ND*nano* Undergraduate Research Fellowship (NURF)
2013 Project Summary

1) Student name: Rachael Bridgman

2) Faculty mentor name: Dr. Zachary Schultz

3) Project title: Determining SERS Cross-section of Amino Acids for Protein Characterization

4) Briefly describe any new skills you acquired during your summer research:
I learned how to create SERS substrates and how to use the Renishaw inVia microscope to take spectra of these and normal Raman samples. I learned how to use Mat Lab and Igor to process my data efficiently and how to create an effective poster to present my research. Finally, I got experience in attending and participating in my first poster session at the Notre Dame SURS event this summer.

5) Please briefly share a practical application/end use of your research:
Quantifying the cross-section of amino acids will enable researchers to better quantify the cross-section of proteins. The cross-section of proteins is an important piece of information in developing single protein detection using normal Raman and surface-enhanced Raman spectroscopy.

Single protein detection has many applications, including in the healthcare industry. For example, having the ability to detect a single protein could lead to early and life-saving diagnoses in patients who are in the initial stages of an illness. These patients would have very low viral or bacterial loads that could require a diagnostic tool sensitive enough to detect a single protein in a biopsy sample.

Project summary:
The chemical structure of proteins is important for understanding their function, which is dictated by the amino acid sequence. Raman spectroscopy is a label free, chemically specific technique based on the inelastic scattering of photons. This scattering occurs when photons from the light source are incident on a molecule, exciting the molecule from its ground state to a virtual energy state. When the molecule relaxes to the ground state, it scatters photons having a difference in frequency that is proportional to the molecule's various vibrational modes corresponding to its chemical bonds. The photons can be detected to create fingerprint spectra that are representative of the vibrational modes.

Raman spectroscopy can be used to identify the amino acids in proteins. Although spontaneous Raman is a weak signal, the resultant signals can be enhanced by gold and silver nanostructures. This phenomenon known as surface-enhanced Raman spectroscopy (SERS) occurs when the localized surface plasmon resonance (LSPR) of a metal is excited by incident photons, creating an electromagnetic field that enhances the Raman signal.

Determining each amino acid's SERS cross-section provides a measure of how likely one amino acid will scatter compared to another. However, it has been difficult for scientists to develop a reliable method for quantifying the SERS cross-section of molecules. The goal of this project is to determine the SERS cross-section of all 22 amino acids and use this information to specify amino acid contributions to the SERS spectrum of the protein Bovine Serum Albumin (BSA).

To accomplish this, the spectra of all 22 amino acids and BSA in powder and aqueous form were taken using normal Raman spectroscopy. Powder forms of BSA and the amino acids were individually deposited onto a silicon chip fixed to a glass microscope slide. Spectral acquisitions of the powders were taken using the Renishaw inVia Raman microscope. The acquisitions varied in acquisition time and laser intensity for all samples. Amino acid solutions were created using various concentrations diluted with 18.2 MΩ cm nanopure water and sonicated at 45°C until fully dissolved. A template of polydimethylsiloxane (PDMS) with a small well was adhered to a glass microscope slide. Seventy microliters of each solution was deposited in the well for spectral acquisitions. In addition, the SERS spectrum of BSA was taken. SERS gold (Au) substrates were created according to Asiala and Schultz, 2011. Fifteen microliters of 1mM BSA was deposited on the substrate's surface and allowed to dry for 30 minutes. Twenty-second spectral acquisitions of the substrates were taken using the Renishaw inVia Raman microscope.

The regular Raman spectrum of each amino acid as a solid and in aqueous solution was measured quantitatively with respect to sample concentration, laser power, and acquisition time. These results were used to deconvolute the BSA spectrum and determine which amino acids contributed to the protein's spectrum. Analysis of the powder BSA spectrum with the powder amino acid spectra suggested that histidine, phenylalanine, tyrosine and tryptophan were detected in BSA. These amounted to 2.80%, 4.94%, 3.45% and 0.49% of the protein's sequence respectively. Analysis of the aqueous BSA spectrum with the aqueous amino acid spectra suggested that phenylalanine, histidine, serine and glycine were detected in BSA. BSA is composed of 5.27% serine and 2.80%. The cross-section of each amino acid is proportional to their respective spectrum. Future work will be to use the information gathered from these spectra to calculate the cross-section of each amino acid.

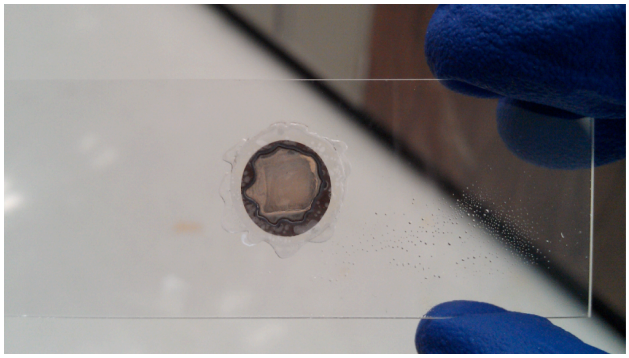
Publications (papers/posters/presentations):

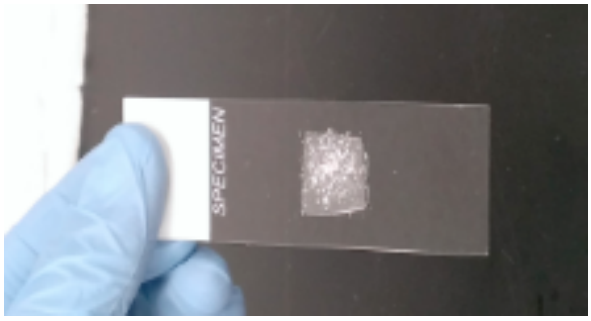
I presented a poster at the Notre Dame SURS event on August 2nd. (See attachment to this email)

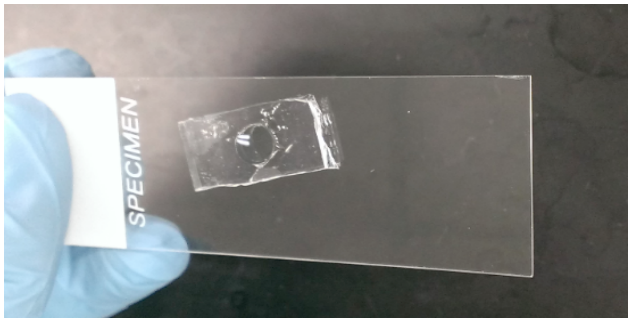
RESEARCH PICTURES:

Figures 3. **a)** Image of SERS Au substrates. **b)** Image of SERS substrate with I mM solution BSA deposited and dried. **c)** Image of BSA powder deposited onto the silicon chip. **d)** Image of PDMS well filled with solution. **e)** Image of Renishaw inVia Raman microscope used to acquire spectra.

a) 

b) 

c) 

d) 

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