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Label-Free Biosensor Technical Solutions from the ForteBio Octet Platform

September 22, 2009

冷泉港生物科技股份有限公司

Agenda

- Label-Free BLI Technology
- Octet Applications in Quantitation
- Octet Applications in Kinetics

Reasons to Adopt Label-Free Technology

- Improve time to result
  - Reduce sample preparation
  - Eliminate long incubation and wash steps
  - Simplify assay development time
- Reduce costs
  - Reduce labeling reagents
  - Reduce required number of Ab pairs
  - Reduce labor time required to run assay
- Increase accuracy and precision of measurement
  - Measure direct binding events
  - Enables real-time detection and high content information
  - Eliminate false results due to labeling interferences

The Octet Platform: Fast, Accurate, EASY!

- Very easy to use with short set-up and run times
  - Label-free and fluidics-free platform
  - Dip and read BLI (biolayer interferometry)-based technology
- Broad spectrum of kinetic applications
  - Screening
  - Characterization
  - Epitope Binning
  - Method Development

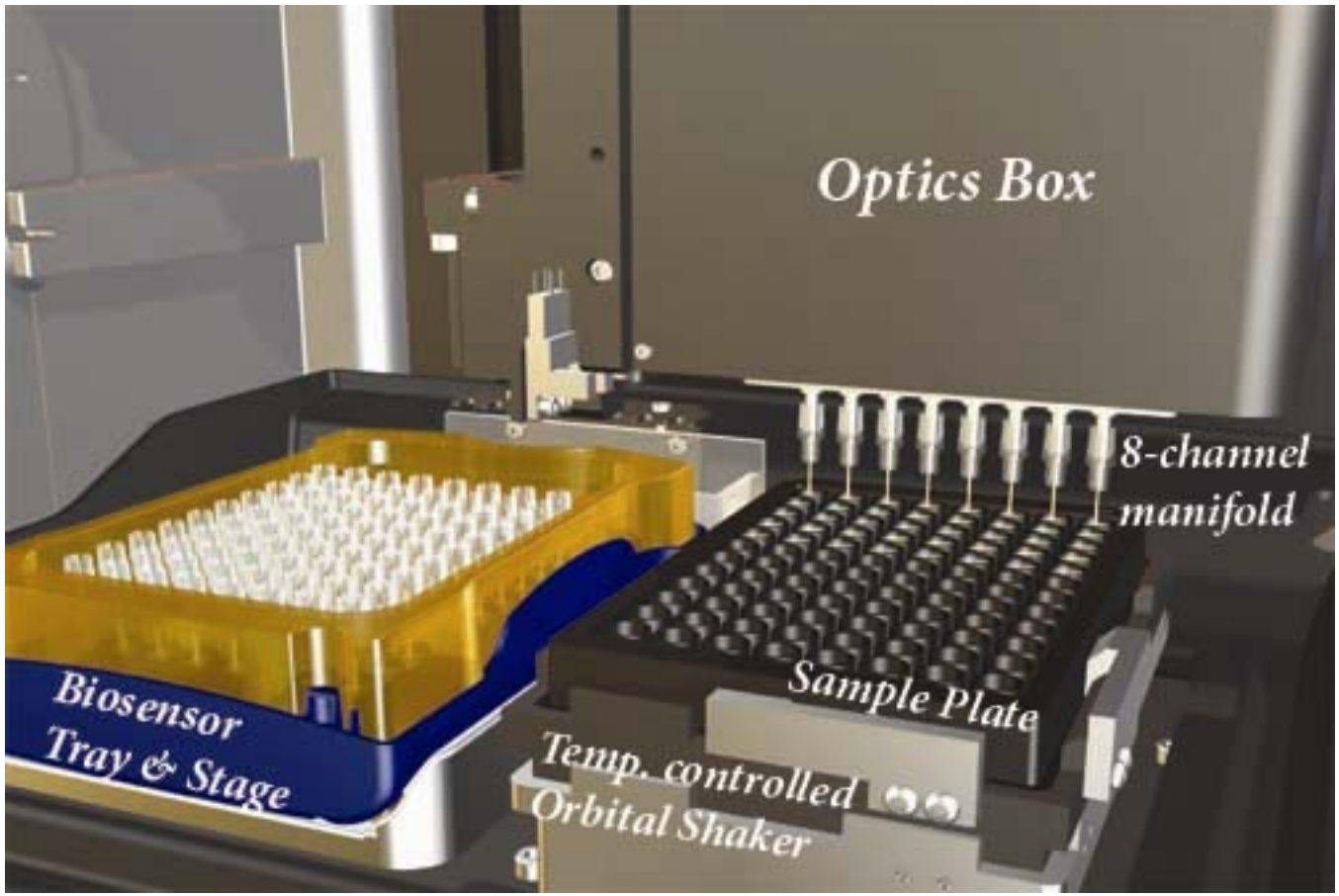
- Automate up to 96 samples at a time
- Ability to measure in crude samples and in DMSO (up to 10%)
- It also does quantitation!

Octet RED = Rapid Extended Detection

- Extended Sensitivity
  - 8 higher resolution spectrometers
  - RMS noise 0.003 nm
  - New Super SA Biosensors
- Faster sampling rate
  - Data taken for 8 channels in parallel
  - Data acquisition 5Hz (0.2 Sec/pt)
- Extended Range
  - mM to pM affinities
  - Small molecules and peptides
  - Dynamic Range LLOD ≥ 10 ng/mL

The Octet Instrument

Enabling rapid sample analysis



Fast. Accurate. EASY.

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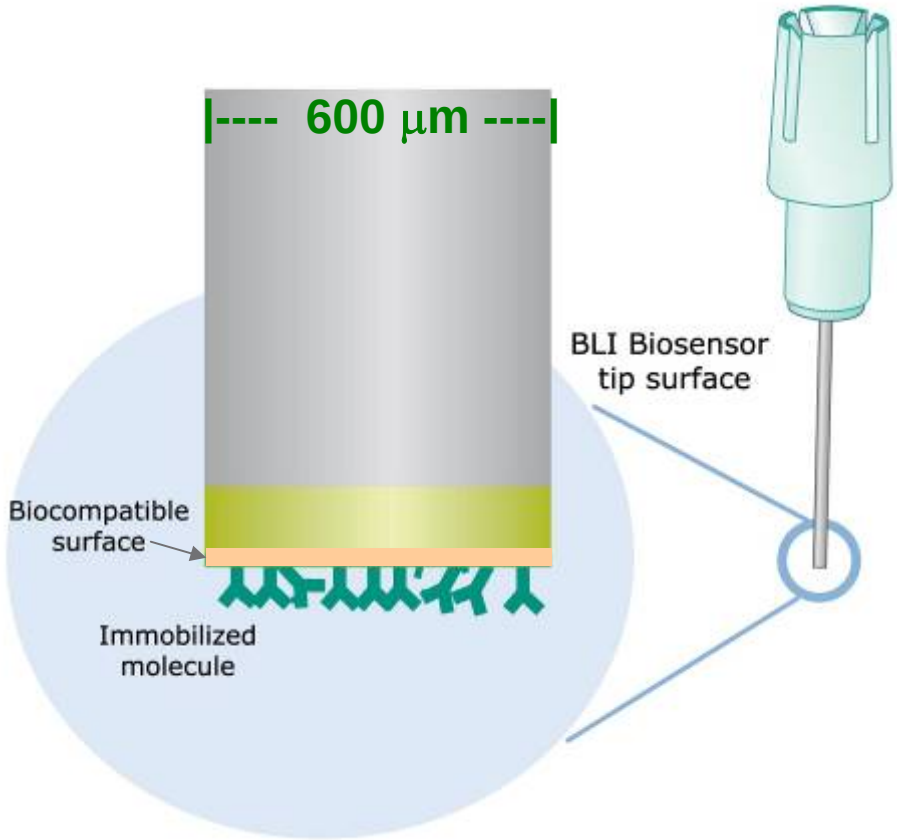
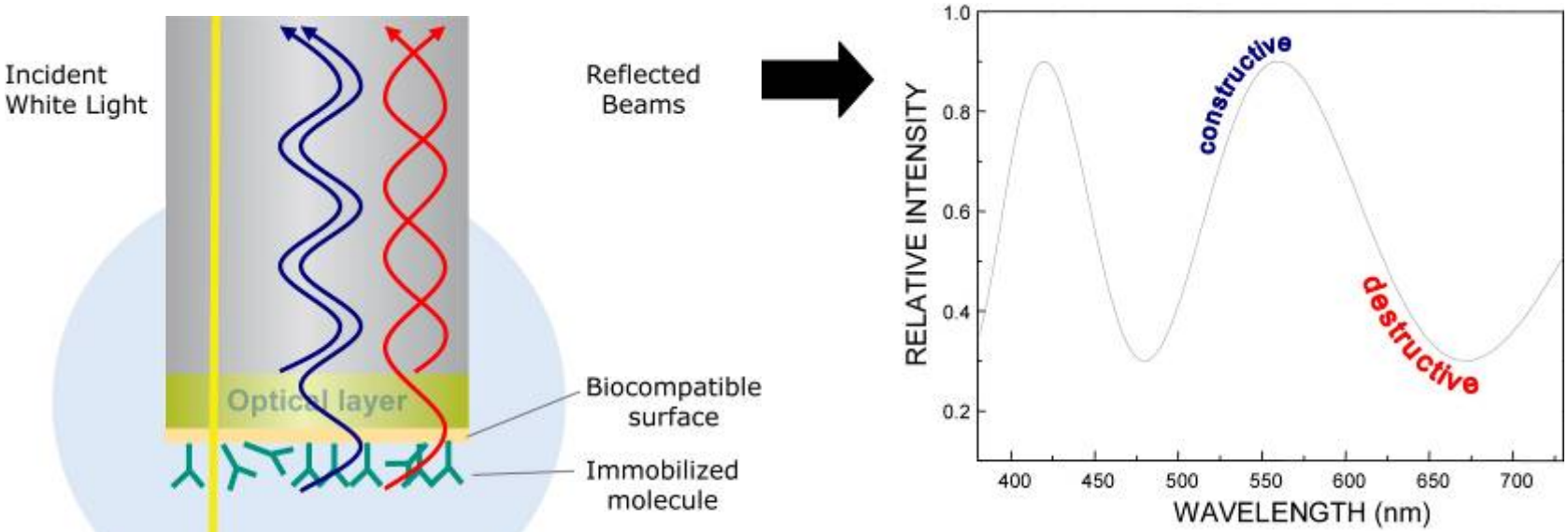
BLI Surface Chemistry
Bio-Layer Interferometry

- **The Octet Biosensor consists of a polished fiber optic embedded into a polypropylene hub with a sensor-specific chemistry at the tip**
  - **Two-dimensional binding surface**
  - **Biocompatible Matrix**
    - minimizes non-specific binding
  - **High uniformity across the sensor surface**
  - **Non-denaturing**



BioLayer Interferometry (BLI)

- **A layer of molecules attached to the tip of an optic fiber creates an interference pattern at the detector.**



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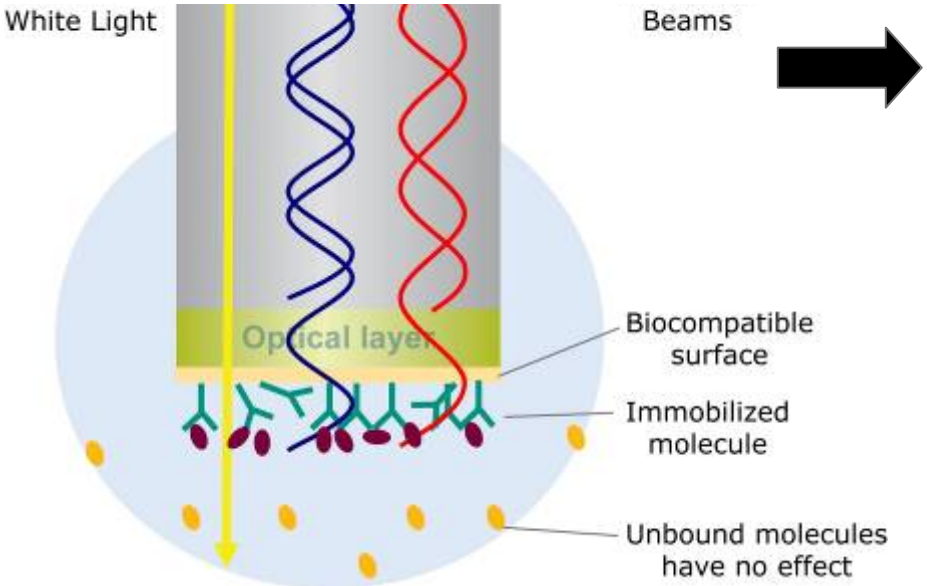
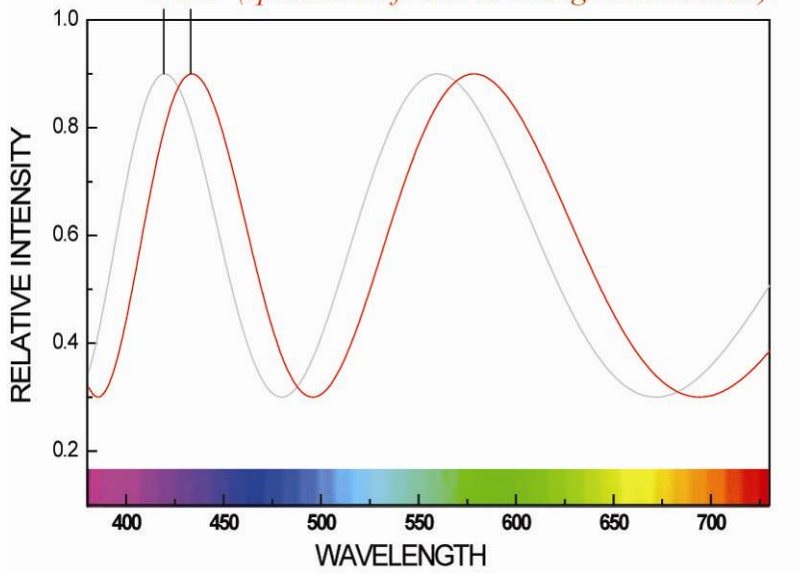
BioLayer Interferometry (BLI)

- **A layer of molecules attached to the tip of an optic fiber creates an interference pattern at the detector.**
- **Any change in the number of molecules bound causes a measured shift in the pattern**



Response = $\Delta\lambda$ *(spectral shift due to change in thickness)*



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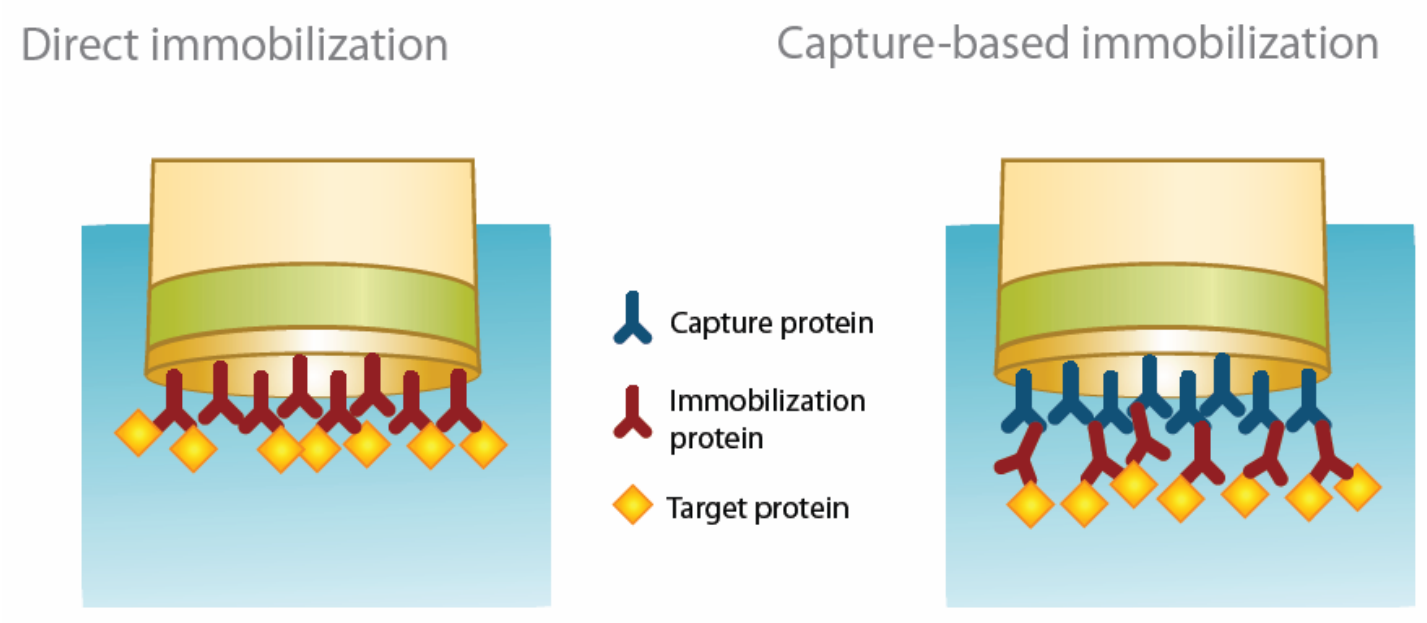
Surface Chemistry of Biosensors



Amine Reactive(AR) or Streptavidin(SA) Biosensors

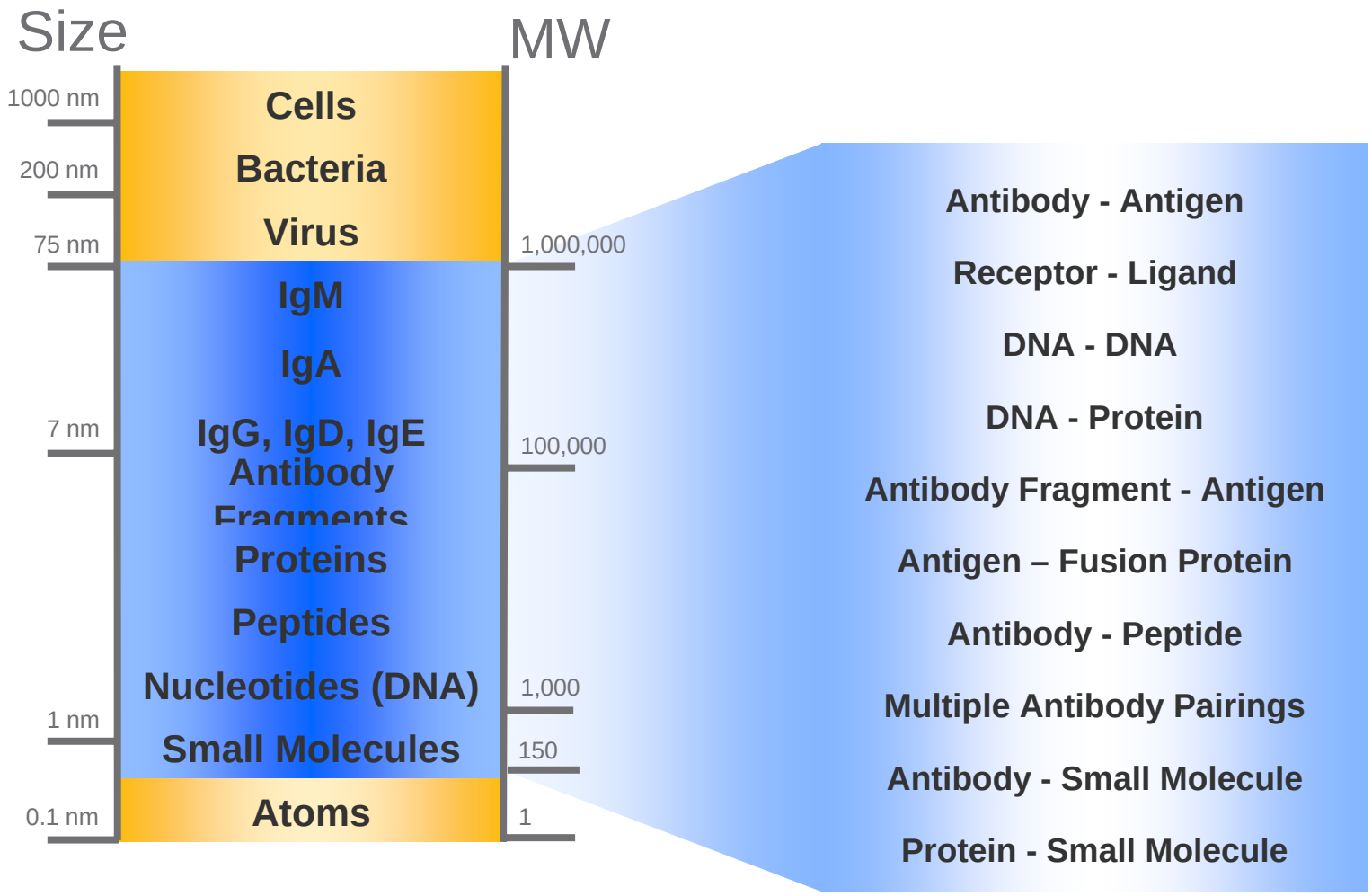
Anti-hIgG Fc Capture Biosensors

Biosensor Chemistry Options

Application	Sensor Type	Regeneration
Quantitation	Anti-Human IgG Fc	No
	Anti-Murine IgG (Fab')2	No
	Protein A	Yes
	Streptavidin (SA)	Yes
Screening or Kinetics	Streptavidin (SA)	Yes
	Super Streptavidin (SSA)	Yes
	Amine Reactive (AR)	Yes
Kinetics	Aminopropylsilane (APS)	Yes
	Anti-hIgG Fc Capture Surface (AHC)	Yes

Some regeneration protocols may be dependent on chemistry of protein attached to the biosensor.

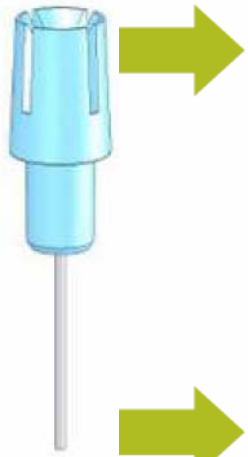
Octet Versatility in Interaction Analysis



What does the Octet do?

Quantitation Applications

- IgG quantitation
- Protein quantitation



Cell line development
Bioreactor process optimization
Production titer monitoring

Kinetics Applications

- Affinity characterization
- Measure kinetic constants
- Rank order affinities

Rank ordering of clone selection

Kinetic characterization

Epitope binning

Antibody pair matching

Octet應用：快速蛋白質定量

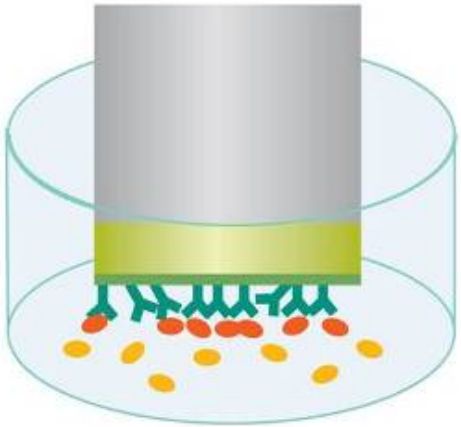
- Rapid and high-sensitivity titer/IgG quantitation

Octet Automated Workflow for Quantitation

Surface Chemistry

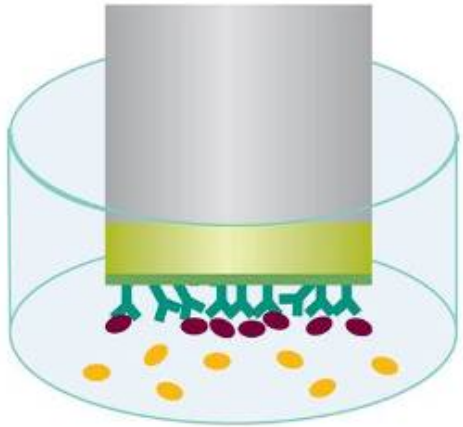
Anti-hIgG Fc sensor or
Anti-Murine IgG Fv sensor
or
Protein A sensor

Standard Curve

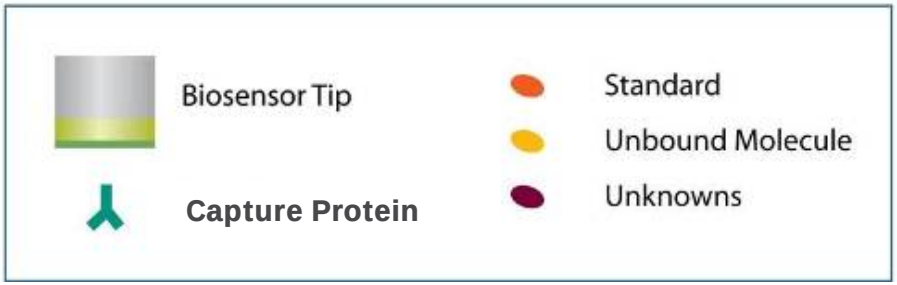


IgG standard

Unknowns



Purified sample,
Cell culture supernatant,
Crude lysate, etc.

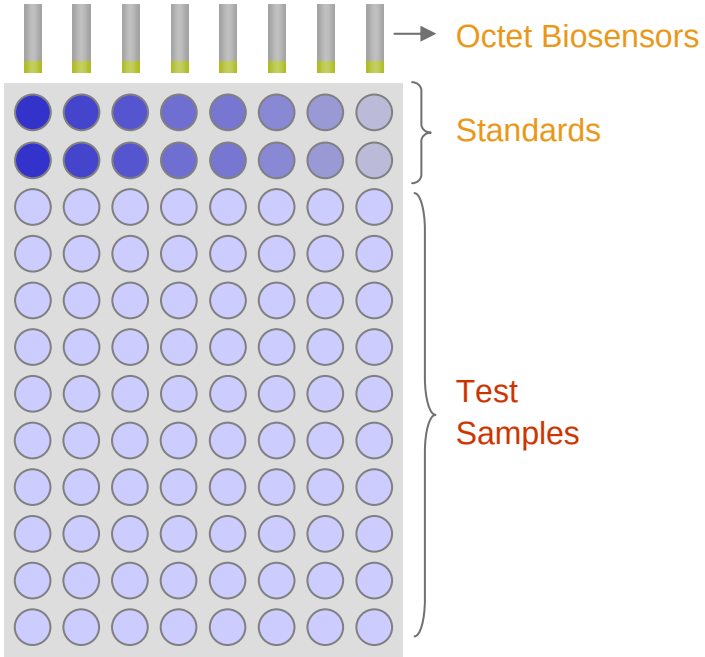


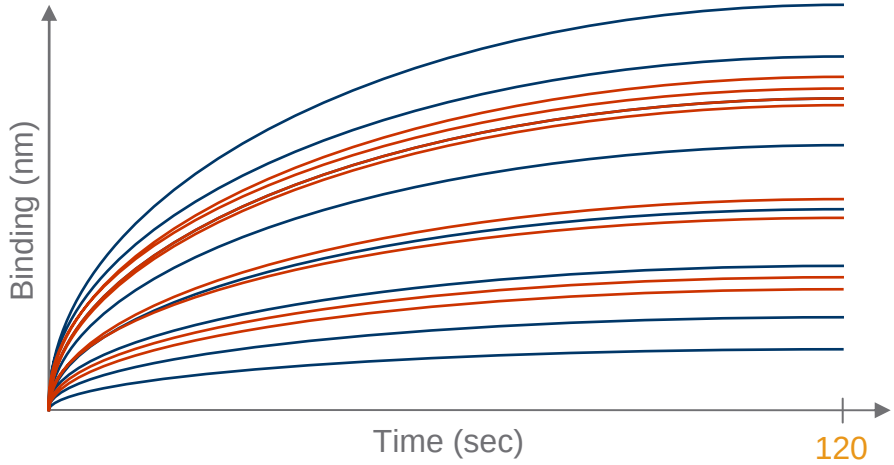
Quantitation biosensors allow flexibility in applications

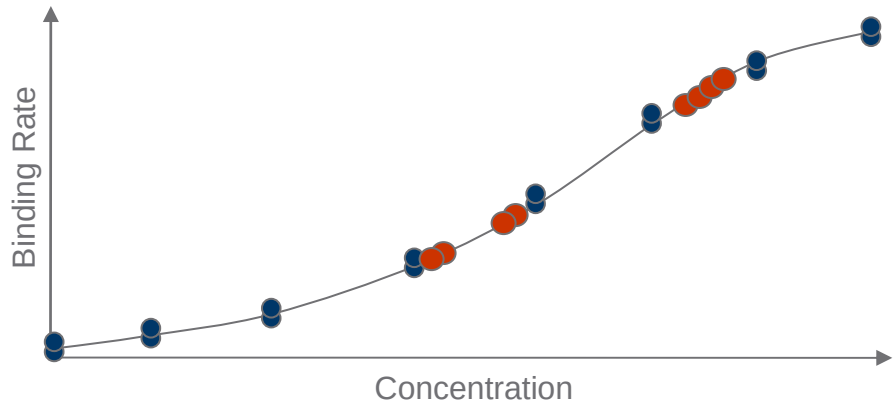
Biosensor Name	Immobilization Chemistry	Potential Applications
Protein A	The protein A immobilized on the biosensor binds to the Fc region of human IgGs. Will also bind to the Fc of many subtypes of mouse and rabbit IgG.	Concentration determination of hIgG in buffer, serum free media, and other crude mixtures which do not contain other species IgGs.
Anti-murine IgG Fv	Binds specifically to the F(ab')2 portion of mouse and rat IgGs.	Concentration determination of mouse or rat IgG in buffer, serum free media, serum containing media and other crude mixtures.
Anti-human IgG Fc	Binds specifically to the Fc portion of human IgGs.	Concentration determination of hIgG in buffer, serum free media, serum containing media and other crude mixtures.
Streptavidin (SA)	Streptavidin coated biosensor with a high binding capacity for biotinylated proteins, peptides and nucleic acids.	As a base sensor for immobilizing a biotinylated antibody or protein that binds to a specific target. The protein functionalized sensor can then be used to construct a tailored quantitation assay.

Octet automated workflow for quantitation

-No plate coating. No sample prep. No washes.







- **Data is taken for 2minutes per 8 wells**

➔ **96 wells in ~30 minutes**

➔ **1 step, no washing**

Fast. Accurate. EASY.

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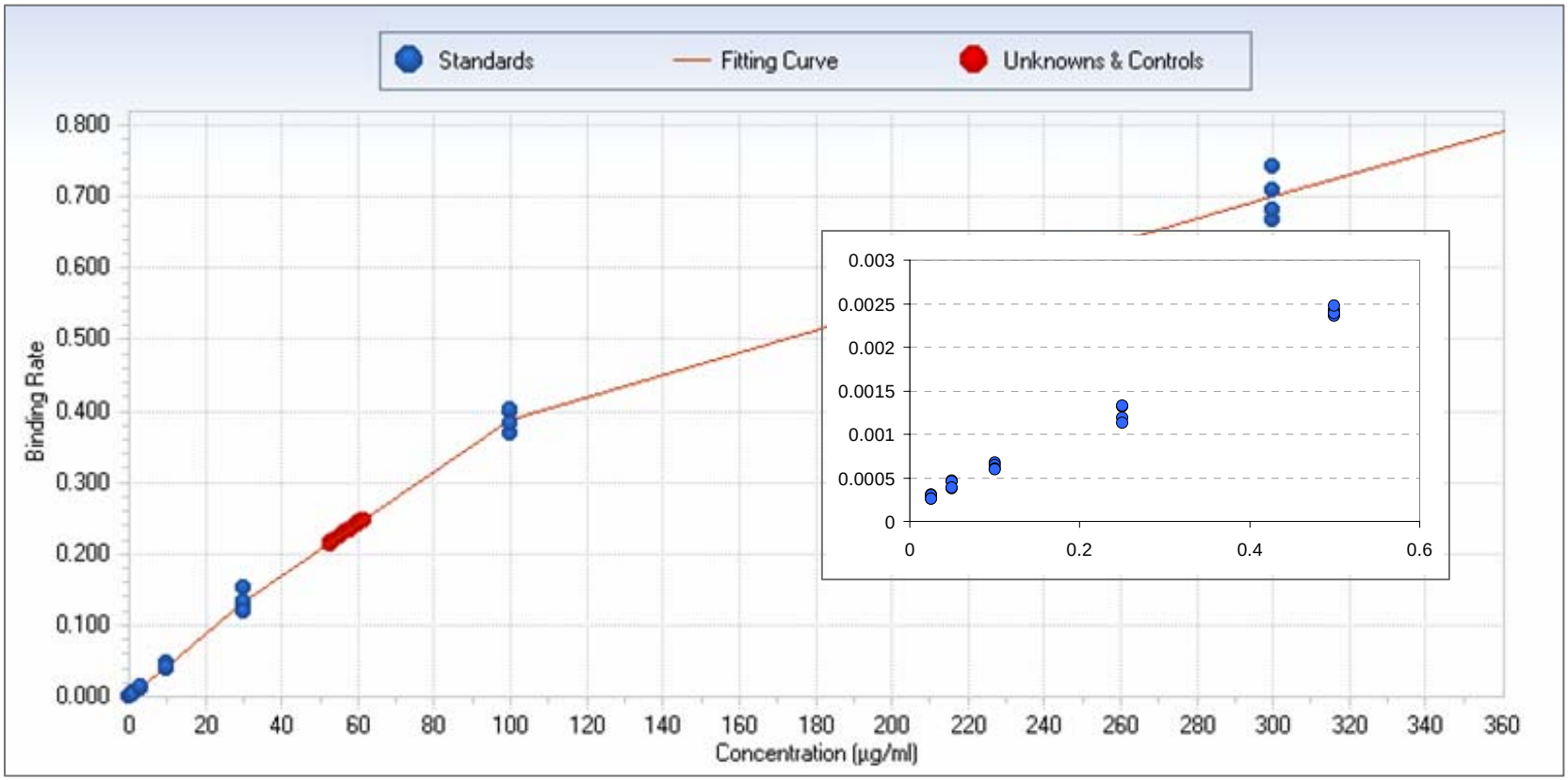
High sensitivity titer analysis of expressed human IgG

- **Issue:**
  - Need a rapid assay for analysis of expression clones early in development
  - Requires high sensitivity since expression levels at this stage are at the 50-1000 ng/mL range

- **Solution:**
  - Use the Octet RED and Protein A biosensors in a higher sensitivity mode (uses higher flow rate and longer assay time)

High sensitivity titer analysis

Calibration curve shown covers 25 ng/mL to 300 µg/mL range



Higher sensitivity mode throughput is a 96 well plate/per hour

Fast. Accurate. EASY.

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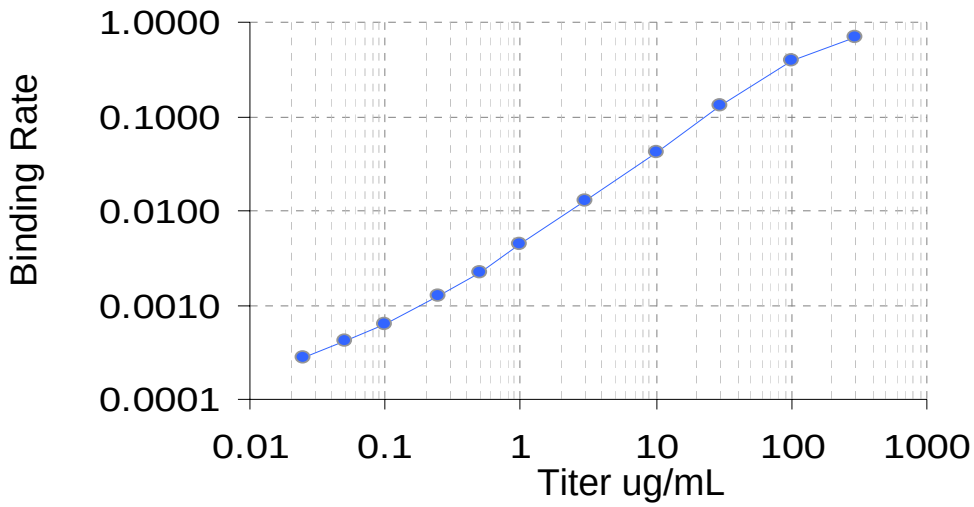
Good precision throughout the range

Calibration curve shown covers 25 ng/mL to 300 µg/mL range

1000 rpm, 5 minutes per column read time, Streptavidin sensor



Sample (nominal µg/mL)	Conc (µg/mL)	CV%	Rec%
0.025	0.026	9.7%	104%
0.05	0.04	9.9%	85%
0.1	0.09	9.7%	88%
0.5	0.50	4.8%	101%
1	1.07	6.9%	107%
10	10.3	6.6%	103%
50	57.4	5.1%	115%

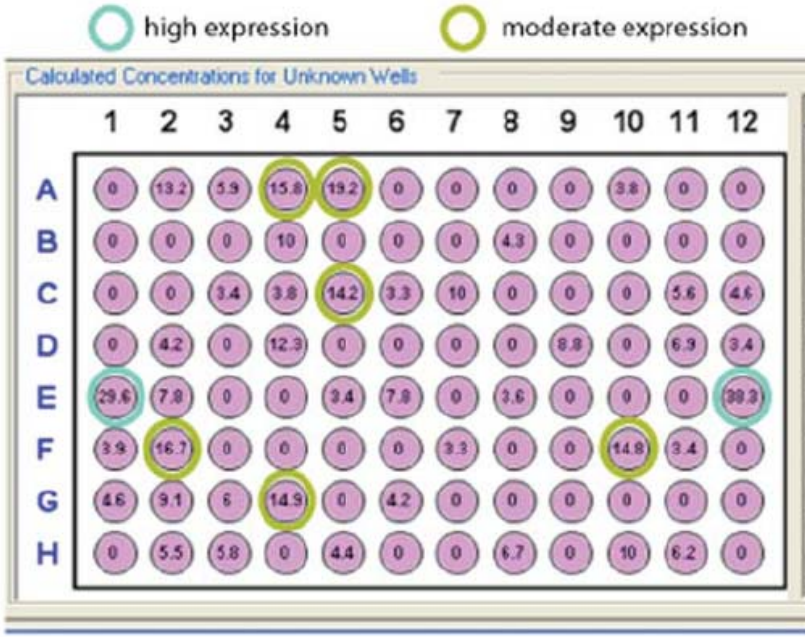
Method parameters are flexible allowing desired dynamic range and sensitivity.

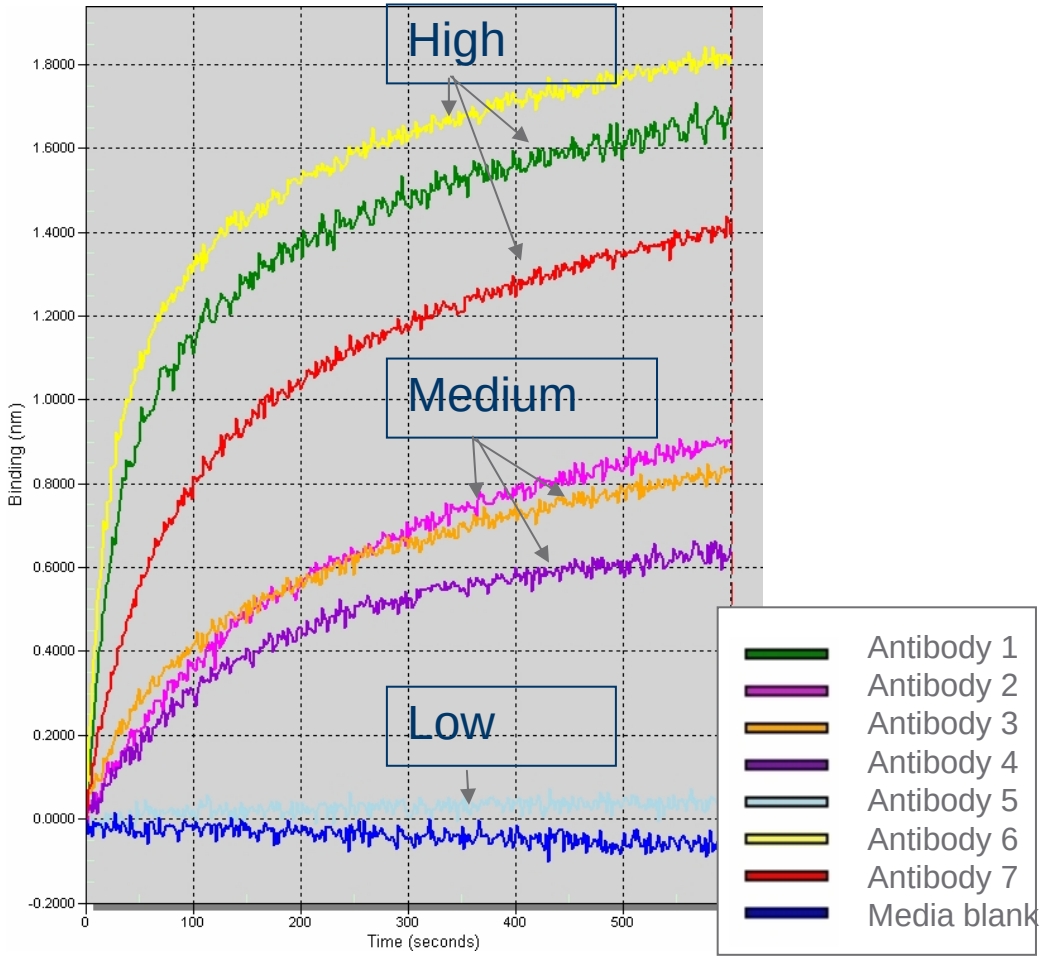
No additional sample, plate, or instrument preparation.

Higher sensitivity can be achieved with longer incubation.

Wide dynamic range allows less dilutions.

Rapid Screening of Antibody Clones for Expression





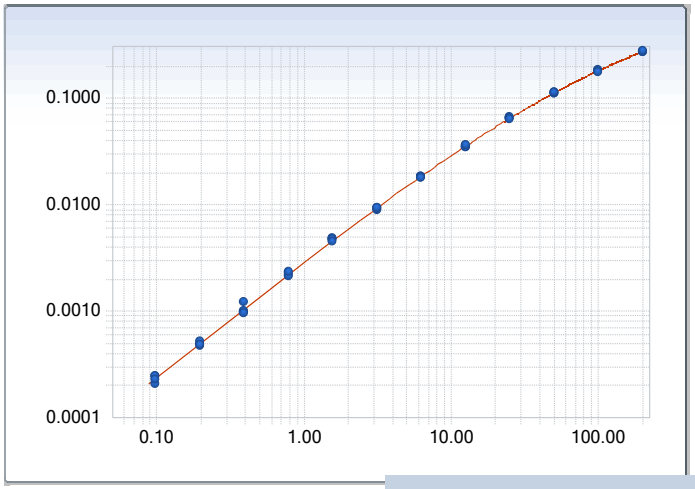
The Octet can be used to monitor the purification process in addition to screening of clones

- **Rank Antibody-Producing Cell Lines**

Higher sensitivity mode for hIgG

- Extends assay range down to ~25 ng/mL
- Throughput is one 96 well plate per hour
- No washes. No plate prep. No sample prep needed
- Good accuracy and precision throughout extended range

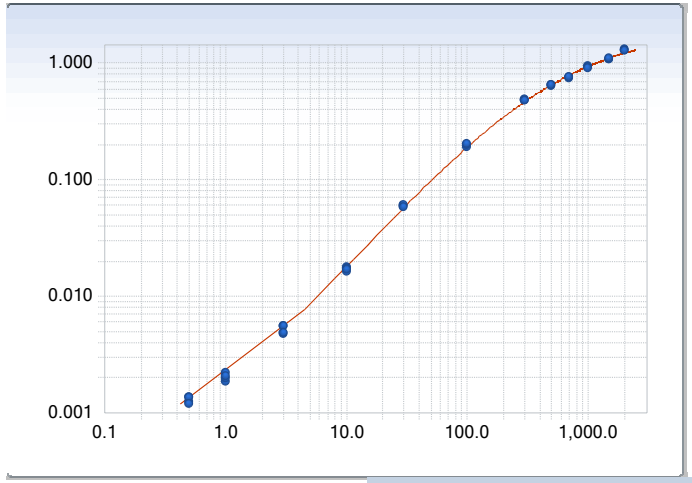
Quantitating IgGs with Octet RED



Anti-Mouse IgG Biosensors

Expected ug/mL	Average calculated ug/mL	%CV N=4
200	200.00	1.8%
100	100.44	3.1%
50	50.12	2.0%
25	25.05	2.6%
12.5	12.52	2.7%
6.3	6.25	1.1%
3.1	3.13	2.3%
1.6	1.56	2.8%
0.8	0.78	3.8%
0.4	0.36	2.6%
0.2	0.20	3.2%
0.1	0.10	7.7%

Binding Rate



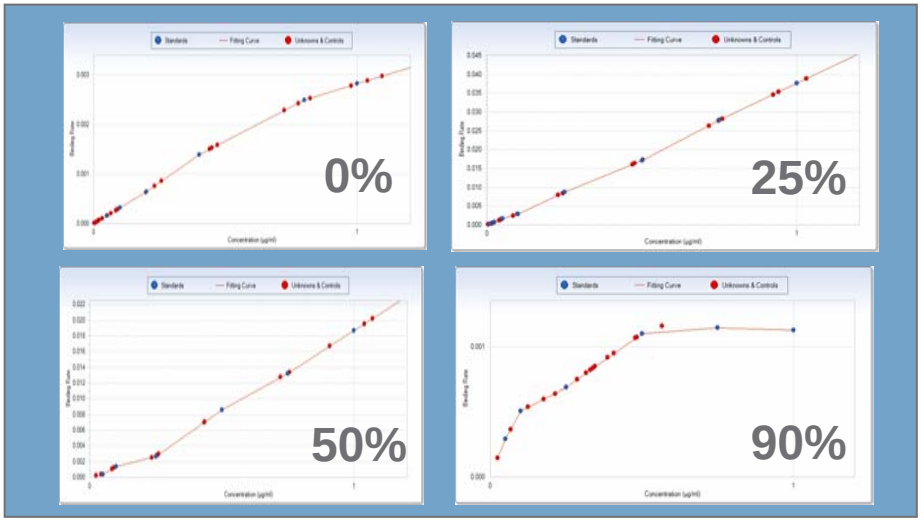
Anti-Human IgG Biosensors

Expected ug/mL	Average calculated ug/mL	%CV N=4
2000	2000.00	2%
1500	1498.37	1%
1000	1009.13	4%
700	699.57	2%
500	501.60	2%
300	301.29	2%
100	100.42	3%
30	30.02	2%
10	9.97	3%
3	2.99	8%
1	1.02	7%
0.5	0.51	9%

Concentration (ug/ml)

Therapeutic Proteins in Human Sera Samples

Biotinylated Capture Antibody on Streptavidin HBC Sensors Detects Therapeutic Protein in the Presence of 25-90% Human Serum



CVs for Therapeutic Protein Levels in Human Serum

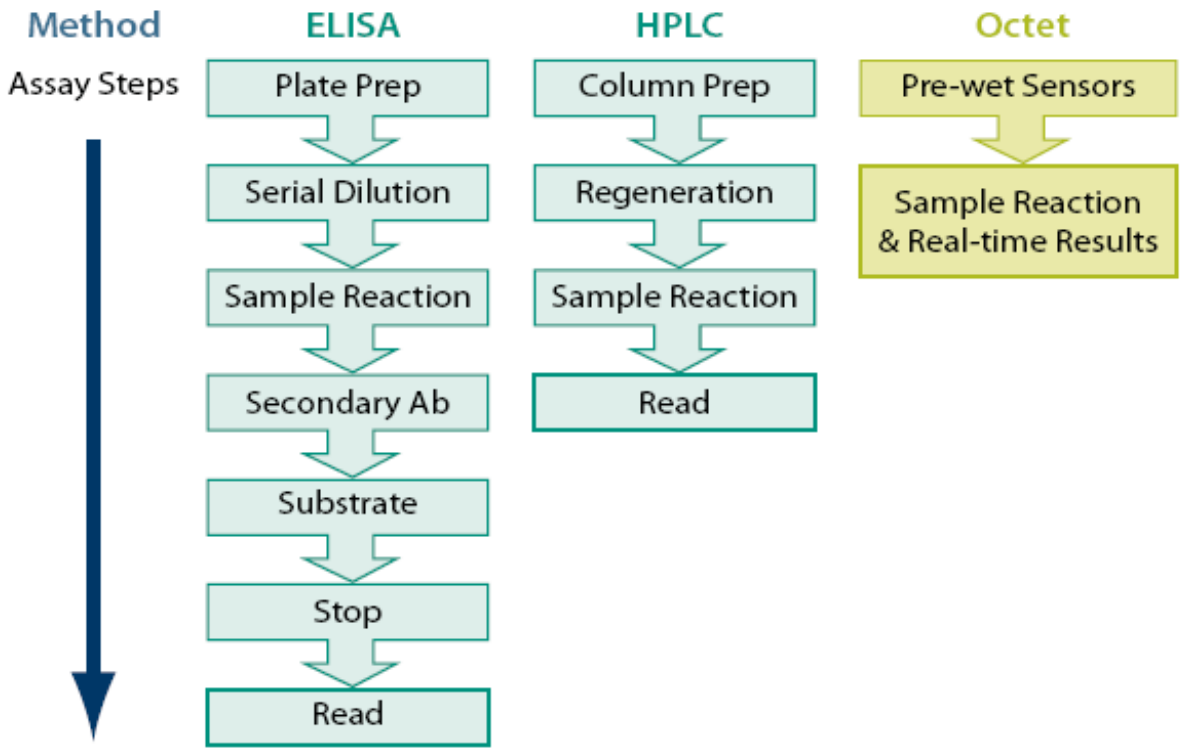
% Human Serum	Dynamic Range Tested	%CV Range
0%	10-1,000 ng/ml	3.7-12%
25%	10-1,000 ng/ml	3.7-10%
50%	10-1,000 ng/ml	1.5-10%
90%	10-1,000 ng/ml	6.0-25%

Octet應用：改善蛋白質定量流程
□ Faster Quantitation for Process Development

Faster Quantitation is Ideal for Process Development

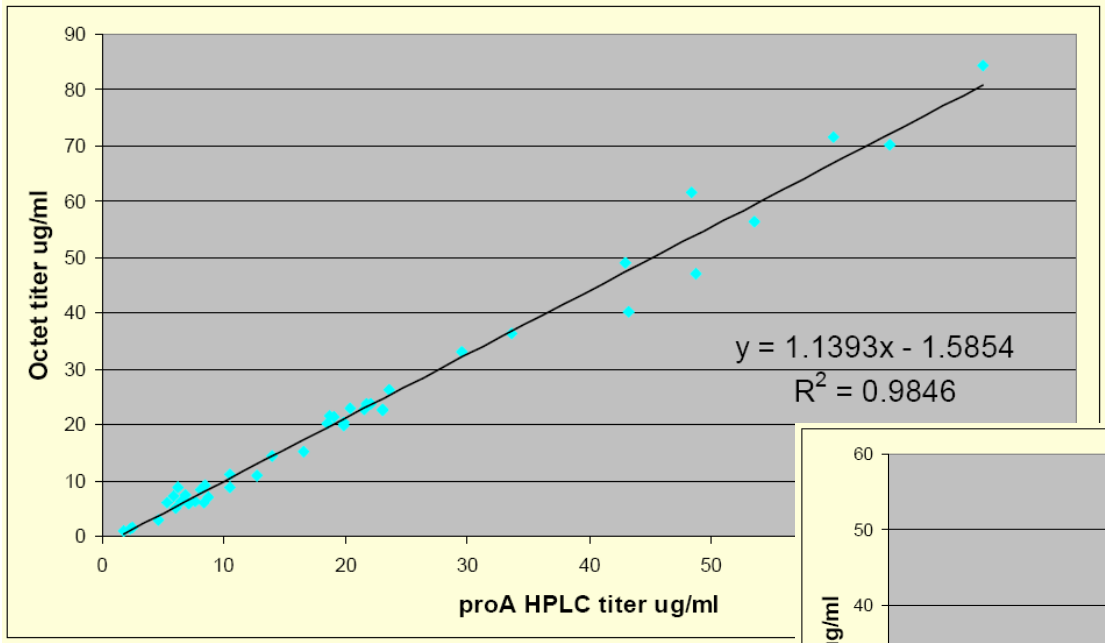
ELISA and HPLC are typically used to monitor antibody expression.

Octet platform allows faster feedback on reactor status and reduces risk of production losses.

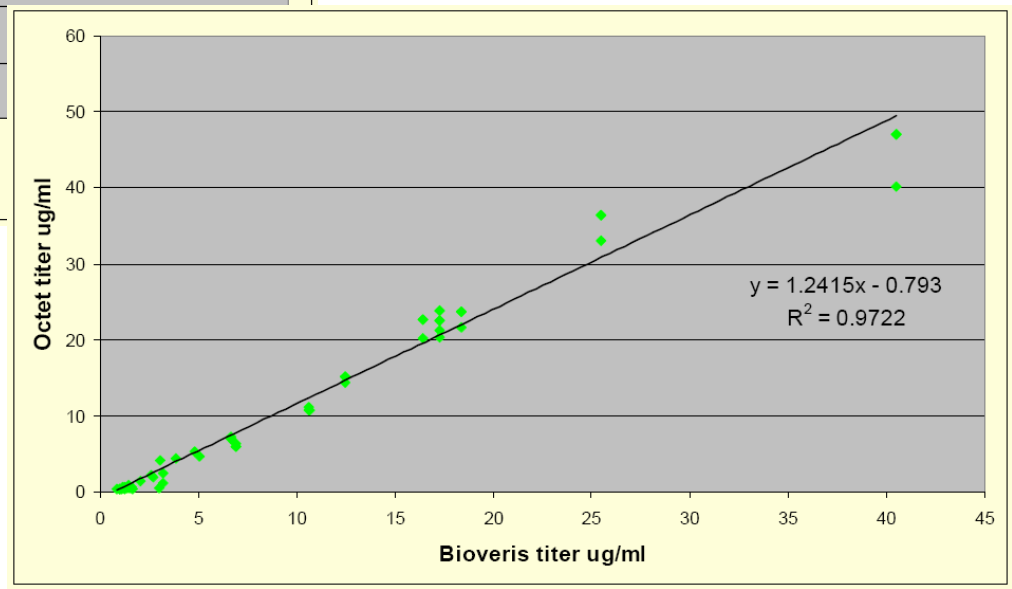


	ELISA	HPLC	Octet
Labor Time	3 hrs	0.5 hrs	<0.2 hrs
Total Time to Results	> 6 hrs	10 hrs	0.5 hrs

Accuracy Correlates Between Octet, HPLC, and ECL



Protein A biosensors were compared to Protein A HPLC and ECL technology.



Data presented at Bioprocessing International 2007 by Sarah Koob from Wyeth

Reproducible Throughout 10 Plate Screening Assay

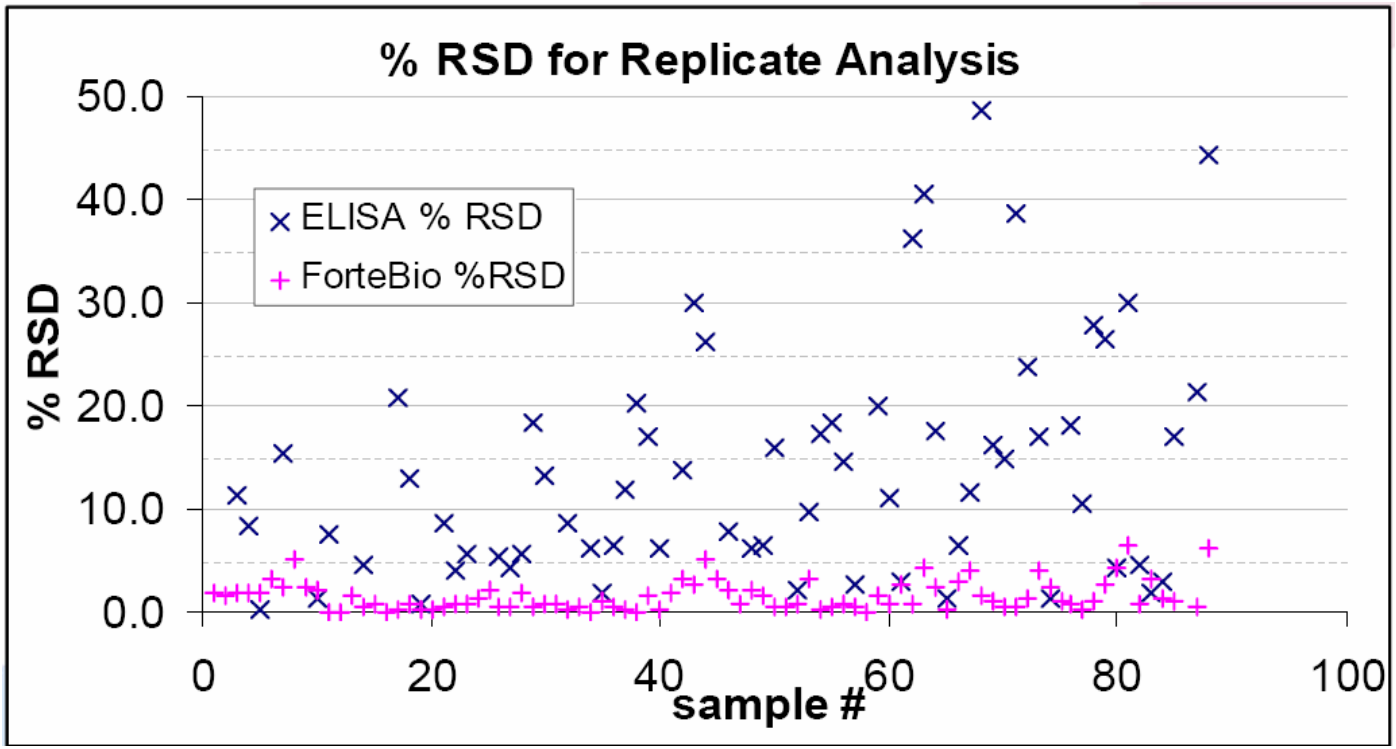
Controls	150	50
P1 row A	129.4	47.8
P1 row H	138.4	53.6
P2 row A	130.2	46
P2 row H	134.3	47.7
P3 row A	138	45.2
P3 row H	152.3	48.6
P4 row A	135.7	44.8
P4 row H	137.9	46.1
P5 row A	147.8	45.3
P5 row H	139.4	48.6
P6 row A	123.4	45.1
P6 row H	141.4	46.2
P7 row A	125.5	47.5
P7 row H	136.2	51.2
P8 row A	131.2	41.9
P8 row H	130	45.1
P9 row A	140.6	44.2
P9 row H	137.8	45.2
P10 row A	133.8	45.2
P10 row H	147.4	46.6

mean	137	47
% recovery	91	93
%CV	5.4	5.5

- **Controls reproducible across all 10 plates**
- **Low variability of 5-6% CV within controls**

Data presented at Bioprocessing International 2007 by S. Koob

Octet Reproducibility Comparison to ELISA



Octet = 30 minutes,
ELISA = 5-6 hours

One 96 plate/hour

Typical RSD Values:

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

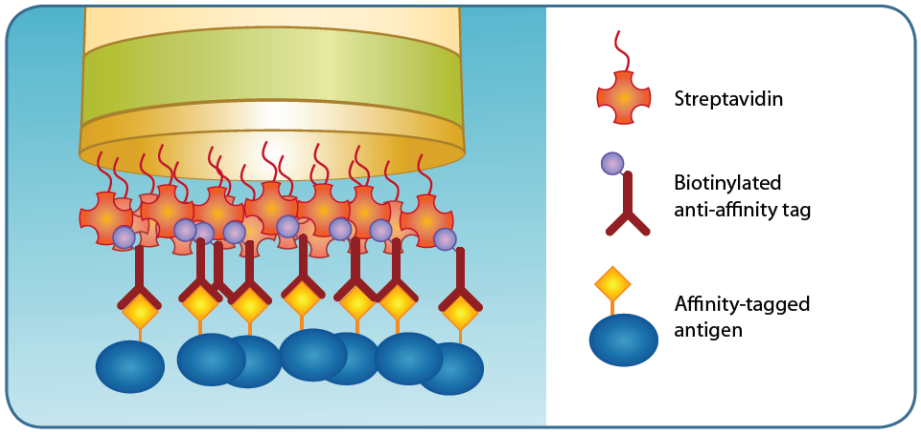
Data presented at IBC Antibody Production, 2008 by Keith Davis from Pfizer MO.

Technology Improvements Achieved by Octet

Improvements over HPLC and ELISA

- **Determine 96 concentrations in 30 minutes**
- **Very little sample prep needed**
- **Fewer dilutions due to wide dynamic range**
- **Crude sample matrices are easily used without interference**
- **Excellent correlation to HPLC and ELISA data**
- **Higher sensitivity than HPLC**
- **Better CVs than ELISA**
- **No fluidics allows no maintenance or instrument set up time**
- **Samples are recovered**

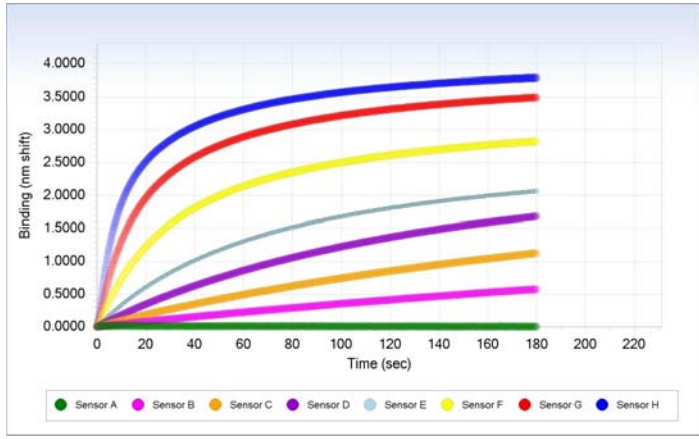
Affinity-tag mediated capture

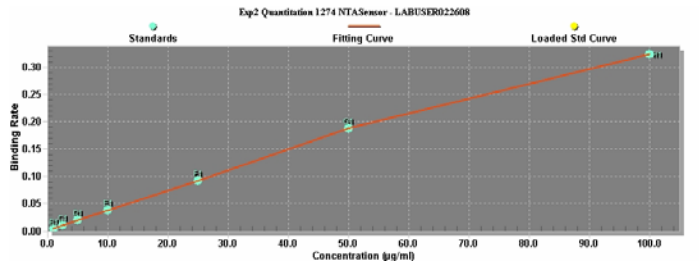


Affinity Tag	Sequence	Length	Binds To
Hexa-histidine, His_6	HHHHHH	6	Nickel NTA, anti-His antibody
FLAG	DYKDDDDKG	8	Anti-FLAG antibody
GST, glutathione S-transferase	Protein	220	Glutathione
Strep-Tag II	WSHPQFEK	8	Streptactin
Protein A	Protein	247	Immunoglobulin

Table 1. Affinity tags successfully used on the Octet RED and Octet QK Systems.

His-Tagged Protein Quantitation





It is possible to create specific biosensors for quantitation of almost any protein.

Fast. Accurate. EASY.

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Octet Applications in Kinetics

- Providing full kinetic characterization of proteins, peptides, and small molecules
- Performing rapid screening for single kinetic parameters
- Enabling fast and easy assay development

Kinetic Characterization Biosensors Allow Flexibility

Biosensor Name	Immobilization Chemistry
Amine Reactive (AR)	Amine functionalized surface to allow for covalent coupling of proteins *via* EDC/NHS mediated amide bond formation
Super Streptavidin (SSA)	Streptavidin coated biosensor with a very high density of biotin binding sites. Immobilizes biotinylated proteins, peptides, and small molecules to form a stable surface
Streptavidin (SA)	High density streptavidin coated biosensor. Immobilizes biotinylated proteins, peptides and nucleic acids to form a stable surface.
Anti-hIgG Fc Capture (AHC)	Immobilization of hIgG or other human Fc containing proteins through the human Fc region.
Aminopropylsilane (APS)	Adsorption of proteins and membrane fractions through hydrophobic moieties.

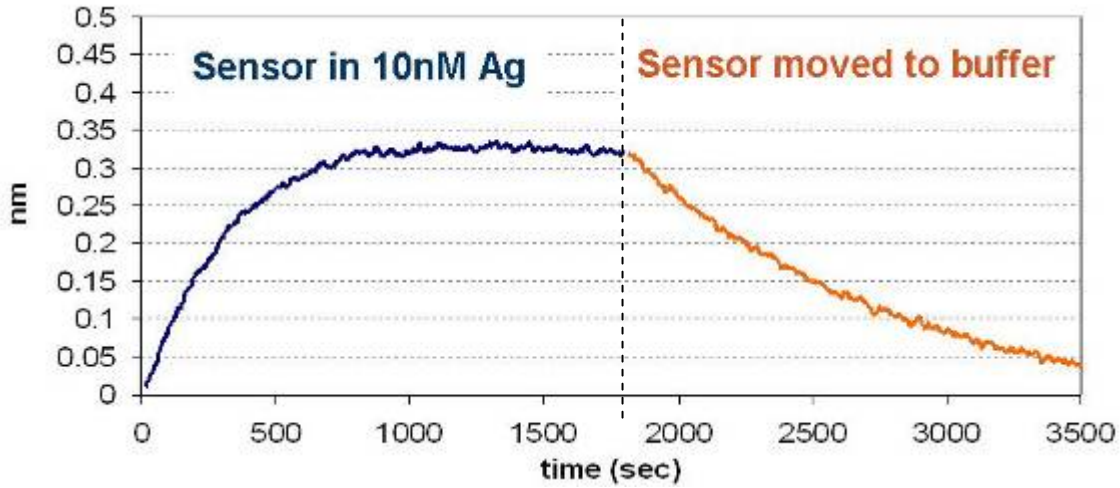
Antibody Binding Kinetics: The Basics

For simple 1:1 binding:

$$Ab + Ag \underset{k_d}{\overset{k_a}{\rightleftharpoons}} Complex$$

k_a = rate of association or "on-rate"

k_d = rate of disassociation or "off-rate"



Non-linear curve fit of data ➔

$$Y = Y_0 + A\,(1 - e^{-kobs*t}) \qquad Y = Y_0 + Ae^{-k_d*t}$$

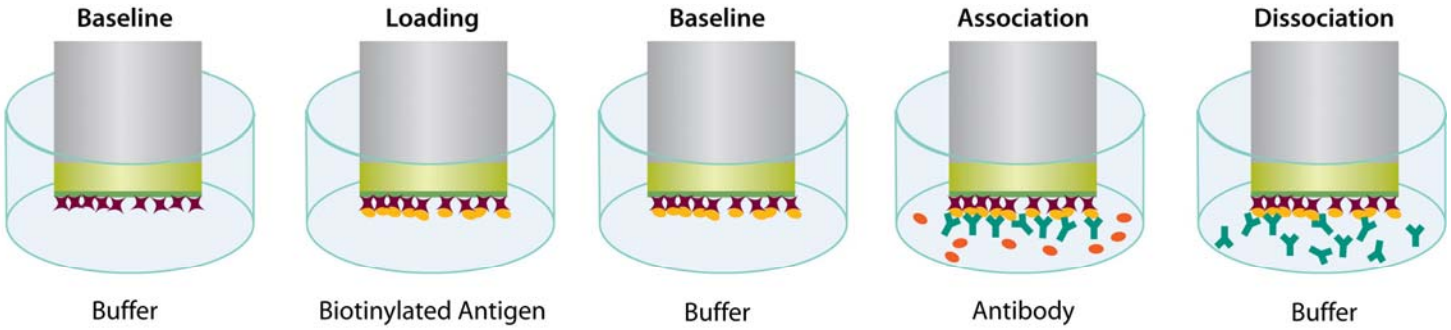
Ka= (1/Ms)

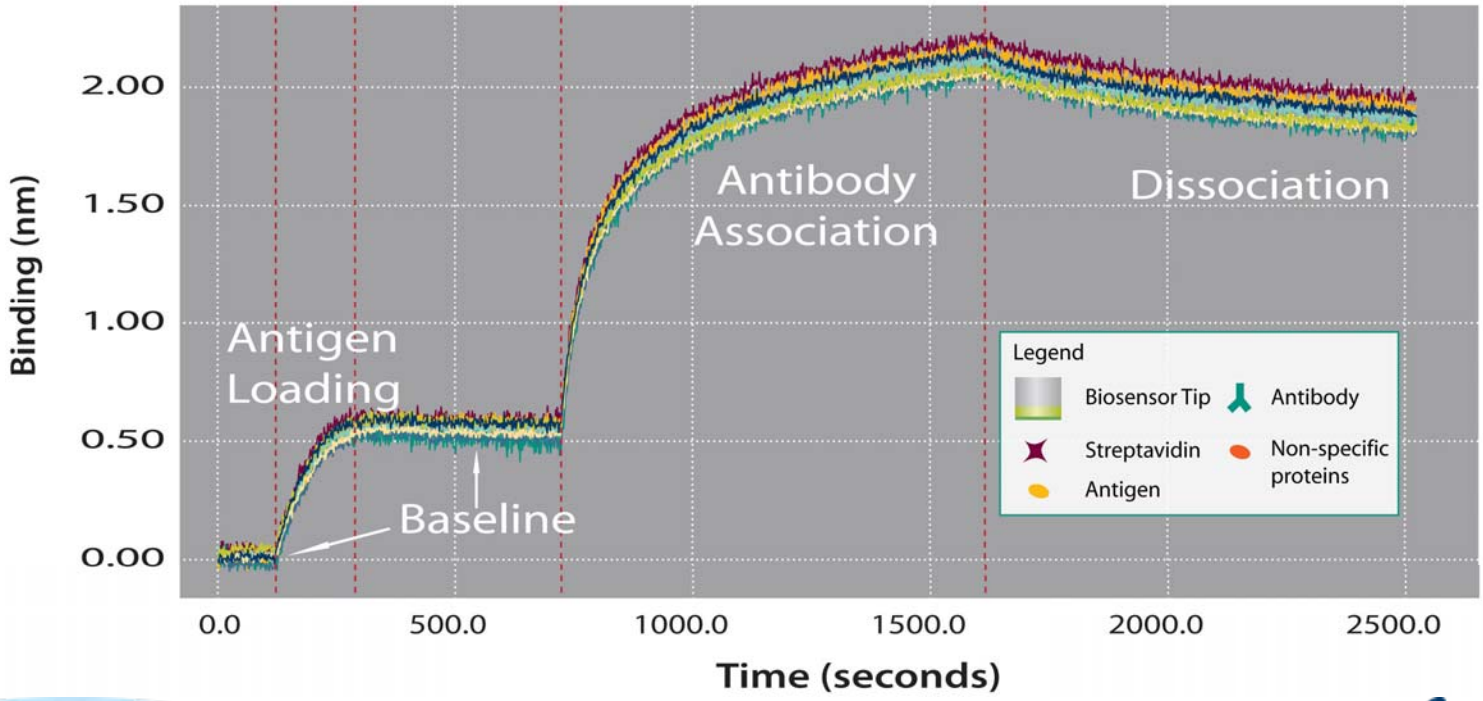
Kd= (1/s)

Kd= (M)

$$k_a = \frac{k_{obs} - k_d}{[\text{Conc Ag (M)}]} \qquad K_D = \frac{k_d}{k_a}$$

Octet Automated Workflow for Kinetics





Fast. Accurate. EASY.

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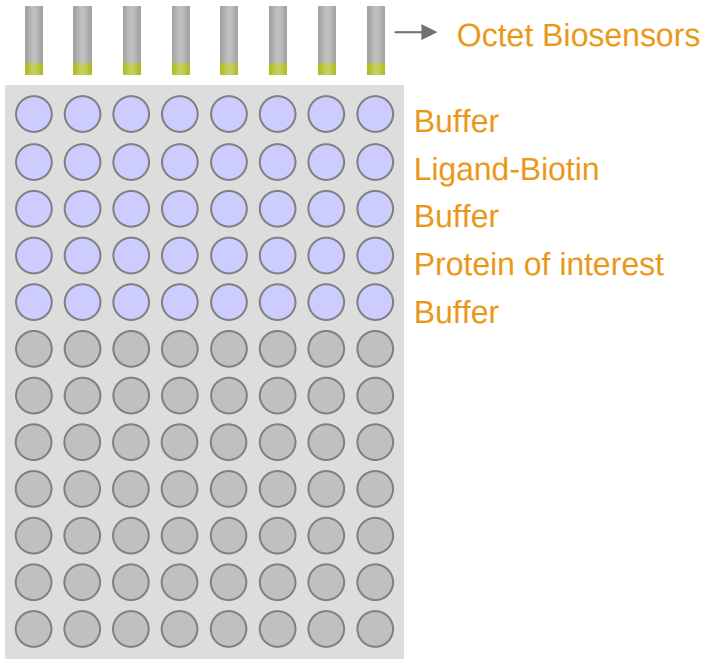
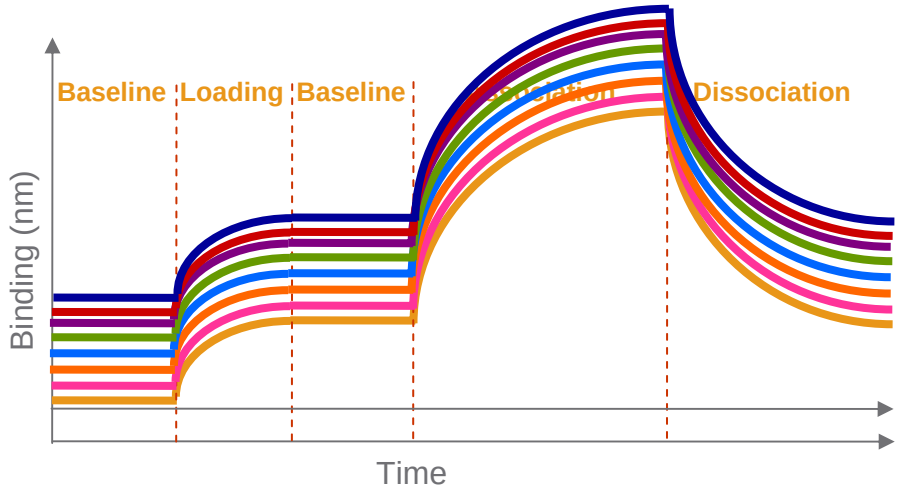
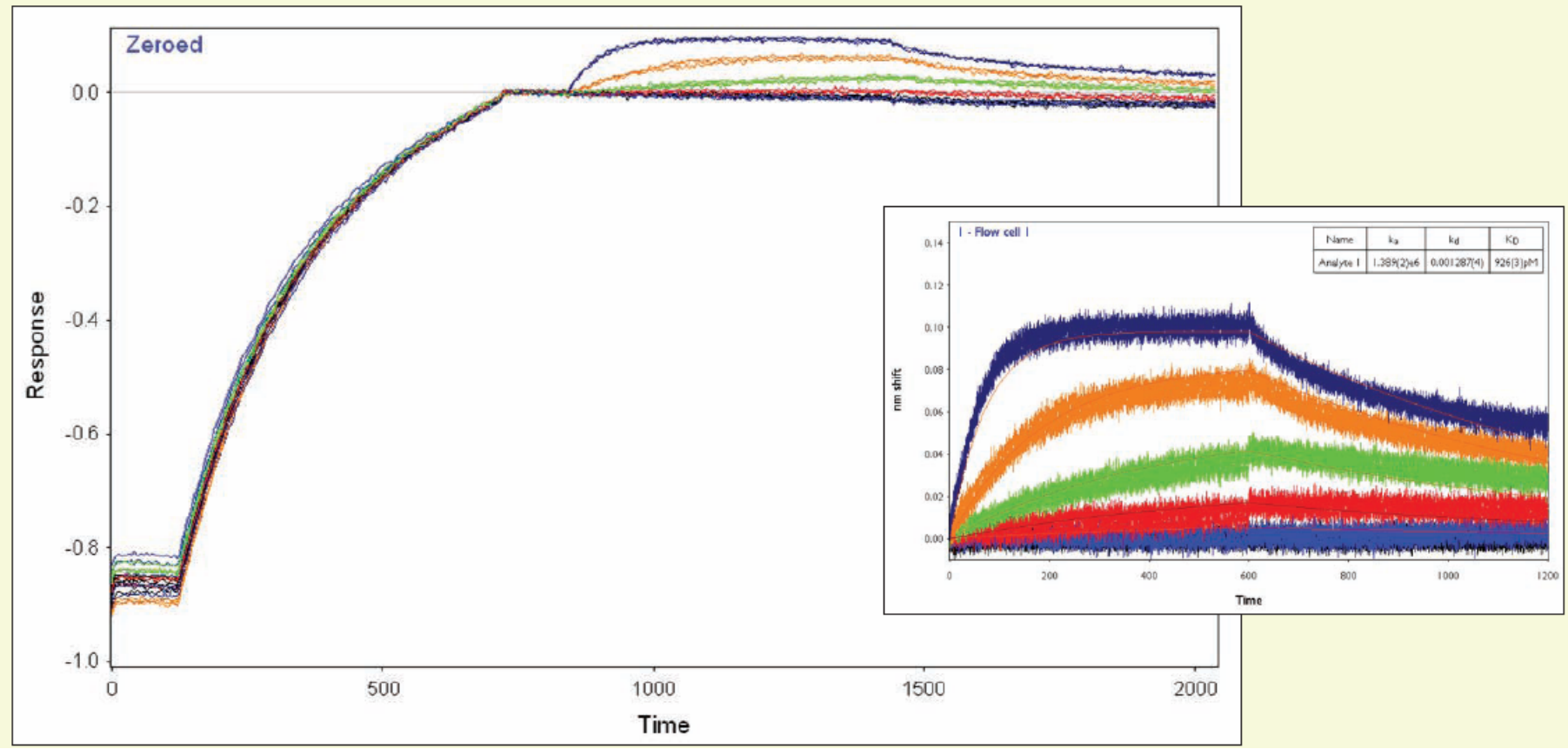
Example Octet Automated Workflow for Kinetics





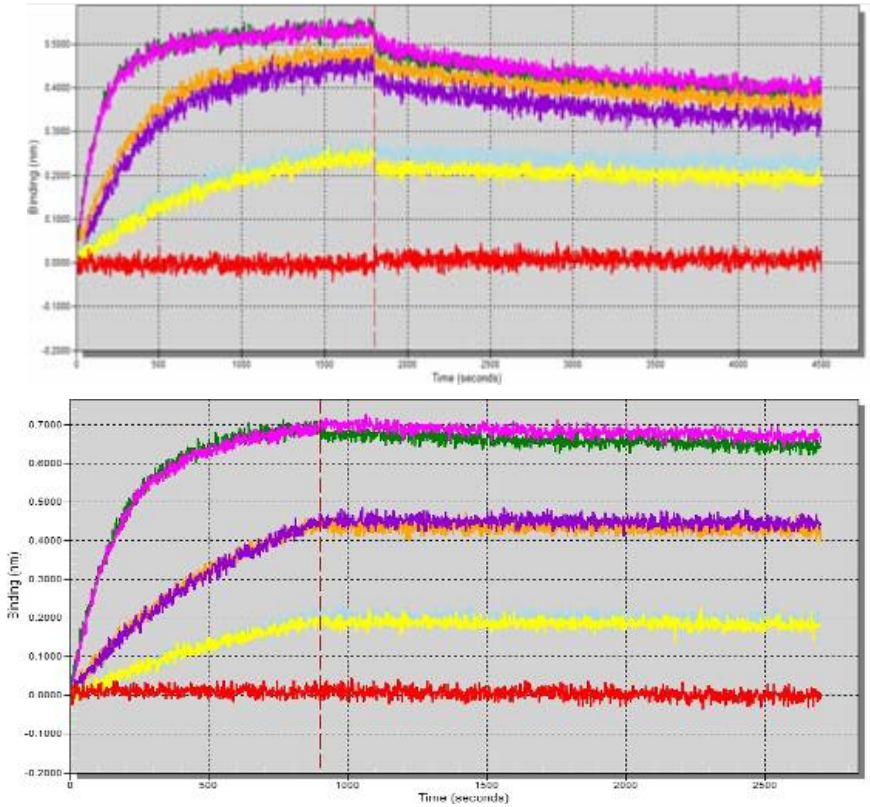
- 8 samples can be analyzed in parallel
- Data is displayed in real-time
- Measure on-rate and off-rate
- Experimental protocols can be customized during sample programming

Reproducibility: Protein Kinetics



- Three replicate runs on the Octet RED. Data shown is for the loading of an mouse anti-IL2 antibody onto an anti-mouse IgG surface and concentration titration (inset) of IL-2. The IL-2 was run at 3, 0.9, 0.3, 0.09, 0.03 and 0 nM in triplicate.

Easily Determine Full Kinetics Characterization



Mutant 1	Full Analysis With 3 Conc	Screening (20nM only in duplicate)
ka (1/Ms)	2.84E+05	3.21E+05
kd (1/s)	8.59E-05	9.46E-05
Kd (M)	3.02E-10	2.95E-10

Mutant 2	Full Analysis With 3 Conc	Screening (20nM only in duplicate)
ka (1/Ms)	2.56E+05	2.73E+05
kd (1/s)	2.41E-05	2.59E-05
Kd (M)	9.41E-11	9.49E-11

Data provided by Genitope

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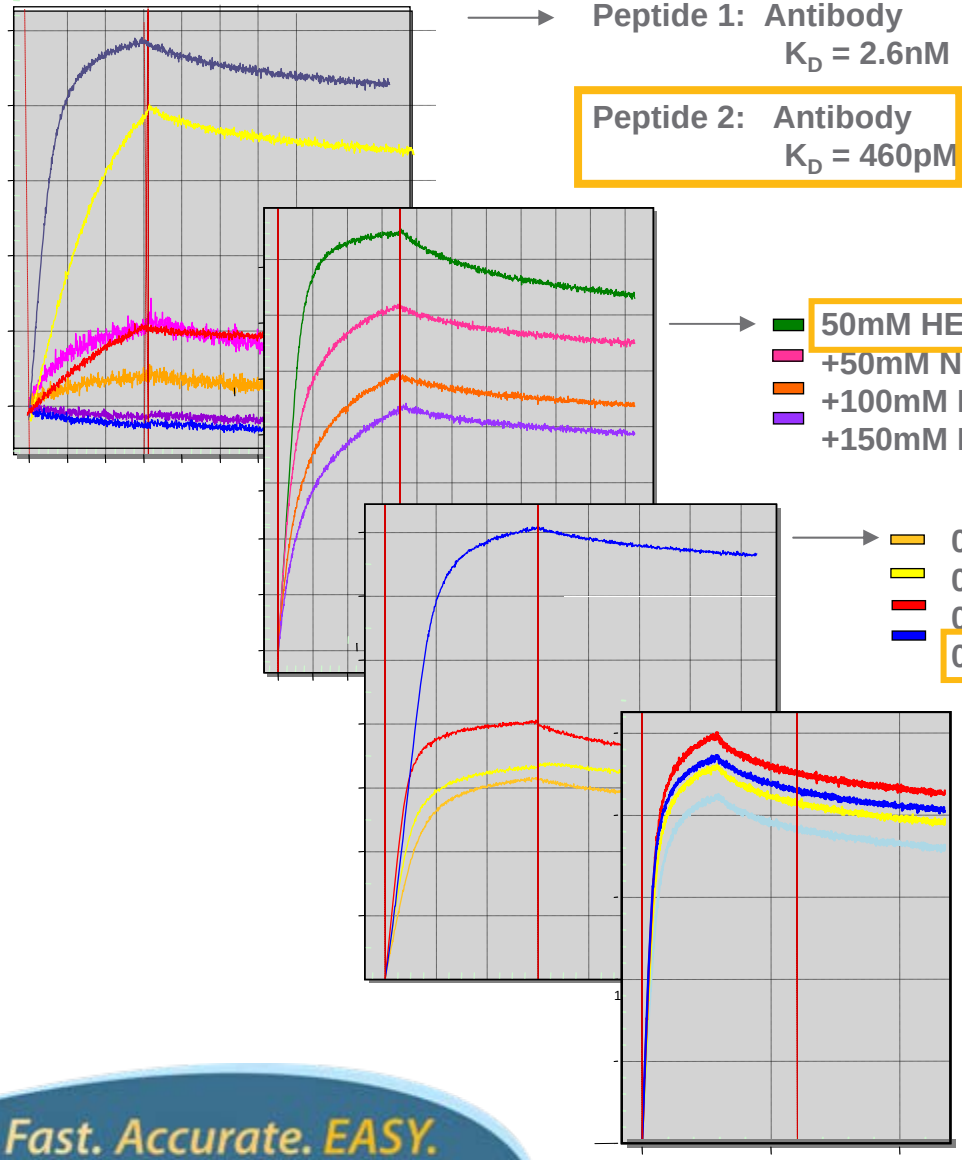
Rapidly Develop Optimal Assay Conditions



Peptide 1: Antibody
K_D = 2.6nM

Peptide 2: Antibody
K_D = 460pM

50mM HEPES + 0mM NaCl
+50mM NaCl
+100mM NaCl
+150mM NaCl

0% v/v Tween
0.001% v/v Tween
0.005% v/v Tween
0.01% v/v Tween

0 mg/ml BSA
0.1mg/ml BSA
0.5mg/ml BSA
1.0 mg/ml aBSA

Step 1:
Test two antibodies at four concentrations for affinity to target

Step 2:
Test optimal NaCl concentration

Step 3:
Test optimal Tween concentration

Step 4:
Test optimal concentration of carrier protein.

All experiments were completed in one afternoon.

Successful conditions were used for HTP bead-based assay.

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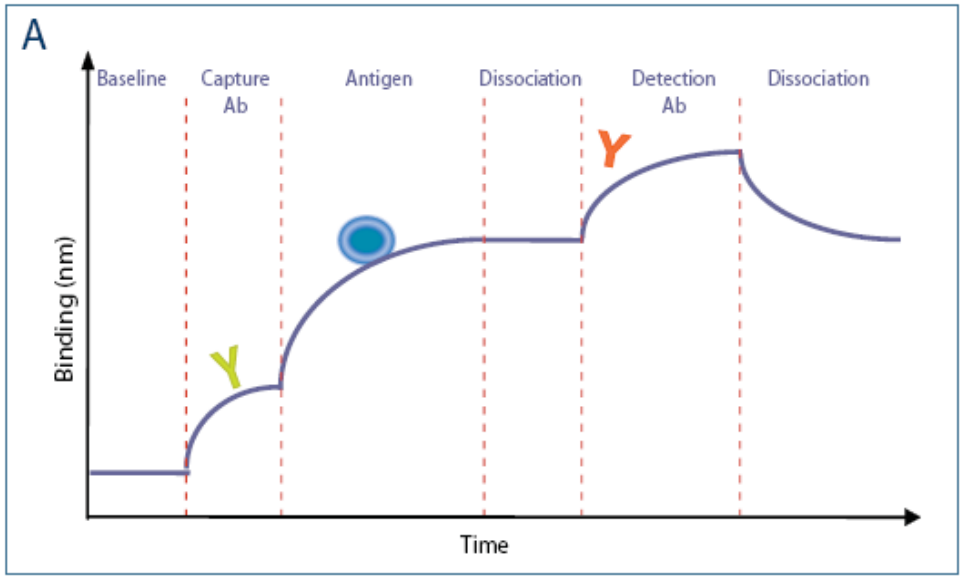
Octet應用：藥物篩選與檢測套組開發

- ☐ Antibody and Antigen Pairs Selection
- ☐ Epitope Mapping

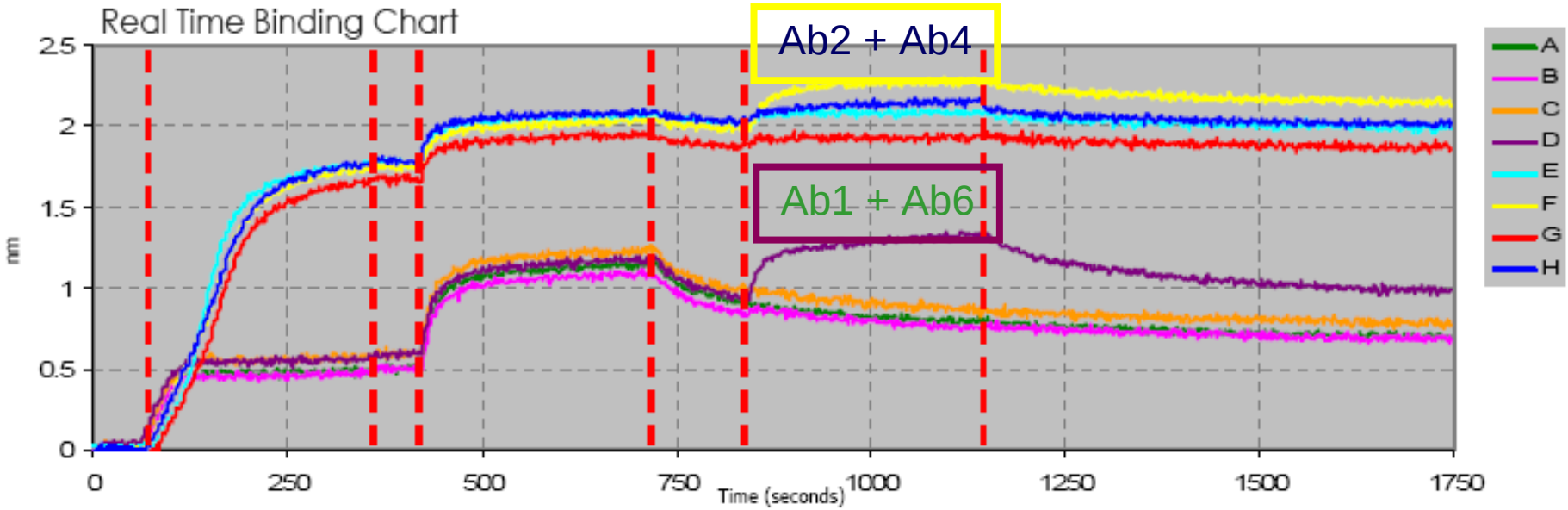
Selection of multiple Antibody and Antigen Pairs for Immunoassay Development

- Capture and detection antibodies must exhibit a high degree of specificity
- Antibody-analyte interactions must be of high enough affinity to withstand wash protocols
- Antibodies must bind to non-overlapping, non-interfering epitopes

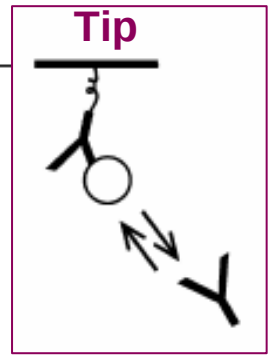


Graphic representation of sequential binding as measured by on the Octet System.

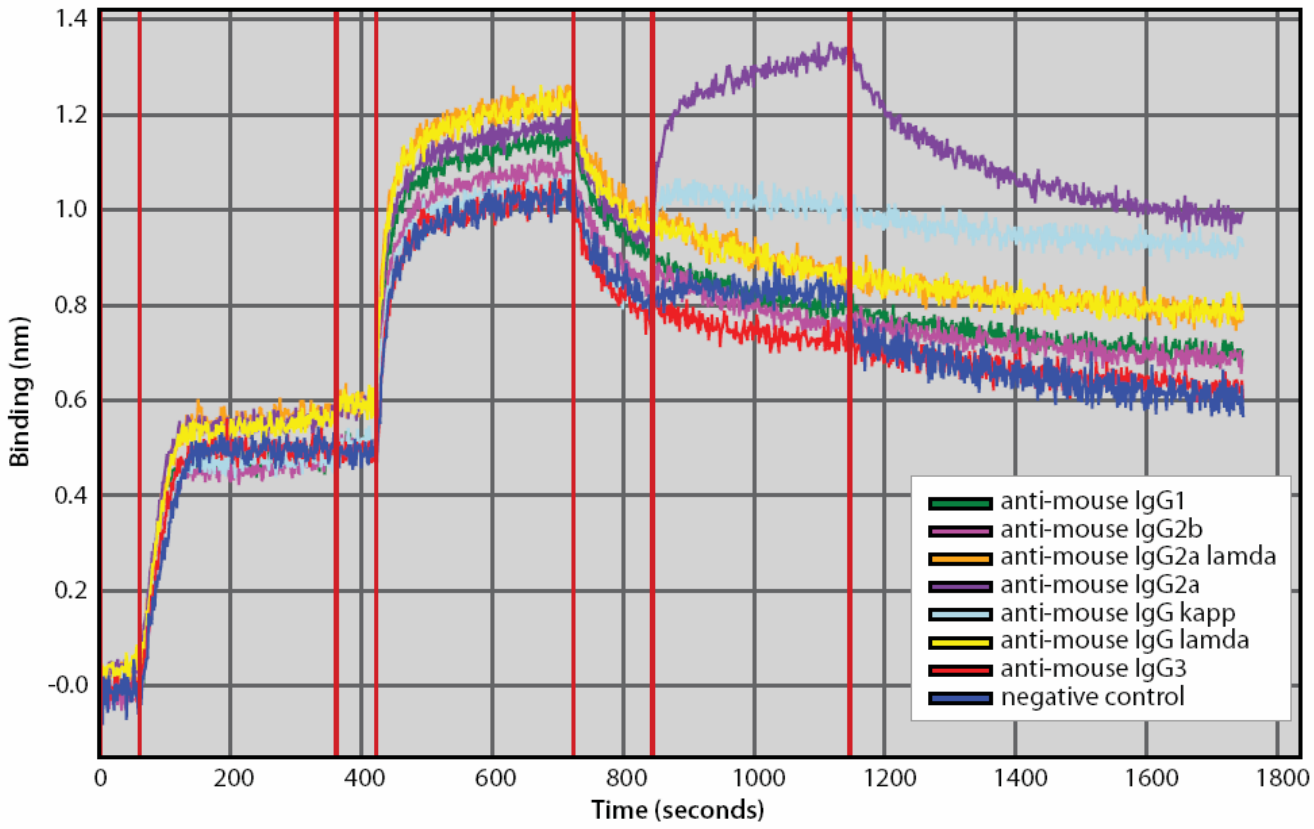
Rapid Screening of Antibody Pairs by using BioLayer Interferometry



STEP 1	2	3	4	5	6	7
PBS	Capture antibodies Ab 1 A, B, C, D Ab 2 E, F, G, H	PBS	Analyte	PBS	Detection antibodies Ab 3 A, E Ab 4 B, F Ab 5 C, G Ab 6 D, H	PBS



Antibody Isotyping Application on the Octet System



From the pattern of the binding, the mIgG sample was correctly identified as mIgG2a kappa.

Case Studies 1: Using Octec Experience from Rinat Pfizer

RINAT NEUROSCIENCE

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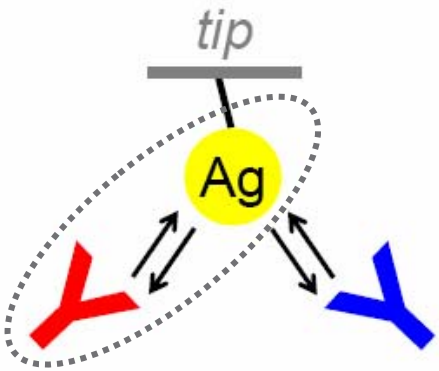
Introducing the Octet as a reliable novel biosensor in characterizing protein/protein interactions

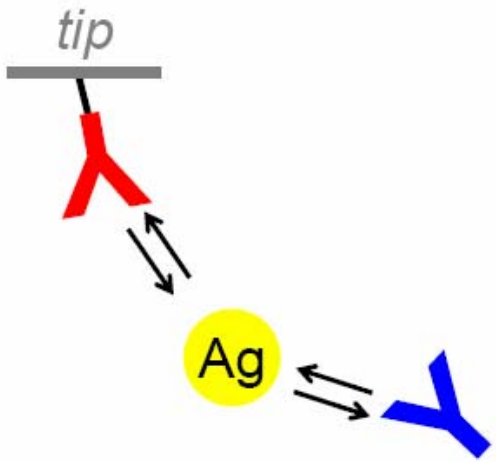
Yasmina Abdiche, PhD
Rinat Pfizer, South San Francisco, CA

Epitope Binning

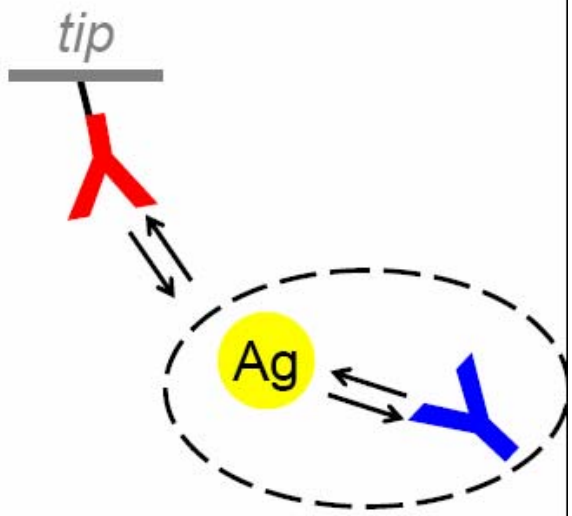
Stepwise blocking



Classical sandwich

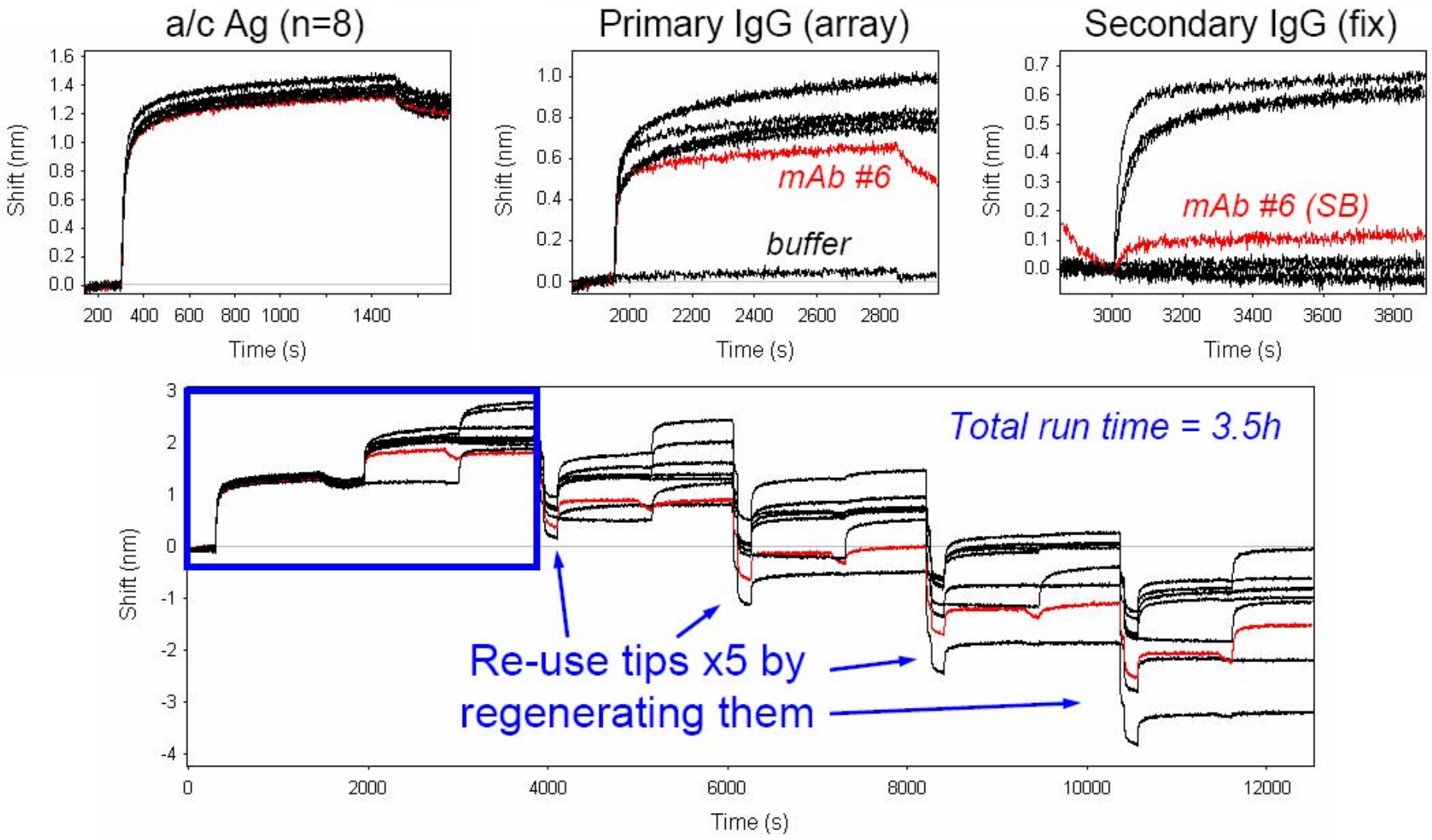


Premix

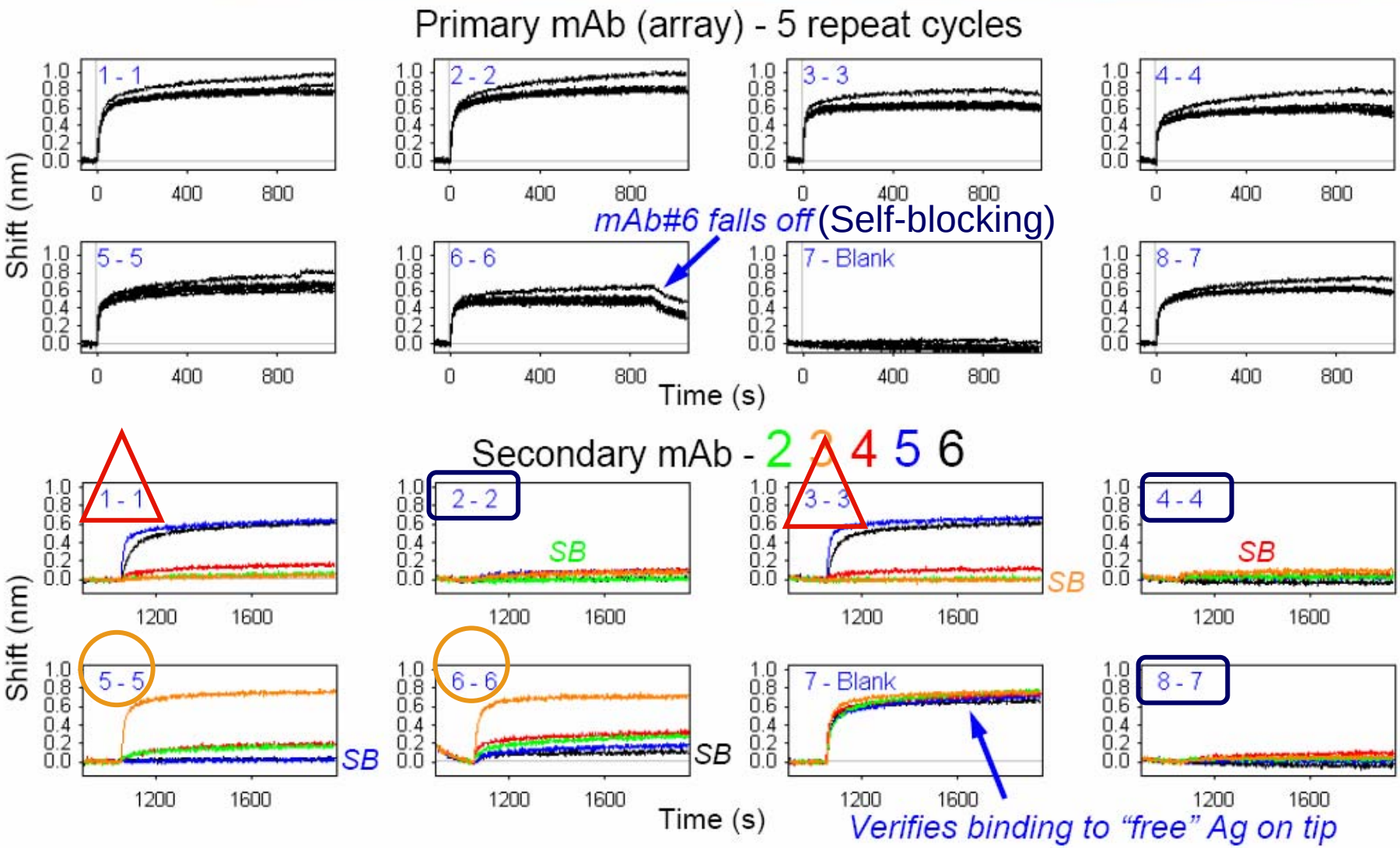


1. Each strategy has its pro's and con's
2. Consider MW and aggregation state of Ag
3. Improve unattended throughput by preparing surfaces offline using "batch immobilization"

Stepwise Blocking



Stepwise Blocking



Confirm 3 Epitope Bins Using Two Blocking Stratagies

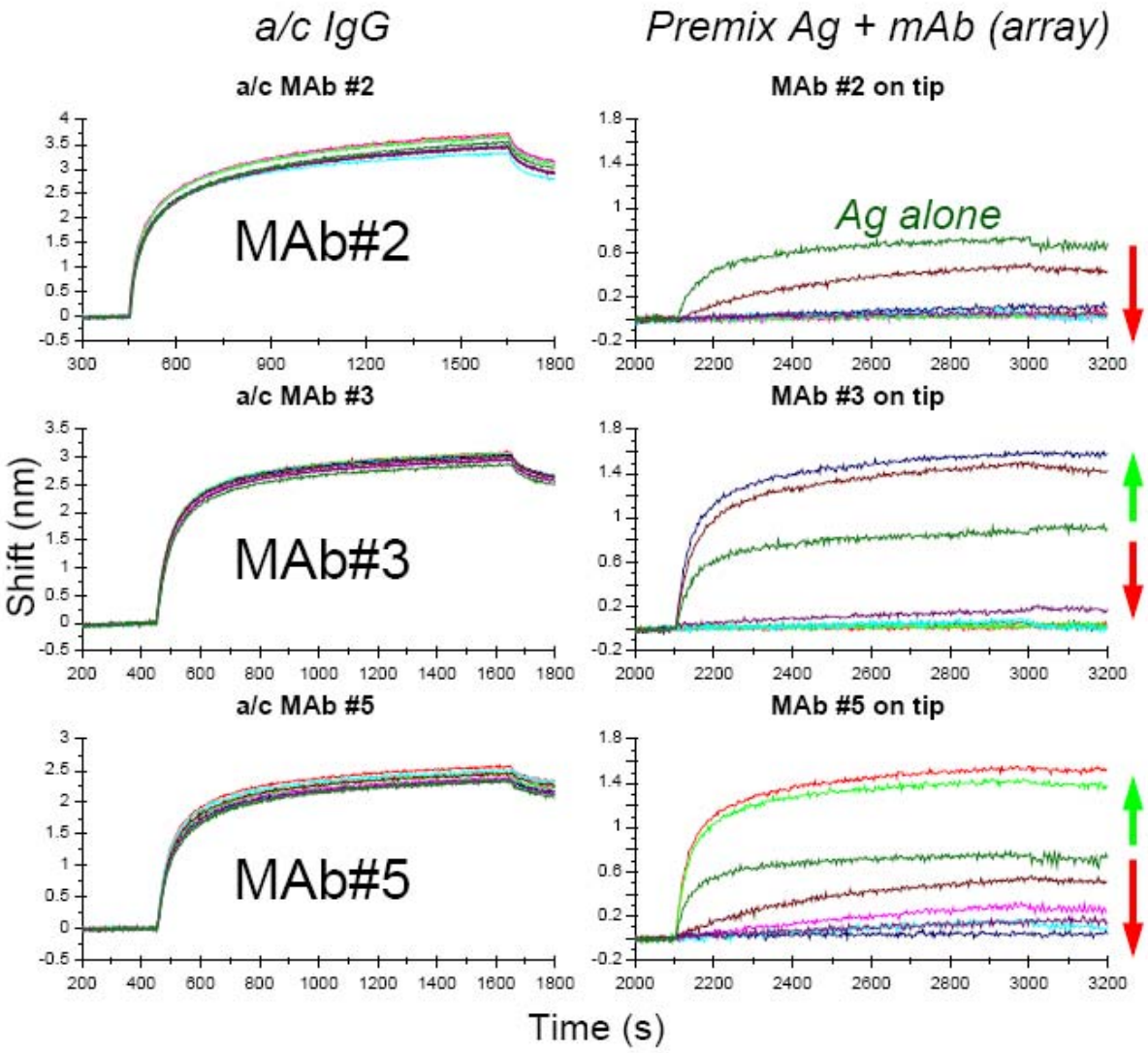
Analytical Biochemistry
Vol. 377(2), 2008, p209-217

Table 1
Pairwise blocking results for the Octet data shown in Fig. 2

Saturating mAb	Competing mAb				
	2	3	4	5	6
1	Y	Y	Y	N	N
2	Y	Y	Y	Y	Y
3	Y	Y	Y	N	N
4	Y	Y	Y	Y	Y
5	Y	N	Y	Y	Y
6	Y	N	Y	Y	Y
7	Y	Y	Y	Y	Y

Note. Y, blocks; N, does not block. Three patterns of blocking activity are discerned whether the table is read from left to right or from top to bottom: bin A = mAbs 1 and 3; bin B = mAbs 5 and 6; and bin C = mAbs 2, 4, and 7.

Premix Approach



"Traffic light" binding matrix

	1	2	3	4	5	6	7
2		SS					
3			SS				
5					SS		

- Binding (sandwich)
- Unclear
- No binding (blocking)

Confirmed 3 epitope bins using two blocking strategies

- BIN 1: #1 and 3
- BIN 2: #5 and 6
- BIN 3: #2, 4, and 7

SS= Self-Sandwich

Summary

1. The Octet is a simple, **low running costs make it accessible**
2. “One-shot” kinetics provides an appealing assay format
3. Returns accurate kinetic rate constants for a wide range of interactions when compared head-to-head with Biacore
4. The direct binding of small molecules is beyond the current detection limit
5. Well-suited to blocking, especially in the context of epitope binning

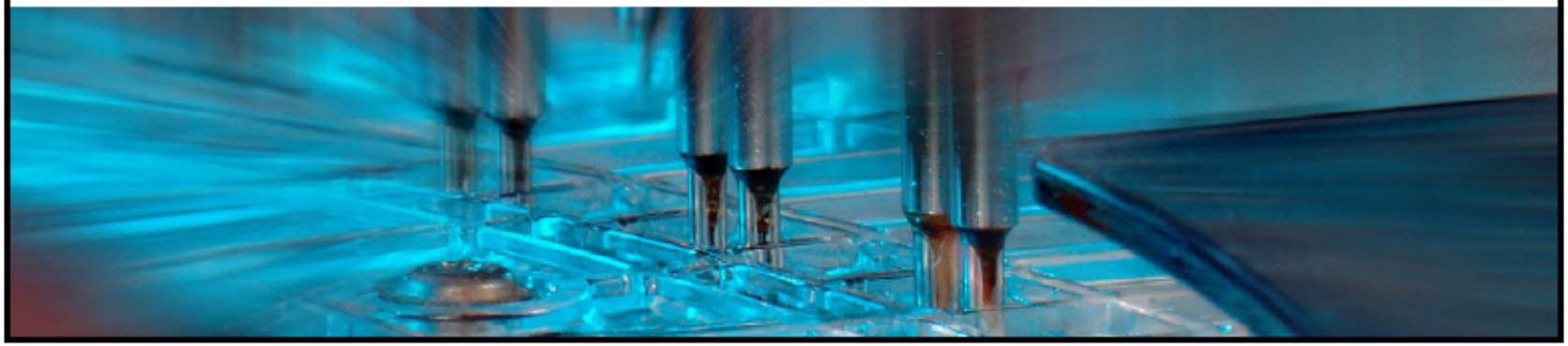
Octet應用：合成化合物與天然藥物篩選

Case Studies 2: Fragment Screening Using the Octet RED System from Roche

Roche

Applying Label-Free Technologies to Validate Screening Hits

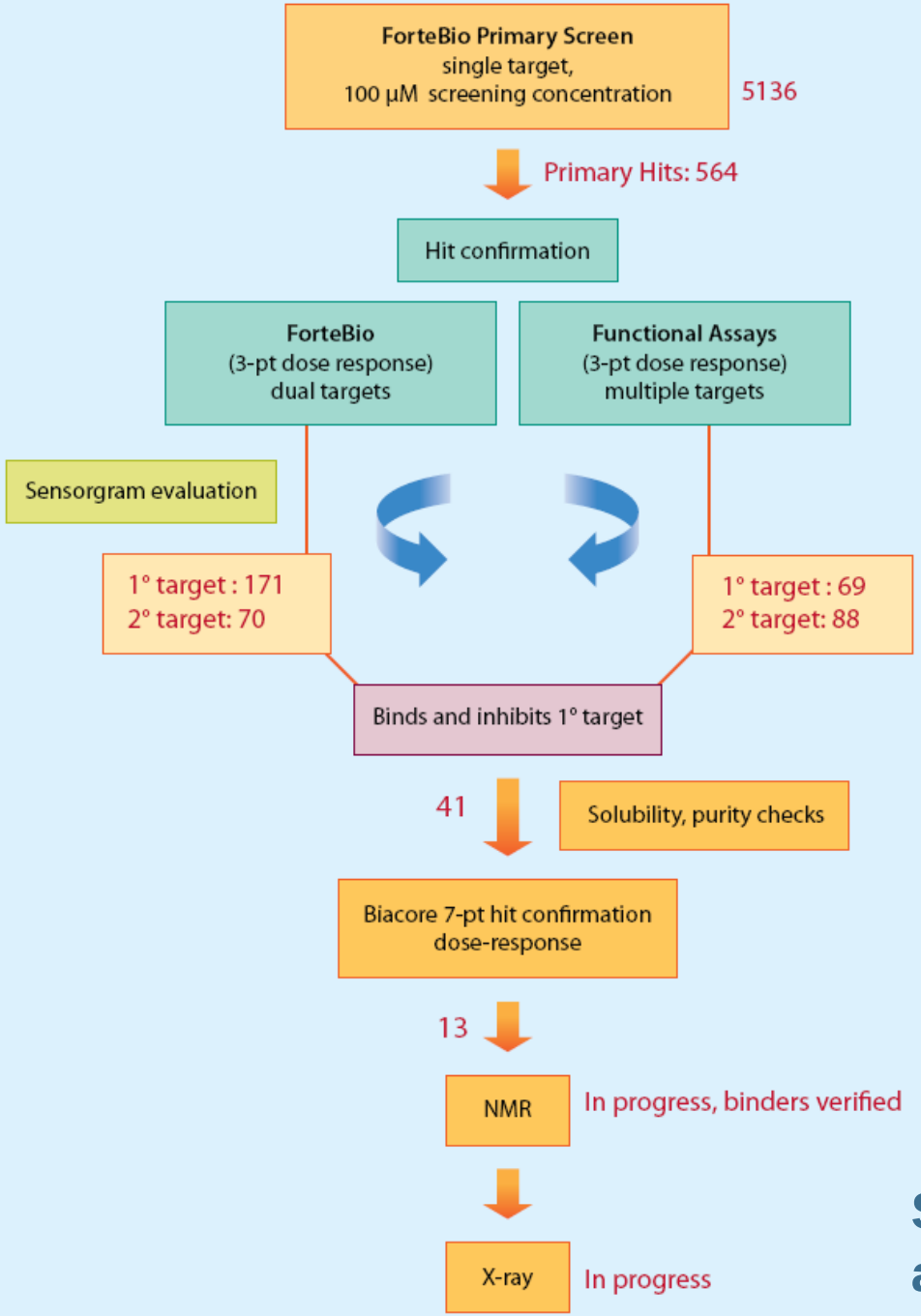
David Mark, Frank Podlaski, Shirley Li, Kuo-Sen Huang
Janette Phi-Wilson*, Charles Wartchow*



Advantages of Label-free Technologies
Binding of small molecules to proteins

- Detect direct binding of small molecules to protein Target
  - – Distinguish between specific binding versus non-specific binding to other reagent components
  - – Determine stoichiometric binding versus aggregation
- Label-free
  - – Simplify reagent preparation and assay development

Fragment Screening Using the ForteBio Octet RED System



Strategy for fragment screening assay.

forteBIO

Optimization for Throughput

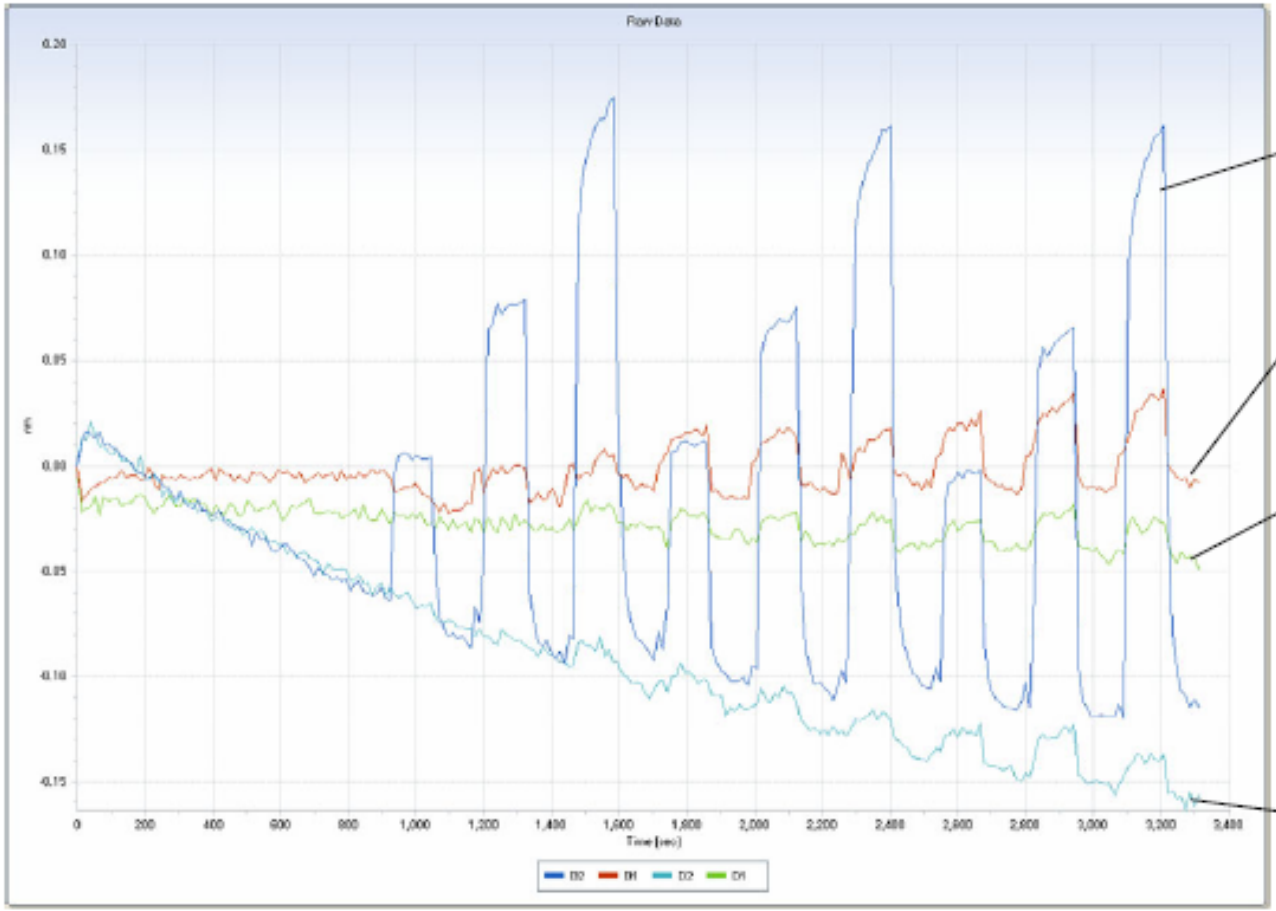
Reusing a sensor for multiple sample wells

Experiment: (Test compound @ 3 concs, 3x in sequence)

1. Prepare target sensors offline
2. Use single dissociation well
3. Test for reproducibility and carryover

			Conc (uM)								
	1	2	3	4	5	6	7	8	9	10	11
A	baseline	dissoc	1.00	5.00	25.00	1.00	5.00	25.00	1.00	5.00	25.00
B	baseline	dissoc	4	4	4	5	5	5	6	6	6
C	baseline	dissoc	7	7	7	8	8	8	9	9	9
D	baseline	dissoc	10	10	10	11	11	11	12	12	12
E	baseline	dissoc	13	13	13	14	14	14	15	15	15
F	baseline	dissoc	16	16	16	17	17	17	18	18	18
G	baseline	dissoc	19	19	19	20	20	20	21	21	21
H	baseline	dissoc	ref	ref	ref	ref	ref	ref	ref	ref	ref

Replicate Testing of Compound 2



Target sensor

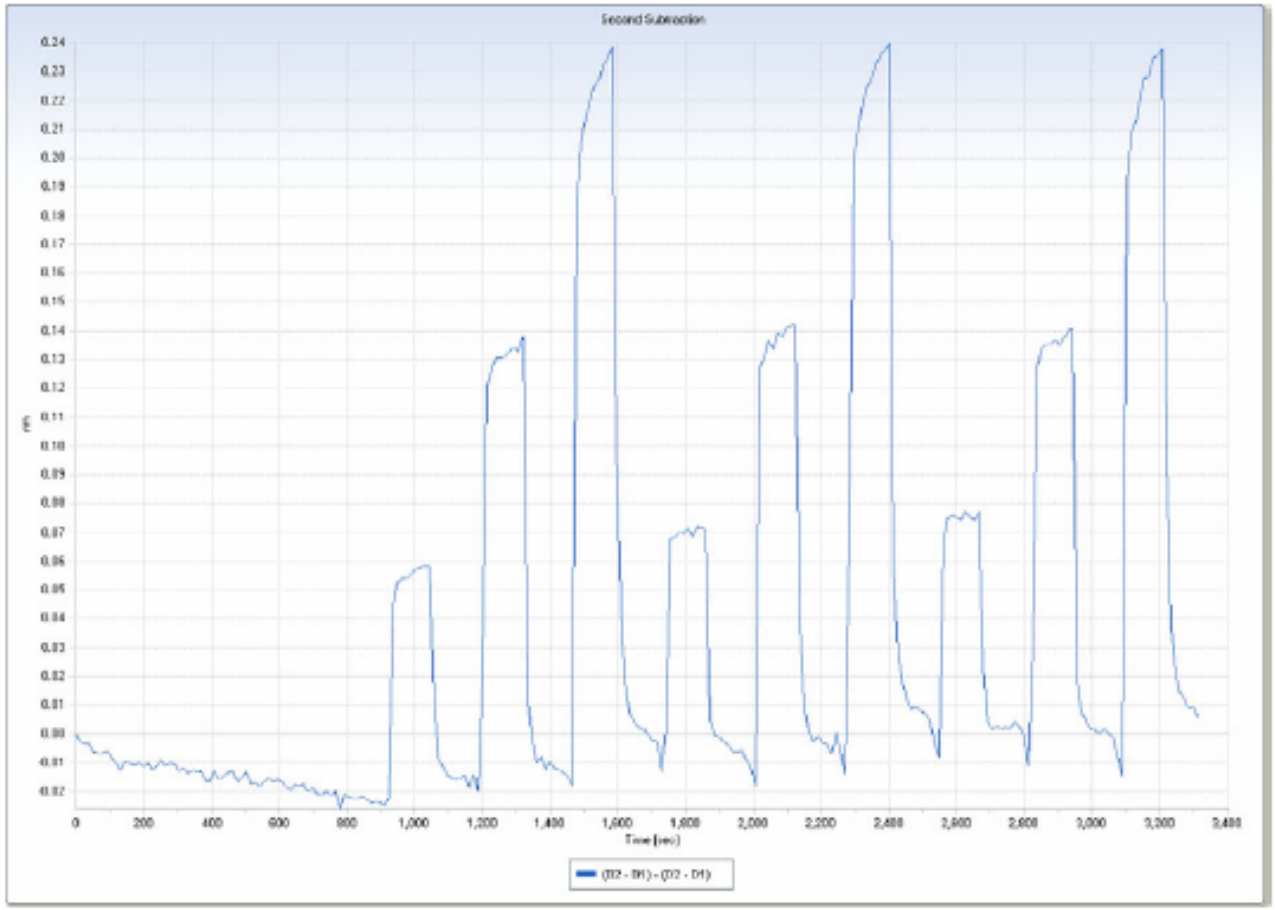
SA ref sensor

SA sensor (Buffer)

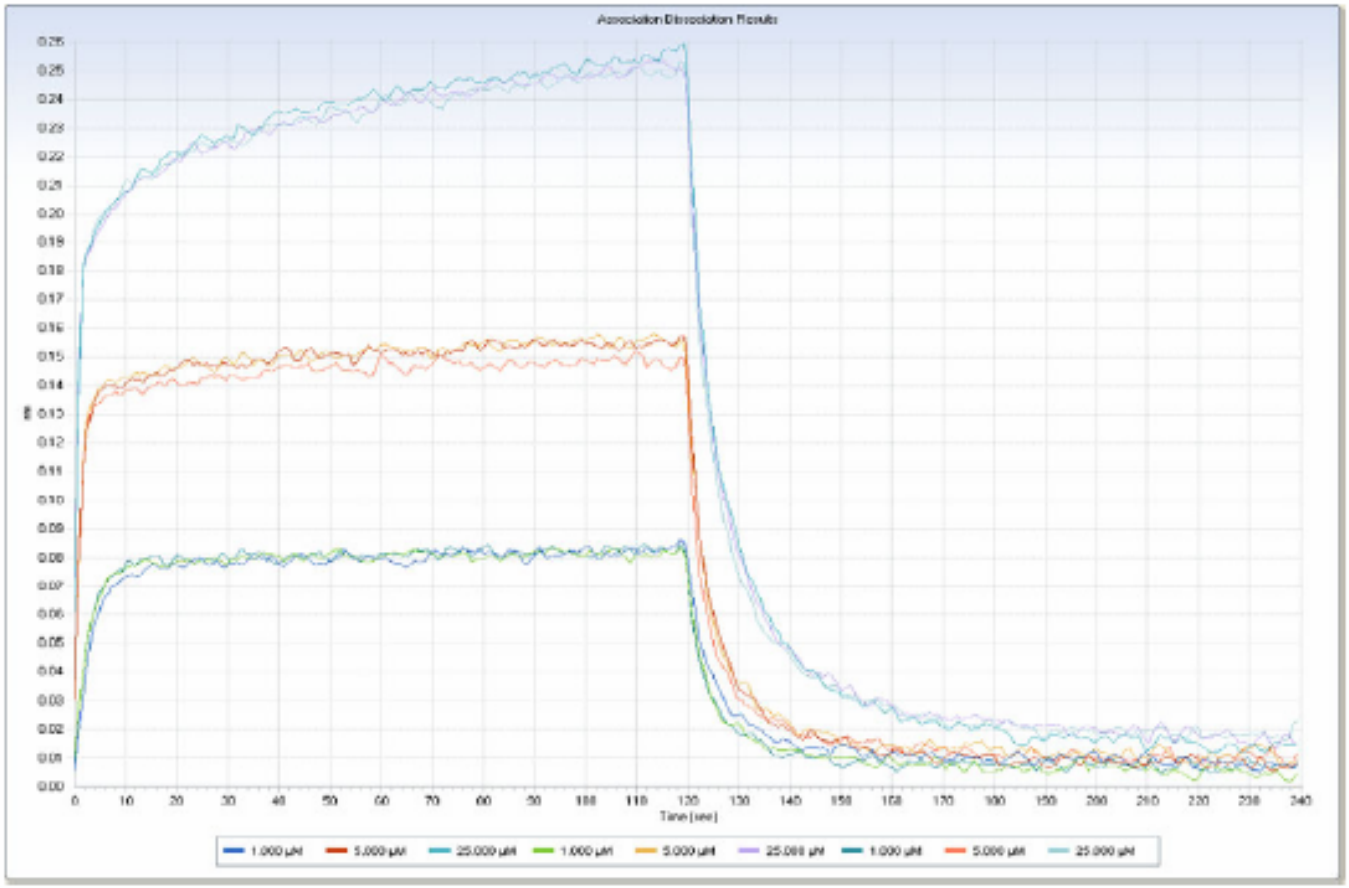
Target sensor (Buffer)

Replicate Testing of Compound 2

1st and 2nd Substraction



Overlay Replicate Response Curves



- Single dissociation well for each row is sufficient

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Fragment Screening Platemap
68 Cpds + 2 Controls

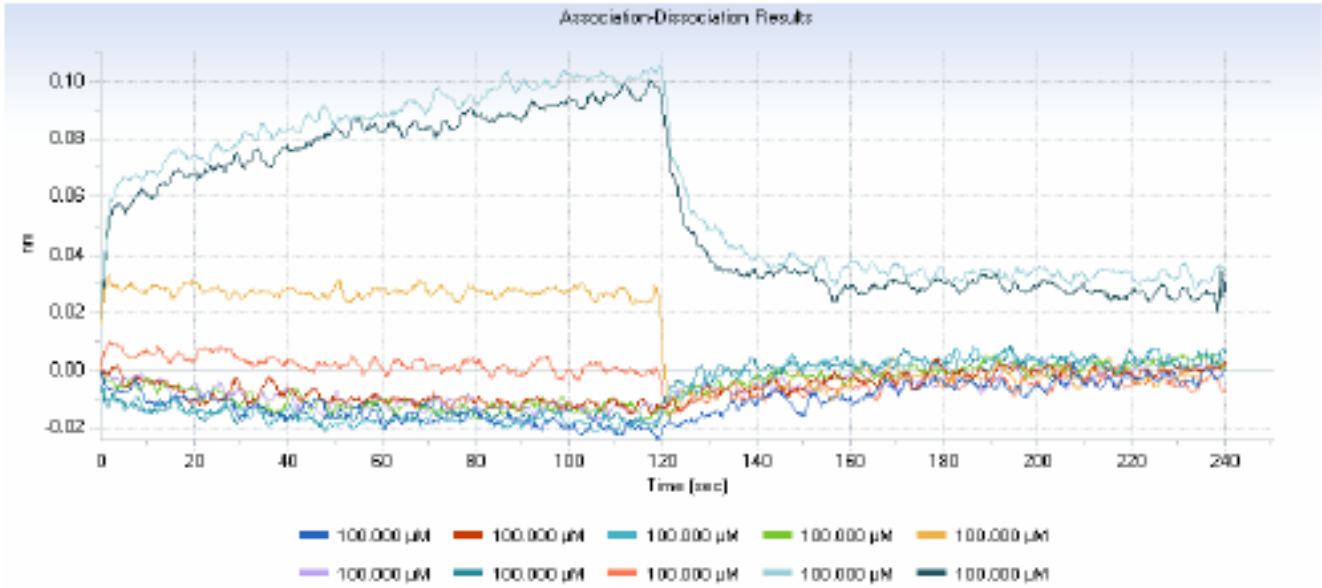
	Equil. Column	Dissociation column										
	1	2	3	4	5	6	7	8	9	10	11	12
A	Running Buffer	Running Buffer	Cpd1	Cpd2	Cpd3	Cpd4	Cpd5	Cpd6	Cpd7	Cpd8	Cpd9	Cpd10
B	Running Buffer	Running Buffer	Cpd11	Cpd12	Cpd13	Cpd14	Cpd15	Cpd16	Cpd17	Cpd18	Cpd19	Cpd20
C	Running Buffer	Running Buffer	Cpd21	Cpd22	Cpd23	Cpd24	Cpd25	Cpd26	Cpd27	Cpd28	Cpd29	Cpd30
D	Running Buffer	Running Buffer	Cpd31	Cpd32	Cpd33	Cpd34	Cpd35	Cpd36	Cpd37	Cpd38	Cpd39	Cpd40
E	Running Buffer	Running Buffer	Cpd41	Cpd42	Cpd43	Cpd44	Cpd45	Cpd46	Cpd47	Cpd48	Cpd49	Cpd50
F	Running Buffer	Running Buffer	Cpd51	Cpd52	Cpd53	Cpd54	Cpd55	Cpd56	Cpd57	Cpd58	Cpd59	Cpd60
G	Running Buffer	Running Buffer	Cpd61	Cpd62	Cpd63	Cpd64	Cpd65	Cpd66	Cpd67	Cpd68	Std Cpd	Std Cpd
H	Running Buffer	Running Buffer	nning Bu	unning Buf	unning Buffe	Running Buffe	unning Buff	unning Buff	unning Buff	unning Buff	Running Buffe	Running Buffer

column 1 = baseline for entire cpd row
column 2 = dissoc for entire cpd row

- Throughput : 1.5 Hours per plate; 5 plates per day (340 compounds)
- Each compound screened at a single concentration (150 to 200 μM)
- Hits confirmed based on concentration dependent response profiles

Fragment Screening Data

Fragment Screening
Report Point Tool for Hit Selection



Sensor Location	Sample Location	Sample ID	Concentration µM	Time 1 (sec)	Binding 1 (nm shift)	Time 2 (sec)	Binding 2 (nm shift)
G2	G3	61	100	115	-0.019588784	235	-0.00152546
G2	G4	62	100	115	-0.010269617	235	-0.000587915
G2	G5	63	100	115	-0.016737366	235	0.005531213
G2	G6	64	100	115	-0.014430019	235	0.005592601
G2	G7	65	100	115	0.023833599	235	-0.001094302
G2	G8	66	100	115	-0.013589256	235	0.001518726
G2	G9	67	100	115	-0.017613245	235	0.0036475
G2	G10	68	100	115	-0.001286287	235	-0.003912368
G2	G11	69	10	115	0.100915789	235	0.030474652
G2	G12	70	10	115	0.092988986	235	0.026241437

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Summary of Octet Red in Hit Validation

Advantages

- ease of use; quick assay development
- low protein consumption (off-line protein loading)
- ability to identify aggregating & reactive compounds
- ability to assay complex or insoluble analytes

Octet Adoption: 18 of Top 20 Pharma/ Biotech

Octet應用：免疫學與癌症生醫標誌研究

Rapid Protein Interaction Study

nature immunology

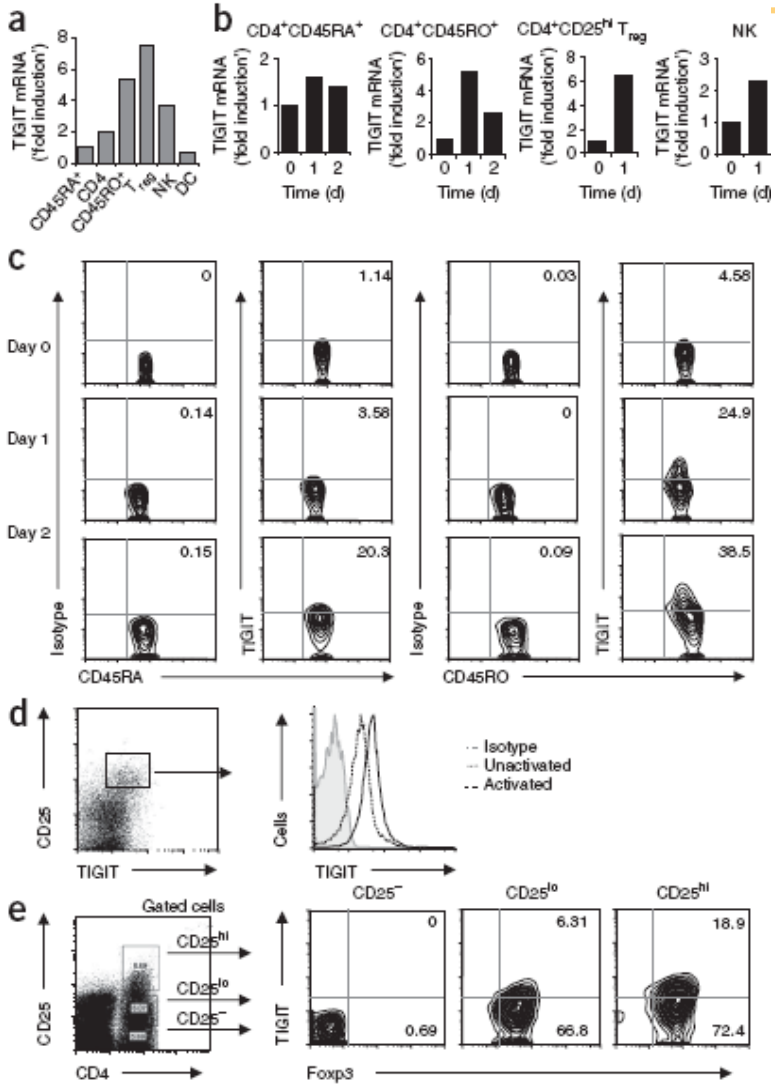
Volume 10, No. 1,
2009 p48-57

The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells

Xin Yu[1,4], Kristin Harden[2,4], Lino C Gonzalez[2], Michelle Francesco[1], Eugene Chiang[1], Bryan Irving[1], Irene Tom[2], Sinisa Ivelja[1], Canio J Refino[1], Hilary Clark[3], Dan Eaton[2] & Jane L Grogan[1]

Here we have identified a surface protein, TIGIT, containing an immunoglobulin variable domain, a transmembrane domain and an immunoreceptor tyrosine-based inhibitory motif that was expressed on regulatory, memory and activated T cells. Poliovirus receptor, which is expressed on dendritic cells, bound TIGIT with high affinity. A TIGIT-Fc fusion protein inhibited T cell activation *in vitro*, and this was dependent on the presence of dendritic cells. The binding of poliovirus receptor to TIGIT on human dendritic cells enhanced the production of interleukin 10 and diminished the production of interleukin 12p40. Knockdown of TIGIT with small interfering RNA in human memory T cells did not affect T cell responses. TIGIT-Fc inhibited delayed-type hypersensitivity reactions in wild-type but not interleukin 10–deficient mice. Our data suggest that TIGIT exerts immunosuppressive effects by binding to poliovirus receptor and modulating cytokine production by dendritic cells.

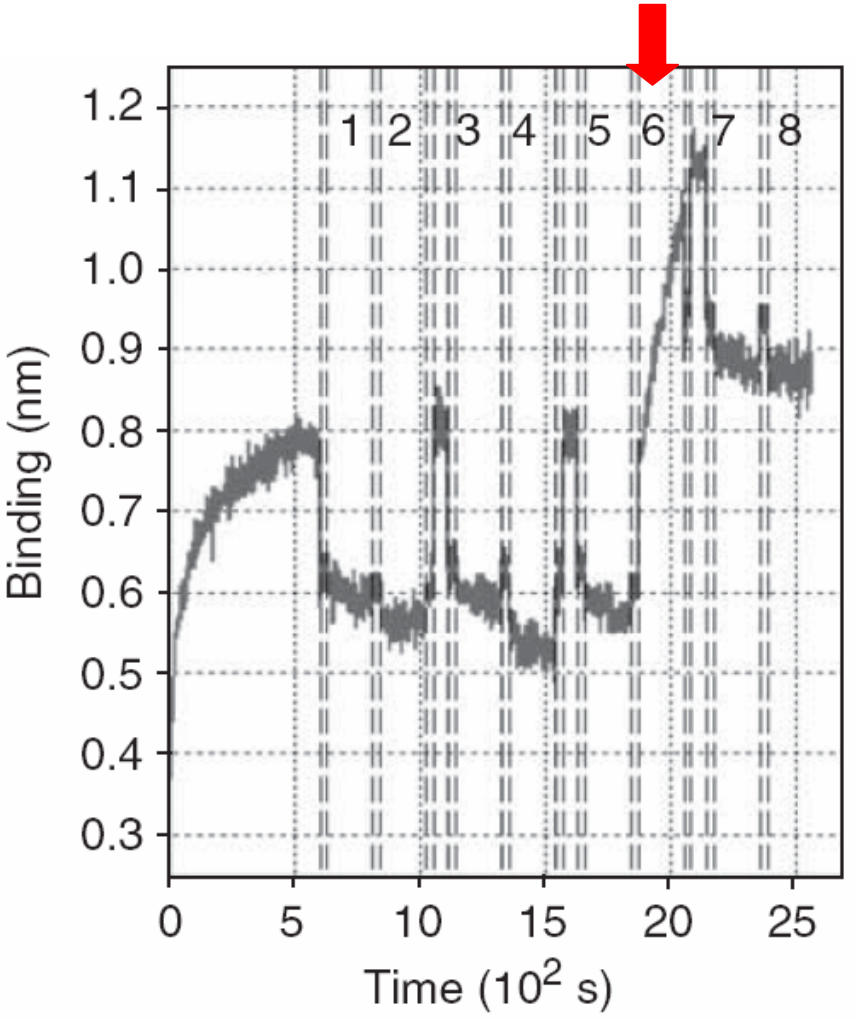
Identification of TIGIT Using Domain Structure Search Strategy



- Figure 1 Expression of TIGIT protein and mRNA in immune cells. (a) RT-PCR of the expression of TIGIT mRNA.

1. 利用Genome-wide domain structure策略，掃瞄所有蛋白質轉譯序列，找出含ITIMs相關的基因
2. 由約1000種分泌型蛋白的In-hosue資料庫中，利用Octet系統鑑定出與TIGIT相作用的PVR (poliovirus recaptor)蛋白

In-house protein library functional screening: using ForteBio for Protein-binding assay



- Figure 2. (a) TIGIT binds to PVR family members.
- Octet sensogram of the screening of TIGIT-Fc against one row of a single plate of the protein library including eight different proteins: endothelial cell adhesion molecule (1); otoraplin (2); TEK tyrosine kinase (3); TNF family member 10c (4); insulin-like growth factor–binding protein 4 (5); **PVR (6);** IL-19 (7); and a second lot of TEK tyrosine kinase (8)

Cancer Biomarker Study: detection DNA in cancer

Current Opinion in Investigational Drugs 2007

8(12):981-986

Editorial overview
Detecting DNA: Getting and begetting cancer

Adam M Farkas, Tina M Kilgore & Michael T Lotze*

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Current Opinion in Investigational Drugs 2007 **8**(12):981-986
 ISSN 1472-4472

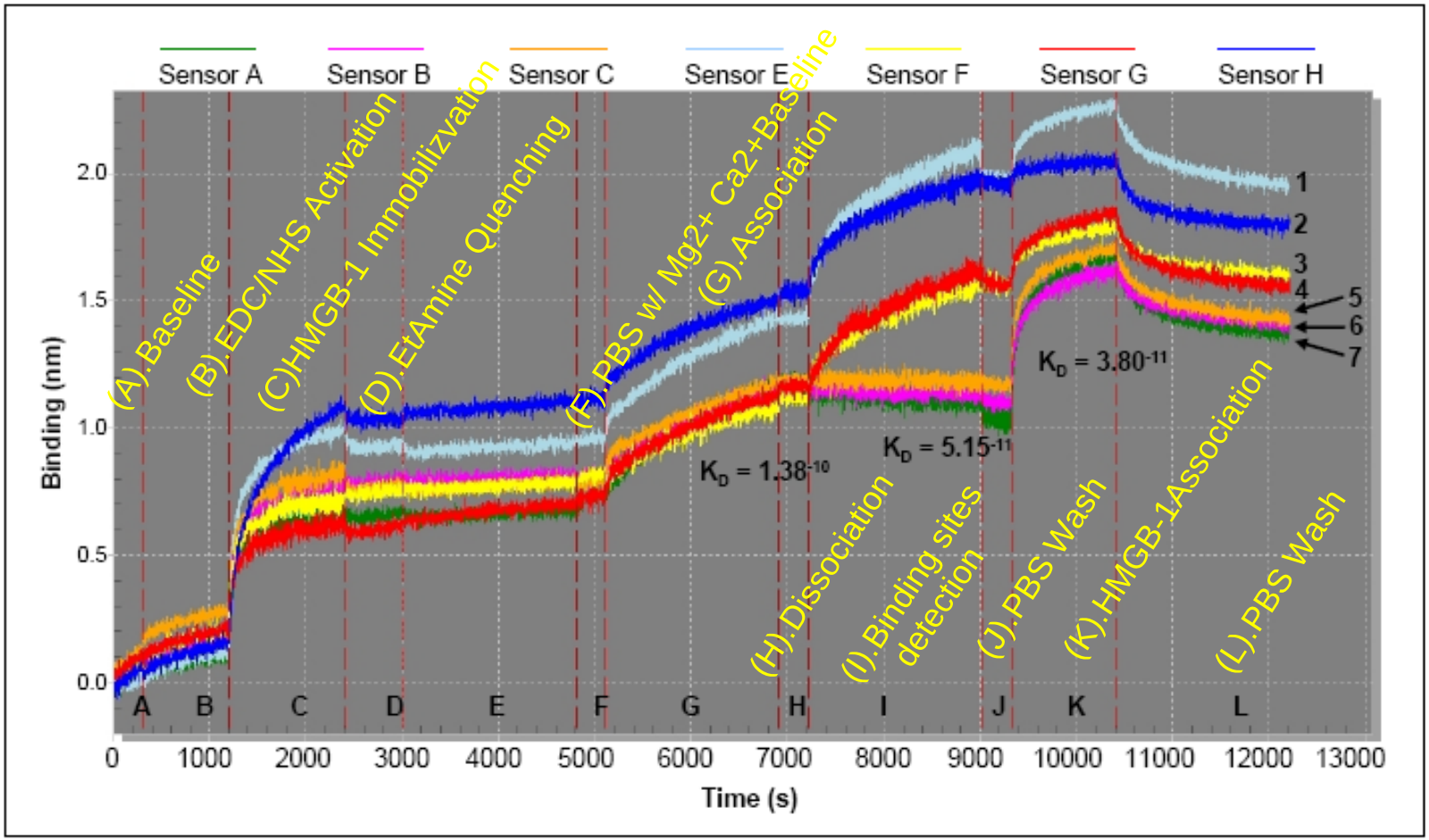
"Ignorance more frequently begets confidence than does knowledge: it is those who know little, and not those who know much, who so positively assert that this or that problem will never be solved by science."

intracellular contents, and the consequences of such release for the induction of an inflammatory response (promoting tissue repair and wound healing in the tumor microenvironment) is important for an integrative understanding of cancer. Efforts to measure and assess such events are central to several novel clinical or experimental applications. In particular, for the diagnostic purposes of tracking progress in cancer therapy, and for considering and evaluating novel therapeutic strategies.

Tumor DNA is not just the target for genomic instability. It may also be defined as a damage-associated molecular pattern molecule (DAMP) [3], signaling the host for repair, and, begetting extracellular instability when found in those compartments.

Using Octet optical Biosensor for detection the Cancer-association DNA in Serum

Figure 1. Measuring DNA with interferometry.



Using Amine Reactive (AR) Biosensor for protein-DNA binding detection:

HMGB-1:nuclear protein high mobility group box-1

Conclusion

- **Optical detection may be used to effectively discern damage-associated DNAs by their binding to HMGB-1 or other macromolecules such as a transcription factor, histone, or an RNA or a DNA aptamer, or chemical agent that can bind DNA with high affinity.**

- **Detecting the disappearance of serum DNA therefore represents a suitable clinical assay for confirming tumor eradication, as well as allowing early detection in the event of recurrence.**

Desirable Kinetic Platform Features

- Flexible Applications
  - Quick on/off rate screening
  - Ability to generate high quality kinetic profiles
  - Fast and easy method development
  - Analyze molecular interactions for size > 150 Da
- High Throughput
  - 96 well assays can be set up and left to run unattended
  - Run multiple samples and conditions simultaneously
- Easy to Use
  - Assays set up is simple and flexible
  - Any user can complete assays with minimal training
  - No maintenance or instrument preparation required
  - Disposable biosensors with optional regeneration

Thanks for your pay attention!!

Fast. Accurate. EASY.

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