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Gold nanorod biochip functionalization by antibody thiolation

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Abstract

Conjugation of biomolecules on gold nanorod (GNR) surfaces is the basis for successful applications in biosensing, imaging, and drug delivery. Current functionalization methods are often problematic, involving multi-step nanoparticle modification to replace surfactant bilayer, delicate nanoparticle protection during surfactant exchange, and material loss due to inevitable aggregation. Instead of intensive surface modification of GNRs, we describe herein a facile method to functionalize gold nanorod surfaces via covalent Au–S bonds by thiolating receptors. The resulting GNR-bioconjugates showed superior dispersion and stability in buffer for months without morphology change and aggregation. ELISA tests confirmed the high biofunctionality of the thiolated anti-IgG moieties immobilized on the GNR surfaces. Furthermore, this simple method facilitated a straightforward functionalization of GNR assembly on glass substrate to construct a specific biochip, which can detect human IgG targets in a label-free fashion with high sensitivity and specificity. Compared to electropolymeric coating to functionalize the GNR, our method exhibited a five-fold enhancement in the spectral sensitivity to refractive index change caused by the target binding. This universal GNR bioconjugation method can be extended to bind different proteins and antibodies for development of biosensors or drug delivery.

Keywords

Gold nanorod biofunctionalization; Antibody thiolation; Nano biochip; Surface plasmon resonance; Biosensing

1. Introduction

Gold nanorod (GNR) exhibits unique optical properties that make it attractive for photothermal therapy [1,2], biosensing [3,4], molecular imaging [5] and controlled drug delivery [6,7]. These applications require the GNRs to be functionalized with different biochemical groups. However, the most common synthesis, for example, seed-mediated growth method results in a tightly packed bilayer of cetyltrimethylammoniumbromide (CTAB) on the nanorod surface. This essential capping agent presents a challenge for

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surface modification to further functionalize the nanorods. Numerous efforts were reported to optimize GNR biofunctionalization strategies [4,8–12]. Most of these works are focused on the modification of the GNR surfaces to facilitate biofunctionalization. For example, various research has demonstrated that the use of alkanethiol molecules such as mercaptopropionic acid, mercaptohexanoic acid, and thiolated poly (ethylene glycol) (SH-PEG) chemisorbed on the GNR surface can displace CTAB [4,13,14]. These molecules provide anchor points for the further immobilization of biological molecules. The most common protocol is the linkage of amino-groups on the biological molecules with carboxyl groups at the free ends of stabilized molecules by using EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-HCl) [15,16]. Though such protocols are relatively well established, the process of GNR bioconjugation is not trivial and characterization of synthesized conjugates is necessary, especially due to the often occurring aggregation during the conjugation. This is due to the fact that most biological molecules such as antibodies or proteins have both primary amine and carboxyl groups, which can act as cross-linking agents between gold nanoparticles [15,17]. Silica layer can also be coated on GNR surfaces to be further chemically modified with another silane molecules (e.g. aminopropyltrimethoxysilane) for target molecule conjugation [18]. We previously demonstrated a label-free nanoplasmon biochip through chemisorption assembly of GNRs on a mercaptosilanized glass substrate followed by a coating of PSS/PAH and conjugation with human anti-IgG for biofunctionalization [19]. However, achieving a uniform silica thickness is highly dominated by the CTAB bilayer density, and solvent/water ratios [20]. The process is often tedious, time-consuming, and easily causes nanoparticle aggregation. Although proteins can be bound to GNR via electrostatic interaction due to the positive charges of CTAB bilayer, the physical adsorption appears unstable at high ionic strengths. All these challenges dramatically impact the functionality of GNRs in biomedical applications [21].

Given that thiol moieties have a high affinity for binding gold surface to form Au–sulfur bond, it is attractive to chemically modify biological receptors such as antibody to enable Au–S interaction. A recent report has shown that antibodies can be directly conjugated to GNRs through modified Fc portions, using a heterofunctional linker with hydrazide and dithiol groups, for specific molecular imaging [17]. In this work, we presented a facile biofunctionalization method suitable for antibody and gold nanostructures by retaining the biological activity. Instead of employing conventionally an intermediary coating step and tedious procedures of nanoparticle surface modification, we carefully modified antibody using Traut's reagent for thiolation. By optimizing the ratio of thiolated antibody to nanorod, an efficient GNR biofunctionalization was achieved with excellent stability without aggregation in physiological buffers. To demonstrate the practical use of the functional nanorods, we developed a label-free, highly selective GNR biochip as a generic biosensor platform to detect proteins (e.g. human IgG) with a high selectivity.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate trihydrate (HAuCl_4 ; 99%), cetyltrimethylammoniumbromide (CTAB), sodium borohydride (NaBH_4 ; 99%), L-ascorbic acid (AA), silver nitrate (AgNO_3 ; 99%), sodium oleate (NaOL, > 97%), (3-mercaptopropyl) trimethoxysilane (MPTMS), poly (ethylene glycol) methyl ether thiol (PEG-SH, MW~ 5000), ethylenediaminetetraacetic acid (EDTA), human serum IgG, rabbit IgG, and goat anti-human IgG were purchased from Sigma Aldrich (St. Louis, MO). Traut's Reagent, Ellman's Reagent, and Zeba™ Spin Desalting Columns (7K MWCO) were obtained from Thermo Scientific (Rockford, IL). Glass slides (7 mm × 50 mm × 0.7 mm) were from Delta Technologies (Loveland, CO).

2.2. Fabrication and characterization of GNRs

Gold nanorods of varying aspect ratio (AR) were chemically synthesized using the seed mediated growth method [22]. To fabricate GNRs with longitudinal surface plasmon resonance (LSPR) wavelengths longer than 850 nm, a bi-surfactant system containing CTAB and NaOL was used in the growth solution (HAuCl_4 , 1 mM; CTAB, 0.037 M; NaOL, 0.0126 M, AgNO_3 , 4 mM; and AA, 64 mM) [23]. GNRs were then purified by centrifugation, twice at 10,000 rpm for 25 min and were redispersed in DI water. Absorption spectra of the synthesized nanorods were measured using UV-vis spectrophotometer (Beckman Coulter). The physical dimensions of the GNRs were characterized by a Hitachi H-7500 transmission electron microscope (TEM) (Hitachi Ltd, Tokyo, Japan), and their surface zeta potential were measured by dynamic light scattering (ZetaSizer NanoZS90, Malvern Instruments, Worcestershire, UK).

2.3. Thiolation of anti-IgG

Anti-Human IgG with or without FITC labels (Sigma-Aldrich) were modified by Traut's reagent, which conjugates thiol (–SH group) to free amines. Briefly, 0.1 mL anti-IgG was mixed Traut's reagent in phosphate buffered saline (PBS, pH 7.4, Invitrogen) containing 2 mM EDTA. The Traut's reagent is 10-fold molar excess per mol protein to ensure full thiolation of the biological moiety. The mixtures were incubated at room temperature for 1 h. Afterwards, excess Traut's reagent was removed via filtration through Zeba™ Spin Desalting Columns. Sulfhydryl groups in the purified thiolated anti-IgG molecules were measured using Ellman's Reagent, according to the manufacturer instructions.

2.4. Biofunctionalization of GNRs with thiolated antibody

50 μL of the above prepared anti-IgG (10 $\mu\text{g}/\text{mL}$) was added dropwise to 1 mL of GNR solution, which facilitated distribution of the molecules on the GNRs surface [24]. After 5 min of incubation at RT with agitation, methoxy-PEG-SH (PEG-SH, 150 μM) was added to the colloidal solution and incubated for 2 h. The human anti-IgG conjugated GNRs were separated from excess free-form antibody by centrifugation at 8000 rpm for 15 min and washed by a washing buffer (1% BSA in 0.01 M PBS at pH 7.4). The pellet was finally resuspended in 0.01 M PBS, followed by characterization using UV-vis spectroscopy and transmission electron microscopy. The biological activity of the antibody molecules on the

nanorod surfaces were confirmed by ELISA tests similar to previous studies [25,26]. The protocol was described in supporting information.

2.5. Label-free LSPR assay based on functional GNR biochip

Immobilization of GNRs on silanized glass substrates was performed as previously described [20]. The nano-chip was then biofunctionalized with a thiolated anti-human IgG (0.1 mg/mL) by incubation for 2 h. The biochip was amply rinsed with a washing buffer (1% PEG-SH in 0.01 M PBS at pH 7.4) to minimize non-specific adsorption of excess antibody on the glass. Since the antibody was FITC-labeled, fluorescence microscope equipped with a Leica DFC 360 FX camera (Leica DMI 6000B; Wetzlar, Germany) was used to characterize the bioconjugation on the GNR chip.

To perform a label-free nanoplasmonic assay, diluted human IgG solution with varying concentration up to 80 nM were applied onto the functionalized GNRs chips and incubated for 1 h. Afterwards, UV-vis absorption spectra were taken to observe red shifts of longitudinal plasmon band maxima. The spectral shift is a function of the target analyte binding on the GNR surface, constituting the optical transduction for biological detection. Compared to conventional immuno-bioassay, the GNR sensor eliminates the need of signaling tags, thus allowing a label-free biosensing.

2.6. Statistical analysis

All studies were performed with at least three measurements. Each data point is presented as mean $\pm$ SD. Statistical analyses were performed using SPSS version 10.1 (Statistical Package for Social Sciences, Chicago, IL). Statistical significance was determined by Student's *t*-test with *P*<0.05.

3. Results and discussion

3.1. Thiolation of biological receptor to conjugate with gold nanorods

For effective biological applications, the fabricated GNRs need to be biofunctionalized with biological receptor such as antibody. It is well known that thiol (–SH) group demonstrates a strong affinity to Au element. As such, if the antibody structures can be modified with –SH derivatives, it will facilitate the biofunctionalization of GNRs through covalent Au–S bonds. Fig. 1 shows the schematic of direct immobilization of thiolated antibody moieties onto the GNR surfaces. The antibody is first modified by Traut's reagent, which reacts with primary amines (–NH₂) to introduce sulfhydryl (–SH) group while maintaining charge properties [27]. It was reported that a 10-fold molar excess of Traut's Reagent would yield antibody molecules modified with at least three sulfhydryl groups [28]. Afterwards, the –SH terminated antibody can directly conjugate with the GNRs in a facile fashion. To minimize non-specific binding, PEG-SH is followed to patch the GNR surfaces as a blocking agent. To further eliminate non-specific adsorption, the functionalized GNRs can be washed by 0.01 M PBS containing 1% BSA (pH 7.4). Due to the weak physisorption, a significant portion of non-specific proteins will be removed after vigorous washing [29].

The unique optical properties of GNRs result in strong absorption peaks in the visible to infrared region of spectrum, where two bands represent their transverse and longitudinal axes, respectively. The absorption band is sensitive to the localized refractive index change caused by deposition of biomolecules [30,31]. As such, UV-vis spectroscopy was used to monitor the bioconjugation process. Fig. S1 shows the absorption spectra of GNRs upon binding with various amounts of thiolated anti-human IgG molecules. Due to the refractive index change caused by antibody binding, a red shift in plasmon peak band of nanorod, especially in the dominant longitudinal peak was observed. An optimal molar ratio of 1:1 resulted in a 6 nm red-shift in the longitudinal SPR and little distortion in the peak morphology. The functionalized nanorods were dispersed in buffer solution without aggregation. Therefore, the following study was carried out using this optimal ratio.

One advantage of nanorod over spherical shape is the wide tunability of the plasmon band. Depending on the aspect ratio (AR) of gold nanoparticle, the longitudinal plasmon maxima is tunable from visible to NIR region which is highly suitable for biomedical application. To investigate the effectiveness of GNR functionalization using thiolated antibody, a variety of GNR sizes with distinct plasmonic peaks were studied. TEM images shows that the mean aspect ratios (length: width) of these nanorods are 2.6, 3.0, 4.5, and 5.0 (Fig. 2 insets). The corresponding LSPR wavelengths are 674, 764, 810, and 916 nm, respectively. Fig. 2 shows the absorption spectra of these varying sized GNRs before and after conjugation. Pronounced red-shifts of 6–19 nm were recorded for all the nanorods upon binding of thiolated anti-IgG. As the GNR aspect ratio increased, the shift magnitude was enlarged because nanorods exhibiting longer LSPR wavelength has a higher spectral sensitivity to local refractive index change [32]. More importantly, the lack of peak broadening and little spectrum change indicated that the spectral shift was not induced by nanorod aggregation for all the cases. The TEM images of the functionalized GNRs confirmed the mono-dispersion before and after bioconjugation (Fig. S2). Successful deposition of anti-human IgG on the GNR surface via covalent Au-S bonds can be further demonstrated by fluorescence study, because the anti-human IgG was pre-labeled with fluorescein isothiocyanate (FITC). Fig. 1E shows the fluorescence of GNR-anti-IgG complexe in the pellet that was redispersed after centrifuge. A dramatic increase in the fluorescence intensity over bare GNRs was exhibited after immobilization of thiolated anti-IgG. Table 1 summarizes the change in the zeta potential of GNR surfaces due to antibody immobilization. At baseline, CTAB-coated GNRs have a highly positive surface charge because of the densely packed cationic surfactant bilayer [33,34]. After bioconjugation, the GNR surface potentials were all decreased due to the replacement of CTAB by the thiolated anti-IgG. These results corroborated with previous studies [35,36], indicating successful coating of biological receptors on the rod surfaces.

CTAB surfactant acts as an essential capping agent on the GNR surface to prevent nanoparticle aggregation. Replacement of CTAB by the thiolated antibody not only immobilizes the biological receptor onto the nanorods, but also helps improve the stability of the nanoparticles. Here, the functionalized GNRs by thiolated anti-IgG were resuspended in 0.01 M PBS buffer (pH 7.4) and stored at 4°C for months. The stability of the mono-dispersed GNRs was superior without aggregation after 4 months as demonstrated in Fig. S3. The absorption spectra (panel A–C) confirmed the strong affinity of the thiolated anti-IgG molecules bound onto the GNR surfaces, as the antibody coating protected the

nanoparticles from aggregation, even though majority of the CTAB bilayer has been replaced. This superior stability implied that the conjugation between the thiolated anti-IgG and the GNRs was covalent binding via Au–S bonds rather than weaker adsorption through electrostatic interaction in this study.

3.2. Confirmation of biological activity of thiolated antibody immobilized on GNRs

ELISA was performed to investigate the biological functionality of the antibody after thiolation and the ensuing immobilization onto the GNR surface. This is very important for the following biosensing application to ensure the desired sensitivity and specificity. As shown in Fig. 3, the absorbance from the thiolated antihuman IgG was comparable to that from the free-form, unmodified antibody (positive control). This result indicated little destruction of the active bio-functionality after antibody thiolation. After further immobilization of the thiolated antibody onto the GNRs, there were slight dips in the absorbance caused by conformational change due to fixation onto solid substrate. However, the activity loss was within the tolerance range against the positive control. While bare nanorods and peglated GNRs (negative control) caused minimal colorimetric change, all the four sized GNR-antibody conjugates with LSPR wavelength from 674 to 916 nm showed a significant increase in the optical density ($P < 0.01$). It suggested little effect of the thiolation and GNR conjugation on the high specificity of the antibody moieties immobilized on the surfaces. In reality, this universal functionalization method is expected to simplify the biological preparation of different sized nanorods for multiplexed biosensing [37,38].

3.3. Evaluation of the functionalized GNR biochip sensing performance

The optical transduction of GNRs in response to local refractive index change can be utilized in a label-free biochip for biomolecular recognition. To demonstrate the practical use of the facile GNR biofunctionalization by thiolated antibody as described above to develop a functional biosensor, we first immobilized nanorods onto mercaptosilanized glass substrates. The absorption spectra of different sized GNRs were comparable to those in solution before assembly, demonstrating the characteristic double peaks with dominant longitudinal band (Fig. 4). Subsequently, thiolated antihuman IgG molecules were incubated with the GNR chip for 1 h to directly bind onto nanorods for biofunctionalization. Upon binding of thiolated anti-human IgG onto the GNR assembly to functionalize, the spectral red-shift in the longitudinal LSPR wavelength of 692 nm (A), 745 nm (B), 870 nm (C), and 908 nm (D) was 3, 8, 9, and 10 nm, respectively. The representative SEM image of gold nanorods functionalized with thiolated anti-human IgG molecules demonstrated monodispersed assembly without aggregation on the glass surface (Fig. S4). Fluorescence microscopy showed further proof of the attachment of thiolated anti-IgG onto the GNR biochip. Sharp scattering of green fluorescent dots (red arrows) from FITC label on the antibodies were observed at the surface of each GNR biochip. As a comparison, non-specific binding of FITC-labeled anti-IgG molecules alone immobilized on glass showed clumps of green fluorescence (Fig. S5). This data clearly indicated that the thiolated antibody was indeed specifically bound onto the GNR assembly through Au–S bonds.

Next, we evaluated the sensing performance of the GNR biochip functionalized with the thiolated anti-human IgG for human IgG detection as a model. Specifically, samples with

spiked human IgG concentration up to 80 nM were applied to the functional GNR biochip. Fig. 5 shows the absorption spectra where perturbation of local refractive index caused by specific target binding induced a typical red-shift in the longitudinal plasmon band maxima. The shift magnitude was directly correlated with the target amount present in the sample (panel A). Fig. 6A shows the LSPR red shift as a function of the human IgG concentration. The standard curve was linear in the range of 10 to 40 nM ($R^2=0.99$) and clearly differentiate the target amount in this region. The increase rate of the shift magnitude decreased for the human IgG concentrations beyond 40 nM, indicating a saturation of the binding sites on the nanochip.

To better understand the advantage, it is interesting to compare the sensing performance of the functional GNR biochip prepared by this new method vs. conventional method. We previously reported the detection of human IgG using a GNR biochip functionalized by electropolymeric coating of poly (sodium 4-styrenesulfonate) (PSS) and poly (allylamine hydrochloride) (PAH) alternatively to immobilize anti-human IgG which is negatively charged [19]. The LSPR red shift of the GNR sensor resulted from the detection of 10 nM human IgG was only ~1.2 nm, while the biochip using the thiolated antibody in this work showed an approximately 6-fold increase in the spectral shift to ~7.5nm. This was the case for the human IgG assay at 20 and 40 nM as well (Fig. 6B). The significant enhancement in the spectral sensitivity of the nanosensor can be attributed to the direct binding of thiolated antibody onto the GNR surface rather than a thick electropolymer coating on the nanorods. It has been shown that the LSPR shift induced by local refractive index (RI) due to biological binding is highly distance dependent as a result of the exponential decrease in field enhancement further from the nanoparticle surface [39]. This distance dependence of sensitivity was elucidated in the following equation [40] and experimentally measured [12,41,42].

$$R=m\Delta RI \left\{ 1 - \exp \left(-\frac{2d}{l} \right) \right\}$$

where R is the plasmon shift, m is the intrinsic RI sensitivity, RI is the difference between the RI of the analyte and that of the surrounding medium, d is the modified layer thickness on GNR surface, and l is the decay length of the resonant electric field. By closely immobilized on the nanorod surface due to the thiol group ($-SH$) derivatives on the antibody, the receptor-analyte binding events occurred almost directly on the optical transducer (in this case, the gold nanorod). On the contrary, because of the PSS/PAH overcoating to accommodate more efficient functionalization than CTAB-capped nanorods, the distance of the human IgG binding was inevitably further away (>6 nm) [43] from the transducer. Due to the evanescent nature of surface plasmon, the LSPR shift exhibits a characteristic decay with increasing distance from the rod surface. As a result, the new functionalization method significantly enhanced the sensing performance of the GNR biochip. Additionally, spontaneous reaction of thiolated molecules with CTAB-capped GNRs leads to attachment of antibody molecules at the end faces of the rod-shaped nanostructure [44]. This is because the CTAB bilayer is less densely packed compared to the side faces of nanorods. However, PSS/PAH overcoating eliminates this preference as the coating is uniformly wrapping

around the rod shape without differentiation in surface charge. It has been shown that the LSPR associated electric field is enhanced near the ends of nanorods [45,46]. Therefore, the preferred attachment of receptors at the ends by the thiolated antibody also contributed to a significant enhancement in the LSPR shift as shown in Fig. 6B.

It has been previously reported that PSS-coated gold nanorod bioprobe could induce more than 100 nm red shift in LSPR for human IgG assay in solution [47]. This detection was much more sensitive than our result. The large wavelength shift was caused by both the refractive index change surrounding the rods and aggregation driven by biological recognition. The anti-human IgG on PSS-GNR surfaces facilitated side-by-side agglomeration upon human IgG binding in the solution. This phenomenon changed not only the spectral position and width of the LSPR band, but also decreased the absorbance in a short time. The large shift augmented by aggregation was not possible in the present study because the nanorods were covalently immobilized on MPTMS-treated glass substrates. As shown in all the absorption spectra, we did not observe widening and significantly reduced intensity of LSPR bands during detection. These results indicated that the spectral change was not due to the aggregation-induced shift. Compared to solution-based LSPR sensors, the GNR biochip is more stable and eliminates artificial readings from nanoparticle concentration fluctuation due to avoidable particle loss from agglomeration. Huang et al. successfully developed a GNR biochip based on the extinction coefficient change with a high repeatability [48]. Instead of the wavelength shift, the LSPR intensity was progressively higher with the increase in the concentration of IgG binding. It should be noted that the amount of immobilized GNR monolayer on substrates is usually limited, thereby leading to a diminished peak intensity. This might present a challenge for maximal sensing range as compared to nanorod solution where abundant particles are available. However, the significant improvement in simplicity, robustness, and versatility of the chip-based gold nanorods together with the facile functionalization by thiolated receptors make the GNR biochip very attractive for a powerful bioanalytical tool.

Finally, the specificity of the GNR biochip functionalized by this new method was evaluated. In addition to the human IgG samples, the GNR biochip was probed by other irrelevant proteins including rabbit IgG. Fig. 5B–D shows the absorption spectra of the nano-sensor in response to rabbit IgG (3 mg/mL), myoglobin (1 μ g/mL), and cardiac troponin I (1 μ g/mL), respectively. Fig. 6C shows the comparison of the LSPR shifts from these proteins with that from human IgG (20 nM; positive control). As expected, rabbit IgG, myoglobin, and cTnI samples only resulted in less than 0.5 nm spectral shift because of the minimal interaction with the anti-human IgG immobilized on the GNR sensor. In contrast, the human IgG sample caused a longitudinal resonant shift of ~9 nm which was in line with the calibration curve. This data confirmed that thiolation modification and further immobilization of antibody moiety onto solid substrates did not affect the intrinsic high selectivity to ensure specific target detection.

4. Conclusions

We demonstrated that a thiolation of antibody using Traut's reagent can efficiently introduce thiol groups onto the antibody structure without compromise in the biological activity and

specificity. The thiolated moiety allows direct immobilization onto gold nanorod surface of varying sizes with LSPR wavelength from 600 to 1000 nm via high affinity of Au–S bonds. This facile biofunctionalization process greatly alleviated the complicated process and eliminated aggregation problems encountered in conventional surface modification of CTAB-capped GNRs. This new method showed a practical use in simplifying the subsequent functionalization of GNR biochip where nanorods were assembled. We demonstrated that the thiolated anti-IgG could easily bind with GNR nanochip to develop a label-free, plasmonic nanosensor with high specificity and sensitivity. More importantly, spectral sensitivity exhibited by the GNR biochip with thiolated antibody functionalization was significantly improved as compared to electropolymeric coating for antibody attachment through electrostatic interaction. In summary, the thiolation of biological receptors to facilitate GNR functionalization in a simple and universal fashion will provide a new paradigm in nanoparticle preparations suitable for biomedical applications such as biosensing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.talanta.2014.11.023>.

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Fig. 1. Schematic of antibody thiolation by Traut's reagent, followed by functionalization of gold nanorods by direct binding through covalent Au-S bonds.

Fig. 2.

Absorption spectra before and after conjugation of the thiolated anti-human IgG molecules onto gold nanorods of varying size exhibiting longitudinal SPR peaks at (A) 674; (B) 764; (C) 810; and (D) 916 nm, respectively. A red shift was observed upon binding. Inset: TEM images of varying sized nanorods. (E) Fluorescence intensity from FITC-labeled anti-IgG confirmed the immobilization of antibody onto the GNRs' surfaces. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3.

ELISA tests showed minimal loss of the biological functionality of the thiolated anti-IgG immobilized on GNR surfaces. Data are expressed as the mean $\pm$ SD from 3 independent tests performed in triplicates. (** $P < 0.01$, compared to the GNRs and GNRs+PEG).

Fig. 4. Direct binding of thiolated anti-IgG moiety onto GNRs that were assembled on glass substrates to construct a functional GNR biochip. Left: absorption spectra of GNRs assembly with (A) 692, (B) 745, (C) 870, and (D) 908 nm longitudinal plasmonic band, respectively. Right: Fluorescence microscopy of GNR biochip functionalized with thiolated anti-IgG exhibiting 2 strong green dots from the FITC label. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5.

Absorption spectra of the GNR biochip functionalized with thiol-tagged anti-human IgG molecules probed by varying concentrations of human IgG up to 80 nM (A), and irrelevant proteins including rabbit IgG (B), myoglobin (C), and cardiac troponin I (D).

Fig. 6.

(A) LSPR shifts as a function of human IgG concentrations detected by thiolated anti-IgG moieties immobilized on the GNR biochip. (B) Comparison of the sensing performance for human IgG detection using different functionalization method. (C) Plasmonic response to different samples shows a high specificity of the GNR biochip functionalized with thiolated anti-human IgG. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table 1

Zeta potential of CTAB-capped GNRs and after functionalized with thiolated anti-IgG molecules.

Samples (LSPR peaks)	@ 674 nm		@ 764 nm		@ 810 nm		@ 916 nm	
	GNRs	GNRs+Anti-IgG	GNRs	GNRs+Anti-IgG	GNRs	GNRs+Anti-IgG	GNRs	GNRs+Anti-IgG
ζ potential (mV)	41.47 $\pm$ 8.38	4.51 $\pm$ 0.59	33.00 $\pm$ 1.31	9.04 $\pm$ 0.85	30.53 $\pm$ 3.44	6.26 $\pm$ 0.84	26.17 $\pm$ 0.61	10.59 $\pm$ 1.92