Electronic Supplementary Information for :

Supramolecular Silk from a Peptide Hydrogel

Jie Zhan, Yanbin Cai, Shenglu Ji, Yuna Shang, Jie Gao* and Zhimou Yang*

Materials and general methods:

Chemicals: Fmoc-amino acids were obtained from GL Biochem (Shanghai, China). All the other Starting materials were obtained from Alfa. Chemical reagents and solvents were used as received from commercial sources.

Peptide Synthesis: The peptide derivative was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 0.8 mmol/g. 20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used during deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, 2-naphthylacetic acid was used to attach on the peptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 mL per gram of resin), followed by five steps of washing using DCM for 1 min (5 mL per gram of resin). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TIS and 2.5% of H_2O for 1 hours. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was centrifuged for 10 min at 4 ˚C at 10,000 rpm. Then the supernatant was decanted and the resulting solid was directly used in the next synthesis steps.

Preparation of the hydrogels (2.0 wt.%): 10 mg of peptide was dispersed in 0.4 mL

of PBS buffer solution (pH = 7.4). Na_2CO_3 (1 M) was added to the above solution to adjust the final pH=7.4, then PBS was pipetted into the solution to 0.5 mL. The solution was heated to dissolve the powders completely and gel was formed after the hot solution being kept at room temperature (22-25℃) for about 1 hour.

Rheology: Rheology test was done on an AR 2000ex (TA instrument) system, 40 mm parallel plates was used during the experiments at the gap of 500 μm. Dynamic frequency sweep was characterized by the mode of in the region of 0.1-100 rad/s at the strain of 0.1%.

Transmission electron microscopy (TEM): TEM samples (2 wt.%) were prepared at 25 °C. A micropipet was used to load 5 μL of sample solution to a carbon coated copper grid. The excess solution was removed by apiece of filter paper. The samples were dyed by 10 μL uranyl acetate for 30 seconds and dried overnight in a desiccator and then conducted on a Tecnai G2 F20 system operating at 200 kV. Samples with external forces were prepared by putting the copper grid into the gel with a tweezers and stirred gently, then pulled the copper grid out.

Scanning electron microscope (SEM): SEM samples were prepared at 25°C, samples were affixed onto aluminum stubs with carbon tape and observed by SEM (Quanta 200, Czech).

Anisotropy birefringence: Anisotropy birefringence test were done on polarization microscope (OLYMPUS BX41) at crossed-field by rotate the plain stage at different angle.

Characterization of Nap-FGG: ^{1}H NMR (400 MHz, DMSO-d6): δ 8.47 – 8.33 (m, 2H), 8.11 (t, J = 5.8 Hz, 1H), 7.86 (d, J = 7.1 Hz, 1H), 7.77 (m, J = 14.5, 8.7 Hz, 2H), 7.61 (s, 1H), 7.47 (t, J = 7.2 Hz, 2H), 7.27 – 7.12 (m, 6H), 4.62 – 4.51 (m, 1H), 3.76 (d,

J = 5.6 Hz, 4H), 3.69 – 3.58 (m, 3H), 3.06 (d, J = 13.7 Hz, 1H), 2.79 (dd, J = 16.6, 7.1 Hz, 1H). HR-MS: calcd. M^+ = 447.1794, obsvd. $(M + H)^+$ = 448.1870.

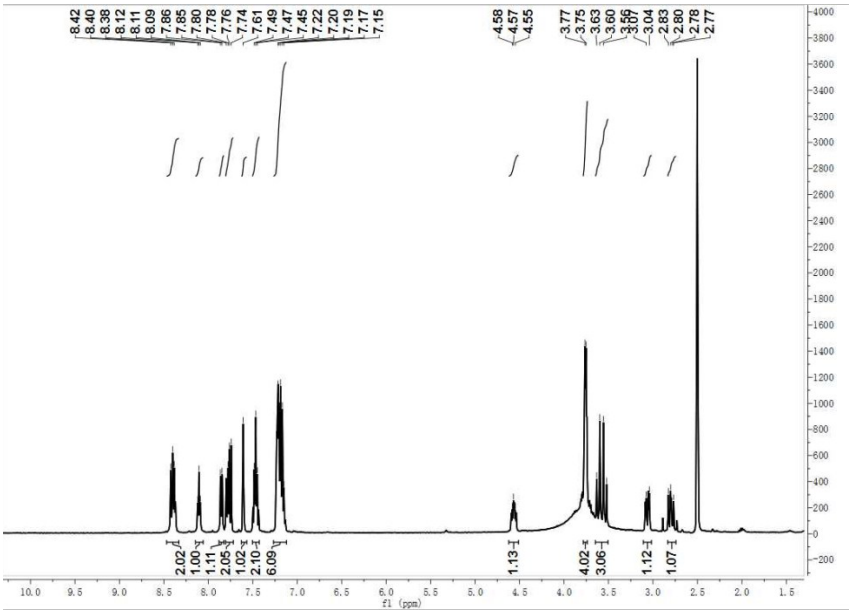


Figure S1. ^{1}H NMR of Nap-FGG

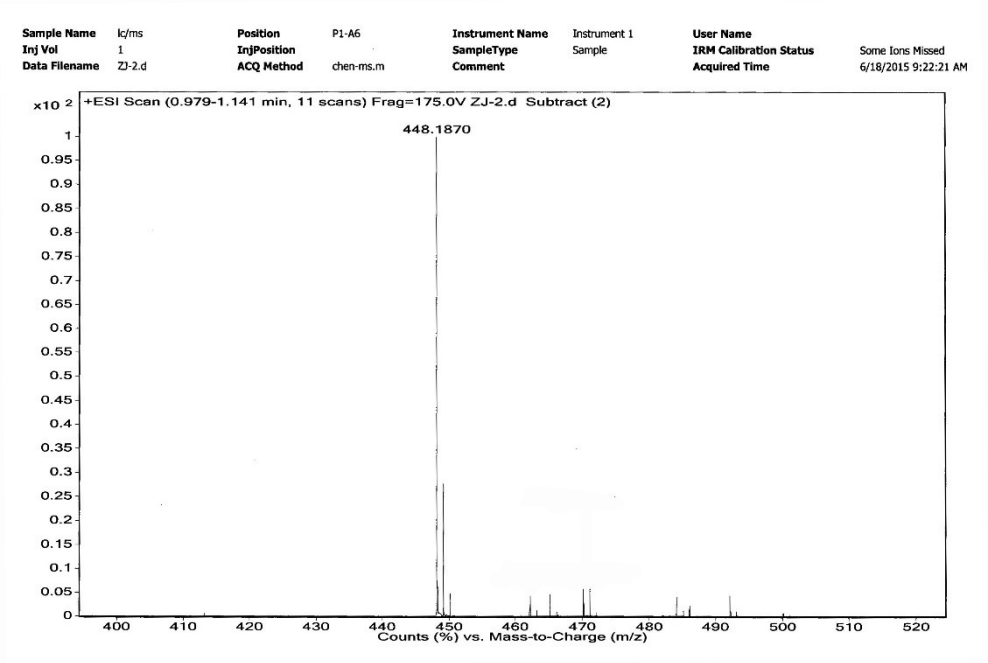


Figure S2. HR-MS of Nap-FGG

Characterization of compound Nap-FG: ^{1}H NMR (400 MHz, DMSO) δ 8.44 – 8.36 (m, 2H), 7.85 (d, J = 8.8 Hz, 1H), 7.76 (dd, J = 15.0, 7.8 Hz, 2H), 7.59 (s, 1H), 7.46 (dd, J = 10.1, 4.4 Hz, 2H), 7.25 – 7.15 (m, 6H), 4.59 (td, J = 10.2, 3.9 Hz, 2H), 3.79

(dd, J = 5.8, 2.5 Hz, 2H), 3.65-3.56 (m, 3H), 3.05 (dd, J = 13.7, 4.0 Hz, 1H), 2.77 (dd, J = 13.7, 10.4 Hz, 1H). HR-MS: calcd. M^+= 390.1580, obsvd. $(M + H)^+$=391.1637.

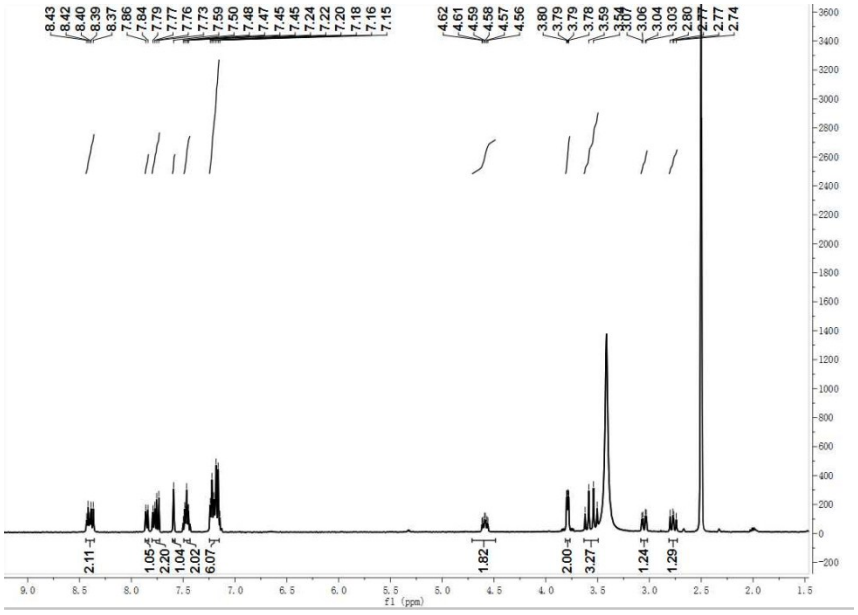


Figure S3. ^{1}H NMR of Nap-FG

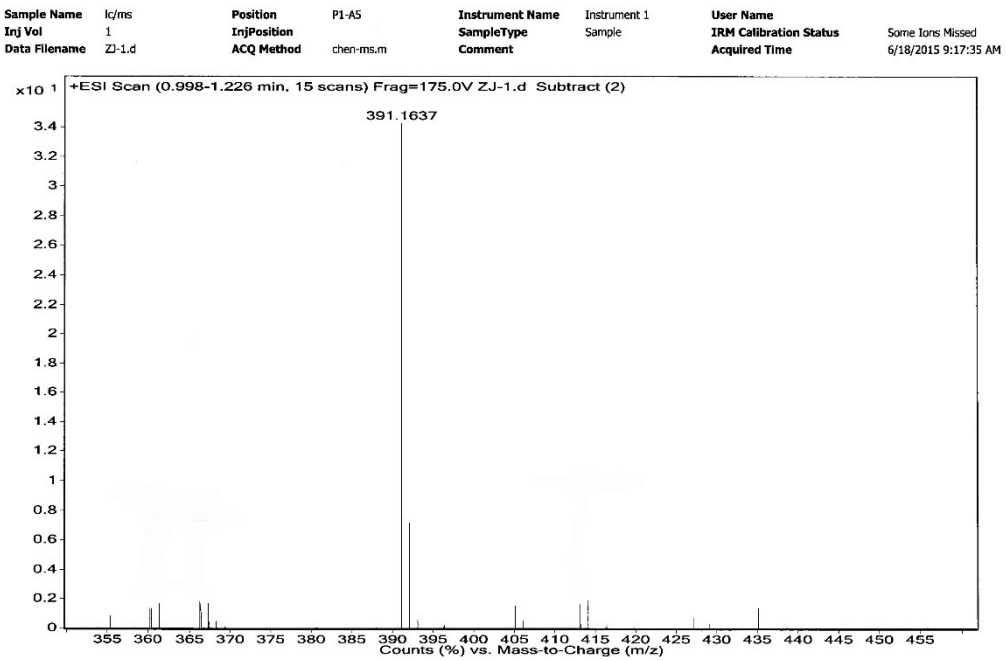


Figure S4. HR-MS of Nap-FG

Characterization of compound Nap-FG$_3$: 1H NMR (400 MHz, DMSO) δ 8.40 (d, J = 8.3 Hz, 1H), 8.36 (t, J = 5.6 Hz, 1H), 8.16 (t, J = 5.8 Hz, 1H), 8.10 (t, J = 5.9 Hz, 1H),

7.85 (d, J = 8.8 Hz, 1H), 7.77 (dd, J = 14.4, 8.5 Hz, 2H), 7.60 (s, 1H), 7.47 (t, J = 6.5 Hz, 2H), 7.27 – 7.07 (m, 6H), 3.78 – 3.73 (m, 5H), 3.64 – 3.53 (m, 4H), 3.06 (dd, J = 13.7, 4.2 Hz, 1H), 2.82-2.79 (m, 1H). HR-MS: calcd. M^+ = 504.2009, obsvd. $(M + H)^+$ = 505.2090.

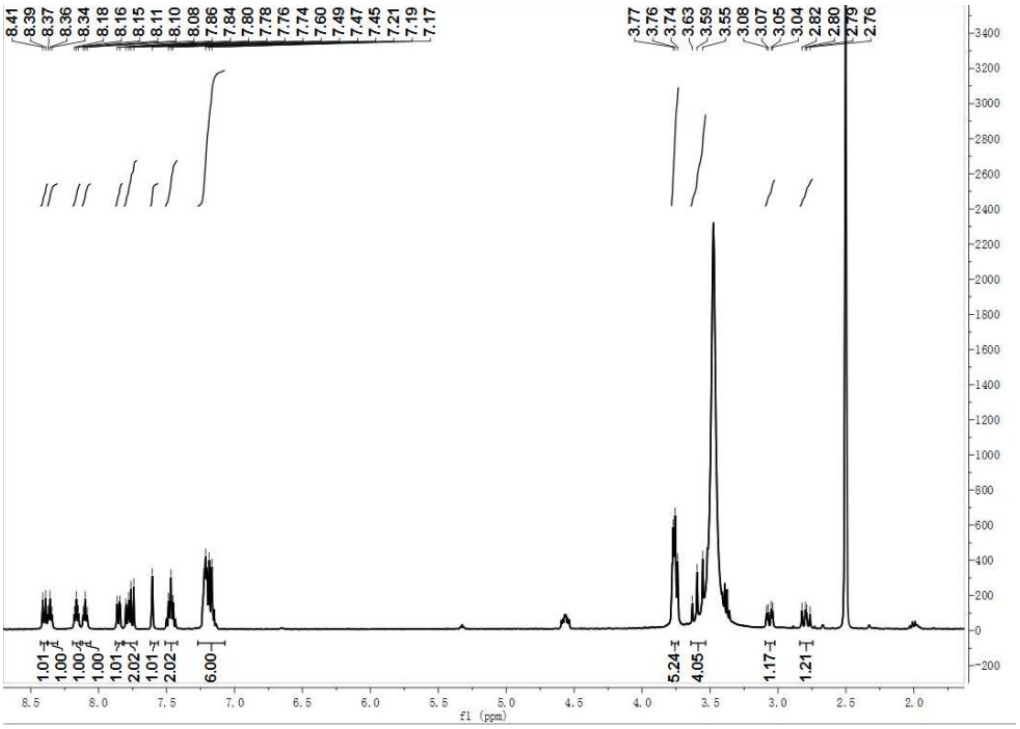


Figure S5. ^{1}H NMR of Nap-FG_3

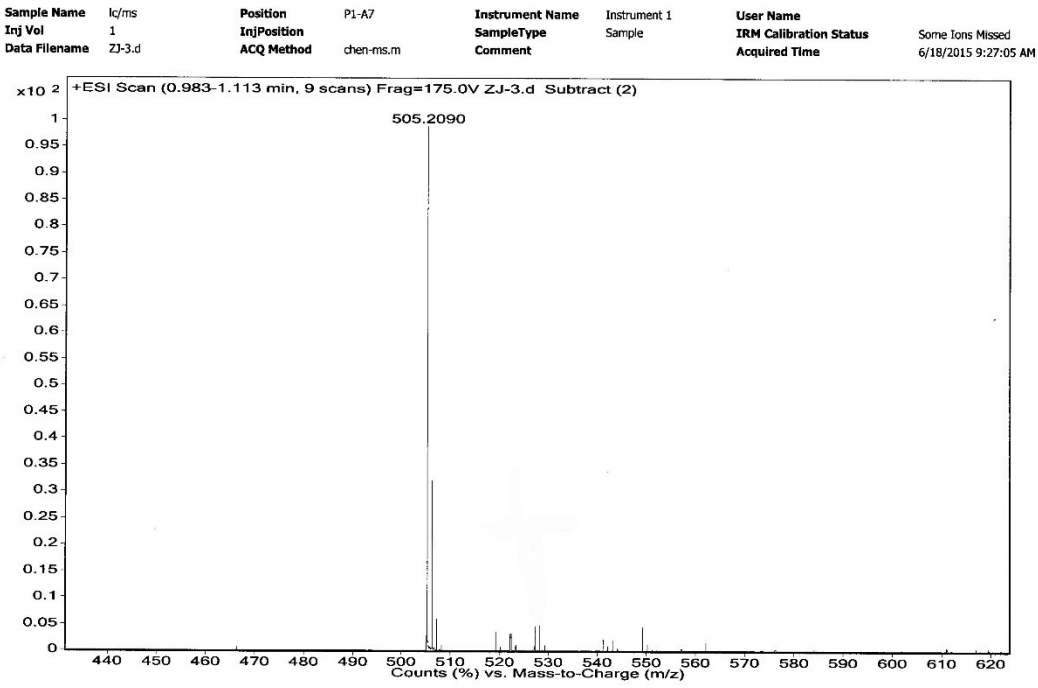


Figure S6. HR-MS of Nap-FG_3

Characterization of compound Nap-FG$_4$: ^{1}H NMR (400 MHz, DMSO) δ 8.42 – 8.34 (m, 2H), 8.16 (d, J = 3.3 Hz, 2H), 8.08 (t, J = 5.6 Hz, 1H), 7.86 (d, J = 7.3 Hz, 1H), 7.77 (dd, J = 13.9, 7.8 Hz, 2H), 7.60 (s, 1H), 7.47 (t, J = 6.5 Hz, 2H), 7.24 – 7.15 (m, 6H), 3.77 (t, J = 6.0 Hz, 8H), 3.72-3.57 (m, 3H), 3.06 (dd, J = 13.8, 4.2 Hz, 1H), 2.79 (dd, J = 13.7, 10.1 Hz, 1H). HR-MS: calcd. M^+ = 561.2223, obsvd. $(M + H)^+$ =562.2308.

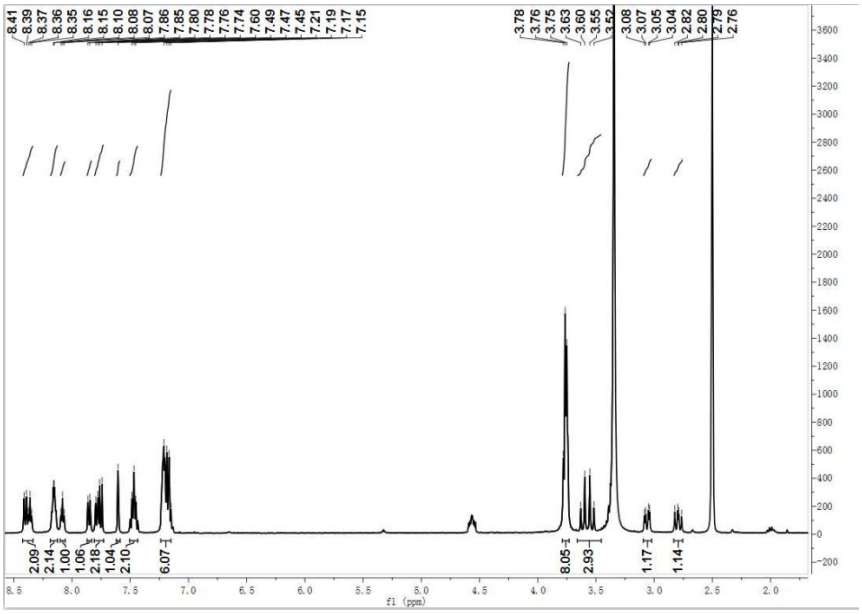


Figure S7. ^{1}H NMR of Nap-FG$_4$

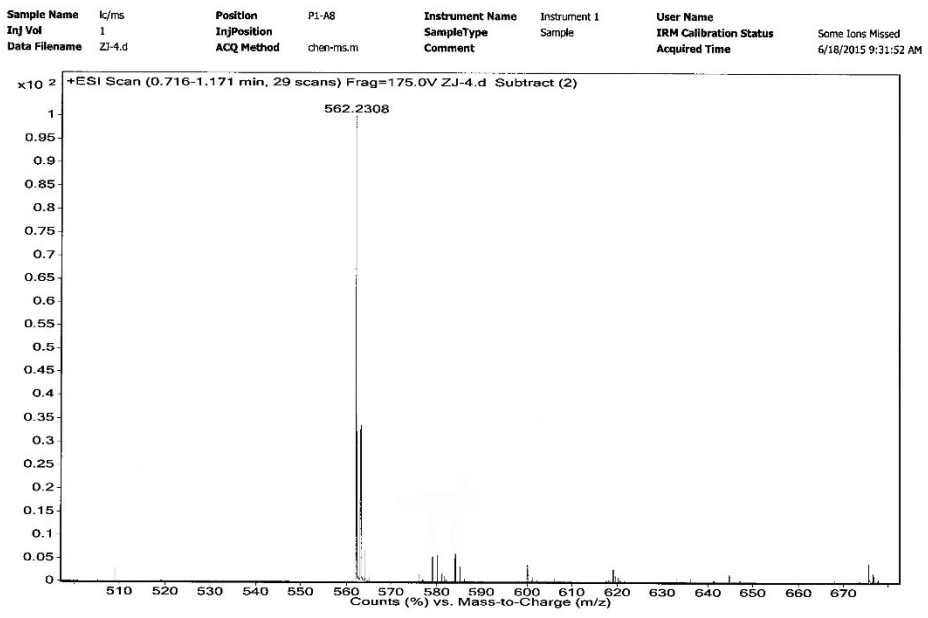


Figure S8. HR-MS of Nap-FG$_4$

Characterization of compound NapF$_2$G: ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 8.50 – 8.05 (m, 3H), 7.99 – 7.65 (m, 3H), 7.57 (s, 1H), 7.51 – 7.34 (m, 2H), 7.31 – 7.03 (m, 11H), 4.53 (dd, *J* = 14.5, 6.1 Hz, 2H), 3.78 (d, *J* = 5.8 Hz, 2H), 3.52 (dd, *J* = 28.0, 14.0 Hz, 2H), 3.10 – 2.92 (m, 2H), 2.76 (ddd, *J* = 28.9, 13.7, 9.8 Hz, 2H). HR-MS: calc. M^+ = 537.2336, obsvd. $(M+H)^+$ = 538.2332.

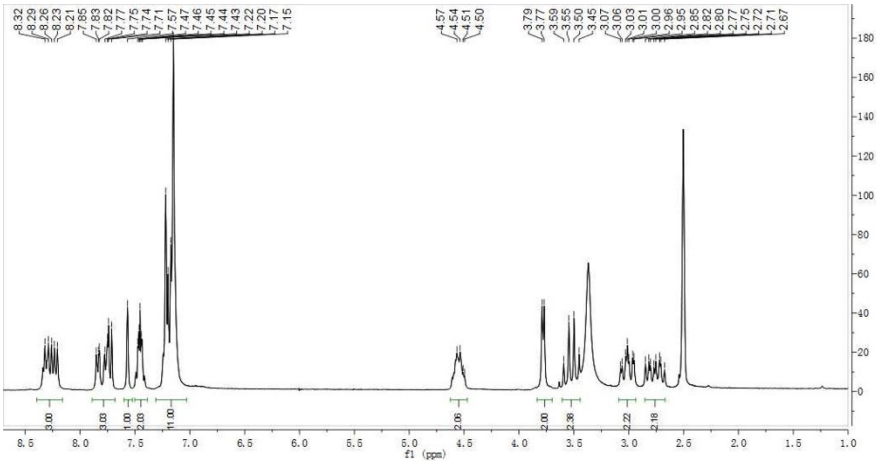


Figure S9. ^{1}H-NMR of Nap-F$_2$G

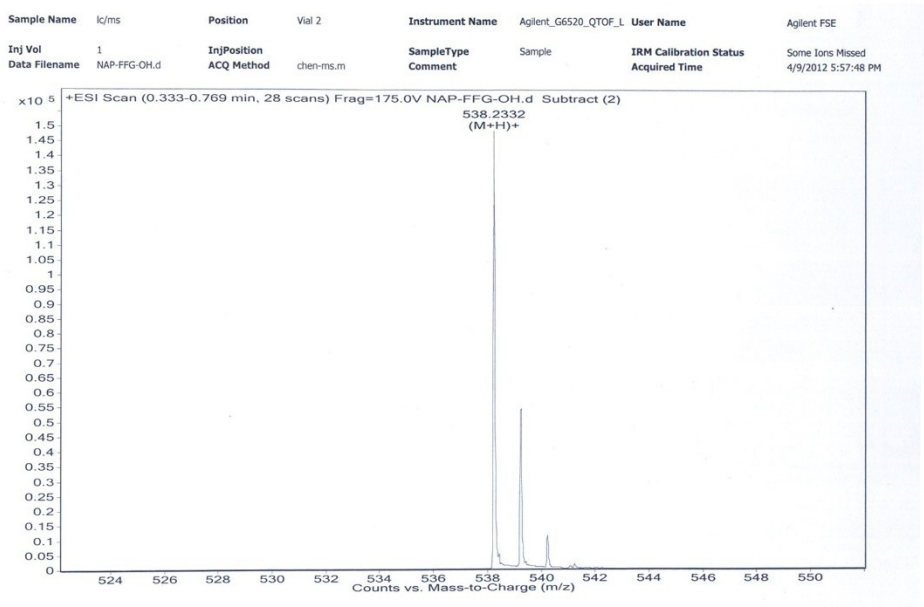


Figure S10. HR-MS of Nap-F$_2$G

Characterization of Nap-F$_3$G: ^{1}H NMR (300 MHz, DMSO-d6): δ 8.20 (m, J = 16.6, 9.6 Hz, 3H), 8.06 (s, 1H), 7.82 (d, J = 9.1 Hz, 1H), 7.73 (dd, J = 12.1, 8.9 Hz, 2H), 7.55 (s, 1H), 7.47 – 7.35 (m, 2H), 7.25 – 7.17 (m, 4H), 7.18 – 7.02 (m, 13H), 4.68 – 4.40 (m,

4H), 3.77 (d, J = 5.8 Hz, 4H), 3.50 (m, J = 29.8, 14.1 Hz, 5H). LC-MS: calcd. M^+ = 684.78, obsvd. $(M + H)^+$ = 685.40.

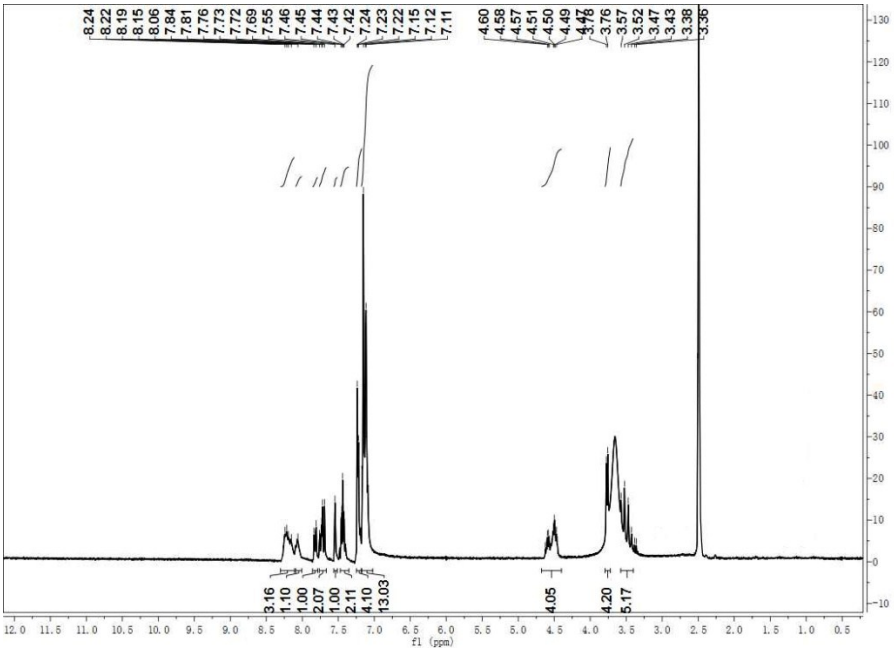


Figure S11. ^{1}H NMR of Nap-F_3G

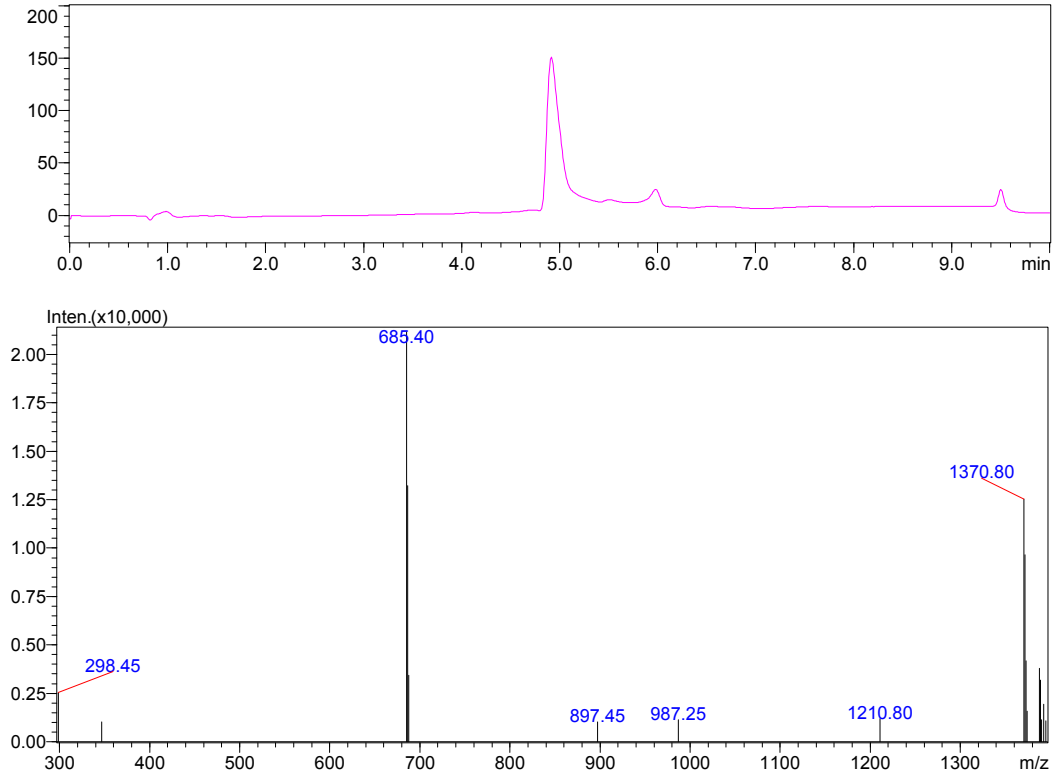


Figure S12. LC-MS of Nap-F_3G

Characterization of Nap-F_2G_2: ^{1}H NMR (300 MHz, DMSO-d6) δ 8.25 (m, J = 16.5,

7.8 Hz, 3H), 8.08 (s, 1H), 7.83 (d, J = 8.8 Hz, 1H), 7.78 – 7.67 (m, 2H), 7.56 (s, 1H), 7.49 – 7.38 (m, 2H), 7.16 (m, J = 16.4, 6.9 Hz, 11H), 4.58 – 4.48 (m, 2H), 3.75 (dd, J = 12.3, 6.0 Hz, 7H), 3.61 – 3.35 (m, 3H). LC-MS: calcd. M^+ = 595.15, obsvd. $(M + H)^+$ = 594.66

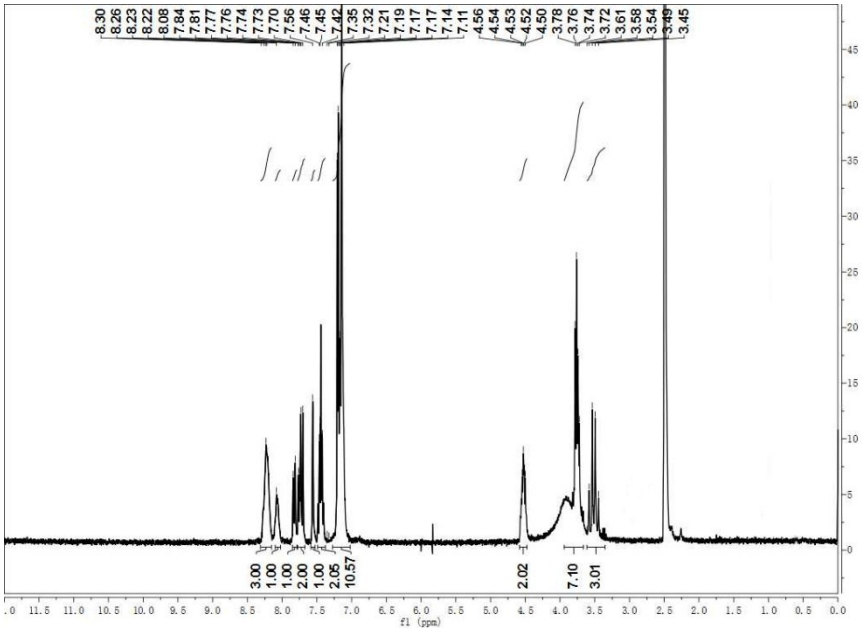
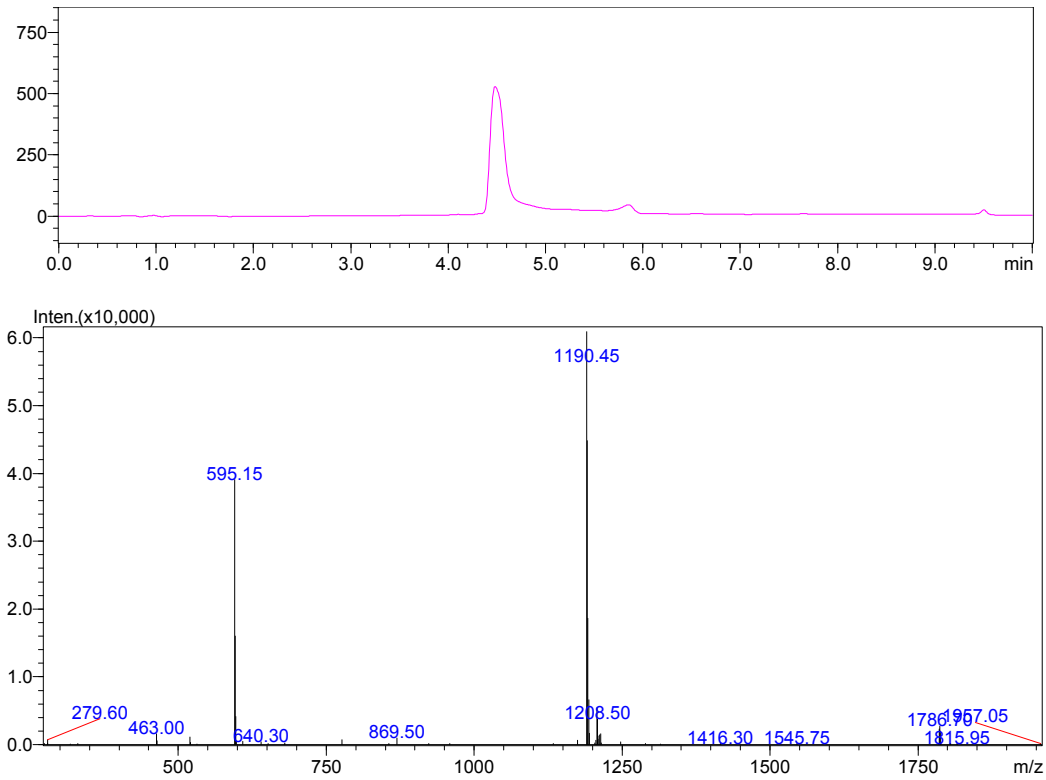


Figure S13. ^{1}H NMR of Nap-F_2G_2



. **Figure S14.** LC-MS of Nap-F_2G_2

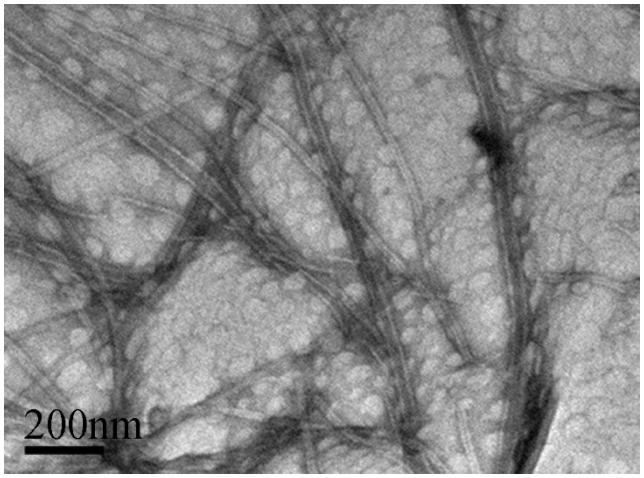


Figure S15. TEM image of the hydrogel formed by 2.0 wt.% of Nap-FGG at day 3 without external forces

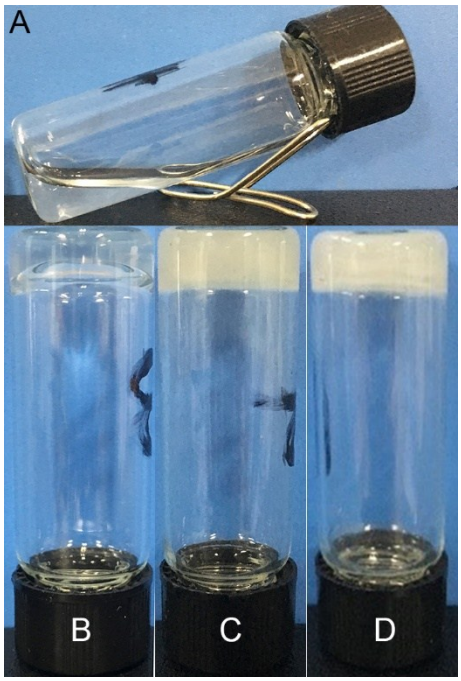


Figure S16. Optical images of hydrogels or solution containing (A) 0.5, (B)1.0, (C)3.0, (D)4.0 wt.% of Nap-FGG

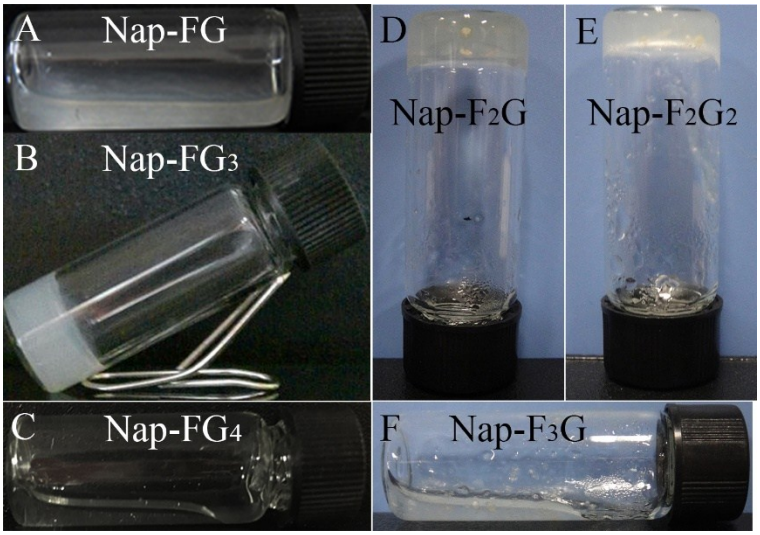


Figure S17. Optical images of hydrogels or solution of (A) Nap-FG, (B)Nap-FG_3, (C)Nap-FG_4, (D)Nap-F_2G, (E)Nap-F_2G_2, (F)Nap-F_3G

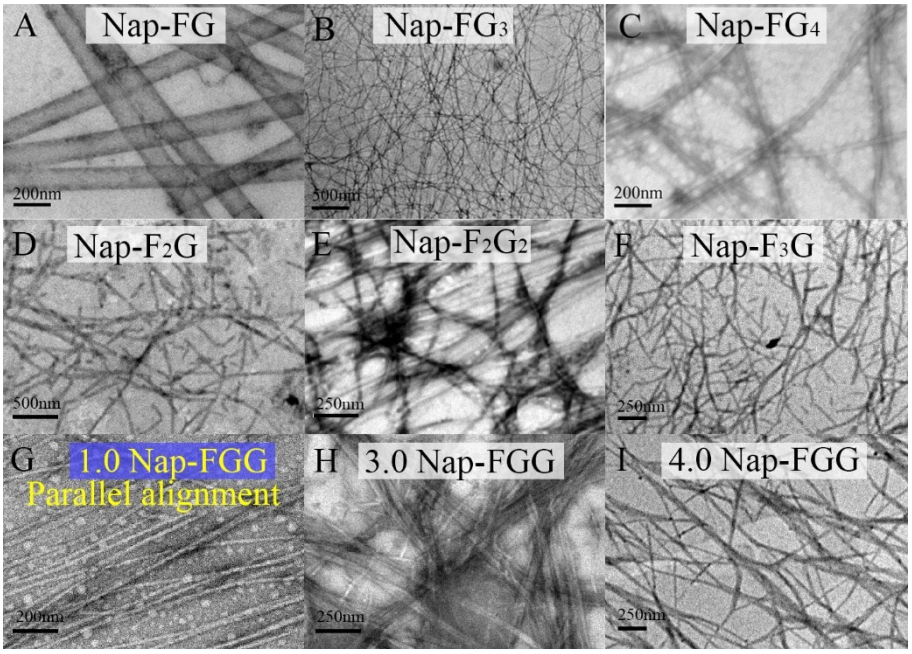


Figure S18. TEM images of nanostructures in samples of (A)Nap-FG, (B)Nap-FG_3, (C)Nap-FG_4, (D) Nap-F_2G, (E) Nap-F_2G_2, (F) Nap-F_3G, (G) 1.0 wt.% of Nap-FGG, (H) 3.0 wt.% of Nap-FGG, (I) 4.0 wt.% of Nap-FGG after external force was applied

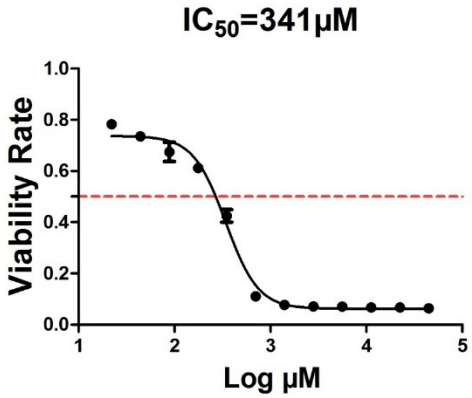


Figure S19. IC_{50} of Nap-FGG against NIH 3T3 mouse fibroblast cells at 24h. (Mean ± SEM, n=5)