Biorecognition-Driven Self-Assembly of Gold Nanorods: A Rapid and Sensitive Approach toward Antibody Sensing

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Gold nanoparticles possess optical properties that make them uniquely suitable for chemical and biological sensing because of their shape and size-dependent optical properties, whose origin is localized surface plasmon resonance (LSPR).[1–3] Most of the existing work utilizing the LSPR mechanism was based on the binding-induced aggregation of spherical gold nanoparticles. Specifically, Mirkin and co-workers demonstrated that the distance-dependent optical properties of aggregation of gold nanoparticles, induced by DNA hybridization, was exploited for developing a one-pot colorimetric sensor for DNA detection, as well as the detection of single-base mismatches in DNA hybridization.[4–6] Spherical gold nanoparticles have extensively been employed as analytical probes in biotechnological systems such as the diagnostic and biological imaging, as well as cancer therapy.[7–11]

It is known that the absorption spectra of rod-shaped gold nanoparticles (known as gold nanorods: GNRs) exhibited two plasmon absorption bands corresponding to the longitudinal band along the long axis (SP_{long}, at a longer wavelength depending on the aspect ratio of GNRs) and the transverse band assignable to the short axis (SP_{trans}, at a shorter wavelength, ca. 520 nm), respectively.[12–15] This unique optical property of GNRs opens up fascinating applications in biological and chemical sensors. GNRs should provide several advantages over spherical gold nanoparticles for LSPR sensing: (1) the absorption spectrum of GNRs shows two peaks assignable to the transverse and longitudinal surface plasmon bands, respectively, (2) the LSPR properties of GNRs can be tuned by adjusting their aspect ratio from the visible to the NIR region, and (3) the SP_{long} band is extremely sensitive to changes in the dielectric properties of the surroundings including solvents, adsorbates, and the interparticle distance of the GNRs. In addition, the sensitivity increases as the aspect ratio of the GNRs increases.[16–18]

Recently the analyte-induced aggregation of GNRs has been utilized for a qualitative and quantitative analysis of amino acids and peptides by employing the significant LSPR wavelength shift.[19] In this system, GNRs bearing thiol and zwitterionic groups anchored to their ends formed a unique end-to-end connection via a two-point electrostatic interaction, resulting in remarkable interplasmon coupling. However, the use of GNRs as biosensors for protein has not been widely pursued. This is because the cetyltrimethylammonium bromide (CTAB) layers on the surfaces of GNRs especially at the flat sides cannot be easily displaced by biomolecules, while displacement is more favorable at the tips, which has been exploited in several recently published end-to-end linkage protocols.[20–22] On the other hand, the large amount of CTAB dispersed in the solution causes denaturation of the proteins and engenders significant cytotoxicity.[23–25] Thus, GNRs have found limited applications in biological sensing, imaging, and therapy.[26–29] More recently, Ma et al. demonstrated a new colorimetric method for protein sensing by LSPR using silica coated GNRs, diminishing CTAB disadvantages, immobilized on the surfaces of glass slides as the biosensing substrates.[30] Therefore, functionalized GNRs with

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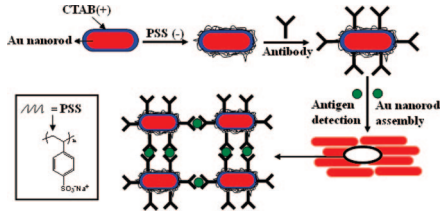

Figure 1. Schematic representation of detection of h-IgG through the assembly of GNRs driven by antibody–antigen recognition.

a variety of molecules will provide a novel nanosensor platform based on LSPR and function as an anisotropic-shaped colorimetric reporter, which originated from the GNRs LSPR properties highly sensitive to the changes in the dielectric constant of the local environment of the GNR surfaces.

Herein, we report a rapid and sensitive method for protein biosensing such as h-IgG as a model using LSPR of GNRs modified by poly(styrene sulfonate) (PSS) based on changes in wavelength in an aqueous solution. The surface of GNRs with average aspect ratio 3.5 functionalized by PSS for the attachment of goat anti-h-IgG yielded GNR molecular probes, which exhibited the rapid and sensitive detection of h-IgG by localized surface plasmon absorption (LSPR). Plasmonic sensors are highly rapid and sensitive and can be used to monitor both changes in refractive index caused by molecular interactions in the vicinity of the GNRs and the biorecognition-driven assembly of GNRs process. Anionic polymer PSS coating on the GNRs surrounded by positively charged CTAB molecules not only diminishes the cytotoxicity of CTAB but makes it stable to the attachment of goat anti-h-IgG, forming GNR molecular probes. More importantly, PSS-capped GNRs, rather than spherical gold nanoparticles with similar diameter, offer a larger surface area (the flat sides and tips) for the adsorption of proteins. Additionally, the aggregation of GNRs which were preferentially oriented in a lateral (side-to-side) fashion driven by specific antibody–antigen binding process was observed, resulting in the marked wavelength shift and the reduction of intensity along with a significant widening of the plasmon for the rapid and sensitive detection purposes. A very small amount (30 ng) quantity of h-IgG added into GNR molecular probe solution (500 μL) leads to a marked red shift of LSPR in a very short time (ca. 100 s). The results show that the limit of detection of h-IgG is 60 ng mL^{-1} using GNR molecular probes. This novel method using GNRs with different aspect ratios is simple and sensitive, may provide a novel optical multiplex biosensor platform, and has broad potential applications in immunoassay and disease diagnosis.

A schematic diagram suggesting the coupling of the goat anti-h-IgG to the GNRs and h-IgG is shown in Figure 1. The GNRs were synthesized using a seed-mediated growth method described by Nikoobakht and El-Sayed (see the Supporting Information for experimental details).[31] The as-prepared GNRs with average aspect ratio 3.5 were fairly

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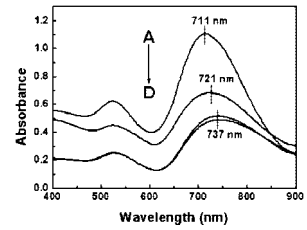

Figure 2. UV–vis absorption spectra at different stages of (A) as-prepared GNRs with average aspect ratio 3.5; (B) PSS-capped GNRs; (C) PSS-coated GNRs/goat anti-h-IgG conjugates; (D) PSS-coated GNRs/goat anti-h-IgG conjugates interacted with BSA prior to exposure to h-IgG.

uniform in shape and highly dispersed in water without aggregation. Initial GNRs were capped with a bilayer of CTAB, which is positively charged. A typical procedure for preparing GNR molecular probes is as follows: The original GNRs were centrifuged at 14 000 rpm twice to get rid of the extra free CTAB molecules in solution. The positively charged surface of GNRs was changed to a negatively charged surface by exposing GNRs to PSS (molecular weight = 15 300) polyelectrolyte solutions. The extra PSS in solution was separated by centrifuging GNRs at 10 000 rpm for 15 min After that, the precipitate of the GNRs was redispersed in phosphate-buffered saline (PBS; pH = 7.4). The PSS-capped GNRs were then mixed with goat anti-h-IgG solution. The GNRs conjugated with goat anti-h-IgG were centrifuged and redispersed into PBS solution, so as to remove unbound antibodies. The resulting GNR molecular probes were incubated in a bovine serum albumin (BSA) blocking solution to preclude nonspecific binding and then used for detection of h-IgG (see experimental details at the Supporting Information).

Figure 2 shows the absorption spectra of as-prepared GNRs (curve A), PSS-capped GNRs (curve B), PSS-coated GNRs/goat anti-h-IgG conjugates (curve C), and those interacted with BSA prior to exposure to h-IgG (curve D). The SP_{long} band of the as-prepared purified GNRs without free CTAB is observed at 711 nm as shown in Figure 2 (curve A). Upon capping these GNRs by a layer of PSS, there is a red shift and broadness in this band to 722 nm (curve B), which reflect the variation of the local dielectric function of PSS-coated GNRs versus as-prepared nanoparticles and possible aggregation of the GNRs. A greater additional red-shift (737 nm; curve C) was observed upon exposing to the excess amount of goat anti-h-IgG solution (50 μg mL^{-1} in PBS solution) for 30 min, because the protein has a higher refractive index than that of PSS. However, the transverse bands of GNRs undergo almost no wavelength shift (curves A–D) which can be attributed to the fact that the SP_{long} band more excellently responds to the binding to the target biomolecules in comparison with that of SP_{trans} band. To avoid the nonspecific binding of the uncovered GNR surfaces during the immunoassay, no significant change in the wavelength (curve D) was observed when using 50 μg mL^{-1} BSA in PBS (pH = 7.4) buffer solution for 30 min as the blocking solution, suggesting saturation coverage of the surface with goat anti-h-IgG.

Figure 3 shows the changes in the absorbance of the GNR molecular probes, PSS-coated GNRs/goat anti-h-IgG conjugates, with various times after the addition of

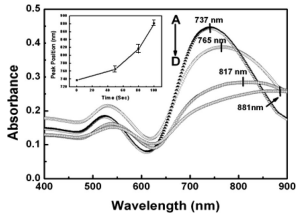

Figure 3. UV–vis absorption spectra of (A) PSS-coated GNR/goat anti-h-IgG conjugates with h-IgG added (30 ng) at various times: (B) 50 s; (C) 80 s; (D) 100 s. Inset shows peak position as a function of time corresponding to curves A–D.

h-IgG. The SP_{long} band of the GNR molecular probes in aqueous medium was observed at 737 nm. After the addition of a very small quantity (30 ng) of h-IgG into 500 μL of GNR molecular probe solution, the intensity was dramatically reduced accompanied with a peak red-shift to much longer wavelength (765 nm) in a short time (ca. 50 s.). This kind of shift was attributed to the preferential assembly of GNRs driven by molecular binding events in a side-to-side fashion. Broadening of the SPR peak was also expected. As the time of h-IgG binding increases (from 50 to 80 to 100 s), the SP_{long} bands of the GNRs decrease in intensity and red shift to longer wavelengths (from 765 to 817 to 881 nm; Figure 3, curves B–D), accompanied by widening the SPR peak. This is due to GNR aggregation induced by the target specific binding events with side-to-side and side-to-end fashions with the increasing time of the molecular recognition of goat anti-h-IgG and h-IgG.[22,32] In all, the presence of h-IgG recognized by GNR molecular probes changes not only the spectral position and width of the LSPR band but also decreases the intensity in a short time. Figure 3 (curves B–D) depicts the detection of h-IgG using GNR molecular probes via successive SP_{long} changes, such as red shift, broadening, and reducing in the intensity. The SP_{trans} peaks near 520 nm are not very sensitive to the refractive index change induced by target binding, and the red-shifts of these peaks are below 10 nm. In contrary, the SP_{long} bands of the GNRs are extremely sensitive to the refractive index changes induced by target binding, suggesting that they are excellent reporters of target specific binding events. The inset in Figure 3 shows the peak position as a function of time. It should be noted that the addition of 30 ng of h-IgG can cause a SP_{long} band obvious change from 737 to 765 nm in less than 1 min, indicating that it is a rapid and sensitive method to detect h-IgG through the LSPR-band changes of GNRs only using a simple optical spectrometer.

Evidence for CTAB-stabilized GNRs functionalized with PSS and subsequently goat anti-h-IgG anchored on the PSS-capped GNRs and then attached to h-IgG can be seen from Fourier transformation infrared (FTIR) spectra (see Supporting Information, Figure S1). Figure 4 represents transmission electron microscopy (TEM) images of as-prepared GNRs (A), PSS-coated GNRs (B), PSS-coated GNRs/goat antihuman IgG conjugates (C), and those after the treatment with h-IgG (D). From the TEM images, one

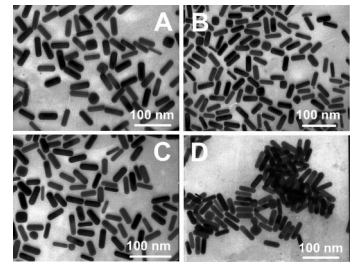

Figure 4. TEM images of (A) as-prepared GNRs; (B) PSS-coated GNRs; (C) PSS-coated GNRs/goat anti-h-IgG conjugates; and (D) those after treatment with h-IgG (30 ng).

can observe that the initial GNRs are uniform and dispersible. The dispersity of GNRs is not changed after being coated with PSS and goat antihuman IgG, respectively. Upon binding GNR molecular probes to h-IgG, however, the aggregation of GNRs, which was preferentially oriented in a lateral (side-to-side) fashion driven by specific antibody–antigen binding process, was observed (Figure 4D). Thus, the position, intensity, and shape of the LSPR band depend upon the changes in combination with both the local refractive index and the aggregation of GNRs. The LSPR-band response is sensitive to both changes in refractive index caused by molecular interactions in the surroundings of the GNRs and the aggregation of GNRs driven by biorecognition.

To address the nonspecific adsorption of other proteins on the PSS-capped GNRs immobilized by goat anti-h-IgG, control experiments have been performed by adding 100 ng mL^{-1} mouse IgG instead of h-IgG. No LSPR-band change was observed. These results indicate that the changes in SP_{long} bands of GNRs arise from specific goat anti-h-IgG and h-IgG binding.

In conclusion, we demonstrated a rapid and sensitive approach to detect protein such as IgG using the localized surface plasmon absorption of the GNRs. The detecting limit of the present method is 60 ng mL^{-1}. The sensitivity of this assay is increased compared to that based on spherical gold nanoparticles as probes with a detection limit of 0.1 μg mL^{-1}. In addition, aggregation of GNRs which were preferentially oriented in a lateral (side-to-side) fashion driven by biorecognition process was observed, which is sensitively reflected in their LSPR bands, especially in the longitudinal bands. These characteristics make them extremely sensitive reporters of molecular binding events with excellent biosensing capability. In principle, the design concept presented herein may be extended to act as the platform using GNRs with various aspect ratios to develop the multiplex assays for the detection of molecular binding events.

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Supporting Information Available: Experimental details and FTIR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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