Measuring protein adsorption using surface plasmon resonance

Application Note #110

Introduction

The way in which biomolecules adsorb and bind on solids is critical to the success of biocompatible surfaces, filters and membranes, and molecular diagnostics. The aim of this note is to demonstrate that SPR-Navi from KSV Instruments Ltd. is an ideal device for the *in situ* investigation of protein adsorption and binding at the solid-liquid interface.

Experimental

The gold-coated glass slides used in the SPR measurements were cleaned by immersion in a boiling 1:1:5 NH_3OH:H_2O_2:H_2O for ten minutes, flushed thoroughly with ion exchanged water and blown dry with nitrogen. One of the gold surfaces was then used directly for the Bovine Serum Albumin (BSA) adsorption measurement, while the second gold surface was further treated by immersion in a 1 mM Octadecylmercaptan/Hexane solution for three hours, which rendered it hydrophobic. Phosphate Buffered Saline (PBS) was used as carrier liquid for the SPR measurements and BSA (0.1 mg/mL, Aldrich) in PBS was used for the adsorption tests.

Results

The characteristic SPR intensity versus incidence angle curve (Figure 1) provides a wealth of information about molecular changes occurring at the surface. In aqueous solution, the SPR absorption peak appears at around 69°. The shape of the peak can be accurately described by theory (WinSPALL Software, Wolfgang Knoll, MPI, Germany), providing the crucial ability to extract the thickness of the adsorbed layer. In this case, the thickness before and after adsorption to the bare and thiol-treated surface was determined to be 0.82 nm and 0.52 nm respectively.

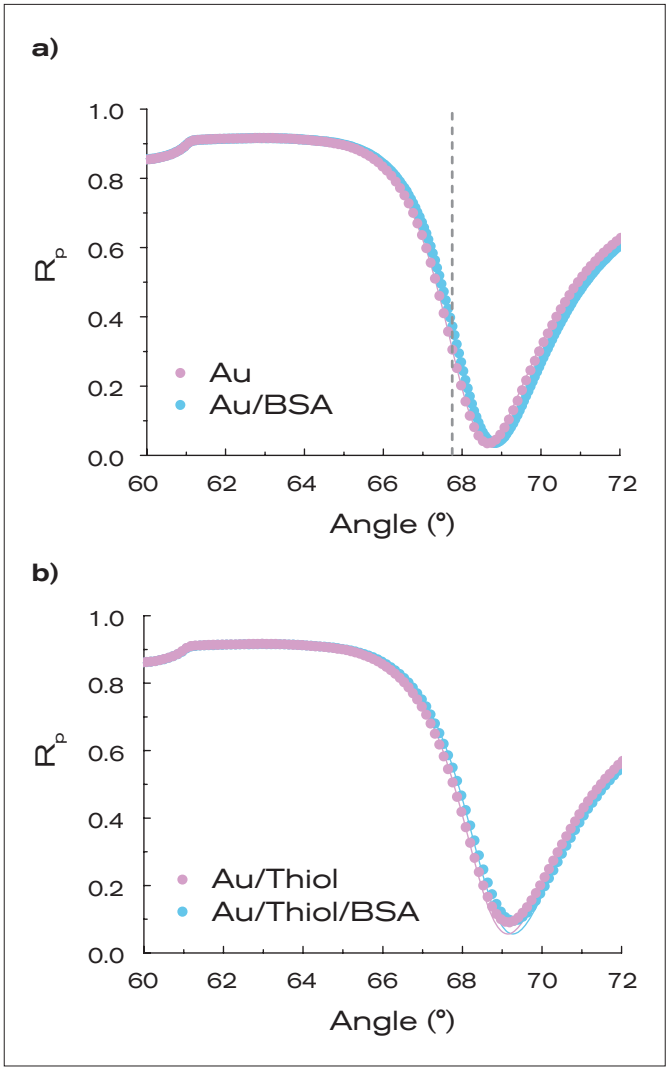

Figure 1. Change in complete SPR curve before and after BSA adsorption onto (a) hydrophilic and (b) hydrophobic gold. The dashed vertical line in (a) highlights the angle used for kinetic measurements in Figure 2.

Monitoring the kinetics of changes in the surface concentration (in this case due to adsorption) are most sensitively monitored by measuring the change in SPR intensity at the steepest part of the intensity versus angle curve (see vertical line in Figure 1a). The intensity versus time signal was then followed, as illustrated in

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Figure 2. BSA solution entered the liquid flow chamber at ca. 30 seconds. It is immediately clear that the surface treatment of the gold surface has a major influence on the adsorption process. About twice as much BSA adsorbed to hydrophilic gold compared with the hydrophobic gold-thiol surface, in good qualitative agreement with the thicknesses extracted by fitting the intensity versus angle curves with theory. A hypothesis that matches this difference in adsorption is that BSA tends to adsorb randomly onto gold with both end-on and side-on orientations, while on hydrophobic surfaces, BSA molecules mainly adopt a side-on orientation.

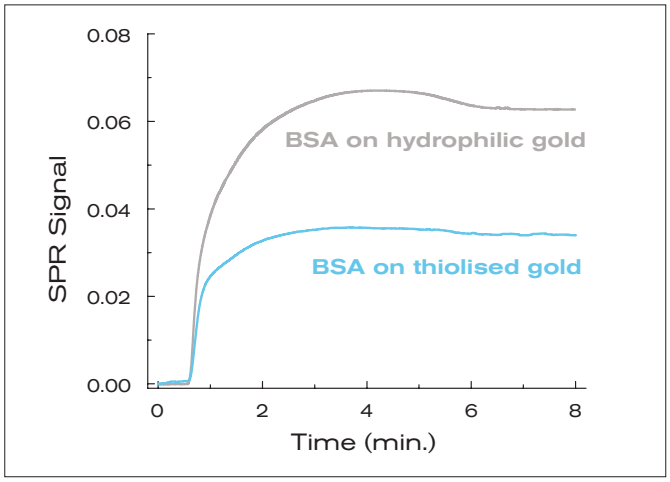

Figure 2. Change in SPR signal during BSA adsorption and desorption to hydrophilic and hydrophobic gold. BSA was rinsed from the cell after about 4 minutes.

Discussion

The layer thickness modeled as in Figure 1 is conspicuously small compared to the length and width dimensions of BSA (8 nm x 4 nm). However, it should be kept in mind that the theoretical fitting assumes a uniform film thickness, whereas it is known that each BSA molecule adsorbs separately, leading to a "patchy" presence on the surface. It is assumed that if the dimensions of the adsorbed protein are known, then the fitted film thickness can be used to calculate the actual surface coverage. This concept is illustrated in Figure 3. The conclusion, therefore, is that, at this concentration, BSA is far from forming a complete monolayer, with an approximate coverage of between 5 and 20% depending on molecular orientation of BSA on the surface.

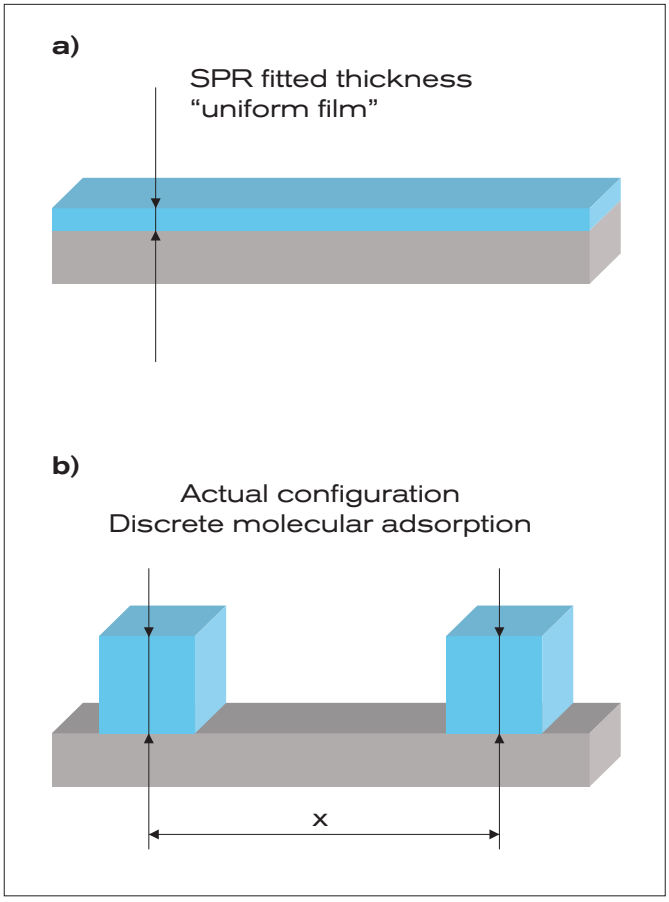

Figure 3. Conceptual illustration of how SPR "sees" a surface with a non-uniform molecular coating.

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