A characteristic feature of the dense phases formed by fiber-shaped molecules is their organization into parallel rods packed in a hexagonal or pseudo-hexagonal lateral network. This is typically the case for the collagen triple helices inside fibrils, as confirmed by recent X-ray diffraction experiments carried out on highly crystallized fibers obtained by immersing the freshly extracted fibers in a salt-controlled medium.
However such diffraction patterns also generally exhibit additional features in the form of diffuse scattering, which is a clear signature of a low degree of lateral ordering. Only few studies have analyzed and modeled the lateral packing of collagen triple helices when the structure is disordered. Some authors have used the concept of short-range order but this approach does not contain any echo of a hexagonal order. In this study, we use an analytical expression derived from the paracrystal model which retains the hexagonal symmetry information and leads to a good agreement with the experimental data in the medium-angle region. This method is quite sensitive to the degree of disorder and to the inter-object distance. One clear result is that the shift in peak positions, generally attributed to variations in intermolecular distances, can also arise from a change in the degree of ordering without any significant modification of the distances. This underlines the importance of evaluating the degree of ordering before attributing a shift in peak position to a change in the unit-cell. This method is generic and can be applied to any system composed of rod-shaped molecules.

Samples - Eight three-month old female C57Black/6 wild-type (WT) mice were used. The mice were sacrificed with CO2 and tails were amputated close to the body attachment. Five tails were frozen and stored at -80 °C and three tails were directly used for experimentation to establish the impact of cold-storage on tendon structure. Under a stereomicroscope, approximately 2–3 tendon fibers were extracted from each tail. Then, single tendon fibers (Nfrozen = 9, Nfresh = 4) were inserted right away into a glass capillary (diameter = 0.7 mm) which was rapidly sealed and placed vertically in the X-ray beam at room temperature and relative humidity.

X-ray scattering experiments - The experiments were performed at the microfocus beamline ID13 (Riekel et al., 2000). The high intensity monochromatic beam (wavelength k = 0.9621 Å), obtained from an in-vacuum undulator and a Si(1 1 1) double-crystal monochromator, was focused with ellipsoidal mirror (focal spot 20(h) x 40(v) μm2) and a size-limited down to a 2 μm diameter section by a Kirpatrick-Baez optics. A two-pieces guard aperture (Pb, about 10 μm square aperture) was used to reduce the diffuse scattering from the exit of the collimator. Samples were mounted with the fiber axis vertical, perpendicularly to the X-ray beam, on a motorized gantry coupled with a microscope allowing to position the sample with a resolution close to 0.1 μm. The experiments were carried out at a sample-detector distance of 344.5 mm, which was calibrated using silver behenate (first order spacing 58.38 Å). Using a 200 μm diameter beamstop, two-dimensional X-ray scattering patterns were recorded 2048 x 2048 pixels; pixel size of 78.94 x 78.94 μm2). The data collection procedure consisted in a series of transverse scans (perpendicular to the fibers), at least three for each sample, with a step size between 2 and 4 μm. Time exposure for recording a pattern was 2 s. No radiation damage effect was detected in the scattering pattern for this exposure time. Data analysis was carried out using the equatorial profiles obtained by azimuthal integration of the intensity within a ±20° angular range.