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SERS-active Ag@Au core-shell NPs assemblies for DNA detection

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An innovative Raman sensor was developed for DNA detection, based on silver@gold (Ag@Au) core-shell nanoparticles (NPs) assemblies fabricated by polymerase chain reaction (PCR). The combination of exponential amplification by PCR and the amplified Raman signal achieved DNA detection of 11.8 aM.

Numerous studies have been carried out on the surface-enhanced Raman spectroscopy (SERS) of geometry-controlled NPs aggregates.[1, 2] The rationale behind the enhancement of SERS is based on the localized surface plasmon resonance (LSPR) of metallic NPs arising from resonant oscillations of the conducting electrons around NPs.[3-5] When an analyte is localized at a junction between NPs ("hot spot"), an enhancement of the electronic and vibrational spectroscopic signals is observed. In comparison to fluorescence, SERS signals are not only free from oxygen, humidity and foreign species, but exhibit 10-100 times narrower spectral bands and possess higher discrimination due to the molecularly specific fingerprint spectra,[6] and are a promising alternative for biosensing in diverse surroundings.[7-9]

Despite the extraordinary limits of detection (LODs) for small organic molecules and ions provided by SERS,[10-14] the quantitative application of ultrasensitive biomacromolecules, particularly related to long DNA detection, are necessary for early disease diagnosis and pharmacogenomics, but have significant challenges in the actual applications: (a) the requirements to label Raman reporters on a DNA probe are complex and time-consuming; (b) the restricted LODs at picomolar, and in some cases, femtomolar levels due to large interparticle gaps (Table 1).[6, 15-20]

The design of an efficient and label-free substrate with a consistently high density of hot-spots is crucial for SERS sensors in sensitive long DNA detection.[21] Anisotropic NPs of various sizes and shapes have recently been engineered to optimize high electromagnetic fields in a controlled arrangement.[22-24] Compared to the widely studied Au NPs,[25, 26] Ag NPs should be of interest in SERS properties, due to their better plasmonic oscillations at visible frequencies. However, the easy oxidation and toxicity of Ag NPs is an intrinsically challenging problem in actual applications. To satisfy the criteria of large-scale stable electromagnetic enhancement, a stable and biocompatible Au shell was deposited on the surface of Ag NPs assemblies. Au shell deposition takes advantage of the excellent plasmon properties of the Ag core, and directly guides the interparticle distance of NPs and engineers the intensity of electromagnetic fields.

Herein, Ag NPs multimers were first assembled by PCR attributing to its advantages of exponential amplification and high specificity benefiting from the heat transfer properties of NPs, as well as its automatic manipulations.[27-29] The enormous enhancement of the SERS signals for Ag NPs assemblies at the sub-3.7 nm gap was exploited by Au shell deposition at different PCR cycles (Fig. 1). Depending on the exponential amplification of interface-PCR and the amplified SERS signal, we determined the bioanalytical potential of Ag@Au core-shell NPs assemblies, which achieved Raman-signal based attomolar DNA detection. Shell driven strong Raman signals allow the fabrication of label-free and highly reproducible SERS-active materials with reliable nanometer gap sizes for biomacromolecule detection.

18.0 ± 2.3 nm Ag NPs were synthesized by the reduction of silver nitrate using sodium borohydride (Fig. S1, ESI†).[30] Forward primers and reverse primers were respectively modified on the surface of Ag NPs by Ag-SH covalent bonds at a molar ratio of 100:1.[28, 29] Under optimized amplification conditions, Ag NPs assemblies were prepared using 1.56 pM target templates (Fig. 1). From 2 to 5 cycles, Ag NPs oligomers involving dimers, trimers and tetramers were gradually assembled; From 10 to 40 cycles, the degree of NPs assemblies increased, resulting in the formation of large sized multimers (Fig. S2, ESI†).[28]

A layer of Au shell was post-deposited on the surface of Ag NPs assemblies by the reduction of $HAuCl_4$ solution to prevent Ag NPs from atmospheric oxidation.[11] Bright-field TEM images clearly demonstrated the Ag core and Au shell attributing to their distinct contrast grades (Fig. 2a, insert). With an increase in the number of cycles from 2 to 40, Ag@Au core-shell NPs number of assemblies was systematically increased (Fig. 2). Note that the interface configuration of Ag@Au core-shell NPs was not as smooth as that of Ag NPs and transformed into Au dendrites.[31] Such branched protrusions enabled Ag@Au core-shell NPs to exhibit extraordinary properties in the intensification of the electromagnetic field.[32-34] The interparticle distance between NPs was decreased from 16.5 nm to 3.7 ± 1.0 nm after Au shell deposition and can be employed to obtain large intensifications in SERS (Fig. S3, ESI†).[35] The underlying shell deposition had a significant influence on the plasmonic properties of the assemblies. Compared to the plasmonic peak at 404 nm of single Ag NPs, only 4 nm red shifts were observed for Ag NPs multimers assembled after 20 cycles, while a significant red shift of 85 nm for Ag@Au core-shell NPs assemblies was seen in the plasmonic resonance peaks which were determined by the LSPR of the encapsulated Ag core and deposited Au shell (Fig. 3).[36] Ag NPs assemblies after Au deposition from 2 to 40 cycles showed distinct plasmon resonance which ranging from 489 nm to 499 nm.

Clear evidence of narrow-gap plasmonic coupling of Au and Ag heterogeneous elements in Ag@Au core-shell NPs assemblies prompted us to explore their potential SERS properties. In comparison, SERS signals obtained from 4-NTP and the control groups (0 cycles) were weak (Fig. 4a). Ag NPs assemblies showed increased Raman signals, and a Raman shift at 1346 cm^{-1} exhibited the highest intensity (Fig. S4, ESI†).[37, 38] From 2 to 20 cycles, the SERS intensity of Ag NPs assemblies correspondingly increased, which was related to the number of hot spots between the gaps of two NPs with increasing degree of aggregation of the Ag NPs. From 20 to 40 cycles, no obvious increase in Raman signals was observed, but a slight decrease was seen, due to the formation of large aggregates which caused sedimentation and induced instability of the solution. Ag NPs multimers assembled at 20 cycles showed the highest Raman signal, but the intensity was

only 1295 due to the large gaps between NPs.[29, 39] However, Ag NPs assemblies after Au shell

deposition exhibited as much as 3 orders of magnitude more intensity than that of pure Ag NPs

assemblies and showed 8-fold higher intensity than pure 4-NTP (Fig. 4b). The variation law of

Raman intensity at different cycles was similar. Particle structure and interparticle distance

markedly affected the "hot spots" between metal nanostructures, which were spatial regions where

the electromagnetic field was extremely intense contributing to the plasmon coupling between

neighboring NPs.[35, 40] After Au shell deposition, neighboring NPs were closer and the

nanofocusing effect was particularly pronounced originating from strong "hot spots" at their

junctions where the electromagnetic field was enormously enhanced, and numerous enhanced

regions containing a high density of hot spots. Of particular importance were the slits between

small Au tips and Ag cores which acted as novel hot spot generating regions and were favorable

for significantly magnifying SERS signals.[41] The highest plasmonic resonance of Ag NPs, the

narrow gaps between NPs and the formation of multi-shaped tips on the NPs surface after Au

deposition were the deciding factors in the response to SERS signals.[23, 32, 42] Assemblies at 20

cycles was not only more stable than that of 30 and 40 cycles, but contained the maximum NPs

number than that of 2, 5 and 10 cycles. Ag@Au core-shell NPs assemblies at 20 cycles possessed

the highest density of hot spots and showed the strongest SERS signal which reached up to 3070.

A particularly exciting sensing application utilizing the resultant high-efficiency SERS-active

Ag@Au core-shell NPs assemblies was exploited. An overview of the experimental data is

depicted in Fig. 4. Ag NPs assemblies at 20 PCR cycles after Au deposition exhibited the highest

Raman signals, with promising application in the detection of biomolecules. The Raman shift at

1346 cm^{-1} was employed as the identification position for the quantitative analysis. As shown in

Fig. 5A, the fewer the target templates applied, the smaller the degree of NPs assemblies was

formed by PCR. The target concentration was in proportion to the Raman intensity of the

assemblies. A standard curve was established between the intensity of the 1346 cm^{-1} peak of

4-NTP and the logarithm of DNA concentration in the range of 1.56 pM to 15.6 aM, which

exhibited a good correlation (R^2 = 0.993) (Fig. 5B). There were no obvious changes in Raman

intensity when the DNA concentration was lowered to 1.56 aM and even 156 zM. The LOD was

calculated to be 11.8 aM which demonstrated at least a three-fold enhancement of sensitivity

compared to previous reports.[19, 20, 43] It was instructive to evaluate plasmonic-dependent Raman

sensors with ultraviolet-visible absorption utilizing the plasmonic properties of NPs. The

decreased concentration of target DNA induced a slight blue shift in the ultraviolet-visible

absorption spectra (Fig. S5, ESI†).

The specificity of the developed method was investigated in the presence of non-target

DNA_{EC} (the genome of Escherichia coli). There were no repeated fragments between the genome

of Escherichia coli and λDNA through standard nucleotide BLAST on NCBI. PCR products

assembled at 1 fM DNA_{EC} after 20 cycles still showed single NPs dispersity in despite of a few

oligomers (Fig. S6, ESI†). No obvious changes of SERS intensity was observed between the PCR

products and the control groups. Therefore, strong SERS signal in our study mainly came from the

nanostructures assembled by target templates attributing to the specific recognition and

amplification between primers and λDNA, and was correspondingly enhanced with the increasing

concentration of λDNA The reliability and reproducibility of this method was evaluated by

investigating the recovery of six different concentrations of target DNA in spiked detection buffer.

The results showed satisfactory recovery in the range of 93.18%-105.24% (Table S1, ESI†). These

data demonstrated that this assay would be an accurate and reliable Raman sensor in real sample

analysis. The novel combination of the exponential amplification of target DNA by interface-PCR

and the amplified intensification of the electromagnetic field after Au shell deposition ensured a

shell driven label-free Raman biosensor with high sensitivity and good selectivity for long DNA

detection (Table 1).[44-46]

In summary, a Raman signal based attomolar DNA detection platform was developed for the

first time. The as-fabricated Ag@Au core-shell NPs assemblies exhibited SERS activities which

were superior to commercial SERS substrates in terms of signal intensity and reproducibility. Au

deposition not only preserved the strongest plasmonic resonance of Ag NPs, but reduced the gaps

between NPs and endowed assemblies with SERS-active tips, which were required to enhance the

electromagnetic field. The amplified Raman signal with the aid of exponential amplification by

interface-PCR significantly improved the sensitivity of the established Raman sensor with a LOD

as low as attomolar level. Post-deposited shell driven amplified SERS signals provide the

opportunity to fabricate ideal and reproducible SERS-active substrates, has potential application

for the ultrasensitive and may single-molecular level detection of biomacromolecules from

biological systems.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Detail experimental section and additional TEM images and Raman spectra of Ag NPs assemblies, statistical analysis of Au shell thickness. See DOI: 10.1039/c000000x/
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Captions:

Fig. 1 Schematic illustration of SERS detection of DNA by PCR-based Ag@Au core-shell NPs assemblies.

Fig. 2 (a-f) TEM images of Ag NPs assemblies by deposition of $HAuCl_4$ solution after 2, 5, 10, 20, 30, 40 cycles. Insert in a, the enlarged TEM images of Ag@Au core-shell NPs assemblies after 2 cycles.

Fig. 3 UV-vis spectra of Ag NPs, Au NPs, Ag NPs assemblies, Ag@Au core-shell NPs assemblies after different cycles.

Fig. 4 (a) SERS spectrum of Ag@Au core-shell NPs assemblies after different cycles compared with pure 4-NTP and control groups. (b) Evolution of spectral features of 4-NTP, control groups and Ag@Au core-shell NPs assemblies with increasing cycles.

Fig. 5 (a) SERS spectrum of Ag@Au core-shell NPs assemblies at different DNA concentration after 20 cycles. (b) Calibration curves between DNA concentration and Raman intensity of Ag@Au core-shell NPs assemblies. Error bars represented the standard deviation of sample measurements.

Table 1. LODs for SERS based DNA detection by different analytical methods.

Ag NPs   Forward Primer   Reverse Primer   Template   4-NTP

Fig. 1 Schematic illustration of SERS detection of DNA by PCR-based Ag@Au core-shell NPs assemblies.

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Fig. 3 UV-vis spectra of Ag NPs, Au NPs, Ag NPs assemblies, Ag@Au core-shell NPs assemblies after different cycles.

Fig. 4 (a) SERS spectrum of Ag@Au core-shell NPs assemblies after different cycles compared with pure 4-NTP and control groups. (b) Evolution of spectral features of 4-NTP, control groups and Ag@Au core-shell NPs assemblies with increasing cycles.

Fig. 5 (a) SERS spectrum of Ag@Au core-shell NPs assemblies at different DNA concentration after 20 cycles. (b) Calibration curves between DNA concentration and Raman intensity of Ag@Au core-shell NPs assemblies. Error bars represented the standard deviation of sample measurements.

1

2 **Table 1.** LODs for SERS based DNA detection by different analytical methods.

General Features	Signal	LODs	Refs
Dye-labeled SERS primers	SERS	1 nM	7, 44
Raman reporter dye-coded Ag NPs	SERRS[a]	1.25 nM	45
SERS-active dye labeled DNA probes, Requires lambda exonuclease	SERS	21.7 pM	6
Graphene with in situ grown Au NPs, Reporter-labeled DNA	SERS	10 pM	16
Au NPs-on-Au nanowires, Reporter DNA with Raman dye	SERS	10 pM	17
Hybridization chain reaction coupled with DNA-mediated Ag NPs growth	SERS	3.4 pM	15
Dye-labeled oligonucleotide probe	SERS	750 fM	43
NPs with Raman spectroscopic fingerprints, Raman dye-labeled DNA	SERS	20 fM	18
The adsorption of a colored molecule onto Ag NPs	SERRS	1.17 fM	20
Silicon nanowires with in situ grown Ag NPs, Dye-attached DNA	SERS	1 fM	46

[a]SERRS, surface-enhanced resonance Raman scattering

1
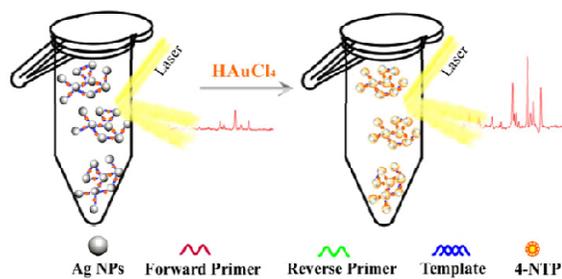
TOC Figure

3　　SERS-active silver@gold (Ag@Au) core-shell nanoparticles (NPs) assemblies were
4　　fabricated by polymerase chain reaction (PCR) for the sensitive DNA detection.

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