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Ultrasensitive SERS detection of nucleic acids *via* simultaneous amplification of target-triggered enzyme-free recycling and multiple-reporter

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ABSTRACT

The development of ultrasensitive and specific methods for facile detection of trace nucleic acids is of great significance to human health and safety. In the present work, an ultrasensitive SERS-based strategy for detecting nucleic acids was proposed by integrating the SERS-active AgNRs array with double signal amplifications, i.e. the primary target-triggered enzyme-free amplification recycling and the secondary signal enhancement of multiple-reporter. By comparing two SERS sensing routes, i.e. solid interface recycling (Route A) and solution recycling (Route B), the superior solution recycling was determined first, and then the sensing strategy was optimized by investigating the immobilization time, surface blocking, and number of reporters utilized in the SERS sensing. The experimental results indicate that an ultrasensitive SERS strategy can be achieved *via* the primary amplification of target-triggered enzyme-free recycling and additional enhancement by the usage of multiple reporters. Under the optimal conditions, the SERS sensing showed good specificity and uniformity, and a linear calibration curve of DNAs in human serum solution, ranging from 1 μM to 1 fM, was obtained with LOD as low as 40.4 aM, and the following recovery rate measurements confirmed that the proposed SERS sensing had good repeatability and reliability, which shows great potential for facile detecting trace DNAs, especially disease-related nucleic acids in the liquid biopsy of early-stage cancer detection.

1. Introduction

Ultrasensitive and specific detection of nucleic acids is of great importance and urgent requirement in a variety of applications such as life research (Muren and Barton, 2013; Rossetti et al., 2018), disease diagnosis (Xu et al., 2016; Guo et al., 2018), forensic detection (Frascione et al., 2013), environmental and food safety monitoring (Tortajada-Genaro et al., 2012; Li et al., 2018). In recent years, a variety of optical methods, including fluorescence (Huang et al., 2012; Liu et al., 2018a), surface plasmon resonance (SPR) (Nie et al., 2018), chemiluminescence (Zeng et al., 2013), electrochemiluminescence (ECL) (Feng et al., 2017; Gao and Li, 2014), surface-enhanced Raman scattering (SERS) (Kang et al., 2010) and so on, have been proposed for detecting nucleic acids and received extensive attention owing to the wide dynamic range and high sensitivity. Among them, as a high-sensitive vibrational spectroscopy obtained near the surface of plasmonic nanostructures (e.g., noble metal nanoparticles), SERS is considered to be a powerful tool for fast, non-destructive, ultrasensitive analysis of trace substances (Zong et al., 2018; Morla-Folch et al., 2016), even a single molecule (Kneipp et al., 1997; Nie and Emery, 1997). In order to develop SERS-based sensing technology to achieve convenient, ultrasensitive and specific detection of nucleic acids, the development of excellent SERS sensing strategy is one of the most feasible methods and has been a research hotspot in recent years (Ye et al., 2014; Ouyang et al., 2017; Guo et al., 2018; Song et al., 2016).

Currently, a variety of nucleic acids-related signal amplification strategies have been developed for SERS sensing by using enzyme-assisted amplifications such as polymerase chain reaction (PCR) (Alemdaroglu et al., 2009), rolling circle amplification (RCA) (Zeng et al., 2013) and so on by using enzymes like polymerases, endonucleases and exonucleases. These enzyme-assisted amplification strategies can effectively increase the detection sensitivity of target

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molecules, however the enzymes-dependent amplifications face many limitations. For example, the activity of enzyme is easily affected by the environment (i.e., temperature, surfactant) and is highly possible to be inhibited by other components in the sample. The susceptible enzyme-assisted amplification can produce a significantly nonspecific effect and lead to failure amplification or false positive detections (MAURER, 2011; Borst et al., 2004). Thus, enzyme-free amplification is very much needed and has been designed for nucleic acid sensing (Zhang et al., 2012, 2016; Liu et al., 2018b; Yan et al., 2016). Very recently, several fluorescence-based target-triggered enzyme-free amplification strategies providing efficient signal amplification for sensitive detection of nucleic acids have been reported and attracted increasing attention due to relatively easy design of nucleic acid sequences, high efficiency of hybridization and target recycling. Although various amplification strategies have been designed to combine with SERS for developing sensitive sensing strategy, to the best of our knowledge, there are few solutions to well-directed design of SERS-based sensing utilizing the amplification of target-triggered enzyme-free recycling for the ultrasensitive nucleic acid detection.

In this work, an ultrasensitive and specific SERS sensing of DNAs was built by integrating the target-triggered enzyme-free amplification recycling with SERS-active AgNRs array. The enzyme-free amplification recycling was operated by adding target DNAs into the mixture of three specially designed DNA hairpins H1, H2 and H3. The selected target DNA was a specially designed single-stranded DNA (ss-DNA) with the same number of nucleotides as miRNA which is well known as a class of disease-related nucleic acid biomarker in blood. The target DNAs can specifically hybridize with H1 hairpins and open their hairpin structures to trigger a chain reaction between hairpins H2, H3 to form the trefoil DNAs and release the target DNAs. In order to obtain the superior sensing strategy, two SERS sensing routes, i.e. solid interface recycling (Route A) and solution recycling (Route B), were conducted and compared, followed by further optimization of the sensing conditions like immobilization time of the trefoil DNAs on the substrate and the MCH blocking time, as well as the number of reporters labeled on the hairpins H2 and H3. Finally, the calibration curves and limit of detections (LODs) of SERS sensing for detecting target DNAs in buffer and human serum were achieved under the optimal conditions and the recovery rate measurements were performed to characterize the repeatability and reliability of the proposed SERS sensing strategy. The proposed ultrasensitive SERS detection of nucleic acids *via* simultaneous amplification of target-triggered enzyme-free recycling and multiple-reporter can provide a universal detection strategy for sensing DNAs and the disease-related miRNAs.

2. Experimental section

2.1. Reagents and materials

Magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$), and tris(hydroxymethyl) aminomethane ($C_4H_{11}NO_8$) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Mercaptohexanol (MCH) was obtained from Sigma-Aldrich (St Louis, MO, USA). Human serum was bought from Biosharp (Shenzhen, China). All ss-DNA sequences were synthesized and HPLC-purified by TaKaRa Biotech Co., Ltd. (Dalian, China). The names, abbreviations and nucleic acid sequences used in this work are listed in Table 1. The DNA hairpin probe H1 (H1) was labeled with thiol group at the 3′-end, and the DNA hairpin probe H2 (H2) and DNA hairpin probe H3 (H3) were labeled with fluorescence molecules ROX at both the 3′-end and 5′-end. The target DNA (T) was regard as a nucleic acid analyte. The mismatched bases of the Single-base mismatched DNA (SM) and Unmatched DNA (UM) are marked in italics. TM buffer solution (20 mM Tris, and 50 mM $MgCl_2$, pH 8.0) was used for DNA hybridization. All solutions were prepared with ultrapure Millipore water (18.2 MΩ cm).

2.2. Target-triggered enzyme-free amplification recycling

The target-triggered enzyme-free amplification recycling was operated by hairpin probes (H1, H2 and H3) and target DNA (T) in TM buffer, as the yellow part shown in Scheme 1. Firstly, H1, H2 and H3 were heated at 95 °C for 5 min respectively, and then cooled to 25 °C slowly to form the hairpin structures. Secondly, the T with a certain concentration was added into the mixture of H1 (1 μM), H2 (1 μM) and H3 (1 μM) in TM buffer at 25 °C for 3 h in order to form trefoil-structured DNA (specified as trefoil DNA in Scheme 1). In this process, the T hybridized with the H1 through the complementary bases marked in blue in Table 1, and triggered the opening of hairpin structure of H1. Then, the green-marked bases of H1 were paired up with the complementary bases of H2 (green-marked) to form double strands, and the purple-marked bases of H2 was then released to hybridize with the same color marked complementary bases of H3, followed by opening the hairpin structure of H3. As a result, the blue-marked bases of H3 could further competitively hybridize with H1 from the 3′-end to form trefoil DNA and release the T into the solution for next target-triggered enzyme-free amplification recycling.

2.3. Protocols of SERS sensing

The SERS sensing utilizing the target-triggered enzyme-free amplification recycling was conducted in two manners, i.e. solid interface recycling (Route A) and solution recycling (Route B), as shown in Scheme 1. The SERS-active AgNRs array substrates were prepared following the reported oblique angle deposition (Song et al., 2014, 2017). Then, the substrate was patterned by a prefabricated premopolydimethylsiloxane (PDMS) film with arrayed 4 × 10 wells (diameter 4 mm, height 1 mm). The representative SEM image of the AgNRs array and the photo of the PDMS wells patterned substrate are shown in Fig. S1.

For the Route A, the AgNRs in each well were rinsed with ultrapure water and TM buffer in sequence. Then, 20 μL of 1 μM H1 was added in each well and incubated with AgNRs for 3 h at 25 °C to immobilize H1 molecules onto the AgNRs by Ag-S bonds, followed by thoroughly TM buffer wash. After that, 20 μL of 1 μM MCH solution was dropped in each well to block H1-uncoated surface of AgNRs for 10 min. After thoroughly TM buffer rinse, equal amounts of 20 μL mixture containing H2 (1 μM), H3 (1 μM) and different concentrations of T were pipetted in H1-modified wells, and incubated for 3 h at 25 °C to conduct the target-triggered enzyme-free amplification recycling and form the trefoil DNAs on the AgNRs substrate. Finally, the sensing wells were rinsed by water and the SERS measurements were performed when the substrate surface in the wells were dry.

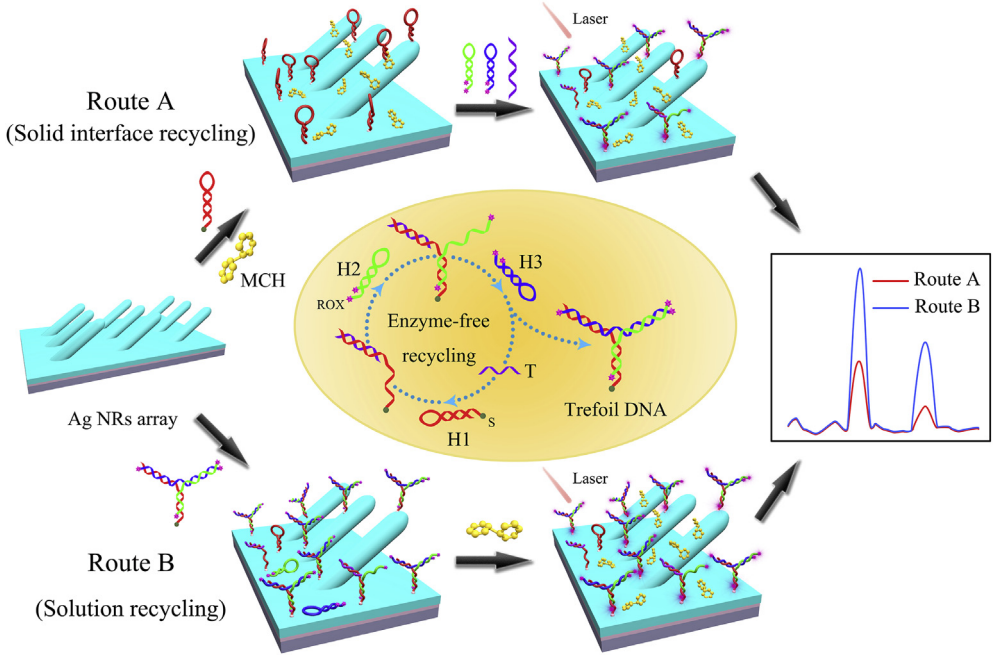
For the Route B, the T molecules with a certain concentration were incubated with H1(1 μM), H2(1 μM) and H3(1 μM) at 25 °C for 3 h in TM buffer to conduct the target-triggered enzyme-free amplification recycling and form the trefoil DNAs first. Then, the final solution containing trefoil DNAs was dropped into the wells on the substrate and incubated for 3 h to assemble the trefoil DNAs onto the AgNRs by Ag-S bonds. After TM buffer wash, the wells were incubated with 20 μL of 1 μM MCH for 10 min to remove nonspecifically adsorbed DNAs, and the SERS spectra from the wells were collected after water wash and air-dry.

2.4. Detection methods and apparatus

The UV-3600 UV-vis spectrophotometer (Shimadzu, Japan) was used to determine the concentrations of the DNA solutions by measuring the absorption at 260 nm. The Bio-Rad electrophoretic apparatus (Bio-Rad Laboratories, USA) was used to perform polyacrylamide gel electrophoresis (PAGE). The InVia confocal Raman microscope (Renishaw, England) was used to measure the SERS spectra with a 633 nm laser (1% power), a 20 × objective and 1 s exposure time.

Table 1
DNA sequences.

Name (abbreviation)	Sequence
Target DNA (T)	5'- GCA CTA CTC CCT AAC ATC TCA AGC -3'
Hairpin probe H1 (H1)	5'- GCT TGA GAT GTT AGG GAG TAG TGC TCC AAT CAC AAC GCA CTA CTC CCT AAC ATC-SH-3'
Hairpin probe H2 (H2)	5'-ROX-AGG GAG TAG TGC GTT GTG ATT GGA AAC ATC TCA AGC TCC AAT CAC AAC GCA CTA-ROX-3'
Hairpin probe H3 (H3)	5'-ROX-GTT GTG ATT GGA GCT TGA GAT GTT GCA CTA CTC CCT AAC ATC TCA AGC TCC AAT-ROX-3'
Single-base mismatched DNA (SM)	5'- GCA CTA CTC CCT AAC ATC TCA ***G***GC -3'
Unmatched DNA (UM)	5'- ***CGT GAT GAG GGA TTG TAG AGT TCG*** -3'



Scheme 1. Schematic illustration of the target-triggered enzyme-free amplification recycling (yellow part) and the SERS sensing strategies of the Route A and Route B.

Unless otherwise specified each spectrum displayed in the manuscript was averaged from 10 measurements at different spots. The strongest Raman band of ROX at 1503 cm^{-1} was selected as the characteristic peak in the research.

3. Results and discussion

3.1. Characterizations of the target-triggered enzyme-free amplification recycling

The target-triggered enzyme-free amplification recycling was analyzed by the PAGE. In order to make the electrophoresis strip clear and narrow without serious "tail-dragging", the PAGE analysis was performed by strict quantifying the concentrations and volumes of the DNA samples and the 10% PAGE image is shown in Fig. 1. The lanes 1 to 4 present the electrophoretic stripes of T and the hairpins H1, H2 and H3, respectively. The mixture of H1, H2 and H3 (lane 5) exhibits a clear band at the similar position of the hairpins and a relatively lighter stripe belonging to the hybrid product of H1, H2 and H3 is also observed, suggesting that only a few of H1, H2 and H3 potentially hybridized together to form trefoil DNAs without the trigger of target DNA. The complex of T, H2 and H3 (lane 6) shows little change of the band position relative to the ones shown in lanes 2 to 4 with a band corresponding to T as shown in lane 1. Once the H1 was incubated with T, a new band appeared in lane 7, which indicates the formation of the T-H1 complex. After mixing H2 with T-H1 complex, several new bands appear in lane 8, which indicates the hybridization of H2 with T-H1 complex. In lane 9, when the target DNA was mixed with H1, H2 and H3, a bright band of trefoil DNAs and a very light band of T can be observed, which indicates the high yield target-triggered formation of trefoil structures and verifies the recycling of T in the target-triggered enzyme-free amplification. The target-triggered enzyme-free amplification recycling was also characterized by SERS. Fig. 1b shows the SERS spectra of the different mixtures on AgNRs substrate, i.e. T + H1 + H2 + H3, T + H1 + H2, T + H2 + H3, and H1 + H2 + H3, respectively, and their corresponding intensities at 1503 cm^{-1} are shown in Fig. 1c. The SERS characterization is consist with the PAGE analysis.

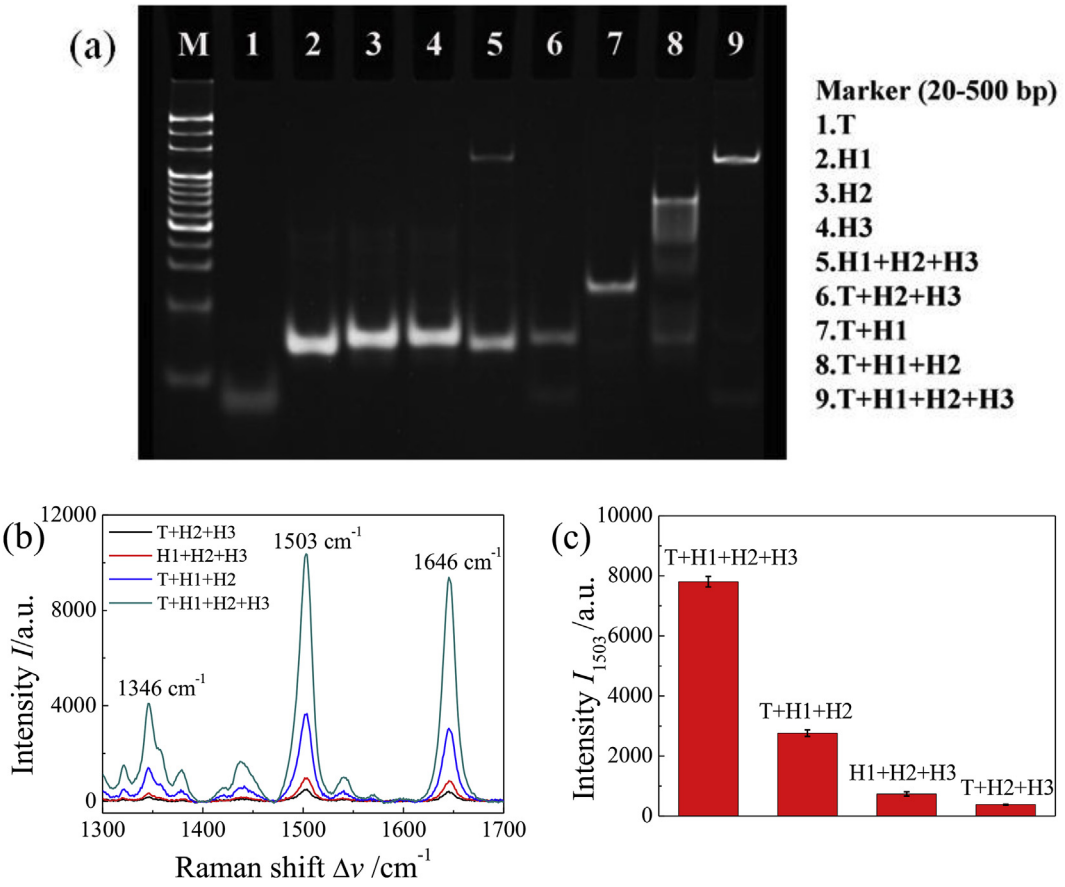


Fig. 1. Characterizations of the target-triggered enzyme-free amplification recycling and the SERS sensing. (a) Marker (20–500 bp), lane 1: T (10 μM, 3 μL); lane 2: H1 (5 μM, 3 μL); lane 3: H2 (5 μM, 3 μL); lane 4: H3 (5 μM, 3 μL); lane 5: H1 + H2 + H3 (1 μM, 5 μL); lane 6: T + H2 + H3 (1 μM, 5 μL); lane 7: T + H1 (1 μM, 5 μL); lane 8: T + H1 + H2 (1 μM, 5 μL); lane 9: T + H1 + H2 + H3 (1 μM, 5 μL). (b) SERS spectra and (c) the corresponding peak intensities obtained from the target-triggered amplification recycling and other DNA ligations following the Route B. The concentration of target DNA was 100 pM.

3.2. Comparison of the SERS sensing strategies of route A and B

Generally, SERS biosensors were designed in two types, interface-based sensing on solid substrate (Gao et al., 2015; Li et al., 2016) or solution phase-based sensing in liquid (Yi et al., 2013). Herein, the SERS sensing strategies based on the solid interface recycling (Route A) and solution recycling (Route B) were investigated and compared by testing 10^{-6}, 10^{-10} and 10^{-14} M target DNAs. Fig. 2a shows the corresponding SERS spectra obtained by detecting the target DNAs following the two strategies, and their SERS signals at 1503 cm^{-1} were plotted in Fig. 2b. The SERS intensities of Route B were obviously stronger than the ones of Route A for detecting each concentration of the T, and the intensity ratio ($I_{Route\ B}/I_{Route\ A}$) increased from 1.78 to 4.60 with the decrease of the target concentration, which indicates that the SERS sensing utilizing Route B strategy is more sensitive than the usage of Route A strategy, especially for detecting target with low

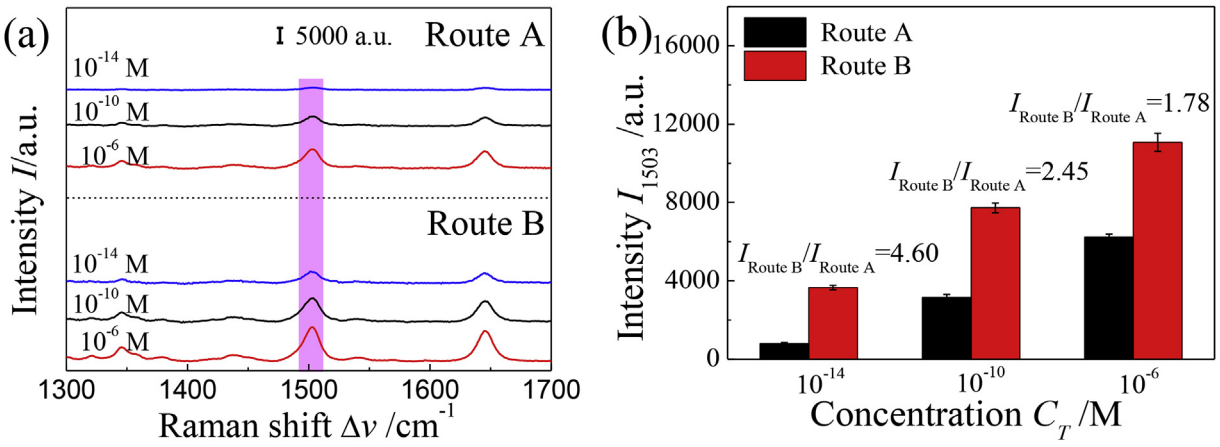


Fig. 2. Comparison of the SERS sensing strategies of the solid interface recycling (Route A) and solution recycling (Route B) by testing 10^{-6}, 10^{-10} and 10^{-14} M target DNAs. (a) SERS spectra of detections following the solid interface recycling (Route A) and solution recycling (Route B), respectively, and (b) corresponding SERS intensities at 1503 cm^{-1}.

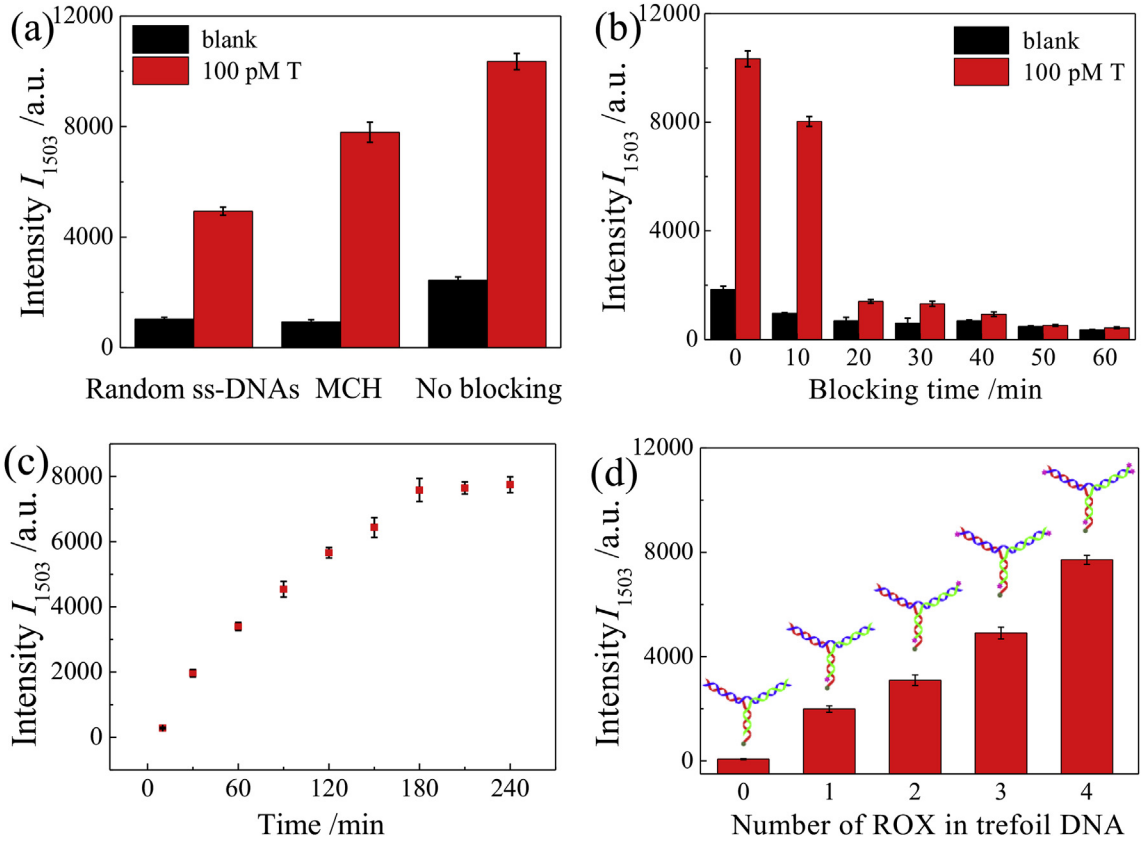


Fig. 3. Optimization of the solution phase-SERS sensing. (a) Comparison of two different surface blocking strategies by using random ss-DNAs and MCH; (b) SERS intensity obtained by blocking procedure of MCH solution from 0 to 60 min; (c) SERS intensity of the trefoil DNAs were immobilized on the substrate for different time, from 0 to 240 min; (d) SERS intensity obtained by using multiple ROX reporters from 0 to 4 on the trefoil DNAs. The concentration of target DNA was 100 pM.

concentration.

3.3. Optimization of the solution phase-SERS sensing

3.3.1. Optimization of surface blocking

During the solution recycling-based SERS sensing (solution phase-SERS sensing), the target-triggered enzyme-free amplification recycling was conducted first and then the trefoil DNAs in the solution were specifically captured onto the AgNRs *via* Ag-S bonds. Actually, the final solution had a very complex composition, such as the initially added single-stranded DNAs and their hybrids containing at least one type of ROX-labeled DNA. All these products can be immobilized on the AgNRs by specific Ag-S bonds or unspecific physical adsorption which may cause significant background. Thus, the reduction of unspecific adsorption is very important for the sensitive and specific SERS detection. For this purpose, two different surface blocking strategies of SERS substrate were utilized. One way was to block the AgNRs by using random ss-DNAs before the incubation of SERS substrate with final solution, and the other way was to replace the physically adsorbed DNAs on the SERS substrate after the incubation with final solution by using MCH for 10 min. As the data shown in Fig. 3a and Fig. S2, both the two ways show obviously reduced background relative to the sensing without any blocking, and there is no significant difference of SERS intensity between the two blocking methods in the absence of target DNAs, but the SERS intensity of MCH treated sample for detecting 100 pM T was almost 1.6 times higher than the random ss-DNAs blocked sample. Thus, MCH was much better for blocking the unspecific absorption by replacing the physically adsorbed DNAs, and the MCH treatments were selected as the optimal method for reducing the unspecific background. The optimal blocking time of MCH treatments was also investigated by adjusting from 0 to 60 min and the results were shown in Fig. 3b. The background was decreased when the blocking time was extended, however the SERS intensity of 100 pM T detection was also decreased significantly since the increase of MCH blocking time could enhance the competitive replacement of previous specifically immobilized DNAs *via* Ag-S bonds (Braun et al., 2007; Sun and Irudayaraj, 2009). Therefore, to balance the minimization of background and preservation of specific detection signals, the blocking time of 10 min was selected as the optimal condition for the following sensing.

3.3.2. Optimization of immobilization time of the trefoil DNAs on the substrate

In order to investigate the optimal immobilization time of the trefoil DNAs on the substrate, the SERS substrate was incubated with the final solution for 0–210 min in order to immobilize the trefoil DNAs on the substrate. As the results shown in Fig. 3c, along with the increasing immobilization time, more trefoil DNAs were captured onto the SERS substrate and outputted stronger SERS signal. After incubating for 180 min, the SERS signal was close to saturation, which means the optimal immobilization time can be selected as 180 min.

3.4. Multiple-reporters based secondary amplification

In order to improve the sensing sensitive, besides the amplification of target-triggered enzyme-free recycling, additional secondary amplification of signal was performed by introducing a multiple-reporter strategy into the sensing system. For achieving the multiple-reporter based secondary amplification, the H2 and H3 used in the present work were specially designed by labeling ROX molecules at both their 3′-ends and 5′-ends, so that four ROX molecules (4-ROX) were included in a single trefoil DNA and the more ROX reporters could output the

stronger SERS signal. The control experiments were conducted by using the H2 and H3 labeled with one (1-ROX) or two ROX molecules to form the trefoil DNAs with one, two or three ROX molecules in a single trefoil DNA. The sensing results of 100 pM T were shown in Fig. 3d and Fig. S3. The SERS intensity was enhanced when the number of ROX was increased from 1 to 4. The sensitivity of 4-ROX included trefoil DNA strategy was approximately 4 times of the 1-ROX sensing, which indicates that a secondary amplification of SERS signals can be obtained by special design of H2 and H3 labeled with more ROX reporters.

3.5. Characterization of the SERS sensing

Basing the optimized sensing strategy, the calibration curve, LOD and selectivity of the solution phase-based SERS sensing were characterized. Fig. S4a shows the SERS spectra for detecting target DNAs in TM buffer with concentrations from 100 aM to 1 μM. A linear calibration curve, $I_{1503} = 1075 \times \lg (C_T) + 18387$ ($R^2 = 0.997$), was fitted according to the relationship between SERS peak intensity (I_{1503}) and the logarithm of T concentration (C_T), as shown in Fig. S4b. Defining the LOD as the mean value of the blank control (in the absence of target) plus 3 times of standard deviation of the mean (Zhao et al., 2015), the LOD of the SERS sensing of target DNAs in TM buffer is 24.4 aM. The selectivity of the solution phase-based SERS sensing was investigated by testing the target DNAs (100 pM) and the nonspecific nucleic acid sequences (10 nM) including single-base mismatched (SM) and unmatched (UM) ss-DNAs, respectively. As the sensing results shown in Fig. S4c and Fig. S5, the blank sample presented very weak SERS signal and slightly increased SERS signals were obtained by detecting the UM and SM ss-DNAs. However, these SERS signals were obviously weaker than the specific detection of target DNAs, which indicates the good specificity of the proposed solution phase-based target-triggered enzyme-free amplification SERS sensing strategy. Besides, the SERS sensing signals collected from 50 random points on the sensing interface and from 10 batches of AgNRs substrates show small relative standard deviations of 4.93% (Fig. S6) and 8.87% (Fig. S7) respectively, which indicates that the proposed SERS strategy has good uniformity.

The similar results were also obtained by detecting the target DNAs in 10% human serum. Fig. 4a shows the SERS spectra of target DNAs with different concentrations. By fitting the concentration dependent-SERS intensity data (Fig. 4b), a linear calibration curve, $I_{1503} = 959 \times \lg (C_T) + 16098$ ($R^2 = 0.998$), was obtained with the LOD as 40.4 aM. The proposed SERS sensing shows ultrahigh sensitivity relative to the previous reported sensing methods as shown in Tables S1–S3. The characterizations of specificity shown in Fig. 4c and Fig. S8 were also confirmed that the proposed solution phase-based target-triggered enzyme-free amplification strategy can achieve specific SERS sensing of the target DNAs in human serum. Furthermore, the recovery rate measurements were carried out by testing the 10% human serum *via* adding different concentrations of target DNAs, in order to characterize the reliability and reproducibility of the proposed SERS strategy. As the data shown in Table 2, the found concentrations are very close to the added ones, and recovery rates were obtained from 97.81% to 103.8% with relative standard deviation (RSD) less than 5.5%, which indicates that the proposed SERS sensing strategy containing simultaneous amplification of target-triggered enzyme-free recycling and multiple-reporter has good repeatability and reliability, and shows great potential for DNAs detection in clinical applications.

4. Conclusions

In the present work, a highly sensitive and specific SERS sensing of DNAs was proposed on the SERS-active AgNRs array by integrating with double amplification strategies, i.e. the primary target-triggered enzyme-free amplification recycling and secondary enhancement of multiple-reporter. Firstly, the feasibility and working mechanism of

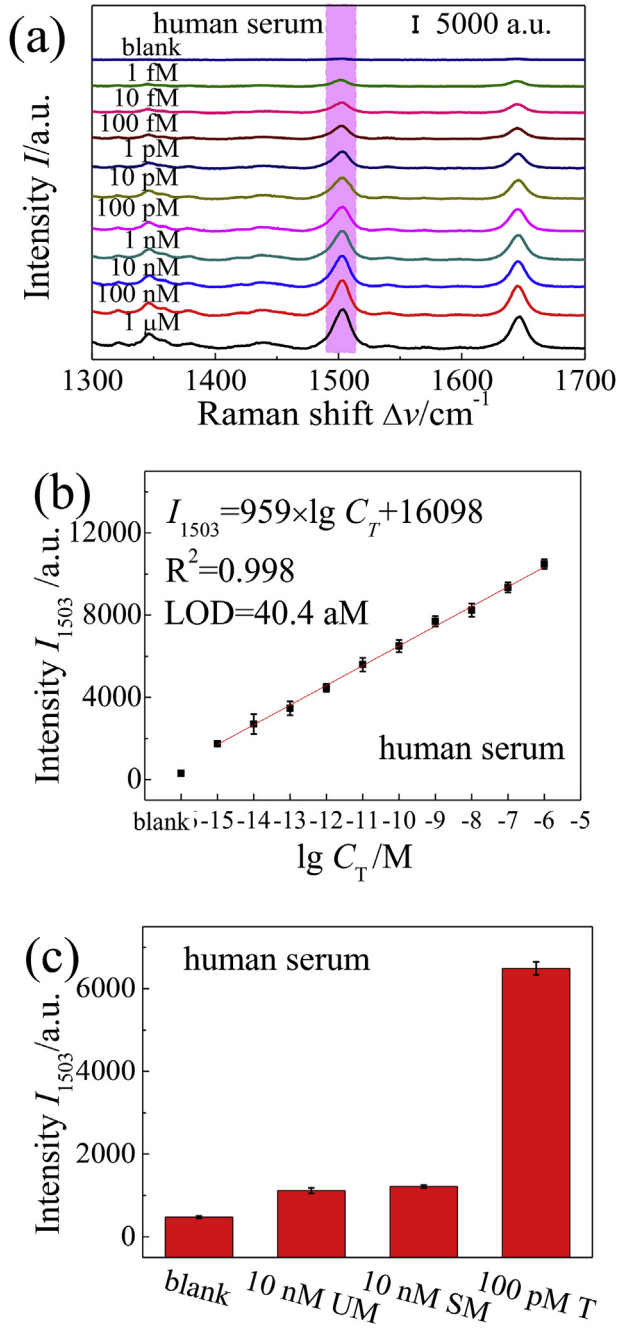


Fig. 4. SERS sensing of target DNAs in human serum. (a) SERS spectra of the detection of target DNAs with different concentrations. (b) The plot of the concentration-dependent SERS intensity I_{1503}. (c) The plots of I_{1503} of SERS detections of 10 nM unmatched ss-DNA (UM), 10 nM single-base mismatched ss-DNA (SM), 100 pM target DNA (T), and in the absence of target DNA (blank). Error bars show the standard deviations (n = 10).

Table 2
Recovery of the proposed SERS sensing for detecting target DNAs in human serum.

Sample number	Added/M	Found/M	Recovery/%	RSD/%
1	3.8×10^{-7}	3.94×10^{-7}	103.8	3.11
2	4.2×10^{-9}	4.16×10^{-9}	99.09	5.42
3	4.8×10^{-11}	4.94×10^{-11}	103.0	1.80
4	5.2×10^{-13}	5.09×10^{-13}	97.81	4.31
5	2.6×10^{-14}	2.56×10^{-14}	98.62	3.87

target-triggered enzyme-free amplification recycling were investigated and confirmed by PAGE and SERS analyses. Then, two SERS sensing routes, i.e. the solid interface recycling (Route A) and solution recycling (Route B), were conducted and the sensing results indicate that the SERS sensing following the Route B is more sensitive than the usage of Route A, especially for detecting target with low concentration. The solution phase-SERS sensing route was then optimized by investigating the immobilization time and the MCH blocking time. The optimal immobilization time of the trefoil DNAs on the substrate was 180 min and the preferred MCH blocking time was 10 min. Besides the primary amplification of target-triggered enzyme-free recycling, additional secondary amplification was realized by the special design of double ROX molecules labeled H2 and H3, and an approximately 4 times enhancement of SERS signal was obtained. Finally, the calibration curves and LODs of SERS sensing for detecting target DNAs in buffer and human serum solutions were achieved respectively, and the linear detection range of human serum sample is from 1 μM to 1 fM with the LOD as low as 40.4 aM. The recovery rate measurement indicates that the proposed SERS sensing has good repeatability and reliability. As a summary, an ultrasensitive SERS detection of nucleic acids *via* simultaneous amplification of target-triggered enzyme-free recycling and multiple-reporter were provided, which shows great potential for DNAs detection in clinical applications, and more in-depth analysis of serum samples of patients should be conducted in the future before clinical diagnosis.

CRediT authorship contribution statement

Jingjing Zhang: Methodology, Investigation, Writing - original draft. **Yanjun Yang:** Formal analysis. **Xinyu Jiang:** Formal analysis, Data curation. **Chen Dong:** Formal analysis. **Chunyuan Song:** Conceptualization, Writing - review & editing, Supervision. **Caiqin Han:** Formal analysis, Writing - original draft. **Lianhui Wang:** Project administration, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.111402.

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