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PAPER

Single-step multiplex detection of toxic metal ions by Au nanowires-on-chip sensor using reporter elimination

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We have developed a Au nanowires (NWs)-on-chip surface-enhanced Raman scattering (SERS) multiplex sensor that can sensitively detect multiple toxic metal ions. Most importantly, the reporter elimination method simplified the detection procedure to a single step, which has been much desired for remote environmental monitoring. This sensor has several notable features. First, it shows high reproducibility based on well-defined single-crystalline Au NWs. Second, single-NW-sensors that can detect a specific metal ion are combined for multiplex sensing of metal ions. Third, when a sample solution is put onto the NWs-on-chip sensor, a decrease in the SERS signal of a specific NW-sensor identifies the target metal ion. Simple, rapid, sensitive and quantitative detection of metal ions becomes possible through the measurement of the SERS signals. We successfully detected ions of mercury (Hg^{2+}), silver (Ag^{+}), and lead (Pb^{2+}) coexisting in the same solution by using this sensor.

1. Introduction

Heavy metal ions such as mercury (Hg^{2+}), silver (Ag^{+}), and lead (Pb^{2+}) ions are severe pollutants that cause serious medical effects on human health.[1] They inhibit physical and mental development in children, and damage nervous, renal, immune, and cardiovascular systems.[1–3] Since toxic metal ions are widely distributed in water, air, soil, and food,[1,4–8] multiplex remote detection of metal ions is highly desirable in order to identify fatal environmental pollution immediately. For remote monitoring of metal ions, it is critical to have the simplest but accurate detection procedure that can be executed by an automated process.

Some nucleotide bases of aptamers can form stable complexes with metal ions.[9–21] Multiplex detection methods for metal ions were recently developed by utilizing these aptamers in fluorescence, surface-enhanced Raman scattering (SERS), and electrochemistry.[1,9,11,14,22] While these assays were effective, they need sample preparation steps and often require discriminative procedures for multiplex detection. For real-time and/or remote metal ion detection, however, it is much desired to simplify such procedures.

We report here an alignment-addressed Au NWs-on-chip SERS sensor for multiplex metal ion detection. This is the first SERS sensor that can detect Hg^{2+}, Ag^{+}, and Pb^{2+} simultaneously, to the best of our knowledge. Single-crystalline Au and Ag nanowires (NWs) offer quite effective methods for multiplex detection of chemical/biological analytes.[23–26] Moreover, NWs have been used for remote sensing and complex plasmonic circuits.[27,28] The use of single-crystalline Au NWs over nanoparticles (NPs) is highly advantageous for quantitative SERS detection of analytes because a single Au NW of well-defined geometry placed on a Au film provides highly reproducible SERS signals whereas the signal intensities of NPs-sensors are strongly dependent on the degree of aggregation.[13,29–32] The detection mechanism of this sensor is based on the binding of a specific metal ion to its corresponding aptamer, leading to elimination of a Raman reporter-attached DNA from the NW by conformational change of the aptamer. On dropping a sample solution onto the SERS sensor, Raman signals from the sensor decrease significantly by the Raman reporter elimination reaction, allowing us to detect the target metal ions.

The core of this study is summarized as follows. First, we developed a metal ion sensor that requires only a single step for detection and provides reproducible signals by combining a Au NWs-on-chip platform and aptamers. Second, a multiplex metal ion sensor was fabricated by assembling multiple Au NWs, of which each can act as a specific metal ion sensor. This sensor successfully detected Hg^{2+}, Ag^{+}, and Pb^{2+} in solution selectively and quantitatively.

The presented sensor with a simple detection step could be employed for real-time and remote on-line environmental monitoring using automated devices. Furthermore, because aptamers can detect targets as large as a cell, we anticipate that this multiplex SERS sensing chip would become a quite versatile sensor that can detect targets with a size from metal ions up to biological cells.[33,34]

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Table 1 Oligonucleotides used in this experiment

Name	Sequence (5′→3′)
A_{Hg}	HS-$(CH_2)_6$-CGGCCGTTCTTTCTTCCCTTGTTTGTT
A_{Ag}	HS-$(CH_2)_6$-CTCTCTTCTCTTCAAAAAACAACACA-ACACAC
A_{Pb}	HS-$(CH_2)_6$-GCCAACGGAAGGTGTGGAAGG
C_{Hg}	Cy5-GAACGGCCG
C_{Ag}	Cy5-AGAAGAGAG
C_{Pb}	Cy5-TCCGTTGGC

2. Experimental section

2.1 Materials

Purified DNAs were obtained from Genotech (Daejeon, Korea) and the metal salts ($Hg(Ac)_2$, $Pb(Ac)_2$, and AgAc) were purchased from Sigma-Aldrich. The sequences of oligonucleotides used in this experiment are listed in Table 1. All DNAs and reagents were used as received without further purification except thiolated DNAs. To prepare Hg^{2+}-specific double stranded DNAs (dsDNAs), 10 μM thiolated Hg^{2+}-specific aptamer (A_{Hg}; Table 1) and 10 μM complementary Cy5-labeled single stranded DNAs (ssDNAs) (C_{Hg}; Table 1) were mixed in a phosphate buffered saline (PBS) solution (pH 7.4) in a 1 : 1 molar ratio. The solution was heated up to 95 °C for 5 min and then cooled down to room temperature slowly. The dsDNAs were treated with 1 M dithiothreitol (DTT, Sigma-Aldrich) and purified using an NAP-5 column (GE healthcare Co.). Ag^+ and Pb^{2+}-specific dsDNAs were prepared *via* the same experimental procedure.

2.2 Preparation of NWs-on-chip SERS sensor

Single-crystalline Au NWs were synthesized on a sapphire substrate in a horizontal quartz tube furnace system using a previously described vapor transport method.[29,35,36] Briefly, the sapphire substrate was placed a few centimetres downstream from an alumina boat filled with 0.03 g of a pure Au lump. Ar gas flowed at a rate of 100 sccm, maintaining the chamber pressure at 1–5 Torr. The high temperature zone of the furnace was heated to 1100 °C. Au NWs were grown on the substrate for ~30 min of reaction time. A smooth Au film chip was prepared on pre-cleaned Si substrates by electron beam assisted deposition of a 10 nm-thick film of Cr followed by a 300 nm-thick film of Au. The surfaces of the Au films were smooth and do not give SERS signals by themselves. The Au film chip was cut to 0.25 cm^2 for NWs-on-chip sensor fabrication.

In order to modify the surface of Au NWs by metal ion-specific dsDNAs, as-grown Au NWs were incubated with 5 μM dsDNAs in 1 M KH_2PO_4 buffer (pH 6.75) at room temperature for 18 h. Excessive DNAs were washed by 0.2% (w/v) sodium dodecyl sulfate (SDS) solution for 5 min. Au NWs were transferred one by one onto the Au film chip by using a homebuilt nanomanipulator and aligned to form an alphabetic character "N" (Fig. 1).[31,36] The patterning of Au NWs enables multiplex detection of metal ions without an additional identification tag.

2.3 Detection of metal ions using NWs-on-chip SERS sensor

50 μL sample solution of toxic metal ions was dropped onto the NWs-on-chip SERS sensor and incubated for 30 min in a closed petri dish at room temperature. After incubation, the NWs-on-chip sensors were washed with PBS containing 0.1% (w/v) SDS for 5 min, rinsed twice with distilled deionized water, and dried under a N_2 stream. Finally, SERS spectra were measured from the Au NWs-on-chip SERS sensor using a micro-Raman system. To measure the SERS signals of the three different NWs on the chip, we focused an excitation laser to each individual NW sequentially. The polarization of the excitation laser was perpendicular to the long axis of the NWs.

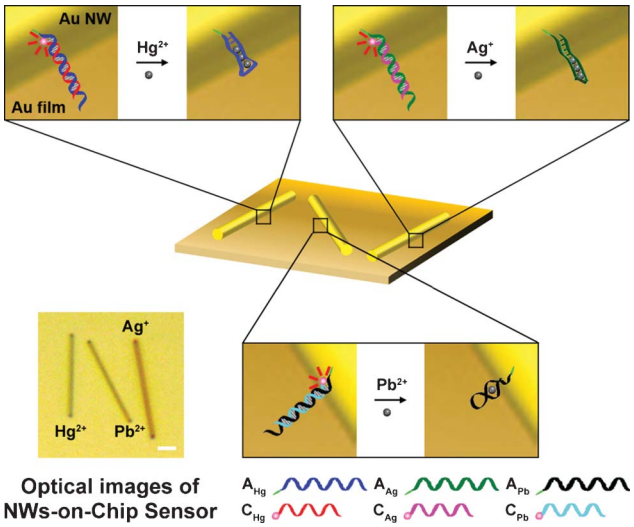

Fig. 1 Schematic representation of the alignment-addressed Au NWs-on-chip sensor. The dsDNAs-modified single Au NW works as a specific metal ion sensor, integration of which enables multiplex detection of toxic metal ions. The inset is an optical image of a fabricated NWs-on-chip sensor for detection of Hg^{2+}, Ag^+, and Pb^{2+}. The scale bar denotes 1 μm.

2.4 Instrumentation

The micro-Raman system was homebuilt based on an Olympus BX41 microscope. The 633 nm radiation of a He–Ne laser (Melles Griot) was focused on a sample through a 100× objective (NA = 0.7, Mitutoyo). The polarization direction of the laser was controlled by rotating a half-wave plate. SERS signals were recorded with a thermoelectrically cooled electron multiplying charge coupled device (EMCCD, Andor) mounted on a spectrometer with a 1200 groove mm^{-1} grating. A holographic notch filter was used. The nanomanipulator consists of a tungsten tip (~100 nm diameter at the end) mounted on a three-dimensional piezoelectric stage (Sigma-Koki).

3. Results and discussion

SERS is a fascinating phenomenon that enormously increases Raman signals compared with normal Raman signals.[29,37,38] This enhancement comes from nm-scale gaps (hot spots) between two metal nanostructures.[39–41] Au NWs-on-chip sensor provides a line of SERS hot spots at the gap between a Au NW and a Au film, enabling sensitive and multiplex detection of toxic metal ions.[29]

Fig. 1 shows a schematic of the multiplex NWs-on-chip SERS sensor. Three Au NWs, each modified by different dsDNAs,

were placed on a Au film chip. The dsDNAs are composed of a thiolated metal ion-specific aptamer (A_{Hg}, A_{Ag}, and A_{Pb}; Table 1) and a complementary oligonucleotide with Cy5 (C_{Hg}, C_{Ag}, and C_{Pb}; Table 1). When a sample solution containing toxic metal ions is added to a NWs-on-chip sensor, metal ion-specific aptamers recognize the target metal ions and form complex structures. A_{Hg} folded into a hairpin structure through thymine (T)–Hg^{2+}–T binding, A_{Ag} also folded into a hairpin structure through cytosine (C)–Ag^{+}–C binding, and A_{Pb} forms a guanine (G)-quadruplex structure.[11,14,19] These conformational changes in the aptamers induce the release of Cy5-labeled oligonucleotides from Au NWs, decreasing SERS signals of Cy5. This scheme enables multiplex and quantitative detection of metal ions through a single step.

The inset of Fig. 1 shows an optical image of the Au NWs-on-chip SERS sensor for the multiplex detection of Hg^{2+}, Ag^{+}, and Pb^{2+}. Each single NW detects a specific metal ion, which is identified by the positional addresses of NWs. Aligned integration of NWs on a chip is the key step toward fabrication of a practical NWs-based multiplex sensor and nanophotonic devices.[26,28,31] The Au NWs are aligned by using a homebuilt nanomanipulator.[31,36] By monitoring the manipulation *in situ* through an optical microscope, we can clearly distinguish which NWs are modified by which dsDNAs and thus which NWs detect which metal ions without additional identification tags.

We first tested Hg^{2+} detection using the NW-on-chip sensor. The sensor was fabricated by placing a single Au NW attached by Hg^{2+}-specific dsDNAs on a Au film chip. SERS signals of a Au NW-on-chip depend on the polarization direction of an incident laser.[29] When the polarization is perpendicular to the NW axis, SERS signals are maximized and when the polarization is parallel, minimized.[29] Therefore, we measured SERS signals of a NW-on-chip sensor with the incident laser perpendicular to the NW axis. When a sample solution containing no Hg^{2+} was dropped onto the sensor, strong SERS signals of Cy5 were observed from the NW (blue spectrum in Fig. 2a). In the presence of Hg^{2+}, the SERS signal was reduced due to elimination of Cy5-labeled ssDNAs caused by binding of Hg^{2+} to its aptamer (magenta spectrum in Fig. 2a), demonstrating the Hg^{2+} sensing ability of the NW-sensor. Since we adopted a strategy that detects metal ions by Raman signal decreases through Raman reporter elimination, Hg^{2+} could be detected simply in a single step and less than an hour without complex pre-treatment steps.

The multiple ion detection ability of the NWs-on-chip SERS sensor was also investigated. We dropped a sample solution containing multiple metal ions onto a NWs-on-chip sensor, incubated for 30 min, and measured the SERS signals of three NWs sequentially after washing. Fig. 2b,c show the reduction of SERS signals upon adding multiple metal ions. I_O and I_M represent the SERS intensity of the 1580 cm^{-1} band in the absence and presence of the metal ions, respectively. When only Hg^{2+} was present in the sample, SERS signals were reduced only at the Hg^{2+}-specific dsDNAs-functionalized NW. When the sample includes Hg^{2+} and Ag^{+}, SERS signals decreased only at the Hg^{2+}- and Ag^{+}-specific dsDNAs-functionalized Au NWs. All three NWs showed a SERS signal decrease when the sample includes all three metal ions. This result clearly demonstrates the multiple ion sensing capability of the alignment-addressed NWs-on-chip SERS sensor. Additionally, we examined the stability of the Au NWs-on-chip sensor. After storing in ambient conditions at room temperature over a week, the as-prepared NWs-on-chip sensor could be used for the multiplex detection of metal ions. Although we measured SERS signals by focusing the laser onto the NWs sequentially for multiplex detection, it may become possible to measure SERS signals of multiple Au NWs at once with remote excitation of SERS and the Raman imaging technique.

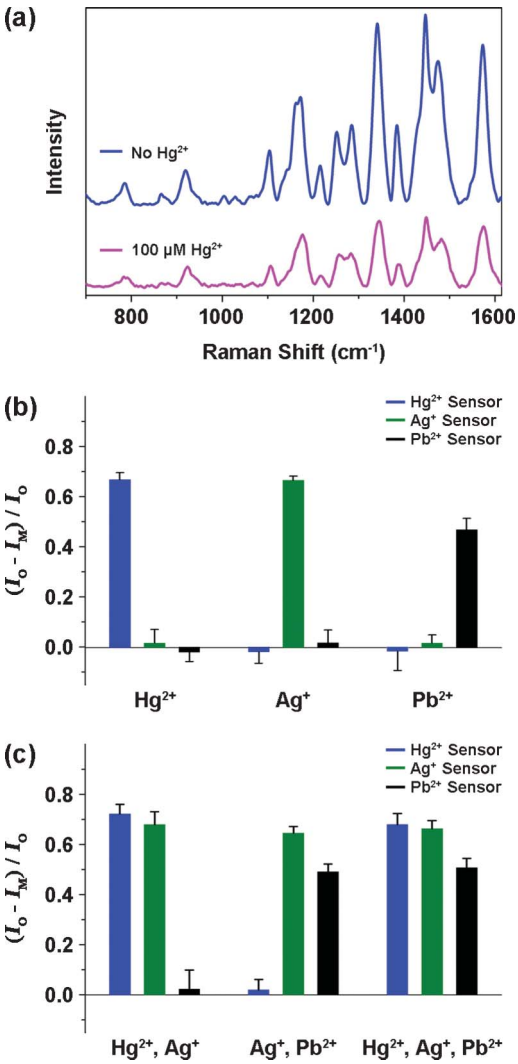

Fig. 2 (a) SERS spectra of Cy5 obtained from the Hg^{2+} specific dsDNAs-modified NW-on-chip sensor for Hg^{2+}-free (blue spectrum) and for a 100 μM Hg^{2+} solution (magenta spectrum). (b,c) Reduction of SERS intensities (at 1580 cm^{-1} peak) measured from the NWs-on-chip sensor in the presence of a single metal ion, (b), or multiple metal ions, (c). I_O is the averaged SERS intensity of a NWs-on-chip sensor in the absence of metal ions. I_M is the averaged SERS intensity from a NWs-on-chip sensor in the presence of a single metal ion or multiple kinds of metal ions. The concentrations of metal ions are 100 μM each. The data were obtained from five measurements for each metal ion and the error bars represent standard deviation of I_M.

Fig. 3 shows detection limit investigation of the NWs-on-chip sensor for Hg^{2+}, Ag^{+}, and Pb^{2+}. SERS intensities of Cy5 decreased with increasing concentrations of metal ions, with detection limits of 500 pM, 1 nM, and 50 nM for Hg^{2+}, Ag^{+}, and

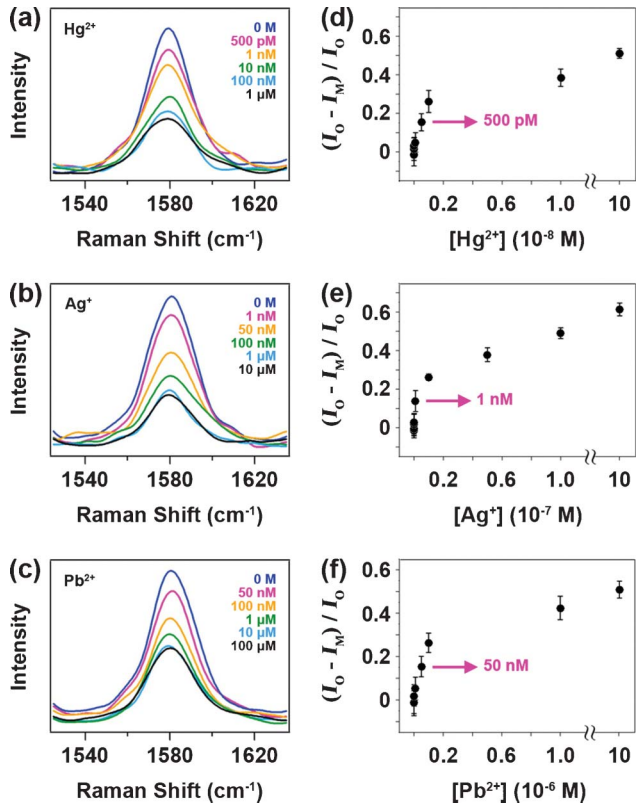

Fig. 3 1580 cm^{-1} peak intensities of Cy5 from the NWs-on-chip sensor at various values of (a) $[Hg^{2+}]$, (b) $[Ag^{+}]$, and (c) $[Pb^{2+}]$. Plots of reduced SERS intensity (at 1580 cm^{-1} peak) *versus* (d) $[Hg^{2+}]$, (e) $[Ag^{+}]$, and (f) $[Pb^{2+}]$. The data were obtained from five measurements and the error bars represent standard deviation of I_M.

Pb^{2+}, respectively (Fig. 3d–f). The detection limit of Ag^{+} is highly improved compared to the previously reported value and that of Hg^{2+} is comparable to what we reported before.[1,9,13,14] Pb^{2+} sensing shows a relatively high detection limit of 50 nM. This low sensitivity can be attributed to the reduced affinity of a Pb^{2+}-specific aptamer. While the aptamer with the GGTTGGTGTGGTTGG sequence can form a G-quadruplex structure through specific binding of Pb^{2+}, it can also bind with Hg^{2+} because of its multiple T bases. Therefore, we modified the Pb^{2+}-specific aptamer to contain the GG*AA*GGTGTGG*AA*GG sequence for selective detection of metal ions. While this modified aptamer can bind only with Pb^{2+}, it has 3–5 times lower affinity than the unmodified aptamer.[19] Although the sensitivity of Pb^{2+} is lower than previously reported sensors, Hg^{2+} and Ag^{+} could be detected in quite high sensitivity. Most importantly, rapid and multiplex detection of all three metal ions was successfully demonstrated by the Au NWs-on-chip SERS sensor.

The key points of the present study can be summarized as follows. First, single-crystalline Au NWs provide superb reproducibility. In order to obtain reproducible SERS signals, well-defined and reproducible nanostructures should be fabricated. In addition, adoption of a single Raman dye is more advantageous for quantitative multiplex sensing than the multi-label based SERS sensors. Second, this is the first SERS demonstration of multiplex detection of Hg^{2+}, Ag^{+}, and Pb^{2+}. Last and most importantly, accurate sensing of toxic metal ions was achieved in a single step and less than an hour without a pre-treatment step by adopting a reporter elimination strategy. Compared to an inductively coupled plasma mass spectrometry, the most widely used method for detection of metal ions, this sensor is simpler, faster, and more cost effective. Therefore, the Au NWs-on-chip sensor could be employed for real-time, on-site, and on-line detection of metal ions.

4. Conclusions

We have developed an alignment-addressed Au NWs-on-chip SERS system using metal ion-specific dsDNAs for sensitive, rapid, and multiplex detection of toxic metal ions. Hg^{2+}, Ag^{+}, and Pb^{2+} in a homogeneous solution could be detected selectively with detection limits of 500 pM, 1 nM, and 50 nM, respectively. The NWs-on-chip SERS sensor developed in this study could be applied in environmental monitoring and medical diagnostics by using numerous kinds of aptamers.

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References

1 Z. Z. Lin, X. H. Li and H. B. Kraatz, *Anal. Chem.*, 2011, **83**, 6896–6901.
2 S. S. Tan, Y. N. Teo and E. T. Kool, *Org. Lett.*, 2010, **12**, 4820–4823.
3 F. Zahir, S. J. Rizwi, S. K. Haq and R. H. Khan, *Environ. Toxicol. Pharmacol.*, 2005, **20**, 351–360.
4 J. O. Nriagu, *Nature*, 1979, **279**, 409–411.
5 S. Zierler, M. Theodore, A. Cohen and K. J. Rothman, *Int. J. Epidemiol.*, 1988, **17**, 589–594.
6 M. E. Mahmoud, M. M. Osman, O. F. Hafez and E. Elmelegy, *J. Hazard. Mater.*, 2010, **173**, 349–357.
7 H. B. Bradl, *J. Colloid Interface Sci.*, 2004, **277**, 1–18.
8 K. K. Krishnani, X. G. Meng, C. Christodoulatos and V. M. Boddu, *J. Hazard. Mater.*, 2008, **153**, 1222–1234.
9 C. Y. Lin, C. J. Yu, Y. H. Lin and W. L. Tseng, *Anal. Chem.*, 2010, **82**, 6830–6837.
10 D. Han, S. Y. Lim, B. J. Kim, L. Piao and T. D. Chung, *Chem. Commun.*, 2010, **46**, 5587–5589.
11 C. W. Liu, C. C. Huang and H. T. Chang, *Anal. Chem.*, 2009, **81**, 2383–2387.
12 J. S. Lee, M. S. Han and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2007, **46**, 4093–4096.
13 T. Kang, S. M. Yoo, I. Yoon, S. Lee, J. Choo, S. Y. Lee and B. Kim, *Chem.–Eur. J.*, 2011, **17**, 2211–2214.
14 R. Freeman, T. Finder and I. Willner, *Angew. Chem., Int. Ed.*, 2009, **48**, 7818–7821.
15 L. B. Zhang, L. Tao, B. L. Li, L. Jing and E. K. Wang, *Chem. Commun.*, 2010, **46**, 1476–1478.
16 G. Q. Wang and L. X. Chen, *Chin. Chem. Lett.*, 2009, **20**, 1475–1477.
17 B. L. Li, Y. Du and S. J. Dong, *Anal. Chim. Acta*, 2009, **644**, 78–82.
18 C. Zhao, K. G. Qu, Y. J. Song, C. Xu, J. S. Ren and X. G. Qu, *Chem.–Eur. J.*, 2010, **16**, 8147–8154.
19 I. V. Smirnov, F. W. Kotch, I. J. Pickering, J. T. Davis and R. H. Shafer, *Biochemistry*, 2002, **41**, 12133–12139.
20 T. Li, S. J. Dong and E. K. Wang, *J. Am. Chem. Soc.*, 2010, **132**, 13156–13157.

21 H. Wang, Y. M. Kim, H. P. Liu, Z. Zhu, S. Bamrungsap and W. H. Tan, *J. Am. Chem. Soc.*, 2009, **131**, 8221–8226.
22 C. S. Wu, M. K. K. Oo and X. D. Fan, *ACS Nano*, 2010, **4**, 5897–5904.
23 C. J. Murphy, T. K. San, A. M. Gole, C. J. Orendorff, J. X. Gao, L. Gou, S. E. Hunyadi and T. Li, *J. Phys. Chem. B*, 2005, **109**, 13857–13870.
24 N. S. Ramgir, Y. Yang and M. Zacharias, *Small*, 2010, **6**, 1705–1722.
25 F. Patolsky, G. F. Zheng and C. M. Lieber, *Anal. Chem.*, 2006, **78**, 4260–4269.
26 B. He, T. J. Morrow and C. D. Keating, *Curr. Opin. Chem. Biol.*, 2008, **12**, 522–528.
27 Y. R. Fang, H. Wei, F. Hao, P. Nordlander and H. X. Xu, *Nano Lett.*, 2009, **9**, 2049–2053.
28 H. Wei, Z. X. Wang, X. R. Tian, M. Kall and H. X. Xu, *Nat. Commun.*, 2011, **2**, 387.
29 I. Yoon, T. Kang, W. Choi, J. Kim, Y. Yoo, S. W. Joo, Q. H. Park, H. Ihee and B. Kim, *J. Am. Chem. Soc.*, 2009, **131**, 758–762.
30 S. M. Yoo, T. Kang, B. Kim and S. Y. Lee, *Chem.–Eur. J.*, 2011, **17**, 8657–8662.
31 T. Kang, S. M. Yoo, I. Yoon, S. Y. Lee and B. Kim, *Nano Lett.*, 2010, **10**, 1189–1193.
32 T. Kang, I. Yoon, J. Kim, H. Hee and B. Kim, *Chem.–Eur. J.*, 2010, **16**, 1351–1355.
33 G. A. Zelada-Guillen, J. Riu, A. Duzgun and F. X. Rius, *Angew. Chem., Int. Ed.*, 2009, **48**, 7334–7337.
34 H. M. So, D. W. Park, E. K. Jeon, Y. H. Kim, B. S. Kim, C. K. Lee, S. Y. Choi, S. C. Kim, H. Chang and J. O. Lee, *Small*, 2008, **4**, 197–201.
35 Y. Yoo, K. Seo, S. Han, K. S. K. Varadwaj, H. Y. Kim, J. H. Ryu, H. M. Lee, J. P. Ahn, H. Ihee and B. Kim, *Nano Lett.*, 2010, **10**, 432–438.
36 T. Kang, I. Yoon, K. S. Jeon, W. Choi, Y. Lee, K. Seo, Y. Yoo, Q. H. Park, H. Ihee, Y. D. Suh and B. Kim, *J. Phys. Chem. C*, 2009, **113**, 7492–7496.
37 M. Moskovits, *Rev. Mod. Phys.*, 1985, **57**, 783–826.
38 K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari and M. S. Feld, *Chem. Rev.*, 1999, **99**, 2957–2975.
39 H. Wei, F. Hao, Y. Z. Huang, W. Z. Wang, P. Nordlander and H. X. Xu, *Nano Lett.*, 2008, **8**, 2497–2502.
40 H. X. Xu, J. Aizpurua, M. Kall and P. Apell, *Phys. Rev. E*, 2000, **62**, 4318–4324.
41 H. X. Xu, E. J. Bjerneld, M. Kall and L. Borjesson, *Phys. Rev. Lett.*, 1999, **83**, 4357–4360.