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Temporal fluctuations in the SERRS spectra of single iron–protoporphyrin IX molecule

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Abstract

Surface enhanced resonance Raman spectra of Fe–protoporphyrin IX, adsorbed on silver colloidal nanoparticles immobilized onto a polymer-coated glass slide have been investigated at very low concentrations. The spectra exhibit drastic temporal fluctuations on a time scale of seconds in both line frequency and intensity; such a trend suggesting that the single molecule limit is approached. Sequences of spectra have been analyzed in terms of an underlying continuum and of Raman peaks superimposed on this continuum. A statistical analysis of the spectrum intensity has allowed us to put into evidence that main contribution to the intensity fluctuations arises from the continuum. In addition, a high correlation between the total integrated intensity and the intensity detected at different Raman peaks has been revealed. Furthermore, the ratio between the intensity detected in correspondence of different FePP vibrational modes shows a temporal variability likely reflecting the intrinsic dynamics of the molecule. All these findings have been ascribed to a desorption–adsorption mechanism of the molecules at the silver surface.
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1. Introduction

The capability to detect spectroscopic signals from single molecules has recently opened the route to the investigation of new aspects normally masked by the ensemble average [1–5]. Moreover, detection and manipulation of single molecules offers the opportunity to develop new devices at the nanometer scale such as biosensors and electronic devices [6–8]. Applications in these emerg-ing fields require, in addition to a high sensitivity detection, a detailed knowledge of the temporal behaviour of single molecules and a great ability in controlling the spatial location of these molecules. On the other hand, the possibility of following the temporal evolution of a single molecule in com-parison with the ensemble behaviour relates to the fundamental question concerning the ergodic be-haviour of molecules [9].

Different spectroscopic approaches have so far been applied and developed up to reach single molecule detection [1,4,5]. One of these is surface-enhanced Raman spectroscopy (SERS) [10]. Ad-sorption of molecules onto both nanometer-sized metallic particles or rough metallic islands may

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result into a dramatic enhancement of the Raman cross-section (up to 10^{14} times) [10,11]. A further increase in sensitivity can be sometimes achieved by coupling SERS with Resonance Raman (SERRS). In such a way, the extremely low efficiency of the Raman spectroscopy can be overcome up to reach single molecule detection for both organic [12–17] and even protein molecules [3,18,19]. Besides single molecule detection, SERS offers the remarkable possibility, in principle, of identifying and characterizing a single molecule by virtue of its vibrational spectrum.

Although the prerequisites for surface enhancement of the Raman signals are only partially understood, it appears clear its dependence on a variety of parameters, such as metallic particles size and composition, aggregation and excitation wavelength [10–12]. The enhancement of the SERS and SERRS cross-sections has been generally attributed to two, likely cooperating, phenomena: the enhancement of the local electromagnetic (EM) field and the charge transfer (CT) phenomenon [10,11]. The EM effect, related to surface plasmon excitations, is due to the pronounced perturbation that some rough metal surfaces can cause to the incident and scattered EM fields [10]. Such an effect, depending on the microscopic shape of the metallic surface and decreasing progressively with the distance from the surface, has been suggested to be very significant in the cavities between adjacent particles [20]. The CT mechanism, generally provides a smaller contribution to the total enhancement and has been attributed to photon-driven CT between the molecule electronic states and the metal; such a process requiring specific interactions of the adsorbed molecule with the metal surface [21].

A peculiar, common feature of single molecule spectra is the appearance of temporal spectral fluctuations that are generally attributed to a deviation from the ensemble average [4,22]. SERS spectra additionally exhibit abrupt intensity, and or frequency changes, or depth spectral jumps and the presence of a broad band white continuum underneath the Raman lines; such a continuum also varying in intensity and shape as a function of time [2,3,13,19,23,24]. All these phenomena, which cannot be simply explained as deviations from the ensemble average, have tentatively been interpreted in terms of a variety of possible mechanisms. For example, it has been suggested that the spectral fluctuations might arise from a variety of CT contribution likely induced by a modulation in the adsorption process of the molecules at the metal surface [13]. In this connection, it has been shown that a H_2O_2 treatment of silver colloidal particles, preventing the adsorption of horseradish peroxidase, causes the loss of the single molecule SERRS signal [18].

Further experimental and theoretical investigations are required for a deeper understanding of the enhancement processes at the origin of single molecule SERRS signals and, at the same time, to clarify the occurrence of the fluctuations. A statistical analysis of these fluctuations represents an appropriate tool to analyze the main features of the enhancement temporal behaviour, offering also a basis for a theoretical interpretation of SERRS mechanisms.

Recently, we have studied the temporal fluctuations appearing in the single molecule SERRS spectra of myoglobin in both solution and immobilized systems [19]. We have found that the fluctuations could be ascribed to the entanglement of the single protein molecule into the local minima of the energy landscape during its dynamical evolution. Here, with the aim to better investigate the features of these fluctuations, and to get rid of the possible contribution from the protein milieu, we have focussed our attention on the SERRS spectra of Fe–protoporphyrin IX (FePP) adsorbed on silver nanoparticles. FePP, besides to being the prosthetic groups of many important proteins, such as hemoglobin, myoglobin and cytochromes, in which it plays a relevant biological role, it could be an appropriate, robust compound for applicative devices [25,26].

The SERRS spectra of located, brightly emitting colloidal particles of about 70 nm diameter, incubated with FePP at the very low concentration of 10^{-11} M, and immobilized onto a polymer-coated glass surface have been registered. The total integrated intensity and the intensity detected in correspondence of the main Raman vibrational modes have been analyzed, for sequences of 100 SERRS spectra, in terms of an underlying continuum and

of Raman peaks appearing over this continuum. A statistical analysis of the vibrational modes has revealed that the largely dominant contribution due to the continuum is highly correlated with the total intensity. Moreover, the Raman peaks emerging over the continuum displays a different temporal behaviour for the in-plane and out-of-plane vibrational modes. The results are discussed in connection with the related data on FePP and in terms of a desorption–adsorption mechanism of the FePP molecules at the silver surface.

2. Experimental

2.1. Sample preparation, apparatus and analysis

Solutions of colloidal silver have been prepared by standard citrate reduction of $AgNO_3$ (Sigma) by following the procedure of Lee and Meisel [27]. The colloidal optical extinction spectrum reveals the same features reported in the literature [3,21,24]. Accordingly, the concentration of silver particles can be estimated to be about 10^{-11} M, corresponding to about 7×10^{12} particles per liter. As activation agent NaCl has been added to reach the final concentration of 0.25 mM.

Fe–protoporphyrin IX (Sigma) has been dissolved into a KOH solution 0.5 M at pH 12 at a concentration of 10^{-4} M. An aliquot of successive dilutions of this solution has been incubated with silver colloidal suspension for 5 h at room temperature to obtain a final concentration of 10^{-11} M with an approximate ratio of 1:1 between molecules and colloidal particles.

A droplet, with an approximate volume of 10 μl, of this solutions has been deposited onto a glass slide, of area 15×15 mm^2, coated with polymerized 3-aminopropyltriethoxysilane (APES, Sigma). The glass slide coating which is able to bind Ag colloids through bonds between the metal particles and the amino-group of APES, have been assembled by following the procedure reported in [28]: cleaned glass slides (3:1 H_2SO_4:H_2O_2, incubated for 30 min), have been immersed in a solution of 1 ml of APES in 15 ml of chloroform for 3 min. Then, the slides have been rinsed with chloroform and rinsed twice with MilliQ H_2O.

A characterization of immobilized silver colloidal particles, performed by atomic force microscopy (AFM) under ambient conditions, has revealed that the colloids consist of single spherical and rod-shaped particles and aggregates of two, three, four up to many particles; the heterogeneous size particle distribution being characterized by an average diameter of about 70 nm [19].

The SERRS spectra have been recorded through a Jobin–Yvon Labram confocal system by exciting with the 632.8 nm radiation line provided by a He–Ne 15 mW laser. The microscope (equipped with a 100× objective with NA = 0.9) is confocally coupled to a 300 mm focal length spectrograph with a 1800 grooves/mm grating (optimized in the red). A CCD detector Peltier-cooled to 223 K has been employed to record the data. The spectral resolution is lower than 5 cm^{-1}. The laser spot size is about 1 μm in diameter.

To estimate the signal to noise level expected in our measurements, it should be taken into account that the CCD-chip has, at a wavelength of 632 nm, a quantum efficiency of about 0.45 and it produces one count per three collected photons. The total noise N_t of a signal can be determined as the root sum of squares of the different noise components: $N_t = [N_{SN}^2 + N_R^2 + N_D^2]^{1/2}$ where the read out noise N_R and the dark charge noise N_D are specified by the chip manufacturer; N_{SN} is the shot noise associated with the signal and whose amplitude is given by the square root of the measured signal. For our CCD-chip, N_R is 4 electrons r.m.s. and N_D is one electron/pixel/min. In a typical single molecule SERRS experiment, a rate of about 50–300 counts/s are usually detected, by taking into account the raw data which include an underlying continuum. In these conditions, the dominant noise source is the inevitable shot noise associated with the signal itself. Accordingly, a total noise of about 7–18 counts is expected for measurements obtained for an integration time of one second.

As long as the shot noise is dominant, a Poisson distribution with a standard deviation equal to N_t, is expected to describe the spread of the Raman intensity around the average value. However, since the quite large number of Raman events, a Gaussian distribution, with a half height width σ given by

N_t, generally well approximates the intensity spread of a Raman signal [14].

3. Results

Immobilized Ag colloids incubated with FePP at a concentration of 10^{-11} M generally reveal, by manually scanning the sample under the microscope objective of the Raman equipment, sites with different average intensity. In particular, we have detected sites where only noise is registered, and spots characterized by brightly emitting particles with these sites representing only a small portion of the total and exhibiting rather similar average intensity.

Fig. 1 shows a selected collection of SERRS spectra recorded with 1 s of integration time from a typical bright site of FePP molecules immobilized onto Ag colloids. A great variability in the spectra can be observed; both the frequency and the relative intensity of the different peaks drastically varying from one spectrum to another. In addition, we note the presence of a continuum whose intensity significantly changes from spectrum to spectrum and over which the Raman peaks are superimposed.

Fig. 2 shows the SERRS spectra, from two different sites, named A and B, of immobilized FePP Ag colloidal sample as obtained by summing 100 successive SERRS spectra, each one recorded with 1 s of integration time. The spectra related to both sites A and B appear to be quite similar between them and, in addition, they result to be stable in time. Remarkably, the two SERRS spectra exhibit vibrational features practically coincident with those characterizing the resonance Raman spectrum of a dry sample from a solution of FePP at a relatively high concentration (10^{-4} M) (see the lowest curve in Fig. 2). This indicates that, when many spectra are summed (which is equivalent to collect spectra for longer integration times [19]) the time-invariant vibrational features of the heme group, as derived from a very large ensemble of molecules, are substantially recovered. We remark that the close similarity between the spectra obtained by summing many single molecule SERRS spectra and that derived from a sample of FePP rules out the presence of contaminating molecules which often affect the SERRS spectra of species at very low concentration [29].
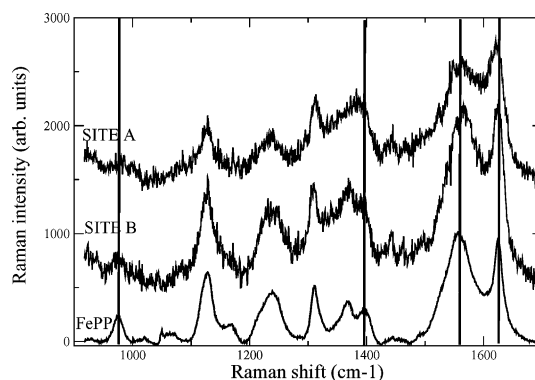
Fig. 2. SERRS spectra (Site A and site B curves) obtained by summing 100 SERRS spectra, each one recorded with 1 s of integration time, of immobilized silver colloidal particles incubated with FePP at a concentration of 10^{-11} M at two different sites and Resonance Raman spectrum (FePP curve) from a dried drop of FePP solution at a concentration of 10^{-4} M obtained with an integration time of 30 s. All spectra have been rescaled to the same relative intensity and a continuum has been subtracted. Line markers are located at: 977, 1394, 1560 and 1625 cm^{-1}. The laser exciting wavelength of 632.8 nm gives rise to a $\pi \rightarrow \pi^*$ optical transition, which lies in the tail of the Q band of the FePP absorption spectrum. The incident power is 15 mM.
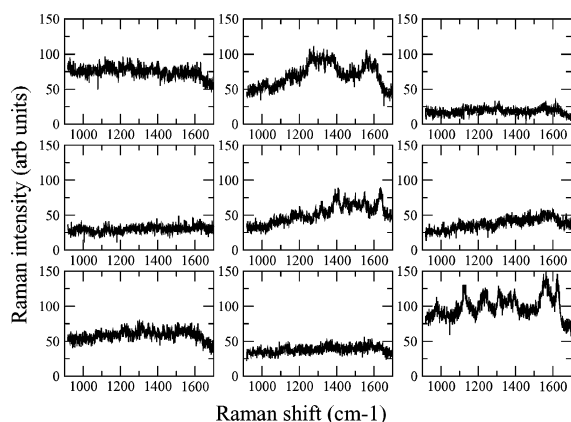

Fig. 1. Selected SERRS spectra as obtained with 1 s of integration time, and lasted 10 s between two successive measurements, from a bright site of immobilized silver colloidal solution incubated with FePP at a concentration of 10^{-11} M (protein to Ag particle ratio of 1:1). The laser spot size is about 1 μm in diameter.

As already mentioned, the appearance of drastic temporal fluctuations is a common phenomenon in the SERRS spectra when the target molecules are present at very low concentration [3,13,24]. Even if only qualitative results are available [2,3,13], it seems that the fluctuations observed in single molecule SERRS spectra are simultaneously present in both the broad continuum and the superimposed Raman peaks [16,24,30]. To deeper investigate such an aspect, we have undertaken a quantitative analysis of these fluctuations in the FePP SERRS spectra. In particular, we have separated the contribution to the overall signal arising from the broad continuum and from the superimposed sharp Raman peaks. First, we have calculated the total intensity by integrating the raw spectrum over the 900–1700 cm^{-1} region for the FePP molecules immobilized onto silver colloids at the sites A and B. The results are shown in Fig. 3 (continuous line) as a function of the spectrum number for a sequence of a hundred spectra. For both the sites, this integrated intensity varies remarkably from spectrum to spectrum. At the same time, the intensity of the continuum, as evaluated by a polynomial fit of the overall spectrum and again integrated over the 900–1700 cm^{-1} region (see dashed curves in Fig. 3) follows exactly the same trend detected for the total intensity. Actually, the intensity maxima appear just in correspondence of the same spectrum number at which maxima are observed for the total integrated intensity. On the other hand, the integrated intensity of the SERRS spectra after subtracting the continuum (see bold curves in Fig. 3) shows in general markedly lower values. Again, a variability from spectrum to spectrum can be observed and maxima are generally detected in correspondence of the maxima of the total integrated intensity.

These results point out that the most relevant contribution to the fluctuations in the spectrum intensity arises from the underlying continuum. However, a variability from spectrum to spectrum still persists after removing such a continuum. On such a ground, it would be interesting to analyze if the intensity detected in correspondence of the various Raman signals follows the same trend of the total intensity. Continuous curves in Fig. 4 show the intensity measured at 1625 cm^{-1} in the
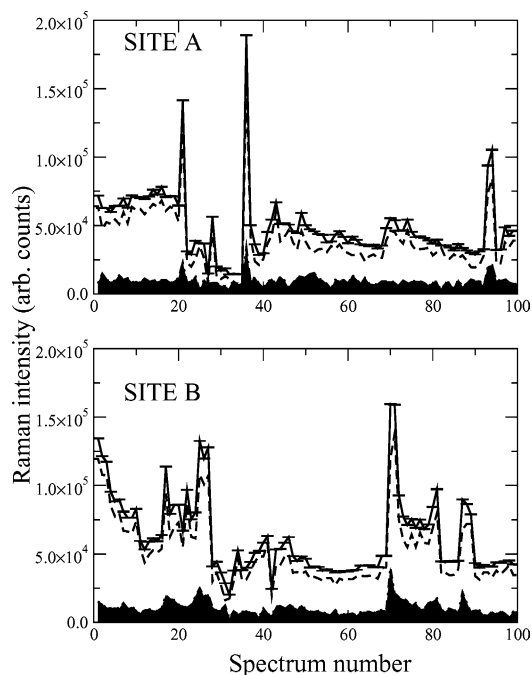

Fig. 3. Total intensity for a sequence of 100 SERRS spectra of single FePP molecule obtained by integrating each spectrum over the 900–1700 cm^{-1} region at two different sites (A and B). *Continuous line*: total intensity of the raw spectra (bars showing experimental errors); *dashed line*: total intensity of the underlying continuum in the spectra; *bold curve*: total intensity of the spectra after removing the underlying continuum. The total intensity of the underlying continuum has been obtained by carefully subtraction from each spectrum the residual background by a polynomial. For the error evaluation see Section 2. Each spectrum having been recorded with 1 s of integration time and about 10 s lasting from one measurement to the successive.

raw spectra as a function of the spectrum number for both the sites A and B. At this position, one of the most intense vibrational mode of FePP occurs. It can be observed a high variability which follows a trend with the spectrum number practically equivalent to that detected for the total intensity (see Fig. 3). After subtracting the continuum (see bold curves in Fig. 4), we note a lower intensity and a variability similar, for both the sites, to that of the total integrated intensity. The same behaviour has been also observed for the frequencies corresponding to the other main vibrational modes (results not shown). Therefore, the time variation of the Raman mode intensity seems to be
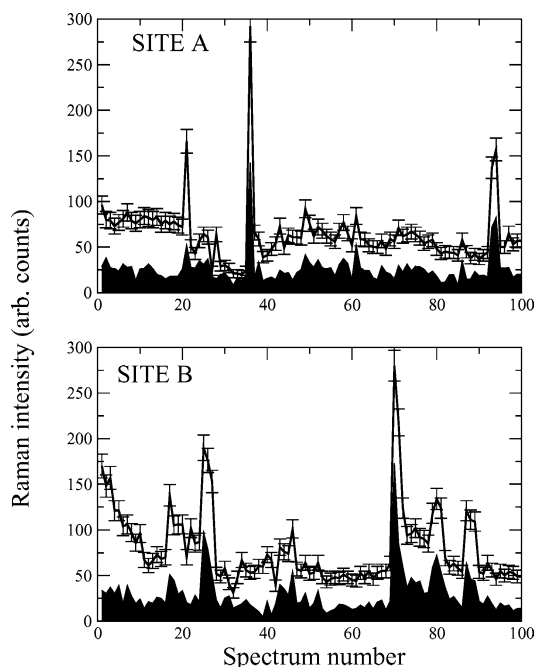
Fig. 4. Intensity of the peak at 1625 cm^{-1} for a sequence of 100 SERRS spectra of single FePP molecule from the raw spectra (*continuous line*) (bars showing experimental errors) and from the spectra after removing the underlying continuum (*bold curve*) at the two different sites A and B. For the error evaluation see Section 2.

strongly correlated to the total intensity. To analyze such an aspect on a more quantitative basis, we have calculated the cross-correlation between the intensity at a fixed frequency v_j and the total integrated intensity, ρ_{v_j}, at a 0 time delay, expressed by

$$\rho_{v_j} = \frac{\sum_i [I_i(v_j) - \overline{I(v_j)}][I_i^0 - \overline{I^0}]}{\{\sum_i [I_i(v_j) - \overline{I(v_j)}]^2 \sum_i [I_i^0 - \overline{I^0}]^2\}^{1/2}}, \quad (1)$$

where $I_i(v_j)$ and $\overline{I(v_j)}$ are the Raman intensity, detected at the wavelength v_j for the spectrum i and that averaged over a series of 100 spectra, respectively; I_i^0 and $\overline{I^0}$ are the total integrated intensity f or the spectrum i and that averaged over a series of 100 spectra, respectively. The calculated cross-correlation ρ_{v_j} values for different frequencies are reported in Table 1 together with the vibrational modes assignment. For the raw data ($\rho_{v_j}^0$), we obtain correlation values higher than 0.90 for all the analyzed lines. Lower values, but still higher than 0.50, are registered from the spectra after subtracting the continuum (ρ_{v_j}) for all the analyzed frequencies with the exception of the 1700 cm^{-1} at which a drastic decrease is observed after removing the continuum. Such a behaviour finds a correspondence with the fact that at 1700 cm^{-1} only noise is present. Indeed, while the total intensity still reflects a high correlation at any frequency irrespectively of the presence of a Raman mode, after removing the continuum, a correlation persists only in correspondence of the FePP vibrational modes. At this point, a crucial question is represented by the relative variability in time of the different vibrational modes. In other words, we wonder if different vibrational modes could be intermittently activated during the dynamical evolution of the FePP molecule. To investigate such an aspect, we have calculated the ratios between the intensities of the following Raman lines: 1394/977, 1560/977, 1394/1560, and 1560/1625 cm^{-1} for the spectra series from both the A and B sites. The analyzed ratios vary from spectrum to spectrum as shown in Fig. 5. Such a variability is particularly evident for the 1394/1560 and 1560/1625 cm^{-1} ratios with respect to the 1394/977 and 1560/977 cm^{-1} ratios. and, in general, exceeds the expected noise level

Table 1
Cross-correlation values between the intensity detected at a given frequency and the total Raman intensity as calculated from Eq. (1) from both the raw data ($\rho_{v_j}^0$) and the data after subtracting the continuum (ρ_{v_j})

Wave number (cm^{-1})		Assignment	$\rho_{v_j}^0$	ρ_{v_j}
977	γ (C$_a$H =)	Vinyl out of plane mode	0.94	0.54
1394	v	In plane skeletal mode	0.97	0.56
1560	v (C$_\beta$C$_\beta$)	In plane skeletal mode	0.96	0.74
1625	v (C$_a$ = C$_b$)	Vinyl in plane	0.94	0.70
1700		No vibrational modes	0.90	0.28

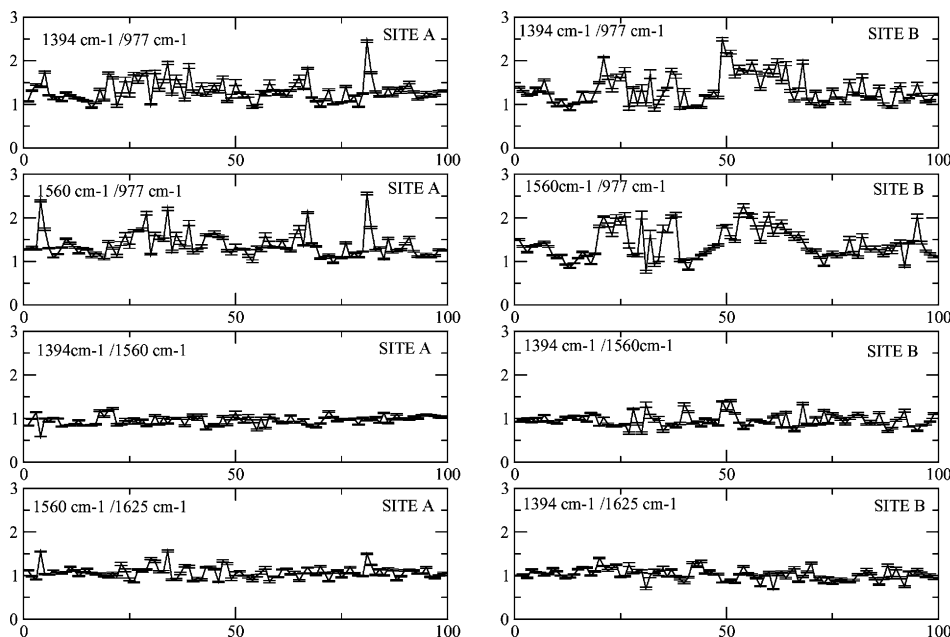Assignment of the vibrational bands of FePP (see also Fig. 2).

Fig. 5. Ratios between the intensities of specific vibrational bands for a sequence of 100 SERRS spectra of single FePP molecule for the two different sites A and B. The errors have been calculated by taking into account the errors at each frequency as evaluated by following procedure in Section 2.

(see Section 2). At the same time, the trend of the ratios does not exhibit any similarity with the total integrated intensity as a function of the spectrum number.

All these findings, confirm, on one hand, that that the continuum generally contributes to all the vibrational modes; on the other, they suggest that a single vibrational mode might be alternatively selected during the dynamical evolution of the molecule. In this connection, it should be pointed out that, while the 1394, 1560 and 1625 cm^{-1} lines correspond to in-plane modes, the 977 cm^{-1} line arises from an out-of-plane mode (see Table 1).

4. Discussion

Generally, the blinking behaviour, and at the same time, the appearance of fluctuations in the SERRS spectra have been assumed as hallmarks for the single molecule detection [2,3,13,16,19]. In our case, these fluctuations, in connection with the extremely low concentration of FePP molecules

present in the initial solution, (the presence of an average number of about 0.4 FePP molecules in the laser-illuminated spot is estimated) and with the fact that only a small fraction of target molecules (about 1%) is expected to yield SERRS effect [15,19], point out that the single molecule limit has been approached.

To reach single molecule detection by SERRS, an extremely large enhancement factor is required [31]. According to recent theoretical and experimental findings, a concomitant action of both the EM and the CT mechanisms is expected to occur [10,11,20]. The largest contribution arises from the EM effect to which a more specific, smaller, effect, related to a chemical interaction between the target molecule and the surface, is to be added [13,21,24]. A modulation of the enhancement due to the CT is assumed to be responsible for the fluctuations in the single molecule SERRS spectra [13]. While the EM enhancement is a nonselective amplifier for Raman scattering, the CT effect is expected to operate only when strong adsorption of the target molecule on the metal surface and specific inter-

actions between the molecule and the metal, involving a CT, takes place; with the electronic states of the adsorbate being shifted and broadened by their interaction with the metal surface or with new electronic states appearing as intermediate states in Raman scattering [11,31]. It is commonly believed that for a molecule–metal system, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the adsorbed molecule are symmetrically located in energy with respect to the Fermi level of the metal [11,30]. The Fermi level of the metal would act as an initial or final state in resonance Raman process which involves adsorbed molecules [11,23]. In this connection, we note that porphyrins are suitable candidates for an effective interaction with the metal surface since they are polarizable molecules characterized by large planar π electronic configurations. Therefore, they are expected to assume, on the metal surface, a flat orientation due to their tendency to maximize the π bonding to the surface; such a configuration having been confirmed by scanning tunnelling microscopy (STM) studies at the surfaces of different metals [25,32–34]. The HOMO and LUMO levels of FePP have been evaluated to be located at −0.48 and 0.40 eV, respectively [34]; while the energy Fermi level for silver is 5.49 eV. On such a basis, however, the related positions among the HOMO, LUMO levels and the Fermi energy of the FePP system do not properly match the above mentioned conditions for the CT mechanism to occur. It should, nevertheless, be considered that a relevant shift of the Fermi energy level could be induced by doping a metal surface with very small amount of either electropositive or electronegative elements [11]. Moreover, the possibility that the HOMO and LUMO levels of FePP adsorbed onto a rough silver surface might be drastically shifted should be taken into account [32]. Alternatively, as very recently suggested, the existence of an intermediate state, between the metal and the molecule, as induced by molecular oxygen, could be hypothesized [35]. On such a ground, the CT process might be assumed as responsible for the observed fluctuations in the SERRS spectra of single FePP molecules.

The evidence that, by summing many SERRS spectra as obtained from a single molecule at different times, the vibrational features of the ensemble averaged spectra can be reproduced, provides a support to the ergodic theorem, in agreement with what recently verified for another small molecule [9]. This means that, by following a single FePP molecule during its temporal evolution, almost all the overall configurational space can be sampled. In this connection, it is interesting to remark that only a partial sampling of the FePP vibrational modes occurs by summing many single molecule SERRS spectra of myoglobin [19]. Such a result could be ascribed to the entanglement of the macromolecule in the local minima of its conformational energy [19] and points out that the sampling of FePP vibrational modes can be restricted when a FePP molecule is embedded within a protein matrix.

Furthermore, our analysis has allowed us to show that the integrated intensity of the single molecule SERRS spectra can be decoupled in two components. One component is due to the Raman peaks and the other one, more intense, arises from the continuum over which the Raman peaks are superimposed; each of them exhibiting drastic fluctuations. A similar behaviour is also detected for the intensity registered in correspondence of a single vibrational mode. Indeed, the occurrence of high values for the correlation between the total Raman intensity and the Raman intensity of a single vibrational mode (by taking into account for the underlying continuum) suggests that the continuum component is strictly related to the enhanced mechanism.

Generally, the continuum component in SERRS spectra is attributed to a byproduct of a nonradiative CT process between the surface and the molecule [36]. Actually, the background signal as due to fluorescence, which commonly affects standard Raman spectra, is expected to be quenched in the SERRS spectra [11]. On such a ground, the evidence of drastic fluctuations in the total intensity, and also in the continuum, suggests a modulation of the enhancement SERRS mechanisms. In agreement with recent findings, it can be then hypothesized that the fluctuations appearing in the SERRS spectra of single FePP molecule could arise from a desorption–adsorption process of the target molecules at the colloidal silver surface [13,17,18]. Such an adsorption process might result into a

slight variation of its distance from the metal surface, determining a modulation of the CT contribution and then of the enhancement factor affecting both the continuum and the line intensity. On the other hand, the fact that the ratios between the in-plane and out-plane modes exhibit a different trend from spectrum to spectrum implies that the mechanisms acting at the metal interface could operate, during the temporal evolution, a sort of selection of the active vibrational modes. Actually, as consequence of successive adsorption–desorption processes, the target molecule might assume slight different orientations with respect to the metal surface; such an orientation variability could result into a variability of the relative enhancement of the in and out plane modes. In this respect, it should be remarked that, for a heme group laying flat on the surface, only vibrational modes with A_{1g} symmetry, are expected to be effectively scattered [37]. The evidence that modes with different symmetry are simultaneously activated provides a support for a heterogeneity in the metal–heme orientation.

Additionally, we note that adsorption or desorption of the target molecule at the metal surface, as induced by electronic transitions [38], or by excitation of vibrational modes has been observed [39]. At the same time, a modulation of surface mobility of porphyrin by the adsorbed–substrate interactions has been put into evidence [25].

Finally, we would like to stress that an adsorption–desorption process could give rise to a modulation of the HOMO and LUMO gap of the adsorbed molecule with respect to the metal surface [13,18]. Therefore, a study of the electronic properties of FePP adsorbed onto rough metal surfaces (e.g. by STM) could allow ones to elucidate in more detail the interactions between the adsorbed molecule and the metal surface.

5. Conclusions

SERRS spectra of immobilized single FePP molecules adsorbed onto a silver colloidal surface have been statistically analyzed. The large spectral fluctuations, usually masked in ensemble SERRS spectra, have been decoupled into a broad, largely dominant, continuum and Raman peaks, over this continuum.

An adsorption–desorption mechanism of the FePP molecules on the metal surface has been proposed to describe these large fluctuations in the spectra intensity. Successive adsorptions and desorptions of the target molecule could modulate the charge transfer between the metal and the molecule by acting on the enhancement mechanism. At the same time, changes in the dynamical behaviour of different vibrational modes can be induced.

These results provide some evidence for the richness of behaviour of molecules, excited by light, adsorbed at a metal rough surface of nanoparticles. In this respect, a synergic effort based on different experimental approaches could be extremely useful to a deeper understanding of the molecule–metal interactions.

Finally, we remark that single molecule SERRS could be an extremely powerful technique to investigate the behaviour of vibrational dynamics and its dependence from the environment of molecules at a metal interface.

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