Surface-Enhanced Raman Spectroscopy of Peptides Adsorbed on Silver and Gold Nanoparticles in Aqueous Solutions

Stephanie L. Stovall

Linfield College Chemistry Department

Abstract

Distinguished from standard techniques of bioanalysis, the high specificity of Raman spectroscopy provides information on a molecular level without tagging the biomolecule. Surface-enhanced Raman spectroscopy (SERS) is a surface-sensitive technique that enhances Raman scattering by a factor up to 10^{11}, increasing sensitivity when molecules are adsorbed to silver (Ag) and gold (Au) nanoparticles. To characterize the physical and chemical properties of di- and tri-peptides, SERS spectra of Gly-Tyr, Phe-Gly-Gly and Val-Tyr-Val were obtained by adsorbing the peptides to Ag and Au nanoparticles in aqueous solutions.

Abstract Continued

The predominance of vibrations and dominant peaks originating in individual amino acid residues and side chains indicated that all the peptides adsorbed to the Ag and Au surfaces at the amino terminus.

Introduction

Raman scattering, the inelastic scattering of photons interacting with a substrate, is associated with the excitation or relaxation of molecular vibrational modes unique to different functional groups. As a result, any given functional group will produce a unique Raman spectrum.[1] The intensification of electromagnetic fields causes an increase in inelastic scattering intensity, known as surface-enhanced Raman (SER) scattering, generally produced via noblemetal substrates.[4] Though Raman spectroscopy has a long established application in solving purely chemical problems, it also provides a noninvasive, nondestructive and water-insensitive analytical technique to resolve problems in molecular biology and biochemistry.[2]

Introduction Continued

Raman spectroscopy, therefore, provides a promising avenue in the development of a systematic approach to label-free biomolecule analysis.[2] Other bimolecular studies utilizing this technology have identified vibrational modes of peptide backbones exhibited by characteristic vibrational bands (amide vibrations) that reflect the bimolecular structures and indicate the presence or non-presence of hydrogen bonding.[1,3] This specific study was designed to investigate the application of SERS in the characterization of physical and chemical properties of di- and tri-peptides adsorbed on silver and gold nanoparticles in aqueous solutions.

Materials and Methods

- *Silver Colloid Preparation*
  - To prepare silver colloids, 0.05-0.06 grams $AgNO_3$ were dissolved in 250 mL of deionized water and brought to a boil in a distillation apparatus. Five mL sodium citrate (1%) was added in one mL increments one minute apart. After boiling for 10-15 minutes, the solution was placed in an ice bath and placed in a storage container.

Materials and Methods Continued

- *Surface Enhanced Raman Spectroscopy Measurements*
  - 0.10 mL peptide (0.010M, 0.05M and 1.0mM Gly-Tyr, Phe-Gly-Gly or Val-Tyr-Val, purchased from Sigma-Aldrich and used as received), 0.10 mL silver or gold colloid and 0.10mLNa_2SO_4 or $NaNO_3$ (0.10 M) were combined to measure their respective SERS spectra utilizing a Raman microscope with a laser excitation at 532 nm.

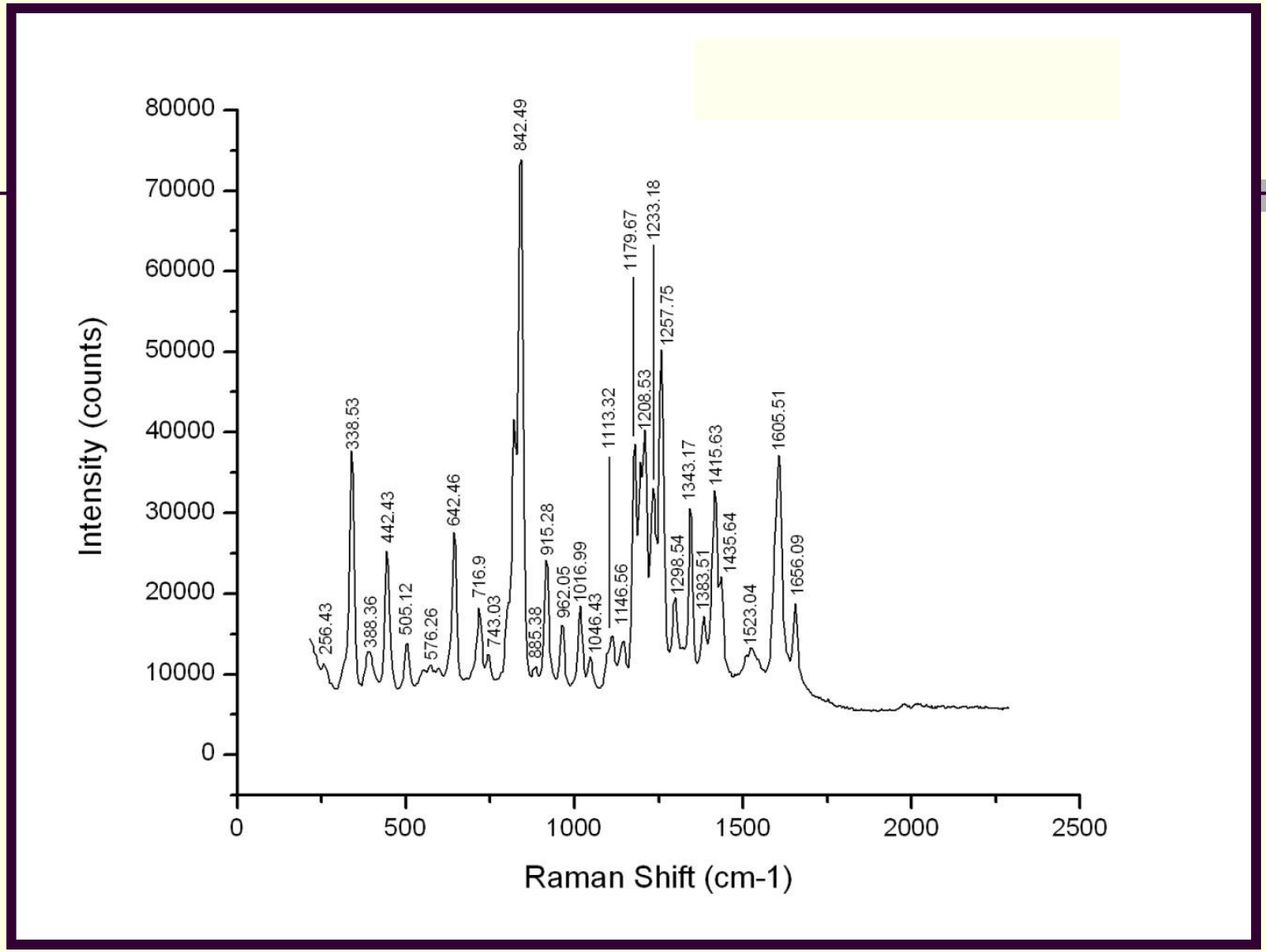

Figure 1: SERS acquisition of solid Gly-Tyr with 10 second exposure at 50μm slits demonstrates peaks primarily corresponding to the Tyr amino acid vibrational modes.

Peak position (cm^{-1})	Proposed band assignment	Proposed residue
1656.09		
1605.51	Sym. ring CC str.	Tyr
1523.04		
1435.64		
1415.63	COO^- sym. str.	Backbone
1383.51		
1343.17	CH_2 wag	Tyr
1298.54-1233.18	Side chain vibrations	Tyr
1179.67		
1146.56	C-N str.	Gly
1113.32		
1046.43		
1016.99		
962.05	C-C str.	Gly
915.28		
885.38		
842.49	Fermi resonance between ring breath and out-of-plane ring bend overtime	Tyr
743.03		
716.90	COO^- def.	Backbone
642.46	Ring def.	Tyr
576.26		
505.12		
442.43		
388.36		
338.53		
256.43		

* Abbreviations: str. = stretch; def. = deformation; sciss. = scissoring; displ. = displacement

Table 1: SERS spectra peak positions and proposed band assignments and residues for solid Gly-Tyr.

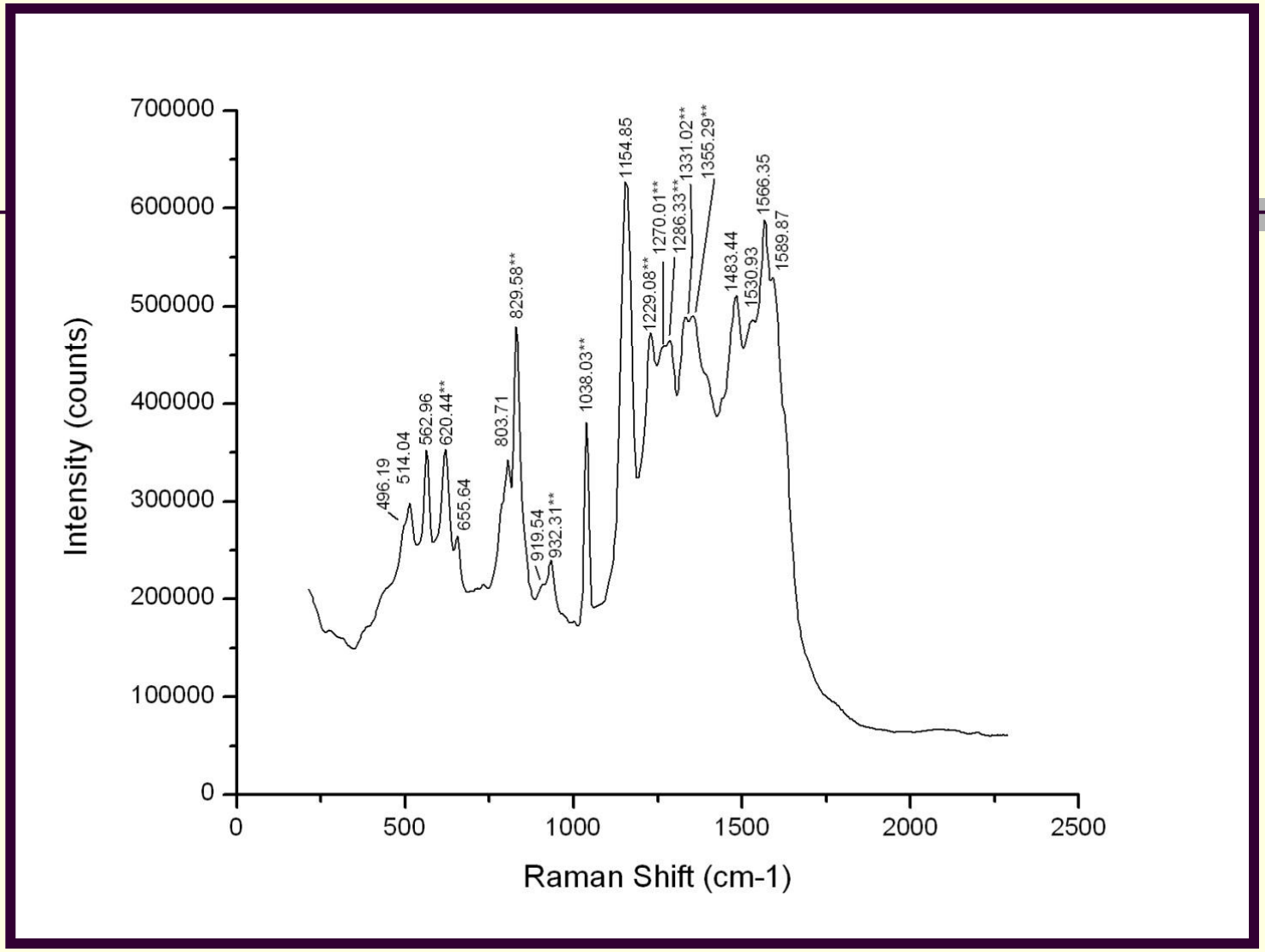

Figure 2: SERS of 0.010M Gly-Tyr adsorbed on Ag nanoparticles with 10 second exposure at 50μm slits demonstrates peaks primarily corresponding to the Gly functional groups.

Peak position (cm^{-1})	Proposed band assignment	Proposed residue
1589.87		
1566.35		
1530.93		
1483.44		
1355.29		
1331.01	CH_2 wag	Tyr
1286.33-1229.08	Side chain vibrations	Tyr
1154.85		
1038.03	C-N str.	Gly
932.31	$C\text{-}COO^-$ str.	Gly
919.51		
829.58		
803.71		
655.64		
620.44	COO^- wag	Backbone
562.96		
514.04		
496.19		

* Abbreviations: str. = stretch; def. = deformation; sciss. = scissoring; displ. = displacement

Table 2: SERS spectra peak positions and proposed band assignments and residues for 0.010M Gly-Tyr adsorbed on Ag nanoparticles.

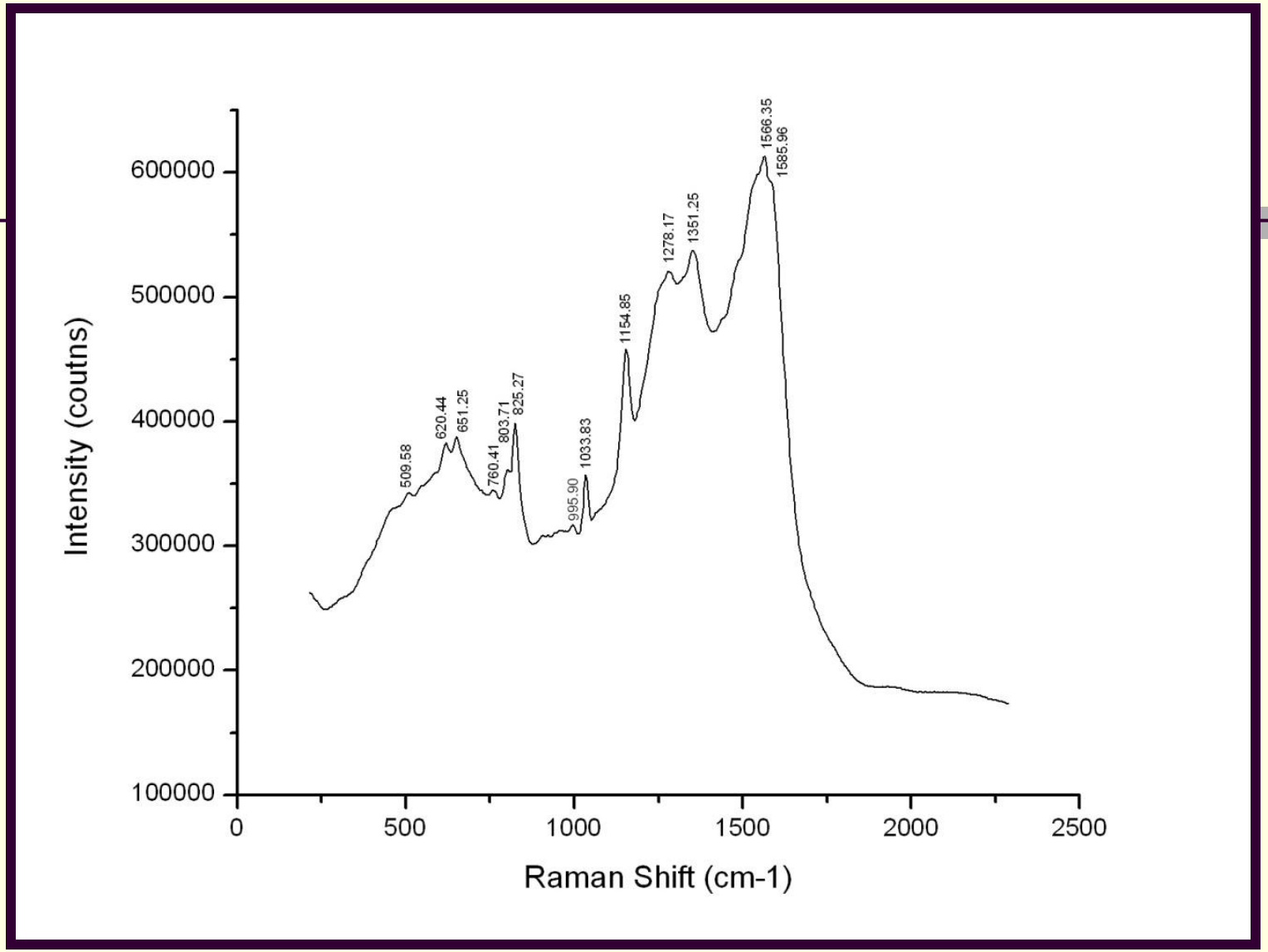

Figure 3: SERS acquisition of 0.010M Phe-Gly-Gly adsorbed on Ag nanoparticles with 10 second exposure at 50µm slits demonstrates peaks primarily corresponding to Phe residues.

Peak position (cm^{-1})	Proposed band assignment	Proposed residue
1585.96		
1566.35		
1351.25		
1278.17	Amide III	
1154.85		
1033.83	In-plane ring CH def.	Phe + gly
995.90	Sym. ring CC str.	Phe
825.27		
803.71		
760.41		
651.25		
620.44		
509.58		

* Abbreviations: str. = stretch; def. = deformation; sciss. = scissoring; displ. = displacement

Table 3: SERS spectra peak positions and proposed band assignments and residues for 0.010M Phe-Gly-Gly adsorbed on Ag nanoparticles.

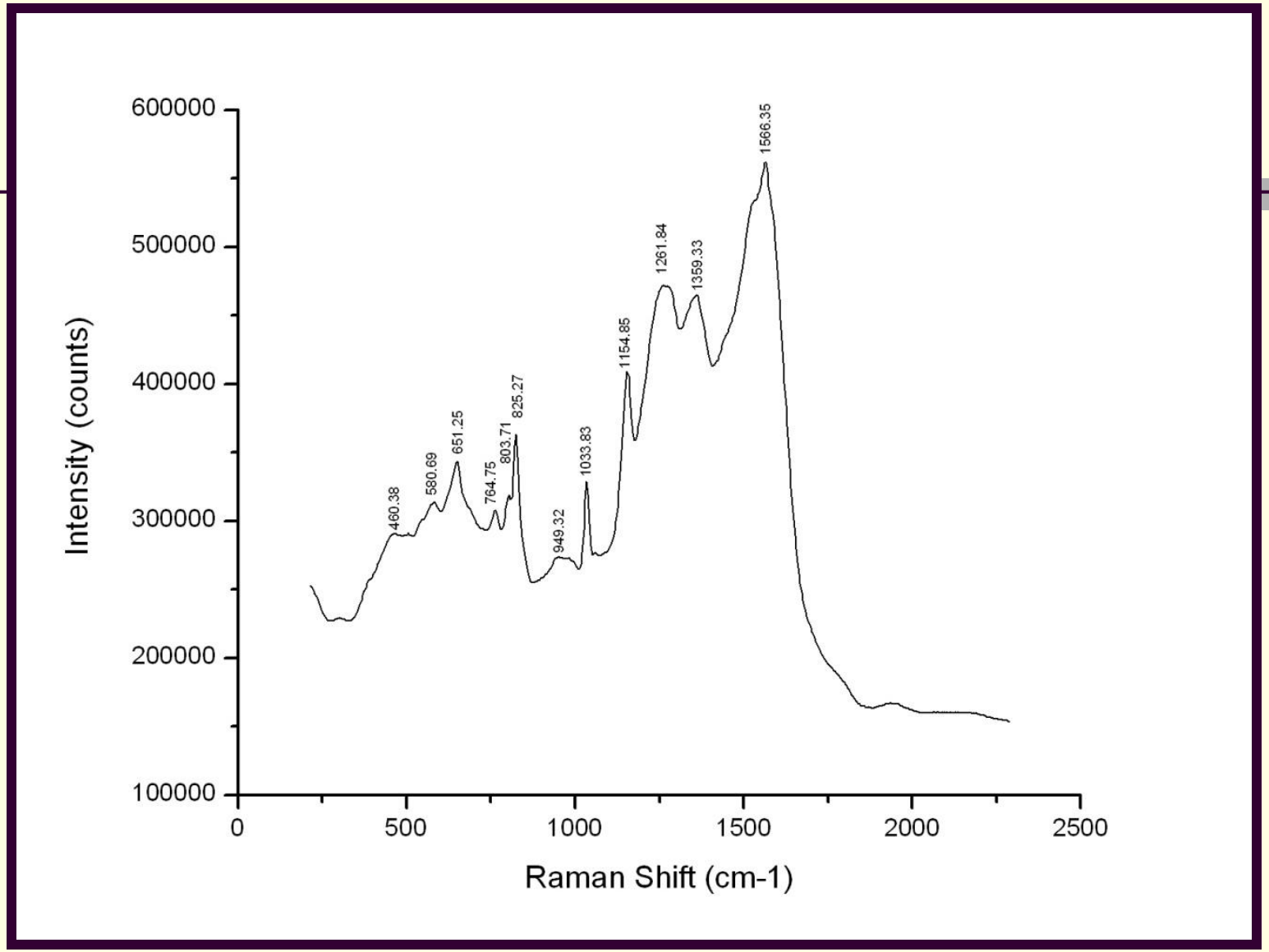

Figure 4: SERS acquisition of 0.010M Val-Tyr-Val with 10 second exposure at 50µm slits.

Peak position (cm^{-1})	Proposed band assignment	Proposed residue
1566.35		
1359.33		
1261.84	Amide III	
1154.85	C-N str.	Gly
1033.83	In-plane ring CH def.	Gly
949.32		
825.27		
803.71		
764.75		
651.25		
580.69		
460.38		

* Abbreviations: str. = stretch; def. = deformation; sciss. = scissoring; displ. = displacement

Table 4: SERS spectra peak positions and proposed band assignments and residues for 0.010M Val-Try-Val adsorbed on Ag nanoparticles.

Conclusion

The observed vibrations in the SERS spectra may be used to identify each peptide through their unique vibrational spectrum. Most vibrational modes detected in the SERS spectra of each peptide (Gly-Tyr, Phe-Gly-Gly and Val-Tyr-Val) were attributed to functional groups relating to the primary peptide in each sequence (Gly, Phe and Val, respectively). It was determined that the peptides adsorbed at the amine terminus because maximum enhancement of vibrational bands occurs in functional groups in direct contact with or located closely to the noble metal substrate.

Conclusion Continued

This adsorption process can be attributed to the attraction of the positively charged amine terminus to the negatively charged (citrate capped) nanoparticles.

Surface-enhanced Raman spectroscopy has the potential to aid in structural investigations of proteins by identifying characteristic bands that are indicative of molecule conformation and hydrogen bonding. Furthermore, the use of Raman biospectroscopy can provide information about intra- and intermolecular interactions with low sample size and minimal preparation.

Conclusion Continued

Thus, further research in Raman biospectroscopy may lead to the discovery of new biomolecules within biological systems and determine their structure and potential interactions with other molecules.

References

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