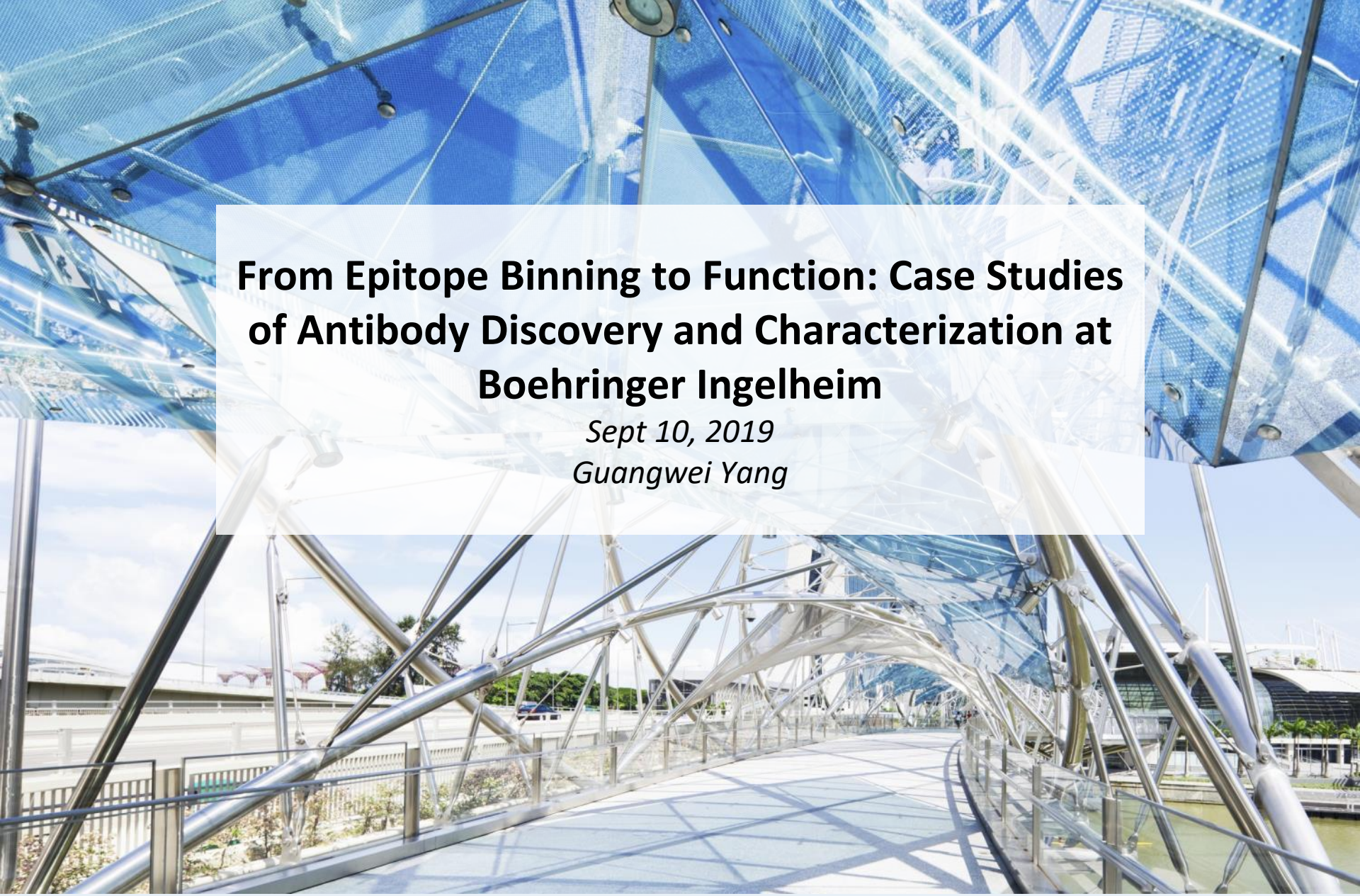


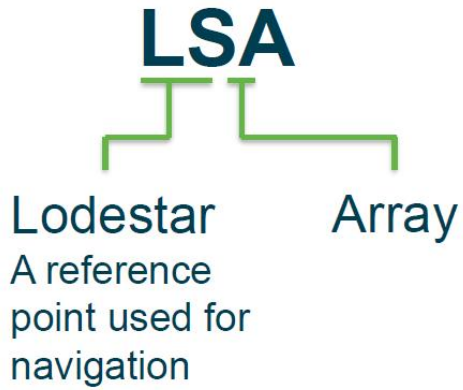
From Epitope Binning to Function: Case Studies of Antibody Discovery and Characterization at Boehringer Ingelheim

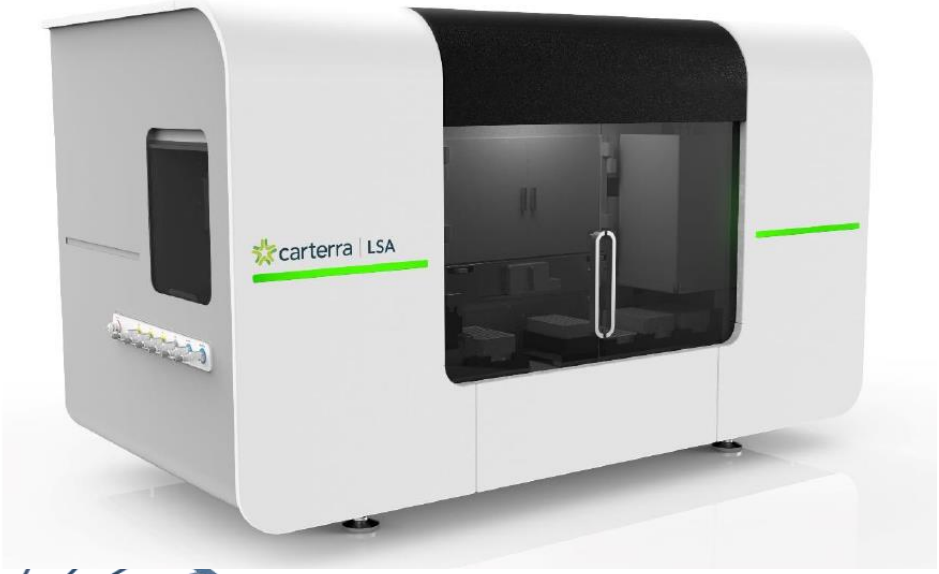
Sept 10, 2019

Guangwei Yang

Boehringer Ingelheim

Outline





- **Overview of Traditional Process for Ab Discovery**
- **Introduction of LSA**
  - **Kinetics**
  - **Epitope Binning**
- **A Case Study**
  - **Overview of the LSA-based New Process**
  - **Kinetics**
  - **Epitope Binning**
  - **Correlation of Epitope Binning with Functional Analysis**

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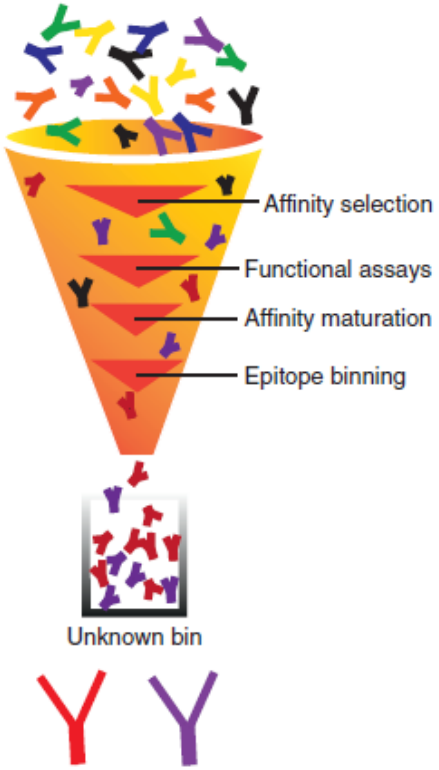
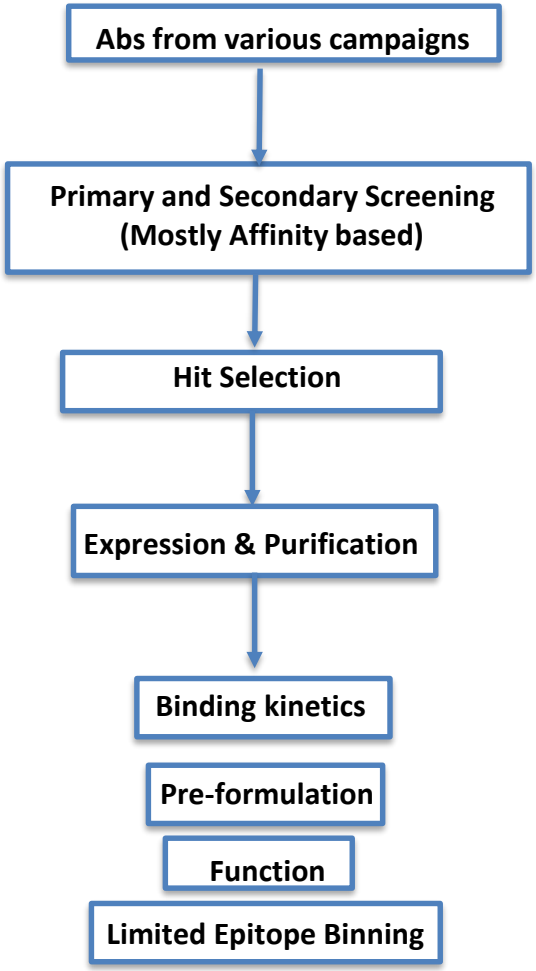
Overview of Affinity-Based Ab Screening & Selection Process



Picture copied from Brooks, et al, 2014



Limitations of the process with epitope binning at late stage:

- Epitope diversity preserved?
- Weak binders binding to right epitope missed?

❖ **Both Affinity AND Epitope are linked to Ab function!**

How to improve of the efficiency of lead identification?

- Increasing throughput
- Moving epitope binning to upstream to allow more molecule to be screened and more information to be collected
- Delivery of a set of well-characterized mAbs with various affinity & epitope profiles for downstream function analysis

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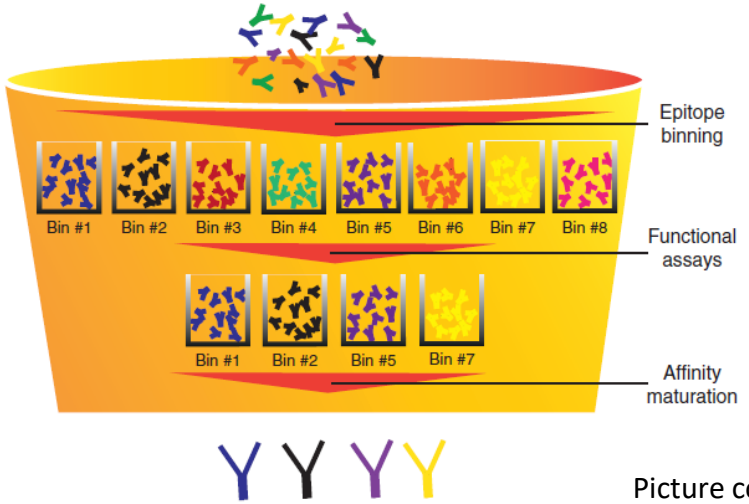
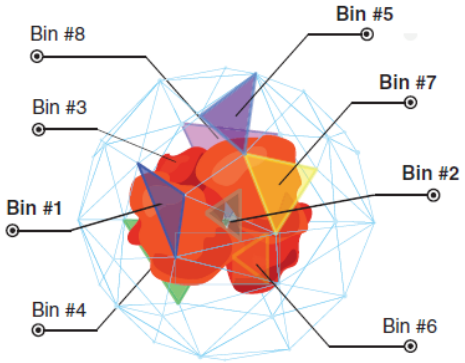
Epitope-Based Ab Screening & Characterization Process





Picture copied from Brooks, et al, 2014

Advantages of Epitope-Based Ab Selection:

- Preserves epitope diversity;
- Identification of additional Abs in the same functional bin, thereby providing multiple leads to choose from;
- Increasing the success of finding therapeutically fitting antibody candidates.

High throughput epitope binning approach is needed!

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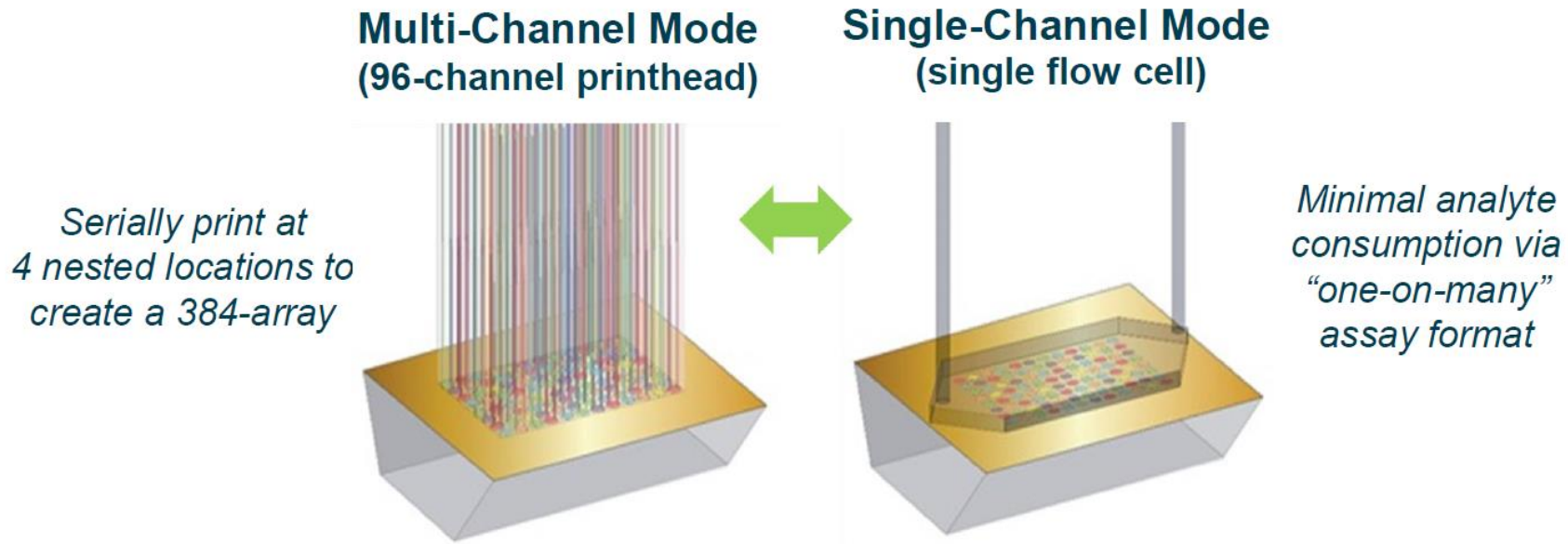
LSA Integrates Flow Printing & Array SPR



- **A combination of flow printing and Surface Plasmon Resonance**
- **Currently the only instrument available on the market for high throughput SPR kinetics and epitope binning purposes**

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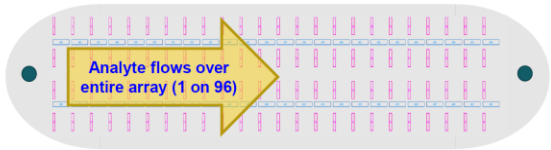
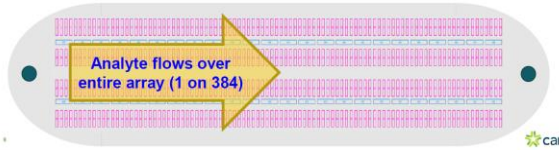
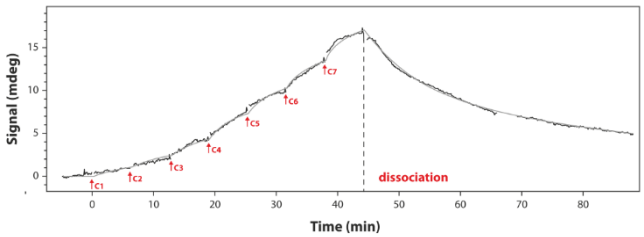
High Throughput Binding Kinetics on LSA: Assay Setup

- **Experiment Setup:**
  - One on Many Format
  - Single Cycle Kinetics
- **Advantages**
  - High Throughput: screening 384 Abs in parallel
  - Powerful analysis Software
  - Replicates when Ab number is less
- **Different instrument design & assay setup from Biacore**
  - Analyte flow path
  - Different setup in “single cycle kinetics”



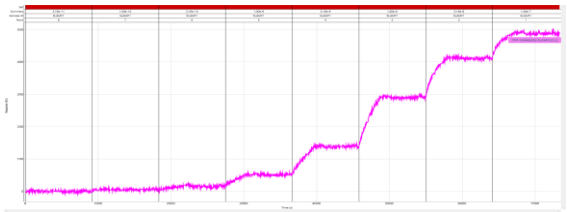


Single Cycle Kinetics on Biacore



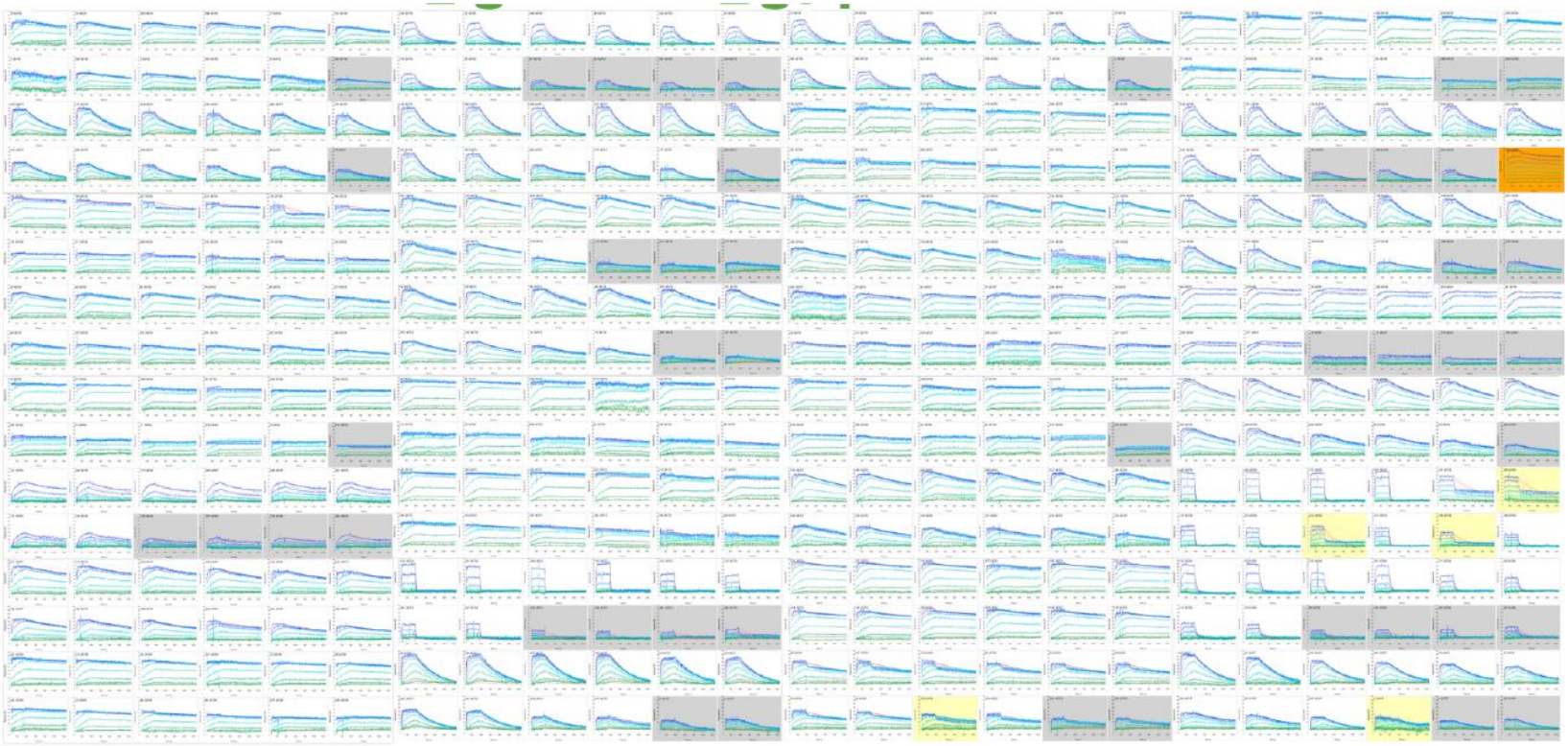
Multiple Association, one dissociation

Single Cycle Kinetics on LSA



Multiple Association, multiple dissociation

High Throughput Binding Kinetics on LSA: Data Output



- 384 ligand kinetics in one parallel run
- Software automatically flags results with unsatisfied quality

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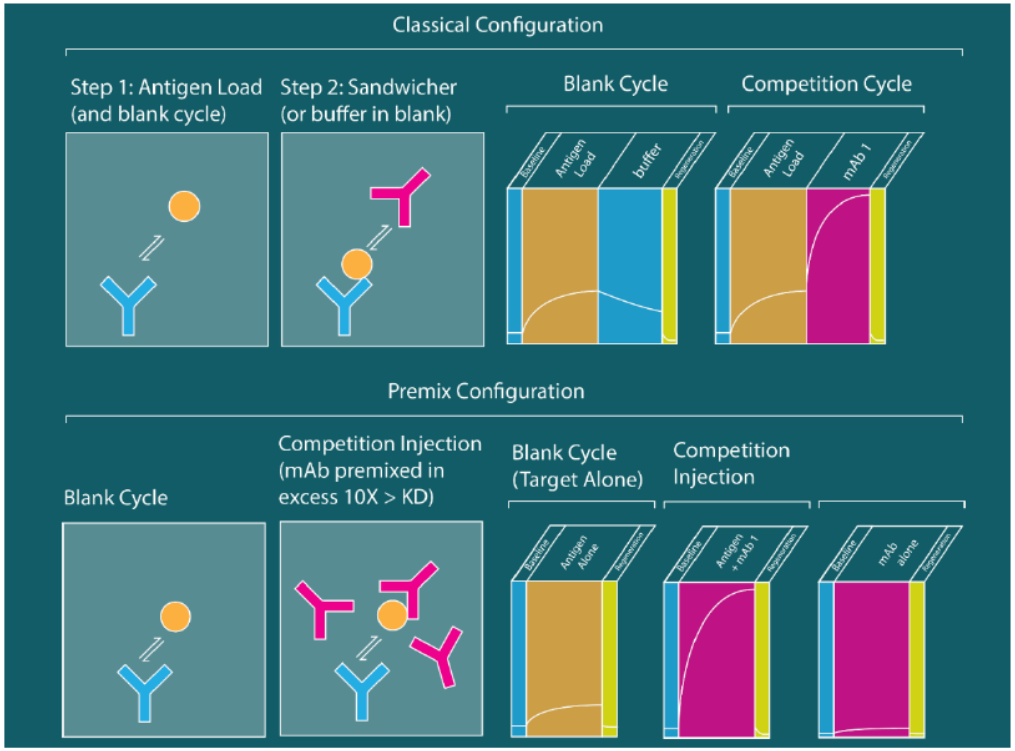
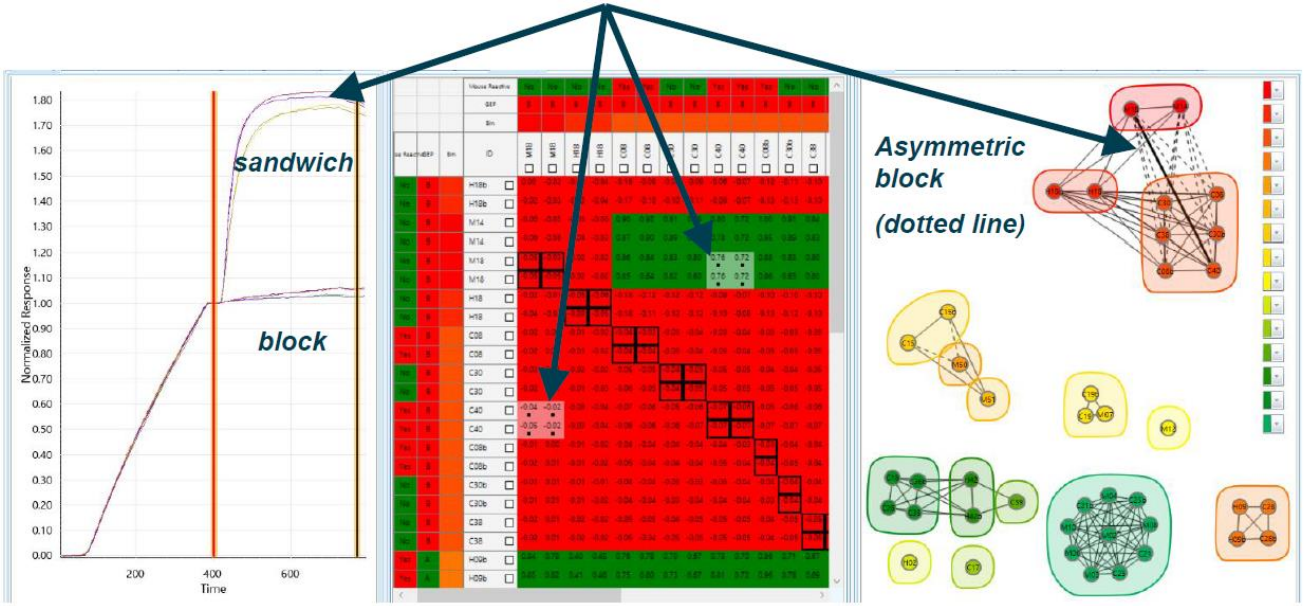
High Throughput Epitope Binning Assay Configurations on LSA

- **Classical Sandwich**
  - Array-based amine-coupling of all Abs
  - Probe with sequential cycles of antigen followed by individual Ab
  - Regenerate and repeat with next Ab
  - Ideal for monovalent antigens
- **Pre-Mix**
  - Array-based amine-coupling of all Abs
  - Inject Ab panel premixed in excess with antigen
  - Regenerate and repeat with next premixed Ab/Ag
  - Ideal for multivalent antigens



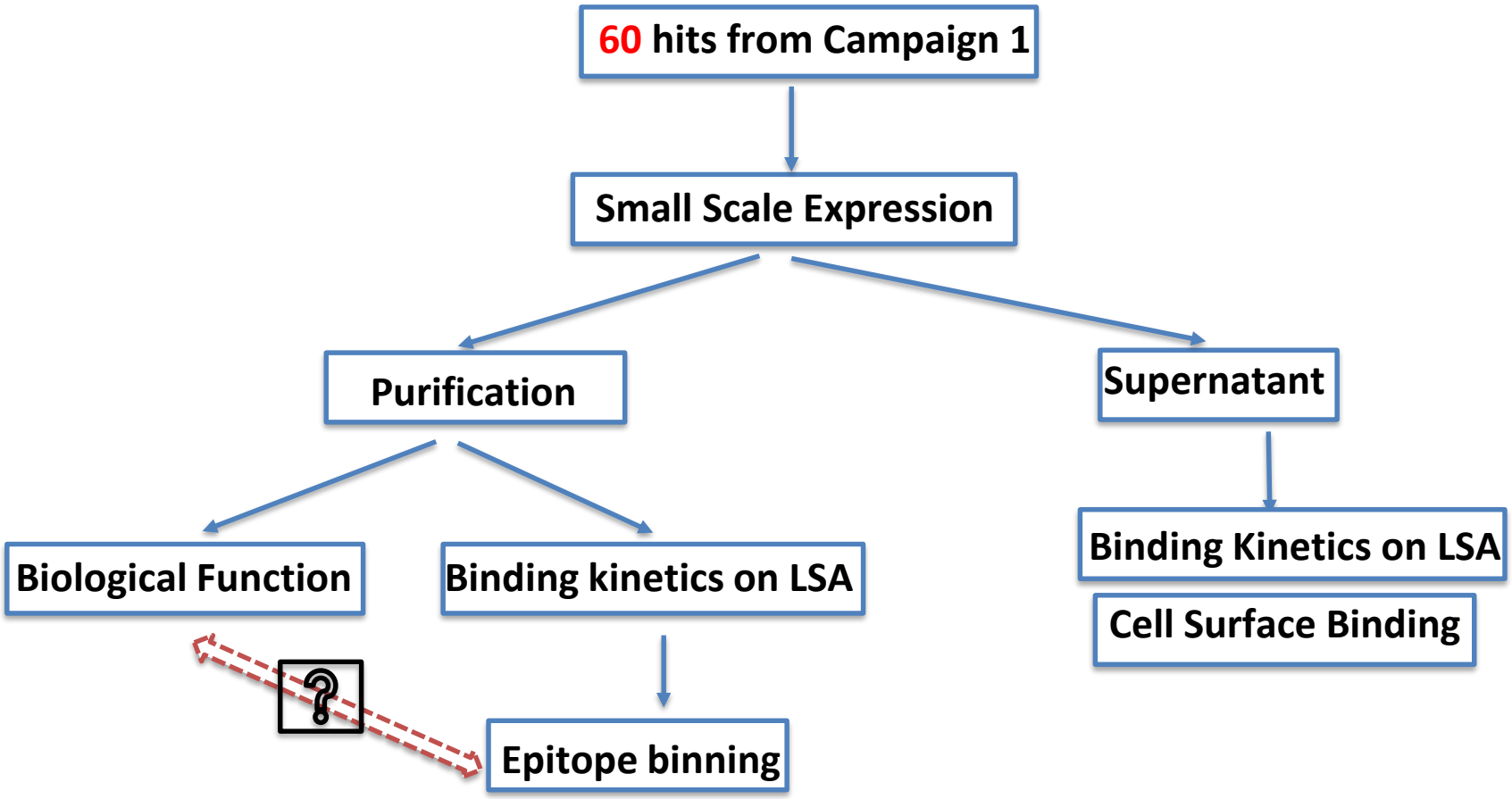
- **The fact of epitope binning: to use competition profile between each pair of antibodies to group them.**

High Throughput Epitope Binning Data Output (classical)



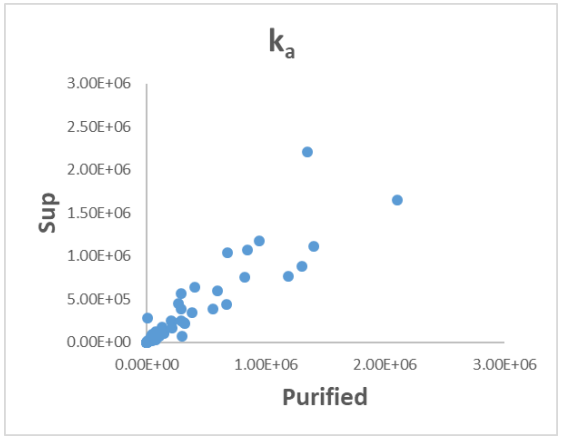
- Automated data output with sensorgrams, heat map and networks
- Data linked across visualization panels

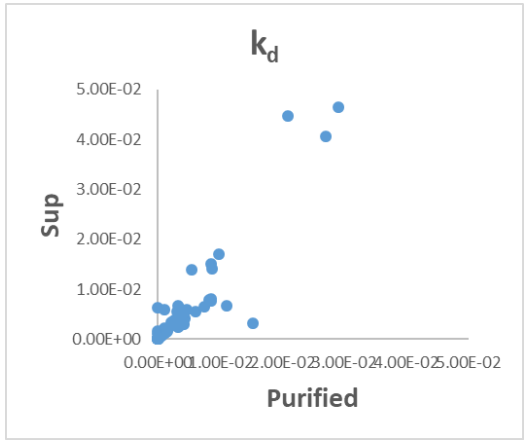
Case Study: Testing a new workflow involving LSA-based kinetic analysis and epitope binning

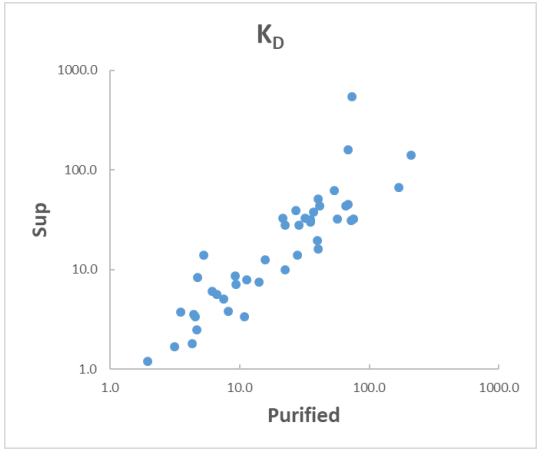


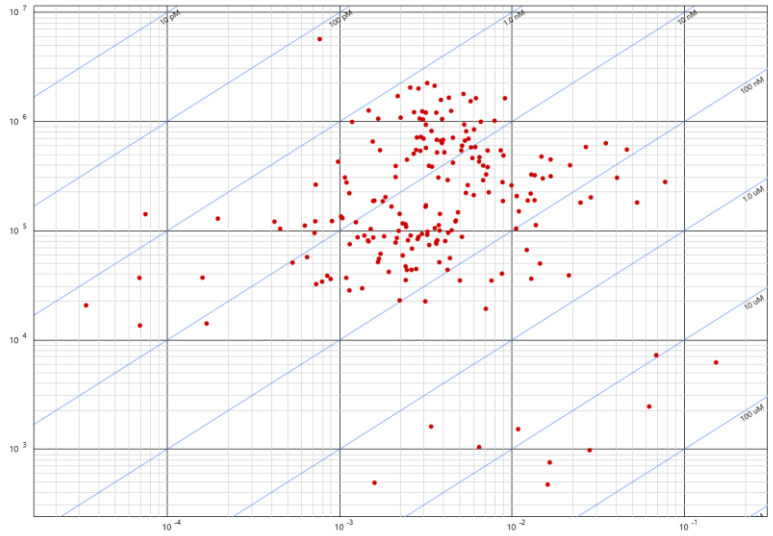
Purpose: To check the correlation between epitope binning with function.

Binding Kinetics (anti-Fc capture) on LSA









Iso-Affinity Plot

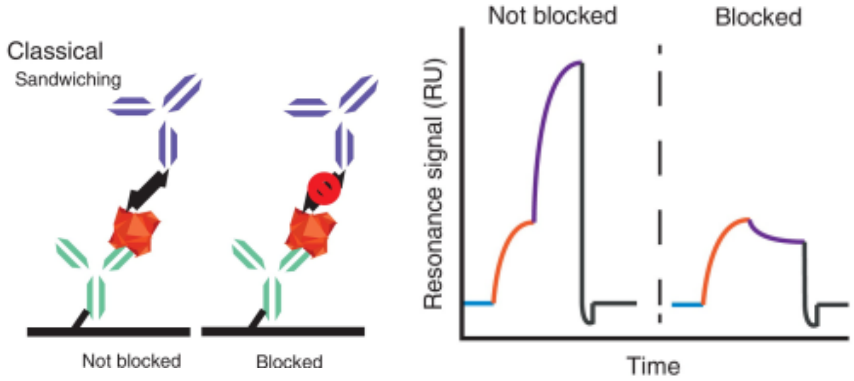
	Purified	Sup	Cell Binding
Binder	48	44	47
Non Binder/Poor Fit	12	16	13
Total	60	60	60

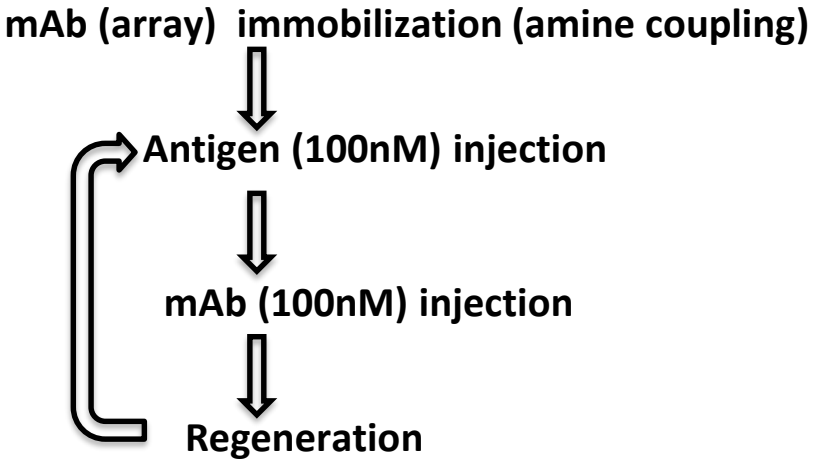
- **Consistent within repeats**
- **Good correlation of binding kinetics between purified mAb and sups**
- **Consistent with cell surface binding results as well as kinetic data generated on Biacore**
- **K_D range: single to triple digit nM**

Epitope Binning Setup (Classical Sandwiching)

Materials:

- **Antigen: Monomer with 6xHis tag, 94% monomer as determined by AUC**
- **Antibody: Total 60 mAbs after one-step protein A purification**





	Kinetics	Binning	
	Purified	Ligand (amine coupling)	Analyte
Binder	48	42	53
Non Binder	12	18	7
Total	60	60	60

- **Total 74 regeneration cycles**
- **Completed in 30 hours**
- **After repeatedly regeneration cycles, Abs are active through the entire experiment**

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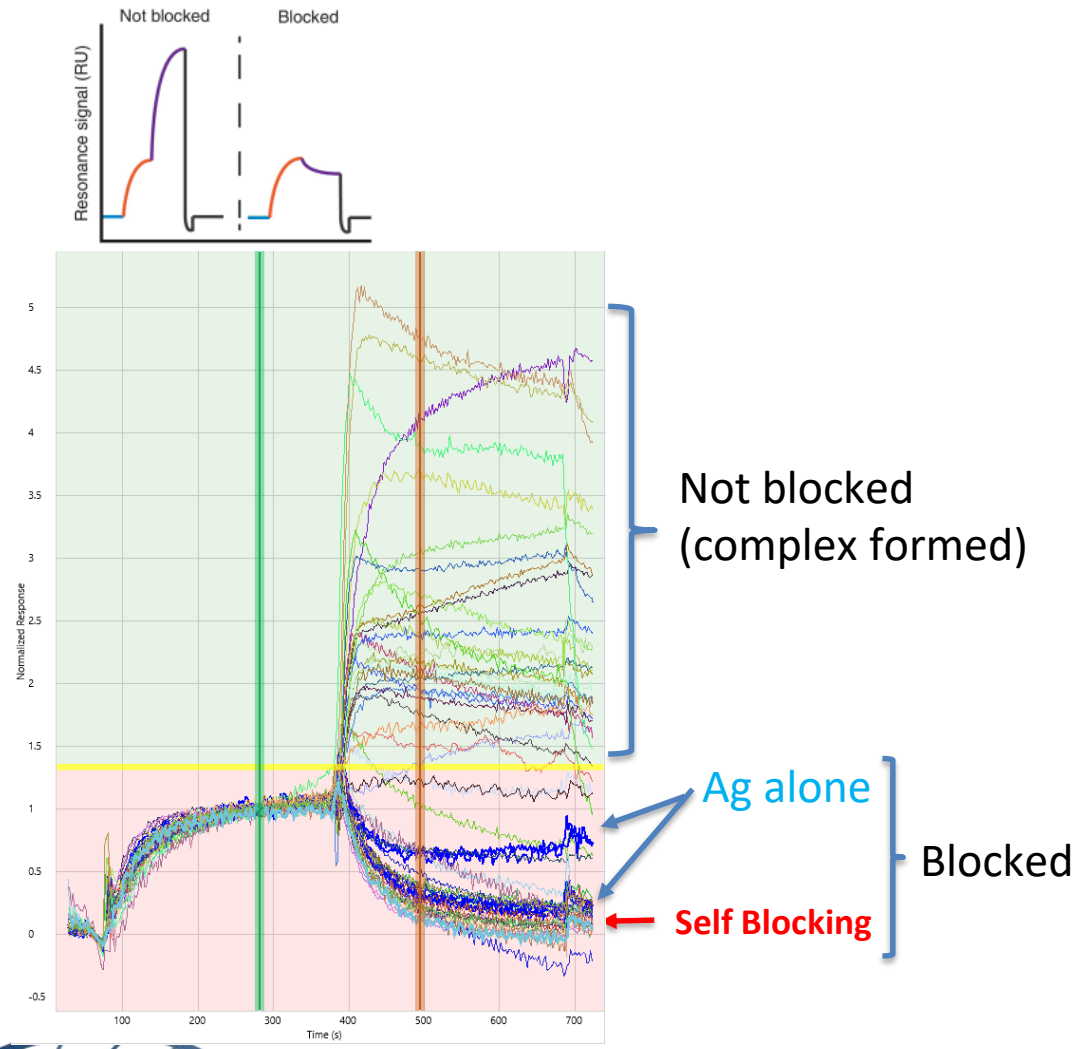
Epitope Binning Sensorgrams



Challenges:

- Weak binders: Ab & Ag concentrations not high enough for complex formation due to low affinity
- Equilibrium binder: unstable complex due to fast off rate

How to distinguish real blocking event from weak binding event?

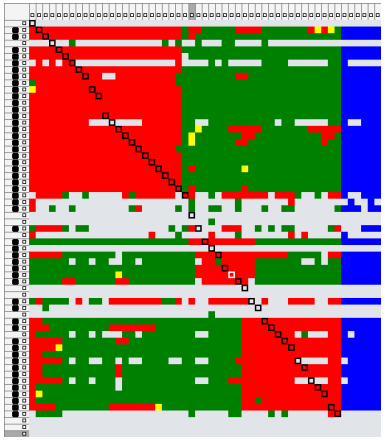
<u>Only</u> blocking events are used for analysis to:

(i) link the Abs;

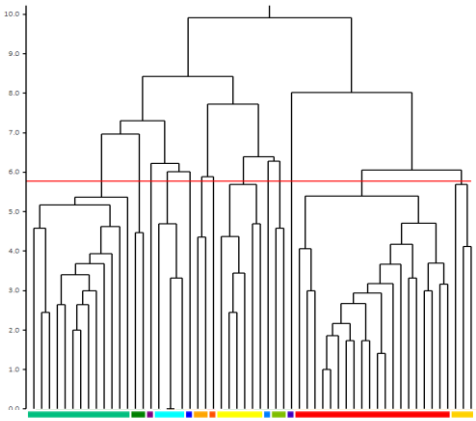
(ii) determine the bin assignments

Epitope Binning Data Output

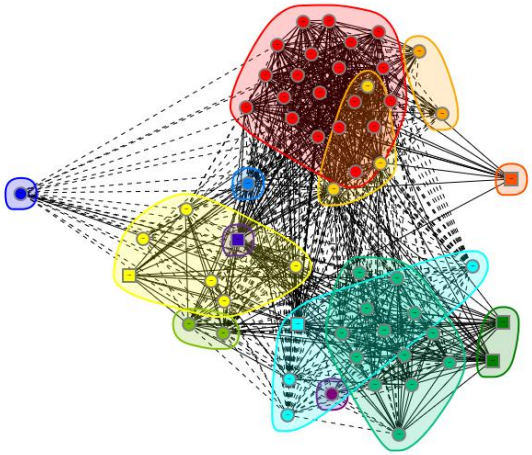
Heat Map



Dendrogram



Community Plots



Report

Group	Id
Group 1	479
	477
	463
	469
	493
	466
	482
	502
	484
	476
	462
	480
	503
	521
	516
	509
	489
	453
	454
	455
Group 2	494
Group 3	497
	485
Group 4	520
	459
	488
Group 5	461
	505
	470
	456
	501
	496
Group 6	467
	515
Group 7	472
	475
Group 8	504
	518
	500
	464
	507
	506
	471
	474
	458
	481
	483
	478
	517
Group 9	498
	491
	490
	457
Group 10	465
Group 11	473
Group 12	487
Group 13	495

Total 56 mAbs were assigned into 13 community groups

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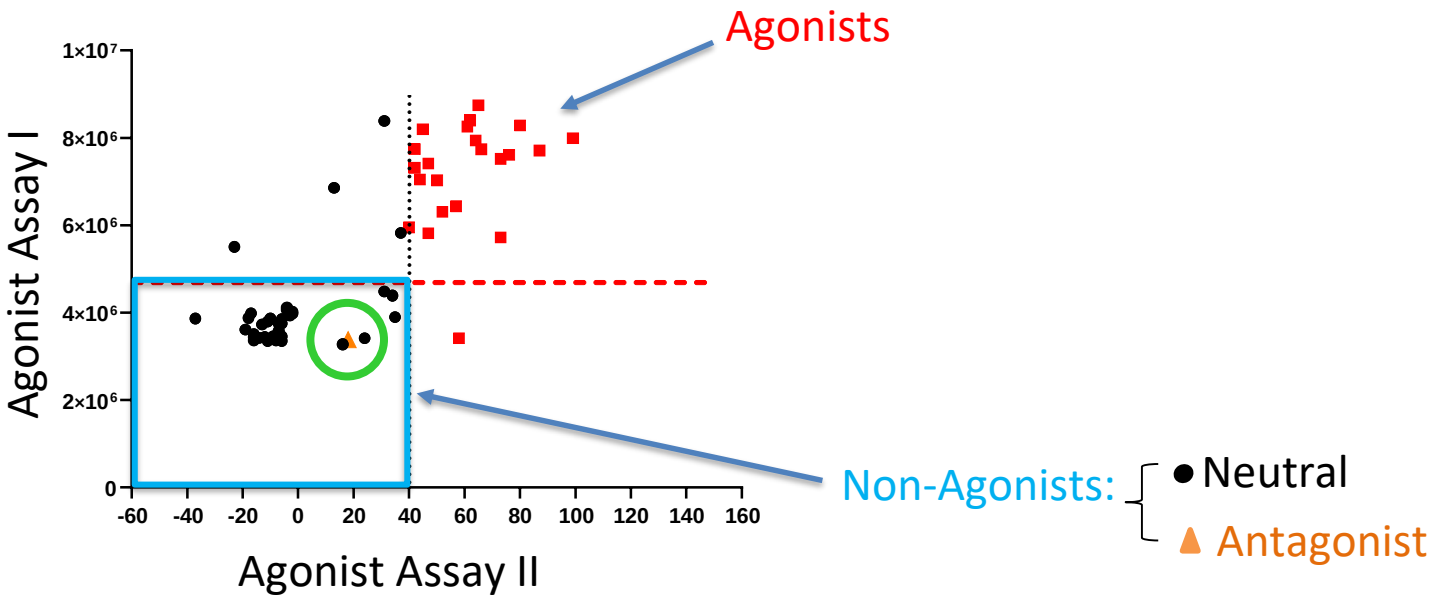
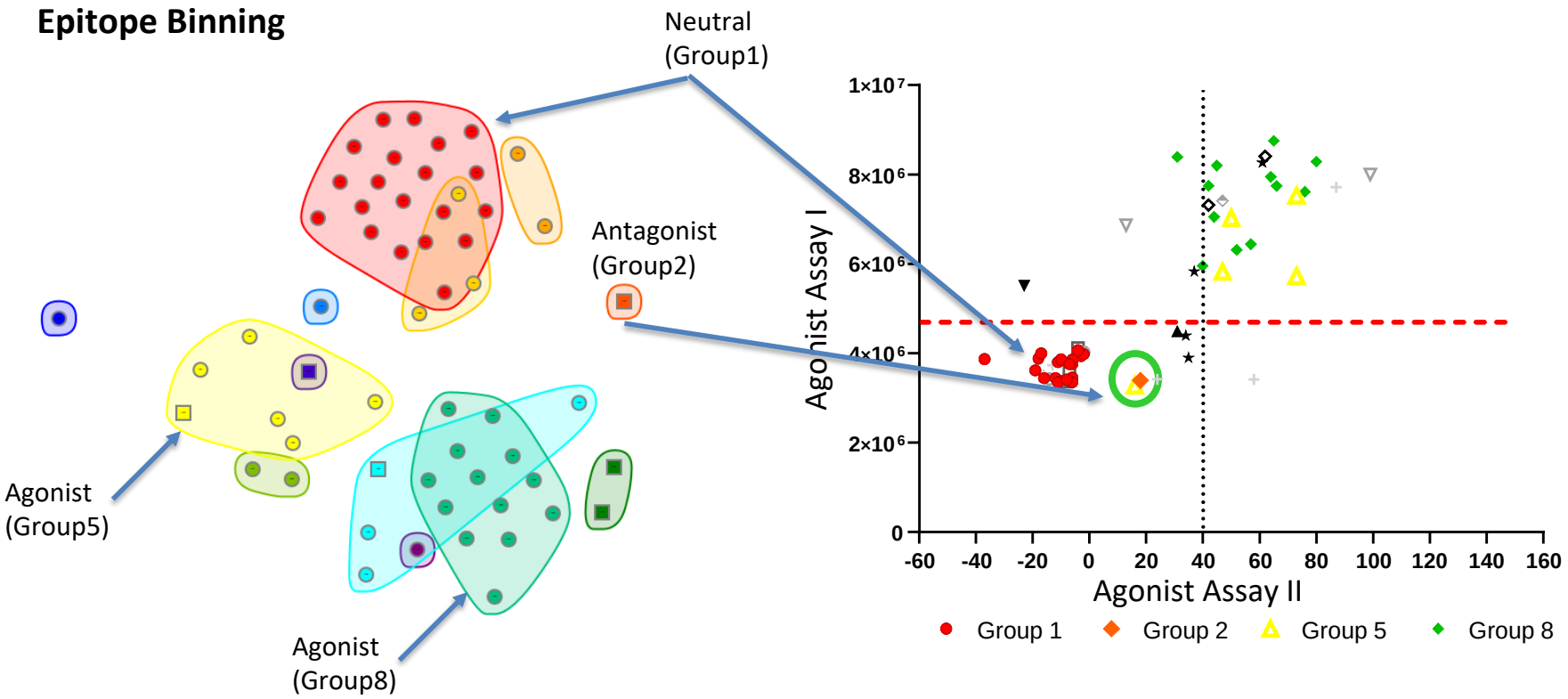
Functional Analysis of mAbs



- ❑ Strong correlation between two agonist assays
- ❑ Substantial number of neutral binders.

Community Assignment and Correlation with Functional Response

Epitope Binning



Initial indication that **specific epitopes** correlate with **functional response** of the receptor.

- Group1: neutral
- Group 5 & 8: agonists
- Group 2: antagonist

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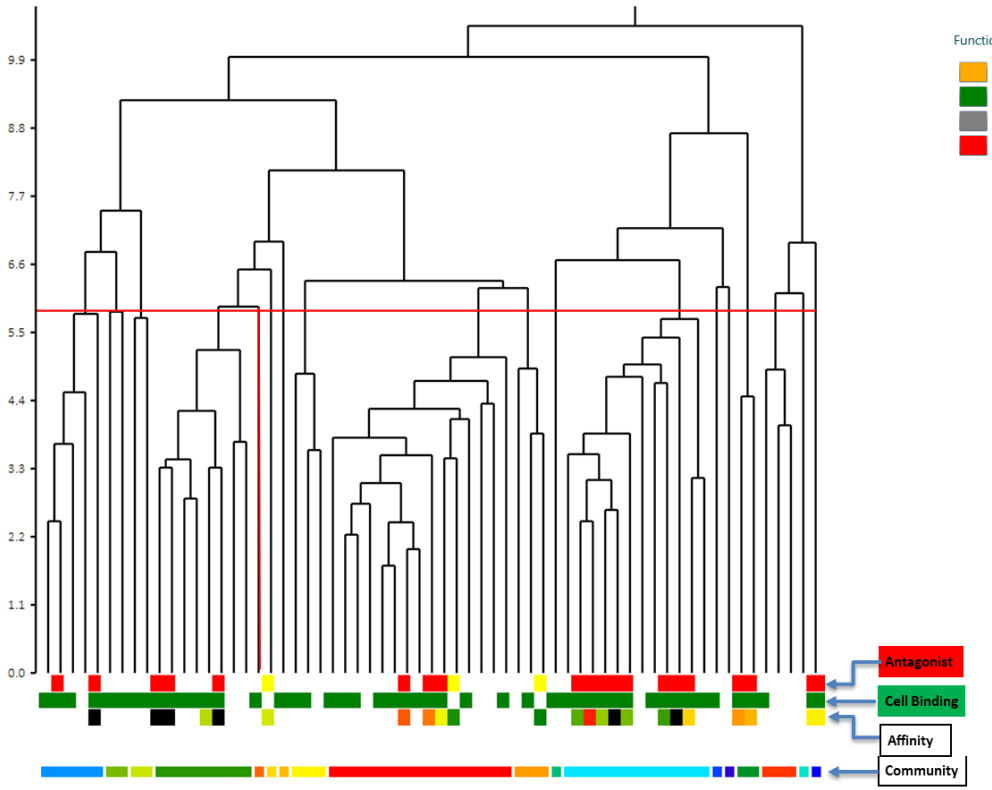
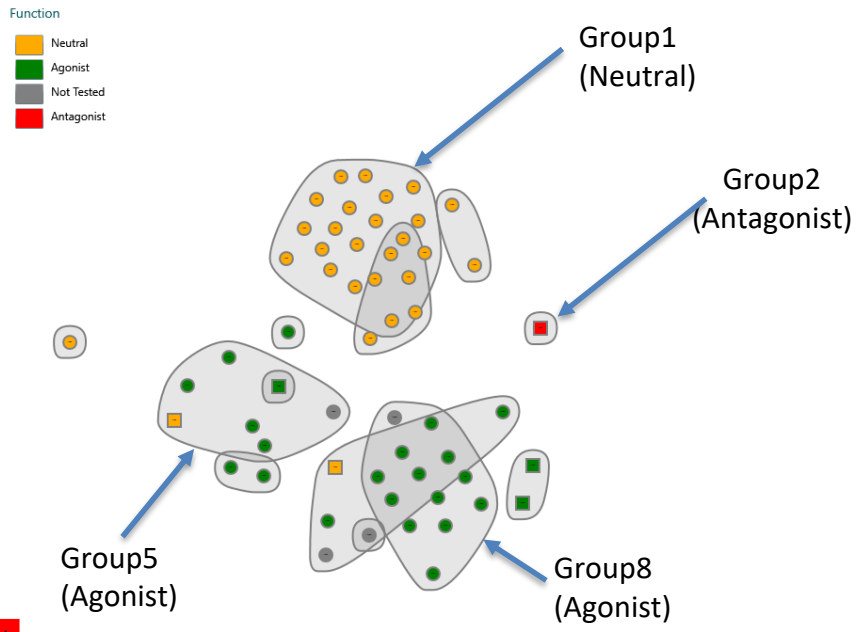
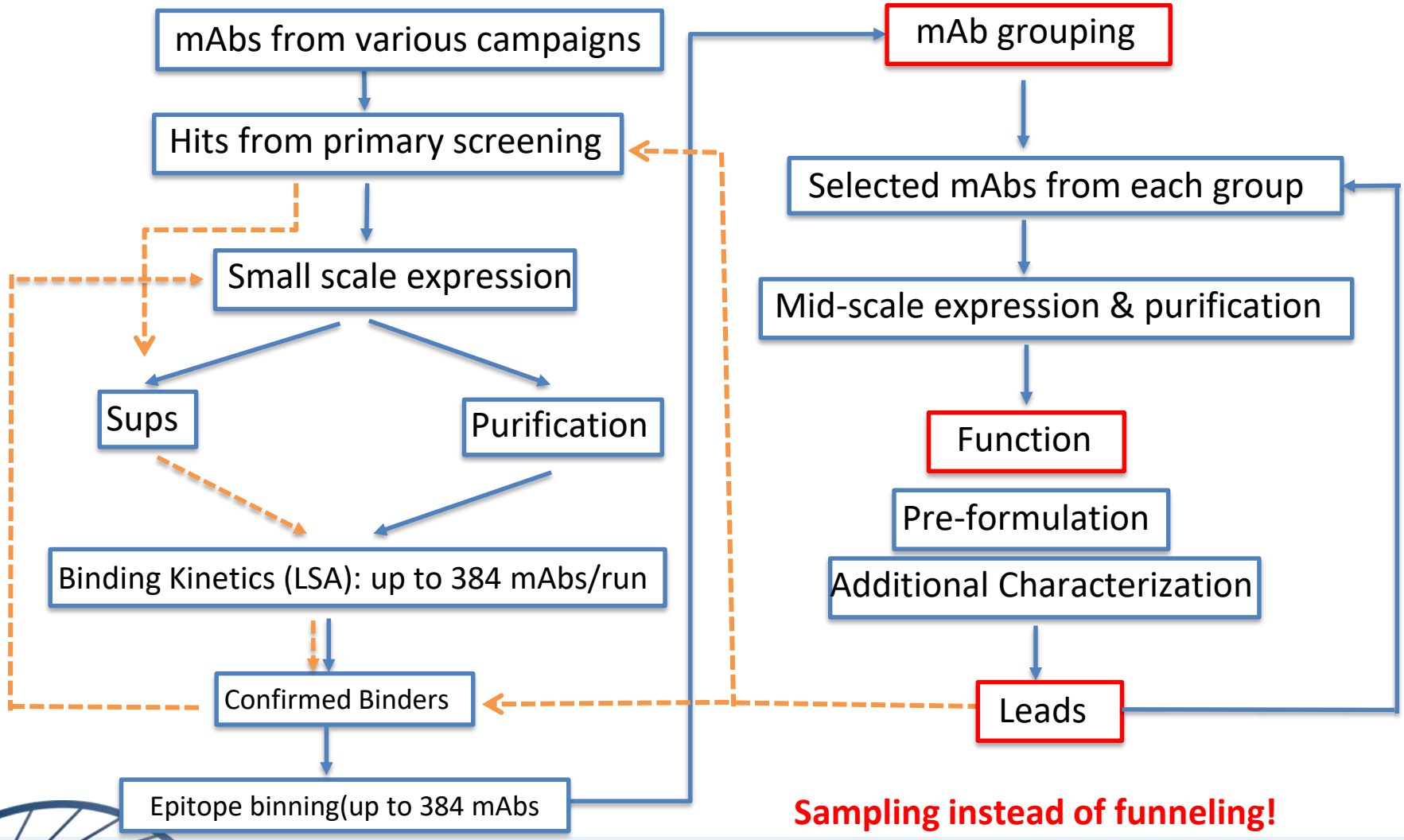
Data Output with Functional Data Incorporated





Current Stage: more mAbs from additional campaigns are under investigation for function and epitope binning correlation

A tentative LSA-based screening & characterization process:



Sampling instead of funneling!

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Proper Preparations Before Starting Epitope Binning Experiments

- Materials and Experimental Design
  - Quality & valency of both antigen and antibodies
  - Antibody Kinetic Profiles
  - Non-specific Binding Profiles of Antigen (and Antibody)

- Project Timeline & Experiment Spacing When Running Multiple Experiments on LSA
  - Project prioritization & coordination
  - Sup: time sensitive
  - Binning: time consuming
  - Instrument maintenance & repair

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Summary

- LSA (together with its analysis software) is a powerful instrument for both high throughput binding kinetics and epitope binning in antibody discovery field
- With proper experiment design and data analysis, epitope binning can provide guidance for quick lead identification

Acknowledgement

- Kinetics Group:
  - Cindy Kenny
  - Gale Hansen
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  - David Cai
  - Joseph Mozdzierz
- Expression Group
- Purification Group
- Reagent Group
- Biology Group
- Rachel Kroe-Barrett
- Andrew Nixon

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