SARTORIUS

Best Practice Guide

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Best Practice Guide: Minimizing the Effects of Non-specific Binding

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Introduction

Non-specific binding (NSB) of biological and non-biological molecules to the sensor chip surface in surface plasmon resonance (SPR) assays can be problematic and cause errors in determining accurate kinetics and affinity. Non-specific binding is characterized by a significant binding to the reference surface of the sensor chip, which does not return to baseline at the end of injection (Fig 1).

In theory, non-specific binding can be 'referenced' out if the active and reference surfaces exhibit very similar levels of non-specific binding but in practice, this is not feasible as ligand immobilization may mask non-specific binding on the active channel, and subsequently lead to higher levels of observed non-specific binding on the reference surface.

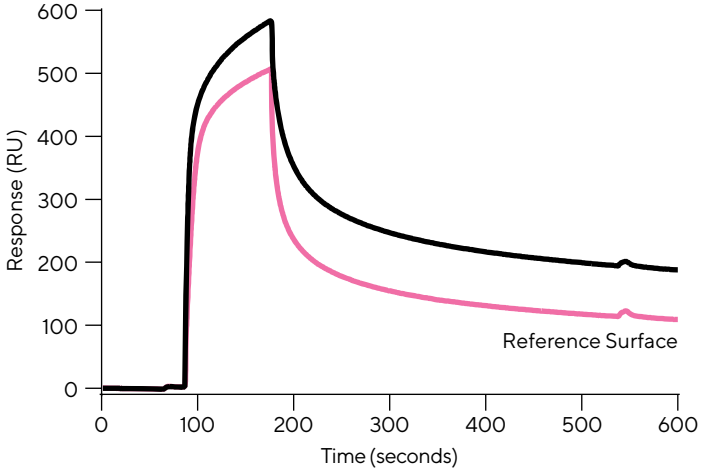

Figure 1

Example of Non-specific Binding

Reference surface (pink) exhibits significant curvature during the analyte injection and does not return to zero at the end of the injection, indicating non-specific binding.

Electrostatic and Non-electrostatic Non-specific Binding

Two common types of non-specific binding are observed in SPR assays:

- Electrostatic NSB
- Non-electrostatic NSB

Electrostatic NSB is characterized by attraction of the positively charged analyte to the negatively charged sensor chip surface (Fig 2). This is often exacerbated when using carboxymethyl dextran-based sensor chips due to their net-negative charge. Attraction of the positively charged analyte to the sensor chip surface often occurs when the analyte has a higher isoelectric point than that the running buffer.

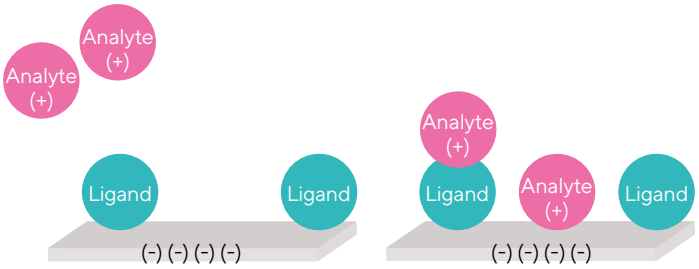

Figure 2

Electrostatic Binding

Electrostatic NSB is characterized by attraction of the positively charged analyte to the negatively charged sensor chip surface.

Non-electrostatic NSB is characterized by interaction of the analyte or components of the sample to the sensor chip through such interactions as hydrophobic, hydrogen bonding or biding to exposed gold patches on the sensor chip (Fig 3).

Non-electrostatic NSB is particularly noticeable with thiol-containing small molecules and 'sticky' or crude samples that contain patches of hydrophobicity or charge on the protein's surface.

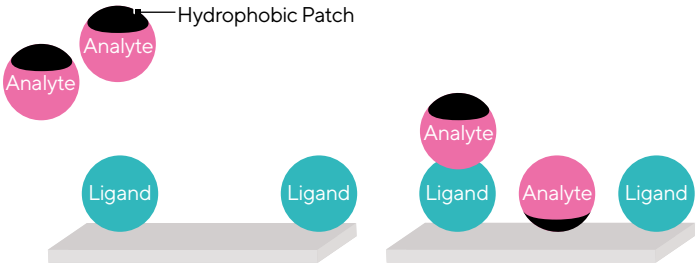

Figure 3

Non-Electrostatic Binding

Analyte containing large hydrophobic patches (black region) are susceptible to non-electrostatic NSB.

Assay Design

To collect accurate data, non-specific binding must be considered and tested during assay design to determine whether non-specific binding is occurring and if yes, what measures can be used to remove the observed non-specific binding.

In general, the simplest way to determine a molecules proclivity for non-specific binding is to inject the highest concentration of the analyte across an unmodified sensor chip surface. It is critical that the same sensor chip that is planned to be used during the assay is tested to ensure an accurate result.

Ideally a "square-shaped sensorgram", reflecting any slight mismatch in bulk refractive index between sample and running buffer should be observed during the association phase (Fig 4).

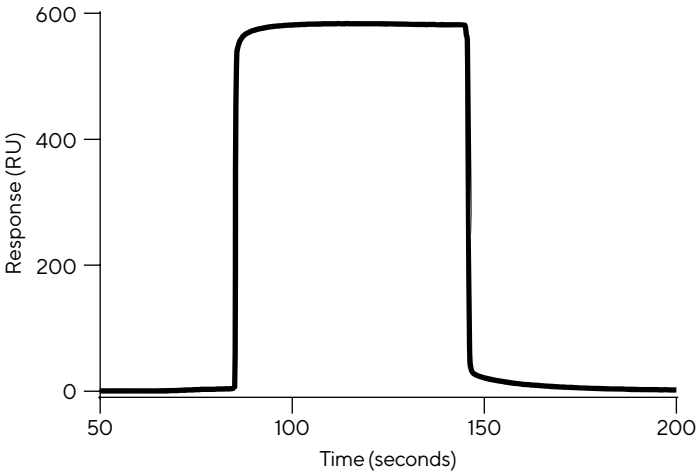

Figure 4

Ideal Sensorgram Shape

Interaction of 20 µM of a small molecule with carbonic anhydrase II shows no observable NSB and a square-shaped sensorgram.

If the sensorgram shows any "tailing" during the dissociation phase, then the analyte may be interacting non-specifically with the surface and strategies for lowering the analyte's non-specific binding with the sensor chip surface must be used.

In general, strategies for dealing with non-specific binding can be separated into two basic strategies:

- Sensor chip optimization
- Buffer optimization

Sensor Chip Optimization

As discussed previously, the net negative charge of carboxymethyl dextran-based sensor chips can cause electrostatic non-specific binding to be observed and therefore, it is recommended that different sensor chip types with fewer functional groups are tested for a reduction in NSB. For example, if an Octet® SPR CDH sensor chip produces an observable level of non-specific binding then an Octet® SPR CDL sensor chip, which contains fewer carboxylic groups should be tested and/or a planar sensor chip such as the Octet® SPR COOH1 sensor chip, which contains no carboxymethyl dextran should be considered (though the user must consider the required immobilization levels when assessing alternative sensor chips).

As discussed in the best practice guide "Minimize Artifacts", correct reference surfaces have a large impact on data quality in addition to having a noticeable effect on non-specific binding. Where possible the reference surface should contain the same immobilization conditions as the active surface and contain a non-interacting protein that should be immobilized to the same response level as the active surface. The non-interacting protein does not directly reduce non-specific binding but masks the charge on the sensor chip surface and aids in allowing non-specific binding to be 'referenced' out.

Where a positively charged analyte is being assessed it is possible to lower the sensor chips net negative charge during amine coupling-based immobilizations by using the same assay parameters and inactivating remaining succinimide esters with ethylenediamine instead of ethanolamine. This reduces the net negative charge on the sensor chip surface and decreases the potential for non-specific binding.

Buffer Optimization

The choice of running buffer is of critical importance in SPR assays as it is the carrier of the analyte and therefore, any composition, pH or other excipient issues can affect the interaction between the analyte and ligand. Assessment of non-specific binding should be performed in a suitable buffer such as HBS-EP+.

In general, it's recommended to start with the 'plus' version of the running buffer as the additional tween (~0.05%) prevents non-specific sticking to the systems micro-fluidics and tubing, which helps reduce both electrostatic and non-electrostatic "stickiness" of samples.

Where non-specific binding is still observed it is then possible to change the buffer choice or add components to the running buffer to reduce non-specific binding. The addition of 0.5 – 2.0 mg/mL (0.05 - 0.2 %) BSA to the running buffer can block potential non-specific binding sites and helps stop proteins stick to assay plates and tubing.

The addition of NaCl up to a final concentration of 500 mM in the running buffer can reduce electrostatic non-specific binding, though care must be taken that the analyte or ligand activity is not affected in non-physiological salt levels.

When dealing with 'sticky' or crude samples it is recommended that the sample is diluted into the running buffer and NaCl levels are investigated. Although a dilution factor of five is a common recommendation, it is advised that the user determines the optimum dilution level and protocol through empirical means.

Where possible the analyte should be as pure as possible to ensure that contaminants that may cause non-specific binding are removed. Where it is not possible to eliminate non-specific binding using the techniques shown here, orientating the assay with the 'sticky' protein as the ligand should be considered. Attention must be paid to the immobilization process where this is attempted as the activity of the immobilized ligand may be affected by its non-specific binding.

Conclusion

Minimizing non-specific binding is often not factored into assay development despite it being a significant factor in the generation of high-quality, accurate data. Therefore, it is recommended that non-specific binding is assessed in assay development and that the best practices outlined in this document are implemented where non-specific binding is observed.

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