Surface-enhanced Raman Scattering for Immunoassay Based on the Biocatalytic Production of Silver Nanoparticles

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We have reported on a novel enzyme immunoassay method for the detection of protein using biocatalytic silver nanoparticles as an enhanced substrate based on surface-enhanced Raman scattering (SERS). First, ascorbic acid was converted from ascorbic acid 2-phosphate by alkaline phosphatase immobilized on polystyrene microwells after a typical sandwich immunoreaction. Then Ag(I) ions were reduced to silver nanoparticles by the obtained ascorbic acid, which would result in a SERS signal when Raman dyes were absorbed. Using human IgG as a model protein, a wide linear dynamic range (1 to 100 ng ml⁻¹) was reached with a low detection limit (0.02 ng ml⁻¹) under the optimized assay conditions. Moreover, the production of an enhanced substrate was chosen as the signaling element in this method, which demonstrates a new way for SERS-based quantitative detection. These results suggest that the application of SERS enhanced by biocatalytic production of metal nanoparticles holds a promising potential for a sensitive immunoassay.

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Introduction

Since being reported in 1928, Raman spectroscopy has been widely used in studies of various chemical and biological systems, and has been expected to be a powerful fingerprint vibrational spectroscopy in qualitative and quantitative analysis.¹⁻¹⁷ Despite having some advantages over the fluorescent counterparts, including richer spectral information and narrower peak widths (*ca.* 20 cm⁻¹), conventional Raman spectra usually lack sufficient sensitivity for routine detection, due to the small Raman-scattering cross sections.³ However, surface-enhanced Raman scattering (SERS) has overcome the weakness of insensitivity for normal Raman spectroscopy with a nearly 10⁴ – 10⁶ fold enhancement in recent years.⁴ It is generally agreed that the large enhancement of the Raman scattering results from electromagnetic (EM) fields at some “hot spots”, which, most often, consist of nanoscale junctions and interstices in interacting with metal nanostructures, such as nanoparticle dimmers or aggregates.^{5,6} In particular, even a single molecule has been detected by SERS, suggesting that an enhancement factor of as much as 10¹⁴ – 10¹⁵ could be achieved.^{7,8} The discovery and study of SERS renewed interest in employing Raman spectroscopy for an ultrasensitive bioassay. An initial trial in SERS-based bioassay was demonstrated by Dou *et al.*, in which an enzyme-reaction product was adsorbed on silver colloids to generate a SERS signal, allowing an indirect assay of the antigen on the basis of the enzyme-linked immunoassay format.⁹ Using colloidal gold with co-adsorbed dyes as a biomarker, Porter and coworkers published a series of reports on SERS-based readout sandwich immunoassay.¹⁰⁻¹² Mirkin's group has proposed SERS-active nanostructures

labeled with different dyes and oligonucleotide probes for the multiplexed detection of nucleic acids and proteins.^{13,14} The common feature of the detections was that any changes of the SERS intensity all resulted from the different quantity of the Raman dye molecules. In fact, the SERS intensity is also dependent on the character of the enhanced substrates according to many studies.^{15,16} Recently, Moskovits' group developed innovative work in which DNA detection was performed through the production of SERS hot spots, which result from the target-dependent hybridization of DNA-coated Ag nanoparticles onto a DNA-coated metal film, where the probe molecules resided.¹⁷ It is interesting that the targets were determined by the production of an enhanced substrate, rather than the Raman dyes, as compared with the traditional SERS-based bioassay. Such an approach opens a new way for SERS-based quantitative analysis.

There has been great interest in the development of new, simple and sensitive methods for the determination of proteins based on the specific recognition of an antigen with a corresponding antibody. Enzyme-linked immunosorbent assay (ELISA) is one of the most commonly used methods for immunoassay, because of its good sensitivity, selectivity, and ease in use. Generally, enzymatic products resulting from the antigen-antibody reactions could be detected by spectrophotometric or electrochemical methods. In recent years, alkaline phosphatase (ALP) has become one of the most commonly used biomarkers for ELISA, due to its high turnover number and broad substrate specificity.¹⁸⁻²⁶ For example, Hwang *et al.* have developed a DNA sensor using an enzyme label, and the accumulation of metallic silver reduced by the product of the ALP-*p*-aminophenyl phosphate reaction, which achieved multiple amplifications and very low detection limits.²³ We have also reported on a series of signal-amplified electrochemical immunoassays on the basis of the biocatalytic deposition of silver nanoparticles by a similar reaction and anodic stripping

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voltammetric detection.²⁴⁻²⁶ Although these methods demonstrated great potential for the determination of their targets, alternative rapid and sensitive detection techniques are still continuously being explored for the development of an immunoassay.

In the present study, a SERS-based enzyme immunoassay technique for the detection of human IgG as a model protein was developed using biocatalytic silver nanoparticles as the enhanced substrate. Ascorbic acid was firstly converted from ascorbic acid 2-phosphate by ALP immobilized on polystyrene microwells *via* a sandwich immunoreaction; it then reduced Ag(I) ions readily to silver nanoparticles, which would result in a SERS signal with Raman dyes absorbed on them. On the basis of this strategy, antigen has been determined at a relatively low concentration under optimized experimental conditions. Herein, the production of an enhanced substrate was chosen as a signalling element, rather than the quantity of Raman dye molecules, as practiced in traditional SERS-based bioassay methods. Besides the inherent advantages over fluorescence or electrochemical detection, the present method only used a common dye as the reporter, which circumvented the labeling of Raman label *via* a specific group to the nanoparticles in traditional SERS-based detection. Transmission electron micrograph (TEM) was utilized to characterize the formed silver nanoparticles. The optimal conditions for the detection of human IgG were investigated.

Experimental

Reagents and chemicals

Goat anti-human IgG antibody, human IgG, and bovine serum albumin (BSA) were purchased from Beijing Dingguo Biotechnology Development Center (Beijing, China). Alkaline phosphatase (ALP) conjugated goat anti-human IgG antibody was obtained from Beijing Zhongshan Biotechnology Reagents. Ascorbic acid 2-phosphate (AA-p) was purchased from Express Technology Co. Ltd. (Japan). Crystal violet (CV), malachite green (MG), basic fuchsin (BF), rhodamine 6G (R6G), rhodamine B isothiocyanate (RBITC), 4-aminothiophenol (4-ATP) were purchased from Sigma-Aldrich. Buffers used in this work included 0.05 M NaHCO₃-Na₂CO₃ (pH 9.6) as a coating medium and 0.05 M sodium phosphate-buffered saline (PBS, pH 7.4) as an incubating and washing buffer. The enzyme reaction solution was a glycine-NaOH buffer containing 1.5 mM AA-p (pH 9.0). Other reagents were of analytical purity, and all solutions were prepared with deionized water (= 18.32 MΩ) purified by a Nanopure Infinity Ultrapure water system (Barnstead/Thermolyne Corp., Dubuque, IA).

Apparatus

Raman spectra were collected using a Jobin Yvon Micro-Raman spectrometer (RamLab-010). The light source was a 12-mW, 632.8-nm He-Ne laser. The slit and the pinhole were set at 100 and 300 μm. A semiconductor-cooled 1024 × 256 pixels charge-coupled device (CCD) detector system was attached to the system. Under this setting, the sampling area was about 10 μm in diameter on the substrate surface. All SERS spectra were acquired with 2 s integration, and processed with software from Jobin Yvon (NGSLabSpec).

Transmission electron micrographs (TEM) were obtained by using a JEM-3010 Electron Microscope (JEOL, Japan) with Digitalgraph software at an accelerating voltage of 100 kV.

Immunoassay procedure

Firstly, 100 μl of 0.25 mg ml⁻¹ goat anti-human IgG antibody

Scheme 1 Schematic diagram of the immunoassay procedure for human IgG (see details in the text).

(dissolved in 0.05 M NaHCO₃-Na₂CO₃ buffer) was added to the polystyrene microwells and incubated at 4°C overnight. After removing the solution, the microwells were rinsed with 0.05 M PBS eight times, and this rinsing procedure was repeated after each following step, unless otherwise described. Then, 150 μl of a 1% BSA solution dissolved in 0.05 M PBS was added to block the active sites of the wells at 37°C for 1 h. Secondly, 100 μl of different concentrations of human IgG solutions (dissolved in 0.05 M PBS) were injected and incubated at 37°C for another 1 h. Thirdly, 100 μl of an ALP-conjugated antibody was added into the microwells at 37°C for 1 h. The microwells were then rinsed with 0.05 M PBS four times and ultrapure water six times after the formation of a sandwich immunocomplex, followed by the addition of 100 μl of an enzyme reaction solution (containing AA-P) for 20 min at 37°C. Finally, 45 μl of the enzyme-catalyzed reduction products was transferred to tubes, followed by the addition of 5 μl of a 10 mM AgNO₃ (pH 7.0) solution and shaking in the dark for another 40 min.

SERS measurement

A 3-μl aliquot of 5 × 10⁻⁵ M R6G was added to 27 μl of the prepared solution in a centrifuge tube. After reagent addition and vigorous shaking for 10 s, the SERS spectrum was recorded. Because the immunoreaction in this work was carried out in polystyrene microwells, about 15 samples could be assayed at one time by parallel detection.

Results and Discussion

Analytical principle

The analytical principle of the SERS-based enzyme immunoassay is depicted in Scheme 1. In short, after the immunocomplex was formed on the bottom of polystyrene microwells, the ALP would catalyze the hydrolysis of AA-p to ascorbic acid. Because the half-wave potential of ascorbic acid is 0.39 V *versus* NHE, and that of Ag(I) ions is 0.7995 V, the ascorbic acid in alkaline solution would spontaneously reduce the Ag(I) ions.²⁵ The whole reaction could be expressed as follows:

This kind of sandwich-type bioassay based on the biocatalytic deposition of silver nanoparticles has been successively developed by several groups.²³⁻²⁵ Furthermore, the size of

Fig. 1 TEM images of metallic silver dropped onto a micro-grid as the final reduction product in response to (A) 0, (B) 10, and (C) 100 ng ml⁻¹ human IgG. The scale bar is 100 nm in each image.

metallic silver particles obtained in these studies was also demonstrated to be nanoscale. According to many published routes for the synthesis of colloidal Au and Ag, the size of the nanoparticles is inversely proportional to the ratio of the reductants to metal.²⁷⁻²⁹ Therefore, the size of Ag nanoparticles should decrease when more ascorbic acid has been produced through enzyme-catalyzed reduction in this work. Herein, the production of ascorbic acid is dependent on the quantity of the analyte, human IgG. On the other hand, Ag nanoparticles reduced by ascorbic acid have been demonstrated to be the enhanced substrate for SERS.^{30,31} Furthermore, the size of nanoparticles has been found to be one of the most important facets to determine SERS activity. Similar results from many groups showed that the enhancement efficiency of silver nanoparticles is proportional to the size at 633-nm excitation.^{15,32} Therefore, the human IgG analyte could be detected quantitatively according to the SERS behavior of Ag nanoparticles reduced by ascorbic acid in this bioassay.

TEM characterization

Figure 1 shows TEM images of metallic silver dropped on to a micro-grid as the final reduction product in response to (A) 0, (B) 10, and (C) 100 ng ml⁻¹ human IgG. One can observe that the silver product is almost monodispersed nanoscale particles with spherical facets. For the three products, average sizes of 59 ± 2 , 20 ± 1 and 12 ± 1 nm were estimated using 100 respective particles in a TEM investigation. It is worth noticing that the biggest nanoparticles were obtained without antigen being added, which is thought to have resulted from unavoidable nonspecific adsorption during formation of the immunocomplex. Furthermore, it can also be observed that smaller silver nanoparticles are obtained by increasing the concentration of antigen, which is consistent with the basic rationale for the size-dependent ratio of the reductant to the metal ion in the synthesis of metal nanoparticles.²⁷ It can be assumed that more antigens would result in an increase of the quantity of ascorbic acid, then generating smaller silver nanoparticles in the next step. Considering that colloidal silver and gold had been demonstrated as being very effective SERS substrates, the nanoparticles obtained in this experiment are expected to show high SERS activity. Although other factors, such as the particle shape and specific adsorption sites, are important for SERS enhancement, the particle size is assumed to be the dominant factor for the SERS performance in the present work. This assumption was subsequently confirmed by SERS experiments.

Response performance in SERS

To investigate the SERS performance of the obtained silver nanoparticles in this work, six dyes were chosen as Raman

Fig. 2 A, SERS spectra of six Raman dyes enhanced with the obtained nanoparticles in response to 10^{-5} mg ml⁻¹ human IgG as targets using 632.8-nm laser excitation at 5×10^{-5} M. B, The ratio of the SERS intensity in response to the PBS buffer (denoted I_{blank}) and 10^{-5} mg ml⁻¹ human IgG (denoted I_{target}) of the six Raman dyes. Each data point represents an average of four measurements (error bars are the relative standard deviations).

reporters, which are all commercially available. Figure 2A depicts the SERS spectra of 5×10^{-5} M Raman dyes enhanced with the obtained nanoparticles in response to 10 ng ml⁻¹ human IgG as the target. It can be observed that most dyes exhibit their typical bands in the region from 1700 to 1100 cm⁻¹. This result indicates that the obtained silver nanoparticles can be used as an efficient enhanced substrate for common Raman dyes. However, the obtained signals are not very strong compared with that enhanced by nanoparticles synthesized by the published routes, which is mainly due to an insufficient aggregation of silver nanoparticles generated during the reduction, so that the hot spots are not sufficient. In this work, the bands at 1641.3, 1504.1 and 1141.7 cm⁻¹ were chosen as marker bands for RBITC, R6G and 4-ATP, respectively, while

Fig. 3 Effect of the concentration of Ag(I) ions on the ratio of the SERS signal at 1504.1 cm^{-1} in response to the PBS buffer (denoted I_{blank}) and 10^{-5} mg ml^{-1} human IgG (denoted I_{target}). The error bars are the relative standard deviations.

Fig. 4 Ratio of the SERS signal at 1504.1 cm^{-1} in response to the PBS buffer (denoted I_{blank}) and 10^{-5} mg ml^{-1} human IgG (denoted I_{target}) as a function of the Ag(I) ions reduction time. The error bars are the relative standard deviations.

the band at 1610.3 cm^{-1} was chosen as the marker band for CV, MG and BF (Fig. 2A). The band was chosen as a marker of each dye due to its ideal peak profile, and its intensity was among the strongest ones. The peaks at 1641.3 and 1504.1 cm^{-1} are assigned to aromatic C–C stretching and those at 1141.7 and 1610.3 cm^{-1} are assigned to aromatic C–H bending and phenyl–N stretch, respectively.^{33–35} To investigate the performance of the strategy in the detection, $I_{\text{blank}}/I_{\text{target}}$ was employed as the measure in the immunoassay. Herein, I_{blank} and I_{target} were the SERS intensities at the marker band in response to the PBS buffer and 10^{-5} mg ml^{-1} human IgG, respectively. As shown in Fig. 2B, the intensity at each marker band in response to the PBS buffer is stronger than that in the response to the 10^{-5} mg ml^{-1} target. This can be explained by the size-dependent enhancement behavior of silver nanoparticles. Considering the correlation between the quantity of human IgG and the size of silver nanoparticles, shown in Fig. 1, smaller nanoparticles would be obtained in response to a 10^{-5} mg ml^{-1} target than that in response to the PBS buffer. According to recent SERS studies on the size-dependent enhancement behavior of silver nanoparticles, a weaker SERS signal would be obtained using smaller silver nanoparticles as an enhanced substrate at 633-nm excitation. As a result, the quantity of the target should be inversely proportional to the SERS intensity in this approach. These results indicate that detection for human IgG could be achieved using the ratio of the signal as the measure based on SERS. Moreover, the biggest response is observed from R6G among all dyes under the same conditions (Fig. 2B). It is assumed that the positive charge of R6G in an aqueous solution would allow good absorption to the negatively charged silver surface, and result in a strong SERS signal.³⁶ Therefore, R6G was selected for a Raman reporter in this SERS-based immunoassay.

Optimization of the immunoassay conditions

In the present work, both the Ag(I) ions concentration and the reduction time played crucial roles for the detection sensitivity. Herein, $I_{\text{blank}}/I_{\text{target}}$ was utilized as a measure to determine the optimum conditions, where I_{blank} and I_{target} were the SERS intensities of R6G at 1504.1 cm^{-1} in response to the PBS buffer and 10^{-5} mg ml^{-1} human IgG, respectively. As shown in Fig. 3, $I_{\text{blank}}/I_{\text{target}}$ was strongly dependent upon the Ag(I) ions concentration, and the optimum ratio was achieved at a concentration of 1.0 mM. It is assumed that either deficient

Fig. 5 SERS response of the immunoassay strategy for human IgG in response to 0, 1, 10 and 100 ng ml^{-1} human IgG.

amount of Ag(I) ions or excess of Ag(I) ions would induce an insufficient size of silver particles for enhancing the Raman scattering, thus resulting in a weak ratio of I_{blank} to I_{target} .²⁵ Figure 4 shows the value of the ratio response to the reduction time of Ag(I) ions added into the product obtained by an enzyme-catalyzed reaction. With an increase of the reduction time, the ratio increased from 10 to 40 min, and decreased obviously. It can be predicted that a shorter silver reduction time would lead to less production of silver nanoparticles, which has a critical effect on the sensitivity of the immunoassay. On the other hand, a larger silver reduction time would prolong the analytical time and, more seriously, result in an enhancement of the signal in response to 10^{-5} mg ml^{-1} human IgG, and decrease the ratio of $I_{\text{blank}}/I_{\text{target}}$ in the immunoassay. Therefore, 1.0 mM and 40 min were selected as the Ag(I) ions concentration and the time for reducing the Ag(I) ions, respectively. Such a condition was used throughout subsequent experiments.

SERS-based detection of human IgG

Under the optimized assay conditions, this SERS-based immunoassay strategy was employed to detect human IgG. As shown in Fig. 5, the SERS signal decreased monotonously with increasing the target concentration range from 0 to 100 ng ml^{-1} , which is consistent with the change of the silver particle size observed in the TEM images (Fig. 1). Herein, 0, 10 and 100 ng ml^{-1} IgG were chosen to demonstrate the assay strategy. As

Fig. 6 A, I_{blank} vs. nanoparticles size (12, 20 and 59 nm). B, I_{target} vs. nanoparticles size (12, 20 and 59 nm). C, I_{target} vs. human IgG concentration (0, 10 and 100 ng ml⁻¹).

shown in Fig. 6, I_{blank} kept in a nearly constant level with an increase of the nanoparticles size (A), while I_{target} increases obviously with an increase of the nanoparticles size (B), and decreased with an increase of the human IgG concentration (C), which is in good agreement with the assay principle. More antigens resulted in an increase in the quantity of ascorbic acid, and generating smaller silver nanoparticles in the next step. As a result, a lower SERS intensity would be obtained. Therefore, the SERS intensity is inversely proportional to the human IgG concentration. Considering that I_{blank} remained at a nearly constant level with an increase of the nanoparticles size, the linearity between $I_{\text{blank}}/I_{\text{target}}$ and human IgG could be obtained. Actually, a linear correlation between $I_{\text{blank}}/I_{\text{target}}$ (SERS intensity of R6G at 1504.1 cm⁻¹) and the IgG concentration was achieved in the range from 1 to 100 ng ml⁻¹, in which each bar is the relative standard deviation (Fig. 7). The calibration equation was $I_{\text{blank}}/I_{\text{target}} = 1.305 + 0.0421C$ (human IgG concentration), with a correlation coefficient of 0.9878. The detection limit was 0.02 ng ml⁻¹, based on the 3 σ -rule (where σ is the standard deviation of a blank, $n = 4$), indicating a high sensitivity for protein detection. The obtained sensitivity demonstrated the favorable competency of a SERS-based immunoassay for human IgG compared with the reported approaches using fluorescence³⁷ or electrochemical signals.^{25,26}

Fig. 7 Detection for human IgG by measuring the ratio of the SERS intensity at 1504.1 cm⁻¹ (each bar is the relative standard deviation (RSD)).

Conclusions

The present study described a SERS-based enzyme immunoassay technique for the detection of protein using biocatalytic silver nanoparticles as an enhanced substrate, which was characterized by TEM and quantified by Raman assays. Utilizing human IgG as a model protein, the SERS response linearly correlated with the concentration of the target over the range from 1 to 100 ng ml⁻¹ with a detection limit of 0.02 ng ml⁻¹, which exhibited good competency compared with an analogous immunoassay based on the fluorescence and electrochemical signals. On the other hand, this SERS-based immunoassay was developed by employing the production of enhanced substrate as the signalling element, but not the quantity of Raman dye in the traditional methods, which demonstrated the potential of the new way for quantitative detection based on SERS. Furthermore, common Raman dyes could be utilized to indicate the production of silver nanoparticles in the present method, which circumvented the labelling of the Raman label *via* a specific group to the nanoparticles in the traditional SERS-based detection. Therefore, it is expected that the proposed approach might hold a promising potential for SERS-based quantitative analysis.

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References

1. C. V. Raman, *Nature*, **1928**, *121*, 501.
2. C. L. Haynes, A. D. McFarland, and R. P. Van Duyne, *Anal. Chem.*, **2005**, *77*, 338A.
3. S. P. Mulvaney, M. D. Musick, C. D. Keating, and M. J. Natan, *Langmuir*, **2003**, *19*, 4784.
4. D. J. Jeanmaire and R. P. Van Duyne, *J. Electroanal. Chem.*, **1977**, *84*, 1.
5. M. Moskovits, *Rev. Mod. Phys.*, **1985**, *57*, 783.
6. M. I. Stockman, *Phys. Rev. E*, **1997**, *56*, 6494.

7. S. Nie and S. R. Emory, *Science*, **1997**, 275, 1102.
 8. K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari, and M. S. Feld, *Chem. Rev.*, **1999**, 99, 2957.
 9. X. Dou, T. Takama, Y. Yamaguchi, H. Yamamoto, and Y. Ozaki, *Anal. Chem.*, **1997**, 69, 1492.
 10. J. Ni, R. J. Lipert, B. Dawson, and M. D. Porter, *Anal. Chem.*, **1999**, 71, 4903.
 11. D. S. Grubisha, R. J. Lipert, H. Y. Park, J. Driskell, and M. D. Porter, *Anal. Chem.*, **2003**, 75, 5936.
 12. J. D. Driskell, K. M. Kwarta, R. J. Lipert, and M. D. Porter, *Anal. Chem.*, **2005**, 77, 6147.
 13. Y. W. C. Cao, R. C. Jin, and C. A. Mirkin, *Science*, **2002**, 297, 1536.
 14. Y. W. C. Cao, R. C. Jin, J. M. Nam, C. S. Thaxton, and C. A. Mirkin, *J. Am. Chem. Soc.*, **2003**, 125, 14676.
 15. S. R. Emory and S. Nie, *J. Phys. Chem. B*, **1998**, 102, 493.
 16. C. J. Orendorff, A. Gole, T. K. Sau, and C. J. Murphy, *Anal. Chem.*, **2005**, 77, 326.
 17. G. Braun, J. S. Lee, M. Dante, T. Q. Nguyen, M. Moskovits, and N. Reich, *J. Am. Chem. Soc.*, **2007**, 129, 6378.
 18. V. F. Ximenes, A. Campa, W. J. Baader, and L. H. Catalani, *Anal. Chim. Acta*, **1999**, 402, 99.
 19. C. M. Ruan, W. Wang, and B. H. Gu, *Anal. Chem.*, **2006**, 78, 3379.
 20. J. Fenoll, G. Jourquin, and J. M. Kauffmann, *Talanta*, **2002**, 56, 1021.
 21. H. J. Kim and J. Kwak, *J. Electroanal. Chem.*, **2005**, 577, 243.
 22. C. M. Ruan and Y. Li, *Talanta*, **2001**, 54, 1095.
 23. S. Hwang, E. Kim, and J. Kwak, *Anal. Chem.*, **2005**, 77, 579.
 24. L. Zhou, L. J. Ou, X. Chu, G. L. Shen, and R. Q. Yu, *Anal. Chem.*, **2007**, 79, 7492.
 25. Z. P. Chen, Z. F. Peng, Y. Luo, B. Qu, J. H. Jiang, X. B. Zhang, G. L. Shen, and R. Q. Yu, *Biosens. Bioelectron.*, **2007**, 23, 485.
 26. Z. P. Chen, Z. F. Peng, J. H. Jiang, X. B. Zhang, G. L. Shen, and R. Q. Yu, *Sens. Actuators, B*, in press.
 27. G. Frens, *Nature*, **1972**, 241, 20.
 28. P. C. Lee and D. Meisel, *J. Phys. Chem.*, **1982**, 86, 3391.
 29. N. Leopold and B. Lendl, *J. Phys. Chem. B*, **2003**, 107, 5723.
 30. L. H. Lu, A. Kobayashi, K. Tawa, and Y. Ozaki, *Chem. Mater.*, **2006**, 18, 4894.
 31. G. W. Slawiński and F. P. Zamborini, *Langmuir*, **2007**, 23, 10357.
 32. P. Hildebrandt and M. Stockburger, *J. Phys. Chem.*, **1984**, 88, 5935.
 33. J. T. Zhang, X. L. Li, X. M. Sun, and Y. D. Li, *J. Phys. Chem. B*, **2005**, 109, 12544.
 34. Q. Zhou, G. Zhao, Y. Chao, Y. Li, Y. Wu, and J. Zheng, *J. Phys. Chem. C*, **2007**, 111, 1951.
 35. K. F. Domke, D. Zhang, and B. Pettinger, *J. Am. Chem. Soc.*, **2006**, 128, 14721.
 36. K. Faulds, W. E. Smith, and D. Graham, *Anal. Chem.*, **2004**, 76, 412.
 37. R. A. Evangelista, A. Pollak, and E. F. G. Templeton, *Anal. Biochem.*, **1991**, 197, 213.
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