Supporting Information for:

Dephosphorylation of D-Peptide Derivatives to Form Biofunctional, Supramolecular Nanofibers/Hydrogels and Their Potential Applications for Intracellular Imaging and Intratumoral Chemotherapy

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Contents.

S1. Experimental materials and instruments.

All of the solvents and chemical reagents were used as received from the commercial sources without further purification unless otherwise noted. Flash chromatography was performed on silica gel 60 (230-400 mesh). Analytical thin layer chromatography (TLC) was performed using silica gel 60 F-254 pre-coated glass plates (0.25 mm) and analyzed by short wave UV illumination. Hydrophilic products were purified with Waters Delta600 HPLC system, which equipped with an XTerra C18 RP column and an in-line diode array UV detector. ^{1}H, ^{13}C, and ^{31}P NMR spectra were obtained on Varian Unity Inova 400. Chemical shifts are reported in δ (ppm) relative to the solvent residual peak (phosphoric acid for ^{31}P NMR). Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and br (broad). LC-MS spectra were obtained on a Waters Acouity ultra Performance LC with Waters MICRO-MASS detector. Rheological data were measured on TA ARES G2 rheometer with 25 mm cone plate. TEM images were taken on Morgagni 268 transmission electron microscope. The PTP1B inhibitor was purchased from BIOMOL. The HeLa cell line (CCL2) was purchased from American Type Culture Collection. All of the media were purchased from Invitrogen. Cytotoxicity tests were measured by DTX 880 multimode detector.

S2. Synthesis and characterizations.

2-(Naphthalen-2-yl)acetyl-(L)-Phe-(L)-Phe-(L)-Lys-(L)-Tyr phosphate (1a). The L-amino acid based hydrogelator precursor was prepared by the standard solid-phase peptide synthesis (SPPS), which used 2-chlorotrityl chloride resin (100~200 mesh and 0.3~0.8 mmol/g) and *N*-Fmoc-protected amino acids with side chains properly protected by *tert*-butoxycarbonyl (Fmoc-Lys(Boc)-OH) group. Fmoc-Tyr(PO_3H_2)-OH was prepared from L-Tyr-OH and directly used in SPPS.[1] The resin was first swelled in dry dichloromethane (DCM) by bubbling it with nitrogen gas (N_2) for 20 minutes, and was washed with 3 mL of dry *N,N*-dimethylformamide (DMF) for three times. Then the first amino acid Fmoc-Tyr(PO_3H_2)-OH was loaded onto resin at its C-terminal by bubbling the resin in a DMF solution of Fmoc-protected amino acid (2 equiv.) and 1 mL of *N,N*-diisopropylethylamine (DIPEA) for 1 hour. After washed with 3 mL of DMF for three times, the unreacted sites in resin were quenched by bubbling the resin with blocking solution (16:3:1 of DCM/MeOH/DIPEA) for 2 x 10 minutes. Then the resins were treated with 20% piperidine (in DMF) for 0.5 hour to remove the protecting group, followed by washing the resin in DMF for five times. Then we conjugated the sequent Fmoc-protected amino acid (2 equiv.) to the free amino group on the resin using DIPEA/*O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (2 equiv.) as the coupling reagent. These coupling and de-protection steps were repeated to elongate the peptide chain, which were carried out by the standard Fmoc SPPS protocol.[2] The resin was washed with DMF for 3~5 times after each step. Finally, we washed the resin with DMF (5 times), DCM (5 times), methanol (5 times), and hexane (5 times) respectively, then we cleaved the peptide with TFA (10 mL) for 2 hours. The resulted crude products were purified by reverse phase HPLC and gave a total yield of 64%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (d, J = 8.3 Hz, 1H), 8.24 (d, J = 8.1 Hz, 1H), 8.12 (d, J = 7.8 Hz, 1H), 8.04 (s, 3H), 7.93 (d, J = 7.5 Hz, 1H), 7.85 (d, J = 7.4 Hz, 1H), 7.78 (d, J = 7.5 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.58 (s, 1H), 7.51 – 7.41 (m, 2H), 7.32 – 7.00 (m, 15H), 4.60 – 4.45 (m, 2H), 4.45 – 4.37 (m, 1H), 4.16 (dd, J = 14.6, 7.0 Hz, 1H), 3.53 (dd, J = 35.9, 14.0 Hz, 2H), 3.13 – 3.02 (m, 2H), 2.97 – 2.87 (m, 2H), 2.80 (dd, J = 13.9, 9.6 Hz, 1H), 2.69 (dd, J = 13.4, 10.3 Hz, 1H), 2.54 (s, 2H), 1.46 – 0.95 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.79, 171.16, 171.03, 170.54, 169.84, 137.83, 137.72, 133.91, 132.90, 131.74, 129.73, 129.27, 129.16, 128.03, 127.90, 127.61, 127.44, 127.38, 127.25, 126.29, 126.14, 125.97, 125.42, 119.25, 119.14, 53.94, 53.69, 53.06, 52.92, 42.25, 37.54, 37.20, 35.53, 31.97, 26.62, 22.01; ^{31}P NMR (162 MHz, DMSO-d_6) δ -4.84; LC-MS (ESI) (m/z): $C_{45}H_{50}N_5O_{10}P$ calcd 851.33; found 852.64 $[M+1]^+$, 850.69 $[M-1]^-$.

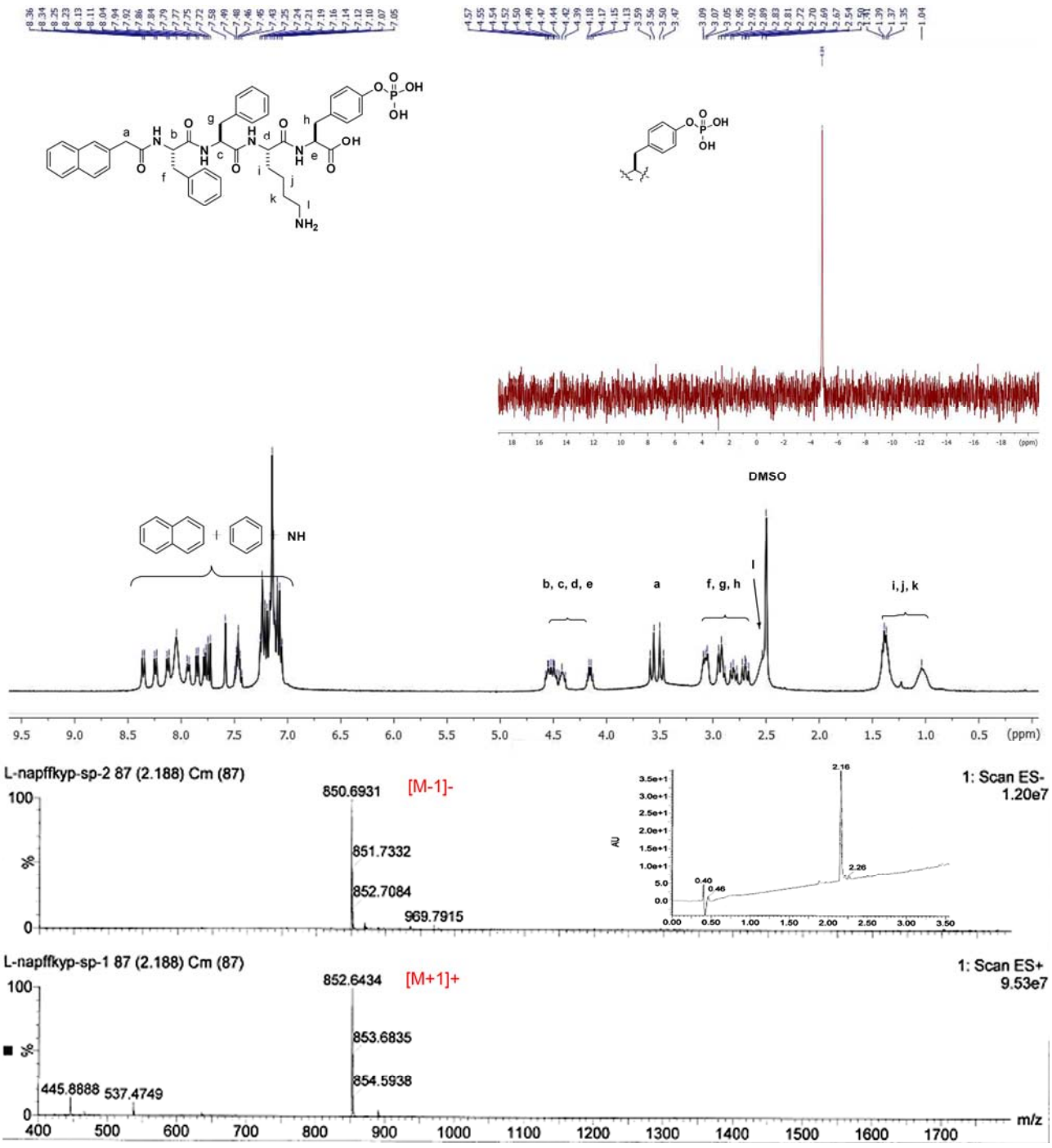

Figure S1. The ^{1}H NMR and ^{31}P NMR of **1a** in d-DMSO and its LC-MS spectra.

2-(Naphthalen-2-yl)acetyl-(D)-Phe-(D)-Phe-(D)-Lys-(D)-Tyr phosphate (1b). The D-amino acid based hydrogelator precursor was also synthesized by solid-phase peptide synthesis described as above. All the *N*-Fmoc-protected amino acids we used here were D-version amino acids, including Fmoc-D-Phe-OH, Fmoc-D-Lys(Boc)-OH, and Fmoc-D-Tyr(PO_3H_2)-OH. Fmoc-D-Tyr(PO_3H_2)-OH was also prepared from D-Tyr-OH and directly used in SPPS. Purification with reverse phase HPLC gave pure white powder in a yield of 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J = 7.8 Hz, 1H), 8.26 (d, J = 6.7 Hz, 1H), 8.16 (s, 4H), 7.91 (d, J = 5.3 Hz, 1H), 7.85 (d, J = 5.9 Hz, 1H), 7.78 (d, J = 6.3 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.59 (s, 1H), 7.46 (s, 2H), 7.29 – 7.03 (m, 15H), 4.61 – 4.46 (m, 2H), 4.42 (s, 1H), 4.13 (s, 1H), 3.53 (dd, J = 35.9, 13.9 Hz, 2H), 3.08 (d, J = 12.4 Hz, 2H), 2.93 (d, J = 11.7 Hz, 2H), 2.87 – 2.76 (m, 1H), 2.75 – 2.64 (m, 1H), 1.47 – 0.91 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.88, 171.19, 171.06, 170.58, 169.82, 152.26, 137.81, 137.76, 133.94, 132.93, 131.73, 130.64, 129.69, 129.30, 129.27, 128.07, 127.94, 127.65, 127.49, 127.43, 127.31, 127.24, 126.25, 126.17, 126.00, 125.48, 119.23, 119.19, 53.92, 53.73, 53.16, 52.96, 42.26, 38.55, 37.59, 37.23, 35.45, 32.02, 26.65, 22.06; ^{31}P NMR (162 MHz, DMSO-d_6) δ -4.87; LC-MS (ESI) (m/z): $C_{45}H_{50}N_5O_{10}P$ calcd 851.33; found 852.64 $[M+1]^+$, 850.69 $[M-1]^-$.

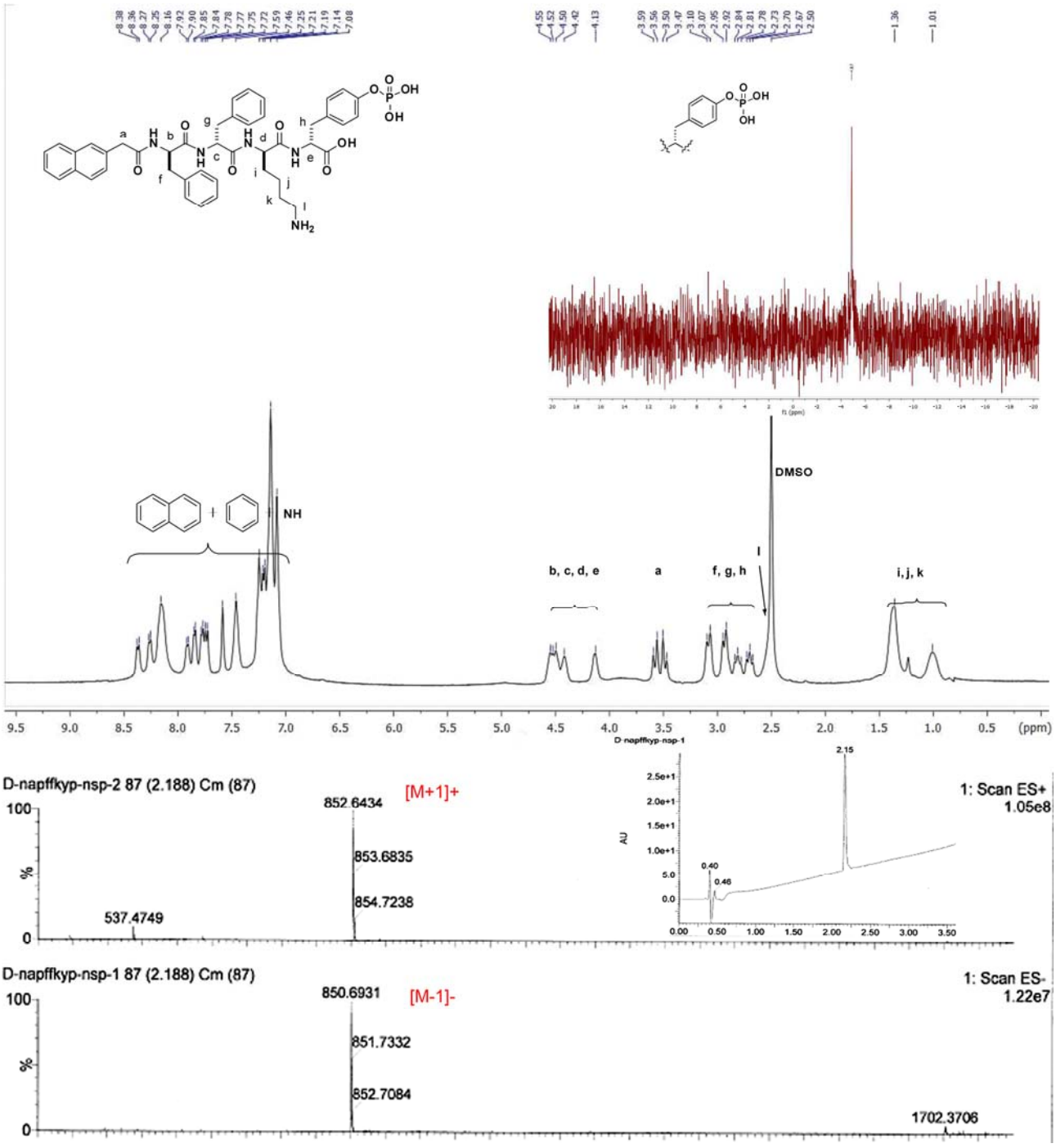

Figure S2. The ^{1}H NMR and ^{31}P NMR of **1b** in d-DMSO and its LC-MS spectra.

2-(Naphthalen-2-yl)acetyl-(D)-Phe-(D)-Phe-(D)-Lys(NBD)-(D)-Tyr phosphate (4b): 112.0 mg (0.13 mmol) of hydrogelator precursor **1b** was dissolved in an aqueous solution with pH adjusted to 9.0 by Na_2CO_3. Then the methanol solution of 26.0 mg (0.13 mmol) of 4-chloro-7-nitro-2,1,3-benzoxadiazole was added into the above solution of **1b** dropwise and the resulting solution was stirred for 2 h at 50 ^oC. After cooled down the solution, we neutralized it with 1M HCl, followed by the purification with HPLC (detected at 430 nm). Pure orange powder was collected in a yield of 48%. ^1H NMR (400 MHz, DMSO-d_6) δ 9.59 (s, 1H), 8.45 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 7.2 Hz, 1H), 8.16 (d, J = 7.9 Hz, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.74 (dd, J = 16.1, 8.2 Hz, 2H), 7.58 (s, 1H), 7.50 – 7.38 (m, 2H), 7.28 – 7.01 (m, 15H), 6.33 (d, J = 9.1 Hz, 1H), 4.53 (s, 1H), 4.47 (s, 1H), 4.43 – 4.29 (m, 2H), 3.53 (dd, J = 34.5, 14.2 Hz, 2H), 3.39 (s, 2H), 3.06 – 2.63 (m, 6H), 1.78 – 1.28 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.81, 171.45, 171.20, 170.68, 169.79, 151.46, 145.11, 144.42, 144.16, 138.05, 137.77, 133.95, 132.90, 131.69, 129.86, 129.26, 127.94, 127.40, 126.15, 125.94, 125.42, 120.49, 119.69, 119.60, 99.12, 53.97, 53.77, 53.66, 52.31, 43.37, 42.26, 37.64, 37.32, 35.91, 31.93, 27.32, 22.62; ^{31}P NMR (162 MHz, DMSO-d_6) δ -5.17; LC-MS (ESI) (m/z): $C_{51}H_{51}N_8O_{13}P$ calcd 1014.33; found 1015.56 $[M+1]^+$, 1013.67 $[M-1]^-$.

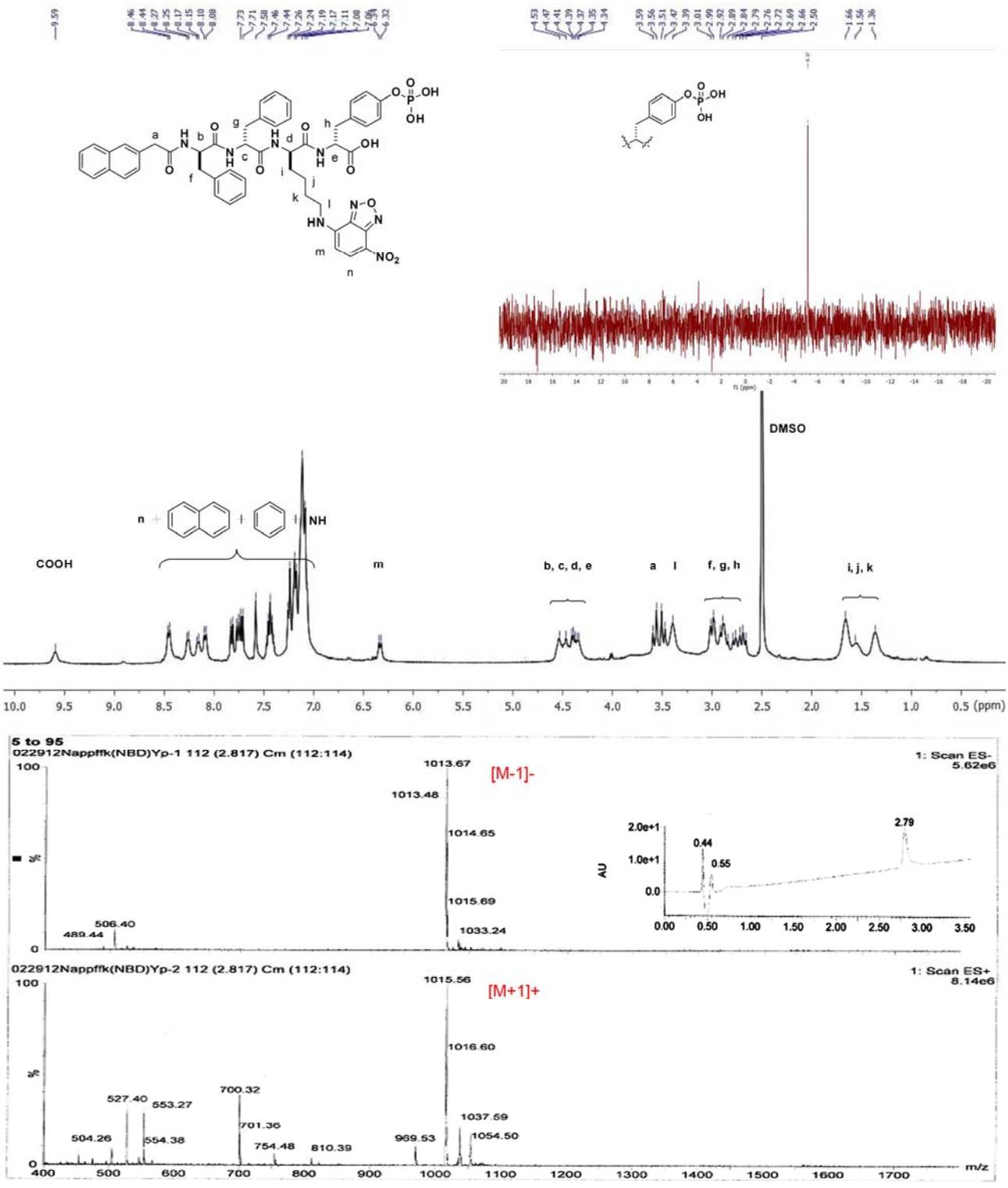

Figure S3. The ^{1}H NMR and ^{31}P NMR of **4b** in d-DMSO and its LC-MS spectra.

2'-NHS-succinyl-paclitaxel (8). 69.6 mg (0.70 mmol) of succinic anhydride and 45.8 mg (0.37 mmol) of 4-dimethylaminopyridine were added to a solution of 170.8 mg (0.2 mmol) of paclitaxel (**6**) in 5 mL of dry pyridine. After stirred for 3 hours at 20 ^{o}C, the mixture was extracted with 20 mL of dry dichloromethane (DCM) and 1 M HCl solution (20 mL×3). Then the organic phase was washed with water (20 mL×3) and brine (10 mL×3), followed by the treatment with anhydrous sodium sulfate and the evaporation under reduced pressure. The residue of 2'-succinyl-paclitaxel (**7**) was then dissolved in 5 mL of chloroform and reacted with 23.0 mg (0.20 mmol) of *N*-hydroxysuccinimide (NHS) and 27.8 mg (0.22 mmol) of *N,N'*-diisopropylcarbodiimide (DIC) without further purification. After stirred for 6 h at 20 ^{0}C, the resulting mixture was filtered to remove *N,N'*-diisopropylurea (DIU) and the filtrate was concentrated by rotary evaporator. Purification with column chromatography over silica gel (1:0 – 20:1 dichloromethane/methanol) gave pure white product of 2'-NHS-succinyl-paclitaxel (**8**) with yield of 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 7.2 Hz, 2H), 7.74 (d, *J* = 7.1 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.56 – 7.45 (m, 3H), 7.45 – 7.29 (m, 7H), 7.16 (d, *J* = 9.2 Hz, 1H), 6.28 (s, 1H), 6.22 (t, *J* = 8.6 Hz, 1H), 5.98 (dd, *J* = 9.1, 3.6 Hz, 1H), 5.68 (d, *J* = 7.1 Hz, 1H), 5.52 (d, *J* = 3.6 Hz, 1H), 5.15 (s, 1H), 4.97 (d, *J* = 8.0 Hz, 1H), 4.44 (dd, *J* = 10.5, 6.7 Hz, 1H), 4.31 (d, *J* = 8.5 Hz, 1H), 4.20 (d, *J* = 8.3 Hz, 1H), 3.80 (d, *J* = 7.0 Hz, 1H), 3.02 – 2.80 (m, 4H), 2.73 (s, 4H), 2.61 – 2.50 (m, 1H), 2.43 (s, 3H), 2.34 (dd, *J* = 15.4, 9.4 Hz, 1H), 2.23 (s, 3H), 2.13 (dd, *J* = 15.2, 8.9 Hz, 1H), 1.91 (s, 3H), 1.90 – 1.83 (m, 1H)1.67 (s, 3H), 1.22 (s, 3H), 1.13 (s, 3H);

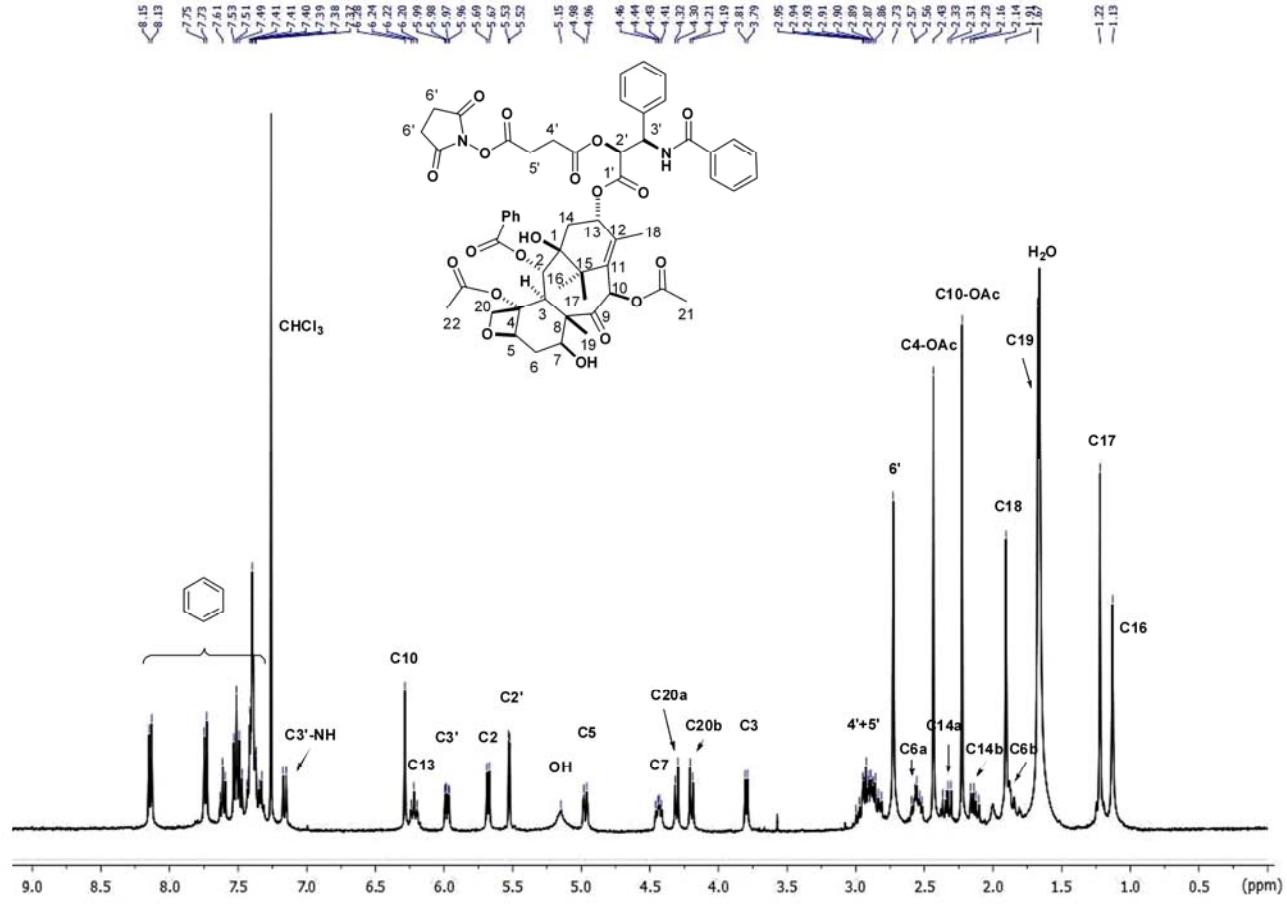

Figure S4. The ^{1}H NMR spectrum of **8** in $CDCl_3$.

2-(Naphthalen-2-yl)acetyl-(D)-Phe-(D)-Phe-(D)-Lys(Taxol)-(D)-Tyr phosphate (9b). 170.2 mg (0.20 mmol) of **1b** was dissolved in 3 mL of water with carefully adding Na_2CO_3 (1.5 equiv.) to adjust the pH of aqueous solution to 8.0. Then the acetone solution (2 mL) of 198.2 mg (0.18 mmol) of 2'-NHS-succinyl-paclitaxel (**8**) added into the weak basic aqueous solution of **1b** dropwise. More acetone and water were added carefully to keep the resulting solution clear. After the mixture was stirred at 20 ^{0}C overnight, it was purified with HPLC and gave pure white powder in a yield of 37%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (d, J = 8.2 Hz, 1H), 8.28 (d, J = 8.2 Hz, 1H), 8.22 – 8.13 (m, 2H), 8.07 (dd, J = 15.5, 7.6 Hz, 1H), 8.02 – 7.93 (m, 2H), 7.89 – 7.81 (m, 4H), 7.80 – 7.69 (m, 3H), 7.69 – 7.62 (m, 2H), 7.61 – 7.52 (m, 3H), 7.52 – 7.38 (m, 8H), 7.26 – 7.10 (m, 13H), 7.07 (d, J = 7.8 Hz, 2H), 6.29 (s, 1H), 5.82 (t, J = 8.9 Hz, 1H), 5.53 (t, J = 8.6 Hz, 1H), 5.41 (d, J = 7.0 Hz, 1H), 5.34 (d, J = 8.8 Hz, 1H), 4.91 (d, J = 9.4 Hz, 1H), 4.65 – 4.47 (m, 3H), 4.42 (dd, J = 13.0, 7.5 Hz, 1H), 4.31 (dd, J = 13.1, 8.2 Hz, 1H), 4.16 – 4.07 (m, 1H), 4.01 (t, J = 10.4 Hz, 2H), 3.57 (d, J = 13.1 Hz, 2H), 3.48 (dd, J = 13.7, 5.7 Hz, 1H), 3.08 – 2.64 (m, 8H), 2.59 (s, 2H), 2.44 – 2.28 (m, 4H), 2.23 (s, 3H), 2.19 – 2.11 (m, 1H), 2.09 (s, 3H), 1.77 (s, 3H), 1.72 – 1.56 (m, 3H), 1.50 (s, 3H), 1.41 – 1.16 (m, 6H), 1.02 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 202.39, 172.70, 172.04, 171.58, 171.14, 170.68, 170.01, 169.76, 169.68, 169.17, 168.80, 166.43, 165.22, 150.13, 139.45, 137.78, 137.62, 137.38, 134.26, 133.89, 133.53, 133.35, 132.90, 131.71, 131.51, 130.21, 130.14, 129.94, 129.60, 129.23, 128.71, 128.34, 128.21, 128.01, 127.91, 127.68, 127.58, 127.46, 127.36, 127.26, 127.22, 126.24, 126.15, 125.97, 125.45, 119.83, 119.78, 83.56, 80.26, 76.74, 75.32, 74.57, 70.72, 70.45, 57.40, 54.03, 53.74, 53.68, 53.54, 52.31, 46.10, 42.95, 42.21, 38.63, 37.52, 37.40, 36.53, 35.89, 34.40, 32.03, 29.54, 28.91, 28.77, 26.36, 22.72, 22.58, 21.42, 20.72, 13.95, 9.81; ^{31}P NMR (162 MHz, DMSO-d_6) δ -5.16; LC-MS (ESI) (m/z): $C_{96}H_{103}N_6O_{26}P$ calcd 1786.67; found 1788.80 $[M+1]^+$, 1786.01 $[M-1]^-$.

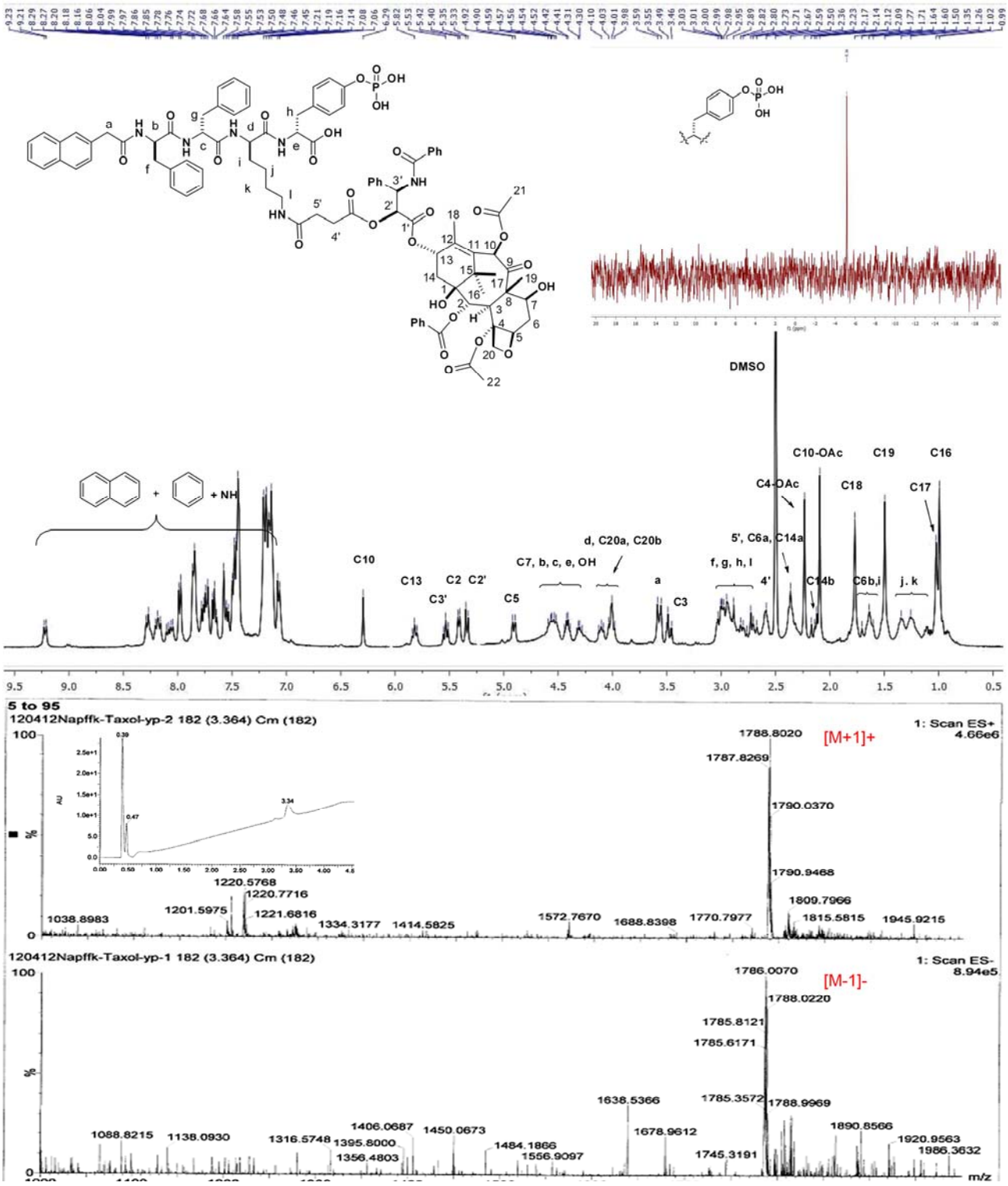

Figure S5. The ^{1}H NMR and ^{31}P NMR of **9b** in d-DMSO and its LC-MS spectra.

S3. Characterization of the properties of self-assembly.

General procedure for hydrogel preparation. All the compounds were dissolved in de-ionized water. We then adjusted pH of the solutions carefully adding 1M of NaOH and 1M of HCl and measured the values by pH paper (pH 6.0 ~ 8.0). After prepared clear weakly basic solutions (pH 7.6 or 7.4), we then formed the hydrogels by adding enzymes (alkaline phosphatase).

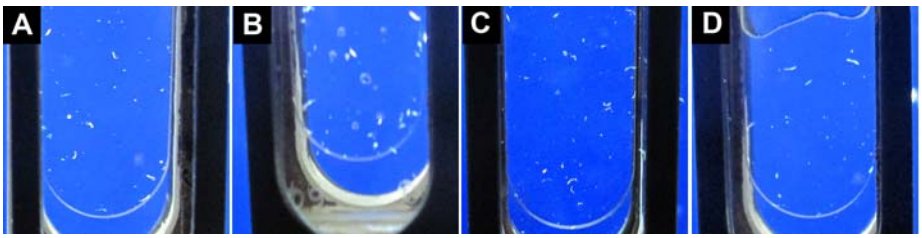

Figure S6. The optical images of hydrogels (between a pair of crossed polarizer) formed by (A) 0.4 wt% of **2a**, (B) 1.0 wt% of **2a**, (C) 0.4 wt% of **2b**, (B) 1.0 wt% of **2b**. The light spots are mainly coming from the bubbles and dusts, which also could be observed without polarized light.

TEM sample preparation. In this paper, we used negative staining technique to study the TEM images. The 400 mesh copper grids coated with continuous thick carbon film (~35nm) were first glowed discharge just before use to increase their hydrophilicity. After sample solution (3 μL) placed onto the grid, which should be sufficient to cover the grid surface, we then rinsed grid by dd-H_2O for three times. In this rinsing step, we first let the grid touch the water drop with the sample-loaded surface facing the parafilm, then gently absorb water from the edge of the grid with the aid of a filter paper sliver. Immediately after rinsing, the grid was stained by UA stain solution (2.0 % (w/v) uranyl acetate) for three times. Similar to the rinsing step, we first let the grid touch the stain solution drop with the sample-loaded surface facing the parafilm, then gently absorb the redundant stain solution from the edge of the grid using a filter paper sliver. Then we allow the grid to dry in air and examine the grid as soon as possible.

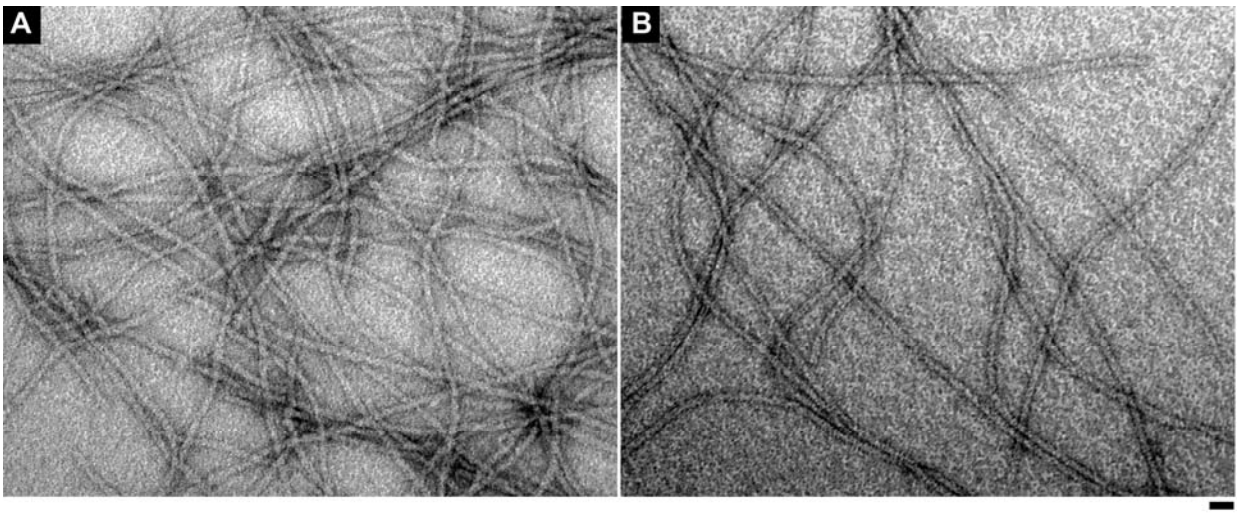

Figure S7. The TEM images of (A) 0.4 wt% of **2a** and (B) 0.4 wt% of **2b** with scale bar of 20 nm.

Rheological measurement. Rheological tests were conducted on TA ARES G2 rheometer with 25 mm cone-plate and TA Orchestrator Software during the experiment. The minimum volume of

hydrogel sample placed on the cone-plate was 0.2 mL. Here we perform both dynamic strain sweep and dynamic frequency sweep on our hydrogels

(1) Dynamic strain sweep: The measurement was performed at the frequency of 6.28 rad/s and temperature at 25 ^{o}C. Carried out with the "log" sweep mode, we applied strain to the hydrogel sample from 0.1 to 100 % (10 points per decade). The critical strain (γ_c) value was determined from the storage-strain profiles of the hydrogel sample. Over a certain strain, a drop in the elastic modulus was observed. Then we determined the strain amplitude (γ_c) at which storage moduli (G') just begins to decrease by 5 % from its maximum value, which was taken as a measure of the critical strain of the hydrogels and corresponded to the breakdown of the cross-linked network in the hydrogel samples.

(2) Dynamic frequency sweep: The frequency ranged from 200 rad/s to 0.1 rad/s, depending on the viscoelastic properties of each sample. A suitable strain, which was the average value around maximum storage moduli during dynamic strain sweep, was used to ensure the linearity of dynamic viscoelasticity.

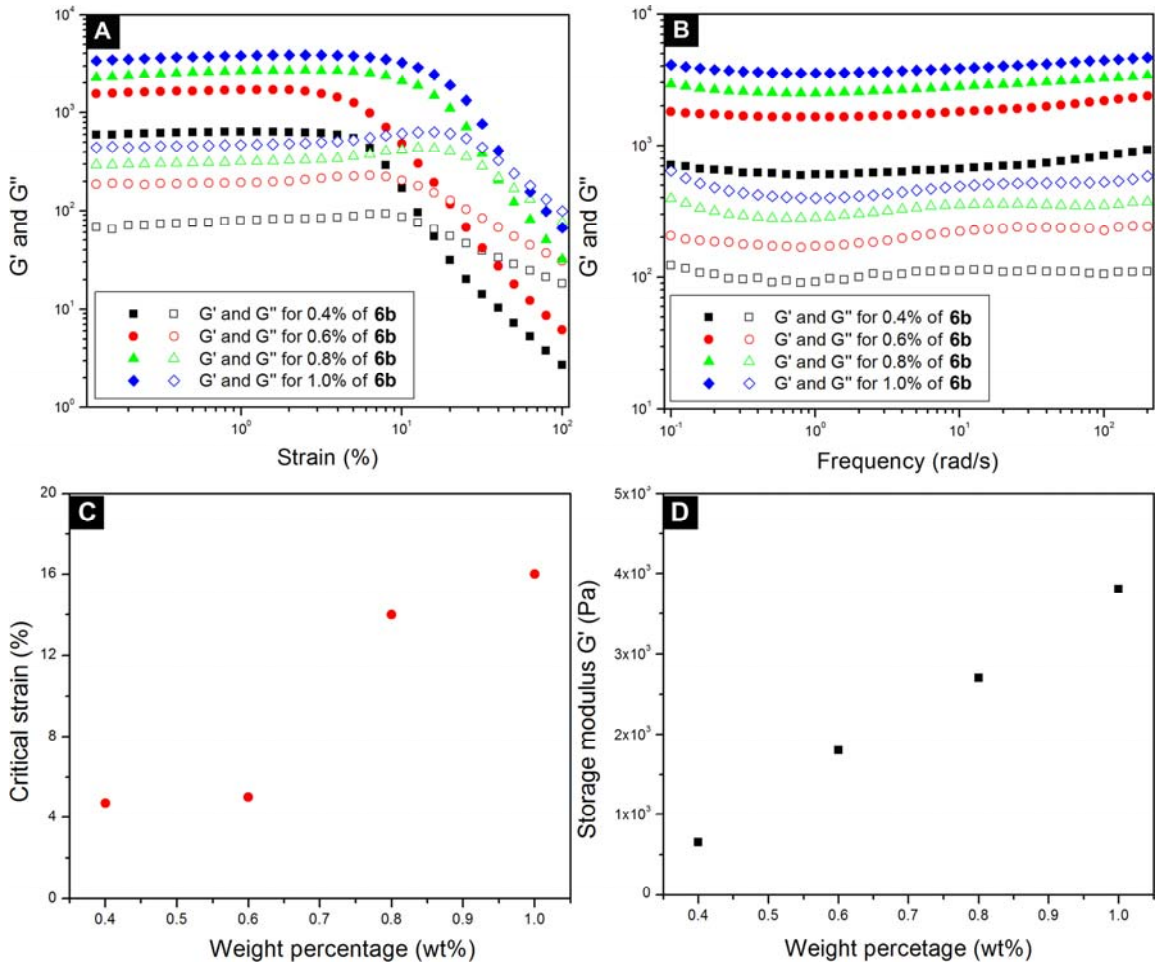

Figure S8. The strain (A) and frequency (B) dependence of dynamic storage modulus G' (solid) and loss modulus G'' (hollow) of the gels formed by **2b** upon the treatment of 1.0 U/mL enzyme at pH 7.6. The values of (C) critical strains and (D) storage moduli at frequency of 6.28 rad/s *vs*. concentrations of hydrogels of **2b**.

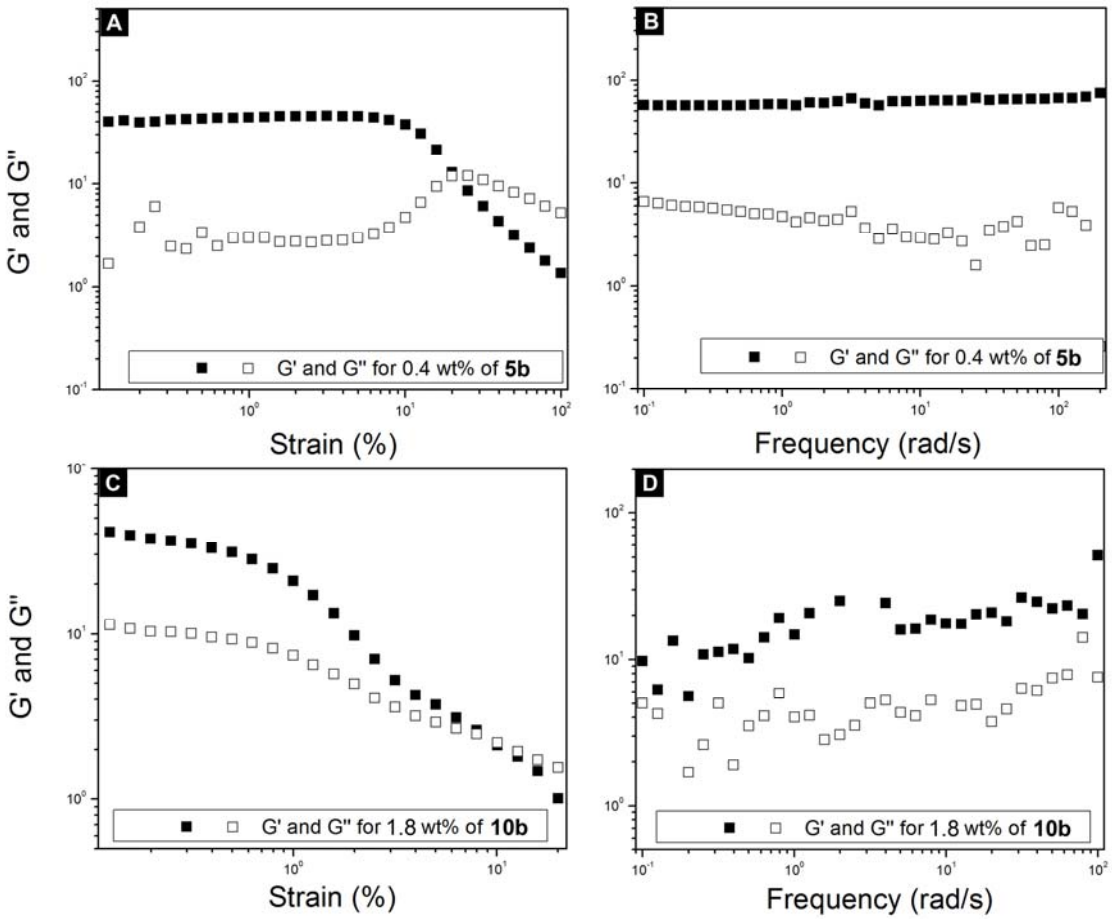

Figure S9. The strain (A) and frequency (B) dependence of dynamic storage modulus G' (solid) and loss modulus G'' (hollow) of the gels formed by 0.4 wt% of **5b** upon the treatment of 20.0 U/ml enzyme at pH 7.4; The strain (C) and frequency (D) dependence of dynamic storage modulus G' (solid) and loss modulus G'' (hollow) of the gels formed by 1.8 wt% of **10b** at pH 7.4.

S4. Biological applications and the *in vivo* tests

MTT assays for cytotoxicity. We seeded 5 x 10^5 (cells/well) of health HeLa cells into 96-well plate with 100 μL of MEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg ml21 streptomycin. The incubation at 37 ^oC and 5% CO_2 for 12 hours allowed HeLa cells to attach the bottom of 96-well plate. Then we replaced the medium by another 100 μL of growth medium that contained serial diluents of our compounds (0.5% DMSO) and then incubated the cells at 37 ^oC and 5% CO_2 for additional 72 hours. During the measurement of proliferation for HeLa cells, which were assayed into three days, we added 10 μL of (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 0.5 mg/mL) into the assigned wells in their corresponding day every 24 hours, which was followed by adding 100 μL of 0.1% sodium dodecyl sulfate (SDS) 4 hours later. Then we collected the assay results after another 24 hours incubation. Since the mitochondrial reductase in living cells reduced MTT to purple fomazan, the absorbance at 595 nm of the whole solution was finally measured by DTX 880 Multimode Detector. With MEM medium as blank and untreated HeLa cells as control, we measure each concentration of these compounds in triplicate. The IC_{50} values of our hydrogelators were read from their activity curves (with the measurement of 8 different concentrations) in day 3.

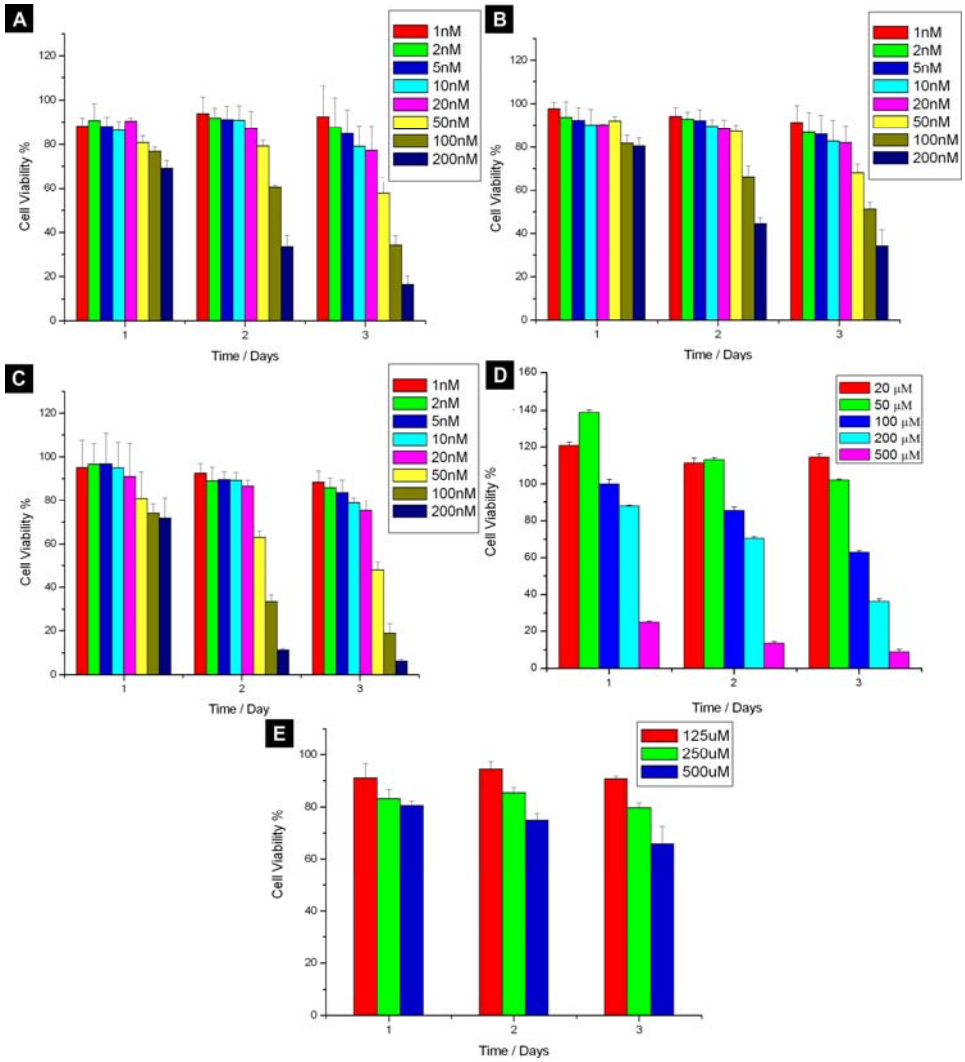

Figure S10. MTT assays for (A) **9b**, (B) **10b**, (C) **6**, (D) **4b**, and (E) **1b** on HeLa cells for 72 hours.

Live cell imaging. We seeded 2 x 10^5 of HeLa cells in Glass Chamber (Thermo Scientific Nunc Lab-Tek, 2-well) with MEM medium (2 ml) that was supplemented with 10% FBS, 100 U/mL penicillin and 100 mg ml21 streptomycin for 4 h to allow the cell attachment. In order to perform the PTP1B Inhibition assay, we then replaced the culture medium in both wells and re-incubated the cells for 1 h, for which one-half of the wells was incubated in the medium containing 25 μM CinnGEL 2Me (novel inhibitor of PTP1B, prepared by reconstitution in DMSO), other well as a control was in the culture medium plus the same volume of DMSO.[3] After that, we replaced the medium and washed the HeLa cells with PBS buffer for three times. Then we fixed the cell-containing glass chamber on the confocal microscope stage, and replaced the PBS buffer with 1 ml solution of our fluorescent hydrogelators (500 μM, dissolved in PBS buffer) for each well. The sample we added into the PTP1B inhibition well also contained 25 μM of CinnGEL 2Me. Thereafter fluorescent images were captured immediately in the xyt mode with a delay of 11.64 s between frames.

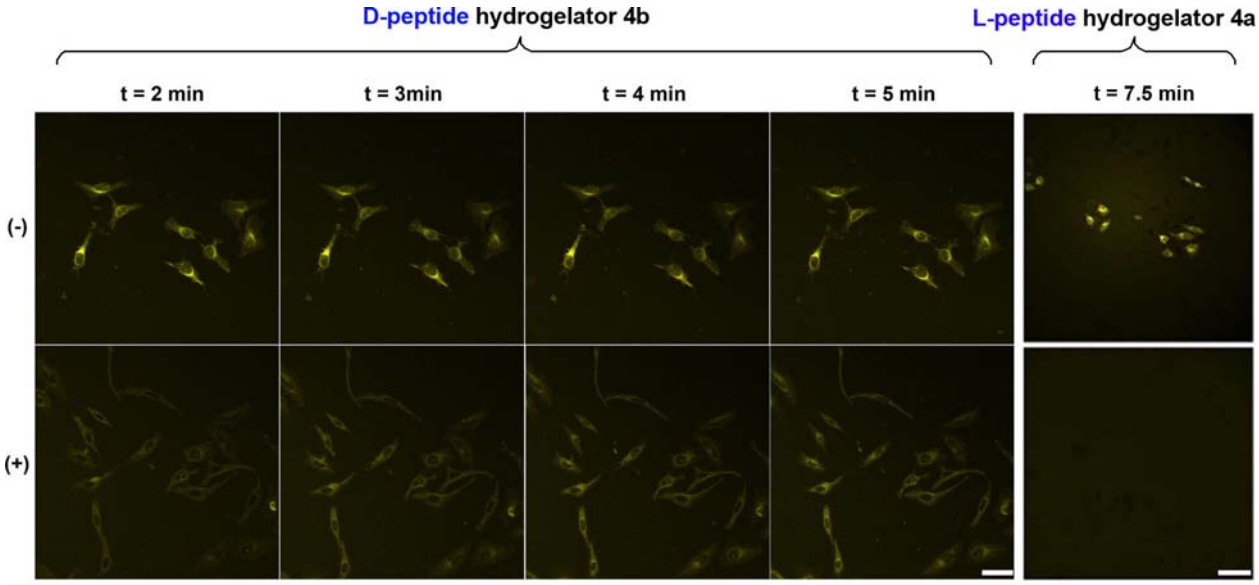

Figure S11 The time course of HeLa cells incubated with 500 μM of **4b** and **4a** without (-) or with (+) the PTP1B inhibitor (25 μM) (scale bar is 50 μm) within 7.5 minutes.

***In vivo* evaluation of antitumor activity.** Female Balb/c mice were incubated with 2 x 10^5 4T1-luciferase cells in the mammary fat pad. Tumor growth was monitored every other day and the tumor volume was calculated by the formula: length x width x (Length + Width)/2. Once tumors size reached around 500mm^3, we randomly divided mice into different treatment groups. (a) 4 x 10 mg/kg of taxol formulated with Cremophor EL was intravenous injected (I.V.) every other day from day 0 (the day giving drugs) for indicated times; (b) 10 mg/kg of our hydrogel in 40 μl volume was intratumoral injected at day 0; (c) the PBS vehicle control was intratumoral injected at day 0. Mice died immediately with injecting 40 mg/kg of taxol in one injection due to its cytotoxicity. The taxol containing hydrogels **10a** and **10b** were prepared by enzyme treatment in PBS buffer (pH 7.4) before their intratumoral injections, which could sustain one month. Mice weight was monitored after receiving treatment and presented as relative weight (%).

Biostability test in the presence of proteinase K. 1 mg of **1a** and **1b** were dissolved in 5 mL of HEPES buffer at pH 7.5, respectively. Then 3.2 U/mL of proteinase K were added into both

solutions, which followed by incubation at 37 °C for 24 hr. 50 μL of sample was taken out at 1, 2, 4, 8, 12, and 24h and analyzed by HPLC.

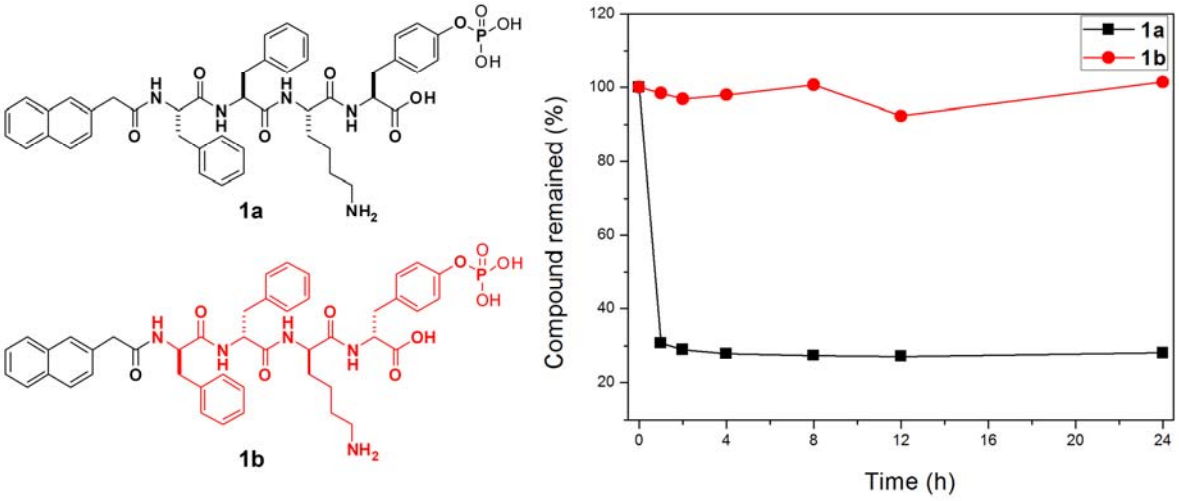

Figure S12. The molecular structures of **1a** and **1b**, and their time-dependent course of the digestions by proteinase K.

References:

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(3) Ishino, Y.; Zhu, C.; Harris, D. L.; Joyce, N. C. *Mol. Vis.* 2008, *14*, 61.