ND*nano* Undergraduate Research Fellowship (NURF)
2014 Project Summary

1. Student name: Colleen Riordan

2. Faculty mentor name: Dr. Zachary Schultz

3. Project title: Surface enhanced Raman detection of carbohydrates

4. Briefly describe any new skills you acquired during your summer research:

I learned how to use surface-enhanced Raman spectroscopy (SERS) substrates and how to add a self-assembled monolayer (SAM) to the surface of the substrate. I also learned how to use the flow cell developed by the Schultz lab, which was developed to obtain Raman spectra of molecules in solution at low concentrations.

5. Briefly share a practical application/end use of your research:

This summer, I worked on showing that simple sugars can be detected using SERS and the flow cell. This work is important because once I achieve good results with simple sugars I am planning on moving on to work with more complicated carbohydrates, glycans associated with glycoproteins. These glycoproteins are important parts of many biochemical processes such as protein folding, stability, and localizations and also in cellular communications between cells, cell matrixes, proteins and sugars. Because glycoproteins are important in so many cell processes, glycans are believed to be related to many diseases including hereditary disorders, immune deficiencies, cardiovascular disease, and cancer. Many researchers are trying to identify glycan structures, because they believe that this will allow for the diagnosis and treatment of many of these diseases. Though there have been many ways developed to try to separate and identify glycan structures, there is still no good method used. I plan to use the skills I learned this summer to examine these glycan molecules in an attempt to find a way to separate the glycan molecules through the different vibrational stretches that I will see in the Raman spectra. This will enable the identification of different glycans, even those that are isomeric.

Project Summary:

The identification and separation of isomeric carbohydrates is often difficult using many types of spectroscopy. Of particular interest to many researchers are glycans, carbohydrates found in nature that are heavily involved in essential cell processes such as protein folding and cell communications. Most research in the separation of glycans has focused on mass spectrometry, but MS is unable to distinguish isomeric glycans. As the specific structure of the glycans correlate to the distinct function of the glycan, it is essential that isomeric structures be identified. Raman spectroscopy is one way in which isomeric carbohydrates could be detected. In Raman, monochromatic light is irradiated on a sample, in which the photons are raised from ground state to some virtual state and then scattered. Most photons are Rayleigh scattered, meaning that the photons enter and leave the sample with the same energy. However, some photons lose or gain energy, and these are stokes or anti-stokes scattered. These photons are

scattered at different wavelengths that correspond to the sample's vibrational frequencies. The advantage of Raman is that the spectra are unique to each chemical, so Raman should be able to distinguish isomeric carbohydrates. The goal of this project was to use Raman and enhanced Raman techniques to identify the differences in the spectra of isomeric carbohydrates.

To begin with, the Raman spectra of solid samples of the simple sugars were taken and compared. Of particular interest were the isomeric sugars, particularly glucose, galactose and mannose which differ only in the axial or equatorial conformation of various hydroxyl groups. The spectra of these three sugars were each unique, showing that Raman can be used to separate isomers. There were also many peaks in common between the three sugars, so the structure and the spectra are clearly related. Though Raman spectroscopy was able to differentiate the sugars in the solid state, this method uses large amounts of sample, which becomes an issue when the sample is expensive or difficult to synthesize or isolate. The issue is that Raman scattering is a relatively weak process, with only 1 out of 10^{13} photons Raman scattering. Because of this, it is very difficult to obtain Raman spectra of molecules in low concentrations. In answer to this problem, surface-enhanced Raman spectroscopy, or SERS, was developed. This method uses metal nanoparticles that when irradiated with a laser produce an electric field, which enhances the signal up to 10^{13} over normal Raman. SERS of the simple sugars also showed both differences and similarities between the sugars, in concentrations as low as 1.0 micromolar. For some of the sugars, these spectra were quite clear, but for others the background of the SERS substrate blocked or overshadowed many of the peaks from the sugar. To attempt to remedy this, and to continue collecting spectra at low concentrations, a self-assembled monolayer (SAM) was used on the surface of the SERS substrate. The SAM was composed of 1.0 mM decanethiol and 1.0 mM mercaptohexanol. The difference in the height of these two thiols is approximately the same size as a glucose molecule, so when a solution of glucose is in contact with the SAM on the SERS surface, the SAM traps the molecules close to the surface. This is important because SERS substrates only can enhance the signal if the analyte is within nanometers of the surface. When using the SAM, some peaks corresponding to those of glucose were picked up in the spectra, but there were fewer peaks than had been found in either the Raman or the SERS spectra. However, the SAM was next tested with galactose as the analyte, as glucose and galactose are basically the same size, and the SAM had more peaks than the SERS for galactose. For both glucose and galactose, the SAM was able to obtain spectra at slightly lower concentrations, 100 nanomolar at the lowest, but still did not give much enhancement over SERS. Using Raman, SERS and SAM enhanced SERS substrates, it is possible to differentiate isomeric carbohydrates, as shown through the use of simple sugars.

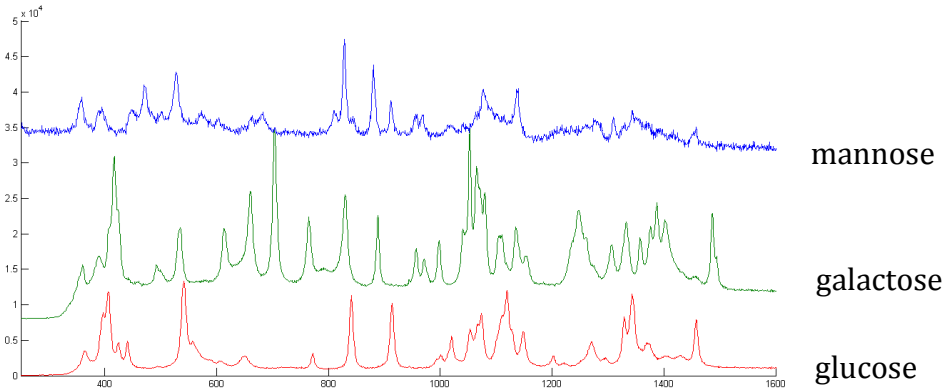

Figure 1: Raman spectra of solid isomeric sugars, showing that Raman can be used to separate isomeric carbohydrates, as these sugars only differ in the axial/equatorial conformation of hydroxyl groups, but the Raman spectra are each unique.

Figure 2: SERS substrate on a glass slide, with a SAM of decanethiol and mercaptohexanol.

Publications (papers/posters/presentations):

Poster presentation at the Undergraduate Research Symposium