Optimizing the Optical Properties of Functionalized Silver Nanoparticles by Size

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Abstract

Many studies have focused on harnessing the extraordinary optical properties of noble metal nanoparticles for the development of biological and chemical nanosensors. Metallic nanoparticles emulate surface roughness, facilitating the coupling of light to surface plasmon polaritons. The excitation of the plasmons enhances the electromagnetic field near the surface so that minute changes in the surrounding nanoenvironment's index of refraction induces a measurable change in the nanoparticles response to the optical field. This study endeavors to quantify the general correlation between the nanoparticle sensitivity and the aspect ratio, specified as the in-plane-width over out-of-plane height. The antidinitrophenyl (antiDNP) immunoassay was used as a model for ligand-receptor systems. The fabrication of silver triangular nanoparticles of three different out-of-plane heights was performed by nanosphere lithography (NSL). The binding of antiDNP to dinitrobenzoic acid (DNBA)-functionalized self-assembled monolayers (SAMs) upon the nanoparticle surfaces was measured with localized surface plasmon resonance (LSPR) spectroscopy. Results demonstrated that increasing the aspect ratio of nanoparticles increased extinction shift. Larger LSPR shifts correspond to greater sensitivity of the nanoparticles to adsorption of molecular analytes. LSPR measurements will allow us to develop model systems for approximating the physical properties of the optical nanosensor conducive to optimal performance.

Introduction

A wide interest in nanomaterials and their properties stems from the high demand for smaller, faster, and cheaper devices. The field of chemical and biological nanosensors proves no exception in striving toward miniaturization of medical devices for implantation, in situ diagnosis, and monitoring of diseases. The optical properties of noble-metal nanoparticles are desirable for the realization of a working biochemical nanosensor encompassing these ideal characteristics.

The key advantage of nanoparticles, in addition to their size, is their ability to sense changes in the dielectric constant of their nanoenvironment. This sensitivity can be quantified by LSPR spectroscopy. The LSPR signal is derived from the collective oscillation of conduction electrons, which absorbs and scatters selective wavelengths specific to the properties of the nanoparticles and their surrounding nanoenvironment. This light absorbance and scattering, which occurs at the nanoparticle surface, generates a locally enhanced electromagnetic field that can be detected by optical extinction spectroscopy. The change in the LSPR λ_{max}, caused by varying dielectric constants of different nanoenvironments, can be used to monitor binding and dissociation events of adsorbates on the nanoparticle surface.

The current study aimed to determine the effects of nanoparticle size on the sensitivity of Ag nanoparticles that have been overlayed by a DNBA-terminated SAM. The functionalized SAM was fashioned to increase nanoparticle specificity to the target immunoglobulin molecule, antiDNP. Properties of the nanoenvironment contributed by this overlayer, including the layer thickness, the dielectric constant, the molecular adsorbate dielectric constant, thickness, and coverage, are expected to contribute effect on the extinction spectra of the nanoparticles. Nevertheless, the trend correlating biological nanosensor sensitivity to nanoparticle size is expected to be the same as that for nanoparticle arrays without the SAM overlayer, for which previous studies have demonstrated a growing extinction wavelength shift for increasing nanoparticle aspect ratio.[1,2] The antiDNP immunoassay was used as a model system for determining the dependence of sensitivity on nanoparticle size, applicable to other specific ligand-receptor systems, including protein-ligand, antibody-antigen, protein-carbohydrate, and protein-DNA interactions.[1]

Background

Achieving a nanoparticle-based biochemical sensor for the detection of biomolecules relies on the capability of the nanoparticles to sense changes in their surrounding environment. General preliminary work has studied the effects of various properties of the nanoparticles (size, shape, interparticle spacing) on the sensitivity to perturbations in the nanoenvironment's dielectric constant using solutions of varying refractive indices as well as analyte-containing buffers.[1,4,5,6] The optical size-dependent properties of nanoparticles can be easily controlled by NSL. NSL is a simple, inexpensive, high-throughput fabrication technique using self-assembly of nanospheres. The monolayer of

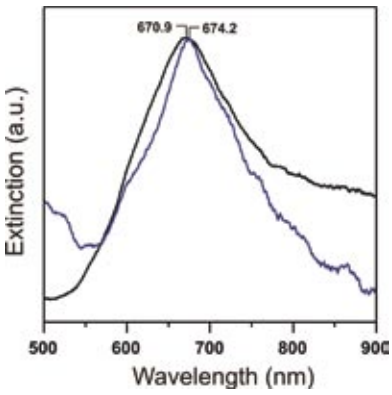


Figure 1: Nonspecific binding study. AntiDNP (1.05 10^{-7} M) was exposed to bare NSL-fabricated nanoparticles.

latex polystyrene nanospheres forms a hexagonal close-packed array that functions as a mask for material deposition via thermal evaporation, electron beam deposition, or pulsed laser deposition. This step controls the out-of-plane height of the nanoparticles depending on the amount of material deposited through the nanosphere mask.

Changes in the concentration of biological molecules are detected by LSPR as a result of the binding action of the biomolecule to the nanoparticle surface. The nanoparticles are functionalized with a SAM designed specifically to target biological molecules. To demonstrate the necessity of the functionalized SAM for LSPR sensing of biomolecules, the LSPR λ_{max} shift of the nanoparticles without the SAM was measured. The negligible LSPR response to the exposure of the Ag nanoparticles to immunoglobulin molecules, antiDNP ($1.05x10^{-7}$ M), and to the nanoparticle surface, depicted in Figure 1, demonstrates that binding of the target biological molecules to the nanoparticles is critical for detection.

Hence, the next step for the realization of nanoparticles as a component of biochemical nanosensors is to characterize the sensitivity of nanoparticles coated with an overlayer of functionalized SAM for specific binding to analytes. It has been previously determined from experimentation and calculation that the decreased out-of-plane nanoparticle height increases detection of molecular binding.[4] Previous work has demonstrated the sensitivity of Ag nanotriangles to varying antiDNP concentrations.[2] This study will use the same antiDNP immunoassay system to determine the optimal aspect ratio for greatest sensitivity.

Approach

The ability of NSL to synthesize monodisperse, size-tunable nanoparticles was exploited to investigate the size-dependent nanoparticle optics of single-layer periodic particle array (SL PPA) structure. This study focused on manipulating the size of triangular nanoparticles by variance of mass deposition, d_m, while keeping other factors constant (in-plane width and interparticle spacing). The amount of metal deposited through the single layer mask of latex polystyrene nanospheres specifies the out-of-plane height of the resulting Ag triangular nanoparticles.

In preparation for NSL, glass substrates were first cleaned with piranha etch comprising a 3:1 H_2SO_4: 30% hydrogen peroxide mixture. Next, the glass substrate was made hydrophilic with base treatment entailing sonication in a 5:1:1 H_20:NH_4OH:30% H_2O_2 mixture for 1 hr. Approximately 2.3 l of 390 nm diameter-sized latex polystyrene nanospheres suspended in water were then drop-coated onto the glass substrates. The nanospheres were left under ambient conditions to dry and naturally settle into a 2D hexagonally close-packed array.

Ag metal was used in this study to produce the nanoparticle sensors because of Ag LSPR spectrum's well-known high sensitivity to changes in the local and external dielectric environment. A study was performed using Ag nanoparticles with fixed in-plane width (~100 nm) and three different out-of-plane heights. A thermal deposition chamber was employed for the silver deposition of nanoparticle arrays with out-of-plane heights 15, 25, and 50 nm. After metal deposition the polystyrene mask was sonicated off in ethanol for 3 min,

leaving the triangular nanoparticles exposed on the substrate. Subsequently, the silver nanoparticles were incubated in a mixture of 2:1 octanethiol: 11-aminoundecanethiol for 48 hr to create an amine-functionalized SAM. DNBA was covalently attached by incubation of 1 mM DNBA and 1 mM EDC for 3 hr to form an amide bond. After forming the SAM complex, the sample was exposed to antiDNP of 1.05 x 10^{-7} M concentration in 10 mM PBS buffer for 30 min. The sample was rinsed with 10 mM PBS buffer and then with pH neutralized millique water via injections through a flow cell to wash away unbound antiDNP molecules. Basic labeling steps of the nanoparticles with SAM are depicted in Figure 2.

Subsequently, UV-Vis extinction measurements of each sample were taken with a UV-Vis spectrometer. The LSPR spectra were obtained in transmission geometry using polarized white light in nitrogen.

Finally, atomic force microscopy was employed to obtain topographic images of the nanoparticle arrays. The images were taken with a Digital Instruments Nanoscope III microscope in tapping mode. Corresponding line scans (Figure 3) of the Ag nanoparticles on images before and after antiDNP binding were taken and analyzed in Grapher 4 to determine the average height of the DNBA-functionalized nanoparticles.

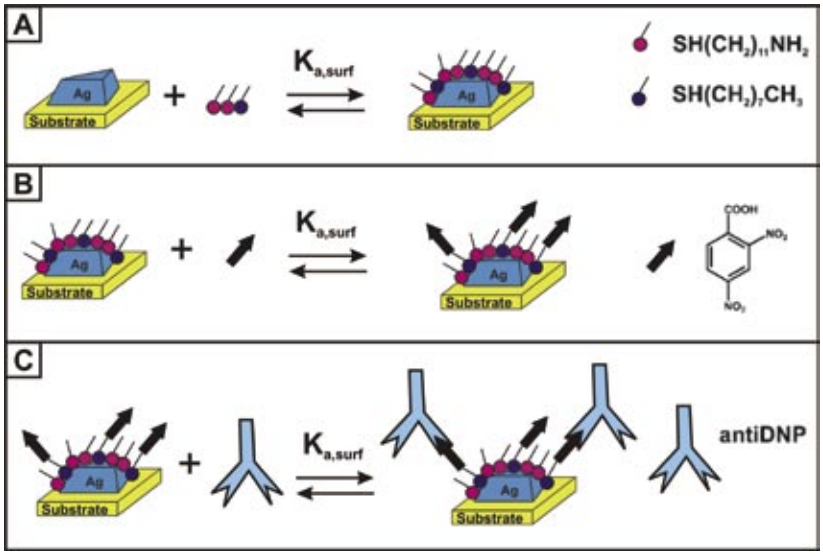


Figure 2: Schematic of the Ag nanoparticles functionalization.

Results and Discussion

The LSPR λ_{max} red moves to longer wavelengths when the Ag nanoparticles are exposed to 1.05 x 10^{-7} M antiDNP. The magnitude of this red shift indicates the nanoparticle array's sensitivity to antiDNP binding. The averaged LSPR λ_{max} shifts for Ag triangular nanoparticles with out-of-plane heights of 15 nm, 25 nm, and 50 nm were 36.9 nm, 13.5 nm, and 10.7 nm, respectively. The depiction of these results in Figure 4 displays a nonlinear trend between $\Delta\lambda_{max}$ and the corresponding out-of-plane heights of the nanoparticles, proportional to the aspect ratio. This demonstrates that sensitivity of this nanosensor grows with increasing aspect ratio.

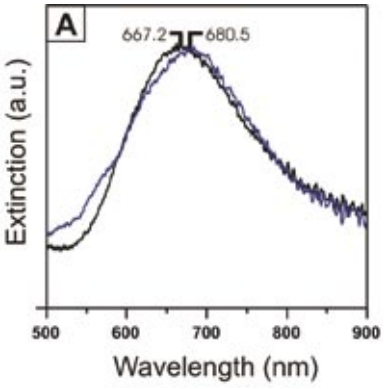
In the present work LSPR shift dependence on nanoparticle shape and size is isolated from the effect of refractive index perturbation to the nanoenvironment from the change in surface concentration of bound molecules. In previous work the assumption that the height of the thickness of the SAM is the same for all three samples in a model system was determined to yield results approximate to experimental observations. Additionally, the surface concentration of bound immunoglobulin is largely independent of varying out-of-plane heights. Therefore, for this study, the magnitudes of $\Delta\lambda_{max}$ must be solely dependent on the particle aspect ratio.

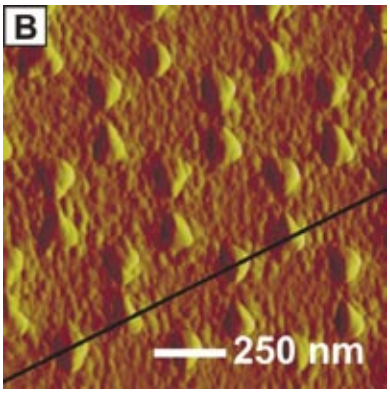
The relationship between the particle aspect ratio and the structure of the surrounding electromagnetic field can be explained by nonuniform wavelength shift of adsorbed molecules at different positions on the nanoparticles.[1] Nanoparticles with larger aspect ratios generate greater average wavelength shift from adsorption than do nanoparticles with smaller aspect ratios, as was observed in this study.[3,7]

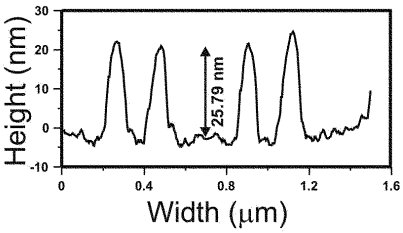
Conclusions

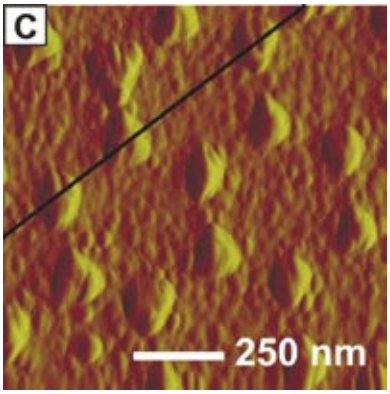
The current work studied the dependence of the LSPR λ_{max} shift on the aspect ratio of noble metal nanoparticles with functionalized overlayers. Nanoparticles with a larger aspect ratio displayed a greater wavelength shift than those with smaller aspect ratios. Therefore, the trend for nanoparticle dissociation response as a function of its aspect ratio observed for nanoparticles without overlayers is applicable to nanosensor arrays in the presence of functionalized complex coating. The correlation of the nanoparticle sensitivity to binding events as a function of its aspect ratio should stay the same regardless of which ligand-receptor system is used. A similar study was done with Concanavalin A to a mannose-functionalized SAM surface, generating the same correlation as observed with the antiDNP immunoassay in the present study.[1]

Future work will endeavor to experimentally verify the dependence of surface enhancement factor on the surface location at which analytes are adsorbed. The next step will be to investigate the mechanism behind anisotropic SAM binding to SL PPA nanoparticles observed in previous studies. The capacity to facilitate SAM linkage to regions of the nanoparticle surface determined to yield high surface enhancement results can greatly improve signal response.[3,4,5]









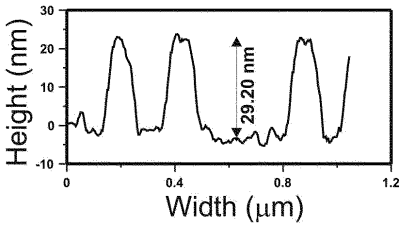


Figure 3: AFM micrographs and corresponding linescans of the Ag nanoparticles. (A) LSPR response before and after AntiDNP was exposed to the DNP-functionalized Ag nanoparticles. (B) AFM micrograph of the DNP-functionalized Ag nanoparticles. (C) AFM micrograph of AntiDNP bound DNP-functionalized Ag nanoparticles.

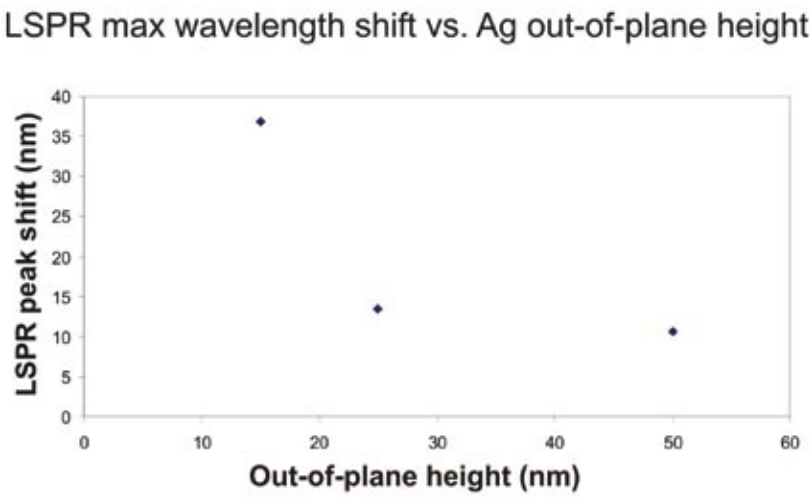


Figure 4: LSPR peak shift vs. nanoparticle out-of-plane height. NSL-fabricated Ag nanoparticles with out-of-plane heights 15 nm, 25 nm, and 50 nm showed LSPR $\Delta\lambda_{max}$ of 36.9 nm, 13.5 nm, and 10.7 nm, respectively.

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