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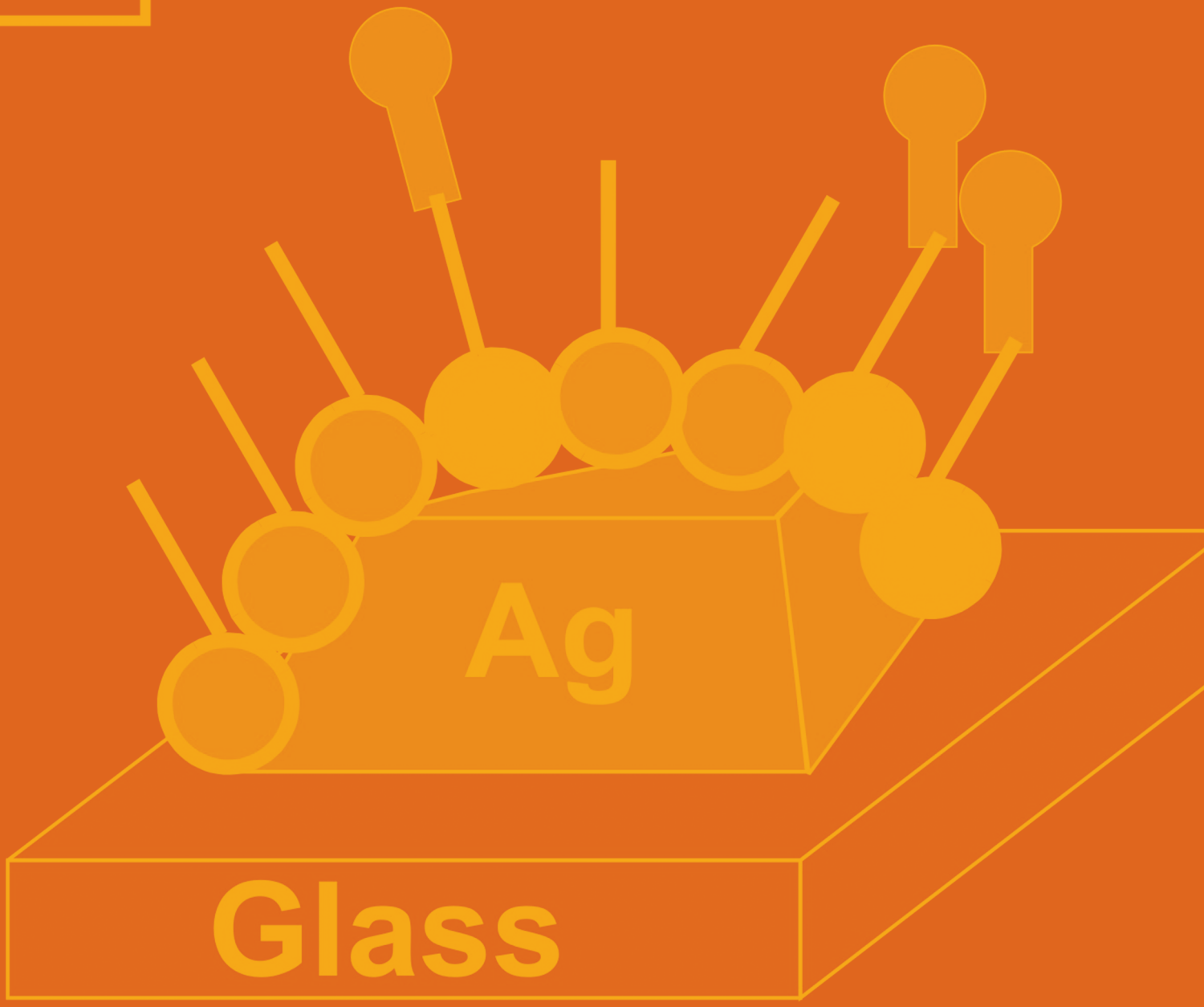

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Colloidal Au–Enhanced LSPR for Sensitive Detection of Antigen/Antibody Interactions

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Abstract

Antibiotin was labeled with colloidal Au nanoparticles as a method of amplifying the localized surface plasmon resonance (LSPR) extinction maximum shift. This shift allows for the monitoring of binding events on the surface of nanosphere nithography (NSL) fabricated silver nanoparticles. A shift of +2.9 nm was observed for the colloidal gold labeled studies, while one of 0 nm was observed for the studies carried out without the antibiotin labeling.

Introduction

The past decade has seen a significant increase in research geared toward the development of devices based on nanoscience and nanotechnology. This research has been partly fueled by the potential to develop highly sensitive and specific sensors for biological targets.[1] One of these sensors is the localized surface plasmon resonance (LSPR) nanosensor. The LSPR nanosensor is a refractive index–based sensing device that relies on the extraordinary optical properties of coinage (gold, silver, and copper) metal nanoparticles.[2] LSPR occurs when the frequency of the incident photon is resonant to the collective oscillation of the conduction electrons found within the individual nanoparticles.[2–3] This collective oscillation occurs only in response to specific wavelengths of light and causes the individual nanoparticles to exhibit selective photon absorption, which can be easily monitored by taking the extinction (absorption plus scattering) spectrum using ultraviolet-visible (UV-vis) spectroscopy.[1–5,7]

The peak of the extinction spectrum, λ_{max}, is highly dependent on the size, shape, interparticle spacing, dielectric properties, and local environment of the nanoparticles.[4] Research has shown that increasing the density and thickness of adsorbate layers produces a red shift of the λ_{max} of nanoparticles fabricated using nanosphere lithography (NSL) by changing the local dielectric environment.[5] The magnitude of the wavelength shift is determined by the size and packing density of the molecules on the surface of the nanoparticles.[1] This sensitivity of the LSPR spectrum to adsorbate-induced changes in the local dielectric environment has proven useful in detecting biological molecules in real time and can therefore be employed in the development of a simple, robust, and accurate biosensor.[4] Van Duyne et al have shown that LSPR technology is applicable in the development of such a biosensor for use in the early diagnosis of AD.[1,2,5] This nanoscale optical biosensor is capable of detecting ADDLs (amyloid-derived diffusible ligands), an Alzheimer's disease (AD) biomarker, at the sub-100-fM range.[2] A sandwich assay was used to monitor the magnitude of the λ_{max} shift of an anti-ADDL antibody in response to varying concentrations of ADDLs.[2,4] The magnitude of the red shift was shown to quantitatively measure ADDL concentration. However, improvements must be made to the current limit of detection (LOD) of the sensor for it to be clinically useful. The following is a proposed method for lowering the LOD using colloidal gold (Au) labeled antibiotin. The method uses biotin/antibiotin as a model. The results from these model studies can then be applied to the AD biosensor.

Background

There is great need for ultrasensitive detection methods in the diagnosis and understanding of diseases such as Alzheimer's disease.[5] AD, the leading cause of dementia in people over 65, is a progressive neurodegenerative disease for which there is neither a good clinical diagnostic tool nor a cure.[3,5,6] The anti-ADDL/ADDL sandwich assay developed by Van Duyne et al uses the LSPR nanosensor, a refractive index–based sensing device[1] to detect changes in the concentration of ADDLs in cerebral spinal fluid (CSF). The LSPR nanosensor's sensitivity is derived from its ability to sense changes in the local refractive index near the surfaces of substrate-confined silver and gold nanoparticles.[5] These changes are then converted into shifts in the peaks of the extinction spectra of the nanoparticles that are observed using UV-vis spectroscopy. The magnitude of the shift in the peak of the extinction spectrum ($\Delta\lambda_{max}$) due to adsorbing molecules onto the surface of the nanoparticles is determined by the size and packing density of the molecules, while the LOD of the system is determined by the surface-confined binding constant between the capture ligands on the surface and the target molecules in solution.[4] This study focuses on the interactions between antigens and their antibodies, specifically the interactions between the well-studied biotin and

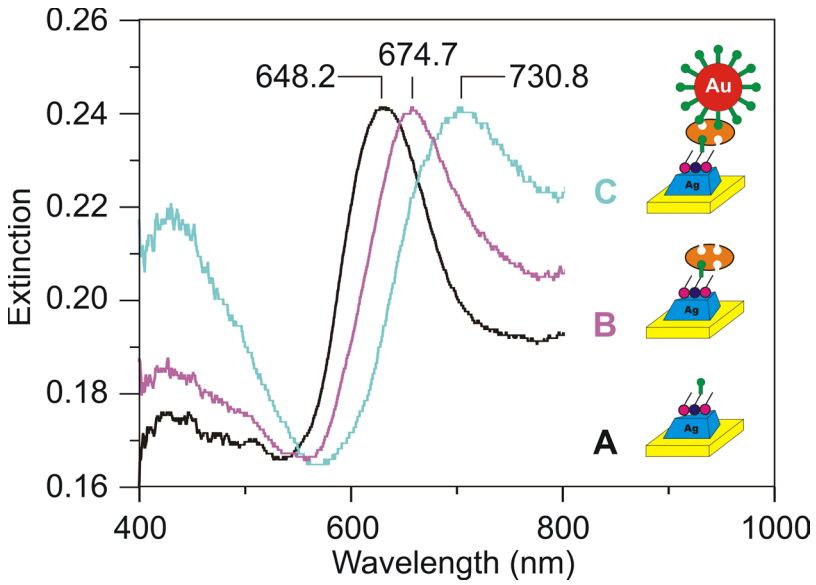

Figure 1: Attaching colloidal Au nanoparticles to the antibody greatly amplifies the LSPR extinction maximum shift due to binding the antibody because the Au nanoparticles have a much larger refractive index than the antibody.

antibiotin pair. Biotin was first covalently linked to the Ag nanoparticles, and then antibiotin in solution was allowed to bind to it. The subsequent shifts in λ_{max} were recorded. Previous studies of this system have shown no nonspecific binding and, therefore, the entire shift in λ_{max} was attributed to the specific interactions between the molecules in question.[4]

After determining the shifts in λ_{max} for low and high concentrations of antibiotin, regular antibodies were exchanged for colloidal gold (Au) labeled ones. It was hypothesized that by labeling the antibodies with colloidal Au nanospheres, the λ_{max} shift seen in the model studies would be greatly amplified because Au nanoparticles have a much larger refractive index than the antibodies to which they are attached. Therefore, in recording the shift in λ_{max}, one would see the shift due to attaching the antibody and also due to the Au nanoparticles (Figure 1).

Approach

Fabrication of Ag Nanoparticles

Nanosphere lithography was used to create monodisperse, surface-confined Ag nanotriangles. Approximately 2 µL of polystyrene nanosphere solution (diameter = 390 nm) was drop-coated onto a piranha-cleaned circular glass substrate and allowed to dry, forming a monolayer in a close-packed hexagonal formation that served as a deposition mask. This was then mounted into a vacuum thermal deposition system and, with a quartz crystal microbalance monitoring the height of the silver being deposited, 25 nm silver was evaporated onto the spheres. Following deposition, the samples were sonicated for 3–5 min

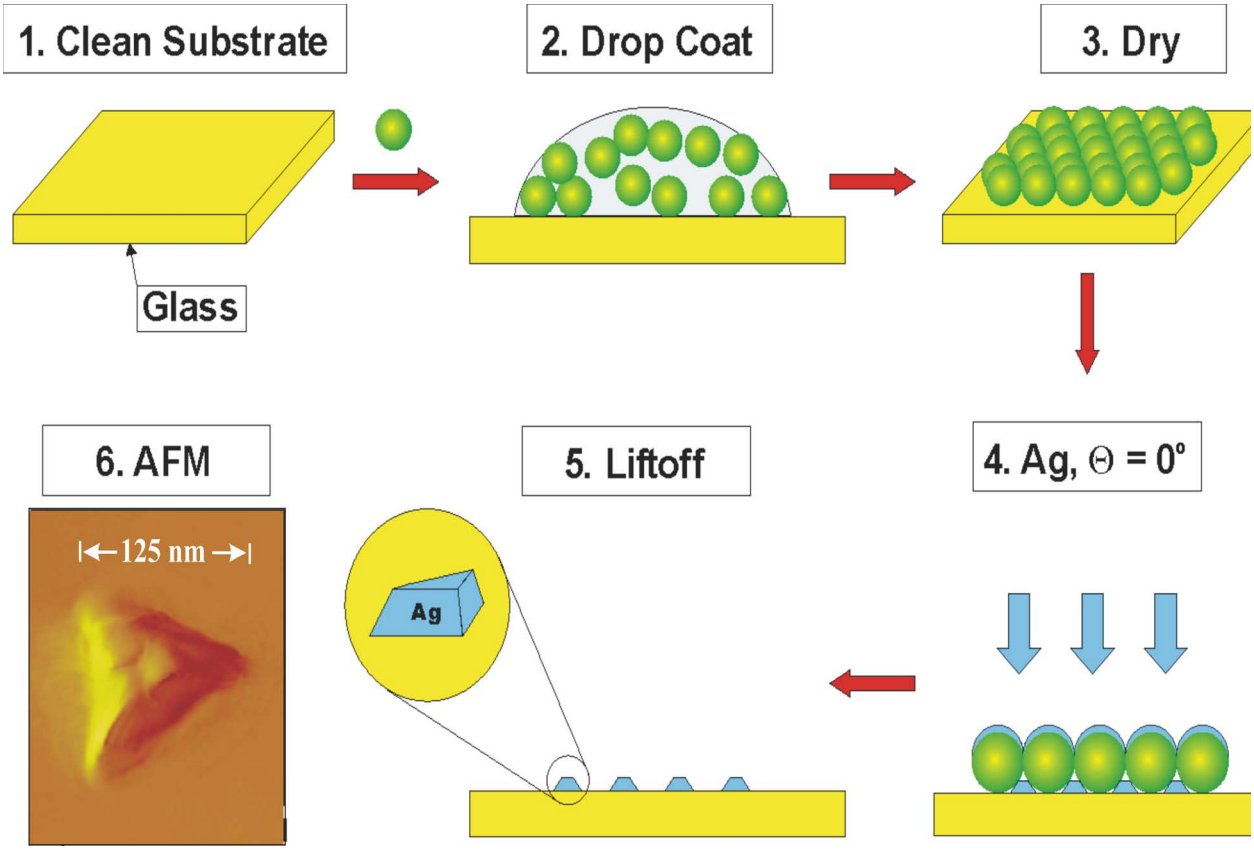

Figure 2: (1) Substrate is cleaned using piranha solution , 5:1:1 MQH_2O/ NH_4OH/ H_2O_2. is used to make the surface hydrophobic, (2) A ~2μL solution of 390nm polystyrene spheres are drop coated onto the surface, (3) The spheres form a monolayer as they dry, (4) 25nm Ag metal is thermally evaporated onto the spheres, (5) The spheres are then sonicated off using ethanol, (6) An AFM image reveals the Ag nanotriangles left behind.

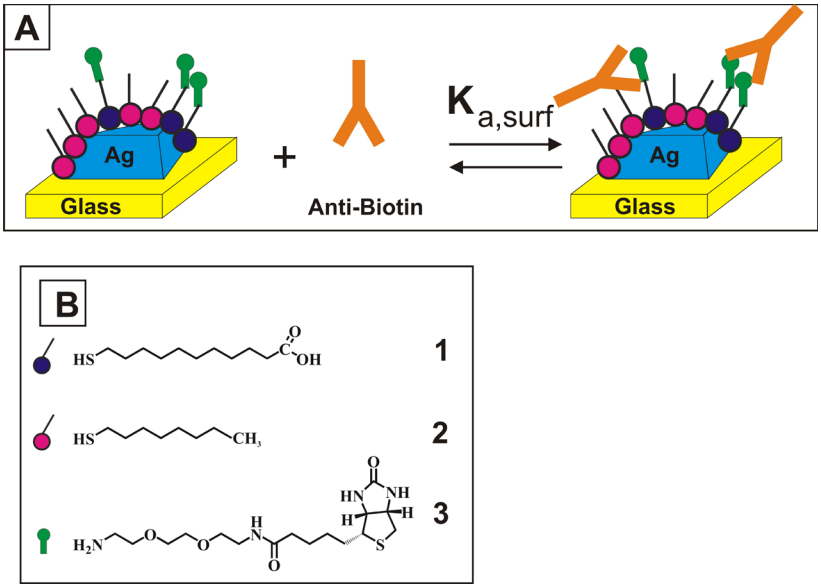

Figure 3: A) This illustrates the binding schematic and surface chemistry of the LSPR nanosensor. The NSL fabricated nanoparticles are functionalized with a SAM layer of 1-OT and 11-MUA. Biotin is then covalently attached to these molecules, after which the antibiotin is introduced and bound to the biotin. B) 1-octane thiol, 11-mercaptoundecanoic acid, biotin.

in 100% ethanol to remove the polystyrene nanosphere mask, thus creating Ag nanotriangles on the surface of the glass (Figure 2).

Nanoparticle Functionalization
Upon removal of the polystyrene nanosphere mask, the sample was incubated for 24–48 hr in 3:1 1 mM 1-Octanethiol (OT)/1 mM 11-Mecarptoundecanoic acid (MUA) solution in ethanol to form a self-assembled monolayer (SAM) on the surface of the particles.[1] The sample was then rinsed thoroughly in ethanol to remove physisorbed alkanethiol molecules and dried in nitrogen (N_2). A zero-length coupling agent, 1-ethyl-3-[3-dimethyl-aminopropyl]carbodiimide hydrochloride (EDC), was used to covalently link 1 mM biotin in 10 mM phosphate-buffered saline (PBS) to the surface-confined carboxyl groups for over 1 hr. The assay was completed by washing the sample with 3 mL MQ water and then incubating it for 1 hr in 200 nM, 10nM antibiotin or 20 nm Au colloid-labeled anti biotin, depending on the experiment (Figure 3). The 20 nm Au colloid-labeled anti biotin was purchased from Sigma-Aldrich.

Ultraviolet-Visible Extinction Spectroscopy
UV-vis extinction measurements were collected for each sample using an Ocean Optics SD2000 fiber-optically coupled spectrometer with a CCD detector. All spectra in this study are macroscopic measurements performed in standard transmission geometry with unpolarized light. The probe diameter was about 2 mm. A homebuilt flow cell was used to control the external environment of the Ag nanoparticle substrates.[7]

Results and Discussion

Due to noise patterns superimposed on the UV-vis spectra, all the data presented here have been smoothed. The λ_{max} of each spectrum was first located by calculating the first derivative. By convention, red shifts in the extinction spectrum are considered positive (+), while blue shifts are considered negative (-). In reporting the results, the relative shift in the peak of the extinction spectrum, $\Delta\lambda_{max}$, was used as a measure of binding events because the absolute value of the extinction maxima varied from sample to sample. This variation can be overlooked, since the absolute value does not affect the relative response shifts of the sensor, as the small differences in λ_{max} are the result of variation in nanoparticle roughness features and in the dielectric environment of the nanoparticles caused by the adsorption of a water layer.[1, 4]

Functionalizing the particles with a SAM layer of 1-OT/ 11-MUA greatly increased the stability of the samples, ensuring that consistent red shifts were produced after incubation in biotin and antibiotin. Given that biotin is a much smaller molecule than antibiotin, it was expected that biotin would give a much smaller LSPR shift at full coverage. However, because the LSPR shift for molecules closer to the surface of the nanoparticles is greater than for those further away, the shift due to the binding of biotin was magnified.

After 24–48 hr incubation in SAM, a representative LSPR extinction wavelength of the nanoparticles was measured to be 754.7 nm (Figure 4). Samples were then incubated in 1 mM biotin/100 mM EDC for 1 hr to ensure that the amide bond between the amine of the biotin

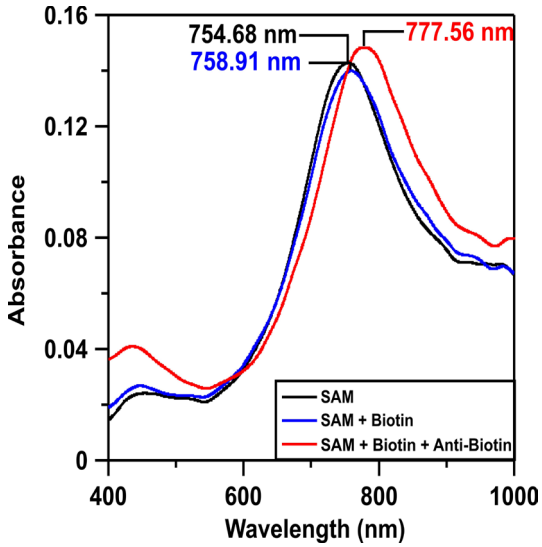

Figure 4: LSPR spectra for each step of the preparation of the Ag nanosensor at high concentration (200 nM) of antibiotin.

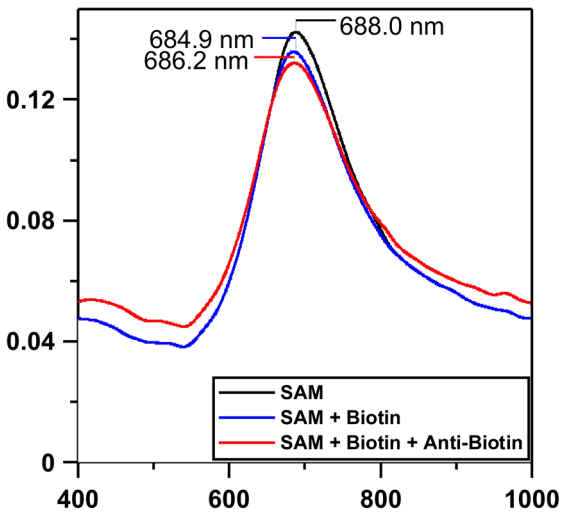

Figure 5: LSPR spectra for each step of the preparation of the Ag nanosensor at low concentration (10 nM) of antibiotin.

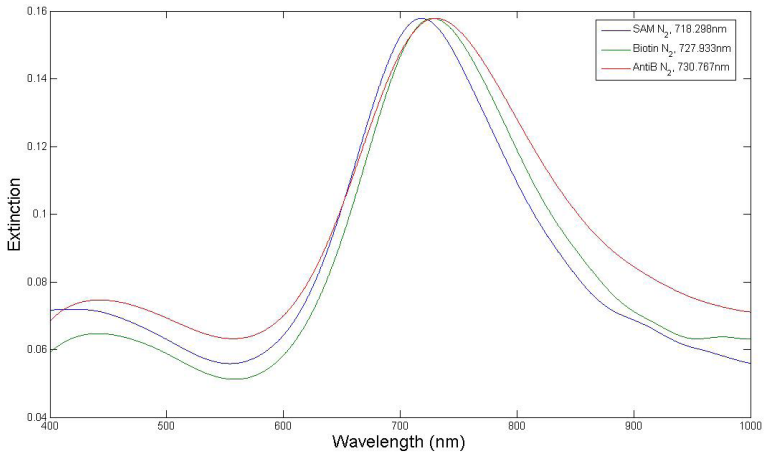

Figure 6: LSPR spectra for each step of the preparation of the Ag nanosensor with colloidal Au labeled antibiotin.

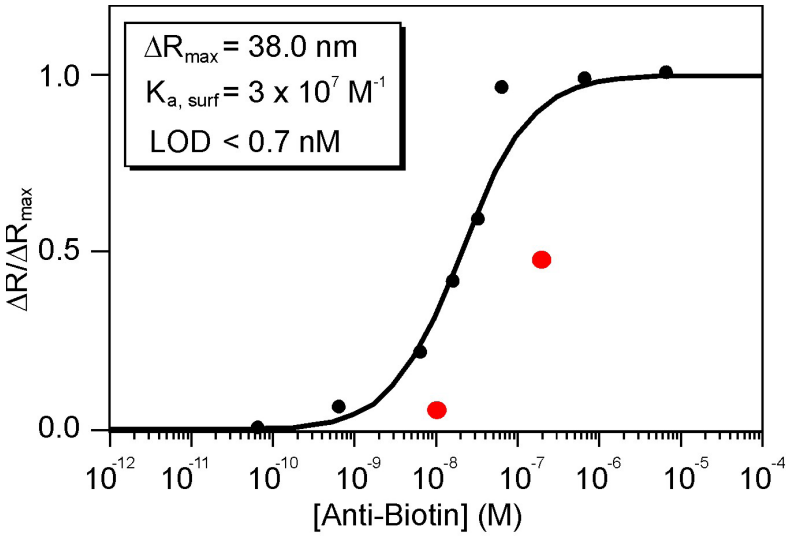

Figure 7: Normalized LSPR shift, $\Delta R/\Delta R_{max}$, versus the concentration of antibiotin response curve for the specific binding of antibiotin to a biotinylated Ag nanosensor as reported by Roboh et al. All measurements were collected in an N_2 environment. The red dots represent the data collected in this study.

and the carboxyl groups of the SAM was formed. The LSPR wavelength shift due to this binding event was +4.2 nm, resulting in an extinction wavelength of 758.9 nm (Figure 4). After this step, the sensor was now ready to detect the specific binding of antibiotin. Incubation in 200 nM antibiotin/10 mM PBS solution for 1 hr resulted in an LSPR shift of +18.7 nm. This meant that the final extinction wavelength was 777.6 nm (Figure 4). The results of this experiment were very different when the concentration of the antibiotin was lowered to 10nM. At this lower concentration, the LSPR shift was +2.2 nm, from 686.2 nm to 688 nm (Figure 5).

The comparison of these results to those achieved by Riboh et al [4] is also represented in a binding curve in Figure 6. As seen, the results obtained from this study vary greatly from those obtained in the previous study. Two explanations arise for this marked difference: 1) In the Riboh study, a longer incubation period for antibiotin was used (3 hr as opposed to 1 hr in this study). This means that the antibiotin had a longer period to bind to the biotin molecules. 2) The study also used a different form of biotin. This might mean different binding mechanisms between the biotin and antibiotin, which would lead to different shifts in λ_{max}.

Experiments using Au colloid-labeled antibiotin were also carried out. By labeling the antibiotin with Au colloids, the LSPR shift upon binding antibiotin to the biotinylated surface was observed to be +2.9 nm, from 727.9 nm to 730.8 nm (Figure 7). From calculations, the concentration of the antibiotin in the gold solution was comparable to 1 nM of the regular antibiotin. After running an experiment using 1 nM antibiotin, an LSPR shift of 0 nm was observed. These preliminary results are promising because they show that there is an amplification of the LPSR shift from binding antibiotin.

Conclusions

This study shows that the amplification of the LSPR shift due to binding antibiotin by labeling it with Au nanoparticles is possible. This conclusion comes from the + 2.9 nm shift in λ_{max} observed when Au colloid-labeled antibiotin was attached to the biotinylated Ag nanoparticle surface, compared with the 0 nm shift observed from attaching 1 nM antibiotin.

Future work will involve determination of the limit of detection for the biotin/antibiotin interactions and biotin/Au colloid-labeled antibiotin interactions and comparison of these results. Once favorable results are obtained, this system can then be applied in studying ADDLs/anti-ADDLs interactions.

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