Supporting Information

Material and Methods

Materials. Fmoc-Lys(Boc)-Wang resin (100-200 mesh, subst.: 0.66 mmole g^{-1}), Rink Amide MBHA resin (100-200 mesh, subst.: 0.34 mmole g^{-1}), Fmoc-Met-OH, Fmoc-Glu (OtBu)-OH, Fmoc-Lys (Boc)-OH, and O-benzotriazole-N,N,N,N-tetramethyl-uroniumhexafluoro-phosphate (HBTU) were purchased from NovaBiochem (Merck Biosciences AG). GC grade N, N-dimethylformamide (DMF), 4-methylmorpholine (NMM), trifluoroacetic acid (TFA), acetic anhydride, pyridine, dichloromethane (DCM, HPLC/SPECTRO), acetonitrile (HPLC), DTT and piperidine 99% were ordered from Merck Schuchardt. Triisopropylsilane 99% (TIPS) was obtained from Aldrich. Other chemicals were obtained from SCRC Co., Ltd.

Peptide Synthesis and Purification. Ionic-complementary peptides: EMK8-II with free (N-EMK8-II-C) and protected termini (Ac-EMK8-II-$CONH_2$). Here, we chose a new kind self-assembling oligopeptide, EMK8-II (MEMEMKMK, where M corresponds to methionine, E to glutamic acid, and K to lysine). EMK8-II contains three kinds of amino acids (Met, Glu and Lys) which constitute an eight amino acids long sequence. The hydrophobic residue Met and hydrophilic residues Glu and Lys are alternately lined in a regular sequence, leading to particular amphiphilic property. For comparison, we also synthesized two more peptides with disordered Lys positions (Ac-MEMEMKKM-$CONH_2$ and Ac-MEMEKMKM-$CONH_2$).

All these four peptides were synthesized using Fmoc chemistry on a Symphony Quartet peptide synthesizer (Protein Technologies Inc., USA). In a 16-mL standard glass reaction vessel, 38 mg (0.025 mmol) of lysine pre-loaded Wang resin with the amino group protected by Fmoc was introduced. The resin was swelled by washing with DMF. Two 15-minute steps of deprotection in DMF containing 20% piperidine were followed by two 1-hour coupling step while adding two solutions in equal volume. One solution was amino acid (0.125 mmol) protected by Fmoc in DMF, the other was the coupling reagent consisting of NMM (0.5 mmol) and HBTU (0.125 mmol) in DMF.

The deprotection and coupling procedures were repeated for all amino acids in the EMK-8II sequence. After two steps of deprotection, we cleaned the resin by washing with DMF and DCM six times and five times respectively in preparation for the cleavage. A solution of 2.22 mL of TFA, 0.186 g of DTT, 0.186 mL of pure water (18.2 MΩ; Millipore Milli-Q system), and 0.096 mL of TIPS were used to perform cleavage from the resin and side-chain protecting groups. After filtration, centrifugation of the solution and treatment with 50 mL (repeated five times) ethyl ether gave the peptide as a precipitate. After dissolving in roughly 5 mL of pure water (18.2 MΩ; Millipore Milli-Q system), the solution was freeze-dried for more than twenty hours.

Compared with EMK8-II, there are some differences in the synthetic procedures of Ac-EMK8-II-$CONH_2$, Ac-MEMEMKKM-$CONH_2$ and Ac-MEMEMKKM-$CONH_2$. Firstly, the resin is rink amide MBHA resin without pre-loaded amino acid. Secondly, before cleavage, an 8-hour coupling step of protection of the amino terminus was carried out in DMF containing

acetic anhydride and pyridine.

Peptide purification was performed via reverse-phase chromatography on a AKTABasic pH/C 10 mL System(Amersham Biosciences) equipped with a Source 5RPC ST4.6/150 column (Amersham Biosciences; Polystyrene matrix, 5 μm particle size, 4.6 × 150 mm). The peptide was eluted from the column via the application of a linear gradient from 10% to 40% solution B over 40 min at 1.0 mL min^{-1}, where eluent A was 0.1% TFA in water and eluent B was 0.1% TFA in 90% acetonitrile. The collected peak of the crude peptide was confirmed by matrix assisted laser desorption time-of-flight (MALDI-TOF) on a Bruker Daltonics Autoflexll mass spectrometer.

Sample Preparation. Lyophilized peptide powder was dissolved in ultrapure water (18.2 MΩ; Millipore Milli-Q system) to get a final concentration of 1.0 mg mL^{-1}. The pH of the solutions is ~6 after the peptide is fully dissolved. The concentrations of all the samples used for AFM imaging, CD measurements were 1.0 mg mL^{-1} unless specified. Unless noted for special purpose, the solutions were stirred at 120 rpm at room temperature overnight before AFM imaging, far-UV CD, FT-IR and ThT measurements.

Atomic Force Microscopy (AFM). About 5 μL of the peptide solution was placed on the surface of a newly peeled mica surface. Each sample was placed on the mica for ten minutes, and then rinsed with 100 μL of pure water to remove unattached peptides. Then the peptide sample on the mica surface was air-dried, and images were acquired immediately.

The images were obtained by scanning the mica surface at room temperature by a NanoWizard II AFM (JPK, Germany) operating in intermittent contact mode (conditions: scan rate, 1 Hz; resonant frequency range of AFM tips, 204-497 kHz ; number of pixels, 512 × 512.) using cantilevers either AC160 from Olympus (tip radius: 10 nm) or SSS-SEIHR from Nanosensors (tip radius: 2nm). To obtain typical images in each case, different samples and three regions in the entire surface were scanned.

For the dimensions of the assemblies, it was found that the widths were broadened due to the convolution effects arising from the finite size of the AFM tip.[1-4] Thus, the observed values for the dimensions had to be corrected. For a fibrillar sample, the real width of the fibrillar can be obtained by the following equation, $W = W_{obs} - 2(2R_tH - H^2)^{1/2}$, where R_t is the radius of the AFM tip and H is the observed height.[5]

Far-UV Circular Dichroism (CD). CD spectra were measured using a JASCO J-810 CD spectropolarimeter (Jasco, Japan). With a 0.1-mm path length quartz cell, a wavelength scan was done from 260 to 190 nm at room temperature. All spectra were baseline corrected before analysis.

Fourier Transform Infrared (FTIR) Spectroscopy. FT-IR spectra were recorded on Bruker VERTEX 80V spectrometers. The spectra, the average of 200 scans, were recorded with a resolution of 2 cm^{-1} in D_2O solution. Samples were inserted between CaF_2 windows with a path length of 100 μm. The decomposition of the spectra was performed by using the Origin software.

Each of samples has an absorption spectrum with a broad amide I' band (Figure S2). A decomposition analysis of the amide I' region of EMK8-II self-assembly (Figure S2A) gives the following distribution of the different types of conformation[6-10]: α-helical conformation (12%), β-sheet structure (13%) and other component. Then, the same method was applied to analyze the FT-IR spectrum of Ac-EMK8-II-$CONH_2$ (Figure S2B), indicating that there are more α-helical content (23%) than EMK8-II.

Congo Red Binding Assay. Congo red binding was assessed essentially as described by Klunk et al.[11]. Briefly, the peptide fibril sample of 1 mg mL^{-1} was added into Congo red solution and incubated for 20 minutes at room temperature. The final concentrations of the peptide and Congo red are 25 μg mL^{-1} and 5 μM, respectively. The absorbance spectra of the resulting solutions were then measured from 700 to 400 nm using a JASCO V-550 UV-Vis Spectrophotometer.

ThT Fluorescence. The fluorescence spectrum of Thioflavin T (ThT) solution at a concentration of 20μM was recorded at room temperature using a Jasco FP-6500 spectrofluorometer (Japan) at an excitation wavelength of 450 nm. Then, the peptide fibril sample of 1 mg mL^{-1} was added into ThT solution and incubated for 30 minutes. The final concentrations of the peptide and ThT are 20μg mL^{-1} and 20μM, respectively. The fluorescence spectrum of this sample was taken under the same condition as the ThT solution.[12]

Transmission Electron Microscopy (TEM). Preparation of samples for negative contrast was performed as described[13]. Briefly, sample was applied to a carbon-coated 200 mesh copper grid, stained with 2% (w/v) uranyl acetate. Samples were observed using a JEOL transmission electron microscope.

Wide-Angle X-ray Scattering. The X-ray powder diffraction spectrum of the Ac-EMK8-II-$CONH_2$ powders was obtained using a Bruker D8 Advance X-ray powder diffractometer with Cu Kα radiation and nickel filter. The spectrum was analyzed by Bragg's law:

$$2 \cdot d \cdot \sin\theta = n \cdot \lambda$$

(where n is an integer, λ is the wavelength of incident wave (0.15406 nm), d is the spacing between the planes in the atomic lattice, and θ is the angle between the incident ray and the scattering planes.)

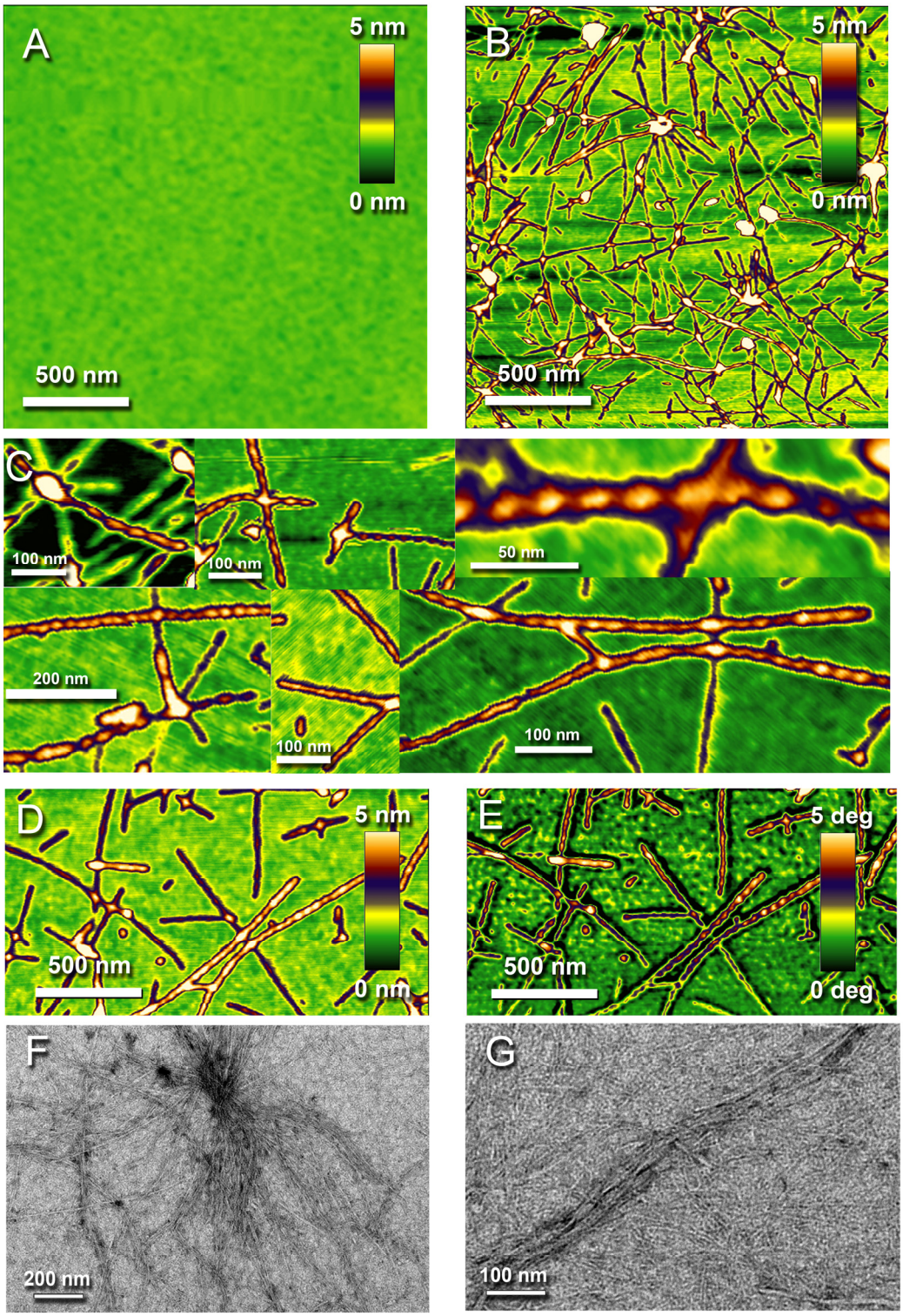

Figure S1. (A) AFM images of EMK8-II in pure water. (B)-(D) AFM height images of the self-assembled nanofibrils of Ac-EMK8-II-$CONH_2$ in pure water. (E) The phase image of (D). (F) and (G), TEM images of the self-assembled nanofibrils of Ac-EMK8-II-$CONH_2$ in pure water.

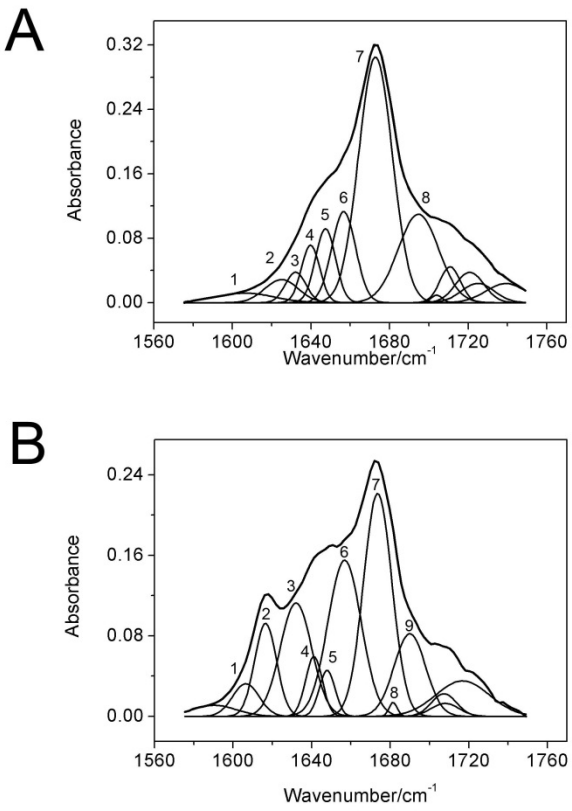

Figure S2. (A) Amide I' region of the FT-IR spectrum in D_2O of EMK8-II. The spectrum was decomposed by fitting with Gaussian curves. Assignments: 1, side chain; 2, 3, 4, β-sheet; 5, α-helix or random coil structure; 6, α-helix; 7, random coil; 8, turns. (B) Amide I' region of the FT-IR spectrum in D_2O of Ac-EMK8-II-$CONH_2$. The spectrum was decomposed by fitting with Gaussian curves. Assignments: 1, side chain; 2, β-sheet; 3, turns; 4, random coil or β-sheet; 5, α-helix or random coil; 6, α-helix; 7, random coil; 8, β-sheet; 9, turns.

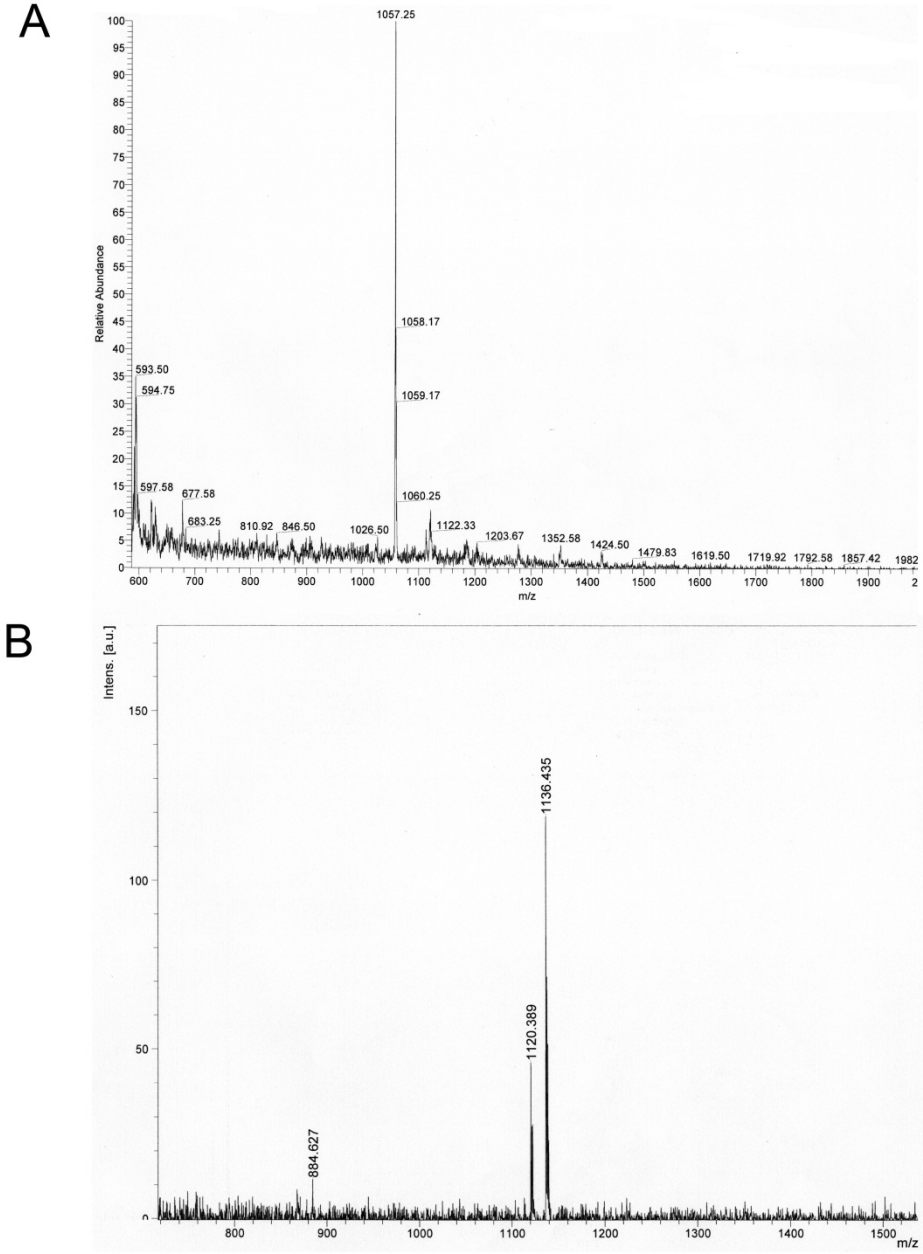

Figure S3. (A) The mass spectrum of EMK8-II. The calculated molecular weight is 1056.4. The peak stands for the $[M+H]^+$ = 1057. (B) The mass spectrum of Ac-EMK8-II-$CONH_2$. The calculated molecular weight is 1097.4. The peaks stand for the $[M+Na]^+$ = 1120 and $[M+K]^+$ = 1136.

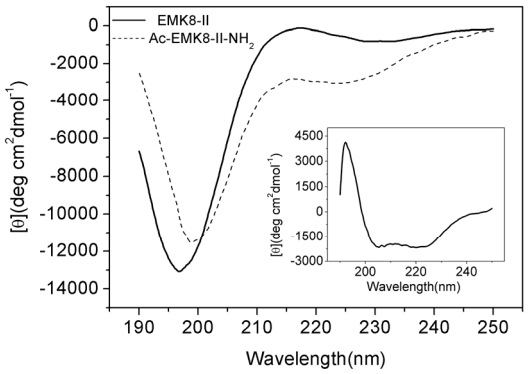

Figure S4. The far-UV CD spectra of EMK8-II (solid line) and Ac-EMK8-II-$CONH_2$ (dashed line) at a concentration of 1.0 mg mL^{-1} in pure water. The inset spectrum is the dashed line subtracted by the solid line.

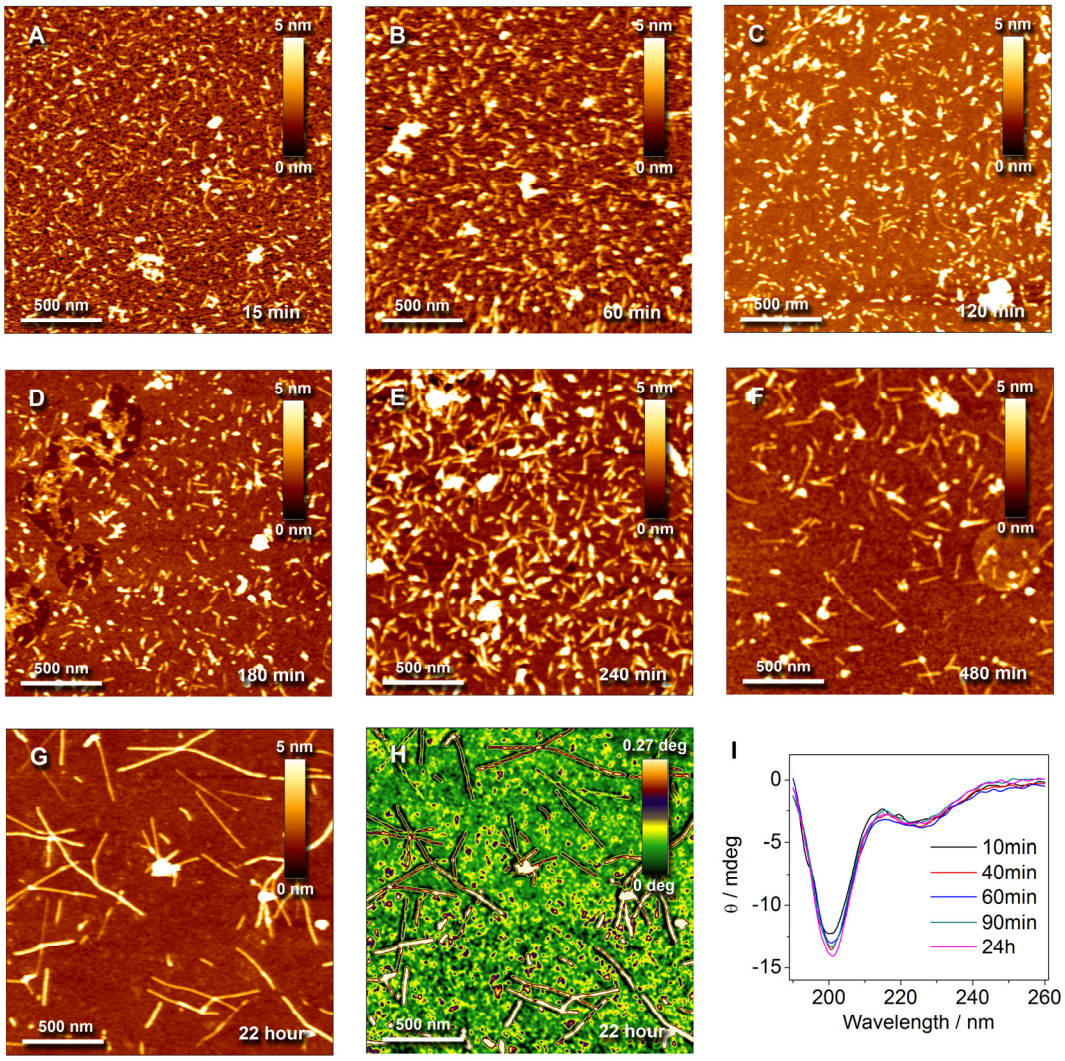

Figure S5. Time-dependent AFM images and CD spectra of Ac-EMK8-II-$CONH_2$. (A) 15 minutes. (B) 60 minutes. (C) 120 minutes. (D) 180 minutes. (E) 240 minutes. (F) 480 minutes. (G) 22 hours. (H).The phase image of (G). (I) CD spectra of the assembled peptide samples after different incubation time.

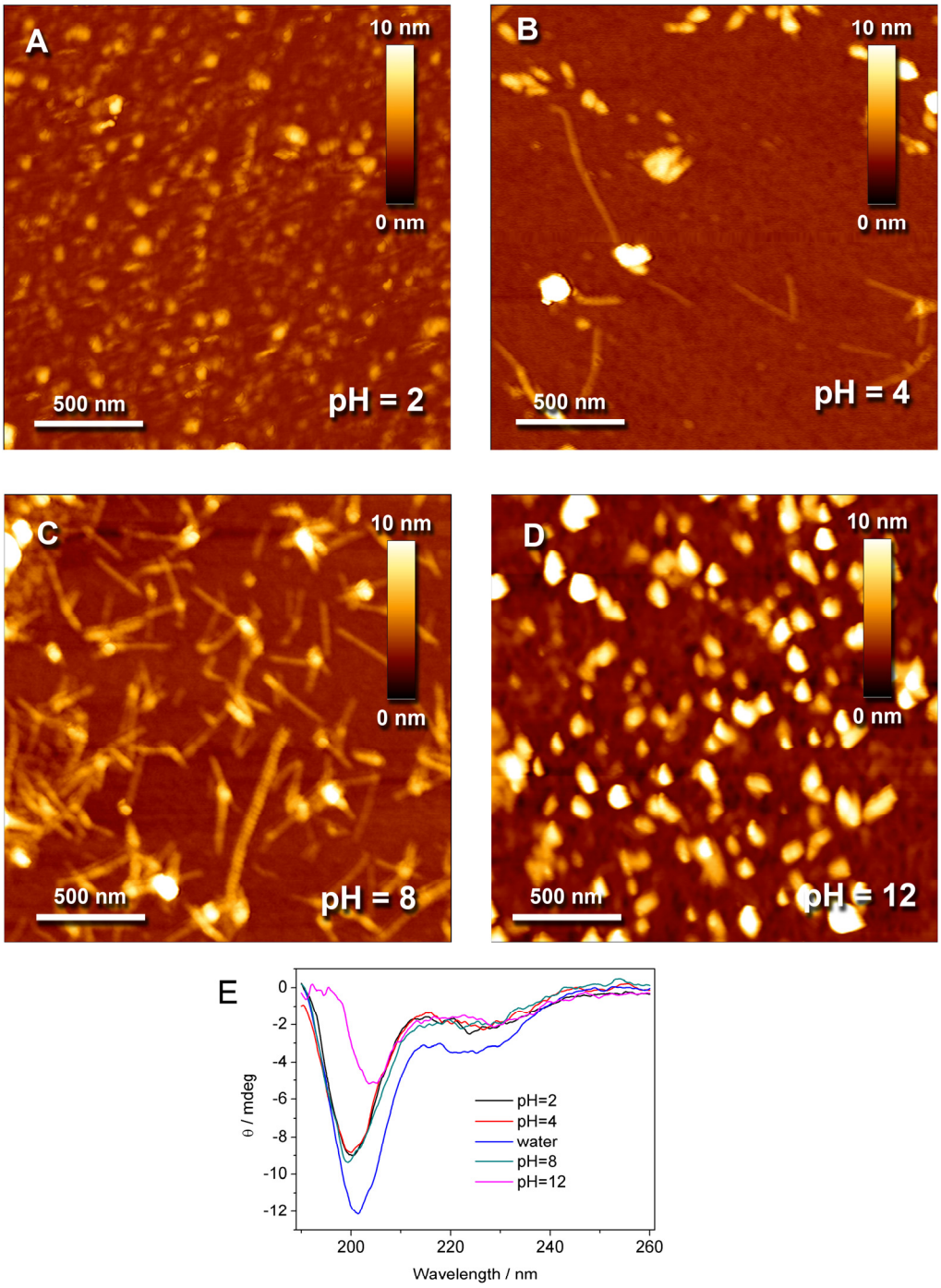

Figure S6. The AFM images and CD spectra of Ac-EMK8-II-$CONH_2$ at different pHs at a concentration of 1.0 mg mL^{-1}.

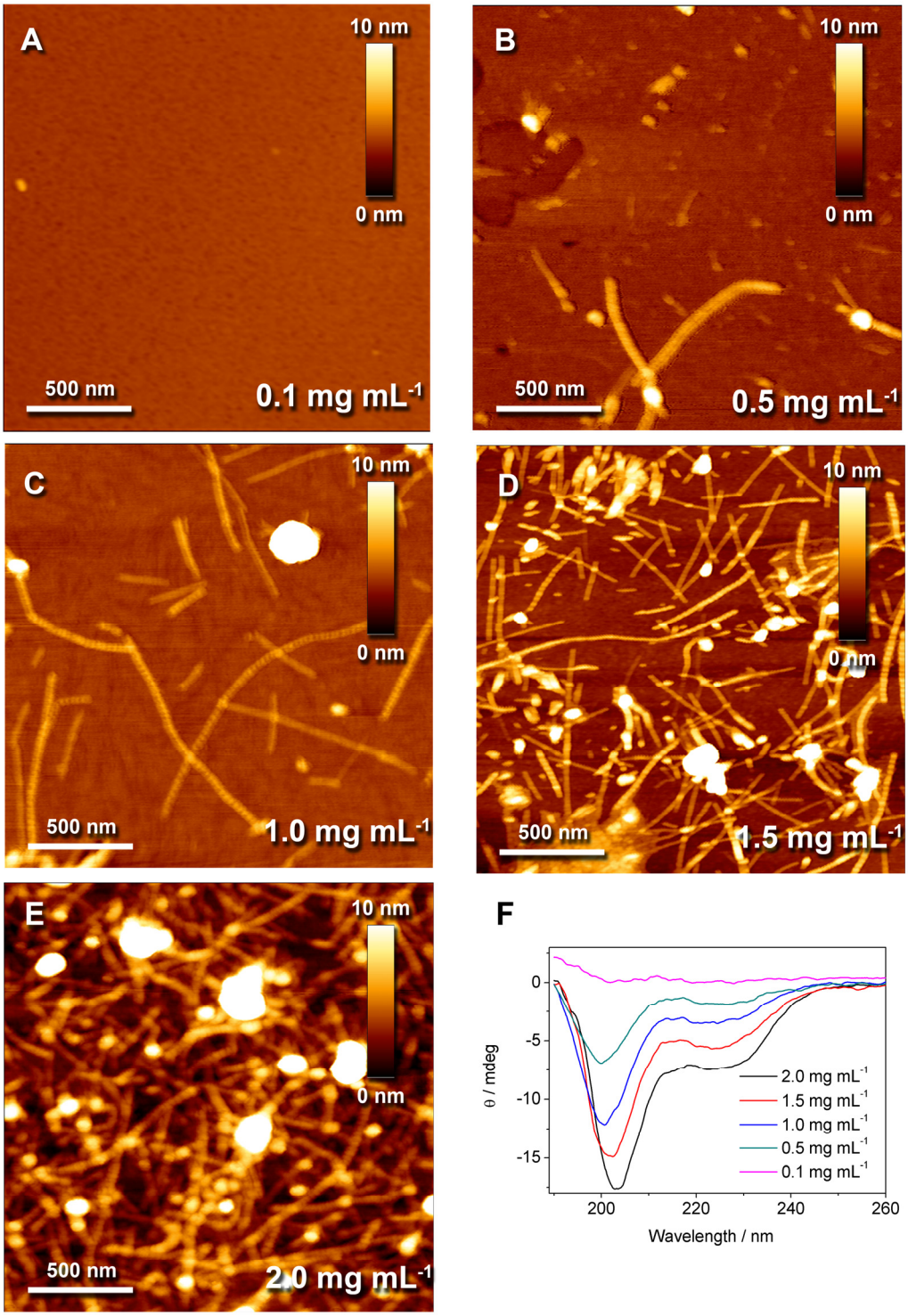

Figure S7. The AFM images and CD spectra of Ac-EMK8-II-$CONH_2$ at different concentrations in pure water.

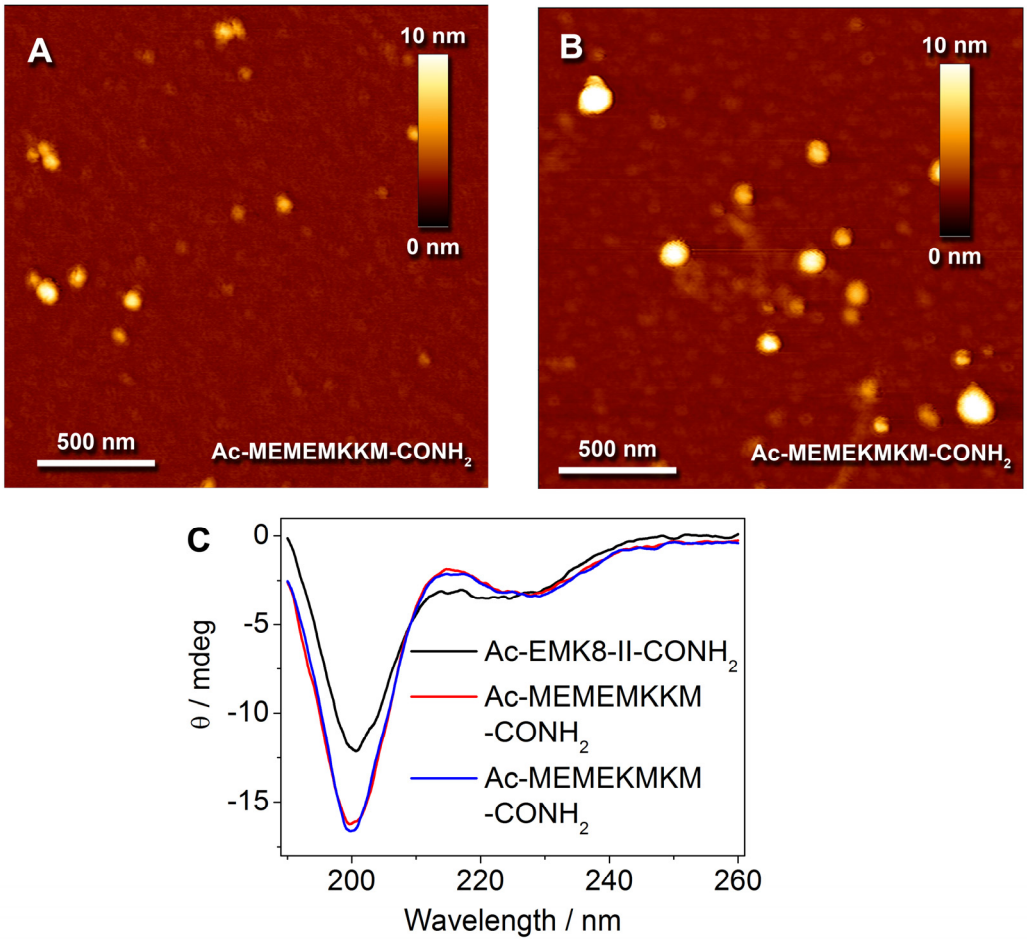

Figure S8. The AFM images and CD spectra of Ac-MEMEMKKM-$CONH_2$ and Ac-MEMEKMKM-$CONH_2$ in pure water.

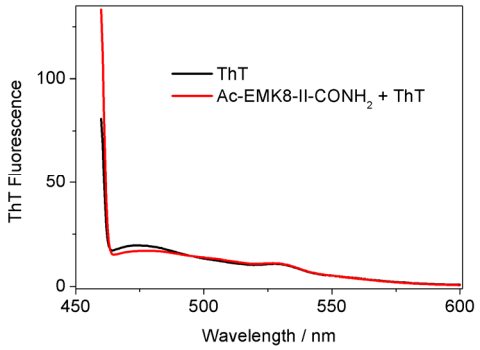

Figure S9. The ThT Fluorescence in the absence (black) and presence (red) of Ac-EMK8-II-$CONH_2$ fibrils.

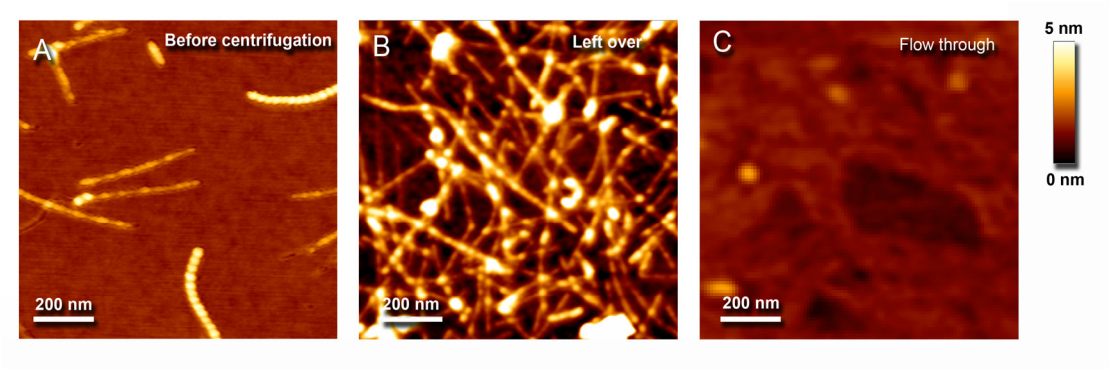

Figure S10. The AFM images of the peptide Ac-EMK8-II-$CONH_2$ sample (1.0 mg/mL) under 16 hours incubation (A), and its left-over (B) and flow-through (C) using a centriplus centrifugal filter (Millipore, Bedford, MA) with a molecular weight cutoff of 3000 Da. The above mentioned fibril sample (2 mL) were centrifuged for 2 hours and the resulted sample in left-over is about 0.5 mL and that in flow-through is bout 1.5 mL.

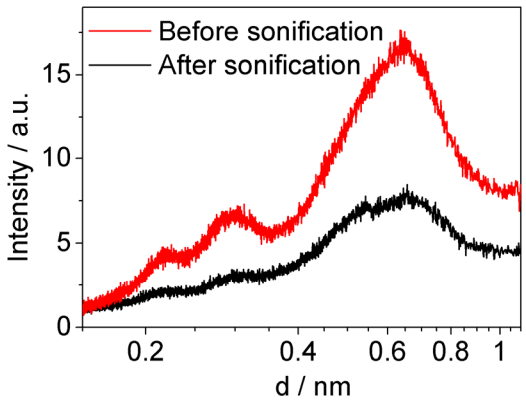

Figure S11. The XRD spectra of the Ac-EMK8-II-$CONH_2$ fibrils before (red) and after sonification for 30 minutes (black). The fibril sample was prepared according to the procedure reported in the experimental section and lyophilized before XRD measurement. The sonificated sample was subjected to sonification (40 KHz, 100W) for 30 minutes and immediately lyophilized for XRD measurement. The breakage of long fibrils has been confirmed by AFM image. However, sonification does not change the CD spectrum of the sample.

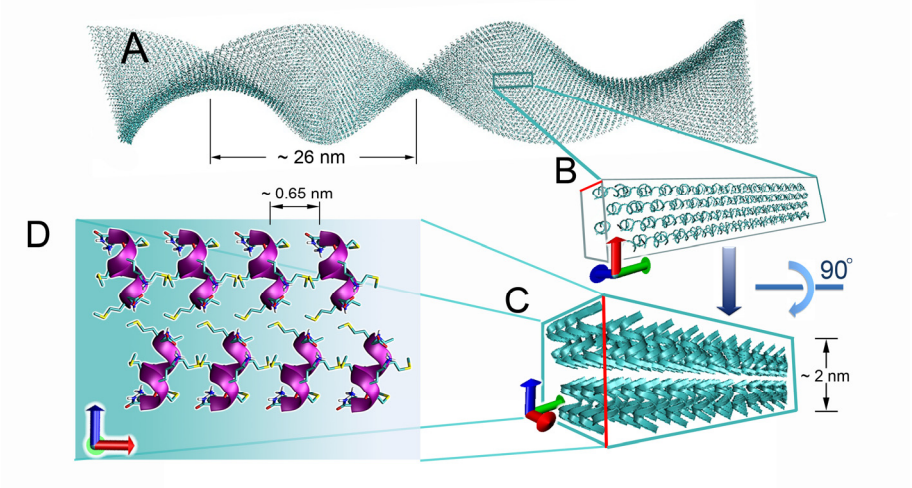

Figure S12. The length scale of the schematic shown in Figure 3. The distance between individual helices is ~0.65 nm, and the period and the height are ~26 nm and 2 nm, respectively.

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