Beyond Epitope Mapping:
Using Deep Mutational Scanning to Generate a Complete Map of SARS-CoV-2 Diagnostic Antibody Escape Mutations

POCTRN Webinar

04/19/2022

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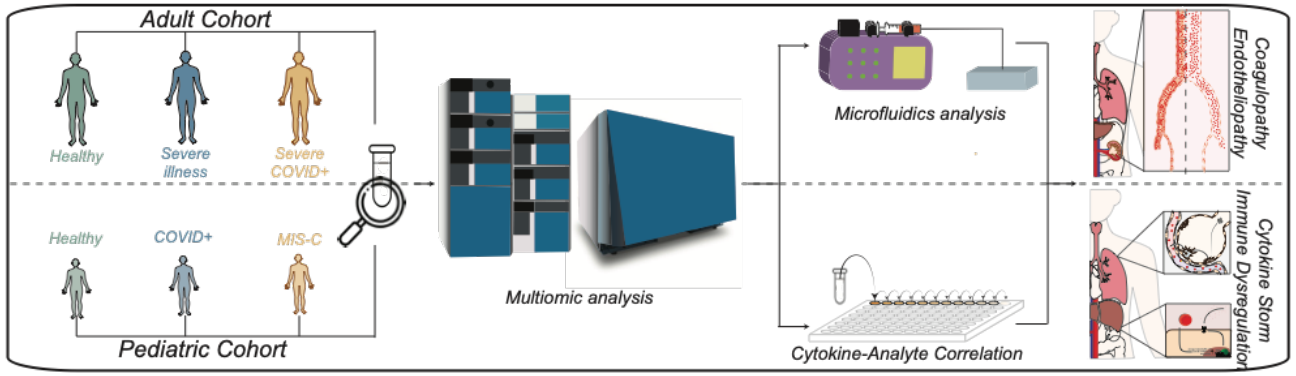
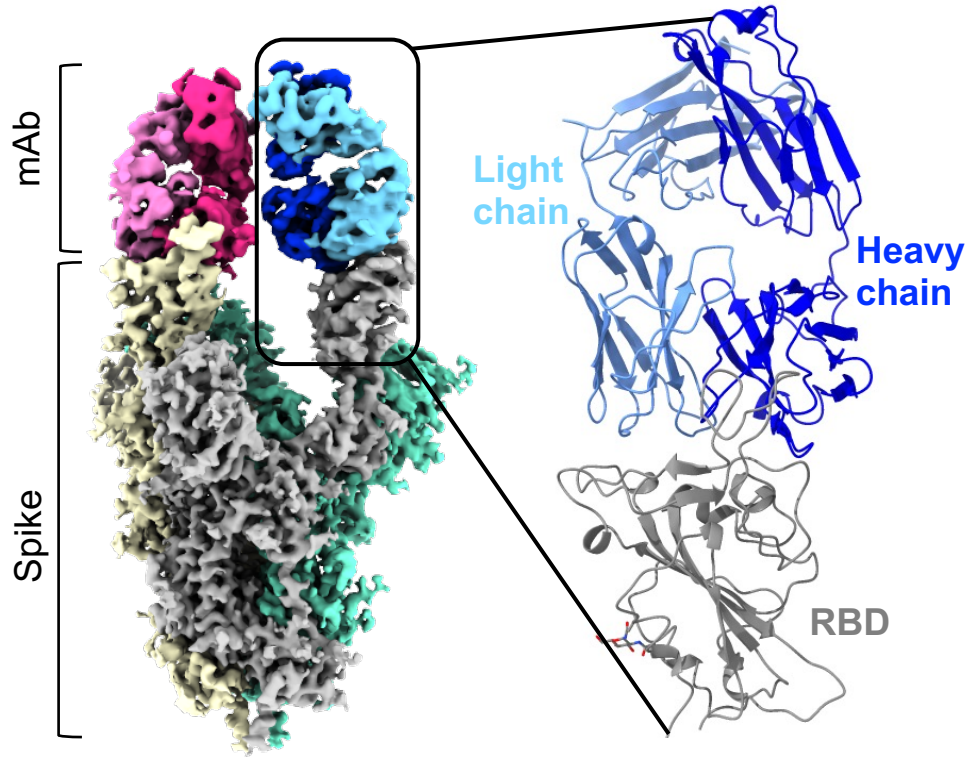
Ortlund lab

Multi-omics to discover pathways driving disease and immune response



Mechanistic and biochemical studies on SARS-Cov-2 proteins to support Emory's efforts to develop in house molecular tests

CryoEM to determine the mechanism of action for antibodies that neutralize SARS-CoV-2



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RADx Tech

Overarching Goal

Establish a robust pipeline of innovative diagnostic technologies to **increase national testing capacity**

Innovate Across the Testing Landscape

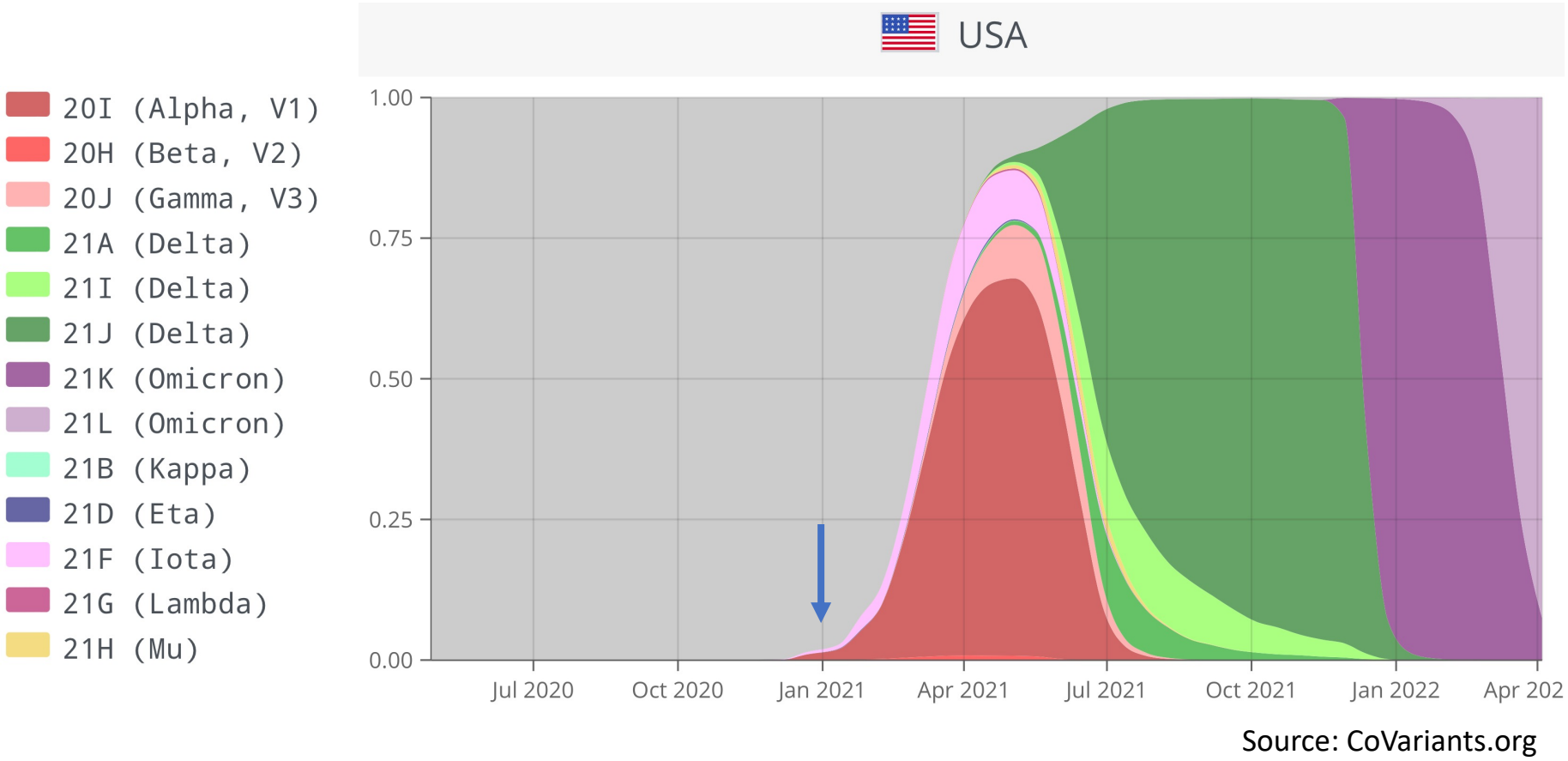
Expand the number, type, access, and throughput of testing technologies

Optimize Technology Performance

Develop technology for a range of essential “Use Cases”

- At-home
- Point of Care (POC)
- Hospital
- Testing Laboratory

The emergence of SARS-CoV-2 Variants



Source: CoVariants.org

Overview: RADx Variant Task Force

Established

January 2021

The RADx Variant Task Force is a cross-discipline and cross-organization group of scientists and industry leaders with expertise in virology and diagnostic testing.

Objective

Rapidly analyze whether the performance of any of RADx's portfolio of diagnostic tests were affected by the mutations in the new variants.

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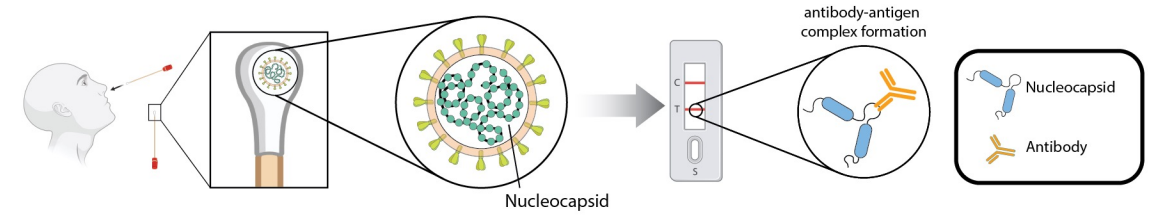
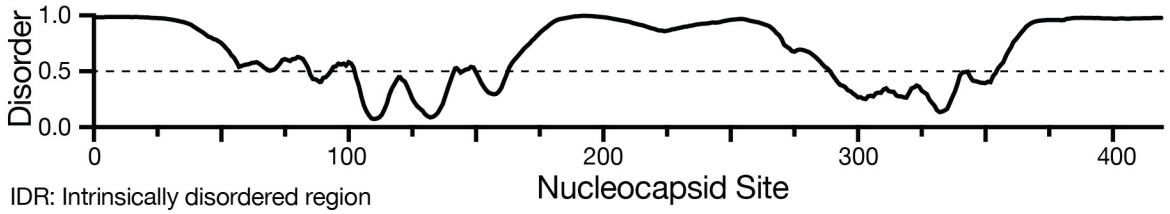
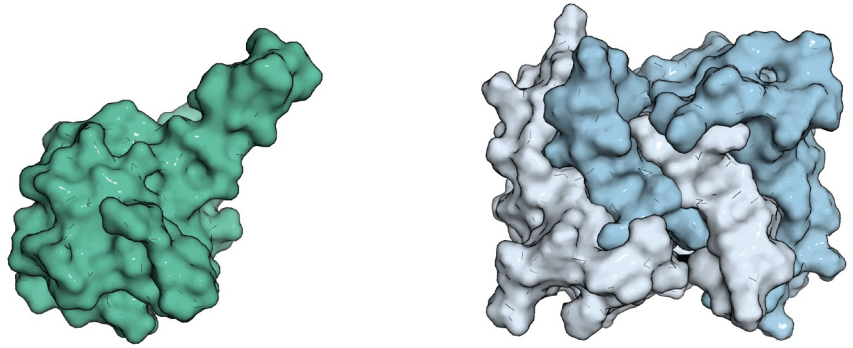
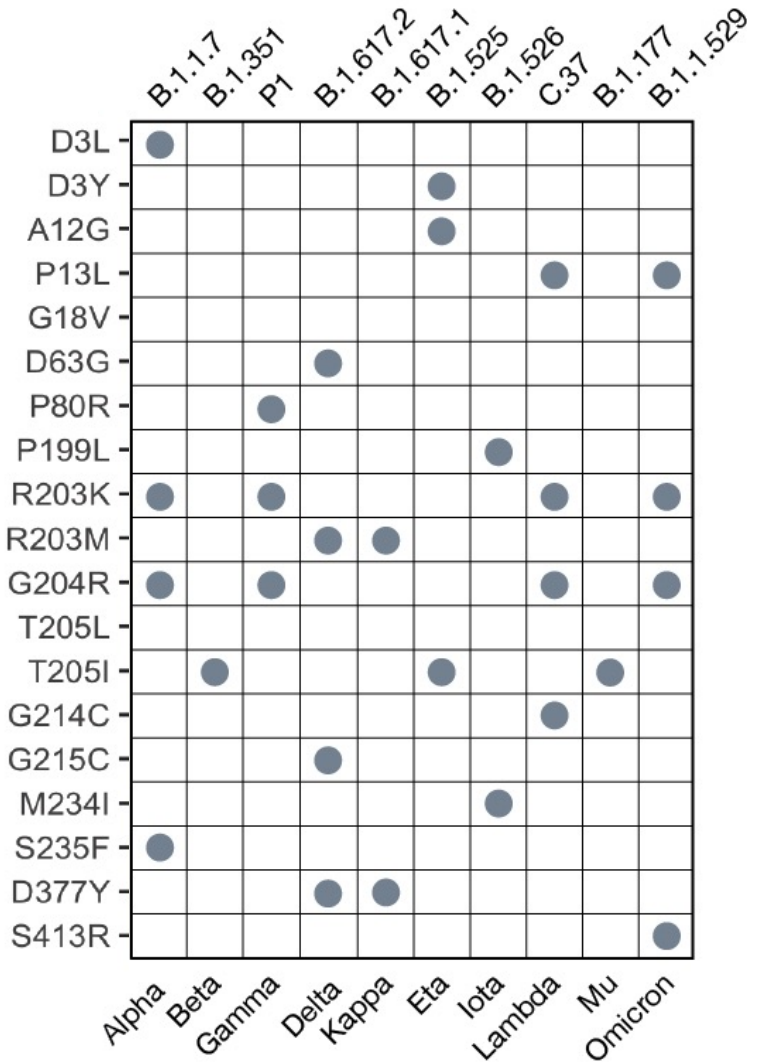
Antigen tests target the SARS-CoV-2 Nucleocapsid protein





IDR: Intrinsically disordered region





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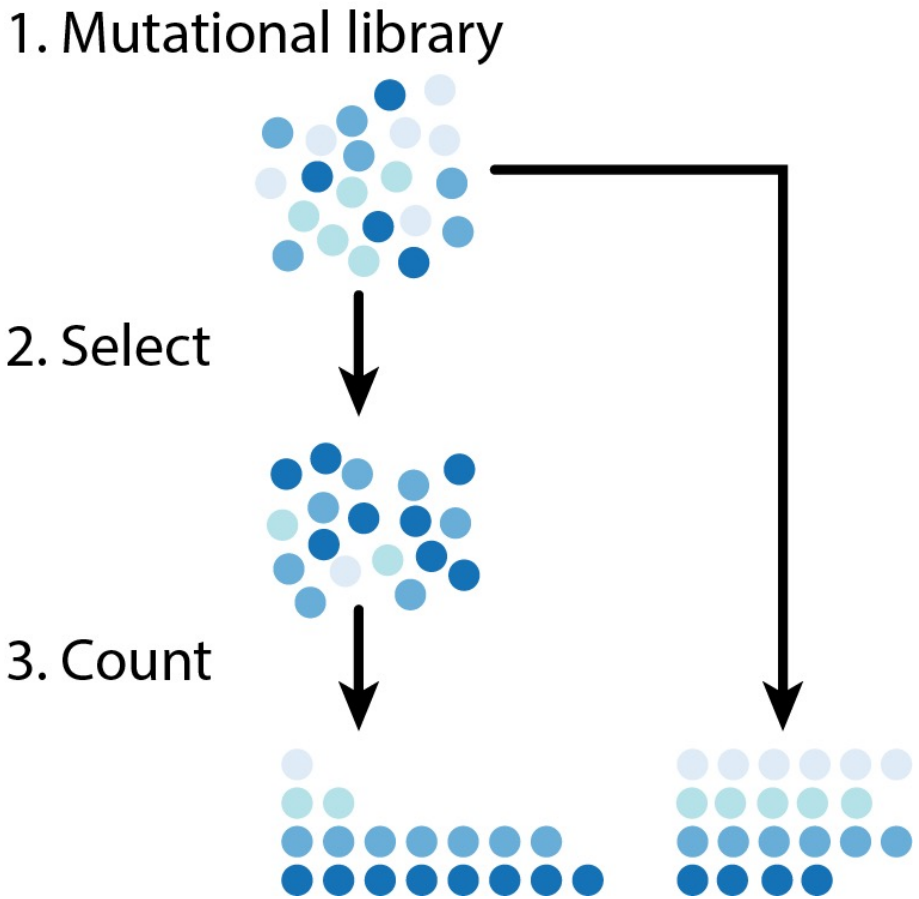
Epitope mapping approaches

- Structural approaches (crystal structures, cryo-EM)
- Peptide-based approaches
- Site-directed mutagenesis (e.g. alanine scanning)

But: No direct measurement of the effect of mutations

Deep mutational scanning



Selection methods:

- Cell growth
- Viral passaging/fitness
- Cellular signaling
- …
- **Surface-display and FACS**

Pioneered at University of Washington:
Fields lab: Fowler *et al.*, Nat Methods, 2010 & 2014
Bloom lab: **Starr *et al.*, *Cell*, 2020**
…

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Mammalian surface-display combined with deep mutational scanning

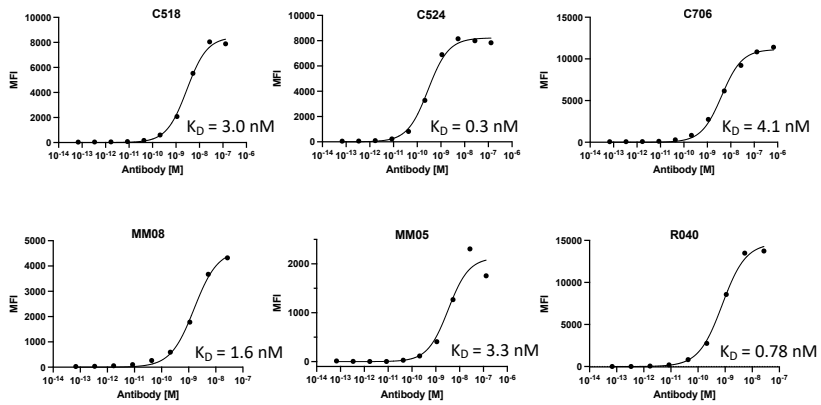
Advantages of this approach:

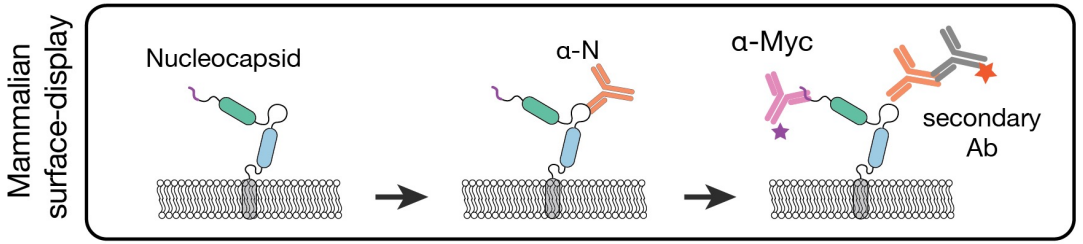
(i) Full coverage of all **past, present, or future mutations** found in variants

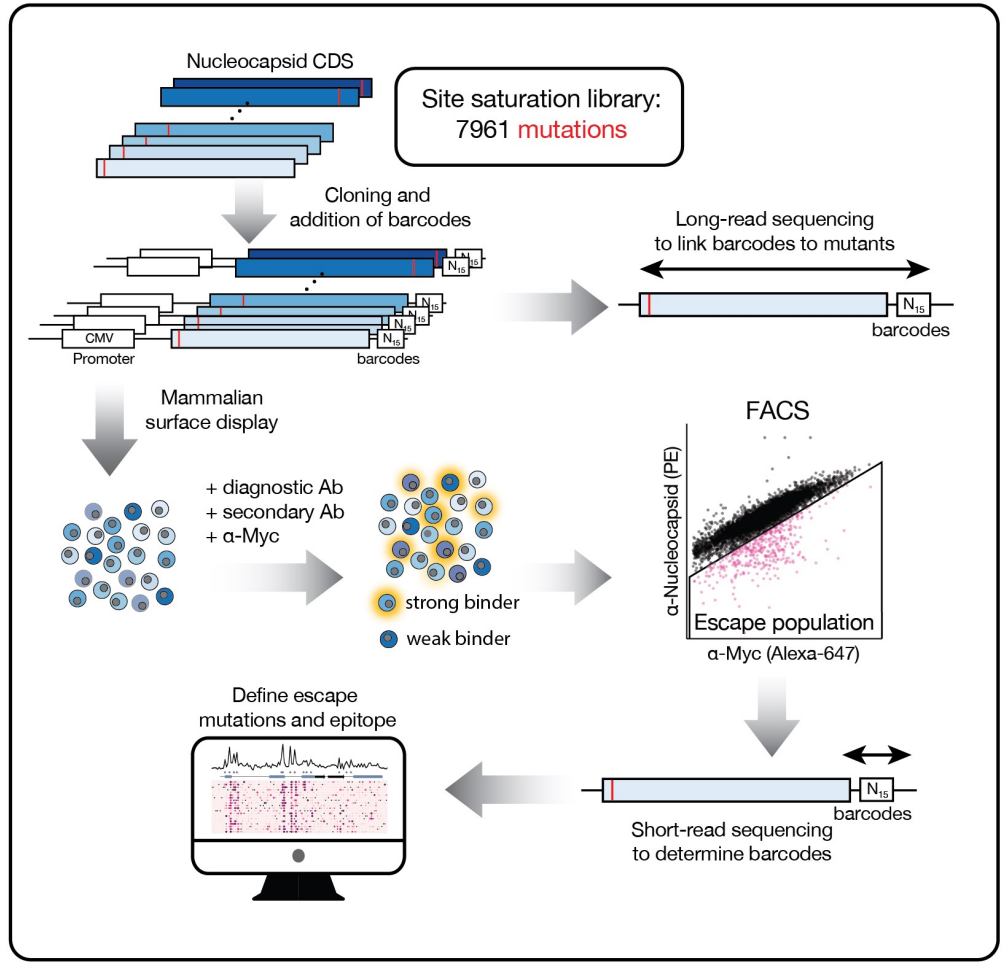
(ii) Direct measurement of antibody-antigen interactions in the context of **functionally folded, full-length N protein**

(iii) Accurate detection of **linear and three-dimensional epitopes** (and more!)

Test binding to Wuhan N:







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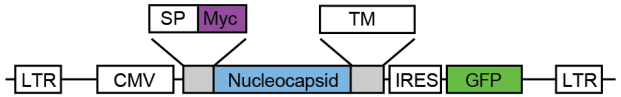
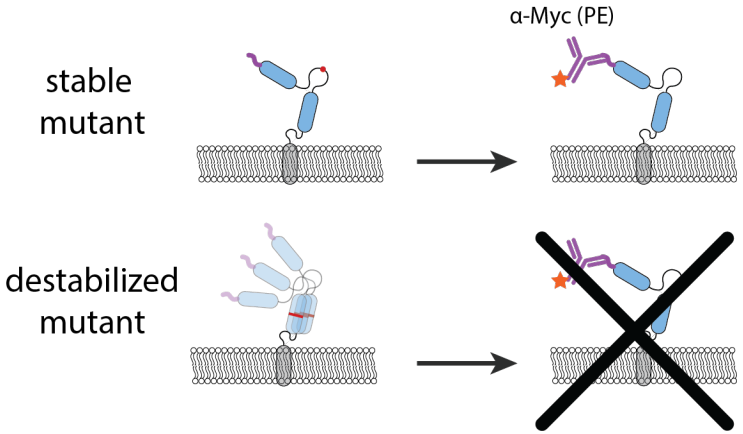
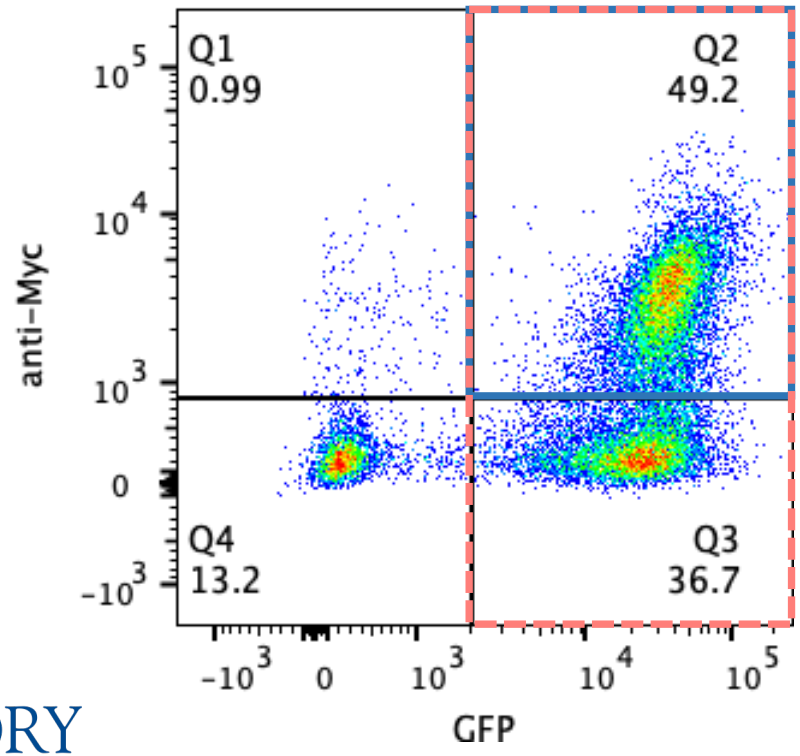
Library generation procedure

Lentiviral expression construct:



1. Generate lentiviral library
2. Infect HEK293 cells
3. Sort GFP⁺ cells (infected)
4. Sort Myc⁺ cells





All possible, **functionally folding** Nucleocapsid protein mutations

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All possible Nucleocapsid protein mutations

$$ER_i = \frac{n_{i,selected} / N_{selected}}{n_{i,input} / N_{input}}$$

ER_i: enrichment ratio of variant i

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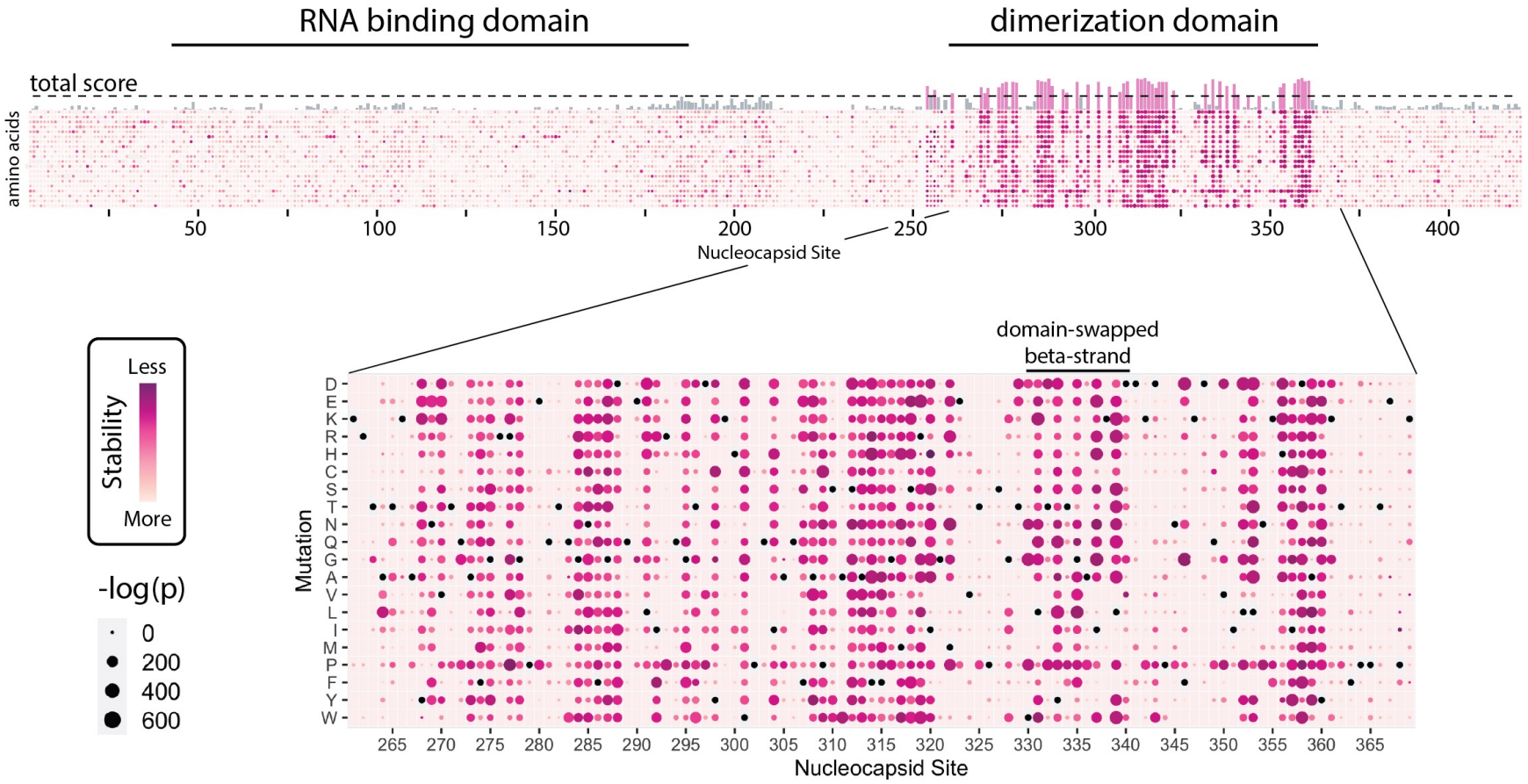
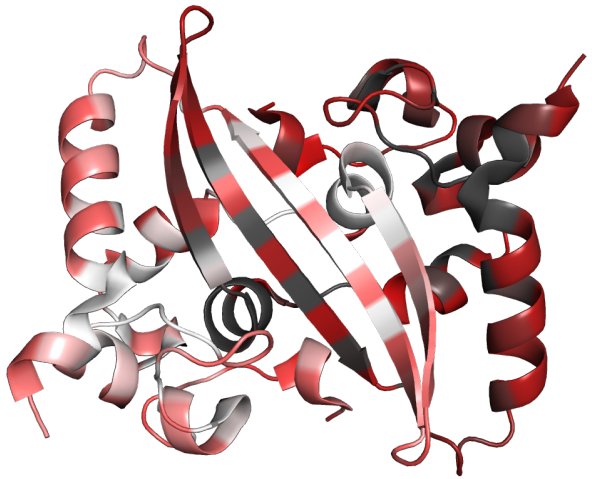
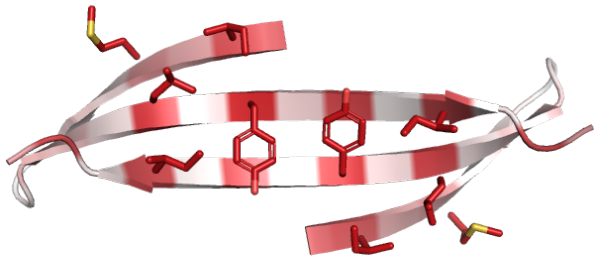
A high-throughput protein folding experiment





Protein side:



Solvent side:

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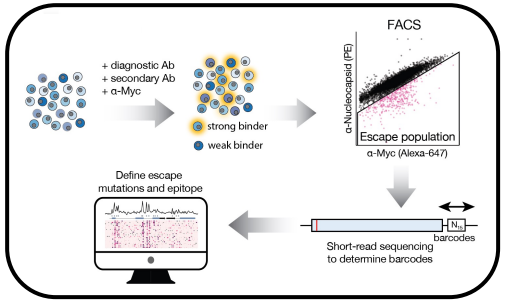
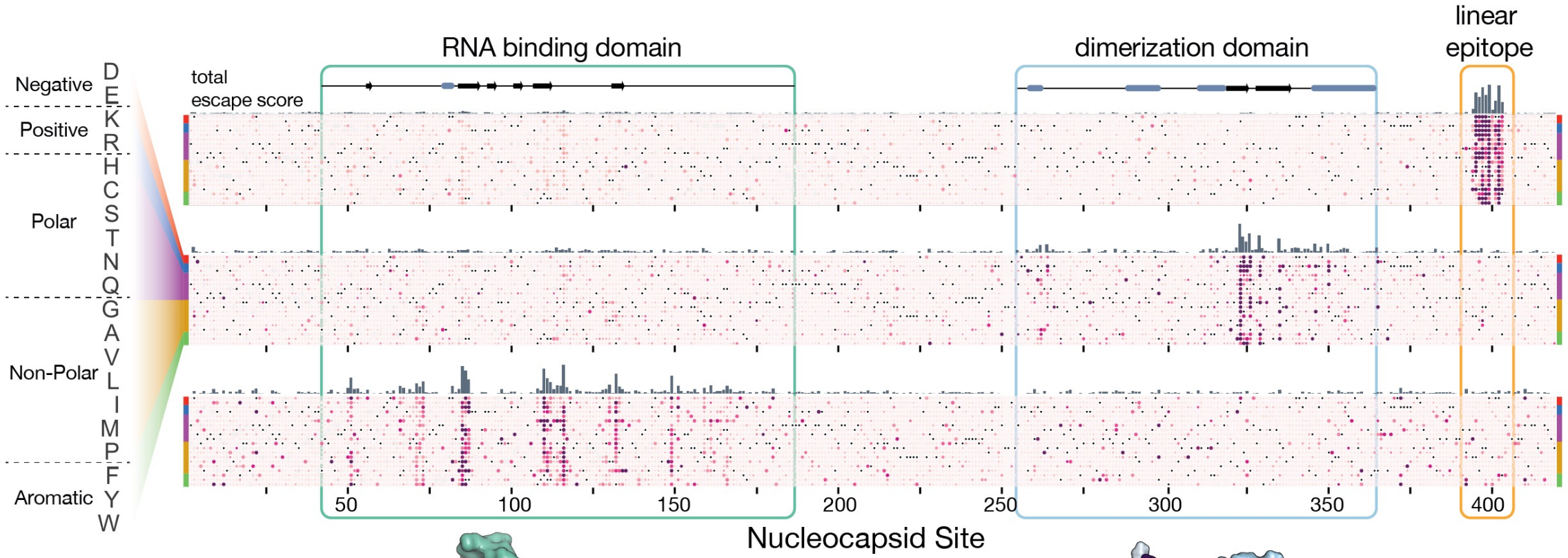
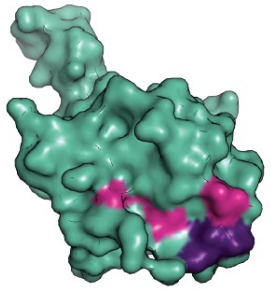
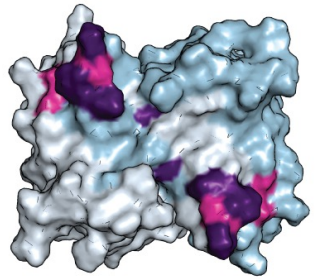
Detailed escape mutation maps of linear and 3-dimensional epitopes









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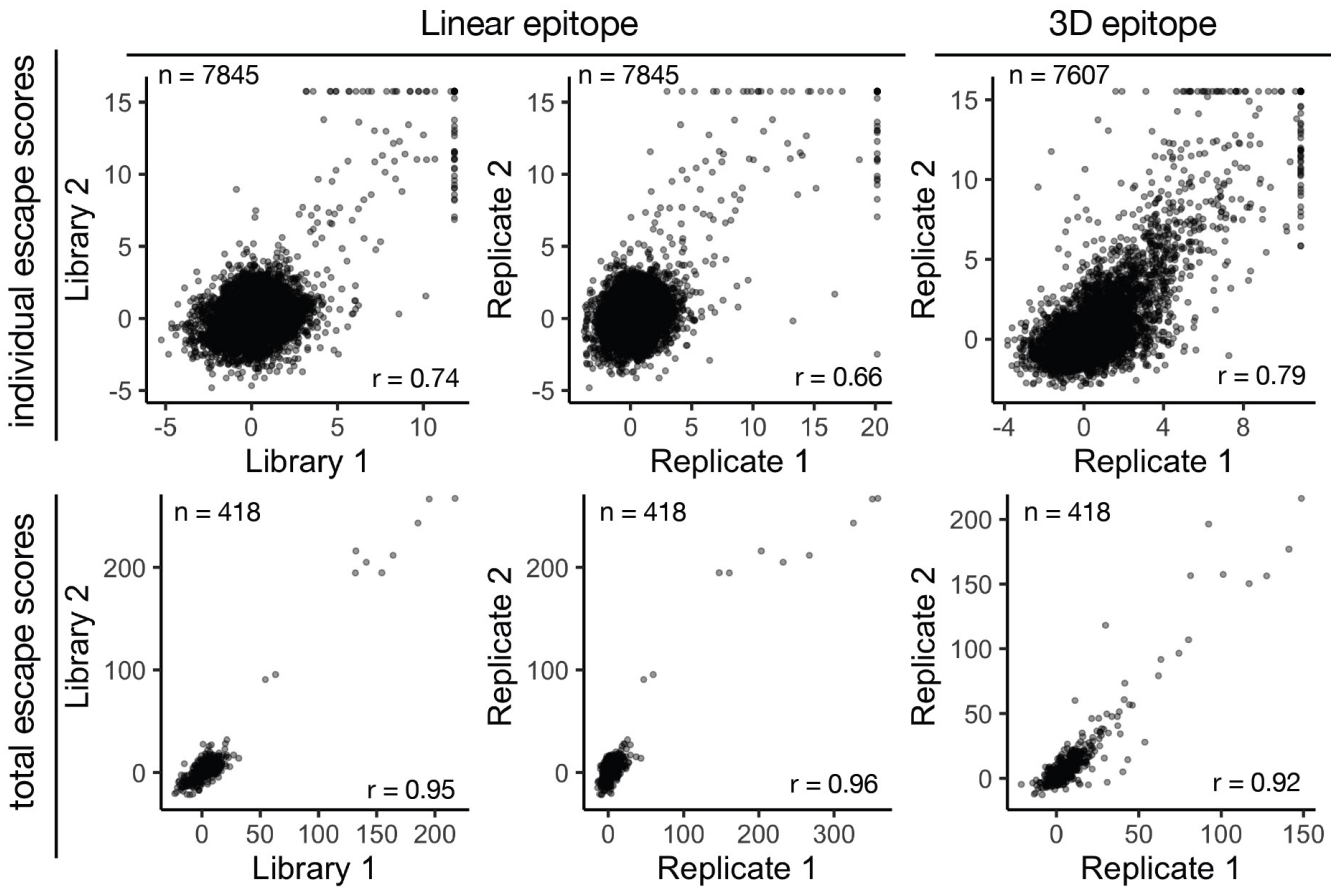
Method validation – replicate comparisons



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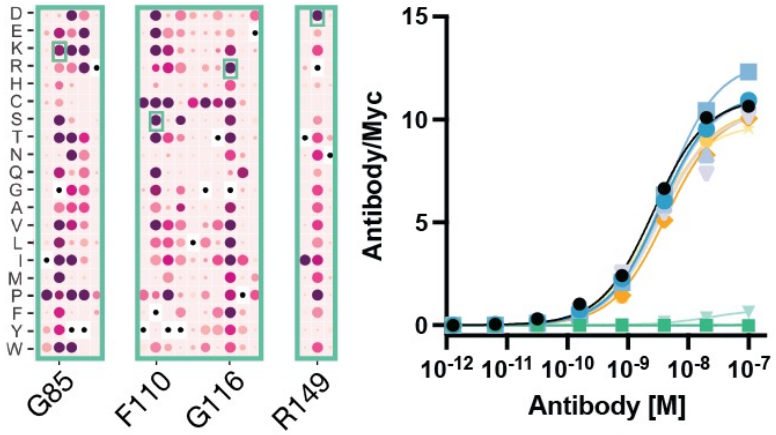
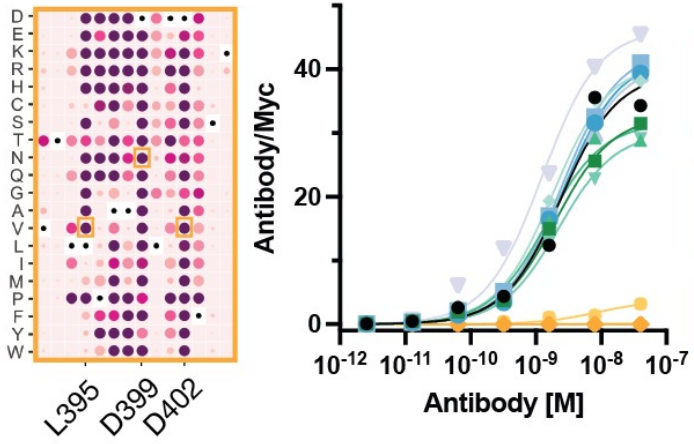
Method validation – individual mutations

Wuhan
G85K
F110S
G116R
R149D
E323V
V324E
P326A
T329G
L395V
D399N
D402V

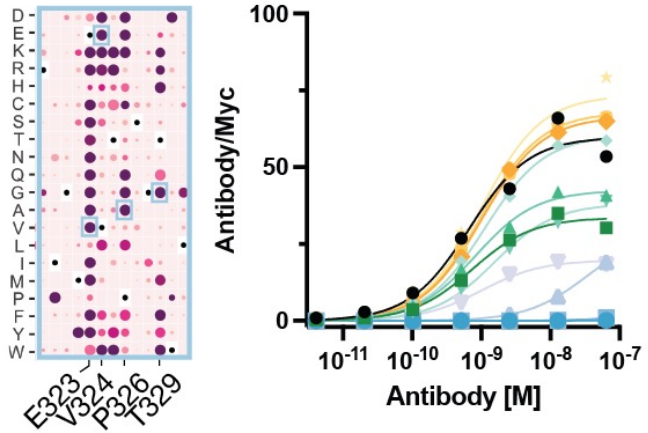
3D epitope – RNA-binding domain



Linear epitope



3D epitope – Dimerization domain



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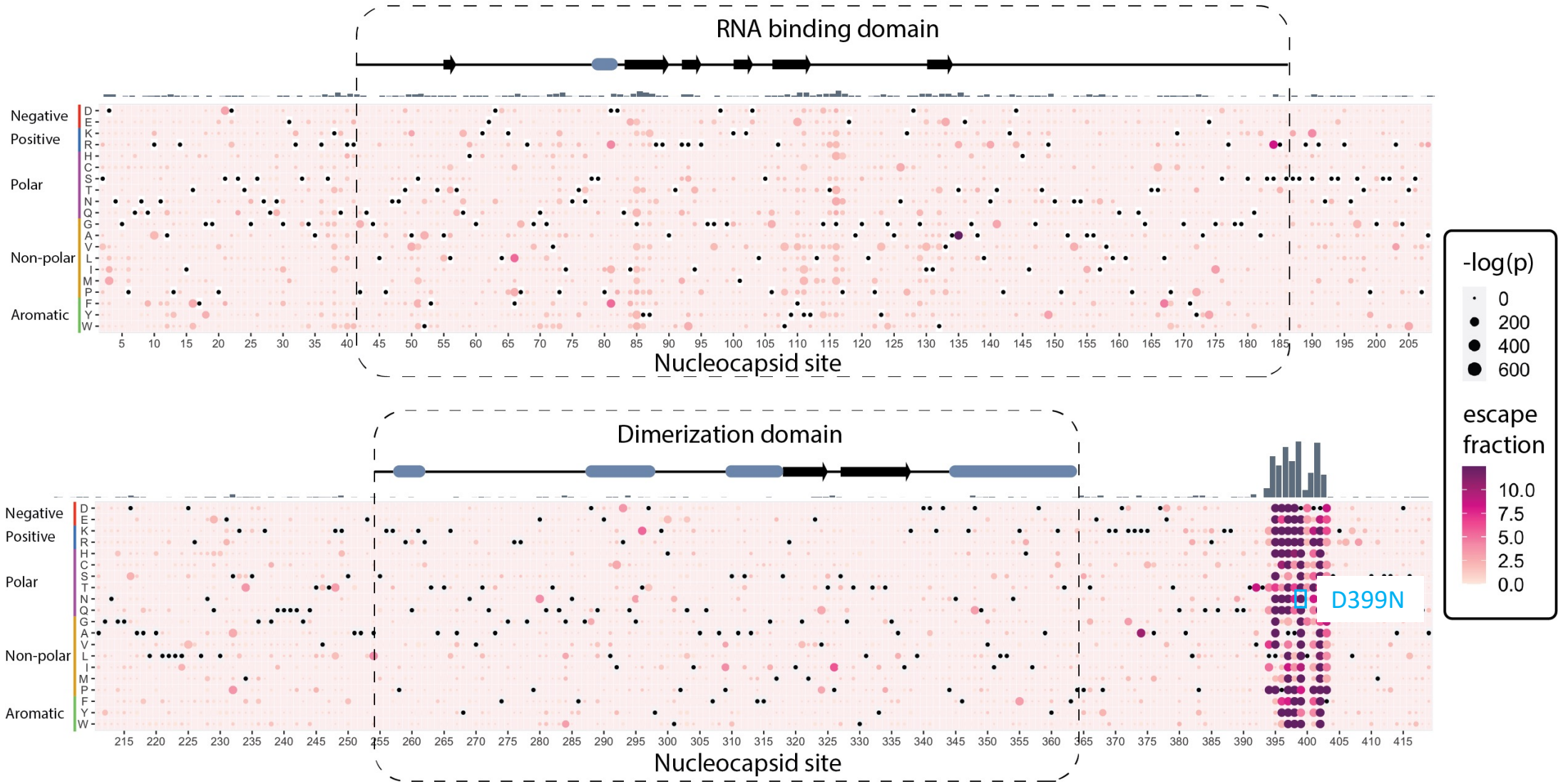
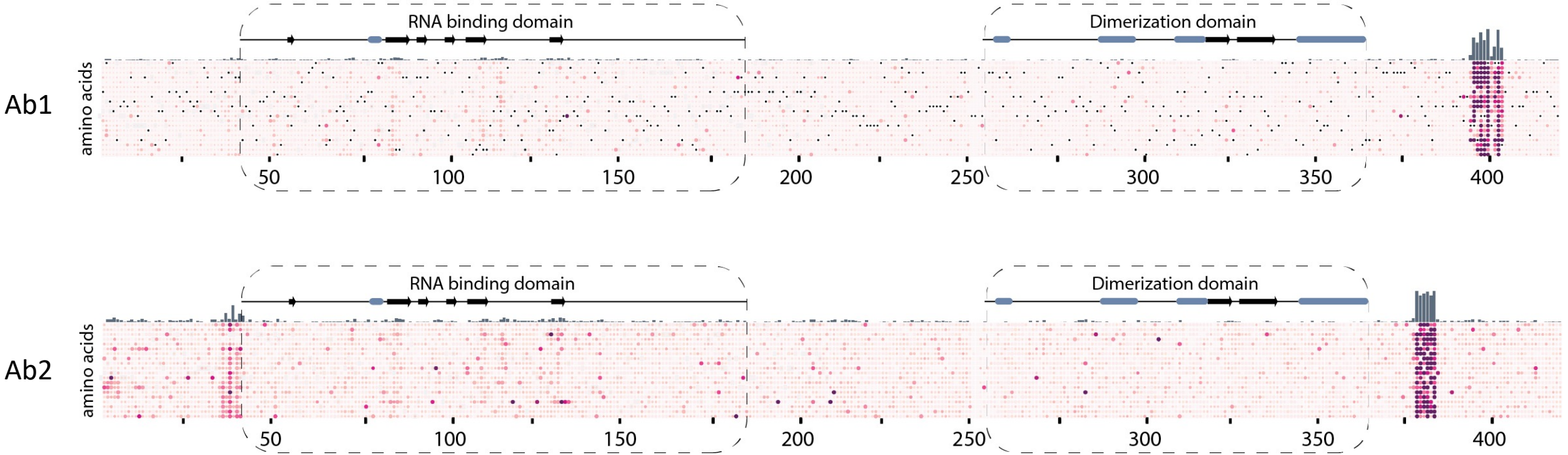
A linear epitope at the C-terminus is highly sensitive to mutations



Bourassa *et al.*, *J Clin Virol*, 2021
("A SARS-CoV-2 Nucleocapsid Variant that Affects Antigen Test Performance")

Linear epitopes are highly sensitive to mutations within the epitope



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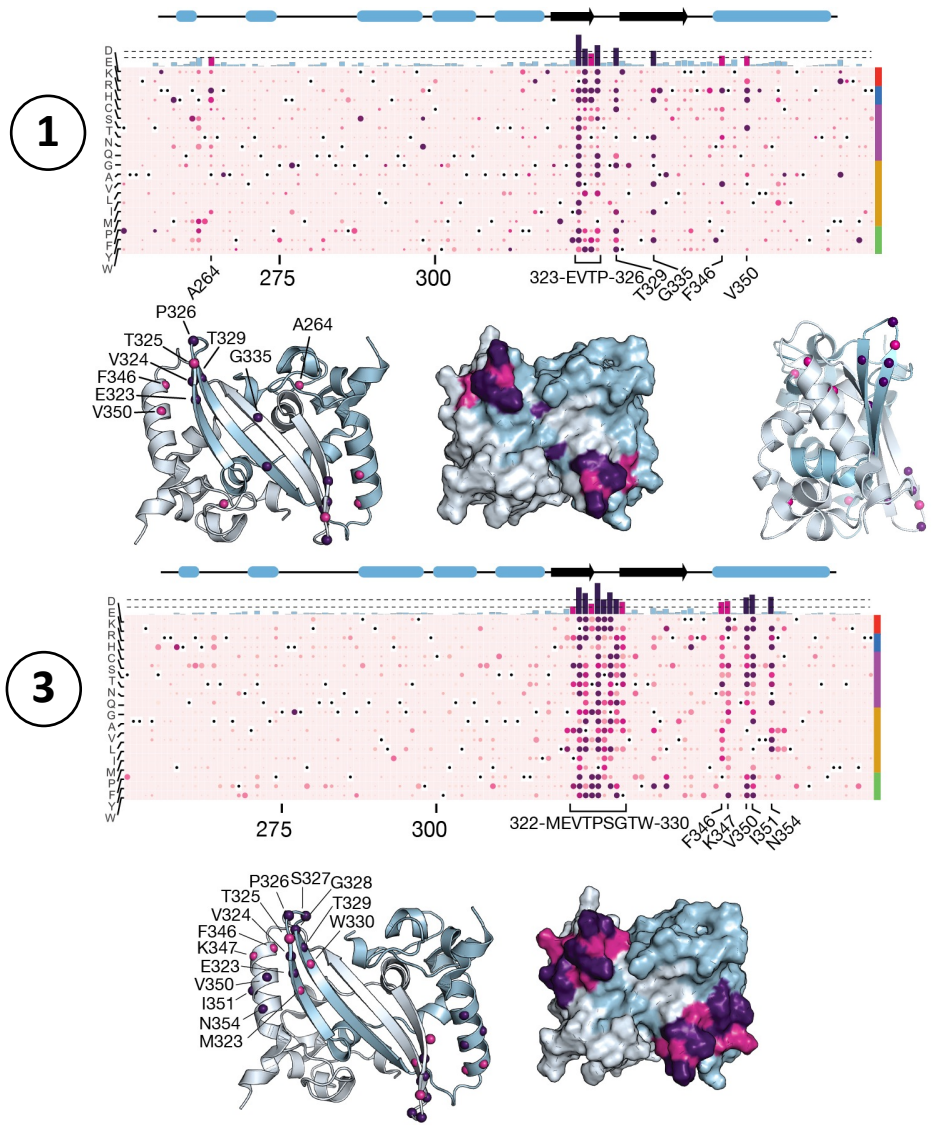
Three-dimensional epitopes in the dimerization domain



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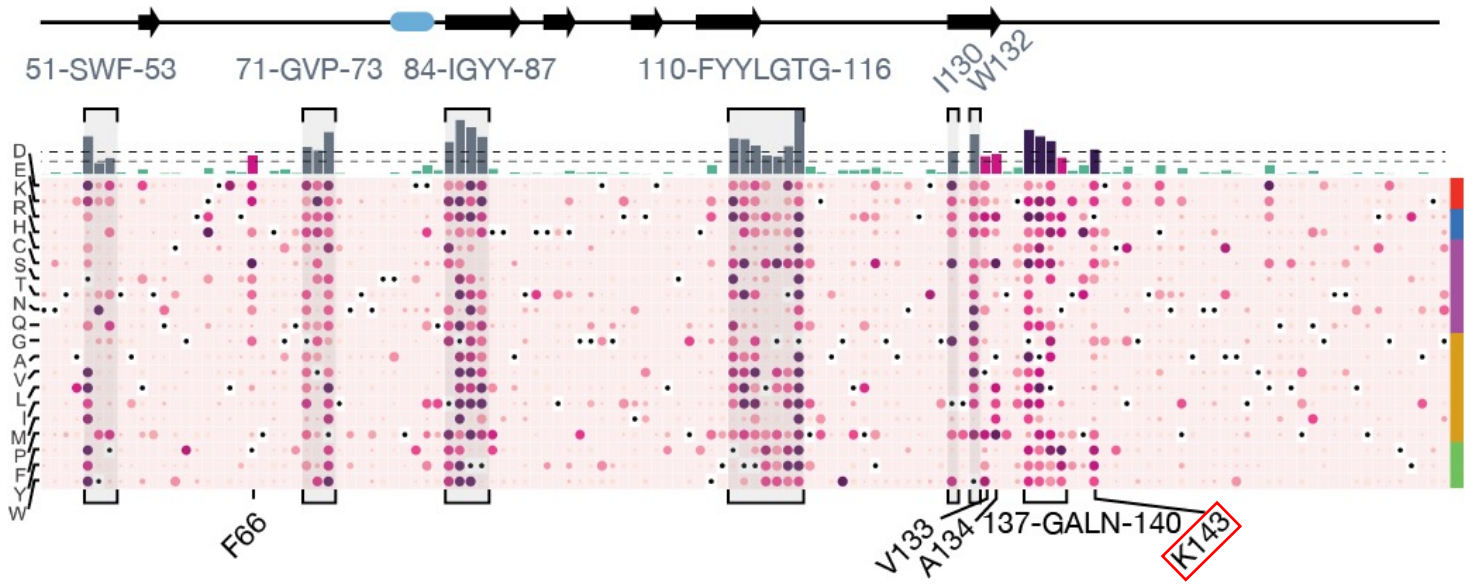
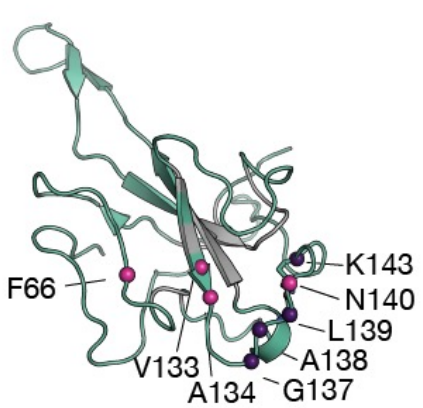
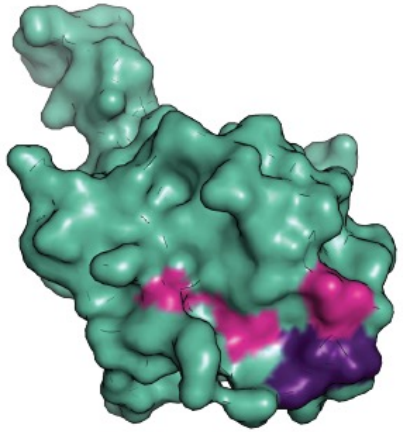
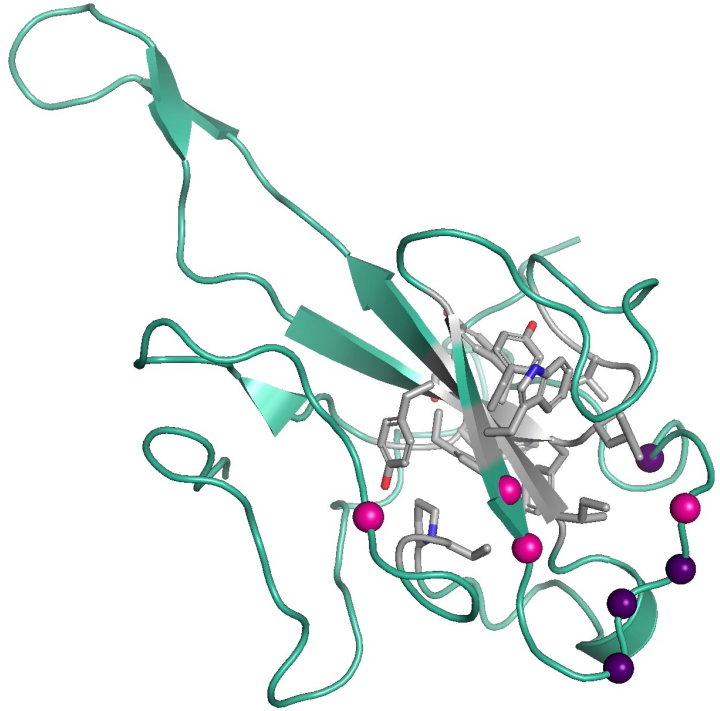
Three-dimensional epitopes in the RNA-binding domain









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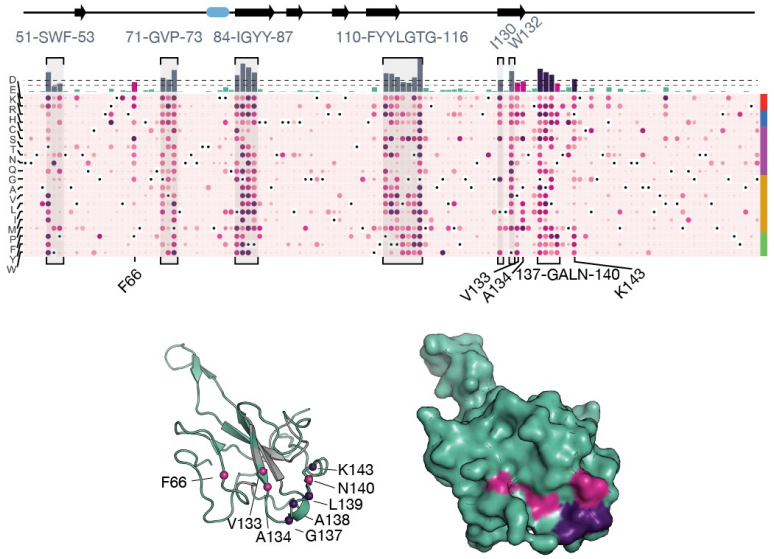
Three-dimensional epitopes in the RNA-binding domain

1



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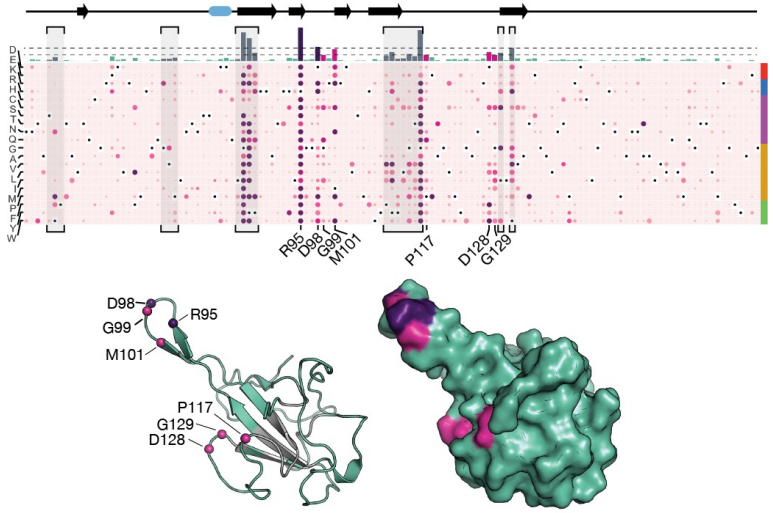

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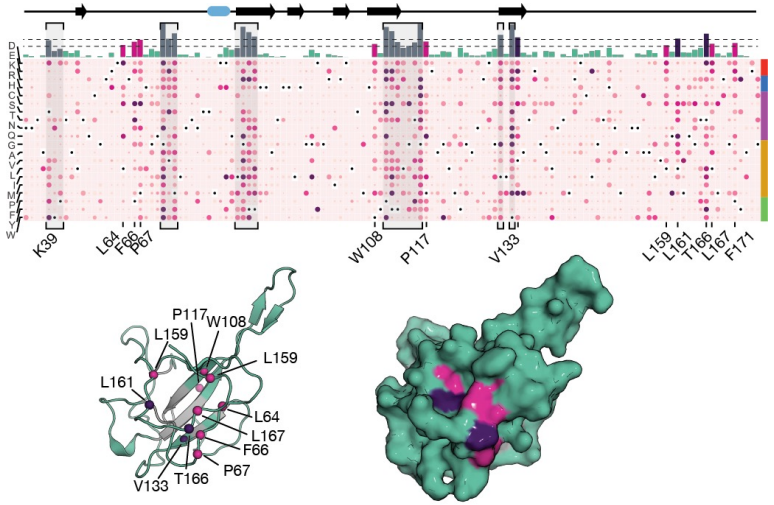

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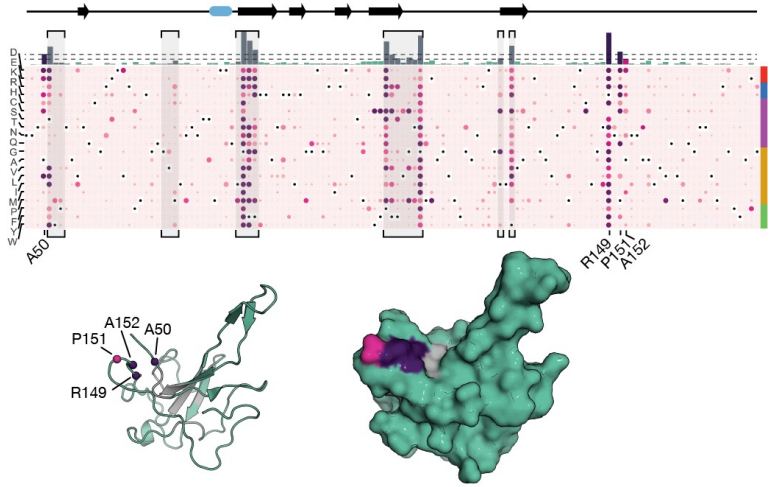

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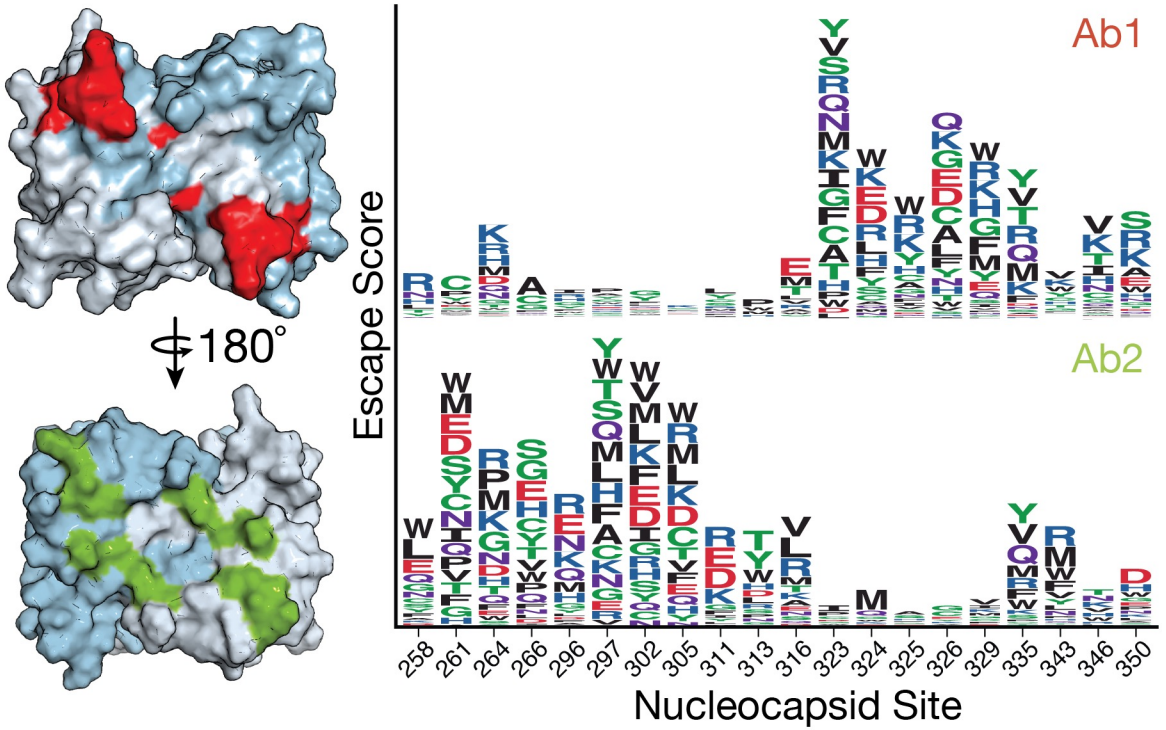
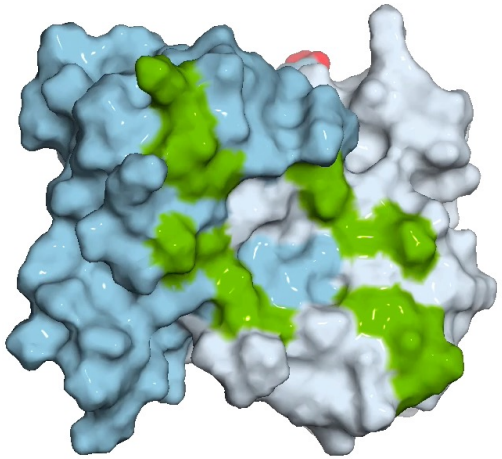
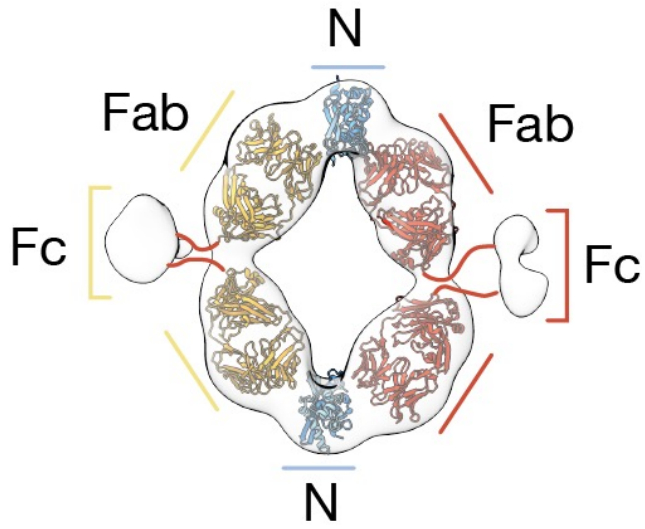
Diagnostic antibody combinations target spatially separated epitopes







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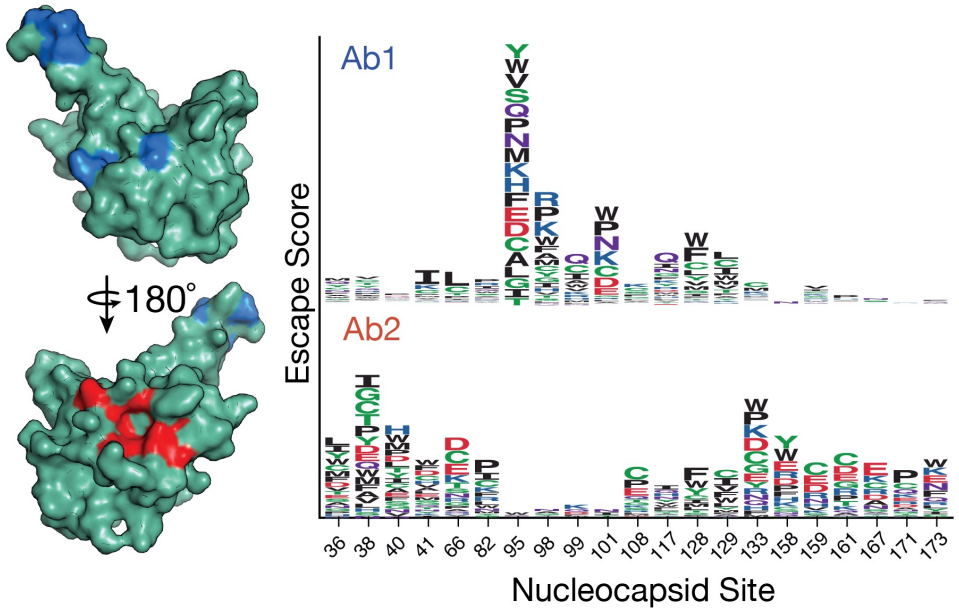

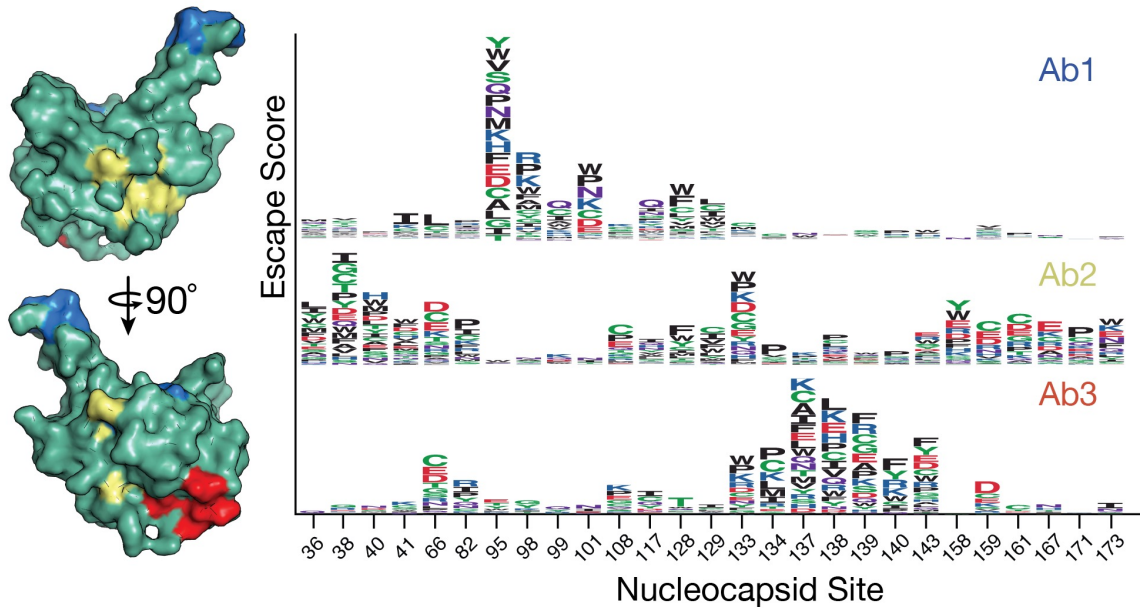

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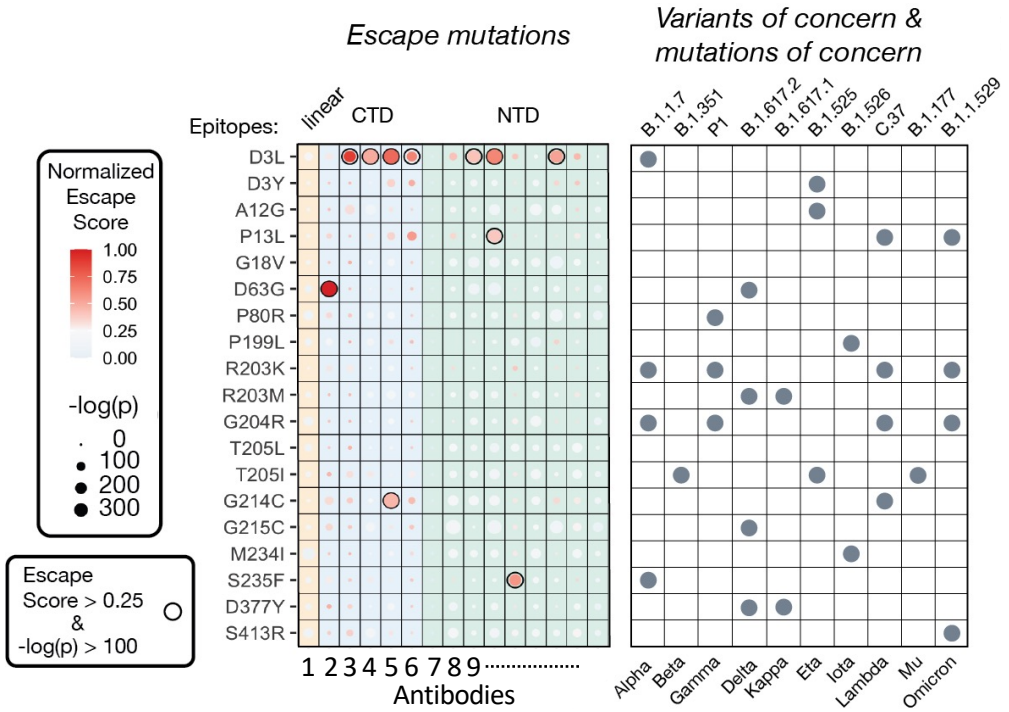
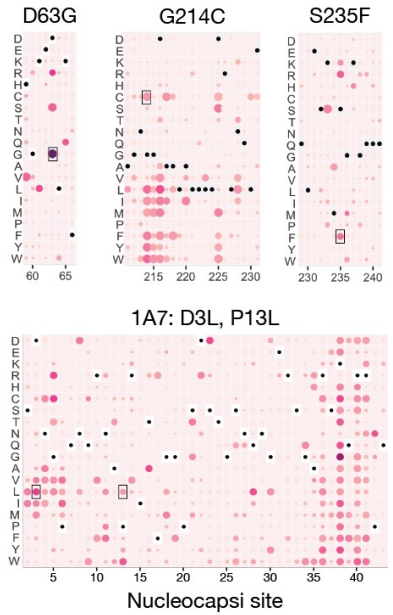
Antibody performance against mutations of concern





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Pediatrics/Winship Flow Cytometry Core
Yerkes Genomics Core

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