

Single-Molecule Raman Spectroscopy: A Probe of Surface Dynamics and Plasmonic Fields

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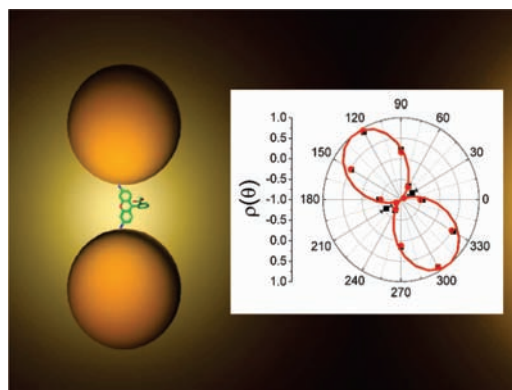
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CONSPECTUS

Single-molecule spectroscopy has opened exciting new realms of research, allowing the exploration of molecular dynamics within heterogeneous media, from live cells to chemical catalysts. Raman spectroscopy of individual molecules is particularly useful because it may provide more detailed information than is available in the typically broad fluorescent spectrum. To overcome the problem of small Raman cross sections, however, enhancement by surface plasmon excitation is necessary. This enhancement is particularly strong in the gaps between noble metal nanoparticles; indeed, it is strong enough for the observation of Raman signals from single molecules.

The electromagnetic fields generated by surface plasmons depend quite intricately on the shape of the nanoparticles, their spatial arrangement, and their environment. Single molecules can serve as the ultimate local probes for the plasmonic fields. Such a “mapping expedition” requires accurate molecular positioning abilities on one hand, and nanoparticle cluster engineering methods on the other hand. This Account describes our first steps toward achieving these goals. It is shown that a molecule can indeed be judiciously positioned within the gap of a nanoparticle dimer and that it can report on the effect of particle size on the plasmon resonance spectrum. When a third particle is added, breaking the dimer symmetry, the electromagnetic field at the gap changes significantly, as manifested by dramatic polarization effects. A combination of electron microscopy, Raman spectroscopy, and theoretical calculations is used to fully understand symmetry breaking in nanoparticle trimers.

As is well-known, the strong interaction of molecules with metallic surfaces may lead to modulation of their excited state energies and even to charge transfer to or from the surface. The impact of charge transfer on surface-enhanced Raman scattering has been debated for many years. Single-molecule spectroscopy offers new opportunities for probing this phenomenology. Charge-transfer excitations may enhance Raman scattering, sometimes also modulating the Raman spectrum in a manner reminiscent of the molecular resonance effect. Two approaches for looking into this effect are described in the Account. First, the observation of spectral dynamics driven by molecular motion provides indirect evidence for the importance of molecule-surface electronic coupling. More direct evidence is offered by single-molecule Raman spectroscopy studies within an electrochemical cell. The surface potential is systematically modulated, and the effect on Raman spectra is studied. It is found that the charge transfer interaction increases the signals by at least 3 orders of magnitude, but it also changes dramatically Raman spectral shapes. A mechanism for this complex behavior is proposed based on the theory of charge-transfer resonance-Raman scattering.



1. Single-Molecule Raman Scattering: Introduction

More than 30 years after its discovery, surface-enhanced Raman scattering (SERS) enjoys a significant revival, which is due to two factors. The

first of these is the realization that SERS might be sensitive enough to detect single molecules.¹ The second factor is the increasing interest in the new field of plasmonics, that is, the study of the interaction of light with nanoscale metallic structures.²

The two are, of course, connected: it is the strong surface-plasmon-related electromagnetic fields within and around metallic nanostructures which facilitate the observation of Raman scattering with single-molecule sensitivity.³

Surface plasmons (SPs) are collective excitations of the electrons within the conduction band of a metal. Electromagnetic radiation does not excite SPs on a smooth metallic surface because of a mismatch of the dispersion relations. However, the formation of structure on the surface whose features are smaller than the wavelength of light facilitates excitation of SPs. Such structure can be simply a roughened surface, or a collection of metallic nanoparticles (NPs). On extended surfaces the coupling of SPs and the electromagnetic field leads to propagating excitations, surface plasmon polaritons. However, on NPs, the SP excitation cannot propagate and is therefore termed "localized surface plasmon (LSP)". The LSP resonance of gold and silver NPs occurs in the visible range of the spectrum, which makes these two metals particularly useful for applications.

The electric field formed by SP excitation at the surface of metallic NPs is much stronger than the external electromagnetic field. This strong local field may couple to molecules on the surface and facilitate Raman scattering. The molecules scatter the field, producing a new field at a shifted frequency due to the Raman process. This new field may also be enhanced by interaction with the metal. The overall enhancement of the Raman scattering cross section may therefore be written as a product of the enhancement at the external frequency and the enhancement at the Raman frequency and is particularly strong within narrow junctions between NPs. A maximal enhancement is achieved when the nanogap between two particles is of the order of 1 nm.⁴ The enhancement drops precipitously as the distance increases. The enhancement also drops when the distance decreases below 1 nm because of increased electron tunneling between the particles.⁵ Thus, a 1–2 nm long molecule bridging two NPs seems to be under the optimal conditions for SERS.

The electromagnetic mechanism is not the only cause for enhancement of Raman scattering from molecules. It has been postulated that the interaction of molecules with metallic surfaces may be behind an additional increase in the Raman scattering, the so-called chemical enhancement (CE).⁶ Over the years, there has been much debate with regard to physical origin and magnitude of CE. Recent experimental and theoretical work starts to clarify this issue. It is likely that CE does contribute 3–4 orders of magnitude to the Raman signal of molecules on some metallic surfaces. This contribution is much smaller than that of the electromagnetic enhancement.

The latter may therefore be seen as a useful means to facilitate the observation of the former. Physically, it has been suggested that CE embodies at least two separate effects.⁷ First, an increase in the static polarizability of a molecule due to adsorption on the metal may increase the Raman cross-section. Second, the appearance of new absorption bands due to light-induced charge transfer between the molecule and the surface may also increase the Raman cross-section in a mechanism akin to molecular resonance Raman scattering.⁸ This effect is sometimes called charge-transfer resonance Raman (CT-RR) scattering.⁹

Clearly, molecule–surface interactions can have a strong impact on the Raman cross-section. It is therefore apparent that single-molecule surface-enhanced Raman spectroscopy (smSERS), with its inherent sensitivity, can be used as a probe of molecular dynamics on surfaces, including charge transfer interactions. This is the subject of section 2 of this Account. But, as will be shown in section 3, individual molecules can also be used to probe the plasmon fields themselves within NP clusters and to understand their intensity and spatial variation in relation to cluster structure.

2. From Spectral Fluctuations to Charge-Transfer Dynamics

2.1. Fluctuations in Raman Spectra of Individual Molecules. It has been recognized from the early days of single-molecule spectroscopy that the signals of individual molecules are characterized by strong fluctuations due to both photon noise and molecular dynamics, which may lead to transiently dark states. Rigorous tests for the identification of fluorescence signals as arising from a single molecule were devised, the most common based on the observation of antibunching in photon correlation functions and single-step photobleaching in intensity trajectories. Neither of these robust tests can be applied to identify Raman scattering from single molecules. The Raman-scattering molecule spends a very short time on the excited state (of the order of a few femtoseconds), so that it is technically very difficult to observe an antibunching signal. The short excited-state lifetime implies that single-step photobleaching is also hard to observe. The validation of single-molecule signals had thus relied on less rigorous criteria, including the use of a very low concentration of molecules and the observation of blinking and intensity fluctuations. Recently, Le Ru et al.¹⁰ and Dieringer et al.¹¹ showed that, by using two analytes whose Raman spectra differ, one can validate single-molecule signals in a statistically rigorous manner. The idea behind their method is that if each

individual NP cluster contains only one molecule, only “pure” spectra are registered, while if more than one molecule is adsorbed on each NP cluster, the spectra may be mixed. It was verified that under the conditions typically used in single-molecule Raman experiments, almost only “pure” spectra are seen, and the single-molecule limit is indeed attained.

Two additional observations made earlier in our lab also strongly indicated that the single-molecule limit is reachable. First, we looked at the intensity of the Raman signals obtained from surface-adsorbed small NP clusters, created in the presence of a range of concentrations of the molecule crystal violet (CV), from 10^{-6} to 10^{-10} M (corresponding to a ratio of molecules to particles ranging from 10^5 down to 10).¹² The average intensity of signals from individual NP clusters was essentially constant below $\sim 10^{-7}$ M. This observation suggests that the signals obtained below that concentration already emanate from single molecules. Second, in experiments described in the following paragraph, we found not only overall intensity fluctuations in R6G spectra but also dramatic temporal changes in the relative intensities of vibrational bands.¹³ Such spectral dynamics are also, we believe, a strong indication that the experiments were conducted at the single-molecule limit, or very close to it. Let us discuss in some detail the spectral dynamics of R6G molecules and their possible physical origin.

Our smSERS experiments with R6G¹³ started with an attempt to reproduce the groundbreaking experiments of Nie and co-workers.¹ R6G molecules at a concentration of 10^{-10} M were added to a silver colloid solution. NPs were adsorbed on a glass surface and imaged under the microscope. Upon excitation at 532 nm we were able to register Raman spectra from individual “hot spots” on the surface. The designation of these spectra as arising from individual molecules was heuristically done based on the molecular concentration used and the appearance of intensity fluctuations or “blinking”. The surprise came when it was noted that strong *spectral fluctuations* (i.e., variation of the *relative* strength of different spectral lines) appeared in many of the spectral series we registered, with a typical correlation time of seconds (Figure 1). It was hypothesized that the origin of the spectral fluctuations was lateral motion of R6G molecules on the surface of the NPs. Indeed, increasing the viscosity of the aqueous solution covering the colloids by addition of glycerol led to a large increase in the correlation time of the fluctuations, suggesting a major slowdown of lateral diffusion.¹⁴

But how does a large molecule such as R6G diffuse on a metallic surface? Since it was found that the fluctuation correlation time depends linearly on laser power (while heating

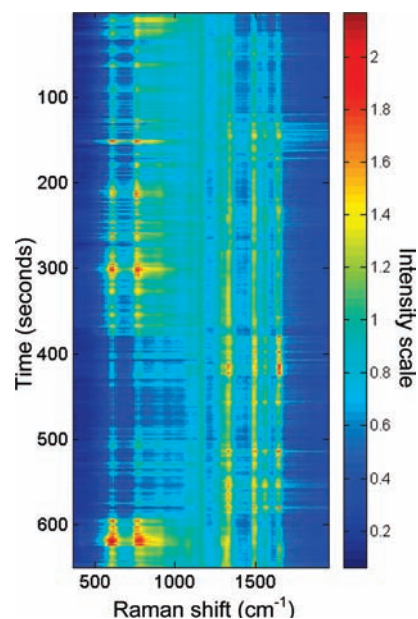


FIGURE 1. Time-dependent spectral trajectory of a single R6G molecule, illuminated with a laser power of 10 W/cm^2 . Each row contains one color-coded spectrum, and the time advances from top to bottom. The strong fluctuations of the bands at 614 and 774 cm^{-1} are particularly conspicuous in this figure.

effects could be ruled out),¹³ it is likely that the motion of R6G is photoinduced. The process of “desorption induced by electronic transitions (DIET)” comes to mind.¹⁵ In the simplest version of DIET, light-induced transfer of a single electron to/from the adsorbed molecule from/to the surface induces vibrational excitation in a mode crucial for surface bonding and provides enough energy to that mode to enable desorption. An electronic transition that involves charge transfer between the metal surface and the molecule is at the heart of the CT-RR process.⁸ It remains to be explained why the motion of the molecule on the surface modulates its Raman spectrum. In fact, when a molecule diffuses on a metal surface, it may well encounter regions with different local potential, or local work function (LWF).¹⁶ A change in the LWF is equivalent to a local change in the position of the Fermi level of the metal near the molecule. This implies that the coupling between the molecule and the surface, necessary for a CT transition, may be modulated, leading to spectral dynamics (Figure 2). One way to prevent variation of the LWF on the surface is to decorate it with adsorbed ions, such as chloride. Indeed, a large concentration of chloride ions reduced dramatically the correlation time of spectral fluctuations.¹³ This presumably happened not since the molecular diffusion was slowed down, but due to the removal of surface heterogeneity by the bound ions.

Thus, there is a likely physical correlation between spectral dynamics and LWF heterogeneity on the surface. It is therefore possible to invert the distribution of spectral fluctu-

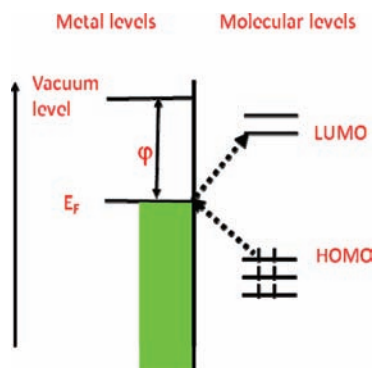


FIGURE 2. Scheme of the energy levels involved in a CT transition. HOMO and LUMO are the highest-occupied and lowest-unoccupied molecular orbitals, respectively. E_F is the Fermi level and φ is the (local) work function. The dashed arrows designate CT from the molecule to the metal or vice versa. Fluctuation of φ may tune the CT transition in and out of resonance with a laser field.

ations and obtain, using some simple assumptions, the distribution of the LWF on the surface.¹⁴ Particularly intriguing was the observation, that the standard deviation of the LWF distribution extracted in this way is as small as 0.1 eV. This suggests that a single molecule of R6G serves as an exquisitely sensitive sensor of LWF variation on a metallic surface. Spectral fluctuations were also observed in our lab in experiments with single molecules of CV¹² and were also reported by several other groups (see, for example, refs 17 and 18).

2.2. Charge Transfer and the Raman Signal. Obviously, the observation of light-induced spectral fluctuations does not constitute a direct proof for an electronic transition involving metal-molecule charge transfer. In the past, the CT-RR mechanism was probed by measuring Raman spectra of molecules adsorbed on electrodes, so that the surface potential could be actively varied, tuning the molecule in and out of a CT resonance (see refs 8 and 9 and references therein). These experiments were marred by surface heterogeneity, as well as by uncertainties with respect to the degree of surface coverage of the probed molecules and their orientation. Experiments at or near the single-molecule limit can overcome some of these problems. We therefore decided to perform single-molecule Raman spectroscopy within an electrochemical cell, which allows the surface potential to be varied.¹⁹ For this experiment, we chose molecules of 4-mercaptopyridine (4MPy), which strongly chemisorb on a silver surface. As a substrate we used silver islands deposited on an indium tin oxide (ITO) slide, which also served as an electrode. 4MPy molecules were allowed to adsorb on the silver islands from a 100 nM solution. We then illuminated the surface with light at either 488, 532, or 632 nm, far from any molecular resonance of 4MPy. Images registered with a back-illuminated CCD camera revealed bright dots of size of the order of the diffraction limit

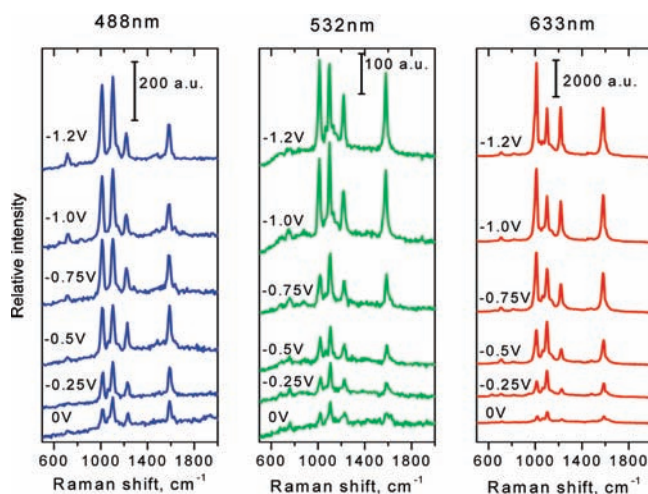


FIGURE 3. Wavelength- and potential-dependent SERS spectra of 4MPy molecules recorded from individual hot spots within silver island structures. Note the strong increase in the overall intensity with electrode potential, accompanied by significant changes in spectral shape.

(smaller than the size of the silver islands). We concluded that the bright dots are individual SERS “hot spots”, and estimated that even in the extreme case that all molecules adsorbed on the surface, not more than 50–100 4MPy molecules could be found in each of these hot spots, with the actual number being most likely significantly lower. Thus our experiment represented a case of single-hot-spot spectroscopy, close to the single-molecule limit.

Typical 4MPy SERS spectra obtained from individual hot spots, excited at the three wavelengths mentioned above and registered at a range of electrode potentials, are shown in Figure 3. Clearly, the spectra vary quite dramatically as the electrode potential changes, both in overall intensity and in the relative intensity of individual lines. This is a manifestation of a CT resonance; analysis of the voltage dependence of the signals suggests a metal-to-molecule charge transfer.¹⁹ The design and results of our experiments allowed us to discard the above-mentioned uncertainties related to surface coverage and molecular reorientation, and show quite unequivocally the existence of a CT resonance. In particular, 4MPy molecules are chemisorbed, not physisorbed, so that there are no surface coverage changes during the experiment. Further, a careful analysis of the potential-dependent spectra, in light of the surface selection rule,²⁰ showed that molecular reorientation cannot be a dominant cause of spectral variations. Let us briefly summarize the argument, which is more fully presented in ref 19. The surface selection rule implies that essentially only vibrational modes with polarizability components perpendicular to the surface are enhanced. The four strong bands in 4MPy spectra are all due to totally symmetric, in-

plane vibrational modes. A molecular reorientation which leads to increased Raman scattering intensity must make the molecular ring plane more normal to the surface. But in this case the ring plane is parallel to the surface at 0 V, when the intensity is minimal, and out-of-plane modes should appear in the spectrum. The absence of out-of-plane modes is therefore an indication that reorientation plays a minor role in reshaping the spectra as electrode potential is changed.

Having convinced ourselves that a CT resonance is responsible for the phenomenology observed in the voltage sweep experiment, we can now estimate the enhancement due to this resonance. To separate the chemical enhancement from the electromagnetic enhancement, we need to assess the latter first, which is difficult. We therefore decided to use R6G molecules as intensity standards. R6G is particularly useful as its resonance Raman cross section upon excitation at 532 nm is known.²¹ We adsorbed R6G molecules on the silver islands at a concentration that guarantees the single-molecule limit and registered their Raman spectra. On the basis of intensity histograms for the 1650 cm^{-1} band of R6G molecules and the 1580 cm^{-1} band of 4MPy molecules (see Supporting Information of ref 19), it was concluded that the Raman cross-section of the strong vibrational bands of 4MPy should be of the order of $\sigma_{4\text{MPy}} \approx 10^{-25} - 10^{-26} \text{ cm}^2$. Thus the CT resonance enhances the Raman cross-section of 4MPy by 3–4 orders of magnitude in comparison to the nonresonant Raman cross-section of similar small organic molecules in solution, such as benzene ($\sigma_{\text{benzene,free}}^{944\text{cm}^{-1}} \approx 10^{-29} \text{ cm}^2$ at 488 nm excitation).²²

A useful theoretical framework for the analysis of CT effects on Raman spectra was developed by Lombardi and Birke.⁸ They used the Herzberg–Teller theory of vibronic transitions to obtain expressions for the Raman polarizability, including the possibility for electronic transitions which involve CT between the molecule and the metal. For the case of a metal-to-molecule CT transition, two terms are relevant. The so-called A term is operative only in case of resonance of the excitation wavelength with the CT transition. The C term mixes the CT transition with all other electronic transitions of the molecule, borrowing intensity from them. It involves the derivatives of the system's Hamiltonian with respect to the various normal modes.

The large contribution of the CT resonance to the enhancement of the Raman spectra (at least 3 orders of magnitude), as well as the fact that all strong bands in the spectrum are totally symmetric, suggest that an A-term resonance Raman process is the main source of the chemical enhancement. However, the changes in the relative intensities of different vibrational band of 4MPy with electrode potential cannot be

readily explained through the A term. Rather, it is possible that tuning the electrode potential affects differently the derivatives of the Hamiltonian with respect to different normal modes, changing the relative intensities of various vibrational bands through the C term. In this process, intensity may be borrowed from molecular electronic transitions or even from the strong plasmon absorption transition of the metallic nanostructure.

3. Raman Probes of Localized Surface Plasmon Fields within Nanogaps

While in the studies described in section 2 smSERS was used as a probe of molecule-surface dynamics, useful because of the strong plasmonic enhancement of the signal, in this section we would like to show that smSERS can also be used to probe the plasmon fields themselves. We focus on simple plasmonic structures, such as dimers or trimers of NPs. A dimer with a small interparticle gap is probably the simplest structure that can sustain large enough plasmon fields for single molecule detection by Raman scattering.²³ NP dimers are usually generated spontaneously, for example by addition of a small concentration of salt to NP solutions to facilitate aggregation (see, for example, our work in refs 12, 13, and 24). In recent years, there have been efforts to systematically produce dimers, and other metallic nanostructures with gaps, for plasmonic applications. Top-down techniques, for example electron-beam lithography,^{25,26} were used to generate such structures on a substrate. Much effort has been devoted to the preparation of dimers and even trimers of metal NPs in solution using DNA duplexes as connectors, starting with the pioneering work of Alivisatos and co-workers.²⁷ It has proven more difficult to prepare dimeric structures, either in solution or on surfaces, with molecules (spectroscopic tags) precisely situated in their gaps.

The LSP fields of plasmonic structures are usually probed in the far field by registering their light scattering spectra, using dark-field microspectroscopy.²⁸ This method, while being sensitive down to the single particle level and allowing much detail on spectral shapes to be registered, does not provide any spatial information on the fields within the plasmonic device. Local information has been obtained using near-field microscopy,²⁹ which employs a sharp metallic tip to directly couple to the local electric fields. The surface plasmons around a nanoparticle can also be mapped using electron energy loss spectroscopy (EELS) within a scanning transmission electron microscope (STEM), and very recently the first demonstration of the application of this method to

dimers appeared.³⁰ STEM-EELS provides complementary information to optical spectroscopy, as optical selection rules are relaxed, leading to the excitation of modes which are optically forbidden. Below we discuss how individual molecules can also serve as sensitive and very local probes of plasmon fields and their properties.

3.1. Probing the LSP Fields through Their Effect on smSERS Signals. In principle, a molecule well-positioned within a nanostructure can probe the electromagnetic field, and report back in the form of fluorescence or Raman scattering. Obviously, the field around and within a plasmonic device shows a strong spatial variation. Therefore, the use of a single molecule (as opposed to many molecules attached to the same device) becomes essential for providing a true mapping of this field. The spontaneous inclusion of emitting molecules within an interparticle junction has been the method of choice for studying single molecules in NP plasmonic devices.^{1,31,32} Obviously, this strategy is limited in its ability to provide spatial information, as the exact molecular location is unknown.

We recently reported one possible strategy for approaching this problem, which puts the molecule right in “the eye of the storm”.³³ We prepared silver dimers using bifunctional molecules that served both as bridges and as Raman reporters. For these experiments we synthesized silver NPs of well-defined size and a narrow size distribution, using small gold NPs as seeds for the growth of a silver shell. Two molecules were used for dimer preparation, a polythiophene with two thiol groups, and rhodamine 123 (Rh123), which contains two amine groups. In both cases we were able to demonstrate the formation of a large fraction of dimers, and to observe Raman spectra from individual ones. Analyzing many such constructs, we showed that the SERS intensity increased with the size of the NPs, which was well-correlated with the changes in calculated near-fields as a function of NP size. Further, the increase in NP size was shown theoretically to lead to a clear change in the shape of near-field spectrum, and therefore to modulate and shape the emitted Raman spectra (Figure 4a). This prediction was verified in the experiment (Figure 4b,c). Plasmonic shaping of optical spectra was already demonstrated in a beautiful study by Ringler et al.³⁴ They showed that the distance between two NPs can be used as a control parameter that modifies the fluorescence spectra of adsorbed molecules.

Our method of dimer-molecule conjugate preparation suffers from lack of flexibility, since the distance between the NPs cannot be varied easily. It has been recently shown that DNA strands can be used to overcome this problem. Lim et al.³⁵ formed silver dimers using double-stranded DNA as a tether.

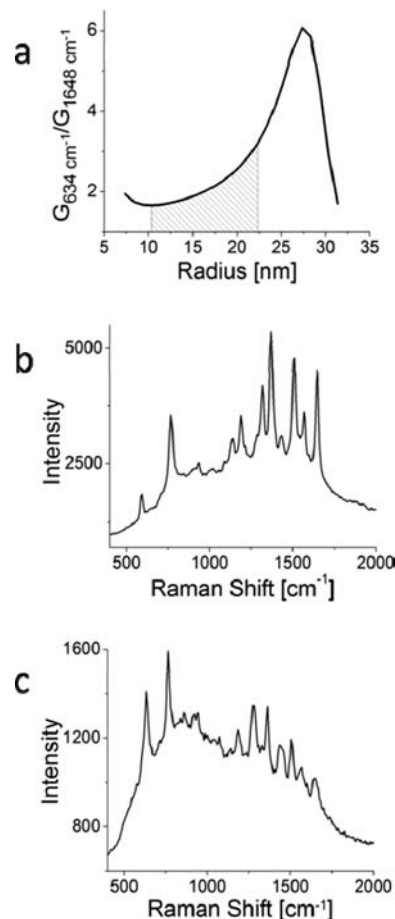


FIGURE 4. Spectral shapes of Raman scattered light from NP dimers bridged by individual rhodamine 123 molecules may be modulated by variation of NP size. (a) Theoretical calculation of the enhancement of the 634 cm^{-1} Raman mode of rhodamine 123 relative to that of its 1648 cm^{-1} mode as a function of NP radius. The dashed area is the one explored in our experiment. (b,c) Spectra taken from dimers of 14 and 22 nm particles, respectively. Changes in spectral shape follow the theoretical prediction.

They were able to locate an individual molecule within the dimeric junction and to observe its Raman spectrum.

3.2. Polarization Spectroscopy and Symmetry Breaking in Trimers. Besides intensity, another important characteristic of the electromagnetic field in the nanogap is its symmetry. A dimer of two equal NPs of the type described above sustains a plasmon field with simple axial symmetry.^{36,37} In principle, more complex symmetries may be supported by higher-order NP clusters. One way to probe the symmetry of plasmon fields is to rotate the sample with respect to the laser beam polarization (or alternatively rotate a waveplate inserted into the optical path of the experiment) and to register the effect of rotation on intensity and polarization of the enhanced Raman scattering of a molecule within the cluster.

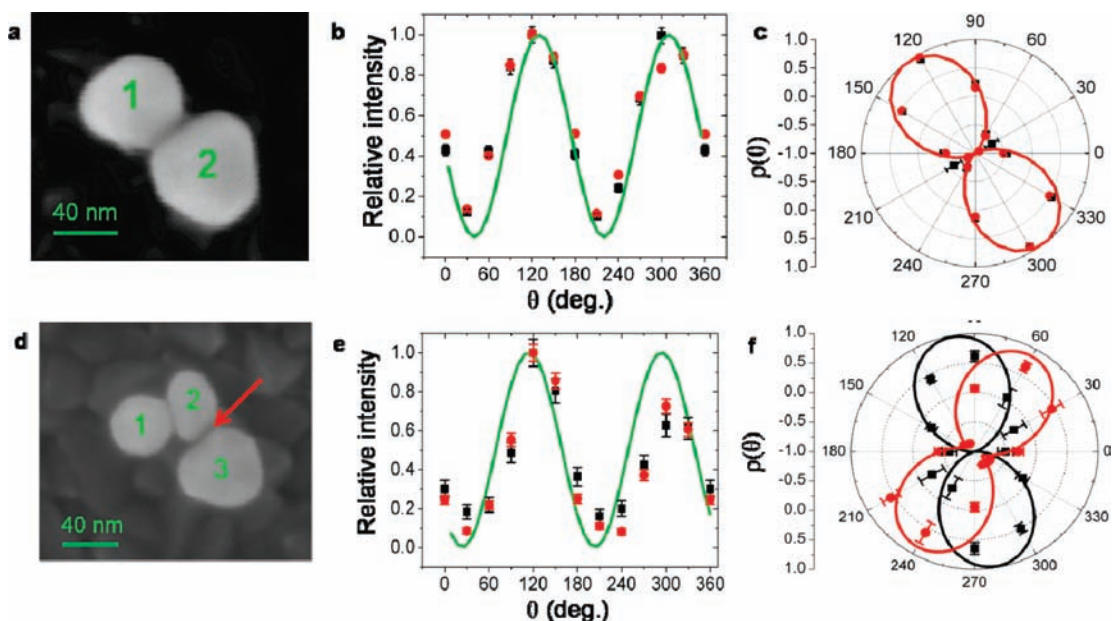


FIGURE 5. Polarization response of an NP dimer (a–c) and an NP trimer (d–f). (a) SEM image of a dimer. (b) Normalized Raman scattering intensity at 555 nm (black squares) and 583 nm (red circles) as a function of the angle of rotation of the incident polarization. Maximal intensity is achieved when the incident field is polarized along the dimer symmetry axis. The green line is the result of a GMT calculation. (c) Depolarization ratios at 555 and 583 nm (symbols as above). No wavelength dependence is observed. Black and red lines show the results of Mie theory calculations performed at 555 and 583 nm, respectively. (d) SEM image of a trimer. A red arrow indicates the position of the molecule that leads to the best agreement between experiment and calculation. (e) Normalized Raman scattering, with symbols as in b. The intensity profile does not peak along the direction of the axis connecting particles 2 and 3. (f) Depolarization profiles are found to be wavelength-dependent, and are aligned differently than the intensity profiles. The black and red lines show the result of calculations at the two wavelengths.

Consider a molecule residing in one of the junctions formed between pairs of nanoparticles in a cluster. The components of the electromagnetic field generated by the molecule–aggregate complex can be written as follows:

$$E = \begin{pmatrix} E_{\parallel} \\ E_{\perp} \end{pmatrix} \propto g'(\omega_0 - \omega_{\text{vib}})\alpha_{\text{vib}}g(\omega_0)E_0 \quad (1)$$

Here, E_0 is the incident field. g is a tensor describing the enhancement of the laser field at the interparticle junction, at frequency ω_0 . g' is a tensor describing the enhancement of the Raman field generated by the molecule, measured at $\omega_0 - \omega_{\text{vib}}$, where ω_{vib} is the frequency of a particular molecular vibrational mode. α_{vib} is the molecular polarizability tensor of this mode. The enhancement tensors are related to each other by optical reciprocity. The simple symmetry of the enhanced local fields in the junction formed between two metal particles simplifies the form of the two tensors, so that only two elements are nonzero in each (for details see ref 38).

The total (relative) intensity of emitted light, $I_{\text{rel}}(\theta)$, and its depolarization ratio, $\rho(\theta)$, can be written as a function of the rotation angle of the sample with respect to the laser beam polarization direction, θ .³⁸ Interestingly, it is found that the relative intensity depends only on enhancement tensor g , while the depolarization ratio depends only on the emission ten-

sor g' . This separation can be obtained only if just one junction in a cluster is populated with molecules; this condition is trivially fulfilled in the case of single-molecule observation. If molecules reside in more than one junction, then the averaging over several geometries will preclude the separation between g -dependent and g' -dependent properties. It is also notable that the molecular polarizability tensor does not enter any of these quantities, so that they do not provide any information about the molecule itself. In other words, the molecule serves purely as a probe of the plasmon field!

Raman spectra from many individual molecules within NP clusters were measured, and $I_{\text{rel}}(\theta)$ and $\rho(\theta)$ were calculated for each.³⁸ The clusters were then imaged using a scanning electron microscope (SEM). The geometry of each cluster was used as an input for a calculation based on the generalized Mie theory (GMT).³⁹ In the case of NP dimers (e.g., Figure 5a–c), both $I_{\text{rel}}(\theta)$ and $\rho(\theta)$ obeyed simple axial symmetry, as expected. In the case of NP trimers, however, more complex behavior was observed. Figure 5d shows one example of a trimer, as observed by the SEM. This trimer has two interparticle junctions, between particles 1 and 2 or 2 and 3, where strong enhancement is expected. Can we find in which of these a molecule resides? The $I_{\text{rel}}(\theta)$ functions at two different scatter-

ing wavelengths are shown in Figure 5e. While the two functions coincide, they do not peak either in the direction of the axis 1–2 or the direction of the axis 2–3. An even richer behavior is seen in the functions $\rho(\theta)$ (Figure 5f), which do not even coincide with each other. The GMT calculation, using only the geometric input, is able to reproduce the experimental results, if it is assumed that the junction 2–3 is occupied with molecules.

The origin of the unusual behavior of the measured functions is now clear: particle 1 couples to the other two particles and influences both the near-field in their junction and the far-field Raman scattering. It breaks the axial symmetry of the dimer, and induces a wavelength-dependent response. Systematic analysis of the axial symmetry breaking of dimers by an additional particle, using the GMT,^{38,40} unearthed some interesting features. First, the degree of symmetry breaking turns out to depend strongly on the position and distance of the third particle with respect to the dimer. Second, the size of the third particle is crucial: the effect is negligible for very small particles. The size at which the polarization rotation is maximal depends on the observation wavelength. Another interesting way of tuning the degree of polarization rotation is by changing the refractive index of the surrounding.⁴⁰ In general, symmetry breaking of plasmonic fields has become an interesting topic in the last year, with diverse manifestations reported, such as Fano resonances in scattering spectra.⁴¹

4. Conclusion

Single-molecule Raman spectroscopy is still a relatively new and not-so-well explored experimental technique. And as with other new experimental methods, it hides some surprises which are waiting to be revealed. smSERS has shed new light on the SERS phenomenon itself, and has led to better understanding of various aspects such as the distribution of hot spots, the possible extent of enhancement etc. However, it is surmised that the main promise of smSERS lies in its application as a sensitive probe for physicochemical dynamics. In this Account we highlighted our own work in this direction, which focused on probing molecule-surface interactions and surface plasmon fields. It is very likely that many additional applications of smSERS will appear in the coming years, in which the sensitivity and spectral resolution of the technique will be used to provide answers to exciting unsolved physical and chemical questions.⁴²

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BIOGRAPHICAL INFORMATION

Gilad Haran is currently in the department of Chemical Physics of the Weizmann Institute of Science. He did his Ph.D. at the Weizmann Institute with Profs. Ephraim Katchalsky-Katzir and Elisha Haas. He was then a postdoctoral fellow with Prof. Robin Hochstrasser at the University of Pennsylvania. Gilad Haran's lab is using single-molecule spectroscopy to study molecular dynamics, from protein folding and association to charge transfer dynamics on metal surfaces.

FOOTNOTES

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