Supporting Information for

Aromatic-Aromatic Interactions Enhance Interfiber Contacts for Enzymatic Formation of A Spontaneously Aligned Supramolecular Hydrogel

Jie Zhou, Xuewen Du, Yuan Gao,[†] Junfeng Shi, and Bing Xu*
Department of Chemistry, Brandeis University, 415 South Street, Waltham, MA 02454, USA

Content

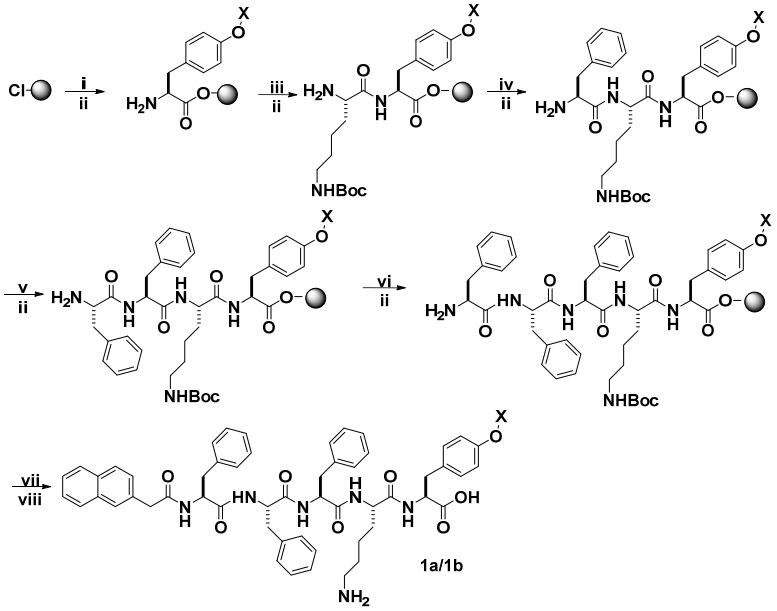
S1. Experimental materials and instruments

We used all the solvents and chemical reagents directly as receiving from the commercial sources without further purification. We purified all the products (**1a**, **1b**, **2a**, and **2b**) with Water Delta600 HPLC system, equipped with an XTerra C18 RP column and an in-line diode array UV detector. We got ^{1}H-NMR spectra on Varian Unity Inova 400, LC-MS spectra on a Waters Acouity ultra performance LC with Waters MICRO-MASS detector, Rheological data on TA ARES G2 rheometer with 25 mm cone plate, TEM images on Morgagni 268 transmission electron microscope, and polarized optical microscopy retardance images on an inverted microscope (Nikon TE-2000), equipped with traditional polarization optics, a DIC module, a fluorescence imaging module and 2D LC-Polscope microscopy.

S2. Synthesis and characterizations

We prepared the hydrogelator precursors (**1a** and **2a**) and corresponding hydrogelators (**1b** and **2b**) by the standard solid-phase peptide synthesis (SPPS)[1], which used 2-chlorotriyl chloride resin (100-200 mesh and 0.3-0.8 mmol/g) and N-Fmoc-protected amino acids with side chains properly protected (e.g., Fmoc-Lys(Boc)-OH). We prepared Fmoc-Tyr(PO_3H_2)-OH, directly used in SPPS, from Tyr-OH based on previous work.[2]

The following scheme illustrates the synthetic procedure of **1a**/**1b**, and the synthesis of **2a**/**2b** is similar to that of **1a**/**1b**.



Scheme S1. The synthetic route of **1a**/**1b**

1a: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 8.1 Hz, 1H), 8.18 (d, J = 7.8 Hz, 1H), 8.11 (d, J = 8.0 Hz, 2H), 8.05 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.74 (dd, J = 8.0, 4.0 Hz, 3H),

7.56 (s, 1H), 7.45 (m, 2H), 7.26 - 7.20 (dd, J = 16.0, 8.0 Hz, 5H), 7.17 - 7.10 (m, 12H), 7.06 (d, J = 8.0 Hz, 2H), 4.51 - 4.39 (m, 5H), 4.29 - 4.23 (dd, J = 16.0, 8.0 Hz, 1H), 3.47 (dd, J = 40.0, 12.0 Hz, 3H), 3.03 (d, J = 4.0 Hz, 2H), 2.96 - 2.87 (m, 3H), 2.81 - 2.65 (m, 5H), 2.60 (s, 2H), 1.54 - 1.21 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.84, 171.42, 170.94, 170.78, 169.87, 150.84, 137.78, 134.02, 133.93, 132.93, 132.32, 131.84, 130.91, 130.10, 129.29, 128.88, 128.81, 128.53, 128.29, 127.37, 127.30, 127.19, 126.89, 126.73, 126.38, 126.28, 125.49, 125.21, 124.78, 120.51, 118.90, 54.11, 53.06, 52.71, 51.74, 43.53, 42.23, 39.94, 39.73, 39.31, 37.55, 35.83, 28.00, 22.14; ^{31}P NMR (162 MHz, DMSO-d_6): δ -5.96; ESI MS(m/z) [M]+ calcd. for $C_{54}H_{59}N_6O_{11}P$ 998.40, found $[M-H]^-$ 997.30.

1b[3]: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.69 (s, 1H), 9.22 (s, 1H), 8.26 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.11 (d, J = 8.0 Hz, 2H), 8.07 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.75 (dd, J = 8.0, 4.0 Hz, 3H), 7.57 (s, 1H), 7.46 (m, 2H), 7.25 - 7.12 (m, 15H), 7.01 (d, J = 8.0 Hz, 2H), 6.66 (d, J = 8.0 Hz, 2H), 4.59 - 4.49 (m, 3H), 4.40 - 4.30 (m, 2H), 3.50 (dd, J = 40.0, 12.0 Hz, 2H), 3.37 (d, J = 4.0 Hz, 2H), 2.93 - 2.84 (m, 3H), 2.81 - 2.65 (m, 5H), 2.54 (s, 2H), 1.69 - 1.29 (m, 6H). ESI MS(m/z) [M]+ calcd. for $C_{54}H_{58}N_6O_8$ 918.43, found $[M-H]^-$ 917.34.

2a: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 7.8 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.73 (dd, J = 8.0, 4.0 Hz, 3H), 7.58 (s, 1H), 7.47 (m, 2H), 7.22 - 7.14 (m, 12H), 7.07 (d, J = 8.0 Hz, 2H), 4.56 - 4.42 (m, 4H), 4.25 - 4.23 (dd, J = 8.0, 4.0 Hz, 1H), 3.53 (dd, J = 40.0, 12.0 Hz, 3H), 3.07 - 2.92 (m, 3H), 2.84 - 2.63 (m, 5H), 2.54 (s, 2H), 1.47 - 1.17 (m, 6H). ESI MS(m/z) [M]+ calcd. for $C_{45}H_{50}N_5O_{10}P$ 851.33, found $[M-H]^+$ 852.64 and $[M-H]^-$ 850.69.

2b: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.69 (s, 1H), 9.22 (s, 1H), 8.26 (d, J = 8.1 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.69 (dd, J = 8.0, 4.0 Hz, 3H), 7.58 (s, 1H), 7.47 (m, 2H), 7.18 - 7.10 (m, 10H), 7.01 (d, J = 8.0 Hz, 2H), 6.65 (d, J = 4.0 Hz, 2H), 4.63 - 4.43 (m, 2H), 4.43 - 4.25 (m, 2H), 3.53 (dd, J = 40.0, 12.0 Hz, 2H), 3.08 - 2.87 (m, 3H), 2.87 - 2.60 (m, 5H), 2.54 (s, 2H), 1.46 - 0.95 (m, 6H). ESI MS(m/z) [M]+ calcd. for $C_{45}H_{49}N_5O_7$ 771.36, found $[M-H]^+$ 773.19 and $[M-H]^-$ 770.46.

S3. General procedures for hydrogel preparation.

Enzymatic gelation: We dissolved **1a**/**2a** (4 mg) into distilled water (450 mL), and adjusted pH of the solution, monitored by pH paper, carefully by adding 1M NaOH. After the pH of the solution reaches 7.4, we then added extra distilled water to make the final concentration of 0.8 wt%, followed by the addition of alkaline phosphatase (ALP).

Gelation by changing pH: We dissolved **1b**/**2b** (4 mg) into distilled water (400 mL) by adjusting the pH of the solution with 1 M NaOH. Monitoring the pH by pH paper, we found the solution is rather basic when it became clear. With careful addition of 0.2 M HCl, we adjusted the pH of the solution to 7.4, leading to the formation of hydrogel, followed by the addition of water to make the final concentration of 0.8 wt%.

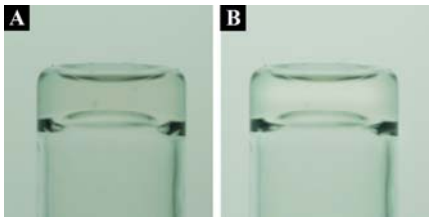


Fig S1. Optical image of **Gel$_{1b}$** (A) and **Gel$_{2b}$** (B), formed by treating the solution of **1a** or **2a** with ALP (2U/mL), at the concentration of 0.8 wt% and pH of 7.4 in water.

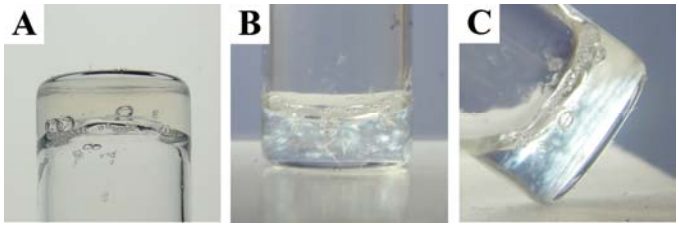


Fig S2. (A) Optical images of **Gel$_{1b}$'**, at the concentration of 0.8 wt% and pH of 7.4 in water, (B, C) the same sample is placed between cross polarizers in two different orientations, illuminating by ambient light.

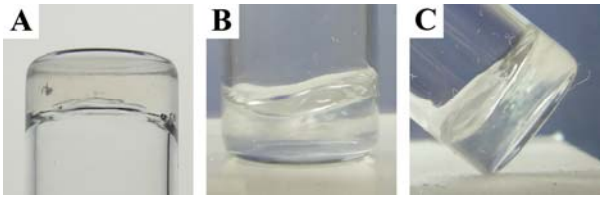


Fig S3. (A) Optical images of **Gel$_{2b}$'**, at the concentration of 0.8 wt% and pH of 7.4 in water, (B, C) the same sample is placed between cross polarizers in two different orientations, illuminating by ambient light.

S4. Polarized optical microscopy sample preparation.

We assembled light microscopy chambers using glass slides and coverslips (Goldseal, Fisher Scientific) with a layer of unstreched Parafilm and two layers of double-sticky film as a spacer with a height of 235 μm. We prepared gel or solution samples in vials and transferred them into the chambers just before image taking. The samples, sealed with ultraviolet-cured glue (Norland Optical) remained good for weeks.

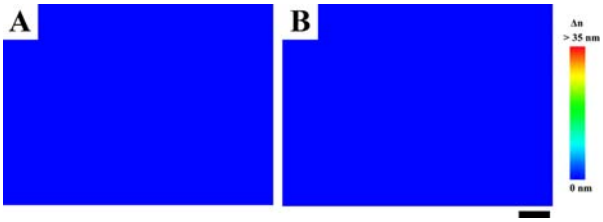


Fig S4. Polarized optical microscopy retardance images (10x magnification) of (A) solution of **1a**, (B) solution of **2a**. The images are taken with a sample thickness of 235μm and concentration of 0.8 wt% (pH = 7.4). The scale bar is 100 μm.

S5. TEM sample preparation.

In this paper, we used negative staining technique to study the TEM images. We first glowed discharge the 400 mesh copper grids coated with continuous thick carbon film (~ 35 nm) prior to use to increase the hydrophilicity. After loading samples (4 μL) on the grid, we then rinsed grid by dd-water for twice or three times. Immediately after rinsing, we stained the grid containing sample with 2.0 % w/v uranyl acetate for three times. Afterwards, we allowed the grid to dry in air.

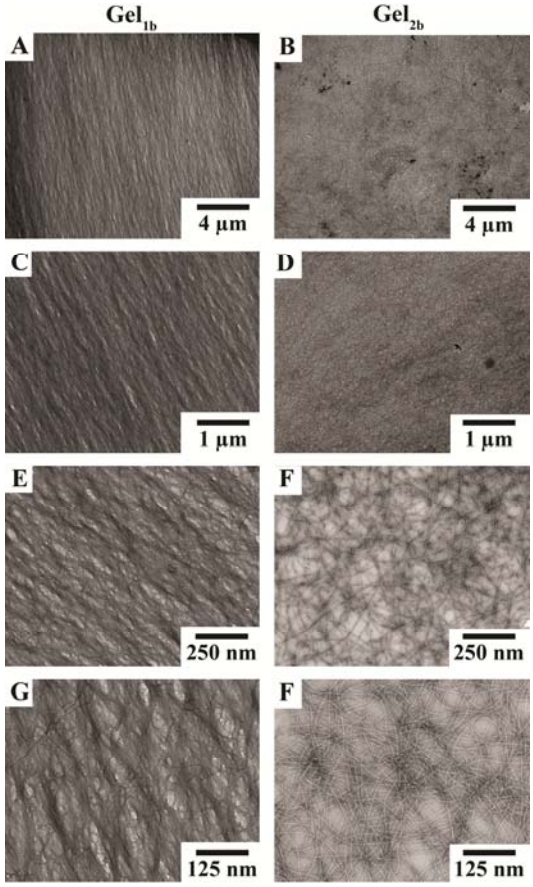


Fig S5. Transmission electron microscopy (TEM) images of the nanofiber matrices of **Gel_{1b}** (on the left) and **Gel_{2b}** (on the right), formed by the treatment of **1a** and **2a** with ALP (2 U/mL) at the concentration of 0.8 wt% and pH of 7.4, respectively.

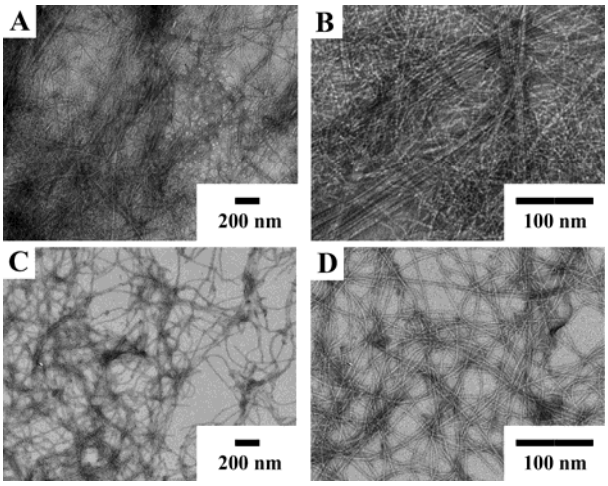


Fig S6. Transmission electron microscopy (TEM) images of the nanofiber matrices of **Gel_{1b}'** (A, B) and **Gel_{2b}'** (C, D), at the concentration of 0.8 wt% and pH of 7.4 in water.

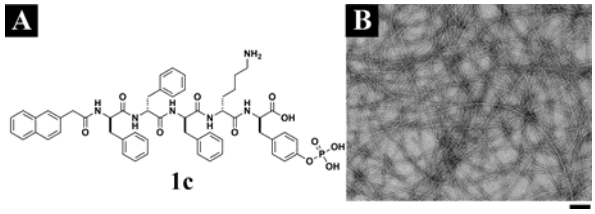


Fig. S7 (A) Chemical structure of **1c** (the enantiomer of **1a**); (B) TEM image of the hydrogel formed by treating the solution of **1c** with ALP at the concentration of 0.8 wt% and pH of 7.4.

S6. Rheological characterization

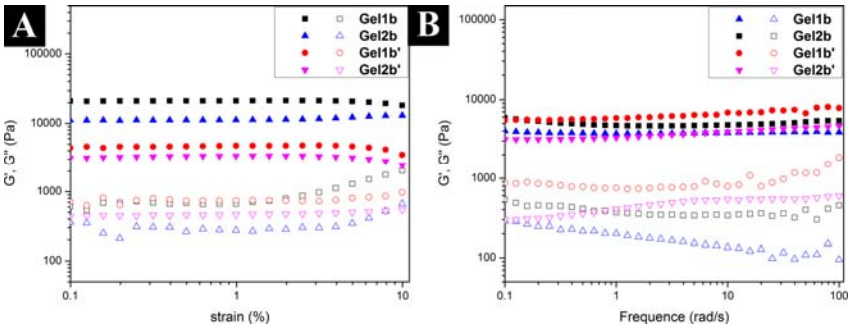


Fig S8. Rheological characterization of **Gel_{1b}**, **Gel_{1b}'**, **Gel_{2b}**, and **Gel_{2b}'**, at the concentration of 0.8 wt% and pH of 7.4. The strain (A) and frequency (B) dependence of the dynamic storage.

Reference

(1) Chan, W. C.; White, P. D.; Editors *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Oxford Univ Press, 2000.

(2) Ottinger, E. A.; Shekels, L. L.; Bernlohr, D. A.; Barany, G. *Biochemistry* **1993**, *32*,

4354-4361.

(3) Li, J. Y.; Gao, Y.; Kuang, Y.; Shi, J. F.; Du, X. W.; Zhou, J.; Wang, H. M.; Yang, Z. M.; Xu, B. *J. Am.Chem. Soc.* **2013**, *135*, 9907-9914.