Is Supramolecular Proto-Filament Chirality the Underlying Cause of Major Morphology Differences in Amyloid Fibrils?

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Supporting Information:

Experimental procedures:

Peptide and protein preparation and aggregation.

Bovine insulin (Sigma-Aldrich, (St. Louis MO)) was aggregated at different pH for 2-24 hours under different temperatures. Solution pH was adjusted by HCl. The fibrillation process was terminated by reducing the temperature to ~25 °C and a sample centrifugation at 14,000g for 20 min.

Chicken-egg lysozyme (Sigma-Aldrich, (St. Louis MO)) was aggregated at different pH for 3 or 5 days at 65 °C. Solution pH was adjusted by HCl.The fibrillation process was terminated by reducing the temperature to ~25 °C and/or a sample centrifugation at 14,000g for 20 min.

A short fragment of transthyretin ($TTR_{105-115}$) with sequence YTIAALLSPYS was obtained through a custom synthesis from GenScript Corporation (Piscataway, NJ). The peptide (2 mg/mL) was dissolved in 10 mMHCl and then lyophilized to remove trifluoroacetate ion, which is a common admixture in peptides obtained by solid-phase peptide synthesis. $TTR_{105-115}$ peptide powder (15mg/ml) was dissolved in 10% acetonitrile/water solutionand incubated for 2 days at 37°C followed by 14 days at room temperature.

HET-s (218-289) prion protein was expressed in *E. coli* cell culture. Cell inclusion bodies were purified and solubilized in 6M GdnHCl buffer and HET-s was purified by Ni-NTA chromatography. The eluted protein was additionally desalted using a Sephadex G-25 superfine column into 20mM citric acid or sodium citrate at pH 2.0, 3.3, and 3.9 for fibrillization. Fibrils weredialyzed against 5 mMsodiumacetate buffer at pH 2.0, 3.3 and 3.9. Protein samples were incubated at room temperature for 2 months.

Apo-α-lactalbumin (Sigma-Aldrich, (St. Louis MO)) was aggregated at different pH at 37 °C in a presence of 150 mMNaCl for 72 hours. The fibrillation process was terminated by reducing the temperature to ~25 °C and a sample centrifugation at 14,000g for 20 min.

Vibrational Circular Dichroism (VCD) spectroscopy.

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 fibril sample was placed in a *Bio*Cell (BioTools, Inc.) with CaF_2 windows and a 6-µm pathlength. During measurements the *Bio*Cellwas rotated at a constant velocity about the beam IR axis using SyncRoCell (BioTools, Inc.)

to eliminate cell and possible sample birefringence. VCD and IR spectra were acquired for 2-8 hours at 8 cm^{-1} spectral resolution. Spectral baselines for VCD and IR were determined from measurements of the corresponding solvent in the same *Bio*Cellfor the same length of time as sample measurements. All subsequent data processing leading to final spectra was carried out in GRAMS/AI 7.0 (Thermo Galactic, Salem, NH).

Microscopy.

Fluid-cell Atomic Force Microscopy (AFM).

Immediately after the fibrillation was completed, an aliquot of fibrillar gel was re-suspended in HCl with the same pH as the analyzed sample. This diluted fibril aliquot (1:400 dilution factor (V/V)) 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, with the same pH as the analyzed sample, 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.

Dry-mode AFM.

After a sample aliquot was incubated for 2 min, the solution excess from the mica surface. Then, mica was gently dried under a nitrogen flow. AFM scanning was performed immediately in tapping mode using the same AFM with Olympus AC-160 tips.

Scanning Electron Microscopy (SEM).
For each sample 20 μL of analyzed solution were diluted in 1:400 ratio by distilled water and deposited on a 200-mesh copper grid. Staining with 1% uranyl acetate was performed in 10 minutes after the deposition. Samples were imaged on Zeiss Supra SEM in InLense mode with 5 kV EHT.

Cryo-SEM
An aliquot of the fibril gel (10uL)was deposited onto a silicon wafer, blottingand immediately plunge freezing using Reichert-Jung KF80 plunge freezer in liquid ethane. Then, samples were briefly dipped into liquid nitrogen and transferred intoBALZER 301 freeze fracture apparatus onto a -170°C stage and pumped down to a vacuum betterthan 10^{-4} mbar. Samples then were brought to room temperature within 1 hour, while stillunder vacuum. While at room temperature, 3 nm layer of platinum was deposited on the sample.Imaging was done in Hitachi S-4800 instrument using secondary electron detector at 2 kVaccelerating voltage.

Deep ultraviolet resonance Raman (DUVRR) spectroscopy.

DUVRR spectra were obtained at the University of Albany using a home-built Raman spectrometer as described elsewhere[1] 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.

DUVRR Results for Lysozyme

A typical protein DUVRR spectrum is dominated by amide bands, which characterize the polypeptide backbone conformation, and aromatic amino acid bands reporting their local environment.[2] The DUVRR signature of the amide chromophore consists of Amide I, II, III and C_α-H bending bands. We found that lysozyme fibrils grown at pH 1.5, 2.3 and pH 2.7 have the same frequencies for all amide bands as well as the C_α-H band. Lysozyme fibrils that were grown at pH 1.5 have a small decrease in the intensity of Amide II band, while intensities of Amide I and C_α-H band are identical. This high similarity of amide bands indicates that all studied lysozyme fibril polymorphs share the same cross-β-core structure. Previously, cross-β-core structure of one of the lysozyme chiral polymorphs was extensively studied in our laboratory.[3, 4] It was found that lysozyme fibrils grown at pH 2.0, 65 °C have antiparallel β-sheet structure with a Ψ angle of 133°.[5] The results obtained here indicate that all lysozyme fibril polymorphs, despite previously demonstrated differences in morphology and chiral filament and fibril structure, share the same cross-β-core structure.

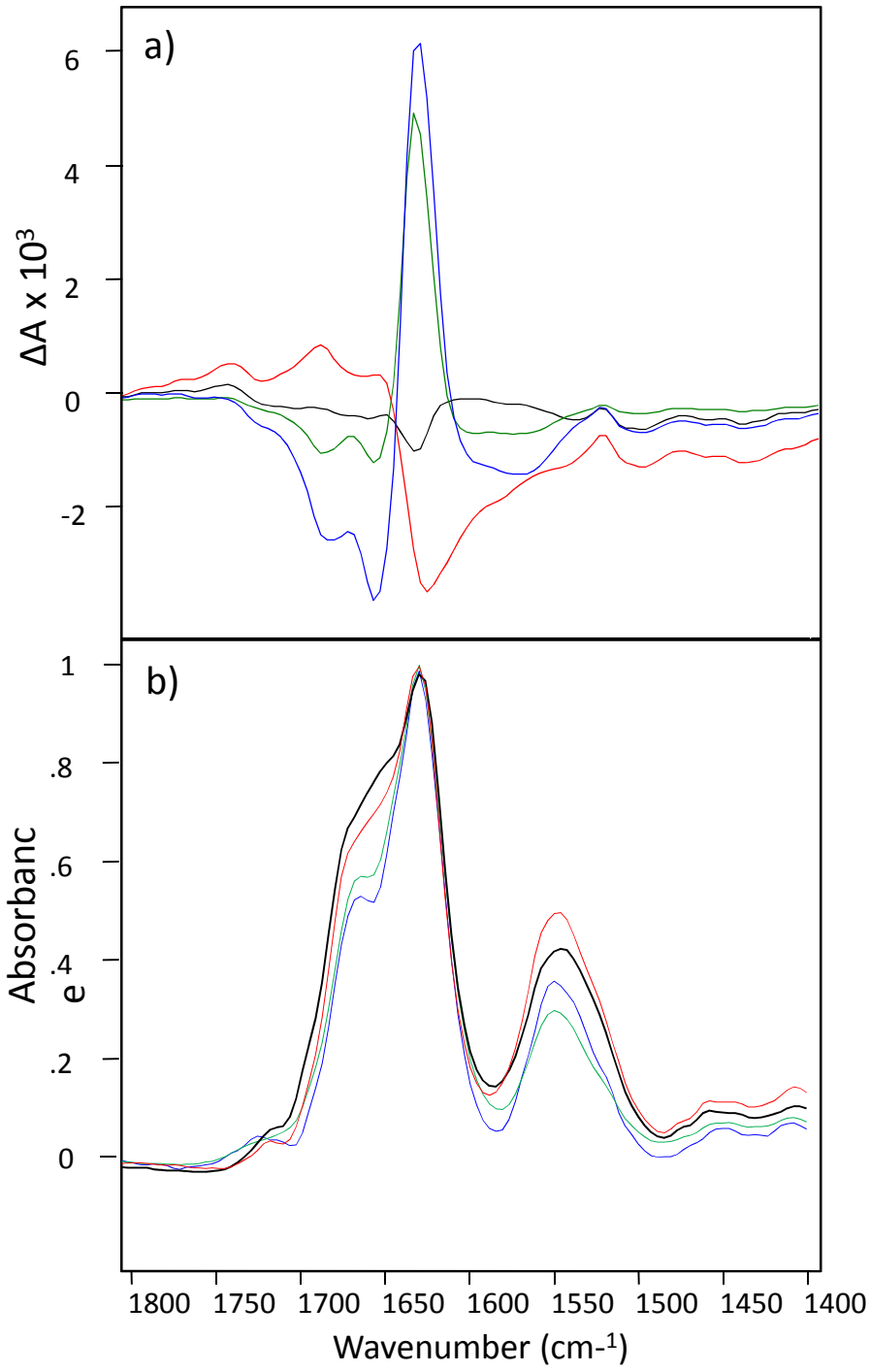
We also found that the intensities of aromatic amino acid bands (Tyr and Phe) are almost identical in lysozyme fibrils grown at pH 2.3 and pH 2.7. However, the intensity of these bands is lower for fibrils grown at pH 1.5. The change in the aromatic band intensities indicates differences in the local environments of these amino acids for reversed (tape-like) and normal (left-twisted) fibril morphologies.

Calculation of protein and peptide isoelectric point (pI).

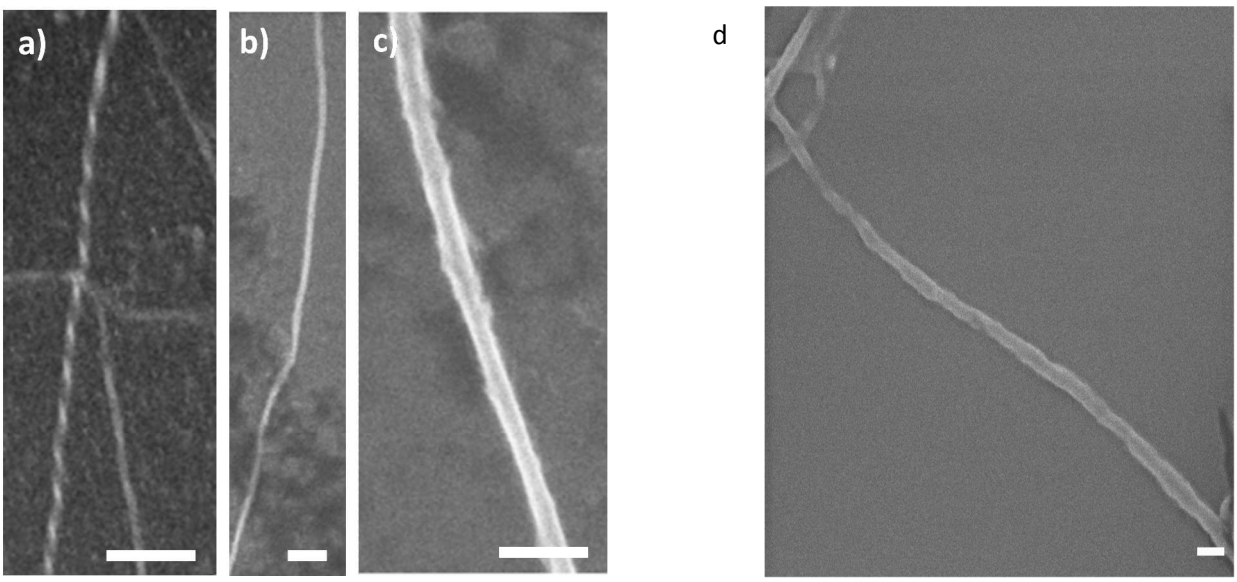
For the calculation of protein isoelectric point (pH) on-line Scripps (protein calculator v 3.3) was used (http://www.scripps.edu/~cdputnam/protcalc.html).

References for Supporting Information

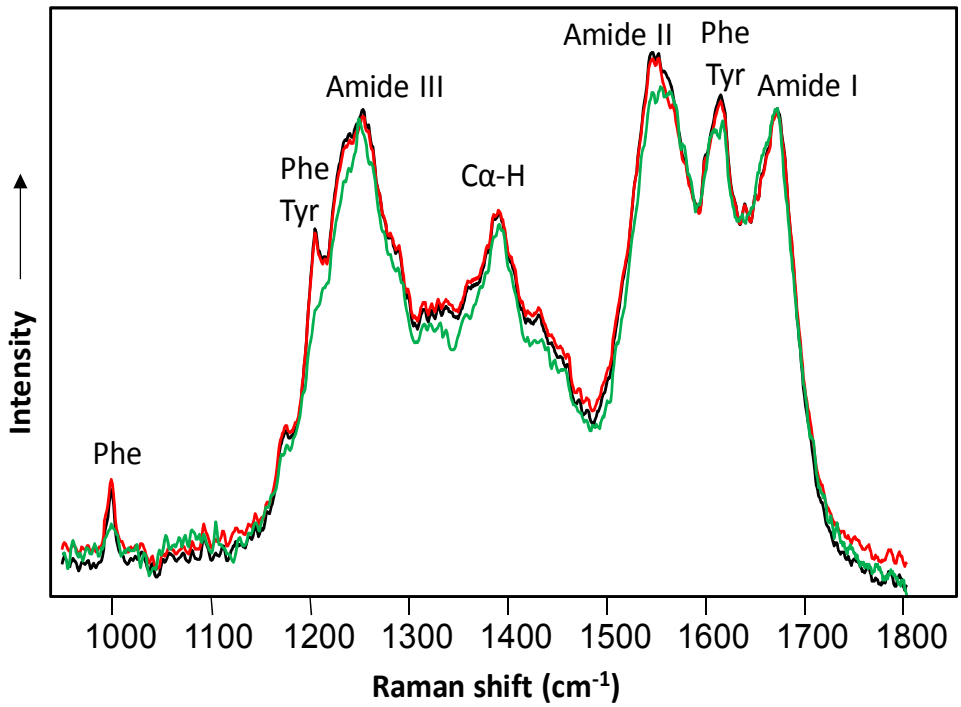
1. Lednev, I. K.; Ermolenkov, V. V.; He, W.; Xu, M.*Anal. Bioanal. Chem.***2005,** 381, 431-437.
2. Lednev, I. K., Vibrational Spectroscopy: Biological Applications of Ultraviolet Raman Spectroscopy. In *Protein Structures, Methods in Protein Structures and Stability Analysis*, Uversky, V. N.; Permyakov, E. A., Eds. Nova Sci. 2007, pp. 1-26.
3. Shashilov, V. A.; Sikirzhytski, V.; Popova, L. A.; Lednev, I. K. *Methods* **2010,** 52, 23-37.
4. Shashilov, V. A.; Lednev, I. K.*Chem. Rev.***2010,** 110, 5692-5713.
5. Xu, M.; Shashilov, V.; Lednev, I. K.,*J. Am. Chem Soc.***2007,** 129, 11002-11003.



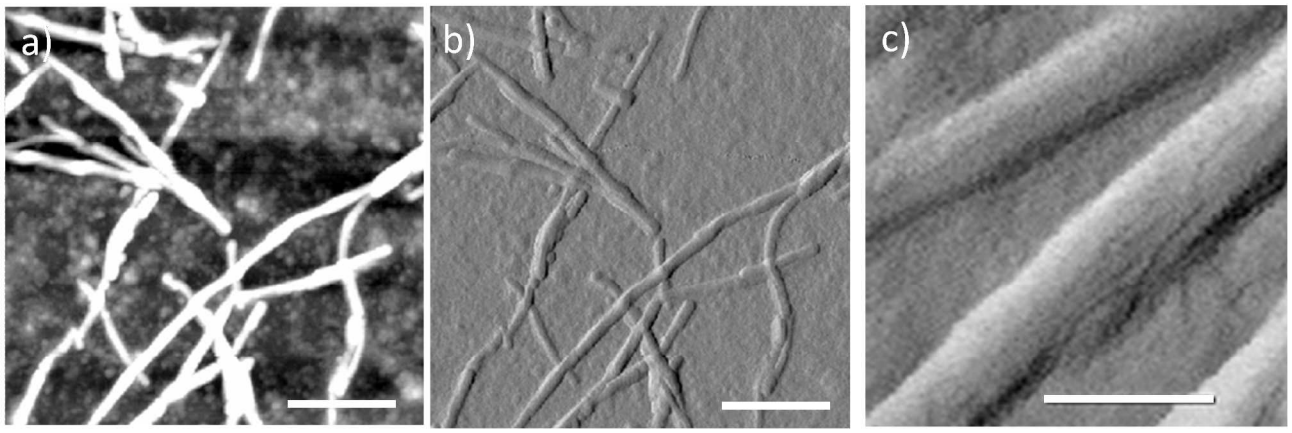
Supplementary Figure S1.IR (left) and VCD (right) spectra of lysozyme fibrils grown at pH 1.0 (blue), 1,5 (green), 2.3 (black) and 2.7 (red) for 5 days at 65 ^{0}C.



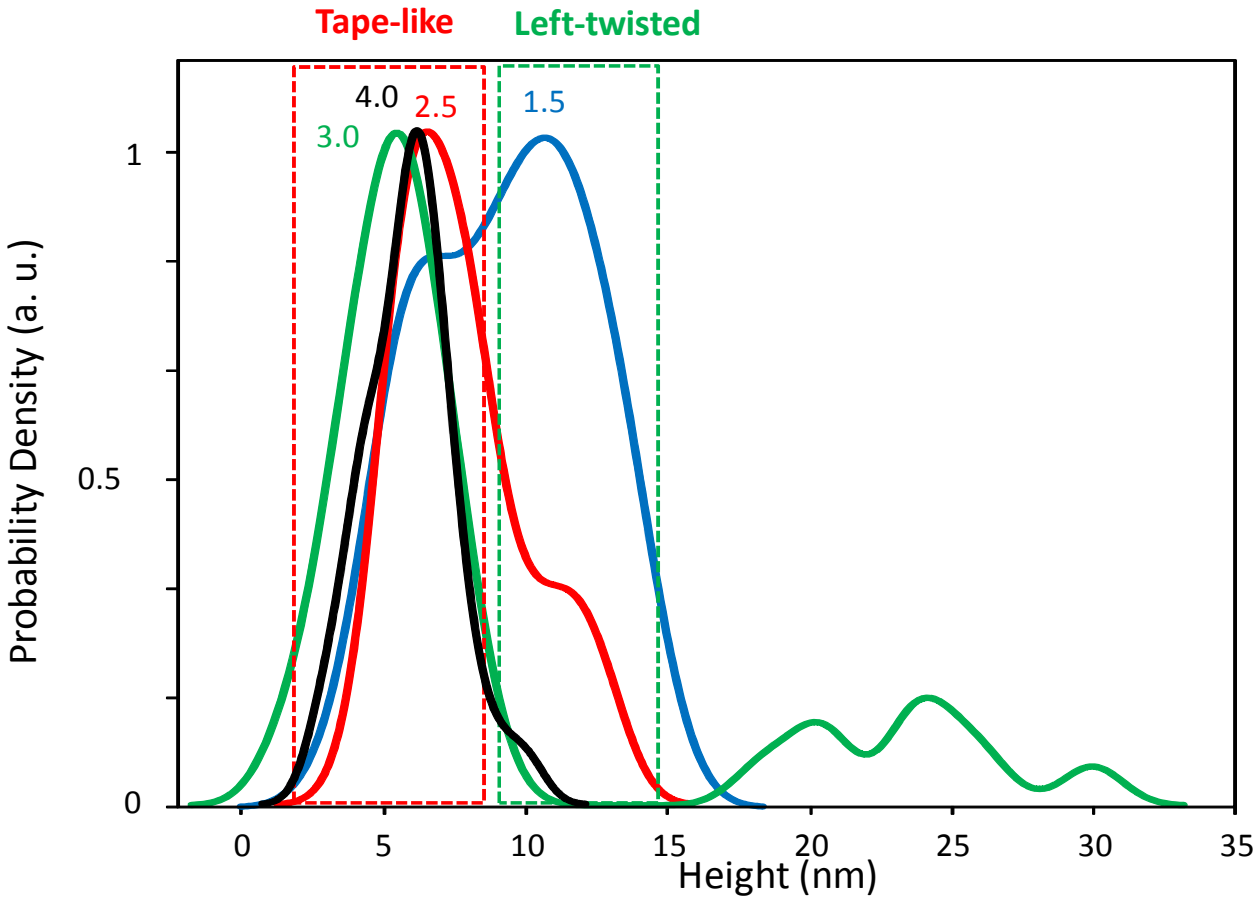
Supplementary Figure S2.Cryo-SEM images of (a) left-handed insulin fibrils that are present in the solution that shownormal VCD; and (b and d) tape-like and (c)binary fibrils that show no right-handed structure but exhibitreversed VCD. Scale bar is 100 nm.



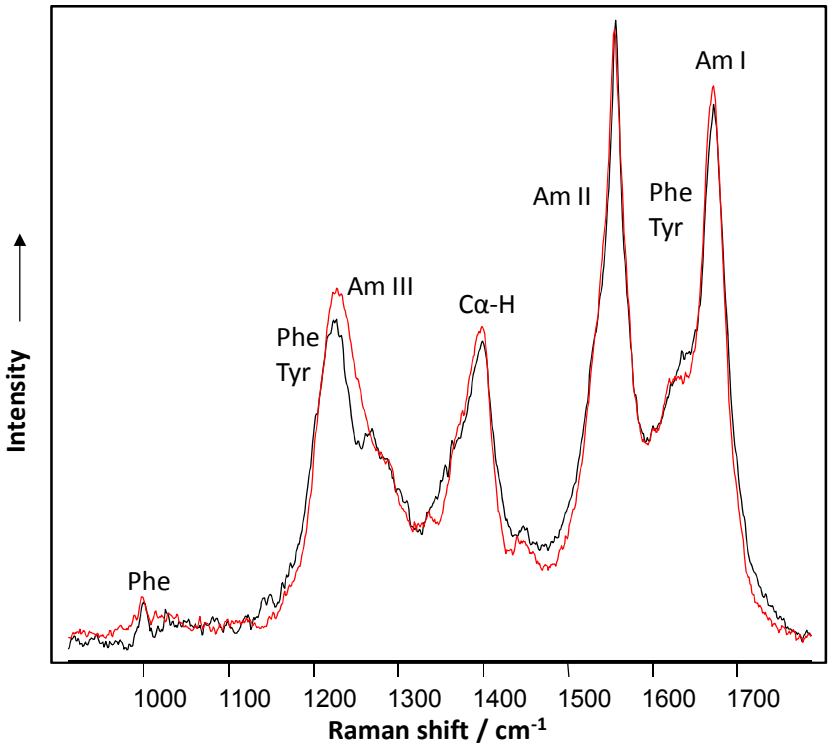
Supplementary Figure S3. DUVRR spectra of lysozyme fibrils grown at pH 1.5 (green), 2.3 (black) and pH 2.7 (red) for 3 days at 65 ºC. Amide I vibrational mode is dominated by C=O stretching, with a small contribution from C–N stretching and N–H bending. Amide II and III bands involve significant C–N stretching, N–H bending, and C–C stretching. The Cα-H bending vibration mode involves Cα-H bending and C–Cα stretching.



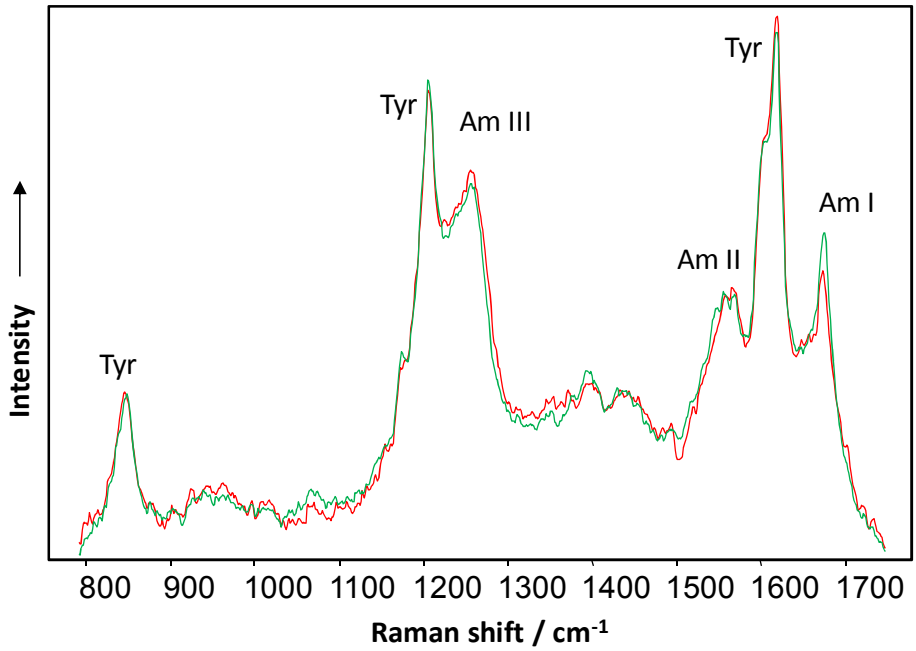
Supplementary Figure S4.AFM images of apo-α-lactalbumin fibrils grown at pH 2.0, 150 mMNaCl at 37 ^{0}C for 3 days. Scale bars are 250 nm (a, b) and 50 nm (c).



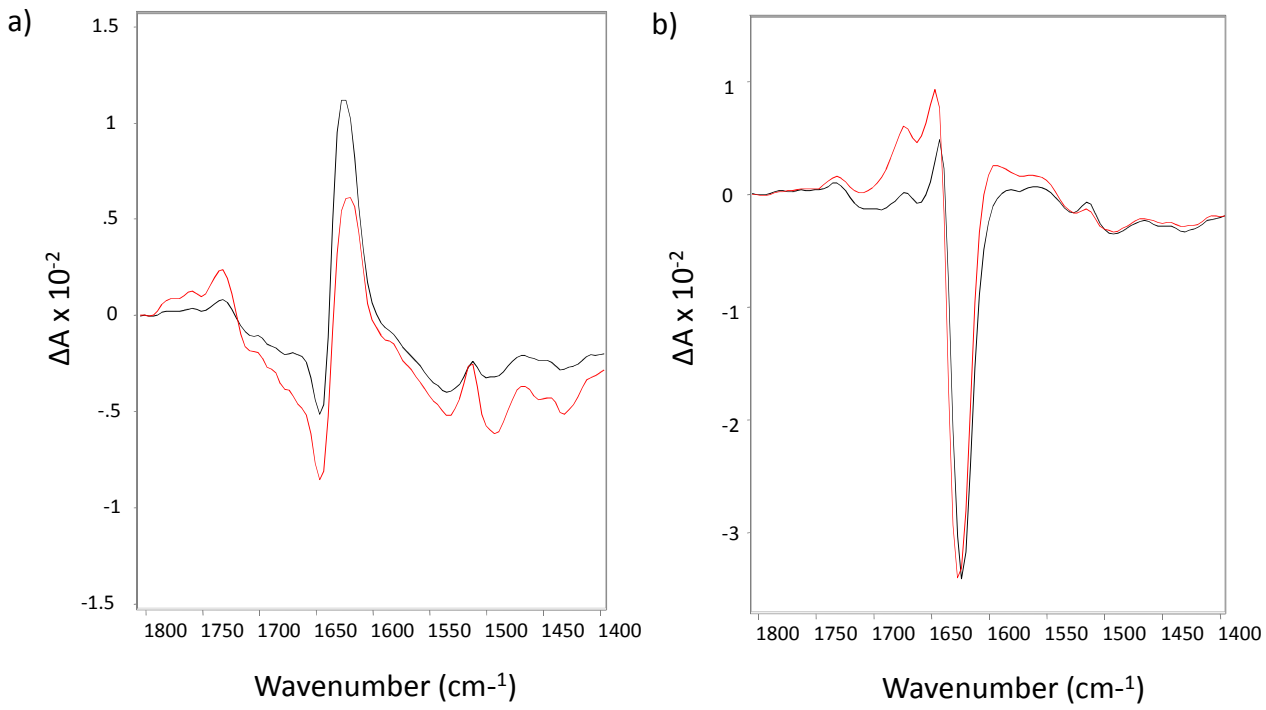
Supplementary Figure S5. Height profiles of apo-*α*-lactalbumin fibrils grown at pH 1.5 (blue), 2.5 (red), 3.0 (green), and 4.0 (black), show presence of two polymorphs: tape-like fibrils with a height of ~6 nm and left-twisted fibrils with a height ~12 nm. The fibril height distributions were calculated using Kernel density statistics. For each calculation 35 individual fibers were measured.



Supplementary Figure S6.DUVRR spectra of HET-s $_{(218-289)}$ prion fibrils grown in pH 2.0 (red) and 3.9 (black).



Supplementary Figure S7.DUVRR spectra of $TTR_{105-115}$ fibrils grown at pH 1.5 (green) and pH 2.5 (red).



Supplementary Figure S8. VCD spectra of insulin fibrils grown in a presence of 0.5 M NaCl (red) and 0 M NaCl (black) at pH 1.4 (a) and 2.6 (b), 70 ºC.