Surface plasmon resonance biosensors

**Introduction**

Surface plasmon resonance (SPR) spectroscopy is a surface-sensitive technique that has been used to characterize the thickness and/or index of refraction of ultra-thin organic and biopolymer films at noble metal (Au, Ag, Cu) surfaces. Due to the following advantages, *(a)* the kinetics of biomolecular interactions can be measured in real time by SPR spectroscopy, *(b)* the adsorption of unlabeled analyte molecules to the surface can be monitored in real time, and *(c)* SPR has a high surface sensitivity that allows weakly bound interactions to be monitored in the presence of excess solution species, this technique attracted worldwide attention in the decade following the appearance of the first few publications.\(^1,2\) In recent years, SPR spectroscopy has been widely used in biochemistry research to monitor events such as antibody-antigen binding,\(^3,4,5\) DNA/RNA hybridization,\(^6,7,8,9\) and protein-DNA interactions,\(^10,11\) and detection of the conformational changes of immobilized proteins.\(^12\)
**Theory**

When a light beam passes an interface from a medium having a higher refractive index to a medium having lower refractive index at an angle above the critical angle, 100% of the light will be reflected back. In SPR, a thin layer of metal film is placed in the middle of two media having different refractive indices. Although this light is totally reflected, a finite electrical field intensity (evanescent field wave) will appear under the interface. This evanescent field wave can penetrate through the metal film (typically 50-150nm thick) into the media having lower refractive index. Generally, the maximum penetration depth is about 200 nm.\

![Diagram of SPR](image)

*Figure 1. Surface plasmon resonance*

When the wavevector for the photon and plasmon are equal in magnitude and direction, surface plasmon will be excited in the interface between the thin metal film and the medium having lower refractive index. This process transfers a significant amount of energy of the marching photon to the plasmon. The reflected light intensity will decrease accordingly. Because the magnitude of the wavevector for the plasmon
depends on the lower refractive index of the medium, the SPR signal is sensitive to the change of the refractive index and thickness of the medium having lower refractive index. It can be used to detect the affinity of an analyte to an immobilized ultra-thin organic or biopolymer film on the metal film.

A schematic of SPR spectroscopy instrument is shown below:

![Figure 2. Surface plasmon resonance detection unit. L: light source, D: photodiode array, P: prism, S: sensor surface, F: flow cell. The two dark lines in the reflected beam projected on to the detector symbolise the light intensity drop following the resonance phenomenon at time = t₁ and t₂. The line projected at t₁ corresponds to the situation before binding of analyte to the target on the surface and t₂ is the position of resonance after binding.](image-url)
Application

I Antibody-antigen binding

SPR can be used to study low-molecular-weight antigen-antibody interactions. The antibody is usually immobilized on the SPR surface and different antigens are dissolved in solution. The most common information can be given by SPR studies include:

a. Surface competition analysis
b. Direct Kinetic analysis

II DNA hybridization

SPR has gained attention as a label-free method for the detection of the binding of biological molecules onto functionalized surfaces. In an SPR imaging experiment, changes in the reflectivity from a thin gold film are used to monitor adsorption onto the surface. Bryce et al. demonstrated a multi-step procedure to create DNA arrays on gold surfaces for use with SPR imaging. These arrays can be used to study affinity interactions for a variety of target molecules, including unlabeled DNA and RNA.

Figure 3. SPR image showing hybridization adsorption site

\[ \Delta \% R = 0.5\% \]

50 nM DNA 18-mer complement
Some known sequence DNA/RNA can be immobilized on the surface of gold, meanwhile, an array was used to flow the solution that contains target DNA/RNA inside. As shown in figure 3, the change of the reflectivity represents the match between the immobilized DNA/RNA and target DNA/RNA.

III Protein-DNA interactions

Protein-DNA interaction governs the faithful replication of the genetic material. The specificity of the binding between the DNA and protein was studied. The mismatch on the protein was also studied to see the binding effect under imperfect conditions.
Reference


