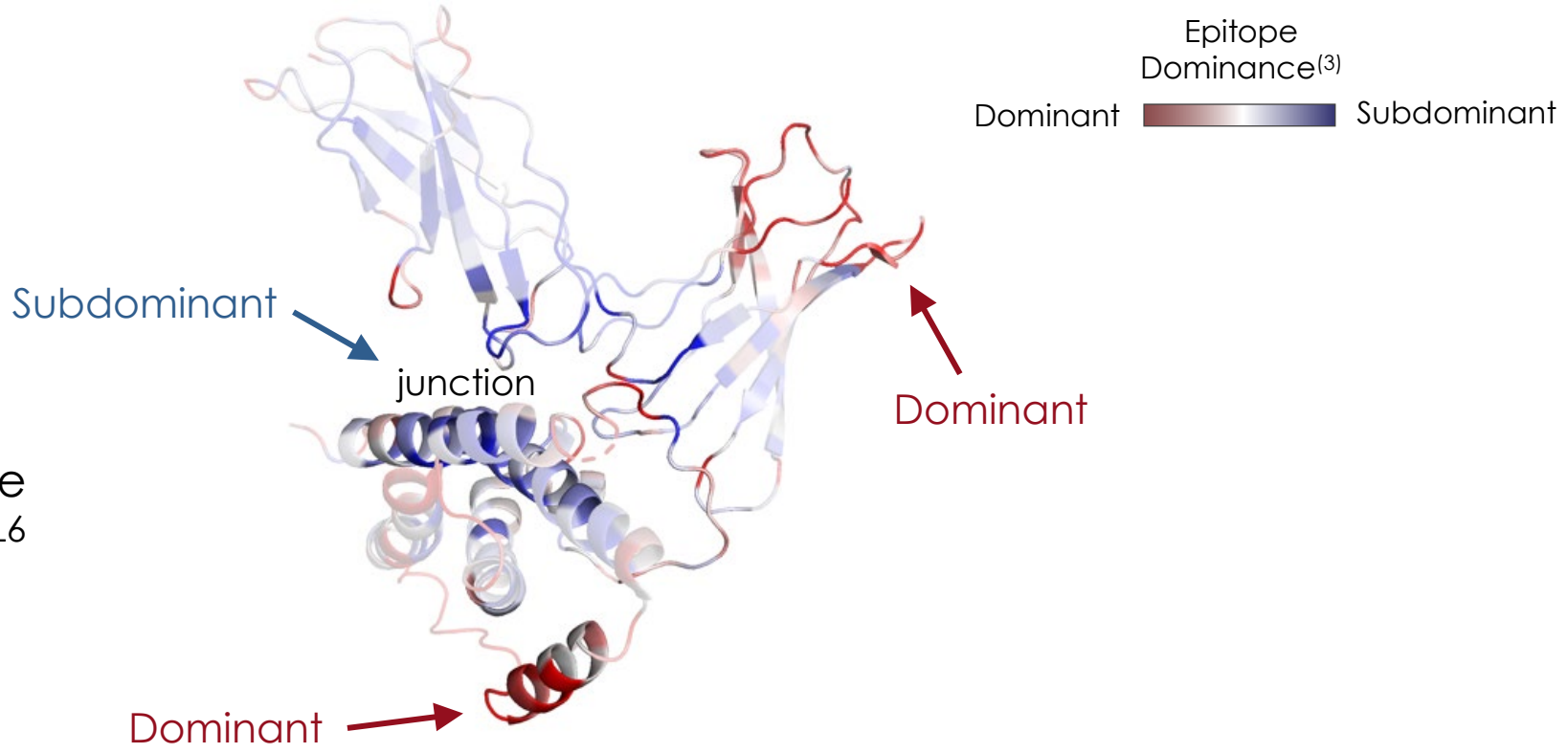
Epitope-Selective Antibody Discovery with Engineered Immunogens

September 2023

Problem #1: Traditional Antibody Discovery Provides Little to No Control Over the Epitope Binding Site

Dominant epitope – generates many antibody hits[1,2]

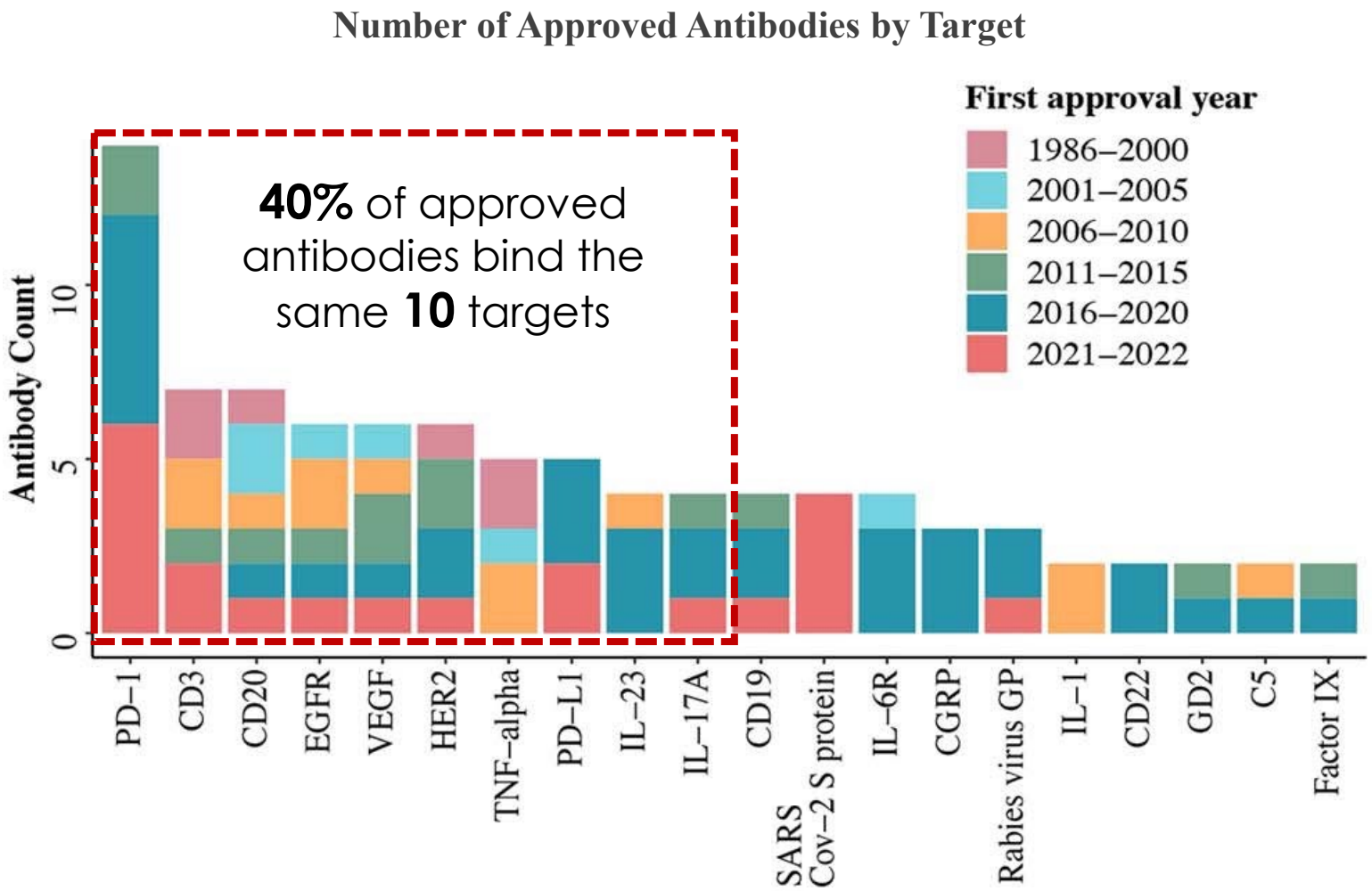
Subdominant epitope – generates few/no antibody hits



Junctional Epitope
sIL6R:IL6

(1) Wicker, et.al. Eur. J. Immunol. 1984, (2) Nakra, et.al. J. Immunol. 2000, (3) Ponomarenko, et.al., BMC Bioinfor. 2008

Problem #2: Traditional Antibody Discovery is Saturated with Conventionally Easier Targets



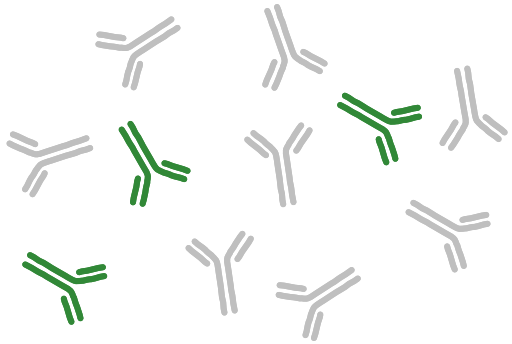
Missed Opportunities

GPCRs,
Membrane Transporters,
Protein-Protein Junctions,
Disease Variants, …

Engineered Epitopes Focus Antibody Repertoires On Desired Binding Sites

1

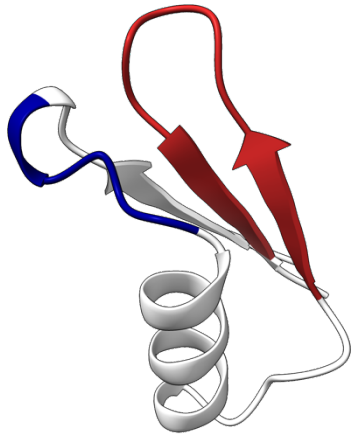
Naïve in vivo or in vitro antibody library



epitope-specific Ab

2

Focus library with engineered epitopes

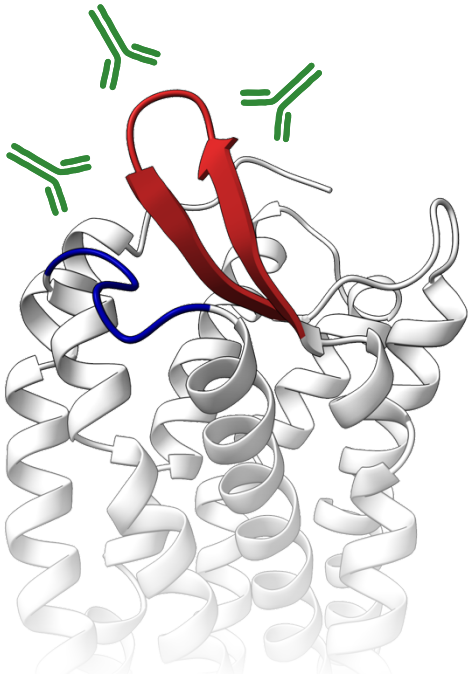


epitope

de novo scaffold

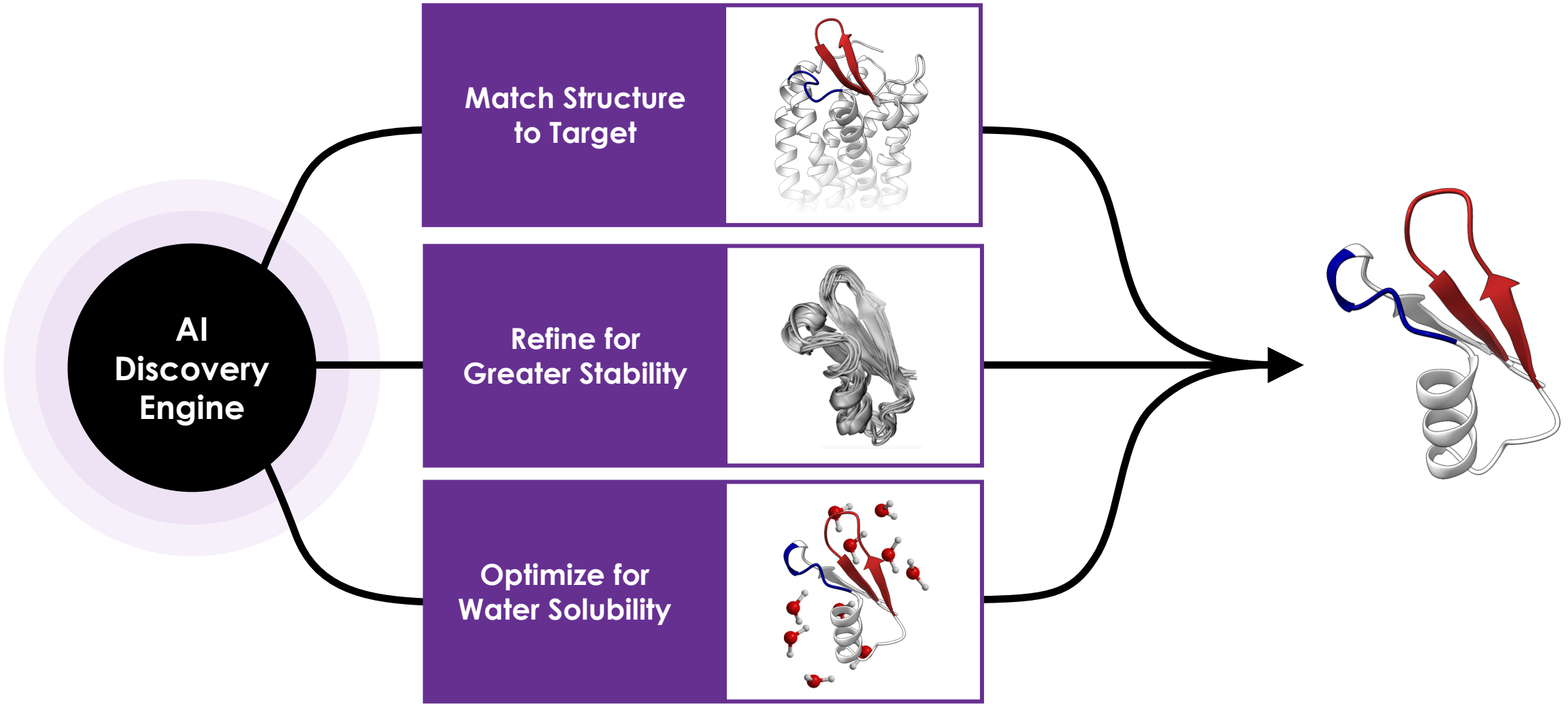
3

Efficient discovery of epitope-specific Abs

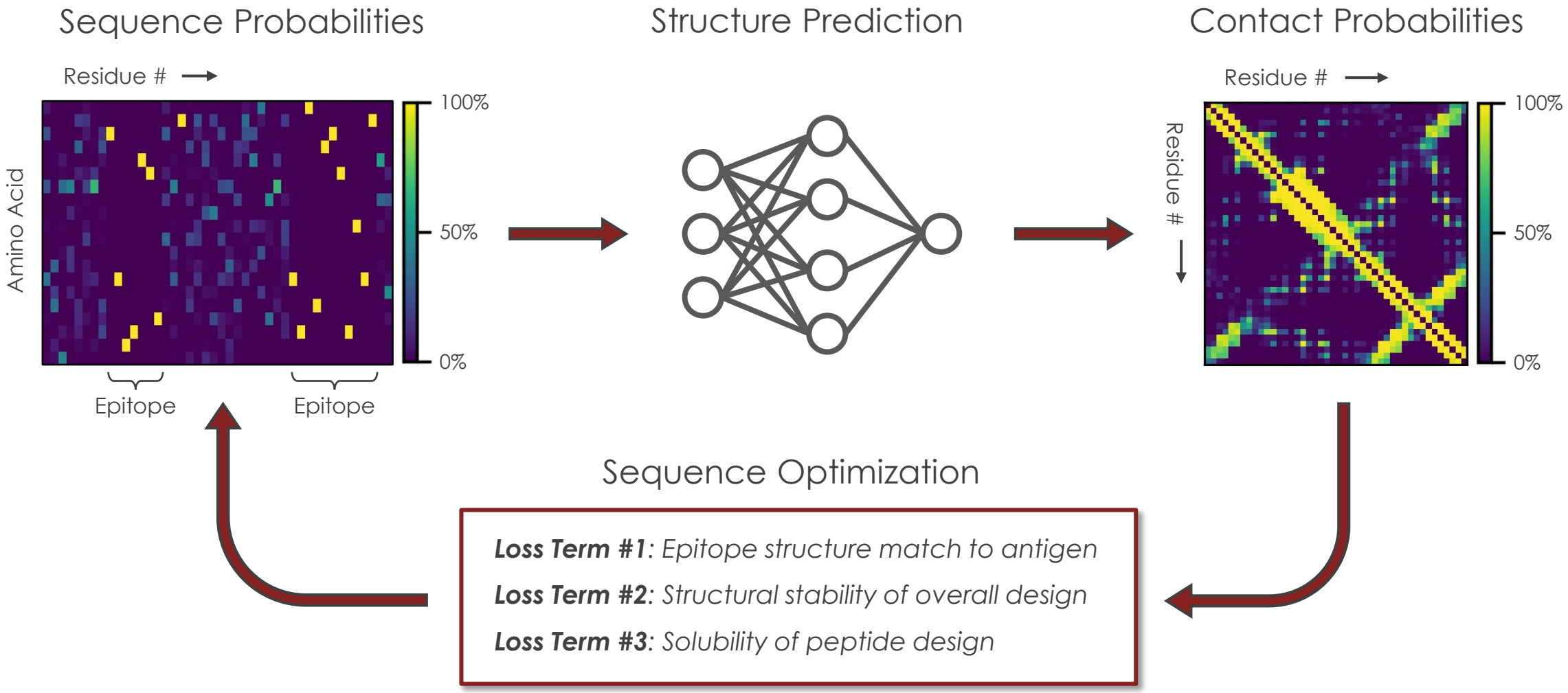


full-length target

AI-Engine Optimizes Engineered Epitope Structure, Stability, and Solubility



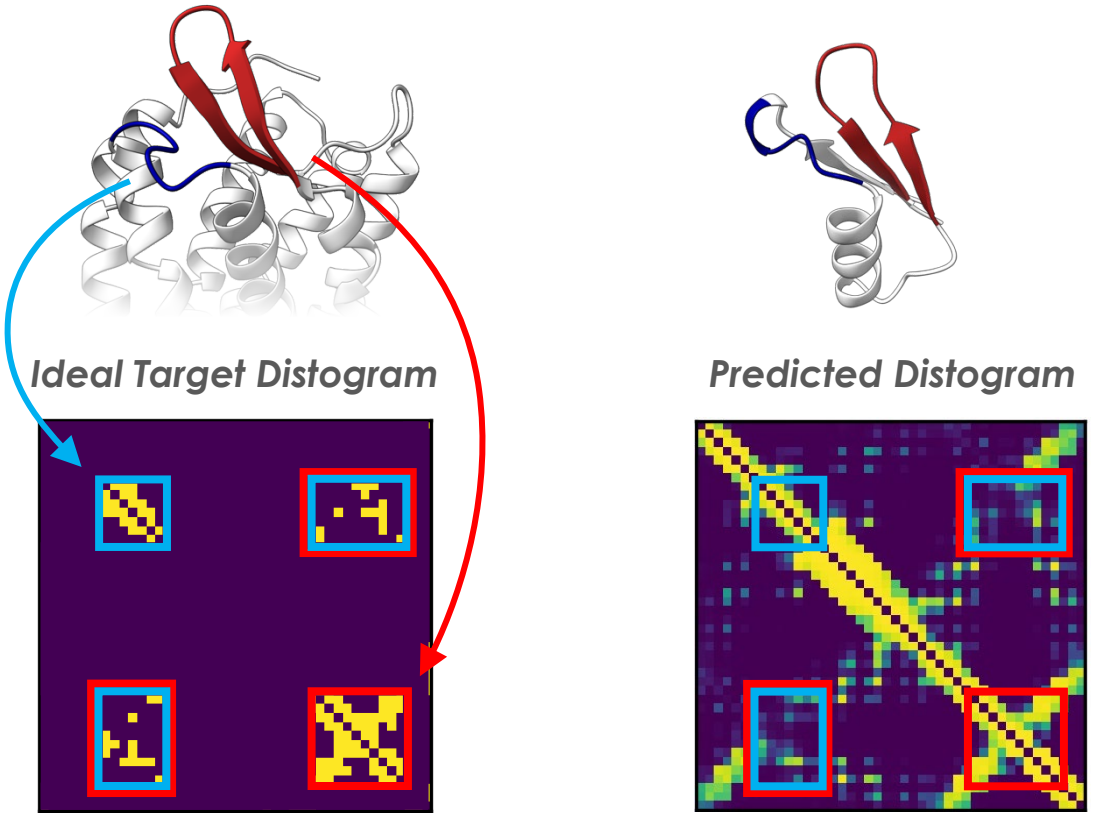
Sequence and Structure are Jointly Refined Until Loss Terms are Satisfied



(1) Anishchenko & Baker et al, bioRxiv 2020, (2) Norn & Ovchinnikov et al, bioRxiv 2020, (3) Tischer & Baker et al, bioRxiv 2020

Multi-Loss Function Enforces Epitope Structure Match and Stability

Loss Term #1



Ideal Target Distogram

Predicted Distogram

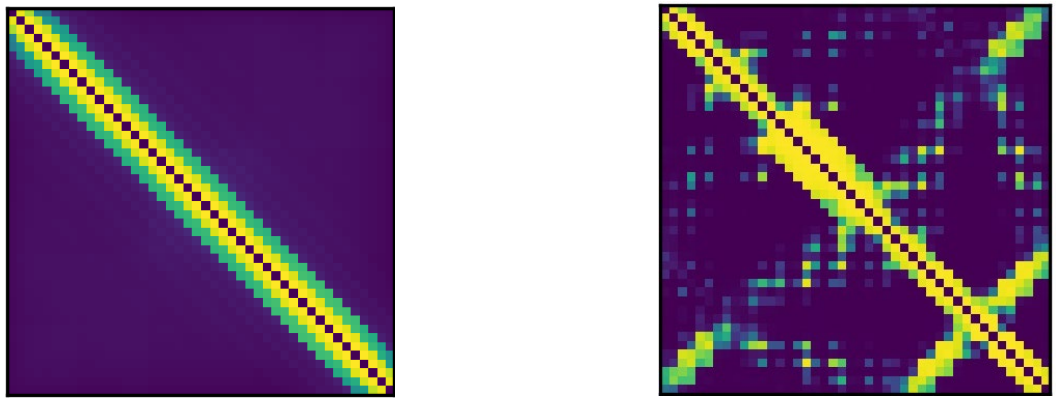
Minimize Cross-Entropy between boxed regions

Loss Term #2

Average predicted distograms across random sequence space

Random Coil Distogram

Predicted Distogram

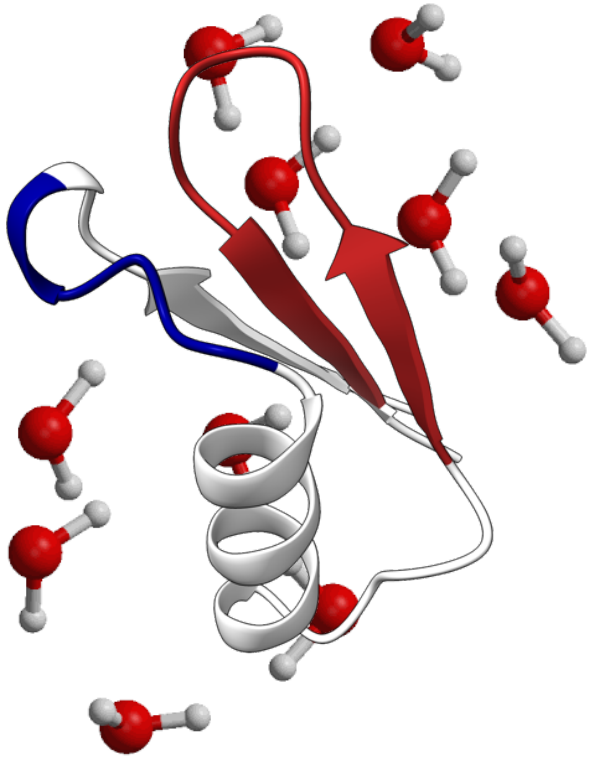


Maximize KL-Divergence between distograms

(1) Anishchenko & Baker et al, bioRxiv 2020, (2) Norn & Ovchinnikov et al, bioRxiv 2020, (3) Tischer & Baker et al, bioRxiv 2020

Multi-Loss Function Optimizes Peptide Solubility

Loss Term #3

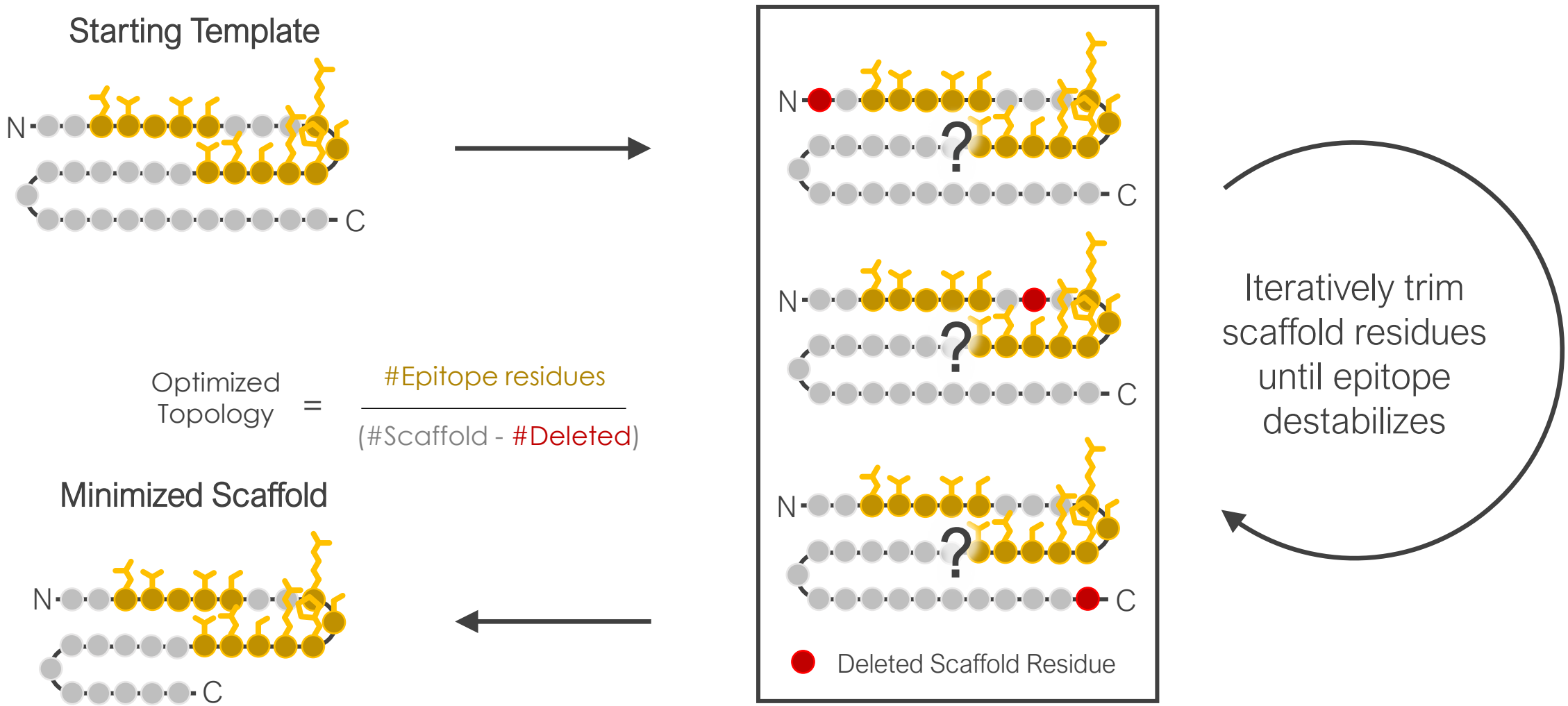


Amino Acid Hydropathies

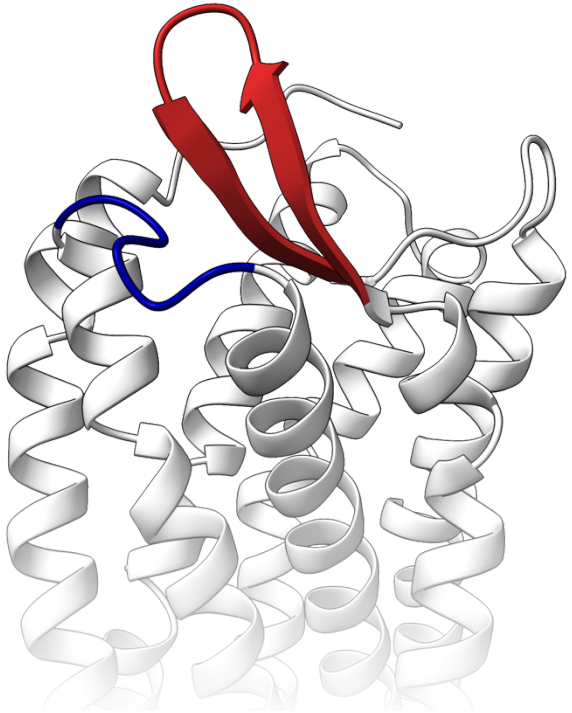
I: 4.5	V: 4.2	L: 3.8	F: 2.8
C: 2.5	M: 1.9	A: 1.8	G: -0.4
T: -0.7	S: -0.8	W: -0.9	Y: -1.3
P: -1.6	H: -3.2	E: -3.5	Q: -3.5
D: -3.5	N: -3.5	K: -3.9	R: -4.5

Average peptide hydropathy is minimized

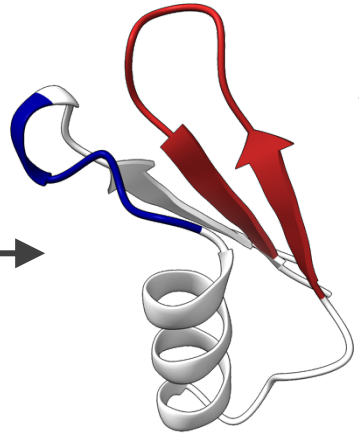
Scaffold Size is Minimized to Reduce Off-Target Immune Response



Engineered Epitopes Undergo In Silico and Experimental Cross Validation

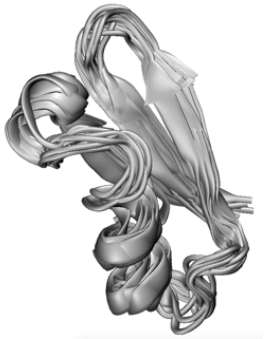


AI Discovery Engine



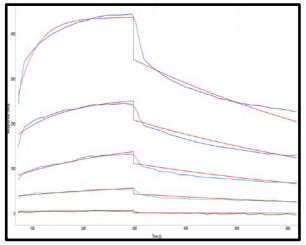
Folding Simulations

e.g. AlphaFold2



Biophysical Characterization

e.g. SPR, thermostability

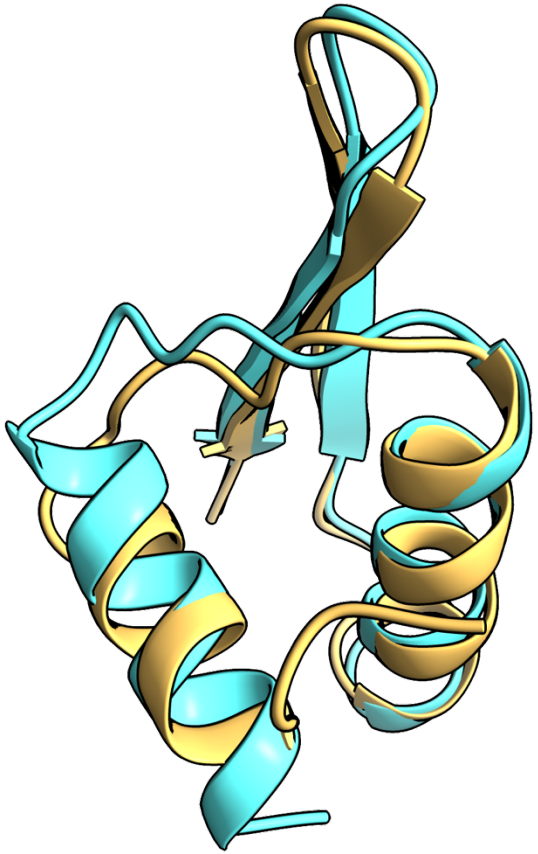


Structure Determination

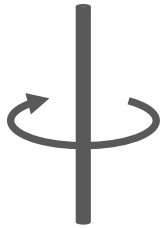
e.g. NMR

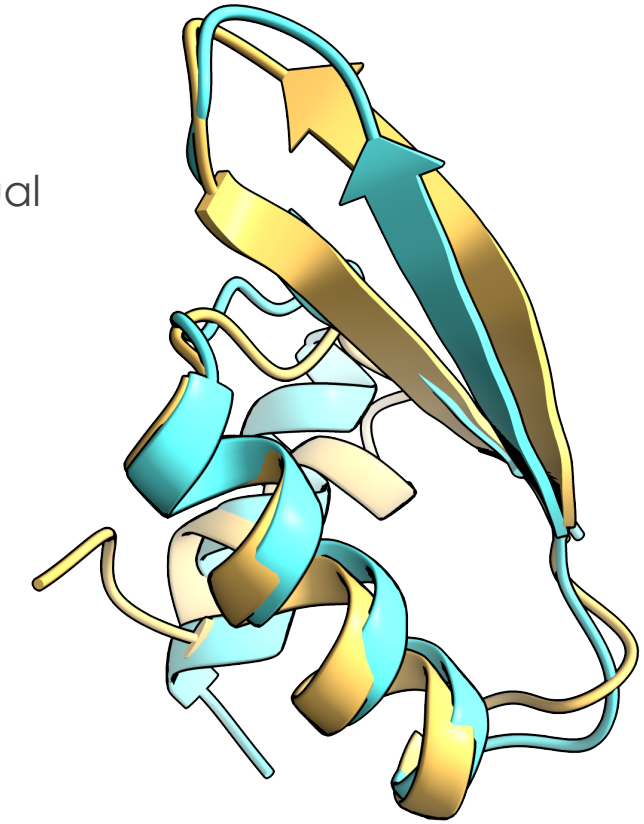
NMR Structure Validates Engineered Epitope Design Engine

NMR Solved Structure
Predicted Structure



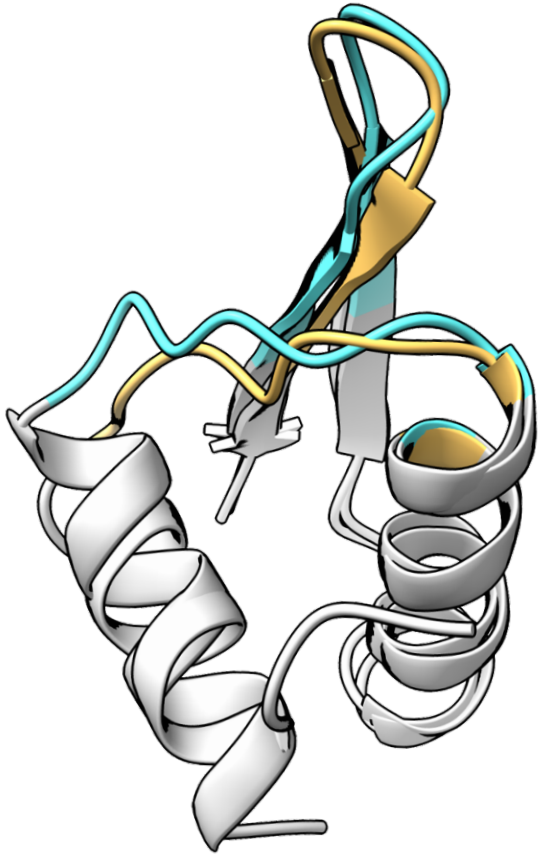
Predicted structure vs. Actual
Backbone RMSD: **3.2 Å**



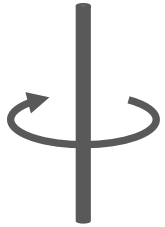


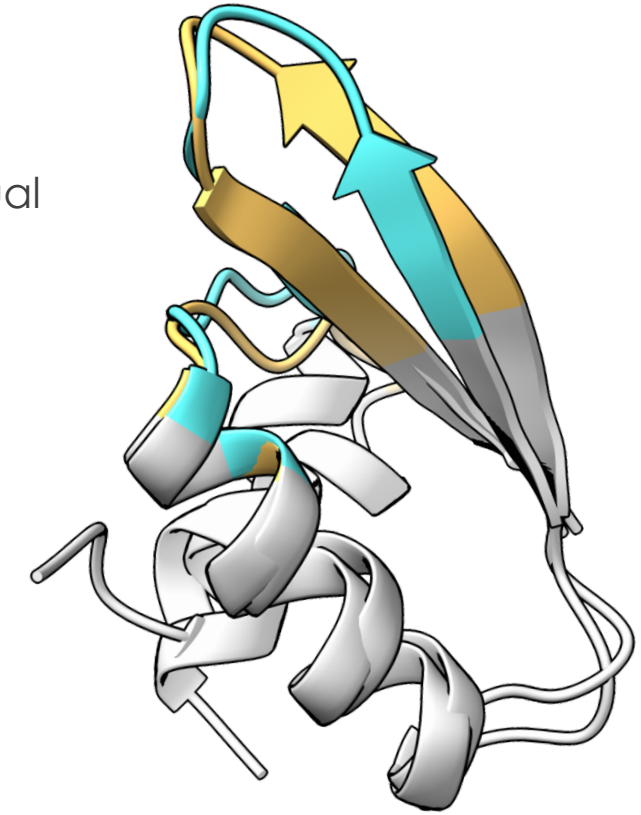
NMR Structure Validates Engineered Epitope Design Engine

NMR Solved Structure
Predicted Structure



Predicted structure vs. Actual
Backbone RMSD: **1.6 Å**

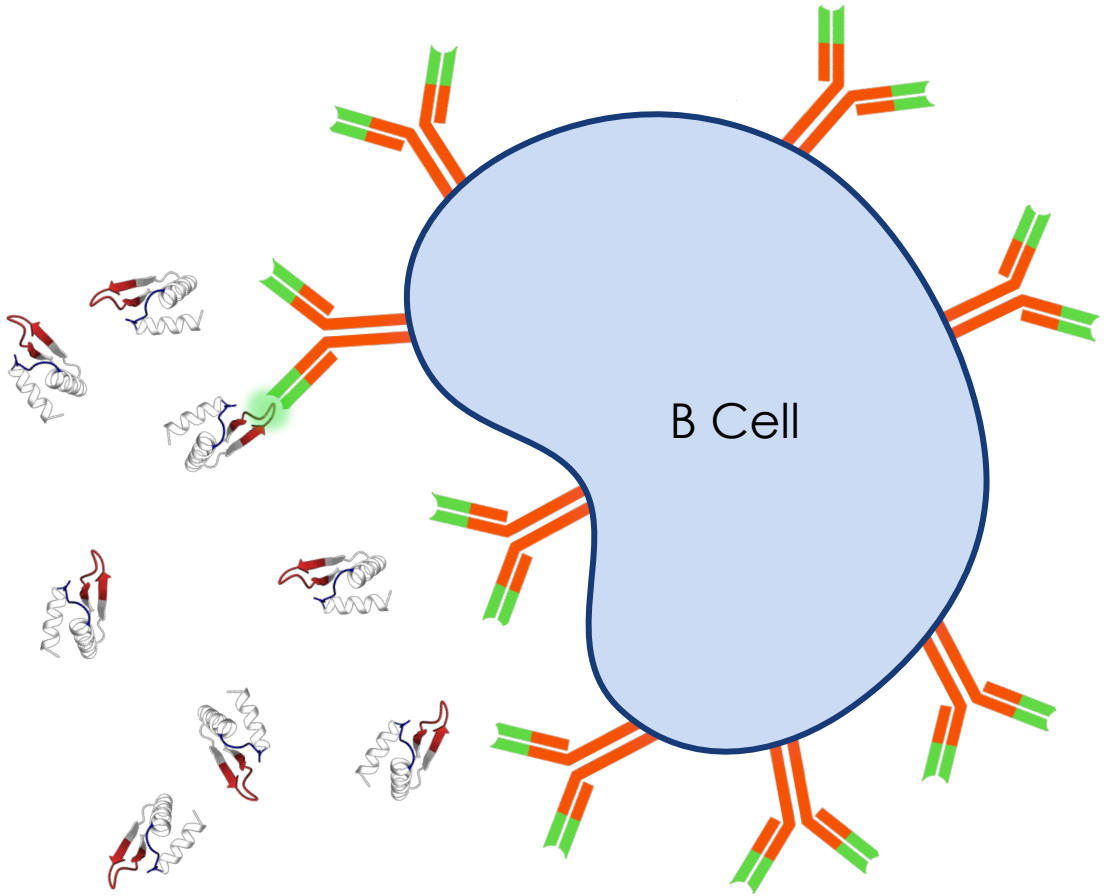




Multivalent Display of Engineered Epitopes Enhances Immune Response

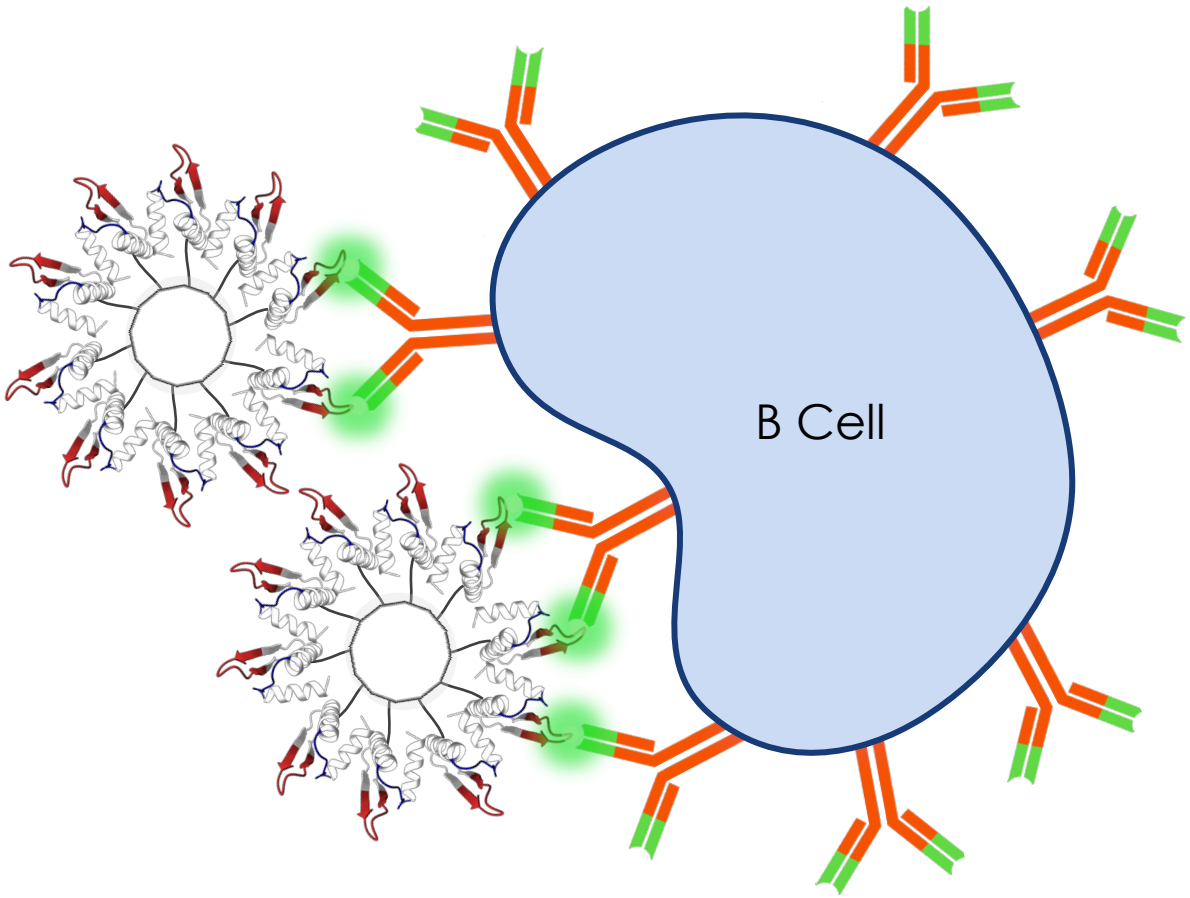
Engineered Epitope Immunization

Weak B cell activation



Nanoparticle Immunization

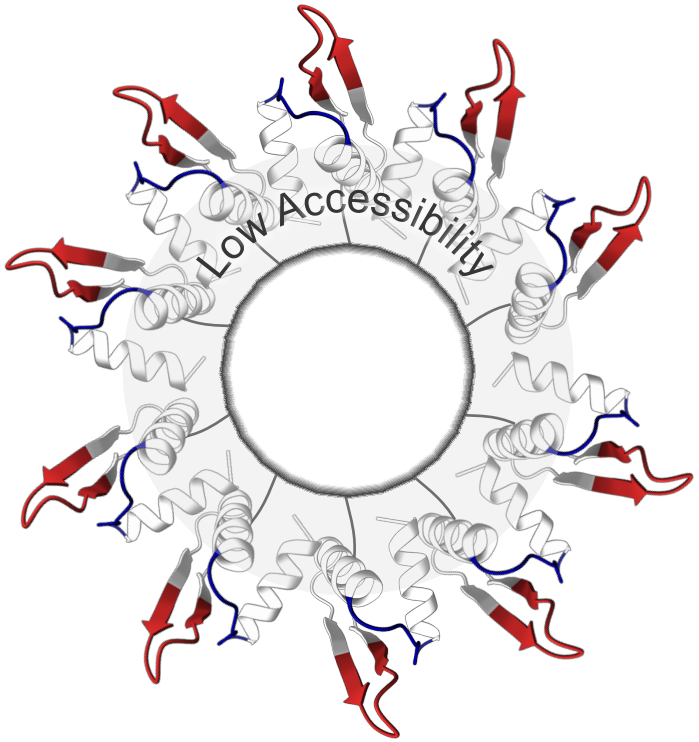
Strong B cell activation



Nanoparticles are Optimized for Correct Epitope Orientation and High Valency

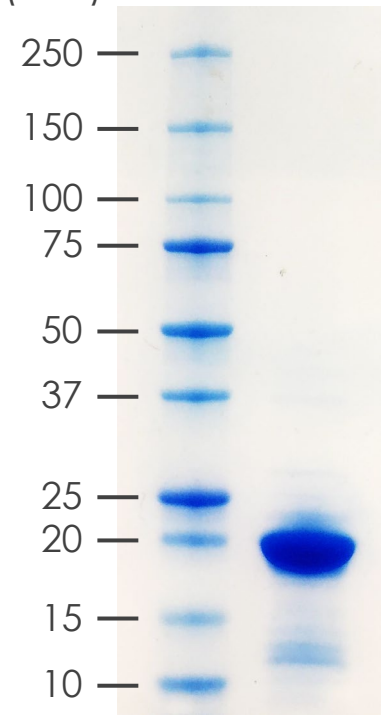
Orientation

- Epitope Residues: Outward
- Scaffold Residues: Inward

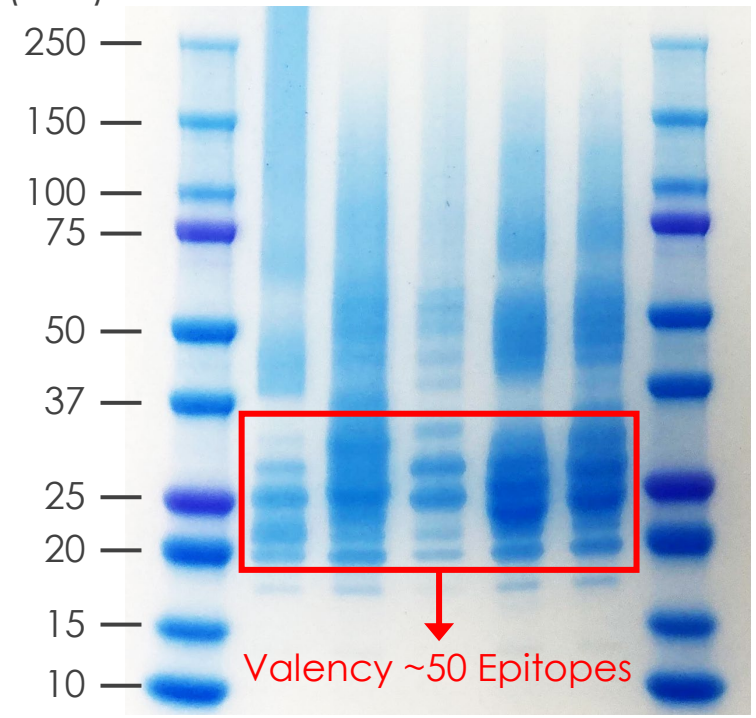


Valency

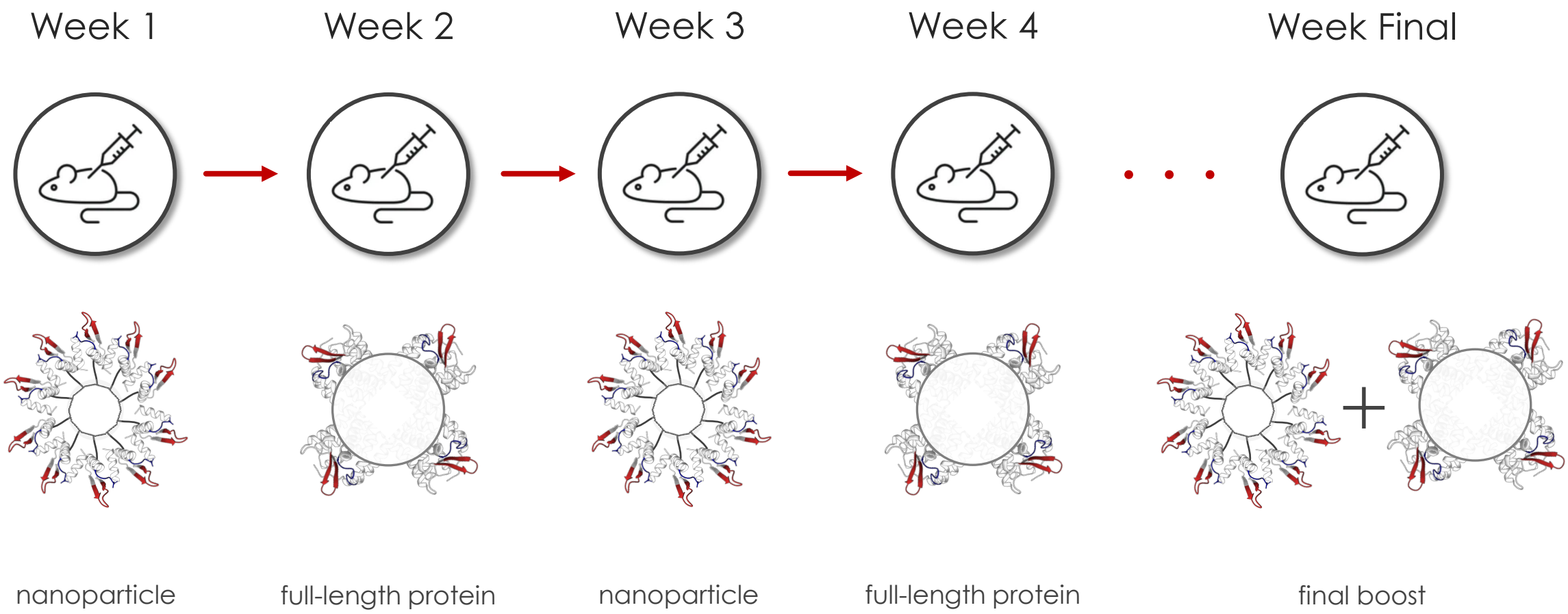
Nanoparticle



Nanoparticle + Engineered Epitopes

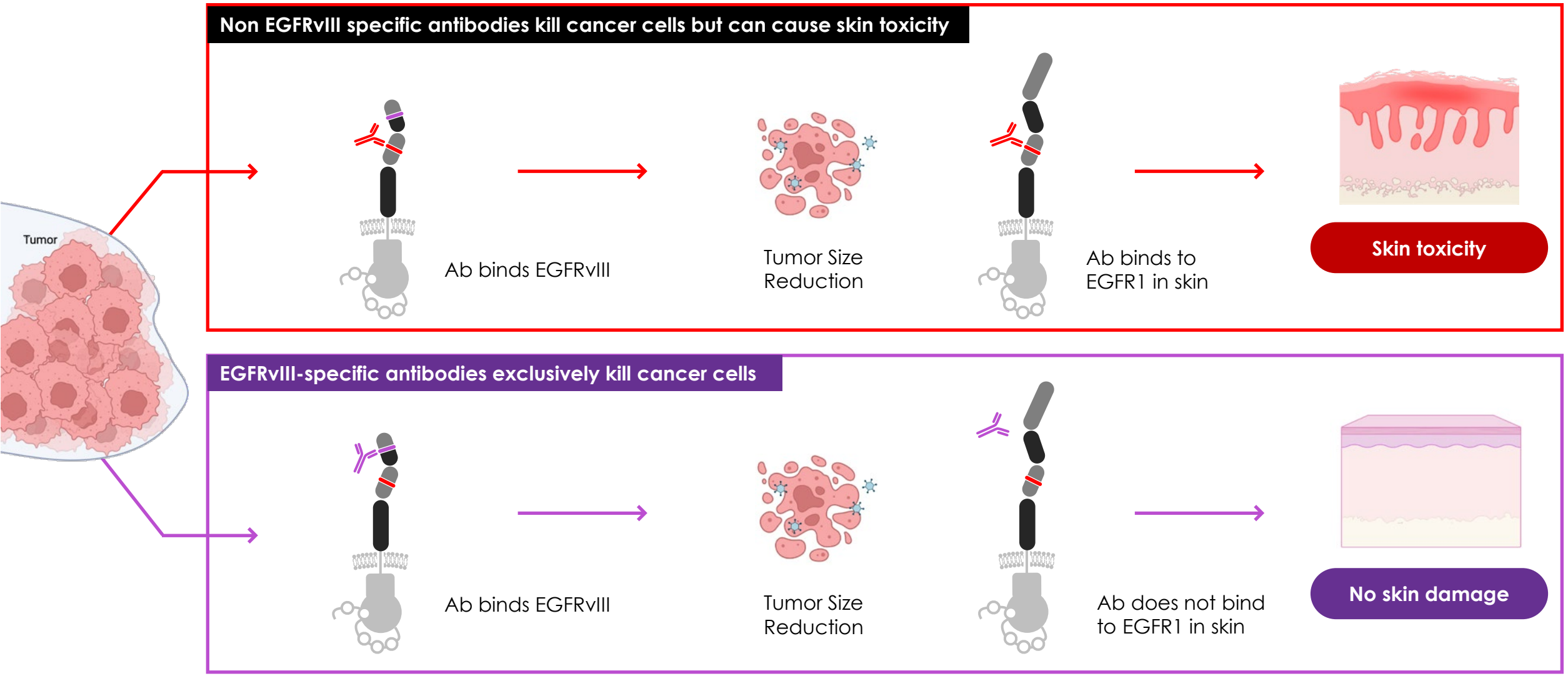


Immunizations Alternate between Nanoparticle and Full-Length Protein



Case Study #1
Target: EGFRvIII
MOA: Tumor-Specific ADCC

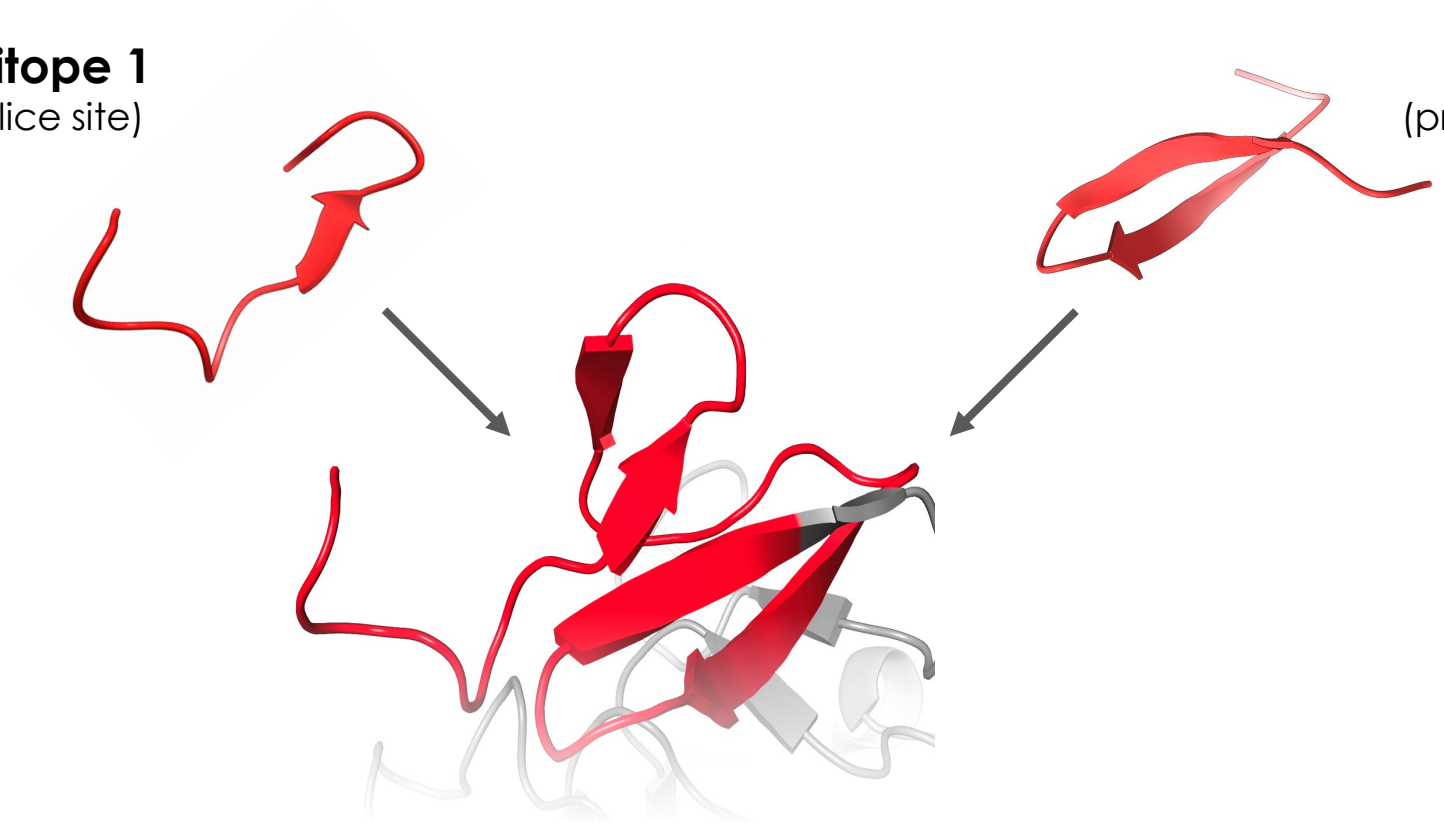
EGFRvIII is a Splice Variant of EGFR1 that Contains a Tumor-Specific Epitope



Engineered Epitopes are Designed for the EGFRvIII Splice Site

Epitope 1
(splice site)

Epitope 2
(proximal to splice site)



Epitope 1+2

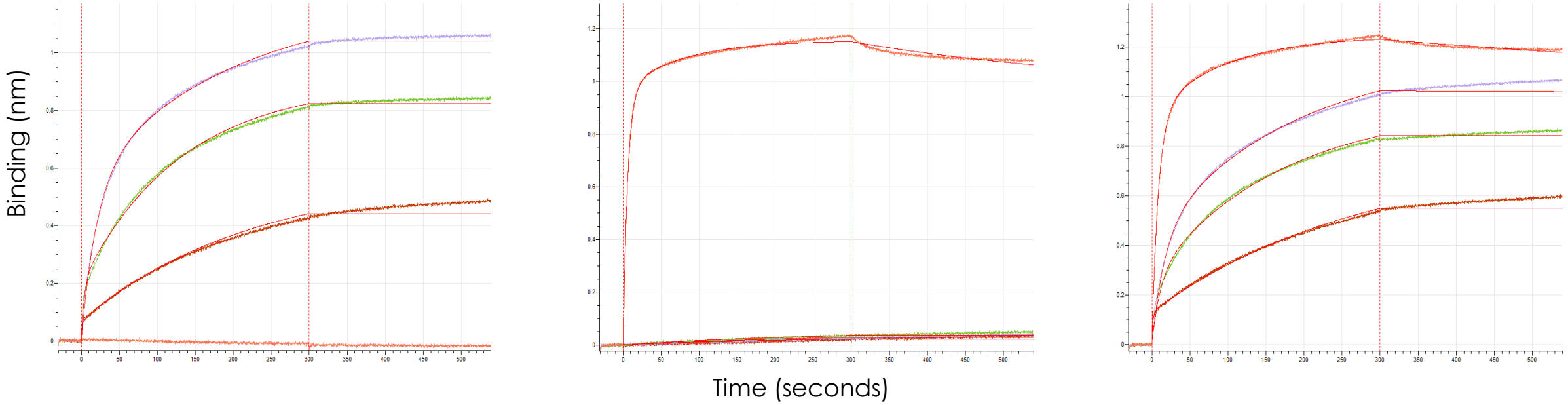
EGFRvIII
Tumor Specific Epitope

Engineered Epitopes Bind to their Corresponding Benchmark Antibodies

Epitope 1

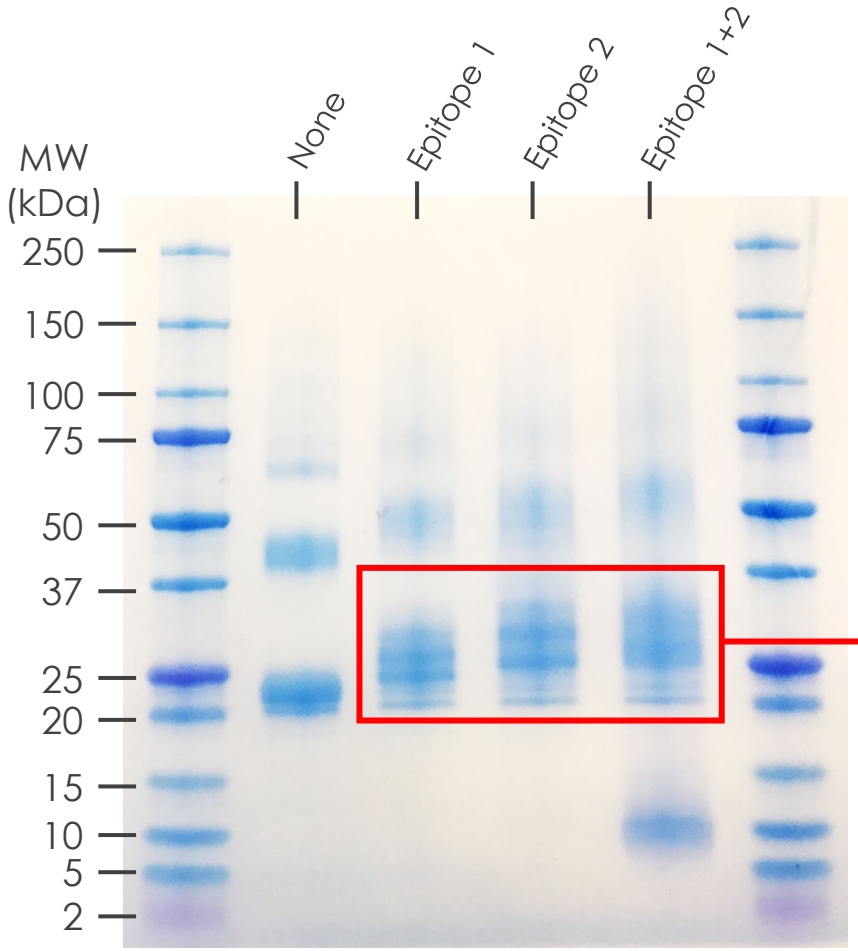
Epitope 2

Epitope 1+2

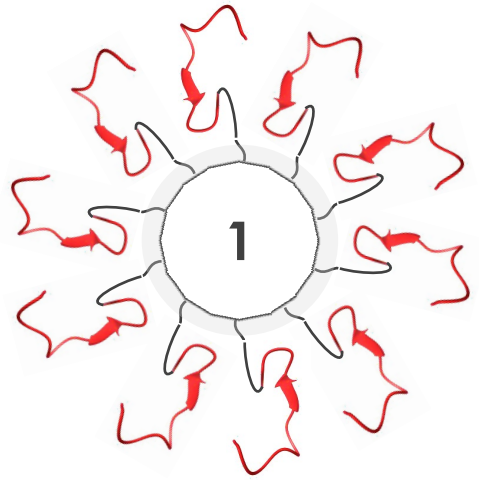


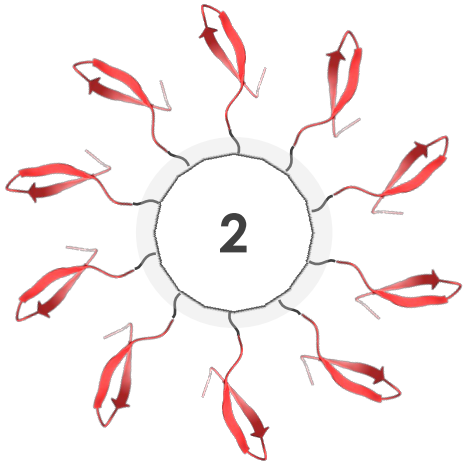
Engineered Epitopes are Conjugated to Nanoparticles

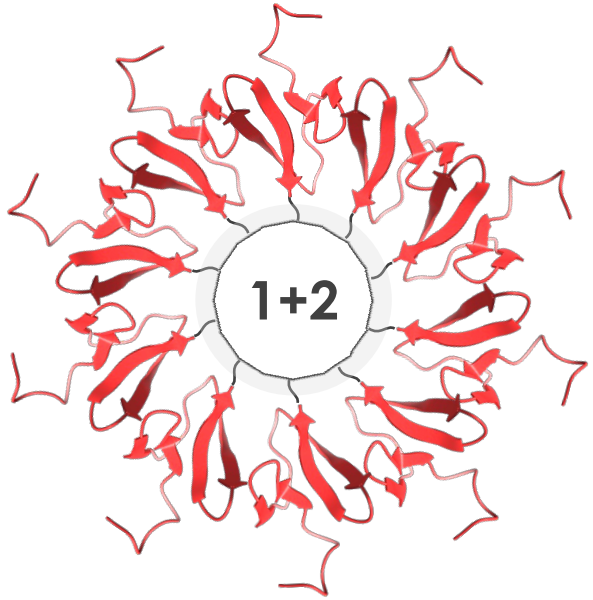
Nanoparticle Conjugations



~50 epitopes per nanoparticle

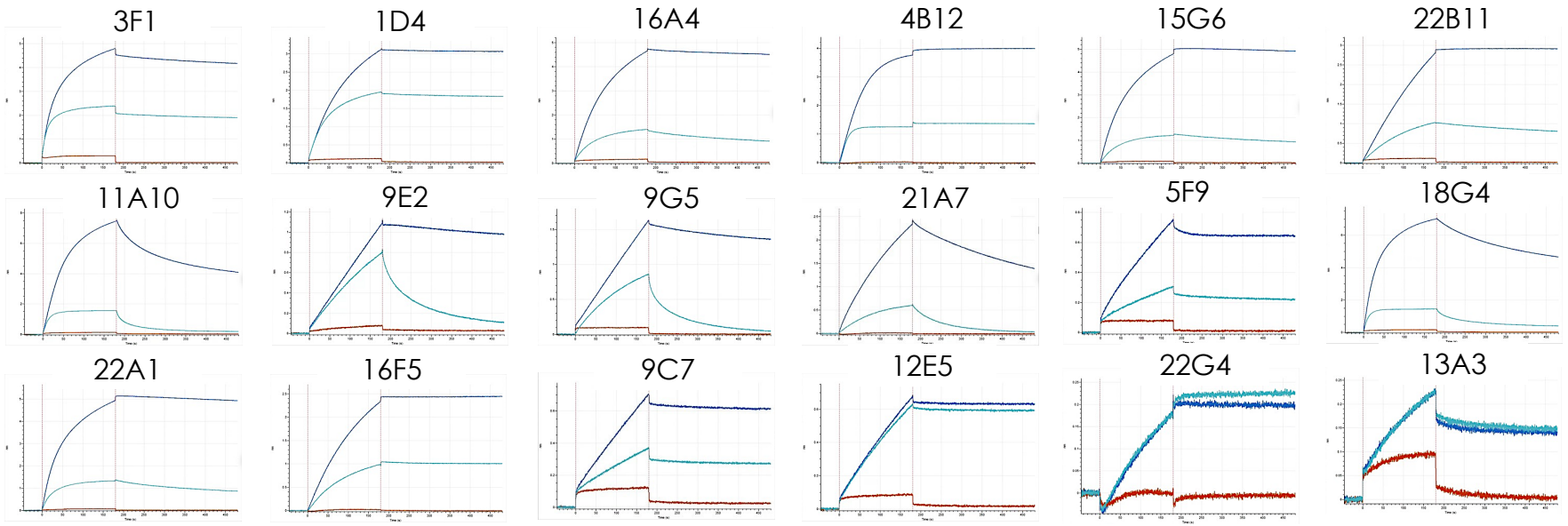






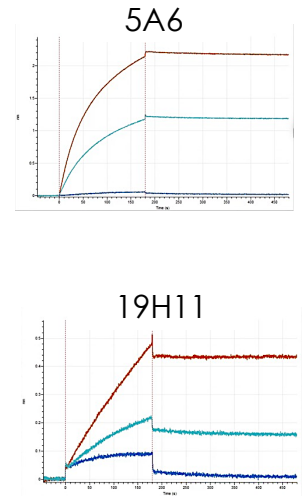
Immunizations Steered Towards Epitope 1 Produce EGFRvIII Specific Binders

Epitope 1 Steered



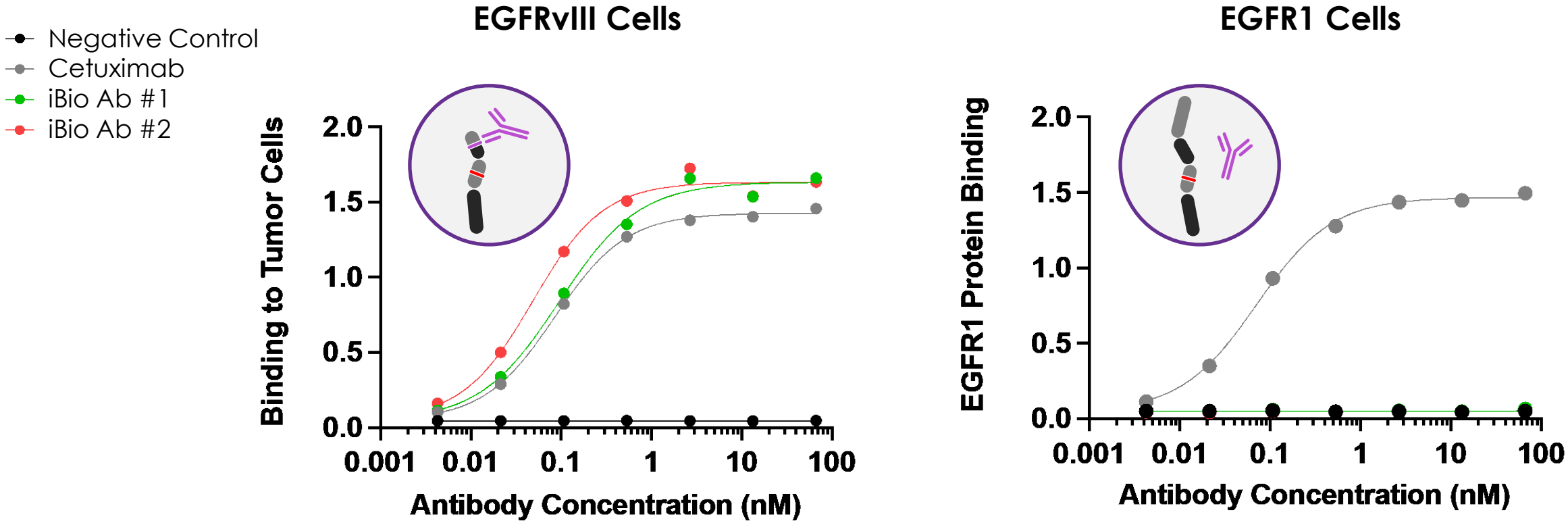
10 bind EGFRvIII and not EGFR1

Epitope 2 Steered

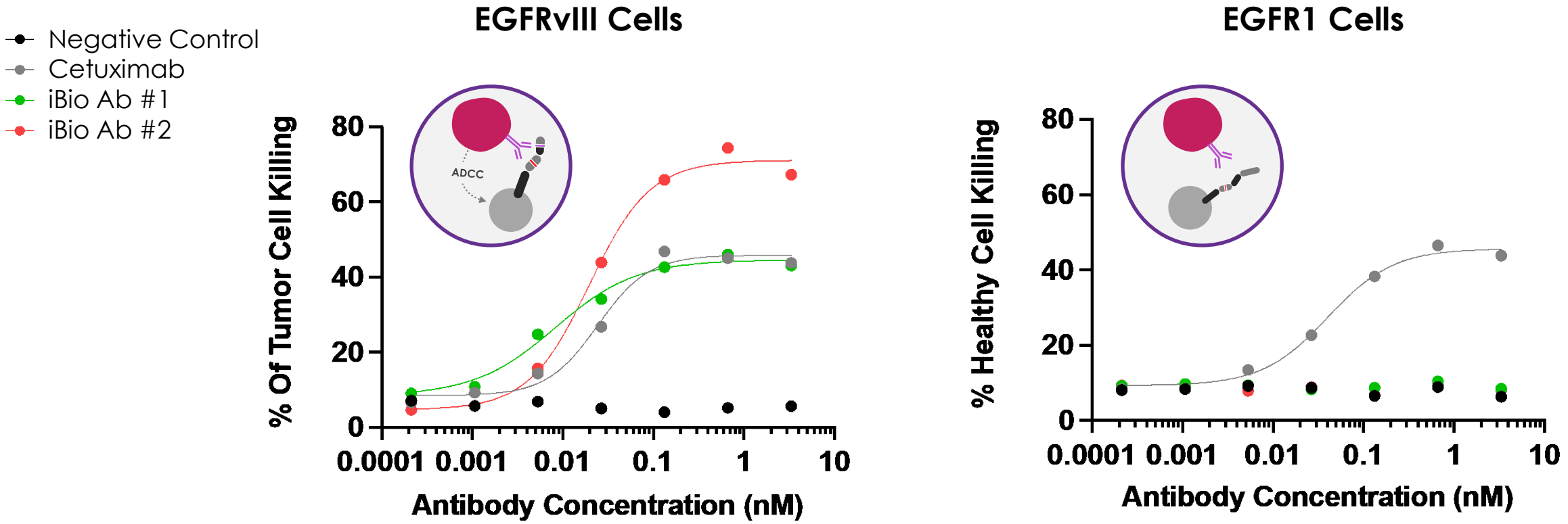


0 bind EGFRvIII and not EGFR1

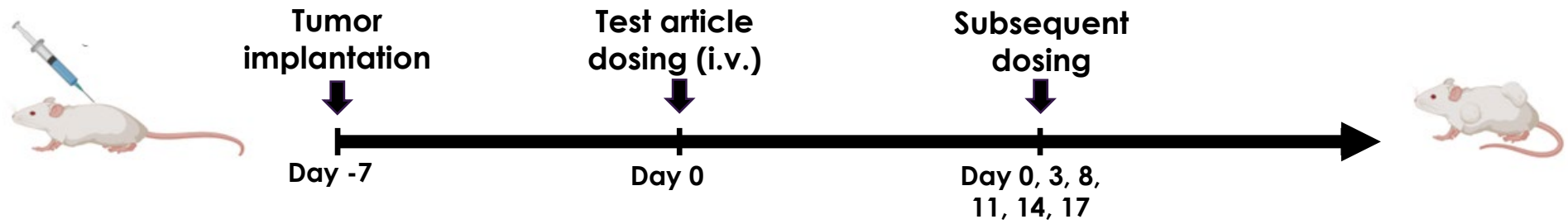
Lead Antibodies Specifically Bind EGFRvIII-Expressing Cells

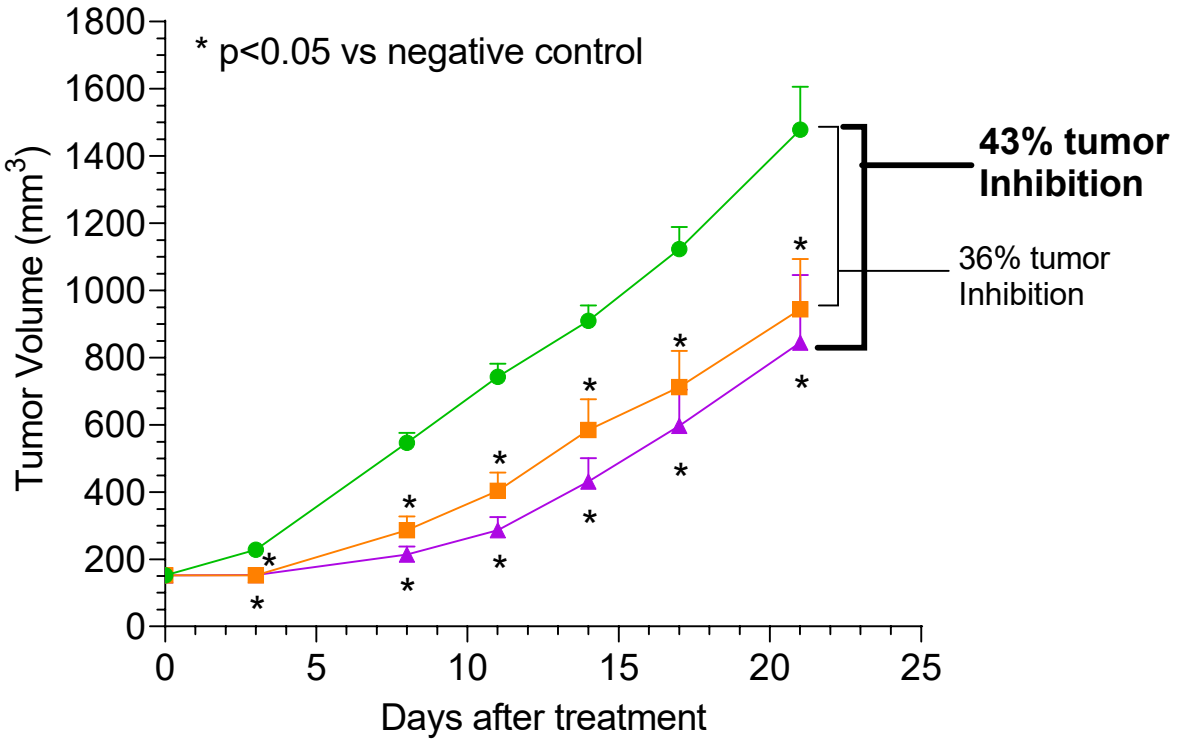


Lead Antibodies Specifically Kill EGFRvIII-Expressing Cells by ADCC



Lead Antibody Inhibits Tumor Growth in EGFRvIII Tumor Xenograft Mouse Model

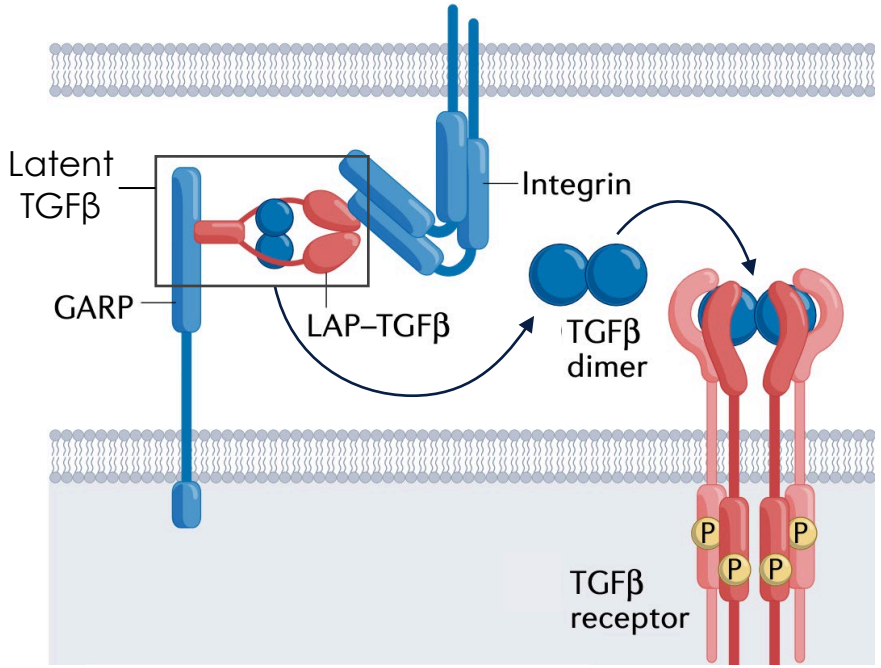




Case Study #2
Target: Latent TGFβ1
MOA: Anti-Immune Suppression in Tumors

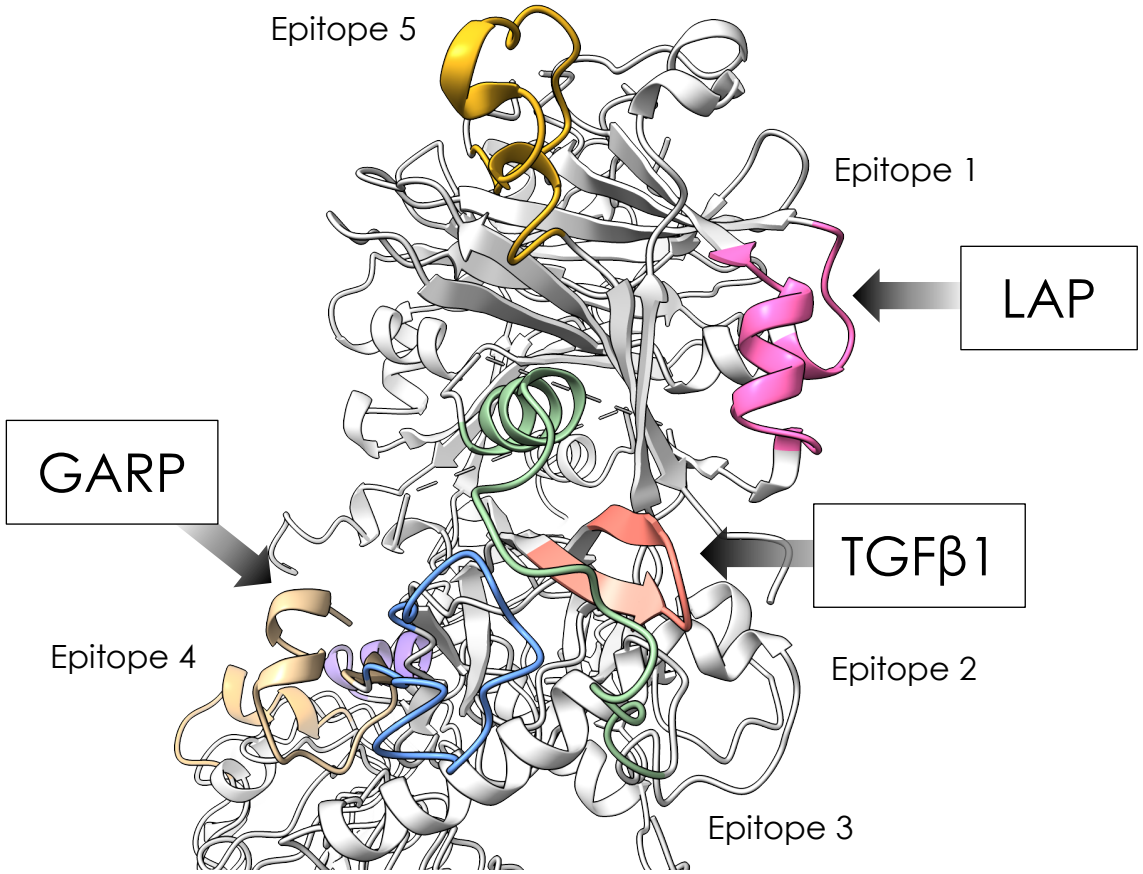
Latent-TGFβ1 is a Potential Oncology Target for Immune Modulation

TGFβ Release is Immunosuppressive



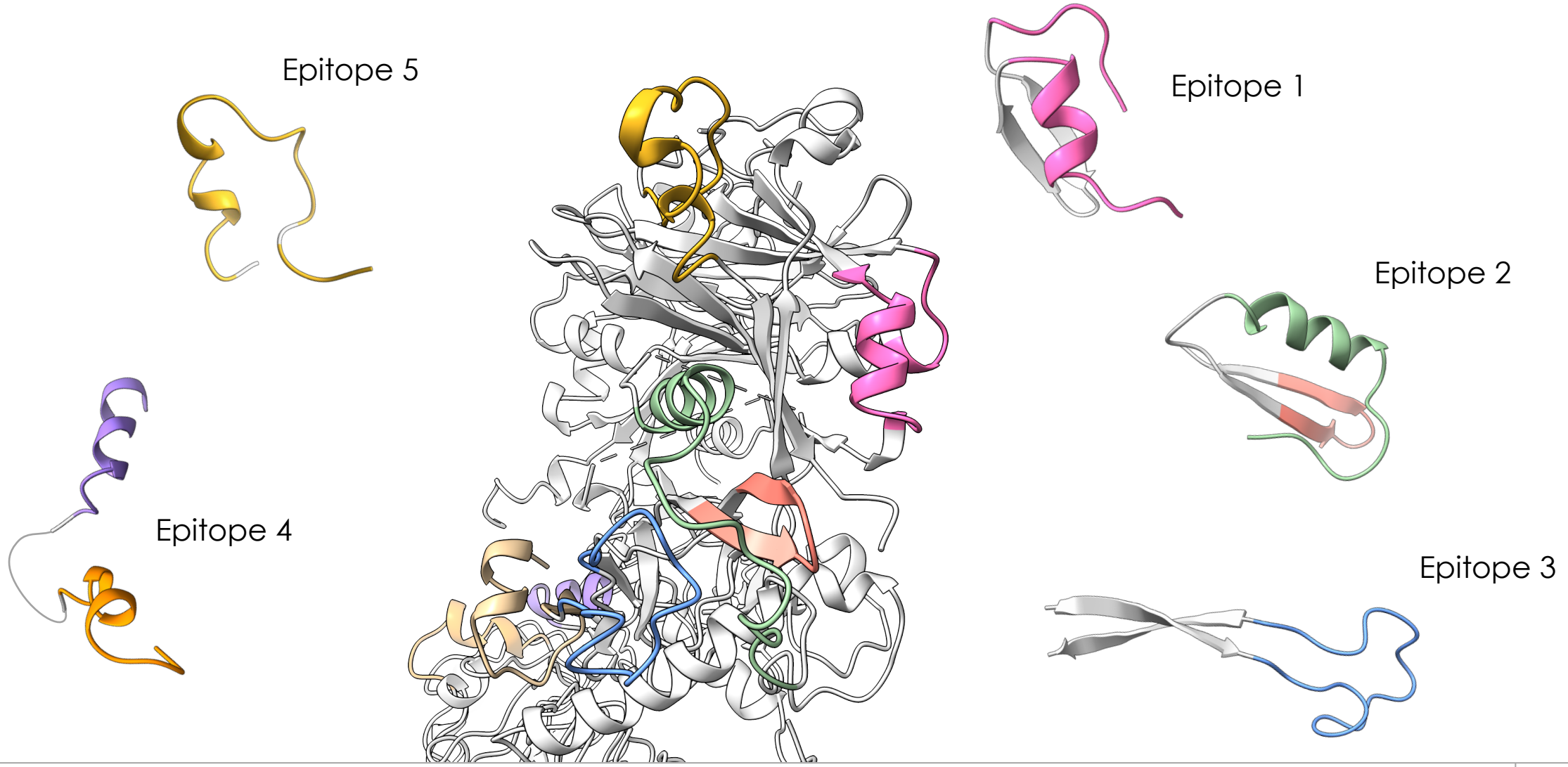
Release mechanisms include protein interations (integrin), protease activity, pH…

Latent-TGFβ1 is a Multimeric Complex



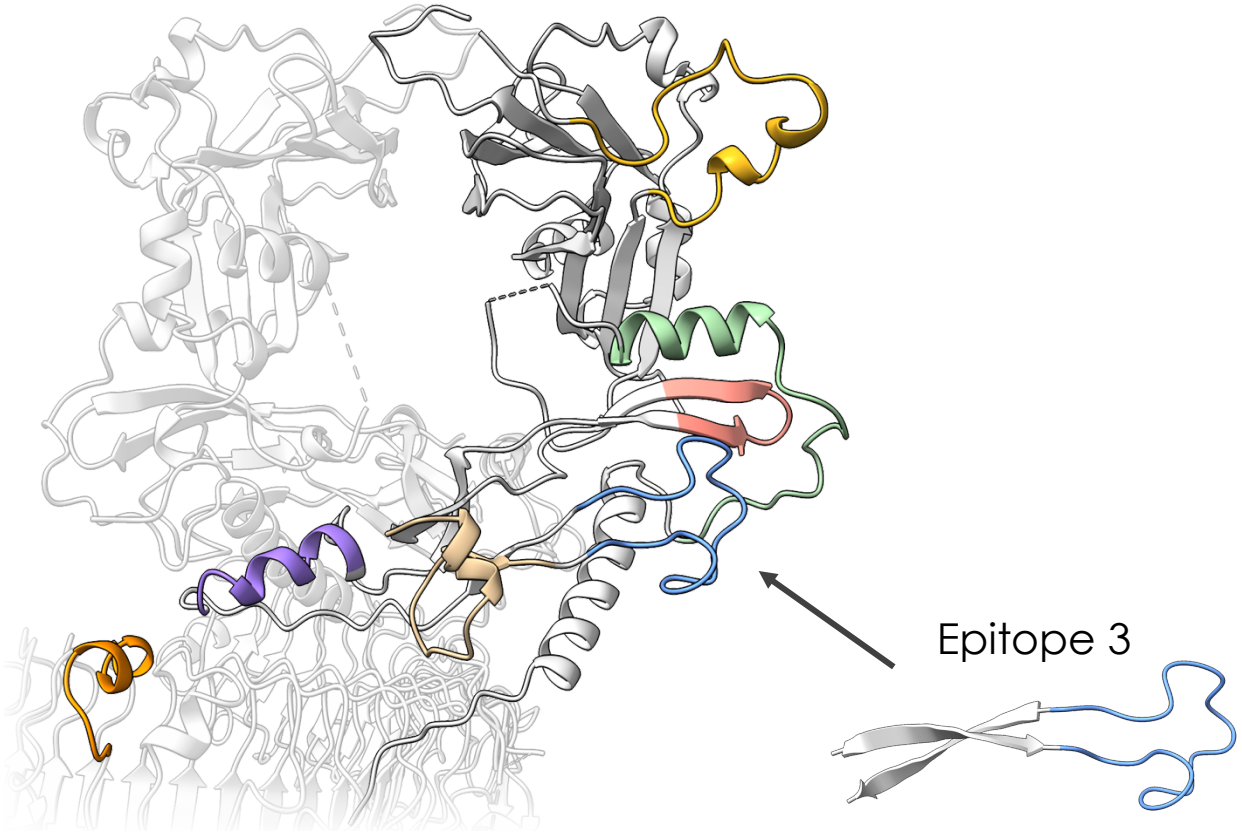
Nixon et al., Nat. Rev. Immunol. 2022

Engineered Epitopes are Designed for Sites Across TGFβ1, LAP, and GARP

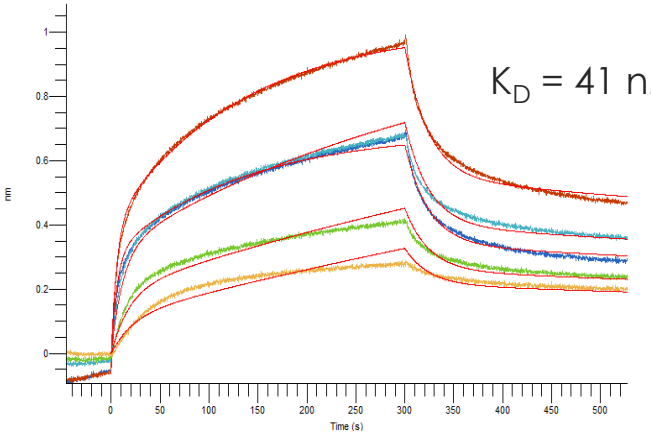


Engineered Epitope 3 Binds to Benchmark Antibody SRK-181

Latent-TGFβ1

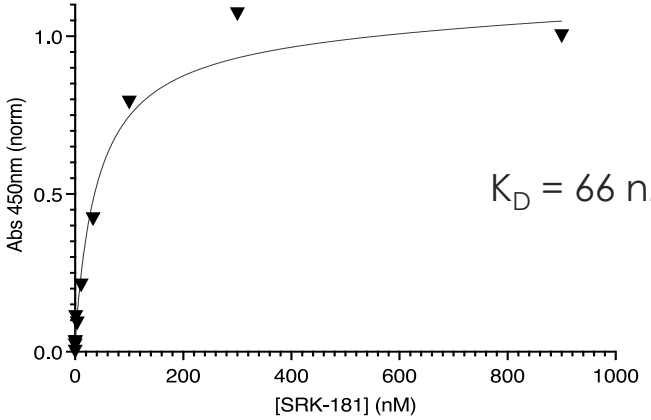


Octet Binding:



K_D = 41 nM

ELISA Binding:



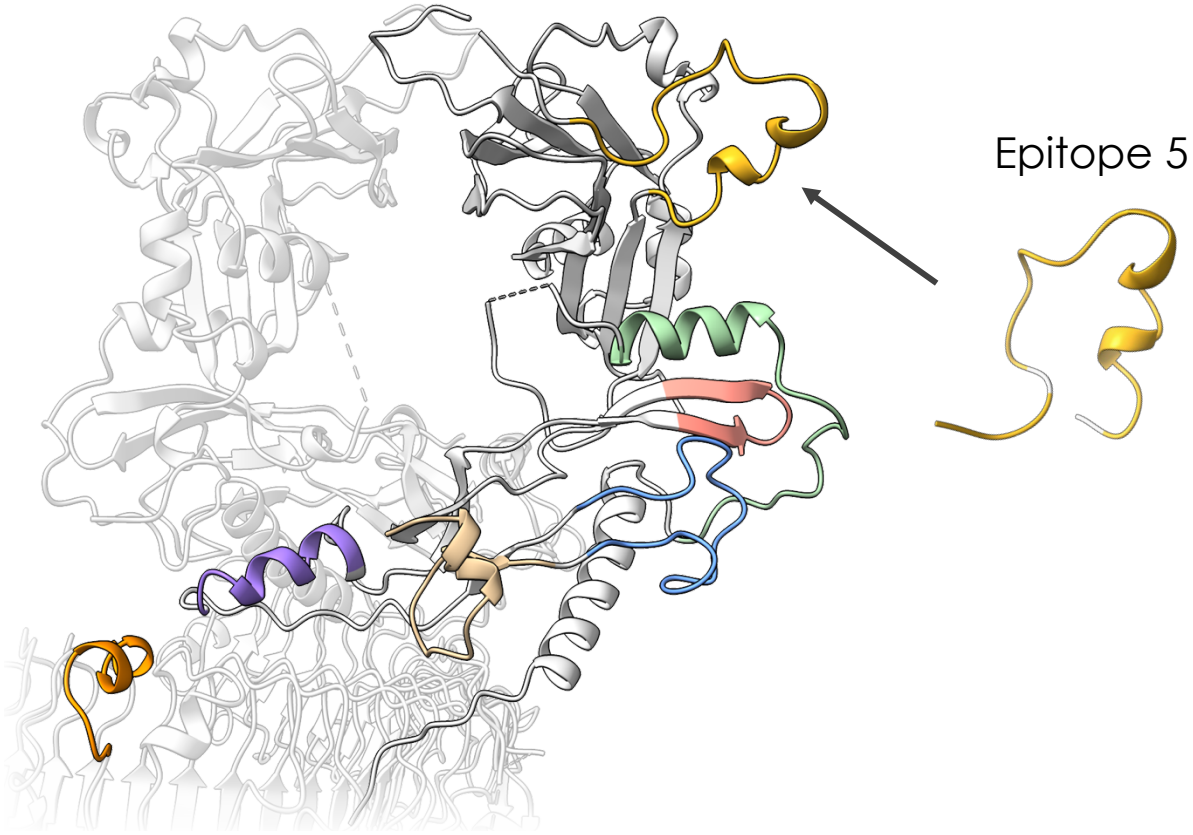
K_D = 66 nM

Consistent with HD exchange data[1]

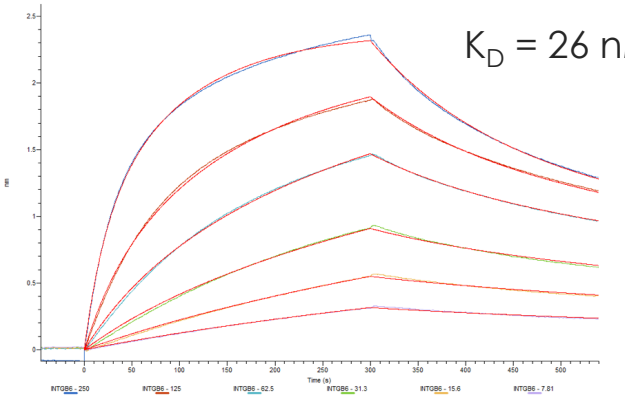
(1) Martin et al., Sci. Transl. Med. 2020

Engineered Epitope 5 Binds Integrin avβ6

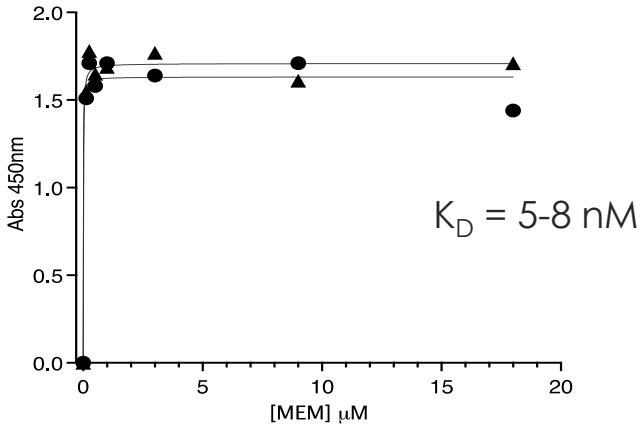
Latent-TGFβ1



Octet Binding:

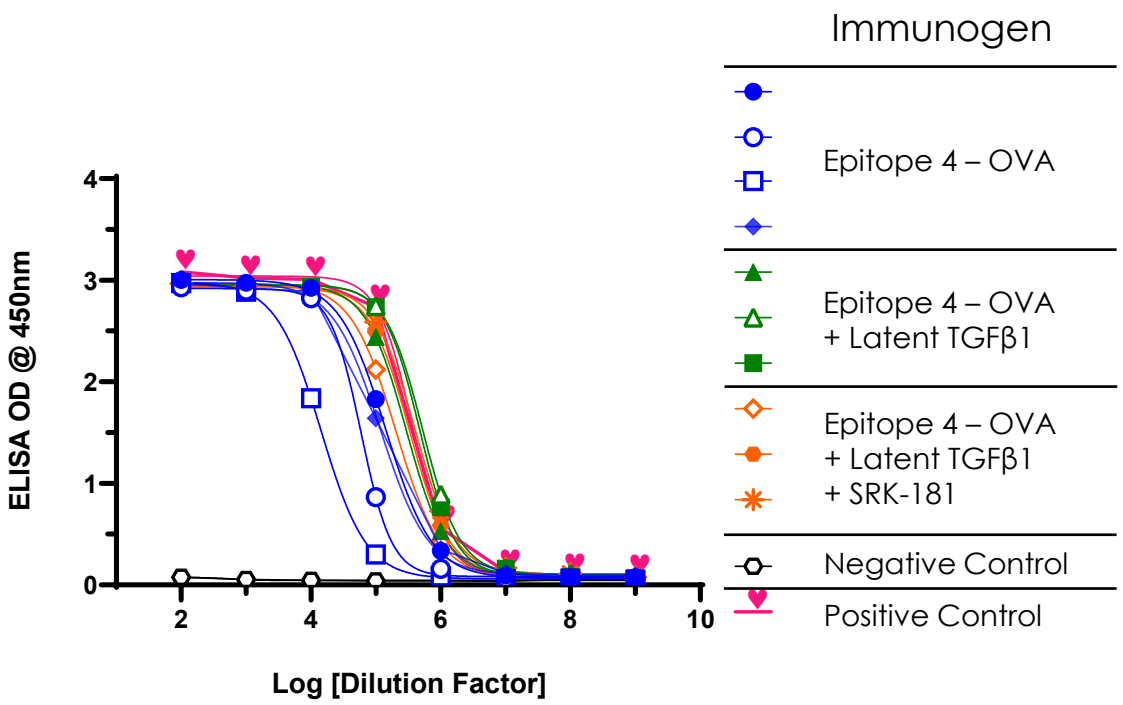


ELISA Binding:

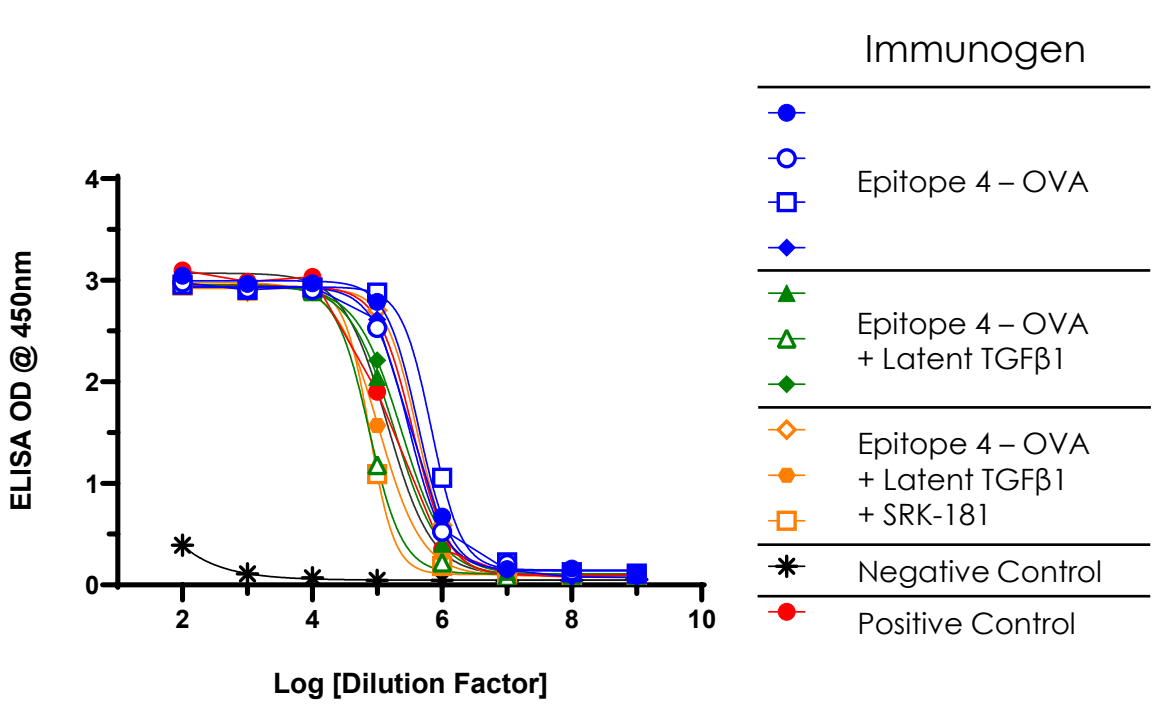


Mice Immunized with Engineered Epitopes are Serum Positive for Latent TGFβ1

Epitope 4 Mouse Serum Titers



Epitope 5 Mouse Serum Titers



Serum is latent TGFβ1 positive even for mice immunized with the engineered epitope only

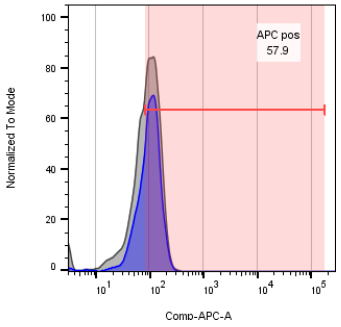
Hybridoma Supernatant Screening Generates Hundreds of Cell Binders

Cell Line

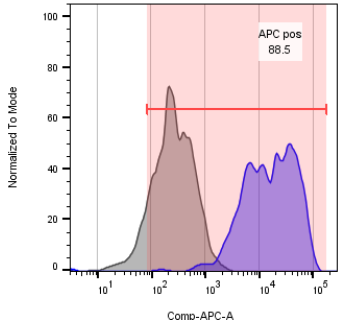
293T (naïve)

293T (huLTGFb1+)

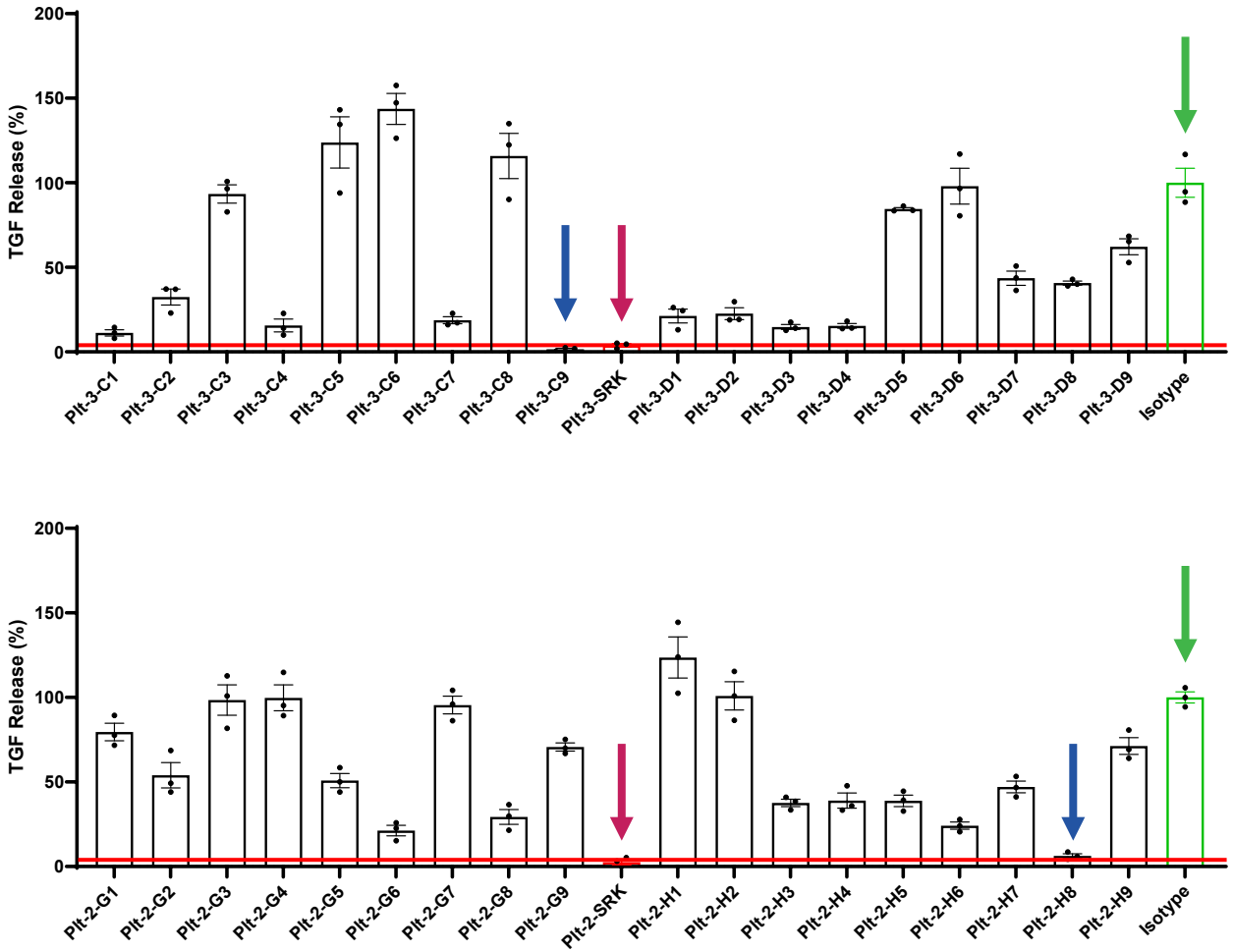
No stain



SRK-181 (1µg/mL)



TGFβ Release Assay Identifies Potential Hits from Hybridoma Supernatants

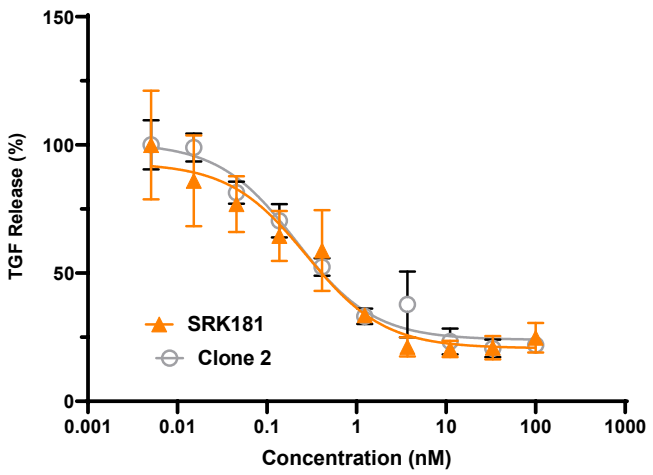


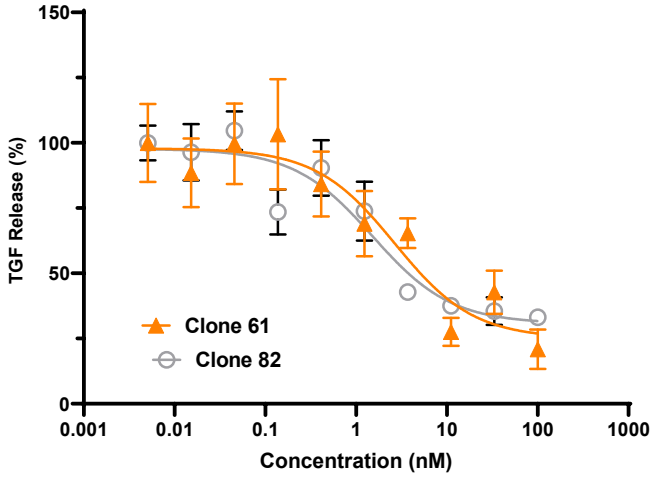
Hit Comparable to SRK-181

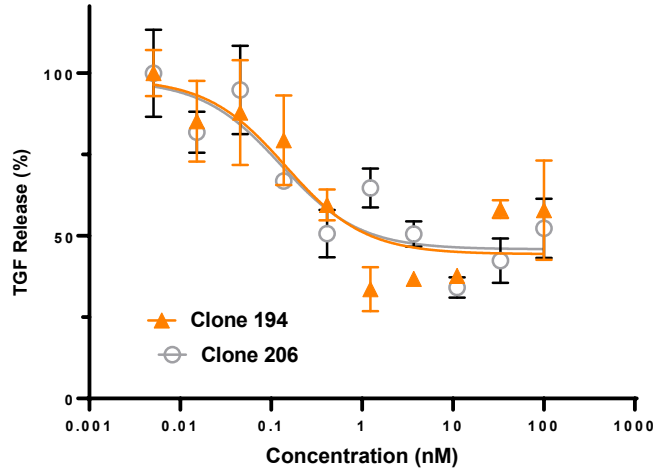
SRK-181 Positive Control

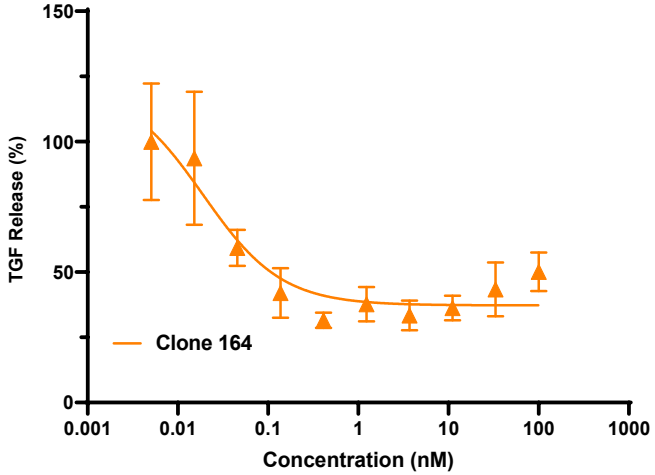
Negative Control

Purified Antibody Hits Show Inhibition of TGFβ Release



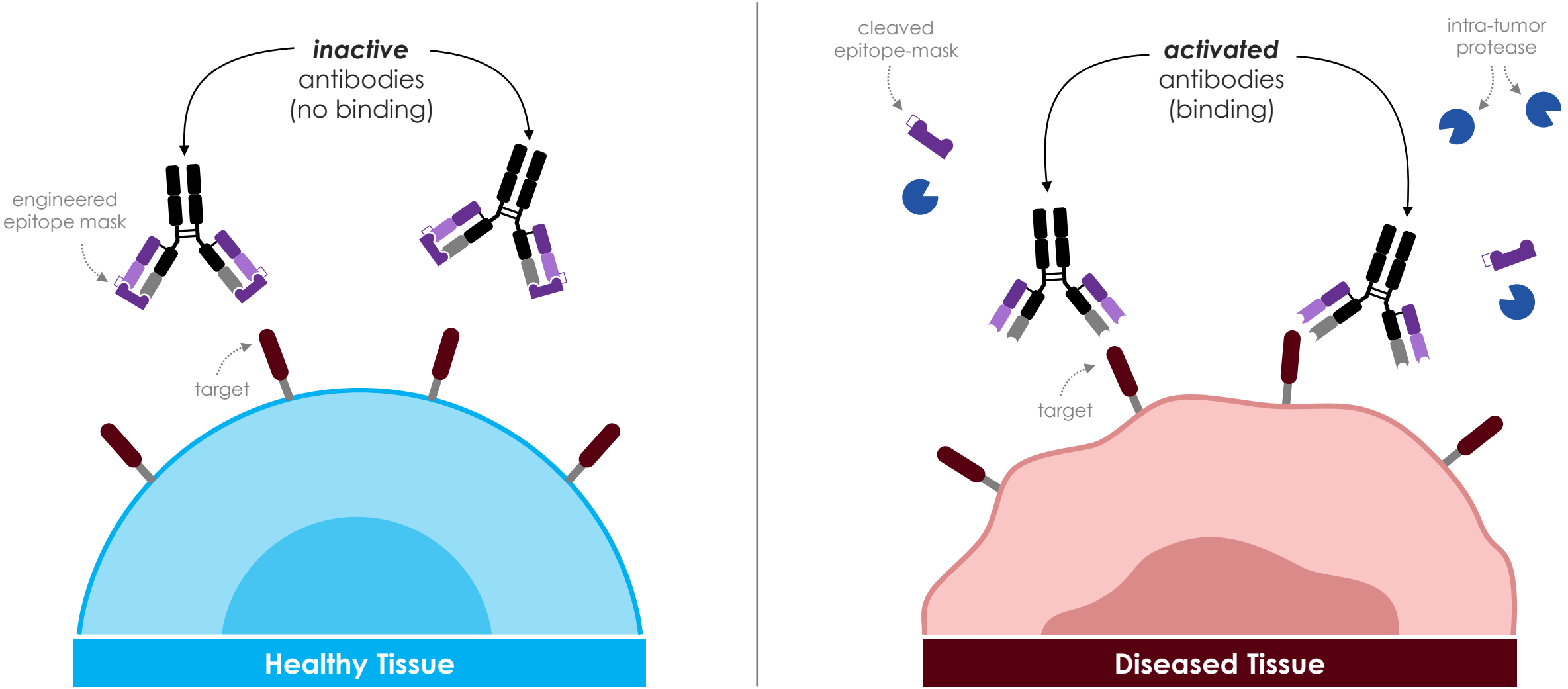






Case Study #3
Target: MUC16
MOA: Tumor-Associated Antigen

Can Engineered Epitopes be Used for Conditionally-Activated Antibodies?

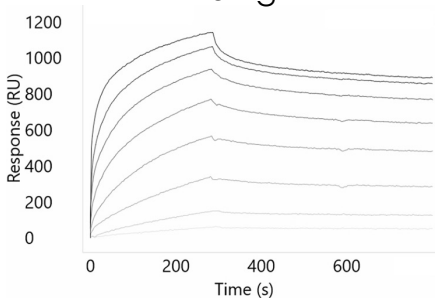


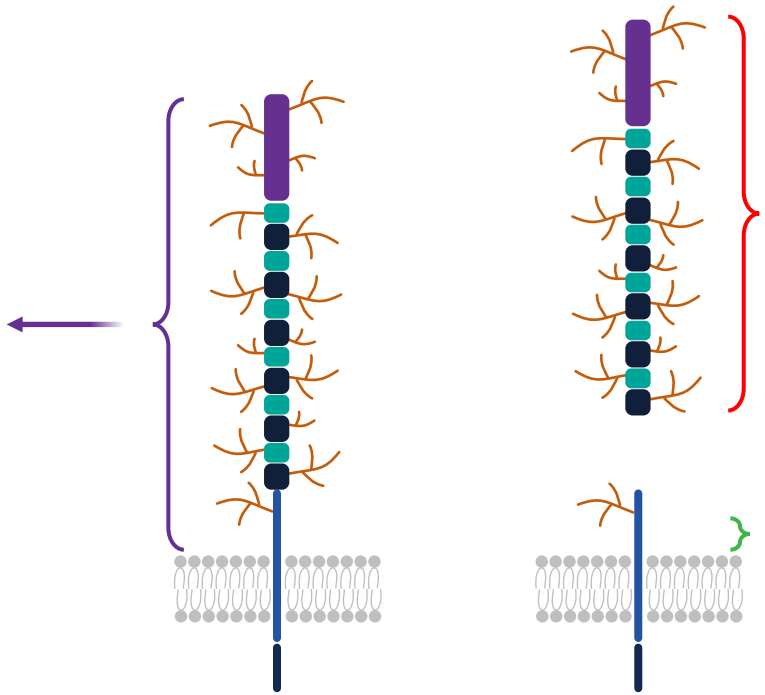
Engineered Epitopes Steer Immunizations to the MUC16 Non-shed Domain

MUC16 can get Proteolyzed

Extracellular 288-mer

Binding

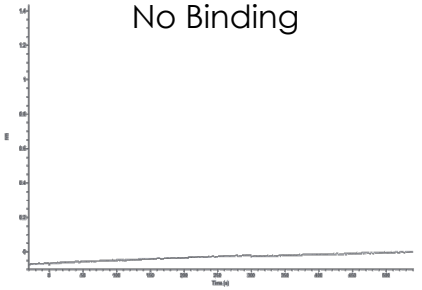




Glycosylation Site

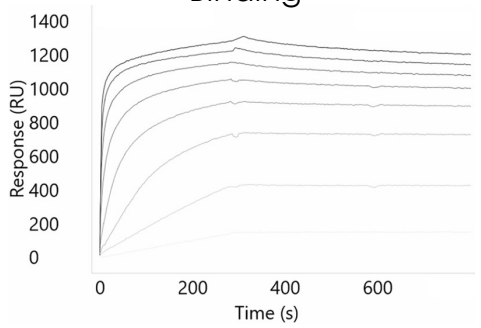
Shed 230-mer

No Binding

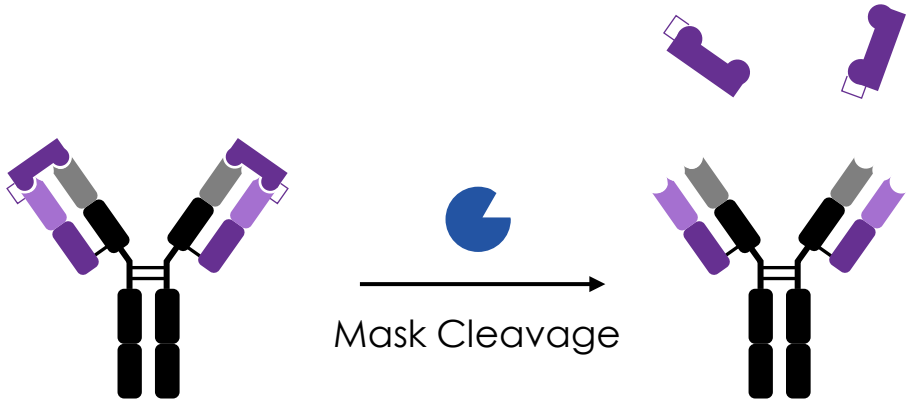


Non-shed Non-glycosylated 29-mer

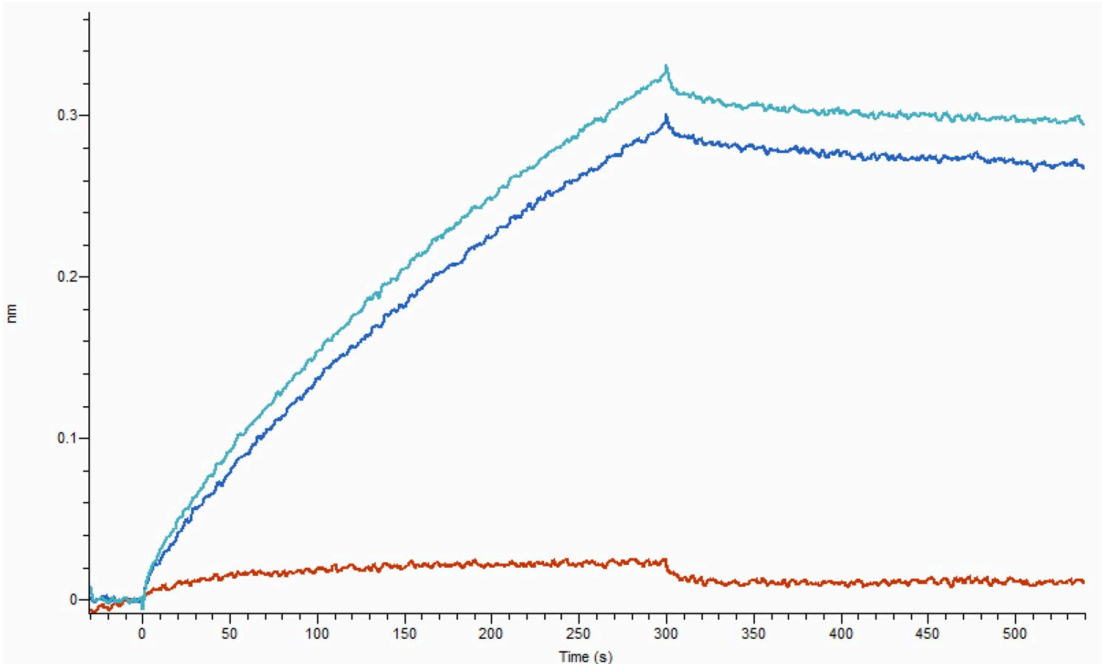
Binding



Engineered Epitope Mask Conditionally Activates Anti-MUC16 Antibody



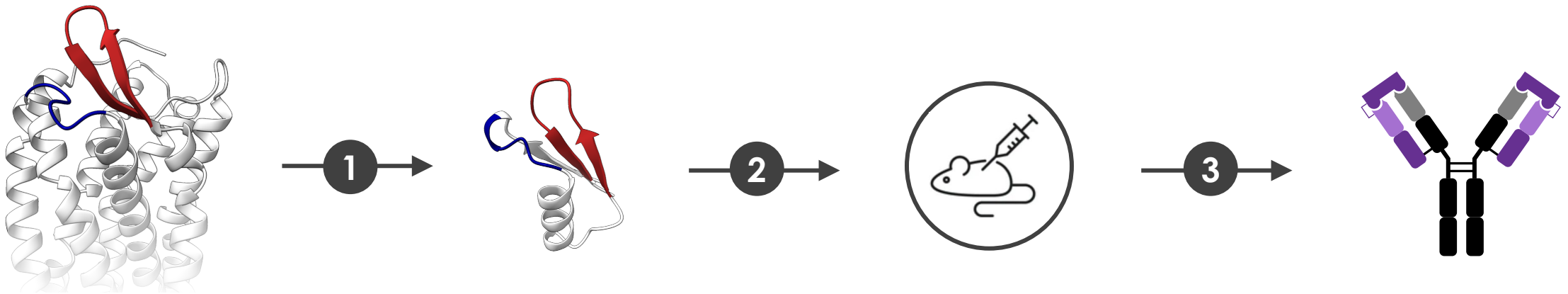
Octet Binding to MUC16 Non-Shed Domain



Summary

iBio Engineered Epitope Platform

1. Engineered epitopes are designed to match the target sequence and structure
2. Epitope-specific antibodies are discovered from immunizations
3. Engineered epitopes are used as masks for improved therapeutic safety



Thanks to the iBio Scientific Team!

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