# SPR Sensorgram Explained: Using P4SPR™ Software for Generating Sensorgram

A Surface Plasmon Resonance (SPR) sensorgram is a plot of SPR response vs. time generated from an SPR instrument. It can reveal whether there is a binding event between an analyte (e.g. antibody) and a ligand (e.g. protein) and whether the binding is specific. Furthermore, it contains valuable kinetic, affinity, and concentration information pertaining to the analyte and ligand of interest (Figure 1). The analyte is the biomolecule or molecule being investigated and the ligand is the recognition element being immobilized on the SPR sensor. This technote will discuss how a sensorgram is obtained, how to qualitatively interpret a sensorgram, and how to distinguish a high quality sensorgram from a poor one by noting specific sensorgram features. Examples of Affinité's sensorgrams will also be shown. But first, an overview of SPR will be introduced.

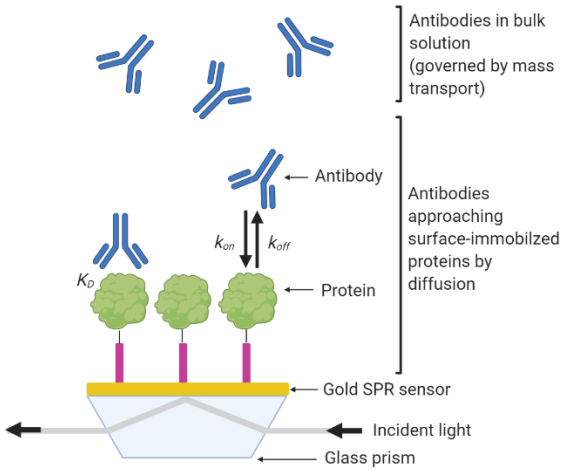


**Figure 1**. Schematic of an SPR sensor and the wealth of information that can be obtained. The reverse setup can be done as well (i.e. immobilized antibody as ligand with protein as analyte). The SPR effect generated by the reflected incident light produces a sensitive region responsive to refractive index changes induced by the binding interaction.

## Overview of Surface Plasmon Resonance

Surface Plasmon Resonance, commonly known as SPR, is an optical, surface-sensitive technique that has been traditionally used for screening, characterization, and bio- and chemical sensing purposes (**Figure 2**).

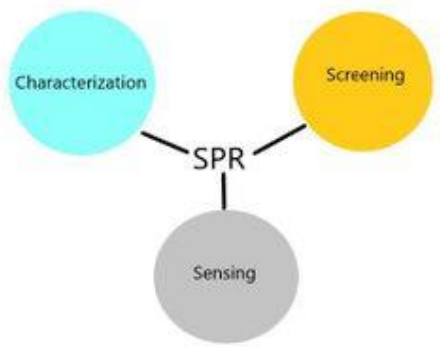


**Figure 2**. Applications of SPR.

In a typical SPR experiment, the interaction between an analyte and a ligand is characterized by kinetic and affinity data and the amount of bound analytes can be calculated as well. The ligands are immobilized on a SPR sensor surface which is exposed to a flowing solution of analytes in a microfluidic channel (**Figure 1**). A plane-polarized, monochromatic incident light shines upon a material of high refractive index (RI) (usually a glass prism) which is coated with a thin metal film (e.g. gold or silver) in total internal reflection conditions. This is known as the Kretschmann configuration. Under these conditions, the light generates surface plasmons (charged oscillations) at the metal surface at a certain wavelength and angle. These surface plasmons are sensitive to the interfacial refractive index by creating an evanescent field (~200 nm) that extends perpendicular to the surface in a sample. If there is a change in refractive index at the surface, for example, when analytes become bound to



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surface-immobilized ligands, the surface plasmon resonance conditions would change, which would result in a change in the reflected angle or wavelength [2].

## From SPR Signal to Sensorgram

In an SPR experiment, a dip or minimum in the reflectance spectrum (intensity of reflection vs. angle or wavelength) signifies the angle or wavelength at which the light has been absorbed by the surface plasmons. Once there is a change in the refractive index at the sensing layer caused by bound molecules, causing surface structural changes, the dip would shift, indicating a change in the angle or wavelength of resonance. **Figure 3** is an example of wavelength interrogation. The increase in refractive index causes an increase in resonance wavelength shift.

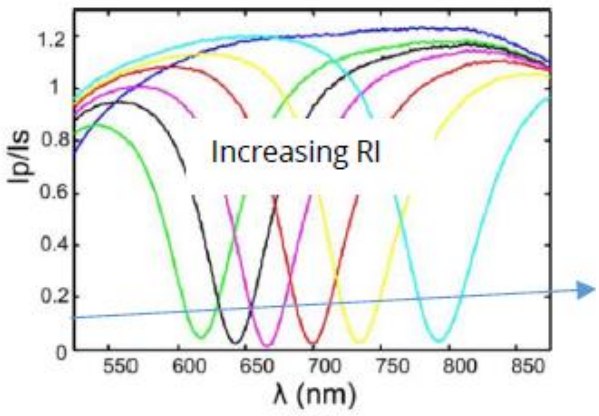


**Figure 3.** Reflectance spectra (light polarizing ratio) of solutions with different RI on an Au SPR sensor in wavelength interrogation mode [2].

The detected change in the reflected angle or wavelength is proportional to the amount of newly bound analytes at the surface due to an increase in mass. When this change in SPR response is plotted with time, a ***sensorgram*** is produced (**Figure 4A)**.

A sensorgram is composed of five phases (see **Figure 4A**): *baseline*, *association*, *steady-state*, *dissociation,* and *regeneration*.
*1. Baseline*: The initial phase is the baseline. A running or flow buffer is used to condition the sensor surface and check for any sensor system instability. It is crucial to have a flat baseline because any drift, injection spike, and high buffer response is an indication that the system should be checked and cleaned [1]. Standard running buffers include phosphate-buffered saline and HEPES-NaCl [1] [3].

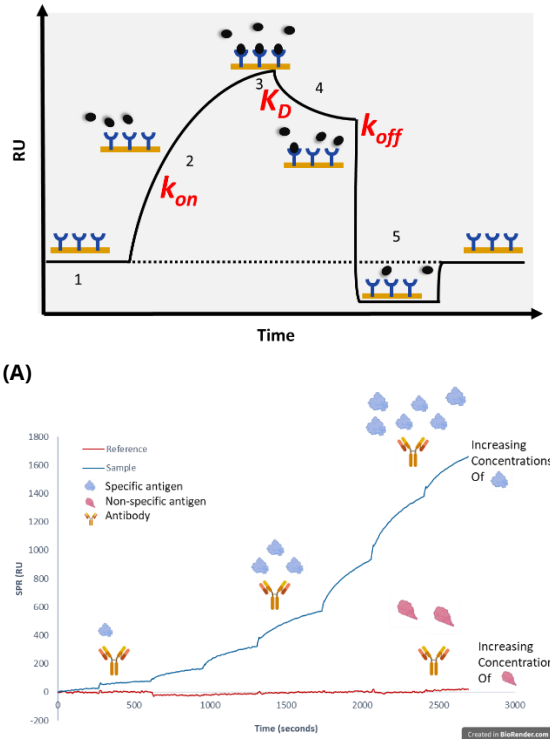


**Figure 4. (A)** A typical sensorgram. On/off rates ($k_{on}$ and $k_{off}$) can be determined from various parts of a sensorgram and dissociation constant ($K_D$) can be determined by the ratio of $k_{off}/k_{on}$. (1) Baseline, (2) Association, (3) Steady-state, (4) Dissociation, (5) Regeneration. RU represents resonance units. **(B)** A typical sensorgram generated by Affinité's P4SPR™ where antibodies are immobilized and increasing protein concentrations are injected into the P4SPR. Dissociation constant ($K_D$) can be determined by fitting a binding curve model of the SPR response against protein concentrations plot.

*2. Association*: The second phase is where analytes (A) begin to bind to immobilized ligands. It is indicated by the initial sharp rise of the SPR signal in the sensorgram and it is ideally a single exponential curve [4]. This step is governed by two events, namely mass transport from bulk solution to surface and movement to surface-immobilized ligands (See **Figure 1** and **Equation 1**).

$A_{bulk} \rightleftharpoons A_{surface} \rightleftharpoons A_{surface}+Li \rightarrow A_{surface}Li$ (Equation 1)

Analytes in bulk solution must move from the bulk solution to the surface and then bind to surface-immobilized ligands. If the binding of surface analytes to ligands is hindered by the movement of analytes from bulk solution to the surface (mass transport limitation), then the interaction between the analyte and ligand is more diffusion than kinetic limited. The association curve would be more linear [4]. Any attempt to obtain kinetic data without accounting for mass transport diffusion would lead to inaccuracy in the rate of association.

*3. Steady-state*: This phase occurs at the top flat portion of the sensorgram where the net rate of bound analytes is zero. This is not to be confused with saturation, where all the ligands have been occupied by analytes. An equilibrium response may not mean saturation, but a response that reaches saturation will be at equilibrium [4].

*4. Dissociation*: This phase begins when the analyte solution is replaced by a wash buffer, which causes the specific interactions between the analytes and ligands to break. It is represented by the downward sloped curve after the steady-state phase. It should be a single exponential decay; however, any mass transport limitation or other factors could affect the shape of this phase [4].

*5. Regeneration*: Finally, a low pH buffer such as glycine is flowed to reset the SPR baseline signal as the beginning of the experiment [1]. It is important to establish a steady baseline signal to indicate that the sensor system is free of bound analytes and non-specifically adsorbed molecules, has stability (having intact and functional ligands), and that no other effects such as temperature or surface chemistry changes would affect the next set of SPR measurement, as SPR sensing surfaces can be reused numerous times.

**Figure 4B** depicts a typical sensorgram obtained from Affinité's P4SPR™. It looks different from Figure 4A because it is achieved by steady-state measurements, which is the measurement of equilibrium binding as a function of analyte concentrations. Increasing analyte (e.g. protein) solutions are *manually* injected into the SPR instrument in one single cycle, i.e. with no regeneration in between injections. Therefore, one can see a continuous string of association curves from the lowest to the highest concentration of protein. Furthermore, the P4SPR does not flow buffer at a fixed rate, hence there is no dissociation curve. **Figure 5** shows a sensorgram generated by the P4SPR that does include a regeneration phase to remove all bound proteins. The immobilized ligands are regenerated and available for the next protein solution. Note that this only applies if the dissociation constant is in the higher μM or mM range.

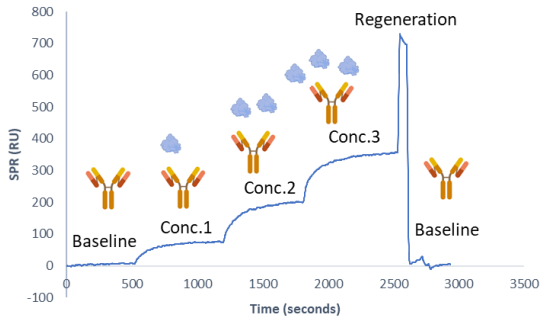


**Figure 5.** A sensorgram obtained from Affinité's P4SPR that shows the regeneration phase after injections of increasing concentrations of a protein.

The relative SPR response in a sensorgram is proportional to the amount of bound analytes. An example can be found in **Figure 6**, where the SPR response increases as the concentration of LacI increases due to binding to LacO.

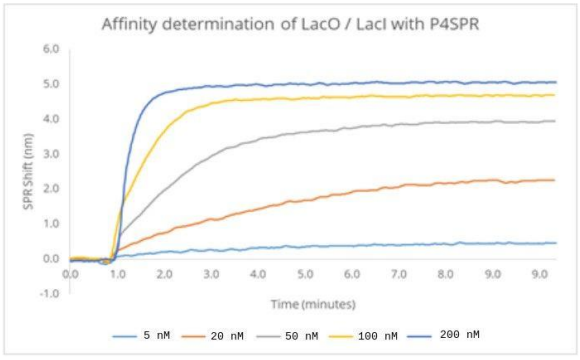


**Figure 6**. Superimposed binding curves, generated by Affinité's P4SPR, of increasing LacI concentrations due to



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binding to LacO (see Application Note - Gene Regulation, Lac Operon/Lac Repressor) [2].

Once the sensorgram data are obtained, the rate of association ($k_{on}$), dissociation ($k_{off}$), and dissociation constant ($K_D$) can be determined by fitting the data into a suitable binding model. Therefore, kinetic and affinity data can be obtained readily from an SPR experiment.

## Sensorgram Shapes

**Figure 7** illustrates some commonly encountered sensorgram shapes that are overlaid for comparison. Figure 7a distinguishes between a binding and non-binding event. It is obvious that there would not be a SPR response changes for the sensorgram with no binding event. Figure 7b differentiates between a strong vs. a weak binding event. The stronger binding interaction would elicit a higher SPR response than the weaker one. Figure 7c shows the sensorgram shape changes for a rapid association and dissociation vs. one with slower phases, which may reflect mass transport limitations.

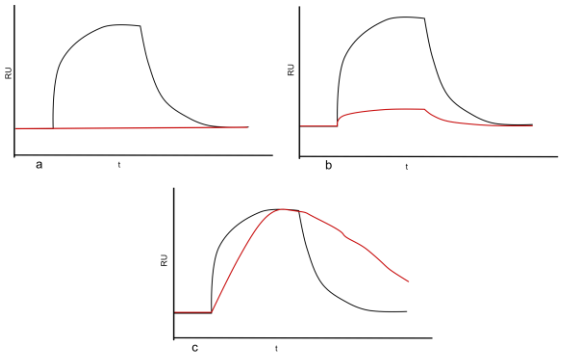


**Figure 7**. Various SPR sensorgram shapes. a) black - binding, red - no binding; b) black - strong binding, red - weak binding; c) black - fast association and dissociation, red - slow association and dissociation.

**Figure 8** is an example of a sensorgram where continuous injections of increasing concentrations of RDX were performed. This is typical for experiments requiring a calibration curve for sample quantitation. Repeated association curves of each newly injected concentration could be observed with water injections in between to establish the baselines.

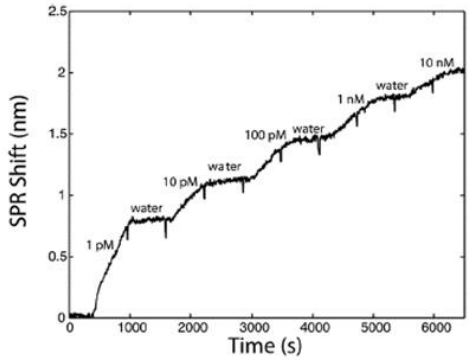


**Figure 8**. Increasing RDX solutions were continuously injected to generate this SPR sensorgram using Affinité's P4SPR for the calibration of the sensor for RDX detection (see Application note 2 - Onsite testing of RDX in environmental waters ) [2].

**Figure 9** demonstrates how analyte concentrations must be optimized for an SPR assay to determine the concentration of an analyte, for example. Figure 9a shows overlaid sensorgrams where the analyte concentrations are too high, and Figure 9b shows what they would look like if the concentrations are too low. Figure 9c represents an ideal set of sensorgrams where the analyte concentration range is wider. Figure 9c is the best set of sensorgrams to use to accurately determine the concentration of the same analyte. Using these sensorgrams, a calibration curve of the change in SPR response after a certain time interval vs. concentration can be constructed [3]. An example of such a calibration curve can be found in Appnote 2-Onsite testing of RDX in environmental waters, Figure 5). Thus, the specific change in SPR response of a sample can be used to find its concentration.

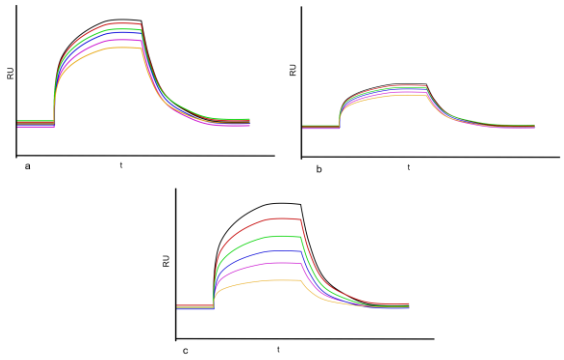


**Figure 9**. Sample concentration optimization. a) sample concentrations are too high; b) sample concentrations are too low; c) range of sample concentrations are optimized.

Another important aspect about sensorgrams is that they can take on different curvature even if the $K_D$ values are the same. This, of course, depends on the type of interaction, hence the rates of association and dissociation [1]. Marquart has illustrated this point by plotting $k_a$ vs. $k_d$, where the slope of each line represents a unique $K_D$ [1]. Along each line graph, different types of sensorgrams can be obtained with varying degrees of $k_a$ and $k_d$, resulting in different curvatures. **Figure 10** is an overlaid plot of four types of curvature expected for different $k_a$ and $k_d$ but with the same $K_D$. The same trend can be expected for different concentrations of the same analyte [1].

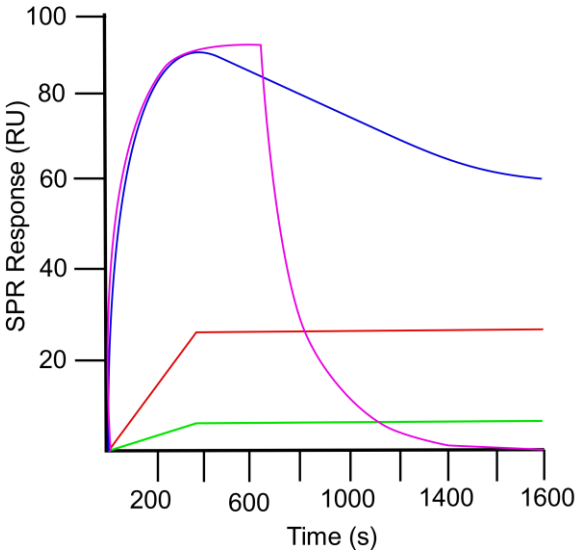


**Figure 10.** Sensorgrams with the same $K_D$ but with different curvature.

## Ideal Features in a Sensorgram

One must look at a sensorgram carefully before mathematical fitting to determine if any experimental modifications are needed. The SPR Pages has shown an excellent example of a high quality sensorgram compared to a poor quality one [1]. **Table 1** summarizes the main points. One of the key points is to start at a lower ligand density and work with analyte concentrations between 0.1-10 times the expected $K_D$ [1].

**Table 1**. Features of a Poor and High Quality Sensorgram [1]

| | **Poor quality sensorgram** | **High quality sensorgram** |
|---|---|---|
| Ligand amount | High | Low |
| Baseline | Drift | Flat |
| Shape of association phase | Linear (mass transport limited) | A single exponential; has curvature |
| Saturation | No | Yes; demonstrated by more than one analyte concentrations |
| Analyte concentration range | Narrow | Wide |
| RI jumps and spikes | Present | Negligible jumps and no spikes |
| Dissociation decay | Short | Long |
| Replicates | Not present or non-superimposable | Present and superimposable |

If there is any indication of a poor quality sensorgram, one should not proceed to fit it with a complex model because it would not give meaningful data [1, 4]. One should try to modify various aspects of the SPR experiment such as: immobilization chemistry, surface density,

regeneration conditions, analyte concentration [4]. It would also be helpful to check the quality of the ligand and analyte, increase flow rate to check for mass transport issues, and verify whether buffers and detergents used are optimal for the experiment [1].

## Generating a Sensorgram with P4SPR™

For an SPR instrument with wavelength interrogation mode such as the P4SPR™, the detector detects the change in wavelength (of the absorption band minimum, or dip) upon introduction of a sample which would change the refractive index near the sensing layer. As the sample continues to flow across the sensing surface, the wavelength (λ) position is continuously detected and recorded in the SPR instrument in real-time. The resulting sensorgram is a plot of change in wavelength (Δλ) (referenced against a solution at a specific time) vs. time. Then, any kinetic and affinity data can be calculated by fitting the sensorgram data into a suitable binding curve model, as mentioned above. Thermodynamic models are integrated with the P4SPR™ system so there is no need to obtain a separate modelling software. **Figure 11** summarizes how P4SPR™ converts the SPR signal into a sensorgram.

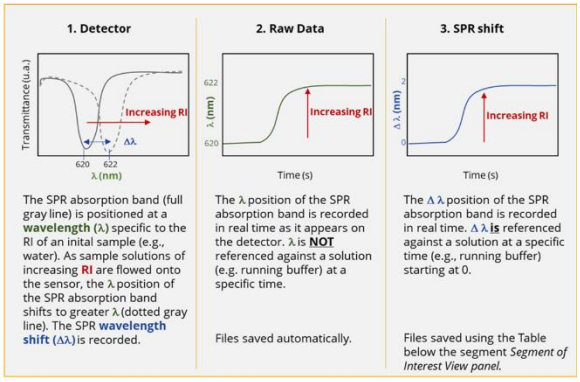


**Figure 11**. (1) The P4SPR™ detects a change in refractive index (RI) in terms of change in wavelength (δλ) or shift in absorption band minimum; (2) Raw data can be saved as the change in wavelength over time. (3) The P4SPR™ plots the SPR shifts (Δλ) with respect to time to provide a sensorgram [3].

## Conclusion

From this technote, there is hopefully a deeper sense of how SPR sensorgrams are generated and how one qualitatively interprets them. Furthermore, tremendous emphasis must be placed on the design of the SPR experiment to produce meaningful sensorgrams.

Finally, Affinité Instruments' P4SPR's portable wavelength-mode instrument provides dependable data acquisition and fitting software to meet your research needs.

## References

1. Arnoud Marquart, "SPRpages," 2006-2020. [Online]. Available: https://www.sprpages.nl. [Accessed 7 September 2020].
2. Affinité Instruments, "Affinité Instruments," [Online]. Available: http://affiniteinstruments.com. [Accessed 7 September 2020].
3. Richard B. M. Schasfoort, Chapter 1:Introduction to Surface Plasmon Resonance, in *Handbook of Surface Plasmon Resonance (2)*, 2017, pp. 1-26.
4. Rebecca L. Rich and David G. Myszka. Survey of the year 2007 commercial optical biosensor literature. *J. Mol. Recognit.* **21**, 355-400 (2008).
5. Affinité Instruments, P4SPR User Manual. 2020, pp. 14.

## About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built on deep knowledge accrued throughout more than a decade of research in SPR. The commercialization of promising innovations is spearheaded by a leadership team experienced in business, science and engineering.

## About the Author

Dr. April Wong is an analytical chemist and has research experience in the biosensor area for over a decade. She obtained her Ph.D. at the University of Toronto.