# Thermal Activation of Blinking in SERS Signal

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Temperature dependence of blinking in SERS (surface enhanced raman scattering) signal, which is abrupt repeated intensity and peak frequency fluctuation, was studied. It was found that the blinking observed at room temperature (RT) was suppressed by cooling to 77 K for one-third to one-fourth of the particles and recovered by warming to RT. Accordingly, the blinking is thermally activated, e.g., by diffusion of molecules between different sites on Ag particles. These sites are possibly attributed to junctions of touching particles with enormously large electric fields and other ordinary sites with modest enhancement, as predicted by numerical simulations of the local electric field. The thermal activation is compatible with photochemical activation for particular molecules with appropriate electronic states for efficient charge transfer against metal surfaces.

## 1. Introduction

Recent progress in SPM (scanning probe microscopy) technologies to manipulate and observe nanoscale structures in addition to a highly sensitive charge-coupled device (CCD) allows us to detect extremely weak Raman signals from individual metal particles or even from single molecules. In fact, several groups reported vast enhancement corresponding to single molecule sensitivity in SERS,[1−5] although details of the mechanism and origin are still obscure. Plenty of information can be obtained in SERS such as molecular structure, orientation, or interaction with neighboring species, in contrast to fluorescence spectroscopy preceded as the single molecule detection (SMD) method. Thus it is valuable to establish the SMD method with SERS to elucidate various elementary reaction processes at solid/liquid interfaces in addition to clarifying the enhancement mechanism in SERS.[6,7] As commonly observed results for vast enhancement in SERS:[1−5] (1) so-called "hot particles" appear with prominent enhancement among many particles with modest enhancement when the surface coverage of the adsorbates is ca. several tens to one hundred per each Ag particle, and (2) at much lower surface coverages, less than ca. 5 molecule/particle, an extremely small number of Ag aggregates show the "blinking" of Raman signal for dye or other biomolecules for which the intensity suddenly and repeatedly changes with a typical frequency of a few hertz. Concerning the blinking, Emory et al. reported complete intermittent (on/off) features in SERS intensity.[1]

The blinking is attributed to a single molecule phenomenon, because it is not plausible for many molecules to move or to change their orientation at the same time. Recently, Weiss and Haran[8] reported that the rate of spectral fluctuation is propor-

tional to laser intensity and suggested a nonthermal process such as photoinduced desorption of adsorbates. Bosnick et al.[9] reported the SERS scattering intensity fluctuates due to motions of the molecule in and out of the hot spot and is highly localized around it. These efforts give us insight into the origin of the blinking and the vast enhancement in SERS from Ag nanoparticles to establish SMD-SERS as an analytical tool. Similarly, thermal activation of the blinking was pointed out as a possible candidate in the literature.[4,10] However, to our best knowledge it has not been investigated in detail. In this short paper, we report the temperature dependence of the blinking in SERS signals to discuss the contribution of thermal activation.

## 2. Experiments

Details in sample preparation and optical measurements were the same as described previously.[5] Briefly, Raman microscopy was combined with AFM measurements to observe a scattered light image, Raman image and Raman spectra for individual Ag particles, of which morphology is characterized similarly to other groups.[1,3,4] SERS images of Ag nanoparticles were obtained using a notch filter and CCD camera at a video rate (30 flames/s), whereas SERS spectra were measured with a Renishow spectrometer. Only a countable number of Ag nanoparticles prepared from $AgNO_3$ and sodium citrate are immobilized onto a Si wafer coated with (3-aminopropyl)-trimethoxysilane, which has a grid like maker with a space of 30 $\mu$m along the two perpendicular directions.[5] With tuning, the concentration of adsorbates, i.e., R6G or adenine, and Ag particle, we realized only a single blinking particle exists in a sampled area of ca. 30 $\mu$m × 30 $\mu$m. For low-temperature measurements in a vacuum, the Ag/Si was fixed onto a copper block, which is thermally connected to a liquid-nitrogen cryostat from Oxford. For temperature dependence, the blinking particles in the Raman image were captured at room temperature for R6G adsorbed Ag nanoparticles. The sample was then cooled to 77 K, while the position of Ag particles was adjusted by a precise X−Y stage. This procedure is crucial to observe the same Ag particle at RT and 77 K, because thermal drift and/or mechanical

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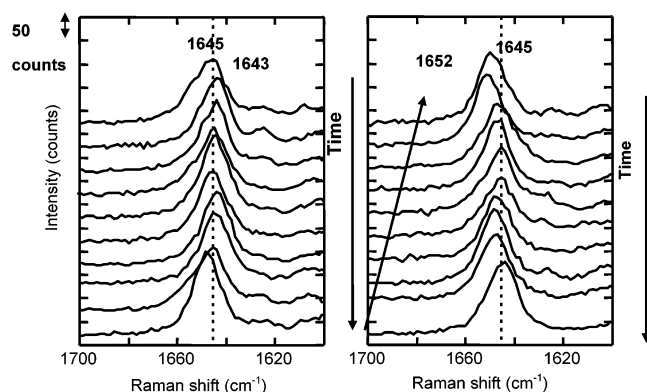|| Nanoarchitectonics Research Center (NARC)-AIST.

**Figure 1.** Peak frequency fluctuation of the SERS spectra from R6G on Ag nanoparticles. The average surface coverage of R6G is 1 molecule per Ag particle. Each spectrum was obtained by sequential measurement of accumulation time of 1 s (from top to bottom and from left to right) at $\lambda = 488$ nm and 70 $\mu$W/$\mu$m$^2$.
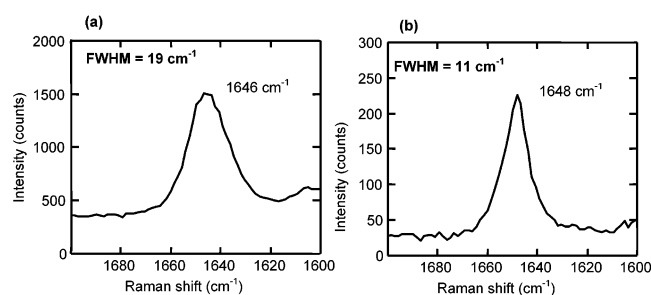


**Figure 2.** SERS peak profile (a) from Ag particles with higher surface coverage and (b) from blinking particles. The average surface coverages of R6G in (a) and (b) are 300 molecules and 1 molecule per Ag particle, respectively. SERS spectra were obtained with the same condition as in Figure 1.

deviation of the sample holder is ordinarily much larger than the observed region (ca. 30 $\mu$m $\times$ 30 $\mu$m). Thus we observed the Raman image from the same particles at room temperature and 77 K.

## 3. Results and Discussion

**3.1. SERS Signal at Room Temperature.** As reported in a previous paper,[5] Ag particles that yield vast enhancement are aggregates with a typical size less than ca. 1 $\mu$m, which give higher enhancement for polarization parallel to the connection axis compared to the vertical direction.[1] Only a few of these particles show the blinking. In addition, the fluctuation of the peak frequency within ca. 10 cm$^{-1}$ and narrower bandwidth (ca. $^1/_2$) than that for higher surface coverage were observed, as shown in Figures 1 and 2. It is noteworthy that these spectra were sequentially measured with an accumulation time of 1 s to give sufficiently high signal-to-noise ratio with the CCD, whereas the actual blinking frequency is several hertz, slightly faster than the accumulation time. Nevertheless, intensity and peak frequency fluctuations were clearly observed, whereas prominent intermittent features were observed by Emory et al.[1] using an avalanche photodiode. These observations suggest the existence of various adsorption sites on Ag particles with different interactions and enhancements. Adsorbed molecules possibly diffuse between these sites.

Before going into the temperature dependence, it is useful to discuss a possible temperature increase of Ag nanoparticles by the excitation laser, which may cause a significant difference in the LSP resonance and enhancement in SERS. At first, in our experiments with a quite weak laser power of 70 $\mu$W/$\mu$m$^2$
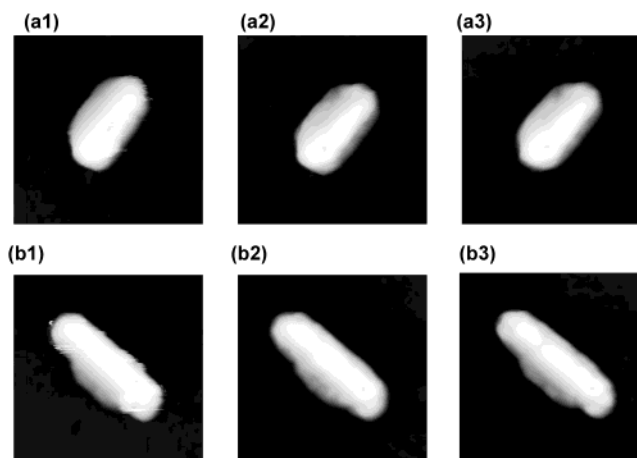


**Figure 3.** AFM images pf SERS active Ag particles (a, b) before and after laser irradiation: (a1, b1) before irradiation; (a2, b2) after 60 min at 0.3 $\mu$W/$\mu$m$^2$; (a3, b3) followed by further 10 min irradiation with 90 $\mu$W/$\mu$m$^2$.
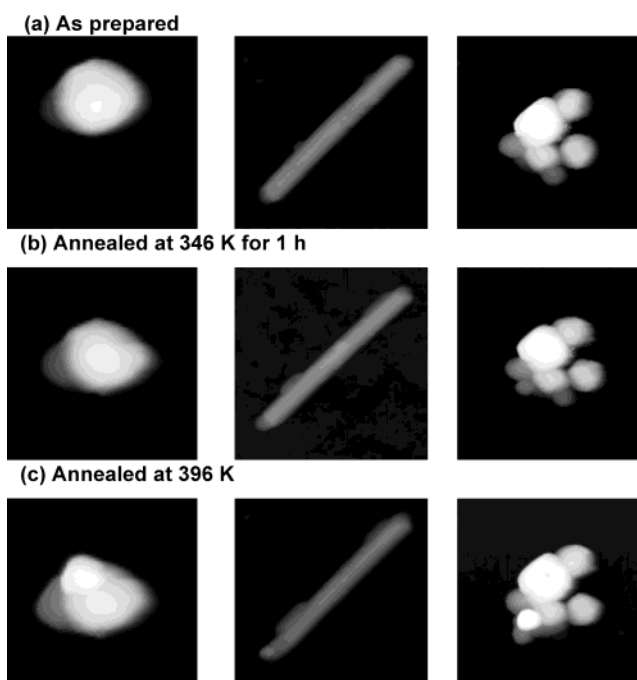


**Figure 4.** AFM images before (a) and after thermal annealing of Ag particles at 346 K for 1 h (b) and followed by further annealing at 396 K for 1 h (c).

at a wavelength of 488 nm, the observed Raman bands are safely assigned to vibrational modes from original species. Accumulated spectra for a long duration of measurement, e.g., for 100 s or longer, do not contain any pronounced Raman bands from plausible contaminants such as amorphous carbon.[11] Therefore, the blinking of SERS signal is attributed to R6G and adenine adsorbates. On the other hand, if the temperature of Ag particles increased even up to ca. 315 K, the morphology of the Ag particles is irreversibly changed, as reported by Semin et al.[12] However, AFM measurement before and after the laser irradiation does not indicate any distinct change in nanoscale morphology of our Ag particles, as shown in Figure 3a−c. The laser power ($\lambda = 488$ nm, 100 $\mu$W/$\mu$m$^2$ for 10 min) adopted here is the maximal intensity in our experiments to measure SERS spectra, whereas only a modest intensity of 1 $\mu$W/$\mu$m$^2$ was used for the SERS image measurements here. In contrast, Ag nanoparticles prepared with the same procedure significantly changed their morphology by annealing to 350 K (for 1 h) or
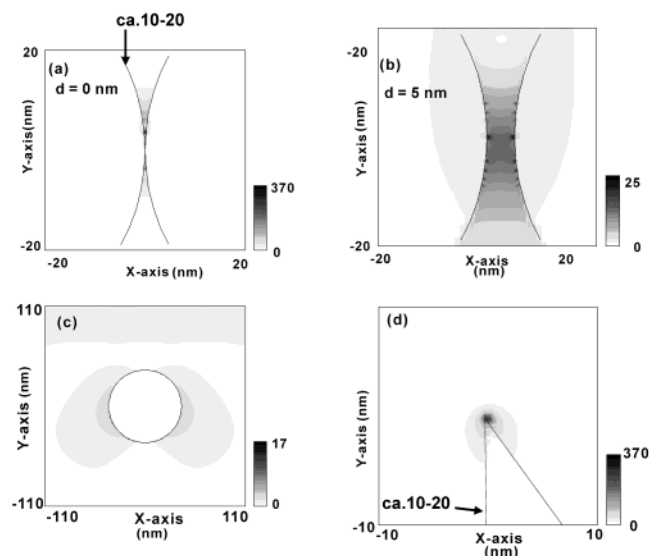
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**Figure 5.** Local electric field (a) on Ag spheres ($r = 40$ nm) at the junction (gap size $d = 0$ nm; touching particles) with a polarization parallel to the touching axis, (b) as in (a) but with the gap size $d = 5$ nm, (c) on an isolated Ag cylinder surface, and (d) on a triangular Ag prism. At the junction of touching Ag particles, vast enhancement ($10^{11}$ as Raman scattering based on $I_{Raman} \approx (I_{amp})^4$) was obtained, which rapidly decreases with the gap size. In contrast, only modest enhancement ($10^4$ to $10^5$ as Raman scattering) was predicted at other ordinary sites of touching and isolated Ag particles.

to 400 K (see Figure 4a−c), in accordance with the observation for Ag island films.[12] Accordingly, invariant morphology of Ag particles under laser irradiation indicates only a negligible temperature increase of the samples during our SERS experiments. Namely, the LSP resonance of Ag nanoparticles and thereby the enhancement is not modulated by the excitation laser. In accordance with these results, the temperature increase of Ag particles was estimated to be <4.5 K for our laser power used for SERS spectral measurements ($\leq 100$ $\mu$W/$\mu$m$^2$) using eq 3 in ref 8 based on the stationary thermal diffusion from Ag nanoparticles to the hydrating water layer. In addition, similar blinking features were observed for SERS spectra of adenine on the Ag nanoparticles at RT,[5] which has no electronic transitions in the visible wavelength region in a bulk solid or solution state. From these observations, it seems that photochemical bleaching, relaxation via triplet electronic state, or morphology changes by laser irradiation are not concerned with the blinking.

The origin of the blinking is possibly due to a thermal process such as thermal diffusion of an individual molecule on the Ag particle presuming physisorbed molecules, of which the binding energy is comparable with the thermal energy. This is rationalized, if (1) the Ag surface has different sites with distinct enhancements and (2) there is sufficiently large thermal energy to overcome an activation barrier for diffusion. Concerning the first point, it was demonstrated by numerical simulations using the finite difference time domain (FDTD) method[13,14] that vast
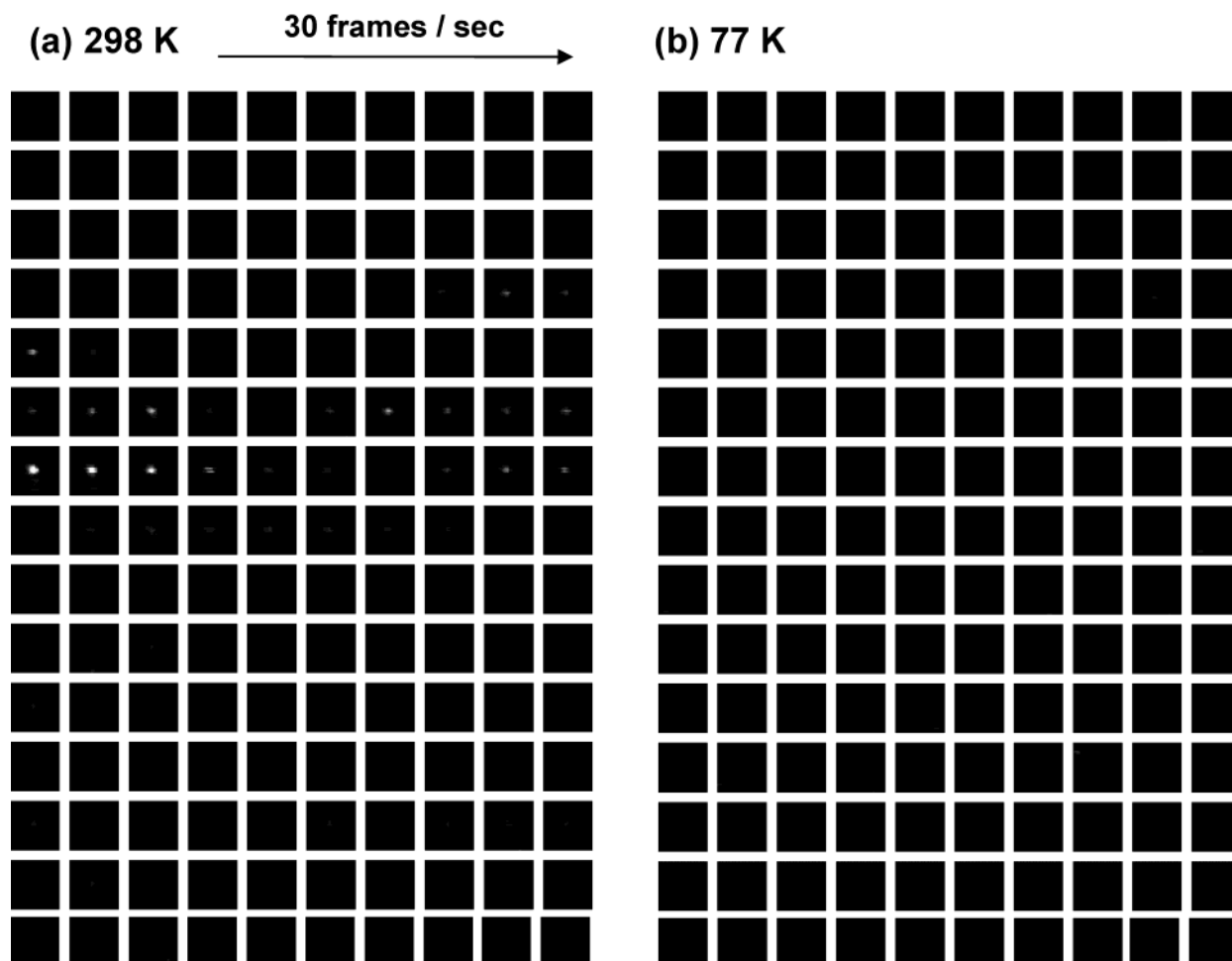


**Figure 6.** Temperature dependence (I) of blinking: (a) at RT; (b) at 77 K. A darkened spot was observed at 77 K through the experiments (for ca. 10 min), indicating blinking is suppressed at inactive sites.
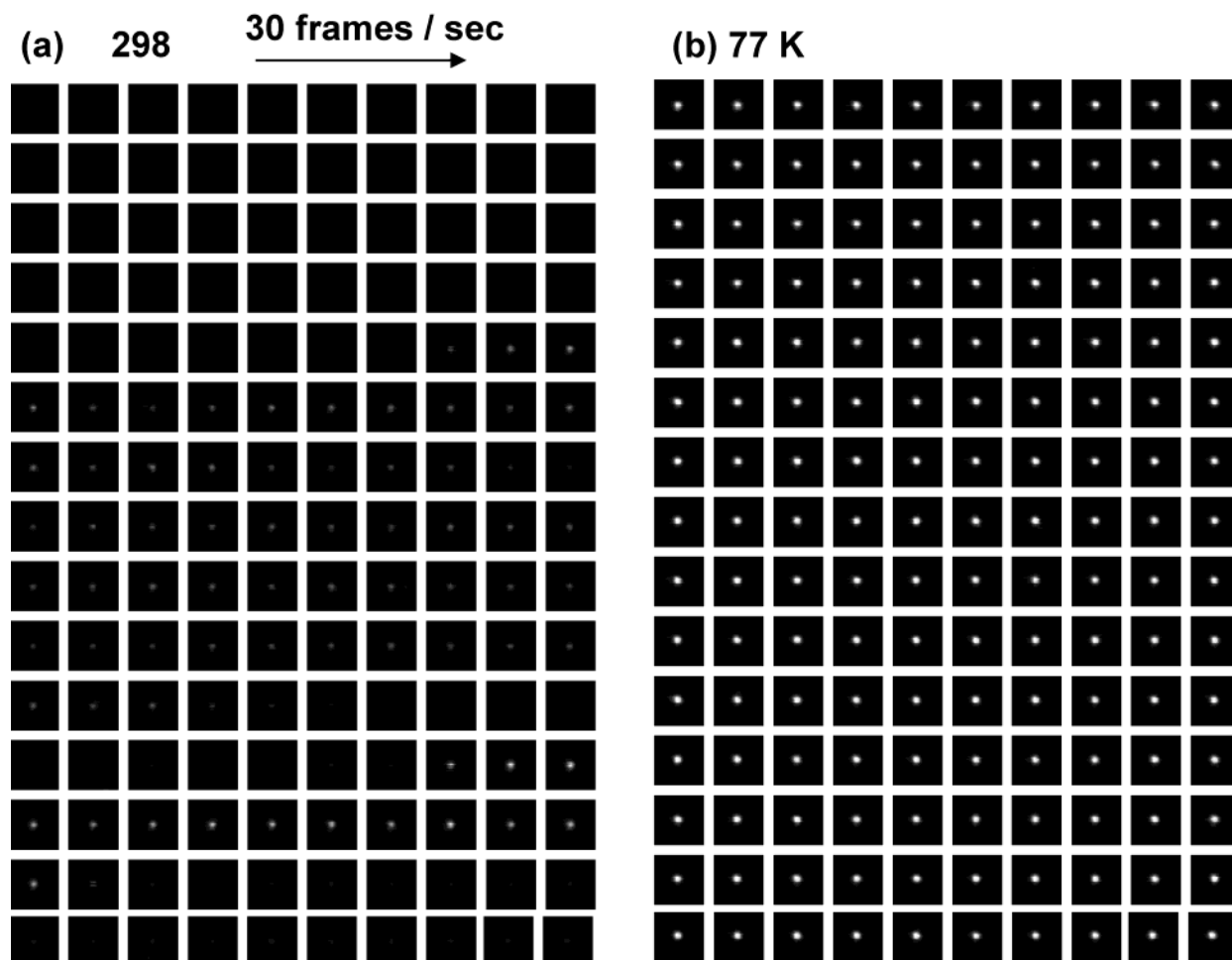
**Figure 7.** Temperature dependence (II) of blinking: (a) at RT; (b) at 77 K. A bright spot was observed at 77 K through the experiments (for ca. 10 min), indicating blinking was suppressed at active sites.

enhancement of $>10^{11}$ in Raman scattering was obtained at a junction between two touching Ag particles with various shapes and sizes in addition to an edge of isolated triangular cylinders under the localized surface plasmon (LSP) resonance (see Figure 5a,d). The vast enhancement sharply decays with increasing gap size (Figure 5b).[14] Other sites apart from the junctions or edges of the touching particles and of isolated triangular prisms gave only modest enhancement of $\leq 30$ (Figure 5c,d). Similar results for metal nanoparticles with different sizes and shapes were reported.[3,15,16] The values of more than 330 in amplitude enhancement at the junction of touching Ag particles are sufficiently large to give single molecule sensitivity in our facility for ordinary molecules without using electronic resonance.[13,14] In addition, significantly larger enhancement by a factor of $100-1000$ was obtained for polarization parallel to the connecting axis compared to vertical direction (not shown, please see ref 14 in detail). These results by numerical simulations agree with the experimental observations, because only Ag aggregates show the vast enhancement with polarization parallel to the touching axis. Thus, we may attribute the blinking to thermal diffusion of adsorbed molecules on the Ag surface between the junctions with vast enhancement and other ordinary sites with modest enhancement. If the blinking arises from thermal diffusion of adsorbates on the Ag particles with respect to the second point, the fluctuation frequency should be decreased or blinking is completely suppressed with decreasing the temperature according to simple consideration of hopping. Therefore, we measured the temperature dependence of the

blinking in the SERS signal from R6G at a surface coverage of ca. 3 molecules/Ag particle.

**3.2. Blinking at Low Temperature.** At first, the blinking particles were found at room temperature and then cooled to 77 K. The precisely adjustable X-Y stage was adopted to compensate thermal and mechanical drift of the sample. As clearly depicted in Figure 6a,b, a bright spot does not change its intensity at 77 K through the measurement, i.e., 5 s ($>10$ s in reality), in contrast to repeated intensity changes at room temperature, e.g., a bright spot with intensity fluctuation between 1.6 and 3.4 s and 3.9 and 4.4 s. We also observed alternate cases where the blinking spot at room temperature, e.g., a bright spot between 1.3 and 2.1 s, became completely darkened and never turned to a bright image at 77 K, as shown in Figure 7a,b. Both of these observations enable us to conclude that the blinking in the SERS signal at RT is suppressed at 77 K, indicating the blinking is a thermally activated phenomenon: when the individual dye molecule is immobilized at the sites with vast enhancement, a bright invariant spot was observed at 77 K, whereas at modest enhancement sites, dark images were given. It should also be noted that roughly one-third to on-fourth of blinking Ag particles were frozen, indicating most of the blinking particles have much smaller activation energies for the process compared to thermal energies at the liquid $N_2$ temperature. This is not surprising, because each adsorbed molecule can possess different bound energies on polycrystalline Ag particles according to different surface electronic states. Moreover, as shown in Figure 8a,b, Raman spectra from R6G on
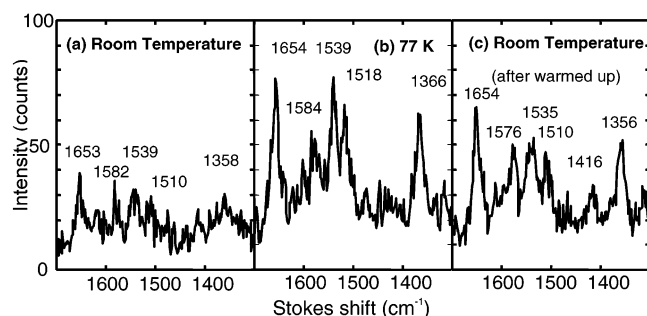
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**Figure 8.** Raman spectra at RT of R6G at (a) 77 K (b) and RT after warming (c). SERS spectra from R6G were observed without significant contamination.

Ag at blinking (room temperature) and at frozen (77 K) conditions, are safely assigned to intramolecular vibrations of R6G, e.g., 1653 ($\phi$ C−C str), 1582 ($\phi$ C−C str), 1539, 1510 ($\phi$ C−C str), 1358 cm$^{-1}$ ($\phi$ C−C str) in good agreement with a former report.[20] The rather poor signal-to-noise ratio of the SERS spectra observed here compared to the previous one[13] is due to lower optical throughput and/or collection efficiency for the sample in a liquid N$_2$ cryostat. The occasional intensity difference of these SERS spectra is due to the rather long accumulation time of 1 s with respect to the blinking frequency of a few Hz.

Interestingly, the frozen particles at 77 K recovered the blinking again, when they were warmed to room temperature as shown in Figure 9. Thus, the suppression of the blinking is

intrinsic and reversible with the temperature variation between RT and 77 K. It clearly suggests that the temperature dependence observed here is not an experimental artifact, such as photo-chemical reaction of adsorbates, which is irreversible for continuum illumination by excitation light, and ordinarily yields much faster spectral changes compared to the experimental time scale (ca. 1−2 h). Consequently, the blinking is thermally activated, most probably due to thermal diffusion of adsorbed molecules between the particular sites with vast enhancement and with modest enhancement on Ag surfaces. These sites are attributed to the junction (ca. 2−3 nm[13,14]) of touching particles and other ordinary sites far from the junctions on the basis of the theoretical simulation, as shown in Figure 5a,b. Relative intensity changes of SERS bands during the blinking[4,9] can also be explained by orientation changes of molecules during diffusion with respect to the anisotropic electric field at the junctions.[14,19]

Weiss and Haran[8] reported for R6G on Ag particles using 532 nm excitation that (1) background intensity as well as the SERS signal shows the intensity fluctuation and (2) the fluctuation rate is proportional to the laser power, whereas the thermal effect is negligible in their experiments (excited at 532 nm with 1 $\mu$W/$\mu$m$^2$), as the sample temperature does not increase at all. Then, they concluded the origin of the increased fluctuation is not a thermal but a photochemical process, possibly due to molecular diffusion that is mediated by desorption triggered by electron tunneling between the metal surface and molecules. To avoid a possible confusion, it should
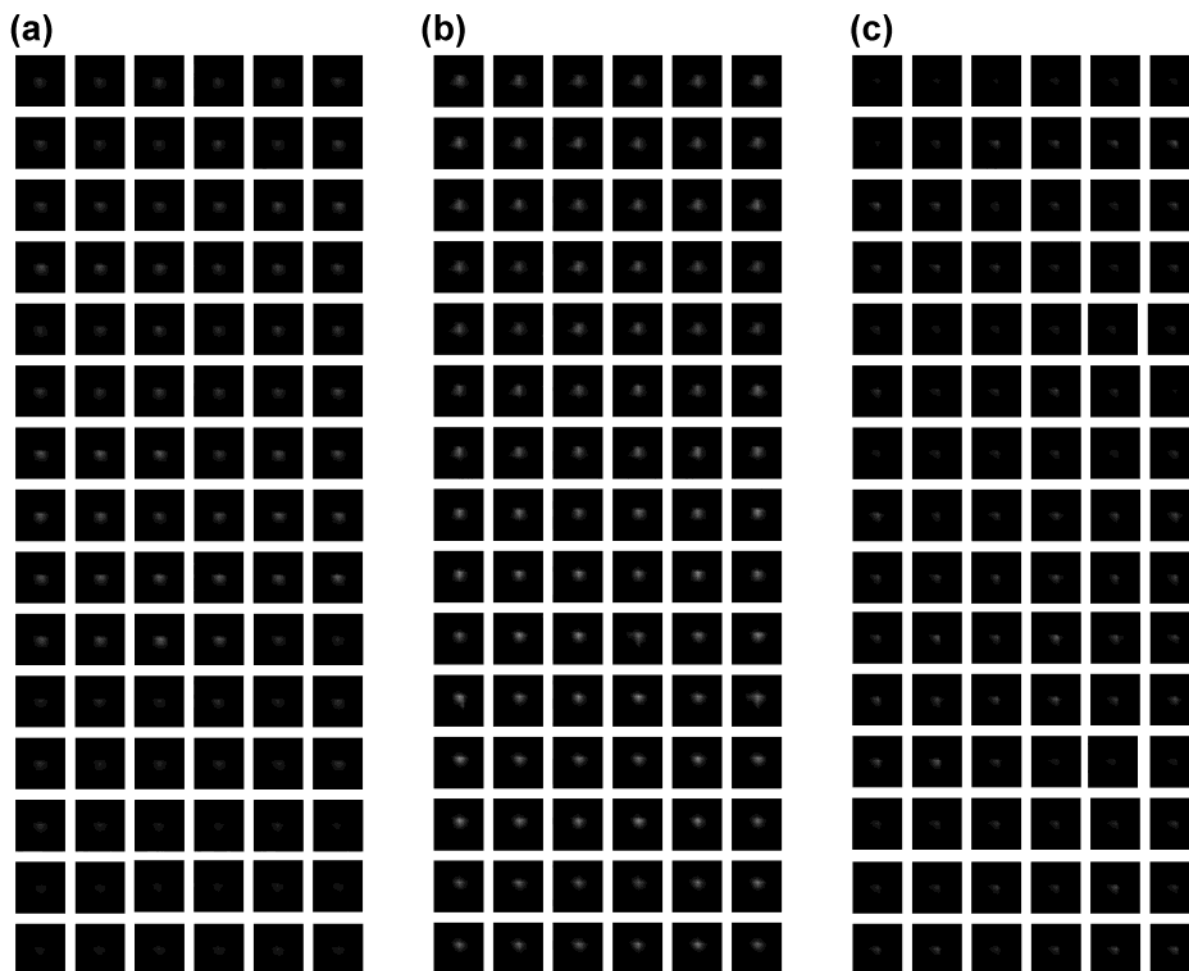


**Figure 9.** Temperature dependence (III) of blinking: (a) at RT, (b) at 77 K, and (c) after warming to RT. Blinking was suppressed at 77 K and recovered at RT after warming, indicating it is thermally activated.

be noted that the laser power dependence was studied by Weiss and Harran at a constant temperature, whereas we explicitly changed the sample temperature at a fixed laser power. As described in section 3.1, in our experiments the temperature of the Ag particles is not significantly raised by the excitation laser (at 488 nm with 100 $\mu$W/$\mu$m$^2$). Clearly, photochemical activation by them and thermal activation suggested here are not exclusive, but compatible for particular molecules such as R6G or other dyes that have an appropriate electronic resonance with the excitation light. Thermal diffusion can also work for other molecules that have no electronic transitions in the visible wavelength at adsorbed states.

With respect to the vast electric field gradient at the junction, an optical confinement of molecules is prospected in analogous to the optically biased diffusion of the R6G molecule in solution.[17] However, as predicted by theoretical simulation for surface plasmon enhanced optical forces,[18] even if the enormously large electric field is formed at the junction of touching Ag particles, only a modest trapping force is induced for R6G adsorbates under our experimental condition ($\leq$100 $\mu$W/$\mu$m$^2$ at 488 nm, see also Figure 1 in ref 18). In fact, they obtained the trapping energy of ca. 0.03 $k_B T$/(100 $\mu$W$\mu$m$^{-2}$) at the junction of two touching Ag particles with a size of 40 nm in diameter at $\lambda = 540$ nm, roughly similar to the above estimation based on the trapping potential in solution.[17] Conclusively, the optical trapping force works to immobilize adsorbates onto the junction, but the value is much smaller than the thermal energy. Thus, thermal diffusion is not frozen by the optical confinement effect.

Apparently, the chemical enhancement based on charge transfer between metals and adsorbates[6,7] is not always necessary to obtain single molecule sensitivity in SERS. Nevertheless, the noble theory of SERS developed by Otto[10] using dynamic charge transfer explicitly accounts for the first layer effect and blinking of single molecule SERS for adsorbates with an appropriate lowest unoccupied molecular orbital or highest occupied molecular orbital with respect to the Fermi level of metal and excitation wavelength. This was well confirmed by experimental results on the first layer SERS and surface enhanced infrared absorption.[6,10] Then, it seems that orientation changes of adsorbed molecules, which yield distinct dynamic charge transfer efficiency and possibly cause the blinking in the SERS signal for particular molecules. In fact, additional enhancement, e.g., the first-layer effect for chemisorbed species or electronic resonance as in dye molecules, may critically contribute to give detectable signal from a single molecule, because the blinking of the Raman signal from R6G or other dye molecules is more feasibly obtained compared to the case of DNA bases.[5] Further study using different adsorbates is underway to obtain a deeper insight into the physical background behind the SMD-SERS mechanism and to establish it as an analytical tool.

## 4. Conclusion

It was found by temperature dependence of SERS signal that part of the blinking Ag particles at room temperature was frozen at 77 K and recovered by rewarming. Accordingly, the blinking is thermally activated, possibly due to thermal diffusion of adsorbed molecules between junctions of touching Ag particles and other ordinary sites with modest enhancement. The attribution was supported by numerical simulation using the FDTD method, because it agrees well with the observation that only aggregated Ag particles show the vast enhancement with polarization parallel to the touching axis. Thermal activation is compatible with photochemical activation suggested by other groups, particularly for dye molecules.

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