Electronic Supplementary Information

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

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Captions:

Experimental Section

Fig. S1 Representative TEM images of single Ag NPs.

Fig. S2 (a-f) TEM images of Ag NPs assemblies at 2, 5, 10, 20, 30 and 40 cycles.

Fig. S3 Statistical analysis of Au shell thickness.

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Fig. S5 UV-vis spectra of Ag@Au core-shell NPs assemblies at different DNA concentration.

Fig. S6 (a, b) TEM images of Ag@Au NPs assemblies in the presence of 1fM DNA_{EC}. (c) SERS intensity of assemblies at different concentration of templates. (1) Control groups (1fM λDNA, 0 cycle); (2) 1fM DNA_{EC}, 20 cycles; (3) 200 aM λDNA and 1fM DNA_{EC}, 20 cycles; (4) 2 fM λDNA and 1fM DNA_{EC}, 20 cycles.

Table S1. Detected results of DNA spiked into PCR buffer.

Experimental Section

Materials and Reagents.

Silver nitrate ($AgNO_3$), poly-N-vinyl-2-pyrrolidone (PVP), sodium borohydride ($NaBH_4$) and hydroxylamine hydrochloride (NH_2OH-HCl) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd., Beijing, China. Chloroauric acid ($HAuCl_4$), trisodium citrate, L-sodium ascorbate and thiolated polyethylene glycol (PEG_{1000}-SH, Mw 1000) were purchased from Sigma-Aldrich. Millipore-Q water used throughout the experiments was purified using the Milli-Q device (18.2 MΩ, Millipore, Molsheim, France). All glassware was cleaned with freshly prepared *aqua regia* (V_{HNO3}: V_{HCl} = 1 : 3) and rinsed several times with Millipore-Q water. PCR buffer, dNTP and Taq DNA polymerase were purchased from Shanghai Sangon, China. All DNA fragments purified by high-performance liquid chromatography were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd, including λDNA as the template, forward primers and reverse primers. The amplification length of the primers was 50 bp.

Amplified Target Sequences: TGG CTG ACC CTG ATG AGT TCG TGT CCG TAC AAC TGG CGT AAT CAT GGC CC

Forward primers : 5′-SH-$(CH_2)_6$-TGG CTG ACC CTG ATG AGT TCG-3′

Reverse primers: 5′-SH-$(CH_2)_6$-GGG CCA TGA TTA CGC CAG TT-3′

Preparation of primer-modified Ag NPs.

18.0 ± 2.3 nm Ag NPs were synthesized as follows: 5 mL 1% (by weight) PVP solution as the stabilizer agent was added to 20 mL Millipore-Q water in a water-ice bath. 0.6 mL 1% (by weight) $NaBH_4$ solution was mixed with the above solution under vigorous stirring. Then, 5 mL 15 mM $AgNO_3$ solution and 5 mL 1% PVP solution were slowly injected into the mixture by two constant-flow pumps at a rate of 30 mL/h. Finally, the reaction solution was kept at 80℃ for 2 h to remove excess $NaBH_4$ before being stored at 4℃.

The as-fabricated Ag NPs (1 mL) were centrifuged at 5400 g for 10 min, and re-suspended in 200 μL Millipore-Q water. 50 μL concentrated Ag NPs were respectively reacted with 50 μL F_{50} and 50 μL R_{50} at a molar ratio of 1:100 for 12 h. The Ag NPs-primer conjugates were centrifuged at 5400 g for 10 min to remove the unreacted primers, and then re-suspended in 50 μL Millipore-Q

water. PEG_{1000}-SH was further modified on the Ag NPs-primer conjugates at a molar ratio of 5:1 to improve their stability in PCR buffer.

PCR-based Ag NPs assemblies.

5 μL 10 × PCR buffer, 1 μL 1 mM dNTP, 0.5 μL λDNA and 0.5 U Taq DNA polymerase were mixed in 37 μL Millipore-Q water. 3 μL Ag NPs-F_{50} conjugates and 3 μL Ag NPs-R_{50} conjugates were added to the above solution. The optimized PCR process was performed as follows: 94ºC (3 min), 94ºC (30 s), T_m 60ºC (30 s), 72ºC (1 min) and 4ºC (10 min) after 2, 5, 10, 20, 30, and 40 cycles. PCR products were centrifuged at 5400 g for 10 min and re-suspended in Millipore-Q water at 1/5 of the original volume.

Construction of Ag@Au core-shell NPs assemblies.

50 μL five-fold concentrated Ag NPs assemblies were added to a mixture containing 200 μL 0.1 M PBS and 100 μL 1% PVP. 50 μL 5 mM $HAuCl_4$ solution and 50 μL 10 mM NH_2OH-HCl solution were injected into the above solution. The mixture was shaken for 3 h at room temperature, and then centrifuged and re-suspended in 50 μL Millipore-Q water.

SERS measurements

4-nitrothiophenol (4-NTP), as a standard Raman reporter molecule, was modified on the surface of Ag NPs assemblies and Ag@Au core-shell NPs assemblies after 2, 5, 10, 20, 30 and 40 cycles through Ag-SH/Au-SH covalent bonds. The final concentration of 4-NTP was 2 μM. The mixtures reacted for 12 h and were centrifuged at 5400 g for 10 min to remove the unmodified 4-NTP. SERS spectrum of assemblies bearing 2 μM 4-NTP was measured through LabRam-HR800 Micro-Raman spectrometer.

SERS signal-based DNA detection.

Ag NPs assemblies were prepared at 20 cycles under different DNA concentrations ranging from 1.56 pM to 156 zM, and then a layer of Au shell at 50 μL 5 mM $HAuCl_4$ solution and 50 μL 10 mM NH_2OH-HCl solution were deposited. After centrifugation, SERS intensity of the Ag@Au core-shell NPs assemblies after modification with 4-NTP was measured. A standard curve was established by plotting the SERS intensity against the DNA concentration. To evaluate the accuracy of the Raman sensor in DNA detection, a recovery test was performed by spiking the PCR buffer with different concentrations of DNA. The SERS signals of interface-PCR products

assembled at known DNA concentrations were measured, and the percent recovery was calculated based on the established standard curve.

Instrumentation and Measurement.

The structures of Ag NPs, Ag NP assemblies and Ag@Au core-shell NPs assemblies were characterized using a JEOL JEM-2100 transmission electron microscope (TEM) operated at 200 kV. The UV-vis spectra of NPs and assemblies were measured by a UNICO 2100 PC UV-vis spectrophotometer. SERS of the assemblies were obtained by a LabRam-HR800 Micro-Raman spectrometer with Lab-spec 5.0 software. The slit and pinhole were set at 100 and 400 mm, respectively, in a confocal configuration with a holographic grating (600 g/mm) and an air-cooled He-Ne laser for 632.8 nm excitation with a power of ca. 8 mW.

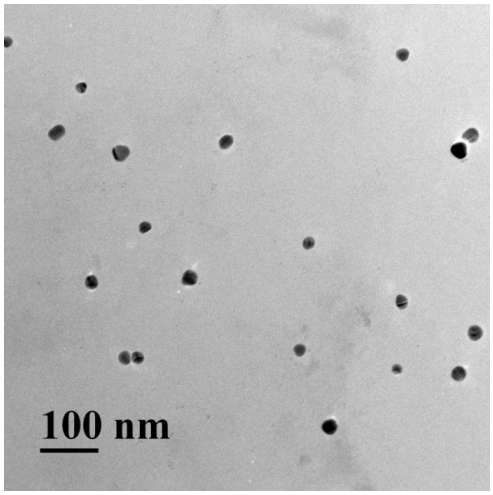
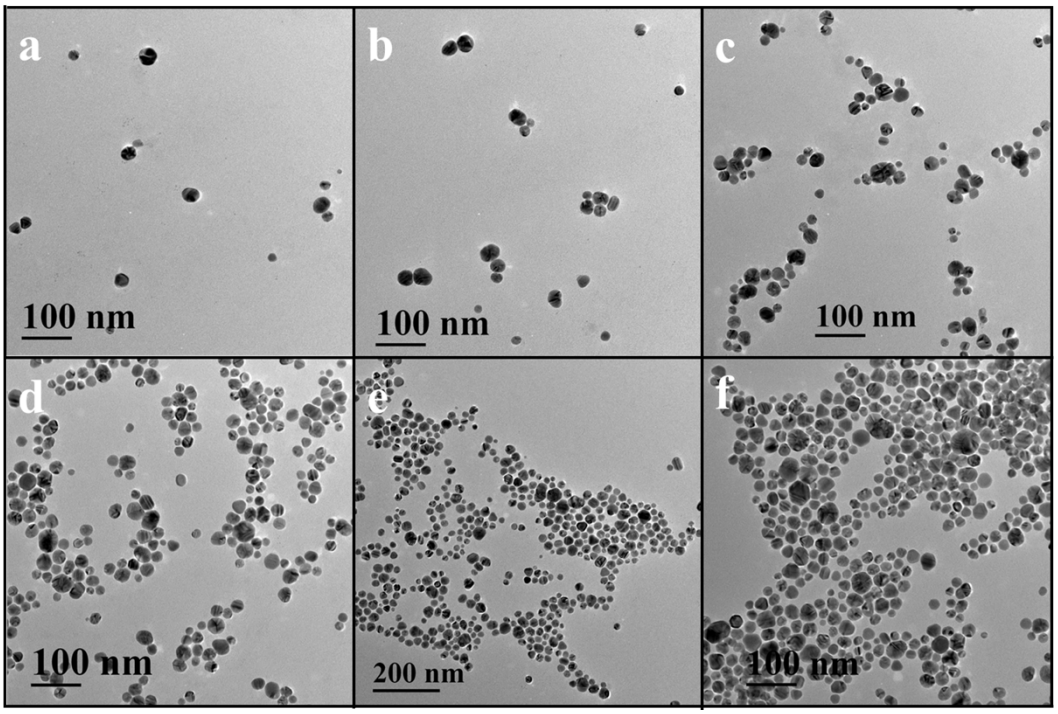


Fig. S1 Representative TEM images of single Ag NPs.



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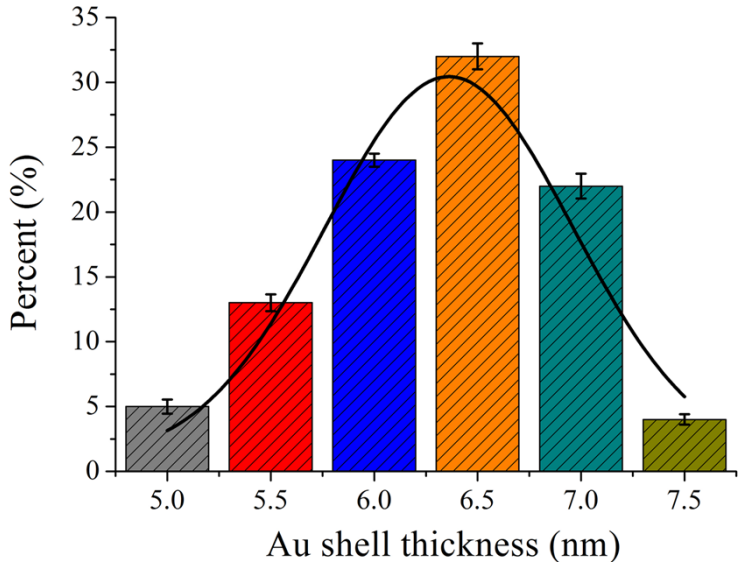

Fig. S3 Statistical analysis of Au shell thickness.

The interparticle distance between Ag NPs was 16.5 nm determined by the length of amplified primers (50 bp, 16.5 nm), and the thickness of Au shell was statistically analyzed to 6.4 ± 0.5 nm, thus the interparticle gap between Ag@Au core-shell NPs was around 3.7 ± 1.0 nm.

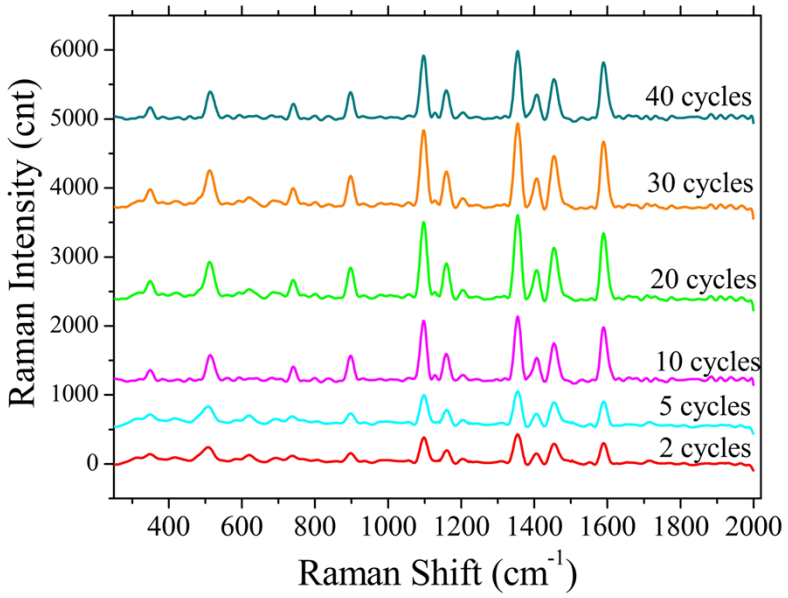

Fig. S4 SERS spectrum of Ag NPs assemblies at different cycles.

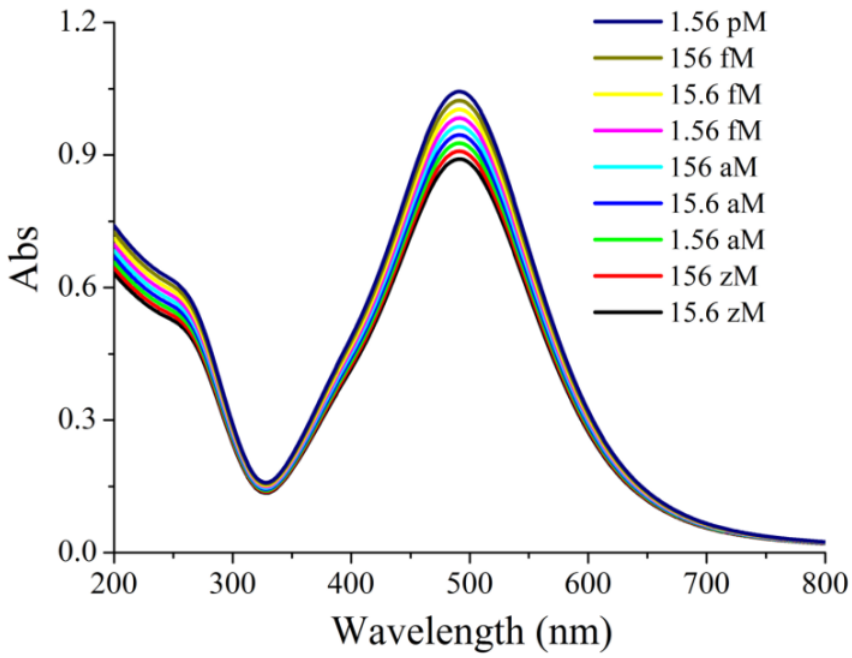

Fig. S5 UV-vis spectra of Ag@Au core-shell NPs assemblies at different DNA concentration.

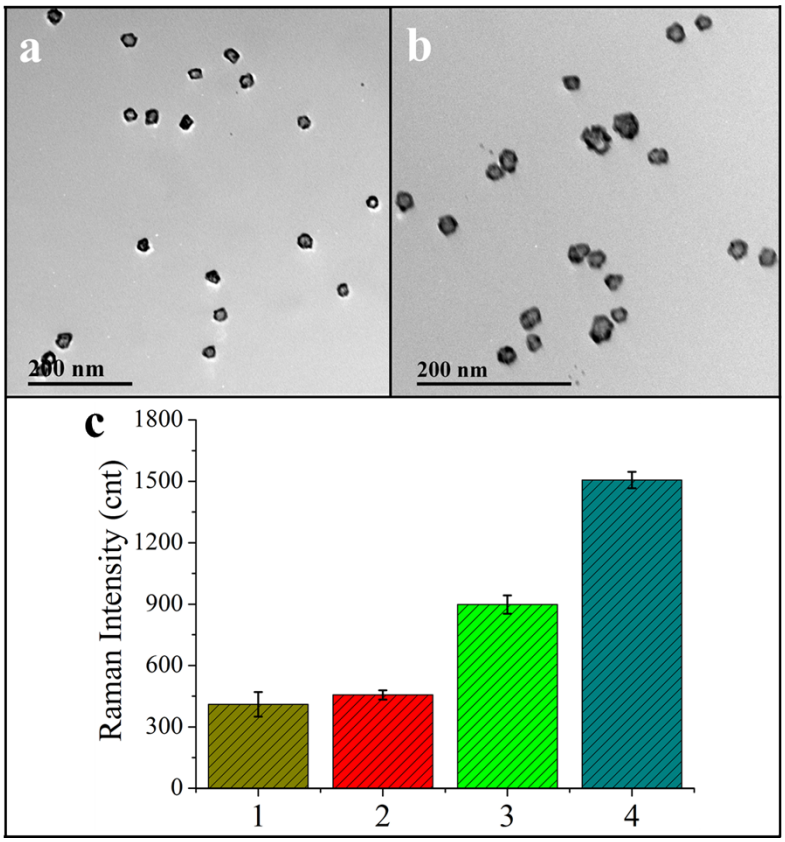

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Table S1. Detected results of DNA spiked into PCR buffer.

Spiked concentration (fM)	Detected Concentration Mean ± SD (fM)	Recovery (%) Mean ± SD[a]
100	107.2 ± 5.35	104.51 ± 2.27
10	11.2 ± 1.89	105.24 ± 3.61
1	0.98 ± 0.085	95.68 ± 2.67
0.5	0.48 ± 0.021	97.53 ± 1.29
0.1	0.095 ± 0.0052	96.49 ± 1.86
0.05	0.047 ± 0.0037	93.18 ± 3.78

[a]SD, standard deviation, was calculated based on three experiments.