

# Study of the Effect of Chromophore Environment on UV-Resonance Raman Scattering Spectroscopy of Tyrosine containing Short Peptides

Jerry Cabalo <sup>1</sup>, Erik Emmons <sup>2</sup>, Semion Saikin <sup>3</sup>, Dmitriy Rappoport <sup>3</sup>, Alan Aspuru-Guzik <sup>3</sup>  
 (1) U.S. Army Edgewood Chemical Biological Center, (2) Leidos, Inc., (3) Dept. Chem. Bio., Harvard University

## Abstract

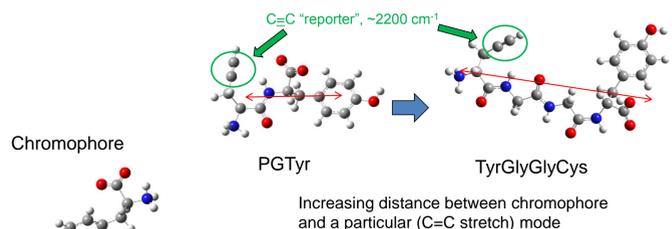
To investigate changes in the resonance Raman response to intermolecular interactions, we used an experimental and theoretical study of two series of peptides containing tyrosine and cysteine, and tyrosine and L-C-propargylglycine (PG), in solution, and in contact with the surface of titania anatase nanoparticles. Four technical barriers were shown in the course of this study. The first barrier was the incorrect assumption that high lying electronic states could be neglected. Our results show that for tyrosine, high energy electronic states out of resonance with the excitation can influence the Raman response and that intermolecular interactions can act on the molecular polarizability through these states. Three of the technical barriers were not overcome: 1) correct preparation of amino acid or peptide samples in close contact with the nanoparticle surfaces where signal predominantly arises from molecules on the surface, 2) correct calculation of peptides in contact with a transition metal oxide including the excited electronic states, 3) correct selection of test molecules that demonstrate change in response to the presence of a surface.



## Introduction

Raman spectroscopy is a very important analytical tool because it has such a high degree of chemical specificity. This spectroscopic approach is highly applicable to a number of Army missions such as the sensing of hazardous materials, including explosives, toxic chemicals, and pathogenic organisms, or obtaining an understanding of how these toxic materials affect biological systems, such as the binding of organophosphates to acetylcholinesterase. However, low sensitivity limits the utility of this powerful technique. In recent years, a number of methods for overcoming sensitivity limitations involving Surface Enhanced Raman Spectroscopy (SERS) or Resonance Raman Spectroscopy (RRS) have been demonstrated. These methods, such as RRS, exploit the selectivity of Raman spectroscopy while taking advantage of the electronic structure of the target molecule to amplify the Raman signal.

RRS depends on resonance between the excitation laser and electronic states within the analyte molecule. Only normal mode vibrations involving the atoms within the chromophore experience enhancement. While this selectivity towards vibrational modes within a chromophore is an advantage for some applications, it is also desirable to permit vibrational modes outside of the chromophore to experience resonant enhancement in a controlled manner. An understanding of how vibrational modes that do not directly involve the chromophore interact with the excited electronic states of that chromophore may permit controlled enhancement of these modes.



## Methods

**Theory:** For the calculation of RRS and RRS excitation profiles, the sum-over-states approach is used. Excitation profiles are the RRS cross section of a given vibrational mode as a function of excitation energy. Within the simplified sum-over-states approach, the resonance Raman cross section is represented as a sum over excited electronic states and is obtained by straightforward differentiation of the sum-over-states expansion of the frequency-dependent polarizability with respect to a vibrational normal mode. The differential Raman scattering cross section for a given normal mode  $Q$  can be written as

$$\left(\frac{\partial\sigma}{\partial\Omega}\right)_Q = \frac{(\omega - \omega_Q)^4}{2\omega_Q c^4} |\langle\sigma_Q(\omega)\rangle|^2$$

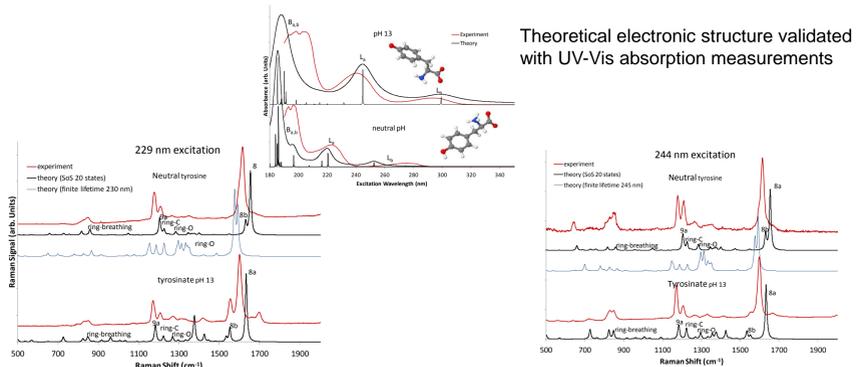
Where  $\omega$  is the excitation frequency,  $\omega_Q$  is the vibrational frequency of mode  $Q$ ,  $c$  is the speed of light, and  $\sigma_Q$  is the angle-averaged Raman scattering tensor. The Cartesian component  $mn$  of the Raman scattering tensor is computed as

$$\sigma_Q^{mn}(\omega) = \sum_k \left[ -\mu_{0k}^m \mu_{0k}^n \left[ \frac{(\Omega_k - \omega)^2 - \gamma_k^2}{(\Omega_k - \omega)^2 + \gamma_k^2} + \frac{2i(\Omega_k - \omega)\gamma_k}{(\Omega_k - \omega)^2 + \gamma_k^2} \right] \frac{\partial\Omega_k}{\partial Q} + \left[ \mu_{0k}^m \frac{\partial\mu_{0k}^n}{\partial Q} + \frac{\partial\mu_{0k}^m}{\partial Q} \mu_{0k}^n \right] \left[ \frac{\Omega_k - \omega}{(\Omega_k - \omega)^2 + \gamma_k^2} + \frac{i\gamma_k}{(\Omega_k - \omega)^2 + \gamma_k^2} \right] \right]$$

where  $\Omega_k$  is the electronic state excitation energy of the  $k^{\text{th}}$  electronic state,  $\mu_{0k}^m$  and  $\mu_{0k}^n$  are the  $m$  and  $n$  components of the  $0 \rightarrow k$  transition dipole moment, and  $\gamma_k$  is its linewidth. The linewidth  $\gamma_k$  of the  $k^{\text{th}}$  excited electronic state cannot be computed from first principles. Thus, we approximated  $\gamma_k$  in resonance Raman spectra by the linewidth fitted from the experimental absorption spectrum.

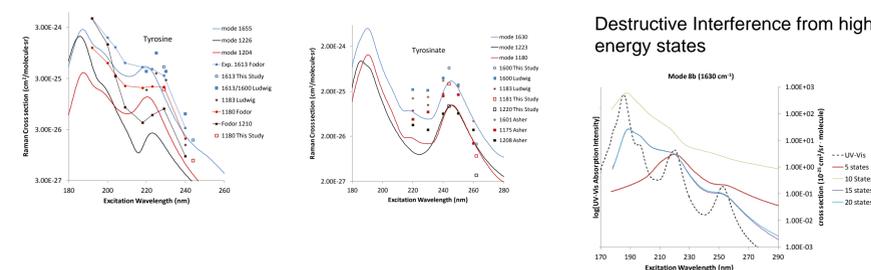
**Experiment:** Non-resonance Raman, RRS, and SERS measurements were performed both for solution and solid phase samples. The solution phase samples for the cross section measurements were prepared by dissolving the material of interest in ultrapure water. To mitigate the photodegradation of the tyrosine solutions, the samples were magnetically stirred in small glass cups during the measurements, so that the laser beam was continuously exposed to fresh sample. The cups were covered with Ultra Violet-transparent quartz cover slips to prevent evaporation of the acetonitrile internal standard.

## Results



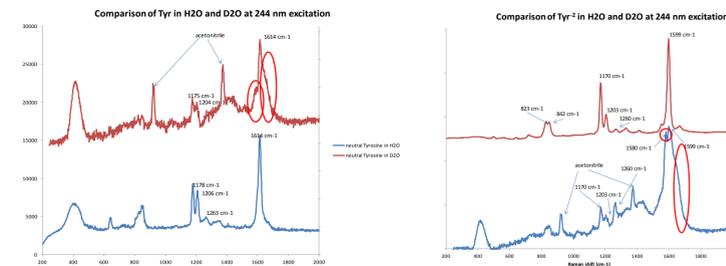
Resonant Raman theoretical spectra and relative peak intensities validated with experimental measurements.

## Experimental validation of theoretical excitation profiles

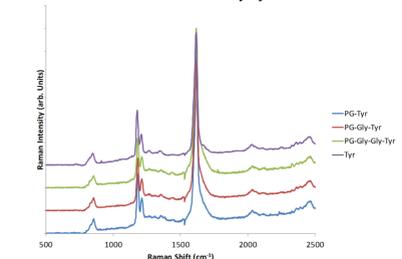


On and off plasmon resonance SERS for different peptide lengths. When on plasmon resonance, all modes enhanced equally by long range electromagnetic mechanism. When off plasmon resonance, tyrosine ring modes enhanced by a chemical mechanism, and ring modes become very sensitive to chain length.

## Measurement of RRS in water and deuterium oxide

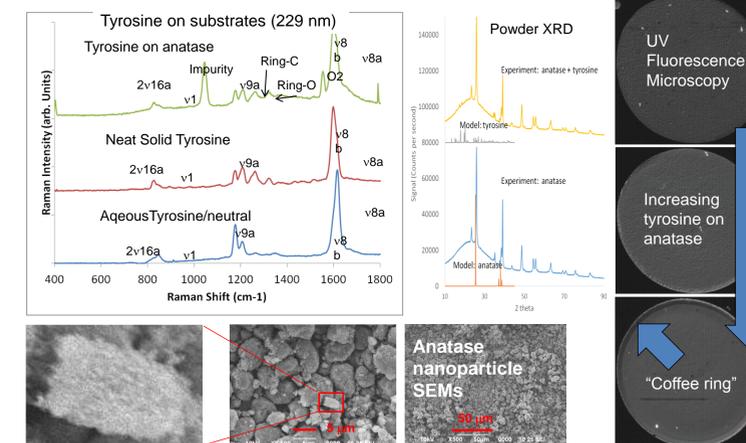


## Measurement of RRS of L-C-propargylglycine in aqueous solution are dominated by tyrosine.



**Acknowledgements:** The authors thank the Edgewood Chemical Biological Center for their assistance in this research and the U.S. Army for funding of this work through the In-House Laboratory Independent Research program. We also acknowledge the Defense Threat Reduction Agency grant HDTRA1-10-1-0046. Dmitriy Rappoport and Alan Aspuru-Guzik acknowledge support through the Cyberdiscovery Initiative Type II (CDI2) grant of the National Science Foundation (NSF), grant number OIA-1125087 and support from the Corning Foundation. The views expressed in this presentation are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government.

## Measurements of RRS of Tyrosine on Titania Nanoparticles



## Conclusions

A study has been performed to further the understanding of the connection between intermolecular induced changes to the electronic structure and the resulting response in the RRS. For the system of tyrosine and tyrosine containing peptides on titania, we encountered four significant technical barriers. The first involved obtaining an understanding of the influence of destructive interference in the sum of states for these systems. The hypothesis used at the beginning of this study had made the assumption that only electronic states in resonance with the optical excitation and that the ground electronic state had significance, and that intermolecular interactions could only significantly affect the RRS through these states. However, our study of destructive interference between states demonstrates intermolecular interactions can affect the RRS through electronic states that are much higher in energy than the excitation. Although there was success in surmounting the first technical barrier, we did not surmount the last three barriers.

For preparation of samples on titania, all methods using aqueous deposition did not produce controlled amounts of peptides evenly distributed on the surfaces. Vacuum techniques are likely necessary to correctly prepare peptides on titania. The third technical barrier not overcome was the correct modeling of the titania surface, where the electronic structure could not be found even for a fixed nuclear geometry. This is likely due to the difficulty in modeling the electronic structure of transition elements such as titanium, and if this is so, simulation with larger basis sets may be necessary. Lastly, PG did not prove to be a suitable "reporter" residue. This is most likely due to the fact that the enhancement coming from the aromatic ring in tyrosine acts over a much shorter distance than anticipated.

In conclusion, although several technical barriers were not overcome, this investigation did shed light on the influence of high energy electronic states through destructive interference. Furthermore, this study revealed the experimental approaches that would be necessary to investigate further the connection between intermolecular interactions, electronic structure, and the RRS spectrum.

