Analyst

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Analyst*, 2013, **138**, 7175

A protein A modified Au–graphene oxide composite as an enhanced sensing platform for SPR-based immunoassay†

Jia Zhang,[a] Ying Sun,[a] Qiong Wu,[a] Hua Zhang,[a] Yu Bai[b] and Daqian Song*[a]

Received 15th August 2013
Accepted 18th September 2013

DOI: 10.1039/c3an01553j

www.rsc.org/analyst

A sensitive and selective wavelength modulation surface plasmon resonance (SPR) biosensor is reported with Au nanoparticle decorated graphene oxide (GO) as an enhanced sensing platform. GO sheets possess favourable water dispersibility, good biocompatibility and high loading capacity. An Au–GO composite with the Au spheres size of 15–20 nm was synthesized and modified with staphylococcal protein A (SPA). The as-prepared composite assembles directly onto the Au film surface of the SPR sensor. Meanwhile, SPA specifically recognizes and binds the Fc portion of antibodies, contributing to highly oriented antibody immobilization on the chip surface without any antibody modification. Consequently, the biosensor based on the SPA modified Au–GO composite exhibits a satisfactory response to rabbit IgG in the concentration range of 0.1–50 μg mL^{-1}, while the biosensor based on the sole SPA layer for antibody immobilization shows a response in the concentration range of 1.6–50 μg mL^{-1}. Experimental results show that the SPA modified Au–GO composite can be successfully used for the signal amplification of immunosensors, thereby improving the sensitivity and obviating the need of chemical modification of the antibody.

Introduction

Over the past decades, surface plasmon resonance (SPR) sensors which measure changes in the refractive index occurring in the field of an electromagnetic wave have made vast advances in terms of both technology and application.[1] The number of reported applications for analytes related to medical diagnostics, environmental monitoring, and food safety has been rapidly growing due to their label-free, high-sensitivity, real-time analysis and flexible system design.[2] One of the main drawbacks that impede further development of SPR applications is the lack of sufficient sensitivity to reliably detect small changes in refractive index associated with the analyte on the sensing surface.[3] The effective immobilization of either the antibody or the antigen on the sensing surface is a crucial step in order to achieve a highly reliable immunosensor. This immobilization step influences the sensitivity, specificity and detection limit of the sensor.[4] Novel SPR biosensor structures are being investigated by many groups to design sensors with higher sensitivity, better performance, and lower limit of detection.[5–10] Despite the above demonstrations, it is still highly desirable to develop more ideal platforms to enhance the performance of SPR biosensors without complicated fabrication procedures.

Graphene, a free-standing two-dimensional crystal with a single layer structure, has become one of the hottest topics in the fields of physics, chemistry, nanotechnology and materials science.[10,11] A label-free, regenerative and sensitive surface plasmon resonance and electrochemical aptasensor based on graphene was first proposed for the detection of α-thrombin by Wang *et al.*[12] Graphene oxide (GO) has also received a great deal of attention because it is conveniently prepared on a large scale and it has unique properties. GO has many attractive features, including good water dispersibility, a large surface area, a high surface electron density, and straightforward surface functionalization.[13,14] In addition, its effectiveness in quenching fluorescence affords advantages over alternative carbon materials such as nanodiamonds and carbon nanotubes that have been investigated for biosensing.[15]

Recently, GO has been used extensively as a substrate to fabricate nanohybrid materials. It is expected that the nanoparticles anchored on the sheet potentially exhibit novel catalytic, magnetic, and optoelectronic properties. A variety of nanoparticles including Pb, Pt, Au, TiO_2, and Fe_3O_4 have been hosted on the surface of GO.[16–18] For example, the nanostructure of GO–Ag nanoparticles fabricated by the self-assembly method was used as SERS substrates, in which Ag nanoparticles supply strong SERS activity and GO concentrates the target molecules.[19] Au nanoparticle decorated GO sheets were synthesized

[a]*College of Chemistry, Jilin University, Qianjin Street 2699, Changchun 130012, China. E-mail: songdq@jlu.edu.cn; Fax: +86-431-85168399; Tel: +86-431-85168399*

[b]*Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, China*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3an01553j

and used for hydrazine detection and catalytic reduction of 4-nitrophenol.[20] The functionalization of GO with magnetic chitosan (Chm) was investigated to prepare a nanocomposite material (GO–Chm) for the adsorption of a reactive dye.[21] However, few papers thus far have reported on the utilization of a composite related to GO sheets and their enhanced performance in SPR biosensors for immunoassays. It is anticipated that a hybrid composed of graphene oxide and AuNPs could become a promising functional material in a traditional SPR system as the enhanced sensing platform.

In this paper, the Au–GO composite was synthesized and applied in the SPR biosensor as a solid support for the direct immobilization of goat anti-rabbit IgG. The composite was modified with staphylococcal protein A (SPA) for self-assembly on the SPR chip and subsequent binding specifically to the Fc region of the immunoglobulin molecules without interacting at the antigen binding sites. The binding of antibody–antigen leads to changes in the refractive index at the sensor surface, which results in the shifts of the resonant wavelength that can be easily measured by the SPR biosensor. The experiment results demonstrated that the sensitivity of the biosensor based on the Au–GO composite was enhanced significantly.

Experimental section

Reagents

Goat anti-rabbit IgG was purchased from Sigma-Aldrich. Rabbit IgG, staphylococcal protein A (SPA), human IgG, bovine IgG were purchased from Beijing Biosynthesis Biotechnology Company. Bovine serum albumin (BSA) was purchased from Ding Guo Biotechnology Company (Beijing, China). Graphite was purchased from Sigma-Aldrich. Hydrogen tetrachloroauratehydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Acrosl. Sodium citrate, potassium permanganate, sulfuric acid, phosphoric acid, hydrochloric acid, ethanol, and all other chemicals were of analytical reagent grade. All the solutions were prepared with ultra pure water, and all the glassware was cleaned with aqua regia before the experiments.

Goat anti-rabbit IgG, rabbit IgG, human IgG and bovine IgG were stored at −20 °C and all other biological reagents were stored at 4 °C. Sodium phosphate buffered saline (PBS, 0.01 mol L^{-1}, pH 7.4) was used as running buffer.

Instrumentation

The wavelength modulation SPR analyzer installed in our laboratory was used.[22] It is working with Kretschmann configuration to achieve the resonant condition by attenuated total reflection (ATR) in a prism. A 2 nm adhesive layer of Cr followed by a 50 nm layer of Au was deposited on a K9 glass slide, then the glass slide with the Au film was put on the base of a prism (K9 glass) using a suitable index matching oil. A halogen tungsten lamp is used as the excitation light source. The light passes through a polarizer and two lenses and becomes TM-polarized parallel light. The parallel polychromatic light beam passes through the optical prism and excites surface plasmon at the interface between the Au film and the sample solution. The output light from another side of the prism passes through the third optical focusing lens. One end of the optical fiber is put at the focal point of the focusing lens and then the light beam has access to optical fiber as much as possible. The opposite end is connected to the spectrophotometer. Thus the output light is guided into the optical fiber and then enters the spectrophotometer (HR2000+, Ocean Optics Inc., USA). The HR2000+ spectrometer integrates a high-resolution optical bench, a powerful 2 MHz analog-to-digital (A/D) converter, programmable electronics, a 2048-element CCD-array detector and a high-speed USB 2.0 port. This combination produces an extremely fast spectrometer and provides resolution to 0.035 nm (full width at half maximum, FWHM).

Synthesis of GO and Au–GO composite

GO was prepared using a modification of Hummers and Offeman's method from graphite powders.[23] In a typical reaction, 0.5 g of graphite, 0.5 g of $NaNO_3$, and 23 mL of H_2SO_4 were stirred together in an ice bath. Next, 3 g of $KMnO_4$ was slowly added. Once mixed, the solution is transferred to a 35 °C water bath and stirred for about 1 h. Next, 40 mL of water was added, and the solution was stirred for 30 min while the temperature was raised to 90 °C. Finally, 100 mL of water was added, followed by the slow addition of 3 mL of H_2O_2 (30%). The warm solution was then filtered and washed with 100 mL of water. The filter cake was then dispersed in water by mechanical agitation. Low-speed centrifugation was done for 3 min. During the centrifugation, the relative centrifugal force (RCF) is 112*g*. It was repeated until all visible particles were removed (about 3–5 times). The supernatant then underwent three more high-speed centrifugation steps for 30 min to remove small GO pieces and water-soluble byproduct (RCF: 7168*g*).

The synthesis of the Au–GO composite was based on the reduction of the gold(III) complex by sodium citrate.[24] Typically, 2.5 mL of a GO aqueous suspension (1.5 mg mL^{-1}) was added to 50 mL of $HAuCl_4$ solution (0.24 mmol L^{-1}). The resultant suspension was aged for 30 min to promote the interaction of gold ions with the GO surface. After that, the solution was heated until 80 °C, after which 940 μL of sodium citrate (0.085 mol L^{-1}) was added dropwise. The reaction was kept under these conditions for 1 h. The resultant composite was washed with distilled water using centrifugation twice to remove the free gold nanoparticles that formed in solution (RCF: 2016*g*). The final precipitate was diluted with 5 mL of ultra pure water. In the application process, 100 μL of 1 mg mL^{-1} SPA was added to 50 μL of the above solution followed by gentle shaking for several seconds and kept for 3 h at 4 °C for complete incorporation. The SPA modified Au–GO composite was diluted with PBS until the final concentration of SPA was 100 μg mL^{-1}.

The modification process to fabricate the SPR sensing surface

The glass slide with the Au film was fixed under the flow cell. After PBS was injected into the flow cell, the SPA modified Au–GO composite solution was injected into the flow cell and incubated for 3 h to form a Au–GO composite modified Au film. Subsequently, PBS was injected as the baseline solution. After

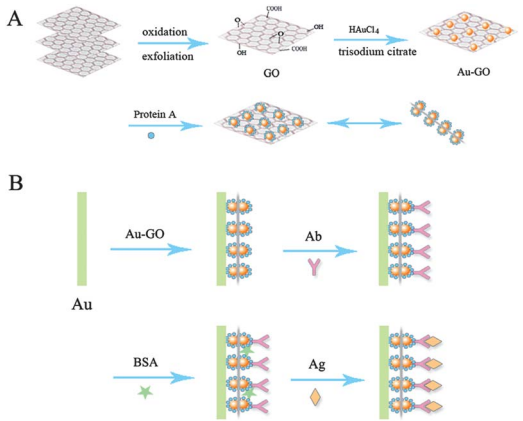

Fig. 1 A schematic diagram of the experimental procedure.

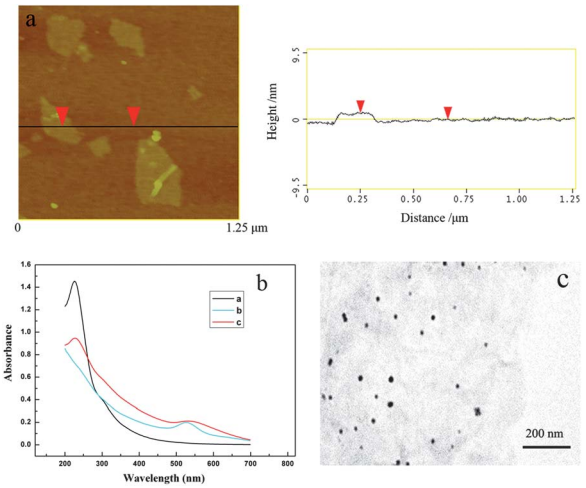

Fig. 2 Atomic force microscopy (AFM) image of GO (a), UV-vis absorption spectra of GO, supernatant and Au–GO composite (b) and TEM image of the Au–GO composite (c).

the resonant wavelength was kept constant, goat anti-rabbit IgG was injected into the flow cell to attach to the SPA surfaces for 12 h and thus the anti-rabbit IgG membrane was stable. The biosensor surface was blocked with BSA for at least 40 min.

For comparison, the SPA covered Au film without the Au–GO composite was also constructed. PBS was used as the baseline solution and 100 μg mL^{-1} SPA was injected into the flow cell. After 3 h, 100 μg mL^{-1} goat anti-rabbit IgG solution was injected. The following processes were the same as those mentioned above.

Immunoassay

Different concentrations of rabbit IgG diluted with PBS were separately injected into the flow cell over the immobilized antibody surface. After the resonant wavelength was kept constant, PBS was injected into the flow cell for 30 min. Due to a large amount of ions in the PBS, the antigen can be dissociated from the antibody immobilized on the surface of the biosensor and removed from the flow cell by impulse injection of the PBS.[25] When the antigen was dissociated from the antibody on the biosensor surface, another sample was injected into the flow cell. All experiments were conducted in triplicate to examine the reliability of the assays. A schematic of the fabricated biosensor is shown in Fig. 1.

To evaluate the selective binding of rabbit IgG, bovine IgG and human IgG were determined by the same procedure as that used to detect rabbit IgG.

Results and discussion

Synthesis and characterization of GO and Au–GO composite

Water-soluble GO was synthesized by oxidizing graphite according to the modified Hummer's method, followed by strong sonication to disperse GO and by centrifugation to remove nondispersed large GO layers. Atomic force microscope (AFM) was used to confirm the formation of the single layer GO sheet. As shown in Fig. 2a, the thickness of the GO sheet is about 0.943 nm, which is typical for a single layered sheet of GO and similar to that reported previously. Au–GO composites were synthesized from the reduction of a diluted solution of Au(III) complex ions with sodium citrate in the presence of GO. The composite was centrifuged twice after the synthesis to remove any Au nanoparticles that were not linked to the GO surface (RCF: 2016*g*). Fig. 2b shows the typical UV-vis absorption spectrum of the GO solution (curve a), the supernatant (curve b) and the final Au–GO composite (curve c). There is an absorption peak at 230 nm for the GO sheets which is in the 227–231 nm range as previously reported for GO.[26] A weak shoulder around 300 nm is observed and can be attributed to n–π* transitions of the carbonyl groups. Au nanospheres typically show a band around 520 nm in the visible spectrum due to the local surface plasmon resonance. As can be observed, the absorbance measurement on the supernatant (curve b) shows the presence of characteristic absorption bands of Au particles and no relevant peaks of GO. The measurement indicates that free Au nanoparticles that are not linked to the GO surface have been removed. In the case of the final GO/Au aqueous suspension (curve c), there is an absorption peak at 531 nm for the aqueous suspensions of the Au–GO composite, which indicated that Au nanoparticles have successfully adhered to the GO surface. The slightly red color of the composite observed is in agreement with dispersed Au nanoparticles. Transmission electron microscope (TEM) analysis of the composite confirmed the above optical results. A distribution of discrete Au nanoparticles with diameters of 15–20 nm was observed at the GO surface with negligible nanoparticle agglomeration (Fig. 2c). The UV-vis absorption spectrum and the TEM image confirmed that the Au–GO composite was synthesized successfully.

Preparation of a sensing substrate based on the Au–GO composite

The effective immobilization of antibody on the sensing surface is a crucial step in order to achieve a highly reliable immunosensor. Covalent amine coupling of antibodies on chemically activated surfaces is the common method for antibody immobilization. However, this approach leads to randomly oriented

antibodies, rendering a low density of antigen-binding sites, and reducing the immunoassay sensitivity.[4] Staphylococcal protein A (SPA) is a polypeptide of *Staphylococcus* and has four binding sites with antibody, which has been frequently used for the immobilization of antibodies. SPA is specific for binding the Fc region of the antibodies, which keeps the fragment antigen-binding (Fab) region free for antigen binding thus obviating the need of any chemical modification of the antibody.[27] As a result, a high degree of orientation for the capture antibody on a sensor surface can be attained with a sub-layer of protein A. In addition, Au–protein A complexes are highly stable, have an association constant of 10^8 M^{-1}, and are believed to be van der Waals in nature.[28,29] After the composite was centrifuged twice to remove Au nanoparticles that are not linked to the GO surface, the final precipitate was diluted with ultra-pure water. In the previous report, the precipitate dissolved well in ultra-pure water and the as-prepared GO–Au composite served as an excellent substrate for surface enhanced Raman scattering.[24] Meanwhile, SPA solution was added to the composite solution followed by gentle shaking for several seconds and kept for complete incorporation. The SPA layer on the surface not only acts as a bridge for access to the antibody but also as a stabilizer of the Au–GO composite due to the interaction between SPA and Au nanoparticles. This is beneficial to the composite stability. In the final determination process of antigen, all experiments were conducted in triplicate. It can be seen from Fig. 5 that the detection signal was significantly enhanced and the error margin of the resonant wavelength shifts is acceptable. The experiment result indicates the reliability of the assays. Therefore, the centrifugation has little influence on the ultimate sensing performance. After SPA was used to modify the Au–GO composite, the as-prepared solution at a SPA concentration of 100 μg mL^{-1} was injected into the flow cell. The composite spontaneously assembles on the bare Au film due to the interaction between the SPA and the Au film. Fig. S2† shows the kinetic adsorption curve of the SPA–Au/GO composite at the Au film surface. About 50 min later, the combination of the composite and Au film was completed and the maximum shift of the resonance wavelength is 14.90 nm.

In the direct immunoassay, the Au film surface was modified with SPA as the platform. The solution containing 100 μg mL^{-1} SPA was flowed over the biosensor surface, and the self-assembly of SPA on the Au film was monitored by the SPR sensor in real time. Exposure of the sensor surface to the SPA solution leads to a 4.20 nm shift in the resonance wavelength within 15 min, and the resonance wavelength remains almost constant as time prolongs. This suggests that the self-assembly monolayer has been completed. It can be concluded from the above experimental result that the Au–GO composite modified with SPA has been successfully immobilized on the bare Au surface and leads to a large resonant wavelength shift compared to the sole SPA layer.

Immobilization of goat anti-rabbit IgG

After the Au film surface was modified with the SPA–Au/GO composite, the goat anti-rabbit IgG diluted with PBS buffer at a concentration of 100 μg mL^{-1} was injected into the flow cell to perform its binding on the SPA surface. Since the SPA selectively binds the Fc region of the antibody, leaving the Fab region available for antigen detection, the goat anti-rabbit IgG can be effectively bound on the surface of the composite. Fig. 3 represents the kinetic adsorption curves for immobilization of goat anti-rabbit IgG. When antibodies are immobilized on the composite surface, the resonant wavelength moves to the longer wavelength. It is noticeable that the resonant wavelength shows an obvious shift at first. With increasing time, the resonant wavelength increased slowly and seemed to be stable at 60 min, which indicates that the immobilization of antibody on the composite surface was completed. When the antibody immobilized on the composite surface reaches saturation, the resonant wavelength remains constant. It can be seen from Fig. 3 that the maximum resonant wavelength shift for the immobilization of goat anti-rabbit IgG at a concentration of 100 μg mL^{-1} is 14.40 nm.

The platform based on a SPA modified Au film was also investigated. Fig. 3 also shows the kinetic adsorption curves of 100 μg mL^{-1} goat anti-rabbit IgG obtained with the biosensor based on the SPA modified Au film (curve a). The maximum resonant wavelength shift for the immobilization of antibodies is 7.60 nm within 80 min, while it is 14.40 nm for the proposed substrate. The SPA–Au/GO composite immobilized on the Au film surface leads to a large resonant wavelength shift and functions as a sensitivity enhancer in immunoassay. When SPA–Au/GO was used as a platform, the signal for antibody immobilization was significantly amplified at the same concentration of goat anti-rabbit IgG. The thickness of the sensing membrane increases and the resonant wavelength moves to longer wavelength, which also lead to the signal amplification of the wavelength-modulation SPR biosensor.[30,31] The experiment results demonstrate that an active interface of the fabricated substrate plays a valuable role in the simple self-immobilization of antibodies and the SPA–Au/GO substrate can be utilized for the development of effective biosensor platforms.

After 12 h, PBS buffer was injected into the flow cell to wash off unbound antibody and to stabilize the baseline. Additional

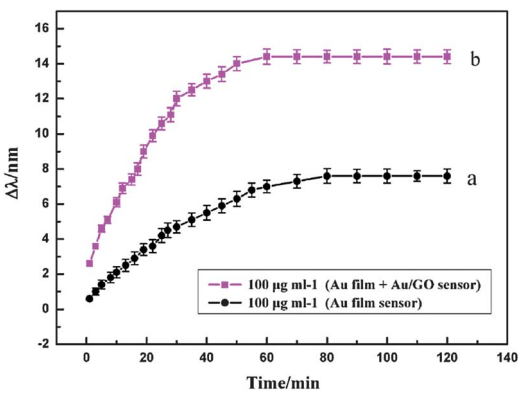

Fig. 3 The kinetic adsorption curve of goat anti-rabbit IgG at a concentration of 100 μg mL^{-1} on a SPA modified Au film (curve a) and SPA–Au/GO modified Au film (curve b).

data points after the injection of PBS are given in Fig. S3.† As expected, the resonance peak shift has a decrease in the spectrum. The injection of PBS caused a 0.4 nm decrease in the resonant wavelength shift on the SPA–Au/GO platform (curve a), and a 0.5 nm decrease in resonant wavelength shift on the SPA platform (curve b). The resonant wavelength shift reaches a firm value in 5 min and remains constant for 25 min. After that, BSA was used to block the non-specific binding sites on the biosensor surface.

Determination of antigen

In this report, the SPR biosensor based on a SPA modified Au film as a platform and a biosensor based on the SPA–Au/GO composite as the enhanced platform were constructed. Prior to the analysis, 5 mg mL^{-1} BSA was used to block the residual binding sites on the biosensor surface. To demonstrate the potential sensitivity of the proposed SPR biosensor based on the SPA–Au/GO composite, rabbit IgG at different concentrations was separately injected into the flow cell at room temperature. The shifts of the resonant wavelength due to antibody–antigen immunoreaction were measured in real time. Fig. 4 shows the SPR spectrum for detection of 25 μg mL^{-1} rabbit IgG in different methods. At the same concentration of goat anti-rabbit IgG, incubation of the SPA–Au/GO substrate with 25 μg mL^{-1} solution of rabbit IgG yields a 7.40 nm shift in resonant wavelength (Fig. 4B). While the exposure of the SPA modified substrate to 25 μg mL^{-1} solution of rabbit IgG results in a 2.2 nm shift in resonant wavelength (Fig. 4A). When the SPA–Au/GO composite platform was used, the signal was significantly enhanced.

Fig. 5 illustrates the variation of the resonant wavelength shifts as a function of different concentrations of rabbit IgG in two types of biosensors. The SPR biosensor based on the SPA–Au/GO composite shows a good response to rabbit IgG in the concentration range of 0.1–50.00 μg mL^{-1}. The minimum shift of the resonant wavelength is 0.20 nm at 0.1 μg mL^{-1} and the maximum shift is 10.60 nm at 50.00 μg mL^{-1}. The SPR biosensor based on the SPA layer shows a response to rabbit IgG in the concentration range of 1.60–50.00 μg mL^{-1}. The shift of the resonant wavelength is 0.20 nm at 1.60 μg mL^{-1} and 3.0 nm at 50.00 μg mL^{-1}. The limit of quantification (LOQ) is defined as the lowest measured concentration adjusted by three times of the standard deviation of the multiple measurements. The LOQ obtained with the biosensor based on the SPA–Au/GO composite is about 16 times lower than that obtained with a sole SPA platform.

When the SPA–Au/GO composite was anchored to the Au film surface as a support to the antibody immobilization, the optical and electronic properties of the substrate changed. The surface plasmon of Au nanoparticles on a GO sheet might be resonantly excited through energy transfer in the near field and create a strong local electromagnetic field, which enhanced the shift of the resonant wavelength. Moreover, the electronic coupling between Au nanoparticles and the surface plasmon wave further leads to the amplification of the SPR response originating from the dielectric changes of the substrate upon binding of the antigen–antibody complexes.[5] In addition, the thickness of the sensing membrane increases and the resonant wavelength moves to longer wavelength, which also lead to the sensitivity enhancement of the wavelength modulation SPR

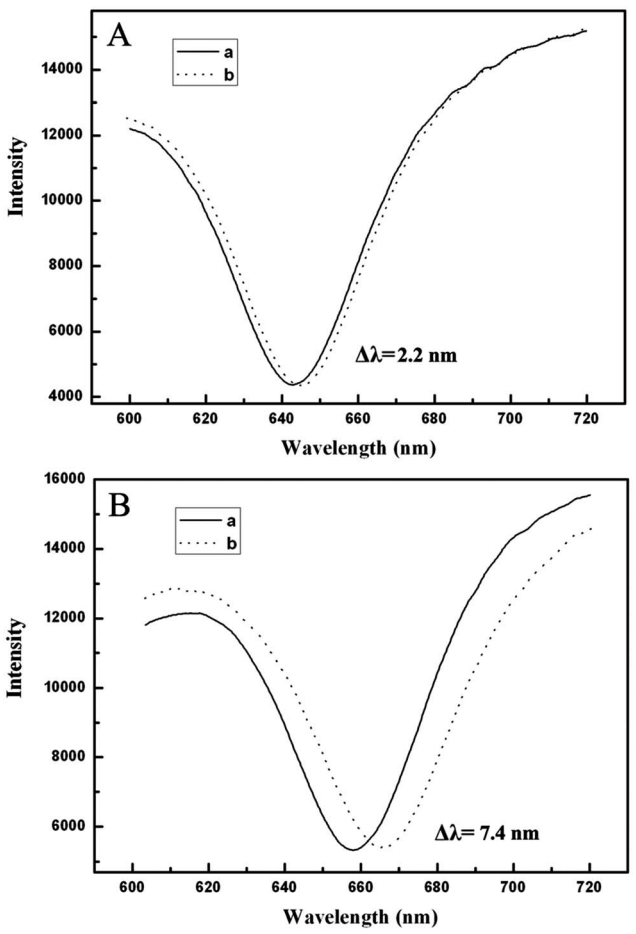

Fig. 4 *In situ* SPR curves obtained before (—) and after (···) determination of 25 μg mL^{-1} solution of rabbit IgG based on different systems: (a) SPA-modified Au film; (b) SPA–Au/GO modified Au film.

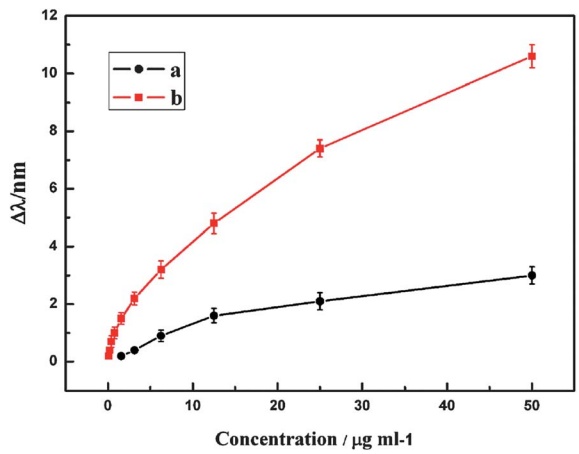

Fig. 5 The relationship between the shifts of the resonant wavelength and the concentrations of rabbit IgG. The biosensor based on a SPA-modified Au film (a); the biosensor based on a SPA–Au/GO modified Au film (b).

biosensor. The experiment results demonstrate that the use of the SPA modified Au–GO composite for antibody immobilization in the SPR biosensor permitted a 16 times enhancement in LOQ.

In the previous reports, the determination of rabbit IgG based on the Hg–Au film and Ag–Au film with a wavelength modulation SPR biosensor was developed.[32] Mercury or silver was electrodeposited on an Au surface to form an Hg–Au or Ag–Au film. The range of concentrations that could be determined was 0.63–40.00 μg mL^{-1} for the Hg–Au film and 0.42–20.00 μg mL^{-1} for the Ag–Au film. A SPR biosensor based on ZnO–Au nanocomposites was also developed for the detection of rabbit IgG.[33] The biosensor exhibits a satisfactory response to rabbit IgG in the concentration range of 0.15–20.00 μg mL^{-1}. The limit of determination of the present biosensor is lower than the previous reports and the upper determination limit has been expanded to 50 μg mL^{-1}. As mentioned above, the experimental results indicated that the performance of the wavelength-modulation SPR biosensor based on a SPA–Au/GO platform was significantly improved and oriented binding of antibodies could be achieved through the SPA layer.

As can be seen in Fig. 5, the shift of the resonant wavelength does not linearly increase with the increase of the concentration of rabbit IgG in a certain concentration range. The reaction of antigen (Ag) and antibody (Ab) can be expressed as follows: Ag + Ab = AgAb, the binding constant is K_A.[22]

$$K_A = \frac{[AgAb]}{[Ag][Ab]} \quad (1)$$

where [AgAb] corresponds to the SPR biosensor signal $\Delta\lambda_{eq}$, [Ag] is the concentration of antigen in the solution, [Ab] corresponds to $(\Delta\lambda_{max} - \Delta\lambda_{eq})$. $\Delta\lambda_{eq}$ is the biosensor response at equilibrium. $\Delta\lambda_{max}$ is the SPR response corresponding to the maximum capacity of binding antigen on the sensor surface. So eqn (1) can be expressed as:

$$\frac{\Delta\lambda_{eq}}{[Ag]} = K_A\Delta\lambda_{max} - K_A\Delta\lambda_{eq} \quad (2)$$

$\Delta\lambda_{eq}$ increases with the increase of the concentration of antigen ([Ag]) in the solution. It is seen from eqn (2) that the $\Delta\lambda_{eq}$/[Ag] decreases with the increase of $\Delta\lambda_{eq}$. So the slope of the relationship curve between the shift of the resonant wavelength and the concentration of rabbit IgG decreases with the increase of the concentration of antigen, which is in accordance with the experimental results.

The specificity is demonstrated by detecting human IgG and bovine IgG under the same conditions. After the goat anti-rabbit IgG was immobilized on the surface of the biosensor, human IgG and bovine IgG at a concentration of 12.5 μg mL^{-1} were separately injected into the reactor for 40 min. There are no observable shifts of the resonant wavelength (Fig. 6), which indicates the selective binding of rabbit IgG. The biosensor retains the specificity of antigen. Therefore, the proposed platform based on the Au–GO composite has sufficient specificity and rabbit IgG can be identified with high selectivity.

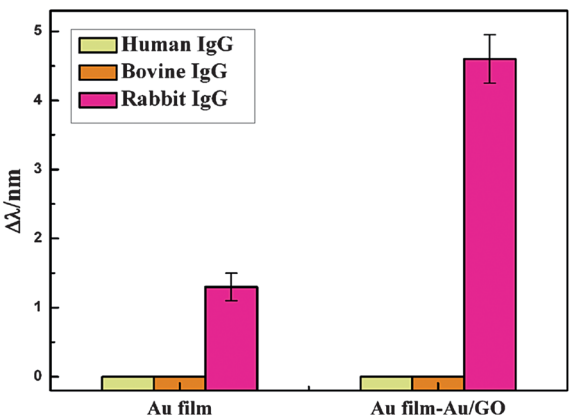

Fig. 6 The shift of the resonant wavelength measured with different antigens (human IgG, bovine IgG and rabbit IgG) based on different sensing membranes.

Conclusions

This work demonstrates the successful application of the Au–GO composite in the wavelength modulation SPR biosensor for rabbit IgG detection. Experimental results show that the SPA modified Au–GO composite was directly immobilized onto SPR chips and the simple and oriented binding of antibodies could be achieved through the SPA without any additional chemical treatment. The biosensor with the SPA modified Au–GO composite as the enhanced sensing platform shows a satisfactory response to rabbit IgG in the concentration range of 0.1–50 μg mL^{-1}. The LOQ obtained with the composite is 16 times lower than that obtained with the SPA modified chip. These results provide a simple and effective approach for the fabrication of a sensitive SPR immunosensor and extend the application of the Au–GO composite in immunoassays.

Acknowledgements

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

References

1 J. Homola, *Chem. Rev.*, 2008, **108**, 462–493.
2 T. L. Chuanga, S. C. Wei, S. Y. Lee and C. W. Lina, *Biosens. Bioelectron.*, 2012, **32**, 89–95.
3 C. F. Gao, Z. D. Lu, Y. Liu, Q. Zhang, M. F. Chi, Q. Cheng and Y. D. Yin, *Angew. Chem., Int. Ed.*, 2012, **51**, 5629–5633.
4 E. D. Juan-Franco, A. Caruz, J. R. Pedrajas and L. M. Lechuga, *Analyst*, 2013, **138**, 2023–2031.
5 L. A. Lyon, M. D. Musick and M. J. Natan, *Anal. Chem.*, 1998, **70**, 5177–5183.
6 M. C. Estevez, J. Belenguer, S. Gomez-Montes, J. Miralles, A. M. Escuela, A. Montoyad and L. M. Lechugaa, *Analyst*, 2012, **137**, 5659–5665.

7 O. Esteban, F. B. Naranjo, N. D. Herrera, S. V. Felip, M. C. Navarrete and A. Gonzalez-Cano, *Sens. Actuators, B*, 2011, **158**, 372–376.
8 Y. Sun, Y. P. Bai, D. Q. Song, X. Z. Li, L. Y. Wang and H. Q. Zhang, *Biosens. Bioelectron.*, 2007, **23**, 473–478.
9 M. Z. Mousavi, H. Y. Chen, S. H. Wu, S. W. Peng, K. L. Lee, P. K. Wei and J. Y. Cheng, *Analyst*, 2013, **138**, 2740–2748.
10 H. Jiang, *Small*, 2011, **7**, 2413–2427.
11 Y. Wang, Z. Li, J. Wang, J. Li and Y. Lin, *Trends Biotechnol.*, 2011, **29**, 205–212.
12 L. Wang, C. Z. Zhu, L. Han, L. H. Jin, M. Zhou and S. J. Dong, *Chem. Commun.*, 2011, **47**, 7794–7796.
13 N. N. Zhang, H. X. Qiu, Y. Liu, W. Wang, Y. Li, X. D. Wang and J. P. Gao, *J. Mater. Chem.*, 2011, **21**, 11080–11083.
14 Y. K. Kim, H. K. Na and D. H. Min, *Langmuir*, 2010, **26**, 13065–13070.
15 K. P. Loh, Q. L. Bao, G. Eda and M. Chhowalla, *Nat. Chem.*, 2010, **2**, 1015.
16 T. Liu, H. C. Su, X. J. Qu, P. Ju, L. Cui and S. Y. Ai, *Sens. Actuators, B*, 2011, **160**, 1255–1261.
17 W. B. Lu, Y. L. Luo, G. H. Chang and X. P. Sun, *Biosens. Bioelectron.*, 2011, **26**, 479.
18 E. Peng, E. Shi, G. Choo, P. Chandrasekharan, C. T. Yang, J. Ding, K. H. Chuang and J. M. Xue, *Small*, 2012, **8**, 3620–3630.
19 W. Ren, Y. X. Fang and E. K. Wang, *ACS Nano*, 2011, **5**(8), 6425–6433.
20 W. B. Lu, R. Ning, X. Y. Qin, Y. W. Zhang, G. H. Chang, S. Liu, Y. L. Luo and X. P. Sun, *J. Hazard. Mater.*, 2011, **197**, 320–326.
21 N. A. Travlou, G. Z. Kyzas, N. K. Lazaridis and E. A. Deliyanni, *Langmuir*, 2013, **29**, 1657–1668.
22 J. Wang, L. Y. Wang, Y. Sun, X. N. Zhu, Y. B. Cao, X. H. Wang, H. Q. Zhang and D. Q. Song, *Colloids Surf., B*, 2010, **75**, 520–525.
23 D. C. Marcano, D. V. Kosynkin, J. M. Berlin, A. Sinitskii, Z. Z. Sun, A. Slesarev, L. B. Alemany, W. Lu and J. M. Tour, *ACS Nano*, 2010, **4**, 4806–4814.
24 G. Goncalves, P. A. A. P. Marques, C. M. Granadeiro, H. I. S. Nogueira, M. K. Singh and J. Gracio, *Chem. Mater.*, 2009, **21**, 4796–4802.
25 R. Pei, X. Cui, X. Yang and E. Wang, *Talanta*, 2000, **53**, 481–488.
26 X. Gao, J. Jang and H. Nagase, *J. Phys. Chem. C*, 2010, **114**, 832–842.
27 S. K. Vashist, C. K. Dixit, B. D. MacCraithad and R. O. Kennedy, *Analyst*, 2011, **136**, 4431–4436.
28 D. P. Tang, R. Yuan and Y. Q. Chai, *Bioprocess Biosyst. Eng.*, 2006, **28**, 315–321.
29 K. A. Davis, *Anal. Chem.*, 1989, **61**, 1227–1230.
30 J. Homola, *Sens. Actuators, B*, 1997, **41**, 207–211.
31 J. Homola, I. Koudela and S. S. Yee, *Sens. Actuators, B*, 1999, **54**, 16–24.
32 Y. Sun, D. Q. Song, Z. Q. Li, Y. Bai and H. Q. Zhang, *Anal. Bioanal. Chem.*, 2007, **387**, 1875–1882.
33 L. Q. Wang, Y. Sun, J. Wang, J. Wang, A. Q. Yu, H. Q. Zhang and D. Q. Song, *J. Colloid Interface Sci.*, 2010, **351**, 392–397.