-HCl at pH 8.8 under sonication. The mixture was stirred for a while and aged for 3 hours.

Rheological Characterization

Dynamic rheological measurements were carried out using a DHR-2 rheometer (Waters, China). The hydrogel sample of 1 ml was laid on the plate of the instrument at room temperature. For the frequency sweep oscillatory shear rheology test, the frequency was varied from 0.01 Hz to 100 Hz while the strain was kept at 1%. The oscillatory strain amplitude sweep measurement was conducted from 0.1% to 100% while setting the frequency to be 1 Hz. In order to investigate the hydrogel viscosity variation at different shear rates, viscosity measurement was measured by controlling shear rate varying from 1-500 Hz. In order to investigate the recovery property of the hydrogel samples, the applied strain amplitude was controlled of 1 % in 60 s and then 100% in 180 s, this procedure was repeated several circles continuously in time.

Transmission Electron Microscopy (TEM)

The samples were observed using a Tecnai G2 F20 transmission electron microscope (FEI Ltd, Holland). To prepare samples for TEM analysis, the hydrogel was diluted to 2 mg/ml ultrasonically with deionized water. Then 10 μL droplets were deposited on the ultrathin carbon-coated copper mesh. After 2 min, excess water from the sample was absorbed by a filter paper (Whatman, UK). Then the sample was negatively stained with 1 wt% phosphotungstic acid.

As shown in Figure S3, the dilution influenced little on morphology when preparing the samples. Admittedly, this method involved of drying and phosphotungstic acid may potentially lead to artefacts.

Polarized Optical Microscopy (POM)

The samples were observed by a polarized-light microscope (ShunYu XP, China) with an attached charge-coupled device video camera.

Raman Microscopy

Raman spectra were measured on Renishaw in Via Reflex spectroscope (Renishaw, UK) in the

wavenumber range of 400–3000 cm^{-1} . The hydrogel samples were first frozen in liquid nitrogen quickly, then incubated in a lyophilizer and vacuum freeze-dried for 24 hours. The resulting samples were detected with the 633 nm line of a He–Ne laser.

Circular Dichroism (CD)

Circular dichroism spectroscopy (CD) was recorded on a JASCO J–810 CD spectropolarimeter (Jasco Inc., Japan) over a wavelength range of 180–350 nm with a 0.1 mm path length quartz cuvette. The CD data were collected three times at a scanning speed of 500 nm min^{-1} with a bandwidth of 2 nm.

It was hard to detect the samples by a CD spectropolarimeter at high concentrations. The highly concentrated peptide solutions showed very strong linear dichroism owing to the alignment of the self-assembled nanofilaments.^{1,2} Besides, the CD signals were observed fluctuated greatly when the wavelength was less than about 220 nm because the HT data were over 600 V (Figure S6). So that the peptide samples used for CD measurements were diluted to a concentration of 2 mM.

Wide-angle X-ray Diffraction (WAXD)

To avoid the influence of drying, in situ wide-angle X-ray diffraction was used to characterize the peptide assembly which was kept on the solution state. The WAXD measurements were performed at beamline 1W2A of the Beijing Synchrotron Radiation Facility (BSRF, Beijing, China). The wavelength of the X-rays was 0.154 nm, and the distance from the sample to the detector was set at 151 mm. The peptide solutions were placed in cuvettes with a 1.0 mm path length. Samples were irradiated for 200 s, and the scattered radiation was detected using a Mar CCD detector. The 1D scattering profiles were obtained by radial integration of the 2D patterns, with scattering from the cuvettes subtracted as the background. Scattering profiles were subsequently plotted on a relative scale as a function of the scattering vector $q = (4\pi/\lambda) \sin(\theta/2)$, where θ is the scattering angle.

High Performance Liquid Chromatography (HPLC)

HPLC measurement was carried out on Agilent1100 (Agilent Technologies Inc., USA) equipped with a UV–vis detector. In the detection, the column used was C18 (5 μm , 4.6 mm \times 250 mm, Tianjin Bonna-Agela Technologies Ltd., China), the eluent was 80% acetonitrile and 20% water (v:v), the flow rate was 0.5 mL min^{-1} , the injection volume was 10 μL , and the UV wavelength detected was 300 nm. All of the samples were prepared at 1 mg/mL for detection. 1 mg Fmoc-FFC powder was dissolved in 1 mL acetonitrile. The Fmoc-FFC solution and the hydrogel were both diluted in water at 1 mg/mL .

To calculate the field of the dimers roughly, the absorption of the dimer was assumed to be two times as that of the monomer of the peptide. The absorption of the deprotected molecules and other impurities was regarded equal to the Fmoc-FFC. Then the field of the dimer was analyzed by the peak area in the HPLC spectra.

Mass Spectrometry (MS)

The experiments were carried out on a micrO-TOF-QII mass spectrometer (Bruker, America), which was equipped with an ESI source and detected in positive ionization mode. The scan mass spectra were recorded in the m/z range from 600 to 1400.

Printing Test

The printing test was carried on a homemade 3-D printer, which consisted of a computer controlled

3-axis movement platform, a screw-driven fluid dispenser, and a nozzle, typically with inner diameter of 1.75 mm. The as-prepared hydrogel was printed into a square pattern (30 mm × 30 mm) with filaments spacing of 0.5 mm. The printing speed was 6 mm s⁻¹.

¹H Nuclear Magnetic Resonance (NMR)

The ¹H NMR was recorded on Bruker AV-400 spectrometer (Bruker, Germany), referenced to Si(CH₃)₄. The solvent used in the measurement was DMSO-d₆. The lyophilized form of the samples were used. The spectrum of the gel were representative of the mixture including of the dimer, the monomer and other impurities.

Fmoc-FFC molecule: δ_H (400 MHz; DMSO-d₆) 12.97 (1 H, s) , 8.36 (2 H, d), 8.19 (2 H, m), 7.88 (3H, m), 7.61 (3H, m), 7.01-7.49 (9H, m), 4.69–4.38 (3H, m), 4.28–4.03 (3 H, m), 3.95 (2 H, s), 3.14-3.04 (2 H, m), 2.97–2.63 (3 H, m).

Measurement of the Number of the Free Sulfhydryl

To determine the consumption of sulfhydryl groups in the gelation process, the Ellman method was conducted. 0.04 g DTNB was dissolved in 10 ml Na₂HPO₄ (50 mM) to form the standard solution. Then 1 ml standard solution was diluted in 100 ml Tris-HCl (0.25 M, pH 8.3) to form the Ellman's reagent. Mixed L-cysteine solutions with different concentration (0.14 mM, 0.17 mM, 0.20 mM, 0.23 mM, 0.26 mM) and the Ellman's reagent at 25 °C. After incubating the resulting mixture was aged at 25 °C for 10 min, the samples were then analyzed spectrophotometrically at 412 nm on a TU-1810 UV-vis spectrophotometer (Persee Instruments Ltd., China) to get a cysteine standard curve (Figure S7).

Then the hydrogel sample, which was incubated for 48 hours, was treated at the same way. The number of the free sulfhydryl groups could be calculated by the cysteine standard curve. According to the adsorption intensity (0.092) of the hydrogel exhibited at 412 nm, the free sulfhydryl of the sample was 0.043 mM. The free sulfhydryl groups of the treated sample was 0.253 mM if there were no crosslinking. As a result, about 83% sulfhydryl groups have been consumed.

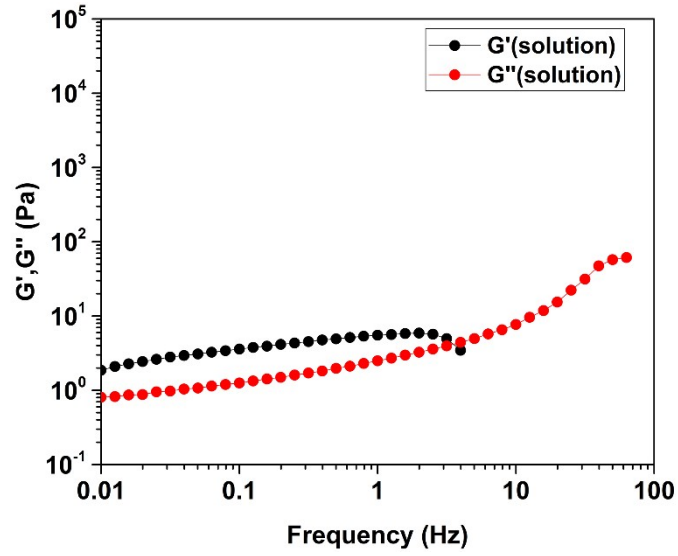


Figure S1. Frequency-dependent (strain of 1%) oscillatory rheology of 2.8 wt% Fmoc-FFC solution.

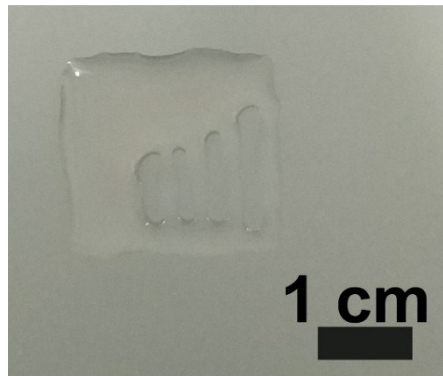


Figure S2. The printing pattern of 2.8 wt% Fmoc-FFC solution.

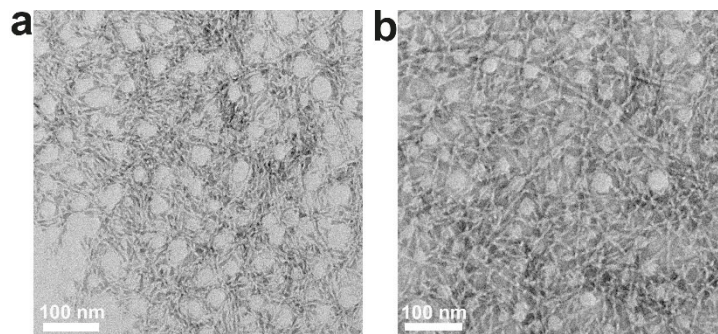


Figure S3 a) TEM images of the Fmoc-FFC hydrogel sample (diluted to 5 mg/mL). b) TEM images of the Fmoc-FFC hydrogel sample with 29.3 mg/mL.

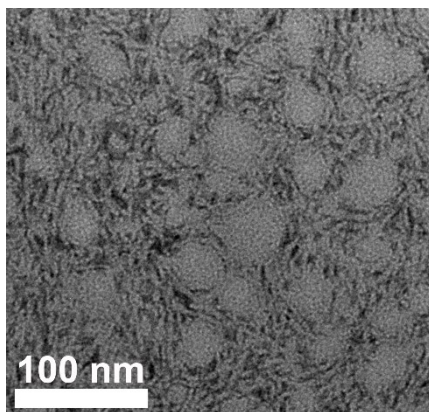


Figure S4. TEM image showing the network of micelles within the Fmoc-FFC hydrogel (diluted to 2 mg/mL).

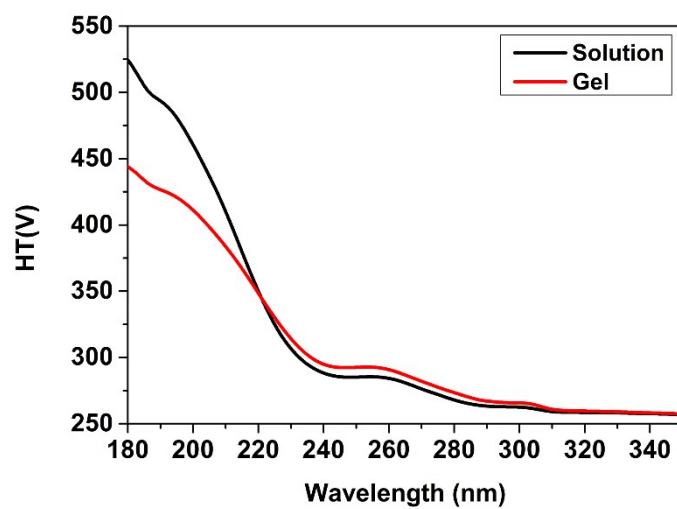


Figure S5. HT data of Fmoc-FFC samples during circular dichroism (CD) analysis.

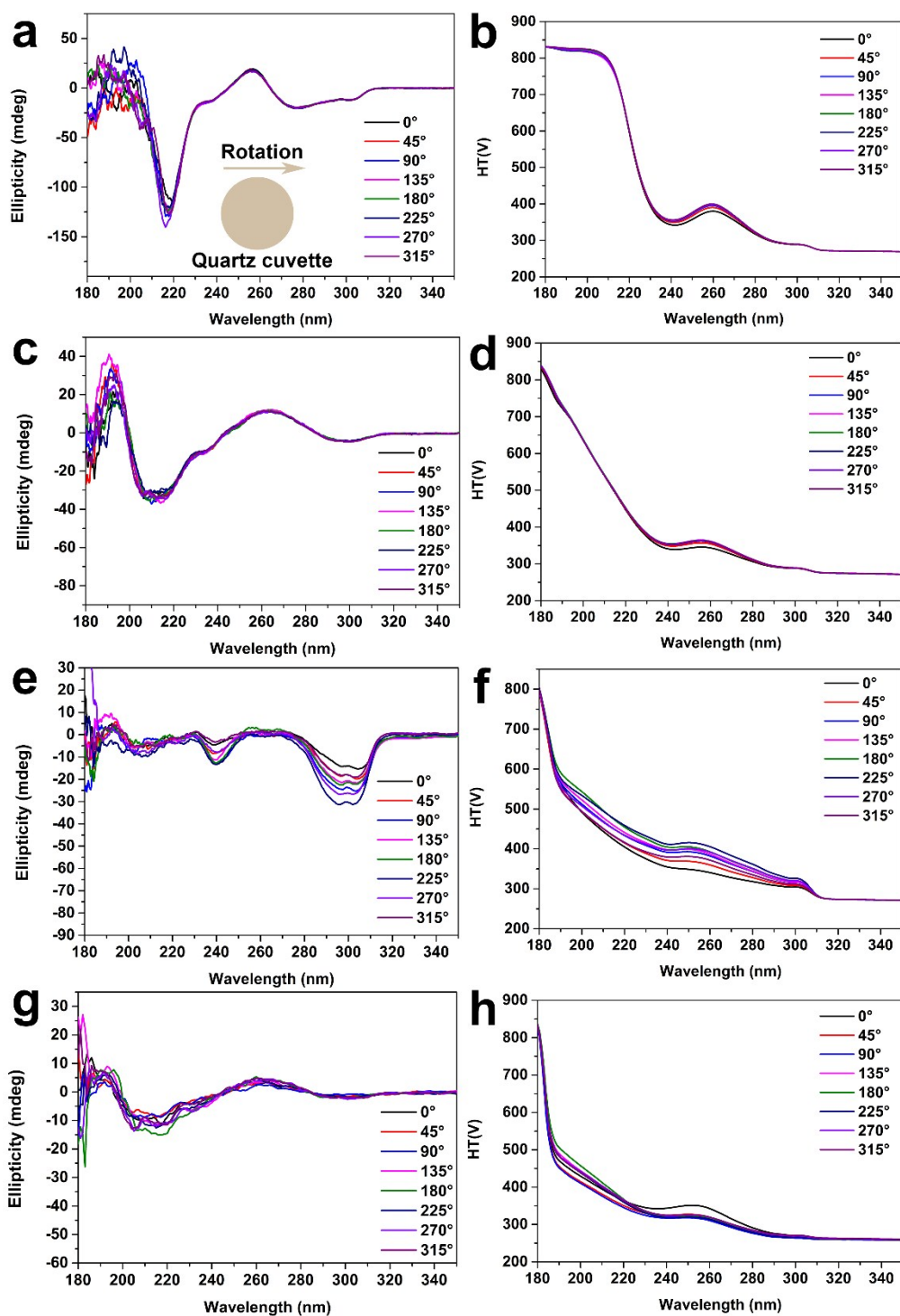


Figure S6. CD spectra of the Fmoc-FFC solution samples which was diluted to 10 mM (a), the gel sample diluted to 10 mM (c), the solution sample with 29.3 mg/mL (e), the gel sample with 29.3 mg/mL (g). HT data of the Fmoc-FFC solution samples diluted to 10 mM (b), the gel sample diluted to 10 mM (d), the solution sample with 29.3 mg/mL (f), the gel sample with 29.3 mg/mL (h). The eight curves in each figure were obtained by rotating the quartz cuvette for 45° clockwise eight times.

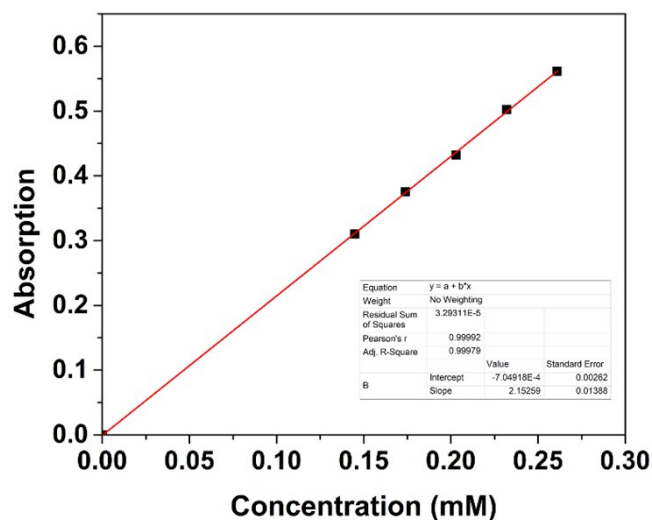


Figure S7. Cysteine standard curve.

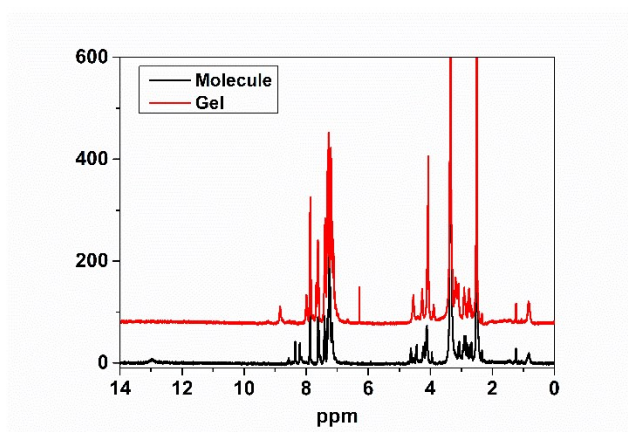


Figure S8. NMR spectra of the Fmoc-FFC molecule and gel.

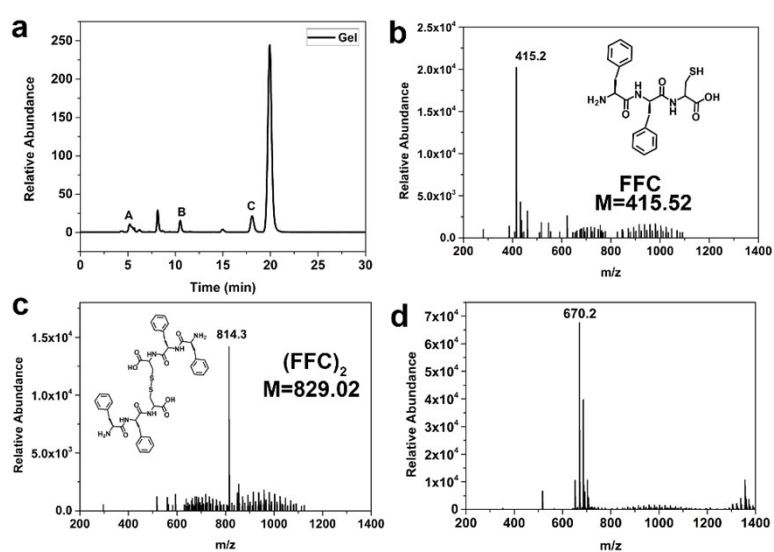


Figure S9. a) HPLC spectrum of Fmoc-FFC hydrogel (incubated for 48 h) sample. b-d) Mass spectra of A, B, C peaks in a.

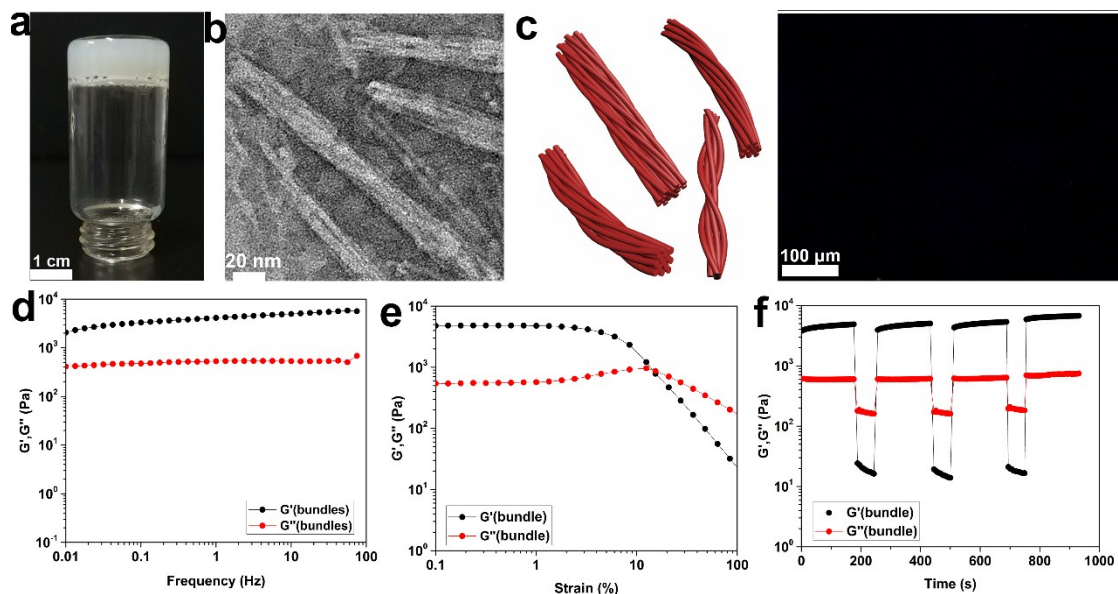


Figure S10. a) Photograph of 2.8 wt% Fmoc-FFC gel at pH 8. b) TEM image of the Fmoc-FFC gel samples (diluted to 2 mg/mL) at pH 8. c-f) Schematic illustration and POM image (c), frequency-dependent (strain of 1%) oscillatory rheology (d), strain-dependent (frequency of 1 Hz) oscillatory rheology (e) and dynamic strain amplitude cyclic test (strain of 1% and 100%, f) of 2.8 wt% Fmoc-FFC hydrogel at pH 8.

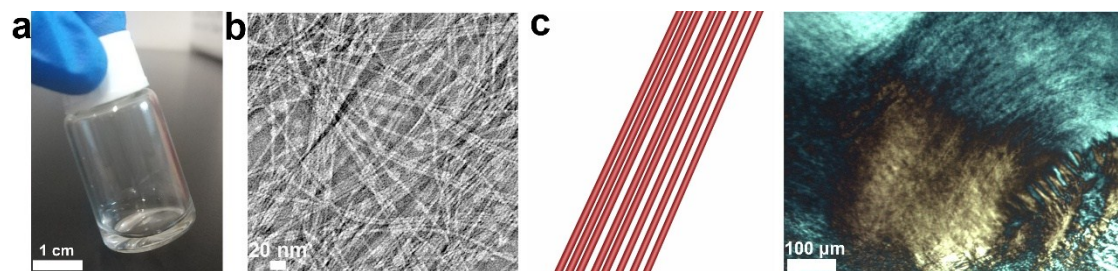


Figure S11. a) Photograph of 2.46 wt% Fmoc-FF solution. b) TEM image of THE Fmoc-FF solution (diluted to 2 mg/mL). c) Schematic illustration and POM image of 2.46 wt% Fmoc-FF solution.

References

1. A. R. Hirst, S. Roy, M. Arora, A. K. Das, N. Hodson, P. Murray, S. Marshall, N. Javid, J. Sefcik and J. J. N. C. Boekhoven, *Nat. Chem.*, 2010, **2**, 1089-1094.
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