Development of SERS substrates for the detection of latent chemicals and the study of noncovalent interactions

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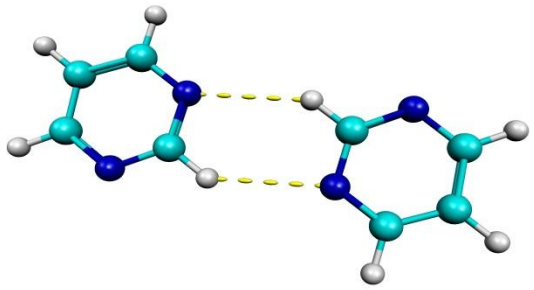
Introduction

As a B. S. Chemistry major at the University of Mississippi, senior research (CHEM 463) is a requirement to graduate. While many students wait until the last year or two of their undergraduate career to begin research, I began the summer after my freshman year. At this point in my undergraduate career, I have conducted research during three summer breaks and three academic years. In this time, many collaborations were made and conferences were attended.

Pyrimidine

Crystalline Pyrimidine

Pyrimidine is a heterocyclic organic benzene-like compound containing nitrogen in the one and three positions of its ring. It serves as a building block within many important biological macromolecules such as the nucleic acids thymine, uracil, and cytosine, the nicotinamide in NADH, and ATP. Here, Raman spectroscopy is used to study the effects of weak CH···N interactions (shown below) on the vibrational spectrum of crystalline pyrimidine. The results of electronic structure computations are used to interpret the experimental results and aid in the assignment of spectral features.

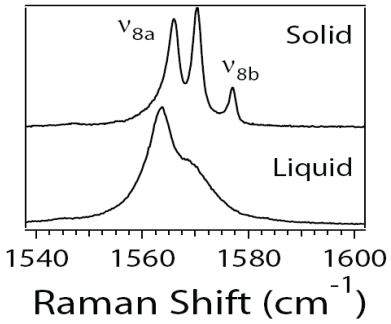


Weak CH···N interactions between neighboring pyrimidine molecules

Weak noncovalent interactions at the molecular level play key roles in determining macroscopic structures and functions of biological macromolecules and are behind many of the important driving forces in nature. For example, from the hydrogen bonding between DNA base pairs to protein structure and folding, the study of these weak interactions between or within biomolecules is crucial for a full understanding of biological function. Weak CH···N interactions exist between the pyrimidine molecules in its crystal structure.[1] Each pyrimidine molecule in its crystalline environment interacts with its eight nearest neighbors with CH···N distances ranging from 2.56 - 2.72 Å. These interactions are typically classified as weak hydrogen bonds and mimic interactions found in larger and more complex biological systems.

The Raman spectrum of crystalline pyrimidine was obtained using a thermoelectric

cooler to keep the sample in the solid state. Peaks in the Raman spectra reveal inconsistent shifts when pyrimidine transitions from the liquid state to the solid state. Shown below, for example, is a comparison of the Raman spectrum of ν_{8a} and ν_{8b} in the liquid and solid states.



Solid and liquid phase spectra of pyrimidine

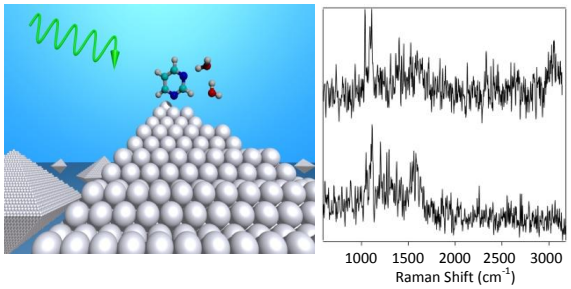
Computations were performed on a cluster of nine pyrimidine molecules (one pyrimidine molecule and its eight nearest neighbors in the crystalline geometry) to help analyze the experimental results. These calculations predicted that the observed shifts are related to the modes concerning the motion of hydrogen atoms. Assignments of both fundamentals and combination bands in both the liquid and solid states are facilitated by the mode-specific nature of the shifts. We recently published these results in the journal *Chemical Physics Letters*.[2]

Pyrimidine/Water Mixture SERS

Weak noncovalent interactions play key roles in determining macroscopic structures and functions of biological macromolecules and are behind many of the important driving forces in nature. It has recently been reported that hydrogen bonding with water results in the blue-shifting of certain normal modes of pyrimidine.[3] By comparing experiment-tal polarized Raman spectra with the results of electronic structure calculations, the origin of this blue-shifting was found to center around charge transfer from pyrimidine to water in the bulk mixtures. Here, we wish to study individual aqueous hydrogen bonded networks involving pyrimidine. By studying the effects of weak noncovalent interactions between water and individual pyrimidine molecules, we can identify the dominant structural motifs present in complex biological systems.

Pyrimidine and its numerous derivatives are important constituents of many biological molecules. The nucleic acids thymine, cytosine, and uracil and the nicotinamide in NADH are all derivatives of pyrimidine as are adenine-containing building blocks of many biologically relevant molecules, including ATP. Pyrimidine is also becoming a significant constituent in many drugs including those used for antiviral, antibacterial and anti-inflammatory purposes.[4] The use of pyrimidine is even becoming important in chemotherapy, considering some derivatives have antitumor characteristics.

We are currently studying how weak, noncovalent interactions affect the surface enhanced Raman spectra (SERS) of neat pyrimidine and pyrimidine molecules in dilute aqueous solutions using silver-island film substrates. Our ultimate goal is to obtain vibrational spectra of individual pyrimidine molecules in an aqueous environment and connect reoccurring spectral signatures to structural motifs. A cartoon illustrating our experiment and two resulting single molecule SERS spectra are shown below.



We analyze the spectroscopic results by comparing them with the results of electronic structure computation on specific pyrimidine/ water clusters.

Vapor Deposition

Au Nanoparticles

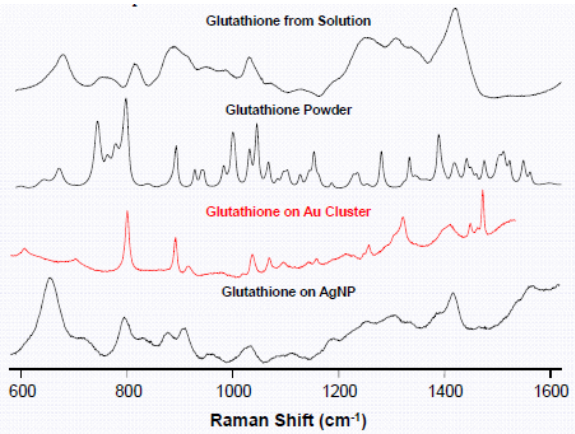
The development of metallic nanomolecules with controllable size and composition is currently an extremely active area of research. Gold nanomolecules such as Au_{25} and Au_{38} exhibit unique electronic properties that are controlled by their size and the ligands that are

incorporated into their core structure. Our collaborative reseach team aims to describe the photophysical and structural properties of this new molecule class using Raman and fluorescence spectroscopies and electronic structure calculations.

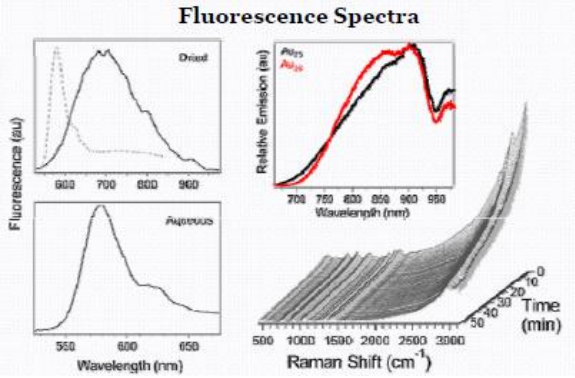
IMAGE

The characterization of the photophysical and structural properties of this new molecular class of metallic nanomolecules is key to engineering materials with specific electronic properties for a myriad of electronic applications. For example, the use of gold nanomolecules in biological applications is rapidly increasing and requires a high level of chemical stability in very specific aqueous environments.

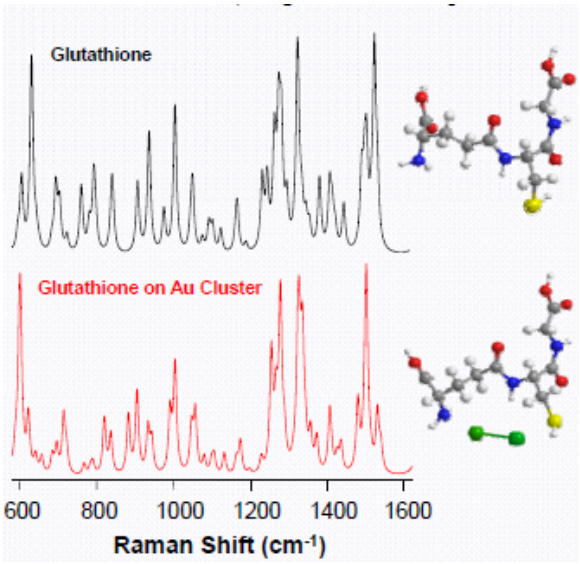
A Coherent Innova Ar^+ laser at 514.5 nm was utilized as the excitation source for acquiring fluorescence spectra. Raman spectra were acquired using either a Horiba LabRam HR Raman microscopy system with 632.8 nm HeNe Lasaer excitation or a Nikon TE2000U inverted microscope with 514.5 or 647.1 nm Kr^+ laser excitation and CCD detection. Raman spectra acquired with a 632.8 nm excitation are shown here. Typical CCD Integration times for the 632.8 nm excitation were 100 s for glutathione solid, 500 s for the Au nanomolecules with glutathione ligands, and 100 s for glutathione SERS on AgNPs. The 632.8 nm laser power was 13 mW for the solid and solution, and 1.3 mW for Au nanomolecules and for glutathione on AgNPs. The objective employed was a 100X (NA 0,95) for glutathione solid and the auNP cluster and for the glutathione solution for SERS on AgNP a 10X objective with NA value of 0.25 was used.



The Raman spectra of glutathione on Au nanomolecules were similar in nature to that of glutathione powder and differed significantly from glutathione deposited from solution. SERS spectra of glutathione on silver nanoparticles resembled that deposited from solution.



The Raman spectra of glutathione and glutathione bonded to a small Au cluster were computed at the B3LYP/LANL$_2$DZ level of theory. The simulated spectrum including interactions with gold exhibits less intense Raman features, in agreement with the experimental data.



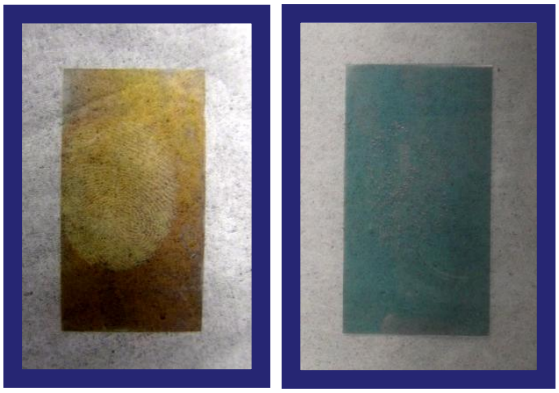
Latent Fingerprints

The use of surface enhanced Raman spectroscopy (SERS) for the analysis of latent fingerprints has recently garnered much attention. Previously, advancement in latent fingerprint analysis has mainly focused on the imaging of the distinct physical patterns unique to individual fingerprints.[5] More recently, researchers have concentrated on retrieving the molecular information embedded in the fingerprints using SERS.[5] An important consideration in the development of such technologies is the choice of SERS substrate. Usually metallic nanostructures composed of silver or gold are used. It has recently been suggested that encapsulation by gold protects latent fingerprints for a long period of time.[6] Here, we present our results from using gold and silver island film substrates deposited directly onto latent fingerprints using vapor deposition. This method offers the advantages of encapsulation and preservation as well as single molecule level detection for the investigation of important trace analytes.

Glass coverslips were cleaned with a 3:1 sulfuric acid/hydrogen peroxide Piranha Solution and rinsed in water. The slides were dried with purified, compressed N_2 gas. A carefully cleaned thumb is coated with or without additional analyte (such as acetaminophen) and lightly pressed on a clean slide to deposit a fingerprint.

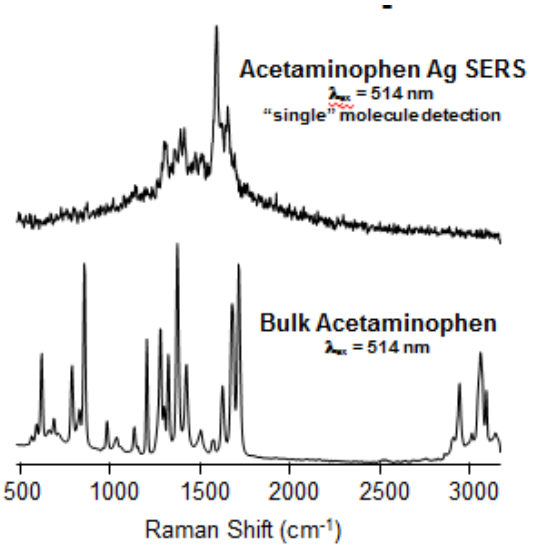
The fingerprinted slide was placed in the vapor deposition chamber (Edwards AUTO 360 Vacuum Coater) and Ag Au at a was deposited at a rate of 0.2 Å/s. Thicknesses of 5 nm (Au and Ag) and 8 nm (au) were coated on the coverslips containing a fingerprint. The slides remained under vacuum for at least one hour after deposition. Once removed from the vacuum coater, the slides were housed in a sealed, plexiglass box pressurized with purified, compressed N_2 gas.

The 514.5 nm line from an Ar^+ laser is used as the SERS excitation source for the Ag-coated slides and the 676.4 nm output from an Kr^+ laser for the Au-coated. Raman scattered light was introduced to the samples using an inverted microscope with an 100x high NA objective. After passing through one or more notch or cutoff filters the scattered light was dispersed using a grating spectrometer and detected using a CCD camera.

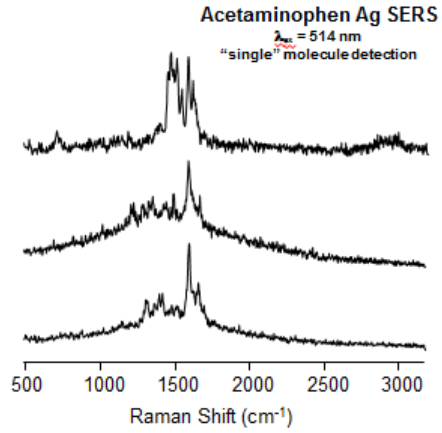


Silver **Gold**

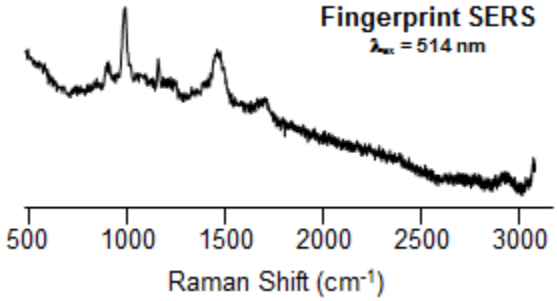
Photographic illustration of substrate coated fingerprints on Piranha cleaned glass cover slips.



Comparison of SERS "Single" molecule detection of acetaminophen on a silver island film and a bulk acetaminophen sample



SERS "Single" molecule detection of acetaminophen on a silver island film with CCD camera image



SERS spectra of only the fingerprint residue

The coating of the fingerprinted coverslips with 5 nm of Ag was successful in providing bulk and single molecule SERS of the chosen analyte. By UV/Vis examination, the 5 nm substrate thickness of Au to coat the fingerprinted coverslips was found to be inadequate in providing SERS data. The 8 nm substrate thickness of Au was proven to be adequate by UV/Vis. However, observations with SERS contradicted. Single molecule SERS spectra of acetaminophen are similar to the bulk SERS measurements with the exception of the ratio of the intensity modes varies between each molecule.

References

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