Revolution in Interactions Analysis

Attila Aranyos Ph.D.
2016 May

PALL Life Sciences
fortéBIO A Division of Pall Life Sciences
Better Lives. Better Planet.SM

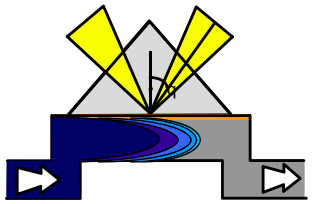
Bio Layer Interferometry

Label Free Revolution in Protein Quantitation and Real-time Kinetics

- Working Principles
- The BLI instrumentation platform
- Difference / Complementarity with SPR
- Key applications with scientific examples

Attila Aranyos, Ph.D

Label-free , Real-time Techniques

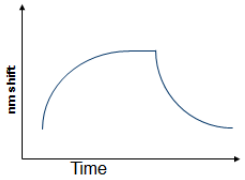


Mainstream

- BLI (Bio Layer Interferometry)
- SPR (Surface Plasmon Resonance)

Others

- QCM (Quartz Crystal Microbalance)
- SAW (Surface Acoustique Wave)



- They differ in decetion method, but they all provide « Sensograms »
- They largely differ in speed, throughput, sample compatibility, usablility

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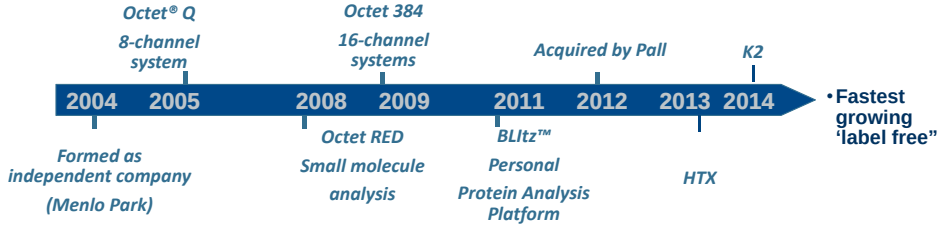

*Pall corporation is part of Danaher Corporation since Aug 2015

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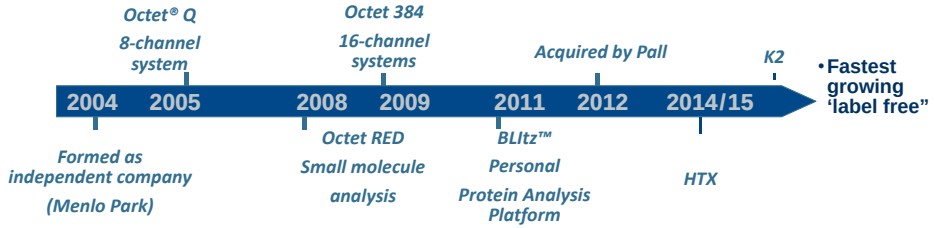

- **What do we Propose**: Proprietary bio-layer interferometry (BLI) technology for label-free bio sensing (Immunoassay, kinetics and quantitation) as a credible alternative to SPR
- **Key to success**: *SPR-quality data without the inconveniences the SPR systems : no fluidics, more channels more flexibility, simpler to use*

- **Success factors 2015:**
  - 9th consecutive year of 25%+ growth
  - 1200 users in user meetings
  - Over 1500 systems in total , 15 million biosensors
  - 300+ publications, in respected papers including Nature
  - Multi-system industrial accounts : 2 out 3 users

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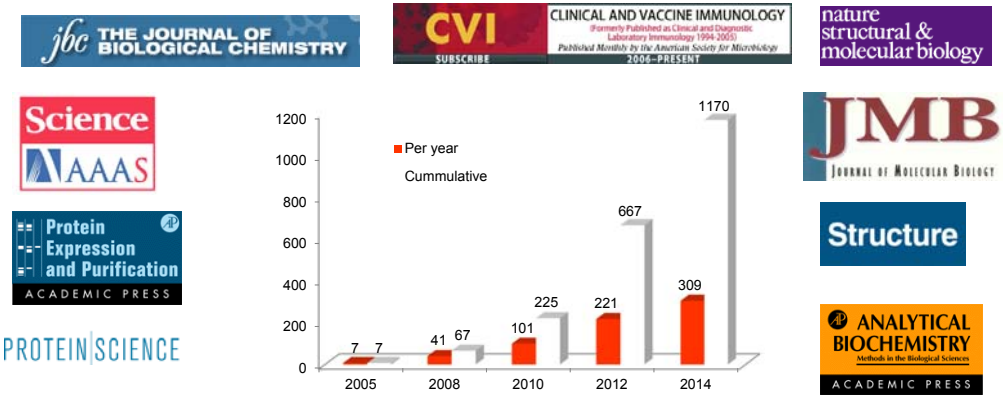
Since the first Octet system shipment, Nearly 1200 articles published in peer reviewed journals



Cancer Research

PALL Life Sciences

Since the first Octet system shipment, Nearly 1200 articles published in peer reviewed journals

LETTER

doi:10.1038/nature13614

HSP70 sequestration by free α-globin promotes ineffective erythropoiesis in β-thalassaemia

Jean-Benoît Arlet[1,2,3,4]*, Jean-Antoine Ribeil[1,3,4,5]*, Flavia Guillem[1,3,4], Olivier Negre[6], Adonis Hazoume[7,8], Guillaume Marcion[7,8], Yves Beuzard[6], Michaël Dussiot[1,3,4,9], Ivan Cruz Moura[1,3,4,9,10], Samuel Demarest[11], Isaure Chauvot de Beauchêne[11,12], Zakia Belaid-Choucair[1,3,4], Margaux Sevin[7,8], Thiago Trovati Maciel[1,3,4,9,10], Christian Auclair[11,12], Philippe Leboulch[6,13], Stany Chretien[6], Luba Tchertanov[11,12], Véronique Baudin-Creuza[14], Renaud Seigneuric[8], Michaela Fontenay[4,15], Carmen Garrido[7,8,16]§, Olivier Hermine[1,3,4,17]§ & Geneviève Courtois[1,3,4]§

Cancer Research

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Very strong Industry acceptance Pharma and Biotech

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Molecular interactions in Biopharma

Discovery & Basic research

- Specificity = Which molecule?
  - Targets
  - Signalling mechanisms
- Affinity = How strong is the binding?
- Kinetics = How fast if will act?
- Mapping = Where it will bind?

Bioprocess development / recombinant proteins

- Concentration = Do I have enough?
  - Clones, cell cultures conditions
- Purity = Safe to use?
- Formulation (Stability & Activity)

Clinical development

- Action or Immune reaction
- ADA

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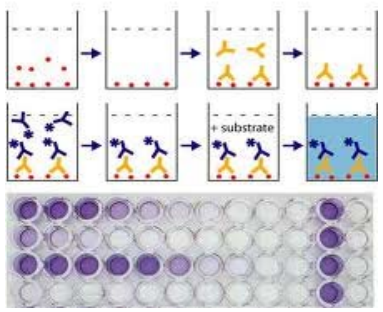
Traditional routes to answers: with label and at equilibrium

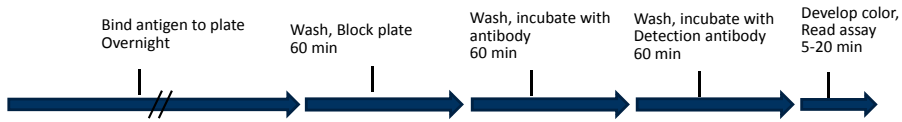
Protein Quantitation with ELISA:



Total assay time: 3-4hours plus overnight incubation

Requires 100-200 ul sample

Target-specific antibodies



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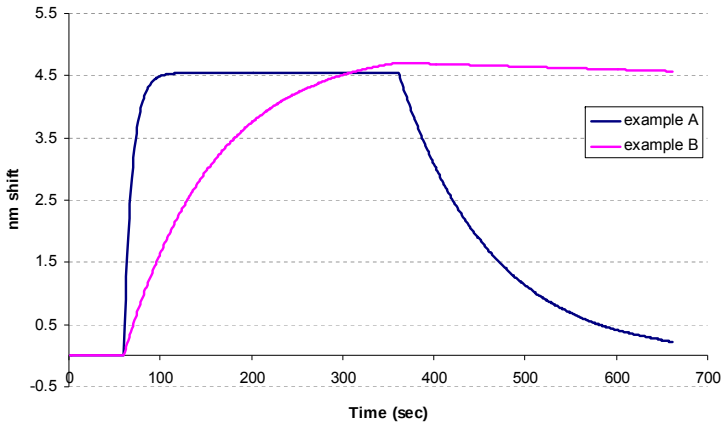
Real time approach : More Infomative

	Association	Dissociation	K_D = Affinty
Example A	1.00E+04	1.00E-02	**1.00E-06**
Example B	1.00E+02	1.00E-04	**1.00E-06**



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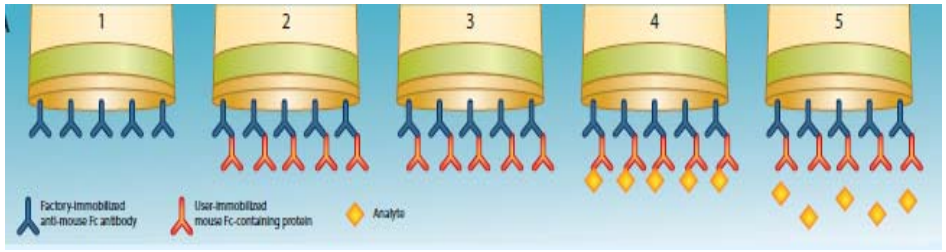
Capabilities of BLI Analytical Instruments



$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

- Protein Concentration
- Association rate
- Dissociation rate
- Binding Affinity

Myszka et. al. *Direct Comparison of Binding Equilibrium, Thermodynamic and Rate Constants Determined by Surface- and Solution-based Biophysical Methods*
Protein Science 2002, 11 (5), 1017-1025.

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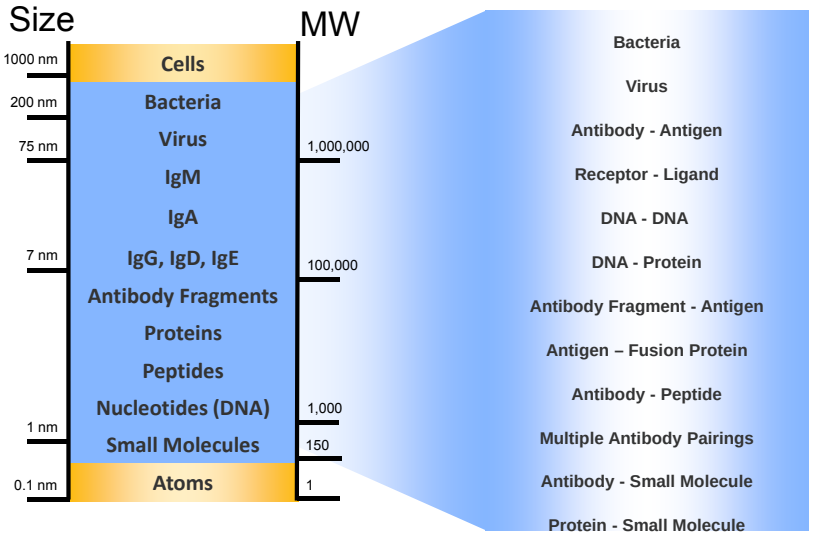
Octet Versatility in Interaction Analysis



Current Octet assays within blue area

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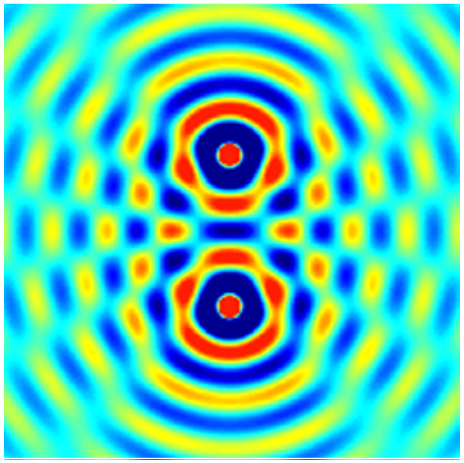
What is Interferometry?



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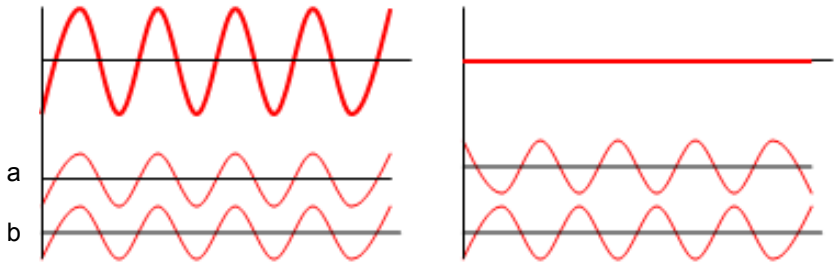
Measuring of interactions between waves



Two waves in phase
➔ Constructive interference

Two waves 180° out of phase
➔ Destructive Interference

http://en.wikipedia.org/wiki/Interference

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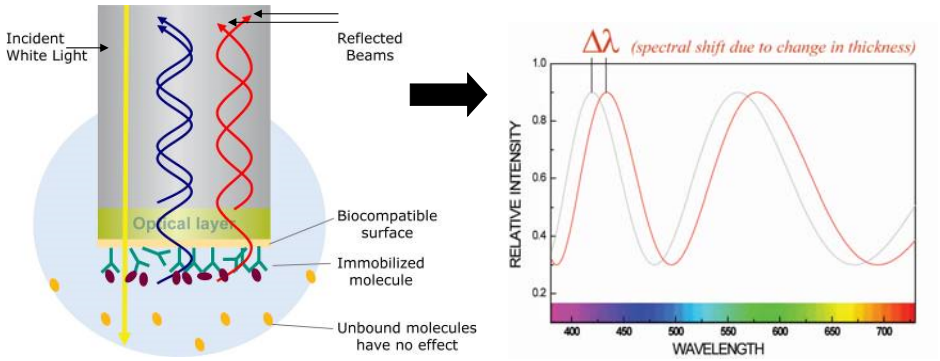
Bio-Layer Interferometry (BLI)

- Much studied physical principle commonly use in surface profiling, semiconductor industry, astrophysics
- 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



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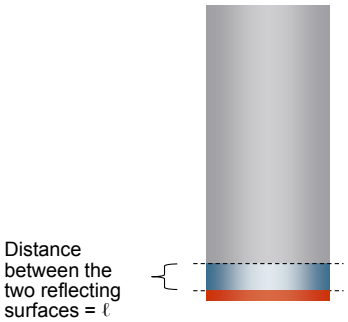
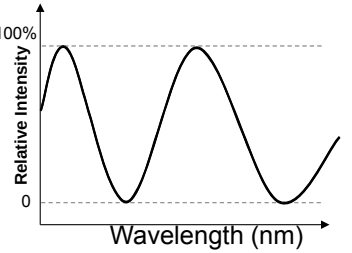
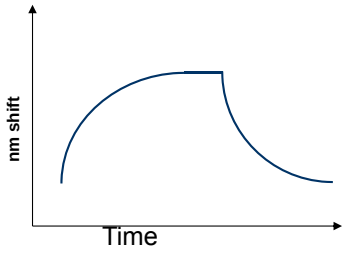
Monitoring the Interference: Real Time





$$\text{Intensity}_{\lambda} = f(\lambda, \ell)$$



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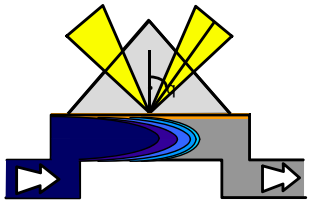
Label-free Techniques



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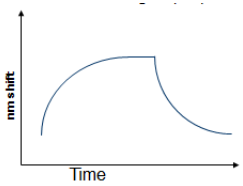
- QCM (Quartz Crystal Microbalance)
- SAW (Surface Acoustique Wave)



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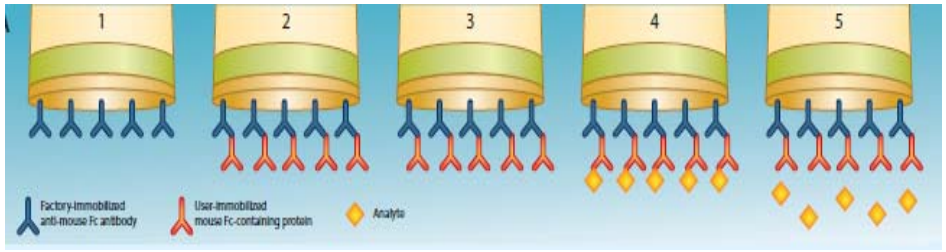

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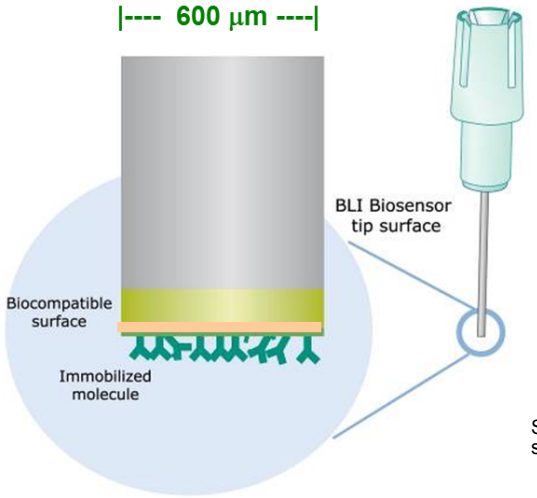
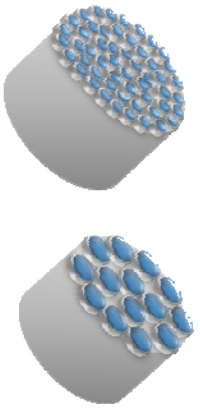
The BLI Sensor





Smaller ligand size allows greater packing on surface – greater binding capacity

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Current Biosensors

Generic Biosensors:
- Streptavidin (SA)
- Super Streptavidin (SSA)
- High Precision Streptavidin (SAX)
- Amine Reactive 2nd Generation (AR2G)
- Aminopropylsilane (APS)

Tag Biosensors:
- Anti-Penta-HIS (HIS)
- Ni-NTA (HIS)
- Anti-GST
- Anti-FLAG

Current Biosensor Kits:
- Immunogenicity (ADA)
- Residual Protein A
- CHO HCP

Antibody Biosensors:
- Anti-Human IgG Fc (AHQ)
- Anti-hIgG Fc Capture Surface (AHC)
- Anti-Murine IgG Fv (AMQ)
- Anti-Murine IgG Fc Capture (AMC)
- Protein A
- Protein G
- Protein L
- Anti human IgG Fab

Custom Sensors
- Custom Capture Sensor
- Custom HCP kit

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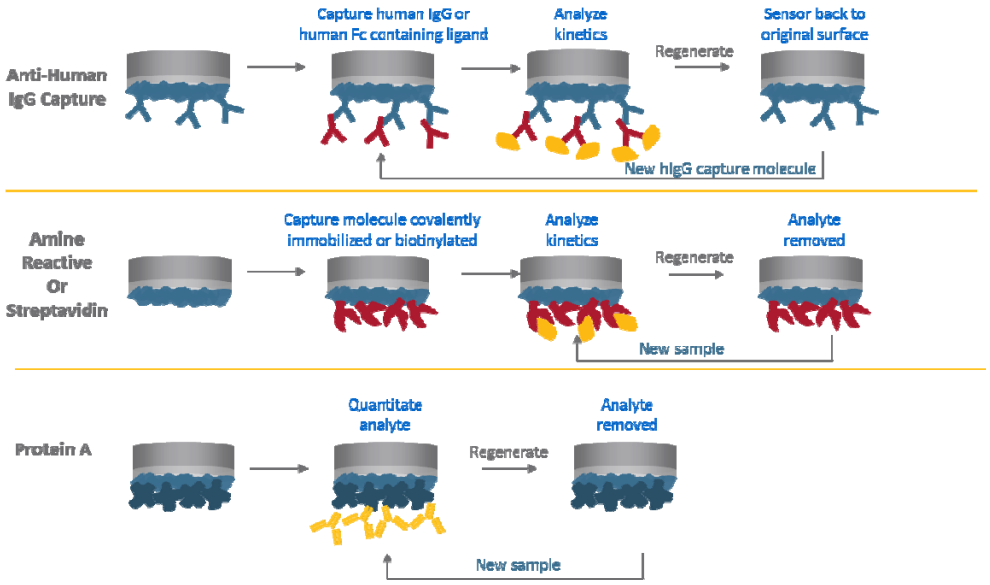
Sensor regeneration



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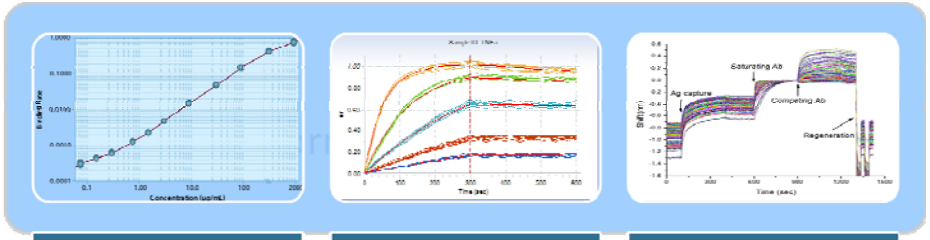
BLI: One Platform, Many Capabilities



Quantitation
- Direct, 1-step
- Sandwich
- ELISA
- mg/mL – pg/mL

Kinetics
- k_a, k_d, K_D
- Proteins
- Peptides, Oligos
- Small molecules

Specificity
- Function testing
- Rank ordering
- Epitope binning
- Isotyping

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BLI solutions
A Wide range of applications

Kinetic Applications
- Protein - protein interactions
- Protein - small molecule interactions
- Lipisome - protein/antibody binding
- Bacteria – antibody binding
- Virus-like particle - protein binding
- DNA aptamer binding
- Glycan – protein binding
- GPCR-Protein binding

Quantitative Applications
- Titer determination
- Rapid protein IgG quantitation
- Quantitation assays for ELISA replacement
- Residual Protein A contamination
- Protein/Antibody Quantitation
- Plant protein quantitation in crude extracts
- Host-cell protein contamination
- Immunogenicity (low and high affinity ADA's)

Screening Applications
- Screening proteins for crystallization studies
- DNA aptamer screening
- Small molecule fragment screening
- Secondary screening and hit validation
- DNA-DNA mismatch detection
- Phage binding (phage display)
- Protein/peptide/small molecule inhibition
- Clone selection in media
- Monitoring protein expression
- Bioreactor monitoring
- Epitope mapping/binning

Assay Development Applications
- Media development
- Process development
- Antibody subtyping
- Antibody pair selection

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FortéBio OCTET application field : Innovator

Target ID & Validation	Lead Screening & Selection	Optimization & Characterizat	Preclinical Development	Process Development	QC & Manufacturing
Mechanism of action – biomolecular interactions	Screening for binders (hybridoma, phage or lysates)	Affinity maturation – binding kinetics	PK assay	Cell line Develop.– titer & growth media assessment	Activity assay
ELISA assay development	Affinity/ on-rate/ off-rate ranking of clones	engineering/ humanization – FcRn binding	PD assay	Dynamic Binding Capacity (DBC) measurement	FcRn binding assay
	Epitope binning	Epitope mapping	Immunogenicity	Chromatography optimization – binding & elution conditions	Binding Kinetics
	Mammalian clone selection for scale up	Pre-formulations	Neutralization assay	Contaminant testing – insulin, HCP &residual protein A	Stability Testing
	Loading concentration for small-scale purification			Formulations	

Historical applications

Second wave

Most Recent

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FortéBio OCTET application field : Biosimilar

Target ID & Validation	Lead Screening & Selection	Optimization & Characterizat	Preclinical Development	Process Development	QC & Manufacturing
		engineering/ humanization – FcRn binding	PK assay	Cell line Develop.– titer & growth media assessment	Activity assay
		Epitope mapping	PD assay	Dynamic Binding Capacity (DBC) measurement	FcRn binding assay
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	Loading concentration for small-scale purification		Neutralization assay	Contaminant testing – insulin, HCP &residual protein A	Stability Testing
				Formulations	

Historical applications

Second wave

Most Recent

Pall ForteBio is a Leader in Label-Free Protein Analysis

- Full life-cycle offering for protein and other biomolecular analysis
  - Instruments, consumables, software, service
  - Label-free assays based on Biolayer Interferometry (BLI)

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Octet Systems

	Analyte MW: >150 D LOD: 25 ng/mL for hIgG on Protein A biosensors in 5 min assay	Analyte MW: >5 kD on Octet QK^e, QK384 LOD: 100 ng/mL for hIgG on Protein A biosensors in 5 min assay	
	Octet RED384	Octet QK384	• 16-channel • 96-, 384-well • ≥ 40 µL sample volume • 2 plate positions • Biosensor re-racking • Automation-friendly
	Octet RED96	Octet QK^e	• 8-channel • 96-well • 1 plate position • Biosensor re-racking* *Not available on Octet QK

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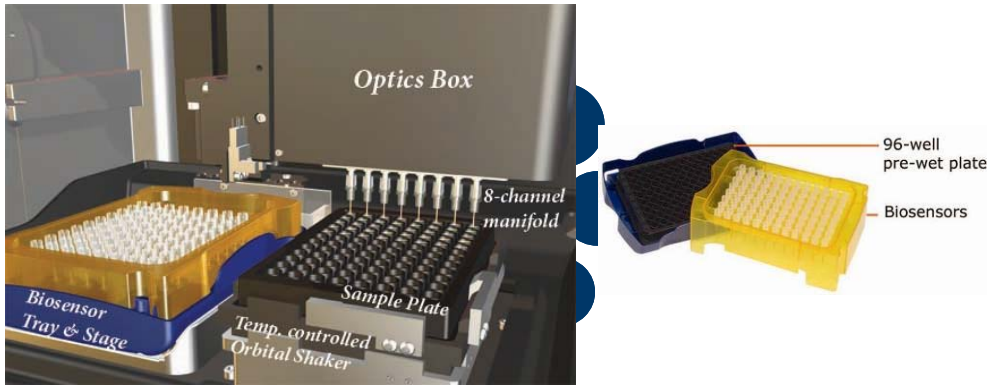
The Classic Octet System



- Optics box moves sensors to samples
- Samples stay inside microplate; non-destructive testing
- "Dip and Read" format allows a large number of interactions to be studied in one experiment

Unattended throughput

supports 96 sensors two 96-well or 384-well sample plates

Octet Systems : K2 – Two channel automated

2 Channel Read Head

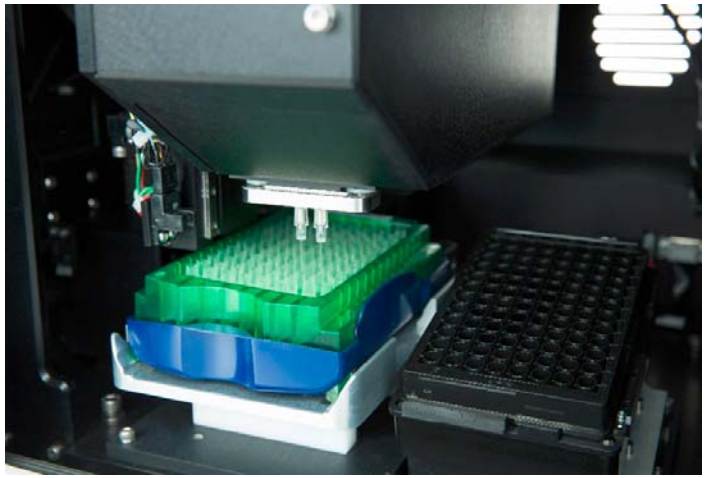
96 Well Microplates

≥180 µL Assay Volume

1 Microplate Position

Biosensor Reracking

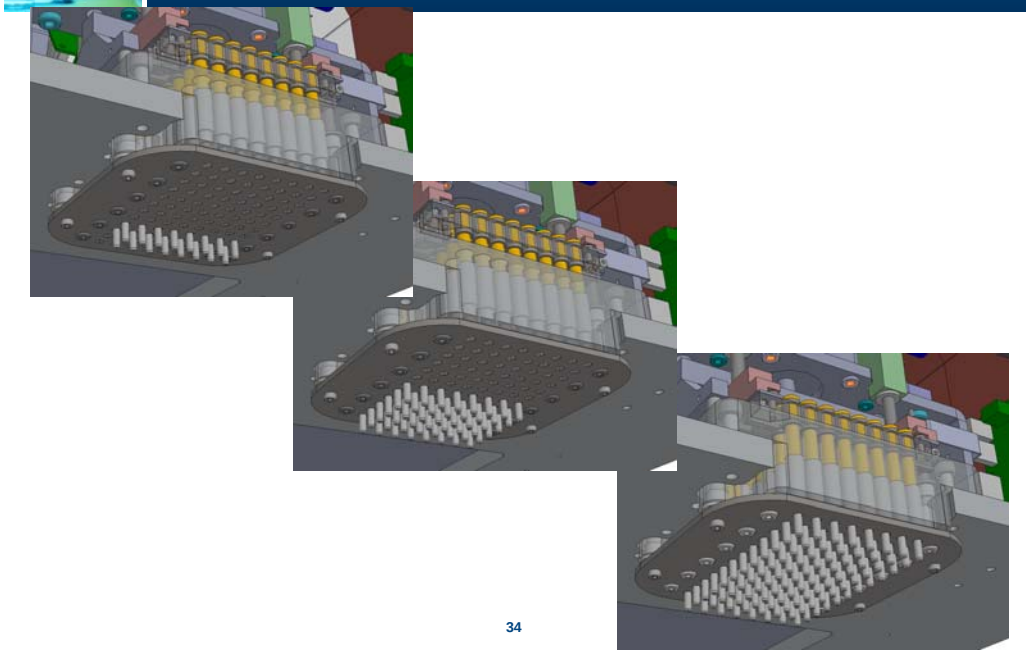
What Is the Octet K2 System?



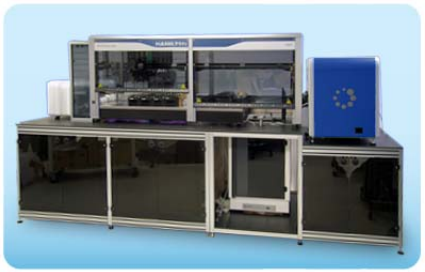
Octet HTX – Unmatched Performance and Speed

- 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

Breakthrough Technology: User-Selectable Read Head



Automated Use of Octet 384 Systems



- Clone selection and screening
- Epitope mapping and binning
- Off-rate screening for antibody ranking
- Host cell protein and residual protein A detection
- Immunogenicity monitoring
- Small molecule and fragment screening

BLItz : The personal Analyzer

Label-free Assays in a Drop

BLITZ: Brilliantly Simple

3 Simple Steps:

1. **Pipet** a drop
2. **Insert** a biosensor
3. **Close** cover to start assay

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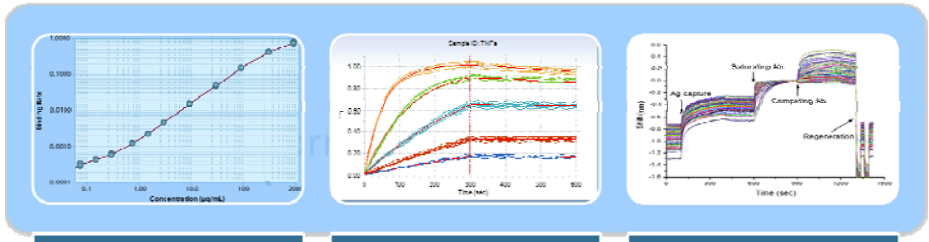
BLI: One Platform, Many Capabilities



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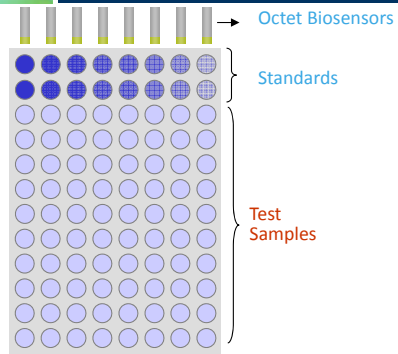
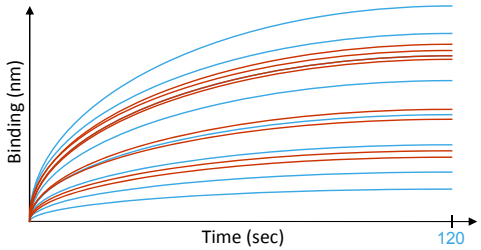
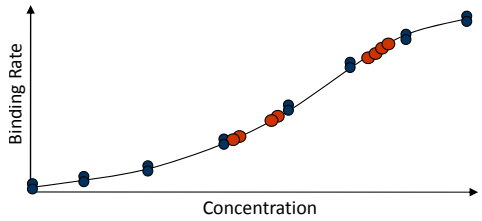
Workflow for Quantitation







- The binding rates of test samples are measured and interpolated from the standard curve to determine concentration
- 96 samples analyzed in 15 - 30 minutes
- Reuse of standard curve is optional

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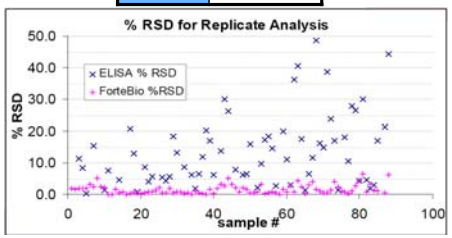
Octet Advantages in Bioprocessing Applications

Method	ELISA	HPLC	Octet
Assay Steps	Plate Prep	Column Prep	Prewet sensors
	Serial Dilution	Regeneration	Sample Reaction & Real-time results
	Sample Reaction	Sample Reaction	
	Secondary Ab	Read	
	Substrate		
	Stop		
	Read		
Labor Time	3 hrs	0.5 hrs	<0.2 hrs
Total Time to results	> 6 hrs	10 hrs	0.5 hrs

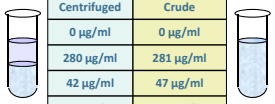
x ELISA	~5-20%
+ Octet	~5%



Data presented at IBC Antibody Production, 2008 by Keith Davis **from Pfizer MO.**

Octet unaffected by crude sample

IgG expressed in E. Coli



Octet Centrifuged	Octet Crude
0 µg/ml	0 µg/ml
280 µg/ml	281 µg/ml
42 µg/ml	47 µg/ml
323 µg/ml	327 µg/ml
132 µg/ml	130 µg/ml

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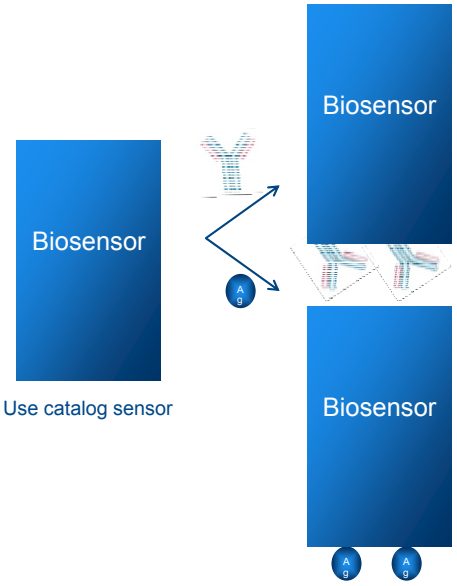
Direct quantification : using catalogue sensors



Antibody Biosensors:
- Anti-Human IgG Fc (AHQ)
- Anti-hIgG Fc Capture Surface (AHC)
- Anti-Murine IgG Fv (AMQ)
- Anti-Murine IgG Fc Capture (AMC)
- Protein A
- Protein G
- Protein L
- Anti human IgG Fab-CH1

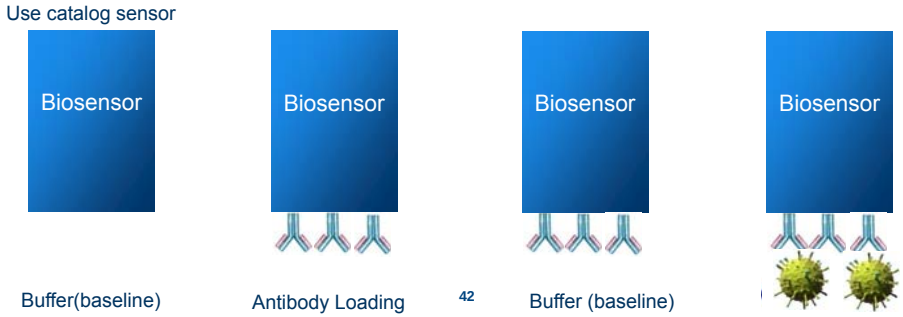
Tag Biosensors:
- Anti-Penta-HIS (HIS2)
- Ni-NTA (HIS)
- Anti-GST
- Anti-FLAG

Generic Biosensors:
- Streptavidin (SA)
- High-precision SA (SAX)
- Super Streptavidin (SSA)
- Aminopropylsilane (APS)

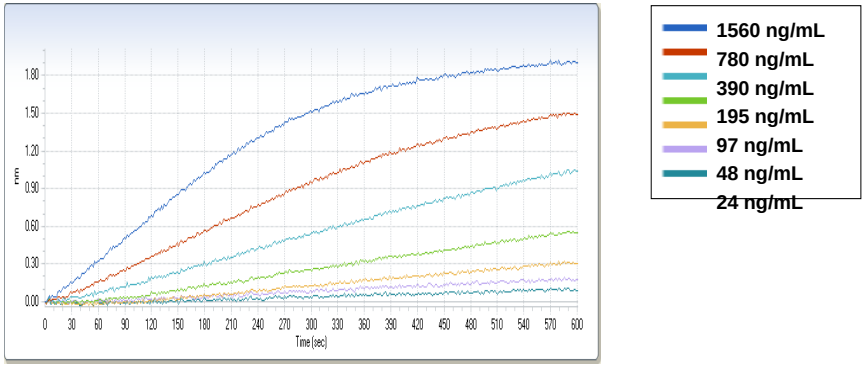
Custom quantification

- A custom quantification consisted in creating a sensor (from one in the sensor catalog) with a surface able to bind the molecule of interest in the media
  - This quantification can be split into two phases (as shown in the schema)
    - Loading of a ligand(often antibody) able to bind the analyte we aim to measure
      - Immunocapture (HIS TAG, AMC, AHC, …) or
      - "Covalent" loading (SA, AR2G)
    - Dipping into the analyte solution and measure of the association

Ex: basic protocol



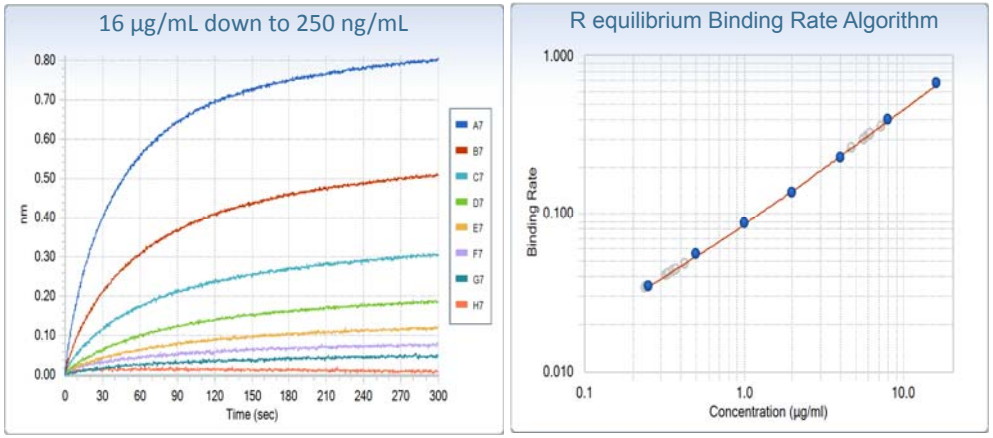
Detection of 24kDa Viral Protein on Octet QKe



Immobilized capture antibody onto Streptavidin Biosensors and looked at binding of sample for ten minutes

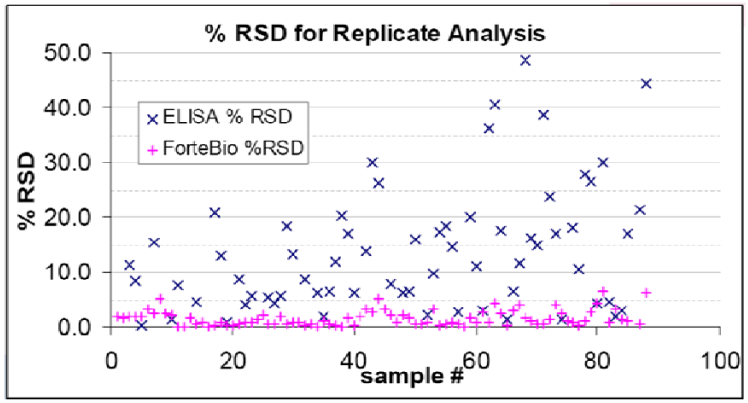
Able to detect binding of protein down to 24 ng/mL with one step quantitation assay

Quantitation of HIS-tagged PAI (43 kDa) on Octet RED



Rapidly quantitate His-tagged proteins on the Octet using Anti-Penta HIS Biosensors

Octet Speed & Reproducibility Comparison to ELISA



Octet = 30 minutes, ELISA = 5-6 hours

Typical RSD values for multiple users and systems:

ELISA	**5 - 20%**
Octet	**< 5%**

Data presented at IBC Antibody Production, 2008 by Keith Davis from Pfizer MO.

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Case Example : Bio Process development

+

Develop the cultures

Analyze the results

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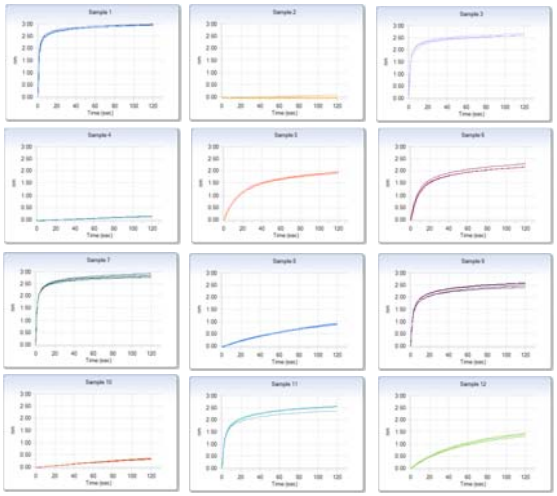
Expression Ranking : what are the best conditions



Rank	Binding Rate	%CV
Sample 1	1.991	10.8%
Sample 7	1.646	2.4%
Sample 3	1.373	3.5%
Sample 9	0.864	3.2%
Sample 11	0.614	2.2%
Sample 6	0.230	3.7%
Sample 5	0.112	0.6%
Sample 12	0.031	4.3%
Sample 8	0.015	2.0%
Sample 10	0.004	4.3%
Sample 4	0.002	8.9%
Sample 2	0.000	NA

Highest expression

Lowest expression

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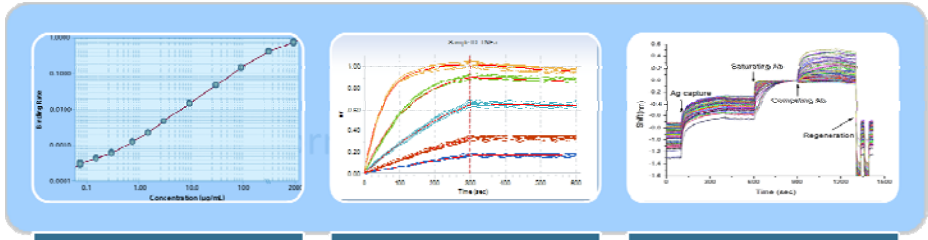
BLI: One Platform, Many Capabilities



Quantitation

- Direct, 1-step
- Sandwich
- ELISA
- mg/mL – pg/mL

Kinetics

- k_a, k_d, K_D
- Proteins
- Peptides, Oligos
- Small molecules

Specificity

- Function testing
- Rank ordering
- Epitope binning
- Isotyping

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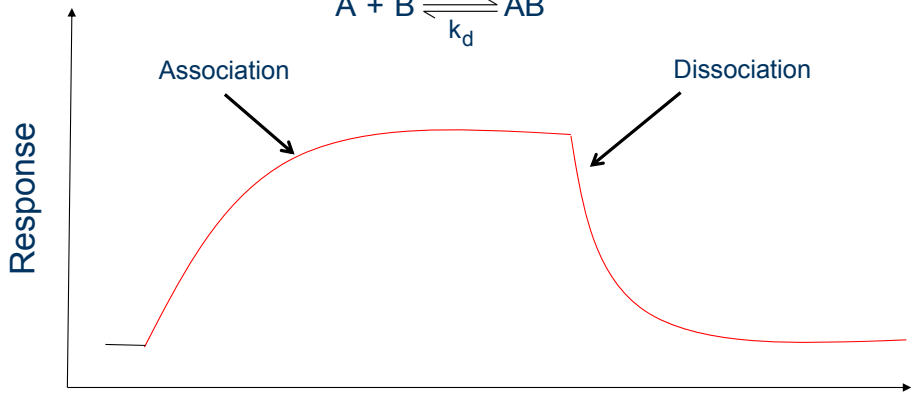
An Ideal Sensorgram

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$



In a simple 1:1 binding model, the association and dissociation phases are described by a single exponential function

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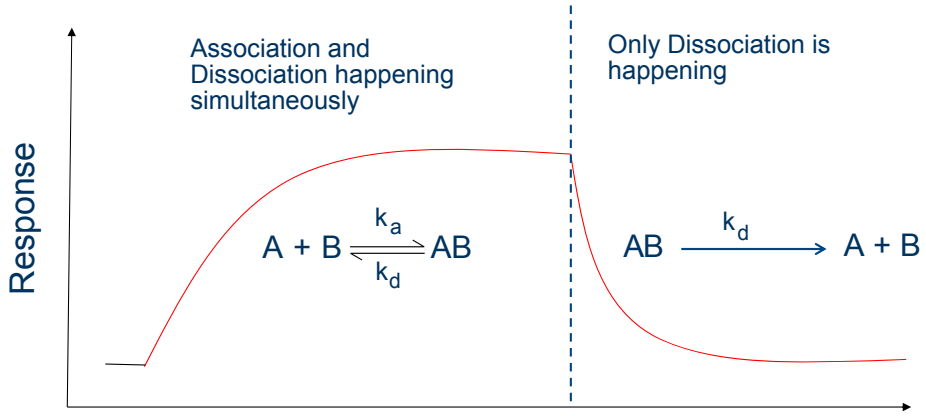
An Ideal Sensorgram



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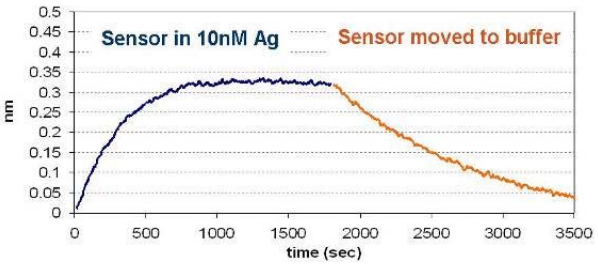
Putting it together : from sensogram to K_D

For a simple 1:1 Interaction: $A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$

Using succssive iterations until a fit is found :



1. $Y=Y_0+Ae^{-k_d*t}$
2. $Y = Y_0 + A\,(1 - e^{-k_{obs}*t})$
3. $k_a = \dfrac{k_{obs} - k_d}{[\text{Conc Ag (M)}]}$
4. $K_D = \dfrac{k_d}{k_a}$

k_a = rate of association or "on-rate"

k_d = rate of disassociation or "off-rate"

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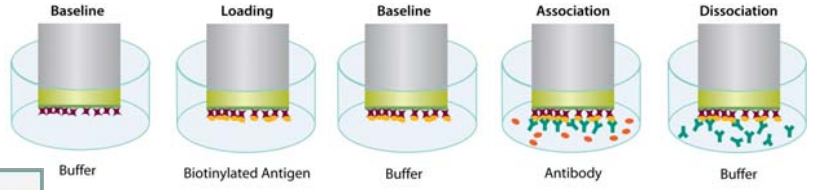
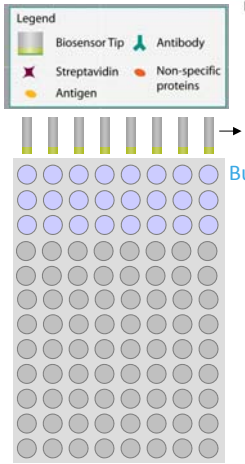
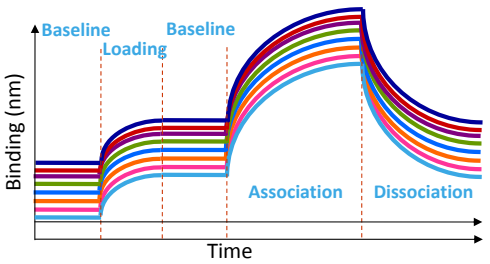
Kinetics Work Flow





Octet Biosensors



- 8 or 16 samples can be analyzed in parallel
- Measure on rates and off rates
- Data is displayed in real-time
- Experimental protocols can be customized

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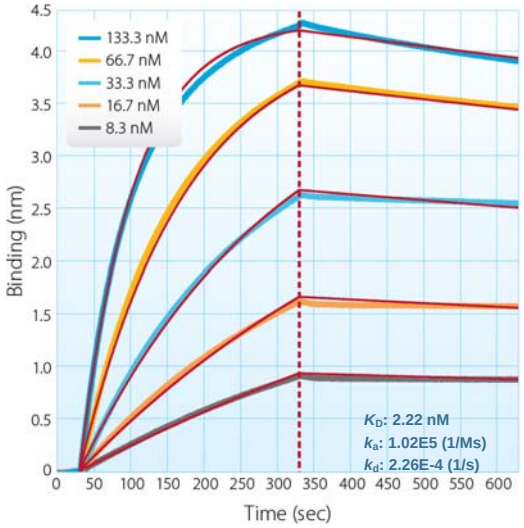
Binding Kinetics: Effortless



Application areas:

- Protein-protein interactions
- Protein quality through binding assays
- Biotherapeutic drug development

Analysis methods complemented/replaced:

- SPR : faster, simpler, more flexible
- ELISA : faster, more accurate
- ITC: faster, different insight

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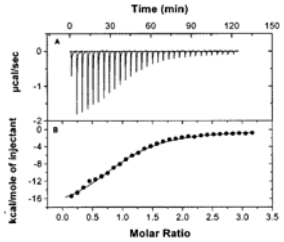
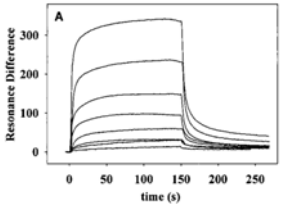
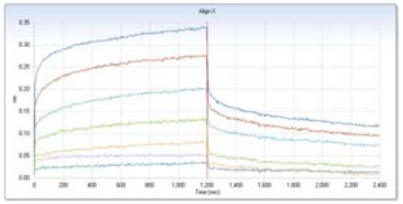
Parathyroid Hormone (PTH) Binding to PTH Receptor

(Octet) Biotin-PTH1R ECD on SA biosensor – PTH(1-34)NH_2 in solution.
(Biacore) PTH1R on CM5 chip – PTH(1-34)NH_2 in solution.







Instrument	K_D
Biacore X	4.9 µM
ForteBio Octet RED	2.8 µM
Microcal iTC	3.4 µM

PTH is of clinical interest for its ability to stimulate bone formation.
(Octet) J. Biol. Chem. **2009**, *284*, 28382-28391.
(Biacore) Biochemistry, **2000**, *39*, 8878-8887.

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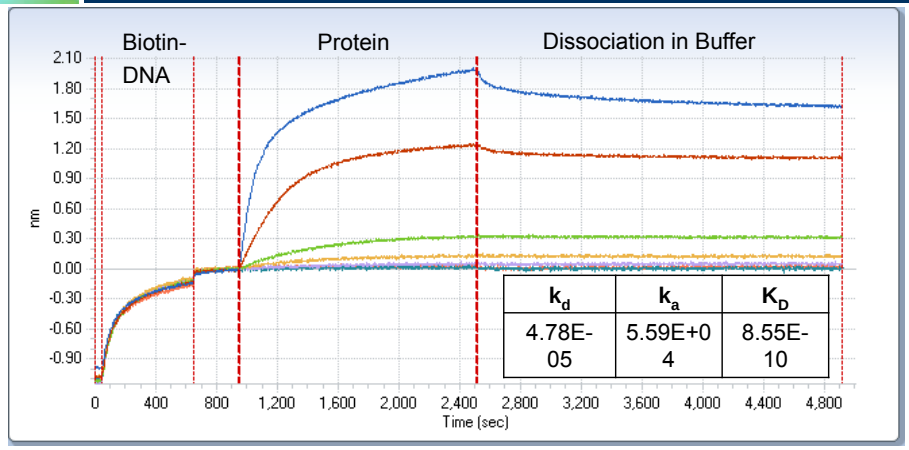
DNA-Binding Protein Kinetic Analysis on the Octet QK



k_d	k_a	K_D
4.78E-05	5.59E+04	8.55E-10

Binding of DNA-Binding Protein to Immobilized Biotinylated ssDNA

Rapid Analysis of Binding of Transcription Factors and Other Promoter Elements to Specific DNA Sequences

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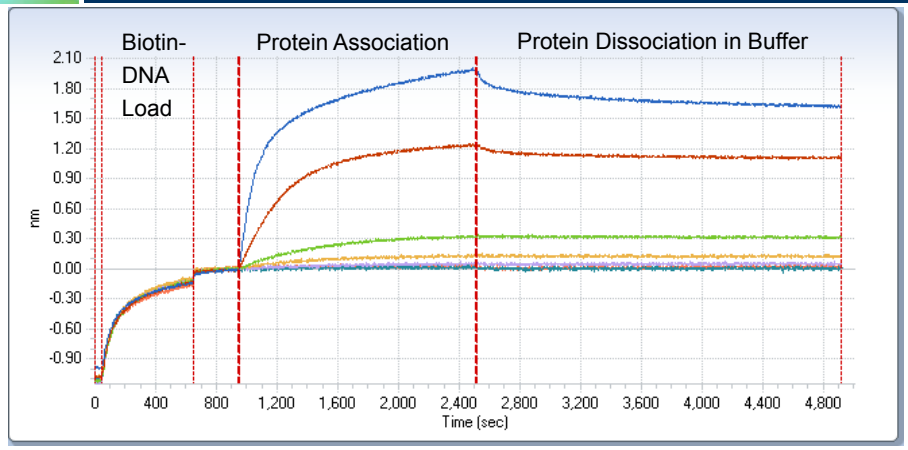
DNA-Binding Protein Kinetic Analysis on the Octet QK



Binding of DNA-Binding Protein to Immobilized Biotinylated ssDNA

Rapid Analysis of Binding of Transcription Factors and Other Promoter Elements to Specific DNA Sequences

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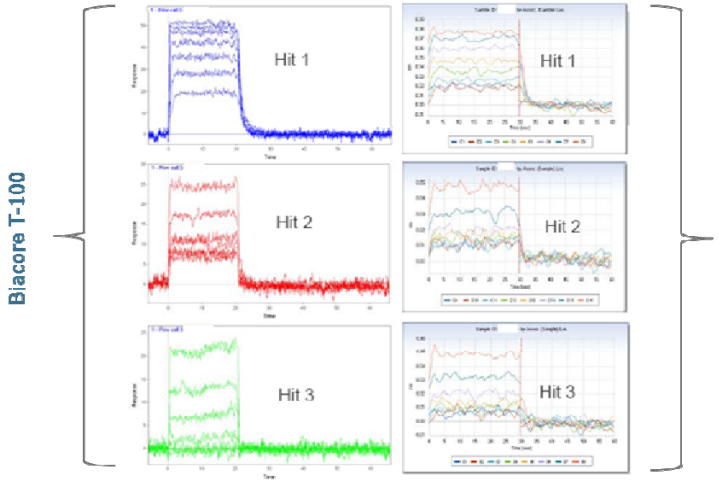
Small molecule screening/binding characterization



Data very consistent across both platforms

* Small Fragments binding to Kinase- courtesy of Roche

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Round Robin on SPR & BLI

David Myszka Publication Biosensor Benchmark Study Comparison of Biacore Models vs. Octet QK

50 kDa Fab binding to a 60 kDa antigen

Instrument	Model	n	ka (M-1 s-1)	kd (M-1 s-1)	KD (nM)
Biacore	A100	3	(1.1 ± 0.3) X 10^5	(0.60 ± 0.26) X 10^4	0.59 ± 0.35
	T100	33	(1.4 ± 0.8) X 10^5	(0.43 ± 0.14) X 10^4	0.36 ± 0.18
	S51	8	(1.7 ± 1.4) X 10^5	(0.86 ± 0.75) X 10^4	1.1 ± 1.2
	3000	77	(1.3 ± 0.9) X 10^5	(0.75 ± 1.2)X 10^4	0.83 ± 1.1
	2000	76	(1.4 ± 1.7) X 10^5	(0.57 ± 1.0) X 10^4	0.62 ± 1.2
	1000	2	(0.82 ± 0.19) X 10^5	(1.3 ± 0.7) X 10^4	1.8 ± 1.2
	X100	2	(1.26 ± 0.08) X 10^5	(0.38 ± 0.03) X 10^4	0.30 ± 0.01
	X	8	(0.87 ± 0.29) X 10^5	(0.65 ± 0.20) X 10^4	0.81 ± 0.28
	Flexchip	4	(1.2 ± 0.2) X 10^5	(0.46 ± 0.10) X 10^4	0.39 ± 0.13
ForteBio	Octet QK	3	(1.7 ± 0.6) X 10^5	(0.70 ± 0.22) X 10^4	0.45 ± 0.26
Study Average		258	(1.4 ± 1.3) X 10^5	(0.61 ± 0.87) X 10^4	0.62 ± 0.98

"These results demonstrate that when this biosensor assay was designed and executed appropriately, the reported rate constants were consistent, and independent of which protein was immobilized and which biosensor was used."

Riche et. al. Analytical Biochemistry 386 (2009) 194–216

Fast. Accurate. EASY.

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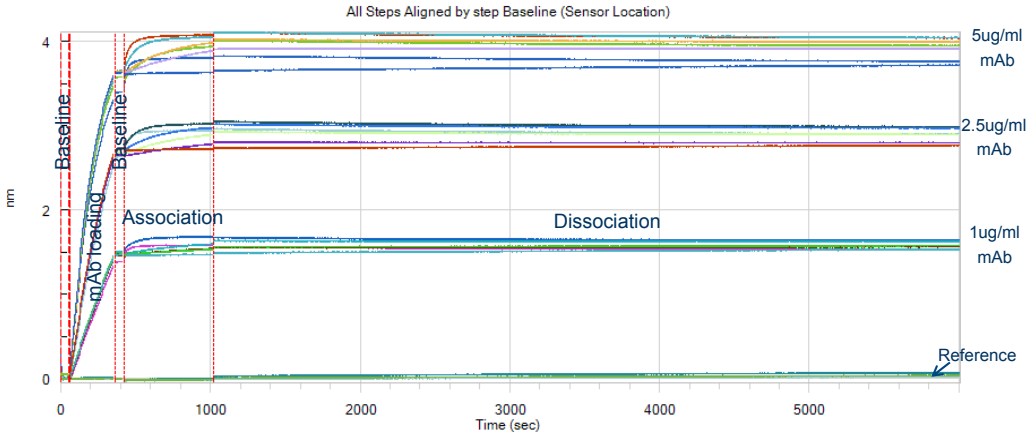
Determination of the KD for Ab – Ag interactions (K2 in SWU)



- Ab: 150kDa
- Ag : 9kDa

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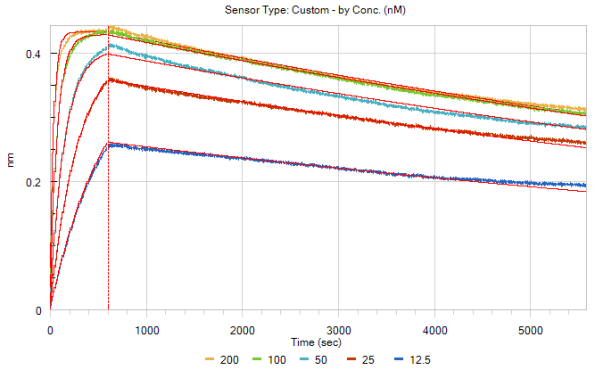
Project 2

Determination of the KD for Ab – Ag interactions

- **Loading at 5ug/ml mAb**



Good fitting (global 1:1). By excluding the highest concentrations, it gets even slightly better.

Sample ID	Conc. (nM)	KD (M)	KD Error	kon(1/Ms)	kon Error	kdis(1/s)	kdis Error	Rmax	Rmax Error	kobs(1/s)	Req	Req/Rmax(%)	Full X^2	Full R^2
	200	5.25E-10	<1.0E-12	1.34E+05	1.61E+02	7.02E-05	4.82E-08	0.4338	0.0001	2.68E-02	0.4327	99.7	0.402971	0.99727
	100	5.25E-10	<1.0E-12	1.34E+05	1.61E+02	7.02E-05	4.82E-08	0.4308	0.0001	1.34E-02	0.4285	99.5	0.402971	0.99727
PG VB26	50	5.25E-10	<1.0E-12	1.34E+05	1.61E+02	7.02E-05	4.82E-08	0.41	0.0001	6.76E-03	0.4058	99	0.402971	0.99727
	25	5.25E-10	<1.0E-12	1.34E+05	1.61E+02	7.02E-05	4.82E-08	0.4199	0.0002	3.41E-03	0.4113	98	0.402971	0.99727
	12.5	5.25E-10	<1.0E-12	1.34E+05	1.61E+02	7.02E-05	4.82E-08	0.4199	0.0003	1.74E-03	0.403	96	0.402971	0.99727

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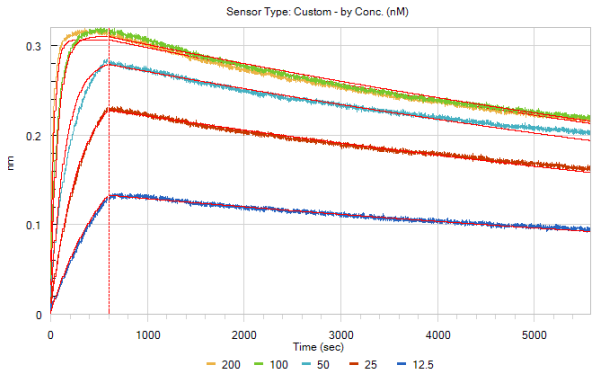
Determination of the KD for Ab – Ag interactions

- **Loading at 2.5ug/ml mAb**



Good fitting (global 1:1). By excluding the highest concentrations, it gets even slightly better.

Sample ID	Conc. (nM)	KD (M)	KD Error	kon(1/Ms)	kon Error	kdis(1/s)	kdis Error	Rmax	Rmax Error	kobs(1/s)	Req	Req/Rmax(%	Full X^2	Full R^2
	200	5.63E-10	1.23E-12	1.30E+05	2.48E+02	7.31E-05	7.80E-08	0.3077	0.0001	2.60E-02	0.3069	99.7	0.489537	0.995908
	100	5.63E-10	1.23E-12	1.30E+05	2.48E+02	7.31E-05	7.80E-08	0.3123	0.0001	1.31E-02	0.3105	99.4	0.489537	0.995908
PG VB26	50	5.63E-10	1.23E-12	1.30E+05	2.48E+02	7.31E-05	7.80E-08	0.2881	0.0001	6.57E-03	0.2849	98.9	0.489537	0.995908
	25	5.63E-10	1.23E-12	1.30E+05	2.48E+02	7.31E-05	7.80E-08	0.2701	0.0002	3.32E-03	0.2641	97.8	0.489537	0.995908
	12.5	5.63E-10	1.23E-12	1.30E+05	2.48E+02	7.31E-05	7.80E-08	0.2177	0.0003	1.70E-03	0.2083	95.7	0.489537	0.995908

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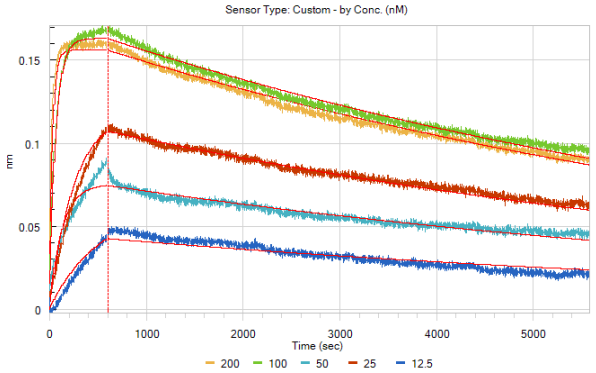
Determination of the KD for Ab – Ag interactions

- **Loading at 1ug/ml mAb**



Good fitting (global 1:1). By excluding the highest concentrations, it gets even slightly better.

Sample ID	Conc. (nM)	KD (M)	KD Error	kon(1/Ms)	kon Error	kdis(1/s)	kdis Error	Rmax	Rmax Error	kobs(1/s)	Req	Req/Rmax(%)	Full X^2	Full R^2
	200	7.86E-10	2.64E-12	1.50E+05	4.72E+02	1.18E-04	1.33E-07	0.1568	0.0001	3.00E-02	0.1562	99.6	0.242063	0.994755
	100	7.86E-10	2.64E-12	1.50E+05	4.72E+02	1.18E-04	1.33E-07	0.1644	0.0001	1.51E-02	0.1632	99.3	0.242063	0.994755
PG VB26	50	7.86E-10	2.64E-12	1.50E+05	4.72E+02	1.18E-04	1.33E-07	0.0765	0.0001	7.60E-03	0.0753	98.4	0.242063	0.994755
	25	7.86E-10	2.64E-12	1.50E+05	4.72E+02	1.18E-04	1.33E-07	0.1228	0.0001	3.86E-03	0.119	96.9	0.242063	0.994755
	12.5	7.86E-10	2.64E-12	1.50E+05	4.72E+02	1.18E-04	1.33E-07	0.0648	0.0001	1.99E-03	0.061	94.1	0.242063	0.994755

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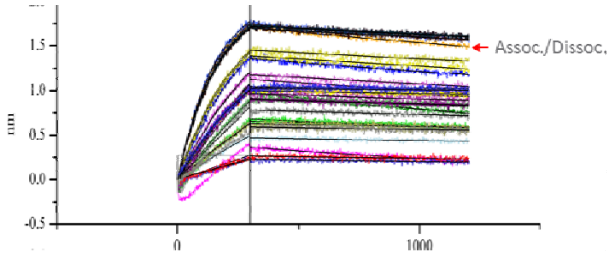
Affinity Characterization of IgG Products (8 channel Octet)

96 supes can be assayed in 10-15 minutes with the HTX

16 mutant culture supernatents were assayed in triplicate on Ag-Fc fusion protein bound to Protein A biosensors in less than 30 minutes



These crude samples were unsuitable for Biacore analysis due to media composition and unfilterable cell debris.

Rapidly eliminated 5 out of 16 mutants by off rate criteria.

Data from Brian Miller at Biogen IDEC

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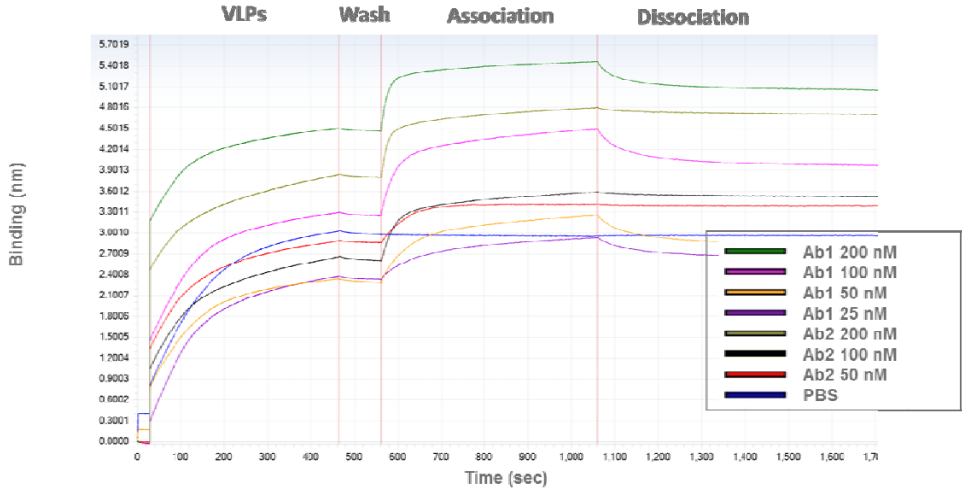
Binding of mAbs in PBS to VLPs on Octet RED



* Data courtesy of Integral Molecular

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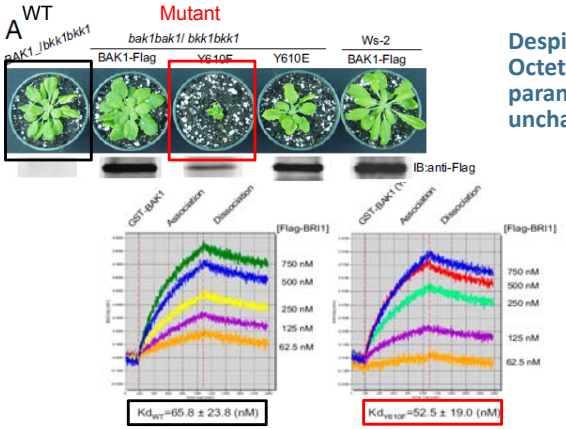
Kinetics of *Arabidopsis* Proteins measured on Octet QK

Autophosphorylation of Tyr-610 in the receptor kinase BAK1 plays a role in brassinosteroid signaling and basal defense gene expression

Man-Ho Oh[a,b], Xiaofeng Wang[c], Xia Wu[d], Youfu Zhao[e], Steven D. Clouse[c], and Steven C. Huber[a,b,1]

[a]Agricultural Research Service, United States Department of Agriculture and [b]Department of Plant Biology, University of Illinois, Urbana, IL 61801; [c]Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695; [d]Physiological and Molecular Plant Biology Program, Department of Plant Biology, University of Illinois, Urbana, IL 61801; and [e]Department of Crop Sciences, University of Illinois, Urbana, IL 61801

Edited by Jianming Li, University of Michigan, Ann Arbor, MI, and accepted by the Editorial Board September 1, 2010 (received for review January 5, 2010)



Despite gross changes in plant growth rate, Octet QK data demonstrates the kinetic parameters of the mutated kinase are unchanged from wild type.

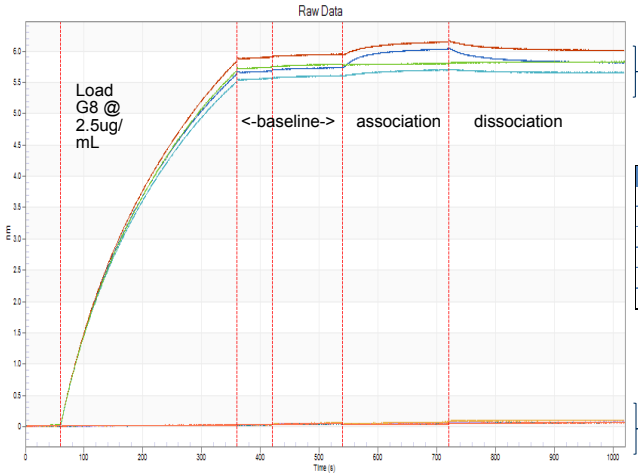
Octet QK parameters:

- Standard 96W plate
- Anti-Murine biosensors
- 30C, 1000 RPM in MOPS buffer
- Loaded anti-GST antibody to capture GST-BAK protein
- 15 minute association w/ BRI1
- 15 minute dissociation

PALL Life Sciences

Galectin 8 vs. VHH-H4 : Assay setup & Raw data

The following assay parameters were used for all other VHH studied vs. Galectin 8



Step	Step Name	Step Type	Step Time
1	Baseline	BASELINE	60
2	Loading	LOADING	300
3	Baseline	BASELINE	60
4	Baseline2	BASELINE	120
5	Association	ASSOC	180
6	Dissociation	DISASSOC	300

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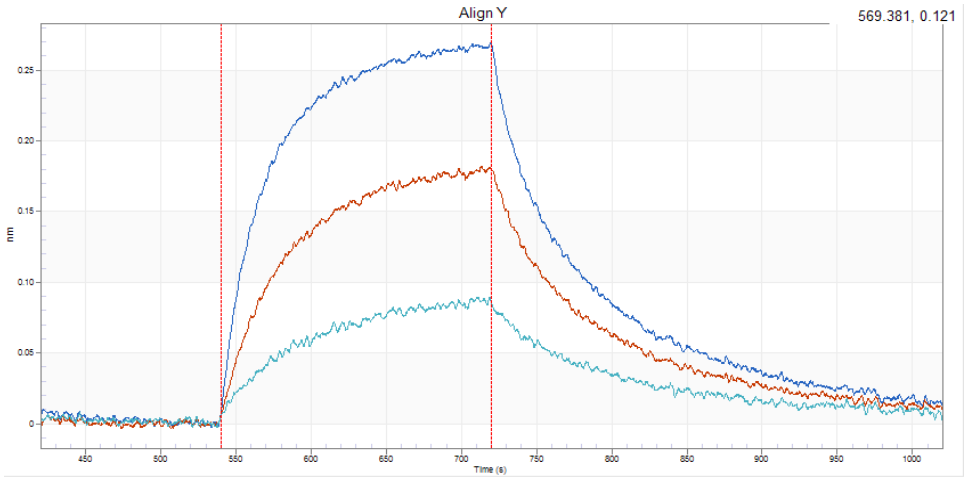
Galectin 8 vs. VHH-H4 : Reference substracted data



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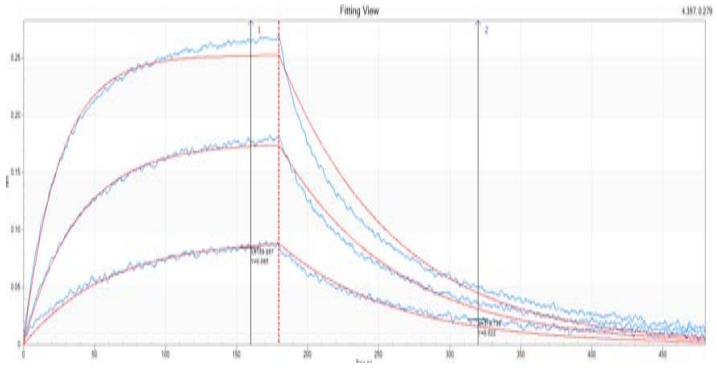
Galectin 8 vs. VHH-H4 : KD calculation



Sample ID	Conc. (nM)	KD (M)	kon(1/Ms)	kdis(1/s)
VHH-H4	500	2.27E-07	5.42E+04	1.23E-02
VHH-H4	250	2.27E-07	5.42E+04	1.23E-02
VHH-H4	83	2.27E-07	5.42E+04	1.23E-02

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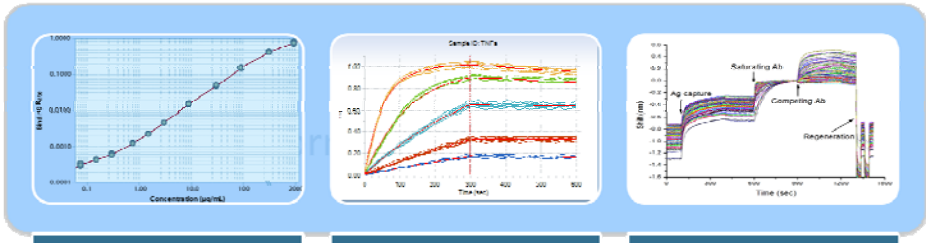
BLI: One Platform, Many Capabilities



Quantitation
- Direct, 1-step
- Sandwich
- ELISA
- mg/mL – pg/mL

Kinetics
- k_a, k_d, K_D
- Proteins
- Peptides, Oligos
- Small molecules

Specificity
- Function testing
- Rank ordering
- Epitope binning
- Isotyping

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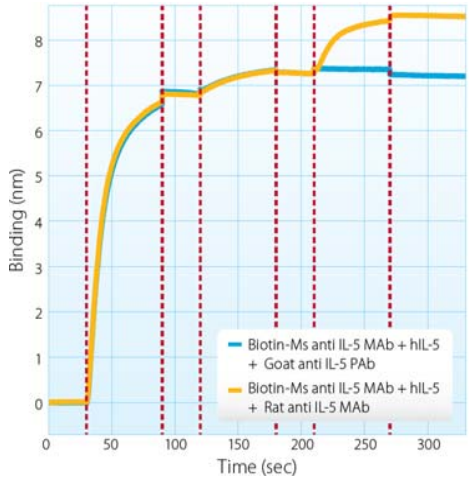
Assay Development: Smarter



Tests performed:
- Binding pair optimization
- Immunoassay development
- Cross-blocking studies

Analysis methods complemented/replaced:
- ELISA
- SPR

Application areas:
- Immunoassay development
- Academic research ELISAs
- Biomarker discovery and development
- Reagent quality analysis
- Antibody manufacturing
- Enzyme and other protein manufacturing

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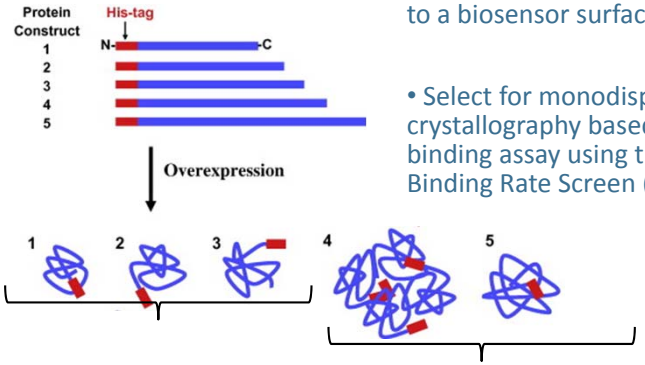
Use of the Octet platform in protein crystallography

Data courtesy of Jiamin Yu from Novartis | CHI The Bioprocess Summit 2011 | ForteBio Workshop



- Aggregated and mis-folded proteins bind slower to a biosensor surface due to larger size
- Select for monodisperse proteins for crystallography based on a simple label-free binding assay using the Octet platform to do a Binding Rate Screen (BRS)

Binding Rate Screen - a high-throughput assay in soluble lysate for prioritizing protein expression constructs
Anal Biochem. 2010 Apr 15;399(2):276-83.

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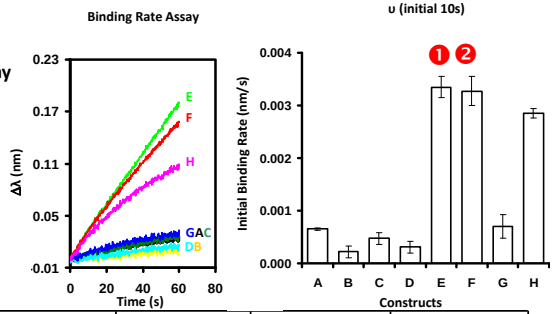
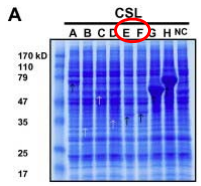
Binding Rate Screen Identifies Constructs That Produce Crystals

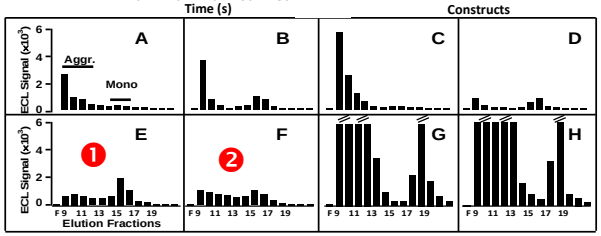
Data courtesy of Jiamin Yu from Novartis | CHI The Bioprocess Summit 2011 | ForteBio Workshop

- **SEC on crude lysate**
- **Analyze fractions with anti-his immuno assay**
- **Compare to Octet binding rate assay**
- **Constructs E & F crystallized**

1 minute Octet crude lysate assay correctly prioritizes protein constructs for crystallization







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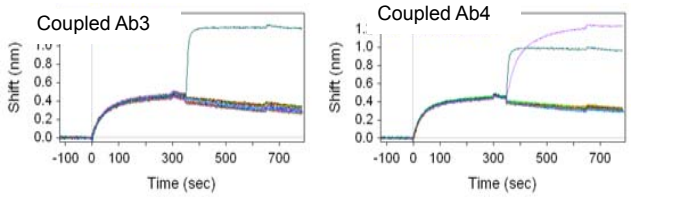
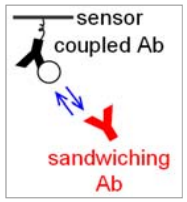
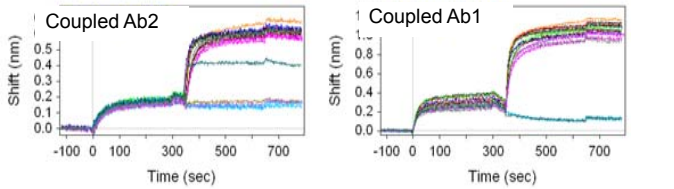
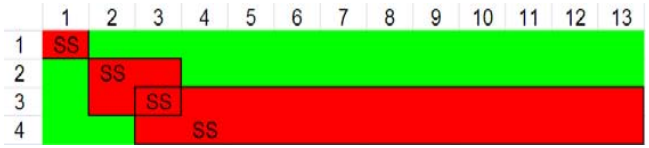
Antibody Epitope binning/pairing

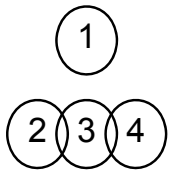






Classical sandwich binning





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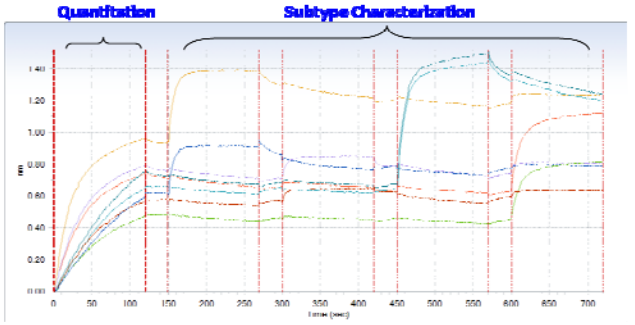
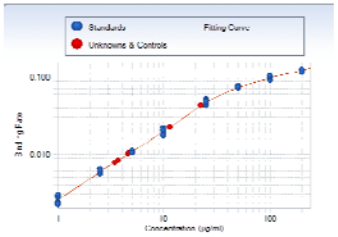
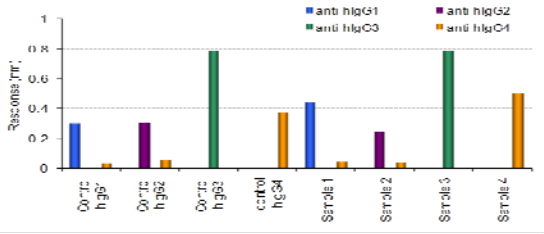
Subtyping human IgG's



- Biosensor
  - Anti-Human IgG Fc
- Subtype specific secondary antibodies
  - Southern Biotech
  - Abcam
- Total assay time = 12 minutes





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Recover for MS : EPO quant trouble shooting

Customer :

Research at CFB

The Novo Nordisk Foundation Center for Biosustainability (CFB) at the Technical University of Denmark aims at developing new knowledge and technologies to help facilitate the transformation from the existing oil-based chemical industry to a more sustainable bio-based society, in which chemicals are produced biologically. Furthermore, CFB aims at accelerating the development of genome-scale science for CHO cell lines for improved bioprocessing.

The center is organized in 11 research sections and a Core which aims at platform strain and technology development purposes:

Yeast Cell Factories (YCF)
CHO Cell Line Engineering and Design (CLED)
Glyco-Engineering of CHO (GEC)
Network Reconstructions and in silico Biology (NRiSB)
Genome-Scale CHO in silico models (GSCiSM)
Yeast Synthetic Biology (YSB)
High-throughput Molecular Bioscience (HTMB)
Bacterial Cell Factories (BCF)
New Bioactive Compounds (NBC)
iLoop
Cho-Core

Application : Customer has developed a custom quant assay for EPO (supernatants / purified)

Issue: in certain eucariote supernatants the sensitivity is unexpectedly low compared to others, with no apparent reason. Teams suspects some issues with (non)specific binding of competing proteins

Solution : Recovery of capture and MS analysis

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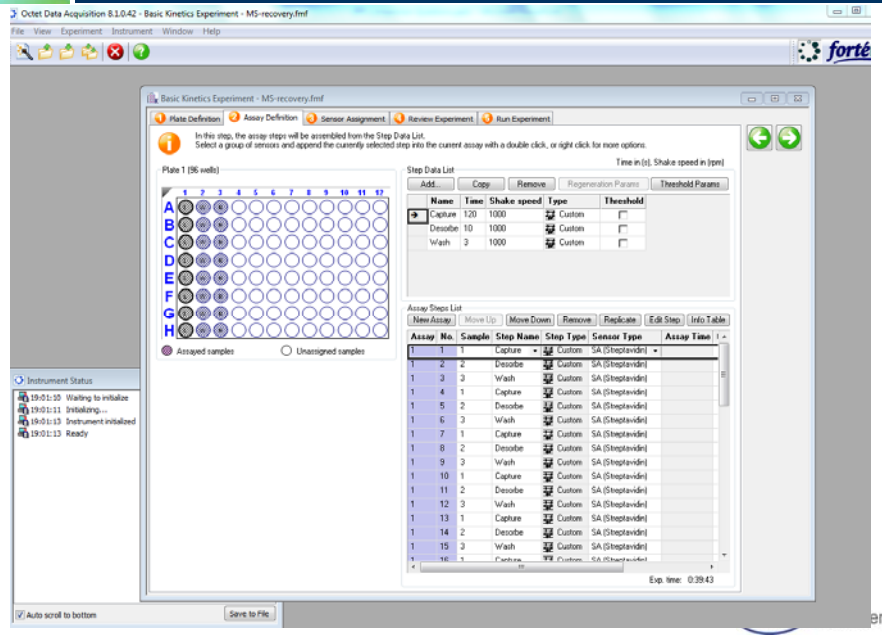
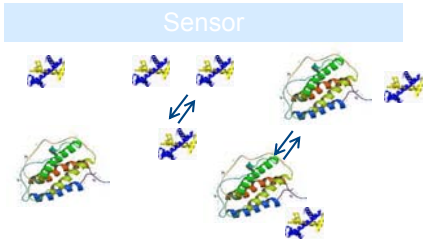
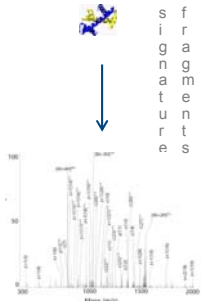
Recovery for MS : ligand fising



LC - MS

1. Neutralize recovery solution
2. Micro UPLC
3. MS-MS analysis





Host cell histones block the EPO binding!

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Octet Platform

fortéBIO
A Division of Pall Life Sciences

Top 10 Applications

1.Kinetic Characterization
2.Ab Titer
3.Cross-blocking and Ab Pairing (Epitopes)
4.Non-Ab Titer
5.Small Molecule
6.Mechanism of Action
7.Reagent Development (Immunoassay)
8.Lipids, Membranes
9.Vaccines
10.Oligonucleotides

Source: North American customer survey of 478 application responses

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High-Throughput Small Molecule Fragment Screening and Hit Validation on Octet RED384

- 16-channels
- 384 tilted-well plate (40µL min volume)
- 2 plate positions

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Full Robotics Compatibility for True High-Throughput Label-Free Measurements

Integration-Ready for All Major Robot Vendors

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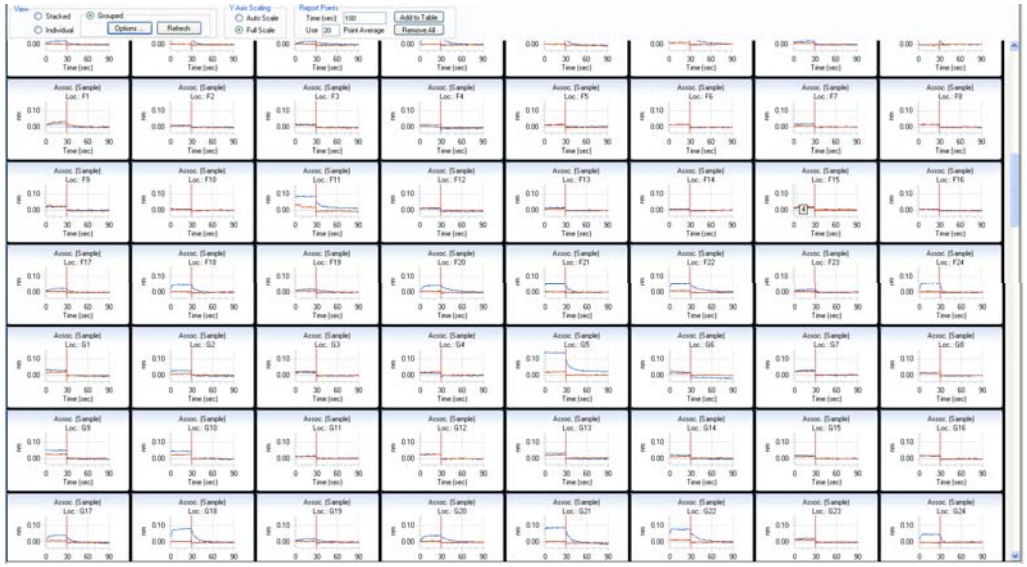
Large Fragment Libraries Screened & Analyzed Quickly and Easily



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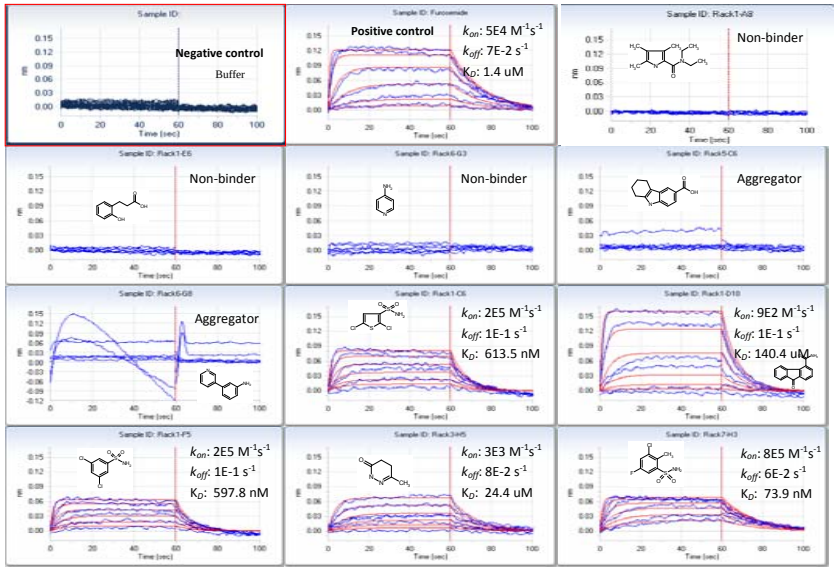
Hit Validation by Titration Series



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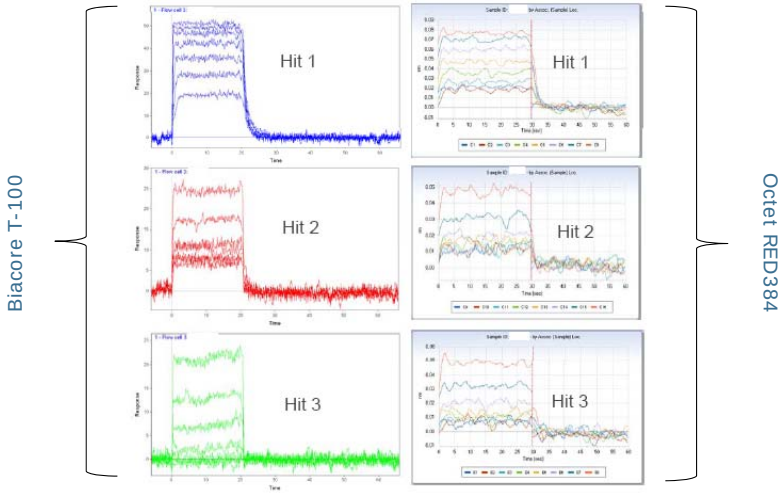
Small Molecule Fragment Screening on Octet RED384 vs Biacore
Small Fragments binding to Kinase



Data very consistent across both platforms

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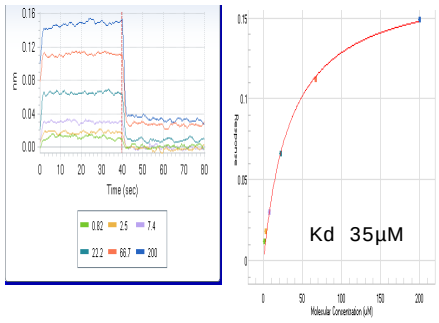
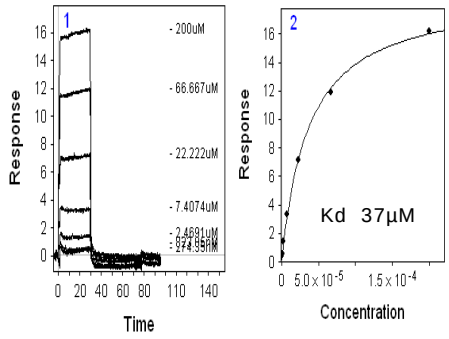
Small Molecule Kinetics on Octet RED384 vs Biacore Roche Validation Study

Octet RED384



Biacore



238 Dalton fragment binding to biotinylated kinase;
6 point dilution series in TBS, 1mM DTT, 0.005%P20, 5%DMSO

Data Courtesy of Frank Podlaski, Discovery Technologies, Roche, Inc.

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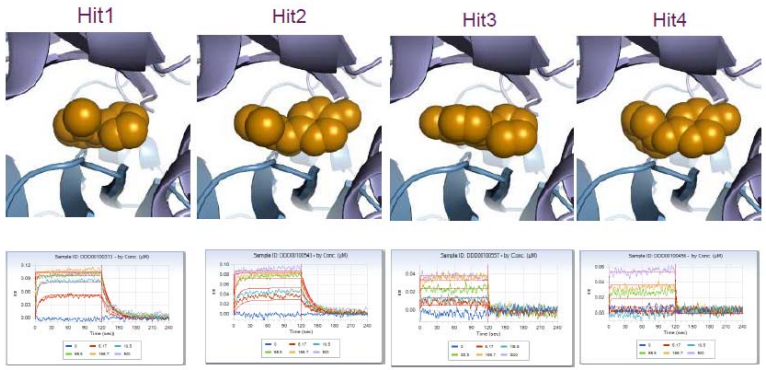
Confirmation of hits by X-Ray

Crystal structures – Orthogonal Method

- Co-crystallisation of top hits with target protein



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Benefits of the Octet Platform

- **Higher throughput, faster workflow**
  - Concentration measurements **faster than ELISA or HPLC**
  - Screen more samples in less time than SPR
  - **Little** dilution or **sample preparation required**
  - **Full sample recovery**
  - Fixed volume allows long on/off rate determinations
- **Unparalleled ease-of-use**
  - Multiple users are easily trained to operate the system
  - **Microfluidics-free f**ormat and disposable biosensors
  - **No maintenance**, cleaning, or instrument prep time required
- **Versatility**
  - **Crude samples** can easily be measured
  - **Wider solvent** tolerance and assay flexibility
- **Price**
  - **Lower capital** acquisition cost than traditional SPR systems
  - Lower usage/maintenace costs SPR systems
  - **Lower cost per sample** than Protein A HPLC or commercial ELISA kits

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