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Application Note

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Futureproof your Lab with the Octet® R Series of Systems – Flexibility, Sensitivity and Accuracy All Rolled into a Single System

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Abstract

BLI-based Octet® systems offer an advanced, fast, robust and fluidics-free approach for protein analysis. The modular Octet® R series of products give you the additional flexibility of choosing from a series of system configurations with 2, 4 and 8 channels, and upgrading to the next level when your throughput needs increase. This overview compares the performance of the three modular Octet® configurations. The results demonstrate that all three configurations show similar performance in the quantitation and kinetics characterization of proteins as well as protein-small molecules. However, there is a significant time saving associated with using the Octet® R8 system over the Octet® R4 and R2 systems given the same throughput requirement.

Find out more: www.sartorius.com/octet

Introduction

The Sartorius Octet® systems are built on the label-free optical technique of Bio-Layer Interferometry (BLI) which provides real-time analysis of biomolecular interactions. Relying on the robust and easy to use Dip and Read biosensor format, they provide faster time to results relative to technologies like ELISA. The technology operates in a non-fluidic format, which eliminates the complexities in operation, analysis and maintenance that fluidics-based technologies such as SPR experience. Octet® systems are robust and require limited maintenance — which minimizes instrument downtime and increases productivity. Octet® systems are compatible with crude samples and can be used with a diverse array of biological molecules.

The Octet® platform is a comprehensive characterization tool across a range of applications in various stages of drug development, including antibody and protein quantitation. It circumvents limitations of ELISA and HPLC platforms, enabling informed decisions to be made earlier in bioprocess development. Octet® systems utilize a standard microplate format, enabling high-throughput, automated binding analysis directly from 96-well plates and greater flexibility in assay design. In addition, sample consumption during analysis is minimal. Precious samples can be recovered for use in other analyses, maximizing process economy. Octet® systems offer the best quantitation and kinetics performance for a diverse range of molecules over a broad dynamic range and with sensitivity to detect molecules as small as 150 Daltons. The instruments can measure both high- and low-affinity interactions and detect fast binding interactions including protein-small molecule binding.

The modular Octet® R series is available in three different configurations of 2, 4 and 8 channels respectively (Table 1). The 2- and 4-channel Octet® R2 and Octet® R4 sytems are field upgradable to the 8-channel Octet® R8 system, while the 2-channel Octet R2 can be field upgraded to the Octet R4 as well, providing the flexibility to upgrade your instrument as your throughput and budgetary needs change. To compare the performance of the three Octet® R Series instruments, a series of quantitation and kinetics experiments were performed. The samples and assay conditions used were identical, however some minor differences in the workflow was necessary due to the differences in systems' through-put.

	Octet® R2	Octet® R4	Octet® R8
Number of spectrometers / channels	2	4	8
Maximum simultaneous reads	2	4	8
Temperature control	15–40°C	15–40°C	15–40°C
Evaporation cover	No	No	Yes
GxP package availability	No	No	Yes

Table 1: Modular Octet® R series features.

Quantitation Performance Comparison

The Octet® sample plate format enables streamlined workflows and rapid quantitation of antibodies and other biologics. In a typical quantitation assay, biosensors coated with capture molecules are simply dipped into the analyte samples. The resultant binding, which is a function of sample concentration, is then analyzed using either the rate of the initial slope of binding or the equilibrium of binding; both of which depend on the sample concentration. To quantify samples, a standard curve is generated using the binding rates of known concentrations of the same analyte as the unknown. The binding rate of test samples can then be used to extrapolate their concentration off the standard curve.

Materials and Methods

Octet® ProA Biosensors (Cat # 18-5010), human IgG standard samples (0.5 µg/mL, 1000 µg/mL, 1500 µg/mL and 2000 µg/mL) (in-house), human IgG test samples (500 µg/mL, 1500 µg/mL) (in-house), human IgG standard samples (1-700 µg/mL) (Cat # 18-1118), Assay buffer (Sample Diluent) (Cat # 18-1104), and Regeneration buffer (10 mM glycine buffer, pH 1.0) were used for the quantitation assay.

Quantitation Assay Setup for Performance Comparison

Figure 1 shows the quantitation method setup parameters. ProA Biosensors were dipped into regeneration buffer and neutralized for 5 sec each for 3 cycles before and in between uses.

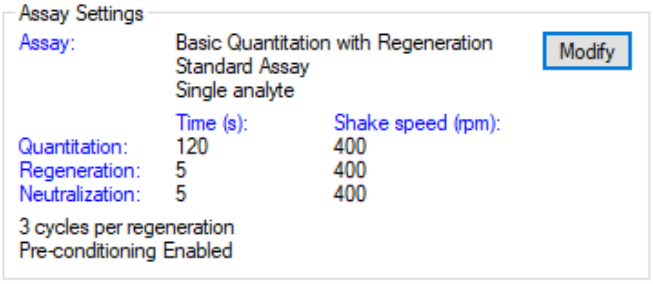

Figure 1: Modular Octet® R series quantitation comparison method setup. The method was developed in Octet® BLI Discovery Software which comes with pre-defined method files.

Figures 2 to 4 show the biosensor tray maps on the left panel and the sample plate maps on the right panel. For the quantitation assay on the Octet® R2 system, (Figure 2 left panel), four sets of biosensors were used to leverage the biosensor re-use times. For assays run on Octet® R4 and R8 systems, two sets of biosensors were used (Figures 3 and 4 left panels). Multiple sets of biosensors were used evenly to exclude any potential biosensor variation. All configurations had the standard samples in 4 replicates in columns 1, 3, 8 and 10 (Figures 2 to 4 right panels). The test samples were added in columns 2 and 9 for the 500 µg/mL concentration and columns 4 and 11 for the 1500 µg/mL concentration, with 16 replicates in total for each concentration. The regeneration buffer was in column 6 and the neutralization buffer in column 7.

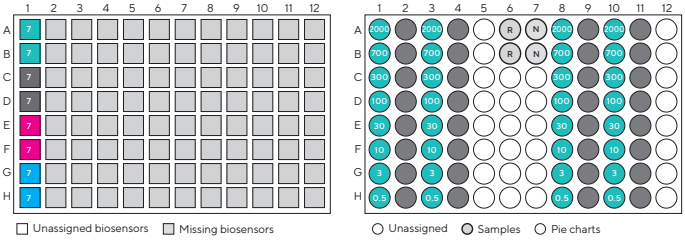

Figure 2: Octet® R2 quantitation assay method setup showing the use of 2 biosensors in-tandem, standard samples (n of 4/concentration), test samples (n of 16/concentration) and biosensor regeneration reagents.

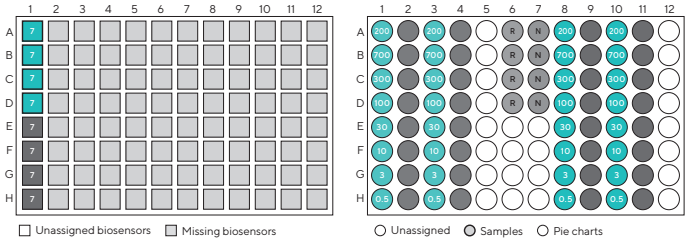

Figure 3: Octet® R4 quantitation assay method setup showing the use of 4 biosensors in-tandem, standard samples (n of 4/concentration), test samples (n of 16/concentration) and biosensor regeneration reagents.

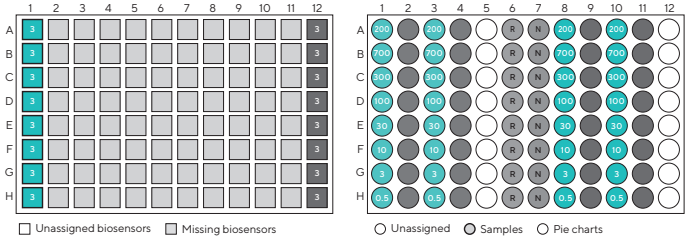

Figure 4: Octet® R8 quantitation assay method setup showing the use of 8 biosensors in-tandem, standard samples (n of 4/ concentration), test samples (n of 16/ concentration) and biosensor regeneration reagents.

Data Analysis for Quantitation Performance Comparison

Octet® Analysis Studio Software version 12.2 was used for data analysis in all experiments. On the Home Tab, the data file was loaded by double clicking the experiment data folder from the Experiment Explorer window. In the Quantitation Analysis Tab, the following default settings were selected:

a. Standard curve equation: 5PL unweighted
b. Processing Parameters
    i. Binding Rate Equation: Initial Slope
    ii. Read Time: 120 sec

Residual Protein A Detection

In addition to monoclonal antibodies and recombinant proteins, the Octet® platform can be used to detect and quantify residual contaminants such as Host Cell Protein (HCP) and Residual Protein A (RPA). The detection and removal of process related residual Protein A from antibody drug molecules is an essential requirement to ensure the safety of antibody-based therapeutics. Sartorius offers Octet® users RPA testing kits that are compatible with Octet® instruments and enable sensitive detection of leached Protein A with a sensitivity as low as 100 pg/mL. Here we compared the detection and quantitation of RPA using the 3 modular Octet® R series instruments.

Materials and Methods

Octet® RPA Kit (18-5128, biosensor and reagent included) and MabSelect Sure (GE Code# 28-4018-60*) were used for this test.

* Product has been discontinued; please contact GE for new product code/number

Residual Protein A Detection Setup for Performance Comparison

Figures 5 to 7 show the general assay setup on the top left panel. RPA Biosensors were used. These biosensors were not regenerated during the assay.

Figures 5 to 7 show the biosensor tray maps on the top right panel and the sample plate maps on the bottom two panels, which were designed to compare residual Protein A detection on the three configurations of the modular Octet® system. Due to the throughput limitation of Octet® R2 system, two sets of detection reagents were needed to enable the total use times to less than 12.

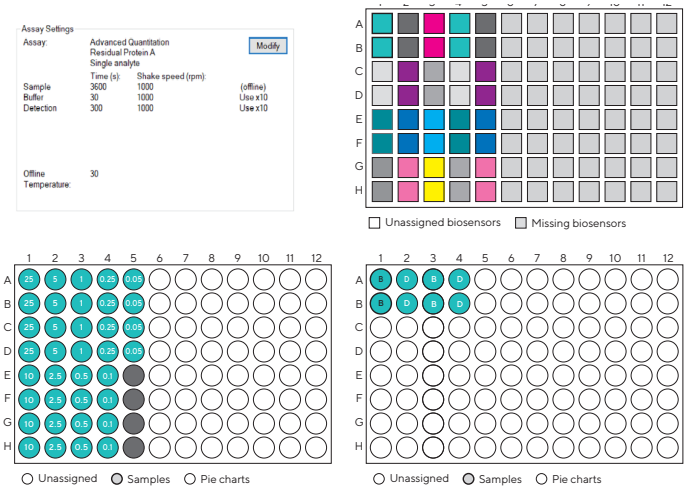

Figure 5: Octet® R2 method and plate map for RPA.

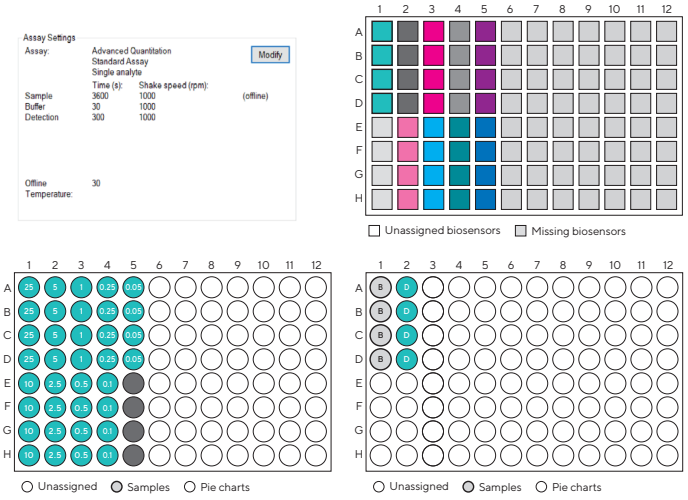

Figure 6: Octet® R4 method and plate map for RPA.

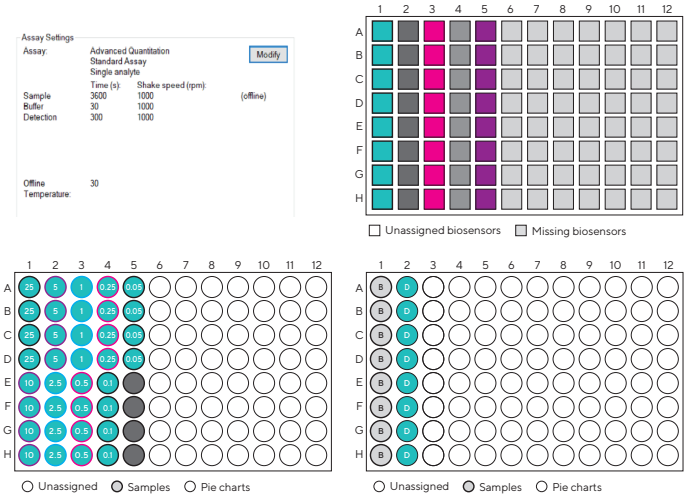

Figure 7: Octet® R8 method and plate map for RPA.

MabSelect Sure standards at different concentrations (n=4 per level) plus reference (Sample Dilution Buffer, n=4). Plate 2: B as Detection Diluent - Protein A; D as Detection Reagent - Protein A.

Kinetics Analysis

The specific recognition and binding of biological molecules by antibodies and other proteins is fundamental to many processes in biology. Kinetics analysis is used to measure association and dissociation rate constants and to determine the affinity of such interactions.

Octet® kinetic analysis begins with the selection of a biosensor from a list of multiple chemistries provided by Sartorius. The process involves the immobilization of one interactant, commonly known as the ligand, on the surface of the biosensor while the other (analyte) remains in solution. The assay begins with an initial baseline or equilibration step where the unlabeled biosensor is dipped in an assay buffer. Next, a ligand molecule such as an antibody is captured on the surface of the biosensor (loading), either by direct immobilization or capture-based methods. After ligand capture, biosensors are dipped into buffer solution to dissociate any weakly immobilized ligand molecules. The baseline in this step is also an indicator of ligand immobilization stability. Then, biosensors are dipped into buffer solution to establish a new baseline that precedes the association step. The biosensors are next dipped into the analyte solution for the association phase. The final step is the dissociation step where the biosensors are dipped back into buffer solution for baseline establishment to measure the rate at which the interaction falls apart. To test the three Octet® modular configurations and compare and contrast with each other during kinetic analysis, the following experiment was designed.

Kinetics Performance Comparison – Large Molecules

Materials and Methods

Protein-protein interaction kinetics was studied for the kinetic assay comparison between the three modular Octet® R series configurations. The following reagents were used: Octet® SAX2 Biosensors (Cat # 18-5136), biotinylated hCD64 (5 µg/mL) (in-house), humanized IgG1k monoclonal antibody (mAb) (1 nM, 2 nM, 4 nM) (in-house), and assay buffer (1 x Kinetics buffer + 0.1% BSA) (in-house).

Assay Workflow Comparison for Protein—Protein Kinetics Method Setup

The Figures 8 to 10 show the general assay setup for three configurations of Modular Octet® systems, respectively. The time required for each step of the kinetic assay is shown. Each assay contains an initial baseline at the beginning (baseline), then a ligand (hCD64) loading step, followed by another baseline step for washing (baseline 2), then a short baseline step used for kinetics analysis (baseline 3), followed by association and dissociation. The total time for one assay was up to one hour and multiple assays were included in one run. The assay was performed at 1000 rpm shaker speed at 25°C. Due to the differences in the three configurations of the Octet® modular systems, different assay setups were used to take the reference subtraction and the total run time into consideration. Global fitting was used with three concentrations of the analyte-humanized IgGk mAbs and the reference. With the Octet® R2 system (Figure 8), you can test three concentrations of the analyte with its own reference in three assays respectively, where a single run only yield one replicate (n=1). With the Octet® R4 system (Figure 9), you can test all three concentrations of the analyte in a single assay with one reference, where a single run can yield two replicates (n=2). With the Octet® R8 system (Figure 10), two sets of three concentrations of the analyte can be studied within the same assay with its own reference, with four replicates each (n=4) while using two references. One run with two assays enables precise evaluation of kinetic rates based on four replicates. The longer baseline after ligand loading can provide additional information for ligand immobilization stability. In the case of the Octet® R4 system, the total run time will be doubled to obtain the same replicates compared to the Octet® R8 system. Therefore, the baseline after ligand loading is shortened for washing only in the current design. For the Octet® R2 system, the total run time will be more than quadrupled to obtain the same replicates compared to Octet® R8 system. Therefore, like the Octet® R4 system, the baseline after ligand loading is shortened for washing only in the current design. Depending on the kinetics model pairs, sample reference subtraction can be done across different assays when the baseline after ligand immobilization is stable and consistent along the experimental time. In this case, kinetics analysis with three concentrations and one reference can be achieved within two assays (data not shown).

Step Data List

Add... | Copy | Remove | Regeneration Params | Threshold Params

	Name	Time	Shake speed	Type	Threshold
➔	Baseline	180	1000	Baseline	☐
	Loading	300	1000	Loading	☐
	Baseline2	180	1000	Baseline	☐
	Baseline3	60	1000	Baseline	☐
	Association	900	1000	Association	☐
	Dissociation	900	1000	Dissociation	☐

Assay Steps List

New Assay | Move Up | Move Down | Remove | Replicate | Edit Step | Info Table

Assay	No.	Sample	Step Name	Step Type	Sensor Type	Assay Time	Comment
1	1	A1	Baseline	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	2	A2	Loading	Loading	SAX2 (High Precision Streptavidin 2.0)		
1	3	A3	Baseline2	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	4	A4	Baseline3	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	5	A5	Association	Association	SAX2 (High Precision Streptavidin 2.0)		
1	6	A4	Dissociation	Dissociation	SAX2 (High Precision Streptavidin 2.0)	0:42:57	
2	1	C1	Baseline	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	2	C2	Loading	Loading	SAX2 (High Precision Streptavidin 2.0)		
2	3	C3	Baseline2	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	4	C4	Baseline3	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	5	C5	Association	Association	SAX2 (High Precision Streptavidin 2.0)		
2	6	C4	Dissociation	Dissociation	SAX2 (High Precision Streptavidin 2.0)	0:42:57	
3	1	E1	Baseline	Baseline	SAX2 (High Precision Streptavidin 2.0)		
3	2	E2	Loading	Loading	SAX2 (High Precision Streptavidin 2.0)		
3	3	E3	Baseline2	Baseline	SAX2 (High Precision Streptavidin 2.0)		
3	4	E4	Baseline3	Baseline	SAX2 (High Precision Streptavidin 2.0)		
3	5	E5	Association	Association	SAX2 (High Precision Streptavidin 2.0)		
3	6	E4	Dissociation	Dissociation	SAX2 (High Precision Streptavidin 2.0)	0:42:57	

Figure 8: Protein-protein binding kinetics method on Octet® R2 system: The method was developed in Octet® BLI Discovery Software.

Step Data List

Add... | Copy | Remove | Regeneration Params | Threshold Params

	Name	Time	Shake speed	Type	Threshold
➔	Baseline	180	1000	Baseline	☐
	Loading	300	1000	Loading	☐
	Baseline2	180	1000	Baseline	☐
	Baseline3	60	1000	Baseline	☐
	Association	900	1000	Association	☐
	Dissociation	900	1000	Dissociation	☐

Assay Steps List

New Assay | Move Up | Move Down | Remove | Replicate | Edit Step | Info Table

Assay	No.	Sample	Step Name	Step Type	Sensor Type	Assay Time	Comment
1	1	A1	Baseline	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	2	A2	Loading	Loading	SAX2 (High Precision Streptavidin 2.0)		
1	3	A3	Baseline2	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	4	A4	Baseline3	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	5	A6	Association	Association	SAX2 (High Precision Streptavidin 2.0)		
1	6	A4	Dissociation	Dissociation	SAX2 (High Precision Streptavidin 2.0)	0:42:57	
2	1	A1	Baseline	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	2	A2	Loading	Loading	SAX2 (High Precision Streptavidin 2.0)		
2	3	A3	Baseline2	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	4	A5	Baseline3	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	5	A7	Association	Association	SAX2 (High Precision Streptavidin 2.0)		
2	6	A5	Dissociation	Dissociation	SAX2 (High Precision Streptavidin 2.0)	0:42:57	

Figure 9: Protein-protein binding kinetics method on Octet® R4 system: The method was developed in Octet® BLI Discovery software.

Step Data List

Add... | Copy | Remove | Regeneration Params | Threshold Params

	Name	Time	Shake speed	Type	Threshold
➔	Baseline	180	1000	Baseline	☐
	Loading	300	1000	Loading	☐
	Baseline2	1200	1000	Baseline	☐
	Baseline3	60	1000	Baseline	☐
	Association	900	1000	Association	☐
	Dissociation	900	1000	Dissociation	☐

Assay Steps List

New Assay | Move Up | Move Down | Remove | Replicate | Edit Step | Info Table

Assay	No.	Sample	Step Name	Step Type	Sensor Type	Assay Time	Comment
1	1	1	Baseline	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	2	2	Loading	Loading	SAX2 (High Precision Streptavidin 2.0)		
1	3	3	Baseline2	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	4	4	Baseline3	Baseline	SAX2 (High Precision Streptavidin 2.0)		
1	5	6	Association	Association	SAX2 (High Precision Streptavidin 2.0)		
1	6	4	Dissociation	Dissociation	SAX2 (High Precision Streptavidin 2.0)	1:00:20	
2	1	1	Baseline	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	2	2	Loading	Loading	SAX2 (High Precision Streptavidin 2.0)		
2	3	3	Baseline2	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	4	5	Baseline3	Baseline	SAX2 (High Precision Streptavidin 2.0)		
2	5	7	Association	Association	SAX2 (High Precision Streptavidin 2.0)		
2	6	5	Dissociation	Dissociation	SAX2 (High Precision Streptavidin 2.0)	1:00:20	

Figure 10: Protein-protein binding kinetics method on Octet® R8 system: The method was developed in Octet® BLI Discovery software.

Figures 11 to 13 show the biosensor tray maps on the left panel and the sample plate maps on the right panel, which were designed to compare kinetic analysis of three concentrations of the humanized IgGk on the three configurations of the modular Octet® system. For the kinetic assay on the Octet® R2 system (Figure 11 left panel), two biosensors in a single assay – one for the sample and one for the reference were used. For the assay on the Octet® R4 system (Figure 12 left panel), four biosensors were used in a single assay — three for the various sample concentrations and one for the reference. For the assay on the Octet® R8 system (Figure 13 left panel), eight biosensors were used in a single assay — six for the various sample concentrations in duplicate and two for the reference in duplicate. All configurations had the same three concentrations of the analytes as shown in Figures 11 to 13 right panels.

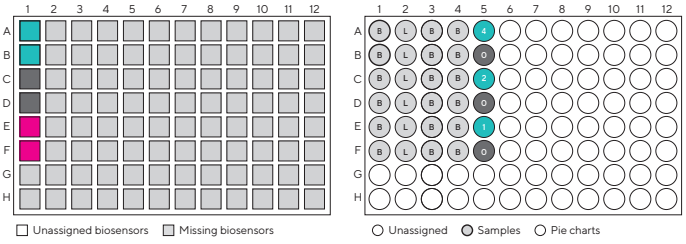

Figure 11: Octet® R2 system protein-protein binding kinetics method showing biosensor and sample trays setup with two biosensors in-tandem. For each sample concentration, one biosensor is used for sample while the other is used for referencing. In this example, 3-concentration point kinetics are determined in one run, three assays and no replicates. A separate run is needed for each replicate. Assay buffer in columns 1, 3, and 4; Biotin-HCD64 at 5 µg/mL in column 2; humanized IgG1k in columns 5 (assay buffer for references).

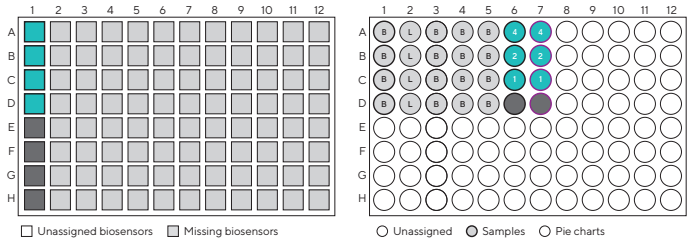

Figure 12: Octet® R4 system protein-protein binding kinetics method showing biosensor and sample trays setup with four biosensors used in-tandem for three concentration points plus reference well. This design can be used for one run, two assays and two replicates. A second run with a second column of biosensors would be needed to achieve four replicates. Assay buffer in columns 1, 3-5; Biotin-HCD64 at 5 µg/mL in column 2; humanized IgG1k in columns 6&7 (assay buffer for references).

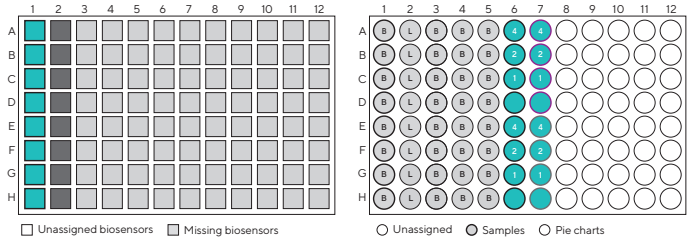

Figure 13: Octet® R8 system protein-protein binding kinetics method showing biosensor and sample trays setup with eight biosensors used in-tandem for three concentration points plus reference wells. The design is for one run, two assays and four replicates. Assay buffer in columns 1, 3-5; Biotin-HCD64 at 5 µg/mL in column 2; humanized IgG1k in columns 6 & 7 (assay buffer for references).

Data Analysis for Kinetics Performance Comparison (Large Molecules)

In the Octet® Analysis Studio Software version 12.2 Home Tab, the data file was loaded by double clicking the experiment data folder in the Experiment Explorer window. In the Preprocessed Data Tab, under Reference Sample Tab, the reference subtraction was done as below by columns, by selecting the samples on the sample plate map:

a. Octet® R8 system: two references were averaged in each assay
b. Octet® R4 and R2 sytem: only one reference was available in each assay

Data was processed and fitted using a 1:1 binding model (Refer to the Data analysis user guide for data processing).

Protein-Small Molecules Characterization Performance Comparison

The binding of small molecules to proteins can also be measured efficiently on Octet® instruments. In a typical experiment, a biotinylated protein target is immobilized onto a high-capacity SSA (Catalog # 18-5057) Biosensor surface followed by an exposure of this surface to a solution of the small molecule in a microplate well. The association of the small molecule to the target protein on the biosensor is measured over time. Assuming that the biosensor is pre-immobilized with the biotinylated target protein, the assay begins with an initial baseline prior to the association step. The dissociation step of the assay has to be acquired off the same initial baseline buffer well. Ideally, successful analysis requires a robust, well-characterized system including a stable, active, and purified protein, and a stable, non-aggregating small molecule compound. Compounds that bind to the target protein and those with no binding should be used as positive and negative controls. Successful analysis also requires a biotinylated target protein; typically with ~1 molar equivalent of biotin per protein (refer to Sartorius Technical Note: "Biotinylation of Protein for Immobilization onto Streptavidin Biosensors"). Optimal results are obtained when the sample matrix is identical to the assay buffer used for baselines and dissociations.

The solubility of the compounds to be tested is also a crucial factor — compounds that form aggregates can produce misleading and anomalous results. Additionally, the sample matrix or buffer used to dilute the samples and the assay buffer used for recording baselines and dissociation data should be identical, to avoid introducing artifacts into the data. Since most small molecules require some amount of organic solvent to dissolve, matrix matching to include an identical amount of the organic solvent used is critical to the success of the assay. The experimental setup requires two sets of assays; one, the binding assay between the target protein and the small molecule and two, the reference assay. The reference assay steps and samples are identical to the binding assay except that no active protein is immobilized on the biosensors. The reference assay data is subtracted from the binding assay data during data analysis to determine binding specificity. The biosensors are typically blocked with biocytin.

Materials and Method

For the protein-small molecule characterization study comparison on the three configurations of modular Octet systems, the following reagents were used. Octet® SSA Biosensors (Cat # 18-5057), biotinylated carbonic anhydrase, biocytin, furosemide (0.1 µM–30 µM) (in-house), assay buffer (PBS+0.5% DMSO) (in-house). For the protocol for carbonic anhydrase-coated biosensors and biocytin-blocked biosensors, refer to Sartorius Technical Note "Small Molecule Binding Kinetics."

Assay Workflow Comparison for Protein—Small Molecules Kinetics Method Setup

Figure 14 shows the general assay setup which applies to either sample binding assay and reference assay. The time required for each step of the kinetic assay is shown. Each assay contains a short equilibrium step at the beginning, followed by six cycles of baseline, association, and dissociation for each concentration level. To enable double reference subtraction for kinetics analysis, two columns of biosensors must go through the same sample plate with the same assay setup sequentially. The first column of biosensors was SSA coated with biotin-carbonic anhydrase while the second column of biosensors are SSA blocked with biocytin. The order can be switched. The total time for one assay was about 30 min and multiple assays (at least two assays) were included in one run. The assay was performed at 1000 rpm shaker speed at 25°C. Sample reference subtraction must be done within each assay, while biosensor reference subtraction must be done within adjacent assays. However, each run can include up to six assays (three pairs) considering the total run time. The overall baseline performance of biosensors will change along the experimental time but double reference subtraction in data analysis will compensate for this change.

Step Data List

Add... | Copy | Remove | Regeneration Params | Threshold Params

	Name	Time	Shake speed	Type	Threshold
→	adjust	600	1000	Custom	☐
	baseline	30	1000	Baseline	☐
	on	60	1000	Association	☐
	off	90	1000	Dissociation	☐

Assay Steps List

New Assay | Move Up | Move Down | Remove | Replicate | Edit Step | Info Table

Assay	No.	Sample	Step Name	Step Type	Sensor Type	Assay Time	Comment
1	1	1	adjust	Custom	SSA (Super Streptavidin)		
1	2	1	baseline	Baseline	SSA (Super Streptavidin)		
1	3	7	on	Association	SSA (Super Streptavidin)		
1	4	1	off	Dissociation	SSA (Super Streptavidin)		
1	5	2	baseline	Baseline	SSA (Super Streptavidin)		
1	6	8	on	Association	SSA (Super Streptavidin)		
1	7	2	off	Dissociation	SSA (Super Streptavidin)		
1	8	3	baseline	Baseline	SSA (Super Streptavidin)		
1	9	9	on	Association	SSA (Super Streptavidin)		
1	10	3	off	Dissociation	SSA (Super Streptavidin)		
1	11	4	baseline	Baseline	SSA (Super Streptavidin)		
1	12	10	on	Association	SSA (Super Streptavidin)		
1	13	4	off	Dissociation	SSA (Super Streptavidin)		
1	14	5	baseline	Baseline	SSA (Super Streptavidin)		
1	15	11	on	Association	SSA (Super Streptavidin)		
1	16	5	off	Dissociation	SSA (Super Streptavidin)		
1	17	6	baseline	Baseline	SSA (Super Streptavidin)		
1	18	12	on	Association	SSA (Super Streptavidin)		
1	19	6	off	Dissociation	SSA (Super Streptavidin)	0:31:27	

Figure 14: Protein-small molecules binding kinetics method developed in Octet® BLI Discovery software.

Figures 15 to 17 show the biosensor tray maps on the left panel and the sample plate maps on the right panel, which were designed to compare protein–small molecule binding kinetics on the three configurations of the modular Octet® system. For the assay on the Octet® R2 system (Figure 15 left panel), three sets of two paired SSA Biosensors were used. The first column of biosensors from each set (protein ligand biotinylated carbonic anhydrase coated SSA) was used to acquire data for binding of the furosemide (small molecule analyte, 330.7 Da) which was plated in increasing concentrations across the plate (Figure 15 right panel). The second column of biosensors from each set (biocytin-blocked SSA) serves as a negative control when used in the same workflow. For the assays on Octet® R4 and R8 systems, two sets of four paired SSA Biosensors and a single set of eight paired SSA biosensors were used (Figures 16 and 17 left panels).

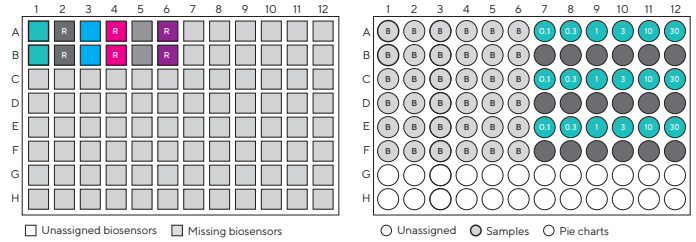

Figure 15: Octet® R2 system protein-small molecules assay setup: One run; six assays and two runs in total. The assay workflow is similar to Octet® R8 system except that only two biosensors are used at a time. In this example, a total of three replicates of data is acquired sequentially using two columns of biosensors for each replicate. Biosensors in column 1 and 2 dip into sample plate rows A-B, biosensors in columns 3 and 4 dip into rows C-D of the sample plate while biosensors in columns 5 and 6 dip into rows E-F of the sample plate.

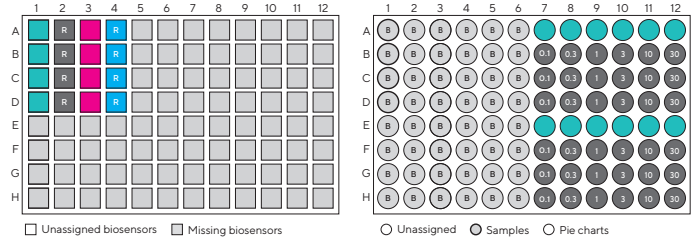

Figure 16: Octet® R4 system protein-small molecules assay setup: One run; four assays. The assay workflow is similar to the Octet® R8 system workflow except that only four biosensors are used at a time. The first two columns of biosensors are used to dip into sample wells on rows A–D while the next two columns are dipped into sample wells E-H to acquire data for the set of replicates.

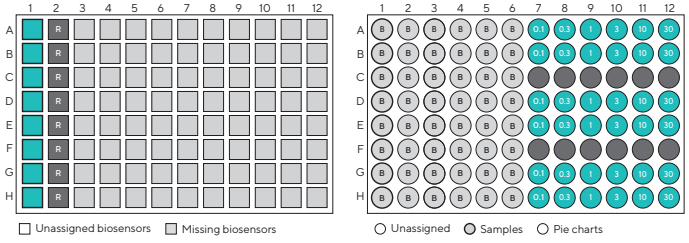

Figure 17: Octet® R8 system protein-small molecules binding kinetics setup: One run; two assays. In assay 1 biosensors in column 1 are first used to acquire data for the binding of furosemide (analyte) to the protein (ligand). The sample plate has furosemide at dose response concentrations across the right half of the plate and as assay buffer across the left half of the plate). Note that in this example, the biosensors come pre-immobilized with the biotinylated carbonic anhydrase already. In the second assay, a second batch of biosensors (with biocytin blocked) are similarly dipped into the samples on the tray with identical assay workflow as the first column of biosensors.

Data Analysis For Kinetics Performance Comparison (Small Molecule)

In the Octet® Analysis Studio version 12.2 Home Tab, the data file was loaded by double clicking the experiment data folder in the Experiment Explorer window. In the Preprocessed Data Tab, under the Reference Sensor Tab, biosensors were selected on the biosensor tray map by right clicking and selecting **Subtract Reference by Pairs** > **Left**.

Under the Reference Sample Tab, samples on the sample plate map were selected by right clicking and selecting **Subtract Reference by Columns**.

a. Octet® R8 system: in each assay, only one reference is used while the other is changed to sample prior to sample reference subtraction. In other words, two references can serve as the back-up for each other in case of abnormal binding
b. Octet® R4 and R2 system: only one reference is available for use in each assay

Data was processed and was fitted using a 1:1 binding model (Refer to the Octet® Software Data Analysis User Guide for data processing)

Results

Quantitation Performance Comparison on Modular Octet® R Series

The comparison of quantitation of two unknown samples on the three configurations of the Octet® R series of systems showed all modules gave comparable results in terms of reproducibility in binding curves from the different standard replicates (Figures 18 A-C). Similarly, a comparison of RPA detection on the modules show good comparability in response signals for all samples tested (Figure 19). Additionally, the IgG quantitation standard curve precision and fit is also comparable across the three modular Octet® R series systems (Table 2). In terms of precision %CV obtained from the unknown samples, the three modules again show cross comparability. The percentage recovery of the unknown sample concentrations plotted on the standard curves generated on the three modular Octet® systems do not show any significant variation (Table 3). Therefore, we conclude that the three Octet® R series modules show comparable quantitation performance.

However, the total assay time required to run the same number of standards replicates and unknown samples during IgG quantitation and in RPA detection varies between each of the Octet® R series modules (Tables 4 and 5).

	Protein A Test		
	Standard Curve Precision and Fit		
Calibrator	Octet® R8	Octet® R4	Octet® R2
0.5 µg/mL	10.0%	1.8%	6.4%
3 µg/mL	0.6%	0.9%	3.1%
10 µg/mL	0.7%	3.7%	3.3%
30 µg/mL	1.0%	0.8%	3.3%
100 µg/mL	1.0%	0.5%	3.7%
300 µg/mL	1.1%	0.6%	3.6%
700 µg/mL	4.4%	1.4%	2.7%
2000 µg/mL	9.2%	5.0%	6.3%
Standard Curve R^2	0.9990	0.9996	0.9994
	Binding Rate Separation		
Conc. Level	Octet® R8	Octet® R4	Octet® R2
10–0.5 µg/mL	0.035	0.033	0.033
2000–0.5 µg/mL	0.918	1.019	0.938
2000–700 µg/mL	2.471	2.533	2.486
	2.472	2.535	2.488

Table 2: Standard curve precision and fit across the Octet® R2, R4 and R8 systems.

	Protein A Test		
	Precision %CV		
Conc. Level	Octet® R8	Octet® R4	Octet® R2
500 µg/mL	1.8%	2.1%	2.8%
1500 µg/mL	5.7%	4.2%	3.6%
	Precision % Recovery		
Conc. Level	Octet® R8	Octet® R4	Octet® R2
500 µg/mL	102.6%	101.5%	100.6%
1500 µg/mL	102.1%	98.2%	105.2%

Table 3: CV and recovery percentages of the two unknown samples as calculated from the standard curves generated on the Octet® R2, R4 and R8 systems.

	Modular Octet® Configuration		
	Octet® R2	Octet® R4	Octet® R8
Number of replicates for each standard/sample concentration	4/16	4/16	4/16
Number of standard/sample concentrations	8/2	8/2	8/2
Number of runs needed	1	1	1
Number of ProA Biosensors	8	8	16
Total run time (hours)	2	1	0.5

Table 4: Comparison of the total number of assay runs, biosensors and time required to test eight concentrations for a standard curve with four replicates and two concentrations for precision samples with 16 replicates on Octet® R2, R4 and R8 systems.

Residual Protein A Detection and Quantitation on the Modular Octet® R Series

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

Table 5: Biosensors, run time and reagent usage time comparisons between the modular Octet® R series instruments for RPA testing.

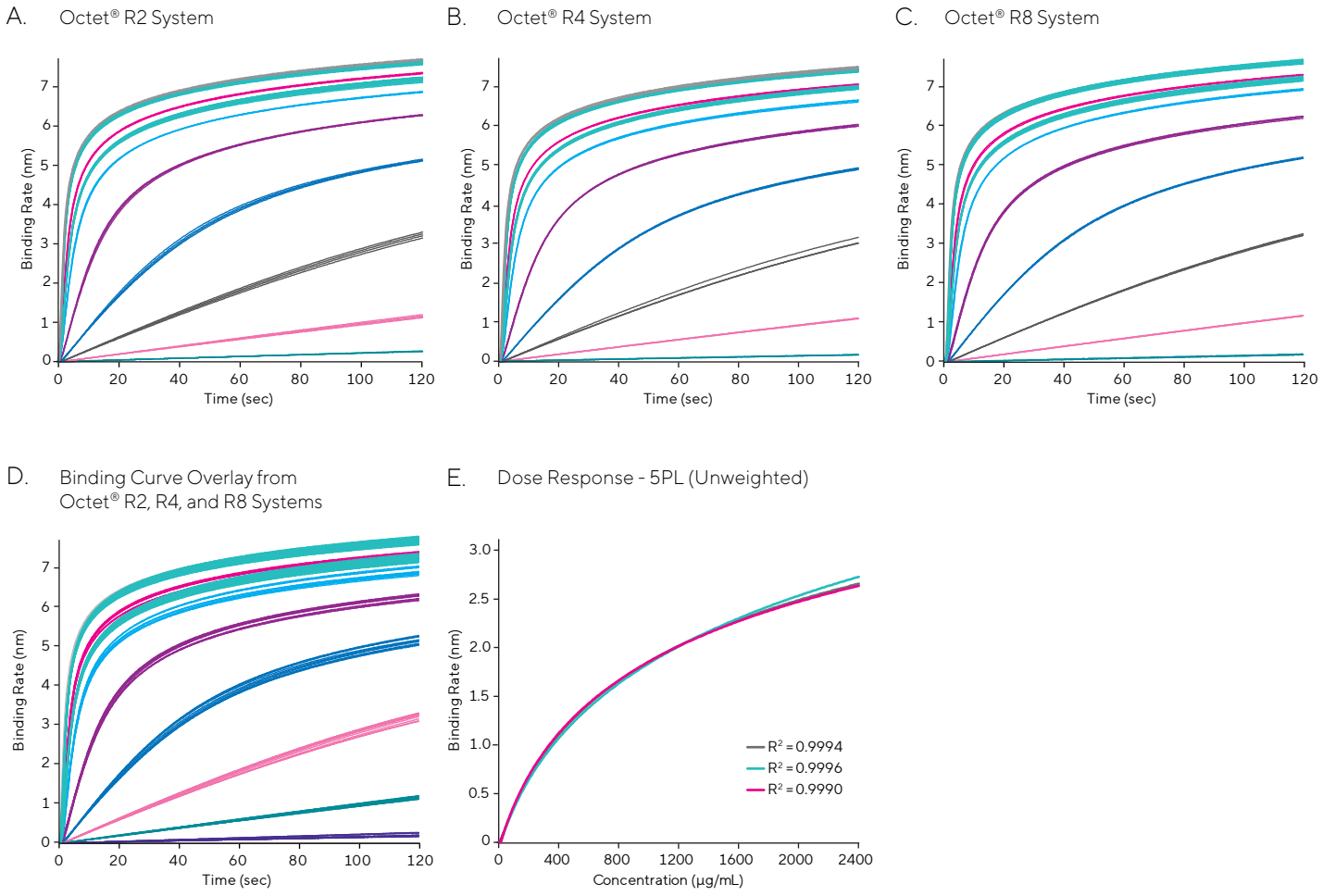

Figure 18: Direct comparison of raw data obtained from the 3 modular Octet® R series. A-C show the standard dose response binding curves (0.5–2000 µg/mL) as well as the 500 and 1500 µg/mL concentration point unknown sample binding curve replicates (in teal).

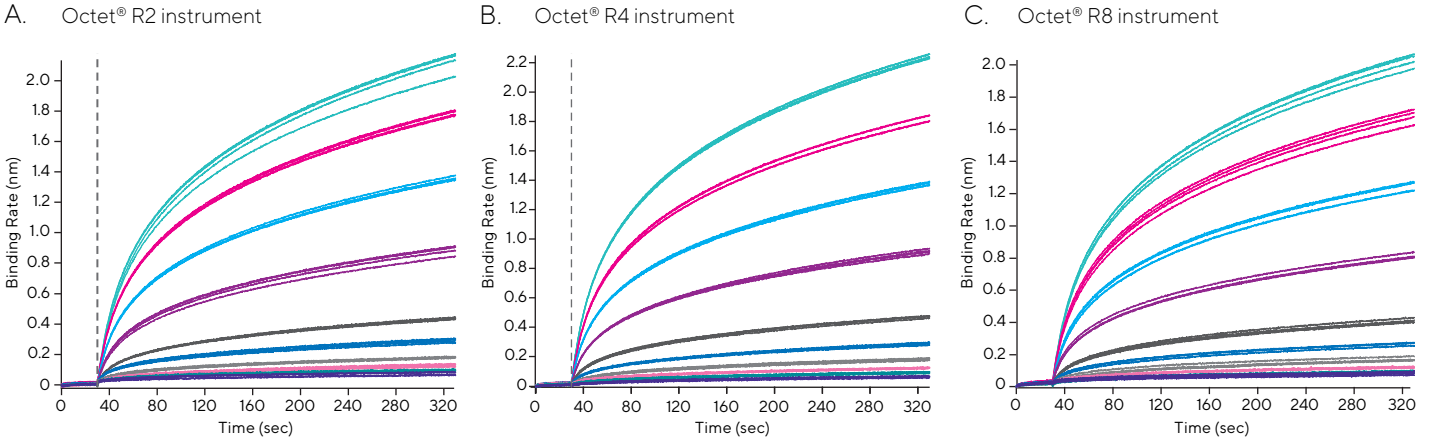

Figure 19: Raw data of RPA for Octet® R2, R4, and R8 systems. Comparison data across the three instruments show near identical signal response.

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

During kinetic analysis on Octet® systems, the binding events can be observed in real time. In the kinetic performance comparison on modular Octet systems, the association and dissociation of the analyte hIgG1K monoclonal antibody on SAX2 Biosensors loaded with hCD64 ligand was studied. As with the quantitation performance, tight reproducibility between the replicates were seen across the three Octet® R series modules in this instance as well (Figure 20). The comparability in the raw data obtained from each of the three analyte concentrations can be seen in Figure 20 A-C. The magnification of just the association and dissociation steps (Figure 20 D-F) shows comparable slow association and dissociation on the three modular Octet® systems. In addition, comparable loading nm shift, K_D, k_{on} and k_{off} as well as their CV%s were seen across the three Octet® R series modules (Table 6). Therefore, we conclude that the three Octet® R series modules show comparable large molecule kinetics performance.

However, the total assay time required to run in the same number of replicates varies between each of the Octet® R series modules (Table 7). This is because one run with three assays on an Octet® R2 system can generate one sample replicate reading, while one run with two assays on an Octet® R8 system can generate all four replicate readings. Therefore, to get the same four replicate readings as an Octet® R8 system, the Octet® R4 system needs double the number of runs and assays, and the Octet® R2 system needs 4x the number of runs and 6x the number of assays.

Kinetics	Octet® R2	Octet® R4	Octet® R8
Loading nm shift	1.06	1.04	0.9
Loading nm shift %CV	4.9%	1.8%	1.2%
K_D	1.65E-10	1.52-10	1.32-10
K_D %CV	7.8%	5.7%	5.3%
k_{on}	4.82E+05	5.18+05	6.29+05
k_{on} %CV	2.6%	5.5%	2.5%
k_{off}	7.97-05	7.98E-05	8.32-05
k_{off} %CV	9.3%	4.3%	3.1%

Table 6: Protein-protein kinetics assay parameters obtained from the 3 modular Octet® R series instruments when used with identical samples. Kinetics parameters were obtained in 4 replicate assays for each instrument .

	Octet® R2	Octet® R4	Octet® R8
Number of replicates for kinetic rates	4	4	4
Number of sample concentrations for global kinetic analysis	3	3	3
Number of runs needed	4	2	1
Number biosensors	24	16	8
Total run time (hours)	8.67	3	2*

Table 7: A comparison of the total number of assay runs, biosensors and time required to test three concentrations of a sample with four replicates with a reference on the Octet® R2, R4 and R8 series (*elongated baseline after ligand loading) systems for the kinetics performance comparison.

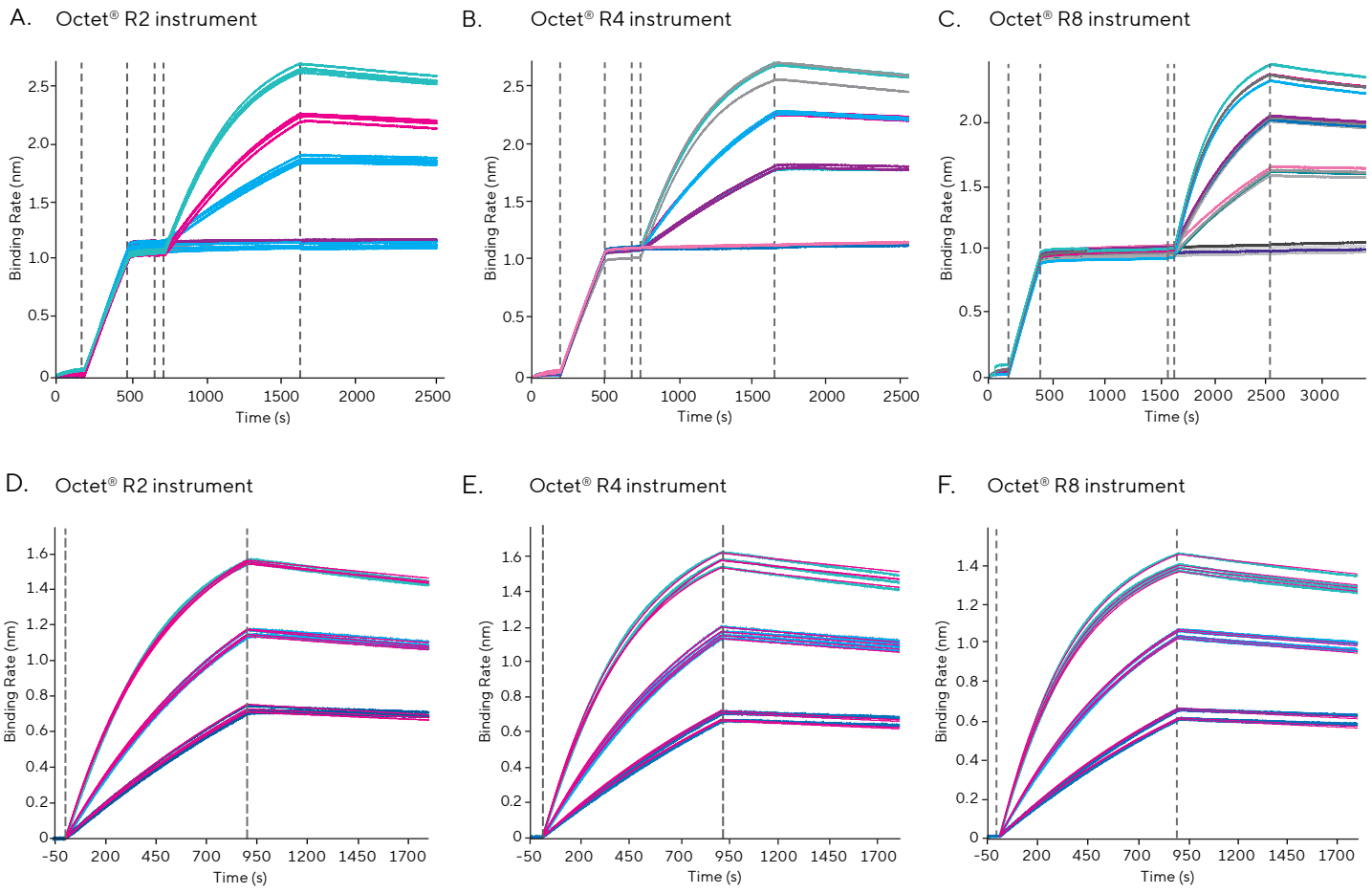

Figure 20: Direct comparison of protein-protein binding raw and analyzed data as obtained from the 3 modular Octet® R Series. A-C show the full dose response assay with replicates overlaid. D-F show the overlay of the association and dissociation steps with replicates for the three instruments. Association and dissociation steps were analyzed using Octet® Analysis Studio software version 12.2, results are shown in Table 5.

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

In the kinetic performance comparison of small molecules on modular Octet® systems, the association and dissociation of the analyte furosemide on SSA Biosensors loaded with carbonic anhydrase ligand was studied. As with the large molecule kinetics performance, tight reproducibility between the replicates was seen across the three Octet® R series modules (Figure 21). In this case, we see the phenomenon of fast association and dissociation as evidenced by the steep on and off curves. In addition, comparable loading nm shift, K_D, k_{on} and k_{off} as well as CV%s were seen across the three Octet® R series modules (Table 8). Therefore, we conclude that the three Octet® R series modules show comparable small molecule kinetics performance as well. This set of experiments reiterate the increased time needed for performing the kinetic analysis on the same number of replicates using the Octet® R2 system relative to Octet® R4 and R8 systems (Table 9).

Furosemide Kinetics	Octet® R8	Octet® R4	Octet® R2
K_D (M)	6.07E-07	6.99E-07	4.93E-07
k_n (1/Ms)	6.67E+04	5.96E+04	8.66+04
k_{dis} (1/s)	4.00E-02	4.16E-02	4.26E-02
pm shift at 30 µM	133.69	118.55	151.77
K_D %CV	12.6%	7.9%	4.4%
k_{on} %CV	14.1%	5.4%	7.7%
k_{dis} %CV	6.6%	5.1%	5.1%
pm shift at 10 µM %CV	4.5%	5.3%	2.8%

Table 8: Protein-small molecule binding characterization (carbonic anhydrase–furosemide) comparison chart for the 3 modular Octet® R series instruments.

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

Table 9: Comparison of the Octet® R series experimental run times for protein-small molecule interaction (carbonic Anhydrase binding to furosemide).

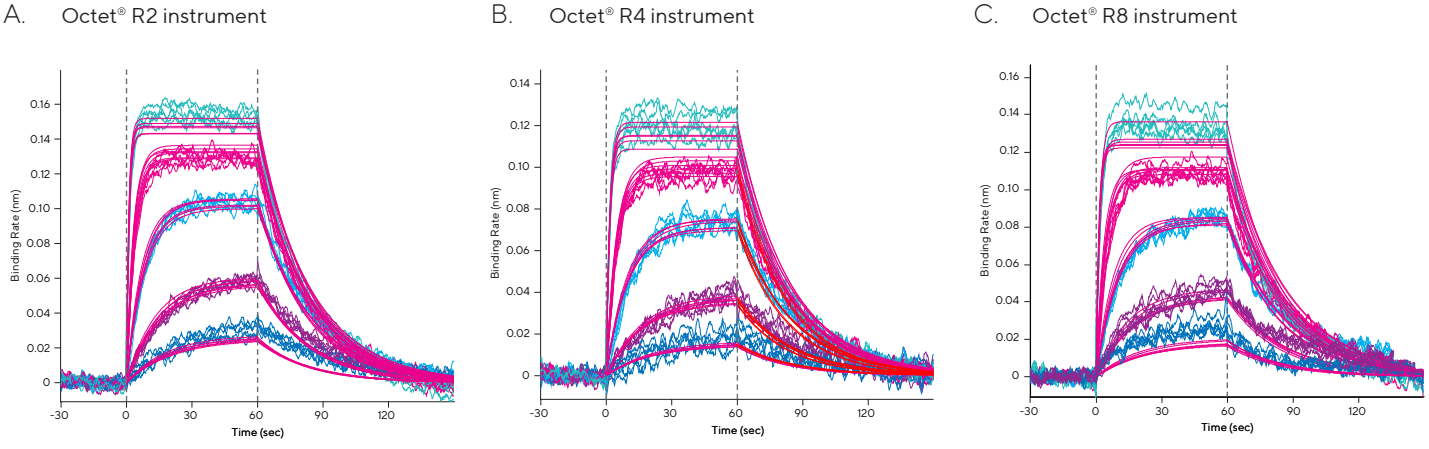

Figure 21: Carbonic anhydrase–furosemide binding characterization comparison data as obtained using the 3 modular Octet® R series instruments. The data represents replicates of a dose response analysis of furosemide dissolved in PBS/0.5% DMSO buffer. Data was later globally fit to a 1:1 kinetics binding model using the Octet® Analysis Studio Software (Table 7).

Conclusion

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

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

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

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