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Raman spectroscopic detection of sub-picomolar DNA by coupling silver catalyzed silver deposition with circular strand-replacement polymerization on magnetic nanoparticles

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Experimental

Materials and reagents: DNA polymerase, and the mixture of deoxyribonucleotides (dNTPs) were obtained from Fermentas Biotechnology Co. Ltd (Canada). $AgNO_3$ and trisodium citrate were obtained from Shanghai Reagent Co. (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (18 MΩ, Milli-Q, Millipore) was used in all assays. Polyethylene glycol sorbitan monolaurate (TWEEN-20), MBA, dithiolthreitol (DTT), tris(2-carboxyethyl)phosphane hydrochloride (TCEP), tris(hydroxymethy) aminomethane (tris), a silver-enhancer kit including enhancement solutions A and B were purchased from Sigma-Aldrich (USA). MNPs (100-200 nm) modified with carboxyl groups were obtained from Base Line Chrom Tech Research Centre (Tianjin, China). DNA hybridization buffer was phosphate-buffered saline (137 mM NaCl, 2.5 mM Mg^{2+}, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4, pH 7.4). DNA storage solution was prepared with Tris-HCl (10 mM, pH 8.0) containing 1 mM

ethylenediaminetetraacetic acid (EDTA). Phosphate-buffered salines (PBS, 0.01 M) of various pHs were prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. The washing buffer was PBS (0.01 M, pH 7.4) containing 0.05% (w/v) Tween-20.

The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. Their sequences were

target: 5'- ***GAA CAG CCA CCG AAC*** -3'

NH_2-modified molecular beacon (MB): NH_2-ATC GAT TAC CGC ***GTT CGG TGG CTG TTC*** TAC GTA ATC GAT-3'

SH-modified primer: SH-AAA AAA AAA ATC GAT TAC -3'

single-base mismatched: 5'- ***GAA CAG CTA CCG AAC*** -3'

non-complementary: 5'- ***TGC ATC GGC AAC CCA*** -3'

Preparation and modification of AgNPs: To provide a suitable metal surface for enhancing the SERS signal, a suspension of citrate-reduced AgNPs was produced using a modified procedure[S1] with the conditions specified by Munro et al.[S2] Briefly, 200 mL aqueous solution of 10^{-3} M $AgNO_3$ was boiled under vigorous stirring, then 5 mL of 35 mM sodium citrate solution was added, and the resulting mixture was kept boiling for 1 h. The colloidal solution was stored at 4 °C and protected from room light. Primer attached AgNPs were then prepared by incubating the mixture of 1 mL of colloidal AgNPs solution and 100 μL of SH-modified primer (10 μM) for at least 18 h. The resulting primer attached AgNPs were purified three times by centrifugation at 8000 rpm for 15 min, redispersed in 10 mM PBS and stored at 4 °C.4 μL of MBA solution (1 mM) was added to 1.0 mL of primer attached AgNPs (25 nM) for reaction under stirring for 12 h. The MBA co-assembled with the primer on AgNP surface by thiol group. The obtained reporter AgNPs were rinsed by centrifugation and redispersed in 1 mL PBS (0.01 M, pH 7.4).

Modification of MNPs with MB: 50 μL carboxyl-modified MNPs suspension was dispersed in 1 mL PBS (0.01 M, pH 7.4) followed by dropping the mixture of 200 μL EDC (0.1 M), 400 μL NHS (0.05 M)

and 150 μL MB (5.4 μM) at room temperature under stirring. After reaction for 4 h, the resulting MNPs were isolated using a permanent external magnetic field and washed with water for several times. The MB modified MNPs were dispersed in 1 mL of PBS (0.01 M, pH 7.4) and stored at 4 °C for latter usage.

Procedure of CSRP and silver Enhancement: 50 μL duplication solution containing the 10 μL target DNA with the designed concentration, 5 μL of reporter AgNPs (0.394 nM) and 2 μL of polymerase (4 U) and dNTPs (12 μM for each component) was added to 10 μL of MB modified MNPs. The duplication process was allowed to proceed for 100 min at 37 °C, and the CSRP product was then separated using a magnetic field. The product attached MNPs were mixed with 10 μL of silver deposition solution containing a 1:1 mixture of the silver enhancement solutions A and B. After silver deposition for 2 min, the MNPs were separated to perform the Raman measurements.

Apparatus and measurements: SERS measurements were performed using a Renishaw 2000 Raman microscope system (Renishaw, U.K.). A Melles Griot He-Ne laser operating at λ=514 nm was used as the excitation source with a laser power of approximately 30 mW. The MNPs deposited with AgNPs on the CSRP product were collected using a capillary tube, concentrated into one spot under an external magnetic field, and then a 10×objective lens was used to focus a laser spot on the concentrated sample pellet.[S3,S4] The UV-vis absorption spectrum was observed with a UV-3600 UV-vis spectrophotometer (Shimadzu). X-ray photoelectron spectroscopic (XPS) measurements were performed using an ESCALAB 250 spectrometer (Thermo-VG Scientific, USA) with an ultrahigh vacuum generator.

Gel electrophoresis: The 20% polyacrylamide gel electrophoresis (PAGE) analysis of the CSRP products was carried out in 1×Tris-Borate-EDTA (pH=8.3) at a constant voltage of 100 V for about 2 h. After ethidium bromide staining, gels were scanned using a Molecular Imager Gel Doc XR (BIO-RAD, USA).

Optimization of detection conditions: The amount of Raman dye on AgNP surface plays an important role in producing the SERS signal. In order to examine the effect of dye on the SERS signal, different

volumes of MBA solution (1.0 mM) were added to 1 mL of primer attached AgNPs (25 nM) for preparation of the reporter AgNPs. The Raman intensity increased with the increasing volume from 1.0 to 4.0 μL and then trended to a constant value (Fig. S1A). Thus 4.0 μL was selected as the optimum volume of dye for the preparation of reporter AgNPs.

The CSRP was affected by a series of factors. Some of them, which were associated with the activity of polymerase, had been standardized in the manual of the polymerase, such as the suitable temperature (37 °C) and suitable pH (7.4, provided by the polymerase buffer). The other conditions that were related to the specific duplication system, including the time duration of the duplication process and the amount of the polymerase used, were subsequently optimized. As shown in Fig. S1B, with the increasing amount of polymerase from 0 to 6 units, the Raman intensity at 1586 cm^{-1} increased with an inflexion at 5 units. After 5 units of polymerase the Raman intensity trended to a constant value. Thus it was chosen as the optimal amount of polymerase.

After the target was added to the mixture containing polymerase, dNTPs and reporter AgNPs, with the increasing duplication time, the Raman intensity increased. However the increase rate decreased (Fig. S1C) due to the limited amount of dNTPs, reporter AgNPs and MB on MNP surface and the decrease of polymerase activity. After the duplication time of 100 min, the signal did not increase obviously. Thus this work performed the CSRP for 100 min.

In order to intensify the Raman signal, a silver deposition process was used to grow the Ag around reporter AgNPs, which led to the enhancement of Raman scattering. From Fig. S1D, the Raman signal increased with the increasing silver deposition time and reached a maximum value at 2 min. However, when the deposition time was longer than 2 min, the Raman signal obviously decreased, which could be attributed to the shield effect of the Ag shell deposited around reporter AgNPs on the Raman dye. The over-deposited Ag shell enwrapped the dye and blocked the penetration of the laser of Raman through the thick shell to irradiate the Raman dye.

Optimization of reaction conditions:

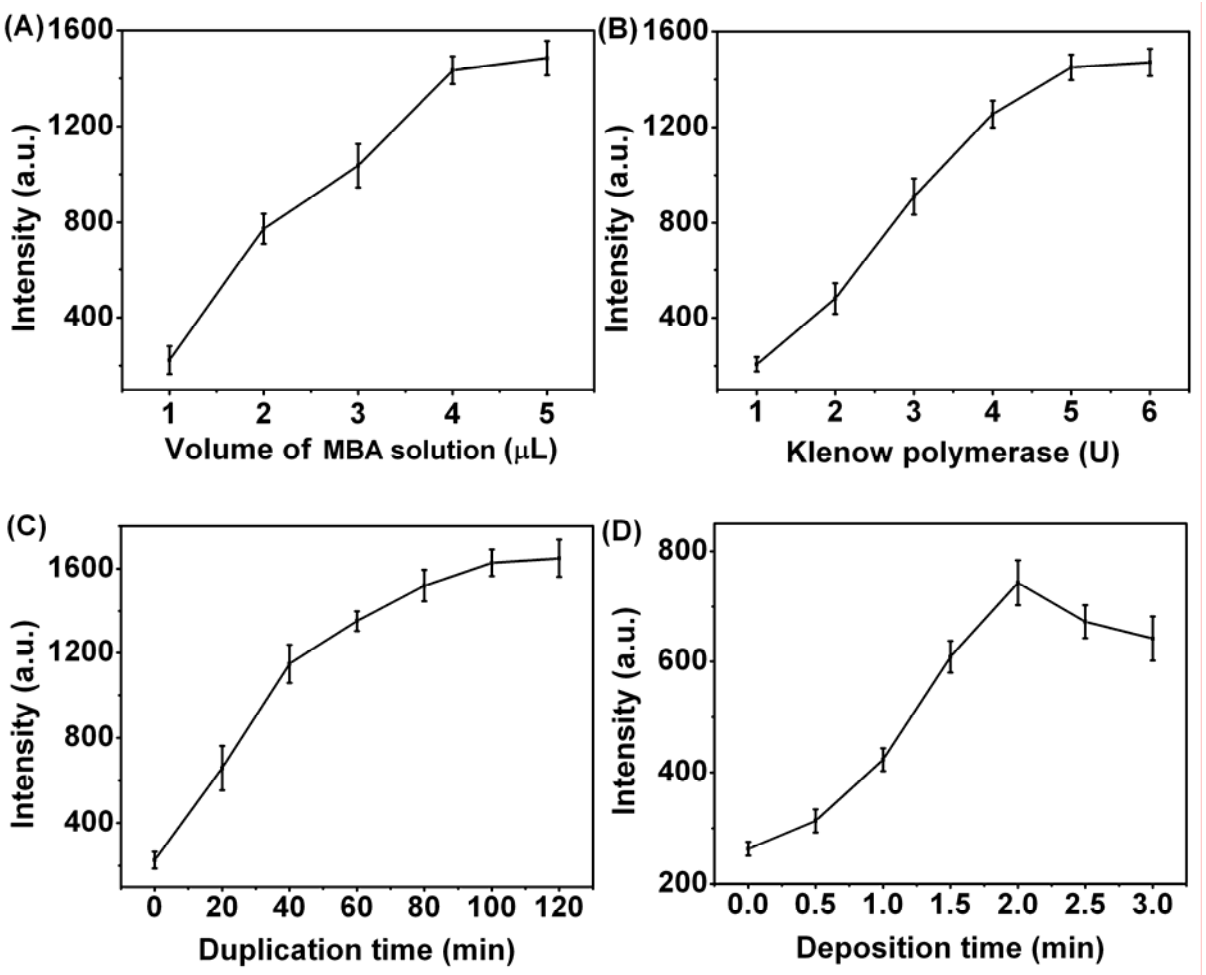

Fig. S1 Dependence of Raman intensity on (A) volume of MBA solution used for preparation of reporter AgNPs, (B) amount of Klenow polymerase, (C) duplication time and (D) silver deposition time.

Control experiment for detection of DNA in absence of polymerase and silver enhancement

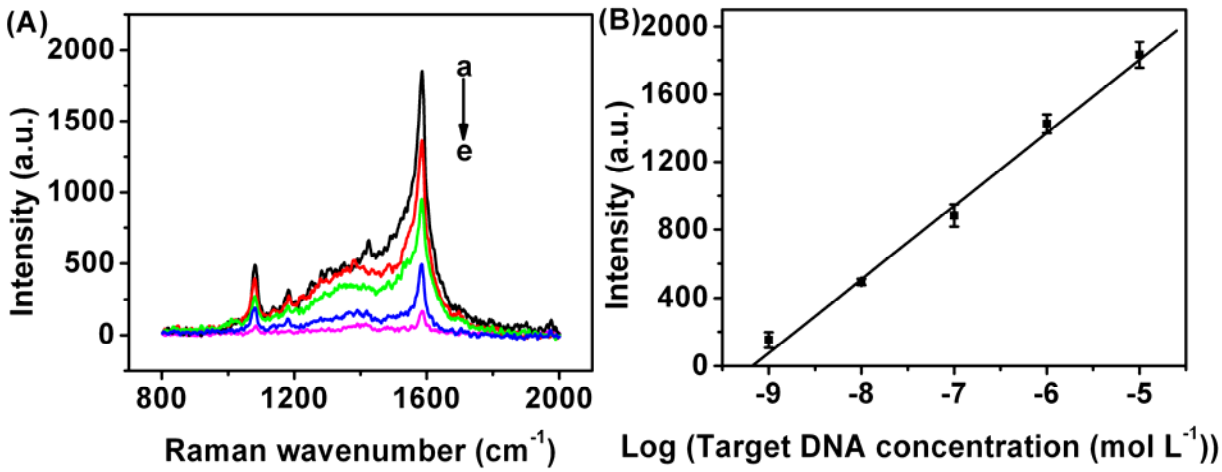

Fig. S2 (A) SERS spectra and (B) Raman intensity before CSRP and silver enhancement at target concentrations from 10^{-9} to 10^{-5} mol L^{-1}.

Control experiments for detection of DNA in absence of only silver enhancement

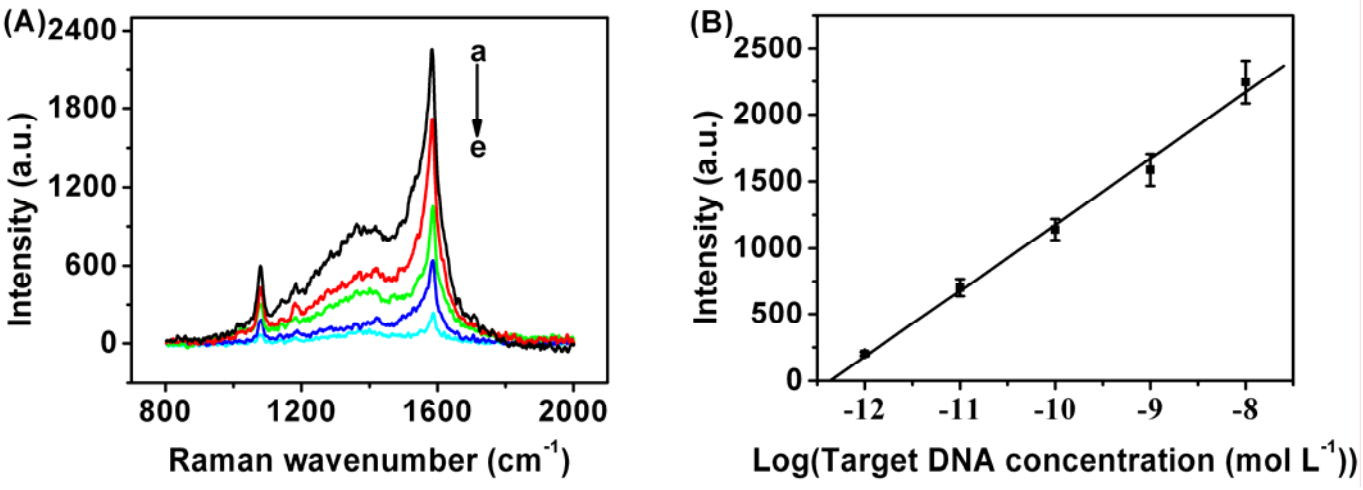

Fig. S3 (A) SERS spectra and (B) Raman intensity after CSRP but in the absence of silver enhancement at target concentrations from 10^{-9} to 10^{-5} mol L^{-1}.

Selectivity of the proposed method

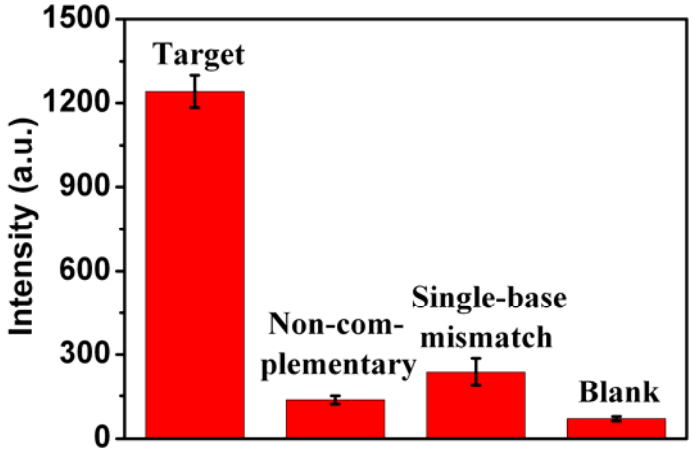

Fig. S4 Histograms for Raman intensity for 10 pmol L-1 complementary, single-base mismatch, non-complementary sequences, and blank.

References

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