



## **Applications of ForteBio Systems in Downstream and GMP Environments**

**October 30th, 2013**

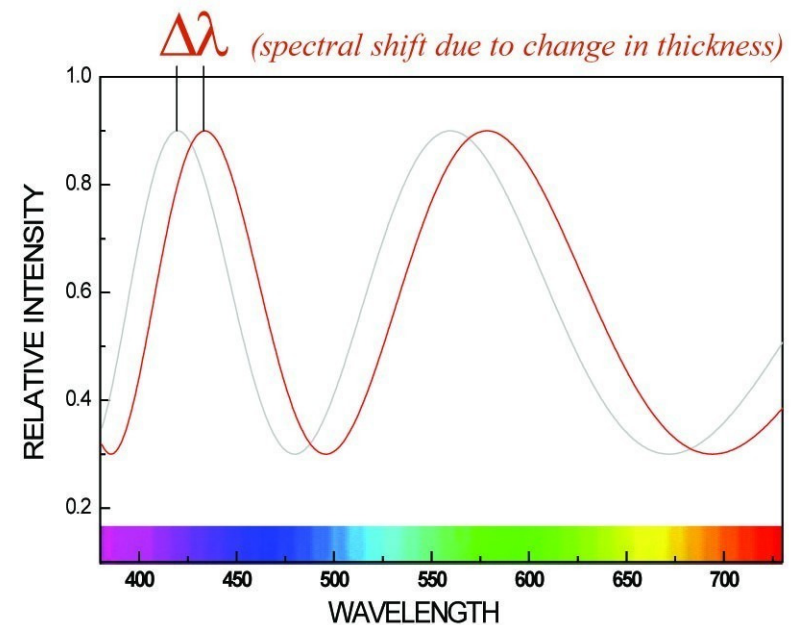
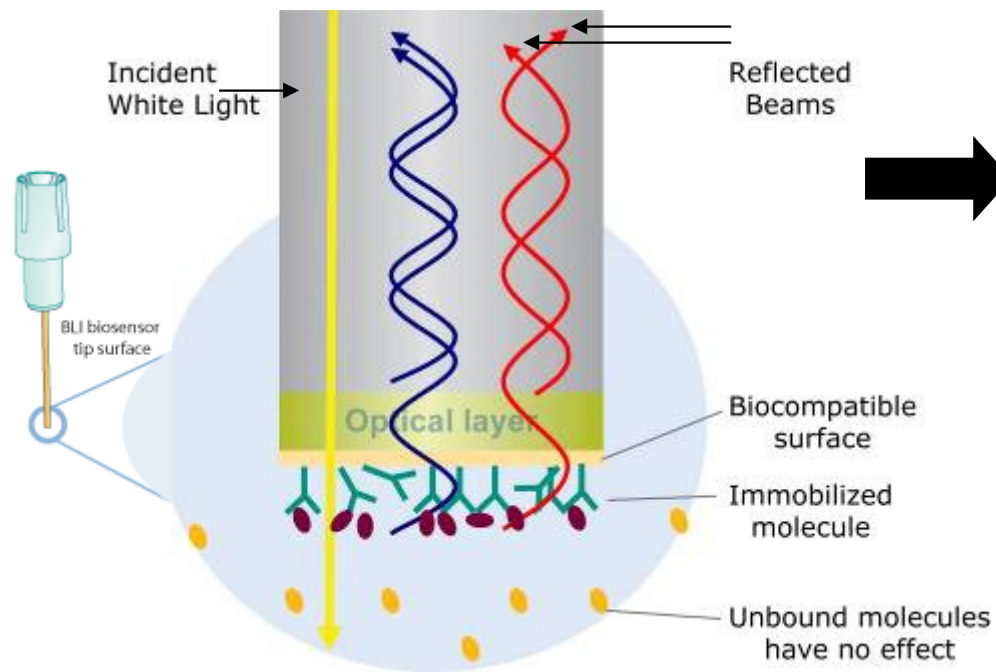
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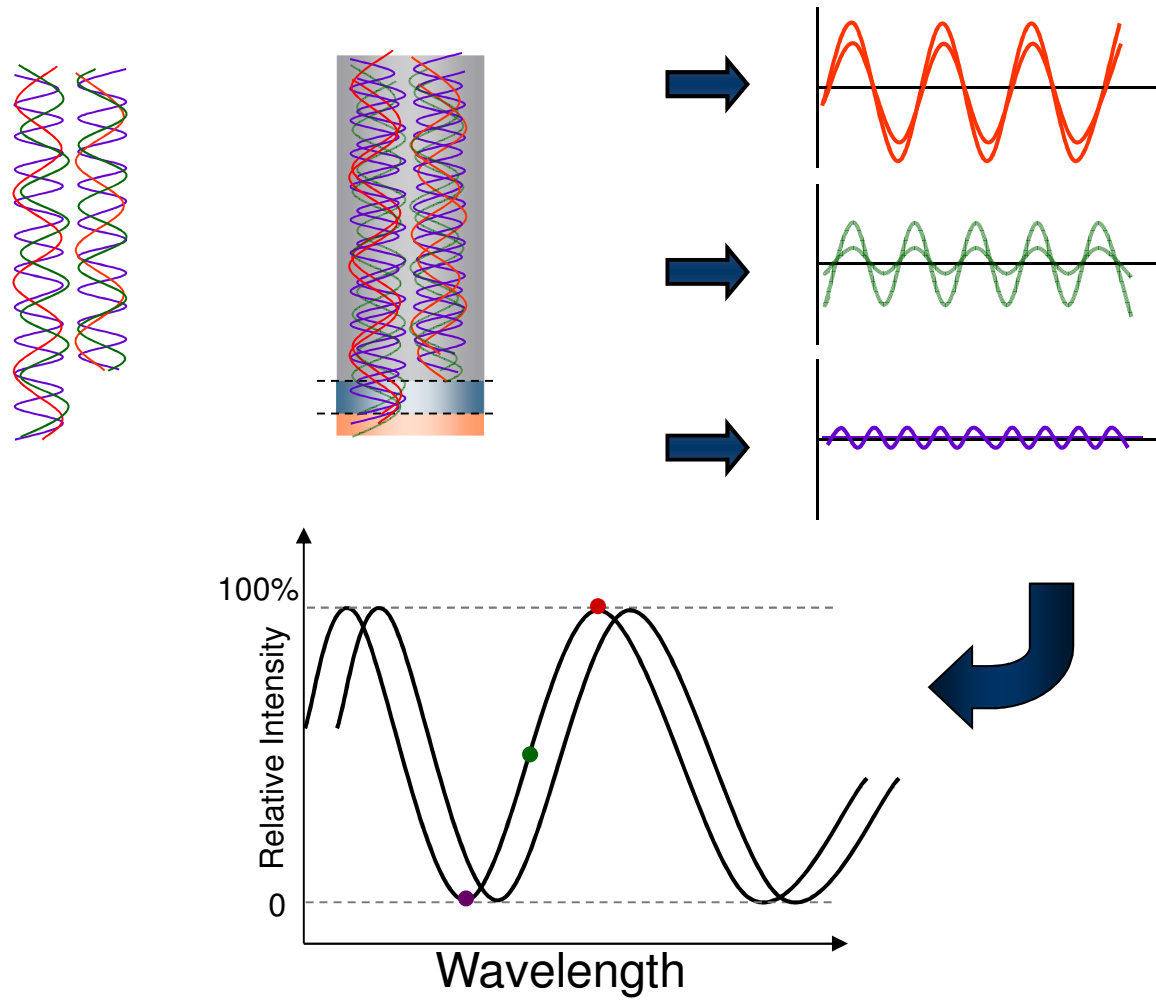
# BioLayer Interferometry

- Optical layer reflects simple white light; second reflection from tip of biosensor, both reach detector
- Analyte binding changes thickness of bio-layer, which is measured at detector



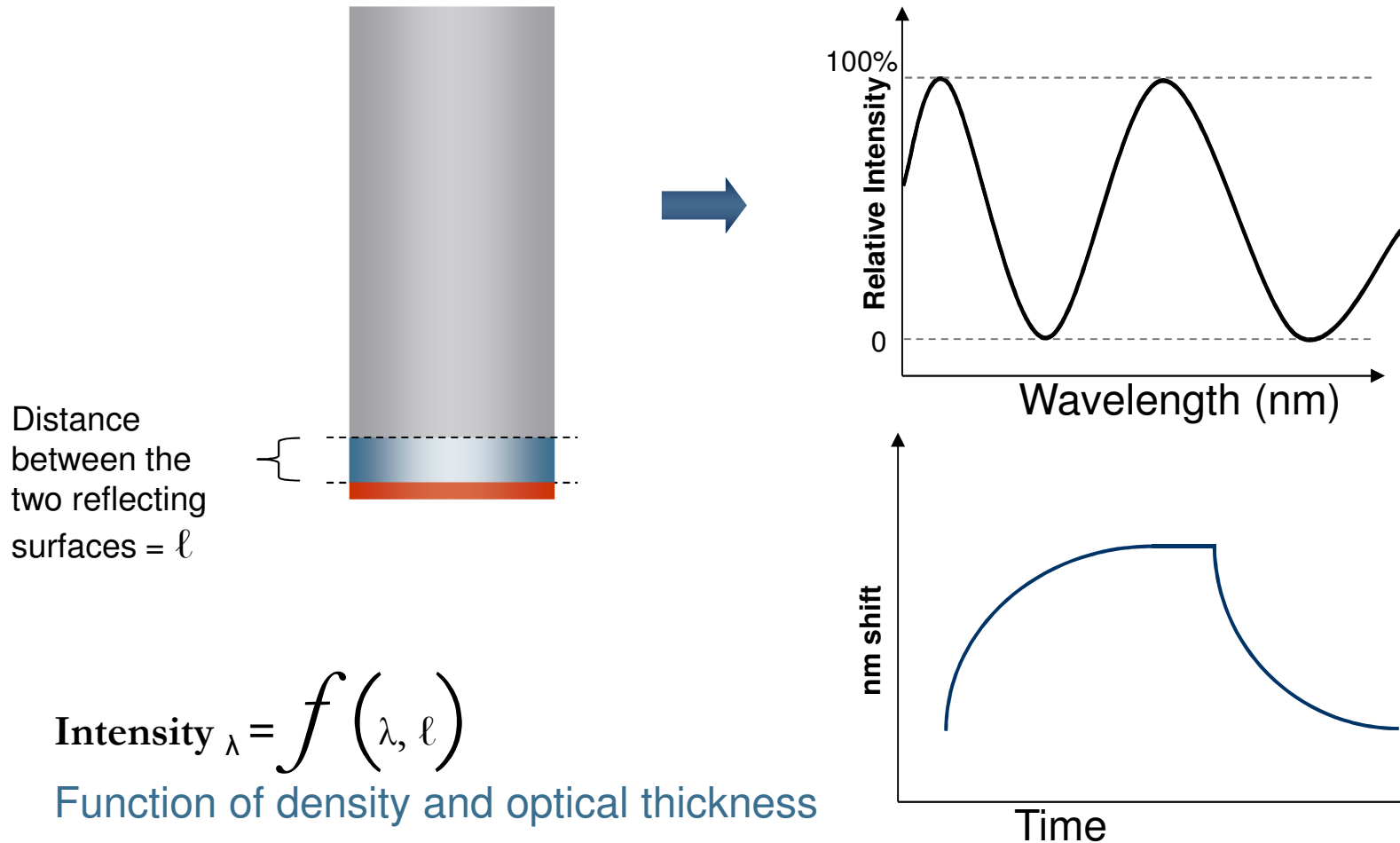


## Increase in Molecular thickness changes the interferometric pattern



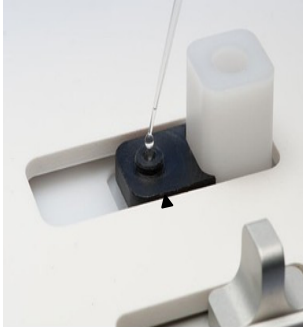


# Monitoring the Interference Pattern vs. Time Provides Kinetic Data on Molecular Interactions





# BLitz : The personal Analyzer



Label-free Assays in a Drop





# Octet Systems and Features

**16 Channel Read Head**  
**96 and 384 Well Microplates**  
 **$\geq 40 \mu\text{L}$  Assay Volume**  
**2 Microplate Positions**  
**Biosensor Reracking**  
**Automation Compatible**

## **Octet Red Series** **Analyte MW: $> 150 \text{ D}$**



**RED384**

## **Octet QKe Series** **Analyte MW: $> 2 \text{ kD}$**



**QK384**

**8 Channel Read Head**  
**96 Well Microplates**  
 **$\geq 180 \mu\text{L}$  Assay Volume**  
**1 Microplate Position**  
**Biosensor Reracking**



**RED96**



**QKe**



# Octet HTX – Unmatched Performance and Speed



Flagship of the Octet Line

Epitope Binning: 2-8 x Faster

Full Plate Quantitation in 2 Min

Full Plate Kinetic Screen in Mins, Not Hrs



# Current Biosensors

## Flavored Biosensors:

Anti-Human IgG Fc  
Anti-hIgG Fc Capture Surface (AHC)  
Anti-Murine IgG Fv  
Anti-Murine IgG Fc Capture (AMC)  
Protein A  
Protein G  
Protein L  
Anti human IgG FabCH1

## Current Biosensor Kits:

Immunogenicity (ADA)  
Custom HCP

## Generic Biosensors:

Streptavidin (SA)  
Super Streptavidin (SSA)  
Anti-FLAG  
Amine Reactive 2nd Generation (AR2G)  
Aminopropylsilane (APS)  
Anti-Penta-HIS (HIS2)  
Ni-NTA  
Anti-GST



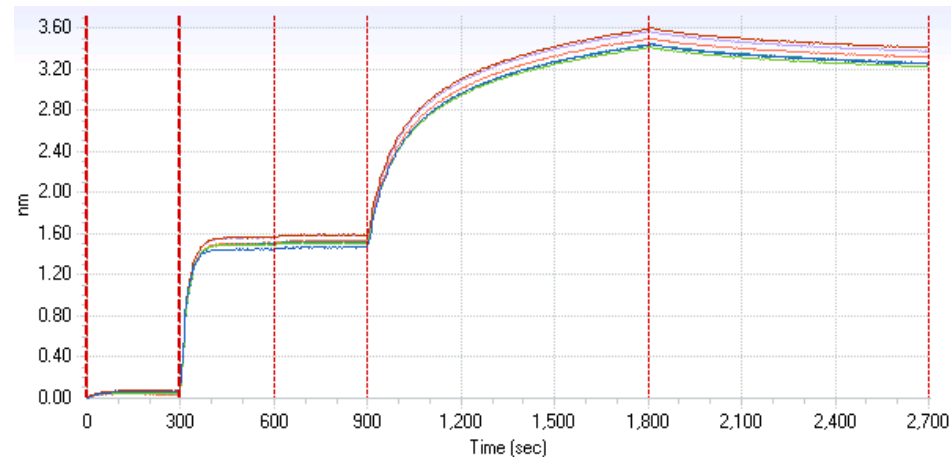
Residual Protein A



# Information Provided by Octet Platform

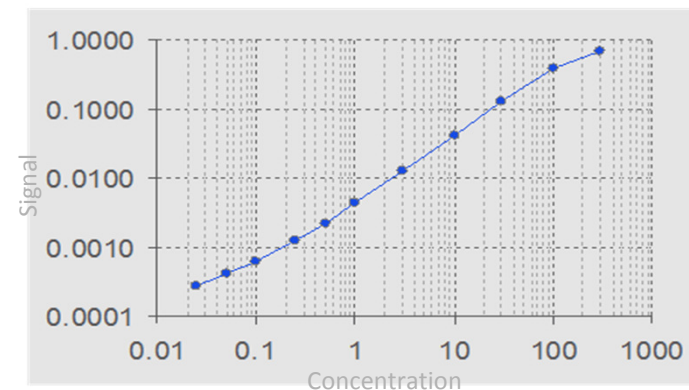
- Affinity characterization of proteins, antibodies, and small molecules

- Binding  $K_D$
- On and off rates
- Rank order
- Yes / No kinetic screening
- Epitope binning / mapping



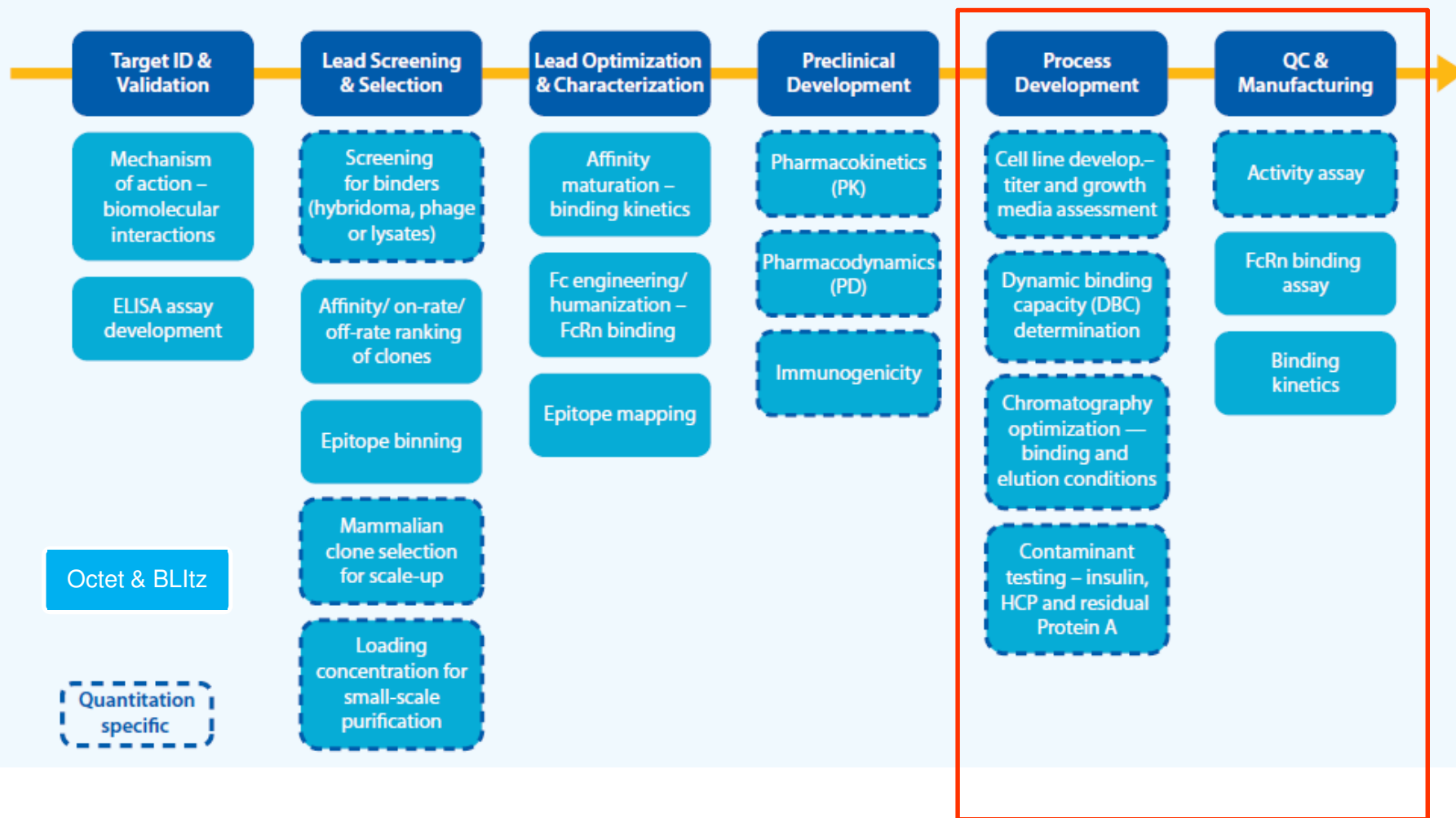
- Protein/Antibody Concentration

- Picogram to milligram dynamic range
- Contaminant testing
  - Immunogenicity
  - Residual Protein A Detection
  - HCP Detection





# Octet & BLItz Applications in the Drug Development Process





# Database of Publications and Presentations

Main navigation  
by application

PRODUCTS APPLICATIONS **BIOPHARM PORTAL** LEARNING ORDERING SUPPORT NEWS/EVENTS COMPANY GET QUOTE

■ Portal Home  
Target Identification  
Lead Identification  
Lead Optimization  
PKPD  
Upstream Processing  
Downstream & Mfg  
Vaccines & Viruses  
Small Molecules  
Publication Search

## Welcome to the Biopharm Learning Portal

*The Latest in Biopharm Discovery, Development, and Bioprocessing*

ForteBio systems have become indispensable tools at biopharmaceutical companies worldwide, resulting in a steady stream of scientific publications and presentations. This online resource will help you stay current on the latest studies featuring data from the Octet® and BLItz® systems. Fresh publications and presentations are captured as they become available, and can be browsed by application area or pinpointed by keyword search.

**SEARCH ARTICLES**

Keyword:

[Browse all publications](#)  
[Search by application](#)

**JUST ADDED**

- Self-Antigen Recognition by Follicular Lymphoma B Cell Receptors  
*Blood*, 120(20), 4182-90, 2012, K.L. Sechen, et al. [\[show details\]](#)
- Cross-Neutralization of Influenza A Viruses Mediated by a Single Antibody Loop  
*Nature*, 489(7417), 526-32, 2012, D.C. Ekiert, et al. [\[show details\]](#)
- Structural Insights into SUN-KASH Complexes Across the Nuclear Envelope  
*Cell Research*, 22(10), 1440-1452, 2012, W. Wang, et al. [\[show details\]](#)

**FEATURED ARTICLES**

**Optimization of Affinity, Specificity and Function of Designed Influenza Inhibitors Using Deep Sequencing**  
*Nature Biotechnology*, 30(6), 543-548, 2012, Whitehead, T.A., et al.  
The authors use sequence-function maps generated from deep sequencing studies to optimize the binding affinities of two computationally-designed protein inhibitors of H1N1 hemagglutinin. High-affinity variants with subnanomolar affinities were obtained using these computational methods. The Octet RED platform was used to study binding specificities for the two inhibitors against a panel of 16 hemagglutinin subtypes. Additional experiments demonstrated that the high-affinity variants bound to group 1 influenza viruses and neutralized H1N1 viruses in cell culture studies. Really impressive work!  
[PubMed](#)

**The Protein Zfx5 Binds and Stabilizes mRNAs with AU-rich Elements in Their 3 prime Untranslated Regions**  
*J Biological Chemistry*, 287(30), 24967-24977, 2012, He, G., et al.  
The first publications featuring the BLItz system are starting to appear. In this work, the BLItz system is used to determine kinetic rate constants for binding of a protein containing two zinc finger domains to AU-rich elements of mRNA. Identification of novel factors that are involved in regulating cytokine mRNA stability and capable of attenuating host

Highlights new  
publications

Search  
functions

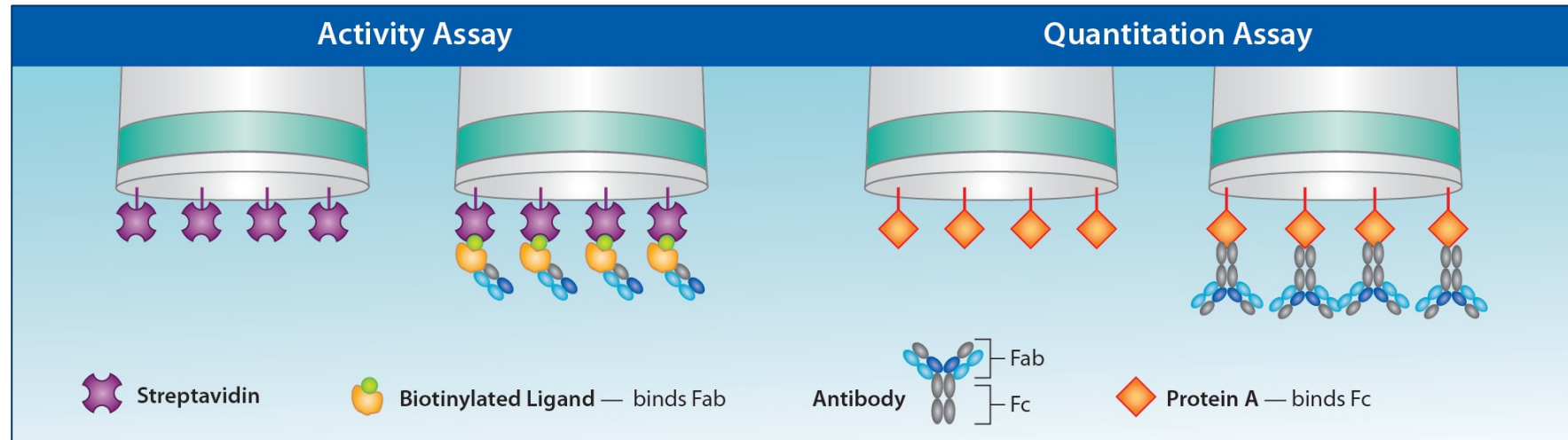


## Today's Topics

- Titer Assays
- Process Development
- Process Troubleshooting
- Bioprocess Impurity Analysis
- Lot Release Activity Assays



## Two Primary Assay Formats

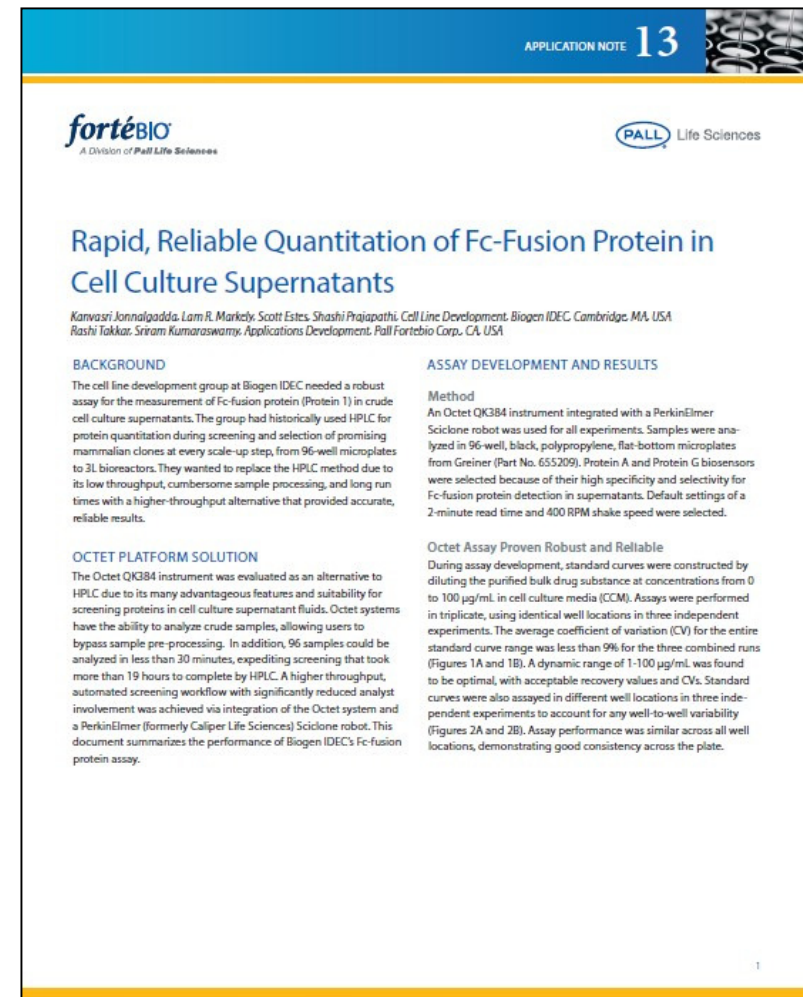


Full menu of biosensor surface chemistries



# Octet Titer Assays Are Migrating Downstream

- Primary reason: <30 minutes per 96-well plate
- Biogen IDEC study:
  - Fc-fusion protein
  - Protein G HPLC protocol required 19 hours/96 samples
  - Application Note 13 presents Octet-HPLC qualification study design
    - Plate location
    - Culture media concentration
    - Dilution linearity
    - Intermediate precision

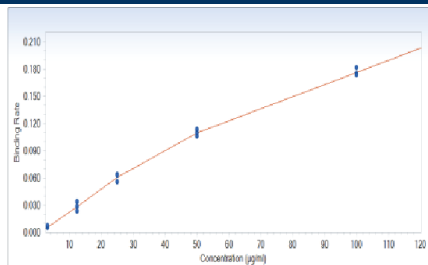




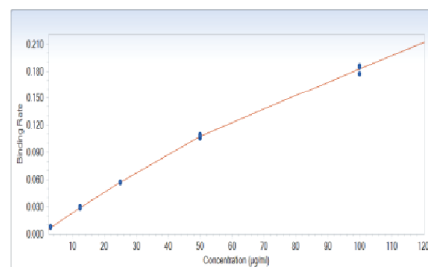
# Cell Culture Media Had No Impact on Quantitation

*Data courtesy of Biogen IDEC*

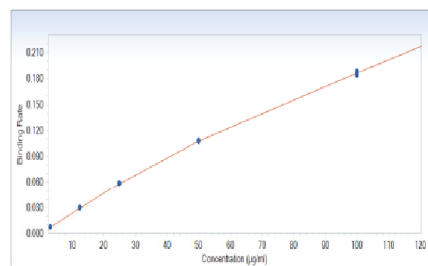
Neat



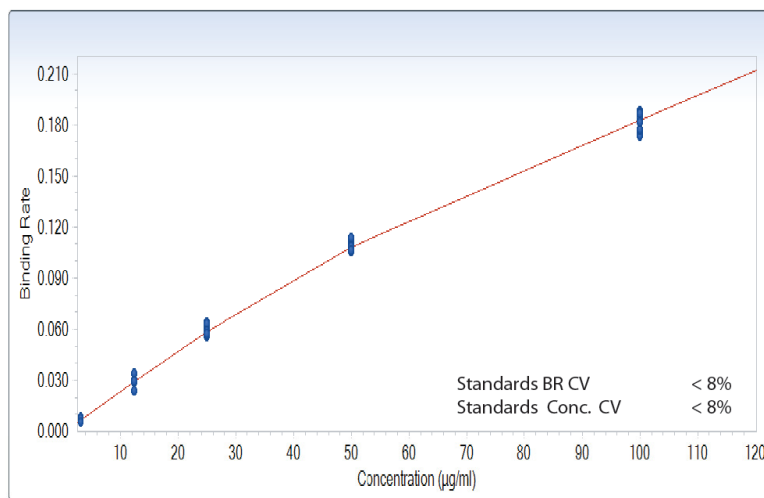
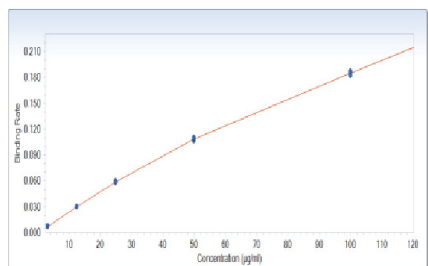
2F dil  
(50% CCM)



4F dil  
(25% CCM)



8F dil  
12.5% CCM

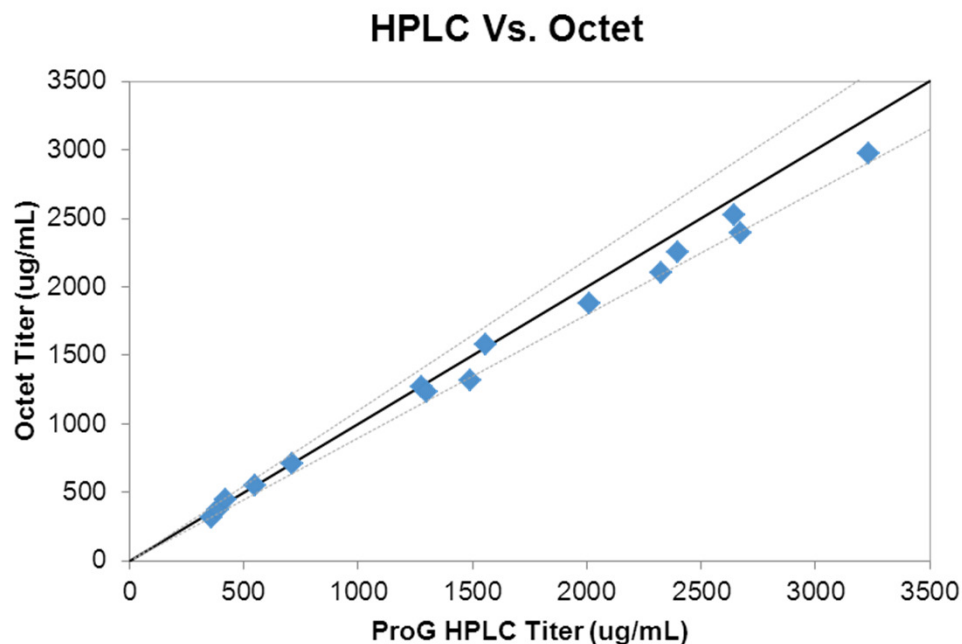


Pooled Data



# <10% Variation Between HPLC and Octet

*Data courtesy of Biogen IDEC*

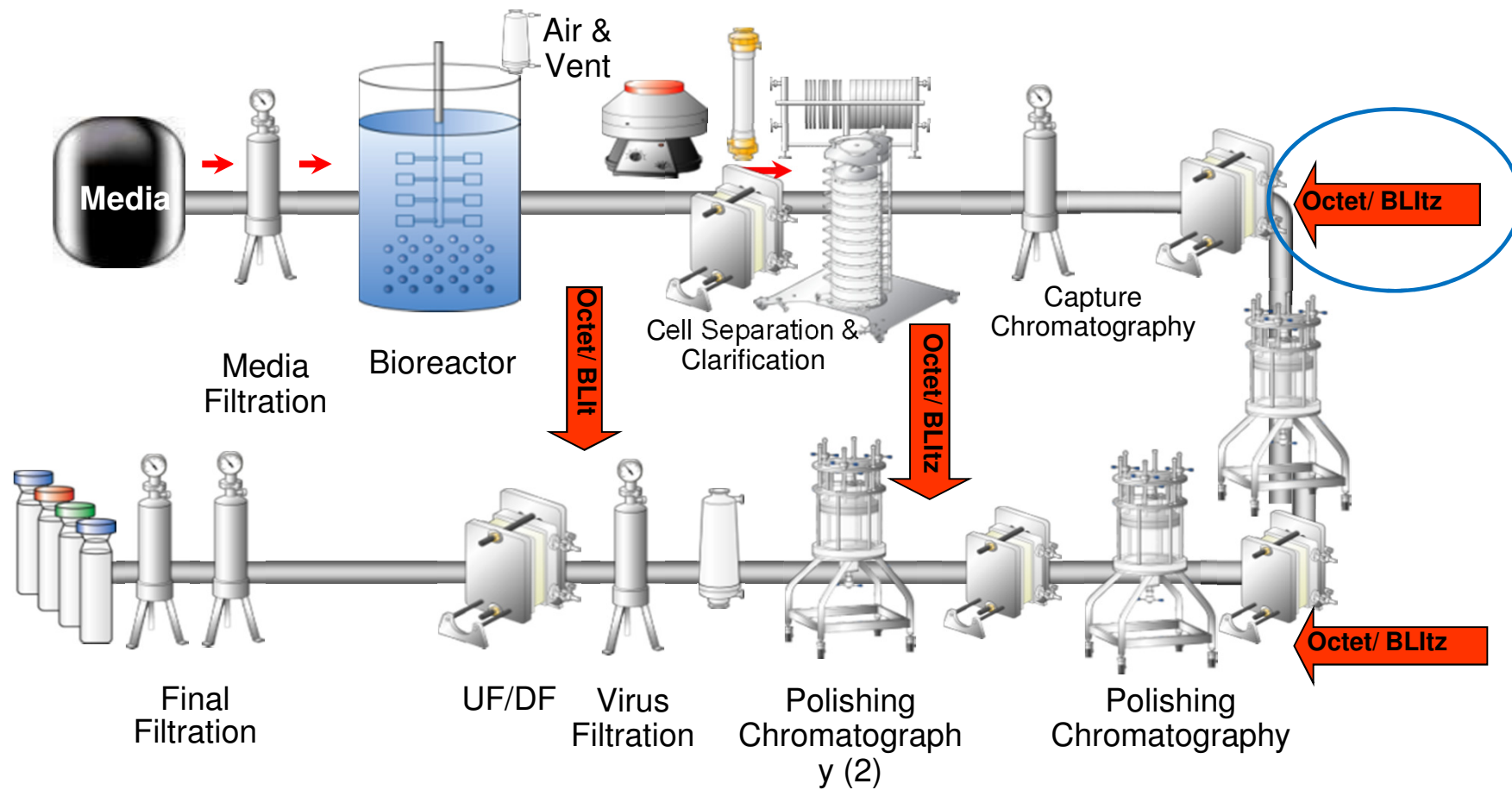


Sample ID	ProG HPLC (ug/mL)	Fortebio Octet (ug/mL)	% Difference
S1	357	318	11%
S2	2672	2395	10%
S3	388	373	4%
S4	421	448	-6%
S5	2397	2254	6%
S6	1275	1266	1%
S7	1299	1235	5%
S8	1493	1313	12%
S9	2645	2521	5%
S10	1555	1578	-1%
S11	2327	2104	10%
S12	2009	1883	6%
S13	710	708	0%
S14	551	545	1%
S15	3233	2971	8%

% variation between LC & Octet <10%



# Optimization of Purification Processes





# Dynamic Binding Capacity (DBC) Determination

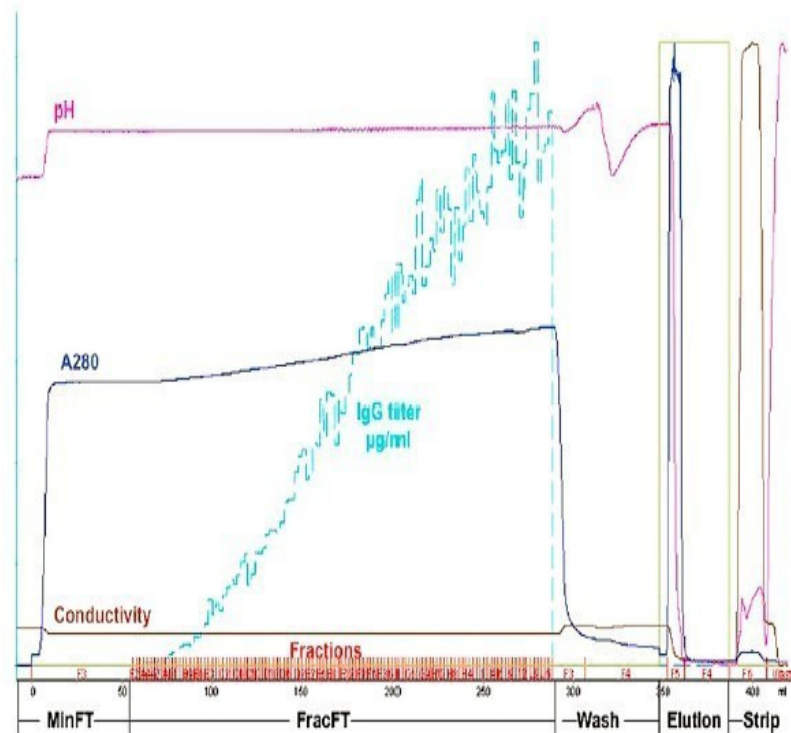


Fig. 3. Typical chromatogram from purification of cell culture supernatant on protein A resin. Fractions were collected and tested by biolayer interferometry for IgG concentration. These concentrations are plotted (in light blue) directly on the chromatogram. Dynamic binding capacity was calculated based on the first fraction for which IgG was detectable. FT, flowthrough; Min FT, earliest flowthrough fraction in which IgG was detectable by biolayer interferometry.

Tri Do et al., *A rapid method for determining DBC of resins for the purification of proteins. Protein Expression and Purification.* 60 (2008) 147-150.

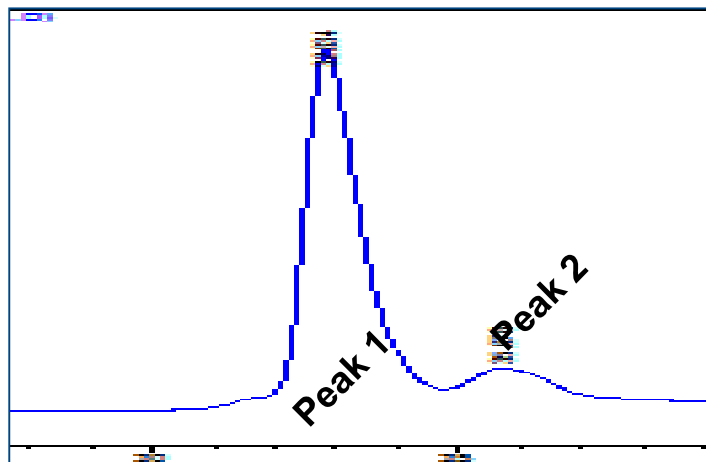


# Process Troubleshooting

*Data courtesy of Aragen Bioscience*

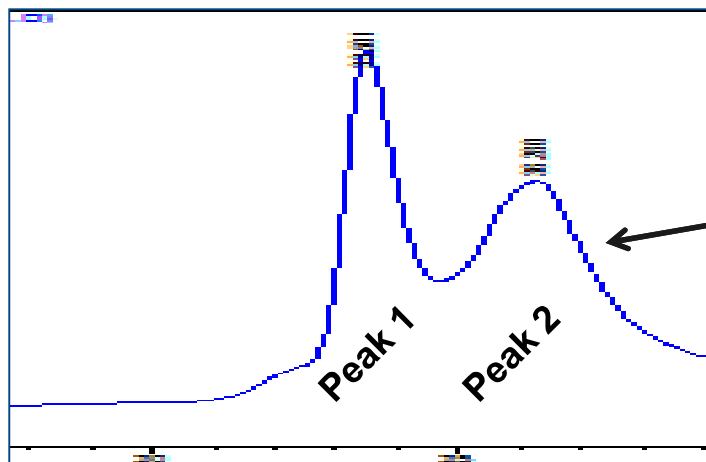
**Lot 1**

**High  
Specific  
Binding  
Activity**



**Lot 2**

**Low  
Specific  
Binding  
Activity**



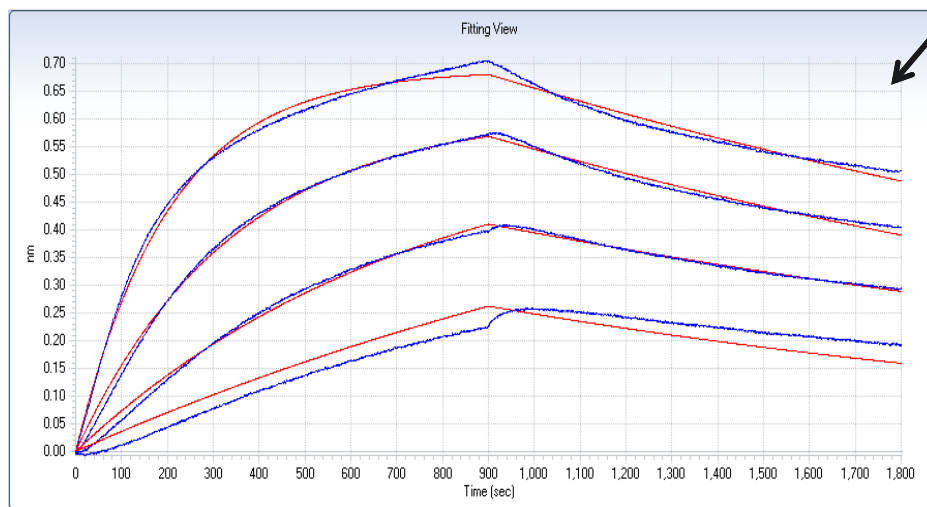
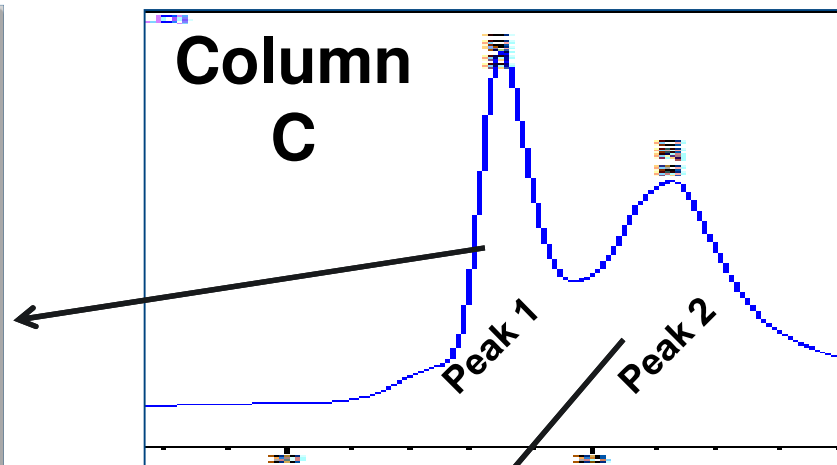
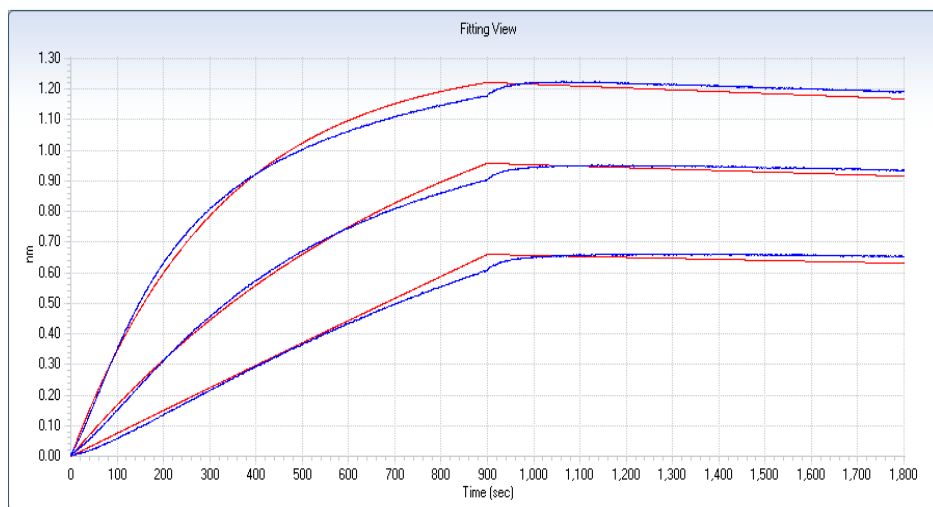
**Presence of large  
second peak**

*Ref: O Beske, The Three A's of Biologicals: Activity, Activity, Activity: Guidung Process Development Using Bioactivity Measurements, Bioprocessing Summit, August 2011*



## Peak 2 in New Lot – Less-active Fraction

*Data courtesy of Aragen Bioscience*

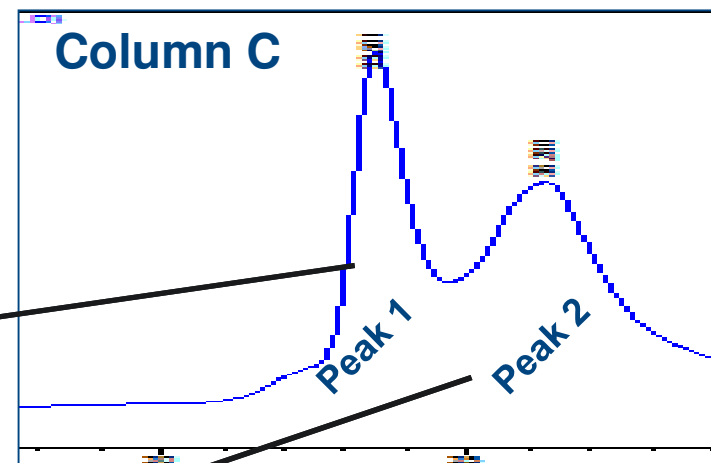
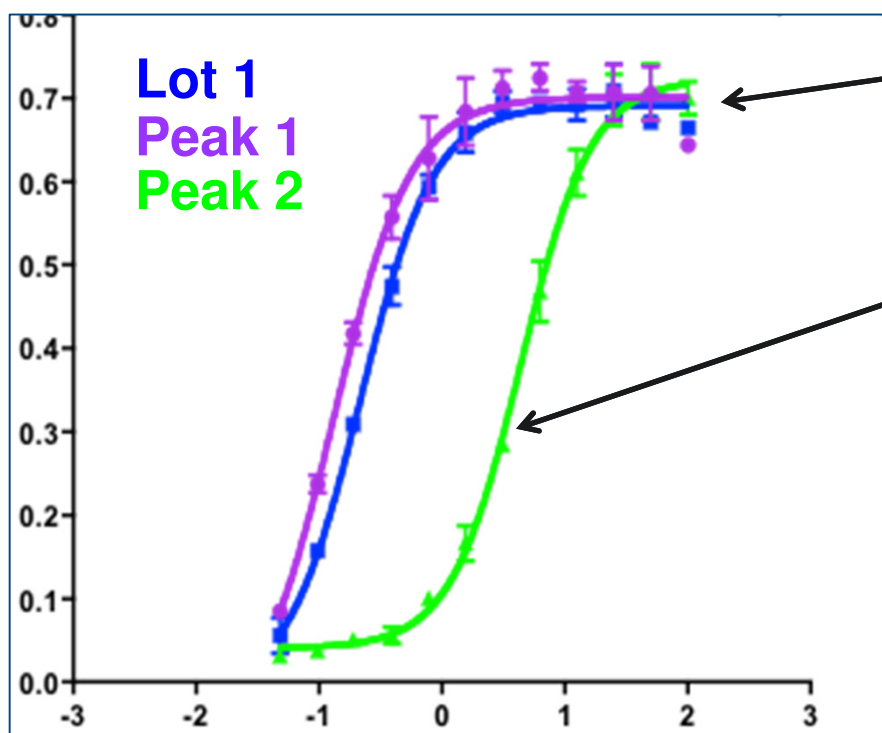


**Less-active  
fraction exhibited a  
lower on-rate and  
higher off-rate**



## Confirmed by Cell-Based Assays

*Data courtesy of Aragen Bioscience*



The reduced binding fraction had 100-fold reduced cell based activity



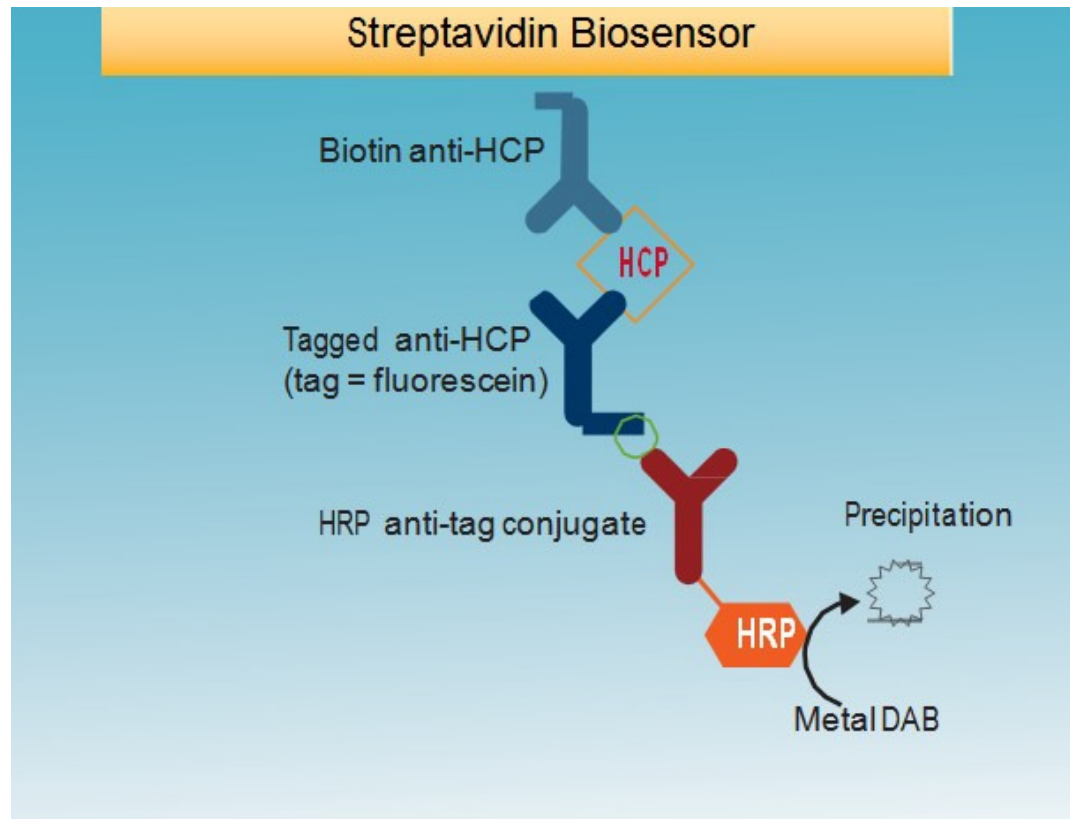
# Bioprocess Impurity Analysis

- Host Cell Proteins (HCP) are produced or encoded by the organisms and unrelated to the intended recombinant product
  - Heterogeneous family
  - Few hundreds daltons to few hundreds kDa
  - In mammalian cells, as CHO cells, >10,000 proteins
  - In bacteria, as E.Coli, >1,000 proteins
  - Safety risk
  - Can be used as a marker of the purification process efficiency
  
- Residual Protein A
  - Derived from affinity chromatography columns
  - Required test for monoclonal antibody products



# Octet HCP Assay Format

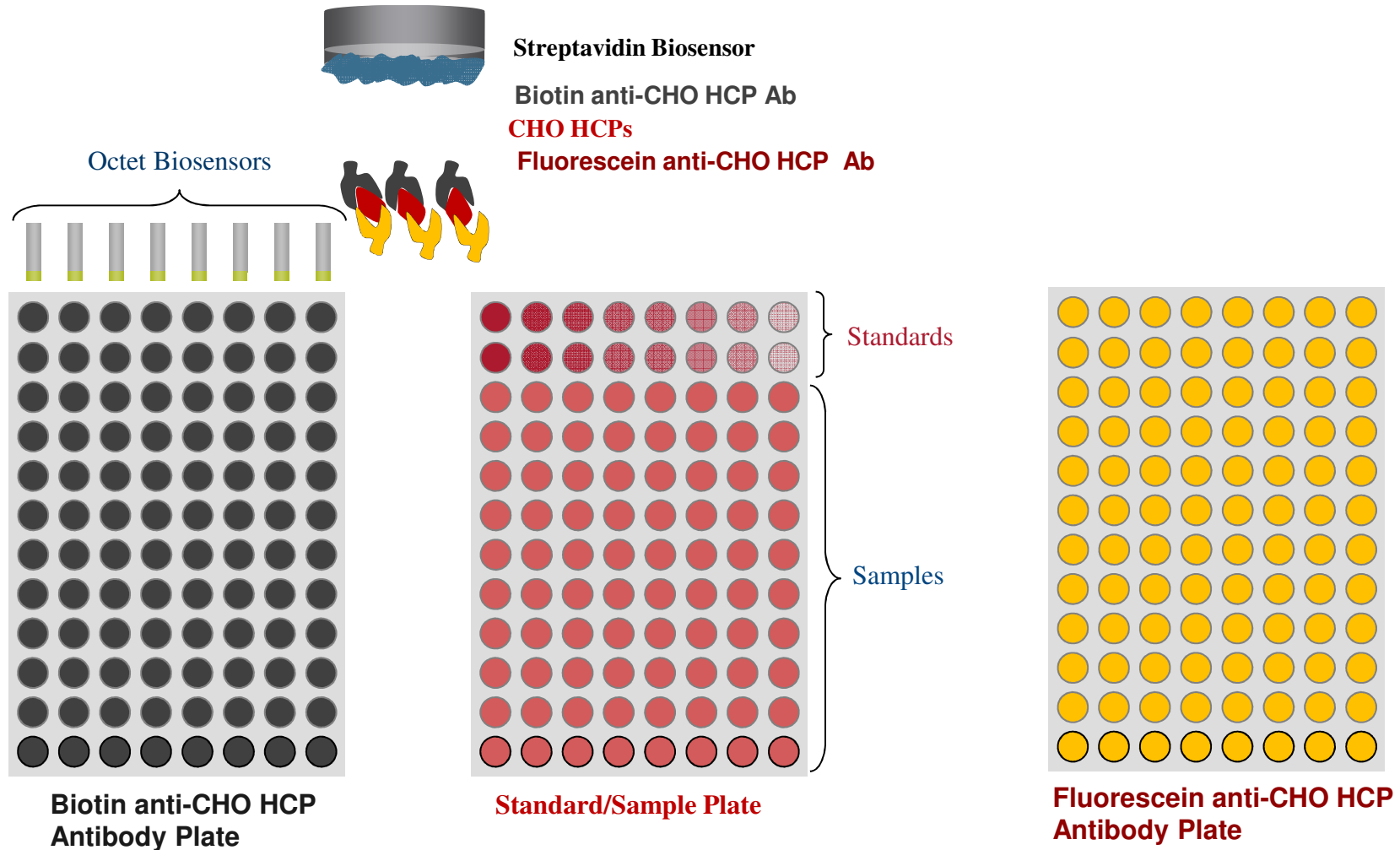
- Multistep
- Amplified detection
- Detection range:
  - 0.5 to 250ng/ml





# CHO HCP Octet Workflow

## Assay Steps on Shakers



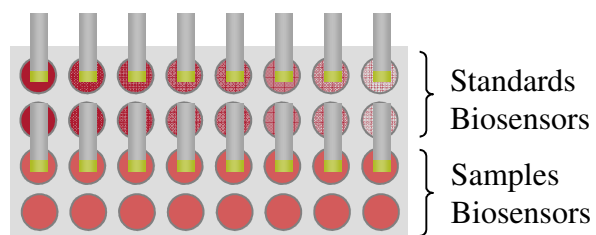
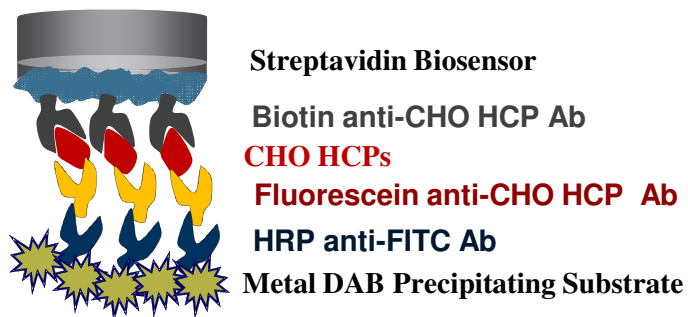
- Entire rack of 96 sensors treated by dipping into plate
  - No washing steps needed between steps

Ref: Dan Schuessler, GSK

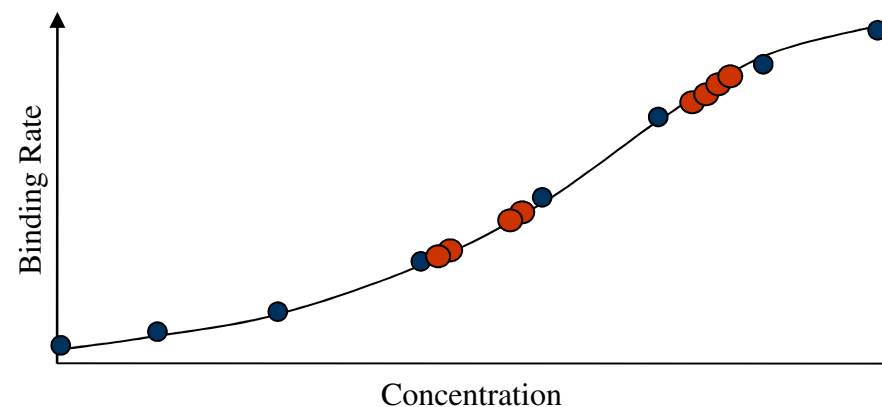
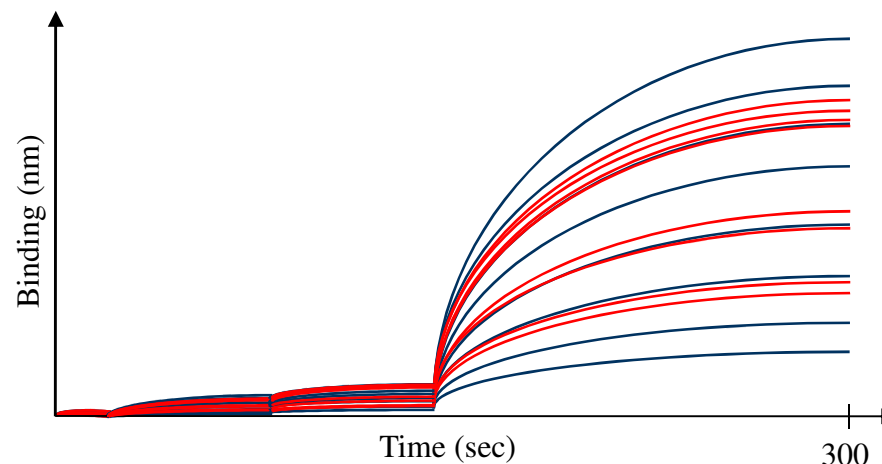
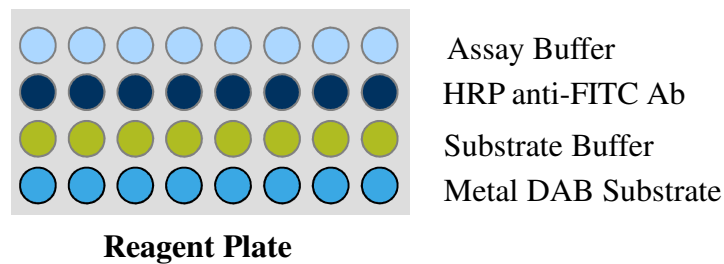


# CHO HCP Octet Workflow

## Assay Steps in Octet QK384



Biosensors from Shakers



Ref: Dan Schuessler, GSK



# Automated Octet Assay for HCP



Switching Assay Plates on Custom Octet Plate Shakers



Octet Instrument Loading

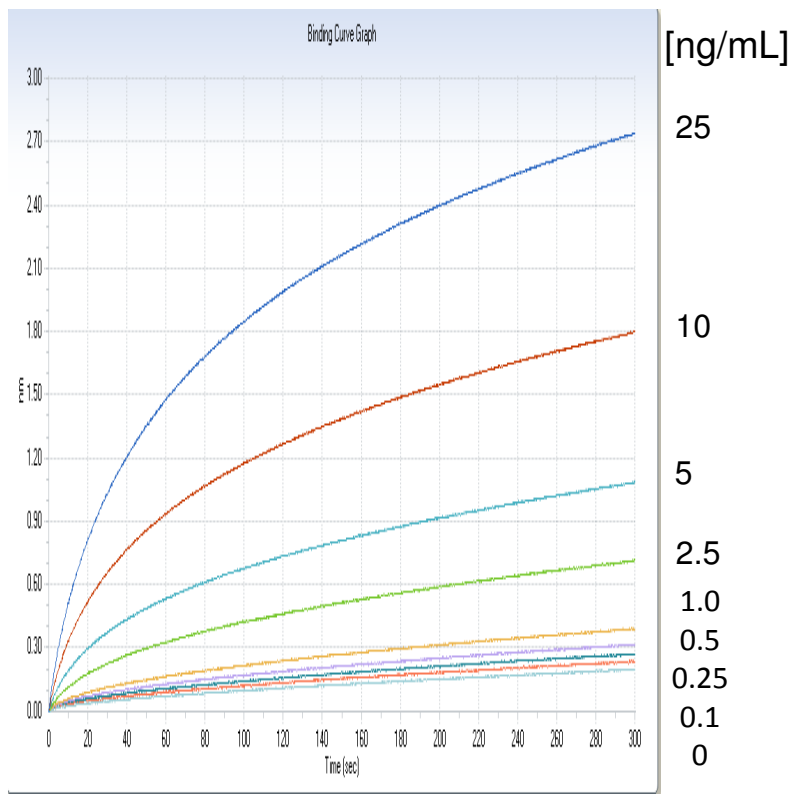


Benefit	Details
<b>Precision</b>	Liquid handling robot reduces pipetting variation inherent in manual pipetting
<b>Reliability</b>	Method performed exactly the same each time
<b>Streamlined Process</b>	Worklist drives robotic method and creates sample plate importation files Robotic method automatically creates & executes Octet method file
<b>Walk Away</b>	No analyst intervention needed to complete method after instrument loaded and diluent volumes are checked
<b>Washing Steps</b>	No washing steps needed & plate washer integration not required
<b>Analyst Involvement</b>	Automated Octet → ~30 minutes for 1-3 assay plates Manual ELISA → ~2.5 hours per assay plate
<b>Throughput</b>	3 assay plates can be run in ~5 hours - 38 samples/plate in duplicate wells → 108 samples in 3 plates
<b>Antibody Consumed</b>	Re-use of coating antibody can significantly reduce consumption over multiple assay plates



# Residual Protein A Contamination Assay

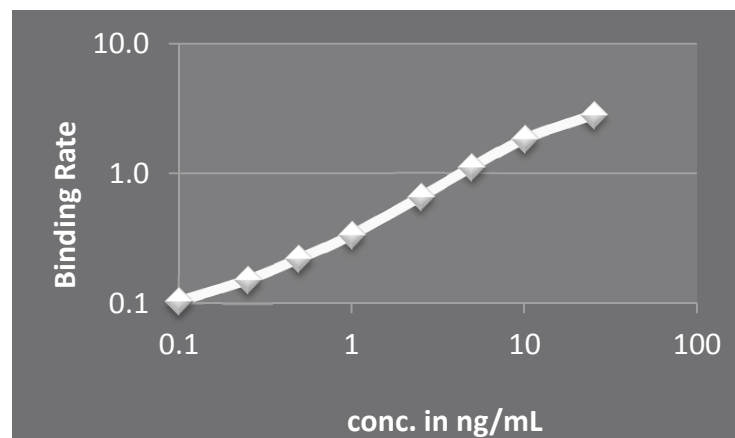
## Standard Curve



All dilutions include 0.5 mg/mL human IgG

Assay LLOD = 43 pg/mL

Concentration of Protein A/ MabSelectSure (N=4)		
ng/ml	Binding Rate	%CV
0.1	0.10	4.2%
0.25	0.15	6.1%
0.5	0.22	3.6%
1	0.33	3.4%
2.5	0.66	5.4%
5	1.11	3.6%
10	1.84	2.2%
25	2.82	1.5%





# Activity Assays in PD and Manufacturing

## Rapid Assessment of Fab Activity Using Octet BioLayer Interferometry (BLI)

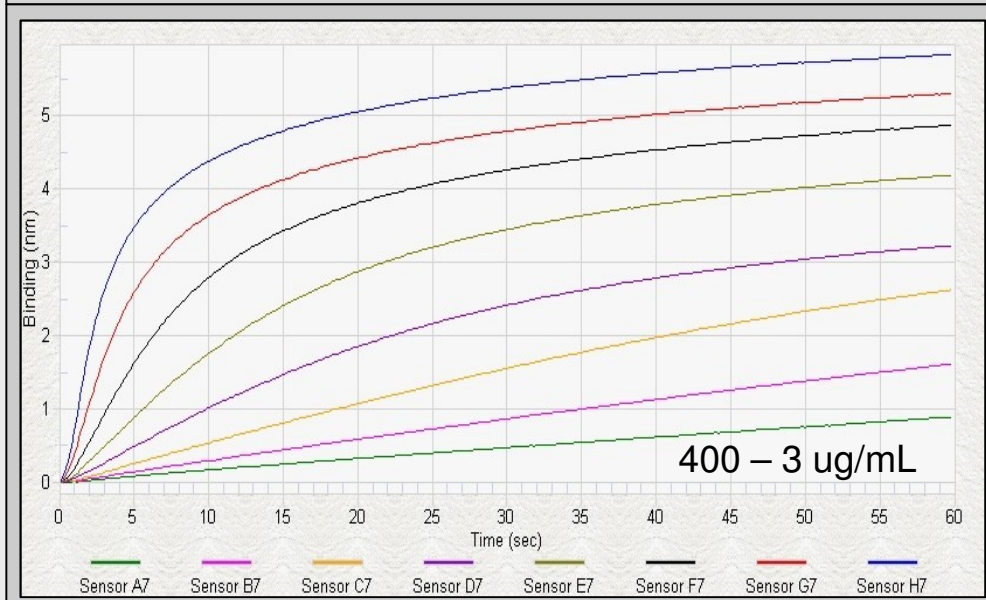
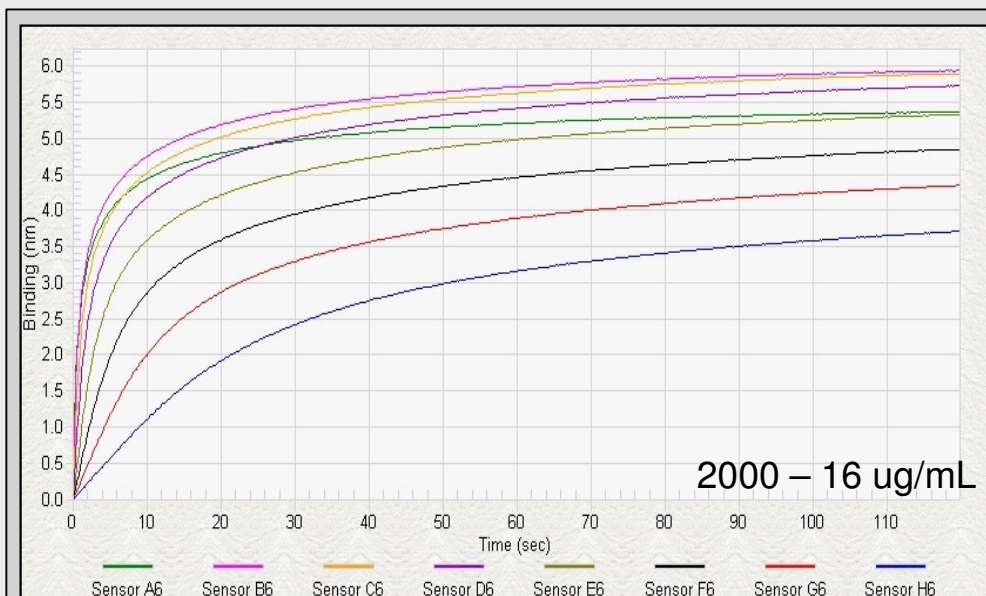
Sydney Zaremba  
Sr. Associate Scientist  
Analytical Science  
Fremont CA



# Urgent Need to Develop Two Assays

- Transferred in a Fab molecule for development at BI Fremont
- Two assays needed:
  - Titer - for samples in cell culture fluid
  - Activity – a surrogate bioassay for in-process and lot release samples
- Options
  - ELISA
  - Octet
- Assay qualities
  - Speed
  - QC friendly

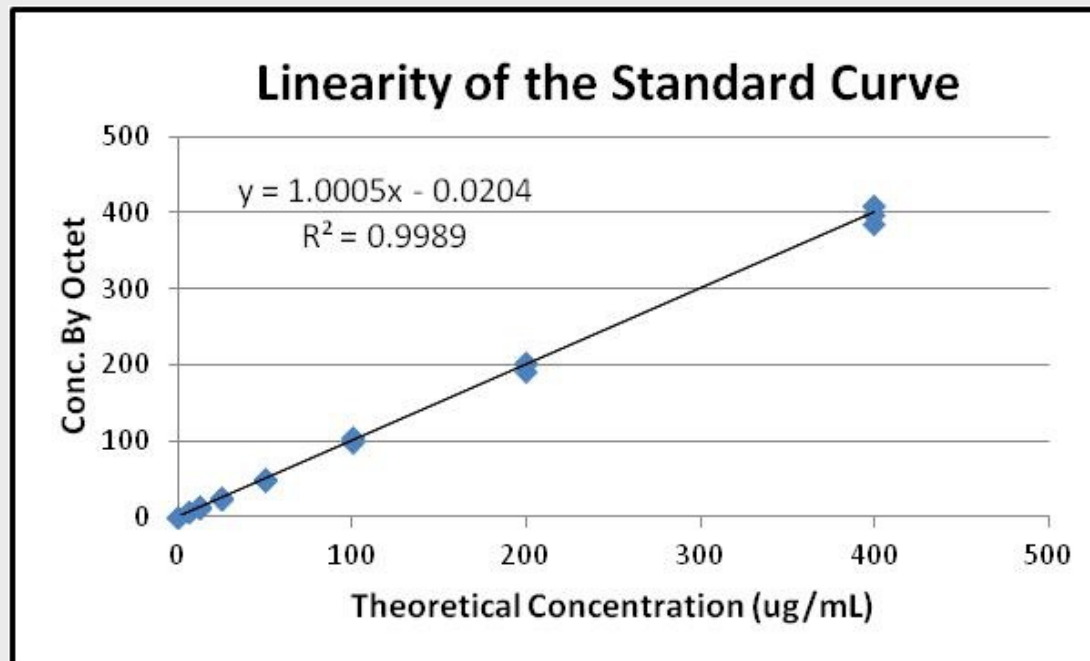
# Standard Curve - Range Determination



[Theoretical] ug/mL	[Octet] ug/mL	%C V	% Accuracy
2000	682	14.3	34%
1000	702	12.2	70%
500	526	11.3	105%
250	265	2.3	106%
125	128	1.3	102%
62.5	64	2.1	102%
31.3	30	2.7	96%

3.13	3.1	2.6	99%
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# Linearity and Repeatability



## Repeatability

n= 8

**97%**

**95%**

**101%**

**99%**

**100%**

**99%**

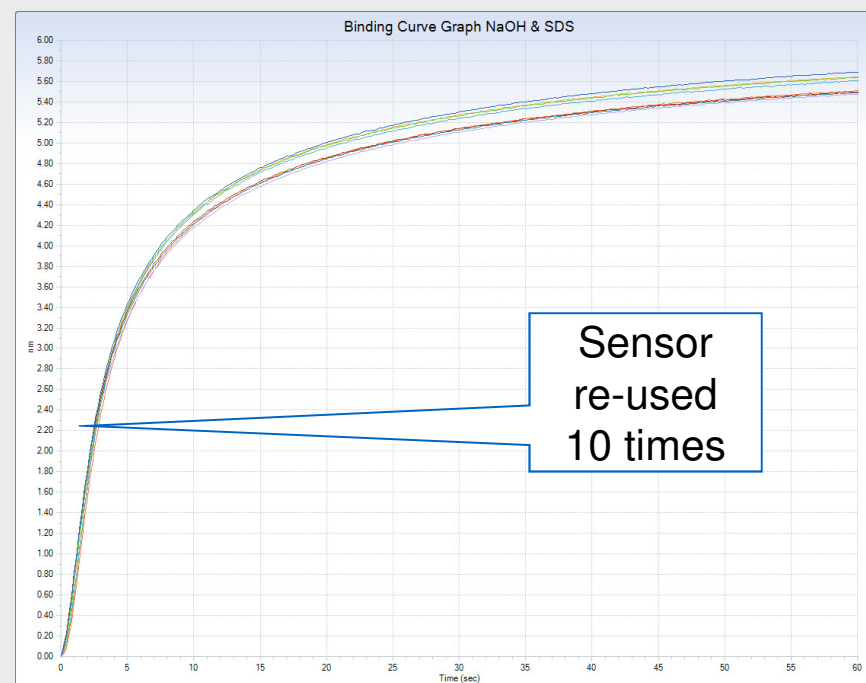
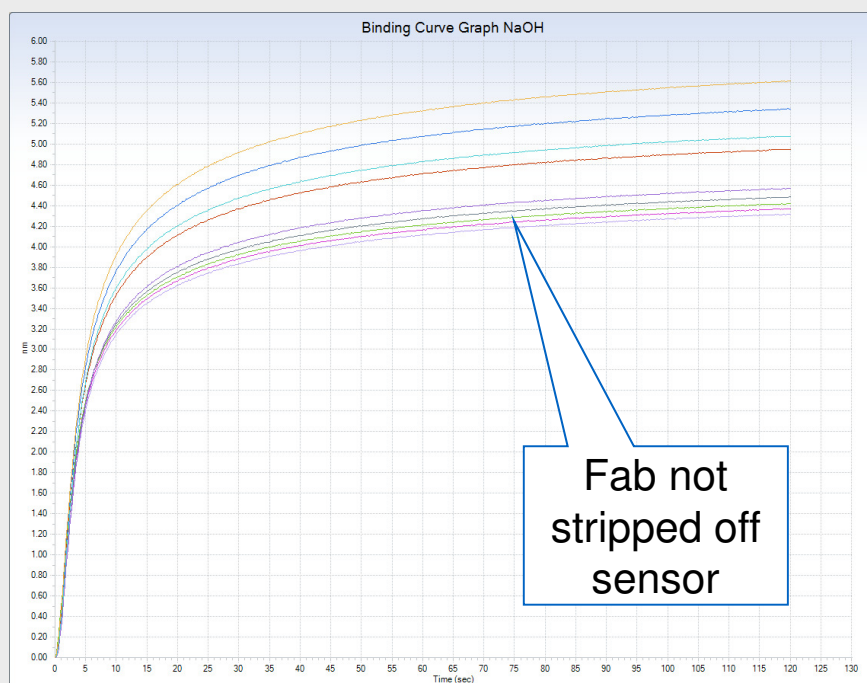
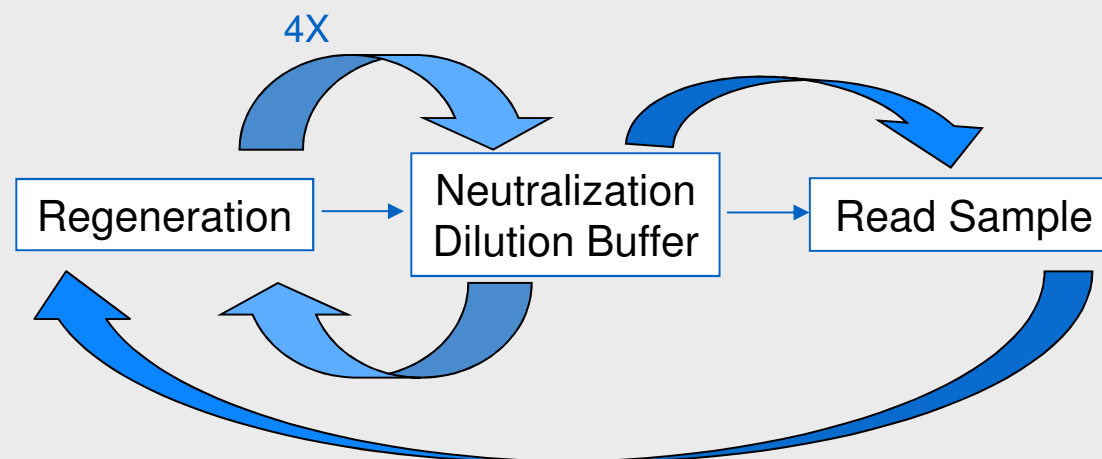
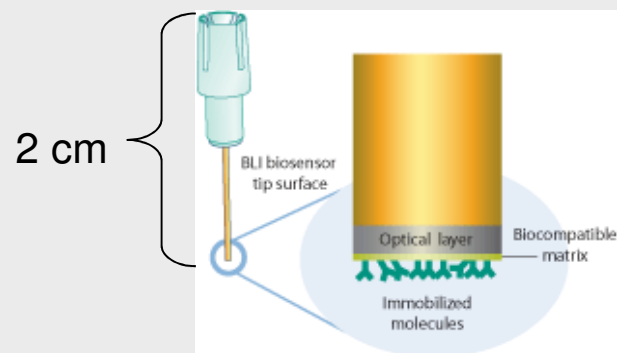
**90%**

**96%**

**Ave = 97 %**

**%CV = 3.6**

# Regenerating Ligand Bound Biosensors



# In-Process Activity Assay Results

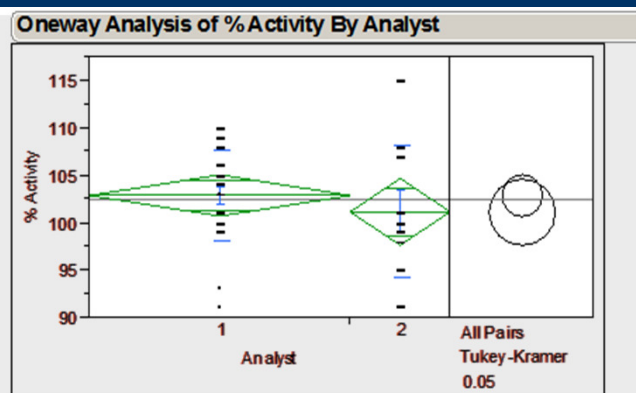
Sample	Octet μg/mL	A280 μg/mL	%C V	% Activity	% Major Impurity
First purification step	7680	9500	2.6	81	15%
First intermediate step	4220	4380	4.4	96	<1%
Second intermediate step	7570	7680	1.8	99	<1%
Final purification step	67880	65790	7.6	103	<1%
BDS	50100	49500	5.0	101	<1%

$$\% \text{ Activity} = \frac{(\text{active concentration})}{(\text{A280 concentration})} \times 100$$

# Accuracy and Precision

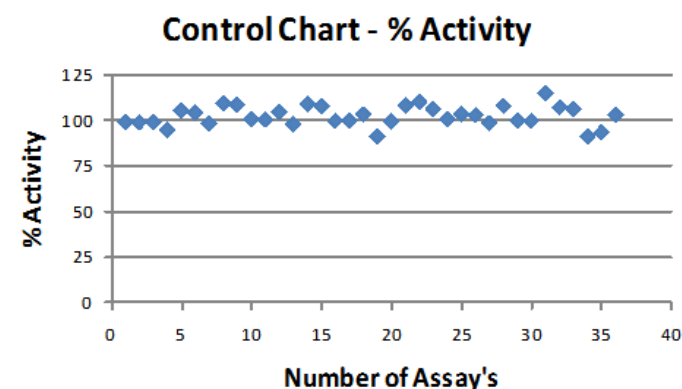
Sample	Octet µg/mL	A280 µg/mL	%CV	% Activit y	% Accuracy
99% Fab	49100	50500	5.0	97	98
50% Fab	430	880	6.7	49	98
5% Fab	46	1100	3.3	4	84

## Intermediate Precision



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
1	26	102.923	4.83258	0.9477	100.97	104.87
2	10	101.200	6.98888	2.2101	96.20	106.20



N = 36	% Activity	%CV
Ave	102	5.3

# Assay Run Time

10 Samples	Octet (Minutes)	ELISA (Minutes)	Biacore (Minutes)
Sample prep	30	30	30
Software data entry	10	10	10
<b>Run time</b>	<b>20</b>	<b>240</b>	<b>270</b>
Data analysis	5	5	5
Total time	65 (~1 hr)	285 (~5 hr)	315 (~5 hr)



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