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SPECIAL REPORT

Accelerating the Development and Manufacture of Therapeutics Using the Octet Platform

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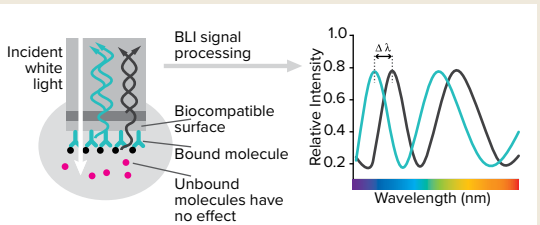
David Apiyo

Identifying a promising biotherapeutic candidate, developing it for a preclinical program, and designing a robust manufacturing process all require complex, multistep workflows. The steps include the determination of titers and cell-line expression levels, assessment of the kinetics of target molecule binding, determination of a therapeutic's potency, and confirmation of critical quality attributes (CQAs). Traditional analytical techniques that measure protein quality and quantity include UV spectroscopy, enzyme-linked immunosorbent assays (ELISAs), and high-performance liquid chromatography (HPLC). To overcome their limitations and increase analytical efficiency, advanced, label-free biosensor-based technologies such as biolayer interferometry (BLI) are being used increasingly. The platform is a fast, robust, and fluidics-free alternative for bioprocessing-based applications. Below, I provide an in-depth exploration of Octet BLI and the benefits it offers in upstream and downstream bioprocessing and in the development of viral vectors and vaccines.

The Principle of BLI

BLI is an optical technology that measures the changes in interference patterns between light waves (**1**). It measures light interference originating from the tip of the 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 interferes 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 (e.g., the number of molecules bound to the biosensor surface). That spectral shift is monitored at the detector and reported on a sensorgram as a change in wavelength (nanometer shift). Monitoring an interference pattern (e.g., spectral shift) provides real-time binding profiles of molecular interactions.

Figure 1: Octet systems with biolayer interferometry (BLI) technology measures the difference in the wavelength of reflected light (Δλ) between two surfaces on the biosensor.



Only molecules binding to or dissociating from a BLI biosensor can cause a signal change. This is a unique characteristic of the technology, and with its fluidic-free design, It enables direct measurements in crude samples. All biosensor tips are coated with a biocompatible matrix that minimizes nonspecific binding and provides a uniform and nondenaturing surface for biomolecules. The biosensor with immobilized ligand moves over a 96- or 384-well plate and is dipped into a sample. Elimination of complex fluidic pathways allows for the monitoring of molecular binding interactions directly in complex matrices and in crude samples.

The design of Octet BLI systems enables users to

- acquire real-time binding kinetics data to measure the rate of association (k_a), rate of dissociation (k_d), and affinity constants (K_D)
- generate 1–96 data curves simultaneously with fully automated assays
- 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 viruses
- recover precious or low-availability samples (because binding reagents are not added directly to the sample and materials are minimally consumed).

Figure 2: Applications of biolayer interferometry

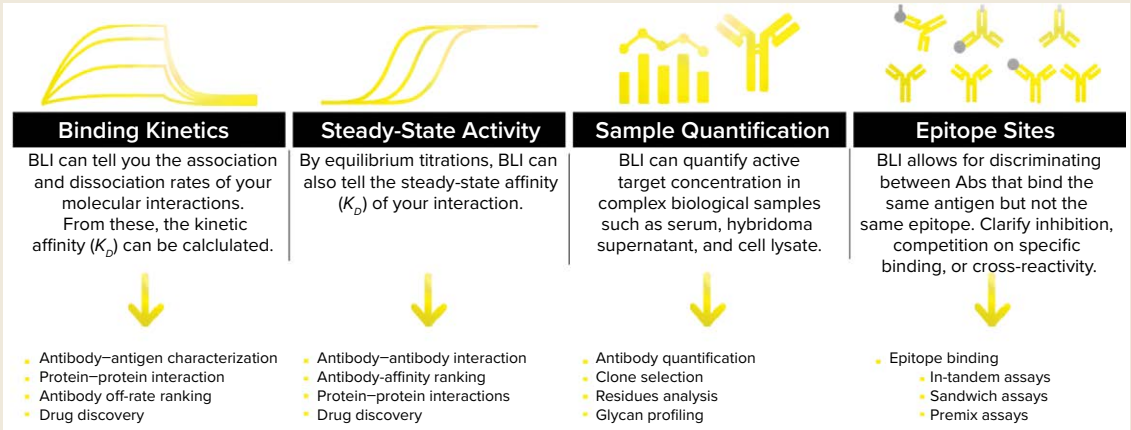
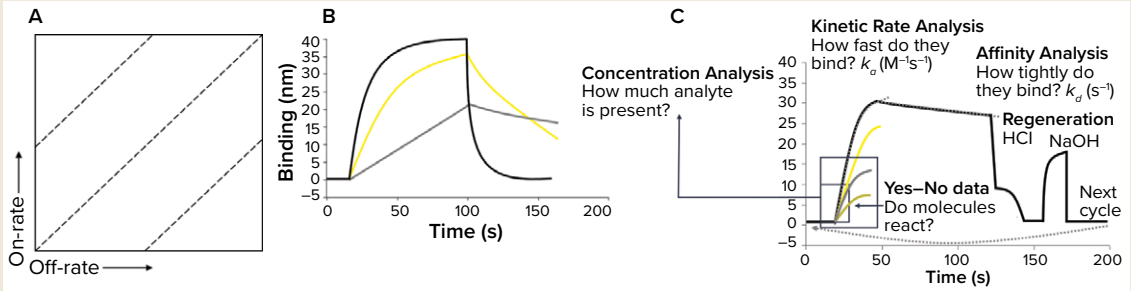


Figure 3: Comparing data provided from single-point (A) or real-time analysis (B and C) using biolayer interferometry



Used across research and development and throughout upstream and downstream workflows, BLI can be applied to a wide range of applications, including binding kinetics, steady-state affinity, quantification, and epitope binning (Figure 2). It provides answers to key questions, including the following:

- Do the molecules interact (binding specificity)?
- How tightly do the molecules bind (binding affinity)?
- How much analyte is present (concentration analysis)?
- What is the speed of interaction (binding kinetics)?

Advantages Over ELISA and HPLC

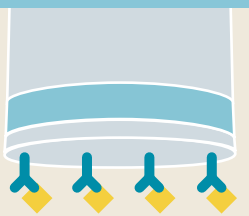
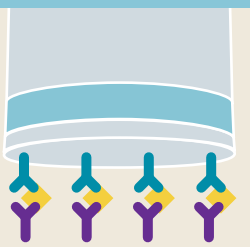
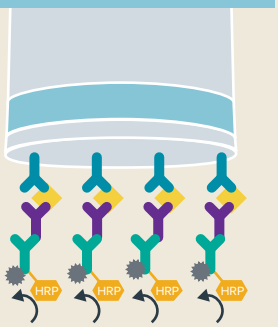
BLI offers the ability to perform real-time assays, whereas end-point assays provide only basic information (e.g., overall binding strength) and no kinetic information (Figure 3). Octet BLI platforms are an excellent alternative to assays that are currently performed using ELISA and HPLC. Octet BLI assays are label free and fully automated. Compared with ELISA and HPLC, Octet BLI assays require significantly lower user intervention and a simplified workflow. The technology provides researchers with the flexibility to choose the most efficient assay format based on assay needs such as sensitivity, dynamic range, and workflow (Table 1).

Protein quantitation can be performed faster with the Octet BLI platform than with ELISA and HPLC. Figure 4 shows the analysis times for 70 samples on an Octet R8 system and with ELISA and HPLC assays. The Octet assay requires as little as 55 minutes, including operator hands-on time, whereas ELISA and HPLC assays require at least 22 hours, including several hours of analyst involvement.

Octet BLI platform also facilitates fast kinetic analysis and the real-time data help analysts select the optimal association, dissociation, and ligand immobilization times for each experiment. That eliminates unnecessarily long or overnight incubation times and enables analysis of biomolecules that are unstable under assay conditions. Low-affinity analytes that tend to be washed away in ELISA workflows can be characterized accurately with biosensor assays.

Octet concentration assays are complemented by the platform's ability to measure functional activity. For example, monoclonal antibody (MAb) titers can be determined using biosensors coated with protein A. Functional activity can be assessed in a second assay

Table 1: Quantitation assay formats and features of the Octet platforms (HRP = horseradish peroxidase; ELISA = enzyme-linked immunosorbent assay)

Assay Features	One Step	Two Steps	Three Steps
Illustration			
Assay steps	1 Bind analyte	1 Bind analyte. 2 Bind secondary analyte.	1 Bind analyte–secondary analyte complex. 2 Bind HRP-loaded antibody. 3 Incubate in precipitating substrate for HRP.
Typical assay time	• 30 min (Octet QK, QKe, RED96) • 15 min (Octet RED384, QK384)	• 1 h 30 min (Octet QK, QKe, RED96) • 1 h 15 min (Octet RED384, QK384)	• 2 h (Octet QK, QKe, RED96) • 1 h 30 min (Octet RED384, QK384)
Typical concentration range	Low mg/mL to low pg/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 reuse of biosensor in most cases	• Two incubation steps — still fast, easy, reduces reagent expenses compared with 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 ELISAs in format — but faster and easier • Excellent sensitivity — down to low pg/mL, depending on assay • Automated and no-wash assay minimizes handling

step involving binding to a specific antigen. By contrast, HPLC and A^{280} spectroscopy determine only a sample's total protein concentration, and separate assays need to be used to measure biological activity.

Upstream Bioprocessing Applications

Optimization of upstream processes is essential for successful and cost-effective production of biotherapeutics. It requires integration of analytical technologies to facilitate clone selection, cell-line development, titer determination, and critical quality attributes (CQAs) assessment. The Octet platform and assay kits offer both intermediate- and high-throughput capabilities for those applications. Because of the system's fluidics-free design, users can implement those assays without purifying samples, providing significant time and cost savings (**2, 3**).

Concentration Measurement: A biotherapeutic molecule's concentration in a sample is determined by a direct binding or by a sandwich assay on the Octet platform. In a typical quantitation assay, a standard curve is generated, and unknown sample concentrations are interpolated. Concentration can be calculated from the interaction's initial binding rate, which is based on the initial slope or from the binding rate at equilibrium.

Titer Determination and Clone Selection: In clone selection, thousands of hybridoma or phage clones are screened, and titer measurements are used to select high-producing clones. The Octet platform can analyze up to 96 samples in two minutes using biosensors precoated with protein A, protein G, or other antibody-binding proteins (**3**).

The platform also can be used in glycan screening with the immobilization of sugar-specific lectins onto the biosensor's surface. Glycosylation profiling in combination with titer determination can identify not only high-producing clones, but also those

Figure 4: Comparing protein quantitation in complex matrices using Octet systems and high-performance liquid chromatography (HPLC) or an enzyme-linked immunosorbent assay (ELISA)

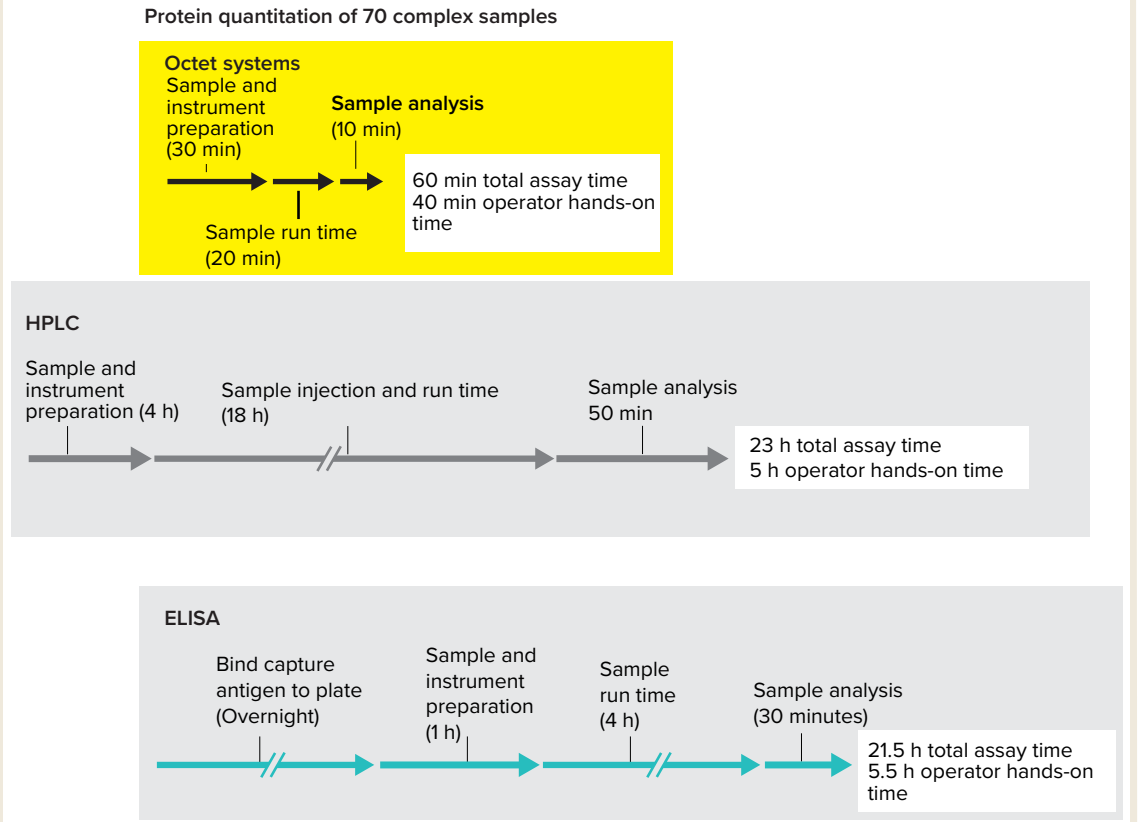
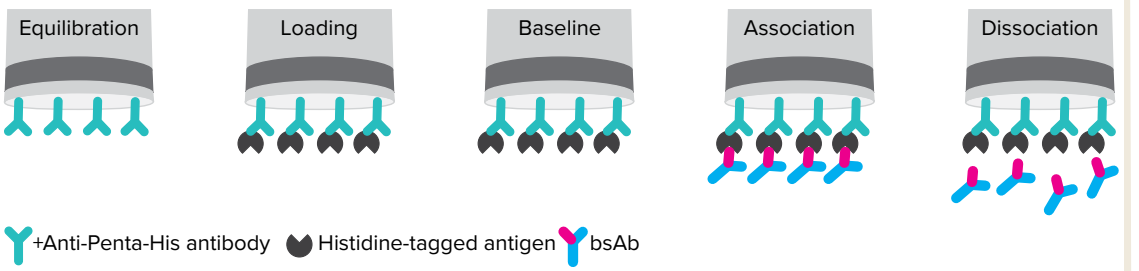


Figure 5: Bispecific antibody (bsAb) quantitative assay workflow

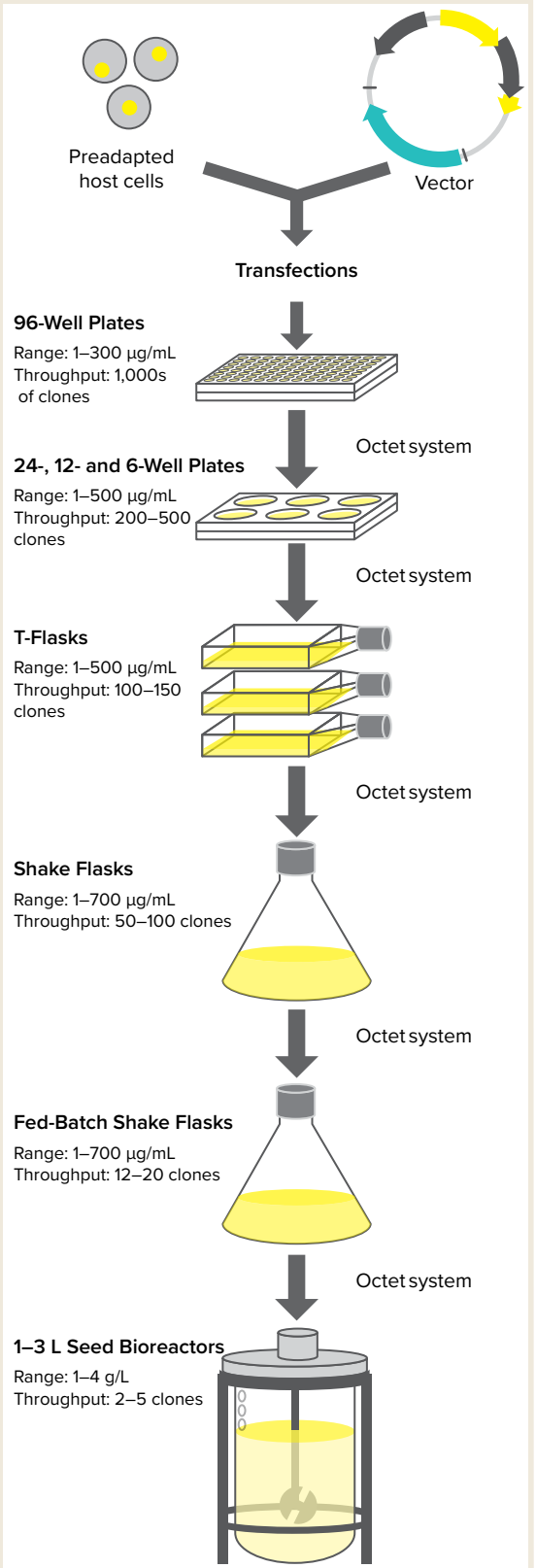


expressing the protein with the correct glycan profile. That determination can be carried out as early as subcloning stage to advance only high-quality candidates downstream, thereby minimizing the number of late-stage failures.

With the Octet platform, researchers can identify pools and clones of bispecific antibodies (bsAbs). Traditional technologies for characterizing antibodies are time sensitive and limited in their ability to provide quantitative functional assessment of two interactions to one bispecific molecule (**4**). Figure 5 shows the simple design for a high-throughput bsAb quantitative assay on the Octet platform used at Celgene. The assay consists of protein A and Anti-Penta-HIS (His1K) biosensors (from Sartorius). A bsAb is captured sequentially by each antigen-binding site region using a correspondent antigen loaded onto the biosensor surface. This approach offers an easy screening method and a workflow that assesses bsAb interactions in a versatile, label-free, and easy-to-use format.

By analyzing specific binding results with IgG titer in a single platform, researchers can assess the binding activity of bsAbs to both antigens to rank the top pools and clones during cell-line

Figure 6: Protein titer assessment and growth media optimization using the Octet platform at different stages of cell-line development



development. Results obtained from the Octet binding assay have been validated by comparing results from other analytical methods such as reversed-phase HPLC (RP-HPLC), capillary electrophoresis sodium dodecyl sulfate (CE-SDS), surface plasmon resonance (SPR), and liquid chromatography tandem mass spectrometry (LC-MS) to show that binding results can be correlated to the purity of a bsAb.

Cell-Line Development: Harvest samples can be screened on the Octet platform to select high-expressing clones across an entire scale-up workflow (Figure 6) (**3**). Octet assays also can be used to determine protein levels during media development by comparing protein-secretion levels following variations in feeding regimes, strategies, and concentrations. Data acquisition and analysis can be performed rapidly for hundreds of samples, eliminating traditional processing bottlenecks (**3**).

Downstream Bioprocessing Applications

Using the Octet platform, researchers quickly can determine the impact of multiple process variables during downstream processing and identify optimal conditions (Figure 7). Preconfigured reagents and protocols are available for rapid quantitation of protein products, host-cell proteins (HCPs), and residual protein A levels.

Dynamic Binding Capacity (DBC) Determination: A key step in optimizing an affinity chromatography step is to determine a column's DBC, defined as the amount of protein that will bind to a column resin under a specified condition (**3**). DBC is determined by continuously loading a sample containing a known concentration of target protein and monitoring that protein in flow-through fractions. Determination of DBC using HPLC or A^{280} spectroscopy is hampered by the presence of large amounts of HCP in the flow-through. Detection of the protein of interest among contaminants is straightforward with the Octet platform because Octet systems are compatible with crude samples, and it reduces the time required to optimize purification conditions (Figure 8) (**5**). Optimization of binding, wash, and elution conditions also can be accelerated. Different buffer compositions, salt, pH, operating temperatures, and sample-injection volumes can be screened rapidly to determine their effects on product titer and quality.

Residual Contaminant Detection: Contaminants such as HCPs can elute with a target drug product during purification. BLI technology offers throughput comparable with that of manual ELISA for

Figure 7: The Octet platform can be used throughout the downstream workflow.

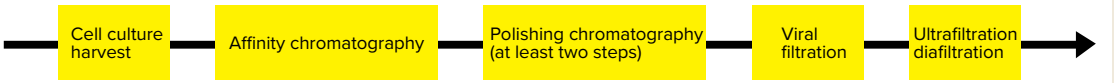


Figure 8: Comparing high-performance liquid chromatography (HPLC) and the Octet platform for determination of dynamic binding capacity (DBC)

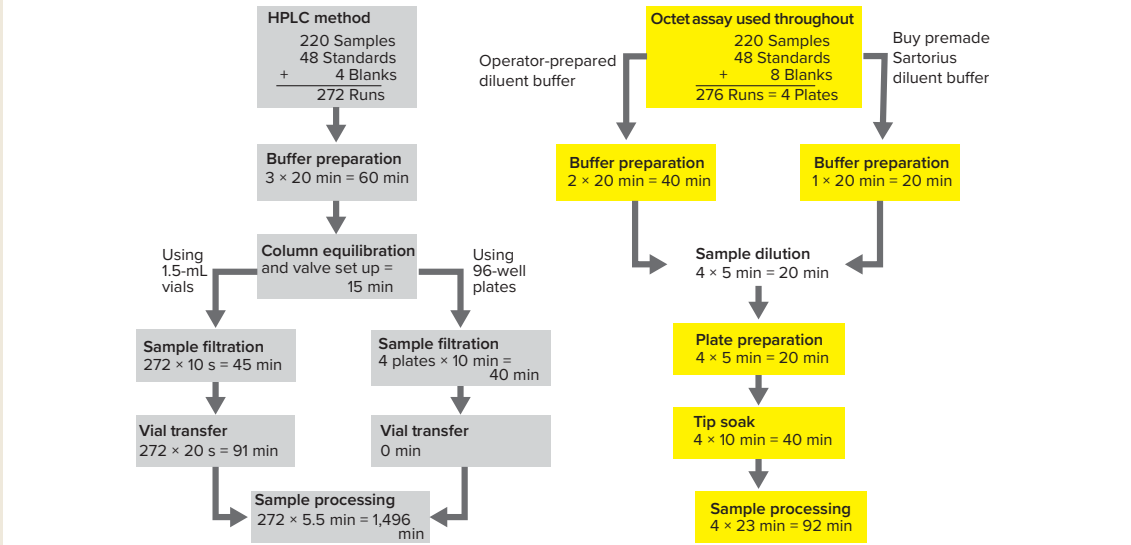
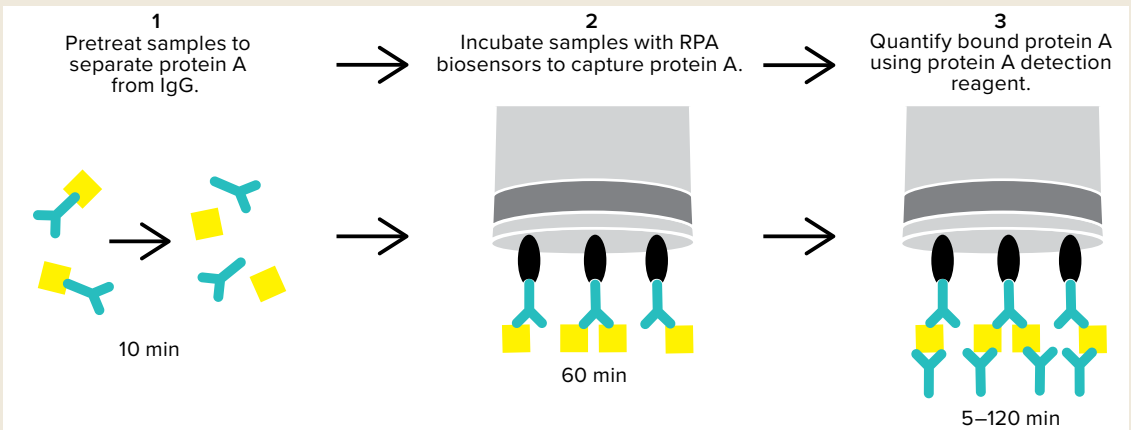


Figure 9: Accelerated residual protein A workflow on the Octet platform

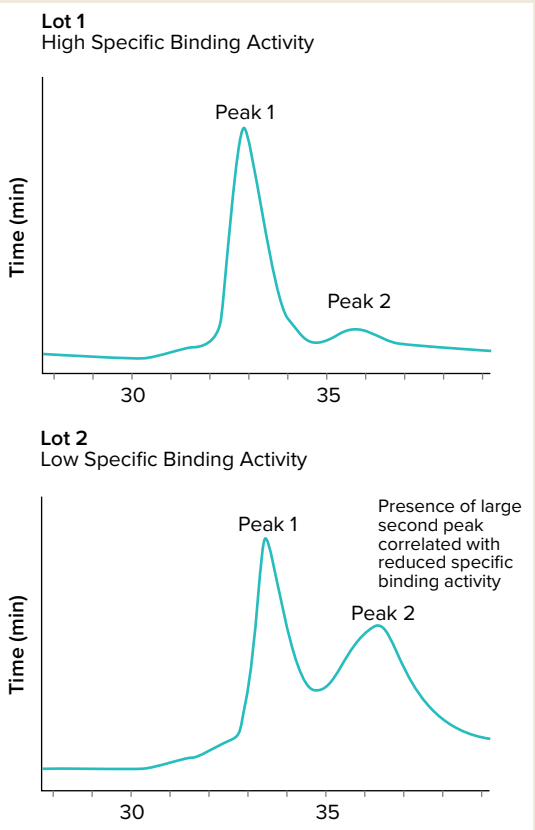


contaminant detection but with better precision. The platform also provides data in real time, thus enabling rapid assay optimization.

The analytical laboratory at GlaxoSmithKline incorporated an HCP assay on the Octet system. The assay required minimal analyst intervention and provided more accurate and precise results than a manual ELISA assay did. Hands-on time for preparing and processing assay plates was reduced to 30 minutes (rather than the 2.5 hours needed previously), and antibody consumption decreased by 40% (**3**).

Protein A leached from purification columns can reduce drug efficacy, so it must be detected and cleared. The Octet residual protein A detection kit has a highly simplified workflow compared with traditional methods that require heat denaturation and centrifugation steps (Figure 9). Using the Octet platform, 96 samples can be analyzed in less than two hours (**3**).

Figure 10: High-performance liquid chromatography (HPLC) spectra of Lot 1 and Lot 2 of a drug molecule



Formulation development is a key step in downstream bioprocessing because production and storage media play critical roles in the activity and stability of biotherapeutics. Unlike traditional methods such as dynamic light scattering (DLS) and circular dichroism (CD) that mainly measure stability parameters such as aggregation or melting temperatures, the Octet platform can evaluate both stability and functional characteristics of biological molecules (**6**).

An automated method using biotinylated GroEL-streptavidin biosensors can detect the formation of transient preaggregate species in MAb samples. The Octet platform helps users rapidly evaluate the effect of oxidation on the functional activity of Herceptin through the binding characterization of the drug product against a neonatal Fc receptor (FcRn) molecule. The drug product is incubated in hydrogen peroxide in the sample plate in a time-staggered way, then the drug activity is analyzed at different time points (**7**).

Quality Control (QC): The Octet platform enables robust and highly reproducible assays for protein concentration and functional activity. The technology is designed for good laboratory/manufacturing practice (GLP/GMP) environments, and the system provides 21 CFR Part 11 compliance tools. Octet BLI-based protein activity and kinetic assays can support in-process testing, drug potency, lot-to-lot variability, and stability studies (**8**).

Typically, an activity assay is used during process development, QC, and manufacturing to compare different lots of a drug molecule and its stability. Activity assays are critical because they differentiate active protein from inactive or clipped variants, which do not bind the ligand. Active protein concentration can be determined using a binding assay, immobilizing a ligand against a target analyte onto a biosensor, and then measuring the binding interaction with the analyte.

Aragen Biosciences developed an Octet assay to compare the activity and quality of a product lot (Lot 2) with a reference lot (Lot 1) throughout their bioprocess and manufacturing processes (**3**). The assay involved loading a biotinylated ligand on streptavidin biosensors and measuring binding interaction of the ligand with the protein analyte. As Figure 10 shows, Lot 2 contained a large second peak that was absent in the Lot 1 reference material. The second peak in Lot 2 exhibited a slower on-rate and much faster off-rate, indicative of a less-active fraction (Figure 11). Octet system activity data results were confirmed with a cell-based assay, and Aragen was able to modify its production conditions to reduce this second peak fraction significantly.

Potency Determination: Ligand-binding kinetics assays can be used as batch lot-release methods. In these studies, the interaction is assessed usually by measuring either the affinity of an analyte–drug product to a receptor or ligand immobilized on the biosensor surface or by monitoring drug-product binding response signals as a function of concentration and comparing them to a control for relative potency assessment (**9**).

Ready-to-use nickel–nitrilotriacetic acid (Ni-NTA) and anti-human Fab-CH1 (FAB2G) biosensors for immobilization of different panels of Fc gamma receptors can used to bind drug products for potency assessment (**8**). An overlay of replicate data from the binding of an FcRn molecule to an IgG shows excellent reproducibility (Figure 12).

Stability Studies: Octet platforms can be leveraged for measuring and distinguishing fully functional drug products from those drug products with binding activities that have been affected by degradation. These systems also can distinguish between native and deactivated antigen (**8**). The affinity of an IgG1

Figure 11: The Octet binding kinetics and functional assay demonstrated that peak 1 was the active fraction.

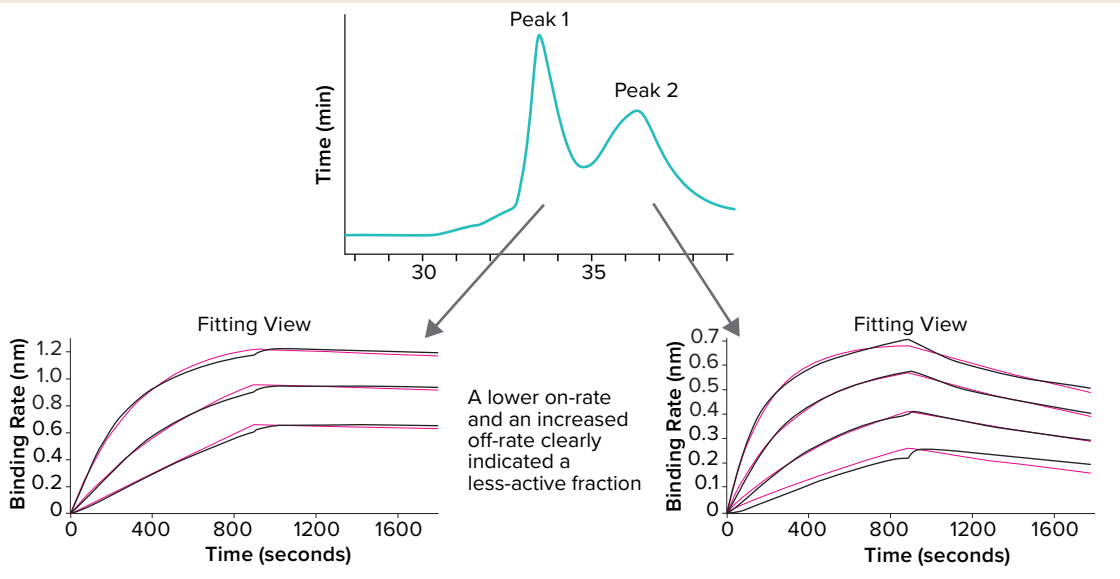


Figure 12: Overlay of several replicates of FcRn–IgG interactions on the Octet platform using FAB2G biosensors

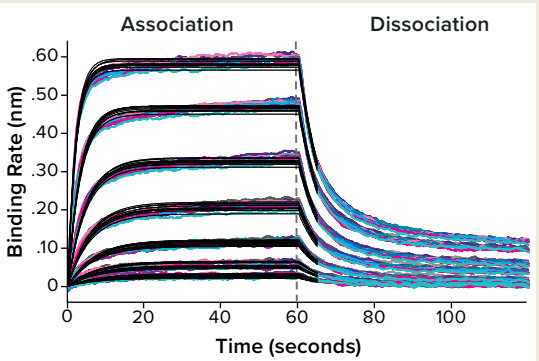
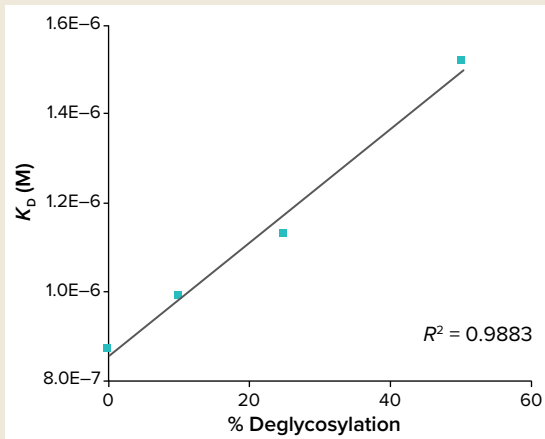


Figure 13: Affinity analysis of the binding of an IgG1 to an Fc gamma receptor as a function of deglycosylation



to an Fc gamma receptor IIIa molecule has been shown to decrease with increasing percent deglycosylation, further indicating that Octet systems are suitable for use in developing stability-indicating methods (Figure 13) (**8**).

Viral Vector Bioprocessing Applications

Analysts use several techniques to quantitate adenoassociated virus (AAV) particles. Typically, viral capsid titer is measured by ELISA, empty–full capsid titer ratios are obtained by analytical ultracentrifugation (AUC), and viral genome titer often is measured with Droplet Digital polymerase chain reaction (ddPCR) technology. These methods generally are time consuming, labor-intensive, and impractical for at-line, rapid measurement of viral titer during production.

The Octet at-line, high-throughput, one-step AAV assay can be used to quantitate different serotypes using a biotinylated capture molecule (**10**). The versatility of this assay is based on an antibody pair, with one antibody recognizing a broad spectrum of AAV serotypes and the other antibody being serotype specific. The assay has a greater quantification range than a commercially available capsid ELISA kits have, and the Octet AAV assay can test hundreds of process samples with minimal matrix interference. Figure 14 shows that the assay reduces time to results by 80%, operator hands-on time by 50%, and sample preparation time by 15–25% (**11**).

Figure 14: Comparing process steps and time required for an Octet adenoassociated virus (AAV) assay and enzyme-linked immunosorbent assay (ELISA) (HRP = horseradish peroxidase , Strep = streptavidin, TMB = 3,3′,5,5′-Tetramethylbenzidine

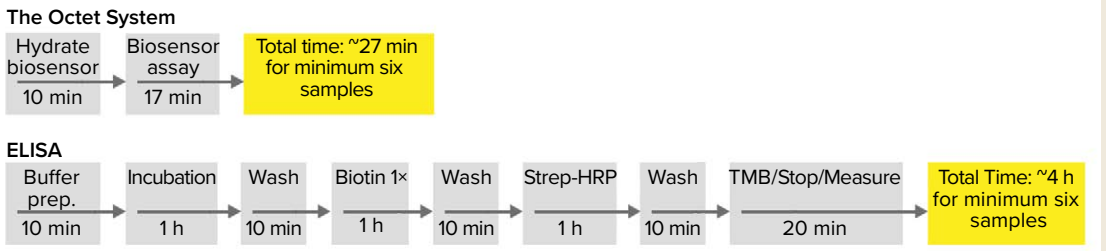
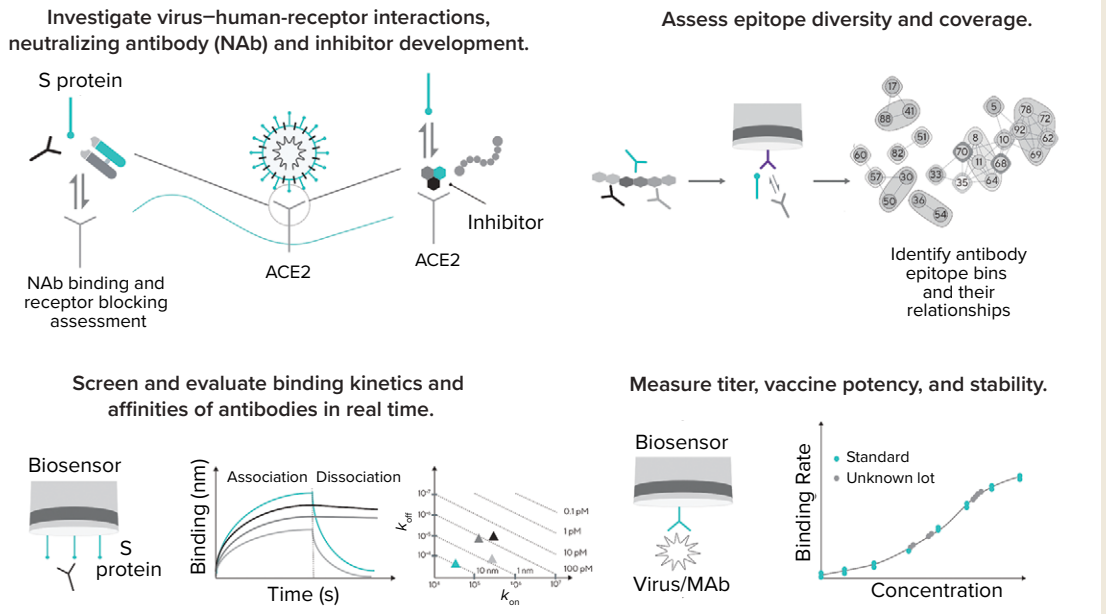


Figure 15: The Octet platform offers a comprehensive set of applications for COVID-19 vaccine and therapeutic research and production. (RBD = receptor-binding domain, ACE 2 = angiotensin-converting enzyme 2)



Vaccine Bioprocessing Applications

The Octet platform is well suited to meet the critical measurement, speed, and reliability needs of vaccine development. Its benefits include the following:

- Binding kinetics and affinities can be evaluated in real time, accelerating selection of lead candidates with preferred properties.
- Epitope diversity and coverage can be assessed easily.
- Titer, potency, and stability measurements can be performed at-line within minutes.

SARS-CoV-2 Vaccine and Therapeutics Development: The Octet platform has been an integral part of strategies to accelerate development of both SARS-CoV-2 vaccines and therapeutics (**12, 13**). Applications include assessment and determination of structural biology, host-receptor recognition properties, affinity characterization, cross-reactivity with SARS-CoV, and MERS-CoV neutralization antibodies (NAbs). The platform also can be used in workflows for developing NAbs (Figure 15).

Influenza Vaccine Potency Assay: Fast and accurate determination of vaccine titer during influenza vaccine manufacture is important for understanding process performance and scale-up. Traditional assays such as single radial immunodiffusion (SRID) and ELISA have their limitations. Although SRID is considered to be the gold standard, it requires highly skilled operators to obtain reproducible results, and it is relatively low throughput. ELISA exhibits low precision and narrow dynamic range.

By contrast, the Octet platform delivers higher accuracy and reproducibility. The assay is based on binding a vaccine to polyclonal antibodies that recognize the influenza epitopes presented by the

vaccine (**14**). The polyclonal antibody is bound to a protein-G– or protein-A–derivatized biosensor. That configuration increases the biosensor flexibility by enabling rapid changes in vaccines derived from different viral strains by simply binding the paired antibody for the new strain to a biosensor — without the need for derivatization. Thus, the assay is suitable for detecting rapid changes in viral strains represented in a vaccine. The Octet assay is applicable to both attenuated and synthetic vaccines, and it can be used for vaccine potency assessment and in different process stages.

Nanoparticle Vaccine Constructs: Antigenic presentation by nanoparticle vaccine constructs depends on several physiochemical properties of the nanoparticles. Those include size, charge, surface chemistry, and composition. Given that variability, methods and assays are needed to optimize such variables for constructing ideal immunogenic antigen presentations. Using the Octet platform, researchers can screen and identify nanoparticle physical properties, evaluate antigenic profiles and antigen stability, and measure humoral responses in vaccine development workflows (**15**).

Dengue Vaccine Avidity Assay: The Octet platform serves as the foundation of a novel dengue virus avidity assay developed by Takeda (**16**). The assay was used to evaluate evolution of polyclonal serum antibody avidity following vaccination with a tetravalent dengue vaccine candidate in two phase 2 trials. This method is expected to facilitate a deeper understanding of the mechanisms of induction of immunity to dengue infection and vaccination, including the relationship betwen affinity matured antibodies and protection against dengue infection.

A Platform for All Bioprocess Steps

The high costs of discovery, development, and manufacture of therapeutics requires improved process efficiencies and economics. Analytical tools that eliminate the need for reagent labeling and enable real-time data visualization save development time and improve efficiencies during process development. The Octet platform and assays can be used throughout process development and manufacturing, including cell-line development, clone selection, and DBC determination for affinity purification columns. The ability of the BLI technology to monitor binding interactions in real time, coupled with its capability to analyze samples in their crude matrix and in high throughput, can help shorten development and analysis times dramatically leading to significant cost savings and process economy.

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David Apiyo, *PhD, is senior manager applications at Sartorius, david.apiyo@sartorius.com.*

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