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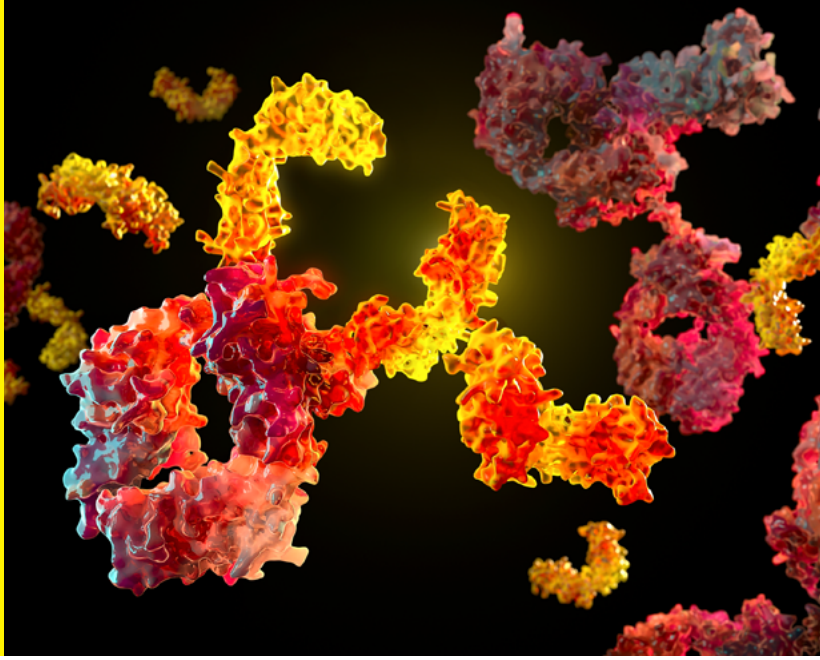
eBook

Label-Free Techniques for Biologics Assessment



Articles Include:

Foreword

Good Data. Rich Insights. More Discoveries.

Biomolecular interactions occur between a wide range of biological molecules and are critical for a variety of biological processes, including signal transduction, enzyme catalysis, and immune response. As such, the ability to study and characterize these interactions is essential for understanding the mechanisms of biological processes and for the discovery and development of new therapeutics.

In the field of antibody discovery and development, label-free biomolecular interaction analysis (BIA) has emerged as a powerful technique for understanding the molecular interactions between antibodies and their targets. Label-free BIA allows researchers to detect and quantify biomolecular interactions, without the need for fluorescent or radioactive labels, resulting in a more accurate and efficient analysis. Surface Plasmon Resonance (SPR) and Bio-Layer Interferometry (BLI) are two of the most widely used label-free BIA techniques, with the potential to accelerate the discovery and development of novel therapeutic antibodies.

SPR is based on the principle of the excitation of electromagnetic waves that propagate along the interface between a metal and a dielectric medium. When an analyte binds to the immobilized ligand on the sensor chip, it causes a change in the refractive index at the surface, resulting in a shift in the angle of total reflected light. This shift can be measured in real-time and is directly proportional to the amount of binding, allowing researchers to quantify the binding kinetics and affinity of the interaction.

BLI is the other industry-leading label-free BIA technique that measures changes in the interference pattern of light waves that pass through a thin film of immobilized biomolecules on a sensor tip. As with SPR, an analyte binding to the immobilized ligand on the sensor tip causes a change in the interference pattern, which can be measured in real time. BLI also enables researchers to measure binding kinetics and affinity, as well as to screen large numbers of potential targets or antibodies in a high-throughput manner.

Both SPR and BLI offer numerous advantages over traditional label-based detection techniques. They can monitor interactions in real time, detect weak and transient interactions, and do not require labeling of the interacting molecules. As a result, they have become essential tools in the field of antibody discovery and development, facilitating the identification of high-affinity and specific antibodies for a wide range of targets, including cancer, infectious diseases, and autoimmune disorders. Moreover, they have been used for the optimization of antibody pharmacokinetics and selection of the best antibody formats for therapeutic applications.

In addition to their applications in antibody discovery and development, SPR and BLI have also been used in a wide range of other biomedical and biotechnological applications, including protein-protein interactions, protein-small molecule interactions, and nucleic acid-protein interactions. They can also be used to study the effects of post-translational modifications and other critical quality attributes (CQAs), such as phosphorylation or glycosylation.

In conclusion, label-free biomolecular interaction analysis has revolutionized the field of antibody discovery and development. It has enabled researchers to assess the binding characteristics of potential therapeutic antibodies quickly and accurately, accelerating timelines and leading to significant advances in the development of more effective and specific biotherapeutics.

Binding Specificity
Do the molecules interact?

Binding Affinity
How tightly do the molecules bind?
(e.g., K_D, k_a relative affinity ranking)

Binding Kinetics
What is the speed of the interaction?
(k_a and k_d)

Concentration Analysis
How much analyte is there?

Simplifying Progress

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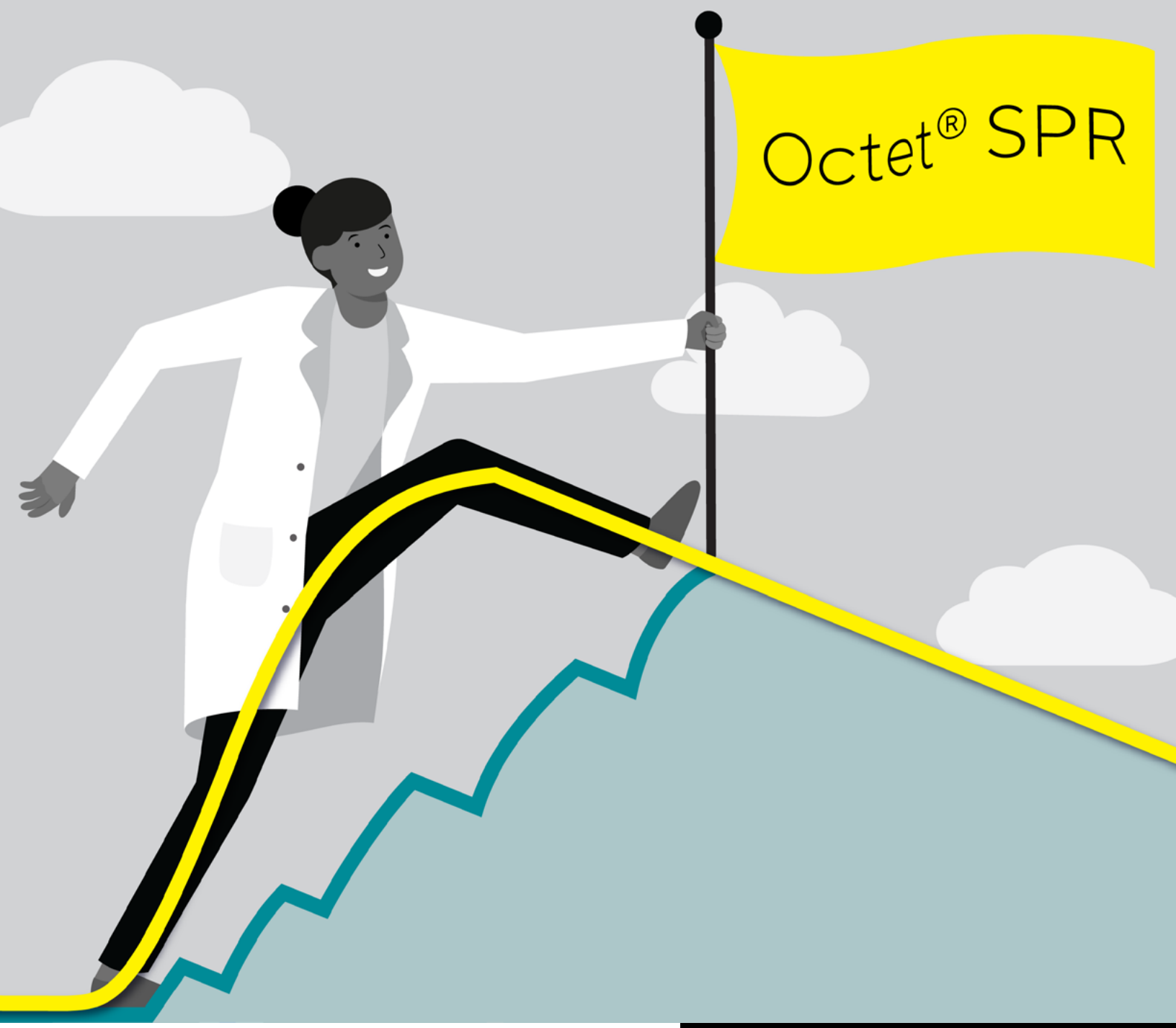

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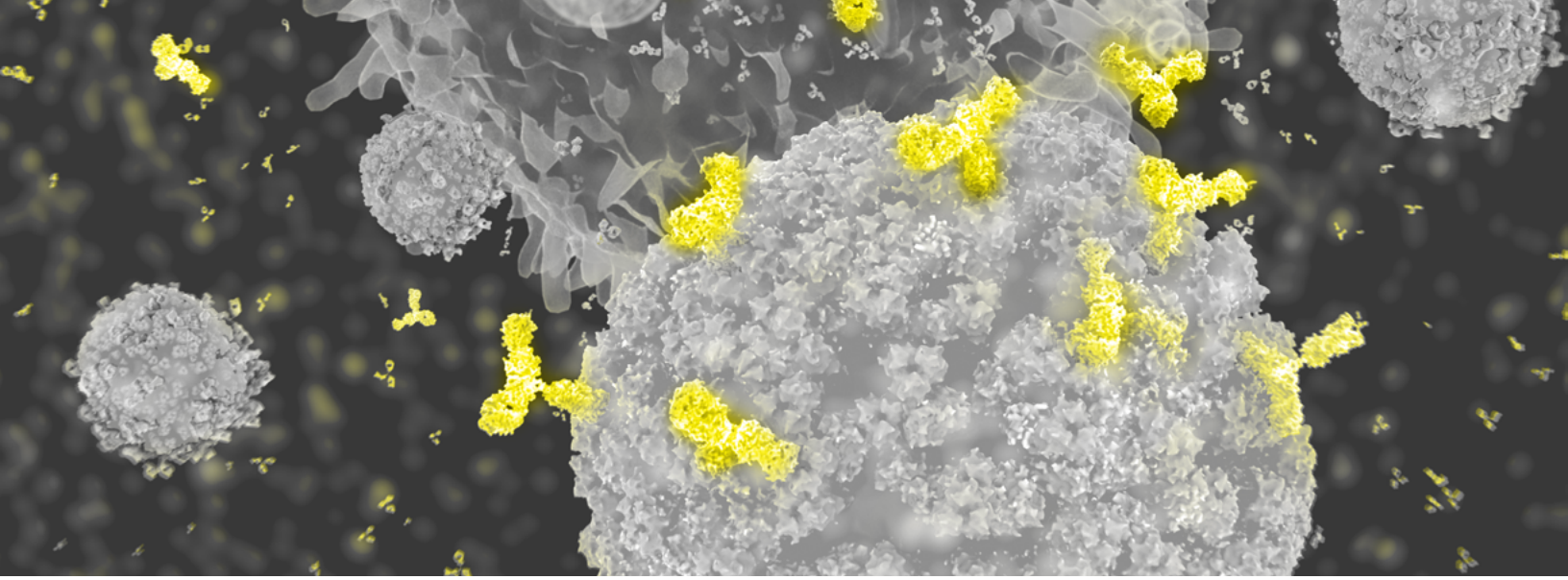

Biologics in All Their Different Shapes and Sizes

Biologics is an umbrella term referring to a number of different products derived from natural sources. The general workflow for biologics production is relatively similar across this range of products, centering on cell culture. For molecular-based biologics, living cells or lysates containing cellular machinery are employed to produce agents of interest such as cytokines, antibodies, or other proteins. Alternatively, the cells themselves can be the end product, with cell culture serving to expand and select for specific phenotypes. Scientists can combine both strategies to generate engineered cells, such as chimeric antigen receptor (CAR) T cells. Biologics manufacturing therefore relies on having the ability to characterize, analyze, and screen large numbers of candidate agents, and then subsequently optimize and produce "leads" for clinical production and use.

Cell-Based Biologics

Cells offer several advantages as therapeutic agents. They are functional, autonomous entities, giving them the potential to persist within biological environments and adapt to changing conditions. This makes them more functionally flexible and more likely to integrate within existing systems. However, these properties also create problems for scientists designing and manufacturing cell-based biologics.[1] Cells are inherently more complex, variable, and heterogeneous than macromolecules.[1,2] They are highly sensitive to environmental changes during production, and their potency and efficacy are time sensitive.[2,3] As such, producing cell-based products in accordance with Good Manufacturing Practices (GMP) can be challenging. Scientists need to take extra precautions when characterizing cells both pre- and post-production and have a solid understanding of the intended mechanism(s) of action while also being cognizant of potential off-target effects. They also need to establish reliable and consistent supply chains for all necessary reagents, as well as ensure no end product contamination, whether by mycobacteria, toxins, residual cell culture reagents, or non-effective cells.[1,2] Ultimately, scientists need to determine the final product quality for each individual lot based on measurable molecular and/or cellular characteristics that are associated with clinical activity.[2]

Molecular Biologics

Generally, producing biologics is simpler than manufacturing cell-based agents. This is one of the

reasons why smaller biological molecules, including recombinant proteins, fusion receptors, and antibodies, make up the majority of developed biologic agents. Most of these agents are produced using host cells, although researchers have developed cell-free methods using cell lysates for smaller-scale applications.[4] As such, the first decision facing manufacturers of protein-based biologics is whether to use mammalian cells, which are more complex but can deliver more intricate proteins and post-translational modifications, or prokaryotic cells, which are more stable and secrete fewer undesired elements during the production process.[5] After this, scientists strive to optimize upstream and downstream processing in their manufacturing workflow. Upstream considerations largely center on cell culture conditions, including bioreactor surface area availability, initial cell seeding density, and media composition, and focus on maximizing yield. Downstream optimization, in turn, focuses on purification.[6] Scientists turn to a variety of methods, including centrifugation, micro/ultrafiltration, and chromatography, to remove impurities such as host cell proteins, DNA, viruses, and endotoxins. These methods have strengths and weaknesses when it comes to resolution, speed, capacity, and recovery. Finally, certain molecules may require multiple purification steps.[6]

Workflows designed for smaller biological molecules can struggle with complex molecules. In particular, engineered antibodies and cell surface receptors typically employ fusion proteins generated by combining two or more genes so that they yield a single protein product. These proteins typically confer the individual effects of their constituent elements, and can possess novel or improved functionality, reduced side effects, more advantageous dosing regimens, and harmonized distribution profiles.[7] However, they are difficult to manufacture. Fusing two proteins together can introduce amino acid sequence compatibility issues, leading to aggregation, misfolding, immunogenicity, and loss of access to active domain sites. Furthermore, it can be difficult to optimize components individually without affecting the remainder of the workflow.[7] As such, scientists can employ additional mid-workflow steps intended to improve yields such as precipitation or flocculation, and extra stringency when assessing final product integrity, purity, and functionality is typically required.[8,9]

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Techniques for Assessing Biologics

Biologic agents are evaluated mostly based on functionality and immunogenicity. High immunogenicity can decrease function, while poor function can necessitate higher dosages leading to immunogenicity. To assess these attributes, scientists look at physical properties such as the presence of unwanted complexes or aggregations, bioavailability, and target-binding affinity, as well as biokinetic parameters such as half-life, degradation, and desensitization. To do this, they use live-cell analysis, as well as a variety of binding assays including enzyme-linked immunoabsorbance assays (ELISAs), bio-layer interferometry (BLI), and surface plasmon resonance (SPR).

Probing for Answers

ELISA has been a constant feature in pharmacology and drug development for decades. In these assays, a target is adhered to a surface and then probed with agents of interest. Binding can be detected and assessed through a colorimetric or fluorescent tag that is either attached to the agent of interest itself (direct ELISA) or bound to a secondary antibody that targets the agent of interest (indirect ELISA).[1] Scientists can use bridging ELISAs to detect targets, such as antibodies, with two or more identical binding sites. Furthermore, simultaneously applying two agents with two different tags is a good method for assessing competitive binding and inhibition. Given this, ELISA is popular for drug candidate screening.[1]

While the technique is convenient and relatively high-throughput (conducted mainly using 96-well plates), it presents a relatively high false positive rate. ELISAs also generate end-point measurements, providing limited data on biomolecular binding characteristics, and often miss very weak or transient interactions due to the required washing steps. Furthermore, ELISA protocols employ numerous wash steps, potentially resulting in the loss of agents of interest that bind with low affinity.[1] Perhaps most problematically, ELISA relies on the pre-existence of antibodies or other detection reagents capable of binding to known targets or agents of interest. As such, it is not well-suited for detecting molecules that have formed unexpectedly or changes in molecular mass, shape, or conformation.[2]

Label-Free Solutions

ELISA, like many other assays, uses probes and/or tags to detect molecules and interactions of interest. These elements are typically affixed onto existing molecules or introduced into host cell genomes, and can change molecular conformations or cause steric hindrance. This is a major impetus for the development of label-free detection techniques.[3,4] Furthermore, because they do not rely on probes, label-free techniques can

potentially identify previously unknown compounds, leading to a more diverse panel of leads with new mechanisms of action.[3,4]

BLI and SPR are the two most well-known label-free techniques. BLI measures biomolecular interactions by analyzing interference patterns of white light reflected from the surface of a biosensor tip. This light is reflected off of two surfaces—a ligand-binding surface and a stationary surface—and binding causes a detectable shift in light wavelength proportional to the optical depth of the binding surface.[4,5] SPR passes white light through a prism where it impacts and is reflected by a thin layer of gold. This light excites the electrons in the metal film, forming surface plasmons and enabling incident light photon absorption by the induced plasmon wave at a specific critical angle. Ligand binding to metal film-bound targets changes the mass of the layer, resulting in a detectable altered refractive index.[4]

In addition to the inherent advantages of being label-free, both BLI and SPR offer real-time results, enabling faster decision-making that is based on data concerning complete biomolecular interactions. Their primary limitation is that one binding partner needs to be immobilized on a surface, and it is therefore imperative that this immobilization does not affect biomolecular conformation or orientation in a way that impacts the binding event. BLI and SPR are versatile, sensitive, specific, and rapid techniques for assessing and characterizing macromolecular interactions with greater confidence than traditional binding assays. They are also not limited to protein-protein interaction analysis, and are widely used in investigations of protein interactions with other small molecules in drug discovery.

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The End of End-Point Assays

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Abstract

The determination of accurate kinetics, affinity, titer, and other critical quality attributes plays an increasingly important role in both up-stream and down-stream bioprocessing. It is important that any systems developed for these purposes match not only the high throughput needs of the user but also their sensitivity needs, allowing assays to be performed earlier in the workflow using minimal amounts of precious samples. This faster time-to-results allows assessment of accurate and precise data earlier in the workflow and therefore quicker decisions can be made on lead candidates to promote.

Octet® bio-layer interferometry (BLI) is widely used and accepted in research and assay development and has been rapidly adopted as an important analytical tool in laboratories that work with biological molecules, either as drug products, vaccines, or diagnostic reagents.

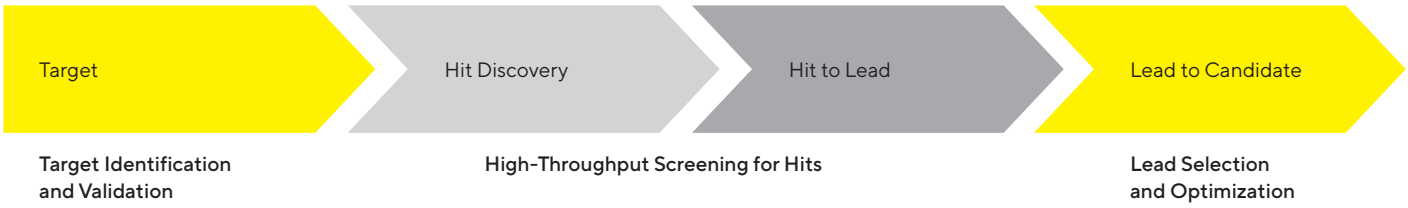
This white paper highlights the recent developments and performance of the three modular Octet® R series configurations with either 2, 4 or 8 channels and shows results demonstrating that all three configurations show similar performance in the quantitation and kinetics characterization of proteins as well as with protein-small molecules interactions. Unique to the Octet® R series is the ability to field upgrade your system configuration to the next modular level when required, allowing you to have full confidence that your system can grow with your future requirements.

Find out more: www.sartorius.com/octet

Introduction

General Introduction

Drug development is predicated on the identification of therapeutic targets, typically proteins or nucleic acids, that play a causal role in a disease and are 'druggable', i.e., amenable to pharmacological action by the drug. The drug development process is generally long, risky, and costly and typically takes longer than 10 years from discovery to approval of a new drug. Moreover, only a very small percentage of the many drug candidates under development each year make it to clinical trials and are approved by regulatory bodies. Identifying and isolating a therapeutic target and characterizing its properties and targeted interactions requires multiple processes and characterization techniques that contribute to long development timelines. Modern label-free and plate-based analytical techniques such as BLI are designed for real-time analysis and high-throughput capabilities, which can significantly reduce the time to the discovery, streamlining the selection of optimal drug candidates with the best chances of success downstream.



BLI is a non-fluidic format and can offer users key distinct advantages over traditional fluorescence-based techniques, including higher throughput, and better sample versatility, including the ability to analyze crude samples and an increased tolerance to diverse sample matrices. Assays that require labeling can contain multiple labeling-specific steps that each require their own optimization, resulting in increased development times and eventual time-to-results. In addition, data quality can be negatively impacted due to false positives that arise due to interference from fluorescent labels. Since they do not generally require labelled reagents, label-free analytical platforms speed up assay development and offer distinct advantages in early drug discovery. Combined with ease of use, low maintenance requirements, intuitive data and analysis software with the option of 21 CFR Part 11 compliant software and generating high-quality data, the Octet® BLI R series platform decreases time-to-results throughout the drug development process.

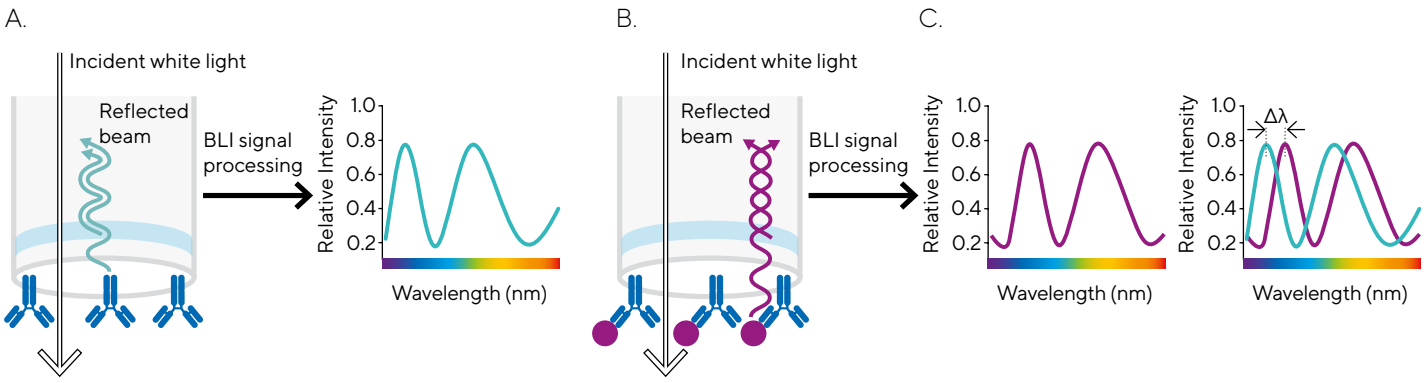
Sartorius' Octet® BLI systems utilize BLI technology to monitor biomolecular interactions in real time. They utilize the robust and easy to use Dip and Read biosensor format and provide faster time-to-results relative to technologies like ELISA and HPLC. Octet® BLI systems are ideal for the quantitation of a diverse array of biological molecules including antibodies and recombinant proteins and are especially suitable for product potency lot release assays. The Octet® R8 platform is particularly well-suited for GxP and QC laboratories.

Bio-Layer Interferometry Technology

Bio-layer interferometry translates biomolecular interactions into response signals in real-time, providing researchers with additional capabilities to characterize binding mechanisms. These systems can be used for kinetics characterization, concentration determination and biomolecular interactions screening among other applications. Of major importance is the ability of label-free technologies to provide on- and off-rates in kinetic characterization experiments which are key determinants in affinity constant derivation and information not available with end-point analysis techniques such as ELISA.
BLI can be applied across a range of applications at various stages of drug development, including antibody and protein quantitation and allows the user to circumvent limitations of ELISA and HPLC platforms, enabling informed decisions to be made earlier in bioprocess development.

BLI analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip and an internal reference layer (Figure 1A). Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real time (Figure 1A and 1B). The binding between a ligand immobilized on the biosensor surface and an analyte in solution produces an increase in optical thickness measured as a wavelength shift, $\Delta\lambda$ (Figure 1C).

Figure 1



Note. Relative intensity of the light reflection pattern from the two surfaces on the bionsensor. Octet® systems with BLI technology measure the difference in the wavelength of reflected light (Δλ) between the two surfaces.

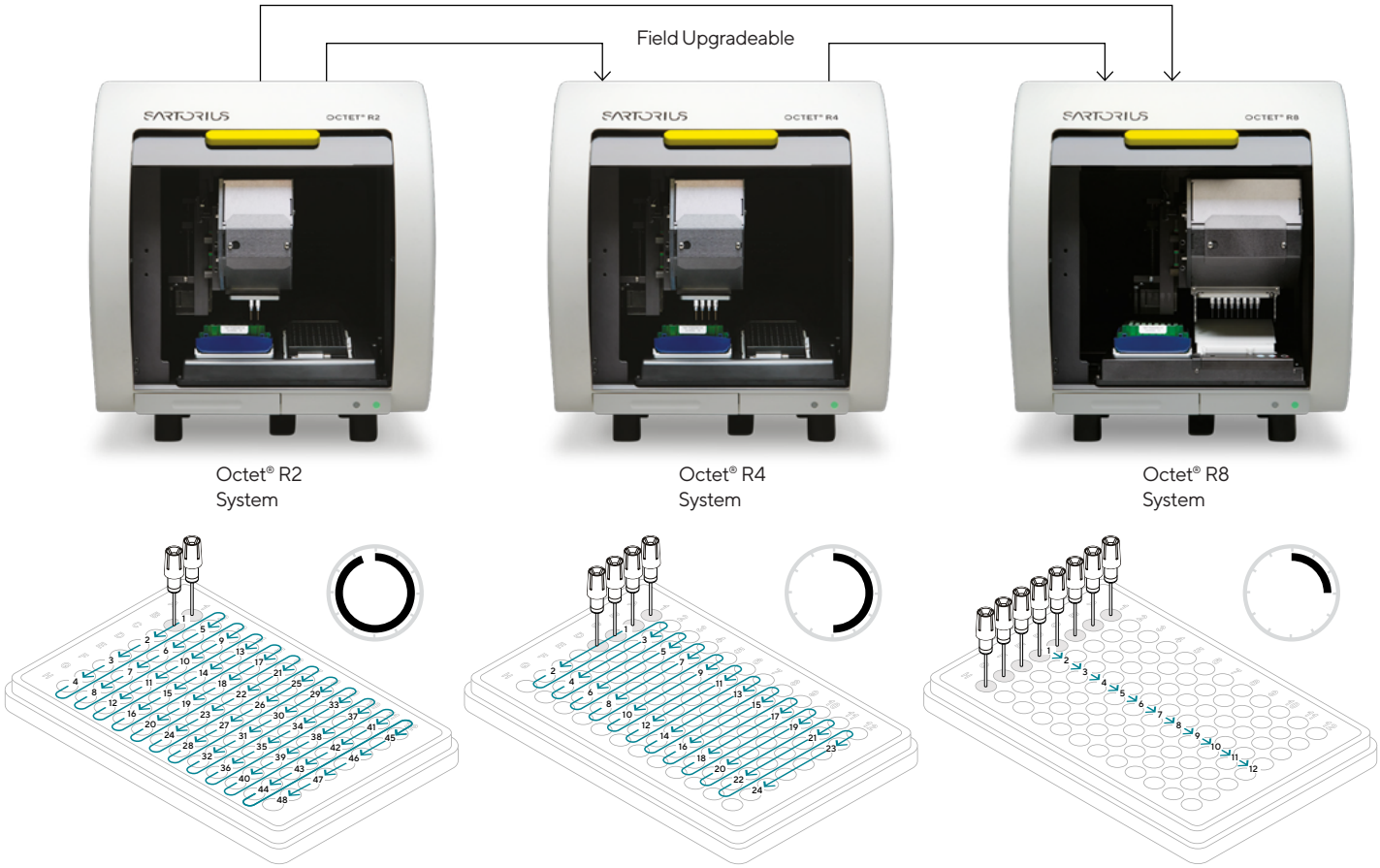
Octet® BLI systems utilize a standard microplate format, enabling high-throughput, automated binding analysis of samples directly from 96-well plates and greater flexibility in assay design. In addition, sample consumption during analysis is minimal and due to its non-destructive technique, precious samples can be recovered for use in other analyses, maximizing process economy. Octet® BLI systems offer the best quantitation and kinetics performance for a diverse range of molecules over a broad dynamic range and with sensitivity to detect molecules as small as 150 Daltons and as large as 1000 KDa. The systems can measure both high- and low-affinity interactions and detect fast binding interactions including protein-small molecule binding.

The newest addition to the Sartorius portfolio of Octet® BLI systems is the modular Octet® R series, available in three different configurations of 2, 4 or 8 channels (Figure 2). Compared to previous versions, all the systems feature improved sensitivity, which allows users to analyze a broader range of molecule sizes and higher data acquisition rates (2, 5 and 10 Hz), meaning the user can analyze faster interactions. The introduction of a cooling sample plate stage to all R series systems allows the user to control sample temperature between 15–40 °C in 1 °C increments; allowing kinetics and affinity to be determined at different temperatures and ensuring that thermodynamics can also be easily studied. In addition, the R8 includes an evaporation cover that allows experiments to be extended to up to 12 hours. Thanks to hardware improvements and the fluidics-free format of the Octet ® R series there are minimal requirements for maintenance and as the 2- and 4-channel Octet® R2 and Octet® R4 systems are field upgradable to the 8-channel Octet® R8 system, no future system trade-in is required, meaning you maintain the value of your initial investment.

As shown in Figure 2, the Octet® R series provides users the flexibility to select a system that satisfies their current requirements and upgrade the system as their needs change and still experience comparative data across each system. This means that users can seamlessly carry methods and workflows across the Octet® R series without having to spend time and money redesigning and qualifying assays and reagents.

To compare the performance of the three Octet R® Series systems, a series of quantitation and kinetics experiments were performed. In all cases the samples and assay conditions used were identical, however some minor differences in the workflow were necessary due to the differences in each system's throughput.

Figure 2



Octet® R2 System | Octet® R4 System | Octet® R8 System

	Octet® R2 System	Octet® R4 System	Octet® R8 System
Throughput	2 channels	4 channels	8 channels
Temperature Control	15–40 °C in 1 °C increments*	15–40 °C in 1 °C increments*	15–40 °C in 1 °C increments*
Upgradeability	To 4 and 8 channels	To 8 channels	No
GMP/GXP Compatibility	No	No	Yes

Differences Are More Than Just In Throughput

	Microplate Compatibility	Sample Volume	Molecular Weight Detection	On-rate (k_a) Range ($M^{-1}s^{-1}$)	Off-rate (k_d) Range (s^{-1})	Affinity (K_D) Range	Data Collection Rate	Orbital Flow Capacity
Octet® R Systems	96-well plate	200 µL/well, non-destructive testing	> 150 Da	10^{1}–10^{7}	10^{-6}–10^{-1}	1 mM–10 pM	2, 5 or 10 Hz	Static or 100–1500 rpm

Note. The new Octet® R series consists of three different configurations that allows you to go from a 2-channel to 4-channel or 8-channel system from a single visit field upgrade. Among the R series, the Octet® R8 system offers the highest throughput, allowing you to analyze a 96-well plate in the least amount of time. The Octet® R4 system strikes the right balance between throughput and price, and the Octet® R2 system provides throughput at the most affordable price. As represented by the clock face, the relative time for measuring the same number of samples and replicates is lowest on the R8 (R8 < R4 < R2).

* The Octet® R8 includes an evaporation cover that allows experiments to be extended to up to 12 hours.

Data Section and Results

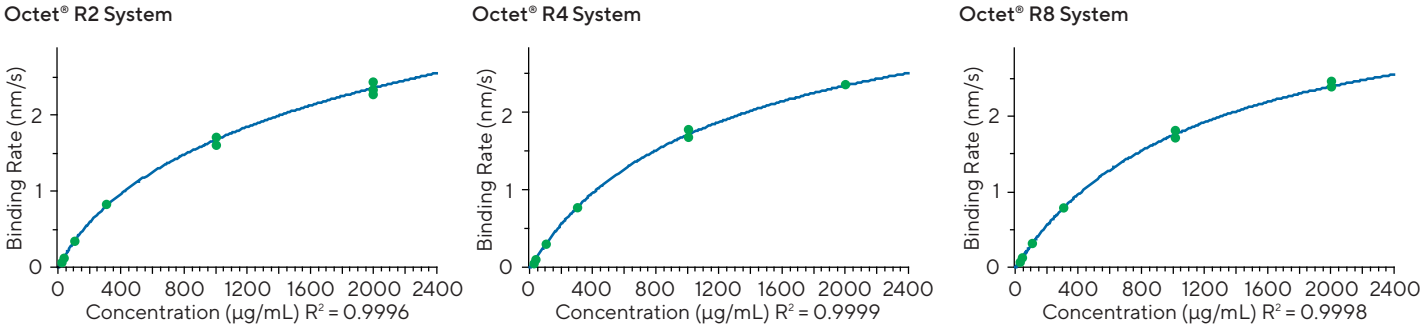
Quantitation Assay Setup for Performance Comparison
Generating accurate kinetics and affinity data requires knowledge about the concentration of your proteins and therefore, quantitation of protein concentration prior to performing kinetic-based assays is a critical step. Thanks to its plate-based format it is possible to rapidly quantitate antibodies and other biologics using a simple assay workflow on the Octet® R series modules.

Here, a standard curve of human IgG (hIgG) reference samples was prepared over a wide range of concentrations (0.025–2,000 µg/mL) at 30 degrees Celsius and a shake speed of 400 RPM using biosensors coated with the capture molecule Protein A (Protein A Biosensors (18–0004)). The initial binding rate of the standards were fitted to a 5PL unweighted curve, and all curves showed an R^2 value > 0.999 across all R series systems (Figure 3A). In a typical quantitation assay, biosensors coated with capture molecules are simply dipped into analyte samples of unknown concentration and the resultant binding, which is a function of sample concentration, is then analyzed using the initial rate of binding (or the equilibrium of binding), which depends on the concentration of the unknown sample.

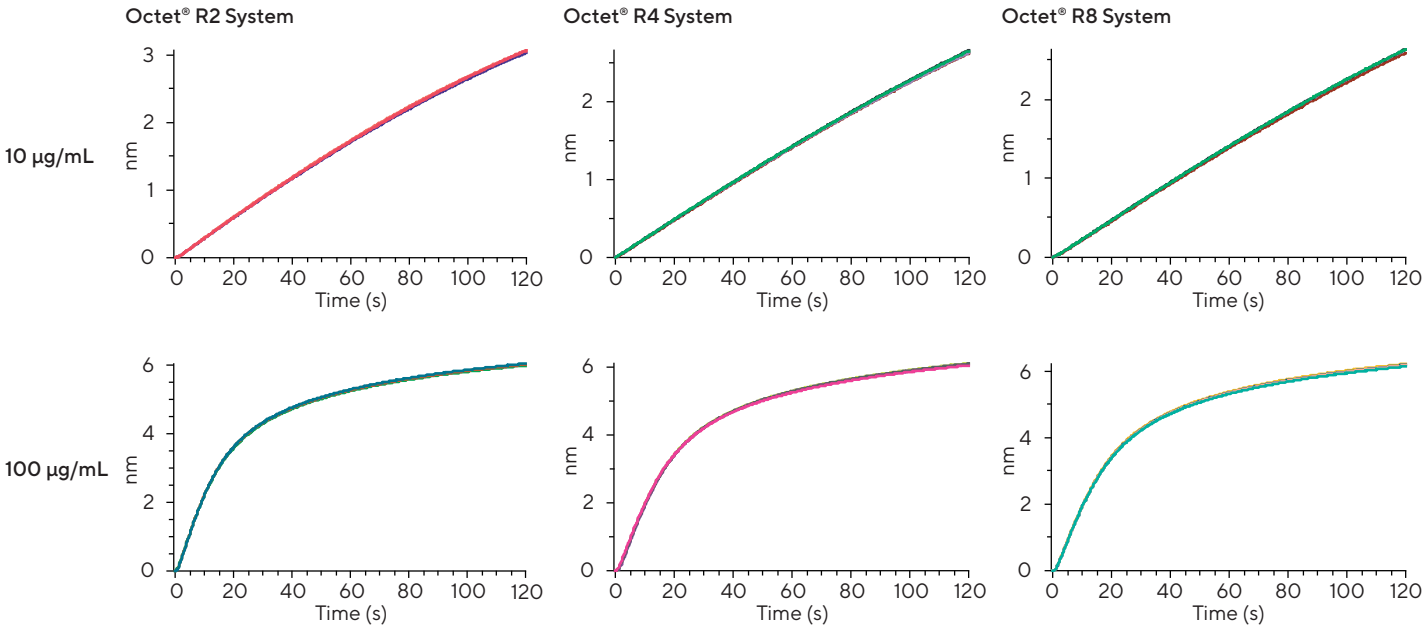
Here, samples of human IgG of known concentrations, 10 µg/mL (n=4) and 100 µg/mL (n=4), were spiked into the assay at 400 RPM and their concentration determined by comparing their initial rate of binding to the standard curve (Figure 3B).

Figure 3

A.



B.



The three R series modules showed excellent precision at 10 µg/mL and 100 µg/mL with % CV < 0.91% and < 1.89%, respectively (Figure 3B, Table 1) with a concentration accuracy range of –7.75 to 11.18% for 10 µg/mL and 1.56 to 3.93% for 100 ug/mL (Table 1). Therefore, all three Octet® R series modules show comparable quantitation performance.

It is important to note that quantitation is molecule specific and assay parameters such as shake speed can influence the assay resolution. This is especially important when low concentrations of analyte are being assessed.

Table 1

	Modular Octet® Configuration		
	Octet® R2 System	Octet® R4 System	Octet® R8 System
Human IgG Concentration	Coefficient of Variation (CV)		
10 µg/mL	0.91%	0.49%	0.75%
100 µg/mL	1.61%	1.89%	0.70%
	Concentration Accuracy*		
10 µg/mL	11.18%	-2.70%	-7.75%
100 µg/mL	3.93%	1.13%	1.56%

* Negative concentration accuracy value denotes a value lower than the accepted value and a positive value denotes a value higher than the accepted value.

Accelerating Cell Line Development

In addition to monoclonal antibodies and recombinant proteins (Read more in the Sartorius Application Note: MAb Quantitation: Protein A HPLC vs. Protein A Bio-Layer Interferometry), the Octet® BLI platform can also be used to accelerate cell line development by quantifying critical quality attributes such as mannose and sialic acid glycan content of Crude and Purified mAb and Non-mAb Protein Samples (GlyM (18-5139) and GlyS (18-5135) kits) and measuring residual contaminants such as host cell protein (HCP (18-5141)) and Residual Protein A (RPA). The detection and removal of process-related Residual Protein A from antibody drug molecules is an essential requirement to ensure the safety of antibody-based therapeutics.

Residual Protein A detection kits (18-5128) are available for all Octet® BLI systems and enable sensitive detection of leached Protein A with a sensitivity as low as 100 pg/mL. Here we compared the detection and quantitation of RPA using of four different MabSelect Sure standards using the three modular Octet® R series systems.

As can be seen in table 2, there is a significant time and reagent saving when comparing the R8 to the R4 and R2 due to the increased number of channels present in the R8 and subsequent throughput. Therefore, it is important to consider which system is most appropriate for current and future assay needs.

Table 2

	Modular Octet® Configuration		
	Octet® R2	Octet® R4	Octet® R8
Number of Residual Protein A Biosensors	40	40	40
Number of detection reagents usage times	10	10	5
Total run time (hours)	2	1	0.5

Accurate Kinetic and Affinity Analysis of Biological Interactions

The specific recognition and binding of biological molecules by antibodies and other proteins is fundamental to many processes in biology and the determination of accurate kinetic rate constants (association (ka) and dissociation (kd)) provides further information about interaction mechanisms, as well as the global affinity constant (KD). This information is critical when characterizing functional properties and is a necessary analytical step in target molecule identification, lead selection, optimization, and subsequent production.

These parameters allow researchers to better understand the potential mechanisms of action (MOA) of the drug candidate against its target and provides information needed to select optimal therapeutic candidates to advance through the development cycle. In general, the full characterization of lead molecules is typically performed using purified molecules; however, lead selection, as well as off-rate ranking in screening experiments, can be performed with non-purified samples.

Kinetics analysis is used to measure association and dissociation rate constants and to determine the affinity of such interactions. Octet® BLI kinetic analysis begins with the selection of a biosensor from a list of multiple chemistries provided by Sartorius. Read more in the Sartorius Application Note: **Biomolecular Binding Kinetics Assays on the Octet® Platform**. The process involves the immobilization of one interactant, commonly known as the ligand, on the surface of the biosensor while the other (analyte) remains in solution.

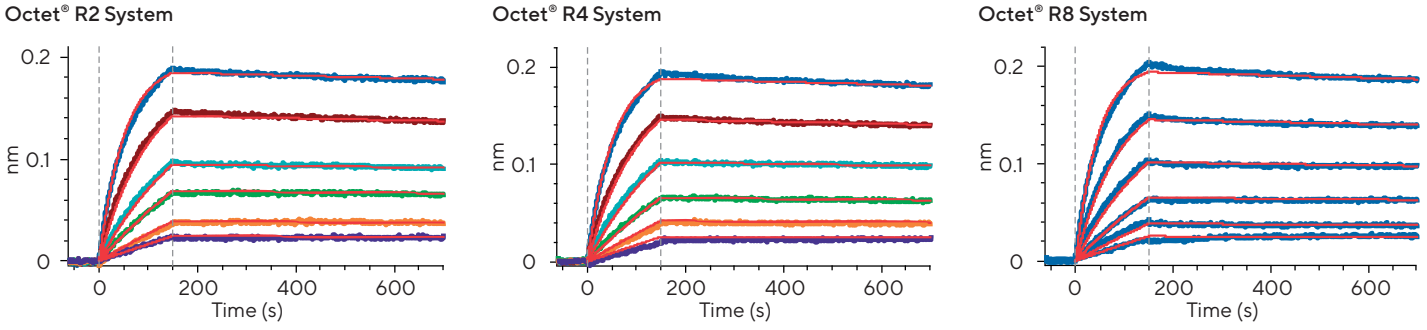
Here, the Octet® R2, R4, and R8 systems were used to assess the binding of a biotinylated anti-HER2 antibody (ligand) to the HER2 receptor protein (analyte) at 25 °C using SAX2.0 biosensors (18-5136). A dissociation time of 1800 seconds was used to ensure an observed decrease in binding > 5% but only the first 700 seconds of the dissociation are shown here in order to highlight the association kinetics.

As shown in Figures 4A, 4B, and 4C the Octet® R series exhibit good visual agreement between systems and analysis of the binding interaction using a 1:1 kinetic model (Table 3) shows a variability of less than 5% CV for the association phase and less than 10% CV for the global affinity. All variability is lower than the levels recommended by regulatory organizations (ICH and FDA).[1,2] Therefore, even if you start with an Octet® R2 system and field-upgrade to an R4 or R8 system, you can be sure that your data will be directly comparable without the need for any assay development.

Table 3

	Modular Octet® Configuration			
	Octet® R2 System	Octet® R4 System	Octet® R8 System	% CV
Ka ($M^{-1}s^{-1}$)	6.83E+05	6.76E+05	6.24E+05	4.9
Kd (s^{-1})	6.57E-05	6.87E-05	5.47E-05	11.7
KD (M)	9.62E-11	1.02E-10	8.76E-11	7.4
χ^2	0.1525	0.2125	0.3397	NA
R^2	0.9991	0.9988	0.9984	NA

Figure 4



Kinetics Performance Comparison of Small Molecules on the Modular Octet® R Series

Of the drugs approved by the FDA in 2021 over 70% were non-protein based and therefore, the ability to measure the kinetics and affinity of a range of molecular sizes is critical. BLI can measure molecular sizes as low as 150 Da and here, carbonic anhydrase II was loaded onto SSA (Super Streptavidin (18-5057)) Biosensors and the association and dissociation kinetics of the analyte Furosemide (330 Da) determined.

As with the large molecule kinetics performance, good reproducibility between the replicates was seen across the Octet® R series modules (Figure 5) and broad agreement with literature values of 0.5 to 1.0 µM for the affinity of Furosemide for carbonic anhydrase II. In addition, comparable loading nm shift, KD, ka and kd were seen across the Octet® R series modules, with all intra- and inter-assay % CV below the recommended acceptable level for ligand binding assays (Table 4).[2]

As shown in Table 5, there is a clear saving of time and reagents when performing multi-cycle kinetic assays using the R4 and R8 systems compared to the R2 system. Fewer biosensors were needed for the R4 and R8 compared to the R2 (16, 16 and 24, respectively) and due to the increased number of channels on the R8 compared to the R2, a 6-fold decrease in time taken to perform the assay was observed. When taken together, this allows the user to generate more data per day with the need for fewer consumable reagents.

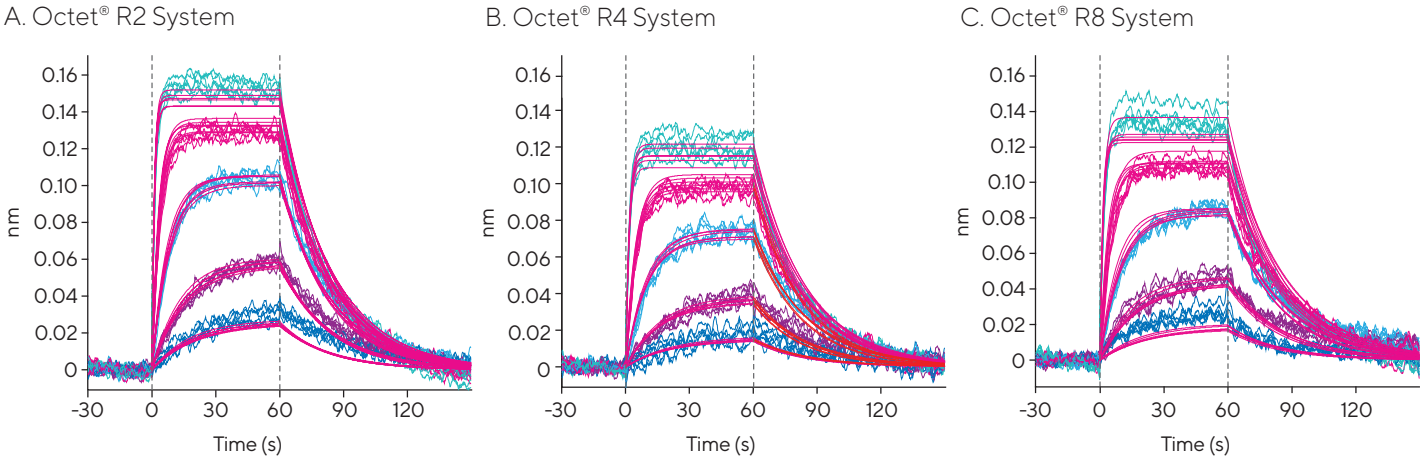
Table 4

Furosemide Kinetics	Octet® R2 System	Octet® R4 System	Octet® R8 System
Ka ($M^{-1}s^{-1}$)	8.10E+04	5.53E+04	6.44E+04
Kd (s^{-1})	4.56E-02	4.48E-02	4.41E-02
KD (M)	5.64E-07	8.13E-07	6.91E-07
pm shift at 30 µM	166.60	135.44	141.19
Ka % CV	7.0%	5.6%	12.3%
Kd % CV	5.7%	3.9%	6.4%
KD % CV	3.3%	7.9%	10.3%

Table 5

	Octet® R2 System	Octet® R4 System	Octet® R8 System
Number of replicates for kinetic rates	6	6	6
Number of sample concentrations for global kinetic analysis	6	6	6
Number of runs needed	2	1	1
Number of biosensors	24	16	16
Total run time (hours)	6	2	1

Figure 5



Note. Carbonic anhydrase II – Furosemide binding characterization comparison data as obtained using the three modular Octet® R series systems. The data represents replicates of a dose response analysis of Furosemide dissolved in PBS/0.5% DMSO buffer. Data was later globally fit to a 1:1 kinetics binding model using the Octet® Analysis Studio Software.

Conclusion

The three modular configurations, Octet® R2, R4 and R8 systems, show very comparable results for quantitation and kinetic analysis of biomolecules irrespective of the size. All three modules can be used for analyzing a whole 96-well plate of samples and come with sample temperature control for stable processing of temperature-sensitive samples.

However, there is significant difference in the total time required to assay the same set of samples and replicates between the three modules. The Octet® R8 system consists of 8 channels which can simultaneously assay eight independent samples with eight biosensors leading to significant time saving. In contrast, the Octet® R4 and R2 systems have four and two channels each, and can only analyze four and two samples simultaneously, leading to increased time requirement respectively. The Octet® R8 system also comes with an evaporation cover, facilitating longer experimental runs (up to 12 hrs) without any significant loss in the sample volume.

Although all the three systems show comparable performance, the Octet® R2 system is most suited to the labs and workflow steps which have low throughput requirements and the Octet® R4 system is suited to labs with higher throughput. Since these systems are modular, they can be upgraded to the highest-throughput Octet® R8 system when the throughput needs increase, thereby futureproofing the lab and maintaining the initial investment within the low-throughput system. Octet® R8 systems are best suited for labs working on high-throughput biomolecule analysis or within workflow steps requiring parallel processing of a large number of samples.

Also highlighted is the significant time saving associated with using the Octet® R8 system over the Octet® R4 and R2 due to its higher throughput ability.

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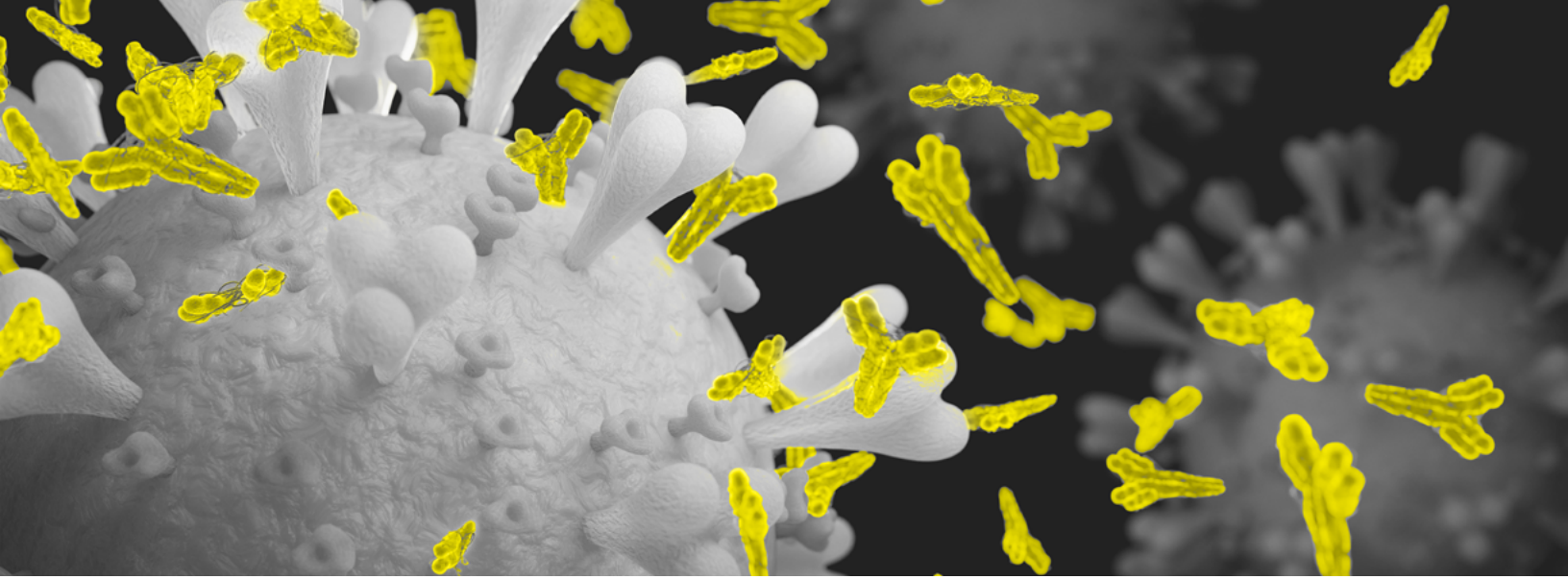

Label-Free Techniques and the COVID-19 Pandemic

The COVID-19 pandemic tested whether existing drug research, development, and manufacturing techniques could handle a rapid crisis situation. Upon identifying the SARS-CoV-2 virus as the cause for COVID-19, researchers immediately investigated its mechanism of action and potential ways to impede viral activity. Bio-layer interferometry (BLI) and surface plasmon resonance (SPR) were instrumental in examining binding interactions between virus and receptor, antibody and receptor, antibody and virus, and antibody and antibody.

Hits, Leads, and Beyond

In 2020, Jinghua Yan's team from the Chinese Academy of Sciences identified two monoclonal antibodies (mAbs) that blocked the SARS-CoV-2 receptor binding domain (RBD) from interacting with the angiotensin-converting enzyme 2 (ACE2) receptor. The researchers subsequently used BLI to investigate the binding kinetics of these two mAbs, dubbed CA1 and CB6, as well as SPR to examine whether CA1 and CB6 competed with each other.[1] No complementary binding was identified and CB6 possessed stronger binding affinity for the SARS-CoV-2 RBD than CA1.[1] These results encouraged further work with CB6, leading to the development of etesevimab, which gained US FDA emergency use approval in 2021.[2]

That same year, a China-wide collaboration headed by Lei Liu investigated the secretory activity of SARS-CoV-2 RBD-targeting B cells extracted from COVID-19 patients. Using BLI to screen the generated antibodies, they found four—B5, B38, H2, and H4—that bound to the RBD of SARS-CoV-2 but not that of SARS-CoV-1.[3] SPR characterization further found dissociation constant (K_D) values ranging from 10^{-7} to 10^{-9} M, as well as neutralizing activity for all four antibodies. Furthermore, a cocktail of B38 and H4 exhibited synergetic neutralizing ability. Finally, BLI-based competition assays demonstrated that only B38 and H4 showed complete competition with ACE2 for binding to RBD and that they recognized different epitopes on RBD with partial overlap.[3] This information eventually led to the development of bsAb15, a bispecific monoclonal antibody based on B38 and H4 with greater neutralizing efficiency than its parental antibodies, especially against variants of concern.[4]

Keeping Pace with the Virus

BLI and SPR helped scientists keep pace with a rapidly evolving virus. Sidi Chen's group at Yale University

used the techniques to characterize SARS-CoV-2-neutralizing mAbs produced by mice immunized with purified SARS-CoV-2 RBD.[5] They used BLI to measure the binding strength of these mAbs, and found two clones in particular where dissociation was not observed in their assay. SPR confirmed the BLI results and further showed that a bispecific clone based on these two clones also expressed high affinity to the SARS-CoV-2 RBD.[5] This data helped demonstrate how novel antibodies could be developed, characterized, and assessed relative to known antibodies, something that is necessary for a virus that mutates as quickly as SARS-CoV-2.[5] Indeed, many research teams employed label-free binding assays to assess K_D for the ACE2 receptor against the RBDs of various SARS-CoV-2 virus variants. In this way, they endeavored to better understand how new variants of concern affected the efficacy of existing vaccines, focusing in particular on how spike protein mutations found in the Omicron variants affected SARS-CoV-2 engagement with host receptors as a means of explaining Omicron immune evasion.[6,7]

Understanding Drug-Virus Interactions

Finally, label-free biomolecular interaction analysis helped scientists better understand the relationship between receptor binding affinity and mechanisms of cell entry and infection. A collaborative effort between Cheng-I Wang's team in Singapore and the QBI COVID-19 Research Group at the University of California, San Francisco scanned existing libraries for antibodies capable of binding the ACE2 receptor but did not show virus neutralizing activity.[8] Examining six candidates with BLI, they found that reformatted immunoglobulin G (IgG) clones showed slower dissociation — likely due to bivalent binding with both arms — than F(ab) fragments. Furthermore, two of the six candidates stood out: 5A6 exhibited far greater viral neutralization potency than other antibodies with superior avidity, while 3D11 was among the least potent regarding neutralization despite displaying the strongest binding.[8] Investigation with SPR showed that 5A6 bound more tightly to spike protein trimers than 3D11, resulting in a greater antibody packing density which in turn inhibited spike-mediated cell fusion. This study helped to elucidate specific modes of action for antibody-SARS-CoV-2 interactions, as well as their functional consequences.[8]

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Exclusive interview with Michael Metterlein – ChromoTek GmbH

A part of Proteintech Group released in addition to the AppNote - **Optimizing Kinetics Assays to Avoid Avidity Effects.**

Tell us a little about yourself and your work.

I have been working for ChromoTek as a Scientist for almost nine years. Since 2020, ChromoTek has been a part of Proteintech. Together we develop nanobodies and conventional antibodies as new tools for research. My main responsibilities are protein analytics using bio-layer interferometry (BLI), nanoDSF and dynamic light scattering. Further, I am strongly involved in developing immunoassays, such as immunofluorescence (IF), immunoprecipitation (IP) or western blotting for antibody validation and in-project management workflows.

What is the difference between (antibody) affinity and avidity?

Affinity is the strength of a single interaction (1:1 binding), such as the interaction between the epitope on an antigen and the antibody at a single binding site. The affinity of an antibody to its antigen is measured by the dissociation constant (K_D), which is the rate constant of dissociation at equilibrium. The K_D is defined as a ratio of k_{off} / k_{on}, where k_{off} describes the rate of dissociation of the antibody from the antigen, and k_{on} is the rate of association of the antibody to the antigen. As such, the smaller the K_D value the greater the binding affinity.

Affinity is influenced by hydrogen bonds, electrostatic bonds, Van der Waals forces, and hydrophobic forces. Avidity, also known as the functional or apparent affinity, describes the cumulative strength of multiple affinities between interacting biomolecules, which arises from two or more interaction sites. It is influenced by binding affinity, valency, and structural arrangements. Avidity can, for example, define the strength of a bivalent antibody to its antigen.

For example, IgG and IgE have two antigen-binding sites, as opposed to a dimerized IgA, which has four binding sites, and IgM with 10 binding sites. In case of

an IgM, it is unlikely that all 10 antigens will disengage from the IgM pentamer simultaneously. Therefore, the avidity of IgM can be relatively high, while the binding affinity of a single binding-site may be low.

What would be the method of choice to measure affinity and avidity: ELISA or a label-free real-time approach?

Analysis of antibody and antigen complexes has traditionally been done using enzyme-linked immunosorbent assays (ELISAs). However, a major shortcoming of this end-point assay is the lack of kinetic, thermodynamic, or stoichiometric information. ELISA cannot accurately describe the affinity or avidity of an antibody. Thus, we use BLI early on in our antibody discovery process to get important information on affinity and rate constants.

ChromoTek provides nanobody-based reagents. Can you explain the role of avidity in this work as avidity is typically associated with multivalent analytes like IgG or IgM?

Most of our nanobody-based products are single-domain antibodies that were derived from Camelid immune libraries and screened for high affinity, which is crucial for example for efficient IP and IF experiments (ChromoTek Nano-Traps, Boosters and Labels). These reagents show efficient 1:1 binding in case of monomeric antigens. Nevertheless, in some cases we exploit the avidity effect by using bivalent nanobody formats to increase the apparent affinity.

In your experimental setup, how would you recognize interactions that contain a bivalent or multivalent component?

In most cases we know our test samples quite well. By comparing monovalent and bivalent formats we can observe significant differences in k_{off}. Bivalent proteins usually show much slower k_{off} values than monovalent proteins – but note that this is only true for bivalent proteins used as analytes (free in solution and not when immobilized).

Can you tell us what your recommendations are to resolve issues arising from avidity effects in your assay setup?

The easiest way to prevent avidity effects is to immobilize the bivalent protein sample. Another approach is titrating down a target protein, but this requires a lot of optimizations.

Can you tell me a bit about your collaboration with Sartorius?

Since we purchased our first BLI instrument in 2015 we have been in close contact with the Sartorius Field Application Scientists and the support center. They helped us a lot to establish a set of assays for screening and characterization of our samples. In 2018, we supplied nanobody-based samples for an industry kinetics workshop that we also participated in. Out of this workshop the idea for a collaboration was born as we recognized that avidity is an underappreciated concept.

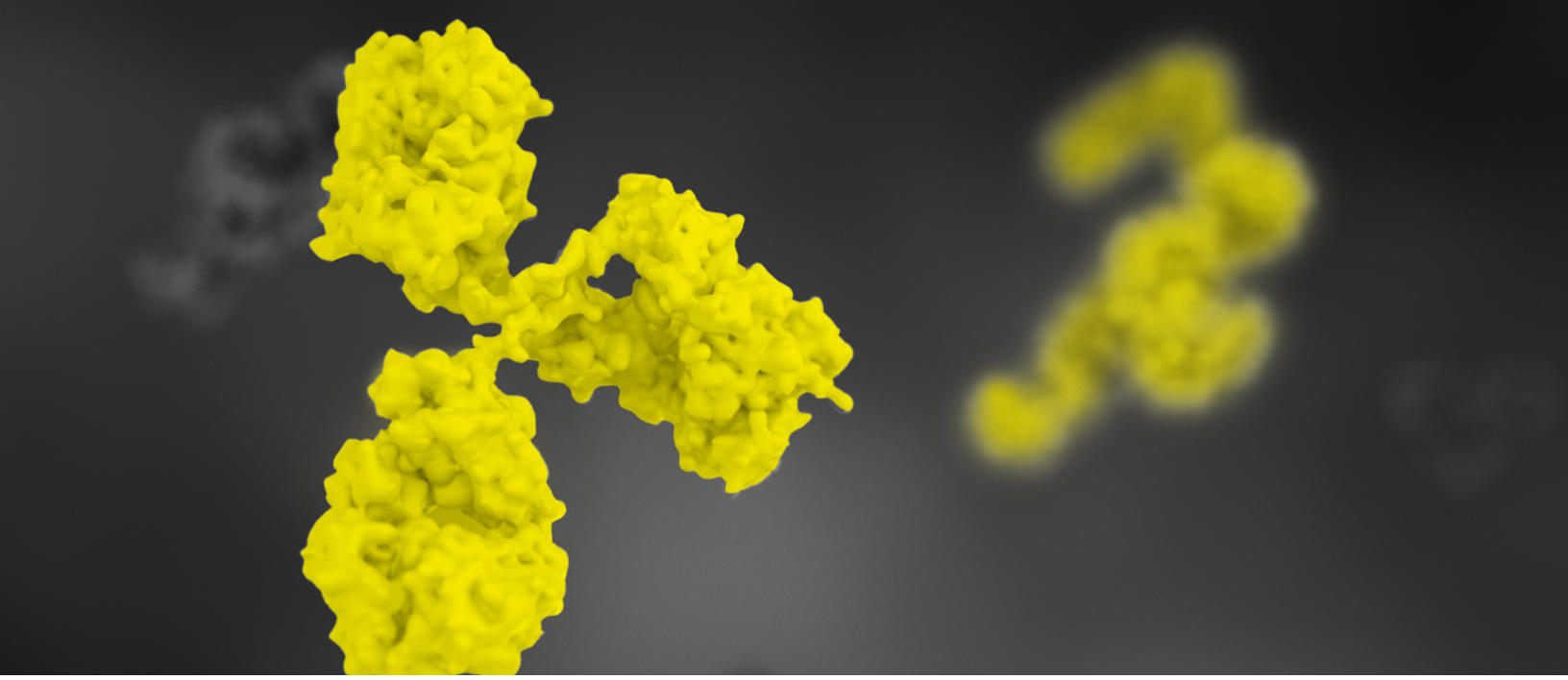
How does the Octet® BLI platform help you to meet your scientific goals?

We use the BLI platform in early development during clone screening after ELISA. A ranking of clones regarding their k_{off} is of high importance for many of our projects. It accelerates our projects by reducing the number of clones of interest and thus saves us money and time. In addition, we use BLI to characterize final candidates regarding their affinity and kinetics, as well as for epitope binning studies. Quantification of hybridoma supernatants or binding specificity assays are further important applications of the BLI platform in our workflows.

Any other final thoughts on everything we discussed today?

A robust assay design helps to limit assay artifacts and helps you measure the desired binding kinetics and affinity data. Having accurate affinity data early on can accelerate your research tremendously!

Thank you very much for the interview!



Converting an ELISA Assay Into an Octet® Quantitation Assay

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Abstract

Enzyme-linked immunosorbent assays (ELISA) are routinely used to quantitate molecules and despite their popularity, these assays are labor- and time-intensive. Quantitation assays on the Octet® platform can be considered automated forms of ELISA but allow a myriad of benefits compared to standard ELISA assays, notably the ability to detect lower affinity interactions and a reduced hands-on time for the scientist. As with ELISA, the signal reported in quantiation assays is either directly or inversely proportional to the amount of bound analyte.

The conversion of many existing ELISA to an Octet® assay typically requires simple re-optimization and validation of the conditions and configurations. This application note describes the key steps for ensuring successful conversion of an ELISA-based assay to the Octet®, including defining the assay requirements, selecting the biosensor type and assay format, minimizing non-specific binding, and optimizing the assay buffer.

Introduction

Quantitation assays on the Octet® platform have many similarities to enzyme-linked immunosorbent assays (ELISA). Both are performed on a solid support on which the capture molecule is immobilized and the analyte is bound from solution. Signal reported in the assay is either directly or inversely proportional to the amount of bound analyte. In fact, Octet® quantitation assays can be considered automated forms of ELISA. The conversion to an Octet® assay often involves simply re-optimizing and/or validating the conditions and configurations of the already existing ELISA assay. However, in some cases where the minimum essential requirements are stringent, more development work is required.

It is often beneficial to convert ELISA assays to the Octet® platform as it allows the scientist to:

1. Choose from a number of assay formats (label-free direct binding, sandwich, sandwich followed by signal amplification, etc.) to suit detection limit requirements
2. Detect low-affinity analytes often missed by ELISA
3. Minimize handling via automated and wash-free steps
4. Fully recover and re-use samples and reagents
5. Regenerate the assay surface and re-use for some binding pairs (e.g., Protein A/human IgG).

This article provides guidance on converting an existing ELISA-based assay to an Octet® assay. The process can be broken down into five steps:

1. Defining assay requirements
2. Selecting a biosensor type
3. Selecting assay format
4. Minimize non-specific binding (NSB)
5. Optimizing assay buffer

Details on each of these steps are presented in the following sections.

Defining Assay Requirements

Conversion activity can start with defining the minimum assay requirements. Sensitivity and throughput requirements often become critical factors in determining the Octet® instrument, biosensor type and assay format, while the sample matrix has the biggest impact on reagent formulation and the blocking protocol. Sample volume requirements also influence the choice of instrument (Octet® QKe, RED, and R8 require 80–200 µL while Octet® RH16 and RH96 require 40–80 µL). Sample volume also indirectly influences the sensitivity of detection when dilution is required to compensate for limited availability of sample. Physical characteristics (pI, size, polymeric status, hydrophobicity, stability, etc.) of the analyte should be considered when selecting the surface and the immobilization protocol.

Selecting a Biosensor Type

There are four types of biosensors to be considered in building quantitation assays on the Octet® platform. They are Streptavidin (SA), Aminopropylsilane (APS), Amine-Reactive (AR) and analyte-specific Biosensors.

SA, APS, and AR Biosensors can be used to build custom quantitation assays for analytes that are process-specific.

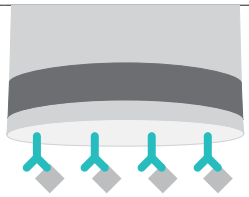
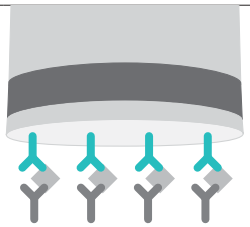
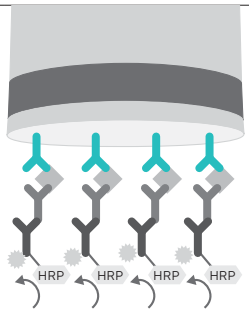
SA Biosensors are most often preferred for their flexibility to accommodate a variety of capture molecules (ligand) through streptavidin/biotin interaction and its superior surface capacity derived from the use of specially designed cross-linked streptavidin conjugates. There are several advantages of immobilizing the ligand that is labeled with a long-chain biotin at a low molar coupling ratio (biotin : ligand):

1. Loss of binding capacity due to cross-linking and steric hindrance are reduced.
2. Biosensors can be prepared in batch mode and stored for later use.
3. The biotin-ligand solution may be used to prepare multiple batches of biosensors.
4. The strong, nearly irreversible binding between the biotinylated ligand and the Streptavidin Biosensor allows easy regeneration of the ligand-loaded biosensor.

APS Biosensors are ideal for immobilizing ligands that are not suitable for covalent linking chemistry. The mode of immobilization is a combination of hydrophobic and/or electrostatic interactions. The APS Biosensor surface is somewhat more hydrophobic in comparison to the surface of ELISA microtiter plates , so the stability of the ligand on the biosensor surface should be tested prior to loading to avoid denaturation.

AR Biosensors can be used to covalently immobilize amine group containing ligands onto carboxylate groups on the biosensors. Batch mode preparation and regeneration of the biosensor are possible.

Table 1: Octet® assay formats and features.

Assay features	1-Step	2-Step	3-Step
Pictorial representation			
Assay steps	1 Bind analyte	1 Bind analyte 2 Bind secondary reagent	1 Bind analyte – secondary antibody complex 2 Bind HRP-loaded antibody 3 Incubate in precipitating substrate for HRP
Typical assay time	▪ 30 min (Octet® QKe, R8) ▪ 15 min (Octet® RH16, RH96)	▪ 1 hr 30 min (Octet® QKe, R8) ▪ 1 hr 15 min (Octet® RH16, RH96)	▪ 2 hr (Octet® QKe, R8) ▪ 1 hr 30 min (Octet® RH16, RH96)
Typical concentration range	▪ Low mg/mL to low ng/mL	▪ Low ng/mL to low pg/mL	▪ Low ng/mL to low pg/mL
Advantages	▪ Single incubation step — fast, easy, reduces reagent expenses ▪ Low affinity analytes detected — even those missed by ELISA ▪ NO labeled reagents ▪ Kinetic parameters can be measured ▪ Allows regeneration and re-use of biosensor in most cases	▪ Two incubation steps — still fast, easy, reduces reagent expenses in comparison to ELISA ▪ Higher sensitivity of detection, down to low pg/mL, depending on assay ▪ NO labeled reagents ▪ Automated and no-wash assay minimizes handling	▪ Similar to most ELISA assays in format — but faster and easier ▪ Excellent sensitivity — down to low pg/mL, depending on assay ▪ Automated and no-wash assay minimizes handling

Analyte-specific biosensors are pre-immobilized with specific capture proteins such as anti-human IgG Fc, anti-murine IgG (Fab')2, Protein A, Protein G, and anti-penta HIS. These ready-to-use biosensors are suited for quantitative analysis of human IgG, mouse IgG, rat IgG, all proteins that bind Protein A, those that bind Protein G, and penta-HIS-tagged proteins, respectively.

Selecting Assay Format

The choice of an assay format is dependent on the concentration range of analyte to be quantified. Octet® systems offer the advantage of a direct binding assay (also called 1-step), that generally affords a dynamic range of detection from low ng/mL to low mg/mL, depending on the analyte. The direct binding assay is fast, easy and eliminates the need for secondary reagents and steps. It also allows regeneration of the biosensor in some cases.

Multi-step assays provide enhanced sensitivity down to low pg/mL, depending on the analyte. Octet® systems measure signal as a function of the thickness and density of the analyte binding layer, so increasing analyte binding translates to bigger signals. When analyte is present in low concentrations and the binding signal is low even after a long incubation, building additional layers of secondary reagents over the analyte binding layer enhances signal.

Such multi-layer assays include sandwich-style assays (also called 2-step assays), which use the biosensor to capture analyte in the first step, followed by use of a second antibody to sandwich the analyte in the second step. For even more signal amplification, an enzyme-linked sandwich assay (also called a 3-step assay) captures analyte bound by two separately-labeled capture molecules to the biosensor in the first step, binds an HRP-conjugated antibody to the complex in the second step, and precipitates a substrate directly onto the biosensor surface in the third step.

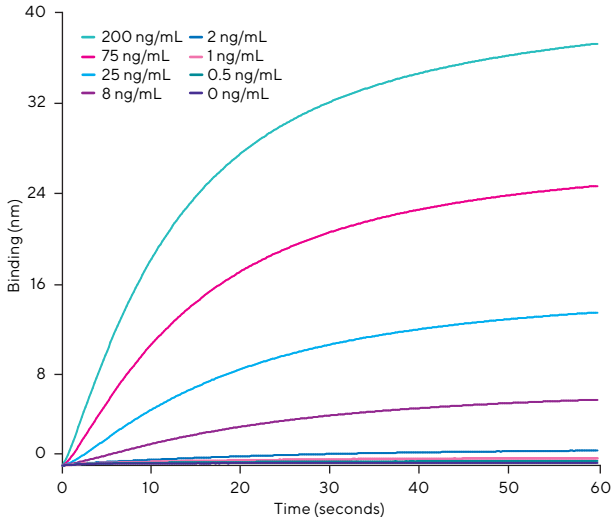


Figure 1: Raw data from the final amplification step of the CHO HCP assay using Octet® R8 system.

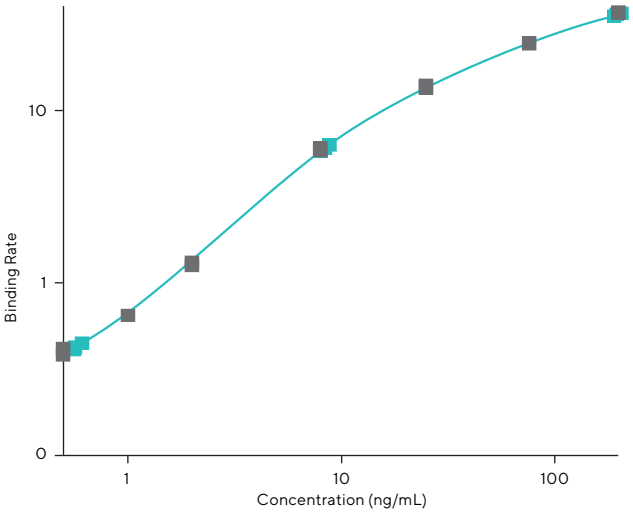


Figure 2: Standard curve showing standards (n = 3 for each standard) and unknown samples (n = 8 for each unknown). The lower limit of detection in this assay was less than 0.5 ng/mL.

Table 2: Accuracy and robustnss of CHO host cell protein quantitation. In this assay, CVs of less than 10% were achievable across the entire dynamic range.

Spike conc. (ng/mL)*	Calculated conc.	%CV of calculated conc.	% Recovery
200	201.6	4	101
8	8.5	4	106
0.5	0.57	7	114

* CHO Cell Protein, n = 8 for eac

Minimize Non-Specific Binding (NSB)

Managing NSB and matrix effects are critical parts of the assay development process that ensure acceptable specificity and sensitivity. The major sources of NSB are hydrophobic, electrostatic, and cross-reactive interactions between the molecules on the biosensor surface and in the solution. In addition to NSB, unrelated proteins and other components of the sample matrix often cause assay interference.

The most effective way to minimize such effects is passivation of the biosensor surface by including blocking step(s) prior to sample incubation. Often, blocking buffer formulation needs to be matched to the sample matrix (e.g., block with the serum for immunogenicity sample in serum).

Optimizing Assay Buffer

Assay buffer formulation has a significant effect on NSB, sample matrix interferences and signal over background. The goal of optimizing reagent formulation is to maximize specific signal and minimize NSB. A direct adaptation of previously developed ELISA formulation may be sufficient in most cases, unless the formulation contains components or conditions that are not compatible with Sartorius biosensors. Examples of such incompatible reagents include those with low pH (<4), high pH (>10), and certain types of organic solvents. The characteristics of the analyte (pI, hydrophobicity and stability) and sample matrix components are important to keep in mind when developing an assay buffer.

On a related note, the rinsing step employed in Octet® assays is often performed in physiological buffers such as TBS and PBS in the presence of a detergent.

The following is a list of common assay buffer components that are useful to keep in mind during optimization.

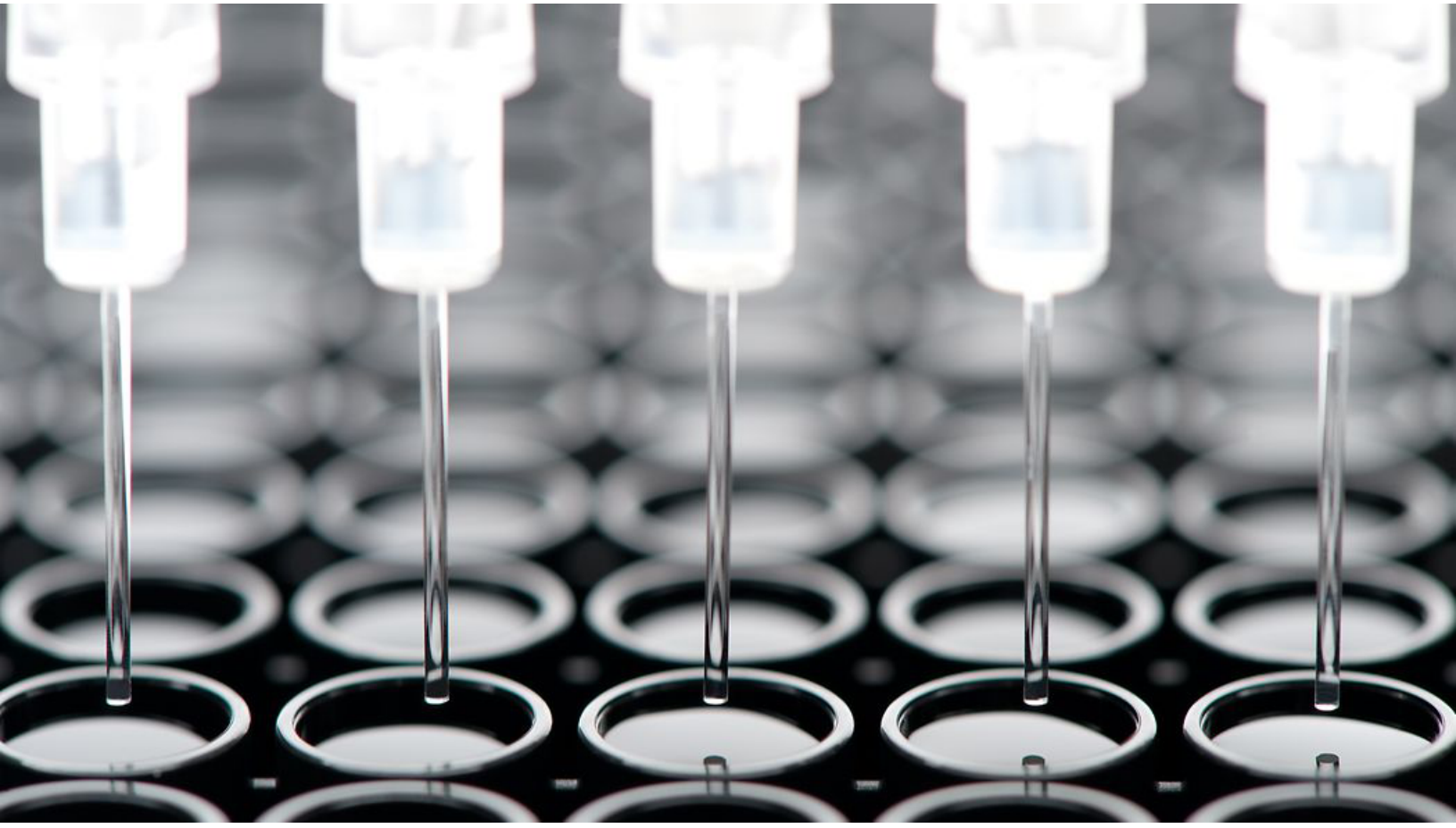
1. Salt: high salt concentrations slow down the reaction, reduce stickiness of antigens with very high/low pI values, reduce charge-induced NSB and denature proteins in solution when needed (e.g., Protein A contamination assay sample pre-treatment buffer).
2. Buffer capacity: higher buffer capacity minimizes pH changes and stabilizes sample solutions.
3. Non-specific antibodies: reduce NSB from multi-species cross-reactions.
4. Detergents: reduce sample aggregation, reduce hydrophobic NSB and reduce sample coating out of very low concentration samples.

5. Bulk proteins (BSA, casein, antibodies): serve as blocking agents, reduce sample coating out of solution, and stabilize analyte proteins in solution in other ways.
6. Sugars (trehalose, dextran, sucrose): stabilize some proteins, enhance signal by increasing effective concentration in solution.
7. PEG: helps reduce non-specific binding and can enhance signal in some cases by increasing the effective concentration of proteins in solution.
8. pH: low pH may often reduce the affinity of competing interactions and improves the proportion of ligand-analyte binding to the biosensor.
9. Dilution: to minimize matrix effects.

Conclusion

ELISA-based assays and Octet® quantitation assays share many similarities. Therefore, conversion of a pre-configured ELISA assay to the Octet® platform may only require transfer of assay conditions. When assay conditions need re-optimization on the Octet® platform, considerations are often similar to those employed in ELISA.

The Octet® platform's direct binding assay method is simple, fast and accurate; the multi-step methods offer high sensitivity and expanded dynamic range. The automated assay formats enhance time-to-results and walk-away time and reduce operating expenses.



A Historical Timeline of Label-Free Techniques Development

Label-free techniques may feel relatively new, but they are the culmination of decades, if not centuries, of theoretical research and technological development

SPR

BLI

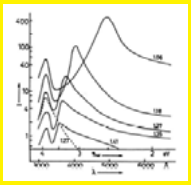
1902

R.W. Wood notices a pattern of unusual dark and light bands in the reflected light when he shines polarized light onto a metal-backed diffraction grating.[1]

1907

J.W. Strutt, 3rd Baron Rayleigh makes the first theoretical treatment of the Wood anomalies, publishing his "dynamical theory of the grating."[2]

1968



E. Kretschmann and H. Raether, as well as A. Otto demonstrate optical excitation of surface plasmons by means of attenuated total reflection.[3,4]

1983

B. Liedberg, C. Nylander, and I. Lundstrom pioneer an SPR method based on attenuated total reflection in prism coupler-based structures.[5]

1984

Pharmacia Biosensor AB is founded with the goal of developing a functional SPR instrument.

1990

Pharmacia Biosensor AB releases the first BIAcore SPR instrument.

1999

S.-W. Kim and G.-H. Kim extend the interferometric method to profiling the thickness of transparent thin-film layers, demonstrating that both the top and the bottom interfaces of a thin-film layer can be independently measured at the same time.[6]

2001

FortéBio is founded, and the Octet® system—the first BLI instrument—is released five years later.[7]

2011

Multi-parametric SPR (MP-SPR) is introduced by scientists at BioNavis Ltd., who develop the first commercial instrument that offered measurements with multiple wavelengths.[8]

2020

Sartorius acquires FortéBio and incorporates the Octet® product line within its Sartorius Lab Products & Services Division.

2021

The Octet® R series, launches under Sartorius.

2022

Sartorius launches the Octet® SF3, a next-generation SPR instrument, thereby becoming the first brand to offer both BLI and SPR.

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