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Catalyzed Deposition of Signal Reporter for Highly Sensitive Surface-Enhanced Raman Spectroscopy Immunoassay Based on Tyramine Signal Amplification Strategy

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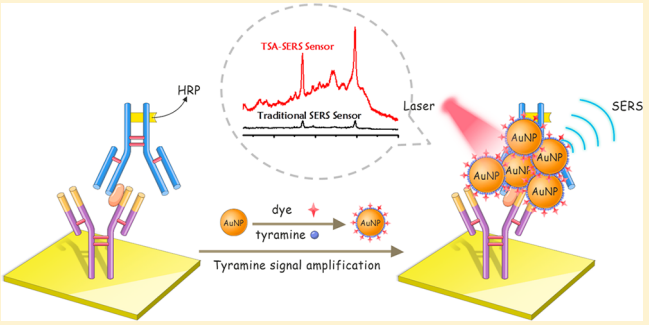
Supporting Information

ABSTRACT: A novel surface-enhanced Raman spectroscopy (SERS) sensor was proposed for an ultrasensitive detection immunoassay based on tyramine signal amplification (TSA) strategy. In this study, an immune sandwich was prepared with a capture antibody and a horseradish peroxidase (HRP)-conjugated antibody upon the addition of a target antigen. In the presence of H_2O_2, HRP can convert tyramine to a short-lived radical intermediate that forms covalent compounds with aromatic amino acids on the surfaces of proteins. By labeling the tyramine with SERS tags in the form of gold nanoparticles (AuNPs) functionalized with a Raman-active probe (4-mercaptobenzoic acid, 4-MBA), AuNPs@4-MBA was deposited and aggregated near the proteins, so the SERS signal of 4-MBA could be detected and amplified. On the basis of the TSA strategy, the developed SERS-based immunoassay can discriminate concentrations as low as 0.01 ng/mL of the target antigen and exhibited approximately 10 times stronger SERS signal intensity than traditional SERS-based immunoassays. These results demonstrated the application potential of this TSA-based SERS biosensor for the detection of important proteins in biomedical research.



Proteins are important components of tissues and cells and are involved in all the biological processes of organisms. In-depth studies of protein systems improve our understanding of the nature of various biological phenomena.[1−3] Research on the molecular structures of protein and protein content is dependent on new and effective analytical technology. Surface-enhanced Raman scattering (SERS) technology can rapidly and sensitively obtain information regarding the chemical structures of molecules in aqueous environments and can allow real-time and in situ detection. SERS is uniquely advantageous for the analysis and detection of biomolecules.[4−6] To improve the detection sensitivity of SERS sensors, many enhancement strategies have been proposed.[7−9] For example, the design of an active substrate with a "hot spot" structure to achieve SERS signal enhancement has been reported.[10−13] In this substrate, the incident light is focused on the gap, crack, or sharp position of the plasma material with nanoscale characteristics to obtain a local enhanced electromagnetic field, producing a "hot spot" effect. Simultaneously, enzymes, nucleic acids, and other biomolecules have been employed to identify and synthesize signal reporters that are placed precisely at the "hot spot" position on the substrate, further improving the detection sensitivity of the system.[14−16] However, the previous strategies are based on a single enhancement step that converts target recognition events into a signal readout, which to a certain extent, limits the high sensitivity of the SERS sensor. To overcome this challenge, Yang and co-workers proposed the use of an intermediate amplification to produce multiple amplified signals for a target recognition event.[17,18] In their work, a silver nanoparticle (AgNP), introduced by a single identification event, was dissolved to yield many silver ions by oxidizing agents. These silver ions act as intermediates to induce the aggregation of gold nanoparticles (AuNPs) modified by probe molecules, forming many SERS "hot spots", resulting in a number of amplified signal outputs. In addition, if the signal reporting unit in the sensing system is enriched on the sensor surface via catalyzed reporter deposition technology, the detection signal can be made to achieve geometric scale amplification. Tyramine signal amplification (TSA) is an enzyme-mediated amplification technology.[19−21] In the presence of hydrogen peroxide (H_2O_2), tyramine can be converted by horseradish peroxidase (HRP) into a highly reactive oxidized intermediate, which

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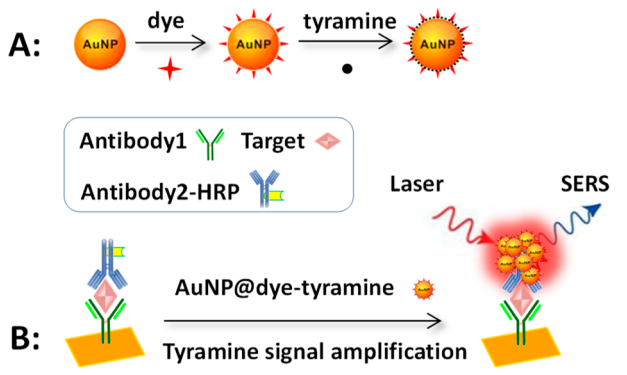
Anal. Chem. XXXX, XXX, XXX−XXX

binds covalently and rapidly to adjacent proteins or to HRP-linked antibodies. By conjugating fluorescent dyes or other tags to tyramine molecules, the fluorescence signal or other corresponding signals can be detected immediately when the molecules are deposited.[22,23] In addition to not increasing nonspecific adsorption, this process reduces the consumption of biomolecules; therefore, this method has received much attention in the field of biosensing. The TSA system has been combined with a variety of detection technologies and has significantly improved the detection sensitivity of biosensors. Liu and co-workers used CdTe quantum dots coupled with tyramine as a marker for an electrochemiluminescence (ECL) immunosensor, and the sensitivity of the immunoassay was improved by 1 order of magnitude.[24] Tang and co-workers studied a combination of the tyramine signal amplification technology and an electrochemical immunoassay for detection of the tumor marker carcinoembryonic antigen (CEA).[25−27] The TSA-based immunosensor can distinguish between two close levels of CEA, exhibiting higher sensitivity than routine enzyme immunoassays. In these methods, however, the ECL and redox probes were always sensitive to the sample and the surrounding environment, leading the response signals to suffer from poor stability and reproducibility. Therefore, a classic TSA strategy coupled with high-potential detection technology for biosensing assays is highly desirable.

In this present study, a novel and sensitive immunoassay protocol for highly sensitive detection of the mouse immunoglobulin G (IgG) antigen as a model target protein by coupling the TSA and SERS techniques was proposed for the first time. The SERS tags used to label tyramine were enriched via a catalyzed reporter deposition technique by following the principles of TSA. By using this method, numerous signal reporters were deposited via a single immune recognition step, thus greatly amplifying the SERS signal intensity. The fabrication of a SERS-based immunosensor based on a TSA-assisted signal amplification strategy is shown in Scheme 1. First, a solution of gold nanoparticles modified by Raman dyes (4-mercaptobenzoic acid (4-MBA)) as SERS tags were prepared and further conjugated to the tyramine molecules (AuNP@MBA-tyramine) (see panel A of Scheme 1). The localized surface plasmon resonance (LSPR) properties of the AuNPs before and after modification with 4-MBA and tyramine were monitored by UV−vis spectroscopy (Figure 1). The UV−vis absorption spectra revealed that the maximum absorption wavelength of these AuNPs was 524 nm, and a redshift of 5 nm was observed after linking the AuNPs with 4-MBA and tyramine, which may be attributed to the interaction between the molecules and AuNPs. As determined from the transmission electron microscopy (TEM) image shown in the inset, the average size of the AuNPs ranged from 20 to 30 nm. For comparison, the TEM image and the average size of AuNP@MBA-tyramine were also displayed in Figure S1. The results indicate that the modification of 4-MBA and tyramine did not destroy the dispersion of AuNPs, and the size of the AuNPs did not significantly change.

Scheme 1. Schematic Illustration of the Sandwich Immunoassay Based on SERS and Tyramine Signal Amplification[a]



[a]Modification of gold nanoparticles with 4-MBA and tyramine (section A) and construction of SERS immunoassay (section B).

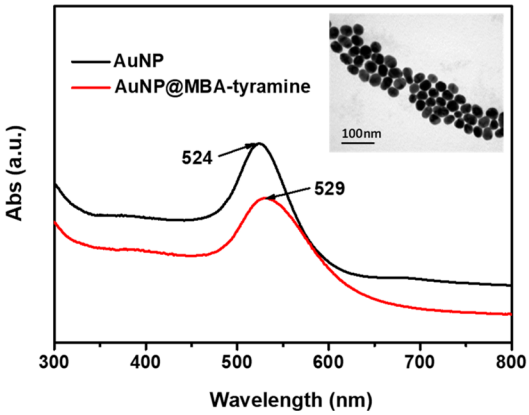


Figure 1. UV−vis spectra of AuNPs (black) and AuNPs functionalized with 4-MBA and tyramine (red). Inset: TEM image of the AuNPs.

The silicon wafer was coated with gold film as the substrate to load the sample. Antibody 1 (goat antimouse IgG) was assembled on the surface of the gold wafer via covalent interaction, and the HRP-conjugated secondary antibodies (rabbit antimouse IgG, antibody 2-HRP) were sandwiched on the sensor surface via immune recognition in the presence of the target antigen (mouse IgG). Then, the TSA process was triggered to catalyze the deposition of tyramine in the presence of H_2O_2, which was followed by AuNP@MBA aggregation on the chip. Finally, the SERS spectra were acquired from the sensor (panel B of Scheme 1). Enhanced sensitivity for the detection of IgG could be achieved by the increasing the amount of SERS tags loaded, leading to the formation of numerous SERS hot spots. The traditional SERS-based immunosensor, without TSA technology, was also tested (details are provided in the Supporting Information).

To demonstrate that the TSA technology could enhance the precipitation of SERS tags, antibody 1 (Ab1) and the antigen (0.5 ng/mL) were initially assembled onto two cleaned gold substrates. Then, the modified gold substrates were employed for the SERS immunoassay in the absence and presence of TSA. The resulting substrates were characterized by using scanning electron microscopy (SEM). As shown in Figure 2, in the traditional SERS immunoassay, immune-gold nanoparticles of Ab2-AuNP@MBA were dispersedly assembled on the substrate surface via an immune sandwich reaction (a), whereas numerous AuNP@MBA-tyramines were aggregated and deposited on the gold film after the HRP catalyzed reaction with the assistance of H_2O_2 (b). This result clearly demonstrates that the TSA process can generate multiple

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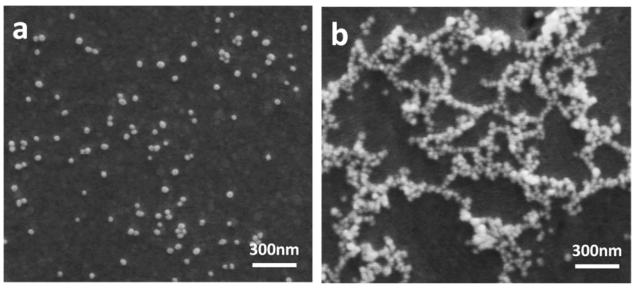

Figure 2. SEM images of the gold substrate after incubation with (a) Ab1 + antigen + Ab2-AuNP@MBA and (b) Ab1 + antigen + Ab2-HRP + H_2O_2 + AuNP@MBA-tyramine conjugate.

signal reporters based on the immune recognition response, which can potentially amplify the Raman signal. In addition, the TSA-based SERS immunosensor formed many nanoparticle aggregates that were abundant in hot spots, which can also cause greatly increased Raman signal intensity.[28] To demonstrate this point, we further recorded the SERS spectra from the samples of (a) and (b), and the results are shown in Figure 3.

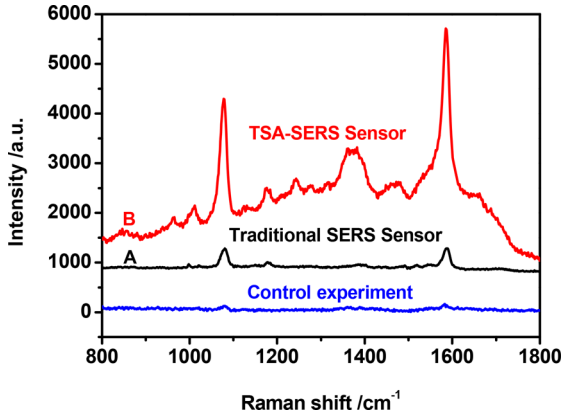

Figure 3. SERS responses of the traditional SERS sensor (A), the TSA-based SERS sensor (B), and the SERS spectrum of the clean Au film-AuNP@MBA-tyramine system as control experiment (blue line).

We conducted a control experiment by measuring the SERS spectrum of a clean Au wafer-AuNP@MBA-tyramine system without immunoassay (Figure 3, blue line). Prior to dropping the AuNP@MBA-tyramine, we blocked out the gold film surface with BSA, which can effectively prevent the nonspecific adsorption in our system. A very weak SERS signal of 4-MBA, which is caused by the physical adsorption of scarce AuNP@MBA-tyramine on the surface of the substrate is observed. The amplification efficiency of the TSA-based immune sensor was evaluated by comparison of the SERS signal intensity before and after the enzyme catalyzed precipitation reaction. As shown in Figure 3A, using Ab2-AuNP@MBA led to a relatively weak SERS signal, whereas the signal obtained by using HRP-Ab2 and AuNP@MBA-tyramine was enhanced significantly (Figure 3B), and the signal intensity of the band at 1583 cm^{-1} was increased approximately 10-fold. The results revealed that TSA technology could lead to amplification of the SERS signal. This finding could be ascribed to the following: (i) HRP converted tyramine to highly reactive intermediates, resulting in covalent attachment of AuNP@MBA-tyramine to the adjacent protein, thus increasing the amount of 4-MBA bound as a signal reporter. (ii) Many SERS hot spots were generated by the aggregation of the AuNPs, which greatly enhanced the SERS signal.

On the basis of the above advantages, we investigated the analytical performance of the developed TSA-SERS immunoassay with different concentrations of the target antigen. As seen in Figure 4A, the intensity of the SERS signal was

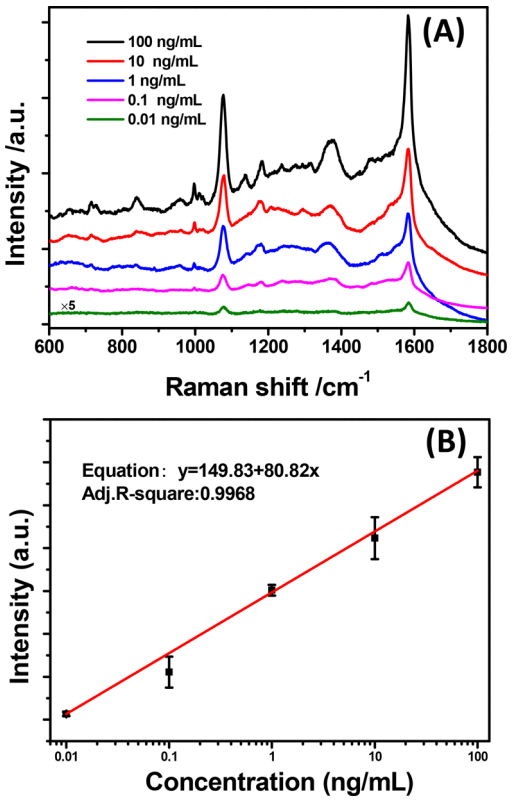

Figure 4. (A) SERS measurements of different concentrations of antigen. (B) The linear relationship between the signal intensity of the band at 1583 cm^{-1} and the antigen concentrations. The SERS spectra were measured with the 633 nm laser excitation, and the integration time was 5 s. The error bars are calculated from three trials on the same substrate.

positively correlated with the concentration of the target antigen. For the band at 1583 cm^{-1}, a linear relationship was achieved between the intensity and concentrations within the range of 0.01−100 ng/mL (Figure 4B). The linear regression equation was $y = 149.83 + 80.82x$ ($R^2 = 0.9968$). The limit of detection (LOD) was 0.01 ng/mL, which was estimated at a signal-to-noise ratio of 3.

To explore the reproducibility of this SERS, we have randomly selected 40 points on the same immunosensor sample to compare the SERS signal according to the methods introduced in refs 29 and 30. The results are shown in Figure S2. We evaluated the relative standard deviation (RSD) values of the signal intensity at 1073 and 1588 cm^{-1}, which were 9.34% and 10.93%, indicating that the proposed immunosensor has acceptable reproducibility.

The stability and selectivity of the developed immunoassay were investigated by utilizing this system for the detection of other interfering species (e.g., glycine, RNA, and Cu^{2+}). As shown in Figure S3, the Raman intensity of band at 1073 cm^{-1} did not exhibit a distinct variation after the introduction of

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Anal. Chem. XXXX, XXX, XXX−XXX

interfering reagents, which confirmed that the immunosensor has excellent stability. The signal intensities of the system that replaces the target antigen with the interference of glycine, RNA, and Cu^{2+} are very weak relative to the target antigen despite the interferences at higher concentrations. This finding also proved that the immune sensing system has excellent selectivity.

In conclusion, we have developed a SERS signal amplification strategy based on a catalytic deposition technology, namely, TSA, for model protein detection. The highlights of this study are described as follows: (i) The TSA process caused a large amount of Raman-active molecules to be deposited on the surface of the sensor, which was beneficial for the amplification of SERS signals. Simultaneously, the TSA system had a role at low concentrations of biomolecules, which reduces the nonspecific adsorption. (ii) Compared with the traditional SERS immunosensor, the introduction of TSA can transform a single immune recognition event into multiple signal reporting units, which improves the sensitivity of the immunoassay. Therefore, highly sensitive detection of mouse IgG was achieved with the TSA-based SERS sensor, and in future studies, this method can be extended to other meaningful targets by applying different recognition elements such as DNA and aptamers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b02419.

Additional experimental details: modification of AuNPs with 4-MBA and tyramine, construction scheme of immune sandwich structure, and initiation of TSA reaction; supplementary results and discussion: TEM characterization of the AuNP@MBA-tyramine, SERS reproducibility of the developed immunosensor, and stability and selectivity study (PDF)

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Notes

The authors declare no competing financial interest.

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Anal. Chem. XXXX, XXX, XXX−XXX