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Multiplexed Gold Nanorod Array Biochip for Multi-Sample Analysis

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Abstract

Optical transduction of biological bindings based on localized surface plasmon resonance (LSPR) of gold nanorods (GNRs) is attractive for label-free biosensing. The aspect ratio (AR) dependence of LSPR band maxima inherently provides an ideal multiplex mechanism. GNRs of selected sizes can be combined to ensure distinct plasmon peaks in absorption spectrum. Monitoring the spectral shift at the dedicated peaks allows for simultaneous detection of the specific analyte. Here, we first transformed the GNR's multiplexed biosensing capability to a robust chip-based format. Specifically, nanorods of AR 2.6 and 4.5 were assembled onto thiol-terminated substrates, followed by functionalization of respective antibodies to construct a GNR multiplex biochip. As a model system, concentrations of human IgG and rabbit IgG were simultaneously measured by correlating red-shifts at distinct resonance peaks caused by specific target binding. The calibration curves exhibited linear relationship between the spectral shift and analyte amount. The sensing performance in multi-analyte mode correlated nicely with those for single analyte detection with minimal cross-reactivity. Moreover, mixed GNRs can be deposited in controllable array pattern on the glass chip to analyze numerous samples at the same time. Each GNRs dot functioned independently as a multiplexed plasmonic sensor. Coupled with microplate reader, this GNR nanoarray chip can potentially result in large scale assay of samples concurrently while for each sample, a multi-analyte detection simultaneously if desired. The concept shown in this work is simple and versatile that will definitely be a new paradigm in high-throughput protein biochip development in the era of nano-biosensing.

Keywords

Multiplex biosensing; Gold nanorod biochip; Nanoarray; High throughput assay; Surface plasmon resonance

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1. INTRODUCTION

Dramatic improvement in instrumentation and advancement in experimental design for surface plasmon resonance (SPR) based optical biosensing resulted in extraordinary expansion of detection applications from proteins to bacteria, cells, and disease-specific mRNA. With the advent of nanotechnology, nanoparticle-based SPR, notably gold nanorods (GNRs), has been at the center of recent studies in label free biosensing (Dreaden et al., 2011). The optical transduction of GNRs relies on the localized SPR (LSPR) phenomenon where biological binding events lead to plasmonic shift caused by changes in dielectric environment in the vicinity of the functionalized nanorods (Mayer and Hafner, 2011; Petryayeva and Krull, 2011; Jain et al., 2006; Chen et al., 2007; Marinakos et al., 2007).

Compared to spherical nanoparticles, anisotropic structure like GNRs is inherently more sensitive to the local refractive index perturbation which is desirable for sensing (Zeman, 2011; Yang, 1995; Malinsky, 2001; Sun and Xia, 2002). The wavelength shift of the plasmonic peak of spherical particles is only 1~2 nm which is too small a value for any realistic detection, while GNR bioprobes have demonstrated 10–50 nm plasmonic shift upon specific target binding (Yu and Irudayaraj, 2007; Marinakos et al., 2007; Nusz et al., 2008; Casas et al., 2013). In addition, GNRs provide additional benefits because the LSPR properties can be conveniently tuned by adjusting the aspect ratio, thereby exhibiting a dominant resonance band maxima ranging from 600 to 1,300 nm. This size- and shape-dependent optical property provides a unique opportunity to develop a multiplexed biosensing by combining selective GNRs with distinct plasmonic bands. Yu et. al. demonstrated the simultaneous detection of three different species of anti-IgG molecules in biological buffers using a mixture of three-sized GNRs (Yu and Irudayaraj, 2007). However, solution-based nanorod bioprobe has intrinsic disadvantages. Due to multiple wash, the inherent artificial fluctuation in optical readings from nanoparticle amount change can not be avoided. Moreover, free-suspended nanoparticles are prone to aggregation because of ionic strength change and excess centrifugation. Therefore, we have developed a chip-based plasmonic assay via chemisorption of gold nanorods onto thiol-terminated glass substrates (Wang and Tang, 2013). Further expansion of this technique to a multiplexed biosensing in a chip format by simultaneously assembly of varying sized GNR bioprobes will indubitably lead to a significant improvement in simplicity, robustness, and versatility of this label-free biosensing modality as a powerful bioanalytical tool.

Protein array systems with high throughput capabilities could enable simultaneous processing of large volume samples, reductions in sample volume, and more cost-effective analysis of target analytes. The concept of microarray-based ligand-binding assays on solid support was introduced to implement high-throughput detection (Mendoza et al., 1999; Ekins, 1998). A chip-based GNR biosensing can facilitate the microarray format, which enables precise pattern arrangement in a controllable and orderly fashion. Furthermore, the direct contact of samples with the deposition of capture molecules (i.e. functional GNR probes here) on substrates can effectively eliminate the analyte diffusion to nanorod surface for binding to occur. This will help alleviate the adverse effect of diffusion-limited reaction which is commonly a problem. So far in the literature, there are only few reports of LSPR sensing based on coupled nanospheres conducted in multi-sample manner (Xie et al., 2014).

To our knowledge, we describe herein for the first time, not only to study the multiplexed sensing of GNR biochip, but also to explore the feasibility of GNR array pattern in solid support for high-throughput detection. The combination of multiplexed biosensing and nanoarray in chip-based format will be capable of simultaneously analyze large numbers of samples as well as multi-analyte detection within a single sample.

2. MATERIALS AND METHODS

2.1 Materials

Gold chloride (HAuCl_4), sodium borohydride (NaBH_4), cetyltrimethylammoniumbromide (CTAB), L-ascorbic acid (AA), silver nitrate (AgNO_3), (3-Mercaptopropyl)trimethoxysilane (MPTMS), human serum IgG, rabbit serum IgG, goat anti-human and rabbit IgG respectively, and sodium oleate (NaOL), Poly(ethylene glycol) methyl ether thiol (thiol - PEG, $M_n \sim 6,000$) were obtained from Sigma-Aldrich (St. Louis, MO). Microscopy glass substrates with ITO coating were from Delta Technologies (Loveland, CO). All reagents were used as purchased without any further treatment.

2.2 Preparation of Au nanorods

AuNRs with aspect ratios up to 8.0 were chemically synthesized by a seed-mediated growth using CTAB and NaOL bisurfactants (Ye et al., 2013). The seed solution was first prepared by adding 0.025 mL of 10 mM HAuCl_4 in 1 mL of aqueous 0.1 M CTAB. This was followed by adding 1 mL of 10 mM ice cold NaBH_4 to immediately result in the solution color change from yellow to brownish yellow. The seed solution was aged at room temperature for 30 min. For preparing the growth solution, 7.0 g of CTAB and 1.234 g of NaOL were dissolved in 250 mL of warm water ($\sim 50^\circ\text{C}$). The solution was allowed to cool to 30°C , and 1.8 mL of 4 mM AgNO_3 was added to the 25 mL of CTAB/NaOL solution. The mixture was kept undisturbed at 30°C for 15 min, after which 25 mL of 1 mM HAuCl_4 was added. The solution became colorless after 90 min of stirring, indicating the reduction of Au^{3+} to Au^+ . After another 15 min, 0.125 mL of 64 mM ascorbic acid and 0.08 mL of the seed solution were added. The resulting mixture was left undisturbed at 29°C for 48 h for full nanorod growth. To fabricate nanorods of varying aspect ratios, we fine tuned the seed volume in the growth solution as well as the pH of the growth solution by adjusting the amount of HCl (37 wt.%). After nanorod synthesis, excess reagents were removed by centrifugation. Briefly, the suspension was spun twice at 8,500 rpm for 30 min. 5 mL MilliQ water was then added to resuspend the solid pellet and centrifuged again at 13,000 rpm for 3 min. The resulting pellet was then redispersed in a total of 5 mL solution to make a final concentrated GNR solution.

2.3 Functionalization of Au nanorods

Fig. 3A shows the schematic of gold nanorod biofunctionalization. 20 μL of Traut's reagent (5 mg/mL) were first added to 0.1 mL of antibodies (i.e. anti-rabbit IgG or anti-human IgG) for 2 hours to conjugate thiol (-SH) groups onto the IgG surface. Then 200 μL of purified AuNRs solution and 100 μL 10 mg/mL thiol-PEG were added into the thiolated antibody solution for incubation overnight. Because of the high affinity of Au-S bonds, the antibody moieties can be covalently functionalized onto the GNR surfaces. The amount of IgG

molecules per unit nanorod was determined by fluorescence intensity of FITC-IgG labeled AuNRs based on the linear relationship between intensity and concentration of FITC-IgG. The UV-vis absorption spectra were used to monitor red shift of plasmon band maxima during the biofunctionalization process.

2.4 Preparation of multiplex AuNRs chip

To effectively immobilize GNRs, glass surfaces were chemically modified with thiolsilane agents before chemisorption of nanoparticles from bulk solution, following the protocol in our earlier work (Wang and Tang, 2013). To develop a multiplexed GNR biochip, 15 μ L of antibody-immobilized GNRs with mixed aspect ratios were dropped onto designated spots on the thiol-terminated glass substrates and incubated for 2 hours. The detection spots were arranged to mimic the layout of a 96 well plate to accommodate an absorption reading by a plate reader in a high-throughput fashion. After the immobilization, the substrates were washed three times with MilliQ water, and then air dried with nitrogen. Weakly adsorption of nanoparticles onto the biochip would have been washed off to ensure a robust multiplexed GNR biochip.

2.5 Multiplex, label-free nanoplasmon biosensing

Upon immobilization of antibody functionalized GNRs which onto glass substrate, a functional GNR biochip is constructed. To minimize non-specific binding, the chip was chemically treated with a blocking solution containing 5 mg/mL thiol-PEG in PBS buffer for 2 hours. To perform a label-free assay, a sample solution (20 l) with respective human IgG concentration up to 60 nM was pipetted onto the nanosensing chip surface and incubated for 30 min until equilibrium. Afterwards, 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. For multiplex detection, different aspect ratio nanorods with distinct longitudinal SPR peak were immobilized with respective antibodies. Upon biological binding, the resulting spectral shift in the targeted LSPR band was examined. A pronounced red shift at 630 nm indicated a detection of rabbit IgG, while plasmonic shift around 840 nm for human IgG assay.

2.6 Instruments

UV-vis absorption spectra and fluorescence intensity were measured by Biotek plate reader. Gold nanorods on substrates were characterized by Hitachi S5500 scanning transmission electron microscope (STEM). The SEM images of substrate assembled GNRs were obtained from indium tin oxide (ITO) coated glass slide.

3. RESULTS and DISCUSSION

The unique optical property of gold nanorod as a signal transducer for multiplexed biosensing is the continuously tunable plasmon resonance band by adjusting its length to width ratio (Liz-Marzan, 2006). Fig. 1A shows the absorption spectra of various aspect ratio GNRs suspension exhibiting longitudinal plasmon peak from 611 to 1,215 nm. The

correlation between aspect ratio (i.e. length to width ratio) and the longitudinal plasmon band maxima (λ_{max}) can be expressed as the following.

$$\lambda_{max}=93.4R+456.7 \quad (1)$$

where R is the nanoparticle aspect ratio. The advantage of bi-surfactant system in the nanorod synthesis is the high purity of longer rods, resulting in a sharp absorption at almost all the desired wavelengths including NIR. This capability provides a great flexibility in selecting appropriately sized nanorods for optimal multiplex sensing. Fig. 1B shows the representative absorption spectrum of mixing two different aspect ratios of GNRs that are assembled onto a glass substrate. The plasmon wavelength measurements were performed in air to be consistent with the following biochip studies. There are three plasmon bands, of which the first peak at 520 nm represents the characteristic Au resonance in transverse direction. The second peak around 630 nm reflects the longitudinal band from shorter rods (AR: 2.6), while the third peak at 840 nm (AR: 4.5) is from the longer rods. It is noteworthy that the two dominate peaks are distinctly separated, thereby enabling a simultaneous monitoring of plasmonic shift at both positions independently for the dedicated target analyte in a single sample. As shown in the SEM image, the nanorod assembly is truly a monolayer with good dispersion over the substrate. Because the glass was modified by MPTMS to make the surface to be thiol (SH) terminated, the gold nanorod was assembled via Au-S bond. This covalent binding allows excellent stability for biofunctionalization with antibody in PBS buffer that contains high ionic species (Fig. S4).

3.1 Chip-based nano-plasmonic biosensing

To develop a specific sensor, the GNR assembly on the glass substrates was functionalized with thiolated antibody moieties (Fig. 3A). We first performed human IgG and rabbit IgG detection individually using the respective functional GNR biochip. Briefly, a series of target IgG sample at a concentration from 10 to 60 nM was probed by specific GNR sensor chip. It is known that resonance shift at the longitudinal plasmon peak due to biological binding provides the label-free biosensing mechanism. As such, UV-vis spectroscopy was used to monitor the spectral shift upon target reaction. Fig. 2A shows the absorption spectra of the GNR sensor in response to rabbit IgG detection. The up-regulation of the analyte concentration resulted in a direct proportional increase in the magnitude of the resonance shift. The inset shows the calibration plot by fitting the red shift vs. concentration of IgG. The nanorod biochip shows a linear response to the analyte in the range from 10 nM to 60 nM with high sensitivity of 0.21 nm/nM ($R^2 = 0.96$) for rabbit IgG (Figure 2A). Similarly, the GNR sensor specific for human IgG shows higher sensitivity of 0.27 nm/nM ($R^2 = 0.99$), Fig. 2B. The sensitivity was increased by more than 300% comparison with the reported literature with the sensitivity of 0.0607 nm/nM (Wang and Tang, 2013). Chip-based nanorod sensor provides not only convenience for operation, but also a robust and reliable platform. The gold particles covalently assembled on the thiolated glass ensures a strong deposition without dislodge. This effectively eliminates the intrinsic problem of solution-based GNR bioprobes where extinction coefficient can be dramatically reduced due to particle loss. Another concern of solution-based assay is aggregation-induced spectral shift not caused by specific target binding. For example, end-to-end or side-by-side aggregation of nanorods

was reported to result in large shift of longitudinal SPR (Jain et al., 2006). An aggregation of streptavidin with biotinylated nanorods can cause a red shift of ~ 100 nm (Yu and Irudayaraj, 2007). These phenomena can easily happen in suspension because of crosslinking nanoparticles. In this study, the GNRs were strongly confined to treated glass surfaces by chemical covalent binding. As such, the chances of red shift caused by GNR aggregation are greatly reduced. We did not observe significant shift in transverse band during our study, which is often the case for aggregation induced shift. Additionally, lack of significant band broadening/distortion further confirmed that the red shifts at the respective plasmon peak observed were indeed caused by specific human and rabbit IgG bindings.

The molecular detection limit of gold nanorod (L_M) is determined by several factors including nanorod geometry, as shown in the following equation (Nusz et al., 2009).

$$L_M l, d = \frac{V_s}{V_a} \cdot \frac{U}{S(r) \cdot \Delta RI} \quad (2)$$

where V_s is the total sensing volume of the nanorod; V_a is the volume of the analyte molecule; U is the uncertainty of the optical system in detecting wavelength shift; $S(r)$ is the refractive index sensitivity dependent on the distance (r) of the bound analyte molecule from the surface of nanorod; and ΔRI , the difference between the refractive index (RI) of the analyte and that of the surrounding medium. Careful comparison of the two GNR sensor performance in Fig. 2 reveals that the human IgG binding usually caused a larger resonance shift. For example, at 50 nM concentration, human IgG caused a ca. 14 nm shift while ca. 10 nm shift for rabbit IgG binding. This observation is consistent with Eq. 2 where detection sensitivity L_M is a function of the length l and diameter d of nanorod. The variation in the nanorod dimension was directly related to the aspect ratio. Thus, the GNRs functionalized for human IgG detection exhibit plasmon band around 840 nm and GNRs dedicated for rabbit IgG have plasmon band at 630 nm. It is generally accepted that the bulk refractive index sensitivity of the plasmon band is linearly correlated with the LSPR peak wavelength so that nanoparticles with a resonance band at higher wavelengths are more sensitive to their local environment than those at shorter wavelengths (Miller and Lazarides, 2005; Nusz et al., 2009). Our data indeed corroborated with the theoretical calculation. Additionally, the more refractive index change caused by target binding on the nanorod sensor as indicated by ΔRI in Eq. 2, the higher magnitude of the plasmon shift will be induced. Therefore, we previously reported that application of magnetic nanoparticle (MNP; RI: 2.42) in LSPR sensing system can significantly enhance the spectral sensitivity in protein detection (Tang et al., 2013). In this work, we found that the magnetic-mediated LSPR biosensing can cause at least 70% more plasmon shift for target IgG binding as compared to without magnetic nanoparticles (Figure S2).

3.2 Label-free, multiplexed biosensing

Fig. 3 shows the schematic of three processes involved in a label-free, multiplexed LSPR biochip. First, GNRs of varying size needs to be functionalized with anti-human IgG and anti-rabbit IgG respectively before assembly onto glass chip. Here, we modified the antibody moiety using the Traut's reagent. The thiolated antibody can then be immobilized

onto the GNR surfaces through covalent Au-S bonds. Upon conjugation, a pronounced absorption around 280 nm and plasmon shift in longitudinal peak were recorded in the spectrum (Fig S1). The amount of antibody bound to the GNRs was determined by fluorescence of GNRs with FITC-labeled IgG conjugates. On average, there were approximately 3 anti-rabbit IgG molecules per nanorod of aspect ratio 2.6 and ~ 7 anti-human IgG molecules per nanorod of larger size (AR: 4.5). Afterwards, by assembly of the two functional GNR sensors on glass substrate, we can design a multiplexed biochip where plasmonic response at distinct band maxima only responds to specific target. Herein, the longitudinal peak around 630 nm represents the smaller nanorod immobilized with anti-rabbit IgG and the larger sized rods with 840 nm peak is dedicated for the human IgG detection.

Fig. 4 shows the spectra of simultaneous detection of rabbit IgG and human IgG at concentrations from 10 to 60 nM. Pronounced red shifts were observed at both dominate bands with progressively larger shift in response to the analyte increase. To investigate the multi-analyte sensing performance, we compared the calibration curve with the single analyte detection respectively. The inset of Fig. 4A shows the calibration plot of the anti-rabbit IgG detection in both single and multiplex GNR sensor, indicating a minimal interference of sample mixture to the sensitivity and specificity. For the anti-human IgG sensor, it is interesting that the sensitivity was increased by larger shift magnitude as compared to single detection. The improved spectral sensitivity, defined as relative shift in resonance wavelength with respect to the refractive index change, is desirable. To ensure the sensitivity enhancement in multiplexed sensing was not due to the cross-reactivity of anti-human IgG with rabbit IgG in the sample, we performed a nonspecific binding study. Fig. 5 shows the response of GNR sensor immobilized with anti-human IgG molecules when probed by both human IgG (positive) and rabbit IgG (negative) samples. At all the tested concentrations, the rabbit IgG molecules consistently caused a 2 nm spectral shift, while the human IgG molecules resulted in significant spectral shifts in line with the expected results. These data clearly indicate minimal cross-reactivity of the antibody moieties for high specificity in the multiplexed biosensing.

Previously, numerous approaches were reported to realize multiplexed assay. Most commonly, multiple analytes are differentiated with distinct labels, such as radioactive markers (Yvert and Delagneau, 1993; Gow et al., 1986), fluorescent molecules (Vuori et al., 1991; Sauer et al., 1993), or different enzymes (Nanjee and Miller, 1996; Choi et al., 1991). However, a loss of sensitivity is usually observed compared to individual assay format, due to the poor discrimination of signals generated by multiple labels. An alternative approach for multi-analyte sensing is spatially separating the assay zone. Discrete areas in one sensor platform can be coated with different antibodies, enzymes, or receptors specific for each of the targeted analytes. Since each analyte is spatially separated, the same or different labels can be used for detection. Therefore, the number of analyte in one sample to be analyzed is not limited by the availability of enzymes or broad fluorophore emission spectrum. However, this strategy may greatly reduce the number of samples that can analyzed in the limited biochip surface, thereby making it less appealing for high-throughput format. In our study, the sensitivity to the refractive index change caused by specific analyte binding at the nanorod surface can provide efficient optical transduction that can be effectively exploited

to develop a label-free detection. The wide tunability of the GNRs offers continuous plasmonic peak combination for optimal multi-analyte sensing design. As such, these features renders GNR probe to be a superior multiplex platform over the conventional multi-label strategy owing to simplicity, minimal crosstalk, and low cost.

3.3 Gold nanorod array biochip

Array technology is a valuable tool for high-sample-throughput biochip development. GNR assembly onto substrates can be an efficient means of nanoarray pattern fabrication owing to its flexibility, simplicity, and low cost. Fig. 6A shows a picture of gold nanorod array biochip fabricated by nanoparticle deposition in spatially resolved arrangement. Each nanorod dot can function as an individual plasmonic sensor after immobilization of functional receptors. Coupled with a high-throughput microplate reader, the nanorods array can expand to potentially analyze large numbers of different biological samples concurrently. In the meantime, each sensing dot can perform multiplexed biosensing within a single sample. In this study, we performed a feasibility testing of simultaneous multi-sample detection using the nanorod array. Fig. 6B shows the absorption spectra of nine GNR deposition in an array pattern. Each dot demonstrated a reproducible nanorod assembly on the solid supports. After biofunctionalization with anti-rabbit IgG molecules, these patterns became multi-sensors specific for rabbit IgG assay. As a first attempt, each of the isolated sensing dots was probed by one sample containing rabbit IgG of varying concentration. This was to mimic the situation where multiple samples with different target amounts were analyzed concurrently. As shown in Fig. 6C, the plasmon shift correlated nicely with the spiked IgG amount in each sample when compared to individual biochip study described above (Fig. S3; supporting information). Individual nanorod dot in the array functioned independently to record expected spectral shift upon exposure to the assigned sample. This data provides a proof of concept design that is capable of clearly quantifying multiple samples simultaneously in a label-free nanoarray biochip fashion. Fig. 6D shows the multiplexed biosensing of respective target analyte in a single sample at the dedicated LSPR peak by each sensing dot on the nanoarray biochip. Compared to the commercial SPR biosensors based on thin film of gold coating, the nanorod array is more easily miniaturized to increase high throughput of detection. The multiplexed biochip also facilitates seamless integration with microfluidic systems on lab-on-a-chip.

4. CONCLUSIONS

We have described a multiplexed biosensing mechanism using gold nanorods as optical transducer in a chip based format. The sensitivity as well as the plasmon resonance wavelength is closely related to geometrical parameters. By adjusting the aspect ratio of the fabricated nanoparticles, the LSPR properties of gold nanorods can be continuously tuned into near infrared region. Combination of appropriately selected nanorods of different size can be effectively assembled onto thiol-terminated glass substrates to construct a robust nanochip. Each sized nanorod sensor was dedicated for a target analyte due to the functionalization of specific antibody moieties on the rod surface. Spectral shift at the distinct plasmon resonance peak showed linear relationship with the increase in the target amount independently with minimal cross-reactivity. The LSPR sensitivity can be further

enhanced by the application of magnetic nanoparticles. In addition to multiplexed biosensing, functional nanorod sensor can be deposited in an array pattern. This feature could result in a high throughput nano-biochip capable of analyzing large volume of samples simultaneously. This proof of concept study is a stepping stone towards the ultimate goal of developing a multiplexed gold nanorod biochip in controllable nanopattern for high throughput biological detections suitable for clinical diagnostics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Casas J, Venkataramasubramani M, Wang Y, Tang L. Replacement of cetyltrimethylammoniumbromide bilayer on gold nanorod by alkanethiol crosslinker for enhanced plasmon resonance sensitivity. *Biosens Bioelectron.* 2013; 49:525–530. [PubMed: 23816849]
- Chen CD, Cheng SF, Chau LK, Wang CR. Sensing capability of the localized surface plasmon resonance of gold nanorods. *Biosens Bioelectron.* 2007; 22:926–932. [PubMed: 16697633]
- Choi MJ, Choe IS, Kang HK, Lee JS, Chung TW. Simple enzyme immunoassay for the simultaneous measurement of whole choriogonadotropin molecules and free beta-subunits in sera of women with abnormal pregnancies or tumors of the reproductive system. *Clin Chem.* 1991; 37:673–677. [PubMed: 1709596]
- Dreaden EC, Alkilany AM, Huang X, Murphy CJ, El Sayed MA. The golden age: gold nanoparticles for biomedicine. *Chem Soc Rev.* 2011
- Ekins RP. Ligand assays: from electrophoresis to miniaturized microarrays. *Clin Chem.* 1998; 44:2015–2030. [PubMed: 9733000]
- Gow SM, Caldwell G, Toft AD, Beckett GJ. SimulTRAC simultaneous radioimmunoassay of thyrotropin and free thyroxine evaluated. *Clin Chem.* 1986; 32:2191–2194. [PubMed: 3779981]
- Jain PK, Eustis S, El Sayed MA. Plasmon coupling in nanorod assemblies: optical absorption, discrete dipole approximation simulation, and exciton-coupling model. *J Phys Chem B.* 2006; 110:18243–18253. [PubMed: 16970442]
- Liz-Marzan LM. Tailoring surface plasmons through the morphology and assembly of metal nanoparticles. *Langmuir.* 2006; 22:32–41. [PubMed: 16378396]
- Malinsky MDKLSGCVDRP. Chain Length Dependence and Sensing Capabilities of the Localized Surface Plasmon Resonance of Silver Nanoparticles Chemically Modified with Alkanethiol Self-Assembled Monolayers. *J Am Chem Soc.* 2001; 123(7):1471–1482.
- Marinakos SM, Chen S, Chilkoti A. Plasmonic detection of a model analyte in serum by a gold nanorod sensor. *Anal Chem.* 2007; 79:5278–5283. [PubMed: 17567106]
- Mayer KM, Hafner JH. Localized surface plasmon resonance sensors. *Chem Rev.* 2011; 111:3828–3857. [PubMed: 21648956]
- Mendoza LG, McQuary P, Mongan A, Gangadharan R, Brignac S, Eggers M. High-throughput microarray-based enzyme-linked immunosorbent assay (ELISA). *Biotechniques.* 1999; 27:778–6. 788. [PubMed: 10524321]
- Miller MM, Lazarides AA. Sensitivity of metal nanoparticle surface plasmon resonance to the dielectric environment. *J Phys Chem B.* 2005; 109:21556–21565. [PubMed: 16853799]
- Nanjee MN, Miller NE. Sequential microenzymatic assay of cholesterol, triglycerides, and phospholipids in a single aliquot. *Clin Chem.* 1996; 42:915–926. [PubMed: 8665684]

- Nusz GJ, Curry AC, Marinakos SM, Wax A, Chilkoti A. Rational selection of gold nanorod geometry for label-free plasmonic biosensors. *ACS Nano*. 2009; 3:795–806. [PubMed: 19296619]
- Nusz GJ, Marinakos SM, Curry AC, Dahlin A, Hook F, Wax A, Chilkoti A. Label-free plasmonic detection of biomolecular binding by a single gold nanorod. *Anal Chem*. 2008; 80:984–989. [PubMed: 18197636]
- Petryayeva E, Krull UJ. Localized surface plasmon resonance: nanostructures, bioassays and biosensing--a review. *Anal Chim Acta*. 2011; 706:8–24. [PubMed: 21995909]
- Sauer M, Han KT, Muller R, Schulz A, Tadday R, Seeger S, Wolfrum J, Arden-Jacob J, Deltau G, Marx NJ, Drexhage KH. New fluorescent labels for time-resolved detection of biomolecules. *J Fluoresc*. 1993; 3:131–139. [PubMed: 24234821]
- Sun Y, Xia Y. Increased sensitivity of surface plasmon resonance of gold nanoshells compared to that of gold solid colloids in response to environmental changes. *Anal Chem*. 2002; 74:5297–5305. [PubMed: 12403584]
- Tang L, Casas J, Venkataramasubramani M. Magnetic nanoparticle mediated enhancement of localized surface plasmon resonance for ultrasensitive bioanalytical assay in human blood plasma. *Anal Chem*. 2013; 85:1431–1439. [PubMed: 23267460]
- Vuori J, Rasi S, Takala T, Vaananen K. Dual-label time-resolved fluoroimmunoassay for simultaneous detection of myoglobin and carbonic anhydrase III in serum. *Clin Chem*. 1991; 37:2087–2092. [PubMed: 1764784]
- Wang Y, Tang L. Chemisorption assembly of Au nanorods on mercaptosilanized glass substrate for label-free nanoplasmon biochip. *Anal Chim Acta*. 2013; 796:122–129. [PubMed: 24016592]
- Xie L, Yan X, Du Y. An aptamer based wall-less LSPR array chip for label-free and high throughput detection of biomolecules. *Biosens Bioelectron*. 2014; 53:58–64. [PubMed: 24121209]
- Yang WHSGCVRP. Discrete dipole approximation for calculating extinction and Raman intensities for small particles with arbitrary shapes. *J Chem Phys*. 1995; 103(3):869–875.
- Ye X, Zheng C, Chen J, Gao Y, Murray CB. Using binary surfactant mixtures to simultaneously improve the dimensional tunability and monodispersity in the seeded growth of gold nanorods. *Nano Lett*. 2013; 13:765–771. [PubMed: 23286198]
- Yu C, Irudayaraj J. Multiplex biosensor using gold nanorods. *Anal Chem*. 2007; 79:572–579. [PubMed: 17222022]
- Yvert JP, Delagneau JF. Simultaneous multiple immunoassays: current data. *Ann Biol Clin (Paris)*. 1993; 51:43–48. [PubMed: 8338255]
- Zeman EJSJC. An accurate electromagnetic theory study of surface enhancement factors for Ag, Au, Cu, Li, Na, Al, Ga, In, Zn, and Cd. *J Phys Chem -US*. 2011; 91(3):634–643.

Highlights

- Chip-based multiplex gold nanorod biosensing is described.
- The GNR nanoarray assembly assembly on glass substrate in a controllable pattern.
- The GNR nanoarray chip could provide a high sample throughput, multi-assay.

Figure 1.

A: UV-vis spectra of fabricated Au nanorods. The longitudinal plasmon peak is tunable by varying rod aspect ratio. B: mixture of two sized nanorods results in two distinct longitudinal SPR band maxima (ca. 630 and 840 nm respectively) in the absorption spectrum. Inset: SEM image of longer (AR: 4.5) and shorter (AR: 2.6) Au nanorod mixture assembled on the glass substrate.

Figure 2.

A: detection of rabbit IgG targets of varying amounts using AuNRs of aspect ratio of 2.6 with anti rabbit IgG antibody. Inset: calibration curve of longitudinal plasmon shift vs. rabbit IgG concentration. B: detection of human IgG targets by anti human IgG-AuNRs of aspect ratio 4.5. Inset: calibration curve of longitudinal plasmon shift as a function of human IgG concentrations. Each data point is presented as mean $\pm$ SD.

A. Thiolation of antibody, followed by GNR functionalization

B. Fabrication of multiplex GNR biochip

C. Optical detection

Figure 3.

Schematic of GNR biofunctionalization and design of label-free, multiplexed biosensing by gold nanorods of different aspect ratios.

Figure 4. Multiplexed biosensing of human and rabbit IgG samples at various concentrations. The shorter wavelength around 630 nm is dedicated for rabbit IgG assay, while the longer one around 840 nm for human IgG assay. Insets A and B: comparison of the multiplexed biosensing performance with single analyte detection.

Figure 5.
Minimal cross-reactivity of antibody moieties immobilized on the gold nanorod sensor.

Figure 6.

A: representative picture of gold nanorod array on glass substrates which can be expanded to a high throughput biosensing chip. B: UV-vis spectra of gold nanorods deposited in nine-dot array pattern on glass chip. C: simultaneous detection of nine samples spiked with varying rabbit IgG concentrations from 10 to 300 nM using respective nanorod sensor in the nanoarray chip. D: Multiplexed biosensing of rabbit and human IgG at varying concentrations by the respective sensing dots on the nanoarray biochip.