Antibody Analytics

Improving Biosensor Analysis: Finding the Common Ground

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Antibody Analytics

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INTRODUCTION

- Back to the future
- Assay setup
- GIGO!
- Putting it all together
- Antibody Analytics - Carterra

A little introduction

UNIVERSITY OF LEEDS

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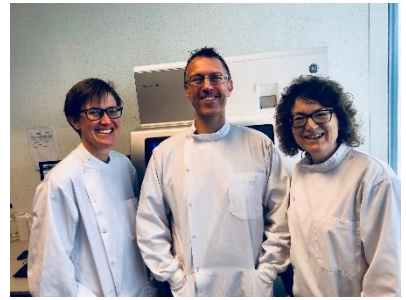
2006 2009 2011 2014 2016 2018



UC San Diego

bio outsource
is part of the
Sartorius Stedim Biotech Group

SPR throughout drug discovery

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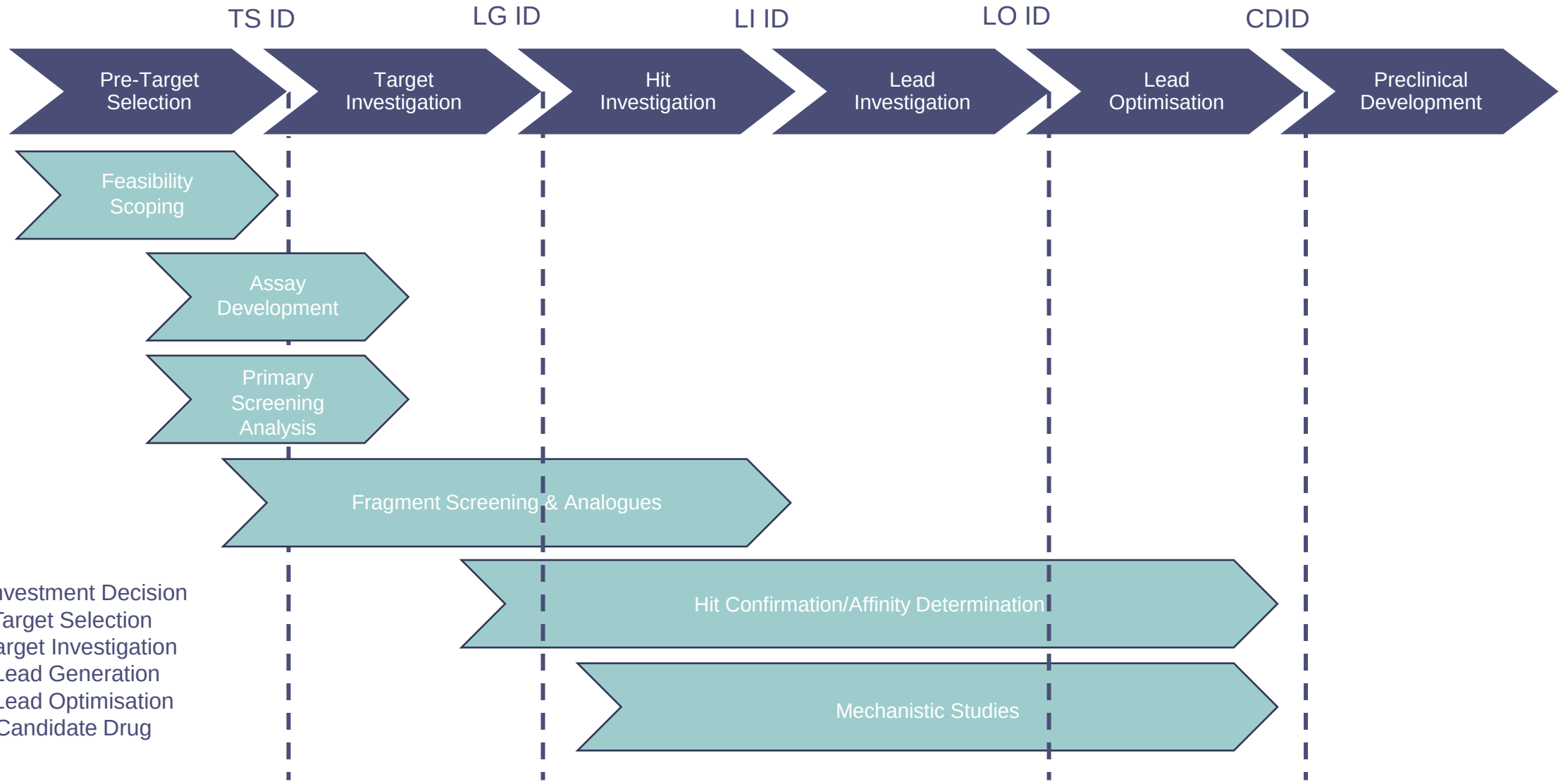

ID – Investment Decision
TS – Target Selection
TI – Target Investigation
LG – Lead Generation
LO – Lead Optimisation
CD – Candidate Drug

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Better, Faster, Stronger

Better

- Imagine your progress if you had characterization-like data earlier on

Faster

- Imagine your progress if you had increased amounts of data faster
- ~1,200 kinetic results in 24 hours

Stronger

- Imagine your progress if you had increased confidence in your data
- Increasing biological relevance

Potential savings of millions of dollars and years of research time

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Back to the future

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1 + 1 ≠ 2!

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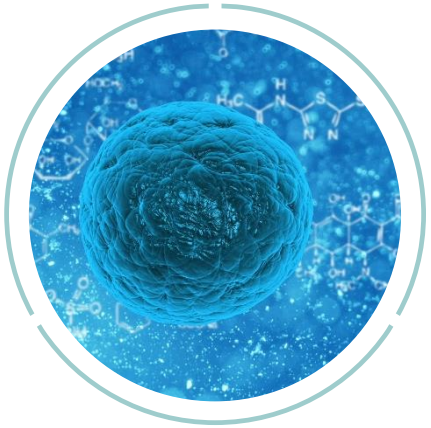
It’s not magic!



Better - assay setup

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Better – it's all about kinetics

- At Antibody Analytics the overriding message is that SPR is not an end point assay like an ELISA, it should be used to get you as much possible information about how the interaction between the analyte and ligand is occurring (ka, kd and KD)
- It's all about controlling the variables to ensure assay are accurate and precise

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Better – the sleeping pill analogy

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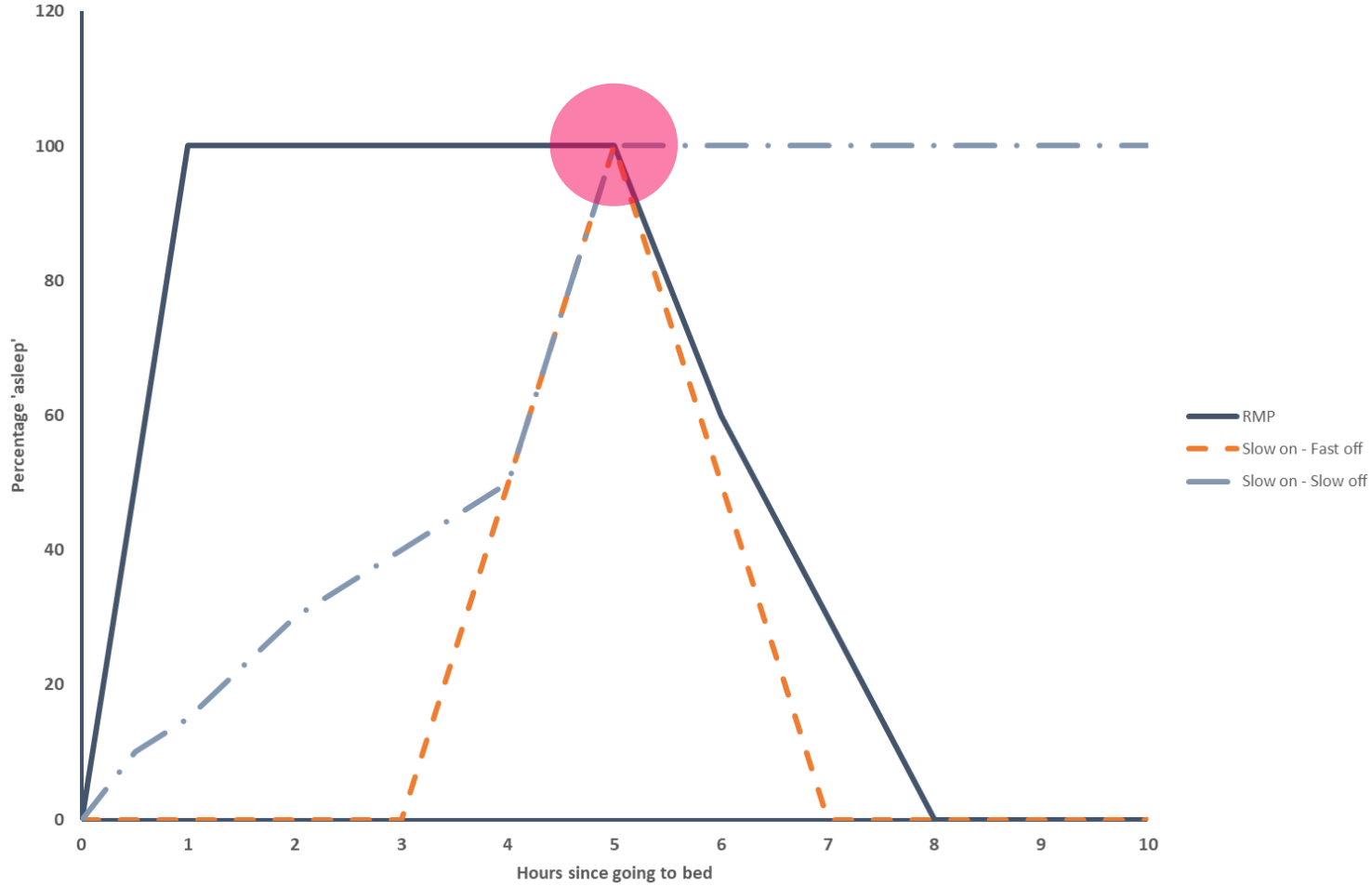
Better – the sleeping pill analogy



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20 years on

JOURNAL OF MOLECULAR RECOGNITION
J. Mol. Recognit. 1999;**12**:279–284

Improving biosensor analysis

David G. Myszka*

Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84132, USA

The quality of optical biosensor data must be improved in order to characterize the mechanism and rate constants associated with molecular interactions. Many of the artifacts associated with binding data can be minimized or eliminated by designing the experiment properly, collecting data under optimum conditions and processing the data with reference surfaces. It is possible to globally fit high-quality biosensor data with simple bimolecular reaction models, which validates the technology as a biophysical tool for interaction analysis.

Keywords: **BIACORE, surface plasmon resonance; kinetics; protein–protein interactions; biosensor**

Received 2 July 1999; accepted 5 July 1999

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It’s not magic!

KISS – **K**eep **I**t **S**imple **S**tupid

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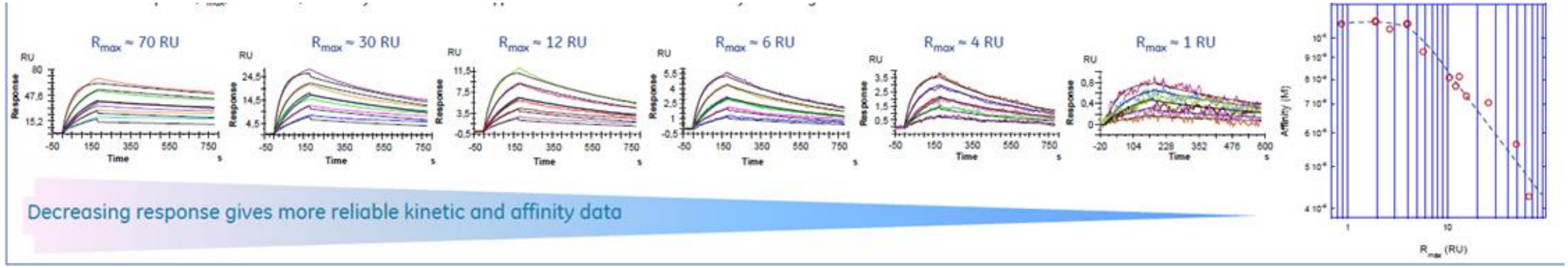
KISS - GIGO!

- *“the inability to fit data to a simple model is often a result of how the experiments are run and not a flaw in the technology”*
- What question are you trying to answer?
  - Start with the end in mind
    - Do you want to know the kinetics of a single interaction or against a large panel of antibodies?
    - Do you just want a yes / no screen of a panel of antibodies or targets?
    - Do you want to know the affinity of an interaction but don’t mind if you don’t know the kinetics?
    - Do you want to toggle-switch epitope select antibodies?… the list is endless!

KISS – Rmax

- 20 years ago Rmax was well defined at levels of <10 RU but still to this day people run assays where the observed responses are in the hundreds, maybe thousands
- This is not only unnecessary but also detrimental to measuring an accurate affinity



KISS – Rmax

- In general when measuring antibodies, their bivalent nature can give rise to avidity effects and kinetic analysis can become challenging
- By decreasing the response levels, these avidity effects start to disappear and a 1:1 binding model dominates
- There is a lower end to this where signals become close to instrument noise and therefore, it's important to find the 'Goldilocks zone' for your assay (and machine)

The Goldilocks Zone

KISS – Baseline drift

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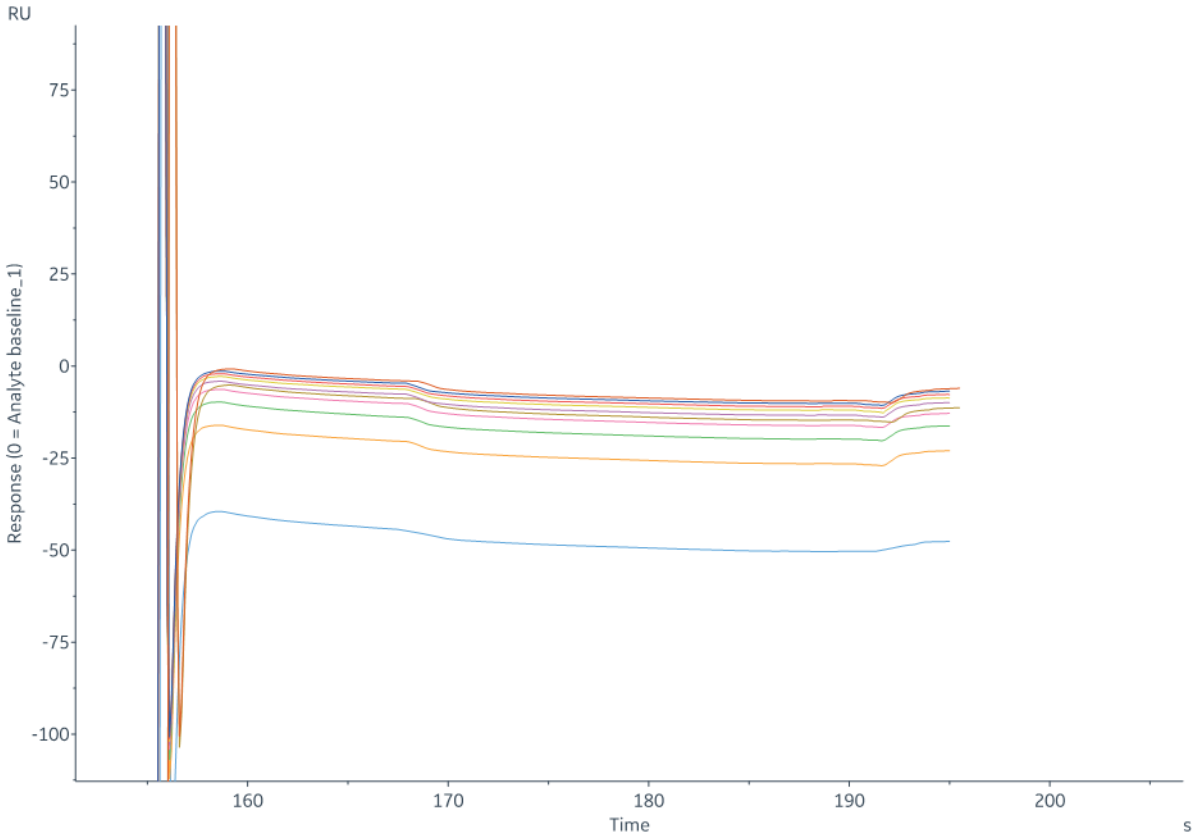
- Keep your ins ’s recommenda
- Hydrate, hydr



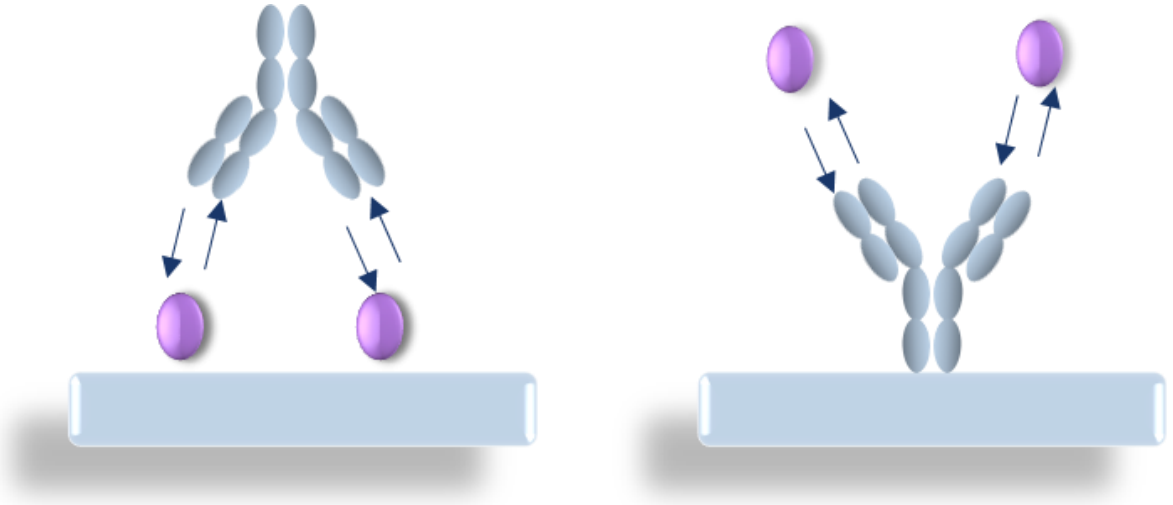
KISS – None Specific Binding

- *Biological macromolecules often show a proclivity to interact with surfaces.*

- Assess the non-specific binding of your analyte to the sensor chip surface prior to performing the assay
- Inject the highest concentration to be assessed across a non-derivatised surface
- Assess the sensorgram, square or tailing?
- Choose assay orientation and buffer based on results

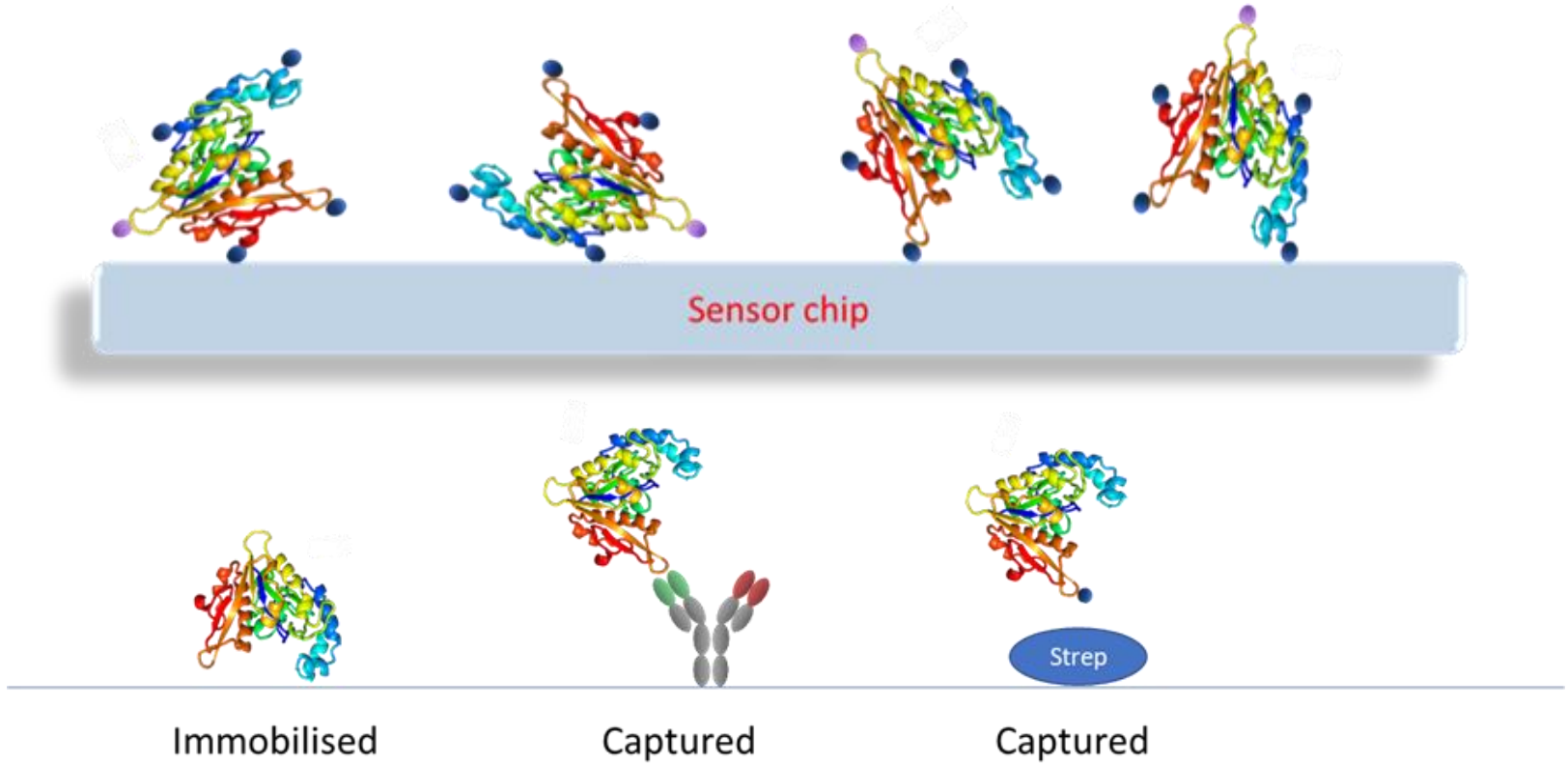
KISS – Avoid Avidity Effects

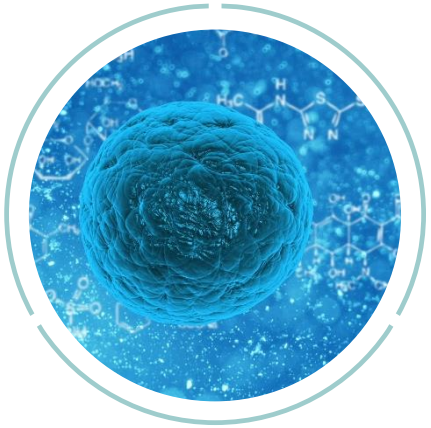
- *"the goal of most SPR assays is to describe the data using the simplest model possible"*
- It is important to minimise the potential avidity effects of multi-valent molecules



KISS – Orientated capture and loading

- Assay orientation allows you to control multiple parameters both intra- and inter-assay





Putting it all together

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The Question

- The Customer is in the process of developing a novel anti-XYZ FAb fragment and have, through affinity maturation and “hot spot removal”, generated 8 different anti- XYZ binders with identical epitopes and nearly identical Complementarity-determining regions (CDRs). In addition, one XYZ binder with different CDRs may be assessed.
- The customer has requested an assay package using SPR to determine the ability of Antibody Analytics to determine the absolute affinities of the FAb fragments and relative active concentrations of stressed samples to prove whether “hot spot removal” has been successful.

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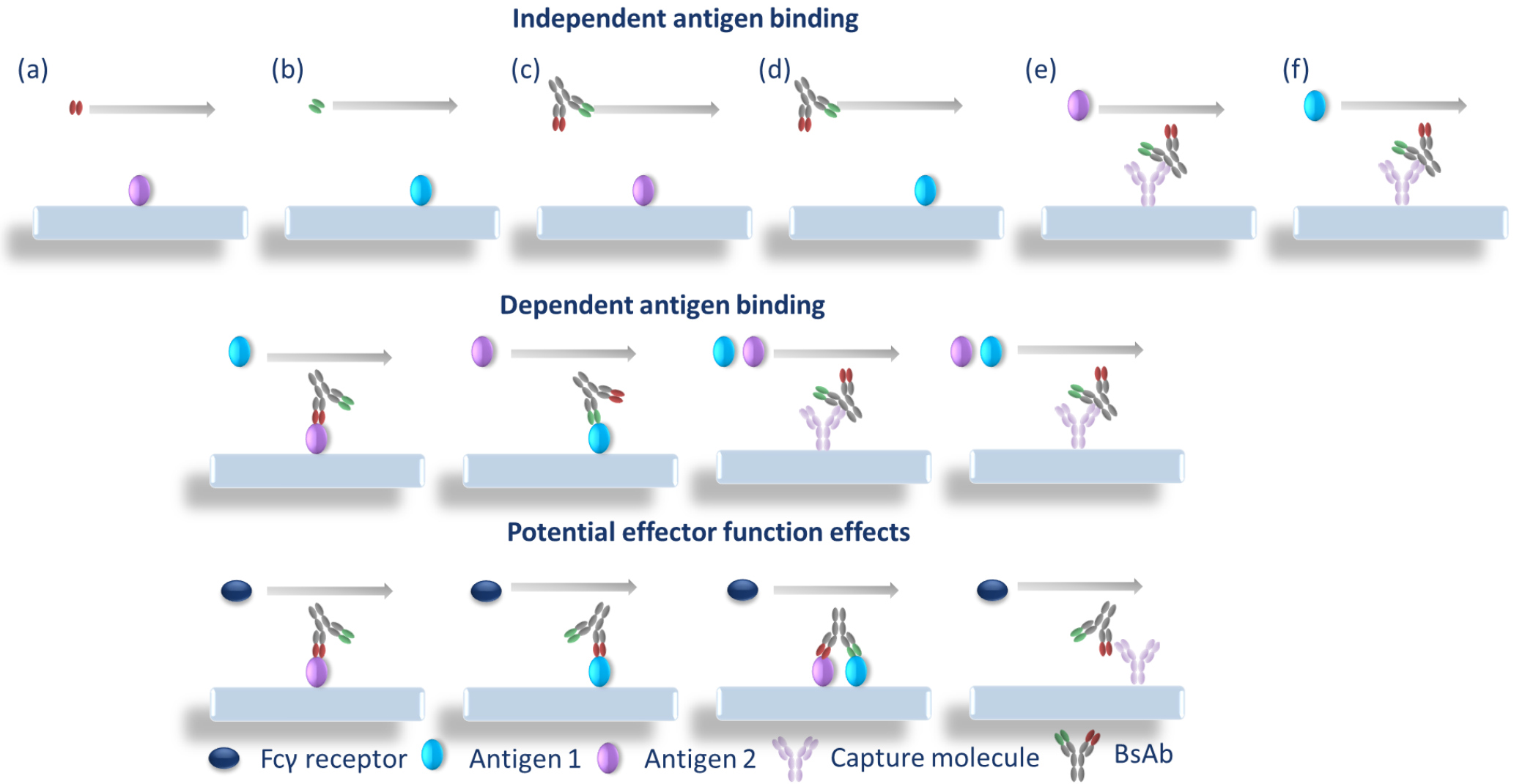
The Rmax

- $R_{ligand} = \frac{R_{max} * Mr_{ligand}}{Mr_{analyte} * Valency_{ligand}}$

- Rligand = (50*16,000) / (50,000 * 1) = 16 RU

The assay setup



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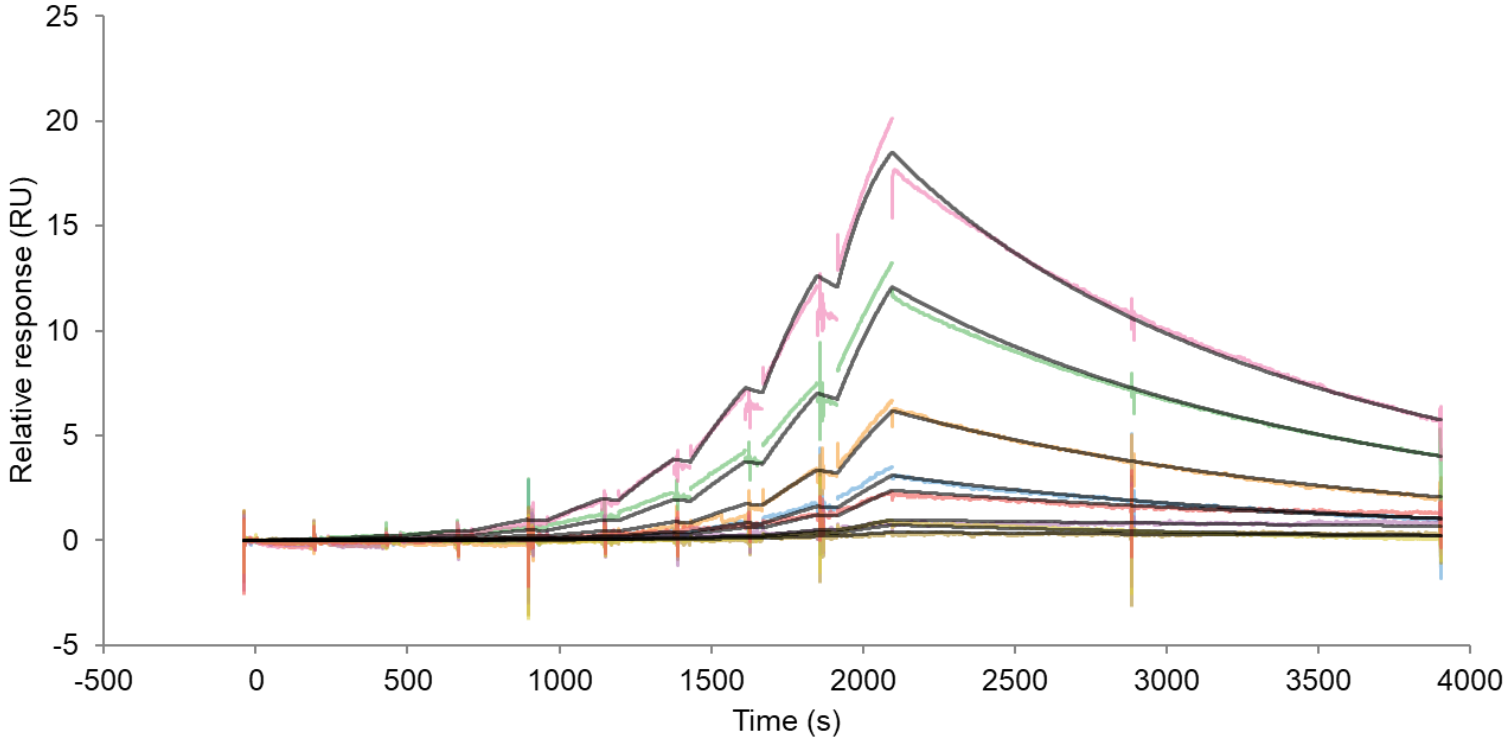
2D kinetics – analyte concentration

- Assess multiple concentration series to find the Goldilocks zone and/or reassess your setup



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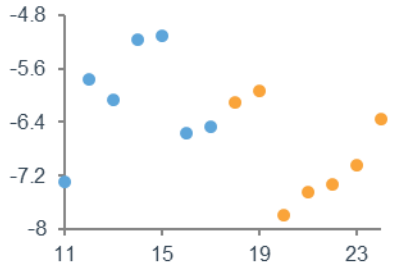
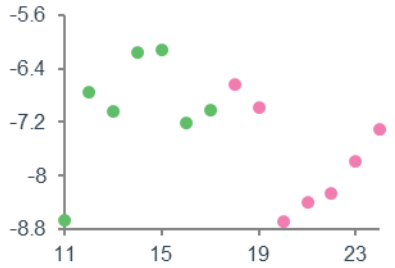
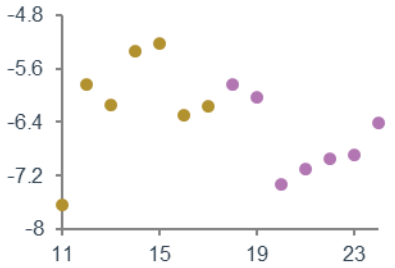
NSB test



Ch 1



Ch 2



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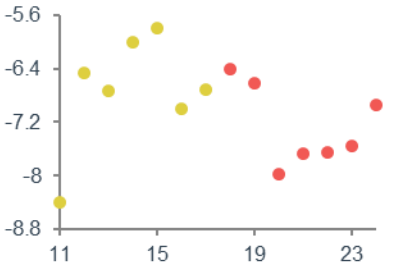

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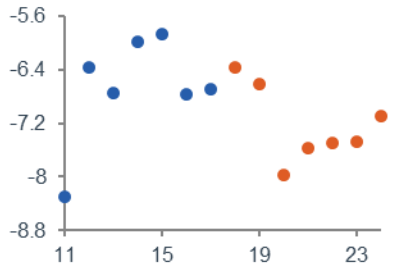

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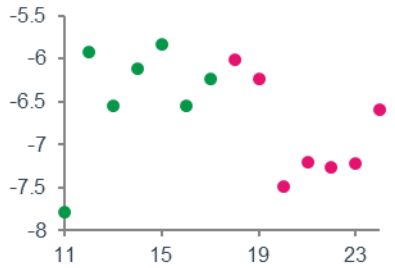

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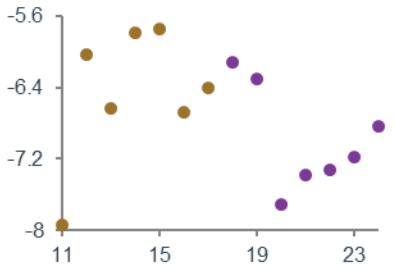

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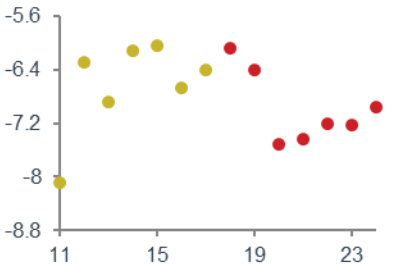

Ch 8

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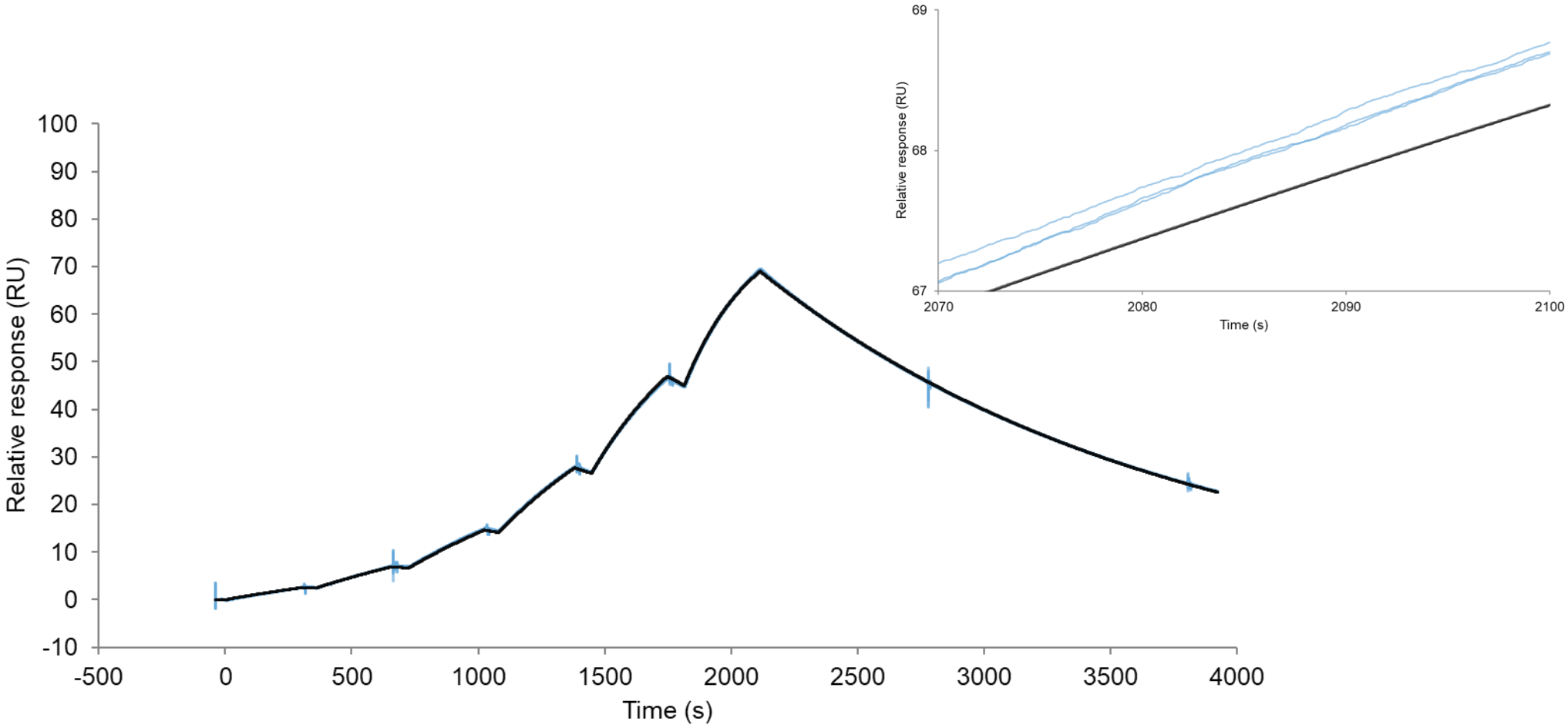
All together



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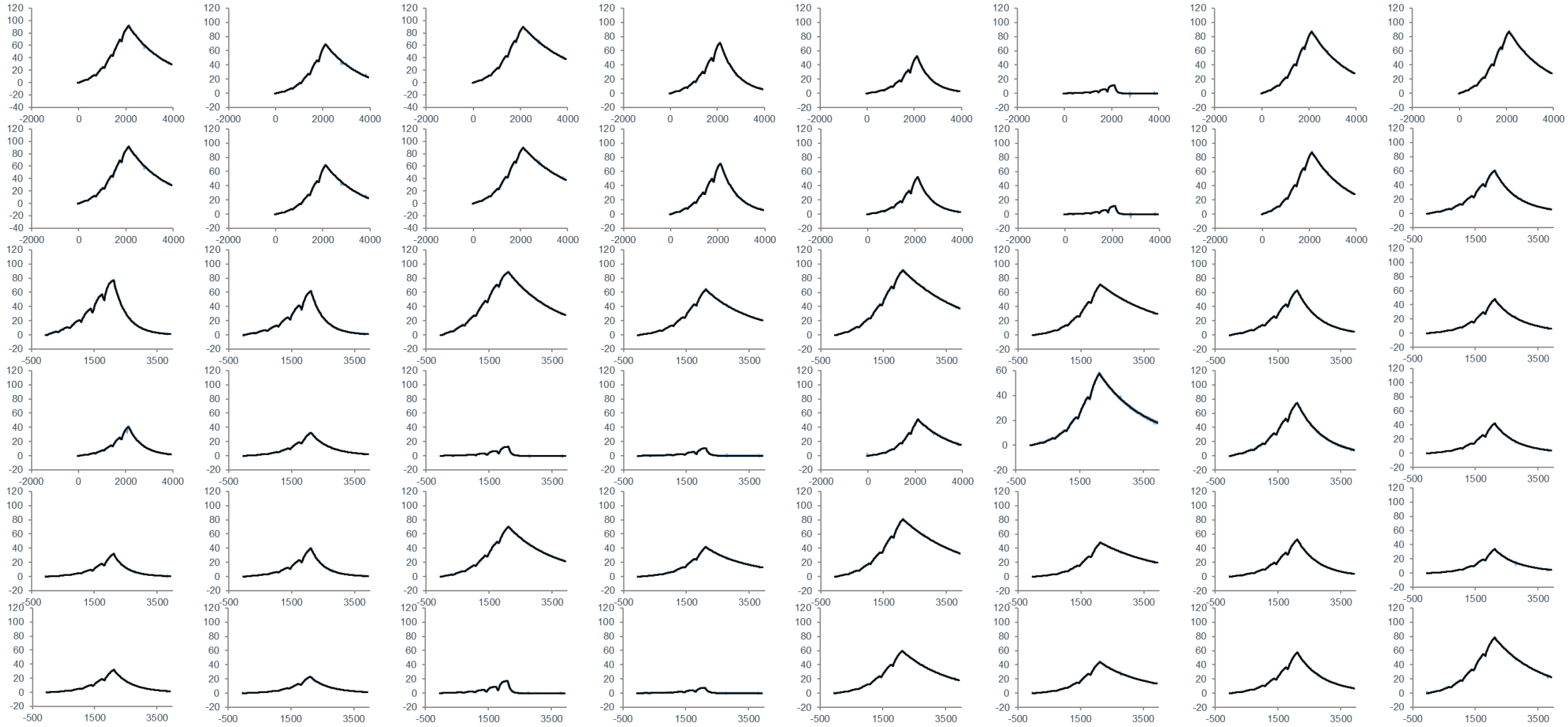
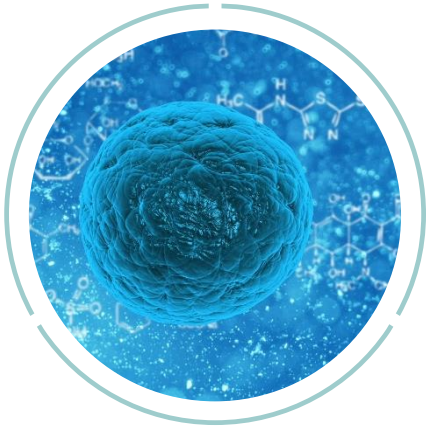
High Throughput



SAM43-31-0



Antibody Analytics - Carterra

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The Question

- The customer was interested to see whether the Carterra LSA machine could produce similar data than my Biacore 8K
- Yas and I decided to do a little test!
- I sent her the samples, some AviTag XYZ and the KD of the reference standard, told her the general setup (assay orientation) but nothing else, the rest was up to her and the LSA

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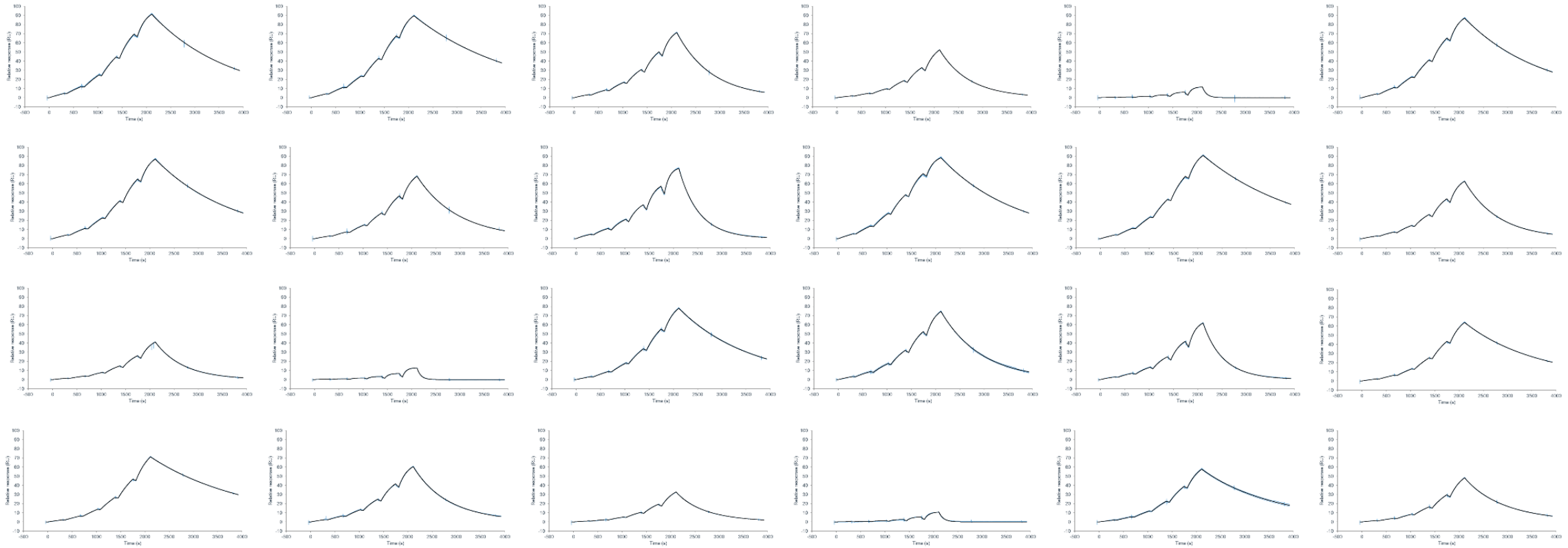
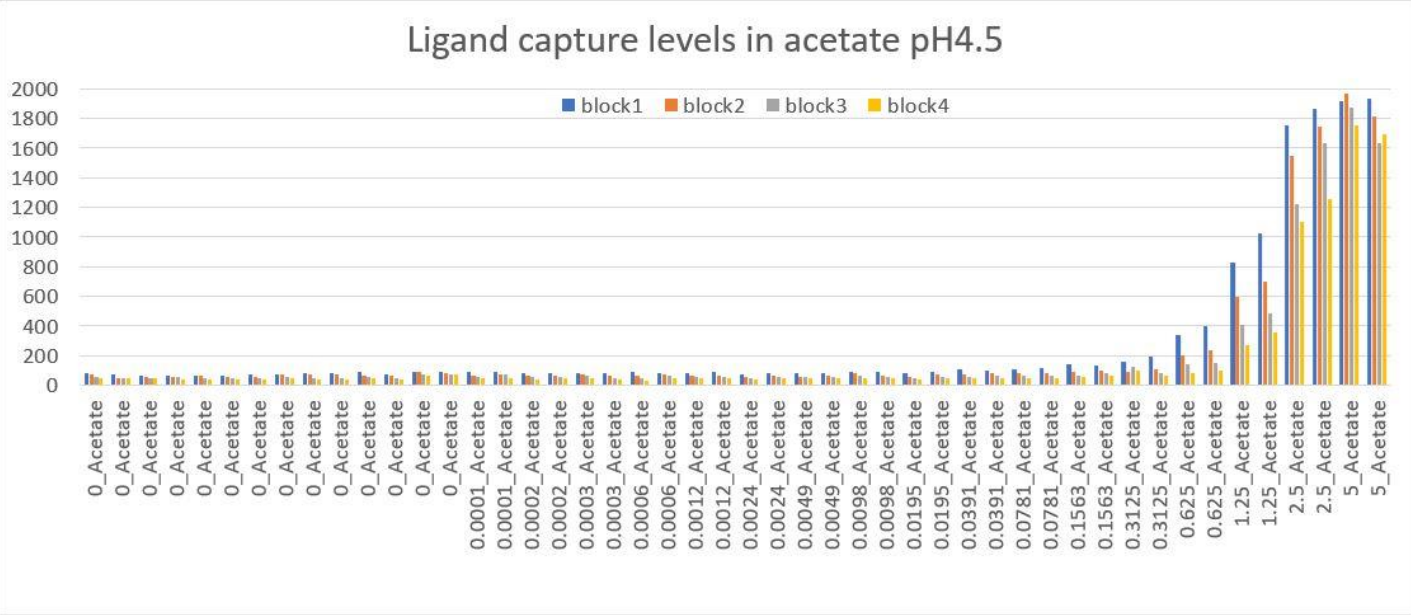
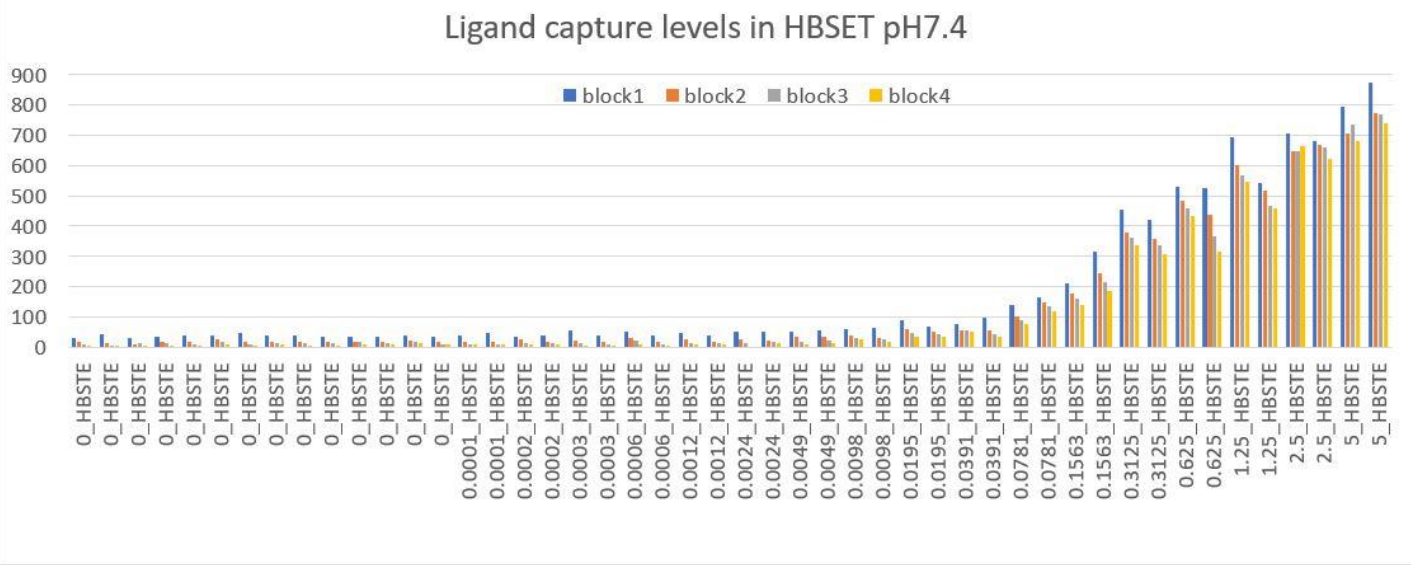
The samples



Surface Prep Array – capture biotin-XYZ in the 96PH





- Prepare biotin-XYZ as 17membered 2fold series (5µg/ml – 76pg/ml) in either pH7.4 (HBSET) or pH4.5 (10 mM sodium acetate), to see how pH affects their preconcentration and capture efficiency
- Dispense each set of samples (pH7.4 and pH4.5) into duplicate wells of a 96plate and fill remaining wells with respective buffer (blanks)
- Capture the 96well plate of samples in parallel using the 96-channel printhead (96PH) onto print blocks 1, 2, 3, and 4 in series by serially docking/undocking the 96PH (re-using the same samples, returned to plate after each draw).
- Final 384-array contains (68x4) 272-ligand coated spots and (28x4) 112 blank spots, with each ligand concentration represented 16x within the array (8x in HBSET and 8x in acetate)
- Reconstituting the biotin-XYZ in pH4.5 yielded 2x higher capture levels for samples >1ug/ml, but levels dropped off faster than pH7.4, possibly due to solubility issues

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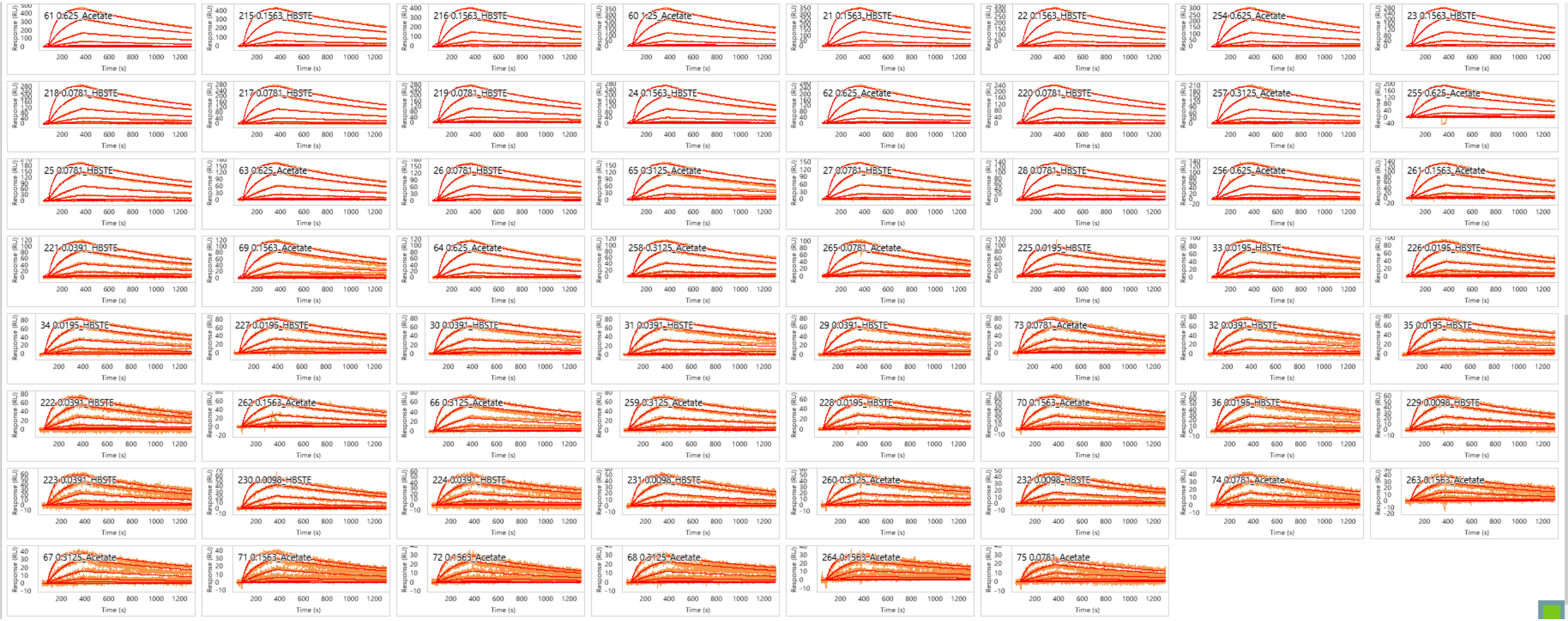
Fab#2 – 126 spots of data ranked by R_{max} (high to low)

490 Rmax



26 Rmax

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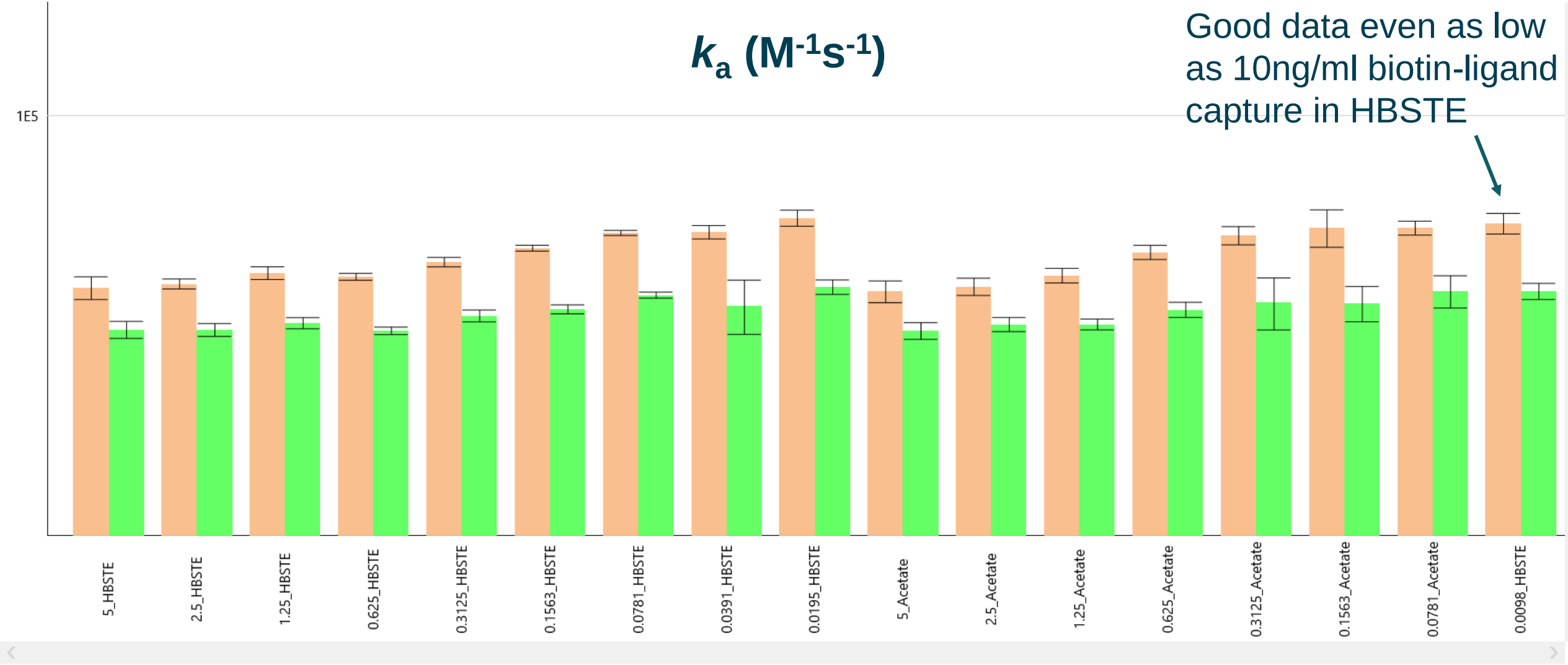
Onrate Stats - surfaces grouped by capture conc (µg/ml) and buffer



Mean +/- StDev or 4-8 reps (spots) per capture condition

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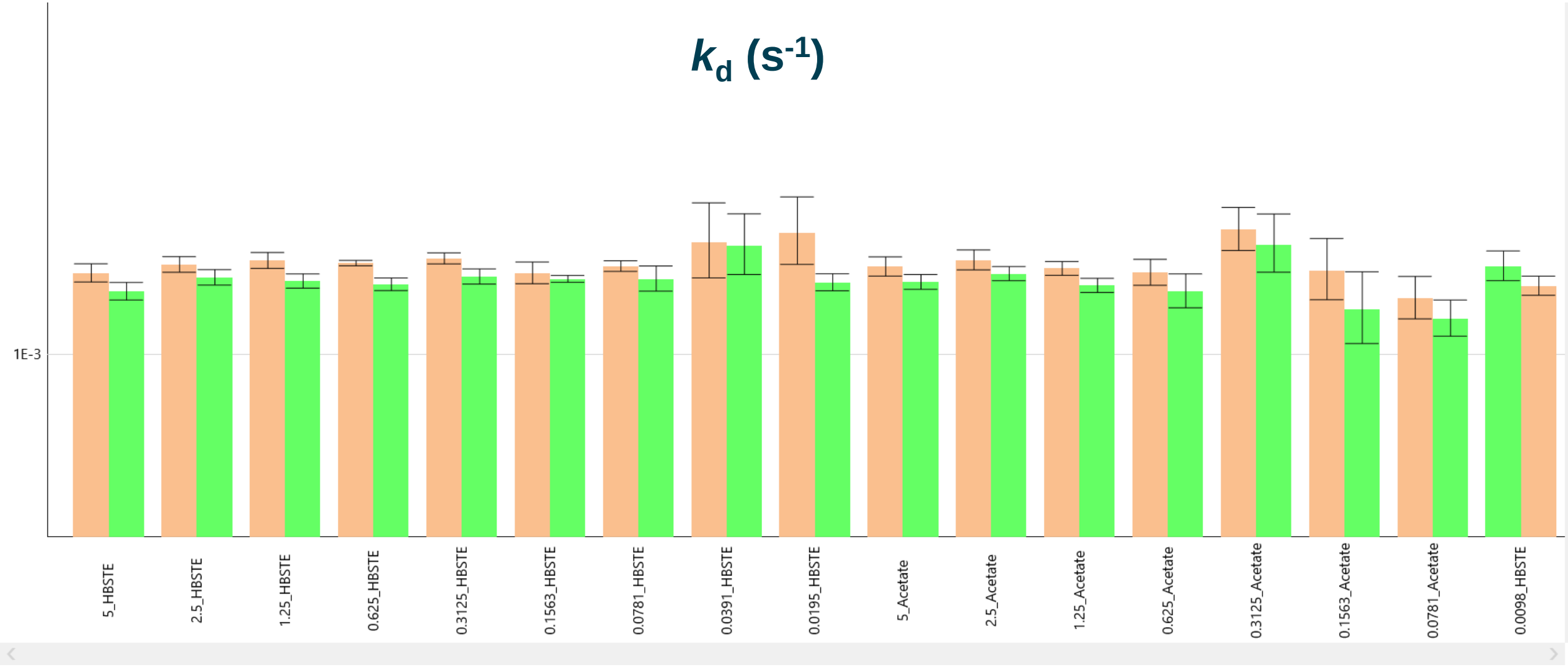
Offrate Stats - surfaces grouped by capture conc (µg/ml) and buffer



Mean +/- StDev or 4-8 reps (spots) per capture condition

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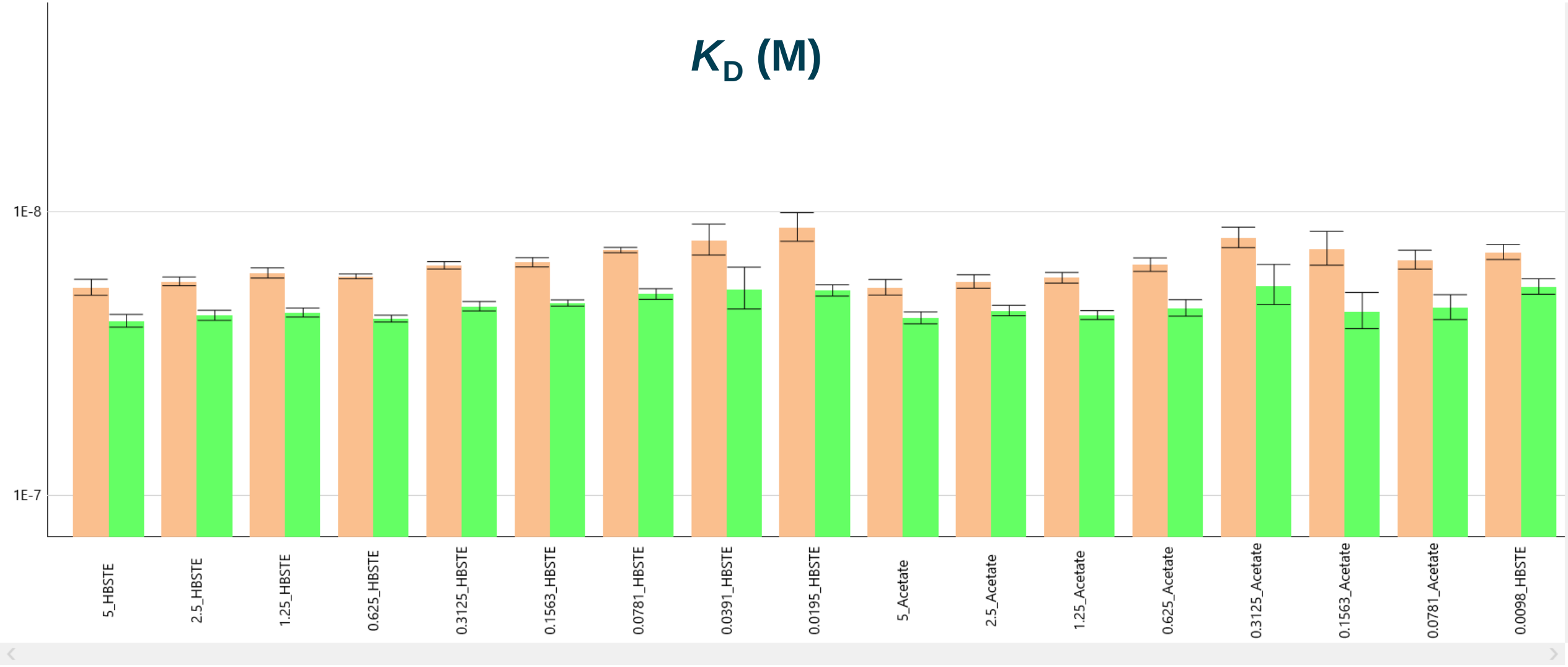
Affinity Stats - surfaces grouped by capture conc (µg/ml) and buffer



Mean +/- StDev or 4-8 reps (spots) per capture condition

carterra

Rank	Antibody Analytic (sample number)	Carterra (Sample number)	Antibody Analytics (KD, nM)	Carterra (KD, nM)	Difference in KD
1	10	10	10.4	8	N/A
2	3	11	10.5	12	0.9
3	11	3	11.1	12	1.5
4	2	7	12.6	13	-0.7
5	7	2	13.7	13	0.4
6					-4.3
7					-2
8					N/A
9					N/A
10					N/A
11	8	4	30.9	29	-2.9
12	4	8	31.9	30	-0.9
13	20	12	32.7	34	-0.4
14	9	20	33.3	34	1.3
15	12	24	34.4	40	-6.7
16	24	21	46.7	52	-22.2
17	17	9	50.6	52	18.7
18	13	17	58.2	55	4.4
19	5	5	58.9	55	N/A
20	21	13	74.2	87	28.8
21	14	6	1160	335	-1140.8
22	6	14	1475.8	383	-777
23	22	22	4330	453	N/A

Average difference between Top 10 is -0.7 nM

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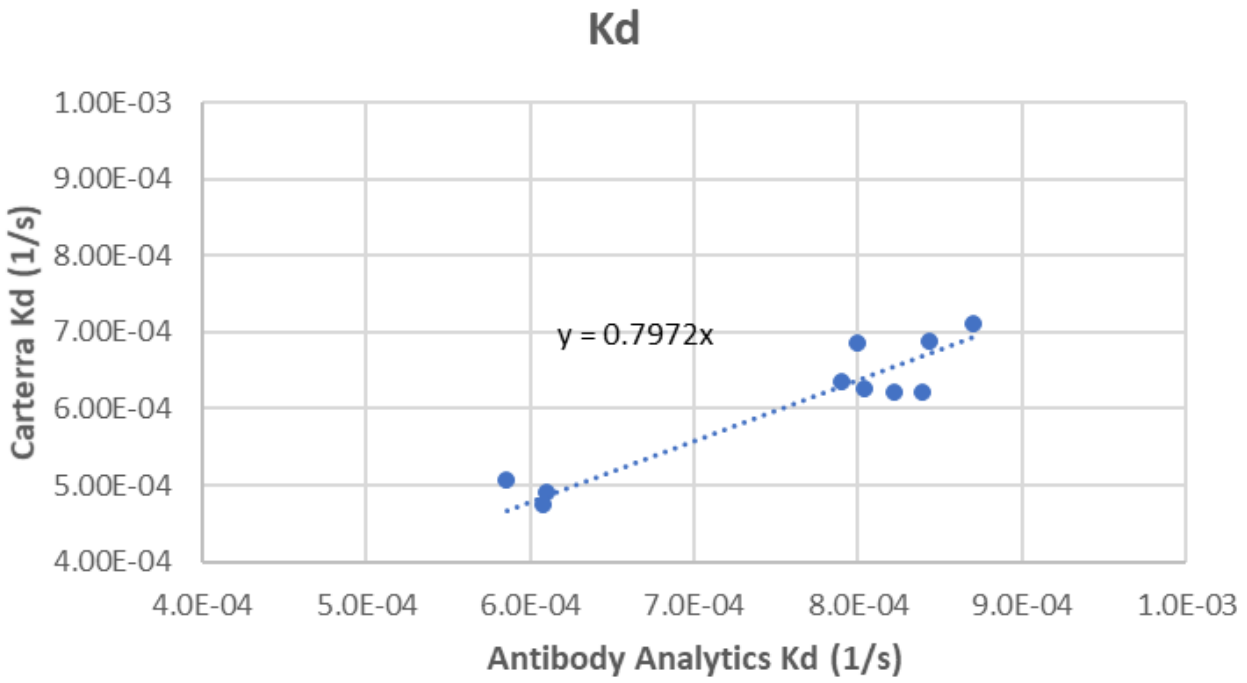
How close?



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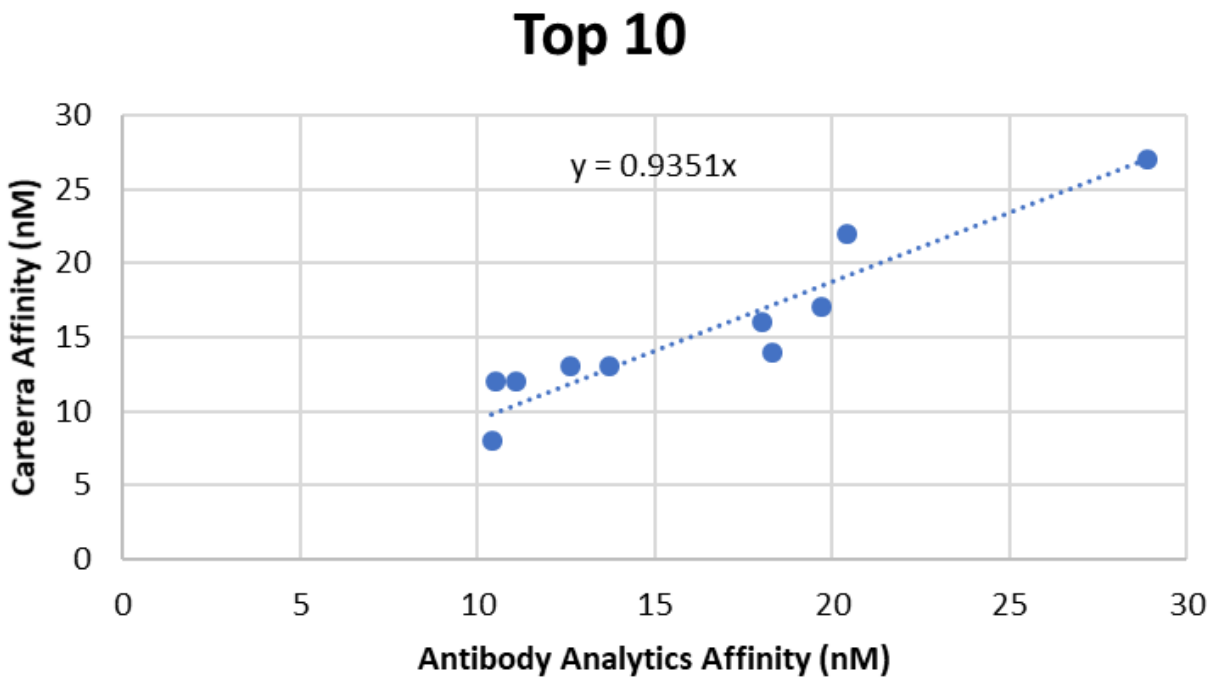
How close?



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Summary

- Following the basics that were laid out over 20 years ago for SPR can still yield excellent data
- KISS – you can always make it more complicated
- In a blind study, Yas and I generated near identical data using different machines but a similar approach

Potential savings of millions of dollars and years of research time

Acknowledgments

Antibody Analytics


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Jennifer Clark


Gillian Goodwin

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