

# Surface-enhanced Raman evidence for Rhodamine 6G and its derivative with different adsorption geometry to colloidal silver nanoparticle

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Some high-affinity functional groups or resonant molecules were often used as probe molecules adsorbed on silver nanoparticles for Surface-enhanced Raman scattering (SERS). However, it is still unclear how the attached molecules interact with the silver nanoparticles' surface, and how the anchoring groups affect the optical and electronic properties of molecules. Here, we report that surface-enhanced Raman studies of two organic compounds; rhodamine 6G (R6G) and its aminated derivative (R-NH<sub>2</sub>) have very different functional groups for surface binding but nearly identical SERS spectroscopic properties at pH = 7 and UV-vis at pH = 3, respectively. A surprise was found that under the same experimental conditions, the SERS signal intensity for R6G is nearly 50-fold higher than that of R-NH<sub>2</sub>. Furthermore, the pH-dependent study reveals that the structure of R6G is irreversibly stabilized or 'locked' in its form and no longer responsive to pH changes. In contrast, R-NH<sub>2</sub> is still sensitive to pH, and can be switched between its open-ring and closed-ring structures. Copyright © 2013 John Wiley & Sons, Ltd.

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**Keywords:** anchoring group; derivatives; nanoparticle; pH-dependent; surface-enhanced Raman spectroscopy

## Introduction

High-affinity function groups for attaching to gold (Au) or silver (Ag) nanostructure have been widely used to form self-assembled structure of monolayer,<sup>[1]</sup> to coat the surface of nanotube,<sup>[2]</sup> and to connect nanoparticles (NPs) for studying molecular junctions.<sup>[3,4]</sup>

The groups of thiol and amino linked with conjugated  $\pi$ -electrons are especially important because they could allow more efficient electron transfer<sup>[5,6]</sup> and change the molecular absorption geometry.<sup>[7]</sup> It is well known that rhodamine 6G (R6G) has been widely used as probe of surface-enhanced Raman scattering (SERS); however, it is not clear how chemical affinity groups interact with the Au or Ag NPs and how the adsorptive groups affect the UV-vis and SERS spectra of organic compound. On the other hand, SERS has the advantage of conforming chemical identity, through analysis of spectral features; thus, the detailed information on interfacial structures, adsorption mechanisms, and surface reactions would be obtained. Recently, the studies on NP-R6G-based aggregation have been further carried out, indicating the agglomerated sizes of the Au(-)NPs were approximately eight times larger than the Au(+)NPs upon the addition of R6G, which would induce a much shorter distance between the dye molecules and NPs, providing the hot spots to induce large SERS enhancement.<sup>[8-10]</sup> Our experiment, however, would eliminate the NPs aggregation and contribution of electromagnetic mechanism, proving the enormous SERS intensity difference between target molecules that were assigned to chemical adsorption or charge transfer.

Here, we study R6G and its aminated derivative (R-NH<sub>2</sub>). Some works have been performed, through the comparison of chemical absorption and spectroscopic characteristic; the rationale is that

such isotopologues have identical surface adsorption properties but distinct spectroscopic features that allow identification of each molecule from their composite SERS spectra. In this work, the opposite reason has been concluded that this pair of organic compounds has identical UV-spectroscopic signatures, different SERS signatures under acid condition, and identical SERS spectroscopic with different UV-spectroscopic signatures under basic condition. Meantime, two molecules have different chemical groups for surface adsorption.

## Experimental

### Reagents

Rhodamine 6G, ethylenediamine (NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), ethanol (EtOH), acetonitrile, Ag nitrate, and trisodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the chemicals used were of analytical grade or better and were used without further purification.

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## Synthesis of R-NH<sub>2</sub>

Synthesis routine for R-NH<sub>2</sub> between precursor R6G and NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub> was synthesized by a similar route as literatures.<sup>[11]</sup> R6G (958 mg, 2 mM) was dissolved in 20 mL of hot EtOH, followed by addition of NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub> (0.7 mL, 10 mM). The reaction mixture was refluxed for 8 h until the color of the solution was changed from red to light pink, which indicated that the fluorescence disappeared. The reaction solution was cooled to room temperature, and the precipitate was collected and washed three times with 30 mL of cold EtOH. Crude product was purified by recrystallization from acetonitrile to produce pure R-NH<sub>2</sub> ( $M + H^+$ ):  $m/z = 457$ .

## Preparation of Ag NPs

Silver NPs were synthesized by adding 10 mL  $1 \times 10^{-2}$  mol/L Ag nitrate into 90 mL distilled water of a 250 mL three-necked flask. When the solution has been heated to boiled, add into 3 mL 1% trisodium citrate solution for heating at 1 h.

## Characterization and instruments

Fourier-transform IR spectra were obtained using a Nicolet-8700 spectrophotometer. Raman spectra were carried out on a LabRAM HR800 confocal microscope Raman system (Horiba Jobin Yvon). The UV-vis absorption spectra for solution phase were recorded with a Shimadzu UV-2550 spectrometer. The structure of R6G-derivative (R-NH<sub>2</sub>) was determined with a ProteomeX-LTQ mass spectrometer employed a regular electrospray ionization source setup.

## Results and discussion

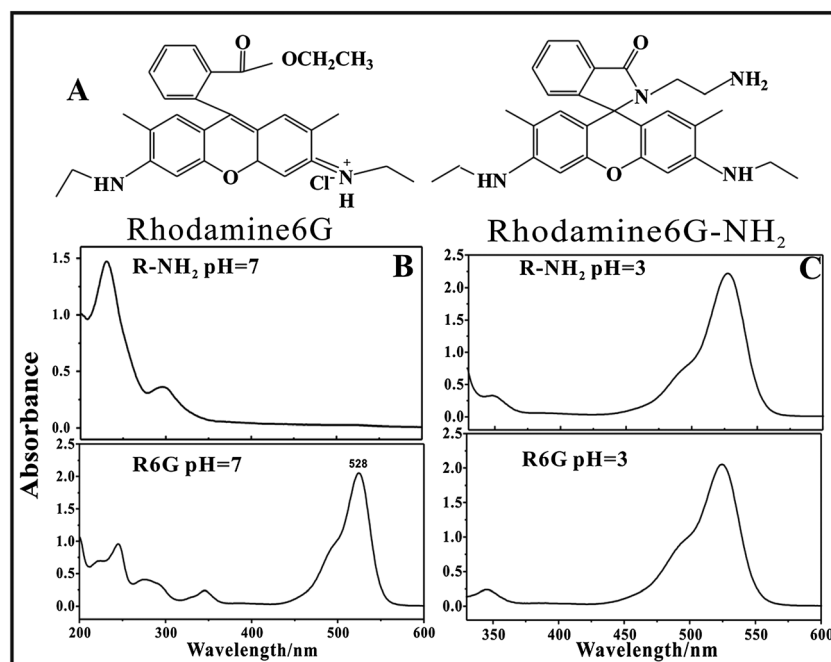
Rhodamine 6G, containing two functional groups, a dibenzopyrene xanthene and a carboxyphenyl group tilted by about 90° with respect to the xanthene ring,<sup>[12]</sup> exhibits a boost of Raman intensity and high fluorescent yield, so this dye molecule has been widely used as SERS<sup>[9,13,14]</sup> spectroscopy and fluorescent probes.<sup>[15–17]</sup> The R-NH<sub>2</sub> was the aminated derivative of dye molecule R6G, and has a weaker Raman intensity than R6G but the free functional groups C=O and NH<sub>2</sub> in comparison to R6G. Comparing the UV spectra of R6G with that of R-NH<sub>2</sub> at different pH condition, as shown in Fig. 1B, the bands at 230 nm and 295 nm of R-NH<sub>2</sub> were also attributed to benzene B band and new spectra band R, which result from benzene linked with auxochromic groups NH<sub>2</sub>, C=O. Because the  $\pi$ -systems in two groups of R6G are not conjugated, the strong absorption in aqueous solution reach a band at 525 nm, which come from the single dye molecule, and a vibration shoulder at around 490 nm that come from the aggregative molecules.<sup>[18–21]</sup> A series of experiments have proved that the single molecule band at 525 nm is still higher than the aggregative molecules band at 490 nm; on the basis of the previous results, we can concluded that the most of dyes existed in solution with single molecule are not in aggregative state. R-NH<sub>2</sub> retains the most structures of R6G; however, the molecule is absent of the absorbance band at 525 nm and the shoulder band in comparison to R6G at pH = 7. It might result from the structure of R-NH<sub>2</sub> breaking the nonrigidity plane of R6G, and the disappearance of nonrigidity plane of R6G results in the presence of transannular conjugate effect of derivative, so the band at 525 nm is not anymore the UV-absorption characteristic band of derivative at pH = 7. However, as shown in Fig. 1C, R6G and its aminated derivative have nearly identical UV-vis spectra at

pH = 3. The main reason for this is that the aminated structure and the effect of transannular conjugate of derivative have been broken, thus derivative recovered the nonrigidity structure of R6G molecular. On the basis of the previous results, two organic compounds have nearly identical UV-spectroscopic at pH = 3 but different UV-spectroscopic characteristics at pH = 7.

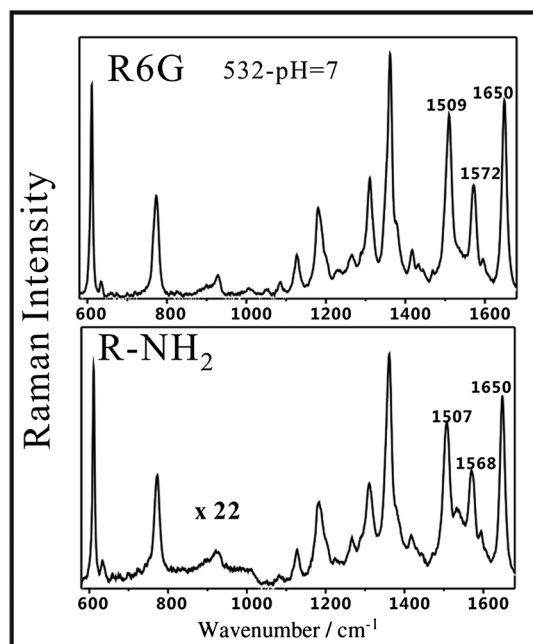
Both R6G and R-NH<sub>2</sub> were found to be stably adsorbed on colloid Ag (90% of the added molecules were adsorbed on to the Ag NPs) and no aggregation with the appropriate volume ratio between molecule and Ag colloid (1:9) was observed. The size of Ag NPs was about 50 nm, and experiment data would prove that this dramatic intensity difference between two organic molecules came from the efficient chemical enhancement, so the NP aggregation and adsorbed molecular amounts have been eliminated. Thus, the number of dye molecules can be calculated within an error of only a few percent (see Figs. S1 and S2 in Supporting information). Under similar condition, the SERS of R6G and R-NH<sub>2</sub> have been detected, as shown in Fig. 2 (laser 532 nm); the absolute SERS intensities of R6G were higher than those of the R-NH<sub>2</sub>. On the other hand, the normal Raman data in Fig. S3 indicated that R6G and R-NH<sub>2</sub> have not only similar vibration modes but also similar scattering cross sections. So, this surprising difference may account for the fact that 525 nm were the electronic transitions band of R6G. In contrast, the band at 525 nm (Fig. 1B) was not the characteristic band of derivative UV-vis spectra at pH = 7 or the observed SERS signal of R6G would contain a resonance, which were the surface-enhanced resonance Raman scattering. To ascertain whether this dramatic SERS spectrum difference between R6G and R-NH<sub>2</sub> should be assigned to resonance, the SERS spectra of these two organic compounds at 633 nm excitation have been obtained (See the Fig. S4 in Supporting information), and the result was that the SERS signals for R6G were still more intense than those of R-NH<sub>2</sub> under non-resonance excitation condition by a large factor. On the basis of the result noted previously, the dramatic SERS intensity difference between R6G and R-NH<sub>2</sub> was possibly attributed to the surface effect or charge transfer, even though this pair of organic compound has similar SERS spectra at pH = 7.

The SERS signals of R6G and R-NH<sub>2</sub> were also researched under acidic condition (pH = 3). Figure 1C showed that both R6G and H<sup>+</sup>-R-NH<sub>2</sub> have UV-vis absorption at 525 nm; the observed signals should have contained similar resonance enhancement. Interestingly, the SERS of R6G were still dramatically higher than those of R-NH<sub>2</sub>, as shown in Fig. 3. Also, to ascertain whether this resonance enhancement effect should be responsible for the observed difference, the SERS spectra of these two compounds at 633 nm excitation have been observed (See the Fig. S5 in Supporting information). A wavelength was not in resonance with the electronic transitions of R6G or R-NH<sub>2</sub>; however, the same result has been obtained that the SERS signals of R6G were also more intense than those of R-NH<sub>2</sub> under non-resonance condition. The R6G and the derivative have not only the same electronic absorption, but also the same molecular structure. In theory, they should obtain the same SERS spectra and intensities; however, in comparison to Figs. 2 and 3, the same result at pH = 3 and pH = 7 has been concluded, respectively and therefore, this dramatic intensity difference was believed to arise from efficient chemical enhancement mediated by the adsorption groups, adsorption geometry, or strong electronic coupling.

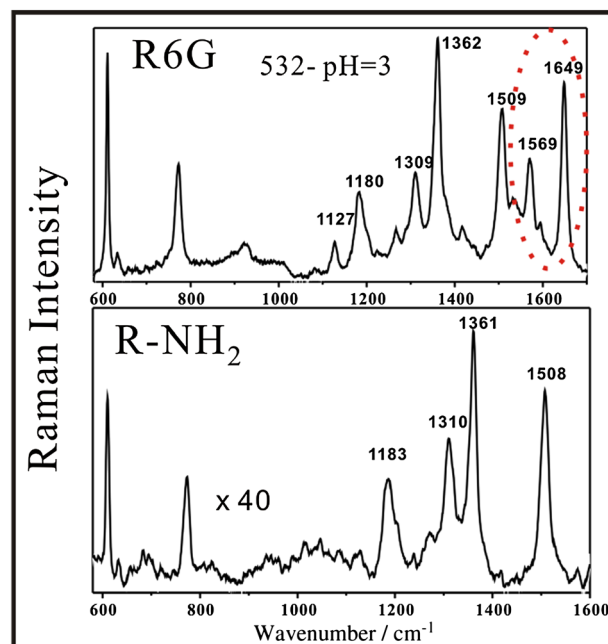
To further investigate how surface absorption could alter the electronic structures of R6G and R-NH<sub>2</sub>, we have taken advantage



**Figure 1.** Rhodamine 6G(R6G) and its aminated derivative (R-NH<sub>2</sub>): two organic compounds that have different functional groups for attaching to Ag NPs but nearly identical spectroscopic at pH = 3 and different spectroscopic characteristics at pH = 7. (A) Schematic chemical structures of R6G and R6G-derivatives. (B) The comparison of UV-vis spectra at pH = 7 (C) at pH = 3 between R6G and derivative, respectively.



**Figure 2.** Surface-enhanced Raman (SERS) spectra of R6G and derivative at pH = 7. The concentration of R6G and derivative are the same (1 ppm) and the volume ratio between molecule and Ag colloid was 1:9. SERS spectra obtained at 532 nm (resonance) laser excitation (integration time = 1 s). In both R6G and derivative, the R6G spectra are expanded by a factor of 22 for spectral details.

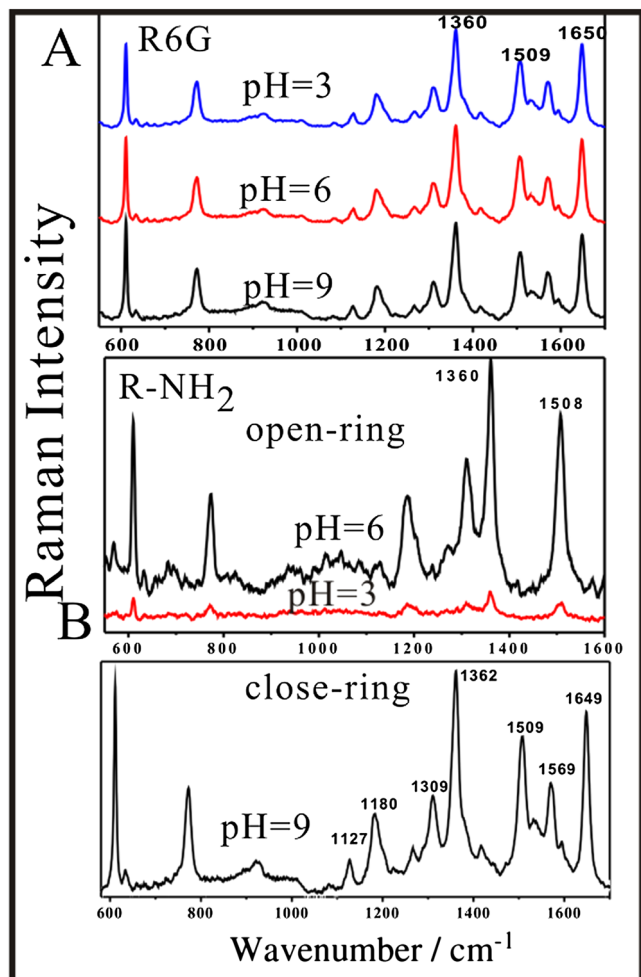


**Figure 3.** Surface-enhanced Raman (SERS) spectra of R6G and derivative at pH = 3. The concentration of R6G and derivative are the same (1 ppm) and the volume ratio between molecule and Ag colloid was 1:9. SERS spectra obtained at 532 nm (in resonance) laser excitation (integration time = 1 s). In both R6G and derivative, the R6G spectra are expanded by a factor of 40 for spectral details.

of their pH-responsive properties to examine how their SERS signals changed as a function of pH. Figure 4 showed the SERS spectra for R6G and R-NH<sub>2</sub> obtained at pH = 3, pH = 6, and pH = 9, respectively. The color of R6G with different pH was similar: strong red-yellow; however, the color of R-NH<sub>2</sub> has appeared a

large alternation and changed from bright red-yellow at pH = 3 to colorless at pH = 9 (because of disappearance of optical absorption in the visible spectrum) (See the Fig. S6 in Supporting information); this was consistent with a result that R6G-derivative existed isomers with different spectroscopic

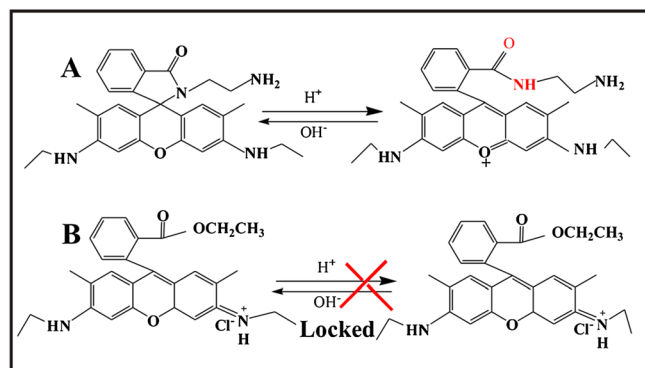
properties, its closed-ring molecular form was colorless and the open-ring molecular form displayed an intense yellow or red color.<sup>[22]</sup> So, R6G-derivatives appeared different molecular structure with different pH value.



**Figure 4.** (A) SERS spectra of R6G and (B) R-NH<sub>2</sub> obtained with 633 nm laser excitation as a function of pH. The pH was changed from 3 to 9. Integration time was 10 s. The concentration of R6G and derivative are the same (1 ppm) and volume ratio between molecule and Ag colloid was 1:9.

For the adsorbed R6G, similar SERS signal intensities and frequencies were observed under both basic and acidic conditions, indicating a stabilized or locked surface structure that was no longer sensitive to pH. However, the adsorbed R-NH<sub>2</sub> was still sensitive to pH, and the SERS signal has illustrated a strong difference within different pH condition. Interestingly, the trace of SERS signals of R-NH<sub>2</sub> remained at pH=3, but the SERS spectrum at pH=9 was similar with that of adsorbed R6G by a delocalized electronic structure (the same structure at neutral and acidic condition). Because pH has an impact on the structure of derivative, the Fourier-transform IR spectra of R-NH<sub>2</sub> was obtained. In free solution, the pH indeed gave rise to completely different structure of closed-ring and open-ring of R-NH<sub>2</sub>, respectively. In the meantime, compared with SERS of R6G, the SERS of R-NH<sub>2</sub> under acidic condition lacked the bands at 1575 cm<sup>-1</sup> and 1651 cm<sup>-1</sup> (Fig. 3), and these two bands were assigned to C=C vibration of aromatic ring. It is likely that although H<sup>+</sup> broke the closed-ring structure of R-NH<sub>2</sub> and recovered the nonrigidity plane of R6G molecule, molecular fine structure have been destroyed by the H<sup>+</sup> or solvent and the charged derivative molecules adsorbed on Ag NPs surface likely induced intermolecular and intercluster interaction.<sup>[23]</sup> Therefore, the open-ring derivate would slantwise be adsorbed on Ag NPs with the free NH<sub>2</sub>, which result in the CC vibration of aromatic ring far away the metal surface. On the other hand, this result was consistent with the SERS characteristics of protoporphyrin that adsorbed on the graphene with different molecule configuration.<sup>[24]</sup> Moreover, the free NH<sub>2</sub> has been protonated; thus, the different SERS spectra have been obtained. Consequently, the aminated R-NH<sub>2</sub> within different condition would be adsorbed on Ag NPs with different adsorptive geometry. To ascertain the experimental results, we collected the random experiment data 15 times, and the relative standard deviation (RSD) of major peaks was often used to estimate the reproducibility of experiment. Figure S7 showed the SERS-RSD spectrum of dyes molecules and the maximal value of RSD of major SERS peaks 1360 cm<sup>-1</sup> was observed to be below 0.2, indicating that Ag dyes have a good replication across the entire experiment.

In comparison to the SERS intensity for R6G and its derivatives within different pH, the SERS signal intensities for R6G were nearly 40-fold higher than those of R-NH<sub>2</sub> in pH=3 and nearly 23-fold higher than those of R-NH<sub>2</sub> in pH=9. On the basis of the previous results, the possible conclusion would be obtained that R6G and its derivative were adsorbed on Ag NPs with different adsorptive geometry even though they have nearly identical SERS-spectroscopic



**Figure 5.** Schematic diagram showing the structure of R6G and the derivative at different pH condition. R6G dye was found irreversibly stabilized or 'locked' in its structure, whereas the derivative was sensitive to pH and could be switched between its open-ring and closed-ring forms.



at pH=7 and identical UV-spectroscopic characteristics at pH=3. On the other hand, on the basis of the surface selection rule, when molecules are perpendicular to the substrate, the enhancement signal is stronger.<sup>[25]</sup> In addition, whether the detective condition approached to acid or not, molecule R6G would possibly be adsorbed on the Ag NPs with an optimum adsorbed geometry with perpendicular to the substrate with two aminoethyl, and the observed strongly SERS vibration was also consistent with this optimum adsorbed geometry. It should be believed that R6G dye was found irreversibly stabilized or 'locked' in its structure and insensitive to pH.<sup>[26]</sup> In contrast, its derivative possibly would be adsorbed on Ag NPs with signal amino group; this adsorptive group did not sufficiently stabilize or lock the electronic structure of R-NH<sub>2</sub>. In addition, the different adsorption should also be considered for the SERS intensity difference between two organic molecules. R6G-NH<sub>2</sub> derivative has several basic groups, thus sensitive to pH, due to the large amount of amino groups in its structure, whereas R6G is less sensitive to pH. Therefore, the protonation and the deprotonation of basic groups should also be considered to account for the differences of both molecules regarding the adsorption on the surface. As a result, R-NH<sub>2</sub> adsorbed on Ag NPs was still sensitive to pH, and its electronic structure can be reversibly switched by pH changes, similar to pH-induced structural changes in solution between its open-ring and closed-ring forms, as schematically illustrated in Fig. 5. We also noted that molecules with stronger binding affinities to metal surfaces were known to give larger enhancement in SERS because of both Franck-Condon and Herzberg-Teller (vibronic) mechanisms, as predicted by theory.<sup>[27,28]</sup>

## Conclusion

In summary, SERS intensity for R6G was higher than that of its aminated derivative R-NH<sub>2</sub> by a large factor, which displayed that R6G was adsorbed on the metal NPs with an optimum adsorptive geometry, perpendicular to the substrate with two aminoethyl, and these two adsorptive groups did sufficiently stabilize or lock the electronic structure of R6G. The pH-dependent study also revealed that the structure of R6G was irreversibly stabilized or 'locked' in its form, and was no longer responsive to pH changes. In contrast, R-NH<sub>2</sub> was still sensitive to pH and can be switched between its open-ring and closed-ring forms. Consequently, SERS would be recognized as one of the most sensitive spectroscopic tools for detection of target molecule, understanding the enhanced mechanism and adsorptive mechanism.

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## Supporting information

Supporting information may be found in the online version of this article.

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