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Stabilizing single-molecular Raman spectrum of a nonbonding molecule on Ag nanoparticles†

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The applicability of single-molecule surface-enhanced Raman spectroscopy to a nonbonding molecular system is demonstrated on a uniformly assembled colloidal Ag nanoparticle substrate.

The possibility of using surface-enhanced Raman scattering (SERS) to probe single-molecular behavior has attracted considerable attentions in recent years.^{1–6} However, single-molecule SERS (SM-SERS) has encountered many problems: fluctuations, non-reproducibility, and a lack of understanding of the origin of the sites suitable for SM-SERS. The existence of SM-SERS has unfortunately been often limited to a few dye molecules and associated with few indirect evidences, such as the use of low concentration, spectral-fluctuation, polarization dependence and statistically quantized intensity.^{4,7} It is argued that the use of the so-called bi-analyte technique could lead to definite single-molecular behavior.⁷ However, such a technique requires the mixture of two different chemical compounds and can thus not be applied to *in situ* detection of single molecules. Although it is possible to obtain stable spectra for certain systems, like the widely used R6G molecule, due to their strong interaction with the substrate, the strong fluctuation of SM-SERS spectra has made it very difficult to determine the structure, behaviour and properties of single molecules. To extend the applicability of SM-SERS, it is thus highly desirable to find an effective means to reduce the spectral fluctuation.

Herein we report a joint experimental and theoretical study on temperature-dependent SM-SERS of a nonpolar organic molecule perylene on colloidal Ag nanoparticles. As expected, at room temperature, the SERS spectra of perylene displays strong spectral-fluctuation, which can, however, be effectively eliminated by lowering the temperature of the substrate, resulting in a stable SERS spectrum over a long period of time. To the best of our knowledge, this study presents the first SM-SERS for a nonpolar molecule, and more importantly the first blinking-free SM-SERS. Our observation supports a previous finding that spectral-fluctuation could not be used

as the intrinsic property of single-molecular spectroscopy.⁸ It shows that the thermal effect is not from laser heating or plasmon excitation but mainly from the local environment, which can be well controlled experimentally. By comparing with first-principles calculations, it can be determined that the molecule is eventually trapped in the gap of nanoparticles, which might be driven by the electric field gradient force generated from the local electromagnetic fields.

We have first optimized the colloidal Ag nanoparticle substrates *via* closely-packed assembly on a cover-glass (details in ESI†). Based on the uniformly assembled Ag nanoparticle substrate (Fig. 1A and B), we carried out concentration-dependent SERS measurements of perylene molecules at room temperature. The measured SERS spectra for concentrations of 10^{-15} , 10^{-12} , 10^{-7} and 10^{-2} M are shown in Fig. 1C, respectively, together with a calculated Raman spectrum of a free single perylene molecule for comparison. A free perylene molecule has a high symmetry of D_{2h} . Among its 90 vibrational modes, only three A_g modes, at frequencies of 1287, 1383 and 1555 cm^{-1} , respectively, show strong Raman intensities. It is noted that the calculated Raman spectrum of the single molecule is in excellent agreement with the experimental spectrum of high concentration (10^{-2} M), which is also identical to the calculated spectrum of a perylene trimer. It indicates that at this concentration the perylene molecules can form ordered aggregates through π - π stacking and each individual perylene molecule behaves the same as that in the gas phase. We refer these three spectral profiles as bulk-like features. By lowering the concentration of the molecule, there are two competing channels that can affect the spectra. Lower concentration results

Fig. 1 (A/B) SEM and AFM images of the colloidal Ag nanoparticles assembled on the cover-glass. (C) Concentration dependent Raman spectra of perylene molecule on colloidal Ag nanoparticles with concentrations of: 10^{-15} (a), 10^{-12} (b), 10^{-7} (c), 10^{-2} (d), and compared with a calculated Raman spectrum of a single free perylene molecule (e); (D) SM-SERS of perylene from other 'hot-spots' on the sample. All the spectra are collected with 20 mW laser power.

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in fewer molecules being excited in a given area and weaker spectral signals, while more metal surface areas that are exposed to the laser excitation can lead to surface enhancement. It can be seen from Fig. 1C that at the lower concentration spectral bands become broader, and on top of the broad bands, sharp spectral features start to appear. At a concentration of 10^{-15} M, many narrow spectral peaks are observed and the spectral distribution is strongly dependent on positions of the excitations. Fig. 1D shows two spectra that are taken from other "hot-spots" for comparison. These two spectra also present very sharp and rich spectral features. For all the three spectra, the overall spectral profiles are very different. It is easy to estimate that at this concentration only one single molecule is statistically probed by the laser beam. Together with the observation of strong spectral-fluctuation, one could argue that the observed Raman spectrum at a concentration of 10^{-15} M can be considered as the result of SM-SERS. It is worth mentioning that to detect a Raman signal from molecules at such low concentration has often been a challenging task. The SERS processes are complicated by the mixture of electromagnetic and chemical enhancements. The former is related to the structures of the metal nanoparticles, and the latter to the molecular resonance and charge transfer through molecule-metal bonding. The use of nonpolar perylene molecules can minimize the effects of chemical enhancement since this molecule can hardly form any chemical bonds with the metal particles and its free motion leads to strong spectral fluctuation. The room-temperature SE-SERS spectra of ultra-low concentration perylene solution thus favours the general view that electromagnetic (EM) enhancement is the dominating effect that contribute to SERS enhancements.

Unfortunately, it is difficult, if not impossible to provide proper assignment for the rich spectral features appearing in these SM-SERS spectra, since for an isolated perylene molecule only three vibration modes are Raman active. The breaking of molecular symmetry upon adsorption and the thermal population of excited vibrational levels could be the possible causes for the generation of a large number of extra Raman peaks, but the presence of strong spectral-fluctuation further complicates the SM-SERS processes. Without a good control of the spectral-fluctuation and reduction of spectral features, the usefulness of SM-SERS is completely lost.

Several possible mechanisms for the origin of Raman blinking have been proposed, including thermal diffusion of the molecules in and out of 'hot-spots',⁸ thermal induced molecular reorientation,⁹ photoionization, and reorganization of the Ag substrate morphology.¹⁰ Perylene molecules do not form strong charge transfer states with the metal substrates due to the nature of the physical absorption and should not be photoionized under the excitation of an off-resonant continuous laser. The fact that all spectra can be obtained at the same 'hot-spot' also rules out the possible rearrangement of the Ag substrate morphology. One of the most possible mechanisms, in particular for the physically adsorbed perylene molecule, is thus the thermal effect generated by the relatively strong laser power, long detection time and relative high temperature of the surroundings.

We can examine laser induced thermal effects by reducing the laser power (Fig. S5, ESI†) and conducting the

Fig. 2 Time-dependent Raman spectra of perylene (10^{-15} M) on an Ag-coated cover-glass at -150 °C, taken sequentially every 1.5 min ((a)–(f)). Laser radiation was stopped for 3 min in between spectra (f) and (g), (h) and (i), (j) and (k). All spectra were performed at the same 'hot-spot' (15 mW).

experiments at lower temperature. Fig. 2 shows time-dependent SM-SERS spectra of perylene (10^{-15} M) on Ag-coated cover-glass at -150 °C. These low-temperature Raman spectra display quite different time evolution in comparison with those taken at the room temperature. They have much higher signal-to-noise ratio and show less spectral-fluctuation. Such observations suggest that the change of temperature has a major impact on the single-molecular behaviour. The heat generated by the laser radiation seems to be effectively dissipated into the environment through the cold Ag nanoparticles. The most interesting observation is that after a certain interval of time the Raman spectrum becomes very stable and contains only one dominant peak around 1400 cm^{-1} . It thus shows that the molecule is eventually relocated to a new equilibrium position. Similar spectra also appeared occasionally during the room-temperature measurements (Fig. S5, ESI†), indicating that the potential surface around the new equilibrium is quite flat and can be easily overcome by thermal heating. Nevertheless, these stable spectra have provided the first evidence that the spectral-fluctuation in the SM-SERS measurements can be effectively eliminated. It is conceptually important because it implies that spectral-fluctuation can no longer be used as an exclusive indicator for the SM-SERS. It is also practically useful since it makes SM-SERS a good tool to analyze the structure of single molecules.

From these experiments, it can be concluded that the molecular motion is not controlled by a spontaneous thermal effect, but by a well-defined force. It is well understood that it is the huge enhancement of local electromagnetic field generated by the metal nanoparticles that has made the observation of SM-SERS possible and such a huge field can lead to a strong electromagnetic force which can in turn control the motion of the molecule. In a recent work of Svedberg *et al.*,⁶ it was demonstrated that a nanoparticle dimer could be manipulated by an optical tweezer because of high electric field gradient

Fig. 3 Calculated Raman activity of perylene based on the model by assuming a single free molecule perylene adsorbed near Ag_{10} clusters (A), and trapped into two pyramidal Ag_{10} clusters (B), in comparison with low-temperature experimental SM-SERS spectra. (C): Schematic diagram for the behaviour of a perylene molecule near a pair of electronegative Ag nanoparticles.

forces.¹¹ The conventional continuous laser used in our experiment does not have enough power to move the nanoparticles in such a dramatic way, however, it can generate enough electric field gradient force among the nanoparticles due to the irregular shapes and distributions of the nanoparticles and this force can drive molecule motion around the nanoparticles.

We have tested many model systems with density functional theory calculations.¹² It is found that only when the molecule is placed in between two Ag clusters, the calculated Raman spectrum can reproduce the most stable experimental spectrum. The agreement between the theory and the experiment is remarkable, as nicely demonstrated in Fig. 3B. It is found that the dominant spectral feature at 1400 cm^{-1} originally belongs to a Raman inactive B_{1u} mode in the gas phase. The presence of Ag nanoparticles breaks the local system of the molecule and makes this mode strongly Raman active. It is noted that in the simulations the molecular structure remains unchanged and the gap distance is less than 1 nm. These results are consistent with the observation of Xu *et al.*⁴ that the molecule prefers to sit in between two nanoparticles with a small gap in SM-SERS, which might due to the fact that in this situation the electric field gradient force for a molecule could become zero and the molecule can safely remain there.

The strong thermally induced molecular motion at room temperature has made it impossible to construct a sensible theoretical model. However, based on the evolutions of laser power dependent and temperature dependent experimental spectra, we could propose a model to describe the dynamic process of a single perylene molecule on Ag nanoparticles under low temperature as shown in Fig. 3. When a perylene molecule is spread on the nanoparticles, it can be shown that the most stable position for the perylene is to lie on the surface of a nanoparticle. It is energetically unfavourable for a molecule to drop in between two nanoparticles with a gap of less than 1 nm. Calculations indicate that when the distance between the molecule and the nanoparticle is sufficiently small, around 0.6 nm, more vibrational modes become Raman active because of the interaction between the molecule and the metal.

As shown in Fig. 3A, the calculated spectrum closely resembles the experimental spectrum taken at the very beginning of the measurement (Fig. 2(a)). The discrepancy between theory and experiment could be attributed to the molecular motion or even the electric field gradient that are not considered in the theoretical models. Under the guidance of the strong electric field gradient force, the molecule eventually moves into a gap between two nanoparticles,¹³ as shown in Fig. 3C.

It is noted in Fig. 2(d)–(k) that the intensity ratio between the two dominating peaks at 1371 and 1400 cm^{-1} show considerable changes, which might be due to the local heating caused by the relatively long time of laser exposure that induces the local movement of the perylene molecule. It should also be mentioned that the calculated Raman intensity of the single molecule in the gap of Ag clusters shown in Fig. 3 has the same order of magnitude as that in the gas phase, indicating clearly that the chemical enhancement is not the necessary condition for the observation of SM-SERS.

In summary, we demonstrated SM-SERS spectroscopy of a small organic molecule physically adsorbed on a uniformly assembled colloidal Ag nanoparticle substrate. The expected strong spectral-fluctuation at ‘hot-spots’ could be effectively eliminated by lowering the local temperature of the samples to generate very stable Raman spectra for the nonbonding molecule. In combination with first principle calculations, the stable structure of molecule is determined to be trapped in the gap of two nanoparticles. Our work has not only widened the scope of practical applications of SM-SERS technique, but also provides a general protocol for controlling single-molecular Raman behaviors of a small organic molecule on metal nanoparticles.

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