INFRARED STUDY OF RECLUSE SPIDER SILK

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I am reporting the study of single strands of Recluse spider silk with an Infrared Microscope. Silk is relevant for study due to its potential for high impact applications, arising from exceptional mechanical properties. Silks are semi-crystalline biopolymers, which consist of folded proteins that exist in amorphous or crystalline domains. These secondary structures relate to the observed macroscopic mechanical properties. Recluse silk is unique, due to a rectangular cross section not present in any other observed silks. The simplified structure of the Recluse silk has led to the hypothesis of it being an ideal model amongst silks. Infrared transmission was performed on single strands of this silk to study the composition and orientation of the crystalline and amorphous domains present. These details could provide optimized parameters for the production of high strength silks.

INTRODUCTION

Materials research on novel space age materials is paramount for advancements in aeronautics and space exploration. Of these novel materials, spider silk has numerous high impact technological implications including ultra-strong light weight fabrics, biomedical technologies such as biosensors, and optical applications¹. Artificial silks could decrease payload for space bound NASA missions as well as provide an increase in strength and extensibility over existing materials. Silk’s durability and relative invariance to temperatures would allow for use in extreme environments where space bound missions occur². These technologies all align with NASA’s research in aeronautics and space technology.

As a recipient of the VSGC graduate fellowship last year, I sought to examine the protein secondary structure present in recluse spider silk. Establishing the connection between secondary structure content/formation and their influence on macroscopic mechanical properties will assist in artificial production of strong extensible silks. Recluse spider silk is special because of its rectangular cross section not present in any other identified silks. Due to this fiber morphology, the fibers are also much thinner, with overall thickness of about ~55nm and width ~10μm as compared to radii of ~1μm for other studied silks with circular cross-section. Since the morphology of the recluse spider silk is simpler, it is hypothesized that this could be a fundamental silk³. If fully understood, this silk could pave the understanding of all other more complicated biopolymers. In the following sections I will present the work that been completed, and how I propose to extend the research for next year.
EXPERIMENT

Infrared polarized FTIR transmission measurements of multiple and single strand recluse spider have been taken. The multiple strand polarized FTIR infrared transmission data was taken previously by S. Wang and Z. Xing. These multiple strand data sets only provide a qualitative measure of secondary structures present. This is due in part to the difficulty of defining the strand geometry relative to the incident beam in the multiple turn system. For this reason the multiple turn data is not discussed in the main text. Single strand microscopic studies in the infrared provide local quantitative secondary structural information by eliminating this geometrical uncertainty. Local structural information is highly relevant in determining the interplay between the protein secondary structure present and silk’s unique macroscopic mechanical properties. Issues arise in obtaining quality microscopic data though, due to the difficulty of attaining high signal to noise for microscope spot sizes in the mid-infrared. This is due to the low throughput of readily available mid-infrared light sources. To circumvent this issue, optimization of the beam path optical alignment, proper choice of limiting aperture size and lengthening acquisition times were necessary to obtain sufficient signal.

We coupled our Bruker 80v FTIR infrared spectrometer to a Spectratech optical microscope with confocal Schwarzschild objectives of numerical aperture 0.58. We designed and implemented moveable sample and detector stages for optimized precision transmission and reflectance measurements. The whole microscope is encased in a chamber which was filled with ~10 psi of compressed air. This compressed air had been scrubbed of CO₂ and H₂O by our Parker Balston gas purge. Both CO₂ and H₂O have large absorption lines in the mid-infrared so they must be removed to obtain reliable results free of artificial absorption lines. Our infrared light source is a water cooled globar, and we used a KBr beamsplitter. We polarized our input beam parallel and perpendicular to the long axis of the silk strands with a wire grid KRS-5 polarizer. Each measurement, involved taking data for both polarization states. Data was taken between 1000 cm⁻¹ - 4000 cm⁻¹ with 4 cm⁻¹ spectral resolution using a liquid nitrogen cooled MCT photoconductor. This spectral range captures the Amide I, II, and III regions of interest. The dimensions for a single strand of our silk is ~55 nm thick, and ~ 10 μm wide. The dimensions of these strands was previously measured using AFM topography scans³.

In order to get absolute transmission values, the spot size at the focus of the object needed to be determined. Data was taken with confocal rectangular 0.25 mm x 3 mm apertures, which with 15 times magnification equates to a ~ 16 μm x 200 μm spot at the focus of the object. This aperture size was chosen to maximize the amount single strand in the field of view. It was natural to elongate the apertures along the length of the strand while reducing the width. This allowed us to get sufficient signal-to-noise while maintaining single strand precision. To corroborate the predicted spot size values we measured the beam size along both axes with the knife edge method. This involved scanning a sharp edged knife perpendicular to the optical axis at the focus and measuring the integrated intensity. The results were fit to the following formula⁴

\[ P(x) = \frac{A}{2} \left[ 1 + \text{erf} \left( \frac{\sqrt{2}(x-x_0)}{w} \right) \right] + C \]
where $A$, $C$ and $w$ in equation 1 are fitting parameters. The spot size is then determined from the fitting parameter $w$ which is the beam waist of the Gaussian beam. The measured spot size is ~2 times larger than the theoretical prediction based on the magnification of our lens, across our spectral range of interest.

Another detail required for absolute data fitting is the incident beam angle. We chose to average the beam angle between our primary aperture axes to simplify the analysis. The angle along the 3 mm axis was determined by measuring the spot size at different stage heights. The half angle for the 3 mm axis was determined to be ~20°. The beam angle along the 0.25 mm axis could not be measured directly since the change in spot size at different heights was indiscernible. Instead a calculation was performed to estimate the beam angle. Using étendue conservation and the spot sizes measured with the knife edge method, the approximate half angle was calculated to be ~6.7°. Averaging the results, the beam angle used in data fitting was ~13°.

A frequency dependent background exists in the transmission spectrum. This background likely occurs as a result of frequency dependent diffraction, reflectance and scattering due to the geometry of the spider silk fiber used for transmission measurements. We are able to remove this background using the following procedure. We initially exclude the spectral range of infrared vibrational modes, and use a polynomial to fit the remaining featureless spectrum which is mainly attributed to transmission background. We then subtract this polynomial fit from the raw transmission data to obtain a background-free transmission spectrum.

Since our confocal apertures were larger than the fiber being sampled, part of the beam did not pass through the sample. This geometrical mismatch was corrected by weighting the experimental spectrum by a ratio of incident beam powers. The incident beam powers are the power transmitted through the fiber and the total power through the aperture. The powers were calculated by assuming a Gaussian

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Figure 1: Experimental and oscillator fitted transmission spectra for both polarization states. a) Polarization parallel to the fiber long axis. b) Polarization perpendicular to the fiber long axis.

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distributed intensity as was done with knife edge measurements. The integrated power over the aperture was done using the previously measured spot size data.

**DATA ANALYSIS**

After accounting for the fact that only a fraction of the total power is incident on the silk strand, the spectra for each of the two polarization states were fit individually. We used a combination of Lorentz and Lorentz-Gauss oscillators to fit the frequency dependent complex dielectric function \( \varepsilon(\nu) \). This fitted function is used to simulate a transmission spectra given in figure 1, for a given thickness and beam angle, which is then compared to the experimental transmission spectra. The oscillator fit parameters are characteristic of the particular type of proteins present, as well as their orientation. The Lorentz model assumes that molecular bonds respond to electrical fields as a driving force on a damped harmonic oscillator\(^5,6\), with the equation of motion

\[
M \left( \frac{d^2\vec{u}}{dt^2} \right) - M\gamma \left( \frac{du}{dt} \right) = -M\omega_T^2\vec{u} + QE_0e^{i\omega t}\hat{\epsilon} \tag{2} 
\]

where \( M \) is the reduced mass, \( \vec{u} \) is the displacement from the equilibrium position, \( \gamma \) is the damping factor, \( \omega_T \) is the natural frequency of the oscillators, \( \omega \) is the (angular) frequency of the electric field, \( Q \) is the charge of the oscillators, \( E_0 \) is the amplitude of the electric field, and \( \hat{\epsilon} \) is the polarization of the incident electric field. The polarization field responds linearly to the displacement, which results in a frequency dependent dispersion relation to govern the propagation of electric fields through the medium. In Tables 1-2 we have referenced our fitted oscillators for both polarization states with published works, Tables 1-2 are provided at the end.

In Table 1 and Table 2, we can see that the most prominent molecular assignments of the vibrational modes include beta sheet with polyalanine domains and amides. Beta sheets are one of the main structures we see in the silk. They are the crystalline regions of the silk and are often poly-Ala structures with alanine residues\(^7\). Beta sheets are very distinct and contain a hydrogen face as well as a methyl and methanolic group comprising another face\(^8\). They exist on the order of a few nanometers, and consist of a dense network of hydrogen bonds\(^9,11\). A study conducted using electron diffraction on spider dragline silk found the space group of beta sheets to be P2\(_1\)\(^12\).

An amide is an organic compound with a general structure that include an O=C—N group. They are amongst the characteristic bands that are regularly found in the infrared spectra of proteins and polypeptides, as they link amino acids. Amide I absorption is associated with

![Figure 2: Fitted infrared absorbance spectra for both polarization states. The absorbance was obtained by A=Log(Tr) where Tr is the fitted transmission spectrum.](image)
stretching vibrations of C=O bonds, while Amide II absorption is associated with bending vibrations of the N—H bond\textsuperscript{13}. Amide III is a broad band that is comprised of C—C stretching, C==O stretching, C—N stretching, and N—H bending\textsuperscript{14}. All of these bonds are important in the hydrogen bonding of the secondary structure of protein. It is important to note that the Amide I band is difficult to resolve because the amplitude is small compared to the intrinsic width of the band—one broad peak is observed instead of many well resolved peaks\textsuperscript{13}. We note that another prominent amide, Amide A, occurs between 3270 and 3310 cm$^{-1}$, depending on the strength of the hydrogen bond\textsuperscript{15}. Amide A comes from the NH stretching vibration, and is localized on the NH group\textsuperscript{15}. It occurs as part of the Fermi resonance doublet\textsuperscript{15}. The second component, amide B, is weaker and occurs between 3030 and 3100 cm$^{-1}$\textsuperscript{15}.

Note that we also converted the transmission spectrum to an absorbance spectrum by using the formula $A = -\log \left( \frac{I}{I_0} \right)$, where $I_0$ is the intensity of the beam transmitted through the reference aperture, and $I$ is the intensity of the beam transmitted through the silk specimen. This formula stems from the Beer-Lambert Law. The absorption spectra are shown in Figure 2. Absorbance allows quantitative characterization of the different chemical quantities in our silk, since it is the absorbance and not the transmission which is proportional to individual concentrations in a sample\textsuperscript{16}.

In both polarizations, the vibrational modes occur in two distinct groups, one from ~700 to 1700 cm$^{-1}$, and one from ~2800 to 3300 cm$^{-1}$. This stems from the $\omega^2 = k/M$ relationship, where $k$ is an effective spring constant that signifies the bond strength. The modes at higher frequencies arise from vibrations of lighter elements, such as hydrogen. Moreover, they originate from stronger chemical bonds with higher effective spring constants and tend to be stretching modes\textsuperscript{17}. Conversely, modes at lower frequencies arise from heavier elements, such as carbon, nitrogen, and oxygen, and are more likely to be bending modes with lower effective spring constants\textsuperscript{17}.

The polarized infrared data shows that the silk of the recluse spider is anisotropic because of differences between the spectra for polarizations parallel and perpendicular to the long axis of the silk. There are fewer vibrational modes apparent in the data obtained with polarization perpendicular to the long axis of the silk. Both polarizations show a large mode at ~1500-1600 cm$^{-1}$ arising from the Amide II structure. However, it is much stronger in the parallel polarization. The parallel polarization shows an additional large mode at ~1660 cm$^{-1}$. Also note that the N—H stretching Amide A mode at ~3300 cm$^{-1}$ appears more strongly in the data obtained with polarization perpendicular to the long axis of the silk.

**CONCLUSION**

In collaboration with Dr. Schniepp’s research group in the applied science department at the College of William and Mary, we are in the process of writing a publication. They have performed polarized single strand Raman spectroscopy which will be compared to our single strand infrared data. Based on the mutual exclusion principle for a crystalline material, Raman and Infrared vibrational modes are at distinct non-overlapping frequencies because of crystal symmetries\textsuperscript{18}. For an amorphous material no translational symmetry exists, so all vibrational modes are allowed and
Raman and Infrared modes will overlap. Therefore by comparison of single strand Raman and infrared spectra, we can infer which features in the spectra are crystalline or amorphous. This allows us to make estimates of the crystalline and amorphous composition in our silk. It is known that crystallinity and strength are correlated quantities\textsuperscript{19}. As previously stated, the recluse spider’s silk is potentially a fundamental member of its material class due to its simplified morphology\textsuperscript{3}. Under this hypothesis, the crystalline content of the recluse silk which has not been previously characterized, could be the optimal quantity for biopolymers in general. Establishing the optimal ratio of crystalline to amorphous composition in biopolymers for a given application, will greatly enhance the ability for the production of artificial silks.

ACKNOWLEDGEMENTS

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<table>
<thead>
<tr>
<th>Center Frequency (cm⁻¹) of infrared modes of recluse spider silk</th>
<th>Molecular Assignment</th>
<th>Frequency from literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>964.21</td>
<td>β-PAla (CH₃ rock, N-Cα stretch)</td>
<td>963²⁰</td>
</tr>
<tr>
<td>1002.2</td>
<td>(AlaGly)ₙ CH₃ chain</td>
<td>1000²⁰</td>
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<tr>
<td>1054</td>
<td>PPro I (Cα-Cβ-Cγ stretch), other amino acids</td>
<td>1049²⁰</td>
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<td>1102</td>
<td>CH₃ rock, Cα-Cβ str.</td>
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<td>β -PAla (Hα bend, CH₃ sym. b., Cα-Cβ str.)</td>
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<tr>
<td>1218</td>
<td>Amide III β-Sheet</td>
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<td>1229.9</td>
<td>Amide III Random Coil</td>
<td>1235²²</td>
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<td>1260.5</td>
<td>Amide III α-helix</td>
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<tr>
<td>1302.2</td>
<td>β -PAla (CN stretch, Hα bend)</td>
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<td>CH₃ sym. bend, Hα bend, NH ipb</td>
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<td>Hα bend, NH ipb, CH₃ sym. bend</td>
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<td>CH₃ sym. bend</td>
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<td>Amide II</td>
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<td>1533</td>
<td>Hα bend, NH ipb, CH₃ sym. bend</td>
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<td>Amide I C=O stretching²⁶ Random coil</td>
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<td>Amide I C=O stretching²⁶ 3₁₀ Helix</td>
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<td>Crystalline Polyglycine I (CαH asym str)</td>
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</table>

**Table 1:** Infrared vibrational modes in the recluse spider silk data for polarization parallel to the long axis of the silk. Molecular assignments are based on values from the literature for similar organic compounds.
Center Frequency (cm$^{-1}$) of infrared modes of recluse spider silk | Molecular Assignment | Frequency from literature
--- | --- | ---
1120 | NC str., C$_\alpha$C$_\beta$ str. | 1116$^{21}$
1168.5 | $\beta$ -PAla (C$_\alpha$-C$_\beta$ stretch, H$_\alpha$ bend, CH$_3$ symmetric angle bend) | 1167$^{23}$
1232 | Amide III Random coil | 1235$^{22}$
1263 | Amide III $\alpha$-helix | 1265$^{22}$
1304 | $\beta$ -PAla (CN stretch, H$_\alpha$ bend) | 1306$^{23}$
1337 | CH$_2$ wagging mode | 1338$^{27}$
1374 | $\beta$ -PAla (CH$_3$ symmetric angle bend, H$_\alpha$ bend) | 1372$^{23}$
1410 | CH$_2$ wagging mode | 1409$^{28}$
1449 | $\beta$ -PAla (CH$_3$ asymmetrical angle bend) | 1444$^{27}$
1518 | Amide II CN stretch, NH bending$^{26}$ | 1520$^{28}$
1549 | Amide II CN stretch, NH bending | 1542$^{20}$
1629.5 | Amide I C=O stretching$^{26}$ | 1620-1640$^{26}$
1644 | Amide I, Random Coil | 1648$^{26}$
1674 | Amide I, $\beta$ -Turn | 1665$^{26}$
2792 | | 

Table 2: Infrared vibrational modes in the recluse spider silk data for polarization perpendicular to the long axis of the silk. Molecular assignments are based on values from the literature for similar organic compounds.
References


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