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Au Nanowire-on-Film SERRS Sensor for Ultrasensitive Hg^{2+} Detection**

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Abstract: We report an ultrasensitive and selective single nanowire-on-film (SNOF) surface-enhanced resonance Raman scattering (SERRS) sensor for Hg^{2+} detection based on structure-switching double stranded DNAs (dsDNAs). Binding of Hg^{2+} induces conformational changes of the dsDNAs and let a Raman reporter get close to the SNOF structure, thereby turning on SERRS signal. The well-defined SNOF structure provides a detection limit of 100 pM with improved accuracy in Hg^{2+} detection. This sensor is stable over a considerable amount of time and reusable after simple treatment. Since this SNOF sensor is composed of a single Au NW on a film, development of a multiplex sensor would be possible by employing NWs modified by multiple kinds of aptamers.

Keywords: DNA · gold · mercury · nanowires · surface-enhanced Raman scattering

Introduction

Mercury is a widespread pollutant with distinct toxicity and causes a number of severe health problems such as brain damage, kidney failure, and various cognitive and motion disorders.[1] Water-soluble mercuric ion (Hg^{2+}), one of the most stable inorganic forms of mercury,[2] is a carcinogenic material with high cellular toxicity[3] and becomes more harmful when it is accumulated in human body through methyl mercury as a result of a microbial biomethylation in aquatic sediments.[4] A sensitive detection method of Hg^{2+} is thus highly important in order to evaluate the safety of aquatically derived food supplies. Several Hg^{2+} detection methods based upon fluorophores,[5] semiconductor quantum dots,[6] cyclic voltammetry,[7] anodic stripping voltammetry (ASV),[8] polymeric materials,[9] proteins,[10] and microcantilevers[11] have been developed. Surface-enhanced Raman scattering (SERS), in particular, can be an attractive method for highly sensitive detection of Hg^{2+} because it can detect the analytes even on a single molecule level.[12] Beside high sensitivity, Raman spectra have advantages such as narrow band widths and alleviated photobleaching.[13] These intrinsic advantages have led to the development of Hg^{2+} SERS sensors based on nanoparticle (NP) aggregations.[14] Although these sensors show high sensitivities, their SERS signal could be strongly affected by degree of aggregation, size distributions of NPs, and inhomogeneous distribution of molecules on the NP surfaces. Recently, we have reported a novel SERS active platform composed of a Au nanowire (NW) on a Au film.[15] Since this SERS platform provides reliable reproducibility with their well-defined structure as well as good time stability and excellent sensitivity,[15] we significantly improved the accuracy, stability, and sensitivity in Hg^{2+} detection.

Herein, we present a highly sensitive and selective single NW-on-film (SNOF) sensor for Hg^{2+} that relies on thymine (T)-Hg^{2+}-T coordination chemistry. As compared to SERS sensors comprising NPs, the SNOF structure is composed of a single-crystalline Au NW having atomically smooth surfaces and thus well-defined in nanoscale. The SNOF sensor provides a detection limit of 100 pM, 50 times lower than that of previously reported aptameric Hg^{2+} SERS sensor.[14b] Furthermore, this sensor has good reproducibility,[15] long-term stability, and reusability. The SNOF structure, a few micrometers long and ~100 nm wide, could prove to be a very effective and practical chemical SERS sensor. We anticipate our assay to be a starting point for a multiplex sensor of various ions, which could be fabricated by combining multiple SNOF sensors.

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[**] Surface-enhanced resonance Raman scattering.

Results and Discussion

Figure 1 a shows the sensing strategy of Hg^{2+} using a SNOF surface-enhanced resonance Raman scattering (SERRS) sensor. Single-crystalline Au NWs were synthesized by vapor transport method.[16] For the construction of SNOF sensor, as-grown NWs were incubated in thiolated probe DNA solution and then transferred onto a Au film by using a custom-built nanomanipulator.[16a] In order to recognize Hg^{2+} specifically and achieve a "signal-on" sensor, we used the structure-switching double stranded DNAs (dsDNAs) which were prepared by the hybridization between single stranded (ss) T-rich DNAs and complementary Cy5-labeled DNAs. Cy5 has an absorption maximum of 647 nm,[17] allowing the incoming light in 633 nm wavelength to excite resonant vibration of molecule. SERRS typically enhances Raman signals by 10^2–10^3 times compared with nonresonant SERS.[17–18] When a drop of the mixture of structure-switching dsDNAs and Hg^{2+} solution is put on a SNOF sensor, T-rich DNAs folded into a hairpin structure in the solution to form stable T-Hg^{2+}-T complexes. As a result, Cy5-labeled DNAs are hybridized with T-rich DNAs only by six base pairs, and can be released easily at room temperature.[19] The released Cy5-labeled ssDNAs are then captured by a SNOF sensor modified by probe DNAs complementary to Cy5-labeled DNAs. Finally, the presence of Hg^{2+} in a sample can be detected by turning on the SERRS signal of a SNOF sensor.

SERRS spectra measured from a SNOF sensor in the absence of and after addition of 1 μM Hg^{2+} solution are presented in Figure 1 b. When Hg^{2+} existed in the sample, four major bands of Cy5 at 1580, 1485, 1360, and 1185 cm^{-1}, which corresponds to $\nu(C{=}N)_{stretch}$, $\nu(C\text{-}C)_{ring}$, $\nu(C{=}C)_{ring}$, and $\nu(C{-}N)_{stretch}$ was clearly observed (bottom spectrum in Figure 1 b).[20] In contrast, when the sample contained no Hg^{2+}, the SERRS signal was hardly observed (top spectrum in Figure 1 b). This result shows that Hg^{2+} in water can be successfully detected by the SNOF sensor.

To determine the detection limit of SNOF SERRS sensor, the Hg^{2+} solutions with concentrations of 10^{-12}–10^{-5} M were examined (Figure 2). The intensity of a prominent Raman peak at 1580 cm^{-1} increased with the concentration of Hg^{2+}

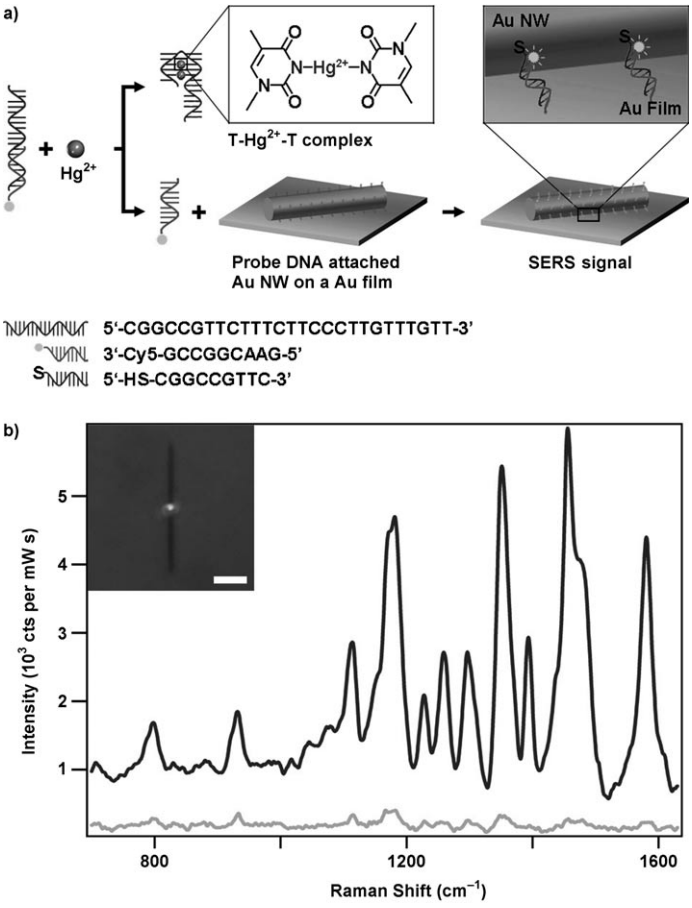

Figure 1. a) Schematic representation of a SNOF SERRS sensor for Hg^{2+} detection based on a structure-switching dsDNAs. b) SERRS spectra of a SNOF sensor in the absence of (bottom spectrum) and addition of 1 μM Hg^{2+} solution (top spectrum). The inset is an optical image of a SNOF sensor. The scale bar denotes 5 μm.

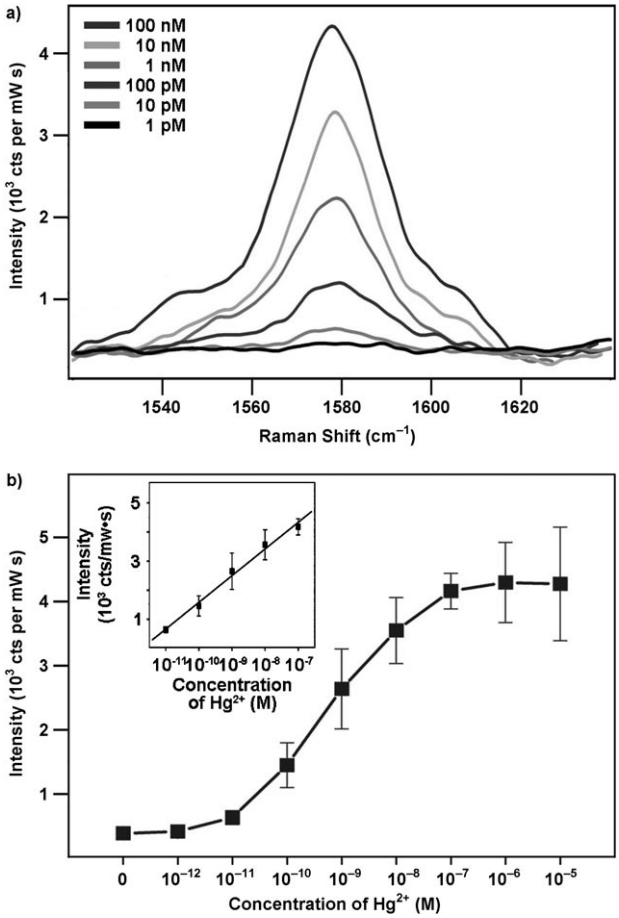

Figure 2. a) 1580 cm^{-1} band intensities of Cy5 from SNOF sensors by varying the concentration of Hg^{2+}. b) Plot of 1580 cm^{-1} band intensities versus concentrations of Hg^{2+}. The inset shows a dynamic range and linearly fitted line. The data was obtained from five measurements and the error bars represent standard deviation.

and a linear relationship between the SERRS intensity and the Hg^{2+} concentration was observed in the range of 10^{-10} to 10^{-7} M (inset of Figure 2b). We estimated the detection limit of this sensor to be 100 pM. In comparison to that of previously reported aptameric SERS sensor for Hg^{2+},[14b] the SNOF sensor improved the detection limit 50-fold. This ultrasensitivity could be achieved by combining SERS enhancement of SNOF structure with resonance Raman scattering of Cy5. Although other Hg^{2+} detection methods such as resonance scattering spectroscopy[21] and ASV[8] provide similar detection limit, they are limited either by the complexity of the assay or rigorous electrode preparation and conditioning procedure. Considering the simple procedure, the detection limit of SNOF sensor is rather impressive.

In order to confirm that the SERRS enhancement is dependent on the specific recognition of Hg^{2+}, 100 μM of various metal ions (Hg^{2+}, Mg^{2+}, Ca^{2+}, Cr^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}) were individually added to SNOF sensors with structure-switching dsDNAs. Figure 3 shows the intensities of a 1580 cm^{-1} Raman band measured after the respective metal ion solution was dropped on a SNOF sensor. The remarkable SERRS enhancement was observed only in the presence of Hg^{2+}. The selectivity of this method was further investigated with mutant structure-switching dsDNAs consisting of ssDNAs having random sequences and Cy5-labeled ssDNAs. Upon adding a solution of 100 μM Hg^{2+} and the mutant dsDNAs to a SNOF sensor, we could not observe any discernible SERRS signals (last column in Figure 3). Taken together, these observations suggest that a nucleic-acid-based SNOF sensor is highly specific for the detection of Hg^{2+}.

Finally, the reusability and long-term stability of the SNOF sensor were investigated. The SNOF sensor once used for Hg^{2+} detection was reused after the incubation in distilled water at 65 °C. At this temperature, the Cy5-labeled DNAs captured in SNOF sensors are released by the thermal melting of dsDNAs,[17,19b] enabling repeated usage as a sensor as many as three times, each with incubation in hot water. The SERRS signal for detecting Hg^{2+} was still clearly observed even after three rounds of use. Moreover, as-prepared SNOF sensors could be used without significant decrease of the signals after storing in ambient condition at room temperature over four weeks (see Figure 4).

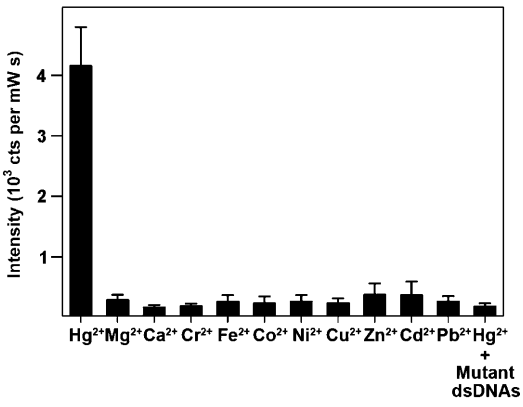

Figure 3. Selectivity of SNOF sensor for Hg^{2+} detection based on structure-switching dsDNAs. Solutions containing a single kind of metal ion were individually tested. The tested metal ions are shown on the *x* axis and the corresponding intensities of 1580 cm^{-1} Raman band are shown in *y* axis. Discernable SERRS signals did not appear from SNOF sensors when the sample has metal ions other than Hg^{2+}. When mutant dsDNAs were used, Hg^{2+} could not be detected. The data was obtained from ten measurements and the error bars represent standard deviation.

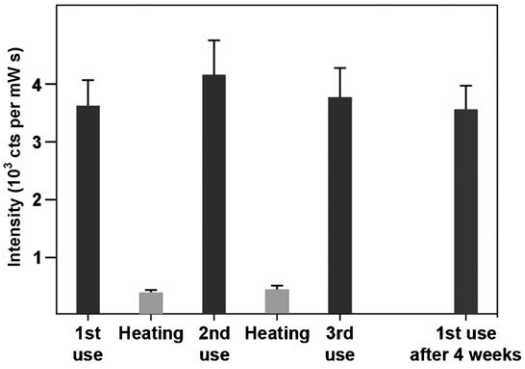

Figure 4. Reusability and long-term stability of a SNOF sensor. The SNOF sensors were used to detect 100 μM Hg^{2+} for three times, followed by the incubation in distilled water at 65 °C. The SERRS intensities of 1580 cm^{-1} Raman band were decreased after heating because of the thermal melting of dsDNAs (gray columns). The SNOF sensors could be used for Hg^{2+} detection without decrease of SERRS signals after three rounds of use (dark gray columns). The last dark gray column shows that strong SERRS signals were still observed from SNOF sensors after four weeks. The data was obtained from five measurements and the error bars represent standard deviation.

Conclusion

We have developed an ultrasensitive and selective SNOF SERRS sensor for Hg^{2+} detection based on structure-switching dsDNAs. This sensor provides several advantages. First, the simple and well-defined SNOF structure made of well-faceted Au NWs and a flat Au film allows us to determine Hg^{2+} concentration in high accuracy. Lack of reproducibility of the nanostructures for SERS platform has been a major barrier for quantitative SERS sensing. Second, the SNOF sensor provides ultra low detection limit of 100 pM. At a concentration 100 times lower than the maximum permitted level of Hg^{2+} (10 nM) defined by US Environmental Protection Agency in drinking water,[14a] the SERRS signal remained very clear. Third, this sensor is stable over a long period of time and can be reused after simple treatment. Additionally, the development of portable and multiplex sensor would be feasible by using multiple kinds of aptamers and correspondingly modified NWs.[22]

Experimental Section

Materials: Purified DNAs were obtained from Genotech (Daejeon, Korea). The metal salts ($Hg(Ac)_2$, $Mg(Ac)_2$, $Ca(Ac)_2$, $CrCl_2$, $FeCl_2$, $Co(Ac)_2$, $NiCl_2$, $CuCl_2$, $Zn(Ac)_2$, $Cd(Ac)_2$, and $Pb(Ac)_2$) were purchased from Sigma–Aldrich. The DNA and reagents were used as received with-

out further purification except probe DNAs. Before use, the thiolated probe DNAs were treated with 1 M dithiothreitol (Sigma–Aldrich) to reduce the sulphide bonds and purified using NAP-5 column (GE healthcare.Co.).

Preparation of SNOF sensors: Au NWs were synthesized on a *c*-sapphire substrate in a horizontal quartz tube furnace system using a vapor transport method.[16b] The sapphire substrate was placed a few centimetres downstream from an alumina boat filled with 0.03 g of pure Au powder as a precursor. Ar gas flowed at a rate of 100 sccm, maintain 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. Smooth Au films were prepared on pre-cleaned Si substrates by electron beam assisted deposition of 10 nm of Cr followed by 300 nm of Au. The surfaces of the Au films were smooth enough to be SERS inactive by themselves.[15] The Au films were cut to 0.25 cm^2 for SNOF sensor fabrication. To prepare the probe DNA-attached Au NWs, 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 removed by 0.2% (w/v) sodium dodecyl sulphate (SDS) solution for 5 min. Finally, the probe DNA-attached Au NWs were transferred one by one onto Au film by using a custom-built nanomanipulator,[16a] constructing the SNOF sensors for Hg^{2+} detection.

Preparation of structure-switching dsDNAs: The 10 μM T-rich DNAs and 10 μM Cy5-labeled DNAs were mixed in phosphate buffered saline (PBS) solution (pH 7.4) with a molar ratio of 1:1. This solution was heated to 95 °C for 5 min and cooled down to room temperature slowly. The mutant structure-switching dsDNAs were prepared by mixing ssDNAs having random sequences (5′-CGGCCGTTCAAACGGCC-CAAGCCCGCC-3′) and Cy5-labeled DNAs via the same procedure as structure-switching dsDNAs.

Detection of Hg^{2+} using SNOF sensors: The 10 μM solution of structure-switching dsDNAs (45 μL) was mixed with Hg^{2+} solution of a fixed volume (5 μL) at a series of concentrations. This mixture was dropped onto the SNOF sensors and followed by incubation for 2 h in closed petri dish at room temperature. Before the measurement of SERRS signal, SNOF sensors were washed with PBS containing 0.1% (w/v) SDS for 4 min, rinsed twice with distilled deionized water, and dried under N_2 stream. For the reuse of SNOF sensor, the used one was incubated in distilled water at 65 °C twice for 5 min. After cleaning with distilled water, the SNOF sensors were used again for Hg^{2+} detection.

Instrumentation: The custom-built nanomanipulator is a tungsten tip (~100 nm diameter at the end) mounted on a three-dimensional piezoelectric stage. SERRS spectra were measured from a micro-Raman system based on an Olympus BX41 microscope. The 633 nm radiation of a He/Ne laser (Melles Griot) was used as an excitation source and the laser was focused on a sample through a ×100 objective (NA = 0.7, Mitutoyo). The laser power on the sample was 0.4 mW. The SERRS signals were recorded with a thermodynamically cooled electron multiplying charge coupled device (EMCCD, Andor) mounted on the spectrometer with a 1200 groove/mm grating. The acquisition time of all SERRS spectra was 60 s. A holographic notch filter was used to reject the laser light.

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