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Multiplexed gold nanorod array biochip for multi-sample analysis

Yanyan Wang, Liang Tang*

Department of Biomedical Engineering, University of Texas at San Antonio, One UTSA Circle, San Antonio, TX 78249, USA

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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 plamonic 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.

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 1300 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 and Irudayaraj (2007) demonstrated the simultaneous detection of three different species of anti-IgG molecules in biological buffers using a mixture of three-sized GNRs. However, solution-based nanorod bioprobe has intrinsic disadvantages. Due to multiple wash, the inherent artificial fluctuation in optical readings from nanoparticle amount change cannot 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 chemisoprtion 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

* Corresponding author. Tel.: +1 210 458 6557; fax: +1 210 458 7007.
E-mail address: liang.tang@utsa.edu (L. Tang).

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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 ~6000) 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 (~50 °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 over night 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 spinned twice at 8500 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 Traunt's reagent (5 mg/mL) were fist added to 0.1 mL of antibodies (i.e. anti-rabbit IgG or anti-human IgG) for 2 h 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, 10 μL of antibody-immobilized GNRs with mixed aspect ratios were dropped onto designated spots on the thiol-terminated glass substrates and incubated for 2 h. 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 h. To perform a label-free assay, a sample solution (10 μl) with respective 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.