Enhancing Bispecific T Cell Engager Discovery with AI and Mammalian Display

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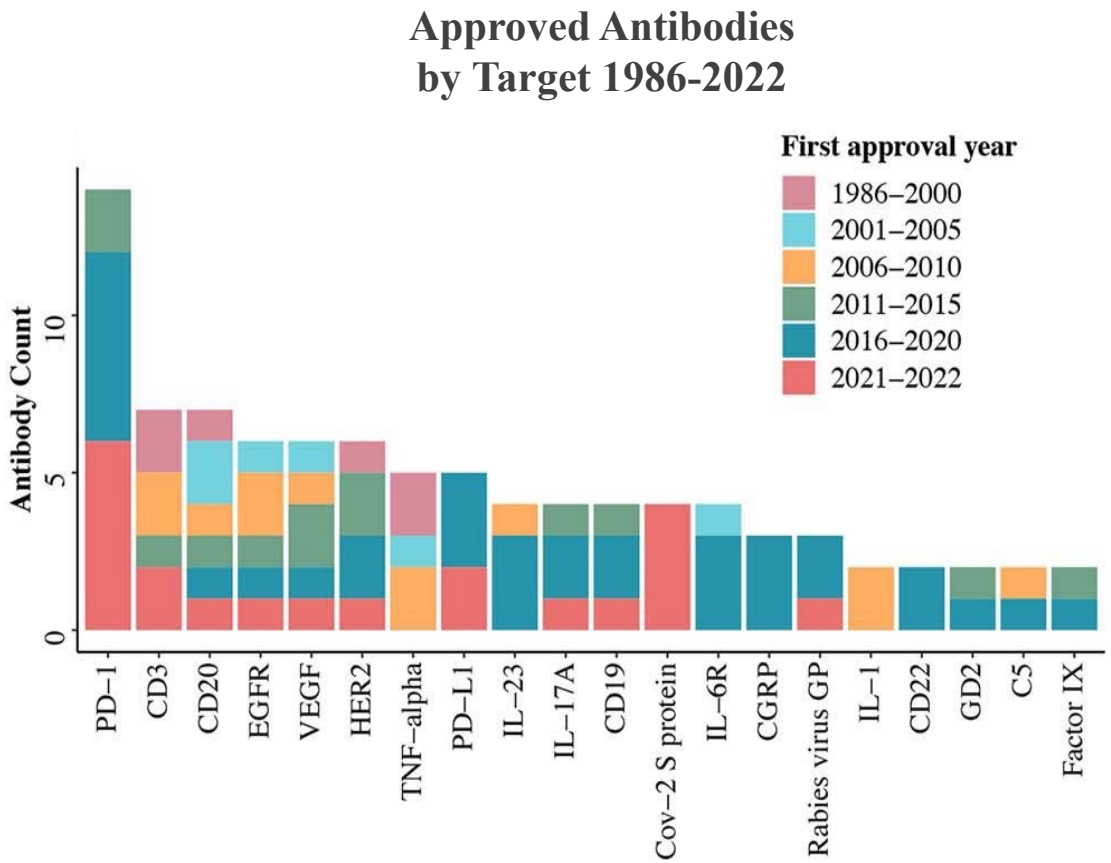
Festival of Biologics
Oct. 2023

Relatively Easy Targets & Modes of Action Dominate Therapeutic Antibodies

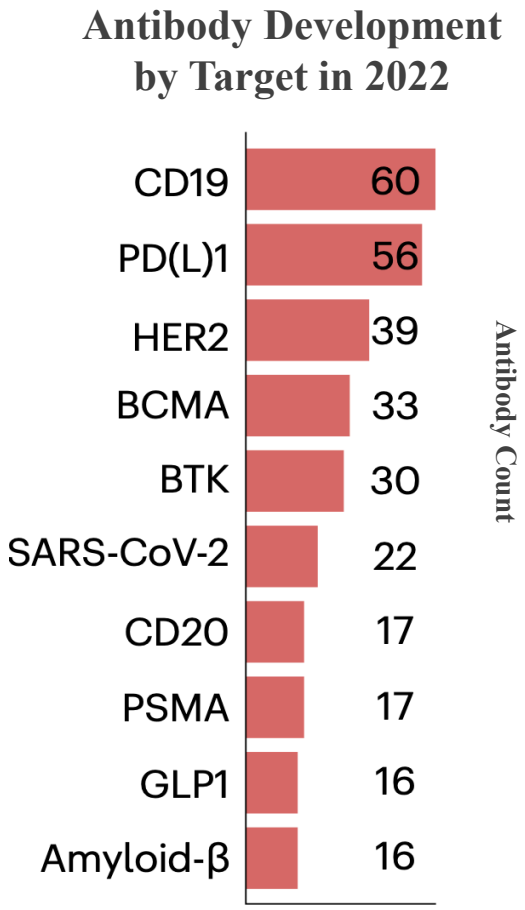
Approved antibodies:
40% bind 10 targets



Lyu et al., *Antibody Therapeutics* Sept. 2022

Relatively Easy Targets & Modes of Action Dominate Therapeutic Antibodies

New antibody development:
Focused on a few targets



Fougner et al., *Nat. Rev. Drug Disc.* Aug. 2023

Untapped Opportunities

Targets

- GPCRs
- Membrane transporters
- Protein-Protein junctions
- Disease-Specific variants

…

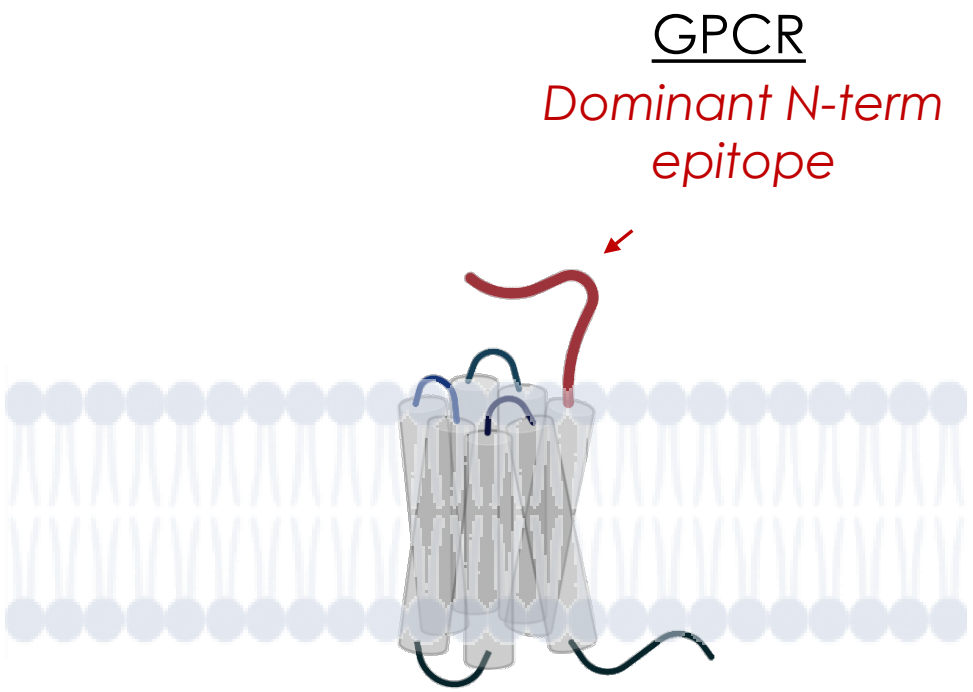
Modes of Action

- Agonism
- Multispecifics
- Dual+ MOA
- Microenvironment activation

…

Traditional full-length antigen discovery is inefficient for challenging targets and MOAs

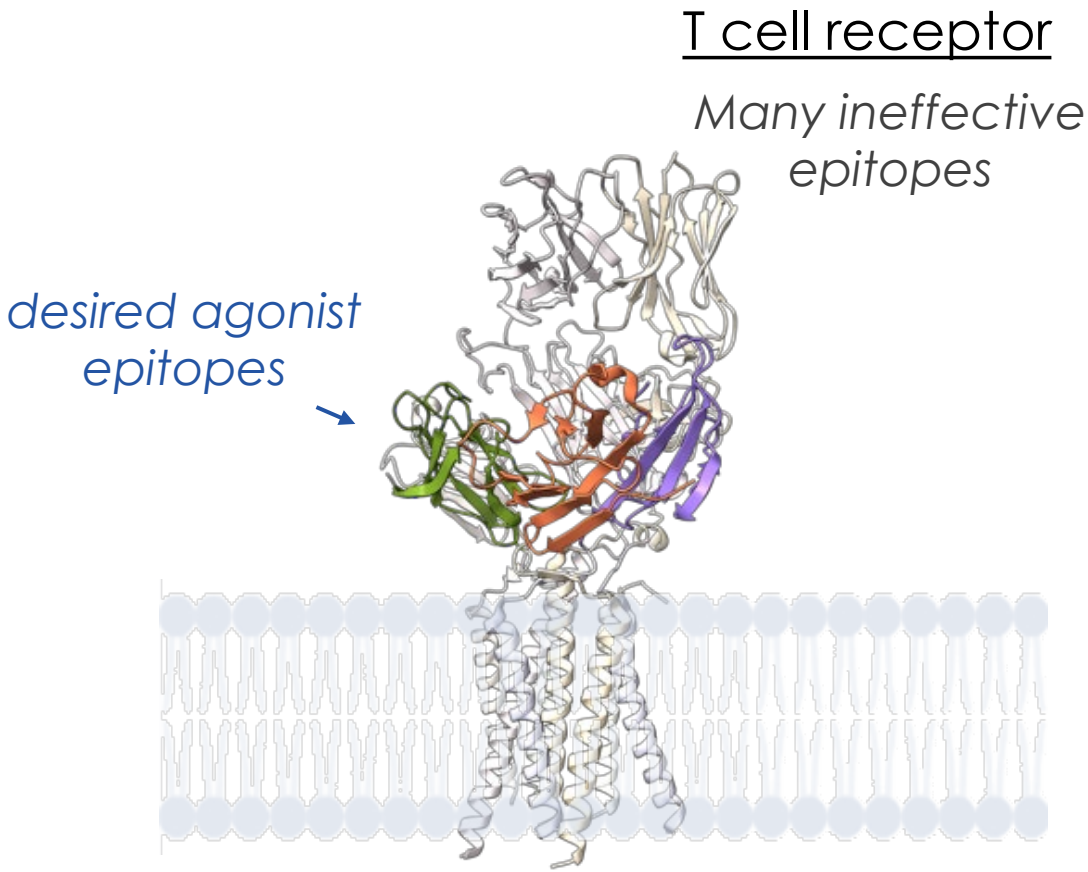
Dominant epitope antibodies overwhelm traditional discovery [1, 2, 3]



(1) Wicker *et al.*, *Eur. J. Immunol.* (1984)14, p.447
(2) Victora *et al.*, *Cell* (2015) 163, p.545
(3) Nakra *et al.*, *J. Immunol.* (2000) 164, p.5615

Traditional full-length antigen discovery is inefficient for challenging targets and MOAs

Low discovery yield for high-value, challenging therapeutic target epitopes [4]



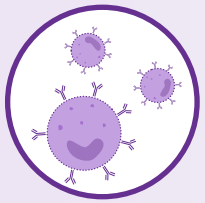
(4) Trkulja *et al.*, *Sci. Adv.* (2021) 7:16, p.eabe6397

Our Solution to Challenging Target and MOA Antibody Discovery: Epitope-Steering and High-Developability Mammalian-Display

1 **Engineered Epitope**
Design Engine

Steer antibody discovery to intended epitopes

2 **Human Diversity**
Antibody Library



Natural diversity in fully human validated frameworks

3 **StableHu™**
Antibody Optimizer

Human diversity mammalian-display optimization

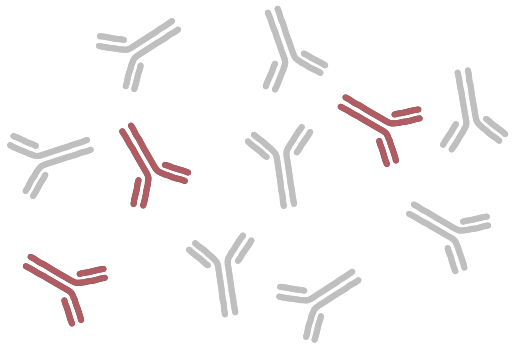
Epitope-Targeted

Antibody Discovery

Engineered Epitopes Focus Antibody Repertoires On Desired Binding Sites

1

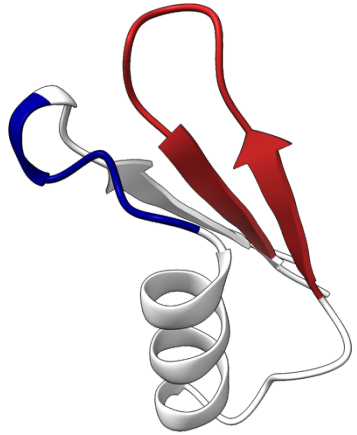
Naïve in vivo or in vitro antibody library



epitope-specific Ab

2

Focus library with engineered epitopes

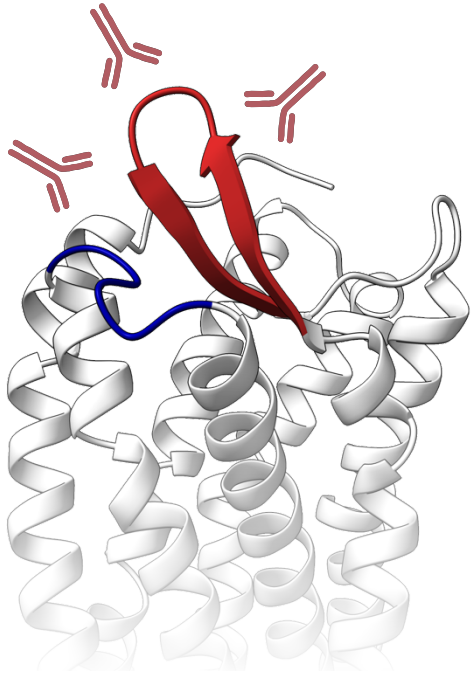


epitope

de novo scaffold

3

Efficient discovery of epitope-specific Abs

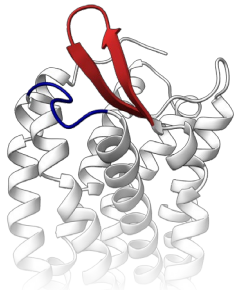


full length target

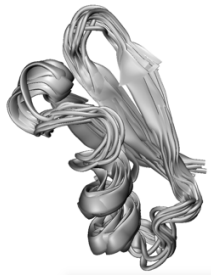
AI-Engine Optimizes Engineered Epitope Structure, Stability, and Solubility

Engineered Epitope Design Objectives

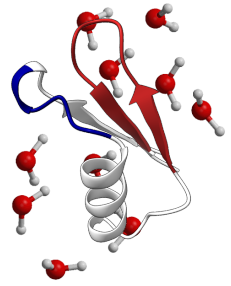
1. **Match Structure to Target**



2. **Refine for Greater Stability**



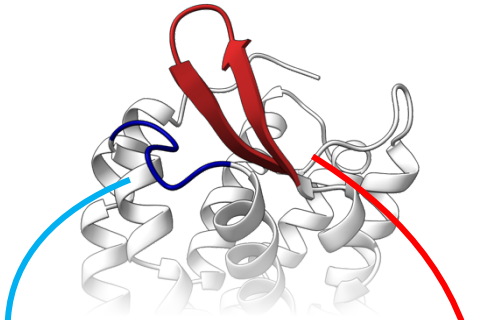
3. **Optimize for Water Solubility**



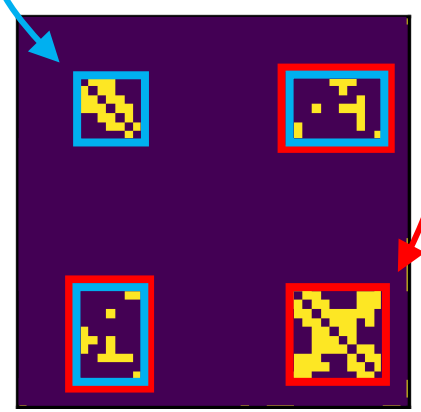
AI Discovery Engine

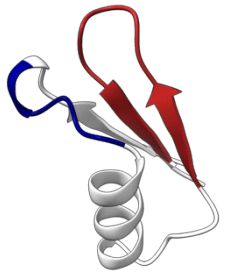
Multi-Loss Function Enforces Engineered Epitope Structure Match to Target and Overall Stability

Loss Term #1

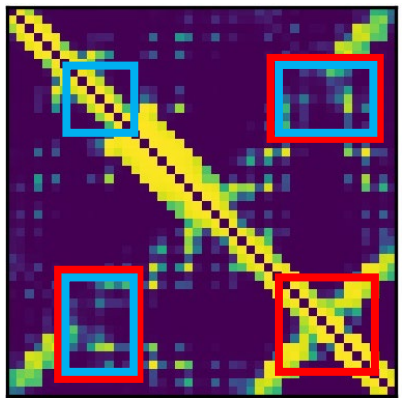


Ideal Target Distogram





Predicted Distogram

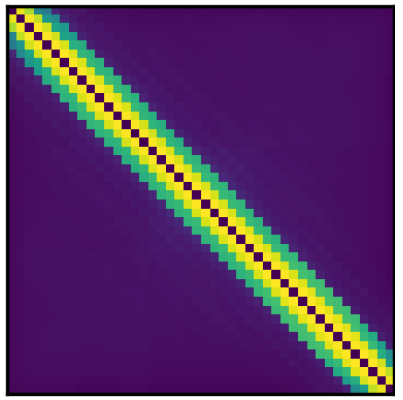


Minimize Cross-Entropy between engineered & target epitope residues

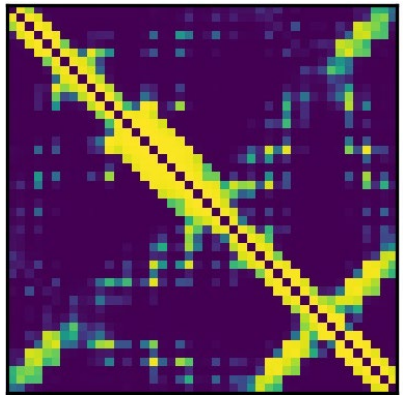
Loss Term #2

Average predicted distograms across random sequence space

Random Coil Distogram

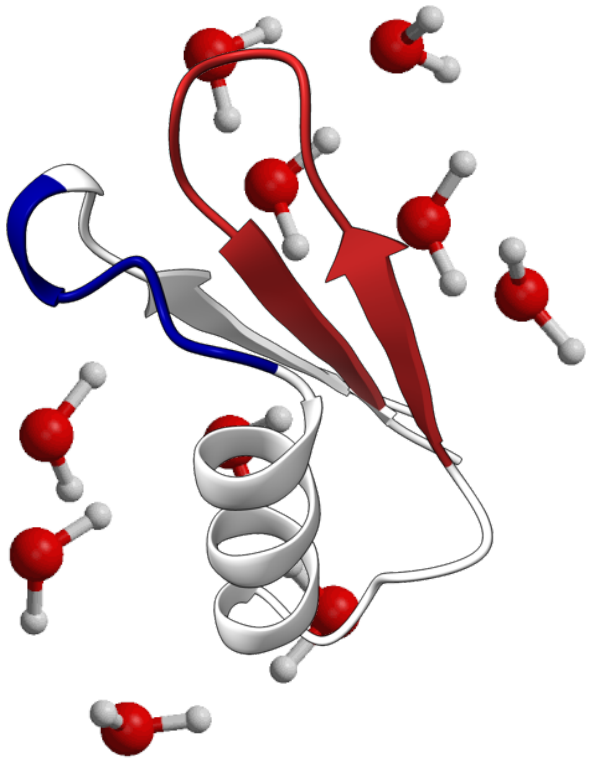


Predicted Distogram



Maximize KL-Divergence between unstructured coil and engineered epitope

Multi-Loss Function Optimizes Engineered Epitope Solubility



Loss Term #3

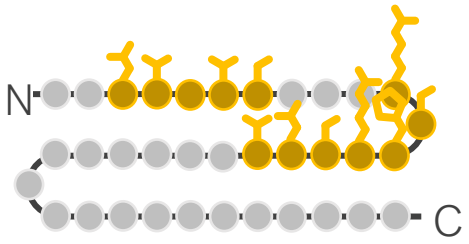
Amino Acid Hydropathies

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

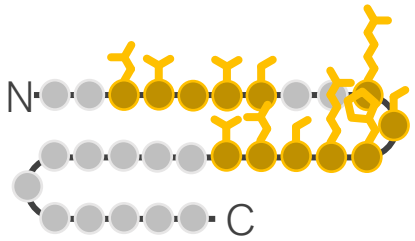
Average hydropathy is minimized

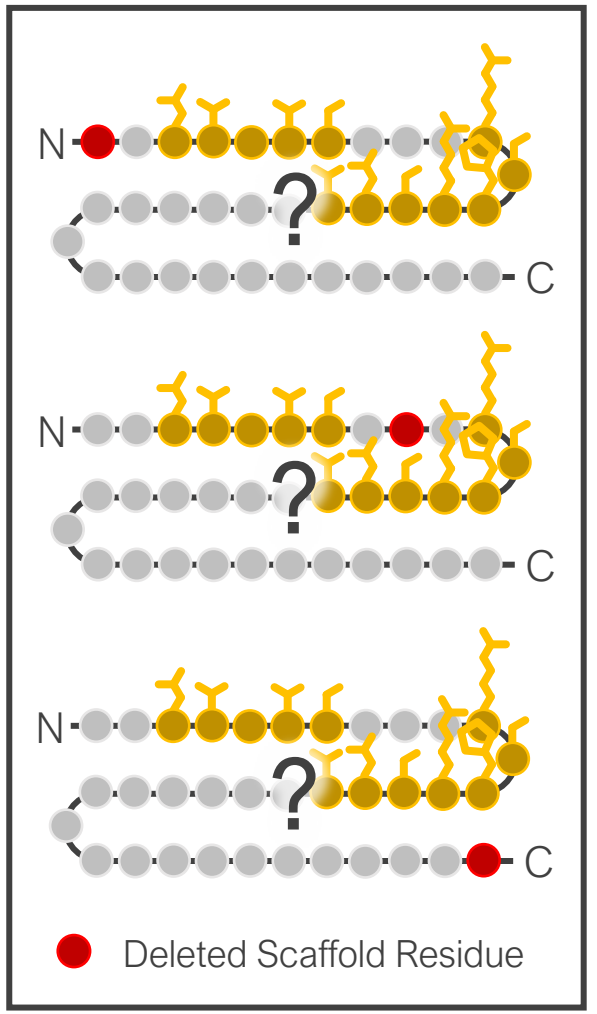
Engineered Epitopes are Further Optimized by Maximizing the Epitope-to-Scaffold Ratio to Reduce Scaffold-Specific Antibodies

Starting Topology = $\frac{\#\text{Epitope residues}}{\#\text{Scaffold residues}}$



Optimized Topology = $\frac{\#\text{Epitope residues}}{(\#\text{Scaffold} - \#\text{Deleted})}$

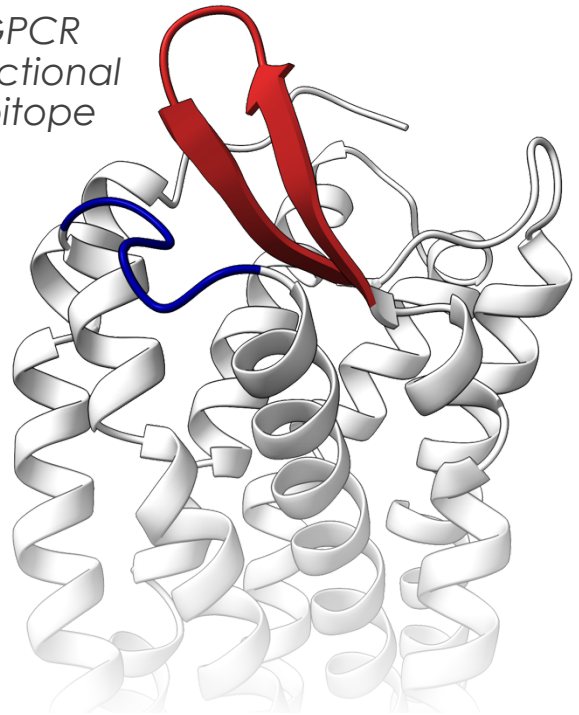




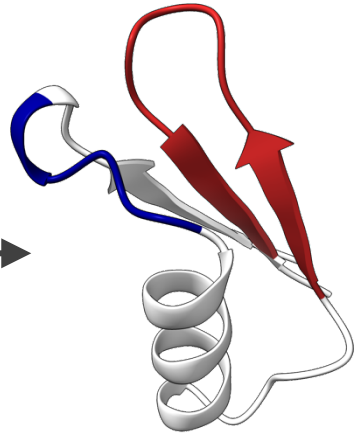
Iteratively trim scaffold residues until epitope destabilizes

Engineered Epitopes are Designed with the AI-Engine and Cross Validated with Folding Simulations, Binding Measurements, T_m, and NMR

GPCR Junctional Epitope

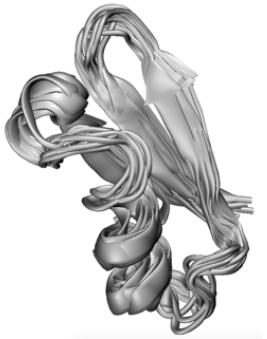


AI Discovery Engine



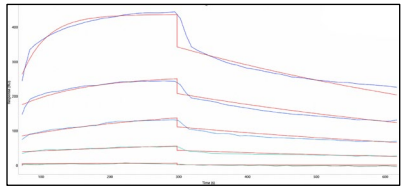
Folding Simulations

e.g. AlphaFold2



Antibody Binding

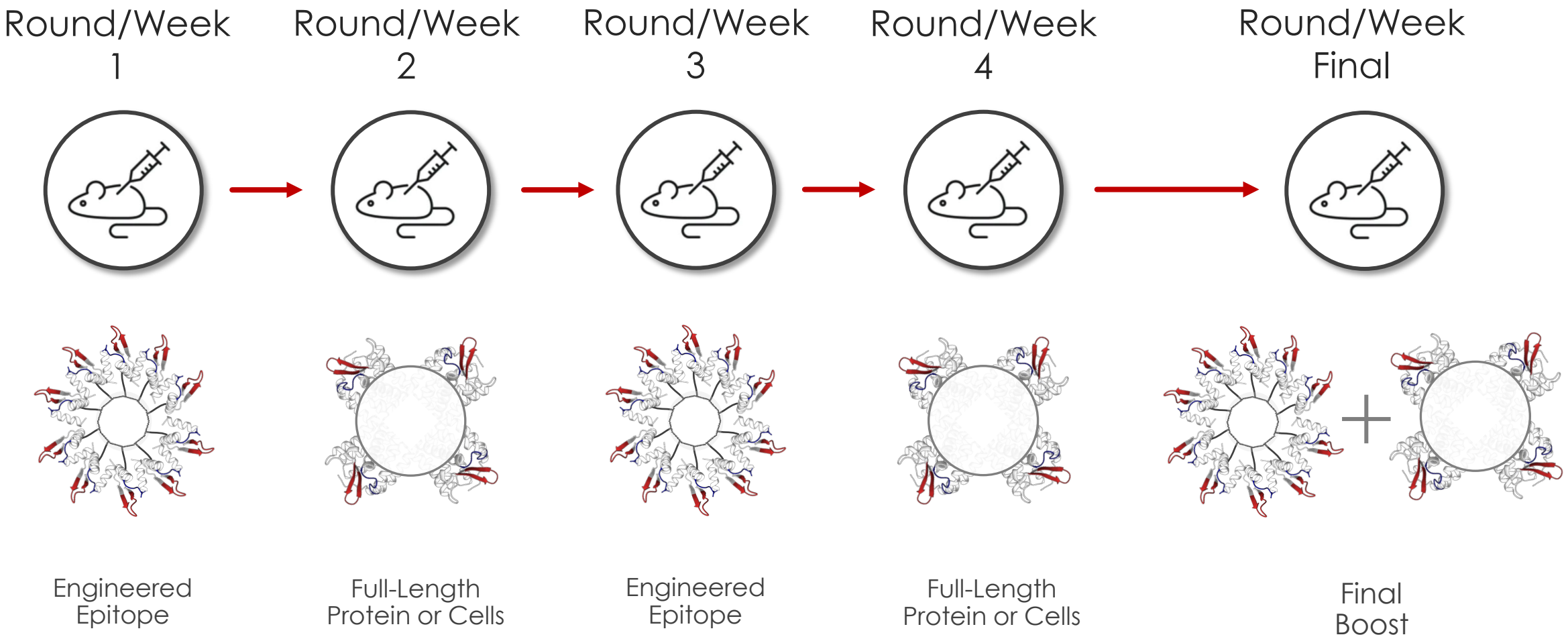
e.g. SPR



NMR, Melting Temp.

Engineered Epitopes Steer Immunization and In Vitro Libraries to Target Epitopes

Engineered epitopes alternated with full length protein/cells steers immunizations and in vitro selections while enforcing full length protein and cell binding



Engineered Epitopes Can Be Used In Primary Screens to Epitope-Map Hits

Positive on Antigen and Engineered Epitopes

CD3

Clone	CD3εδ/εγ	Epitope-1	Epitope-2	Epitope-3
13A5	0.93	0.75	0.23	0.21
13A12	0.49	0.59	0.25	0.88
13B4	0.53	0.87	0.29	0.25
13B6	0.34	0.85	0.24	0.24
13B7	0.90	0.80	0.25	0.24
13C4	0.51	0.88	0.26	0.25
13D3	0.34	0.59	0.24	0.24
13D10	0.41	0.63	0.46	0.27
13F1	0.67	0.92	0.26	0.26
13F3	0.32	0.27	0.24	0.24
13G5	0.43	0.90	0.31	0.27
13H7	1.01	0.91	0.28	0.58
13H9	0.97	0.84	0.26	0.25
14A11	0.73	0.79	0.24	0.30
14A12	0.95	0.82	0.24	0.23
14B11	0.59	0.86	0.23	0.24
14D11	0.48	0.77	0.24	0.25
14E4	0.30	0.31	0.22	0.23
14E7	0.91	0.86	0.23	0.23
14E9	0.95	0.87	0.25	0.88
Media	0.32	0.29	0.20	0.19

MUC16

Clone	Epitope 1
5B7	1.06
6B7	1.04
5G5	0.30
13F11	1.01
13F9	1.10
14A6	0.33
15D9	1.14
16B12	0.91
18A8	0.35
18B1	0.69
19C12	1.11
18A8	0.31
Media	0.30

High Developability, Human Diversity

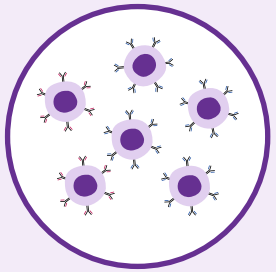
Antibody Libraries

Naïve In Vitro Library Uses Human Diversity to Minimize Immunogenicity Risk

Learn diversity from Hu Ig databases

Library Display

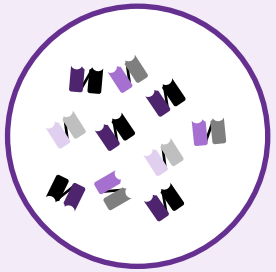
Mammalian



High developability

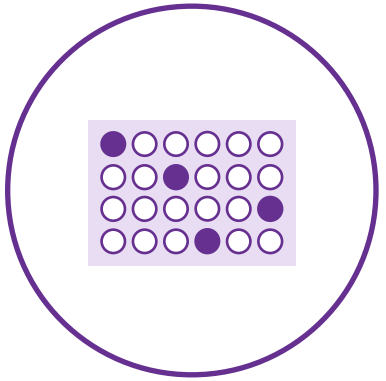
10^6 diversity

Phage



High diversity

$>10^{10}$ diversity



Selection or sorting to identify hits

Naïve Library Diversity Matches Natural Framework-Specific Distribution

Learn framework-specific CDR sequence distributions

Observed CDR sequences in clinically-validated frameworks

cAb-Rep & OAS Hu Ig databases

Natural Human Sequence Distribution	
QQSYSTPRT	2.799%
QQSYSTPLT	2.645%
QQSYSTPWT	1.565%
QQSYSTPYT	1.444%
QQSYSTPPT	1.227%
...	
QQALGP	0.001%
QQSYSTRTFT	0.001%
QQSCTIPRT	0.001%
QQTYNTPPPT	0.001%
QQSYSTPPGPWT	0.001%

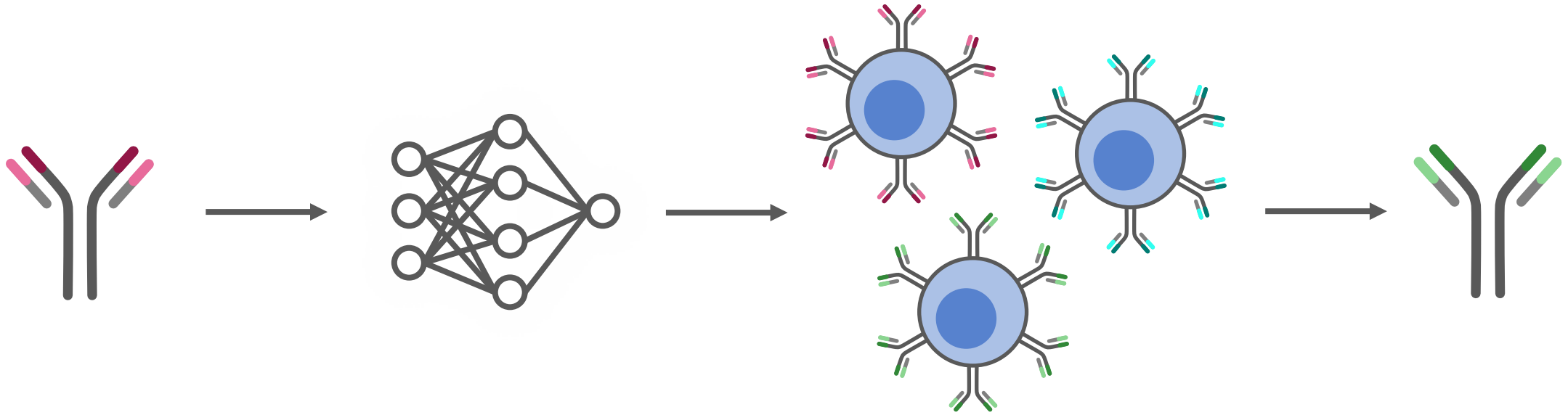
StableHu™ Optimizer Generates Focused Library Diversity Within the Capacity of Mammalian Display

Input Antibody

StableHu Optimizer AI-Engine

Mammalian Display

Output Antibody



Template CDR

Predict library of human CDR variants

HT screen mammalian display CDR library

Optimized antibody with fully human CDRs

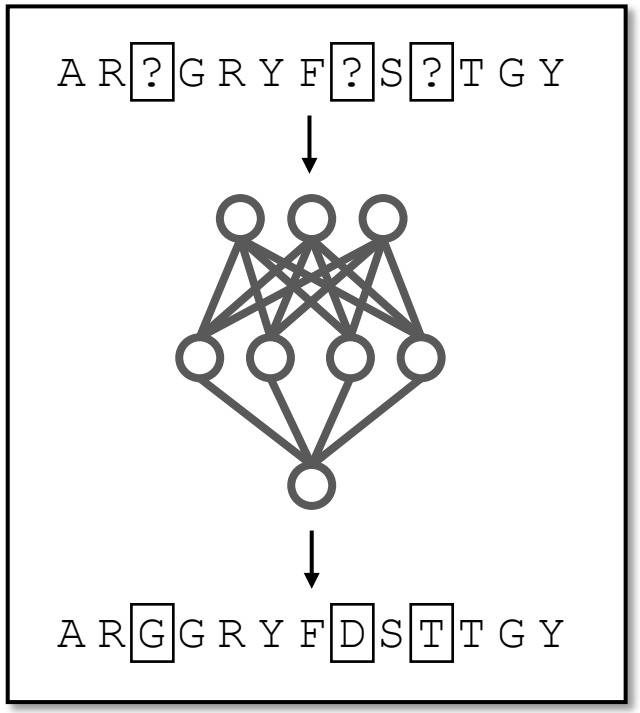
Optimizer AI Model is Trained to Predict Fully Human CDR Sequences

Antibody Database

cAb-Rep & OAS
Hu Ig databases

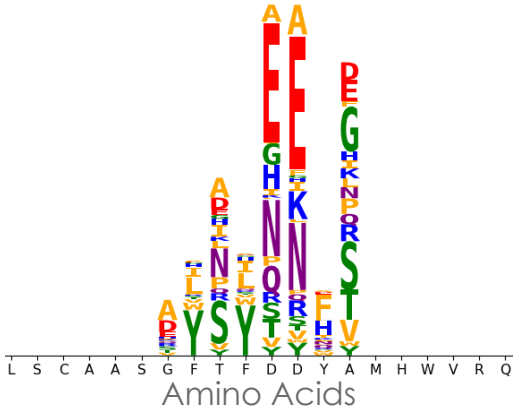
>1 billion curated human antibody sequences

Optimizer AI



AI trained to predict fully human CDR from masked CDR

Trained Model

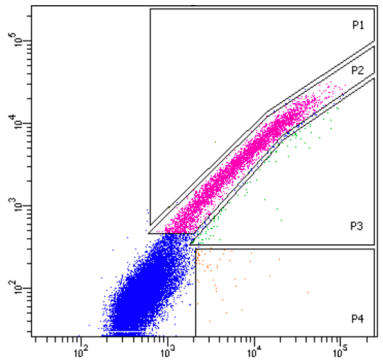


Predict library of fully human CDRs from template CDR

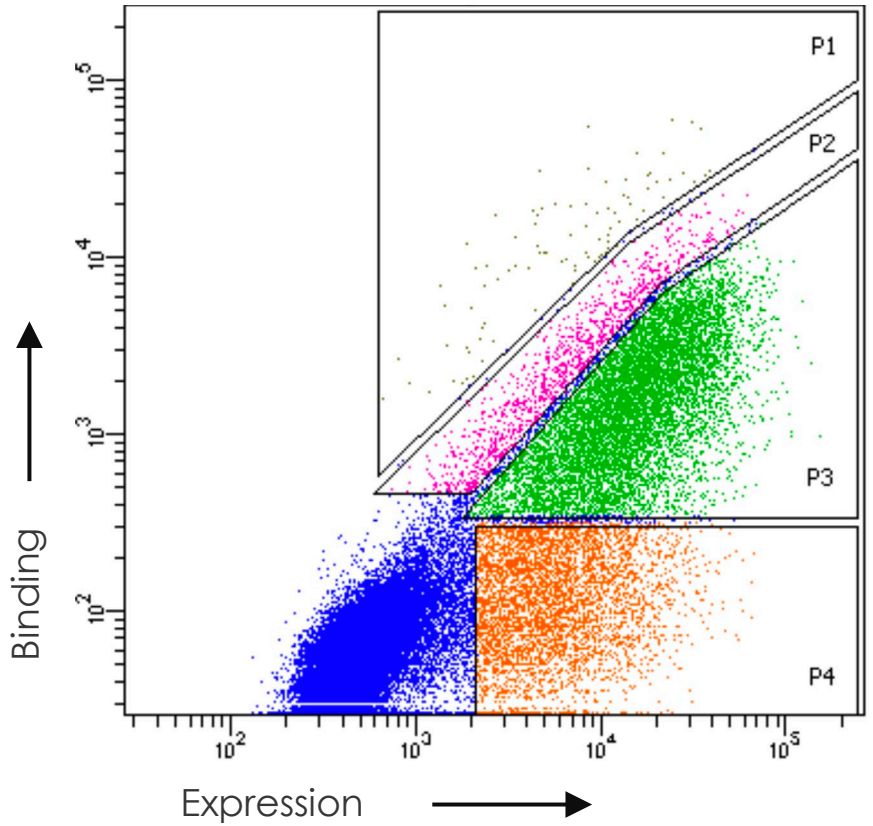
StableHu Library Sorting and NGS Identify Improved Human CDR Variants

Mammalian Display Single-Cell Sorting

Template Ab



StableHu Library



Sorting Bin

- Binding > Template
- Binding ≈ Template
- Binding < Template
- No Binding

Sorted Bins
NGS &
HT SPR Screen

Binding Scores Are Used to Rank Hits and Train Predictive Models for Further Optimization if Needed

Mammalian-Display sorted clones →

Scored Sequences

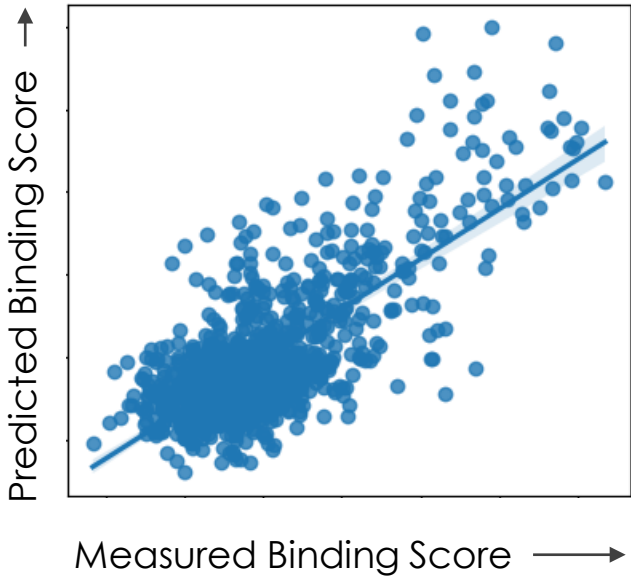
Rank	Sequence	Score
1	QQSYSTGPPT	2.45
2	QQSYGNPPT	2.35
3	QQGYSSPAT	2.32
4	QQSYSEPTT	2.31

⋮

Score: sorting bin and/or affinity

→

Predictive Model



Train, then test model on hold-out set

→ HT Screen for further optimized variants

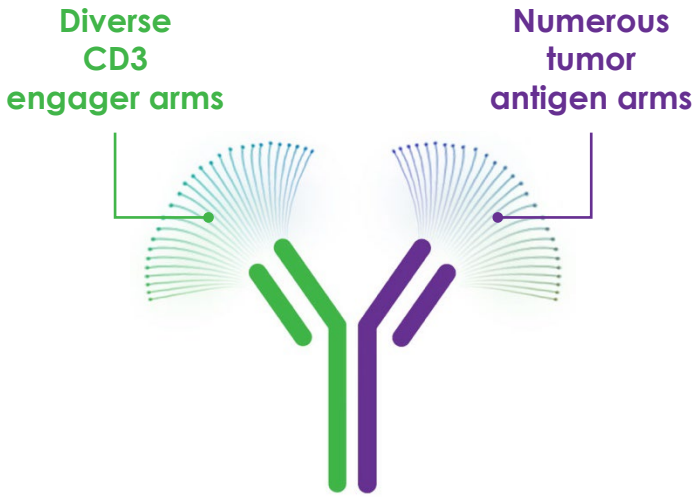
CD3 T Cell Engager Arm

Anti-CD3 T Cell Agonist

Key Challenges of CD3 T Cell Engager Discovery

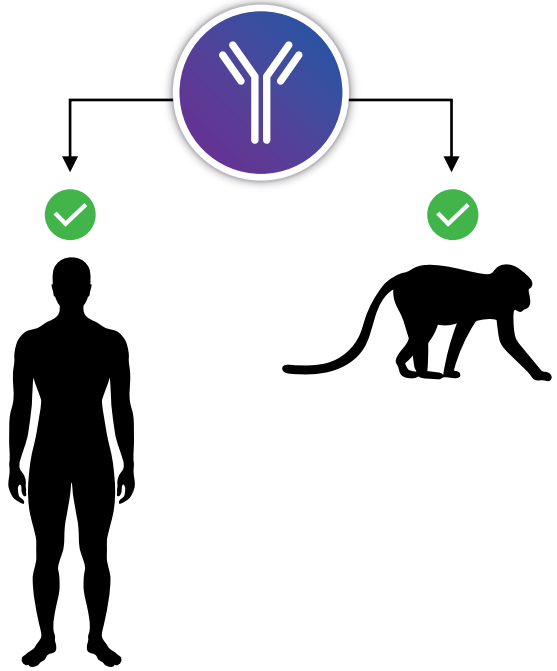
1 Sequence Diversity

Broad CD3 activity for optimized paring with tumor antigen arms



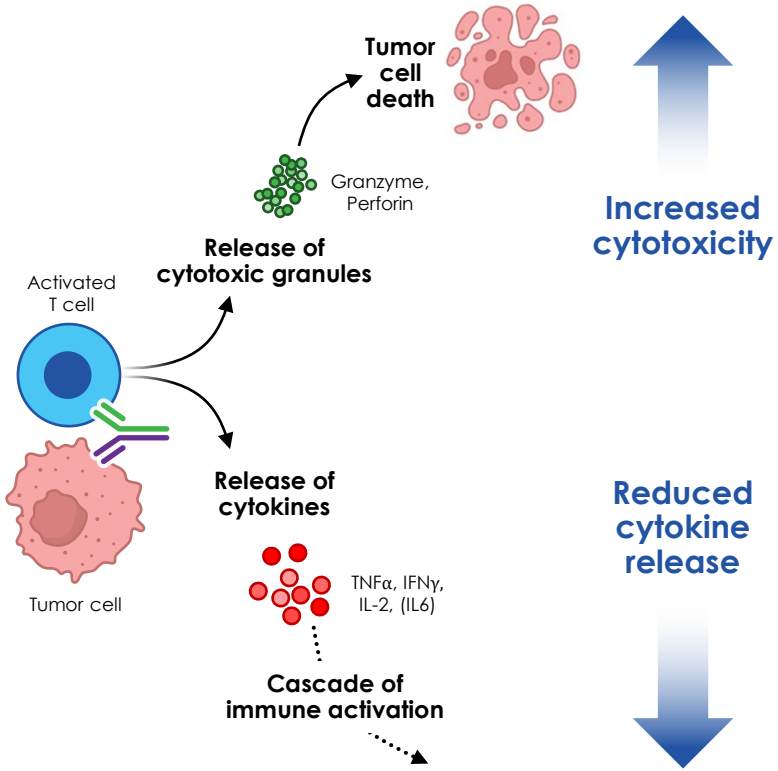
2 Hu + Cyno Cross-Reactivity

Risk reduction via cyno monkey toxicity study compatibility



3 Range of Cytokine Release

Tailored cytokine release for expanded therapeutic window



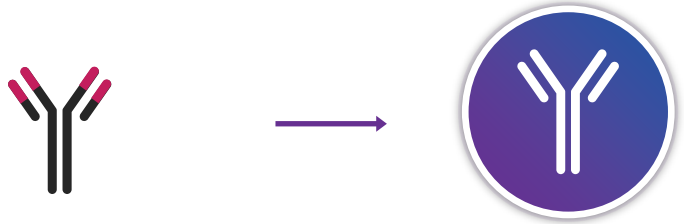
Dual Approaches to a Diverse Panel of Anti-CD3 Antibodies

AI Discovery Engine

Engineered-Epitope Immunization & Screening

AI-Engineered Immunogens → Epitope & T Cell Immunization → Epitope & CD3 Screen

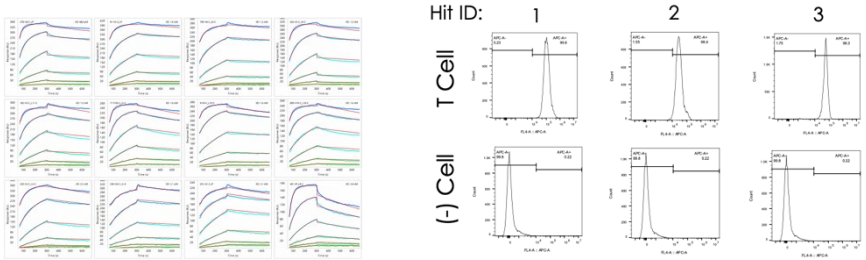
StableHu Optimizer



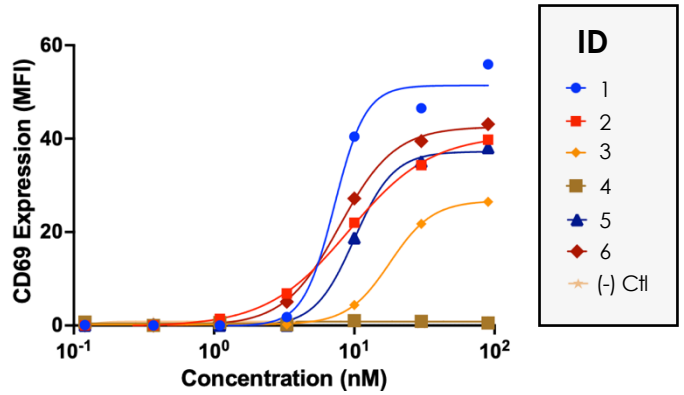
Template Antibody → Optimized Antibodies

SCREEN

Hu + Cyno CD3 & T Cell Binding



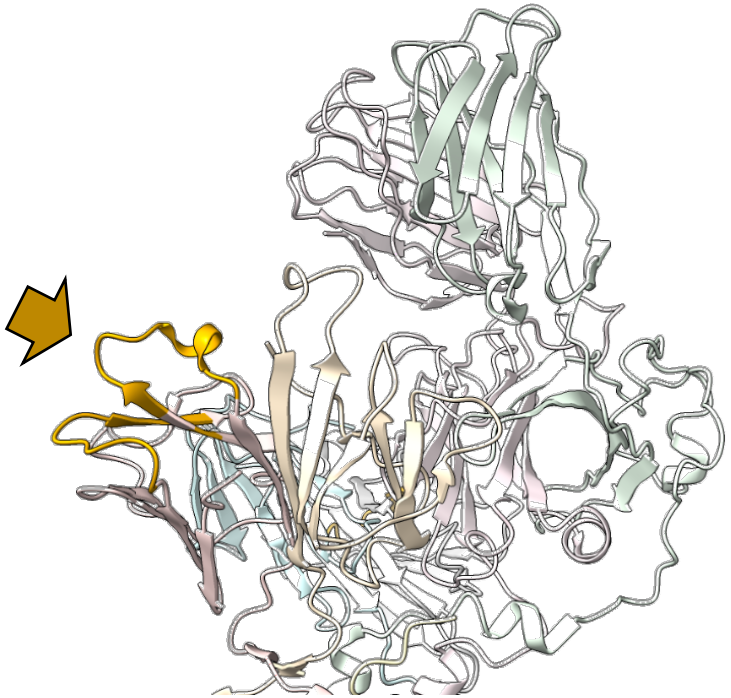
T Cell Activation



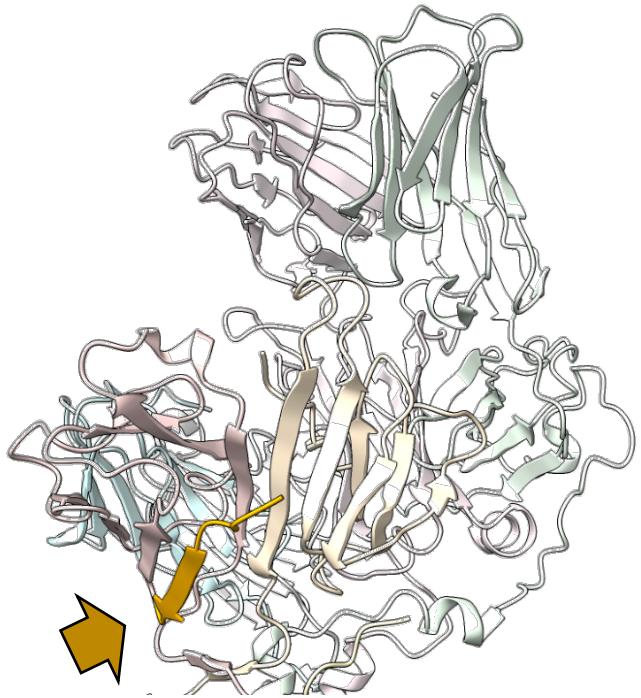
Epitope Engineering for TCR Accessibility & Hu + Cyno Cross-Reactivity

CD3 target epitopes in the context of the full TCR

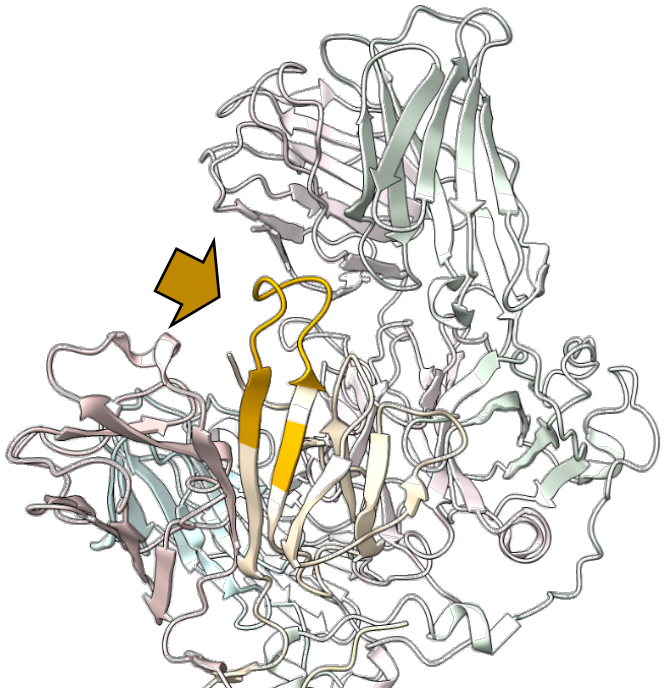
Epitope 1



Epitope 2

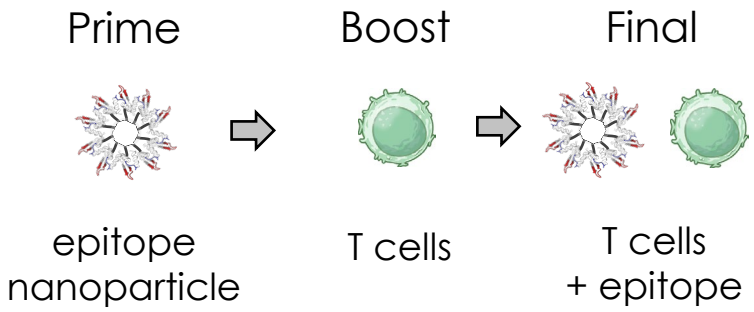


Epitope 3



Immunized CD3 Repertoires Were Cloned and Screened in Mammalian Display

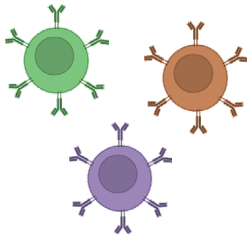
1. Epitope-Steered Immunization



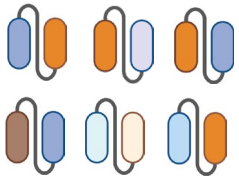
Epitope-Steered Immunized Repertoire

repertoire cloning

2. Mammalian Display



Phage Display 2X Rounds



1-sorting round

3. Multi-Modal Screening

Single-Cell Screen:
1. Epitope binding
2. CD3 binding
3. Ab expression
4. Poly-Specificity

NGS,
SPR,
In vitro activity

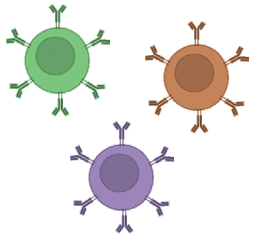
Mammalian Display Sorting for Human + Cyno CD3 Binding & Enhanced Ab Expression

Epitope-Steered Immunized Repertoire

repertoire cloning

2 libraries

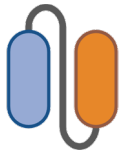
Mammalian Display



Phage Display 2X Rounds

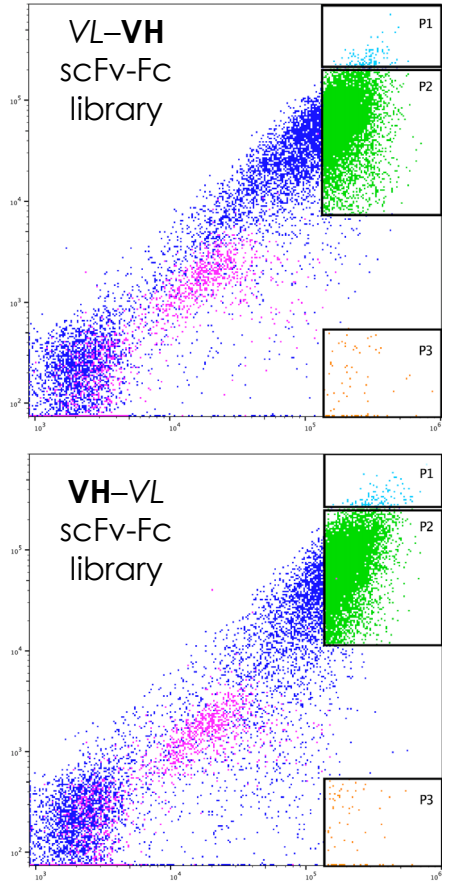
VL–**VH**

VH–*VL*



cell sorting

CD3 Binding



Antibody Expression

P1: High-expression, high-binder

P2: High-expression, mid-binder

P3: High-expression, non-binder

CD3 Reference Ab (SP34 KD = 10 nM)

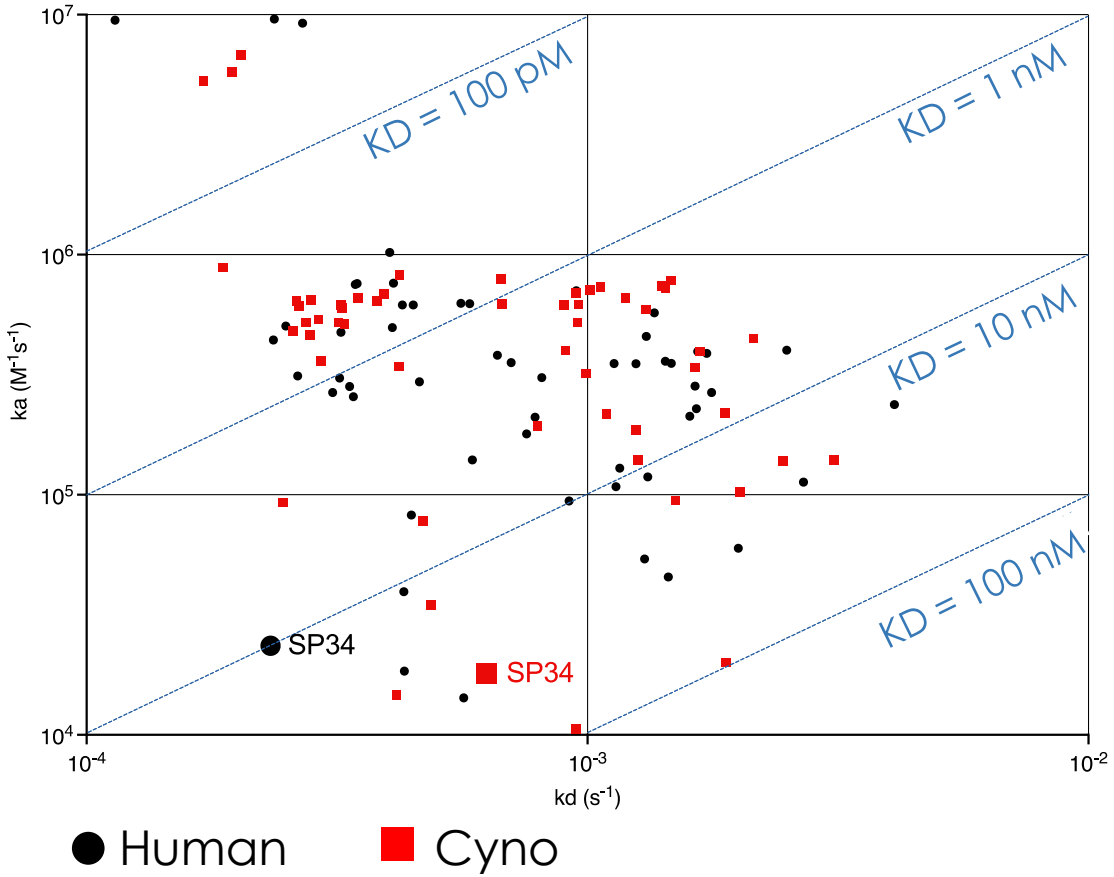
Hit: P1 NGS enrichment ≥ 5

P1 NGS Enrichment =
(P1 Clone Count) / (P3 Clone Count)

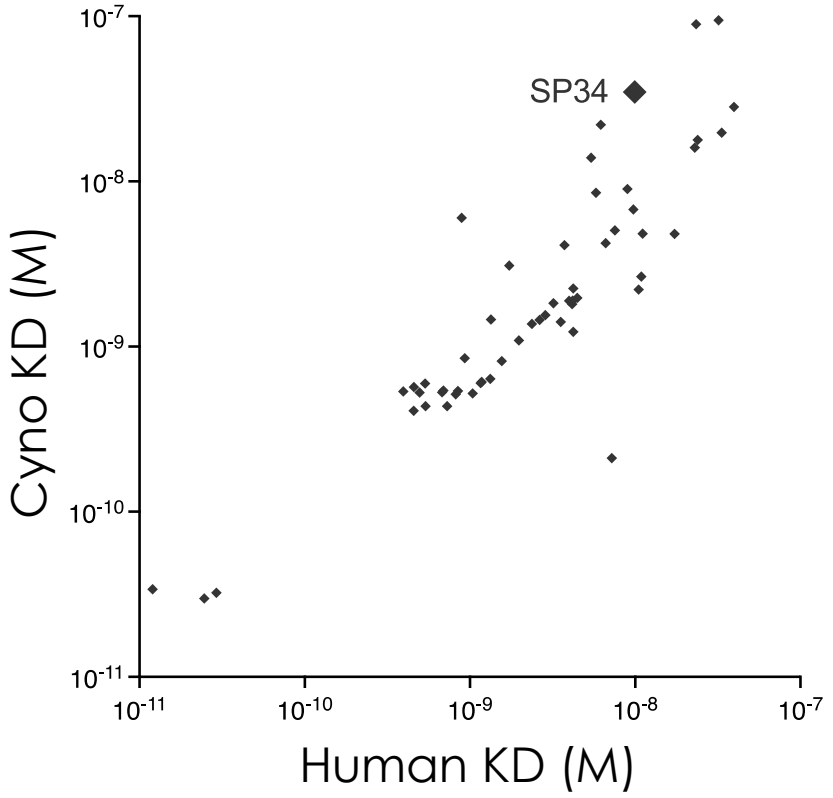
Epitope-Steered Immunization Identifies Human+ Cyno CD3 10^4 Affinity Range Binders

Human vs Cyno CD3ED HT-SPR Affinity

54 hits bind human and cyno CD3
Affinity range KD = 10s pM ~ 100 nM

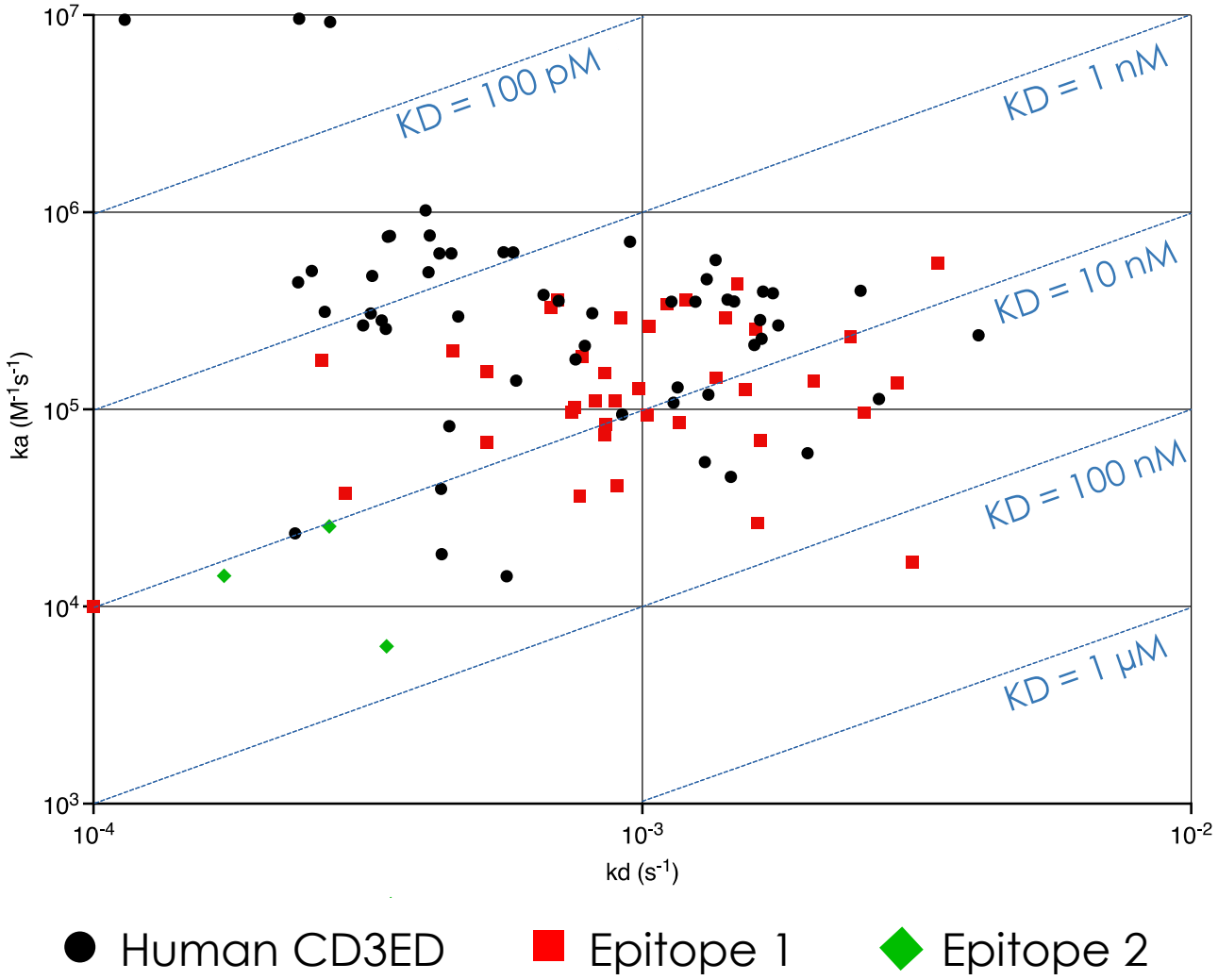


Most hits have comparable affinity for human and cyno CD3



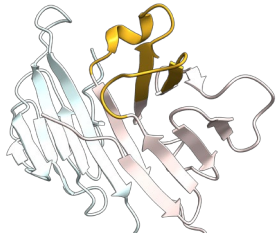
39/54 = 72% Human + Cyno CD3 Cross-Reactive Hits Bind Engineered Epitopes

Human **CD3ED**, **Epitopes 1, 2** HT-SPR Affinity

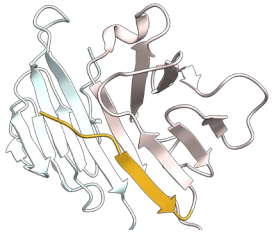


- All engineered-epitopes identified epitope-specific antibodies
- Epitopes 1 & 2 identified Hu + Cyno cross-reactive antibodies meeting affinity threshold of KD ≤ 100 nM
- Epitope 1 is the most productive, potentially due to high accessibility

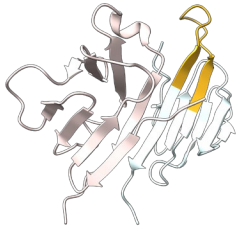
Epitope 1



Epitope 2

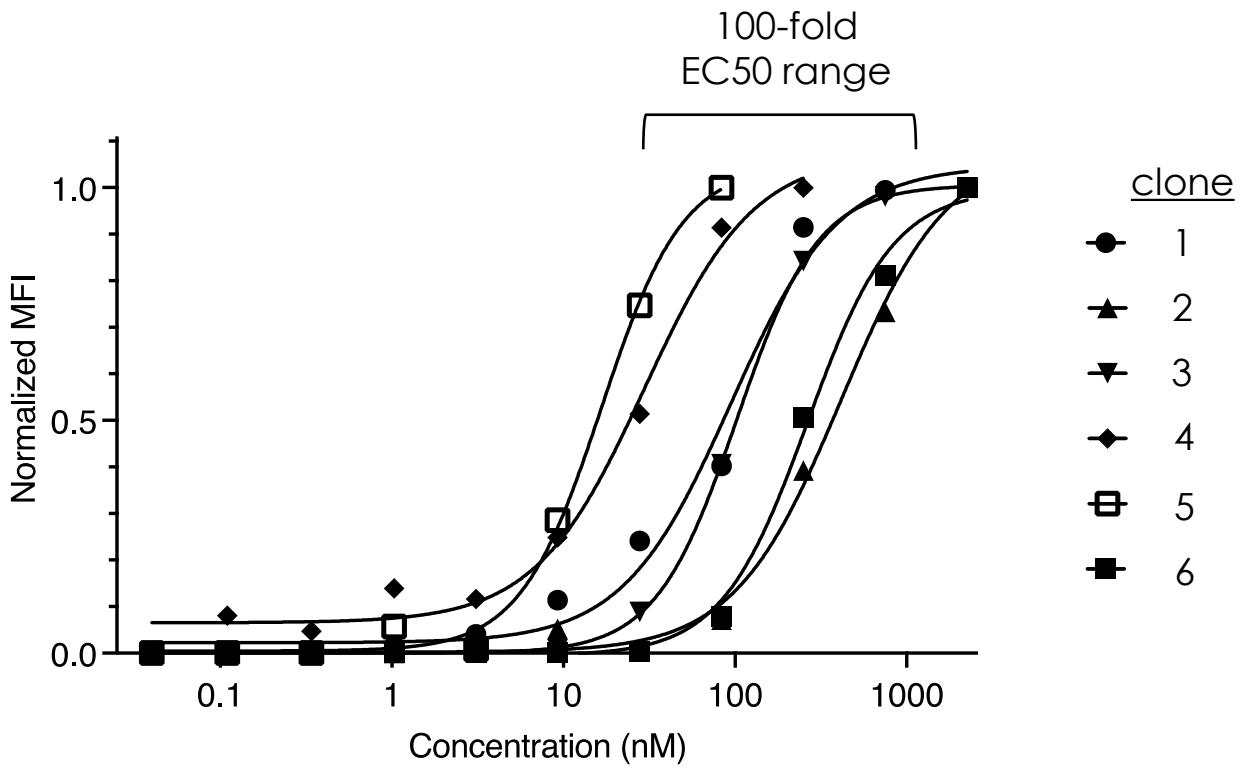


Epitope 3

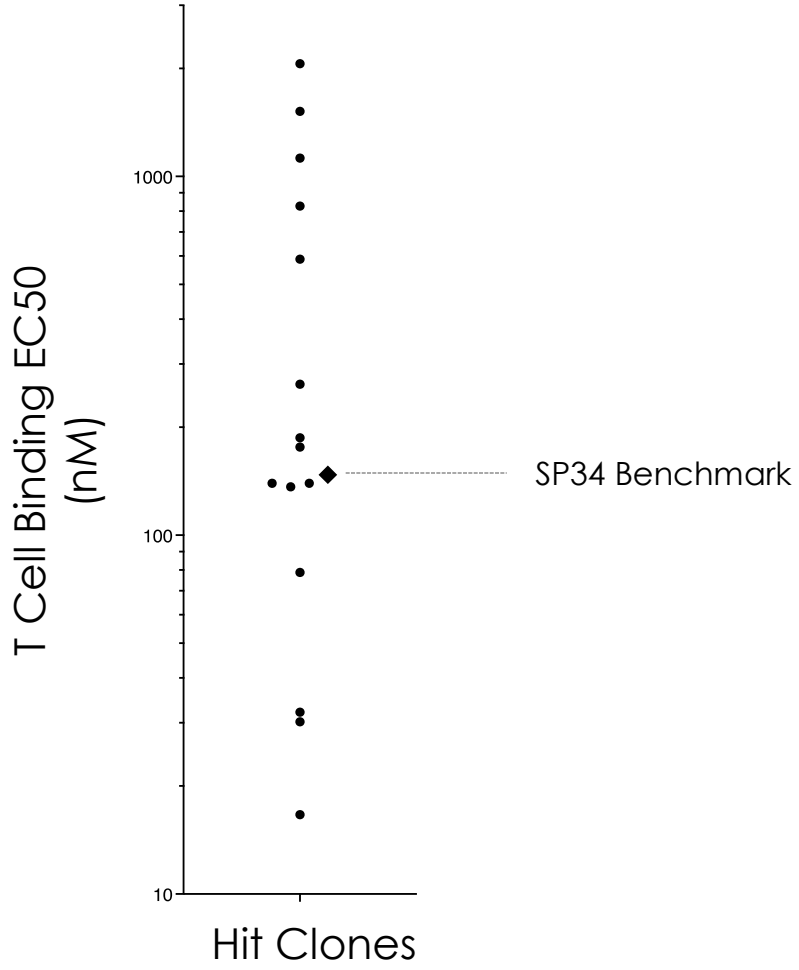


Human T Cell Screen Identifies 22/54 Hits That Bind Cells Across a Broad EC50 Range

Representative Subset of
T Cell Binding Hits



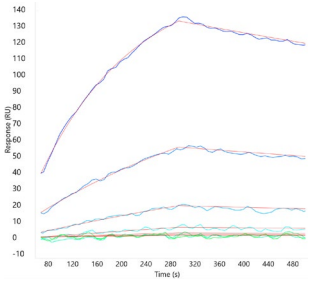
T Cell Binding
Hit Panel

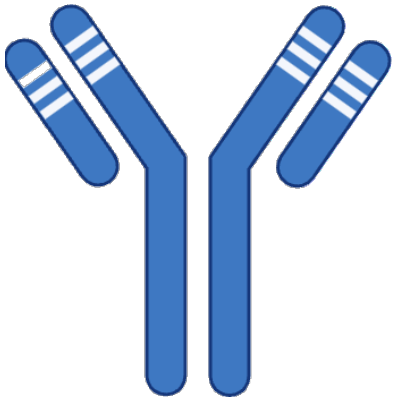


Anti-CD3 Template Antibody Human Diversification with StableHu AI

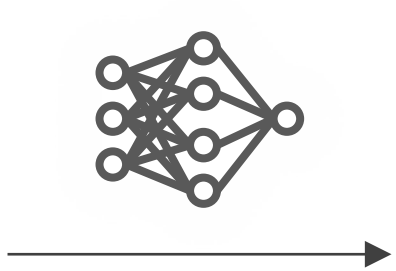
1. Anti-CD3 Ab template with mouse CDRs

KD =
10 nM



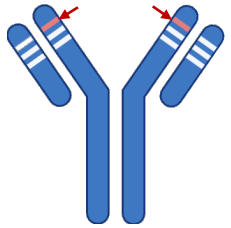


2. AI-model predicts human CDRs

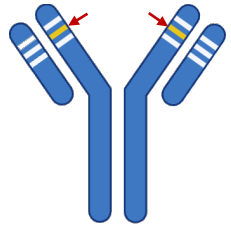


3. Human heavy & light chain CDR diversity libraries

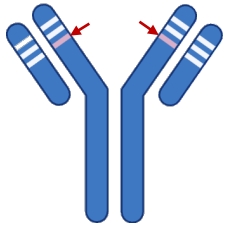
HCDR1
2000



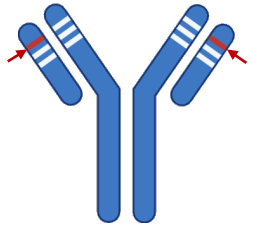
HCDR2
2000



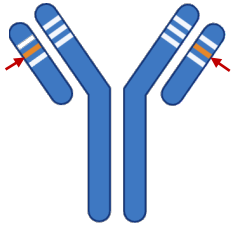
HCDR3
2000



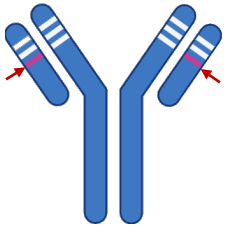
LCDR1
2000



LCDR2
1000

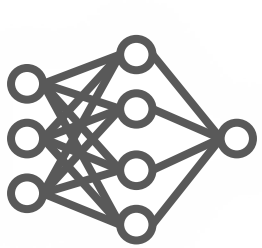


LCDR3
2000



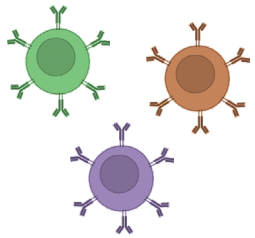
Mammalian Display Sorting for Human + Cyno CD3 Binding & Enhanced Ab Expression

StableHu AI



repertoire

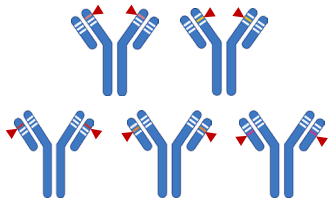
Mammalian Display



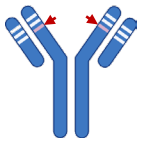
two libraries

cell sorting

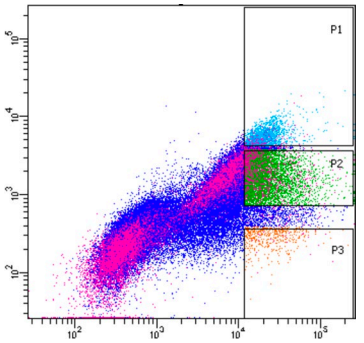
HCDR1,2
LCDR1,2,3
pooled



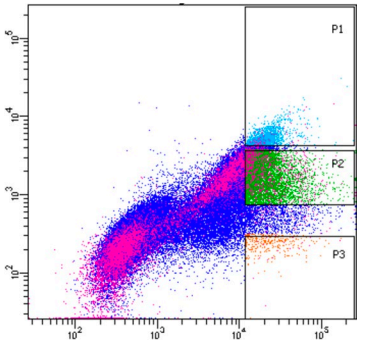
HCDR3



HCDR1,2 LCDR1,2,3
pooled diversity library



HCDR3
diversity library



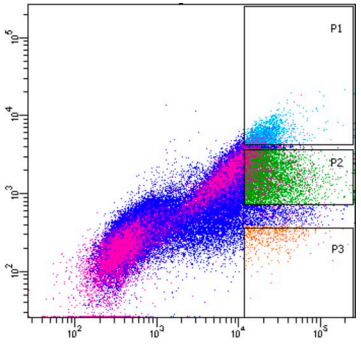
CD3 Binding

Antibody Expression

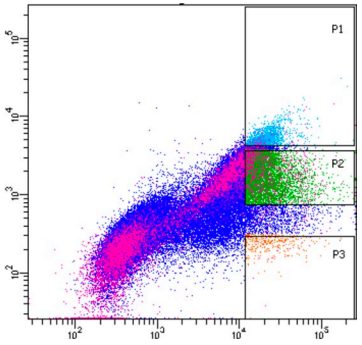
- P1: High-expression, high-binder
- P2: High-expression, mid-binder
- P3: High-expression, non-binder
- CD3 Template Ab (KD = 10 nM)

Individual CDR Hits from First Cell-Sort Generate Combinatorial Multi-CDR Diversity Library

HCDR1,2 LCDR1,2,3
pooled diversity library



HCDR3
diversity library



CD3 Binding

Antibody Expression

Cell sorting
NGS

P1: High-expression, high-binder

P3: High-expression, non-binder

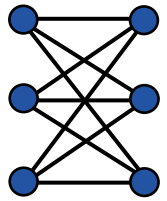
CDR hit: P1 NGS enrichment ≥ 5

P1 NGS Enrichment =
(P1 Clone Count) / (P3 Clone Count)

Generate & screen Mammalian-Display combinatorial library of CDR hits

Heavy Chain CDR Hits

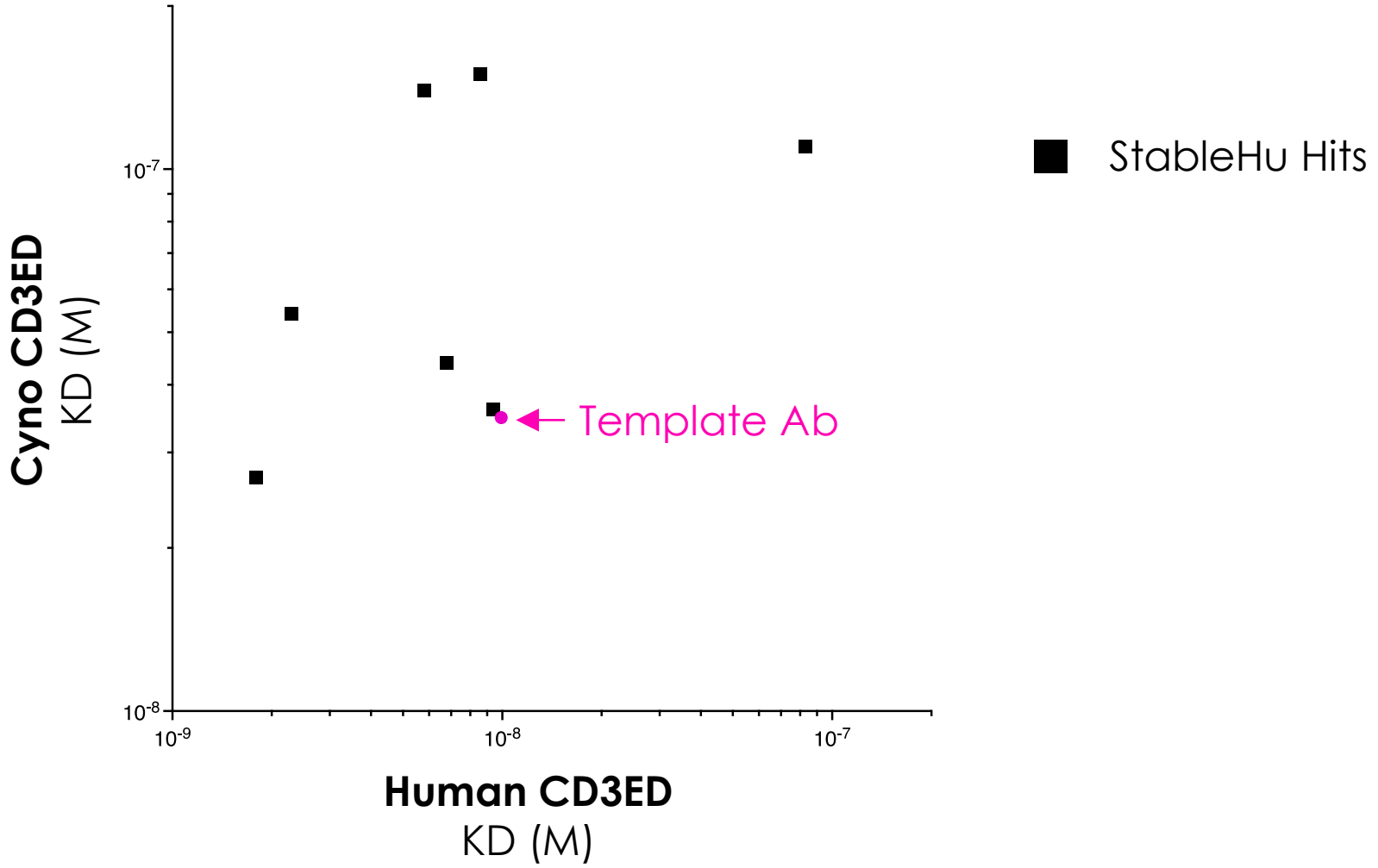
HCDR1	HCDR2	HCDR3
GFTFTSRGVS	IIPIFGTI	ARGGNWNQFDY
GFTFTSRGVS	IIPIFGTI	TRRGNWNPFEN
GFTFTSRGVS	IIPIFGTI	TRRGNWNPFDY



Light Chain CDR Hits

LCDR1	LCDR2	LCDR3
QSIGSY	SAS	QQSYSTPPT
QSVSSG	DAS	QQSYSTPPT
QSVSSG	AAS	QQSYSTLPT

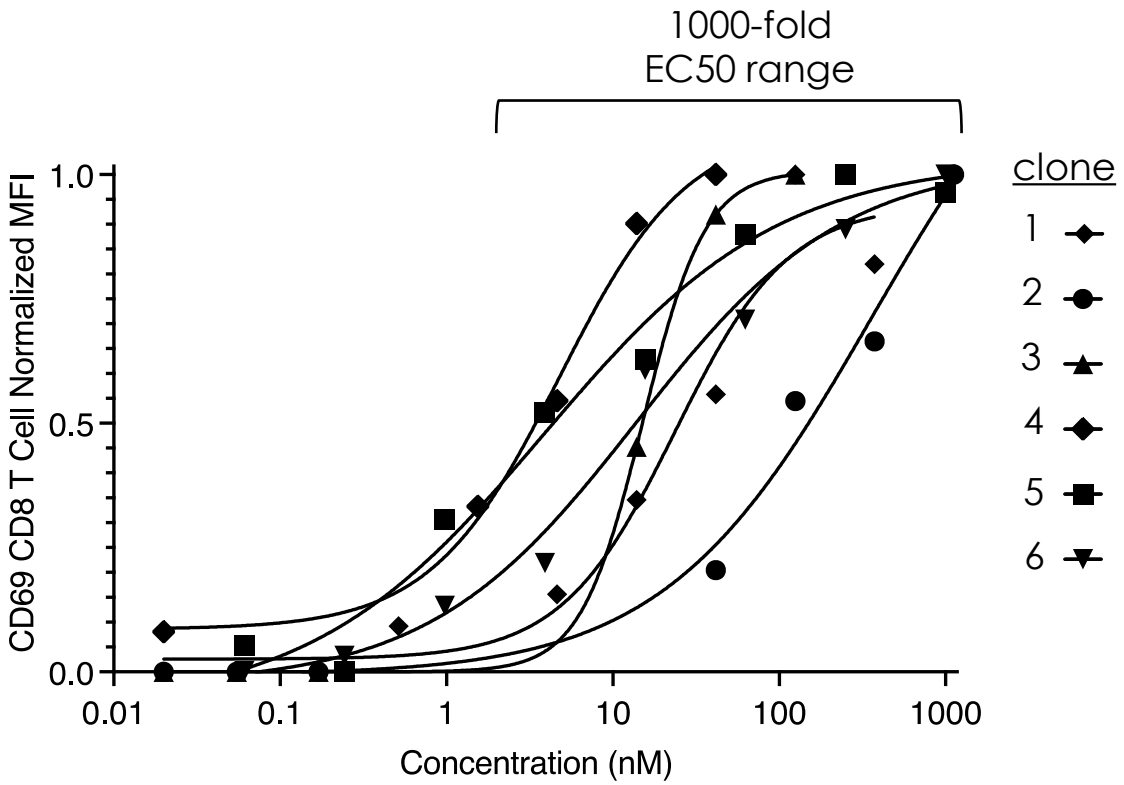
StableHu Library Screening Identifies
7 Hu + Cyno CD3 Cross-Reactive Hits Across a Broad Range of Affinity



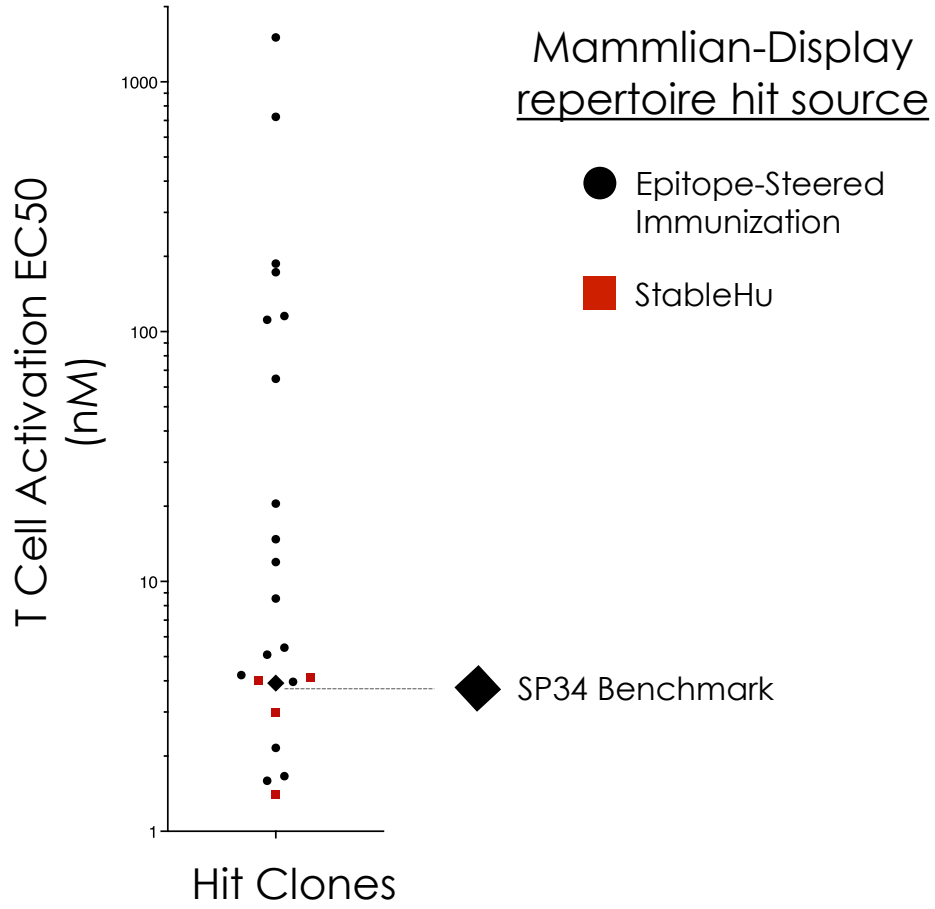
Dual-Track Discovery Identifies 22 Hits That Activate T Cells Across a Broad EC50 Range

Combined mammalian-display hit panel: Epitope-steered immunization and StableHu

Representative Subset of
T Cell Activation Hits



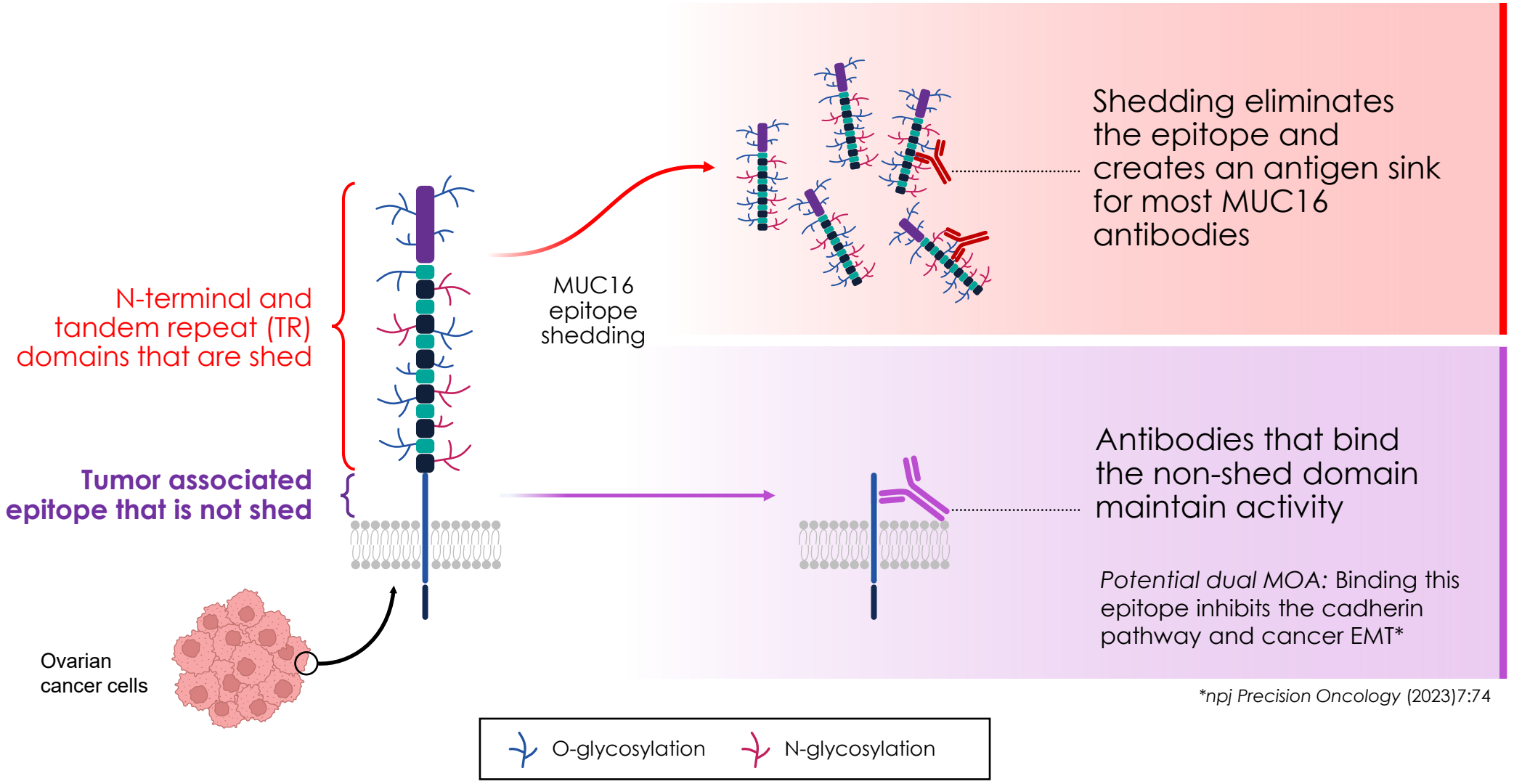
T Cell Activation
Hit Panel



Tumor Associated Antigen Arm

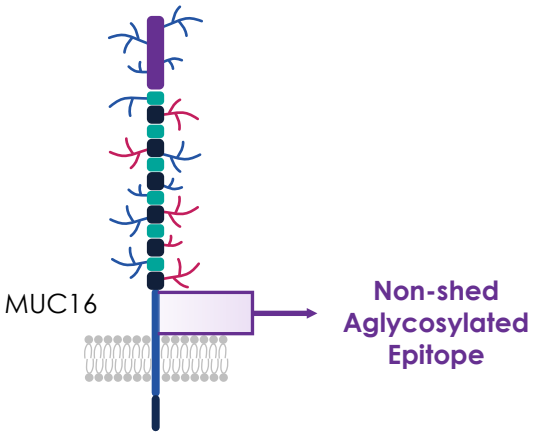
Non-Shed Epitope Anti-MUC16 Antibody

MUC16 Is Overexpressed and Shed by Tumor Cells



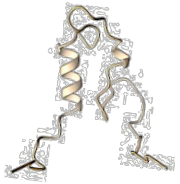
**npj Precision Oncology* (2023)7:74

Immunizations Were Steered to a MUC16 Epitope that Avoids Epitope Shedding

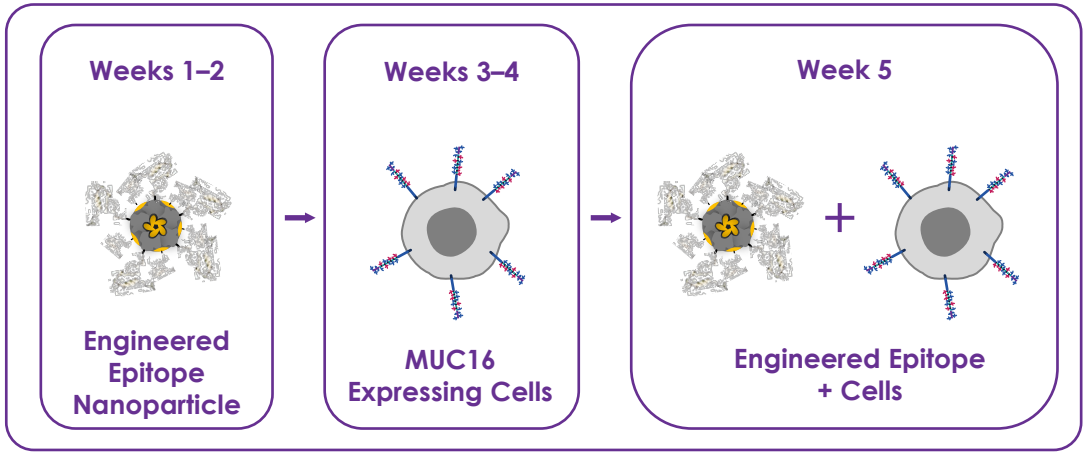


AI Discovery Engine

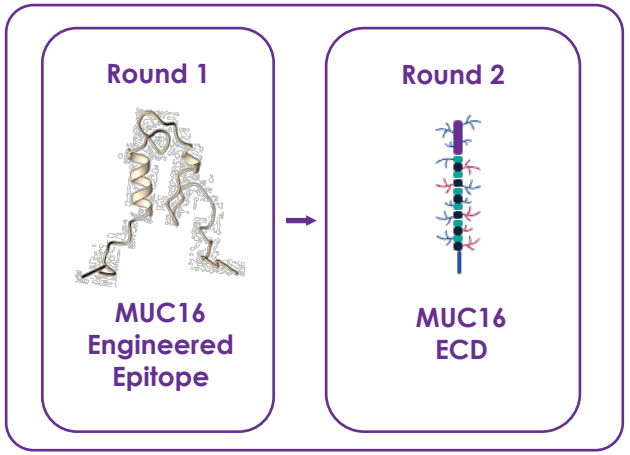
MUC16 Engineered Epitope



Epitope-Steered Immunization & Screening



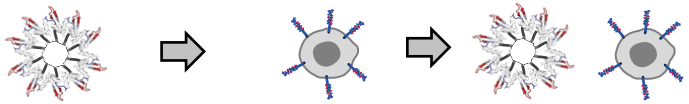
Epitope-Steered Naïve In Vitro Selection



Immunized MUC16 Repertoires Were Cloned and Screened in Mammalian Display

1. Epitope-Steered Immunization

Prime → Boost → Final



epitope nanoparticle

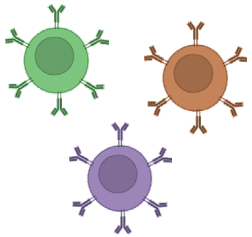
MUC16 cells

cells + epitope

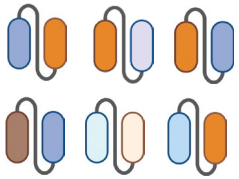
Epitope-Steered Immunized Repertoire

repertoire cloning

2. Mammalian Display



Phage Display 2X Rounds



1-sorting round

3. Multi-Modal Screening

Single-Cell Screen:

1. Epitope binding
2. MUC16 binding
3. Ab expression
4. Poly-Specificity

NGS,
SPR,
In vitro activity

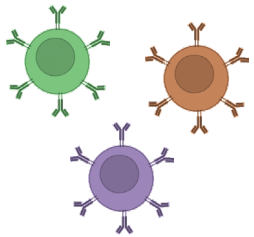
Mammalian Display Sorting for MUC16 Epitope Binding & Enhanced Ab Expression

Epitope-Steered Immunized Repertoire

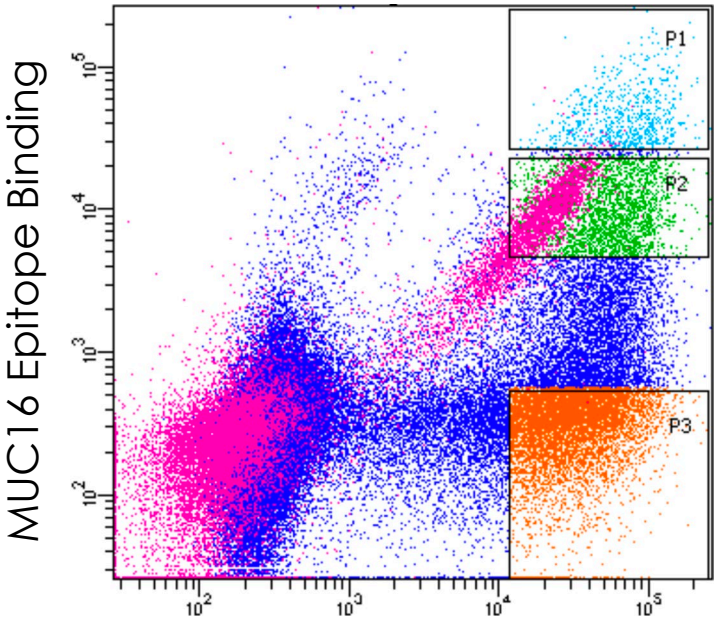
repertoire cloning

Phage Display 2X Rounds

Mammalian Display



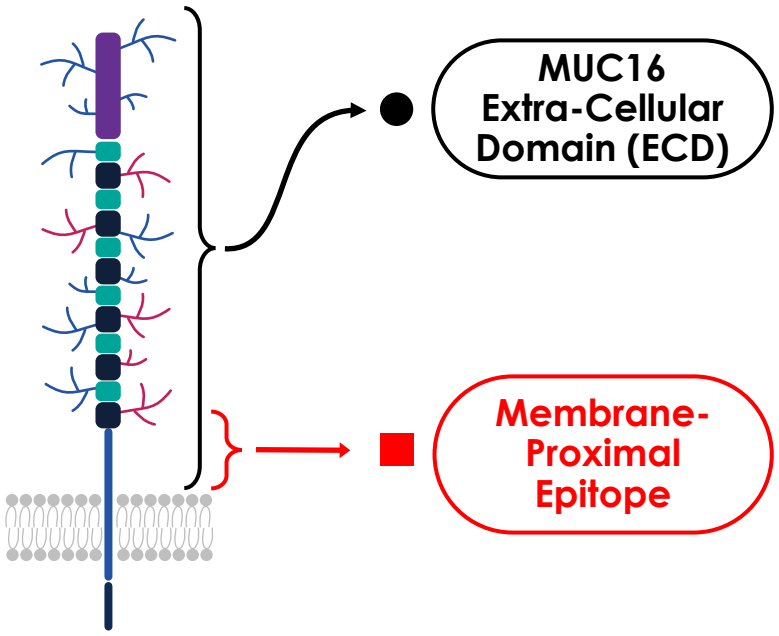
cell sorting

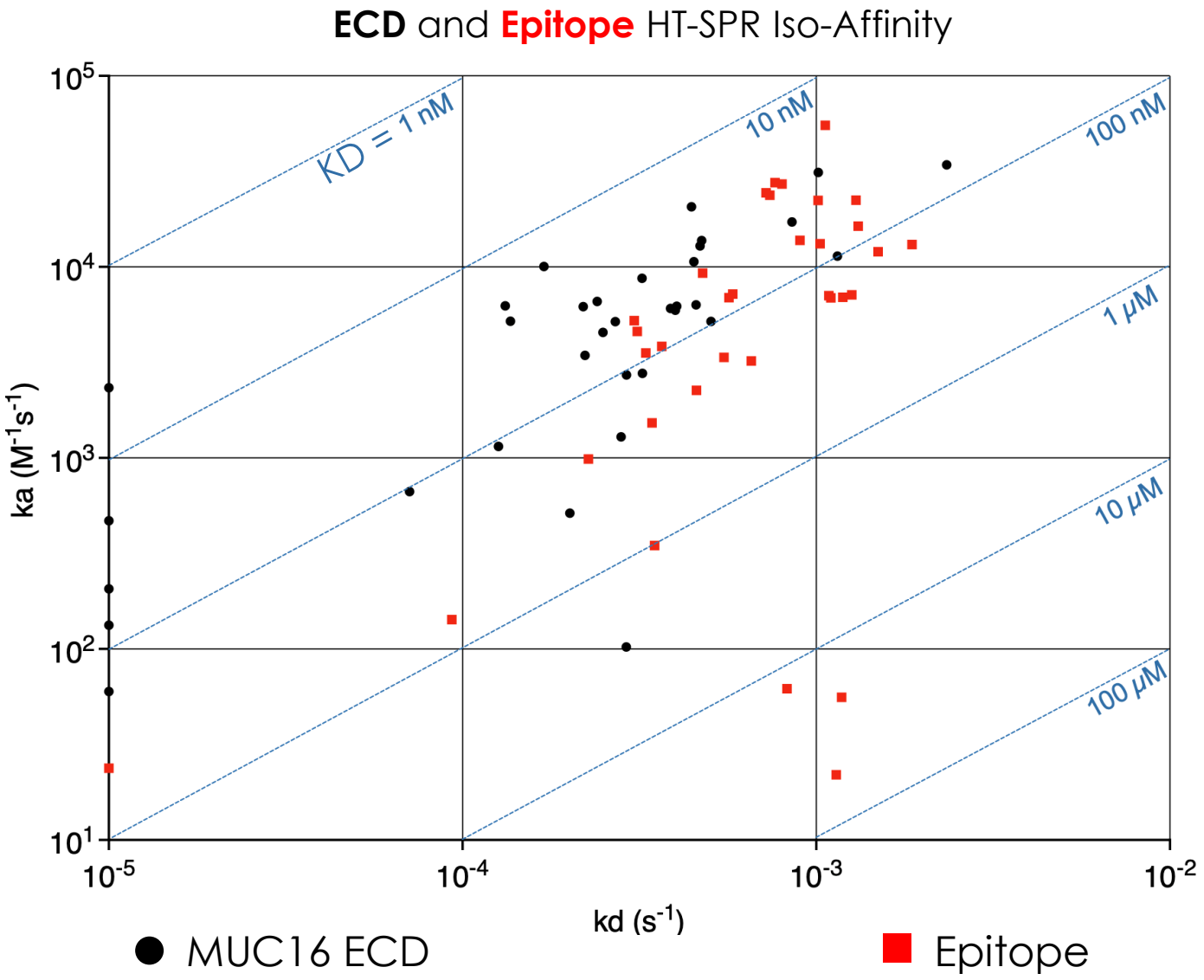


- P1: High-expression, high-binder
- P2: High-expression, mid-binder
- P3: High-expression, non-binder
- MUC16 Reference Ab (KD = 23 nM)

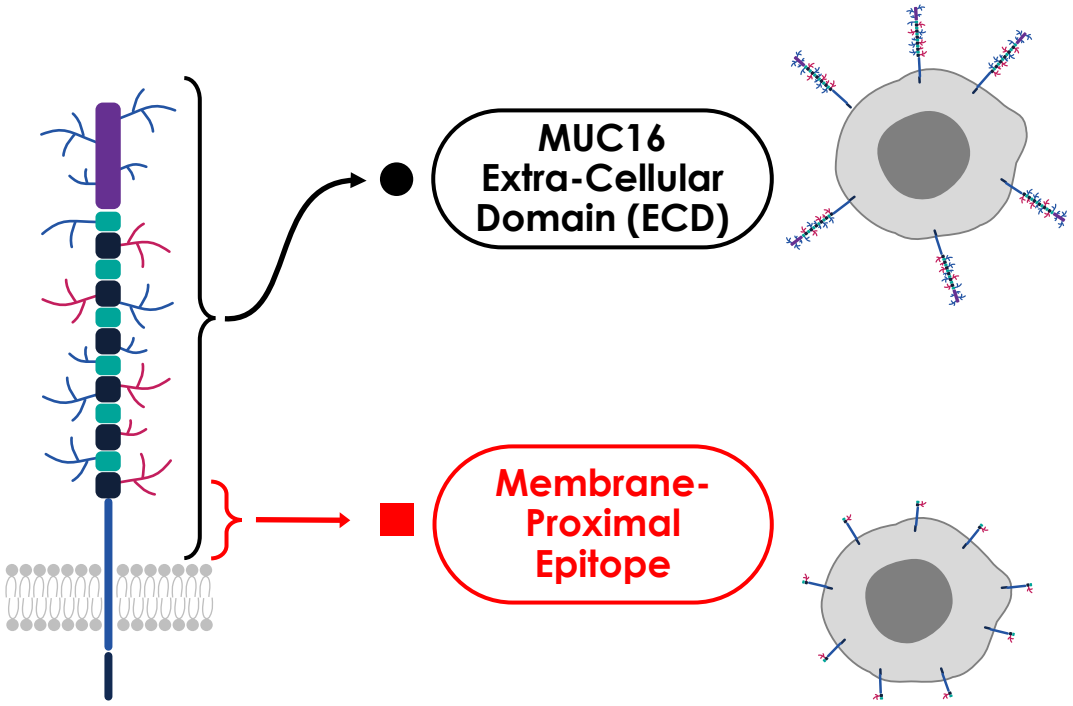
P1 NGS Enrichment =
(P1 Clone Count) / (P3 Clone Count)

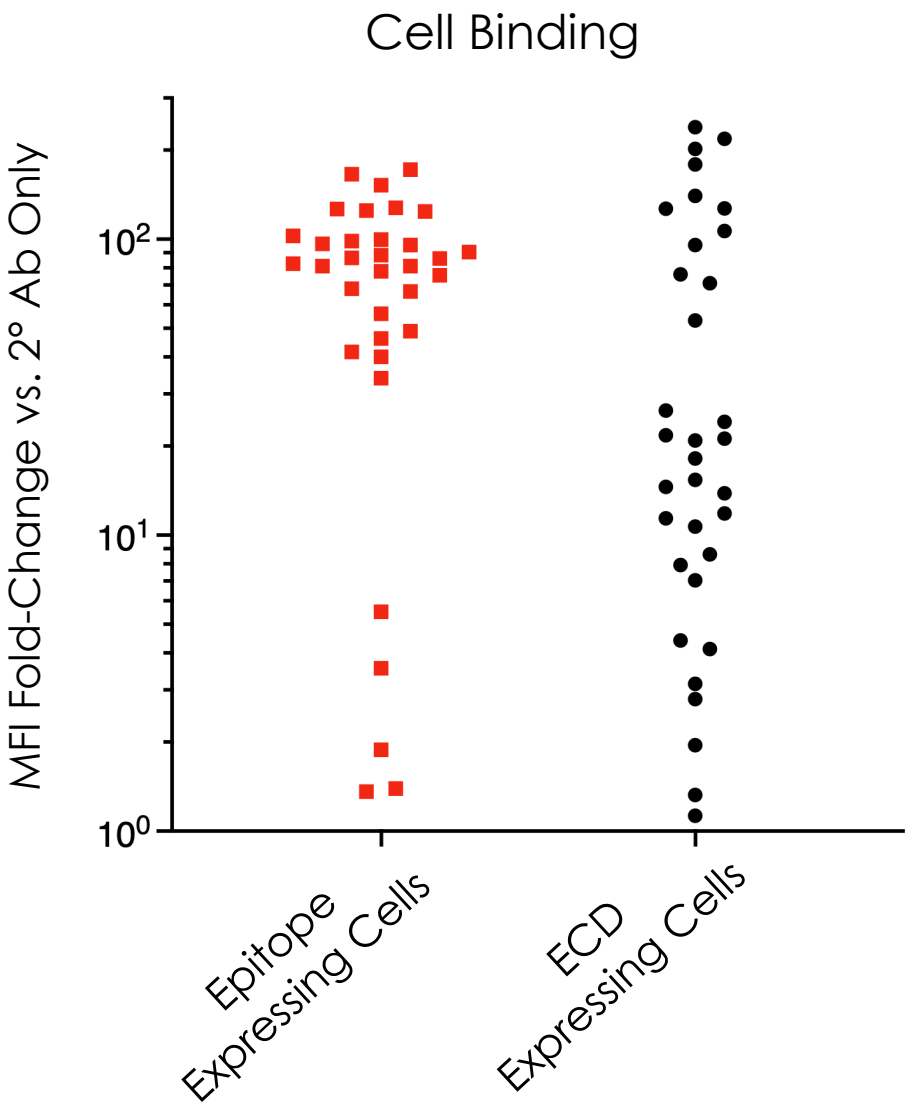
Dual-Track Discovery Identifies 34 Hits that Bind the MUC16 Epitope and ECD





34/34 Hits Bind MUC16 Membrane-Proximal Epitope and ECD Expressing Cells





Combining Arms: Anti-CD3 X Anti-MUC16

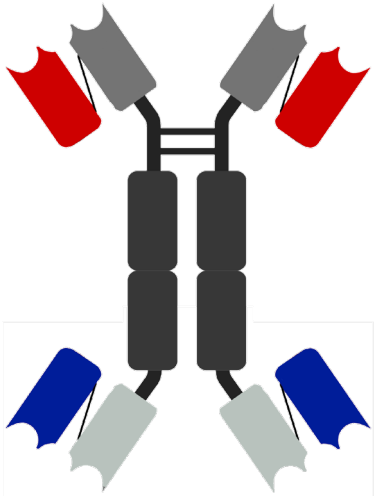
Bispecific T Cell Engager

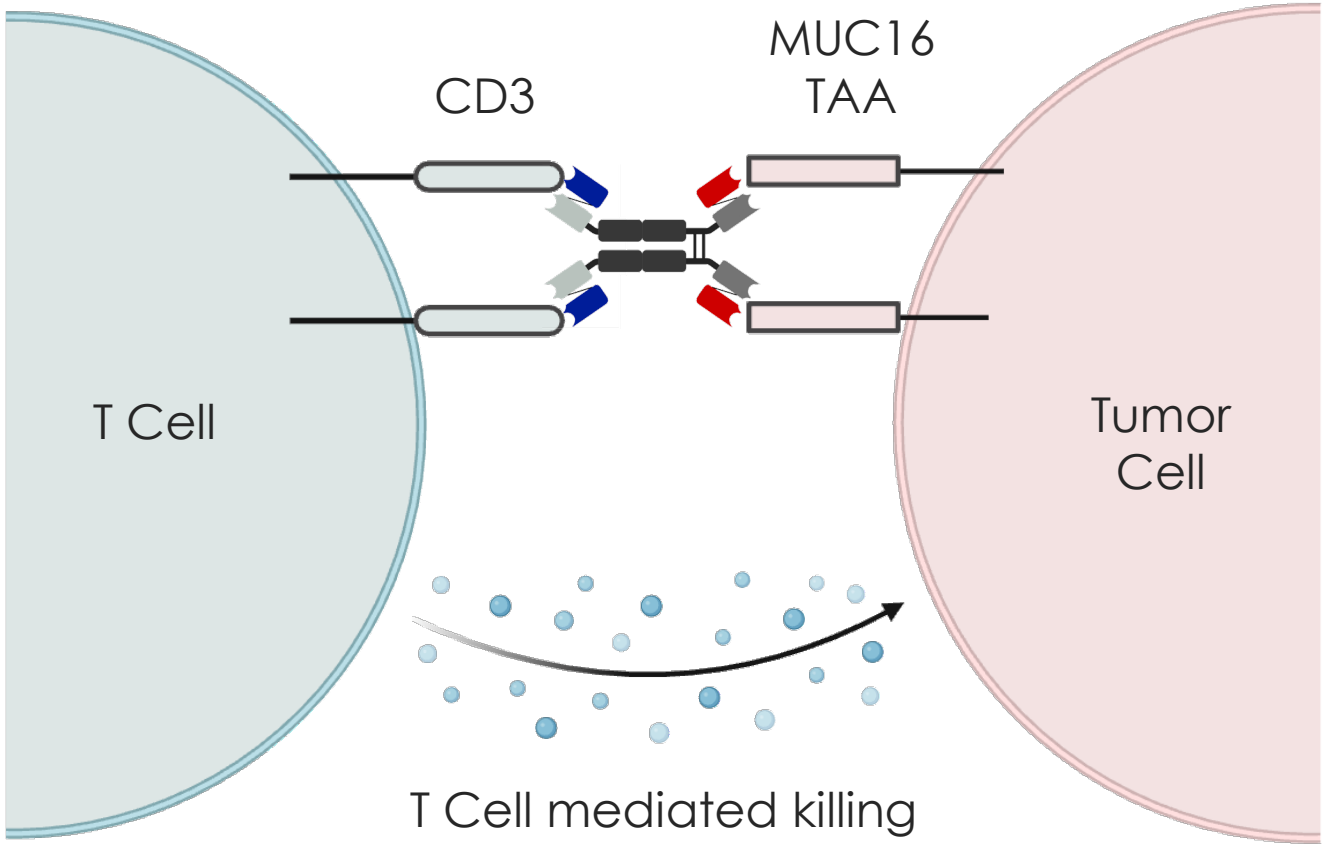
Anti-CD3 X MUC16 Bispecific T Cell Engagers Were Evaluated in 2x2 Format

scFv-Fc 2x2

Anti-MUC16 TAA

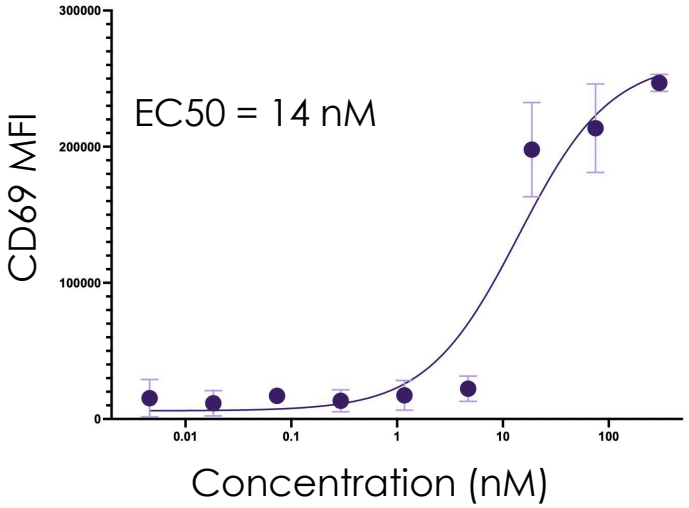
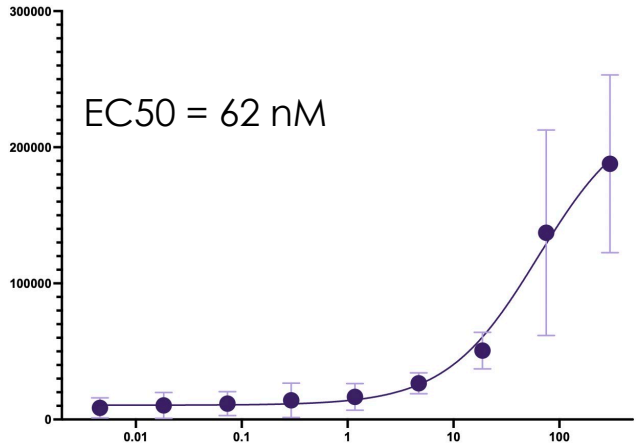
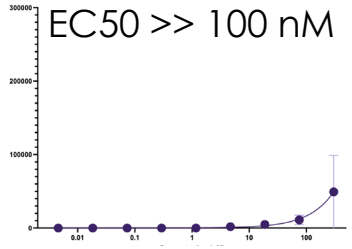
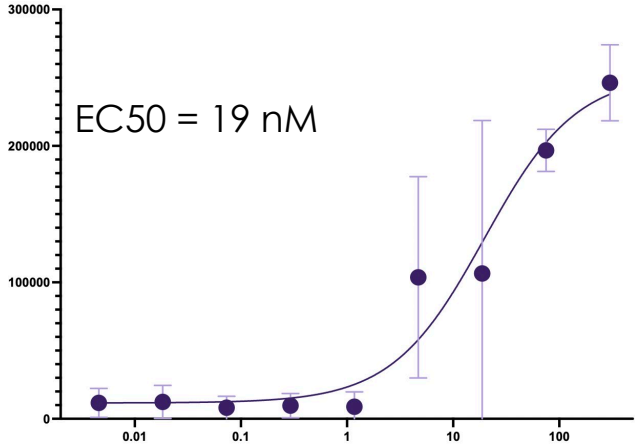
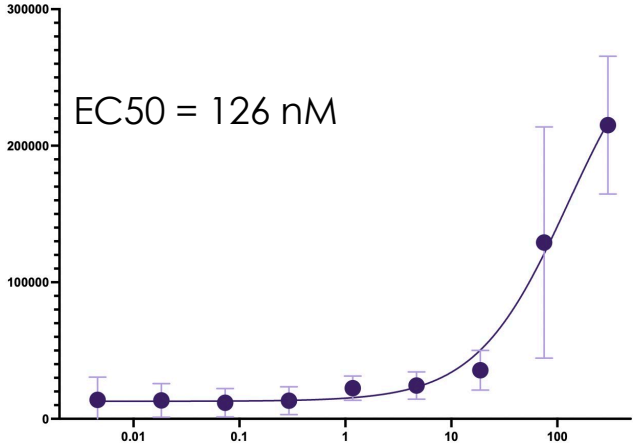
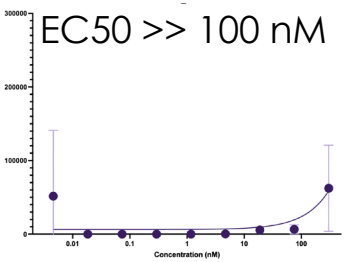
Anti-CD3





2X2 Anti-CD3 X MUC16 T Cell Engagers Stimulate T Cells in Donor PBMCs

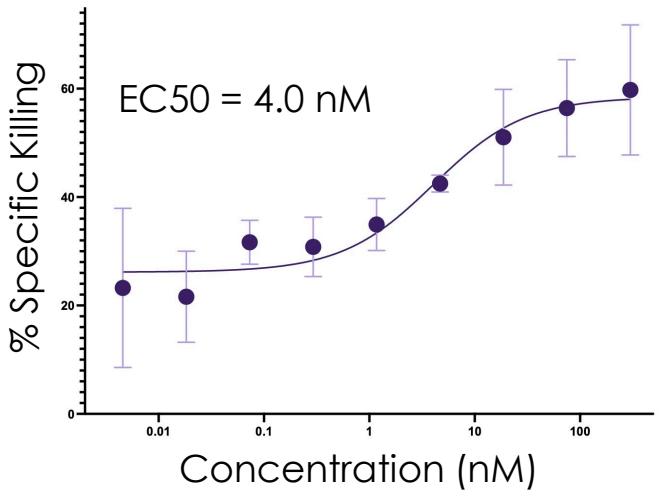
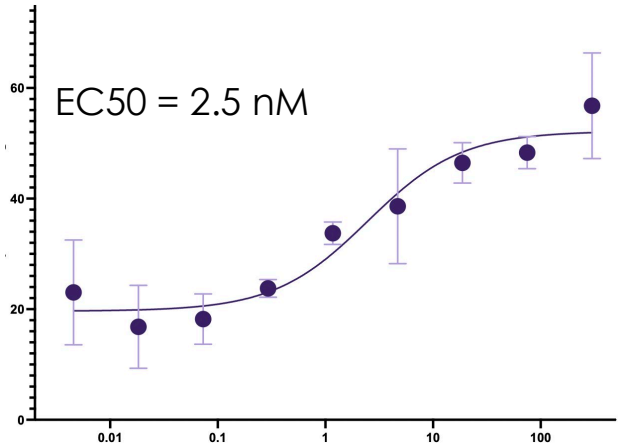
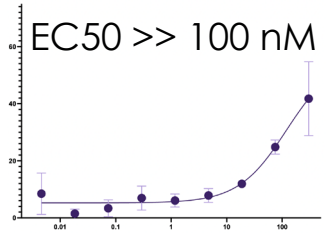
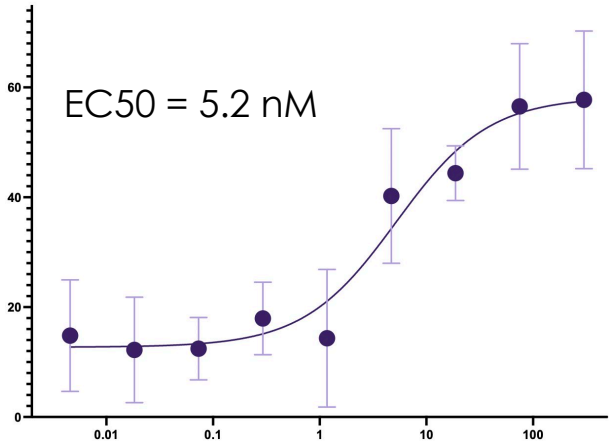
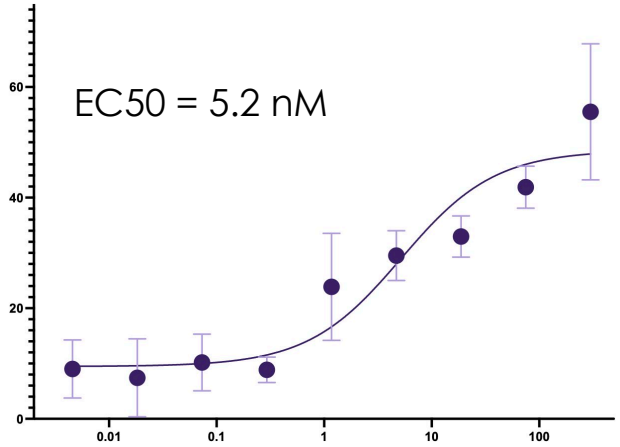
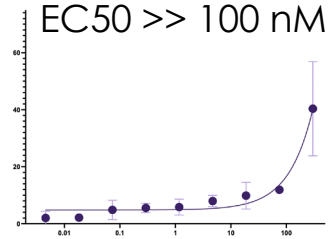
MUC16 Arms

CD3 Arms	MUC16 Arm 1	MUC16 Arm 2	(-)CD3 Arm only
Epitope-Steered Immunized Hit			
StableHu Hit			

2X2 Anti-CD3 X MUC16 T Cell Engagers Kill OVCAR-3 Ovarian Cancer Cells in Donor PBMCs

MUC16 Arms

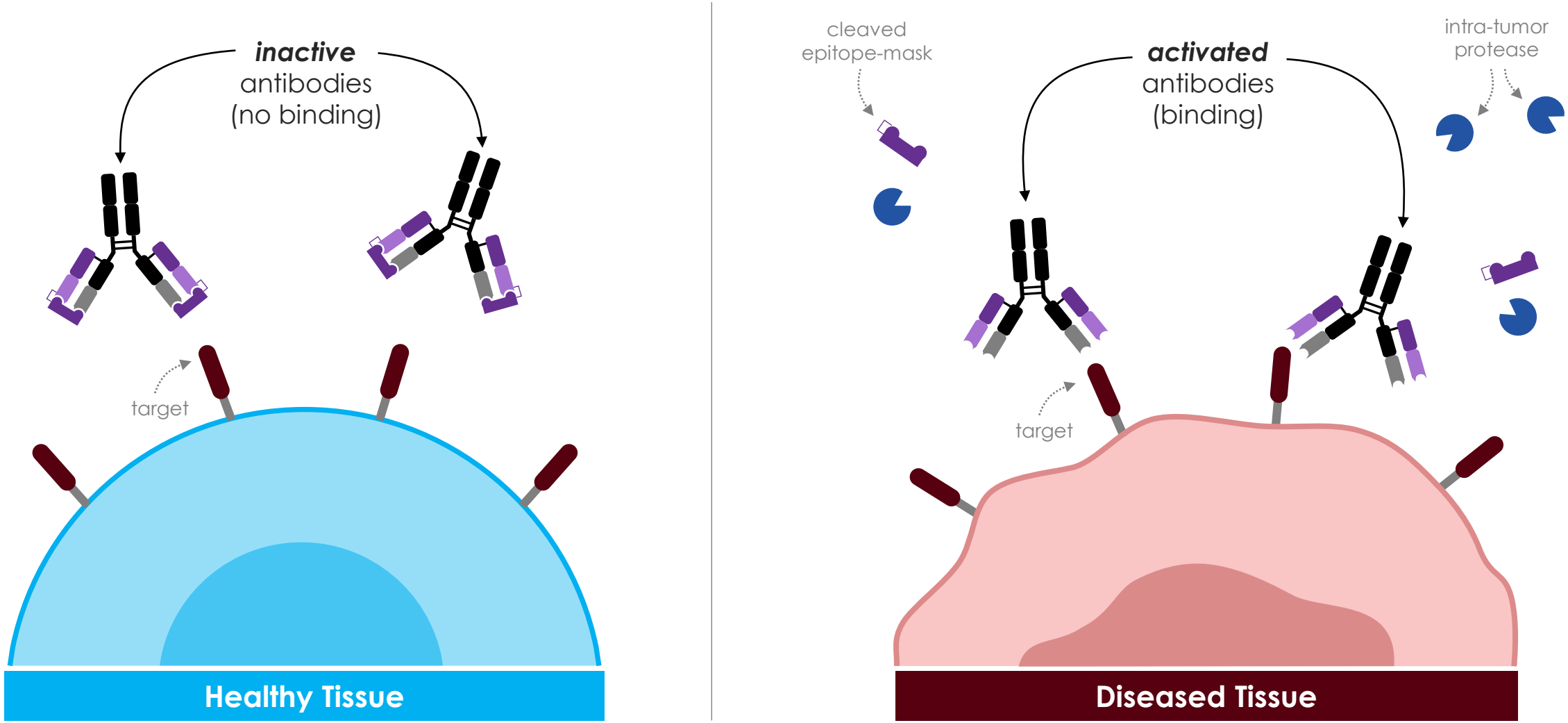
CD3 Arms

	MUC16 Arm 1	MUC16 Arm 2	(-)CD3 Arm only
Epitope-Steered Immunized Hit			
StableHu Hit			

Epitope-Targeted & Conditionally-Activated Anti-CD3 X MUC16

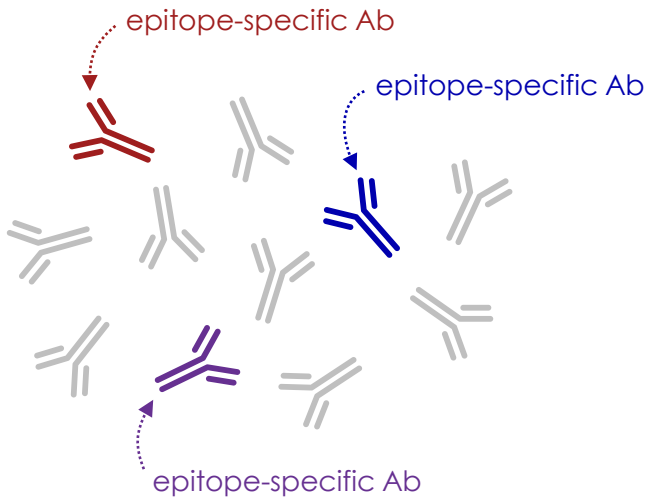
On-Target & On-Tissue T Cell Engager

Conditionally-Activated Antibodies Minimize On-Target, Off-Tissue Risks

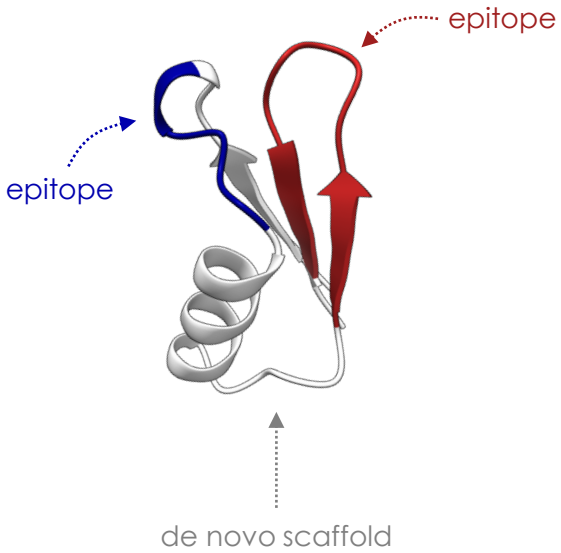


Single-Cycle Discovery of Conditionally-Activated Antibodies via Engineered Epitopes

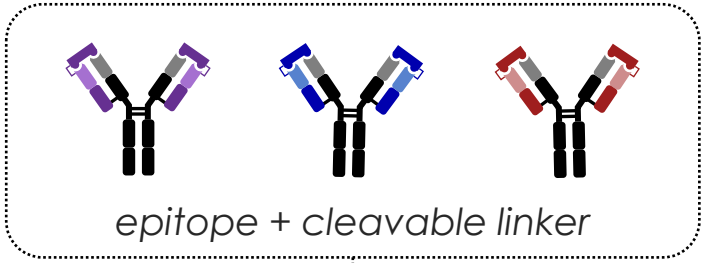
1 Naïve in vivo or in vitro antibody library

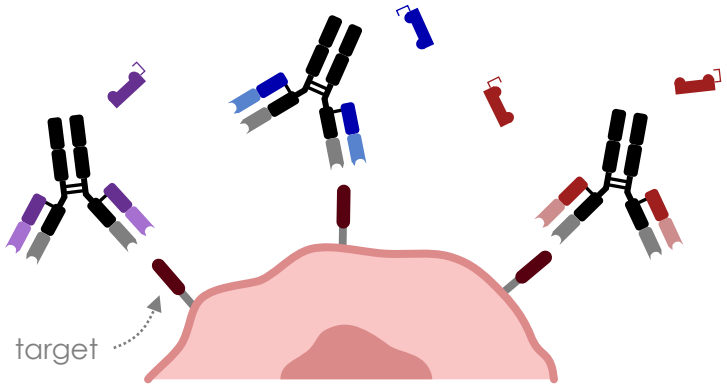


2 Focus library with engineered epitopes



3 Engineered-epitope conditionally-activated Ab



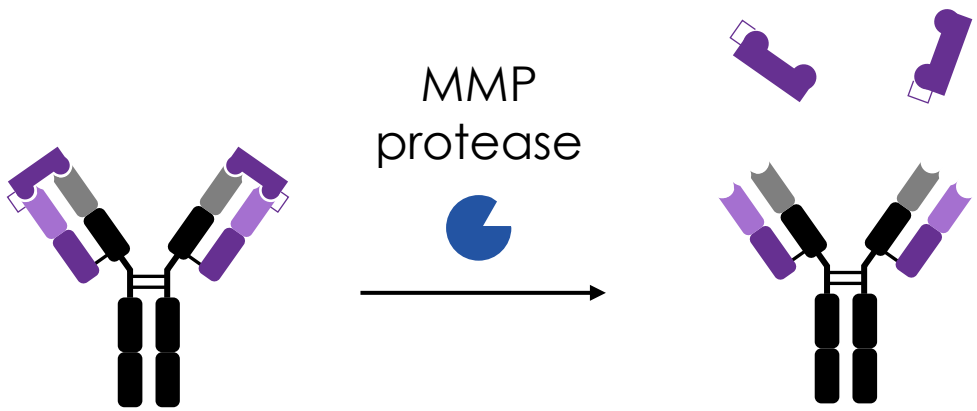


tumor microenvironment cleavage and Ab activation

Engineered Epitope Mask Conditionally Activates MUC16 and CD3 Hits

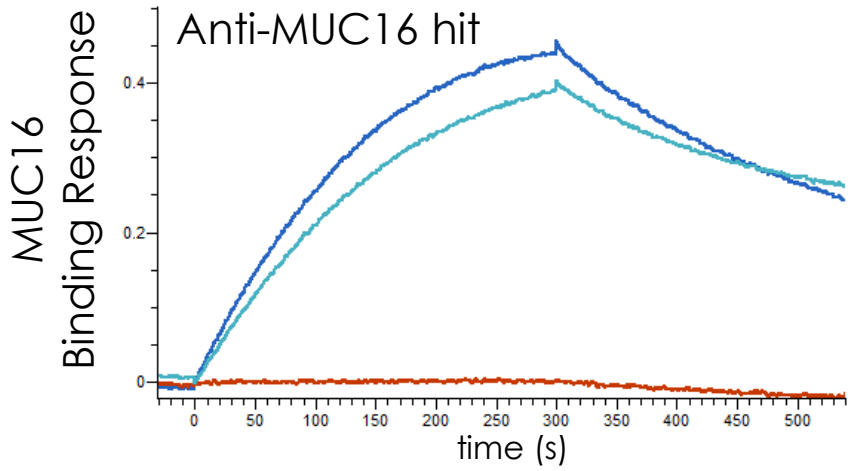
Engineered Epitope Mask Intact

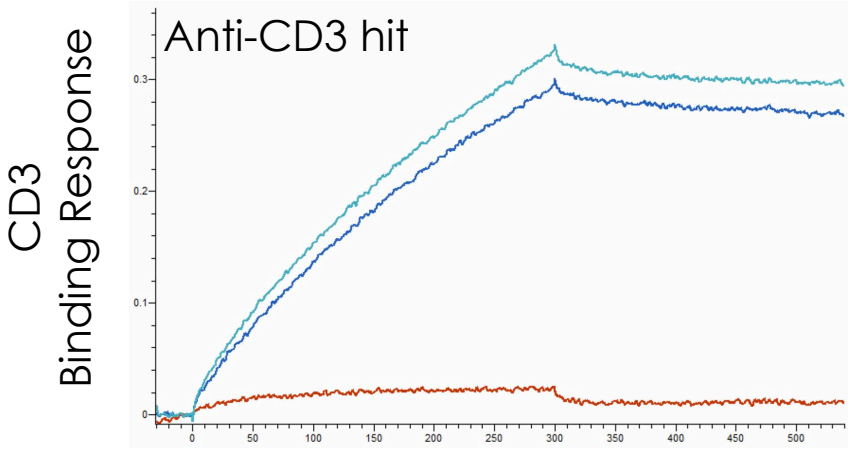
Mask Cleavage



Inactive antibody

Active antibody





No Mask

Mask (–MMP9)

Mask (+MMP9)

Conclusions

Epitope-Steering + Mammalian-Display Bispecific T Cell Engager Discovery

Epitope Steering

- Engineered epitopes direct and enrich antibody discovery to intended epitopes
- Reveals per-antibody-epitope activity via a multi-epitope target survey
- Antibody-engineered epitope binding enables epitope-mapping in early screens
    - Efficient single-cycle discovery of antibody-conditional masks

Mammalian-Display

- Multi-dimensional assessment at 10^6 library diversity scale:
    - CHO cell expression level
    - 1+ desired target binding (e.g. Hu & Cyno target)
    - Specificity (e.g. poly-specificity reagent, undesired target)
- Sufficient data per-dimension for AI model training and refinement

Thanks to the iBio Scientific Team!

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Alex Taguchi

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