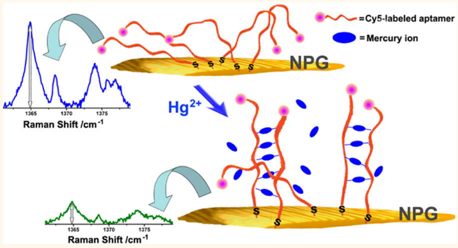
Nanoporous Gold Based Optical Sensor for Sub-ppt Detection of Mercury Ions

Ling Zhang,[†,‡] Haixin Chang,[†,‡] Akihiko Hirata,[‡] Hongkai Wu,[‡,§] Qi-Kun Xue,[‡,∥,*] and Mingwei Chen[‡,⊥,#,*]

[‡]WPI Advanced Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan, [§]Department of Chemistry, Hong Kong University of Science and Technology, Hong Kong, [∥]Department of Physics, Tsinghua University, Beijing 100871, People's Republic of China, [⊥]State Key Laboratory of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiao Tong University, Shanghai 200030, People's Republic of China, and [#]CREST, Japan Science and Technology Agency (JST), Saitama 332-0012, Japan. [†]These authors contributed equally to this work (L.Z. and H.C.).

ABSTRACT Precisely probing heavy metal ions in water is important for molecular biology, environmental protection, and healthy monitoring. Although many methods have been reported in the past decade, developing a quantitative approach capable of detecting sub-ppt level heavy metal ions with high selectivity is still challenging. Here we report an extremely sensitive and highly selective nanoporous gold/aptamer based surface enhanced resonance Raman scattering (SERRS) sensor. The optical sensor has an unprecedented detection sensitivity of 1 pM (0.2 ppt) for Hg^{2+} ions, the most sensitive Hg^{2+} optical sensor known so far. The sensor also exhibits excellent selectivity. Dilute Hg^{2+} ions can be identified in an aqueous solution containing 12 metal ions as well as in river water and underground water. Moreover, the SERRS sensor can be reused without an obvious loss of the sensitivity and selectivity even after 10 cycles.



KEYWORDS: nanoporous gold (NPG) · surface enhanced resonance Raman scattering (SERRS) · aptamer · mercury ion · optical sensor

Mercury ions (Hg^{2+}) as environmental pollutants have serious medical effects since mercury accumulation in human body through food chains and drinking water cause irreversible damage of brain and central nervous system as well as other chronic diseases.[1−4] Therefore, highly sensitive and on-site detection of Hg^{2+} ions in aqueous media is important in molecular biology and environmental and food monitoring, as well as in clinical toxicology.[5,6] Nondestructive optical detection is very attractive for on-site analysis of Hg^{2+} ions because of the obvious merits in remote and fast acquisition in natural environments, high toxic sources, and even in living organisms. Several optical methods have been developed for the detection of mercury ions,[7] such as colormetric assays,[8] fluorescence detection,[9−11] Plasmon resonance spectroscopy,[12] and surface enhanced Raman scattering (SERS).[13] Because Hg^{2+} is not active to most optical spectroscopy, the measurements are usually through Hg^{2+} sensitive molecular probes and labeling tags.[14,15] Aptamers and metal ions-specific organic ligands have been often used for this purpose.[9,16−24] However, the sensitivity of all these optical methods is still insufficient to detect dilute mercury ions with a concentration less than a few picomolars (sub-ppt level), limiting their applications in precise measurements of Hg^{2+} ions from drinking water, foods, and living organisms. In this study, we report a novel surface enhanced resonance Raman scattering (SERRS) method for ultrasensitive optical detection of Hg^{2+} ions by using dealloyed nanoporous gold (NPG) as a plasmonic substrate and Cy5-labeled aptamer as optical tags. The NPG/aptamer based hybrid SERRS sensor has an unprecedented sensitivity of 1 pM (0.2 ppt) for Hg^{2+} ion detection, which is ∼1000 times more sensitive than conventional optical sensors and about 4 orders of magnitude lower than U.S.A. EPA-defined maximum level of drinking water.[25] To the best of our knowledge, this is the most sensitive optical sensor for the Hg^{2+} detection in water. The NPG/aptamer sensor also shows excellent selectivity and can be directly used to detect Hg^{2+} ions in river and underground water that contains complicated organic and inorganic interferents.

RESULTS AND DISCUSSION

NPG has been demonstrated to be an excellent plasmonic substrate because both nanosized gold ligaments and nanopores

* Address correspondence to
qkxue@mail.tsinghua.edu.cn,
mwchen@wpi-aimr.tohoku.ac.jp.

Received for review March 19, 2013
and accepted April 16, 2013.

10.1021/nn4013737

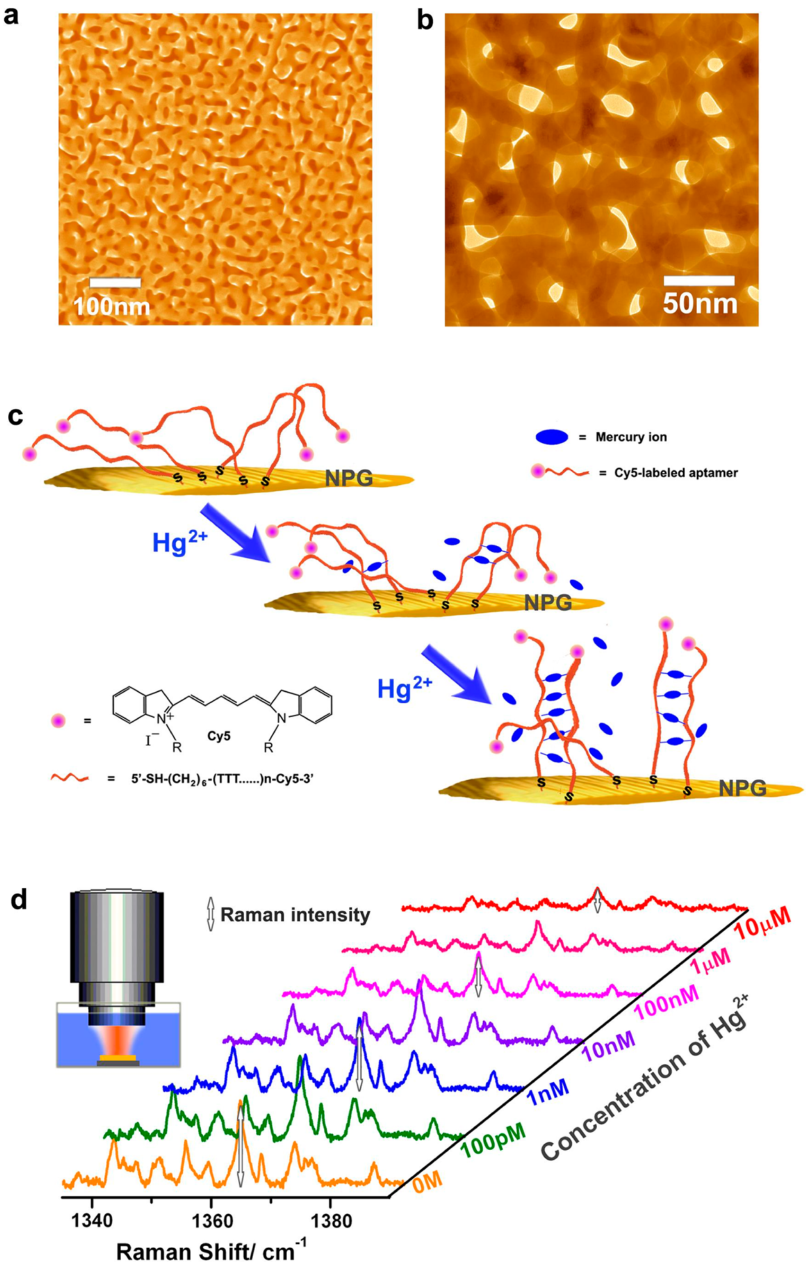


Figure 1. Characterization of NPG/aptamer hybrid SERRS sensor. (a, b) Typical SEM micrograph (a) and TEM image (b) of NPG used in this study. (c) Schematic description of SERRS sensing of Hg^{2+} based on the aptamer modified NPG. (d) Quantitative detection of Hg^{2+} by measuring the SERRS signal drop of Cy5 tags using Apt15@NPG sensor. The inset shows the experimental setup for the Hg^{2+} detection.

can generate intense localized surface plasmon fields for amplifying the Raman scattering of molecules.[26,27] A steady SERS enhancement can be well reproduced from anywhere of a centimeter-sized NPG film owning to their uniform nanoporous structure spanning from tens of nanometer to centimeters. This analytical feature is extremely valuable, compared to heterogeneous nanoparticle-based substrates, for sensor applications that require unvarying and reproducible Raman signals from any detected regions of each sample. Figure 1a shows a typical scanning electron microscope (SEM) image of the NPG substrate used in this study. The characteristic length of nanopores is ∼20 ± 2 nm, measured by a fast Fourier transform method.[28] The uniform nanoporous structure is further confirmed by transmission electron microscopy (Figure 1b).

The SERRS sensor comprises a single-strand, multi-thymines oligonucleotide probe with an alkanethiol moiety (-SH) at the 5′-terminus and a Cy5 tag at 3′-end. The aptamer is immobilized on the gold ligament surface of NPG by the thiol anchor. Quantitative and sensitive detection of Hg^{2+} ions is achieved by monitoring the intensity changes of Cy5 SERRS peaks with Hg^{2+} concentrations. The length of the aptamer can be tuned by controlling the number of thymine (T) bases. In this study, the aptamer with 15 T (Apt15) and 8 T

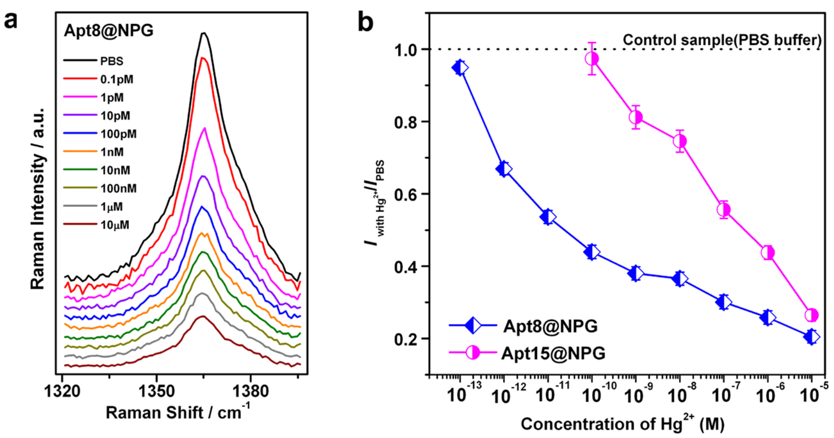


Figure 2. Performance of the Apt8@NPG SERRS sensor. (a) The most prominent SERRS peak of Cy5, at 1365 cm^{-1}, with different concentrations of Hg^{2+}. The SERRS intensity of Cy5 decreases with increasing Hg^{2+} concentration. (b) The normalized Intensity of 1365 cm^{-1} peak by comparing the intensity with various Hg^{2+} ions and with PBS buffer only. The data from the Apt15@NPG SERRS (round dots) are also plotted for comparison.

(Apt8) are used. The Cy5 tags have a $S_0 \rightarrow S_1$ transition mode centered at ~650 nm and, thus, a 632.8 nm excitation laser can yield a resonant vibration of Cy5. The resultant SERRS signals of Cy5 are ~3−5 orders of the magnitude stronger than the regular SERS ones for a low detection limit and ultrahigh sensitivity. The Hg^{2+} sensitivity of the NPG/aptamer based SERRS sensor relies on the changes of the Cy5 Raman intensity caused by the cooperative coordination of a pair of poly-T oligonucleotides with Hg^{2+} ions as shown in Figure 1c. In the absence of Hg^{2+}, the single strand poly-T oligonucleotides display a flexible and random structure. Most Cy5 tags lay on the NPG surface and the Raman signals of Cy5-tags can be maximally enhanced by the local surface plasmon resonance originating from NPG. In the presence of Hg^{2+} ions, Hg^{2+} can specifically bind in between two DNA thymine bases and lead to the formation of T− Hg^{2+}−T pairs though N−Hg^{2+}−N *J*-coupling bonding.[29] Circular dichroism (CD) spectra of Apt8 with various Hg^{2+} additions (see Figure S1 in the Supporting Information) indicates the formation of T−Hg^{2+}−T pairs,[30,31] and single strand poly-T oligonucleotides undergo a structural change to form a duplex-like structure.[32] The Hg^{2+} mediated T−T base pairs are as stable as a normal Watson−Crick base pair and can trigger a conformational reorganization of the poly-T oligonucleotides from flexible single strands to relatively rigid duplex-like complexes (Figure 1c). However, the T−Hg^{2+}−T coordination complex may not be the exact antiparallel or parallel duplex. As a result, the Cy5 tags are pulled away from the NPG substrate, resulting in a decrease in the SERRS signals of the Cy5 tags. At a low concentration, the number of Hg^{2+} ions is not sufficient to form a complete duplex-like structure and some aptamers still flexibly lay on the NPG surface. Thus, the SERRS signals from Cy5 tags only partially decrease. Because the fraction of the duplex-like structure has a linear correlation with the number of Hg^{2+}, the Hg^{2+} concentrations can be determined from the decrement of the SERRS intensity of the Cy5 tags.

Figure 1d illustrates SERRS spectra taken from the Apt15 functionalized NPG substrate (Apt15@NPG) with different Hg^{2+} concentrations. The inset is the schematic of the experimental setup for the measurements. The relative intensity of the SERRS signals from Cy5 tags decreases with increasing concentration of Hg^{2+} ions. The strongest Cy5 Raman band at 1365 cm^{-1} was chosen for quantitative analysis based on the intensity dependence of the characteristic Raman peak on Hg^{2+} concentrations. With 100 pM Hg^{2+} addition, the detectable Raman peak intensity drop of ~3% can be measured. Further increasing the Hg^{2+} to 1 nM and 1 μM, the intensity drops ~20% and 60%, respectively. The detection sensitivity of the Apt15@NPG sensor for Hg^{2+} is better than 1 nM with a dynamic detection range from 1 nM to 10 μM.

The sensitivity of Hg^{2+} detection is associated with the distance between Cy5-tags and the plasmonic surface of NPG. The more thymine bases are in the oligonucleotide, the more Hg^{2+} ions are required to form a duplex-like structure that hauls up Cy5-tags from the substrate surface for the corresponding SERRS signal reduction. Therefore, a short aptamer with less thymine bases is expected to be more responsive to Hg^{2+}. To test this idea, a short Apt8 aptamer (Apt8, 5′-SH-(CH$_2$)$_6$-TTT TTT TT-Cy5−3′) was synthesized and immobilized on NPG (Apt8@NPG). Figure 2a shows the decreased intensity of the 1365 cm^{-1} Raman band with the increase of Hg^{2+} concentrations. Apparently, the short Apt8 aptamer dramatically improves the sensitivity of the SERRS sensor, compared to the Apt15. The normalized intensity of the 1365 cm^{-1} bands acquired by the Apt8@NPG sensor is plotted in Figure 2b. For comparison, the data from Apt15 are also shown in the figure. It can be seen that ~5% intensity drop, corresponding to 100 fM Hg^{2+}, can be captured by the ultrasensitive Apt8@NPG optical sensor. The intensity drops almost 30% with 1 pM Hg^{2+} and more than 70% with 1 μM Hg^{2+}. The Hg^{2+} detection sensitivity of the Apt8@NPG sensor is estimated better

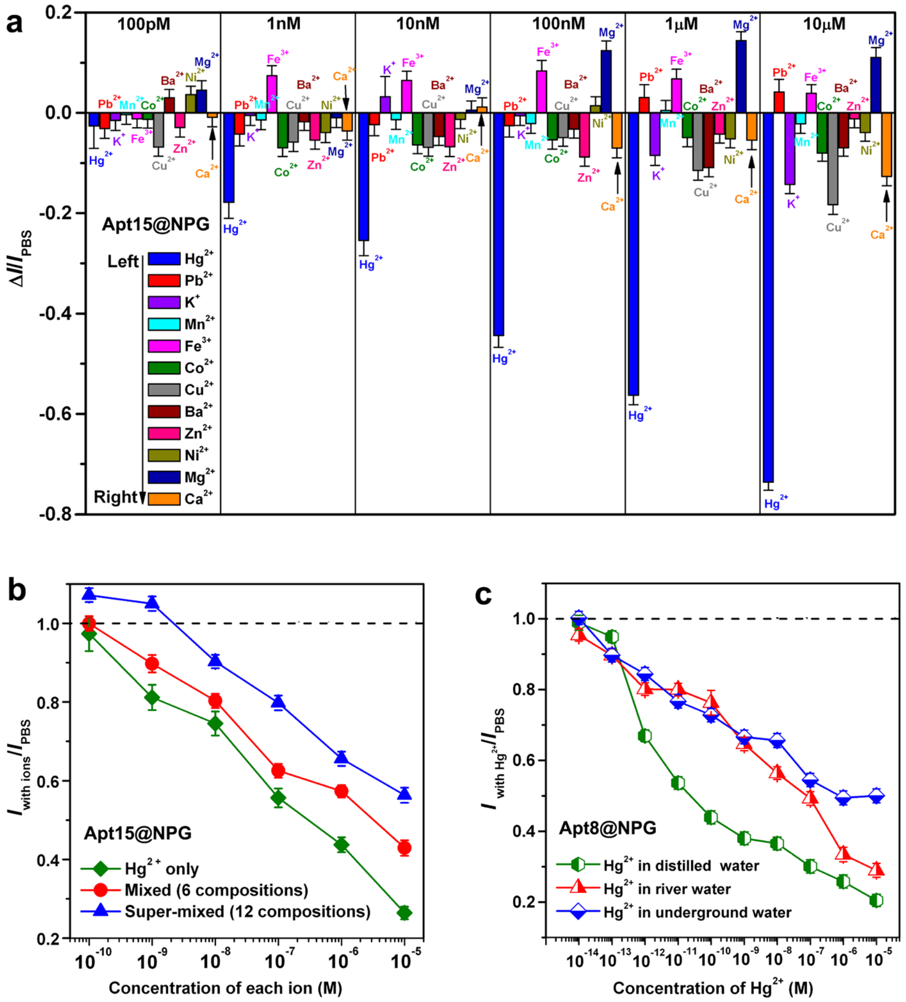


Figure 3. Selectivity test of the NPG/aptamer SERRS sensor. (a) Normalized Raman intensity changes of Cy5 1365 cm^{-1} band with individual metal ions at various concentrations. (b) The normalized peak intensities of the 1365 cm^{-1} band with various mixed ions at different concentrations. The square dots indicate the solution only contains Hg^{2+}, the round dots (mixed) indicate the solution contains Hg^{2+}, Pb^{2+}, Ni^{2+}, Fe^{3+}, Co^{2+}, and Mn^{2+} with the same concentrations, and the triangle dots (super-mixed) indicate the solution contains Hg^{2+}, Pb^{2+}, K^{+}, Fe^{3+}, Co^{2+}, Mn^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Mg^{2+} with the same concentration. The lines guide for eyes. (c) Hg^{2+} ion detection in distilled water, river water and underground water using the Apt8@NPG sensor.

than 1 pM. To the best of our knowledge, this is the most sensitive optical sensor for mercury ions detection in water environments known so far.

To verify that the SERRS signal change corresponds solely to the specific recognition of Hg^{2+}, we replaced Hg^{2+} by other metal ions (Pb^{2+}, K^{+}, Fe^{3+}, Co^{2+}, Mn^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Mg^{2+}). Figure 3a depicts the SERRS response of the Apt15@NPG sensor to various metal ions, respectively. The sensor shows appreciable intensity change in the response to Hg^{2+}. The more Hg^{2+} ions are introduced into the solution, the lower the SERRS intensity is. For Pb^{2+}, Fe^{3+}, Co^{2+}, Mn^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, and Zn^{2+}, the SERRS intensity does not change perceptibly (less than ±10%) over the entire concentration range from 1 nM to 10 μM. The intensity increases slightly for Mg^{2+}, but the variation less than 10% even with a concentration larger than 1 μM. For K^{+} and Cu^{2+}, small intensity decrease can be observed. Although Cu^{2+} and thymine can form a concomitant compact conformation,[8] the presence of Cu^{2+} does not severely influence the SERRS signals of the Cy5 tags. Moreover, the sensitivity limit for Cu^{2+} detection is approximately 10 μM, which is about 4 orders of magnitude higher than that of Hg^{2+}.

An essential feature of a chemical sensor is its selectivity not only to isolated targets but also to mixtures that are analogous to the natural environments in field measurements. We first mixed Hg^{2+} with five other cations (Pb^{2+}, Ni^{2+}, Fe^{3+}, Co^{2+}, and Mn^{2+}) that are not response to the sensor during individual tests (Figure 3a). As shown in Figure 3b (round dots), the interference from the five metal cations only gives rise to a slight decrease in the sensitivity. About 20% reduction still remains in the presence of 10 nM Hg^{2+}. Subsequently, we further added six more cations (Ca^{2+}, Ba^{2+}, K^{+}, Cu^{2+}, Zn^{2+}, and Mg^{2+}) into the solution, and each of them has the same concentrations as Hg^{2+}. The reduction of the Cy5-tag SERRS

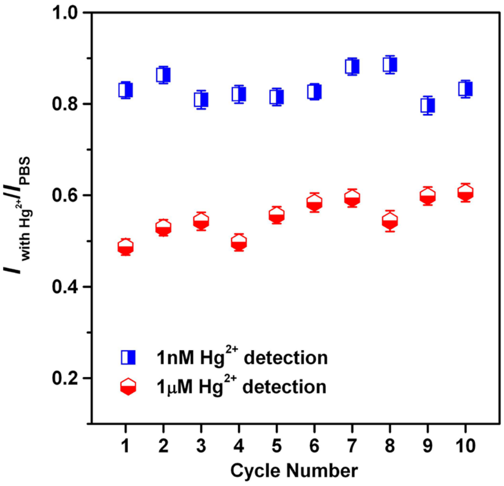


Figure 4. Recyclability test of the NPG/aptamer SERRS sensor. Normalized peak intensities of the 1365 cm^{-1} band with 1 nM or 1 μm Hg^{2+} in the aqueous solution for 10 cycles.

intensity, corresponding to 10 nM Hg^{2+}, keeps at ~10% even in such a complex solution.

The high sensitivity and selectivity of the SERRS sensor even can be retained in the natural environments during field measurements. When we replaced the laboratory distilled water by river water and underground water, the intensity drop remains ~20% with 1 pM Hg^{2+} using Apt8@NPG sensor (Figure 3c, triangle and quadrangle dots). For our SERRS sensor, the detection limit is better than 1 pM (0.2 ppt), which is at least 3 orders of magnitude lower than that of the conventional optical methods (see Table S1 in the Supporting Information), for example, UV−vis optical absorption (1 nM, ref 8), fluorescence method (2.4 nM, ref 9; 5 nM, ref 21), surface Plasmon resonance spectroscopy sensor (0.1 ppb, equal to 0.5 nM, ref 12), gold nanoparticle enhanced fluorescence sensor (0.2 ppb, equal to 1 nM, ref 22), and gold nanoparticle based SERS sensor (5 nM, ref 15; 5 ppb, equal to 25 nM, ref 16). In addition, the NPG/aptamer hybrid sensor is very stable and can be kept in the PBS buffer for one month without the loss in the ultrahigh sensitivity (see Figure S2 in the Supporting Information).

The optical sensor is readily regenerated in 100 mM ascorbic acid solution for 1 h, followed by washing with a 33 mM PBS buffer (pH 6.9) solution containing 0.5 M NaCl and 0.1 M $NaClO_4$ for 15 min.[32] Figure 4 displays 10 typical cycles of the regeneration of the SERRS sensor for detecting 1 nM and 1 μM Hg^{2+} in aqueous solutions. It can be observed that the SERRS intensity decreases to 80−90% of the original one with 1 nM Hg^{2+}, while 1 μM Hg^{2+} gives rise to 40−50% decrease of the original intensity. Although the relative intensity of the SERRS signal from the immobilized aptamers can not be fully recovered, the variation ratio is less than 10% within 10 cycles. Moreover, the used NPG substrates can be recycled for aptamer decoration after fully washed by distilled water and the sensor can be completely recovered to the original state for sensing applications.

CONCLUSION

In this study we report a Cy5-labeled aptamer@NPG SERRS sensor for the optical detection of Hg^{2+} with ultrahigh sensitivity and excellent selectivity. Amplified by the plasmonic NPG substrate and resonant excitation laser, the hybrid SERRS Hg^{2+} sensor can achieve an unprecedented 1 pM (0.2 ppt) Hg^{2+} sensitivity with excellent selectivity. It provides a powerful tool to detect dilute Hg^{2+} pollutions in water, foods and living organisms for environmental and health monitoring and clinical toxicology. It is worth noting that the optical detection approach developed by this study can also be utilized to detect other heavy metal ions by changing the metal ions-specific organic ligands and aptamers since the basic principle of the SERRS sensor is the same.

EXPERIMENTAL METHODS

Fabrication of Nanoporous Gold. Nanoporous gold (NPG) films were prepared by selectively etching Ag from 100 nm thick $Ag_{65}Au_{35}$ (at. %) leaves using 69% nitric acid at room temperature.[26,33] The as-prepared NPG films were carefully rinsed with distilled water (18.2 MΩ·cm) to remove the remained nitric acid, and then stabilized on polymer sheets for sensor applications. Microstructure of the NPG films was characterized by using a scanning electron microscope (SEM; JEOL, JIB-4600F) and a transmission electron microscope (JEOL, JEM-2100F).

Aptamers and Oligonucleotide Immobilization. Aptamers (5′-SH-$(CH_2)_6$-(TTT...)*n*-Cy5−3′) with an alkanethiol moiety (-SH) at the 5′ terminal and a Cy5-tag at 3′ end were synthesized by TAKARA Bio Inc., Japan. The length of the aptamer was tuned by the number of thymine (T) bases. NPG substrates (5 × 2 mm^2) were incubated in the 0.5 mL probe solution containing 10 nM aptamer, 1.0 M NaCl in 10 mM PBS buffer (pH = 7.4) for 24 h at room temperature to allow aptamers self-assemble on the surface of NPG films. The substrates then were rinsed with a 1 mL of PBS buffer (pH = 6.9) solution containing 0.5 M NaCl and 0.1 M $NaClO_4$ for 2 min to remove unstable aptamers and then kept in the PBS buffer at 4 °C, where the biosensors can maintain the activity for more than 1 month.

Raman Spectroscopy. A micro-Raman spectrometer (Renishaw InVia RM 1000) with an excitation laser wavelength of 632.8 nm was used for SERRS measurements. The laser power was set at a low value of 0.03 mW to avoid possible fluorescence decay and molecular damage. A water immersed objective lens (Nikon, 60 × / 1.00W, WD2.0) was used for *in situ* measurements in aqueous solutions.

To detect Hg^{2+} and other metal ions, the SERRS sensor was immersed by 2 mL PBS buffer (33 mM, pH = 6.9) solution containing 0.5 M NaCl, 0.1 M $NaClO_4$, Hg^{2+}, and other metal ions for 30 min at room temperature, followed by *in situ* Raman measurements. Metal ion solutions were added in the container at different concentrations, and the SERRS signal was collected from the substrate surfaces in the solution using the water immersed long distance objective lens. Each SERRS spectrum was averaged by the Raman signals collected from ten sites from the NPG/aptamer substrate and displayed after subtracting the fluorescence background from the original signals. To investigate the selectivity of the hybrid sensor, various metal ions including $Hg(NO_3)_2$, $Cu(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$, $Ni(NO_3)_2$, KCl, $FeCl_3$, $CoCl_2$, $CaCl_2$, $MgCl_2$, $BaCl_2$, and $Mn(CH_3COO)_2$ were analyzed in this study.

Conflict of Interest: The authors declare no competing financial interest.

Acknowledgment. This work was supported by "World Premier International Research Center (WPI) Initiative" by the MEXT, and JST-CREST "Interface Science for Highly Efficient Energy Utilization", JST, Japan.

Supporting Information Available: Sensitivity comparison of various optical methods, circular dichroism (CD) spectra of Apt8 with various Hg^{2+} additions, and stability test of the sensor are shown. This material is available free of charge *via* the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1. Choi, Y.; Park, Y.; Kang, T.; Lee, L. P. Selective and Sensitive Detection of Metal Ions by Plasmonic Resonance Energy Transfer-Based Nanospectroscopy. *Nat. Nanotechnol.* **2009**, *4*, 742–746.
2. Cho, E. S.; Kim, J.; Tejerina, B.; Hermans, T. M.; Jiang, H.; Nakanishi, H.; Yu, M.; Patashinski, A. Z.; Glotzer, S. C.; Stellacci1, F. Ultrasensitive Detection of Toxic Cations through Changes in the Tunnelling Current across Films of Striped Nanoparticles. *Nat. Mater.* **2012**, *11*, 978–985.
3. Clarkson, T. W. The Three Modern Faces of Mercury. *Environ. Health Perspect.* **2002**, *110*, 11–23.
4. Onyido, I.; Norris, A. R.; Buncel, E. Biomolecule-Mercury Interactions: Modalities of DNA Base-Mercury Binding Mechanisms. Remediation Strategies. *Chem. Rev.* **2004**, *104*, 5911–5929.
5. Nriagu, J. O.; Pacyna, J. M. Quantitative Assessment of Worldwide Contamination of Air, Water and Soils by Trace-Metals. *Nature* **1988**, *333*, 134–139.
6. Jensen, S.; Jernelov, A. Biological Methylation of Mercury in Aquatic Organisms. *Nature* **1969**, *223*, 753–754.
7. Nolan, E. M.; Lippard, S. J. Tools and Tactics for the Optical Detection of Mercuric Ion. *Chem. Rev.* **2008**, *108*, 3443–3480.
8. Li, D.; Wieckowska, A.; Willner, I. Optical Analysis of Hg^{2+} Ions by Oligonucleotide-Gold Nanoparticle Hybrids and DNA-Based Machines. *Angew. Chem., Int. Ed.* **2008**, *47*, 3927–3931.
9. Liu, J.; Lu, Y. Rational Design of "Turn-On" Allosteric DNAzyme Catalytic Beacons for Aqueous Mercury Ions with Ultrahigh Sensitivity and Selectivity. *Angew. Chem., Int. Ed.* **2007**, *46*, 7587–7590.
10. Lee, J.; Jun, H.; Kim, J. Polydiacetylene-Liposome Microarrays for Selective and Sensitive Mercury(II) Detection. *Adv. Mater.* **2009**, *21*, 3674–3677.
11. Yang, Y. K.; Ko, S. K.; Shin, I.; Tae, J. Synthesis of a Highly Metal-Selective Rhodamine-Based Probe and its Use for the *In Vivo* Monitoring of Mercury. *Nat. Protoc.* **2007**, *2*, 1740–1745.
12. Wang, S.; Forzani, E. S.; Tao, N. Detection of Heavy Metal Ions in Water by High-Resolution Surface Plasmon Resonance Spectroscopy Combined with Anodic Stripping Voltammetry. *Anal. Chem.* **2007**, *79*, 4427–4432.
13. Alvarez-Puebla, R. A.; Liz-Marzán, L. M. SERS Detection of Small Inorganic Molecules and Ions. *Angew. Chem., Int. Ed.* **2012**, *51*, 11214–11223.
14. Wang, G.; Lim, C.; Chen, L.; Chon, H.; Choo, J.; Hong, J.; deMello, A. J. Surface-Enhanced Raman Scattering in Nanoliter Droplets: towards High-Sensitivity Detection of Mercury(II) Ions. *Anal. Bioanal. Chem.* **2009**, *394*, 1827–1832.
15. Lee, C.; Choo, J. Selective Trace Analysis of Mercury(II) Ions in Aqueous Media Using SERS-Based Aptamer Sensor. *Bull. Korean Chem. Soc.* **2011**, *32*, 2003–2007.
16. Senapati, T.; Senapati, D.; Singh, A. K.; Fan, Z.; Kanchanapally, R.; Ray, P. C. Highly Selective SERS Probe for Hg(II) Detection Using Tryptophan-Protected Popcorn Shaped Gold Nanoparticles. *Chem. Commun.* **2011**, *47*, 10326–10328.
17. Wang, H.; Wang, Y.; Jin, J.; Yang, R. Gold Nanoparticle-Based Colorimetric and "Turn-On" Fluorescent Probe for Mercury(II) Ions in Aqueous Solution. *Anal. Chem.* **2008**, *80*, 9021–9028.
18. Forzani, E. S.; Zhang, H. Q.; Chen, W.; Tao, N. J. Detection of Heavy Metal Ions in Drinking Water Using a High-Resolution Differential Surface Plasmon Resonance Sensor. *Environ. Sci. Technol.* **2005**, *39*, 1257–1262.
19. Liu, X.; Tang, Y.; Wang, L.; Zhang, J.; Song, S.; Fan, C.; Wang, S. Optical Detection of Mercury(II) in Aqueous Solutions by Using Conjugated Polymers and Label-Free Oligonucleotides. *Adv. Mater.* **2007**, *19*, 1471–1474.
20. Liu, J.; Lu, Y. Preparation of Aptamer-Linked Gold Nanoparticle Purple Aggregates for Colorimetric Sensing of Analytes. *Nat. Protoc.* **2006**, *1*, 246–252.
21. Liu, C.; Huang, C.; Chang, H. Highly Selective DNA-Based Sensor for Lead(II) and Mercury(II) Ions. *Anal. Chem.* **2009**, *81*), 2383–2387.
22. Ye, B.-C.; Yin, B.-C. Highly Sensitive Detection of Mercury(II) Ions by Fluorescence Polarization Enhanced by Gold Nanoparticles. *Angew. Chem., Int. Ed.* **2008**, *47*, 8386–8389.
23. Hollenstein, M.; Hipolito, C.; Lam, C.; Dietrich, D.; Perrin, D. M. A Highly Selective DNAzyme Sensor for Mercuric Ions. *Angew. Chem., Int. Ed.* **2008**, *47*, 4346–4350.
24. Zhang, X.; Xiao, Y.; Qian, X. A. Ratiometric Fluorescent Probe Based on FRET for Imaging Hg^{2+} Ions in Living Cells. *Angew. Chem., Int. Ed.* **2008**, *47*, 8025–8029.
25. Lee, J.-S.; Mirkin, C. A. Chip-Based Scanometric Detection of Mercuric Ion Using DNA Functionalized Gold Nanoparticles. *Anal. Chem.* **2008**, *80*, 6805–6808.
26. Qian, L. H.; Yan, X. Q.; Fujita, T.; Inoue, A.; Chen, M. W. Surface Enhanced Raman Scattering of Nanoporous Gold: Smaller Pore Sizes Stronger Enhancements. *Appl. Phys. Lett.* **2007**, *90*, 153120.
27. Lang, X. Y.; Guan, P. F.; Zhang, L.; Fujita, T.; Chen, M. W. Characteristic Length and Temperature Dependence of Surface Enhanced Raman Scattering of Nanoporous Gold. *J. Phys. Chem. C* **2009**, *113*, 10956–10961.
28. Fujita, T.; Chen, M. W. Characteristic Length Scale of Bicontinuous Nanoporous Structure by Fast Fourier Transform. *Jpn. J. Appl. Phys.* **2008**, *47*, 1161–1163.
29. Tanaka, Y.; Oda, S.; Yamaguchi, H.; Kondo, Y.; Kojima, C.; Ono, A. 15N−15N J-Coupling Across Hg(II): Direct Observation of Hg(II)-Mediated T−T Base Pairs in a DNA Duplex. *J. Am. Chem. Soc.* **2006**, *129*, 244–245.
30. Miyake, Y.; Togashi, H.; Tashiro, M.; Yamaguchi, H.; Oda, S.; Kudo, M.; Tanaka, Y.; Kondo, Y.; Sawa, R.; Fujimoto, T.; et al. Mercury^{II}-Mediated Formation of Thymine−Hg^{II}−Thymine Base Pairs in DNA Duplexes. *J. Am. Chem. Soc.* **2006**, *128*, 2172–2173.
31. Gruenwedel, D. W. Effect of Hg(II) on the Spectroscopic Properties of DNA Bases: Circular Dichroism of Deoxyadenosine and Thymidine Monomers and Dimers. *J. Inorg. Biochem.* **1994**, *56*, 201–212.
32. Liu, S.-J.; Nie, H.-G.; Jiang, J.-H.; Shen, G.-L.; Yu, R.-Q. Electrochemical Sensor for Mercury(II) Based on Conformational Switch Mediated by Interstrand Cooperative Coordination. *Anal. Chem.* **2009**, *81*, 5724–5730.
33. Erlebacher, J.; Aziz, M. J.; Karma, A.; Dimitrov, N.; Sieradzki, K. Evolution of Nanpoporosity in Dealloying. *Nature* **2001**, *410*, 450–453.