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Electronic Supplementary Information

Surface-enhanced Resonance Raman Scattering (SERRS) Simulate PCR for Sensitive DNA Detection†

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EXPERIMENTAL SECTION

Chemicals, Biochemicals and Materials. Sodium hydroxide (NaOH), sodium choride (NaCl), hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) and silver nitrate ($AgNO_3$) were purchased from Sigma-Aldrich (Taufkirchen, Germany) and used without further purification. SYBR Green I and Thermo-Start DNA Polymerase were purchased from Thermo Scientific Absolute. 5× Colorless GoTaq® Flexi Buffer, TaqDNA Polymerase and 25 mM $MgCl_2$ were purchased from Promega. cDNA were generated from mice lung tissue. Glass slides (26 mm×76 mm×1 mm) were purchased from Carl Roth (Karlsruhe, Germany). MilliQ water (18.2 MΩ cm) was produced using a Millipore water purification system.

Colloid Synthesis. As a reference, standard AgNPs were synthesized as reported in earlier experiments.[1-3] The preparation follows a modified procedure of Leopold and Lendl.[4,5] Briefly, 17 mg (0.1 mM) $AgNO_3$ were dissolved in 10mL of MilliQ water. 100 mL of 11.6 mg (0.17 mM $NH_2OH \cdot HCl$ solution containing 3.3 mL NaOH (0.1 M) was prepared and divided into 9 mL batches in centrifuge tubes. To the reducing agent, 1 mL of $AgNO_3$ was added at a flow rate of 0.67 mL s^{-1} without stirring. Finally, the centrifuge tube was inverted once to complete the mixing. The size distribution of the silver colloid suspension was tested using a UV-vis spectrometer and TEM (procedure details are described later). The yellow/greenish colloid sols were stored in the dark at 4 °C.

Common PCR and qRT-PCR Preparation. Both common PCR and qRT-PCR amplification for Actb were prepared using a 25 µL reaction volume. For common PCR, 5 µL 5× Colorless GoTaq® Flexi Buffer, 0.25 µL TaqDNA Polymerase, 4 µL 25 mM $MgCl_2$, 1 µL dNTP's, 1 µL of 10 µM forward and reverse primer mixture and 1 µL cDNA from mice lung tissue together with 12.75 µL H_2O were added into a PCR tube to create a total 25 µL reaction mixture. The cycle conditions for common PCR were 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 60 °C for 1 min, 72°C for 1 min and 72°C for 1 min using the Eppendorf PCR system.

For qRT-PCR, 10 μL of 2× master Mix containing TaqDNA Polymerase, SYBR Green I, $MgCl_2$ inside, proprietary reaction buffer and dNTP's together with 9.5 μL H_2O, 1 μL of 10 μM forward and reverse primer mixture, 1 μL cDNA from mice lung tissue were added into a 96 well plate to create a total 25 μL reaction mixture. Then this mixture in a 96 well plate underwent a PCR thermal cycling program using an ABI PRISM®7000 system. The cycling profile used was 95 °C for 15 s, 60 °C for 1 min for total 40 cycles amplification.

Table S1 Oligonucleotide sequences

Target gene	**Acc. No.**	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
Actb	NM_007393	TCCATCATGAAGTGTGACGT	GAGCAATGATCTTGATCTTCAT

Gel electrophoresis. 2 g agarose was added to 100 mL 1× TBE to prepare a 2% agarose gel for the Actb PCR product electrophoresis. Gel electrophoresis was carried out using a Bio-Rad system at a voltage of 120 V for 45 min, and MassRuler Low Range DNA Ladder was used to determine the size of product, then the gel was imaged by a Sony system.

SERS Measurements. 3 μL of a sample suspension, already treated with colloids, was pipetted onto normal glass slides. The recording of the Raman spectra was started immediately after this sample preparation, using the 633 nm line of a He-Ne with 14 mW power at the sample and a 10× objective. The Raman spectra were continuously collected (one spectrum every 40 s) with the auto repeat function until the droplet was dried. If not explicitly stated, the exposure time and the accumulation number were 1 s and 10, respectively, and the confocal slit width was 100 μm, detecting spectra in the region of 50 to 3000 cm^{-1}. The same parameters and procedures were used for 532 nm and 785 nm laser line excitation.

Colloid diagnostics. UV-vis absorption spectra were recorded with a Specord Plus spectrometer (Shimadzu UV-2550 spectrometer). All of the UV-vis spectra in the suspension were obtained at room temperature using water as solvent with a path-length of 1 cm. TEM images were taken with a JEM 2010 (TEM, JEOL 2010) with an accelerating voltage of 200 kV. SERRS measurements were conducted with a Raman microscope (LabRAM HR, HORIBA Scientific, Japan).

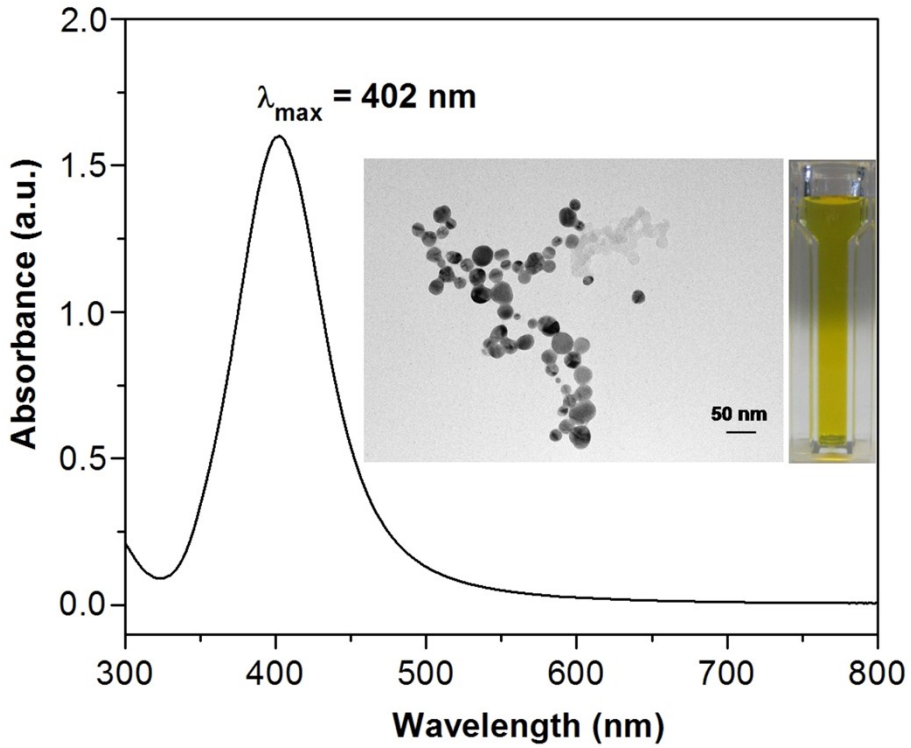

Fig. S1 UV-vis spectrum and TEM images of AgNPs, optical images of AgNPs suspension.

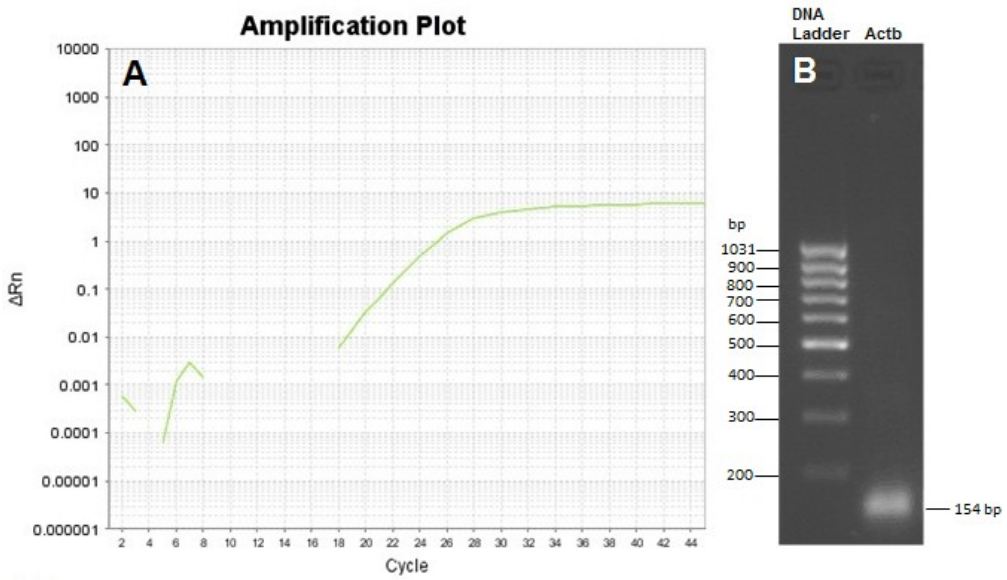

Fig. S2 (A) qRT-PCR amplification curve. (B) Gel electrophoresis images. The MassRuler Low Range DNA Ladder was used to determine the size of product.

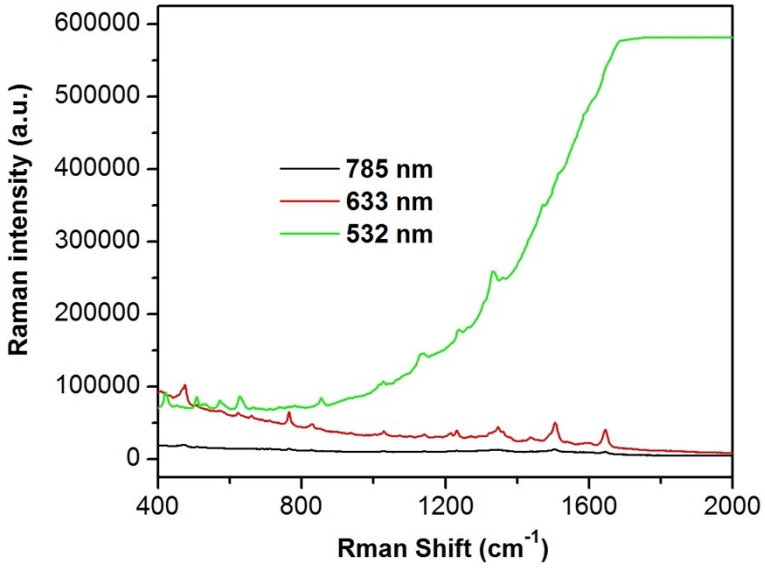

Fig. S3 SERRS spectra of SYBR Green I/DNA/AgNPs suspension were measured under 532 nm, 633 nm and 785 nm laser line excitation.

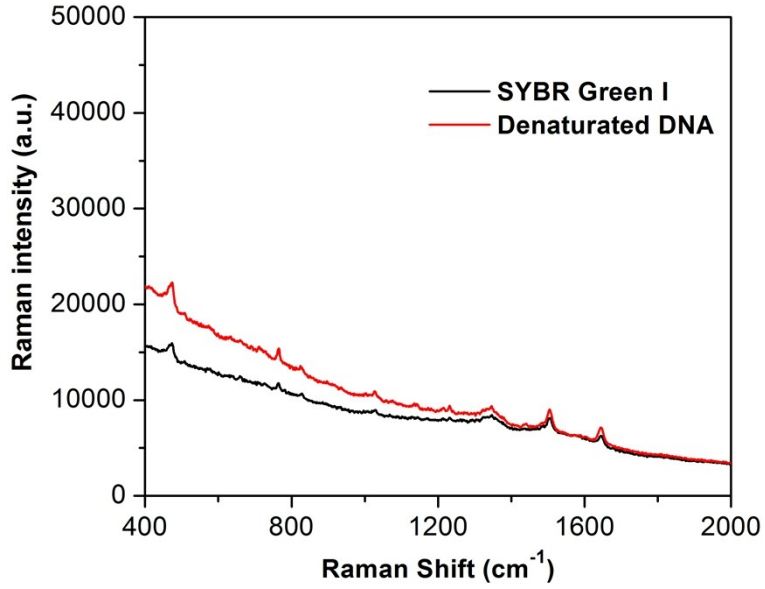

Fig. S4 SERRS spectra of denaturated DNA/SYBR Green I and SYBR Green I with the AgNPs.

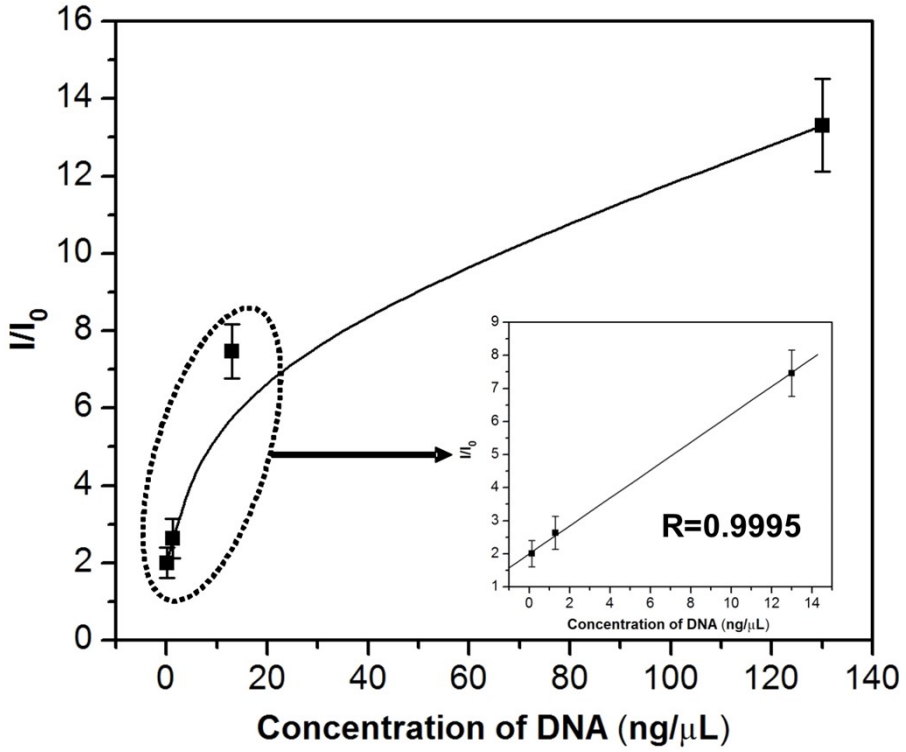

Fig. S5 The SERRS enhancement (I/I_0) of SYBR Green I/DNA/AgNPs suspension at 1507 cm^{-1} vs the different concentrations of DNA. The inset is the plots of linear scale vs DNA concentrations in the range of 0.13 ng/μL ~ 13 ng/μL. The linear regression equation is y = 1.9972 + 0.422x, thus deduced a theoretical detection limit of 0.0066 ng/μL (6.6×10^{-12} g/μL) based on 3σ of the blank signals. I and I_0 represent Raman intensities at 1507 cm^{-1} after the addition of DNA and without the DNA, respectively.

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