High throughput SPR characterization of multi-specific antibodies

New capabilities and workflow approaches advancing discovery

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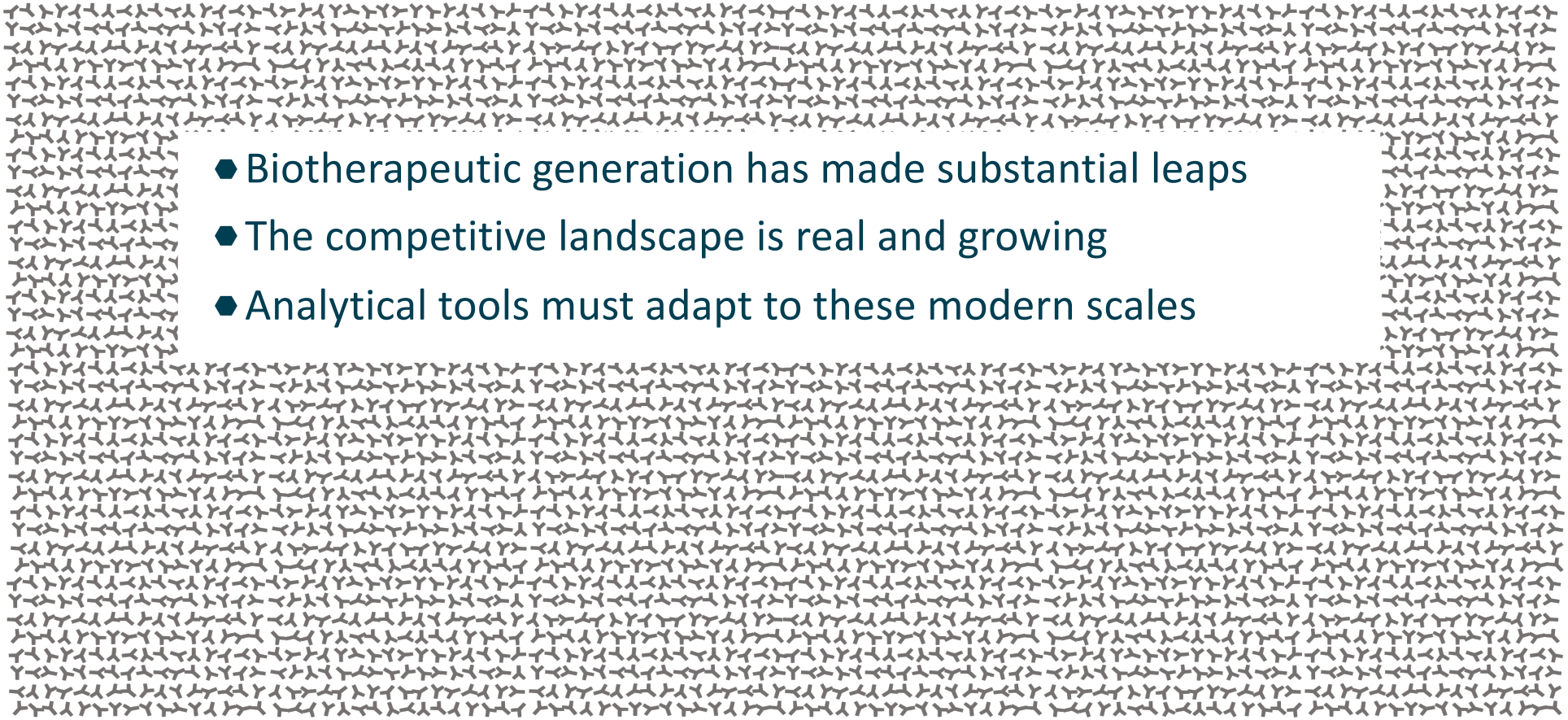
Outline

- The need for high throughput SPR-based biophysical characterization and how it accelerates discovery
- Carterra's HT-SPR technology
- Epitope binning and its role in bispecific antibody discovery
  - An example from a recent publication
- Assay formats for high throughput SPR characterization of bispecifics
  - Independence or non-interference of each binding site
  - Bridging assays

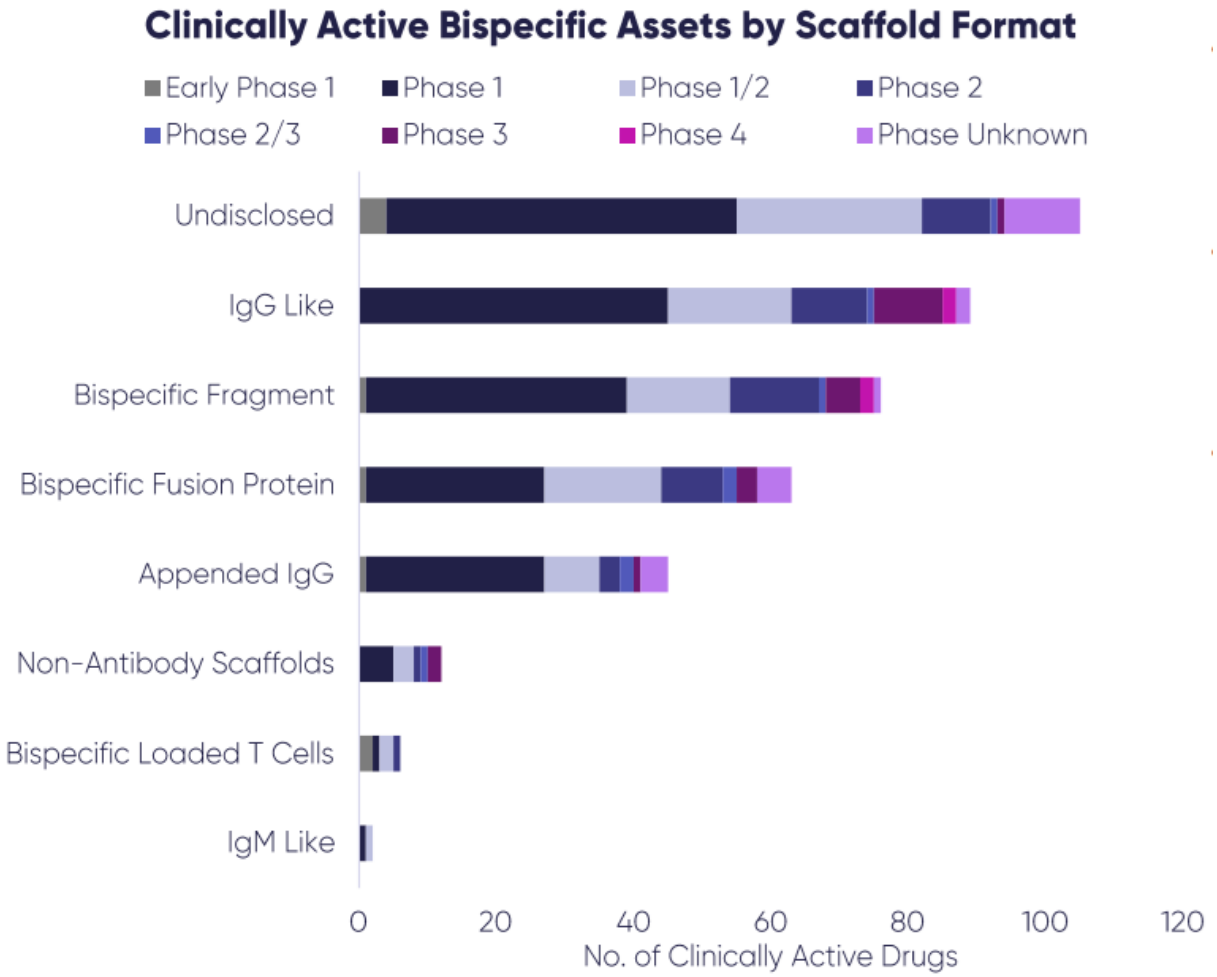
Which one(s)?



- Biotherapeutic generation has made substantial leaps
- The competitive landscape is real and growing
- Analytical tools must adapt to these modern scales

Bispecific/multispecifics development landscape

- 9 bispecifics have been approved
- 5 of them in 2022!
- The pace of research, number of disease areas, and MOAs are growing exponentially
- Hundreds of molecules are in late stage preclinical or clinical development

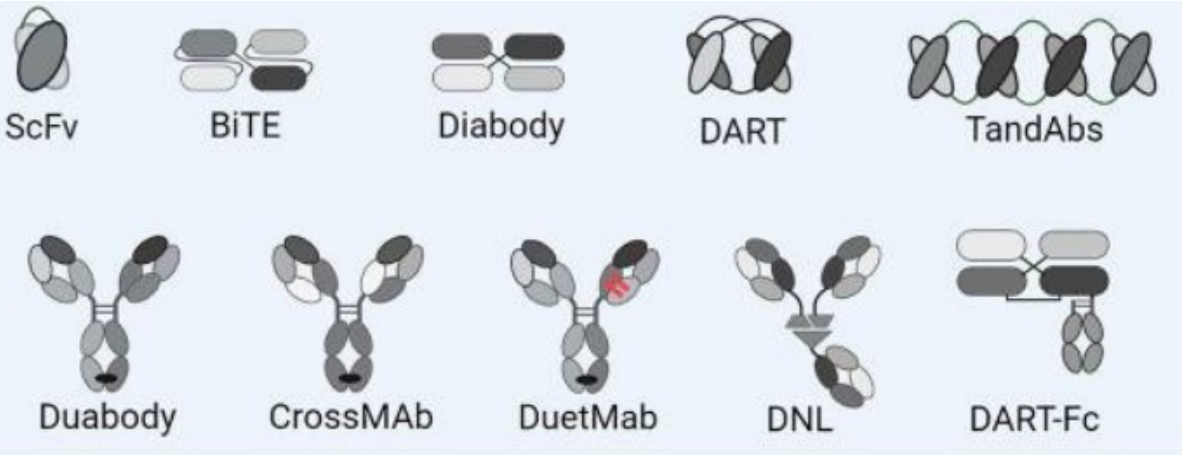


Multispecific Biotherapeutics- A wide open frontier!

Incomplete list of MOAs

- T-Cell engagers
  - NKs, APCs
- PROTABS
  - protein homeostasis
- Bi(+)paratopic
  - Avidity enhancement
- Half life extension
- Blood Brain Barrier

small sample of possible formats



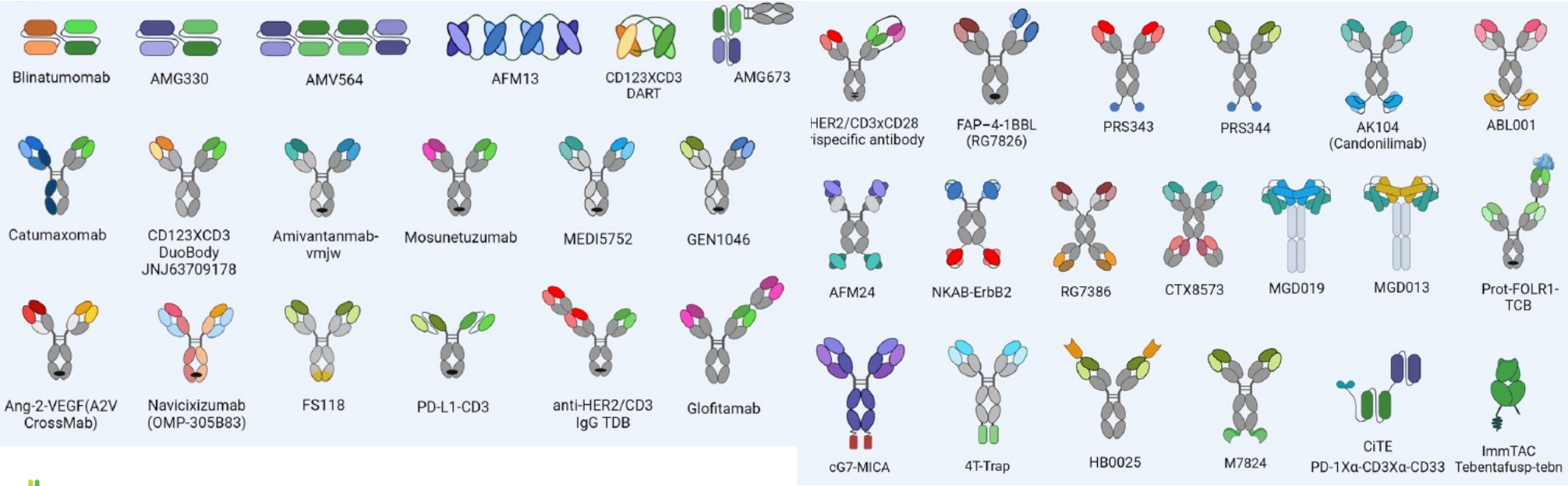
Current landscape and future directions of bispecific antibodies in cancer immunotherapy

Jing Wei [1], Yueyao Yang [2], Gang Wang [2], Ming Liu [1]

Front Immunol. 2022 Oct 28;13:1035276. doi: 10.3389/fimmu.2022.1035276.

A few specific examples

- Most contain some antibody or antibody like binding domain(s)
- Protein mimetic domains are common
- Evolving role for ADCs, enzymes, and pro-drugs as well



https://doi.org/10.3389/fimmu.2022.1035276

Characterization Needs for Mutlispecifics

- Multi-specific drug development requires additional characterization
- In some ways, several lead binder drug programs must converge into a single molecule, creating layers of analysis
- Each interaction has some degree of independent screening and characterization requirement plus the need to test and validate combined formats
- LSA enables key binding assay formats for multi-specifics:
  - Independent affinity and kinetics
  - Alternative site occupied binding and kinetics
  - Bridging assays

Carterra’s HT-SPR Technology

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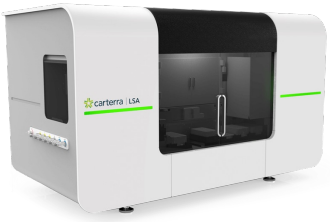
Fully integrated HT-SPR package



LSA

LSA^{XT}

Instrumentation

Control and analysis software

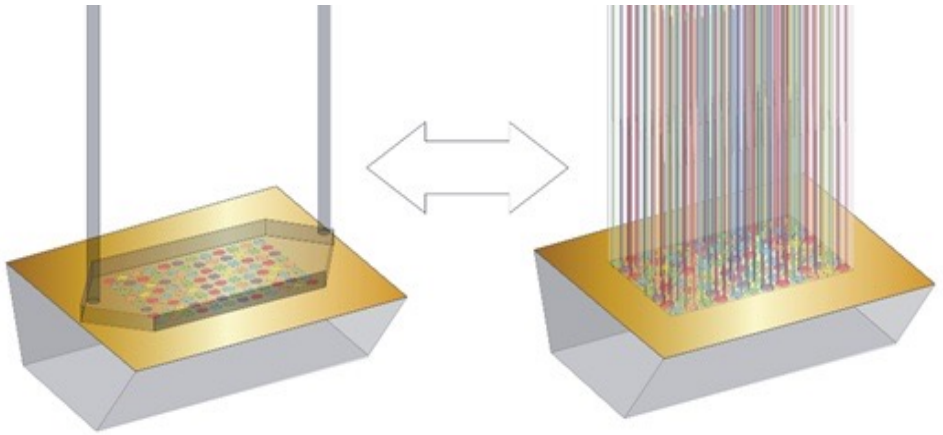
Biosensor chips and consumables

LSA integrates flow printing & array SPR.

Single-channel mode
(single flow cell)

Multi-channel mode
(96-channel printhead)

Minimal analyte consumption via "one-on-many" assay format

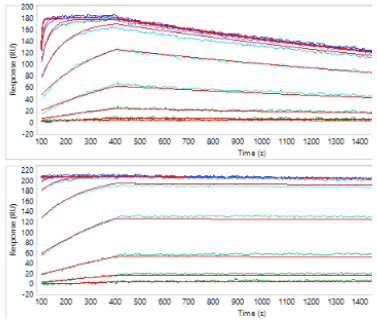


Serially print at 4 nested locations to create a 384-array

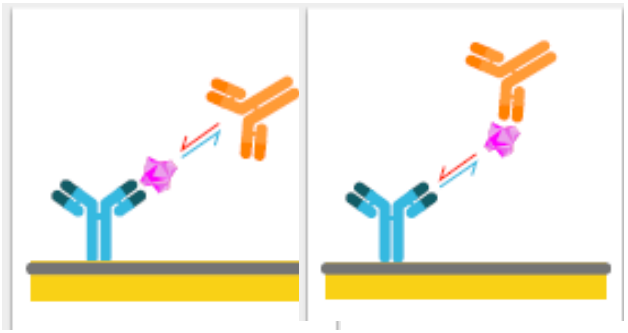
- Automated flow cell switching between multi- and single-channel modes
- 384 reaction spots + reference interspots per array
- In-line reloading of array
- Supports capture formats and standard amine coupling

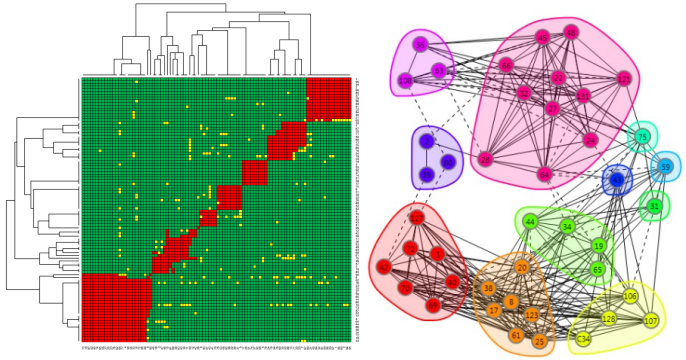
LSA's Core Applications

Kinetics/Affinity

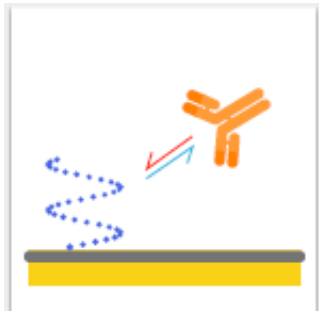


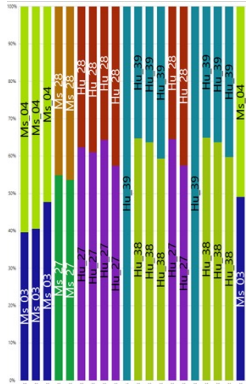
Epitope Binning



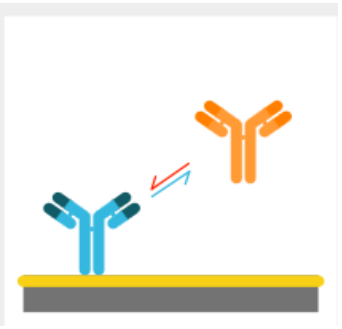


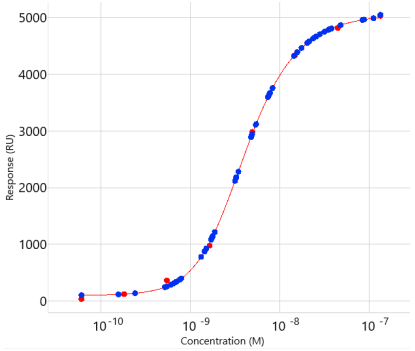
Mapping





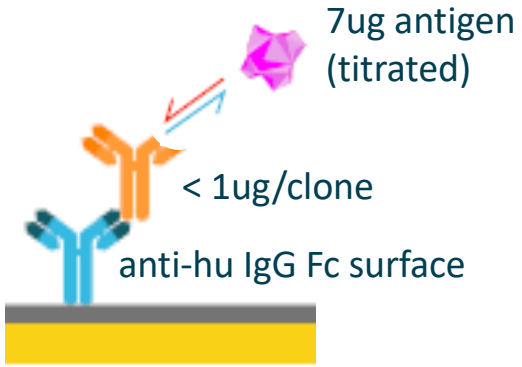
Quantitation





Unmatched assay throughput

- Up to 1152 clones in a single experiment
  - 3 x 384 well plates
  - One or multiple forms of antigen
  - Crude or purified
- Highly parallel – simultaneous binding to same antigen by all 384 clones
- Leverage array capacity
  - Capturing each clone on multiple spots to provide replicates
  - Titrate ligands to optimize density on the fly



Experience true HT-SPR kinetic analysis with the LSA

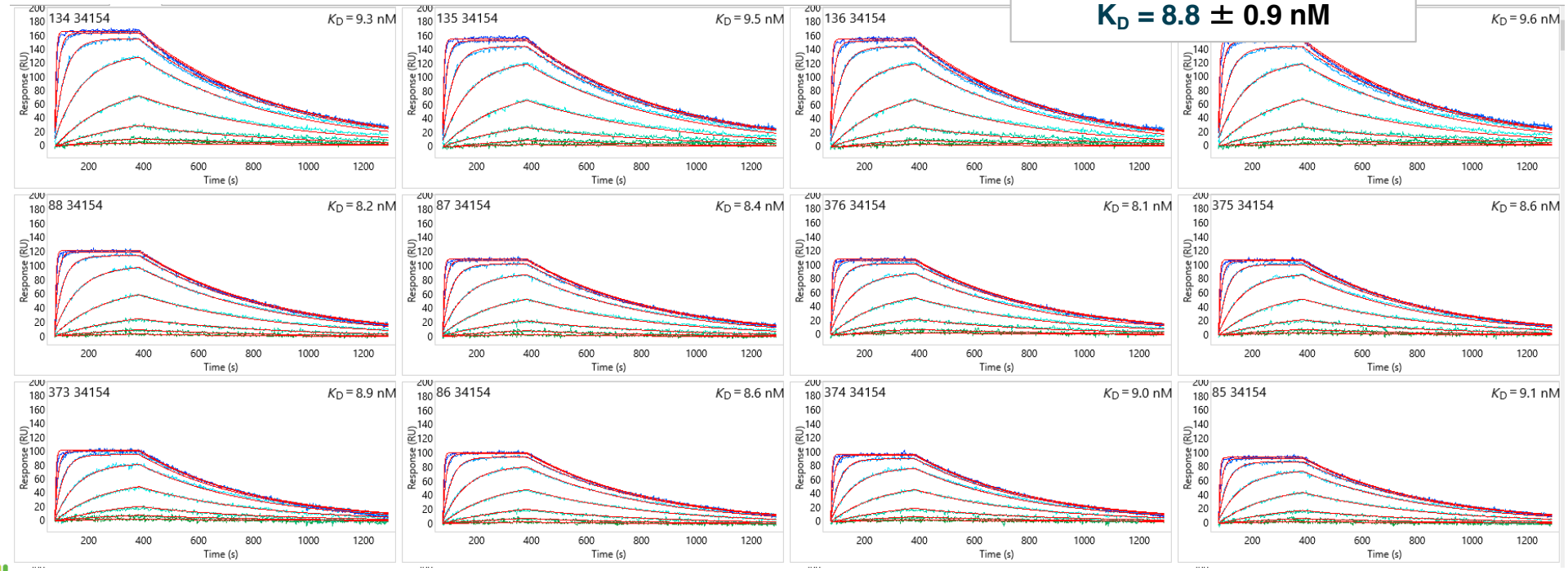
384 ligand kinetics | Single run | 7 µg Antigen | 8 Concentrations

Software automatically flags the data needing more investigation

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



Epitope Binning

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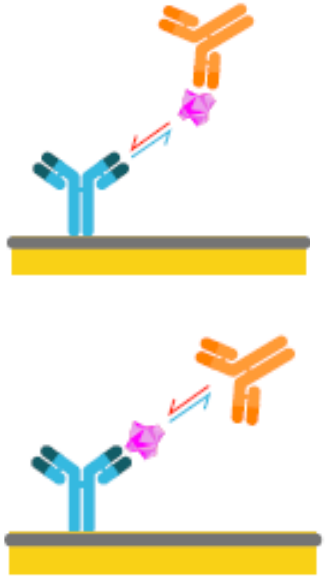
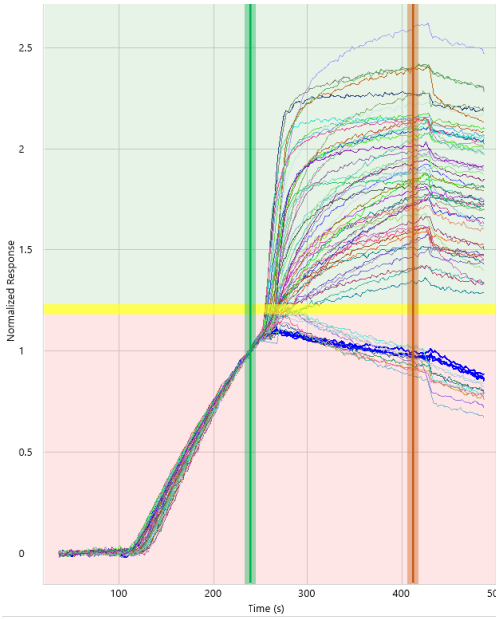
High-throughput/high-resolution epitope binning enables better selection of clones for bispecifics

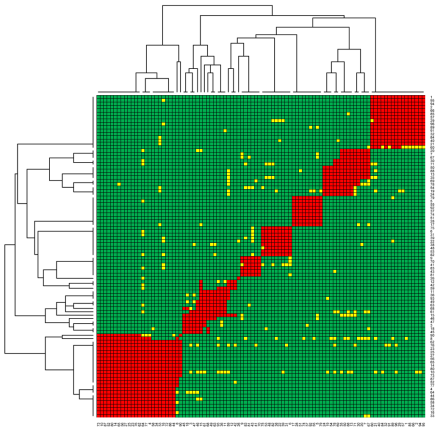
- Numerous high impact publications have emphasized epitope analysis and early epitope characterization as key elements in rapid mAb drug discovery workflows
- Epitopic diversity is a surrogate for functional diversity
- The binding epitope is fundamental for the activity and MOA of antibodies
  - Different binding epitopes and geometries can have a profound impact on activities of bi and multispecifics
  - Different arrangements are useful for cis and trans activation or neutralization
- Understanding the competition profiles between clones is essential for the creation of cocktails or biparatopic binders

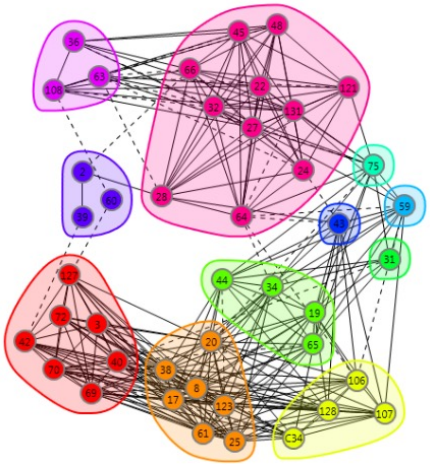
LSA Epitope binning workflow

Compete Clones → Define Competitive Thresholds → Represent Epitope Relationships using Heat Maps, Network Plots, etc.







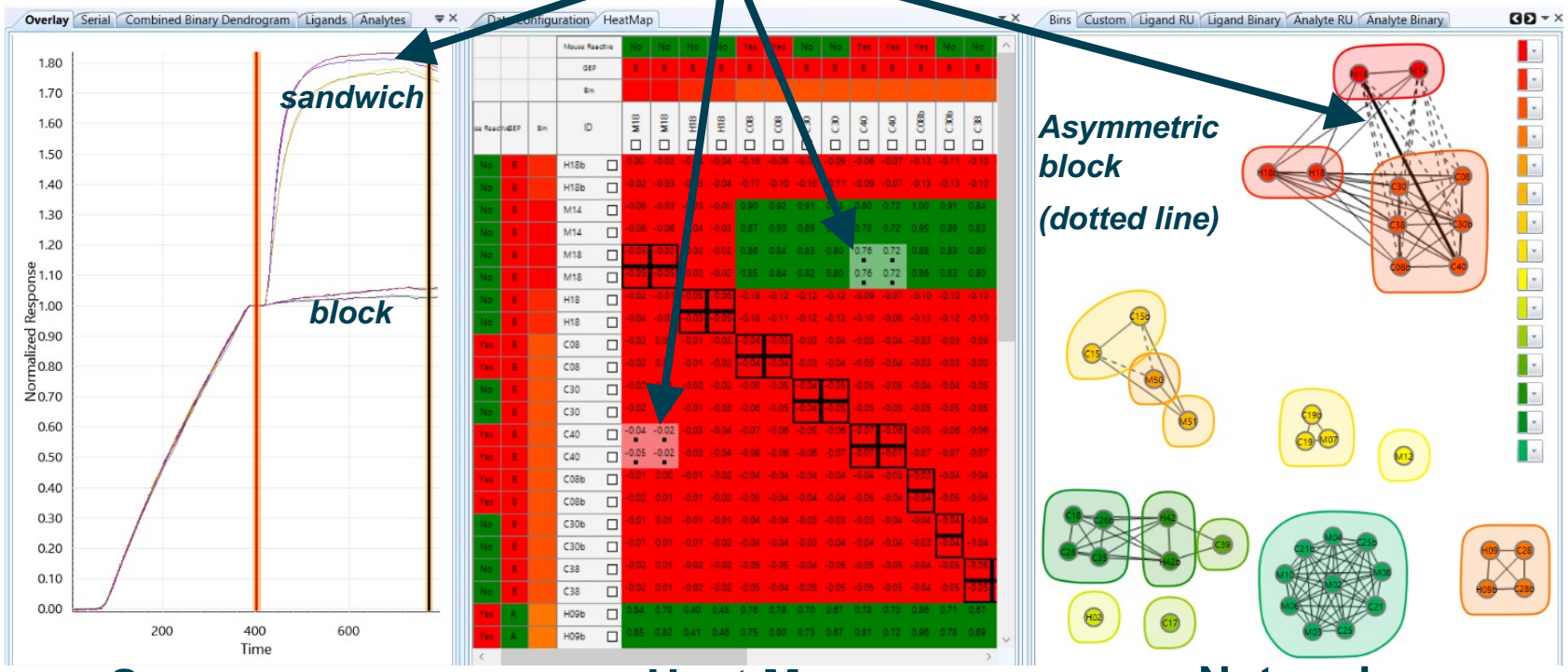


Larger and more diverse binning sets add resolution

- Assay set up complexity on LSA scales linearly
- Large heat maps provide many pair-wise interactions
  - 96 x 96 = 9,216 interactions
  - 192 x 192 = 36,824 interactions
  - 384 x 384 = 147,456 interactions
- Each unique interaction can be thought of as a probe
  - Each unique sample has the potential to elucidate new behaviors or subtle differences present in the epitope
  - The more diverse set you have, the more resolution you get!
- The LSA is the only platform to allow epitope binning assays to scale efficiently

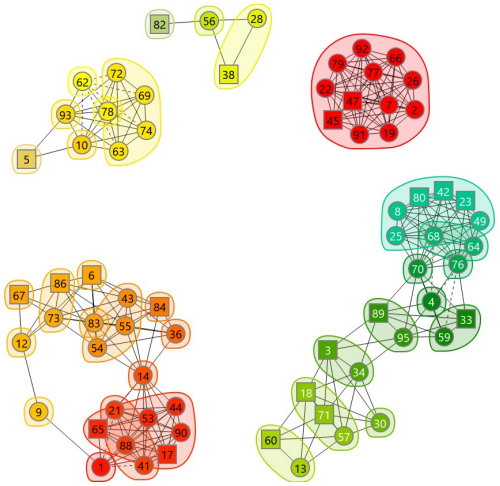
Epitope binning software user interface

Data Linked Across Three Visualization Panels

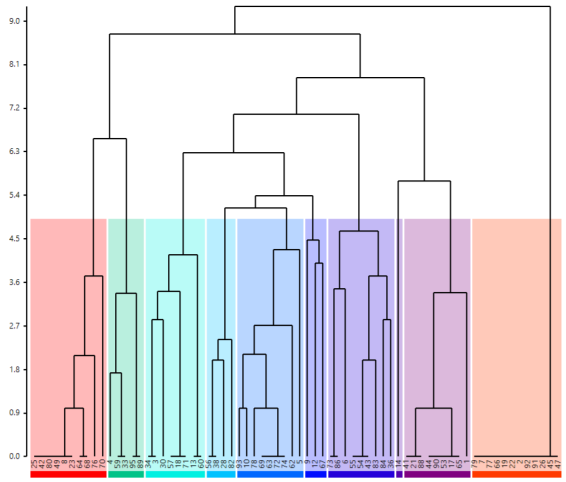


Sensorgrams **Heat Map** **Networks**

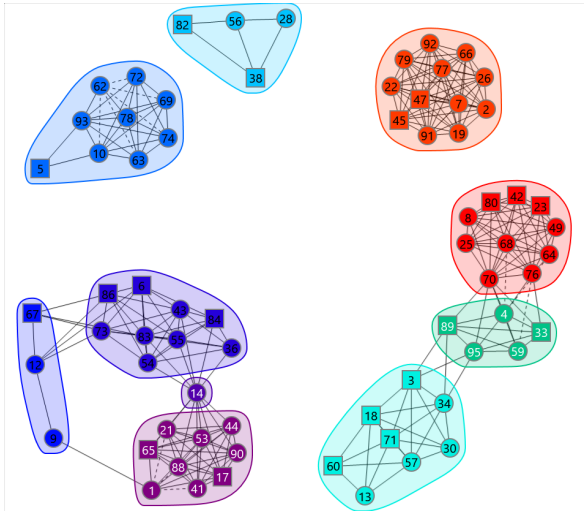
Three informative views to interpret epitope binning results.



Networks
(most granular bins)



Dendrogram



Communities
(user-defined cut-height)

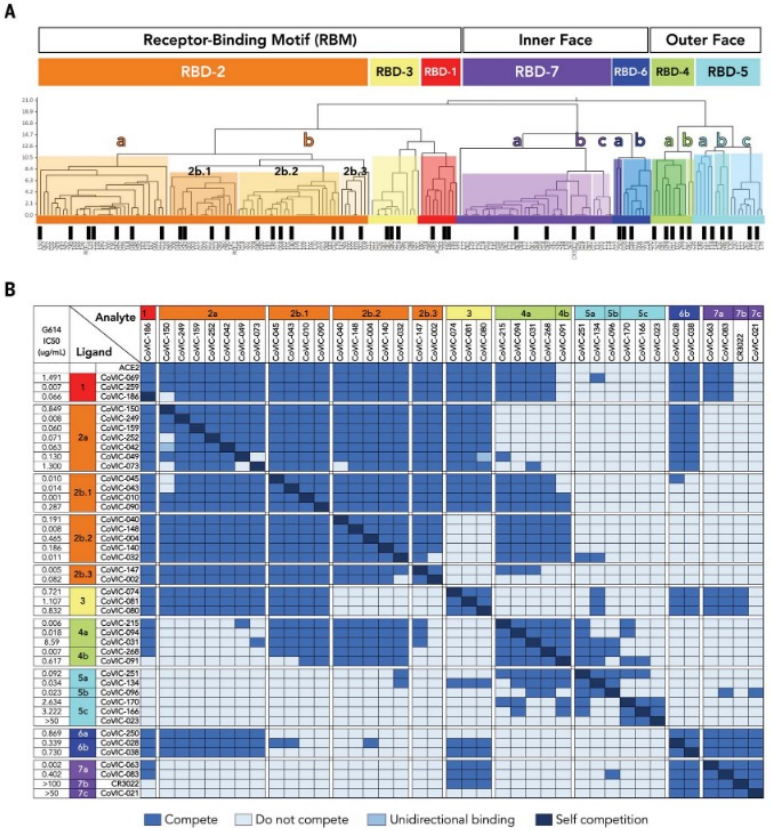
Coronavirus Immunotherapy Consortium- CoVIC

Science

CORONAVIRUS

Defining variant-resistant epitopes targeted by SARS-CoV-2 antibodies: A global consortium study

Kathryn M. Hastie[1]†, Haoyang Li[1]†, Daniel Bedinger[2], Sharon L. Schendel[1], S. Moses Dennison[3], Kan Li[3], Vamseedhar Rayaprolu[1], Xiaoying Yu[1], Colin Mann[1], Michelle Zandonatti[1], Ruben Diaz Avalos[1], Dawid Zyla[1], Tierra Buck[1], Sean Hui[1], Kelly Shaffer[1], Chitra Hariharan[1], Jieyun Yin[1], Eduardo Olmedillas[1], Adrian Enriquez[1], Diptiben Parekh[1], Milite Abraha[3], Elizabeth Feeney[3], Gillian Q. Horn[3], CoVIC-DB team[1], Yoann Aldon[4], Hanif Ali[5], Sanja Aracic[6], Ronald R. Cobb[7], Ross S. Federman[8], Joseph M. Fernandez[9], Jacob Glanville[10], Robin Green[8], Gevorg Grigoryan[8], Ana G. Lujan Hernandez[11], David D. Ho[12], Kuan-Ying A. Huang[13], John Ingraham[8], Weidong Jiang[14], Paul Kellam[15,16], Cheolmin Kim[17], Minsoo Kim[17], Hyeong Mi Kim[17], Chao Kong[18], Shelly J. Krebs[19], Fei Lan[9,20], Guojun Lang[18], Sooyoung Lee[17], Cheuk Lun Leung[8], Junli Liu[14], Yanan Lu[9,21], Anna MacCamy[22], Andrew T. McGuire[22], Anne L. Palser[15], Terence H. Rabbitts[5,23], Zahra Rikhtegaran Tehrani[24], Mohammad M. Sajadi[24], Rogier W. Sanders[4], Aaron K. Sato[11], Liang Schweizer[25], Jimin Seo[17], Bingqing Shen[25], Jonne L. Snitselaar[4], Leonidas Stamatatos[22], Yongcong Tan[18], Milan T. Tomic[26], Marit J. van Gils[4], Sawsan Youssef[10], Jian Yu[12], Tom Z. Yuan[11], Qian Zhang[25], Bjoern Peters[1,27], Georgia D. Tomaras[3], Timothy Germann[2], Erica Ollmann Saphire[1,27]*



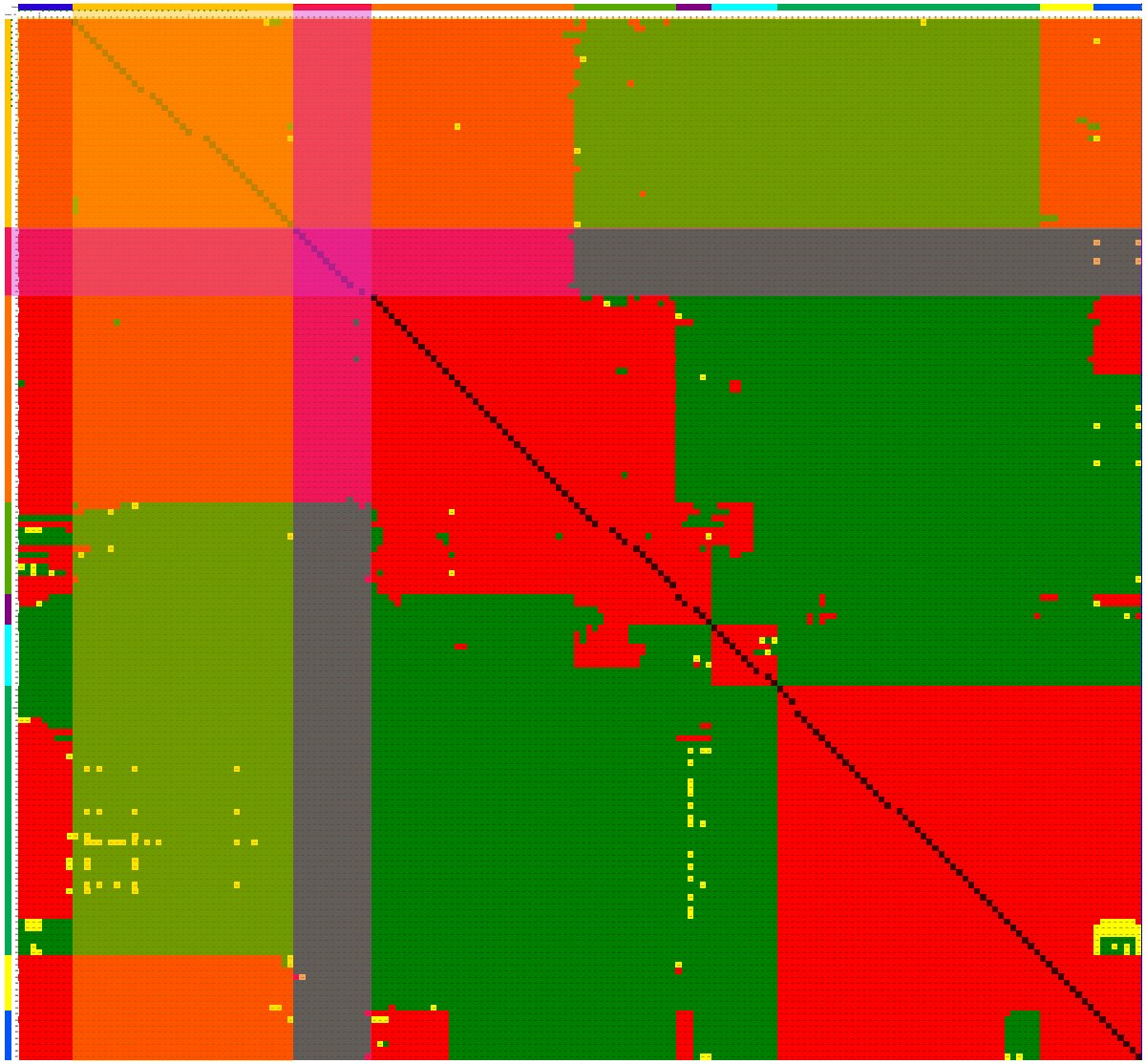
https://doi.org/10.1126/science.abh2315

CoVIC

La Jolla Institute FOR IMMUNOLOGY

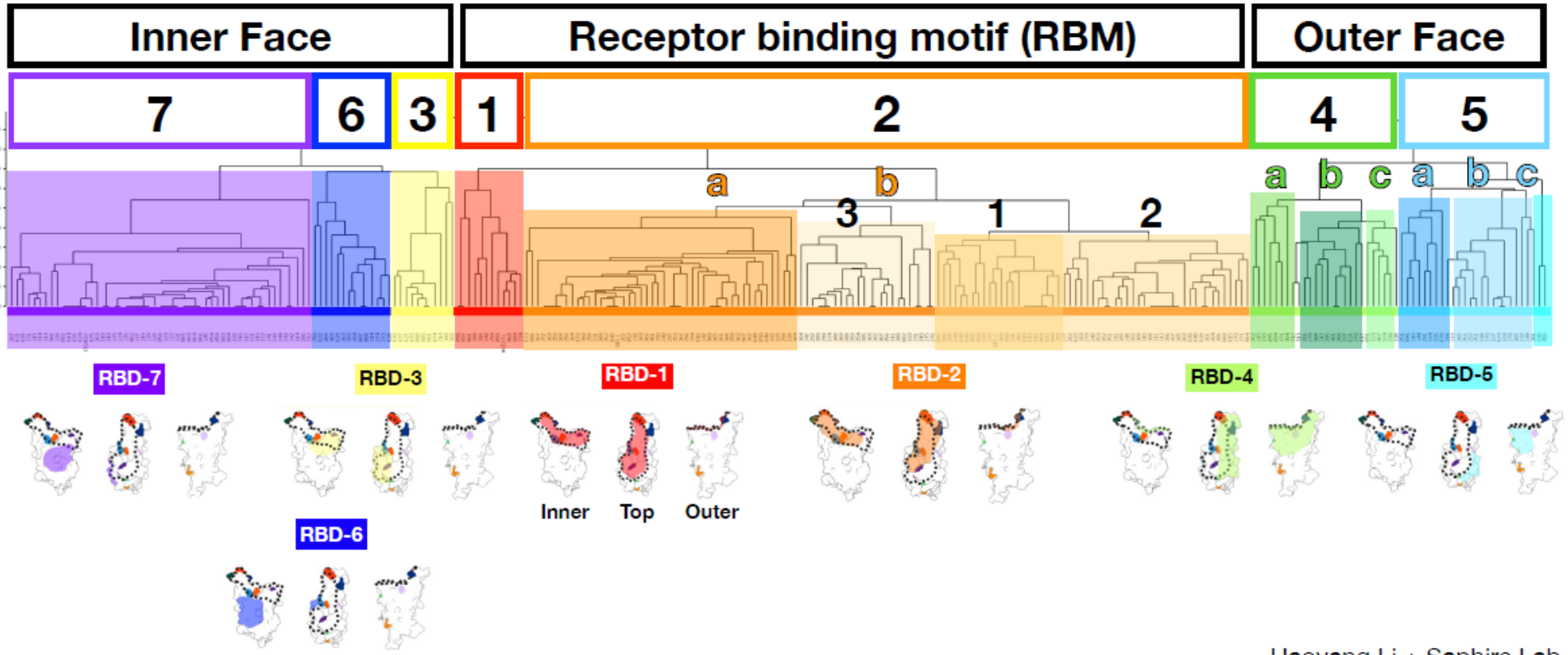
Representative heat map of a CoVIC panel assay

- 170 ligands x 188 analytes 31,960 interactions
- Single experiment
- Binning map is highly symmetric
- All ligands show self-versus self blockade
- Clear shared relationships and complex overlapping, but differentiated competition profiles



CoVIC

7 core RBD-directed communities and 10 sub communities



Haoyang Li + Saphire Lab

La Jolla Institute FOR IMMUNOLOGY

Adapted from K. M. Hastie et al., *Science* 10.1126/science.abh2315 (2021)

Bispecific and Multi-specific Antibody Characterization

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NIAID developed potent and broadly neutralizing bi-specifics

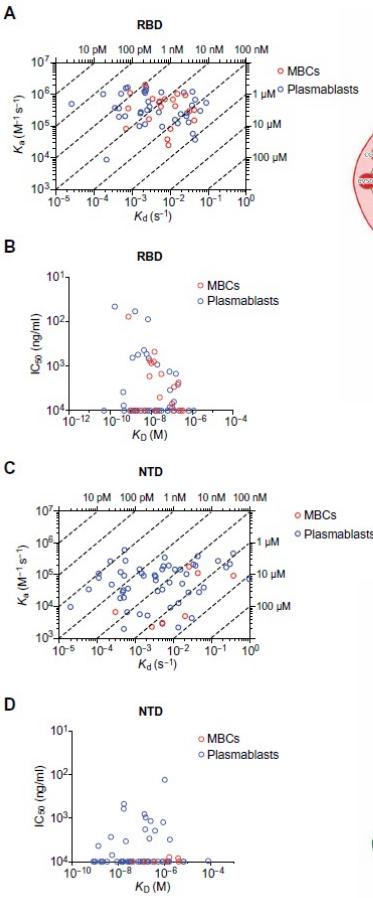
Science Translational Medicine

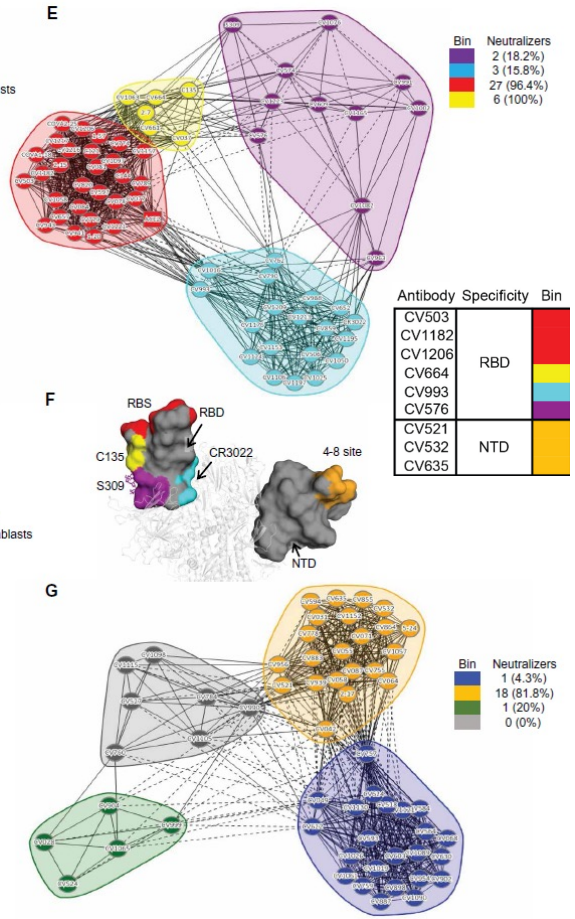
CORONAVIRUS

Bispecific antibodies targeting distinct regions of the spike protein potently neutralize SARS-CoV-2 variants of concern

Hyeseon Cho[1†], Kristina Kay Gonzales-Wartz[2†‡], Deli Huang[3†], Meng Yuan[4†], Mary Peterson[1†], Janie Liang[5], Nathan Beutler[3], Jonathan L. Torres[4], Yu Cong[5], Elena Postnikova[5], Sandhya Bangaru[4], Chloe Adrienna Talana[6], Wei Shi[6], Eun Sung Yang[6], Yi Zhang[6], Kwanyee Leung[6], Lingshu Wang[6], Linghang Peng[3], Jeff Skinner[1], Shanping Li[1], Nicholas C. Wu[4†§], Hejun Liu[4], Cherrelle Dacon[2], Thomas Moyer[7], Melanie Cohen[7], Ming Zhao[8], Frances Eun-Hyung Lee[9], Rona S. Weinberg[10], Iyadh Douagi[7], Robin Gross[5], Connie Schmaljohn[5], Amarendra Pegu[6], John R. Mascola[6], Michael Holbrook[5], David Nemazee[3], Thomas F. Rogers[3,11], Andrew B. Ward[4], Ian A. Wilson[4,12‖], Peter D. Crompton[1*‖], Joshua Tan[2*‖]

- Generated neutralizing monoclonals from patient PBMCs
- Evaluated for potency, affinity, and epitope diversity
- Used epitope binning to design likely synergistic RBD bispecific DVD-Ig
- Found highly potent, broadly neutralizing bispecific mAbs

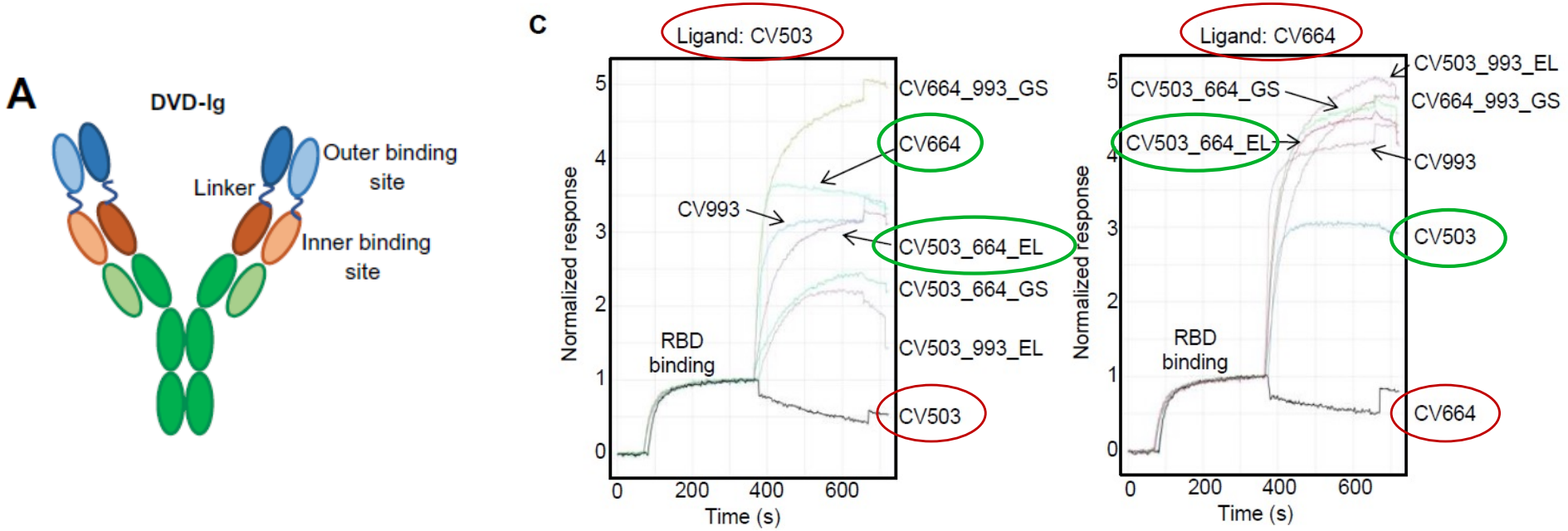




https://doi.org/10.1126/scitranslmed.abj5413

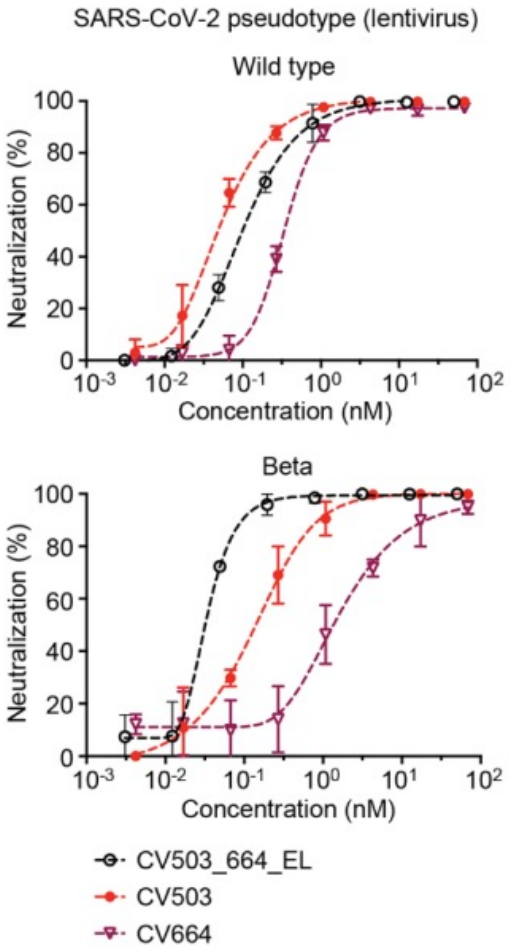
Bispecifics retained both binding specificities as DVD-Ig

- Using classical sandwich binning format, DVD-Ig bispecifics sandwiched as expected against monospecific parent clones

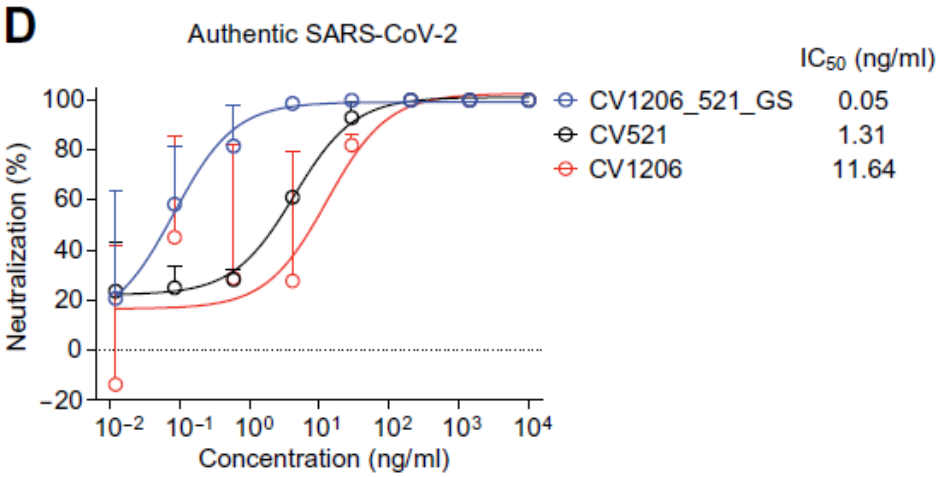


https://doi.org/10.1126/scitranslmed.abj5413

DVD-Ig Bispecifics are highly potent



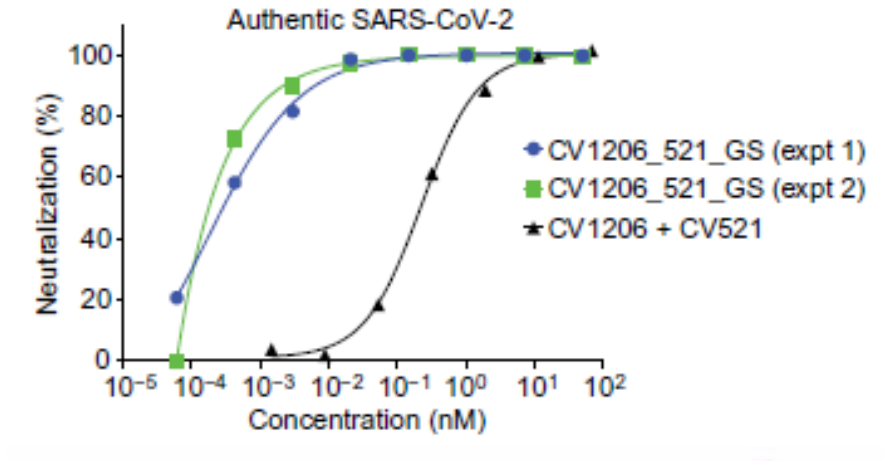
- Most bispecific clones demonstrated much higher potency than monospecific constructs
  - Retain potency against variants

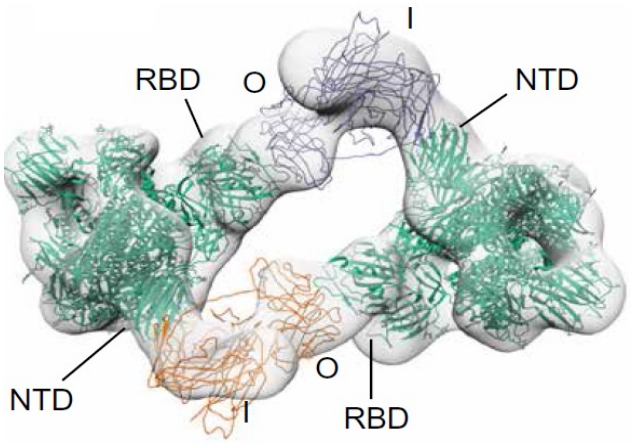


https://doi.org/10.1126/scitranslmed.abj5413

Additive or Synergistic?

- Potency of mixed monospecific clone cocktails was generally more additive than synergistic
- In some cases the bispecific improved the potency of the clones more than 100-fold versus the cocktail of the two IgGs
- Synergy of CV1206_521 was likely due to a spike protein cross-linking mechanism
- Additional mechanism of enhanced apparent affinity/avidity also for the dual RBD binders





https://doi.org/10.1126/scitranslmed.abj5413

Bispecific Assay Methods

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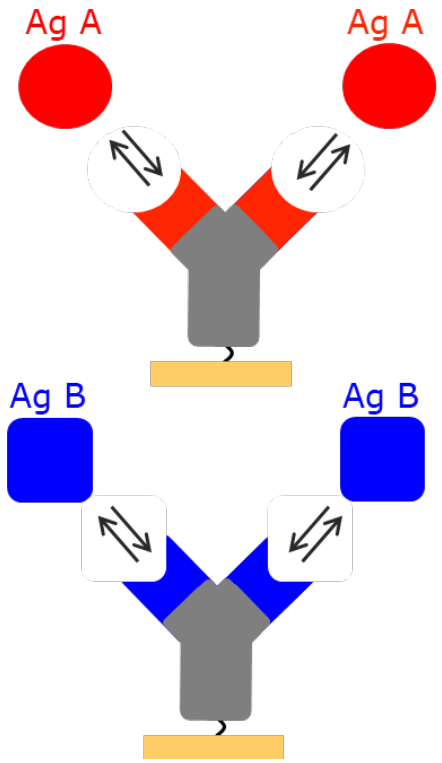
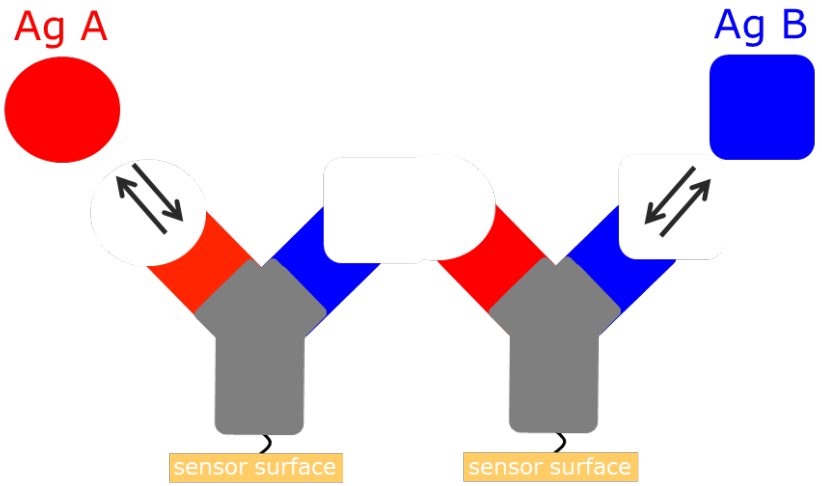
Assay Type 1: Bispecific kinetic screen of each target

- Do antigens bind the same to bispecifics as monospecific control Abs?

Monospecific Controls

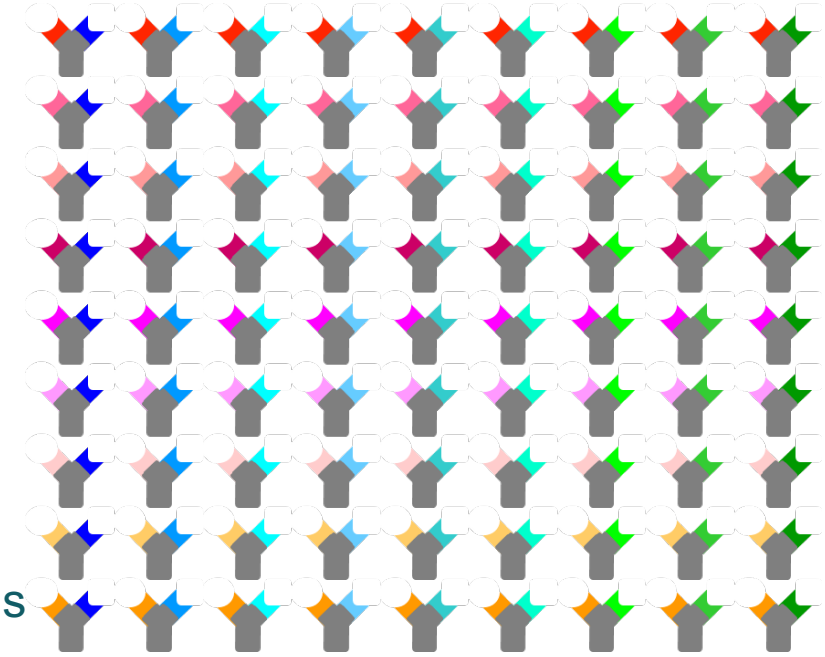


Bispecific Compounds

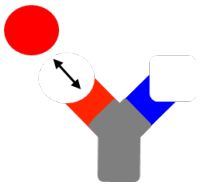


Binding kinetics and affinity to each target of a Bispecific

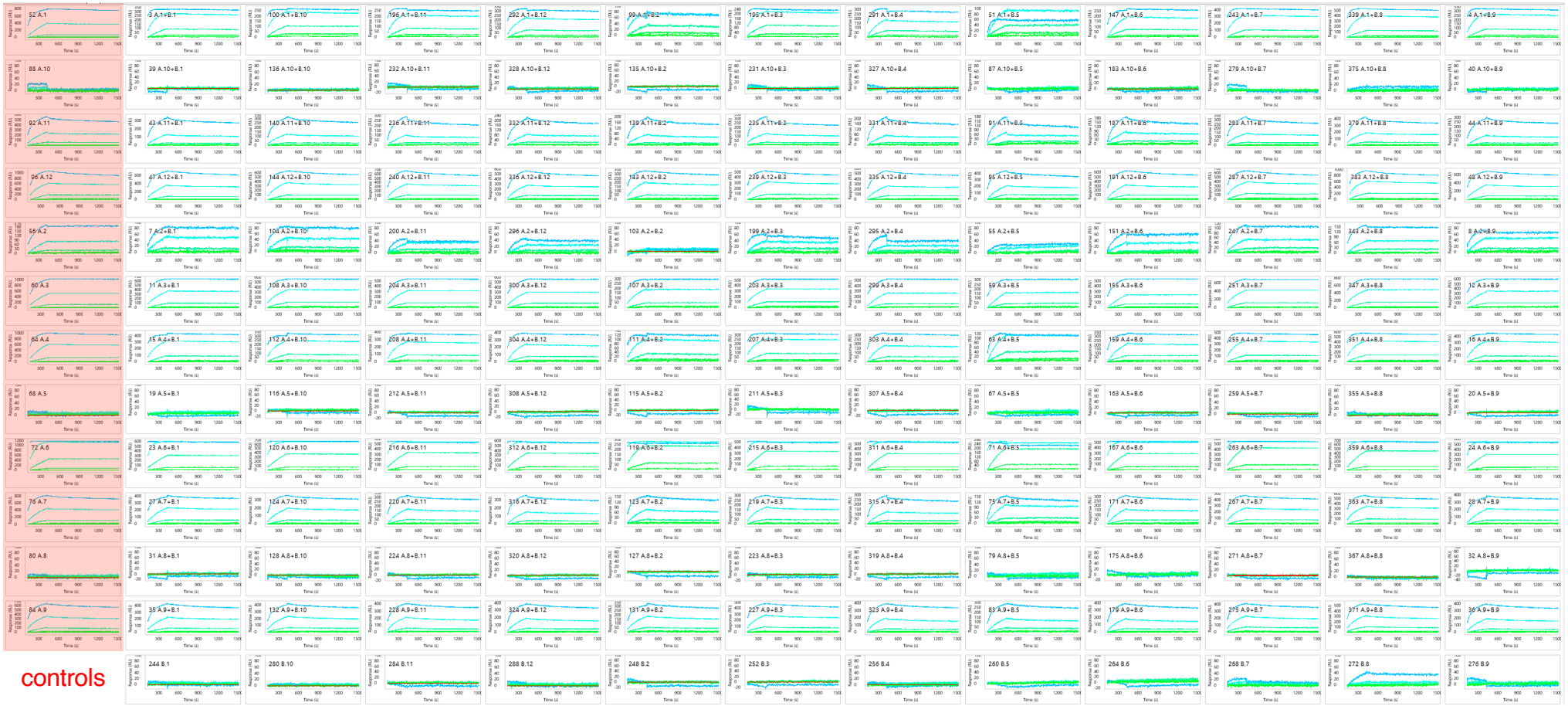
- The LSA is ideally suited for routine kinetic characterizations of large panels of ligands versus multiple antigens.
  - 1152 ligands x 2 Analytes is an unattended 48 hour run- full kinetic profiles
- Example is a matrix of ligands with mixed specificities to two targets.
  - 12 unique sequences targeting Ag A and 12 sequences targeting Ag B
  - Samples are prepared in a matrix creating 144 unique combinations plus monospecific controls
  - A single capture kinetics assay was run overnight to assess the kinetics of all 144 ligands to each target.



Ag A binding

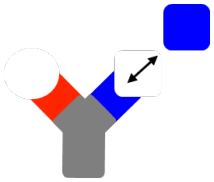


- 144 combinations of A and B binders
- All combinations appear to retain binding activity of A analyte

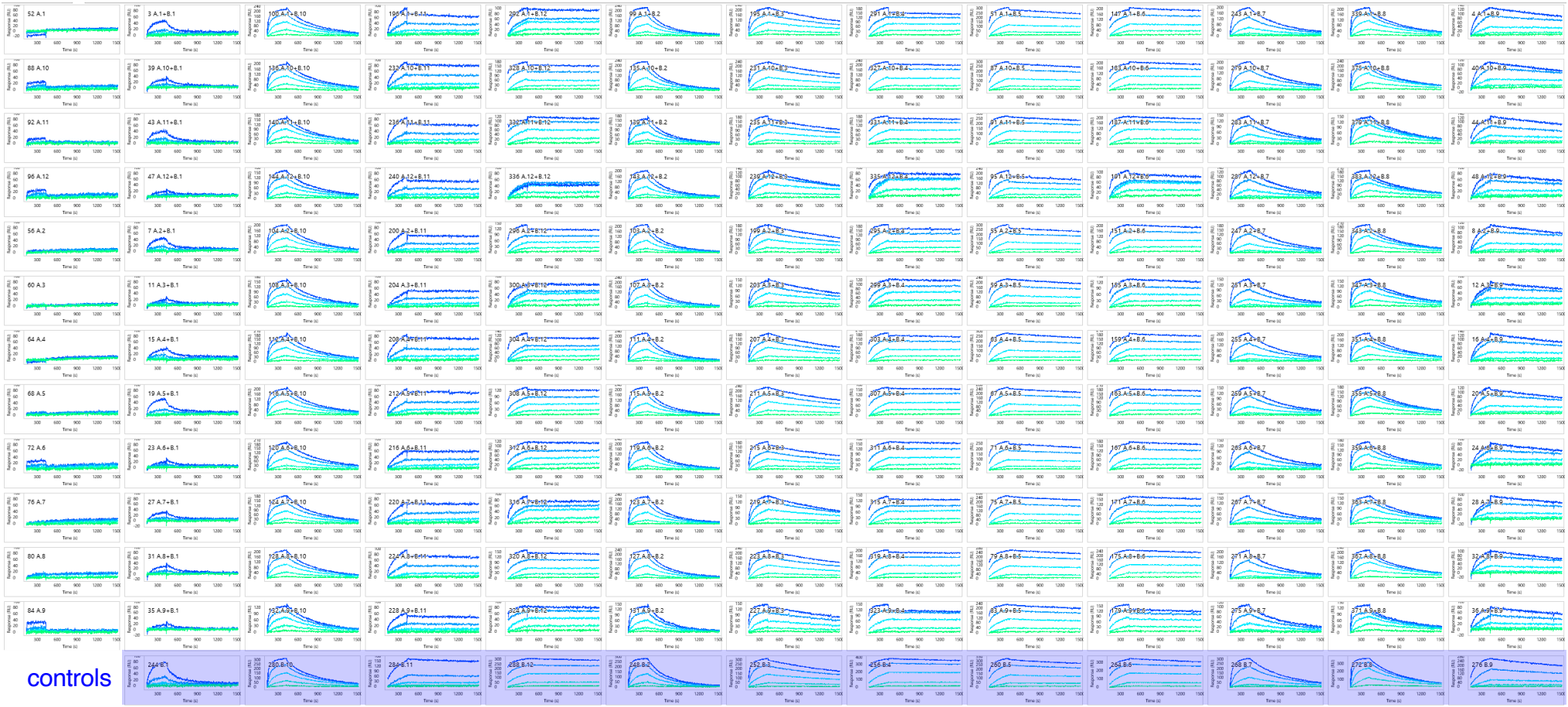


controls

Ag B binding

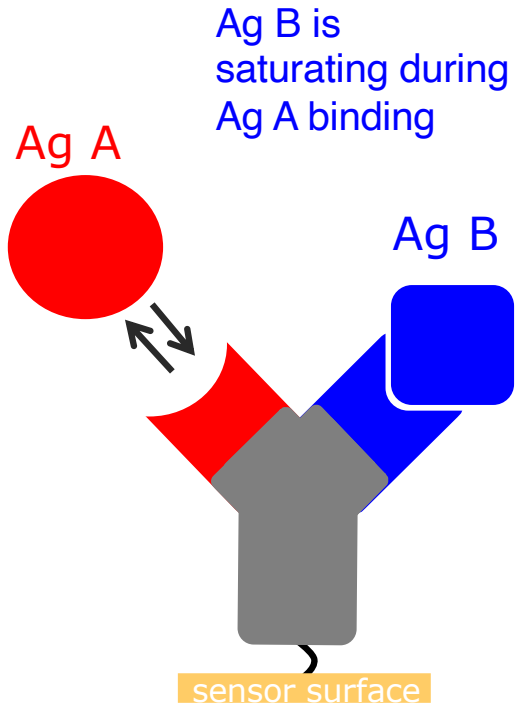


- 144 combinations of A and B binders
- All combinations appear to retain binding activity of B analyte



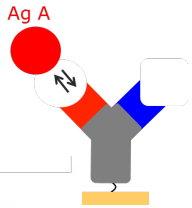
Assay Type 2: Alternate Site Saturation Kinetics

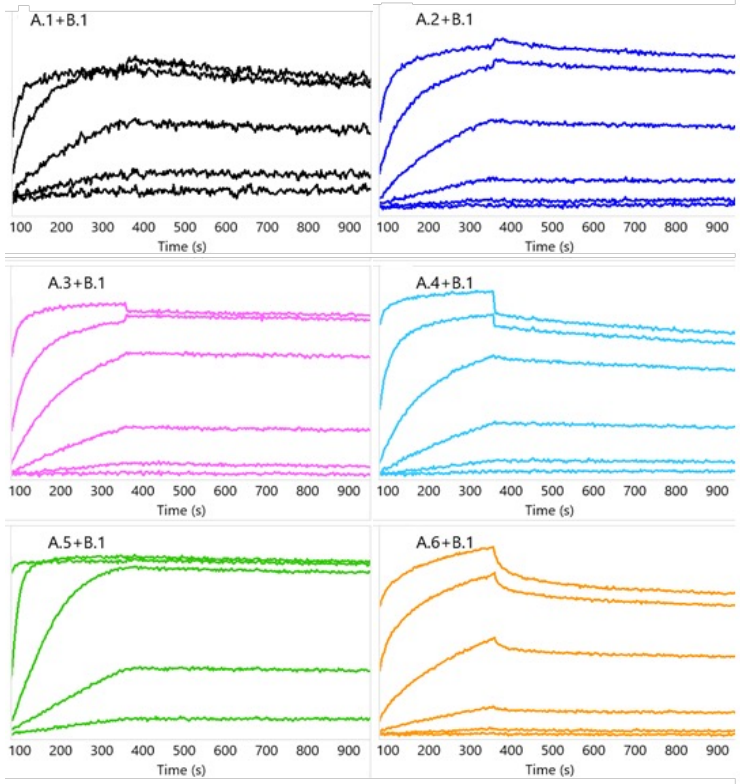
- Establish independence (non-interference) of target binding
- Assay procedure
  - Immobilize or capture array of mAbs
  - If interaction B is high affinity:
    - Inject saturating conc. of Ag B then inject kinetic series of Ag A
  - If interaction B is lower affinity:
    - Inject saturating concentration of Ag B as blanks
    - Inject a titration series of Ag A diluted into a background of the same saturating concentration of Ag B.
    - Blank can be used for double referencing to extrapolate specific binding curves
- Double referenced data allows for comparison to original independent affinity measurements



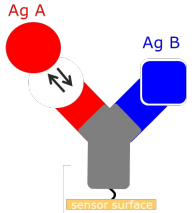
Alternate Site Saturation Kinetics

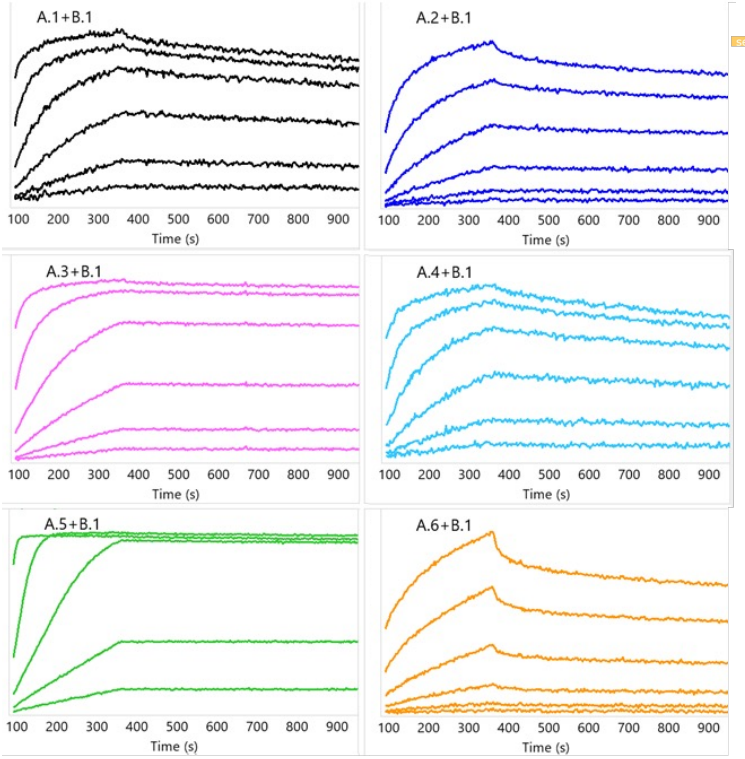
Binding site B not occupied





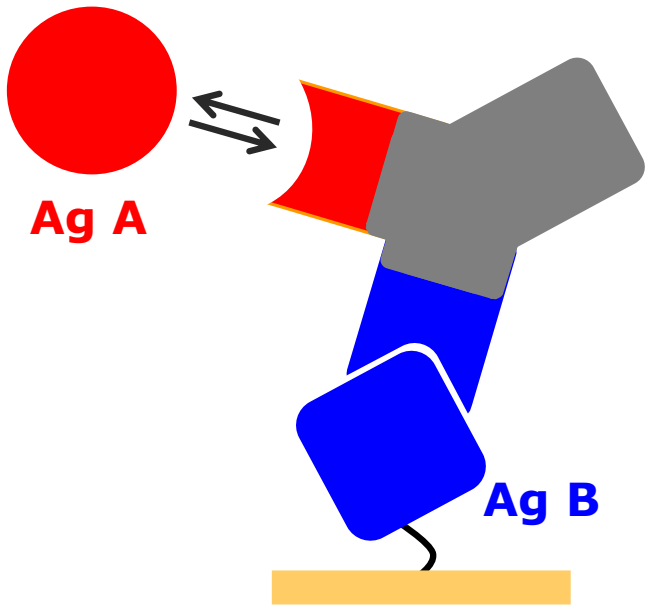
Binding site B fully occupied





Assay Type 3: Bispecific Bridging Assay Format

- In addition to the saturation method, the bridging assay format isolates the analyte binding to only antibody molecules which are already bound to the other target
  - For systems where the binder is high affinity this can enable kinetics evaluation of the analyte
  - For low affinity systems bridging assay may be more of a qualitative assessment, validating results from the saturation method
- Assay Steps:
  - Immobilize or capture Ag B on the surface
  - Use 96 Channel flow cell to load bispecifics
  - Use SFC to inject concentration series of analyte A



Summary

- Bispecific antibody discovery can be supported at an early stage by HT-SPR
- Epitope binning and high throughput kinetics add significant value to bispecific discovery workflows
- The LSA enables several workflows to address the special needs for binding characterization of bispecific molecule panels
  - Saturation binding and bridging assay formats
- Carterra is interested in collaborators with projects aimed at near term publication and presentation of these methods

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- Erica Ollman Sapphire

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- Joshua Tan

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Appendix

carterra

Coronavirus Immunotherapy Consortium- CoVIC

Science

CORONAVIRUS

Defining variant-resistant epitopes targeted by SARS-CoV-2 antibodies: A global consortium study

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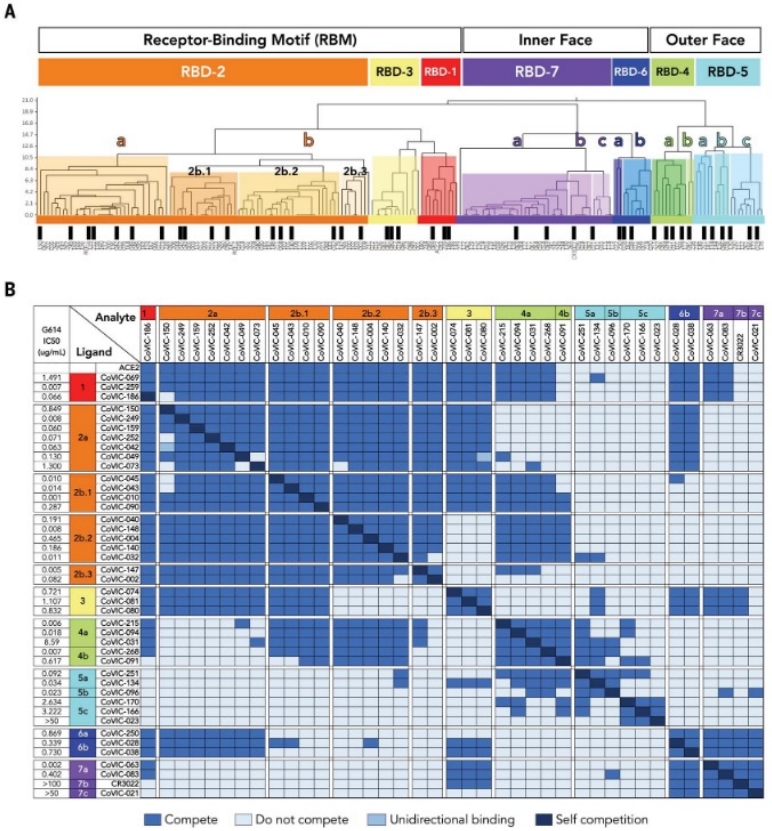

https://doi.org/10.1126/science.abh2315

CoVIC

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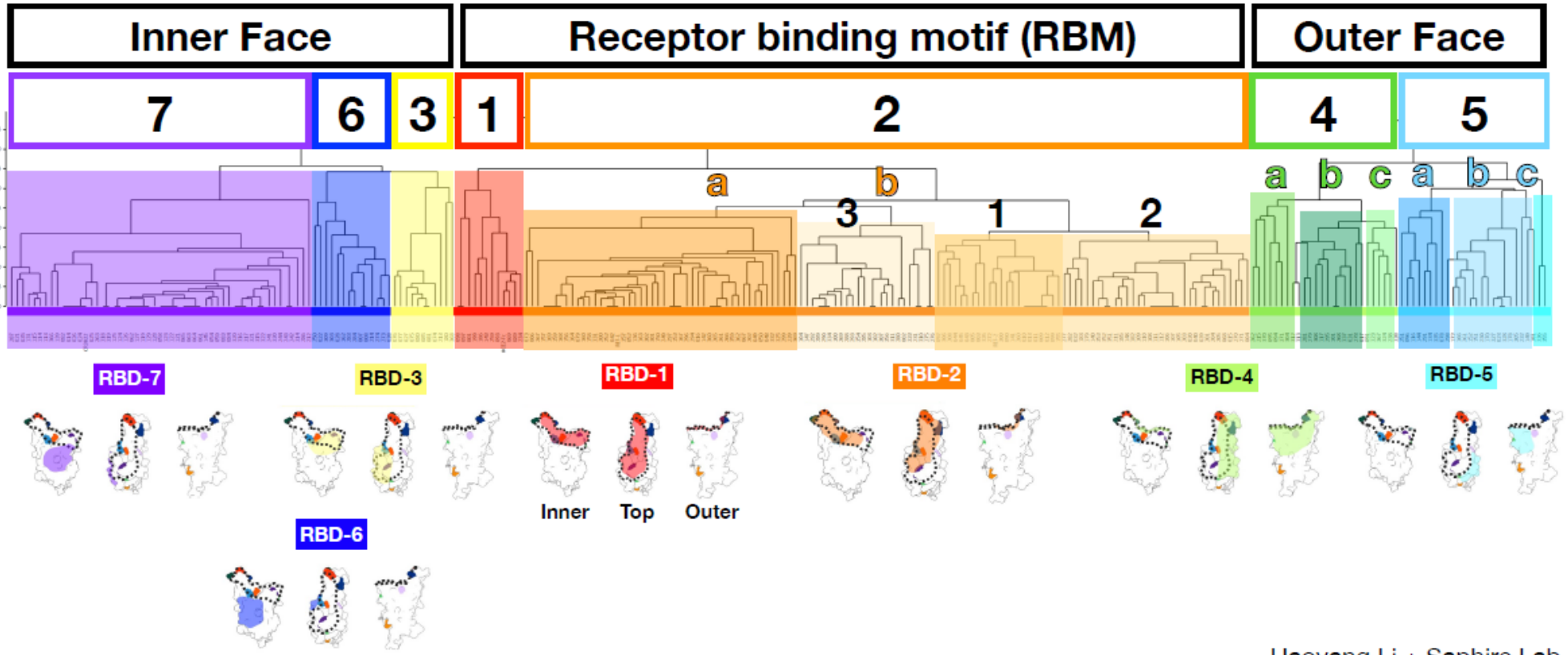
7 core RBD-directed communities and 10 sub communities



Haoyang Li + Saphire Lab

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Adapted from K. M. Hastie et al., *Science* 10.1126/science.abh2315 (2021)