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Surface Enhanced Raman Spectroscopy of Proteins: Implications for Drug Designing

Invited Review Article

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Abstract In this review article we present a general overview of the recent progress in the newly developing area of the study of protein-ligand interaction by surface enhanced Raman spectroscopy (SERS). Since its first observation in 1977, SERS have been fast developing into an analytical tool for trace detection of molecular entities, particularly in the area of bio-molecule sensing and characterization. Also, with the development of the ability to design a variety of plasmonic structures and to be able to control and tune their plasmonic properties, we have been able to use them as SERS substrates for probing complex materials. Here we describe yet another application of SERS, mainly protein-ligand interaction and its future into drug designing. We start with a general description of the SERS phenomenon. Subsequently we discuss the key spectral features of amino acids, peptides and proteins, and their structural aspects that can be elucidated from the SERS spectra. In the final sections we discuss the application of SERS to the study protein-ligand interaction and its potential role in the area of drug designing.

Keywords Surface enhanced Raman spectroscopy, protein- ligand interaction, drug designing.

1. Introduction

Surface enhanced Raman scattering is an emerging tool for studying the vibrational fingerprints of biomolecules [1,2]. Understanding the behaviour and function of biomolecules at the molecular level is key to the discovery and development of new drugs, as well as diagnostic techniques. Development in the field of structural biology has changed the way drugs are being designed and developed nowadays. Techniques like X-ray crystallography and NMR have helped to determine the 3D structure of complex protein molecules, as well as protein-ligand complexes [3,4]. These techniques, coupled with sophisticated molecular visualization tools, have opened up new areas in structure-based drug design and development. X-ray crystallography can generate structures of a variety of proteins and protein complexes, but one of the drawbacks of this technique is the inability to crystallize all proteins [5]. It is difficult to crystallize some proteins and even if they are crystallized, their crystal structure cannot be solved or they do not look the same as those in the cellular environment [6]. NMR has been an important alternative, being able to determine protein structures in its physiological conditions, as well as providing the opportunity to study protein kinetics and dynamics [7]. But the major disadvantage of using

NMR is that it is very complicated to use for bigger proteins (bigger than 150 kD) [8]. Another promising tool, which can provide useful information about complex protein structures, is vibrational spectroscopy [9-12]. Raman and IR spectroscopy can provide information about the secondary structure of proteins, unlike X-ray crystallography and NMR, which provides information about the tertiary structure [13,14]. Raman and IR are complementary techniques. Since most of the proteins and other biomolecules are found in aqueous solutions, Raman spectroscopy is more advantageous to use as it is a water insensitive technique. Other advantages of Raman spectroscopy are that it is a non-destructive and non-invasive method, and can be performed in physiological conditions with a variety of light sources [15-17]. Raman spectroscopy has been used for characterization of various proteins like wool [18], keratin in nails and hair [19], ivory [20], kidney and gallstones [21] and many other proteins. Raman spectroscopy has also been used to detect and identify microscopic pathogenic organisms like bacteria and viruses [22,23]. It has also been used to detect cancerous tissues, both in vitro and in vivo [24]. In addition, Raman spectroscopic techniques have been used to study intermolecular interactions and the dynamics of proteins and nucleic acids [25]. Even though Raman spectroscopy has been known about for over 80 years, it had to wait for the arrival of lasers and sophisticated instrumentations to realize its potential as a spectroscopic tool. One of the limitations of Raman spectroscopy is the low scattering cross-section (only one in a million photons is Raman scattered) limiting its use in biomolecule characterization. With the discovery of surface enhanced Raman spectroscopy (SERS) and the development of this technique over the past three decades, it has been possible to use it in biological applications. In SERS, the analyte molecule is absorbed onto a metallic, nanoscale surface to achieve enhanced Raman signals [26]. The enhancement depends upon the effective adsorption of the analyte molecules to the nanoscale surface and also on the nature and dimensions of the nanoscale surface [27]. SERS is a highly sensitive technique and in combination with resonant Raman (usually referred to as surface enhanced resonant Raman spectroscopy [SERRS]) can even detect molecules at single molecular level [28]. Other advantages of SERS include the need for very low concentrations of samples and the ease of sample preparation. Therefore, it is gaining in importance in the field of medicine and pharmacology. Our group has demonstrated the use of SERS to understand protein-ligand interactions in therapeutically important proteins with no available structural information and this will be discussed later in this review. These techniques, once fully developed, would have therapeutic importance and provide valuable information about protein-ligand systems. The high sensitivity of SERS makes it useful in both labelled, as well as non-labelled, schemes.

2. Surface enhanced Raman spectroscopy

2.1 Role of substrates

SERS is a form of Raman spectroscopy in which we achieve an unusually high Raman scattering cross-section when an analyte molecule gets adsorbed to a metallic surface, which has nanoscale roughness [29]. There are many forms of SERS substrates depending on the purpose they are used for [30,31]. These include metal nanostructures immobilized or developed on solid substrates, as well as different kinds and forms of metal nanoparticles stabilized in solution phase [32]. SERS was first observed on the roughened surface of electrodes [33]. Subsequently, the Raman spectrum of pyridine was enhanced to almost more than 10^6 times in SERS on metal colloids [34]. This phenomenon was called SERS and it was realized that the nature of the substrate plays an important part in the enhancement [35]. Nanostructures from noble metals like gold, silver and copper exhibit SERS and aluminium, lithium and sodium also show enhancement of Raman spectra, but to a lesser extent [36]. Every material has a characteristic plasmon (collective oscillations of electrons) associated with it, which is size dependent. When the mean free path of the electron exceeds the size of the structure (10 to 100 nm), the plasmon is mostly associated with the surface. When a light matching the plasmon frequency of the nanostructure is incident on it, it excites the surface plasmons of the nanostructure. This is called the surface plasmon resonance [37]. The excited surface plasmon produces an oscillating dipole leading to generation of source, thus producing a local electromagnetic

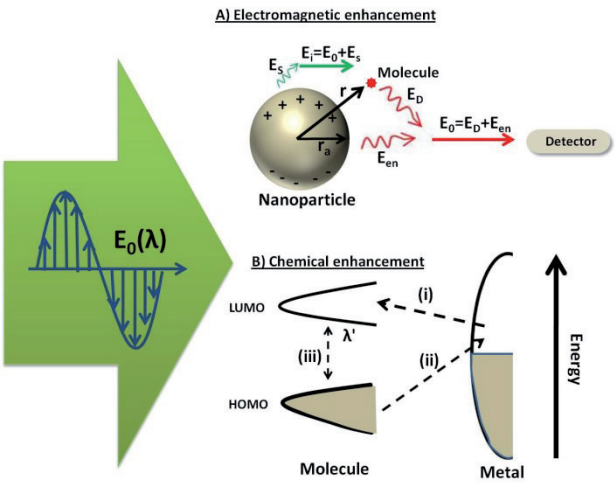

Figure 1. (A) shows the electromagnetic enhancement mechanism. The metal nanoparticle experiences a time varying electromagnetic field E_0 which produces an oscillating dipole moment in the nanoparticle. The resultant incoming field is E_i and the resultant outgoing field is E_o. (B) Shows the chemical enhancement mechanism. HOMO and LUMO interact with the metal surface and are broadened into resonances. Charge transfer happens through paths (i) and (ii), and path (iii) shows the resonant Raman process [40].

electromagnetic (EM) radiation matching the excitation enhancement very close to the surface (within ~ 1 nm of the nanostructure). When a molecule is in the proximity of the surface of such a nanostructure, the Raman signal is enhanced due to the increase of the EM field because of resonant plasmons, leading to the phenomenon of SERS [38]. There are two proposed mechanisms for SERS enhancement, electromagnetic enhancement and chemical enhancement [39]. The intensity of Raman scattering is directly proportional to the square of the dipole moment $\vec{P} = \alpha.\vec{E}$, where α is the molecular polarizability and $\vec{E}$ is the electric field due the incoming radiation. Electromagnetic enhancement is associated with $\vec{E}$ and chemical enhancement is associated with α [40]. The two types of enhancement will be described in detail below. A pictorial representation of the enhancement mechanisms is shown in Fig. 1.

2.2 Electromagnetic enhancement

The surface plasmons of the nanoparticles can be resonantly excited by the time-varying electric field of the incident light in the visible region of the electromagnetic spectrum in the case of noble metals [41,42]. Metals can be considered to consist of periodic static positive charges surrounded by a sea of electrons. A momentary displacement of the electrons causes a dipole to be formed and this dipole oscillates with its own characteristic frequency. The dipole oscillation in turn generates an electric field which is a function of distance from the surface of the nanostructure and that decays down as $1/r^{12}$, where r denotes the distance between the centre of the spherical nanoparticle and the molecule. Fig. 1(A) shows the scheme for electromagnetic enhancement mechanism. The resultant field experienced by the adsorbed molecule near the surface is $E_i = E_0+E_s$, where E_0 is the incident field and E_s is due to the surface Plasmon [31]. E_s depends on the sphere's radius r_a and the distance d (=r-r_a) from the molecule. It also depends on the metal's dielectric constant ε and the incident field E_0. E_s, using a drude model, is given by [42]:

$$E_s = \frac{\varepsilon - \varepsilon_0}{\varepsilon + 2\varepsilon_0}\left(\frac{r}{r+d}\right)^3 E_0$$

The resultant E_i produces an induced dipole μ_i in the molecule. The μ_i is directly proportional to the E_i and the proportionality constant, α, is the molecular polarizability tensor, as both of them are vectors. The α is a function of the molecular vibrations and hence would combine with the oscillating E_i to produce three kinds of frequencies of oscillations of the μ_i, namely, ω_0 (Rayleigh scattering, the same as the incident electric field), $\omega_0 + \omega_R$ (anti-Stokes) and $\omega_0 - \omega_R$ (Stokes), where ω_0 is the angular frequency of the incoming light and ω_R is the angular frequency of normal mode. In the case of Stokes scattering, the dipole radiation E_D is red-shifted with respect to the incident light. The resulting outgoing field is given by $E_o=E_D+E_{en}$, where E_{en} is the additional component of enhancement due to the elastic scattering producing further induced dipole in the molecule. The field enhancement, A(ν) at the molecule, is given by the ratio of the field amplitudes, E_i/E_0 and E_o/E_0 for laser and Raman scattered field respectively. The SERS electromagnetic enhancement (G_{SERS}) is the product of the enhancement factors for the laser and Raman scattered fields which are $|A(\nu_l)^2|$ and $|A(\nu_s)^2|$respectively. Thus G_{SERS} scales as fourth power of the local field at the vicinity of the metallic nanostructure and is strong in the case where the scattered and the plasmon field are in resonance. G_{SERS} is maximum when the real part of ε(ν) is equal to -2ε_0 and the imaginary part is very small, deduced from the equation given above. These are the conditions for resonant excitation of surface plasmons in the metal sphere. The electromagnetic factor accounts for 10^4-10^6 of the total enhancement factor.

There are instances when the enhancement factor reaches more than 10^{12}, especially, when the molecule is localized in between two or more nanoparticles, forming a cluster-like situation [28]. These regions of intense electromagnetic field, having a large electromagnetic enhancement, are called "hot spots" [43, 44]. There is also interplay between enhancement of Raman signals and fluorescence when the distance of the molecule to the surface of the nanostructure varies, leading to the change in their respective cross-sections [45]. The increase in Raman scattering in the hot spots makes single molecule detection through SERS viable. The role of hot spots in SERS has been proven theoretically, as well as experimentally [42-46]. It has been shown that these hot spots contribute almost 24% of the total intensity of SERS, even though only 63 hot spot sites contribute to every million SERS sites [47]. Thus, most of the SERS signal is contributed by the hot spots. There have been studies to understand the nature of the hot spots and the intensity of SERS signals emerging from them, and this helps in designing highly efficient SERS substrates. This is a challenging task as the hot spots are generally heterogeneous, distributed in experimental studies and also there is possible chemical bonding, which has to be accounted for. Yoshida et al. studied the quantitative nature of the electromagnetic enhancement effect on SERRS and showed its correspondence with the optical anisotropy of plasma resonance and the nanostructure morphology [46]. Weber et al. studied the shape of SERS hot spots and the spatial distribution of SERS' intensity by combining SERS with scanning electron microscopy [48]. The hot spots are the regions where behaviour in the single molecule domain is observed like spectral blinking and inhomogeneous broadening of Raman peaks [49, 50]. There have been attempts to control these hot spots' geometries in an attempt to introduce reproducibility and make the technique quantitative [51, 52].

2.3 Chemical enhancement mechanism

Chemical enhancement accounts for 10-100 (relatively small, but nevertheless important) of the total enhancement factor and requires the molecule to be in direct contact (bonded) with the surface of the metallic nanostructure [26]. When there is an electronic coupling between the molecule and the metal, the Raman cross-section of the molecule increases due to charge transfer from the metal to the molecule. A charge transfer between the molecule and the metal leads to the broadening and shifting of the electronic level in the adsorbed molecule resulting in a 'resonance' Raman effect which gives SERS enhancement. A dynamic charge transfer takes place when a photon is absorbed by a metal which results in a hot electron state. The hot electron gets transferred to the Lowest Unoccupied Molecular Orbital (LUMO) of the molecule. The increase in the LUMO electrons leads to increased Raman signal due to increased probability of electron-phonon coupling in the Raman scattering tensor. The excited electron is later transferred back to the metal from the LUMO and this results in the emission of Stokes photons. This mechanism is called the first layer effect. The direct attachment of the molecule to the surface gives a higher enhancement factor [54]. Fig. 1(B) shows the schematic representation of chemical enhancement mechanism.

3. SERS of amino acids, peptides and proteins: an overview

Amino acids are the basic building block of peptides and proteins. A detailed analysis of the Raman and SERS spectra of the amino acids is useful to assign bands of complex and much larger protein structures. Amino acids are linked together by peptide bonds to form peptides. Longer peptides are known as oligo-peptides and polypeptides. Repeating units of polypeptide backbone form the secondary structure, which constitutes the backbone of the protein. A typical protein has a secondary structure consisting of α-helix, β-sheet and random coils among the major components. These secondary structures combine together to give a three dimensional conformation of the protein called the tertiary structure. The polypeptide chains assemble into an oligomeric protein structure called the quaternary structure. All these structures of bio-molecules have been probed by Raman spectroscopy [54]. Several reviews can be found in the literature to elucidate this [55-58]. Considering some of the drawbacks of normal Raman-like low scattering cross-section, fluorescence background and difficulty in producing therapeutically important proteins in substantial amounts, SERS is being considered as a potential method for the study of bio-molecules [59,60] and a considerable amount of work has been carried out in this direction. We list a few here for completeness. SERS of amino acids, homopeptides, heteropeptides and proteins have been studied in detail by Podstawka et al. [61-66]. SERS of peptides and proteins on electrochemically prepared silver surfaces have been studies by Stewart et al. [67]. Hu et al. studied the SERS of lysozyme on silver colloids [68]. Suh and Moskovitz studied the SERS of amino acids and nucleotide bases on silver colloids, and have derived orientational information of the molecules with respect to the surface of the silver colloid [69]. Gullekson et al. studied the surface sensitive Raman spectroscopy of collagen I fibrils, one of the main constituents of connective tissues in humans [70]. SERS has been employed for highly sensitive detection of proteins by means of label-free or labelled techniques [1]. Several articles have been published on protein bio-sensing and bio-recognition [71-77]. Further studies of different proteins include flavoproteins [78], glucose oxidase [79], lactate oxidase, p-hydroxybenzoate hydroxylase, old yellow enzyme [80], IgG [81], haemoglobin [82], myoglobin and cytochrome C [83]. SERS has also been used to detect molecular signatures of viruses and bacteria [84,85]. Chumanov et al. reported on the SERS of water soluble proteins, dipeptides and amino acids [86,87]. Nabiev et al. have studied the SERS of aromatic amino acids and proteins on the surface of silver hydrosols [88]. Kneipp et al. demonstrated SERS in living cells, opening up the possibility of in vivo and real-time applications [89]. These studies become interesting provided the instruments used are adaptable to biological methods and if they are inexpensive to undertake. We provide the schematics of the system (see Fig. 2) developed by the one of the authors on a fluorescence microscope [90].

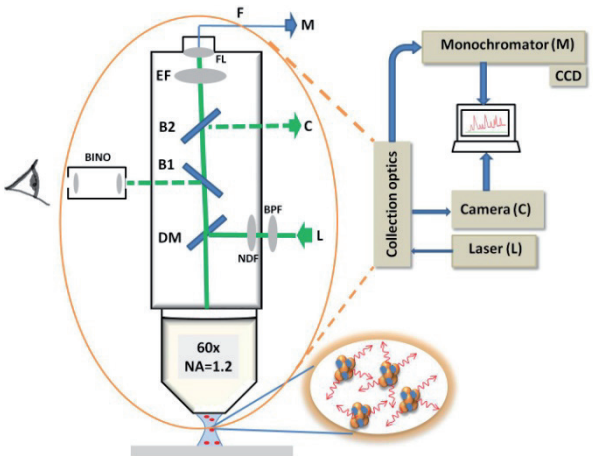

Figure 2. shows the SERS microscopy setup. The scattered light from a sample in aqueous solution is collected by a water immersion objective and directed to the monochromator via the collection optics. The collection optics is connected to a monochromator and CCD, camera and a laser input. The camera and the monochromator are connected to a computer. The collection optics consists of the following components. NDF - neutral density filter, BPF - band pass filter, DM - dichroic mirror, B1 and B2 - beam splitters, BINO - binocular, EF - edge filter, FL - focusing lens and F - optical fibre.

4. p300 structure probed by SERS

In our work we have studied biologically important proteins such as p300 (Fig. 3A) and CARM1 (coactivator associated arfinine methyltransferase 1) [91,92]. A custom made Raman setup used for the SERS experiment is shown in Fig. 2. All SERS experiments were performed in aqueous phase to probe the protein structure close to physiological conditions. p300 is a 300 kDa multi-functional protein, which also functions as transcriptional co-activator and is involved in multiple, signal dependent transcriptional events. Due to its diverse functional importance, any alteration in the function leads to several diseases, including cancer and diabetes to name but a few [93]. In order to study the structure-function relation of p300, it is very important to investigate its structural aspects through SERS due to the absence of any x-ray diffraction data for full-length p300. Therefore, the structural information obtained from SERS is therapeutically significant. SERS studies have also been conducted on the p300 HAT domain to look at the effect of auto-acetylation (see Fig. 3B) [94].

SERS, in general, contains information about the secondary structure of the protein and the relative abundance of the different aromatic amino acids present in the protein. The position and intensities of these bands depend on the orientation with respect to the surface of the nanostructure, distance, as well as the local environment of the residues. Bands from carboxylic groups and other side chain vibrations, including those containing sulphur, can also be observed in the spectra of proteins. Characteristic bands found in the SERS spectra of peptides and proteins include amide I, II and III bands. The amide band arises due to bond vibrations in the peptide units. The amide I band consists of C=O stretching vibrations with some contribution from N-H bending, out of phase CN stretching vibration and CCN deformation. The amide II mode is the combination of the NH in plane bending and CN stretching vibration with contributions from CO in-plane bending and CC stretching vibration [85]. The C=O and N-H bonds take part in the hydrogen bonding between different residues inside the protein framework, therefore, their position and intensities can be directly correlated to the secondary structure of the protein. The amide I bands for α-helix, β sheet and random coil lie in the regions 1640-1658 cm^{-1}, 1665-1680 cm^{-1} and 1660-1665 cm^{-1} respectively in Raman spectra. On the other hand, in the case of SERS, due to the strong interaction of the –CO-NH- with the silver surface, the C=O bond weakens and thus the amide I band shifts to lower frequencies. The amide II mode, which is a Raman inactive mode, appears in the SERS spectrum because of a modification of surface selection rules. This band occurs in the region around 1550 cm^{-1}. The amide III band lies in the region from 1200 cm^{-1} to 1400 cm^{-1}. In the case of normal Raman, the amide bands can be resolved to obtain the fractions of the secondary structures in the proteins, as the spectra are the average of the total structure of the proteins. But in the case of SERS, preferential enhancement takes place depending on the way the protein binds to the nanoparticle surface.

In full-length p300, the amide I, II and III bands lie at 1623, 1540 and 1296 cm^{-1} respectively. In the SERS of p300 HAT domain (p300HD), as shown in Fig. 1B, the amide bands lie at 1660 cm^{-1} (amide I, α-helix), 1632 cm^{-1} (amide I, random coil), 1518 cm^{-1} (amide II) and 1294cm^{-1} (amide III) [94]. The positions of the amide bands were confirmed by deuteration of the proteins. Upon deuteration, most of the labile hydrogen in the amide groups gets replaced by the heavier deuterium. The reduced mass of the vibrating unit increases, which is inversely proportional to the vibrational frequency. The amide I band in both p300 and p300HD shifts by around 7 cm^{-1} on deuteration. The amide II band also shifts on deuteration by around 5 cm^{-1} and the amide III band shifts from 1296 cm^{-1} to 950 cm^{-1} [91]. The SERS of proteins are also dominated by bands from the aromatic side chain vibrations of amino acids like tyrosine (Tyr), tryptophan (Trp) and phenylalanine (Phe). As a general rule, in SERS, a mode is strongly enhanced if its polarizability component is perpendicular to the surface of the nanostructure. Therefore, the intensities of the ring modes greatly depend on the orientation of the ring to the surface of the nanostructure. The modes which have polarizability components in all x, y and z axes will always be enhanced irrespective of their orientation with respect to the nanoparticle surface. The other modes, which have polarizability component preferentially oriented, will be enhanced when they are oriented in a perpendicular direction with respect to the metal surface. A conspicuous absence of a mode in the spectrum, in spite of having the polarizability component in all three axes, is due to the distance of this moiety from the metal surface. The Raman intensity of a mode decays drastically with distance. Hence, not all modes that present in the normal Raman of the proteins get enhanced in the SERS spectra. Modes due to aliphatic chain vibrations can also be seen in the SERS spectrum of proteins. Mostly the carboxylic group or the amino groups in the protein interact with the metal surface to give strong enhancements of these bands. In p300 SERS spectra, the carboxylate group of the aspartic acid (Asp), glutamate (Glu) and the C-terminus group in protein interact with the silver surface. The enhancement of modes corresponding to the symmetric stretching of $C_\alpha N$ and the asymmetric stretching of $CC_\alpha N$ groups suggest that the p300 absorbs to the silver nanoparticles through the nitrogen groups. SERS can be used as a tool to generate spatially localized surface spectra of proteins. Thus, a great amount of information about the protein structure, orientation and environment of the amino acid

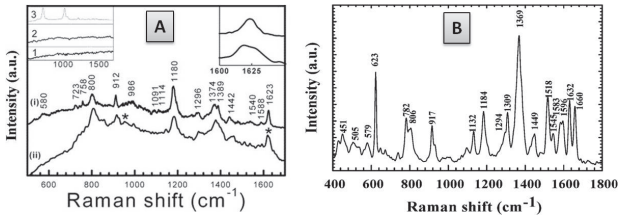

Figure 3. (A) inset on left - Raman spectra of pure p300 (curve 1), nanoparticles (curve 2) and buffer with nanoparticles (curve 3). Curve (i) - SERS spectra of p300. Curve (ii) - SERS of deuterated p300, *denotes the new or modified bands. Inset on right - amide I band region. (B) SERS of p300 histone acetyltransferase domain. Reprinted with permission from The Journal of Physical Chemistry B, vol. 110, pp.16787-16792. Copyright 2006 American Chemical Society.

side chains can be deduced through SERS, making it a promising tool for investigation of protein structure. Vibrational spectroscopic techniques, like Raman spectroscopy, are very sensitive to bond strength changes. These kinds of techniques are also used to monitor bond distortions in catalytic reactions with great accuracy. We will discuss further in this review how the SERS technique can be used to monitor protein-ligand interactions and provide valuable information about drug development.

5. SERS for studying protein-ligand interactions

One of the most important advantages of SERS is the ability to detect protein-ligand interactions at very low concentrations, much lower than that of NMR and x-ray crystallography [96]. It is possible to detect and interpret protein-ligand interactions without prior information about the protein's functions and the nature of the ligand. There are three main approaches to studying protein-ligand interactions by SERS. The first one constitutes the Raman dye-labelled methods. SERS have been employed to study protein-ligand interactions using Raman dye-labelled silver or gold nanoparticle probes [1]. This method needs specially designed fluorophores or chromophores and other substrate analogues. The second method is to study the difference spectra of the ligand and the protein-ligand complex. This gives the mode of binding of the ligand to the protein. The third method constitutes the change in spectra of proteins on the binding of the ligand. This gives an insight into the protein conformation change and the interaction of the ligand with various residues in the protein. In our studies we have used this label-free method to study the interaction of ligand with proteins.

5.1 Detection of protein-ligand interactions by dye-labelling methods

This method can be used in high-throughput studies in proteomics. Fluorescence is generally used to conduct such studies, but SERS is also emerging as a promising tool in this area due to multiplexing possibilities [97]. There are two major drawbacks of the fluorescence method which limit its application in certain areas. Firstly, its broad emission spectra make multiplexing difficult and complicated. Secondly, there is also a possibility of photo-bleaching, which affects the detection limit. Ozaki et al. have devised a method to study the protein-ligand interaction using the dye-labelling method [98]. Ligands like small molecules and antibodies were labelled with TRITC and Atto610 [96]. They were used as Raman and fluorescence reporters for determinations of the interactions between human IgG and TRITC-anti-human IgG and those between avidin and Atto 610-biotin. Silver staining techniques were used to obtain SERS spectra. Surface-enhanced fluorescence (SEF) could also be seen in cases when the dye molecules were far from the surface of the nanoparticles due to the protein structure. Han et al. have also used coomassie brilliant dyes, like brilliant blue R-250 (BBR) and brilliant blue G-250 (BBG), as SERS labels for studying protein-ligand interactions [98]. These dyes have strong affinity for proteins without any need for separation and purification. The dyes are not directly labelled to the nanoparticles, making the procedure simpler and faster. This method can also be used to detect protein-protein interactions and protein-small molecule interactions either in solution phase or on a solid substrate. Protein-ligand or antibody interactions can also be studied by dye displacement method by the use of SERS [99].

5.2 Study of protein-ligand interactions by label-free methods

This method is used to predominantly study protein-drug or protein-small molecule interactions. Miškovský et al. have studied the binding of the antiretrovirally active drug hypericin to the IIA subdomain of human serum albumin (HSA) by SERS and resonance Raman [100]. SERS enabled the study of hypericin in the complex structure as it was strongly enhanced and also the fluorescence was quenched on adsorbance of the molecule to the silver colloidal surface. The SERS spectra of hypericin and hypericin-HSA complex were obtained, and the difference spectrum was studied. The main features observed in the complex spectrum in relation to that of hypericin were the decrease in relative intensity and change in spectral profile. From the SERS spectra of hypericin and the protein complex it could be interpreted that there is a disruption of the intermolecular H-bonds existing between the drug molecules with the solubilizing of hypericin molecule and formation of an H-bond between the carbonyl groups of hypericin and a hydrogen donor of albumin leading to a protonated carbonyl in the drug. Fabriciova et al. studied the interaction of the antitumor drug emodin with human serum albumin [101]. It was shown that emodin could interact with human serum albumin through two binding sites, I and II sudlow binding sites. It was shown how the drug binds to the protein in defatted conditions and also in

the presence of other ligands like fatty acids. Jurasekova et al. have studied the binding of flavonoid luteolin to human serum albumin [102]. It was shown by SERS that luteolin interacts with protein in the neutral form and through the B and C ring of the ligand molecule.

5.3 Protein-drug interactions in p300: a case study

The other approach to studying protein-ligand interactions is to study the change in protein spectra on binding of ligand or small molecules to the protein. In the subsequent sections we show how the change in structure of the proteins brought about by covalent attachment or other modes of binding of small molecules can be probed by SERS. It can be a quick and easy method to find the efficiency of binding of molecules to the target protein indicated by the associated structural changes. A cartoon representation of the study of protein-ligand interaction through SERS is shown in Fig. 4.

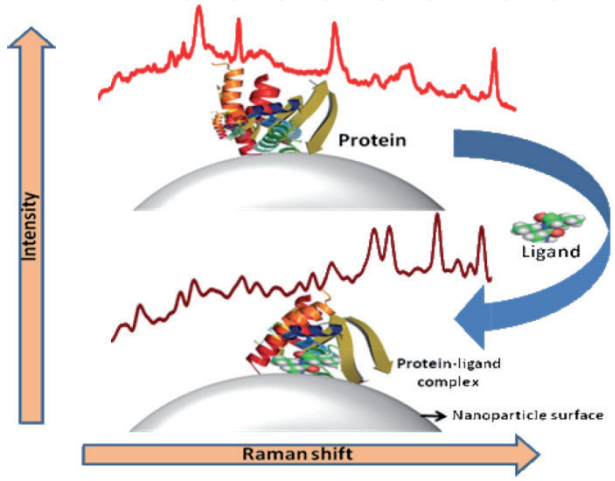

Figure 4. Cartoon representation of detection of protein-ligand by SERS. The change in the SERS spectrum is a direct indication of ligand binding to a protein forming a protein-ligand complex. On the binding of the ligand, the protein may undergo structural changes which results in change in the orientation of the amino acid moieties with respect to the nanoparticle surface. Due to the ligand binding disruptions of hydrogen, bonding happens and some of the residues are distanced from the nanoparticle surface. These lead to changes in the spectrum of SERS which indicate the formation of a protein-ligand complex.

5.3.1 Acetylation of histone acetyltransferase (HAT) domain of p300

SERS was used to probe the acetylation-induced specific structural changes in the histone acetyltransferase (HAT) domain of p300. On comparing the normal HAT and the acetylated HAT domain, there was a marked change in the modes corresponding to the symmetric stretching of COO^- (1369 cm^{-1}) and amide III (1294 cm^{-1}), ν_{9a} of Phe (1184 cm^{-1}) and asymmetric stretching of $C_\alpha CN$ (1132 cm^{-1}) [84]. The intensity of these modes changed and also shifted in position to lower frequencies, indicating softening of

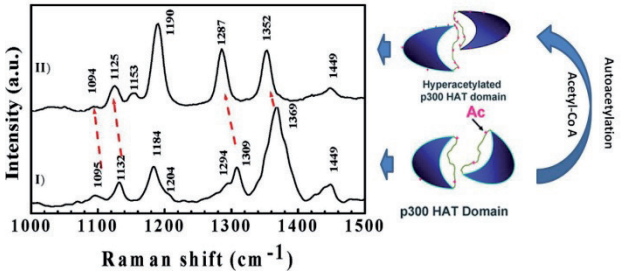

Figure 5. (I) shows the SERS of HAT domain and (II) shows the SERS of acetylated HAT domain. Softening of some modes due to acetylation is shown by red arrows.
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modes (Fig. 5). The shifts in band positions is because of acetylation, which effects hydrogen bonding causing softening and the changes in intensities of bands. This also indicates change in the orientation of the protein on the nanoparticle surface due to local conformational changes of the HAT domain on complete acetylation.

5.3.2 Interaction of small molecule activators with p300

SERS was used to probe activation of p300 histone acetyltransferase by small molecules altering the enzyme structure [103]. SERS was performed on p300 protein and p300 complex with CTPB and CTB (Fig. 6a). Both CTPB and CTB (structure is given in Fig. 6a) are the only known activators for the histone acetyl transferase (HAT) and are insoluble in water. In addition, CTPB has a very large penta-decayl alkane chain. Hence, both of these molecules would go into a hydrophobic domain of the HAT. In addition we can anticipate a strong steric hindrance from the penta-decayl chain of CTPB, hence CTPB binds weakly to the protein compared to CTB, again confirmed from the activity of these enzymes [103]. This has been reflected in the SERS spectra of p300 when complexed with CTPB and CTB. In the case of CTPB, the intensities of bands at 1654, 1335 and 960 cm^{-1} change, indicating binding of the molecule to the amide groups of α helix and β sheet. The changes are greater when CTB binds to p300 with complete disappearance of the 1623 cm^{-1} band. The binding of molecules to the protein distances the binding sites from the nanoparticle surface also changing their orientation with respect to the nanoparticle surface. These result in the change in intensity of Raman modes, as well as complete disappearance. The change in the spectrum of protein on binding of molecules indicates protein structure change. This also gives an idea about the probable binding sites of the molecules. Since the molecules are hydrophobic, it binds to the hydrophobic region of the protein. Secondly, when we look at the CTPB and CTB, we find that the halide groups give the hydrophobic envelop and the ethoxy and amide region of the molecule is the

hydrophilic region. The molecule hence would form a micelle by combining together to get into the hydrophobic region. Interestingly, looking at the protein sequence, we find that the most hydrophobic and most hydrophilic region resides in the HAT domain itself. Secondly, we find that the HAT domain should be full of α-helix and β-sheets as the spectral change shows a large change due to the binding of the small molecule. It is interesting to point out here that x-ray data of synthetically prepared HAT domain carried out later indeed confirm these observations [104]. In addition, the modes related to aromatic amino acids also underwent changes in intensity and position on binding of CTPB and CTB (for example disappearance of 802 cm^{-1} related to the tyrosine residue [81]). These kinds of binding are brought on by strong hydrogen bonding, as well as hydrophobic interactions and the Raman peaks being shifted by 10-35 cm^{-1}.

Derivatives of the compounds CTPB and CTB were made to find the structural basis of enhancement of enzyme activation. The positions of the hydrophobic groups, namely para pointing –Cl and meta pointing -CF_3 in CTB, were changed to disturb the hydrophobic influence and it was observed that the activity of these derivatives was strongly correlated to their position. In one such situation, the activation of the HAT was not achieved at all and we find that the SERS immediately captures this, showing no change in spectra when there were no functional groups in the para position of the phenyl ring (Fig. 6b). It was observed that substitution in the meta and para positions of the phenyl ring with strong hydrophobic groups leads to large structural changes evident from SERS, confirming that the distance between the hydrophilic to hydrophobic group is critical to the activity of this molecule itself. It was observed that only the molecules, which activated p300, showed structural alteration of the enzyme as shown by SERS (Figures 6a and 6b). There was also a one to one correspondence between the degree of activation and the extent of change in the SERS spectra of the enzyme-molecule complex.

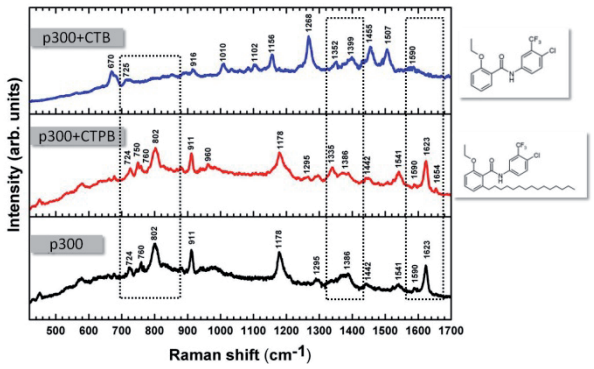

Figure 6a. SERS spectra of p300 (a) and p300 complexed with CTB(a) and CTPB(b). The SERS spectra of p300 with CTPB and CTB both show changes with appearances of new modes. The change in the case of CTB is larger. The dotted areas show the modes which undergo changes [93].

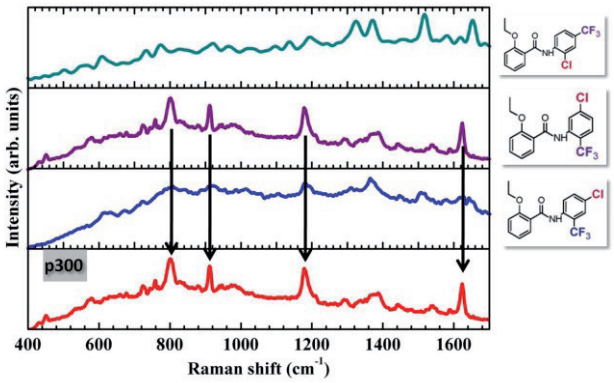

Figure 6b. SERS spectra of p300 (a) and p300 complexed with derivatives of CTB with the electronegative groups –Cl and –CF_3 groups at different positions of the phenyl group. This shows the role of the hydrophobic group of CTB molecule, namely, –CF_3 and –Cl. The molecular structure is given along with the spectra. There is no effect on the SERS spectra in the case of the 3rd curve from bottom, concurrent with its activity on p300 (arrows show all the peaks existing in both the spectra) [93].

5.3.3 Interaction of small molecule inhibitor with p300

SERS was also used to probe p300-specific inhibitors, inducing specific alteration in the enzyme conformation. SERS was performed on p300 in complex with garcinol, isogarcinol (IG) and isogarcinol derivatives [105]. These aromatic ligands form hydrophilic and hydrophobic interactions with p300 resulting in structural changes in protein. Garcinol brought about changes in the amide bands in p300. IG is structurally different from garcinol in having an oxygen bridge instead of a hydroxyl group in garcinol. It causes large-scale changes to the structure of p300. The modes, which were affected in the case of garcinol, were also affected in the case of IG. In addition to that it also affects the modes related to Tyr, Trp, Phe and His. SERS of protein complex with the derivatives of IG like LTK-13, -14, -15 and -19 (the structure of these molecules are given in the [105]) were performed (see Fig. 7b). LTK-15 is an inactive IG derivative and showed no change in SERS spectrum (see Fig. 7b H). LTK-13 brought about changes in the SERS spectrum of p300 only in the areas of amide modes and certain carboxylic groups of the aliphatic amino acids (see Fig. 7b E). But in the case of LTK-14 and LTK-19 there were large-scale changes in the SERS spectra (see Fig. 7b F & 7b G), not only in amide modes, but also in modes corresponding to Tyr, Trp, Phe and His. SERS was therefore useful in elucidating the differential structural changes brought about by specific and non-specific inhibitors on binding to p300. Detailed docking studies would considerably help in the understanding the SERS response to elucidate the binding difference of specific and non-specific inhibitors of p300. The large size of p300 is in fact a practical difficulty in this case.

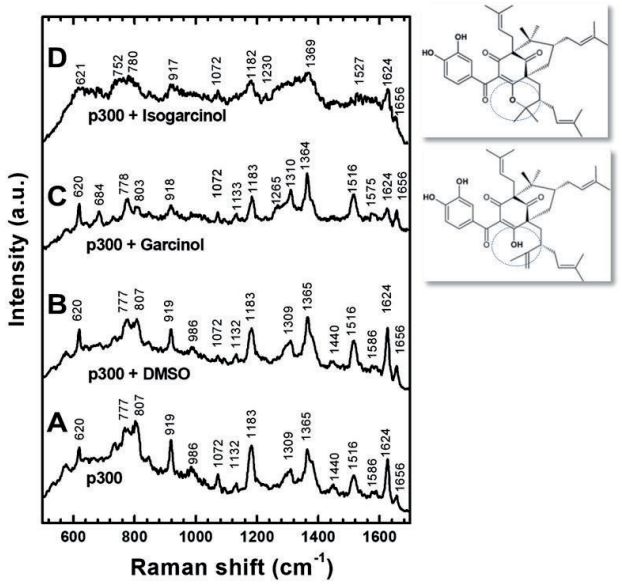

Figure 7a. SERS of (A) p300 and p300 complexed with (B) DMSO, (C) Garcinol and (D) isogarcinol. [95] The dotted circles show the altered functional groups in the molecules.
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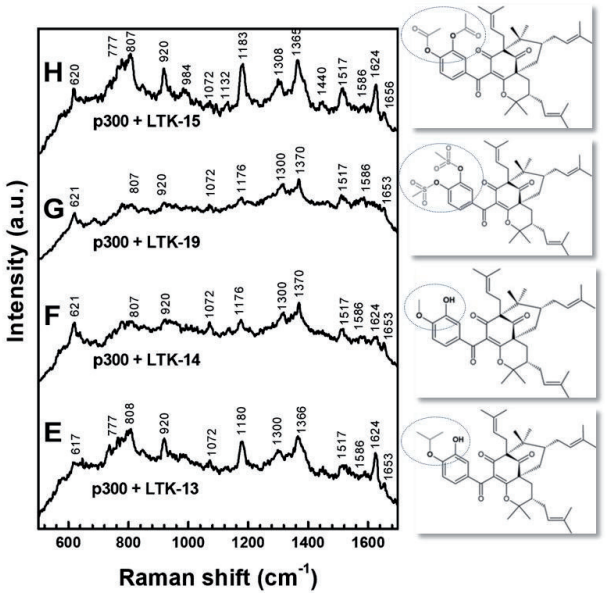

Figure 7b. SERS of p300 complexed with (E) LTK-13, (F) LTK-14, (G) LTK-19, and (H) LTK-15 [95]. The dotted circles show the altered functional groups in the molecules.
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6. Conclusion

In conclusion, SERS, hitherto being used for diagnostics, can now be looked at as a potential tool to understand small molecule protein interactions, without the knowledge of the structure of the protein in physiological conditions - very close to the biological conditions used in kinetic studies.. Therefore, this technique can soon be used by biologists and pharmacologists for studies on therapeutic proteins. Future of use of SERS in drug designing will depend on improvements in computational techniques to perform accurate docking studies and the development of computational techniques to understand the physics of SERS. Small steps have already been taken in this direction and there are a few publications coming in the immediate future to propel this field further.

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8. References

[1] X. X. Han, B. Zhao, Y. Ozaki, "*Surface-enhanced Raman scattering for protein detection*" Analytical and Bioanalytical Chemistry, vol. 394, pp 1719-1727, 2009.

[2] Hering et al. "*SERS: a versatile tool in chemical and biochemical diagnostics*", Analytical and Bioanalytical Chemistry, vol. 390, pp 113-124, 2008.

[3] D. Wishart, "*NMR spectroscopy and Protein Structure Determination: Applications to Drug Discovery and Development*", Current Pharmaceutical Biotechnology, vol. 6, pp 105-120, 2005.

[4] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, "*The Protein Data Bank*", Nucleic Acids Research, vol. 28, pp 235-242, 1999.

[5] S. D. Durbin, G. Feher, "*Protein crystallization*", Annual Review of Physical Chemistry, vol. 47, pp 171-204, 1996.

[6] A. M. Davis, S. J. Teague, *G. J. Kleywegt, "Application and Limitations of X-ray Crystallographic Data in Structure-Based Ligand and Drug Design*", Angewandte *Chemie International Edition, vol. 42, pp 2718- 2736, 2003.*

[7] *D. Staunton, J. Owen and I. D. Campbell, "NMR and Structural Genomics*", Accounts of Chemical Research, vol. 36, pp 207-214, 2003.

[8] G. Wagner, "*Prospects for NMR of large proteins*", Journal of Biomolecular NMR, vol. 3, pp 375-385, 1993.

[9] R. Vogel and F. Siebert, "*Vibrational spectroscopy as a tool for probing protein function*", Current opinion in Chemical Biology, vol. 4, pp 518-523, 2000.

[10] W. K. Surewicz, H. H. Mantsch and D. Chapman, *"Determination of protein secondary structure by Fourier transform infrared spectroscopy*", Biochemistry, vol. 32, pp 389-394, 1993.

[11] Z. Chi, X.G. Chen, J.S.W. Holtz and S.A. Asher, "*UV Resonance Raman-Selective Amide Vibrational Enhancement: Quantitative Methodology for Determining Protein Secondary Structure*", Biochemistry, vol. 37, pp 2854-2864, 1998.

[12] E. Goormaghtigh, J. M. Ruysschaert and V. Raussens, "*Evaluation of the information content in infrared spectra for protein secondary structure determination*", Biophysical Journal, vol. 90, pp 2946-2957, 2006.

[13] R. W. Williams, A. K. Dunker, "*Determination of the secondary structure of proteins from the amide I band of the laser Raman spectra*", Journal of Molecular Biology, vol. 152, pp 783-813, 1981.

[14] J.T. Pelton, L.R. McLean, "*Spectroscopic Methods for Analysis of Protein Secondary Structure*", Analytical Biochemistry, vol. 277, pp 167-176, 2000.

[15] P. Carey, "*Raman spectroscopy for the analysis of biomolecules*", TrAC Trends in Analytical Chemistry, vol. 2, pp 275-277, 1983.

[16] R. Petry, M. Schmitt and J. Popp, "*Raman spectroscopy - A Prospective Tool in the Life Sciences*", ChemPhysChem, vol. 4, pp 14-30, 2003.

[17] S. P. Mulvaney and C. D. Keating, "*Raman Spectroscopy*", Analytical Chemistry, vol. 72, pp 145R-157R, 2000.

[18] L. J. Hogg, H. G. M. Edwards, D.W. Farwell, A.T. Peters, "*FT Raman spectroscopic studies of wool*", Journal of Society of Dyers and Colourists, vol. 110, pp 196-199, 1994.

[19] A. C. Williams, H.G. M. Edwards, B. W. Barry, "*Raman spectra of human keratotic biopolymers: Skin, callus, hair and nail*", Journal of Raman Spectroscopy, vol. 25, pp 95-98, 1994.

[20] H.G.M. Edwards, *D.W. Farwell, J.M. Holder, E.E. Lawson, "Fourier-transform Raman spectroscopy of ivory: II. Spectroscopic analysis and assignments"*, Journal of Molecular Structure, vol. 435, pp 49-58, 1997.

[21] V. R. Kodati, G. E. Tomasi, J. L. Turumin and A.T. Tu, "*Raman Spectroscopic Identification of Phosphate-Type Kidney Stones*", Applied Spectroscopy, vol. 45, pp 581-583, 1991.

[22] S. A. Overman , G. J. Thomas Jr., " *Raman Spectroscopy of the Filamentous Virus Ff (fd, f1, M13): Structural Interpretation for Coat Protein Aromatics*", Biochemistry, vol. 34, pp 5440-5451.

[23] C. Xie and Y. Li, "*Confocal micro-Raman spectroscopy of single biological cells using optical trapping and shifted excitation difference techniques*", Journal of Applied Physics, vol. 93, pp 2982-2986, 2003.

[24] R. E. Kast, G. K. Serhatkulu, A. Cao, A. K. Pandya, H. Dai, J. S. Thakur, V. M. Naik, R. Naik, G. W. Auner, R. Rabah, "*Raman spectroscopy can differentiate malignant tumors from normal breast tissue and detect neoplastic changes in a mouse model*", Biopolymers, vol. 89, pp 235-241, 2008.

[25] G. J. Thomas Jr., "*Raman spectroscopy of protein and nucleic acid assemblies*", Annual Review of Biophysics and Biomolecular Structure, vol. 28, pp 1-27, 1999.

[26] A Campion and P. Kambhapati, "*Surface-enhanced Raman scattering*", Chemical Society Reviews, vol. 27, pp 241-250, 1998.

[27] K. Kneipp, M. Moskovits, H. Kneipp, "*Surface-Enhanced Raman Scattering*", Topics in Applied Physics (Physics and Applications Series, Springer), vol. 103, 2006

[28] S. Nie, S. R. Emory, "*Probing Single Molecules and Single Nanoparticles by Surface-Enhanced Raman Scattering*", Science, vol. 275, pp 1102-1106.

[29] S. L. McCall, P. M. Platzman, P.A. Wolff, "*Surface enhanced Raman scattering*", Physics Letters A, vol. 77, pp 381-383, 1980.

[30] X. M. Lin, Y. Cui, Y.H. Xu, B. Ren, Z. Q. Tian, "*Surface-enhanced Raman spectroscopy: substrate-related issues*", Analytical and Bioanalytical Chemistry, vol. 394, pp 1729-1745, 2009.

[31] S. Schlucker, "*SERS microscopy: Nanoparticle Probes and Biomedical Applications*", ChemPhysChem, vol. 10, pp 1344-1354, 2009.

[32] T. A. Laurence, G. Braun, C. Talley, A. Schwartzberg, M. Moscovitz, N. Reich and Thomas Huser, "*Rapid, Solution-Based Characterization of Optimized SERS Nanoparticle Substrates*", Journal of American Chemical Society, vol. 131, pp 162-169, 2009.

[33] M. Fleischmann, P. J. Hendra and A. J. McQuillan, "Raman spectra of pyridine adsorbed at a silver electrode", Chemical Physics Letters, vol. 26, pp. 163-166, 1974.

[34] J.Hu, B. Zhao, W. Xu, B. Li, Y. Fan, "*Surface-enhanced Raman spectroscopy study on the structure changes of 4-mercaptopyridine adsorbed on silver substrates and silver colloids*", Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 58, pp 2827-2834, 2002.

[35] P.L. Stiles, J. A. Dieringer, N.C. Shah and R. P. Van Duyne, "*Surface-Enhanced Raman Spectroscopy*", Annual Review of Analytical Chemistry, vol1, pp 601-626, 2008.

[36] E. J. Zeman, G. C. Schatz, "*An accurate electromagnetic theory study of surface enhancement factors for silver, gold, copper, lithium, sodium, aluminum, gallium, indium, zinc and cadmium*", Journal of Physical Chemistry, vol. 91, pp 634-643, 1987.

[37] J. M. Pitarke, V. M. Silkin, E. V. Chulkov and P. M. Echenique, "*Theory of surface plasmons and surface-plasmon polaritons*", Reports on Progress in Physics, vol. 70, pp 1-87, 2007.

[38] G. A. Baker, D. S. Moore, "*Progress in plasmonic engineering of surface-enhanced Raman-scattering substrates toward ultra-trace analysis*", Analytical and Bioanalytical Chemistry, vol. 382, pp 1751-1770, 2005.

[39] M. I. Stockman, "*Electromagnetic Theory of SERS*", Surface-enhanced Raman scattering-Topics in Applies Physics, vol. 103, pp 47-65, 2006.
[40] M. K. Hossain and Y. Ozaki, "*Surface-enhanced Raman scattering; facts and inline trends*", Current Science, vol. 97, pp 192-201, 2009.
[41] R. Aroca, "*Surface-Enhanced Vibrational Spectroscopy*", Wiley, New York, 2006.
[42] K. Kneipp, M. Moskovitz, H. Kneipp, Surface-Enhanced Raman Scattering: Physics and Applications, Topics in Applied Physics;Springer: Berlin, vol. 103, , 2006.
[43] P. Alonso-González, P. Albella, M. Schnell, J. Chen, F. Huth, A. García-Etxarri, F. Casanova, F. Golmar, L. Arzubiaga, L.E. Hueso, J. Aizpurua and R. Hillenbrand, "*Resolving the electromagnetic mechanism of surface-enhanced light scattering at single hot spots*", Nature Communications, vol. 3, 684, 2012.
[44] H. Cang, A. Labno, C. Lu, X. Yin, M. Liu, C. Gladden, Y. Liu and X. Zhang, "*Probing the electromagnetic field of a 15-nanometre hotspot by single molecule imaging*", Nature, vol. 469, pp. 385-389, 2011.
[45] P. Johansson, H. Xu and M. Kall, "*Surface-enhanced Raman scattering and fluorescence near metal nanoparticles*", Physical Review B, vol. 72, 035427, 2005.
[46] K. Yoshida, T. Itoh, H. Tamaru, V. Biju, M. Ishikawa and Y. Ozaki, "*Quantitative evaluation of electromagnetic enhancement in surface-enhanced resonance Raman scattering from plasmonic properties and morphologies of individual Ag nanostructures*", Physical Review B, vol. 81, 115406, 2010.
[47] Y. Fang, N.-H. Seong and D. D. Dlott, "*Measurement of the distribution of site enhancements in surface-enhanced Raman scattering*", Science, vol. 321, pp. 388–392, 2008.
[48] M.L. Weber and K. A. Willets, "*Correlated Super-Resolution Optical and Structural Studies of Surface-Enhanced Raman Scattering Hot Spots in Silver Colloid Aggregate*", The Journal of Physical Chemistry Letters, vol. 2, pp. 1766-1770, 2011.
[49] P. G. Etchegoin and E. C. Le Ru, "*Resolving Single Molecules in Surface-Enhanced Raman Scattering within the Inhomogeneous Broadening of Raman Peaks*", Analytical Chemistry, vol. 82, pp. 2888-2892.
[50] Z. Wang, S. Pan, T. D. Krauss, H. Du and L. J. Rothberg, "*The structural basis for giant enhancement enabling single-molecule Raman scattering*", Proceedings of the National Academy of Sciences, vol. 100, pp. 8638-8643, 2003.
[51] L. Qin, S. Zou, C. Xue, A. Atkinson, G. C. Schatz and C. A. Mirkin, "*Designing, fabricating and imaging Raman Hot spots*", Proceedings of the National Academy of Sciences, vol. 103, pp. 13300-13303, 2006.
[52] D.-K.. Lim, K.-S, Jeon, H. M. Kim, J.-M. Nam and Y. D. Suh, "*Nanogap-engineerable Raman-active nanodumbbells for single-molecule detection*", Nature Materials, vol. 9, pp. 60-67, 2010.
[53] A. Otto, I. Mrozek, H. Grabhorn and W. Akermann, "*Surface-enhanced Raman scattering*", Journal of Physics: Condensed Matter, vol. 4, pp 1143-1212, 1992.
[54] J. De Gelder, K. De Gussem, P. Vandenabeele, L. Moens, "*Reference database of Raman spectra of biological molecules*", Journal of Raman Spectroscopy, vol. 38, pp 1133-1147, 2007.
[55] R. Schweitzer-Stenner, "*Structure and dynamics of biomolecules probed by Raman spectroscopy*", Journal of Raman Spectroscopy, vol. 36, pp 276-278, 2005.
[56] R. Tuma, "*Raman spectroscopy of proteins: from peptides to large assemblies*", Journal of Raman Spectroscopy, vol. 36, pp 307-319, 2005.
[57] T. G. Spiro and B. P. Gaber, "*Laser Raman Scattering as a Probe of Protein Structure*", Annual Review of Biochemistry, vol. 46, pp 553-570, 1977.
[58] Z. Wen, "*Raman Spectroscopy of Protein Pharmaceuticals*", Journal of Pharmaceutical Sciences, vol. 96, pp 2861-2878, 2007.
[59] C. L. Haynes, A. D. McFarland, R. P. Van Duyne, "*Surface Enhanced Raman Spectroscopy*", Analytical Chemistry, pp 338A-346A, 2005.
[60] E. Koglin, J. Sequaris, "*Surface enhanced Raman scattering of biomolecules*", Topics in Current Chemistry, Springer, Berlin, vol. 134, pp 1-57, 1986.
[61] E. Podstawka, Y. Ozaki and L. M. Proniewicz, "*Part I: Surface Enhanced Raman Spectroscopy of Amino Acids and Their Homodipeptides Adsorbed on Colloidal Silver*", Applies Spectroscopy, vol. 58, pp 570-580, 2004.
[62] E. Podstawka, Y. Ozaki and L. M. Proniewicz, "*Part II: Surface Enhanced Raman Spectroscopy Investigation of Methionine Containing Heteropeptides Adsorbed on Colloidal Silver*", Applies Spectroscopy, vol. 58, pp 581-590, 2004.
[63] E. Podstawka, Y. Ozaki and L. M. Proniewicz, "*Part III: Surface-Enhanced Raman Scattering of Amino Acids and Their Homopeptide Monolayers Deposited onto Colloidal Gold Surface*", Applied Spectroscopy, vol. 59, pp. 1516-1526, 2005.
[64] E. Podstawka, Y. Ozaki and L. M. Proniewicz, "*Adsorption of S-S Containing Proteins on a Colloidal Silver Surface Studied by Surface- Enhanced Raman Spectroscopy*", Applied Spectroscopy, vol. 58, pp. 1147-1156, 2004.
[65] E. Podstawka, E. Sikorska, L. M. Proniewixz, B. Lammek, "*Raman and surface-enhanced Raman spectroscopy investigation of vasopressin analogues containing 1-aminocyclohexane-1-carboxylic acid residue*", Biopolymers, vol. 83, pp. 193-203, 2006.
[66] E. Podstawka, "*Investigation of molecular structure of bombesin and its modified analogues nonadsorbed and adsorbed on electrochemically roughened silver surface*", Biopolymers, vol. 89, pp. 506-521, 2008.
[67] S. Stewart, P. M. Fredericks, "*Surface-enhanced Raman spectroscopy of peptides and proteins adsorbed on an electrochemically prepared silver surface*", Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 55, pp. 1615-1640, 1999.

[68] J. Hu, R. S. Sheng, Z. S. Xu, Y. Zeng, "*Surface enhanced Raman spectroscopy of lysozyme*", Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 51, pp. 1087-1096, 1995.

[69] J. S. Suh, M. Moskovitz, "*Surface-enhanced Raman spectroscopy of amino acids and nucleotide bases adsorbed on silver*", Journal of American Chemical Society, vol. 108, pp. 4711-4718, 1986.

[70] C. Gullekson, L. Lucas, K. Hewitt, L. Kreplak, "*Surface-sensitive Raman spectroscopy of collagen I fibrils*", Biophysical Journal, vol. 100, pp. 1837-1845, 2011.

[71] N. P. W. Pieczonka, P. J. G. Goulet and R. F. Aroca, "*Chemically Selective Sensing through Layer-by-Layer Incorporation of Biorecognition into Thin Film Substrates for Surface-Enhanced Resonance Raman Scattering*", Journal of American Chemical Society, vol. 128, pp. 12626-12627, 2006.

[72] A. J. Bonham, G. Braun, I. Pavel, M. Moskovits and N. O. Reich, "*Detection of Sequence-Specific Protein-DNA Interactions via Surface Enhanced Resonance Raman Scattering*", Journal of American Chemical Society, vol. 129, pp. 14572-14573, 2007.

[73] J. D. Driskell, J. M. Uhlenkamp, R. J. Lipert and M.D. Porter, "*Surface-Enhanced Raman Scattering Immunoassays Using a Rotated Capture Substrate*", Analytical Chemistry, vol. 79, pp. 4141-4148, 2007.

[74] F. Domenici, A.R. Bizzarri and S. Cannistraro, "*SERS-based nanobiosensing for ultrasensitive detection of the p53 tumor suppressor*", vol. 6, pp. 2033-2042, 2011.

[75] B. C. Galarreta, P.R. Norton and F. L. Labarthet, "*SERS detection of Streptavidin/Biotin Monolayer Assemblies*", Langmuir, vol. 27, pp. 1494-1498, 2011.

[76] H. Li, J. Sun, B. M. Cullum, "*Label-Free Detection of Proteins Using SERS- Based Immuno- Nanosensors*", NanoBiotechnology, vol. 2, pp. 17-28, 2006.

[77] F. Domenici, A.R. Bizzarri and S. Cannistraro, "*Surface-enhanced Raman scattering detection of wild-type and mutant p53 proteins at very low concentration in human serum*", Analytical Biochemistry, vol. 421, pp. 9-15, 2012.

[78] R. A. Copeland, S. P. A. Fodor, T. G. Spiro, "*Surface-enhanced Raman spectra of an active flavoenzyme: glucose oxidase and riboflavin binding protein on silver particles*". Journal of American Chemical Society, vol. 106, pp. 3872-3874, 1984.

[79] R. E. Holt, T. M .Cotton, "*Surface-enhanced resonance Raman and electrochemical investigation of glucose oxidase catalysis at a silver electrode*", Journal of American Chemical Society, vol. 111, pp. 2815-2821, 1989.

[80] N. S. Lee, Y. X. Hsieh, M. D. Morris, L. M. Schopfer, "*Reinterpretation of surface-enhanced resonance Raman scattering of flavoproteins on silver colloids*", Journal of American Chemical Society, vol. 109, pp. 1358-1363, 1987.

[81] E. S. Grabbe, R. P. Buck, "*Surface-enhanced Raman spectroscopic investigation of human immunoglobulin G adsorbed on a silver electrode*", Journal of American Chemical Society, vol. 111, pp. 8362-8366, 1989.

[82] M. Casella, A. Lucotti, M. Tommasini, M. Bedoni, E. Forvi, F. Gramatica, G. Zerbi, "*Raman and SERS recognition of β-carotene and haemoglobin fingerprints in human whole blood*", Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 79, pp. 915-919, 2011.

[83] T. M. Cotton, S. G. Schultz, R. P. Van Duyne, "*Surface-enhanced resonance Raman scattering from cytochrome c and myoglobin adsorbed on a silver electrode*", Journal of American Chemical Society, vol. 102, pp. 7960-7962, 1980.

[84] J. D. Driskell, S. Shanmukh, Y. Liu, S. Chaney, S. Hennigan, L. Jones, D. Krause, R. A. Tripp, Y. P. Zhao, R. A. Dluhy, "*Novel Nanoarray SERS Substrates Used for High Sensitivity Virus Biosensing and Classification*", ACS Symposium Series, ch. 8, pp. 99-114, 2009.

[85] W. R. Premasiri, D. T. Moir, M. S. Klempner, N. Krieger, G. Zones and L. D. Ziegler, "*Characterization of the Surface Enhanced Raman Scattering (SERS) of Bacteria*", The Journal of Physical Chemistry B, vol. 109, pp. 312-320, 2005.

[86] G. D. Chumanov, R. G. Efremov, I. R. Nabiev, "*Surface-enhanced Raman spectroscopy of biomolecules. Part I.- water-soluble proteins, dipeptides and amino acids*", Journal of Raman Spectroscopy, vol. 21, pp. 43-48, 1990.

[87] G. D. Chumanov, R. G. Efremov, I. R. Nabiev, "*Surface-enhanced Raman spectroscopy of biomolecules. Part II. Application of short- and long-range components of SERS to the study of the structure and function of membrane proteins*", Journal of Raman Spectroscopy, vol. 21, pp. 49-53, 1990.

[88] R. Nabiev, V. A. Savchenko, E. F. Efremov, "*Surface-enhanced Raman spectra of aromatic amino acids and proteins adsorbed by silver hydrosols*", Journal of Raman Spectroscopy, vol. 14, pp. 375-379, 1983.

[89] J. Kneipp, H. Kneipp, A. Rajaduraim R. W. Redmond and K. Kneipp, "*Optical probing and imaging of live cells using SERS labels*", Journal of Raman Spectroscopy, vol. 40, pp. 1-5, 2009.

[90] G.V. P. Kumar, C. Narayana, "*Adapting a fluorescence microscope to perform surface enhanced Raman spectroscopy*", Current Science, vol. 93, pp. 778-781, 2007.

[91] G. V. P. Kumar, B. A. A. Reddy, M. Arif, T. K. Kundu and C. Narayana, "*Surface-Enhanced Raman Scattering Studies of Human Transcriptional Coactivator p300*", Journal of Physical Chemistry B, vol. 110, pp. 16787-16792, 2006.

[92] G. V. P. Kumar, R. Selvi, H. Kishore, T. K. Kundu, C. Narayana, "*Surface enhanced Raman spectroscopic studies of coactivator associated arginine methyltransferase 1*", Journal of Physical Chemistry B, vol. 112, pp. 6703-6707, 2008.

[93] N. G. Iyer, H. Ozdag and C. Caldas, "*p300/CBP and Cancer*", Oncogene, vol. 23, pp. 4225-4231, 2004.

[94] M. Arif, G.V. P. Kumar, C. Narayana and T. Kundu, "*Autoacetylation Induced Specific Structural Changes in Histone Acetyltransferase Domain of p300: Probed by Surface Enhanced Raman Spectroscopy*", The Journal of Physical Chemistry B, vol. 111, pp. 11877-11879, 2007.

[95] A. Barth and C. Zscherp, "*What vibrations tell us about proteins*", Quarterly Reviews of Biophysics, vol. 35, pp. 369-430, 2002.

[96] X. X. Han, Y. Kitahama, Y. Tanaka, J. Guo, W. Q. Xu, B. Zhao and Y. Ozaki, "*Simplified Protocol for Detection of Protein-Ligand Interactions via Surface-Enhanced Resonance Raman Scattering and Surface-Enhanced Fluorescence*", Analytical Chemistry, vol. 80, pp. 6567-6572, 2008.

[97] T. Vo-Dinh, F. Yan and M.B. Wabuyele, "*Surface-enhanced Raman scattering for medical diagnostics and biological imaging*", Journal of Raman Spectroscopy, vol. 36, pp. 640-647, 2005.

[98] X. X. Han, L. Chen, J. Guo, B. Zhao and Y. Ozaki, "*Coomassie Brilliant Dyes as Surface Enhanced Raman Scattering Probes for Protein-Ligand Recognitions*", Analytical Chemistry, vol. 82, pp. 4102-4106, 2010.

[99] V. P. Drachev and V. M. Shalaev, "*Biomolecule Sensing with Adaptive Plasmonic Nanostructures*", Surface-Enhanced Raman Scattering: Physics and Applications (eds: K. Kneipp, M. Moskovitz, H. Kneipp), Topics in Applied Physics, vol. 103, Springer, Berlin, 2006.

[100] P. Mišková*ský*, D. Jancura, S. Sanchez-Cortes, E. Kocisova and L. Chinsky, "*Antiretrovirally Active Drug Hypericin Binds the IIA Subdomain of Human Serum Albumin: Resonance Raman and Surface-Enhanced Raman Spectroscopy Study*", Journal of American Chemical Society, vol. 120, pp. 6374-6379, 1998.

[101] G. Fabriciova, S. Sanchez-cortes, J. V. Garcia-Ramos, P. Miskovsky, "*Surface-enhanced Raman spectroscopy study of the interaction of the antitumoral drug emodin with human serum albumin*", Biopolymers, vol. 74, pp. 125-130, 2004.

[102] Z. Jurasekova, G. Marconi, S. Sanchez-cortes, A. Torreggiani, "*Spectroscopic and molecular modeling studies on the binding of the flavonoid luteolin and human serum albumin*", Biopolymers, vol. 91, pp. 917-927, 2009.

[103] K. Mantelingu, A. H. Kishore, K. Balasubramanyam, G. V. P. Kumar, M. Altaf, S. N. Swamy, R. Selvi, C. Das, C. Narayana, K. S. Rangappa and T. K. Kundu, "*Activation of p300 Histone Acetyltransferase by Small Molecules Altering Enzyme Structure: Probed by Surface-Enhanced Raman Spectroscopy*", The Journal of Physical Chemistry B, vol. 111, pp. 4527-4534, 2007.

[104] X. Liu, L. Wang, K. Zhao, P. R. Thompson, Y. Hwang, R. Marmorstein and P. A. Cole, "*The structural basis of protein acetylation by p300/CBP transcriptional coactivator*", Nature, vol. 451, pp. 846-850, 2008.

[105] K. Mantelingu, B. A. A. Reddy, V. Swaminathan, A. H. Kishore, N. B. Siddappa, G. V. P. Kumar, G. Nagashankar, N. Natesh, S. Roy, P. P. Sadhale, U. Ranga, C. Narayana and T. K. Kundu, "*Specific Inhibition of p300-HAT Alters Global Gene Expression and Represses HIV Replication*", Chemistry and Biology, vol. 14, pp. 645-657, 2007.