A Novel Graphene Oxide-Based Surface Plasmon Resonance Biosensor for Immunoassay

*Hua Zhang, Ying Sun, Shang Gao, Jia Zhang, Hanqi Zhang, and Daqian Song**

Recently, graphene has received significant interest due to its outstanding properties including prominent electrical conductivity, extraordinary mechanical strength, facile surface modification, and good biocompatibility.[1] As a promising precursor for graphene, graphene oxide (GO) is a monolayer of two-dimensional carbon-based material that contains multifunctional groups, such as carboxyl, epoxy, ketone, and hydroxyl groups, in its basal and edge planes.[2] Due to the large surface area (theoretical limitation: 2630 m^2 g^{-1}), flat surface, and rich π-conjugation structure, GO can serve as a good support for biomolecules. What is more, GO has shown great potential for use in biosensors because of its versatile surface modification, good water dispersibility, and photoluminescence.[3,4] However, until recently there have been limited studies on GO-based surface plasmon resonance (SPR) biosensors. The SPR sensor chip surface is usually coated with sulfhydryl compounds forming a sensing layer to immobilize an antibody.[5] Then the antigen can be detected through antibody–antigen immunoreaction. But the sensitivity of this method is limited. Herein, a GO-based SPR biosensor for detection of human immunoglobulin G (IgG) is reported. When GO is fabricated on the Au film surface of a SPR biosensor, the special property of GO enables a greater refractive index change near the GO/sensing medium interface. Meanwhile, the coating of Au film with GO also modifies the propagation constant of the surface plasmon polariton, which influences the sensitivity to refractive index change.[6]

The surface plasmon wave is sensitive to the change in refractive index or thickness of the medium close to the Au film.[7,8] Metal nanoparticles, such as Au or Ag nanoparticles, have been used to enhance the sensitivity through the inductance coupling interaction between metal nanoparticles deposited on sensors and Au films.[9,10] Another way to enhance the sensitivity results from the fact that Au or Ag nanoparticles couple with antigen and the conjugates bind to the antibody immobilized on the SPR biosensor surface.[11] Au nanorods (AuNRs) are elongated nanoparticles that exhibit two absorption bands: one is the transverse band around 520 nm, similar to the absorption band of spherical gold nanoparticles; the other is the longitudinal band, which is tunable from the visible through near-infrared region with nanorod aspect ratio.[12] It has been proved that AuNRs show higher sensitivity to the local dielectric environment than similar-sized spherical gold nanoparticles owing to their stronger SPR characteristics.[13] Considering these facts, AuNRs have been widely applied for biosensing.[14] When human IgG is coupled with AuNRs, the sizes increase resulting in a refractive index change. As mentioned above, it is possible to design a highly sensitive and selective SPR biosensor. As shown in **Figure 1**, GO has many negatively charged groups, such as carboxylic acids, hydroxyl groups, and epoxides, so GO sheets can be assembled on a positively charged SPR Au film (p-Au film) through electronic interactions, which are strong enough to ensure that the GO is retained during a washing step. Goat anti-human IgG is immobilized on the GO through covalent attachment between the amine group and the carboxyl group. Thus, human IgG can be detected based on specific antibody–antigen interaction.

The GO used in this study was synthesized from natural graphite powder by using a modified Hummers and Offeman's method.[15] X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) were applied to the characterization of GO. As shown in **Figure 2**, the C 1s XPS spectra can be deconvoluted into four peaks at 284.6, 285.9, 286.9, and 288.7 eV, which are associated with C–C, C–OH, C(epoxy/alkoxy), and C=O, respectively.[16] AFM data show that the prepared GO sheets are mono- or bilayers and the surface is flat. The subsequent assembly of GO onto Au film was investigated by monitoring the wavelength shift with the SPR biosensor (Supporting Information, Figure S2). When 3-mercaptopropionic acid (MPA), poly(allylamine hydrochloride) (PAH), and GO were assembled on Au film step by step, the wavelength shifted about 2.66, 4.87, and 6.87 nm, respectively. This suggests that GO was successfully immobilized on the Au film surface.

After the carboxyl group of GO was activated, goat anti-human IgG was injected into the flow cell to covalently attach on GO. To select the optimum concentration of antibody, different concentrations of goat anti-human IgG were tested experimentally. The effects of the concentration of goat anti-human IgG on the shift of the resonant wavelength λ are shown in **Figure 3**A. It was found that the resonant wavelength exhibits an evident shift at first and then increases slowly as time goes on. Eventually, the resonant wavelength is almost unchanged when the time is longer than 120 min,

Dr. H. Zhang, Dr. Y. Sun, Dr. S. Gao, Dr. J. Zhang, Dr. H. Zhang, Dr. D. Song
College of Chemistry
Jilin University
Qianjin Street 2699, Changchun 130012, PR China
E-mail: songdq@jlu.edu.cn

DOI: 10.1002/smll.201202958

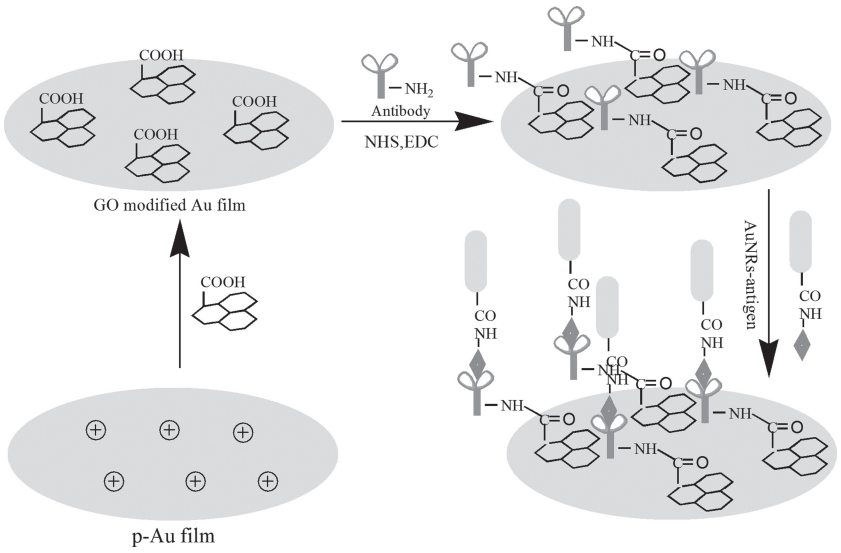

Figure 1. Schematic diagram of the experimental procedure. NHS = *N*-hydroxysuccinimide, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

which indicates that the immobilization of goat anti-human IgG on the sensor surface was accomplished. With an increase of the concentration, more antibodies were immobilized on the sensor surface. It resulted in a slight change of the resonant wavelength when the antibodies immobilized on the sensor surface reached saturation. As shown in Figure 3A, when the concentrations of goat anti-human IgG are 50, 75, and 100 μg mL^{-1}, the shifts of the resonant wavelength are 5.31, 8.62, and 9.72 nm, respectively. As a consequence, 75 μg mL^{-1} was chosen as the optimum concentration of goat anti-human IgG for the detection of human IgG.

As shown in Figure 3B, when the concentration of goat anti-human IgG is 75 μg mL^{-1}, the antibody immobilized on the sensor surface formed a sensing layer very well. The covalent attachment between the amine group and the carboxyl group is a good stratagem for the immobilization of antibody on the Au film. For the biosensor based on GO, the shift of the resonant wavelength increases quickly at first and then slowly and reaches about 86% of its total shift within 1 h. The maximum resonant wavelength shift for the immobilization of goat anti-human IgG is 8.62 nm for the biosensor based on GO within 150 min. However, when 75 μg mL^{-1} goat anti-human IgG was also immobilized on the surface of the biosensor based on MPA to detect human IgG, the kinetic adsorption curves of the antibody immobilization shown in Figure 3B indicate that the maximum resonant wavelength shift is 5.52 nm within 150 min. The immobilization of antibody is related to both the number of binding sites and proper space between the binding sites. When MPA was assembled on the smooth surface of Au film to immobilize antibody, it was arranged very closely and the steric hindrance was increased. The surface of GO is relatively rough because of the abundant functional groups. When GO was assembled step by step, the space of the sensor surface was expanded. Owing to the large surface area and the space effect, it is easier for the antibody to be immobilized. It is clear that the maximum resonant wavelength shift based on GO is much bigger than that based on MPA, which indicates the amount of goat anti-human IgG immobilized on the sensor based on GO is much more significant than that immobilized on the sensor based on MPA.

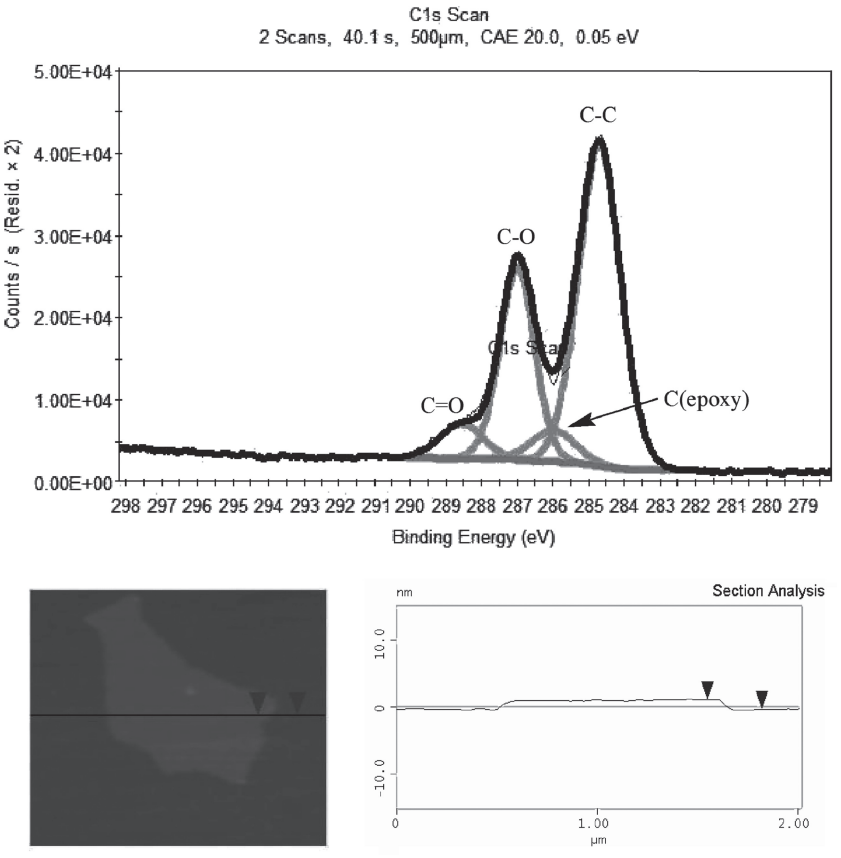

Figure 2. XPS spectra (top) and AFM image (bottom) of GO.

At room temperature, after the goat anti-human IgG was immobilized on the sensor surface, human IgG was injected into the flow cell. The shifts of resonant wavelength caused by antibody–antigen interaction were monitored in real time. For the direct detection of antigen, human IgG was diluted with sodium phosphate-buffered saline (PBS) to obtain solutions of

NANO MICRO small

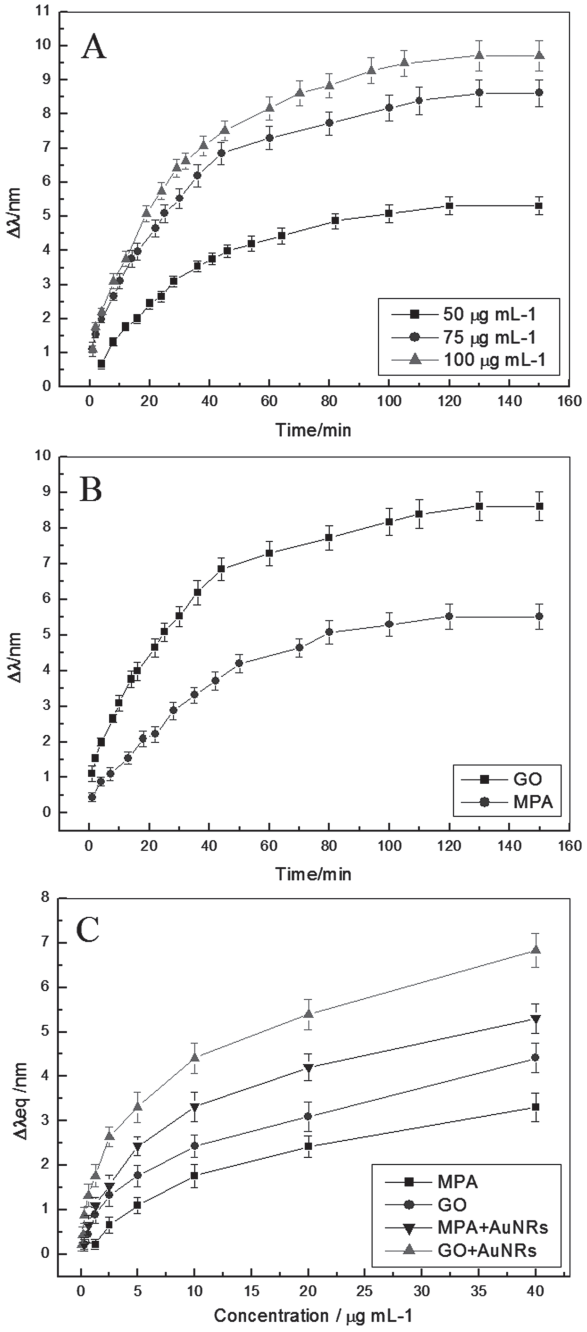

Figure 3. A) Kinetic adsorption curves of immobilized goat anti-human IgG on a GO-based SPR biosensor. B) Kinetic adsorption curves of immobilized goat anti-human IgG at the concentration of 75 μg mL^{-1} on SPR biosensors based on MPA and GO. C)Relationship between the concentrations of human IgG and the shifts of resonant wavelength obtained with the biosensor based on MPA and GO; relationship between the concentrations of AuNR–human IgG conjugates and the shifts of resonant wavelength obtained with the biosensor based on MPA and GO.

different concentration. As shown in Figure 3C, the biosensor based on MPA shows a satisfactory response to human IgG in the concentration range of 1.25–40.00 μg mL^{-1}. The minimum shift of resonant wavelength is 0.22 nm at 1.25 μg mL^{-1} and the maximum shift of resonant wavelength is 3.31 nm at 40.00 μg mL^{-1}. The biosensor based on GO shows a satisfactory response to human IgG in the concentration range of 0.30–40.00 μg mL^{-1}. The minimum shift of resonant wavelength is 0.22 nm at 0.30 μg mL^{-1} and the maximum shift of resonant wavelength is 4.41 nm at 40.00 μg mL^{-1}. By contrast, the application of GO improves the performance of the wavelength modulation SPR biosensor.

For the sake of further sensitivity enhancement, when antibody was immobilized on the sensor surface the antigen coupled with AuNRs was detected. Covalent attachment was demonstrated to be more stable to construct the AuNR–antigen conjugates. The effect of ratio of AuNRs to human IgG was investigated to select the optimum experimental conditions. A 0.5 mL volume of AuNRs solution was selected as the optimum volume to bind with 5 μg of human IgG (Supporting Information, Figure S3). AuNR–antigen conjugates were diluted to different concentrations with PBS and injected separately into the flow cell. As shown in Figure 3C, when the human IgG was modified with AuNRs, the biosensor based on MPA showed a satisfactory response to human IgG in the concentration range of 0.30–40.00 μg mL^{-1}. The minimum shift of resonant wavelength is 0.22 nm at 0.30 μg mL^{-1} and the maximum shift of resonant wavelength is 5.31 nm at 40.00 μg mL^{-1}. The biosensor based on GO shows a satisfactory response to human IgG in the concentration range of 0.075–40.00 μg mL^{-1}. The minimum shift of resonant wavelength is 0.22 nm at 0.075 μg mL^{-1} and the maximum shift of resonant wavelength is 6.84 nm at 40.00 μg mL^{-1}. The limit of quantification (LOQ) is defined as the lowest concentration of an analyte that can be determined quantitatively by the present approach. Under identical conditions, the LOQ obtained with the biosensor based on GO was about 4 times lower than that obtained with the biosensor based on MPA. When the human IgG was modified with AuNRs, the LOQ obtained with the biosensor based on GO was about 16 times lower than that obtained with unmodified human IgG for the biosensor based on MPA.

These experimental results indicate that when GO and AuNRs were used, the sensitivity of the SPR biosensor was remarkably enhanced. Since GO has a large surface area and contains carboxyl groups, it is favorable for the immobilization of antibody. The amount of the antibody immobilized on the sensor surface does indeed have an effect on the sensitivity of the SPR biosensor. The more antibodies that are immobilized on the sensor surface, the more antigens are detected. Then the resonant peak shifts to a longer wavelength. Based on theoretical analysis, the shift of the resonant wavelength towards a long wavelength could increase the sensitivity of the wavelength modulation SPR biosensor.[17] Besides, GO will also modify the propagation constant of the surface plasmon polariton, which results in the increase of the refractive index and the enhancement of sensitivity. AuNRs exhibit two absorption bands: the transverse band and the longitudinal band. The longitudinal band of AuNRs has been found to be more sensitive to changes in the local environment. The incident light field coupling to the local surface plasmon

might resonantly excite the surface plasmon of AuNRs through energy transfer and induce a strong local electromagnetic field, which further increases the shift of the resonant wavelength. On the other hand, when AuNRs couple with antigen, the sizes of the antigen increase, which results in a change of refractive index that is observed as a shift of resonant wavelength. Based on the above factors, the application of GO and AuNRs can enhance the sensitivity of the wavelength modulation SPR biosensor significantly. The proposed biosensor extends the application of GO and AuNRs in immunoassay.

Evaluation of the specificity was performed by detecting bovine IgG and bovine serum albumin (BSA). After the goat anti-human IgG was immobilized on the surface of the biosensor, bovine IgG and BSA at the concentration of 10 μg mL^{-1} were injected separately into the flow cell for 40 min. There were no observable shifts of the resonant wavelength, which indicated selective binding with human IgG (Supporting Information, Figure S4).

In conclusion, we have developed a novel GO-based wavelength modulation SPR biosensor for highly sensitive and selective detection of human IgG. The GO-based SPR biosensor has been demonstrated to be a promising platform for the sensitive detection of biomolecules. Considering the low cost of preparing GO and facile fabrication of GO onto the sensor surface, it indicates the great potential of GO for applications in biotechnology and the biomedical fields.

Experimental Section

Synthesis of GO: GO was synthesized through a modified Hummers and Offeman's method. When the reaction finished, the resulting solution was centrifuged several times to completely wash the graphite oxide flakes and then dispersed into individual sheets in distilled water under sonication.

Synthesis of AuNR–Antigen Conjugates: AuNRs were first synthesized following a seed-mediated method. The AuNR–antigen conjugates were prepared by adding human IgG to the activated AuNRs solution and different ratios of AuNRs to human IgG were prepared to select the optimum experimental conditions.

Instrumentation: The wavelength modulation SPR biosensor installed in our laboratory was used (Supporting Information, Figure S1). All SPR measurements were carried out at room temperature. XPS (KRATOS, AXIS ULTRA) measurements were carried out by using a monochromatized Al Kα (1486.7 eV) X-ray source. AFM was performed on a Nanoscope III scanning probe microscope (from Digital Instruments) in tapping mode under ambient conditions.

Immunoassay: After the Au film modified with GO was fixed on the SPR instrument, a solution containing *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was injected into the flow cell to activate the carboxyl groups of GO. Then the goat anti-human IgG solution was injected to covalently attach on GO. Finally, different concentrations of AuNR–IgG diluted with PBS buffer were detected.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 20727003, 21075049, 21105037), Program for New Century Excellent Talents in University (No. NECT-10-0443), Special-funded Programme on National Key Scientific Instruments and Equipment Development (Nos. 2012YQ090194) and Science and Technology Developing Foundation of Jilin Province (Nos. 20100356, 20110162).

[1] a) H. Chen, M. B. Muller, K. J. Gilmore, G. G. Wallace, D. Li, *Adv. Mater.* **2008**, *20*, 3557; b) M. Zhou, Y. Zhai, S. Dong, *Anal. Chem.* **2009**, *81*, 5603; c) J. F. Wu, M. Q. Xu, G. C. Zhao, *Electrochem. Commun.* **2010**, *12*, 175; d) K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva, A. A. Firsov, *Science* **2004**, *306*, 666; e) A. K. Geim, K. S. Novoselov, *Nat. Mater.* **2007**, *6*, 183; f) Z. W. Tang, H. Wu, J. R. Cort, G. W. Buchko, Y. Y. Zhang, Y. Y. Shao, I. A. Aksay, J. Liu, Y. H. Lin, *Small* **2010**, *6*, 1205.
[2] a) D. R. Dreyer, S. Park, C. W. Bielawski, R. S. Ruoff, *Chem. Soc. Rev.* **2010**, *39*, 228; b) S. Stankovich, D. A. Dikin, R. D. Piner, K. A. Kohlhaas, A. Kleinhammes, Y. Jia, Y. Wu, S. T. Nguyen, R. S. Ruoff, *Carbon* **2007**, *45*, 1558.
[3] A. K. Geim, *Science* **2009**, *324*, 1530.
[4] S. Stankovich, D. A. Dikin, G. H. B. Dommett, K. M. Kohlhaas, E. J. Stach, E. A. Zimney, R. D. Piner, S. T. Nguyen, R. S. Ruoff, *Nature* **2006**, *442*, 282.
[5] L. Y. Wang, Y. Sun, J. Wang, X. N. Zhu, F. Jia, Y. B. Cao, X. H. Wang, H. Q. Zhang, D. Q. Song, *Talanta* **2009**, *78*, 265.
[6] L. Wu, H. S. Chu, W. S. Koh, E. P. Li, *Opt. Express* **2010**, *18*, 14395.
[7] Y. Li, H. J. Lee, R. M. Corn, *Anal. Chem.* **2007**, *79*, 1082.
[8] X. N. Shan, U. Patel, S. P. Wang, R. Iglesias, N. J. Tao, *Science* **2010**, *327*, 1363.
[9] L. A. Lyon, M. D. Musick, M. J. Natan, *Anal. Chem.* **1998**, *70*, 5177.
[10] Y. L. Chen, B. Y. Dong, W. Y. Zhou, *Appl. Surf. Sci.* **2010**, *257*, 1021.
[11] J. Mitchell, *Sensors* **2010**, *10*, 7323.
[12] C. J. Orendorff, C. J. Murphy, *J. Phys. Chem. B* **2006**, *110*, 3990.
[13] H. W. Liao, J. H. Hafner, *Chem. Mater.* **2005**, *17*, 4636.
[14] a) H. J. Parab, C. Jung, J. H. Lee, H. G. Park, *Biosens. Bioelectron.* **2010**, *26*, 667; b) N. S. Eum, S. H. Yeom, D. H. Kwon, H. R. Kim, S. W. Kang, *Sens. Actuators B* **2010**, *143*, 784; c) H. Y. Liu, D. Chen, L. Q. Yang, X. L. Ren, F. Q. Tang, J. Ren, *Nanotechnology* **2010**, *21*, 185504.
[15] W. S. Hummers, R. E. Offeman, *J. Am. Chem. Soc.* **1958**, *80*, 1339.
[16] S. Park, J. An, R. D. Piner, I. Jung, D. Yang, A. Velamakanni, S. T. Nguyen, R. S. Ruoff, *Chem. Mater.* **2008**, *20*, 6592.
[17] J. Homola, S. S. Yee, G. Gauglitz, *Sens. Actuators B* **1999**, *54*, 3.

Received: November 29, 2012
Revised: January 21, 2013
Published online: February 22, 2013

