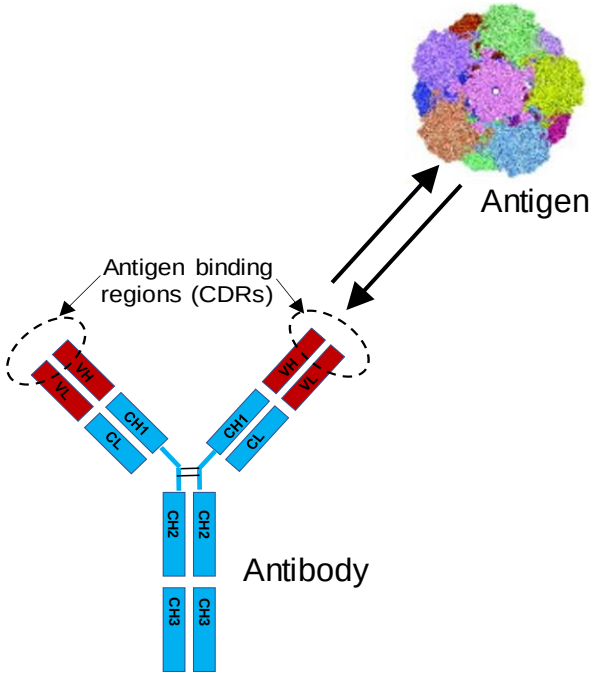
High-throughput biophysical characterization of monoclonal antibodies via array-SPR for identifying epitope diversity

Carterra Symposium
May 31st, 2024

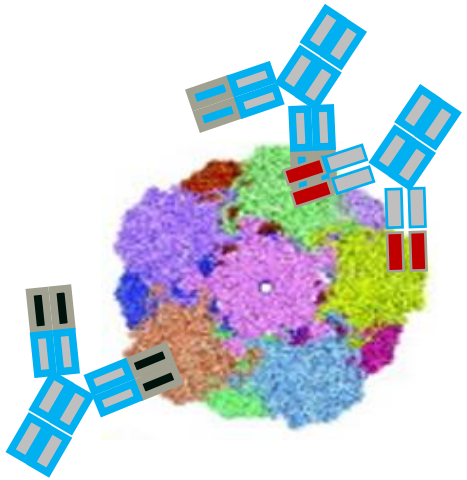
Zimple Matharu, Ph.D.
Principal Scientist
NGM biopharmaceuticals, South San Francisco

Antibody binding assessment

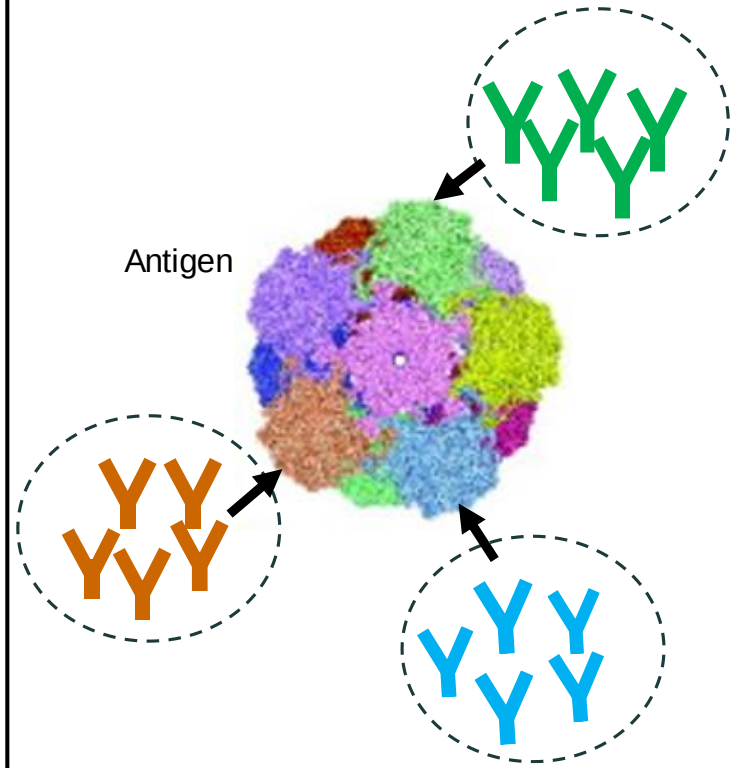
Kinetics and affinity:
How fast is the Ab-Ag interaction
Strength of interaction



Antibody's epitope:
mAbs binding region on Ag



Epitope binning:
Identifying diversity of a mAb panel

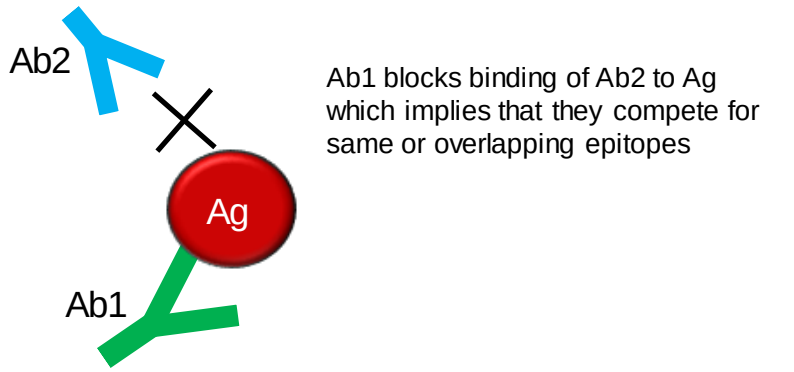


- Affinity can be tuned by engineering, but antibody's epitope is an innate property that cannot be rationally designed/altered.
- Only particular epitopes influence the function of the target protein.

Epitope binning for identifying epitope diversity

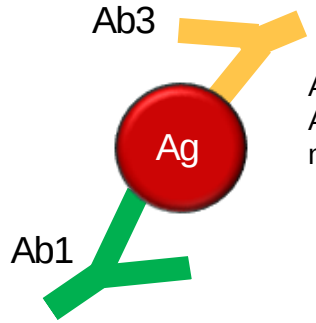
- Epitope binning is a competitive immunoassay in which mAbs are tested for simultaneous binding to their specific antigen in a pairwise fashion.
- A “HT-binning” experiment identifies diversity of the mAb panel by sorting them in different epitope groups (“Communities” or “Bins”) based on their similar or identical blocking relationships.
- **“Communities”:** Ab sorting based on closely related pairwise binding/blocking patterns. **“Bins”:** Ab sorting based on identical pairwise blocking/binding patterns.
- mAbs assigned to a “Bin” or “Community” and are likely to share similar functional characteristics.

Scenario 1



Ab1 blocks binding of Ab2 to Ag which implies that they compete for same or overlapping epitopes

Scenario 2



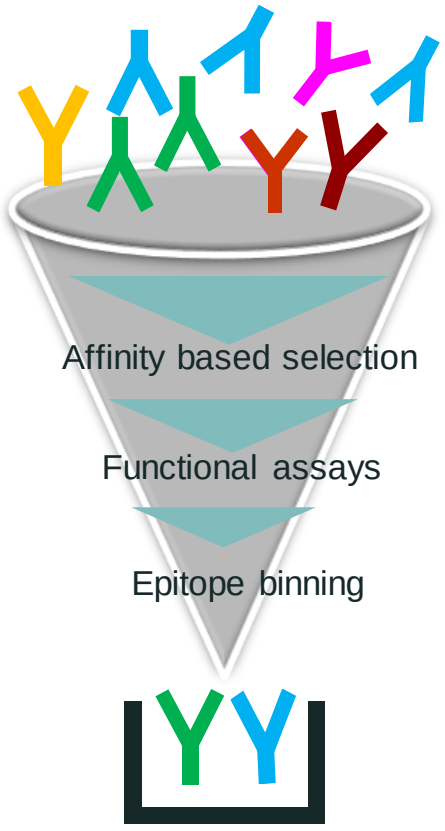
Ab1 does not block binding of Ab3 to Ag which implies that they target non-overlapping epitopes

Antibody screening workflows

Antibody generation

- Hybridoma
- B-cell cloning
- Display technologies (phage, yeast)

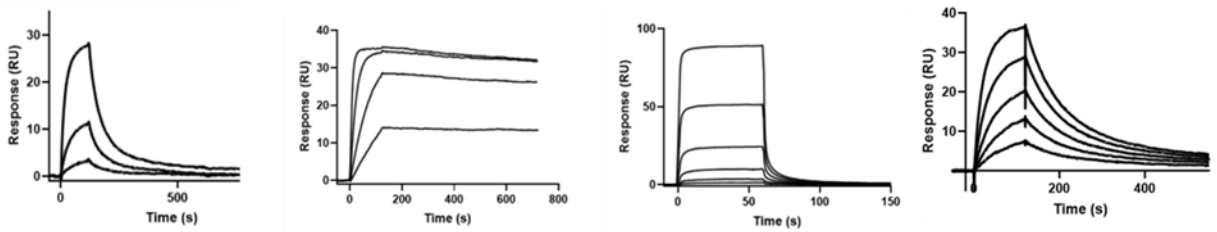
1. Traditional screening funnel



less diverse lead panel

2. New HT screening approach

HT- kinetics



HT-epitope binning

Pick diversified mAbs

↓

Functional assays

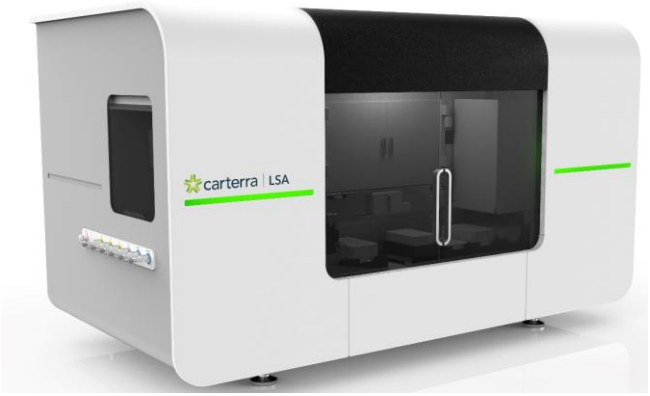
↓

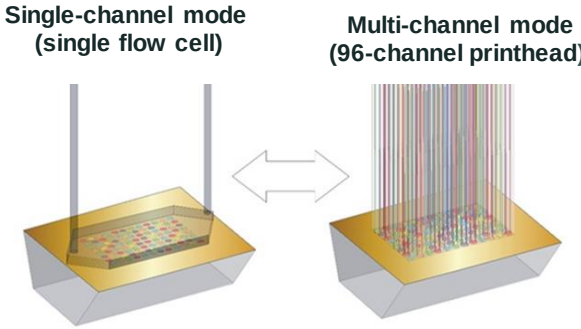
Diverse panel of leads

Prioritizing "affinity-based selections" over "epitope-based selections", may result in lead antibody panels lacking epitope diversity.

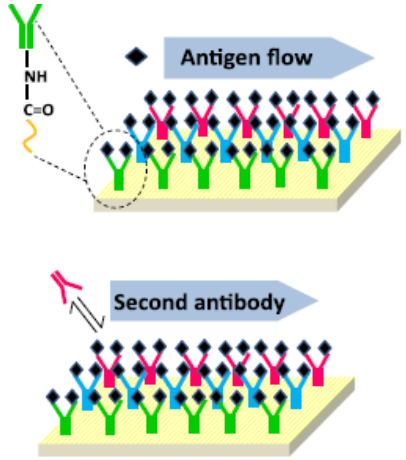
Real-time label-free biosensors for epitope binning: Array-SPR

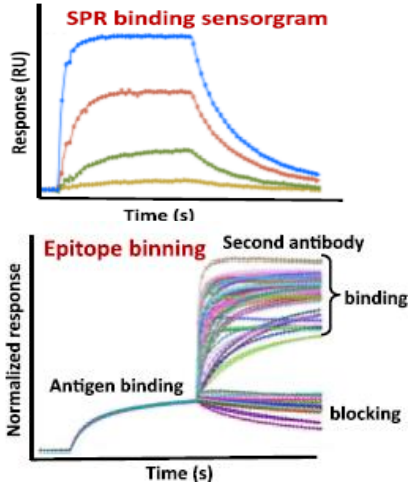
Array-SPR (Carterra, LSA)





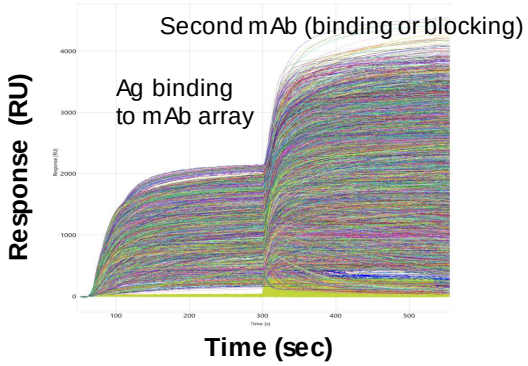
"One-on-many" format



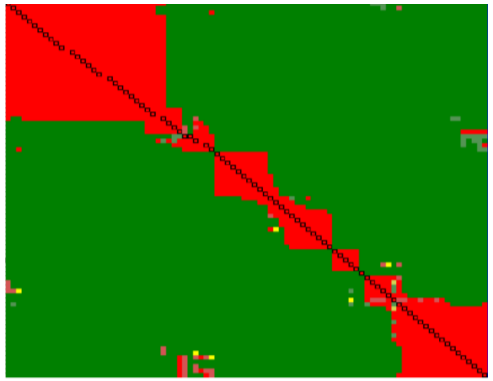


Data visualizations via epitope software

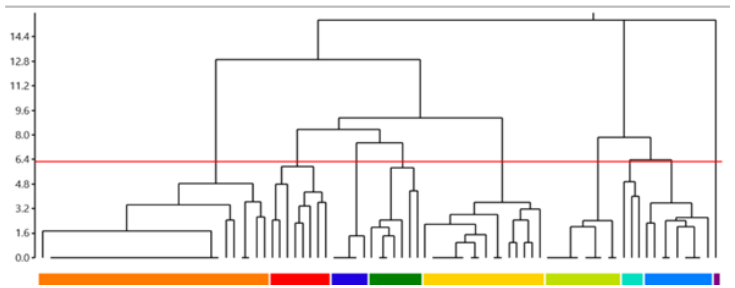
Real-time signals



Sorted heat-map

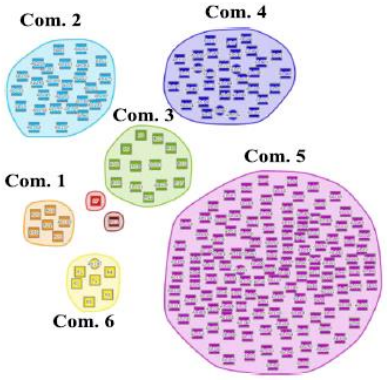


Dendrogram



Clustering of the mAbs based on their closely related blocking profiles

Network plots



Curated network plot

High throughput epitope binning case studies

Study 1:

Identifying epitope diversity and sample redundancy in a large mAb panel for technology development by using benchmark controls and representatives of known epitopes.

-a total of 250 mAbs were selected (via hybridoma and B-cell encapsulation) for an antigen “X”.

-mAbs covered 7 different mouse strains.

-Ag size ~22kDa.

-Matharu et al. Anal. Chem. 2021, 93, 16474−16480 (studies performed at BMS, redwood city)

Study 2:

Full symmetric bi-directional epitope binning of a panel of mAbs to identify domain specific binders.

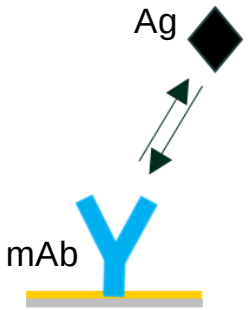
-96 mAbs were selected via hybridoma for an antigen “Y”.

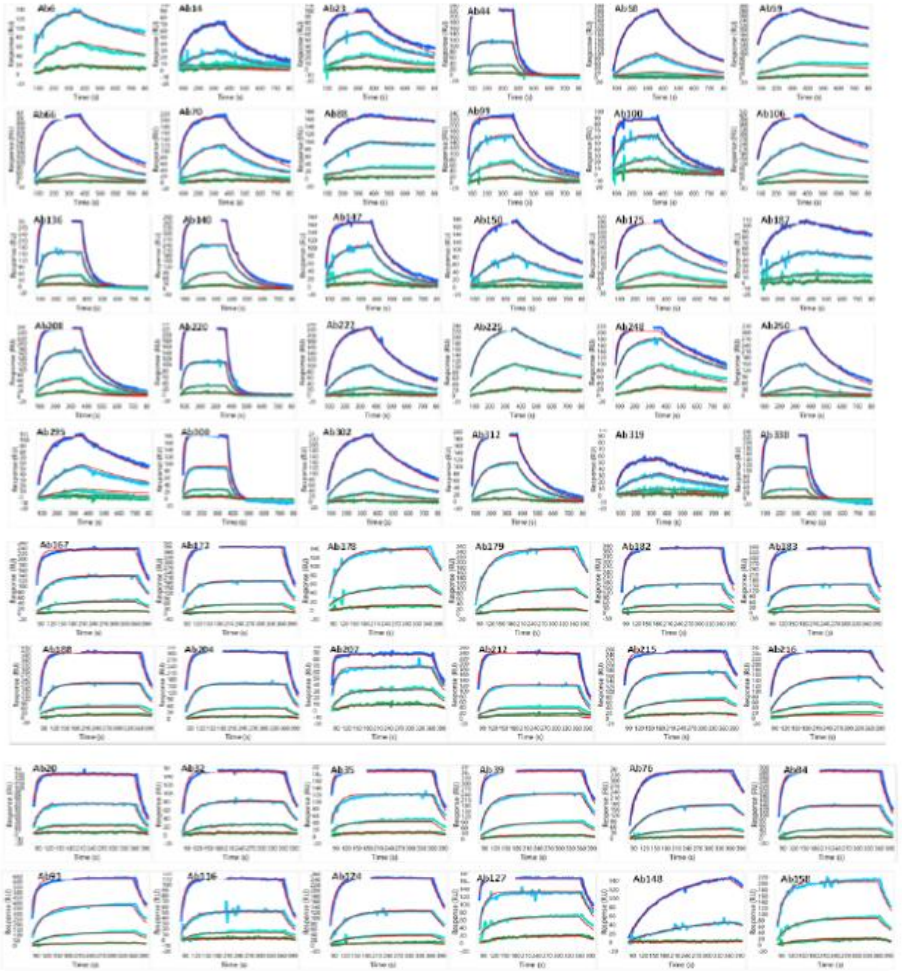
-Ag size ~55kDa.

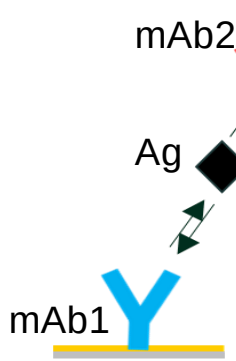
-full symmetric binning results compared with FACs based cell binding data.

Coupled mAb array

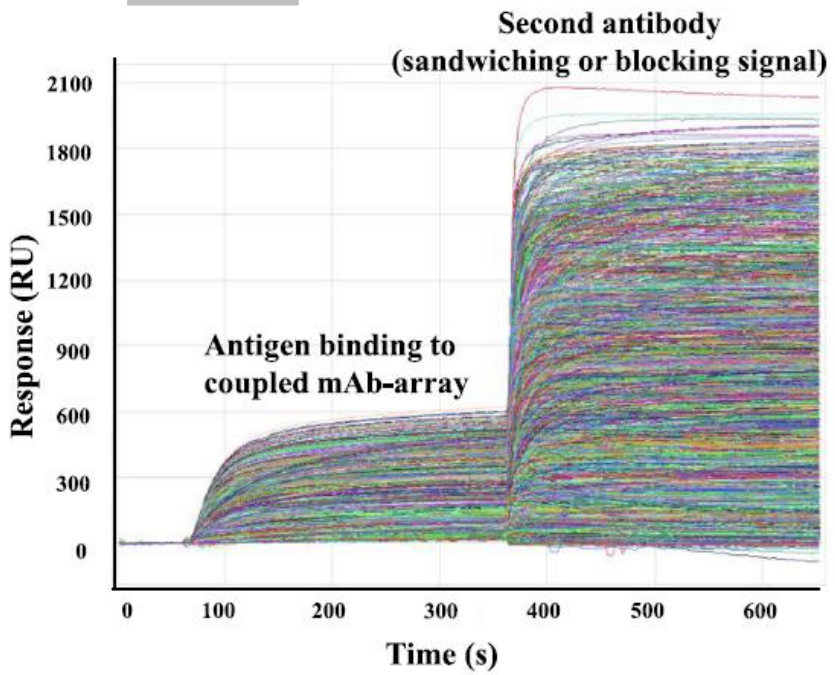
Study 1: HT-kinetics and binning of mAbs for antigen X







190 mAbs (Hybr.& BCE) epitope binned against 35 control antibodies

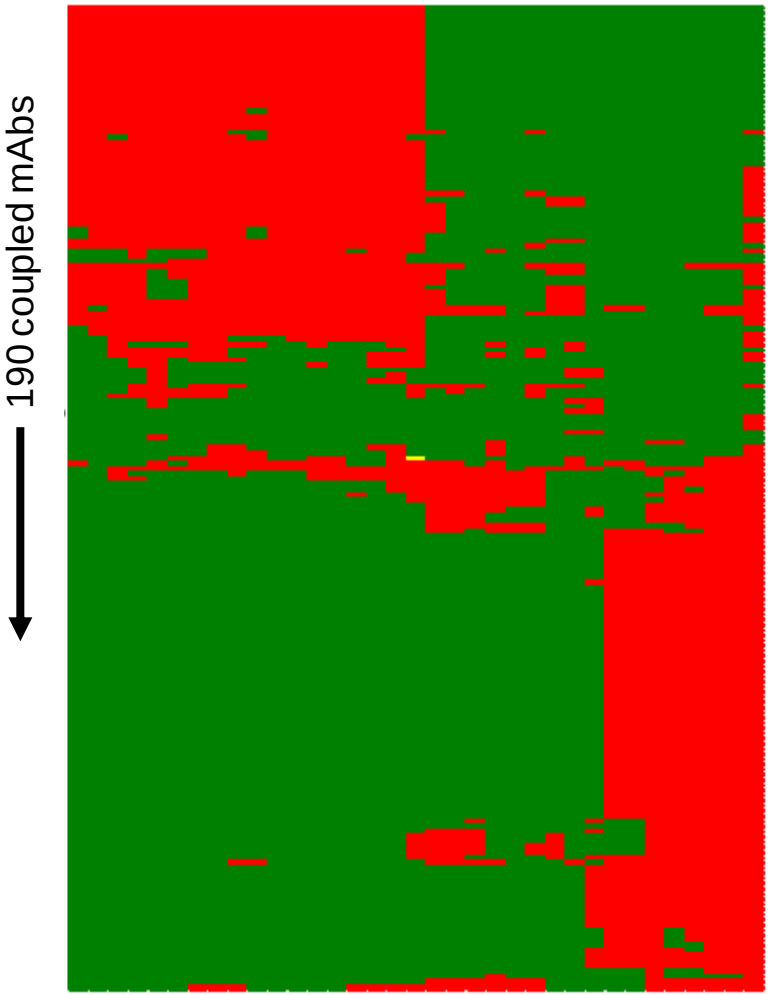


Matharu et al. Anal. Chem. 2021, 93, 16474−16480

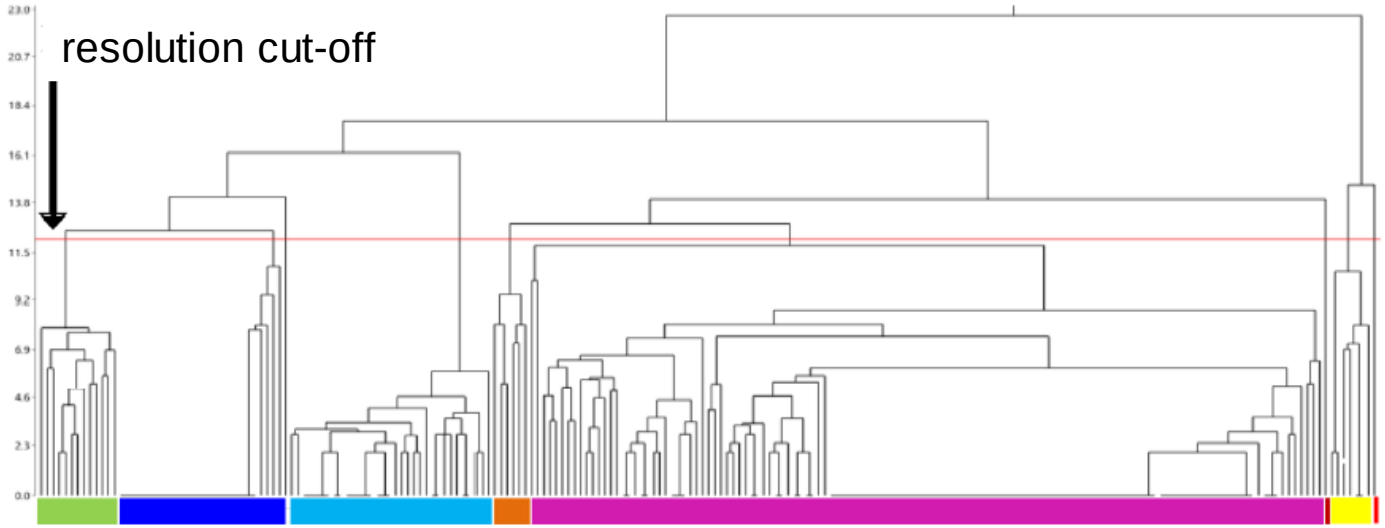
Binning analysis

Heat-map

35 second mAbs (sandwiching/blocking)



Combined dendrogram

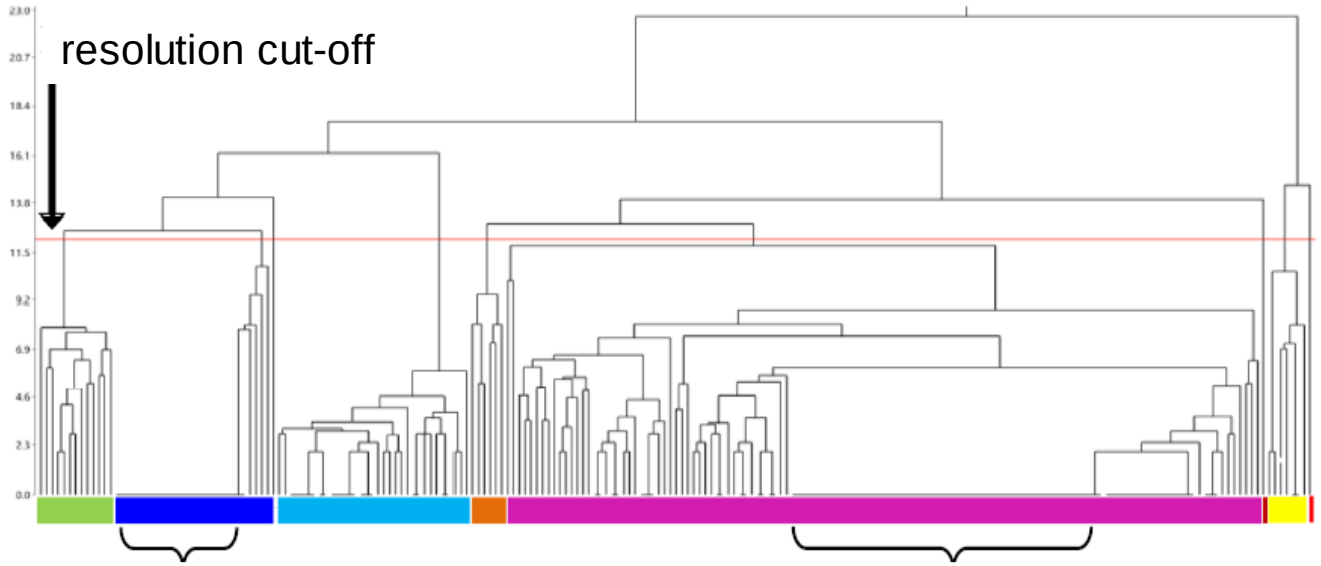


- The clustering algorithm of the analysis software converts the generated heat-map into a dendrogram tree.
- Dendrogram shows clustering of the mAbs based on their closely related blocking profiles. The placement of the “resolution cut-off” dictates curation of network plot (next slide) to illustrate panel diversity.

Figures adapted from Matharu et al. Anal. Chem. 2021

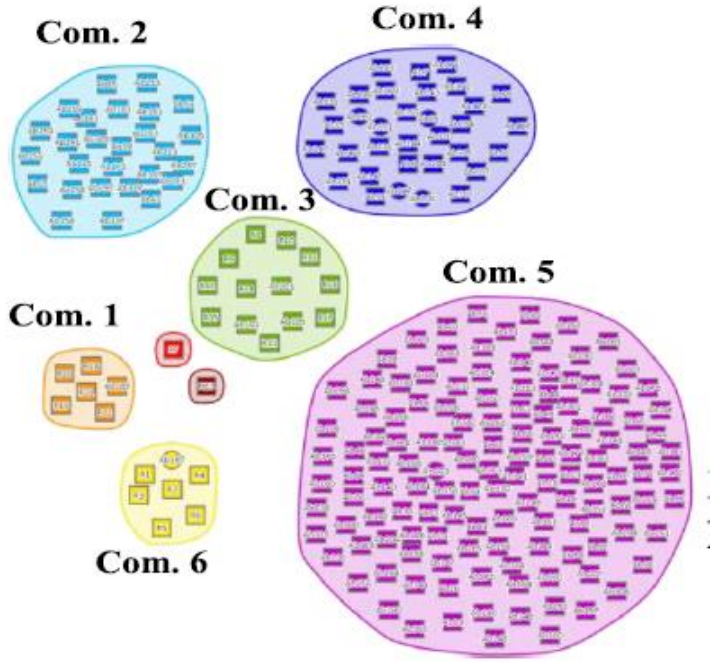
Epitope communities, panel diversity and redundancy

Combined Dendrogram



Dendrogram could help in identifying unknown sample redundancy when analyzed at highest level of resolution.

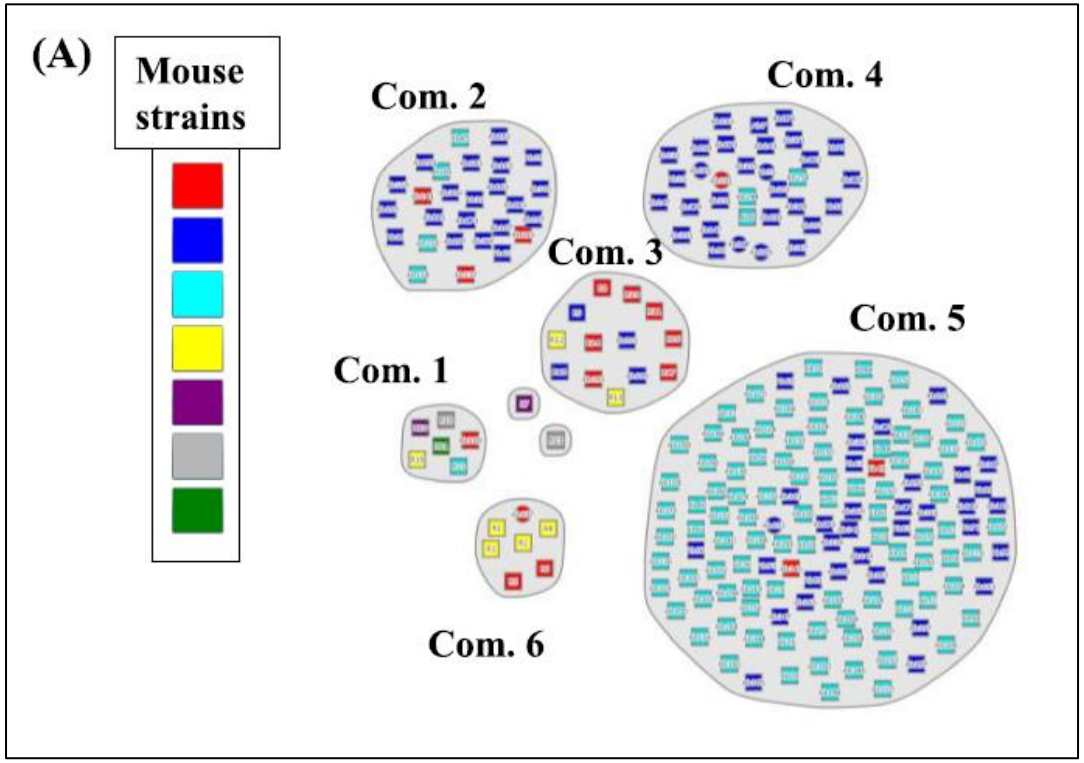
Curated network plot (“Communities”)

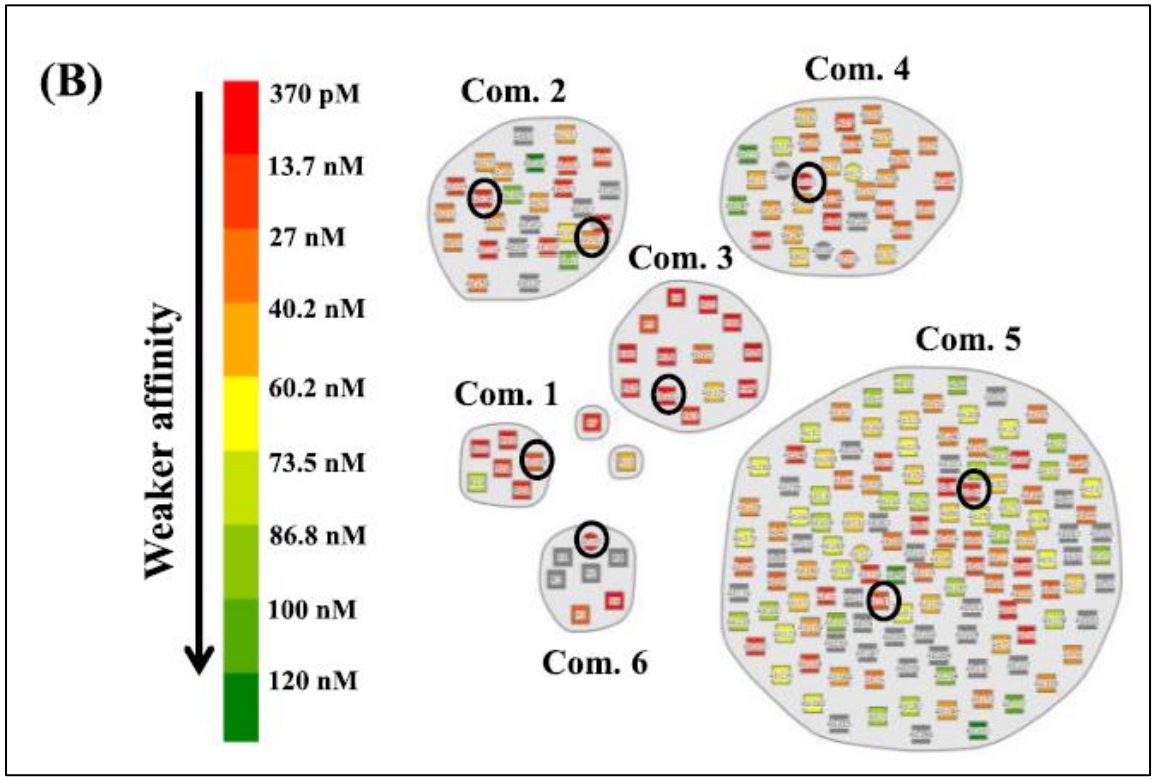


Network plot depicting 6 different epitope communities and 2 singlets

Large number of mAbs in Com.5 (in pink color) were found to be replicates

Decoding performance of different transgenic mouse strains





Figures adapted from Matharu et al. Anal. Chem. 2021

Mouse strain depicted in red color produced higher diversity and high affinity mAbs

Capturing full diversity in sequence and epitope

Multiple sequence alignment by bin:

- Harness full diversity by combining sequences and binning data.
- Choose low sequence liability upfront.

Combine with affinities:

- Explore epitope specific binding characteristics.
- Performance of transgenic mice.
- Understand affinity maturation.

Studies expanded by functional assay

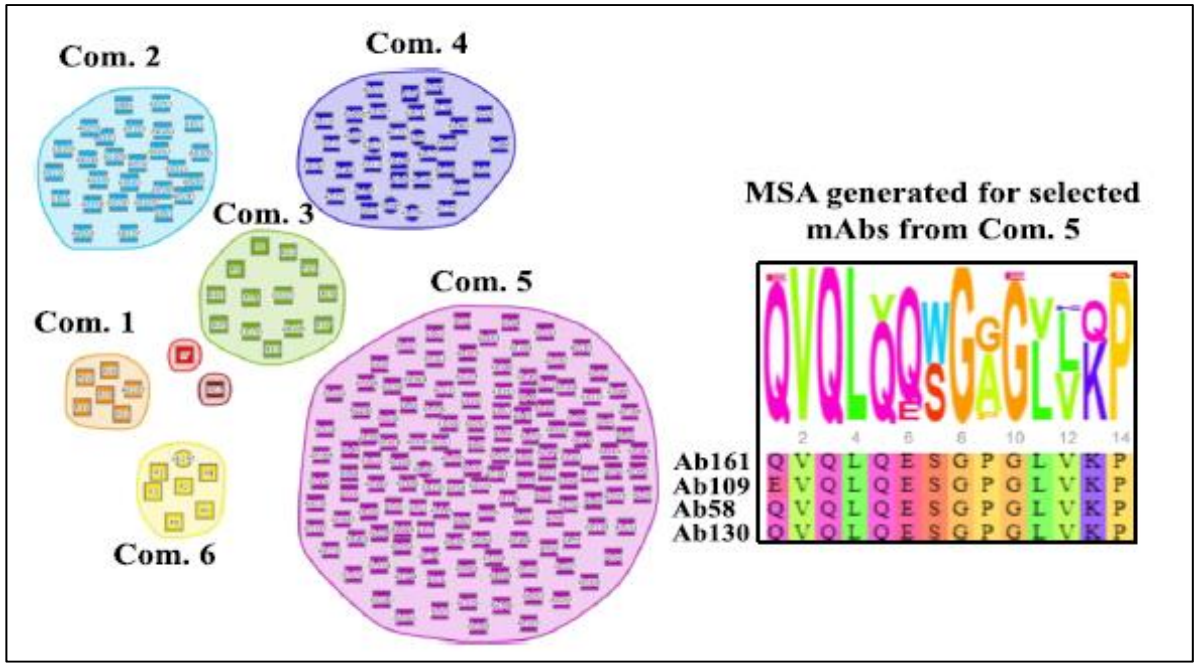
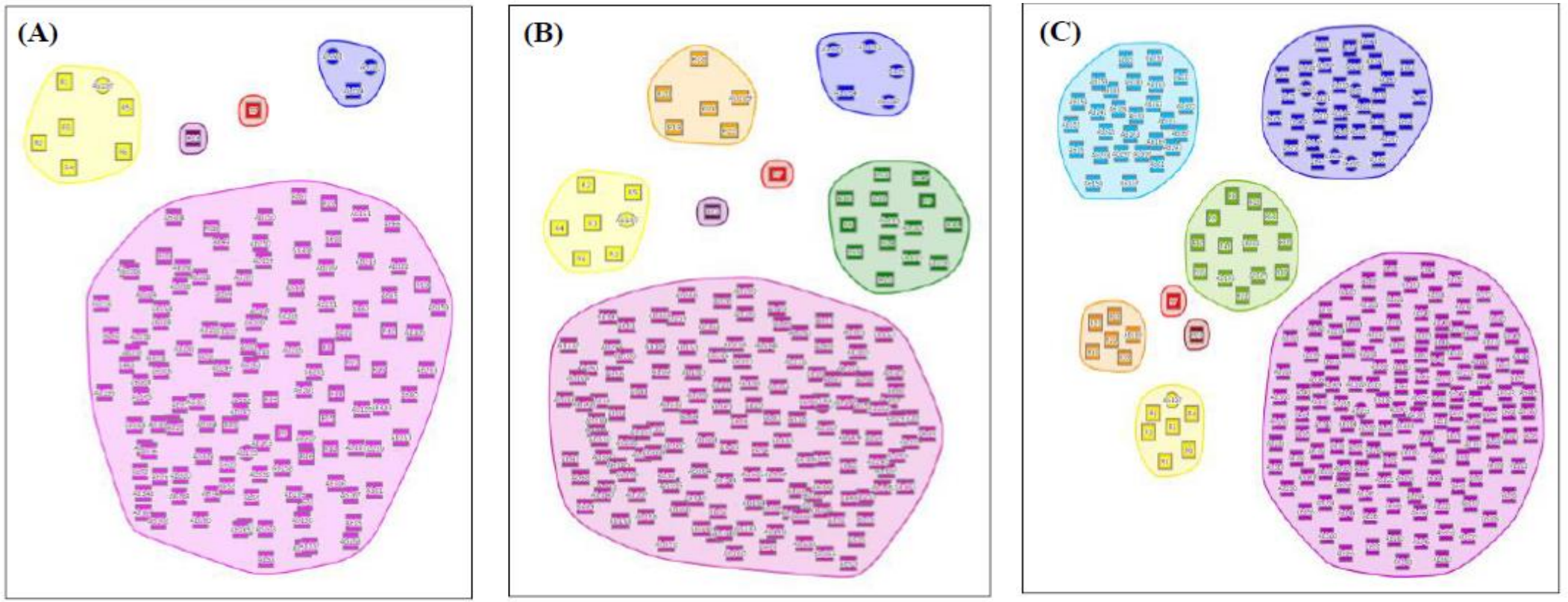


Figure adapted from Matharu et al. Anal. Chem. 2021

Pick an optimal panel of diversified leads

Effect of mAb panel size on epitope binning and diversity

Communities generated by a panel of 100 mAbs, 140 mAbs and 190 mAbs



Figures adapted from Matharu et al. Anal. Chem. 2021

Important epitopes could go unidentified with smaller binning panels resulting in less diversity

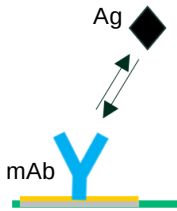
Study 2:

Full symmetric bi-directional epitope binning of a full hybridoma panel of mAbs to identify domain specific binders.

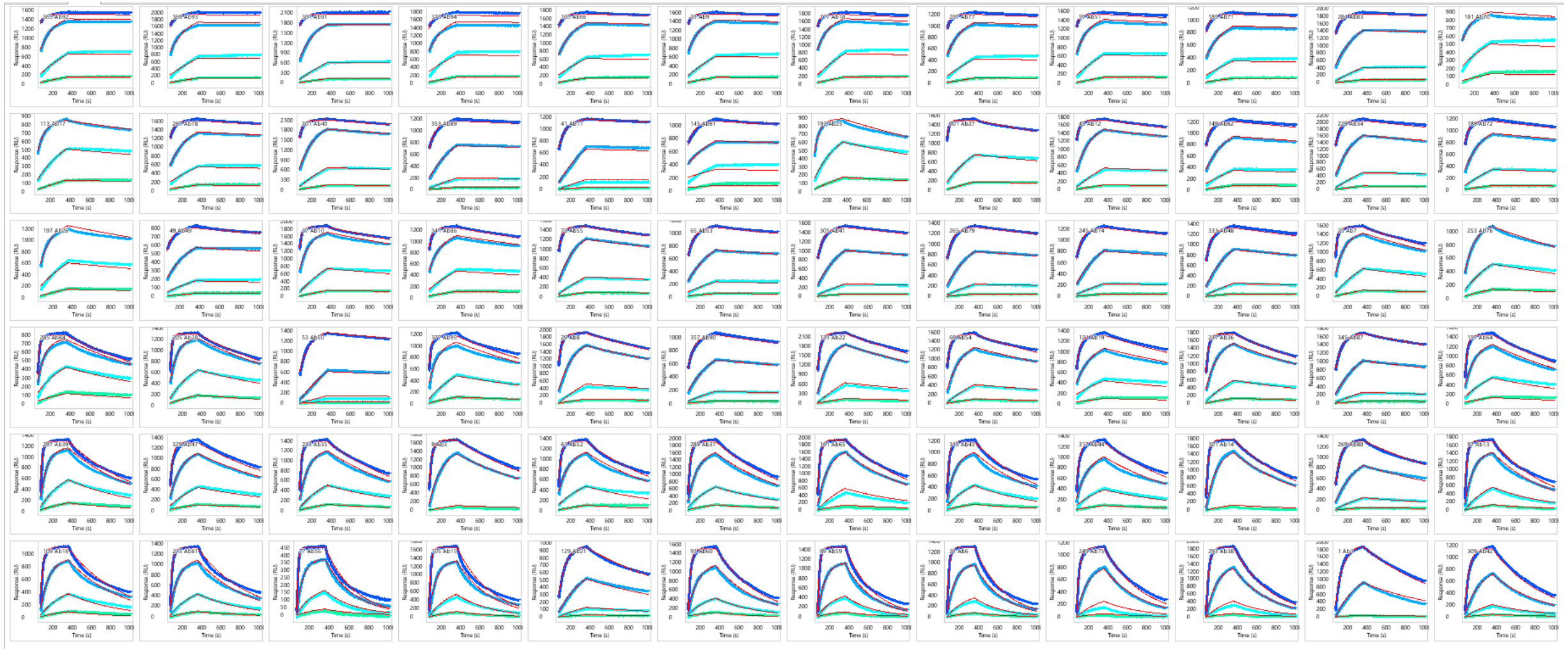
-96 mAbs were selected via hybridoma for an antigen “Y”.

-Ag size ~55kDa.

-full symmetric binning results compared with FACs based domain mapping data.

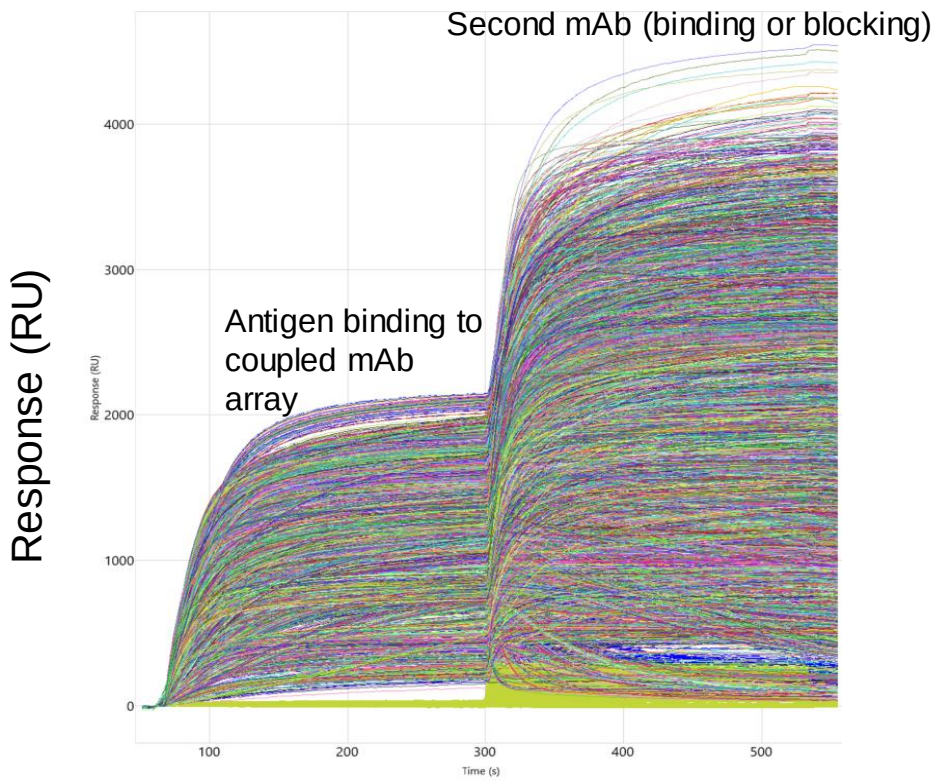


HT-Kinetics of 96 antibodies against antigen Y

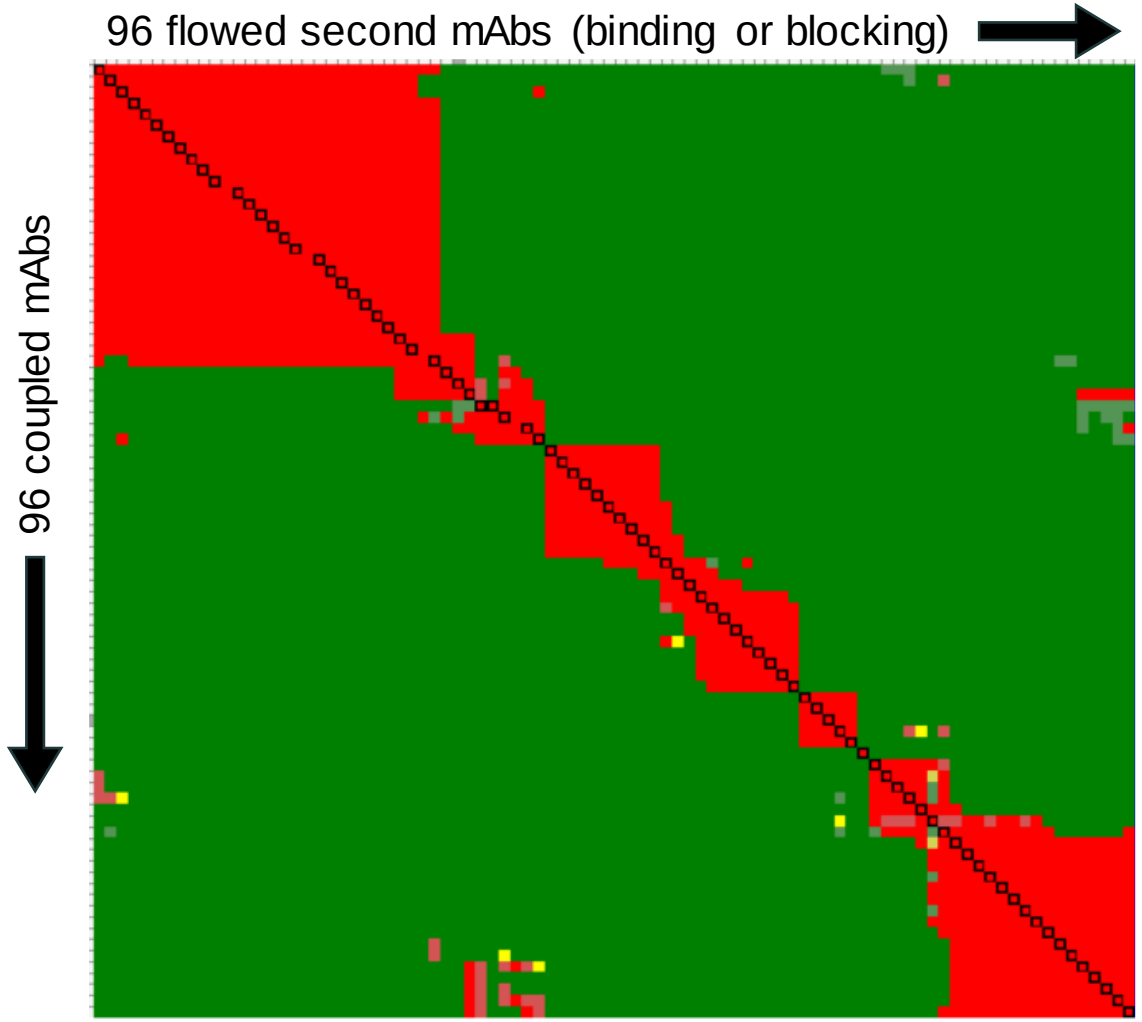


Subset of SPR sensorgrams

Full-symmetric binning of antibodies against antigen Y



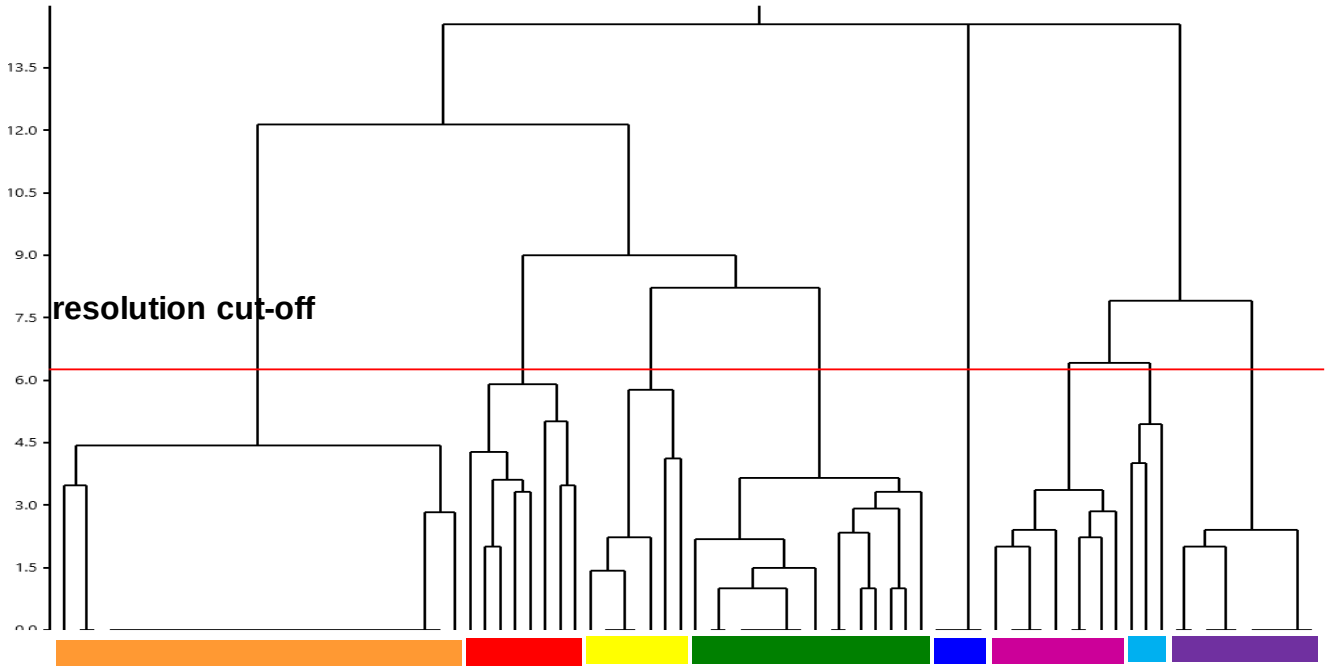
Raw binning signals on coupled mAb array



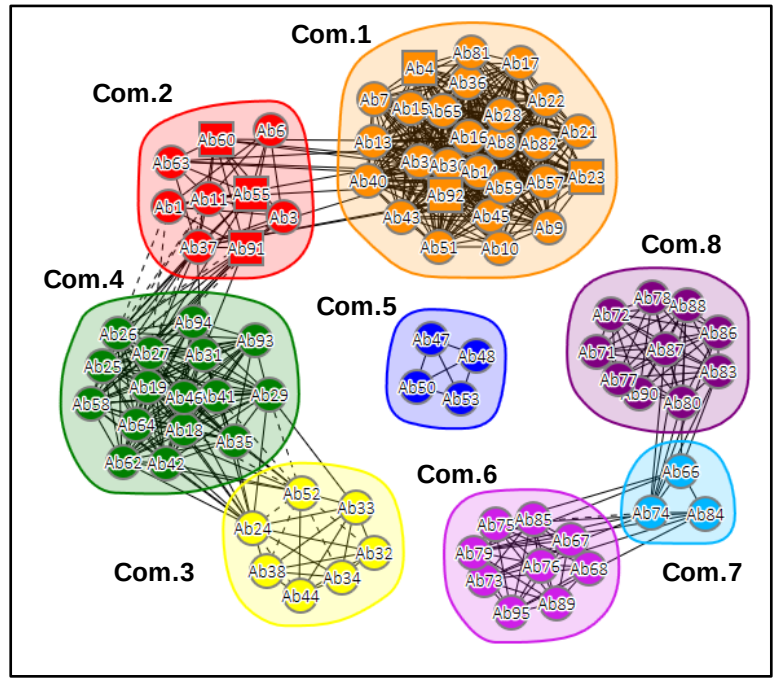
Sorted heat-map

Epitope diversity analysis

Combined dendrogram



Curated network plot ("Communities")

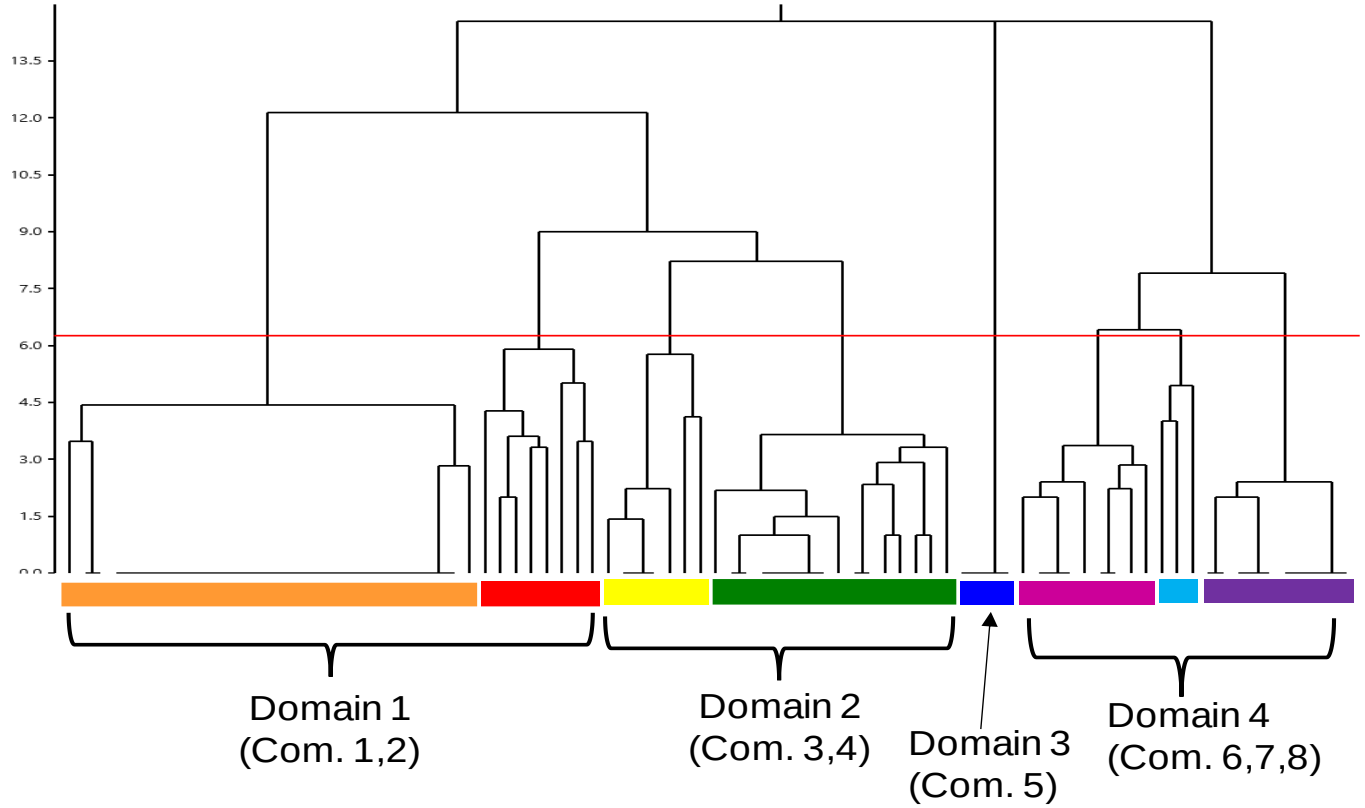


mAb panel diversity represented by different communities

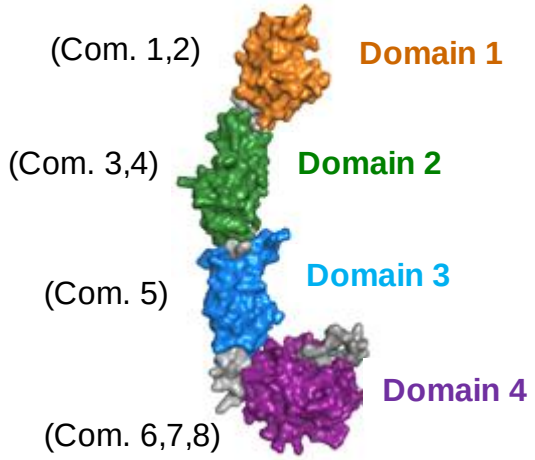
- Several blocking relationships between communities (1 and 2), (2 and 4), (4 and 3). These communities also fall next to each other in the dendrogram.
- Similarly, epitope communities 6, 7, and 8 are connected to each other by several blocking relationships and fall next to each other in the dendrogram.
- Epitope Com 5. represents a separate binding epitope in the middle.

Binning communities and domain mapping

Combined dendrogram



Structure of antigen "Y"



FACS study

Four domain constructs of Ag (Y) were transiently expressed in mammalian cells to determine the binding domain of each mAb via FACS.

Ag domain deletion	mAb binding domain on Ag			
	Dom.1	**Dom.2**	**Dom.3**	**Dom.4**
FL	Yes	Yes	Yes	Yes
Del1	---	Yes	Yes	Yes
Del12	---	---	Yes	Yes
Del123	---	---		Yes

Epitope communities map well with FACS data which helps with identification of domain specific binders

Summary

- Early characterization of large mAb panels via high-throughput SPR can revamp lead Identification and technology development.
- Collective HT-kinetics, HT-binning and sequence analysis enables selection of high-affinity binders and helps in harnessing the full epitope diversity of large mAb panels.
- It also provides a way of assessing antibody generation technologies and various transgenic mouse strains.
- Campaigns generating large antibody panels with broad epitope coverage enhance the chances of identifying mAbs with epitopes that influence the function of target protein.
- The integration of affinities and binning results with domain mapping analysis may provide an effective way of identifying high affinity domain specific mAbs.

THANK YOU!

NGM Colleagues

Alison Lucero
Sara Medfisch*
Vicky Lin*
Jonathan Aguayo
Carmence Ho
Mark Humphrey
Gyasu Bajracharya*
Yadong Yu
Mark Humphrey
Peter Lee*
Betty Li*
Noha Elabed
Chichi Li
Sindhuja Ramakrishnan
Alan Kutach
Bin Fan

NGM leadership

Alessandro Palumbo
Dan Kaplan
Rick Feldman
Kathy Miller*
Arthur Hsu

Previous BMS Colleagues

Christine Bee
Flavio Schwarz
Haibin Chen
Matthew Tomlinson
Gabriel Wu
Ginger Rakestraw
Michael Hornsby
Winse Morishige
Ralston Barnes
Andrew Drake
Pavel Strop
Arvind Rajpal
Gavin Dollinger