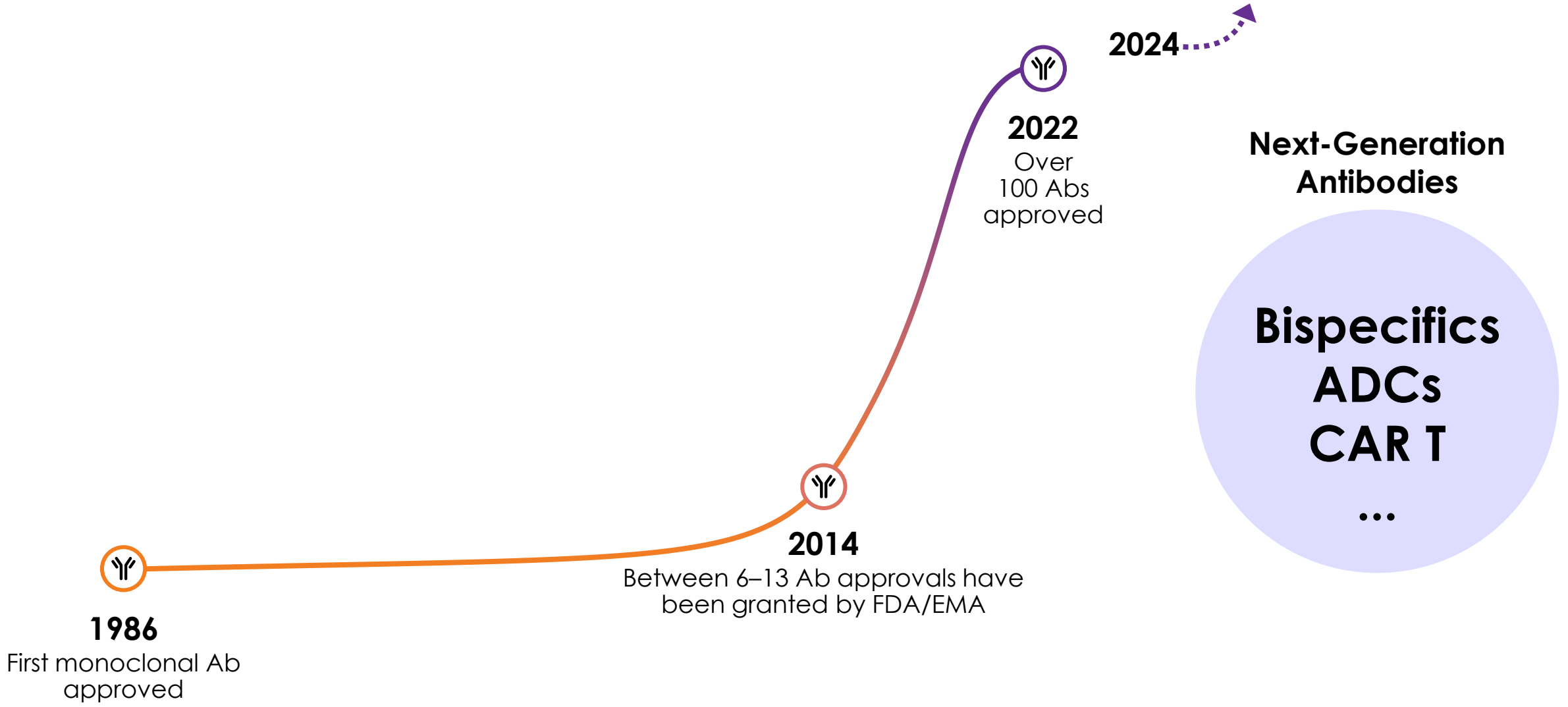
Enhancing Bispecific T Cell Engager Discovery with AI and Mammalian Display

Matthew Greving, Ph.D.
VP, Head of AI & Platform Technologies, iBio

PepTalk, San Diego
Jan. 16, 2024

Innovation is Key to the Next Era of Antibody Therapeutics



Current Ab Discovery Challenges: Complex Targets, Safety & Developability

Complexity

- Targets
  - Multi-pass membrane
  - Protein-Protein junctions
- Modes of action
  - Agonism
  - Conditional activation

Safety

- On-target, Off-tissue effects
  - ADCs
  - Immune-engagers
- Cytokine release
  - T Cell engager bispecifics
- Immunogenicity

Development

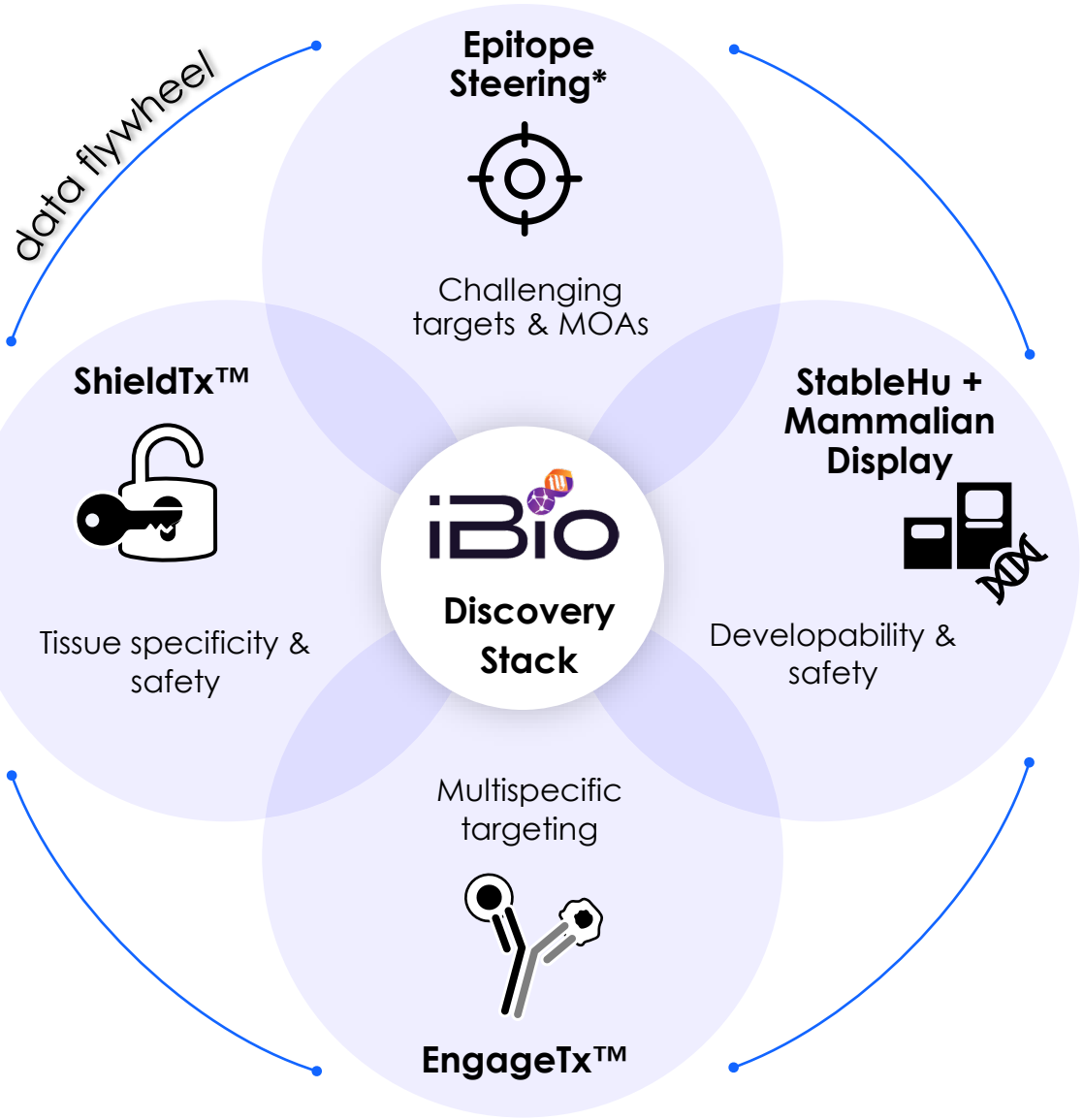
- Mono and Multi-Specifics
  - Low yield
  - Instability
  - Aggregation

Antibody Discovery

Technology Stack

Our antibody discovery stack advantage:

Generating safe, developable antibodies for challenging targets and modes of action



* U.S. Patent No. 11,545,238

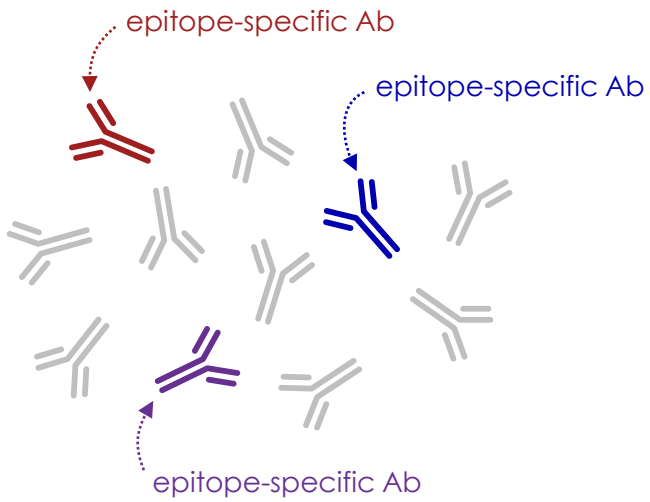
Epitope-Targeted

Antibody Discovery

Engineered Epitopes Focus Antibody Repertoires On Desired Binding Sites

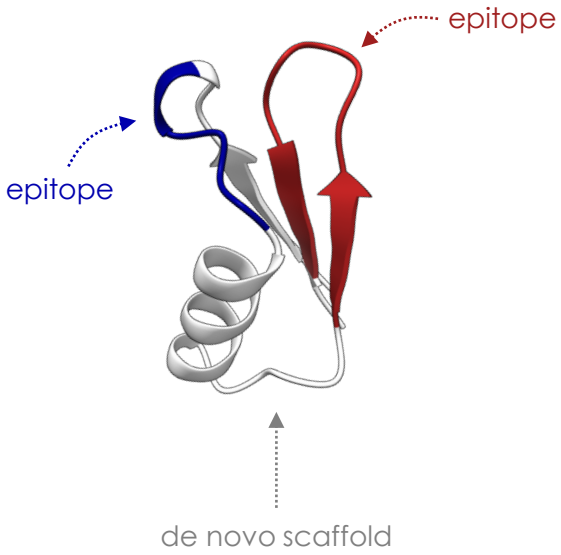
1

Naïve in vivo or in vitro antibody library



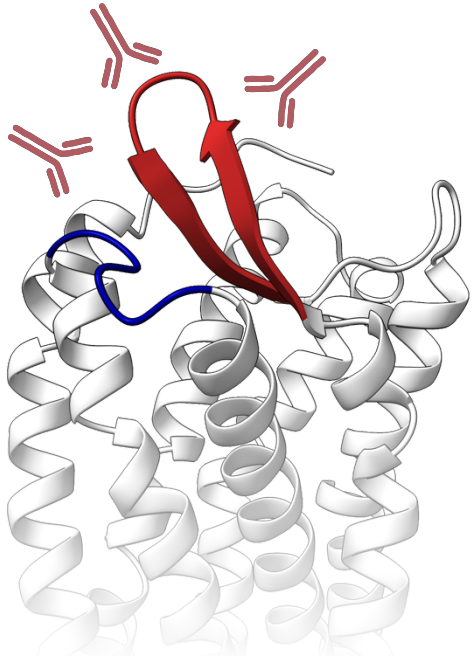
2

Focus library with engineered epitopes



3

Efficient discovery of epitope-specific Abs

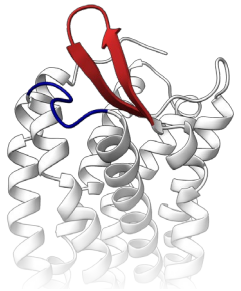


full length target

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

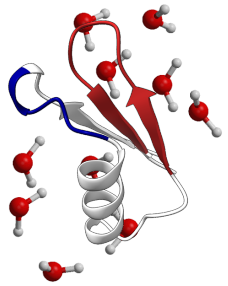
Engineered Epitope Design Objectives

1. **Match Structure to Target**



2. **Refine for Greater Stability**

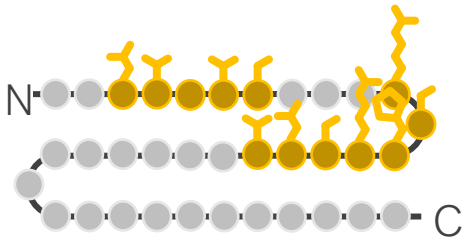
3. **Optimize for Water Solubility**



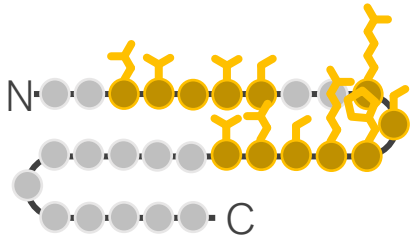
AI Discovery Engine

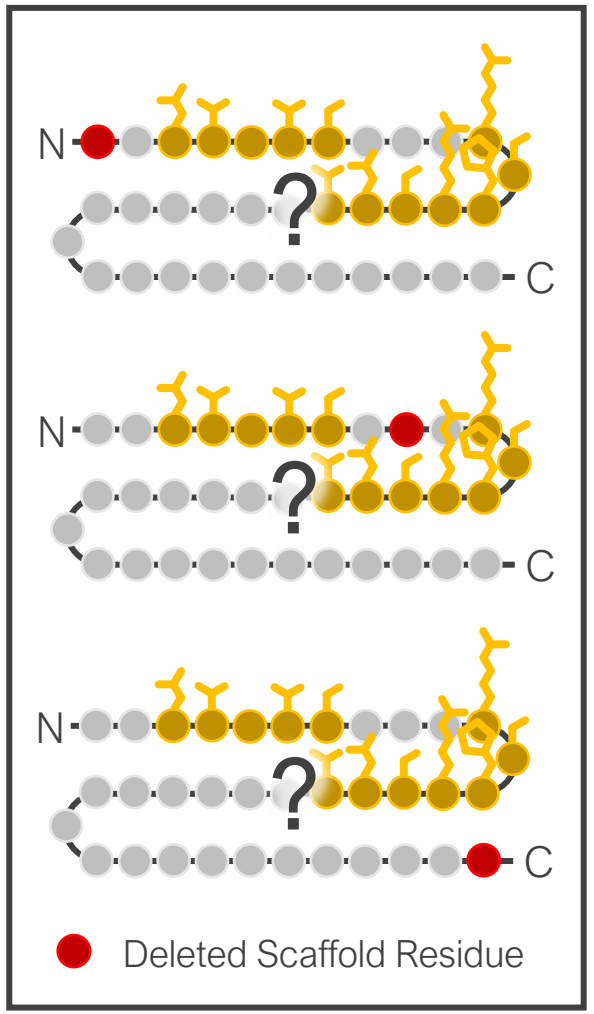
Engineered Epitopes are Further Optimized to Minimize Designed Scaffold

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



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

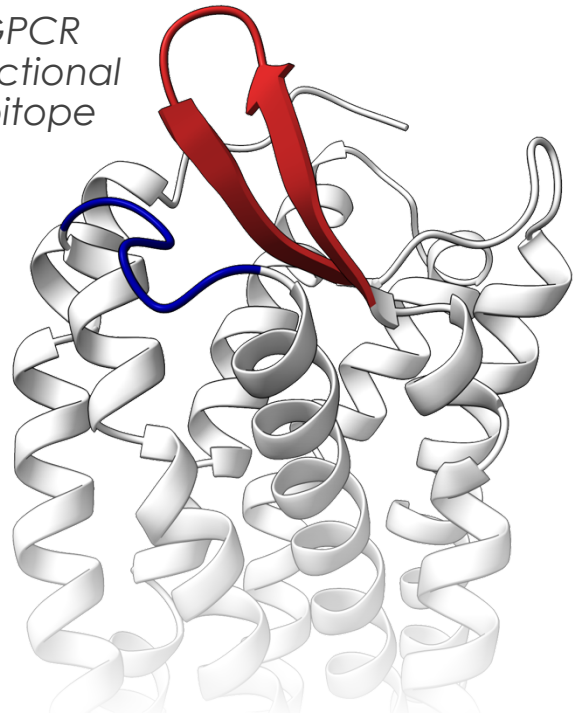




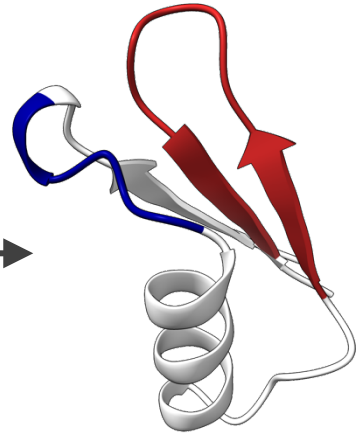
Iteratively trim scaffold residues until epitope destabilizes

Engineered Epitopes are Cross Validated *In Silico* and *In Vitro*

GPCR Junctional Epitope

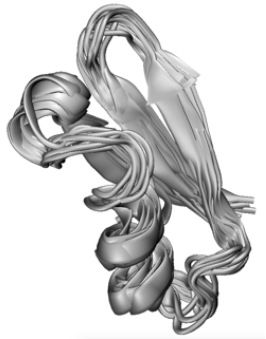


AI Discovery Engine



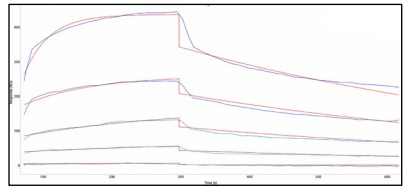
Folding Simulations

e.g. AlphaFold2



Antibody Binding

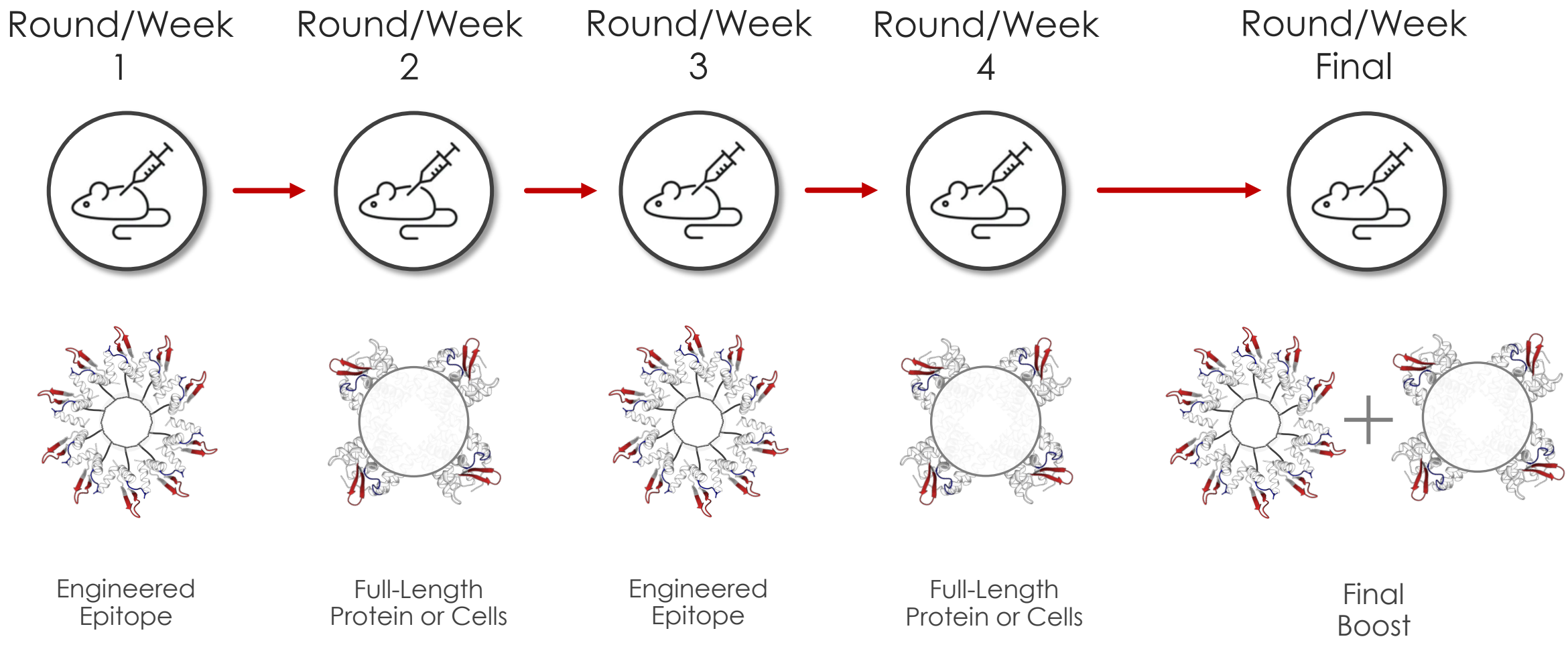
e.g. SPR



NMR, Melting Temp.

Engineered Epitopes Steer Immunization & *In Vitro* Libraries to Target Epitopes

Engineered epitopes alternated with full length native target protein and/or cells



Engineered Epitope-Steering Proven with Diverse Targets & Modes of Action

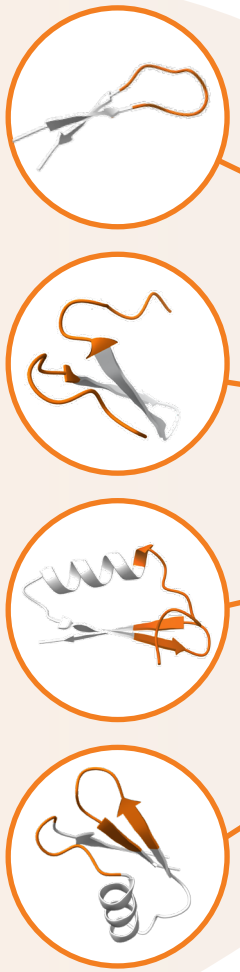
EPITOPE STRUCTURES

Loop Display

Junctional Epitopes

Complex Conformations

Membrane Proteins



AI Discovery Engine

SOLUTIONS

T-Cell Agonism
(CD3, PD1)

Tumor Specific Epitopes
(EGFRvIII)

Protein Complex Stabilization
(Latent TGFb)

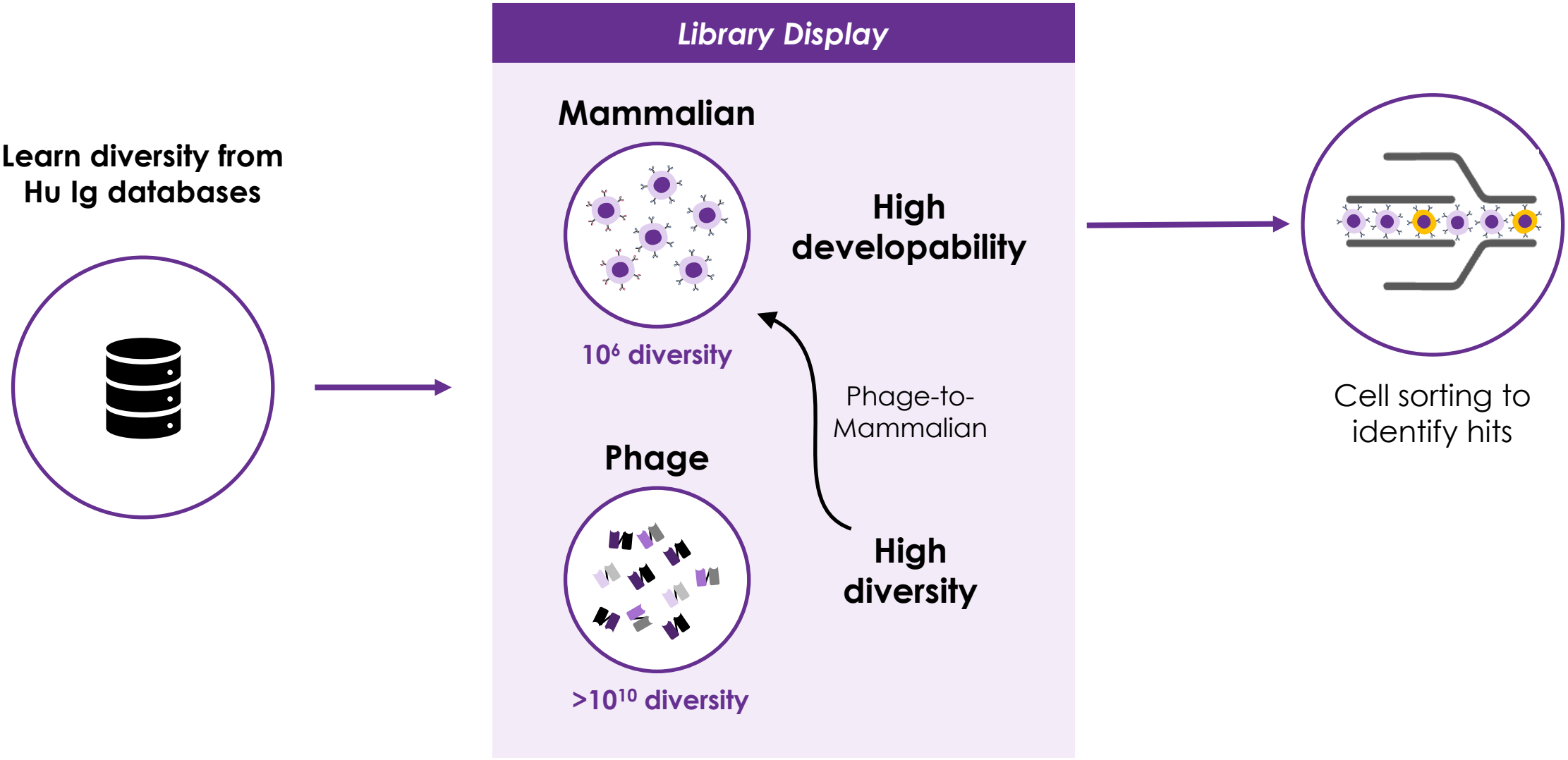
Antagonism
(MUC16, CCR8)

Conditional Activation
(MUC16, CD3)

High Developability, Human Diversity

Antibody Libraries

Naïve *In Vitro* Library Uses Human Diversity to Minimize Immunogenicity Risk



Naïve Library Diversity Matches Natural Framework-Specific Distribution

Learn framework-specific CDR sequence distributions

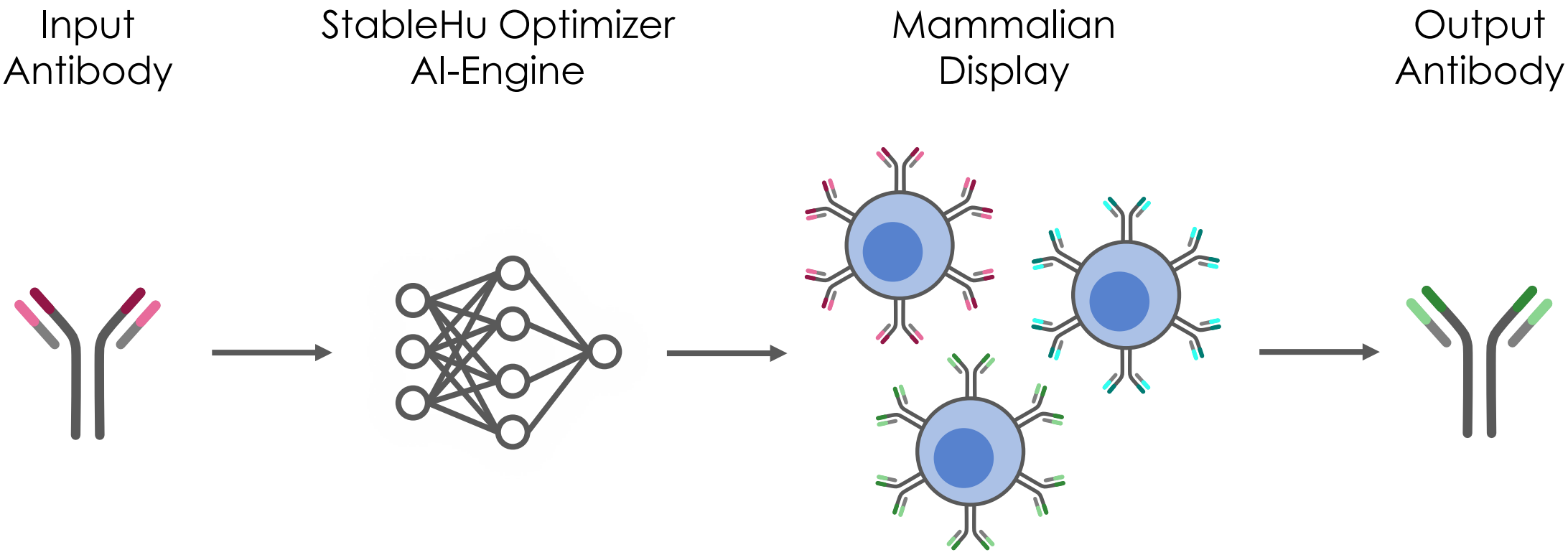
Observed CDR sequences in clinically-validated frameworks

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 AI Model Generates Focused Diversity for Mammalian Display

AI generated diversity is enriched in functional variants with fully human sequences



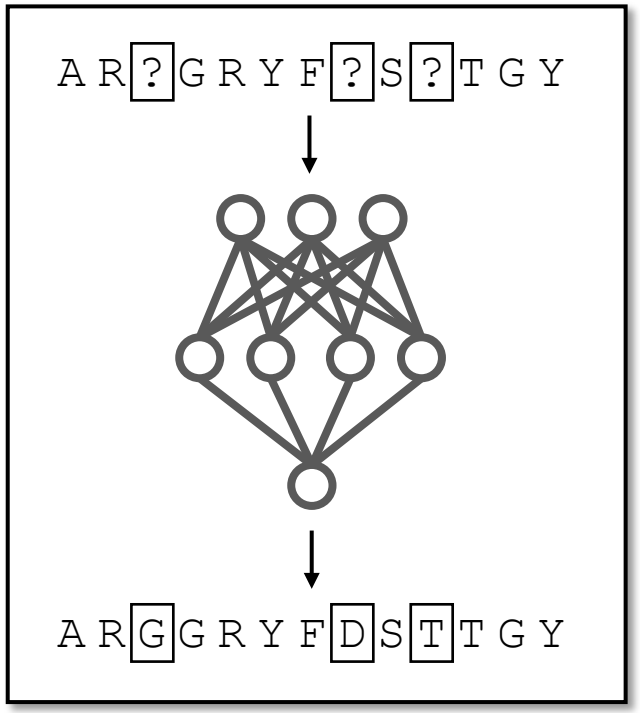
StableHu AI Model is Trained to Predict Fully Human CDR Sequences

Antibody Database

cAb-Rep & OAS
Hu Ig databases

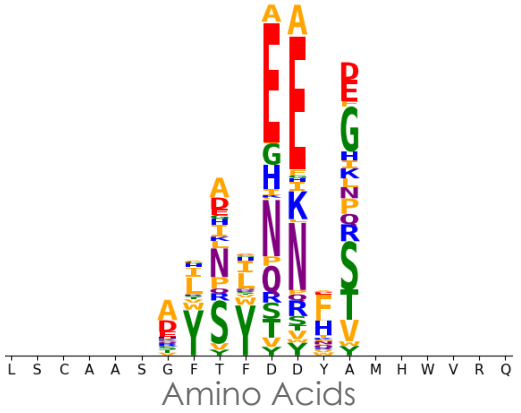
>1 billion curated human antibody sequences

Optimizer AI



AI trained to predict
fully human CDR from masked CDR

Trained Model

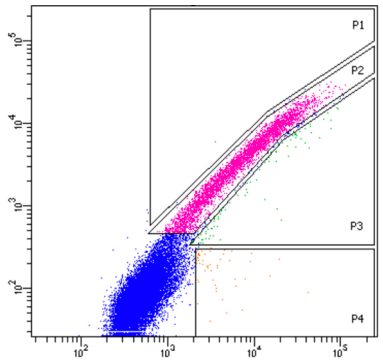


Predict library of fully human CDRs from template CDR

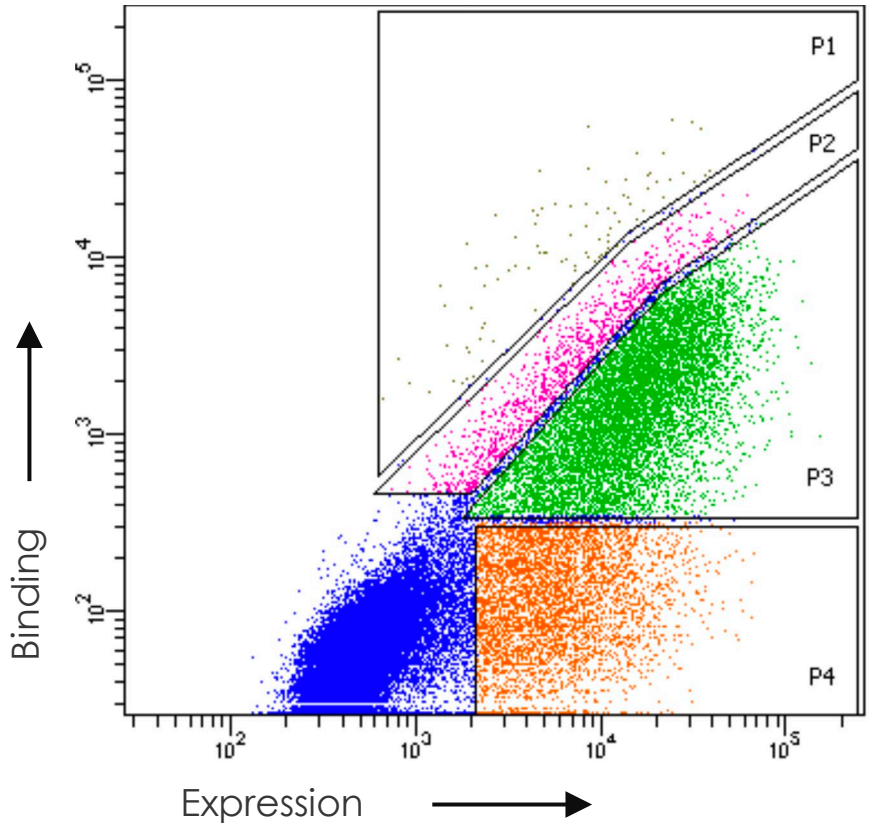
StableHu Library Sorting and NGS Identify Improved Human CDR Variants

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

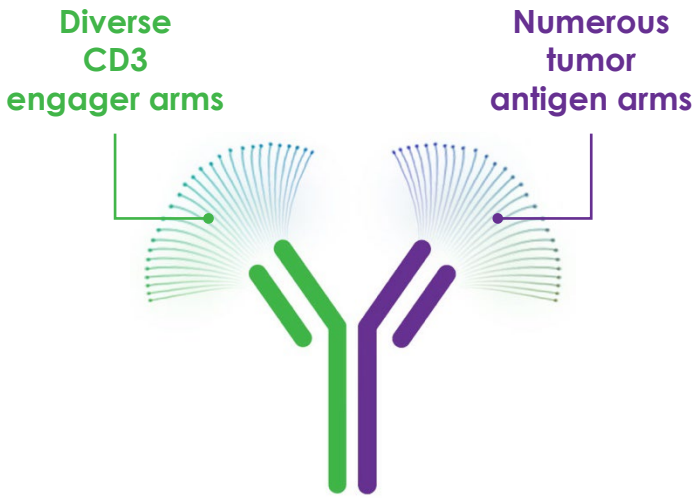
CD3 T Cell Engager Arm

Anti-CD3 T Cell Agonist

Key Challenges of CD3 T Cell Engager Discovery

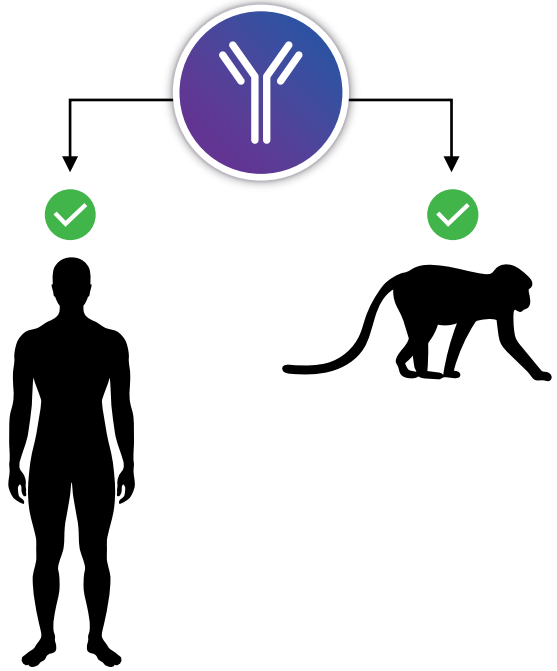
1 Sequence Diversity

Broad CD3 activity for optimized paring with tumor antigen arms



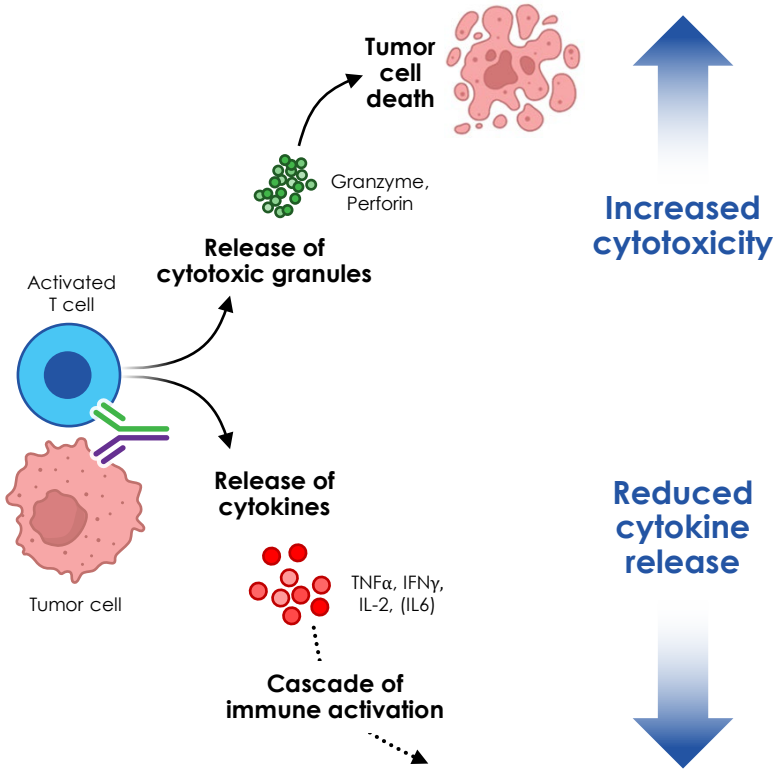
2 Hu-Cyno Cross-Reactivity

Risk reduction via cyno monkey toxicity study compatibility



3 Range of Cytokine Release

Tailored cytokine release for expanded therapeutic window



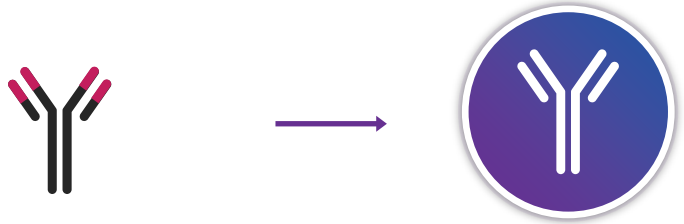
Dual-Tracks to Discover a Diverse Panel of Anti-CD3 Antibodies

AI Discovery Engine

Engineered-Epitope Immunization & Screening

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

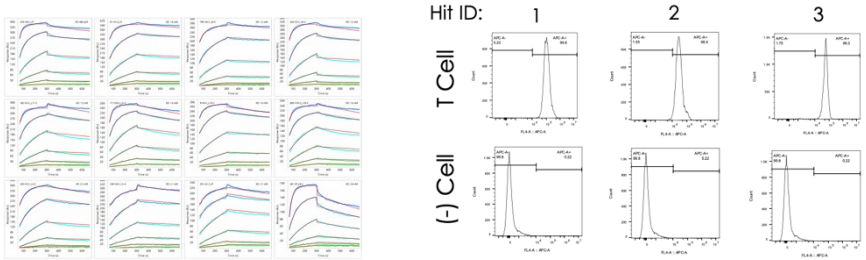
StableHu Optimizer



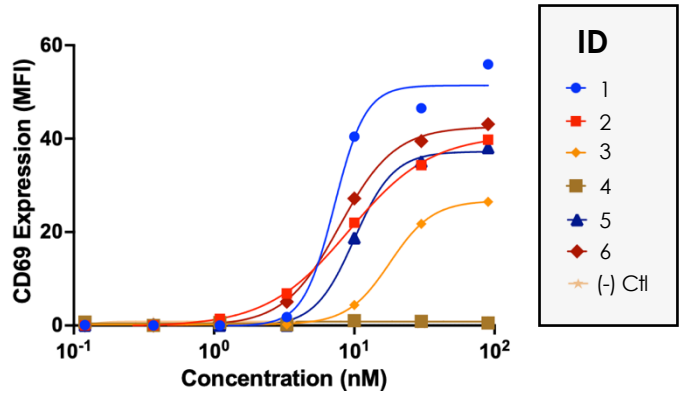
Template Antibody → Optimized Antibodies

SCREEN

Hu-Cyno CD3 & T Cell Binding



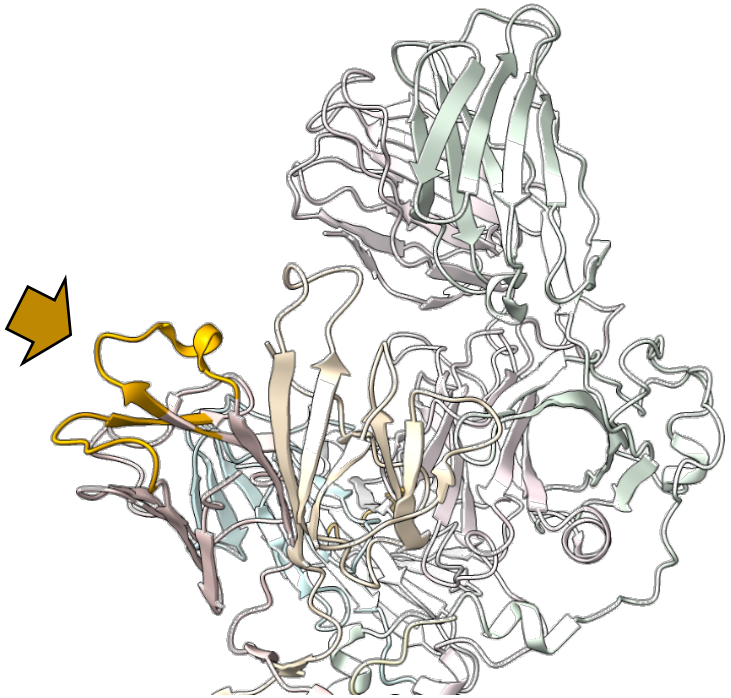
T Cell Activation



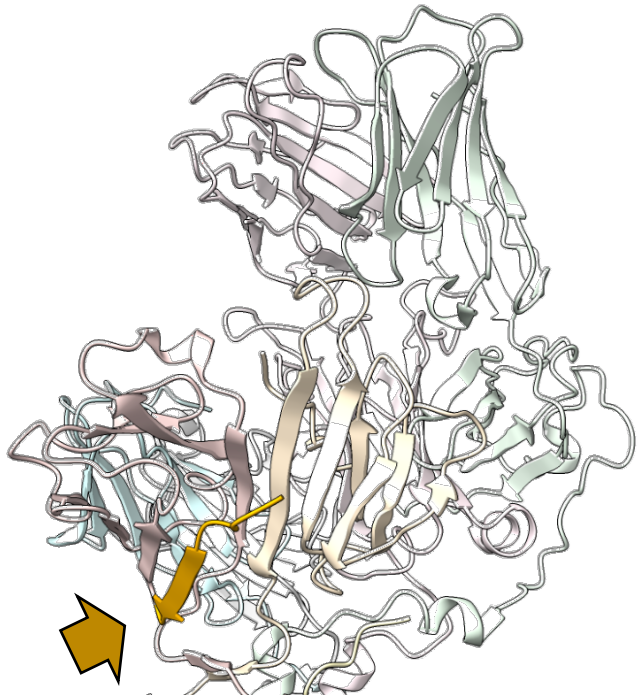
Epitope Engineering for TCR Accessibility & Hu-Cyno Cross-Reactivity

CD3 target epitopes in the context of the full TCR

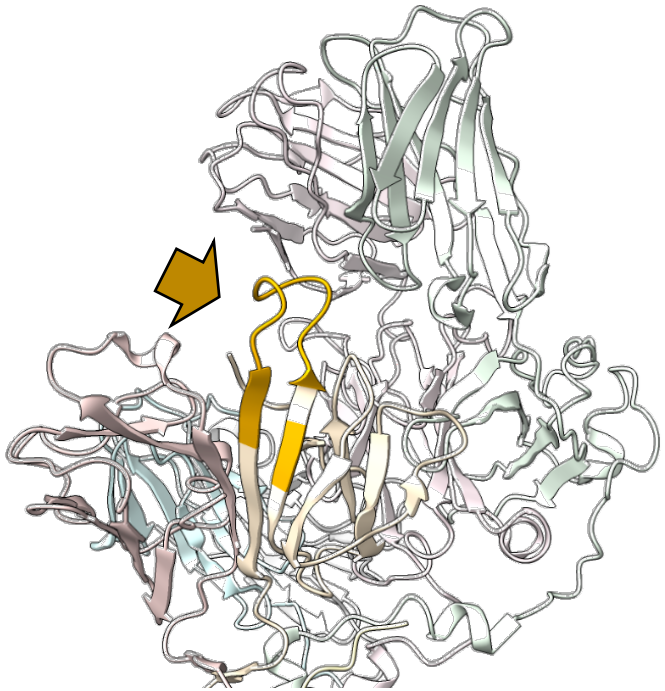
Epitope 1



Epitope 2

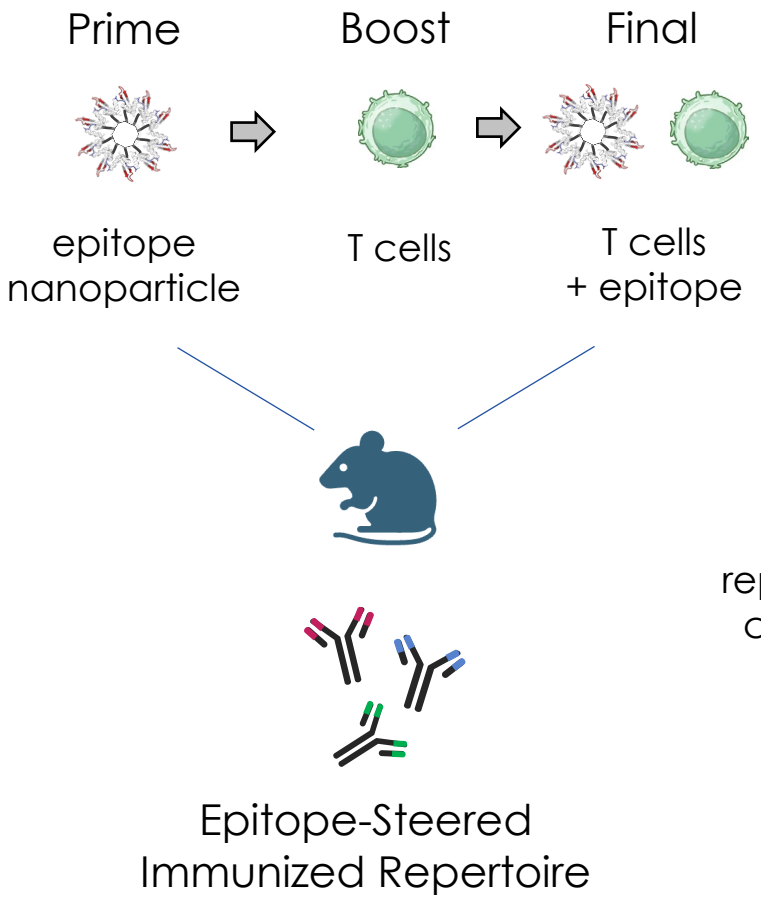


Epitope 3



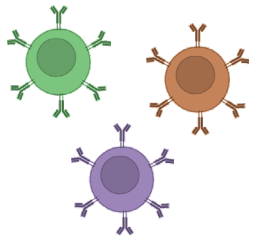
Immunized CD3 Repertoires Were Cloned & Screened in Mammalian Display

1. Epitope-Steered Immunization

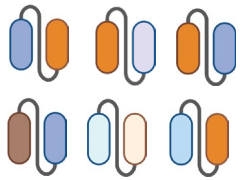


repertoire cloning

2. Mammalian Display



Phage Display 2X Rounds



1-sorting round

3. Multi-Modal Screening

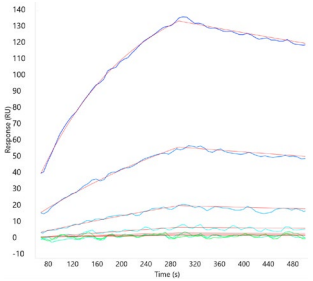
Single-Cell Screen:
1. Epitope binding
2. Hu-Cyno CD3 binding
3. Ab expression
4. Poly-Specificity

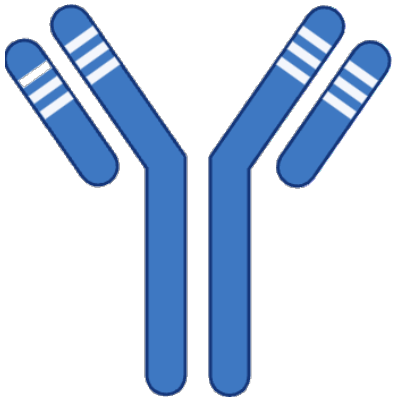
NGS,
SPR,
In vitro activity

Anti-CD3 Template Antibody Human Diversification with StableHu AI

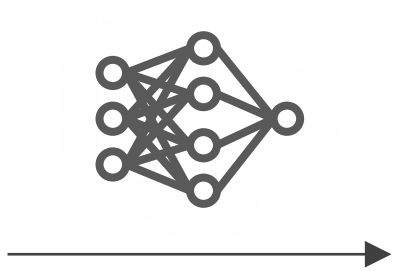
1. Anti-CD3 Ab template with mouse CDRs

KD =
10 nM



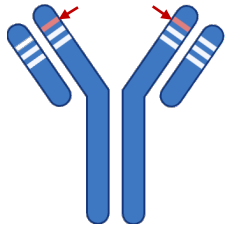


2. AI-model predicts human CDRs

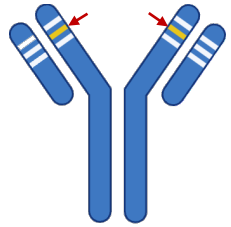


3. Human heavy & light chain CDR diversity libraries

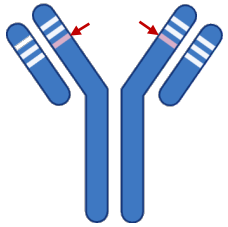
HCDR1
2000



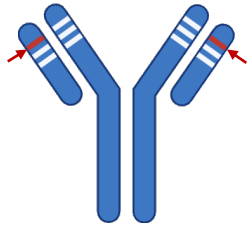
HCDR2
2000



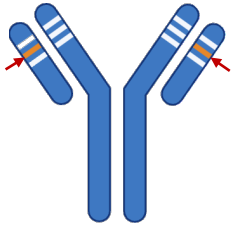
HCDR3
2000



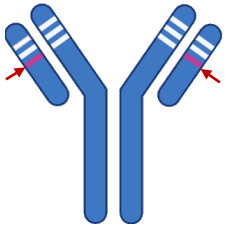
LCDR1
2000



LCDR2
1000



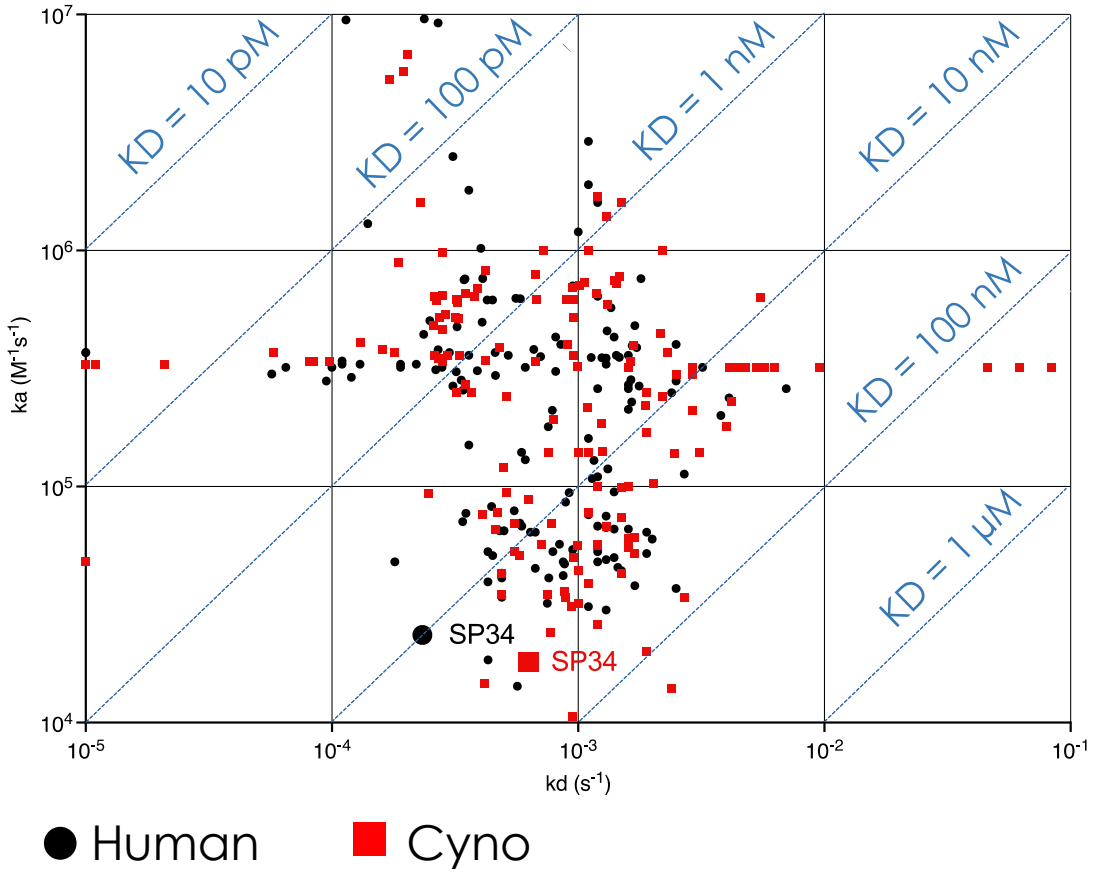
LCDR3
2000



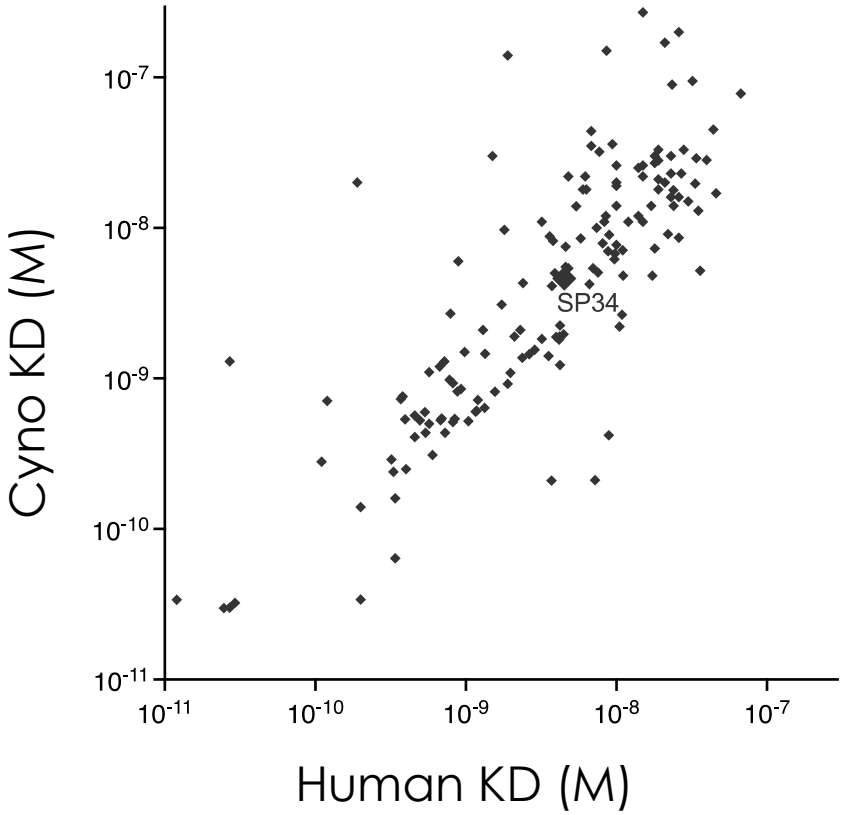
Dual-Track Discovery Identifies Hu-Cyno CD3 10^4 Affinity Range Binders

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

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

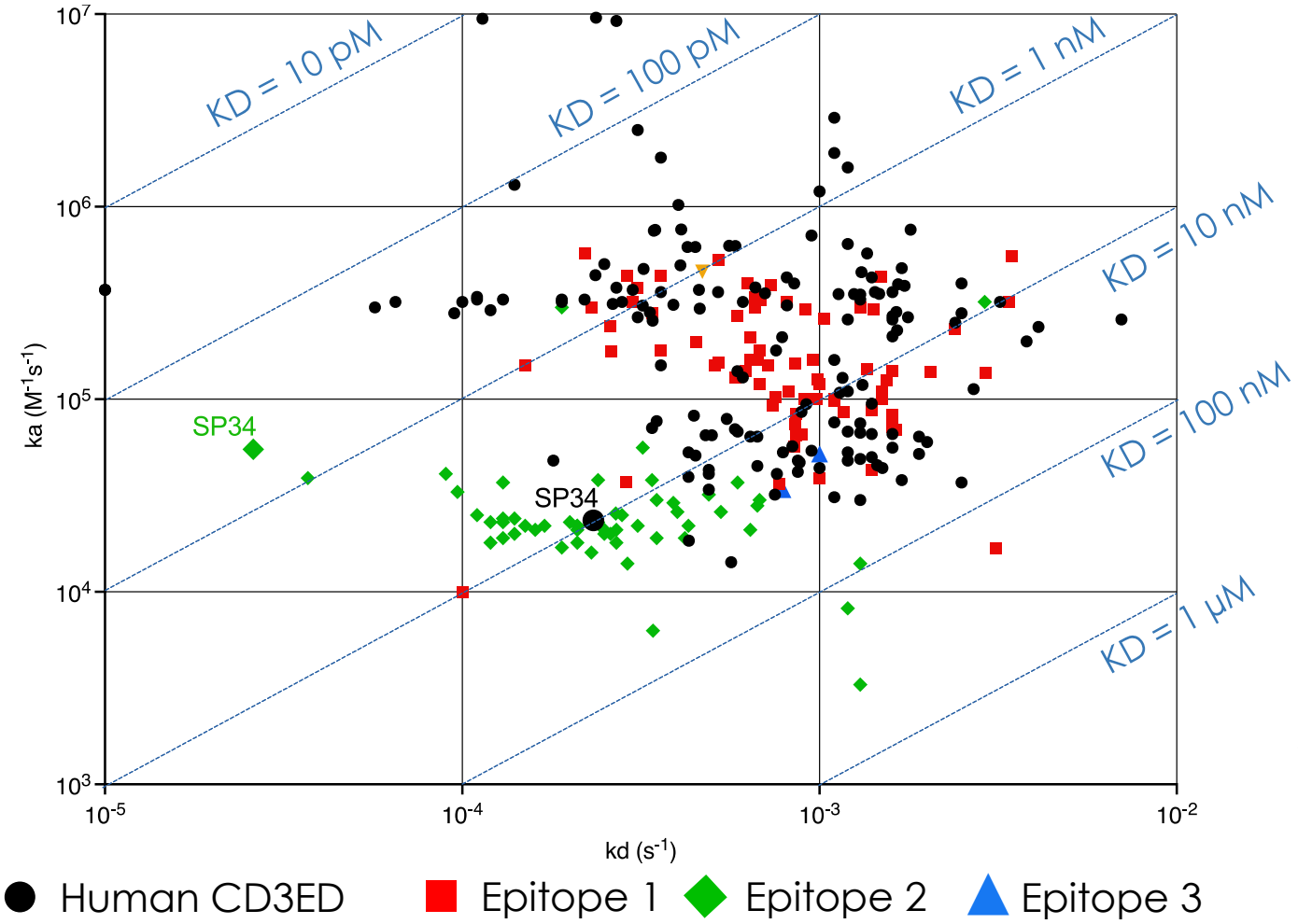


Most hits have comparable affinity for human and cyno CD3



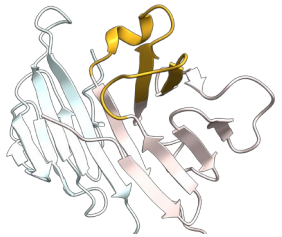
127/150 = 85% Hu-Cyno CD3 Cross-Reactive Hits Bind Engineered Epitopes

Human **CD3ED**, **Epitopes 1, 2, 3** HT-SPR Affinity

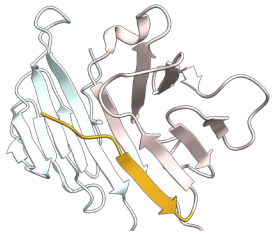


- All engineered-epitopes generated epitope-specific antibodies
- Epitope 1 is the most productive in immunization, potentially due to high accessibility, larger binding surface

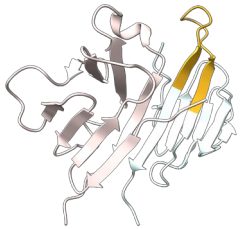
Epitope 1



Epitope 2

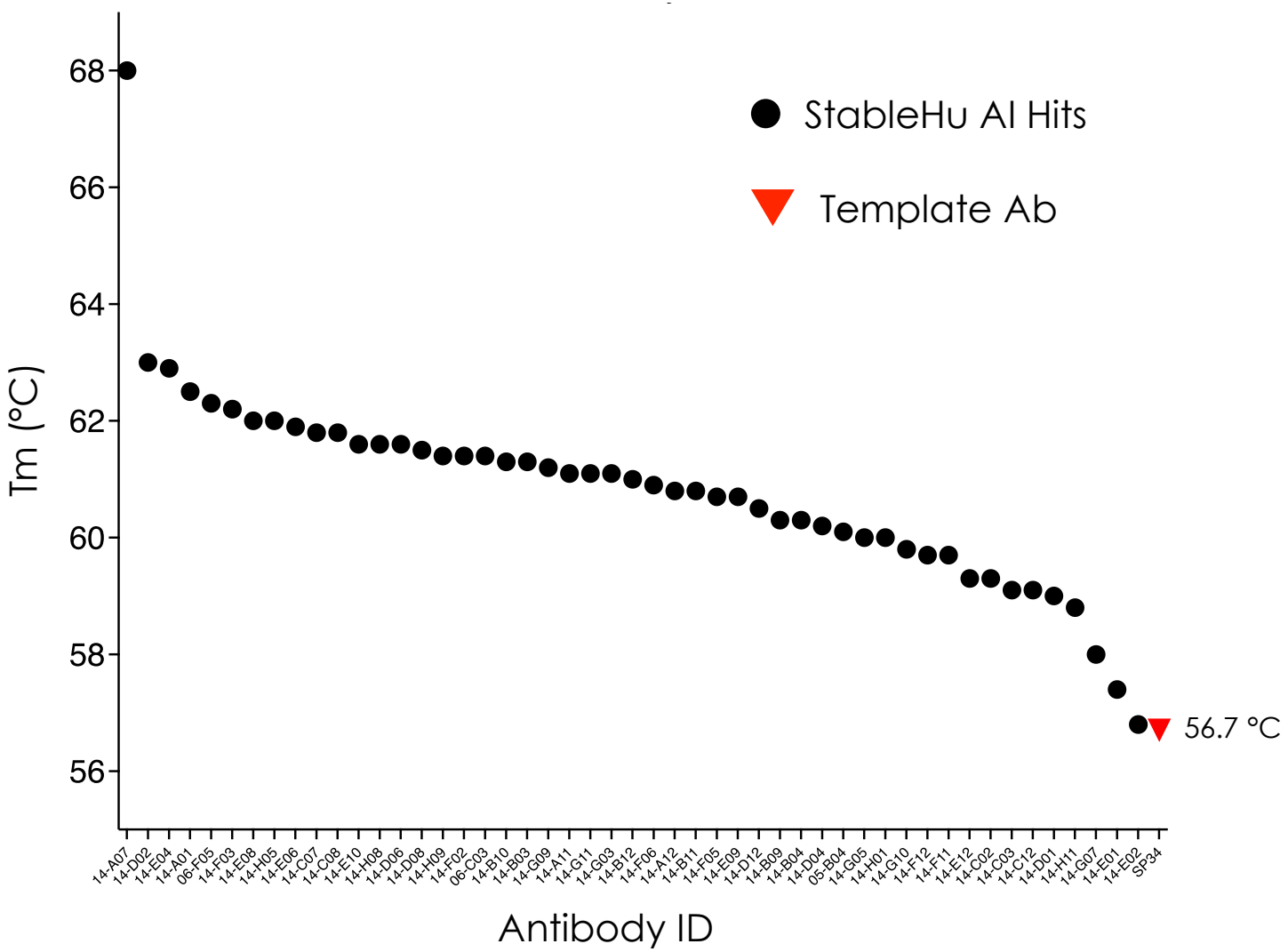


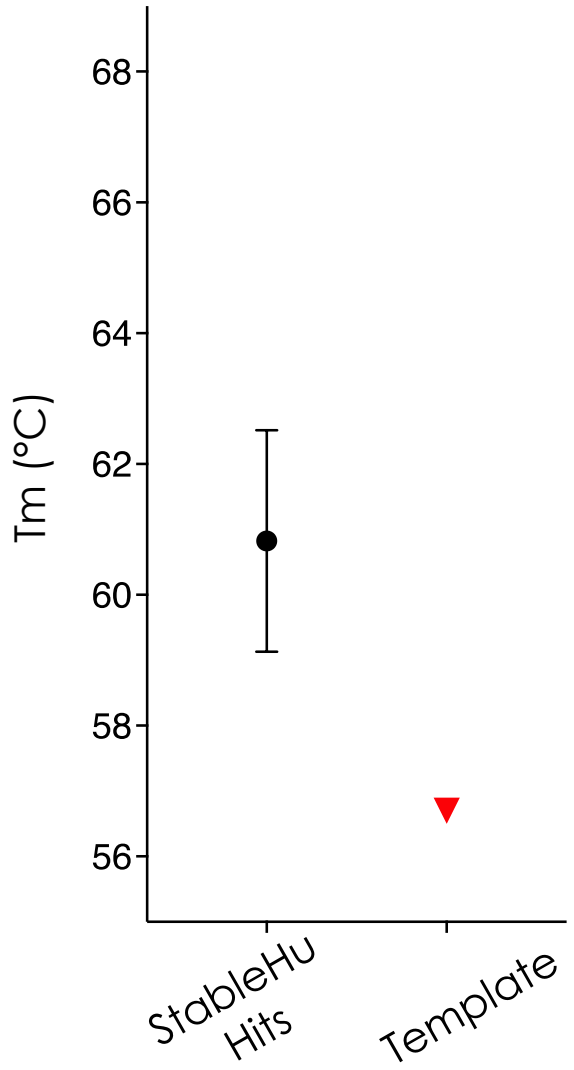
Epitope 3



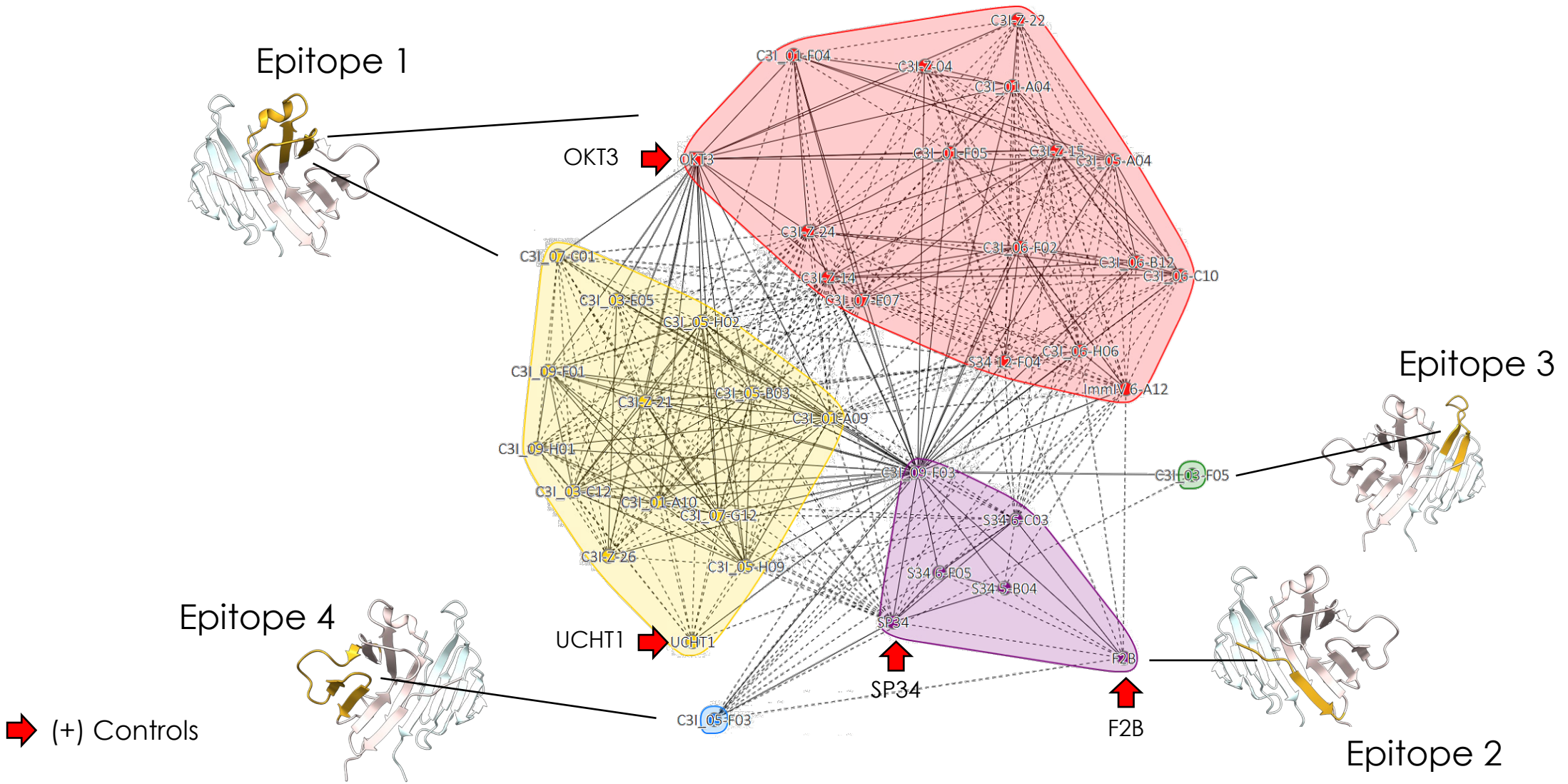
StableHu AI Derived Hits Have Significantly Higher Tm vs. Template Antibody

Melting temperatures of CD3 Hu-Cyno & epitope triple-positive StableHu AI hits

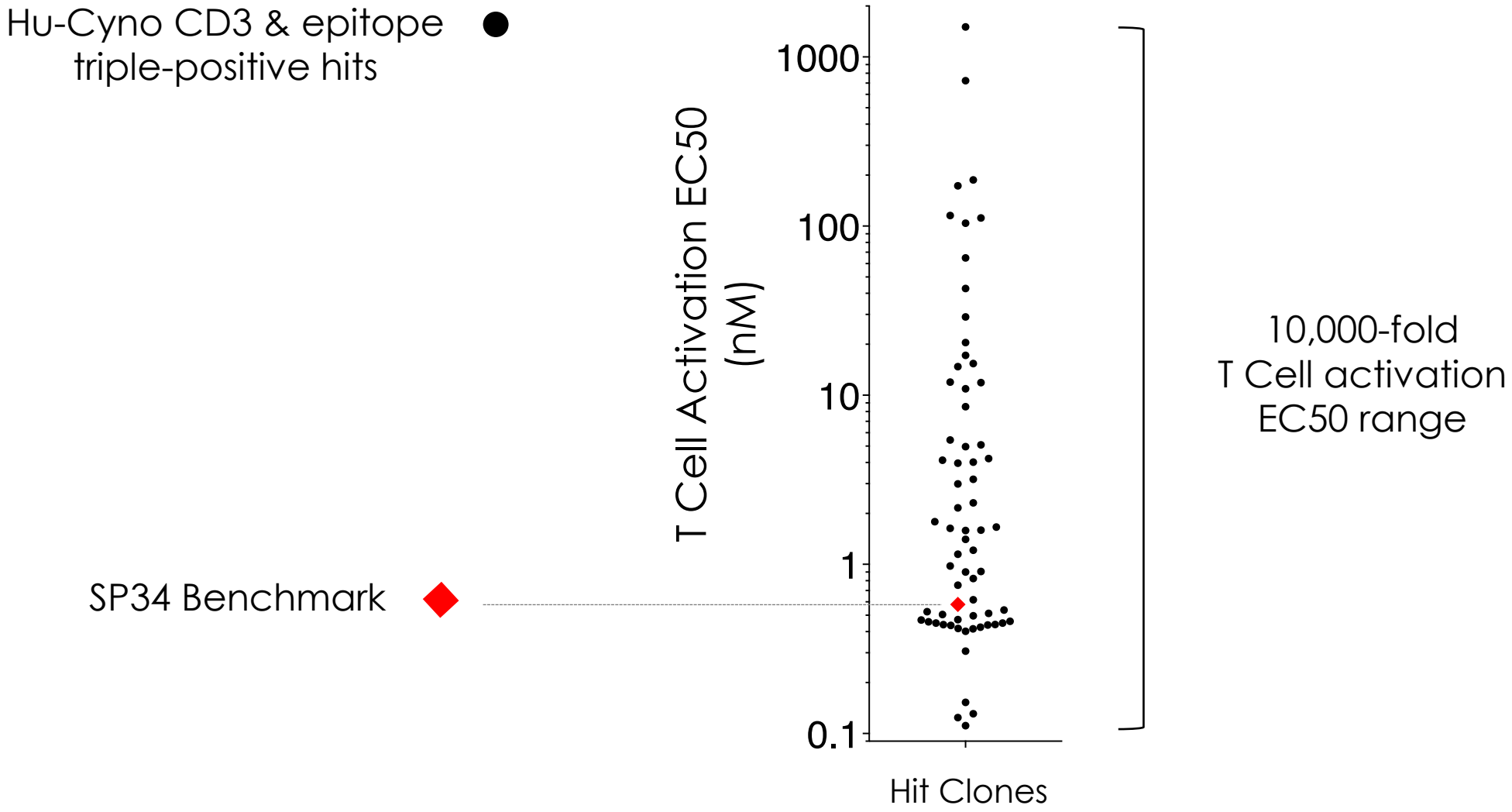




Dual-Track Discovery Hits Cover 4 Engineered Epitopes and 5 Epitope Bins



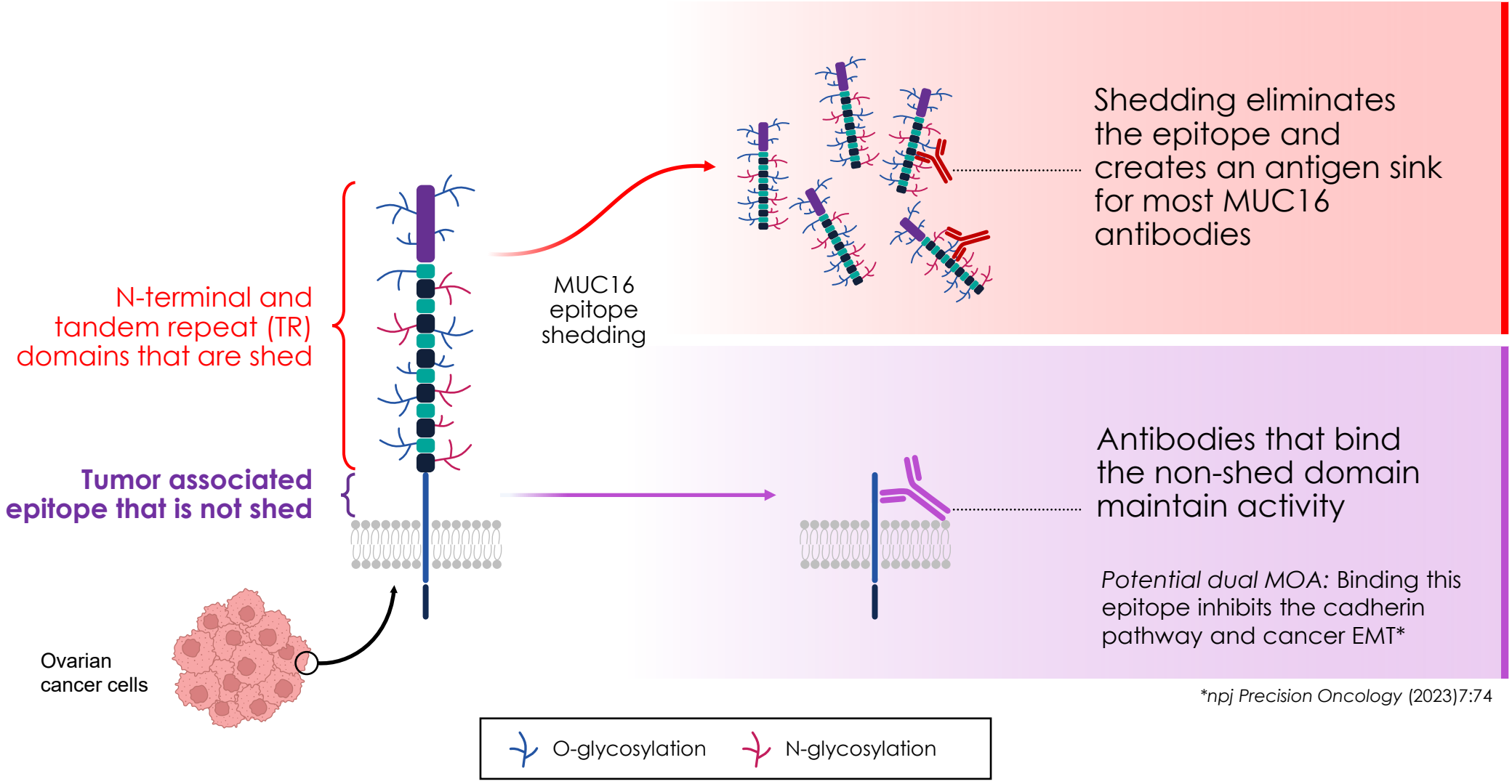
Dual-Track Discovery Hits Activate T Cells Across a 10^4 Range



Tumor Associated Antigen Arm

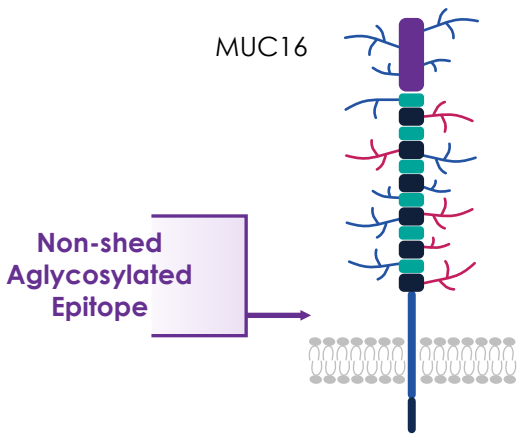
Non-Shed Epitope Anti-MUC16 Antibody

MUC16 Is Overexpressed and Shed by Tumor Cells



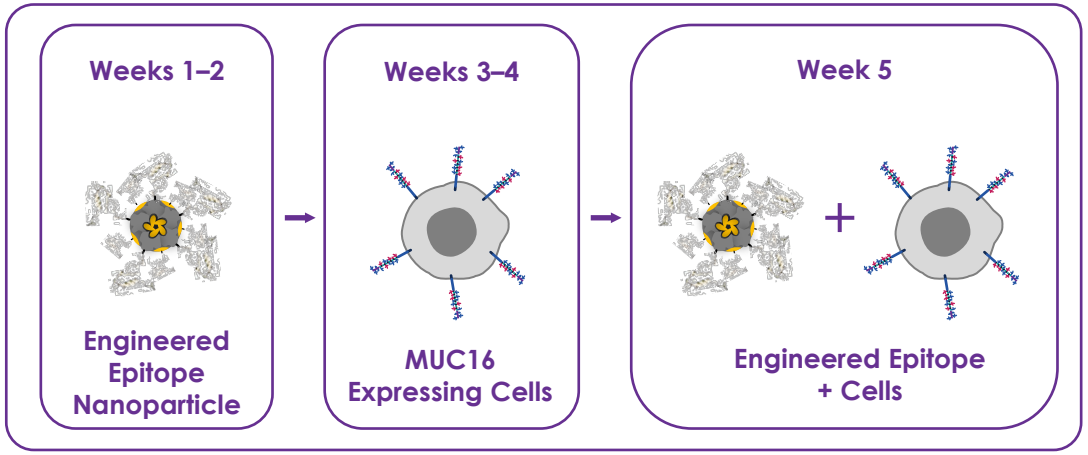
*npj Precision Oncology (2023)7:74

Dual-Discovery Tracks Were Steered to a MUC16 Epitope that Avoids Shedding

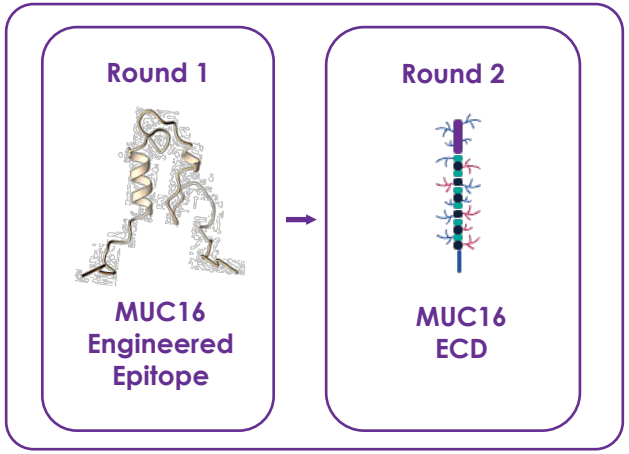


AI Discovery Engine

Epitope-Steered Immunization & Screening



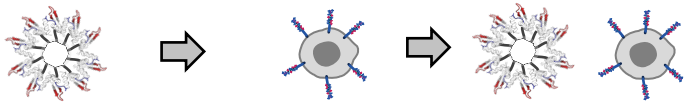
Epitope-Steered Naïve In Vitro Selection



Immunized MUC16 Repertoires Were Cloned and Screened in Mammalian Display

1. Epitope-Steered Immunization

Prime → Boost → Final

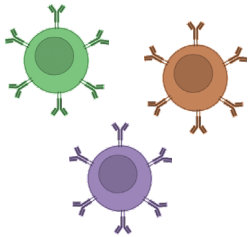


epitope nanoparticle → MUC16 cells → cells + epitope

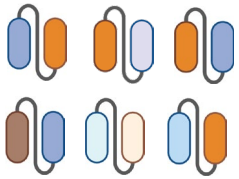
Epitope-Steered Immunized Repertoire

repertoire cloning

2. Mammalian Display



Phage Display 2X Rounds



1-sorting round

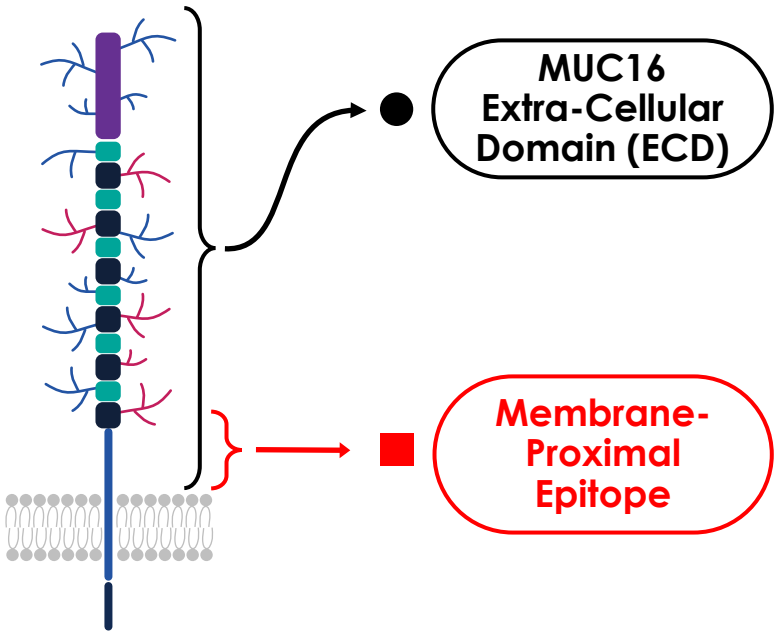
3. Multi-Modal Screening

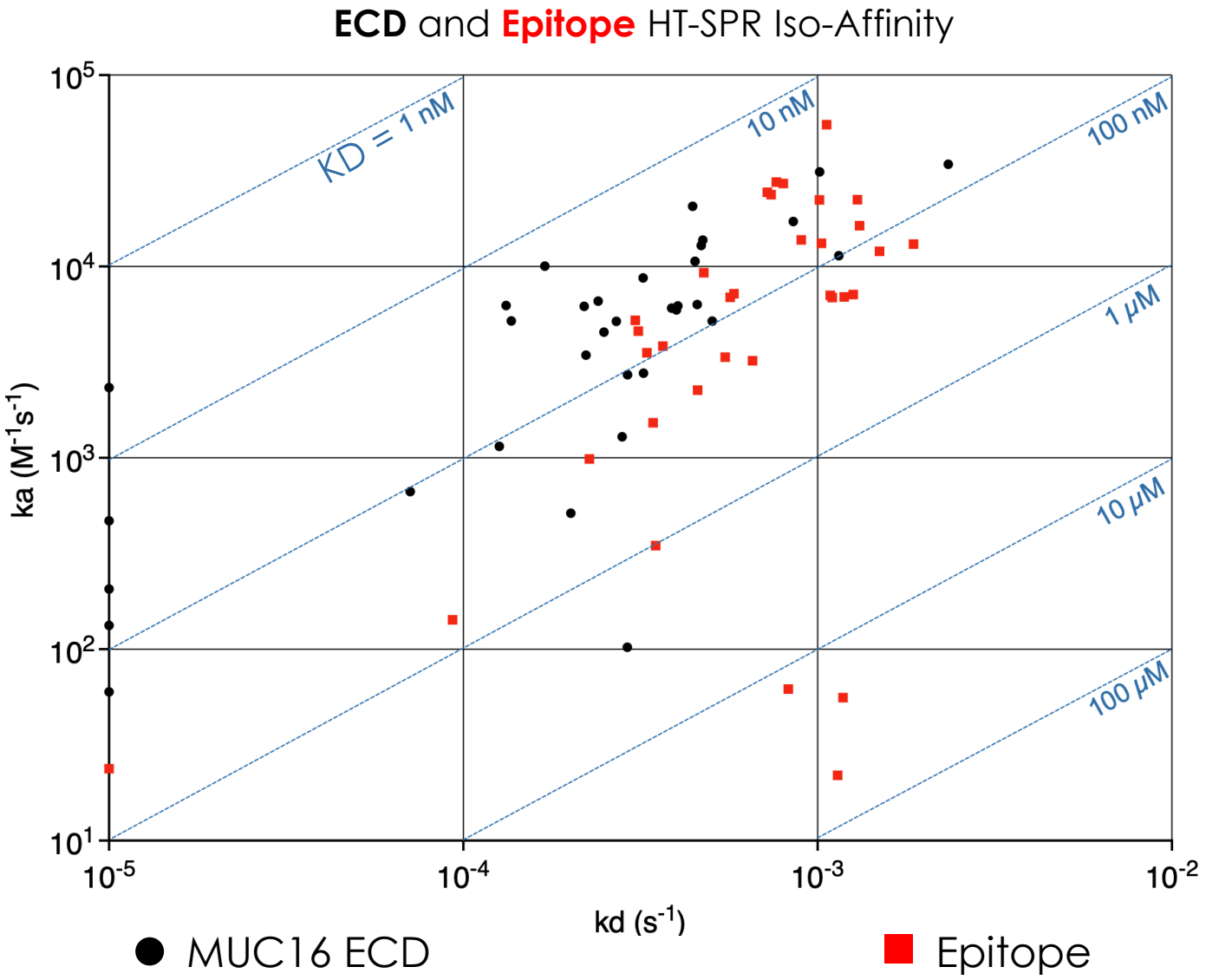
Single-Cell Screen:

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

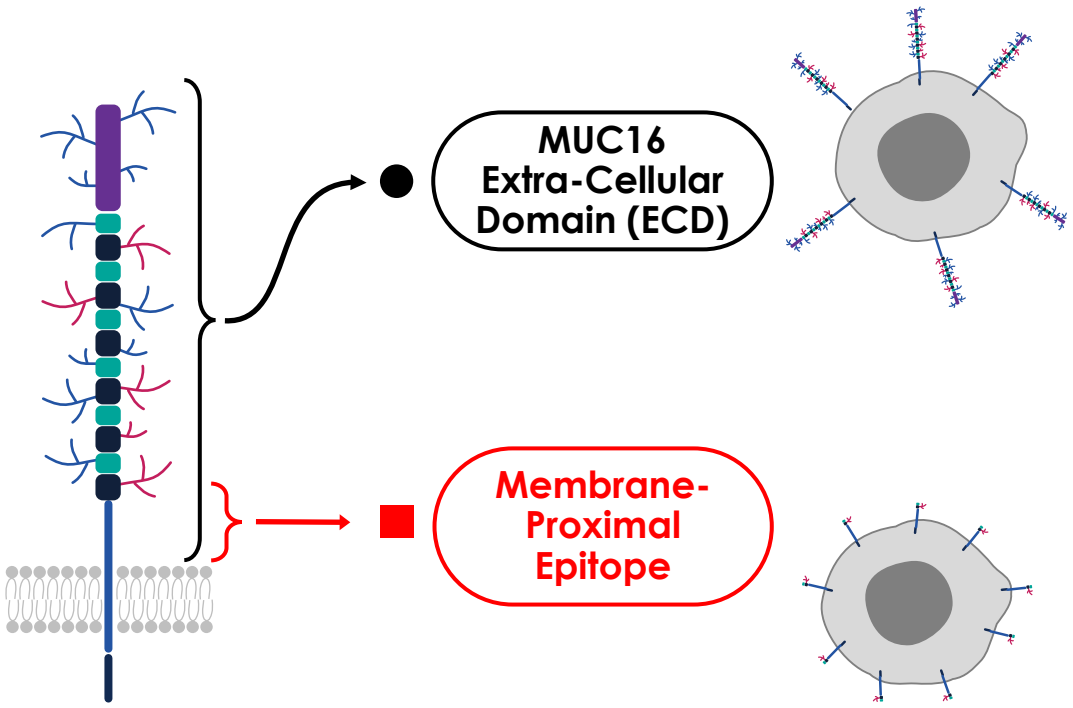
NGS,
SPR,
In vitro activity

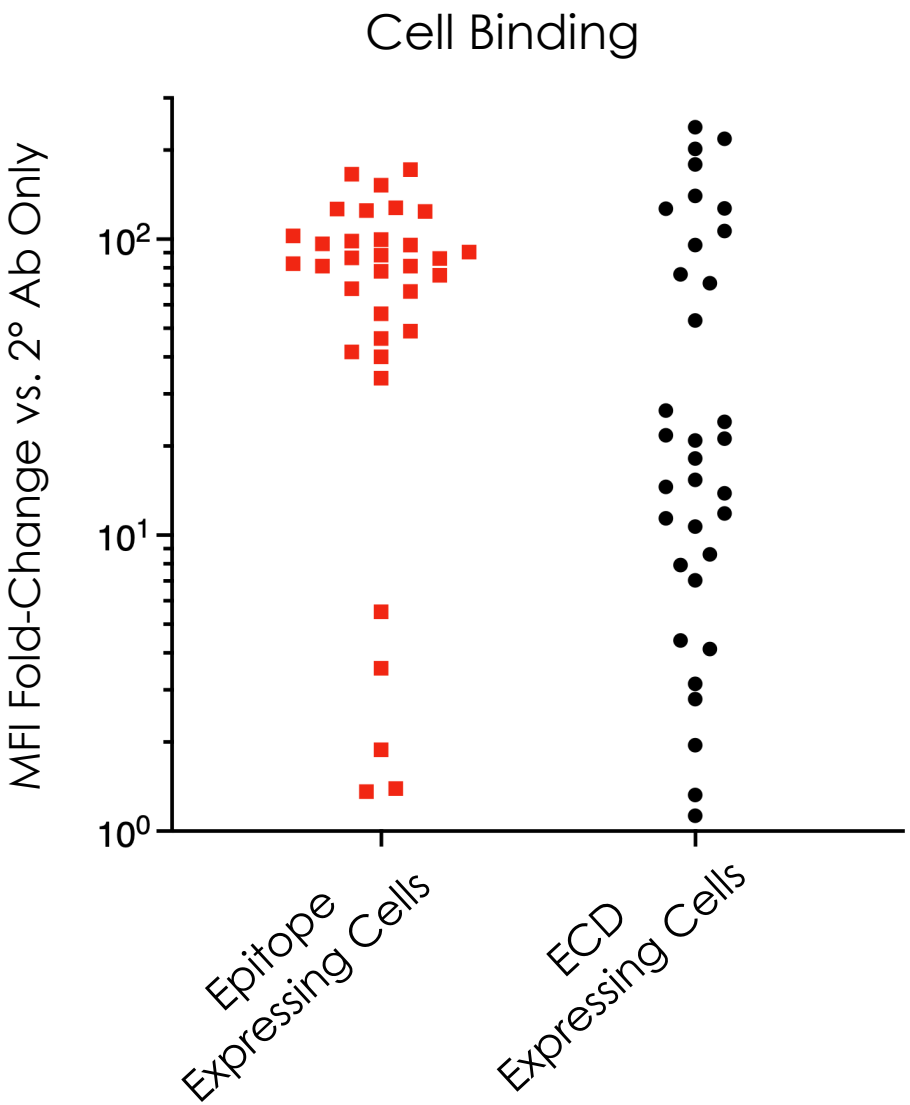
Dual-Track Discovery Identifies 34 Hits that Bind the MUC16 Epitope and ECD



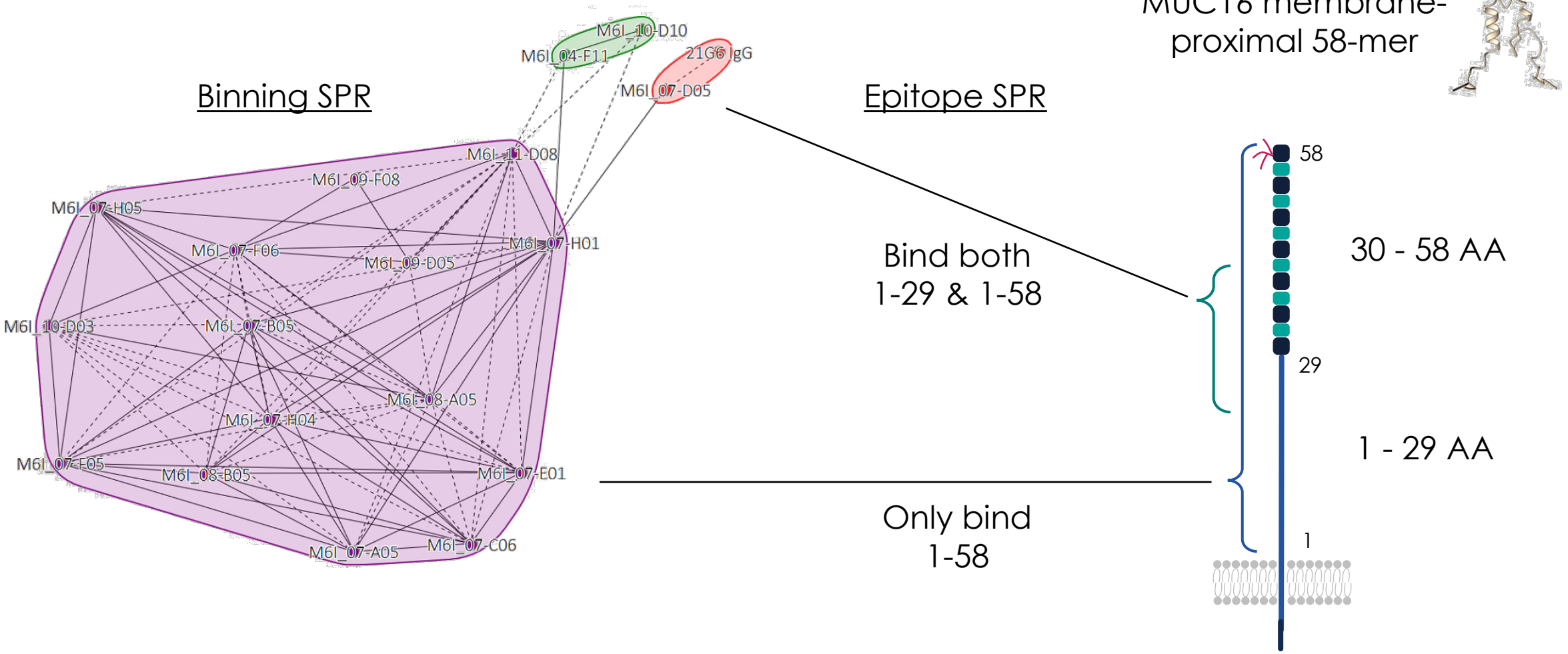


34/34 Hits Bind MUC16 Membrane-Proximal Epitope and ECD Expressing Cells





MUC16 Hits Cover 3 Epitope Bins on Distinct Membrane-Proximal Epitopes



Combining Arms: Anti-CD3 X Anti-MUC16

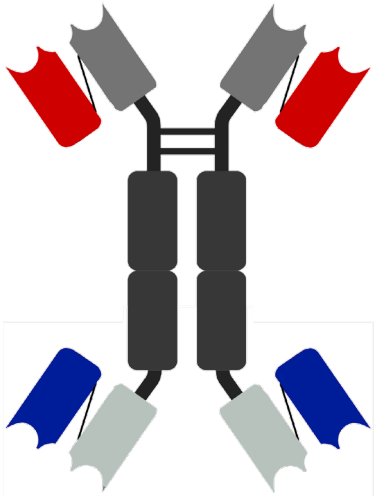
Bispecific T Cell Engager

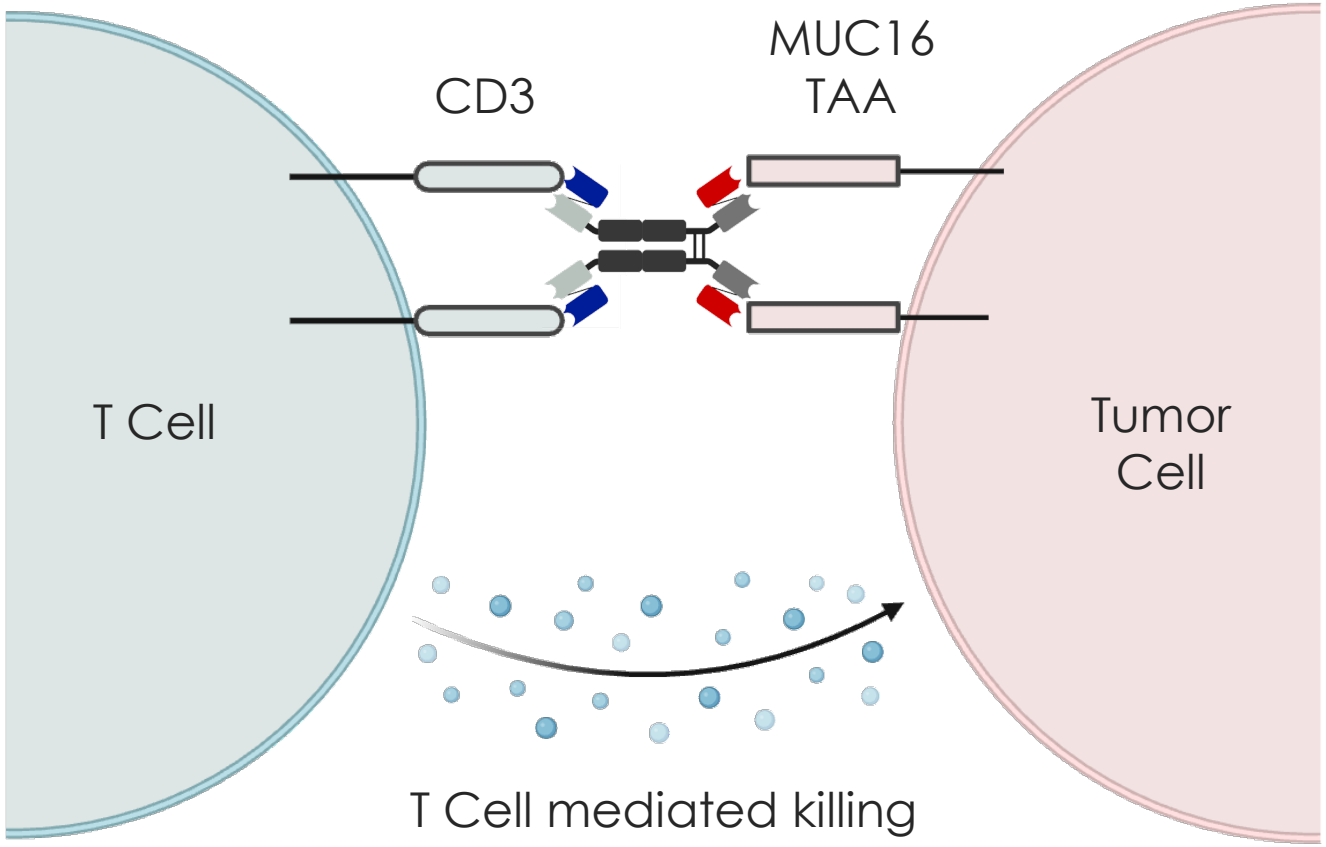
Anti-CD3 X MUC16 Bispecific T Cell Engagers Were Evaluated in 2x2 Format

scFv-Fc 2x2

Anti-MUC16 TAA

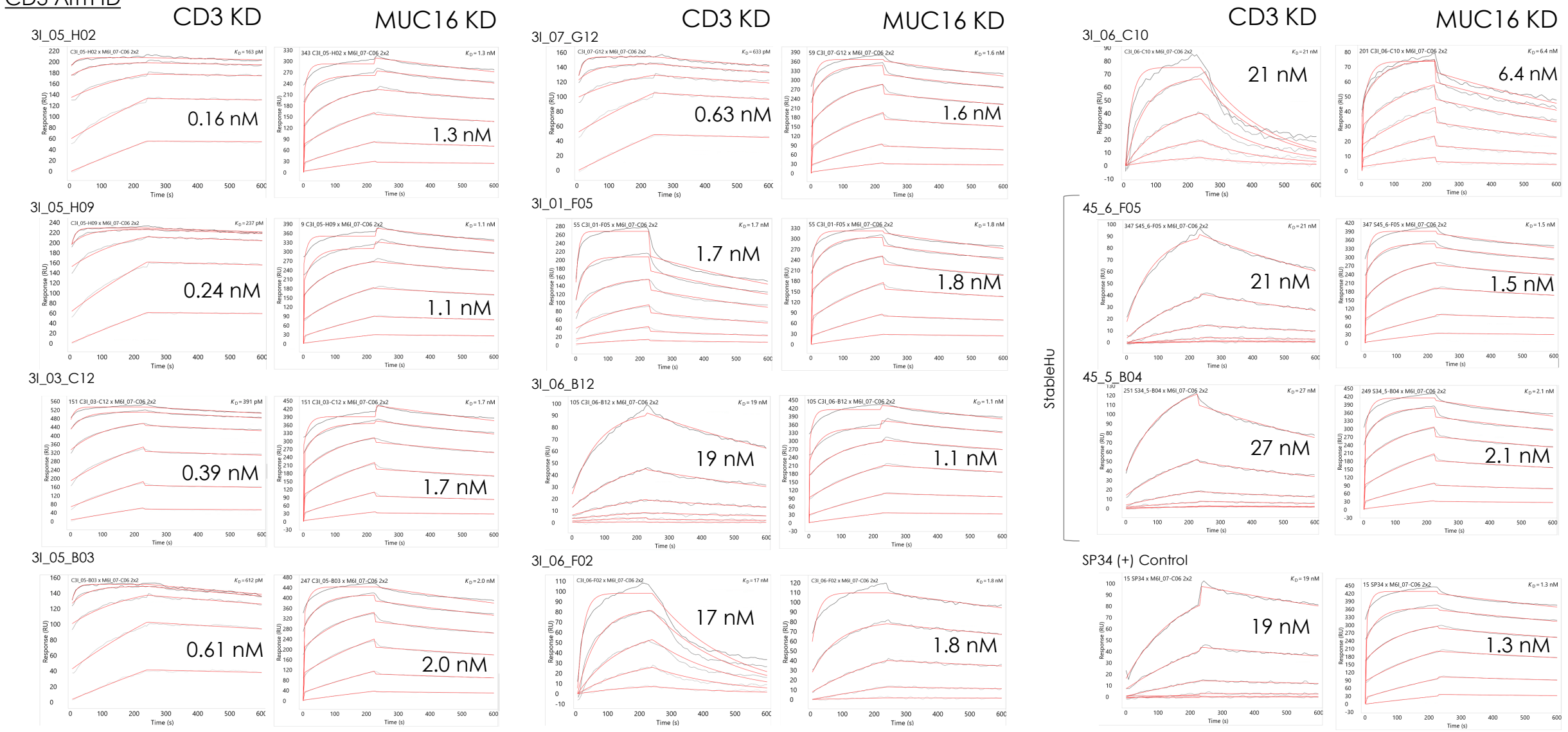
Anti-CD3





11 Epitope-Steered and StableHu CD3 Arms Were Tested with a MUC16 Arm

CD3 X MUC16 bispecifics SPR binding to each antigen



CD3 X MUC16 T Cell Engagers Kill OVCAR3 Cells with a Range of Cytokine Release

CD3 X MUC16 bispecifics cell killing and cytokines

	Killing EC50	TNF-a EC50	IFN-G EC50
ID: 3I_05_H02	13 nM	41 nM	> 100 nM
3I_05_H09	13 nM	17.5 nM	40.6 nM
3I_03_C12	9.0 nM	> 10 nM	> 50 nM
3I_05_B03	16 nM	17.9 nM	61.3 nM

	Killing EC50	TNF-a EC50	IFN-G EC50
3I_07_G12	45 nM	> 100 nM	> 100 nM
3I_01_F05	44 nM	> 100 nM	N.D.
3I_06_B12	11 nM	17.8 nM	35.0 nM
3I_06_F02	1.6 nM	19.2 nM	13.1 nM

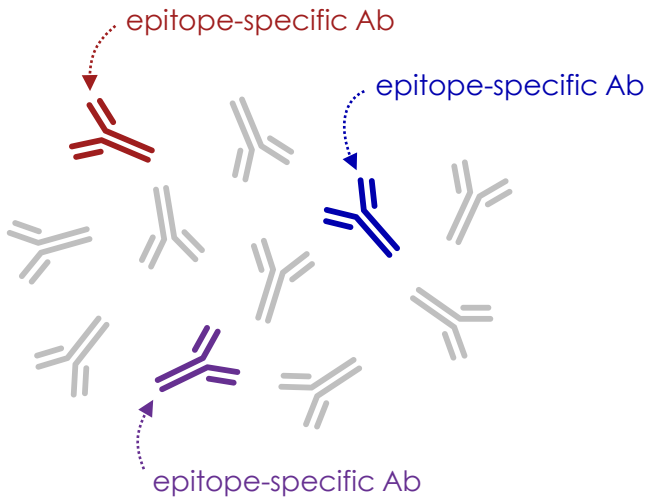
	Killing EC50	TNF-a EC50	IFN-G EC50
3I_06_C10	3.2 nM	36.8 nM	> 20 nM
StableHu 45_6_F05	6.0 nM	10.6 nM	34.1 nM
StableHu 45_5_B04	26 nM	> 100 nM	N.D.
SP34	4.1 nM	10.0 nM	31.0 nM

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

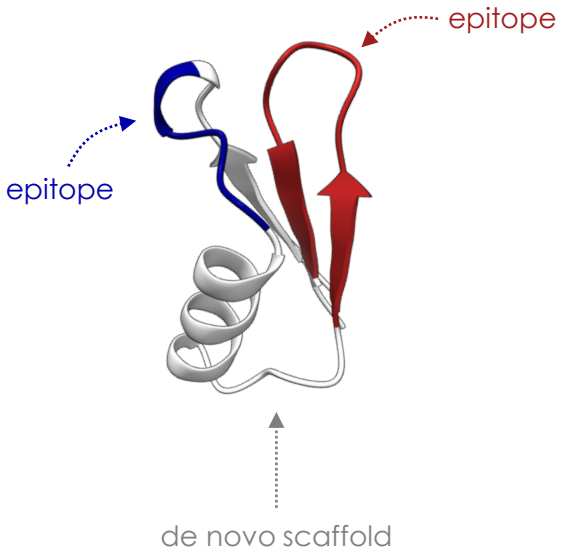
On-Target & *On-Tissue* T Cell Engager

Efficient, Single-Cycle Discovery of Conditionally-Activated Antibodies

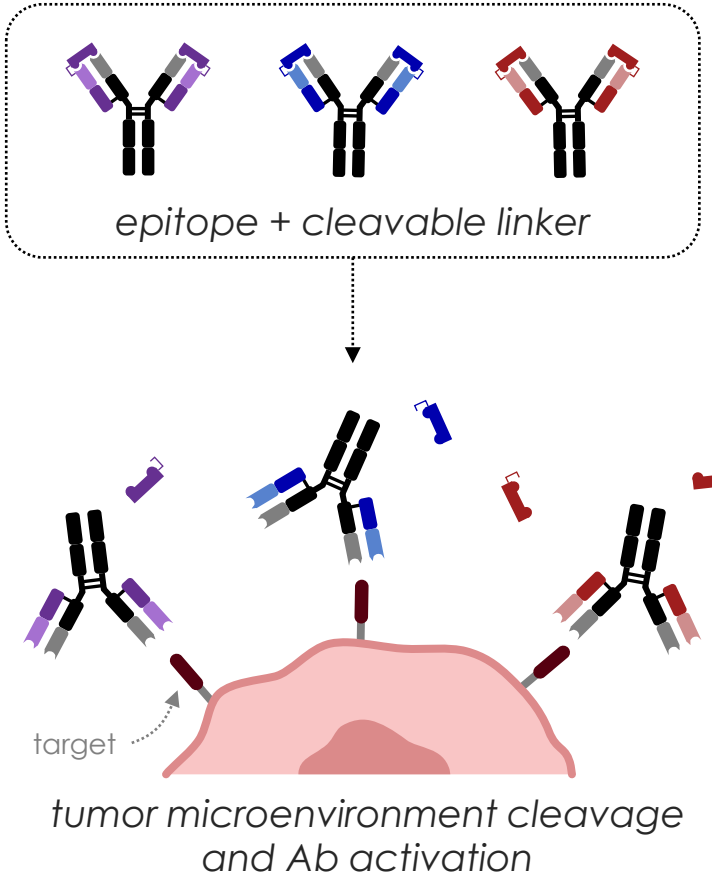
1 Naïve in vivo or in vitro antibody library



2 Focus library with engineered epitopes



3 Engineered-epitope conditionally-activated Ab

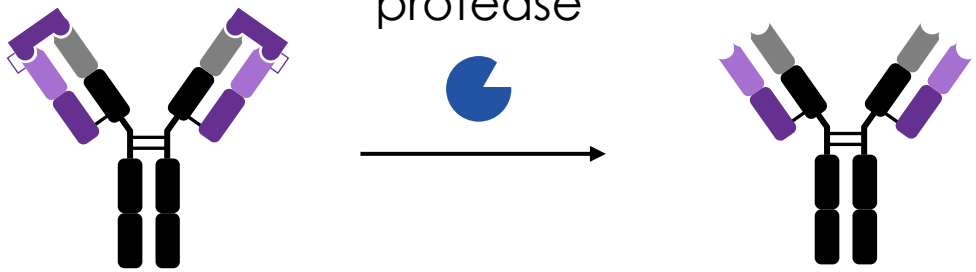


Engineered Epitope Mask Conditionally Activates MUC16 and CD3 Hits

Engineered Epitope Mask Intact

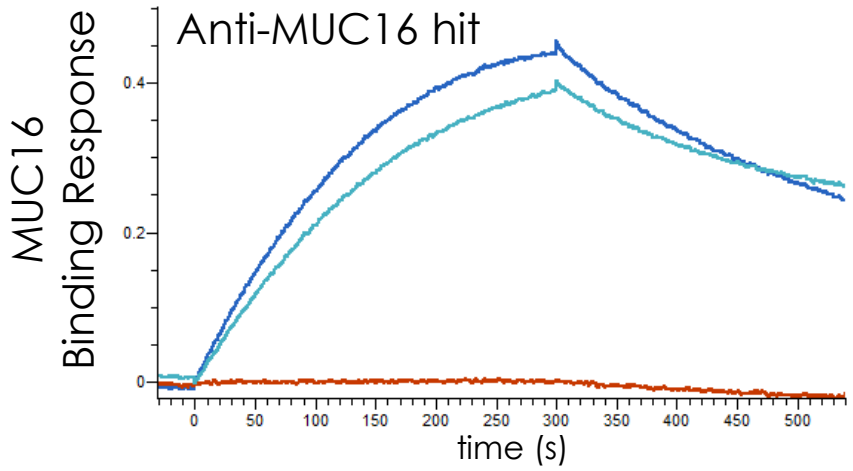
Mask Cleavage

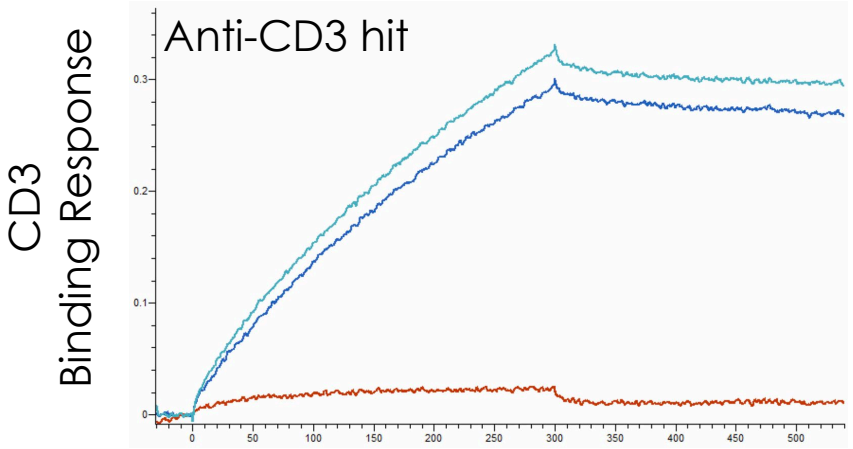
MMP protease



Inactive antibody

Active antibody





No Mask

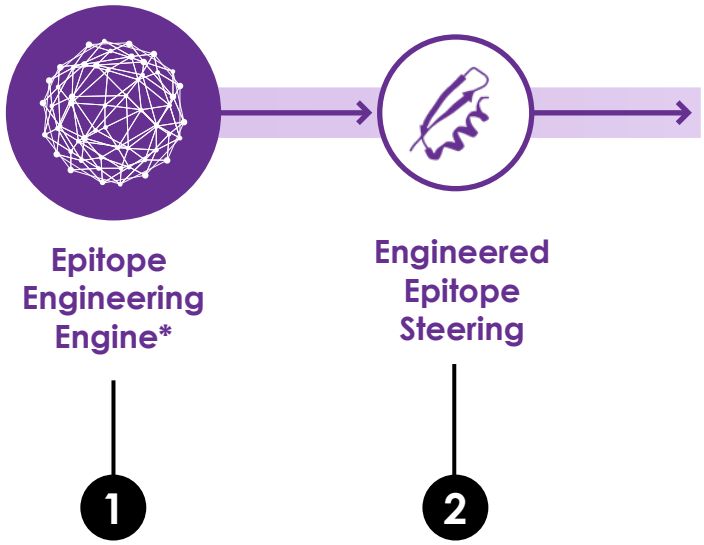
Mask (–MMP9)

Mask (+MMP9)

Conclusions

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

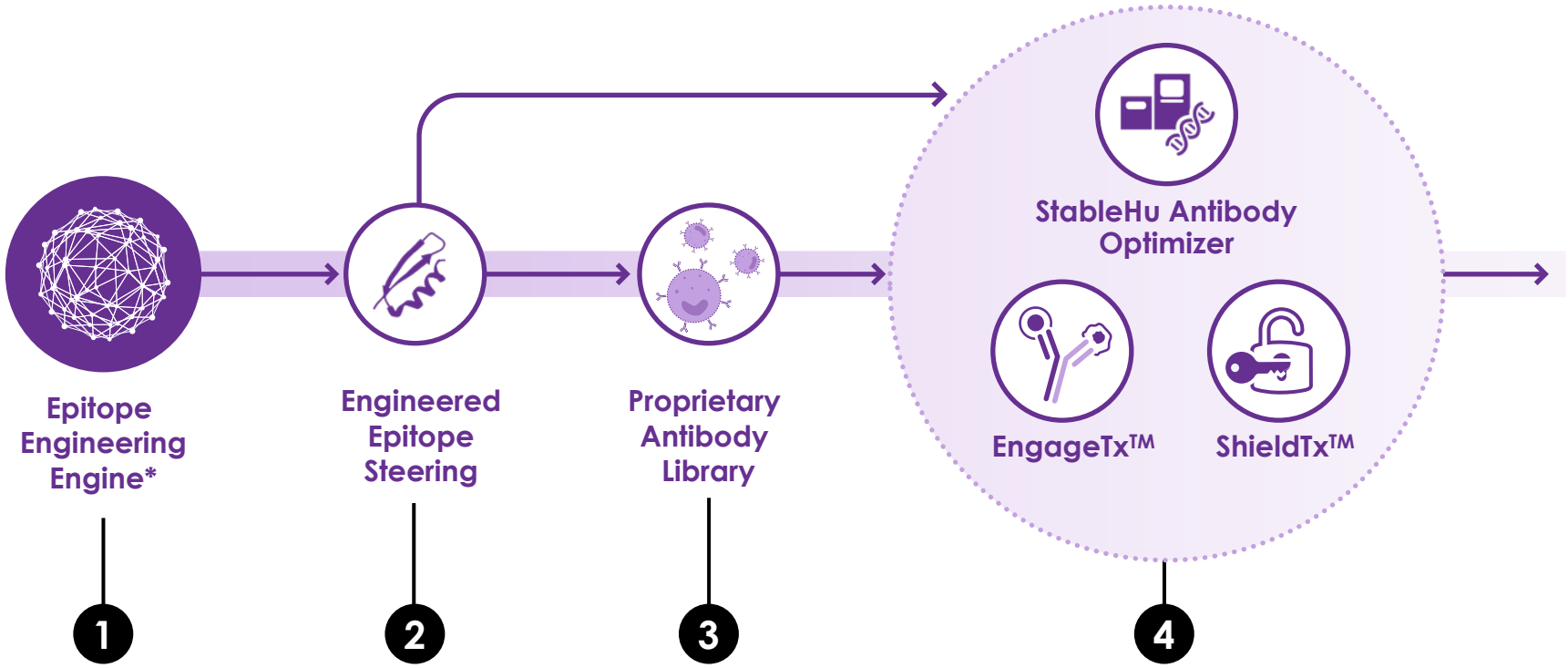
Epitope-Steering Enhances Difficult Target and MOA Discovery Productivity



- Strategically steer antibody discovery to intended epitopes
- Investigate multiple epitopes to reveal per-epitope activity
- Co-discovery of antibody and antibody-mask for on-target & on-tissue activation

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

Mammalian-Display Selects for Developability – Including Advanced Formats



1 AI-powered engineering of **conformational epitopes**

2 Epitope-specific antigens that steer discovery to intended epitopes

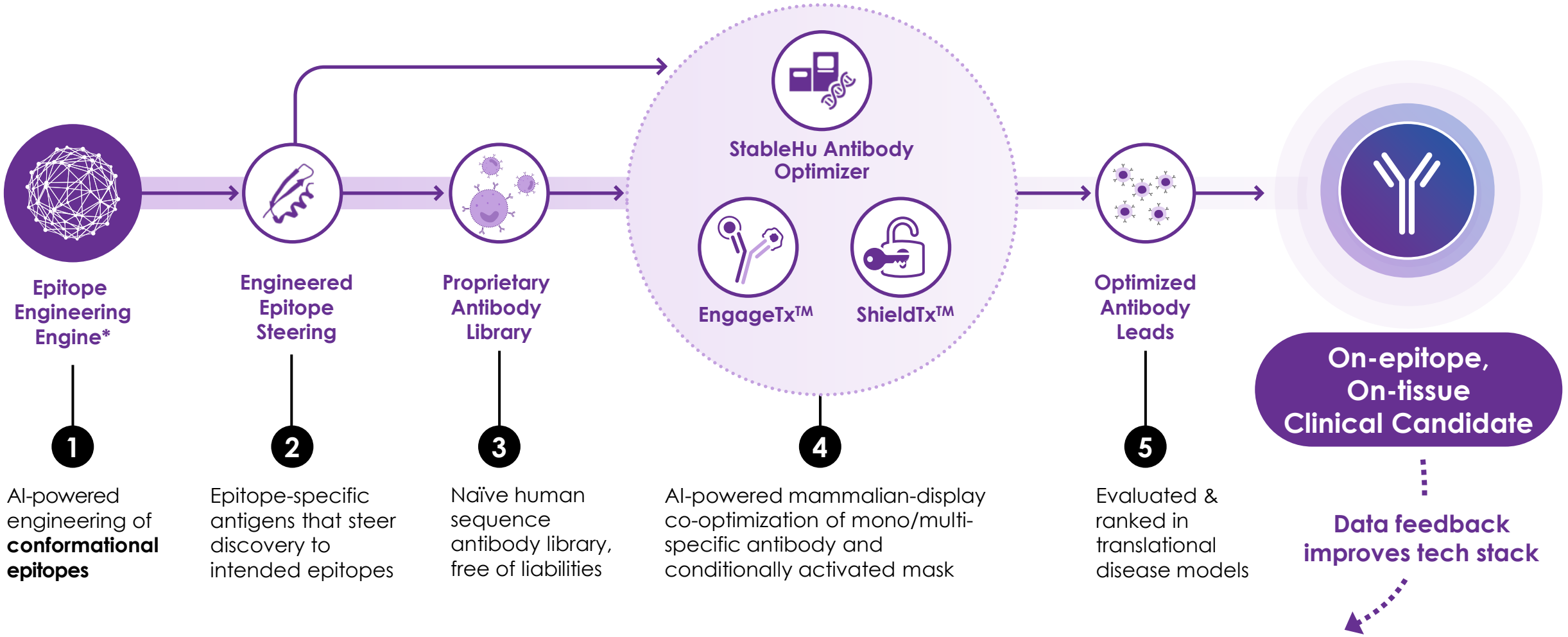
3 Naïve human sequence antibody library, free of liabilities

4 AI-powered mammalian-display co-optimization of mono/multi-specific antibody and conditionally activated mask

- High developability bi-specific masked antibodies

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

Epitope-Steering and Mammalian-Display Tackle Discovery Challenges



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