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Protein-Metal Interactions Probed by SERS: Lysozyme on Nanostructured Gold Surface

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Abstract
Surface-enhanced Raman scattering is a well-established technique for molecular detection at low concentration, which is becoming increasingly popular in the field of biotechnology and health sciences. Since the process is understood in depth, the technique is becoming reliable. In this contribution, we consider another aspect of SERS besides molecular detection, focusing on the binding mechanisms of a complex system such as a protein to the noble metal substrates required by the technique itself. We also show that using a solid nanostructured substrate produced by controlled pulsed laser deposition SERS enables label-free detection of a protein. This is checked on lysozyme as a well-known prototype. Use of solid substrates with controlled morphology proves advantageous over colloidal systems for SERS applications. Moreover, such substrates are superior in terms of shelf life, packaging and ease of shipment.

Keywords Surface-enhanced Raman scattering · Label-free detection · Laser ablation · Nanostructured substrates · Lysozyme · Protein binding

Introduction

Since several years, the research on SERS activity of various nanoparticle systems shifted from simple test molecules such as Rhodamine dye to complex molecular systems like proteins or drugs that should be identified and detected in complex fluids like human blood or plasma [1]. While small test molecules bind well and show reproducible SERS spectra, complex molecular systems such as proteins are often more difficult to deal with through SERS. State-of-the-art detection of proteins in clinical applications employs fluorescent markers, such as in ELISA test [2, 3] for HIV recognition [4]. Such a process is time-consuming as well as expensive. Time becomes even more a vital factor in medical emergency, where first aid is the provision of initial care and it is important to give the appropriate treatment following the right diagnosis. In such diagnostic procedures, a first quick screening to confirm selected disorders is a must. While Raman spectroscopy is not yet a very popular technique in medical diagnostics, it has the potential to positively contribute to the molecular-sensitive diagnostic techniques currently adopted in the biological and medical fields [5–8]. Indeed, promising results were obtained in therapeutic drug monitoring applications of SERS [9]. In this work, we show the molecular sensitivity of SERS through its dependence on the protein-metal interactions at the nanostructured substrate surface used to drive the SERS action. This label-free application of SERS may become a rapid, cheap and easy screening procedure aimed at probing selected biomolecules through their specific interaction sites.

The Au substrates used in this work consist of mutually assembled Au nanoparticles synthesized in a controlled way on suitable inert rigid supports by pulsed laser deposition (PLD), and such substrates demonstrated to be efficient SERS sensors for both the mechanisms responsible for

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enhancement effects (i.e. charge transfer and covalent bonding) [10, 11]. These substrates are sensitive towards dye molecules like Rh6G [12], drugs such as apomorphine [13, 14] and carbamazepine [15] and volatile compounds such as 2-naphthalenethiol [16]. As a further development, the potential for applications of these substrates should also be tested towards complex biological molecules such as proteins.

In the field of proteomics [17, 18] and metallomics [19, 20], protein structures, functions and their interaction with metals in their free or ionic form are the focus of study. Protein purification [21] is an essential step in this research. However, the low yield of the synthesis and purification stage leads in most cases to ultra-low amounts of proteins. Hence, it is quite costly and laborious to manufacture sizeable quantities for analysis and studies with conventional methods and instrumentation such as X-ray diffraction, NMR spectroscopy, RNA analysis and other spectroscopy techniques [22]. Thus, a sensitive technique such as SERS, which is able to detect and investigate low quantities of proteins, is welcome in this field of applications.

Point-of-care (POC) diagnostics is an emerging field which relates to sensing low amounts of biological material such as proteins in blood plasma in a quick and reliable fashion, by operating with microfluidic channels [23]. POC would be an effective diagnostics at a doctor's office within few minutes. One of the technological mechanisms of microfluidics for POC is by metal-protein interaction [24]. For this, it is necessary to understand the binding process of proteins on metallic nanostructures on flat supports. Hence, nanostructured metal surfaces synthesized on flat rigid supports, such as obtainable by PLD, provide a reliable tool for this study through the SERS effect.

We point at investigating in detail lysozyme as a prototypical example because it is a well characterized protein for which useful information is available in the literature, both on the SERS response and on the interaction with noble metal nanostructures [25, 26] as probed by X-ray diffraction [27]. Lysozyme is an enzyme protein often found in secretions. Its role in the innate immune system is to break the cell walls of certain pathogens [28]. Deficiency of lysozyme can lead to increased incidence of diseases, such as bronchopulmonary dysplasia, conjunctivitis and loss of protection against pathogens like *Streptococcus* [29]. In certain kinds of cancer like leukemia [30], excessive production of lysozyme takes place, which is toxic to body and may lead to kidney failure and other organ disorders [31]. To the best of our knowledge, the first study on SERS of lysozyme by Hu et al. dates to 1995 [32]. Hu et al. dealt with Ag NPs in colloidal state produced by silver borohydride used as a reducing agent along with lysozyme solution to obtain SERS spectrum of lysozyme. Although Hu et al. could probe by SERS the binding of lysozyme with Ag NPs in colloidal state, no indications were proposed about the binding of specific amino acid residues to the NPs. In a more recent study, Chandra et al. [33] use the same synthesis procedure of Ag NPs to characterize lysozyme and they find indication of interaction of silver with the Trp123 residue. A more detailed study by Wei et al. investigates the binding site of lysozyme to Au NP with the help of X-ray diffraction [27]. Wei et al. propose the binding of Au NP to the His15 residue of lysozyme.

Here, we concentrate on label-free SERS detection of lysozyme. The SERS spectrum was obtained by working in acidic media since lysozyme is known to show affinity towards metal NPs at a pH of about 4 [34]. We also propose a binding site for the protein with the Au NP on a locally flat substrate. To the best of our knowledge, this is the first time that moving from the interpretation of the SERS spectrum of a protein a proposal is offered for the binding site of the protein on the metal surface. In perspective, this approach may foster the application of SERS to the label-free characterization of proteins. In fact, similarly to the case of lysozyme, the features of the SERS spectrum of proteins are expected to be the fingerprint of specific molecular dependent interactions with the selected metal nanostructures.

It is also worth mentioning that the SERS substrates considered in this work are thin films deposited on solid supports. This is different from other popular SERS systems such as colloids which have the mobility to probe the molecule from all directions [35] and in some cases can even unfold the protein and probe out its insides. Thus, the protein-metal interaction using colloidal systems (Ag or Au) is not easily controllable which leads to poor molecular-structure sensitivity and scarcely reproducible SERS signal. Different proteins may denature and show similar SERS profiles or the same protein may show different SERS spectra with substantial variations. In this respect, the PLD substrates are constraint systems making them a more controlled SERS system able to probe in a more reproducible way the protein-metal interactions, so that even minor differences in SERS spectra can be taken as a possible fingerprint of the protein structure.

Experimental Methods

Preparation of PLD Au Substrates

Gold nanostructured substrates were produced using pulsed laser deposition (PLD) technique using for the deposition a KrF excimer laser ($\lambda = 248$ nm, pulse width 25 ns, repetition rate 10 Hz). The laser was focused on the target surface by a quartz lens. Pure gold targets were placed on a rotating holder in order to avoid surface cratering. The support slides (made of 7059 Corning glass) were placed at a distance of 35 mm from the target. The PLD chamber was kept at a constant pressure of 70 Pa of Argon. The deposition of Au nanoparticles was fixed at 10000 laser shots and 2 J cm^{-2} of laser fluence [36, 37].

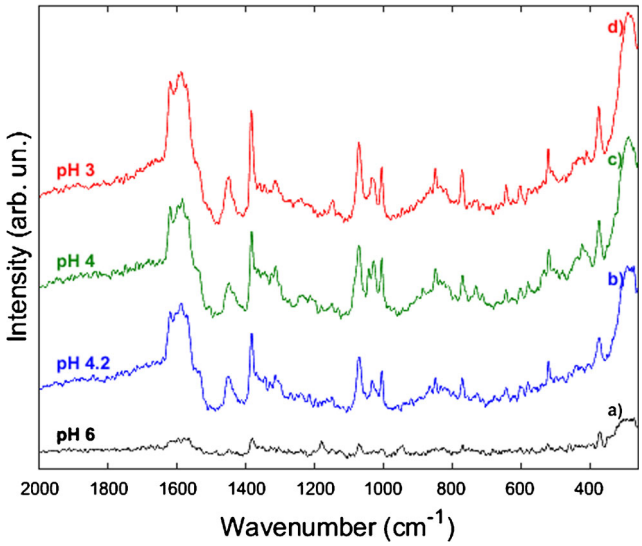

Fig. 1 SERS spectra of 7×10^{-4} M lysozyme on PLD Au substrates with different pH at (a) 6 (black) (b) 4.2 (blue), (c) 4 (green) and (d) 3 (red) (colour figure online)

Preparation of Ag Colloids

Silver colloids were prepared using chemicals of analytical reagent grade. Distilled water was used for the reaction. Silver colloids were prepared by adding dropwise 5×10^{-3} M silver nitrate (100 ml) into ice-cold 6×10^{-3} M sodium borohydride (300 ml) solution under vigorous stirring. The resulting solution was kept at 40–50 °C for about 20 min, and then, the colloidal solution was diluted to a volume of 500 ml with twice distilled water. Solutions prepared by this technique were aged for 2 or 3 weeks before use.

Preparation of Lysozyme Solution

Lysozyme from chicken egg white was purchased in powder form from Sigma-Aldrich (molecular weight of ~ 14.3 kDa). Ten milligrams of lysozyme was dissolved in 1 ml of distilled water to obtain the concentration of 7×10^{-4} M. A water solution of nitric acid prepared at pH 2 was used to modify the pH of the lysozyme solution by careful dropwise mixing.

Raman and SERS Measurements

The Raman/SERS spectra were acquired through a Labram Jobin Yvon HR800 instrument in backscattering mode. The 785 nm excitation line was focused on the sample through a × 50 objective and the power at the sample was 0.8 mW. The Raman spectrum of solid lysozyme was collected over 5 min. SERS measurements with Au substrate were performed by immersing it in the lysozyme solution and spectra were accumulated over 600 s. After every spectrum, the pH was changed from 6 down to 3 to observe the effect of pH on the peak intensities of the SERS spectrum, which reflects the binding strength of the protein with the Au substrate. SERS spectrum with Ag colloidal solution was obtained by mixing in an NMR tube the Lysozyme solution (0.1 ml) with the Ag colloid (0.5 ml) after which SERS spectra were collected. The integration time of the measurements on colloids was 600 s

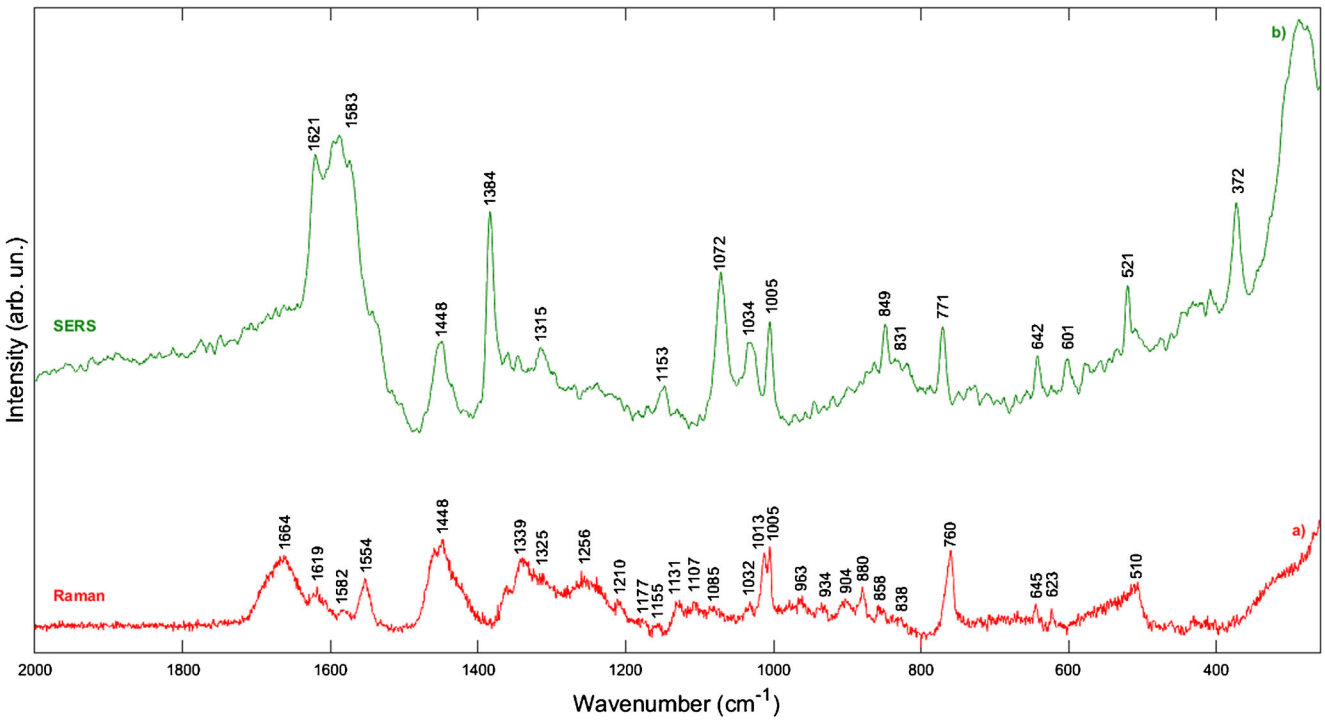

Fig. 2 Raman (a, red) and SERS spectra (b, green) of lysozyme from PLD Au substrates with major peak assignments (colour figure online)

Table 1 Assignment of experimental Raman and SERS (from PLD Au substrate) signals of lysozyme with brief description of observed differences

Raman peaks (cm^{-1})	Raman assignment	SERS peaks (cm^{-1})	SERS assignment	Description (observed differences)	
				Raman	SERS
		372	Proline • Au (s)	Absent	
510	SS bridge stretching	521	SS bridge	Broader	
623	Phenylalanine	601	Phenylalanine		Shifted
645	Tyrosine	642	Tyrosine		Shifted
760	Tryptophan (s)				Absent
		771	Arginine • Au (s)	Absent	
838	Tyrosine doublet	831	Tyrosine doublet		Shifted
858		849			
880	Tryptophan				Absent
904					Absent
934	CC stretching				Absent
963	Tyrosine				Absent
1005	Phenylalanine (ring breathing)	1005	Phenylalanine (ring breathing)		Weaker
1013	Tryptophan				Absent
1032	Phenylalanine	1034	Phenylalanine		Slight shift
		1072	Arginine • Au (s)	Absent	
1085	Tyrosine				Absent
1107	Tryptophan				Absent
1131	Tryptophan				Absent
1155	Phenylalanine	1153	Phenylalanine		Slight shift
1177	Tyrosine				Absent
1210	Tyrosine				Absent
1256	Amide III				Absent
1325	Phenylalanine + histidine	1315	Histidine + phenylalanine		Shifted
1339	Tryptophan				Absent
		1384	COO^- symmetric stretch (s)	Absent	
1448	Amide II + histidine (CH2 scissoring) (w)	1448	Amide II + histidine (CH2 scissoring) (s)		Stronger
1554	Tryptophan				Absent
1582	Histidine (C=C stretching)	1583	Histidine (C=C stretching)		Strong and broad
1619	Tryptophan + phenylalanine	1621	Tryptophan + phenylalanine		Slight shift
1664	Amide I				Absent

with 0.8 mW power. The spectra shown are subtracted from the background signal which slowly increases with decreasing wavenumber.

Results and Discussion

SERS Spectra on Au PLD Substrates of Lysozyme

The SERS spectrum of lysozyme at 7×10^{-4} M (10 mg ml^{-1}) was acquired as a function of the pH of the lysozyme solution, from slightly alkaline to acidic, i.e. from pH 6 to pH 3 (Fig. 1). At pH 6, the SERS spectrum of lysozyme is very weak. As the pH becomes acidic, the spectrum becomes more prominent and detailed. This indicates that lysozyme can interact with the Au substrate at acidic pH, which was observed in previous studies [38].

Comparison of Raman and SERS Spectra on Au PLD Substrates of Lysozyme

The Raman spectrum of lysozyme as solid powder was obtained with the 785 nm excitation line (Fig. 2). The Raman peaks were assigned based on literature studies [39–41] on the Raman activity of solid proteins based on their constituent amino acids. The main Raman features arise from the aromatic residues, i.e. tryptophan, tyrosine, phenylalanine and histidine. The same scenario applies to describe the Raman

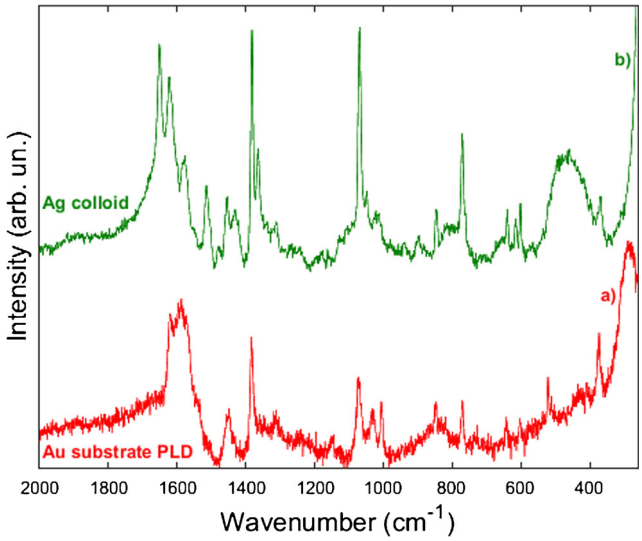

Fig. 3 Comparison of SERS spectra of lysozyme obtained from PLD Au substrate (a, red) and from Ag colloid (b, green) (colour figure online)

response of lysozyme as well. Other important features of the Raman spectrum come from the vibrations of the peptide bonds, which is divided into three regions specific to certain amide vibrations known as amide I, amide II and amide III. In particular, amide I is the in-plane vibration of the peptide bond with a major contribution from the C=O stretching (observed here at 1664 cm^{-1}) while amide II is the stretching of the CN group and in-plane bending of the NH group (observed here at 1448 cm^{-1}).

Comparing the Raman spectrum of lysozyme to its SERS counterpart, we observe several differences. New peaks emerge in the SERS spectrum compared to Raman as shown in Fig. 2. Indeed, moving from high to low wavenumbers in SERS, there is a very strong peak at 1384 cm^{-1}, assigned to COO^- symmetric stretching, which is absent in Raman. We assign some of the peaks in the region from 1300 to 1700 cm^{-1} to originate from histidine in the SERS while only one histidine peak is evident in the Raman spectrum. Again, a couple of strong peaks emerge in the SERS spectrum at 1072 and 771 cm^{-1} which were assigned [42] to arginine residues bound to gold and are absent in the Raman spectrum. A broad peak assigned to the disulphide bridge stretching (due to cysteine-cysteine binding and due to various conformations of SS bridge like GGG (glycine), TGG (tryptophan) and TGT (cysteine)) in the Raman around 510 cm^{-1} transforms into a sharp narrow peak at 521 cm^{-1} in the SERS spectrum. The peak at 372 cm^{-1} is assigned to the proline amino acid [43] which was interpreted as evidence of binding of proline with gold. Table 1 summarizes the assignments of the observed Raman and SERS peaks.

Comparison of SERS Spectra from Au PLD Substrates and Ag Colloid

Colloidal systems are straight forward for SERS detection of proteins since they can be easily produced and are mobile. We used Ag colloids since Ag is known to provide often higher SERS activity than Au. In a colloidal system, NPs are mobile and hence have the possibility to probe the protein from all directions. Thus, it is likely that the SERS spectrum obtained from a colloidal system will slightly differ from the one obtained on a solid substrate as can be seen from Fig. 3. Comparing the two spectra, we find minor differences. Major peaks assigned to arginine, histidine, aspartic acid, phenylalanine and proline remain the same. Slight changes in the

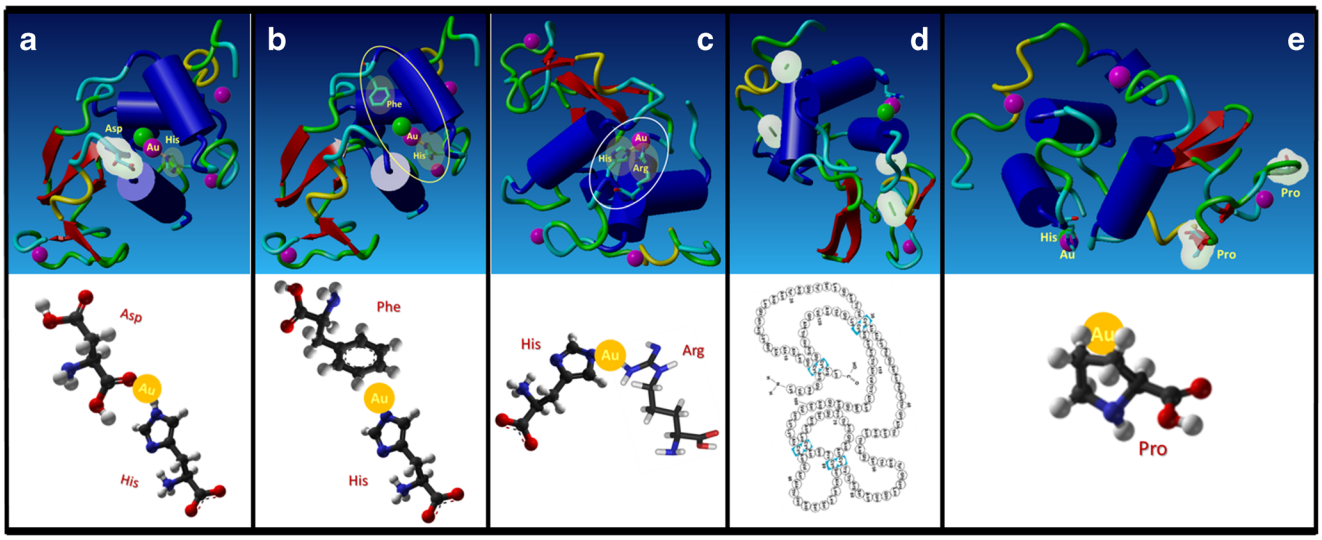

Fig. 4 Row 1: Interactions of the amino acid residues with the Au substrate responsible for peaks in SERS of lysozyme assigned to; row 2: schematic representation of the interaction of relevant amino acids with Au NP. Sequence of lysozyme in which **a** histidine and aspartic acid, **b** histidine and phenylalanine, **c** histidine and arginine, **d** four di-sulphide bonds due to cysteine-cysteine interaction and **e** proline bind to the Au NP. Reference pdb file: 3P64

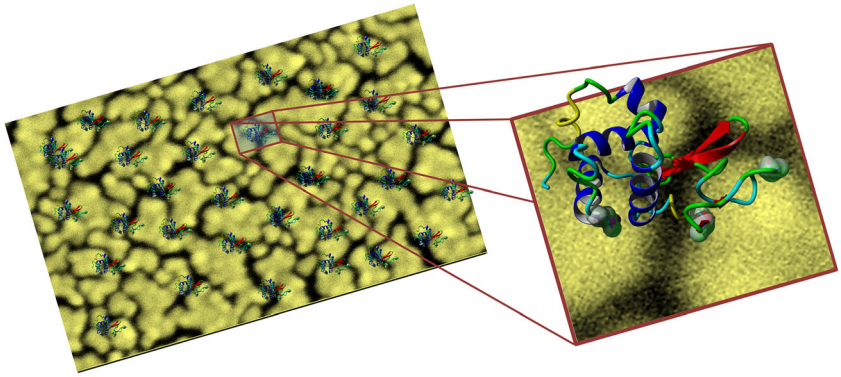

Fig. 5 Schematic representation of lysozyme lying flat on the Au substrate with indications of the binding position. The scale lengths of the substrate and lysozyme protein are not proportional

spectrum from the Ag colloid concern the intensity ratios. A peak at 1650 cm^{-1} differs from that of the spectrum from the Au substrate pertaining to the amide I band. This implies that the colloidal system is in fact able to probe the protein even in regions not accessible to the solid substrate. A study based on X-ray diffraction carried out on lysozyme in silver colloid [44] elucidated the binding site of lysozyme on Ag colloid and supported our interpretation of data.

Proposal for Lysozyme Binding Site with the Au Substrate

With the help of available structure of lysozyme interacting with gold obtained from the protein data bank 3P64 [27], we make a proposal for the possible binding site for lysozyme with the gold substrate, based purely on our assignments of the SERS peaks (see previous sections). Firstly, the strongest and most evident peak at 1384 cm^{-1} peak assigned to COO^{-} stretching region comes from the carboxylic residue of aspartic acid which is bound to gold as depicted in Fig. 4a. Also, the histidine peaks in the region 1300–1700 cm^{-1} of the SERS spectra are due to the binding of the histidine residue to gold as shown in Fig. 4a, b. Moving to lower wavenumbers, we assigned several peaks to phenylalanine. This can be explained by the presence of the aromatic residue of the phenylalanine amino acid in proximity of Au as schematized in Fig. 4b.

The peaks at 1072 and 771 cm^{-1} assigned to arginine bound to Au NP are well explained by the binding of arginine residues to the Au atom as shown in Fig. 4c. In fact, the peak at 1072 cm^{-1} is specifically assigned to CN vibrations of the arginine residue and the peak at 771 cm^{-1} is assigned to NH wagging and torsion modes. Taking into account the above peak assignments, we are confident that the Au surface is in contact with residues of histidine, aspartic acid and phenylalanine.

Next, the narrow peak at 521 cm^{-1} assigned to disulphide bridge stretching can be ascribed to the presence of TGG conformation of the disulphide bonds which is in proximity to Au NP [45, 46] or to conformational changes in lysozyme due to which most of the disulphide bonds change structure. The shoulder in the 521 cm^{-1} peak which is approximately at 510 cm^{-1} is assigned to GGG conformation of the disulphide bonds [47, 48]. There are four disulphide links in the lysozyme protein in which one out of the four has a TGG conformation while the remaining three have the GGG conformation [49]. In Fig. 4d, lysozyme is shown with the four disulphide bridges in blue.

Lastly, a probable marker can be identified to justify the binding region of lysozyme to Au at 372 cm^{-1} which is assigned to proline. In Fig. 4e, we observe two proline amino acids in plane with gold. As such, the two proline amino acids can be visualized as lying flat on the solid Au substrate (see Fig. 5) implying proximity of the proline with Au, thus originating this low frequency SERS peak.

We remark that our proposal for the binding location agrees with lysozyme binding sites to Au by X-ray diffraction [27]. This is a robust indication of the reliability of the SERS spectrum to this end. We achieved a good comparison showing that one Au atom of a NP attaches conveniently with the solo histidine moiety of the lysozyme protein. Our result proves that SERS spectra of complex molecules are reliable to a certain extent to understand binding sites and conformations of a protein, even in the absence of any functionalization of the metal surface.

Conclusions

In conclusion, SERS of a test protein (lysozyme) was obtained with Au NP substrates deposited using PLD. The substrates, which are solid in nature and hence lack the characteristic mobility of colloids, being a more constraint system, were used for SERS of a protein which has been proved to be difficult to obtain in a controlled and reproducible way. While SERS spectra of many proteins in literature were accomplished using labels, i.e. either fluorescent markers or aliphatic thiols to bind the NP with the protein, with our substrates, SERS of a protein is performed as a label-free technique. Lastly, a binding site of the protein to the NP is proposed based solely on the analysis of the SERS spectrum.

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References

1. Han XX, Zhao B, Ozaki Y (2009) Surface-enhanced Raman scattering for protein detection. Anal Bioanal Chem 394:1719–1727
2. Engvall E, Perlmann P (1971) Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobin G. Immunochemistry 8:871–874
3. Weemen BKV, Schuurs AHWM (1971) Immunoassay using antigen-enzyme conjugates. FEBS Lett 15:232–236
4. Constantine NT, Zink H (2005) HIV testing technologies after two decades of evolution. Indian J Med Res 121:519–538
5. Harz M, Rösch P, Popp J (2009) Vibrational spectroscopy—a powerful tool for the rapid identification of microbial cells at the single-cell level. Cytometry Part A 75A:104–113
6. Lentini G, Fazio E, Calabrese F, De Plano LM, Puliafico M, Franco D, Nicolò MS, Carnazza S, Trusso S, Allegra A, Neri F, Musolino C, Guglielmino SPP (2015) Phage-AgNPs complex as SERS probe for U937 cell identification. Biosens Bioelectron 74:398–405
7. Fazio E, Trusso S, Franco D, Nicolò MS, Allegra A, Neri F, Musolino C, And C, Guglielmino SPP (2016) A micro-Raman spectroscopic investigation of leukemic U-937 cells in aged cultures. Spectrochim Acta A Mol Biomol Spectrosc 159:21–29
8. Lentini G, Franco D, Fazio E, De Plano LM, Trusso S, Carnazza S, Neri F, Guglielmino SPP (2016) Rapid detection of Pseudomonas aeruginosa by phage-capture system coupled with micro-Raman spectroscopy. Vibr Spectrosc 86:1–7
9. Jaworska A, Fornasaro S, Sergo V, Bonifacio A (2016) Potential of surface enhanced Raman spectroscopy (SERS) in therapeutic drug monitoring (TDM). A critical review. Biosensors 6:47
10. Moskovits M (1985) Surface-enhanced spectroscopy. Rev Mod Phys 57:783–826
11. Kneipp K, Kneipp H, Kneipp J (2006) Surface-enhanced Raman scattering in local optical fields of silver and gold nanoaggregates-from single-molecule Raman spectroscopy to ultrasensitive probing in live cells. Acc Chem Res 39:443–450
12. Agarwal NR, Fazio E, Neri F, Trusso S, Castiglioni C, Lucotti A, Santo N, Ossi PM (2011) Ag and Au nanoparticles for SERS substrates produced by pulsed laser ablation. Cryst Res Technol 46: 836–840
13. Agarwal NR, Neri F, Trusso S, Lucotti A, Ossi PM (2012) Au nanoparticle arrays produced by pulsed laser deposition for surface enhanced Raman spectroscopy. Appl Surf Sci 258:9148–9152
14. Zanchi C, Lucotti A, Tommasini M, Trusso S, De Grazia U, Ciusani E, Ossi PM (2015) Au nanoparticle-based sensor substrates for apomorphine detection in plasma. Beilstein J Nanotechnol 6: 2224–2232
15. C. Zanchi, A. Lucotti, M. Tommasini, S. Trusso, U. De Grazia, E Ciusani. and P. M. Ossi, Laser tailored nanoparticle arrays to detect molecules at dilute concentrations, Appl Surf Sci, 396, 1866–1874 (2017)
16. Agarwal NR, Lucotti A, Tommasini M, Neri F, Trusso S, Ossi PM (2016) SERS detection and DFT calculation of 2-naphthalene thiol adsorbed on Ag and Au probes. Sens Actuators B Chem 237:545–555
17. Anderson NL, Anderson NG et al (1998) Proteome and proteomics: New technologies, new concepts, and new words. Electrophoresis 19:1853–1861
18. Blackstock WP, Weir MP (1999) Proteomics: quantitative and physical mapping of cellular proteins. Trends Biotechnol 17:121–127
19. Haraguchi H (2004) Metallomics as integrated biometal science. J Anal At Spectrom 19:5–14
20. Mounicou S, Szpunar J, Lobinski R (2009) Metallomics: the concept and methodology. Chem Soc Rev 38:1119–1138
21. Berg JM, Tymoczko JL, Stryer L (2002) Biochemistry, chapter 4. W. H. Freeman, New York
22. Wang H, Chu C, Wang W, Pai T (2014) A local average distance descriptor for flexible protein structure comparison. BMC Bioinformatics 15:1471–2105
23. Yager P, Edwards T, Fu E, Helton K, Nelson K, Tam MR, Weigl BH (2006) Microfluidic diagnostic technologies for global public health. Nature 442:412–418
24. Sun J, Xianyu Y, Jiang X (2014) Point-of-care biochemical assays using gold nanoparticle-implemented microfluidics. Chem Soc Rev 43:6239–6253
25. Yang T, Li Z, Wang L, Guo C, Sun Y (2007) Synthesis, characterization, and self-assembly of protein lysozyme monolayer-stabilized gold nanoparticles. Langmuir 23:10533–10538
26. Yuan X, Luo Z, Yu Y, Yao Q, Xie J (2013) Luminescent noble metal nanoclusters as an emerging optical probe for sensor development. Chem Asian J 8:858–871
27. Wei H, Wang Z, Zhang J, House S, Gao Y, Yang L, Robinson H, Tan LH, Xing H, Hou C, Robertson IM, Zuo J, Lu Y (2011) Time-dependent, protein-directed growth of gold nanoparticles within a single crystal of lysozyme. Nat Nanotechnol 6:93–97
28. Saurabh S, Sahoo PK (2008) Lysozyme: an important defence molecule of fish innate immune system. Aquac Res 39:223–239
29. Nester EW, Anderson DG, Roberts CE, Nester MT (2007) Microbiology: a human perspective, 5th edn. McGraw-Hill Higher Education, Boston
30. Krugliak L, Meyer PR, Taylor CR (1986) The distribution of lysozyme, alpha-1-antitrypsin, and alpha-1-antichymotrypsin in normal hematopoietic cells and in myeloid leukemias: an immunoperoxidase study on cytocentrifuge preparations, smears, and paraffin sections. Am J Hematol 21:99–109
31. Prockop DJ (1964) A study of urinary and serum lysozyme in patients with renal disease. N Engl J Med 270:269–274
32. Hu J, Sheng RS, Xu ZS, Zeng Y (1995) Surface enhanced Raman spectroscopy of lysozyme. Spectrochim Acta A 51:1087–1096
33. Chandra G, Ghosh KS, Dasgupta S, Roy A (2010) Evidence of conformational changes in adsorbed lysozyme molecule on silver colloids. Int J Biol Macromol 47:361–365
34. Cheng AKH, Ge B, Yu H (2007) Aptamer-based biosensors for label-free voltammetric detection of lysozyme. Anal Chem 79: 5158–5164
35. Kneipp J, Kneipp H, McLaughlin M, Brown D, Kneipp K (2006) In vivo molecular probing of cellular compartments with gold nanoparticles and nanoaggregates. Nano Lett 6:2225–2231
36. D'Andrea C, Neri F, Ossi PM, Santo N, Trusso S (2009) The controlled pulsed laser deposition of Ag nanoparticle arrays for surface enhanced Raman scattering. Nanotechnology 20:245606
37. Fazio E, Neri F, Ossi PM, Santo N, Trusso S (2009) Growth process of nanostructured silver films pulsed laser ablated in high pressure inert gas. Appl Surf Sci 255:9676–9679
38. Espinoza L, Hower JC, Jiang S (2007) Influence of salt and pH on the adsorption of fibrinogen and lysozyme to self-assembled monolayers using a surface plasmon resonance sensor. Journal of Undergraduate Research in Bioengineering 7:73–79
39. Kitagawa T, Hirota S (2006) Raman spectroscopy of proteins, handbook of vibrational spectroscopy. Wiley

40. Fabian H, Mäntele W (2006) Infrared spectroscopy of proteins, handbook of vibrational spectroscopy. Wiley
41. Siebert F, Hildebrandt P (2008) Structural studies, vibrational spectroscopy in life science. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
42. Aliaga AE, Garrido C, Leyton P, Diaz G, Gomez-Jeria JS, Aguayo T, Clavijo E, Campos-Vallette MM, Sanchez-Cortes S (2010) SERS and theoretical studies of arginine. Spectrochim Acta A 76:458–463
43. Podstawka E, Ozaki Y, Proniewicz LM (2005) Part III: surface-enhanced Raman scattering of amino acids and their homodipeptide monolayers deposited onto colloidal gold surface. Appl Spectrosc 59:1516–1526
44. Panzner MJ, Bilinovich SM, Youngs WJ, Leeper TC (2011) Silver metallation of hen egg white lysozyme: X-ray crystal structure and NMR studies. Chem Commun 47:12479–12481
45. David C, Foley S, Enescu M (2009) Protein S-S bridge reduction: a Raman and computational study of lysozyme interaction with TCEP. Phys Chem Chem Phys 11:2532–2542
46. Kudryavtsev AB, Mirov SB, DeLucas LJ, Nicolete C, van der Woerd M, Bray TL, Basiev TT (1998) Polarized Raman spectroscopic studies of tetragonal lysozyme single crystals. Acta Crystallogr D Biol Crystallogr 54:1216–1229
47. Sugeta H, Go A, Miyazawa T (1973) Vibrational spectra and molecular conformations of dialkyl disulfides. Bull Chem Soc Jpn 46: 3407–3411
48. Van Wart HE, Scheraga HA (1986) Agreement with the disulfide stretching frequency-conformation correlation of Sugeta, Go, and Miyazawa. Proc Natl Acad Sci U S A 83:3064–3067
49. Carpentier P, Royant A, Weik M, Bourgeois D (2010) Raman-assisted crystallography suggests a mechanism of X-ray-induced disulfide radical formation and reparation. Structure 18:1410–1419