

Nanoparticle enhanced surface plasmon resonance biosensing: Application of gold nanorods

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Abstract: In this report, we demonstrate the use of functionalized gold nanorods as amplification labels for ultra-sensitive surface plasmon resonance biosensing. Drastic sensitivity enhancement, owed to the electromagnetic interaction between the nanorod and the sensing film, was maximized using longitudinal plasmonic resonance of gold nanorods. The detection sensitivity of the nanorod-conjugated antibody is estimated to be ~40 pg/ml, which is 25 – 100 times more sensitive than the current reported values in the literature. This work paves the way to a new generation of ultra-sensitive nanoparticles-based biosensor platforms with maximized enhancement of sensitivity for ultra-fast screening and real-time detection of “hard-to-identify” biomolecules.

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1. Introduction

Over the last decade, the surface plasmon resonance (SPR) sensing technique has proven to be indispensable for biomolecular interaction studies, immunosensing, and early stage cancer detection [1–3]. Information about biomolecular interactions is obtained by measuring the optical characteristics (spectral intensity and phase) of light reflected from the metal/analyte interface. Therefore, the SPR biosensor enables real-time monitoring of analyte-analyte interactions with high sensitivity [4,5]. To date, conventional SPR biosensors, based on monitoring intensity of the reflected light, have been successfully used to obtain high detection sensitivity, ranging from pM to fM of analyte concentration. However, their major drawback is their inability to detect low molecular weight biomolecules such as DNA or hormones. To overcome this limitation, several research groups have developed phase-sensitive [6–10] and nanoparticle-enhanced [11–14] SPR biosensors, which can amplify the signal of ultra-small biomolecules. Specifically, the use of nanoparticle tags/labels results in a dramatic enhancement of sensitivity due to three main factors: (i) an increase of the absolute mass in each binding event, (ii) an increase in the bulk refractive index of the analyte, and (iii) coupling between the localized surface plasmon resonance (LSPR) of metallic nanoparticles and surface plasmon resonance (SPR) of the sensing film. The role of plasmonic coupling in sensitivity enhancement is still an open question. In order to obtain a better understanding of this phenomenon, we investigate how the shape and the size of metallic nanoparticle labels correlate with the enhancement factor. To date, there do not appear to be any published studies of this correlation behavior. In this letter, we report a more than 20-fold sensitivity enhancement of phase-sensitive SPR biosensing using gold nanorods (Au-NRs) as amplification labels. Nanorods are superior to spherical particles for this application. First and foremost, the easy tunability of LSPR peak via changing the synthesis parameters to control the aspect ratio (AR) enables fine tuning and therefore optimization of the enhancement. Moreover, in a typical SPR system with 50 nm thick Au sensing film, longer wavelength laser sources (785 – 1064 nm) lead to higher detection sensitivity. Longer wavelength of the incident light requires non-spherical nanotags for resonant coupling with the film (at a given angle of incidence). Other aspects, such as extension of the sensing field farther into the analyte and high longitudinal polarizabilities together with excellent colloidal stability and bio-compatibility make nanorods a versatile nanotag. This study will provide a solid foundation for developing an ultra-sensitive biosensor.

2. Materials and methods

2.1 Synthesis of Au nanorods with different aspect ratio

Colloidal Au-NRs were synthesized using a typical seed-mediated solution phase approach as previously described with slight modifications [15]. Firstly, Au nanoparticle seeds were synthesized in advance for fabrication of gold nanorods. In a 10 mL glass vial, 5 mL of 0.2 M cetyltrimethylammonium bromide (CTAB) solution was mixed with 5 mL of 0.96 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, resulting in the formation of a light-brown 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 by mixing 400 μL of 25 mM HAuCl_4 and 10 mL of 0.2 M CTAB in 10 mL of HPLC-grade water. Subsequently, 100 μL of 4.0 mM silver nitrate (AgNO_3) solution was added to this solution at room temperature. 200 μL of 0.08 M ascorbic acid was then added to the mixture and gently stirred for 30s. After the solution color changed from orange to transparent, 24 μL of the seed

solution was injected to the growth solution. The resulting mixture was left undisturbed for 24 hrs at room temperature. Au NRs were purified from the excess surfactant solution by centrifugation (13000 rpm for 15 min). By manipulating the AgNO_3 concentration in the growth solution, different aspect ratios of gold nanorods can be obtained.

2.2 Preparation of antibody-conjugated Au nanorod

To prepare bioconjugated Au-NRs, the as-synthesized Au-NR solutions were purified by centrifugation to remove excess surfactant (CTAB). To ensure complete removal of CTAB, centrifugation was performed 2 to 3 times. Bioconjugation of Au-NRs with anti-rabbit immunoglobulin G antibody (anti-IgG, Sigma-Aldrich, USA) was carried out as reported previously [16]. Briefly, Au-NR solutions with different aspect ratios were diluted to the same concentration by unifying the optical density value of the longitudinal peak of Au-NR (~ 0.25 , stock solutions). Then, 100 μL of anti-IgG (100 ng/ml) solution was added to 100 μL of Au-NR stock solution. The solution was mixed for 30s. The mixture was left undisturbed for conjugation for 30-40 min and followed by centrifugation and redispersion in 1mL of PBS solution. Here, the final concentration of the bioconjugate (Au-NR-antiIgG) was estimated to be ~ 10 ng/ml (10 ng of anti-IgG dispersed in 1 mL of PBS).

2.3 Functionalization of Au sensing film with IgG antibody

The sensing film with a 50 nm thick gold coating was purchased from Platyus Technologies. Rabbit immunoglobulin G antibody (IgG, Sigma-Aldrich, USA) was conjugated on the sensing film using a standard carbodiimide chemistry method. The sensing film was first immersed in a 20mM of 3-mercaptopropyl acid solution ($\text{pH} = 10 - 11$) for 0.5 – 1 hr in order to modify the Au surface with carboxyl functional group. A typical biomolecule immobilization procedure was accomplished by using traditional carbodiimide strategy. 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride (EDC): N-hydroxysuccinimide (NHS) 1:1 (molar ratio, 1 mmol) solution was added to the carboxylated surface for 1 hr and then rinsed with buffer solution for 5 min. IgG solution (1mg/mL) was incubated in the flow cell for 40 min accompanied by rinsing with buffer solution for 5 min.

3. Results and discussions

Highly mono-dispersed Au-NRs were systematically characterized using UV-vis absorption spectroscopy (Shimadzu UV-3600 spectrophotometer) and transmission electron microscopy (TEM) (JEOL JEM-100). Figure 1 shows the TEM images of Au-NRs and their corresponding absorption spectra. The estimated diameter and the length are 22 and 22 nm for NR-530, 21 and 50 nm for NR-642, 15 and 48 nm for NR-718 and 16 and 58 nm for NR-772 respectively. Different aspect ratios (length vs diameter) of Au-NRs can be achieved by manipulating the synthesis parameters. In general, when the aspect ratio of NRs increases, a red shift of longitudinal LSPR peak is observed.

The measurements have been carried out using our home-made phase-sensitive SPR biosensor [8,17]. For detail experimental setup procedures, please refer to Ref [8]. The sensing film was functionalized with IgG antibody and the signal was monitored using our setup. As shown in Fig. 2, a sharp increase in phase (3rd harmonic) signal occurred upon the addition of IgG-containing PBS solution. The system was left to settle for 40 min. to allow a layer of IgG to form on the gold surface. PBS was rinsing again to confirm the functionalization.

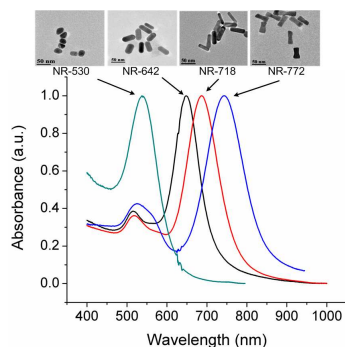


Fig. 1. TEM pictures and UV-vis absorption spectra of Au-NRs with the LSPR peak at 530, 642, 718 and 772 nm.

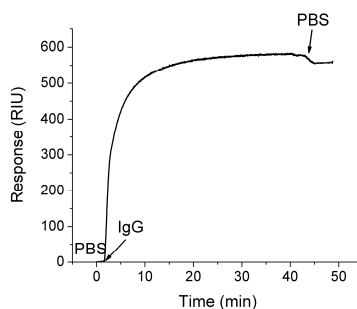


Fig. 2. Response curves obtained from immobilization of IgG on the sensor surface.

Figure 3 illustrates the bio-specific interaction of nanorod-conjugated anti-IgG (NR642-anti-IgG) and un-conjugated anti-IgG with the IgG-modified sensing film. In this case, the baseline was initially set and maintained by running phosphate buffered saline (PBS) through the system for 2-3 min. The PBS buffer containing NR642-anti-IgG (10 ng/ml) was then introduced into the sensor head. Upon introducing the sample solution to the phase-sensitive SPR biosensor, a progressive change of the phase signal was immediately observed (Fig. 3, black curve). PBS was running again to confirm the binding reaction only occurred between the analyte and the sensing film. Control experiment was conducted by simply introducing free anti-IgG (10 ng/ml) to the SPR system under the same experimental conditions (Fig. 3, red curve). An insignificant response was obtained in comparison with NR-conjugated sample. Detection sensitivity for the NR642-anti-IgG and the un-conjugated anti-IgG samples are estimated to be 40 pg/ml and 0.9 ng/ml, respectively. Thus, a 23-fold increase in sensitivity was demonstrated, thereby justifying our strategy of using Au-NRs as powerful labels to amplify the biomolecular interaction signals. The non-specific binding was also investigated by flowing unconjugated Au-NRs sample onto the sensing film. As expected, a minimal response was observed, indicating the specificity of the bioconjugated Au NRs with the functionalized sensing film, see Fig. 4.

In order to clarify and identify the mechanism of sensitivity enhancement, we performed a systematic study of the plasmonic coupling. Same concentrations of NR-530, NR-642, NR-718 and NR-772 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 5(a) shows the magnitude change of the enhancement factor as a function of the Au-NRs LSPR peak wavelength. Each set of experiments was repeated five times and the error bar, representing the standard deviation, is shown in Fig. 5.

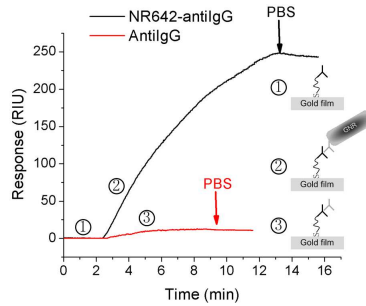


Fig. 3. Response curves obtained from immobilization of NR-642 conjugated anti-IgG (black curve) and unconjugated anti-IgG (red curve) to the IgG-modified sensor surface.

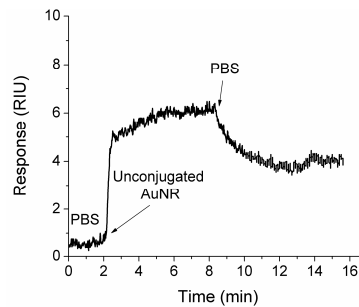


Fig. 4. Response curves obtained from the nonspecific binding between unconjugated Au NR and IgG-modified sensing film.

It is worth noting that the errors can be attributed to the variations of the film thickness, uneven coverage of bioconjugates on the sensor surface, random orientation of Au-NRs and variations of the Au-NRs–film distance. As shown in Fig. 5, the maximum enhancement effect was obtained when NR-642 sample was used as the amplification labels. It should be also noted that a laser operating at 785 nm was used as the excitation source 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 maximum enhancement effect can be achieved when the LSPR peak wavelength of Au-NRs functionally matches the wavelength of the excitation source. It is well known that the excitation of surface plasmons in the Kretschmann configuration requires projection of the incident optical wave vector, k_x , parallel to the film, to be matched with the surface plasmon wave vector, k_{sp} :

$$k_x = k_0 n_{\text{glass}} \sin \theta_{\text{inc}} = k_{sp} \quad (1)$$

Here k_0 is the free space wave vector of the optical wave, $n_{\text{glass}} = 1.62$ is the refractive index of the prism and $\theta_{\text{inc}} = 50.4^\circ$ is the incidence angle. Hence, from Eq. (1), the wavelength of surface plasmon, λ_{sp} , is estimated to be 629 nm. Since the LSPR of NR-642 is very close to this surface plasmon wavelength, resonant coupling between the nanorods and the film occurs, resulting in maximum enhancement. Indeed, the evanescent field of the sensing film, spanning approximately 200 nm into the analyte, resonantly excites the plasmon oscillations of the nanorods. As a consequence, the sensitivity of biosensor gets enhanced due to (i) extension of the sensing field further into the analyte, and (ii) local field enhancement in the gap between the film and the Au-NRs, presumably leading to non-linear response of the biomolecules. To further examine this speculation, we repeated the experiments using a shorter laser wavelength (632 nm). The data in Fig. 5(b) shows that the maximum enhancement at 642 nm is no longer the case. Instead, the use of the 632 nm light source led to the coupling of NR-530 with the film.

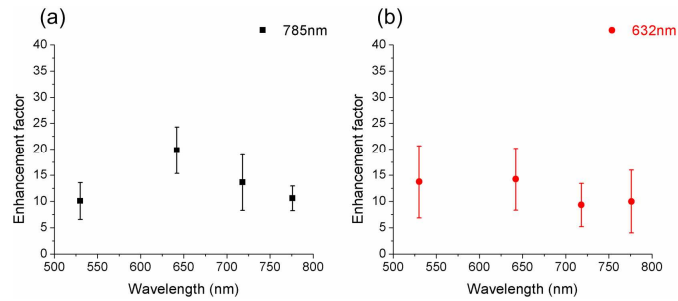


Fig. 5. Effect of LSPR of Au-NRs on the enhancement factor using laser source operating at (a) 785nm and (b) 632nm.

As an additional check-up, we used finite element analysis (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 gold nanorod with AR = 2 (Fig. 6 (b)). The field is greatly enhanced in the gap between the rod and the film, and spans beyond the outer end of the rod. These findings demonstrate that tuning the longitudinal LSPR of gold nanorods enables to achieve maximum enhancement of biosensor sensitivity owed to resonant plasmonic coupling between the nanorods and the sensing film.

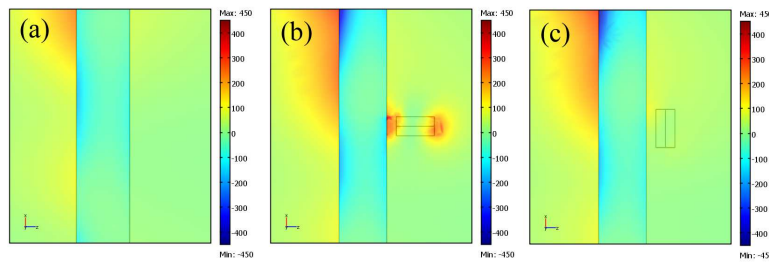


Fig. 6. FEA simulations of resonant Au-NR (radius is 10 nm, height is 40 nm, distance from the film is 10 nm) coupling to the film. The distribution in XZ-plane of time averaged electric energy density (J/m^3) for (a) the bare film, (b) the film and the Au-NR perpendicular to the film, (c) the film and the Au-NR parallel to the film. The film and the rod are shown with gray lines.

5. Conclusion

In summary, we have demonstrated greater than 20-fold enhancement of sensitivity of a phase-sensitive SPR biosensor with the use of gold nanorods as powerful amplification labels. The correlation between the enhancement factor and the longitudinal LSPR of nanorods has been observed. The nanorod-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 bio-conjugates. In the future, we envision the development of an ultra-sensitive oncological biosensor for multiple clinical real-time diagnoses using Au-NR bio-conjugates formulation as amplification labels.

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