

Supporting Information.

Fibrillar Superstructure from Extended Nanotapes formed by a Collagen-Stimulating Peptide Amphiphile

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Experimental Section

Materials. Peptide amphiphile Matrixyl, Palmitoyl-Lys-Thr-Thr-Lys-Ser was purchased from CS Bio (Menlo Park, California). Purity was 97.6% by analytical HPLC, MS 802.47 (expected) 802.05 (measured), acetate content was 11% (by HPLC).

Laser Scanning Confocal Microscopy (LCSM). Experiments were performed on a Leica TCS SP2 confocal system mounted on a Leica DM-IRE2 upright microscope, using an objective x20 with 0.4NA numerical aperture. For LCSM, Matrixyl solutions were dyed using Rhodamine B (RhoB), such that each sample examined had a 2.6×10^{-4} wt % RhoB content. The excitation wavelength generated by an Argon laser was 514 nm, while the emission detection was in the range 558-617 nm. Samples were examined in the flow cell, before and after shear, or they were put between a glass slide and a coverslip.

Cryogenic Transmission Electron Microscopy (Cryo-TEM). Samples (0.1% and 1%) were prepared manually in a controlled environment vitrification system (CEVS).³⁸ Lower concentration solutions were more fluid, and prepared either in the CEVS or using the commercial environmentally-controlled automated Vitrobot (FEI, Netherlands). In both procedures, cryo-TEM samples were prepared at a controlled temperature of 25 °C and at saturation. A 4- μ l drop of the solution was placed on a 200-mesh TEM copper grid covered with a perforated carbon film. The drop was blotted, manually in the CEVS and automatically in the Vitrobot, and the sample was plunged into liquid ethane (-183 °C) to form a vitrified specimen, then transferred to liquid nitrogen (-196°C) for storage. Most vitrified specimens were examined in a Tecnai T12 G² TEM (FEI) at 120 kV, at temperatures below -175 °C. Images were recorded digitally on a Gatan UltraScan 1000 cooled CCD camera using DigitalMicrograph (Gatan, U.K.) in the low-dose imaging mode to minimize beam exposure and electron-beam radiation damage. Some cryo-TEM samples were examined at similar conditions and procedure in a Philips CM120 TEM instrument.

Negative Stain TEM. High resolution TEM (HR-TEM) was performed using a JEOL JEM-4000 microscope operated at 200 kV. Droplets of Matrixyl solution (1 wt% in water) were placed on Cu grids coated with a carbon film (Agar Scientific, UK), stained with uranyl acetate (1 wt %) (Agar Scientific, UK) and dried. Additional TEM was performed using a Philips CM120 transmission electron microscope operating at 120 kV. Images were recorded digitally on a Gatan MultiScan 791 camera using the DigitalMicrograph software (Gatan, U.K.).

Scanning Electron Microscopy (SEM). High-resolution SEM images were obtained using a Zeiss Ultra Plus microscope equipped with a Schottky field-emission electron gun. Specimens were examined at low electron acceleration voltage (1 to 3.5 kV) and short working distance (~3 mm), using both InLens and the Everhart–Thornley secondary electron imaging detectors.

Atomic Force Microscopy (AFM). Images were acquired using DI3100 Nanoscope IIIa (Digital Instruments, Santa Barbara, CA). Imaging was done in tapping mode under ambient conditions. Silicon nitride cantilevers (Mikromasch) of 10 nm typical tip radius were used.

Small-Angle X-ray Scattering (SAXS). SAXS data were collected on the SWING beamline at SOLEIL,³⁹ the French synchrotron facility. A few microlitres of sample (0.95 wt% in water) were injected at a slow and very reproducible flux into a quartz capillary, which is placed in front of the X-ray beam. Although the quartz capillary is enclosed in a vacuum chamber, in order to avoid parasitic scattering, the sample itself

circulates at atmospheric pressure within a quartz capillary. After the sample is injected in the capillary and placed in front of the X-ray beam, the flow is stopped during the SAXS data acquisition. The sample is thermostated throughout its entire travel from the injector to the quartz capillary. SAXS experiments were performed at 20 and 65 °C. The wavenumber q range was set to 0.004-0.6 Å⁻¹, with $q = 4\pi\sin(\theta)/\lambda$, where 2θ is the scattering angle and $\lambda = 1.03$ Å (12 keV) is the X-ray wavelength. The images captured by the AVIEX170170 CCD detector were radially averaged and corrected for transmitted intensity and water background using *ActionJava*, a home-made dedicated application.

Fourier Transform Infrared (FTIR) Spectroscopy. FTIR spectra were recorded on a Nicolet FTIR Nexus spectrometer equipped with a DTGS detector. Solutions of Matrixyl in D₂O were sandwiched in ring spacers between two CaF₂ plate windows (spacer 0.006 mm). Spectra were scanned 128 times over the range of 4000-400 cm⁻¹.

Circular Dichroism (CD). Spectra were recorded using a Chirascan spectropolarimeter (Applied Photophysics, UK). CD was performed using matrixil dissolved in water (0.013, 0.075, 0.13, 2.3, 5.4 and 9 wt.%) and on a peptide film dried from a 0.13 wt % solution. The liquid samples were loaded into quartz cover slip cuvettes (0.1 or 0.01 mm thick) or into 1 mm thick quartz bottles. The peptide film was dried onto a quartz plate. Spectra are presented with absorbance $A < 2$ at any measured point with a 0.5 nm step, 1 nm bandwidth and 1 second collection time per step. CD spectra were collected at 20 °C for all the samples investigated. A temperature ramp experiment was run for the 0.013 wt % solution, using a 3 °C step ramp, within the interval (20-80) °C.

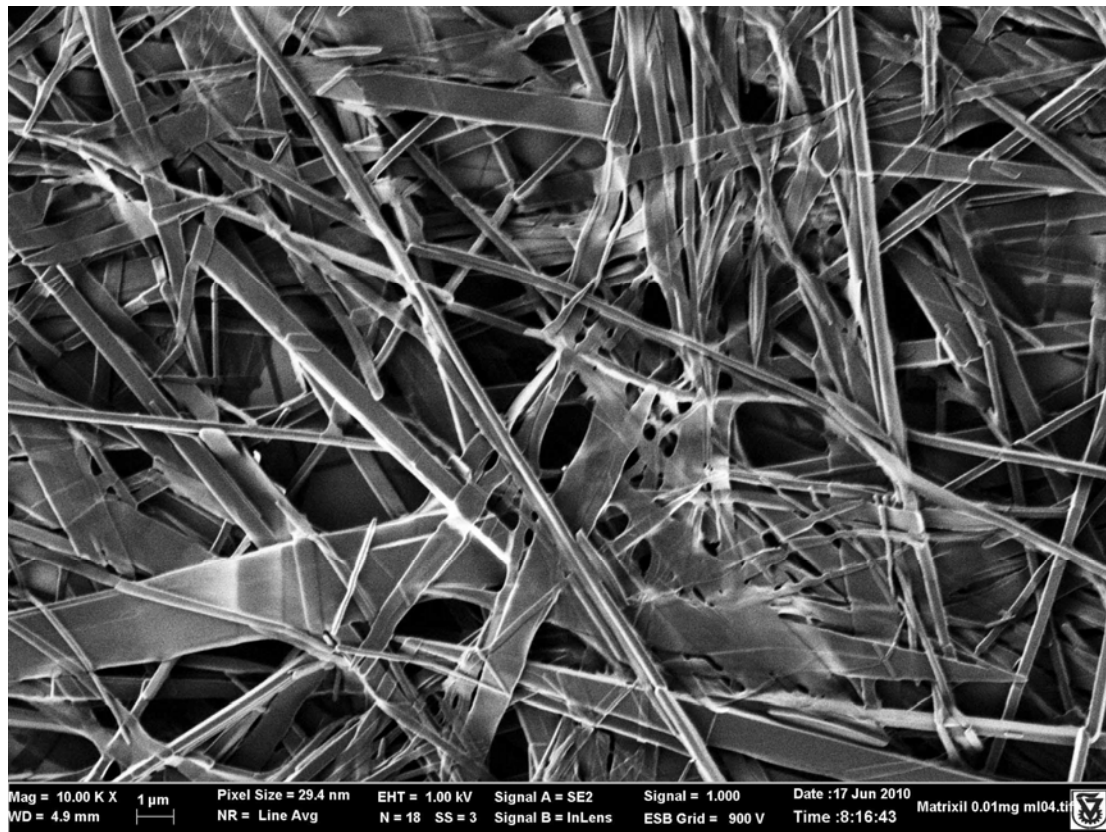
X-Ray Diffraction (XRD). X-ray diffraction was performed on stalks prepared by drying filaments of the peptide. Aqueous gels (8.9 wt.%) of peptide was suspended between the ends of wax-coated capillaries and dried. The stalks were mounted (vertically) onto the four axis goniometer of a RAXIS IV++ x-ray diffractometer (Rigaku) equipped with a rotating anode generator. The XRD data was collected using a Saturn 992 CCD camera.

Congo Red Assays. A 8.9 wt % Matrixyl sample was stained using a freshly prepared and filtered Congo red/NaCl solution. The Congo red/NaCl solution contained 0.5 wt % Congo red and 0.2 wt % NaCl diluted in a MeOH/water mixture (80% MeOH, 20% water). A drop of the stained sample was placed onto a glass microscope, under a cover slip. The sample was then observed with the microscope through crossed polarizers in an Olympus BX41 polarized microscope.

Polarized Optical Microscopy. Images were obtained with an Olympus BX41 polarized microscope by placing the sample between crossed polarizers. Samples were placed between a glass slide and a coverslip before capturing the images with a Canon G2 digital camera.

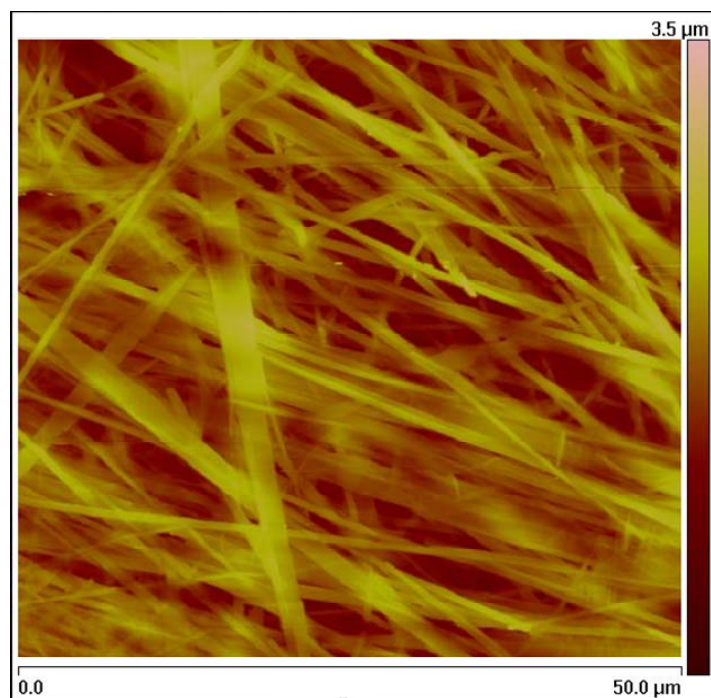
Results Section

SEM



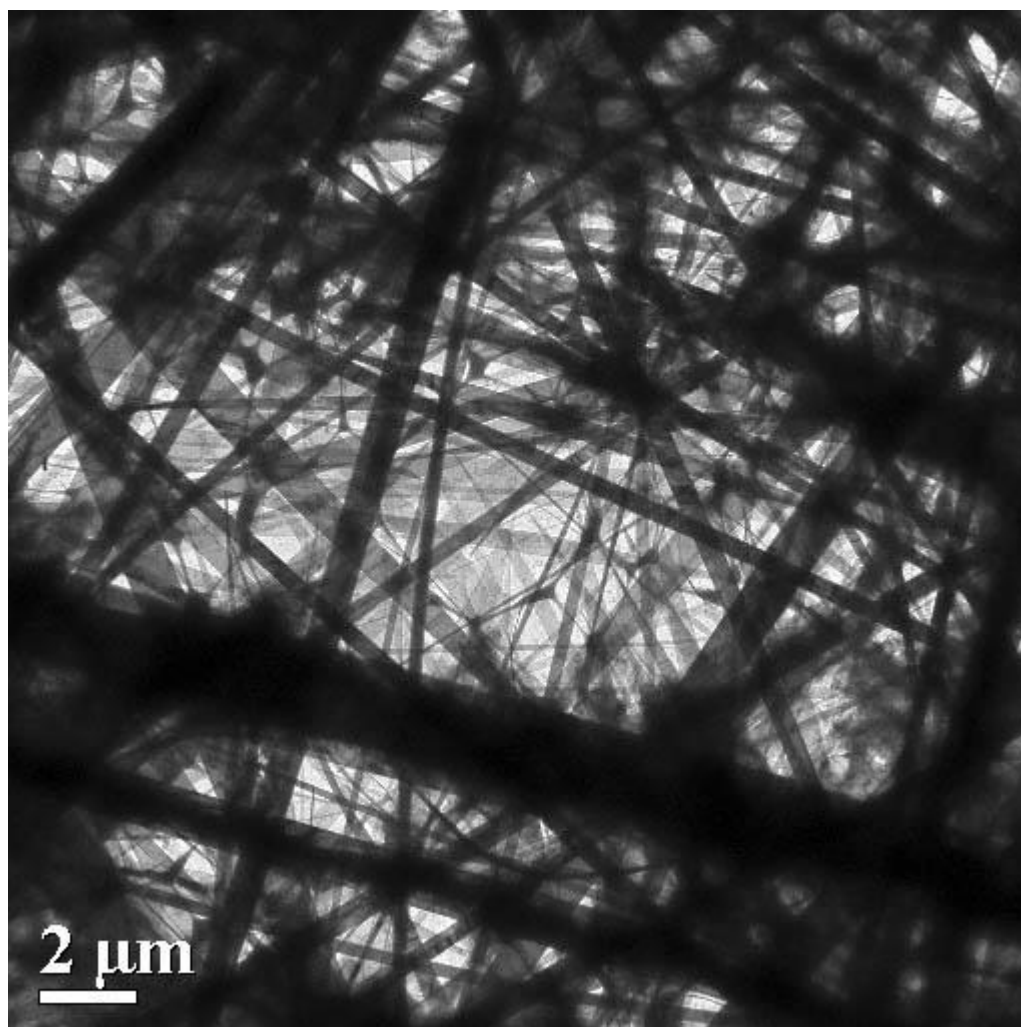
SI Fig. 1. Representative SEM image of Matrixyl showing tapes and fibrils.

AFM



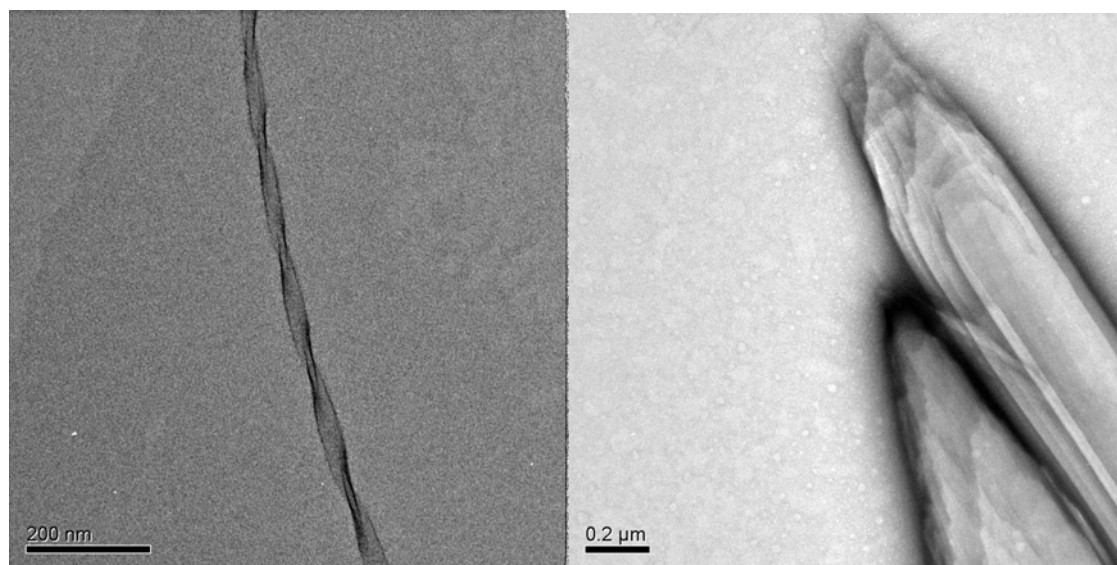
SI Fig.2. AFM image (topography, scale indicated) showing superstructure of Matrixyl.

Cryo-TEM



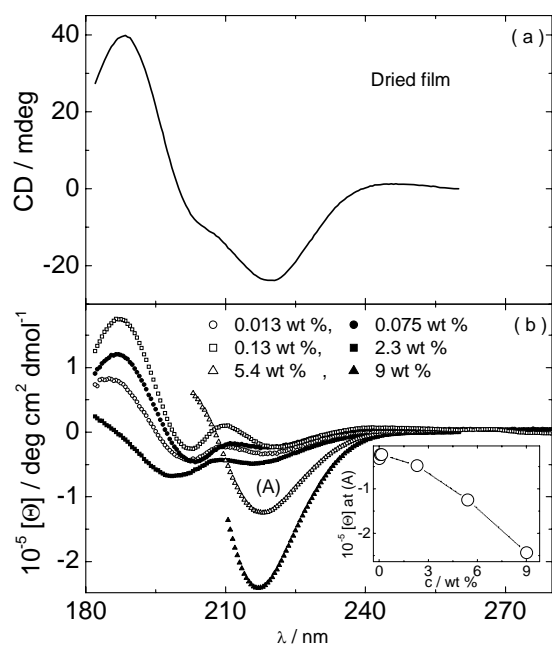
SI Fig.3. Low magnification cryo-TEM image obtained for a 1 wt% sample of Matrixyl.

TEM



SI Fig.4. Negative stain TEM images (a) Example of twisted tape (0.075 wt% matrixyl), (b) Multi-layer crystal formed by matrixyl in an unfiltered solution (0.075 wt % matrixyl) solution. Thermal treatment was as follows: The sample was heated at 50 °C for 30 minutes, strong vortex for 2 minutes and sonicated in a water bath at 50 °C for a further 30 minutes.

CD



SI Fig.5. CD spectra for matrixyl at 20 °C, (a) Dried film, (b) Comparison of spectra at several concentrations. The inset shows the increase in the β -sheet minimum at (A) i.e. 217 nm.

SI Table 1. Position of peaks in XRD pattern

Equatorial	Meridional and off-meridional (M/OM)
26.4(4)	4.78(1) (M)
13.92(2)	4.34(1) (OM)
11.28(3)	4.14(1) (OM)
9.18(3)	
7.70(3)	
6.69(1), 6.49(1)	
3.93(1)	
3.92(1)	