Quantified DUV Raman Analysis for Detecting Organic Biosignatures. J. Razzell-Hollis[1], H. Sapers[1,2,3], M. Fries[4], R. Bhartia[1], L. Beegle[1], [1]NASA Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA, [2]California Institute of Technology, Pasadena, California, USA, [3]University of Southern California, Los Angeles, California, USA, [4]NASA Johnson Space Center, Houston, Texas, USA.

Introduction: Searching for life on other planets has, primarily, focused on the detection of structurally complex organic molecules that form the basic building blocks of cellular life as we know it. Raman spectroscopy is a well-established, non-destructive technique for identifying complex organic molecules by the pattern of Raman scattering peaks produced by the molecule's unique vibrations, but is only now being considered for use in biosignature detection and in planetary science missions beyond Earth.[1] The presence of a complex organic is not itself a biosignature, but the conjunction of different organics in the same location is unlikely to occur spontaneously. Biosignature detection then becomes a process of identifying the distribution of complex organics and the search for their co-incidence. If detected, it is essential that the mixed signal can be sufficiently deconvolved into its component parts such that they are all identifiable and their concentrations measurable, before any comment on the likelihood of abiotic vs biological origins can be made.

SHERLOC is a deep ultraviolet (DUV) Raman spectrometer designed to search for chemical biosignatures in the Martian surface and near sub-surface, and has been selected to be part of the scientific payload aboard the Mars 2020 rover.[2] DUV Raman exploits the molecular resonance enhancement of high-energy excitation to provide unparalleled sensitivity to aromatic molecules, even at low concentrations, and is capable of distinguishing chemically similar compounds by the patterns of their vibrational modes, all without damaging the sample, making SHERLOC ideal for identifying potentially biosignificant samples for return to Earth. However, DUV Raman is a sufficiently novel development that the exact behavior of many molecules under such excitation is not well-known, and a fully quantitative analysis of complex, mixed spectra requires significant work in building accurate libraries, detailed analysis of known samples and careful consideration of the technique's selectivity on limits of detection.

To this end, we have developed a model to describe the Raman intensities of different component molecules in a mixed sample, in terms of their relative concentration and the optical properties of the instrument, with the aim of accurately calculating sample parameters from spectroscopic data. We then conducted studies of two extreme examples of potential biosignatures: intact microbial cells, and the carbonaceous material that is left after ancient organic matter is subjected to extensive geothermal alteration.

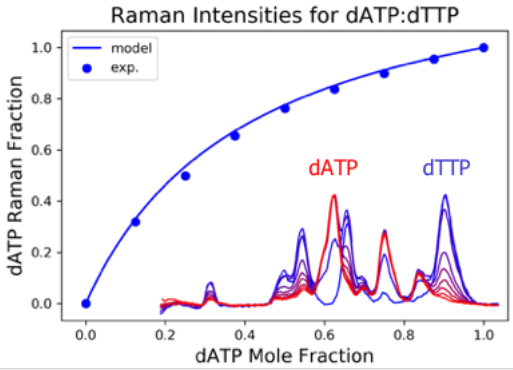

Figure 1. Modelled and experimental Raman ratio for a solution of adenosine (dATP) and thymidine (dTTP), with a total concentration of 1 mM.

Optical Model: We constructed a mathematical model to predict Raman intensities from any given sample based on the incident laser power provided by the laser, the optical constants of the molecules in question (Raman scattering cross-sections, absorption coefficients), and the optical throughput parameters of the spectrometer. Aqueous solutions of single organics at varying concentrations were measured to obtain intensity curves and test the model. For strongly UV-absorbing molecules such as DNA/RNA nucleotides, intensity was found to be absorption-limited at concentrations above 1 mM, and so we ensured that 'standard' organic spectra were taken of 1 mM solutions, or lower if necessary. When mixed solutions were measured, their spectra were found to be a weighted combination of peaks from both components, and could be spectrally deconvoluted by linear least-squares fitting with the appropriate standard spectra. The fitted intensities were consistent with the predicted values from the model, confirming that (provided the samples were homogenous) the ratio of intensities determined solely by the ratio of Raman scattering cross-sections. The standard spectra obtained so far will be used as the basis of a detailed spectral library for future data analysis from SHERLOC measurements.[2]

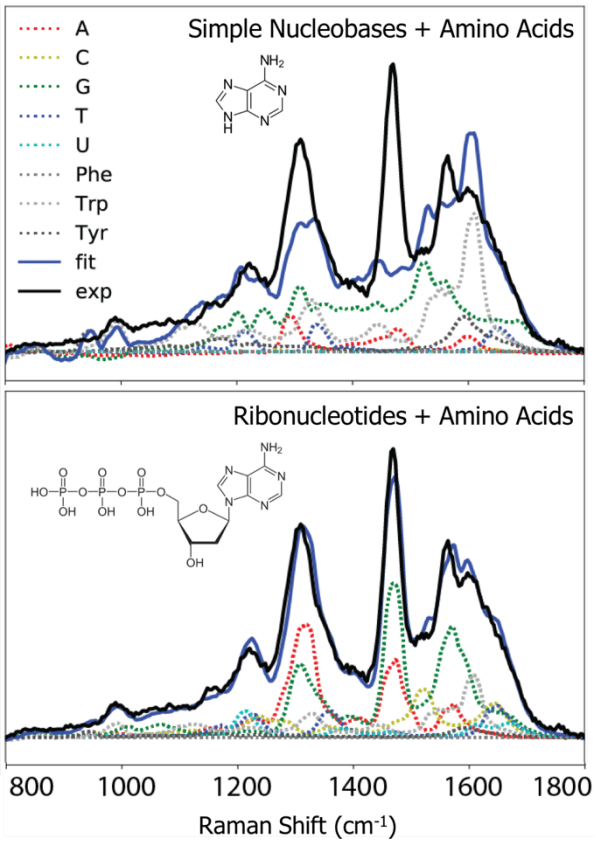

Figure 2. Deconvolution of the spectrum of *E. coli* based on standard spectra.

Microbial signatures: to represent an intact microbial signature, we measured the DUV Raman spectra of *E. coli* cells. Selective enhancement of aromatic components leads to a cell spectrum dominated by only eight molecules: five nucleobases (adenine, cytosine, guanine, thymine, uracil) and three amino acids (phenylalanine, tryptophan, tyrosine), due to resonance with aromatic rings.[3] These overlap significantly in the 'fingerprint region' (800 – 1800 cm^{-1}) that contains the most distinctive modes for each component, hindering clear identification and making estimation of individual contributions impossible. By deconvolution using linear combination of individual component spectra, it is possible to distinguish the relative contribution of each molecule to the total intensity, determined by molecular concentration and relative Raman cross-section. Deconvolution with standard spectra for the 5 simple nucleobases (and the 3 aromatic amino acids) leads to a poor fit, inadequately describing the vibrational spectra of the entire cell. However, deconvolution with standard spectra for the 5 ribonucleotide triphosphates instead (adenosine, cytidine, guanosine, and uridine), plus the 3 aromatic amino acids, leads to an excellent fit.[1] Furthermore, the necessity of a minimum level of structural complexity to obtain a good fit is a biosignature in of itself, as ribonucleotide triphosphates have yet to be observed in abiotic synthesis,[4] and may be specific to biological systems as we know them. Although we do not expect to observe *E. coli* on Mars, this serves to demonstrate the effectiveness of DUV Raman to accurately detect the presence of several biosignificant molecules in a complex system, obtain their relative concentrations, and provide detailed information without the need to destroy the sample.[1]

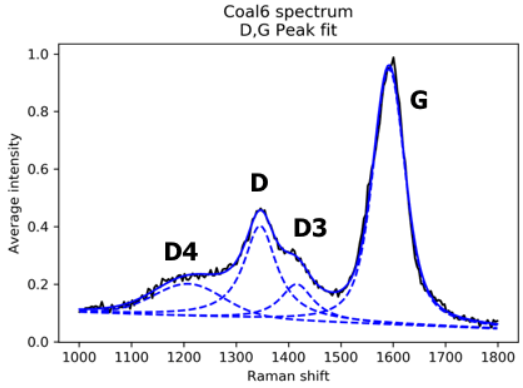

Figure 3. Fitting of the DUV Raman spectrum of carbonaceous material using Voigt functions, peaks assigned to either Graphitic or Defective bands.

Carbonaceous signatures: the use of Raman spectroscopy to study carbonaceous materials has already been established on samples from both terrestrial and extra-terrestrial sources,[5] but information on the spectra obtained under DUV excitation has so far been limited. Using terrestrial coals (Penn. State) to represent carbonaceous matter across a range of geothermal maturities, we demonstrate that compared to visible-light excitation, DUV Raman is particularly sensitive to the more ordered, graphitic components of the material, as opposed to the more disordered, defective component. Through Voigt-function fitting the spectral parameters of the 'G' band can be accurately measured, and correlates well to defect concentration, and thus may be used as an indicator for the integrity of the organic matter, or the geothermal history of the material.

References:

[1] Sapers, H.; et al. *Frontiers Microbiology*, submitted 2018. [2] Beegle, L.; et al. (2015) *IEEE Aerospace Conference Proceedings*; Vol. 2015–June. [3] Wu, Q.; et al. (2001) *Anal. Chem.* 73 (14), 3432–3440. [4] Burton, A. S., et al. (2012). *Chem. Soc. Rev.* 41, 5459–5472. [5] Rahl, J. M.; et al. (2005) *Earth Planet. Sci. Lett*, 240 (2), 339–354.