A Supramolecular Hydrogel Self-assembled from Pentafluorobenzyl-dipeptide

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Supporting Information

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Synthesis of PFB-YF. This peptide derivative was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-tyrosine and pentafluoro benzeneacetic acid. The resin (1.20 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (0.78 g, 2.0 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide (DMF) and *N*,*N*-diisopropylethylamine (DIEA; 0.83 mL, 5.0 mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc-L-tyrosine (2.30 g, 5.0 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.758 g, 2.0 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.830 mL, 5.0 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, pentafluoro benzeneacetic acid (0.45 g, 2.0 mmol) was coupled to the free amino group using HBTU (0.76 g, 2.0 mmol) and DIEA (0.83 mL, 5.0 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried under a stream of air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual

solvent (0.33 g). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 2.60-2.70 (m, 1H), 1.85-3.20 (m, 3H), 3.59 (s, 2H), 4.40-4.55 (m, 2H), 6.65 (d, *J* = 9.0 Hz, 2H), 7.04 (d, *J* = 9.0 Hz, 2H), 7.20-7.35 (m, 5H), 8.4 (d, *J* = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 28.6, 36.6, 36.9, 53.5, 54.1, 110.4, 114.7, 126.4, 127.6, 128.1, 129.1, 130.0, 136.7, 137.4, 139.3, 144.8, 155.7, 166.2, 171.1, 172.7; MS [ESI$^-$]: calcd. m/z 536.45, obsvd. 535.1 $[M - H]^-$.

Synthesis of PFB-YpF. The peptide derivative was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding Fmoc-L-phenylalanine, Fmoc-Yp-OH and Pentafluoro benzeneacetic acid. The resin (1.2 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (0.38 g, 1.0 mmol) was loaded onto the resin in anhydrous DMF and DIEA (0.41 mL, 2.5 mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc-Yp-OH (1.2 g, 2.5 mmol) was coupled to the free amino group using HBTU (0.94 g, 2.5 mmol) and DIEA (1.03 mL, 6.25 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 m the

in). Finally, pentafluoro benzeneacetic acid (0.68 g, 3.0 mmol) was coupled to free amino group using HBTU (1.14 g, 3.0 mmol) and DIEA (0.62 mL, 3.75 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried under a stream of air and then Et_2O was added to precipitate the crude compound. The resulted crude product was purified by reverse phase HPLC and gave the target product. The solid was dried under vacuum to remove residual solvent (55 mg). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 2.70-2.80 (m, 1H, CH_2), 2.85-2.90 (m, 2H, CH_2), 3.05-3.15 (m, 1H, CH_2), 3.624 (s, 2H, CH_2), 4.40-4.50 (m, 1H, CH), 4.50-4.60 (m, 1H, CH), 7.05 (d, *J* = 8.0 Hz, 2H, CH), 7.16 (d, *J* = 8.0 Hz, 2H, CH), 7.20-7.35 (m, 5H, CH), 8.35 (d, *J* = 8.0 Hz, 1H, NH), 8.46 (d, *J* = 8.5 Hz, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d$_6$): δ = 29.5, 37.7, 38.0, 54.6, 55.0, 111.2, 120.7, 127.4, 129.2, 130.1, 130.9, 133.3, 137.7, 138.4, 140.3, 145.8, 151.7, 167.4, 171.7, 173.8; MS [ESI$^-$]: calcd. m/z 616.1, obsvd. 615.0 $[M - H]^-$.

Inverted Tube Method. Gelation was performed by weighing a compound (2.0 mg) in a screw-capped 2-mL vial (diameter: 10 mm). Sodium hydroxide solution was added to the suspension to adjust pH; alternating vortex and ultrasonication were applied until a clear solution was obtained. This solution was then neutralized by a dropwise addition of hydrochloric acid for gelation.[S1]

Transmission Electron Microscopy. Images were obtained with a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 100 kV. Hydrogels were applied directly onto 200 mesh carbon-coated copper grids. Excess amount of the hydrogel was carefully removed by capillary action (filter paper), and the grids were then immediately stained with uranyl acetate for 30 s. Excess stain was removed by capillary action, and the grids were allowed to air dry.

Rheological tests. Rheological tests were conducted using an Anton Paar rheometer and a 25-mm parallel plate. The hydrogel sample (400μL, 1 wt %) was placed on the parallel plate for the angular frequency sweep test (test range: 0.25 to 100 $rads^{-1}$; 13 points per decade; sweep mode, "log"; temperature, 25 °C).

Computational Methods. The ground-state structure of **PFB-YF** was calculated by the semiempirical AM1 method. The geometric optimization of the four-molecule model was carried out using DREIDING force field.[S2]

Cell viability tests. The biocompatibilities of different peptides were measured by the MTT cell viability test. The cells were seeded in 24-well plates at a density of 50000 cells per well with 0.5 mL medium (DMEM for CTX TNA2, HeLa, MCF-7 and MEM for WS1) contained 10% FBS and 1 % Penicillin-streptomycin solution and incubated for 24 h. Compounds at different concentrations (10, 50, 100, 200, 500 μM) were added when cells were plated. 24 and 48 h later, replaced the medium with

fresh medium supplemented with 0.5 mL of MTT reagent (4 mg mL^{-1}) per well. After another 4 h, the medium containing MTT was removed and DMSO (0.5 mL/well) was added to dissolve the formazan crystals. Each 24-well was transferred to 96 well plate. The optical density of the result solution was measured at 595 nm, using an absorbance microplate reader (Infinite F50, TECAN). Cells without the treatment of the compounds were used as the control. The cell viability percentage was calculated by the following formula: The cell viabilitypercentage (%) =$OD_{sample}/OD_{control}$.

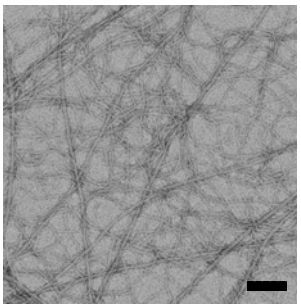

Fig. S1. Negative-stained TEM image of a 1 wt% freeze-dried gel of **PFB-YF** (scale bar: 100 nm).

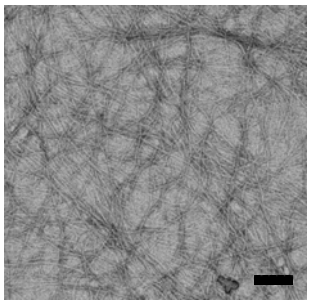

Fig. S2. Negative-stained TEM images of 2 wt% air-dried gel of **PFB-YF** (scale bar: 100 nm).

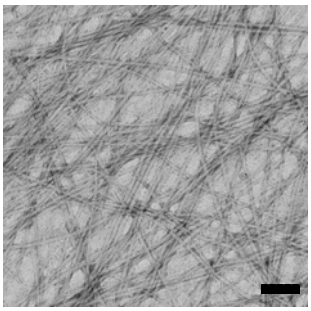

Fig. S3. Negative-stained TEM images of 5 wt% air-dried gel of **PFB-YF** (scale bar: 100 nm).

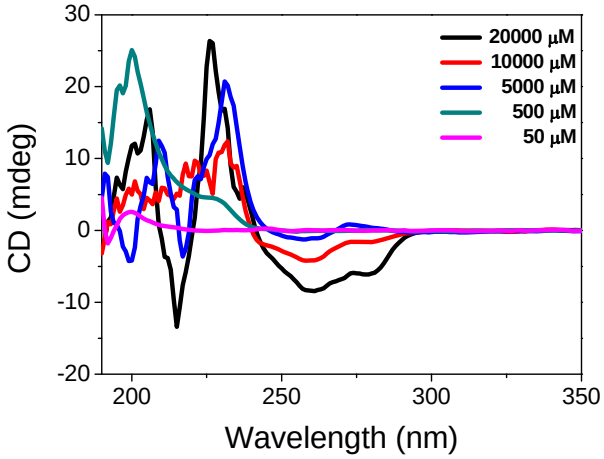

Fig. S4. Concentration-dependent CD spectra of **PFB-YF** in water .

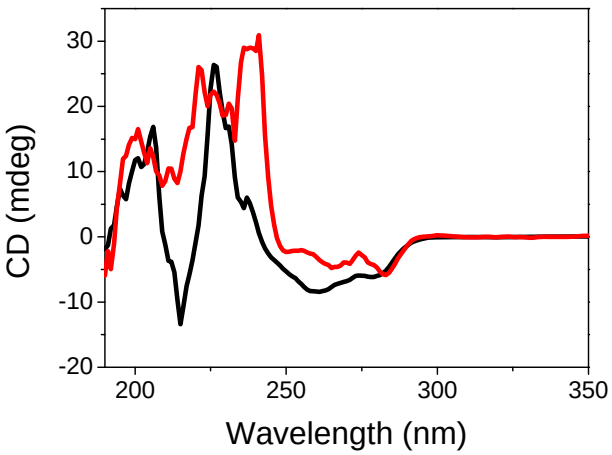

Fig. S5. CD spectra of **PFB-YF** at 1 wt% in water (black) and in methanol (red).

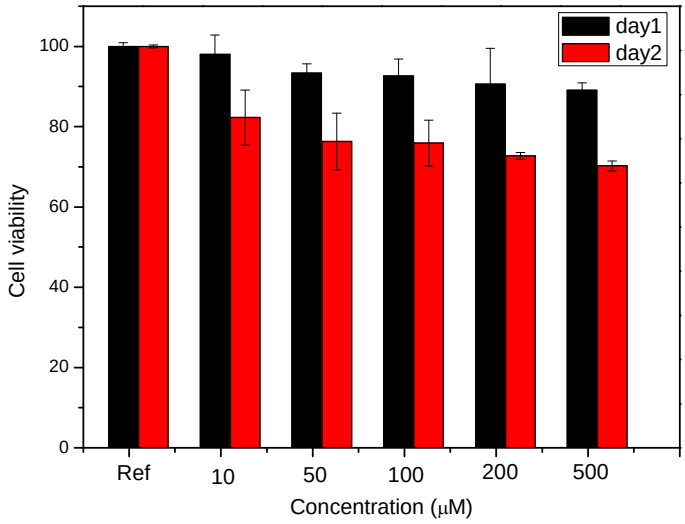

Fig. S6. Viability ratios of CTX TNA2 cells incubated with 10-500 μM of **PFB-YpF** after 24 (black) and 48 h (red). Ref: cell culture without the hydrogelators in the medium.

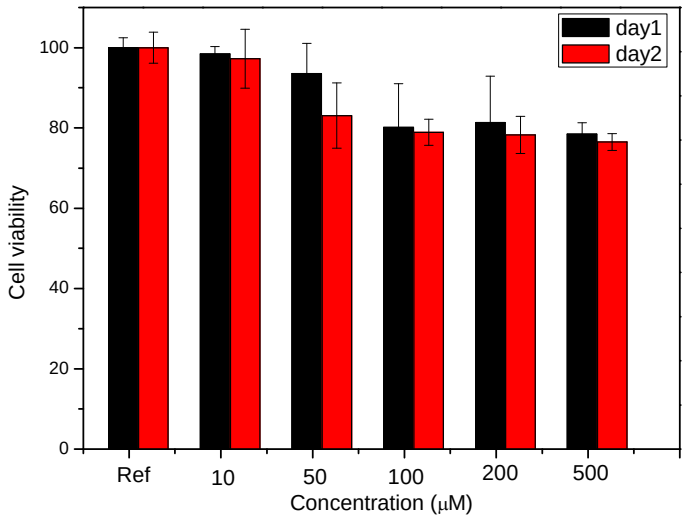

Fig. S7. Viability ratios of WS1 cells incubated with 10-500 μM of **PFB-YpF** after 24 (black) and 48 h (red). Ref: cell culture without the hydrogelators in the medium.

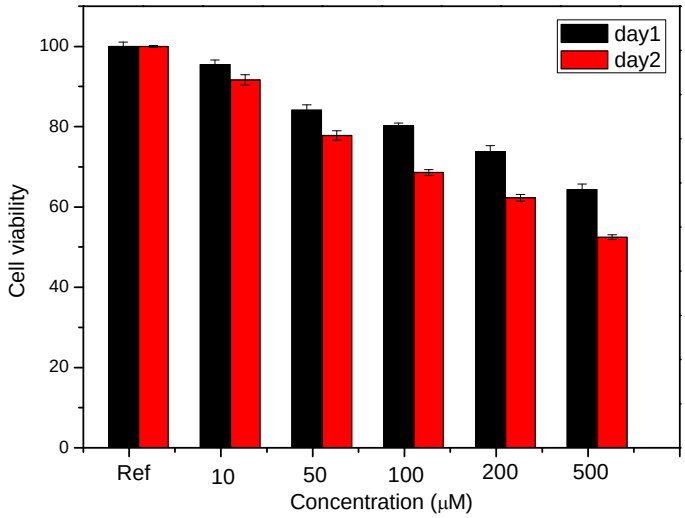

Fig. S8. Viability ratios of HeLa cells incubated with 10-500 μM of **PFB-YpF** after 24 (black) and 48 h (red). Ref: cell culture without the hydrogelators in the medium.

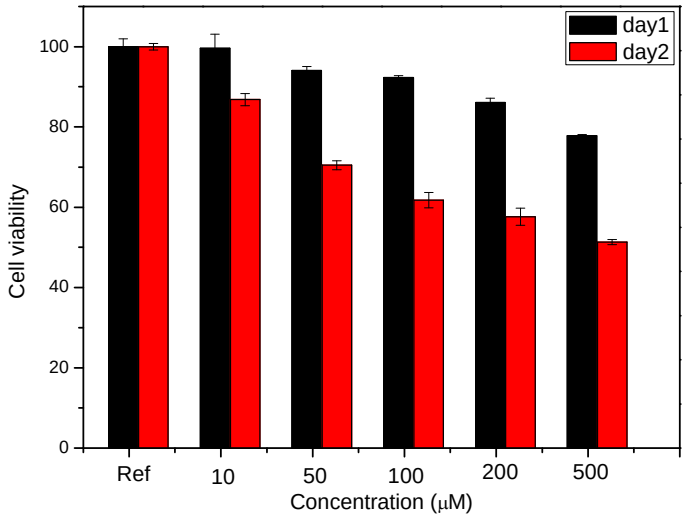

Fig. S9. Viability ratios of MCF-7 cells incubated with 10-500 μM of **PFB-YpF** after 24 (black) and 48 h (red). Ref: cell culture without the hydrogelators in the medium.

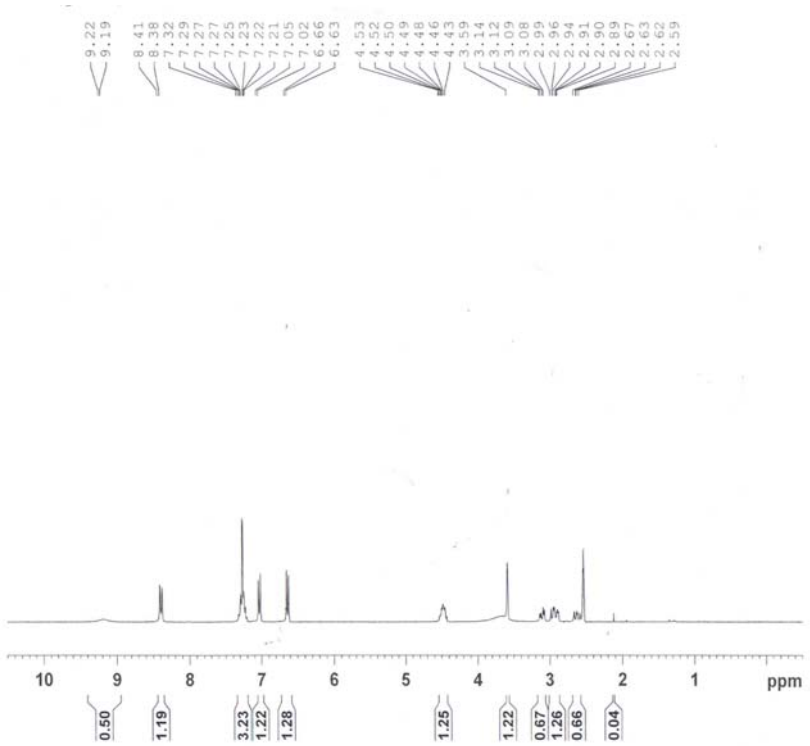

Fig. S10. ^{1}H NMR spectrum of **PFB-YF** in [D_6]DMSO.

Reference:

S1. Wang, X. Z.; Li, X. Q.; Shao, X. B.; Zhao, X.; Deng, P.; Jiang, X. K.; Li, Z. T.; Chen, Y. Q. *Chem. Eur. J.* **2003**, *9*, 2904.

S2. Mayo, S. L.; Olafson, B. D.; Goddard III, W. A. *J. Phys. Chem.* **1990**, *94*, 8897.