Exploring Blocking Assays Using a Parallel Real-time Label-free Biosensor, the Octet

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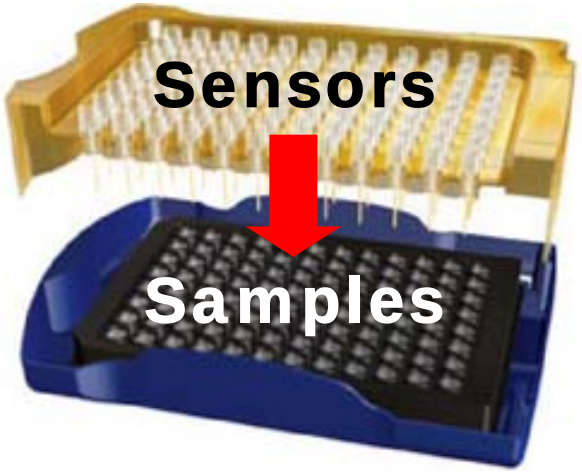
Overview

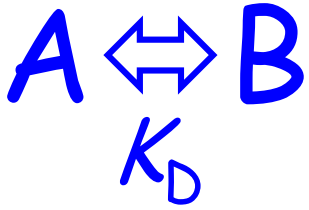
- Basic Octet Set-Up
- Significance of Blocking to Drug Discovery, Focusing on Epitope Binning
- Orienting a Blocking Assay:

  In Tandem, Premix, and Classical Sandwich
- Extending Scope of Premix Blocking to Quantitative Assays Aimed at Determining Solution Affinity and Active Concentration

Basic Octet Set-Up

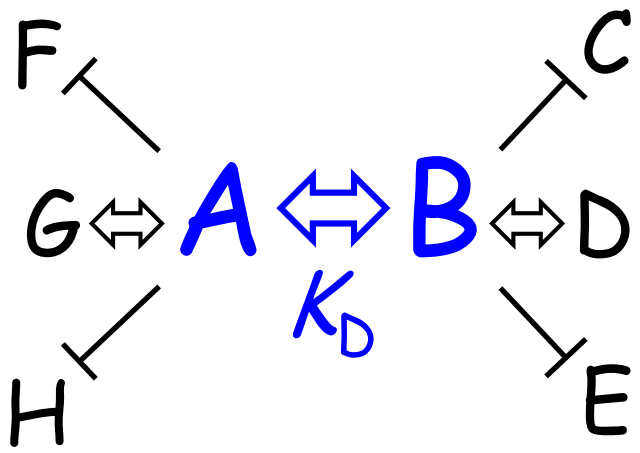
- **Bio**Layer **I**nterferometry detection
- Single-use/disposable sensors on fiber optic tips
- Samples are addressed in parallel by moving a column of 8 sensors to samples held in an open shaking microplate
- Dip-and-read format does not require any microfluidic handling
- Samples are not consumed, so can be re-used or recovered
- Ligands can be immobilized *offline* to increase throughput





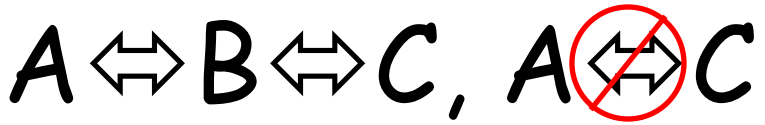
- Knowing how tightly two biomolecules bind one another helps to characterize them…to a point.

Competitive Binding



- Knowing how tightly two biomolecules bind one another helps to characterize them…to a point.
- Understanding how that binding event impacts others may elucidate the **functional significance** of the interacting biomolecules.
- Probing blocking is key to drug discovery, *e.g.*, identifying inhibitors of ligand/receptor interactions, probing allosteric sites, epitope binning, and much more…

Assay Orientations



1. Any binding partner can be on sensor
2. You can preform A+B or B+C
3. The preformed complex can be on sensor or in solution
4. A and C can swap roles

Assay Orientations

$A \Leftrightarrow B \Leftrightarrow C$, $A \not\Leftrightarrow C$

1. Any binding partner can be on sensor
2. You can preform A+B or B+C
3. The preformed complex can be on sensor or in solution
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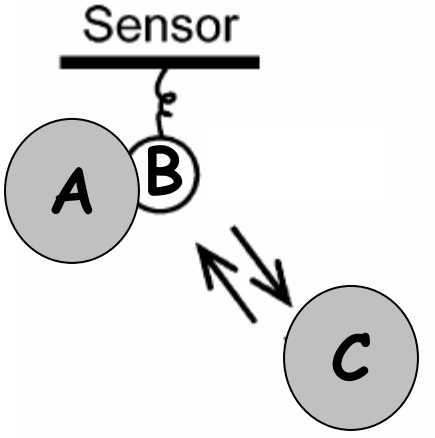
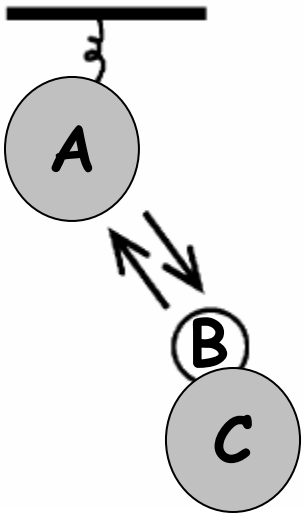
In Tandem

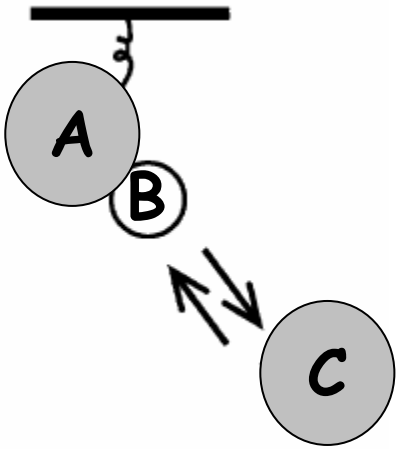


Yes/No Read Out

Premix



Classical Sandwich

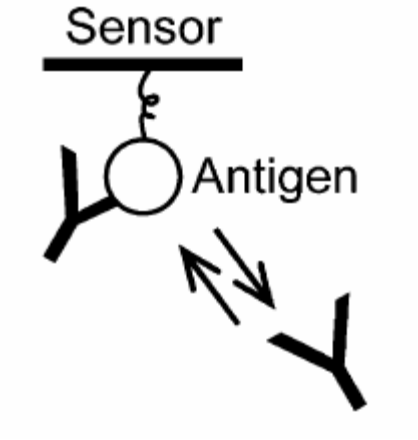


Epitope Binning

Ab⇔Ag⇔Ab, Ab⃠⇔Ab

By competing Abs against one another in a pairwise and combinatorial format, we discriminate Abs with distinct blocking behaviors and assign them to “bins”.

In Tandem



Yes/No Read Out

Premix

Classical Sandwich

Significance of Binning

- You are more likely to discover a lead biotherapeutic compounds by epitope-screening than affinity-screening
- Discriminates Abs in a functional context without knowing any contact-level detail about the epitopes being targeted
- Identifies Abs likely to share functional characteristics and thus lowers the “hits” taken forward into low-throughput functional cell-based or *in vivo* assays
- When the natural blocking partner is unknown, establishing a set of Abs that binds a repertoire of epitopes increases the chance of targeting a functional epitope
- Identifies “sandwiching” pairs for use in other assays
- Broadens the claims of a patent

Analytical Biochemistry 386 (2009) 172–180

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Exploring blocking assays using Octet, ProteOn, and Biacore biosensors

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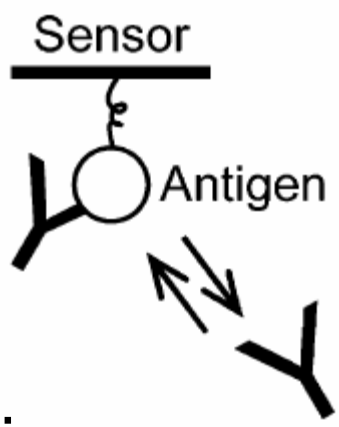
In Tandem

(1) Couple Ag

(2) Saturate with Ab

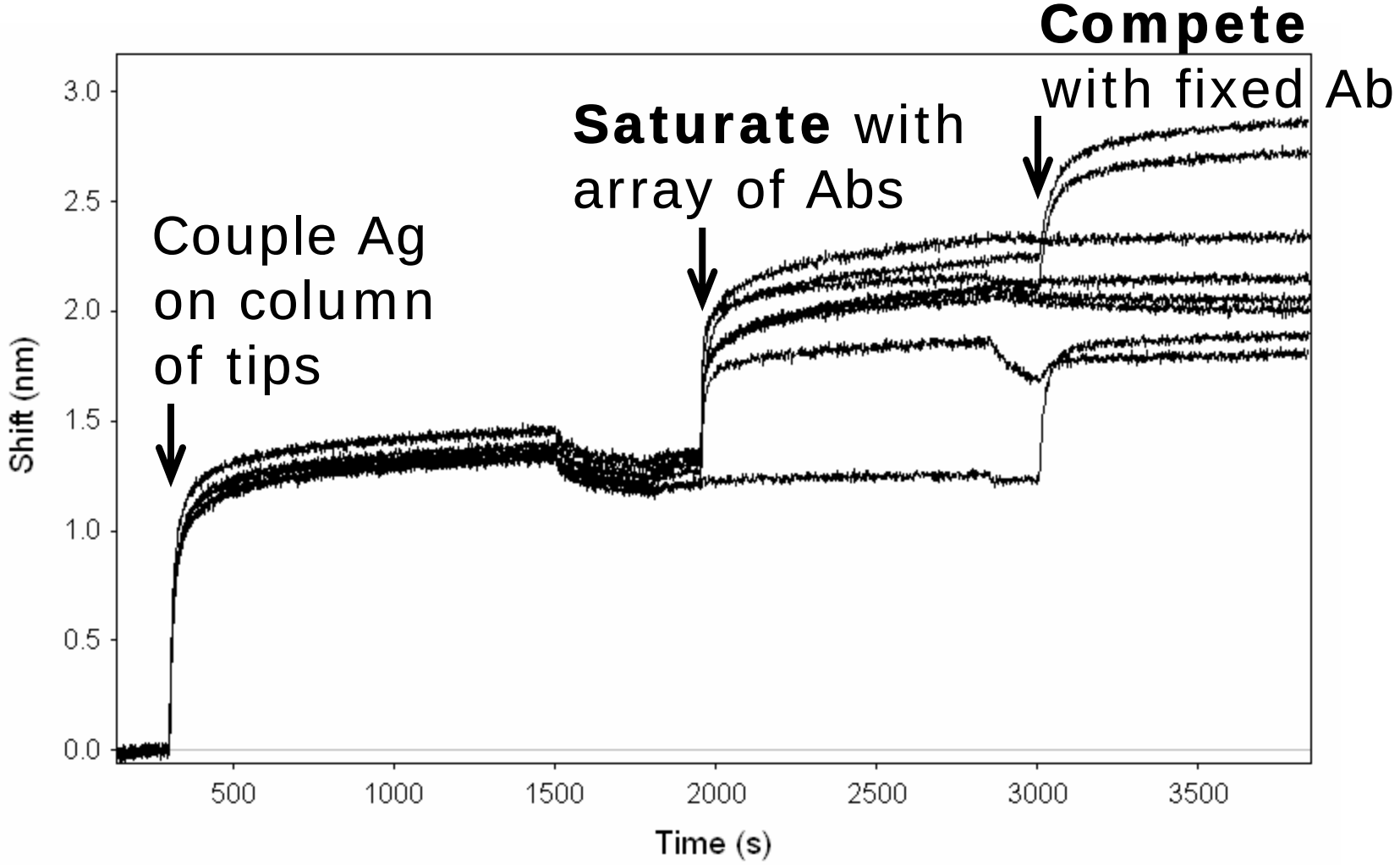


(3) Compete with another Ab

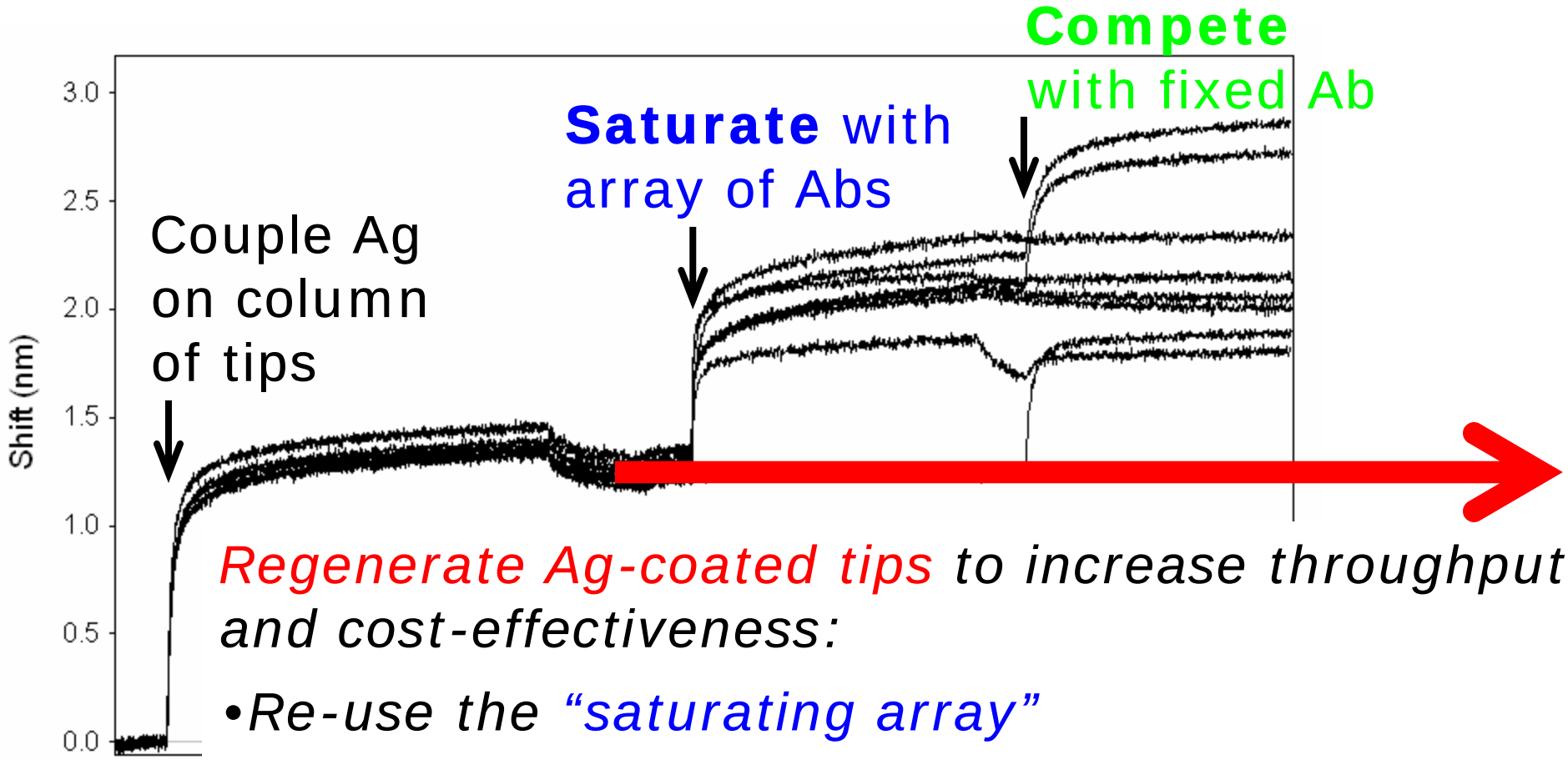
Caveats for **clean** assay:

- The order of binding matters. The “saturating” Ab must remain bound to prevent exposing “free Ag” to the “competing” Ab
- Coupling Ag may inactivate it or mask epitopes and may not be economic if Ag is precious. If the Ag is tagged or fused, an oriented capture may be preferable as it also allows Ag to be re-used.
- Simple format for screening large panels of Ab’s

In Tandem


Compete
with fixed Ab
Saturate with
array of Abs
Couple Ag
on column
of tips
Shift (nm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
500
1000
1500
2000
2500
3000
3500
Time (s)

In Tandem



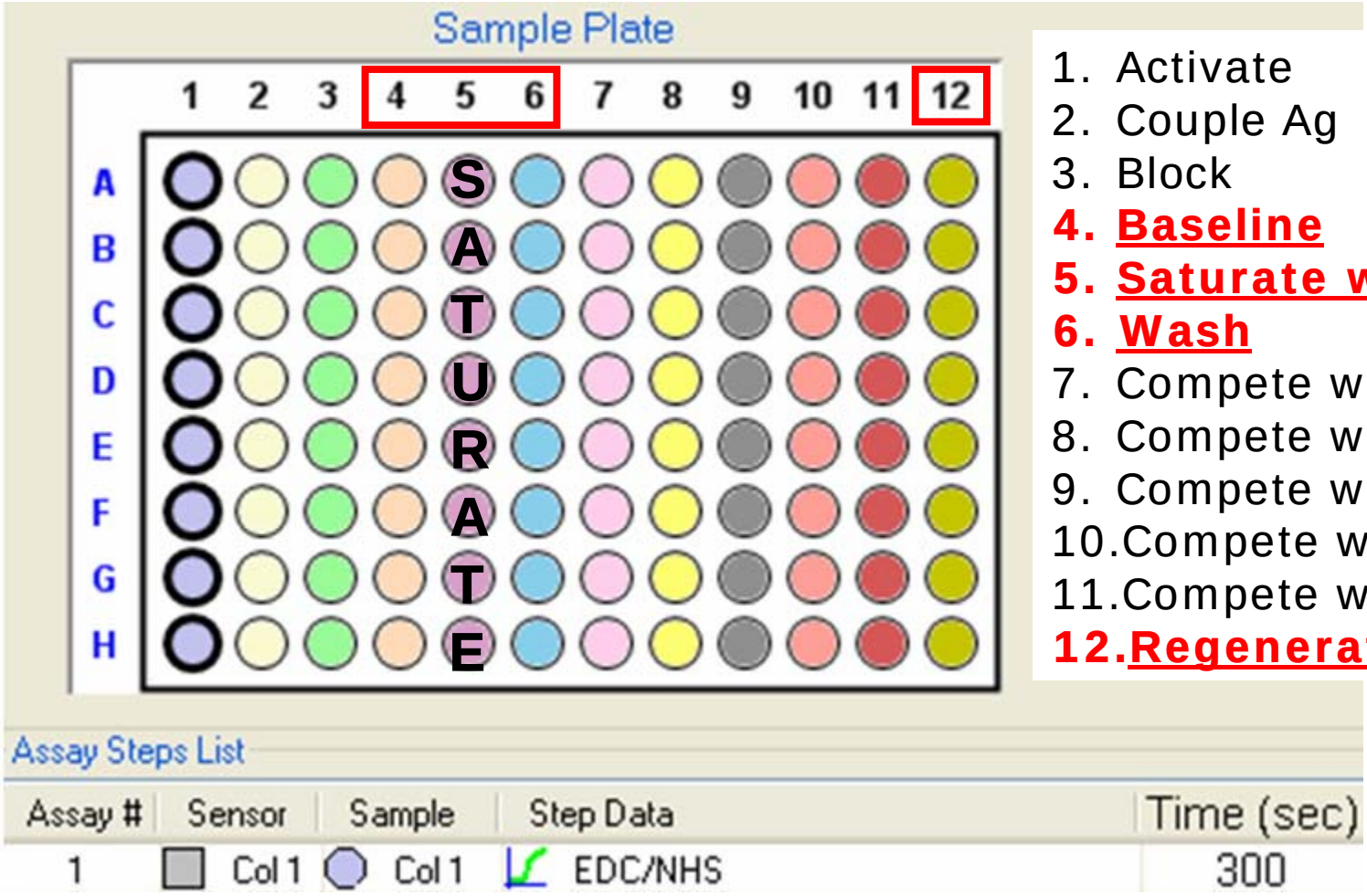
Regenerate Ag-coated tips to increase throughput and cost-effectiveness:

- *Re-use the "saturating array"*
- *Screen a different "competing Ab" in separate binding cycles allowing each its own column in plate*
- *Measure 40 pairs of Abs in 5 cycles in 3.5h*

In Tandem Method

Single-use column

Re-used column



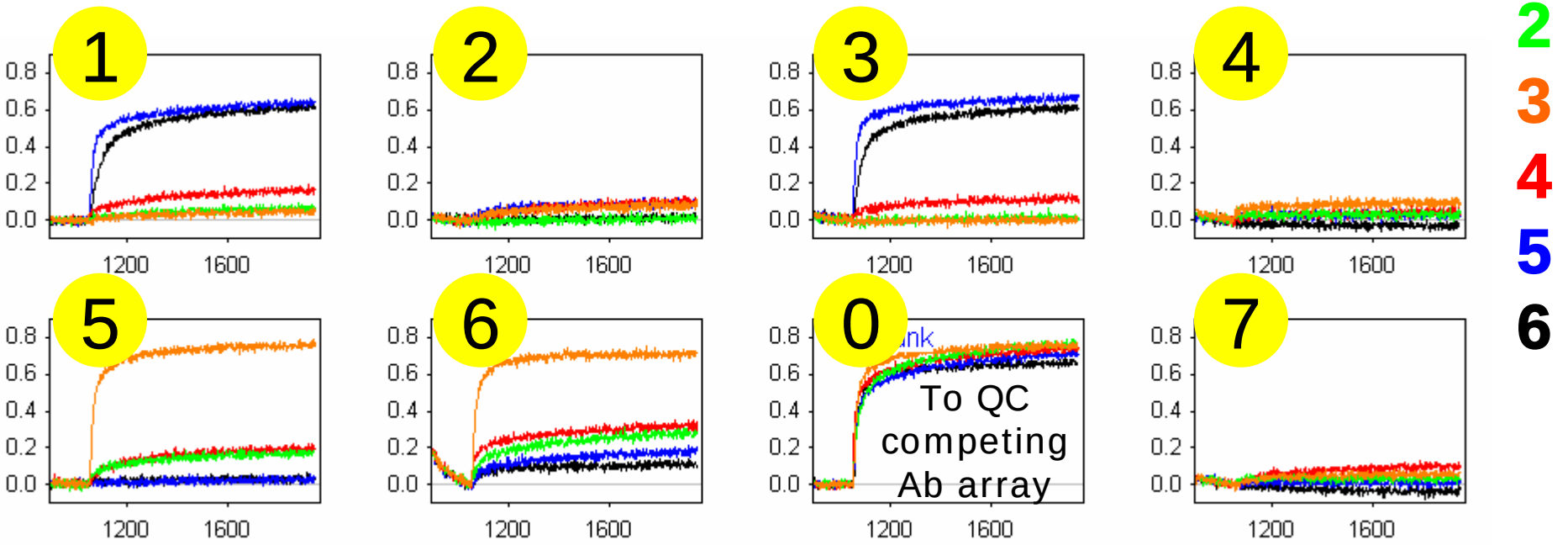
1. Activate
2. Couple Ag
3. Block
4. **Baseline**
5. **Saturate with Ab array**
6. **Wash**
7. Compete with Ab#2
8. Compete with Ab#3
9. Compete with Ab#4
10. Compete with Ab#5
11. Compete with Ab#6
12. **Regenerate**

Competing Step

Saturate with a different Ab along a column of tips

Competing Ab:

2
3
4
5
6

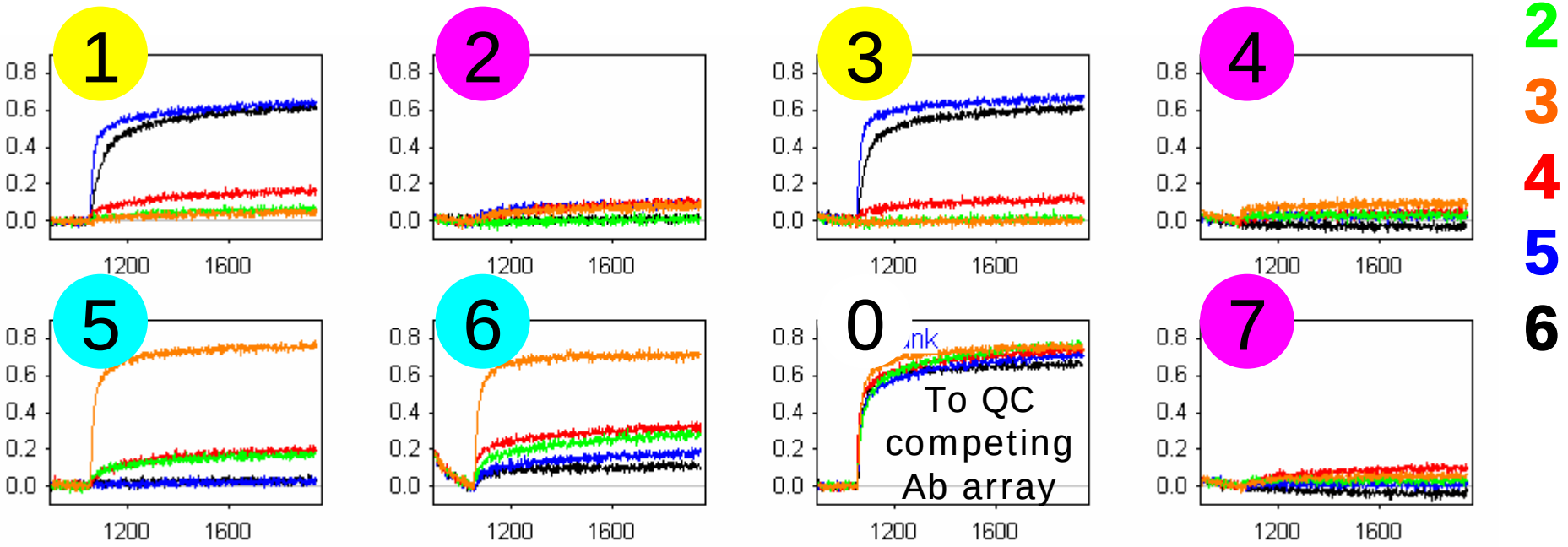


Competing Step

Saturate with a different Ab along a column of tips

Competing Ab:

2
3
4
5
6



3 bins emerge:

Sandwich with 5,6

1 3

Sandwich with 1,3

5 6

Universal blocker

2 4 7

Traffic Light Matrix

Saturating Ab

Competing Ab

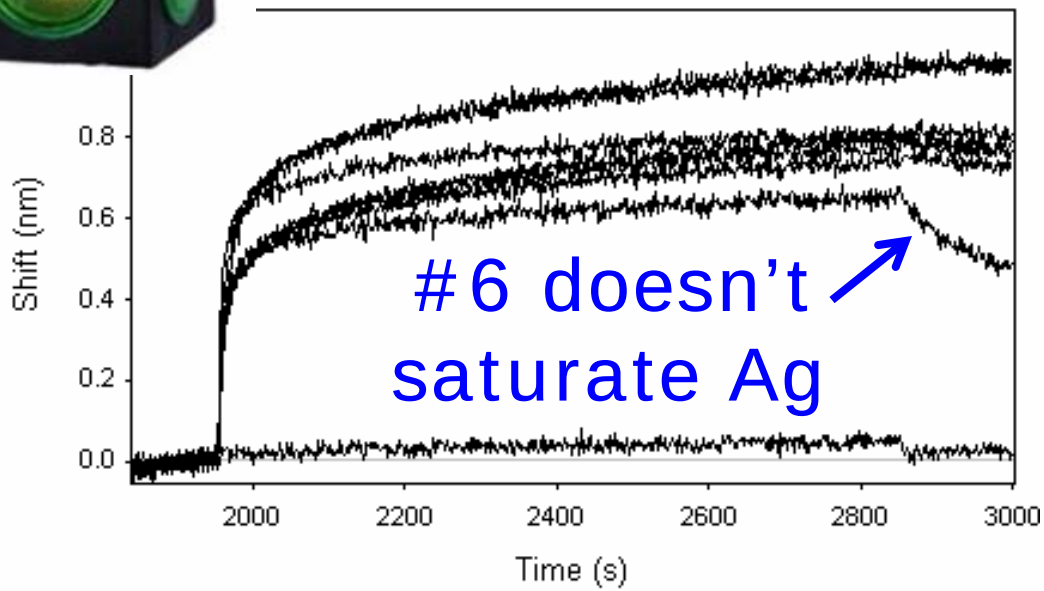
	3	2	4	5	6
1	Y	Y	Y	N	N
3	SS	Y	Y	N	N
2	Y	SS	Y	Y	Y
4	Y	Y	SS	Y	Y
7	Y	Y	Y	Y	Y
5	N	Y	Y	SS	Y
6	N	Y	Y	Y	SS

Read table across or down to discern 3 bins

Blocks (SS=self-sandwich)

Unclear

Does not block



Premix Blocking

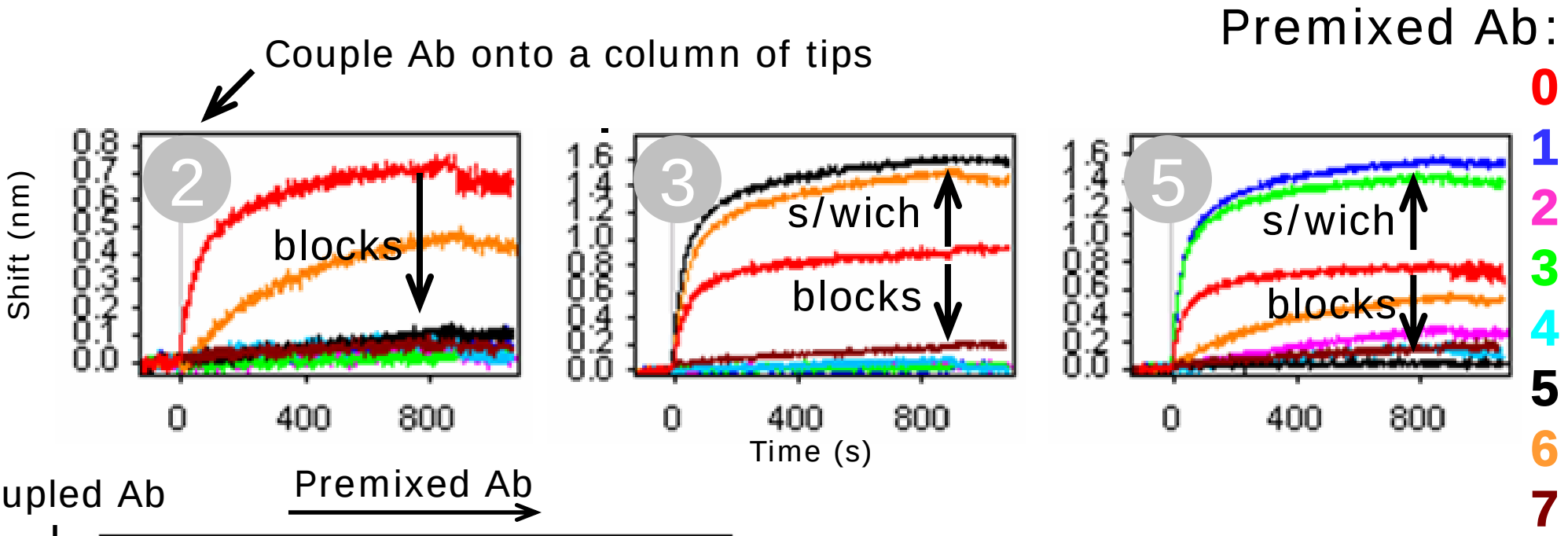
(1) Couple Ab

(2) Bind Ag±Ab premixes

Caveats for **clean** assay:

- Premixed Ab must be in large mol excess over Ag
- Ag's conc must be $>K_D$ for each premixed Ab
- Premixed Abs that give intermediate responses should be titrated into Ag to verify that they block or sandwich in a dose-dependent manner
- Premixed Abs must not cross with coupled Ab

Premix Blocking



Coupled Ab

Premixed Ab

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

Premix corroborates In Tandem data:

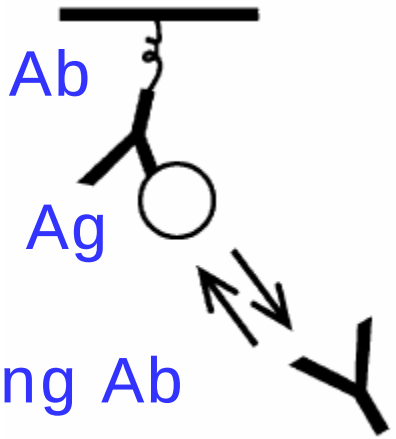
- BinA (#1,3) Sandwiches with BinB
- BinB (#5,6) Sandwiches with BinA
- BinC (#2,4,7) Universal blocker

Classical Sandwich

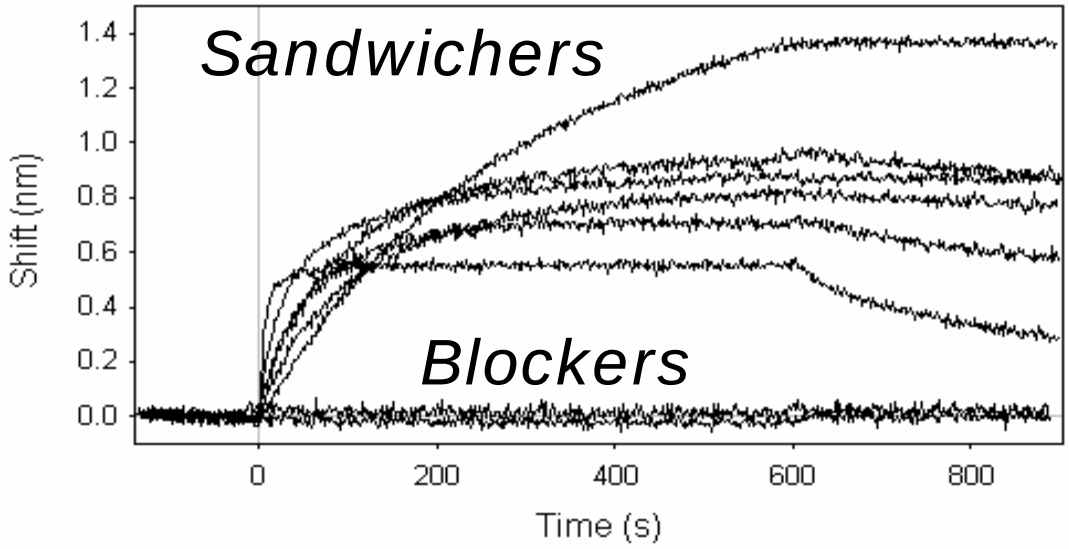
(1) Couple Ab

(2) Capture Ag

(3) Sandwiching Ab



Example of sandwiching array



Caveats for **clean** assay:

- Ag must be a monomer
- Ag cannot dissociate rapidly from coupled Ab
- Trust blockers only if they sandwich elsewhere. If they do not, then reverse the assay and couple them to verify their binding to “free” Ag
- Solution Abs must not cross with coupled Abs

Orienting the Assay

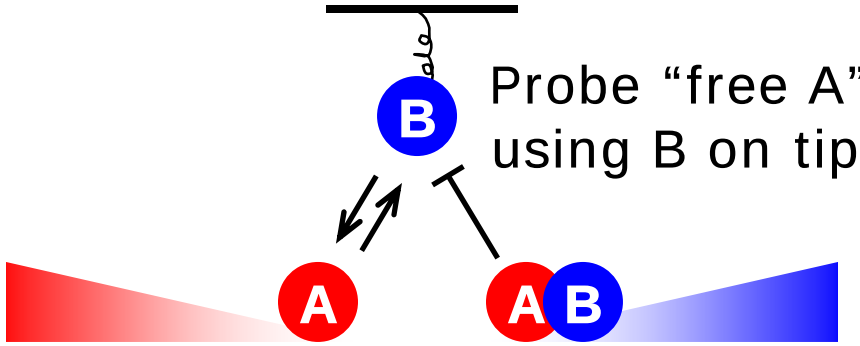
Relies upon an interplay of factors:

- Is Ag a monomer or multivalent/aggregated?
- Can Ag be coupled easily to tips?
- How many Abs are being screened?
- How tightly do the Abs bind Ag?
- How precious are the reagents?
- Do I obtain intermediate responses?

Often, it is prudent to corroborate data from various assay orientations

Quantitative Premix Blocking

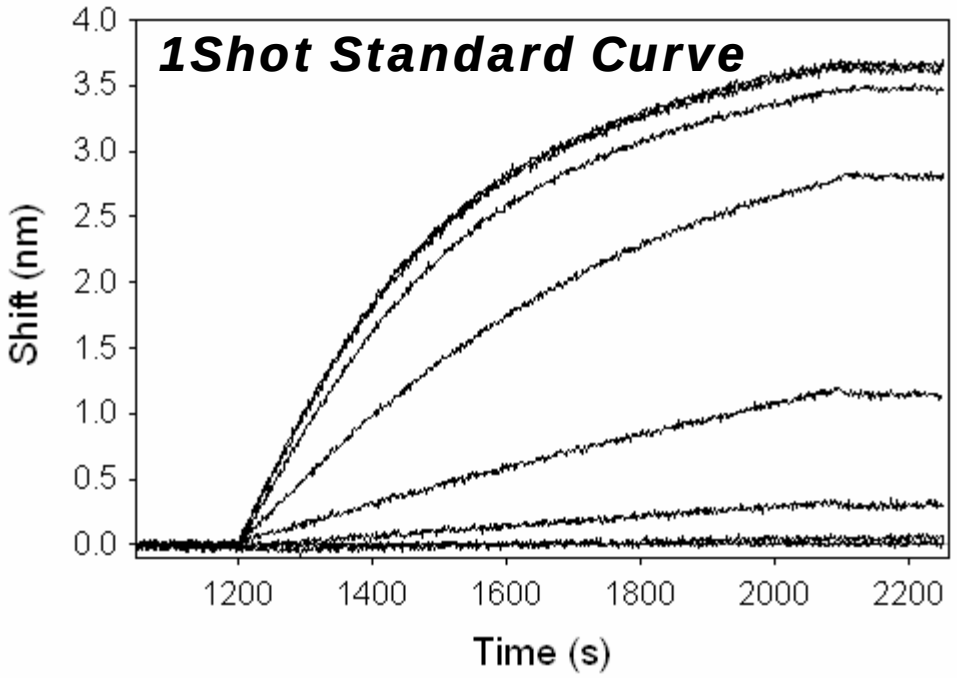
Use to convert inhibition curve shift values to conc's of "free" A

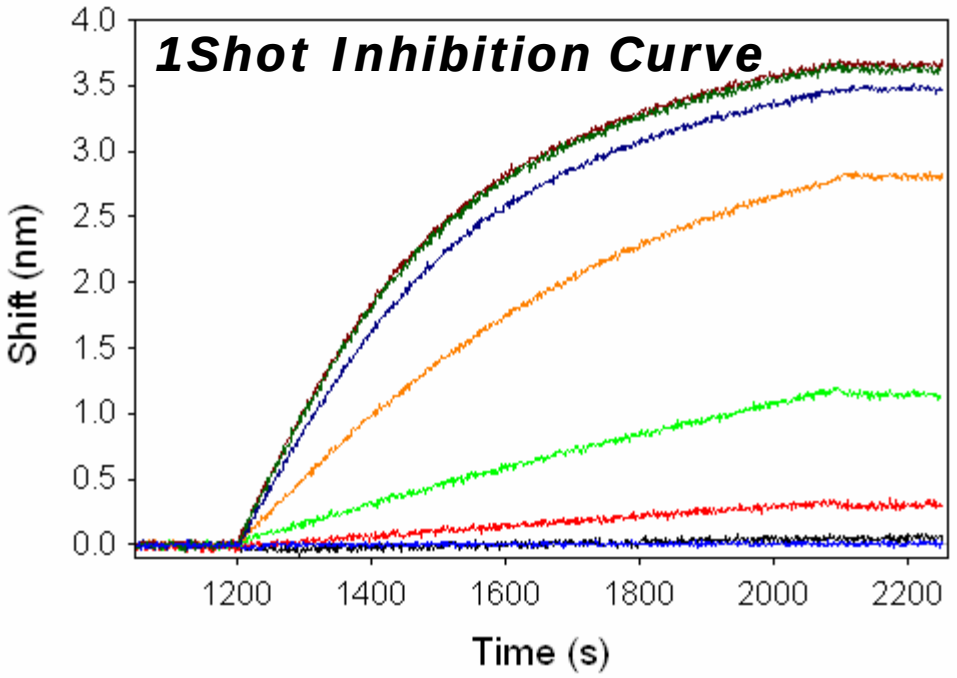


Probe "free A" using B on tip

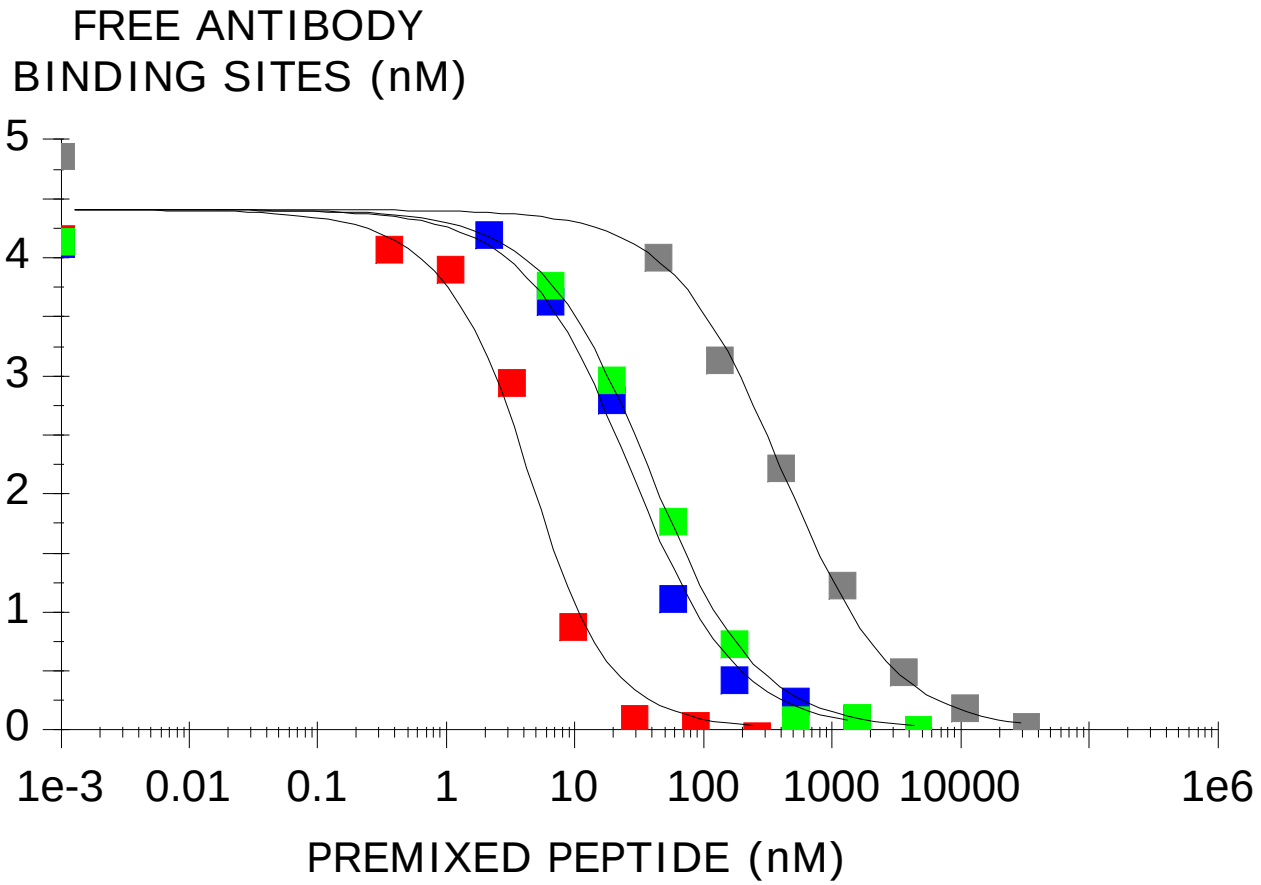
Titrate A alone

Titrate B into fixed A





Solution Affinities



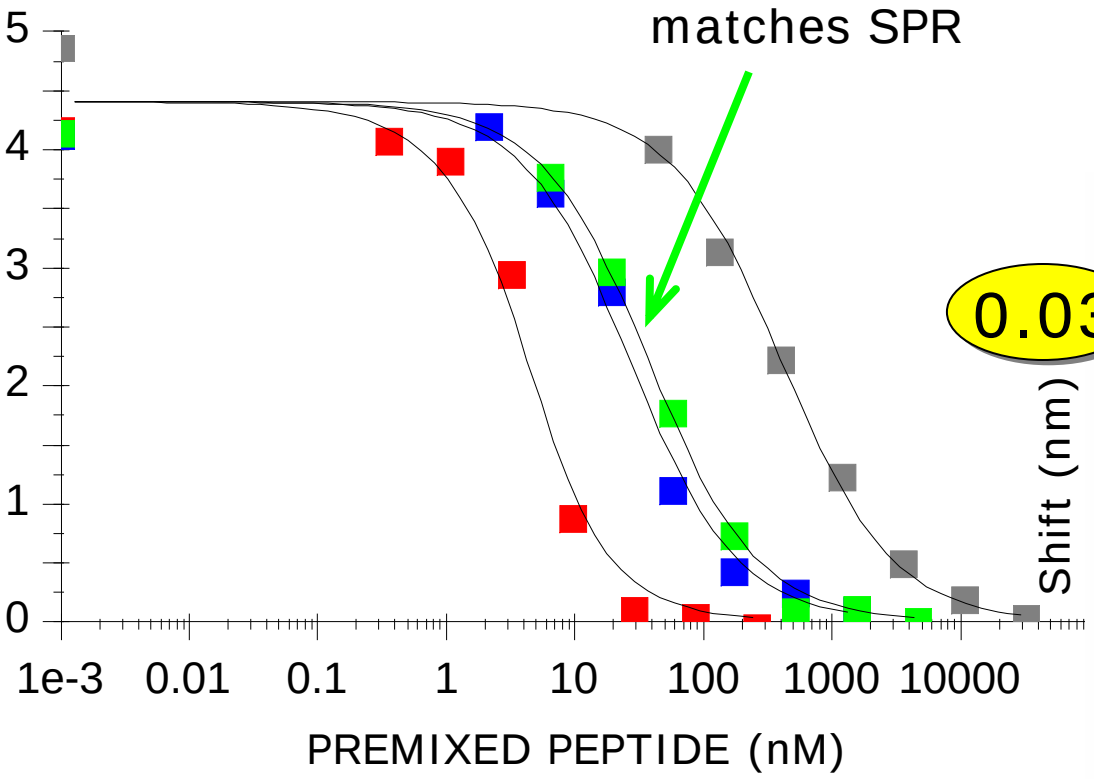
Abdiche *et al*, Anal. Biochem. 377 (2008) 209–217

Octet Red v. QK

Via solution comp. on Octet QK

$K_D = 40nM$

matches SPR

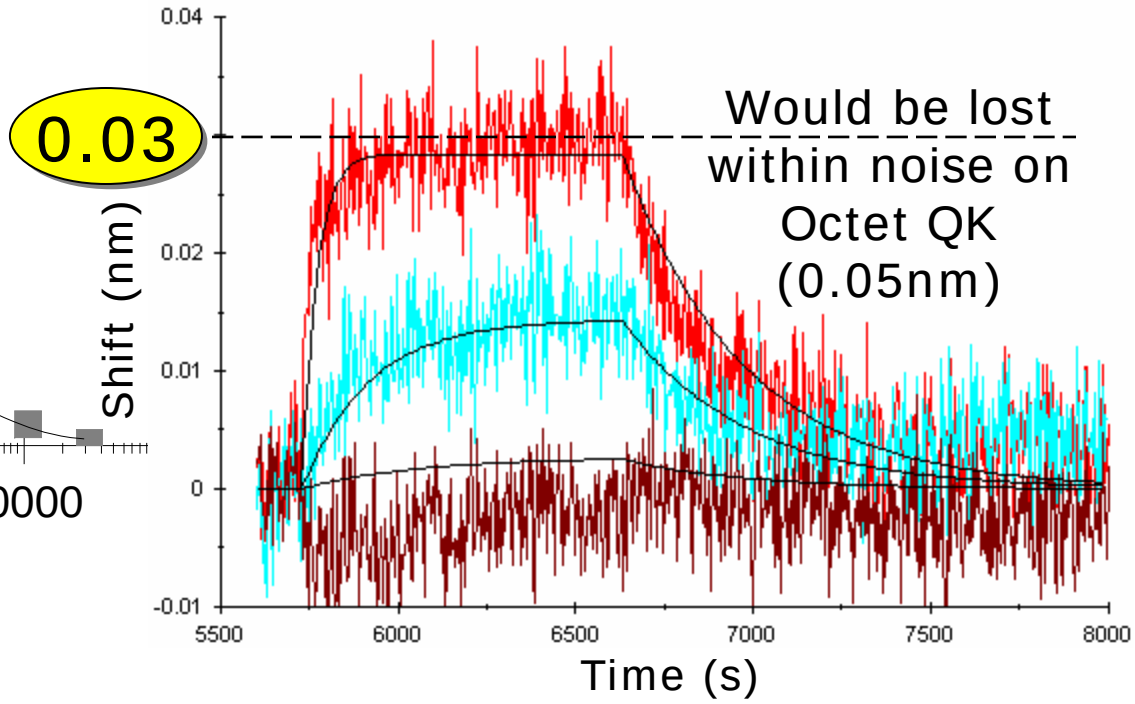


Abdiche *et al*, Anal. Biochem. 377 (2008) 209–217

Unpublished observation on **Octet Red**

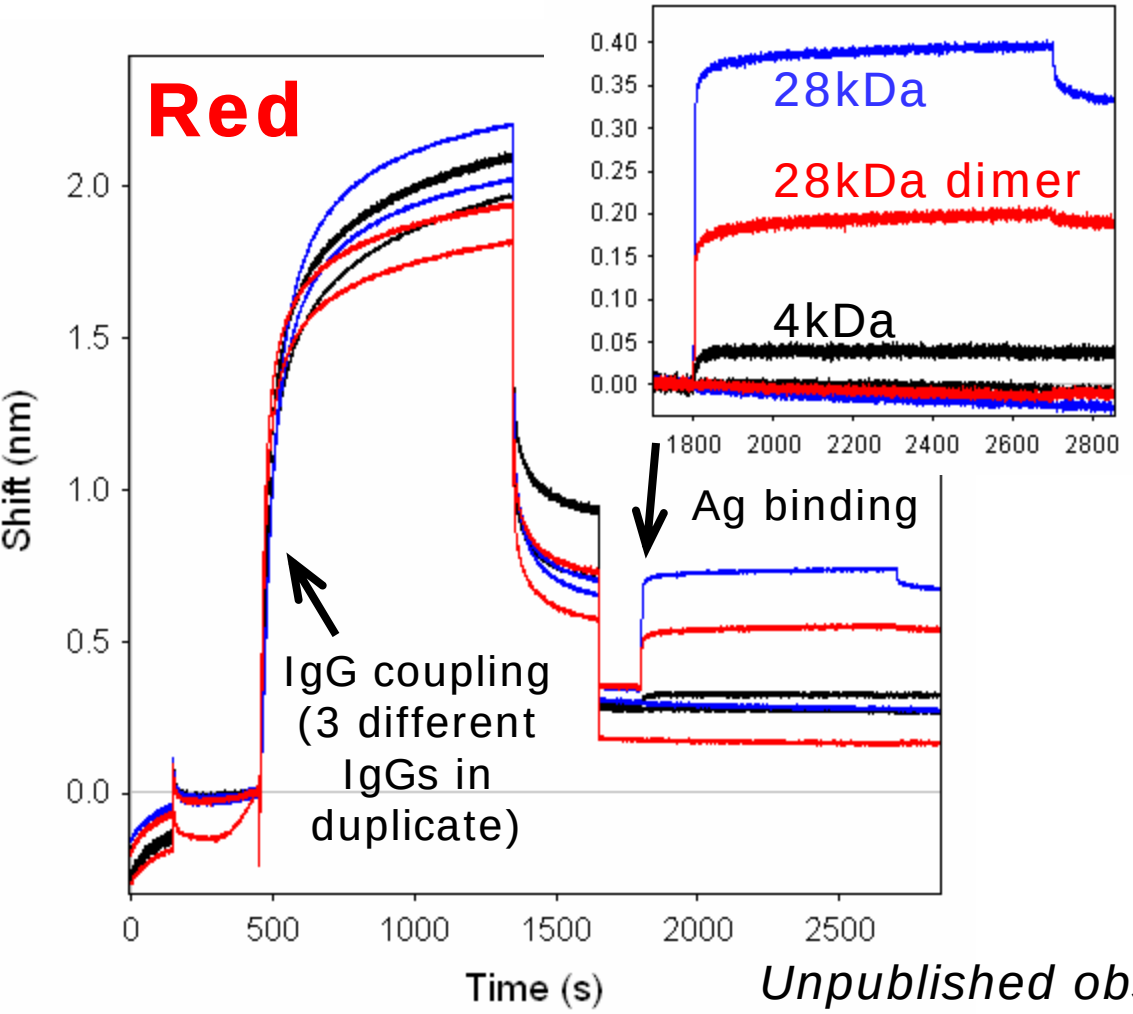
$K_D = 42nM$

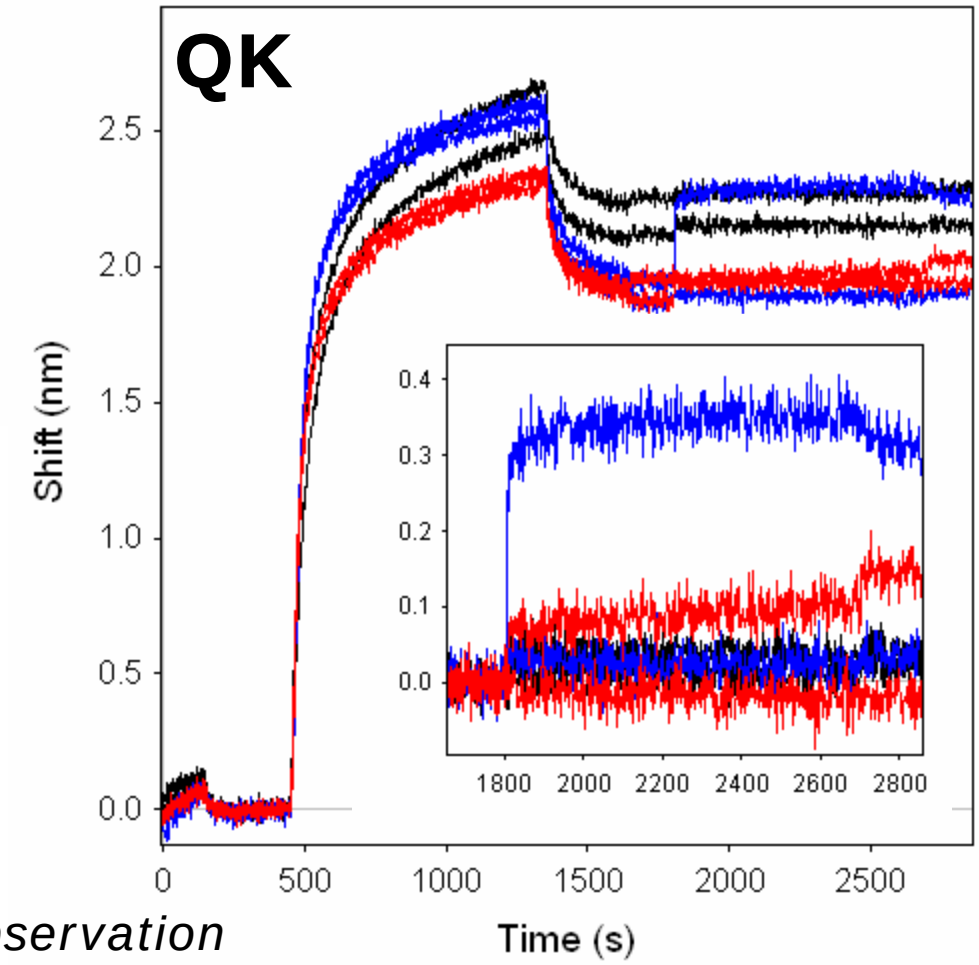
Direct binding of 1.3kDa peptide Ag to IgG captured via Anti-Murine tips



Octet Red v. QK

The 10fold smaller noise on the **Red** enables direct detection of molecules that were masked within the noise of the QK





Unpublished observation

Summary

1. The Octet is a reliable and versatile biosensor that is simple to use and is especially well-suited to blocking assays that are valuable to drug discovery
2. In tandem blocking assays are particularly appealing due to the Octet's ability to re-use samples and process 8 interactions at once
3. Epitope binnings from Octet matched those from SPR, so that the choice of assay orientation was driven by the specifics of the interaction system, not the biosensor used
4. Solution affinities from Octet matched those from SPR
5. The Red's smaller noise enables assays that were impossible on the QK

Thank You!

Dan Malashock, PhD
Alanna Pinkerton

Amisha Kamal Kizhakkedathu, PhD

Arvind Rajpal, PhD (Director, Protein Engineering)
Jaume Pons, PhD (VP and CSO, Rinat-Pfizer)