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# Mapping molecular orientation in dry and wet *Nephila clavipes* dragline spider silk

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**Abstract**. The alignment of β-sheets within spider dragline silk fibers is an important factor in their tensile strength and extensibility. We are using linear dichroism of the C 1s $\rightarrow \pi^*_{amide}$ transition measured using scanning transmission X-ray microscopy (STXM) to generate quantitative maps of the $<P_2>$ orientation parameters with 30 nm spatial resolution. Here we have extended these measurements from dry samples to samples with partial or full hydration. A device for monitoring and controlling the humidity of a sample in the STXM is described and used to measure the effect of saturated humidity on a section of *N. clavipes* dragline spider silk. The microstructure and distributions of molecular orientation change considerably with hydration in ways consistent with the supercontraction observed in free standing dragline spider silk. The STXM results are compared to infrared and Raman microscopy results.

## 1. Introduction

Spider silk is among nature's most highly engineered structural materials, in some cases achieving combinations of strength and toughness that are unmatched by high-performance synthetic fibers. Spiders produce several different types of silk with mechanical properties that are optimized for specific functions. Dragline silk is a remarkable high toughness fiber that combines both stiffness and extensibility. These properties are due to its block copolymer structure with alternating alanine-rich hard segments containing β-sheets and glycine-rich soft segments. The alignment of β-sheets within the fiber is an important factor in the tensile strength of spider dragline silk. To establish fundamental structure-property relationships in spider silks, it is necessary to characterize the microstructure by measuring the degree of orientation of the β-sheets throughout the fiber.

Scanning transmission X-ray microscopy (STXM) provides quantitative maps of chemical species and orientation with 30 nm spatial resolution. We have shown that the spatial distribution of the linear dichroism of the C 1s $\rightarrow \pi^*_{amide}$ transition in silk fibers can be used to investigate the degree of orientation of the β-sheets in various types of silk fibers [1-3]. Here we extend these measurements

 

from dry samples, to samples with partial or full hydration. A device for varying and monitoring the humidity of a sample in the STXM microscope is described and first results shown.

## 2. Experimental

Although wet cell designs have been published [5-7] and used in many soft X-ray spectromicroscopy studies, to our knowledge this is the first device for *in situ* humidity control. Figure 1a is a photo of the first version of the humid cell used to carry out the measurements described in this paper. Fig. 1b shows the second implementation of the cell, which integrates a miniature relative humidity sensor (Honeywell Hycal HIH-4000) directly with the sample, provides much more flexibility for sample mounting, and includes a thermocouple to monitor temperature. Custom electronics were fabricated to display and control the relative humidity in the sample cell. The signal of the humidity sensor is compared to the humidity set point and the difference is used to control a miniature pneutronics proportional valve (Parker VSONC-5S11-VD-F8) which admits humid helium into the sample cell. The electronic circuit is calibrated to a particular humidity sensor and valve. In the first controller, that was used for the results reported herein, the humidity in the cell could be decreased only by allowing humid gas to pass from the cell to the exterior of the STXM chamber, via a return line. The newer version of the control system is much more responsive since it allows simultaneous control of a second valve that admits dry helium into the cell if the humidity set point is below the sensor reading.

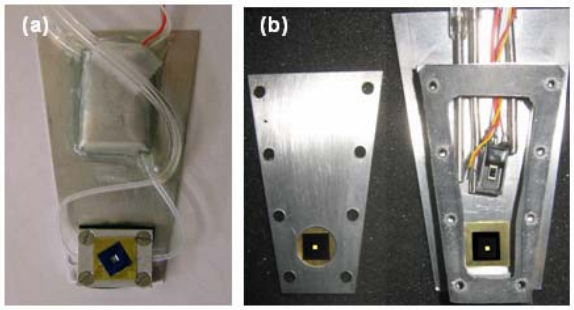


**Figure 1.** (a) First version of humid cell with the humidity sensor only connected to sample compartment via thin tubing. (b) Mark II humid cell with sensor, dry and humid He ports and thermocouple integrated into the sample region.

The humid cell is built on a standard STXM sample plate which gives reproducible kinematic mounting in the STXM microscopes at ALS, CLS and SLS. So far the cell has only been used at the STXM on CLS beamline 10ID1 but a similar device is available at ALS STXM532. The CLS STXM source is an elliptically polarizing undulator (EPU) in which all 4 quadrants can be displaced, thus allowing tuned X-rays with 99% linear polarization to be produced with arbitrary inclination from -90$^{o}$ to +90$^{o}$ relative the plane of the ring [8]. This facilitates studies of linear dichroic signals since a complete and quantitative study can be performed without having to rotate the sample.

## 3. Results

Figure 2 displays an optical image of the longitudinal microtomed section of a full hydrated *N. clavipes* dragline silk fiber embedded in epoxy, along with STXM images recorded at 288.5 eV of parts of this fiber in the low humidity ('dry') and saturated humidity ('wet') states.

$\langle P_2 \rangle$ orientation maps for the dry and wet fiber were derived from 3-image sequences measured with the E-vector aligned parallel and perpendicular to the fiber axis as described elsewhere [3,4]. Figure 3 plots the derived $\langle P_2 \rangle$ maps on the same scale. Figure 3 also compares the histograms of the $\langle P_2 \rangle$ values for the dry and wet states. $\langle P_2 \rangle$ is 1.0 for perfect parallel, -0.5 for perfect perpendicular, and 0 for fully random orientation. The negative $\langle P_2 \rangle$ values observed indicate that the carbonyl groups of the silk proteins are preferentially oriented perpendicular to the fiber axis so that the peptide chains of the β-sheets are mostly aligned along the

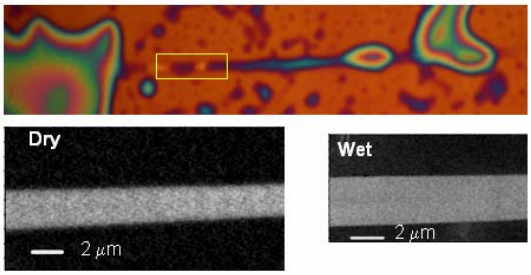


**Figure 2** (upper) Optical image and (lower) STXM OD images (at 288.2 eV) of dry and fully water saturated *N. clavipes* dragline silk fiber embedded in epoxy.

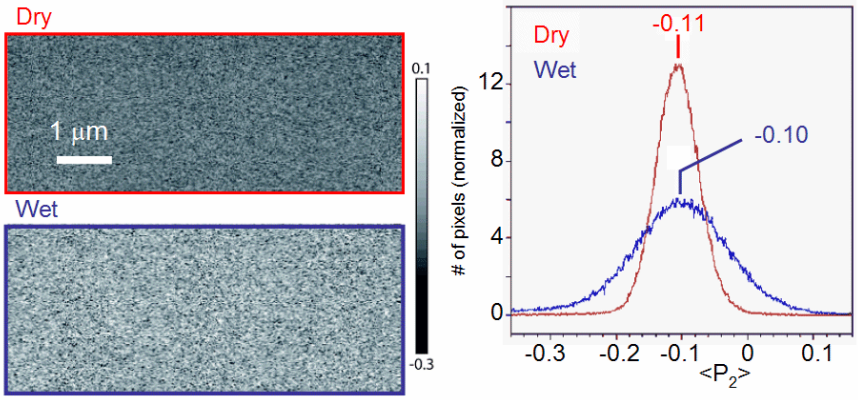


**Figure 3.** (left) $\langle P_2 \rangle$ orientation maps of dry and wet fiber sections displayed on the same intensity scale. (right) Comparison of histograms of $\langle P_2 \rangle$ values for the dry (blue) and wet (red) fibers.

fiber axis, as known from other studies. The dry fiber presents a very fine homogeneous microstructure of moderately oriented domains. The addition of water to the fiber drastically affects the microstructure of the fiber. The microstructure is still homogeneous throughout the sample, but it has a much coarser texture and a broader range of $\langle P_2 \rangle$ values. This effect is clearly observed by comparing the distribution of $\langle P_2 \rangle$ values for the fiber in the dry and wet states (Figure 3). Both distributions display a Gaussian shape centered at a $\langle P_2 \rangle$ value of about -0.1. However, the distribution is much broader for the fiber in the wet state compared to that observed for the dry state. These results show that water induces a major reorganization of the protein chains, with some carbonyl groups displaying higher and some lower orientation than in the dry fiber.

When unrestrained dragline silk is immersed in water, it undergoes supercontraction, shrinks by approximately 40–50%, and its mechanical properties change markedly. The initial stiffness drops by three orders of magnitude, and the material becomes rubber-like [9]. This effect has been associated with the decrease of inter-chain interactions in the amorphous matrix in the presence of water, leading to a loss of rigidity. Polarized Raman spectromicroscopy studies at Université Laval on unrestrained *N. clavipes* dragline silk supercontracted by about 30% show that supercontraction does not alter significantly the average orientation of the silk proteins but changes slightly their conformation. The present STXM results show that, even for a thin section of dragline silk embedded in a polymer matrix, water induces effects similar to those resulting from supercontraction.

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