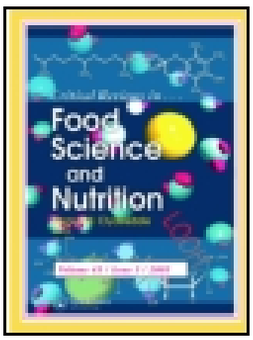

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Recent Advances in Nanofabrication Techniques for SERS Substrates and Their Applications in Food Safety Analysis

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Abstract

The ability to analyze food safety and quality in a quick, sensitive, and reliable manner is of high importance in food industry. Surface-enhanced Raman scattering (SERS), which is popular for its significant enhancement, excellent sensitivity, and the fingerprinting ability to identify special molecules, has shown vast potential for rapid detection of chemical constitutes, chemical contaminants, and pathogens in food sample. For SERS, the enhancement of Raman signals is related to not only the SERS-

active substrates, but also the interactions between sample and substrates. In the current review, colloidal and solid surface-based substrates are briefly described, fabrication techniques for SERS substrates are presented, and applications of SERS for food matrixes, correlation between substrates and food samples are also introduced. Finally some outlook on further developments is presented. The current review is therefore intended to provide a comprehensive overview on the nanofabrication of SERS substrates, and the potential of applying SERS as an important food analysis platform.

Keywords

Surface-enhanced Raman scattering, food safety analysis platform, nanostructure, nanofabrication, small molecules, pathogens, proteins

1. Introduction

Recent years, food quality and safety have received more and more attention, and high level of standardization in terms of quality and safety of food commodities has been imposed. It is therefore significant to detect and control the quality and safety of food commodities to satisfy the corresponding criteria or consumer requirements. As a result, many techniques have been proposed to investigate food quality and safety, such as spectrophotometry, chromatography and spectrometry (McGorrin, 2009). Of the many technologies available, Raman technology has been shown as a powerful alternative tool, providing non-destructive, rapid, and accurate detection. In addition, the minimum requirement for sample handling and preparation also makes Raman technology stand out of other techniques. The inelastic scattering of Raman offers rich spectroscopic information on molecules, and the unique spectra obtained enable the specific identification of individual components in complex matrixes, such as foods, organisms and materials (Cao *et al*., 2015). However, one major disadvantage with traditional Raman is the weak process in the inelastic scattering. The weak signal leads to difficult measurement and limits the application of Raman. On the other hand, this relatively weak signal makes the spectra suffer little interference from water, which exhibits large potential in its application to aqueous systems (Li *et al*., 2014a). In order to widen the application of Raman technology, several methods have been introduced to increase the signal intensity. One of the most significant methods in amplifying the Raman scattering is the surface-enhanced Raman scattering (SERS) (Haynes *et al*., 2005), which possesses a significantly high Raman signal intensity and presents remarkable enhancement factors (EFs) that can reach 10^{11} (Le Ru *et al*., 2007).

Two main mechanisms have been proposed to explain SERS: the electromagnetic enhancement and the chemical enhancement. Electromagnetic enhancement, as the predominant mechanism, refers to the excitation of localized surface plasmon (Jeanmaire *et al*., 1977). The very high local electric fields that

result from the localized surface plasmon resonance (LSPR) are responsible for the remarkably enhanced Raman signal, in addition, small regions of highly enhanced electromagnetic field are known as hot spots. Chemical enhancement is related to the charge transfer between the analyte molecule and metal substrate, which is chemically selective and has been verified in different SERS intensities of CO and N_2 (Campion *et al*., 1998). Overall, these two mechanisms are in action simultaneously for the increase of total SERS enhancement factors (EFs). EF is described as the magnitude of increase in Raman scattering of SERS (Stiles *et al*., 2008). In order to achieve the enhancement, two common types of widely used SERS substrates are available: the colloidal metal nanoparticles and the solid surface-based metal nanostructures. With the advent and progress of nanotechnology, substrates of diverse size, shape, and composition that present different EFs have been extensively applied to food analysis. SERS is relatively mature in aqueous solutions of simple systems, and have been applied for detecting melamine (Hu *et al*., 2015) and protein (He *et al*., 2011a) in milk matrix, aspartame (Peica, 2009) in soft drink, chloromycetin (Gao *et al*., 2014) in honey, *etc*. The SERS enhancement is not only related to the SERS-active nanostructures, but also to the interactions between the target analyte and the substrates, and the microenvironment of analytes (Willets *et al*., 2007). Even the same analyte in different sample will yield different spectral. As food matrixes are complex, it is therefore necessary to choose proper substrates according to different food samples, both the physico-chemical properties of substrates and food samples should be taken into considerlation.

Although SERS has been widely applied for detection of food safety including chemical and microbial contaminants, few publications on detecting food components have been reported and no reviews on the nanofabrication of SERS substrates and their applications for food safety analysis are available. Therefore, the current review intends to show the importance of SERS for detecting a variety of analytes and pathogens in complex food matrixes. It is hoped that the review will provide researchers a

comprehensive understanding on the nanofabrication of SERS substrates and encourage more applications of SERS for food safety analysis and control.

2. SERS-active nanostructures

With the development of nanotechnology, structures in nanoscale roughness have been introduced to SERS technique. Generally, SERS-active nanostructures contain two types of substrates: colloidal substrates and solid surface-based substrates. Using colloid-based substrates is the most straightforward method to obtain enhanced Raman signal, however in the solution-based system, it is difficult to control its assembly and to target the location of the analytes. On the contrary, the solid surface-based substrates can have fine control of the formation of hot spots and the location of analytes, thus exhibiting a better enhancement and reproducibility. The characteristics of some SERS substrates in discussion are shown in Table 1.

2.1 Colloidal substrates

Gold (Au) and silver (Ag) colloids are typical SERS-active substrates, these metallic nanoparticles are stable, easy to fabrication, and excellent in SERS performance. The shape, size, and surface morphology of nanostructures can strongly influence the signal intensity. Consequently, metal nanoparticles of different sizes and morphology have been developed. There are two categories of metal nanoparticles available: one possessing smooth surface morphology that contains nanorod, nanobar, nanowire and others, while the other containing nanostar, nanocube and nanoprism has sharp edges, which has greater electric fields than the smooth surface nanostructures (Wang *et al*., 2015a), with the nanostars attracting much research attention. Rycenga *et al*. (2009) confirmed the stronger enhancement effect of sharp features by calculating the EFs of the synthesized nanocubes and nanospheres, showing that the nanocubes generated a greater SERS intensity. On the other hand, the degree of the aggregation

of the colloidal substrates also dramatically affects the SERS efficiency. Since the colloidal suspension is unstable and the aggregation of metal nanoparticles is unpredictable, the enhancement obtained lacks good reproducibility. Nevertheless, proper aggregation is in favor of the observation of SERS (Sánchez-Cortés *et al*., 1995). In order to control the random aggregation, researchers have proposed several solutions, including bifunctional linker molecules, stimuli-responsive polymers, short single-stranded DNA chains, and aptamers. Since the Au and Ag nanoparticles are instable under ambient conditions, especially the Ag nanoparticles, core/shell nanostructure is fabricated to overcome the drawbacks. These core/shell nanostructures have an effective tuning ability to adapt to diverse purposes, which result from their tenability of the chemical and physical properties of both the core and shell.

The fabrication of colloidal SERS substrates is relatively simple and inexpensive, and can be readily adopted in standard laboratory settings. Colloidal substrates can be applied to detect pesticides (Zhang *et al*., 2014), antibiotics (He *et al*., 2010), melamine (Zhao *et al*., 2015), food additives (Gao *et al*., 2013), toxins (Huai *et al*., 2013), and illegal food dyes (Gao *et al*., 2015). Liu *et al*. (2012a) proposed a rapid method for fabricating Ag nanoparticles on the surface of vegetables and artificial materials for the in-situ detection of paraquat and fenthion. Mixing the bacteria with the preformed substrates and producing the substrates in the presence of the bacteria could generate different SERS spectrum (Efrima *et al*., 2009). However, since colloids are stabilized by the electrostatic repulsions on the metal nanoparticle surface, the charges of liquid food samples can largely impact the adsorption between the targets and nanoparticles. In addition, the pH of metal colloid is easily influenced by food sample. Therefore, the reproducibility and stability of the colloidal substrates need to be improved.

2.2 Solid surface-based substrates

The real-world application requires strongly enhanced, highly reproducible, and long-term stable SERS substrates. Compared to colloidal substrates, solid surface-based substrates are easier to satisfy

these requirements (Alvarez-Puebla *et al*., 2012). Nanodot arrays are typical 0D nanostructures, the size of the metal nanodots and the layer thickness of the metal material are crucial to the SERS enhancement. Compared with 0D nanostructures, the "hot line" 1D structure exhibits stronger SERS activity with typical 1D nanostructures of nanorod arrays, and both monometal and bimetal nanorod arrays have been currently obtained. In addition, in order to satisfy their increasing applications for different purposes, functionalized nanorod arrays have been introduced (Sun *et al*., 2016). Besides the nanorod arrays, nanoneedle arrays also provide strong SERS activity (Yang *et al*., 2012). Yang *et al*. (2012) indicated that in theory Ag nanoneedle arrays could provide EFs greater than 10^{10} due to the forming of very sharp structures. On the other hand, 2D nanostructures such as parallel Ag nanoplate (Xu *et al*., 2014), cross-linking Ag nanoplate arrays and sandwich arranged Ag nanoplate film exhibit high and homogeneous SERS activities (Bi *et al*., 2013a; Bi *et al*., 2013b; Liu *et al*., 2010). Bi *et al*. (2013b) prepared Au nanoplate films with the Neil Blue A sulfate (NBA) being used as the reporter, and showed that the EFs could reach 5.4×10^{7}. Moreover, despite the difficulty in fabrication, complicated 3D nanostructures have attracted great research interests in providing high density "hot spots" in a 3D space in recent years. For example, Huang *et al*. (2015) fabricated Ag nanoparticles decorated ZnO/Si hetero structured nanomace array, which was employed to detect rhodamine 6G (R6G), achieving an extremely low detection limit (10^{-16} M) and high EFs (8.7×10^{7}). The array has also been used to successfully detect melamine in pure milk, exhibiting its great potential in food analysis.

Metal nanoparticles or nanostructures have excellent performance upon enhancing Raman intensity, but they are relative cost-prohibitive and difficult to fabricate, hence they are still short of increasing demand for sensing applications. Thus, further exploration of novel substrates should break new grounds for SERS applications. For example, graphene-mediated SERS substrates and more complicated metal-graphene systems have been shown to have good SERS performance (Kang *et al*., 2015). In addition, silicon nanohybrid-based SERS substrate has received much attention, compared with Au and Ag

nanoparticles-based SERS substrates, as Au/Ag nanoparticles-decorated silicon nanostructures exhibit higher EFs and reproducibility (Wang *et al*., 2014a). Besides, another alternative is to develop substrates with semiconductor materials as they possess controllable photoelectrical properties with high chemical stability (Ji *et al*., 2016). Thus, integrating metals with semiconductors into hybrid nanostructures also merits investigation.

Although the solid surface-based substrate is relatively hard to be fabricated, there is no issue of aggregation. Therefore solid surface-based substrates possess better stability, reproducibility, and sensitivity. Hu *et al*. (2015) applied the Ag dendrites as the SERS substrates to determine melamine in milk and obtained a limit of detection at 0.012 mmol L^{-1}, while Sivashanmugan *et al*. (2013) fabricated a well-ordered Au-nanorod array to detect melamine in milk solution, with the limit of detection being 10^{-12} M. Furthermore, the colloidal substrates are mainly prepared in aqueous solutions, therefore solid surface-based substrates are more favorable for the detection of water-insoluble substances.

3. Fabrication techniques of SERS-active substrates

The top-down approaches and the bottom-up approaches are two main strategies for the fabrication of SERS-active substrates, and their combination is also a common case to produce SERS-active substrates. The top-down method refers to the controlled reduction from larger dimensional materials to ideal nanoscale structures (Gates *et al*., 2005); on the contrary, in the bottom-up method, complex nanoscale structures are constructed from reacting, growing or self-assembly of molecular or atomic (Ariga *et al*., 2011). Top-down fabrication involves a variety of lithographic techniques including E-beam lithography, nanosphere lithography, optical lithography and nanoimprint lithography and some chemical-based processes, while bottom-up fabrication is closely related to the deposition techniques, nucleation and growth of nanocrystalline, and molecular self-assembly processes. A comparison of the material parameters for various nanofabrication techniques are summarized in Table 2.

3.1 Top-down techniques

Top-down approaches, which prepare SERS substrates through direct fabrication process, can readily reproduce the desired array. Thus top-down approaches play a special role in SERS substrates development. Electron beam (e-beam) lithography (EBL) is a commonly used technique to generate periodic SERS nanostructures (Chirumamilla *et al*., 2014), a number of studies have confirmed the EBL to be a powerful tool in fabricating nanostructures of diverse morphologies. Figure 1a shows the 3D nanostar dimers with a sub-10-nm gap, which were fabricated by EBL and reactive-ion *etc*hing (Chirumamilla *et al*., 2014) and Figure 1b illustrates a more complex nanostructure called the 3D-nanostar-dimer-in-ring, which was also prepared by EBL and possessed good homogeneity and fine controllable hot-spot locations. This complex structure presented EFs of 4×10^{10}, and exhibited a high sensibility for rhodamine and adenine (Gopalakrishnan *et al*., 2014). However, time-consuming and high cost of the EBL procedure have hindered the wide spread uses of EBL. Similar to EBL, focused ion beam (FIB) lithography possesses the ability for controlling the size and shape of the nanostructure. However, FIB is also a rather time-consuming process. On the other hand, nanoimprint lithography (NIL) is another important top-down method to prepare SERS substrates. As NIL can fabricate nanostructure with high scalability in size and throughput simultaneously, NIL exhibits a better way in generating patterns than EBL and FIB (Yao *et al*. 2010). Therefore, NIL has been applied to the fabrication of some interesting SERS substrates, such as doubly-periodic gratings and nanocrescents.

In order to avoid the drawbacks of top-down lithographic methods, some low-cost and simple top-down-like patterning techniques have been developed. The colloidal lithography can offer fine patterns with much lower cost, and nanosphere lithography (NSL) is probably one of the most popular colloidal lithography methods. NSL is inherently parallel, low-cost and high-throughput and is a desirable method for periodic nanofabrication (Haynes *et al*., 2001). Wang *et al*. (2016a) combined NSL and anisotropic

wet *etc*hing to fabricate pyramid, ridged-hexagon, and quasi-triangle nanostructures (Figure 2) and showed that these orientation-dependent nanostructures generated high EFs of 10^6--10^7 in their high-density tips and/or gaps.

3.2 Bottom-up techniques

The range of bottom-up approaches is relatively wide, relating to sorts of materials and techniques. They are superior to most top-down synthesis methods in many aspects, bottom-up approaches are simpler, lower-cost, shorter preparation times, and high throughput. Chemical reduction is a commonly used approach to synthesize noble metal nanoparticles. Due to its simple and low-cost nature, chemical reduction has gained popularity in food safety analysis (Zheng *et al*., 2014). For example, the use of chemical reduction has been demonstrated in the detection of tricyclazole residue in paddy rice (Tang *et al*., 2012), phosmet and thiabendazole residues in apples (Luo *et al*., 2016), melamine in milk powder (Lou *et al*., 2011), *etc*. However, overcoming poor stability and reproducibility of the resultant nanoparticles still remains a challenge. Compared with the typical citrate reduction method, polyol process can fabricate nanoparticles with various shapes, and polyol process has thus been extensively employed to fabricate silver nanowires (Zhang *et al*. 2014). Another popular chemical synthesis method is Seed-mediated growth. Various morphologies of nanostructures can be obtained by the seed-mediated growth method, including gold nanorod, nanocages, and silver nanoplates. Successful size control of the seed-mediated growth method has also been reported. Wang *et al*. (2016b) prepared Au nanorings with controllable outer diameter and wall thickness and showed that the nanorings presented excellent performance in detecting low level rhodamine 6G. More recently, concave gold nanobars were synthesized, showing much higher SERS performance for 4-aminothiophenol than rectangular nanobars and truncated gold nanobars (Zhang *et al*., 2013). Self-assembly is one of the most extensively used bottom-up methods. The assembling is generally driven by chemical attachment (Song *et al*., 2012) or physical forces such as electrostatic interaction (Lee *et al*., 2011a) and capillary force (Martin *et al*.,

2010). The advantages of this method lie in several points: it is able to construct complex structures with low cost, it is easy to manipulate, and it has fine adoption on different surfaces (Cecchini *et al*., 2013). The self-assembly method has been widely used in obtaining nanochain. Lee *et al*. (2011b) developed a controlled assembly of polystyrene-terminated cetyltrimethylammonium bromide-coated nanorods (Figure 3) by adding dimethylformamide/water mixture and determined the relationship between SERS intensity and the length of nanorods chains. Lee *et al*. (2011a) used the method to fabricate arrays of gold nanospheres based on the electrostatic interactions and achieved the substrates with time-stability, tenability, and fine reproducibility. Furthermore, the self-assembly method has the advantages of fabricating complicated nanostructures, particularly some 3D nanostructures, and is one of the easiest ways to create SERS substrates. Though the bottom-up approaches have many advantages, it suffers from low reproducibility which is a large challenge for practical application.

3.3 Other techniques

Besides the aforementioned methods for fabricating SERS substrates, there have been other methods, such as combination of top-down and bottom-up approaches and template-assisted approaches.

3.3.1 Combined techniques

The combination of top-down and bottom-up approaches integrates the advantages of both approaches, thus should be a more flexible technique. Yan *et al*. (2011) combined the EBL and self-assembly techniques to fabricate nanoparticle cluster arrays. In their study, EBL was used to define binding sites, which functioned with positively charged polylysine, and the negatively charged nanoparticles could then be efficiently targeted at the binding sites. Recently, Nguyen *et al*. (2015) combined NSL, controlled radical polymerization, and colloidal assembly to create a smart hybrid platform of GNT@PNIPAM@GNR. In this process, the gold nanotriangles obtained through NSL were grafted with poly (N-isopropylacrylamide), and the gold nanorods synthesized by seed-mediated growth

were then assembled on the poly (N-isopropylacrylamide). The resultant nanostructures exhibited thermoresponsive properties and excellent SERS performance for Nile Blue A. On the other hand, Wang *et al*. (2015b) combined photosensitive sol-gel and electrochemical reaction to make flower-like Au arrays substrates for the detection of trace amount of Rhodamine 6G with detectable concentration reaching 10^{-10} M. Overall, the combination of top-down and bottom-up approaches offers a versatile way to prepare various substrates with good reproducibility and uniformity, and therefore it has a wide range of applications in food safety analysis (Galarreta *et al*., 2013; Sivashanmugan *et al*., 2013).

3.3.2 Template-assisted techniques

Template-assisted method provides another way to create SERS structures with well-defined size and shape. It has been extensively used to fabricate SERS-active substrates for its relatively simple manipulation and its high efficiency on a large scale (Jahn *et al*., 2016). There are various templates available, among them, anodized aluminum oxide (AAO) is one of the most widely used templates, which has advantages of high-efficiency, controllable in configuration shapes and readiness for preparation, thus has been used to produce various nanostructure arrays. Habouti *et al*. (2011) used the AAO template to prepare different morphology nanorods with corrugated and smooth topologies in order to demonstrate the crucial role of nanorod's morphology in SERS performance and showed that the EFs of the corrugated arrays were almost three orders of magnitude stronger than that of the smooth arrays. Most recently, Sui *et al*. (2016) developed a novel SERS substrate (Au-$CuCl_2$-AAO) using AAO template (Figure 4) and showed that the developed substrate displayed a tenfold improved SERS activity in detecting Rhodamine 6G as compared the conventional Au-AAO substrate. In addition, the Au-$CuCl_2$-AAO substrate also possessed high homogeneity and excellent reproducibility. Besides the above, there are many other templates available for SERS-active substrate fabrication. To date, increasing studies have been observed on producing SERS-active substrates based on the template-assisted techniques, in particular for the

fabrication of 2D and 3D nanostructures (Jahn *et al*., 2016). However, some existing templates have an issue of stability, and further study on developing high quality and robust template is needed.

4 Applications of SERS substrates in food safety analysis

Since Raman bands are relatively narrow and each entity has its unique Raman peaks (Xie *et al*., 2011), SERS can be used to detect a specific molecule in complex food matrices. Therefore, SERS presents excellent potential in detecting food chemical contaminants, foodborne pathogens, and other analytes. Figure 5 summarizes the SERS technology in food safety analysis, and the common existing SERS-active substrates are listed in Table 3. In addition, Table 4 shows some typical Tandem SERS application examples.

4.1 Applications of SERS-active substrates in small molecules

SERS has been increasingly used in quantitative detection as a modern technique in small molecules analysis correlating to food safety analysis.

4.1.1 Pesticides

Pesticides play a crucial role in modern agriculture and the majority of pesticides are toxic to human health at different extents, therefore, the use of pesticides is strictly controlled by governments (Gilden *et al*., 2010). However, overusing or misusing of pesticides occurs and attention should be paid to pesticide residues. As a rapid, sensitive, and accurate analytical tool, SERS is an attractive technique to detect trace amount pesticides. Most of the present studies have focused on the adsorption reaction between targets and SERS substrates, including the adsorption orientations and sites. The structures and concentration of analytes, the pH value, as well as many other parameters in the microenvironment can make the difference to the SERS performance (Sun *et al*., 2011). Li *et al*. (2014b) applied the polystyrene/Ag nanoparticles as the SERS substrate to conduct a dynamic-SERS for the detection of two kinds of

organophosphorus pesticides (paraoxon and sumithion), and demonstrated that analytes that possessed high affinity to the surface of the prepared substrates could generate stronger SERS signals. Müller *et al*. (2014) characterized the thiabendazole (TBZ) in a wide range of pH and concentration, and demonstrated different adsorption behaviors when TBZ was dissolved in ethanol and aqueous solution. It should be noticed that in the detection of food samples, the spectra of non-target compounds can negatively interfere with the Raman signals of the target analytes, therefore some pretreatment on samples such as sample extraction and purification is essential. With simple sample preparation, Fan *et al*. (2015) used gold-coated Klarite^{TM} substrates (Renishaw Diagnostics Ltd., Glasgow, Scotland) to detect carbaryl in apples. Although the apple extract displayed lower SERS intensity than standard solution, it was still sensitive enough to meet the maximum residue level set by FAO/WHO. On the other hand, He *et al*. (2014) used a swab-SERS method to detect pesticides in real-food matrices, in which thiabendazole was swabbed from the apple surface by the swab stick and the obtained analytes were added to the solution of Ag dendrites. They finally achieved a fairly quantitative detection of thiabendazole on apple surfaces, indicating good sensitivity and accuracy for the pesticide detection. Furthermore, a "surface spray" method was introduced to detect paraquat on the peels of pears and apples and the detection limit with SERS using Ag nanoparticles as substrate could reach 10^{-9} M (Fang *et al*., 2015).

Generally, both the colloidal substrates and the solid surface-based substrates can be applied to the detection of pesticides, but the colloidal substrate seems to be more popular for its easy fabrication and suitability for various food matrixes. Nevertheless, with the development of solid surface-based substrates and the sample pretreatment, it is anticipated that more solid surface-based substrates will be used in the near future.

4.1.2 Antibiotics and other veterinary drug residues

Illegally using of antibiotics has caused concern for their negative effects on public health. He *et al*. (2010) demonstrated that Ag dendrites substrates synthesized through the displacement reaction could be used to detect antibiotics in solution including enrofloxacin, ciprofloxacin, and chloramphenicol. Zhang *et al*. (2012) also confirmed that commercial solid substrates could be used to detect furazolidone and malachite green in tilapia fillets but the enrofloxacin showed no obvious signal even in the standard solution. These studies suggested that it is not possible to apply one substrate to all analytes due to the fact that the reaction between the molecules and the substrate varies from one analyte to another. However it could be possible to find the regular pattern between the molecules of different structure, weight or other parameters and substrates.

Veterinary drugs such as crystal violet, methylene blue, and malachite green are often illegally used in aquaculture. Resulting from the high polarize ability of their conjugated, these dye molecules have relatively strong Raman SERS intensity, and they can be effectively detected. For example, the malachite green in tilapia was detected at 200 ng/g (Zhang *et al*., 2012), the minimum detectable concentration of crystal violet in tilapia was found to be 1 ng/g (Li *et al*., 2014b), and the limit of detection of methylene blue in tilapia could reach 10 ng/g (Li *et al*., 2016). Due to the strong Raman intensity, dye molecules are often used as the Raman label molecules.

4.1.3 Food additives

Food additives are essential items in the production of food products, however their incorrect, excessive or illegal uses can cause serious food quality and safety issues. SERS can be used to effectively detect food additives such as aspartame (Peica, 2009) and food colorants (Xie *et al*., 2012), and most of the current studies are focused on the adsorption mechanisms on the metal surface and on improving the detection limit of additives. In SERS detection, the performance is closely related to the interaction between the substrates and certain group of analyte molecules. Gao *et al*. (2013) investigated the

interaction of aromatic carboxylic acids by SERS substrate Au nanoparticles and revealed that even similar locations could result in different spectral features in the related intensities. With gold-coated Klarite^{TM} SERS-active substrate, Pan *et al*. (2014) could successfully detect tert-butylhydroquinone (TBHQ), however, no Raman signal was found for butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). On the other hand, the detection of BHA was achieved by using gold nanoparticles with the detection limit of 10^{-5}g/mL (Yao *et al*., 2011). Therefore it is essential to choose suitable SERS substrates according to different analytes. In addition, SERS substrates commercially available are very limited, further development of commercial substrates should significantly benefit analytical chemists and food scientists.

4.1.4 Other small molecules

Toxins such as mycotoxins and biotoxins can cause severe health hazards to human and animals, which have also been studied by SERS approach. To date, SERS detection has been applied to fumonisin (Lee *et al*., 2016), saxitoxin (Huai *et al*., 2013) and aflatoxin (Lee *et al*., 2014). Both Huai *et al*. (2013) and Pearman *et al*. (2008) have detected saxitoxin through SERS, but they found different SERS peaks of saxitoxin. Different physical and chemical factors in the analysis systems were responsible for the differences, including the electronic condition of solvents and the interactions between the targets and different sized Ag nanoparticles. Galarreta *et al*. (2013) exploited using aptamer (single stranded oligonucleotide) coupled with SERS to detect ochratoxin A, in which the SERS platform fabricated by EBL was integrated into a microfluidic channel. The results showed that efficiently binding to a specific target molecule could make the strong selectivity of the aptamer, which was in favor of the SERS detection, indicating that the aptamer-based SERS system was promising in food safety analysis.

Among the many small molecules detectable with SERS, illegal food dyes such as Sudan I, Sudan III and Rhodamine B stand out for their ultra-sensitivity and distinctive SERS patterns. Notably, the

substrates used in the dyes detection can be not only noble metal nanostructures, but also aluminum (Al) foils (López *et al*., 2013). Jahn *et al*. (2015) confirmed the detection of water-insoluble molecules (Sudan III) in silver colloidal under the assistance of lipophilic sensor layer (LSL), which gave an example to the detection of water-insoluble molecules with aqueous colloidal substrates.

SERS has been extensively applied in melamine detection, the general detection limit of melamine can reach 10^{-7}M (Zheng *et al*., 2014). Zhao *et al*. (2015) and Li *et al*. (2015a) developed highly SERS-active starch-coated Ag nanoparticles and chitosan-modified popcorn-like Au-Ag nanoparticles for melamine detection, and achieved a LOD of 0.600 μg/L and 8.51 nM, respectively. When the SERS technique is integrated with other techniques, such as immunological separation (Li *et al*., 2015b) and molecularly imprinted polymers (Hu *et al*., 2015), the detection limit can be further improved to reach approximately10^{-9}M. Compared to other analytes, the detection of melamine conducted by SERS is relatively mature, and efforts should be made on developing portable and in-situ melamine detection devices in near future.

Currently SERS applications mainly focus on the detection and characterization of hazardous substances in food matrixes. However, information on using SERS for analyzing food components and/or changes in food components related to food quality is limited. Since SERS enables the screening at micro level, studies on micro-level properties of food should provide better understanding on food quality analysis and control.

4.2 Applications of SERS substrates in big molecules and bacteria

Besides the successful applications in small molecules, SERS has also been applied to the detection of big molecules and bacteria, such as proteins and foodborne pathogens.

4.2.1 Bacteria

Foodborne pathogens pose great risks to human and thus identification of pathogenic bacteria is critical for food safety assurance. The use of SERS for detecting foodborne pathogens in many aspects is superior to other methods, including simplicity, sensitivity, selectivity, and stability. Contrary to the small molecules, detecting pathogens with SERS presents relatively low sensitivity. In order to improve the sensitivity in foodborne pathogen detection, SERS can be combined with membrane filter (Cho *et al*., 2015) and magnetic separation methods (Najafi *et al*., 2014). It can also be combined with other methods to achieve better selectivity. For example, Duan *et al*. (2016) coupled SERS with aptamers for sensitive and rapid detection of *Vibrio parahaemolyticus*. The thiolated aptamer immobilized on the SiO_2@Au core-shell nanoparticles was used to selectively capture the *Vibrio parahaemolyticus*, resulting in a limit of detection of 10 cfu/mL. In addition, antibody is another labeled recognition element widely used in the pathogen detection (Najafi *et al*., 2014). SERS based detection of pathogens can also be label-free, which is dependent on the spectrum of the pathogens themselves (Kowalska *et al*., 2015). Label-free method is simple, but has issues with high detection limit. However, in-situ preparation of Ag nanoparticles and the addition of Triton X-100 were used to improve the label-free detection of *Staphylococcus aureus* (MRSA) and *Listeria spp* (Chen *et al*., 2015).

It should be noted that coexisting of several pathogens in food matrixes is very common. Therefore, simultaneous detection of different pathogens is useful. Zhang *et al*. (2015) carried out simultaneous detection of *Salmonella typhimurium* and *Staphylococcus aureus* on Au colloidal substrate and obtained a high level of sensitivity with a limit detection of 35 cfu/mL and 15 cfu/mL for *S. aureus* and *S. typhimurium*, respectively.

4.2.2 Proteins

Proteins are big molecules, which have weak Raman scattering as mentioned before. Therefore, SERS can be combined with other techniques to improve the weak signal intensity. He *et al*. (2011a)

introduced immunomagnetic separation (IMS) coupled Ag dendrites based SERS method to detect ovalbumin in milk sample, where the ovalbumin was captured by IMS without purification of the sample. Similarly, the combined method was used to detect ricin in milk with the limit of detection of ricin being 4 μg/mL (He *et al*., 2011b). Although IMS is efficient in separating target antigen from complex food matrices, the antibodies have issues with stability and synthesis (Duan *et al*., 2016). Another common agent exploited in molecule-specific capture is aptamer. Compared to antibodies, aptamers are more stable and accessible (Song *et al*., 2008). He *et al*. (2011c) applied aptamer-conjugated Ag dendrites to detect ricin in milk and orange juice and showed that the Ag aptamer assay presented a lower detection limit compared with the Ag antibody assay. However, it should be aware that aptamer with a relatively large size may sometimes hinder the target protein absorbing to the SERS substrate, which decreases the SERS intensity.

To date, SERS has been applied not only for the detection of the presence of certain protein in food matrices, but also for the determination of protein conformation changes. Wang *et al*. (2013) analyzed the structural changes of whey protein under glycosylation reaction as affected by different pH and heat treatments. In their work, different band intensities of whey protein isolate and partially glycosylated whey protein were observed on the Ag dendrites SERS substrate. In addition, Wang *et al*. (2013) also established the intrinsic correlation between the conformation and function of partially glycosylated whey protein. Due to the biological properties of proteins, SERS analysis on proteins should have more choices such as IMS-SERS than other techniques. However, protein denaturation may have negative effects on the SERS detection.

5 Conclusions and outlook

SERS-active substrate is the most important part of SERS technique, and its creating of hot spots plays a key role in element detection. The development of colloidal substrates depends largely on the

advanced synthetic colloidal chemistry. On the other hand, extensive efforts have been devoted to the fabrication of solid surface-based substrates, which have the advantages of stability and reproducibility.

Due to the different adsorption mechanisms, it is essential to fabricate proper substrates for food samples. The bottom-up and top-down approaches are the two main approaches to fabricate SERS-active substrates. The top-down approach has been proved to be effective in fabricating nanostructure with controllable geometries, excellent reproducibility and uniformity, whereas the bottom-up approach can produce ordered structures without the need for expensive facilities. In addition, synergistically integrating these two methods has shown promise in obtaining desired SERS-active substrates with low-cost.

With the advances in nanotechnology, researchers have found effective solutions to fabrication complexity of SERS-active substrates for trace detection and determination of food samples, thus SERS-active substrates are expected to gain more attention in combining with separation methods such as antibody, aptamer, and thin layer chromatography. As a powerful tool, SERS enables rapid, simple, sensitive detection of both small molecules and big molecules in complex food matrices. A wide range of food chemical contaminants, proteins, foodborne pathogens, *etc*. can thus be detected in trace amount by SERS. However, few studies on detecting food components have been reported, and efforts should be made to explore the further applications of SERS in this area.

Future researches on SERS-active substrates and application of SERS for food analysis may focus on the following aspects:

- Integrating metals with semiconductor materials as the novel SERS-active substrates can be used to achieve the desired enhancement characteristics;

- According to the high cost of nanostructure substrates, it is necessary to fabricate cost-effective, high reproducible, recyclable, and readily scalable substrates;
- As food matrices are complex, SERS combining with selection techniques of the targeted molecules has large potential in practical applications;
- Complex 2D or 3D nanostructures have more hot spots, however they are rarely applied in food safety analysis, more studies can be conducted in this area;
- Since SERS enable the screening at micro level, studies on micro-level properties of food should provide intrinsic information for possible improvement on food processing and preserving technologies.

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References

Alvarez-Puebla, R. A., & Liz-Marzan, L. M. (2012). Traps and cages for universal SERS detection. *Chemical Society Reviews, 41*(1), 43-51.

Ariga, K., Li, M., Richards, G. J., & Hill, J. P. (2011). Nanoarchitectonics: A Conceptual Paradigm for Design and Synthesis of Dimension-Controlled Functional Nanomaterials. *Journal of Nanoscience and Nanotechnology, 11*(1), 1-13.

Aswathy, B., Sony, G., & Gopchandran, K. G. (2014). Shell Thickness-Dependent Plasmon Coupling and Creation of SERS Hot Spots in Au@Ag Core-Shell Nanostructures. *Plasmonics, 9*(6), 1323-1331.

Bi, L., Dong, J., Xie, W., Lu, W., Tong, W., Tao, L., & Qian, W. (2013a). Bimetallic gold--silver nanoplate array as a highly active SERS substrate for detection of streptavidin/biotin assemblies. *Anal Chim Acta, 805*, 95-100.

Bi, L., Rao, Y., Tao, Q., Dong, J., Su, T., Liu, F., & Qian, W. (2013b). Fabrication of large-scale gold nanoplate films as highly active SERS substrates for label-free DNA detection. *Biosensors and Bioelectronics, 43*, 193-199.

Campion, A., & Kambhampati, P. (1998). Surface-enhanced Raman scattering. *Chem. Soc. Rev., 27*(4), 241-250.

Cao, Y. Q., Zhang, J. W., Yang, Y., Huang, Z. R., Long, N. V., & Fu, C. L. (2015). Engineering of SERS Substrates Based on Noble Metal Nanomaterials for Chemical and Biomedical Applications. *Applied Spectroscopy Reviews, 50*(6), 499-525.

Cecchini, M. P., Turek, V. A., Paget, J., Kornyshev, A. A., & Edel, J. B. (2013). Self-assembled nanoparticle arrays for multiphase trace analyte detection. *Nature materials, 12*(2), 165-171.

Chen, L., Mungroo, N., Daikuara, L., & Neethirajan, S. (2015). Label-free NIR-SERS discrimination and detection of foodborne bacteria by in situ synthesis of Ag colloids. *Journal of Nanobiotechnology, 13*.

Chirumamilla, M., Toma, A., Gopalakrishnan, A., Das, G., Zaccaria, R. P., Krahne, R., Rondanina, E., Leoncini, M., Liberale, C., De Angelis, F., & Di Fabrizio, E. (2014). 3D Nanostar Dimers with a Sub-10-nm Gap for Single-/Few- Molecule Surface-Enhanced Raman Scattering. *Advanced Materials*, *26*(15), 2353-2358.

Cho, I.-H., Bhandari, P., Patel, P., & Irudayaraj, J. (2015). Membrane filter-assisted surface enhanced Raman spectroscopy for the rapid detection of E. coli O157: H7 in ground beef. *Biosensors and Bioelectronics, 64*, 171-176.

Doering, W. E., Piotti, M. E., Natan, M. J., & Freeman, R. G. (2007). SERS as a foundation for nanoscale, optically detected biological labels. *Advanced Materials, 19*(20), 3100-3108.

Duan, N., Yan, Y., Wu, S., & Wang, Z. (2016). Vibrio parahaemolyticus detection aptasensor using surface-enhanced Raman scattering. *Food Control, 63*, 122-127.

Efrima, S., & Zeiri, L. (2009). Understanding SERS of bacteria. *Journal of Raman Spectroscopy, 40*(3), 277-288.

Fan, Y., Lai, K., Rasco, B. A., & Huang, Y. (2015). Determination of carbaryl pesticide in Fuji apples using surface-enhanced Raman spectroscopy coupled with multivariate analysis. *LWT-Food Science and Technology, 60*(1), 352-357.

Fang, H., Zhang, X., Zhang, S. J., Liu, L., Zhao, Y. M., & Xu, H. J. (2015). Ultrasensitive and quantitative detection of paraquat on fruits skins via surface-enhanced Raman spectroscopy. *Sensors and Actuators B-Chemical, 213*, 452-456.

Feng, S., Gao, F., Chen, Z., Grant, E., Kitts, D. D., Wang, S., & Lu, X. (2013). Determination of α-Tocopherol in Vegetable Oils Using a Molecularly Imprinted Polymers--Surface-Enhanced Raman Spectroscopic Biosensor. *J Agric Food Chem, 61*(44), 10467-10475.

Galarreta, B. C., Tabatabaei, M., Guieu, V., Peyrin, E., & Lagugné-Labarthet, F. (2013). Microfluidic channel with embedded SERS 2D platform for the aptamer detection of ochratoxin A. *Analytical and bioanalytical chemistry, 405*(5), 1613-1621.

Gates, B. D., Xu, Q., Stewart, M., Ryan, D., Willson, C. G., & Whitesides, G. M. (2005). New approaches to nanofabrication: molding, printing, and other techniques. *Chemical Reviews, 105*(4), 1171-1196.

Gao, F., Feng, S. L., Chen, Z. W., Li-Chan, E. C. Y., Grant, E., & Lu, X. N. (2014). Detection and Quantification of Chloramphenicol in Milk and Honey Using Molecularly Imprinted Polymers: Canadian Penny-Based SERS Nano-Biosensor. *Journal of food science, 79*(12), N2542-N2549

Gao, J., Hu, Y., Li, S., Zhang, Y., & Chen, X. (2013). Adsorption of benzoic acid, phthalic acid on gold substrates studied by surface-enhanced Raman scattering spectroscopy and density functional theory calculations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 104*, 41-47.

Ghosh Chaudhuri, R., & Paria, S. (2011). Core/shell nanoparticles: classes, properties, synthesis mechanisms, characterization, and applications. *Chemical Reviews, 112*(4), 2373-2433.

Gilden, R. C., Huffling, K., & Sattler, B. (2010). Pesticides and health risks. *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 39*(1), 103-110.

Glotzer, S. C., & Solomon, M. J. (2007). Anisotropy of building blocks and their assembly into complex structures. *Nature materials, 6*(8), 557-562.

Gopalakrishnan, A., Chirumamilla, M., De Angelis, F., Toma, A., Zaccaria, R. P., & Krahne, R. (2014). Bimetallic 3D Nanostar Dimers in Ring Cavities: Recyclable and Robust Surface-Enhanced Raman Scattering Substrates for Signal Detection from Few Molecules. *ACS Nano, 8*(8), 7986-7994.

Guo, S., Dong, S., & Wang, E. (2009). Rectangular Silver Nanorods: Controlled Preparation, Liquid−Liquid Interface Assembly, and Application in Surface-Enhanced Raman Scattering. *Crystal Growth & Design, 9*(1), 372-377.

Habouti, S., Mátéfi-Tempfli, M., Solterbeck, C.-H., Es-Souni, M., Mátéfi-Tempfli, S., & Es-Souni, M. (2011). On-substrate, self-standing Au-nanorod arrays showing morphology controlled properties. *Nano Today, 6*(1), 12-19.

Haynes, C. L., McFarland, A. D., & Duyne, R. P. V. (2005). Surface-enhanced Raman spectroscopy. *Analytical Chemistry, 77*(17), 338 A-346 A.

Haynes, C. L., & Van Duyne, R. P. (2001). Nanosphere Lithography: A Versatile Nanofabrication Tool for Studies of Size-Dependent Nanoparticle Optics. *The Journal of Physical Chemistry B, 105*(24), 5599-5611.

He, L., Chen, T., & Labuza, T. P. (2014). Recovery and quantitative detection of thiabendazole on apples using a surface swab capture method followed by surface-enhanced Raman spectroscopy. *Food Chem, 148*, 42-46.

He, L., Deen, B., Rodda, T., Ronningen, I., Blasius, T., Haynes, C., Diez-Gonzalez, F., & Labuza, T. P. (2011b). Rapid Detection of Ricin in Milk Using Immunomagnetic Separation Combined with Surface-Enhanced Raman Spectroscopy. *Journal of food science, 76*(5), N49-N53.

He, L., Haynes, C. L., Diez-Gonzalez, F., & Labuza, T. P. (2011a). Rapid detection of a foreign protein in milk using IMS--SERS. *Journal of Raman Spectroscopy, 42*(6), 1428-1434.

He, L., Lamont, E., Veeregowda, B., Sreevatsan, S., Haynes, C. L., Diez-Gonzalez, F., & Labuza, T. P. (2011c). Aptamer-based surface-enhanced Raman scattering detection of ricin in liquid foods. *Chemical Science, 2*(8), 1579-1582.

He, L., Lin, M., Li, H., & Kim, N. J. (2010). Surface-enhanced Raman spectroscopy coupled with dendritic silver nanosubstrate for detection of restricted antibiotics. *Journal of Raman Spectroscopy, 41*(7), 739-744.

Hrelescu, C., Sau, T. K., Rogach, A. L., Jackel, F., & Feldmann, J. (2009). Single gold nanostars enhance Raman scattering. *Applied Physics Letters, 94*(15).

Hu, Y., Feng, S., Gao, F., Li-Chan, E. C., Grant, E., & Lu, X. (2015). Detection of melamine in milk using molecularly imprinted polymers--surface enhanced Raman spectroscopy. *Food Chem, 176*, 123-129.

Huai, Q.-y., Gao, C.-l., Miao, J.-l., Yao, H.-l., & Wang, Z.-l. (2013). Fast detection of saxitoxin using laser tweezers surface enhanced Raman spectroscopy. *Analytical Methods, 5*(23), 6870-6873.

Huang, J., Chen, F., Zhang, Q., Zhan, Y., Ma, D., Xu, K., & Zhao, Y. (2015). 3D Silver Nanoparticles Decorated Zinc Oxide/Silicon Heterostructured Nanomace Arrays as High-Performance Surface-Enhanced Raman Scattering Substrates. *Acs Applied Materials & Interfaces, 7*(10), 5725-5735.

Jahn, M., Patze, S., Bocklitz, T., Weber, K., Cialla-May, D., & Popp, J. (2015). Towards SERS based applications in food analytics: Lipophilic sensor layers for the detection of Sudan III in food matrices. *Anal Chim Acta, 860*, 43-50.

Jahn, M., Patze, S., Hidi, I. J., Knipper, R., Radu, A. I., Muehlig, A., Yueksel, S., Peksa, V., Weber, K., Mayerhoefer, T., Cialla-May, D., & Popp, J. (2016). Plasmonic nanostructures for surface enhanced spectroscopic methods. *Analyst, 141*(3), 756-793.

Jeanmaire, D. L., & Van Duyne, R. P. (1977). Surface Raman spectroelectrochemistry: Part I. Heterocyclic, aromatic, and aliphatic amines adsorbed on the anodized silver electrode. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry, 84*(1), 1-20.

Ji, W., Zhao, B., & Ozaki, Y. (2016). Semiconductor materials in analytical applications of surface‐enhanced Raman scattering. *Journal of Raman Spectroscopy, 47*(1), 51-58.

Jung, M., Kim, S. K., Lee, S., Kim, J. H., & Woo, D. H. (2013). Ag nanodot array as a platform for surface-enhanced Raman scattering. *Journal of Nanophotonics, 7*.

Kang, L., Chu, J., Zhao, H., Xu, P., & Sun, M. (2015). Recent progress in the applications of graphene in surface-enhanced Raman scattering and plasmon-induced catalytic reactions. *Journal of Materials Chemistry C, 3*(35), 9024-9037.

Kleinman, S. L., Ringe, E., Valley, N., Wustholz, K. L., Phillips, E., Scheidt, K. A., Schatz, G. C., & Van Duyne, R. P. (2011). Single-Molecule Surface-Enhanced Raman Spectroscopy of Crystal Violet Isotopologues: Theory and Experiment. *Journal of the American Chemical Society, 133*(11), 4115-4122.

Kowalska, A. A., Kaminska, A., Adamkiewicz, W., Witkowska, E., & Tkacz, M. (2015). Novel highly sensitive Cu-based SERS platforms for biosensing applications. *Journal of Raman Spectroscopy, 46*(5), 428-433.

López, M. I., Ruisánchez, I., & Callao, M. P. (2013). Figures of merit of a SERS method for Sudan I determination at traces levels. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 111*, 237-241.

Le Ru, E., Blackie, E., Meyer, M., & *Etc*hegoin, P. G. (2007). Surface enhanced Raman scattering enhancement factors: a comprehensive study. *The Journal of Physical Chemistry C, 111*(37), 13794-13803.

Lee, A., Andrade, G. F., Ahmed, A., Souza, M. L., Coombs, N., Tumarkin, E., Liu, K., Gordon, R., Brolo, A. G., & Kumacheva, E. (2011b). Probing dynamic generation of hot-spots in self-assembled chains of gold nanorods by surface-enhanced Raman scattering. *Journal of the American Chemical Society, 133*(19), 7563-7570.

Lee, K.-M., & Herrman, T. J. (2016). Determination and Prediction of Fumonisin Contamination in Maize by Surface--Enhanced Raman Spectroscopy (SERS). *Food and Bioprocess Technology, 9*(4), 588-603.

Lee, K.-M., Herrman, T. J., Bisrat, Y., & Murray, S. C. (2014). Feasibility of surface-enhanced Raman spectroscopy for rapid detection of aflatoxins in maize. *J Agric Food Chem, 62*(19), 4466-4474.

Lee, W., Lee, S. Y., Briber, R. M., & Rabin, O. (2011a). Self-Assembled SERS Substrates with Tunable Surface Plasmon Resonances. *Advanced Functional Materials, 21*(18), 3424-3429.

Li, C., Huang, Y., Lai, K., Rasco, B. A., & Fan, Y. (2016). Analysis of trace methylene blue in fish muscles using ultra-sensitive surface-enhanced Raman spectroscopy. *Food Control, 65*, 99-105.

Li, C., Huang, Y., Pei, L., Wu, W., Yu, W., Rasco, B. A., & Lai, K. (2014c). Analyses of trace crystal violet and leucocrystal violet with gold nanospheres and commercial gold nanosubstrates for surface-enhanced Raman spectroscopy. *Food Analytical Methods, 7*(10), 2107-2112.

Li, J., Zhang, G., Wang, L., Shen, A., & Hu, J. (2015a). Simultaneous enzymatic and SERS properties of bifunctional chitosan-modified popcorn-like Au-Ag nanoparticles for high sensitive detection of melamine in milk powder. *Talanta, 140*, 204-211.

Li, P., Dong, R., Wu, Y., Liu, H., Kong, L., & Yang, L. (2014b). Polystyrene/Ag nanoparticles as dynamic surface-enhanced Raman spectroscopy substrates for sensitive detection of organophosphorus pesticides. *Talanta, 127*, 269-275.

Li, X., Feng, S., Hu, Y., Sheng, W., Zhang, Y., Yuan, S., Zeng, H., Wang, S., & Lu, X. (2015b). Rapid detection of melamine in milk using immunological separation and surface enhanced Raman spectroscopy. *Journal of food science, 80*(6), C1196-C1201.

Li, Y. S., & Church, J. S. (2014a). Raman spectroscopy in the analysis of food and pharmaceutical nanomaterials. *Journal of food and drug analysis, 22*(1), 29-48.

Liu, G., Cai, W., Kong, L., Duan, G., & Lu, F. (2010). Vertically cross-linking silver nanoplate arrays with controllable density based on seed-assisted electrochemical growth and their structurally enhanced SERS activity. *Journal of Materials Chemistry, 20*(4), 767-772.

Liu, X. J., Zong, C. H., Ai, K. L., He, W. H., & Lu, L. H. (2012). Engineering Natural Materials as Surface-Enhanced Raman Spectroscopy Substrates for In situ Molecular Sensing. *Acs Applied Materials & Interfaces, 4*(12), 6599-6608.

Livingstone, R., Zhou, X., Tamargo, M. C., Lombardi, J. R., Quagliano, L. G., & Jean-Mary, F. (2010). Surface Enhanced Raman Spectroscopy of Pyridine on CdSe/ZnBeSe Quantum Dots Grown by Molecular Beam Epitaxy. *The Journal of Physical Chemistry C, 114*(41), 17460-17464.

Luo, H. R., Huang, Y. Q., Lai, K. Q., Rasco, B. A., & Fan, Y. X. (2016). Surface-enhanced Raman spectroscopy coupled with gold nanoparticles for rapid detection of phosmet and thiabendazole residues in apples. *Food Control, 68*, 229-235.

Lou, T. T., Wang, Y. Q., Li, J. H., Peng, H. L., Xiong, H., & Chen, L. X. (2011). Rapid detection of melamine with 4-mercaptopyridine-modified gold nanoparticles by surface-enhanced Raman scattering. *Analytical and Bioanalytical Chemistry, 401*(1), 333-338.

Marqués-González, S., Matsushita, R., & Kiguchi, M. (2015). Surface enhanced Raman scattering of molecules in metallic nanogaps. *Journal of Optics, 17*(11), 114001.

Martin, M. N., Basham, J. I., Chando, P., & Eah, S.-K. (2010). Charged gold nanoparticles in non-polar solvents: 10-min synthesis and 2D self-assembly. *Langmuir, 26*(10), 7410-7417.

McGorrin, R. J. (2009). One hundred years of progress in food analysis. *J Agric Food Chem, 57*(18), 8076-8088.

Müller, C., David, L., Chiş, V., & Pînzaru, S. C. (2014). Detection of thiabendazole applied on citrus fruits and bananas using surface enhanced Raman scattering. *Food Chem, 145*, 814-820.

Mungroo, N. A., Oliveira, G., & Neethirajan, S. (2016). SERS based point-of-care detection of food-borne pathogens. *Microchimica Acta, 183*(2), 697-707.

Najafi, R., Mukherjee, S., Hudson, J., Sharma, A., & Banerjee, P. (2014). Development of a rapid capture-cum-detection method for Escherichia coli O157 from apple juice comprising nano-immunomagnetic separation in tandem with surface enhanced Raman scattering. *International journal of food microbiology, 189*, 89-97.

Nguyen, M., Kanaev, A., Sun, X., Lacaze, E., Lau-Truong, S., Lamouri, A., Aubard, J., Felidj, N., & Mangeney, C. (2015). Tunable Electromagnetic Coupling in Plasmonic Nanostructures Mediated by Thermoresponsive Polymer Brushes. *Langmuir, 31*(46), 12830-12837.

Nie, S., & Emory, S. R. (1997). Probing Single Molecules and Single Nanoparticles by Surface-Enhanced Raman Scattering. *science, 275*(5303), 1102-1106.

Orendorff, C. J., Gole, A., Sau, T. K., & Murphy, C. J. (2005). Surface-Enhanced Raman Spectroscopy of Self-Assembled Monolayers: Sandwich Architecture and Nanoparticle Shape Dependence. *Analytical Chemistry, 77*(10), 3261-3266.

Pan, Y., Lai, K., Fan, Y., Li, C., Pei, L., Rasco, B. A., & Huang, Y. (2014). Determination of Tert-Butylhydroquinone in Vegetable Oils Using Surface-Enhanced Raman Spectroscopy. *Journal of food science, 79*(6), T1225-T1230.

Parisi, J., Dong, Q. C., & Lei, Y. (2015). In situ microfluidic fabrication of SERS nanostructures for highly sensitive fingerprint microfluidic-SERS sensing. *Rsc Advances, 5*(19), 14081-14089.

Pearman, W. F., Angel, S. M., Ferry, J. L., & Hall, S. (2008). Characterization of the ag mediated surface-enhanced Raman spectroscopy of saxitoxin. *Applied Spectroscopy, 62*(7), 727-732.

Peica, N. (2009). Identification and characterisation of the E951 artificial food sweetener by vibrational spectroscopy and theoretical modelling. *Journal of Raman Spectroscopy, 40*(12), 2144-2154.

Pietrobon, B., McEachran, M., & Kitaev, V. (2008). Synthesis of size-controlled faceted pentagonal silver nanorods with tunable plasmonic properties and self-assembly of these nanorods. *ACS Nano, 3*(1), 21-26.

Rycenga, M., Kim, M. H., Camargo, P. H. C., Cobley, C., Li, Z.-Y., & Xia, Y. (2009). Surface-Enhanced Raman Scattering: Comparison of Three Different Molecules on Single-Crystal Nanocubes and Nanospheres of Silver.*Journal of Physical Chemistry A, 113*(16), 3932-3939.

Sánchez-Cortés, S., García-Ramos, J. V., Morcillo, G., & Tinti, A. (1995). Morphological Study of Silver Colloids Employed in Surface-Enhanced Raman Spectroscopy: Activation when Exciting in Visible and Near-Infrared Regions. *Journal of Colloid and Interface Science, 175*(2), 358-368.

Sivashanmugan, K., Liao, J.-D., Liu, B. H., & Yao, C.-K. (2013). Focused-ion-beam-fabricated Au nanorods coupled with Ag nanoparticles used as surface-enhanced Raman scattering-active substrate for analyzing trace melamine constituents in solution. *Anal Chim Acta, 800,* 56-64.

Song, J., Zhou, J., & Duan, H. (2012). Self-assembled plasmonic vesicles of SERS-encoded amphiphilic gold nanoparticles for cancer cell targeting and traceable intracellular drug delivery. *Journal of the American Chemical Society, 134*(32), 13458-13469.

Song, S., Wang, L., Li, J., Fan, C., & Zhao, J. (2008). Aptamer-based biosensors. *TrAC Trends in Analytical Chemistry, 27*(2), 108-117.

Stiles, P. L., Dieringer, J. A., Shah, N. C., & Van Duyne, R. P. (2008). Surface-enhanced Raman spectroscopy. *Annu. Rev. Anal. Chem., 1*, 601-626.

Sui, C., Wang, K., Wang, S., Ren, J., Bai, X., & Bai, J. (2016). SERS activity with tenfold detection limit optimization on a type of nanoporous AAO-based complex multilayer substrate. *Nanoscale, 8*(11), 5920-5927.

Sun, K., Huang, Q., Meng, G., & Lu, Y. (2016). Highly Sensitive and Selective Surface-Enhanced Raman Spectroscopy Label-free Detection of 3,3 ‘,4,4 ’-Tetrachlorobiphenyl Using DNA Aptamer-Modified Ag-Nanorod Arrays. *Acs Applied Materials & Interfaces, 8*(8), 5723-5728.

Sun, M., Huang, Y., Xia, L., Chen, X., & Xu, H. (2011). The pH-Controlled Plasmon-Assisted Surface Photocatalysis Reaction of 4-Aminothiophenol to p,p′-Dimercaptoazobenzene on Au, Ag, and Cu Colloids. *The Journal of Physical Chemistry C, 115*(19), 9629-9636.

Tang, H. R., Fang, D. M., Li, Q. Q., Cao, P., Geng, J. P., Sui, T., Wang, X., Iqbal, J., & Du, Y. P. (2012). Determination of Tricyclazole Content in Paddy Rice by Surface Enhanced Raman Spectroscopy. *Journal of Food Science, 77*(5), T105-T109.

Wang, A. X., & Kong, X. (2015a). Review of Recent Progress of Plasmonic Materials and Nano-Structures for Surface-Enhanced Raman Scattering. *Materials, 8*(6), 3024-3052.

Wang, C., Cheng, F. S., Wang, Y. H., Gong, Z. J., Fan, M. K., & Hu, J. M. (2014b). Single point calibration for semi-quantitative screening based on an internal reference in thin layer chromatography-SERS: the case of Rhodamine B in chili oil. *Analytical Methods, 6*(18), 7218-7223.

Wang, H. Y., Jiang, X. X., Lee, S. T., & He, Y. (2014a). Silicon Nanohybrid-based Surface-enhanced Raman Scattering Sensors. *Small, 10*(22), 4455-4468.

Wang, C. G., Wu, X. Z., Di, D., Dong, P. T., Xiao, R., & Wang, S. Q. (2016a). Orientation-dependent nanostructure arrays based on anisotropic silicon wet-*etc*hing for repeatable surface-enhanced Raman scattering. *Nanoscale, 8*(8), 4672-4680.

Wang, Q., He, L., Labuza, T. P., & Ismail, B. (2013). Structural characterisation of partially glycosylated whey protein as influenced by pH and heat using surface-enhanced Raman spectroscopy. *Food Chem, 139*(1), 313-319.

Wang, W., Yan, Y., Zhou, N., Zhang, H., Li, D., & Yang, D. (2016b). Seed-mediated growth of Au nanorings with size control on Pd ultrathin nanosheets and their tunable surface plasmonic properties. *Nanoscale, 8*(6), 3704-3710.

Wang, X. Y., Wang, N., Gong, T. C., Zhu, Y., & Zhang, J. (2016c). Preparation of graphene-Ag nanoparticles hybrids and their SERS activities. *Applied Surface Science, 387*, 707-719.

Wang, Z., Feng, Z., Lin, L., Huang, P., & Zheng, Z. (2015b). Top-down patterning and self-assembly of flower-like gold arrays for surface enhanced Raman spectroscopy. *Applied Surface Science, 356*, 1314-1319.

Willets, K. A., & Van Duyne, R. P. (2007). Localized surface plasmon resonance spectroscopy and sensing. *Annu. Rev. Phys. Chem., 58*, 267-297.

Xie, Y., Li, Y., Niu, L., Wang, H., Qian, H., & Yao, W. (2012). A novel surface-enhanced Raman scattering sensor to detect prohibited colorants in food by graphene/silver nanocomposite. *Talanta, 100*, 32-37.

Xie, Y., Wang, X., Han, X., Song, W., Ruan, W., Liu, J., Zhao, B., & Ozaki, Y. (2011). Selective SERS detection of each polycyclic aromatic hydrocarbon (PAH) in a mixture of five kinds of PAHs. *Journal of Raman Spectroscopy, 42*(5), 945-950.

Xu, B.-B., Wang, L., Ma, Z.-C., Zhang, R., Chen, Q.-D., Lv, C., Han, B., Xiao, X.-Z., Zhang, X.-L., Zhang, Y.-L., Ueno, K., Misawa, H., & Sun, H.-B. (2014). Surface-Plasmon-Mediated Programmable Optical Nanofabrication of an Oriented Silver Nanoplate. *ACS Nano, 8*(7), 6682-6692.

Yan, B., Boriskina, S. V., & Reinhard, B. M. (2011). Design and Implementation of Noble Metal Nanoparticle Cluster Arrays for Plasmon Enhanced Biosensing. *The Journal of Physical Chemistry C, 115*(50), 24437-24453.

Yang, Y., Li, Z.-Y., Yamaguchi, K., Tanemura, M., Huang, Z., Jiang, D., Chen, Y., Zhou, F., & Nogami, M. (2012). Controlled fabrication of silver nanoneedles array for SERS and their application in rapid detection of narcotics. *Nanoscale, 4*(8), 2663-2669.

Yao, J., Le, A.-P., Gray, S. K., Moore, J. S., Rogers, J. A., & Nuzzo, R. G. (2010). Functional Nanostructured Plasmonic Materials. *Advanced Materials, 22*(10), 1102-1110.

Yao, W., Sun, Y., Xie, Y., Wang, S., Ji, L., Wang, H., & Qian, H. (2011). Development and evaluation of a surface-enhanced Raman scattering (SERS) method for the detection of the antioxidant butylated hydroxyanisole. *European Food Research and Technology, 233*(5), 835-840.

Zhang, H., Ma, X., Liu, Y., Duan, N., Wu, S., Wang, Z., & Xu, B. (2015). Gold nanoparticles enhanced SERS aptasensor for the simultaneous detection of Salmonella typhimurium and Staphylococcus aureus. *Biosensors and Bioelectronics, 74*, 872-877.

Zhang, L.-f., & Zhang, C.-y. (2013). Controlled growth of concave gold nanobars with high surface-enhanced Raman-scattering and excellent catalytic activities. *Nanoscale, 5*(13), 5794-5800.

Zhang, Y., Huang, Y., Zhai, F., Du, R., Liu, Y., & Lai, K. (2012). Analyses of enrofloxacin, furazolidone and malachite green in fish products with surface-enhanced Raman spectroscopy. *Food Chem, 135*(2), 845-850.

Zhang, Y., Wang, Z., Wu, L., Pei, Y., Chen, P., & Cui, Y. (2014). Rapid simultaneous detection of multi-pesticide residues on apple using SERS technique. *Analyst, 139*(20), 5148-5154.

Zhao, Y., Tian, Y., Ma, P., Yu, A., Zhang, H., & Chen, Y. (2015). Determination of melamine and malachite green by surface-enhanced Raman scattering spectroscopy using starch-coated silver nanoparticles as substrates. *Analytical Methods, 7*(19), 8116-8122.

Zheng, J., Fang, X., Cao, Y., Xiao, H., & He, L. (2013). Monitoring the chemical production of citrus-derived bioactive 5-demethylnobiletin using surface-enhanced Raman spectroscopy. *J Agric Food Chem, 61*(34), 8079-8083.

Zheng, J. K., & He, L. L. (2014). Surface-Enhanced Raman Spectroscopy for the Chemical Analysis of Food. *Comprehensive Reviews in Food Science and Food Safety, 13*(3), 317-328.

Table 1. Characteristics of SERS hot spot substrates discussed in the text

Substrates	EF range	Sensitivity	Analyte specifity	Reproducibility of SERS signal	Cost ($ - $ $)	Reference
Aggregated colloids	10^6-10^{10}	++	No	++	$	Kleinman *et al*. (2011) Nie *et al*. (1997)
Nanocubes	~10^9	+++	No	+++	$	Orendorff *et al*.(2005)
Nanorod	5.06×10^8	++	No	+++	$	Guo *et al*. (2009)
Nanostar	10^7	+++	No	+++	$ $	Hrelescu *et al*. (2009)
Nanowire	1.2×10^7	+++	No	++++	$ $	Zhang *et al*. (2014)
SiO_2 encapsulated cores	10^6-10^8	++++	No	++++	$ $ $	Doering *et al*. (2007)
Binary metal core-shell	1.1×10^5	++	No	+++	$	Aswathy *et al*. (2014)
Nanodot array	-	+++	No	++++	$ $	Jung *et al*. (2013)
2D periodic array	5.4×10^7	++++	No	+++	$ $ $	Bi *et al*. (2013b)
3D nanostructure	8.7×10^7	+++++	No	++++	$ $ $ $	Huang *et al*. (2015)
Graphene-metal hybrid	10^7	+++	No	++	$	Wang *et al*. (2016c)
semiconductor quantum dots	10^4-10^5	++	No	++++	$ $ $ $	Livingstone *et al*. (2010)

Table 2. A comparison of the materials parameters for the nanofabrication techniques.

Nanofabrication approach	Time of procedure	Cost	Difficulty degree	Nanostructural defectivity	Materials range
Electron beam lithography	++++	$ $ $ $	+++++	Very low	Au, Ag
Focused ion beam lithography	+++++	$ $ $ $	++++	Very low	Au, Ag
Nanoimprint lithography	+++	$ $ $	+++	Low	Au, Ag, semiconductor
Nanosphere lithography	+++	$ $ $	+++	Low	Au, Ag, semiconductor
Polyol process	++	$ $	++	High	Au, Ag
seed-mediated growth	++	$ $	+	High	Au, Ag, semiconductor
Self-assembly	+++	$ $	+	Low	Au, Ag, semiconductor
Langmuir--Blodgett	+++	$ $ $	++	Low	Au, Ag,
Template techniques (AAO)	++++	$ $ $	++	Low	Au, Ag, semiconductor, grapheme

table 3. Existing SERS-active substrates for food safety analysis

Substrates	Approach	Nanofabrication technology	Nanofabrication complexity
Ag/Au colloid	Bottom-up fabrication	Citrate reduction	Low
Ag dendrite	Bottom-up fabrication	Displacement reaction	Low
Ag film	Template-assisted method	AAO template method	High
Ag nanorod array	Bottom-up fabrication	Oblique angle deposition	High
Hexagonal lattice of Au nanotriangles	Combined fabrication	Self-assembly and electron beam lithography	High
Fractal-like Au nanostructure	Bottom-up fabrication	Self-assembly	low
Au@Ag nanoparticles	Bottom-up fabrication	Seed-mediated method	Low
Fe_3O_4/Au nanoclusters	Bottom-up fabrication	Seed-mediated method	Low
SiO_2@Au nanoparticles	Bottom-up fabrication	Seed-mediated method	Low

Table 4. Typical application examples of tandem SERS methods for food analysis

SERS methods	Analyte	Sample	Limit of detection	Analytical time	Reference
IMS-SERS	Ricin	Milk	4 μg/mL	Within 20 min	He *et al*. (2011b)
	Ovalbumin	Milk	1μg/mL	Less than 20 min	He *et al*. (2011a)
	Melamine	Milk	0.79 × 10--3 mmol/L	20 min	Li *et al*. (2015b)
Aptamer -SERS	Ricin	Milk and orange juice	100 ng/mL in milk, 50 ng/mL in orange juice	Less than 40 min	He *et al*. (2011c)
	Vibrio parahaemolyticus	Binding buffer	10 cfu/mL		Duan *et al*. (2016)
MIP-SERS	Chloramphenicol	Honey and milk		Within 15 min	Gao *et al*. (2014)
	α-Tocopherol	Vegetable Oils		Less than 15 min	Feng *et al*. (2013)
	Melamine	Whole milk	0.012 mmol /L	Less than 20 min	Hu *et al*. (2015)
Microfluidics-SERS	Carbonfuran and Alachlor	Water	5 ppb		Parisi *et al*. (2015)
Thin layer chromatography(TLC)-SERS	Rhodamine B	Chili oil			Wang *et al*. (2014b)

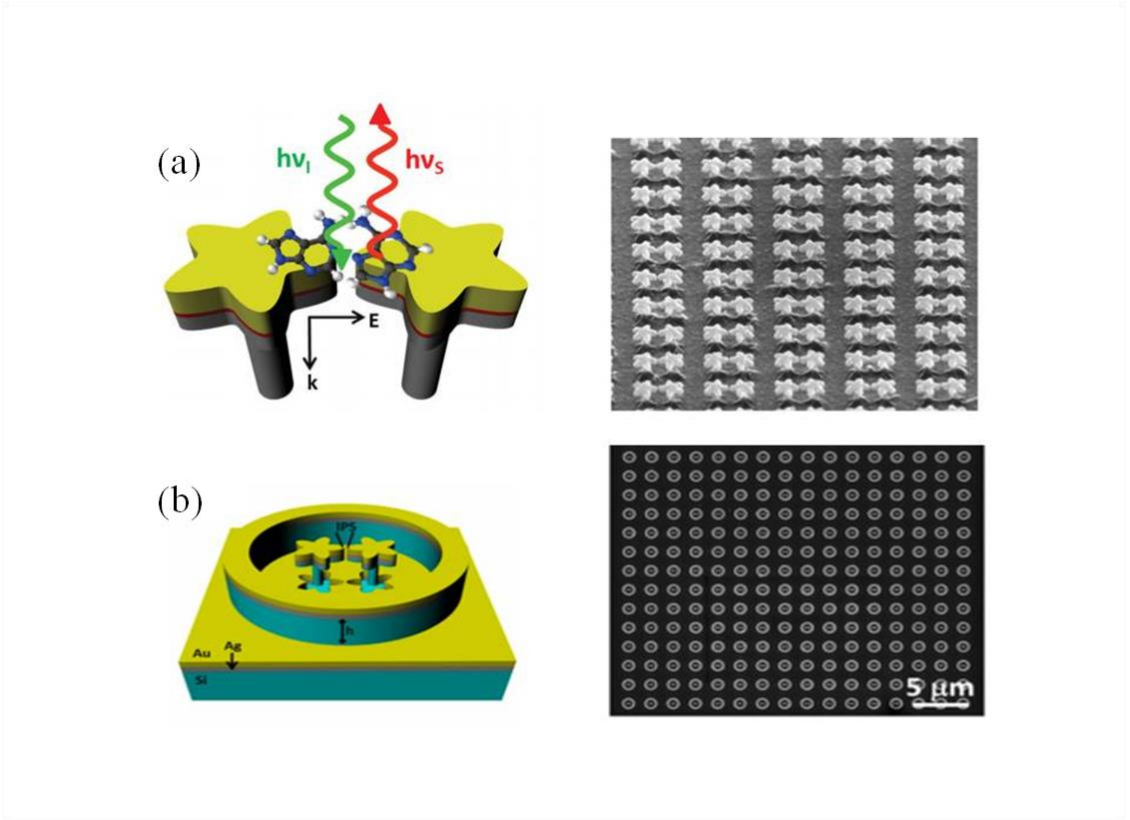

Fig. 1. (a) free-standing 3D nanostar dimer fabricated by EBL and the SEM image of the nanostructure (Chirumamilla *et al*., 2014). (b) 3D nanostar dimer in ring structures obtained through EBL and the SEM image of this nanostructure (Gopalakrishnan *et al*., 2014).

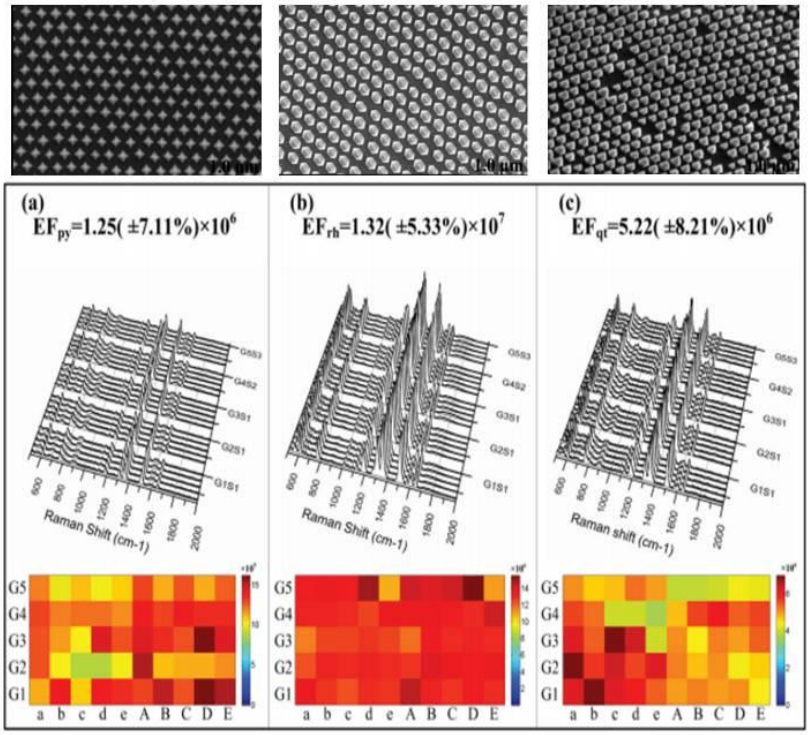

Fig. 2. SEM images of pyramid (a), ridged-hexagon (b), and quasi-triangle (c) nanostructures fabricated by the combination of NSL and anisotropic wet etching techniques and the R6G spectra and EF color maps acquired (Wang *et al*., 2016a).

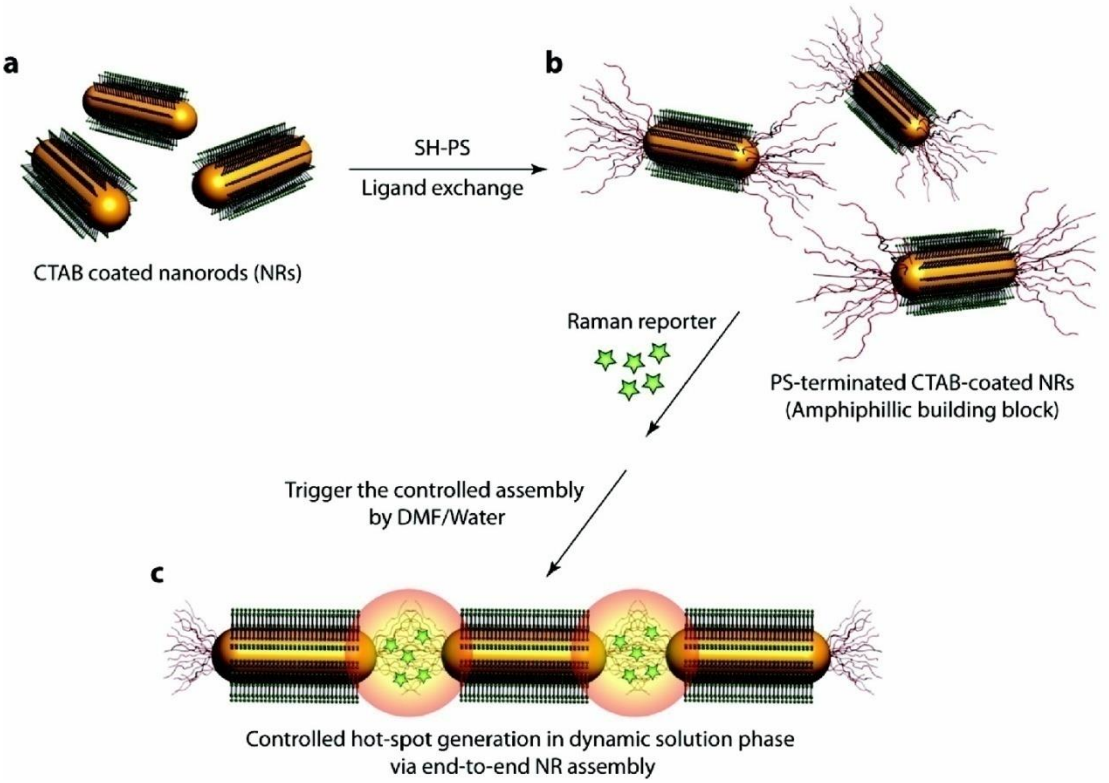

Fig. 3. Gold nanochains prepared by the self-assembly of gold nanorods (Lee *et al*., 2011b).

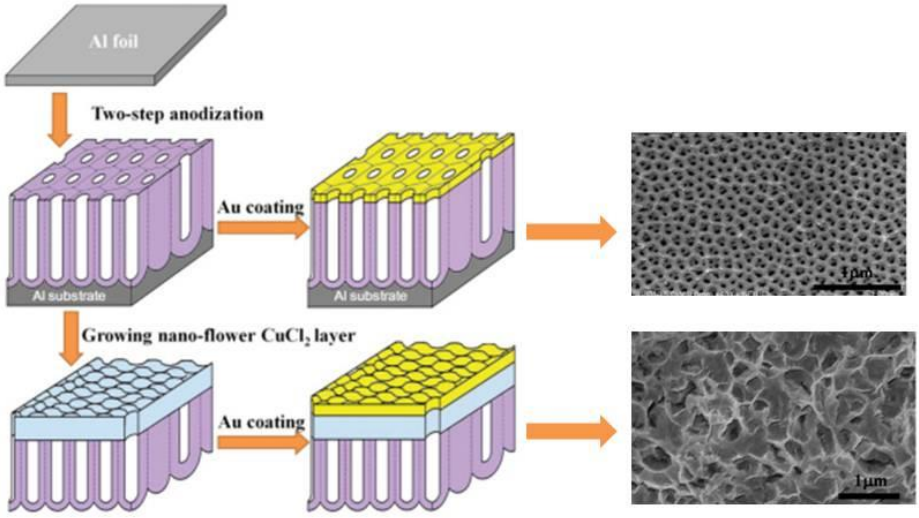

Fig. 4. Preparation of Au-AAO and Au-CuCl2-AAO substrates, and the SEM images of the obtained substrates after 25 nm gold coated (Sui *et al*., 2016)

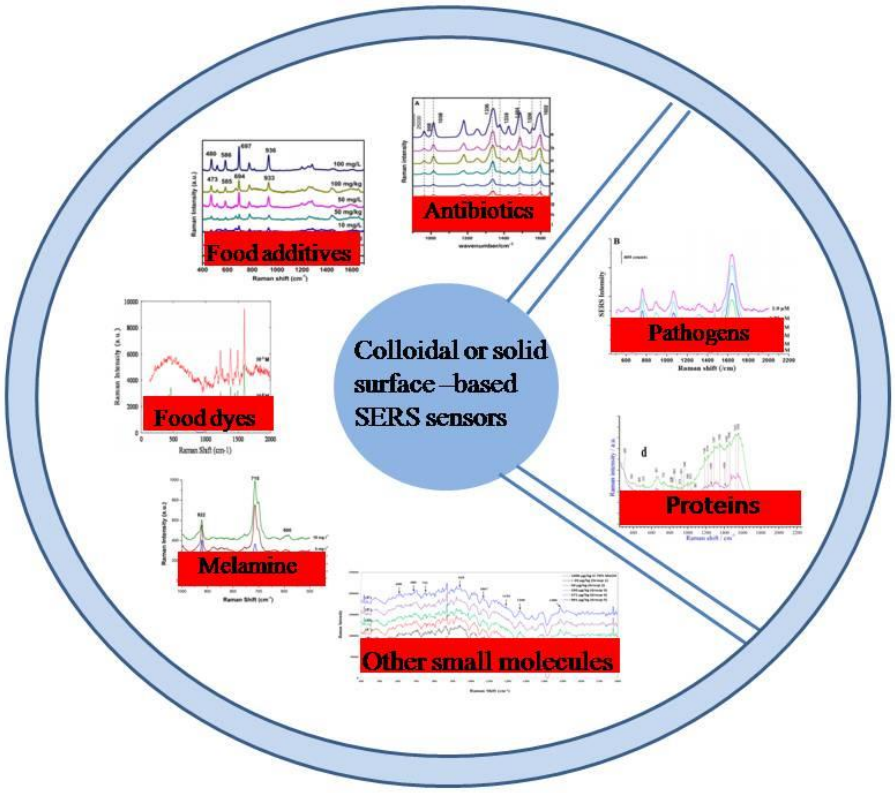

Fig. 5. Summary of SERS used in food analysis