Part III: Surface-Enhanced Raman Scattering of Amino Acids and Their Homodipeptide Monolayers Deposited onto Colloidal Gold Surface

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Surface-enhanced Raman scattering (SERS) spectra were measured for monolayers of various amino acids: L-methionine (Met), L-cysteine (Cys), L-glycine (Gly), L-leucine (Leu), L-phenylalanine (Phe), and L-proline (Pro) and their homodipeptides (Met-Met, Cys-Cys, Gly-Gly, Leu-Leu, Phe-Phe, and Pro-Pro) deposited onto a colloidal gold surface. Orientation of amino acids and their homodipeptides, as well as specific-competitive interactions of their functional groups with the gold surface, were predicted by detailed spectral analysis of the obtained SERS spectra. The analysis performed allowed us to propose a particular surface geometry for each amino acid and homodipeptide on the gold surface. In addition, we compared the structures of these molecules adsorbed on colloidal gold and silver surfaces.

Index Headings: **Surface-enhanced Raman scattering; SERS; Gold colloid; Raman spectroscopy; Amino acids; Homodipeptides.**

INTRODUCTION

During the last few decades, applications of *in situ* surface-enhanced Raman scattering (SERS) spectroscopy for probing molecular structure at metal–solution interfaces have aroused an increasing interest. Controlled deposition of a variety of biomolecules is important, for example, for biomolecular device architecture,[1] separation of proteins by chromatography,[2] tissue cultures,[3] electron-dense tags for transmission electron microscopy (TEM),[4] and in diagnostic immunoassay.[5,6] In addition, the SERS technique is also used in the development of new enzyme-based biosensors[7] and delivery agents for biomolecules.

Surface-enhanced Raman scattering is based on the enormous enhancement of the electromagnetic field occurring in the vicinity of metallic nanoparticles;[8,9] however, it has also been suggested that it is the chemical enhancement that contributes to the total enhancement effect.[10,11]

Most of the SERS studies have used a variety of silver surfaces because those give the strongest SERS signal;[12,13] however, gold surfaces also produce a significant SERS enhancement.[14–17] Compared with silver surfaces, gold surfaces display several desirable properties including the following: (1) they prevent surface oxidant formation, (2) the oxidant potential of Au is higher than that for Ag, and thus, gold can be used in various redox studies on electrodes, (3) gold is suitable for chemical modification by deposition both on metallic and nonmetallic materials, and (4) biomolecules bound to colloidal gold particles are known to retain their biological activity.

Amino acids and peptides contain different functional groups. Therefore, they are suitable for the investigations of the competitive interactions of these functional groups with metal surfaces. Their properties and electrodynamical behavior depend strongly on the pH of solution (formation of anionic, zwitterionic, or cationic species), as well as on the type and charge of metal surfaces, i.e., Ag has a positive while Au has a negative charge. In addition, enhancement of the SERS signal strongly depends upon the laser line used in the experiment. The silver and gold colloids show different optical properties that one has to consider from the point of the electromagnetic mechanism of the SERS effect. Whereas λ_{max} of the solution of adsorbed species on the silver colloid surface is observed usually in the 500–600 nm region (red shift from about 420 nm for Ag aggregate), the species adsorbed on Au substrate shift from λ_{max} at about 520 nm to the 650–900 nm region after adsorption. Thus, to meet resonance conditions and get the strongest signal from a sample, discrete laser lines in the range of 480–550 nm are used for SERS measurements on the species deposited onto Ag (a 514.5 nm Ar-ion laser line is usually used), while laser lines close to the absorption maximum (see above) observed for species adsorbed on Au are used (usually 647.1 or 676.4 nm from a Kr-ion laser).[18]

Surface-enhanced Raman scattering investigations on amino acids and small peptides deposited on gold surfaces are rather limited;[19–25] however, the adsorption process of these molecules on silver surfaces has been investigated actively, providing very valuable information regarding the structure of the adsorbed molecules.[25–34] Amino acids have been found to interact with metal surfaces in a similar way to that present in aqueous solutions of organometallic complexes.[35–37] It was shown that Gly and Ala deposited onto copper surfaces give infrared spectra comparable to those obtained for the solutions of equivalent metal–amino acid complexes.[35–37] Moreover, the reactions of metal ions in the solutions of amino acids reveal that the most common sites for their coordination to metal are the amino and *C*-terminal groups, as well as the sulfur atom in the case of Cys and Met. In the case of peptide binding to a metal surface, it was additionally shown that the –NH– fragment does not take part in the bonding to the metal surfaces as this would require a tetrahedral configuration that is energetically and geo-

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metrically unfavorable.[36,37] However, if the nitrogen atom is neutral or deprotonated, it can be involved in the interaction with the metal surface, giving rise to a large range of metal–peptide species.[37,38] On the other hand, the oxygen atom of an amide bond does not interact with a metal surface if such a chelate structure is not formed. It has to be emphasized that binding geometry of amino acids and peptides to the metal surfaces depends strongly upon pH of the solution. At pH around 2.5 (gold colloid), all the investigated compounds form cationic species, i.e., $-NH_3^+$ and –COOH groups are present in the structure, while at pH about 8.3 (silver colloid) these groups are deprotonated ($-NH_2$ and $-COO^-$); thus, amino acids and homodipeptides investigated in this work appear in the solution as anionic species.

In the preceding paper, we used SERS spectroscopy to characterize the orientation and stability of monolayers formed from L-cysteine (**Cys**), L-glycine (**Gly**), L-leucine (**Leu**), L-methionine (**Met**), L-phenylalanine (**Phe**), and L-proline (**Pro**) and their homodipeptides on colloidal silver particles.[13] The analysis of the obtained SERS spectra allowed us to propose a particular surface geometry for each amino acid and their homodipeptides on silver surfaces. Additionally, using ''time-dependent'' SERS measurements we solved the existing controversy regarding the binding specificity of Gly-Gly on the silver surface.

In the present study, we have investigated SERS of monolayers of amino acids (**Cys**, **Met**, **Gly**, **Leu**, **Pro**, and **Phe**) and their homodipeptides (**Cys-Cys**, **Met-Met**, **Gly-Gly**, **Leu-Leu**, **Pro-Pro**, and **Phe-Phe**) on the gold surface. The aim of this study is to explore the effects of metal substrate substitution on the molecular orientation and interaction of amino acids and their dipeptides. This is crucially important for a better understanding of the mechanisms of interactions between biological materials with a metal surface, as well as their biological activity in different physicochemical conditions, and thus it will become one of the key issues in the near future.

EXPERIMENTAL

Preparation of Gold Colloid. $HAuCl_4$ and $C_6H_5Na_3O_7$ were purchased from Sigma-Aldrich Co. (Poznań, Poland) and used without further purification. A solution of the colloidal gold was prepared twice according to the standard procedure.[39] First, 5 mg of $HAuCl_4$ dissolved in 50 mL of doubly distilled water was brought to a boil. Then, 0.75 mL of 1% solution of sodium citrate was added. The yellow solution immediately turned dark blue, turning dark red after 2 min of boiling. The colloid prepared by this method (pH ~ 2.5) was aged for 4 weeks. The obtained solution shows characteristic absorption bands with the maximum at 520 nm, which is in agreement with the literature data.[25,40,41]

Samples. **Cys**, **Gly**, **Leu**, **Met**, **Phe**, and **Pro** were purchased from Sigma Co. **Met-Met**, **Cys-Cys**, **Gly-Gly**, **Leu-Leu**, and **Phe-Phe** were purchased from Wako Chemical Co. (Osaka, Japan), while **Pro-Pro** was purchased from Bachem Bioscience Inc. (Geneva, Switzerland). All of the samples were used without further purification. Solutions of 10^{-4} M concentration were prepared by dissolving the respective samples in redistilled water. The final sample concentrations after mixing with the gold colloid were ~10^{-5} M. Additionally, KCl used as an aggregation agent was present in the solutions at a concentration of 10^{-3} M. Adsorption of the investigated samples on the colloidal gold was confirmed by UV-VIS measurements that show a red shift of λ_{max} from 520 to 700 nm that is in agreement with previously published data.[25,40,41]

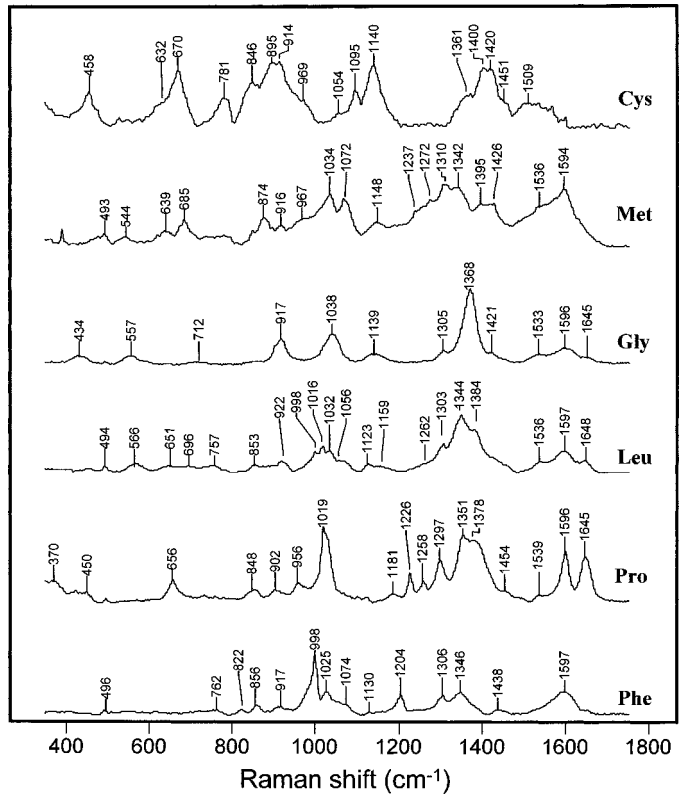

FIG. 1. SERS spectra of **Cys**, **Met**, **Gly**, **Leu**, **Pro**, and **Phe** adsorbed on colloidal gold in aqueous solutions of pH ~2.5. Measurement conditions: sample concentrations in gold colloid, 10^{-5} M; excitation line, 647.1 nm; laser power at the sample, 20 mW.

Instrumentation. Surface-enhanced Raman scattering spectra were obtained with a triple grating spectrometer (Jobin Yvon, T 64000). A liquid-nitrogen-cooled charge-coupled device (CCD) detector (Jobin Yvon, model CCD3000) was used in these measurements. A spectral resolution of 4 cm^{-1} was used. The 647.1 nm line of a Kr-ion laser (Coherent, model Innova 200) was used as the excitation source. Laser power at the sample was set at 20 mW. Special care was taken to monitor whether the laser power has damaged the sample or caused desorption from the gold sol.

The SERS spectra of all amino acids and homodipeptides investigated here were collected twice for each batch of the two gold colloids. The obtained spectra were almost identical, except for small differences (up to ~10%) in band intensities.

RESULTS AND DISSCUSION

Amino Acids. Figure 1 presents SERS spectra, in the range of 350–1750 cm^{-1}, of **Cys**, **Met**, **Gly**, **Leu**, **Pro**, and **Phe** deposited onto colloidal gold surfaces. The observed frequencies, together with the proposed SERS

TABLE I. Proposed band assignments for SERS spectra of Cys, Met, Gly, Leu, Pro, and Phe adsorbed on gold colloid.

Assignment	Frequency [cm^{-1}]					
	Cys	**Met**	**Gly**	**Leu**	**Pro**	**Phe**
$\delta_{as}(NH_3^+)$			1645	1648	1645	
Phe ν_{8a} and/or ν_{as}(COOH)						1597
ν_{as}(COOH)		1594	1596	1597	1596	
$\delta_s(NH_3^+)$	1509	1536	1533	1536	1539	
Phe ν_{19b} and/or $\delta(C_{\alpha 1}H_2)$						1438
$\delta(C_{\alpha 1}H_2)$	1451				1454	
$\delta(C_{\alpha 2}H_2)$	1420	1426	1421			
ν_s(COOH)	1400	1395		1384	1378	
$\nu(C–NH_3^+)$ and/or $\omega(C_{\alpha 1}H_2)$	1361	1342	1368	1344	1351	1346
$\omega(C_{\alpha 2}H_2)$		1310	1305	1303	1297	1306
Pro ring and/or $\delta(C–C_\alpha–H)$						
$\delta(C–C_\alpha–H)$		1272		1262	1258	
Pro ring					1226	
$\delta(C–C_\alpha–H)$		1237				
Phe ν_{7a}						1204
$\omega(NH_3^+)$	1140	1148	1139	1159	1181	1130
				1123		
$\nu_{as}(C_\alpha–C–N)$	1095					
$\tau(NH_3^+)$ and/or $\omega(CH_2)$	1054	1072		1056		1074
$\nu_{as}(C–NH_3^+)$		1034	1038	1032		
Phe ν_{18a}						1025
ν(C–C)	969	967		1016	1019	
				998	956	
Phe ν_{12}						998
ν(C–COOH)	914	916	917	922		
Phe ν_5						917
Pro ring and/or ν(C–COOH)					902	
ν_{as}(C–S–C)	895	874				
ν(C–C), $\tau(CH_2)$, and/or $r(NH_3^+)$	846			853	848	856
						822
δ_b(COOH)	781			757		762
			699	696		
ν(C–S) P_C–G		685				
ν(C–S) P_H–T	670					
ω(COOH)				651	656	
ν(C–S) P_H–G and/or ω(COOH)	632	639				
				566		
		544	557			
τ(CO) + δ(C=O)		493		494		496
skeletal	458		434		450	
					370	

band assignments, are summarized in Table I. The allocation of the SERS bands to the normal vibrations was done referring to the previously reported assignments of the SERS bands of: **Gly** and **Lys** (L-Lysine) and their oligomers;[42] **Phe** and **Pro** residues of DOPA-containing peptides;[20] and IRAS bands of **Cys** deposited on a gold surface.[43] Also, the allocation of RAIR bands of L-**Ala**,[33] L-**Gly**,[34] and tri-L-**Ala** and tri-L-**Leu**[36] adsorbed on a Cu{110} surface, as well as *in situ* scanning tunneling microscopy (STM) characterizations of **Cys** adlayers on Au(III), were very helpful in the analysis of SERS spectra presented in this work.[44,45] We obtained additional information about band assignments from SERS studies on tiophenol,[46] phenylacetylene,[47] aromatic thiols and disulfides,[48] 1,2-ethanedithiol,[39] and 4-cyanobiphenyl[49] deposited on different gold surfaces. It has to be noted that in pH around 2.5 all the investigated compounds form cationic species, i.e., $–NH_3^+$ and –COOH groups are present in the structure, while at pH about 8.3 (silver colloid) both this groups are deprotonated ($–NH_2$ and $–COO^-$); thus, investigated compounds appear in the solution as anionic species.

The orientation of molecules adsorbed on a metal surface can be estimated from the enhancement of the relevant SERS bands with the help of the surface selection rules based on the image dipole theory, as predicted by Creighton et al.[50] and developed further by Moskovits.[51] In particular, the conformation and orientation of **Cys** and **Met** on gold surfaces can be investigated by studying the frequencies and intensities of the spectral features in the 630–720 cm^{-1} range, where bands due to the C–S stretching modes (ν(C–S)) are expected to appear. There are several notations proposed to describe structurally the relationship between internal rotations around the $–CH_2–CH_2–$ and $–CH_2–S–$ bonds of **Cys** and **Met**. In the present study, we have adopted the notation given by Shimanouchi and co-workers[52] and Miyazawa and co-workers.[53] This includes: P_C-T, P_C-G, P_H-T, and P_H-G, where P_C and P_H refer to the two possible conformations of the $–CH_2–CH_2–S–$ group with the carbon and hydrogen atoms at the *trans* position with respect to the sulfur atom, respectively, while T and G stand for the *trans* and *gauche* internal rotation around the $–C(H_2)–S–$ bond, respectively. A ν(C–S) band due to the $–H_2C–S–$ group is expected to appear in the 640–680 cm^{-1} region for the P_H conformer and in the 740–760 cm^{-1} region for the P_C

conformer. On the other hand, the ν(C–S) of the –S–CH_3 group of the **Met** residue appears in the vicinity of 725 cm^{-1}.[53]

In the SERS spectra of **Cys** and **Met** adsorbed on colloidal gold surfaces (the top two traces of Fig. 1), two ν(C–S) bands are observed. In the case of **Cys**, one of the most intense bands of the spectrum at 670 cm^{-1} and a low-frequency shoulder at ~632 cm^{-1} are due to the P_H-T and P_H-G conformers of the –C–S– bond, respectively. For **Met**, medium-intensity bands at 685 and 639 cm^{-1} correspond to the P_C-G and P_H-G rotamers, respectively. The presence of these bands in the SERS spectra suggests that both **Cys** and **Met** adsorb on the colloidal gold surfaces through the sulfur atom; moreover, this occurs in two different conformers. Based on the comparison of intensity between these bands, we may conclude that the thiol group of **Cys** interacts more strongly with the gold surface than the –S–CH_3 group of **Met**, or that the sulfur atom of **Met** occurs near the metal surface. This conclusion is confirmed by the appearance of an intense band at 895 cm^{-1} due to the antisymmetric stretching vibration of the C–S–C linkage (ν_{as}(C–S–C)) in the SERS spectrum of **Cys**, and a corresponding weaker band at 874 cm^{-1} in the **Met** SERS spectrum. A similar conclusion was drawn for the same two amino acids adsorbed on silver surfaces. It was stated that in the SERS spectra, the ν(C–S) vibrations are feebly enhanced for **Met**/Ag and more strongly for **Cys**/Ag.[13]

In addition to ν(C–S), bands due to carboxyl group vibrations (the SERS spectra in Fig. 1 were measured at pH 2.5, where amino acids exist in their cationic forms) are markedly enhanced in the SERS spectra of **Cys** and **Met**, suggesting that this group is also involved in the adsorption process of these two amino acids on colloidal gold surfaces. These modes encompass the symmetric (ν_s(COOH)) and antisymmetric (ν_{as}(COOH)) stretching, bending (δ_b(COOH)), and wagging (ω(COOH)) vibrations. A band due to the ν_s(COOH) mode is observed relatively strongly at 1400 cm^{-1} in the SERS spectrum of **Cys**, while in the **Met** SERS spectrum, the corresponding band appears at 1395 cm^{-1} with medium strength. Bands due to the ν_s(COOH) vibration of **Leu** and **Pro** have similar intensities to that of **Cys**, but they are downshifted to 1384 (**Leu**) and 1378 cm^{-1} (**Pro**), respectively. The observations of the intensities of the ν_s(COOH) bands demonstrate slightly different orientations and/or coordination geometries for the COOH group on the gold surface for **Met** in comparison to those for **Cys**, **Leu**, and **Pro**. Worthy of note is that this band is missing in the **Gly** and **Phe** spectra (see Fig. 1). The absence of this band in these two spectra implies that this group does not assist in the binding of **Gly** and **Phe** to the gold surface. **Gly** and **Phe** assume different configurations on the gold surface from those on the silver surface, since previously we showed that **Gly** and **Phe** interact strongly with the colloidal silver surface through the carboxylate group.[13] The enhancement of ν_s(COOH) of **Cys**, **Met**, **Leu**, and **Pro** adsorbed on the gold silver surface indicates that these amino acids adsorb on both metal surfaces by the *C*-termini group.

In SERS spectra of **Leu** and **Pro**, the ν_s(COOH) band overlaps with the –$C_{\alpha 1}H_2$– wagging and/or the C–NH_3^+ stretching vibrations (1344–1351 cm^{-1}), whereas in the case of **Cys** and **Met**, the corresponding band overlaps with the –$C_{\alpha 2}H_2$– deformation (δ($C_{\alpha 2}H_2$)) (1420–1426 cm^{-1}). The ν_{as}(COOH) band is observed in the range of 1594–1597 cm^{-1} in the SERS spectra of **Met**, **Gly**, **Leu**, and **Pro** (see Table I for detailed frequencies). This band in the **Pro** SERS spectrum is clearly observed as an isolated, relatively intense band, while in the other three spectra, the corresponding band exhibits a weaker intensity and overlaps with the –NH_3^+ group antisymmetric (δ_{as}(NH_3^+)) and symmetric (δ_s(NH_3^+)) deformations observed at ~1645 and 1509–1539 cm^{-1}, respectively. This band is missing in the **Cys** spectrum in Fig. 1 (the top trace). This is expected for a unidentate coordination, in which the antisymmetric orientation of the non-coordinated C=O bond may be favorable for the SERS enhancement. The necessarily symmetric environment for the oxygen atoms of the carboxyl group on the gold surface gives the **Cys** SERS spectrum in which the ν_s(COOH) band is not observed.

The other two vibrations of the –COOH group, i.e., δ_b(COOH) and ω(COOH), appear in the ranges of 757–781 and 632–656 cm^{-1}, respectively. The former band is seen in the SERS spectra of **Cys**, **Leu**, and **Phe** only, while the latter is observed in the SERS spectra of **Cys**, **Met**, **Leu**, and **Pro** (see Fig. 1 and Table I). In addition, this band probably overlaps with the ν(C–S) band in the SERS spectra of **Cys** and **Met**. One more band is associated with the carboxyl group vibrations. In all of the SERS spectra shown in Fig. 1, except that of **Phe**, this band is observed between 902–922 cm^{-1} (relatively weak intensity) and is ascribed to the stretching vibration of the C–C bond adjacent to the carboxyl group (ν(C-COOH)). However, in the **Phe** SERS spectrum, the 917 cm^{-1} band is assigned to the out-of-plane C–H bending vibration of the Phe ring, i.e., the ν_5 mode.

In summary, the appearance of the relatively strong ν_s(COOH) band in the **Cys**, **Leu**, and **Pro** SERS spectra indicates that these amino acids bind on the colloidal gold surface via the *C*-termini group as in the case of the silver surface. However, the weak enhancement of this band in the **Met** SERS spectrum points out that its –COOH group is in close proximity to the gold surface rather than interacting with it. In addition, no band due to ν_s(COOH) in the **Gly** and **Phe** SERS spectra suggests that the carbonyl moiety of these amino acids does not take part in the adsorption on the gold surface. In the case of **Gly**, only bands arising from the ν_{as}(COOH) and ν(C–COOH) modes are enhanced. This result indicates different methods of **Gly** and **Phe** interactions on the gold and silver surfaces.

In connection with the above observations, it is interesting to trace the amine group and the side-chain interactions of all the amino acids investigated here with the colloidal gold surfaces. Generally speaking, the protonated amino and imino groups tend to exhibit different vibrations. Therefore, it is not surprising that in the SERS spectra in Fig. 1, symmetric (δ_s(NH_3^+)) and antisymmetric (δ_{as}(NH_3^+)) deformations, twisting (τ(NH_3^+)) and rocking (r(NH_3^+)) vibrations of the amino group are observed. In the high-frequency region of the **Pro** SERS spectrum, bands due to the symmetric (δ_s(C–NH_2^+–C)) and antisymmetric (δ_{as}(C–NH_2^+–C)) deformations of the protonated imino group are clearly seen at 1539 cm^{-1}

(weak) and 1645 cm^{-1} (intense), respectively. At similar frequencies, bands due to the antisymmetric and symmetric deformations of the $–NH_3^+$ group appear in the **Gly** and **Leu** SERS spectra (see Table I for detailed frequencies). However, they exhibit weak intensity and overlap with other bands expected in this range. The $\omega(C–NH_3^+)$, $\nu_{as}(C_\alpha–C–N)$, $\tau(NH_3^+)$, and $r(NH_3^+)$ modes appear at 1123–1181, 1095, 1054–1074, and 846–856 cm^{-1}, respectively. The first of these bands is observed for all of the amino acids investigated here, but the second one only for **Cys**. The 1054–1074 cm^{-1} band is seen for **Cys**, **Met**, **Leu**, and **Phe**, and may overlap with $\omega(CH_2)$. In addition to these bands, the stretching vibrations of the C–N bond adjacent to the $–NH_3^+$ group are enhanced in the SERS spectra. The $\nu(C–NH_3^+)$ band is observed in the range of 1342–1368 cm^{-1} in the SERS spectra of all the amino acids investigated here, probably overlapping with the band assignable to the $\omega(C_{\alpha1}H_2)$ mode. For **Gly**, **Leu**, and **Pro**, this band is the most intense band in the spectra, whereas for **Cys**, **Met**, and **Phe** it exhibits medium intensity. On the other hand, the $\nu_{as}(C–NH_3^+)$ band is observed at ~1034 cm^{-1} for **Met**, **Gly**, and **Leu** only.

The above observations concerning the amino and imino groups indicate that **Gly** and **Leu** adsorb on the colloidal gold surface also through the protonated amino group, with **Pro** adsorbing through its protonated imino group. In addition, the $–NH_3^+$ group of **Cys**, **Met**, and **Phe** is either in close proximity to the gold surface or interacts with it weakly. These results show that on the gold surface, the *N*-termini groups of the amino acids investigated take place in the adsorption/interaction with the colloidal gold surfaces as in the cases of **Cys**, **Met**, **Gly**, **Leu**, and **Pro** on the colloidal silver surface. However, the strength of these interactions is slightly different between the gold and silver surfaces. Previously we showed that **Cys** and **Met** adsorb on silver surfaces with the *N*-termini group,[13] while in the present paper we notice that this group either interacts with the surface weakly or is in its close proximity. On the other hand, it was stated that the $–NH_2$ group of **Gly**, **Leu**, and **Pro** on the silver surface is in close proximity of the latter but **Phe** does not interact with it.[13] However, in this study we imply that **Gly**, **Leu**, and **Pro** directly interact with the gold surfaces through their *N*-terminals, while **Phe** remains in close proximity to the gold surface.

The additional occurrence of SERS bands corresponding to the $–CH_2–$ deformation and wagging vibrations and the C–C stretching vibrations is also of interest. Spectral features at 1450 cm^{-1} for **Cys**, **Pro**, and **Phe**, at ~1421 cm^{-1} for **Cys**, **Met**, and **Gly**, in the region of 1342–1368 cm^{-1} for all, and in the region of 1297–1310 cm^{-1} for all except **Cys** are ascribed to the $\delta(C_{\alpha1}H_2)$, $\delta(C_{\alpha2}H_2)$, $\omega(C_{\alpha1}H_2)$, and $\omega(C_{\alpha2}H_2)$ modes, respectively.

On the basis of the SERS excitation profiles, it was shown that the Phe aromatic ring vibrations may be enhanced by electromagnetic (EME) and chemical mechanisms (CE).[54]

The selection rule of the EME mechanism allows one to enhance the totally symmetric ring modes, A_1 (containing only the α_{zz} tensor component perpendicular to the surface), when the Phe ring adopts a nearly perpendicular orientation to a metal surface.[46] However, the contribution of the CE mechanism in the enhancement of the Phe ring modes is manifested by the strong enhancement of the ν_{8a} mode.

In the SERS spectrum of Phe, the 1594 cm^{-1} band is broad and exhibits medium intensity. Therefore, it can be concluded that the CE mechanism plays some role in the band enhancement.

In addition, it is accepted that the enhancement of the Phe ring modes is due to the formation of the π-complex of the aromatic ring with the metal surface.[12,13,55–57] If the Phe ring lies horizontally on the surface, mainly the A_1 (~1000 cm^{-1}, ν_2), A_2 (~1256 cm^{-1}, ν_3), and B_1 (~730 cm^{-1}, ν_1) symmetry modes are enhanced, while if it is oriented perpendicularly with respect to the surface, mainly the bands due to the A_1, B_1, and B_2 (~622 cm^{-1}, ν_{6b}) symmetry are enhanced.[27,58]

As mentioned in the previous paper, **Phe** has a number of characteristic aromatic ring bands.[13] These bands appear at 917 (ν_5), 998 (ν_{12}), 1025 (ν_{18a}), 1204 (ν_{7a}), and 1597 cm^{-1} (ν_{8a}) in the SERS spectrum of **Phe** adsorbed on the colloidal gold, i.e., at frequencies lower by a few wavenumbers than those in the corresponding Raman spectrum of **Phe** in an aqueous solution. The corresponding bands of **Phe** adsorbed on a colloidal silver surface occur at nearly the same frequencies, i.e., at 1005, 1031, 1203, and 1603 cm^{-1}. In addition, in this spectrum we observed two other bands at 621 (ν_{6b}) and 1585 cm^{-1} (ν_{8b}). This suggests that although the phenyl ring of **Phe** interacts with the gold or silver surface, the interaction has a minimal effect on the vibrations. Worthy of note is that the band intensity of the ν_{12} mode is dramatically different between the SERS spectra of **Phe** adsorbed on the gold and silver surfaces. In the case of the silver surface, this band is only slightly enhanced, implying that **Phe** adsorbs on the silver particles with the ring perpendicular or slightly tilted towards the surface, while in the case of **Phe** deposited onto the gold surface, the relative intensity of the 998 cm^{-1} band (and some of the other modes) approaches the intensity for the free Phe residue in an aqueous solution. This phenomenon suggests that the surface species closely resemble the parallel orientation to the gold sphere.

Homodipeptides. Figure 2 shows the SERS spectra of the homodipeptides **Cys-Cys**, **Met-Met**, **Gly-Gly**, **Leu-Leu**, **Pro-Pro**, and **Phe-Phe** adsorbed on the colloidal gold at pH 2.5. As discussed above, at this pH the investigated dipeptides form cationic species, i.e. *N*- and *C*-terminal groups are protonated ($–NH_3^+$ and –COOH). In the case of the silver colloid (pH 8.3) these groups are deprotonated ($–NH_2$ and $–COO^-$); thus, investigated compounds appear in the solution as anionic species.

The observed frequencies, together with the proposed SERS band assignments, are summarized in Table II. The allocation of SERS bands to the normal vibrations for these homodipeptides was done on the grounds of previously reported assignments cited for amino acids.[20,33,34,36,39,42–49]

By analogy to the SERS spectra of the amino acids previously investigated, we were able to propose band assignments (see Table II for details) for most bands of their homodipeptides. However, we should expect that the SERS spectra of the homodipeptides show additional bands due to the amide bond vibrations, except the **Cys**

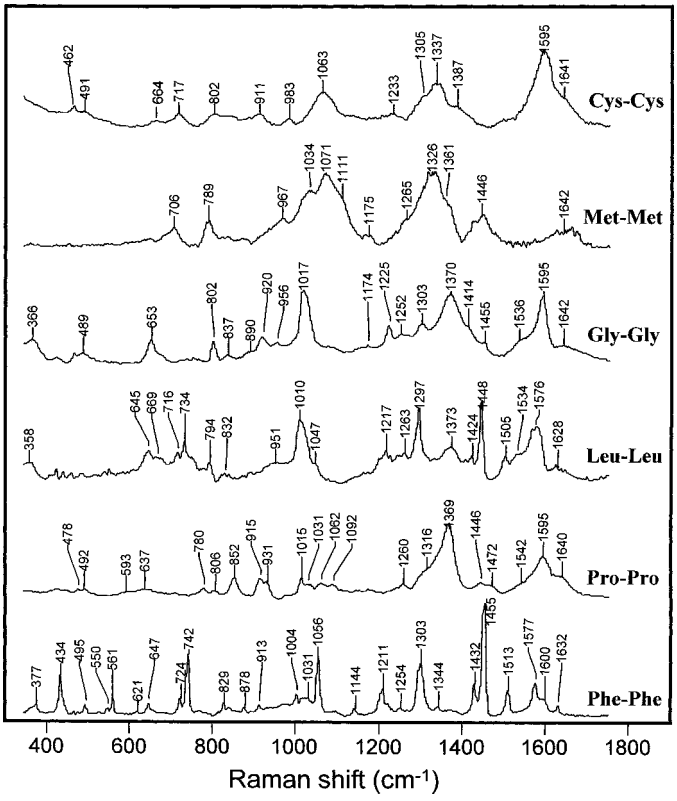

FIG. 2. SERS spectra of **Cys-Cys**, **Met-Met**, **Gly-Gly**, **Leu-Leu**, **Pro-Pro**, and **Phe-Phe** adsorbed on gold colloid in aqueous solutions of pH ~2.5. Measurement conditions as in Fig. 1.

dimer. Cystine (**Cys-Cys**) is the only dipeptide that contains a disulfide bridge in place of the amide bond that gives rise to the ν(S–S) mode at 504 cm^{-1} in the Raman spectrum of **Cys-Cys** in an aqueous solution (pH 9.8). In the SERS spectrum of **Cys-Cys** adsorbed on the colloidal gold surface, there is a weak band at 491 cm^{-1}. This might be due to the stretching vibration of the S–S bond. The appearance of this band may suggest that **Cys-Cys** adsorbs on the gold surface via the electron lone pairs of the disulfide bridge without its cleavage. Moreover, the S–S bond seems to be nearly perpendicular with regard to the gold surface because of the weak intensity of the S–S stretching vibration. The lowering of its frequency in comparison to the SERS spectrum of **Cys-Cys** on the colloidal silver surface, i.e., 518 cm^{-1}, may also be explained by the differences in the bonding with metal surfaces, which may lead to the elongation of the S–S bond on the gold surface in comparison with that on the silver surface. The assignment of the 491 cm^{-1} band is ambiguous, as the C–O bond twisting and the C=O bond deformation vibrations are expected to appear around this frequency. In the SERS spectra of **Gly-Gly**, **Pro-Pro**, and **Phe-Phe**, a weak band in the 489–495 cm^{-1} region is assigned to the ν(C–O)+δ(C=O) mode.

As in the case of the SERS spectrum of **Cys-Cys** adsorbed on silver nanoparticles that show two bands arising from the C–S–S–C fragment (P_H-T and P_H-G rotamers),[13] we expect that bands ascribed to the C–S–S–C linkage appear in the SERS spectrum of **Cys-Cys** deposited onto the gold surface. Two bands observed at 664 and 717 cm^{-1} are assigned to the ν(C–S) modes (Fig. 2, top trace). The P_H-G conformer of the C–S bond yields to the former band, while the P_H-T one yields to the latter band. The presence of these bands supports the statement that **Cys-Cys** interacts weakly with the gold surface via the disulfide bridge.

By analogy with the SERS spectrum of **Met-Met** adsorbed on the silver surface showing two bands at 632 and 673 cm^{-1} of the P_H-G and P_H-T conformers, respectively, the band at 706 cm^{-1} in the SERS spectrum of **Met-Met** adsorbed on gold nanoparticles may be ascribed to the P_H-T rotamer. Thus, it may be concluded that **Met-Met** also interacts with the gold surface through the sulfur atom. As in the case of ν(S–S) the assignments of the 706 or 717 cm^{-1} band may be a subject for discussion. In the range of 706–734 cm^{-1} of the SERS spectra of all homodipeptides investigated here, except that of **Gly-Gly**, vibrations of their other fragments are observed. Thus, this spectral feature may be alternatively assigned to the amide V vibrations, the –COOH group deformation (δ(COOH), and/or the $-CH_2-$ group out-of-plane deformation ($\gamma(CH_2)$), as well as to the $-CH_3$ group rocking vibration ($r(CH_3)$).

In the SERS spectra of **Met-Met** and **Phe-Phe** deposited onto the colloidal gold surface, the enhancement of bands due to the carbonyl group is not observed, as in the cases of these dimers adsorbed on silver particles. Thus, it seems obvious that this group does not interact with the gold surface. Instead, we observe spectral features at 1632–1642, ~1513, 1326–1361, 1144–1175, and 1056–1071 cm^{-1} that may be assigned to the $-NH_3^+$ antisymmetric ($\delta_{as}(NH_3^+)$) and symmetric ($\delta_s(NH_3^+)$) deformations, C–NH_3^+ stretching (ν(C–NH_3^+)), and $-NH_3^+$ wagging + C_α–C–N antisymmetric stretching ($\omega(NH_3^+)$ + $\nu_{as}(C_\alpha$–C–N)) modes, respectively. The fact that the SERS spectra of **Met-Met** and **Phe-Phe** show the bands associated with the $-NH_3^+$ group indicates that their protonated amino groups are involved in their adsorption process on gold particles. However, these bands for **Met-Met** are not as much enhanced as those for **Phe-Phe**, suggesting a weaker interaction of its amino group with the gold surface or its longer distance from the surface. It is worth pointing out that the same method of interaction of the *N*-termini group of these two dimers with the silver surface was found earlier.[13]

As in the case of **Phe** adsorbed on the gold surface, the aromatic rings of **Phe-Phe** give rise to several well-defined SERS bands, i.e., 1600 (ν_{8a}), 1577 (ν_{8b}), 1455 (ν_{19b}), 1211 (ν_{7a}), 1031 (ν_{18a}), 1004 (ν_{12}), 913 (ν_5), 742 (ν_{11}), 647 (ν_4), and 621 (ν_{6b}) cm^{-1}. All of these bands, except the 1455 (A_1) and 742 cm^{-1} (A_1) bands, exhibit low intensity. The lack of a strong enhancement for most of the Phe modes and the strong enhancement of the ν_{19b} mode may suggest that both phenyl rings of **Phe-Phe** are oriented nearly vertically to the gold surface. This orientation of the Phe rings on the gold surface is different from their orientations on the silver surface, on which **Phe-Phe** adopts rather a perpendicular or slightly tilted geometry.[13] Thus, we conclude that the properties and electrodynamical behavior of **Phe-Phe** depend strongly on the pH of the solution, as well as the type and charge of the metal surface. It is worth mentioning that two other medium-intensity bands observed at 1303 and 1056 cm^{-1}

TABLE II. Proposed band assignments for SERS spectra of Cys-Cys, Met-Met, Gly-Gly, Leu-Leu, Pro-Pro, and Phe-Phe adsorbed on gold colloid.

Assignment	Frequency [cm^{-1}]					
	Cys-Cys	**Met-Met**	**Gly-Gly**	**Leu-Leu**	**Pro-Pro**	**Phe-Phe**
$\delta_{as}(NH_3^+)$ and/or amide I	1641	1642	1642	1628	1640	1632
Phe ν_{8a}						1600
ν_{as}(COOH)	1596		1595	1576	1595	
Phe ν_{8a}						1577
$\delta_s(NH_3^+)$ and/or amide II			1536	1534	1542	1513
				1505		
$\delta(C_{\alpha 1}H_2$		1446	1455	1448	1472	
					1446	
Phe ν_{19b} and/or $\delta(C_{\alpha 1}H_2)$						1455
$\delta(C_{\alpha 2}H_2)$			1414	1424		1432
ν_s(COOH)	1387		1370	1373	1369	
$\nu(C–NH_3^+)$ and/or $\omega(C_{\alpha 1}H_2)$	1337	1361				
		1326				1344
$\omega(C_{\alpha 2}H_2)$	1305		1303	1297	1316	1303
Amide III and/or $\delta(C–C_\alpha–H)$		1265	1252	1263	1260	1254
$\delta(C–C_\alpha–H)$	1233		1225	1217		
Phe ν_{7a}						1211
$\omega(NH_3^+)$		1175	1174			1144
$\nu_{as}(C_\alpha–C–N)$		1011			1092	
$\tau(NH_3^+)$ and/or $\omega(CH_2)$	1063	1071		1047	1062	1056
$\nu_{as}(C–NH_3^+)$		1034	1017	1010	1031	
					1015	
Phe ν_{18a}						1031
Phe ν_{12}						1004
ν(C–C)	983	967	956	951		
ν(C–COOH)	911		920		931	
Pro ring and/or ν(C–COOH)					915	
Phe ν_5						913
ν(C–C)			890			878
ν(C–C), $\tau(CH_2)$, and/or $r(NH_3^+)$			837	832	852	829
δ_b(COOH)	802	789	802	794	806	
					780	
Phe ν_{11}						742
δ(COOH), $\gamma(CH_2)$, Am V, and/or $r(CH_3)$				734		724
				716		
ν(C–S) P_H–G	717	706				
ν(C–S) P_H–T	664					
Phe ν_4 and/or δ(CH)						647
ω(COOH)			653	669	637	
				645		
Phe ν_{6b}						621
ω(COOH) and/or amide VI					593	561
						550
ν(C–S) and/or τ(CO) + δ(C=O)	491					
τ(CO) + δ(C=O)			489		492	495
skeletal	462		366	358	478	439
						377

in the **Phe-Phe** SERS spectrum are due to the $\omega(C_{\alpha 2}H_2)$ and $\tau(NH_3^+)$ + $\omega(CH_2)$ modes, respectively.

A careful comparison of the SERS spectra of **Cys-Cys**, **Gly-Gly**, **Leu-Leu**, and **Pro-Pro** shows that similar vibrations of the carboxyl and amine groups are enhanced for these homodipeptides deposited onto the colloidal gold surface. The SERS spectra of both **Gly-Gly** and **Pro-Pro** homodimers show rather intense bands due to the carboxyl group vibrations. A band observed at ~1370 cm^{-1} is due to the ν_s(COOH) mode in these spectra. Bands observed at 1595 cm^{-1} are assigned to ν_{as}(COOH). The intensities of the 1387 and 1373 cm^{-1} bands for **Cys-Cys** and **Leu-Leu**, respectively, are much weaker than those for **Gly-Gly** and **Pro-Pro**. On the other hand, the intensity of the 1596 cm^{-1} band in the **Cys-Cys** SERS spectrum is comparable to that exhibited by **Gly-Gly**, while the intensity of the 1576 cm^{-1} band of **Leu-Leu** is similar to that of **Pro-Pro**. This phenomenon probably demonstrates different orientations and/or coordination geometry for the COOH groups on the gold surface between **Cys-Cys**, **Gly-Gly**, **Leu-Leu**, and **Pro-Pro**. Moreover, the significant enhancement of ν(C–COOH) at 911, 920, and 915 cm^{-1} in the SERS spectra of **Cys-Cys**, **Gly-Gly**, and **Pro-Pro**, respectively, supports the above observations.

Additionally, in the range of 1344–1010 cm^{-1} a few bands are observed that could be due to the $–NH_3^+$ or $C–NH_3^+$ group vibrations. For example, in the **Cys-Cys** SERS spectrum, bands of medium intensity at 1337 and 1063 cm^{-1} are assigned to the $\nu(C–NH_3^+)$ + $\omega(C_{\alpha 1}H_2)$ and $\pi(NH_3^+)$ modes. In the SERS spectra of **Pro-Pro**, the band at 1092 and the bands at 1031 and 1015 cm^{-1} due to the C_α–C–N and $C–NH_2^+$– antisymmetric stretching vibrations, respectively, are weakly enhanced. The splitting of the band of the $\nu_{as}(C–NH_2^+)$ mode probably derives from different orientations of the two proline

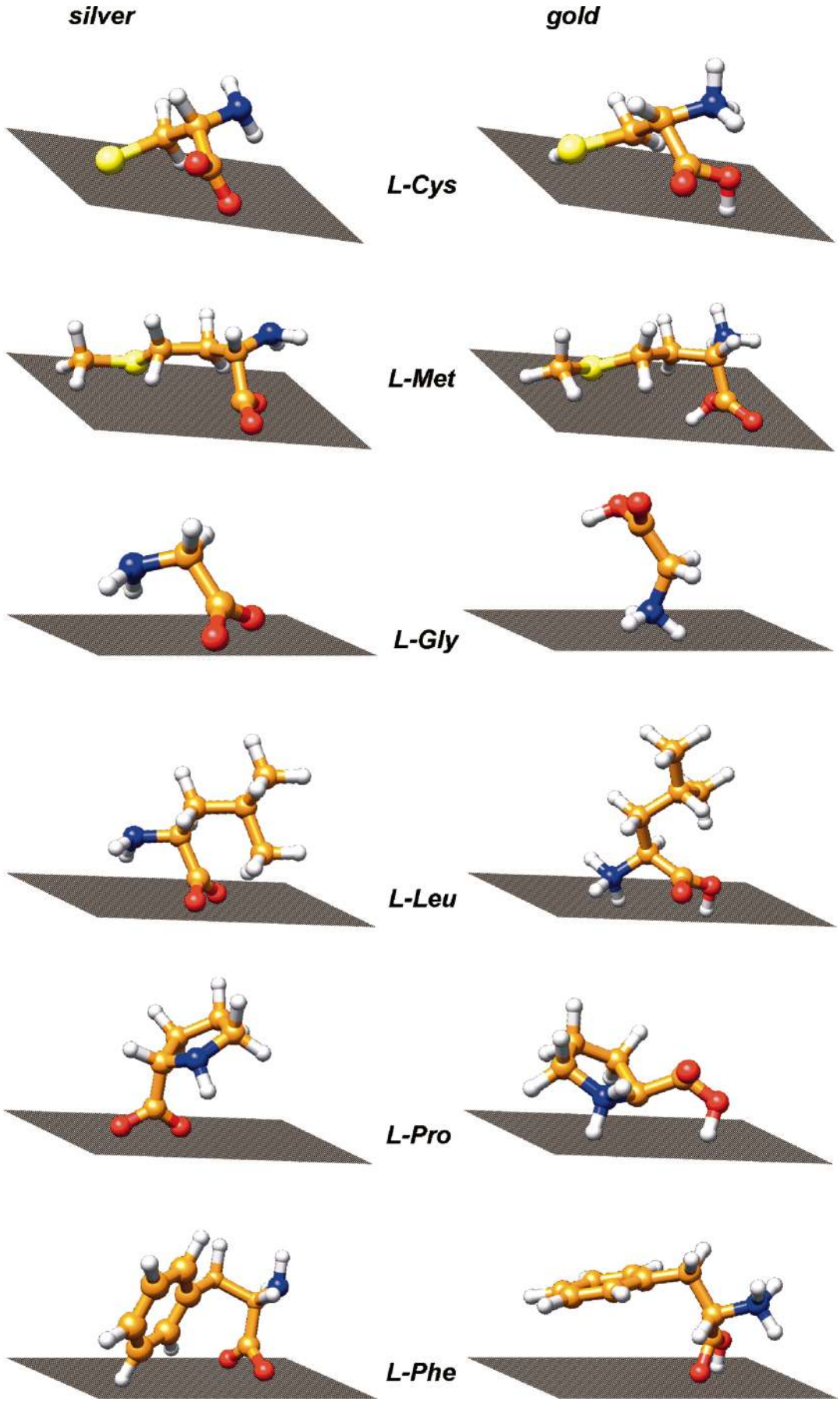

FIG. 3. Possible manner of binding of L-methionine (**Met**), L-cysteine (**Cys**), L-glycine (**Gly**), L-leucine (**Leu**), L-proline (**Pro**), and L-phenylalanine (**Phe**) to colloidal silver and gold surfaces.

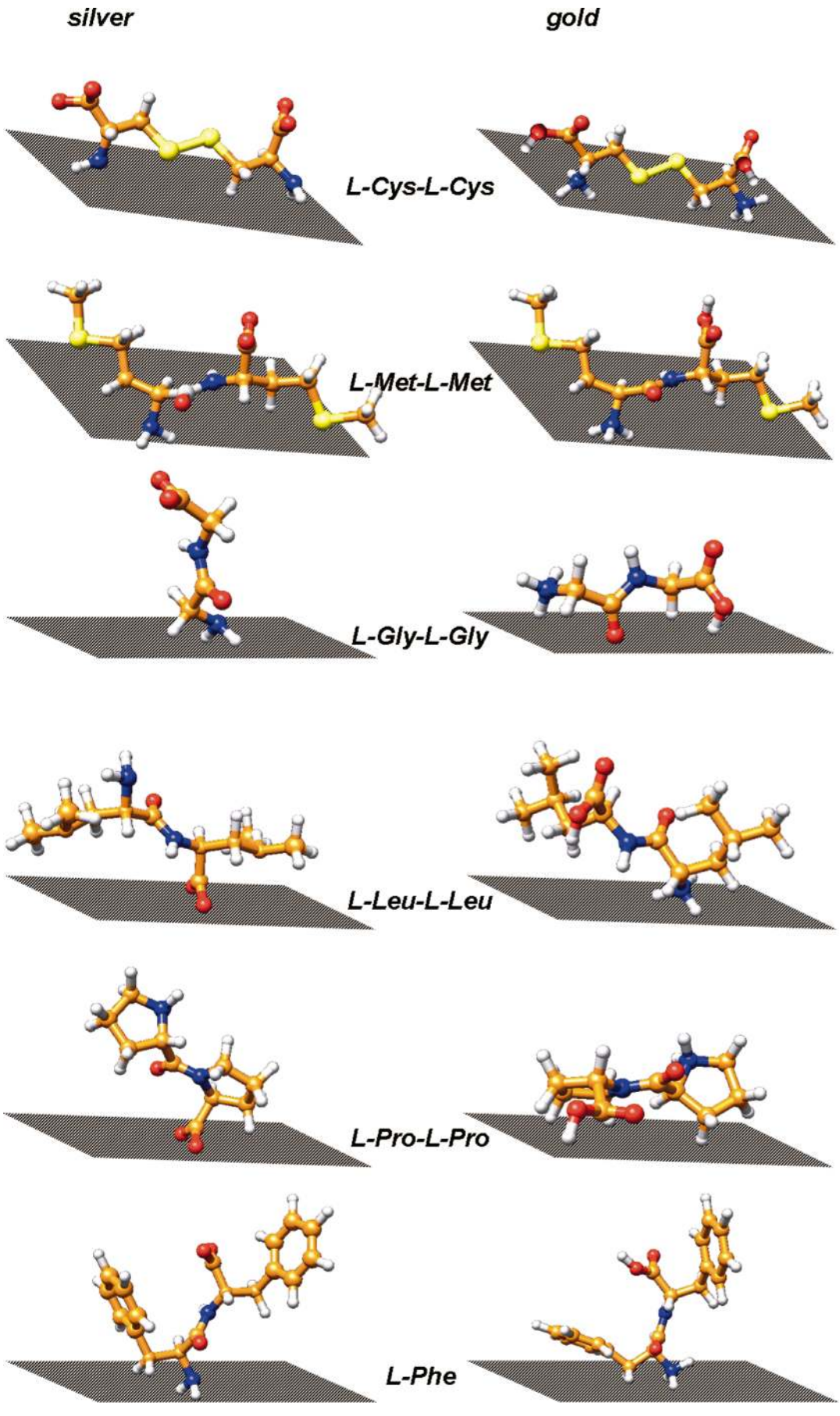

FIG. 4. Possible manner of binding of homodipeptides: **Met-Met**, **Cys-Cys**, **Gly-Gly**, **Leu-Leu**, **Pro-Pro**, and **Phe-Phe** to colloidal silver and gold surfaces.

rings in the proximity of the gold surface. On the other hand, in the SERS spectra of **Gly-Gly** and **Leu-Leu** $\nu_{as}(C–NH_2^+)$ is relatively strongly enhanced, suggesting that these two homodipeptides adsorb on the gold surface mainly via the protonated amino group.

It is worth pointing out here that the adsorption process of **Gly-Gly** on the silver surface was the most controversial point raised in the literature. The most important change in the SERS spectra of this homodimer is the fact that **Gly-Gly** molecules are adsorbed on the silver first through the *C*-termini group. Then, with time, the carboxylate group leaves the silver surface, probably due to rearrangements of the silver colloid, molecular rearrangements, and conformational changes, and **Gly-Gly** becomes attached by its *N*-termini group.[13] In the case of **Gly-Gly** on the gold surface, these changes do not take place, and the pattern of the SERS spectrum does not change with time. On the basis of the above observations, we suggest that **Gly-Gly** is attached to the gold particles through its carbonyl and amine groups, while **Pro-Pro** is attached mainly through the carbonyl group; however, the amine group is in close proximity to the gold surface. In addition, **Cys-Cys** interacts with the surface via both $–NH_3^+$ and COOH groups as well as via the disulfide bridge, with **Leu-Leu** interacting mainly through the amino group, although its carbonyl group is in rather close proximity to the gold surface. A similar result to that for **Pro-Pro** on the gold surface was obtained for **Pro-Pro** adsorbed on the colloidal silver, whereas for **Cys-Cys** and **Leu-Leu** the metal substrate conversions change the manner of their interaction, i.e., **Cys-Cys** deposited onto silver binds to the metal surface by the disulfide bridge and the amine group, while **Leu-Leu** binds mainly by the *C*-termini group.

The amide vibrations of **Phe-Phe** deposited on the colloidal gold surface are difficult to assign because the spectrum is dominated by the ring mode vibrations. A weak band at 1632 cm^{-1} in the **Phe-Phe** SERS spectrum is due either to amide I or to $\delta_{as}(NH_3^+)$. Similarly, a band observed at 1513 cm^{-1} may be alternatively assigned to $\delta_s(NH_3^+)$ or to amide II. A very weak band at 1254 cm^{-1} probably contains contributions from the amide III vibrations.

The SERS spectra of the **Met-Met**, **Gly-Gly**, **Leu-Leu**, and **Pro-Pro** dimers yield a weak spectral feature due to the amide III vibrations around 1254–1265 cm^{-1}, which is detected as a single band, except the **Met-Met** SERS spectrum where amide III overlaps with other bands present in this region. The amide III band arises mainly from a combination of the in-phase and out-of-phase NH bending and CN stretching.[57] A broad band at around 1640 cm^{-1} is assigned to the overlapping vibrations, amide I and $\delta_{as}(NH_3^+)$. Amide I mainly involves the C=O stretching, C_α–C–N deformation, and CN stretching oscillations.[57] In addition, two other amide bands, i.e., amide V and VI, may be traced in the considered SERS spectra. The amide V mode is identified as a band around 706–734 cm^{-1}, while the amide VI mode is identified as a band around 550–593 cm^{-1}.

CONCLUSION

Surface-enhanced Raman scattering spectra of the cationic species (pH 2.5) of **Cys**, **Met**, **Gly**, **Leu**, **Pro**, and **Phe** and their homodipeptides deposited onto the gold surface were presented in this work. Their adsorption patterns were investigated from the intensities and frequencies of the enhanced bands. The role of pH of the solution (gold versus silver colloid) on the orientation of the investigated amino acids and their homodipeptides at the gold surface using SERS spectroscopy was discussed in the paper.

We showed that **Cys** and **Met** adsorbed on the gold surface through the lone electron pair of the sulfur atom. In addition, the pattern of the SERS spectra provided strong evidence that the carbonyl group (–COOH) of **Cys** strongly interacts with the gold surface, while its protonated NH_3^+ group is in its close proximity, and both termini groups of **Met** are in proximity to the gold surface. This observation is similar to that previously reported for **Cys** and **Met** adsorbed on the silver surface.[13] Thus, we concluded that the silver/gold substitution did not change substantially the manner of the interactions of these molecules with the metal surface. As was evident (*vide supra*), **Phe** interacted with gold particles with the ring slightly tilted towards the surface and with the $–NH_3^+$ group lying near this surface. On the other hand, as we showed previously, **Phe** adsorbed on the colloidal silver through the carboxylate ($–COO^-$) group with the phenyl ring perpendicular or slightly tilted to the surface, only. In contrast, **Gly**, **Leu**, and **Pro** were bound to the gold surface primarily through the protonated amino ($–NH_3^+$) moiety; however, the SERS spectra of **Leu** and **Pro** showed that their *C*-terminal groups were also involved in the binding process.

Changes in the SERS spectra of the **Gly-Gly** homodimer adsorbed on the gold surface showed that adsorption appeared via both termini groups ($–NH_3^+$ and COOH) similarly to the results obtained for the **Cys-Cys** homodimer. However, additionally, in the latter case the S–S bond was also involved in **Cys-Cys** adsorption on the gold particles. In the case of **Met-Met**, the *N*-termini group was mainly involved in adsorption on the surface; however, the sulfur atom also took part in this process. On the other hand, **Phe-Phe** adsorbed on the gold surface through the $–NH_3^+$ group and the phenyl ring(s) lying perpendicular to the surface. A different behavior was observed for **Leu-Leu** and **Pro-Pro**, where the main interaction between these homodimers with colloidal gold took place mainly through the amino and carbonyl moieties, respectively, with the *C*-terminal group being in close proximity to the surface.

A proposed manner of binding to the colloidal gold surface of amino acids (**Cys**, **Gly**, **Leu**, **Met**, **Pro**, and **Phe**) and their homodipeptides investigated here is given in Figs. 3 and 4 (right), respectively, and compared with the manner of binding to the colloidal silver surface (left).

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