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

Taejoon Kang,[†,§] Seung Min Yoo,[†,§] Ilsun Yoon,[†] Sang Yup Lee,*[,‡] and Bongsoo Kim*[,†]

[†]Department of Chemistry and [‡]Department of Chemical and Biomolecular Engineering (BK21 Program), KAIST, Daejeon 305-701, Korea

ABSTRACT A Au particle-on-wire system that can be used as a specific, sensitive, and multiplex DNA sensor is developed. A pattern formed by multiple Au nanowire sensors provides positional address and identification for each sensor. By using this system, multiplex sensing of target DNAs was possible in a quantitative manner with a detection limit of 10 pM. Target DNAs from reference bacteria and clinical isolates were successfully identified by this sensor system, enabling diagnostics for infectious diseases.

KEYWORDS DNA, multiplex, pathogen, pattern, surface-enhanced Raman scattering

Multiplex, sensitive, and specific DNA detection is of great demand for various biological and biomedical studies including gene profiling, drug screening, and clinical diagnostics because it has a potential to provide the most information from a small sample volume at low cost.[1] In this regard, simple, reliable, and high-throughput methods that allow detection of multiple DNAs in one assay have been developed by taking various sensing approaches such as the measurement of fluorescence,[1−3] surface plasmon resonance (SPR),[4] electric signals,[5] and mass changes.[6] Among these DNA sensing methods, fluorescence-based assay is currently the most preferred technique for multiplex DNA detection.[7] Surface-enhanced Raman scattering (SERS) has also been considered as an attractive method for label-free multiplex DNA detection because of its single molecule level sensitivity,[8−10] molecular specificity,[11] and insensitivity to quenching.[7,12] These distinct advantages have led to the development of a number of ingenious SERS sensing platforms.[13−16] However, achieving optimum reproducibility of SERS signals and detecting various target molecules with a very small sample volume in one assay still remain as challenging tasks for practical multiplex SERS sensors. It has been shown that hot spots at nanoscale gaps between a nanowire (NW) and nanoparticles (NPs) can become highly SERS-active.[17−22] We have recently reported a new biomolecule detection method that provides reproducible SERS signals by using nanoscale gaps between Au NW and NPs.[23]

Here we present a multiplex DNA detection method employing multiple Au particle-on-wire systems as a SERS sensing platform. The system operates by the self-assembly of Au NPs onto Au NW in the presence of target DNAs,

providing reproducible SERS signals in proportion to the concentrations of target DNAs. Multiple pathogen DNAs could be successfully detected by employing this method, demonstrating that this multiplex SERS sensor can be used a convenient system for clinical diagnostic and biomolecular interaction studies.

Raman signal can be dramatically enhanced by placing the signal carrying molecules in the interstices between the assembled nanostructures.[24−29] To fabricate a SERS-active nanostructure that can be turned on by biomolecular binding, we adopted an Au particle-on-wire structure constructed by self-assembly of Au NPs onto Au NW through DNA hybridization. The Au NW and NPs have attracted great interest as SERS-active platforms because of their superb physicochemical properties and well-defined geometry.[30−33] Figure 1a shows the design of Au particle-on-wire SERS sensor and its operating principle. Au NWs with a diameter of ∼150 nm were synthesized on a c-sapphire substrate using a vapor transport method.[34,35] The synthesized NWs are highly single-crystalline without twins and have atomically smooth surfaces. The NWs were transferred to a Si substrate using a nanomanipulator after the surfaces of Au NWs were modified with the thiolated probe DNAs.[34,35] Modification of Au NPs with the reporter DNAs was carried out by the salt aging method.[36] Si substrate was blocked with methoxy-polyethylene glycol silane to prevent nonspecific adsorption by DNAs and Au NPs before the experiments.[37] Au NWs on the substrate were incubated with a target DNAs and subsequently immersed into a solution of Au NPs attached with the reporter DNAs to construct Au particle-on-wire structure through the sandwich hybridization of probe-target-reporter DNAs. If the target DNAs contains sequences complementary to the probe DNAs and the reporter DNAs, the Au particle-on-wire structure is formed, creating SERS hot spots at the gaps of NW and NPs. The 5′-termini of reporter DNA were labeled with Cy5 as a Raman dye to enhance the sensitivity.

* To whom correspondence should be addressed. Fax: +82-42-350-2810. E-mail: (B.K.) bongsoo@kaist.ac.kr; (S.Y.L.) leesy@kaist.ac.kr.
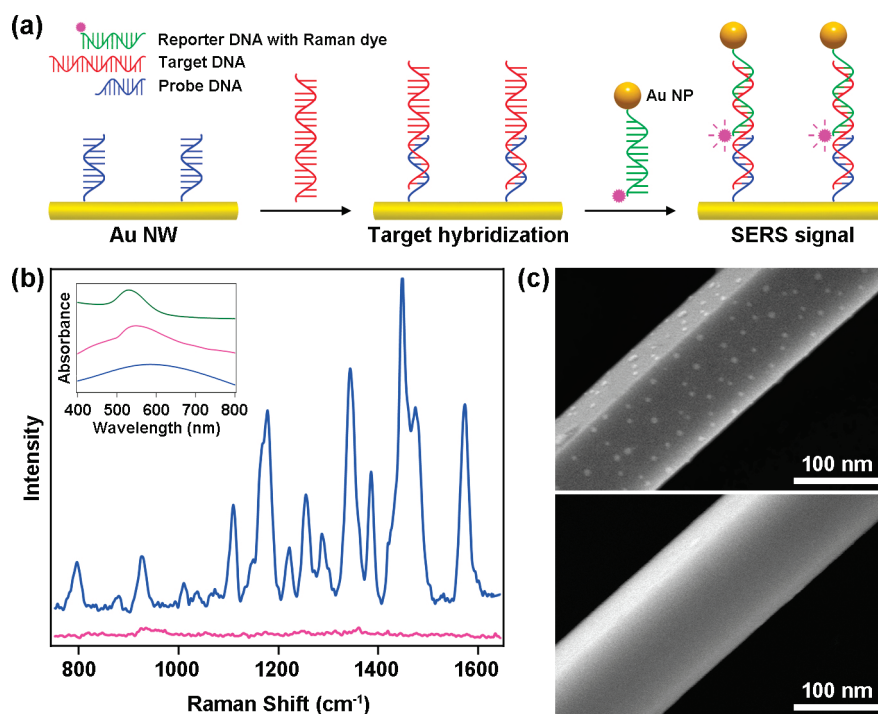§ These authors contributed equally to this work.

FIGURE 1. (a) Schematic representation for the detection of target DNAs by Au particle-on-wire system. (b) SERS spectra of Au particle-on-wire system prepared by probe-complementary target-reporter DNAs hybridization (blue spectrum) and that of a single Au NW prepared by noncomplementary target DNAs in place of complementary target DNAs (magenta spectrum). The inset is UV−vis absorption spectra of Au NPs (green spectrum), Au NWs (magenta spectrum), and Au particle-on-wire systems (blue spectrum). (c) SEM image of a typical Au particle-on-wire structure constructed by adding complementary target DNAs (top) and a clean NW by noncomplementary target DNAs (bottom).

To verify if this system operates correctly, SERS signals were obtained from the Au NW after adding complementary and noncomplementary target DNAs. The spectra were measured with a laser perpendicularly polarized to the long axis of NW because the SERS signals are maximized when the polarization is perpendicular to the NW axis (Supporting Information Figure S4).[20,23] Strong SERS signal from Cy5 is observed only when the complementary target DNAs were added as seen in Figure 1b (blue spectrum), indicating high specificity to DNA sequences. The experimental SERS enhancement factor (EF) of the Au particle-on-wire system with respect to a NW system is evaluated to be 2.6×10^3 (Supporting Information). To understand and optimize the SERS response of this sensor, UV−vis absorption spectra of Au NPs, NWs, and particle-on-wire systems were measured (inset of Figure 1b). The SERS excitation would become most efficient when the laser excitation wavelength coincides with the maximum plasmon absorption wavelength.[38] The absorption spectrum of Au NPs shows a peak near 530 nm and that of NWs shows a slightly red-shifted band. The spectrum of Au particle-on-wire systems displays a broad absorption band associated with the plasmon coupling of NW and NPs.[17] Note that the 633 nm laser for excitation of the particle-on-wire systems is near the absorption maximum and thus allows effective SERS excitation.

DNA hybridization can be directly observed by scanning electron microscope (SEM). Figure 1c shows a typical Au particle-on-wire structure constructed by adding complementary target DNAs (top) and a clean NW without NPs by adding noncomplementary target DNAs (bottom). The SERS spectra and SEM images verify that the formation of NW-NP nanogap is critical for SERS detection. It should be noted that the NPs are evenly distributed over the whole NW surface with each separated NPs clearly discerned on the NW surface without aggregation (Supporting Information Figure S5), contributing to the generation of reproducible SERS signals.

The detection limit of the Au particle-on-wire sensor system was determined by observing the 1580 cm^{-1} band intensity of Cy5 as the concentration of target DNAs is changed (Figure 2a). The SERS intensity versus DNA concentration is plotted at a broader concentration range (10^{-13} ∼ 10^{-6} M) in Figure 2b. The inset shows that the SERS intensity increases linearly with the increase of target DNA concentration between 10^{-11} and 10^{-8} M. SERS signal could be observed even at a DNA concentration as low as 10 pM. Thus, the detection limit of this sensor is 10 pM, which corresponds to 300 amol (1.8×10^8 molecules) if a typical assay volume of 30 μL is used. The control experiment by noncomplementary target DNAs showed no discernible SERS signals (magenta line of Figure 2b).

To confirm multiplex DNA detection by the Au particle-on-wire system, two Au NWs that were modified with different probe DNAs (Efm003-20 and Sau001-20, Supporting Information Table S1) were employed. The substrate carrying two Au NWs were sequentially incubated in mixed
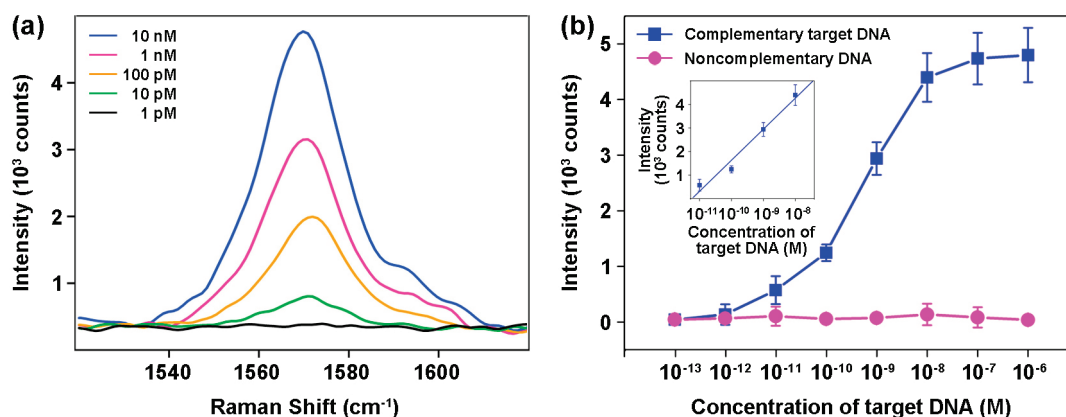
FIGURE 2. (a) SERS intensities of 1580 cm^{-1} band from Cy5 obtained by varying the concentration of target DNAs. (b) Plot of SERS intensities of 1580 cm^{-1} band versus concentration of target DNAs. Control experiment was carried out with noncomplementary target DNAs. The inset shows a dynamic range of about 3 orders of magnitude and a linearly fitted line. The data were obtained from five measurements and the error bars represent standard deviation.
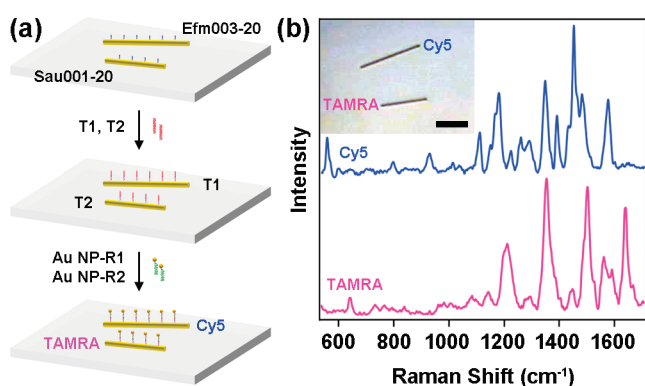

FIGURE 3. (a) Schematic representation for the detection of two different target DNAs by Au particle-on-wire systems. Two Au NWs functionalized with different probe DNAs (Efm003-20 and Sau001-20) are incubated in mixed solution of target DNAs (T1 and T2) and immersed into a solution of Au NPs modified with reporter DNAs (R1 and R2). Because R1 and R2 contains Cy5 and TAMRA as a Raman dye, respectively, the upper particle-on-wire structure gives SERS signal of Cy5 and the lower one gives SERS signal of TAMRA, respectively. (b) SERS spectra of Cy5 and TAMRA from Au particle-on-wire systems after DNA hybridization. The inset is an optical microscope image of particle-on-wire structures from which we obtain SERS. The scale bar denotes 5 μm.

solution of target DNAs (T1 and T2, Supporting Information Table S1) and a solution of Au NPs modified with reporter DNAs (R1 and R2, Supporting Information Table S1) (Figure 3a). The resulting two particle-on-wire sensors provide distinguishable SERS spectra of Cy5 and TAMRA, respectively, because one target DNA (T1) is complementary to Efm003-20 and Cy5 reporter (R1), while the other target DNA (T2) is complementary to Sau001-20 and TAMRA reporter (R2). The blue spectrum in Figure 3b shows SERS spectra of Cy5 obtained from the NW modified by Efm003-20, indicating that only T1 and R1 are attached to this sensor. The magenta spectrum obtained from Sau001-20 modified NW shows SERS band of TAMRA, confirming that only T2 and R2 are attached. No cross hybridization between different target DNAs is observed.

The above experiments clearly demonstrate that quantitative detection of target DNAs is possible by the particle-on-wire system. To evaluate practical applicability, the system was employed for pathogen diagnosis using the target DNAs prepared by polymerase chain reaction (PCR) amplification of the genomic DNAs extracted from four reference pathogenic strains (Supporting Information). *Enterococcus faecium* and *Staphylococcus aureus* are the most prevalent pathogens in bloodstream infections with high morbidity and mortality.[39] *Stenotrophomonas maltophilia* is an important nosocomial pathogen in immunocompromised patients, transplant recipients, and persons with cystic fibrosis.[40] *Vibrio vulnificus* causes gastroenteritis and septicemia, showing a rapid progress and resulting in a high mortality rate of 50% or more within days.[41]

Figure 4a shows a schematic illustration of a four-way multiplex bacterial DNA detection. The sensing platform was fabricated by four Au NWs, each attached with four different probe DNAs (Efm003-20, Sau001-20, Smal03-20, and Vvul02-20, Supporting Information Table S1), respectively, placed on a single substrate. Since the NWs attached by different probe DNAs are indistinguishable, they have been aligned at the specific positions of substrate for identification. The NW alignment and integration on the substrate are key steps toward fabrication of the practical biosensor chip that can realize a NW based sensor for multiplex detection of biomolecules.[42] In this experiment, we used a custom-built nanomanipulator for patterning of different probe DNA attached Au NWs (Supporting Information Figure S1). Since the Au NW can be monitored in situ through an optical microscope while being transferred, the NWs carrying different DNAs are clearly distinguished without additional identification tag. As shown in Figure 4b, the four NWs are assembled to form an alphabetic character M. The orientations of individual NWs provide positional addresses for each NW (Figure 4b), identifying the pathogen DNA that the particle-on-wire sensor has detected. Four NWs on a sub-
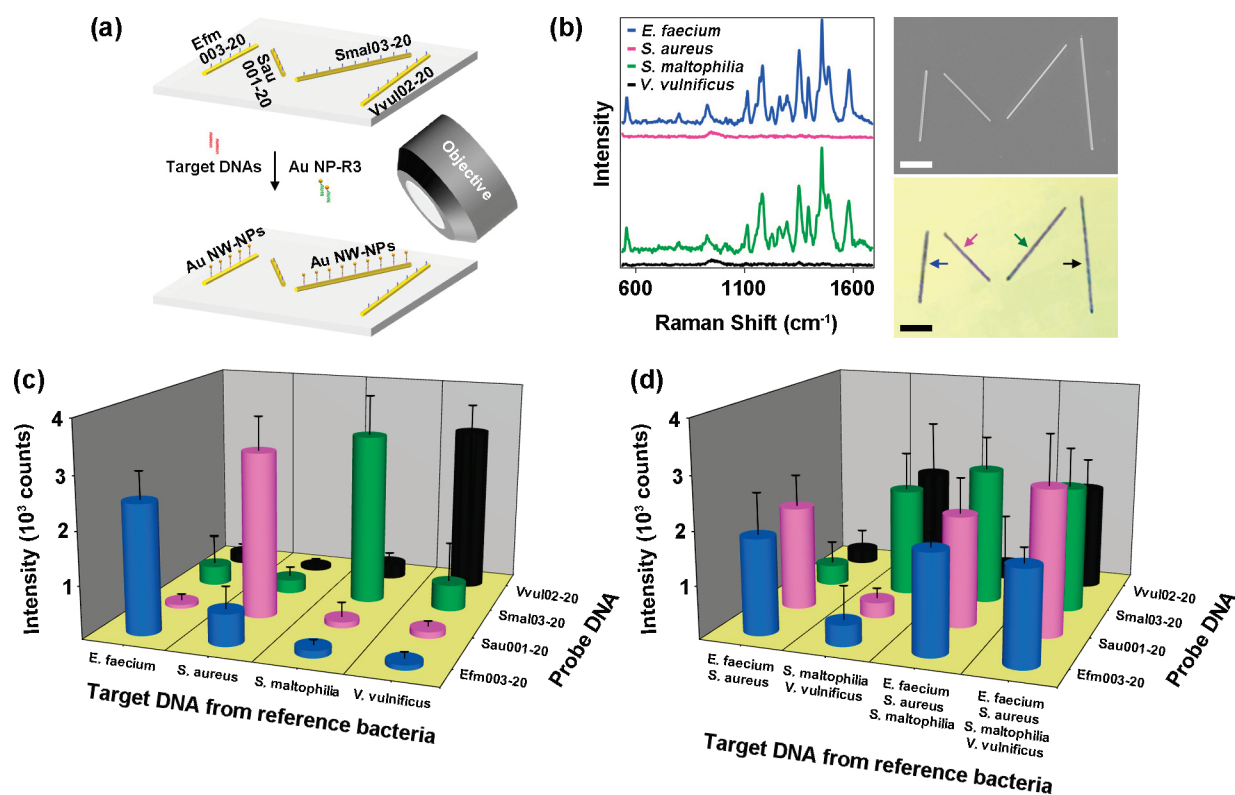
FIGURE 4. (a) Schematic representation for the patterned multiplex pathogen DNA detection using a particle-on-wire SERS sensor. (b) SERS spectra from each of the four particle-on-wire system when the sample included two kinds of target DNAs (*E. faecium* and *S. maltophilia*). SERS spectra of Cy5 were detected only at the blue- and green-tagged sensors. The right figures are an SEM image (upper) and an optical microscope image (lower). The scale bar represents 5 μm. (c) SERS intensities of 1580 cm^{-1} band measured on each particle-on-wire sensor with a sample containing only one kind of target DNA of a concentration of 10^{-8} M. (d) SERS intensities of 1580 cm^{-1} band when the sample contains two, three, and four kinds of target DNAs of which concentrations are 10^{-8} M each.

strate were hybridized with various mixtures of target DNAs and then incubated with reporter DNAs having Cy5 at 5′-termini and Au NPs at 3′-termini.

The results of multiplex bacterial DNA detection are shown in Figure 4b−d. From the addressable positions of the particle-on-wire sensor, we could identify the NWs and detect multiple target DNAs in a single assay. The SERS spectra from each of the four particle-on-wire system when the sample included two target DNAs (*E. faecium* and *S. maltophilia*) are shown in Figure 4b. The SERS spectra of Cy5 were detected only at the blue- and green-tagged sensors. Figure 4c shows SERS intensities of 1580 cm^{-1} band measured on each particle-on-wire sensor with a sample containing only one kind of target DNA. When only *E. faecium* DNA was present in the sample, SERS signal was detected only at the blue-tagged sensor. Figure 4d shows SERS spectra when the sample contains two, three, and four kinds of target DNAs. When the sample included *E. faecium* and *S. aureus* DNAs, the SERS signals were observed only at the blue-tagged and magenta-tagged sensors. All four sensors showed SERS spectra when four target DNAs are included. When multiple pathogen DNAs were added, the SERS intensities decreased slightly compared to that obtained when a single pathogen DNA was included. Decrease of SERS signal can be attributed to reduction of affinity of target

DNAs to the probe DNAs due to increased concentration of multiple DNAs. This may be improved by the optimization of probe DNA design and reaction conditions. Nevertheless, the Au particle-on-wire sensors unambiguously identified the correct pathogen DNAs in each sample. These results demonstrate that the particle-on-wire sensor system developed here can be used for multiplex pathogen diagnostics.

It should be noted that the target bacterial DNAs (119 bp for *S. maltophilia*, 131 bp for *V. vulnificus*, 194 bp for *S. aureus*, and 347 bp for *E. faecium*) are much longer than the synthetic 35-mer oligonucleotides. The detection of label-free DNAs by particle-on-wire sensor is independent of the length of target DNAs because DNAs collapse in a dry condition, and thus reducing the NW−NP gap distance for SERS enhancement.[12,43]

Finally, the particle-on-wire sensor system was used for the identification of pathogenic bacteria in real clinical samples. The target DNAs extracted from various clinical specimens including cerebrospinal fluid, stool, pus, and sputum (Supporting Information) were amplified by PCR and then detected by SERS sensor developed in this study. As shown in Figure 5, four different bacteria from seven clinical isolates (*E. faecium*, 2 isolates; *S. aureus*, 2 isolates; *S. maltophilia*, 2 isolates; *V. vulnificus*, 1 isolate) could be
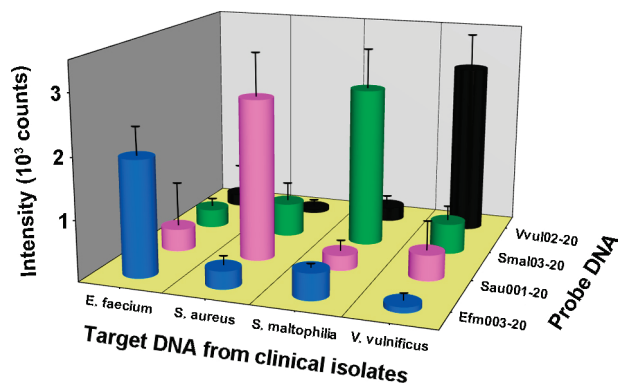
FIGURE 5. Identification of pathogenic bacteria from clinical samples. The target DNAs obtained from various clinical specimens were amplified by PCR and then detected by the particle-on-wire sensor.

successfully identified; the identification results agreed well with those obtained by conventional culture-based assays.

In summary, we have demonstrated that the Au particle-on-wire system employing probe, target, and reporter DNAs can be used as a specific, sensitive, and multiplex DNA sensor. The particle-on-wire sensor provides reproducible SERS signals only in the presence of target DNAs in proportion to the DNA concentration spanning from 10 pM to 10 nM. The Au particle-on-wire SERS sensor system developed in this study can be employed in various biosensing applications as demonstrated for the successful diagnosis of clinical pathogens.

Supporting Information Available. Experimental details including sample preparation, instrumentation, experimental results, and estimation of EF. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

(1)   Li, Y.; Cu, Y. T. H.; Luo, D. *Nat. Biotechnol.* **2005**, *23*, 885–889.
(2)   Nicewarner-Pena, S. R.; Freeman, R. G.; Reiss, B. D.; He, L.; Pena, D. J.; Walton, I. D.; Cromer, R.; Keating, C. D.; Natan, M. J. *Science* **2001**, *294*, 137–141.
(3)   Stoermer, R. L.; Cederquist, K. B.; McFarland, S. K.; Sha, M. Y.; Penn, S. G.; Keating, C. D. *J. Am. Chem. Soc.* **2006**, *128*, 16892–16903.
(4)   Nelson, B. P.; Grimsrud, T. E.; Liles, M. R.; Goodman, R. M.; Corn, R. M. *Anal. Chem.* **2001**, *73*, 1–7.
(5)   Basuray, S.; Senapati, S.; Aijian, A.; Mahon, A. R.; Chang, H.-C. *ACS Nano* **2009**, *3*, 1823–1830.
(6)   Ross, P.; Hall, L.; Smirnov, I.; Haff, L. *Nat. Biotechnol.* **1998**, *16*, 1347–1351.
(7)   Mahajan, S.; Richardson, J.; Brown, T.; Bartlett, P. N. *J. Am. Chem. Soc.* **2008**, *130*, 15589–15601.
(8)   Nie, S.; Emory, S. R. *Science* **1997**, *275*, 1102–1106.
(9)   Kneipp, K.; Kneipp, H.; Itzkan, I.; Dasari, R. R.; Feld, M. S. *Chem. Rev.* **1999**, *99*, 2957–2976.
(10) Kneipp, K.; Kneipp, H.; Kneipp, J. *Acc. Chem. Res.* **2006**, *39*, 443–450.
(11) Huang, W.; Qian, W.; Jain, P. K.; El-Sayed, M. A. *Nano Lett.* **2007**, *7*, 3227–3234.
(12) Braun, G.; Lee, S. J.; Dante, M.; Nguyen, T.-Q.; Moskovits, M.; Reich, N. *J. Am. Chem. Soc.* **2007**, *129*, 6378–6379.
(13) Cao, Y. C.; Jin, R.; Mirkin, C. A. *Science* **2002**, *297*, 1536–1540.
(14) Faulds, K.; Smith, W. E.; Graham, D. *Anal. Chem.* **2004**, *76*, 412–417.
(15) Qin, L.; Banholzer, M. J.; Millstone, J. E.; Mirkin, C. A. *Nano Lett.* **2007**, *7*, 3849–3853.
(16) Sun, L.; Yu, C.; Irudayaraj, J. *Anal. Chem.* **2007**, *79*, 3981–3988.
(17) Gunawidjaja, R.; Peleshanko, S.; Ko, H.; Tsukruk, V. V. *Adv. Mater.* **2008**, *20*, 1544–1549.
(18) Ko, H.; Singamaneni, S.; Tsukruk, V. V. *Small* **2008**, *4*, 1576–1599.
(19) Wei, H.; Hao, F.; Huang, Y.; Wang, W.; Nordlander; Xu, H. *Nano Lett* **2008**, *8*, 2497–2502.
(20) Lee, S. J.; Baik, J. M.; Moskovits, M. *Nano Lett.* **2008**, *8*, 3244–3247.
(21) Hutchison, J. A.; Centeno, S. P.; Odaka, H.; Fukumura, H.; Hofkens, J.; Uji-I, H. *Nano Lett* **2009**, *9*, 995–1001.
(22) Fang, Y.; Wei, H.; Hao, F.; Nordlander, P.; Xu, H. *Nano Lett.* **2009**, *9*, 2049–2053.
(23) Kang, T.; Yoon, I.; Kim, J.; Ihee, H.; Kim, B. *Chem.—Eur. J.* **2010**, *16*, 1351–1355.
(24) Moskovits, M. *Rev. Mod. Phys.* **1985**, *57*, 783–828.
(25) Xu, H.; Bjerneld, E. J.; Käll, M.; Börjesson, L. *Phys. Rev. Lett.* **1999**, *83*, 4357–4360.
(26) Xu, H.; Aizpurua, J.; Käll, M.; Apell, P. *Phy. Rev. E* **2000**, *62*, 4318–4324.
(27) Xu, H.; Käll, M. *ChemPhysChem* **2003**, *4*, 1001–1005.
(28) Graham, D.; Thompson, D. G.; Smith, W. E.; Faulds, K. *Nat. Nanotechnol.* **2008**, *3*, 548–551.
(29) Cho, H.; Baker, B. R.; Wachsmann-Hogiu, S.; Pagba, C. V.; Laurence, T. A.; Lane, S. M.; Lee, L. P.; Tok, J. B.-H. *Nano Lett* **2008**, *8*, 4386–4390.
(30) Talley, C. E.; Jackson, J. B.; Oubre, C.; Grady, N. K.; Hollars, C. W.; Lane, S. M.; Huser, T. R.; Nordlander, P.; Halas, N. J. *Nano Lett.* **2005**, *5*, 1569–1574.
(31) Murphy, C. J.; Sau, T. K.; Gole, A. M.; Orendorff, C. J.; Gao, J.; Gou, L.; Hunyadi, S. E.; Li, T. *J. Phys. Chem. B* **2005**, *109*, 13857–13870.
(32) Lee, S. J.; Morrill, A. R.; Moskovits, M. *J. Am. Chem. Soc.* **2006**, *128*, 2200–2201.
(33) Mohanty, P.; Yoon, I.; Kang, T.; Seo, K.; Varadwaj, K. S. K.; Choi, W.; Park, Q. —H.; Ahn, J. P.; Suh, Y. D.; Ihee, H.; Kim, B. *J. Am. Chem. Soc.* **2007**, *129*, 9576–9577.
(34) Yoon, I.; Kang, T.; Choi, W.; Kim, J.; Yoo, Y.; Joo, S. —W.; Park, Q. —H.; Ihee, H.; Kim, B. *J. Am. Chem. Soc.* **2009**, *131*, 758–762.
(35) Kang, T.; Yoon, I.; Jeon, K. —S.; Choi, W.; Lee, Y.; Seo, K.; Yoo, Y.; Park, Q. —H.; Ihee, Y.; Suh, Y. D.; Kim, B. *J. Phys. Chem. C* **2009**, *113*, 7492–7496.
(36) Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. *Anal. Chem.* **2006**, *78*, 8313–8318.
(37) Lan, S.; Veiseh, M.; Zhang, M. *Biosens. Bioelect.* **2005**, *20*, 1697–1708.
(38) McFarland, A. D.; Young, M. A.; Dieringer, J. A.; Van Duyne, R. P. *J. Phys. Chem. B* **2005**, *109*, 11279–11285.
(39) Wisplinghoff, H.; Bischoff, T.; Tallent, S. M.; Seifert, H.; Wenzel, R. P.; Edmond, M. B. *Clin. Infect. Dis.* **2004**, *39*, 309–317.
(40) Whitby, P. W.; Carter, K. B.; Burns, J. L.; Royall, J. A.; LiPuma, J. J.; Stull, T. L. *J. Clin. Microbiol.* **2000**, *38*, 4305–4309.
(41) Gerhard, G. S.; Levin, K. A.; Price, G. J.; Wojnar, M. M.; Chorney, M. J.; Belchis, D. A. *Arch. Pathol. Lab. Med.* **2001**, *125*, 1107–1109.
(42) He, B.; Morrow, T. J.; Keating, C. D. *Curr. Opin. Chem. Biol.* **2008**, *12*, 522–528.
(43) Qian, X.; Zhou, X.; Nie, S. *J. Am. Chem. Soc.* **2008**, *130*, 14934–14935.