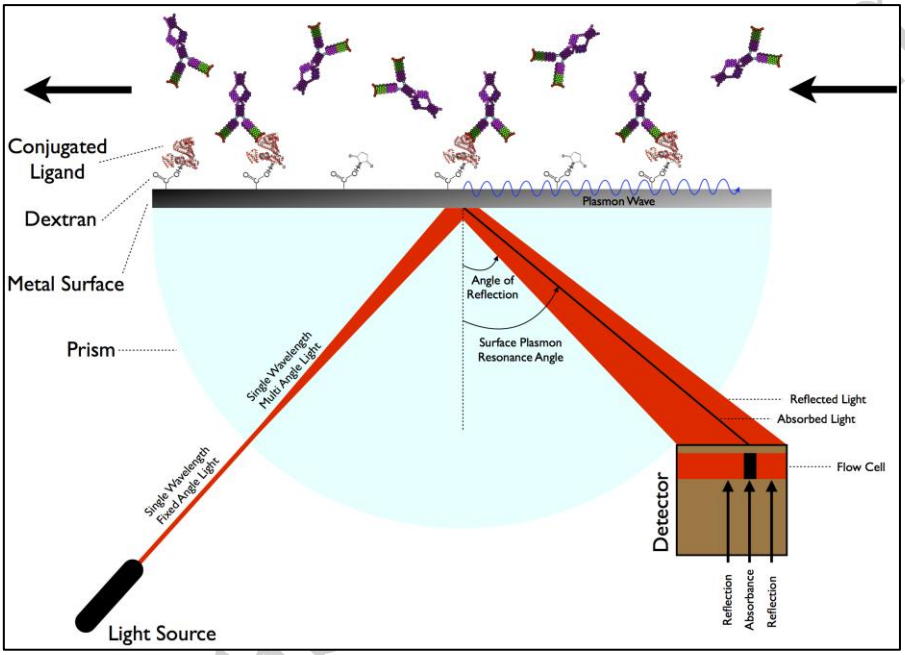
Surface plasmon resonance

Surface plasmon resonance (SPR) is the resonant oscillation of conduction electrons at the interface between negative and positive permittivity material stimulated by incident light. SPR is the basis of many standard tools for measuring adsorption of material onto planar metal (typically gold or silver) surfaces or onto the surface of metal nanoparticles. It is the fundamental principle behind many color-based biosensor applications, different lab-on-a-chip sensors and diatom photosynthesis.



Surface plasmon resonance (SPR).

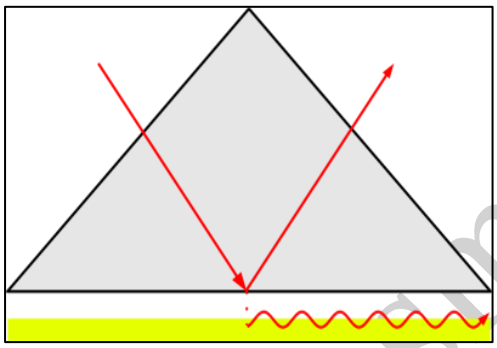
The surface plasmon polariton is a non-radiative electromagnetic surface wave that propagates in a direction parallel to the negative permittivity/dielectric material interface. Since the wave is on the boundary of the conductor and the external medium (air, water or vacuum for example), these oscillations are very sensitive to any change of this boundary, such as the adsorption of molecules to the conducting surface.

To describe the existence and properties of surface plasmon polaritons, one can choose from various models (quantum theory, Drude model, etc.). The simplest way to approach the problem is to treat each material as a homogeneous continuum, described by a frequency-dependent relative permittivity between the external medium and the surface. This quantity, hereafter referred to as the materials' "dielectric function", is the complex permittivity. In order for the terms that describe the electronic surface plasmon to exist, the real part of the dielectric constant of the conductor must be negative and its magnitude must be greater than that of the dielectric. This condition is met in the infrared-visible wavelength region for air/metal and water/metal interfaces (where the real dielectric constant of a metal is negative and that of air or water is positive).

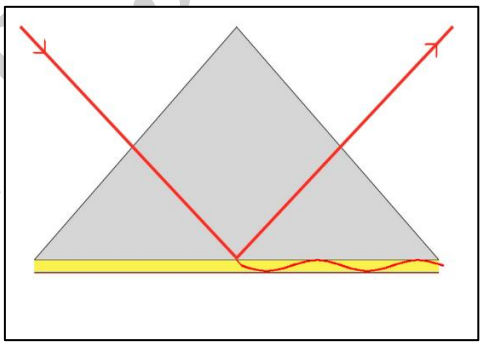
LSPRs (Localized SPRs) are collective electron charge oscillations in metallic nanoparticles that are excited by light. They exhibit enhanced near-field amplitude at the resonance wavelength. This field is highly localized at the nanoparticle and decays rapidly away from the nanoparticle/dieletric interface into the dielectric background, though far-field scattering by the particle is also enhanced by the resonance. Light intensity enhancement is a very important aspect of LSPRs and localization means the LSPR has very high spatial resolution (subwavelength), limited only by the size of nanoparticles. Because of the enhanced field amplitude, effects that depend on the amplitude such as magneto-optical effect are also enhanced by LSPRs.

Implementations

In order to excite surface plasmons in a resonant manner, one can use electron bombardment or incident light beam (visible and infrared are typical). The incoming beam has to match its momentum to that of the plasmon. In the case of p-polarized light (polarization occurs parallel to the plane of incidence), this is possible by passing the light through a block of glass to increase the wavenumber (and the momentum), and achieve the resonance at a given wavelength and angle. S-polarized light (polarization occurs perpendicular to the plane of incidence) cannot excite electronic surface plasmons.



Otto configuration



Kretschmann configuration

Typical metals that support surface plasmons are silver and gold, but metals such as copper, titanium or chromium have also been used.

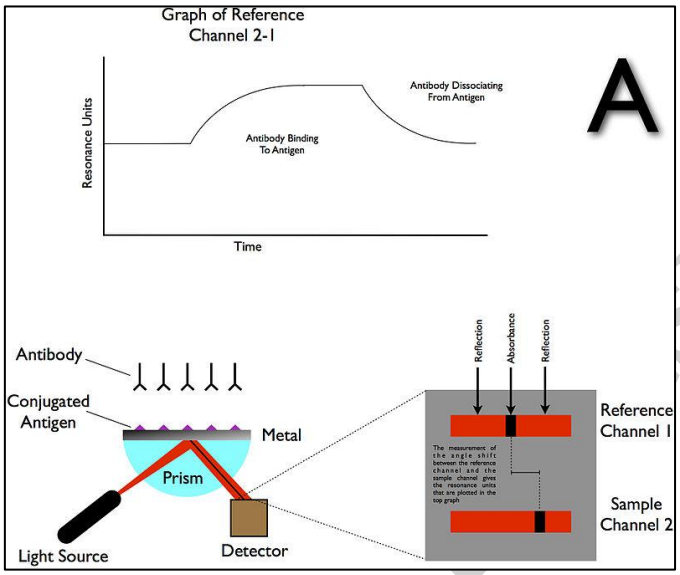
When using light to excite SP waves, there are two configurations which are well known. In the Otto setup, the light illuminates the wall of a glass block, typically a prism, and is totally internally reflected. A thin metal film (for example gold) is positioned close enough to the prism wall so that an evanescent wave can interact with the plasma waves on the surface and hence excite the plasmons.

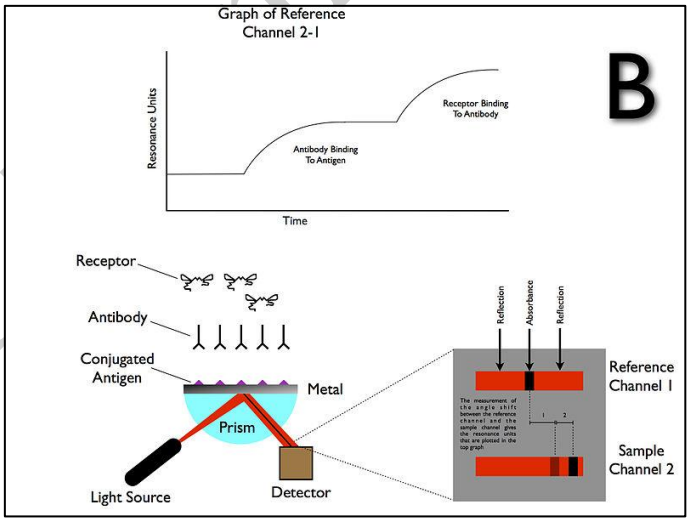
In the Kretschmann configuration, the metal film is evaporated onto the glass block. The light again illuminates the glass block, and an evanescent wave penetrates through the metal film. The plasmons are excited at the outer side of the film. This configuration is used in most practical applications.

SPR emission

When the surface plasmon wave interacts with a local particle or irregularity, such as a rough surface, part of the energy can be re-emitted as light. This emitted light can be detected behind the metal film from various directions.

Applications





Surface plasmons have been used to enhance the surface sensitivity of several spectroscopic measurements including fluorescence, Raman scattering, and second harmonic generation. However, in their simplest form, SPR reflectivity measurements can be used to detect molecular adsorption, such as polymers, DNA or proteins, etc. Technically, it is common to measure the angle of minimum reflection (angle of maximum absorption). This angle changes in the order of 0.1° during thin (about nm thickness) film adsorption. (See also the Examples.) In other cases the changes in the absorption wavelength is followed. The mechanism of

detection is based on that the adsorbing molecules cause changes in the local index of refraction, changing the resonance conditions of the surface plasmon waves. The same principle is exploited in the recently developed competitive platform based on loss-less dielectric multilayers (DBR), supporting surface electromagnetic waves with sharper resonances (Bloch surface waves).

If the surface is patterned with different biopolymers, using adequate optics and imaging sensors (i.e. a camera), the technique can be extended to surface plasmon resonance imaging (SPRI). This method provides a high contrast of the images based on the adsorbed amount of molecules, somewhat similar to Brewster angle microscopy (this latter is most commonly used together with a Langmuir–Blodgett trough).

For nanoparticles, localized surface plasmon oscillations can give rise to the intense colors of suspensions or sols containing the nanoparticles. Nanoparticles or nanowires of noble metals exhibit strong absorption bands in the ultraviolet-visible light regime that are not present in the bulk metal. This extraordinary absorption increase has been exploited to increase light absorption in photovoltaic cells by depositing metal nanoparticles on the cell surface.[7] The energy (color) of this absorption differs when the light is polarized along or perpendicular to the nanowire.[8] Shifts in this resonance due to changes in the local index of refraction upon adsorption to the nanoparticles can also be used to detect biopolymers such as DNA or proteins. Related complementary techniques include plasmon waveguide resonance, QCM, extraordinary optical transmission, and dual polarization interferometry.

Data interpretation

The most common data interpretation is based on the Fresnel formulas, which treat the formed thin films as infinite, continuous dielectric layers. This interpretation may result in multiple possible refractive index and thickness values. However, usually only one solution is within the reasonable data range. In Multi-Parametric Surface Plasmon Resonance, two SPR curves are acquired by scanning a range of angles at two different wavelengths, which results in a unique solution for both thickness and refractive index.

Metal particle plasmons are usually modeled using the Mie scattering theory.

In many cases no detailed models are applied, but the sensors are calibrated for the specific application, and used with interpolation within the calibration curve.

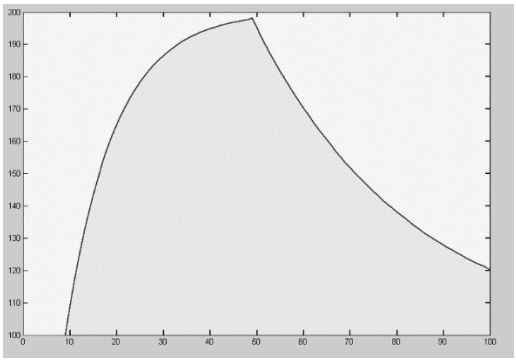
Examples

1. **Binding constant determination**

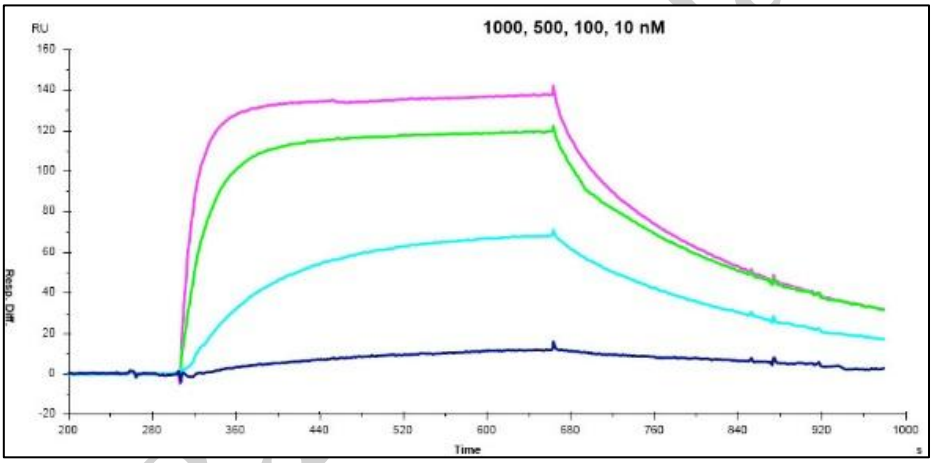
When the affinity of two ligands has to be determined, the binding constant must be determined. It is the equilibrium value for the product quotient. This value can also be found using the dynamic SPR parameters and, as in any chemical reaction, it is the dissociation rate divided by the association rate.

For this, a bait ligand is immobilized on the dextran surface of the SPR crystal. Through a microflow system, a solution with the prey analyte is injected over the bait layer. As the prey analyte binds the bait ligand, an increase in SPR signal (expressed in response units, RU) is observed. After desired association time, a solution without the prey analyte (usually the

buffer) is injected on the microfluidics that dissociates the bound complex between bait ligand and prey analyte. Now as the prey analyte dissociates from the bait ligand, a decrease in SPR signal (expressed in resonance units, RU) is observed. From these association ('on rate', ka) and dissociation rates ('off rate', kd), the equilibrium dissociation constant ('binding constant', KD) can be calculated.



SPR curves measured during the adsorption of a polyelectrolyte and then a clay mineral self-assembled film onto a thin (ca. 38 nanometers) gold sensor.



Example of output from Biacore

The actual SPR signal can be explained by the electromagnetic 'coupling' of the incident light with the surface plasmon of the gold layer. This plasmon can be influenced by the layer just a few nanometer across the gold–solution interface i.e. the bait protein and possibly the prey protein. Binding makes the reflection angle change;

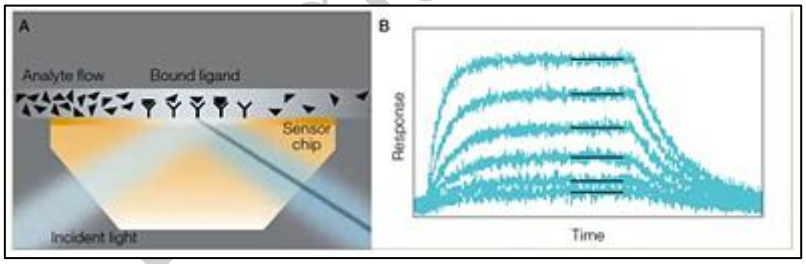
$$KD = kd/ka$$

2. Thermodynamic analysis

As SPR biosensors facilitate measurements at different temperatures, thermodynamic analysis can be performed to obtain a better understanding of the studied interaction. By performing measurements at different temperatures, typically between 4 and 40 °C, it is possible to relate association and dissociation rate constants with activation energy and thereby obtain thermodynamic parameters including binding enthalpy, binding entropy, Gibbs free energy and heat capacity.

Surface Plasmon Resonance and the ProteOn XPR36 System

Surface plasmon resonance is an optical phenomenon that is used in the ProteOn system to monitor the binding of any two unlabeled molecules in real time. The SPR signal is based on changes in the refractive index at the surface of a gold sensor chip as an analyte flows in a microfluidic channel and binds to a ligand immobilized on the sensor chip. Monitoring the change in the SPR signal over time produces a sensorgram, a plot of the binding response versus time. Fitting the sensorgram data to a suitable kinetic binding model allows for the calculation of kinetic parameters such as the association (ka) and dissociation (kd) rate constants.



Schematic illustration of the conversion of shifts in SPR angle into sensorgrams. A, as analyte binds to ligand molecules on the sensor chip surface, the intensity minimum (shadow) produced by the surface plasmon resonance effect undergoes an angular shift. This intensity shift is measured in real time for 36 interaction spots and 42 interspot references; B, each of the resulting sensorgrams is fitted to an appropriate mathematical model to quantitatively characterize the interaction (here shown for one set of six).

In addition to obtaining kinetic data, the ProteOn system can be used to investigate the specificity and thermodynamics of binding, screen compound libraries for affinity, or characterize epitopes of an antigen. The novel XPR™ technology of the ProteOn system is a unique application of SPR that uses enhanced microfluidics and a 6 × 6 interaction array on the ProteOn sensor chip to characterize the binding of multiple analytes to multiple ligands, in a single step, on a single chip.

How Do SPR Technology and the ProteOn system Work?

SPR is an optical phenomenon that occurs when p-polarized light at a certain wavelength and angle is reflected off a thin metal film (the gold film coated on the sensorchip) under the condition of total internal reflection (TIR). The light excites surface plasmons in the metal at a certain incident angle. The TIR field generates an evanescent wave in the thin metal film that extends hundreds of nanometers from the surface into the medium above, in this case the molecules in contact with the chip surface. The excited surface plasmons are very sensitive to the refractive index change at the surface of the thin metal film. Thus the incident angle of the light required for SPR is impacted by the refractive index change of the molecules in contact with the chip surface. In an SPR binding experiment, this refractive index change is brought about by binding of analyte in solution to ligand immobilized on the chip surface; therefore, tracking the change in the incident angle required for SPR allows one to monitor biomolecular interactions in real-time. The change of the incident angle required for SPR is defined as SPR signal in the unit of response unit (RU). 1 RU is 1/1,000,000 of a refractive index unit, and is roughly equivalent to a surface density of protein at approximately 1 pg/mm2. For a more in-depth discussion of SPR, see a recent review that offers an overview of SPR theory and different SPR configurations (Daghestani et al. 2010).

Plotting the SPR signal over time during the interaction between an analyte and a ligand results in a sensorgram, a visual representation of the interaction over time. Figure 2 shows an example sensorgram for an antibody-antigen interaction. The binding response initially increases as analyte is flowed over the sensor chip and associates with the immobilized ligand and then decreases as the analyte solution is replaced with buffer and the binding complex dissociates. If binding equilibrium is reached during the association phase, the sensorgram will reach a constant plateau before the analyte solution is replaced with buffer and the binding complex dissociates. Fitting the sensorgram data to a binding model allows for the calculation of the association (ka) and dissociation (kd) rate constants and determination of the binding affinity. Traditionally, kinetic measurements with SPR usually involve sequential injections of analyte at increasing concentrations over the same ligand surface, which requires complete removal of the analyte, or regeneration of the ligand surface, between analyte injections. In an ideal case, regeneration of the ligand surface is seen in the sensorgram as a sharp drop in RU after dissociation and a return to the original baseline. Regeneration is usually done with a combination of dilute surfactants, salts, and acids or bases; however, care must be taken during regeneration to avoid denaturing the immobilized ligand or removing ligand from the sensor chip.

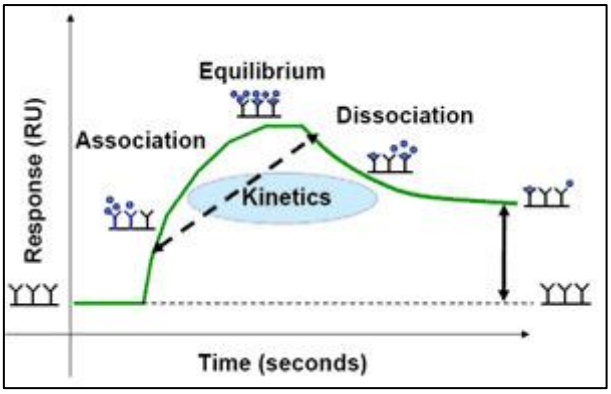


Fig. 2. Example SPR sensorgram of an antibody/antigen interaction showing the establishment of an initial baseline, an increase in response during association, a constant response after equilibrium is reached, and a decrease in response during dissociation.

The ProteOn system offers a distinct advantage over other SPR biosensor platforms because the unique 6 x 6 interaction array of the ProteOn sensor chips enable the One-shot Kinetics approach measuring the interaction of one ligand with a six-concentration series of one analyte in a single injection. This approach eliminates the need for traditional regeneration of the sensor chip between analyte injections that often deteriorates the ligand surface. Using enhanced microfluidic delivery and XPR technology, the ProteOn system can immobilize up to six separate ligands on a single sensor chip in six separate flow cells and then rotate the sensor chip 90 degrees to flow up to six separate analytes over the ligand surfaces (Figure 3).

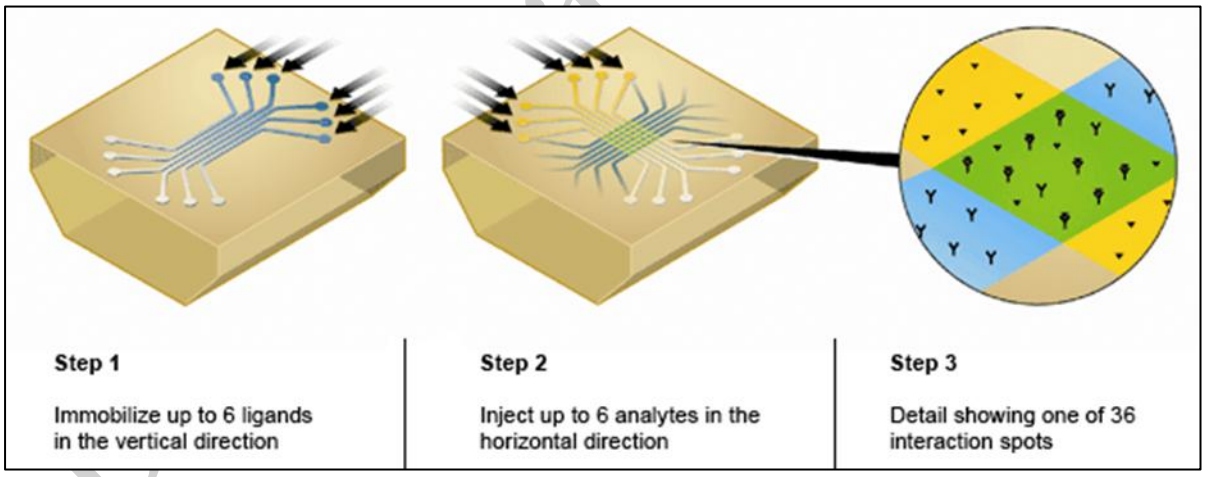


Fig. 3. ProteOn sensor chip 6 x 6 interaction array.

This unique feature of the ProteOn allows for the detection of up to 36 separate interactions on a single sensor chip and significantly increases the throughput of SPR biosensing. In a recent study, the ProteOn was used to immobilize 36 different ligands in a stepwise immobilization procedure designed for the high-throughput epitope mapping and binning of antibody/antigen interactions (Abdiche et al. 2011). The 6 x 6 interaction array of the ProteOn sensor chips also allows for in-line referencing, whereby data from unmodified spots in between the immobilized ligand spots on the sensor chip are used to subtract out artifacts such as noise and baseline drift. This in-line referencing is superior to referencing with a

separate flow cell and means the ProteOn can collect high-quality SPR data at low signal-to-noise ratios, as often the case with small molecule analytes.

What Kinds of Information Can Be Obtained with the ProteOn system?

Using SPR, the ProteOn XPR36 system can provide a wide variety of important information on biomolecular interactions such as the specificity, affinity, qualitative ranking, kinetics, and thermodynamics of binding.

The ProteOn system can be used in pharmaceutical drug discovery, antibody characterization, immunogenicity testing, the development and manufacture of biologics, or for clinical research. However, this is not a comprehensive list and nearly any research field can benefit from using the ProteOn system if there is a need for label-free characterization of a biomolecular interaction. Key applications include:

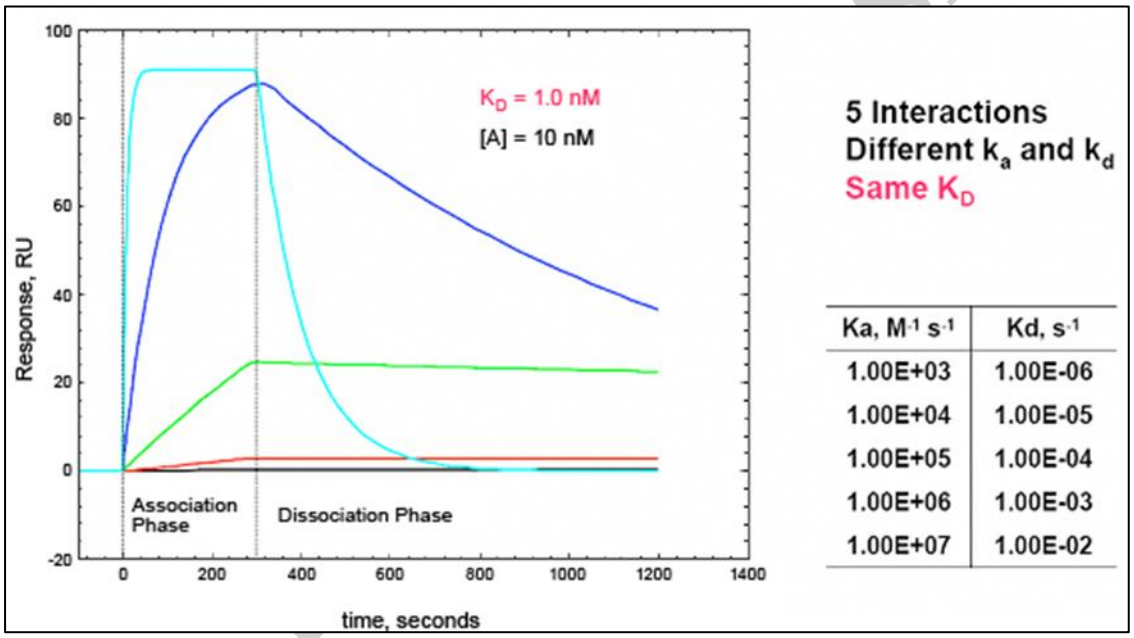
- Quantification of binding affinity and kinetics
- Determination of binding specificity and the number of binding sites
- Characterization of the mechanism of action
- Confirmation of biomolecule binding to a target
- Screening of fragment libraries
- Validation of IC50/EC50 values during hit-to-lead optimization
- Characterization of immune responses

Some classic applications of the ProteOn are in antibody engineering. Epitopes on an antigen can be characterized by epitope mapping, a process by which the affinities of an antibody to site-directed mutants of a single antigen help pinpoint the location of an epitope. An investigation of the epitope specificity, or epitope binning, of different antibodies can be done on the ProteOn system using the sandwich assay. In this assay, a second antibody is injected over a previously-formed antigen-antibody complex to see whether or not the second antibody can still bind. Binding of the second antibody to the antigen-antibody complex, or the formation of a 'sandwich' is an indication that the second antibody recognizes a different epitope than the first antibody.

SPR can also be used to determine the active concentration of an analyte in a crude or impure sample by probing the sample of interest under mass transport control using a low flow rate and/or a high-capacity sensor chip bearing an analyte-specific ligand. Under such mass transport limited conditions the association rate of binding, or the initial binding rate, is proportional to the concentration of analyte in solution. The concentration of analyte in a crude sample can be calculated by comparing the initial binding rate to a standard curve of initial binding rates for known concentrations.

How are Kinetic Parameters Obtained?

By fitting sensorgram data from a ProteOn system experiment to a suitable binding model, kinetic parameters such as the association (ka) and dissociation (kd) rate constants and the affinity (KD) can be extracted. Kinetic data are crucial for characterizing an interaction as they allow for a thorough understanding of the nuances of binding. Interactions with the same affinity (KD) can have markedly different association and dissociation rates, as seen in Figure 5. An antibody or small molecule that has a high affinity (low KD value) for a protein target may be a poor drug in-vivo if it has a very high dissociation rate and thus can be easily displaced by another molecule. This kind of information is easily obtained from an SPR experiment but would not be uncovered using a method such as isothermal calorimetry (ITC) that measures binding affinity based on binding at equilibrium. In addition, knowing the kinetics of a small molecule interaction allows for the more accurate analysis of quantitative-structure activity relationships as different structures can be evaluated by their separate effects on association and dissociation as opposed to affinity alone.



Ka, M^{-1} s^{-1}	Kd, s^{-1}
1.00E+03	1.00E-06
1.00E+04	1.00E-05
1.00E+05	1.00E-04
1.00E+06	1.00E-03
1.00E+07	1.00E-02

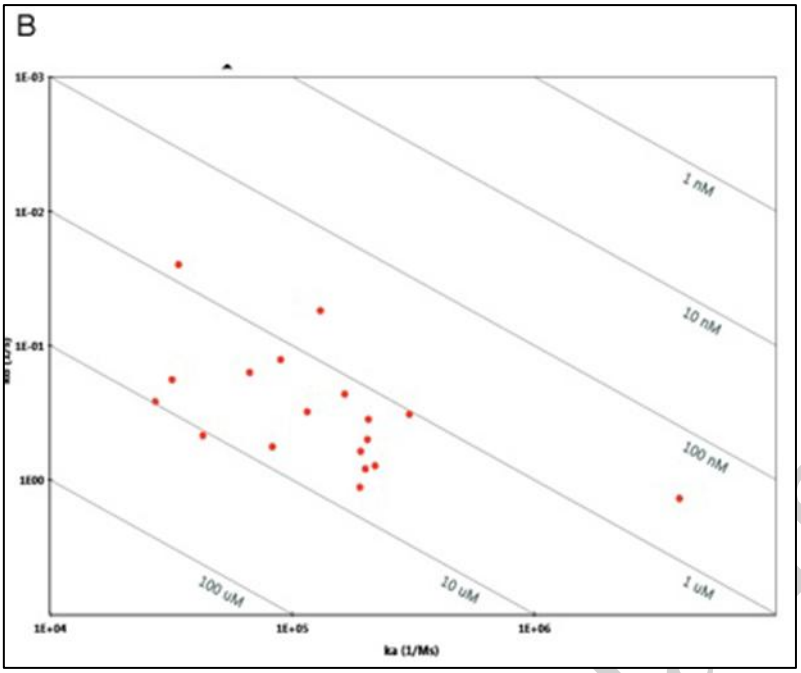


Fig. 5. A. Sensorgram plots showing the response (in RU) versus time for five different interactions with the same affinity (KD = 1.0 nM) but markedly different association (ka) and dissociation (kd) rate constants. The software model for this figure was kindly provided by Mohammed Yousef. Isoaffinity plot from ProteOn Manager™ software illustrating the importance of understanding ka, kd, in addition to KD. B. Isoaffinity plot from ProteOn Manager™ software illustrating the importance of understanding ka and kd in addition to KD. Here the affinity values cluster between 1 and 10 uM but with markedly different on and off rates.

The ProteOn Manager software gives you the option of using seven different binding models to analyze your sensorgram data:

- Langmuir: Simple 1:1 bimolecular interaction

o Simultaneous fitting of ka and kd

o Fitting of dissociation (kd) only

- Langmuir with drift: Simple 1:1 biomolecular interaction with a constant baseline drift taken into account

- Langmuir with mass transport limitations: Simple 1:1 biomolecular interaction that takes into account the rate of diffusion of analyte from the bulk to the surface

- Bivalent analyte: The analyte has two binding sites to one ligand.

- Heterogeneous analyte: Two analytes compete for binding to the same ligand site

- Heterogeneous ligand: One analyte binds to two separate ligand binding sites

- Two-State Conformation: Accounts for a change in conformation of the binding complex that occurs after the analyte binds in addition, it is possible to calculate the affinity value (KD) using equilibrium analysis, in which the equilibrium responses at different analyte concentrations are filledto a simple saturation binding model

Langmuir Model

The most commonly used binding model is the Langmuir model, which describes a simple 1:1 interaction where one ligand molecule interacts with one analyte molecule (O'Shannessy DJ et al. 1993). The complex that forms following pseudo first order kinetics and it is assumed all binding sites are equivalent and act independently of one another. In this simple 1:1 binding model, mass transport of analyte from the bulk to the surface is not taken into account. Fortunately, many interactions investigated using SPR adhere to this model and can be described the following equation:

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

where B represents the ligand and A is the analyte. The rate of formation of the complex is governed by the association rate constant (ka) and the rate of complex dissociation is governed by the dissociation rate constant (kd). Using the above equation, one can describe the expected binding response for the association phase, equilibrium (if it is reached during the experiment), and the dissociation phase using a set of three equations. Figure 6 shows an example sensorgram for a binding interaction that can be described with a simple Langmuir model, with the relevant equations for each phase outlined.

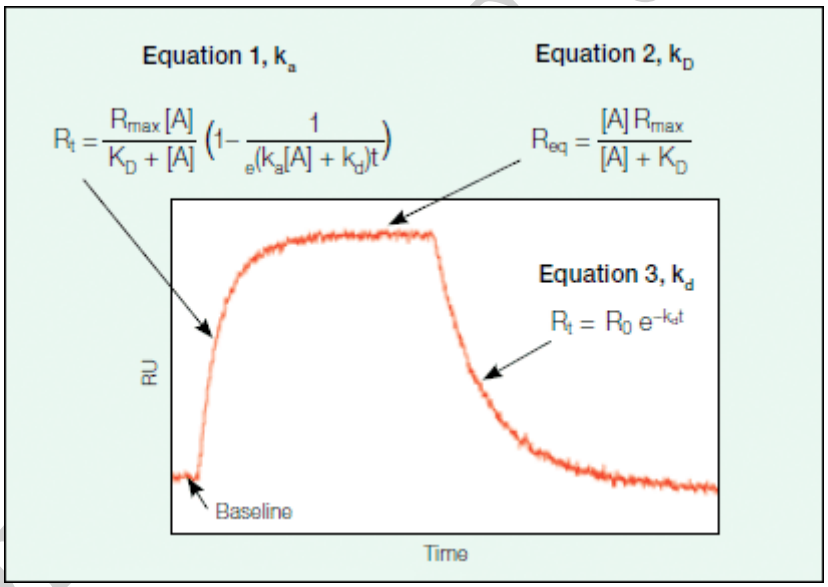


Fig. 6. The sensorgram phases.

The association phase describes the formation of the binding complex over time as analyte is flowed over the ligand surface. As analyte binds to the ligand immobilized on the sensor chip, there is an associated increase in response units as detected by the change in the SPR signal. Figure 7 provides an explanation of how Equation 1 describing the association phase (as seen in Figure 6) was derived.

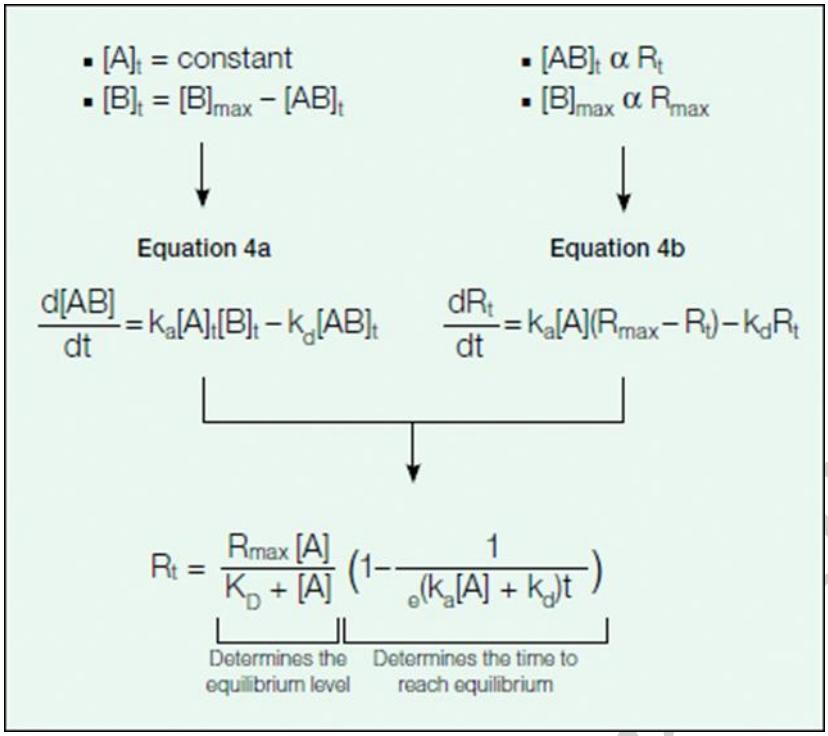


Fig. 7. Rate of complex formation.

As can be inferred from Equation 4a in Figure 7, the change in the amount of bound complex formed over time is proportional to the association (ka) and dissociation (kd) rate constants with the amount of analyte (A) in excess. Because the binding response in the sensorgram is a measure of the amount of bound complex on the sensor chip, the binding response (in RU) is proportional to the amount of bound complex and complex formation can be further described in terms of response units by Equation 4b in Figure 7. Upon integration, Equation 4b becomes Equation 1 in Figure 6, which describes the binding response with time.

During the dissociation phase, analyte is replaced with a buffer solution and the bound complex dissociates with time. This dissociation can be described by simple first order kinetics, as seen in Figure 8. When integrated, this expression becomes a simple exponential decay that describes the decrease in the binding response over time.

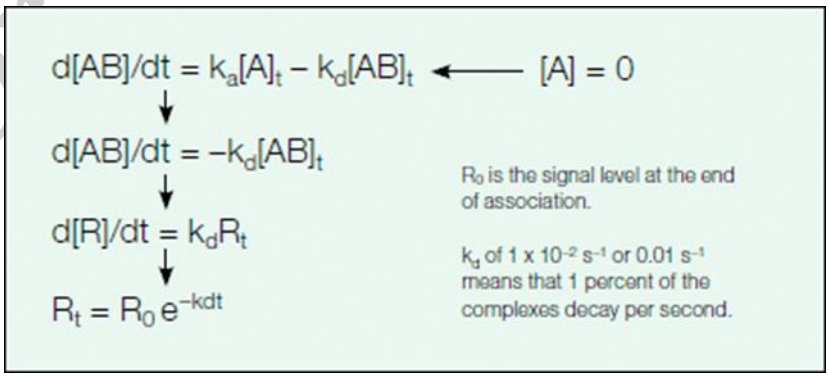


Fig. 8. Pre-steady-state dissociation

By fitting the relevant equations for the association and dissociation phases to the sensorgram data, one can obtain the kinetic parameters ka and kd for the association and dissociation rate constants, respectively, and the affinity (KD = kd/ka) of the interaction can be calculated.

Alternatively, one can find the affinity of a given interaction by equilibrium analysis. The equilibrium binding responses at different concentrations can be fit to a simple saturation binding model, as seen in Figure 9, to extract the affinity (KD). Using this analysis method, there is no need to find kinetic parameters by fitting the association and dissociation phases to a binding model; however, the SPR data must reach equilibrium, as seen as a plateau in the sensorgram, during the experiment.

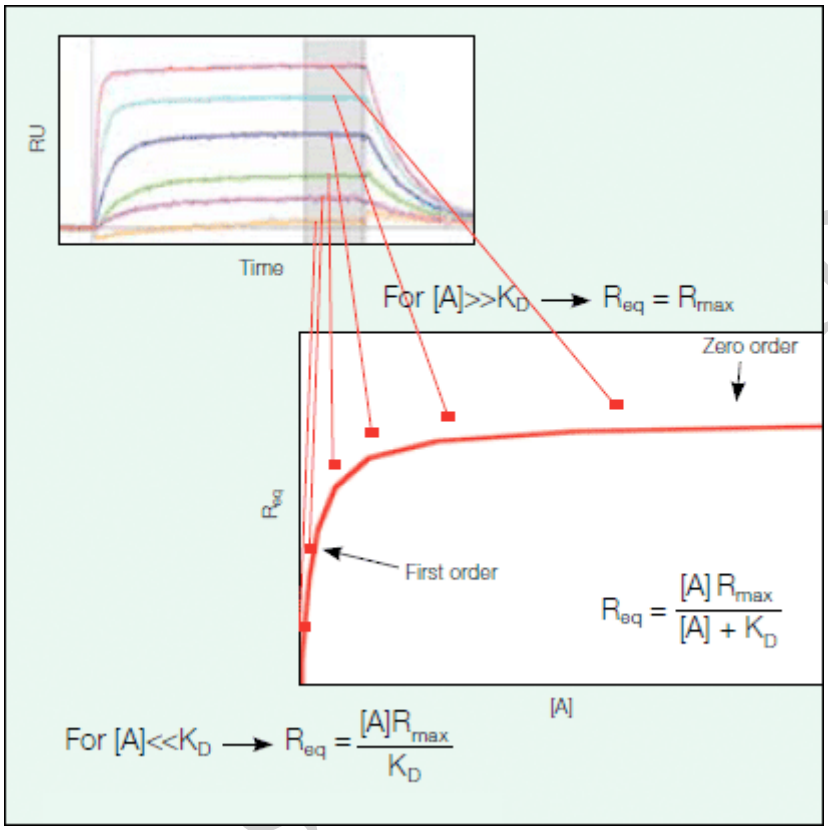


Figure 9. Steady state at equilibrium

Langmuir model with drift

The Langmuir model with drift is used when a biomolecular interaction follows simple 1:1 binding but exhibits a persistent baseline drift that interferes with data interpretation. This is applied in SPR experiments using capturing agents, as the captured ligand may leach from the surface over time. The Langmuir model with drift uses the same kinetic equations as the simple Langmiur model but calculates the drift as a linear drift with time, D*t, where D is the slope of the drift. It should be noted that this model should be applied to the experiments with slow baseline drift because fast baseline drift caused by the rapid decay of the captured ligand usually shows an exponential curvature and does not fit with this model. The optimal solution is correcting the baseline by the subtraction of a blank buffer reference (reference of blank analyte buffer over ligand surface).

Langmuir model with mass transport limitations

The Langmuir model with mass transport limitations assumes a 1:1 binding model, as is the case with the simple Langmuir model, but it takes into account the rate at which analyte is brought from the bulk solution to the sensor chip surface, which is governed by mass transfer. Some biomolecular interactions may be mass transport limited if the rate of association is faster than the rate at which analyte diffuses to the sensor chip surface. The following equation describes Langmuir binding with mass transport limitations:

$$A_{bulk} \underset{k_m}{\overset{k_m}{\rightleftharpoons}} A_{surface} + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

where k_m is the mass transfer rate constant for the diffusion of analyte A from the bulk solution to the surface. A good test of whether an interaction is mass transport limited is to run the experiment at different flow rates and calculate the association rate constant. Diffusion to the surface of the sensor chip will be faster at higher flow rates; thus, if the association rate of a given interaction increases with higher flow rates and decreases with low flow rates most likely the interaction is mass transport limited. Usually one can get around a mass transport limited interaction by running the ProteOn system at high flow rates or by using low ligand density; however, there are certain situations when even these adjustments cannot eliminate the mass transport effect, and modeling the interaction using a Langmuir model with mass transport limitations is more attractive.

Bivalent analyte model

The bivalent analyte model is used when an analyte has two separate binding sites. The following equation describes binding of a bivalent analyte:

$$A + B \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AB + B \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} ABB$$

where A is the analyte and B is the ligand. The association and dissociation of the first binding event is described by k_{a1} and k_{d1}, respectively, while k_{a2} and k_{d2}, respectively, describe the association and dissociation of the second binding event. The first event will yield a traditional 1:1 kinetic fit where the second binding event will cause the ligand-analyte complex to stabilize, thus changing the kinetics of the reaction. Therefore, a sensorgram of a bivalent analyte binding to ligand is the result of two separate kinetic processes occurring in tandem.

Heterogeneous analyte model

When an analyte is heterogeneous, analyte may bind to the ligand in two different locations. This can occur naturally if a sample is not completely pure or if there are two different types of analyte in solution. Thus, a sensorgram of a heterogeneous analyte binding to immobilized ligand represents the sum of two separate binding interactions. If one analyte has a naturally higher affinity than the other analyte, the two may compete for binding of the ligand and the

sensorgram data will reflect the binding kinetics of the higher affinity ligand. The following equations are used to describe and model the binding of a heterogeneous analyte:

$$A1 + B \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} A1B;\ A2 + B \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} A2B$$

Heterogeneous ligand model

A heterogeneous ligand model assumes that there are two sites on the ligand that bind analyte. This can occur if ligand binds to the sensor chip in different orientations, resulting in different binding faces being presented to the analyte. Polyclonal antibodies recognize different epitopes on the same antigen and thus would be considered a heterogeneous ligand. The following equation describes binding of analyte to a heterogeneous ligand:

$$A + B1 \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AB1;\ A + B2 \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} AB2$$

where B1 and B2 are the two separate binding sites on the ligand and A is the analyte. Note that there are two separate sets of association and dissociation rate constants (k_{a1}/k_{d1} and k_{a2}/k_{d2}) to describe each binding event. The binding response of a sensorgram from a heterogeneous ligand then, is the sum of the binding response of two separate binding events.

Two-State Conformation Model

The two-state conformation model accounts for the existence of two-conformations of the bound complex. This can happen if binding of the analyte to ligand triggers a change in conformation of the bound complex. The following equation describes the two-state confirmation binding model:

$$A + B \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AB \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} (AB)^*$$

where AB is the first conformation of the bound complex and (AB)* is the second conformation of the bound complex. Once the complex AB forms it can either dissociate to unbound ligand (B) and free analyte (A) or change to the new conformation (AB)*. However, the complex (AB)* must return to the first complex AB before dissociating into unbound ligand and free analyte. The two-state conformation model is very useful for describing an allosteric binding effect where binding of analyte to ligand (a substrate or inhibitor binding to an enzyme, for example) results in a conformational change.

ADVANTAGES AND DISADVANTAGES OF SPRi

Advantages:

The major advantage of SPRi over standard SPR is its multiplexing capabilities (ability to observe hundreds of reactions simultaneously). SPRi can be applied to perform interaction studies in an HT manner, which is not possible by conventional SPR. Additionally; other advantages of SPR-based biosensors are also applicable for SPRi.

- Label-free detection eliminates the requirement of any secondary reactants and long labeling process.
- Real-time measurements of biomolecular interaction and binding kinetics.
- Highly potential for multiplex analysis; so very well suited for high-throughput analysis.
- Both quantitative and qualitative measurements are possible.

Disadvantages:

Due to its HT capabilities, SPRi is promising for clinical research, but there are many limitations as well; such as

- Requires sophisticated instrumentation. Restricted to choice of metal (gold/silver surfaces).
-Changes in temperature affects refractive index and thereby efficiency of the measurement.
- Non-specific interactions affect the signal.
- Heterogeneous sample surface affects sensitivity.
- Not very effective in handling complex biological samples