Surface sensitive Raman spectroscopy of Collagen I fibrils

Corinne Gullekson, Leanne Lucas, Kevin Hewitt and Laurent Kreplak

SUPPLEMENTARY MATERIALS

Di-phenylalanine Tube Spectra

Phe-Phe nanotubes were assembled by diluting 100mg/ml of di-phenylalanine (FF) in HFR (1,1,1,3,3,3-hexafluoro-2-propanol) to 1mg/ml (10ul FF 990ul water). The solution was then deposited on a glass bottomed Petri dish and dried with nitrogen. The Phe-Phe nanotube Raman spectrum was taken by exposing the sample to a 532nm laser adjusted to 5mW for 25 seconds.

Assignment of enhanced Raman peaks to Phenylalanine and Tyrosine Residues

Although present in small amounts it is reasonable to focus on phenylalanine and tyrosine when assigning peaks. These are the only two amino acids in our sequence that have residues including benzene derivatives. Benzene derivatives produce very strong Raman signals and dominate the spectra of proteins in near field Raman (1). Although glycine, proline and alanine are the three most common amino acids, have relatively weak spectra with non-unique features. In the SERS spectra of these individual amino acids presented by Stewart et al.(2), the strongest bands in each amino acid was the COO^-deformation at 720cm^{-1} followed by the symmetric COO^- stretch at 1410cm^{-1}. Since COO^- groups are present in all amino acids, assignment of these bands to glycine, proline or alanine would not be possible. The only way to distinguish between amino acids is with modes unique to the amino acid's residue. Unfortunately, the proline residue does not produce any strong unique peaks that would make assignment possible (2), and the residues for glycine and alanine, –H and $–CH_3$ respectively, do not have distinguishable spectra neither. Finally, amino acids with C-S bonds are also known to have intense Raman spectra. The methionine residue (in 0.8% of the collagen I sequence) exhibits a distinguishable C-S peak at 700cm^{-1} (2). In the gold SERS experiments there was a common peak around 685cm^{-1}. It is possible that this peak could have arisen from the C-S vibration however; it is also possible that it is from the phenyl ring vibration at 690±10cm^{-1} or a tyrosine vibration at 700±35cm^{-1}. There is not another strong C-S peak present to justify the assignment of this peak to the methionine residue. Considering the prominence of the other ring vibrations in the spectra, it is reasonable to assign this peak to phenylalanine or tyrosine.

The strongest phenylalanine and tyrosine bands were observed around 1585 and 1605cm^{-1}. It is possible other modes could produce bands in this region. That is true with all vibrational spectroscopy of organic materials. However, it is very likely that these bands do arise from phenylalanine and tyrosine. In the SER spectra of non-aromatic amino acids examined by Stewart et al., no bands in that region were observed (2). COO^- asymmetric stretch and a NH_2 scissors vibrations appear in this region in IR. However, these modes exhibit weak or no Raman scattering. It is thus reasonable to assign these bands to the phenylalanine and tyrosine rings as opposed to other organic groups.

References

1. Stewart, S., and P. M. Fredericks. 1999. Surface-enhanced Raman spectroscopy of peptides and proteins adsorbed on an electrochemically prepared silver surface. Spectrochim Acta A 55:1615-1640.
2. Stewart, S., and P. M. Fredericks. 1999. Surface-enhanced Raman spectroscopy of amino acids adsorbed on an electrochemically prepared silver surface. Spectrochim Acta A 55:1641-1660.

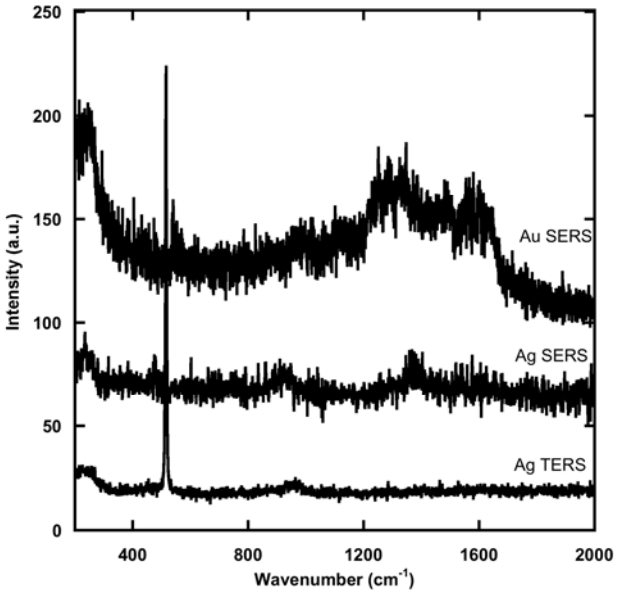

FIGURE S1:
Background spectra of the various enhancement techniques. Spectra of 65nm Au and 30nm Ag nanoparticles were taken on clean glass in water. Spectra of a 30nm of a CONT cantilever in air on clean glass after use in TERS experiments.

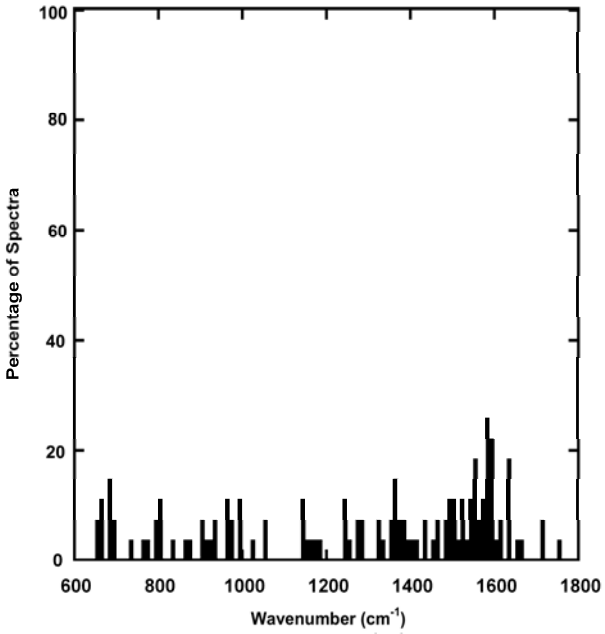

FIGURE S2:
Histogram of peak positions in 27 TERS spectra between 600 and 1800cm^{-1}.

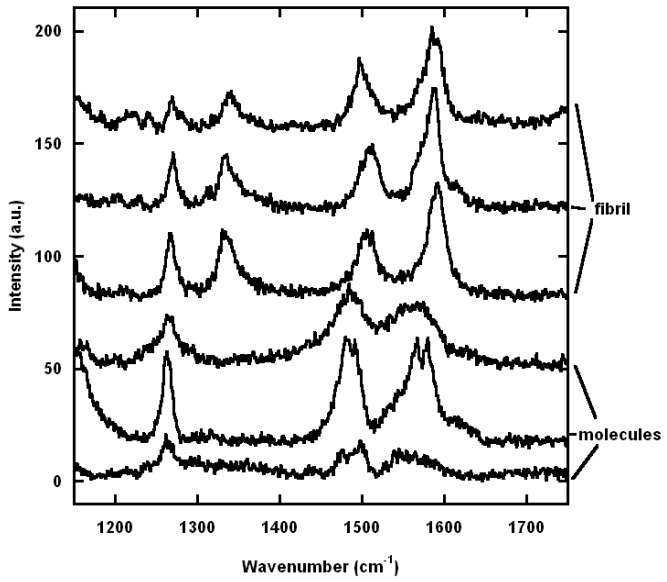

FIGURE S3:
Spectra collected at 3 points on a fibril and 3 points near a potential aggregate of collagen molecules probed in the line scan shown in figure 5.

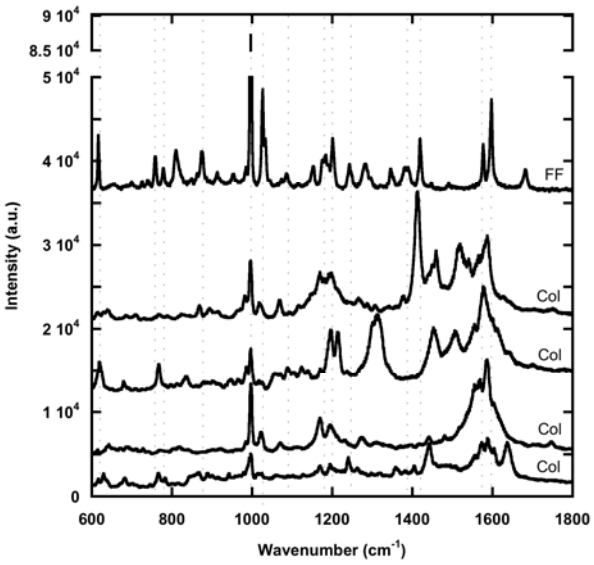

FIGURE S4:
A comparison of Gold SERS spectra and the spectra of Phe-Phe nanotubes. FF: Spectrum of a large clump of Phe-Phe tubes. Col: Gold SERS spectra that resemble the Phe-Phe spectrum. Positions of Phe peaks seen in the gold SERS spectra are marked with dotted lines.