Optimizing the Localized Surface Plasmon Resonance Biosensor through Self-Assembled Monolayers

Undergraduate Researcher
Shenille T. Straker
South Carolina State University, Orangeburg, SC

Faculty Mentor
Richard P. Van Duyne
Department of Chemistry
Northwestern University

Graduate Student Mentor
Windsor Paige Hall
Department of Chemistry
Northwestern University

Abstract

This paper investigates the use of self-assembled monolayers (SAMs) to optimize the localized surface plasmon resonance (LSPR) biosensor for disease detection. Various experiments were done with 1-(9-mercaptononyl)-3,6,9-trioxaundecan-11-ol (TDT) and heptaoxatricosanoic acid (HSA), a new SAM; these included a solvent study to determine refractive index sensitivity and nonspecific binding and specific binding studies to determine selectivity. It has been demonstrated that observing the amount of nonspecific binding with the SAM will facilitate determination of the SAM's sufficiency for detecting ADDLs. Higher ratios of TDT:HSA proved to have less nonspecific binding while still maximizing the binding of amyloid β-derived diffusible ligands (ADDLs). It was shown that ADDLs specifically bound to the antibody, producing a red shift in λ_{max} of 12.17 nm. Further work with TDT:HSA may involve advancing this SAM in order to detect ADDLs in living patients.

Introduction

Alzheimer's disease (AD) is the seventh-leading cause of death in the United States. Roughly 5.3 million Americans are living with the disease, and the figures are increasing with the ever-growing number of older adults.[1] Currently, there is no diagnosis for AD, but localized surface plasmon resonance (LSPR) shows promising results in detecting the AD biomarker, amyloid β-derived diffusible ligands (ADDLs). ADDLs are protein oligomers that bind to the surface of neurons, disrupting neuron signals in the brain.[2] This study optimized LSPR biosensors through self-assembled monolayers (SAMs) for better detection of AD biomarkers. Current limitations in diagnosis and treatment of AD are driving development of biosensors to advance understanding of the disease.

Background

To accurately diagnose a particular disease requires assays that are extremely sensitive and highly discerning. Sandwich assays are used in this study to maximize sensitivity and reduce nonspecific binding. The ADDLs are sandwiched between a surface-bound antibody and a solution-phase detection antibody.[2] LSPR, which occurs in noble metal nanoparticles such as silver (Ag) and gold (Au), refers to the collective oscillation of conduction electrons that is induced by exposure to specific wavelengths of light.[3] The λ_{max} is defined as the wavelength at which the extinction spectrum is most intense. When proteins (such as ADDLs) are bound to the surface of a nanoparticle, a red shift in the λ_{max} of the nanoparticle extinction spectrum occurs due to increases in refractive index (RI). Changes in the λ_{max} indicate changes in the RI due to binding events at the nanoparticle surface.[4] The Van Duyne group has used LSPR sensing for the detection of AD.

However, difficulty in eliminating nonspecific binding when studying ligand-receptor systems necessitates optimization of the SAMs for the LSPR biosensor.[5] In 2005 Haes et al performed a study using human samples such as cerebral spinal fluid (CSF) and demonstrated that a small sensor response is observed even in the absence of ADDLs biomarker.[4] The slight shift resulted from nonspecific binding between SAMs and the biomolecules in CSF. If the SAM proved to have a lower affinity for the bovine serum albumin (BSA) used in this work, then it might be possible to avoid nonspecific binding. In addition, the SAM must immobilize the antibody in a way that maximizes specific binding with ADDLs. This study outlined ways to improve the sensitivity and selectivity of LSPR assays by optimizing SAMs.

Approach

Nanosphere Lithography

Nanosphere lithography (NSL) is used to prepare nanoparticle samples. A #2 glass substrate was cleaned with Piranha, which removed organic residue from the glass surface. Approximately 2.5 µl of 390 nm diameter latex polystyrene nanospheres were dropped coated onto the #2 glass substrate to produce a close-packed nanosphere mask. To dry, the nanosphere solution samples were left in ambient conditions for no more than 1 hr. Au (50 nm) was deposited onto the nanosphere mask using thermal evaporation. Once the metal was deposited, nanospheres were removed by tape lift-off.

Specific/Nonspecific Binding Study

The nanoparticles were incubated in a mixed solution of 1 mM 1-(9-mercaptononyl)-3,6,9-trioxaundecan-11-ol (TDT) and 23-(9-mercaptononyl)-3,6,9,12,15,18,21-heptaoxatricosanoic acid (HSA) for at least 24 hr to form a SAM. The SAM shields the particles from phosphate buffer solution (PBS) and enables the antibodies to bind to the surface of the nanoparticles. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), a coupling agent, was used to catalyze the antibody binding to the surface of the nanoparticles.

UV- vis Spectroscopy

The UV-vis spectrometer consists of a lamp that emits white light, a flow cell mount, and optical lenses that focus the white light onto the LSPR substrate in the flow cell. The flow cell allows one to change the surrounding media around the silver or gold nanoparticles. The nanoparticle extinction is collected with a spectrometer. Changes in

the optical properties of the nanoparticles due to analyte binding are reflected in the changes in the λ_{max} of the extinction spectrum.[5] If there are changes in the refractive index (RI) value, then a shift in the λ_{max} will occur, which in this case is caused by the binding of proteins and antibodies. Once BSA is present, if a red shift is identified, then this can be interpreted as the protein or antibody binding to the surface of the silver or gold nanoparticles nonspecifically.[4] The ADDLs that are used in this experiment are synthetically made.[2] All of the LSPR spectra data were collected in real time to measure the response.

Solvent Study

The aim of this study was to assess the effectiveness of common solvents when observing the λ_{max} of the sample. The five different solvents used were ethanol, methanol, purified water, isopropanol, and dimethyl sulfoxide (DMSO). Between applications of each solvent, nitrogen was used to dry the sample and stabilize the nanoparticles.

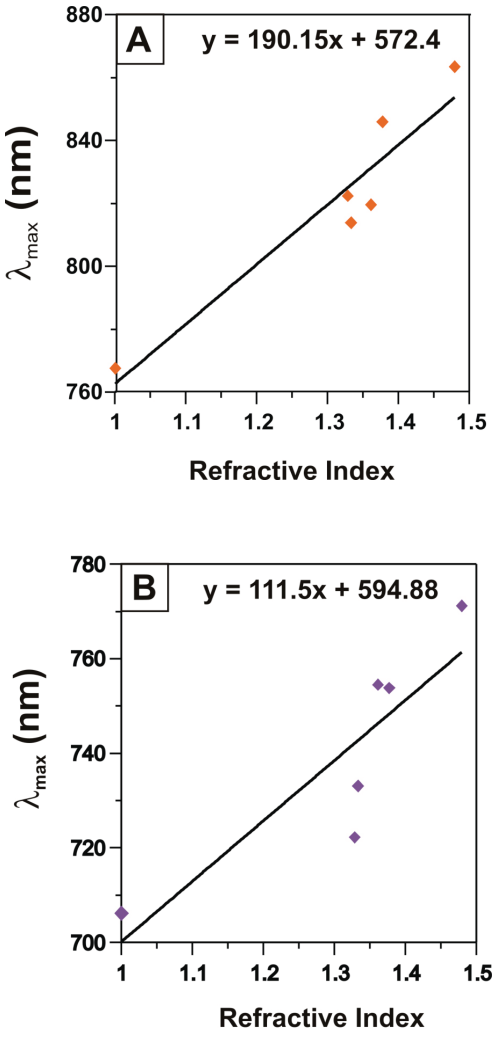

Figure 1. Solvent Study. The λ_{max} vs. refractive index values for each solvent were plotted for an Au nanoparticle sample with a SAM of (a) 3:1 TDT:HSA and (b) 3:1 OT:MUA. The data was fit to a linear curve, yielding the equations displayed in the plots. The slope of the line displayed the sensitivity of the LSPR biosensor.

Results and Discussion

Solvent Study

The primary purpose of the solvent study was to observe changes in the λ_{max} of the nanoparticles in response to the surrounding medium, and consequently to determine the sensitivity of the nanoparticle sample. To produce a diverse array of λ_{max} values, this study used different media with diverse RI values. The shift in λ_{max} is caused by differences in the RI of the medium surrounding the nanoparticles. This relationship is described by the equation

$$\Delta\lambda_{\max} = m(n_1 - n_2)(1 - e^{-2t_{SAM}/l_d})$$

where m is the sensitivity of the nanoparticles in nm/RI unit; n is the refractive index; t_{SAM} is the thickness of the self-assembled monolayer; and l_d is the decay length of the electromagnetic field at the nanoparticle surface. The media used for the study included ethanol, nitrogen, methanol, isopropanol, purified water, and DMSO. The λ_{max} value in each medium was plotted in a graph versus RI and then fit to a linear curve (Figure 1). The slope expressed the sample's sensitivity for detection. For the 3:1 TDT:HSA sample (Figure 1a) the sensitivity was 115.5 nm/RIU, and for the 3:1 OT:MUA sample (Figure 1b) the sensitivity was 190.15 nm/RIU.

The equation presented shows a linear correlation among the λ_{max} values for each media. The slope for the TDT:HSA (111.5 nm/RIU) was low compared with OT:MUA (190.5 nm/RIU) or a typical slope, which ranges between 200 and 300 nm/RIU. It is possible that annealing ("rounding out") of the nanoparticles by the solvent over time resulted in smaller red shifts, decreasing the slope of the line. Strong solvents such as isopropanol and DMSO can increase the rate of nanoparticle annealing on the sample. The results for both experiments demonstrated the sensitivity of these particular SAMs on Au substrates, specifically that the OT:MUA has a higher sensitivity value than the new SAM, TDT:HSA.

Nonspecific Binding Study

This study was performed to observe whether nonspecific binding occurs, and by how much, with each SAM. Figure 2a illustrates the procedures of the experiment, and Figure 2(b–d) shows plots of each nonspecific binding study graphed as extinction intensity versus wavelength. Here it can be seen that the $\Delta\lambda_{max}$ of OT:MUA due to nonspecific binding was 1.99 nm (Figure 2b), the lowest of the three samples. The 3:1 concentration of TDT:HSA had the highest level of nonspecific binding, with a $\Delta\lambda_{max}$ of 7.45 nm (Figure 2c). The 9:1 concentration of TDT:HSA has a $\Delta\lambda_{max}$ of 3.58 nm (Figure 2d), which is an improvement over the 3:1 concentration of TDT:HSA. However, the increase of TDT to HSA ratio in the 9:1 concentration diminishes the amount of antibody binding ($\Delta\lambda_{max}$ = 0.69 nm) compared with the other SAMs. The diminished antibody binding could result from the lower amounts of HSA, which allowed the antibody to bind to the surface of the SAM through formation of an amide bond. The increase in TDT, which resists nonspecific binding, could very well be the reason for lower amounts of nonspecific binding present in the 9:1 concentration verses the 3:1 concentration of TDT:HSA.

The data accumulated for the changes in the λ_{max} after each binding step can be seen in Table 1. This chart displays the antibody shift along with the BSA shift when various mixed SAMs are used. There was a shift for each sample upon binding of the antibody to the SAM-functionalized nanoparticles and a shift upon binding of BSA to the antibody.

Specific Binding Study

This study was performed to observe how the nanoparticle λ_{max} changes when ADDLs bind specifically to an antibody functionalized surface. The 9:1 concentration of TDT:HSA was used for this experiment in order to determine whether the lower ratio of carboxylic acid–terminated HSA, and therefore lower levels of surface-bound antibody, would affect the sensitivity (i.e., magnitude of $\Delta\lambda_{max}$) upon ADDLs binding. Data were collected continuously at about 1 point/sec in real time. Figure 3a displays the procedure for the experiment; Figure 3b shows the change in λ_{max} in relation to time. The spectrum collected after each binding step is displayed in Figure 3c.

The $\Delta\lambda_{max}$ of the nanoparticles was 4.21 nm when the antibodies bound to the SAM and 12.17 nm when the ADDLs bound to the antibody. There was a $\Delta\lambda_{max}$ of 16.38 nm during the entire experiment. This demonstrates that the 9:1 TDT:HSA SAM was sensitive to specific binding of ADDLs and might function well for the detection of AD.

Conclusions

The goal of this study was to optimize the LSPR biosensor by using a different SAM. Specifically, this research explored the issue of nonspecific binding occurring with the SAM. A current aim is to optimize the LSPR biosensor for detection of AD. Since brain damage is irreparable, the earlier AD can be detected, the better — meaning that development of a highly selective and sensitive sensor is crucial. A solvent study was completed to determine the SAM's sensitivity, a nonspecific binding study to determine selectivity, and a specific binding study to observe the behavior of the SAM when exposed to ADDLs. When the surface of a nanoparticle was functionalized with anti-ADDLs antibody, it was shown that ADDLs specifically bound to the antibody, and a red shift in λ_{max} could be detected.

The sensitivity of the TDT:HSA, expressed as the slope of the linear fit, is lower in value compared with usual SAMs, possibly due to the annealing of the nanoparticles. A ratio of 9:1 TDT:HSA showed better

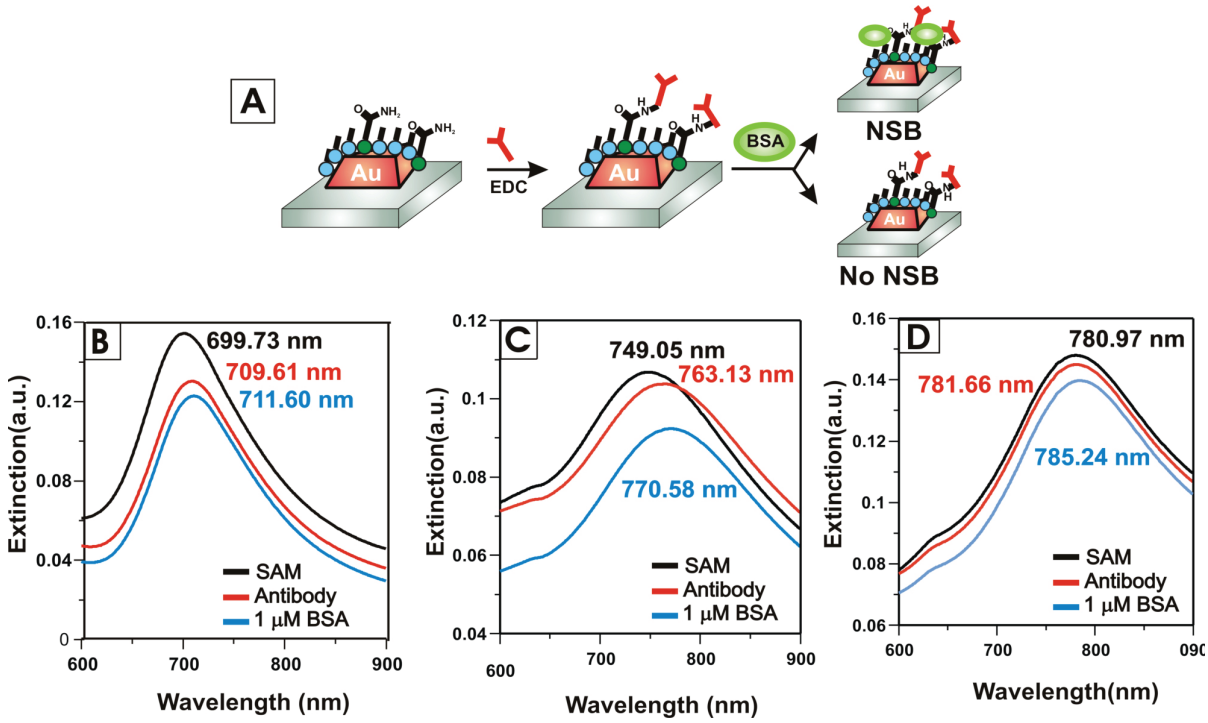

Figure 2. Nonspecific Binding Study. (a) A schematic illustration of the experimental procedure. After formation of a SAM on the Au nanoparticles, antibodies were covalently attached using EDC as a linker. Lastly, the nanoparticles were incubated with 1 μM BSA to test for nonspecific binding (NSB). Extinction spectra for each step in the experiment were plotted: (b) 3:1 ratio of OT:MUA had a $\Delta\lambda_{max}$ of 1.99 nm due to NSB, (c) 3:1 ratio of TDT:HSA had a $\Delta\lambda_{max}$ of 7.45 nm due to NSB, (d) 9:1 ratio of TDT:HSA had a $\Delta\lambda_{max}$ of 3.58 nm due to NSB.

BSA Assay		
	λ_{max} (nm)	
Sample	Antibody	BSA
3:1 SAM: OT: MUA	9.88	1.99
3:1 SAM: T. DOT: HSA	14.08	7.45
9:1 SAM: T. DOT: HSA	0.69	3.58

Table 1. Nonspecific Binding Study. The LSPR response ($\Delta\lambda_{max}$) upon binding of 500 nM anti-ADDLs antibody, followed by 1 μM BSA.

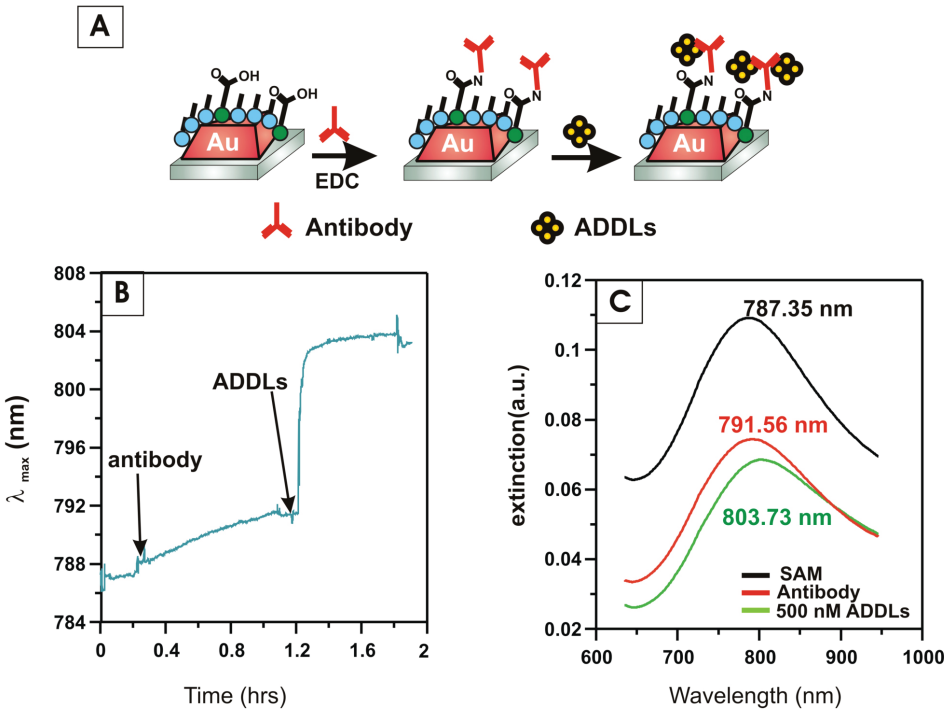

Figure 3. Specific Binding Study. 9:1 TDT:HAS: (a) A schematic illustration of the experimental procedure. After formation of a SAM on the Au nanoparticles, antibodies were covalently attached using EDC as a linker. Lastly, the nanoparticles were incubated with 500 nM ADDLs. (b) Changes in λ_{max} in relationship to time during the specific biding experiment. The first red shift was caused by the antibody, and the second shift was caused by the ADDLs. (c) The extinction spectra for each step in the specific binding experiment. The values shown were the extinction maxima for each step. The $\Delta\lambda_{max}$ due to specific binding of ADDLs was 12.17 nm.

results for nonspecific binding compared with the 3:1 TDT:HSA. It is known that experiments done with varying concentrations of TDT:HSA can produce lower amounts of nonspecific binding. The sensitivity of the LSPR biosensors will continue to improve as nanoparticle designs and nanofabrication methods advance. The work done in this study showed possible progression toward helping monitor potential Alzheimer's patients.

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