Supporting Information

Two Distinct β-Sheet Fibrils from Silk Protein

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Experimental:

Preparation of regenerated silk fibroin (RSF) solution: Raw silk consists of fibroin fibers that are bound together by several hydrophilic gum-like coat proteins, the sericin. The sericin was removed by thoroughly degumming raw silk by boiling it for two 20 min cycles with changes of 0.5% (w/w) $NaCO_3$ solution. After exhaustive washing in distilled water the degummed fibers were allowed to air dry at room temperature.The degummed dry silk was then dissolved in 9.5 mol/L LiBr aqueous solution. After being filtered, the fibroin solution was dialyzed against deionized water for 72 h at room temperature with a 20,000 molecular weight cutoff dialysis membrane to remove the salt. The dialyzed solution was then clarified by spinning in a centrifuge at 6,000 g for about 5 min. The supernatant which was an aqueous RSF solution was collected and carefully stored at 4 °C for no more than 3 days.

Cryo transmission electron microscopy: Cryo transmission microscopy (cryo-TEM) measurements were performed on a CM12 (Philips). A Gatan cryo-holder operating at ~ -170 °C was used for the cryo- TEM measurements. The CM12 is equipped with a LaB_6 filament operating at 200 kV. Images were recorded under standard low dose conditions on 35 mm film (Kodak Technical Pan). The sample (0.75 wt% RSF incubated in 50% (v/v) ethanol-water for 50min) vitrification procedure was carried out using an automated vitrification robot (FEI Vitrobot Mark III) (20 °C and 100% RH). TEM grids, (R2/2 Quantifoil Jena) were purchased from Aurion. The grids were plasma surface treated using a Cressington 208 carbon coater operating at 5 mA for 40 seconds prior to the vitrification procedure. The blot time for the sample was 2s and drain time 5s.

Transmission electron microscopy: RSF at 0.75 wt% was incubated in 50% (v/v) ethanol solution. To observe the formation of nanofibrils, the incubated solution was diluted 20 times with 50% (v/v) ethanol solution and 10 μl diluted solution was placed on a carbon-coated Cu grid with the excess blotted, negatively stained with 1 wt% uranyl acetate, air-dried and examined in a JEOL 2011 transmission electron microscope (JEOL, Japan).

Atomic force microscopy: The incubated RSF solutions were diluted 1000 times with 50% (v/v) ethanol solution, and 20 μl diluted solution was applied onto freshly cleaved mica (~1cm^2) attached to a magnetic steel disc (serving as sample holder). The samples were subsequently air-dried at room temperature overnight. Imaging was recorded on a Nanoscope IV Digital Instruments atomic force microscope (Veeco Metrology Group, USA) in tapping mode, using a Si3N4 cantilever with a spring constant of 50 N m^{-1} and a resonance frequency of 340 kHz.

Preparation and characterization of shear-induced fibrils: 0.75 wt% RSF solution was stirred using a glass rod (or a magnetic stirrer bar) for a period of time (~ 30 second). The generated white fibrous flocs were deposited directly on a glass microscope slide for scanning electron microscopy (SEM) using a Philips XL 30. The stirred solution with shear-induced fibrils was diluted 100 times with distilled water and 10 μl diluted solution was placed on a carbon-coated Cu grid with the excess blotted, negatively stained with 1 wt% uranyl acetate, air-dried and examined in a JEOL 2011 transmission electron microscope.

2D X-ray diffraction: Two-dimensional (2D) X-ray diffraction patterns of RSF fibrils and degummed *Bombyx mori* silk fibers were obtained using a Bruker D8Discover diffractometer (Bruker, Germany) with a GADDS detector calibrated with silicon powder and silver behenate. Fibril samples were prepared by air-drying the RSF solution (at the endpoint of the incubation reaction in ethanol) between two wax-plugged capillary ends. The distance between the capillaries was increased slowly to facilitate the alignment of the fibrils while drying occurred. This procedure produced a small stalk of fibrils protruding from the end of one of the capillaries, which was mounted vertical to the X-ray beam. The degummed silk fibers were wound on a frame to produce well-alligned fiber bundles, which were also mounted vertical to the X-ray beam.

Thioflavin T fluorescence: Thioflavin T binding was carried out at room temperature. The incubated RSF solutions or shear-induced fibrils were diluted 10 times with 50% (v/v) ethanol solution, which contained 80 μM Thioflavin T. The spectra were recorded using a FLS-920 fluorescence spectrophotometer (Edinburgh Instruments, U.K.). A 1.0 cm path length quartz cell was used for the fluorescence measurement. The excitation wavelength was set at 450nm and the emission spectrum was taken from 460nm to 600nm.

Congo red (CR) binding: A fresh CR solution was prepared by dissolving the dye in deionized water at concentration of 500 μM and filtering it three times with a 0.22 μm filters. Aliquots of incubated 0.75 wt% RSF solutions or shear-induced fibril solution (500 μl) were diluted in the distilled water containing 200 μl of the freshly prepared dye solution (5 ml final reaction volume). The reaction samples were thoroughly mixed and incubated at room temperature for 5 min before recording the absorbance spectrum.

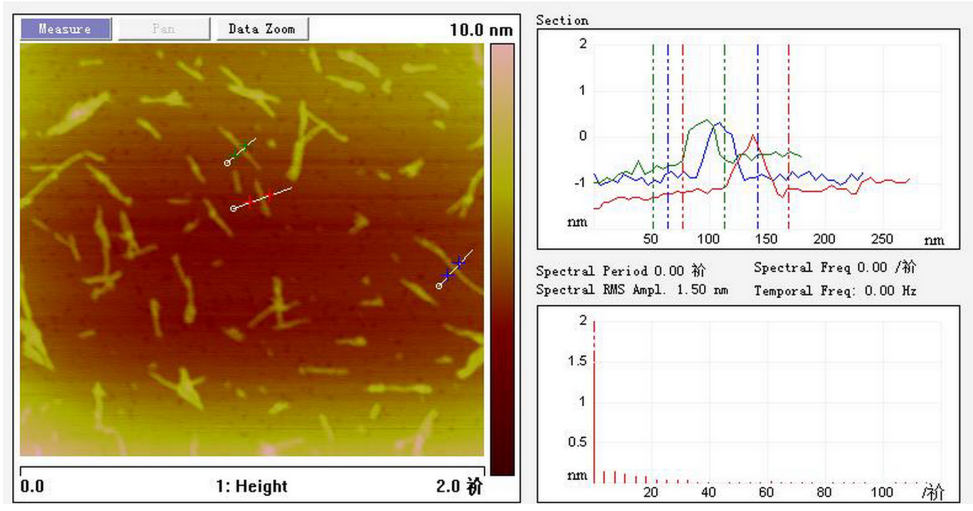

Figure S1 AFM section analysis of the RSF nanofibrils marked by the white lines in the height image (2μm × 2μm).

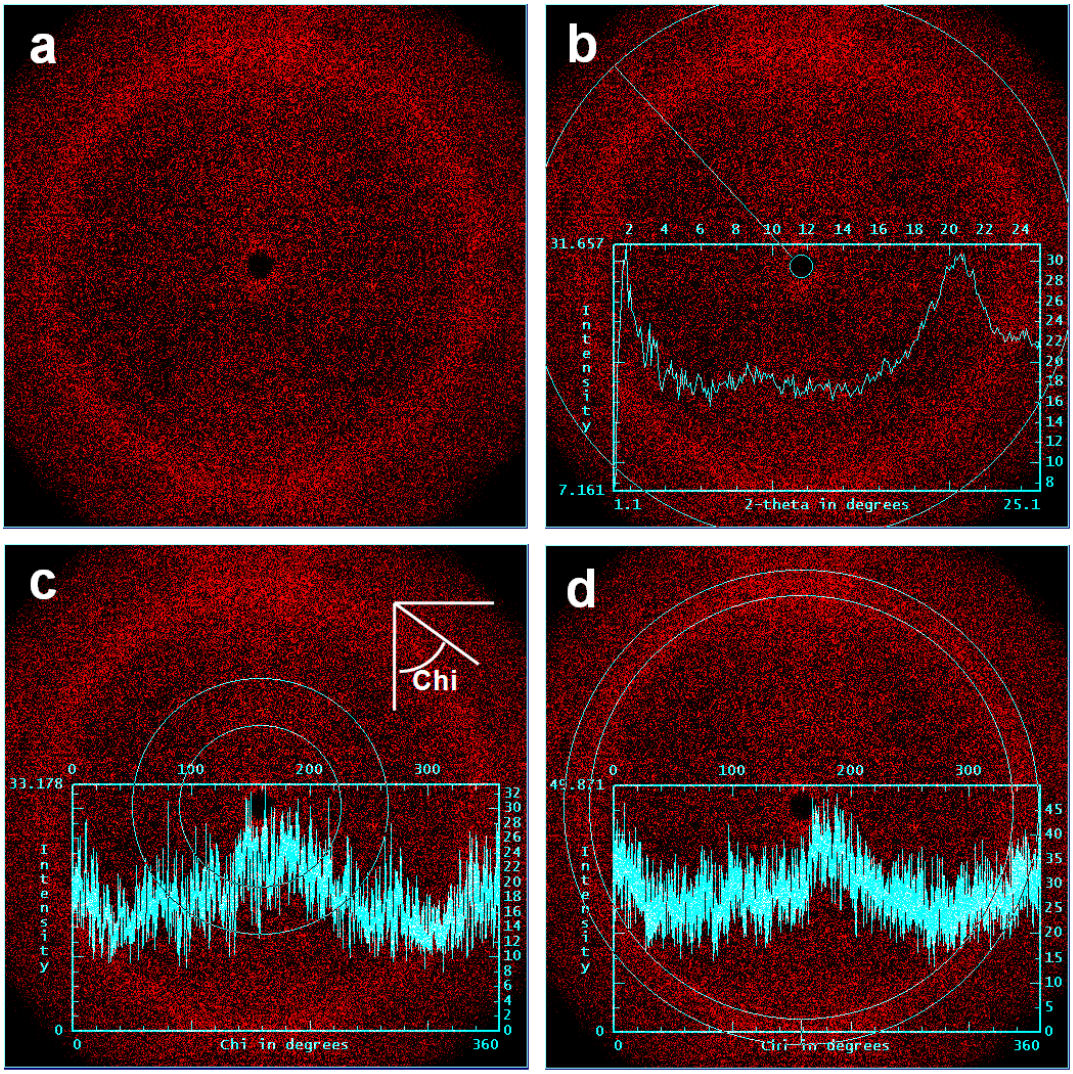

Figure S2 (A) 2D WAXD of shear-induced fibrils measured at 90 degrees (the fiber axis is along the equator); (B) 1D X-ray diffraction patterns after radial averaging; (C) and (D) Azimuthal (angle chi defined in C) intensity variation in the 10 A and 4.4 A diffraction rings.