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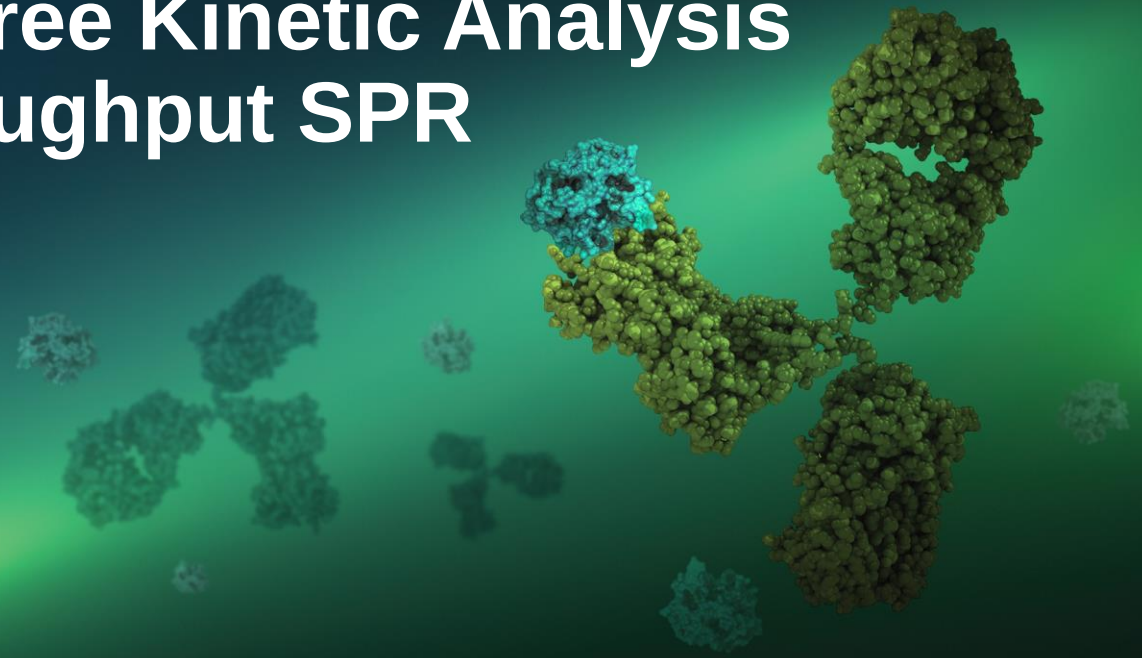
Strategies and Approaches for Detailed Label Free Kinetic Analysis using High Throughput SPR



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San Diego Seminar September 2019

High Throughput Kinetics

- **Detailed kinetic profiling has typically been relegated to a down stream point of characterization in drug discovery**
  - The time of analysis and sample usage is commonly limiting
  - Kinetic screening is simplified to expedite the process and reduce sample consumption
  - 1 or 2 antigen concentrations leading to inaccurate determination of R_{max}, k_a, and K_D

- **High throughput kinetics on the LSA is massively parallel**
- **Solves both time and reagent consumption issues**

Assay Considerations for High Throughput Kinetics

- **Preparation of the capture surface**
- **Antigen concentration series**
- **Power the analysis - “n”**
- **Data Analysis**
- **Data presentation**

It is important to note that while the LSA brings a new scale to kinetic analysis of mAbs, nothing has changed in 20 years regarding how to properly design an SPR experiment.

Improving biosensor analysis.
Myszka DG. J Mol Recognit. 1999 Sep-Oct;12(5):279-84. PMID:10556875

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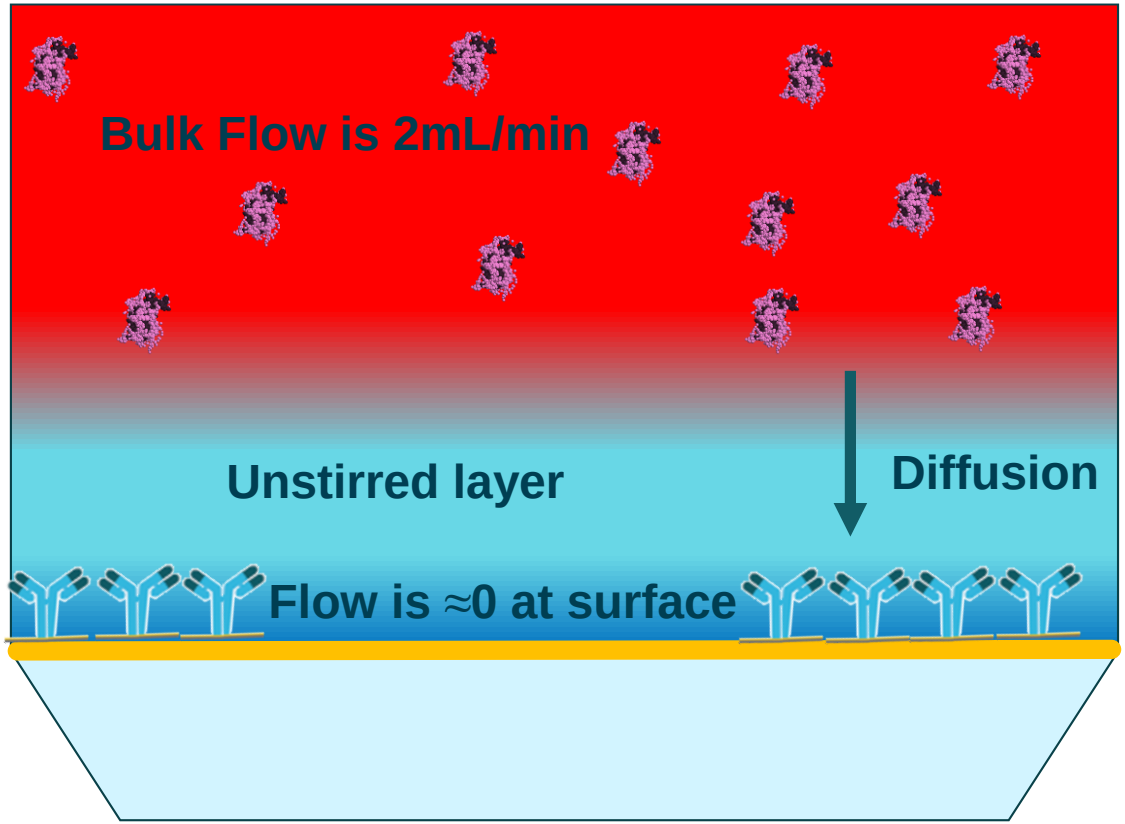
Considerations When Designing Your Assay

- **Does your ligand have a high affinity capture option?**
  - IgG or Fc construct, Fab, V5 tagged protein, other?
  - Will the capture surface reliably regenerate?
- **Do you have a monovalent antigen construct?**
- **Is the antigen prep sufficiently soluble?**
- **What is the molecular weight?**
- **What starting material for ligands?**
  - Purified mAbs, Hybridoma supernatants, transient transfection supernatants, B-cell supernatants, yeast supernatants, bacterial periplasmic extracts?

Chip Surface Considerations

- **Linear polycarboxylate (HC) surfaces have better transport dynamics than 3D carboxymethyldextran (CMD) hydrogel surfaces**
- **HC30M is the go-to for IgGs against protein antigens**
  - Excellent transport dynamics, relatively high capacity
- **Lower concentrations ligand sources or panels targeting very small antigens may benefit from the HC200M**
  - roughly 50% higher binding capacity
- **CMDP (planar CMD) is the most planar surface**
  - Can reduce avidity at low ligand densities
  - roughly 1/3 total capacity of HC30M

Ligand Density and Transport Limitations



- **ALL flow cells contain an unstirred layer through which analytes must diffuse to reach the chip surface.**
- **Low ligand densities reduce mass transport effects.**

Fick's Law:

$$J = -D \frac{d[A]}{dx}$$

J = analyte flux
D = diffusion coefficient
X = diffusion distance

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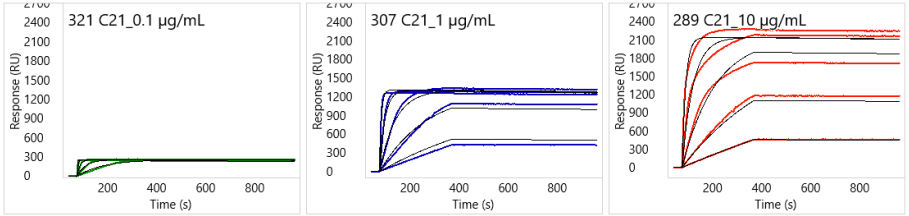
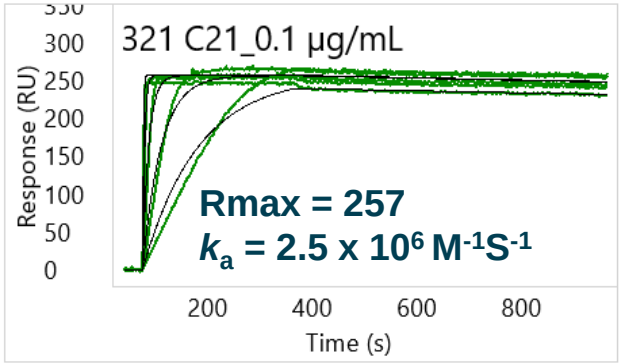
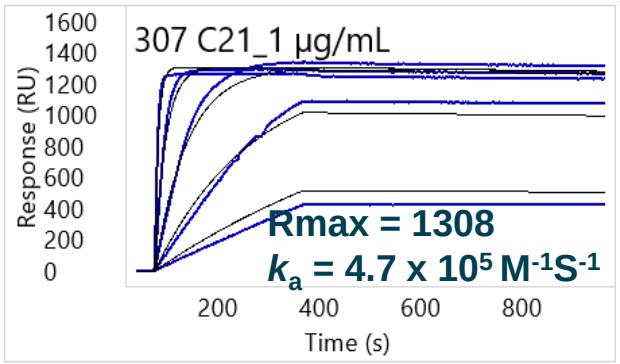
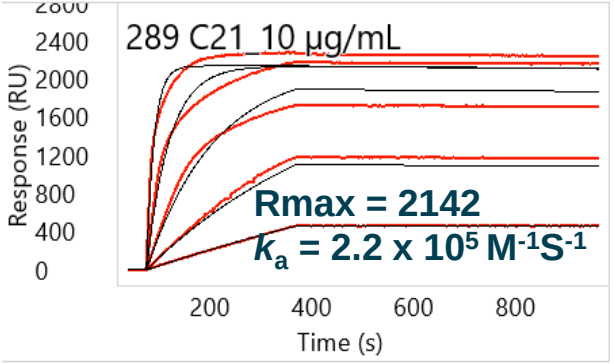
Surface Density and Transport Limitations









- **Fast on-rate clones show clear density dependent transport limitations**

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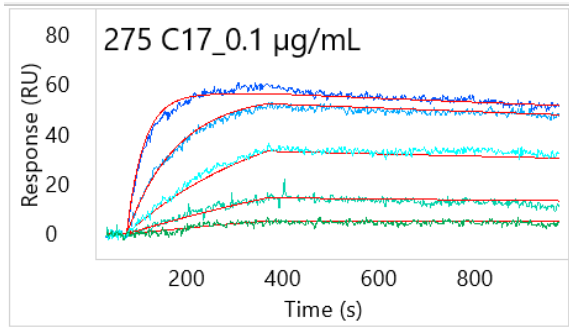
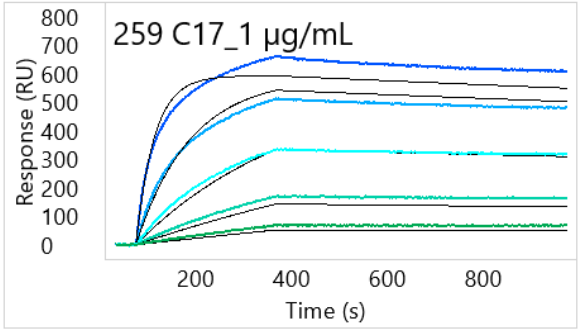
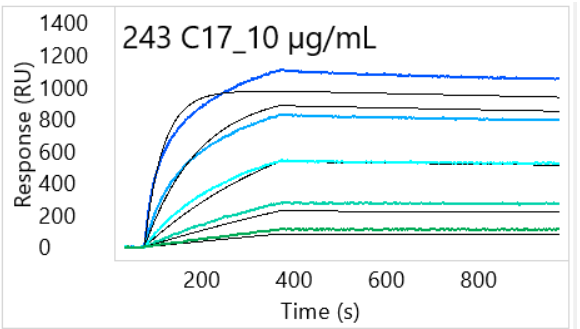
Transport limitations manifest differently in clones with slower on-rates







Rmax = 57
k_a = 9.3 x 10^4 $M^{-1}S^{-1}$

Rmax = 597
k_a = 8.4 x 10^4 $M^{-1}S^{-1}$

Rmax = 1000
k_a = 8.2 x 10^5 $M^{-1}S^{-1}$

- **In this clone with an order of magnitude slower on-rate, kinetic estimates show only a modest trend with density.**
  - The transport limitation does affect the goodness of fit

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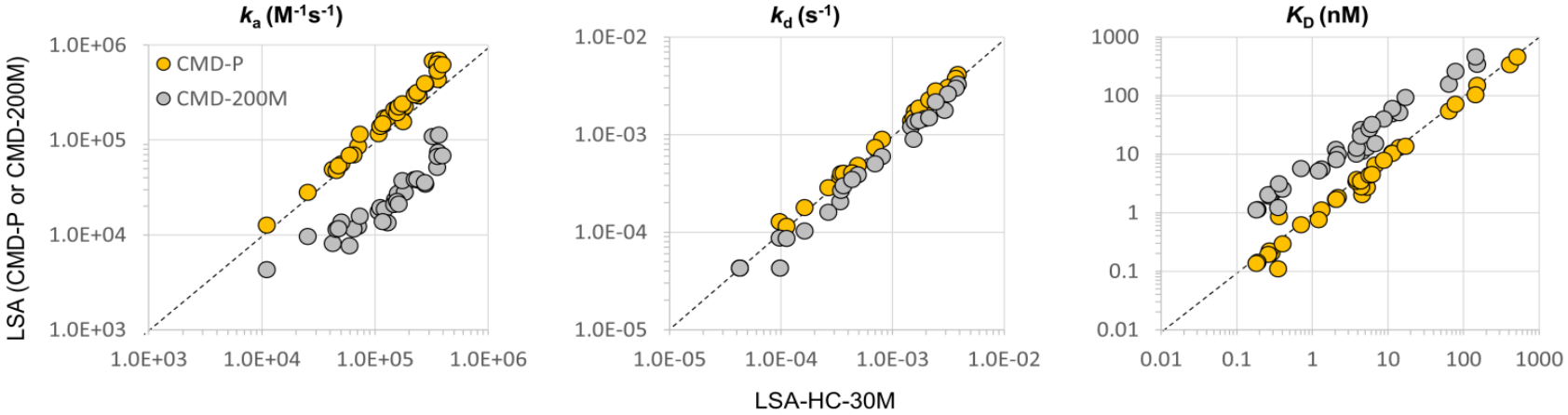
Surface matrix and transport limitation

- **3D hydrogels (CMD) act as a diffusion barrier**
  - The apparent on-rate is reduced several fold compared to the more planar chip types
- **Note that this effect is common to all flow cell based biosensors and has been previously reported:**
  - Expanding the ProteOn XPR36 biosensor into a 36-ligand array expedites protein interaction analysis. *Anal Biochem*. 2011 Abdiche YN, Lindquist KC, Pinkerton A, Pons J, Rajpal A. PMID: 21168382



Above data Submitted to PLOS ONE

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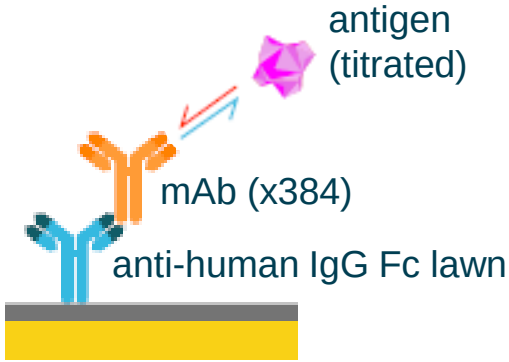
Capture Kinetics Assay Example

- **Screen 384 mAbs in parallel for binding to specific Ag**
  - Unattended analysis of up to 1152 mAbs, 3 x 384well plates
- **Highly parallel analysis enables exquisite kinetic discrimination of clones**
- **Binding to multiple antigens e.g. orthologs and homologs of target**

Assay Steps:

- **Prepare capture surface (lawn)**
- **Capture mAbs**
- **Inject kinetic series of antigen(s)**



Minimal sample requirements:

- 13 µg anti-human IgG Fc (coupled)
- <1 µg/mAb (captured)
- 7 µg antigen (analyte)

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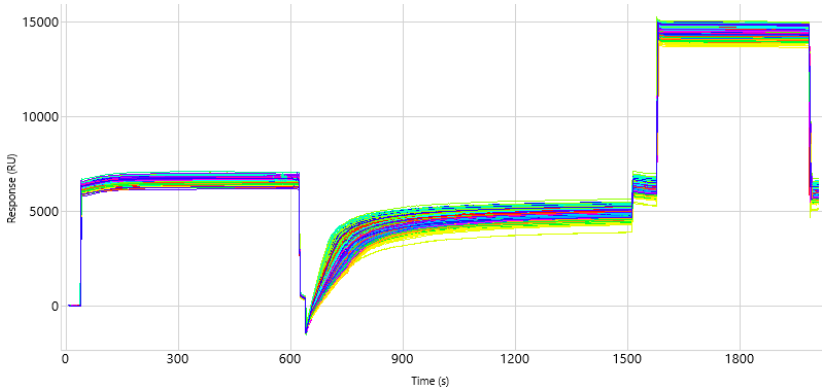
Preparing the capture surface

- **Capture Ligand Concentration- Go high**
  - To immobilize a homogenous capture surface or “lawn” use 30-100 µg/mL
  - Reduce capture capacity by activation, not concentration
- **Capture Ligand Formulation:**
  - 10 mM Acetate pH 4.25 is typical
  - Recommend to perform a pre-concentration test for new ligands
  - If the capture ligand is formulated in a non-amine free buffer, with high salt, or at low concentration, buffer exchange into the appropriate coupling buffer



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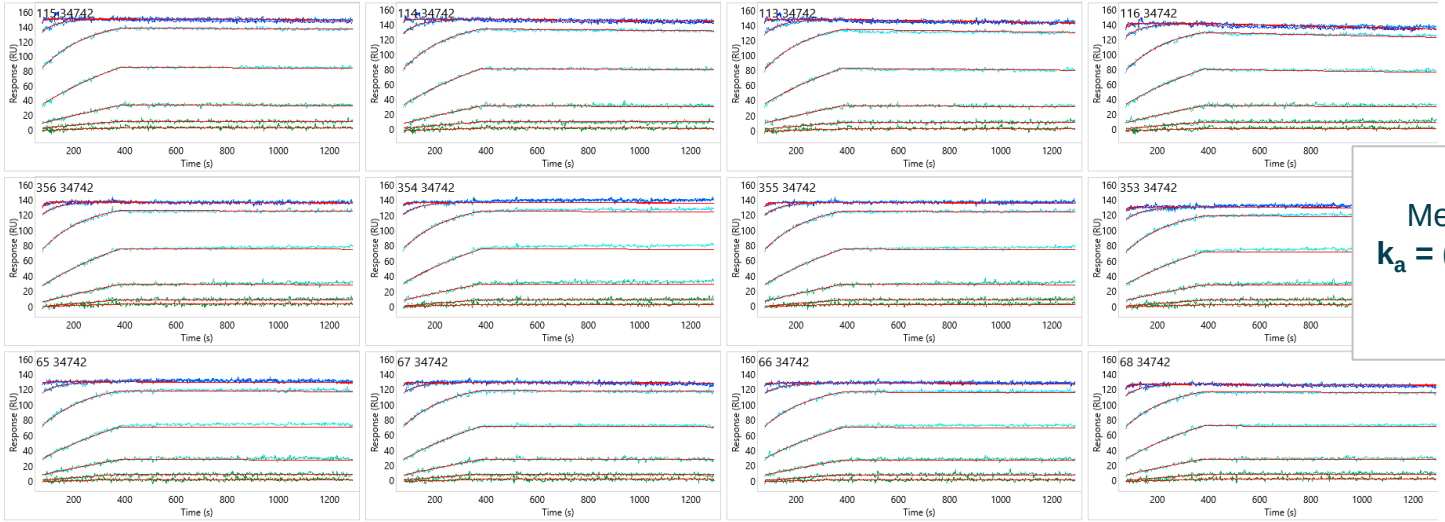
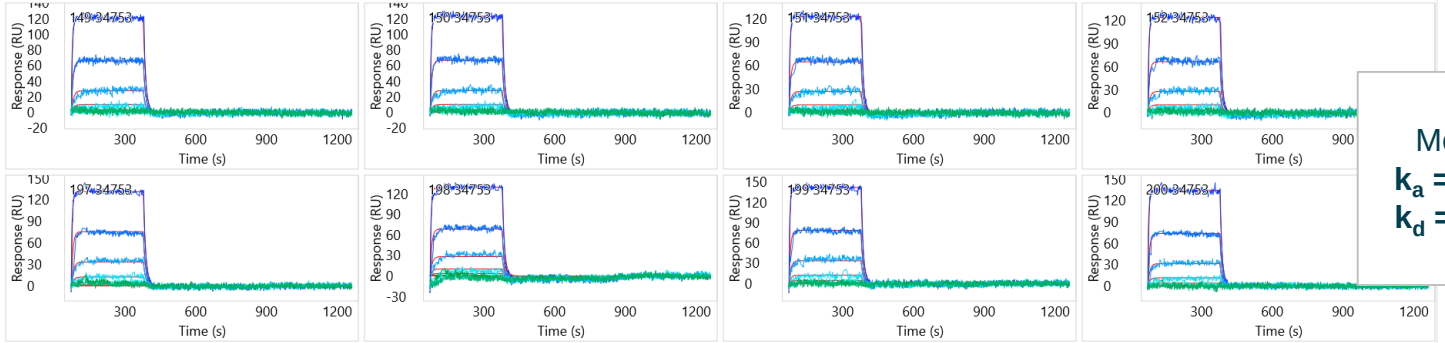
Antigen Concentration Series

- **The LSA uses one injected volume per concentration over the entire 384 ligand array**
- **Even with high concentration antigen usage for kinetic assays is typically not limiting.**
- **This allows for the use of a robust dilution series starting at a high initial concentration.**
  - Typically 1 µM with an 8-point serial dilution, 457 pM lowest concentration.
- **This allows for effective characterization over a broad kinetic range > 10,000 fold**

Both Slow and Rapid Kinetics are Well Described



mAb 34742
Mean ± StDev of 12 spots
$k_a = (1.48 \pm 0.06) \times 10^5$ (1/Ms)
$k_d < 1 \times 10^{-5}$ (1/s)
$K_D < 67 (\pm 3)$ pM



mAb 34753
Mean ± StDev of 8 spots
$k_a = (1.5 \pm 0.1) \times 10^5$ (1/Ms)
$k_d = (1.0 \pm 0.06) \times 10^{-1}$ (1/s)
$K_D = 726 \pm 81$ nM

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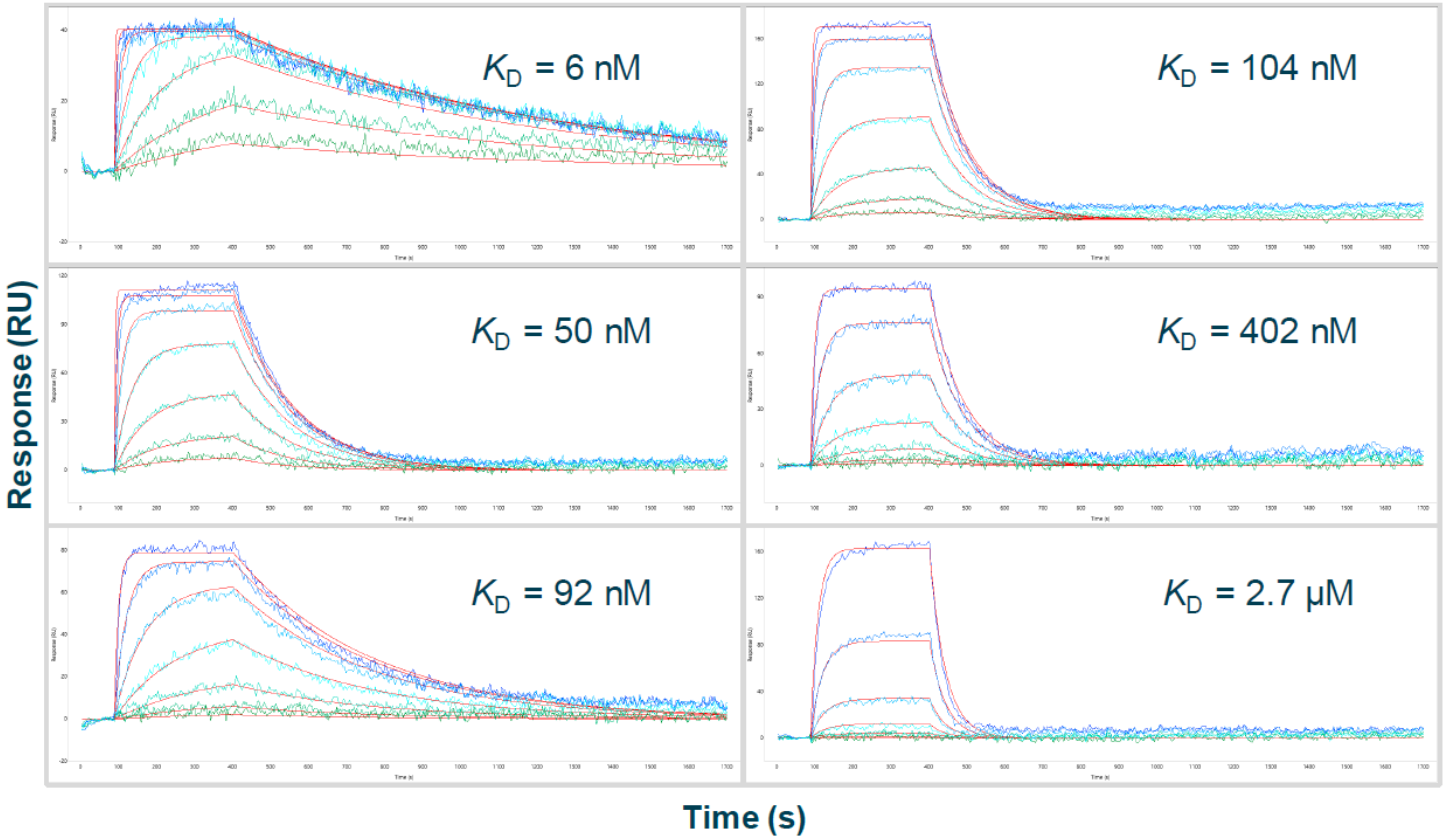
Capture kinetics of a 192-scFv array from crude periplasmic extracts via anti-V5 mAb coated chip



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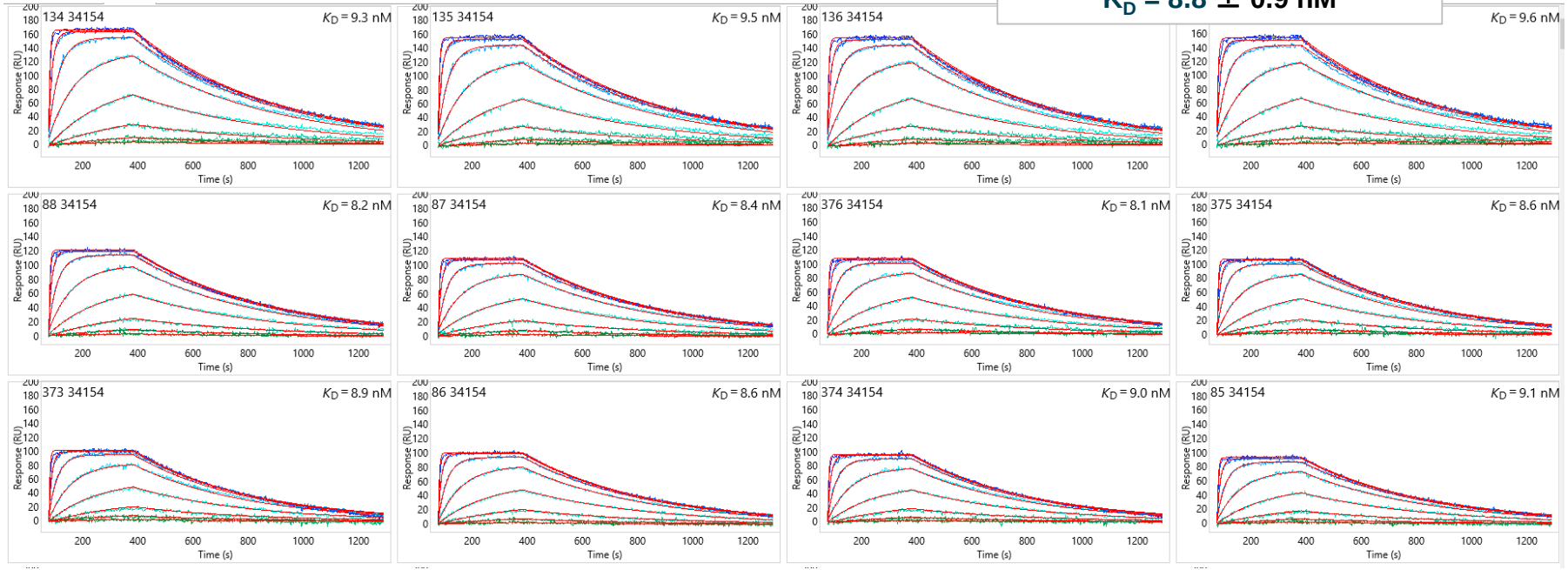
Screen Many or Increase Your N

- **Reproducibility across the array allows you to screen with confidence**
- **If <384 unique mAbs, why not increase your n**
  - Allows statistical analysis of the reported kinetic parameters

mAb 34154
Mean ± StDev of 12 spots
$k_a = (2.4 \pm 0.2) \times 10^5$ (1/Ms)
$k_d = (2.1 \pm 0.1) \times 10^{-3}$ (1/s)
$K_D = 8.8 \pm 0.9$ nM



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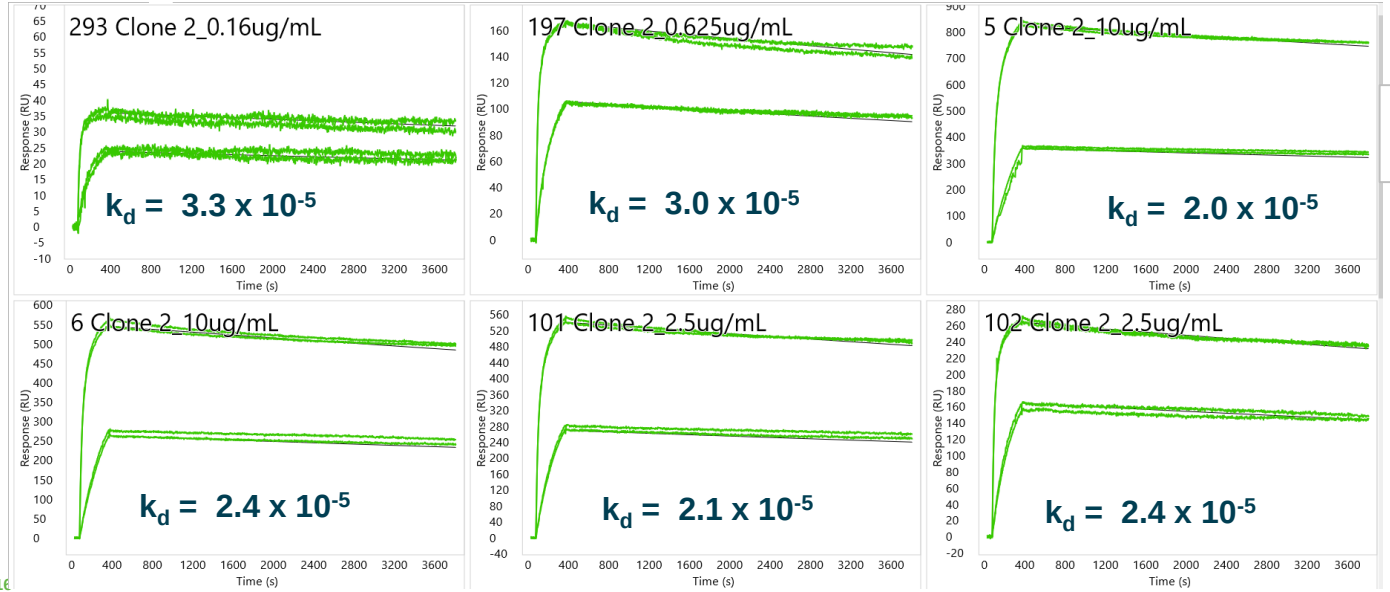
Power the Analysis

- **Use of high replication adds confidence to small differences**
  - Example: Slow off-rates can be estimated with better statistical power if the data is collected with significant replication.
  - 6 ligand densities, duplicates of 2 concentrations with 60 minute dissociation
  - Total collected off-rate time is 24 hours from only 6 spots of an available 384, and 4.5 hours of collected data.



Mean ± StDev of 6 spots

$k_d = (2.5 \pm 0.4) \times 10^{-5}$ (1/s)

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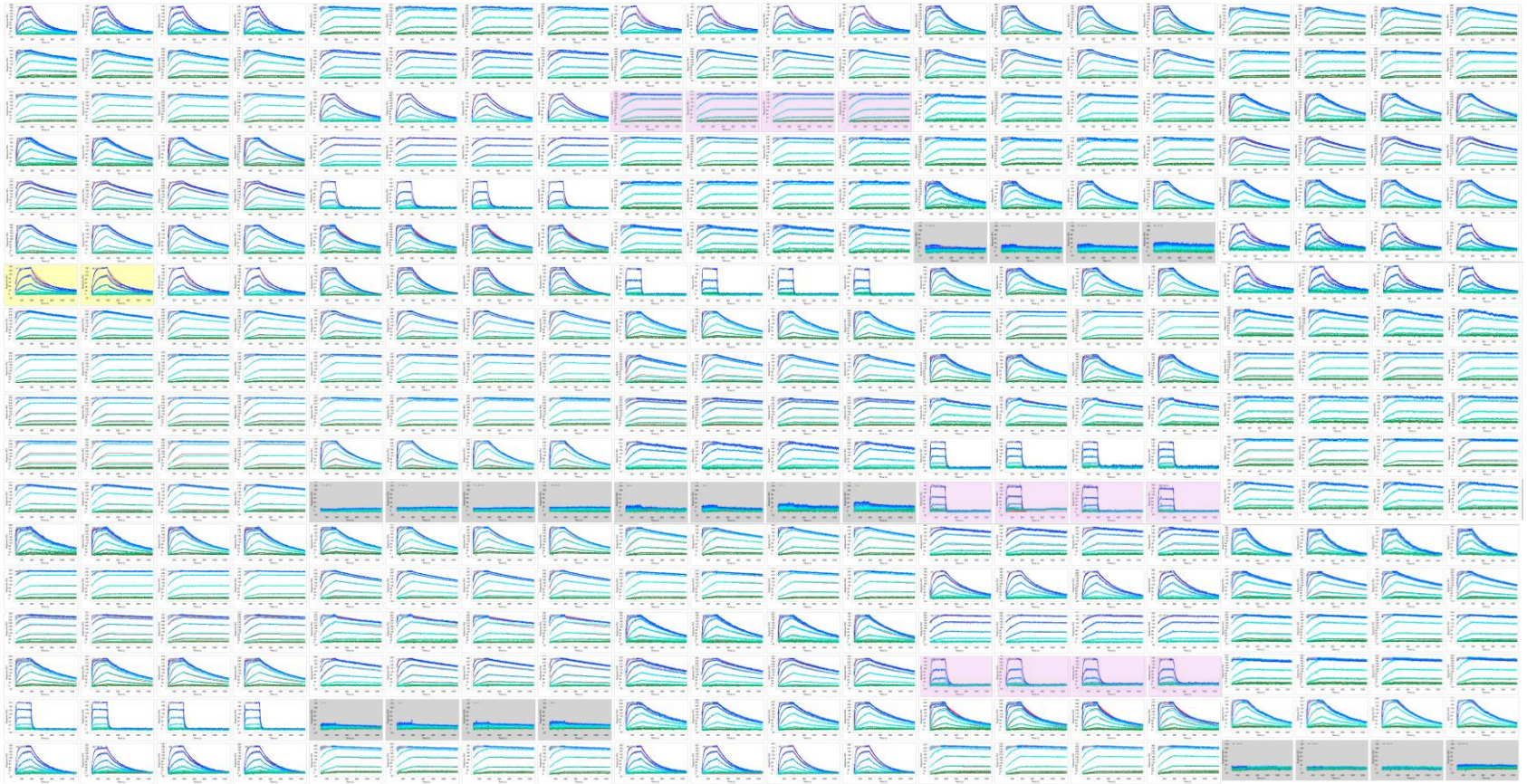
Data Analysis: LSA Kinetics Software

- **Built to handle 1000’s of interactions**
- **Batch-processing**
- **Automatically data QC to flags**
- **Facilitates multiplexed**
- **Results export to an Excel file**

What Does High Throughput Kinetics Look Like?



- **384 Ligand Kinetics, 1 Parallel Run, 8 Concentrations, 7 µg Antigen**

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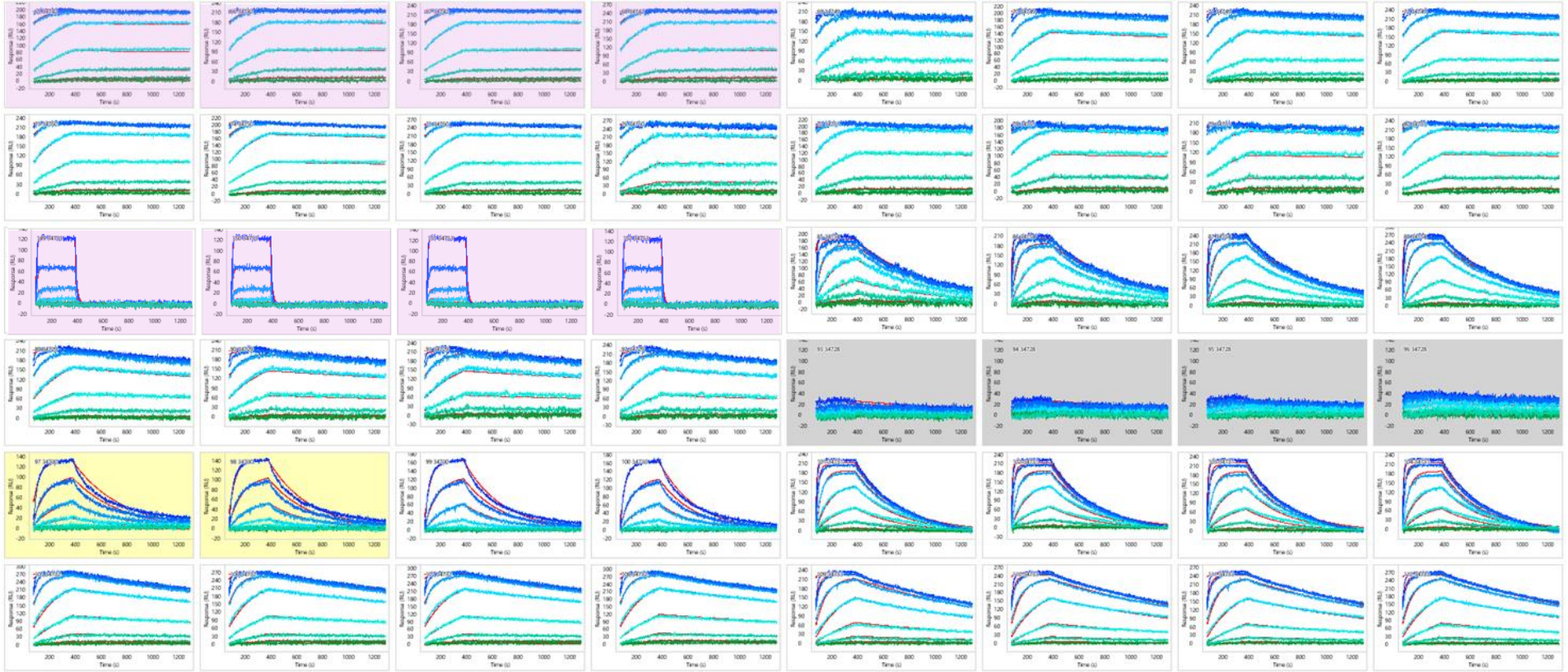
• Software automatically flags the Good, Bad, and Ugly



ROIs with no/low binding, poor fit, or with kinetics pushing the assay limitations are identified automatically.

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General considerations for data analysis

- **Use of blank cycles for double referencing should be approached thoughtfully.**

- **Inclusion or omission of analyte cycles-**
  - Sometimes higher is not better

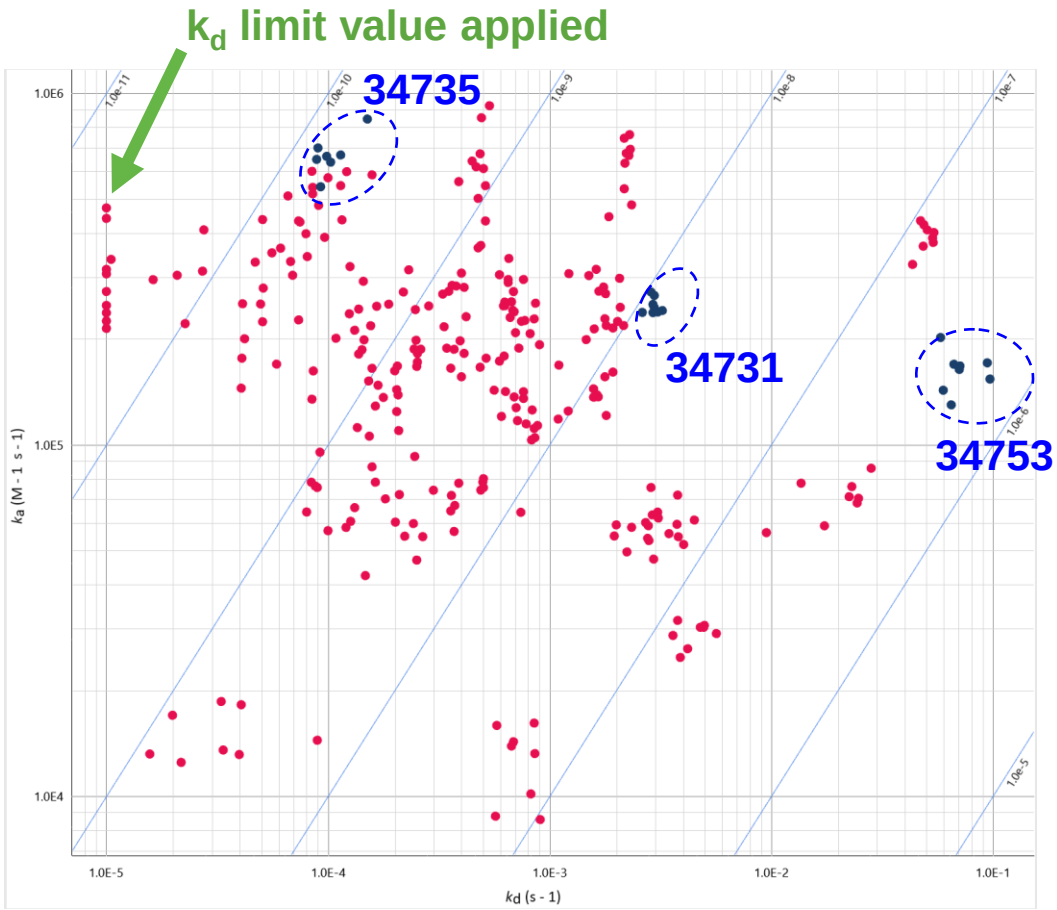
- **Zeroing: If regeneration is not complete, a serial Y-alignment can be applied**
  - This allows for standard kinetic model fitting, but the T_0 parameter can be floated

Data Presentation

- **The kinetics software has a variety of useful tools to visualize data effectively**
- **Array view: striking and highly customizable visualization of your library's kinetic diversity**
  - Color by ligand set, color by analyte set
- **Iso-affinity plots**
- **Statistics page reports and plots mean and standard deviations for key fitted parameters**

Iso-Affinity Plot

- Iso-affinity plots facilitate assessment of kinetic diversity within a mAb panel
- The blue highlights weak, medium, and high affinity clones
- Kinetics are well described covering a 10,000 fold K_D range



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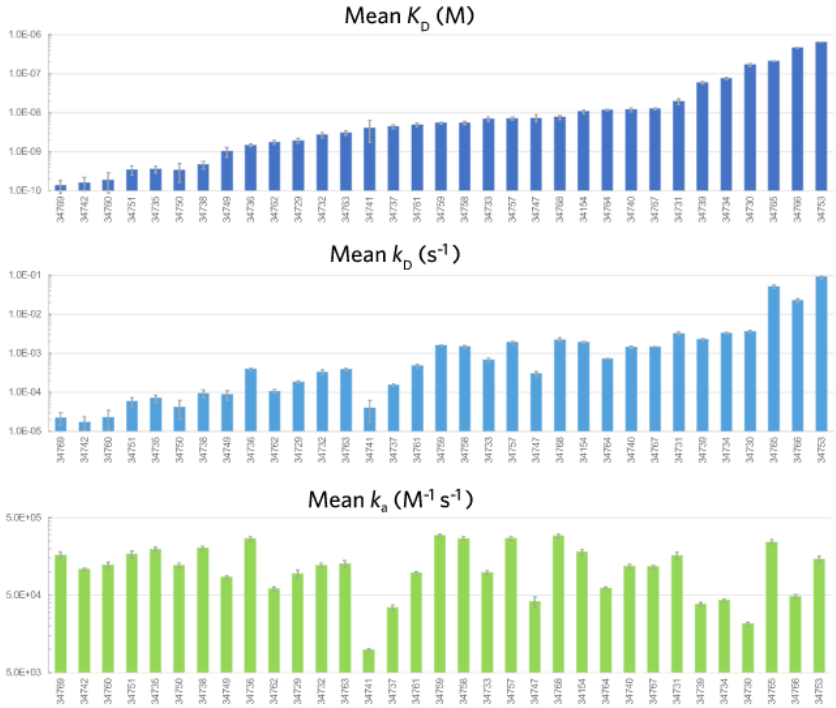
Statistics Page

- **Statistics page reports and plots mean and standard deviations for key fitted parameters**



Ligand	n	Mean k_a (M-1 s-1)	k_a Std. Dev.	Mean k_d (s-1)	k_d Std. Dev.	Mean K_D (M)	K_D Std. Dev.	Mean Rmax (RU)	Rmax Std. Dev.	Mean Res sd	Std. Dev. Res sd
34154	12	1.77E+05	1.26E+04	1.90E-03	8.36E-05	11 nM	895 pM	236	26	52	5.2
34728	12	1.41E+05	2.94E+05	2.34E-04	2.85E-04	12 nM	29 nM	17	11	8	4.7
34729	12	9.15E+04	5.22E+03	1.80E-04	1.18E-05	2.0 nM	172 pM	228	15	129	8.9
34730	12	1.96E+04	1.44E+03	3.14E-03	3.10E-04	160 nM	20 nM	167	14	14	1.4
34731	12	1.56E+05	1.33E+04	3.17E-03	2.56E-04	20 nM	2.4 nM	206	15	22	2.6
34732	12	1.20E+05	6.83E+03	3.22E-04	3.29E-05	2.7 nM	317 pM	236	32	129	20
34733	12	9.81E+04	3.43E+03	6.55E-04	7.04E-05	6.7 nM	755 pM	228	20	98	11
34734	12	4.09E+04	1.75E+03	3.37E-03	1.44E-04	83 nM	5.0 nM	214	26	16	2.8
34735	12	1.91E+05	1.14E+04	6.80E-05	1.62E-05	359 pM	88 pM	269	29	181	21
34736	12	2.64E+05	1.54E+04	4.00E-04	1.91E-05	1.5 nM	114 pM	247	22	141	13
34737	12	3.55E+04	2.04E+03	1.50E-04	9.93E-06	4.2 nM	372 pM	246	21	123	12
34738	12	1.99E+05	9.04E+03	9.78E-05	1.95E-05	493 pM	101 pM	263	18	175	14
34739	12	3.79E+04	2.12E+03	2.15E-03	1.15E-04	57 nM	4.4 nM	224	17	33	4
34740	12	1.13E+05	6.11E+03	1.45E-03	1.21E-04	13 nM	1.3 nM	202	17	56	7.8
34741	12	9.86E+03	3.79E+02	2.98E-05	1.59E-05	3.0 nM	1.6 nM	226	18	89	7.9
34742	12	1.11E+05	1.14E+04	1.44E-05	5.68E-06	128 pM	52 pM	238	25	154	16
34747	8	4.16E+04	6.35E+03	2.97E-04	3.61E-05	7.2 nM	1.4 nM	233	10	109	6.4
34749	12	8.43E+04	4.11E+03	8.39E-05	2.32E-05	991 pM	278 pM	228	32	137	20
34750	12	1.22E+05	5.53E+03	4.22E-05	2.26E-05	343 pM	184 pM	249	37	162	25
34751	12	1.68E+05	1.90E+04	5.97E-05	1.77E-05	351 pM	111 pM	229	39	152	26
34753	8	1.50E+05	1.46E+04	1.08E-01	6.02E-03	726 nM	81 nM	222	12	2.9	0.64
34757	8	2.71E+05	1.64E+04	1.85E-03	1.32E-04	6.9 nM	641 pM	239	21	59	11
34758	8	2.57E+05	8.16E+03	1.48E-03	1.11E-04	5.8 nM	469 pM	220	27	66	12
34759	8	2.91E+05	1.25E+04	1.59E-03	4.42E-05	5.5 nM	281 pM	232	26	66	7.8
34760	8	1.19E+05	8.80E+03	2.48E-05	1.44E-05	212 pM	124 pM	232	18	151	13
34761	8	9.58E+04	4.04E+03	4.62E-04	4.42E-05	4.8 nM	505 pM	240	21	117	13
34762	8	6.82E+04	4.53E+03	1.09E-04	1.69E-05	1.6 nM	272 pM	308	17	176	11
34763	8	1.29E+05	1.37E+04	3.75E-04	3.19E-05	2.9 nM	399 pM	262	30	141	16
34764	12	6.13E+04	2.29E+03	7.01E-04	2.02E-05	11 nM	541 pM	282	17	110	7.8
34765	8	2.03E+05	1.72E+04	4.31E-02	1.38E-03	214 nM	19 nM	259	10	4.8	1.2
34766	12	4.42E+04	2.15E+03	2.01E-02	1.72E-03	455 nM	45 nM	226	14	3.8	0.59
34767	12	1.15E+05	3.53E+03	1.52E-03	1.00E-04	13 nM	964 pM	234	24	62	8.2
34768	12	2.79E+05	2.60E+04	2.16E-03	1.59E-04	7.8 nM	925 pM	225	20	50	6.9
34769	12	1.75E+05	1.94E+04	2.41E-05	6.76E-06	141 pM	43 pM	248	25	169	17

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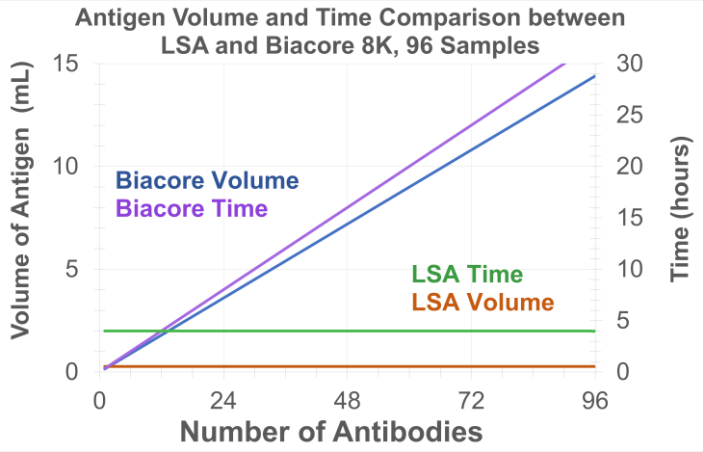
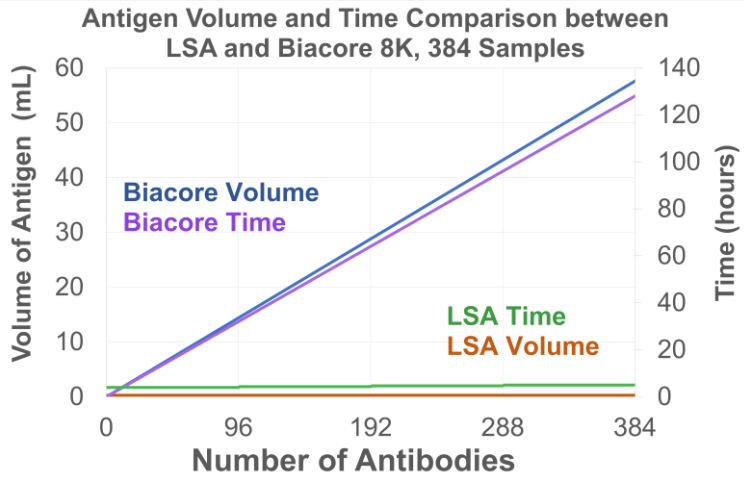
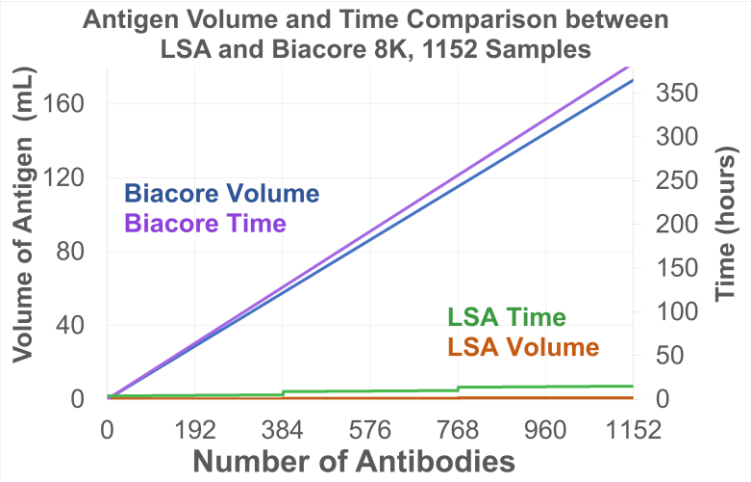
Capture Kinetics Comparison, Time and Volume: LSA vs. 8K







This represents a capture kinetics assay with equivalent number of injections (7 concentrations + blank) for assay timing on both systems.

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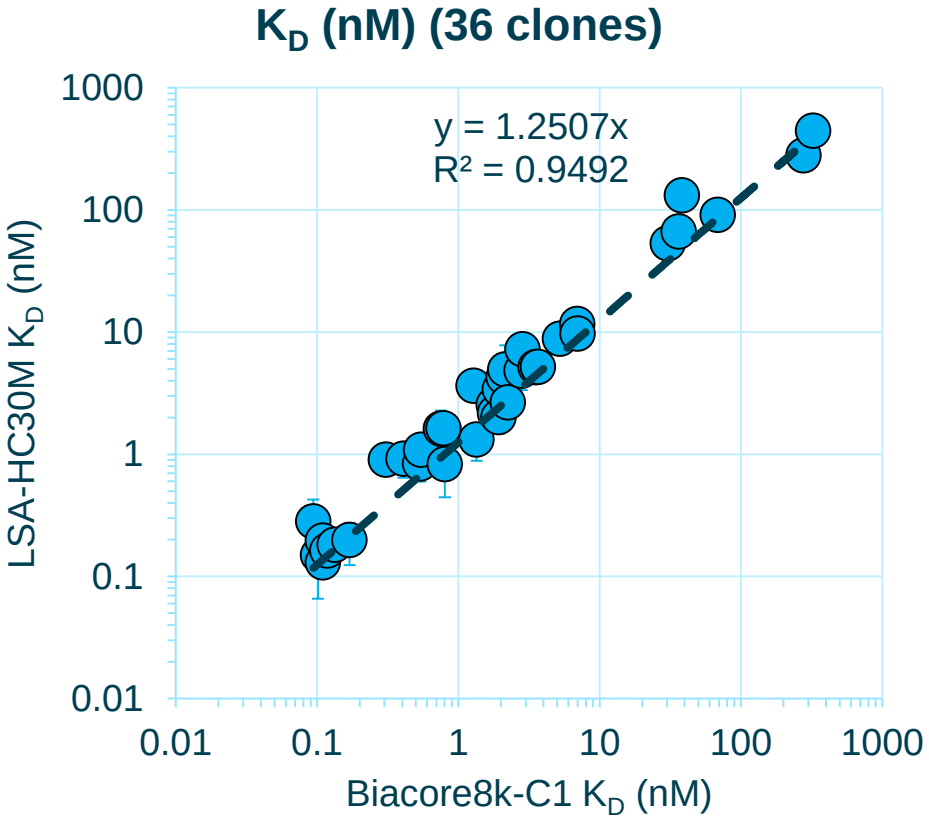
Benchmark LSA vs Biacore 8K



Above data Submitted to PLOS ONE

- **Excellent agreement in kinetic rate constants (within 2 fold, when match chip types)**
- **Excellent agreement across wide affinity range <100pM to >100nM**

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Summary

- The LSA provides a unique approach to high throughput kinetic screening and characterization
- The LSA enables detailed characterization assays at the initial screening stages by removing sample and time constraints
- High quality kinetic analysis can be easily performed on large numbers of crude samples
- The same rules apply for good experimental design as with other SPR biosensors, and attention to detail is important for good results