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Improved properties of gold nanorods coated with thin multilayer of small organic molecules by fast and facile method for surface enhanced Raman scattering

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Abstract We introduce a thin multilayer-shell-coated gold nanorods (GNRs) that we have been able to achieve by way of coating them with two thin layers of small organic molecules in a new, efficient, effective, time-saving, and easy-to-control multilayer coating method. Compared to the thick shell, due to the small size of the coating molecules, this new type of GNR, by simply mixed with the sample solution, has shown that it can act as a highly effective and improved surface enhanced Raman scattering (SERS) enhancement agent without the need of any sample connecting or doping. This enhancement is found not only related to the off-resonance surface enhancement, but also to the increased signal-to-noise ratio brought by the low level of fluorescence emission. It showed excellent stability in strong acids, bases and some organic solvents as examined by comparing the longitudinal surface plasmon resonance peak values. A living body experiment on a tumor-bearing nude mouse injected with the Raman reporter dye loaded onto our new GNRs showed strong surface enhanced Raman scattering signal in the tumor, suggesting its high potential use in many related optical applications.

Keywords Surface enhanced Raman scattering · Gold nanorods · Surface plasmon resonance · Multilayer · Tumor · Fluorescence quench

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1 Introduction

Gold nanorods (GNRs) have been given much interest in recent years in diverse fields such as optics, material science and biomedicine (Pérez-Juste et al. 2005; Vigderman et al. 2012; Alkilany et al. 2012; Chen et al. 2013a, b). Among all the valued properties of GNRs, the most frequently-mentioned one is the effect of surface plasmon resonance (SPR; Zijlstra et al. 2012; Chen et al. 2013a, b; Khatua et al. 2014). The incident light of a suitable wavelength can lead to the collective oscillation of the surface electrons of the GNRs, which can subsequently modulate many optical signals such as fluorescence, Raman scattering, and nonlinear signals (Qian et al. 2011; Zhang et al. 2012; Wang et al. 2005). With the assistance of GNRs, Raman signals, for example, could be enhanced by a factor of 10^{14}, which makes the surface enhanced Raman scattering (SERS) detection technique highly useful in many optical applications (Eustis and El-Sayed 2006).

The function of the GNRs in optical and photonics fields is closely related to their surface modification because (1) the optical properties of GNRs are delicately affected by their shape and monodispersity, which are related to their stability and compatibility and (2) in many surface-enhancement applications such as SERS detection, the signal-reporter agents need to stay within a close proximity from the gold surface (Caswell et al. 2003; Guo et al. 2009; Li et al. 2010). Researchers have found that a great stability of nanoparticles could be achieved by recapping them with a multilayered shell (Hu and Gao 2010; Yang et al. 2014). For example, we have demonstrated a silica–octadecyltrimethoxysilane–polyethylene glycol (silica–OTMS–PEG) coating method which would help the GNRs to survive in water solutions with pH ranging from 1 to 12, in the animal serum and in the living bodies (Zhang et al. 2013). However, in spite of all the benefits of the multilayer coating, some drawbacks have been found to limit its use. In many methods, large molecules, such as polyethylene glycol (PEG), would make the shells relatively thick and thus would prevent the efficient interactions between the SPR field and the external samples. Another problem is the long time and the fine control required in each step of the shell coating procedures.

In our present study, in order to overcome the drawbacks of thick-shell coating, we investigated the properties of GNRs coated with thin molecular multilayers using a new, fast, simple, and well-controllable modification method. In the test of our GNR samples, simply mixing the new GNRs and the sample solution without any complicated doping methods, we observed highly effective strong surface enhancement of the Raman signals and much reduced level of fluorescence from outside the thin layer of the molecules of the shell, leading to a strong Raman signal with a high signal-to-noise ratio. The GNRs of this thin shell coating has been found to have an excellent stability in strong acid, base or organic solvents. Moreover, as the SERS agent could also be simply modified onto the GNRs through an easy process, this technique has been found to be highly useful in biological applications, too, as proven effectively in our study of an in vivo experiment with a tumor-baring mouse, to be described later.

2 Methods of GNR shell modification using thin layers of molecules

GNRs were first synthesized using a seed-mediated method (Pérez-Juste et al. 2004; Zhan et al. 2010). A drop of 0.6 mL of the ice-cold sodium borohydride (10 mM) was quickly added into a 10 mL water solution containing $HAuCl_4$ (0.25 mM) and hexadecyltrimethylammonium

bromide (CTAB; 100 mM). After 20 min stirring, the gold seeds were formed in the solution. Next, a drop of 100 mL CTAB (200 mM) was added into a 100 mL water solution containing $HAuCl_4$ (1 mM) and $AgNO_3$ (0.128 mM). Then 0.9 mL ascorbic acid (90 mM) was added and the solution was stirred sufficiently until becoming colorless. Finally, a 0.24 mL seed solution was added into the solution. After 18 h of GNR growth, the solution was centrifuged once and re-dispersed in water.

For the bilayer coating of the GNRs, 10 mL of the as-prepared GNR solution was mixed with 100 μL (3-mercaptopropyl)trimethoxysilane (MPTMS, 2 % in ethanol) and was kept for 20 min under vigorous stirring. The solution was centrifuged once and re-dispersed in 8 mL water. Then 200 μL dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride (DMOAP, 60 % in methanol) was quickly added. The solution was kept under vigorous stirring for another 10 min, centrifuged twice and re-dispersed in water.

The structure of our new GNR is illustrated in Fig. 1a. (3-mercaptopropyl)trimethoxysilane (MPTMS) are connected onto the gold surface via the –SH group and subsequently a thin layer of –O–Si–O– was formed by way of silane condensation process in water. Then the DMOAP molecules were attached to the MPTMS layer by way of the same condensation process. It is noted that the whole process takes only a very short time of <1 h. In comparison, in many other researches using large molecules such as PEG, the reaction time usually takes more than 15 h to form a PEG single layer, while for layer-by-layer polyelectrolytes, the reaction time usually takes as long as 3 h for each layer

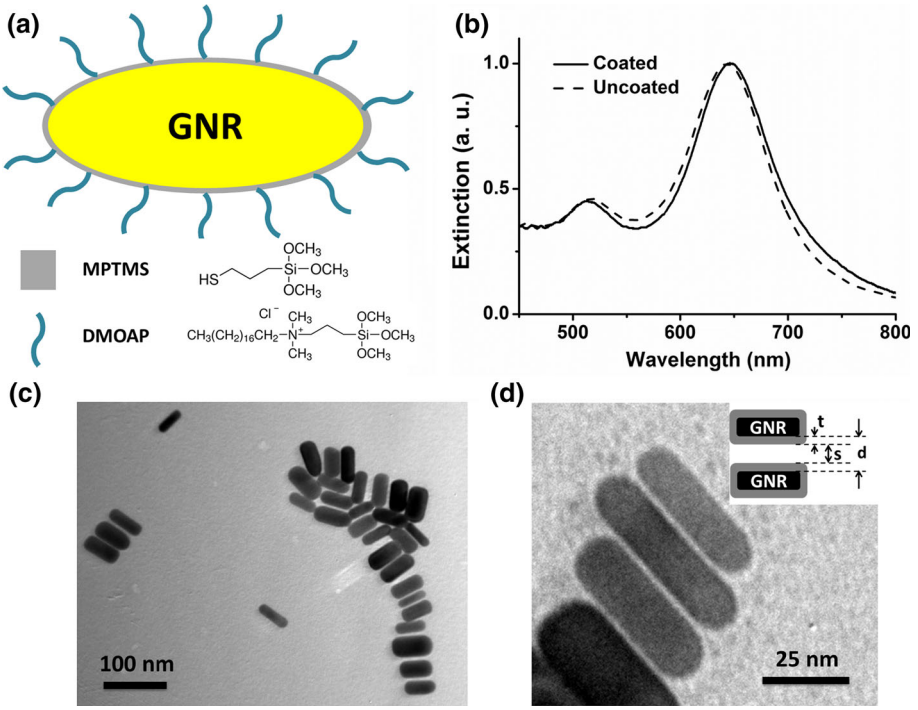

Fig. 1 **a** A schematic view of the DMOAP-MPTMS bilayer shell of the GNRs. **b** The extinction spectra before and after the coating procedures. **c** The TEM image of the coated GNRs. **d** A typical case where several GNRs are seen separated in close proximity on a same focal plane. The *inset* is a schematic illustration of the relationship between the thickness and the distance of the adjacent GNRs

formation (Qian et al. 2011; Jiang et al. 2011). MPTMS on different GNRs can also crosslink with each other, thereby causing a severe aggregation if staying for a long time in water, so DMOAP should be quickly added to prevent this crosslinking. These crosslinking-related phenomena can also be used to check the successful coating of each layer. Figure 1b shows that, after the coating procedures, the extinction curve did not broaden, indicating a good monodispersity for the GNRs. The slight redshift of the curve is due to the change of the localized surface refractive index, which is also an evidence of the bilayer coating.

From those new TEM images, we can evaluate the thickness according to the measurements and calculation based on the figure, schematically illustrated in the inset of Fig. 1d. It can be seen from this inset that $d = 2t + s$ or $d > 2t$, in which d is the distance between the surfaces of two adjacent GNRs (the gap between the dark rods in the TEM image), t is the thickness of the shell, and s is the distance between the shells of these two GNRs. According to the scale bar of Fig. 4d, we can measure that d can be as small as 3–4 nm, and that thickness $t < 2$ nm.

3 Experiments and discussion

We previously demonstrated in our earlier study the useful effect of a multi-layered GNR structure to achieve SERS enhancement (Zhang et al. 2013). In this study, however, the shell was relatively thick so that the SERS signal agents had to be doped into the inner silica layer to keep a close distance from the gold surface. In many applications, it is very complicated and takes cumbersome procedure to dope the samples. In our present work, however, since the bilayer shell is relatively so thin due to the small size of the molecules, it has a strong potential to function as an effective SERS enhancer without any complex doping. In order to prove this, we applied 3,3′-diethylthiatricarbocyanine iodide (DTTC), a commonly used SERS agent, as the sample (Qian and Nie 2008; Chang et al. 2012). A 1 mL coated GNR solution was mixed with a 10 μL DTTC solution (1 mM), and the SERS detection was immediately conducted using a fiber-based Raman probe (BWTEK) with the excitation at 785 nm (300 mW). For comparison, we also tested 1 mL solution of the above-mentioned thick multilayer-coated GNR structure (Zhang et al. 2013, without

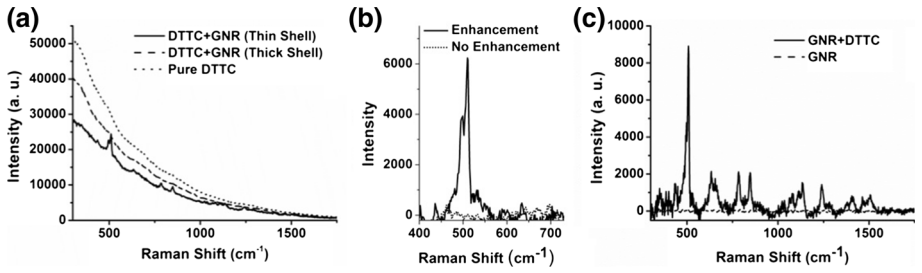

Fig. 2 **a** The combined fluorescence and SERS spectra of DTTC in GNR solution with thin shell (introduced in this paper) and thick shell (demonstrated in our previous paper, Zhang et al. 2013) and pure DTTC solution. The spectrum was captured immediately after the mixing of DTTC and GNRs. The integrating time is 200 ms. **b** The fluorescence-subtracted Raman spectra of the Thin Shell group (enhanced) and the Pure DTTC group (not enhanced) around the 508 cm^{-1} peaks. **c** The SERS spectra of the mixture of DTTC and thin bilayer-coated GNRs, compared with that of pure coated-GNRs. The spectrum was captured immediately after the mixing of DTTC and GNRs. The integrating time is 1 s

DTTC doping) in DTTC with the same concentration and the pure DTTC solution. Their combined fluorescence and SERS spectra are shown in Fig. 2a.

In Fig. 2a, a series of Raman peaks from DTTC could be seen from the thin-shell-GNR-assisted group, while no Raman signals in the thick shell group was observed. It shows that after our coating procedures, the GNRs can act as a highly effective SERS enhancement agent by simply mixing it with the sample solution and that this enhancement is related to the thin thickness of the shell. In our experiments, the GNRs showed a longitudinal SPR peak (Fig. 1b) at a shorter wavelength compared to the Raman excitation (785 nm). This off-resonance mismatch was due to the trade-off between the SPR-induced absorption to the signals and the surface enhancement effect as demonstrated by our previous work (Zhang et al. 2013; Jiang et al. 2011) as well as other researches (Nikoobakht et al. 2002). It is also noted that the fluorescence background of the thin shell-coated GNR group is much weaker than the pure DTTC group. The similar phenomena have been attributed to the fluorescence quenching effect (Schneider et al. 2006; Li et al. 2009), which can lead to a better signal-to-noise ratio for the SERS. To measure the enhancement factor in our case, we subtract the fluorescence background from the curves of the "DTTC + GNR (Thin Shell)" group (after enhancement) and the "Pure DTTC" group (before enhancement) in Fig. 2a to obtain their Raman spectra, shown in Fig. 2b. The enhancement factor can then be obtained from it, dividing the enhanced intensity at the 508 cm^{-1} shift position by the intensity before the enhancement at the same position. This calculation based on our data yields the enhancement factor of 142. We also compared the SERS signals from the solution of our GNRs with and without DTTC, as shown in Fig. 2c. A series of Raman peaks of DTTC can be clearly seen from the curve of the mixture of DTTC and GNRs, while no Raman signals could be found from the pure coated GNR solution, indicating that the signals were from DTTC, not from the bilayer structures of the GNRs. The above results shows that our thin shell coated GNRs can act as a highly effective SERS enhancement agent without any complicated treatment to the sample.

The importance of the stability in solution with different pH and in organic solution has been reported by many previous researches (Auffan et al. 2009; Wu et al. 2011; Cepak and

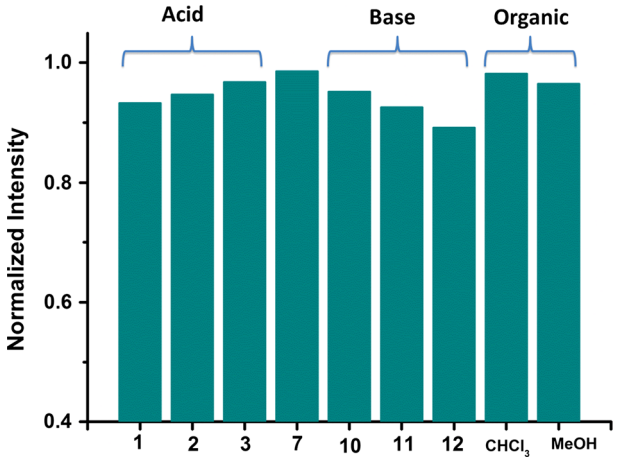

Fig. 3 Intensity of the longitudinal SPR peak value 24 h after mixing in different solvents, normalized by the values that just after mixing. The numbers under the x-axis are the pH values of the groups

Martin 1998). For the unstable GNR structures, their aggregation would cause the broadening of the extinction curve and the decrease of the longitudinal peak value. To test the stability of our GNR structure, we measured the extinction curves and compared their longitudinal peak values. The bilayer-coated GNR solution was centrifuged and re-dispersed into 1 mL of the sample solution (water solutions with different pH, chloroform and methanol). After 24 h, the longitudinal extinction peaks of the mixture were measured and normalized by the value before the re-dispersing. As opposed to most of other groups, Fig. 3 shows that the peak values remained almost unchanged. In strong base solution with pH 12, the peak value dropped a bit but was still about 90 %. This result proved the excellent stability of our bilayer coating in different environment.

GNR nanoparticles are widely used in bio-imaging and bio-detection, and many researches have applied them to the tumor-related study such as tumor diagnosis and therapy (Brannon-Peppas and Blanchette 2012; Choi et al. 2012). In our study, too, we used the GNRs for the study of their function on the tumor. All the in vivo experiments were conducted in compliance with the requirements for the care and use of laboratory animals in research established by the Zhejiang University Animal Study Committee. The male nude mice were purchased and raised by the Animal Experimentation Center of Zhejiang University. To set up the tumor model, Hela cells (5 million cells in 0.2 mL 10 m M PBS, pH 7.4) were injected hypodermically and monitored until the tumor was seen. 3 mL of the as-prepared bilayer-coated GNRs was mixed with 200 μL of the DTTC solution (1 mM) and kept for overnight. The mixture was centrifuged three times and re-dispersed in Phosphate Buffered Saline (PBS). Then 30 μL of the solution was directly injected into the tumor growing on a nude mouse (Fig. 4a). This direct injection method could help us to quickly put the GNRs inside the tumor avoiding any complicated tumor-targeting agents or bio-compatible surfactants, so that we could promptly and conveniently check their SERS performance inside the tumors.

After 10 min, the SERS detection was conducted and the signals from the tumor and the normal skin were compared, as shown in Fig. 4b. Some of the previous work showed that with the enough reaction time, DTTC could be attached onto the gold surface by way of S–Au binding, which was the reason why the SERS signal could be kept even after three times of centrifugation. This result not only showed the strong potential use of our structure in living bodies, but also a strong proof that the modification of the SERS agent is also very simple and effective.

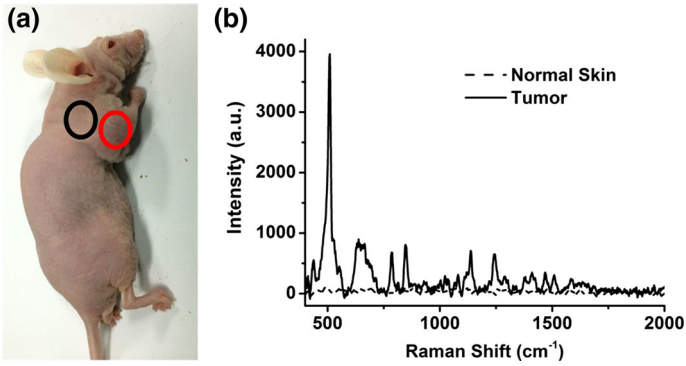

Fig. 4 **a** A tumor-baring nude mice. The *right* and the *left circles* show the injected area (the tumor) and the normal skin, respectively. **b** The Raman signal obtained from the two area in **a**). The integrating time is 10 s

4 Conclusion

We have introduced GNRs coated with thin small molecule shell by way of fast, simple, highly effective, time-saving, easy-to-control, and stable coating method and have shown the improved properties that can be effectively used not only for SERS but also for other applications, including bio-study. The shell consists of two thin layers of small organic molecules attached onto the gold surfaces. The successful coating could be easily confirmed by the process of the crosslinking of the molecules. The new GNRs have shown that, due to thin molecular shell layers, they can interact with the external sample more effectively than the thick shells, thereby making them highly effective SERS enhancement agent without any need of complicated doping procedures. They also displayed excellent stability in different environments of strong acids, bases and organic solvents. In vivo experiment on a tumor-bearing living nude mouse injected with these GNRs showed strong enhanced Raman scattering signal in the tumor. These results collectively suggest that this new type of GNRs, formed by way of fast and facile coating method, would be used for broad and effective practical optical applications.

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