Supramolecular Chirality in Peptide Microcrystals

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Supporting Information

Crystal Sample Preparation

The monomeric peptides for this study were synthesized by GenScript and used as received without further purification. Crystallization was monitored with optical microscopy; NNFGAIL crystals formed within 3 days of incubation while SSTNVG crystals appeared after 5 days.

Microcrystals of $IAPP_{21-27}$ peptide with sequence NNFGAIL were prepared by hanging-drop vapor diffusion; a 2 µl drop contained a mixture of equal volumes of peptide monomer solution (5 mg/mL peptide in unbuffered double distilled water) and reservoir solution (20% v/v ethylene glycol).

Microcrystals of $IAPP_{28-33}$ peptide with sequence SSTNVG were prepared by hanging-drop vapor diffusion; a 2 µl drop contained a mixture of equal volumes of peptide monomer solution (20 mg/mL peptide in unbuffered double distilled water) and reservoir solution (30% v/w PEG-3000, 0.2 M ammonium sulfate and 0.1 M Na cacodylate buffer with pH 6.5).

Fibril Sample Preparation

Fibrils were formed under the same solution conditions as crystallization. Unlike in the crystallization setup, fibrils were formed in microcentrifuge tubes.

Fibrils of NNFGAIL peptide were prepared by incubation of 5 mg/mL peptide solution in double distilled water for 3 days at room temperature (23˚C).

Fibrils of SSTNVG peptide were prepared by incubation of 20 mg/mL peptide solution in 30% v/w PEG-3000, 0.2 M ammonium sulfate and 0.1 M Na cacodylate buffer with pH 6.5 for 3 days at room temperature (24˚C).

Vibrational Circular Dichroism Instrumentation

VCD and IR spectra were measured at BioTools, Inc, Jupiter, FL using Chiral*IR-2X* Fourier transform VCD (FT-VCD) spectrometer equipped with an MCT detector and the Dual*PEM* option for enhanced VCD baseline stability. For each measurement, ~10 µl of sample was placed in a Bio*Cell* with CaF_2 windows and a 6-µm pathlength. During measurements the Bio*Cell* was rotated at a constant velocity about the bean IR axis using *Sync*RoCell (BioTools, Inc.) to eliminate cell and possible sample birefringence, and the resulting VCD and IR spectra were acquired for 2-4 hours at 8 cm^{-1} spectral resolution. Spectral baselines for VCD and IR were determined from measurements of water in the Bio*Cell* of for the same length of time as sample measurements. GRAMS/AI 7.0 (Thermo Galactic, Salem, NH) was used for spectral data processing.

Scanning Electron Microscopy (SEM)

A 200-mesh copper grid (Ted Pella, Ink.) was immersed into mother liquor containing polypeptide microcrystals for 2-3 min, rinsed with 1% uranyl acetate and dried under room temperature. Prior to drying, the excess of uranyl acetate solution was gently removed using Kimwipe tissue. Samples were imaged on Zeiss Supra SEM in InLense mode with 5 kV EHT.

Atomic Force Microscopy (SEM)

A 5 μL sample of fibril suspension or 2 μL sample of microcrystal suspension was deposited on a freshly cleaved mica slide and incubated for 5 min, gently blotted with filter paper on the side and dried under nitrogen flow. The sample was imaged using MFP-3D™ Bio Asylum Research microscope (Asylum Research, CA, USA) with Olympus AC160TS silicon cantilever with tip diameter <10 nm.

Imaging of Fibrillar and Crystalline Samples

Optical imaging of fibrillar samples was performed in mother liquor using Leica DMLM confocal microscope equipped with 20X and 50X objectives. The optical images of SSTNVG and NNFGAIL microcrystals are displayed in Figure S1.

Figure S1 – Optical images of NNFGAIL microcrystals (left) and SSTNVG microcrystals (right).

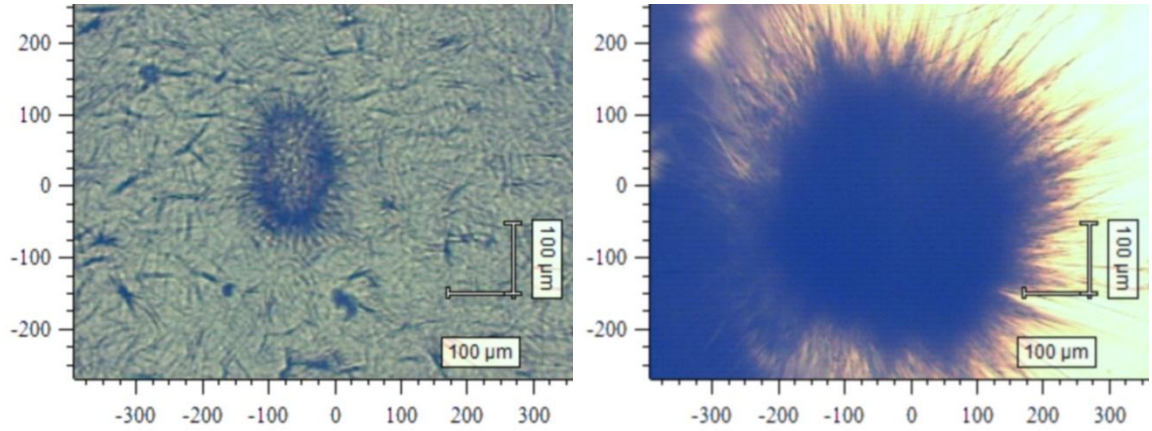

Figure S2 SEM images of NNFGAIL microcrystals. Scale bars are 2 microns for A and 200 nm for B and C.

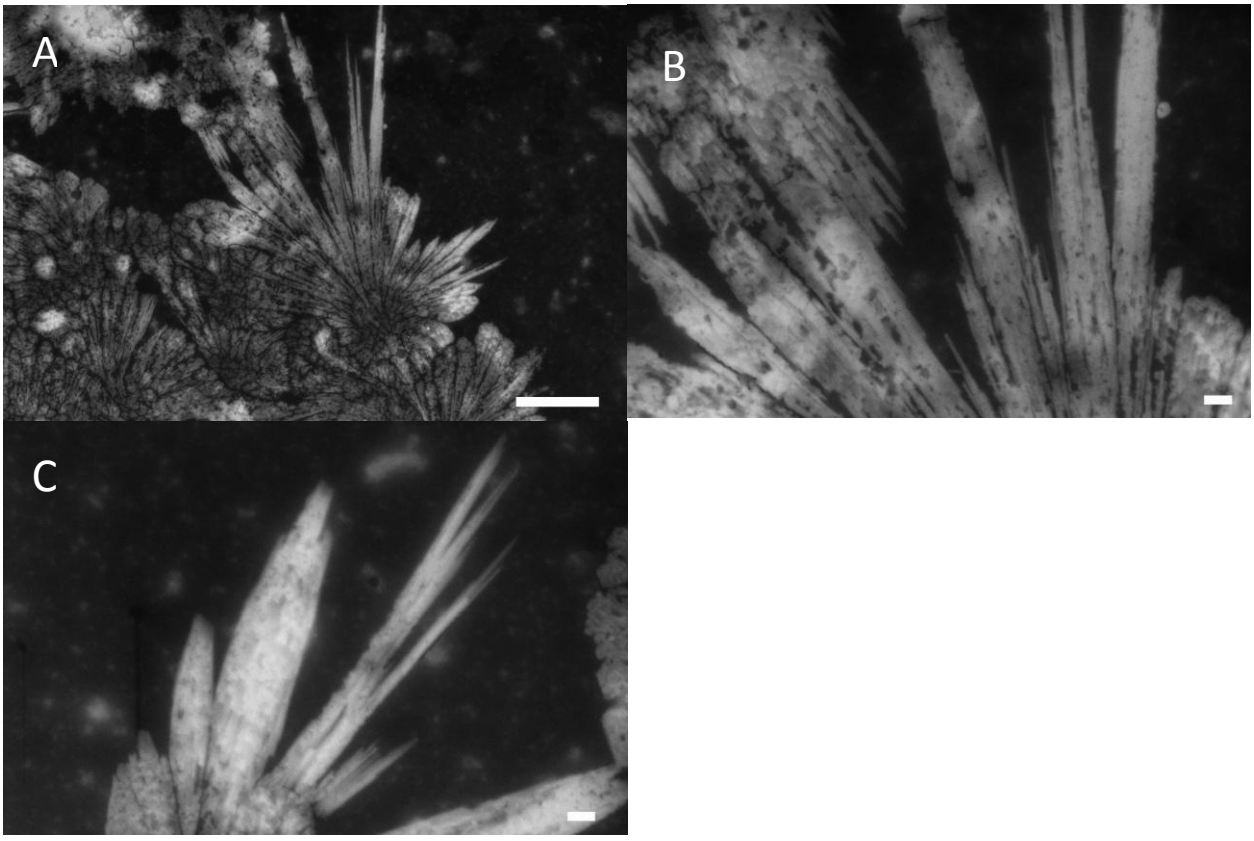

Figure S3 SSTNVG microcrystals. Scale bars are 2 microns for A and 200 nm for B.

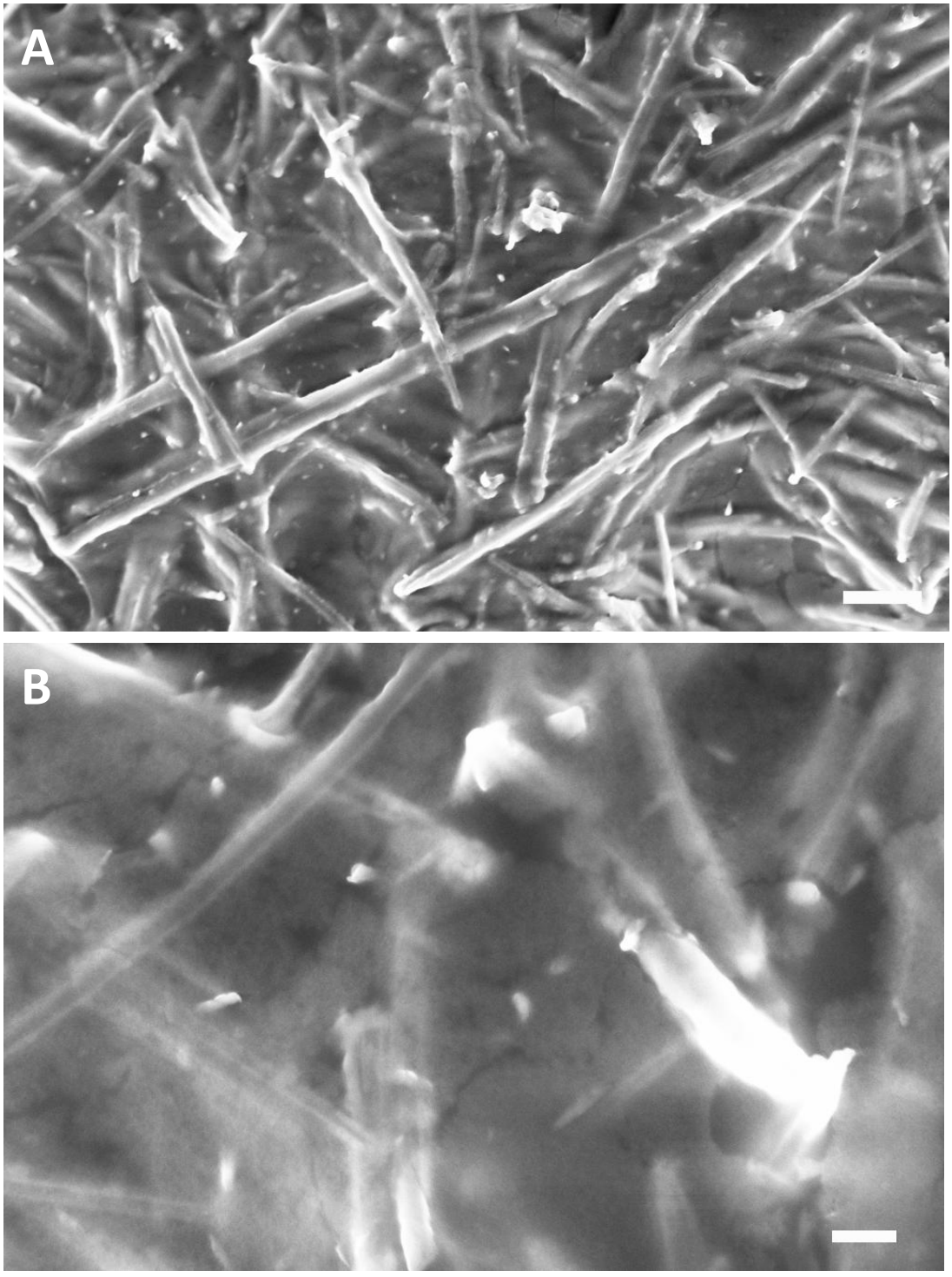

Figure S4 AFM topology of NNFGAIL microcrystals (left panel) and fibrils (right panel).