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Chemisorption Assembly of Au Nanorods on Mercaptosilanized Glass Substrate for Label-free Nanoplasmon Biochip

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Abstract

The fabrication of a localized surface plasmon resonance nanosensor in a chip based format that utilizes Au nanorods (GNRs) as the optical transducer were systematically studied. (3-Mercaptopropyl)trimethoxysilane (MPTMS) modified glass substrate offers GNR deposition with maximal sensitivity to local refractive index changes, which subsequently results in better optical recognition of receptor-analyte binding. Kinetics governing the mass transport and chemisorption of nanorods from bulk to solid surface can be dynamically controlled in a predictable fashion. We demonstrate that slight aggregation induced by a low ionic strength (5 mM NaCl) can facilitate the nanorod assembly to result in a dense, well-distributed surface monolayer. In high ionic media (e.g. 40-80 mM), anions present electrostatically bind with the positively charged cetyltrimethylammonium bromide (CTAB) surrounding nanorod surfaces, thereby leading to instability with heavy aggregation in solution. However, once chemically bound on silanized substrates, the nanorods exhibit excellent stability in physiological buffer where high amount of ionic species are present. The fundamental study is followed by demonstration of a practical application of the fabricated biochip in label-free detection based on GNR wavelength shift of the longitudinal plasmon maxima as the optical signature of human IgG model detection.

Keywords

Au nanorod; Self assembly; Biosensing; Surface plasmon resonance; Nanochip

1. Introduction

The optical transduction by gold nanorod (GNR) is based upon the phenomenon of localized surface plasmon resonance (LSPR). Its sensitivity to changes in the local refractive index exhibits a shift of plasmon band maxima in extinction in proportional to target binding on the nanorod surface [1-4]. This unique size and shape dependent interfacial phenomenon provides the basis of their biosensing utility in a label free approach. Numerous studies have reported biological detection with picomolar sensitivity for buffered solution [5-11]. We recently demonstrated a magnetic nanoparticle enhanced GNR sensor for ultra-sensitive protein analysis in physiological samples [12]. However, solution-based biosensing has intrinsic disadvantages. When the gold nanoparticles are used in the form of a liquid

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solution, the inherent artificial signals from the changes in nanoparticle concentrations due to multiple wash cannot be avoided. This induces the extinction intensity changes, thereby making it inaccurate to monitor specific biological interaction events. There is no doubt that the ability to organize the nanoparticles in a controllable way onto substrates in a chip format will significantly strengthen the utility of this label-free biosensing modality as a powerful bioanalytical device.

Glass will be an ideal substrate because it is optically transparent, inexpensive, and available of well-studied surface modification methodology. To attain GNR assembly with highly ordered morphology and density on substrates, solvent evaporation has been employed [13]. However, this method is not straightforward as a consistent evaporation rate must be strictly controlled throughout the preparation. In addition, undesirable organic crystallites and nanoparticle aggregation leading to decreased intrinsic SPR effect are issues related to self-assembly based on solvent evaporation. Recent studies showed that immersion of chemically modified glass chip in carefully designed GNR solutions can provide a much more convenient coating of large substrate surface area [9, 14]. Scheme 1 summarizes the process of Au nanorod immobilization from bulk solution to mercaptosilane-modified glass substrate upon immersion. Nanorods are usually stabilized in the colloidal solution due to the repulsion between positively charged particles capped by cetyltrimethylammonium bromide (CTAB) bilayer. In favor of substrate deposition, the attractive Au-S chemical binding must be able to overcome the repulsive inter-particle force for the GNRs to move towards interface. Studies have demonstrated various ionic media to reduce the repulsive forces to cause nanoparticle instability [15-17]. To obtain optimal nanochip sensitivity, we investigated how the free CTAB surfactant concentration and ionic strength in the GNR solution are dynamically involved to facilitate maximal substrate assembly. Furthermore, there was limited discussion on optimal silanization of substrates to retain a high LSPR sensitivity of the assembled nanorods to local refractive index changes. The stability of the GNRs chemically bound on silanized glass substrate subjected to physiological buffers has not been systematically studied. Such effects are fundamental to their robust applications in biosensing, which are commonly performed in buffered solutions that contain high concentrations of ionic species.

In this work, we systematically characterize GNR assembly kinetics on the surface of thiol-activated substrates for biosensing application using UV-vis spectroscopy, scanning electron microscopy, and fluorescence microscopy. As a demonstration of the utility of the Au nanorod chip for label-free biomolecular recognition, a model system using human IgG was used to evaluate the sensing performance in terms of sensitivity and dynamic range.

2. Materials and Methods

2.1 Materials

Hydrogen tetrachloroaurate trihydrate (HAuCl_4 ; 99%), sodium borohydride (NaBH_4 ; 99%), cetyltrimethylammoniumbromide (CTAB), silver nitrate (AgNO_3 ; 99%), (3-Mercaptopropyl)trimethoxysilane (MPTMS), (3-Mercaptopropyl)triethoxysilane (MPTES), (3-Aminopropyl)triethoxysilane (APTES), 11-mercaptopundecanoic acid (MUDA), Poly(ethylene glycol) methyl ether thiol (SH-PEG, Mn ~5,000), poly (sodium 4-styrenesulfonate) (PSS, Mw ~70,000), poly (allylamine hydrochloride) (PAH, Mw ~58,000), human serum IgG, and goat anti-human IgG were obtained from Sigma-Aldrich (St. Louis, MO). Glass slides ($7 \times 50 \times 0.7$ mm) were from Delta Technologies (Loveland, CO). All the experiments were carried out at room temperature unless otherwise noted.

2.2 Immobilization of GNRs on glass substrates

GNRs were prepared by the seed-mediated growth method with modifications [18], followed by purification using centrifugation. Briefly, the synthesized nanorod suspension (200 mL) was distributed evenly to six centrifuge tubes and spun at 8,500 rpm for 30 min. Supernatant was carefully discarded without disturbing the nanorods pellet at the bottom. 1.5 mL MilliQ water was then added to each tube to resuspend the solid pellet and centrifuged again at 13,000 rpm for 10 min. The resulting sediments were combined and dispersed in a total of 6 mL, 0.5 mM CTAB solution to achieve a concentrated GNR sample (15–20 nM).

To effectively immobilize GNRs onto glass substrates, 200 μ L of the as prepared GNR solution was mixed with 1 mL of MilliQ water and further centrifuged at 13,000 rpm for 10 min. The pellet was redispersed in 2 mL water containing NaCl at various concentrations. Glass slides were cleaned in a freshly prepared piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$; 3:1 v/v) for 40 min to maximize hydroxyl groups. The substrates were then immersed in three different types of thiosilane solution: MPTMS, MPTES or APMES (10% v/v in ethanol) for overnight, resulting in a thiol- or amine- terminated silane monolayer on the glass surface. The modified glass slides were dipped into GNR suspension to construct a nanorod chip. After the immobilization, the substrates were washed three times with MilliQ water, and then air dried with nitrogen. This step would wash off weakly bound nanoparticles to ensure a robust and reliable density of the GNRs on the substrate. To evaluate the sensitivity of three different silanization of GNR chips to the change in refractive indexes in the surrounding medium, sucrose solution (0.2, 0.4, 0.8, 1, and 1.2 g mL⁻¹) with increasing RI value (1.355, 1.374, 1.407, 1.420, and 1.428 respectively) were prepared, GNRs chips were immersed in these sugar solution and their UV-vis spectra were measured [19].

2.3 Functionalization of GNRs chip and Label-free receptor-analyte sensing study

To construct a specific nanosensor, biological receptors such as antibody need to be efficiently immobilized on the GNR surfaces. CTAB bilayer on the nanorod surface is known to be problematic for biofunctionalization. As such, PSS/PAH deposition was first performed on the GNRs chip by alternating the surface charges. Since CTAB is positively charged, a negative polymer such as PSS can be coated on the CTAB-GNR surface through electrostatic interaction, followed by positive PAH assembly. Briefly, the GNRs chip was incubated in a solution of 2 mg mL⁻¹ PSS for half an hour, followed by water rinse and immersion in 2 mg mL⁻¹ PAH solution to reform a positively charged coating. Afterwards, the PAH coated nanochip was incubated in anti-human IgG solution (0.1 mg mL⁻¹) overnight to immobilize the negatively charged antibody onto the chip. Upon biofunctionalization, the GNR biosensor was chemically treated with a blocking solution containing 1% SH-PEG in PBS buffer for 2 hours to minimize non-specific binding.

To perform a label-free assay, diluted human IgG solution concentration up to 1,000 nM were applied onto the biosensing chip surface and incubated for 30 mins. At the end of the incubation, the UV-vis absorption spectra were taken to observe red shift of plasmon band maxima in proportional to target binding on the nanorod surface. Since the transverse peak is much less sensitive to local refractive index changes, longitudinal wavelength shift was focused for the label-free LSPR biosensing.

2.4 Characterization

UV-vis absorption spectra were measured by Beckman-Coulter UV-NIR spectrophotometer (Fullerton, CA). Absorbance was obtained for wavelength ranging from 400 to 1000 nm. Electron microscopy images of the fabricated GNRs were taken using Hitachi S5500 scanning transmission electron microscope (STEM). The SEM samples were prepared by

placing 2 μL of diluted GNRs solution on a 3 mm 400-mesh Formvar coated copper grids (Ted Pella Inc; Pedding, CA) and air dried at room temperature. The SEM images of substrate assembled GNRs were obtained from indium tin oxide (ITO) coated glass slide.

3. Results and Discussion

The optical properties of Au nanorod arise from localized surface plasmon resonance caused by collective oscillations of the electrons surrounding nanorods [20-21]. Fig. 1a shows a typical UV-vis absorption spectrum of monodispersed GNRs, exhibiting a transverse plasmon resonance peak at ca. 515 nm and a longitudinal resonance peak at ca. 765 nm (panel A). TEM image (inset) shows the rod-shaped nanostructure with 36 ± 6 nm in length and 11 ± 2 nm in width, resulting in an average aspect ratio of 3.2.

3.1 Silanization of glass surface for optimal GNR assembly

Surface modification of glass surface with thiol-terminals is critically important for self-assembly of Au nanostructures. To effectively maximize surface coverage of GNR assembly, three different silane chemicals (MPTMS, MPTES, and APTES) were compared. Fig. S1 illustrates the configuration of assembled GNRs on glass. The silane groups are hydrolyzed to liberate silanols, which condense with the surface residues to form siloxane linkages [22]. Afterwards, the thiol ($-\text{SH}$) groups in MPTMS and MPTES conjugate with rods via Au-S covalent bonding, while the amine ($-\text{NH}_3^+$) groups of APTES capture nanorods via weaker Au-N bonds [23-24].

The UV-vis absorption spectra clearly show that MPTMS-modified glass substrate achieved the most efficient GNR activity (Fig. 2a). Intensity of absorbance, in general, corresponds to the density of deposited nanorods. MPTMS substrate exhibits not only a higher absorbance maximum, but also retains peak position and shape for both transverse and longitudinal plasmons. The effectiveness of MPTMS was also confirmed by its high sensitivity to the refractive index (RI) change in the surrounding medium. Fig. 2b summarizes the longitudinal surface plasmon spectral shift when nanorod chips were probed by each of the following sugar solutions (0.2, 0.4, 0.8, 1, and 1.2 g mL⁻¹) with increasing RI value (1.355, 1.374, 1.407, 1.420, and 1.428 respectively). Their sensitivity, defined as the relative change in longitudinal peak wavelength per refractive index unit (RIU) was calculated by linearly fitting the red shift as a function of RI . The chip fabricated using MPTES modified glass exhibited a wavelength sensitivity of 175 ($R^2=0.95$) nm RIU⁻¹. As a comparison, the sensitivity from MPTMS substrates was increased by 60% to 284 ($R^2=0.98$) nm RIU⁻¹. The enhancement of the sensitivity can be attributed to the nanorod assembly pattern on the glass surfaces. As shown in the SEM images in Fig. S2, MPTMS modified substrate resulted in an even and well distributed GNR monolayer while MPTES showed a patched nanorod assembly with aggregation on the surface. In an actual biological sensing, analyte binding at the surface of nanorods typically perturbs the local RI, thereby providing optical transduction of interfacial binding events. As such, MPTMS is more appropriate for substrate silanization to develop a functional GNR chip. For this reason, MPTMS was used for substrate modification in all subsequent measurements.

3.2 Influence of CTAB on the immobilization of GNRs onto substrate

Contrary to the initial thought that GNRs would readily attach to thiol-modified substrates, almost zero deposition was observed (Fig. 1b, dotted line). We attributed the failure to a high amount of excess CTAB in the solution. To experimentally confirm, additional centrifuge at 13,000 rpm for 10 min was carried out to achieve a more purified GNR solution with slightly excess CTAB concentration. UV-vis analysis of the substrate after immersion in the resulting solution showed distinct two absorptions, resembling the shape

and location of GNR-specific plasmon bands (Fig. 1b, solid line). A major concern was the extremely low surface density as the optical density (O.D.) of the longitudinal SPR peak is only 0.07. This represents a dramatic diminish from that in solution (~ 1.7). Excess CTAB surfactant can adversely affect substrate assembly due to electrostatic shielding effect to stabilize the colloidal solution so that attraction of nanoparticles to the surface becomes secondary [17, 25-27]. Therefore, we had logically attempted to further purify the GNRs to reduce CTAB concentration in favor of the nanorod-substrate interaction. However, nanorod structure is known to be susceptible to an exchange of surfactant bound to the metallic surface with uncoordinated surfactant in solution. When the purity of GNR increases by removing too much free CTAB micelle, the on-off equilibrium of the CTAB shifts to release the surfactant from the rod surface, leading to the aggregation of the unprotected nanoparticles [27-28]. Our result corroborated to find that further centrifuge after three times caused an insoluble black pellet, suggesting irreversible aggregation and deformation.

3.3 Effect of ionic strength on GNR and its immobilization on glass

It was demonstrated that density of GNRs assembled on negatively-charged APTES-modified substrate through electrostatic attraction can be predictably adjusted by NaCl concentration over a wide range [17]. As such, we performed a systematic study on covalent chemisorption of GNRs at surface of MPTMS-treated glass in various ionic strength media to seek a maximal density (i.e. higher absorbance). The ionic strength was controllable by addition of varying salt concentrations to GNRs in solution with minimally excess surfactant. At each concentration, the UV-vis spectra were repeatedly taken at certain time points following the salt addition (time 0). Absorption spectroscopy was primarily used as it is proven to be a reliable tool to determine nanoparticle aggregation state by the peak position and morphology of surface plasmon bands [7, 29]. At 5 mM NaCl, only reduction in absorbance at consistent transverse and longitudinal peak wavelengths were observed during the course of the incubation, indicating that slight aggregation progressively occurred without deformation. After 7 hours, the nanorod solution was still well dispersed with distinct two plasmon peaks retained (Fig. 3a). When the salt concentration was increased to 10 mM, the initial drop of the longitudinal plasmon extinction after 30 min of incubation became larger, accompanied by peak broadening as a result of more aggregations without specific arrangement (Fig. 3b). Eventually after 10 hours, bulk material formation appeared to precipitate. With a further increase in the salt concentration up to 40 mM, the absorption spectra were significantly broadened with diminished plasmon bands. Severe nanoparticle aggregation was observed immediately after the salt additions while the solution turning from reddish purple to bluish gray color (Fig. 3c-d).

In a colloidal solution which is highly stabilized by repulsive forces between charged nanorods, manipulation of ionic strength is an independent way to alter the repulsion distance. Addition of NaCl was reported to induce instability of nanorods, as layer of negatively charged Cl^- ions surrounding the positive rod surface caused a reduction in the Debye length between particles [27, 30]. When nanorod colloids become less stable, their surface energy tends to decrease and therefore particles in proximity readily approach. As nanorods approach each other, attractive Van der Waals force dominates over the repulsive force to result in aggregation. The overall effect of the salt concentration on the stability and aggregation of CTAB-capped nanorods is illustrated in Fig. 3e. Low to moderate ionic media usually induce a quasi-stable situation with slight aggregation of nanoparticles, while increasing salt concentrations such as 20 and 40 mM cause severe aggregation as indicated by distorted extinction spectra.

Slight aggregation in the presence of low salt was postulated to favor surface assembly on the glass since the attractive force imparted by the nanorod-substrate chemical binding easily overwhelms the repulsion between nanorods. It was later found that adjustment of

ionic strength within a specific range of salt concentration can significantly increase the density of surface GNR deposition. As shown in Fig. 4a, while keeping all other conditions constant, introduction of salt at 5 mM significantly facilitated nanorod assembly, leading to a 6-fold increase in the surface density as compared to control study without salt (based on longitudinal surface plasmon spectral absorbance). The absorption spectrum of the GNRs chemisorbed on glass surface resembles the morphology of characteristic plasmon bands in solution with little aggregation. A red shift in the longitudinal plasmon peak wavelength from ca. 765 nm in solution to ca. 785 nm on substrate was observed. It is known that an increase in the local dielectric function results in a red shift of nanoparticle plasmon peak [31]. Our observation is consistent with the change in the dielectric constant of the surrounding medium from water ($n = 1.33$) to glass ($n = 1.46$). This data also indicates the longitudinal surface plasmon sensitivity to the refractive index change in close proximity, thereby enabling a label-free detection of biological binding events which will be discussed later.

Due to the unique anisotropic shape, Au nanorods possess two separate plasmon absorption bands, known as transverse and longitudinal plasmon bands [32]. The ratio of the transverse to longitudinal (T/L) plasmon bands absorbance intensity is generally a good indicator of nanorod percentage in material. A high T/L ratio implies rod aggregation to form bulk cluster. GNR deposition by immersion of the silanized glass slide into rod solution without salt showed a characterized spectrum with T/R ratio of 0.32. SEM image demonstrated a randomly distributed monolayer of GNRs (Fig. 4c). It is clear that the GNR coverage on the glass surface was scattered, which is consistent with other report in the literature [9]. With the addition of a low NaCl concentration at 5 mM, although the ratio of T/L was slightly increased to 0.48 due to some side-by-side and end-to-end nanorod coupling, the resulting deposition showed a much denser yet generally well distributed GNR monolayer on the substrate (Fig. 4d). Similar to the phenomena in solution, increasing amount of salt induced more aggregation in the GNR deposition onto glass substrates. At 10 mM NaCl, the van der Waals interaction between attracting nanorods created a quasi-stable colloidal system. As a result, more particles aggregated and the ratio of T/L was elevated to 0.78 as consistently shown in the spectrum. SEM image confirmed heavy agglomeration of multilayer GNRs deposited on the substrate (Fig. 4e). With 20 mM NaCl, the ratio was almost doubled to 0.92 as compared to that from 5 mM NaCl. Because severe aggregation in the nanorod solution caused precipitation by 40 mM NaCl, there was almost zero observable GNR deposition on the substrate as demonstrated in the absorption spectrum (Fig. 4a). Both plasmon bands were diminished without direct correlation with the surface density. Further elucidation of the chemisorption process of GNR assembly on substrates by fully dipping MPTMS-modified glass into nanorod solution with 5 mM NaCl for 8 hours found that the GNR assembly is diffusion limited. Since the absorbance corresponds to the surface density, an increase in the optical density was observed within the first hour, followed by a slowly progressive deposition (Fig. S3).

3.4 Stability of GNR assembly on substrate in physiological buffer

Knecht and coworkers found that electrostatic aggregation is of concern for colloidal gold in physiological Tris buffer [27]. Since most biological applications will be exposed to a dynamic environment, it is essential to evaluate the stability of the GNRs assembled on substrates subjected to high ionic strength buffered solutions such as PBS buffer. Here, we investigated the GNR retention by comparing the absorption spectra of nanorod chips in water and subsequently in 10 mM PBS buffer containing 138 mM NaCl for 40 min (Fig. 5). Although ionic strength change can cause GNR aggregation slightly in solution at low salt concentration (5-10 mM) and heavily at high concentration (40 mM; Fig. 3), nanorods were much more stabilized on substrate once covalently bound via Au-S chemisorption. Upon

exposure to a much higher NaCl amount beyond 40 mM, it is noteworthy that no significant peak shift and broadening caused by possible nanoparticle aggregation occurred to ensure that any subsequent detectable longitudinal surface plasmon peak shifts are indeed solely caused by specific biomolecular binding. Drop in the absorbance of longitudinal peak albeit small seems to be the only parameter adversely affected by PBS buffer. The inset of Fig. 5 shows the comparison of longitudinal plasmon peak absorbance in water and PBS buffer over time. The intensity was lowered by ~ 9% in saline after switched from water immersion. This may be due to desorption of weakly bound nanorods from the substrate surface in high salt condition. Subsequently, a minimal absorbance dip was present during incubation in PBS buffer, implying a continual detachment of GNRs through weak electrostatic attraction. After 20 min, the nanorod chip was stabilized with little change in absorbance maxima and peak location. Presumably only nanoparticles that were tightly bound by strong covalent binding remained on the thiol-modified substrate. The results are noteworthy as in an electrostatic assembly of nanorods on oppositely charged glass substrate, ~ 30% of the weakly adsorbed particles were washed off by rinse[17]. It was also shown that nanorod assembly by solvent evaporation method cannot withstand dynamic fluid environments which induce those particles deposited on surface to aggregate. Clearly, chemisorption of GNRs on substrate is much stronger and is suitable for further surface modification with biological receptors and biosensing applications.

3.5 Evaluation of GNR biochip sensing performance

The immunosensor was developed by incubating the positively charged PSS/PAH coated GNR chip in the solution of anti-human IgG to immobilize via electrostatic interaction. The sensor was then incubated with human IgG sample at 167 nM and the absorption spectra were collected every 5 min to monitor real-time binding kinetics. Upon binding, there was a red shift in the longitudinal surface plasmon band maxima as human IgG molecules were transported by diffusion from bulk solution to react with antibody on the chip surface. Since the functionalized GNRs were first treated with SH-PEG molecules to block non-specific binding, the resulting wavelength shift was truly caused by the specific binding. Fig. 6a shows the longitudinal surface plasmon wavelength shift as a function of incubation time. The shift rose quickly during the first 30 min and then became saturated with nearly zero observable longitudinal surface plasmon wavelength shifts. This data indicates that the majority of the binding occurs within 30 min which is sufficient for this model detection.

The longitudinal surface plasmon peak shift in response to the human IgG concentration was also studied to investigate the dynamic sensing range. To minimize batch-to-batch variation, each nanorod biochip was probed with a series of human IgG concentration up to 1,000 nM for 30 min. After washing off weakly adsorbed analytes from substrates, absorption spectra were taken to examine the spectral peak wavelength. At least three replications were measured to calculate the mean $\pm$ standard deviation for each concentration. Fig. 6b shows the steady state response of the nanorod chip as a function of the human IgG concentration. A constant increase in the red-shift of plasmon peak showed a typical longitudinal surface plasmon sensor response to local refractive index change caused by increasing amount of human IgG binding. The sensitivity of the LSPR nanochip was evaluated by fitting the wavelength shift vs. concentration of human IgG (inset). The nanosensor shows a linear response to the analyte amount in the range from 33.4 to 233.4 nM with a high sensitivity of 60.7 pM ($R^2 = 0.99$). The rate of the shift in resonance wavelength decreased with increase in the concentration > 250 nM, probably caused by saturation or steric hindrance of the binding sites on the sensor chip.

It should be noted that when nanorods are randomly assembled on the substrate, they tend to come close to each other to form densely aligned, parallel assembly. This random deposition may result in decreased intrinsic SPR effect [33]. A self-assembly with controlled

nanopattern on substrate without aggregation is believed to dramatically extend the use of the GNRs in the practical applications utilizing the SPR effect. Although silver nanoparticles show sharper and more intense resonance peaks, they are more difficult to handle than Au because of oxidation. Moreover, the longitudinal surface plasmon of Au nanorod is tunable from visible to NIR region depending on the overall size. As longer plasmon wavelength is more sensitive to local refractive index change, choosing a nanorod with higher aspect ratio could further improve the LSPR detection limit. Our efforts are focused on these aspects of sensor chip design.

4. Conclusions

We have demonstrated that label-free optical transduction based on surface plasmon resonance shift of Au nanorod can be utilized in a chip-based format by chemisorption assembly on thiol-silanized glass substrates. In order to develop a stable GNR assembly for ultra-sensitive assay, glass substrates are preferred to be silanized with MPTMS. We found the ionic strength are determining factors in the self-assembly of GNRs on thiol-modified substrates. Introduction of salt at low concentration (e.g. 5 mM) to induce slight aggregation in nanorod solution by reducing inter-particle repulsion is desirable to facilitate chemisorption of GNR monolayer on solid surface. Once deposited, the chemically-bound monolayer of Au nanorods exhibit excellent structural stability without aggregation when exposed to physiological buffer with high ionic strength, providing a solid foundation for further biological applications. Herein, the utility of the Au nanorod biochip was demonstrated by label-free detection of human IgG with a high sensitivity. The dynamic results reported for glass substrate in this model study should most likely be applicable to any solid surfaces where nanorods are assembled, which will open up a vast variety of chip-based applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Transfer the label-free gold nanorod sensor to substrate in a chip based format.
- Systematic study of GNR self assembly via chemisorption onto silanized glass substrate
- Dynamic effects of ionic strength on maximal chip density of nanorod monolayer.
- Chemisorption of nanorods on MPTMS-modified glass substrate results in high sensitivity.

Scheme 1.

Schematic illustrating the deposition of CTAB-capped Au nanorods from bulk solution to thiol-modified glass surface.

Figure 1.

(A) UV-vis absorption spectrum of chemically synthesized gold nanorods showing a transverse surface plasmon resonance at 515 nm and longitudinal resonance at 765 nm. Inset: TEM image of monodispersed GNRs in solution. (B) Effect of CTAB concentration on the GNR deposition onto glass substrate. Absorption spectra of nanochips fabricated from GNR solution with low (red) and high (dotted) CTAB concentrations.

Figure 2. (A) UV-vis absorption spectra of GNRs immobilization on MPTMS, MP TES and APTES modified glass surface. (B) MPTMS modified Au-nanochip shows a higher surface plasmon sensitivity in terms of longitudinal spectral shift vs. refractive index change in the surrounding medium.

Figure 3.

Effect of ionic strength controllable by NaCl concentration on stability of GNR colloidal solution. UV-vis absorption spectra of Au nanorods in the presence of salt concentration at (a) 5 mM and (b) 10 mM and (c) 20 mM and (d) 40 mM. (e) Schematic summary of GNRs aggregation under influence of various salt concentrations.

Figure 4.

Effect of the salt concentration on the surface density and pattern of GNR self-assembly on glass substrate. (A) UV-vis absorption spectra of nanochips fabricated from GNR solution with increasing amount of NaCl. (B) Comparisons of absorbance at peak position of transverse SPR and longitudinal SPR (T/L) for GNR chips at different salt concentrations. (C) - (E) SEM images of surface coverage of GNR assembly on glass substrate fabricated with 0, 5, and 10 mM NaCl.

Figure 5. Robustness of GNR assembly by chemisorption on silanized glass substrates subjected to PBS buffer (pH: 7.4) containing high ionic species.

Figure 6.

(A) Binding kinetics between human IgG (167 nM) and anti-human IgG immobilized on the nanorod chip surfaces as a function of longitudinal surface plasmon wavelength shift vs. incubation time. (B) Dynamic sensing range of the nanorod biochip based on shift in the longitudinal surface plasmon peak wavelength. Each data point was shown as the mean $\pm$ standard deviation of at least three replicates. Inset: calibration plot showing a linear relationship of the spectral shift as a function of human IgG concentration up to 233 nM.