Gold Nanoparticle Bioconjugates for Biomolecular Detection

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ABSTRACT

We recently reported the development of a highly sensitive homogeneous one-step immunoassay for cancer biomarker detection.[1] We applied this technology for the detection and assay of free PSA (prostate specific antigen), a biomarker associated with prostate cancer. Two gold nanoparticle probes, one spherical gold nanoparticle with a diameter around 35 nm, and a gold nanorod with a dimension of 10 by 40 nm, are coupled with a pair of monocolonal antibody anti-PSA, respectively. Upon mixing the two nanoparticle probes with sample solutions, free-PSA antigen in sample solution caused a nanoparticle aggregation due to antibody-antigen binding. By measuring the degree of nanoparticle aggregation using a dynamic light scattering technique, the antigen concentration in the sample was quantified.

Keywords: gold nanoparticles, homogeneous immunoassay, dynamic light scattering, antibody, cancer biomarker

1 INTRODUCTION

Gold nanoparticles in the size range of 10-100 nm have exceptionally large scattering cross sections at their surface plasmon resonance wavelength region.[2] This property has enabled many important applications of gold nanoparticles for biomolecular imaging and detection.[3] Dynamic light scattering (DLS) is a technique that has been used routinely for particle size and size distribution study. This technique is based on the Brownian motion of spherical particles which causes a Doppler shift of incident laser light. The diffusion constant of particles are measured and the size of the particles is calculated according to the Stokes-Einstein relation.[4] Because of the intense light scattering from gold nanoparticles, we hypothesized that DLS may be combined with gold nanoparticle probes to develop a highly sensitive immunoassay. Following this hypothesis, we designed a homogeneous immunoassay as illustrated in Figure 1. Two gold nanoparticle probes, Nanoprobe 1 and Nanoprobe 2, are coupled with a pair of monocolonal antibodies, capture and detector antibodies towards a specific analyte. By mixing the two nanoprobes with a sample solution, the antigen in the sample solution will introduce a nanoparticle aggregation. By measuring the degree of nanoparticle aggregation using dynamic light scattering, the concentration of antigen in sample can be obtained. This immunoassay is named as *nano*DLSA.

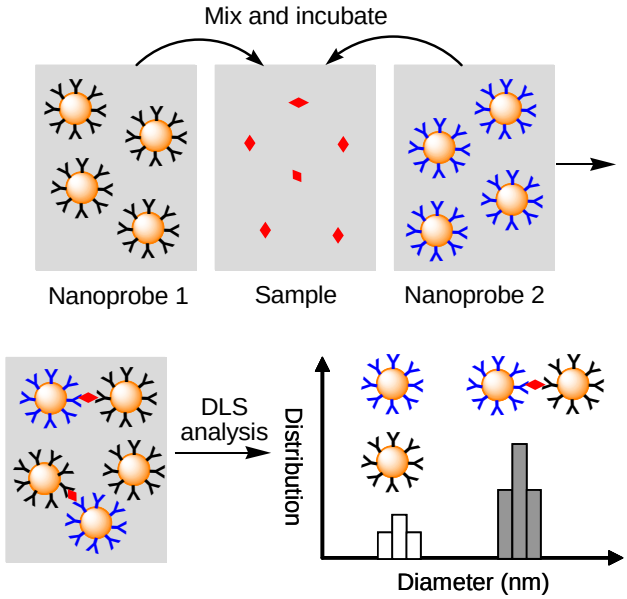

Figure 1: Illustration of a homogeneous immunoassay using gold nanoparticle probes and dynamic light scattering.

2 EXPERIMENTAL

Experimental details for this study can be found in a recently published paper[1] and the electronic Supporting Information.

3 RESULTS AND DISCUSSION

Our initial development of the immunoassay was started at the detection and quantitative analysis of free-PSA, a biomarker associated with prostate cancer.[5] Free-PSA is an unbound form of PSA. The total PSA, which means PSA bound to carrier proteins and free PSA, has been approved by FDA as a biomarker for in-vitro diagnosis of prostate cancer. When cancer occurs and starts to develop, the total PSA level will increase. The established normal threshold for total PSA is about 4 ng/mL. When the total PSA level exceeds 10 ng/mL, the chance for prostate malignancy increases substantially. However, it has been noticed that other non-cancerous conditions may also cause the increase of total PSA level. It

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is now generally recognized the relative ratio of free versus total PSA, instead of total PSA level alone, can provide valuable information for more accurate diagnosis of prostate cancer. The average percentage of free PSA versus total PSA is around 10-25%. Cancer patients typically have lower percentage of free-PSA.

To examine the potential detection limit of our new immunoassay, nanoDLSA, we first examined the detection limit of gold nanoprobes by DLS. Two nanoparticle probes were prepared used in this study, one is a spherical gold nanoparticle (GNP) with a core diameter of 37 nm, and another one is a gold nanorod with a dimension of 10 by 40 nm. Figure 2a and b are the TEM images of these two nanoparticle probes. The light scattering intensity of these two probes at different concentrations was then measured by a DLS. The detection limit determined by DLS for GNP and GNR is 0.02 and 0.4 pM, respectively. This low detection limit suggests that indeed DLS is a very sensitive technique for gold nanoparticle probe detection.

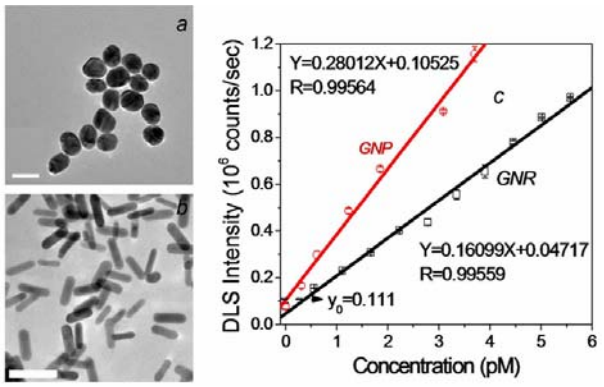

Figure 2: TEM micrographs of (a) gold nanoparticles (scale bar: 50 nm), (b) gold nanorods (scale bar: 60 nm), and (c) their dynamic light scattering intensities and linear regression curves (With a copyright permission from the American Chemical Society).

In the next step, the two nanoparticle probes, GNP and GNR, were coupled with an anti-PSA monoclonal antibody pair, detector and capture antibody, respectively. Both conjugates (GNP-dAb) and GNR-cAb, were prepared by electrostatic adsorption of the antibody to the charged surface of gold nanoparticle probes. While the citrate-protected GNP is surface negatively charged, the GNR is positively charged. In our study, it was found that the detector antibody is more effectively coupled with negatively charged GNP, while the capture antibody is more effectively coupled with positively charged GNR. Figure 3 is the UV-Vis absorption spectra of GNP, GNR and their corresponding bioconjugates, GNP-dAb and GNR-cAb. From the UV-Vis absorption spectra, dynamic light scattering study, and TEM analysis, we confirmed the effective coupling of antibodies to the nanoparticle probes. The hydrodynamic dimension of the GNP-dAb and GNR-cAb became 57, and 37 nm, respectively.

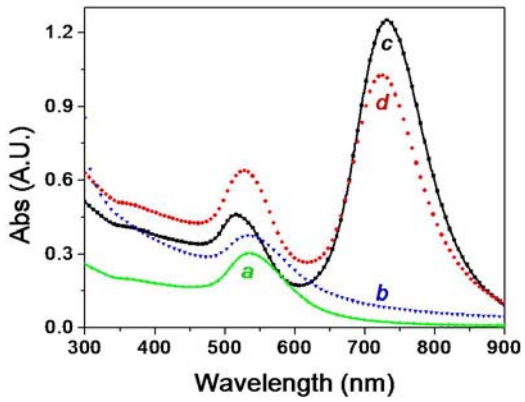

Figure 3. UV-Vis spectra of GNP and GNR and their conjugates with primary antibodies: (*a*) GNP; (*b*) GNP-dAb; (*c*) GNR; and (*d*) GNR-cAb (With a copyright permission from the American Chemical Society).

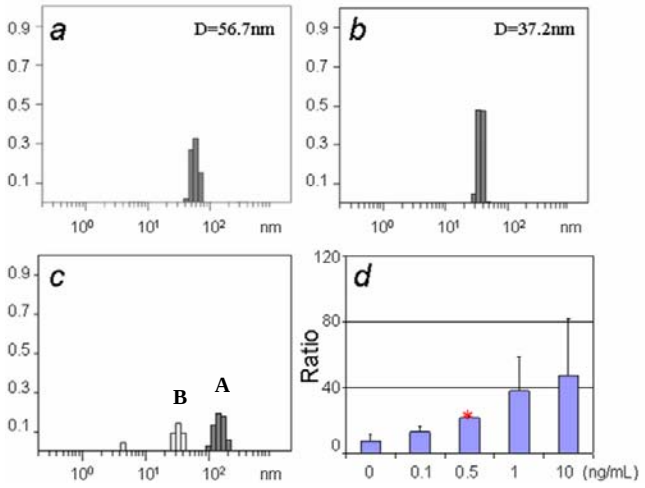

Figure 4: Hydrodynamic diameter distribution plots as determined by DLS measurements: (a) GNP-dAb (5 pM); (b) GNR-cAb (5 pM); (c) a 1:2.5 mixture of GNP-dAb:GNR-cAb in the presence of f-PSA (1.0 ng/mL); and (d) the numerical ratio of nanoparticle oligomers (peak area A) versus individual particles (peak area B) at different concentrations of f-PSA (With a copyright permission from the American Chemical Society).

To conduct the immunoassay for free-PSA, we simply mix the two nanoparticle probes, GNR-cAb and GNP-dAb in a ratio of 2.5:1, with a sample solution. The excessive amount of GNR-cAb is used as an internal standard for antigen quantification. From DLS analysis as shown in Figure 4a, b and c, we can identify two sets of nanoparticle sizes from the assay solution: one belongs to individual nanoparticles and one is attributed to nanoparticle aggregates. For pure individual nanoparticle probe

solutions, only one set of nanoparticle size was observed. The numerical ratio (here we used the intensity ratio) of nanoparticle aggregates (peak area A) versus individual particles (peak area B, the excessive GNR probes) was calculated. This ratio corresponds well to the antigen concentration (f-PSA) in the assay solution. We also prepared an unknown sample. The concentration of the unknown sample as determined by *nano*DLSA corresponds very well to the true concentration of this unknown sample (data labeled with an asterisk).

4 CONCLUSION

This study demonstrates the feasibility of a highly sensitive homogeneous immunoassay technology using gold nanoparticle probes coupled with dynamic light scattering technique. There are several important advantages of *nano*DLSA compared to other immunoassay technologies:

*nano*DLSA is extremely easy to conduct. The assay is a one-step and completely washing free immunoassay. The assay result is read as soon as the sample-nanoprobe incubation is completed, and the reading takes as little as one minute or less. *nano*DLSA requires extremely small amount of samples and antibody conjugates to conduct the analysis. Each assay takes 0.1-5 μL of sample or even less to conduct. As a comparison, a typical ELISA required 50-100 μL of sample solution for each assay. This small amount of sample and antibody conjugates leads to tremendous cost reduction to the immunoassay. Furthermore, the concept demonstrated in our initial work is not limited to the analysis of cancer protein biomarkers, but also can be easily extended to the development of bioassays for fast detection and quantitative analysis of DNAs, therapeutic drugs, and many other biological targets.

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