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Optimizing Protein-Streptavidin Interactions: Effects of Biotinylation Ratios Across Biosensor Types

Technical Note

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Octet®, Bio-Layer Interferometry, BLI, protein-protein interaction, biotinylation, optimization, streptavidin, kinetics, custom quantitation

Abstract

This technical note examines the effects of varying biotinylation ratios across different biosensor types used in Octet® Biolayer Interferometry (BLI) systems. Streptavidin biosensors, including Octet® SA, SAX, SAX2, and SSA, are designed for capturing biotinylated ligands, with each type intended for specific applications. This study highlights that while a 1:1 molar coupling ratio (MCR) is ideal to minimize cross-linking in kinetic assays, increasing the MCR can enhance ligand loading and extend the dynamic range for custom quantitation assays. Experiments demonstrate that higher MCRs lead to increased binding responses and improved detection capabilities for Protein A, with SAX biosensors outperforming SAX2 in signal magnitude and dynamic range. The findings emphasize the importance of tailoring biotinylation strategies and biosensor selection to optimize analytical performance in biologics development.

Introduction

During the development of biologics, accurate detection and quantification of the expressed drug and its interaction with its target are crucial to facilitate the selection of lead candidates. Octet® Biolayer Interferometry (BLI) systems offer a label-free analytical approach, providing a rapid and straightforward method to accurately quantify these molecules and assess binding specificity and affinity. Streptavidin has traditionally been employed as a key biosensor surface to facilitate both kinetic and quantification assays. For these assays, ligands must be biotinylated to serve as capture molecules. This document explores how the optimal molar coupling ratio (MCR) for ligand labelling can vary between kinetic and quantitative applications and compares ligand loading between different types of Octet® streptavidin-based biosensors.

Types of Streptavidin Biosensor

A variety of streptavidin biosensors have been developed at Sartorius for the capture of biotinylated ligands. While these biosensors (Octet® SA, SAX, SAX2 and SSA) are all coated with streptavidin molecules and intended for use with biotinylated ligands, they differ in the intended applications. Table 1 highlights key features of the different types of streptavidin-coated biosensors that are available. For more details, please refer to the **biosensor selection guide.**[1]

Table 1: Range of streptavidin-coated Octet® biosensors for immobilization of biotinylated molecules.[1]

Biosensor	Description	Intended use*	Suggested molecule	Product Number (one tray)
SA	Streptavidin	Kinetics and Quantitation	Proteins and antibodies	18-5019
SAX	High Precision Streptavidin	Kinetics and Quantitation	Proteins and antibodies	18-5117
SAX2	High Precision Streptavidin 2.0	Kinetics and Quantitation	Proteins and antibodies	18-5136
SSA	Super Streptavidin	Kinetics	Small molecules	18-5057

* Biosensors are developed, manufactured, and QC is performed for their intended applications; using biosensors outside their intended purpose requires user validation.

Ligand Biotinylation

The interaction between streptavidin and biotin is widely used as a system for the stable, and irreversible non-covalent binding of biological molecules. Proteins can be biotinylated *in vitro* using biotin labelling reagents, which can be targeted against a variety of functional groups such as primary amines and sulfhydryls.

Biotinylation is simple to perform, gentle on proteins and performed at neutral pH. Free, un-reacted, excess biotin must be removed following the biotinylation reaction. Due to its low molecular weight, biotin can be simply removed using spin columns such as Sartorius Vivaspin® 500 (this process is discussed in more detail in the technical note 'Optimizing Ligand Biotinylation: Conditions for Molecular Interactions Analysis'.)[2] Long chain linkers (reactive biotins with different lengths of PEG molecules) should be incorporated into a biotin tag to minimize steric effects, especially when immobilizing smaller molecules such as peptides. A biotinylated ligand can be prepared in batches and stored for use in multiple experiments.

In a biotinylation reaction, the MCR describes the ratio between moles of biotin used for labelling to the moles of protein being labelled. The optimal ratio for labelling a ligand prior to loading to an Octet® biosensor can differ depending on factors such as the type of ligand and the desired application.

Kinetics Applications

For measuring kinetic interactions between molecules using streptavidin biosensors, it is recommended to use a 1:1 MCR, which should lead to on average one biotin per ligand molecule. This minimal biotinylation level is desirable to reduce the risk of cross-linking on the biosensor surface.[3] Minimal biotinylation can lead to increased wastage due to loss of unbiotinylated ligands, however, it is necessary to ensure that accurate kinetic data can be obtained. Biotinylation of ligands for kinetics assays is explored in more detail in **'Optimizing Ligand Biotinylation: Conditions for Molecular Interactions Analysis'.**[2]

Quantitation Applications

Quantitation applications using streptavidin biosensors have to be customized for specific analytes. In contrast to kinetics assays, for custom quantitation, it can be beneficial to increase the MCR above a 1:1 ratio. This can lead to a higher proportion of biotinylated ligand within the sample, resulting in increased magnitude loading onto the biosensor. This in turn can lead to an increased signal and dynamic range for the detection of the analyte.
To investigate the optimal biotinylation MCR for a custom quantification assay, an experiment was set up to look at binding of Protein A to biotinylated rabbit anti-IgG antibody. The antibody was biotinylated at a range of MCR and loaded at a single concentration onto SAX and SAX2 biosensors. SAX biosensors are similar to SA but with enhanced precision requirements (CV specification of 4%) for high-precision applications. Therefore, data for SAX biosensors in these experiments could be assumed to also apply to SA biosensors. SSA biosensors are not suitable for assessment of large molecules, such as proteins and antibodies, and so were not evaluated in these experiments. Biotinylation of the anti-IgG antibody was performed at a range of 1:1 to 15:1 MCR.

As shown in Figure 1, there was an increase in the binding response for loading of the antibody to both sensor types as the MCR increased from 1:1 to 15:1. The magnitude of antibody loading at each MCR was higher for SAX compared to SAX2, with the loading response with SAX only starting to plateau at later timepoints (400-500 seconds) for the 15:1 and 10:1 MCR. For SAX2, the plateau was reached much earlier (around 200 seconds) for the 10:1 and 15:1 MCR and, by later timepoints, the 5:1 MCR also reached the same plateau.

Following antibody loading, sensors were dipped in a range of concentrations of Protein A (Figure 2). At each concentration of Protein A, and at each MCR, binding to the biotinylated antibodies loaded onto SAX biosensors was higher than to those loaded to SAX2. This correlates with the increased loading of antibody to SAX that was seen in Figure 1. For both sensor types, the highest magnitude binding response, particularly at the highest Protein A concentrations, is seen with the higher MCR ratios (5:1, 10:1 and 15:1). Again, this relates back to the sensors in which the highest level of antibody loading was observed.

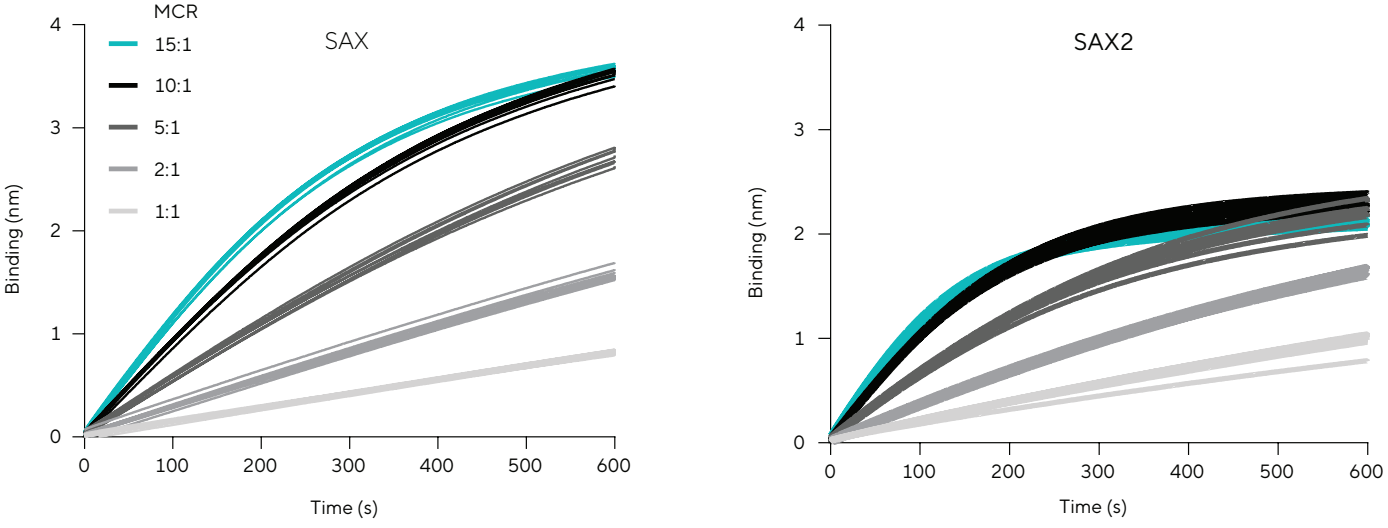

Figure 1: Loading of biotinylated antibody to SAX and SAX2. An antibody was biotinylated at a range of MCR (from 1:1 to 15:1) and loaded at a single concentration onto SAX and SAX2 biosensors for 600 seconds (n=7).

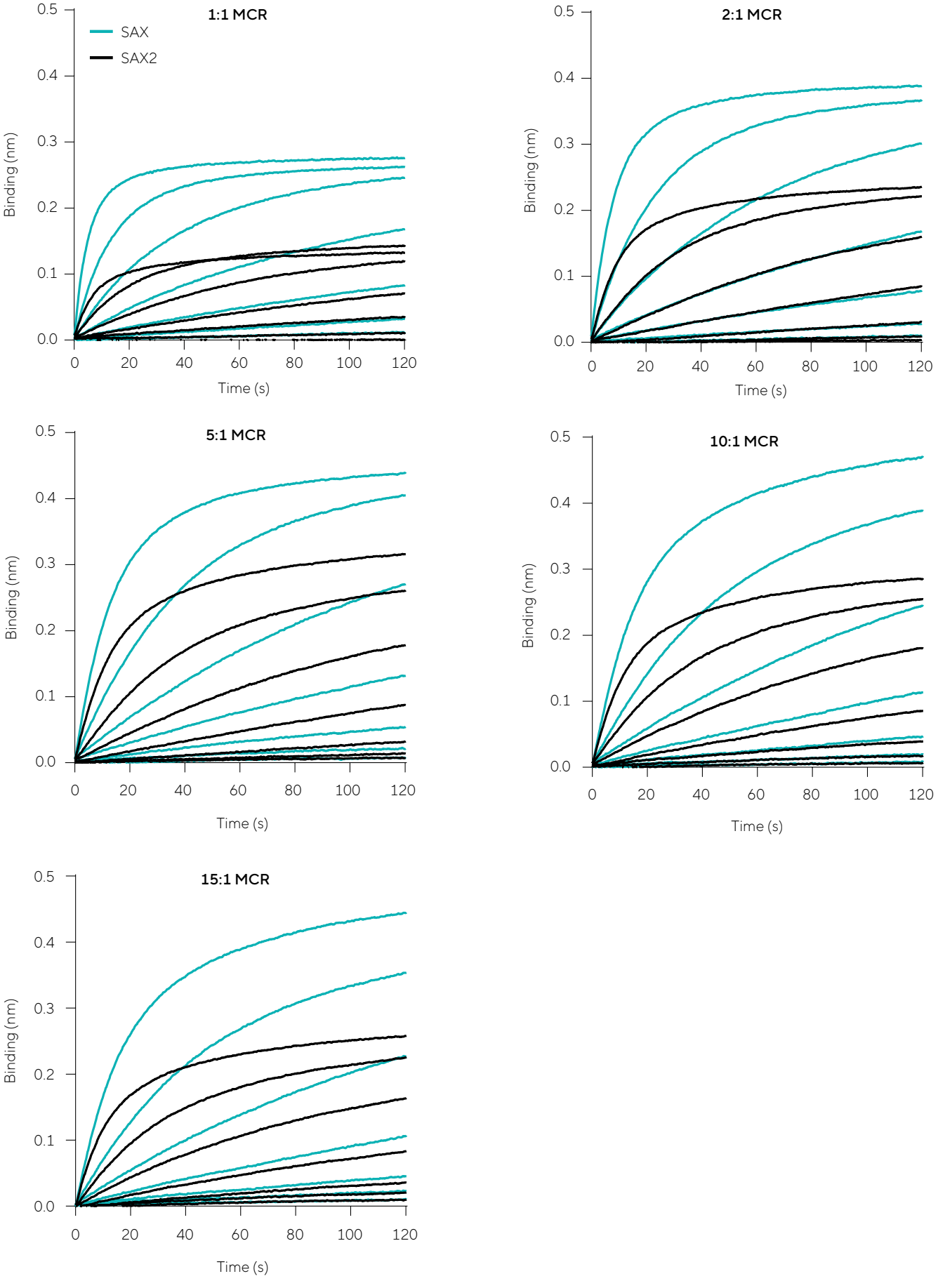

Figure 2. Binding of Protein A to loaded antibody. Association (120 seconds) of a range of concentrations of Protein A (2.7 - 2,000 ng/mL) to antibody-loaded sensors from Figure 1 (n=1). Traces shown for SAX (teal) and SAX2 (black) at each MCR.

To examine the impact of MCR and biosensor type on the dynamic range of a quantitation assay, the traces were fit with the R equilibrium binding rate equation in the Octet® Analysis Studio (representative traces shown in Figure 3A and B). The resulting concentration response curves were calculated using a 5PL unweighted equation (Figure 3C and D). For both sensor types, at the 1:1 and 2:1 MCR there is a plateau in the standard curve at high Protein A concentrations (222 - 2,000 ng/mL), with minimal difference in calculated binding rate leading to these concentrations being poorly distinguished.

In contrast, at higher MCR (5:1, 10:1 and 15:1) the linear range of the assay is extended with better definition of the top concentrations in the curve. Separation of the binding rates for the low Protein A concentrations (2.7 – 74 ng/mL) is evident across both the high and low MCR curves. These data indicate the potential for increasing the dynamic range for quantitation of Protein A by increasing the MCR used for conjugation of the capture ligand. The dynamic range of the assay is also enhanced by the use of SAX biosensors over SAX2, evidenced by increased separation between the high concentrations of Protein A and an overall higher magnitude of response.

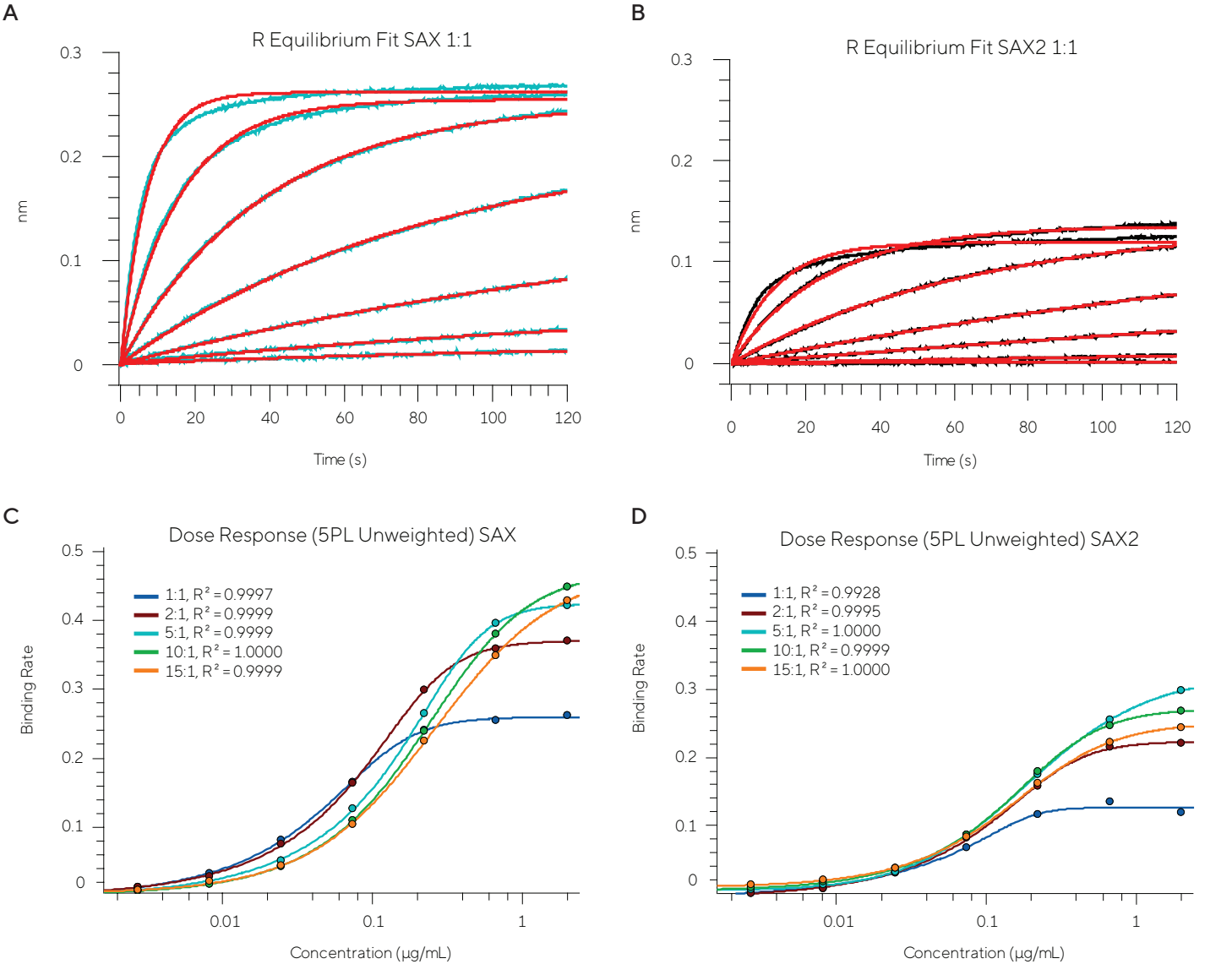

Figure 3. Binding rate and dose response curve fits for Protein A binding. R equilibrium fit binding rate equation applied to Protein A association traces from Figure 2. Representative traces for (A) SAX and (B) SAX2 with antibody biotinylated at a 1:1 MCR is shown. (C and D) Dose response curves based on R equilibrium fit data for the full range of MCR (1:1 to 15:1) on SAX and SAX2. Fit using a 5PL unweighted standard curve equation.

Conclusions

It is widely accepted that a 1:1 MCR for biotinylation is optimal for kinetic BLI assays. For quantitation assays, the data in this technical note provides evidence that increasing the biotinylation MCR above this level, for example to 5:1 or 10:1, can lead to higher ligand loading onto the biosensor at a given concentration. This shows that a greater proportion of ligand is biotinylated during the labelling reaction which leads to less wastage of unbiotinylated ligand and conservation of precious reagents.

Higher ligand loading leads to increased binding of analyte, which can improve the dynamic range for a custom quantitation assay. This was evidenced in these data due to improved separation and detection of higher concentrations of Protein A with a higher magnitude of antibody loading. These data also support the use of SAX rather than SAX2 biosensors for a custom quantitation application, particularly where detection of low or high abundance analytes is required, due to greater dynamic range and signal magnitude. Moreover, the extended linear range of the assay with higher MCR and SAX biosensors could support more accurate data output and increased reliability of detection of analytes, for example from crude samples.

References

1. **Octet biosensor selection guide. Sartorius. 2025**
2. **Technical Note- Optimizing Ligand Biotinylation: Conditions for Molecular Interactions Analysis. Sartorius. 2025**
3. Giuseppe Papalia, David Myszka: Analytical Biochemistry 403 (2010) 30–35

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