DOI: 10.1002/chem.201003372

Detection of Single Nucleotide Polymorphisms by a Gold Nanowire-on-Film SERS Sensor Coupled with S1 Nuclease Treatment**

Seung Min Yoo,[a] Taejoon Kang,[b] Bongsoo Kim,*[b] and Sang Yup Lee*[a]

Abstract: Single nucleotide polymorphisms (SNPs) can serve as important biomarkers for genetic diseases, for which accurate detection of SNPs is essential for early diagnosis. We have developed a novel SNP sensor by combining a Au nanowire-on-film surface-enhanced Raman scattering (SERS) platform with S1 nuclease reaction. The combined sensor system provides reproducible SERS signals only in the presence of perfectly matched target DNAs, to probe DNAs as a result of single-stranded DNA-specific degradation by S1 nuclease. Furthermore, point mutations in DNA causing Wilson disease and Avellino corneal dystrophy were successfully identified by this sensor, thereby indicating its practical ability to diagnose genetic diseases.

Keywords: DNA · enzymes · nanowires · polymorphism · Raman spectroscopy

Introduction

Single nucleotide polymorphisms (SNPs) are the most abundant genetic variations that account for more than 90% of all sequence variations, as 3–10 million of them exist throughout the human chromosome.[1,2] SNPs can provide important clues for tracking disease-related genes, particularly those affecting disease development at various stages of life as well as disease susceptibility and drug sensitivity,[3–5] and therefore the ability to detect SNPs is of great clinical importance. Disease-associated point mutations can change amino acid sequences of proteins, which can in turn cause different levels of protein production and different splicing, thereby resulting in various genetic disorders.[1,2] Accordingly, detection of mismatched DNA sequences is vital in early diagnosis, prevention, and treatment of human diseases, and will become more important for developing personalized medicine. Thus, much effort has been exerted to develop a rapid, accurate, and multiplex SNP detection system.

Traditionally, SNPs have been detected mainly by employing diverse sequencing-based methods such as gel-based sequencing, restriction fragment length polymorphism (RFLP), and single-strand conformation polymorphism (SSCP). Recently, advanced techniques such as a conformation-sensitive gel electrophoresis (CSGE),[6,7] capillary electrophoresis (CE),[8] and DNA microarrays[9] were used in practice for high-throughput detection of single-base mismatches in DNA.

Recently, novel detection methods have been developed that employ rapidly advanced nanotechnology. For example, a variety of sensing techniques, such as fluorescence,[10,11] electrochemistry,[12] surface plasmon resonance,[13] colorimetry,[14,15] and surface-enhanced Raman scattering (SERS)[16–22] utilizing nanostructures such as nanowires (NWs) and nanoparticles, were reported for effective detection of SNPs. For real-world applications, however, these nanotechniques need further improvement.

Herein, we report an advanced Au NW-on-film SERS sensor that utilizes S1 nuclease treatment to identify single-base mismatches in double-stranded DNA (dsDNA). This system has the following distinct merits. First, the Au NW-on-film platform provides highly reproducible SERS signals due to its well-defined geometric architecture (Figure 1 and Figure S1 in the Supporting Information).[23] As minimizing detection error is critically important in SNP sensing, the NW-on-film sensor significantly reduces the detection error of single-base mismatches. Second, by employing S1 nuclease, which recognizes and cleaves the single-base mismatches in dsDNA, detection error is decreased further relative to the SNP detection methods utilizing thermal melting of DNA hybridization. Additionally, multiply combining these NW-on-film sensors would enable the multiplex detection that is required for efficient SNP detection. As the present

[a] Dr. S. M. Yoo,[+] Prof. S. Y. Lee
Department of Chemical and Biomolecular Engineering (BK21 Program)
KAIST
Daejeon 305-701 (Korea)
Fax: (+82) 42-350-8800
E-mail: leesy@kaist.ac.kr

[b] Dr. T. Kang,[+] Prof. B. Kim
Department of Chemistry
KAIST
Daejeon 305-701 (Korea)
Fax: (+82) 42-350-2810
E-mail: bongsoo@kaist.ac.kr

[+] These authors contributed equally to this work.

[**] SERS: surface-enhanced Raman scattering.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201003372.

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

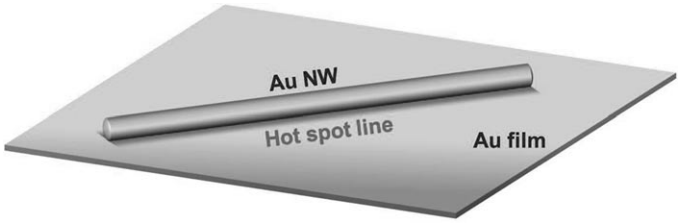

Figure 1. Schematic representation of the Au NW-on-film SERS-active platform constructed by placing a single-crystalline Au NW on a Au film. This sandwich nanostructure provides a SERS hot line at the gap between the NW and the film.

system only requires a single S1 nuclease reaction even for multiplex SNP detection, the experimental process is simpler than other methods employing multiple DNA hybridizations or separation of the resultant products.[16,17,21,22]

To develop disease diagnostic sensors, it is important to verify that the sensor can identify SNPs in clinical practice. Clinical tests demonstrated that the NW-on-film sensor successfully identified mutations in the relevant DNAs in Avellino corneal dystrophy (ACD) and Wilson disease (WD). We expect that high-throughput SNP sensors that can diagnose various genetic diseases in a single assay can be developed by combining multiple Au NWs, each modified with the corresponding DNAs.

Results and Discussion

SERS is a fascinating phenomenon that enormously increases Raman signals of molecules near the gap of noble-metal nanostructures.[23] It provides unique advantages, such as single-molecule-level sensitivity,[24–26] molecular specificity,[27] and insensitivity to quenching,[18,28] for DNA detection including SNPs. However, fluctuating and irreproducible signal enhancement by SERS sensors has been hampering quantitative and reliable sensing. We overcame this problem by newly fabricating a Au NW-on-film SERS-active platform composed of a single Au NW on a Au film. This Au NW-on-film structure has a simple and well-defined sandwich architecture, which provides a line of SERS hot spots (i.e., a SERS hot line) at the gap between the NW and the film upon optical excitation (Figure 1).[23] Because the hot spots are created at the NW position, by focusing the excitation laser on the NW through the optical microscope we obtained high-quality SERS spectra with reliable reproducibility, good time stability, and excellent sensitivity (Figure S1, Supporting Information). By adopting this efficient NW-on-film nanoplatform for SERS, we detected SNPs in a highly quantitative and sensitive manner.

Figure 2 shows a schematic representation of the SERS-based single-base-mismatched sequence detection by using S1 nuclease that specifically hydrolyzes single-stranded DNA (ssDNA). The probe DNA is attached to a Au NW through a thiol group. When a target DNA is immersed in a solution with a probe DNA, the probe DNA can hybridize the target DNA even in the presence of SNPs in the target

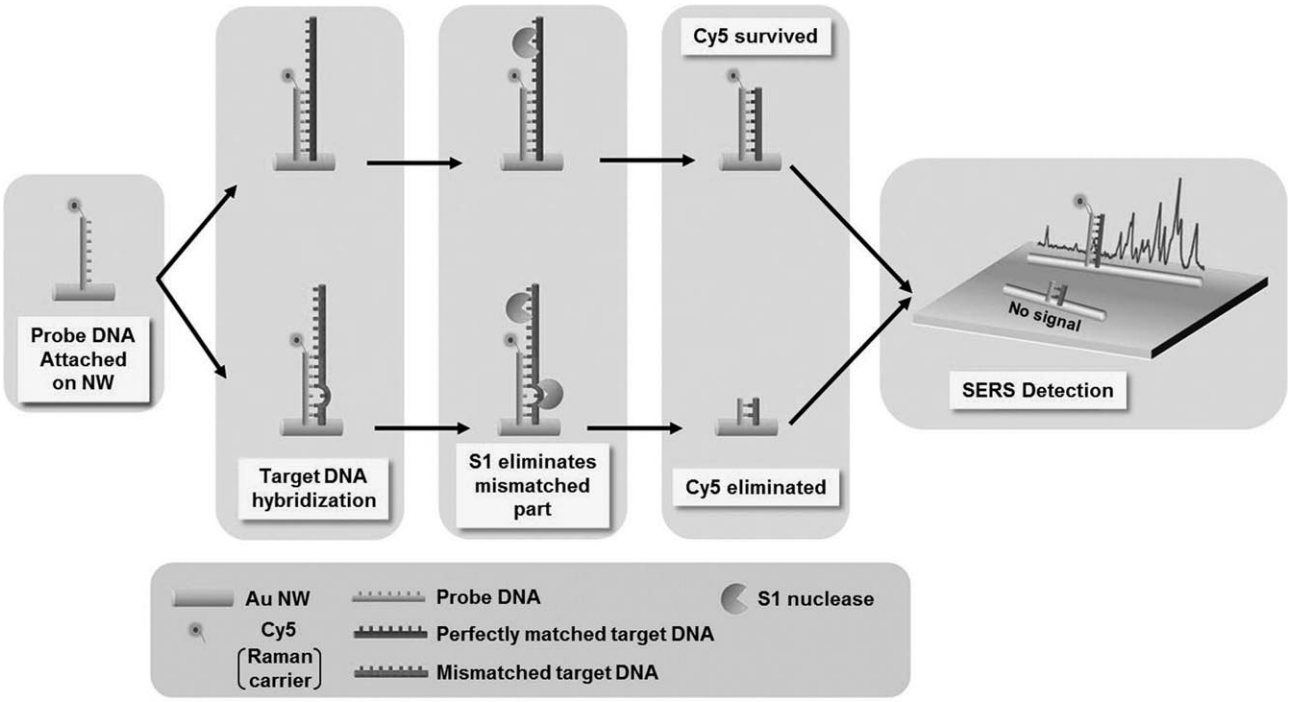

Figure 2. Schematic representation of the strategy for the detection of single-base-mismatched sequences in DNA by a Au NW-on-film SERS sensor combined with S1 nuclease reaction.

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

DNA. If perfectly matched (PM) target DNA is hybridized with a probe DNA, it protects the probe DNA from S1 nuclease attack so that the Raman carrier Cy5 remains at a probe DNA near the Au NW, thereby providing a strong SERS signal. If the target DNA hybridized with probe DNA has a mismatch (MM), then Cy5 is removed by the hydrolysis of S1 nuclease and the SERS signal disappears. From the difference of SERS signals before and after treatment with S1 nuclease, the presence of SNPs can be clearly identified. This specific enzymatic digestion is a key step for increasing the specificity and reducing the error of SNP detection. The specificity of S1 nuclease has been well known and effectively employed in various DNA sensing applications, such as short tandem repeat detection,[29] quantification of the level of alternatively spliced mRNAs,[30,31] and single-base mismatch discrimination.[9,15,32–35] To enhance discriminatory power, the probe DNAs were designed to have a point mutation in the middle (eighth) of the 15-mer sequences, which is known to be destabilized more than a location near the extremities.[36]

To maximize the discriminating ability, we optimized the concentration and reaction time of S1 nuclease. First, two sets of Au NWs modified by Cy5-labeled probe DNA (A_1; Table S1, Supporting Information) were prepared and hybridized with two kinds of target DNA (B_1 for PM and B_4 for MM; Table S1). These two different sets of hybridized Au NWs were incubated in solutions containing various concentrations of S1 nuclease (20, 40, 60, 80, and 100 U mL^{-1}) at 37 °C for 20 min. After washing and fabricating two NW-on-film structures with these two sets of Au NWs, SERS spectra were measured. The SERS intensity difference between the two sets of Au NW samples hybridized with PM or MM target DNAs was maximized at an S1 nuclease concentration of 40 U mL^{-1} (Figure 3a). At S1 nuclease concentrations lower or higher than 40 U mL^{-1}, incomplete cleavage due to insufficient enzyme or overdigestion by excessive enzyme, respectively, were observed. Second, the S1 nuclease reaction time was optimized to maximize the SERS intensity difference (Figure 3b). Same as above, two sets of Au NWs modified by Cy5-labeled probe DNAs were hybridized with PM and MM target DNAs, respectively, and treated with 40 U mL^{-1} S1 nuclease at 37 °C for various incubation times (10, 15, 20, 30, and 40 min). The discriminatory power of the assay was maximum at an incubation time of 20 min. Figure 3c shows SERS spectra measured before and after the treatment with S1 nuclease at an optimized reaction time of 20 min, concentration of 40 U mL^{-1}, and 37 °C. Before S1 nuclease treatment, the SERS signal of Cy5 was observed with all Au NW sets. After treatment with S1 nuclease, the SERS spectra from Au NWs hybridized with a PM target DNA remain strong, whereas those from a MM target DNA almost disappear. Note that comparison of the SERS signal intensities after S1 nuclease treatment is enough for the identification of SNPs under these test conditions.

To identify the resultant mutated nucleotide among the four bases of adenine (A), thymine (T), guanine (G), and cytosine (C), four Au NWs modified by four different probe DNAs (A_1, A_2, A_3, and A_4; Table S1, Supporting Information) having the same sequences except the mutated point as T, C, G, or A, were hybridized with four kinds of target DNAs (B_1, B_2, B_3, and B_4; Table S1) with point mutations; B_i was complementary to A_i. Then, the SERS spectra were measured after incubation with S1 nuclease. Figure 4a indicates that SERS signals were observed only when NWs modified by A_i probe DNA were immersed together with complementary B_i target DNA in a sample solution, thereby demonstrating the superb SNP detection ability of this sensor.

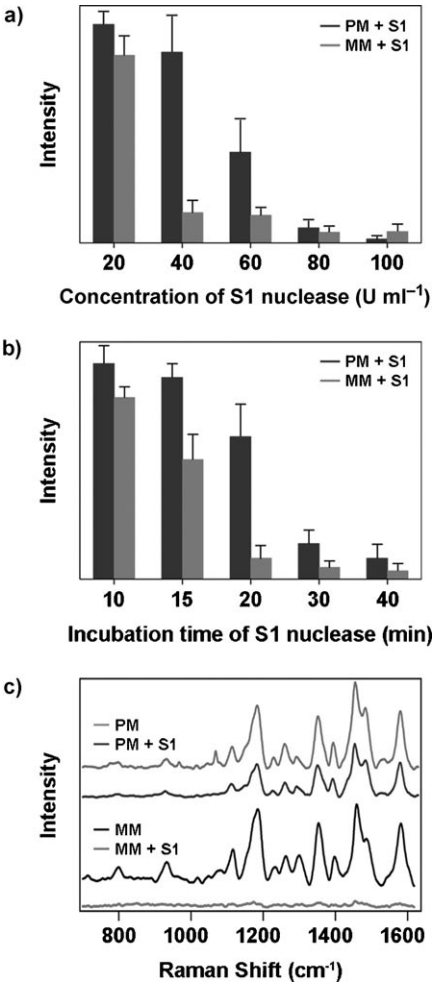

Figure 3. Determination of the optimal conditions for S1 nuclease reaction on the Au NW-on-film SERS sensor. a) Effect of the concentration of S1 nuclease on the SERS intensity of the 1580 cm^{-1} band. The Au NWs assembled with probe DNAs were hybridized with PM or MM target DNAs, and then exposed to various concentrations of S1 nuclease (10, 20, 40, 60, 80, and 100 U mL^{-1}) at 37 °C for 20 min. After the transport of Au NWs on Au film, SERS spectra were measured. b) Effect of the exposure time of S1 nuclease on the SERS intensity. The Au NWs assembled with probe DNAs were hybridized with PM or MM target DNAs, and then exposed to S1 nuclease (40 U mL^{-1}) at 37 °C for various incubation times (10, 15, 20, 30, and 40 min). c) SERS spectra obtained before and after the exposure to S1 nuclease. The enzyme reaction was performed at a concentration of 40 U mL^{-1} at 37 °C for 20 min.

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

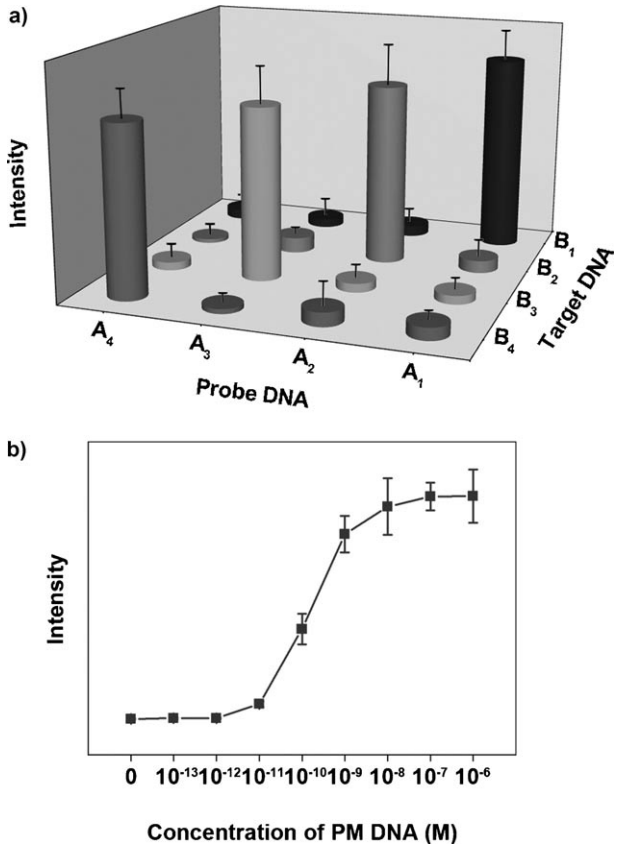

Figure 4. a) SERS intensities of the 1580 cm^{-1} band from probe DNA-immobilized NWs (A_1, A_2, A_3, and A_4; Table S1, Supporting Information) after hybridizing with different target DNAs (B_1, B_2, B_3, and B_4; Table S1) and exposure to S1 nuclease. A_i is perfectly complementary to B_i. b) Plot of 1580 cm^{-1} band intensity versus concentration of PM target DNAs.

The detection limit of the sensor was then determined. Figure 4b shows a plot of the intensity of the 1580 cm^{-1} Raman band versus concentration of PM target DNAs. The SERS intensity is linearly proportional to concentrations of DNA from 10^{-11} to 10^{-9} M. At concentrations below 10^{-11} M, a Raman signal was not detectable, which indicates that the detection limit of this sensing system is about 100 pM.

The diagnostic ability of this sensor was then clinically tested. Most genetic disorders are divided into two types: autosomal dominant and autosomal recessive disorders. Humans have two copies of DNA for each gene. Autosomal dominant requires only a single mutated copy of DNA for disease expression, whereas autosomal recessive requires two copies of mutated DNA. For effective diagnosis and treatment of genetic disorders, therefore, genetic mutations in both copies of DNA should be identified.

Firstly, we chose WD, a representative autosomal recessive disorder, and examined the Arg778Leu mutation (C***G***G→C***T***G) in the *ATP7B* gene, the most frequent mutation in Korean WD patients.[37] The four NWs modified by four different probe DNAs (A_1, A_2, A_3, and A_4; Table S1, Supporting Information), which have sequences of C***T***G, C***C***G, C***G***G, and C***A***G, respectively, were hybridized with unknown target DNAs (91 base pairs (bp) in size; Figure S2, Supporting Information). The four NWs were then treated by S1 nuclease and laid on a Au film. Figure 5 shows the SERS spectra obtained from these four NWs. Figure 5a displays a target DNA showing a SERS signal only at A_3 probe DNA (C***G***G), thus indicating that both copies of human DNA have a nonmutated sequence of C***G***G and the person is healthy. Figure 5b displays a target DNA showing a SERS signal only at A_1 probe DNA (C***T***G) and no signal at A_3 probe DNA, which means that both copies of DNA are mutated from C***G***G to C***T***G and the person is ill. Figure 5c shows that a SERS signal was obtained from A_1 and A_3 probe DNAs, meaning that one copy is mutated and the other is not and the person is healthy. These diagnoses agree with those from direct sequencing.

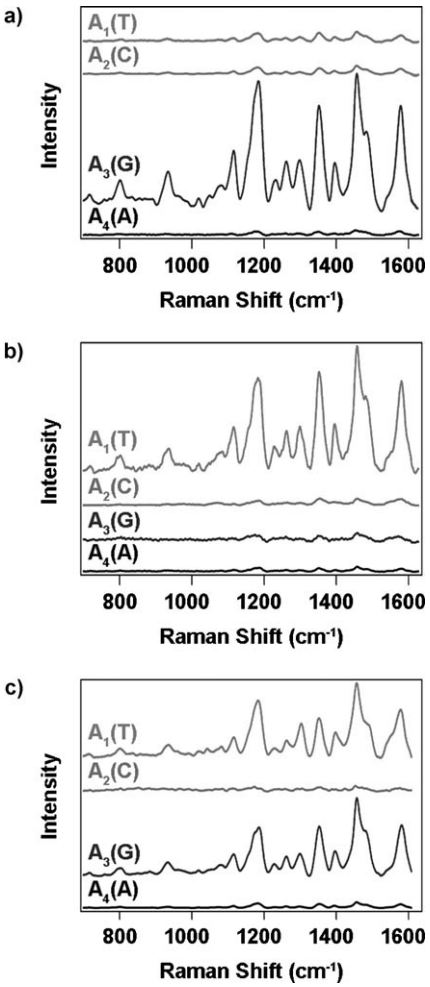

Figure 5. SERS spectra for validation of WD-related mutation. Target DNA has the a) nonmutated sequence of C***G***G, b) mutated sequence of C***T***G, and c) both sequences of C***G***G and C***T***G.

Secondly, we examined the Arg124His mutation (C***G***C→C***A***C) in the human transforming growth factor-β-induced (*BIGH3*) gene responsible for ACD.[38–40] ACD is an autosomal dominant disorder that makes the cornea become

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

blurry, finally leading to eyesight loss. In particular, when an ACD patient receives in situ keratomileusis (LASIK) surgery, ACD develops rapidly, causing blindness. To detect point mutation in the *BIGH3* gene, target DNAs (147 bp in size) from six clinical samples were incubated with Au NWs carrying four different probe DNAs (C_1, C_2, C_3, and C_4; Table S1, Supporting Information). The C_1 probe contained a nonmutated sequence (G) at position 371 in the *BIGH3* gene whereas C_2, C_3, and C_4 contained mutations A, T, and C, respectively, at the same nucleotide position. A SERS signal appearing at C_1 probe DNA indicates that both copies of DNA are not mutated, whereas a signal only at C_2 probe DNA indicates that both copies of DNA are mutated. SERS signals at both C_1 and C_2 probe DNAs indicate that only a single copy of DNA is mutated. Among the six clinical samples examined by the SERS sensor, two samples showed a SERS signal at the C_1 probe DNA, two samples at the C_2 probe DNA, and the remaining two samples at the C_1 and C_2 probe DNAs (Figure S3, Supporting Information). The SERS diagnosis results were consistent with those obtained by DNA sequencing.

By employing the Au NW-on-film SERS sensors, the mutations causing the genetic disorders WD and ACD have been successfully identified. These clinical tests demonstrate that this sensor provides accurate and convenient diagnosis of both autosomal dominant and autosomal recessive disorders. Even though the sequences of the two disease-relevant DNAs are different, the correct diagnosis was accomplished by using a simple enzymatic reaction without requiring complicated processes of finding optimal conditions for sequence-specific hybridization.

Conclusion

We have developed a Au NW-on-film SERS sensor coupled with S1 nuclease treatment for the efficient and accurate detection of single-base mismatches in DNA. A well-defined NW-on-film nanostructure and careful optimization of the S1 nuclease reaction minimize the detection error of SNPs synergistically. Successful identification of SNPs causing disease in real patients demonstrated the efficiency of this system as an effective SNP diagnostic sensor. Generally, about tens or hundreds of different mutations have been known to cause ACD or WD. As only a single mutation can cause the disease, a multiplex SNP sensor that can handle a large number of DNAs simultaneously is highly desirable. The very small size of this NW-on-film sensor of a few micrometers would make it possible to detect SNPs in high-throughput systems by employing a large number of Au NWs modified by the corresponding DNAs.

Experimental Section

Synthesis of Au NWs: Single-crystalline Au NWs were synthesized by direct evaporation of pure Au powder (99.99%, Sigma–Aldrich), by using a horizontal furnace system.[23] The NWs were grown on a c-sapphire substrate that was placed a few centimeters downstream from an alumina boat filled with Au powder (0.02 g). Argon gas was provided at a rate of 100 sccm, maintaining a total pressure of 1 to 5 Torr. The higher-temperature zone of the furnace was heated to 1100 °C.

Materials: All oligonucleotides were synthesized and purified by Genotech, Daejeon, Korea (Table S1, Supporting Information). Thiol-functionalized probe DNAs were treated with 1 M dithiothreitol (DTT, Sigma–Aldrich) to reduce disulfide bonds and were purified by using a NAP-5 column (GE Healthcare Co.). Genomic DNAs from clinical samples were kindly provided by Yonsei Severance Hospital for ACD samples (Seoul, Korea).

Immobilization of probe DNAs on the Au NWs: The 5 μM probe DNAs in 1 M KH_2PO_4 (pH 6.75) were immobilized on the Au NWs at room temperature for 24 h, and excess DNA was removed by washing with 0.2% (w/v) sodium dodecyl sulfate (SDS) for 5 min.

Preparation of target DNAs: Genomic DNA samples from normal human chromosomes were extracted by using the JETQUICK Blood & Cell Culture DNA Spin Kit (GENOMED GmbH, Löhne, Germany). The region containing the Arg778 sequence was amplified in reaction mixture (50 μL) containing 1× Taq buffer, (Takara Shuzo Co., Shiga, Japan), 0.2 mM deoxyribonucleotide triphosphates (dNTPs), Taq polymerase (2 units; Takara Shuzo Co., Shiga, Japan), genomic DNA (5 ng), forward primer (5 pmol; 5′-GCCCTGTGACATTCTTCGA-3′), and reverse primer (5 pmol; 5′-GCTGCTGTTACCTTTGCCA-3′) under the following amplification conditions: 94 °C for 4 min, followed by 40 cycles of 94 °C for 1 min, 57 °C for 1 min, and 72 °C for 30 s, and a final extension to 72 °C for 5 min. The polymerase chain reaction (PCR) product was cloned into the pTop V2 vector (Topcloner kit, Enzynomics Co. Inc., Daejeon, Korea) and the resulting WT plasmid was used as a template for mutagenesis. By using the EZchange site-directed mutagenesis kit (Enzynomics Co. Inc.) and the mutagenic primer pair (forward primer: 5′-GTGGCTGGAACACTTGGCA-3′ and reverse primer: 5′-AGGCCCAGGGCAATGAACACA-3′), the product was cloned into pTop V2 vector, thereby resulting in WD plasmid. The target DNAs for hybridization were prepared from WD and WT plasmid in reaction mixtures (50 μL) containing 1× Taq buffer, 0.2 mM dNTPs, Taq polymerase (2 units; Takara Shuzo Co., Shiga, Japan), genomic DNA (5 ng), forward primer (5 pmol; 5′-GCCCTGTGACATTCTTCGA-3′), and reverse primer 25 pmol; 5′-GCTGCTGTTACCTTTGCCA-3′) under the following amplification conditions: 94 °C for 4 min, followed by 40 cycles of 94 °C for 1 min, 57 °C for 1 min, and 72 °C for 30 s, and a final extension to 72 °C for 5 min. Genomic DNA samples from ACD patients and non-ACD controls were extracted using the JETQUICK Blood & Cell Culture DNA Spin Kit from the peripheral blood of participants who provided their informed consent before the tests, and were used as template DNAs for the PCR reaction. The region containing the ACD-associated mutation was amplified in reaction mixtures (50 μL) containing 1× Taq buffer, 0.2 mM dNTPs, Taq polymerase (2 units; Takara Shuzo Co.), genomic DNA (5 ng), forward primer (5 pmol; 5′-AGCCCTACCACTCTCAAACC-3′), and reverse primer 25 pmol; 5′-CAGGCCTCGTTGCTAGGG-3′) under the following amplification conditions: 94 °C for 4 min, followed by 40 cycles of 94 °C for 30 s, 58 °C for 1 s, and 72 °C for 30 s, and a final extension to 72 °C for 5 min.

Hybridization and S1 nuclease reaction: The synthesized oligo and amplified products (5 mM) were directly added to the hybridization solution containing 6× SSPE (0.9 M NaCl, 10 mM NaH_2PO_4·H_2O, 1 mM EDTA, pH 7.4), 20% (v/v) formamide (Sigma–Aldrich), and 0.1% (w/v) SDS and hybridized with NWs at 30 °C for 6 h. After washing with 2× SSPE buffer containing 0.1% (w/v) SDS for 5 min, the NWs were immersed into 1× S1 nuclease buffer for pre-equilibration at 37 °C for 5 min and then S1 nuclease (Takara Shuzo Co.) was added to the buffer with agitation. After nuclease treatment, the NWs were sequentially washed with 2× saline sodium citrate (SSC) containing 0.1% (w/v) SDS for 5 min and dried. The Au NWs were then transferred onto Au film for the construction of the Au NW-on-film architecture by using a nanomanipulator.

Instrumentation: The nanomanipulator consisted of a tungsten tip (≈100 nm diameter at the end) mounted on a three-dimensional piezo-

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

electric stage. SERS spectra were measured from a micro-Raman system based on an Olympus BX41 microscope. The 633 nm radiation of a He–Ne laser was used as an excitation source and the laser light was focused on the 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).

Acknowledgements

The work of S.Y.L. was supported by the IT Leading R&D Project from the Ministry of Knowledge Economy through IITA, and the World Class University Program of MEST. Work on the detection of Avellino mutations was supported by Avellino Co. We thank Prof. E. K. Kim at Severance Hospital for providing clinical samples. The work of B.K. was supported by NRF of Korea Grants (NRL progam: 2010-0018868) and the "Center for Nanostructured Materials Technology" under "21st Century Frontier R&D Programs" (2011K000210) of the MEST, Korea.

[1] D. G. Wang, J. B. Fan, C. J. Siao, A. Berno, P. Young, R. Sapolsky, G. Ghandour, N. Perkins, E. Winchester, J. Spencer, L. Kruglyak, L. Stein, L. Hsie, T. Topaloglou, E. Hubbell, E. Robinson, M. Mittmann, M. S. Morris, N. P. Shen, D. Kilburn, J. Rioux, C. Nusbaum, S. Rozen, T. J. Hudson, R. Lipshutz, M. Chee, E. S. Lander, *Science* **1998**, *280*, 1077–1082.
[2] R. Sachidanandam, D. Weissman, S. C. Schmidt, J. M. Kakol, L. D. Stein, G. Marth, S. Sherry, J. C. Mullikin, B. J. Mortimore, D. L. Willey, S. E. Hunt, C. G. Cole, P. C. Coggill, C. M. Rice, Z. M. Ning, J. Rogers, D. R. Bentley, P. Y. Kwok, E. R. Mardis, R. T. Yeh, B. Schultz, L. Cook, R. Davenport, M. Dante, L. Fulton, L. Hillier, R. H. Waterston, J. D. McPherson, B. Gilman, S. Schaffner, W. J. Van Etten, D. Reich, J. Higgins, M. J. Daly, B. Blumenstiel, J. Baldwin, N. S. Stange-Thomann, M. C. Zody, L. Linton, E. S. Lander, D. Altshuler, *Nature* **2001**, *409*, 928–933.
[3] N. Risch, K. Merikangas, *Science* **1996**, *273*, 1516–1517.
[4] L. Kruglyak, *Nat. Genet.* **1999**, *22*, 139–144.
[5] S. M. Yoo, J. H. Choi, S. Y. Lee, N. C Yoo, *J. Microbiol. Biotechnol.* **2009**, *19*, 635–646.
[6] M. Acquila, F. Bottini, A. Valetto, D. Caprino, P. G. Mori, M. P. Bicocchi, *Haemophilia* **2001**, *7*, 416–418.
[7] J. Korkko, I. Kaitila, L. Lonnqvist, L. Peltonen, L. Ala-Kokko, *J. Med. Genet.* **2002**, *39*, 34–41.
[8] P. S. Andersen, C. Jespersgaard, J. Vuust, M. Christiansen, L. A. Larsen, *Hum. Mutat.* **2003**, *21*, 455–465.
[9] H. Y. Mun, A. Girigoswami, C. Jung, D. Y. Cho, H. G. Park, *Biosens. Bioelectron.* **2009**, *24*, 1706–1711.
[10] R. L. Stoermer, K. B. Cederquist, S. K. McFarland, M. Y. Sha, S. G. Penn, C. D. Keating, *J. Am. Chem. Soc.* **2006**, *128*, 16892–16903.
[11] Y. Xiao, K. J. I. Plakos, X. H. Lou, R. J. White, J. R. Qian, K. W. Plaxco, H. T. Soh, *Angew. Chem.* **2009**, *121*, 4418–4422; *Angew. Chem Int. Ed.* **2009**, *48*, 4354–4358.
[12] Y. Xiao, X. H. Lou, T. Uzawa, K. J. I. Plakos, K. W. Plaxco, H. T. Sohn, *J. Am. Chem. Soc.* **2009**, *131*, 15311–15316.
[13] S. Y. Yoo, D. K. Kim, T. J. Park, E. K. Kim, E. Tamiya, S. Y. Lee, *Anal. Chem.* **2010**, *82*, 1349–1357.
[14] X. J. Xue, W. Xu, F. Wang, X. G. Liu, *J. Am. Chem. Soc.* **2009**, *131*, 11668–11669.
[15] M. Komiyama, S. Ye, X. G. Liang, Y. Yamamoto, T. Tomita, J. M. Zhou, H. Aburatani, *J. Am. Chem. Soc.* **2003**, *125*, 3758–3762.
[16] Y. W. C. Cao, R. C. Jin, C. A. Mirkin, *Science* **2002**, *297*, 1536–1540.
[17] D. Graham, D. G. Thompson, W. E. Smith, K. Faulds, *Nat. Nanotechnol.* **2008**, *3*, 548–551.
[18] S. Mahajan, J. Richardson, T. Brown, P. N. Bartlett, *J. Am. Chem. Soc.* **2008**, *130*, 15589–15601.
[19] Y. S. Huh, A. J. Lowe, A. D. Strickland, C. A. Batt, D. Erickson, *J. Am. Chem. Soc.* **2009**, *131*, 2208–2213.
[20] B. Moody, G. McCarty, *Anal. Chem.* **2009**, *81*, 2013–2016.
[21] D. G. Thompson, K. Faulds, W. E. Smith, D. Graham, *J. Phys. Chem. C* **2010**, *114*, 7384–7389.
[22] A. J. Lowe, Y. S. Huh, A. D. Strickland, D. Erickson, C. A. Batt, *Anal. Chem.* **2010**, *82*, 5810–5814.
[23] I. Yoon, T. Kang, W. Choi, J. Kim, Y. Yoo, S. W. Joo, Q. H. Park, H. Ihee, B. Kim, *J. Am. Chem. Soc.* **2009**, *131*, 758–762.
[24] S. M. Nie, S. R. Emery, *Science* **1997**, *275*, 1102–1106.
[25] K. Kneipp, Y. Wang, H. Kneipp, L. T. Perelman, I. Itzkan, R. Dasari, M. S. Feld, *Phys. Rev. Lett.* **1997**, *78*, 1667–1670.
[26] T. Kang, S. M. Yoo, I. Yoon, S. Y. Lee, B. Kim, *Nano Lett.* **2010**, *10*, 1189–1193.
[27] M. Moskovits, *Rev. Mod. Phys.* **1985**, *57*, 783–826.
[28] G. Braun, S. J. Lee, M. Dante, T. Q. Nguyen, M. Moskovits, N. Reich, *J. Am. Chem. Soc.* **2007**, *129*, 6378–6379.
[29] J. T. Kemp, R. W. Davis, R. L. White, S. X. Wang, C. D. Webb, *J. Forensic. Sci.* **2005**, *50*, 1109–1113.
[30] L. Sun, J. Irudayaraj, *Biophys. J.* **2009**, *96*, 4709–4716.
[31] L. Sun, J. Irudayaraj, *J. Phys. Chem. B* **2009**, *113*, 14021–14025.
[32] B. J. Till, C. Burtner, L. Comai, S. Henikoff, *Nucleic Acids Res.* **2004**, *32*, 2632–2641.
[33] N. A. Desai, V. Shankar, *FEMS Microbiol. Rev.* **2003**, *26*, 457–491.
[34] B. J. Till, T. Zerr, L. Comai, S. Henikoff, *Nat. Protoc.* **2006**, *1*, 2465–2477.
[35] B. J. Till, T. Zerr, E. Bowers, E. A. Greene, L. Comai, S. Henikoff, *Nucleic Acids Res.* **2006**, *34*, e99 [Corrigendum: B. J. Till, T. Zerr, E. Bowers, E. A. Greene, L. Comai, S. Henikoff, *Nucleic Acids Res.* **2006**, *34*, 5352–5352].
[36] L. M. Wick, J. M. Rouillard, T. S. Whittam, E. Gulari, J. M. Tiedje, S. A. Hashsham, *Nucleic Acids Res.* **2006**, *34*, e26.
[37] E. K. Kim, O. J. Yoo, K. Y. Song, H. W. Yoo, S. Y. Choi, S. W. Cho, S. H. Hahn, *Hum. Mutat.* **1998**, *11*, 275–278.
[38] N. A. Afshari, J. E. Mullally, M. A. Afshari, R. F. Steinert, A. P. Adamis, D. T. Azar, J. H. Talamo, C. H. Dohlman, T. P. Dryja, *Arch. Ophthalmol.* **2001**, *119*, 16–22.
[39] E. M. Stone, W. D. Mathers, G. O. D. Rosenwasser, E. J. Holland, R. Folberg, J. H. Krachmer, B. E. Nichols, P. D. Gorevic, C. M. Taylor, L. M. Streb, J. A. Fishbaugh, T. E. Daley, B. M. Sucheski, V. C. Sheffield, *Nat. Genet.* **1994**, *6*, 47–51.
[40] S. Y. Yoo, T. I. Kim, S. Y. Lee, E. K. Kim, K. C. Keum, N. C. Yoo, W. M. Yoo, *Br. J. Ophthalmol.* **2007**, *91*, 722–727.

Received: November 23, 2010
Published online: June 16, 2011

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim