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Unveiling Hidden Insights: Enhanced Analytics with New Heights of Sensitivity

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Abstract

Biolayer interferometry (BLI) is a widely used technique for real-time, label-free kinetic measurement of biomolecular interactions. However, existing instruments often lack the sensitivity needed to analyze small molecules or low-analyte concentrations. Additionally, there is increasing demand to improve workflow efficiencies and reduce the cost per sample. This document presents compelling data on how the Octet® R8e system effectively addresses these challenges with 384-well microplate compatibility, minimal sample volumes, and increased sensitivity. These improvements are crucial for detecting low-concentration samples and reducing sample usage while expanding the analyte range to small molecules. With a new sample evaporation control for extended experimental run time, the Octet® R8e streamlines workflows and reduces costs at every stage of biopharmaceutical research, from drug discovery to drug development, bioprocessing, and quality control.

Introduction

Label-Free, Real-Time Analysis: A Critical Tool for Biotherapeutic Discovery

Direct measurement of biomolecular interactions is crucial in biotherapeutic drug discovery and development. Label-free technologies offer precise insights into biomolecular complex formation and stability, which are essential for understanding drug-target interactions. Interaction affinity influences the effective dose of a biopharmaceutical, impacting therapeutic efficacy and desirability.

Real-time data on binding specificity, affinity, and kinetics significantly enhance every stage of biopharmaceutical development, from initial discovery to manufacturing. Early identification of promising therapeutic candidates can save both time and resources, reducing the risk of late-stage failures. Detailed characterization of specificity, selectivity, stability, and binding rates under various conditions is essential for making informed process decisions. Although kinetic analysis using label-free technology is invaluable, it presents challenges in producing consistent, high-quality kinetic binding profiles from biological samples.

Real-time, label-free analysis offers rapid, sensitive, and accurate measurements of kinetics, affinity, and activity of complex formations, avoiding artifacts common in traditional methods. Unlike standard endpoint assays, Octet® BLI systems provide detailed insights into molecular interaction mechanisms. While equilibrium binding assays determine affinity constants, they lack binding rate information.

Real-time kinetic analysis reveals complex interactions, allowing early elimination of suboptimal candidates. Additionally, it measures binding affinities up to the millimolar range, unlike ELISA or immunoprecipitation assays, which may lose weaker binders during washing steps.

BLI Technology Principles

Biolayer interferometry (BLI) is an optical analytical technique that measures interference patterns between light waves. It involves directing light down a fiber-optic biosensor towards two interfaces at the tip: a biocompatible layer on the surface and an internal reference layer (Figure 1). Light reflects from these layers, and the resulting interference pattern is detected by a CCD array detector. When a biosensor tip coated with a ligand is immersed in a sample containing the target molecule, the molecules bind to the coated surface, forming a molecular layer. As more molecules bind, the layer's thickness increases, altering the effective distance between the reflective layers and causing a shift in the interference pattern. This spectral shift, indicative of the optical thickness of the molecular layer, is monitored and reported as a wavelength change (nm shift) on a sensorgram. By observing these interference patterns in real time, BLI provides kinetic data on molecular interactions, offering insights into binding events.

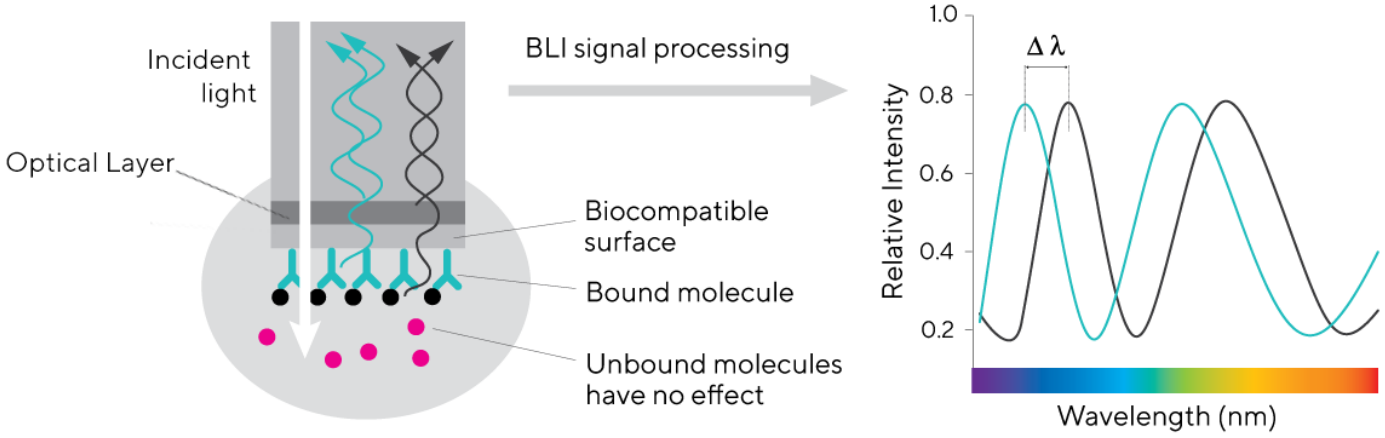


Figure 1: Octet® BLI assays utilize dip and read biosensors, which contain two optical interfaces at the biosensor tip, the internal reference layer (optical layer) and the surface biocompatible matrix on which ligand molecules are immobilized. BLI is an optical analytical technique that analyzes the interference pattern of light reflected from two surfaces. Changes in the number of molecules bound to the biosensor causes a shift in the interference pattern that is measured in real time.

Introducing the Octet® R8e — Enhanced Analytics with New Heights of Sensitivity

The Octet® R8e system is an enhanced version of the well-known 8-channel Octet® R8, offering the most advanced detection capabilities in BLI. Its greater sensitivity expands the analyte range to include small compounds as well as large biological molecules, even in low concentrations. Additionally, faster data acquisition speed allows for the capture of more data points, which is necessary for studying fast kinetic interactions.

The availability and cost of samples are critical factors throughout all phases of drug discovery. While existing 8-channel Octet® instruments are only compatible with 96-well microplates, the Octet® R8e instrument allows users to choose between 96-well or 384-well sample microplates, reducing sample use and enabling highly parallel processing in volumes as low as 40 µL.

Octet® BLI platforms use a fluidic-free Dip and Read format that offers several advantages over other label-free technologies. The measurement is non-destructive and samples therefore can be recovered once the assay is complete.

To increase data generation capabilities, many labs conduct long-term experiments, which offer a more comprehensive understanding of slow or complex kinetics. A notable feature of the system is its new evaporation cover, which reliably maintains sample concentration for up to 16 hours. This ensures sample integrity, resulting in accurate data generation during extended operations.

The Octet® R8e is designed for use in all laboratory environments and offers extensive product support for system performance validation, qualification services and is 21 CFR Part 11-compliant for easy implementation in GxP environments.

This document presents compelling data highlighting the groundbreaking advantages of the Octet® R8e system for applications demanding maximum sensitivity and optimal sample integrity during long experiments. Conveniently, using the same catalog of biosensors across the portfolio ensures seamless transfer of methods developed on other Octet® BLI systems to the Octet® R8e, without compromising data integrity.

Octet® R8e Performance Enhancement Features Include:

Best-in-Class Sensitivity
Improved signal-to-noise ratio for the precise kinetics characterization of low-molecular weight analytes, such as compounds and small proteins, and for the detection of low-concentration analytes.

High-Speed Data for Precise Kinetic Insights
Accurately capture fast kinetics with the enhanced data acquisition feature.

Evaporation Control
Boost accuracy and precision in kinetics, affinity, and quantitation assays by maintaining analyte concentrations over time (16 hours) with enhanced evaporation control capabilities.

16 Hours of Walk-Away Time
Maximize system usage and lab efficiency with hands-free operation for 16 hours.

384-Well Plate Compatibility
Supports high-throughput workflows, increasing sample capacity and maximizing data output.

Reduced Sample Volume
Achieve reliable results with as little as 40 µL of analyte, conserving precious samples and cutting reagent costs.

Increased Sensitivity — Quantification of Even Lower Analyte Concentrations

Sensitivity plays a crucial role in kinetics, affinity, and quantitation assays. High sensitivity allows for the accurate detection of low concentrations of analyte and ensures that kinetic or quantitation parameters can be determined for even the lowest ligand and analyte concentrations, which is vital for determining accurate affinities or concentrations of an interaction.

Quantitation assays on the Octet® BLI platform are similar 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. The signal measured in the assay can be directly or inversely proportional to the amount of bound analyte.

Improved signal-to-noise ratios on the Octet® R8e provides enhanced sensitivity compared to previous systems, providing a broader dynamic range but also decreasing the limit of detection (LOD) and the limit of quantitation (LOQ). Parameters such as LOD and LOQ are essential in assay validation, ensuring the assay's ability to detect and quantify analytes at low concentrations, which is vital for regulatory compliance and scientific credibility. In clinical and research contexts, understanding these limits aids in making informed decisions regarding analyte presence and concentration, influencing diagnosis, treatment, and research outcomes

As shown in Figure 2, Human IgG standards of known concentration binding to ProA Biosensors (Item No.: 18-5010) reveal that analyte concentrations as low as 3.9 ng/mL (26 pM) are easily assessed on the Octet® R8e with a single step quantitation assay extending the limit of quantitation.

When assessed using binding report point analysis, the Octet® R8e shows improved precision (percentage CV (%CV)) between all replicates at all time points. Meaning that quantitation assays can be performed in shorter time periods. When average binding responses are calculated for low analyte concentrations, the response levels stay above the LOD and LOQ for longer periods. This increased resolution is highlighted by the 3.9 ng/mL sample, which is discernable above the LOD (Baseline Average + 3SD) from 25 seconds on the Octet® R8e but from only 100 seconds on the Octet® R8.

Increased resolution in data collection plays an important part in accurately identifying sample concentrations, and all averaged sample concentrations exhibit a decrease in percentage CV relative to the Octet® R8 BLI system. This allows best-in-class sensitivity with an assay timeframe that was previously unattainable.

Improved sensitivity enhances the reliability and applicability of Octet® R8e assays in research, diagnostics, and various industrial applications. Therefore, the Octet® R8e system is well placed for upstream drug development where the detection of low analyte concentrations is essential in many biological and chemical processes. This saves time and reduces costs, as smaller batches can be produced for assessment and data-driven decisions can be made earlier.

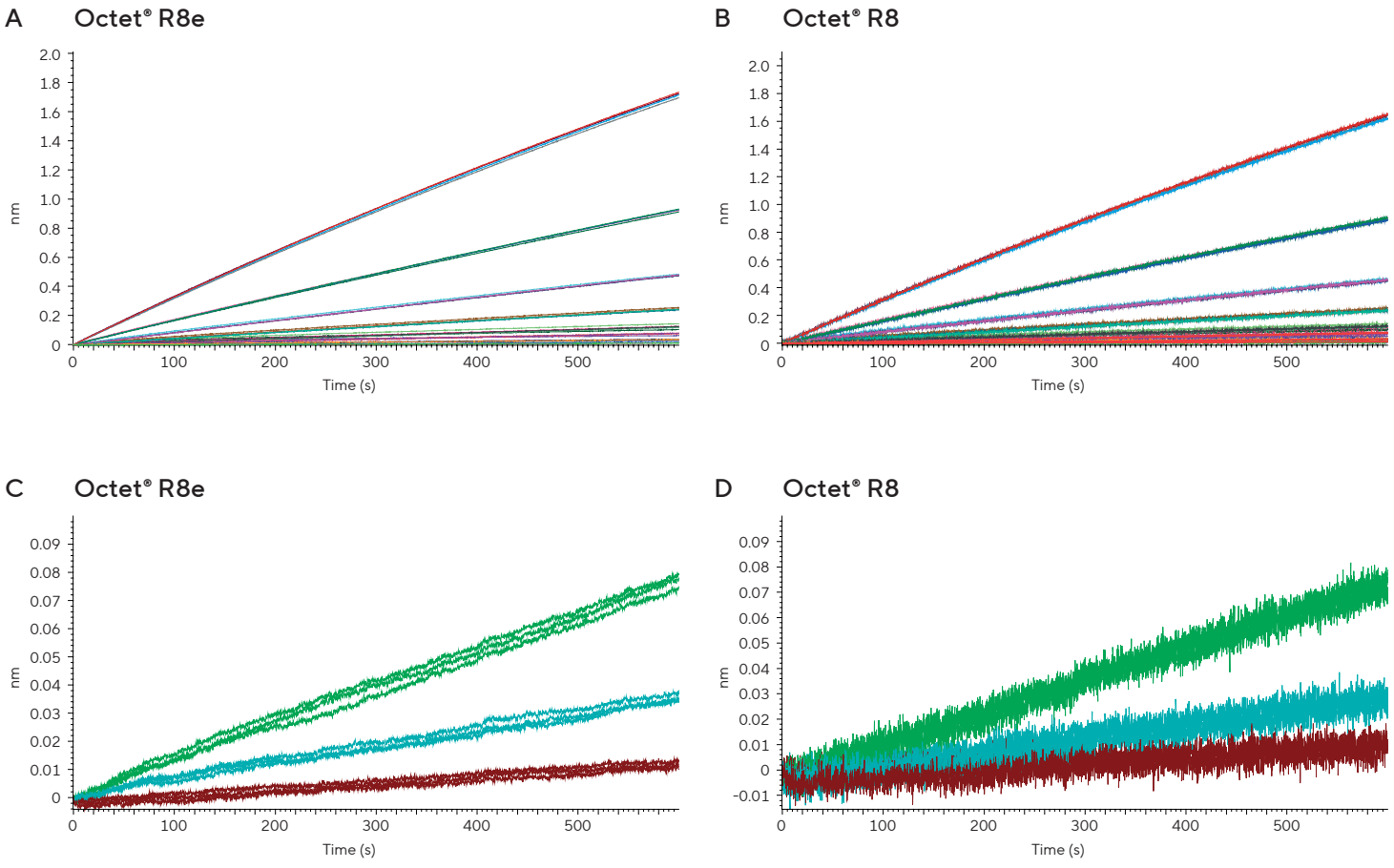


Figure 2: (A) Reference Subtracted data showing binding of human IgG (3.9 – 500 ng/mL) to Octet® ProA biosensors on the Octet® R8e (A) and Octet® R8 (B). The improved signal to noise of the Octet® R8e is highlighted when comparing the lowest concentrations of hIgG (3.9, 7.8 and 15.6 ng/mL). (C) Octet® R8e displays decreased noise levels and corresponding separation of data points which allows accurate determination of analyte concentrations in a shorter period of time compared to the Octet® R8 (D).

Flexible Assay Orientation Addresses Avidity Concerns

Accurate assessment of analyte interactions is crucial for understanding kinetics and affinity, especially when measuring binding interactions at low response levels. This precision is vital for determining microscopic rate constants and ensuring that affinity is measured rather than avidity.

Assay orientation plays a significant role in influencing kinetics and affinity, particularly with smaller molecules that produce weaker signals. To enhance signal detection, peptides are often immobilized on biosensors, allowing larger binding partners to remain in solution. However, this approach can complicate the assessment of bivalent molecules like antibodies, as it amplifies avidity effects.

The Octet® R8e system, with its enhanced sensitivity, addresses these challenges by allowing peptides to remain in solution and be detectable at very low response levels. Traditionally, standard data filtering algorithms such as Savitzky-Golay filtering have been used to remove high-frequency noise from data sets, but as shown in Figure 3, the increased signal-to-noise ratio of the Octet® R8e compared to the Octet® R8 offers a better data resolution at all concentrations and accuracy in data fitting. For instance, using the peptide Insulin (5,808 Da) binding to a capture antibody at a ligand concentration of 1.25 µg/mL, the Octet® R8e provides distinct responses for each analyte concentration below 0.05 nm, unlike the Octet® R8, where responses are less defined (Figure 3).

Therefore, the Octet® R8e allows users to mimic biological interactions better through increased flexibility in assay orientation. Combined with enhanced sensitivity of the Octet® R8e, users can confidently analyze interactions at extremely low responses and ensure that affinity rather than avidity is measured.

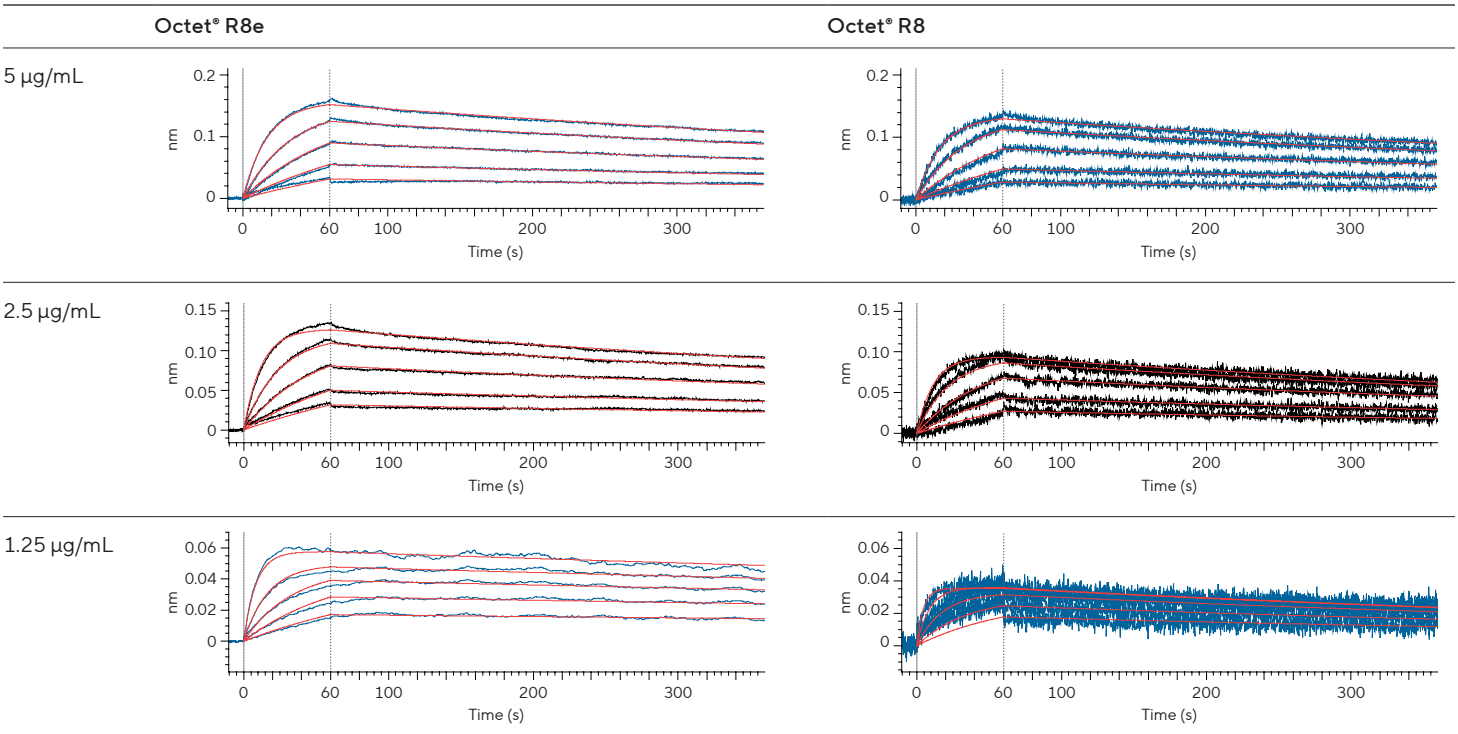


Figure 3: The increased signal-to-noise on the Octet® R8e enables accurate analysis of binding events at responses below 0.1 nm. Here, Insulin peptide (5,808 Da) binding to a capture antibody shows distinct responses for each analyte concentration below 0.06 nm (1.25 µg/mL ligand concentration) when assessed on the Octet® R8e. For all figures, Savitzky-Golay filtering is not used, highlighting the improvement in signal-to-noise on the Octet® R8e system.

Reliable Characterization of Small Molecules and Fast Kinetics

Small molecules account for over 60% of FDA novel drug approvals in the last five years, highlighting their significance in drug discovery. Octet® BLI systems offer distinct advantages over SPR-based methods for studying small molecule binding kinetics. BLI has reduced sensitivity to changes in sample refractive index and viscosity, and this characteristic is crucial for accurate data collection and analysis, especially when working with small molecules.

Screening is a common approach in the screening of drug-like compounds due to its superior coverage of chemical space. The Octet® BLI R8e system enhances this process due to its increased sensitivity allowing for the confident detection and characterization of small molecules. While SPR is often regarded as the 'gold standard' for small molecule analysis, the Octet® BLI R8e provides kinetics and affinity data with high precision, comparable to values reported in literature.

Octet® BLI assays are known for their high assay flexibility, and the Octet® R8e further simplifies drug discovery by enabling parallel high throughput assays in a flexible format. This is achieved without compromising data accuracy or reproducibility. The Octet® R8e system can also accommodate 384-well plates which extends the assay flexibility to another plate format with reduced sample volume.

A key advantage of the Octet® BLI R8e system is its improved signal-to-noise ratio. For instance, data collected at 5Hz in a tilted 384-well microplate on the R8e system shows a better signal-to-noise ratio compared to 5Hz in a 96-well microplate on the R8 system (Figure 4). The newly introduced enhanced data collection feature is especially useful for interactions where the standard data acquisition rate on BLI instruments may miss the initial and fast binding events. The enhanced data collection feature on the Octet® R8e initially shows similar responses to the 5 Hz data but closer assessment reveals key insights that was previously hidden during BLI assays, providing deeper insights into

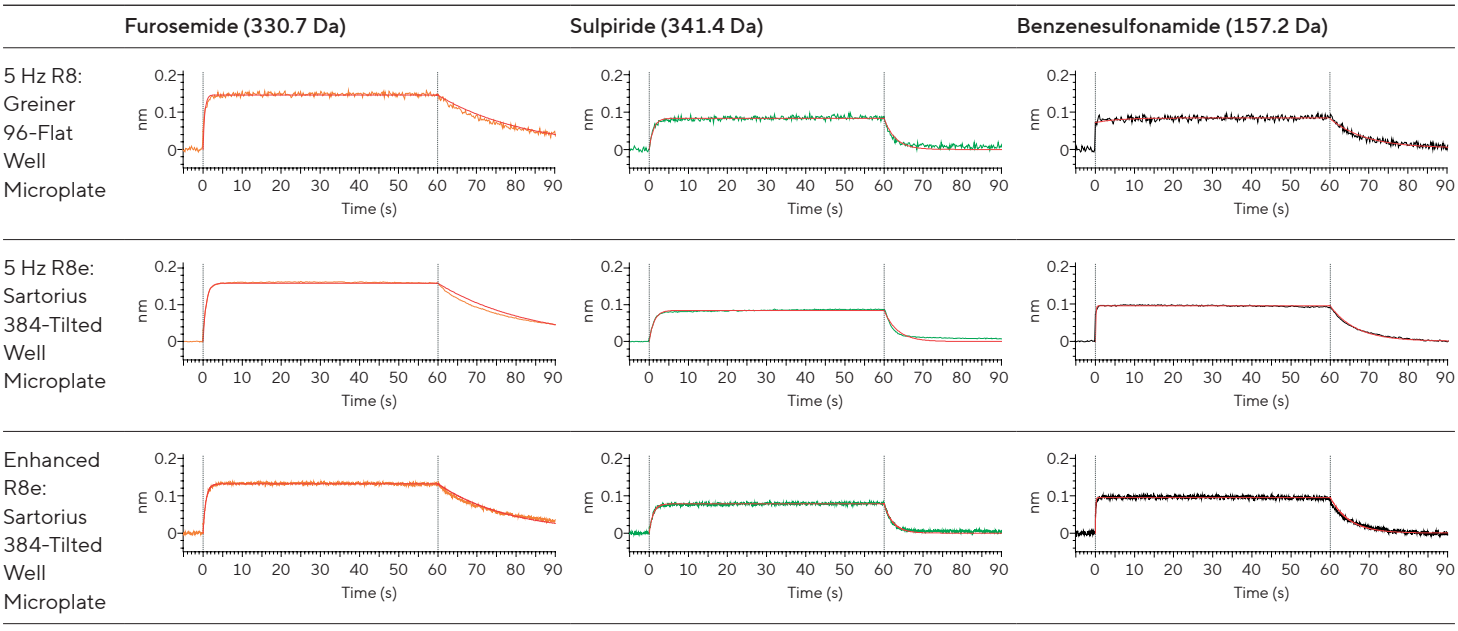


Figure 4: Kinetic analysis of the interaction between carbonic anhydrase and a single concentration of small molecule analytes - furosemide (30 µM), sulpiride (300 µM), and benzenesulfonamide (100 µM) using Super Streptavidin (SSA) Biosensors at 1000 rpm and 25°C. For all sensorgrams, Savitzky-Golay filtering was not used.

molecular interactions.

As shown in Figure 5, assessment of the first 10 seconds highlights the extra data captured during the initial binding phases. For example, a single concentration assessment of furosemide (30 µM) binding to carbonic anhydrase II shows an increase in early binding data at t=0, where 5 Hz data show an initial response of 0.03 nm, but the enhanced data collection initial response is 0.02 nm. The same effect is also observed for benzenesulfonamide, with an initial response of 0.072 nm at t=0 for the 5 Hz data but 0.048 nm for the enhanced data collection. As can be seen for the 1:1 kinetic data fit, for both furosemide and benzensulfonamide, the enhanced data collection allows a more accurate fit to the observed initial data and a resultant increase in accuracy for rate constant determination.

In addition to increasing accuracy for association rate constants, enhanced data collection can also be used to increase accuracy in determining dissociation rate constants. As shown in Figure 5, a 1:1 kinetic fit for 5 Hz sulpiride dissociation data produces a poor fit compared to the enhanced data collection, where an improved 1:1 kinetic fit to the data can be observed.

Overall, the Octet® BLI R8e system offers a powerful tool for small molecule assays, providing enhanced sensitivity, flexibility, and data collection capabilities that reveal previously unseen molecular interactions, thereby advancing the field of drug discovery.

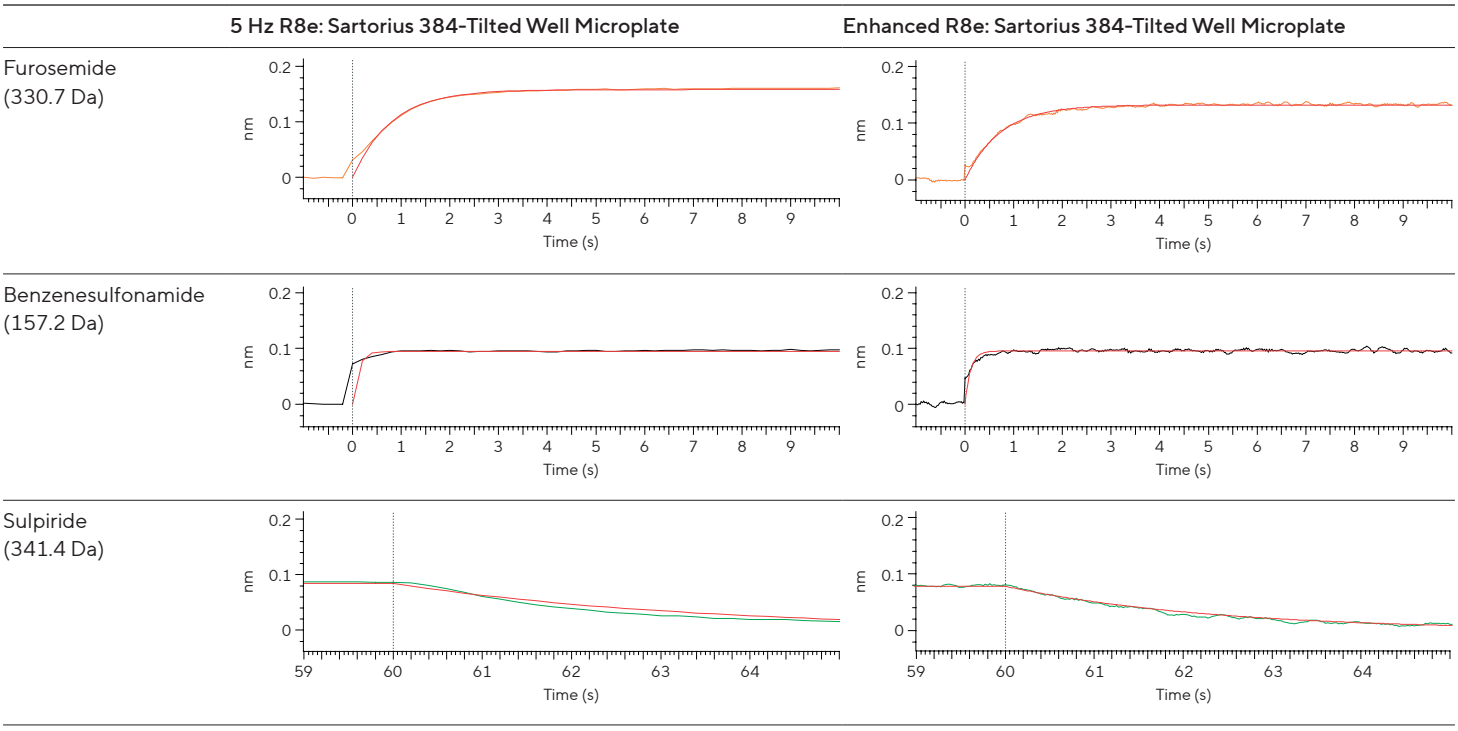


Figure 5: Enhanced data collection captures additional data during initial binding and dissociation phases, revealing previously hidden molecular interactions. It improves accuracy in determining association and dissociation rate constants, offering deeper insights into molecular interactions. The Octet® BLI R8e system enhances sensitivity and flexibility, advancing drug discovery by revealing unseen interactions.

More Data From a Single Assay With Only 20% of the Sample

The capabilities of the Octet® R8e system is extended to accept both 96- and 384-well microplate formats. 384-well microplates offer an instantly recognizable benefit over 96-well microplates by increasing cost efficiency through use of smaller sample volumes (40 µL versus 200 µL, respectively). By using smaller sample volumes, the need for reagents and materials is reduced, significantly lowering the overall assay cost. As shown in Figure 6 and Table 1, assessment of the Performance Qualification Kinetics Kit (PQ-K) on the Octet® R8e shows excellent precision across microplate formats, irrespective of the assay volume. This ensures seamless transferability of current Octet® BLI assays to different microplate formats.

	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
Greiner 96-Flat Well Microplate	5.42×10^5	9.44×10^{-5}	1.74×10^{-10}
Greiner 384-Flat Well Microplate	5.67×10^5	9.61×10^{-5}	1.70×10^{-10}
Sartorius 384-Tilted Well Microplate	5.71×10^5	9.78×10^{-5}	1.71×10^{-10}

Table 1: Comparison of 1:1 global kinetics and affinity assessment results generated using the Octet® Performance Qualification Kinetics Kit (PQ-K) performed on the Octet® R8e.

Sample conservation is not only a money saving exercise but also increases the scope of assays that the Octet® R8e can be used for, including where sample availability may be the limiting factor such as in clinical settings with patient samples (Advances in Immunogenicity Evaluation - Paving the Way for Safer Biologic Therapies) or when assessing smaller upstream grows of biologics, allowing more samples to be assessed earlier due to lower sample requirements.

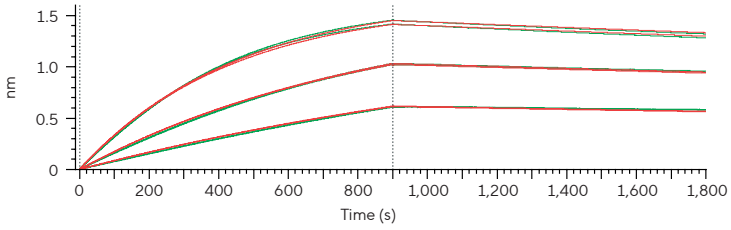
Therefore, 384-well microplates are ideal for first-pass selection where lots of candidates or buffer conditions are available to screen increasing the throughput and efficiency of the experimental process.

The combination of improved sensitivity and 384-well microplates make the Octet® R8e system ideal for detection of low analyte concentration and the determination of accurate kinetics. Smaller volumes allow the user to enhance the sensitivity of their assays by concentrating low abundancy analytes, making them easier to detect and measure.

Smaller volumes also offer an advantage in measuring faster reactions by reducing diffusion distances, making them ideal for assessment of fast kinetics.

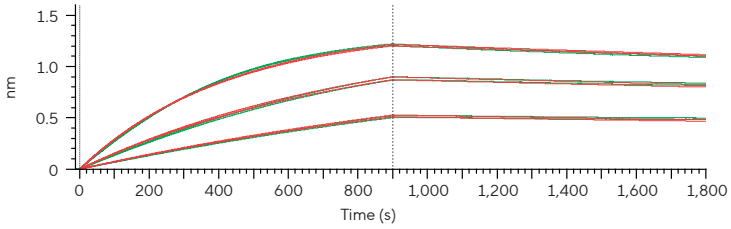
Overall, the use of lower sample volumes can lead to more efficient, cost-effective, assays while maintaining or enhancing the quality and reliability of the results.

96- Flat Well Microplate



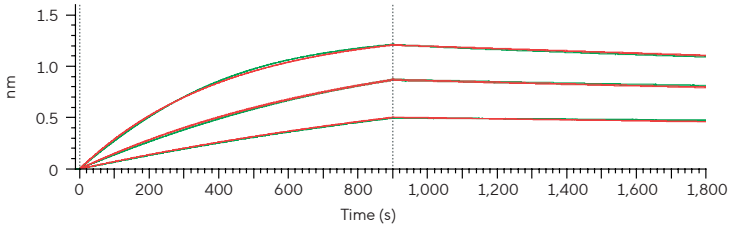
k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
5.42×10^5	9.44×10^{-5}	1.74×10^{-10}

384-Flat Well Microplate



k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
5.67×10^5	9.61×10^{-5}	1.70×10^{-10}

384-Tilted Well Microplate



k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
5.71×10^5	9.78×10^{-5}	1.71×10^{-10}

Figure 6: Comparison of sensorgrams for the Performance Qualification Kinetics Kit (PQ-K) performed on the Octet® R8e using different microplate formats.

Improved Accuracy and Precision With Up To 16 Hours of Evaporation Control

During long or overnight experiments, or when working with small volumes, BLI assays may face evaporation issues. This can change the sample concentration, affecting the accuracy, precision, and reliability of the measurements. Minimizing sample evaporation is essential in experiments to ensure accurate kinetics, affinity, and concentration measurements of samples while increasing replicate precision.

A new microplate evaporation cover, Octet® AE, available for use on the Octet® R8e, RH16, and RH96 systems (PN 18-5152), minimizes sample evaporation from 96-well microplates. It works by maintaining a saturated vapor layer above the sample wells, thereby reducing the evaporation rate, maintaining >90% of the sample volumes for up to 16 hours at 25 °C.

The new evaporation cover increases baseline stability between replicates and allows users to maintain control of sample concentrations for up to 16 hours, leading to improvements in intra- and inter-assay precision. As shown in Figure 7A, anti-Human IgG Goat Fab binding to FAB2G biosensors (Item No.: 18-5125) exhibits excellent data precision over 16 hours with percentage CVs of 6.8% and 7.7% for the determined association and dissociation rate constants, respectively.

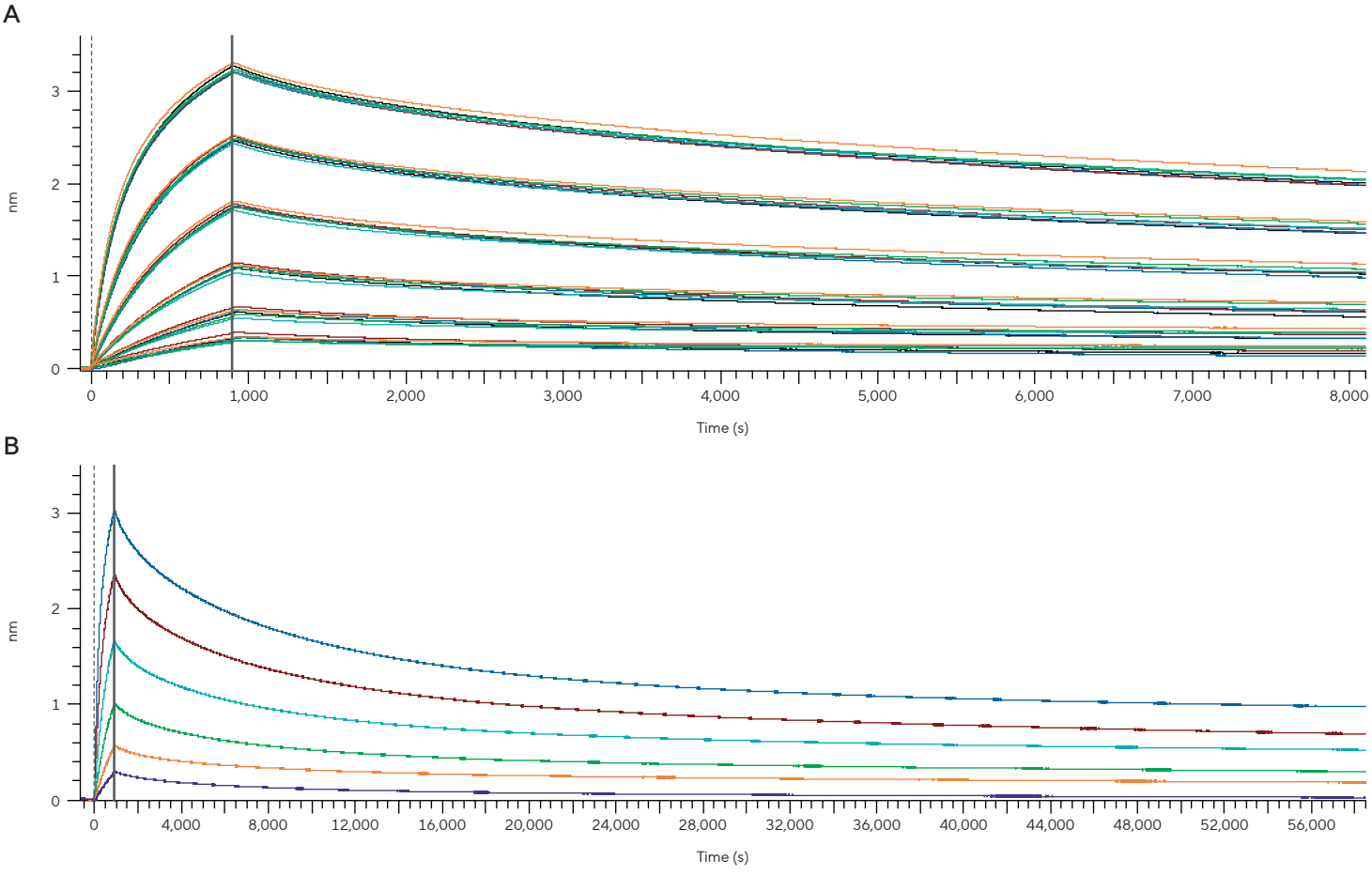


Figure 7: (A) Detection of Human IgG Goat Fab using FAB2G Biosensors on the Octet® R8e system. Six replicates with assay parameters of 1000 rpm speed, 900 s association time and 7,200 s dissociation minutes per sample were performed at 30 °C. Across the 16-hour assay, association and dissociation rate constants were determined with percentage CVs below 7.7% for all replicates. (B) Single replicate of Human IgG Goat Fab detection using FAB2G Biosensors with an extended dissociation time of 16 hours (57,600 s) allows accurate determination of dissociation rate constants due to minimized evaporation.

In addition to increasing precision, the evaporation cover allows accurate assessment of long association and dissociation times, which is essential in determining the correct kinetics and affinity of high-affinity interactions.

Accepted guidelines for accurate determination of dissociation kinetics require a decrease of 5% or more in response. However, the total response must be considered when applying this 5% rule as a 5% may fall within assay noise and drift. For high-affinity interactions, it can take up to 14 hours to see a 5% decrease in response and therefore, evaporation of sample must be minimized to ensure an accurate k_d is determined. As shown in Figure 7B, the Octet® AE evaporation cover enables dissociation times up to 16 hours (57,600 s) with no evaporation effects.

Summary

The Octet® R8e system is a groundbreaking advancement in real-time, label-free biomolecular interaction analysis. It delivers unmatched sensitivity and precision, crucial for biopharmaceutical research and providing research scientists with the most powerful capabilities BLI can offer.

In this document, we have presented data demonstrating accurate detection of analyte concentrations as low as 3.9 ng/mL (26 pM) on the Octet® R8e system. Additionally, the improved signal-to-noise ratios allow for more flexibility in assay orientation, where avidity effects are a concern, resulting in more physiologically relevant insights.

Compatibility with 384-well microplates enables high-throughput assays with minimal sample volumes as low as 40 µL, which enhances efficiency and reduces reagent costs for labs seeking operational efficiency.

While SPR has historically been considered the gold standard of label-free assays for biomolecular interaction analysis, the next-level sensitivity of the Octet® R8e system unlocks the potential of BLI as an alternative for applications with small molecules or low-analyte samples. Moreover, unlike SPR platforms, the Octet® R8e eliminates the need for microfluidics, makes loading times and cleaning redundant, reduces the risk of clogging and contamination, and simplifies maintenance.

The innovative evaporation control feature for the 96-well microplate format maintains sample concentration stability for up to 16 hours, ensuring reliable data collection for long-term kinetic measurements required to observe slow binding interactions or dissociations, enhancing data precision and reliability.

The innovative evaporation control feature for the 96-well microplate format maintains sample concentration stability for up to 16 hours, ensuring reliable data collection for long term kinetic measurements required to observe slow binding interactions or dissociations, improving data precision and reliability. The classic values of Octet®, such as assay flexibility, user-friendly design and 21CFR Part 11 compliance for GxP laboratory settings, are now complemented by enhanced sensitivity and faster data acquisition rate capabilities. These advancements empower researchers to uncover insights previously inaccessible using BLI, accelerating the path to therapeutic breakthroughs.

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