SARTORIUS

Best Practice Guide

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Best Practice Guide: Minimize Artifacts

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Introduction

Surface plasmon resonance (SPR) is an established technique for characterizing the mechanism and rate constants associated with molecular interactions. To determine meaningful kinetic rates and affinity constants and characterize the mechanism associated with molecular interactions, high-quality SPR data must be generated.

It has long been known that many of the artifacts associated with binding data and the inability to fit data to a simple model can be minimized or eliminated by the use of careful assay design, which allows the user to collect data under optimum conditions and process the data with reference surfaces.

Although the application of SPR is often described as an art, adhering to some basic "good practices" along with hands-on experience is fundamental to producing high-quality SPR data that can be readily interpreted to deduce meaningful kinetic rate and affinity constants.

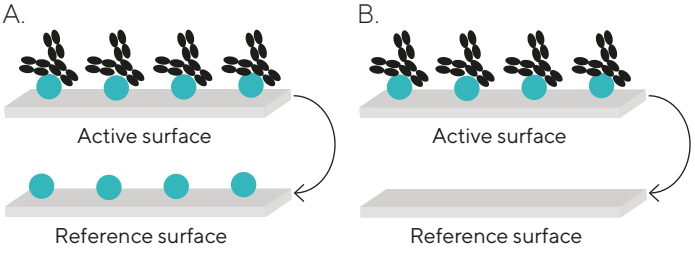
Whether using low-throughput SPR as a secondary characterization tool to study binding interactions, or high-throughput SPR as a preliminary screening tool to survey hundreds of binding interactions in a single assay, there are some established best practices that aid in improving the quality of biosensor data and minimizing artifacts. When the goal is determining the kinetic rate and affinity constants of a biomolecular binding interaction, a checklist of a few key points should be followed to minimize assay artifacts. Here, we provide a synopsis of those recommendations.

Reference Surface

One of the most critical parameters in assay design can be the inclusion of a suitable reference surface in addition to the active surface where binding measurements will be performed. It has been shown that a suitable reference surface can help correct for multiple artifacts such as refractive index changes, non-specific binding, assay drift and injection noise.

Where possible, the reference surface should contain the same immobilization conditions as the active surface. As shown in Figure 1A, the active and reference surface both have the capture molecule immobilized on their surfaces, providing a more relevant reference surface for data analysis than Figure 1B, where the reference surface is left either unmodified or activated | deactivated.

Figure 1



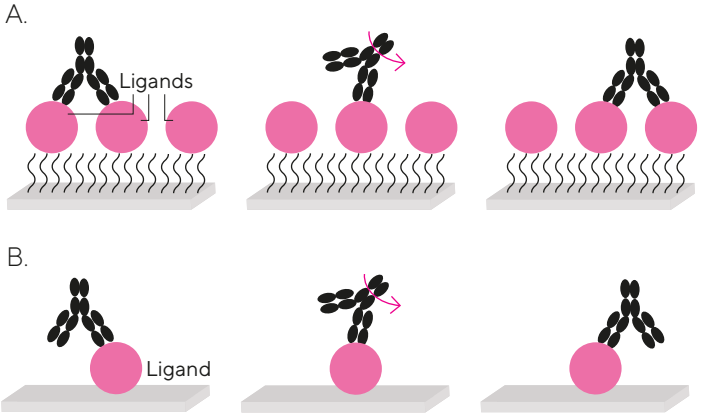
Note. Correct reference surfaces have a large impact on data quality. The capture molecule (teal) should ideally be present on both the active and reference surface to ensure the best data quality possible.

Where a capture assay is not possible, a non-interacting protein, such as a non-binding variant, should be immobilized on the reference surface to the same response level as the active surface. The Octet® SF3 allows the user to specify which channel acts as the reference surface and due to the design of the flow cell, a single channel can act as a parallel reference surface for two active surfaces, which aids in generating high-quality data through double reference subtraction.

Work at an Appropriate Surface Capacity

In addition to correct assay orientation (see best practice guide Assay Orientation), low surface capacities minimize artifacts associated with mass transport, steric hindrance, crowding and aggregation, and subsequently simplify data analysis. However, determining how low is "low enough" is an empirical exercise, although for kinetic assays, an Rmax of ~50 RU will generally yield excellent results.

Figure 2



Note. (A) When using a 3D-hydrogel, the potential to crosswalk to another ligand exists if the surface density is too high and will result in an incorrect rate constant being determined during the dissociation phase. (B) Use of a planar sensor chip reduces the surface density and can minimize | remove the avidity effects observed with a 3D-hydrogel sensor chip.

In addition to exploring a range of surface capacities, the use of planar chips such as the Octet® SPR COOH1 Sensor Chip (Cat. No. 19-0053) instead of the commonly used 3D-hydrogels such as the Octet® SPR CDH Sensor Chip (Cat. No. 19-0128) enables the more rigid tethering of ligand molecules, minimizing ligand crosstalk that may persist even in sparsely coated 3D-hydrogels (Figure 2). While low surface capacities are generally advisable, binding responses should be sufficiently above the instrument noise to be meaningful and not "lost within the noise."

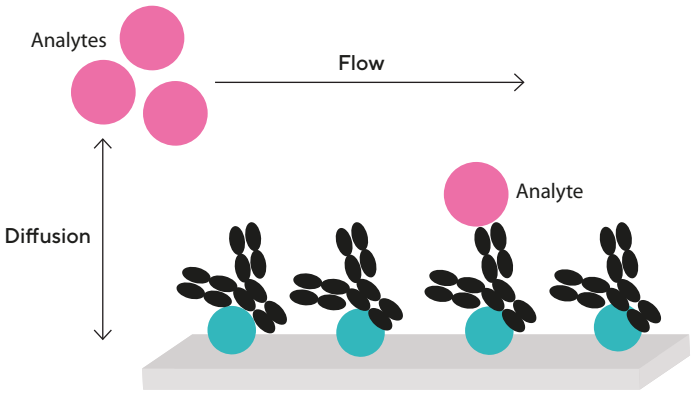
Mass Transport and Flow Rates

For binding to occur in SPR assays the analyte must be transported laterally (diffusion) in the flow cell from the bulk solution to the surface before any interaction with a ligand can take place (Figure 3). This is referred to as mass transport and is a critical component of SPR as a technique.

As discussed above, low surface capacities minimize multiple artifacts associated with SPR but also play a key role in minimizing the risk of mass transport limitation. Mass transport limitation (MTL) occurs when the analyte cannot diffuse to the sensor chip surface rapidly enough, and the measurement becomes diffusion limited rather than kinetics limited, where the analyte can diffuse to the sensor chip surface rapidly enough and there is an abundance of analyte for accurate kinetics determination.

It is important to not only consider the surface capacity during assay development but also the analyte flow rate so as to ensure that MTL is minimized.

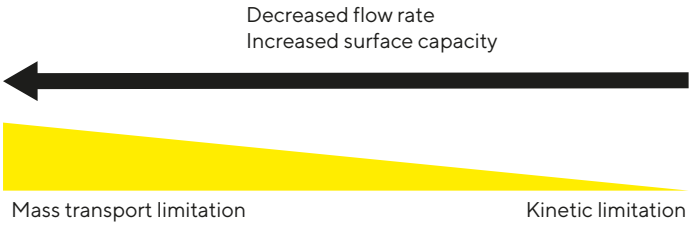
Figure 3



Note. Measurement of binding interactions requires diffusion of the analyte from the bulk flow to the sensor chip surface before binding can be observed.

When designing an assay, it is important to consider that at low flow rates and high surface capacity there is an increased risk of experiencing MTL (Figure 4). Although this assay setup can have a role in determining the active concentrations of the analyte, it is not suitable for determining accurate kinetics as with high immobilization levels transport can be limiting and the analyte concentration at the surface varies with the flow rate. This means the analyte concentration is different at the sensor chip surface than the analyte concentration in the bulk of the sample being used in the assay. Since observed binding rates are proportional to analyte surface concentration, this reduction in surface concentration will result in slower than expected observed binding rates.

Figure 4

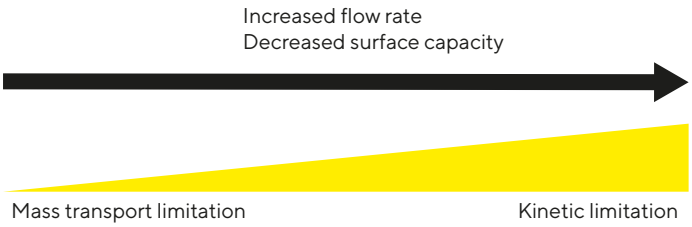


Note. Low flow rates and high immobilization levels can drive assays towards mass transport limitation and lead to incorrect kinetics and affinity.

As discussed above, use of a planar sensor chip reduces the surface density and can minimize | remove the avidity effects observed with a 3D-hydrogel sensor chip. This is due to a reduced immobilization level (and subsequent decrease in Rmax) but it's clear that flow rate also plays an important role in preventing MTL and allowing accurate kinetic data (Figure 5) to be derived.

In general, for biologics assays, a flow rate of 50 µL/min should be used (with 30 µL/min being the lowest) although higher flow rates can be tested if required. Higher flow rates should generally be used for small molecule assays.

Figure 5



Note. Decreasing the surface capacity and increasing the flow rate moves the assay away from MTL and towards measuring accurate kinetics.

Conclusion

In summary, in kinetic limitation, the transport is efficient and the analyte concentration at the surface is identical to the analyte concentration in the bulk of the sample being used in the assay. As observed binding rates are proportional to analyte surface concentration, kinetic limitation means that accurate binding rates will be observed for the interaction being assessed.

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