End-to-End Discovery of Antibodies with Dual Epitope and Tissue Specificity

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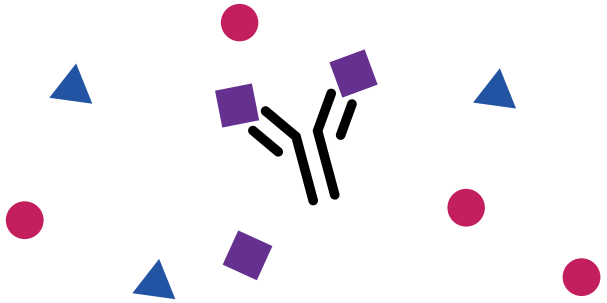
Director of Machine Learning, iBio

What Makes a Good Antibody Therapeutic?

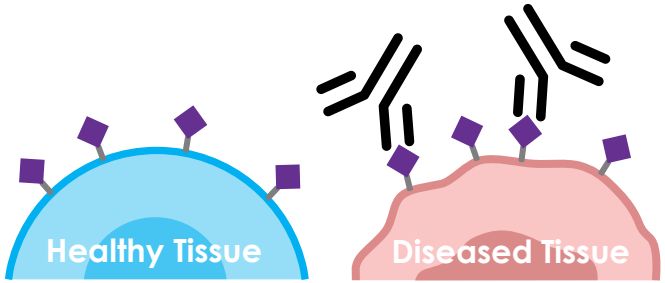


Specificity

- High affinity
- No off-target binding
- Optimal binding site (epitope)

Developability

- High stability
- High expression
- No aggregation

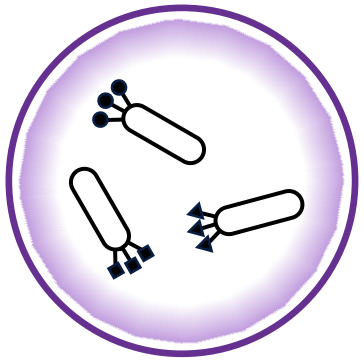


Safety

- Low/no healthy tissue toxicity
- Low immunogenicity

Traditional Approaches Generate Many Hits, But Very Few Translate into Viable Antibody Therapeutics

Animal Immunizations

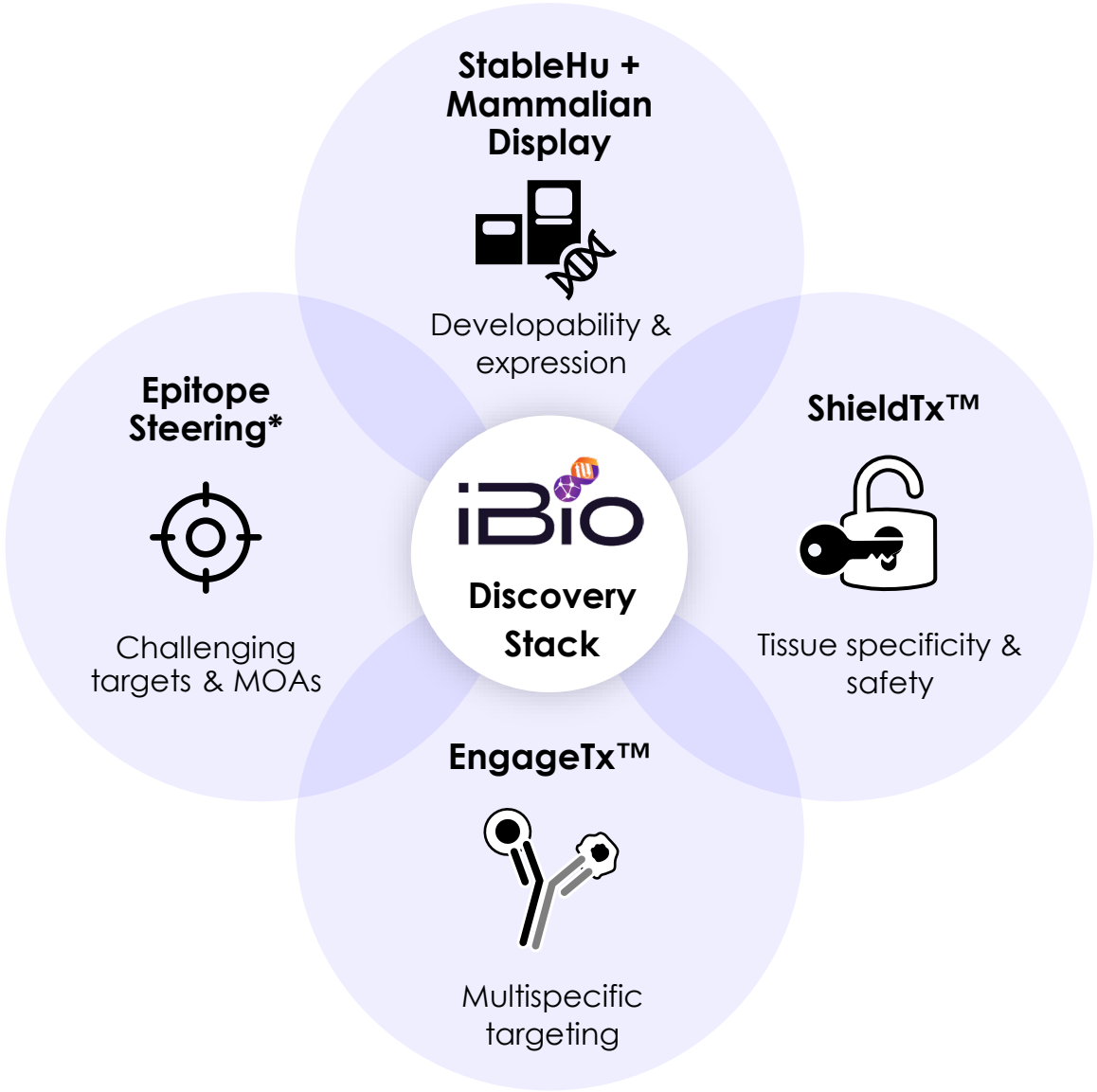


Phage Display

*Time-consuming and expensive screening efforts are required to discover antibodies with the desired **specificity**, **developability**, and **safety** profile of a drug molecule*

Our antibody discovery stack advantage:

Generating epitope-specific, developable antibodies for challenging targets and modes of action



* U.S. Patent No. 11,545,238 (issued January 3, 2023)

iBio Pipeline Improves Efficiency of Therapeutic Antibody Discovery

Engineered Epitope Discovery Engine*

StableHu Antibody Optimizer

ShieldTx Antibody Masking

Efficient discovery of on-epitope, on-tissue antibody drugs

1

Specificity

Filter out antibodies that bind off-epitope with generative AI engine

2

Developability

Optimize stability and expression with AI library design and mammalian display

3

Safety

Reduce off-tissue toxicity by masking antibodies with engineered epitopes

*U.S. Patent No. 11,545,238 (issued January 3, 2023)

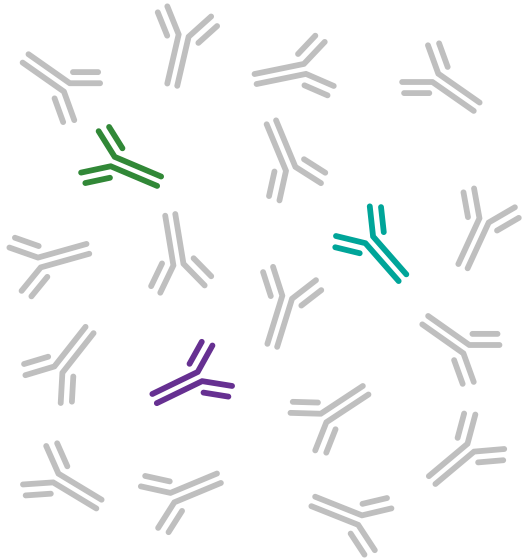
Epitope-Selective Antibody Discovery

Engineered Epitopes

Engineered Epitopes Focus Antibody Repertoires On Desired Binding Sites

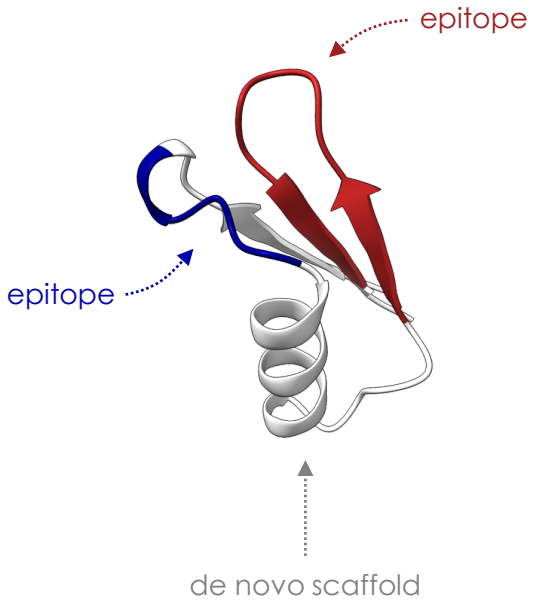
1

Naïve in vivo or in vitro antibody library



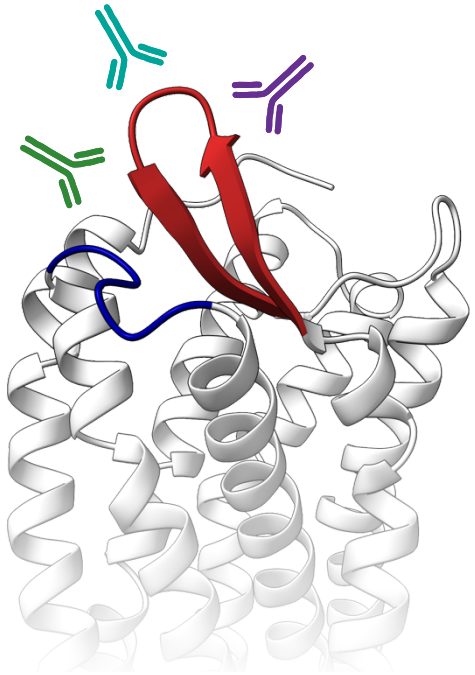
2

Focus library with engineered epitopes



3

Efficient discovery of epitope-specific antibodies

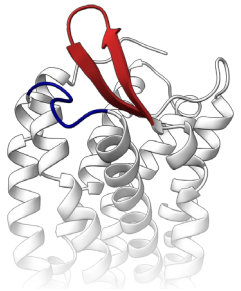


full length target

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

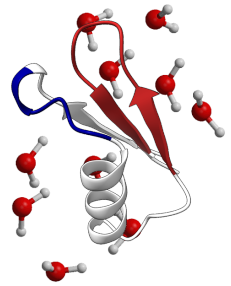
Engineered Epitope Design Objectives

1. **Match Structure to Target**



2. **Refine for Greater Stability**

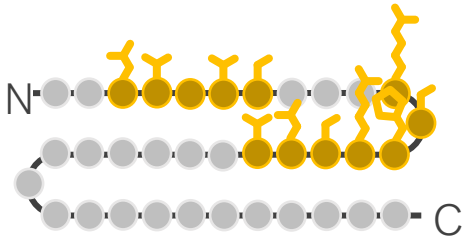
3. **Optimize for Water Solubility**

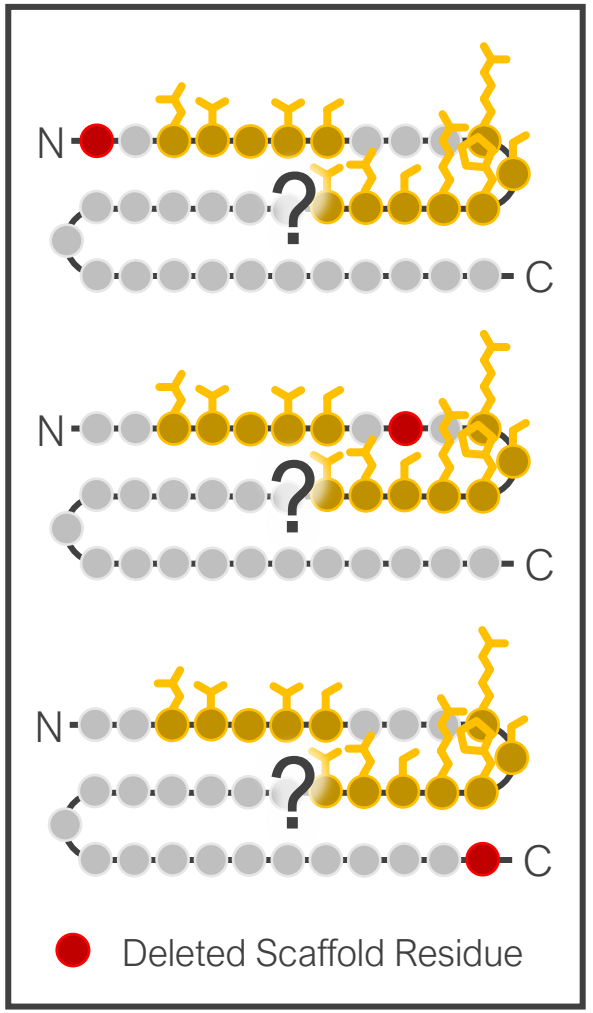


AI Discovery Engine

Engineered Epitopes are Further Optimized to Minimize Designed Scaffold

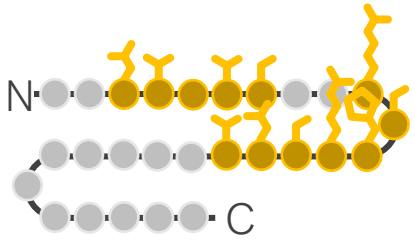
Starting Topology = #Epitope residues / #Scaffold residues





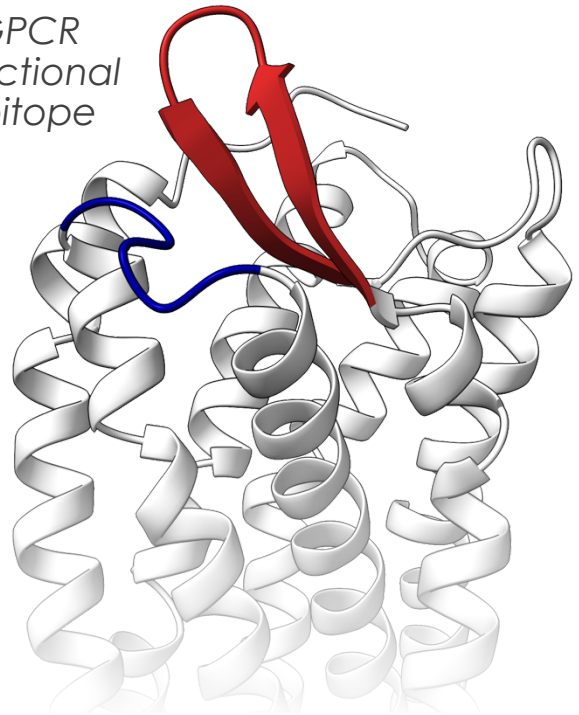
Iteratively trim scaffold residues until epitope destabilizes

Optimized Topology = #Epitope residues / (#Scaffold - #Deleted)

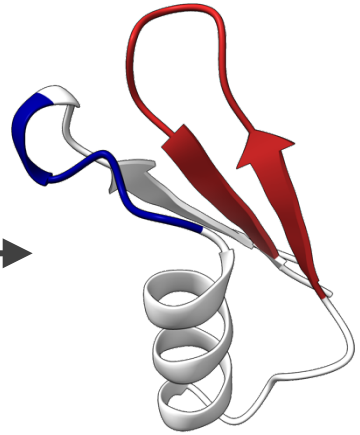


Engineered Epitopes are Cross Validated In Silico and In Vitro

GPCR Junctional Epitope

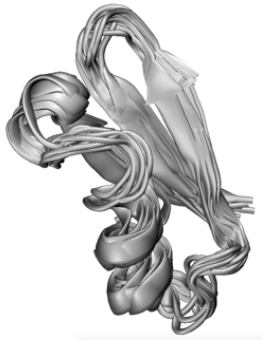


AI Discovery Engine



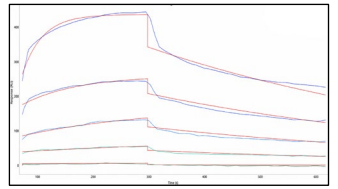
Folding Simulations

e.g. AlphaFold2



Antibody Binding

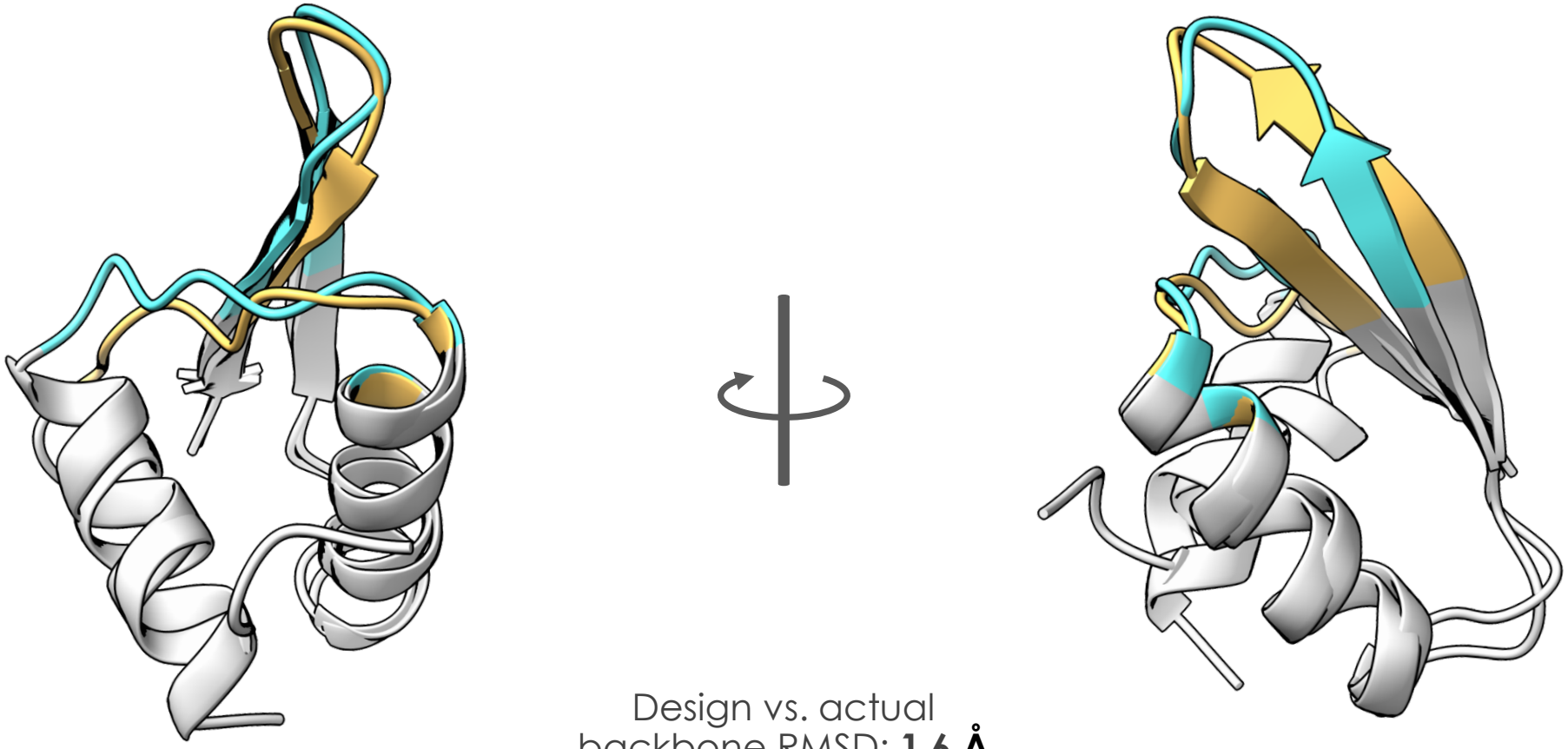
e.g. SPR



NMR, Melting Temperature

NMR Structure Validates Engineered Epitope Design Engine

Designed Epitope Structure
NMR Solution Structure

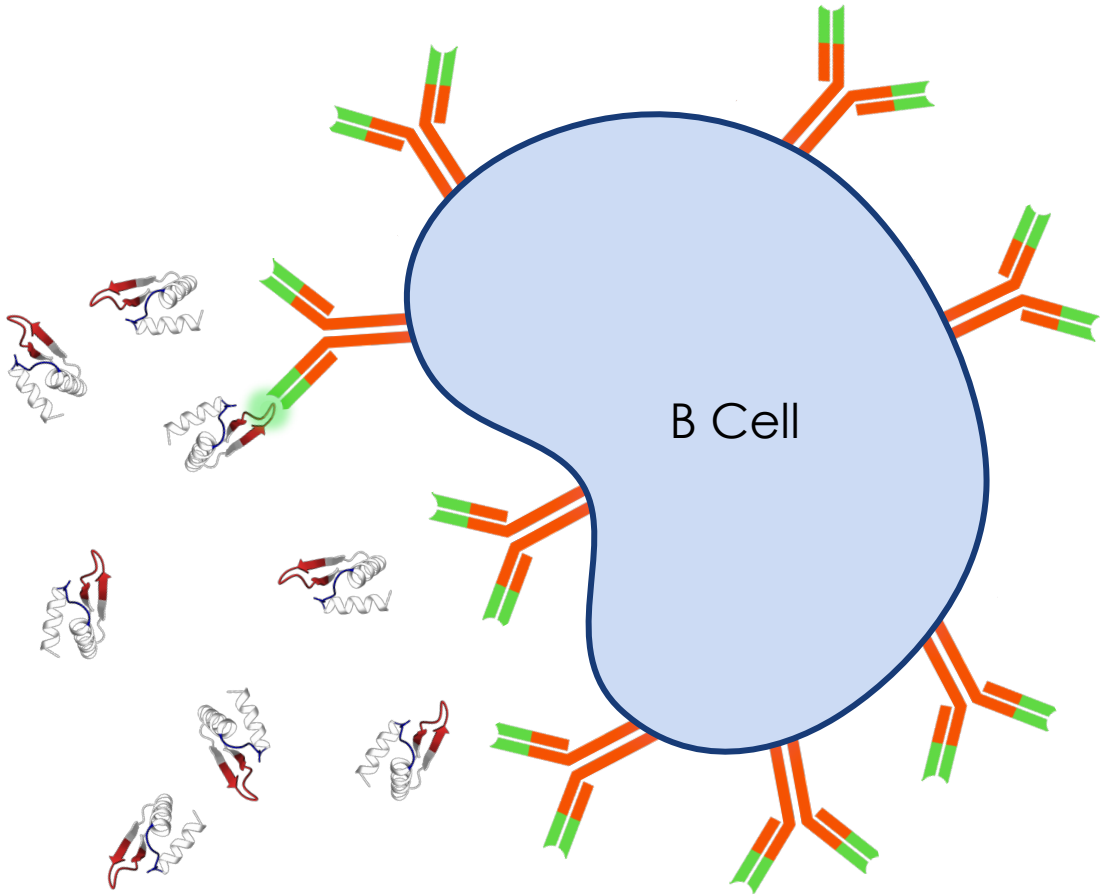


Design vs. actual
backbone RMSD: **1.6 Å**

Multivalent Display of Engineered Epitopes Enhances Immune Response

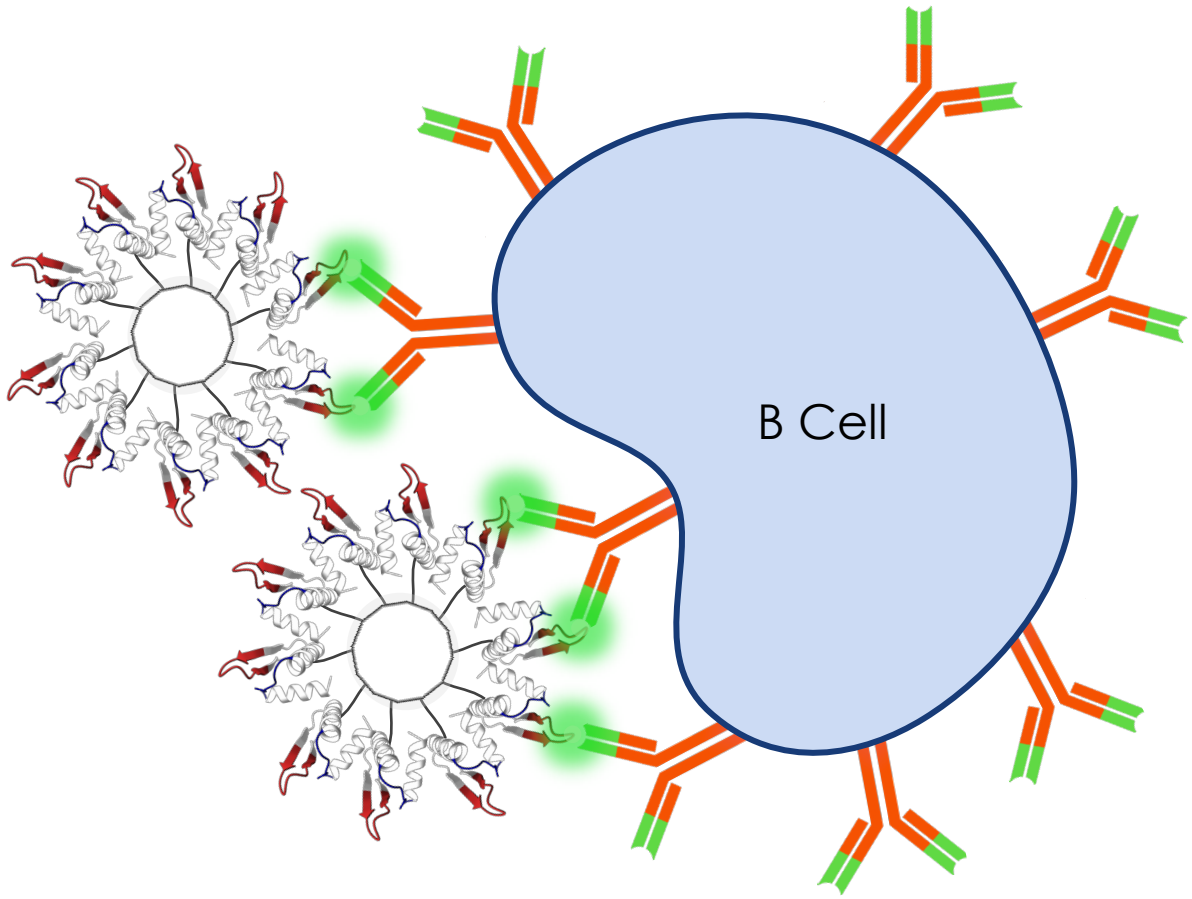
Engineered Epitope Immunization

Weak B cell activation



Nanoparticle Immunization

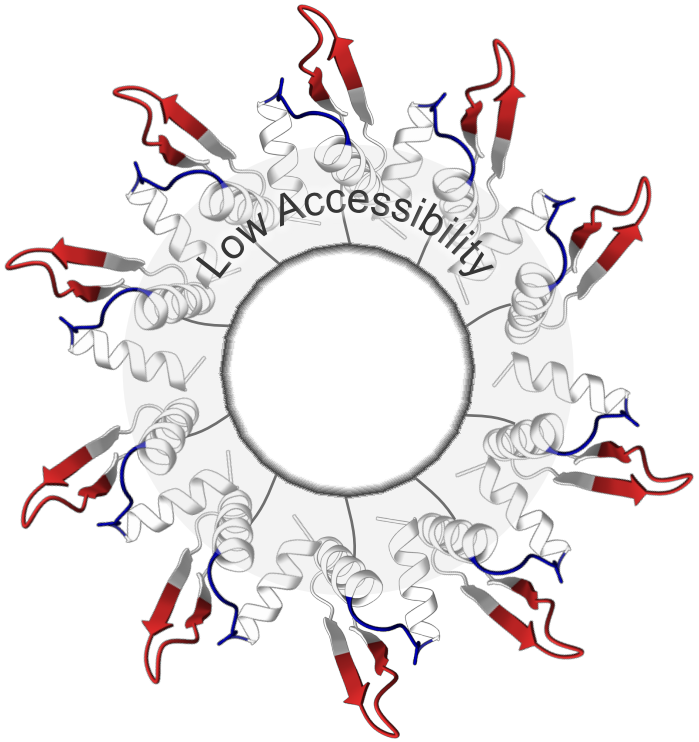
Strong B cell activation



Nanoparticles are Optimized for Epitope Orientation and High Valency

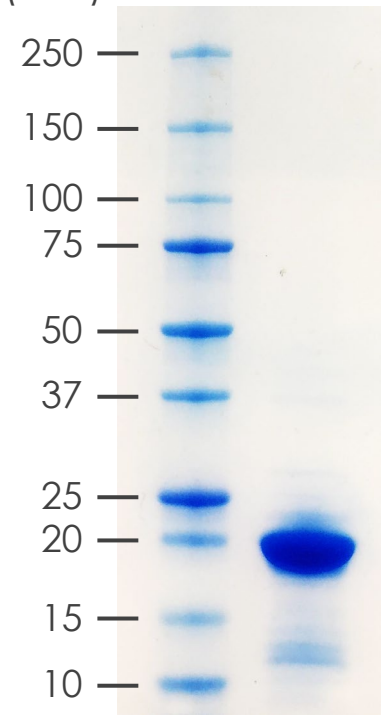
Orientation

- Epitope Residues: Outward
- Scaffold Residues: Inward

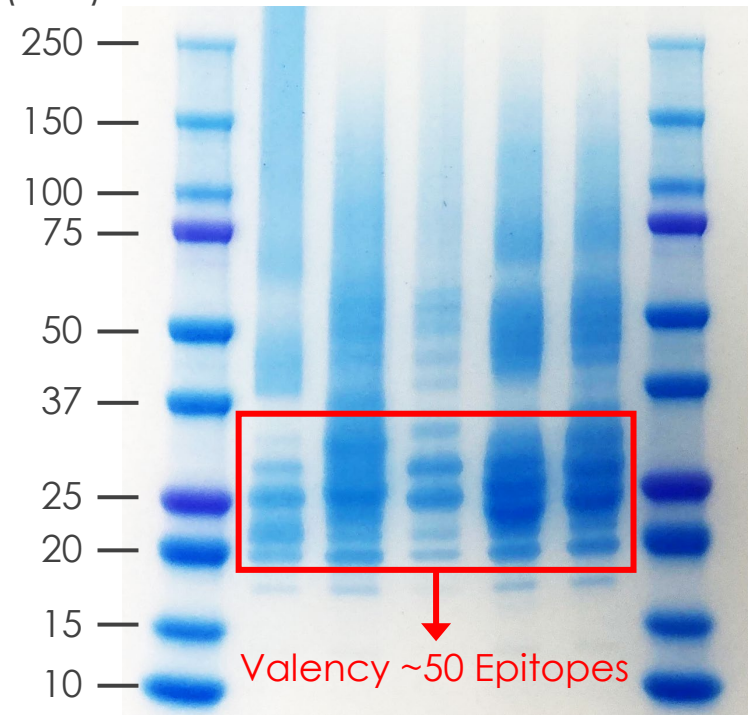


Valency

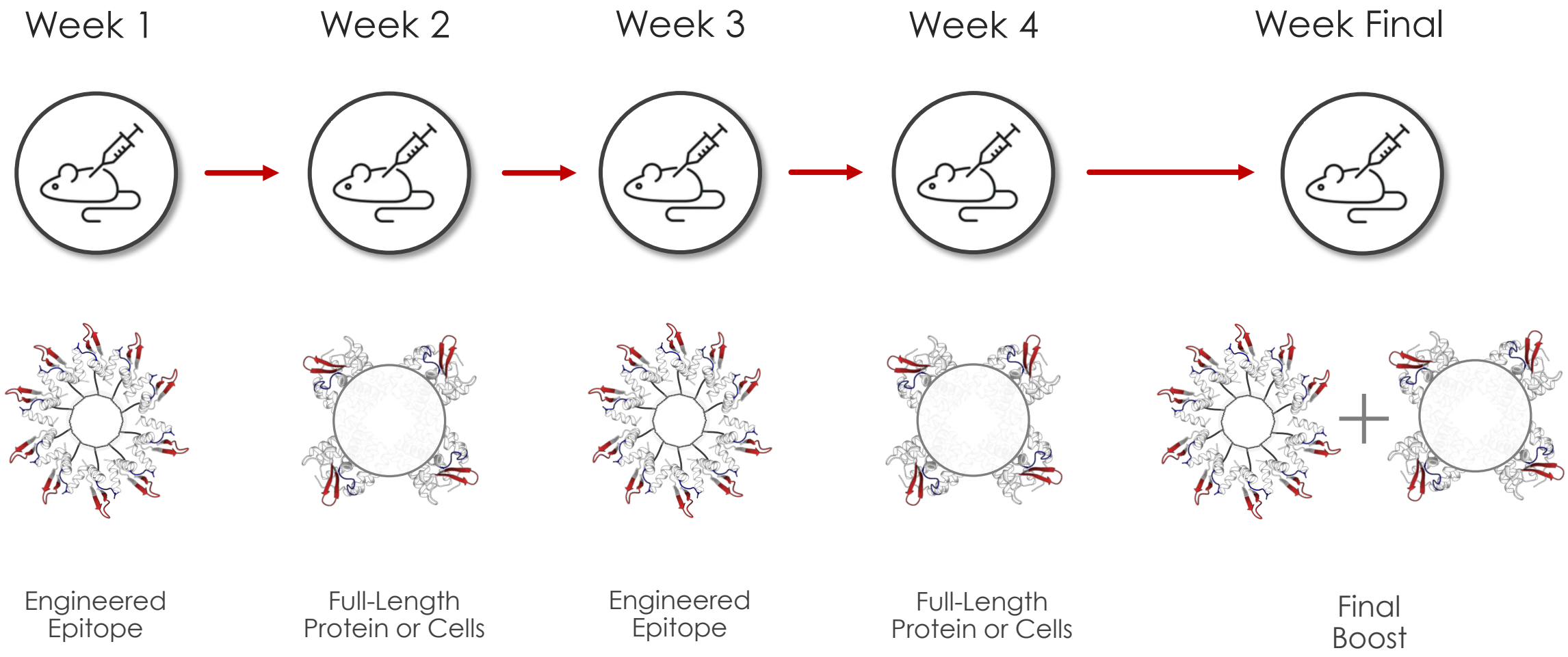
Nanoparticle



Nanoparticle + Engineered Epitopes



Immunizations Alternate Between Nanoparticle and Full-Length Injections



Developable Antibody Libraries

StableHu AI

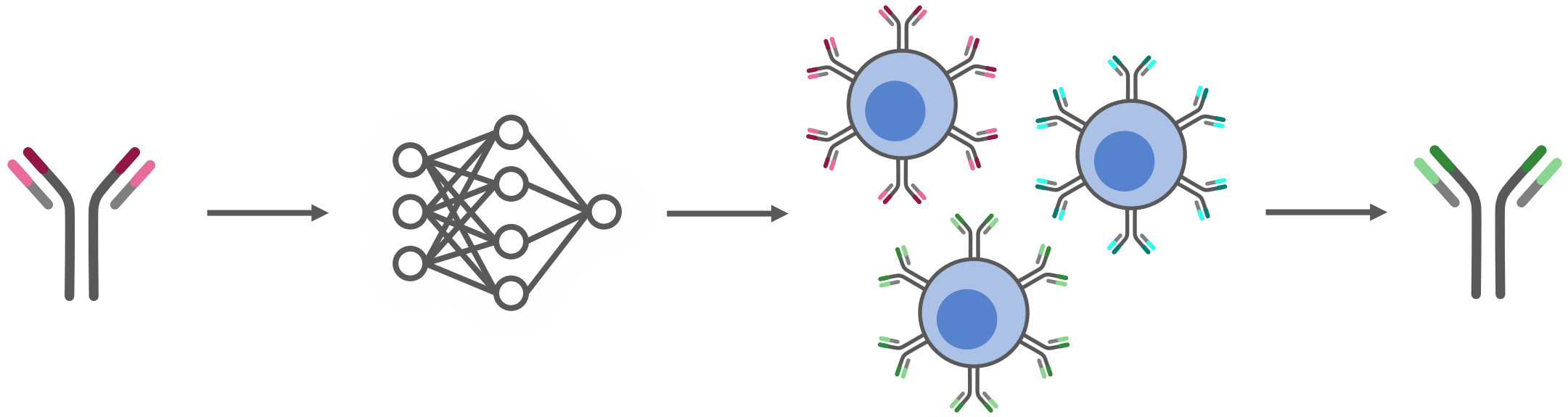
StableHu AI + Mammalian Display Optimize Antibody Developability

Input Antibody

StableHu Optimizer AI-Engine

Mammalian Display

Output Antibody



Template CDRs (red)

Predict library of human CDR variants

Single-cell screen CDR library

Optimized human CDRs (green)

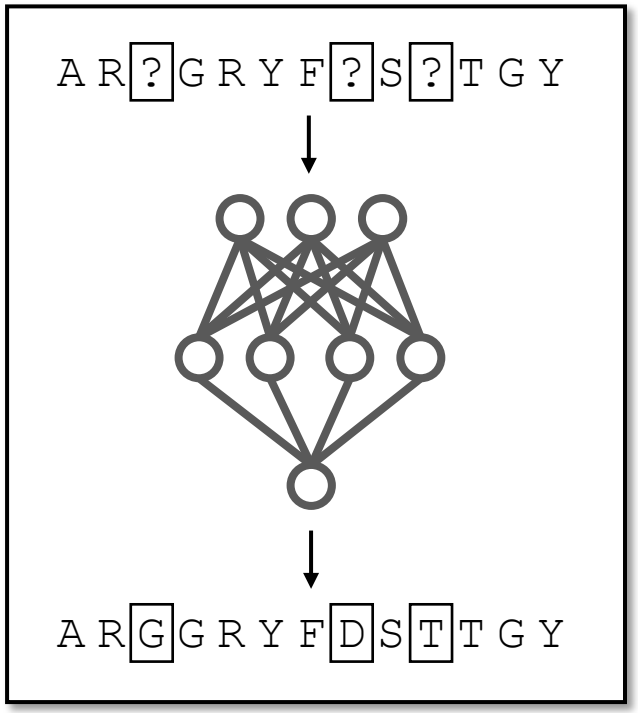
AI Model is Trained to Predict Fully Human CDR Sequences

Antibody Database

cAb-Rep & OAS databases

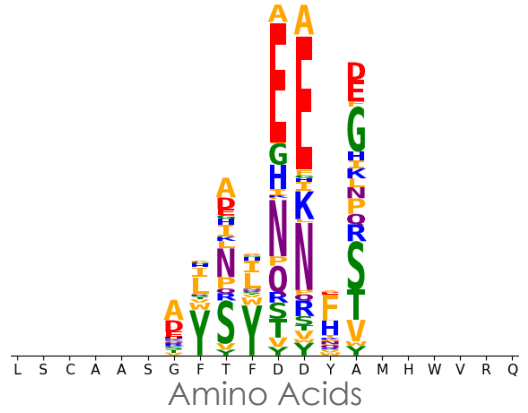
>1 billion curated human antibody sequences

Optimizer AI



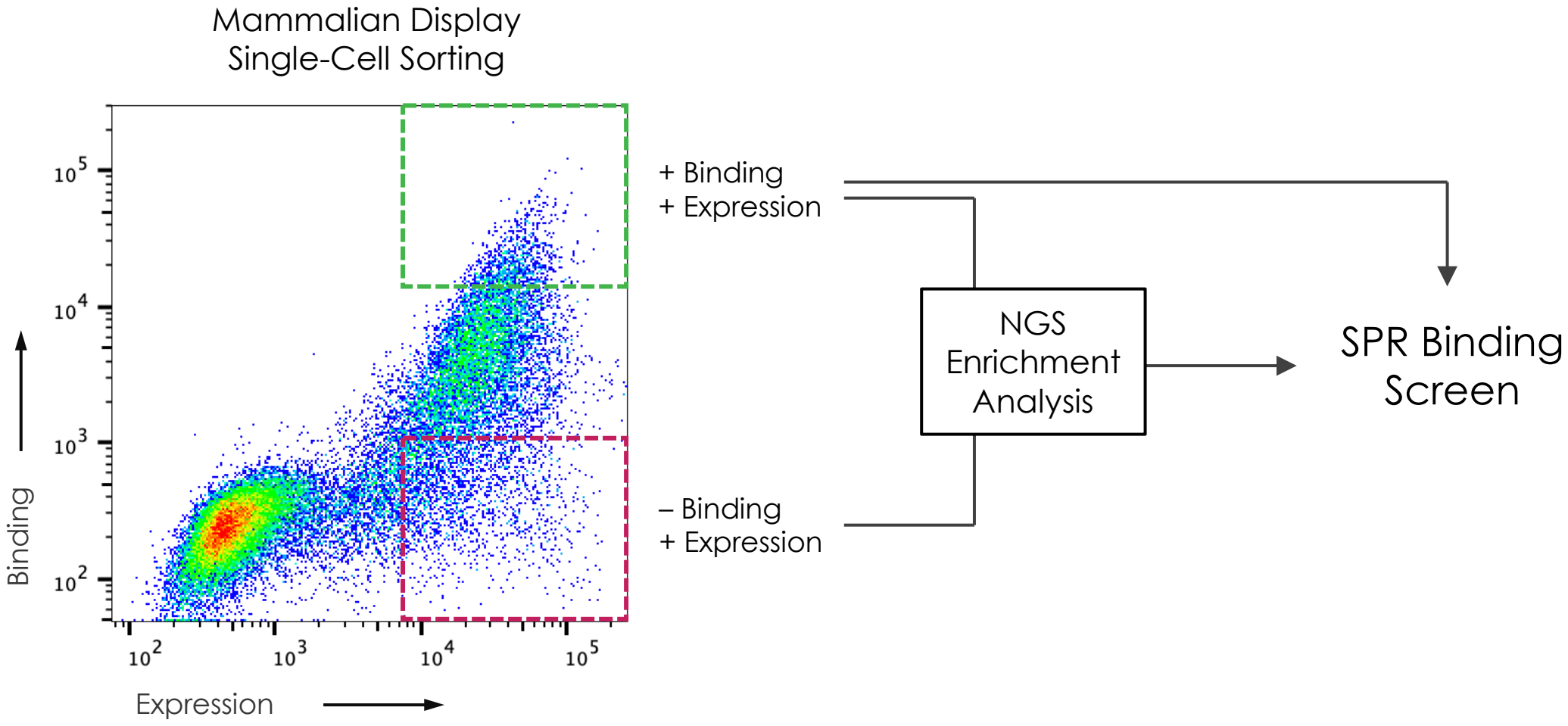
AI trained to predict fully human CDR from masked CDR

Trained Model



Predict library of fully human CDRs from template CDR

StableHu Library Sorting and SPR Screening Identify Improved Human CDR Variants



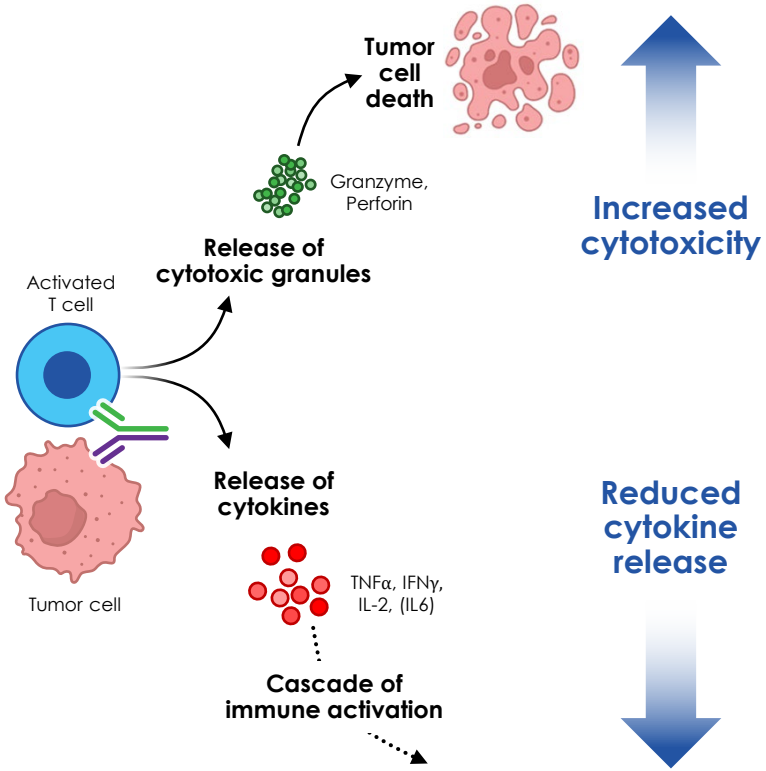
T Cell Engager Antibody Discovery

Anti-CD3 T Cell Agonist

Key Challenges of CD3 T Cell Engager Antibody Discovery

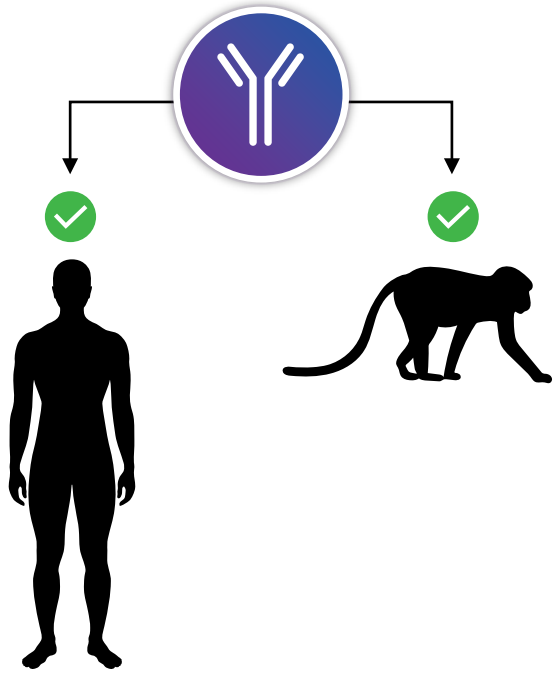
1 Healthy Tissue Toxicity

Optimization of cytokine release for local killing of tumor cells



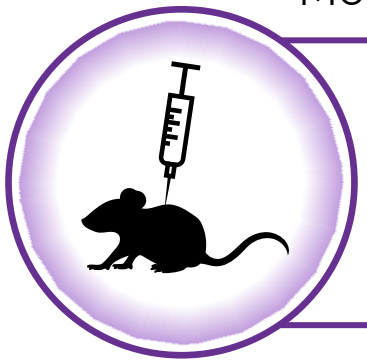
2 Human-Cyno Cross-Reactivity

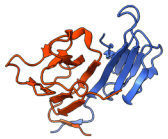
Risk reduction via cyno monkey toxicity studies



Traditional Approaches Failed to Produce T Cell Engagers

Mouse Immunizations

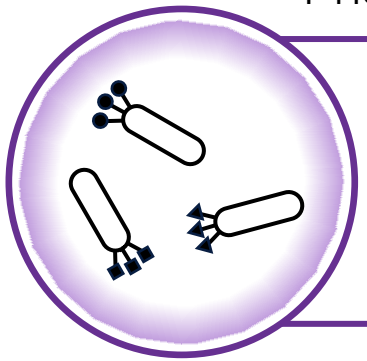




Immunized with CD3εδ

Screened against T Cells

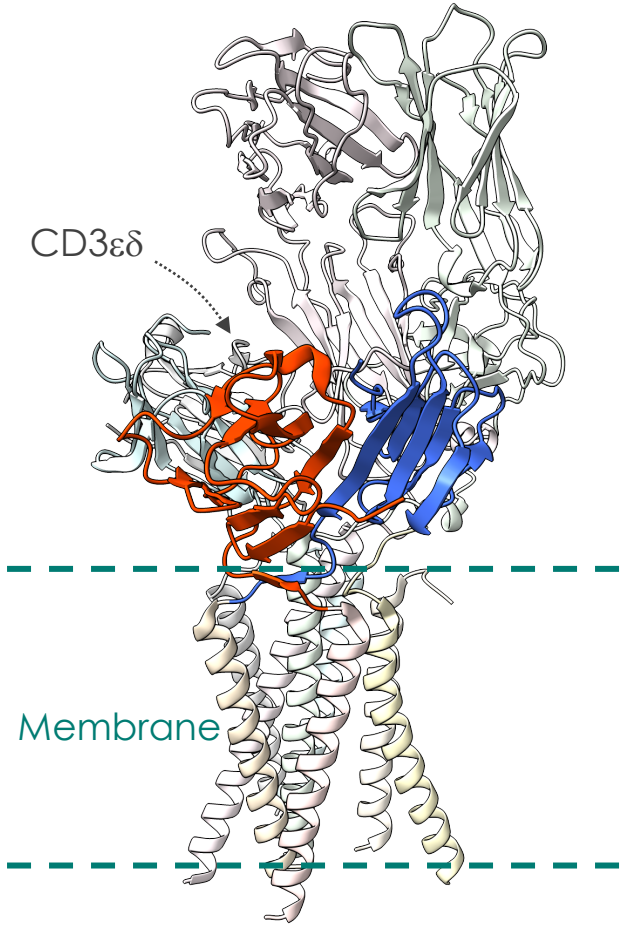
Phage Display



Panned against CD3εδ and T Cells

Many CD3εδ binders, no productive T cell binders

T Cell Receptor (TCR)

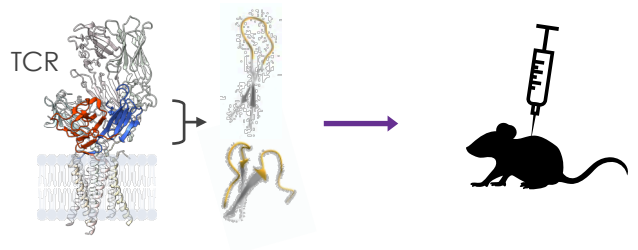


CD3εδ antibodies may bind inaccessible epitopes on T cells

Anti-CD3 Discovery with Epitope-Selective Discovery Engine

Discovery Engine

Engineered Epitope Immunizations



Engineered Epitopes

Epitope & T Cell Immunization

StableHu Optimizer

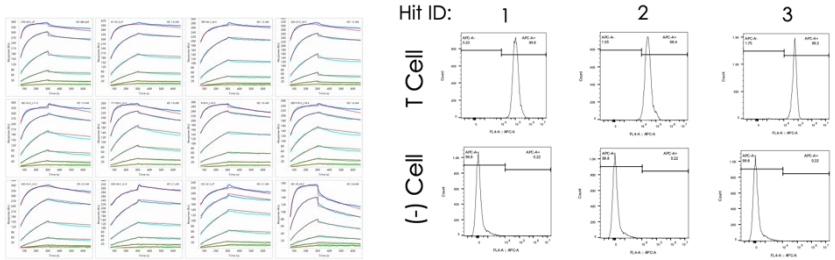
Template Antibody

Optimized Antibodies

Screen

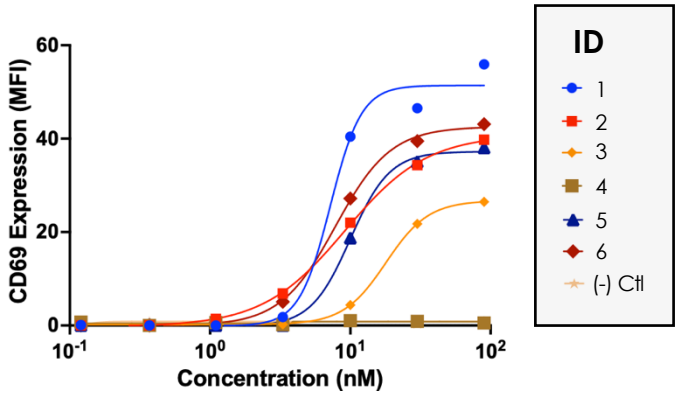
Human-Cyno CD3 & T Cell

Binding

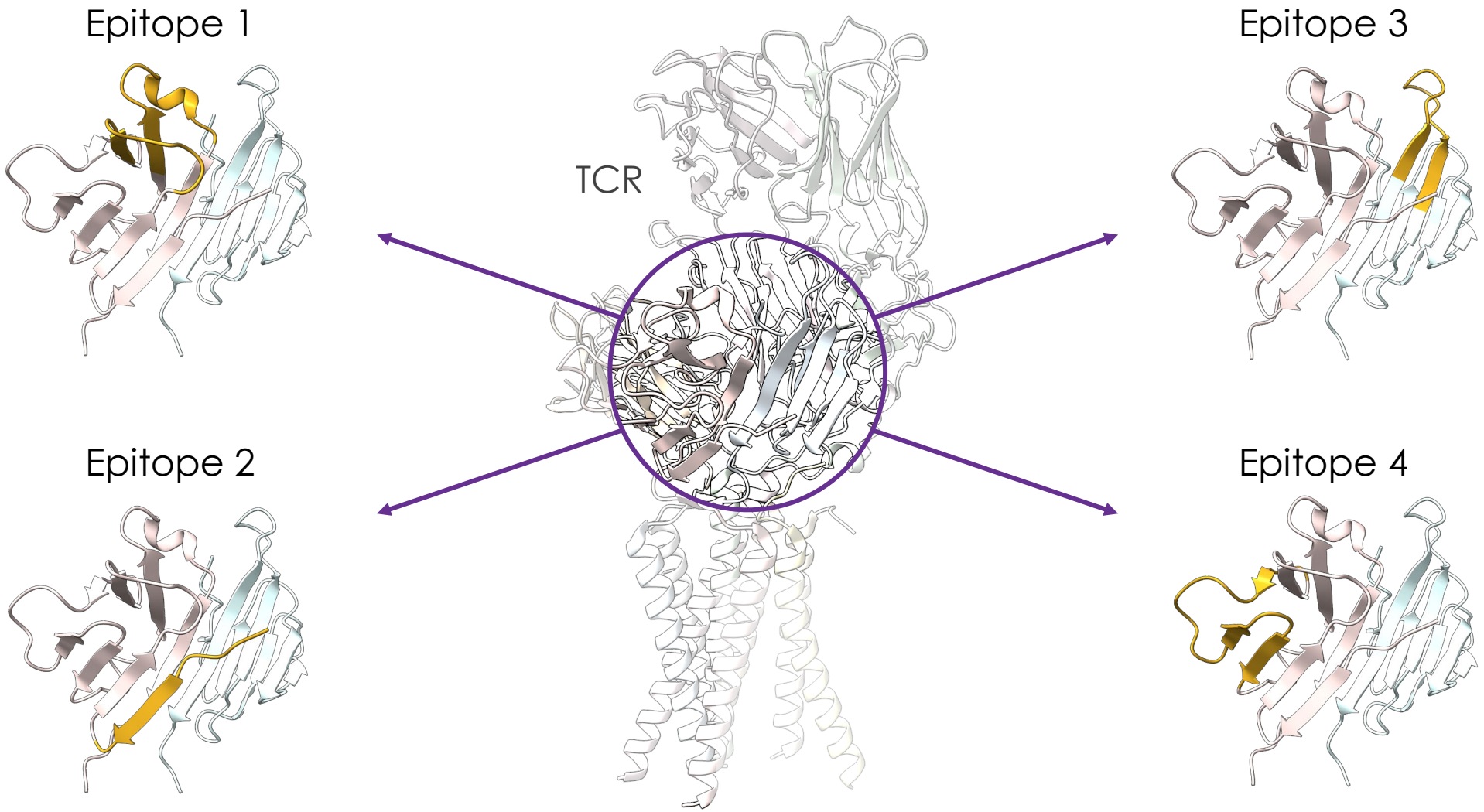


T Cell

Activation



Epitopes Engineered for TCR Accessibility & Human-Cyno Cross-Reactivity

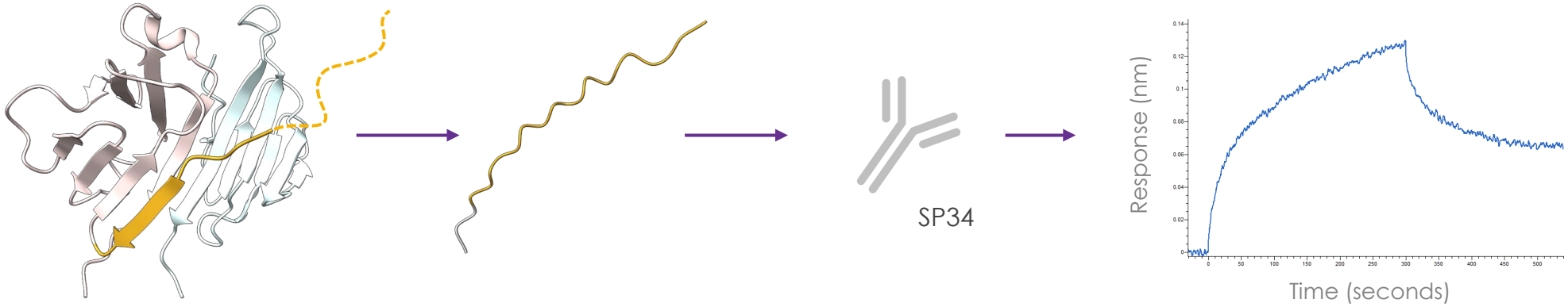


Engineered Epitopes Bind Benchmark Antibodies

Native Structure *Engineered Epitope* *Antibody* *Octet Binding*

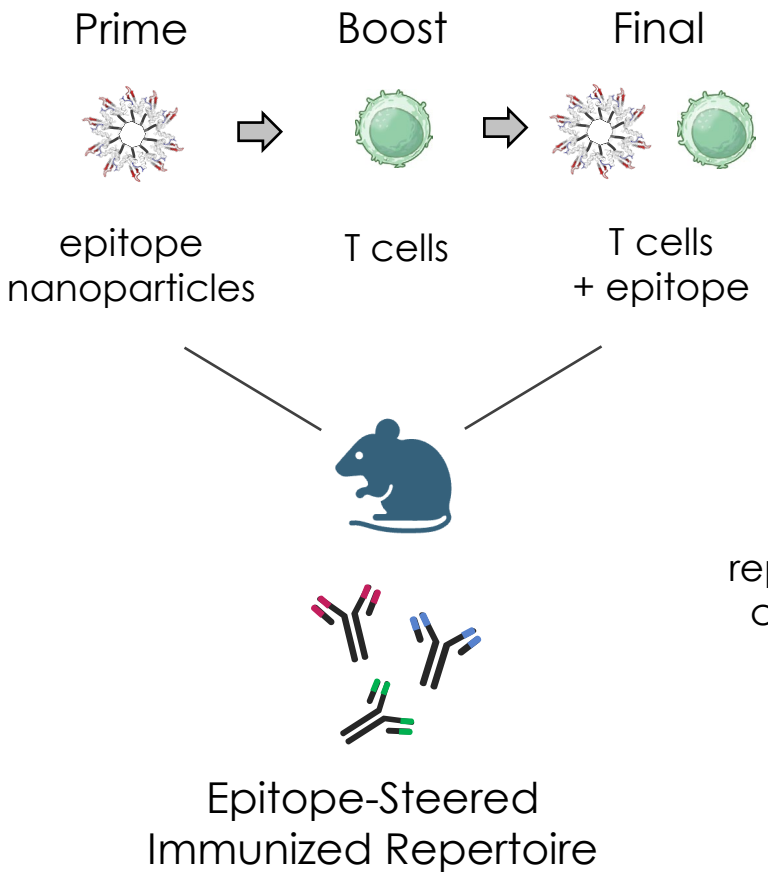
Epitope 1

Epitope 2



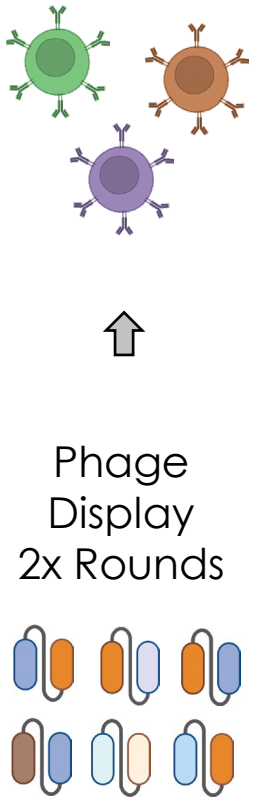
Epitope-Steered Immunizations are Screened by Mammalian Display

1. Epitope-Steered Immunization



repertoire cloning

2. Mammalian Display



1-sorting round

3. Multi-Modal Screening

Single-Cell Sorting

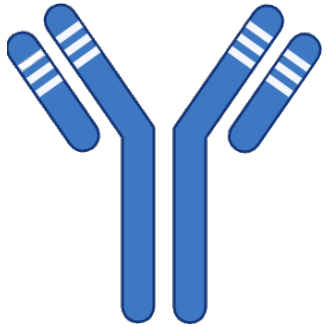
1. Epitope binding
2. Human CD3 binding
3. Cyno CD3 binding
4. Expression

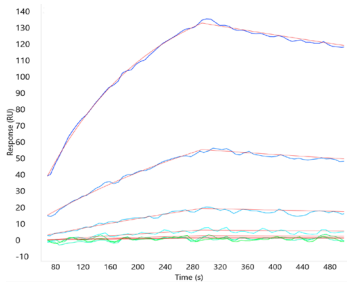
SPR and T cell activation measurements

Anti-CD3 Template Antibody is Diversified with StableHu AI

AI model predicts optimal human CDRs to replace mouse CDRs

Template mouse antibody



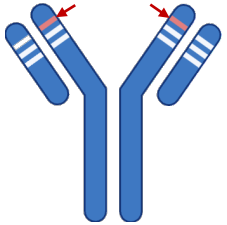


K_D = 10 nM

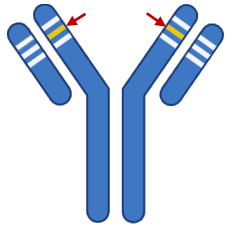
StableHu AI

Predicted libraries of ~10^3 sequences per CDR

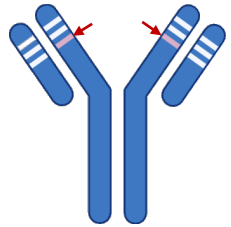
HCDR1
2000



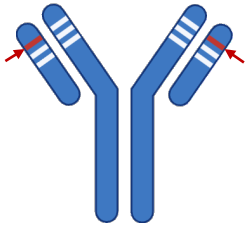
HCDR2
2000



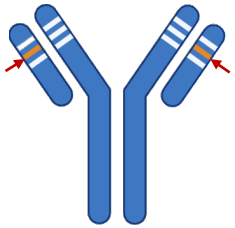
HCDR3
2000



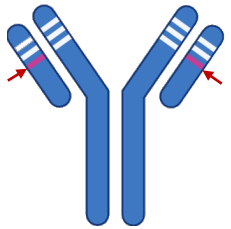
LCDR1
2000



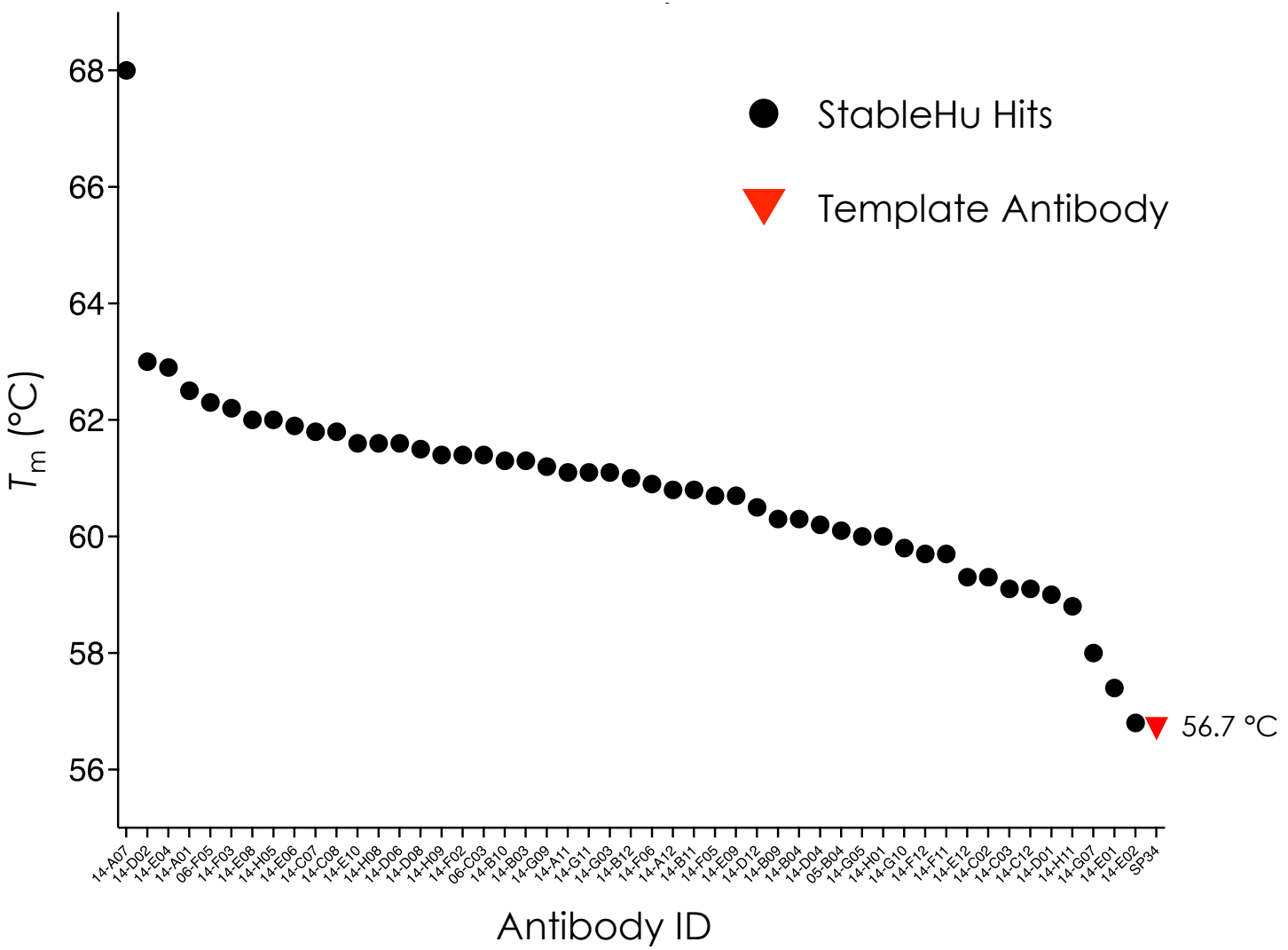
LCDR2
1000

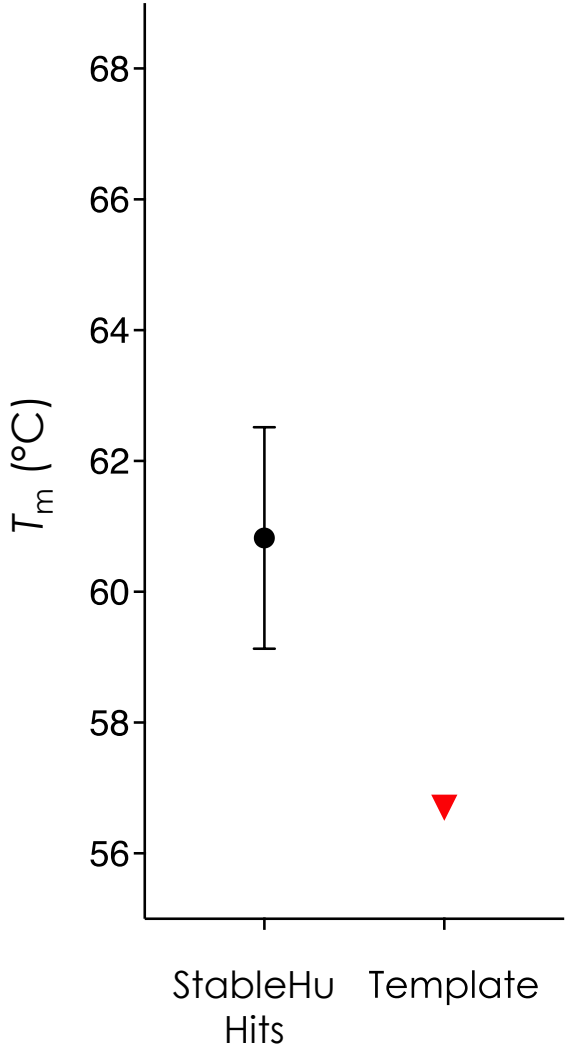


LCDR3
2000



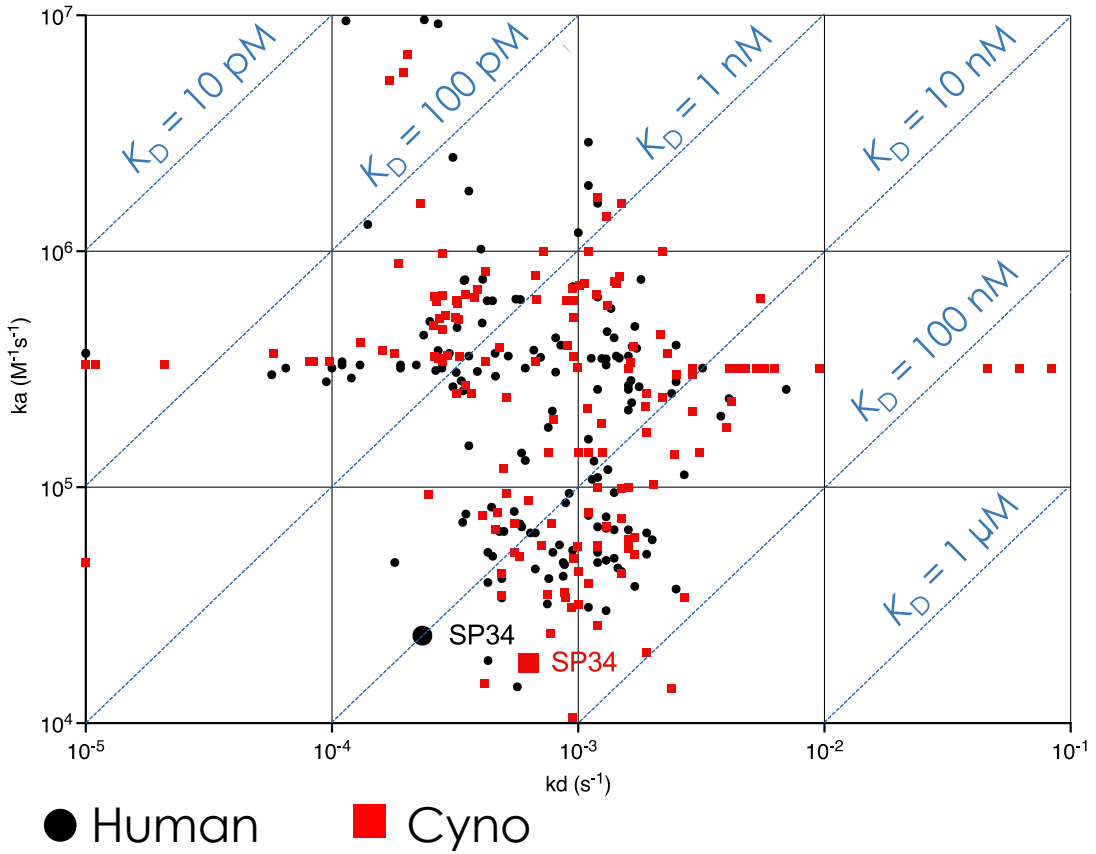
StableHu Improves Melting Temperatures Relative to Template Antibody



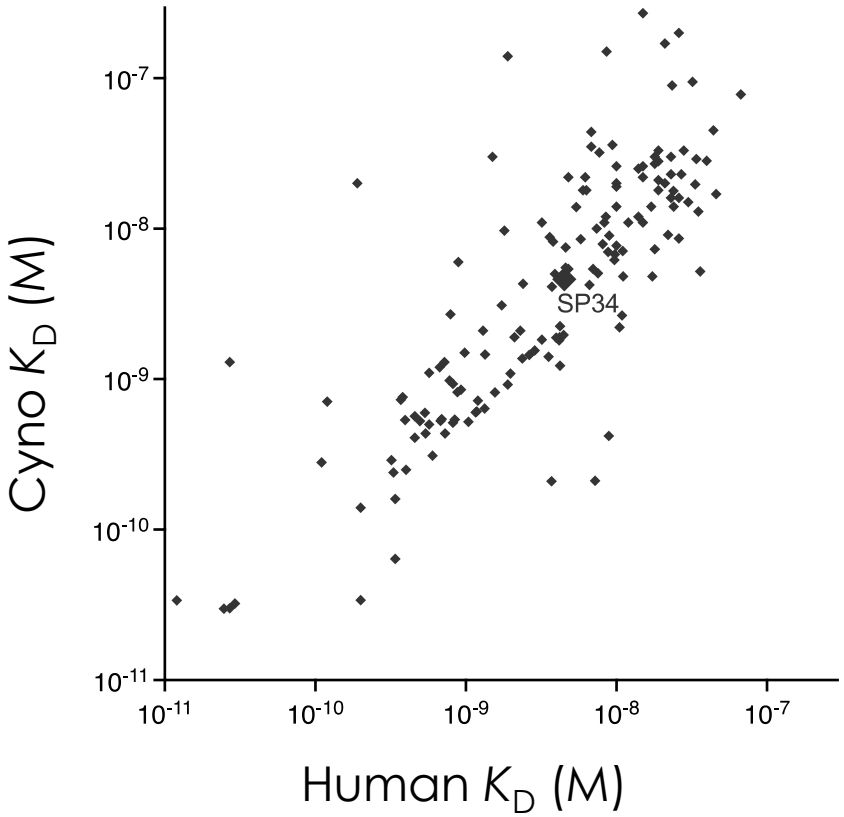


Epitope-Steered Immunizations and StableHu AI Identify Human-Cyno CD3 Cross-Reactive Binders

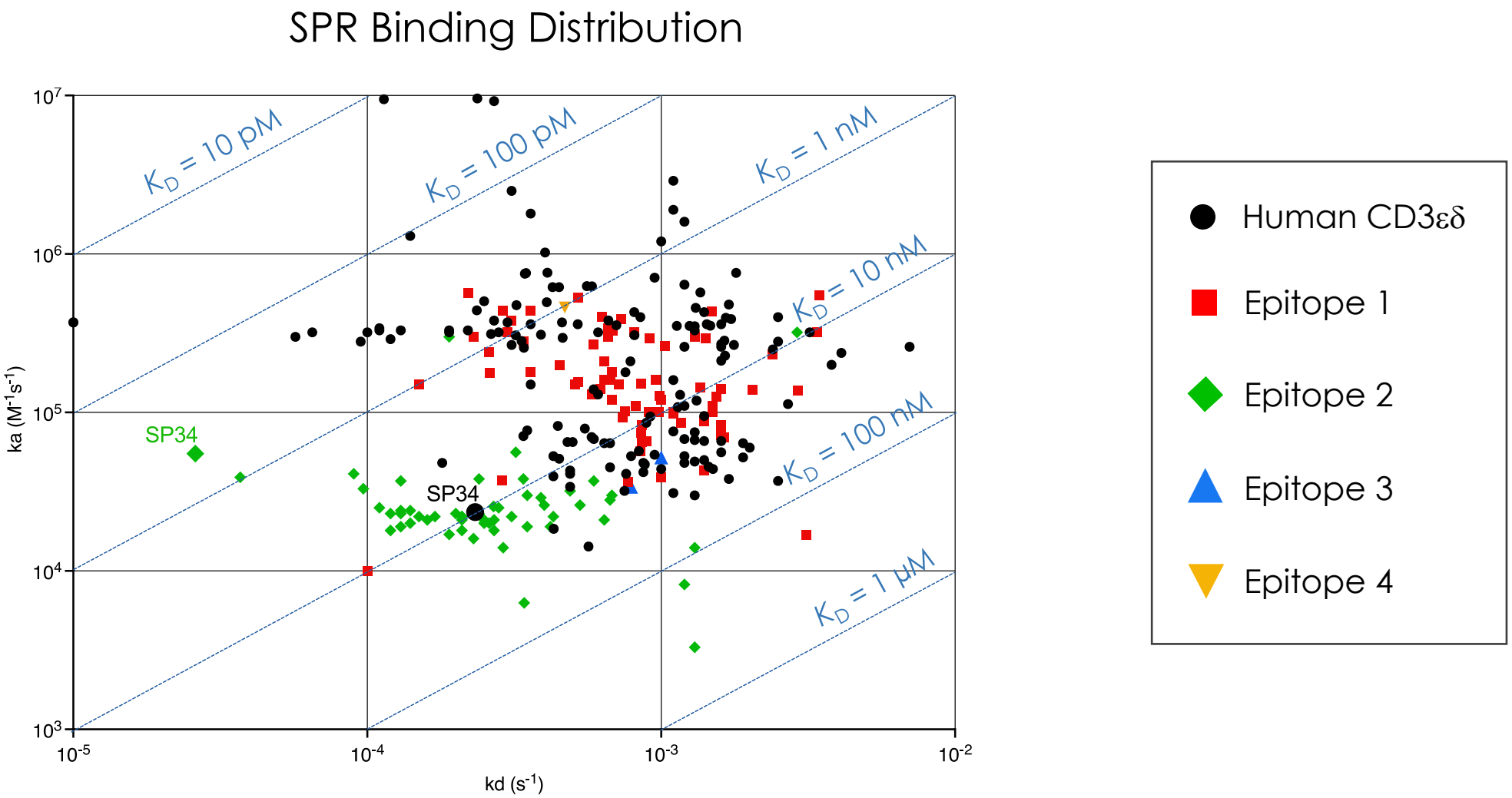
Cross-reactive hits span a diverse range of K_D ~10^{-11}-10^{-7} M



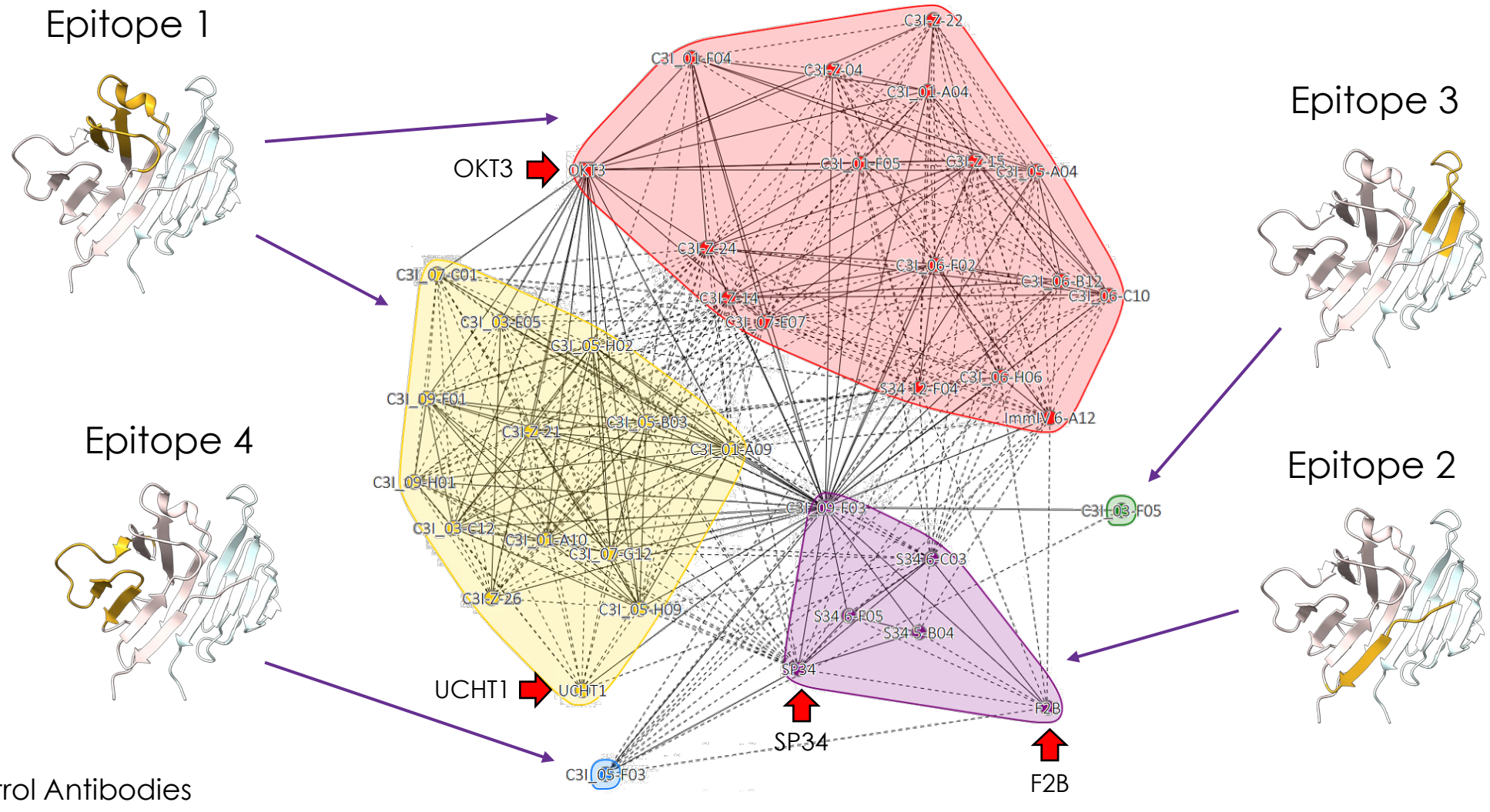
Most hits bind human and cyno CD3 comparably



Most Human-Cyno CD3 Cross-Reactive Hits Also Bind the Engineered Epitopes

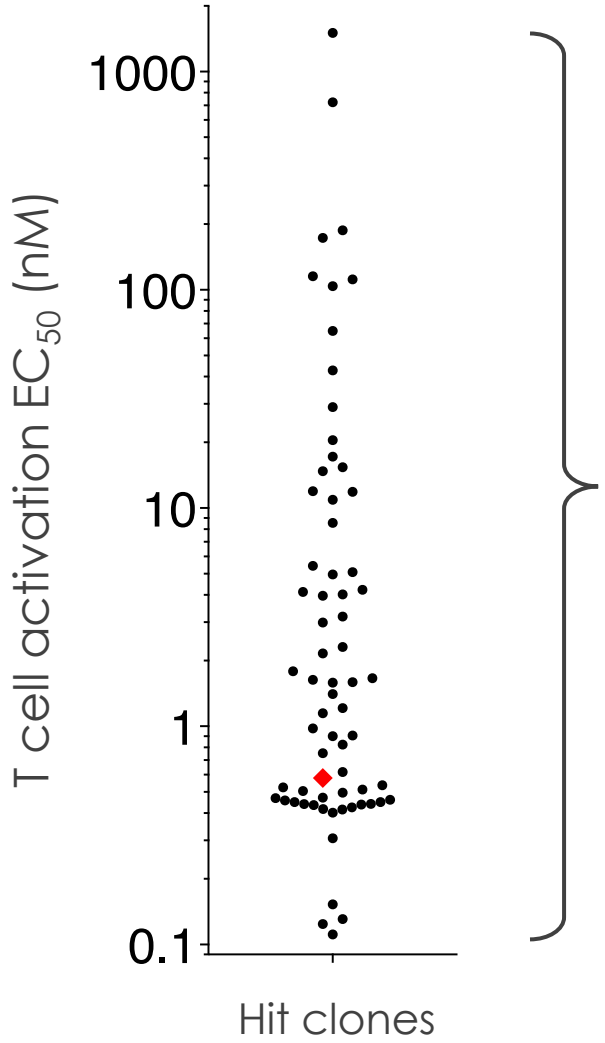


Cross-Reactive Hits Cover 4 Engineered Epitopes and 5 Epitope Bins



(+) Control Antibodies

Cross-Reactive Hits Span a Diverse Range of T Cell Activities



~10,000-fold
T Cell activation
EC_{50} range

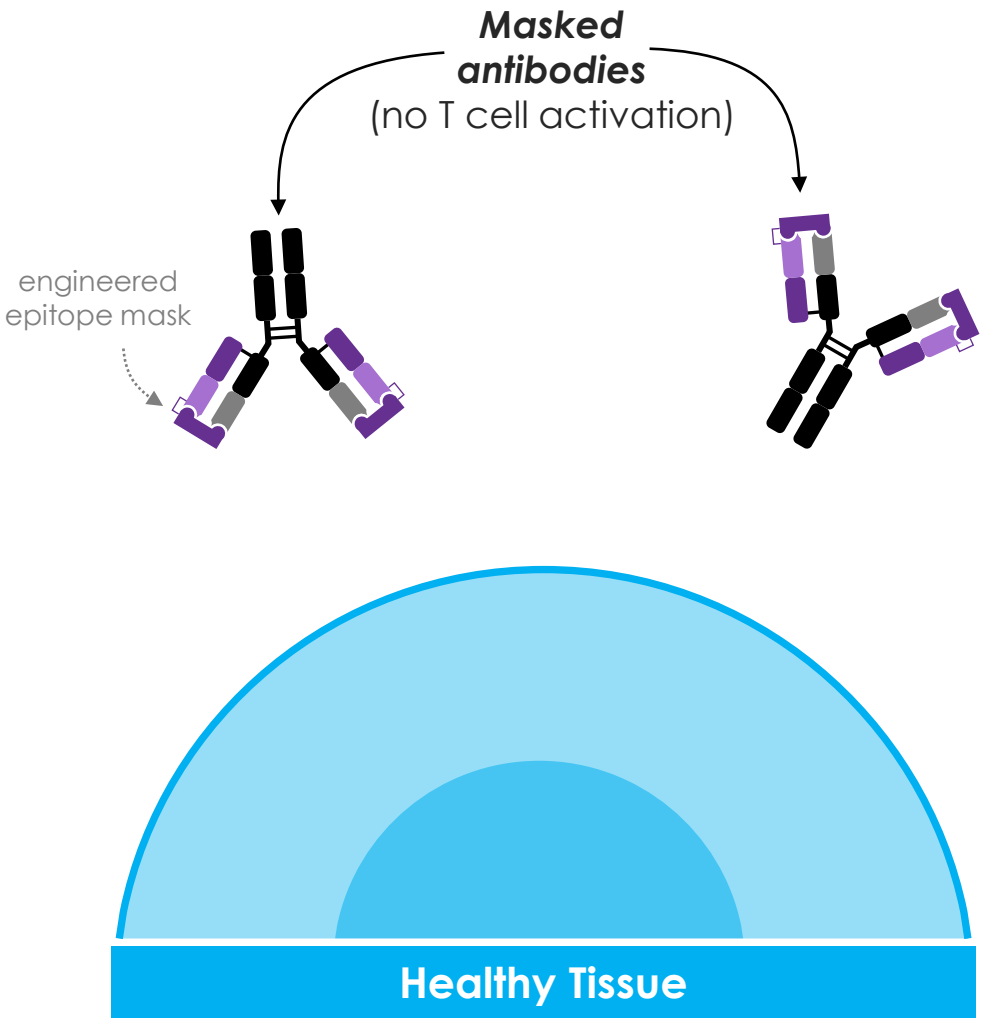
Human-Cyno CD3 & epitope triple-positive hits

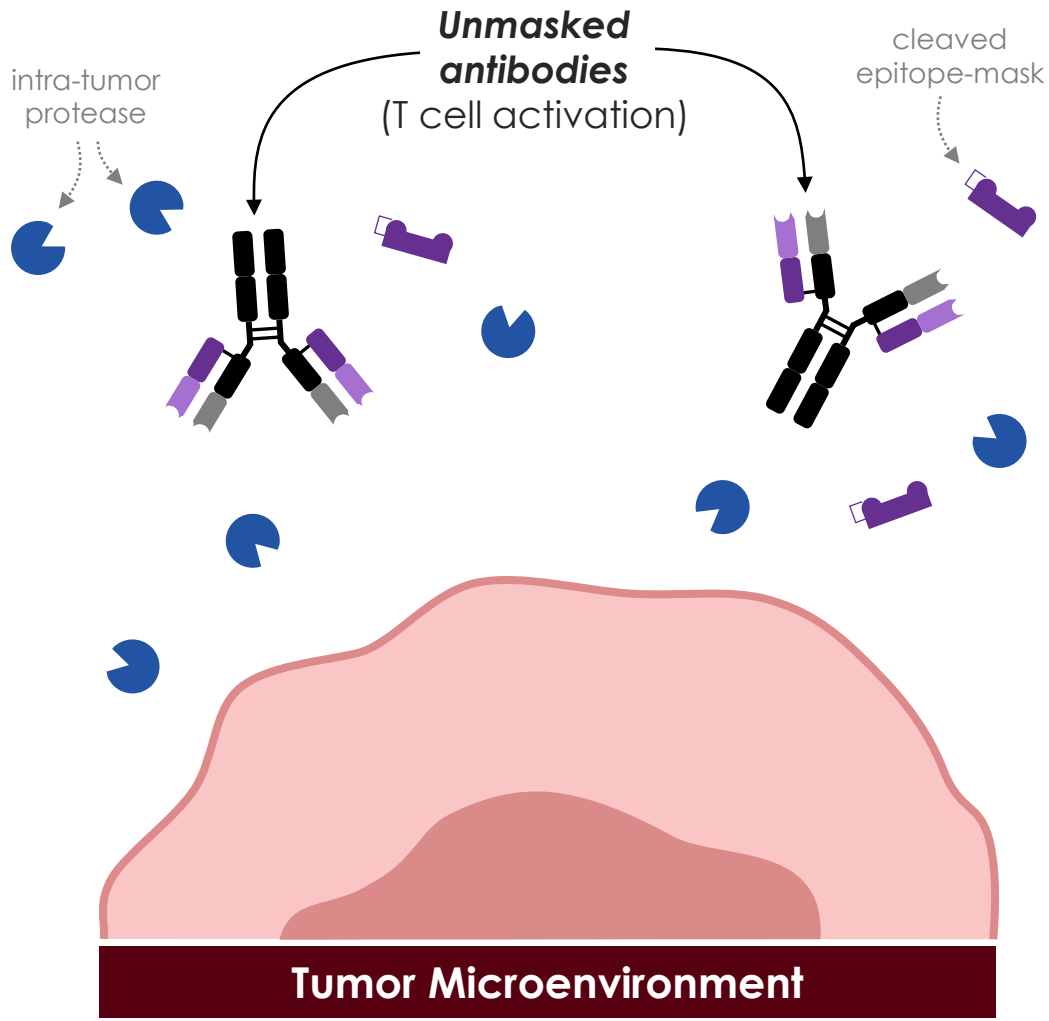
SP34 Benchmark

Conditionally Activated T Cell Engager

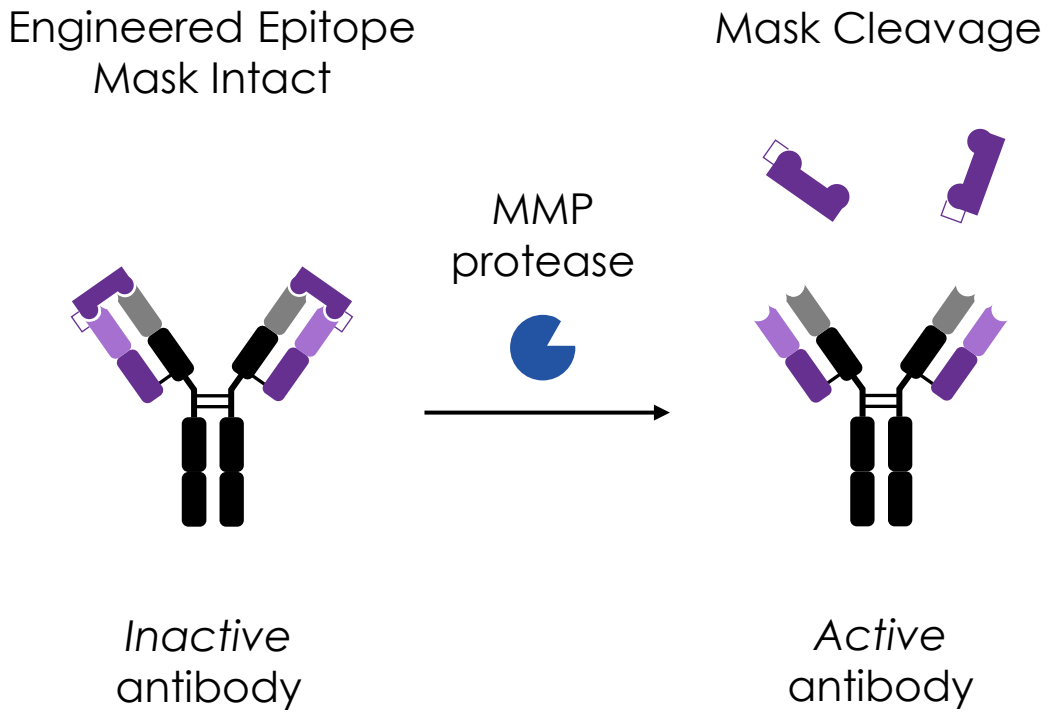
Engineered Epitope Masking

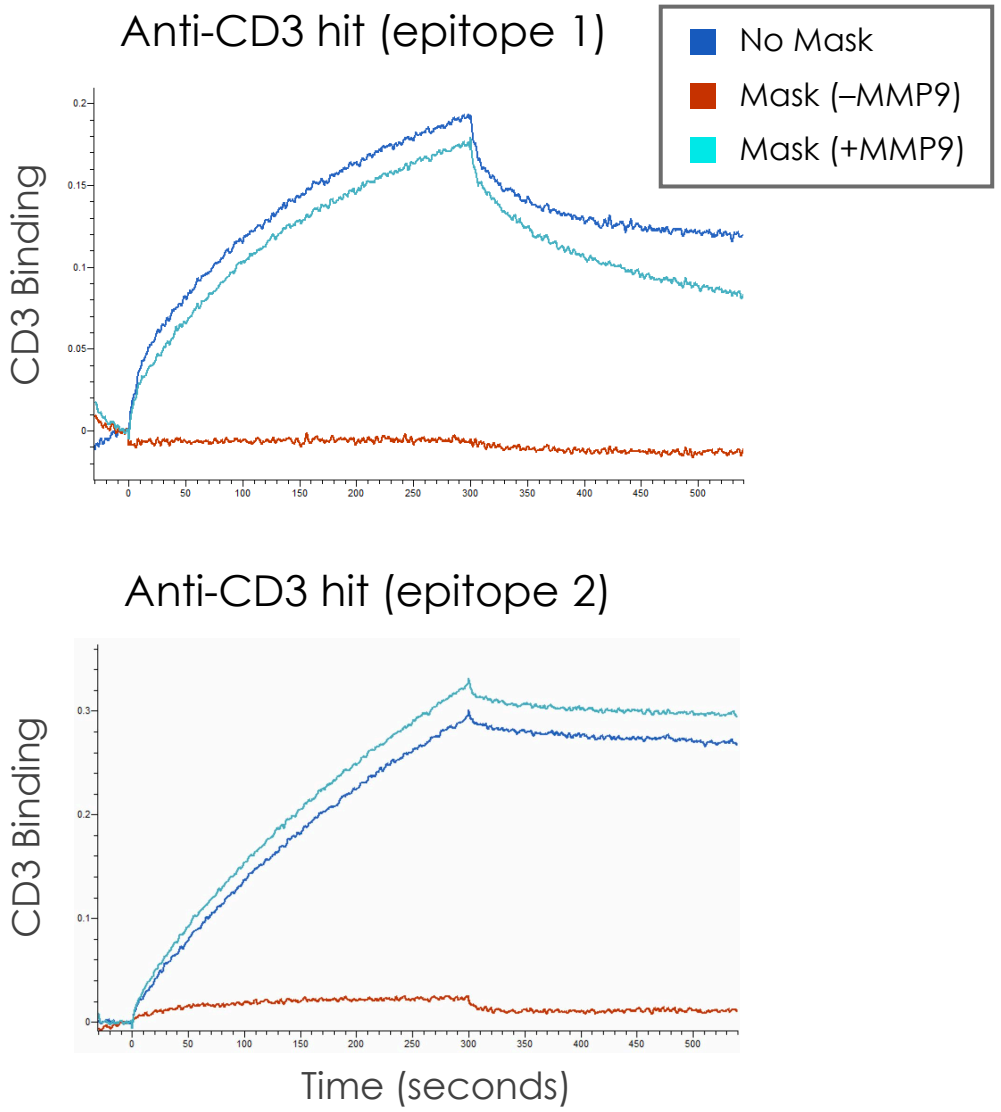
Can Engineered Epitopes be Used for Conditionally Activated Antibodies?





Engineered Epitope Masks Conditionally Activate CD3 Hits





Anti-Tumor Bispecific Antibody

Masked Anti-CD3 X Anti-TROP2

TROP2 is Expressed at Elevated Levels in Multiple Types of Cancers

Breast Cancer

- High TROP2 correlates with poor prognosis and metastasis[1,2]
- TROP2 is overexpressed in all known breast cancer subtypes[3]

Non-Small Cell Lung Cancer

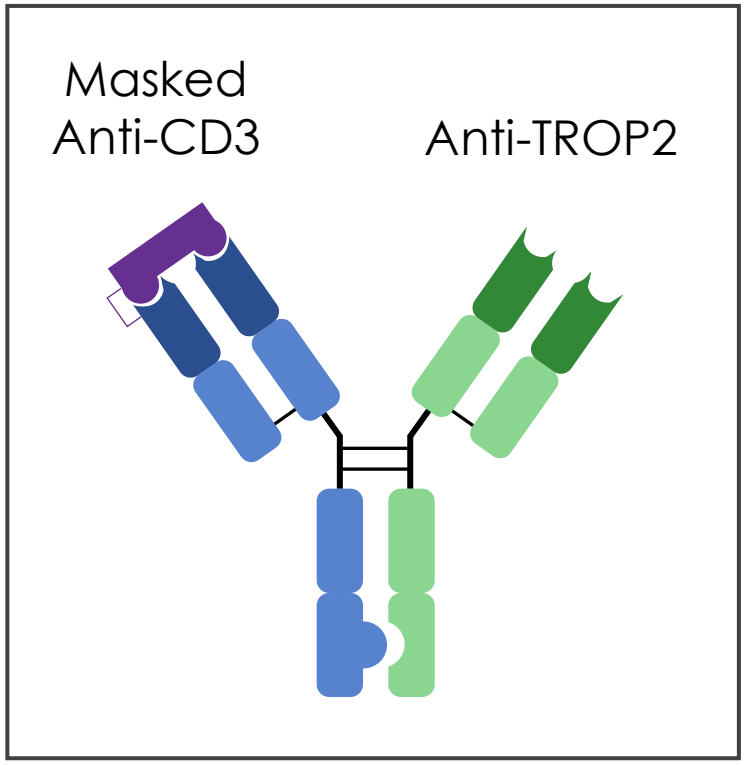
- TROP2 expression correlates with reduced survival probability[4]
- 64-90% of cancers have high levels of TROP2 expression[4,5]

Ovarian Cancer

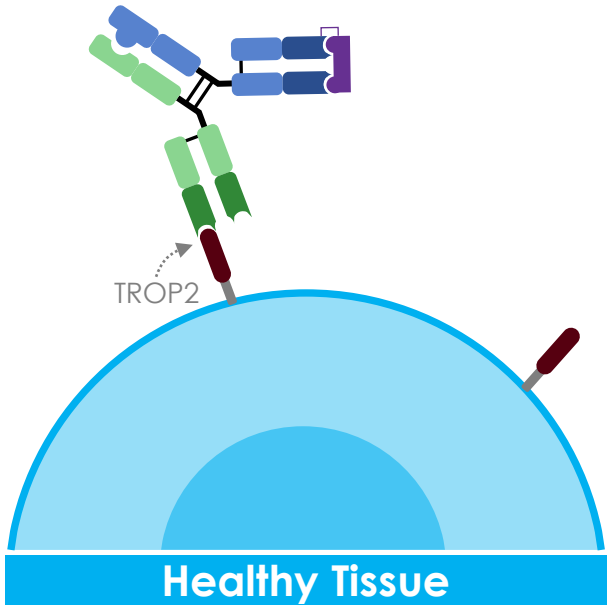
- Higher TROP2 expression is correlated with poor survival[6]
- 47-92% of cancers have moderate to high TROP2 expression[6,7,8]

1. DOI: 10.1016/j.jare.2023.05.012
2. DOI: 10.21037/atm-22-5976
3. DOI: 10.3390/cancers14235936
4. DOI: 10.18632/oncotarget.15647
5. DOI: 10.1200/JCO.2016
6. DOI: 10.1016/j.ejca.2009.12.019
7. DOI: 10.1016/j.ygyno.2011.03.002
8. DOI: 10.3389/fonc.2020.00118

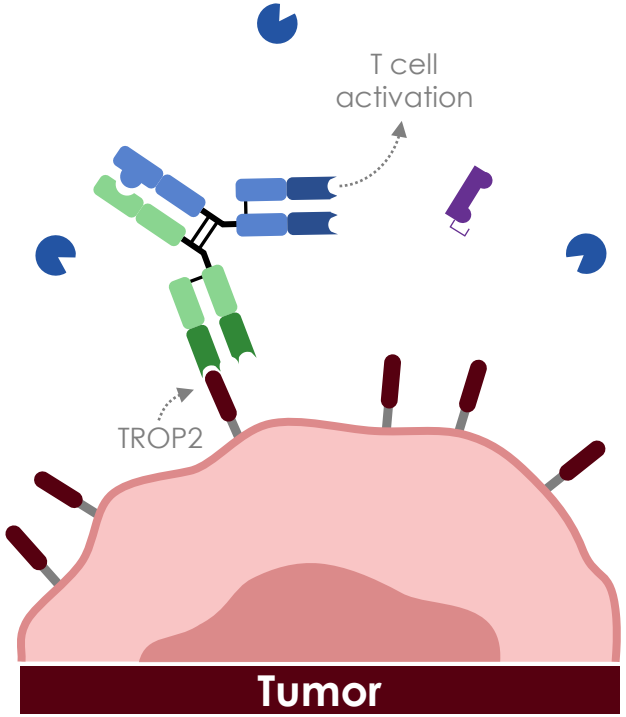
Masked Anti-CD3 X Anti-TROP2 Bispecific Construct



T cell activation suppressed by mask



T cells activated by tumor proteases



MMP9 Conditionally Activates CD3 Binding

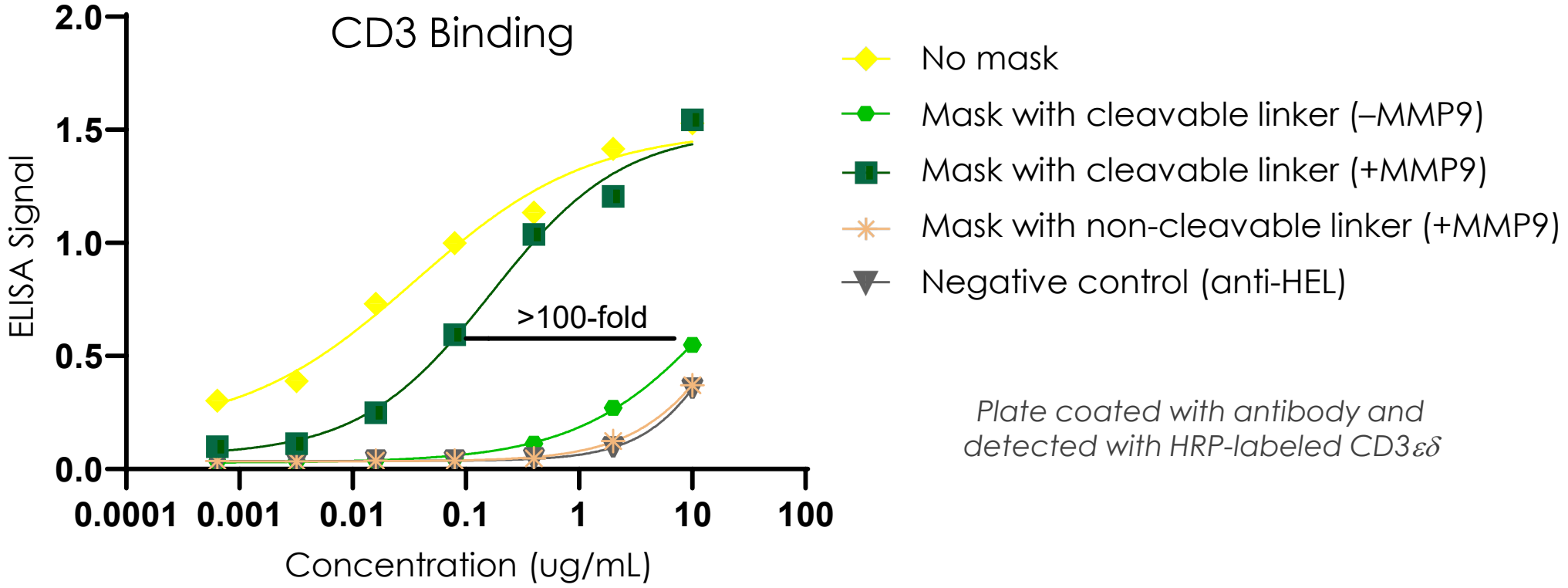
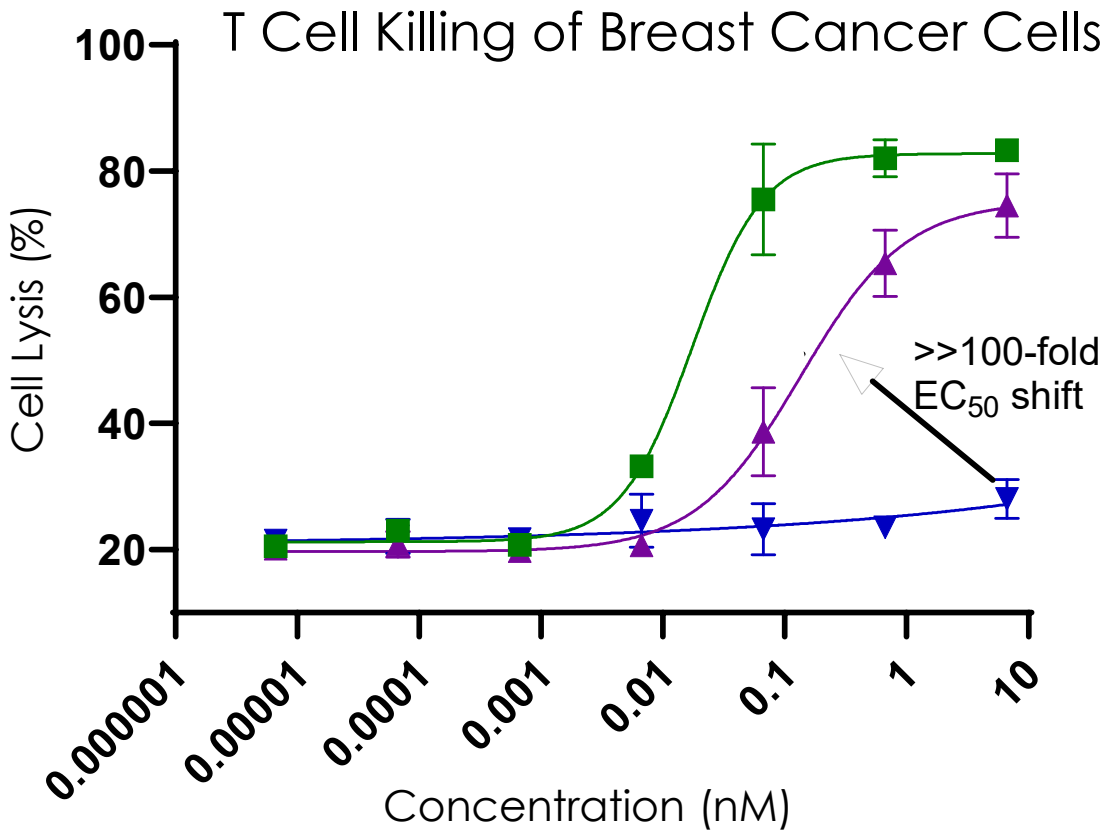


Plate coated with antibody and detected with HRP-labeled CD3εδ

>100-fold inhibition and recovery of CD3 binding with engineered epitope mask

Tumor Proteases Activate T Cell Killing



No mask

Mask with cleavable linker

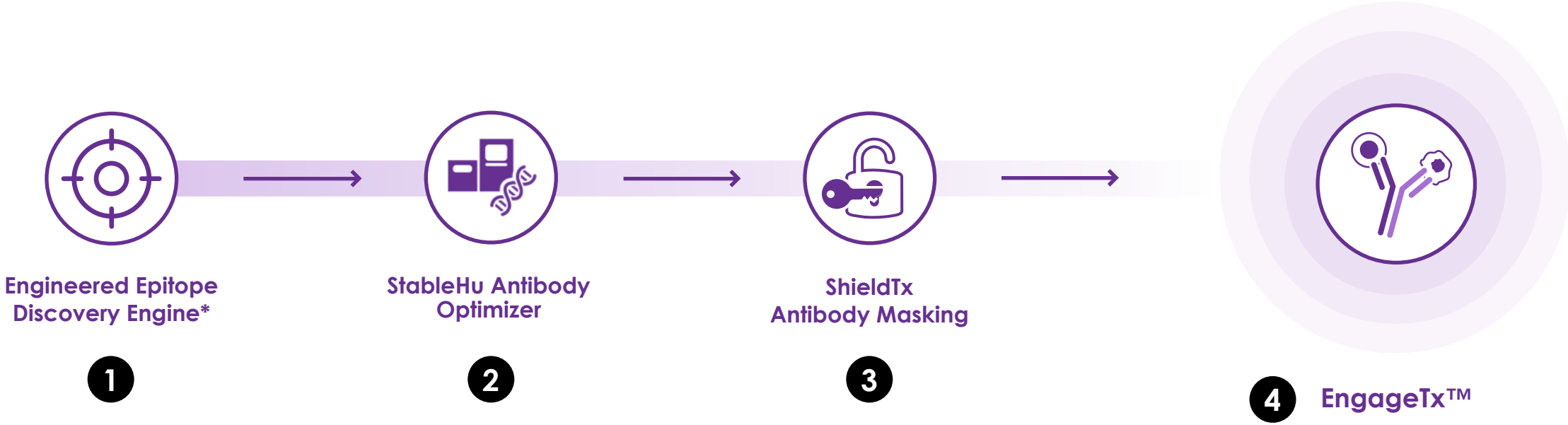
Mask with non-cleavable linker

- *Breast cancer cell line: MDA-MB-231*
- *Antibodies are added undigested, so mask cleavage is due to cancer cell proteases*

>>100-fold greater cell killing with cleavable linker compared with non-cleavable linker

iBio Discovery Stack Summary

1. Epitope-selective antibodies can be efficiently discovered with engineered epitopes
2. StableHu AI generates antibody libraries for optimized developability and expression
3. Engineered epitopes can be used to conditionally activate antibodies
4. Masked anti-CD3 antibodies can be combined with anti-tumor antibodies into bispecifics



*U.S. Patent No. 11,545,238 (issued January 3, 2023)