Best Practices for Designing and Analyzing Epitope Binning Experiments Using the Carterra LSA and Epitope Software

Why Competitive Epitope Binning?

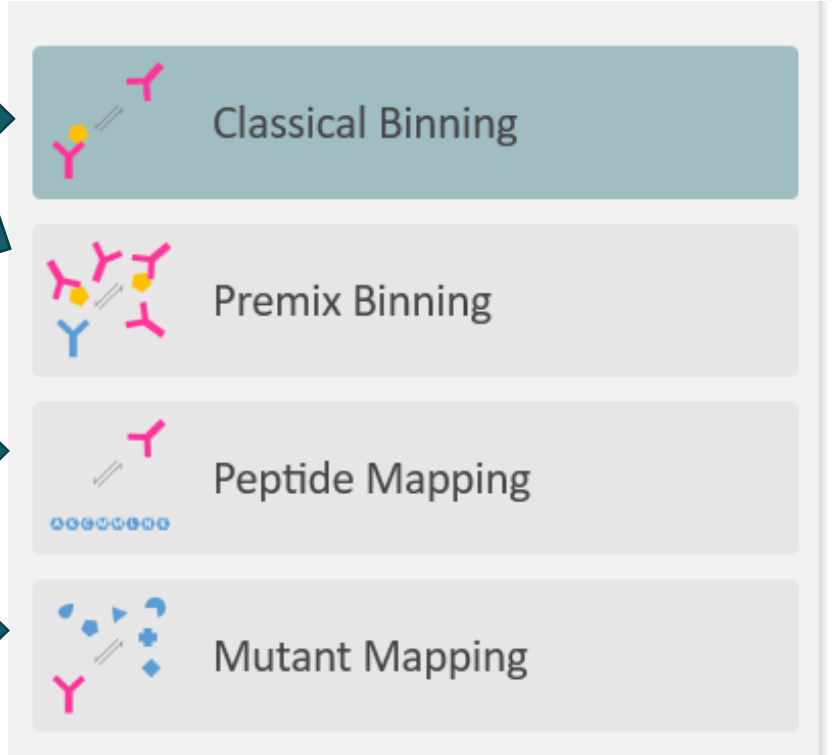
- Process which tests the ability of mAbs to simultaneously bind antigen in a pairwise fashion
- Establishes clusters or “bins” of mAbs that engage similar antigen epitopes
- Binning of mAbs allows meaningful diversification of early stage candidates
- Epitope binning data can be readily mined to identify preferred leads as therapeutic objectives evolve
- A key byproduct of binning is detailed information on sandwiching pairs for downstream supporting assays

Binning is Just One Way to Characterize Epitope Using the LSA

Competitive binning: Determine if two mAbs can bind simultaneously to the same antigen

Epitope mapping: Identify residues on antigen peptides that mAbs bind

Mutant mapping: Use antigen mutants to determine what residues/regions mAbs recognize



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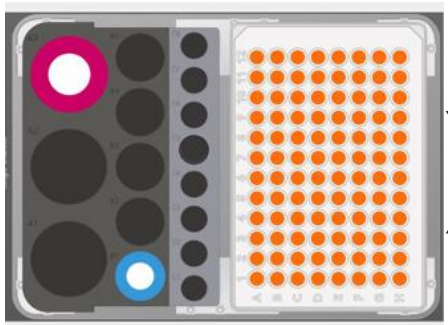
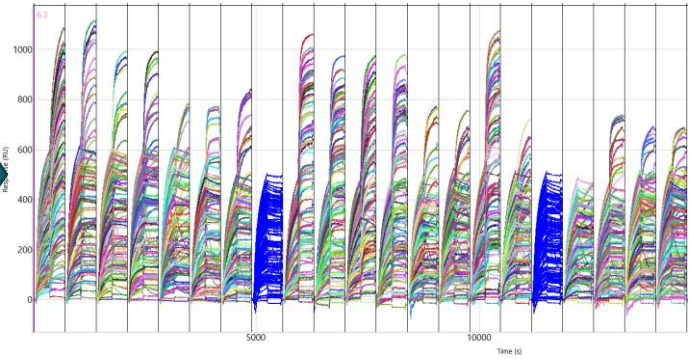
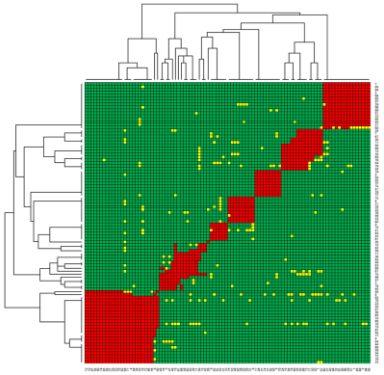
The LSA Epitope Binning Workflow







Prepare Reagents
- 7ug total each Ab
- 0.2ug Ag per cycle

Execute Experiment
- Array Up to 384 mAbs
- Run Premix or Classical Binning

Analyze Data
- Create Heat Maps and Network Plots
- Import Orthogonal Data

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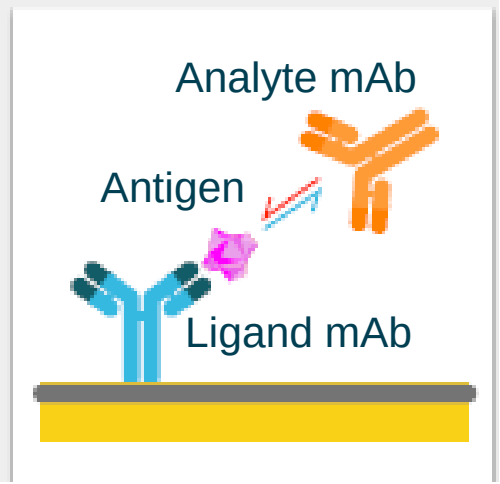
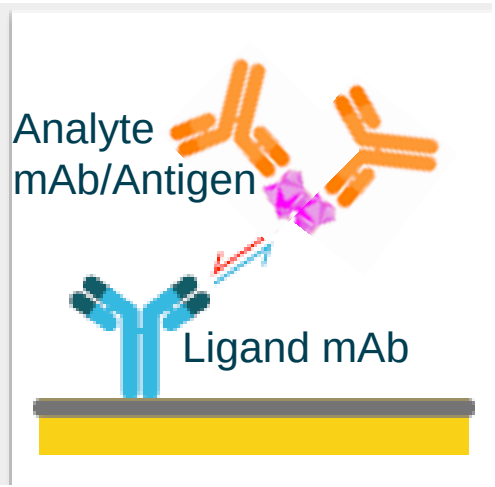
Antigen Valency Dictates Epitope Binning Assay Format

Two formats best suited for the “One-On-Many” design of the LSA



Monovalent Antigen: Classical Binning



Multivalent Antigen: Premix Binning

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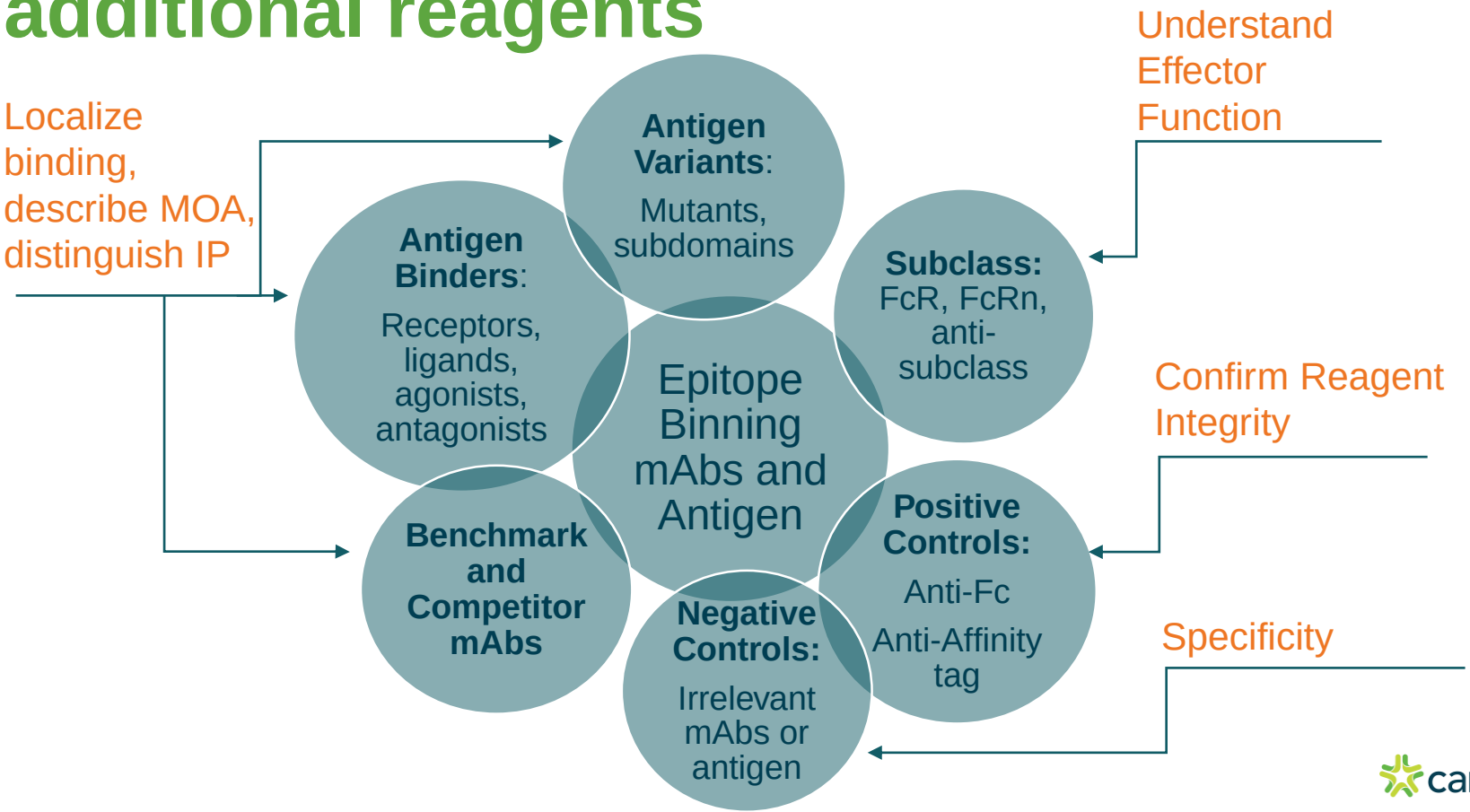
Chip Surface: Higher Capacity Is Preferred

- **Any decrease in surface coupled mAb (ligand) activity during the assay is automatically corrected for in the analysis software**
- **Linear polycarboxylate (e.g. HC200M) generally has improved capacity and more robust regeneration than carboxymethyldextran (e.g. CMD200M)**
- **Surface transport characteristics are generally not a primary concern, though data collected for off-rates in this assay format should be treated as apparent**

Supplement the binning assay with additional reagents



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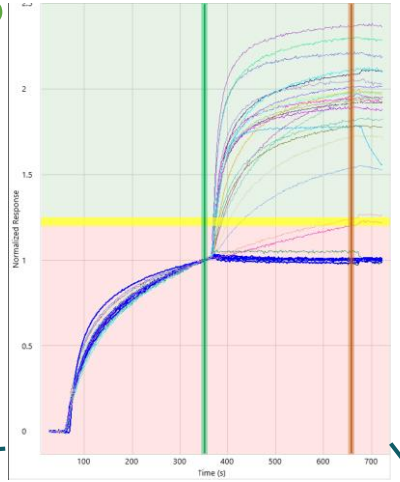
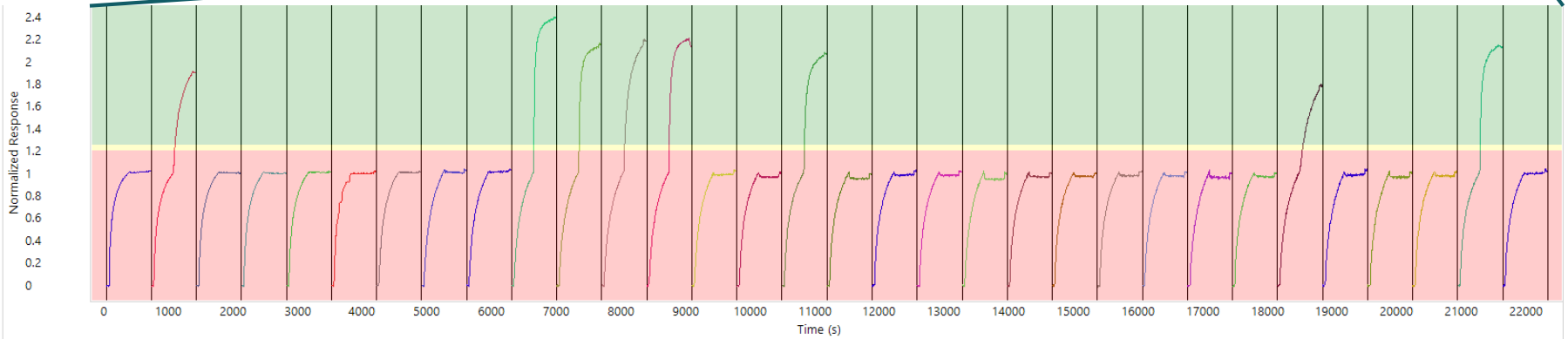
More Challenging Sample Types

- **Crude samples present complexities**
  - Typically must be captured (on-chip enriched) followed by covalent crosslinking and blocking of the capture surface
  - Low protein media expression systems (e.g. Expi293™) greatly improve the potential for success
  - As injections, variable concentrations can make antigen saturation difficult for premix binning

Defining Sandwiching Behaviors Clearly Is Important

- **Customizable threshold delineates blocking/non-blocking**
- **Poor resolution between sandwichers and blockers may suggest antigen or mAb issues**





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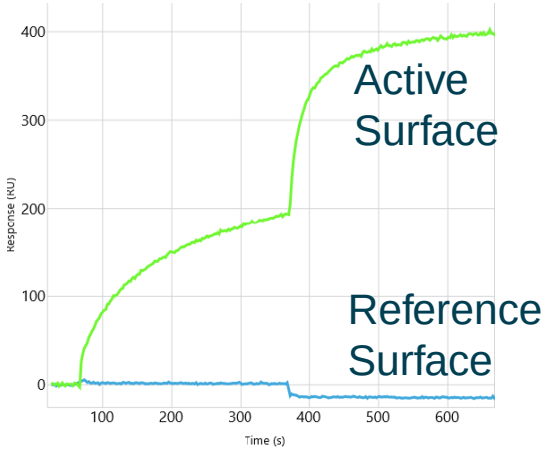
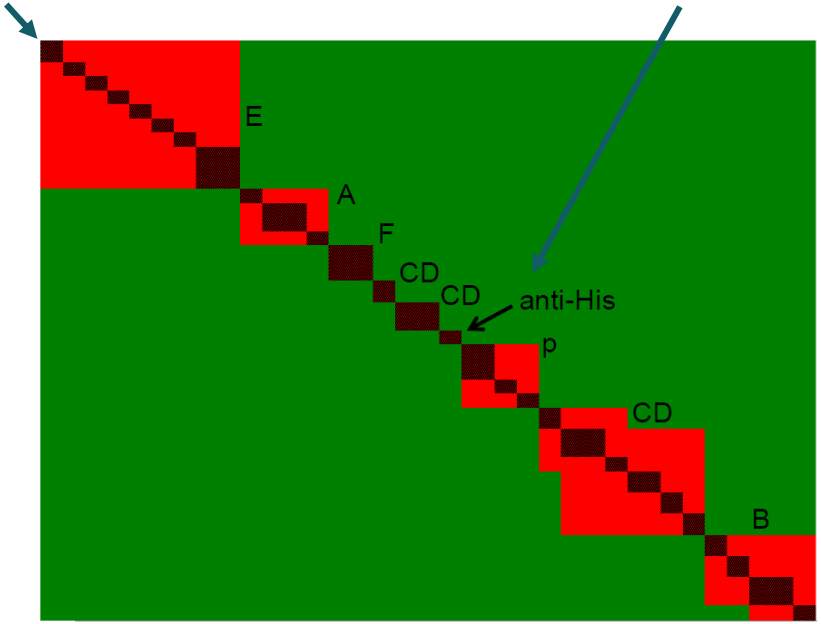
Leveraging Epitope Software to Monitor Data Fidelity



Specificity (lack of non-specific binding) for antigen and mAbs

Self-self blocking confirmed via diagonal highlighting in heat map

Anti-His confirming monomericity of antigen



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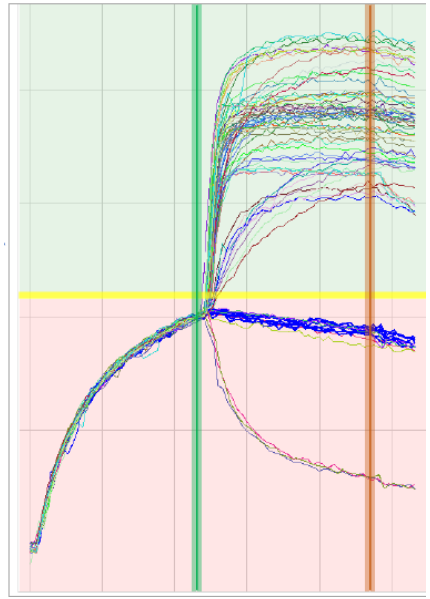
Pulling Out Nuanced Behaviors

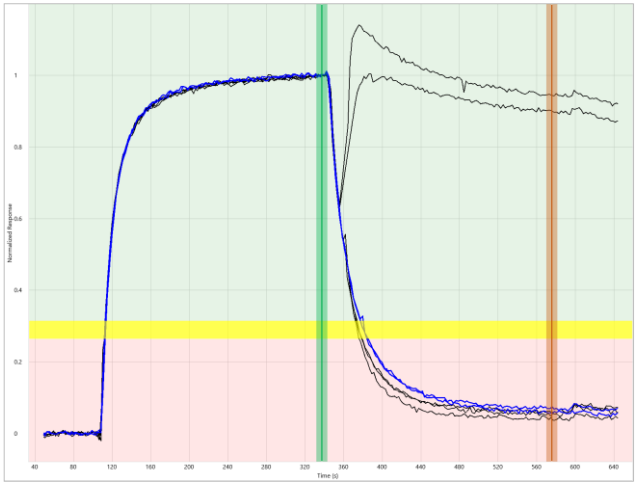
Real-time sensorgram signals provide a wealth of information



Sandwiching mAbs

Blocked mAbs

“Kick-off” mAbs which disrupt antigen binding to the surface mAb



Measurable sandwiching for a rapidly dissociating surface

Abdiche, et al. Antibodies Targeting Closely Adjacent or Minimally Overlapping Epitopes Can Displace One Another. PLoS One. 2017 Jan 6;12(1):e0169535. doi: 10.1371/journal.pone.0169535

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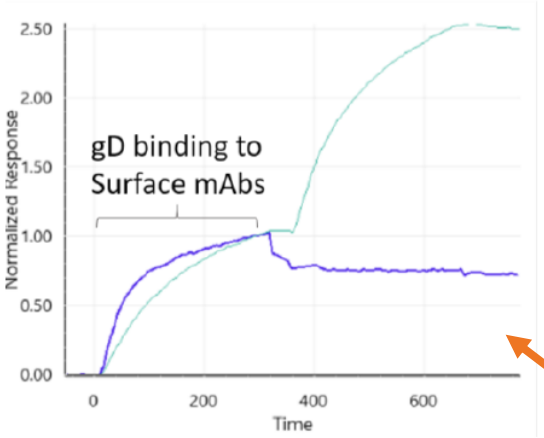
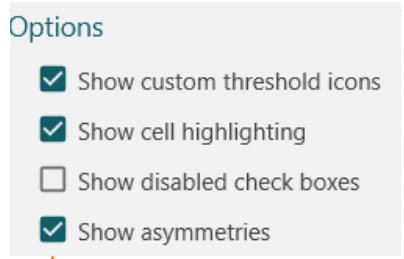
Pulling Out Nuanced Behaviors, Cont'd

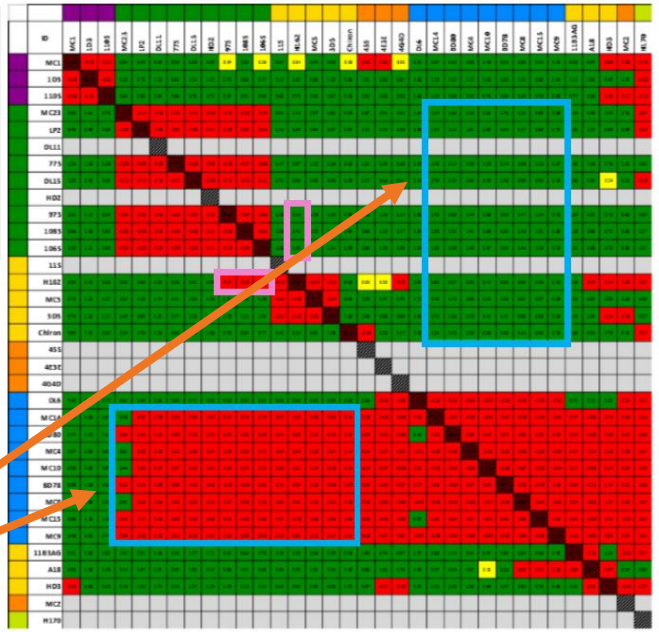
Evaluating symmetry in the heat map



MC8 Injection on 97S Surface

97S Injection on MC8 Surface





Asymmetrical competition is easily highlighted and can be explored at the sensorgram level

See Carterra Allosteric Competition App Note

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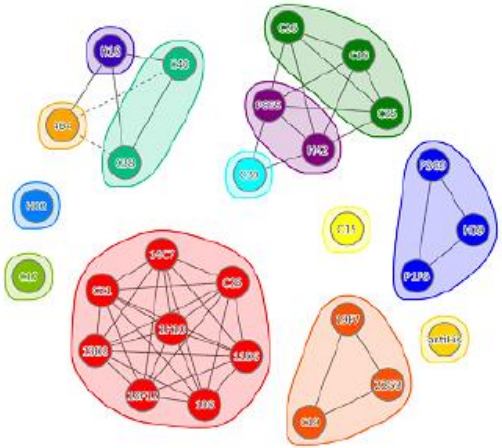
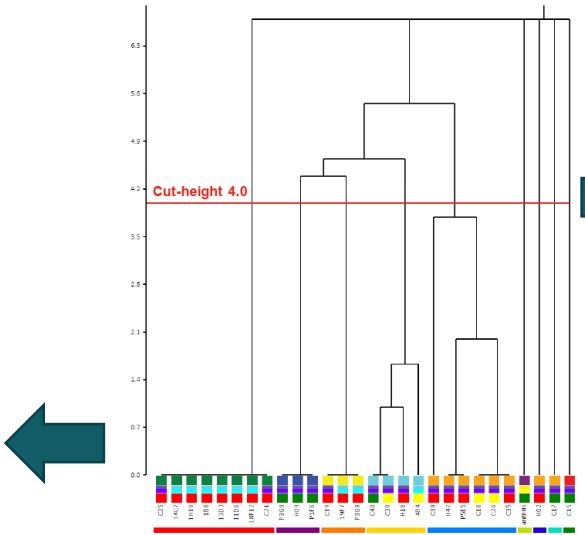
Flexible exploration of epitope clusters

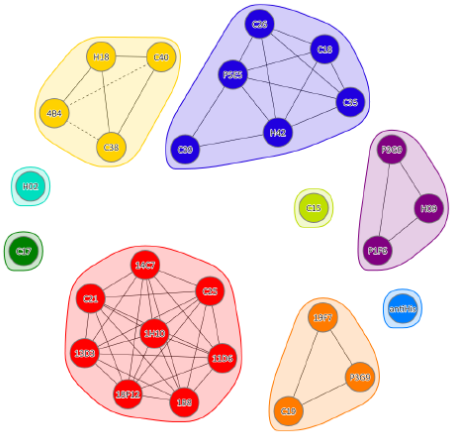
- Lines (chords) represent blocking; distance typically represents degree of epitope similarity
- Visualize competitive relationships dynamically using hierarchical clustering
- Community view aids interpretation of clusters with partial epitope overlaps

Objective Groupings:
Bin-Level Network





Subjective Groupings:
Community-Level Network



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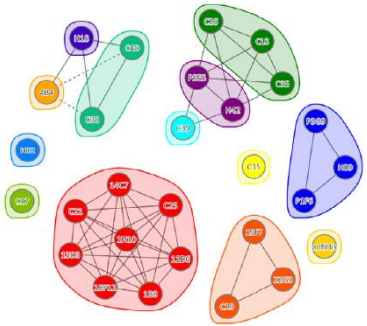
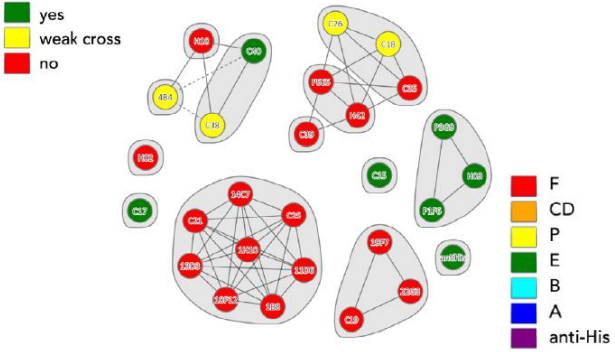
Inform and Refine Lead Selection with Orthogonal Data

Bin

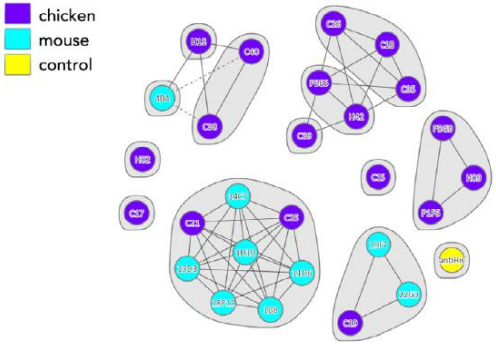


Import orthogonal data as a pasted column of descriptors

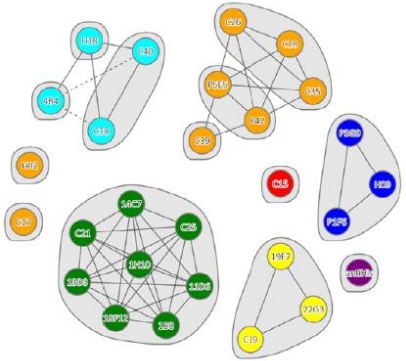
Mouse Cross-reactivity



Library



Mapping



See Carterra Epitope Diversity App Note

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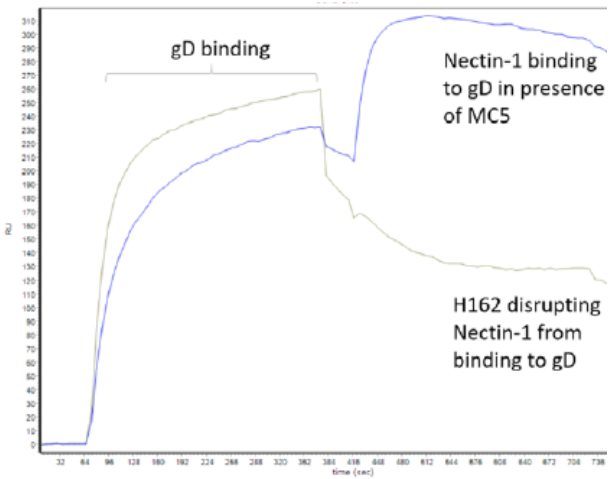
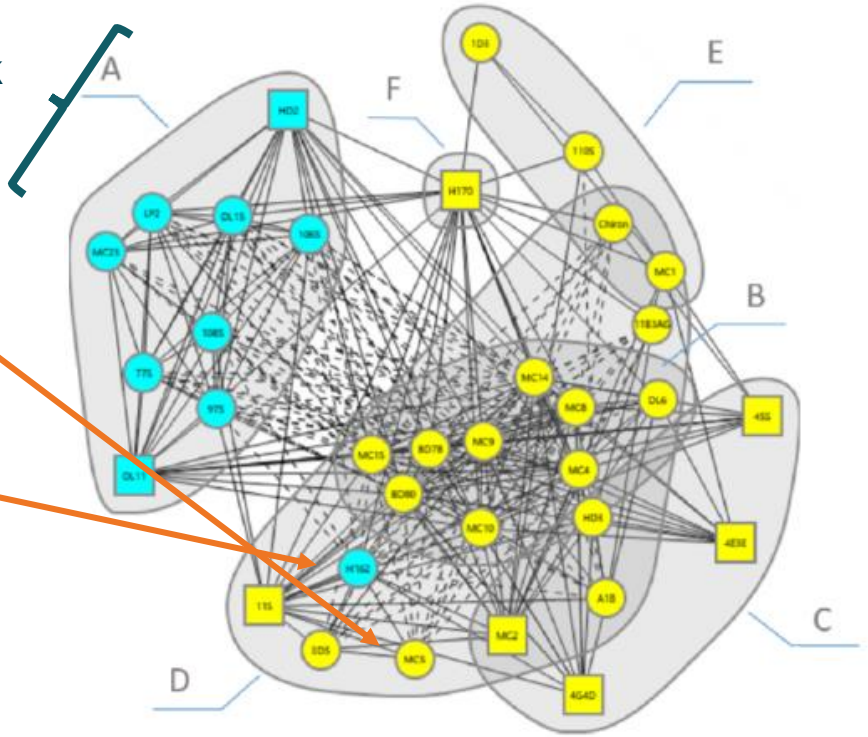
Studying Indirect Competition MOA

Blue: Blocks receptor

Yellow: Does not block receptor





See Carterra Allosteric Competition App Note

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Takeaways

- **The LSA facilitates epitope binning on up to 150,000 pairwise binning interactions (384x384)**
- **Inclusion of additional reagents provides insight into binding behaviors and MOA with minimal sample and effort**
- **Additional data that can be gleaned from a binning experiment includes off-rate, blocking, mAb influence on antigen confirmation, and sandwiching antibody pair selection**
- **Carterra's Epitope software allows easy importation and display of orthogonal data within the heat map and network plots, providing multifaceted insights for each clone**