Spontaneous inter-conversion of insulin fibril chirality

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Supporting Information

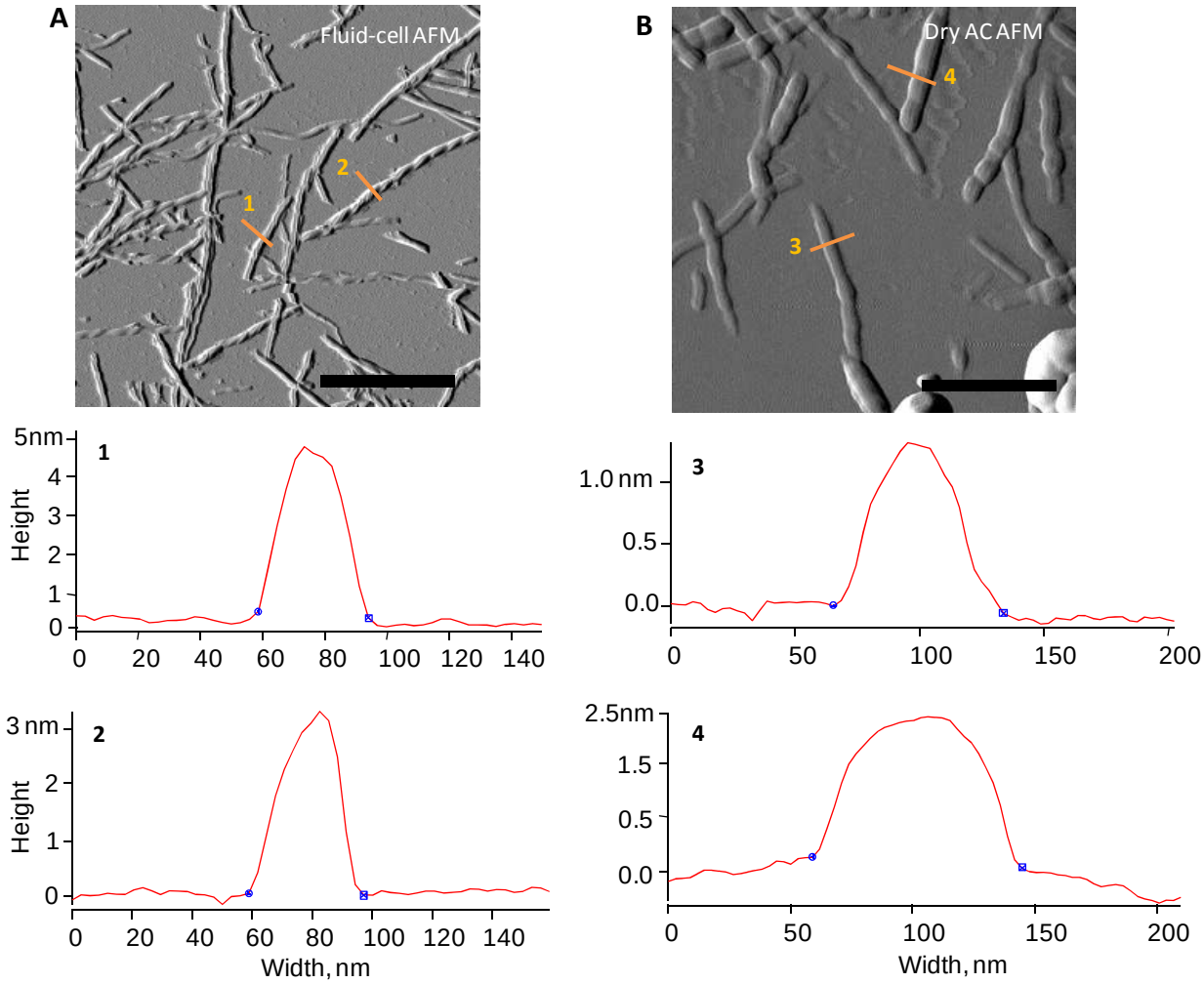

Figure S1. Drying process dramatically deforms insulin fibrils flatters them. Height AFM images and profiles of insulin fibrils imaged in fluid-cell mode (a) and the same fibril solution after drying by nitrogen flow (b). Scale bar is 500 nm. In both cases mica was used as a substrate. Lateral dimensions of insulin fibrils: 1. 35.2 nm (width) x 4.2 nm (height); 2. 38.3 nm (width) x 3.0 nm (height); 3. 68.4 nm (width) x 1.2 nm (height); 4. 82.3 nm (width) x 2.2 nm (height).

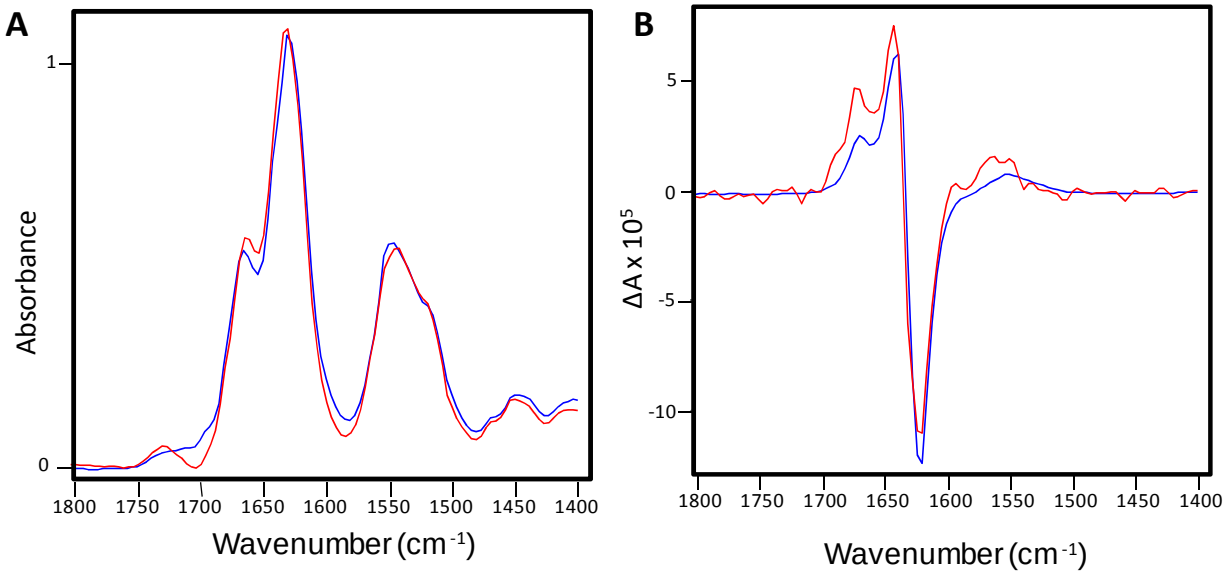

Figure S2. Normal VCD insulin fibrils are stable and do not over turn the chirality as a result of pH decrease. IR (A) and VCD (B) spectra of NF (blue) and the same solution after 8 hours pH was decreased to 1.0 (red).

Preparation of insulin fibrils.

A pH of bovine insulin (60mg/ml (Sigma-Aldrich, (St. Louis MO; I5500, lot 069K0982 and I1882, lot 120M8702V)) solution was adjusted to 1.5 or 2.5 by concentrated HCl (Sigma-Aldrich, (St. Louis MO)). Samples were heated at 70 ^{0}C for 2 hours. Then, the fibrillation process was terminated by reducing the temperature to ~25 °C and a sample centrifugation at 14.000g for 20 min. A gelatinous phase, mostly dominated by well-developed fibrils, was re-dispersed in solution with pH 1.5 or 2.5 for VCD measurements.

Vibrational circular dichroism instrumentation.

VCD and IR spectra were measured at BioTools, Inc, Jupiter, FL using a dual-source, Dual*PEM* Chiral*IR-2X* Fourier transform VCD spectrometer equipped with an MCT detector with spectral coverage from 800 to 2000 cm^{-1}. GRAMS/AI 7.0 (Thermo Galactic, Salem, NH) was used for spectral data processing.

Atomic force microscopy methodology.

Immediately after the fibrillation was completed, an aliquot of fibrillar gel was re-suspended in HCl, pH 1.5 or 2.5 solution with a 1:400 dilution factor (V/V). A drop of this solution was placed onto freshly cleaved mica into AFM fluid chamber and incubated for 2 min followed by removing of the solution excess. To avoid mica surface drying, 2 ml of pure HCl solution, pH 1.5 or 2.5 were placed on top of the mica. AFM scanning was performed immediately in tapping mode using MFP-3D™ Bio Asylum Research microscope (Asylum Research, CA, USA) with Olympus TR400PSA tips. Surface profiles were plotted using MFP3D software. The AFM technique reports accurately the species height, while width dimensions are subjected to broadening due to the finite size of the tip. For the direct observation of fibril inter-conversion a small aliquot of sodium hydroxide (1 μL) was injected into the fluid chamber after the image of RF was obtained. To avoid the effects of temperature change the tip was withdrawn from the mica surface during the pH adjustments. After the solution pH and chamber temperature were equilibrated scanning was continued.

Deep ultraviolet resonance Raman spectroscopy.

DUVRR spectra were obtained at the University of Albany using a home-built Raman spectrometer as described elsewhere (23) with 199-nm excitation wavelength. A spinning NMR tube with a magnetic stirrer inside was used for sampling. All reported Raman spectra are an average of at least three independent measurements. GRAMS/AI 7.0 (Thermo Galactic, Salem, NH) was used for spectral data processing.