Accelerate Your Antibody Discovery with High throughput Array SPR

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Senior Field Applications Scientist, Carterra

Carterra

- **Founded in 2005, Salt Lake City, UT, as Wasatch Microfluidics**
  - Licensed Continuous Flow Microspotter (CFM) technology from the University of Utah Research Foundation
  - Combined the CFM with the IBIS MX96 SPR detector
- **Received significant funding in 2016—rebranded as Carterra**
- **Expanded management, R&D, and commercial teams**
- **Commercial launch in Jan 2018;**
  - Now with sales in North America, Europe, and Asia-Pacific

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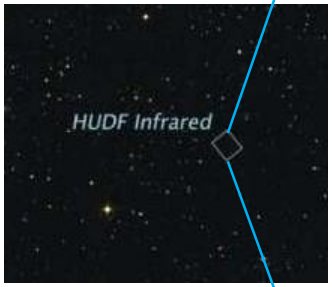
Hubble eXtreme deep field infrared



A disruption in Array SPR

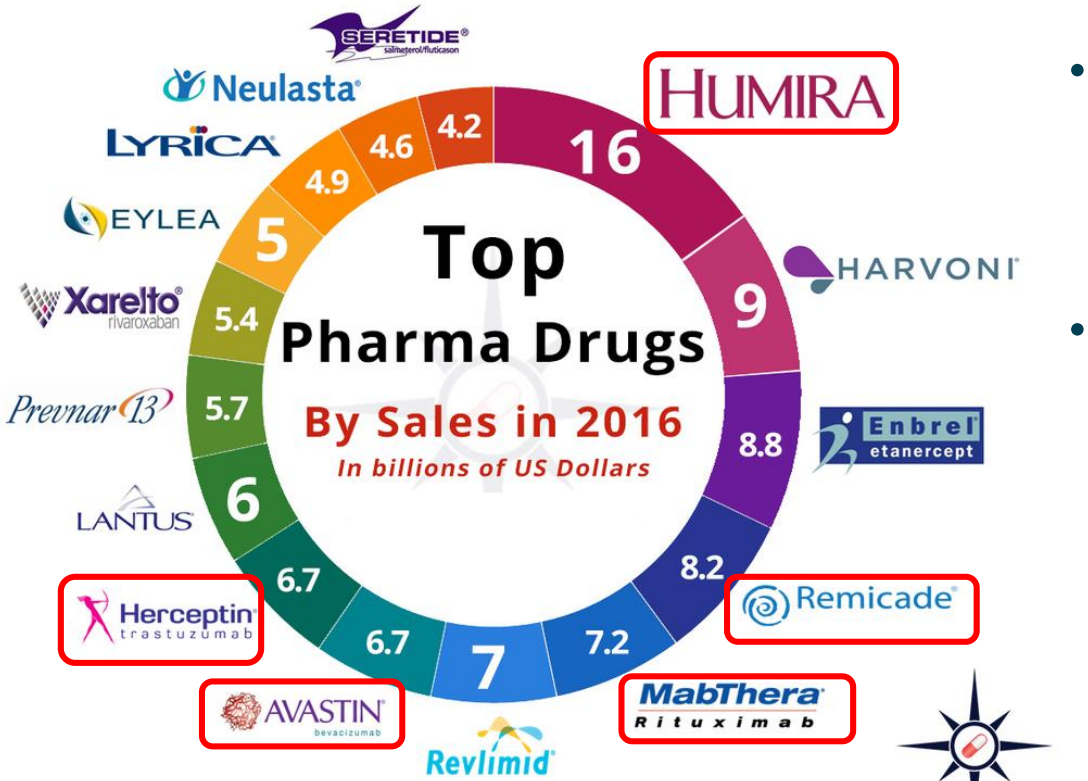
LSA

Lodestar

A reference point used for navigation

Array

Therapeutic mAbs Comprise About Half Pharma Sales



www.pharmacompass.com

PHARMA COMPASS

- Therapeutic mAbs are a lucrative and growing segment of the pharma industry's portfolio
- Biophysics is agnostic of therapeutic area because it studies ***molecular-level binding interactions*** to better understand a drug's ***mechanism of action*** to guide the design of next-generation medicines

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Which One?

- Large investments made in mAb generation
- Analytical tools lag orders of magnitude behind
- Don’t throw away a blockbuster candidate!

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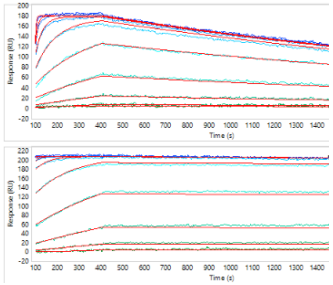
High Throughput SPR – a Disruptive Technology

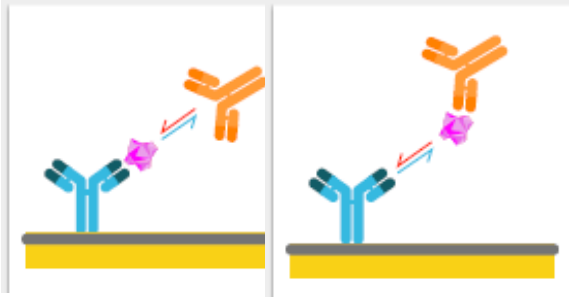
- Array SPR shifts the paradigm in antibody screening enabling detailed kinetic, affinity, and epitope characterization of your library at the earliest stages of research
- Increases the chances you'll find leads within your library that require minimal engineering
- Accelerates library-to-lead selections

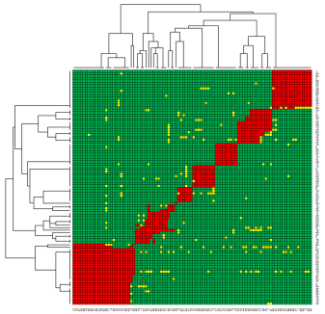
LSA Core Applications

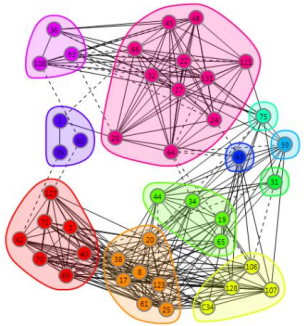
Kinetics/Affinity



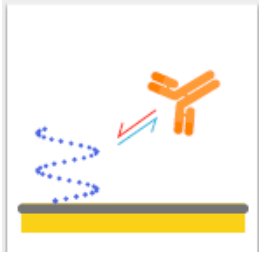
Epitope Binning

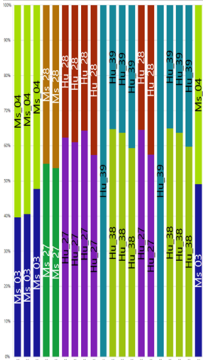






Mapping





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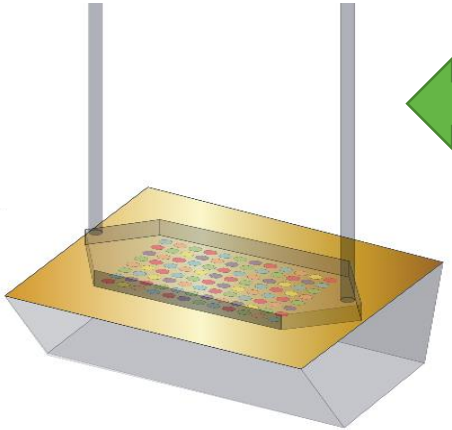
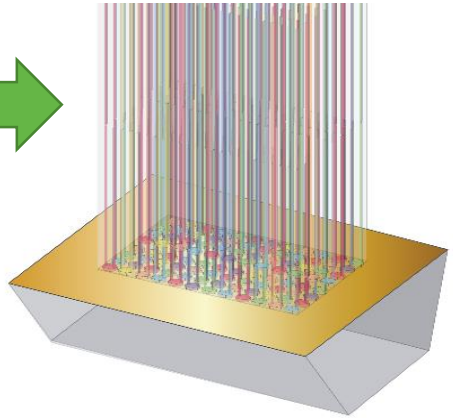
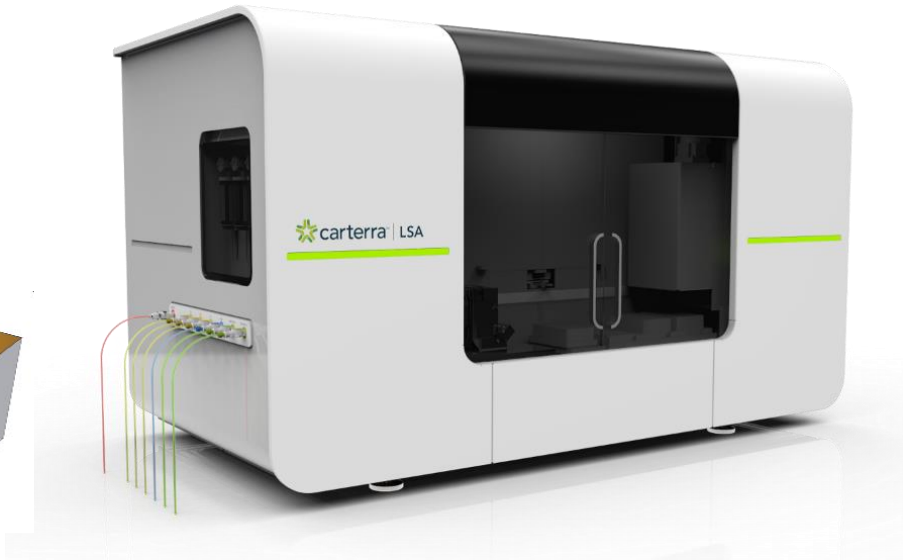
Carterra's LSA™ – A Disruptive Technology

Single-channel mode (large flow cell)



Multi-channel mode (96-channel printhead)





- Integrates Array SPR with Flow Printing
- Automated switching between single- and multi-channel Flow cells
- 384 reaction spots + interspots per array
- In-line reloading of array, up to 1152 ligands per run (3x 384)
- Supports standard amine coupling and capture formats

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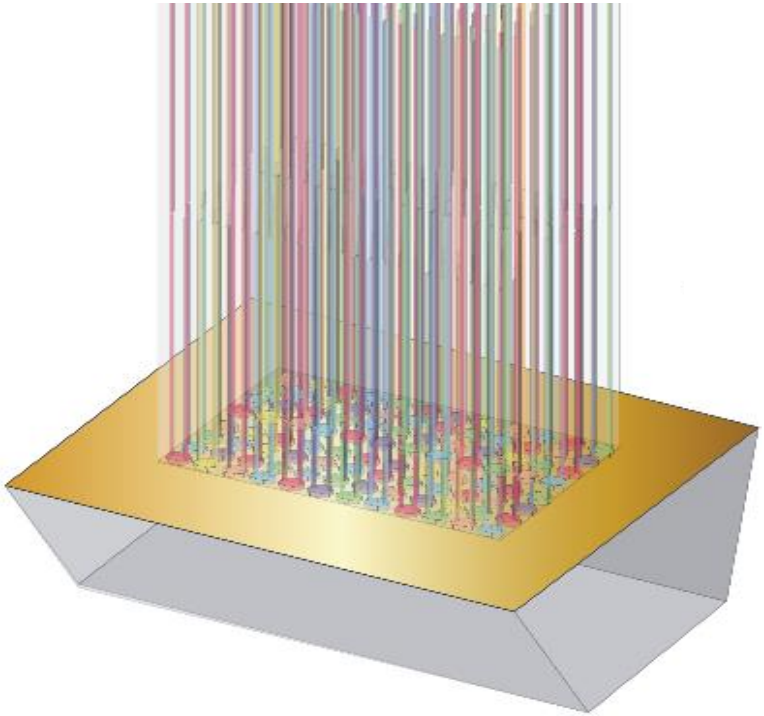
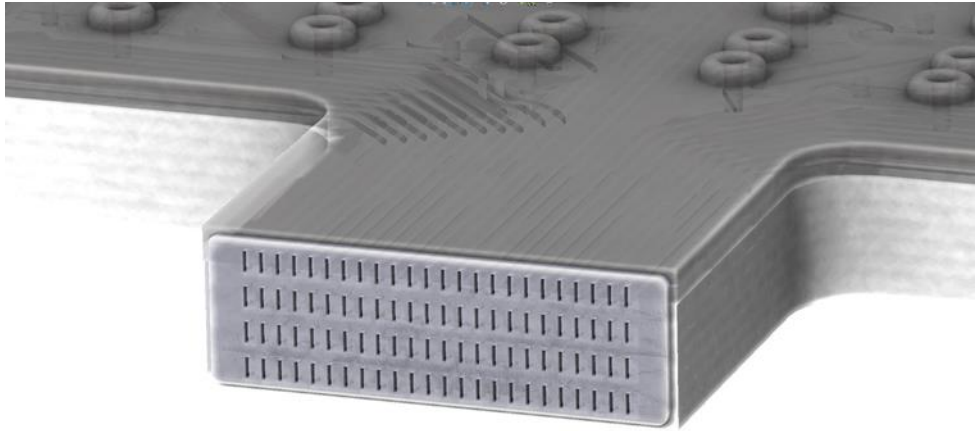
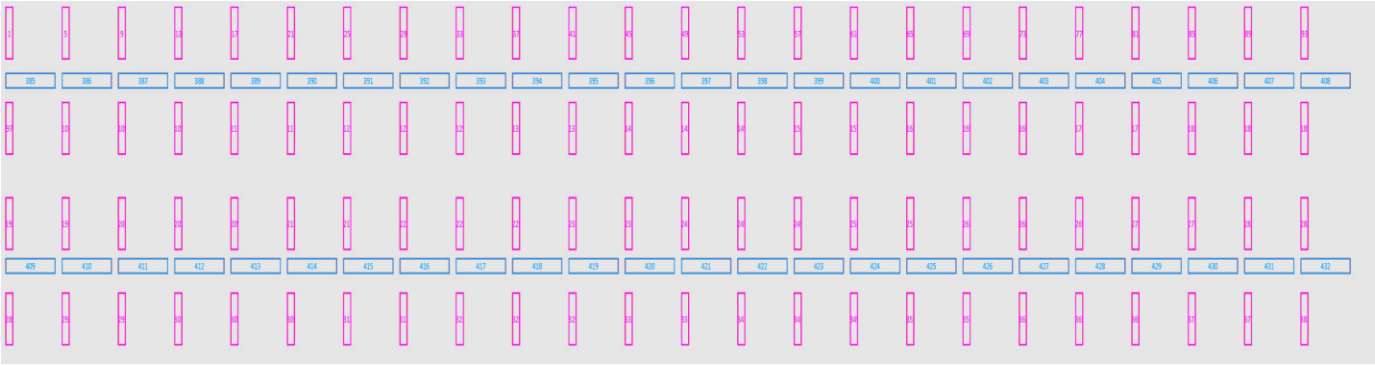
LSA Printhead-96 spots

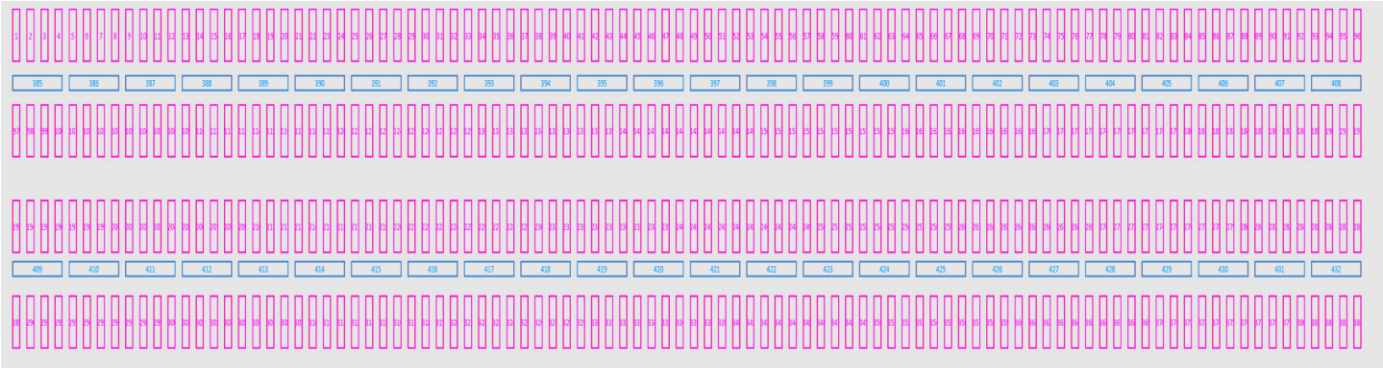




Flow Print a 384-Ligand Array on the LSA



Serially print 4 nested 96-spot arrays to generate a 384-spot array



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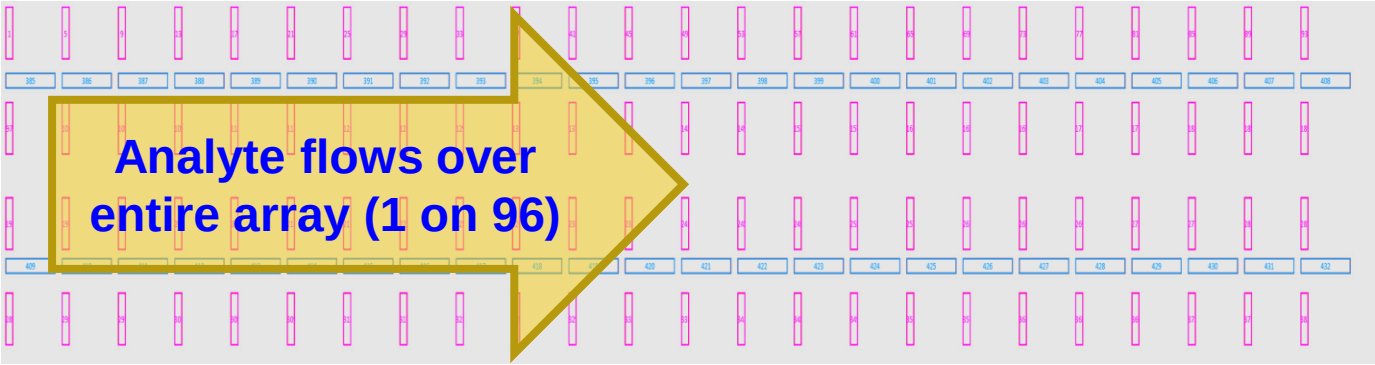
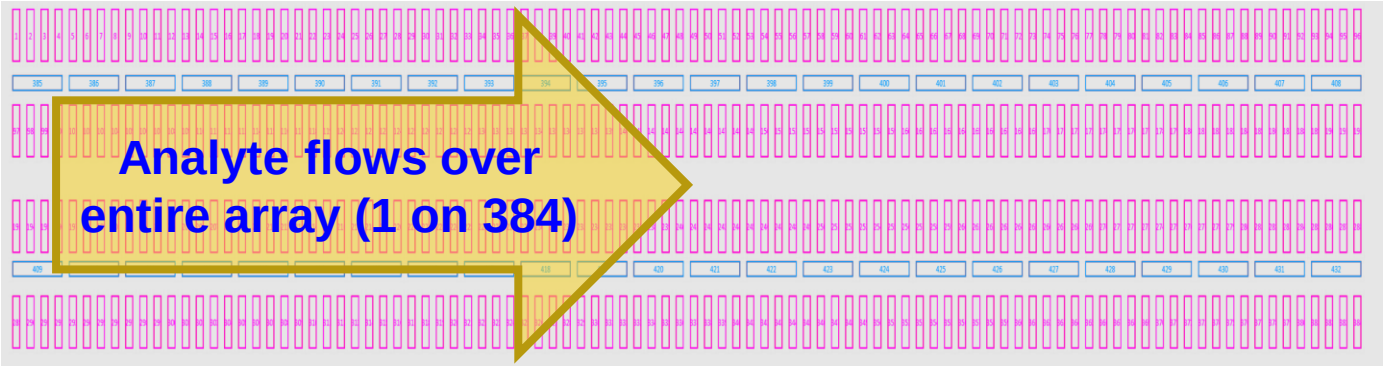
Minimal Analyte Consumption—One-on-Many Assay Format





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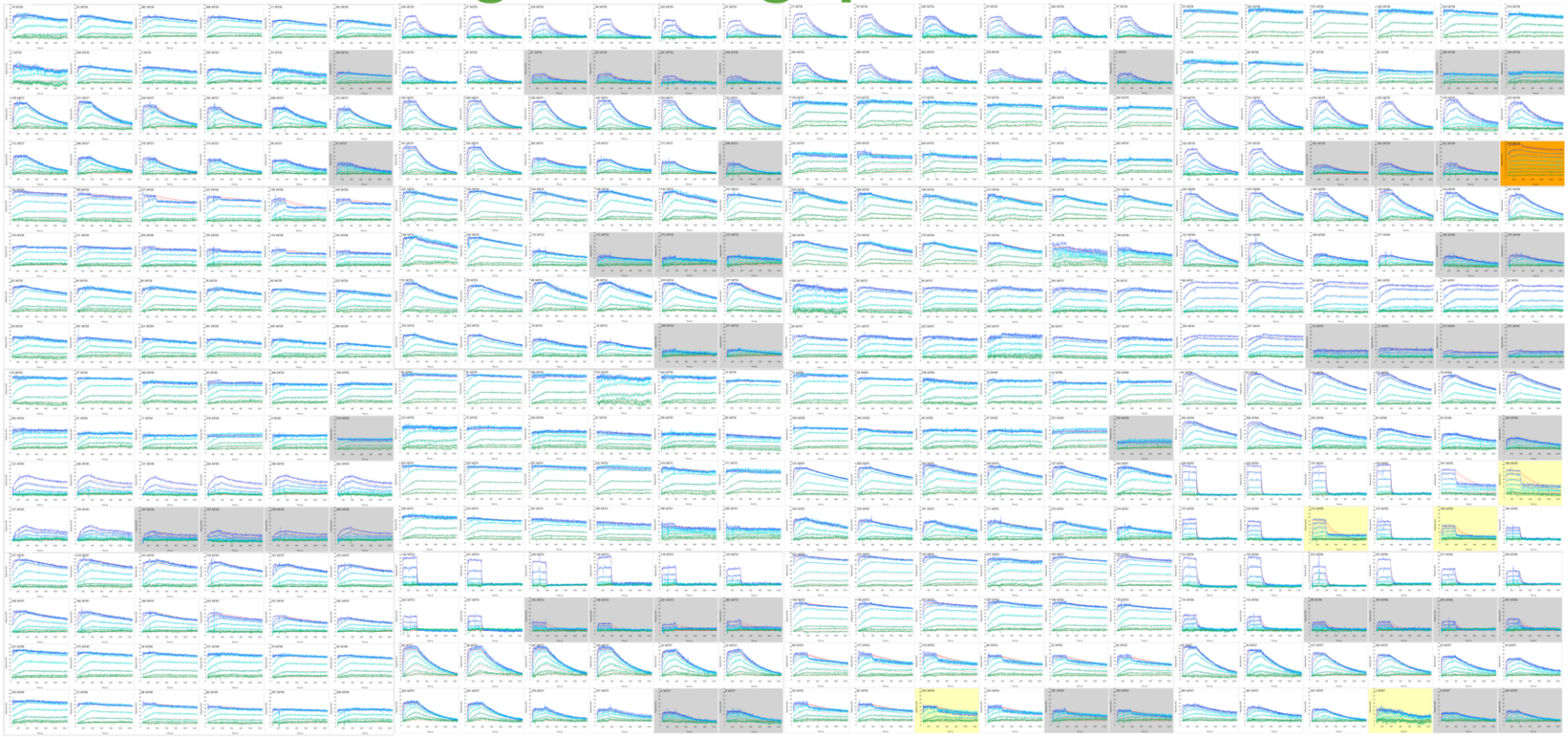
What Does High Throughput Kinetics Look Like?



- **384 Ligand Kinetics, 1 Parallel Run, 7 µg Antigen, 8 Concentrations**
- **Software automatically flags the Good, Bad, and Ugly**

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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

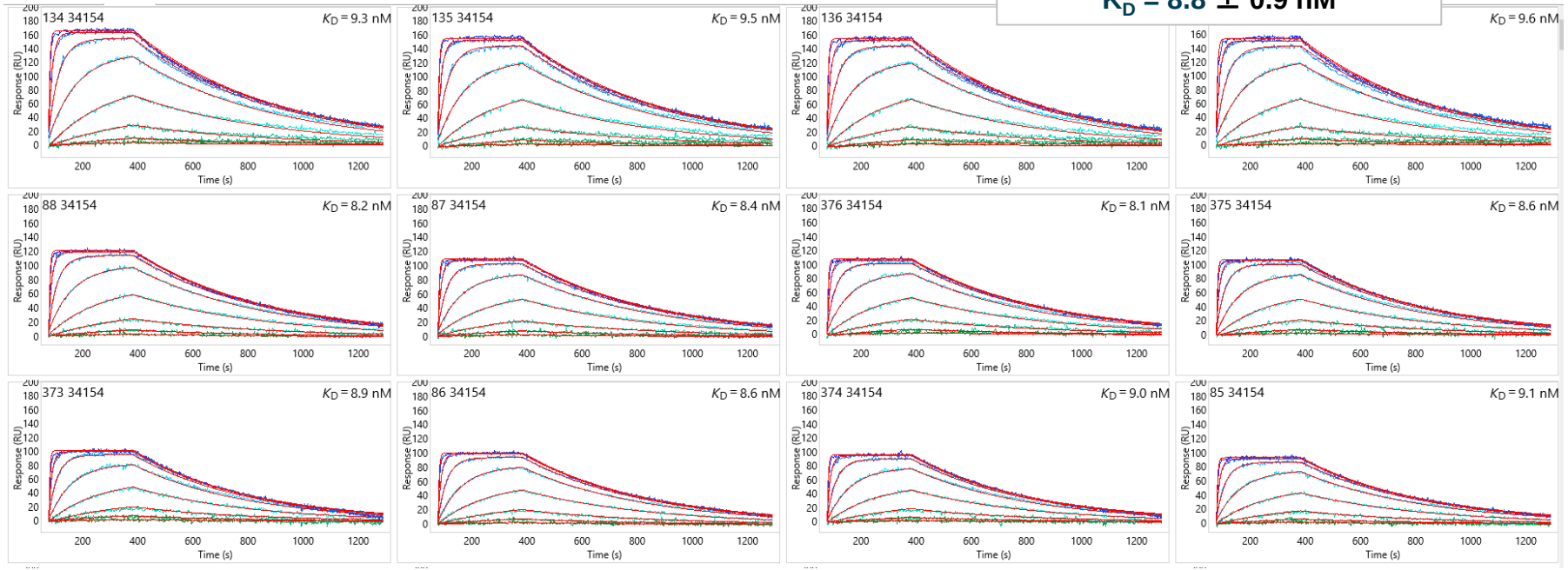
mAb 34154

Mean ± StDev of 12 spots

k_a = (2.4 ± 0.2) x 10^5 (1/Ms)

k_d = (2.1 ± 0.1) x 10^{-3} (1/s)

K_D = 8.8 ± 0.9 nM



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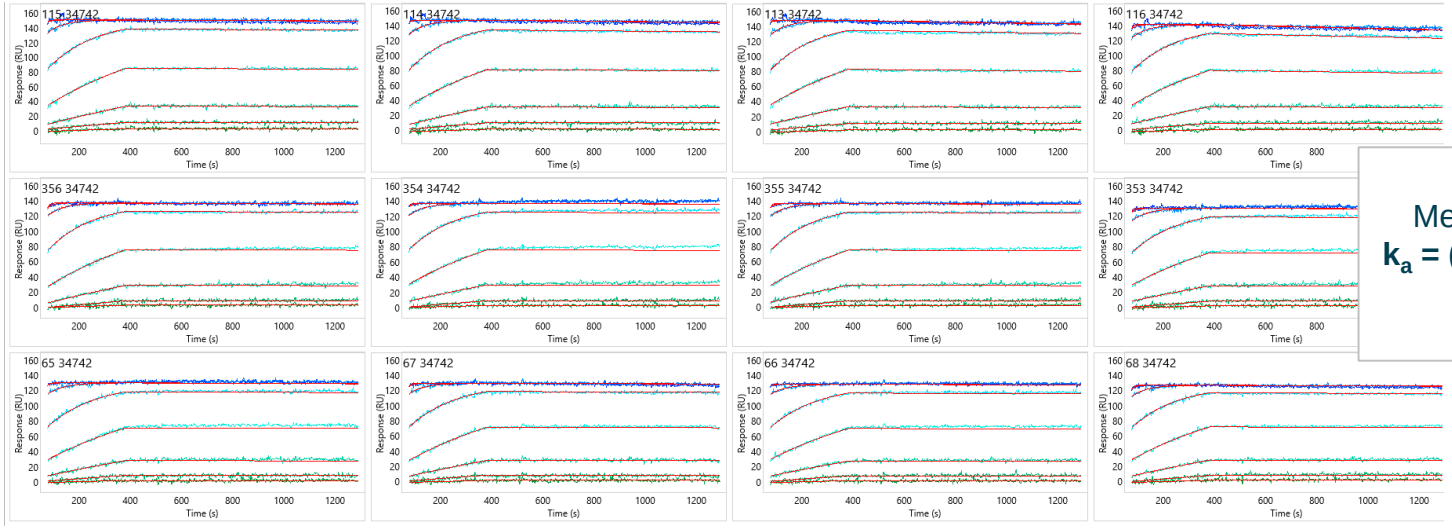
Both Slow and Rapid Kinetics are Well Described



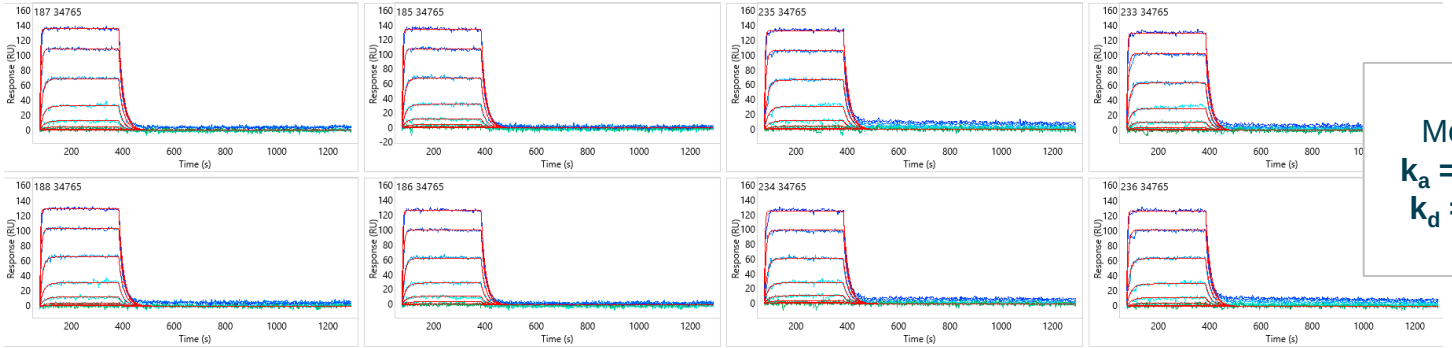
mAb 34742

Mean ± StDev of 12 spots

k_a = (1.48 ± 0.06) x 10^5 (1/Ms)

k_d < 1 x 10^{-5} (1/s)

K_D < 67 (± 3) pM



mAb 34765

Mean ± StDev of 8 spots

k_a = (3.6 ± 0.2) x 10^5 (1/Ms)

k_d = (5.2 ± 0.3) x 10^{-2} (1/s)

K_D = 145 ± 13 nM

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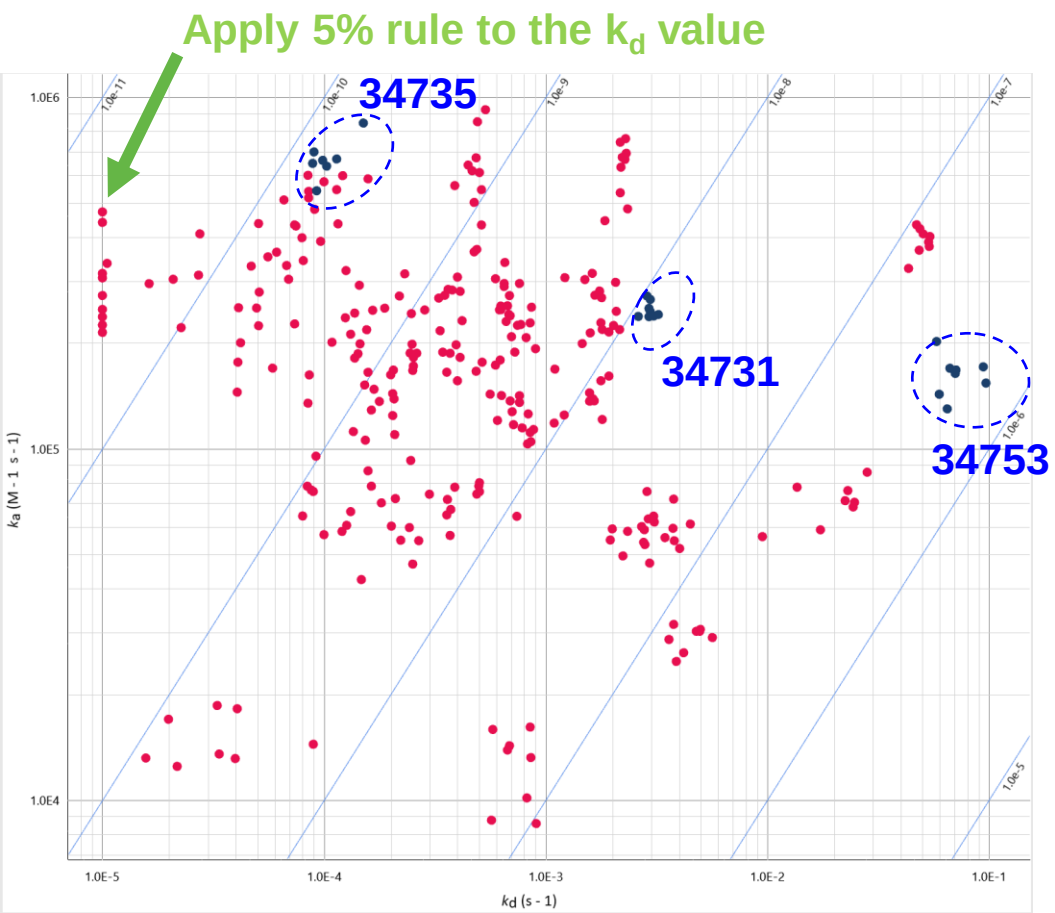
Iso-Affinity Plot

- **Iso-affinity plots facilitate assessment of kinetic diversity within a mAb panel**
- **The blue clones indicate three clones with different affinities**
  - Weak, medium, high
- **Apply the 1 x 10^{-5} k_d limit automatically in the fitting routine to prevent inappropriate affinity estimates when minimal dissociation is observed**
- **Kinetics are well described covering a 10,000 fold K_D range**



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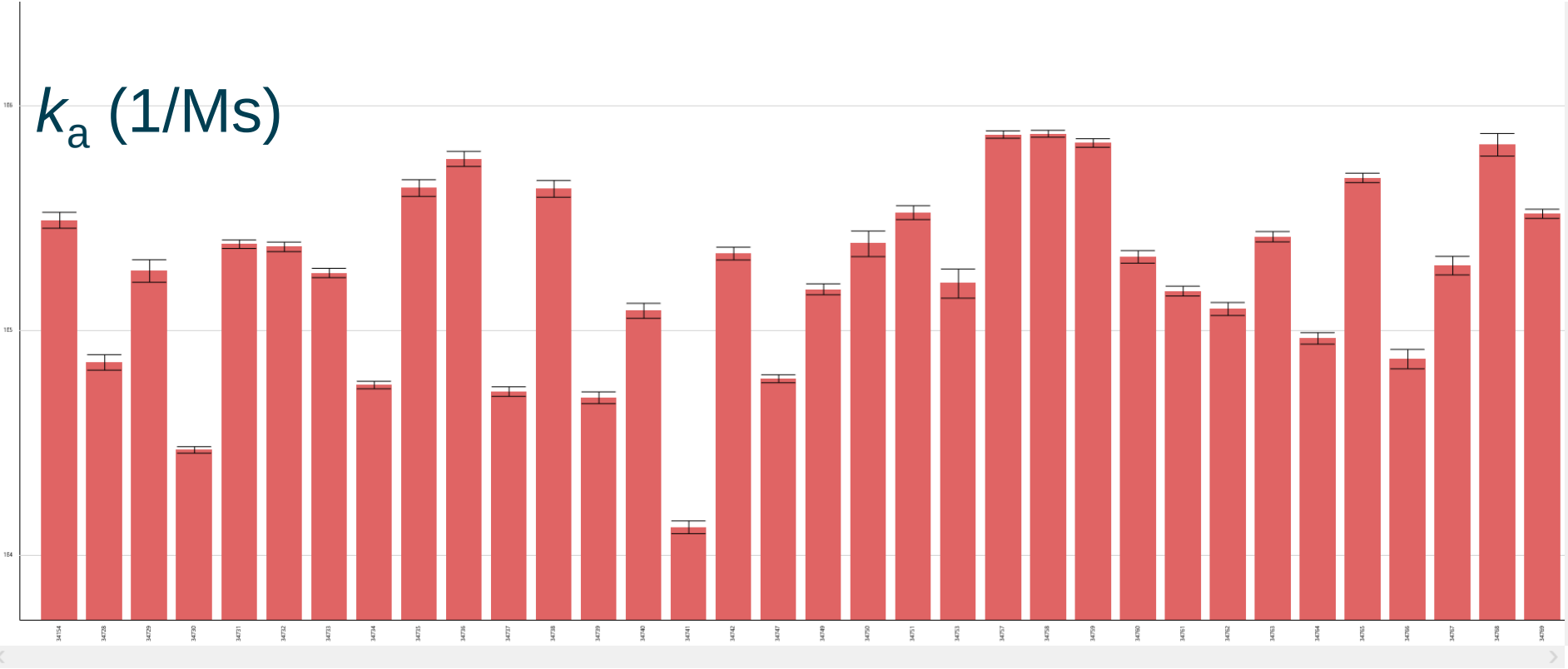
Get Stats on Small MAb Panels By Arraying Clones on Replicate Spots



34 unique mAbs, each on 8 or 12 spots

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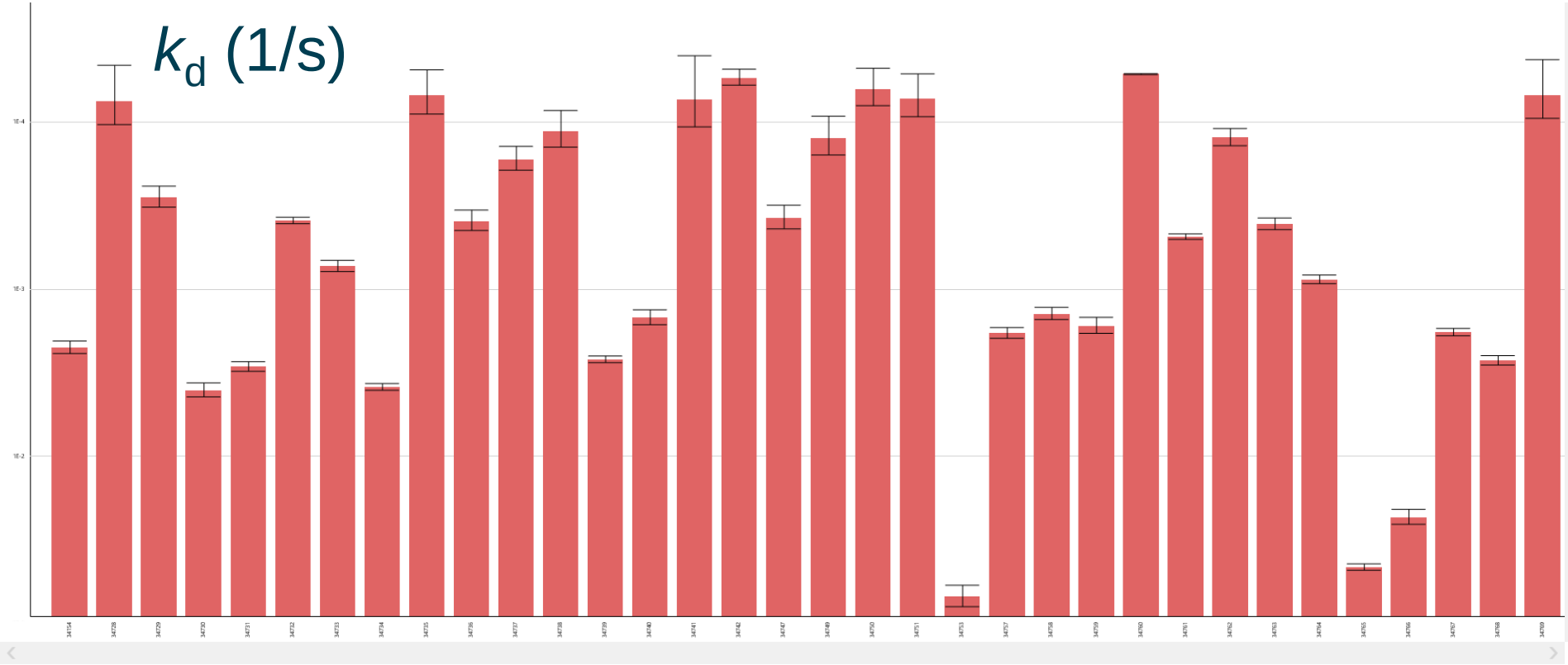
Get Stats on Small MAb Panels By Arraying Clones on Replicate Spots



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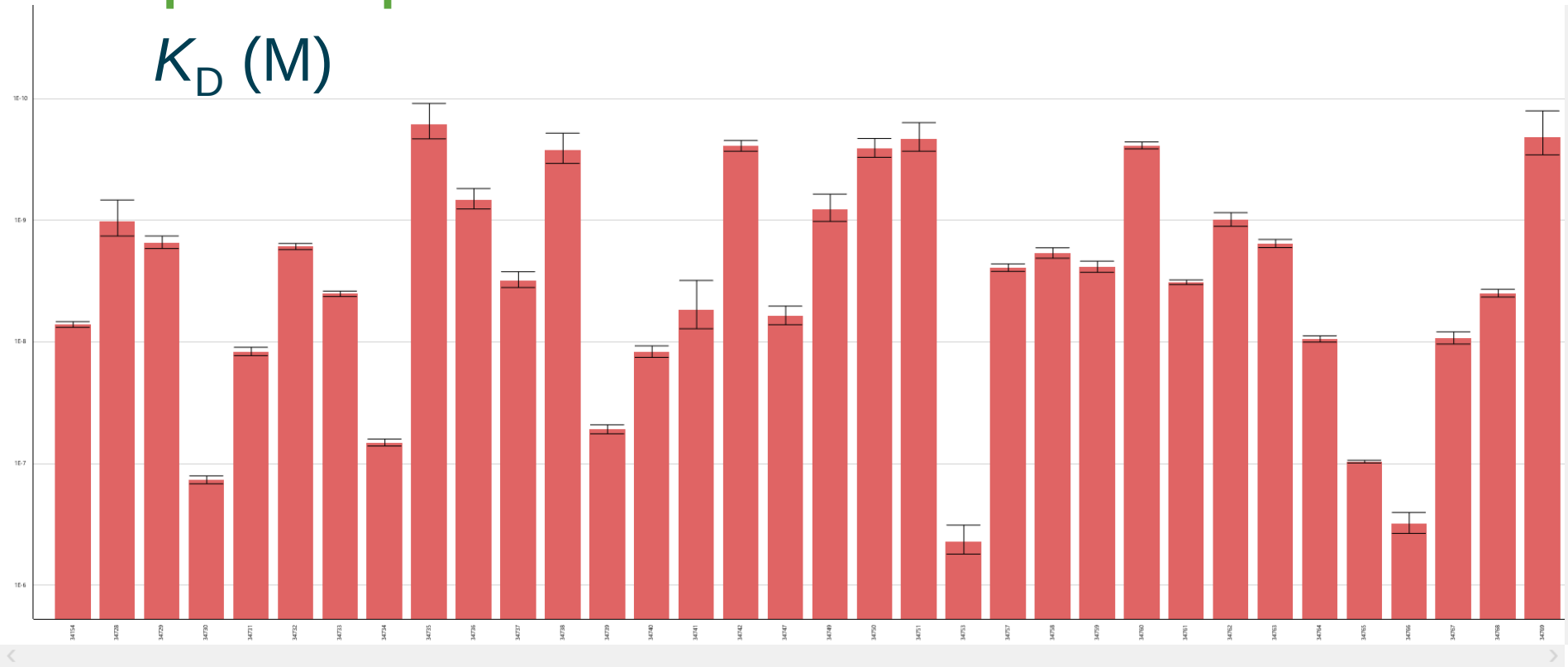

34 unique mAbs, each on 8 or 12 spots

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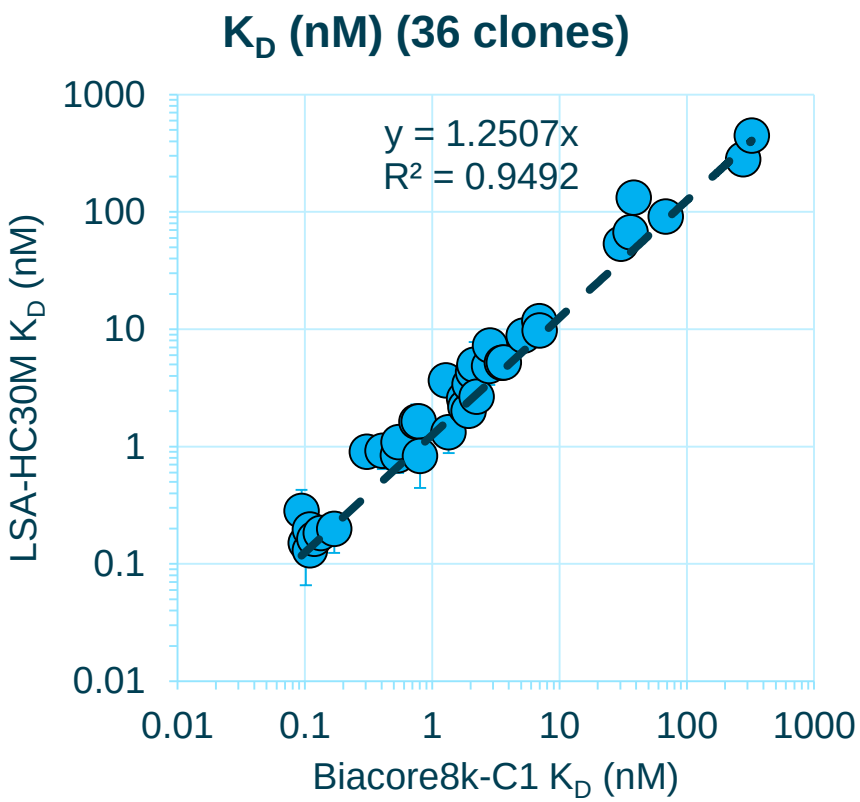
Benchmark LSA vs Biacore 8K



- **Excellent agreement in kinetic rate constants (within 2fold, when match chip types)**
- **Excellent agreement across wide affinity range <100pM to >100nM**
- **LSA is 50x faster than Biacore (384 vs 8 interactions at once)**
- **LSA consumes 1% sample of Biacore**
- **LSA analyzes 384 binding interactions in a single work day's run vs multiple runs/days to achieve similar throughput on Biacore**
- **LSA has powerful batch-mode fitting software to facilitate analysis**

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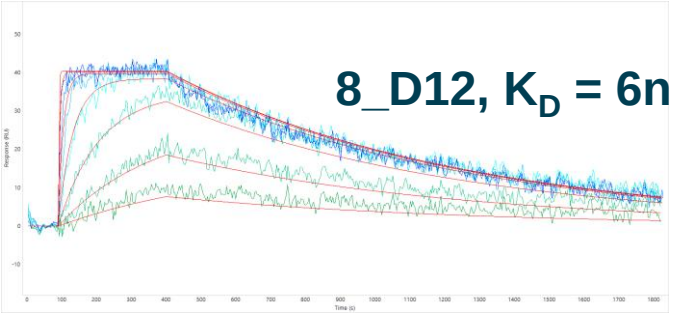
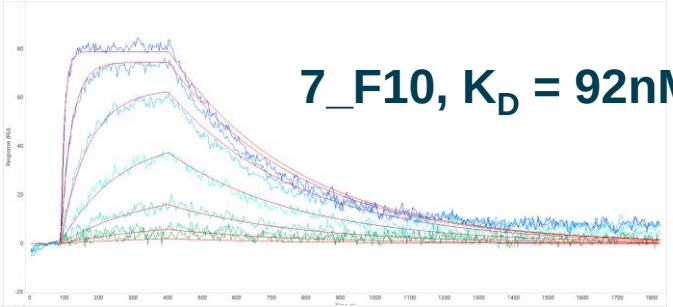
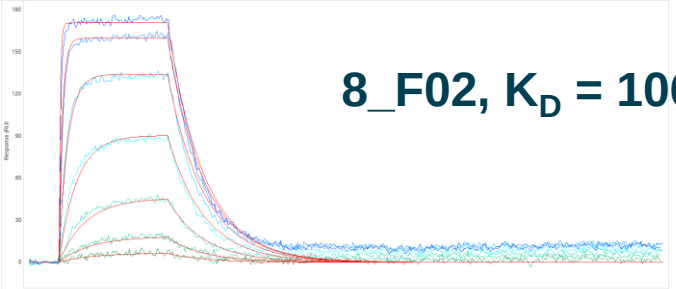
Capture kinetics of a 192-scFv array from crude periplasmic extracts via anti-V5 mAb coated chip



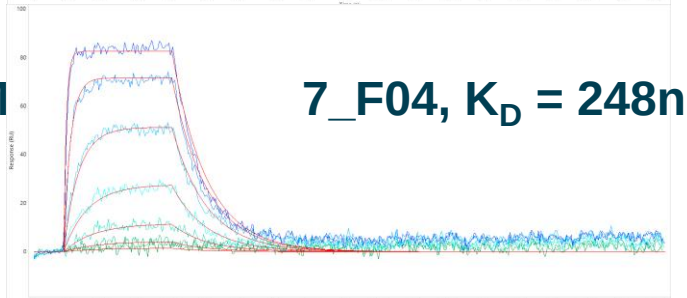
8_D12, K_D = 6nM



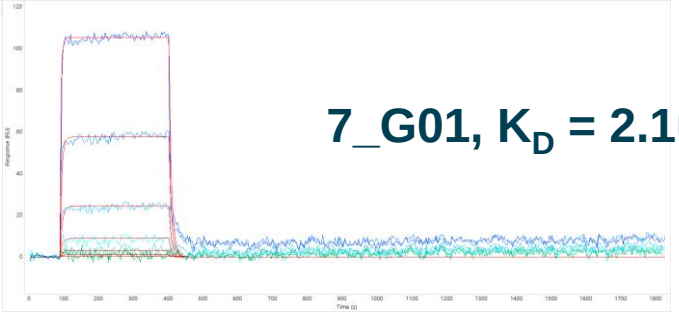
7_F10, K_D = 92nM



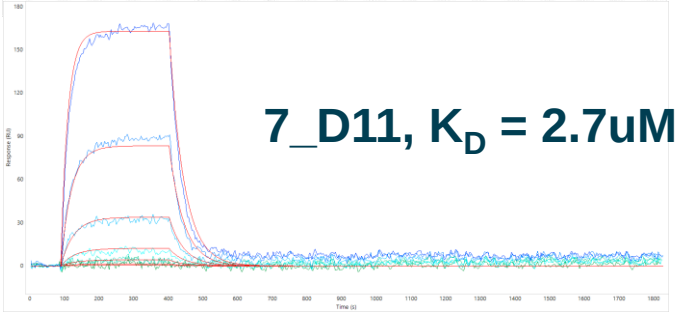
8_F02, K_D = 106nM



7_F04, K_D = 248nM



7_G01, K_D = 2.1uM



7_D11, K_D = 2.7uM

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Why It's All About The Epitope

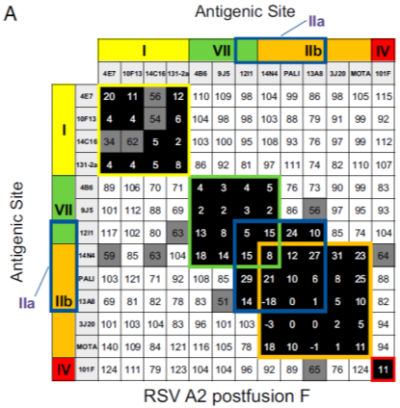
- Understanding a mAb's **mechanism of action (MOA)** is fundamental to the discovery of efficacious and safe therapeutics
- A mAb's epitope largely dictates its biological function, so sorting mAbs by their epitope is more relevant than affinity ranking them
- A mAb's epitope is an **innate** property that can be neither shifted rationally by engineering nor predicted/designed *in silico*, so relies on **empiric selection**
- Identifying mAbs targeting **unique** epitopes is highly desirable because they may offer differentiated MOA's and associated IP opportunities
- Surveying the epitope landscape of your mAb campaign at early-stage research where large numbers of clones are available in low quantities provides **functionally-relevant** information for triaging hits and prioritizing resources
- ***Array SPR can rapidly sort mAbs into epitope families using mapping and binning methods***

High Throughput Epitope Binning

384x384 mAb competition matrix reveals exquisite epitope differentiation

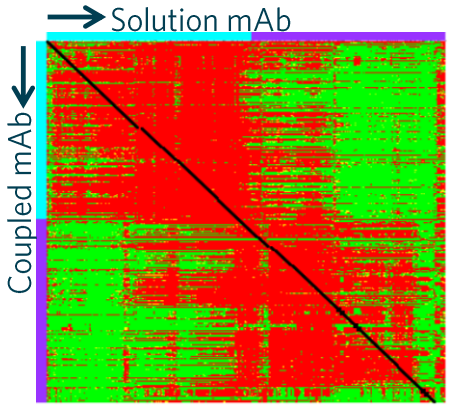
- Triage large mAb panels, identify unique/nuanced binders
- Low sample consumption - facile setup - streamlined analysis
  - Only 5 µg/mAb required regardless of array size
  - One sample for array printing, one sample as analyte
  - ≈30 - 50 µg antigen for 384 x 96 array, 120-200 µg for 384 x 384 binning (147,000 Interactions)

13 x 13 (BLI)

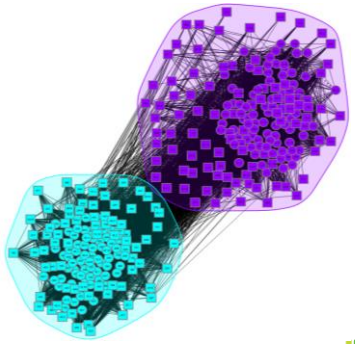


Mousa *et al*, PNAS 2016

384 x 384 (Array SPR)



Community plot



Sivasubramanian *et al*, MABS 2016

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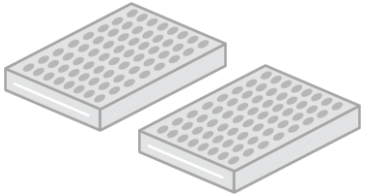
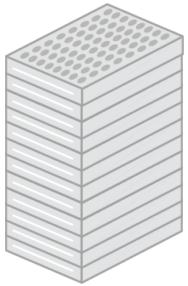
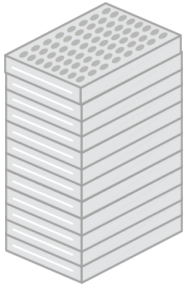
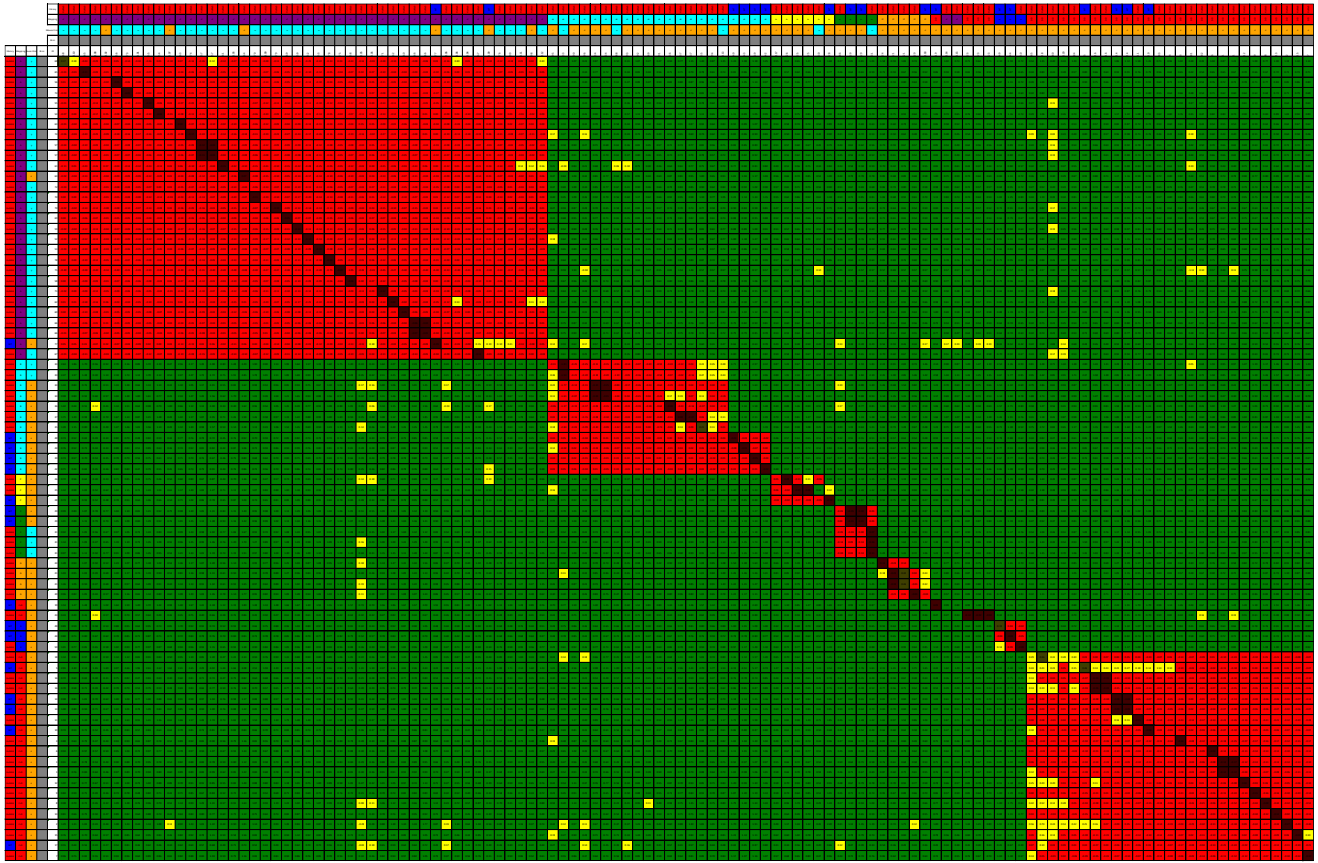
Transforming the Binning Paradigm

	LSA	8K	HTX
Antibody consumption per mAb	 5 µg	 >1000 µg	 >1000 µg
384-well plates required	 2 plates / 4 days	 10 to 100+ / 1 month	 10 to 100+ / 1 month

192 mAb Binning: Anti-PGRN



Ching et al, MABS 2017

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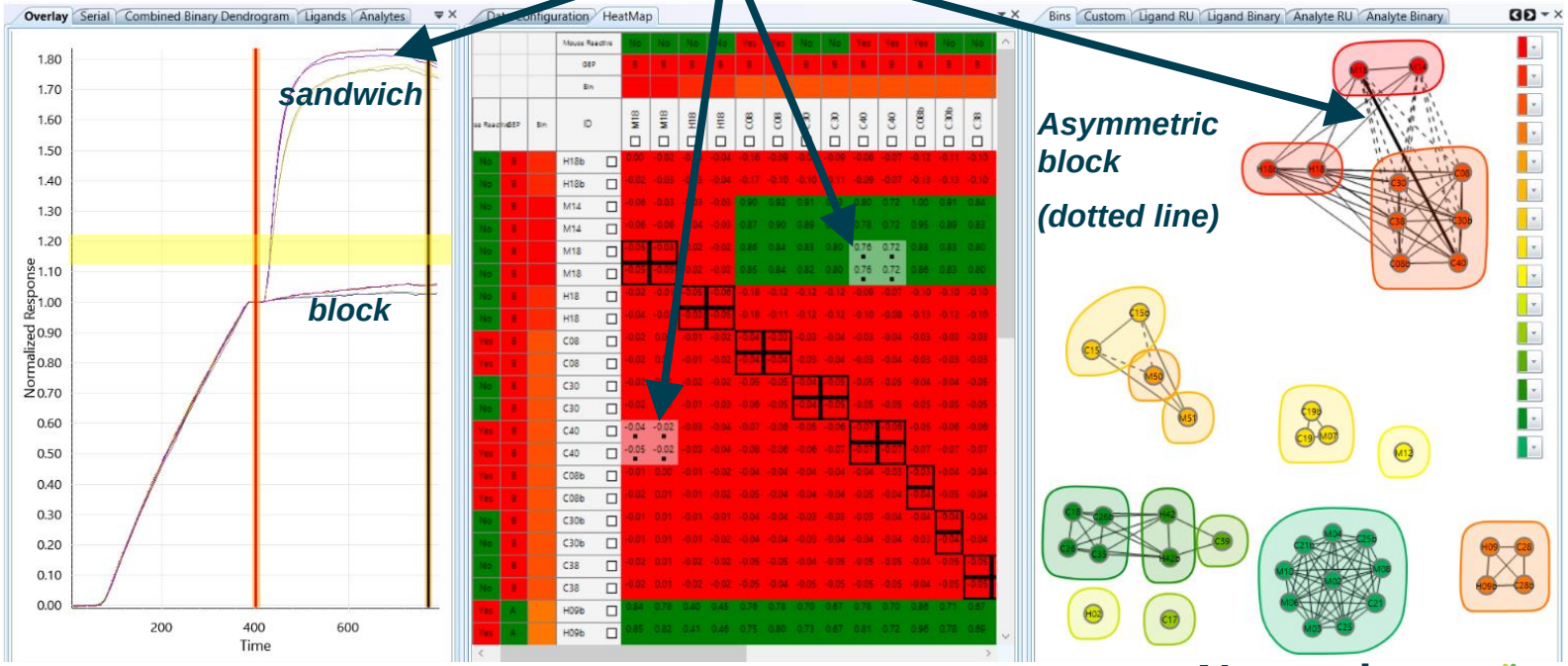
Epitope Binning Software User Interface

Data Linked Across Three Visualization Panels



Sensorgrams **Heat Map** **Networks**

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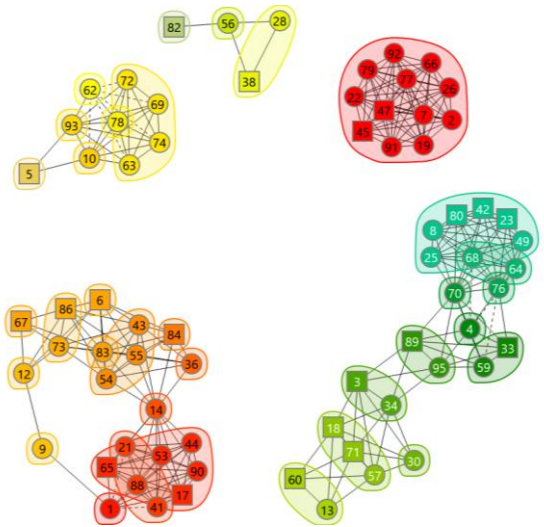
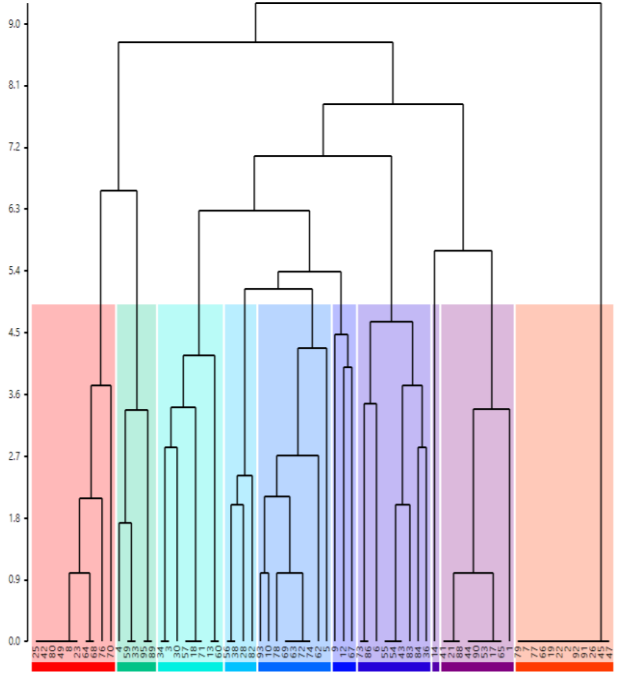
Dendrograms and Community Plots

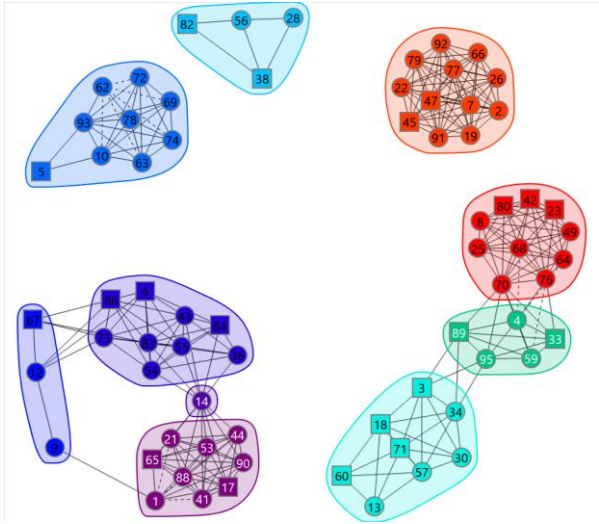
Networks (most granular bins)



Dendrogram



Communities (user-defined cut-height)



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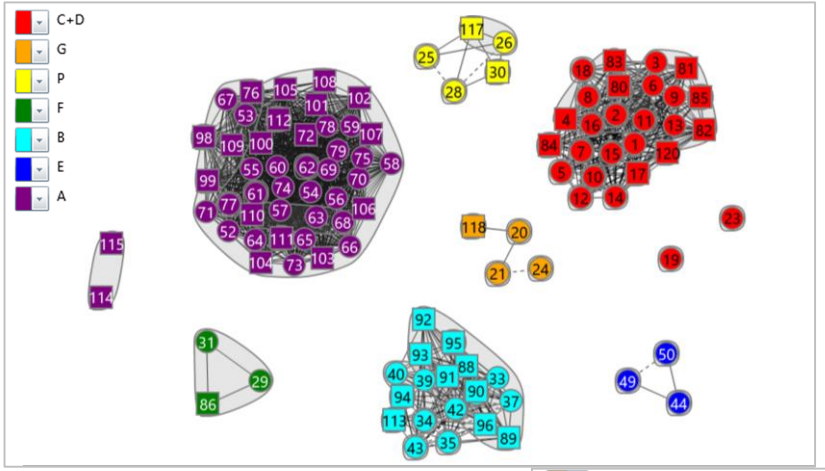
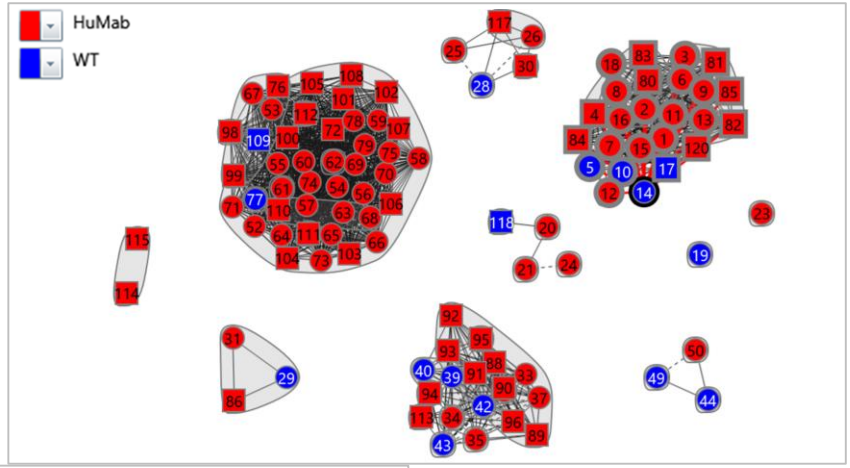
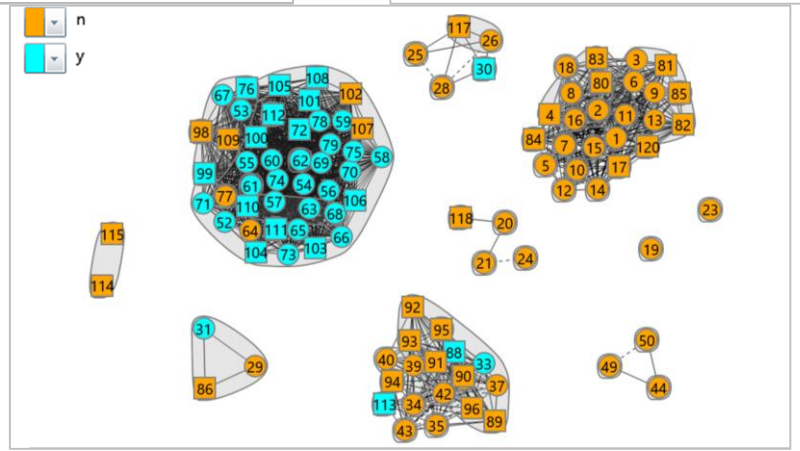
Color By Orthogonal Data

Library





Subdomain



Mouse Cross-Reactivity

Ching *et al*, MABS 2017

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Summary of the LSA

- **High Throughput Array SPR is a disruptive technology**
- **Shifts SPR upstream to screening, compared with its traditional role as a secondary tool**
- **Screening and characterization are *THE SAME STEP***
- **Enables detailed kinetic, affinity, and epitope characterization to be obtained earlier in drug discovery, accelerating library-to-leads**
- **Kinetic rate constants agree closely with Biacore's but are generated 48x faster and with only 1% sample consumption**