Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemical Physics 326 (2006) 356–362
Chemical Physics
www.elsevier.com/locate/chemphys

Time-dependent study of single-molecule SERS signal from yeast cytochrome *c*

Ines Delfino *, Anna Rita Bizzarri, Salvatore Cannistraro

Biophysics and Nanoscience Centre, CNISM, Dipartimento Scienze Ambientali – Università della Tuscia, Largo dell'Università, I-01100 Viterbo, Italy

Received 26 July 2005; accepted 17 February 2006
Available online 6 March 2006

Abstract

A study of cytochrome *c* from *Saccharomyces cerevisiae* adsorbed on silver colloids at very low concentration is carried out by surface-enhanced Raman scattering. Spectra acquired at different times exhibit dramatic fluctuations in both line frequency and intensity indicating that single molecule detection is approached. The intensity fluctuations are investigated by means of a second order time correlation analysis. Such an approach has allowed us to put into evidence the presence of two distinct dynamical phenomena. The results are discussed in connection with diffusion processes to which the protein undergoes with respect to the surface of the Ag nanoclusters and with a modulation of the enhancement of the Raman signal.

Keywords: Single-molecule; Cytochrome *c*; SERS; Correlation analysis

1. Introduction

In recent years, an exponentially growing interest has been recorded in the field of single molecule (SM) spectroscopy. Next to the most obvious advantage of this approach – the possibility of studying minute quantities of sample molecules – several phenomena and rare events, such as dynamical or decaying processes, infrequent reactions, otherwise hidden in the ensemble-averaged properties of the system under study, can be followed.

Different spectroscopic approaches have so far been applied and developed up to reach single molecule detection [1–4]. One of these is surface-enhanced Raman scattering (SERS) [5–7] that exploits the dramatic enhancement of the Raman cross-section (up to 10^{14} times) [6,8] obtained by adsorption of molecules onto both nanometer-sized metallic particles or rough metallic islands. Such an enhancement of Raman signal is due to electromagnetic (EM) and charge transfer (CT) mechanisms [5–7]; the largest contribution arising 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 [9,10]. While the EM enhancement is a non selective amplifier for Raman scattering, the CT effect being sensitive to the relative orientation of the molecule and the metallic surface [3,5].

In SERS experiments, the extremely low efficiency of the Raman spectroscopy can be overcome up to reach single molecule detection for both organic and even protein molecules [8,11–17]. Therefore SERS spectroscopy is currently the only tool capable of combining chemical information, offering the remarkable possibility of identifying and characterizing molecular species by virtue of their vibrational spectrum, with SM sensitivity. At SM level, SERS spectra recorded as a function of time reveal blinking, spectral diffusion, and intensity fluctuations of vibrational lines [16,18]. These fluctuations contain information about different phenomena, such as dynamic processes, charge transfer between protein and metallic surface, modification of protein orientation and configuration, micro-environmental changes, entanglement of the single protein molecule into local minima of the energy landscape, etc. Various experimental and theoretical efforts

* Corresponding author. Tel.: +39 0761357026; fax: +39 0761357179.
E-mail address: delfino@unitus.it (I. Delfino).

0301-0104/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chemphys.2006.02.020

have been addressed to extract detailed information embedded in the temporal fluctuation of SM–SERS signals [15,19]. Boosted by the successful application in the analysis of a wide range of spectroscopic data including Raman measurements [20], correlation methods have been very recently employed in the analysis of SERS data. Time-correlation analysis, providing key insights into dynamics, local and long range interactions, of SERS signals even at SM level has lead to disclose some photo-induced processes [15,21,22]. Furthermore, frequency and time correlation analysis of SEHRS (Surface-enhanced hyper raman scattering, a technique based on the surface-enhancement of a scattering signal that is Raman shifted relative to the second harmonic of the excitation frequency) has enabled to go deeper inside the SERS mechanism [18,19,23,24]. However, some methods based on correlation functions, usually applied in other correlation techniques, have not yet been extended to SERS experiments, thus suggesting that the applicability of time-correlation approach to SERS data can be further increased.

We are interested on the behaviour of single molecule of cytochrome *c* from *Saccharomyces cerevisiae* (Yeast cytochrome *c* – Ycc) near metallic surface even in view of its application in molecular electronics [25]. Ycc is a small single-domain electron transfer (ET) heme–protein, with an additional free sulphur-containing group appropriate for covalent binding to gold, that makes it suitable for applications in hybrid submicrometer-sized electronic components. Actually, the ability to connect functional redox proteins to the metallic electrode (Au or Ag), preferably via chemical bond, is fundamental in order to achieve good electrical contact between the molecule and the conducting substrate. The biotechnological potentialities of Ycc have been confirmed by previous studies showing that when adsorbed on electrodes Ycc preserves its morphological properties and redox functionality with a good electrical coupling with the electrode [26]. The ensemble behaviour of Ycc near metallic surfaces has been widely investigated also by using SERS spectroscopy, evidencing that SERS technique is very sensitive to conformation, oxidation and orientation states of Ycc [21,27]. We have recently exploited the capabilities of SERS in the study of Ycc near metallic surfaces down to SM level [28]. In that study, the temporal fluctuations of specific Ycc Raman lines were widely investigated. To go deeper inside the origin of this variability and to underpin the possible dynamical processes which may be encoded in the temporal behaviour of fluctuations, we have applied an intensity correlation study to the intensity of individual Ycc spectral lines. In particular, we have employed, for the first time, a second order approximation for the description of the autocorrelation functions of SM–SERS data. This approach has allowed us to discuss the signal fluctuations in terms of some dynamical processes, such as translational motion of the protein respect to the surface, surface relaxations and metal-protein interactions.

2. Materials and methods

All chemicals ($AgNO_3$, Ycc, Trizma, APES) were purchased from Sigma Chemical Co. Solutions of colloidal silver were prepared by Lee–Meisel standard citrate reduction method [29]. The produced colloids consisted of an heterogeneous size and shape (spheres and rods) particles with a distribution characterized by an average size of about 70 nm [18]. The concentration of silver particles, estimated by optical absorption, was about 10^{-11} M.

Ycc from *S. cerevisiae* (M.W. 12.6 kDa) is a small single-domain heme-containing protein, which is an essential component of the mitochondrial respiratory chain, playing a major role in the ET between cytochrome *c* reductase and cytochrome *c* oxidase. As in other heme cytochromes, in Ycc the heme group is covalently bound to the protein matrix through thioether linkages involving two cysteine residues (Cys14 and Cys17). In addition, Ycc bears a free sulphur-containing group (Cys102).

Ycc solutions, used without further purification, were prepared dissolving the powder in 1 mM TRIS buffer (pH 8.0) at a concentration of 30 μM. An aliquot of a solution obtained after successive dilutions was incubated with colloidal particles for 1 h at room temperature in order to obtain a ratio of 6:1 between the number of colloidal particles and the number of cytochrome molecules at 1.7×10^{-12} M concentration of Ycc. After the deposition of a droplet (20 μl) onto a glass slide, previously coated with polymerized 3-aminopropyltriethoxysilane (APES) [30], the sample was left in a dryer for 1 h at room temperature. Atomic force microscopy (AFM) put into evidence the presence of nanoparticle aggregates with features similar to those previously reported in Ref. [18].

Raman spectra were recorded using a Jobin–Yvon Super Labram system equipped with a liquid nitrogen-cooled CCD (EEV CCD10-11 back illuminated; pixel format: 1024×128) detector and a spectrograph with a 1800 gr/mm grating allowing a resolution of 4 cm^{-1}. Excitation and collection of Raman scattering were done in confocal geometry using a 0.9 numerical aperture 100× objective and an Argon ion laser (MellesGriot) providing a wavelength at 514.5 nm preresonant with the Q band of Ycc. The laser spot on the sample was about 1 μm^2 and, to avoid photo-degradation of the protein, the laser power was kept below 1 mW [19,31]. The deposition of a 20 μl solution droplet, covering an approximate area of 0.5 cm^2, results in the presence, in average, of about one Ycc molecule in this laser spot.

During measurements, Raman scattering sites were identified by manually scanning (on *x*–*y* plane) the sample under the microscope objective and locating brightly emitting colloidal particles. Highly inhomogeneous Raman intensity over individual hot spots was found, like usually reported in SM–SERS from immobilised particles [8,21]. We focused our attention on those active sites having the lowest Raman intensity and showing Ycc specific vibrational features. The selection of sites by intensity, together

with the expectation of an average single molecule in the laser spot, are the experimental requirements to have the single molecule regime. Twenty active sites with similar Raman intensity were investigated.

For each selected site, series of 500 1s-integration time spectra were acquired; each spectrum being separated by the successive by 1.4 s.

3. Results and discussion

A typical set of Ycc SERS spectra is shown in Fig. 1a as a time- and wavenumber-resolved map. Notably, the spectra reveal intensity fluctuations and spectral diffusion as usually observed in SM–SERS measurements [3,28]. These evidences together with the low number of Raman active particles support the hypothesis that single molecule regime has been reached. On the other hand, we note that such a behaviour is practically the same as observed in our previous work on single Ycc molecule [28]. We have also followed the signal for long time periods (up to 1600 s) observing sometimes its disappearance; only occasionally the signal appears again. Fig. 1b shows that almost all the spectral features of Ycc can be recovered by averaging over all the spectra of a sequence [27,32]. A slight shift (3–6 cm^{-1}) with respect to Raman counterparts is observed for some vibrational features; similar shifts having been observed in other SERS measurements [8,19,28]. Starting from the evidence that we are dealing with a single Ycc molecule, we have analyzed the SERS data to extract information on the dynamics of the single protein near the Ag surface. In particular, the temporal behaviour of SERS signal of three Ycc vibrational fingerprints (1130 cm^{-1} – assigned to in plane vibrational mode of pyrrole half-ring, 1310 cm^{-1} – due out of plane bending modes of C_mH bond, and 1408 cm^{-1} – related to in plane vibrational mode of pyrrole quarter-ring [27,32]) have been taken into account. Fig. 2 shows the time trajectories and the related distributions of the intensity for the selected Raman lines. For comparison, the time evolution and distribution at 400 cm^{-1} of the detected intensity, where no specific Raman features are observed, is shown in the same figure. Regarding Ycc-specific modes, the lines undergo intensity jumps which sometimes occur simultaneously for different frequencies. Their intensity histograms can be described by just one single mode Gaussian distribution, with similar central values (around 100 counts) but different widths (20–40 counts); the widths being higher than the spread expected from the detector noise (13 counts for the present experimental conditions). Among the investigated lines, the lowest intensity value and the narrowest distribution (17 counts) is revealed for the 400 cm^{-1} line. All the distribution widths are found to be higher than that obtained from bare colloids (around 15 counts, see Fig. 2). These results are similar to those recently reported for protoporphyrin [19] and cytochrome [28] in which blinking, frequency shift and intensity fluctuations have been put into evidence. These phenomena have been tentatively attributed to various processes, such as the sampling of different configurations that the system experiences in time, the occurrence of some molecule-specific events (desorption–adsorption process of the target molecules at the colloidal silver surface [19], electron charge-transfer [12]) or modulation of the enhancement effect itself, but their origin is still under debate.

To get some insight into the dynamical processes occurring on single Ycc molecule at the silver surface, a correlation analysis of the SERS total intensity has been recently applied [14,15,21]. We have extended the intensity correlation study to temporal behaviour of single spectral lines. In particular, the autocorrelation function of intensity has been calculated from experimental data for 400, 1130, 1310 and 1408 cm^{-1} lines by $g_\nu(\tau) = \langle \Delta I_\nu(0) \Delta I_\nu(\tau) \rangle$ with $\Delta I_\nu(\tau) = I_\nu(\tau) - \langle I_\nu(\tau) \rangle$; the brackets indicating the time averaging, and $I_\nu(t)$ being the peak intensity of the selected frequency ν at time τ. The resulting curves are shown in Fig. 3. The temporal trends of $g_{1130}(\tau)$, $g_{1310}(\tau)$, $g_{1408}(\tau)$ are quite similar in shape with an high correlation level as emerging from the values at short delays. At variance,

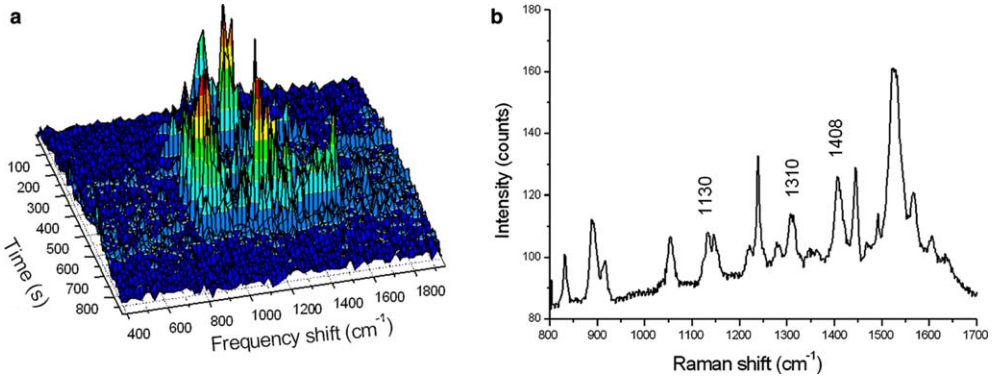

Fig. 1. (a) 3D representation of the temporal behaviour of Ycc SM–SERS spectra. (b) Spectrum obtained by averaging over all the 500 spectra of the selected series recorded from a bright site of immobilized silver colloids incubated with Ycc at a concentration of 1.7×10^{-12} M. The frequencies of the peaks whose time behaviour have been investigated (see text) are labelled.

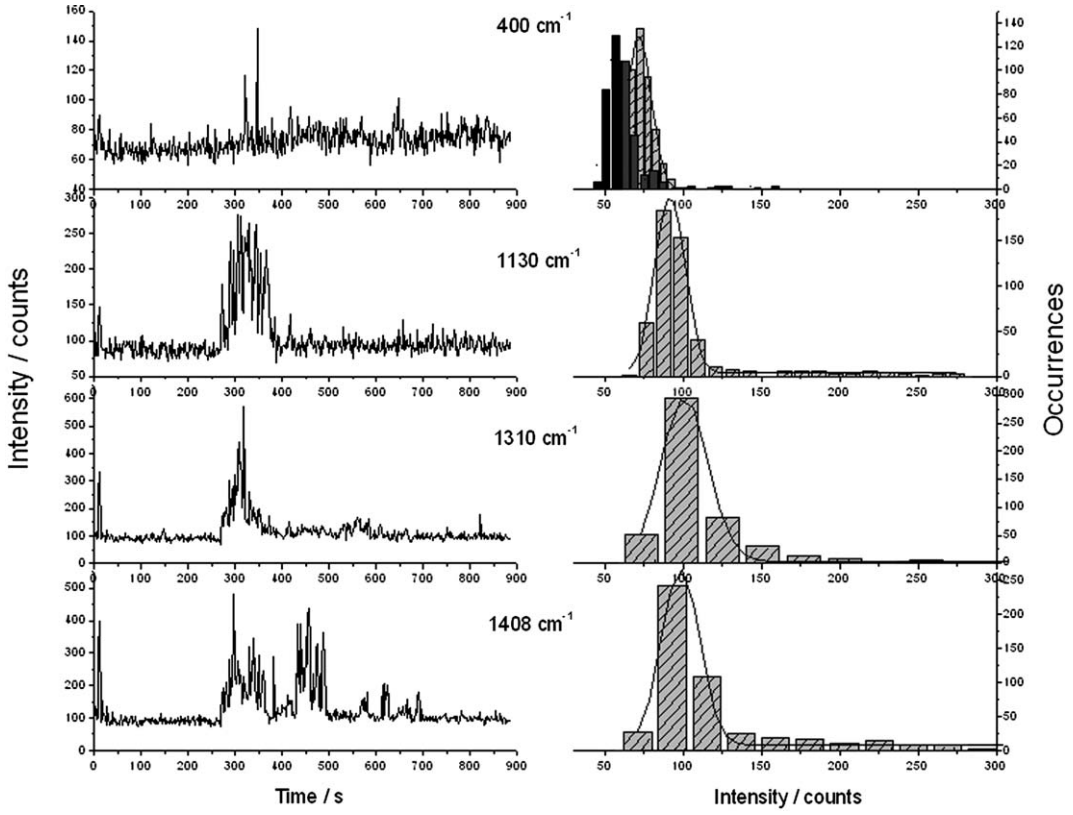

Fig. 2. Time behaviour of SERS intensity, and the corresponding intensity distribution, for four different frequencies of the selected series as obtained from a bright site of immobilized silver colloids incubated with Ycc at a concentration of 1.7×10^{-12} M. The values of the cross-correlation, calculated with the method described in Refs. [18,19], between the intensity detected at 1130 cm^{-1} and that at 400, 1310, and 1408 cm^{-1} are less than 0.5, thus indicating a weak correlation. The histograms have been obtained using 20 bins whose widths are 5% of maximum intensity. Each distribution has been described by a Gaussian fit with the following central values – X_c- and widths –w (in counts): $X_{c400} = 72$, $w_{400} = 17$; $X_{c1127} = 94$, $w_{1127} = 20$; $X_{c1310} = 101$, $w_{1310} = 32$; $X_{c1408} = 98$, $w_{1408} = 26$. Intensity distribution of signals from bare colloids for 400 cm^{-1} line (dark gray): $X_c = 58$; $w = 15$.

the temporal trend of $g_{400}(\tau)$ presents a low correlation; small time values of g_{400} being around the half of the initial value of the other autocorrelation functions. The trend of $g_{400}(\tau)$ is well described by a simple exponential decay [20,33]:

$$g_v(\tau) - 1 = A_0 \exp\left(-\frac{\tau}{\tau_1}\right) \tag{1}$$

where A_0 is the amplitude and τ_1 the decay time. In Fig. 3 the fitting curves, together with extracted parameters, are shown. The quality of the fit has been evaluated by using the χ^2-test with 0.8 as probability threshold [34]. It comes out that the 400 cm^{-1} line fluctuations are dominated by a single relaxation process with a characteristic time of 300 s. Such fluctuations can be ascribed to the continuum background, a common feature of SERS spectra which has been supposed to be related to the enhancing mechanism [7] and/or spurious contributions due to carbon contaminations [35]. On the other hand, the analytical model of Eq. (1) does not well describe the other autocorrelation functions. A satisfactory description of $g_{1130}(\tau)$, $g_{1310}(\tau)$, and $g_{1408}(\tau)$ has been, in turn, obtained by the introduction of a second cumulant term [33,36] in the correlation functions within an expansion in the second order of the Taylor series [20,24,37–40]:

$$g_v(\tau) - 1 = A_0 \exp\left(-\frac{\tau}{\tau_1} - \frac{\tau^2}{\tau_q^2}\right) \approx A_1 \exp\left(-\frac{\tau}{\tau_1}\right) + A_q \exp\left[-\left(\frac{\tau}{\tau_q}\right)^2\right] \tag{2}$$

where τ_1 and τ_q are the first and second cumulant decay times, respectively.

The fitted curves and the retrieved τ_1 and τ_q values related to $g_{1130}(\tau)$, $g_{1310}(\tau)$, and $g_{1408}(\tau)$ are shown in the Fig. 3. All the three curves are characterized by the same value of the τ_1 decay time (about 300 s). Notably, such a time finds a correspondence with that extracted for $g_{400}(\tau)$. At variance, we found that τ_q is dependent on the specific Ycc vibrational mode. In particular, the longest correlation time ($\tau_q = 72$ s) characterizes the 1408 cm^{-1} line, while the shortest one ($\tau_q = 42$ s) is observed for 1310 cm^{-1} line intensity fluctuations.

Second order correlation analysis of single line SERS intensity has been performed for 20 hot sites having comparable intensity and similar results have been obtained for all the sites. In particular, the $g_{400}(\tau)$ has a single decay time (τ_1), while $g_{1130}(\tau)$, $g_{1310}(\tau)$, and $g_{1408}(\tau)$ have two characteristic times each, τ_1 and τ_q, related to the simple exponential decay and to the second order term in Eq.

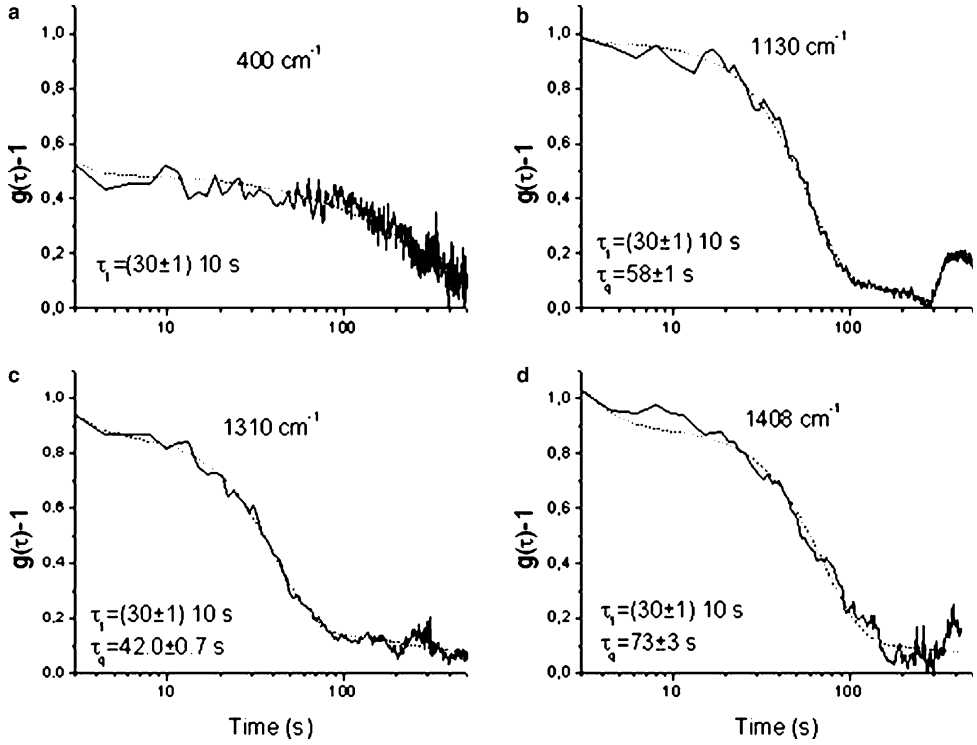

Fig. 3. Autocorrelation functions calculated from experimental data (continuous line) and related fitted curve (dotted line) for each selected frequency: (a) 400 cm^{-1}, (b) 1130 cm^{-1}, (c) 1310 cm^{-1}, (d) 1408 cm^{-1}. The good quality of each fit has been verified by a χ^2 test.

(2), respectively. Notably, τ_1 is quite the same for all the Raman lines (i.e., 400, 1130, 1310 and 1408 cm^{-1}); conversely, τ_q is specific of each Ycc vibrational mode. We note that the absolute values of τ_1 and τ_q are site dependent. As an example, Fig. 4 shows the frequency distribution of the τ_1 values obtained for all the investigated sites ranging from 150 up to 350 s with a mean of 270 ± 50 s. For completeness, we can say that the τ_q values vary in the range 30–90 s with a mean of 63 ± 20 s.

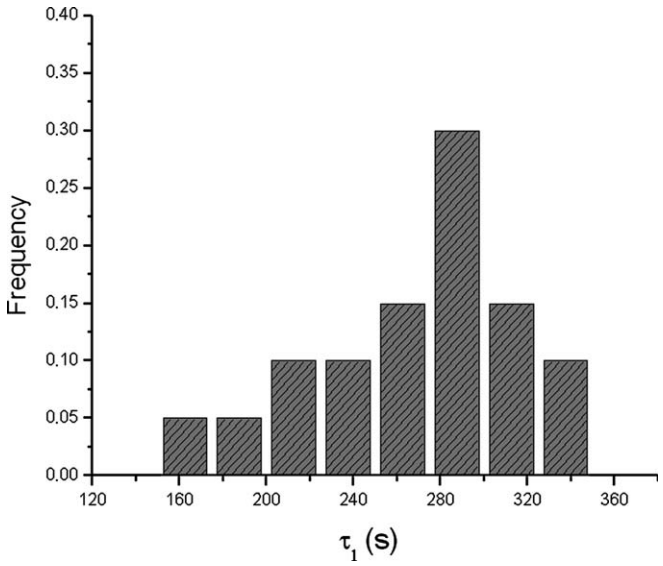

Fig. 4. Frequency distribution of the retrieved τ_1 value obtained by fitting data with Eq. (2) (see text). The histogram has been obtained by using 8 bins whose widths are 6% of the maximum value.

The physical interpretation of these results can be attempted in connection with similar findings obtained with other spectroscopic correlation techniques. The introduction of a second cumulant in the correlation function essentially accounts for a deviation of the real process from the ideal model of single Brownian diffusion [33], likely due to a superimposed process. The second order analysis of correlation function obtained by dynamic light scattering enabled to disclose an intensity dependent translation process superimposed to a particle Brownian diffusion [37]. In this framework, it can be hypothesized that two different processes are governing the time behaviour of Ycc SERS signals. One process, correlated over about 300 s and affecting the whole spectrum, can be tentatively ascribed to a relative translational motion of the Ag-molecule system by assuming a motion of the protein over metal surface. Similar diffusive processes have been recently suggested for rhodamine 6G by the analysis of the time correlation of the overall SERS intensity [21]. Alternatively, a translational motion of the colloids respect to the glass surface can be responsible of the phenomenon and the decay time τ_1 would be the characteristic time of the translational diffusion of Ag colloids on glass surfaces. In such a picture, the disappearing of SERS signals occasionally detected for some sites could be ascribed to the escaping of the Ag colloids

from the scattering volume, being the time scale of such process in reasonable agreement with our experimental findings. In fact, a 70 nm particle is characterized, in solution, by a diffusion coefficient of around 10^{-8} cm^2/s [33], thus about 1 s is needed for escaping from the 1 μm^2 region. However, in dry sample the diffusion is slowed down also because of the colloid-surface interaction and of the aggregation of colloids. Considering all these effects, some hundreds of second as escaping time for Ag colloids should be reasonable. Accordingly, the appearance again of the signal might be related to the entering of another Ag-molecule system in the scattering volume. In our experimental conditions, the probability to have such an event is really low as due to (i) the low number of molecules in the volume (1 molecule as average) and (ii) the low percentage of SERS active molecule-colloid nanoparticle over the whole number of protein-colloid systems. Also the characteristic time of the translational process (300 s) responsible for the escaping/entering of the molecules suggests that the signal could rarely appear again.

Relaxation of the Ag surface roughness on the nanometer scale [41] could be invoked as another possible origin of the long-time process. In such a case, the occasional vanishing of SERS signal might be ascribed to a significant decrease of the enhancement factor due to the flattening of the silver surface. In this frame, the appearance again of the signal might be due to a rearrangement of the protein respect to the modified surface. On the other hand, the dependence of enhancing mechanisms on the orientation and on the position of the molecule with respect to the metal surface might be related even to a modulation of the specific Ycc vibrational mode intensity. This is in agreement with very recent results showing that molecules near metallic surfaces undergo molecular re-orientation [42] with a characteristic time up to seconds [21,43,44]. Notably, all the hypothesized processes, spontaneous or photoinduced, can affect the SERS enhancement by modulating both the EM and CT mechanisms [5,6,39,41].

The observed variability of the characteristic times over different single Ycc molecules could be due to an heterogeneity of the SERS sites. In fact, the Raman enhancement is a very sensitive effect depending on the relative position of the molecule with respect to the metallic surface and on the local EM field that are dependent on the peculiar properties of the sites (molecule-colloid nanoparticle systems).

4. Conclusions

SERS signal fluctuations of individual Ycc molecules near metallic surface (Ag) have been examined in terms of a second order time correlation approach in the analysis of single Ycc specific lines. This has allowed us to decouple two kinds of processes regulating signal fluctuations. One process, characterized by long time correlation (300 s), affects the whole Ycc SERS spectrum and could be interpreted in terms of a relative translational diffusion (protein with respect to the Ag colloids or colloids themselves in the light field). The other process, in the time scale of tenths of seconds, appears to be peculiar of the protein Raman active modes and could be tentatively put into relationship to a modulation of the mode specific CT enhancing effect probably due to a rearrangement of the molecular orientation. These results have been confirmed by a statistical analysis of the behaviour of various SERS hot sites showing an homogeneous occurrence of the two processes together with a site dependence of their characteristic times. The reported data suggest that the assessment of a well suited analysis procedure could help to deeper investigate the time behaviour of single molecule by SERS. The use of correlation studies in SERS field is very promising and its applicability looks very wide, allowing an insightful analysis of protein dynamics and a deeper understanding of SERS mechanism itself. The applicability of this approach to SERS data from various proteins acquired in different experimental condition (temperature, solvent, laser power) is now under study.

Acknowledgements

This work has been partially supported by the FIRB-MIUR Project Molecular Nanodevices and a PRIN-MIUR 2004 Project.

References

[1] T. Baschè, W.E. Moerner, M. Orritt, U.P. Wild (Eds.), Single-Molecule Optical Detection Imaging and Spectroscopy, VCH, Germany, 1997.
[2] N.F. van Hulst, J.A. Veerman, M.F. Garcia-Prajo, L.K. Kuipers, J. Chem. Phys. 112 (2000) 7799.
[3] K. Kneipp, H. Kneipp, I. Itzkan, R.R. Dasari, M.S. Feld, J. Phys.: Condens. Matter. 14 (2002) R597.
[4] A.M. Kelley, X. Michalet, S. Weiss, Science (Washington, DC) 292 (2001) 1671.
[5] A. Campion, P. Kambhampati, Chem. Soc. Rev. 27 (1998) 241.
[6] M. Moskovits, Rev. Mod. Phys. 57 (1985) 783.
[7] B.N. Persson, Chem. Phys. Lett. 82 (1981) 561.
[8] K. Kneipp, Y. Wang, H. Kneipp, L.T. Perelman, I. Itzkan, R.R. Dasari, M.S. Feld, Phys. Rev. Lett. 78 (1997) 1667.
[9] P. Hildebrandt, M. Stockburger, J. Phys. Chem. 88 (1984) 5935.
[10] A.M. Michaels, M. Nirmal, L.E. Brus, J. Am. Chem. Soc. 121 (1999) 9932;
A.M. Michaels, J. Jiang, L. Brus, J. Phys. Chem. B 104 (2000) 11965.
[11] S. Nie, S.R. Emory, Science 275 (1997) 1102.
[12] A.R. Bizzarri, S. Cannistraro, Chem. Phys. Lett. 395 (2004) 222.
[13] A.R. Bizzarri, S. Cannistraro, Phys. Rev. Lett. 94 (2005) 068303.
[14] S. Habuchi, M. Cotlet, R. Gronheid, G. Dirix, J. Michiels, J. Vanderleyden, F.C. De Schryver, J. Hofkens, J. Am. Chem. Soc. 125 (2003) 8446.
[15] A. Weiss, G. Haran, J. Phys. Chem. B 105 (2001) 12348.
[16] H. Xu, E.J. Bjerneld, M. Kaell, L. Boerjesson, Phys. Rev. Lett. 83 (1999) 4357.
[17] A.M. Michaels, M. Nirmal, L.E. Brus, J. Am. Chem. Soc. 121 (1999) 9939.
[18] A.R. Bizzarri, S. Cannistraro, Appl. Spectrosc. 56 (2002) 1531.
[19] A.R. Bizzarri, S. Cannistraro, Chem. Phys. 290 (2003) 297.
[20] W. Schrof, J.F. Klingler, S. Rozouvan, D. Horn, Phys. Rev. E 57 (1998) 2523.

[21] Y.D. Shu, G.K. Schenter, L. Zhu, H.P. Lu, Ultramicroscopy 97 (2003) 89.
[22] C. Eggeling, J. Schafer, C.A.M. Seidel, J. Korte, G. Brehm, S. Schneider, W. Schrof, J. Phys. Chem. A 105 (2001) 3673.
[23] G. Brehm, G. Sauer, N. Fritz, S. Schneider, S. Zaitsev, J. Mol. Struct. 735–736 (2005) 85.
[24] A.A. Moore, M.L. Jacobson, N. Belabas, K.L. Rowlen, D.M. Jonas, J. Am. Chem. Soc. 127 (2005) 7292.
[25] B. Bonanni, D. Alliata, L. Andolfi, A.R. Bizzarri, S. Cannistraro, in: C.P. Norris (Ed.), Surface Science Research Development, Nova Science Publishers, Inc., 2005.
[26] B. Bonanni, D. Alliata, A.R. Bizzarri, S. Cannistraro, Chem. Phys. Chem. 4 (2003) 1183;
R.G. Nuzzo, B.R. Zegarski, L.H. Dubois, J. Am. Chem. Soc. 109 (1987) 733;
A. Szucs, M. Novak, J. Electroanal. Chem. 383 (1995) 75;
C.M. Lett, J.G. Guillemette, Biochem. J. 362 (2002) 281.
[27] H. Wackerbarth, P. Hildebrandt, Chem. Phys. Chem. 4 (2003) 714.
[28] I. Delfino, A.R. Bizzarri, S. Cannistraro, Biophys. Chem. 113 (2005) 41.
[29] P.C. Lee, D. Meisel, J. Phys. Chem. 86 (1982) 3391.
[30] R.G. Freeman, K.C. Grabar, K.J. Allison, R.M. Bright, J.A. Davis, A.P. Guthrie, M.B. Hommer, M.A. Jackson, P.C. Smith, D.G. Walter, M.J. Natan, Science 267 (1995) 1629.
[31] A. Otto, J. Raman Spectr. 33 (2002) 593.
[32] S. Hu, I.K. Morris, J.P. Singh, K.M. Smith, T.G. Spiro, J. Am. Chem. Soc. 115 (1993) 12446.
[33] R. Pecora, Dynamic Light Scattering. Application of Photon Correlation Spectroscopy, Plenum Press, New York, 1985.
[34] J.R. Taylor, An Introduction to Error Analysis, University Science Books, 1982.
[35] A.A. Moore, M.L. Jacobson, N. Belabas, K.L. Rowlen, D.M. Jonas, J. Am. Chem. Soc. 127 (2005) 7292.
[36] D.E. Koppel, J. Chem. Phys. 57 (1972) 4814.
[37] S. Schneider, G. Brehm, P. Freunscht, Phys. Status Solidi (b) 189 (1995) 37.
[38] X.-M. Lin, G. Wang, C.M. Sorensen, K.J. Klabunde, Appl. Opt. 38 (1999) 1884.
[39] B.J. Friskenm, Appl. Opt. 40 (2001) 4087.
[40] H. Xu, J. Aizpurua, M. Kaell, P. Apell, Phys. Rev. E 62 (2000) 4318.
[41] D.B. Lukatsky, G. Haran, S.A. Safran, Phys. Rev. E 67 (2003) 062402-1.
[42] Z. Wang, L.J. Rothberg, J. Phys. Chem. B 109 (2005) 3387.
[43] A.G.T. Ruiter, J.A. Veerman, M.F. Garcia-Parajo, N.F. van Hulst, J. Phys. Chem. A 101 (1997) 7318.
[44] A.P. Bartko, K. Xu, R.M. Dickson, Phys. Rev. Lett. 89 (2002) 026101-1.