Supporting Information

One-step isothermal detection of multiple KRAS mutations by forming SNP specific hairpins on a gold nanoshell

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Materials and methods

Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) except when mentioned specifically and used without further purification. The oligonucleotides were purchased from Bioneer (Daejeon, South Korea).

Synthesis of AuNS

AuNS (diameter: ~128 nm) with 26 nm shell thickness was synthesized according to the previous method. [1] First, silica nanoparticles were prepared by the Stöber method. 1. 5 mL of tetraethyl orthosilicate (TEOS) was added into 45 mL of ethanol containing 2.8 mL of NH_4OH (14.8 N). After 8-hour reaction, 126 μL of APTES was added into the mixture. After 8-hour incubation, the APTES treated silica cores were boiled for 2-hour. For removing unreacted reagents, centrifuge the silica cores for 30 minutes at 2000 g. After discarding supernatant, the pellet was suspended in 50 mL of fresh ethanol using a probe sonicator (VC 750, Sonics) for 10 minutes. The centrifugal purification of the silica core was repeated two times more. Then access seed gold colloids (2~3 nm) were added into silica cores. After overnight incubation, the mixture was suspended in 10 mL of ultra-pure water followed by centrifugal removal of the unbounded seed gold at 2000 g for 30 minutes. The centrifugal removal steps were repeated two more times. The seed gold colloids attached silica cores were mixed with 1mL of 0.0148 % $HAuCl_4$. Growth of nanoshell was initiated by addition of 6.7 μL of 30 % formaldehyde followed by vigorous vortexing for 10 minutes. Thickness of nanoshell could be controlled by varying the amount of silica cores. In order to measure size, and shell thickness of the nanoshell, high resolution images of the synthesized nanoparticles were obtained using TEM (JEM-2100F, JEOL).

Immobilization of Oligonucleotides on AuNS

All the oligonucleotides (TabeS1) were purchased from Bioneer (Daejeon, South Korea). Thiols of FP1 (5′(OP)-CAGCTCCAACA-(SH)3′) were activated by treatment with 10 mM TCEP in 10 mM sodium phosphate buffer (pH 7.0) for 30 minutes at room temperature. 200-fold molar excess of the thiol activated FP1 were mixed with AuNS in 1 mL of ultra-pure water for 16 hours at room temperature. To increase the density of FP1 coverage on the gold surface, NaCl concentration was gradually increased up to 0.1 M. After an additional 48 hours, the excess free FP1 was removed by centrifugation at 8,000 rpm for 25 minutes in ultra-pure water, repeated four times. The primer modified AuNS were redispersed in 200 μL of the water at room temperature.

Gel electrophoresis of oligonucleotides

Electrophoresis of the used oligonucleotides and ligated products was performed by loading the samples into TBE polyacrylamide gel (4~20%) and running the gel at 180 V for 50 minutes. After electrophoresis, the gel was stained in 1× TBE buffer (pH 8.0) containing 1× SYBR Green I Nucleic Acid Gel Stain (Thermo Fisher Scientific, USA) for 40 minutes. UV excited fluorescence images of the stained gel were taken using a Davinchi-Gel Imaging System (Davinchi-K, South Korea)

Isothermal Ligase Reaction and SERS measurement

FP2A (final 0.2 μM) or FP2T (final 0.2μM) and Taq DNA ligase (final 3U/μl) was added into a solution containing FP1 modified AuNS (0.2 nM) in 20 μL reaction buffer containing 20 mM Tris-HCl (pH 7.6), 25 mM $Mg(CH_3COOH)_2$, 10 mM KCH_3COOH, 1mM NAD and 0.1% Triton X-100 in the presence of various amount of target. [2] The mixture was incubated at 45 °C for 1 hr. 10 μl of the reacted samples were dried carefully on a cleaned glass by nitrogen gas. A spot (the diameter of the focused 633 nm laser is 1.03 μm) of the sample was scanned 10 times using 3 seconds of integration time for each scan using a confocal Raman microscope (LabRAM HR Evolution, HORIBA).

References

1. T. Pham, J. B. Jackson, N. J. Halas and T. R. Lee, *Langmuir*, 2002, **18**, 4915-4920.
2. Martin Wiedmann, Wendy I. Wilson, John Czajka, Jianying Luo, Francis Barany, and Carl, A. Batt, Ligase chain reaction (LCR)--overview and applications., *Genome Res*. 1994, **3**, S51-S64

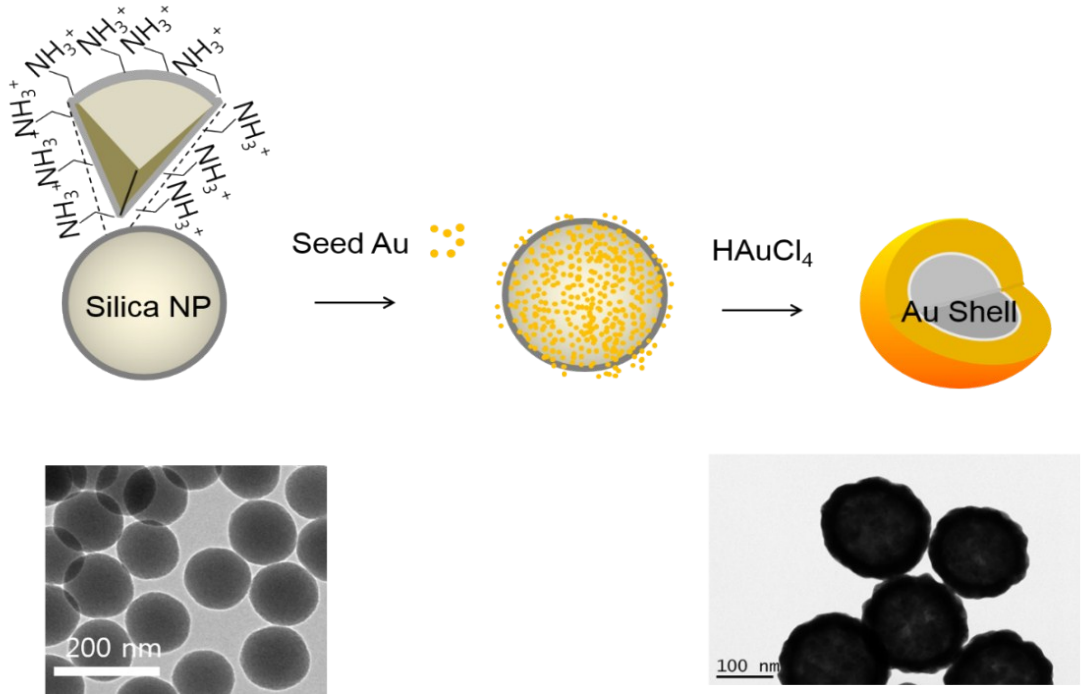

Figure S1 Schematic illustration of the synthesis of AuNS. TEM image of amino-modified silica spheres(left) and AuNS(right).

Table S1 Sequences of oligonucleotides used in the study.

Name	*Sequence*
FP1	5'- (OP)- CAGCTCCAACA-(SH)-3'
FP2A	5'-TAMRA-AAG GAGCTGGTGGACCTACGCCAT-3'
FP2T	5'-Cy3-AAG GAGCTGGTGGACCTACGCCAA-3'
FP2AC	TTTTACGCCAT
35G>A	TGG AGCTGATGGCGTA
35G>T	TGG AGCTGTTGGCGTA
LPA (ligased FP1 and FP2A)	AAGGAGCTGGTGGACCTACGCCATCAGCTCCAACA
35G (wild target)	TGG AGCTGGTGGCGTA

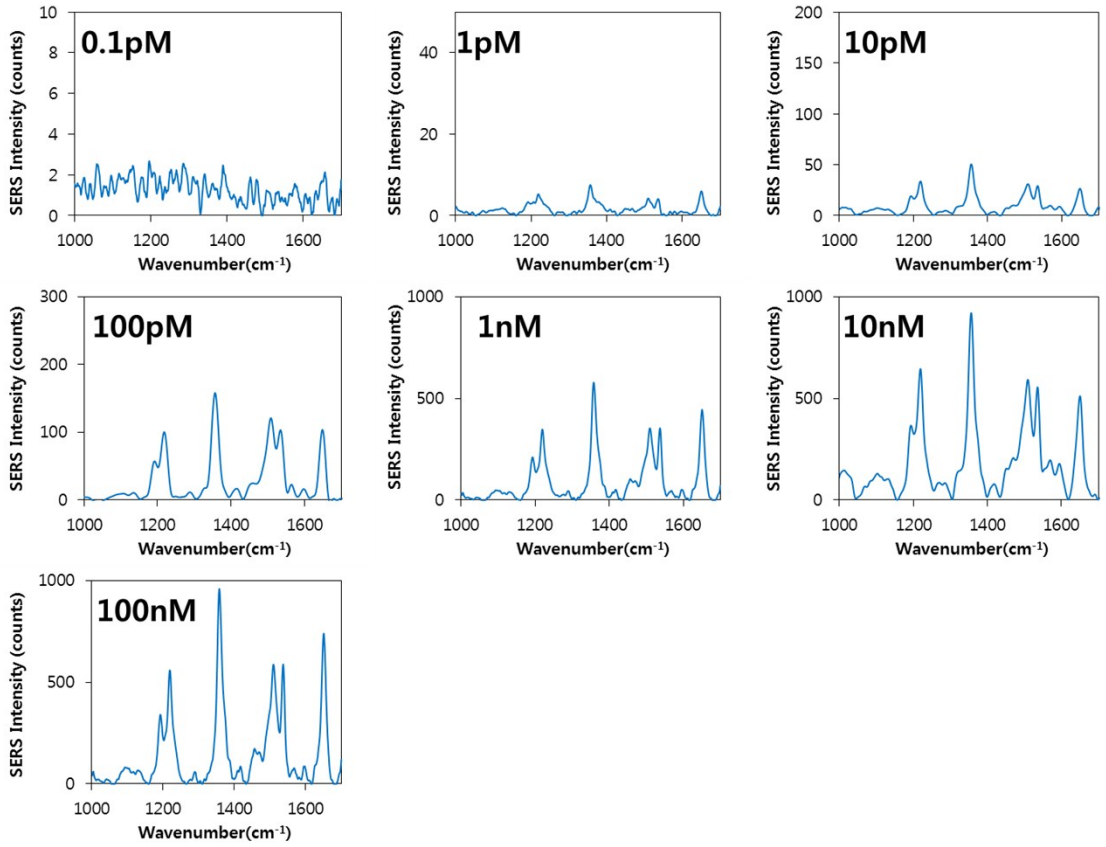

Figure S2. Obtained SERS in our assay depending on concentration of KRAS mutation (35G>A) as indicated on each graph.

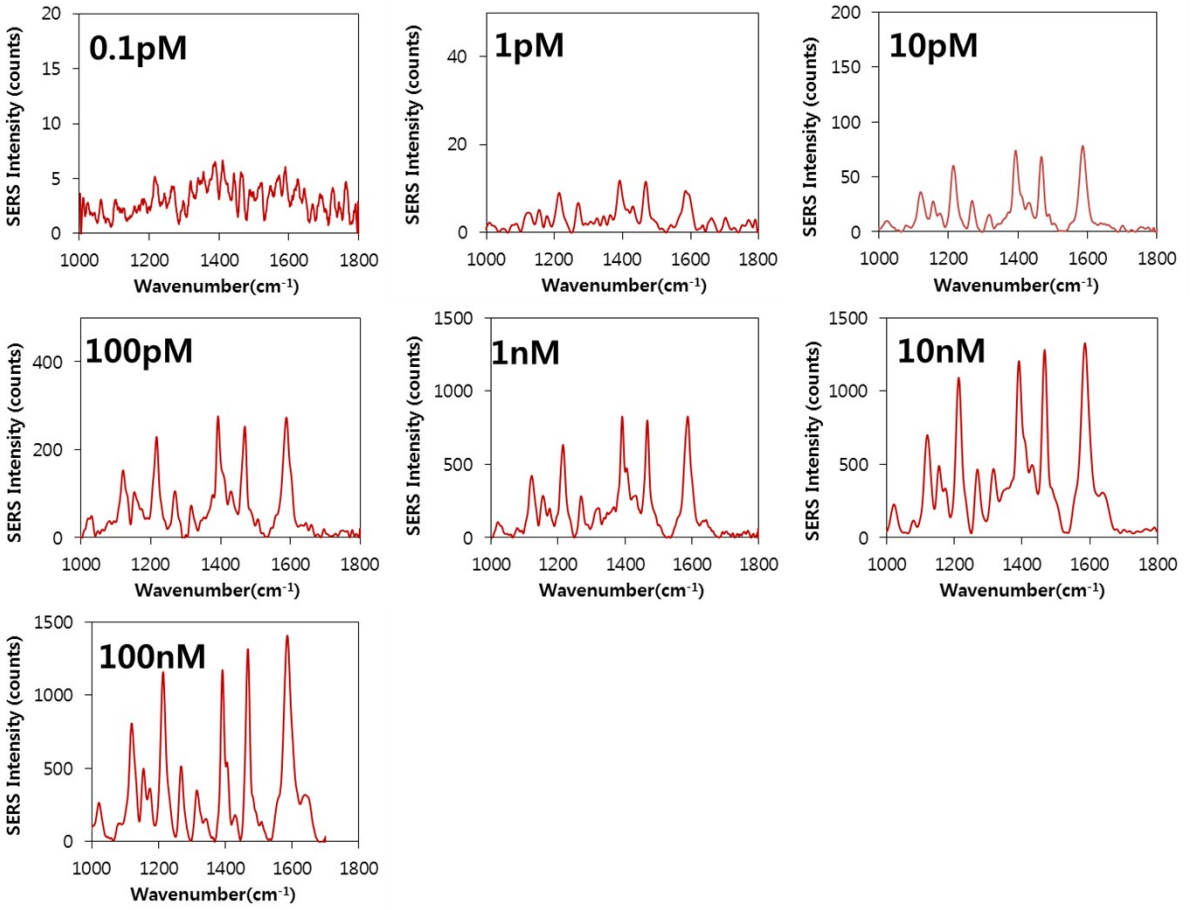

Figure S3. Obtained SERS in our assay depending on concentration of KRAS mutation (35G>T) as indicated on each graph