Introducing a New BLI Instrument Focused on Throughput and Flexibility: The Octet® HTX System

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Abstract

Biolayer Interferometry (BLI) technology has become well established over the past decade for protein quantitation and kinetic characterization of molecular interactions. The non-fluidics based Dip and Read™ format of the Octet instrument line lends itself to highly multiplexed reading, and thus to higher throughput than other label-free techniques. As with the entire Octet line of instruments, the Octet HTX system is compatible with the full range of 18 biosensor types including protein A, streptavidin, anti-His_6-tagged and anti-species coated biosensors. In this poster, we present key applications using the newly developed Octet HTX instrument.

The new Octet HTX instrument monitors up to 96 biosensors simultaneously. The high-throughput read-head, in conjunction with new Data Analysis software features, opens up new applications such as epitope binning and allows higher throughput for standard applications such as quantitation. The flexibility to perform demanding applications such as small molecule kinetics is addressed by a high-sensitivity 16 sensor mode within the same instrument. Additionally, the ability to use both modes of acquisition in a single experiment provides a straightforward workflow for immobilization of capture protein onto a full tray of 96 biosensors, followed by high sensitivity quantitation or kinetic experiments without user intervention.

Developing the Octet HTX System

The goal of this development project was to create a high-throughput 96 channel BLI system that provides high quality data for quantitation and kinetics applications. In addition, there was a goal to allow for flexibility in assay modes for demanding applications such as small molecule kinetics and high sensitivity quantitation assays. In order to accomplish these goals, the Octet HTX system was engineered with two modes of operation: a mode that can operate with 32, 48 or 96 biosensors simultaneously at a data acquisition rate of 0.6 Hz and a mode for 8 or 16 biosensors with a 5 Hz data acquisition rate. Data from the two modes are shown below for various applications.

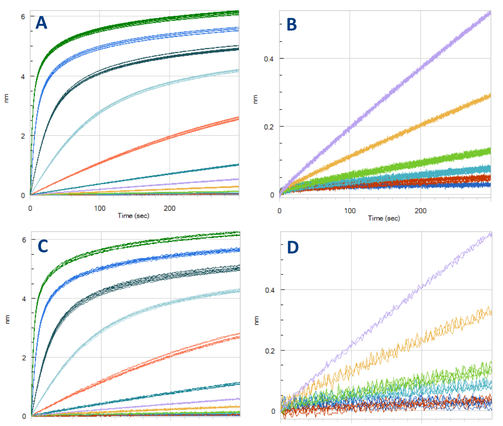
Rapid Quantitation

The speed and performance of the HTX system in quantitation was demonstrated using a simple 1-step quantitation assay. In this assay, Protein A coated biosensors available from ForteBio were used to quantitate human IgG from solution.

Figures 1A-D show real-time data charts for the high sensitivity quantitation of human IgG using Protein A coated biosensors. Human IgG concentrations for this assay ranged from 300 µg/mL to 25 ng/mL with 8 replicates at each concentration. By comparing figures 1B (16 biosensor mode at 5 Hz) and 1D (96 biosensor mode at 0.6 Hz) one can see a difference in the signal-to-noise of the low end of 25 ng/mL (red) and the negative control (blue). Nevertheless, both modes show good precision and recovery throughout the range (**Table 1**). Figures 1A and 1C show the full dynamic range for each mode.

Table 2 shows the total assay time for quantitating hIgG in 576 samples for several techniques. Using the Octet HTX system in a 16 biosensor mode, one can analyze 576 samples in 2.5 hours. Using the 96 biosensor mode, analysis of the same 576 samples requires only 30 minutes.

FIGURE 1A-D: Real time data for binding of hIgG to Protein A biosensors using the 2 acquisition modes. Human IgG concentrations range from 300 µg/mL (dark green) down to 25 ng/mL (red) plus a negative control (blue). The right 2 graphs are enlargements of the lower end of the range (500 ng/mL to 25 ng/mL) to aid in visual analysis. The top two graphs (1A and 1B) show data from the 16 biosensor mode and 1C and 1D show data from 96 biosensor mode. All data was acquired using a 5 minute assay time and a shaking rate of 1000 rpm on the Octet HTX system.



Flexibility for Multi-step Assays

The Octet HTX system allows for a great amount of assay design flexibility. One can program the instrument to perform sequential steps of loading and processing biosensors without user intervention. An example assay for quantitating human IgM down to sub ng/mL levels is shown in **Table 3.** This assay uses the standard ELISA type reagents but in a fully automated, walk-away assay. By taking advantage of the ability to perform some steps (loading, buffer and sample exposure) in a 96 biosensor mode, a full 96 well plate of hIgM samples can be quantitated in just under 2 hours, compared to 4.5 hours for a 16 biosensor mode assay and 5 hours for a plate-based ELISA.

Figure 2 shows the real time data of all steps outlined in Table 3 from the human IgM quantitation assay. This assay covers the dynamic range of 20 ng/mL down to 313 pg/mL of human IgM with 12 replicates at each concentration. Excellent precision and recovery are seen for the entire dynamic range as shown in **Table 4.**

TABLE 1: Recovery and precision for data shown in Figure 1. Each hIgG concentration was run in replicates of 8.

Known conc. (µg/mL)	16 biosensor mode		96 biosensor mode	
	Ave conc. (µg/mL)	%CV	Ave conc. (µg/mL)	%CV
0.025	0.025	7%	0.025	5%
0.05	0.05	6%	0.05	6%
0.1	0.10	2%	0.10	3%
0.25	0.25	2%	0.25	8%
0.5	0.50	3%	0.50	2%
1	1.00	2%	1.00	4%
3	3.00	2%	3.00	3%
10	9.99	2%	10.02	2%
30	30.05	2%	30.10	3%
100	100.39	2%	100.48	3%
300	300.00	2%	300.00	2%

TABLE 2: Calculated assay times for quantitation of human IgG using various techniques.

Assay to quantitate hIgG	Assay time for 576 samples	Details on assay
ELISA	4-5 hours	6 ELISA plates handled simultaneously
HPLC	148 hours	576 injections (15 min each)
Octet HTX system in 16 biosensor mode	**2.5 hours**	**1 X dual 384 well plate assay (2 min read time, 400rpm)**
Octet HTX system in 96 biosensor mode	**30 minutes**	**1 X dual 384 well plate assay (2 min read time, 400rpm)**

TABLE 3: Assay times for the IgM assay in 16 biosensor mode and in the faster mixed mode available with the Octet HTX system.

Step	All in 16 channel mode		Mixed mode	
	# of sensors in parallel	Time (sec)	# of sensors in parallel	Time (sec)
Load biotin-anti-IgM	16	600	96	600
Buffer	16	30	96	30
IgM containing sample	16	1200	96	1200
Buffer	16	30	16	30
Anti-IgM- HRP	16	300	16	300
Buffer	16	30	16	30
Metal DAB	16	300	16	300
TOTAL TIME		**4.5 hours**		**1.75 hours**

FIGURE 2: Real-time data for all steps.

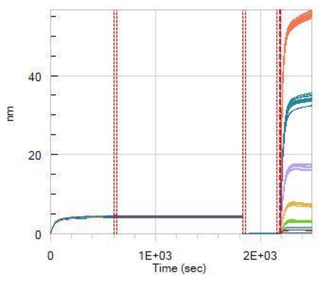


TABLE 4: Recovery and precision for data shown in Figure 2.

Sample of human IgM (ng/mL)	Precision (%CV n=12)	% Recovery
20	1%	100%
10	3%	101%
5	3%	100%
2.5	3%	100%
1.25	4%	100%
0.625	4%	100%
0.313	4%	100%

Large Scale Epitope Binning

The cross-competition matrix between a panel of 63 different monoclonal antibodies (mAbs) was generated using the 32 channel mode of the Octet HTX system in just over 24 hours. An in-tandem assay format was adopted, wherein the antigen was first captured onto the anti-His biosensor and then a panel of monoclonal antibodies against the antigen (subsequently referred to as mAb-1) was allowed to saturate the antigen surface. This was followed by the binding of second monoclonal antibody (mAb-2) to the antigen-mAb-1 complex. If mAb-2 binds to antigen-mAb-1 complex, then mAb-1 and mAb-2 have distinct binding epitopes on the antigen. If mAb-2 does not bind, then both the mAbs have similar or overlapping epitope.

The real time binding data from a single assay with 32 anti-His biosensors is shown in **Figure 3**. This data shows repeated cycles of His-tagged antigen loading, mAb-1 binding and mAb-2 binding followed by regeneration to remove the captured antigen from the sensor surface. Using new features in the Data Analysis software for the HTX system, the binding signal for each step was determined and cross-competition matrix was generated (**Table 5**) where mAb-1 ID is listed as column on the left and mAb-2 ID is represented in row on the top of the matrix. Antibodies competing in both directions, independent of the order of binding are highlighted with red boxes and white font, while uni-directional competitions are highlighted in orange. No competition between antibodies suggestive of distinct binding epitopes is represented as white box with black font while self-self competition is colored in light grey with black font.

FIGURE 3: Example real time data from the 64X64 epitope binning matrix. Each assay was a repeat of the following steps: Loading of antigen for 90 seconds; saturation with mAb-1 for 210 seconds, competing with mAb-2 for 120 seconds. The anti-His sensors were regenerated between each cycle with 10 mM Glycine pH 2.0. All steps were performed at 25°C in HBSP kinetics buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 0.05% v/v Surfactant P20, 0.1 mg/mL BSA) with the shaking rate of 1000 rpm.

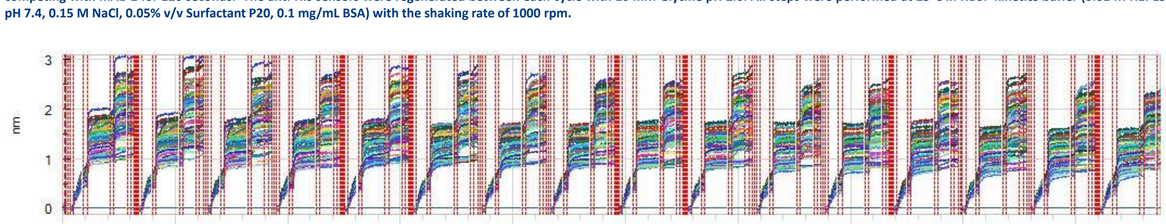


TABLE 5: The real time binding response was monitored during the course of the experiment and the binding response at the end of every step was recorded with new Data Analysis features. The response of mAb-2 binding to antigen pre-complexed with mAb-1 was compared and competitive/non-competitive behavior of different monoclonal antibodies was determined as shown in the matrix below. Cells highlighted in red indicate bi-directional competition, while uni-directional competitions are highlighted in yellow. Self-self competition is colored in grey, and white cells represent non-competing mAbs.

New mAbid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
1	0.22	0.00	0.00	0.02	0.01	0.01	0.02	0.00	0.44	0.47	0.51	0.48	0.49	0.54	0.38	0.43	0.44	0.48	0.46	0.50	0.49	0.48	0.51	0.45	0.48	0.44	0.47	0.49	0.50	0.45	0.41	0.37	0.48	0.46	0.47	0.37	0.50	0.47	0.44	0.36	0.42	0.42	0.41	0.41	0.44	0.46	0.44	0.43	0.45	0.43	0.43	0.40	0.42	0.39	0.38	0.33	0.37	0.40	0.38	0.40	0.39	0.17	0.21	-0.01
2	0.01	0.21	0.01	0.01	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.02	0.01	0.01	0.01