Octet BLI systems – unmatched versatility for discovery, development and quality control

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Abstract

Traditional methods for analyzing the kinetics of biomolecular interactions such as ELISA are often cumbersome and may require rigorous sample preparation. In addition, ELISA may not provide the information required for the confident understanding of a drug molecule's MOA. In contrast the 96-well sample plate based Octet® family of instruments (Figure 1) such as the Octet RED96e system provide researchers with an easy to use real time label free system that can detect the interactions of a diverse range of biomolecules; from small molecules to proteins to mammalian cells. The Octet platform offers an advanced fluidics-free approach with a wide variety of off-the-shelf Dip and Read™ biosensors for rapid binding kinetics and quantitation analysis, enabling direct detections of not only purified biomolecules, but even those in complex media such as cell culture supernatants and lysates. The 96-channel Octet HTX system performs quantitation of 96 samples in as little as 2 minutes, and kinetic screening of 384 samples in 15 minutes. Analysis can be done using a single channel or up to 96 channels, enabling more flexibility in sample throughput when needs change. In addition, the Octet systems have been developed to operate reliably in a regulated environment. Fortebio offers 21 CFR Part 11 software and a full line of GxP products and services as part of the GxP Package. To demonstrate the performance of the BLI technology, applications in protein-protein and protein-small molecules interactions will be described.

Key features

- High-quality kinetic screening and affinity characterization
- Microfluidics-free Dip and Read format reduces assay time and maintenance cost
- Up to 96 parallel, independent channels for maximum speed and flexibility
- Versatility to detect anything from small molecules to mammalian cells
- Non-destructive sampling allows full sample recovery
- Up to 12 hours of unattended run time
- Perfectly suited to operate in GxP-regulated environments

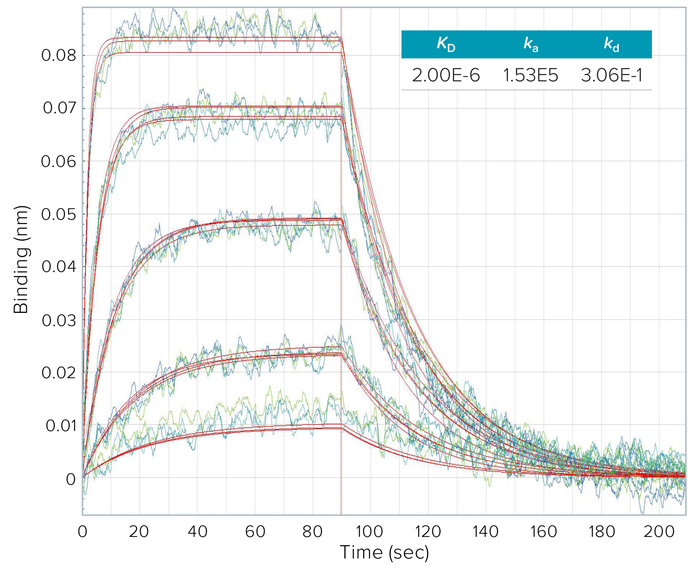

Figure 3: Small molecule kinetics. Example data from benzenesulfonamide (MW 157 Da) binding to biotin-carbonic anhydrase loaded on Super Streptavidin biosensors. Binding was performed at 25°C, with a shake speed of 1000 rpm. A 100 µM benzensulfonamide solution was prepared and serially diluted 1:4.

Figure 1: Range of Octet instruments utilizing label-free and fluidic-free BLI technology.

Bio-Layer Interferometry (BLI)

Octet instruments utilize Bio-Layer Interferometry (BLI) technology and are a suitable replacement for both ELISA and HPLC techniques for quantitation of therapeutic proteins.

Bio-Layer Interferometry is an analytical technique that monitors 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 2). The binding of an analyte in solution to the immobilized protein (ligand) on the biosensor results in an increase in optical thickness which in turn causes a shift in the interference pattern resulting in a wavelength shift. This shift in wavelengths (nm) is reported in real time. Only molecules that associate or dissociate with a change in optical thickness elicit a response.

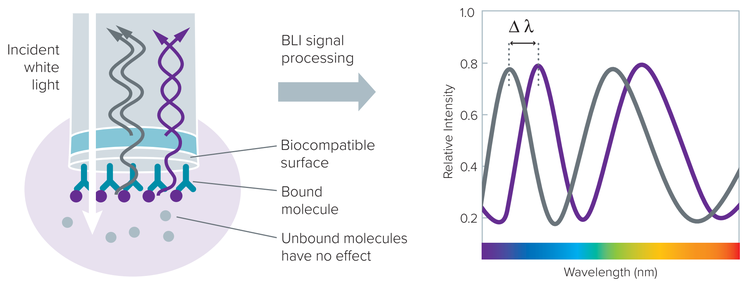

Figure 2: Relative intensity of the light reflection pattern from the two surfaces on the biosensor. The BLI systems measure the difference in reflected light's wavelength (Δλ) between the two surfaces.

Kinetic assays

The Octet systems monitor binding events in real time to calculate on rates (k_a), off rates (k_d), and affinity constants (K_D). The superior sensitivity of the Octet RED96e system enables measurement of small organic molecules (Figure 3) and kinetic constants over a broad range. The temperature of one 96-well sample plate can be controlled in this instrument from 15–40°C, which enables reliable kinetic determinations from low up to physiological temperatures for temperature sensitive proteins (Figure 4). Additional advantages afforded by sample cooling include the ability to rapidly determine binding rate constants at multiple temperatures to extrapolate thermo-dynamic measurements. The Octet RED96e system's eight channels can be used independently to measure samples for screening purposes or in tandem, pairing the sample read with a dedicated reference for high-quality kinetic characterization. An optional microplate evaporation cover minimizes losses in sample volume, allowing full post-analysis sample recovery even after a 12 hour experiment.

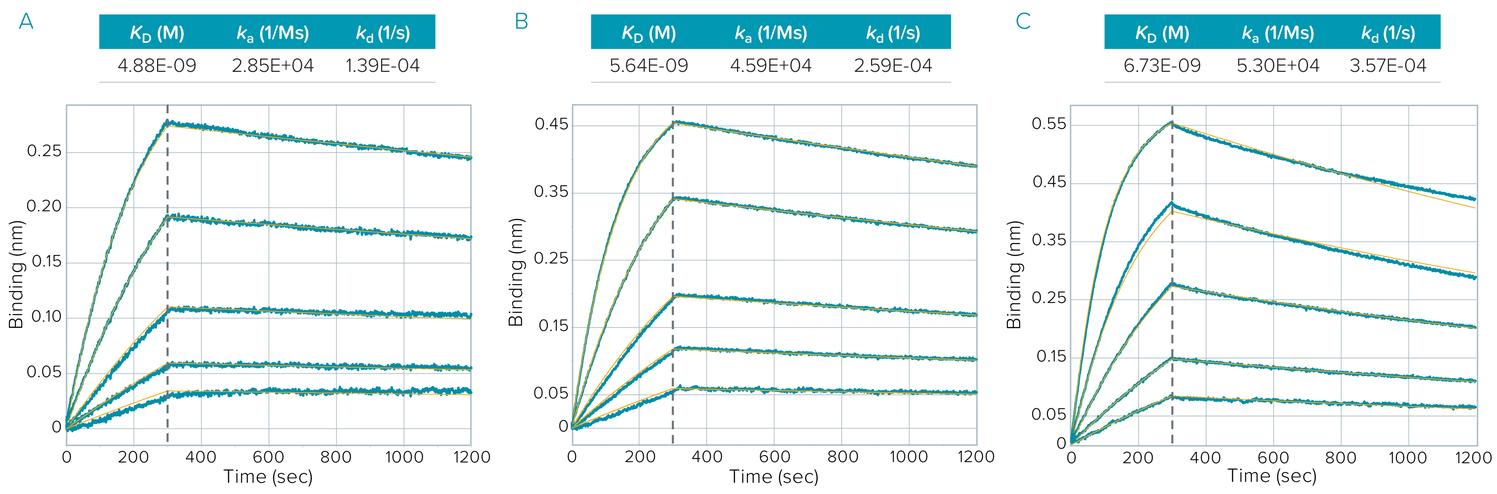

Figure 4: Large molecule characterization. Example data from human Prostate Specific Antigen (PSA, MW 30 kDa) binding to a biotinylated anti-human PSA mouse monoclonal antibody loaded onto Streptavidin biosensors. Binding was performed at 15°C (A), 25°C (B) and 30°C (C), with a shake speed of 1000 rpm. A 200 nM PSA solution was prepared and serial diluted 1:2 to obtain the 5 concentrations run.

Quantitation assays

The Octet systems directly measure the presence of specific proteins and other molecules in solution with minimal interference from complex matrices, allowing rapid analysis of bioreactor samples during cell line development. Protein concentration determination requires the development of a standard reference curve. A standard reference curve is obtained using a purified standard sample of the respective protein. Known concentrations of the purified standard sample are spiked into the matrix of interest and are serially diluted (often two-fold) within the desired concentration range using an analyte-depleted assay matrix.

Accurate and reproducible concentrations can be determined in as little as two minutes per sample or 32 minutes for a whole plate using a simple, one-step assay (Figure 5). High sensitivity in quantitation can be achieved to sub-ng/ml levels with 2-step and 3-step assay formats, allowing automated measurement of contaminants such as host cell proteins and residual protein A faster and more precisely than ELISA. Process economics can be improved further by regenerating and re-using the biosensors.

Figure 5: Human IgG Quantitation. Example data from human IgG analyte binding to Protein A biosensors. Binding was performed at 30°C, with a shake speed of 1000 rpm and a two-minute read per well. Human IgG solution was prepared at 0.025 µg/mL up to 300 µg/mL and the standard curve shown on a log-log scale was generated using the initial slope algorithm and fitted with the unweighted 5-parameter logistic (5PL) regression model.