Surface plasmon resonance (SPR) is one of the standard techniques for measuring adsorption of molecules and chemicals onto surfaces. Surface plasmon resonance is a powerful technique because it provides detailed information on the interaction of molecules with each other, such as how strongly or weakly they interact and how specific those interactions are. A major advantage of surface plasmon resonance is that it is label-free, so unlike techniques like ELISA, no label molecule is required to detect the target of interest. However, surface plasmon resonance systems are expensive, typically costing upwards of $100,000, making them inaccessible to the vast majority of scientists and researchers. OpenSPR™ by Nicoya Lifesciences is different— it doesn’t use a planar gold film, prism, or any of the complicated optics you would find in classic surface plasmon resonance systems. Instead, it uses gold nanoparticles to generate what is known as localized surface plasmon resonance (LSPR).

**Overview of Localized Surface Plasmon Resonance**

Localized surface plasmon resonance is generated by metal nanoparticles, typically gold and silver. LSPR produces a strong resonance peak in the visible range of light, with its position being highly sensitive to the local refractive index surrounding the particle. Using localized surface plasmon resonance simplifies the design of the system and makes the sensors more affordable compared to conventional SPR.

![Fig. 1. Illustration of the LSPR biosensing principle](image-url)
Gold nanoparticles that generate localized surface plasmon resonance are highly stable and sensitive, so they can be used to produce interaction data of similar quality to conventional surface plasmon resonance, but at a fraction of the cost.

As in conventional SPR, the surface of the nanoparticles can be functionalized with a wide variety of different chemicals, molecules, antibodies, DNA, and more. Because we use gold nanoparticles, standard functionalization protocols are applied to immobilize the ligand. Once the analyte of interest interacts with the functionalized surface, the plasmon resonance peak changes position, which can be measured to high degree of accuracy using our optical analysis platform (Fig. 2).

**Sensitivity and Performance of LSPR**

Although SPR sensors have a much higher refractive index sensitivity (m or RIS) than LSPR sensors, their sensitivity towards biomolecular binding events is similar. This is due to the much shorter electromagnetic decay length ($l_d$) of nanoparticles compared to gold films, which confines the response to a smaller sensing volume. An LSPR’s sensor response ($R$) to an absorbed layer can be modeled with the following equation:

$$R = m \Delta n (1 - e^{-2d/l_d})$$

Where $\Delta n$ is the difference in refractive index between the absorbed layer and the surrounding medium, and $d$ is the thickness of the absorbed layer. An example of the LSPR response to absorbed polyelectrolyte bilayers is shown in Fig. 3, along with the sensitivity and decay length. The smaller decay length and sensitivity associated with LSPR reduces artifacts caused by external variables such as temperature drift or buffer refractive index changes. This allows use of a single channel device and eliminates the need for precise temperature control, while still providing a high level of data quality and confidence.
Another important parameter that helps define LSPR sensor performance is the LSPR figure of merit (FOM) defined as:

\[ FOM = \frac{m}{\Delta\lambda} \]

Where \( \Delta\lambda \) is the spectral line width or full width at half maximum of the LSPR peak. The FOM is related to the nanoparticle material, size, shape, and uniformity.

**Biomolecular Kinetics with LSPR**

LSPR can be used for a wide variety of applications. One of the most common is the determination of binding constants between two different molecules. Much like conventional surface plasmon resonance, localized surface plasmon resonance is very well suited for determining these kinetic constants.

Binding kinetics describe the interaction between one or more molecular species. Interaction includes the association and dissociation of the two species. The chemical formula describing a simple 1:1 interaction is as follows:

\[ A + B \leftrightarrow AB \]

\[ k_a \quad \quad k_d \]

Where A and B are the binding molecular species that associate to form AB. The rate of association is governed by the rate constant \( k_a \) and the dissociation rate by \( k_d \).

Standard rate law chemistry applies to this interaction, thus, at steady state the above equation yields:

\[ \frac{d[AB]}{dt} = k_a [A][B] - k_d [AB] = 0 \]

\[ \frac{k_d}{k_a} = \frac{[A][B]}{[AB]} = K_D \]

Where \( K_D \) is the equilibrium dissociation constant defined in concentration units (nM). In defining the system this way, a quick quantification of the strength of the binding interaction can be stated simply by reporting the order of magnitude of the \( K_D \) value. The smaller the \( K_D \) the stronger the interaction.
Steady State Affinity Experiments

The convention for defining the interacting species where one is immobilized, as in the case with OpenSPR, the immobilized species is called the ligand (B) and the free species is called the analyte (A). Some refer to this type of kinetic analysis as equilibrium analysis, using the concepts of steady state and equilibrium interchangeably.

The steady state equation defined above can be adjusted to incorporate the refractive index measurements (R) made by OpenSPR. The amount of AB in the system at any time is measured directly by R. The maximum change in R (R_{max}) is defined by the total amount of B that is immobilized onto the sensor surface and is constant. Therefore, the rate equation can be written completely in terms of measureable quantities determined by OpenSPR:

\[
\frac{dR}{dt} = k_a C R_{max} - k_a CR - k_a R
\]

Where C is the injected analyte concentration. At Steady State (R=R_{ss}):

\[
R_{ss} = \frac{C R_{max}}{C + K_D}
\]

From this equation it can be seen that when R_{ss} is equal to R_{max}/2, K_D is equal to C. Therefore when R_{ss} is plotted against C, K_D can be easily determined as shown in Fig. 4 below.

![Affinity isotherm for steady state analysis to determine equilibrium constant using LSPR](image)

---

Fig. 4. Affinity isotherm for steady state analysis to determine equilibrium constant using LSPR
Kinetic Affinity Experiments

There are some limitations that may make steady state experiments undesirable. For strong interactions, the amount of analyte required to reach steady state conditions may be excessive. Also, steady state experiments can be used to determine equilibrium constants $K_D$ and $K_A$ but not the binding constants $k_d$ and $k_a$. Kinetic affinity experiments can be utilized to determine the binding constants and equilibrium constants and works effectively for more strongly binding systems.

Kinetic analysis is done by monitoring binding and dissociation of the analyte to the ligand over time and at different concentrations, then applying an interaction model to the data generated through experiment. There are two popular ways of performing kinetic analysis: regeneration and titration.

Regeneration

The primary method of performing kinetic affinity experiments is to regenerate the sensor surface between injections. Regeneration refers to the disruption of the binding interaction between the analyte and the ligand. Regenerating the surface leaves the ligand on the surface free for a new injection of analyte at a different concentration. To regenerate the binding surface an injection of either an acidic, basic, salt or surfactant solution is used to disrupt the binding between the ligand and the analyte. The most effective regeneration buffer to use is specific to each binding pair and must be determined by the user. Sometimes cycles of different solutions are required to fully remove the analyte and the user must be careful not to use overly harsh treatments that will damage the ligand and decrease the sensor’s ability to bind during future injections. Data can be post processed in kinetic analysis software such as TraceDrawer.

Kinetic Titration

A second method of performing kinetic affinity tests is to inject increasing concentrations of analyte over a single sensor without the need for regeneration. Post-processing software can then be used to extract the kinetic constants. This method is advantageous as a regeneration protocol does not have to be developed, and results can be obtained much faster.

References

