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Application Note

March 11, 2022

Keywords or phrases:
Process development, dynamic binding capacity, protein quantitation, antibody fragments, analyte activity, host cell protein, quality control, protein aggregation, formulations, potency, protein stability, viral titer, GMP, GxP, regulatory compliance, lot release, ligand binding, residual protein A

Simplifying Biotherapeutic Manufacturing and Quality Control with Label-Free Biosensor Technology

Introduction

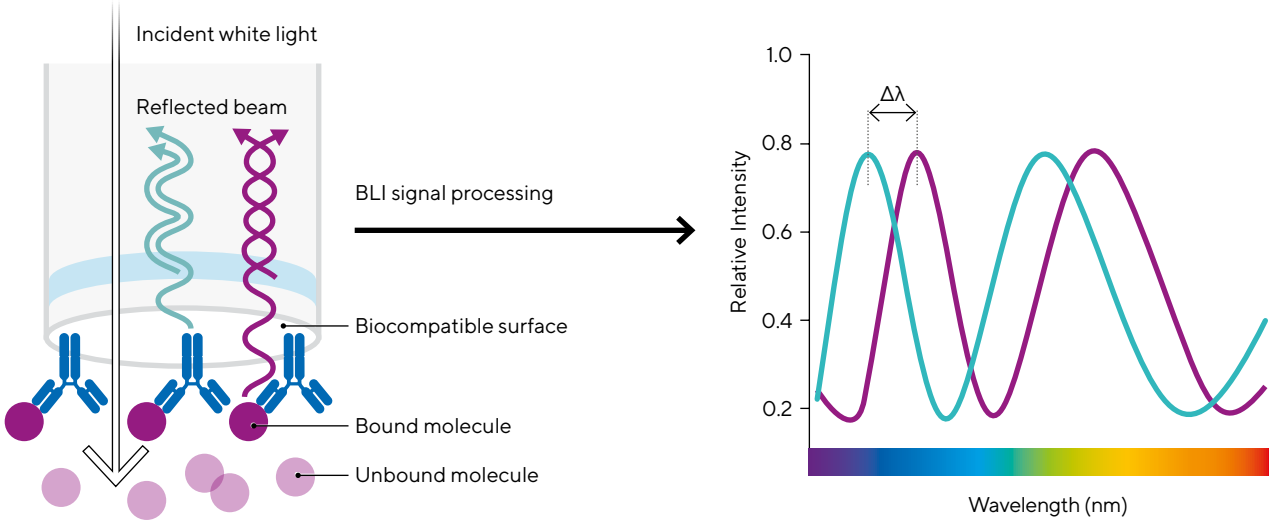
Bio-Layer Interferometry (BLI) systems are used throughout biotherapeutic discovery, development, manufacturing and quality control (QC) workflows to simplify and streamline measurement of process and product attributes. They offer an excellent alternative to assays performed using time- and labor-intensive methods such as ELISA and HPLC. These label-free assays can be run fully automated, require much less user intervention, and provide a simplified workflow.

Following an introduction to the principles of BLI, we highlight how the Octet® BLI platform has been used in a wide range of analytical methods to assess binding kinetics, titer, impurities, aggregation, and activity for in-process testing, stability and lot release applications.

The Principle of BLI

BLI is an optical technology that measures the changes in interference patterns between light waves. Sartorius's BLI based Octet® platforms measure light interference originating from the tip of a biosensor surface where light wavelengths are made to reflect from two layers: a biocompatible layer at the end of the biosensor surface and an internal reference layer (Figure 1). Incident white light reflecting from the two layers interfere constructively or destructively depending on the thickness of the molecular biolayer at the biosensor tip. The spectral pattern of the reflected light changes as a function of the optical thickness of the molecular layer, i.e. the number of molecules bound to the biosensor surface. This spectral shift is monitored at the detector and reported on a sensorgram as a change in wavelength (nm shift). Monitoring the interference pattern (i.e. spectral shift) in real-time provides kinetics data on molecular interactions.

Figure 1



Note. Octet® systems with BLI technology measure the difference in the wavelength of reflected light (Δλ) between the two surfaces on the biosensor.

Only molecules binding to or dissociating from the biosensor can shift the interference pattern and generate a response profile on the BLI system. Unbound molecules, changes in the refractive index of the surrounding medium, or changes in flow rate do not affect the interference pattern. This is a unique characteristic of BLI and extends its capability to perform with crude samples.

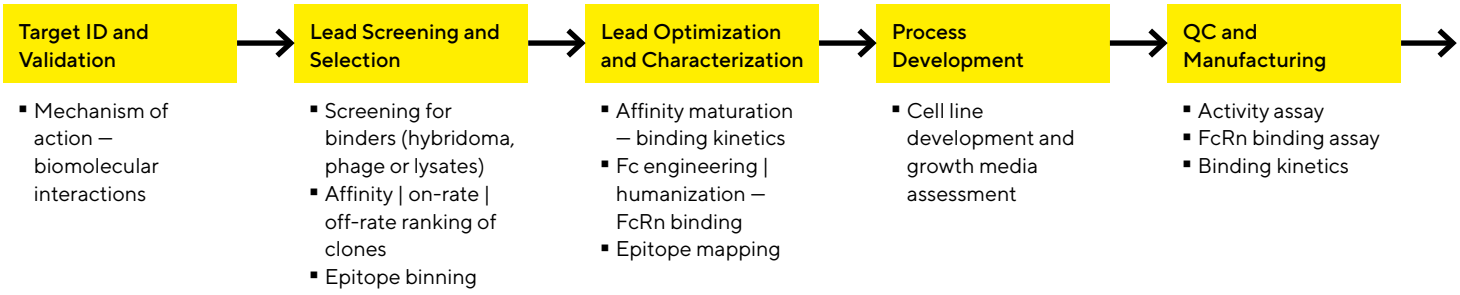
The advanced technology of Octet® BLI systems enables the user to:

- Acquire real-time binding kinetics data to measure the rate of association (k_a), the rate of dissociation (k_d) and affinity constants (K_D).
- Generate up to 96 data curves simultaneously with fully automated assays depending on the Octet® system model.
- Rapidly identify optimal conditions using up to 96 channels to assay multiple conditions and reaction configurations in a single run.
- Generate data from quantitation assays in real time.
- Detect binding of a wide range of analytes, from small molecules to whole cells.
- Recover precious or low-availability samples as binding reagents are not added directly to the sample and materials are minimally consumed.

Determining Binding Kinetics

The Octet® BLI platform offers a rapid, streamlined approach to accurately determine the rate of biomolecular complex formation and stability, key components of drug-target interactions. The affinity of this interaction directly affects the dose at which a biopharmaceutical is effective and elucidating the mechanism of binding has implications related to the efficacy and desirability of a therapeutic candidate. Access to real-time data on specificity, affinity, and kinetics of binding interactions is invaluable throughout discovery, development, and manufacturing (Figure 2).

Figure 2

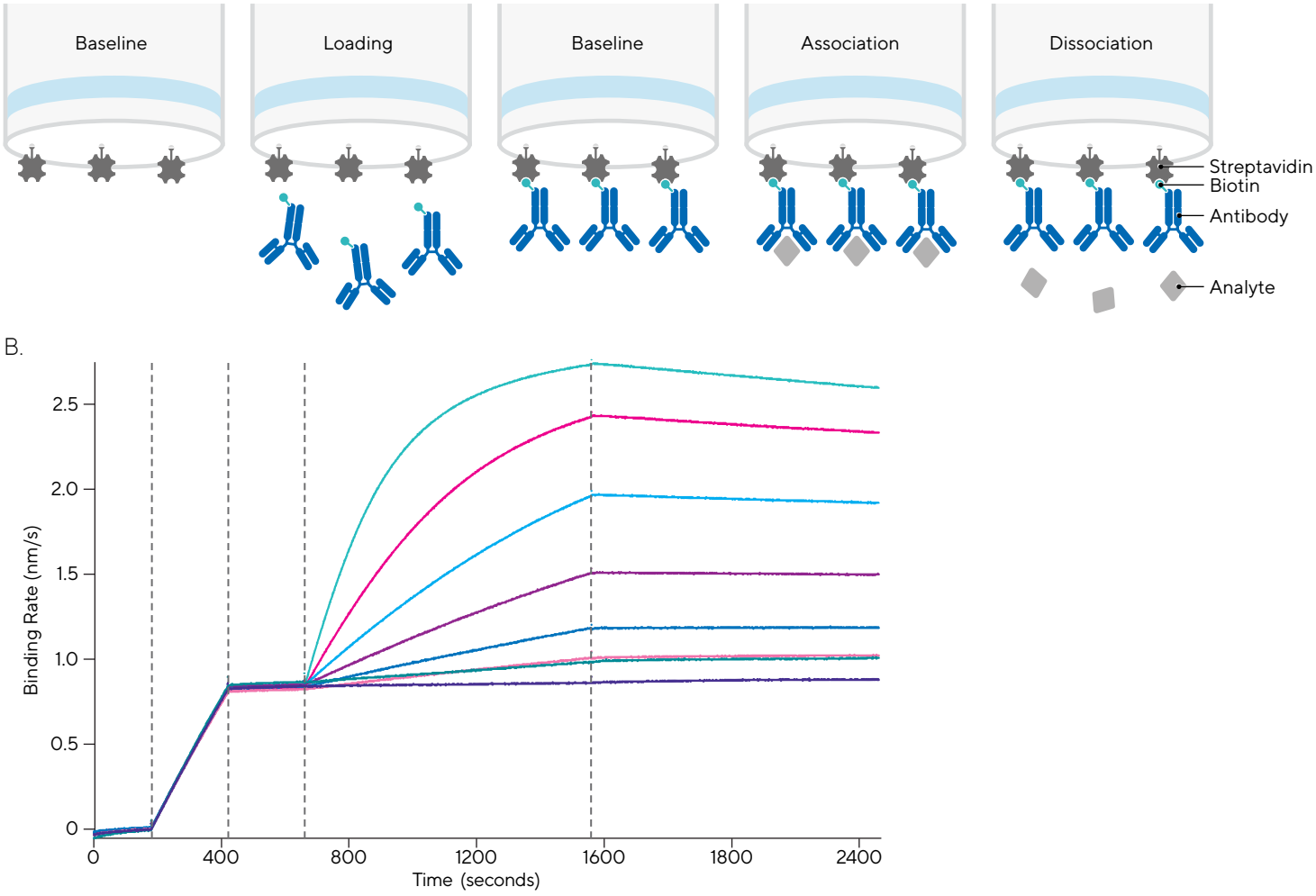


Note. Kinetic applications of the Octet® platform in biopharmaceutical research and development.

While the value of kinetic analysis of molecular interactions using label-free technology is clear, there are many aspects involved in producing consistent, high quality kinetic binding profiles from biological samples. In the application note Biomolecular Binding Kinetic Assays on the Octet® Platform, we provide detailed guidance on options, techniques, and considerations for developing and performing kinetic characterization assays and interpreting results. Use of proper assay development and experimental design help ensure that analyses performed on the Octet® system consistently yield accurate, reproducible data and reliable kinetic and affinity constants.

A typical kinetics experiment setup on the Octet® is shown in Figure 3A. The assay begins with an initial baseline or equilibration step using assay buffer. A ligand molecule such as an antibody is then captured on the surface of the biosensor (loading), either by direct immobilization or capture-based method. Following ligand capture, biosensors are dipped into the buffer solution for a baseline to assess assay drift and determine ligand loading level. Biosensors are then dipped into a solution containing the ligand's analyte (association). In this step, the binding interaction of the analyte to the captured ligand is measured. Next, the biosensor is dipped into buffer solution without analyte, and the bound analyte is allowed to come off the ligand (dissociation). Anywhere from two to 96 analytes or concentrations of analyte can be run simultaneously on the Octet® R2, R4, R8 and Octet® RH16 and RH96 systems respectively depending on the assay goals; for example, a characterization experiment may need a dose response analysis of multiple concentrations of the same analyte while a kinetics screening experiment may utilize one concentration but multiple different analytes. The series of assay steps is then repeated on new or regenerated biosensors for each analyte being tested. Each binding response is measured and reported in real time on a sensorgram trace (Figure 3B).

Figure 3



Note. Binding kinetics experiment using Streptavidin Biosensors. A) After an initial baseline step in buffer, biosensors are dipped into solution with biotinylated ligand (antibody). A second baseline step is performed followed by association and dissociation of analyte molecule in solution. B) Raw data sensorgram showing real-time data acquisition for each step of a kinetic assay.

Determining Concentration and Titer

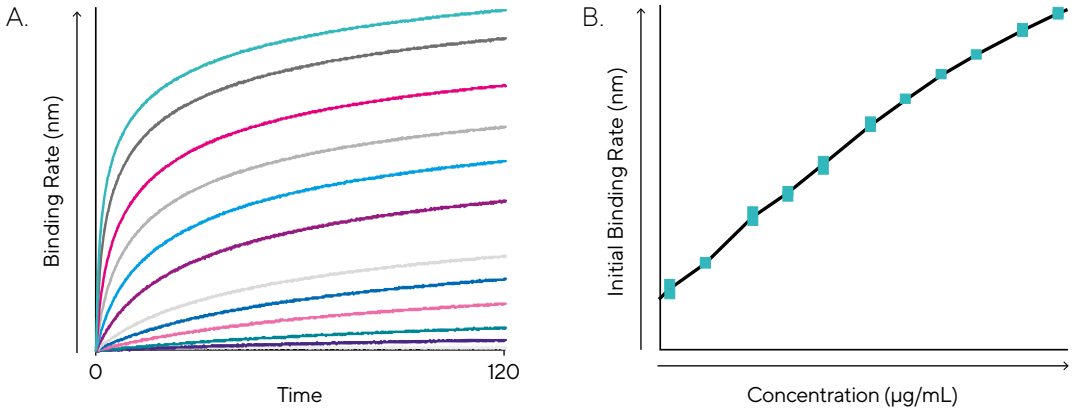
The application note Enhancing Efficiency and Economics in Process Development and Manufacturing of Biotherapeutics describes the use of the Octet® platform for determination of protein concentration and titer in biological samples.

Despite their limitations, UV spectroscopy, ELISA and HPLC have been used for decades for protein quantitation in physiological and process samples. The principles of concentration measurement with an Octet® system are similar to ELISA and HPLC but offer several advantages. The Octet® platform monitors binding of proteins from solution to a biosensor surface in real time, without the need for labels or other detection reagents. This real-time monitoring of binding interactions enables clear discrimination between specific and non-specific binding signals, which can dramatically shorten assay development times. Octet® assays are also much faster; quantitation of a 384-well plate requires 60 minutes compared to more than twenty hours when using ELISA or HPLC.

Samples run on Octet® systems are also recoverable, so that researchers may save and reuse precious samples for other experiments. In addition, Octet® assays are not affected by absorption interferences in colored samples or by light scattering with turbid samples, enabling measurement of analyte concentration in crude matrices such as cell culture supernatant, cell lysate and serum.

Figure 4 shows a typical quantitation assay. Biosensors dip into standards run in duplicate to obtain binding rate (nm) vs. time (sec) curves. The initial slope of the interaction is used to create the standard curve of the binding rate vs. concentration. The concentration of an unknown sample is then interpolated from the standard curve.

Figure 4



Note. A typical quantitation experiment, measuring a series of concentrations (dilution series) (A) to generate a stabdard curve used to determine the analyte concentration of unknown samples (B).

Impurity Testing

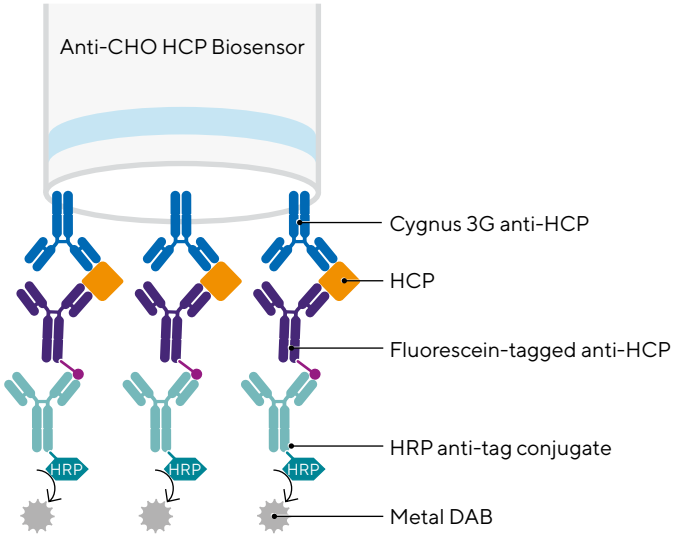
A variety of impurities must be detected and subsequently removed from the biotherapeutic manufacturing workflow; common impurities include host cell proteins (HCPs) from cell lines used to produce the drug molecule and residual Protein A which can leach from purification columns. The Octet® platform offers an accurate, sensitive, and rapid method to detect these contaminants.

The technical note CHO Host Cell Protein Detection outlines a protocol for measuring HCPs from Chinese hamster ovary (CHO) cells generated during production of recombinant protein therapeutics. Residual HCPs can reduce the efficacy of a therapeutic and cause adverse immunogenic reactions in patients. Hence, their detection and reduction to the lowest acceptable levels have become critical aspects of drug safety.

While ELISA is commonly used for detection of HCPs, there are several inherent problems with this technique, resulting from its reliance on highly manual processing steps that introduce variability, time-consuming The Octet® platform offers better precision, better or equivalent sensitivity and dynamic range, low user intervention, rapid assay development, and much faster time-to-results. The measurement involves a sandwich-type assay on an anti-CHO HCP biosensor coated with anti-CHO HCP antibody (Figure 5). A walk-away HCP assay analyzing 96 samples in parallel can be set up to run automatically on the Octet platform with results obtained in one hour. Figure 6 shows data for three samples containing unknown concentrations of HCPs, each having eight replicates.

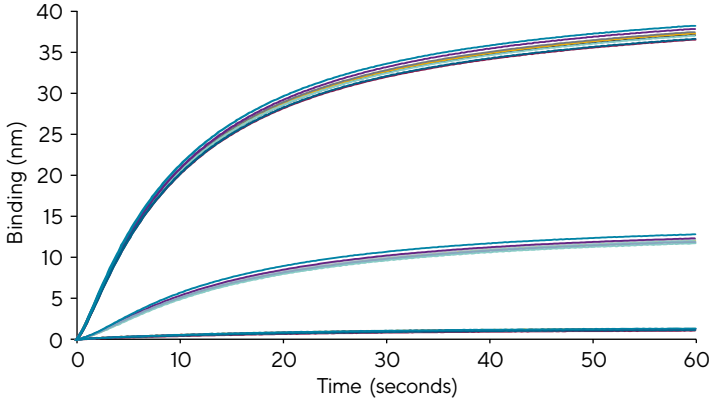
Use of an Octet® assay by GlaxoSmithKline to streamline detection of HCP in process development is described in the

Figure 5



Note. Biosensor-based assay for the detection of CHO HCP.

Figure 6



Sample	Calc. Concentration (mg/mL)	% CV
Sample 1	137.5	4.1%
Sample 2	10.6	4.0%
Sample 3	0.43	5.1%

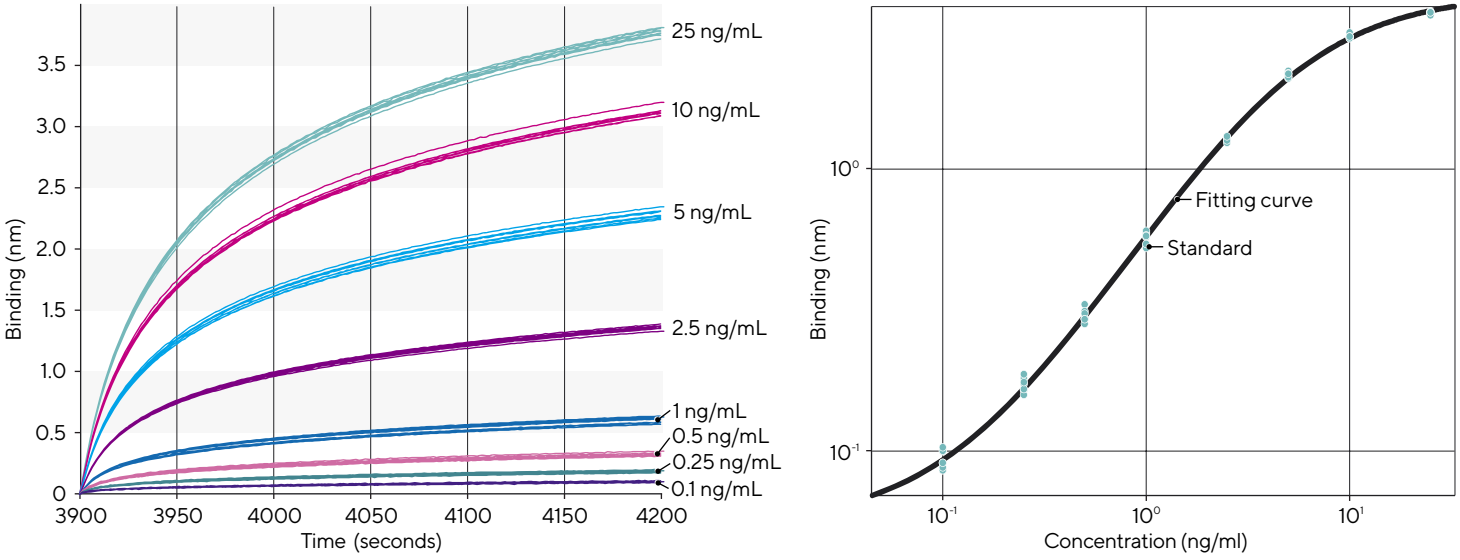
Note. Graph showing data for three unknown samples, each having eight replicates. The calculated concentrations of HCP and %CV are shown in the accompanying table.

application note Enhancing Efficiency and Economics in Process Development and Manufacturing of Biotherapeutics. The automated Octet® HCP assay required minimal analyst intervention and provided more accurate and precise results than their manual ELISA. Hands-on time for preparation and processing of one to three assay plates was reduced to 30 minutes from the previous 2.5 hours with manual ELISA, and antibody consumption decreased by 40%.

Detection of residual Protein A that has leached off the chromatography matrix and co-elutes with the drug substance is also a critical quality control step in the manufacturing of antibody therapeutics. Testing for leached Protein A is performed in several stages of biologic development and production including purification process development, manufacturing, and finished product release testing.

Residual Protein A detection using the Octet® platform can accurately measure down to 100 pg/mL of the contaminant in samples containing up to 5 mg/mL of antibody. Development and use of this assay is described in Residual Protein A Detection Kit. The assay uses a validated sample treatment method for dissociating Protein A from antibodies without boiling, neutralization, or centrifugation steps. Following sample treatment, Dip and Read™ residual protein A biosensors with pre-immobilized chicken anti-Protein A antibody are sequentially dipped into treated samples, buffers, and detection antibody arrayed in 96-well plates to create an immunoassay on the biosensor tip. Signal detection occurs in real time at the secondary antibody step without need for conjugates or enzymatic reactions. Figure 7 shows the standard curve developed using the residual protein A kit in conjunction with the Octet® platform.

Figure 7



Note. Standard curve made with the residual Protein A detection kit standard.

Developing Validation Assays

Real-time data on the kinetics and affinity of binding provide critical information at every stage of biopharmaceutical development. Understanding the mechanism of binding can provide insights into the desirability of a drug candidate during development, including implications for the drug's stability upon complex formation with its binding target. Binding kinetics assays and specifically affinity constant (KD) analysis are increasingly being used for biological product lot release. Regulatory requirements necessitate that such products be QC tested using methods that have been appropriately developed, qualified, and validated under GMP conditions.

Determination of drug analyte characteristics can be affected by test method parameters such as the amount of ligand immobilized for assessing affinity to the drug molecule, assay temperature, flow rate, and shaking speed (for plate-based platforms). These factors can affect binding behavior and thus accuracy of the method and should be evaluated in the pre-qualification stage of analytical method development. While each factor can be evaluated as single variables, an ideal analytical platform is one that allows the user to evaluate multiple factors in-tandem in a design of experiment (DOE) to enable an understanding of the effect of their interactions to the output parameters. The Octet® platform is highly suited for method validation as it offers rapid evaluation of the interactions between potential key assay inputs and allows for relatively fast time to results due to its high-throughput and ease of use.

We describe strategies for development and validation of a percentage relative potency assay for evaluating binding of an Fc gamma receptor III molecule (FcγRIIIa) to the widely characterized National Institute of Standards and Technology (NIST) mAb in the application note Octet® Potency Assay: Development, Qualification and Validation Strategies. The method was shown to be capable of supporting early phase comparability studies and lot release.

The application note also highlights use of the Octet® platform for titer, potency, kinetics, and identity testing by KBI Biopharma. The company has developed more than thirty methods on the platform, many of which are used in manufacturing, drug substance and drug product release testing, and long-term stability testing in a GxP environment.

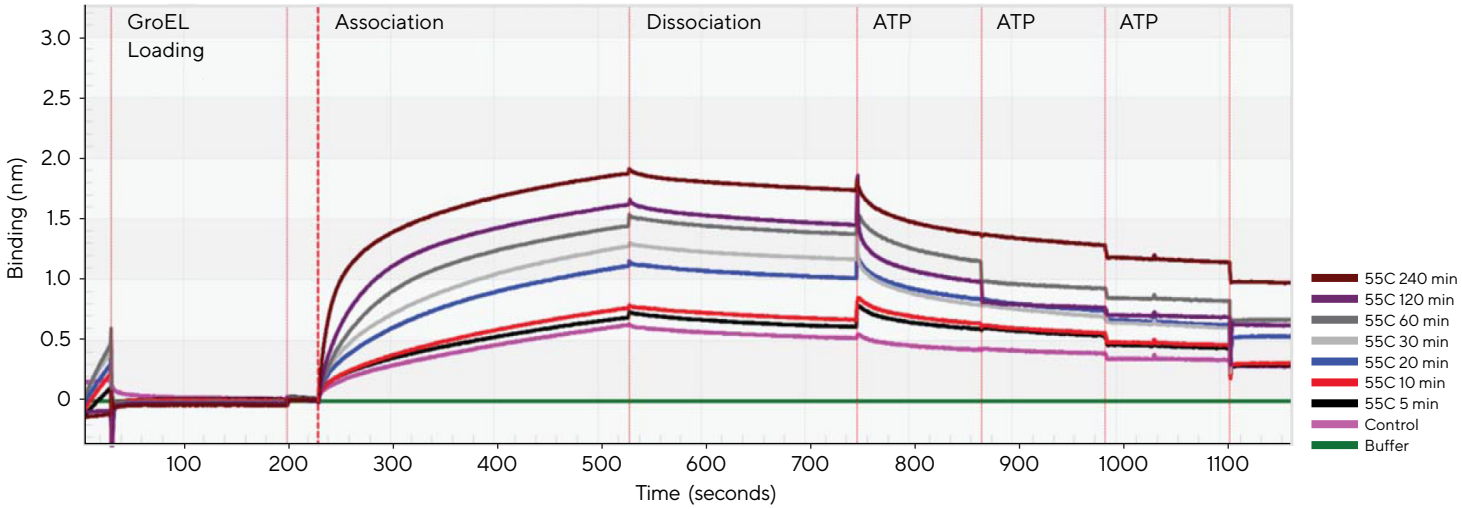
Aggregation and Protein Stability Testing

Structural alterations in a drug substance or product can result from protein mis-folding, denaturation or unfavorable conditions, and lead to formation of aggregate species that can affect efficacy and safety.

Traditional technologies for downstream assessment of biological drug candidate stability include dynamic light scattering (DLS), multiple-angle light scattering (MALS) and circular dichroism (CD). These techniques predict stability through a range of biophysical parameters but cannot also provide a functional assessment.

As described in Expanding Octet® Applications in Downstream Biologics Characterization: Stability, Formulations, and Aggregation Studies, the Octet® platform can detect the presence of aggregated species while monitoring the functional activity of a biologic. A hydrophobic-based probe can be used to screen for and to evaluate molecular structure including unfolded proteins, and differentiate between pre-aggregates and aggregate formation. In addition, the plate design and the use of multiple biosensors in parallel enables screening of buffers or formulations to help determine the optimal formulation conditions. Figure 8 demonstrates the ability of a method developed on the Octet® platform to rapidly detect pre-aggregate/aggregate formation in protein solutions during slightly elevated (40–45°C) to moderate (55°C) thermal stress.

Figure 8



Note. Monitoring the evolution of polyclonal IgG binding amplitude with increasing temperature.[1] Data courtesy of University of Kansas.

Find out more: Probing structurally altered and aggregated states of therapeutically relevant proteins using GroEL coupled to bio-layer interferometry

https://doi.org/10.1002/pro.2515

Developing Activity Assays

Activity assays are used for in-process testing, to assess stability of the drug molecule and for lot release. Using the Octet® platform, active protein concentration can be determined with a binding assay by immobilizing ligand against the target analyte onto the biosensor, and then measuring its binding interaction with the analyte. Below, we outline several activity assay applications developed and performed on the Octet® platform.

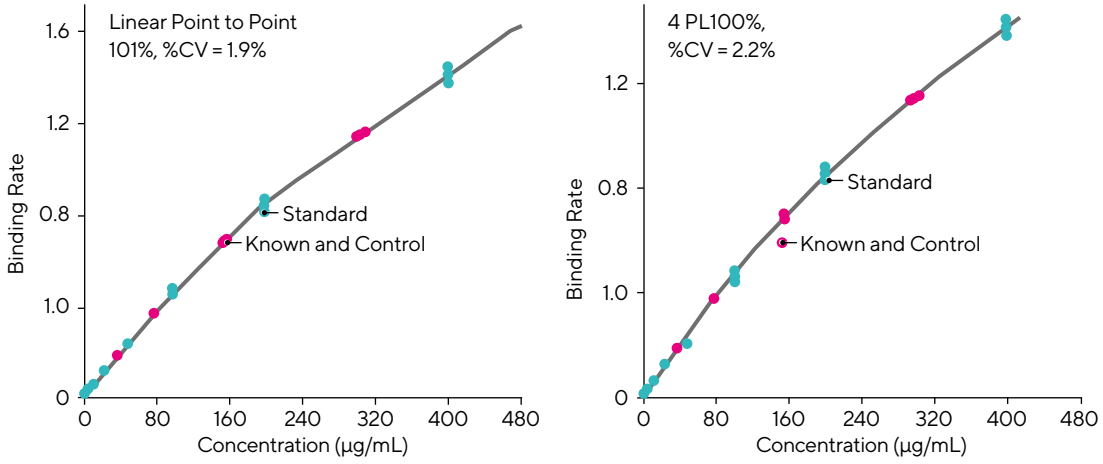
Antibody Fragment (Fab) Activity Assay

The analytical group at Boehringer Ingelheim used the Octet® platform to develop a robust assay to measure the biological activity of an antibody fragment (Fab) molecule and applied it for in-process testing, stability testing, and lot release. The Fab activity assay, which was developed in one week, is accurate and robust, with intermediate and intramediate precision of less than 10% (Figure 9). Drug activity measurement using the Octet® system has become a critical parameter for their product evaluation and has increased Fab drug product consistency and quality. Development and initial validation of this assay is described in the application note Validated Quantitation and Activity Assay of Antibody Fragment Molecule (Fab) for Process Development and Quality Control.

Fc Receptor Binding Assays

The interactions of therapeutic antibodies with Fc receptors and neonatal Fc receptors (FcRn) are measured in vitro as indicators of antibody functional performance. The Octet® platform was used at the University of Kansas Medical Center to rapidly evaluate the effect of oxidation on the functional activity of Herceptin through characterization of binding to an Fc receptor (FcRn). In this study, highlighted in Octet® Systems: Modernize Biopharmaceutical QC Testing to Increase Efficiency, the Dip and Read plate format of the Octet® platform allowed time-staggered incubation of the drug product in hydrogen peroxide (H_2O_2) directly in the sample plate followed by analysis of drug activity at various time points (Figure 10).

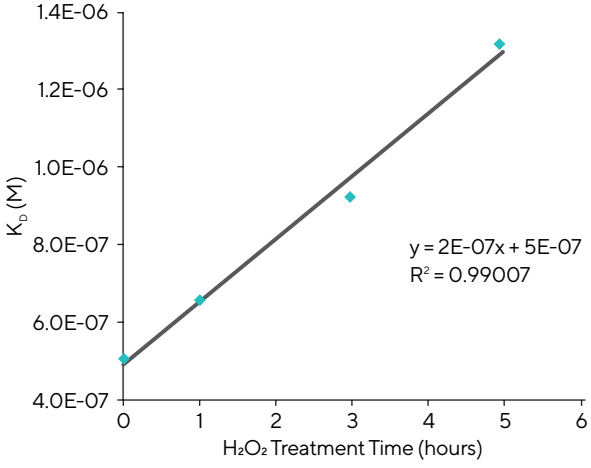
Figure 9



Note. Known and control samples recovered within ±10% of expected values and the eight replicates produced very low CVs (<5%) for all curve fits evaluated. Data courtesy of Boehringer Ingelheim.

Figure 10

K_D (M) Increase with H_2O_2 Treatment



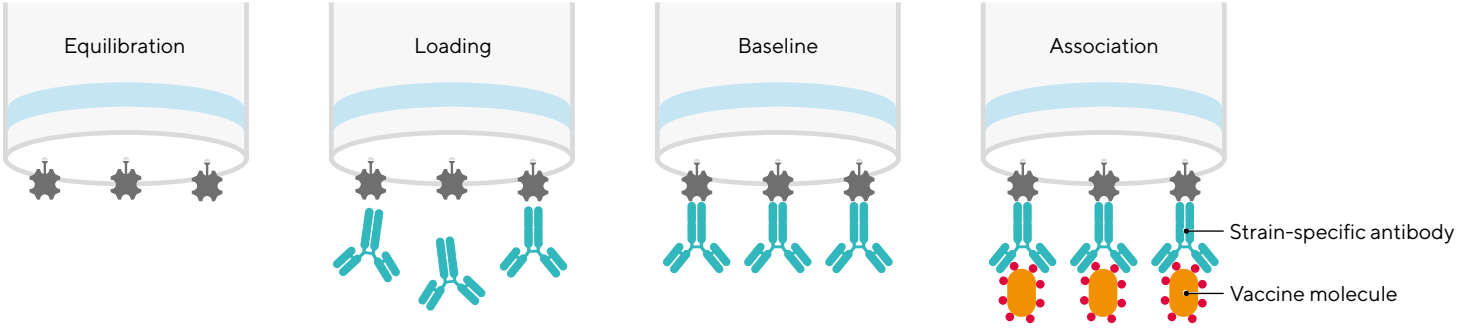
Note. Impact of methionine oxidation on the binding of Herceptin to FcRn. Data indicates clear inactivation of the human IgG over time[2].

Influenza Vaccine Potency Assay

Fast and accurate determination of vaccine titer during production is essential for informing process performance and scale-up. Traditional assays such as single radial immunodiffusion (SRID) and ELISA have their limitations. While it is considered the gold standard, SRID requires very skilled operators to obtain reproducible results and is relatively low throughput. ELISA exhibits low precision and a low dynamic range.

In contrast, the Octet® platform delivers improved accuracy and reproducibility. Development of an assay based on binding of a vaccine to polyclonal antibodies that recognize influenza epitopes presented by the vaccine is described in A Fast and High Precision Influenza Vaccine Potency Assay. The polyclonal antibody is bound to a Protein G or Protein A derivatized biosensor (Figure 11); this configuration offers increased flexibility by allowing rapid changes between vaccines derived from different viral strains by simply binding the paired antibody for the new strain to a biosensor without the need for derivatization. The assay is thus suitable for detecting rapid changes in the viral strains represented in a vaccine. The assay is applicable to both attenuated and synthetic vaccines and can be used for vaccine potency assessment and in various process stages.

Figure 11



Note. Octet® assay workflow for the influenza vaccine titer assay.

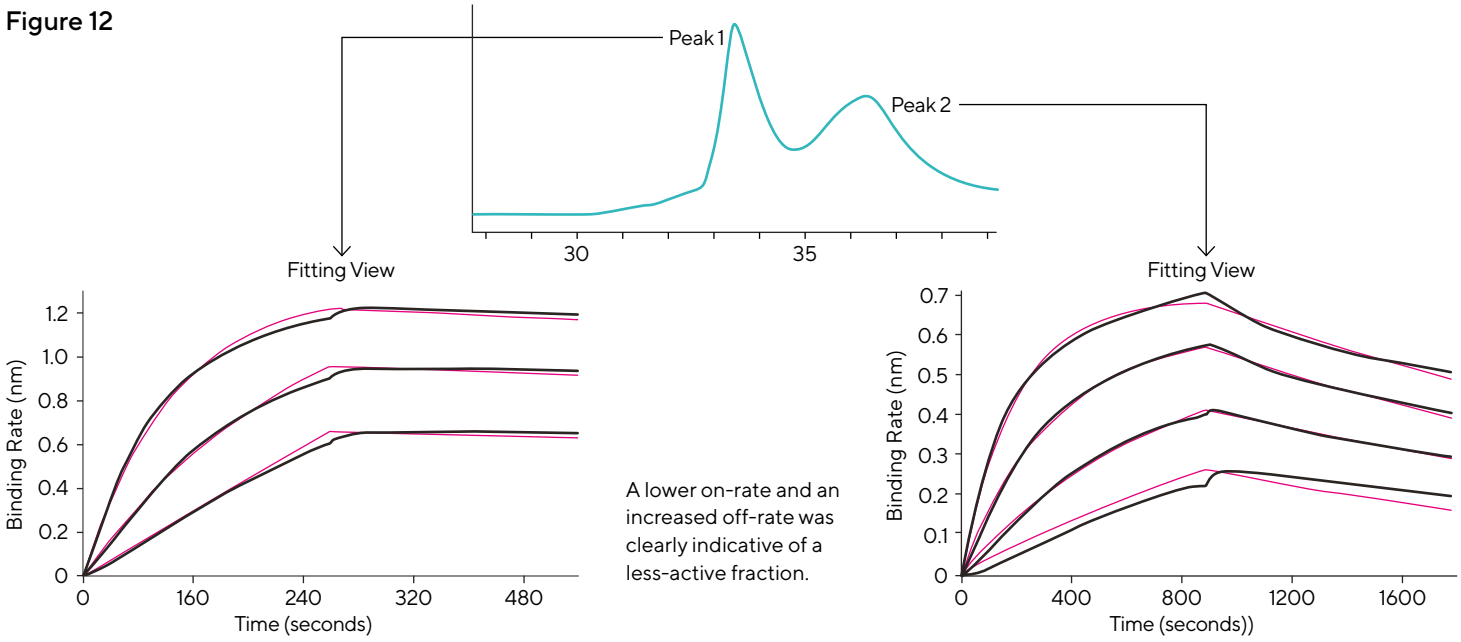
Lot Release Testing

Lot release testing is an essential step to ensure the quality and safety of the drug product. Octet® systems provide GxP users with enhanced productivity and labor efficiency for lot release and in-process testing of biologics. In addition, Octet® instruments can be equipped with 21 CFR Part 11 software for compliance with regulatory requirements. Compliance is further enhanced with the availability of instrument qualification kits including installation qualification and operational qualification (IQOQ) and performance qualification (PQ) kits that ensure the platform performs as expected.

The whitepaper Enhanced Productivity and Labor Efficiency in Lot Release and In-Process Testing of Biologics in GxP Laboratories provides examples of how Octet® systems have been used under GxP compliance for different applications and shows the relative benefits over alternate technologies.

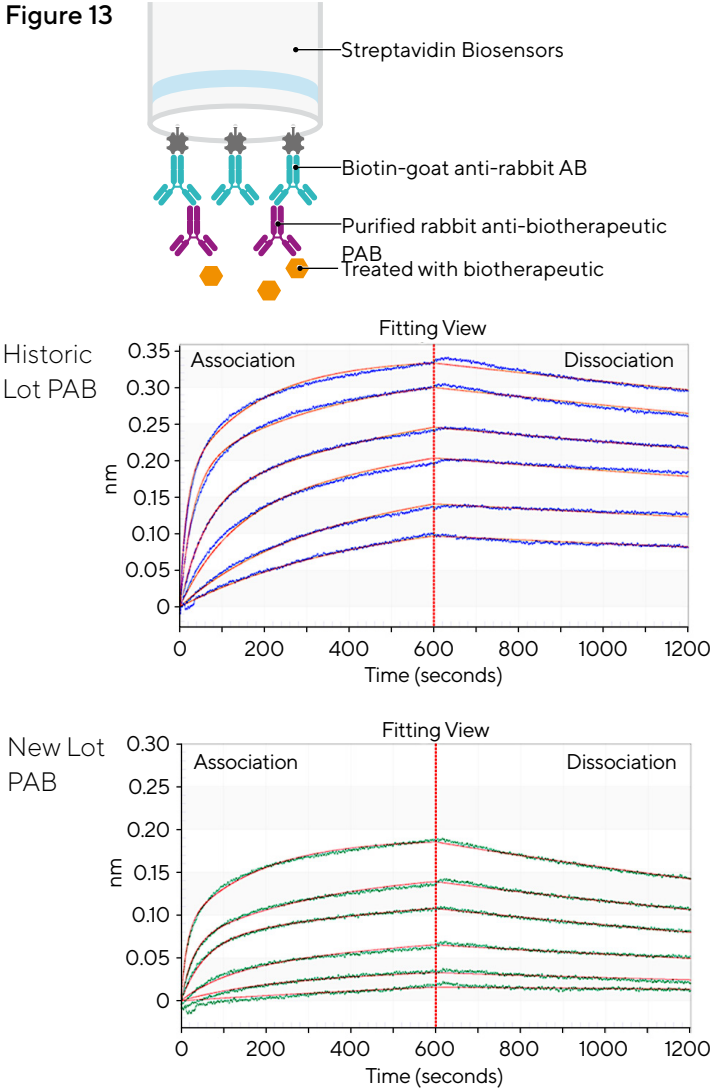
Aragen Biosciences developed an Octet® assay to compare the activity and quality of product lots and reference lots throughout their manufacturing processes. The assay, described in Enhancing Efficiency and Economics in Process Development and Manufacturing of Biotherapeutics, involved loading a biotinylated ligand on streptavidin biosensors and measuring binding interaction of the ligand with the protein analyte. The product lot contained a large second peak that was absent in the reference material; the second peak exhibited a slower on-rate and much faster off-rate, indicative of a less-active fraction (Figure 12). Activity data results provided by the Octet® system were confirmed with a cell-based assay, and Aragen Biosciences was able to modify their production conditions to significantly reduce this second peak fraction.

Figure 12



Note. Octet® binding kinetics demonstrated that Peak 1 was the active fraction. Data courtesy of Aragen Biosciences.

Figure 13

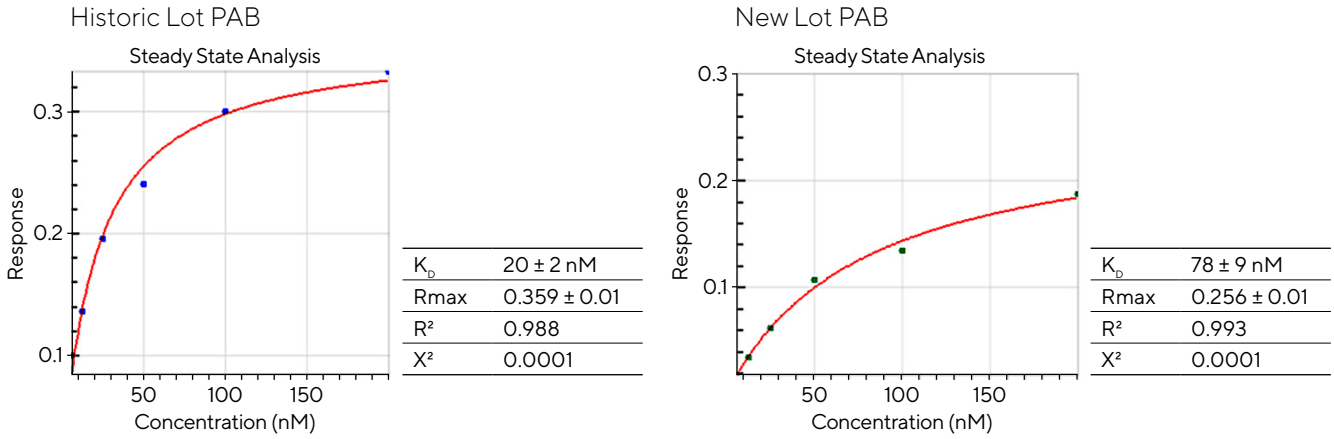


Note. Schematic of assay (A) and a comparison of two drug lots. Data is courtesy of B2S Life Sciences, Franklin, Indiana.

The Octet® platform was used by B2S Life Sciences to support generation of well-characterized anti-biotherapeutic antibodies for a ligand binding assay. In a presentation entitled "Utilizing Octet BLI Platform to Characterize Large Molecule Critical Reagents in Support of Drug Development for Contemporary Biotherapeutics", a case study is shown where an anti-biotherapeutic polyclonal antibody is generated, purified and characterized using the Octet and the MSD platforms as orthogonal techniques. The Octet platform is used to compare the binding characteristics of the new lot of polyclonal antibody against a historic lot. Streptavidin biosensors are used to capture biotinylated goat anti-rabbit antibody followed by sensors dip into the different lots of the rabbit-anti-biotherapeutic antibodies. The assay is completed with a titration of the biotherapeutic the with association and dissociation curves (Figure 13) fitted to derive affinity constants (K_D) that are then used to compare the quality of the new lot against the historic lots.

It was determined that the historic lot of positive control had roughly a 3-fold higher binding affinity toward the analyte of interest (Figure 14).

Figure 14



Note. Steady state analysis was performed as the binding interactions reached equilibrium. A three-fold difference in steady state binding affinities was observed between historic and new lot polyclonal antibodies. Data is courtesy of B2S Life Sciences, Franklin, Indiana.

Conclusion

Based on the principles of label-free BLI, the Octet® platform is a game-changing analytical tool that eliminates the need for reagent labeling while allowing for real time visualization of binding data across a wide variety of assays. This combination of speed and flexibility reduces assay development time and provides unprecedented efficiencies throughout manufacturing and quality control workflows.

Application Note (click to view document)	Binding Kinetics	Concentration and Titer	Impurity Testing	Validation Studies	Activity Assays	Aggregation and Protein Stability	Lot Release
Biomolecular Binding Kinetic Assays on the Octet® Platform	■						
Enhancing Efficiency and Economics in Process Development and Manufacturing of Biotherapeutics		■		■			■
CHO Host Cell Protein Detection			■				
Residual Protein A Detection Kit			■				
Octet® Potency Assay: Development, Qualification and Validation Strategies				■	■		
Validated Quantitation and Activity Assay of Antibody Fragment Molecule (Fab) for Process Development and Quality Control				■	■	■	■
Octet® Systems: Modernize Biopharmaceutical QC Testing to Increase Efficiency					■		■
A Fast and High Precision Influenza Vaccine Potency Assay					■		
Expanding Octet® Applications in Downstream Biologics Characterization: Stability, Formulations, and Aggregation Studies						■	
Enhanced Productivity and Labor Efficiency in Lot Release and In-Process Testing of Biologics							■

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2. Sangeeta B. Joshi, Reza Esfandiary, Robert Stadelman, Steven M. Bishop, C.R. Middaugh, Mark T. Fisher, David B. Volkin, *J Pharm Sci. 2018 107*(2):559-570. doi: 10.1016/j.xphs.2017.10.010.

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Octet-Manufacturing-App-Note-en-L-4060A-Sartorius Status: 03 | 2022