

Surface Plasmon Resonance Used to Monitor Biological Interactions

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Introduction

This paper will describe how the excitation of surface plasmons between a chemically modified thin metal surface and a sample media can be used to probe the interactions between substrates attached to the metal surface and biological molecules (proteins, enzymes) in a sample. Free electrons in an array of metal atoms can be treated as an electron liquid having a density of 10^{23} cm^{-3} . It follows that longitudinal density fluctuations, plasma oscillations, will propagate through the volume of the metal (1). Ritchie noted that there exists not only bulk plasma oscillations in the interior of the plasma, but also surface plasma oscillations, the quanta of which can be called surface plasmons (2). These surface plasmons can be excited by shining light on a layered system that consists of a transparent medium (glass or quartz) on one side, a metal film (usually silver or gold (3)), and a dielectric on the other side of the metal film. Surface plasmons are excited at resonance when the velocities of the evanescent wave from the light and the surface plasmon waves coincide. Maximum excitation is seen as a minimum when the reflected light is plotted against the angle of incidence of the light. The angle of incidence at which this minimum is observed will shift due to changes in the absorption layer density on the metal surface. Hence, surface plasmon resonance spectroscopy (SPR) permits the measurement of changes in the adsorption layer of the metal surface (4).

Theory

Although two different methods have been used to monitor SPR, the grating coupler and the attenuated total reflection (ATR) technique, the ATR technique is more commonly employed (4). This technique, which is also referred to as the Kretschmann-Raether configuration, is shown in figure 1. Light enters the prism with a dielectric of ϵ_2 and strikes the metal surface at an angle of incidence θ_0 . The prism surface is in direct contact with the metal film whose dielectric is ϵ_1 and thickness is d_1 . The surface of the metal is in contact with a medium such as air or another film that has been deposited on the metal surface. In the case of a deposited substance, this layer has a dielectric ϵ_3 and a thickness d_3 . The

intensity of reflected light from the metal is monitored relative to the angle of incidence θ_0 (4, 5).

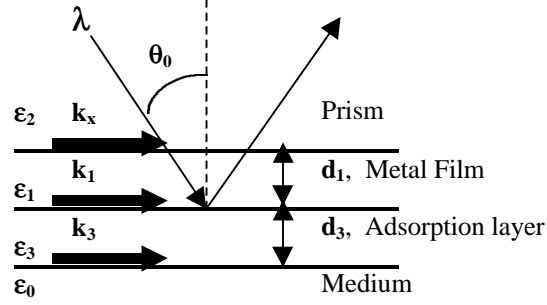


Figure 1. Kretschmann-Raether configuration used to monitor SPR

Surface plasmons have a wave vector in the plane of the interface between two surfaces. The wave vector between the prism and the metal surface, k_x (figure 1), is related to the wavelength of light in vacuum (λ), the dielectric of the prism (ϵ_2), and the angle of incidence θ_0 in the following equation:

$$\mathbf{k}_x = (2\pi/\lambda)(\epsilon_2)^{1/2} \sin \theta_0 \quad \text{Eq. 1.}$$

The following equation relates the reflection coefficient \mathbf{R} with wave vector, dielectric, and thickness of the different layers in figure 1 (4):

$$\begin{aligned} \mathbf{R} = |\mathbf{r}_{210}|^2 &= \left| \frac{\mathbf{r}_{21} + \mathbf{r}_{130} \exp(2i\mathbf{k}_1 d_1)}{1 + \mathbf{r}_{21} \mathbf{r}_{130} \exp(2i\mathbf{k}_1 d_1)} \right|^2, \quad \mathbf{r}_{21} = \frac{\mathbf{z}_{21}}{\mathbf{n}_{21}}, \\ \mathbf{r}_{130} &= \frac{\mathbf{z}_{10} - i\mathbf{z}_{43} \tan(\mathbf{k}_3 d_3)}{\mathbf{n}_{10} - i\mathbf{n}_{43} \tan(\mathbf{k}_3 d_3)}, \quad \mathbf{z}_{i,j} = \mathbf{e}_j \mathbf{k}_i - \mathbf{e}_i \mathbf{k}_j, \\ \mathbf{n}_{i,j} &= \mathbf{e}_j \mathbf{k}_i + \mathbf{e}_i \mathbf{k}_j, \quad \mathbf{k}_j = \left[\mathbf{e}_j \left(\frac{2p}{I} \right)^2 - \mathbf{k}_x^2 \right]^{1/2} \\ \mathbf{e}_4 &= \frac{\mathbf{e}_0 \mathbf{e}_1}{\mathbf{e}_3}, \quad \mathbf{k}_4 = \frac{\mathbf{k}_0 \mathbf{k}_1}{\mathbf{k}_3} \end{aligned} \quad \text{Eq. 2}$$

The subscript numbers in eq. 2 refer to the layers in figure 1. For a given wavelength, \mathbf{R} reaches a minimum at a particular angle of incidence, θ_p , referred to as the plasmon angle in which the surface plasmons have absorbed a maximum amount of photons (3). Knowing the different dielectrics and

thickness of the layers in the setup in figure 1, the theoretical R vs. θ curves can be produced (4).

Conversely, experimentally monitoring R relative to the change in θ , and knowing the dielectric of the different layers and the thickness of the metal film would allow one to calculate the thickness of the absorption layer, allowing quantification of absorbed analyte (6).

Varying the film thickness, d_1 , changes the minimum value of R . This is demonstrated in figure 2 where the thickness of silver is varied on a prism. It has been shown that sensitivity increases with deeper and narrower resonance spectra (R vs. θ). According to the results in figure 2, a film thickness around 500\AA for silver should be used in SPR (1). Silver, followed by gold, is normally the metal of choice due to its deeper and narrower resonance spectra relative to other metals (7, 3).

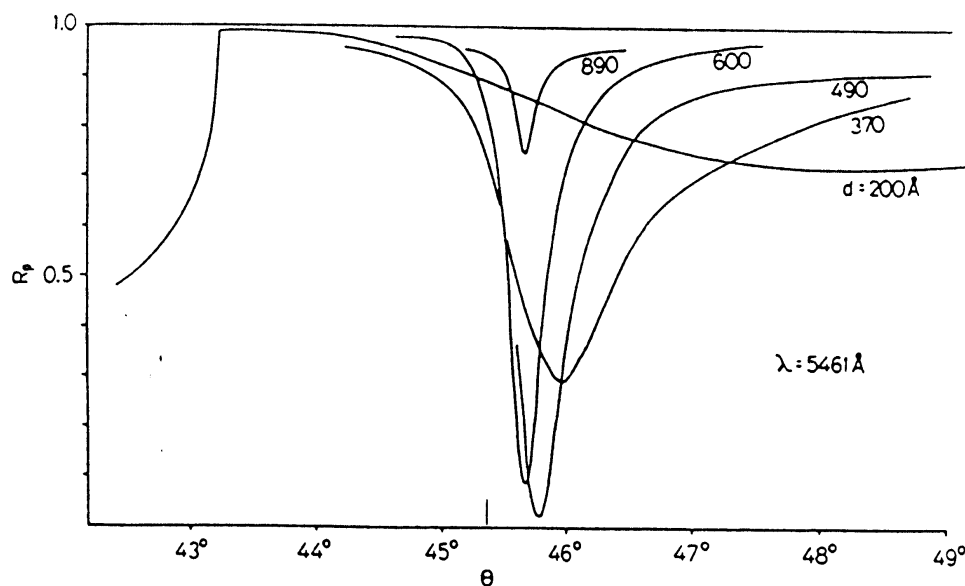


Figure 2. Reflection measured at different silver film thickness (200-890 \AA) with an ATR device; the wavelength used is 5461 \AA (1).

When a layer of LiF of varying thickness was coated onto the silver film, the plasmon angle shifted to higher degrees and the width at half the peak depression increased (figure 3) (1). Similar experimental results of absorbed molecules on a metal film correspond well with theoretical calculations based on eq. 2. This demonstrates the ability of SPR to monitor changes in the thickness of a layer next to a metal film (d_3). When both the shift in the position of the dip in the SPR curve and the change in the

width at half the peak depression is monitored, the lowest detection limit for the change in d_3 is obtained (4, 5).

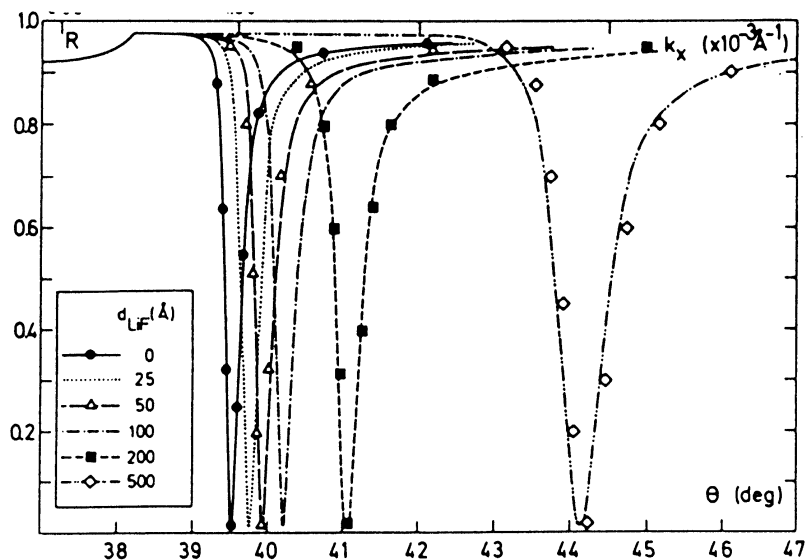


Figure 3. Observed displacement of the plasmon angle of silver with increasing thickness of a LiF coating. The wavelength used is 6471 \AA , and the silver film thickness is 496 \AA (1).

Instrumental Setup for SPR

Four principle components make up a SPR instrument: a source of p-polarized light, a prism coupling light photons to plasmons, a thin metallic or semiconductor film on the surface of which plasmons can be excited, and a light detector (see figure 4). The source is generally a He/Ne laser. The light from the laser ($\lambda = 633 \text{ nm}$) is modulated at a specific frequency by a beam chopper and passed through a polarizer P to obtain a polarization in the plane of incidence (p-polarized beam). This light is then split with part being sent to a detector to normalize the SPR signal. A mirror reflects the other part of the beam onto the prism. A lens focuses the reflected light from the metal/prism surface onto a second detector. The detectors used are photodiodes, and lock-in amplifiers amplify their signal. The angle of incidence is changed by either rotating mirror M2 in figure 4, or by rotating the prism/sample cell setup. A personal computer is used to record the result (4-6).

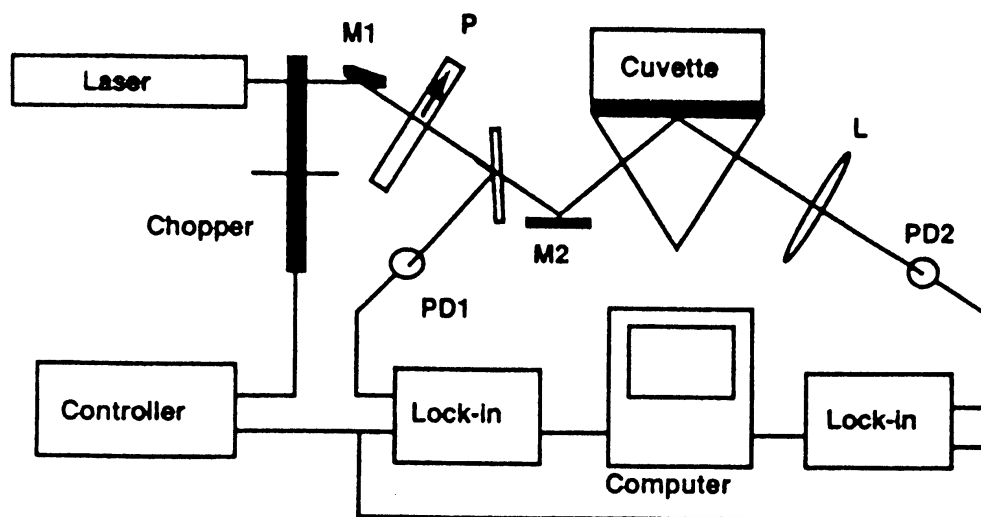


Figure 4. SPR experimental setup where M1 and M2 are mirrors, P is the polarizer, PD1 and PD2 are the photodiodes.

Generation of Surface to Monitor Biomolecular Interactions

Assembly techniques that directly form either monolayers or bilayers of the absorption film on a metal surface for SPR include chemical modifications of the metal surface and spontaneous formation of freely suspended lipid bilayer membranes. A substrate (antibody, peptide, etc.) can then be incorporated into these molecular layers to interact with a sample. Although chemically modified metal surfaces are useful in coupling different ligands and proteins, this is not well suited for to the immobilization of integral membrane proteins that require a lipid bilayer. For membrane protein proteins, the freely suspended lipid bilayer is used, which has been covered by Salamon et. al. (6, 8, 9).

Chemical modifications of metal surfaces can be carried out by either the derivatization of commercially available carboxymethyl dextran-coated substrates (10) or by the covalent coupling of ligands and proteins to self-assembled monolayers (SAM) of alkanethiolates on gold films. Although the dextran substrates are convenient to use, they can have nonspecific binding properties and are not useful with large proteins. SAM, however, can be tailored to have highly resistant nonspecific adsorption and do not have ambiguities associated with interfacial partitioning of large proteins (11).

Lahiri et. al. describe the incorporation of SAM on gold films to monitor biospecific recognition of immobilized benzenesulfonamide with bovine carbonic anhydrase II (CA) (figure 5). The chemisorption of alkanethiols on gold films is carried out by immersing the films in mixed alkanethiols overnight, rinsing with ethanol, and drying under a stream of nitrogen. Coupling of the substrates to the SAM takes place when an aqueous solution containing *N*-hydroxysuccinimidyl (NHS) and ethylene dichloride (EDC) is injected over the SAM in the sample cell (step 1 in figure 5). This converts the carboxylic acid of the SAM into the activated NHS esters. The substrate to be bound is then injected over the SAM (step 2 in figure 5). The sample is then washed with phosphate buffered solution and unreacted NHS esters are hydrolyzed by washing with pH 8.6 phosphate buffer. The sample cell is now ready to monitor bimolecular interactions (11).

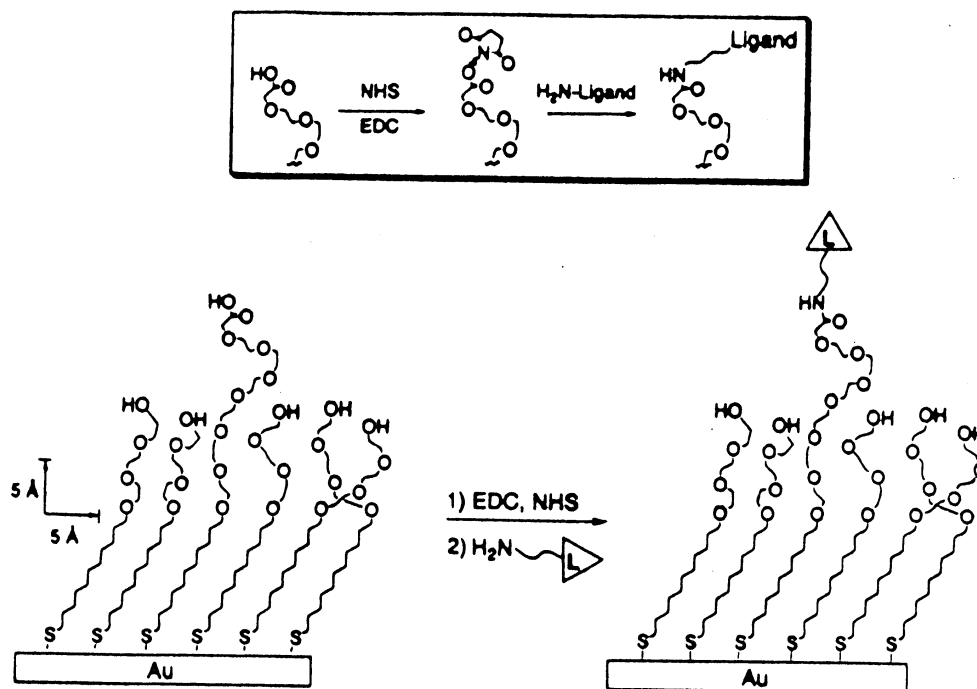


Figure 5. Coupling of substrates to SAM.

Interpretation of Result

A report by O'Shannessy et. al. demonstrated the successful modifications of a commercially available BIAcore sensing surface that had a carboxylated dextran layer over a gold film. The streptavidin/biotin interaction was used to probe the chemical modifications of the sensing surface. SPR can be used to monitor both the derivatization of the surface, the coupling of biotin to the surface, and the interactions of biotin with streptavidin (figure 6). As described above, the NSH esters are made from the reaction between the carboxylic acid of the modified gold surface and NHS and EDC. After making the amine derivative by reaction the NSH esters with ethylenediamine, sulfosuccinimidyl-6-(biotinamido)-hexanoate (NHS-LC- biotin) or sulfosuccinimidyl 2-(biotinamido)ethyl-1,3-dithiopropionate (NHS-SS-biotin) are covalently attached to the chemically modified gold surface. SPR spectra are used to monitor both the coupling of the ligands and the interaction of streptavidin with these ligands (figure 6).

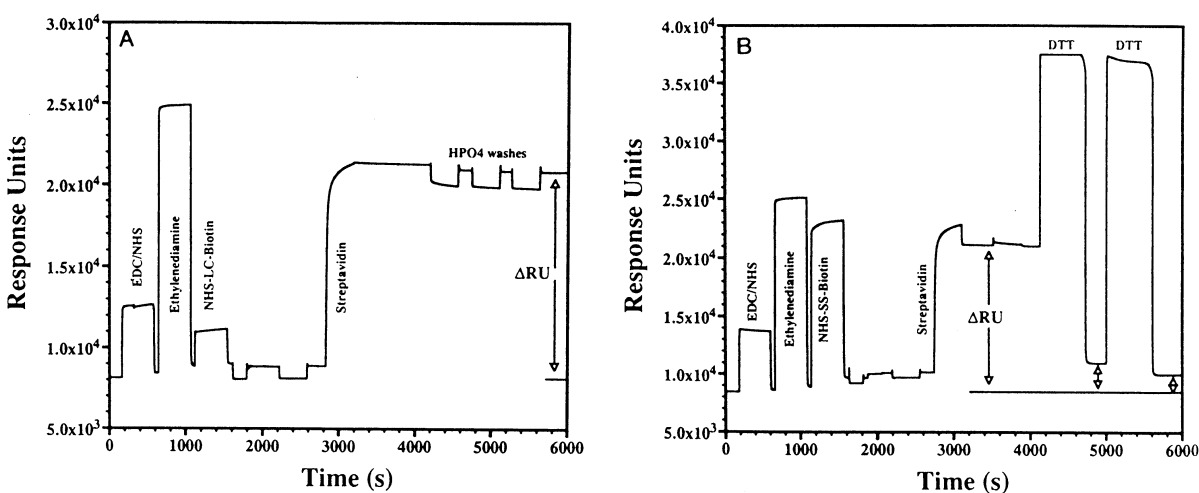


Figure 6. Modification of carboxylated dextran gold surface and interaction of streptavidin/biotin monitored by SPR.

Both spectra demonstrate an interaction of streptavidin with the ligands. Even after multiple injections of 0.1 M phosphoric acid (HPO₄ washes), this interaction is still seen with a positive change in the response from baseline (figure 6a). When NHS-SS-biotin is used, the streptavidin/biotin complex can be cleaved from the modified gold surface by injecting dithiothreitol, allowing the surface to be “regenerated” for

further coupling of other ligands. The removal of the complex is seen in a drop of the SPR response back to the baseline in figure 6b (10).

Work by Lahiri and coworkers demonstrated how SPR could be used to monitor competitive binding of carbonic anhydrase II (CA) with 4-carboxybenzenesulfonamide (CBS) in solution and benzenesulfonamide covalently linked to the SAM on a gold surface (described in the previous section above). This was done by obtaining SPR sensorgrams of a mixture of $0.5 \mu\text{M}$ of CA with varying concentrations of CBS (1, 2, 4, 5, 10, and $50 \mu\text{M}$) (figure 7). It can be seen that with increasing concentrations of CBS, CA binds to the CBS, decreasing the amount of free CA to bind with the immobilized benzenesulfonamide and lowering the change in the SPR response (11).

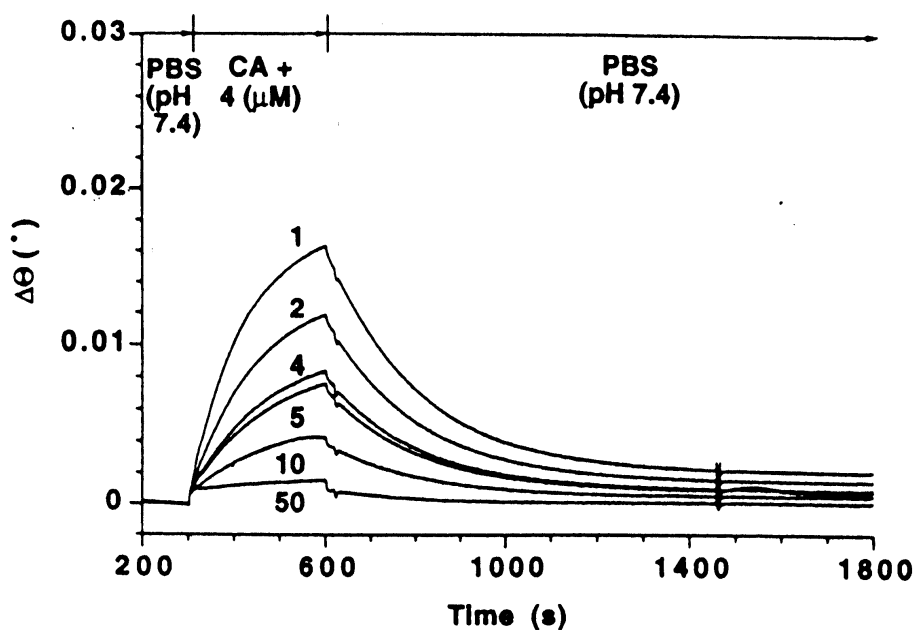


Figure 7. SPR used to monitor the competitive binding of carbonic anhydrase with 4-carboxybenzenesulfonamide in solution and benzenesulfonamide covalently linked to the SAM on a gold surface. Numbers 1-50 represent μM concentrations of 4-carboxybenzenesulfonamide.

Conclusions

Surface plasmon resonance spectroscopy has been demonstrated as a technique to monitor biomolecular interactions between ligands immobilized on a thin metal film with other biomolecules in a

sample cell (cuvette or flow cell). One advantage to this technique is the lack of sample solution interference with the spectroscopic measurement. The light in SPR is not passed through the sample solution, eliminating many problematic matrix effects that take place with other spectroscopic methods. This is particularly important when monitoring interaction in biological fluids such as blood. This method can also be applied to isolating structurally relevant compounds in a complex mixture based on an affinity with the substrate attached to the metal film. When this technique is incorporated with mass spectrometry, the compounds retained by the SPR metal film can be identified by molecular and fragment mass ions (12). Work continues to be done to increase the sensitivity of this technique to monitor physiological levels of different compounds, allowing this to be used as remote fiber optic sensor for *in vivo* measurements (4).

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