Improved sensitivity of wavelength-modulated surface plasmon resonance biosensor using gold nanorods

Peng Hao,[1,*] Yihui Wu,[1,2] and Fengjie Li[1]

[1]State Key Laboratory of Applied Optics, Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun, Jilin Province 130033, China

[2]e-mail: yihuiwu@ciomp.ac.cn

*Corresponding author: hp312@126.com

Received 6 May 2011; revised 4 July 2011; accepted 5 July 2011;
posted 8 July 2011 (Doc. ID 147055); published 30 September 2011

In this report, gold nanorods (GNRs) were used to enhance the sensitivity of the wavelength-modulated surface plasmon resonance (SPR) biosensor. The GNRs were designed and fabricated through seed-medicated growth and surface activation by a layer of a weak polyelectrolyte, poly(acrylic acid) for the attaching antibody. Rabbit anti-goat IgG was immobilized on GNRs, and sandwich assays were carried out to detect goat IgG using a wavelength-modulated SPR biosensor. The detection sensitivity of the nanorod-conjugated antibody is 25–100 times more sensitive than the SPR biosensor without GNRs. Drastic sensitivity enhancement, owing to the electromagnetic interaction between the nanotag and the sensing film, was maximized using the longitudinal plasmonic resonance of the GNRs. GNRs could significantly enhance the sensitivity of the SPR biosensor, and the maximum enhancement effect can be achieved when the longitudinal SPR peak wavelength of GNRs functionally matches the surface plasmon wavelength.

OCIS codes: 240.6680, 120.5050, 280.4788.

1. Introduction

The use of surface plasmon resonance (SPR) biosensors has evolved into a promising technique for biological analysis applications, because they possess an extreme sensitivity to plasmon resonance caused by environmental changes and they remove the requirement for extrinsic biomolecular labeling [1]. However, these researches mainly focus on studying the binding processes between large biomolecules; small molecules are seldom detected by conventional SPR technique directly because the changes in the refractive index resulting from the binding processes of small biomolecules are often small. In order to extend the application of the SPR biosensor and develop a novel SPR sensor for detection of general small molecules with high sensitivity and selectivity, several research groups have been carried out to improve the resolution of the SPR-based measurement of refractive index changes [2,3] as well as toward the amplification of the sensor response.

Now, the nanoparticle-enhanced method has proven to be convenient for the SPR biosensor, which can amplify the signal of ultrasmall biomolecules [4]. Specifically, the use of nanoparticle tags/labels results in a dramatic enhancement of SPR biosensor sensitivity due to two main factors: (i) an increase in the bulk refractive index of the analyte and (ii) coupling between the localized surface plasmon resonance (LSPR) of metallic nanoparticles and the SPR of the sensing film [5,6]. Gold nanorods (GNRs) possess two principal plasmon absorption bands: one is the transverse plasmon band, corresponding to light absorption and scattering along the short axis of the particle, and the other is the longitudinal plasmon band, corresponding to light absorption and scattering along the long axis of the particle. The former is located in the visible region of the electromagnetic spectrum at ≈520 nm, while the latter is aspect-ratio-tunable from the visible to the near-IR region

0003-6935/11/285555-04$15.00/0

of the electromagnetic spectrum [7]. In this study, a GNR-enhanced wavelength-modulated SPR biosensor is proposed. We believe that the nanorod (NR) surface will provide more surface area for analyte absorption, thus increasing the dynamic range; more importantly, the localized surface plasmons (LSPs) will be generated inside the NRs under certain structural conditions; therefore, the SPR properties can be enhanced due to the interaction between the LSPs and surface plasmons. The NRs will be more sensitive and convenient compared to using nanoparticles having a sphere structure resonantly coupled with the gold film of the wavelength-modulated SPR.

2. Materials and Methods

A. Materials

$HAuCl_4 \bullet 3H_2O$, cetyltrimethylammoniumbromide (CTAB), the polyelectrolyte poly(acrylic acid, sodium salt) (PAA), sodium chloride (NaCl), ascorbic acid, and silver nitrate were purchased from Beijing Chemical Reagents (Beijing, China). N-hydroxysuccinimide (NHS), N-ethyl-N-[(dimethylamino) propyl]carbodiimide (EDC) were obtained from the Shanghai Medpep Co., Ltd. (Shanhai, China). Mercaptoundecanoic acid (MUA) was obtained from Sigma-Aldrich. Goat IgG and rabbit anti-goat IgG were obtained form Biodee Biotechnology Co., Ltd. (Beijing, China). All of the chemicals, unless mentioned otherwise, were of analytical reagent grade and used as received.

B. Preparation of Gold Nanorods

Colloidal Au-NRs were synthesized using a typical seed-mediated solution phase approach, as previously described [8–11]. Au nanoparticle seeds were synthesized in advance for fabrication of the GNRs. In a 10 ml glass vial, 5 ml of 0.2 M cetyltrimethylammonium bromide (CTAB) solution was mixed with 5 ml of 0.5 mM gold (III) chloride hydrate ($HAuCl_4$). Then 0.6 ml of ice-cold 0.01 M sodium borohydride ($NaBH_4$) solution was quickly injected at room temperature, leading to the formation of a brownish-yellow solution. The seed solution was vigorously stirred for 30 min and then kept at room temperature. We refer to this mixture as the seed solution. Next the growth solution was prepared—1.5 ml of 0.02 M $HAuCl_4$ and 1.0 ml of 0.01 M $AgNO_3$ were added to 30 ml of 0.1 M CTAB, followed by the addition of 0.8 ml of 0.08 M ascorbic acid. The ascorbic acid here worked as a mild reducing agent and changed the solution from dark yellow to transparent. Finally, 70 μl of the seed solution was added to the growth solution. The resulting mixture was left undisturbed for 24 h at room temperature. Au-NRs were purified from the excess surfactant solution by centrifugation (13,000 rpm for 15 min). The aspect ratio of the GNRs can be controlled by changing the amount of seed solution, ascorbic acid solution, and $AgNO_3$ solution.

C. Preparation of Antibody-Conjugated Au Nanorod

Synthesized GNRs have a bilayer of surfactant hexadecyltrimethylammonium bromide (CTAB) on their surface acting as a stabilizer to prevent aggregation. Excess CTAB in the solution is removed by centrifugation at 5900 rpm for 60 min. After removing excess CTAB, the GNRs form a pellet at the bottom of the tube.

The GNRs are finally dispersed in deionized water to achieve a molar concentration of 0.01 M of GNRs in the solution. A layer of is adsorbed onto the surface of the GNRs by adding 1.5 ml of 10 mg ml^{-1} PAA solution to 1 ml of the GNR solution. The mixture is stirred for 3 h followed by two cycles of centrifugation and redispersion to remove excess PAA in the solution. The layer of PAA provides the –COOH functional group required for the conjugation. The PAA-coated NRs are dispersed in 1 ml of PBS 6.0 buffer solution followed by the addition of 100 μl of 0.2 M EDC [N-ethyl-N -(3-dimethylaminopropyl) carbodiimide] and 100 μl of 0.2 M NHS (N-hydroxy-succinimide).

After 20 min, the reaction mixture was added to 20 μl of rabbit anti-goat IgG. The EDC/NHS mixture forms an active ester intermediate with the GNRs, which undergoes an amidation reaction with the $–NH_2$ group in the rabbit anti-goat IgG to yield the conjugate. The reaction mixture is stored in a refrigerator at 4 °C overnight following centrifugation and redispersion in the buffer to remove the unconjugated drug.

D. Procedure of Wavelength-Modulated SPR Biosensor Measurement

The measurements have been carried out using our homemade wavelength-modulated SPR biosensor (Fig. 1). The sensing film was functionalized with goat IgG antibody, and the signal was monitored using our setup. As shown in Fig. 2, a sharp increase in the wavelength signal occurred upon the addition of IgG-containing PBS solution. The system was left to settle for 60 min to allow a layer of IgG to form on the gold surface. The PBS was rinsed again to confirm the functionalization.

3. Results and Discussion

Highly monodispersed Au-NRs were systematically characterized using UV-VIS absorption spectroscopy (Lambda 850, PerkinElmer, U.S.) and transmission

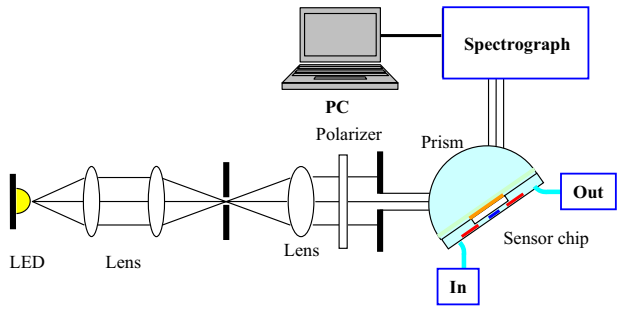

Fig. 1. (Color online) SPR sensor with wavelength modulation.

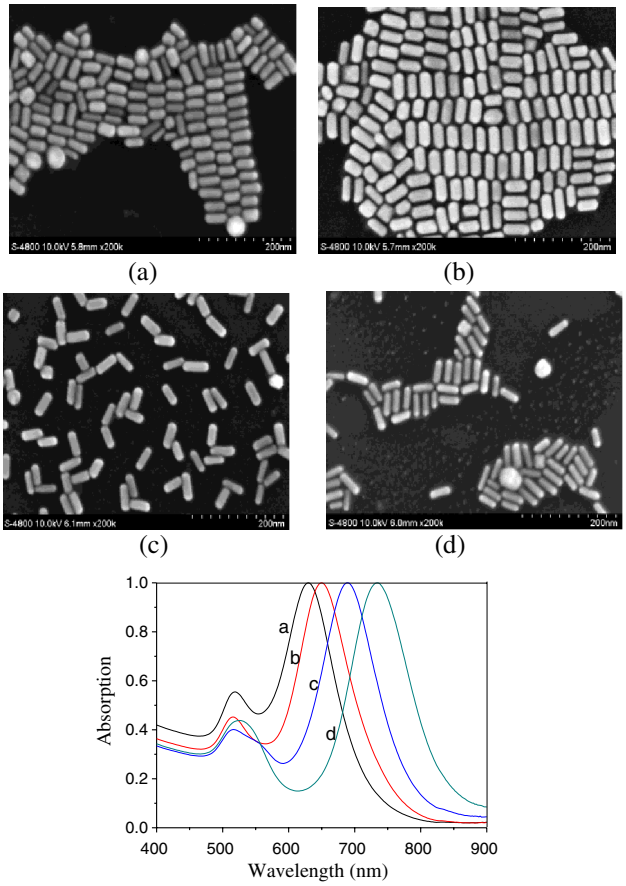

Fig. 2. (Color online) TEM pictures and UV-vis absorption spectra of Au-NRs with the LSPR peak at (a) 630, (b) 650, (c) 690, and (d) 734 nm.

electron microscopy (TEM) (s4800). Figure 2 shows the TEM images of Au-NRs and their corresponding absorption spectra. The estimated diameter and the length are 20 and 42 nm for NR-630, 20 and 47 nm for NR-650, 18 and 52 nm for NR-690, and 16 and 50 nm for NR-734, respectively. Different aspect ratios (length versus diameter) of the Au-NRs can be achieved by manipulating the synthesis parameters. In general, when the aspect ratio of the NRs

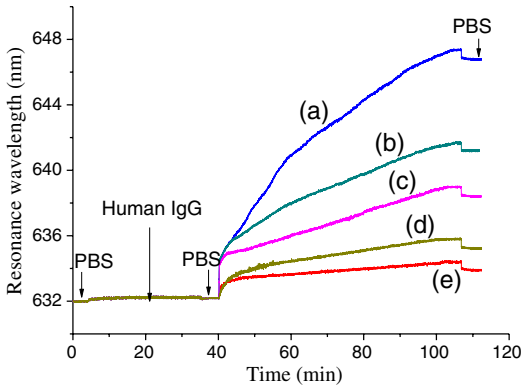

Fig. 3. (Color online) Response curves obtained from immobilization of GNR-conjugated anti-IgG: (a) GNR630-anti-IgG, (b) GNR650-anti-IgG, (c) GNR690-anti-IgG, (d) GNR734-anti-IgG, (e) colloidal Au-anti-IgG.

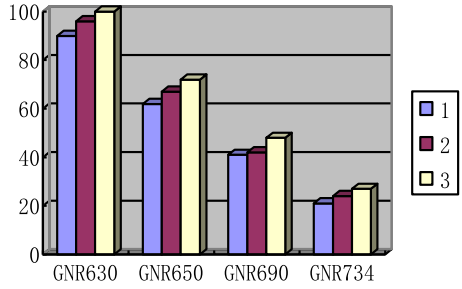

Fig. 4. (Color online) Amplification effect of Au-NRs.

increases, a redshift of the longitudinal LSPR peak is observed.

In order to clarify and identify the mechanism of sensitivity enhancement, we performed a systematic study of the plasmonic coupling. The same concentrations of NR-630, NR-650, NR-690, and NR-734 were conjugated to anti-IgG using the above-described protocol. Plasmonic coupling was then examined by introducing these bioconjugates onto the IgG-modified sensing film. Figure 3 shows the magnitude change of the enhancement factor as a function of the Au-NRs' LSPR peak wavelength, and each set of experiments was repeated three times (Fig. 4). It is worth noting that the errors can be attributed to the random orientation of Au-NRs, uneven coverage of bioconjugates on the sensor surface, and variations of the Au-NRs–film distance.

As shown in Fig. 4, the maximum enhancement effect was obtained when the NR-630 sample was used as the amplification labels. It should also be noted that the resonance wavelength is 632 nm in this study. Based on these facts, we hypothesize that the LSPR wavelength dependence of the enhancement factor is due to the presence of plasmonic coupling between the Au-NRs and the Au sensing film, and the maximum enhancement effect can be achieved when the LSPR peak wavelength of the Au-NRs functionally matches the resonance wavelength. A simplified representation of a plasmonic Au-NR interaction with a gold film is shown in Fig. 5. Assuming the Au-NRs as a polarizable dipole, for the longitudinal mode, an image dipole is induced in the film in response to the presence of an Au-NR. The in-plane (parallel to the film) dipole moments of the image are opposite to those of the Au-NR, so that maximum plasmonic coupling between the Au-NRs and the Au sensing film will happen when the LSPR peak wavelength of Au-NRs matches the resonance wavelength of the SPR.

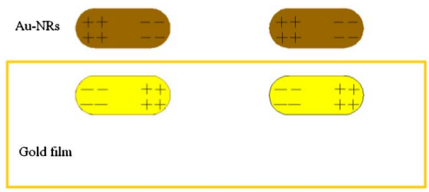

Fig. 5. (Color online) Simplified representation of a plasmonic Au-NR interaction with a gold film.

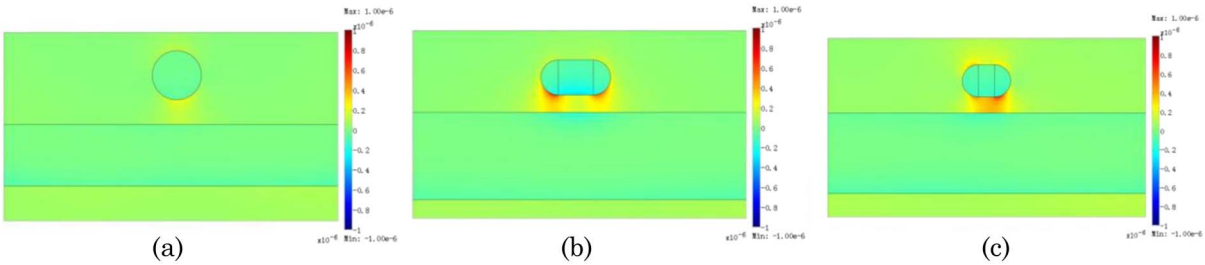

Fig. 6. (Color online) FEA simulations of resonant Au-NR (distance from the film is 10 nm) coupling to the film. The distribution in the X–Z plane of the time-averaged electric energy density (J/m^3) for (a) the film and the 20 nm radius gold nanosphere particle, (b) the film and the 20 nm diameter, 40 nm long GNR particle, and (c) the film and the 20 nm diameter, 30 nm long GNR particle. The film and the rod are shown with gray lines.

To confirm it, we used finite element analysis (FEA, COMSOL Multiphysics 3.5) to simulate the coupling. The results presented in Fig. 6 clearly show that the evanescent field of the film resonantly excites the longitudinal plasmonic resonance of a GNR with 40 nm length, 20 nm diameter [Fig. 6(b)] and 30 nm length, 20 nm diameter [Fig. 6(c)]. The field is greatly enhanced in the gap between the rod and the film. These findings demonstrate that tuning the longitudinal LSPR of GNRs enables achievement of the maximum enhancement of biosensor sensitivity owing to resonant plasmonic coupling between the NRs and the sensing film.

4. Conclusion

In summary, we have demonstrated greater than 25- to 100-fold enhancement of sensitivity of a wavelength-sensitive SPR biosensor with the use of GNRs as powerful amplification labels. The correlation between the enhancement factor and the longitudinal LSPR of NRs has been observed. When the longitudinal LSPR peak wavelength of Au-NRs matches the resonance wavelength of the SPR, the biosensors have achieved maximum enhancement of the biosensor sensitivity. The NR-to-film plasmonic coupling has been identified to be the main reason for the enhancement. This finding will allow us to significantly improve the detection sensitivity by using appropriately engineered bioconjugates.

We thank the National Natural Science Foundation of China (NSFC) (grant 60971025) and the Knowledge Innovation Programs of the Chinese Academy of Sciences (CAS) (grant KJCX2-YW-H18) for financial support.

References

1. J. Homola, "Surface plasmon resonance sensors for detection of chemical and biological species," Chem. Rev. **108**, 462–493 (2008).
2. G. D. VanWiggeren, M. A. Bynum, J. P. Ertel, S. Jefferson, K. M. Robotti, E. P. Thrush, D. M. Baney, and K. P. Killeen, "A novel optical method providing for high-sensitivity and high-throughput biomolecular interaction analysis," Sens. Actuators B Chem. **127**, 341–349 (2007).
3. S. Slavik and J. Homola, "Ultrahigh resolution long range surface plasmon-based sensor," Sens. Actuators B Chem. **123**, 10–12 (2007).
4. J. S. Mitchell, Y. Wu, C. J. Cook, and L. Main, "Sensitivity enhancement of surface plasmon resonance biosensing of small molecules," Anal. Biochem. **343**, 125–135 (2005).
5. E. Hutter, S. Cha, J. F. Liu, J. Park, J. Yi, J. H. Fendler, and D. Roy, "Role of substrate metal in gold nanoparticle enhanced surface plasmon resonance imaging," J. Phys. Chem. B **105**, 8–12 (2001).
6. W.-C. Law, K.-T. Yong, A. Baev, R. Hu, and P. N. Prasad, "Nanoparticle enhanced surface plasmon resonance biosensing: application of gold nanorods," Opt. Express **17**, 19041–19046 (2009).
7. C. Yu and J. Irudayaraj, "Multiplex biosensor using gold nanorods," Anal. Chem. **79** 572–579 (2007).
8. J. Pérez-Juste, I. Pastoriza-Santos, and L. M. Liz-Marzan, "Gold nanorods: synthesis, characterization and applications," Coord. Chem. Rev. **249**, 1870–1901 (2009).
9. B. Nikoobakht and M. A. El-Sayed, "Preparation and growth mechanism of gold nanorods (GNRs) using seed-mediated growth method," Chem. Mater. **15**, 1957–1962 (2003).
10. T. K. Sau and C. J. Murphy, "Seeded high yield synthesis of short Au nanorods in aqueous solution," Langmuir **20**, 6414–6420 (2004).
11. A. Gole and C. J. Murphy, "Biotin–streptavidin-induced aggregation of gold nanorods: tuning rod–rod orientation," Langmuir **21**, 10756–10762 (2005).