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Supporting Information

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Supporting Information for

Mechanical Force-Induced Nucleation and Growth of Peptide Nanofiber at Liquid/Solid Interfaces

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1 Materials and Methods

1.1 Materials

The ionic-complementary self-assembling peptides EAK16-II ($C_{70}H_{121}N_{21}O_{25}$, Mw = 1657 g/mol) and EFK16-II ($C_{118}H_{153}N_{21}O_{25}$, Mw = 2265.6 g/mol) have sequences of AEAEAKAKAEAEAKAK and FEFEFKFKFEFEFKFK, respectively; where A and F correspond to alanine and phenylalanine, E to glutamic acid and K to lysine. At neutral pH, A and F are neutral hydrophobic residues, while E and K are negatively and positively charged, respectively. These peptides were purchased from CanPeptide Inc (Québec, Canada). The N-terminus and C-terminus of these peptides were protected by acetyl and amino groups, respectively, to minimize end-to-end electrostatic attraction between peptides.[1] The purity of EAK16-II is above 95% (purified by reverse-phase high-performance liquid chromatography), while that of EFK16-II is ~60% (crude).

EAK16-II and EFK16-II stock solutions were prepared in pure water (18.2 MΩ; Millipore Milli-Q system) at a concentration of 30 μM and 44 μM, respectively, and stored at 4 °C for at least 2 weeks (allowing peptide self-assembly to reach equilibrium) before use. Reagent grade sodium hydroxide with purity of 99+% and hydrochloric acid (36-38% by weight) were obtained from BDH Chemicals Ltd. (Toronto, Canada) and from Fisher Scientific (Nepean, Canada), respectively.

Grade V-4 muscovite mica ($KAl_2(AlSi_3)O_{10}(OH)_2$) and HOPG (ZYB grade), both obtained from SPI Supplies (West Chester, PA, USA), were used as the two model surfaces for AFM imaging. Mica and HOPG were fixed on the AFM liquid sample plate using double-sided tape. An adhesive tape was used to remove the outer layer of the mica and HOPG surfaces and expose fresh layers prior to the addition of peptide solution.

1.2 *In-situ* AFM imaging

To monitor the effect of the mechanical force applied by the AFM tip on EAK16-II nanofibers on substrates in real-time, *in-situ* AFM imaging was performed on a PicoScan™ AFM (Molecular Imaging, Phoenix, AZ) in a liquid cell (custom-made). The liquid cell was mounted on freshly cleaved mica or

HOPG and filled with 500 μl pure water. Peptide stock solution was then injected into the liquid cell with a micro-syringe. The final peptide concentration in the liquid cell was 2, 4 or 6 μM, as indicated in the figure captions and the main text. After certain time periods, such as 1 hr, the peptide nanofibers formed on the surfaces which were then scanned with the AFM tip to apply a mechanical force. For the experiments on the effect of solution conditions, a 1 mM HCl, 10 mM HCl 1 mM NaOH or 10 mM NaOH solution was injected into the liquid cell followed by the peptide stock solution with a volume ratio of 5:1, and the final solution pH was 3.4, 2.2, 9.9 or 10.5, respectively.

The amplitude of the AFM tip was calculated with the following equation from the AC mode user's manual (Molecular Imaging, Phoenix, AZ):

$$Amplitude\ \ (nm) = \frac{Display\ \ Voltage\ \ (V)}{20 \times Input\ \ Gain} \times 4.3 \times Sensitivity\ \ (\frac{nm}{V}) \qquad (1)$$

The sensitivity of the AFM tip operating in liquid was obtained from amplitude-distance spectroscopy and was found to be 18.0 ± 0.8 and 14.7 ± 1.5 nm/V for the HOPG and mica substrate, respectively. The display voltage was ~1 V during imaging. Thus, the amplitude of silicon nitride cantilever oscillation in liquid was calculated to be ~3-4 nm.

During AFM scanning, the number of taps (m) that an oscillating AFM tip applies to a given nanofiber is inversed proportional to the scan area (see supporting information) can be calculated using the following equation:

$$m = \frac{w \cdot f}{a \cdot v \cdot \sin\alpha} \qquad (2)$$

where w is the width of the nanofiber, which is typically 7 nm, ca. the length of an EAK16-II molecule; f is the oscillation frequency of the AFM tip, usually set to 80% of the tip resonant frequency; α is the angle formed between the nanofiber and scan direction; a is the scan distance in the horizontal direction and v is the scan speed (line/s). Thus, a given scan speed and oscillation frequency, the tip taps more times on a given location of a nanofiber as the scan area becomes smaller. For example, m is estimated to

be ~69-77, ~138-155 and ~275-310 at a scan area of 2000 × 2000 nm^2, 1000 × 1000 nm^2 and 500 × 500 nm^2, respectively (f = 16-18 kHz, v = 0.814 line/s, w = 7 nm and α = 90°).

Atomic force microscopy images were taken using a scanner with a maximum scan area of 6 × 6 μm^2. All AFM experiments were conducted in an environmental-controlled chamber at room temperature to avoid evaporation of the working solution. Silicon nitride cantilevers with a nominal spring constant of 0.32-0.58 N/m (DNP-S, Digital Instruments) and typical tip radius of 10 nm were used for tapping mode in liquid. To obtain the best imaging quality, the typical tapping frequency was usually set between 16 and 18 kHz and the scan rates were set to 0.814 Hz (~5 min for each AFM image). All AFM images were obtained at a resolution of 256 × 256 pixels.

In general, the indicated times in the figures refer to the moment when the EAK16-II stock solution was injected into the liquid cell containing the desired solutions. The indicated concentrations for the acid and alkaline solutions refer to the original concentrations added to the liquid cell. An initial image of a newly cleaved mica/HOPG surface under the working solution was taken to confirm the absence of contaminating particles.

The surface coverage of EAK16-II nanofibers on the HOPG surface at different time periods was measured with the Image J software (http://rsb.info.nih.gov/ij/). The width and height of EAK16-II nanofibers were determined by the cross section analysis tool of the PicoScan software. The peptide nanofiber widths reported herein were obtained using the deconvolution method reported by Fung et al.[2]

1.3 Surface tension measurement

The experimental set-up for the Axisymmetric Drop Shape Analysis-Profile (ADSA-P) technique to study the dynamic surface tension of 30 μM EAK16-II in various solutions was described in more detail in an earlier publication.[3] A pendant drop of the peptide solution was formed at the tip of a vertical Teflon needle (0.92 mm inner diameter) connected to a motor-driven micro-syringe (1 ml, release speed of 0.04 ml/s). The sample was placed in a temperature-controlled environmental chamber, saturated with the corresponding solvent vapor to maintain a constant humidity. The entire system was placed on a

vibration-free table. The images of the pendant drop were magnified by an optical microscope and then captured by a CCD camera before being transferred to a computer. The surface tension of every sample was measured for 2 hours. During each run, images were acquired at 0.5 s intervals for the first 70 s and then at 20 s intervals for the remaining time. Software was used to digitize the images and generate a profile of the pendant drop. A theoretical curve governed by the Laplace equation of capillarity was then fitted to the profile, generating the surface tension value as a fitting parameter.[4] The standard deviation of the surface tension values obtained was less than 0.2 mJ/m^2.

2 Supporting Results and Discussions

Figure S1 shows the self-assembled nanostructures of EAK16-II on HOPG in various solutions: (a) pure water (pH = ~6), (b) 1 mM NaOH (pH = 9.9), (c) 10 mM NaOH (pH = 10.5), (d) 1 mM HCl (pH = 3.4) and (e) 10 mM HCl (pH = 2.2). EAK16-II formed a hexagonal nanofiber pattern on HOPG surface when immersed in pure water and NaOH solutions; these nanofibers had similar width (~6 nm after deconvolution) and height (~0.9 nm). However, no nanofibers were observed in 1 and 10 mM HCl solutions. This indicated that EAK16-II nanofibers did not adsorb on the HOPG surface and EAK16-II molecules did not assemble on the HOPG surface under acidic solution conditions.

The observation that EAK16-II did not assemble on HOPG in 1 and 10 mM HCl solutions may be due to the decrease in hydrophobicity of the peptides. In acidic solution, the EAK16-II molecule becomes highly positively charged. This makes the peptides more hydrophilic and less likely to adsorb and assemble on the hydrophobic HOPG surface. To further confirm this speculation, experiments were carried out with the peptide EFK16-II, which contains 8 phenylalanine (F) residues, replacing the alanine (A) in EAK16-II; thus, EFK16-II is expected to be more hydrophobic than EAK16-II. As shown in Figure S1f, EFK16-II can still form a hexagonal pattern on HOPG surface in 1 mM HCl solution, whereas EAK16-II cannot (Figure S1d). This observation supports the idea that the hydrophobic interaction between the peptide and the HOPG surface dominates its adsorption and assembly on the surface.

The above arguments are further supported by measurements of the surface activities of the peptide molecules/assemblies in the various solutions. Figure S2 shows the dynamic surface tensions of EAK16-II assemblies in various solutions. The peptide has higher surface tension under acidic conditions than in basic solutions and pure water. The surface tensions of the peptide molecules/assemblies in different solutions follow the order: 1 mM HCl ≈ 10 mM HCl > 10 mM NaOH > 1 mM NaOH > pure water. This order is consistent with the AFM observation of EAK16-II assembly on the HOPG surface. A higher surface tension indicates a more hydrophilic structure of the peptide molecules/assemblies and less affinity to the hydrophobic surface. Therefore, the peptide would not be expected to assemble on HOPG under acidic solutions. On the other hand, a lower surface tension indicates a more hydrophobic nature, enabling peptide adsorption onto HOPG. This may explain why EAK16-II is observed to assemble on HOPG in pure water and basic solutions, but not in acidic solutions.

The peptide nanofiber patterns on HOPG were fabricated by utilizing AFM tip nanolithography through a combination of bottom-up and top-down approaches. First, EAK16-II molecules (30 μM) were allowed to assemble into nanofibers on the HOPG surface in a neutral solution until the surface was almost fully covered by the peptide (Figure S3a). The EAK16-II-covered surface was then submerged in a 10 mM HCl solution. Tapping mode AFM was applied to break the nanofibers in a particular region of the surface through repeated scanning. In the acidic solution, nanofibers preferentially detached from this scanned portion of the substrate until the underlying HOPG surface appeared (Figure S3b-d). A representative peptide nanofiber pattern on HOPG was shown in the larger scale image in Figure S3e. Four parallel stripes were made by repeated scanning in 10 mM HCl solution. Since the nanofiber-covered area is hydrophilic while the bare HOPG surface is hydrophobic, alternating hydrophilic and hydrophobic patterns can be created via this method.

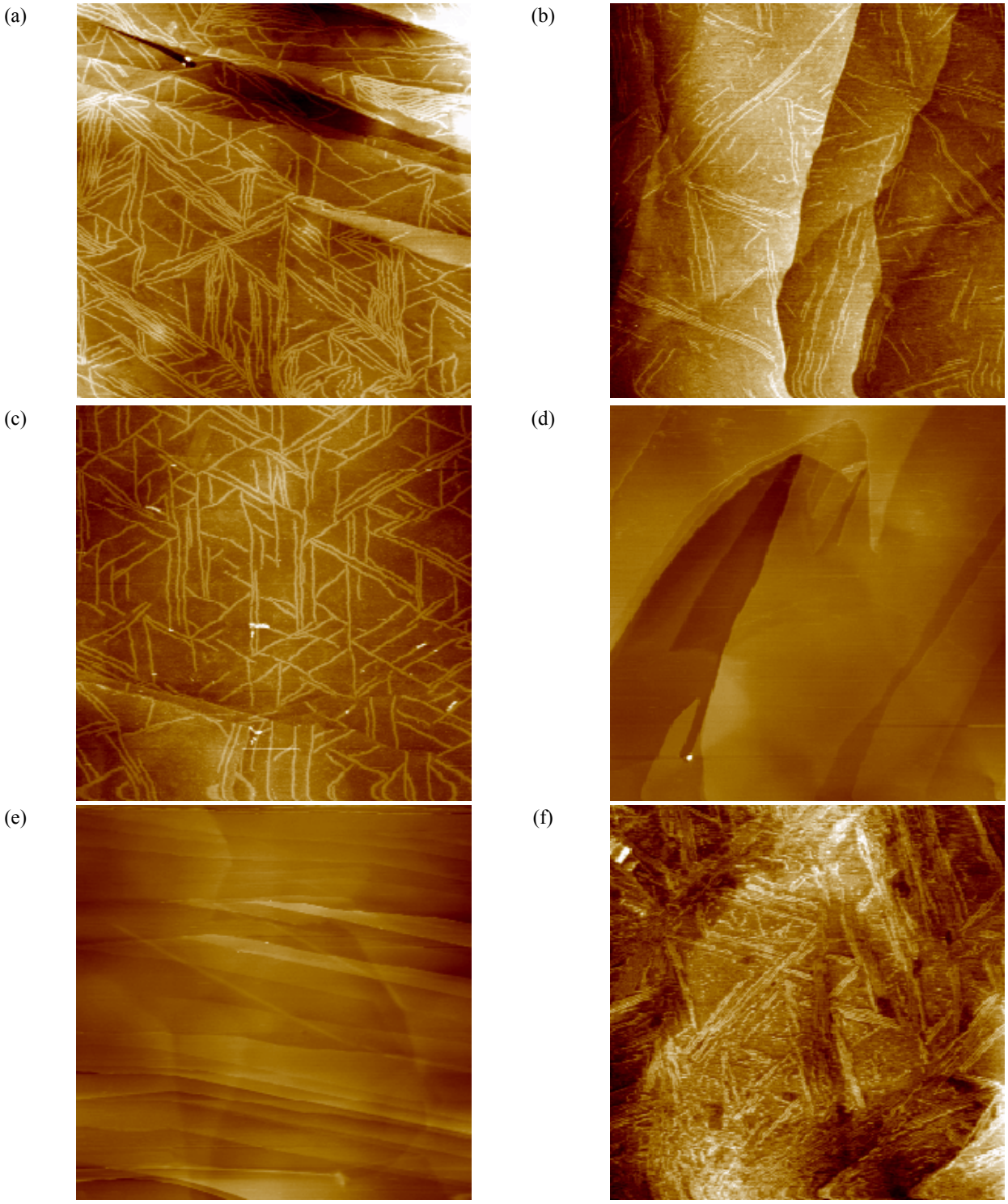

Figure S1. AFM images of 6 μM EAK16-II assemblies on the HOPG surface under different environments: (a) pure water (pH = ~6); (b) 1 mM NaOH (pH = 9.9); (c) 10 mM NaOH (pH = 11.5); (d) 1 mM HCl (pH = 3.4); (e) 10 mM HCl (pH = 2.2). (f) 6 μM EFK16-II on the HOPG surface in 1 mM HCl (pH = 3.4). All images were taken at 1000 × 1000 nm^2.

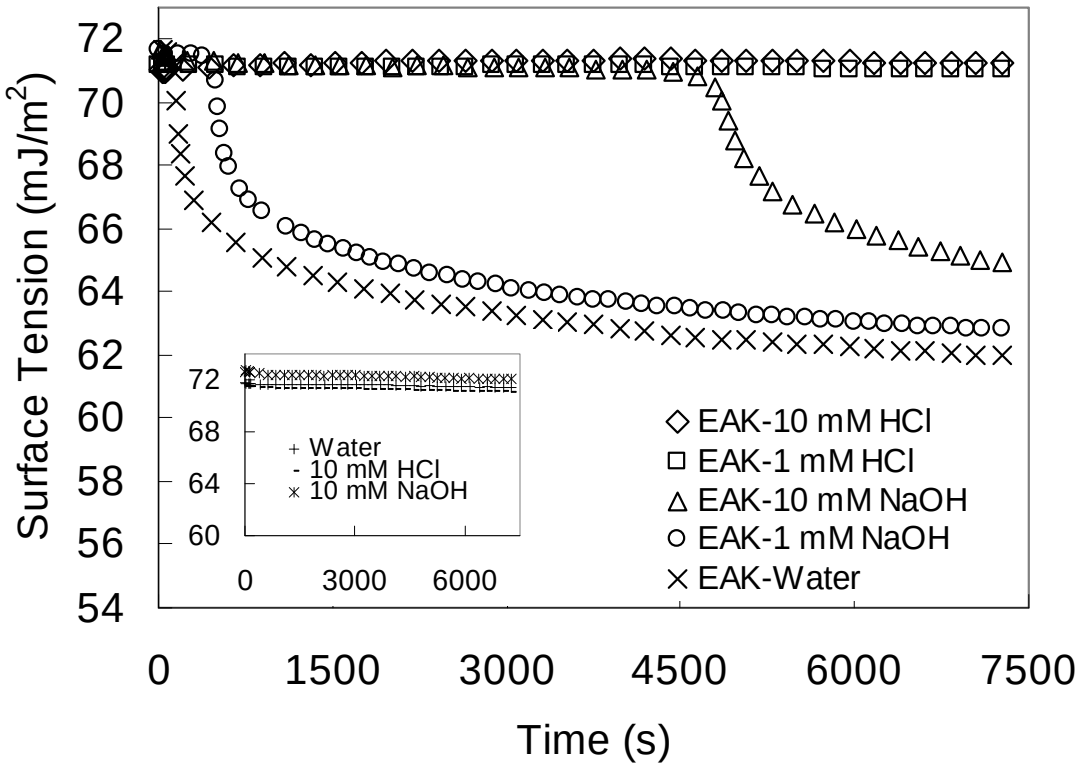

Figure S2. Dynamic surface tensions of 30 μM EAK16-II in various solutions: pure water (×);10 mM NaOH (Δ); 1 mM NaOH (○); 1 mM HCl (□); 10 mM HCl (◇). The inset shows the dynamic surface tensions of the solvents: water (+); 10 mM HCl (-); 10 mM NaOH (∗).

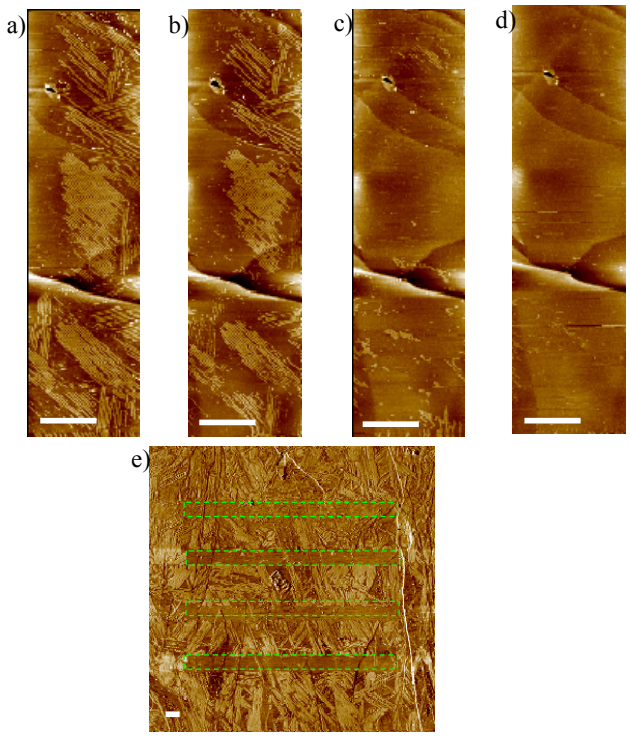

Figure S3. Results of AFM tip nanolithography performed in 10 mM HCl solution to fabricate EAK16-II nanofiber-patterned HOPG surface: (a-d) repetitive AFM scanning to break and remove peptide nanofibers to expose bare HOPG surface; (e) representative AFM phase image of EAK16-II nanofiber patterned HOPG surface; peptide nanofibers were removed within the four stripes (dotted area). Scale bar corresponds to 200 nm.

Full reference for [7b]: C. Whitehouse, J. Fang, A. Aggeli, M. Bell, R. Brydson, C. W. G. Fishwick, J. R. Henderson, C. M. Knobler, R. W. Owens, N. H. Thomson, D. A. Smith, N. Boden, *Angew. Chem.* **2005,** *117*, 2001-2004; *Angew. Chem. Int. Ed.* **2005,** *44*, 1965-1968.

Full reference for [7c]: F. Zhang, H.-N. Du, Z.-X. Zhang, L.-N. Ji, H.-T. Li, L. Tang, H.-B. Wang, C.-H. Fan, H.-J. Xu, Y. Zhang, J. Hu, H.-Y. Hu, J.-H. He, *Angew. Chem.* **2006,** *118*, 3693-3695; *Angew. Chem. Int. Ed.* **2006,** *45*, 3611-3613.

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