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Time-Dependent Single-Molecule Raman Scattering as a Probe of Surface Dynamics

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Large spectral fluctuations, on a time scale of many seconds, are demonstrated in the surface-enhanced Raman scattering of single rhodamine 6G molecules adsorbed on silver nanocrystals and excited at 532 nm. These fluctuations are especially evident in the case of the vibrational bands at 614 and 774 cm^{-1} . A definite connection between spectral fluctuations and changes in the shape of the diffuse background emitted with the SERS spectrum is seen, pointing to the possible origin of both in a molecule-surface charge transfer phenomenon. The rate of spectral fluctuations is shown to depend linearly on laser intensity through a nonthermal mechanism. The rate of fluctuations can also be modified by changing the anion concentration in the solution on top of the silver colloids. Analysis of the results suggests that the fluctuations are due to motion of the adsorbed molecule on the surface, triggered by photodesorption events that are mediated by electron tunneling between the molecule and surface. Our results show that single-molecule Raman scattering can serve as a sensitive local probe for the dynamics of adsorbed molecules under ambient conditions.

Introduction

Exciting opportunities are opened by the recent discovery that surface-enhanced Raman scattering (SERS)^{1–6} is sensitive enough to measure spectra from individual molecules. These new prospects stem from the much more detailed information provided by the vibrational spectrum, compared to the relatively unstructured room-temperature fluorescence spectrum. Efficient utilization of this new phenomenon requires a thorough knowledge of its mechanism and the parameters that can be used to modify it. Unfortunately, a full theoretical and quantitative understanding of the SERS mechanism is lacking even after almost three decades of investigation.

Enhancement of Raman scattering cross-section of molecules adsorbed on rough metal surfaces has been attributed to two major effects (for reviews see refs 7 and 8). The main and foremost contribution to SERS comes from the enhancement of electromagnetic (EM) fields close to the surface through interaction with surface plasmon excitations. The local EM field depends on the microscopic shape of the metallic surface, such as the presence of a sharp edge. A particularly rich example of such local EM enhancement is provided by metal colloids and colloidal aggregates. Calculations by several authors^{5,9–13} suggest that protrusions on the surface of a colloidal particle, as well as “cavities” formed between adjacent particles in an aggregate, lead to a giant enhancement of the local field, up to a factor of 10^{11} .

The second (and smaller) contribution to the enhancement of Raman scattering is a specific interaction of the adsorbed molecule with the metal surface.⁸ In some instances, this molecular chemisorption effect leads to the formation of a charge-transfer interaction, whereby an electron is transferred from the molecule into the empty levels on the metal surface or from occupied surface levels to the molecule. The electron transfer can in fact be viewed as an electronic excitation of the coupled molecule-surface system and should therefore lead to

the appearance of a new band in the electronic spectrum of the molecule. Charge transfer (CT) bands have been demonstrated in optical¹⁴ and electron energy loss spectra¹⁵ of adsorbed molecules, and their relevance to SERS was discussed by Persson¹⁶ and others. Lombardi et al.¹⁷ used Albrecht's Raman scattering theory to discuss the contribution of CT transitions to SERS enhancement through a resonance Raman process. An immediate prediction of this resonance Raman charge-transfer (RR-CT) theory, stemming from the dependence of the energy of a CT band on surface potential, is the ability to modify the Raman enhancement by tuning this potential. This has been verified in many systems, the classical examples being those of pyridine and pyrazine.^{18,19} The CT enhancement mechanism is restricted, by its nature, to molecules adsorbed directly on the metal, as opposed to the EM effect, which extends a certain distance beyond the surface. The role of anions (in particular halide ions) in the formation of specific SERS active sites was discussed by Furtak and co-workers.^{20,21} They showed that the CT resonance of pyridine molecules on an electrode is communicated to coadsorbed chloride ions, indicating the formation of common complexes.

The occurrence of giant SERS cross sections on colloids facilitated the recent measurement of SERS spectra of single molecules of R6G,^{1,6} crystal violet,^{2,22} hemoglobin,²³ and tyrosine.⁴ It was shown by Xu et al.²³ and by Michaels et al.²⁴ that the strongest SERS signals are obtained from molecules adsorbed on small colloidal aggregates, as opposed to single colloidal particles. It was further suggested that it is at the junction between two nanocrystals that the electromagnetic field can reach the enormous values needed for the observed enhancement.

Temporal fluctuations (in both spectrum and intensity) of the fluorescence emitted by individual molecules are one of the hallmarks of single-molecule spectroscopy.^{25,26} Evidence for such fluctuations has emerged also in single-molecule SERS (smSERS) studies. Emory and Nie¹ reported abrupt but infrequent intensity and frequency changes in R6G spectra. Käll and co-workers demonstrated spectral changes in hemoglobin²³ and analyzed in depth spectral jumps in single tyrosine⁴ smSERS

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data, which were attributed to changes in the mode of adsorption of the molecules to the surface.

In this paper we show that SERS spectra of single Rhodamine 6G (R6G) molecules excited at 532 nm show dramatic fluctuations of the relative intensities of particular vibrational bands, while maintaining the overall spectral position of these bands. The rate of fluctuations is modulated in a nonthermal fashion by the intensity of illumination and by the concentration of salt in the solution covering the colloids. Our results provide further strong evidence for the role of charge transfer in the Raman enhancement process. We suggest that the spectral fluctuations are due to modulation of the CT contribution through lateral diffusion of the Raman scattering molecule. SERS of single molecules is thus established as a unique new probe for molecular dynamics on metal surfaces.

Materials and Methods

Silver nanocrystals were prepared by the Lee–Meisel citrate-reduction method²⁷ and shown by transmission electron microscopy (TEM) to be of average size of 50 nm. Samples for SERS were prepared by incubating an aliquot of the colloid solution with 1 mM NaCl for 4 h, then adding Rhodamine 6G at a concentration of 10^{-10} M, which gives a ratio of 1:1 between molecules and colloids. No significant aggregation of the colloids was seen under these conditions, although small aggregates containing several silver nanocrystals each were evident in TEM images, even in the absence of salt. The colloids were allowed to adsorb on polylysine-coated microscope cover slides for 30 min before a thorough rinsing. Experiments were conducted either on samples dried in air or on samples covered with water or salt solutions. It should be noted that samples dried in air contain a hydration layer due to the hydrophilic nature of the exposed surfaces. Indeed, no significant difference was found in the behavior of samples of the two types.

Our Raman spectrometer is built around an inverted microscope equipped with a 0.15 m spectrograph (Acton) attached to its side port. The sample is illuminated by the 532 nm beam of a diode-pumped frequency-doubled Nd:YVO laser (Verdi, Coherent). An area of 15 μm diameter is epi-illuminated by focusing the laser beam at the back focal plane of a 100 \times , N. A. 1.3 objective (Zeiss). Raman-scattered light is collected by the same objective and passes through a dichroic mirror (Chroma) and a Kaiser super-notch filter (to filter out light at the excitation wavelength) before being focused on the entrance slit of the spectrograph. The 30–40 cm^{-1} resolution of our spectrometer is enough to resolve all major peaks of the SERS spectrum of Rhodamine 6G. The dichroic mirror limits the spectra to frequencies larger than 500 cm^{-1} . The spectra are registered by a back-illuminated, thermoelectrically cooled CCD camera (Princeton Instruments).

Results

We identified strong Raman scattering molecules by manually scanning the sample under the microscope and locating brightly emitting colloids. Typically, not more than one “hot” nanocrystal was found in each illuminated area. This observation is similar to previous reports. We assumed that each of the “hot” nanocrystals contained only a single R6G molecule, based on the 1:1 ratio of R6G molecules to colloids, together with the low number of Raman-active particles. Further credence to the identification of the spectra as coming from single molecules is provided by the strong spectral fluctuations, as well as the frequent observation of a sudden loss of the Raman signal, both of which will be discussed in this paper.

Raman spectra were collected continuously, with an integration time of 1 s, until the signal was irreversibly lost. From a comparison of the average total intensity of Raman scattering from single molecules to the fluorescence intensity of single R6G molecules adsorbed directly on glass, we estimate the SERS cross section of a single molecule to be as high as 10^{-13} cm^2 . This is ~ 10 times higher than previous estimates.⁶ The difference can be partially explained by a difference in excitation wavelengths: Hildebrandt and Stockburger²⁸ showed that the SERS excitation profile of R6G follows the absorption spectrum of the dye, and we excite close to the peak of absorption, which is around 530 nm, while other workers have used 514 nm excitation. However, the excitation spectrum accounts for only a factor of 3 between the two wavelengths,²⁸ and so additional and unknown factors, unique to the single-molecule SERS phenomenon, must be in play. In any case, it is this huge single-molecule enhancement that allows us to collect high quality spectra in 1 s (compared to 10–60 s in previous publications^{1,6}).

Total Intensity Fluctuations. As already noted by other workers,^{1,6,22} the total SERS intensity of all molecules studied showed fluctuations, although the stability varied from one molecule to another. Figure 1 presents the time dependence of the total intensity of Raman scattering for 45 molecules illuminated with a laser intensity of 18 W/cm^2 . Each row represents one molecule, and the intensity trajectory was obtained by integration of a series of Raman spectra. Each trajectory is normalized to its highest value. Note that the overall collection time for different molecules is not equal; data collection was usually stopped when the signal became smaller than a certain value.

A variety of behaviors is displayed. Some molecules show several strong intensity jumps, while others show more gradual reduction of intensity. A large fraction of the molecules are seen to lose most of their scattering intensity in a single, sharp step. In general, the loss of the Raman signal is reminiscent of a similar loss discussed in both electrochemical^{29–32} and ultrahigh vacuum SERS systems,^{20,33} where it was attributed to diffusion of silver adatoms, a point to which we will return in the discussion. The strong intensity fluctuations, and particularly the abrupt signal loss, are indicative of single molecule behavior. The latter is probably not due to a photobleaching reaction, as the low fluorescence quantum yield of R6G on silver implies very short excited-state dwell time and therefore very low overall probability for excited-state chemistry.

Spectral Fluctuations. In addition to total intensity jumps, all R6G SERS spectra showed large and dramatic temporal changes in the intensities of individual bands. Figure 2 shows four spectra taken from a trajectory of a single R6G molecule, each labeled by its relative time in the trajectory. Modulations in the intensities of the low-frequency bands at 614 and 774 cm^{-1} are especially evident, although other bands change as well. The diffuse background under the vibration bands is a familiar phenomenon in SERS, and it was already observed that this background exists only in the presence of the spectrum above it and vice versa.⁶ As is evident in Figure 2, the intensity of the background has an intimate connection to the intensity of Raman bands above it, as its shape fluctuates simultaneously with them. The whole time-dependent spectral trajectory of another molecule is shown in Figure 3, where each spectrum, collected in 1 s, is represented by one colored strip. As Figures 2 and 3 show, two major groups of Raman bands emerge: The low-frequency bands at 614 and 774 cm^{-1} , which fluctuate significantly, and the high-frequency bands at 1363, 1509, 1575, and 1650 cm^{-1} , which show much smaller fluctuations.

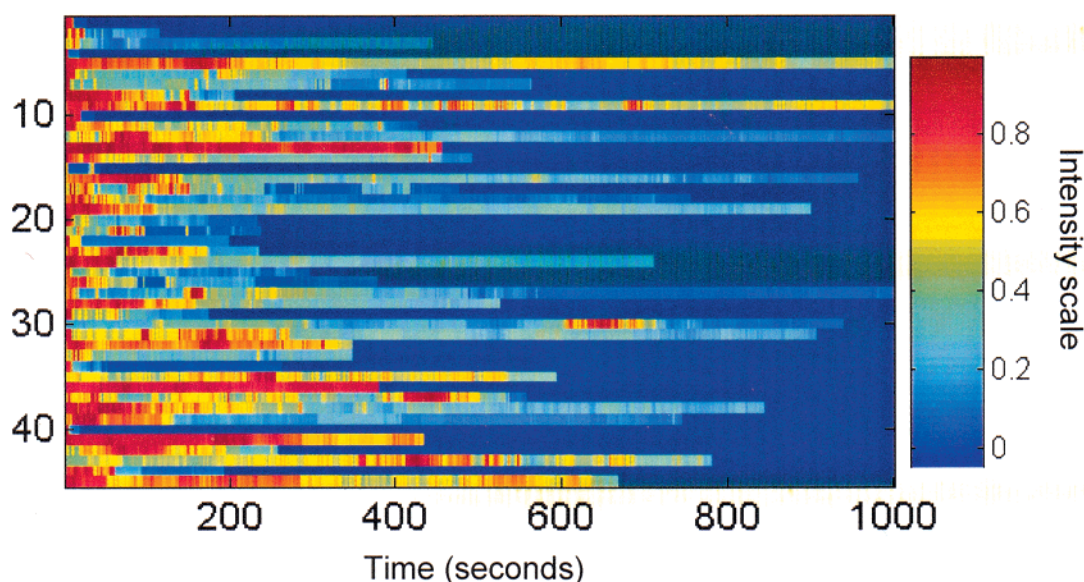


Figure 1. Total intensity fluctuation for 45 R6G molecules illuminated with a laser power of 18 W/cm². Each row shows the intensity trajectory of a single molecule, obtained by integration of time-dependent Raman spectra, and normalized to the highest value. The total data collection time was not equal for all molecules. An abrupt loss of a large fraction of the Raman signal (but usually not all of it) is evident in many of the molecules.

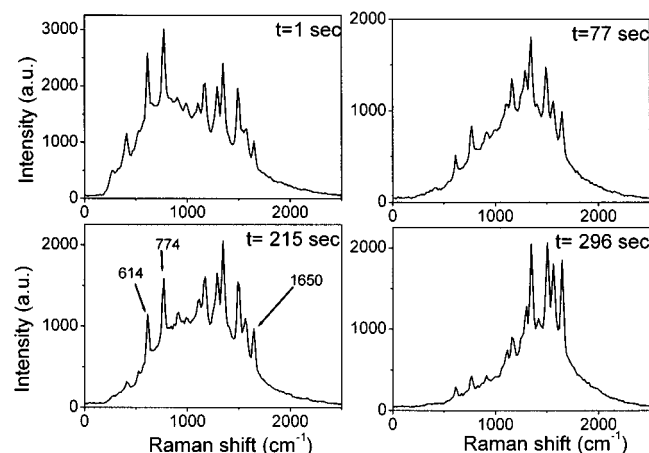


Figure 2. Sample spectra taken from a SERS trajectory of a single R6G molecule adsorbed on a silver nanocrystal and illuminated with a laser power of 18 W/cm². Each spectrum, collected in 1 s, is labeled with its relative time in the sequence. Variations in the intensities of individual vibrational bands are evident, and are especially noted for the two lowest frequency bands seen here, at 614 and 774 cm⁻¹, which can be assigned to bend vibrations.

To quantify the extent of the spectral fluctuations, and to characterize them independently of the total intensity changes, we calculated time-dependent intensity ratios of Raman bands, after carefully subtracting the background from each band. Figure 4 shows these ratios, as calculated from one long single molecule trajectory and presented as fractional fluctuations from the mean. The ratio of the bands at 614 and 774 cm⁻¹, $I_{614\text{cm}^{-1}}/I_{774\text{cm}^{-1}}$, is shown in Figure 4A. These two bands maintain their relative intensity essentially constant. A similar picture arises for the high-frequency bands (data not shown). The extent of fluctuation of the low-frequency vs high-frequency bands is demonstrated in Figure 4B with the intensity ratio of the 614 cm⁻¹ and the 1650 cm⁻¹ bands, $I_{614\text{cm}^{-1}}/I_{1650\text{cm}^{-1}}$. Note that this ratio varies by hundreds of percents! To find the characteristic time for the spectral dynamics observed here we calculated the autocorrelation function for the $I_{614\text{cm}^{-1}}/I_{1650\text{cm}^{-1}}$ ratio. It was found that the correlation function obtained from one molecule was too noisy to interpret, and we therefore

averaged it over 10 molecules. The correlation function is shown in Figure 4C, and it decays with a characteristic time of ~ 18 s.

To find out whether the spectral fluctuations are correlated with the total intensity fluctuations, we performed the following procedure. Series of spectra obtained for more than a hundred molecules were split into three groups according to their total Raman intensity: weak, medium, and strong. The spectra in each group were averaged and normalized. No significant changes were found between the three averaged spectra obtained in this way, indicating a lack of correlation between total intensity and spectral fluctuations.

Nonthermal Effect of Illumination on Spectral Fluctuations. Since in our experiment the sample is continuously illuminated by the laser, it is interesting to test whether the fluctuations are light-induced, either through a thermal effect or some other mechanism. Series of single-molecule spectral trajectories at different laser power densities (30 to 50 molecules in each group) were collected. In this case we found it useful to characterize the spectral dynamics not by using the ratio of specific vibrational bands as above, but by introducing a global measure for the fluctuations through a whole-spectrum correlation function. The spectra were all normalized (to get rid of the total intensity fluctuation contribution), and a correlation function was calculated for each molecule according to eq 1,

$$C(\tau) = \sum_{\bar{\nu}} (\langle I_{\bar{\nu}}(t) \cdot I_{\bar{\nu}}(t + \tau) \rangle - \langle I_{\bar{\nu}}(t) \rangle^2) \quad (1)$$

in which $I_{\bar{\nu}}(t)$ is the intensity at wavenumber $\bar{\nu}$ at time t , including the diffuse background, and the angular brackets denote temporal averaging. Inclusion of the background is not only a numerical convenience, but is justified by the strong relation of background and spectral fluctuations, the implication of which will be further discussed below. Correlation functions were first calculated for each molecule and then averaged over all molecules belonging to the same series. Figure 5 shows the correlation functions obtained at different laser power densities, from 6 to 100 W/cm². Each function is normalized by its first point, which is the mean squared fluctuation amplitude (variance). In addition, the figure includes the “zero laser power” fluctuation rate, which was obtained as follows. Single-molecule

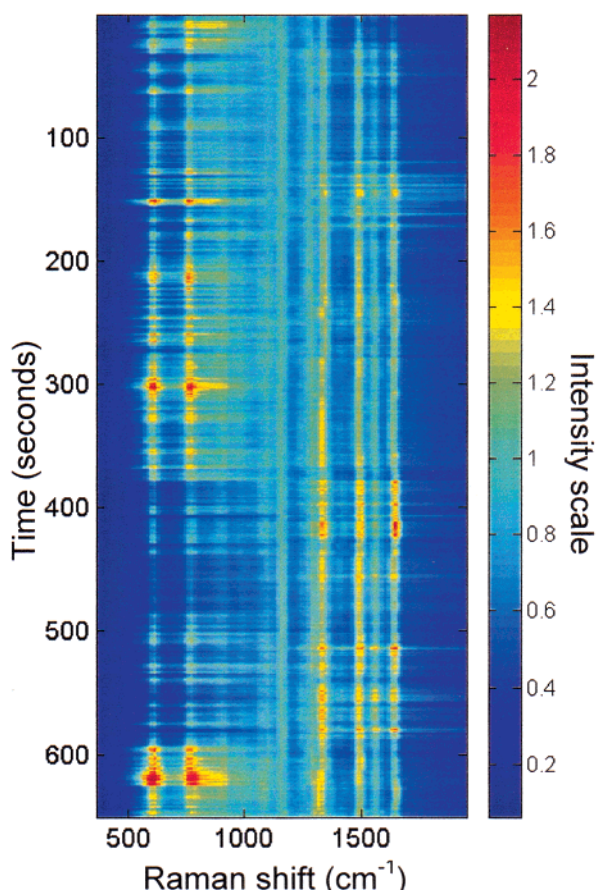


Figure 3. Entire time-dependent spectral trajectory of a single R6G molecule, illuminated with a laser power of 10 W/cm². Each row contains one color-coded spectrum, and the time advances from top to bottom. The strong fluctuations of the bands at 614 and 774 cm⁻¹ are particularly conspicuous in this figure.

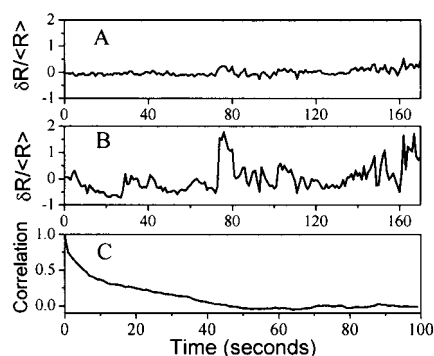


Figure 4. Temporal variation of ratios between vibrational band intensities in the SERS spectrum of a single R6G molecule, presented as fractional fluctuations from the mean, $\delta R/\langle R \rangle$. (A) $R = I_{614\text{cm}^{-1}}/I_{774\text{cm}^{-1}}$. (B) $R = I_{614\text{cm}^{-1}}/I_{1650\text{cm}^{-1}}$. The strong fluctuations of this ratio reflect mainly large intensity changes of the 614 cm⁻¹ band. (C) Autocorrelation function of the ratio $I_{614\text{cm}^{-1}}/I_{1650\text{cm}^{-1}}$, averaged over 10 molecules.

SERS spectral trajectories were registered with increasing dark intervals (i.e., intervals during which the sample was not illuminated) between consecutive spectra. It was found that for all trajectories taken with a dark interval exceeding 10 s, the correlation functions are essentially superimposable. This implies that under these conditions there is essentially no light effect on the fluctuations, and their rate is therefore assigned to “zero laser power”. The rate of fluctuations at each laser power density, k , was calculated by integrating the area under each

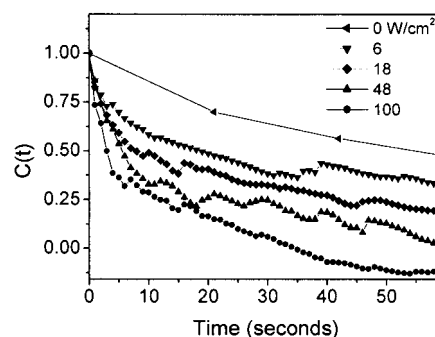


Figure 5. Whole-spectrum correlation functions of R6G molecules under different illumination intensities, given in the legend in units of W/cm². Correlation functions were calculated according to eq 1 in the text, and each function was averaged over several tens of molecules.

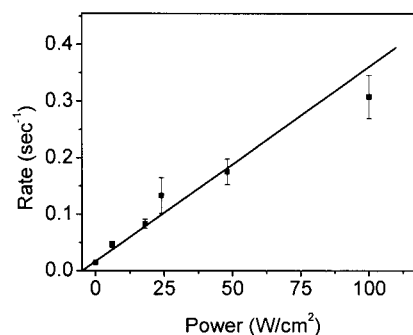


Figure 6. Dependence of the rates of fluctuations on laser power. The rates were determined by integrating the correlation functions of Figure 5, according to eq 2 in the text.

correlation function:

$$k^{-1} = \int (C(\tau)/C(0)) d\tau \quad (2)$$

The rates are plotted in Figure 6, and the linear dependence on laser power density is evident.

An important question is whether this linear relation is mediated by a thermal or a nonthermal mechanism. The colloids are adsorbed to a glass surface and are immersed in water (or covered by a hydration layer — we verified that the behavior is the same under both conditions). The thermal contact with the environment is thus quite good. We can use heat conduction theory to estimate the steady-state temperature at the colloid surface under illumination. Since the surface plasmon resonance dominates the interaction of the colloids with light, we can take a maximal guess and assume that all the energy absorbed by surface plasmon excitation is converted to heat. This is of course a large exaggeration, as most of the surface plasmon energy is re-emitted, but it provides an upper limit to the possible effect of laser light on the temperature. The steady-state temperature rise at the surface of a silver sphere of radius R immersed in water when an amount of heat Q is supplied to the surface per unit time is given by

$$\Delta T = \rho c Q / 4\pi \kappa R \quad (3)$$

where ρ and c are the density and specific heat of silver, respectively, and κ is the heat diffusivity in water.³⁴ By introducing the proper parameters, we calculate that the temperature rise under illumination at 100 W/cm², which is the maximal power density used in our experiment, is <0.1 K. It is concluded that no significant heating is caused by laser illumination, and therefore the effect of illumination is non-thermal.

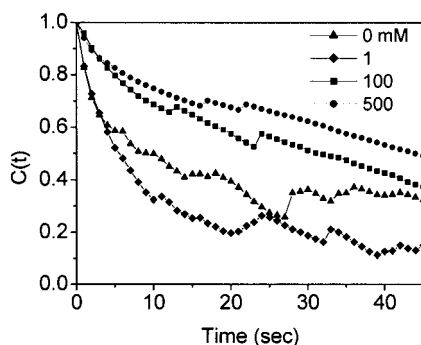


Figure 7. Whole-spectrum correlation functions of R6G molecules under different concentrations of NaCl, given in the legend in mM. Correlation functions were calculated according to eq 1 in the text, and each function was averaged over several tens of molecules. A laser power of 20 W/cm² was used for this series.

Effect of Salt Concentration on the Fluctuations. As noted in the Introduction, anions have been shown to play an important role in the SERS mechanism, forming active sites in complex with adsorbed molecules.^{20,21} Specific enhancement of R6G Raman scattering by halide ions, which reached its maximum at a relatively low ionic concentration, was demonstrated by Hildebrandt and Stockburger.²⁸ For example, chloride ions enhanced the SERS cross section by a factor of ~ 170 at a saturating concentration of ~ 1 mM. We were interested in checking whether anions exert any influence on the spectral fluctuations of single Raman scattering molecules. Series of spectral trajectories were collected under different concentrations of NaCl, with ~ 30 molecules at each concentration. The fluctuations were analyzed in the same way as described before, and the correlation functions are plotted in Figure 7. A remarkable reduction in the rate of the fluctuations is observed as salt concentration is increased.

Discussion

The outline of the discussion section is as follows. We first show that selection rules related to the EM enhancement mechanism are unlikely to be involved in formation of spectral fluctuations. We then discuss the role of CT selection rules and explain how modulation of the CT contribution to SERS can arise from variations of the local work function of the metal surface at the molecular adsorption site. Such variations also explain the intimate connection of the diffuse background to the Raman spectrum. Several causes for temporal changes of the local work function are analyzed, and it is concluded that only lateral motion of the molecule, mediated by laser-assisted desorption, can account for all our experimental findings.

EM Selection Rules and the Spectral Fluctuations. As noted in the Introduction, the main contribution to the giant SERS cross section of R6G must come from the EM enhancement. Can the spectral fluctuations be related to this mechanism? Selection rules related to the EM enhancement are similar in spirit to the surface selection rules of infrared spectroscopy.³⁵ In brief, the component of the electromagnetic field perpendicular to the surface is much larger than the components parallel to the surface. Heuristically, this implies that normal modes polarized perpendicular to the surface will be enhanced significantly more than modes polarized parallel to the surface. This selection rule provides, in principle, a connection between the orientation of a molecule on a surface and the relative intensity of different bands of its Raman spectrum. In view of this, it is feasible that changes in the orientation of a single molecule relative to the colloidal system to which it is attached will lead

TABLE 1: Assignment of Vibrational Bands Seen in the SERS Spectrum of R6G^a

wavenumber (cm ⁻¹)	assignment
614	C—C—C ring in plane bend
774	C—H out of plane bend
1363	aromatic C—C stretch
1509	aromatic C—C stretch
1575	aromatic C—C stretch
1650	aromatic C —C stretch

^a Based on refs 28, 38–40.

to fluctuations such as those registered in our experiment. While useful expressions for the surface selection rules of ensembles of molecules on colloids can be derived,^{36,37} it is not as simple to do the same for a single molecule, as the local configuration of the surface, as well as the orientation of the molecule, are not known. It is likely though that while a molecule may adopt a range of orientations on a surface, the exchange between them will be fast. For example, a molecule can rotate around the bond it forms with the surface, a motion which will occur on the nanosecond time scale. Any spectroscopic signal taken on the time scale of seconds will therefore be averaged essentially over the whole configurational space of the molecule-surface complex. However, if an energy barrier exists between two different average surface orientations, a transition between the two can change the relative enhancement of different Raman tensor elements and lead to the fluctuations seen in the spectra. In this case, the laser intensity dependence registered in the experiment can be attributed to enhancement of barrier crossing by a nonthermal mechanism, as will be discussed below. Clearly, a whole range of surface orientations has to be invoked to explain the spectra of Figures 2 and 3. Further, it is difficult to explain the effect of salt concentration through an orientational mechanism. Thus it is probably safe to rule out the involvement of orientational aspects in causing the spectral fluctuations.

Charge Transfer and Spectral Fluctuations. The important contribution of CT enhancement at specific active sites to the SERS cross section of R6G was already demonstrated in 1984 by Hildebrandt and Stockburger.²⁸ These authors not only demonstrated the specific effect of halide ions on SERS enhancement, but also presented Raman excitation profiles that point to the role of the CT mechanism. While the excitation profiles of most vibrational bands essentially mimic the absorption spectrum, the 774 cm⁻¹ band presents an extra shoulder in its profile, which could be due to a second excitation band, peaking around 570 nm. This band may be attributed to a CT resonance between the molecule and the silver surface.

We therefore suggest, as was also proposed by Brus and co-workers,⁶ that a proper description of the SERS spectrum of R6G should also include, in addition to EM enhancement, the contribution from *resonance Raman scattering* involving both the molecular absorption band and the CT band. The theoretical description of a Raman process involving two resonances of this type and their vibronic coupling is complicated.¹⁷ However, it is obvious that the molecular and CT resonance can each enhance a different set of vibrations. The molecular resonance of R6G is expected to enhance mainly bands polarized along the direction of the electronic transition dipole moment, i.e., in the plane of the aromatic ring system of the molecule. The CT resonance, which involves an ionic excited state of the molecule, might also enhance bands polarized out-of-plane.

Table 1 shows the assignment of the main bands in the R6G SERS spectrum.^{28,38–40} The four bands that are relatively stable in our spectral trajectories are seen to be due to in-plane stretch vibrations, while the two fluctuating bands involve bend

vibrations. This is a qualitative difference that might indicate the disparity in the behavior of the two groups. The excitation profiles mentioned above suggest that the out-of-plane C–H bend vibration at 773 cm^{-1} is indeed enhanced by the CT resonance more than other bands, and this behavior could be shared by the 614 cm^{-1} vibration.

What physical process can modulate the extent of CT enhancement? The energy of the CT resonance depends on the position of the HOMO or LUMO levels of the molecule (for CT to or from the metal, respectively), but these levels are not expected to fluctuate. The CT energy also depends on the local work function (or charge density) of the metal at the molecular adsorption site. Variations in the local work function (LWF) at steps on metal surfaces are well documented^{41,42} and stem from the Smoluchowski electron smoothing effect.⁴³ Such variations can also occur at sites of surface defects. Thus it is tempting to infer that the spectral fluctuations we observe are due to variations of the LWF at the molecular adsorption site, which modify the extent of CT involvement and with it the shape of the spectrum. It is possible to estimate the amplitude of these variations: The excitation spectra of Hildebrandt and Stockburger indicate a CT band at 570 nm, or 2.2 eV. To get this band into full resonance with the laser at 532 nm, the LWF has to change by 0.15 eV. This is certainly within the range of LWF changes measured by Wandelt⁴¹ at different step sites (up to 1.0 eV on a Pt(111) surface).

LWF Variations and the Diffuse Background. Our data show that the Raman spectrum of single R6G molecules always appears on top of a diffuse background signal. Further, fluctuations in the intensity of specific bands in the vibrational spectrum are accompanied by similar fluctuations in the intensity of the background signal. We therefore infer that a common process is responsible for these two wavenumber-specific responses. A diffuse background has been one of the hallmarks of the SERS phenomenon for many years.^{44–46} It was proposed by Otto⁸ that the background is a byproduct of a nonradiative CT process between the surface and the molecule. The origin of the electrons participating in this process is an electron–hole pair formed directly on the surface by the interaction with the light. Contrary to the RR–CT mechanism, the transfer of an electron to the molecule and back is nonradiative. The tunneling electron can lose energy to the molecule through a vibrational excitation, as it is transferred back to the surface. Light will then be emitted by a recombination of the electron with a hole on the surface. The spectral bandwidth of the emitted light will depend on the fast dephasing rate of electron–hole pairs, contrary to the RR–CT mechanism, where it depends on the much slower dephasing rate of vibrational excitations. Therefore, no sharp spectral bands are expected, and this explains the diffuse nature of the spectrum of the background. The nonradiative CT process should be similar to the RR–CT mechanism in the way it depends on the value of the LWF. The strong correlation between the intensity fluctuations of Raman bands and the diffuse background is thus due to their mutual dependence on variations of the LWF.

Microscopic Origin of Local Work Function Variations. What could be the origin of the fluctuations that allow a single molecule of R6G to sample a range of LWF values? Three possible mechanisms are discussed here, and it is found that only surface diffusion of the adsorbed molecule can account both for the laser power dependence and the anion concentration dependence of the fluctuation rate.

Electrostatic Fluctuations. The effect of chloride ions on the rate of fluctuations is suggestive of a role for electrostatic screening. If the electrostatic potential at the double layer around

a colloidal particle fluctuates over time, it will affect the LWF and through it the SERS spectrum. At high salt concentrations these potential fluctuations should be strongly damped, leading to a reduction of the rate of spectral changes. However, calculations by D. Lukatsky and S. Safran (private communication) show that the rate of fluctuations of the double layer potential around a colloid is much faster than the rate of fluctuations registered in our SERS experiment. Furthermore, there is no reason to believe that laser illumination will affect the double layer potential.

Diffusion of Silver Adatoms. The irreversible loss of the SERS signal over time, and its relation to a similar loss which is well-known in ensemble SERS spectroscopy, were already mentioned in the Results section. It should be added here that there is a rough correlation between laser power and the rate of signal loss, although it is difficult to quantitatively characterize this correlation. Since there is also a correlation between laser power and the rate of spectral fluctuations, this implies that the two phenomena arise from the same mechanism. Otto and co-workers pioneered the idea of an involvement of “atomic scale roughness” in the SERS signal formation mechanism,⁴⁷ and suggested that efficient coupling of electromagnetic radiation to molecule-surface complexes at sites of point defects can explain at least part of the SERS enhancement. While this idea leads quite naturally to the CT enhancement mechanism already mentioned, its relevance here stems from the connection made to motion of metal adatoms around the molecular adsorption site. It is possible that such motion leads to modulation of the CT contribution, and as the adsorption site is remodeled by diffusion it gradually loses its initial structure and concomitantly loses its ability to enhance Raman scattering. Estimates of the diffusion constant of silver adatoms on a silver surface vary widely. For example, Pai et al.⁴⁸ obtain a value of $18\text{ Å}^2/\text{s}$ for the diffusion constant at room temperature, extrapolated from measurements of island motion by STM. Hirai et al.,⁴⁹ on the other hand, use electrochemical atomic force microscopy to determine silver diffusion constants at the liquid/metal interface and find a value as high as $\sim 200\text{ Å}^2/\text{s}$. While the process measured by these methods might be more complicated than self-diffusion of a single adatom (they might actually report on a collective mode of surface motions), the time scale of this process does match the time scale of spectral dynamics.

The main caveat in this adatom diffusion mechanism is that it is not straightforward to envision how it couples with light to give a nonthermal dependence on illumination intensity. To the best of our knowledge there are no indications in the literature for laser-assisted, nonthermal enhancement of diffusion rates on a metal surface. It is also not known how anion concentration can affect adatom diffusion.

Diffusion of the Adsorbed Molecule. There is sparse information in the literature regarding the diffusion of large molecules adsorbed on metal surfaces, and the existing information pertains to vacuum conditions.⁵⁰ It is therefore not possible to estimate the possible diffusion rate of R6G on the nanocrystal surface. Further, the diffusion mechanism of a molecule covered with liquid can be quite different than the diffusion mechanisms of a molecule on a clean surface under vacuum. For example, it could be that diffusion is facilitated through a series of desorption-readsorption steps. The role of desorptive events was also implied by the simultaneous appearance of Raman and fluorescence signals from the same particle in the recent single-molecule SERS study of Seidel and co-workers.⁵¹ In fact, it is quite likely that the desorption of R6G molecules is assisted by light. The involvement of hot electrons tunneling between a

metal surface and an adsorbed molecule in the induction of photodesorption is well documented,^{52,53} and the process is known as “desorption induced by electronic transitions (DIET)”. In the simplest case, the transfer of a single electron to/from the adsorbed molecule induces vibrational excitation in a mode crucial for surface bonding and provides enough energy to that mode to enable desorption. The laser intensity dependence of this single electron process is therefore linear. Since our experiments are not carried out under vacuum, the friction exerted on the desorbing molecule by the liquid can force it to readsorb at the same site from which it was detached or a very nearby one. If the new adsorption site has a slightly different LWF from the original site, a change in the Raman spectrum can be induced. The linear dependence of rate of spectral changes on laser power is well explained by the DIET mechanism.

The role of anion concentration under this model is slightly more speculative. The concentration of anions very close to the metal surface should depend on the local surface potential of the bare metal. The modification of anion concentration parallel to the metal surface due to the occurrence of steps and other defect sites should lead in turn to a reduction in the variation of the LWF along the surface. A more uniform LWF translates into a lower rate of fluctuations following motion of the R6G molecule on the surface.

Conclusion

SERS spectral dynamics of individual R6G molecules were examined under various conditions and statistically analyzed. Large spectral fluctuations, which are completely masked in ensemble SERS studies, were clearly identified. It was found that the time scale of these fluctuations depends on the power of illumination and on electrolyte concentration. The most likely mechanism leading to this behavior involves lateral motion of the adsorbed molecule. This diffusive motion is possibly mediated by desorption events triggered by electron tunneling between the metal surface and the molecule. An immediate implication of this mechanism is that our experiment directly follows photodesorption-readsorption cycles of a single molecule. One way to verify this exciting possibility is by measuring fluctuations of the normal mode related to the molecule-surface bond, i.e., the Ag–N vibrational band. Our current instrument does not allow us to resolve this low-frequency band, but we plan to modify it in order to enable this study soon.

The single-molecule study presented here provides a novel view on the involvement of charge-transfer processes in Raman scattering enhancement at metal surfaces. The study also suggests how single-molecule SERS can be used to investigate surface processes with very high spatial resolution under ambient conditions. Such investigations will be facilitated by the establishment of a more direct connection between spectral fluctuations and surface motion. Engineered surfaces, such as silver island films, may allow a systematic variation of surface conditions and test their influence on spectral fluctuations. Overall, this study contributes evidence that single-molecule Raman spectroscopy is emerging as a powerful technique that promises to shed new light on the dynamics of molecules at interfaces.

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