Supporting Information

Patterned Multiplex Pathogen DNA Detection by Au Particle-on-Wire SERS Sensor

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Experimental Details

DNA sequences. Table S1 shows the probe, target, and reporter DNA sequences (Genotech, Daejeon, Korea) used in this experiment. The four probe DNAs are species-specific sequences for the detection of *Enterococcus faecium*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, and *Vibrio vulnificus*, respectively. They were designed from the most variable regions with the bacterial 23S ribosomal DNA (rDNA),[1] which provides good discrimination of pathogenic bacteria and is widely employed for designing bacterial species-specific probes.[2] The label-free target DNAs were synthesized complementary to probe and reporter DNAs. The reporter DNAs have Raman dyes at the 5'-termini and thiol groups at 3'-termini. Therefore, they can be attached to Au nanoparticles (NPs) and gives the surface-enhanced Raman scattering (SERS) signals of Raman dyes.

Table S1. DNA sequences used in the experiments.

Name	Length (-mer)	Sequence (5'→ 3')
Efm003-20	20	HS-$(CH_2)_6$-ACATAGCACATTCGAGGTAG
Sau001-20	20	HS-$(CH_2)_6$-CAAAGGACGACATTAGACGA
Smal03-20	20	HS-$(CH_2)_6$-GCCATTCCAGTGAAGACGAG
Vvul02-20	20	HS-$(CH_2)_6$-GTAGTTGACGATGCATGTTC
T1	40	AGTACCGTGAGGGAAAGGCGCTACCTCGAATGTGCTATGT
T2	40	TGTTACGATTGTGTGAATACTCGTCTAATGTCGTCCTTTG
T3	35	TTCCCTCACGGTACTCTACCTCGAATGTGCTATGT
T4	35	TTCCCTCACGGTACTTCGTCTAATGTCGTCCTTTG
T5	35	TTCCCTCACGGTACTCTCGTCTTCACTGGAATGGC
T6	35	TTCCCTCACGGTACTGAACATGCATCGTCAACTAC
R1	20	Cy5-CGCCTTTCCCTCACGGTACT-$(CH_2)_3$-SH
R2	20	TAMRA-GTATTCACACAATCGTAACA-$(CH_2)_3$-SH
R3	15	Cy5-AGTACCGTGAGGGAA-$(CH_2)_3$-SH

Preparation of target DNAs from reference strains and clinical isolates. Table S2 shows the bacterial species used in this experiment. Reference bacteria were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), Korean Collection for Type Cultures (KCTC, Daejeon, Korea) and Korea Culture Center of Microorganisms (KCCM, Seoul, Korea). All clinical isolates were provided from Yonsei University College of Medicine, Seoul, Korea.[1]

Table S2. Microbial species used in this study.

Species	Source
E. faecium	KCCM 12118
S. aureus	KCTC 1621
S. maltophilia	ATCC 13637
V. vulnificus	KCTC 2962

DNAs from reference bacteria were isolated using the DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Clinical samples from infected patients were collected for routine culture detection, and then bacterial colonies were isolated on the plates for the identification of bacteria. DNAs from clinical isolates were extracted using the QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer's protocol.

Polymerase chain reaction (PCR) for target DNAs preparation was performed with the universal and species-specific primers by using the genomic DNAs from reference bacteria and clinical isolates. PCR was performed in 50 μl reactions containing 1× *Taq* buffer, 0.2 mM dNTPs, 2 units of *Taq* polymerase (Takara Shuzo Co., Shiga, Japan), 5 ng genomic DNA, 5 pM forward primer, and 25 pM reverse primer. Species-specific primers have the same sequences as those of species-specific probes. PCR using species-specific (Efm003-20 for *E. faecium*, Sau001-20 for *S. aureus*, Smal03-20 for *S. maltophilia* and Vvul02-20 for *V. vulnificus*) and universal primer (23BR, 5'-TTCGCCTTTCCCTCACGGTACT-3')

was performed under the following amplification conditions: 94 ^{o}C for 4 min, followed by 40 cycles of 95 ^{o}C for 30 s, 52 ^{o}C for 30 s, and 72 ^{o}C for 30 s, and a final extension at 72 ^{o}C for 5 min. PCR amplicons were then purified using PCR purification kit (iNtRON Co., Ltd, Gyeongido, Korea) according to the manufacturer's protocol.

Synthesis of Au Nanowires. Free standing single-crystalline Au nanowires (NWs) were synthesized by evaporation of Au powder (99.99%, Sigma-Aldrich), using the horizontal furnace system that was described previously.[3,4] The NWs were grown on a *c*-sapphire substrate placed a few cm downstream from an alumina boat filled with 0.02 g of Au powder. Argon gas flowed at a rate of 100 sccm, maintaining the total pressure at 1 to 5 Torr. The higher temperature zone of the furnace was heated to 1100 ˚C. The reaction time was about 30 min.

Preparation of probe DNA functionalized Au Nanowires. The thiolated probe DNAs were treated with 1 M dithiothreitol (DTT) to reduce the disulfide bonds and purified using NAP-5 column (GE healthcare Co.). The as-grown Au NWs on a sapphire substrate were incubated with 5 μM probe DNAs in 1 M KH_2PO_4 buffer (pH 6.75) at room temperature for 24 h. Excessive DNAs were washed by 0.2% (w/v) sodium dodecyl sulfate (SDS) solution. The probe DNA functionalized Au NWs were then transferred onto Si substrate which was modified with methoxy-polyethylene glycol (M-PEG) silane via a self-assembly technique before the transport of NWs. Briefly, the Si substrates were cleaned by piranha solution and then reacted with M-PEG solution in the nitrogen-filled reaction flask at 60 °C for 18 h. The M-PEG solution was prepared by adding 3 mM M-PEG silane (Sigma-Aldrich) in deoxygenated toluene (Sigma-Aldrich) containing 1 % triethylamine (Sigma-Aldrich) as catalyst. After the reaction, the substrates were sonicated in toluene and ethanol for 5 min, respectively, rinsed with distilled water, and dried under nitrogen stream.

In order to provide the positional addresses of each Au NWs, the patterning of Au NWs to which different probe DNAs are attached was accomplished by using a nanomanipulator. The nanomanipulator

is a tungsten tip (~ 100 nm diameter at the end) mounted on a three-dimensional piezoelectric stage. The Figure S1 shows optical microscope images of the patterning procedure of Au NWs. The nanomanipulator picked up a single NW by softly touching it and transferred the NW to the Si substrate. Finally, the four different probe DNA attached Au NWs are assembled to form an alphabetic character M (Figure S1d). This patterning of Au NWs provides positional addresses of each NW, enabling the multiplex pathogen DNA detection without additional identification tag.

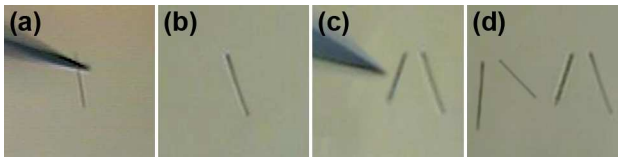

Figure S1. Optical microscope images of the pattering procedure of prove DNA attached Au NWs by using a nanomanipulator.

Preparation of reporter DNA functionalized Au Nanoparticles. The Au nanoparticles (~10 nm) were purchased from Sigma-Aldrich and used after centrifugations (13200 rpm, 30 min). The reporter DNAs with Raman dye (Cy5 and TAMRA) were also purified by the same method with probe DNAs. Au NPs were mixed with the reporter DNAs (5 μM) in 0.01 M phosphate buffer (PB) containing 0.01 % (w/v) SDS and incubated at room temperature for 12 h. In order to gradually increase the concentration of NaCl, the 2 M NaCl solution was added to Au NPs solution maintaining PB concentration of 0.01 M and SDS of 0.01% (w/v). This process was repeated at one more increment of 0.05 M NaCl until a concentration of 0.7 M NaCl was reached. The salting process was followed by incubation overnight at room temperature. To remove excess reporter DNAs, NPs solution was centrifuged and the supernatant was removed, leaving a pellet of Au NPs at the bottom. The NPs then were washed twice with 0.01% (w/v) SDS and finally suspended in phosphate buffered saline (PBS). By this preparation procedure we successfully made the reporter DNA attached NPs suspended in high salt buffer in stable condition for over 6 months. The inset of Figure S2 is the reporter DNA attached NPs solution showing clear red

color and no precipitation. This suggests that the nonspecific electrostatic aggregation of Au NPs does not occur in a buffer solution. Measurement of SERS spectra further confirmed that there is no appreciable aggregation of NPs. The blue spectrum of Figure S2 obtained from the NP solution does not show the SERS signals of Cy5 because the fluorescence is much stronger. Since Cy5 is located sufficiently far from the Au NPs in the solution phase, fluorescence from Cy5 is not quenched and observed dominantly. If there were aggregated Au NPs in a significant concentration in this solution, SERS signals of Cy5 should have been observed upon the fluorescence continuum.[5,6] The magenta spectrum in the figure shows a SERS spectrum of Cy5 that was measured from the intentionally aggregated NPs by centrifugation and still in a solution phase. Taken together, these results suggest that the NPs exist as a separated particle form without aggregation in the solution phase.

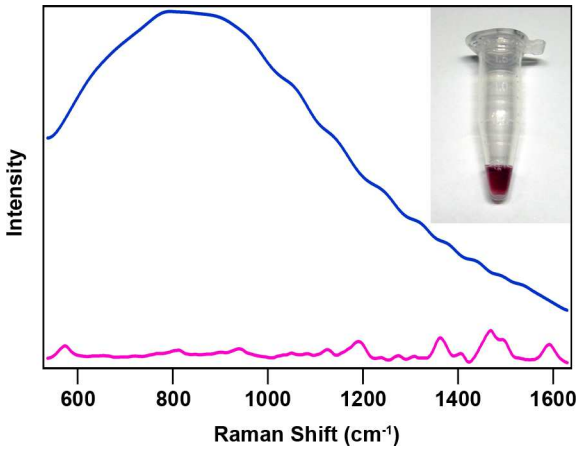

Figure S2. SERS spectra measured form the reporter DNA attached NPs solution (blue spectrum) and intentionally aggregated NPs by centrifugation (magenta spectrum). The inset is an image of reporter DNA attached NPs solution after stored at 4 °C over 6 months.

Hybridization. The target DNAs were added in hybridization buffer that is a mixture of 6× SSPE (0.9 M NaCl, 10 mM $NaH_2PO_4 \cdot H_2O$, 1 mM EDTA, pH 7.4), 20% (v/v) formamide solution (Sigma-Aldrich), and 0.1% (w/v) SDS. The probe DNA functionalized Au NWs were hybridized with hybridization solution at 30 ˚C for 6 h, and then washed with 2× SSPE buffer. The resultant NWs were incubated with

reporter DNA attached Au NPs in PBS containing 0.1 % (w/v) SDS for 6 h. After washing with PBS containing 0.1 % (w/v) SDS for 5 min, rinsing twice with distilled deionized water, and drying under nitrogen stream, SERS spectra from the Au particle-on-wire sensor were measured in ambient conditions.

Instrumentation. SERS spectra were measured from a micro-Raman system based on an Olympus BX41 microscope (Figure S3). The 633 nm radiation of a He-Ne laser (Melles Griot) was used as an excitation source and the laser light was focused on a sample through a ×100 objective (NA = 0.7, Mitutoyo). The SERS signals were recorded with a thermodynamically cooled electron multiplying charge coupled device (EMCCD, Andor) mounted on the spectrometer with a 1200 groove/mm grating. A holographic notch filter was used to reject the laser light.

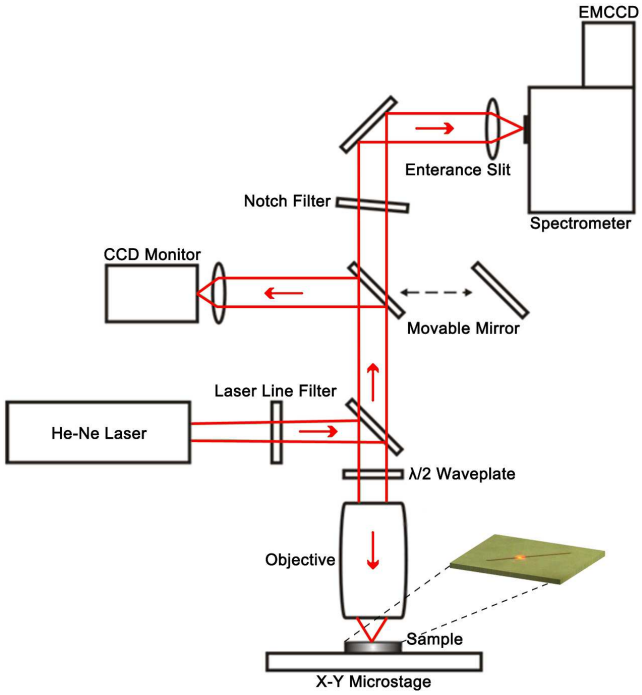

Figure S3. Schematic illustration of micro-Raman system for the measurement of SERS from Au particle-on-wire structure.

The scanning electron microscope (SEM) images were taken on a Hitachi S-4800 operated at 10 kV. The UV-vis absorption spectra were measured using a spectrometer (JASCO, Japan). The absorption spectrum of Au NPs was collected in a colloidal solution state and that of Au NWs was obtained after transferring on a quartz substrate. The absorption spectrum of particle-on-wire structures was acquired from the same Au NWs on a quartz substrate after Au NPs are attached to the NWs through DNA hybridization.

Polarization dependence. The polarization dependence of Au particle-on-wire structures was presented in Figure S4. The SERS signals are maximized when the polarization is perpendicular to the NW axis and minimized when the polarization is parallel.[7] Since we controlled the polarization direction of excitation laser perpendicular to the NW axis by carefully rotating a half-wave plate, the similar SERS signals could be obtained from various sensors which have different orientations.

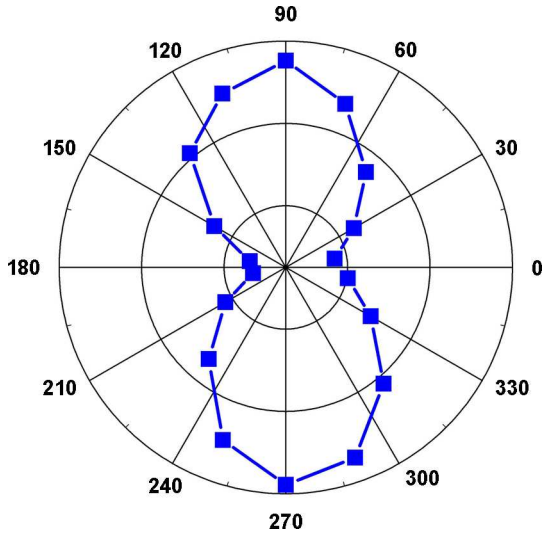

Figure S4. Polar plot of SERS intensities of 1580 cm^{-1} Raman band with respect to the angle between the long axis of NW and polarization direction of incident laser light.

Scanning electron microscope images. Figure S5 shows the SEM images of the NPs on NW surface at four different positions. The attachment of single and separate NPs on the surface of NW without aggregation is clearly seen.

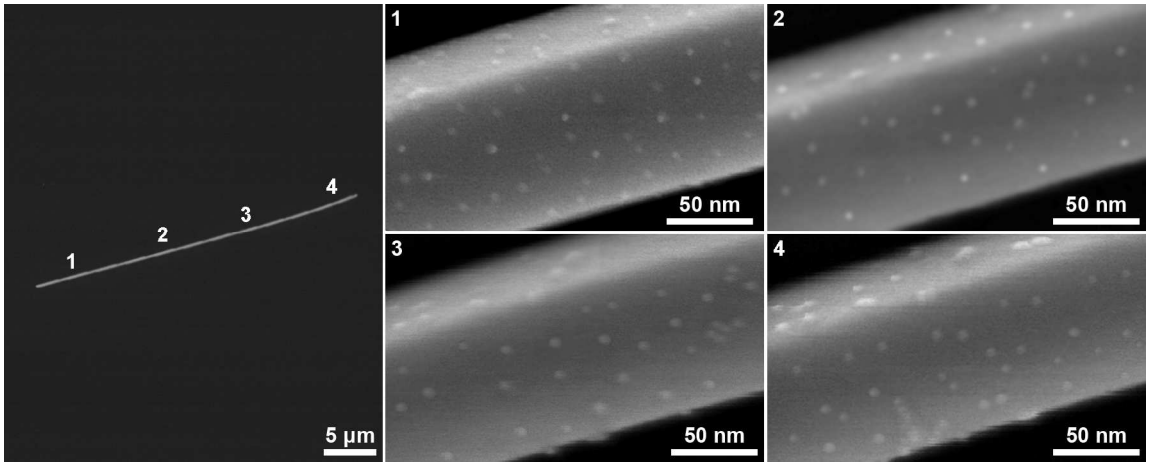

Figure S5. Low and high magnified SEM images of a typical Au particle-on-wire sensor.

Reproducibility. Figure S6a shows the SERS spectra of Cy5 measured at four different positions on a single particle-on-wire sensor. The measured spectra are fairly consistent in intensity as well as shape. This result indicates that the NW-NP hot spots are uniformly created along the whole Au NW. In addition, we tested the reproducibility for a number of Au particle-on-wire systems. The SERS spectra taken from ten particle-on-wire structures and a histogram of the intensities of 1580 cm^{-1} band show good reproducibility (Figure S6b). The slight intensity fluctuations can be ascribed to variations in Au NW diameters. This result demonstrates the reliable reproducibility of Au particle-on-wire sensor for multiplex DNA detection.

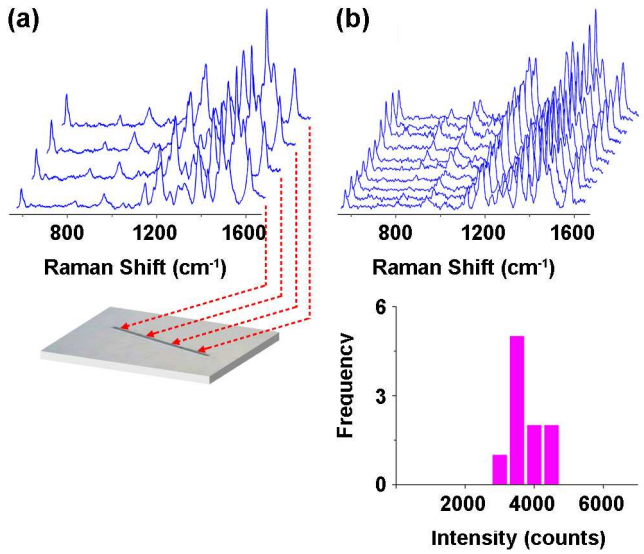

Figure S6. (a) SERS spectra of Cy5 at four different positions on a single Au particle-on-wire sensor (top) and corresponding positions of the sensor (bottom). (b) SERS spectra of Cy5 from ten different Au particle-on-wire sensors (top) and histogram of the SERS intensities of the 1580 cm^{-1} band.

Multiplex DNA detection. Figure S7 shows the SERS results of multiplex DNA detection by using Au particle-on-wire sensor. Unmodified 35-mer oligonucleotides (T3, T4, T5, and T6 in Table S1) were used as target DNAs. The SERS signals were enhanced only at the Au NWs coated with probe DNAs complementary to the target DNAs.

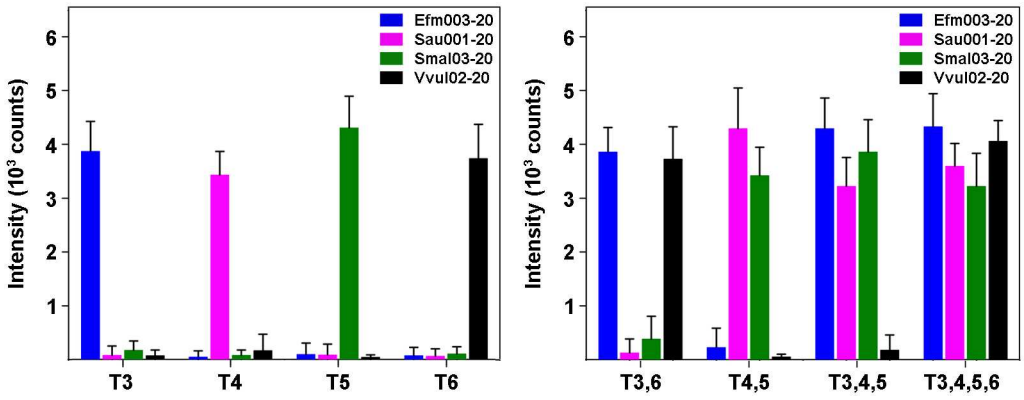

Figure S7. Multiplex DNA detection by using Au particle-on-wire SERS sensor.

Enhancement factor. We estimated the enhancement factor (EF) of the Au particle-on-wire sensor with respect to the single Au NW through the following expression:[7]

$$EF = (I_{SERS} \times N_{Single})/(I_{Single} \times N_{SERS})$$

Where I_{SERS} is the peak intensity for SERS spectra of 1580 cm^{-1} band and I_{Single} is noise intensity which is the maximum possible signal from the reporter DNA attached single Au NW. N_{Single} is the number of molecules on single Au NW and is proportional to the laser illuminated area. N_{SERS} is the number of molecules at the NW-NPs hot spot regions and is proportional to the effective surface area of Au NPs. Assuming uniform adsorption of reporter DNA, we get

$$EF = (I_{SERS} \times A_{Single})/(I_{Single} \times A_{SERS})$$

The A_{Single} indicates the laser illuminated area of a single Au NW and was determined based on the laser spot diameter (~ 500 nm) and NW diameter (~ 150 nm), so that A_{Single} is 2.4×10^5 nm^2. A_{SERS} was estimated by calculating the surface area of a single Au NPs in the hot spot region and multiplying the number of NPs in the laser spot. We choose the hot spot region for which the NW-NP gap is less than 3 nm and the number of Au NPs illuminated by the laser light was determined about 100 from Figure 1c, therefore, the calculated A_{SERS} is about 9.4×10^3 nm^2. The ratio of I_{SERS} and I_{Single} is about 100. Finally, the EF of Au particle-on-wire SERS sensor was calculated to be 2.6×10^3.

References

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