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Short communication

A label-free biosensor based on gold nanoshell monolayers for monitoring biomolecular interactions in diluted whole blood

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Abstract

Gold nanoshells (GNSs) were self-assembled on the surface of transparent glasses modified with 3-aminopropyltrimethoxysilane (APTES) to form GNS self-assembled monolayers (SAMs). Because the localized surface plasmon resonance (LSPR) of GNSs can be controlled in the near-infrared (NIR) region of the spectrum, where the optical transmission through tissue and whole blood is optimal, GNSs would be used as an effective signal transduction in whole blood. Accordingly, after modified with cystamine and biotin-NHS (N-hydroxy succinimide), GNS SAMs were used as a novel optical biosensor for real-time detection of streptavidin–biotin interactions in diluted human whole blood within short assay time, without any sample purification/separation. An UV–vis–NIR spectrophotometer was used to monitor the absorbance changes at 730 nm as a function of time for different concentrations of streptavidin in 20% whole blood, and the results showed that the biosensor displayed low detection limit of ~3 μg/mL and wide dynamic range of ~3–50 μg/mL. This approach provides an opportunity to construct LSPR biosensor for protein sensing and cellular analysis in diluted whole blood.
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Keywords: Gold nanoshells; Self-assembled monolayer; Biosensor; Localized surface plasmon resonance; Streptavidin; Biotin

1. Introduction

Metal nanoparticles (MNPs)-based biosensors have been developed to detect a variety of biomolecular binding events, including DNA and RNA hybridizations (Cai et al., 2003; Elghanian et al., 1997; Rosi et al., 2006; Hamad-Schifferli et al., 2002; Souza and Miller, 2001), antibody–antigen interactions (Schultz et al., 2000; Mann et al., 2000), carbohydrate–protein interactions (Lin et al., 2002), enzyme–substrate interactions (Hong et al., 2004) and cell–ligand interactions (Gu et al., 2003). The key challenge to the successful application of biosensor is transforming molecular recognition events into quantifiable signals. Many of biosensor researches have been devoted to the evaluation of the relative merits of various signal transduction methods including electrochemical (Xiao et al., 2003; Yang et al., 2006), magnetic (Perez et al., 2003), optical absorption (Wilson, 2002), fluorescent (Niethammer et al., 2004; Kalab et al., 2006), interferometric (Yu et al., 2000; Cross et al., 2003; Lin et al., 1997), and others (Shekhawat et al., 2006). Recently, the methods of MNPs-based localized surface plasmon resonance (LSPR) have made an important contribution to the quantification of biomolecular interactions. This is mainly because of the unique LSPR of MNPs, which is strongly dependent on the size, shape, dielectric properties, spatial arrangement of MNPs, as well as refractive index (RI) of the local environment (Malinsky et al., 2001; Jain et al., 2006; Liu and Lu, 2006). For instance, the changes of LSPR ascribed to the growth (Pavlov et al., 2004, 2005), the aggregation (Liu and Lu, 2003, 2004, 2006; Mann et al., 2000) and the local RI changes (Haes et al., 2005; Nath and Chilkoti, 2002) of MNPs were used to achieve the transduction of biomolecular or chemical binding events into optical signals.

Many of these optical biosensors were employed to detect analytes in the media of buffered solution or simulated body fluid. However, whole-blood samples analyses were rarely carried out, because of the complex biological system and the high absorption intensity of whole blood which often strongly interferes with the optical signal interest. For the highly sensitive and widely applied SPR biosensor, analytes measurement in

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whole blood has not yet been reported. The only SPR-based measurements in blood samples currently are the coagulation of both whole blood (Hansson et al., 2002) and blood plasma (Vikinge et al., 2000). Conventional approaches of immunoassay, such as enzyme-linked immunosorbent assays (ELISAs), are time-consuming for sample preparation/separation and labeling procedures, as well as repeated rinsing processes. Optical test of conventional blood immunoassays are performed at visible wavelength, which needs a purification step to separate out a variety of unwanted biomaterials that absorb visible light, and the whole procedure can take several hours or days (Brongersma, 2003). In addition, considering a number of relevant analytes that are bound to blood cells (e.g. cyclosporine), the values measured in plasma/serum will differ from that in whole blood. Thus, it would be amenable to develop biosensors for biomolecular detections or immunoassays in whole blood assay without any sample preparations. In order to minimize the background interference, several approaches have been developed to construct biosensors based on near-infrared (NIR) nanomaterials for analysis in whole-blood samples (Ting et al., 2006; Hirsch et al., 2003).

In this paper, we demonstrate a novel biosensor based on the LSPR of gold nanoshell (GNS) self-assembled monolayers (SAMs) for real-time detection of analytes in diluted whole blood. The LSPR of GNSs (consisting of a silica core covered with a thin gold shell) can be varied systematically by changing the relative dimensions of the core and the shell layers. The optical resonance can be controlled in the NIR regions of the spectrums (700–1300 nm), where optical transmission through tissue is optimal (Hirsch et al., 2003). Consequently, there are three advantages of this biosensor as follows: (1) The LSPR peaks of monodisperse gold nanoparticles (GNPs) with the diameter ranged from 8 to 99 nm are always limited in the spectra region from 518 to 575 nm (Kim et al., 2006), indicating that the LSPR of GNPs may be interfered severely at the presence of whole blood which shows strong absorption at this spectra region. In contrast, owing to the NIR optical properties, GNSs can avoid the interference and achieve the signal transduction in whole-blood samples. (2) Compared with conventional approaches of immunoassay, such as ELISAs, the LSPR biosensor enables label-free detection of biomolecular interactions within a short assay time, without any sample purification/separation. (3) Compared with optical complexity of SPR spectroscopy, this biosensor can be implemented using extremely simple, small, robust, low-cost equipment for unpolarized, UV–vis–NIR spectrophotometer.

2. Experimental

2.1. Materials

All reagents were used as received without further purification except silica colloids: 3-aminopropyltrimethoxysilane (APTES, Sigma), cystamine-hydrochloride·$2H_2O$ (Fluka), *N*-hydroxysuccinimide-biotin (NHS-biotin, Sigma), streptavidin (Sigma), bovine serum albumin (BSA, Sigma), silica colloids (~100 nm in diameter, from Nissan Chemical America Corporation), hydrogen tetrachloroaurate-(III) hydrate, sodium borohydride ($NaBH_4$), sulfuric acid (H_2SO_4, 98%), hydrogen peroxide (H_2O_2, 30%), dipotassium hydrogenphosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium carbonate (K_2CO_3, 99%), ethanol (99.7%) and toluene (all from Nanjing Sunshine Biotechnology Ltd.). Phosphate buffered saline (PBS, 0.05 mol/L, pH 7.4) was prepared by mixing stock standard solutions of K_2HPO_4 and KH_2PO_4. Human blood samples collected in EDTA tubes were supplied by Hospital of Southeast University and stored at 4 °C until needed. Whole blood was diluted with PBS (pH 7.4).

2.2. Synthesis of GNPs

By the reduction of chloroauric acid with sodium borohydride, the aqueous suspensions of small GNPs with ~5 nm in diameter were prepared. Typically, 3 mL of 1% $HAuCl_4$ was mixed with 200 mL of triply distilled water with vigorous stirring, followed by the addition of 1 mL of an aqueous solution of K_2CO_3 (0.2 M). Afterwards, 9 mL of $NaBH_4$ (0.5 mg/mL) was quickly added to the mixture. The wine-red solution was stored at 4 °C until use. Assuming that all chloroauric acid was reduced to ~5 nm GNPs and using the bulk density of gold, we calculated that the concentration of the obtained GNP aqueous solutions was $\sim 2 \times 10^{14}$ particles mL^{-1}.

2.3. Synthesis of GNSs

GNSs were fabricated as described in our previous work (Wang et al., 2007; Wang et al., 2006). Silica colloids were purified by centrifuging and redispersing for five times in ethanol. And then 0.275 mL of APTES was added to 35 mL of purified silica colloids (0.12 g/mL in ethanol) under vigorous magnetic stirring at 45 °C for 3 h. APTES-functionalized silica colloids were purified by centrifuging and redispersing in ethanol. APTES-functionalized silica colloids ($\sim 5.3 \times 10^{11}$ particles/mL in ethanol) were added dropwise to the 200 mL of gold sol under vigorous magnetic stirring (600 rpm) to form gold-attached silica colloids (Au/SiO_2 nanoparticles). After centrifuged and removing the supernatant for three times, 200 mL of Au/SiO_2 nanoparticles were redispersed in 45 mL of water.

Under continuous stirring, 0.7 mL of gold-attached silica colloids were added to 60 mL of the aged $HAuCl_4/K_2CO_3$ solution, followed by 0.15 mL of formaldehyde to form the GNSs. The amount of the added gold-attached silica colloids was depended on the intended thickness of the gold shells. The suspensions were centrifuged to concentrate the GNSs for further use and to remove excess reagents.

2.4. Fabrication of GNS SAMs

Glass slips (9 mm × 55 mm) as substrates were cleaned by sonication for 10 min in hot RBS 35 detergent and washed with triply distilled water at least five times. The slips were further cleaned in a 7:3 solution of H_2SO_4 (98%) and H_2O_2 for 40 min, washed extensively with triply distilled water, and dried

overnight at 70 °C. The cleaned glass slips were immersed in a 1% (v/v) solution of APTES in anhydrous ethanol at 80 °C for 20 min, rinsed five times in ethanol with sonication, and dried at 120 °C for 3 h. The silanized glass slips were subsequently immersed overnight in a GNS solution to form SAMs on both sides of the glass slips.

2.5. Preparation of functional interfaces

The GNS SAMs were amino modified by immersing the slides in a solution of 10 mM cystamine in water for 2 h. Biotinylated surfaces were obtained by reaction with biotin-NHS (200 μg/mL in DMSO) for 1.5 h. The resulted interfaces were blocked with BSA by immersing it in a PBS (pH 7.4) with 400 μg/mL of BSA for 5 h. Exposure to streptavidin was carried out by immersion the interface in 20% whole blood with different concentrations of streptavidin for 1 h. The schematic fabrication process was illustrated in Fig. 1.

2.6. Characterization of GNS SAMs

The morphology of GNS SAMs was characterized with a LEO 1530 VP scanning electron microscopy (SEM). All samples for SEM observation were sputtered with thin gold films. Absorption spectra were employed to investigate the optical properties of these samples by using a Shimadzu UV3150 UV–vis–NIR spectrophotometer in a transmission mode. All transmission measurements were performed at constant regions.

3. Results and discussion

3.1. Preparation and characterization of GNS SAMs

Some of the GNSs are aggregated when self-assembled on glass substrate (see supporting information, Fig. S1), it is possibly ascribed to the high concentration of GNSs in solution before self-assembly and the polarization of GNSs aroused by the positive charged amino group of APTES (Tan et al., 2006). After immersed in different solvents, GNS SAMs show no distinct changes in absorption spectra (data not shown), suggesting that the solvent did not cause spectroscopically detectable detaching of GNSs on the glass substrate. The ability of GNS SAMs to transduce the changes of surrounding RI into that of LSPR was investigated. The result shows that the LSPR of GNS SAMs exhibited a red-shift at the peak wavelength (λ_{max}) along with an increase in the peak intensity as the RI of the environment increased from 1.000 to 1.495 (Fig. 2A). The split of the two absorption maximum, as shown in Fig. 2A, may be ascribed to the different sensitivities between quadrupole and dipole resonance of GNSs to RI of solvent (Tam et al., 2004). These results are consistent with the Mie theory as shown by Halas et al. (Tam et al., 2004). The plot of the absorbance at 730 nm as a function of RI shows a sensitivity of 0.31 absorbance unit (a.u.)/RIU. Thus, we were encouraged to investigate the possibility whether the biomolecular binding events could also be transduced into detectable changes in the absorbance spectrum. We chose the absorbance changes at the fixed wavelength of 730 nm to monitor the biomolecular binding events in real-time.

3.2. Optical properties of GNS SAMs in diluted whole blood

Owing to the complex biological system, whole blood as well as the baseline has strong and ruleless absorption in the spectra region under 600 nm, however, it has no substantial absorption in the region above 600 nm (Deng et al., 2006), as shown in Fig. 2Ba. The LSPR peak of GNSs can be present obviously in 20% whole blood (Fig. 2Bc). Compared with LSPR of GNS SAMs in water, the LSPR peak of GNS SAMs in 20%

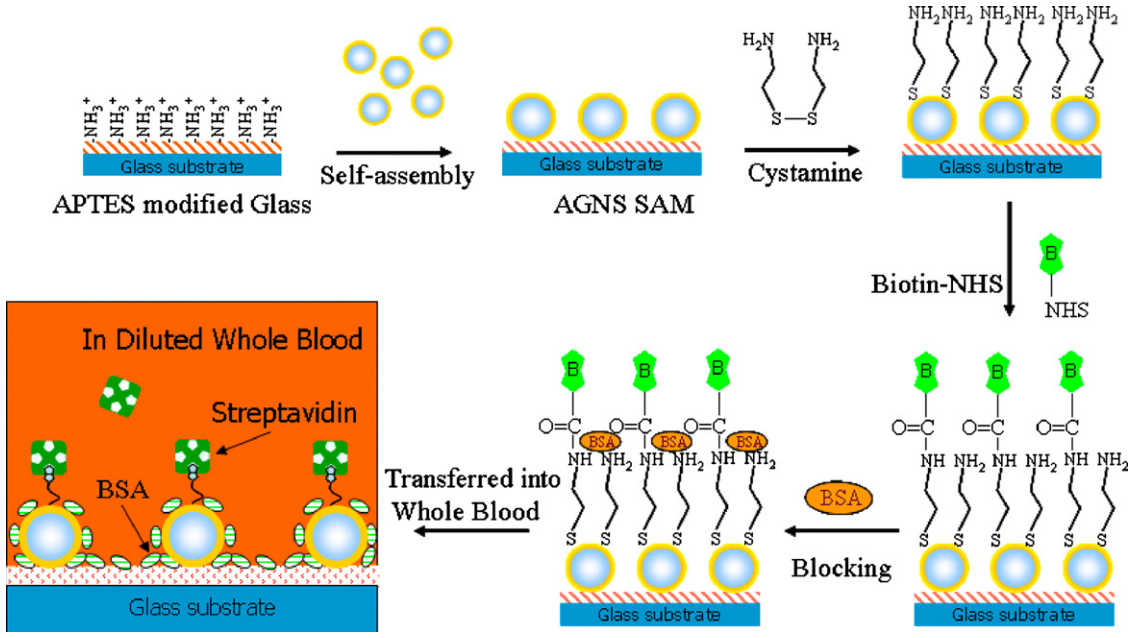

Fig. 1. Schematic fabrication process of the gold nanoshell SAMs and the analysis of streptavidin.

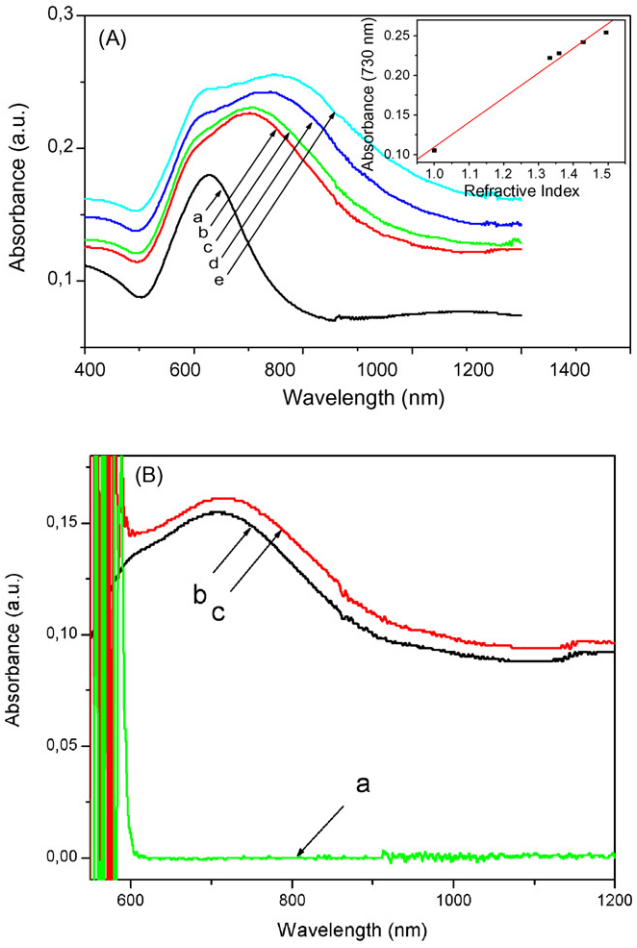

Fig. 2. (A) Absorption spectra of a GNS SAM in the following: (a) air ($n = 1.000$), (b) water ($n = 1.333$), (c) ethanol ($n = 1.360$), (d) 1:1 (v/v) ethanol–toluene ($n = 1.429$) and (e) toluene ($n = 1.495$). The inset shows the dependence of the peak wavelength as well as the absorbance at 730 nm on the refractive index of the surrounding medium. (B) Absorption spectra of (a) baseline of 20% whole blood, (b) a GNS SAM in water, and (c) in 20% whole blood.

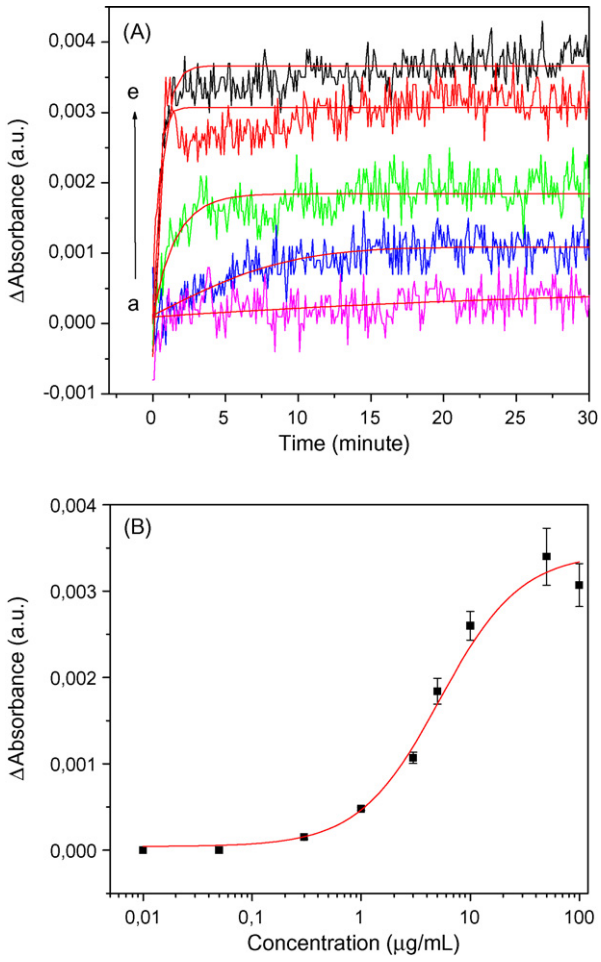

Fig. 3. (A) Time-dependent change of the LSPR intensity at 730 nm as a result of specific binding of streptavidin to the surface of biotin-modified GNS SAMs in 20% whole blood: (a) 0.3, (b) 3, (c) 5, (d) 50, and (e) 100 μg/mL. (B) Absorption change at 730 nm as a function of streptavidin concentration in 20% whole blood.

whole blood red-shifts with about 0.008 a.u. increase in intensity, ascribed to the larger RI of blood.

3.3. Biosensor of streptavidin in diluted whole blood

Proteins such as streptavidin and BSA (negative charged at the working pH, ~7.4) could adsorb non-specifically to APTES molecules, unreacted cystamine molecules (positive charged) or to bare GNSs surface of the SAMs. Considering the possible non-specific binding of the streptavidin and proteins in blood, which may influence the specific analysis, we used BSA as a blocking agent prior to streptavidin binding. After blocking with BSA in PBS (pH 7.4), the intensity of the GNSs LSPR at $\lambda = 730$ nm increases by about 0.003 a.u. (Fig. S2). After immersed in 20% whole blood for more than 0.5 h, the BSA-blocked, biotin-modified GNS SAM shows no detectable changes in the absorbance intensity at $\lambda = 730$ nm (data not shown). This means that BSA is an effective agent for blocking non-specific absorption.

We further investigated the concentration-dependent absorbance change to study the dynamic range and sensitivity of this sensor. The BSA-blocked, biotin-modified GNS SAM was incubated in 20% human whole blood with different concentrations of streptavidin ranging from 0.01 to 100 μg/mL. Fig. 3A shows the absorbance changes at 730 nm as a function of time for different streptavidin concentrations in 20% whole blood. Boltzmann fit was used to fit the time-dependent curve, as shown in Fig. 3A. The steady time would be less than 15 min when the concentration of the streptavidin is higher than 3 μg/mL. Three different replicates made independently show acceptable reproducibility for the absorbance changes at the same concentration of streptavidin. A calibration plot of the absorbance change at 730 nm, after 30 min incubation, versus streptavidin concentration yielded a detection limit of ~3 μg/mL, when considering the signal-to-noise ratio (S/N) as 3 for the samples. The dynamic range of the sensor is ~3–50 μg/mL, as shown in Fig. 3B. These results indicate that GNS SAMs are considerably sensitive to transduce ligand–receptor bindings on the surface into an absorbance change. Compared with the biosensor based on GNP SAMs, which was carried out to detect streptavidin in buffer solution

(Nath and Chilkoti, 2002), GNS-based biosensor presents wider dynamic range. Although the noise of the time-dependant absorbance change is relative larger, we believe that a much lower detection limit can be achieved by optimization of both the surface chemistry (e.g. surface density, particle size and core/shell ratio) and the detector. We believe that the proposed method employing diluted whole blood would be used for detection of proteins, cellular analysis and measurement of analytes bound to blood cells. In addition, this method has potential to detect analytes of different substance classes in complex media like milk, or body fluids.

4. Conclusion

In summary, based on the LSPR of GNSs presented in the NIR region of the spectrum, a simple and low-cost way was achieved to monitor biomolecular interactions in diluted whole blood, on a convenient UV–vis–NIR spectrophotometer. Considering the large background noise of the time-dependant absorbance change for this biosensor, we will try to optimize the surface chemistry of the GNS SAMs. Furthermore, the construction of biosensors to detect the disease biomarkers or other biomolecules in human diluted whole blood will be our future goals. We believe that our methods could offer an opportunity to construct LSPR biosensors based on NIR nanomaterials, a variety of which would be the candidates, for applications in protein sensing and cellular analysis in diluted whole blood.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.bios.2007.10.020.

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