

*forté*BIO™

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

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# Overview

1. The technology
2. Octet v. Biacore
3. “One-shot” kinetics
4. Blocking as applied to epitope binning
  - a. Stepwise
  - b. Classical sandwich
  - c. Premix

# Array Biosensor Platforms

Manufacturer	Technology <sup>a</sup>
Biacore	Biacore, 2000, 3000, T100, Flexchip, S51, and A100
Axela Biosensors	dotLab
Toyobo	MultiSPRinter
Lumera	Proteomic Processor
GenOptics	SPRi-Plex
SRU Biosystems	BIND
Corning	Epic
FortéBIO	Octet
Bio-Rad	ProteOn XPR36

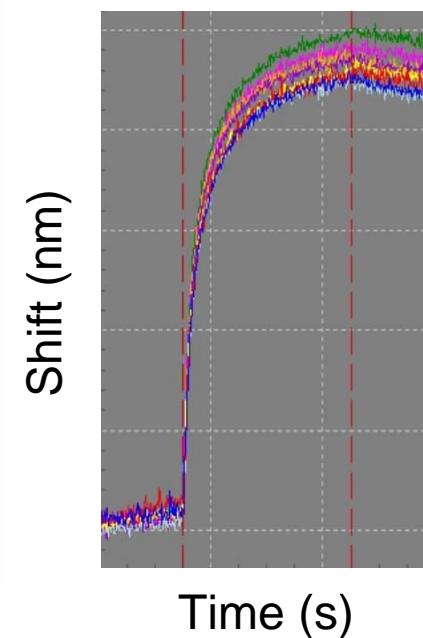
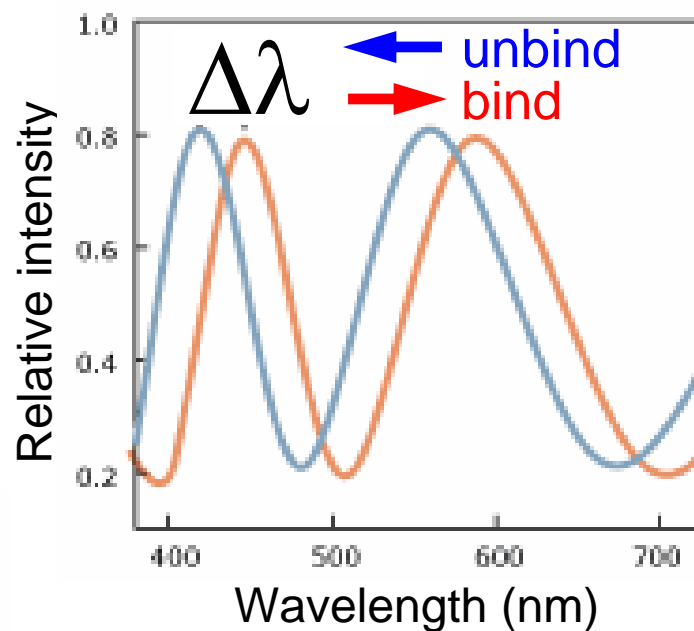
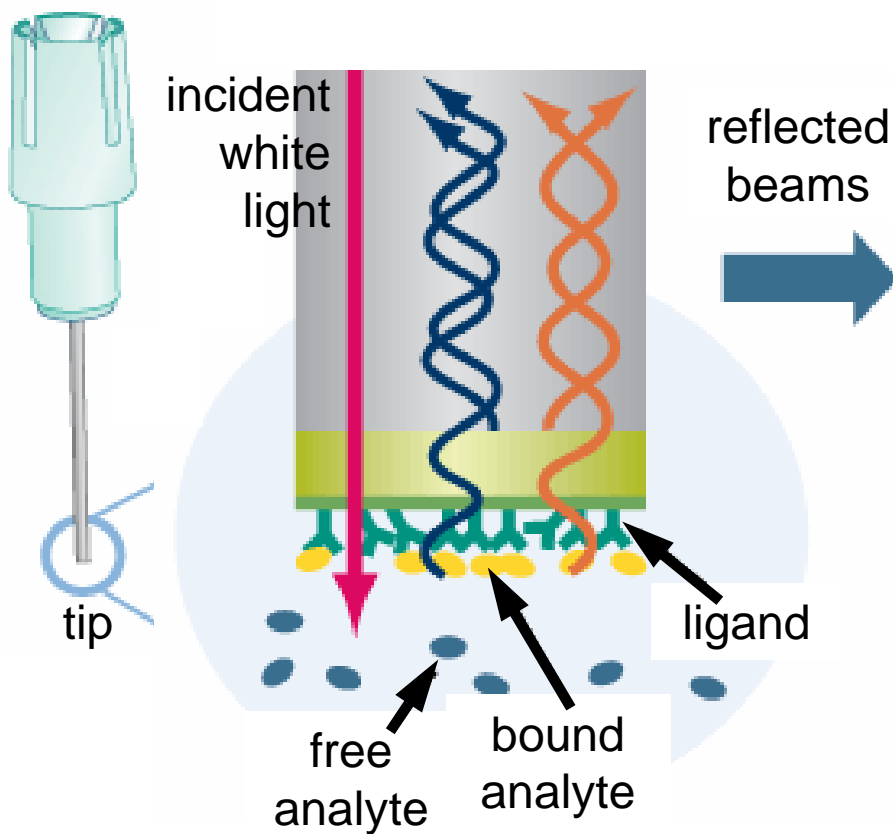
*Rich & Myszka, Anal Biochem 361 (2007) 1-6*

# The Technology

Dip-and-read assay using disposable fiber optic biosensors

Bio-Layer Interferometry (BLI)

Parallel processing of 8 samples

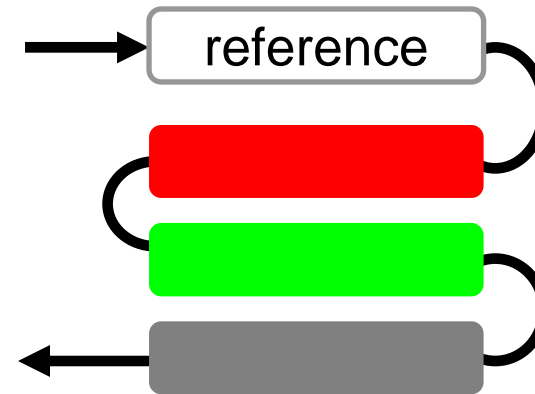


# Octet v. Biacore



## BLI

- 8 parallel sensors
- Single-use tips
- Dip into open shaking plate
- No microfluidics
- Need 200uL/well
- Sample is reusable and recovered
- Heat > room temp.

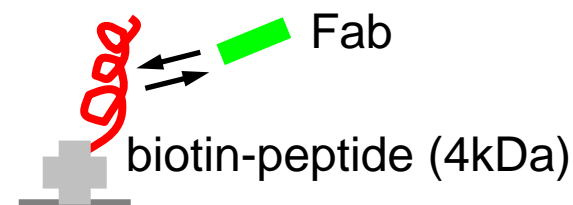
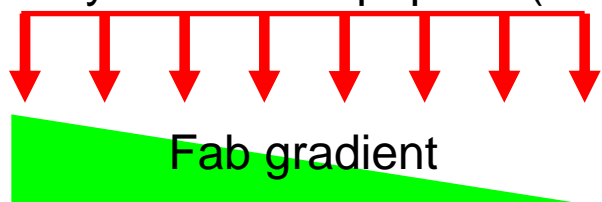


## SPR

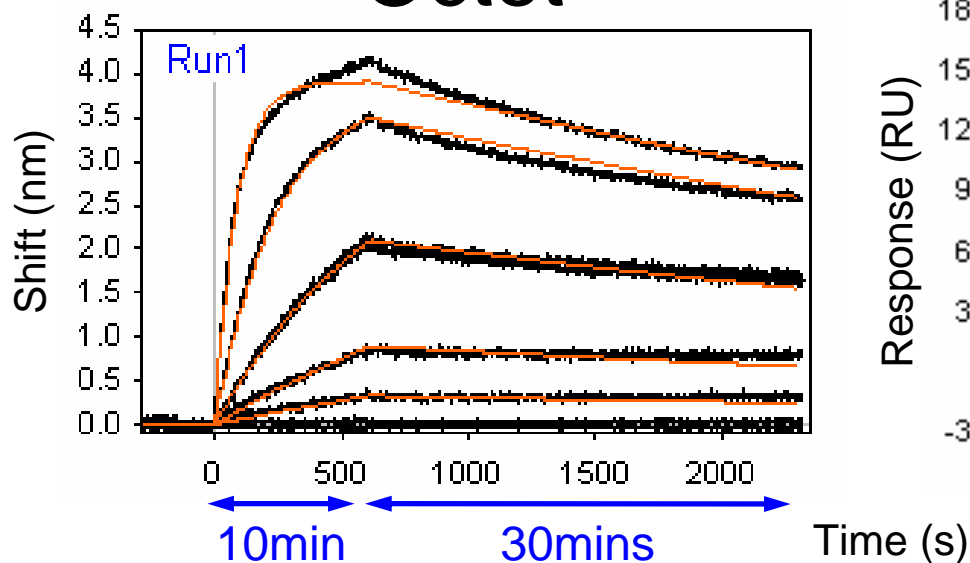
- 4 serial flow cells
- Re-usable chips
- Inject from sealed vials
- Microfluidics
- >25uL/injection
- Sample is consumed
- 4-40°C

# “One-Shot” Kinetics

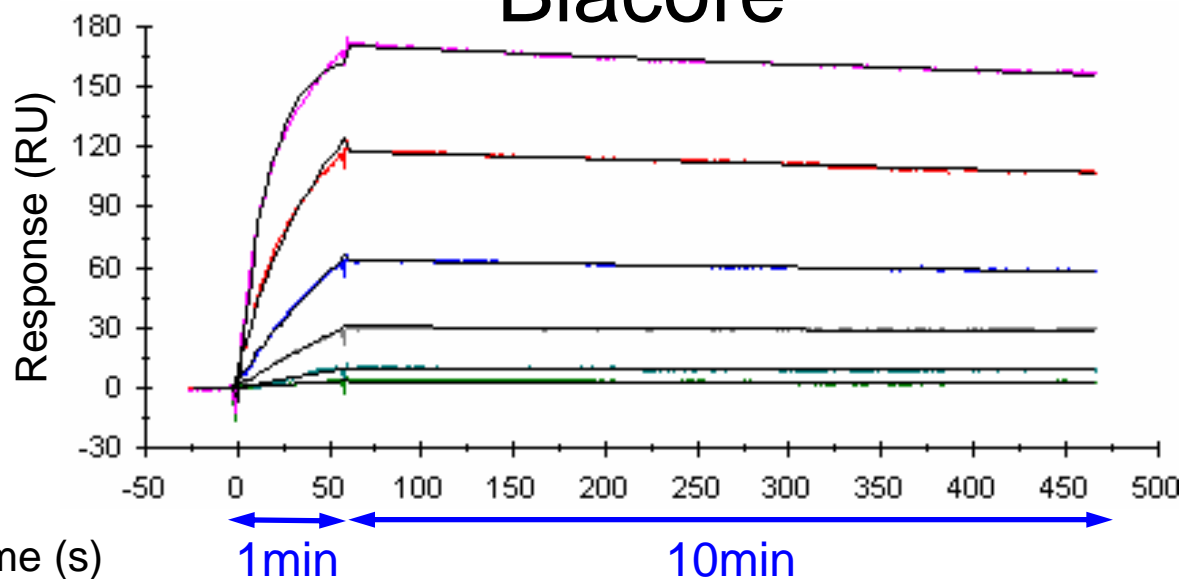
Uniformly load biotin-peptide (on tips)



## Octet



## Biacore



### Method

**Octet** - 6 repeats over 5h with regen.

**Biacore** - 7 independent analyses

### kon (1/Ms)

1.14e5

2.43e5

### koff (1/s)

2.06e-4

2.16e-4

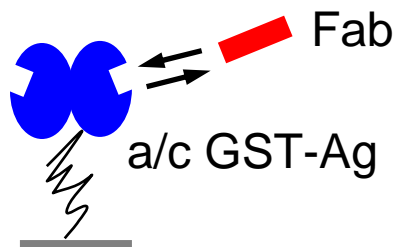
### KD (nM) 25°C

1.81 ± 0.25 (14%)

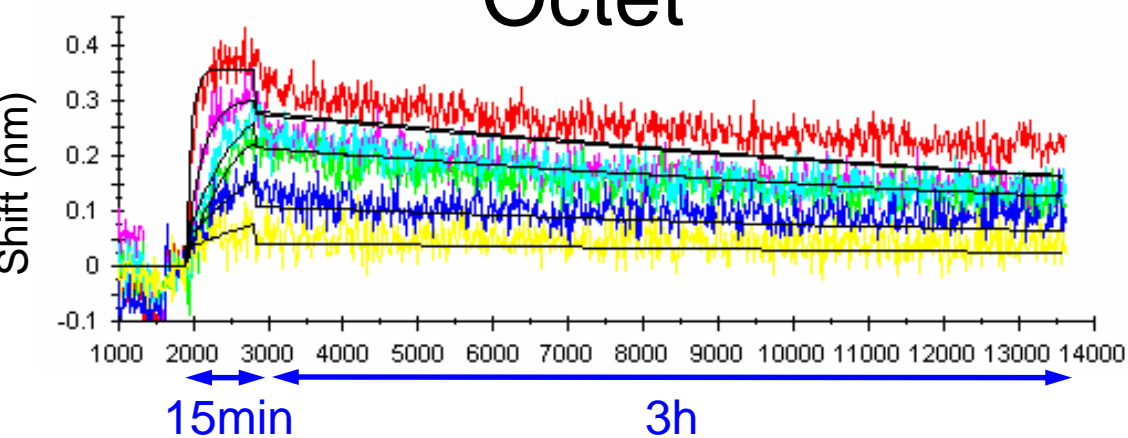
0.97 ± 0.27 (28%)

# Benchmark Study

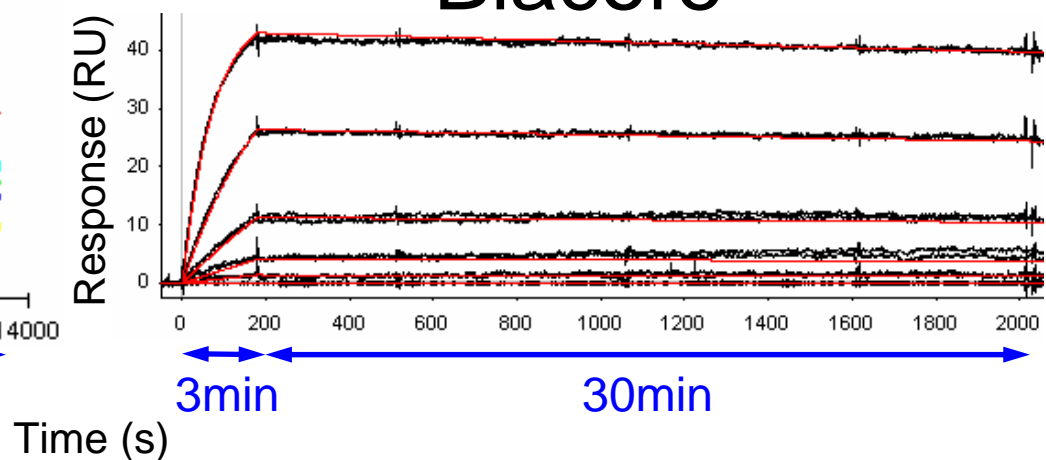
DIPIA, Phoenix AZ, 2007



## Octet



## Biacore



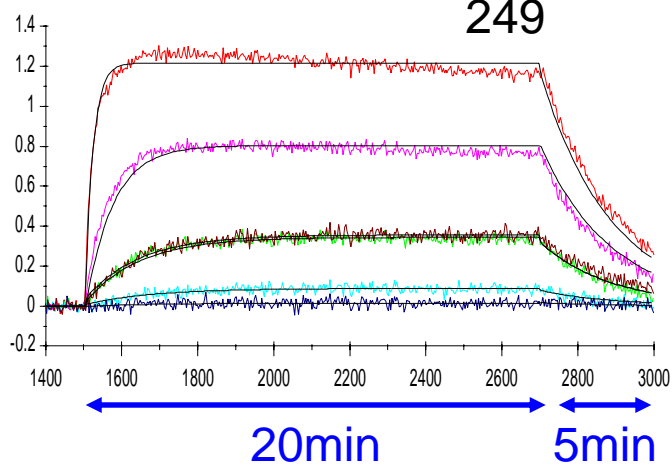
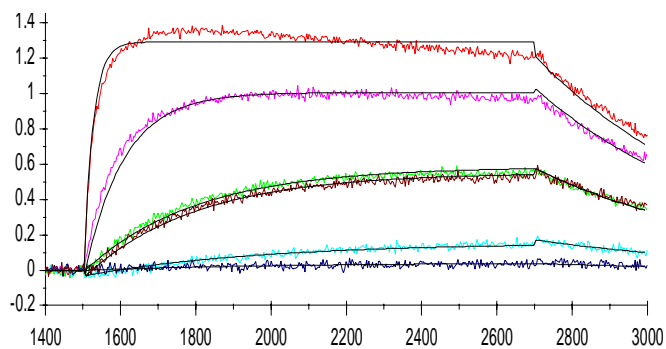
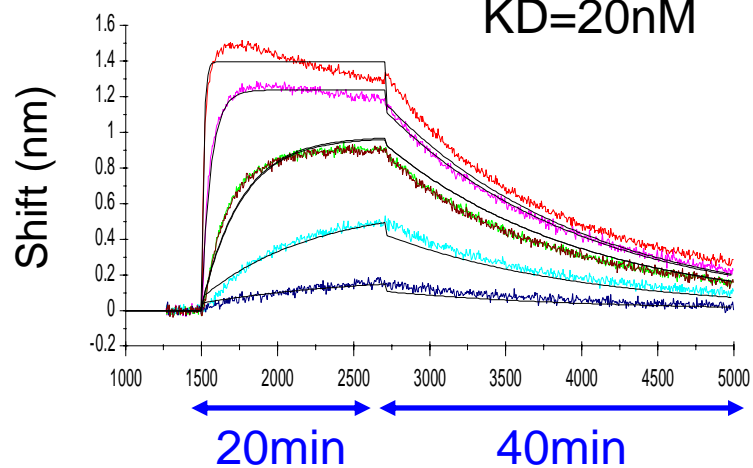
Method	$k_{on}$ (1/Ms)	$k_{off}$ (1/s)	KD (pM) 25°C
Myszka <i>et al.</i> (n~150)	1.2e5	4.9e-5	470 (95% = 210-652pM)
Octet	1.51e5	4.97e-5	329
Biacore (n=3)	1.39e5	4.28e-5	307 ± 3%

# Anti-Id Fab Kinetics

$k_{on}=3.83e4$  (1/Ms)  
 $k_{off}=7.64e-4$  (1/s)  
 $KD=20nM$

**Octet**  $1.96e4$   
 $1.81e-3$   
**92**

$2.15e4$   
 $5.36e-3$   
**249**

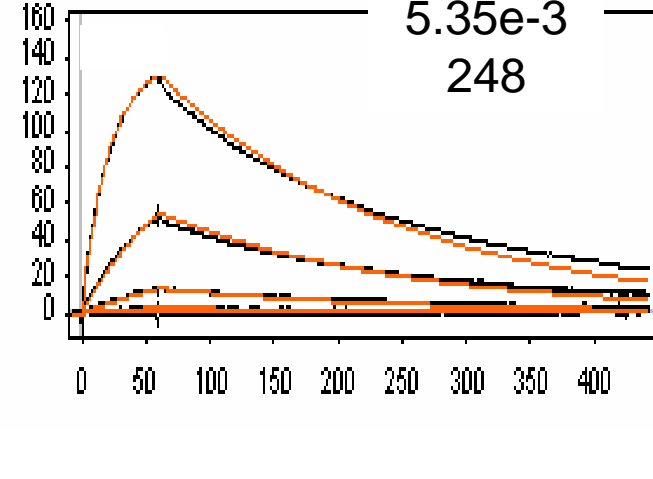
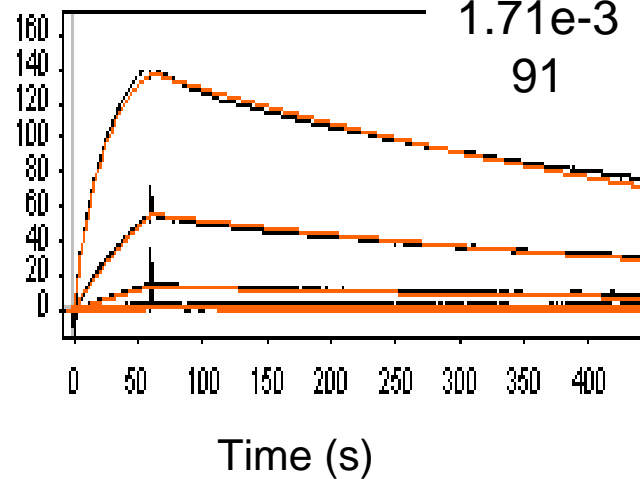
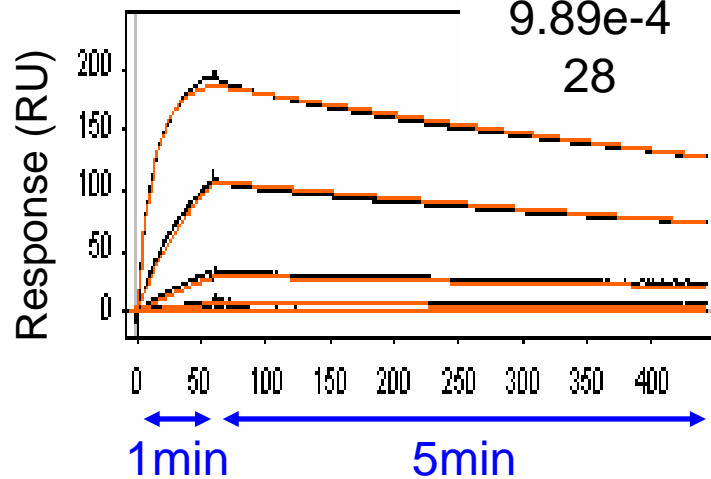


## Biacore

$3.52e4$   
 $9.89e-4$   
**28**

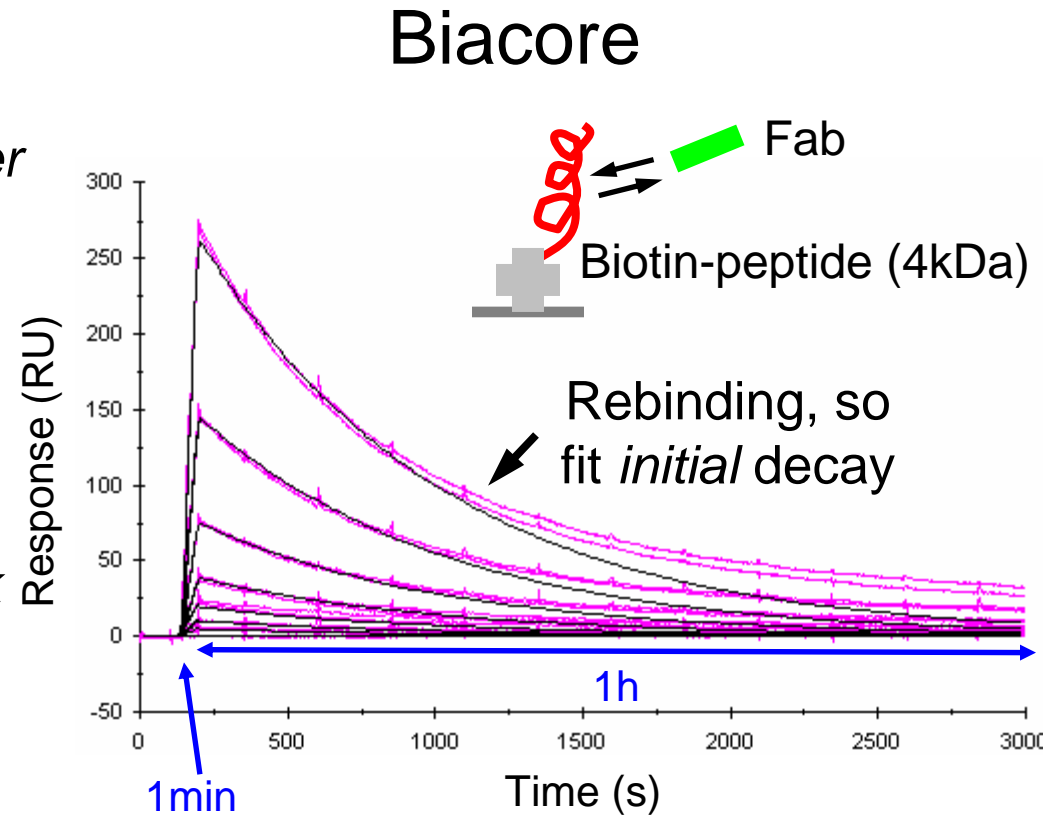
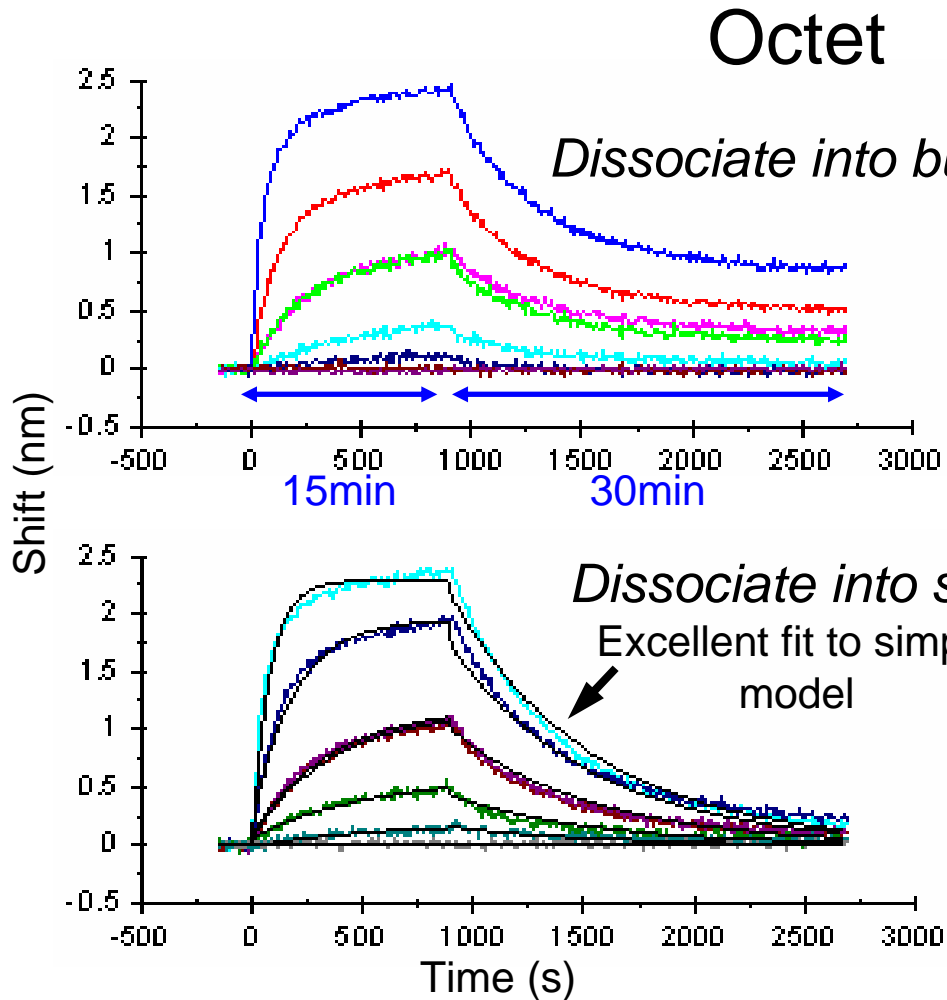
$1.89e4$   
 $1.71e-3$   
**91**

$2.15e4$   
 $5.35e-3$   
**248**



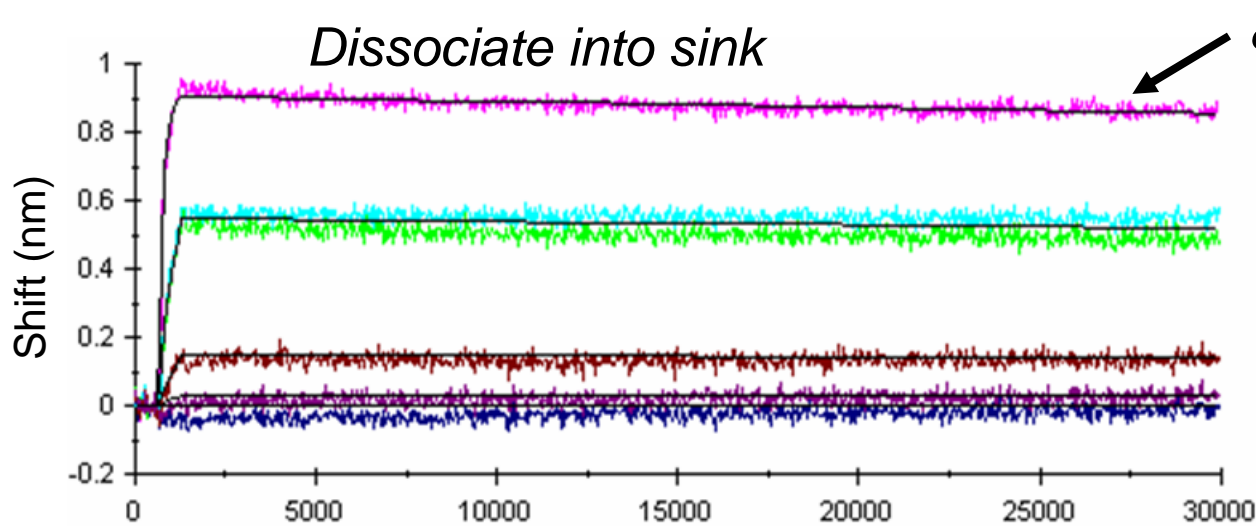


# Minimizing Rebinding Using a Sink

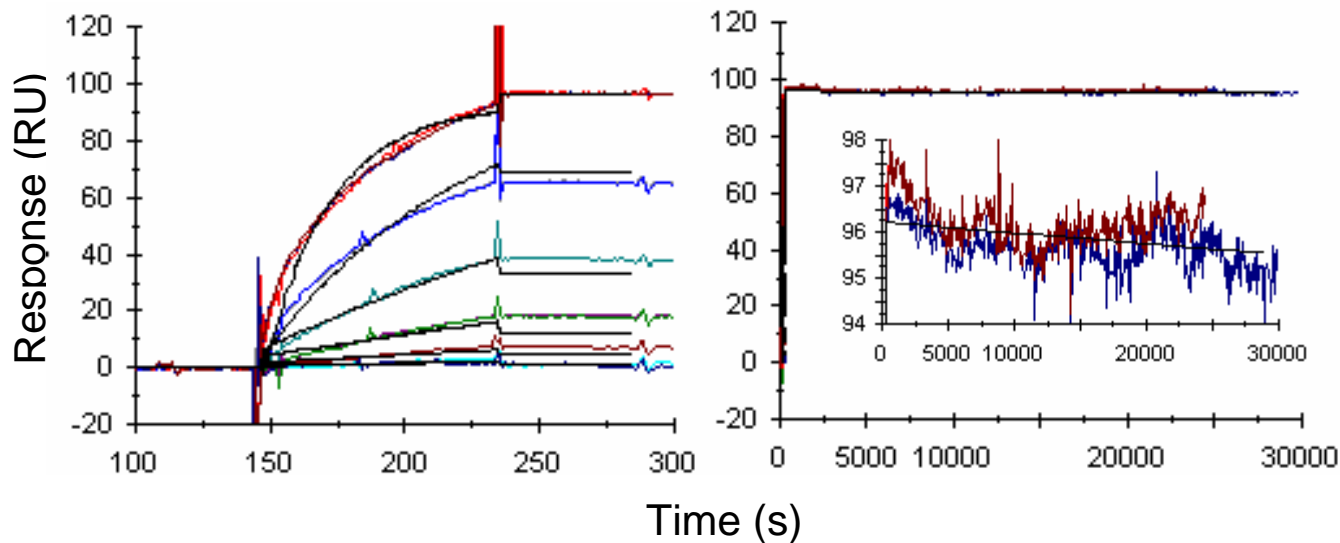


Method	$k_{on}$ (1/Ms)	$k_{off}$ (1/s)	KD (nM)
Octet	$3.76e4$	$1.57e-3$	42
Biacore	$4.91e4$	$1.25e-3$	$26 \pm 9$ (n=3)

# Resolving a Tight Interaction



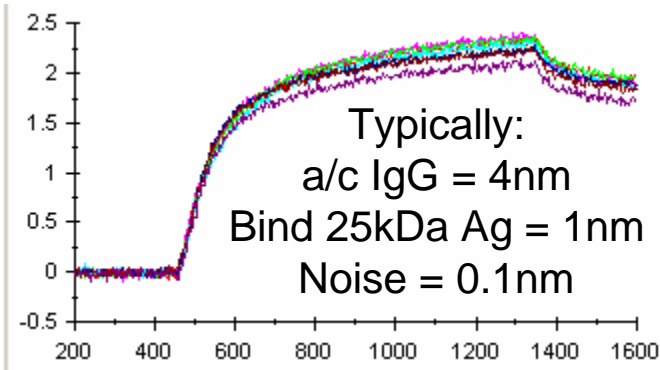
**Octet**  
*biotin-Ag on tip*  
 $k_{on}=3.36e5$  (1/Ms)  
 $k_{off}<2e-6$  (1/s)  
 Within noise ( $\pm 0.1$ nm)  
 $KD<6$ pM



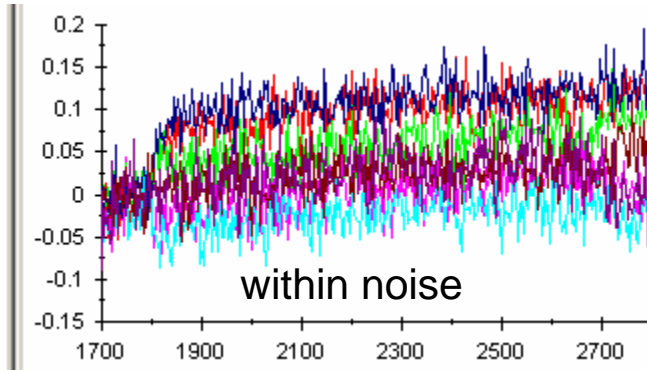
**Biacore**  
*a/c Ag on chip*  
 $k_{on}=1.07e5$  (1/Ms)  
 $k_{off}<2e-6$  (1/s)  
 Within noise ( $\pm 1$ RU)  
 5% rule  
 $KD<19$ pM

# Direct Binding of Small Molecules

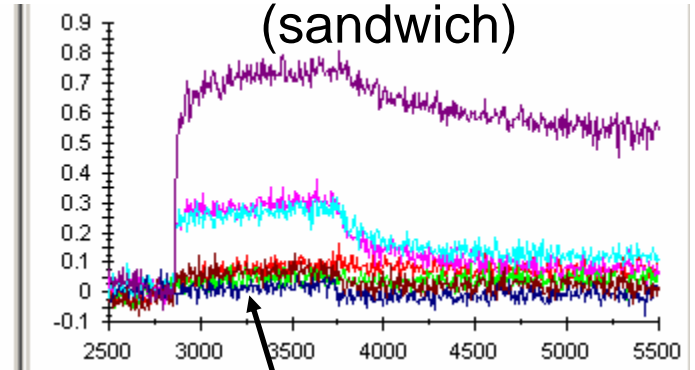
a/c IgG on AR tips



Bind 4kDa-peptide

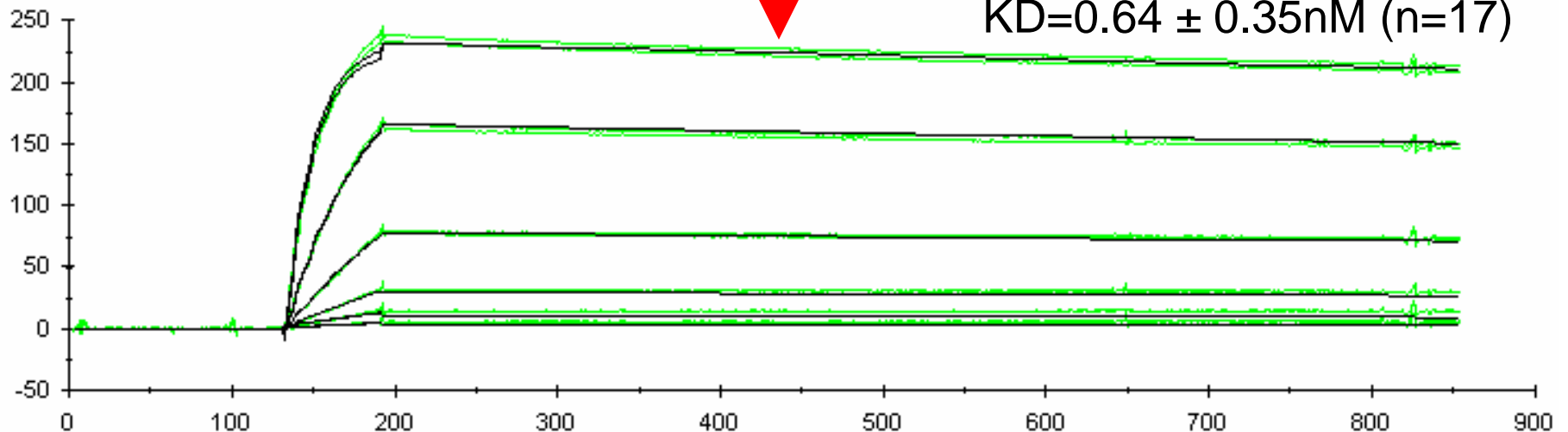


Detect with another mAb (sandwich)



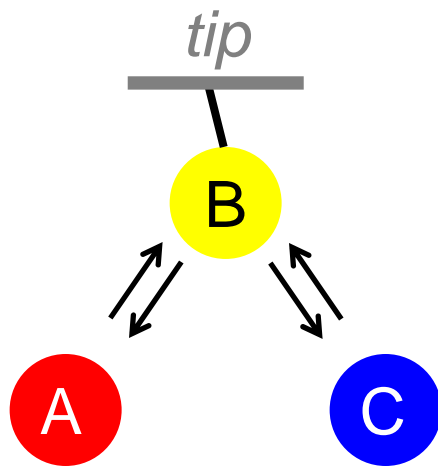
Octet is 100fold less sensitive than Biacore

$KD = 0.64 \pm 0.35 \text{ nM}$  (n=17)

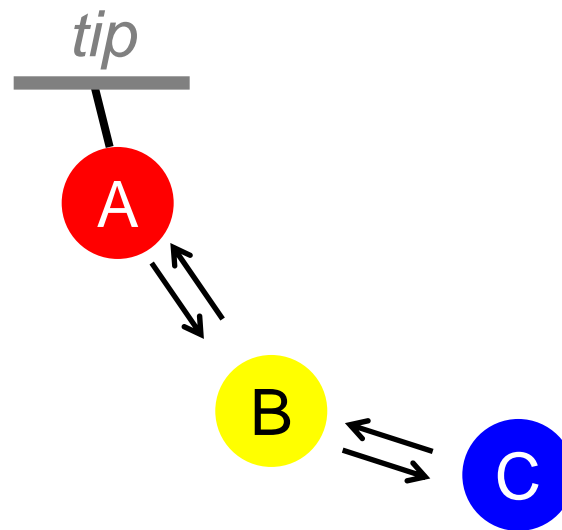


# Blocking Strategies

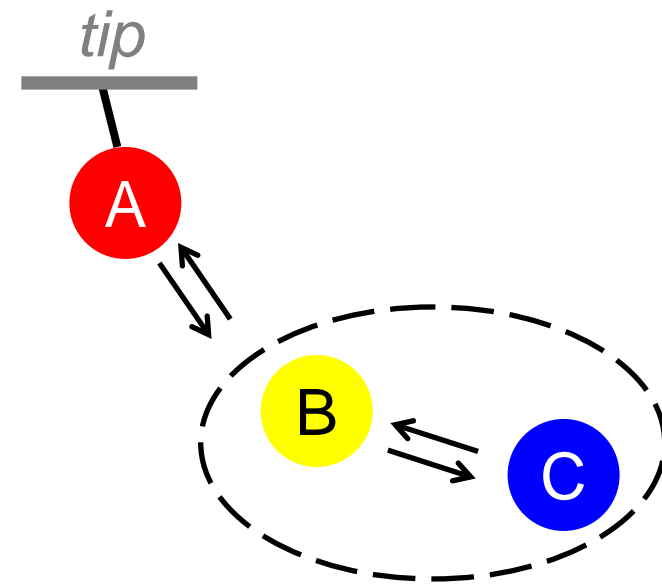
Stepwise blocking



Classical sandwich



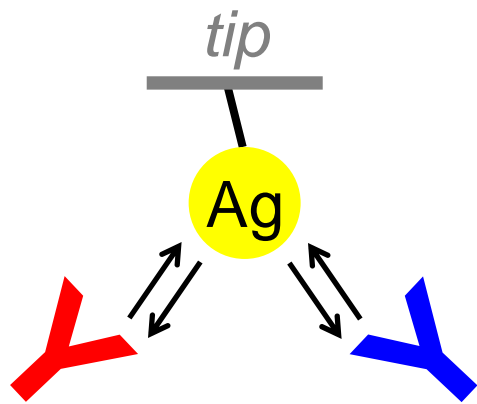
Premix



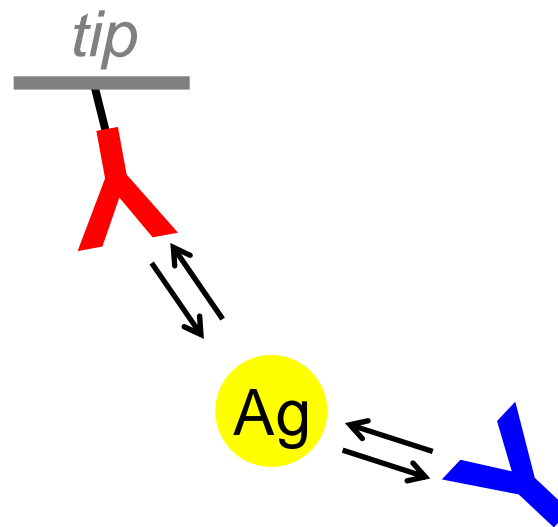
1. “ABC” sandwiches where A does *not* bind C directly
2. Choose assay orientation and surface chemistry
3. Run proper controls, e.g., buffer, irrelevant partner, “self-sandwich”
4. Where a mAb binds is often more important than its KD

# Epitope Binning

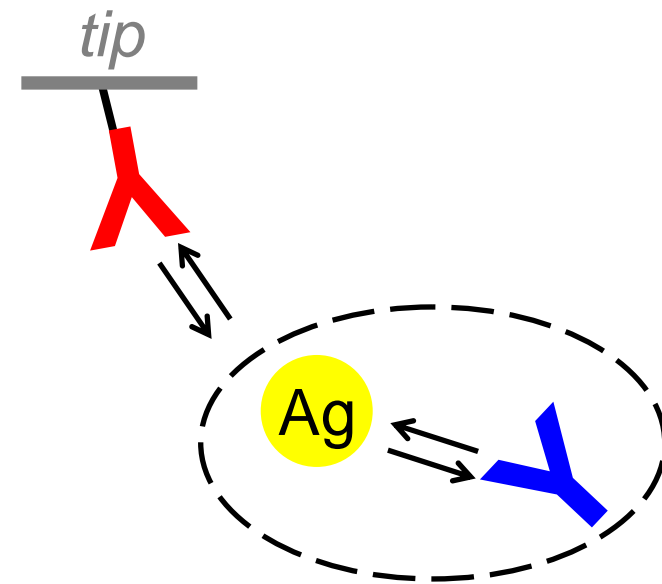
Stepwise blocking



Classical sandwich



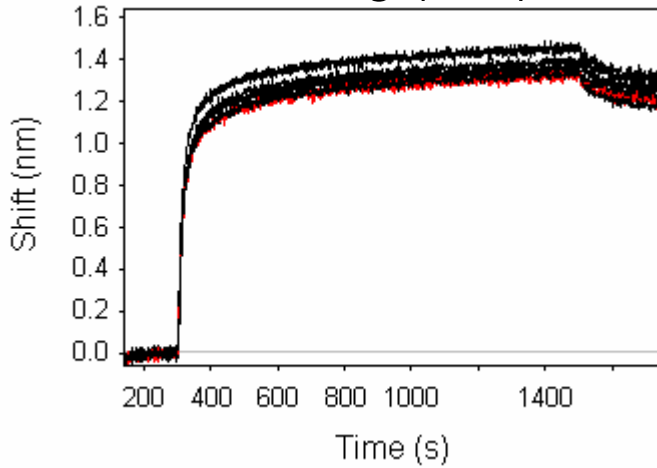
Premix



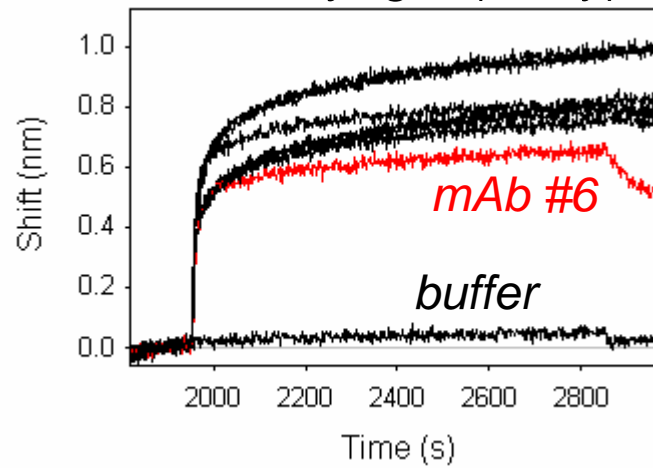
1. Each strategy has its pro's and con's
2. Consider MW and aggregation state of Ag
3. Improve unattended throughput by preparing surfaces offline using "batch immobilization"
4. Can often transfer known regeneration cocktail from Biacore

# Stepwise Blocking

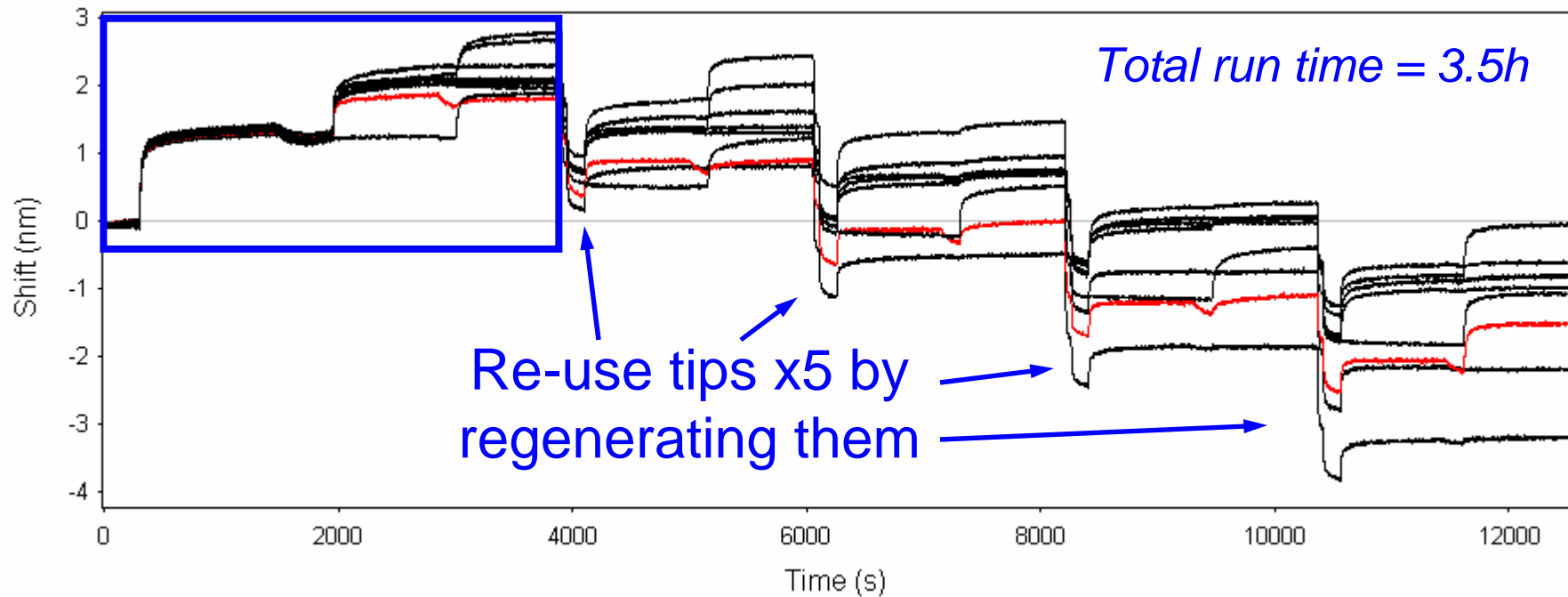
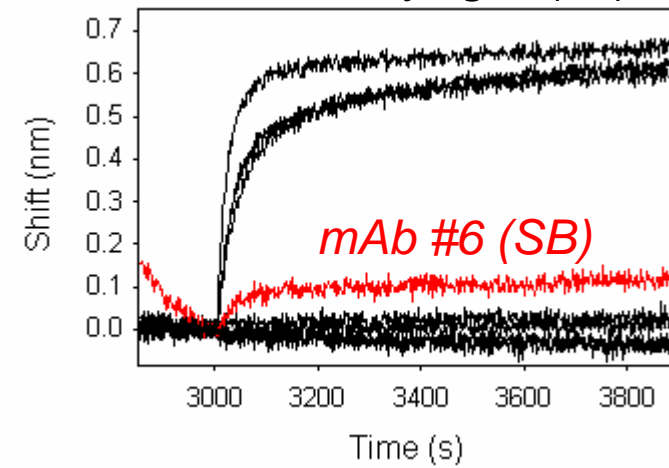
a/c Ag (n=8)



Primary IgG (array)

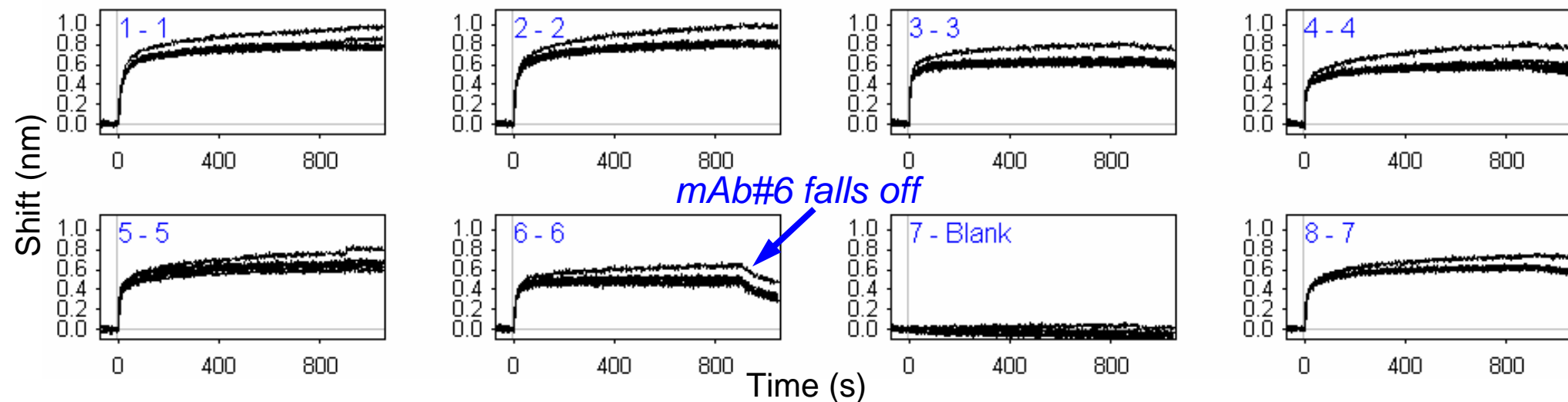


Secondary IgG (fix)

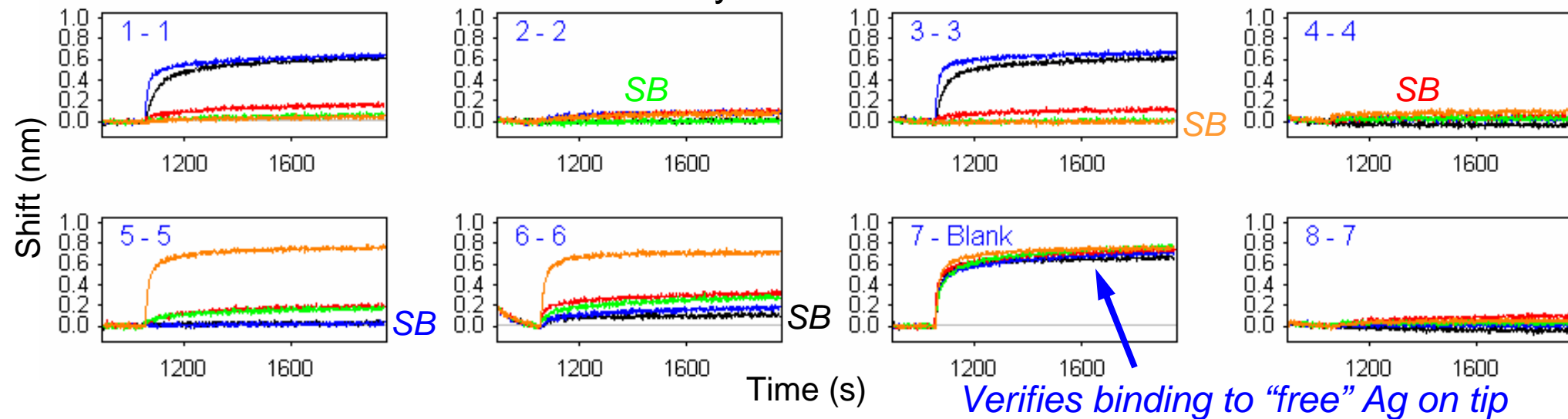


# Stepwise Blocking

Primary mAb (array) - 5 repeat cycles



Secondary mAb - 2 3 4 5 6



# Premix Approach

a/c IgG

a/c MAb #2

MAb#2

a/c MAb #3

MAb#3

a/c MAb #5

MAb#5

Premix Ag + mAb (array)

MAb #2 on tip

Ag alone

MAb #3 on tip

MAb #5 on tip

“Traffic light” binding matrix

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

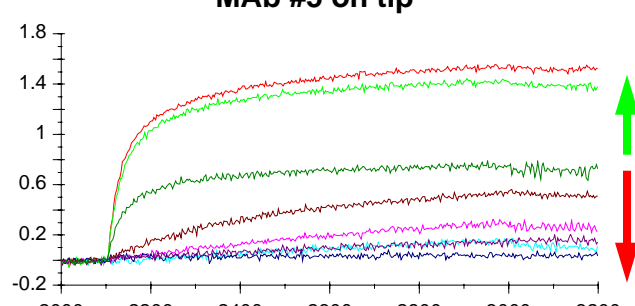
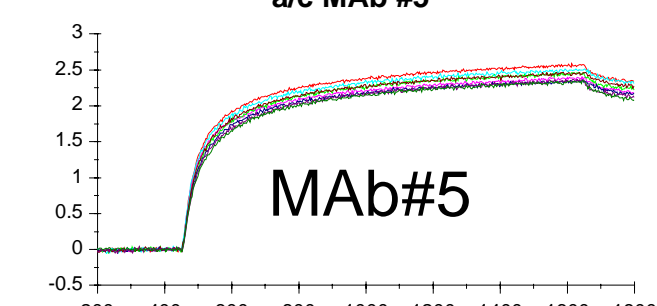
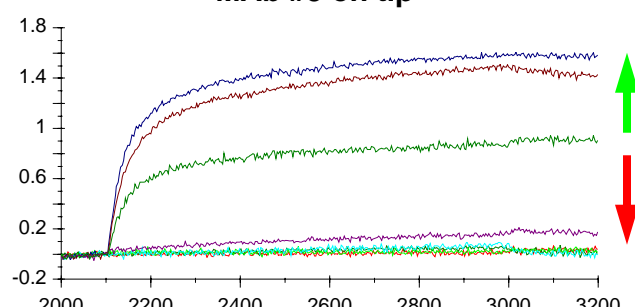
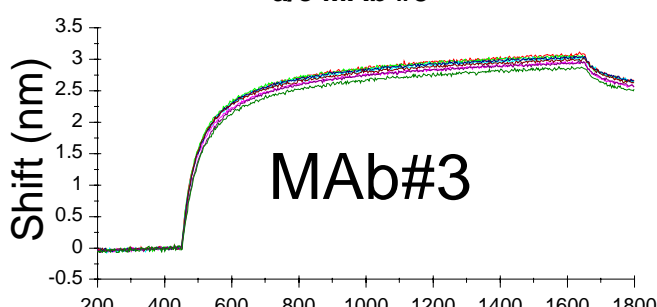
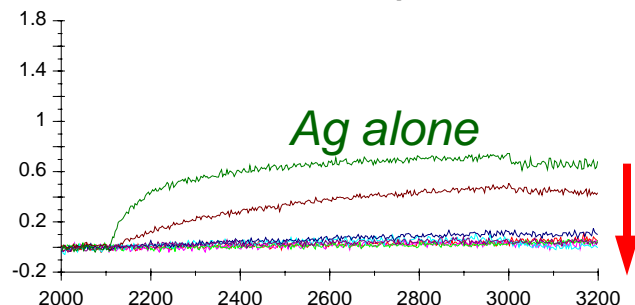
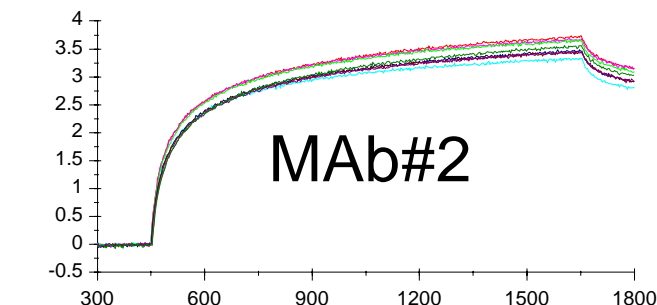
- Binding (sandwich)
- Unclear
- No binding (blocking)

Confirmed 3 epitope bins using two blocking strategies

BIN 1: #1 and 3

BIN 2: #5 and 6

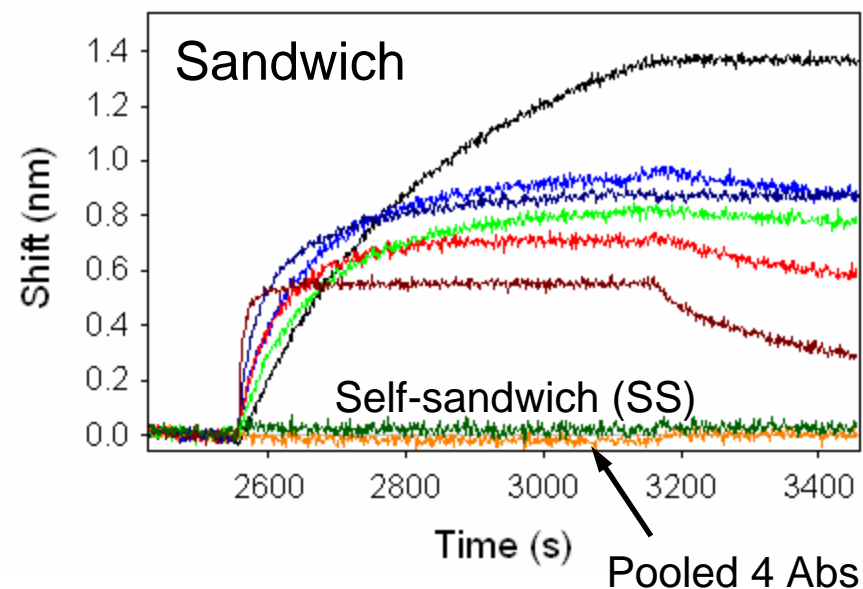
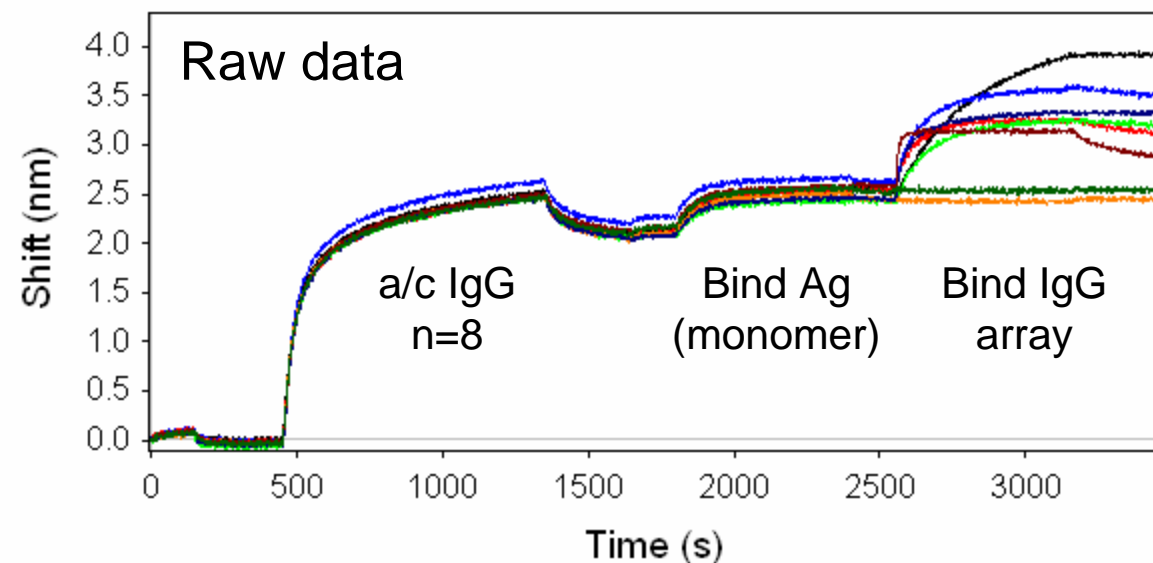
BIN 3: #2, 4, and 7



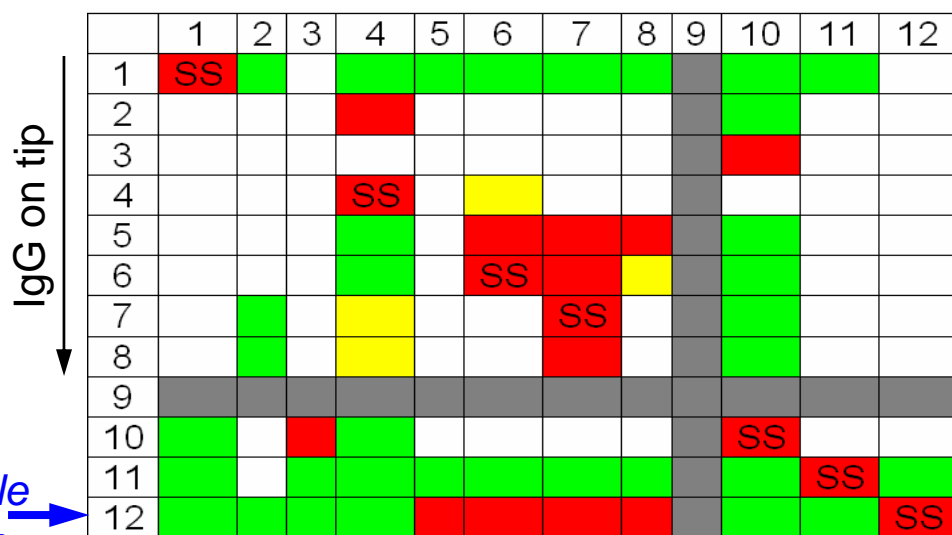
Time (s)



# Classical Sandwich



IgG in solution →



Example shown →

Bin	IgG#
1	1
2	2 and 4
3	3 and 10
4	5, 6, 7, 8, and 12
5	11

# Summary

1. The Octet is a simple, yet versatile complement to Biacore
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

# Acknowledgements



Alanna Pinkerton



Dan Malashock



Jaume Pons  
*Director*  
*Protein Engineering*