Supplementary material

Unexpected β-sheets and molecular orientation in flagelliform spider silk of as revealed by Raman spectromicroscopy

Thierry Lefèvre and Michel Pézolet

Département de Chimie – CERMA - PROTÉO
Université Laval
Pavillon Alexandre-Vachon
Québec (Québec) G1V 0A6
Canada

Raman spectra of the native Flag silk fibers before washing with water

The spectra have a lower signal to noise ratio than after washing, but have the same spectral features. Therefore, the conclusions drawn from the washed fibers can be extended to the native fiber in the presence of the sticking glue.

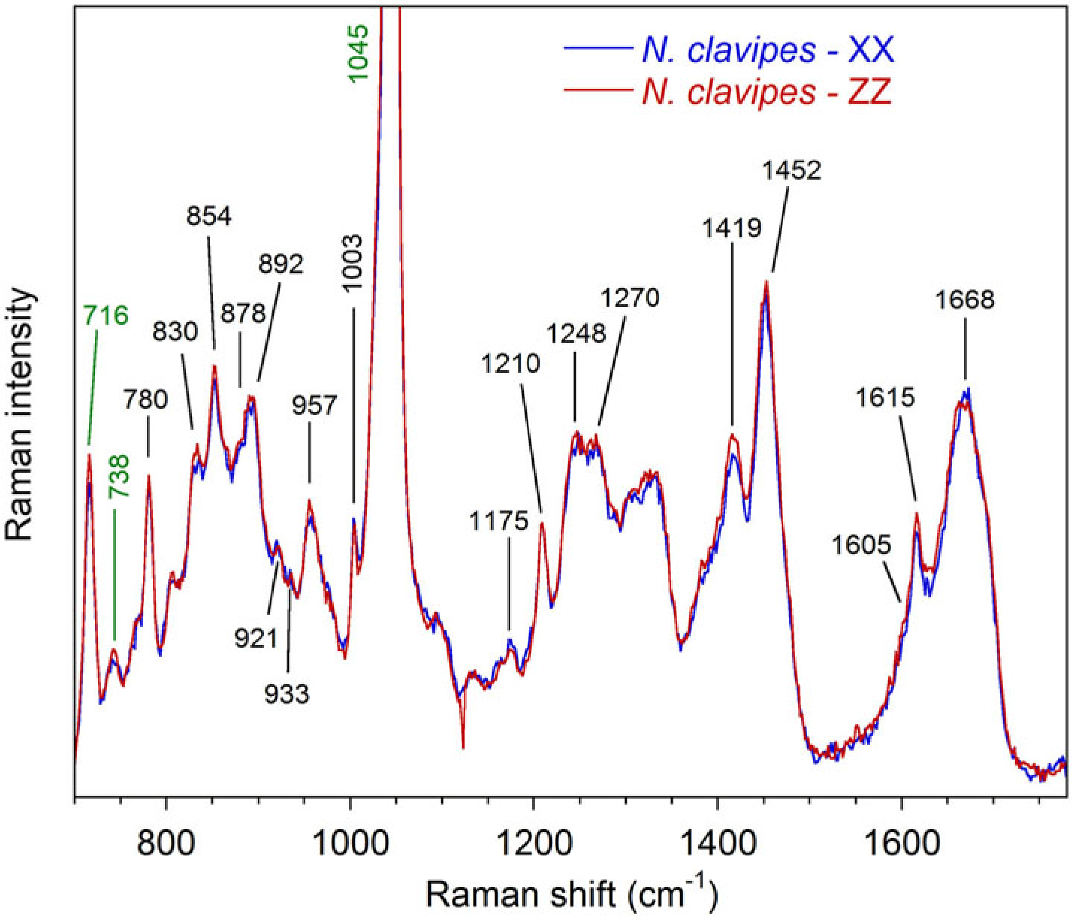

Figure S1

XX- and ZZ-polarized Raman spectra of the native spiral fiber of the web of the spider *N. clavipes* before washing with water. Green values correspond to bands due to the glue.

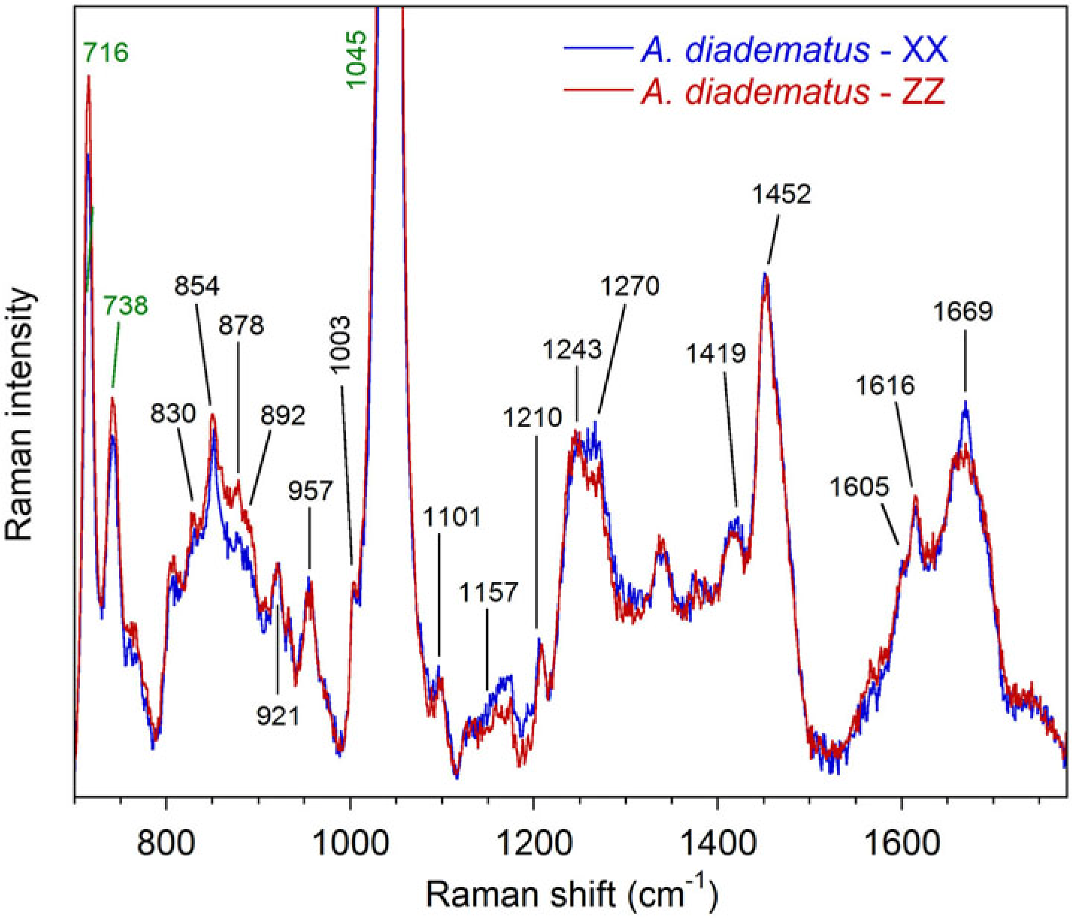

Figure S2

XX- and ZZ-polarized Raman spectra of the native spiral fiber of the web of the spider *A. diadematus* before washing with water. Green values correspond to bands due to the glue.

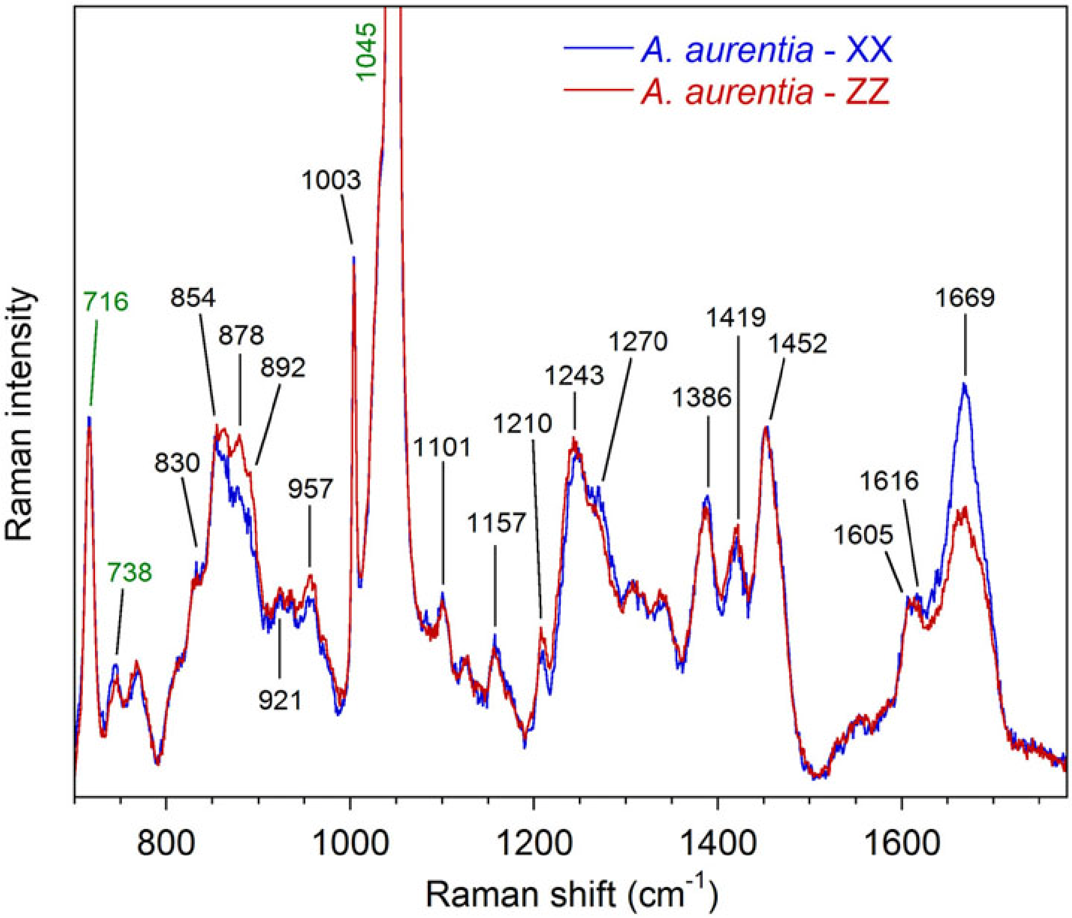

Figure S3

XX- and ZZ-polarized Raman spectra of the native spiral fiber of the web of the spider *A. aurentia* before washing with water. Green values correspond to bands due to the glue.

The curve-fitting procedure in the amide I region

The first criterion to assess the number of bands in the amide I region consists in favoring the minimum number of components. The second criterion is dictated by the shape of the polarized spectra. A careful inspection of the polarized spectra of the Flag silk fibers revealed the contribution of four bands in the amide I region: one located near 1640 cm^{-1} (shoulder on the XX spectrum), a second one located at 1668-1669 cm^{-1} due to β-sheets (maximum of the XX spectrum), a third one located at 1663-1665 cm^{-1} (maximum of the XZ and ZX spectra) and a last one located near 1685 cm^{-1} (assessed from the width of the amide I band and necessary to obtain a reasonable fit).

To validate these observations, a preliminary curve-fitting attempt was undertaken for *N. clavipes* with four bands, two bands being initially located at 1669 cm^{-1}, the others at 1640 and 1685 cm^{-1}. The width of one 1669-cm^{-1} component was limited to 16 cm^{-1} since the β-sheet is a well defined secondary structure whose bandwidth does not exceed this value. The bandwidth of the second 1669-cm^{-1} component was limited to 30 cm^{-1}. The initial intensity of the β-sheet component was arbitrarily 2.5 times smaller than the second one because the spectra of the *N. clavipes* Flag silk obviously contains very few β-sheets. Interestingly, the calculation leads to the separation of the bands, with final positions arising at 1671 and 1661 cm^{-1}, consistently with the values observed in the above assignment. It is noteworthy that the spectral decomposition, with only three bands at 1685, 1668 and 1645 cm^{-1} (and a maximum width of 32 cm^{-1}) was unsuccessful as this method clearly overestimated the content of β-sheets. The reason is that the width of the band at 1668 cm^{-1} was overestimated, showing that two bands at 1671 and 1661 cm^{-1} are necessary.

Apart from the β-sheet component at 1669-1671, the assignments of the other components are unclear and can originate from turns, left-handed 3_1-helices, disordered and/or other irregular structural elements. Two bands at 1615 and 1605 cm^{-1} are associated with tyrosine and phenylalanine side-chain vibrations, respectively. The bandwidths of these bands, especially the one at 1615 cm^{-1}, were limited to 14 cm^{-1} in order to avoid undesirable overlapping with the amide I band.

To be able to decompose the polarized and the orientation-insensitive spectra with the same decomposition model, constraints on the parameters (position, bandwidths) have to be applied during the calculation. Thus, the variation of the band position (1685, 1671, 1661 and 1640) was limited to ±1 cm^{-1}. The narrowest bands that gave a reasonable fit were used. After various attempts, it appeared that the widths of the 1685, 1671, 1661 and 1640 cm^{-1} components have to be limited to 30, 16, 22 and 24 cm^{-1}, respectively. Initial values were 26, 15, 22 and 22 cm^{-1}, respectively. Initial intensities were manually adjusted to obtain the best representation of the experimental spectrum before calculation.

As mentioned above, the procedure was first tested on *N. clavipes* which should provide very small amount of β-sheets and level of orientation. As reasonable results were obtained, these parameters were applied on the ZZ spectra of *A. aurentia* and *A. diadematus* because the contribution of the β-sheets is very small in this polarization due to their perpendicular orientation with respect to the fiber axis. Then, the XX spectra of *A. aurentia* and *A. diadematus* were fitted from their respective ZZ spectra. It has to be noticed that the XX spectrum of *A. aurentia* and *A. diadematus* cannot be fitted from the ZZ spectrum by only increasing the intensity of the β-sheet band. The intensity of the other bands has to be increased too, indicating that the disordered phase of the Flag silk has also a preferential orientation.

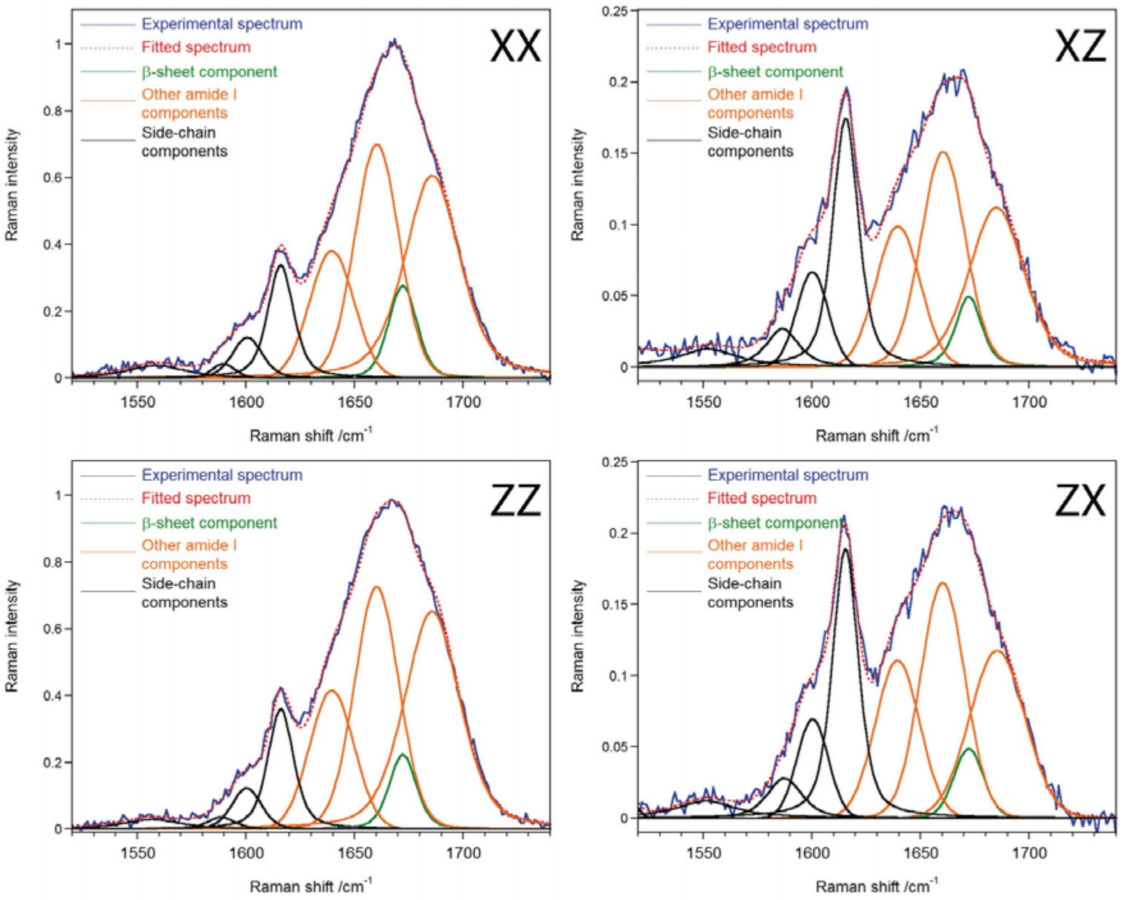

Figure S4
Spectral decomposition of the Raman spectra of the Flag silk of *N. clavipes* in the amide I region.

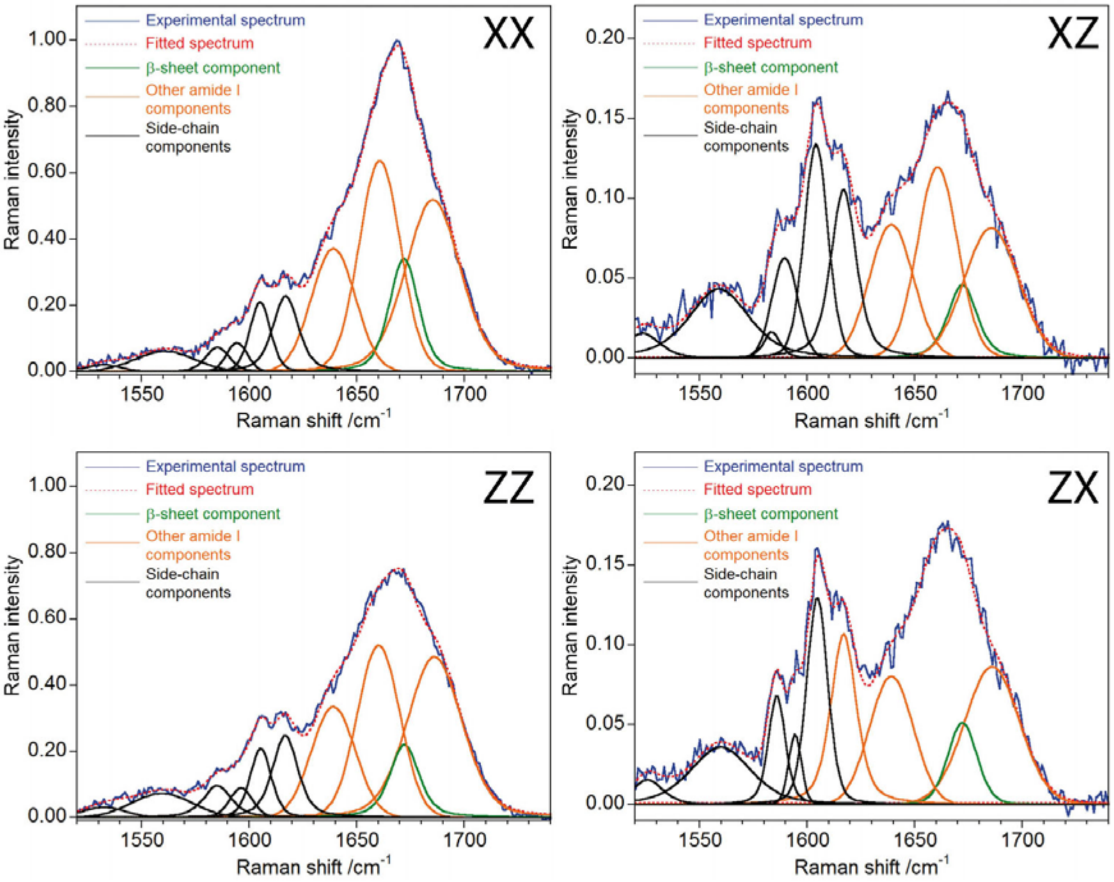

Figure S5

Spectral decomposition of the Raman spectra of the Flag silk of *A. diadematus* in the amide I region.