A beginner's guide to surface plasmon resonance

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Surface plasmon resonance (SPR) has emerged as a powerful optical detection technique for studying the binding behaviour of immobilized ligands and analytes in solution. The technique makes it possible to measure interactions in real time with high sensitivity. Over the past two decades, SPR has become the gold standard for studying biomolecular interactions in biomedical research and drug discovery. The interactions that can be studied are diverse and include protein–protein, protein–small molecule, protein–nucleic acid, protein–carbohydrate, lipid–protein, hybrid systems of biomolecules and non-biological surfaces. SPR allows researchers to determine which molecules interact, how strongly they bind and inform experiments using mutants, truncations or other variations to probe specificity. This article summarizes the principle and experimental set-up and illustrates the utility of SPR using the example of lipid–protein interactions.

What is SPR?

Surface plasmon resonance (SPR) relies on the concept of total internal reflection. In this set-up, light passes through a prism and reflects off the sensor chip surface (typically gold) into a detector at a specific incident angle, known as the resonant angle. Light is absorbed by electrons in the sensor chip surface. The result is an intensity loss in the reflected beam which can be detected as a dip in the SPR reflection intensity curve. The shape and location of the dip can then be used to provide information about the surface (Figure 1). The binding of biomolecules results in changes in refractive index on the sensor chip. When a ligand is immobilized on the sensor chip and the binding of the analyte is measured, there is an increase in mass associated with the binding event. The increase in mass causes a proportional increase in the refractive index, which is observed as a change in response. When this change occurs, a response is plotted with respect to time and a sensorgram is produced (Figure 1).

Experimental setup

In a typical SPR experiment, the first step is to immobilize the ligand to the sensor surface. It is important to choose the appropriate sensor chip and determine optimal conditions for ligand immobilization (pH, ligand concentration and buffers). The ligand can be either permanently or temporarily immobilized on the sensor surface prior to the measurement of the analyte interaction. There are several different types of sensor surfaces and methods for immobilization (Table 1). A good sensor chip surface should provide a biocompatible environment for the ligand, preserving its biological activity. The surface should also minimize non-specific interactions with the chip surface and enable efficient immobilization chemistry and yield.

Once the ligand is immobilized on the sensor surface, any remaining binding sites are blocked and the interaction analysis sensorgram is generated. This step is composed of five phases: baseline, association, steady state (if reached), dissociation and regeneration (Figure 2). A flow buffer is used to condition the sensor chip and check for any sensor chip instability. It is important to have a baseline with minimal drift. Any injection spikes are an indication that SPR should not be run, and the system should be checked and cleaned. Standard running buffers include phosphate-buffered saline and HEPES containing NaCl. During the injection of the analyte, the binding response increases as the analyte–ligand complex is formed, defining the association phase. After a certain time of injection, the steady state is reached, which is the flat portion of the sensorgram where the net rate of binding analyte is zero. When the analyte is replaced by a wash buffer, there is a decrease in analyte due to dissociation of the complexes, defining the dissociation phase. Depending on the dissociation rate, some assays may require a regeneration step; here a low pH buffer is flowed to obtain the same SPR baseline as at the beginning of the experiment. It is important to establish a steady baseline to indicate that the sensor chip is free of bound analyte and has stability.

Analyte concentration plays a critical role in fitting and determining the kinetic rate constants. To generate a full kinetic profile for the interactions and obtain binding constants, one must measure the interaction of multiple analyte concentrations with the ligand. A minimum of six concentrations is suggested and eight or more data

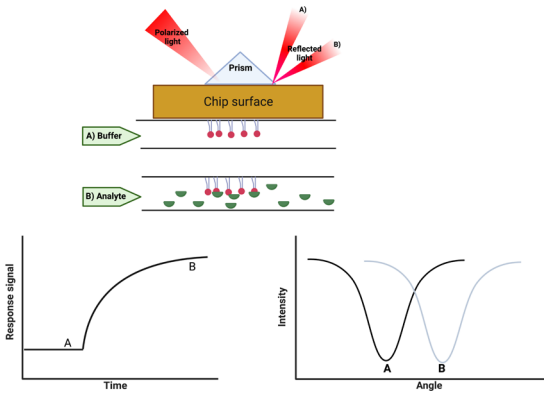

Figure 1. The concept of surface plasmon resonance. Polarized light emanating from a light source passes through a prism prior to contacting the sensor chip surface. The reflected light intensity is measured on the detector at a specific angle of incidence resulting in a dip in the intensity of the reflected light. A change in refractive index caused by a binding event (increased mass on the surface) will cause an angle shift from A to B. The response is plotted against time on a sensorgram as shown in the right panel. Created using Biorender. Figure adapted from https://cmi.hms.harvard.edu/surface-plasmon-resonance.

points are useful for robust data and curve fitting. Several SPR experiments should be run first to determine the analyte concentration necessary for detectable binding before the final SPR experiments. The concentration range should typically span 10× below the K_d to 10× above the K_d of the interaction. If you are starting with an unknown system where you have no prior knowledge of the K_d, search the literature to discover if a similar interaction system has been previously studied. If this is not possible, then consider the type of interaction being

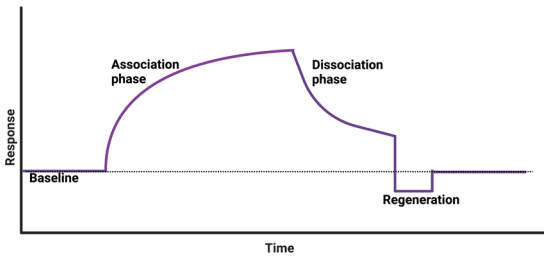

Figure 2. Example sensorgram showing the phases of an analysis cycle. Created using Biorender.

studied. To determine kinetic constants, the steady-state response level can be used. Response value can be plotted against the concentration to determine the affinity using the equation $R_{eq} = R_{max}/(1+K_d/C)$ where R_{eq} is the binding signal obtained at steady state for each concentration (C) and R_{max} is the binding signal at saturation of the analyte–ligand system.

Studying lipid–protein interactions with the L1 sensor chip

Lipids are an important class of biological molecules that have many roles in different processes. They are the building blocks of all cells; they are the major component in the plasma membrane and cellular compartments. Cells express thousands of different lipids to satisfy these different functional requirements, yet lipid biochemistry is not yet wholly understood. As nearly half of the proteins are located within or on membranes, it is essential to characterize lipid–protein interactions to discern the role these proteins and lipids play in cells. Increasingly, SPR is being used as a tool to study the affinity and specificity of lipid–protein binding interaction. Here we will discuss

Table 1. Overview of commonly used SPR sensor chips

Chip name	Application
CM5	The most versatile dextran-based sensor chip. For immobilization of molecules via $-NH_2$, –SH, –CHO, –OH or –COOH groups
HPA	Used when working with model membrane systems. Hydrophobic sensor surface allows for a supported lipid monolayer to form. Useful for the study of membrane-associated interactions
L1	Used for the high-capacity capture of lipid vesicles while retaining the lipid bilayer structure. Useful for the study of lipid–protein interactions and incorporation of transmembrane receptors (e.g., to replicate the membrane environment)
NTA	For the capture of his-tagged molecules via metal chelation. Useful for the interaction of immobilized proteins with a wide range of analyte molecules. It is the first choice for experiments where low molecular weight analytes are being studied.
SA	Harbours a surface enriched with streptavidin to immobilize biotinylated molecules such as carbohydrates, peptides, proteins and nucleic acids

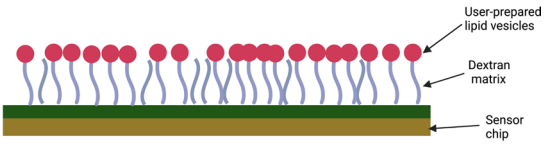

Figure 3. Schematic of the L1 chip, commonly used for coating lipid vesicles. The L1 chip allows for the coating of lipid vesicles through insertion of alkane groups present on the chip surface. Created using Biorender. Adapted from https://content.iospress.com/articles/biomedical-spectroscopy-and-imaging/bsi045.

how the L1 chip can be used to study the binding affinity in lipid–protein interactions. The L1 chip has lipophilic groups that embellish the dextran matrix allowing the capture of intact lipid vesicles or the formation of a bilayer (Figure 3).

First, one must prepare a reliable lipid surface that resembles membranes that the protein of interest would encounter in a biological system. It is important to ensure that the concentrations of lipids being used are close to physiological conditions and can be detected by the instrument. If one is studying how a protein interacts with the plasma membrane, research or literature search should be done to determine the lipid composition of that membrane. In a two-flow cell system, it is recommended that flow cell 1 (FC1) be prepared as the control surface and flow cell 2 (FC2) as the test surface. To coat the L1 chip, first the surface is washed with 50 µL of 20 mM 3-[(3-cholamidopropyl) dimethylammonium]-1-propanesulfonate (CHAPS) and 50 µL of 40 mM octyl glucoside at a flow rate of 30 µL/min. These wash steps are followed by injecting liposomes at a flow rate of 5 µL/min. Always coat FC2 before coating FC1. This allows one to adjust the control FC1 coating so that it is matched to FC2. It is best to have relative response levels (RU) to be within 3–5% between the two channels so as not to bias data collection. The closer the channels match, the better. The coating ability of the lipid vesicles can vary depending on the lipid content; they can go as high as 9000 RU. A coating level of 2500 RU is sufficient for an SPR run but anything lower than this is not coated sufficiently for appropriate analysis of the binding interaction. The lipid surface is then stabilized by a 10 µL injection of 50 mM sodium hydroxide (NaOH) at a flow rate of 30 µL/min. This will wash off any loosely bound lipid vesicles. After the lipid surface has been prepared, it is necessary to block any exposed chip surface by injecting 0.1 mg/mL of fatty acid-free bovine serum albumin (BSA). This is also a good way to assess the coating efficiency of the chip. A well-coated surface is signified by a <100 RU change upon the injection of BSA. BSA left on the chip will not affect lipid-binding abilities and will reduce non-specific binding to the chip by blocking the exposed chip surface.

After this immobilization, the chip is ready to be used for assessing lipid–protein binding interactions. First, the surface must be equilibrated with the flow buffer until the baseline is stable. Prepare the protein dilutions just prior to injection. It is important to use fresh, active protein for the best results. Before use, make sure to spin the protein down at 50,000 g for 20 minutes to remove any precipitated protein (filtration of protein through a 0.45 µm or 0.22 µm membrane is also an option if a protein can tolerate this). Next, plan the protein dilutions to be used. Choose protein concentrations that range between 0.1 times to 10 times the predicted K_d, starting with the lowest protein concentration first in case protein binds or sticks to chip or is hard to remove from the lipid vesicles. The protein should be injected at flow rate of 5 µL/min with enough time to reach saturation (e.g., steady state if possible) for

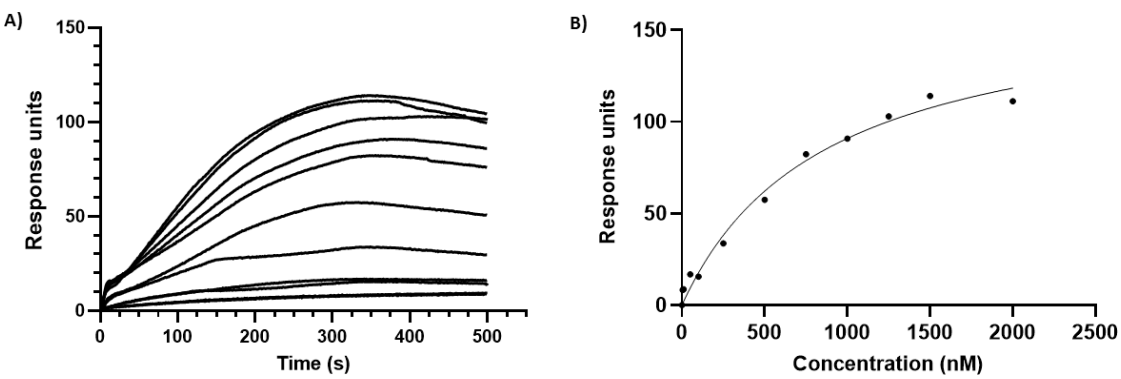

Figure 4. (a) A *POPC:POPE:POPS* surface coated on an L1 chip was used to test the binding of the Ebola virus matrix protein VP40. A *POPC:POPE* surface was used as a control. WT eVP40 was injected at increasing concentrations (5–2000 nM) at a flow rate of 5 µL/min to determine the saturation response in signal (RU) at each respective protein concentration. (B) Saturation values were plotted and fit to determine K_d. Experiments were repeated in triplicate to determine the average apparent K_d (340 ± 65 nM) for binding PS-containing lipid vesicles.

Table 2. Summary of common problems during an SPR experiment

Problem	Possible cause	Solution
Bulk shifts or spikes	Running and analyte buffers are not matched resulting in a shift in a curve at the beginning and the end of an injection	Analyte and running buffers are the same for the best runs. If analyte has been prepared in a different buffer, it is beneficial to dialyse the analyte in the running buffer or perform a buffer exchange with size exclusion
Baseline drift	Sensor surface has not been properly equilibrated	Make sure that the system is properly equilibrated. Use sufficient buffer in the prime and wash steps. Flow the running buffer at the flow rate for the experiment until a stable baseline is observed
Non-specific binding	Buffer mismatch and non-specific binding of the analyte to the reference surface	Supplement your running buffer with additives like a surfactant or BSA to block the sensor chip surface. Another approach is to couple (immobilize) a compound that does not bind the analyte on the reference cell

each concentration. To analyse the data, steady-state RU values are then plotted against the protein concentration to calculate the apparent K_d using the equation $R_{eq} = R_{max}/(1+K_d/C)$ (Figure 4). The lifetime of a lipid surface on an L1 chip can be up to 2 days. During this time, it is possible to collect robust reproducible data. After all protein–lipid binding measurements have been done, the lipid vesicles should be stripped off the chip surface before storage. To remove the liposomes, 50 µL of 20 mM CHAPS is injected at a 30 µL/min flow rate followed by a 50 µL injection of 40 mM of octyl glucoside. The chip can be removed from the SPR machine and stored according to the manufacturer's protocol.

Recognizing problems in the experimental set-up is important. Drifts, jumps and spikes in the SPR curve make analysis difficult(Table 2). It is important to identify problems in the experimental set-up and solve them before attempting to analyse the data. Table 2 summarizes the most common problems that may be encountered during an experiment and possible ways to trouble shoot them.

What's next

SPR is now one of the most used label-free biophysical techniques and there have been many successful commercial applications for this technology. Since its conception, SPR has made great advances in terms of technology and continuous efforts have been made to improve the overall performance of SPR sensors. Over the last decade, SPR has been coupled with other techniques such as electrochemistry and mass spectrometry and has been used for gas phase detection. One of the most significant developments in recent years is surface plasmon imaging (SPRi), also known as surface plasmon resonance microscopy (SPRM). SPRi combines high-resolution optical microscopy and real-time measurements of binding affinity and kinetics.

It has been challenging to study membrane protein binding to ligands using conventional SPR because these proteins are not easy to extract and their immobilization on a sensor surface changes the native environment of the protein and cannot guarantee the proper orientation for ligand binding. SPRi has bridged this gap because it allows for the attachment of whole cells directly onto the sensor keeping proteins in their native state. Only a few SPR imaging and microscopy commercial platforms are available, which limit the development of new applications and the impact of this technology. Even though SPR has been used for decades, there is still a need for more sensitive and accurate SPR sensors and, with advances in technology, this technique will continue to evolve and have an increasing impact on the fields of biology, biophysics, drug discovery and food safety and security.

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Further Reading & Viewing

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