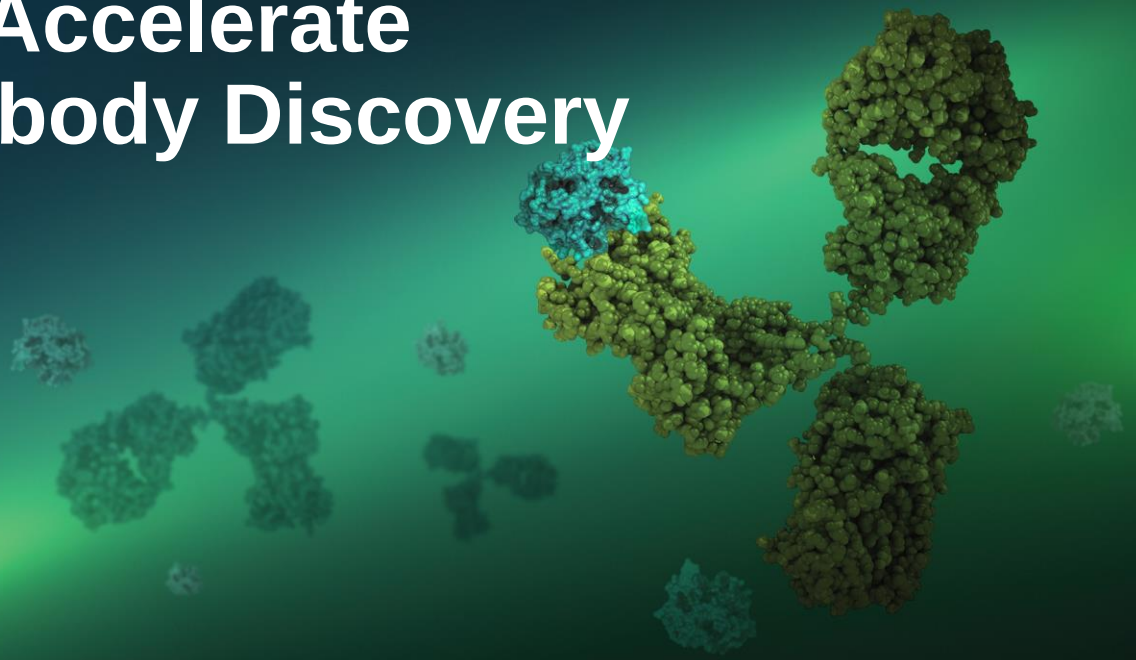
Expanding SPR Throughput Orders of Magnitude to Accelerate Therapeutic Antibody Discovery

Yasmina Noubia Abdiche, PhD
Chief Scientific Officer, Carterra

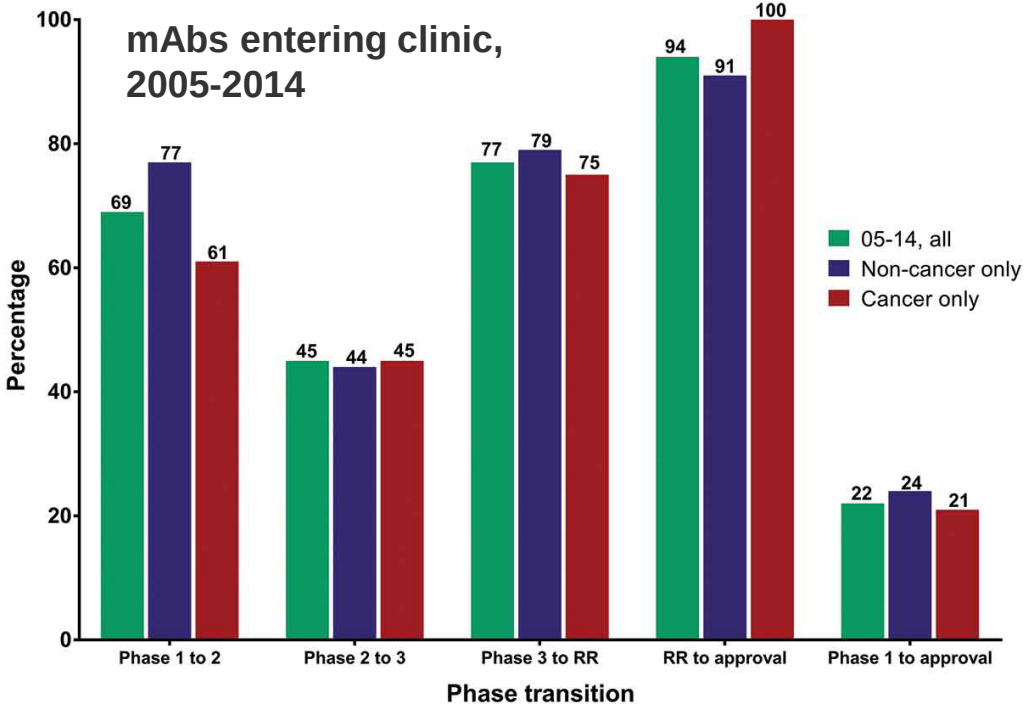


The Therapeutic Antibody Market is Lucrative and Growing

- mAbs are bringing transformative medicines, even “cures”, to patients
- mAbs dominate the top 10 blockbuster drugs
- A diverse, innovative and robust clinical pipeline ensures market growth
- Also, mAbs provide biosimilars, companion diagnostics, and reagents

carterra

Drug Discovery is Costly, Tedious, and Fraught with Failure



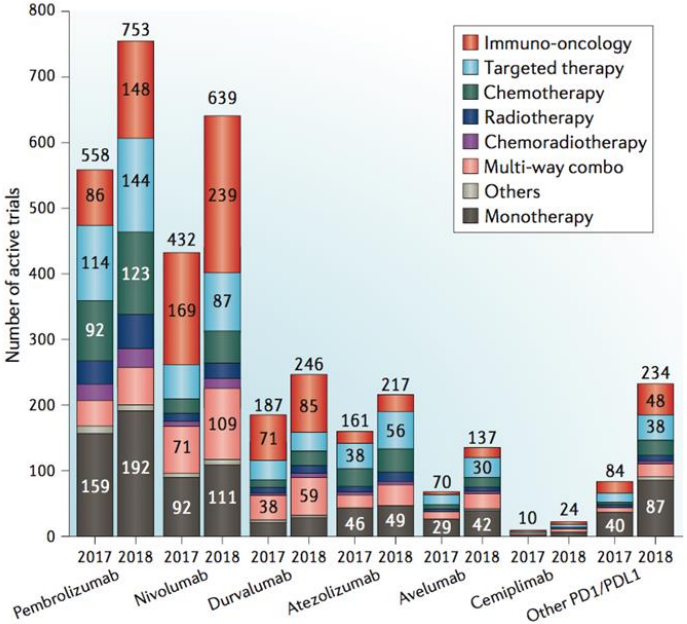
Antibodies to watch in 2019, Kaplon and Reichert, MABS 2018

- **Only 20% Phase I drugs achieve market approval**
- **On average, US$ 1 billion and 12 years from bench to market**
- **Understanding Mechanism of Action (MOA) is key to clinical success**

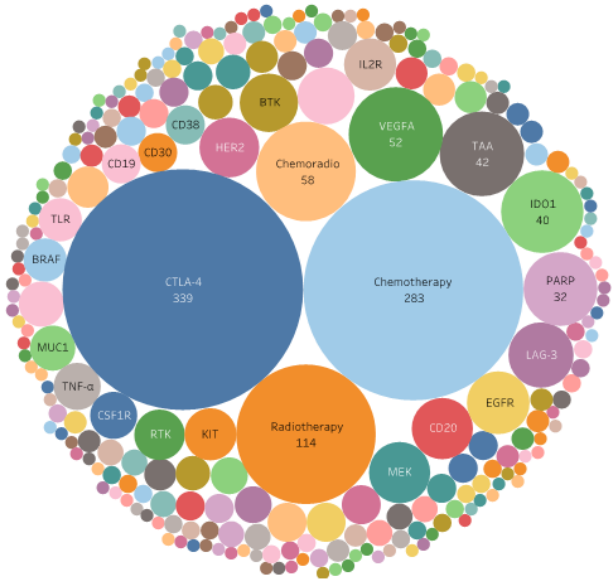
carterra

Prominence of Immuno-Oncology: PD-1/PD-L1 Clinical Landscape

Breakdown of trials (total 2,250 in 2018) by mAb and their combination with other targets/therapies



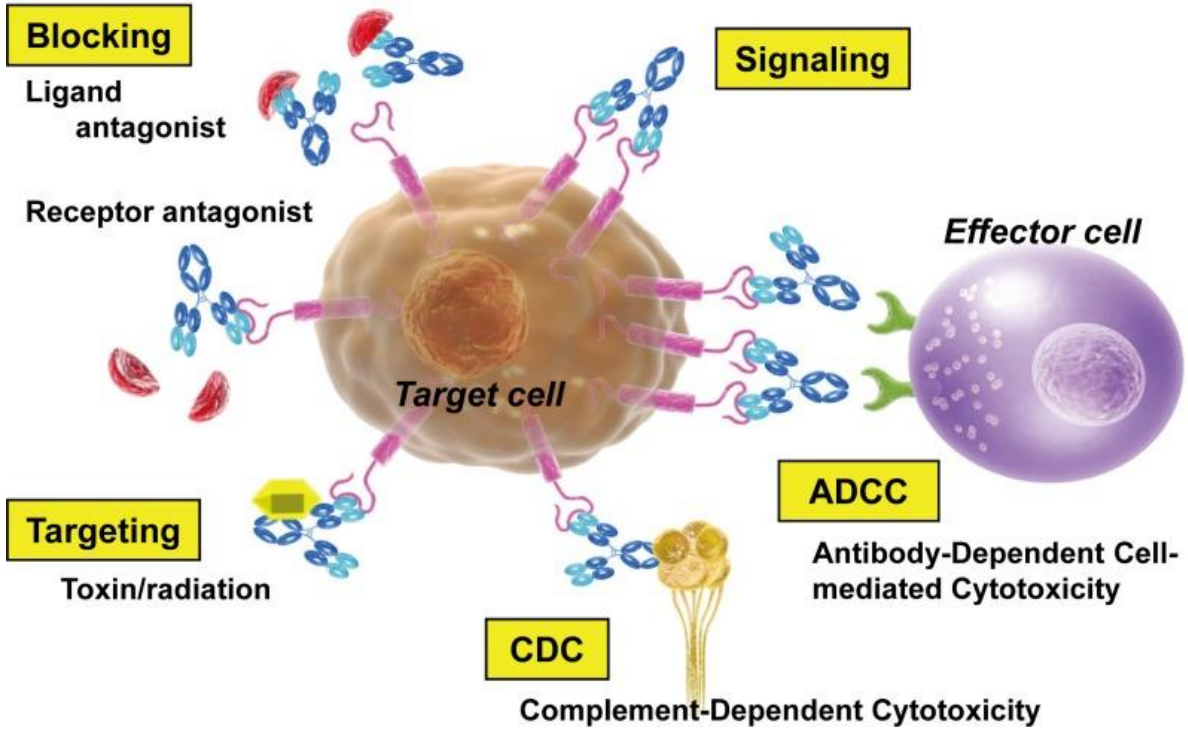
Distribution of trials (total 1,716) in combo with other (250) targets/therapies



The clinical trial landscape for PD1/PDL1 immune checkpoint inhibitors
Tang et al, Nature Reviews Drug Discovery 2018

carterra

Therapeutic mAbs Work via Different MOAs



Therapeutic antibodies: their mechanisms of action and the pathological findings they induce in toxicity studies (review), Suzuki et al, J Toxicol Pathol 2015

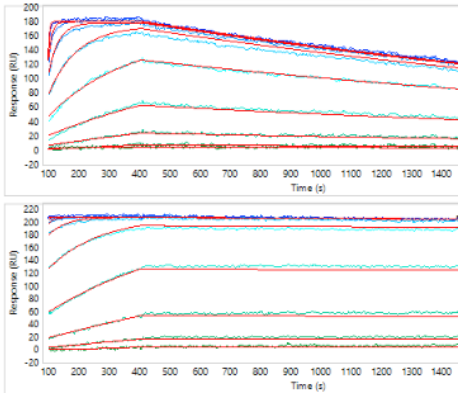
carterra

High Throughput SPR: A Disruption in Antibody Characterization

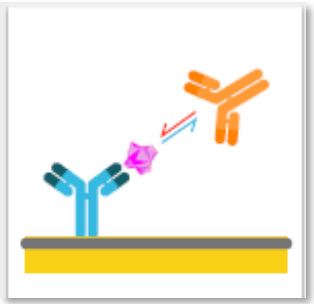
- MAbs can achieve high specificity and affinity - leveraged in drug discovery
- Binding kinetics, affinity, and epitope are crucial parameters in assessing a mAb's quality and fitness for a given therapeutic application, because they inform **Mechanism Of Action**
- SPR is the *de facto* technique for measuring binding kinetics and affinity
- Carterra's LSA expands the throughput of SPR orders of magnitude compared with other platforms, shifting the role of SPR upstream to screening stages where it can streamline the library-to-leads triage
- Additionally, the LSA's 384-ligand capacity enables high throughput epitope binning studies, transforming the binning paradigm and revealing exquisite epitope resolution to guide lead selection and secure IP
- Minimal sample consumption and facile assay set-up
- Fast and intuitive data analysis via dedicated Kinetic and Epitope software

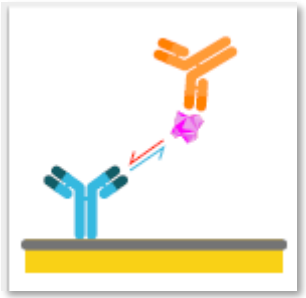
LSA's Core Applications

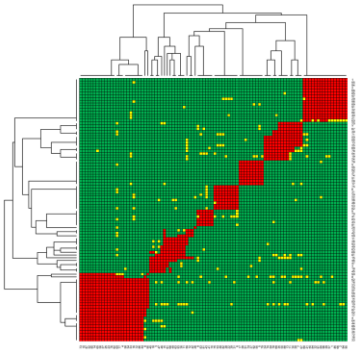
Kinetics/Affinity

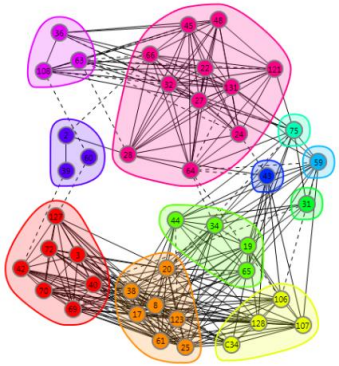


Epitope Binning

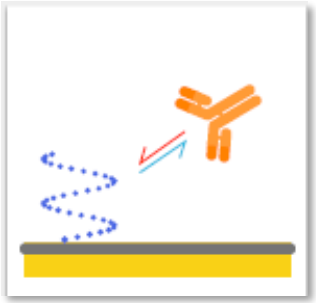


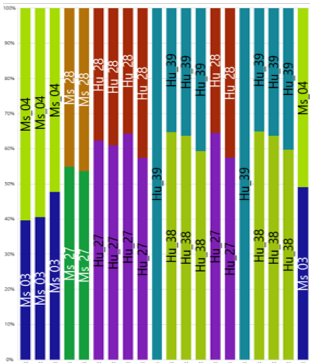






Mapping



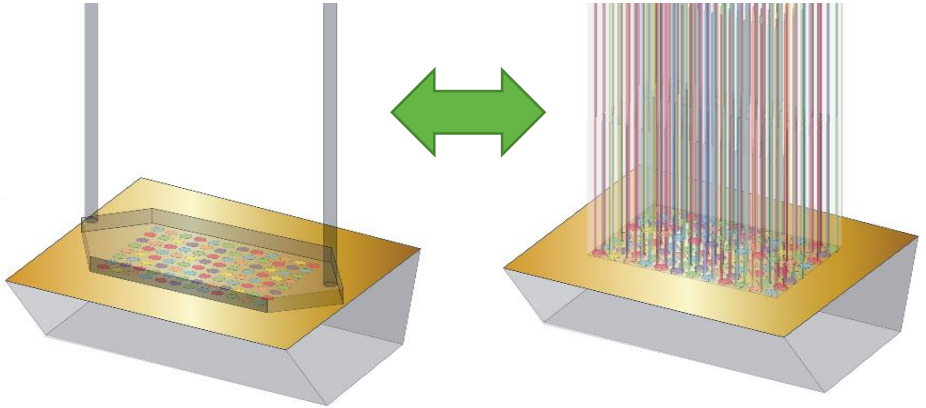


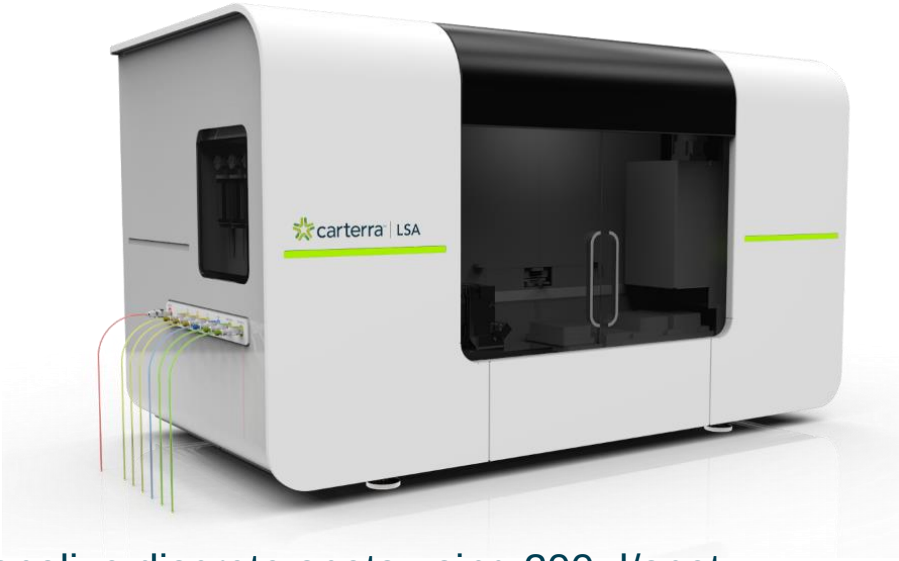
carterra

The LSA Automates a Choreography between Two Microfluidic Modules

Single Flow Cell (SFC)

96-Channel Printhead (96PH)





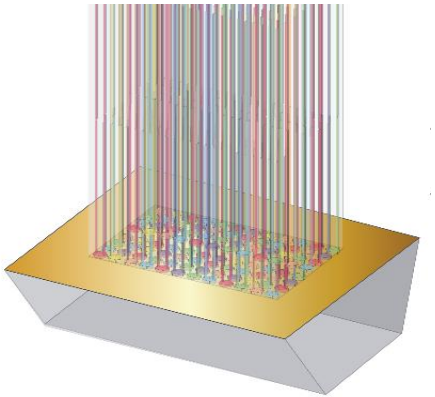
- Inject 250µl over entire array
- 1-on-384 (analyte-on-ligand) mode
- Coat chips as a “lawn”
- Exceptional sample efficiency

- Functionalize discrete spots using 200µl/spot
- Create 384-ligand array using 4 serial docks of 96PH
- Recover samples (-15µl/print)

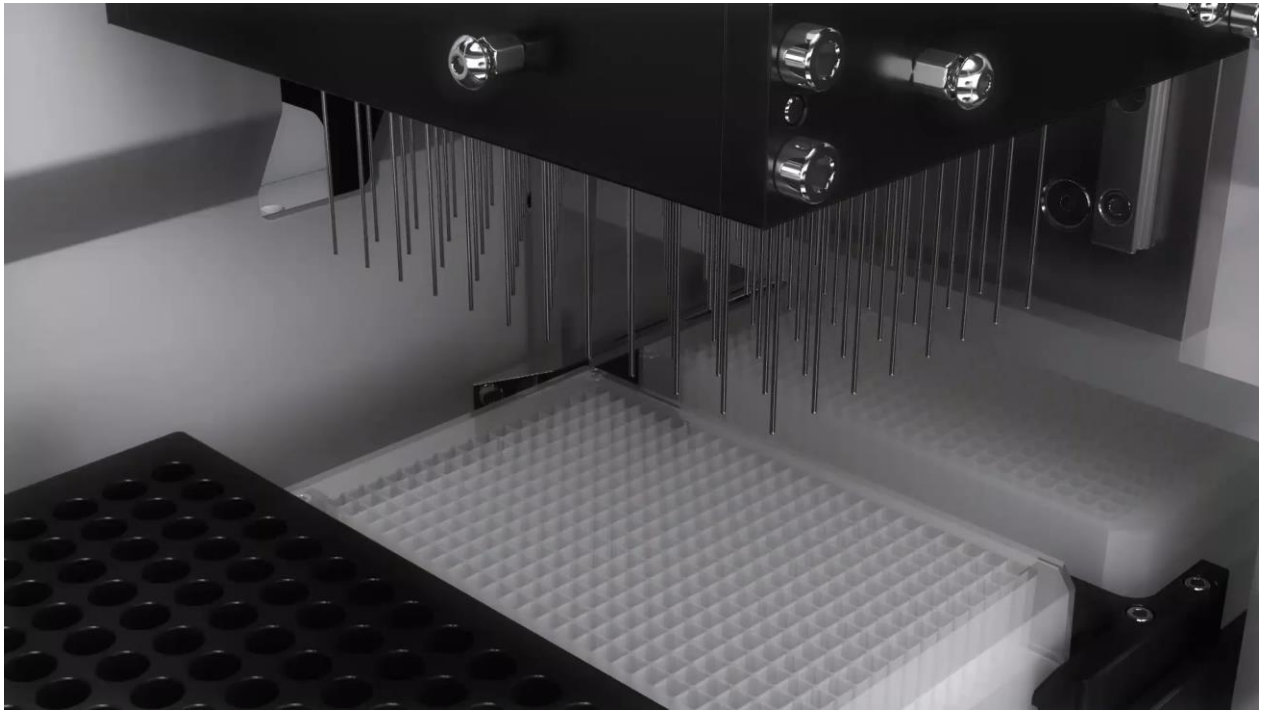
carterra

LSA Integrates Flow Printing

96-Channel Printhead (96PH)



Create 384-ligand array via 4 serial docks of 96PH

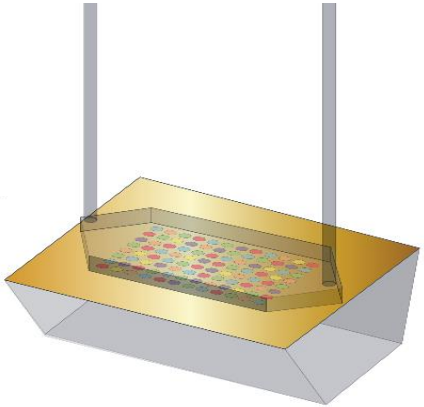


Deck accommodates 3x 96/384 plates

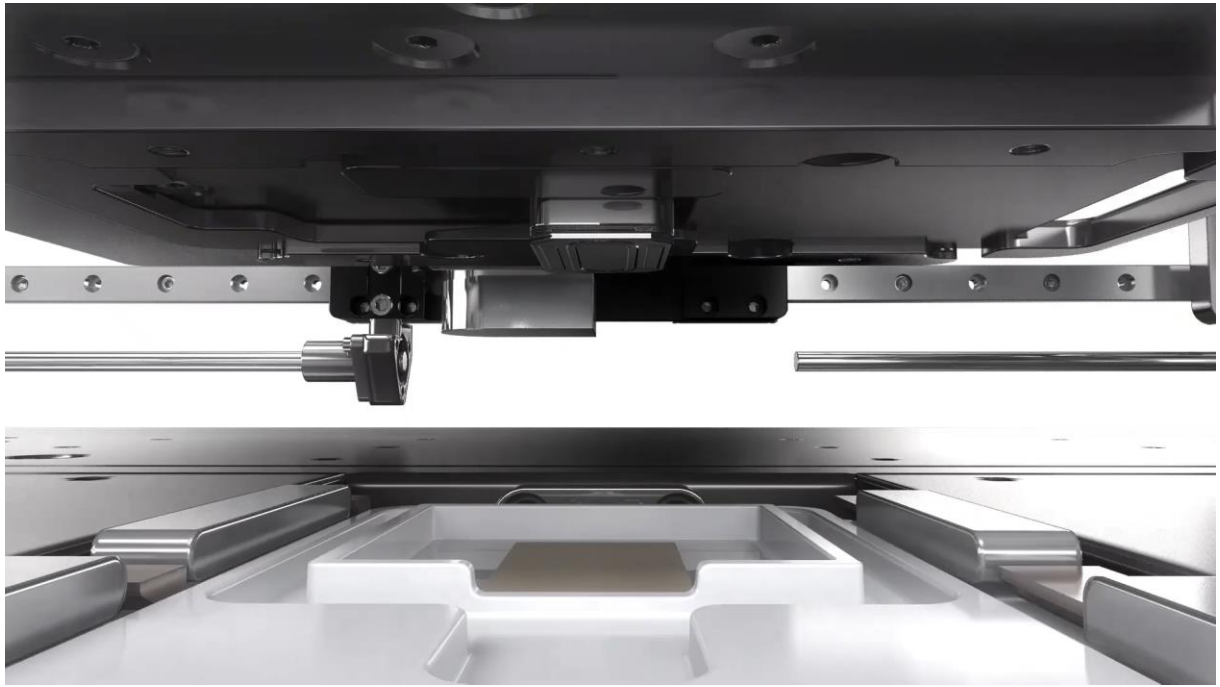
carterra

LSA Integrates High Throughput SPR

Single Flow Cell (SFC)



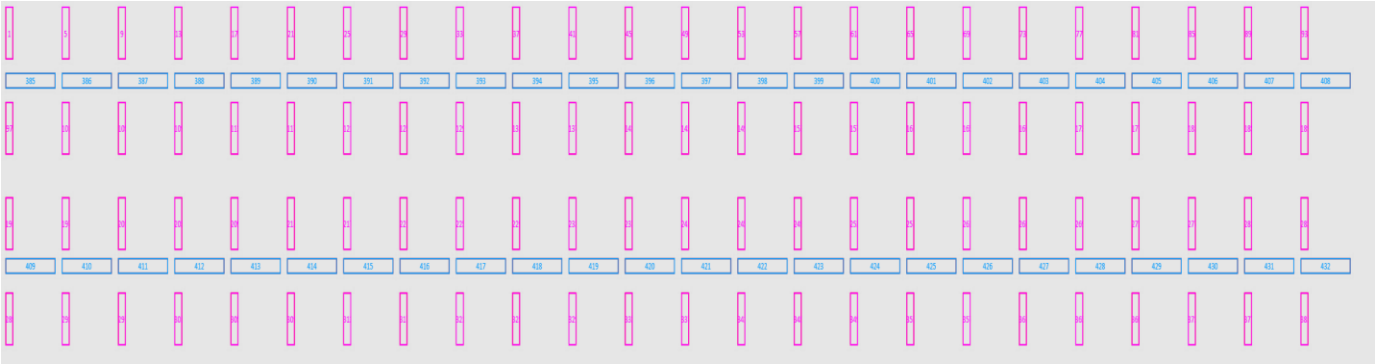
Inject 250µl analyte over entire array in a “1-on-384” analyte-on-ligand mode



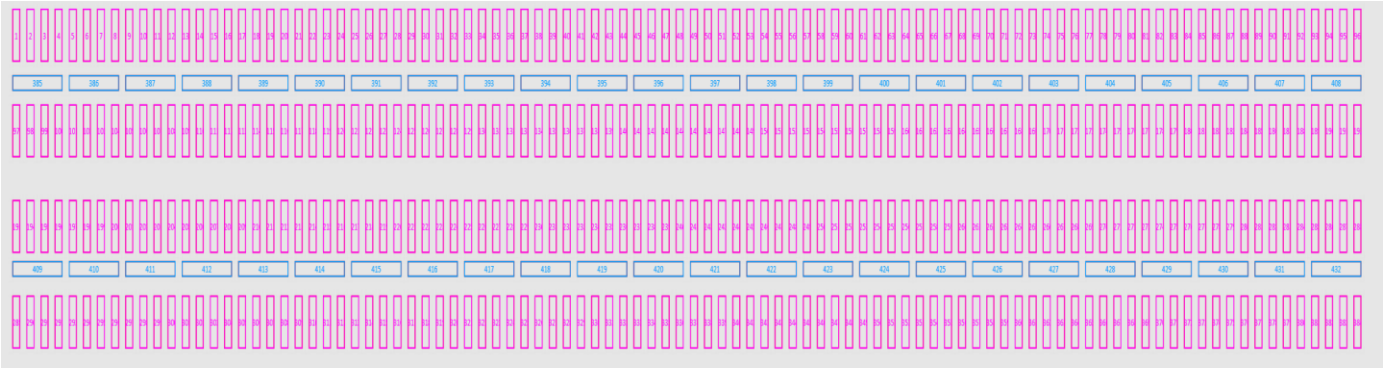
Deck accommodates 2 plate positions: sample block and 96/384 plate

carterra

Flow Print a 384-Ligand Array on the LSA

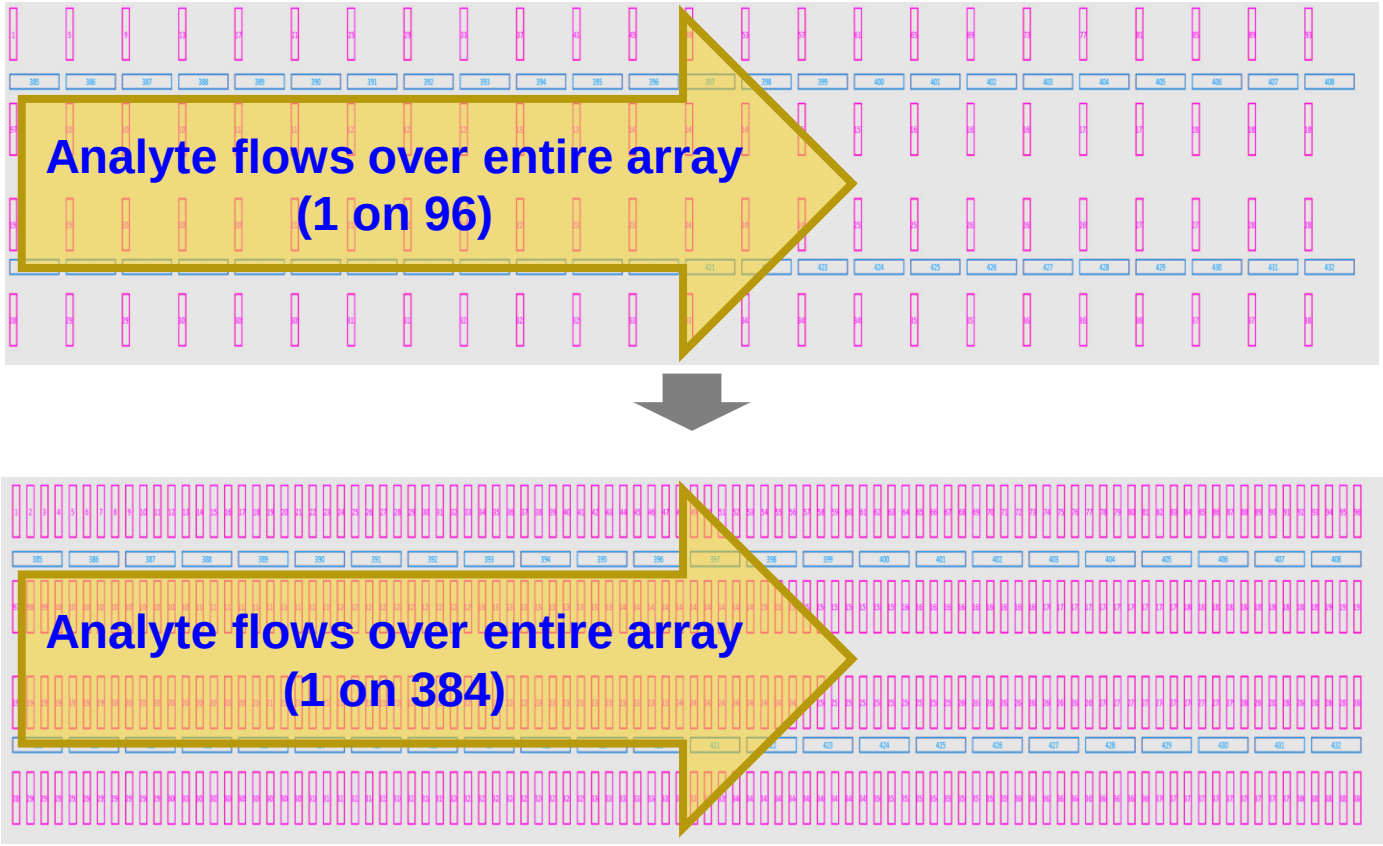


Serially dock the 96PH at 4 nested locations to create a 384-ligand array



carterra

Minimal Sample Consumption

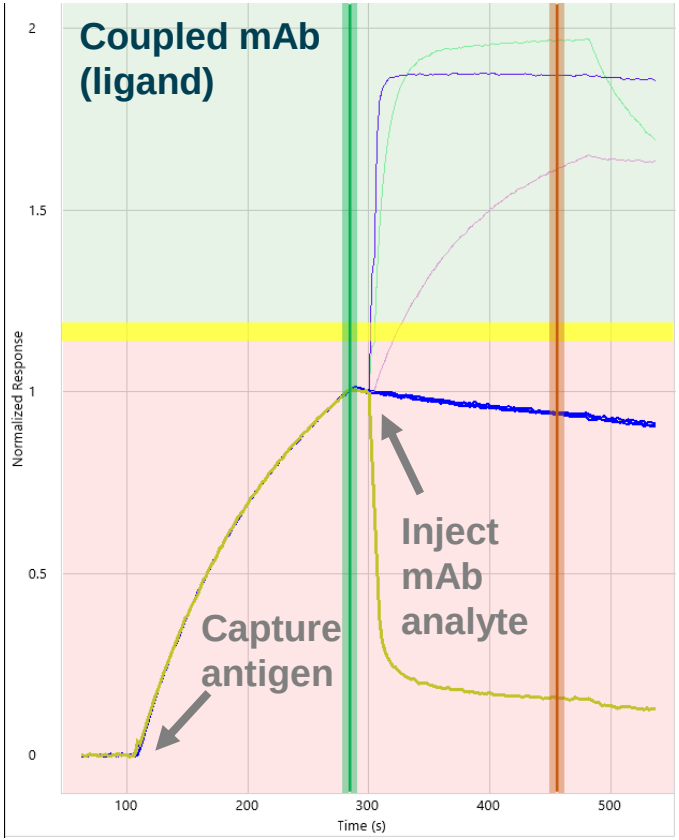


carterra

Why It's All About The Epitope

- Epitope influences a mAb's MOA
- Epitope is innate; it can neither be predicted by *in silico* methods, nor rationally shifted/optimized by engineering, so must be SELECTED
- Epitope can be used to secure IP
- Combining different MOA's often produces superior therapeutic outcome
- High throughput binning can reveal the epitope landscape of an antibody library, and when merged with functional data, can identify biologically-significant epitope clusters.
- **Epitope diversity is a surrogate for functional diversity**

Epitope Binning on the LSA



Analytes that sandwich pair with ligand

Buffer (no sandwiching/ blocked)

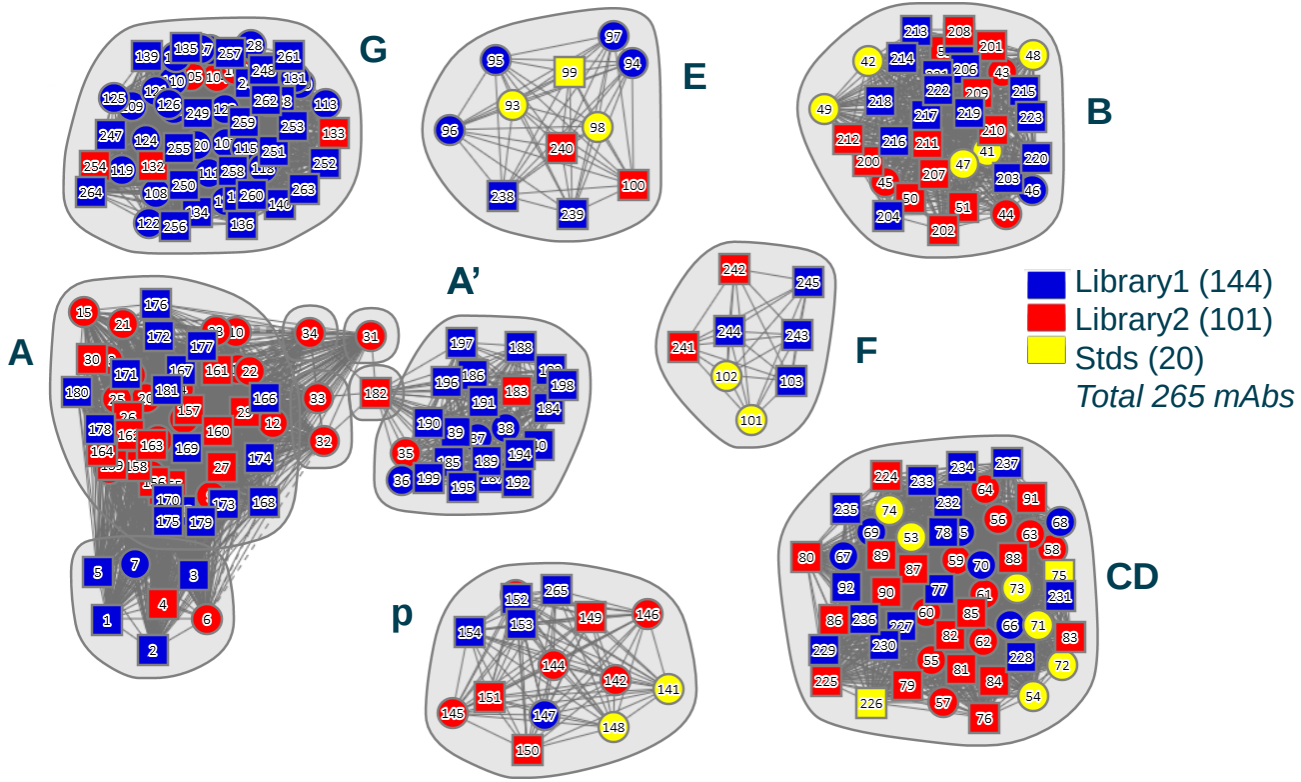
Displacement (< buffer baseline)

antigen + mAb analyte captures are cycled over the array

- A comprehensive epitope binning experiment scales geometrically with the size of the mAb panel
- The LSA provides a simple way to explore a full 384 x 384 competition matrix on a single chip using <10µg/mAb and <200µg antigen
- Powerful analysis software

carterra

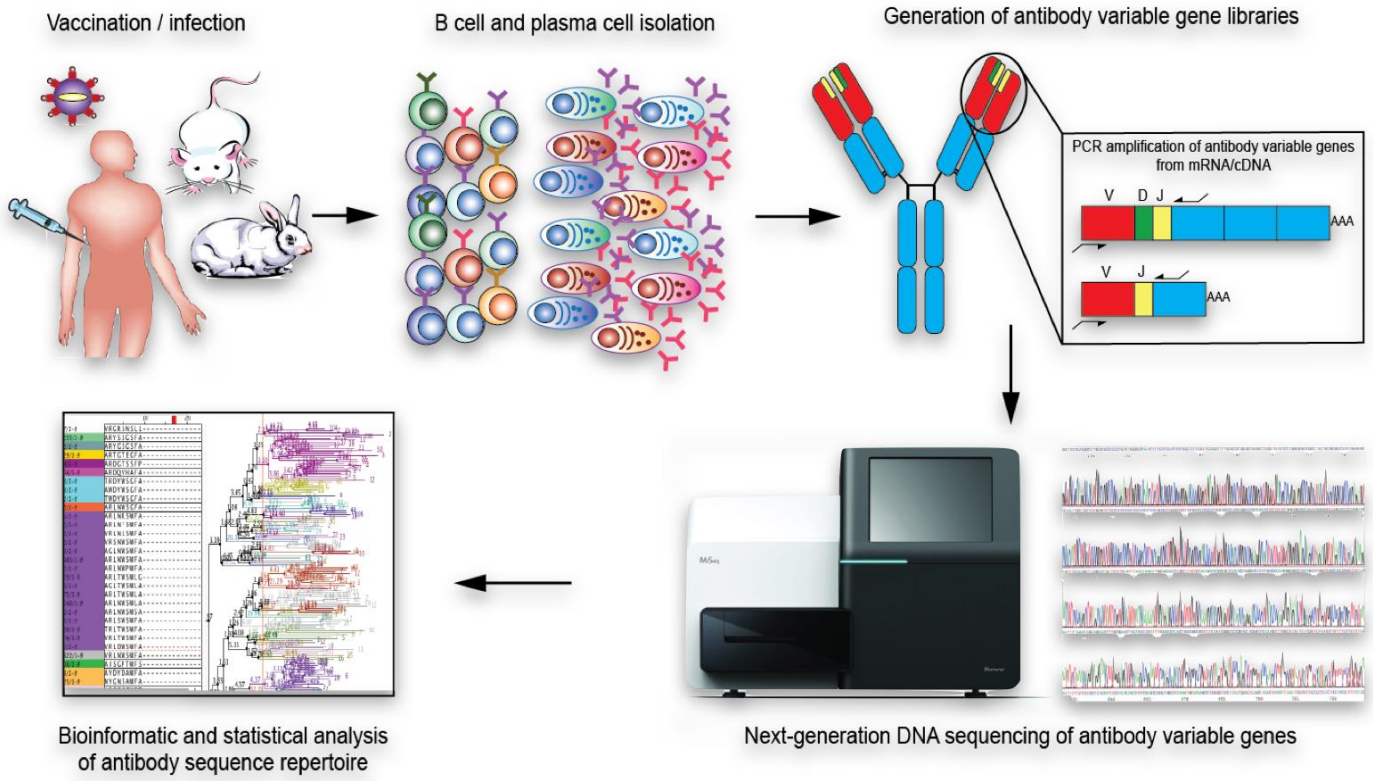
Using Epitope Binning to Benchmark Discovery Platforms – OmniChicken®



Ching et al, submitted to MABS 2019

carterra

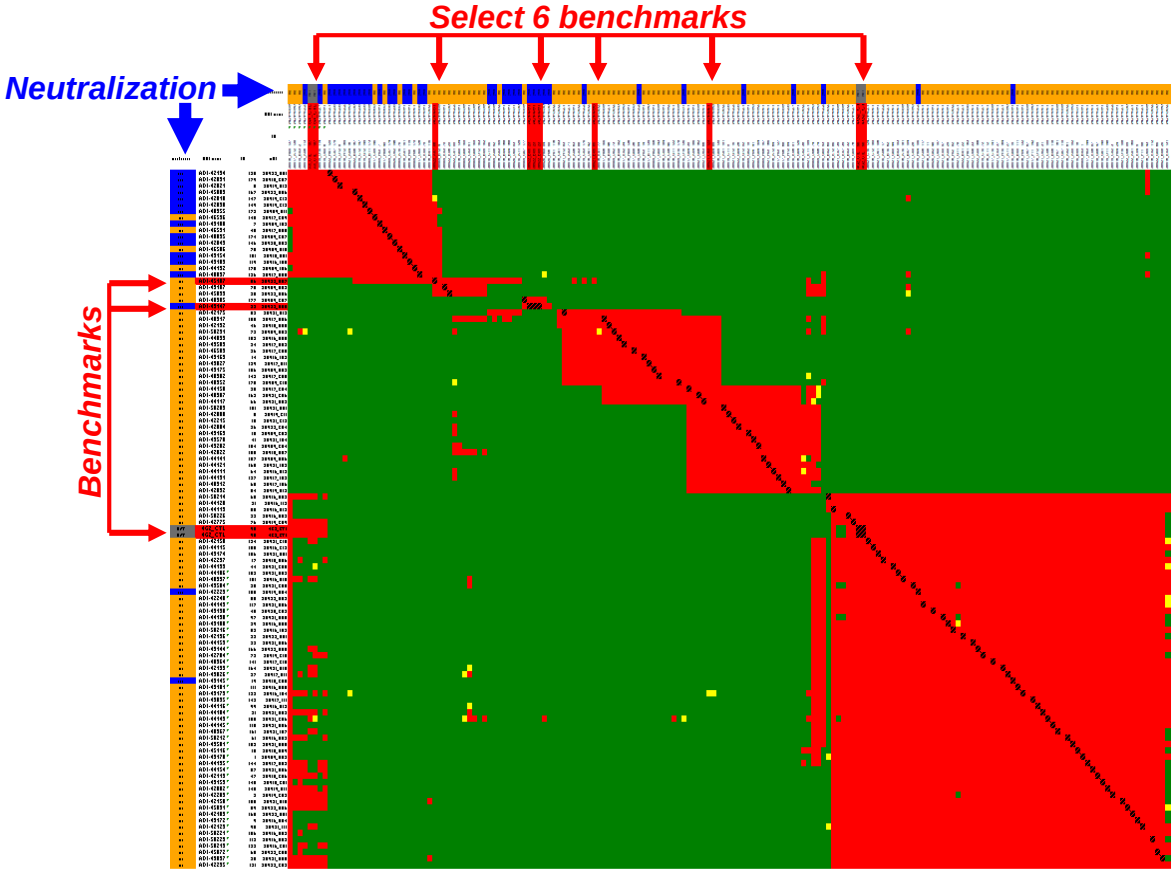
Therapeutic mAb Cocktails for Infectious Disease



- The human immune response can be used to source therapeutic mAbs to treat infectious disease
- Isolate mAbs by B-cell cloning of vaccinated/infected individuals
- Sequence diversity is necessary ***but not sufficient*** to produce functional diversity
- Epitope diversity is a surrogate for functional diversity
- Epitope binning can reveal the epitope landscape

carterra

Therapeutic mAb Cocktails for Infectious Disease



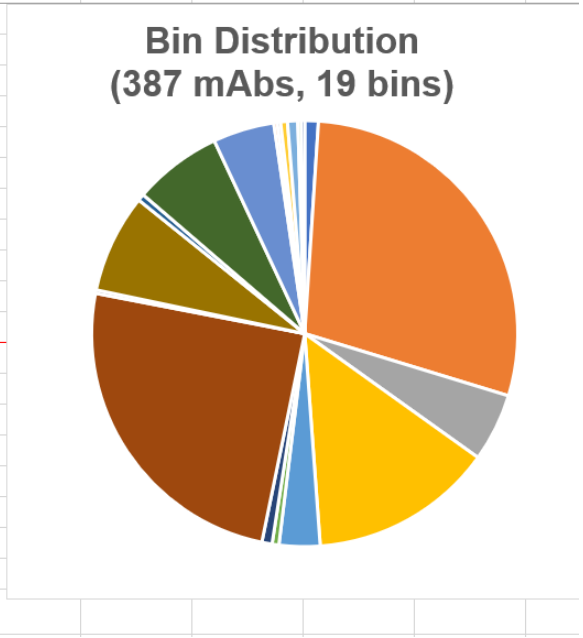
- High throughput binning revealed the epitope landscape of the human immune response
- Inform selection of a few benchmark clones to probe a bigger library
- Merge with neutralization data to understand the **functional space**

Manuscript submitted to Nature Immunology

carterra

Therapeutic mAb Cocktails for Infectious Disease

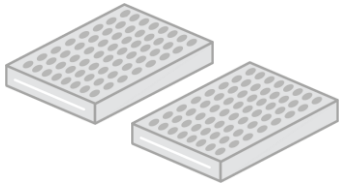
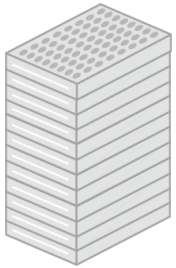
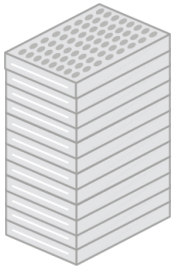
Bin name	CTL-1	CTL-2	CTL-3	CTL-4	CTL-5	CTL-6	Tally	#Neutralizers
0	0	0	0	0	0	0	4	0
1	1	0	0	0	0	0	111	5
2	0	1	0	0	0	0	20	8
3	0	0	1	0	0	0	54	2
4	0	0	0	1	0	0	12	1
5	0	0	0	0	1	0	2	0
6	0	0	0	0	0	1	3	2
12	1	1	0	0	0	0	96	9
23	0	1	1	0	0	0	1	1
24	0	1	0	1	0	0	29	16
34	0	0	1	1	0	0	2	0
35	0	0	1	0	1	0	26	1
45	0	0	0	1	1	0	18	2
135	1	0	1	0	1	0	1	0
245	0	1	0	1	1	0	1	0
345	0	0	1	1	1	0	2	1
1235	1	1	1	0	1	0	3	0
2345	0	1	1	1	1	0	1	0
12345	1	1	1	1	1	0	1	0
						total	*387*	*48*



- Multiple bins were functional
- Some rare bins showed exceptionally ***potent*** neutralization
- Inform the design of a broadly neutralizing cocktail

carterra

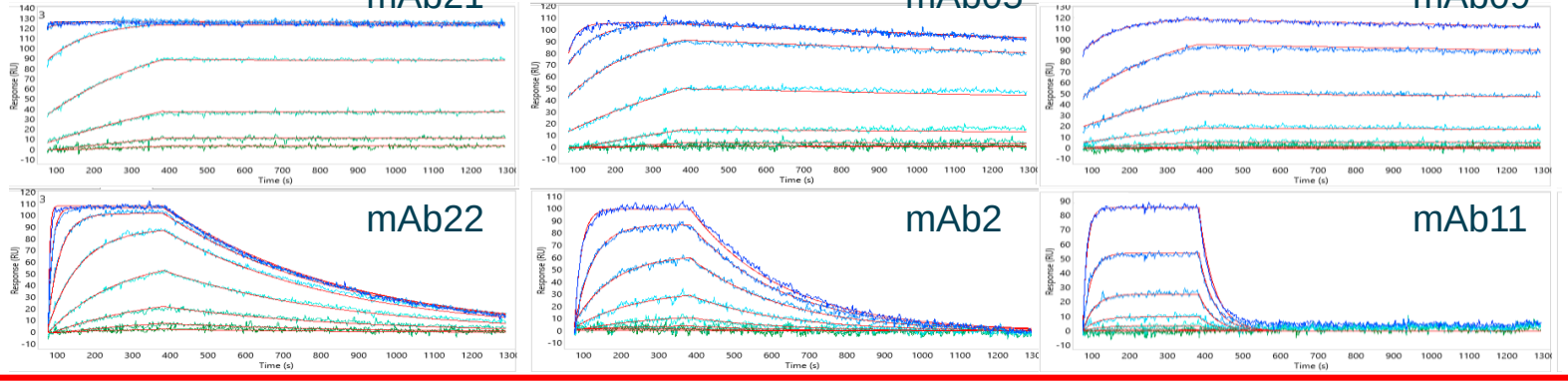
Transforming the Binning Paradigm

	LSA	8K	HTX
Antibody consumption per mAb	 5 µg	 >1000 µg	 >1000 µg
384-well plates required	 2 plates / 6 days	 10 to 100+ / 1 month	 10 to 100+ / 1 month

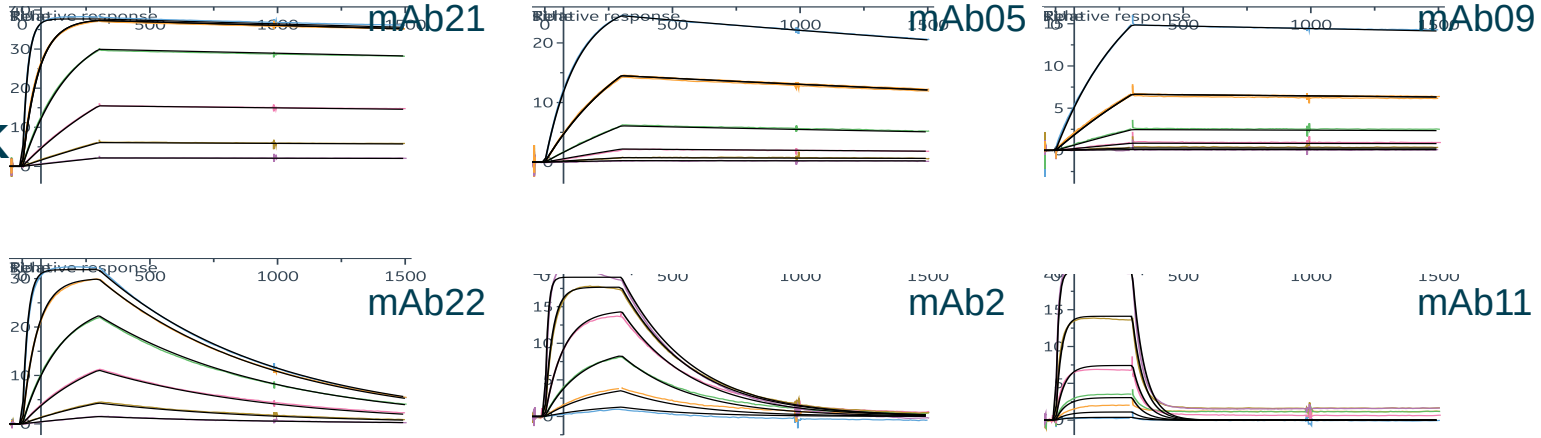
carterra

LSA Kinetics Benchmarked against Biacore 8K

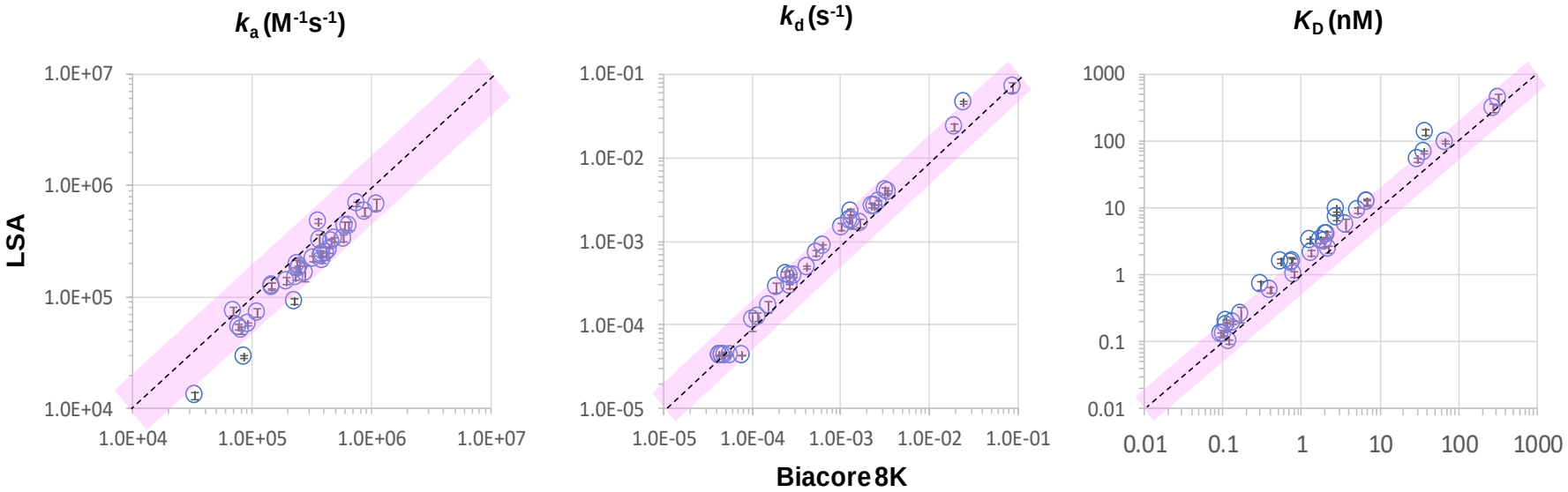
LSA



Bia8K



LSA Kinetics Benchmarked against Biacore 8K

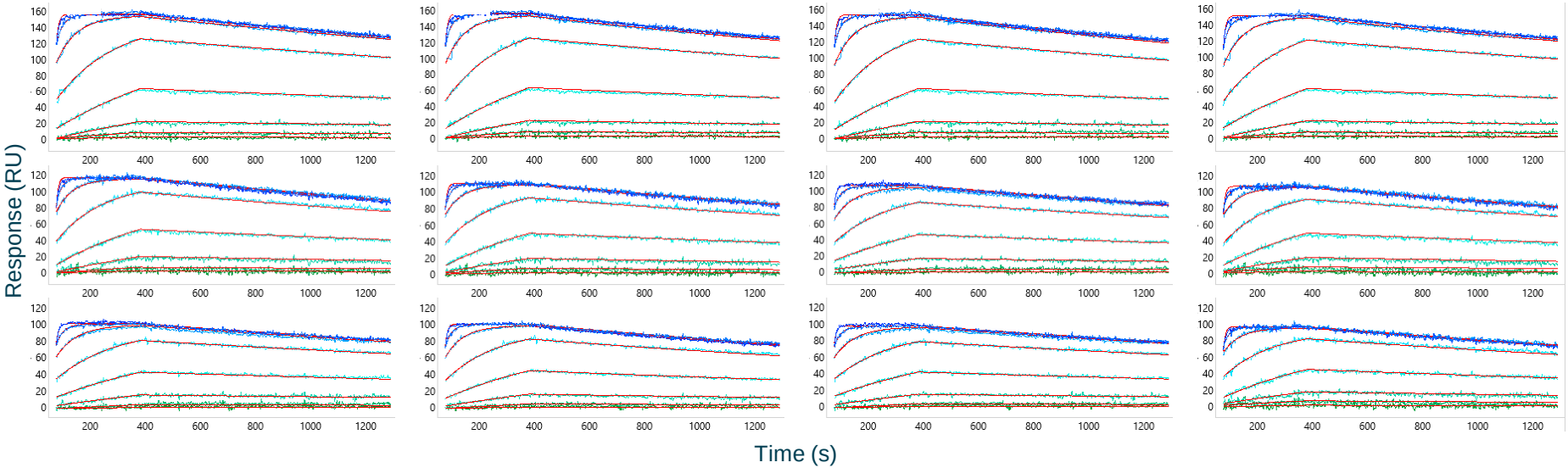


- Near-identical kinetic rate and affinity constants (within 2fold) across platforms
- Tested a panel of 32 kinetically-diverse mAbs
- LSA measurements are Mean ± StDev for 8-12 reps (spots) per mAb
- Biacore are single measurements

carterra

LSA Kinetics: Intra-Assay (Spot-To-Spot) Reproducibility

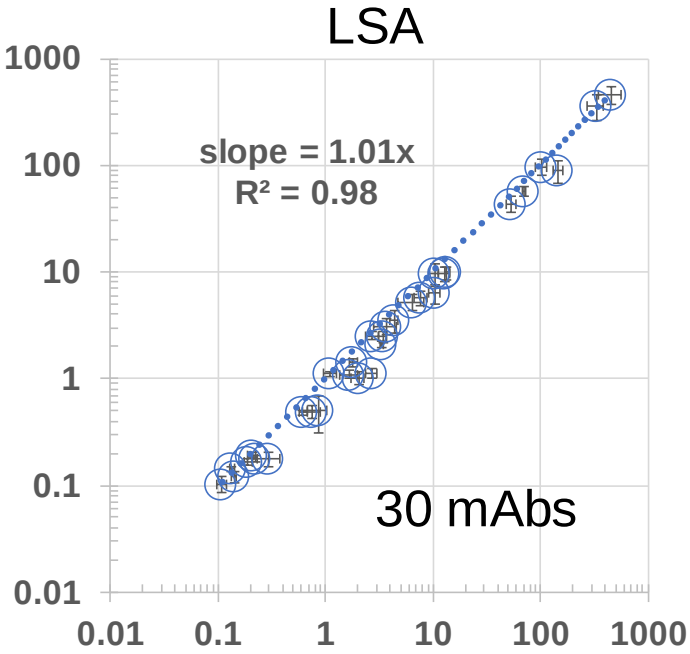
mAb 23, K_D = 1.5 ± 0.3 nM for N = 12



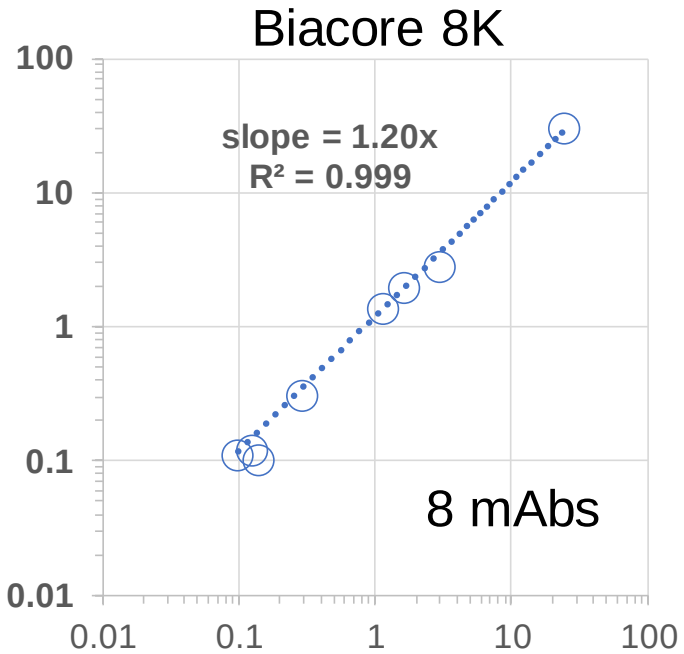
- For small panels of mAbs, use the full capacity of the 384-ligand array to explore surface capacity
- Combine measurements from optimum capacity spots and report parameters with statistical confidence (Mean ± StDev)

carterra

Inter-Assay Reproducibility: K_D values



Each measurement is Mean ± StDev (error bars) for 8-12 reps per interaction



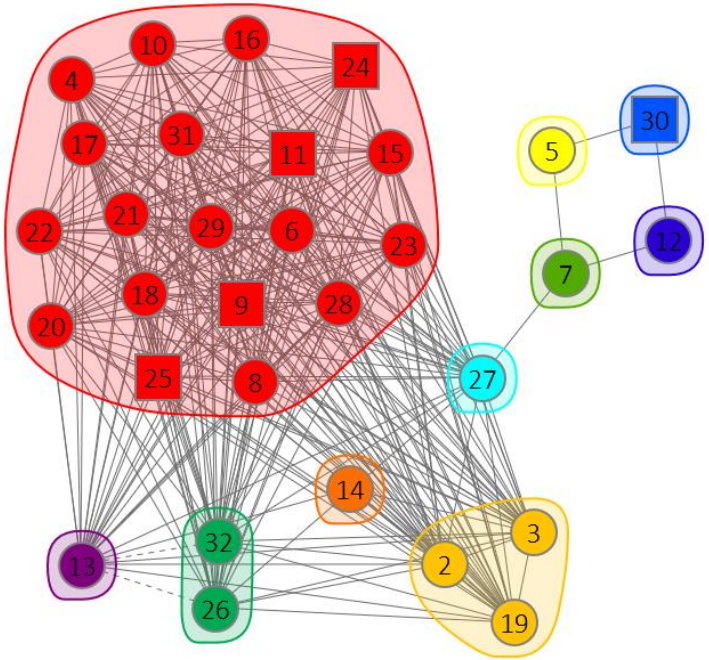
Single measurements

Manuscript submitted to PlosOne, Aug 2019

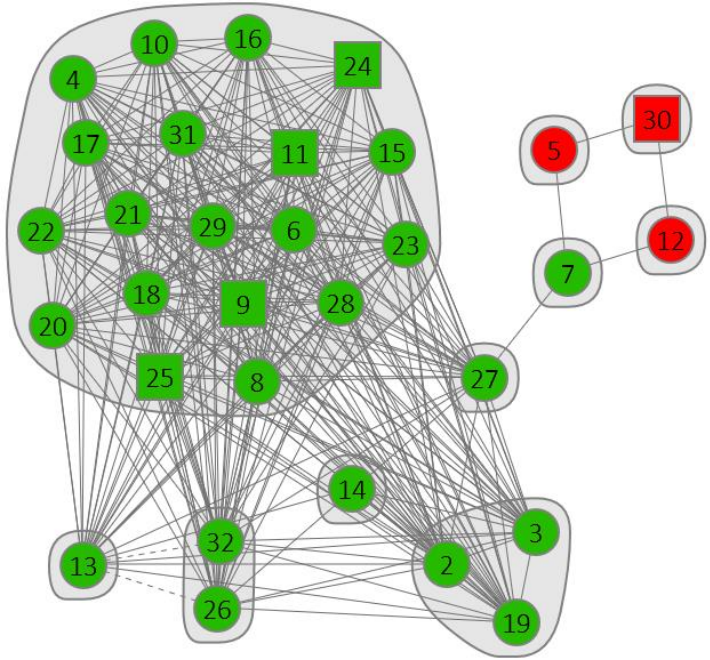
carterra

High Throughput Binning Provides Exquisite Epitope Resolution

Color by bin



Color by ligand blockade

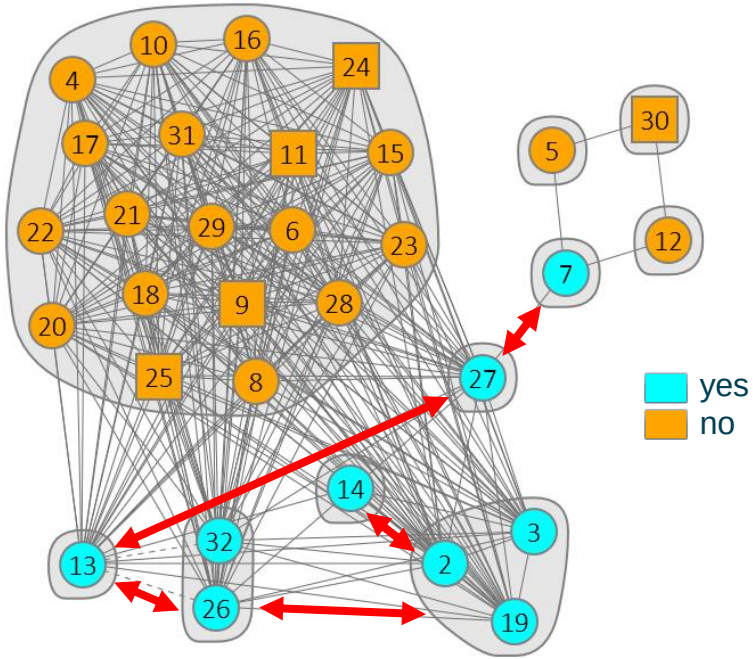


Manuscript submitted to PlosOne, Aug 2019

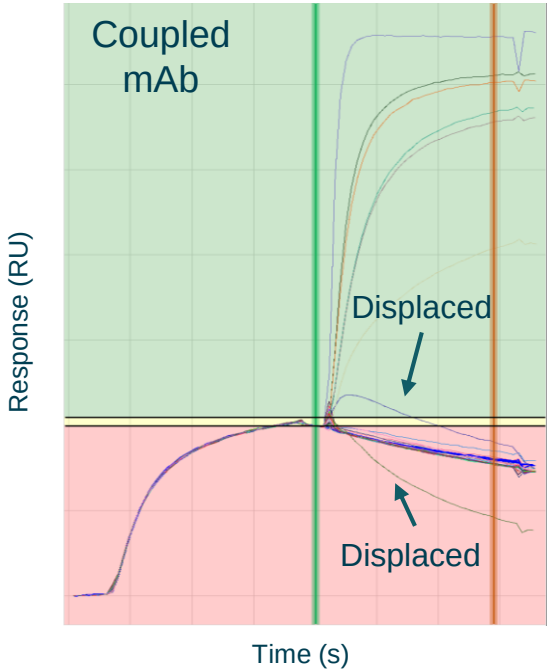
carterra

High Throughput Binning Provides Exquisite Epitope Resolution

Color by displacement



Sensorgram Overlay Plot



Sandwiching analytes

Blocked or displaced analytes

Manuscript submitted to PlosOne, Aug 2019

carterra

The LSA is Disrupting Antibody Analytics

- **Our value proposition:**
  - unprecedented throughput (50x Bia8K)
  - minimal sample consumption and facile assay set-up
  - high quality, reliable kinetics (within 2fold of Bia8K)
  - industry-leading analysis software to expedite getting to the results
- **The LSA's high throughput shifts the role of SPR upstream in drug discovery, where it can impact the library-to-leads triage**
- **High throughput binning reveals the epitope landscape quickly, enabling exquisite epitope resolution and the identification of mAbs with unique and mechanistically-differentiated MOA's**