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Lévy Statistics of Vibrational Mode Fluctuations of Single Molecules from Surface-Enhanced Raman Scattering

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Surface-enhanced Raman spectra of a single Fe-protoporphyrin IX molecule display drastic fluctuations in frequency and intensity. When analyzed in their temporal evolution, the vibrational modes of the molecule undergo an on-off switching behavior that is shown to follow a Lévy statistics. Such a time dependent process may encode both the dynamics of the molecule-environment interactions and the intrinsic gating or activation of the mode emission mechanism.

Ultrasensitive spectroscopy to detect single molecules is of utmost interest in different disciplines for both applicative and theoretical aspects [1]. Signals from single molecules exhibit drastic fluctuations in intensity and shape. Each molecule is in a unique static and dynamic environment, with distinct optical properties. Hence, the single molecule approach offers the possibility to follow the real dynamical behavior which is usually masked in the ensemble average. Moreover, some fundamental questions connected with the ergodicity [2], statistical aging [3], heterogeneity in the line shape [4], etc. can be addressed.

Raman spectroscopy may reach single molecule detection potentiality when the molecules are adsorbed onto nanometer-sized metallic particles, or rough metallic islands [5–11]. In surface-enhanced Raman spectroscopy (SERS), the cross section may hugely increase (even of 14 orders of magnitude) because of two, likely cooperating, mechanisms: an electromagnetic local field enhancement (EM), associated with surface plasmon resonance, and a chemical enhancement requiring a tight interaction between the molecule and the metal surface [12]. SERS therefore constitutes an ultrasensitive spectroscopic probe and couples a high degree of structural and dynamical information with a rewarding chemical specificity [13]. In a single molecule regime, an intermittent activation of vibrational modes may occur due either to intrinsic factors or to interaction with the environment [11]. Such a phenomenon deserves much interest also for understanding the mechanisms regulating the molecule-metal interaction under light excitation. Indeed, the dynamics of both the molecule and the host might be encoded in the temporal behavior of the spectral emission [10]. However, while the intensity fluctuations in single molecule SERS spectra have been analyzed in detail [11], the statistical properties of the vibrational mode temporal emission have not yet been explored. Such properties might reflect the activation or gating dynamics of the molecule vibrational modes as a consequence of its interaction with the environment [10]. An analysis of the intermittent appearance and disappearance of the vibrational modes could give some insight on the properties of single molecule SERS signals; this aspect

deserves much interest also in comparison with that encountered in single molecule fluorescence studies [3].

On such a ground, we focused our attention on the temporal emission of some vibrational mode appearing in the SERS spectra of protoporphyrin IX (FePP) collected in the single molecule limit. FePP constitutes the prosthetic group of many proteins where it performs several functional roles, from electron transfer to ligand binding. On the basis of its electron transfer capability, FePP could be exploited as an appropriate, robust compound for bioelectronic hybrid devices [14,15].

FePP at a concentration of $10^{-11}M$ was incubated for 5 h with Ag colloidal particle solution (with an average diameter of 70 nm [9], and a density of 10^{12} particles/liter), prepared by the procedure of Lee and Meisel [16], at about 1:1 ratio (molecules:colloidal particles). A droplet, with an approximate volume of 10 μL, of this solution was deposited onto a glass slide coated with polymerized 3-aminopropyltriethoxysilane (APES, Sigma) [11]. Raman spectra were collected, at room temperature, with Jobin-Yvon Raman equipment using an irradiation wavelength of 514.5 nm. The collection system consists of a confocal microscope coupled to a single grating spectrometer and a low noise liquid nitrogen cooled charge coupled device (CCD) detector. The microscope objective is a 100X with NA = 0.9 producing laser spot size of about 1 μm in diameter. The laser power at the sample was kept below 1 mW.

Scanning of the sample area reveals, at random, sites where only noise, down to the CCD detector level, is registered, and a few spots characterized by brightly emitting particles, the latter spots being characterized by similar average intensity [11]. At variance, samples of FePP at $10^{-11}M$, without addition of colloids, do not reveal any signal above the noise level. Figure 1(a) shows a sequence of 100 SERS spectra with 1 s of integration time from a typical bright site of immobilized FePP-colloids sample as excited at 514.5 nm. From spectrum to spectrum, there is a great variability in both the relative peak intensity as well as in the peak frequency. The presence of drastic fluctuations is usually assumed as a hallmark for the single
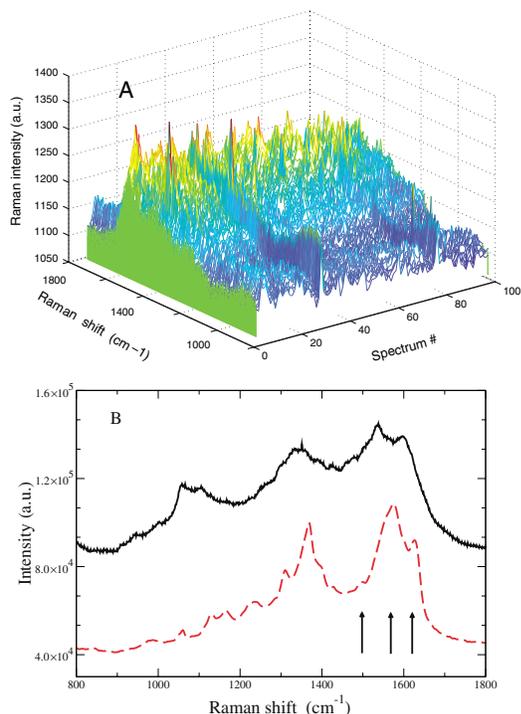
FIG. 1 (color).   (a) A sequence of 100 SERS spectra with l s of integration time from a bright site of immobilized silver colloidal particles incubated with FePP at a concentration of $10^{-11}M$. (b) Continuous line: SERS spectrum as obtained by summing 600 SERS spectra [as in (a)], recorded in sequence lasting 1 s between two successive measurements. Dashed line: Raman spectrum from a dry drop of FePP ($10^{-4}M$ in alkaline aqueous medium) with 30 s of integration time. The arrows mark the frequencies: 1480, 1570, and 1620 cm^{-1}. The spectral resolution is lower than 5 cm^{-1}.

molecule limit [7,8]. In our case, the occurrence of fluctuations, together with the fact that an average number of about 0.4 FePP molecules is expected in the laser-illuminated spot, provides a confirmation to single molecule detection [11]. We cannot rule out the possibility that clustering of silver colloids may sometimes lead to the presence of more than one FePP molecule in the illuminated spot. However, the results are consistent with the fact that only one FePP molecule in the cluster is strongly resonant. Even if a complete recovery of all the vibrational modes of FePP is not reached, the spectrum as obtained by summing up several spectra as obtained in sequence [see continuous line in Fig. 1(b)] reveals the main spectral features of FePP in bulk [see dashed line in Fig. 1(b)]. The peaks are mainly located in the 1000–1700 cm^{-1} region where the main vibrational modes of the porphyrin ring of FePP are expected.

Once verified in such a way that the bright sites contain single FePP molecules, we have concentrated our attention on the temporal emission of three specific vibrational modes of FePP: the iron spin marker ν_3 at 1480 cm^{-1}, the in-plane porphyrin skeletal mode at 1570 cm^{-1}, and the vinyl stretching mode ν_{10} at 1620 cm^{-1} [see markers in

Fig. 1(b)] [17]. We have analyzed series of 600 spectra, each spectrum being recorded with 1 s of integration time and separated from the successive one by 1 s. Figure 2 shows the signal intensity, as a function of the spectrum number, and hence of time, at the three frequencies. Drastic intensity jumps are observed, from spectrum to spectrum, at all three frequencies. At visual inspection, the intensity detected at the three frequencies seems to be strongly correlated. Such a correlation is generally observed in single molecule SERS spectra [8,11]. It can be mainly attributed to the presence of the background underlying the Raman lines; such a background could arise from interactions between the molecule and the metallic surface [12,18].

We now focus on the temporal occurrence of the peaks in the collected spectra. Vertical lines in Fig. 3 indicate, for the three frequencies, the spectrum number at which a peak is detected; the corresponding intensity overcoming a fixed threshold (above 2σ of the background level of the spectrum under analysis). Peaks are randomly observed in the sequence and such an intermittent behavior can be put into relationship to a binary trace of each vibrational mode. This means that the molecule randomly switches from a bright (on) state to a dark (off) state under continuous laser excitation. Although such an on-off behavior is closely reminiscent of that observed in single molecule fluorescence experiments [3], the richness of the spectral information encoded in the SERS spectra makes it particularly intriguing. Notably, no correlation (or anticorrelation) among the temporal emission of peaks at the three frequencies is registered (see legend of Fig. 3). This is indica-
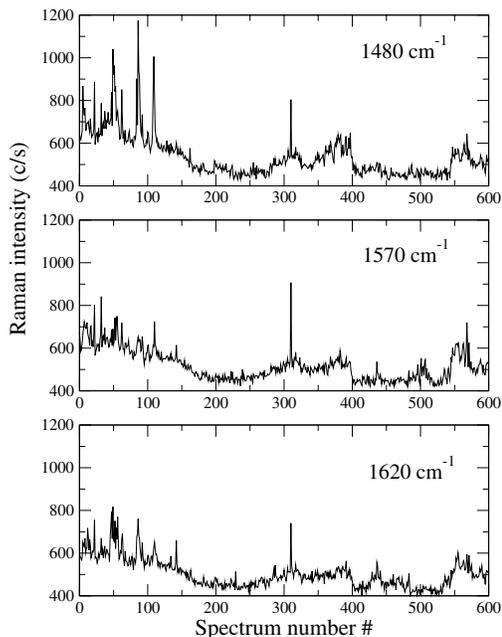

FIG. 2.   Raman intensity of signals as a function of spectrum number (detected at the 1480, 1570, and 1620 cm^{-1} frequencies) from immobilized silver colloids incubated with FePP at $10^{-11}M$.
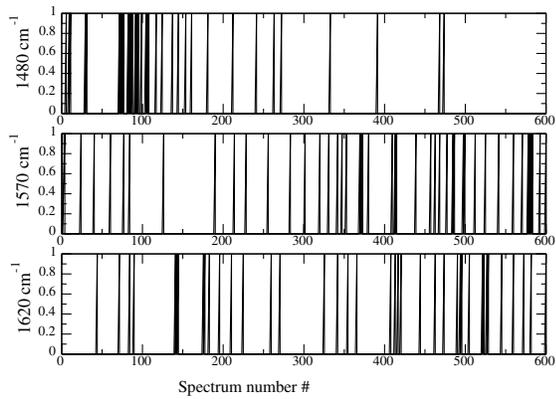
FIG. 3. On-off behavior of signals at the 1480, 1570, and 1620 cm^{-1} frequencies as a function of the spectrum number. The vertical lines mark the spectrum number at which a signal overcoming a threshold of 2σ above the noise level is detected. The values of the cross correlation at a delay 0, ρ_{ν_i,ν_j}, calculated between the appearance of a peak at the two different frequencies ν_i and ν_j are $\rho_{1480,1570} = 0.03$, $\rho_{1480,1620} = 0.05$, $\rho_{1570,1620} = -0.05$; these extremely low values for ρ indicate that there is no cross correlation.

tive of the fact that the various vibrational modes may follow independent activation channels during the temporal evolution of the molecule.

We are interested in analyzing the statistical properties of the on-off state occurrence for the selected vibrational emission modes. We define τ_{off} as the time interval during which no peak, at the given frequency, is revealed in the series of spectra. We have extracted these τ_{off}'s for the peaks at 1480, 1570, and 1620 cm^{-1} by collecting data for ten spectra series, acquired in sequence, to improve the statistics. We have then calculated, for each analyzed frequency, the distributions P_{off} by evaluating the occurrence of the corresponding τ_{off} within the acquisition time. The remarkable nonexponential nature of the obtained P_{off} distributions can be seen in the semilogarithmic plots of Fig. 4. Indeed, the experimental data are well described by a power law [the continuous lines in Fig. 4]:

$$P_{off} \sim 1/\tau_{off}^{(1+\alpha)}, \qquad (1)$$

with α values ranging from 0.46 to 0.56. To rule out the possibility that such an effect could be due to irreversible photoinduced processes, the independence of the α exponents on the laser intensity has been checked. From the statistical point of view, a power law distribution for P_{off} with an exponent between one and two (corresponding to α between 0 and 1) can be interpreted in the framework of the Lévy statistics [19,20].

Lévy statistics is a natural generalization of the Gaussian distribution when analyzing sums of independent, identically distributed, random variables and it is characterized by a diverging variance and broad distributions with power law tails [21–23]. Lévy statistics is encountered in a variety of fields (economy, physics, biology, ecology, etc.)

and it has been applied to analyze many different phenomena, such as laser cooling of atoms, relaxation processes of glasses, inhomogeneous line broadening in defected crystals, anomalous diffusion, mechanics of sandpiles, etc. [4,19,20,23]. Indeed, Lévy statistics is known to well describe several long-range interaction systems for which Gaussian distributions significantly understimate the occurrence probability of large and long time events. Recently, single molecule emission from fluorescent samples has been shown to obey Lévy statistics [3,4]. In our case, the observed intrinsically nonexponential behavior for the P_{off} distribution suggests some heterogeneity in the off (dark) state organization of the FePP vibrational modes [24]. The presence of a single off state would have given rise to a simple exponential decay for P_{off} [25]. On the other hand, the rather similar temporal decay observed for the three lines suggest that, although the vibrational modes may follow different activation channel, a common mechanism is expected to regulate the sampling of the FePP vibrational modes, and then of the on-off process.

Now we would like to discuss the microscopic origin of the peculiar temporal behavior observed in our single molecule system. The occurrence of Lévy statistics in single molecule signals has been generally put into relationship to relaxation phenomena arising from some disorder around the molecule [4]. The disorder could be static, arising from a heterogeneous distribution of traps (off states) from which the molecules should escape. Indeed, the power law distribution so far observed in single molecule fluorescence signals can be put into relationship to a multiple pathway for a recovery from the dark (off) states [25]. Alternatively, it could be due to dynamical changes in the molecule environment, likely in connection with a random walk of the molecule onto the metallic surface, characterized by an universal value of 0.5 for α [26].

The drastic fluctuations in SERS signals of single FePP molecules are expected to reflect either the intrinsic molecule dynamics determining the gating or activation of the different vibrational modes or its interaction with the external environment. It is well ascertained that SERS signals arise from a strong interaction between a molecule and its substrate. Indeed, both the EM and the chemical Raman enhancements drastically depend on the details of the arrangements of the molecule on the substrate (position, orientation, distance, etc). Therefore, due to the strong coupling between the electronic states and the vibrational modes in FePP, even small changes in the arrangement of the molecule on the silver surface (e.g., flipping or twisting) could yield a temporal modulation of the electronic states responsible for the enhancement effects.

Diffusive processes of FePP on the colloidal surface could occur during the measurements, as experimentally verified for similar molecules [14,27]. On such a basis, FePP molecules may experience continuously changing interaction forces (e.g., through changes in the FePP-surface distance) that could also modulate the emission and the gating of the vibrational modes. Additionally, FePP
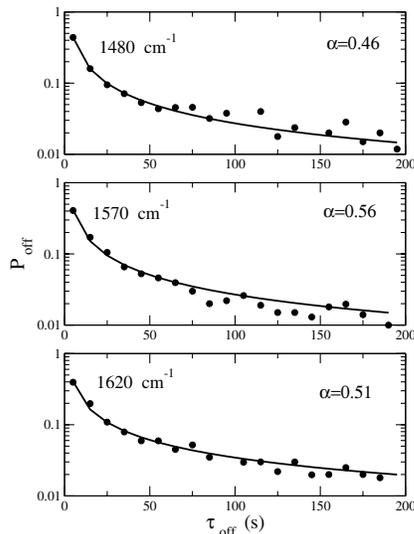
FIG. 4.   Distribution of the time interval τ_{off}; the latter represents the time intervals between the appearance of two successive peaks, from a collection of ten spectra series (each series containing 600 spectra) at the 1480, 1570, and 1620 cm^{-1} frequencies. Continuous curves give the fit by $1/\tau_{off}^{1+\alpha}$. The distributions have been normalized in order to provide the actual probability of measuring a given τ_{off}.

molecules, in the single molecule regime, are expected to be located near a fractal-like surface [28], where a high SERS enhancement takes place. Such a complex topology might give rise to anomalous diffusive processes, as suggested for particles moving on solid surfaces [29], or, more specifically, to Lévy flights [30]. From this picture, it could be hypothesized that the Lévy statistics, observed into the emission of FePP vibrational modes, originates from the disorder in the FePP molecule-colloid interactions likely involving a strong modulation of the electronic states coupled to the vibrational modes of the system.

In summary, the statistical analysis of SERS spectra from a single FePP molecule points out the occurrence of drastic spectral fluctuations governed by a nonexponential temporal behavior that can be traced back to a Lévy statistics. Such a finding constitutes a new insight into elucidating both the dynamical behavior of the molecule and its interaction with the nanoenvironment, also in the perspective to fully exploit the SERS capabilities in analyzing single molecules at the metallic interface.

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