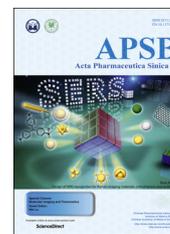




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ORIGINAL ARTICLE

# Design of SERS nanoprobe for Raman imaging: materials, critical factors and architectures



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**Abstract** Raman imaging yields high specificity and sensitivity when compared to other imaging modalities, mainly due to its fingerprint signature. However, intrinsic Raman signals are weak, thus limiting medical applications of Raman imaging. By adsorbing Raman molecules onto specific nanostructures such as noble metals, Raman signals can be significantly enhanced, termed surface-enhanced Raman scattering (SERS). Recent years have witnessed great interest in the development of SERS nanoprobe for Raman imaging. Rationally designed SERS nanoprobe have greatly enhanced Raman signals by several orders of magnitude, thus showing great potential for biomedical applications. In this review we elaborate on recent progress in design strategies with emphasis on material properties, modifying factors, and structural parameters.

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## 1. Introduction

The initial observation of the surface-enhanced Raman scattering (SERS) effect originated from the remarkably enhanced Raman spectra of pyridine on the surface of a roughened silver electrode in 1974<sup>1</sup>, where the researchers did not realize that the enhanced signals arose from the roughened surface of silver rather than from the increased number of molecules adsorbed on the electrode. Since it was discovered in 1977<sup>2</sup> that the Raman signals of molecules attached to the roughened metal could be enhanced by several orders of magnitude, SERS has been extensively researched. Especially over the past decade, SERS as a highly-sensitive and noninvasive technique that could provide specific fingerprints of molecules has been exploited in various fields such as molecule imaging<sup>3</sup>, medical diagnosis<sup>4</sup> and biological trace detection<sup>5</sup>. However, all of these above-mentioned applications are based on appropriate SERS-active nanostructures, which can enhance the Raman signals of target molecules dramatically. Up to now, the amplification of Raman scattering intensities are mainly attributed to two mechanisms—electromagnetic field enhancement<sup>6</sup> and chemical enhancement<sup>7</sup>. The electromagnetic enhancement originates from the collective oscillation of free electrons of metallic nanoparticles (NPs), which are excited by a particular frequency of incident light, resonating with the incident optical field, the so-called localized surface plasmon resonance (LSPR)<sup>8</sup>. LSPR enhances the electromagnetic field in the proximity of the metallic NPs. The regions with extraordinarily amplified electromagnetic field intensity are referred to as hot spots. The Raman scattering of molecules can be dramatically enhanced with theoretical enhancement factors (EFs) even up to  $10^{11}$  in these hot spots<sup>9</sup>. The other contribution to SERS is chemical enhancement, which arises from the charge transfer between adsorbed molecules and metal. The chemical enhancements are weaker than electromagnetic enhancement with EFs of  $10^2$ – $10^{37}$ .

It is critical to develop highly-active SERS substrates capable of providing numerous hot spots for increased EFs. With the rapid development of nanotechnology, a lot of effort has been devoted to achieving improved SERS substrates for various applications. In this review, we give a brief introduction of SERS-active materials and focus on the design strategies and factors for SERS. Subsequently, we discuss the impact of size, shape, symmetry and surface topography on the SERS properties of NPs. Finally, we illustrate the strategies to fabricate nanostructures with optimal SERS performance, outstanding stability, good reproducibility as well as multiple functionalities. This review may contribute to rational design of SERS-active nanostructures with great SERS performance for a wide arrange of applications.

## 2. SERS-active materials

SERS performance is mainly dependent on the SERS-active materials. Up to now, considerable research has been dedicated to exploring SERS-active materials. Apart from the classic metallic substrates such as Ag and Au, novel materials such as semiconductors and graphene have also been reported to show SERS activity. This section will give a concise summary of the SERS-active materials.

### 2.1. Metals

Noble metals are the most common substrates for SERS, including Au, Ag and Cu. In contrast to Cu, Au and Ag have been widely

used due to their better chemical stability. Ag plays an important role in the field of SERS because of its large scattering cross section and low price. However, Ag still has limitations due to its cytotoxicity and non-degradability, thus hindering *in vivo* applications. Au is comparatively more stable and biocompatible than Ag, while it's more expensive and shows lower SERS activity. Bimetals are composed of two distinct metals in which at least one is a SERS-active material. Different metals with various optical and chemical properties exhibit diverse functions as SERS substrates. Bimetals with distinct components can achieve tunable plasmonic properties over a wide range so as to further broaden the applications field. Numerous bimetallics have been reported for SERS applications: the most commonly used metals are Ag and Au in that the combination of Ag and Au integrates the merits of both—high SERS activity of Ag and the favorable surface chemical property of Au. For instance, Yang et al.<sup>10</sup> have prepared Ag@Au nanocubes by directly depositing Au atoms on the surface of Ag nanocubes. The resultant ultrathin Au shell of 0.6 nm is capable of protecting Ag in a harsh environment without decreasing the SERS activity of the original Ag nanocubes. Conversely, the stronger binding of the Raman reporters to the Au surface results in much stronger SERS performance than pure Ag owing to the improved chemical enhancement. In addition to the most popular combination of the noble metals Au and Ag, some transition metals such as Pd<sup>11</sup> and Pt<sup>12</sup> also have been introduced to bimetallic nanostructures. These transition metals are able to endow the bimetallic nanostructures with excellent catalytic properties for specific applications.

Apart from the noble metals and bimetallics, some alkali metals such as Li, Na, K have also been explored as SERS substrates<sup>13</sup>. However, most of these materials are chemically active and would not be favorable for medical applications. Even so, the alkali metals may have great potential as the metallic SERS substrates if their activities could be well-controlled.

### 2.2. Semiconductors

Semiconductors have been reported to show SERS activity over the past two decades, including Cu<sub>2</sub>O<sup>14</sup>, CuO<sup>15</sup>, TiO<sub>2</sub><sup>16–18</sup>, ZnS<sup>19</sup>,  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub><sup>20</sup>. The application of semiconductors as SERS substrates has enormously broadened the range of SERS-active materials while the EFs of these semiconductors vary from approximately  $10^2$  to  $10^4$ , which is much lower than that of the noble metals. Moreover, the enhancement mechanisms of semiconductors are still under debate. Some researchers attribute the enhancement mechanism to the charge transfer between the adsorbed molecules and the semiconductors<sup>17</sup>, which is known as chemical enhancement. However, others hold a different view and have systematically investigated the mechanism by density functional theory (DFT) and three-dimensional finite difference time domain (3D-FDTD)<sup>14</sup>. DFT calculations suggest a charge transfer between semiconductor and adsorbents, while 3D-FDTD simulations have been conducted to confirm the electromagnetic field enhancement around the semiconductor. Simulation results are consistent with the experimental ones. In this regard, the SERS performance may be attributed to both charge transfer and electromagnetic enhancement. This may provide us with a better understanding of the enhancement mechanisms of semiconductors.

Compared to studies on the enhancement mechanism, studies on the enhancement factors attract more attention. Excitingly,

recent research shows that the non-stoichiometric sea urchin-like W18O49 nanowire can serve as a novel SERS substrate with the EF up to  $3.4 \times 10^5$ , reaching the enhancement level of noble metals without hot spots<sup>21</sup>. Therefore, semiconductors deserve more attention in the field of SERS. Although the enhancement mechanism of semiconductors is ambiguous, the potential use of these materials as SERS substrates should be highlighted.

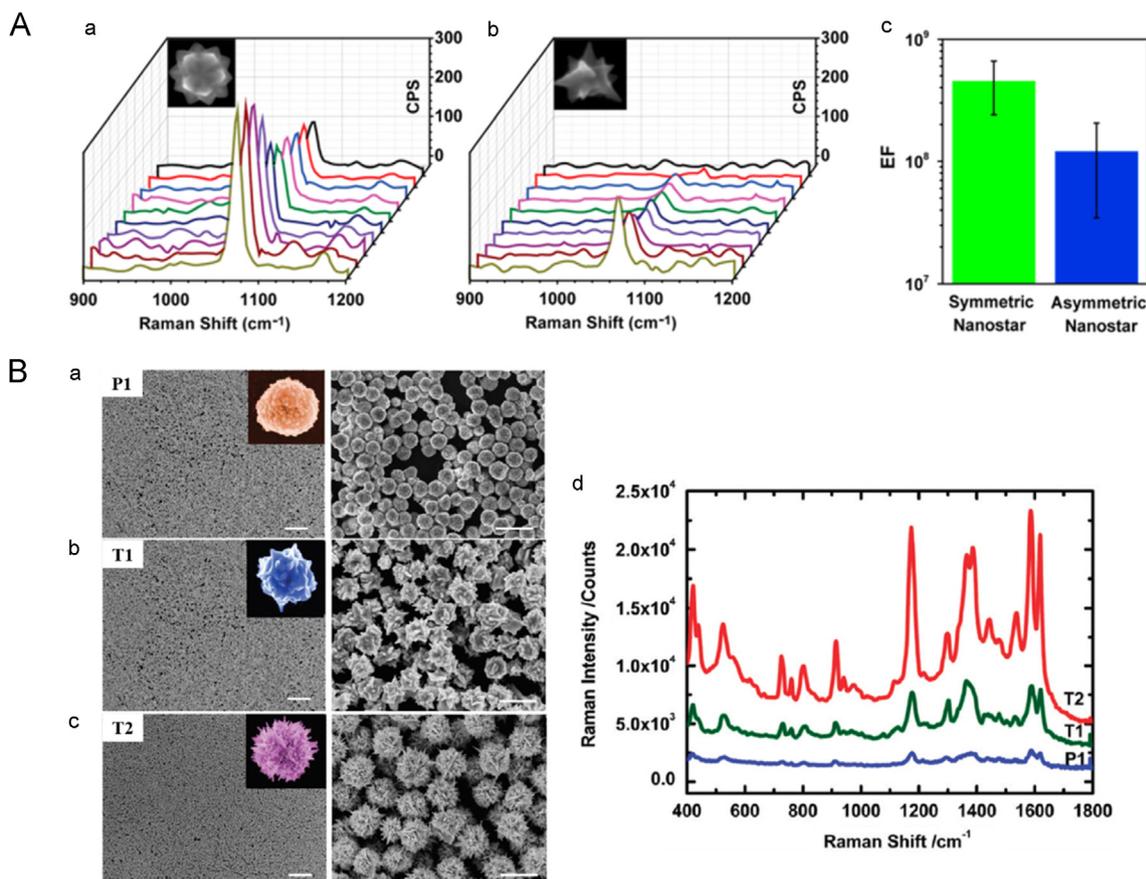
### 2.3. Graphene

Graphene is a monolayer of carbon atoms in a 2D honeycomb crystal structure that exhibits many beneficial properties. Initially, Xie et al.<sup>22</sup> observed fluorescence quenching of fluorescent dyes adsorbed on graphene and thus acquiring their Raman signals. After that, Ling et al.<sup>23</sup> discovered that the Raman signals of phthalocyanine deposited on graphene are much stronger than those on a SiO<sub>2</sub>/Si substrate. Subsequent systematic experiments have confirmed that graphene is capable of serving as a substrate—Graphene-enhanced Raman Scattering (GERS). Although the EFs of GERS are less than  $10^2$ , which is even lower than that of semiconductors, the detection limit of GERS can be at the nanomolar level for some analytes in practical applications. Graphene in other forms such as graphene oxide<sup>24</sup> and graphene quantum dots<sup>25</sup> also exhibits the GERS effect. The discovery of GERS opens new avenues in the development of materials for SERS substrates.

## 3. Factors affecting SERS enhancement

### 3.1. Shape, symmetry and surface roughness

A library of colloidal NPs with various geometries (nanorods<sup>26,27</sup>, nanotriangles<sup>28</sup>, nanocages<sup>29</sup> and nanostars<sup>30–32</sup>) and different sizes are under research. For instance<sup>26</sup>, nanorods with similar aspect ratios but different sizes show distinct SERS properties. Larger sized nanorods are more efficient in light scattering and less efficient in light absorption on account of their appropriate LSPR spectral position. Therefore, they could be more stable and perform better in Raman response. While the nanostars have attracted more widespread interest than nanorods due to their sharp tips, which could enhance the electromagnetic field on the surface of the particle by several orders of magnitude and thereby providing a basis for improved SERS. Recent research shows that highly symmetric nanostars exhibit a superior SERS effect compared to asymmetric ones<sup>30</sup> (Fig. 1A), with nearly four-fold larger EFs. Asymmetric nanostars have varying EFs because of different field enhancements at different orientations. In this regard, highly symmetric nanostars have a more reproducible SERS performance. In addition to the size and shape, surface topography of the NPs can be a significant factor with regard to SERS. Currently, NPs with a highly roughened surface, such as a meatball-like<sup>33</sup> or sea urchin-like<sup>34</sup> morphology, have been confirmed to show excellent SERS performance. Especially, the sea urchin-like<sup>34</sup> NPs (Fig. 1B), which are decorated with about 200



**Figure 1** (A) (a,b) SERS spectra of MBA from symmetric and asymmetric Au nanostars; (c) Average EFs of symmetric and asymmetric Au nanostars, respectively<sup>30</sup>. (B) (a–c) SEM images of Au NPs with increasing surface texture (a: P1, b: T1, c: T2); (d) SERS spectra of crystal violet on individual particles of P1, T1 and T2<sup>34</sup>. Adapted with permission from Ref. 30 and Ref. 34. Copyright 2015 and 2010 American Chemical Society.

“spikes”, are extremely suitable for SERS application as they exhibit high EFs up to  $10^7$  for single particle, arising from a large number of hot spots. The SERS intensity of the sea urchin-like NPs has been significantly improved (Fig. 1B: d) with increasing surface spikes (Fig. 1B: a–c). Generally speaking, NPs with different parameters of structure (including shapes, symmetry and surface roughness) have distinct SERS activities. All of these parameters can be controlled by the selection of surfactant (*e.g.*, SDS or CTAB), reductant, stabilizer and other reaction conditions, so as to achieve optimal SERS performance.

### 3.2. Shell protection

Although the colloidal NPs have been widely used for SERS, the disadvantages of these SERS-active NPs, such as instability and poor reproducibility which result from desorption of Raman reporters or competitive adsorption of other molecules, cannot be ignored. Consequently, nanoshells are introduced to cover these NPs so as to circumvent these problems, as well as providing improved SERS performance and other functions. The protective shells are usually composed of polymers<sup>4,35,36</sup> and SiO<sub>2</sub><sup>37–39</sup>. Qian et al.<sup>4</sup> have prepared Au NPs encoded with Raman reporters and covered with a layer of thiol-PEG (Fig. 2a). These NPs are stabilized by thiol-PEG which can link to the Au surface firmly by an Au-S bond and result in stable and excellent SERS activity. Yang et al.<sup>35</sup> have developed a novel class of SERS probes named polymer-encapsulated metal NPs (Fig. 2b) in which the analyte-tagged Au core is encapsulated by an amphiphilic diblock copolymer denoted as PS154-*b*-PAA60. The hydrophobic PS layer can protect the Au core while the hydrophilic PAA layer can inhibit aggregation of NPs. In addition to the above-mentioned polymers, thermally responsive microgels can also be introduced as the shell<sup>36</sup>. These microgels can trap molecules and position them close to the metal core for improved SERS signals through swelling or collapsing with temperature variation. In addition to polymers, SiO<sub>2</sub> is another important material that can serve as a shell. Doering et al.<sup>37</sup> have prepared dye-embedded colloidal Au

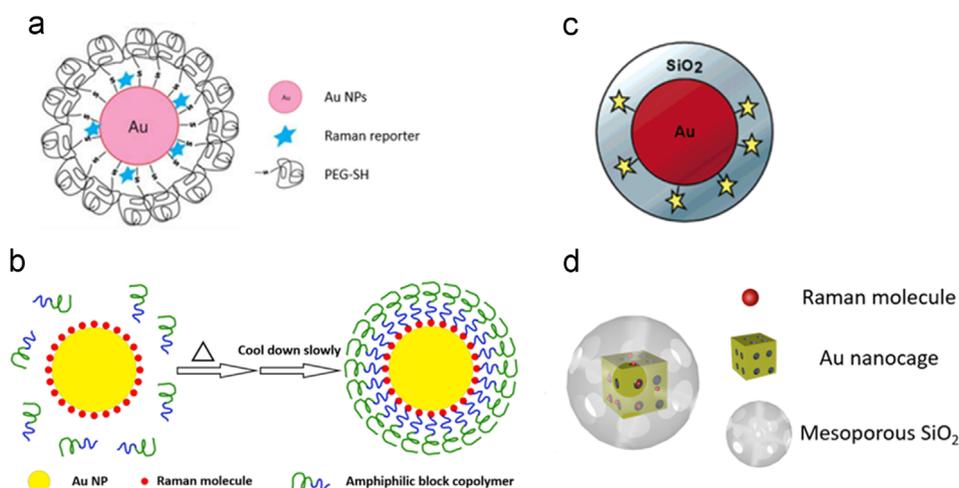
NPs coated with a silica shell (Fig. 2c). The SiO<sub>2</sub>-covered NPs are impermeable to Raman reporters and stable in both aqueous electrolytes and organic solvents with EFs up to  $10^{13}$ – $10^{14}$ . Hu et al.<sup>39</sup> have also covered Raman reporter-loaded Au nanocages with mesoporous SiO<sub>2</sub> (Fig. 2d), which can protect the core and load drugs simultaneously.

### 3.3. Shell-isolated nanoparticle-enhanced Raman spectroscopy

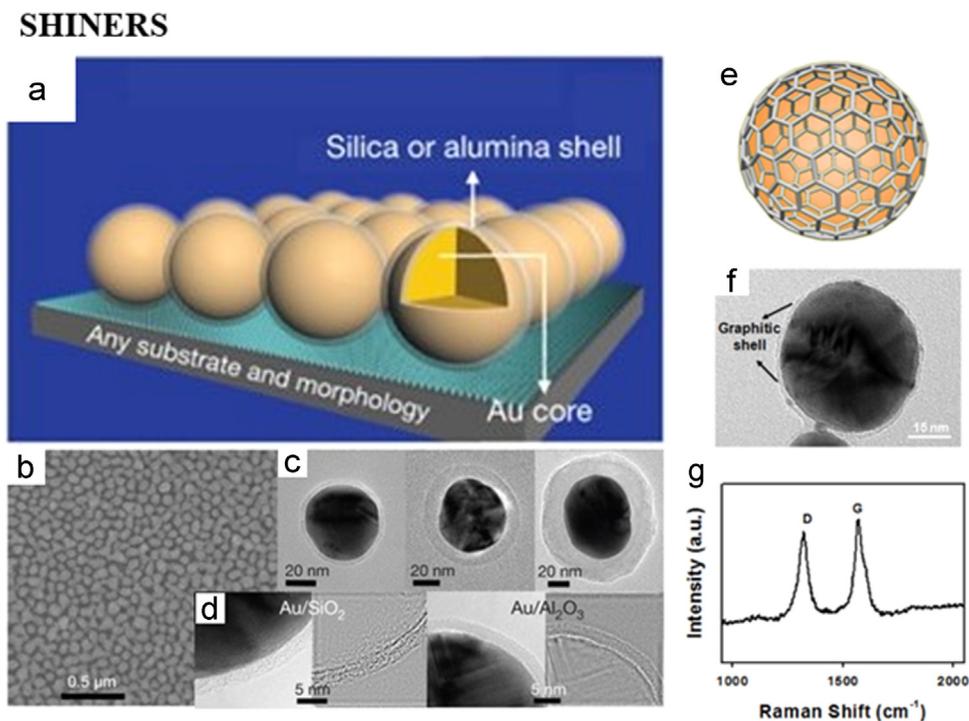
Shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) is a new strategy of Raman signal amplification which has been exploited by Tian's group<sup>40–42</sup>. SHINERS is based on Au NPs covered with an ultrathin (less than 5 nm) layer of silica or alumina shell (Fig. 3a–d). The ultrathin shell is capable of keeping the NPs from aggregating and protecting them from directly contacting the targets. Those NPs could spread as “smart dust” over the surface of analytes with diverse morphologies and achieve stable and intense SERS signals. This approach expands the applications of SERS to a large degree, although it may be difficult to coat the extremely thin dielectric layer of silica or alumina around the metal core consistently<sup>43</sup>. Therefore, some other materials have been utilized as the ultrathin shell, such as graphene<sup>44–46</sup> and polymers<sup>43</sup>. Bian et al.<sup>45</sup> have successfully fabricated graphene-isolated-Au-nanocrystals (GIANs, Fig. 3e–g) via a chemical vapor deposition (CVD) process. The thin graphene shells not only keep the GIANs robust in a harsh environment but also exhibit unique and strong Raman bands, which can be used for Raman imaging. Lee et al.<sup>43</sup> have synthesized gelatin-coated Ag NPs through reduction of AgNO<sub>3</sub> by gelatin. The gelatin-isolated Ag NPs display greatly improved stability in terms of physical and optical properties for more than one year, as well as high SERS activity.

### 3.4. Choice of Raman reporters

The choice of Raman reporters is another significant aspect related to SERS performance. Raman reporters are required to be near or



**Figure 2** (a) Thiol-PEG protected Au NPs encoded with Raman reporters<sup>4</sup>. Adapted by permission from Ref. 4. Copyright 2008 Nature Publishing Group. (b) Schematic of polymer-encapsulated Au NPs by self-assembly. (c) Silica-coated gold particles with Raman spectroscopic reporters embedded at the core-shell boundary<sup>37</sup>. Reprinted with permission from Ref. 37. Copyright 2003 American Chemical Society. (d) Mesoporous SiO<sub>2</sub> covered Au nanocages.



**Figure 3** (a) SHINERS: shell-isolated mode. (b) SEM image of a monolayer of Au/SiO<sub>2</sub> nanoparticles on a smooth Au surface. (c) HR-TEM images of Au/SiO<sub>2</sub> core-shell nanoparticles with different shell thicknesses. (d) HR-TEM images of Au/SiO<sub>2</sub> nanoparticle and Au/Al<sub>2</sub>O<sub>3</sub> nanoparticle with a continuous and completely packed shell about 2 nm thick. (a–d)<sup>40</sup> Used with permission from Ref. 40. Copyright © 2010 Nature Publishing Group. (e) Schematic diagram of GIAN. (f) TEM image of GIANS. (g) Raman spectrum (excitation at 632 nm) of GIANS showing the G and D bands of graphitic carbon. (e–g)<sup>45</sup>. Copyright © 2014 Nature Publishing Group.

adsorbed on the surface of SERS-active substrates as the electromagnetic enhancement mainly depends on the distance between the reporters, and substrates and the chemical enhancement arises from the adsorption. As a consequence, molecules which contain sulfur or nitrogen atoms, such as DTTC, R6G, crystal violet and some small molecules containing thiol are preferable as Raman reporters due to their high affinity for Au and Ag. The Raman reporters with large Raman scattering cross section, which can provide relatively strong enhanced signals, are also a good choice for SERS. In addition, Raman reporters which have absorption spectra that match the excitation wavelength are able to induce the surface-enhanced resonant Raman scattering (SERRS) and further enhance the Raman signals<sup>47</sup>. Apart from using the existing Raman reporters, designing improved molecules for SERS should be advocated. Maiti et al.<sup>48</sup> have introduced lipoic acid to a triphenylmethane dye and the resultant molecule exhibits better stability than malachite green isothiocyanate. Samanta et al.<sup>49</sup> also synthesized lipoic acid-containing ultra-sensitive NIR Raman reporter named CyNAMLA, which shows 12-fold higher sensitivity than the popular molecule DTTC. Despite some progress have been made in designing Raman reporters, new strategies for designing more and improved molecules need to be further explored.

## 4. Architectures

### 4.1. Gap-based hot spots

The nanoscale gaps in the metallic core-shell structures named nanomatryoshkas (NMs) are capable of creating plenty of hot spots

for SERS due to the extremely enhanced electromagnetic fields (Fig. 4a). Usually, the nanogap is made up of a dielectric layer such as DNA<sup>50,51</sup>, SiO<sub>2</sub><sup>52</sup> and some small organic molecules<sup>52,53</sup> such as 1,4-benzenedithiol and 4-methylbenzenethiol, which are able to serve as Raman reporters simultaneously. The Raman reporters embedded in the gap can yield remarkably enhanced and stable Raman signals due to the built-in SERS hot spots. Normally, the plasmonic coupling between the core and shell increases as the gap decreases. However, when the gap size is shrunk to subnanometer level the quantum mechanical effects may play an increasingly important role and lead to a reduction in the electromagnetic enhancement<sup>52</sup>. Therefore, the SERS effect decreases when the gap reaches a subnanometer distance. In addition to gap size, the shell thickness also can be a significant factor related to SERS. Recently, Hu et al.<sup>54</sup> deliberately synthesize Au NMs with thirteen sizes that varied from 40.7 to 99.64 nm. All of these NMs have a uniform Au core of 15 nm and a nanogap of about 1.2 nm which is made up of SH-DNA. These nanogaps are embedded with Raman reporters. The SERS performance increased when the shell thickness increased up to a threshold size, about 61 nm, where the SERS performance decreased. The mechanism of the size-dependent SERS has been explained by some researchers such that with an increase in shell thickness, the internal and external energy modes are closer, resulting in an increase in the intensity of antibonding plasmons. However, when the resonance of the metallic shell increases to that of the core, the energy will dissipate and the plasmonic resonance decreases. It can be concluded that the thickness of shell should match well with the size of core so as to achieve optimal Raman enhancement.

Although the single-shell NM has been most intensively studied, it has only one gap per single nanoparticle. To further

improve SERS activity, a new strategy is to develop multi-shell NMs, which have more than just one gap, thus obtaining a larger gap area for Raman reporters (Fig. 4b). Lin et al.<sup>53</sup> have successfully synthesized NMs with single, double and triple shells with sizes of 58.9, 117.3 and 186.3 nm, respectively. The experimental results suggest that the Raman intensity of reporters in the gap of double-shell NMs should be 22 times higher than that of single shell NMs. Triple shell NMs embedded with more molecules seem to exhibit similar SERS intensity compared to the double shell ones. This may be attributed to the decrease of light penetration as the shell thickness increases. Consequently, an increase of shells enhances Raman signals only to a limited extent.

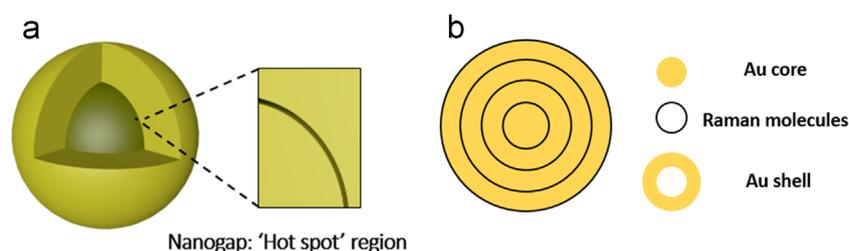
#### 4.2. Assembled nanostructures

Interactions between adjacent plasmonic NPs enable the electromagnetic field in the gap of these NPs to increase drastically, thus creating sufficient hot spots for SERS. Assembled plasmonic nanostructures are a kind of nanoparticle aggregate achieved by covalent binding<sup>55</sup>, electrostatic adsorption<sup>56,57</sup> and hydrophobic interaction<sup>58,59</sup> (as depicted in Fig. 5). Representative assembled nanostructures include dimmers, trimers, multimers, and core-

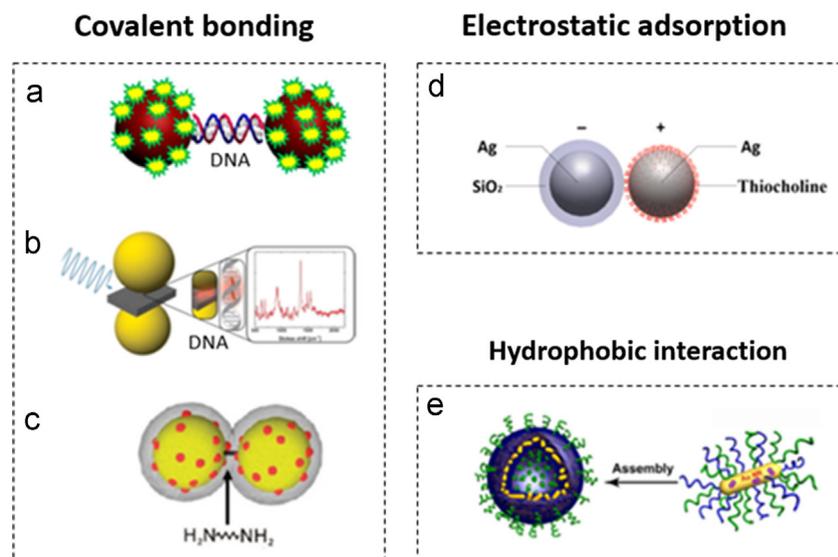
satellites; they display intriguing SERS properties and functionalities for multiple applications, such as biodetection<sup>60</sup> and theranostics<sup>58</sup>.

##### 4.2.1. Dimmers, trimers and multimers

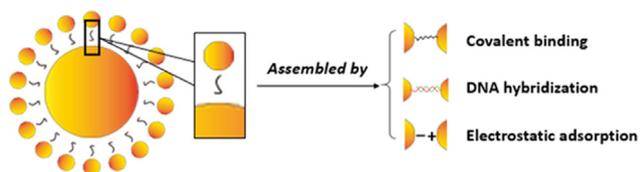
The nanogap junction of dimmers or trimers of plasmonic NPs can generate an enormous electromagnetic field for SERS<sup>61</sup>. Raman signals of reporters embedded in the gap of these assembled NPs can be amplified over two orders of magnitude greater than the signals of reporters adsorbed on the surface of NPs<sup>55</sup>. Initially, dimmers or trimers were prepared *via* salt-induced agglomeration<sup>62</sup>. Chen et al.<sup>62</sup> used NaCl to induce the plasmonic NPs to aggregate and then covered them with polymer for protection. The dimmers and trimers could be obtained by differential centrifugation. However, salt-induced agglomeration could not offer any control over the interparticle distance and thus the assembled NPs obtained by this method may be non-uniform in terms of interparticle spacing despite exhibiting excellent SERS. As a result, some progress has been made in controlling the gap spacer. DNAs<sup>63,64</sup>, polymers<sup>65</sup> and some small organic molecules<sup>55</sup> are employed as linkers to assemble the metallic NPs into dimmers or trimers. Lan et al.<sup>63</sup> have prepared Au nanodimers



**Figure 4** (a) Schematic of gap-based hot spots. (b) Schematic of multi-shell nanomatryoshkas.



**Figure 5** Formation mechanism of assembled nanostructures. (a) Double-stranded DNA-connected Au dimer<sup>63</sup>. (b) DNA origami-assembled Au NP hybrid structure<sup>64</sup>. (c) 1,6-Hexamethylenediamine linked Ag dimer<sup>55</sup>. (d) Ag dimer assembled by electrostatic adsorption<sup>57</sup>. (e) Self-assembly of AuNR@PEG/PLA into plasmonic vesicles by hydrophobic interaction<sup>58</sup>. Reprinted with permission from Ref. 63, Ref. 64, Ref. 55 and Ref. 58 and adapted with permission from Ref. 57. Copyright 2013, 2014, 2009, 2013 and 2014 American Chemical Society.



**Figure 6** Schematic and assembly methods of core-satellite structures.

by dsDNA with a dithiol group at each ends (Fig. 5a). The interparticle distance of the dimmers could be precisely controlled by changing the molecule length of DNA bridge in order to generate optimal SERS activity. Kuhler et al.<sup>64</sup> use DNA origami as a template to make plasmonic dimmers (Fig. 5b). The rigid DNA origami block provided a reliable scaffold for delivering Raman reporters to the hot spot located between the two NPs and improved the stability simultaneously. However, despite these merits, DNAs still have the limitation that the gap size could not be controlled at an extremely small level (below 3 nm)<sup>57</sup>, probably because insufficient base pairs are available for firm connection between NPs. Smaller gaps may be easily generated by small organic molecules<sup>66</sup> or polymers<sup>61</sup>. Small organic molecules, such as dithiols<sup>67,68</sup> and diamines<sup>55,69</sup>, have been used to fabricate dimmers by covalent binding. Braun et al.<sup>55</sup> have utilized 1,6-hexamethylenediamin to assemble plasmonic NPs into dimmers (Fig. 5c), which are then encapsulated with polymer to quench the aggregation process. After that, Raman reporters are infused through the polymer coat and locate at the hot spot areas. The resultant SERS nanocapsules had good uniformity and stability and show SERS enhancement over 300 times greater than that of single NPs. In another example, polymers such as polystyrene are also employed to make dimmers. The Au nanorods are end-functionalized with thiolated polystyrene, which enables end-to-end assembly of dimmers by hydrophobic interaction. The dimmers are encapsulated with phospholipid to quench the self-assembly and exhibit excellent stability.

Besides the organics, inorganic materials such as silica have also been utilized. Zohar et al.<sup>57</sup> have covered Ag nanospheres with an ultrathin silica shell and the silica shell can be further functionalized with Raman molecules. The resultant negatively charged Ag@SiO<sub>2</sub> is capable of adsorbing the thiocholine-covered positively charged Ag NPs into dimmers by electrostatic attraction (Fig. 5d). The nanogaps can be precisely controlled by the shell thickness of silica and even limited to 1.4 nm for improved SERS performance.

In addition to dimmers or trimers, multimers have been explored recently. A particular example is assembled plasmonic vesicles. Song et al.<sup>58</sup> have decorated Au nanorods with hydrophilic PEG and hydrophobic polylactide as well as Raman reporters. The amphiphilic Au nanorods are capable of self-assembling into well-defined plasmonic vesicles in aqueous medium (Fig. 5e) and producing greatly improved SERS signals.

#### 4.2.2. Core-satellites

Core-satellites are another special type of assembled NPs in which the metallic core is covered by some small satellite metallic NPs with part of the core surface left unoccupied<sup>70</sup> (Fig. 6). Similar to other assembled NPs, the fabrication methods of core-satellite structures are mainly covalent binding<sup>70,71</sup>, DNA hybridization<sup>5,72</sup> and electrostatic adsorption<sup>73</sup>. On the one hand, the core-satellite

structure may provide a large amount of hot spots in multiple gaps for SERS. On the other hand, this special structure can broaden the practical applications of SERS. Xu's group<sup>5,72,74,75</sup> has fabricated numerous core-satellite assemblies for ultrasensitive biodetection. In their research<sup>5</sup>, aptamers specific to the biomolecules and their corresponding complementary DNA are employed to assemble the core-satellite NPs. The satellites can be combined with the core by DNA hybridization in the absence of the target biomolecules and thus acquiring high SERS signals. The SERS intensity decreases by introducing the target biomolecules into the core-satellite due to the release of satellites from the core. Moreover, the variation of SERS intensity is directly related to the numbers of satellites around the core. Therefore, these core-satellite NPs may be utilized to detect biomolecules quantitatively.

## 5. Summary and future directions

SERS is a highly-sensitive and noninvasive technique which could provide specific fingerprints of molecules. During the past decade significant progress has been made in the research of SERS-active nanomaterials. In this review, we briefly introduced the development of SERS materials. We discussed the impact of size, shape, symmetry and surface topography on SERS properties of nanostructures and illustrated some strategies to fabricate nanostructures with optimal SERS performance, outstanding stability and good reproducibility.

Increasing interest has been focused on the development of SERS nanoprobes for biomedical applications, such as image-guided surgery, and ultrasensitive detection and tracking of metastatic tumor cells and stem cells. Looking into the future, it would be desirable to develop structurally uniform SERS nanoprobes with equalized SERS signal intensities, thus enabling quantitative detection of molecular biomarkers and live cells. Notably, current SERS materials for medical applications are mainly limited to noble metals (Au, Ag and Cu), which are non-degradable and cannot be eliminated from the imaged tissues. New materials with favorable SERS performance and degradability need to be explored in the future. Furthermore, low-cost and miniaturized devices will facilitate the adoption of SERS for medical applications. Notably, the clinical translation of SERS nanoprobes remains a bottleneck, and it is of great importance to carry out systematic nanotoxicology including pharmacokinetics and pharmacodynamics studies. The research on SERS-based Raman imaging for medical applications will continue to thrive over the next decade, and tremendous opportunities lie ahead.

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