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Label-free optical biosensor based on localized surface plasmon resonance of immobilized gold nanorods

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ABSTRACT

We describe the fabrication and characterization of a localized surface plasmon resonance (LSPR) biosensor that utilizes gold nanorods immobilized as the optical transducer which requires the intensity change at a single wavelength to be monitored as a function of receptor–analyte binding at the nanorod surface. In contrary to free gold nanorods suspended in an aqueous solution with high sensitivity to the longitudinal plasmon wavelength to the surrounding environment, the intensity of the longitudinal plasmon band based on immobilized gold nanorods is more sensitive to changes in the surrounding dielectric properties than the change in the longitudinal plasmon wavelength. Quantitative calculation gives a linear equation between the concentration (X) of the test sample and intensity of LPB (Y) as $Y = 0.0881 + 12.9502X$ and 0.1 pM anti-goat can be detected using this IgG probe in this study. This sensor chip made of immobilized gold nanorods is very stable. The immobilized gold nanorods preserved under 4 °C for 1 year yield almost the same extinction spectrum as the original nanorods. This study reveals a reliable and sensitive method to measure the intensity of longitudinal plasmon bands based on the highly stable LSPR substrate. Moreover, the performance is comparable to dynamic SPR measurements in immunoassays and can monitor the receptor–analyte reactions in real time.

1. Introduction

Nanoparticles have attracted much attention because of their size- and shape-dependent optical properties. Due to the coupling of conduction electrons to the electromagnetic field of the incident light, the optical response of noble metal nanoparticles is typically characterized by the presence of strong absorption and scattering peaks that are not present in the spectrum of the bulk metal, a phenomenon named localized surface plasmon resonance (LSPR). Potential applications of these nanoparticles exploit these unique plasmonic properties including imaging [1,2], photothermal tumor therapy [3,4], antibody/antigen detection [5–8], and DNA detection [9,10]. The wavelength at which the LSPR peak appears depends on the composition, size, and shape of the nanoparticles. In spherical gold particles, a strong absorption band around 520 nm arising from the excitation of plasmon by the incident light can be readily observed. Gold nanorods are quite different from spherical particles in the appearance of a surface plasmon band at lower energy. There are two plasmon resonance absorption bands. One is around 520 nm caused by transverse oscillation of electrons and is thus termed the transverse plasmon band (TPB). The other one lies in the visible or near infrared (NIR) region and is known as the longitudinal plasmon band (LPB). Gold nanoparticles, including spherical and rod-like nanoparticles, are of great interest to label-free sensing because their extinction spectra are highly sensitive to the dielectric constant of the surrounding medium [11–13]. The anisotropic nanorods are potentially useful as molecular probes since significant changes occur in the plasmon spectra in response to changes in the refractive index in the vicinity of the gold nanorods. El Sayed and co-workers [14–16] have demonstrated both experimentally and theoretically that the longitudinal plasmon wavelengths (LPWs) are highly sensitive to changes in the dielectric properties of the surroundings and the sensitivity increases with the aspect ratio of the nanorods. As a result, this optical biosensor based on LPW is very suitable for sensing specific target binding events.

Currently, biosensors utilizing LSPR based on gold nanorods monitor the longitudinal plasmon wavelength shift upon environmental perturbation of the particles, or alternatively the change in the plasmon absorption intensity at a fixed wavelength. Most of existing work adopts the former methodology which can be easily performed by inducing changes in the dielectric properties in the vicinity of the particles by first connecting biological receptor molecules to activated gold nanoparticles with com-

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patible chemical tethers and subsequently reacting with the fabricated molecular probes to their corresponding targets. However, when gold nanoparticles are used in the form of a liquid solution, regardless of their shape and size, the inherent artificial signals from the change in particle concentration cannot be avoided. When multiple washing steps are incorporated to remove the unbound analytes, uncertain changes in the nanoparticle concentration can occur and contribute to changes in the absorption intensity, thus making the monitoring of biospecific interactions events inaccurate. Alternatively, one effective strategy is to chemically immobilize the gold nanorods on the medium surface instead of a solution. In this way, the concentration of the nanorods can be kept constant throughout the experimental process [17–19].

In this work, a LSPR biosensor based on immobilized gold nanorods is fabricated. The process is technologically simple only requiring the intensity change at a single wavelength to be monitored as a function of receptor–analyte binding at the nanorods surface. A series of gold nanorods immobilized with different aspect ratios are investigated. The results show that these immobilized nanorods are insensitive to the aspect ratio of the gold nanorods and the behavior is different from that in the solution. The various gold nanorods almost have the same sensitivity to changes in the dielectric properties of the surroundings.

2. Materials and methods

2.1. Materials

$HAuCl_4 \cdot 3H_2O$, cetyltrimethylammonium bromide (CTAB), ascorbic acid and silver nitrate were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Goat IgG and rabbit anti-goat IgG were obtained form Biodee Biotechnology Co. Ltd. (Beijing, China). *N*-Hydroxysuccinimide (NHS) was acquired from ACROS (NJ, USA), *N*-ethyl-*N*-[(dimethylamino) propyl]carbodiimide (EDC) from Avocado Research Chemicals Ltd. (Lancashire, UK), mercaptoundecanoic acid (MUA) from Aldrich (Milwaukee, USA) and 3-mercapto-propyl-trimethoxy-silane (MPTMS) from Alfa Aesoar (UK). All of the chemicals, unless mentioned otherwise, were all of analytical reagent grade and used as received. Aqueous solutions were prepared in doubly distilled water.

2.2. Preparation of gold nanorods

The seed solution of gold nanorods was prepared according to the previous methods [20,21]. The CTAB solution (1.5 mL, 0.1 M) was mixed with 100 μL of 0.02 M $HAuCl_4$. 100 μL ice-cold 0.01 M $NaBH_4$ was added to the stirred solution leading to the formation of a brownish yellow solution. Vigorous stirring of the seed solution was carried out for 2 min. Afterwards, the seed solution was kept at room temperature (25 °C) and used at least 2 h after preparation.

To grow the gold nanorods, 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 0.08 M ascorbic acid. The ascorbic acid here worked as a mild reducing agent and changed the solution from dark yellow to colorless. Finally, 70 μL of the seed solution was added to the growth solution. The color of the solution gradually changed within 15 min. The gold nanorods fabricated in the CTAB solution were encapsulated in the micelles and thus inhibited from chemical immobilization on the glass slides. Prior to immobilization of the gold nanorods on the mercaptoterminated silane SAM on glass, excess CTAB was removed from the suspension of the gold nanorods by careful centrifugation at 16,000 rpm for 15 min. The supernatant was then removed and the nanorods were resuspended in 0.005 M CTAB solution via sonication.

2.3. Immobilization of gold nanorods on glass slides

Glass slides (2.2 cm × 1.2 cm) were cleaned in a piranha solution that causes vigorous oxidation under extreme caution at 70 °C for 30 min. After cooling to room temperature, the slides were removed, rinsed repeatedly with water, and sonicated for 10 min. Subsequently, the slides were rinsed repeatedly with ethanol and then incubated in a MPTMS 5% ethanol solution for 4 h. Afterwards, the slides were rinsed with ethanol and water sequentially and air-dried at room temperature prior to further modification. Finally, the mercaptosilane-modified glass slides were incubated in the suspension for 30 min to prepare the gold nanorod chips. AFM image were obtained on a PicoScan SPM microscope (Molecular Imaging, Phoenix, AZ) equipped for MAC Mode in which the magnetically coated probe oscillated near its resonant frequency under an alternating magnetic field.

2.4. Surface attachment chemistry

Functionalization of the gold nanorods took advantage of the well-known affinity between gold and thiol compounds [17]. The gold nanorods were easily modified by alkanethiols to form SAMs for attachment to recognition agents (antibodies in this work). The MUA monolayers were formed on the gold nanorods by immersing the glass slides immobilized with nanorods into a 5 mM ethanol solution for at least 18 h, followed by thorough rinsing with ethanol and water in order to remove unbounded MUA. The modified nanorods films were bonded to NHS by immersion in an aqueous solution of 75 mM EDC and 15 mM NHS for 30 min. In order to attach biological macromolecules on the surface, the glass slides with NHS-terminated nanorods were incubated in the goat IgG solution (0.01 g/L). After the reaction proceeded for 30 min, the glass slides were rinsed with 10 mM phosphate buffer saline (PBS) and then blocked the unreacted MUA by 1 M ethanolamine (pH 8.5) for 10 min to prohibit possible nonspecific adsorption in the subsequent analytical process. Finally, the unreactive ethanolamine was removed and the glass slides were rinsed exhaustively with water.

2.5. Experimental setup of LSPR

The digital picture of the LSPR experimental setup is depicted in Fig. 1 in which the glass slide with immobilized gold nanorods acts as the sensor chip. This setup is easy to fabricate and requires only a UV–visible spectrometer (Lambda 35, PerkinElmer, US) for detection. A specially modified cuvette was fabricated for the flow injection of the samples and the detecting glass slide was inserted into the cuvette.

Fig. 1. Digital picture of real LSPR setup.

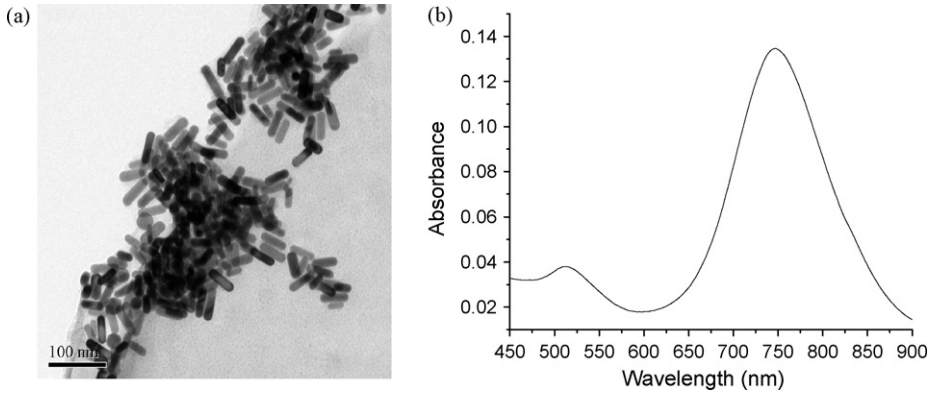

Fig. 2. (a) TEM image of gold nanorods and (b) corresponding extinction spectrum.

3. Results and discussion

3.1. Immobilization of gold nanorods on glass and the acquisition of extinction spectra

The TEM image of the nanorods in Fig. 2a illustrates size consistency. The corresponding extinction spectrum of the nanorods in Fig. 2b shows a transverse LSPR peak at 520 nm and a longitudinal LSPR peak at 747 nm. An AFM image of the gold nanorods immobilized on a mercaptosilanated glass substrate is shown in Fig. 3. The nanorods are randomly distributed on the surface and parallel to the substrate. This is as expected considering the maximum area of contact resulting from adsorption of the nanorods from the side. Although most of these nanorods immobilized on the glass are separated from each other, some aggregation of the gold nanorods can indeed be observed on the surface. It may contribute to a slightly broader peak band, but the same batch of glasses with immobilized gold nanorods has nearly the same LPW.

In fact, acquisition of the extinction spectra from the gold nanorods immobilized on the glass slide is crucial to the detection of biological targets in this research. One of the major requirements is to ensure that it has sub-monolayer coverage to reserve the longitudinal absorption band features for the optical sensing purpose. As aforementioned, the glass slide was transformed into a sensor firstly by immobilizing gold nanorods on a thiol-terminated glass slide. The glass slide with immobilized gold nanorods in the cuvette is mounted vertically on an optical bench and the transmitted light is detected by a detector, as illustrated in Fig. 1. The extinction spectrum acquired is nearly the same as that of the nanorods in the form of liquid, with the exception of a red shift of the LPW from 656 nm to 660 nm. The red shift observed from the LPW after immobilization of the gold nanorods on the glass slide may depend on the following factors: one is the effect of interparticle coupling and another is the existence of the high refractive index of the glass slide compared to water. When the gold nanorods are supported in a uniform medium, a homogeneous external field induces a dipole in the nanorods. However, when the nanorods are supported on a substrate which has a different refractive index than the ambient, the field that acts on the particle is no longer homogeneous due to the field created by the image dipole induced by the substrate. Consequently, multipoles are induced in the nanoparticles giving rise to a red shift in the plasmon resonance. This multipole effect has been predicted theoretically [22–24].

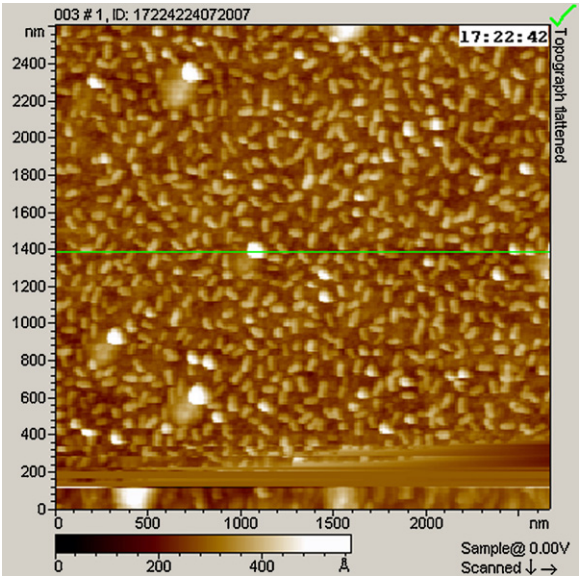

Fig. 3. AFM image of gold nanorods immobilized on a glass slide.

During the process of immobilization of gold nanorods including rod-like and spherical nanoparticles, we observed that the rod-like nanoparticles were more easily immobilized on the mercaptosilane-modified glass slides than spherical nanoparticles. As reported previously [25,26], shortened gold nanorods might be obtained by heating or adding different additives into the as-synthesized gold nanorods. In contrary to the starting nanorods with size consistency, a considerable fraction of spherical nanorods exists in the resulting shortened nanorods, and as a result, a higher intensity is observed from the transverse plasmon band in the extinction spectrum in Fig. 4a. Interestingly, the extinction spectrum obtained from the immobilized nanorods shows that the intensity ratio of the longitudinal plasmon band to transverse plasmon band increases compared to original gold nanorods, as illustrated in Fig. 4b.

3.2. Detection of biological targets based on immobilized gold nanorods

The gold nanorods work as the plasmonic transducer of receptor–analyte binding due to the peaks of LSPR spectra of nanorods being centered in the visible or near infrared region at which the background absorption and scattering of endogeneous chromophores from biological mixtures (e.g., serum and blood) and of water are minimal. Using the immobilized gold nanorods as the LSPR sensor, it was found that a red shift of about 15 nm occurred after the NHS-terminated nanorods were incubated in the goat IgG solution. This is strong evidence that the goat IgG was successfully bonded onto the surface of nanorods. Furthermore, the utility of the gold nanorods immobilized as a biomolecular sensor was investigated using a model receptor–analyte system. The opti-

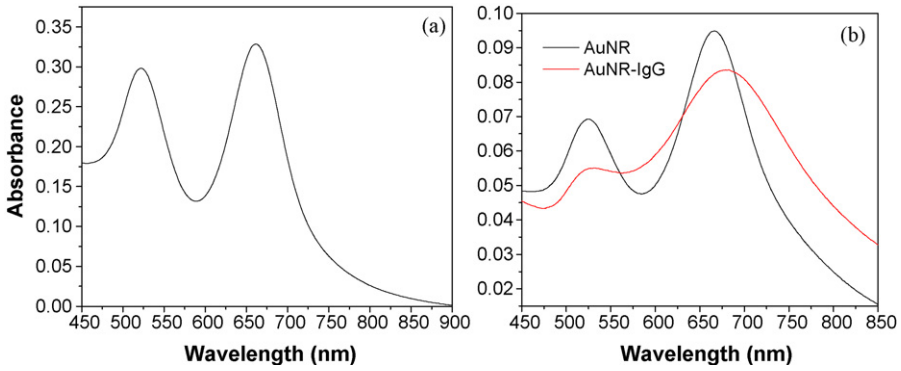

Fig. 4. (a) Extinction spectra acquired from shortened gold nanorods resulting from heating the as-synthesized gold nanorods shown in Fig. 2a and (b) immobilized shortened gold nanorods before and after the immobilization of goat IgG.

cal characteristics evaluation of the anti-IgG was carried out using the IgG probe immobilized on the LSPR-based optical biosensor. The fabricated biosensor chip was first immersed in PBS and the extinction spectrum was recorded. The sample containing anti-IgG was subsequently added and afterwards, a significant increase in the absorption intensity instead of the obvious wavelength shift was observed, along with the interaction between acceptor and ligand. With the rabbit anti-goat IgG binding to goat IgG attached onto the gold nanorods, the refractive index in the vicinity of the gold nanorods was changed and as a result, the absorption intensity increased in response to a change in the refractive index. This indicates that the rabbit anti-goat IgG reacted specifically with the goat IgG attached on the gold nanorods at lower concentrations. As reported in our previous work [27], like other polyclonal antibodies, detection of polyclonal rabbit anti-goat IgG by immunological methods is often hampered by cross-reactivity. A higher concentration of the rabbit anti-human IgG will lead to the cross-reactivity. The steady-state responses of the nanorod sensor as a function of sample concentration are shown in Fig. 5. Since the shift of the plasmon peak was only 1–2 nm in our experiments and too small for detection purposes (low signal/noise ratio), the intensity change at 679 nm was used as an indicator of the target binding events. Quantitative calculation yields a linear equation between concentration (X) of rabbit anti-goat and intensity of LPB (Y) as follows:

$$Y = 0.0881 + 12.9502X$$

The linear regression coefficient is 0.9959 and 0.1 pM rabbit anti-goat can be detected readily. Fig. 6 shows the kinetic process when the rabbit anti-goat IgG is added in the optical biosensor elucidating that the majority of the binding occurs within the first 10 min of incubation. In addition, desorption of the anti-IgG can be carried out readily by washing the sensor chip with elution reagents such as acid, base, and salt with high ion strength solution and then can be used repeatedly.

3.3. Comparison of free and immobilized gold nanorods

The LSPR sensitivity is nearly linear with the aspect ratio of free gold nanorods according to a previous report [28]. Herein, three kinds of gold nanorods with LPW of 650, 712, and 847 nm were chosen to investigate the effects of the aspect ratio of the immobilized gold nanorods. These immobilized gold nanorods exhibit changes in the absorption intensity accompanied by the interactions between ligands and acceptors, and no significant wavelength shift occurs in the LSPR peaks. Interestingly, the various immobilized nanorods with different aspect ratios show similar responses to changes in the sample concentration. When the same type of immobilized nanorods was immersed in various solvents, distinctive changes in the longitudinal plasmon wavelength could be observed as shown in Fig. 7. It is obvious that different solvents lead to different responses in both the LPW and intensity of LPB. Although apparent changes in the intensity may occur in various solvents, the ratio of the intensity of the longitudinal plasmon band to that of transverse plasmon band is almost constant. In comparison, the effects of a series of concentrations of glucose on the free gold nanorods in the form of solution are shown in Fig. 8, which shows that the wavelength shift varies with the increase in the concentration. Gradually diminishing intensities in the extinction spectra arise from decreasing nanorod concentrations due to the addition of the glucose solution leading to reduced concentrations

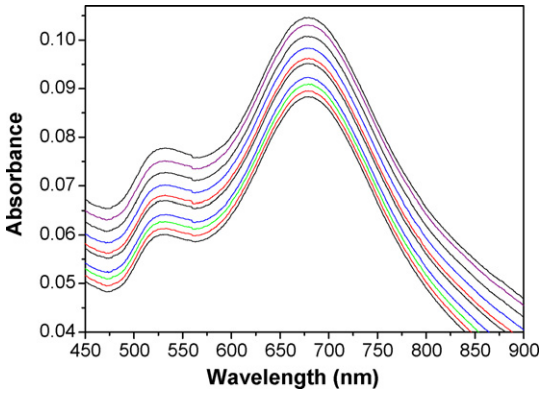

Fig. 5. Extinction spectra acquired by the addition of increasing concentration rabbit anti-goat IgG (from bottom to top).

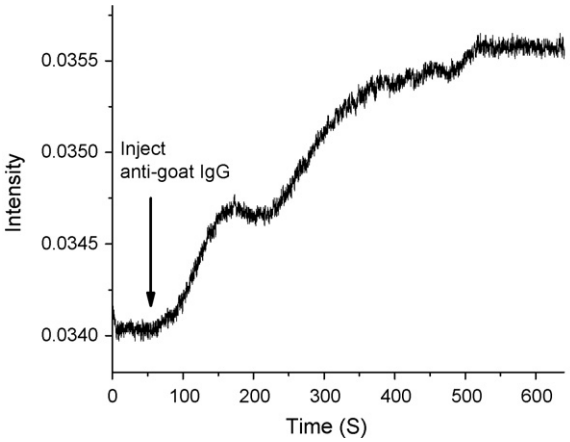

Fig. 6. LSPR kinetic curve for rabbit anti-goat IgG interacting with immobilized goat IgG at a wavelength 679 nm.

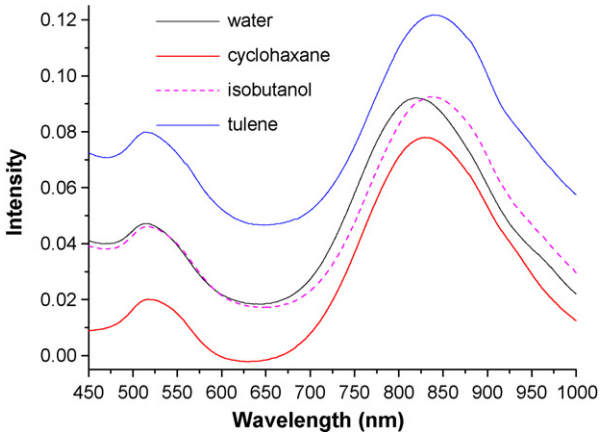

Fig. 7. Dependence of LSPR spectra of gold nanorods immobilized on various solvents.

of the gold nanorods dispersed in the solution. In practice, the wavelength of the longitudinal band is more sensitive than the intensity change in the longitudinal band with the variation in the dielectric environment surrounding the gold nanorods if the addition of sample volume is very small, as described in previous reports [8,29]. Therefore, contrary to immobilized gold nanorods, the wavelength change in the longitudinal band is preferred in order to monitor the binding events, instead of monitoring the change in the absorption intensity at a fixed wavelength as in the case of functionalized gold nanorods in the liquid form.

At present, the two modes based on gold nanorods exhibit unique characteristics when monitoring the ligand–acceptor binding events. The free gold nanorods exhibit a high LPW sensitivity to the dielectric environment and basically prove their practicality as optical sensors. However, this approach is hampered by the uncertain changes in the nanoparticle concentration during the process of multiple washing steps to remove unbound analytes thus making it difficult to monitor biospecific interactions events. On the other hand, observation of the minimal wavelength shift of the plasmon bands is found whereas the intensity is determined by changes in the dielectric properties (i.e., refractive indexes) in the immediate vicinity of the nanoparticles. However, the concentration of immobilized gold nanorods is constant throughout the experiments and thus the inherent problem resulting from free gold nanorods in a solution can be avoided. In addition, immobilized gold nanorods

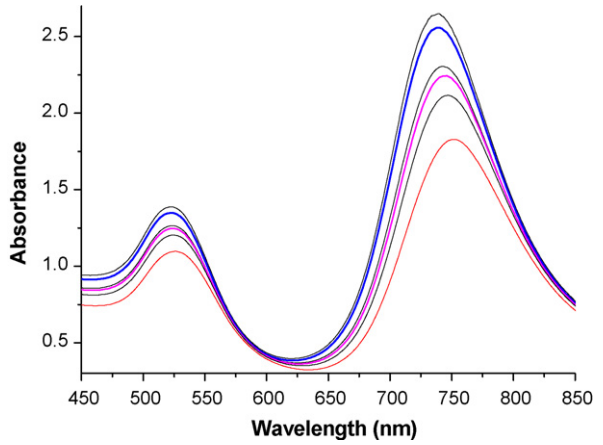

Fig. 8. Effects of a series of concentrations of glucose on free gold nanorods. The concentration of glucose increases gradually from top to bottom, and inversely, the concentration of gold nanorods decreases gradually with addition of the glucose solution.

have a stability advantage over free gold nanorods. In our experiments, the immobilized gold nanorods preserved under 4 °C for 1 year yield nearly the same extinction spectra as the original nanorods. On the contrary, free gold nanorods are unstable and gradually blue shift even being stored at 4 °C [30]. Therefore, immobilized gold nanorods are expected to have broader applications due to their stability and a variety of solvents including many organic solvents can be used. On the contrary, free nanorods are limited to a few solvents because many factors including solvent and surfactant may compromise the stability of the gold nanorods suspended in water.

Our investigation on the optical biosensor based on the free gold nanorods shows that the sensitivity and LPW shift are almost linearly related to the aspect ratio of the gold nanorods. The larger aspect ratio of the gold nanorods, the more sensitive is the response to a change in the refractive index in the vicinity of gold nanorods. Nevertheless, the optical biosensor based on immobilized gold nanorods shows that these immobilized nanorods are insensitive to the aspect ratio of gold nanorods of different forms when in a solution. The various gold nanorods have almost the same sensitivity to changes in the dielectric properties of the surroundings.

4. Conclusion

The fabrication and characteristics of an LSPR biosensor utilizing immobilized gold nanorods used as the optical transducer are described. The changes in the intensity of the longitudinal plasmon band of the nanorods are also studied as the optical signature of receptor–analyte binding. In contrast to free gold nanorod LPW that is highly sensitive to the dielectric environment, a sensitive method to measure the intensity of longitudinal plasmon band has been developed based on the highly stable LSPR substrate. More importantly, the performance is comparable to dynamic SPR measurements in immunoassays and it can monitor the reactions of receptor–analyte in real time. Such an LSPR sensor has significant and broad applications in proteomics, system biology, and *in vitro* diagnostics.

Acknowledgments

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