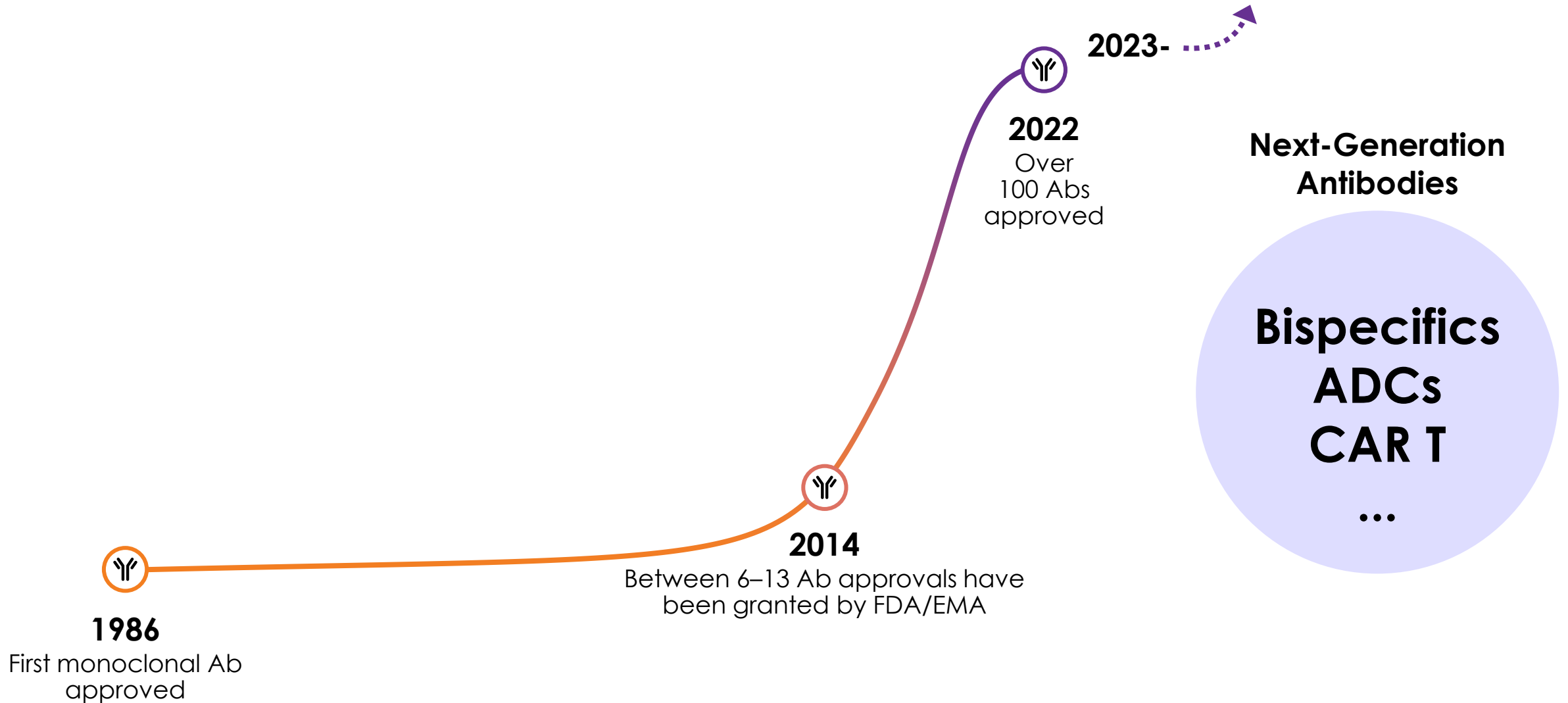# Enhancing Bispecific T Cell Engager Discovery with AI and Mammalian Display

Matthew Greving, PhD
VP, Head of ML and Platform, iBio

Drug Discovery 2023
Nov. 1-3

Innovation is Key to the Next Era of Antibody (Ab) Therapeutics

2023-

2022
Over
100 Abs
approved

Next-Generation Antibodies

**Bispecifics
ADCs
CAR T
...**

2014
Between 6–13 Ab approvals have
been granted by FDA/EMA

1986
First monoclonal Ab
approved

High Antibody Target Saturation – Vast Target Space Untapped

Approved Antibodies[2]

162

Antibody Target Saturation[2]

40% on Only 10 targets

Current Estimates of the Potential Target Space

>6,500
membrane and secreted proteins[1]

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
- Immunogenecity

Development

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

Antibody Discovery

Technology Stack

Core Tech Stack is Built for Challenging Targets and High Developability
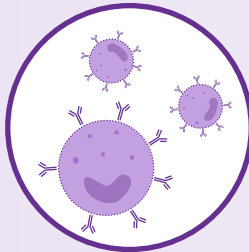
1 **Engineered Epitope***

Epitope-steered antibody discovery

Difficult target productivity enhancement

2 **Antibody Library**

Human diversity, validated frameworks

Reduce immunogenicity risk

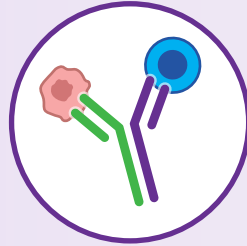**3** **StableHu™**

Mammalian-display optimization

High developability & improved activity

Core Tech Stack Enhances Discovery of Advanced Antibody Modalities

4 **EngageTx™**

Multispecific Immune Engager

Diverse arms for *optimal pairing*

5 **ShieldTx™**

On-Tissue Conditional Activation
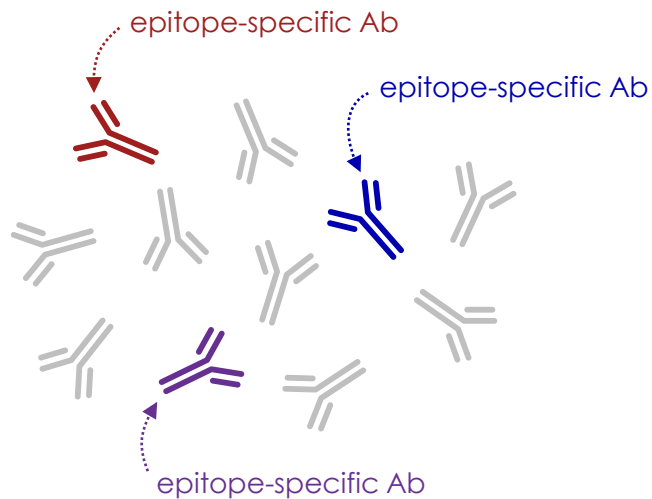
High-efficiency antibody-mask co-discovery

Epitope-Targeted

Antibody Discovery

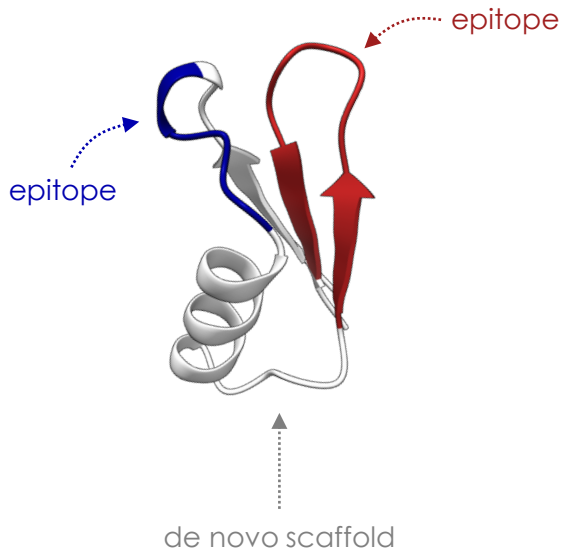# Engineered Epitopes Focus Antibody Repertoires On Desired Binding Sites

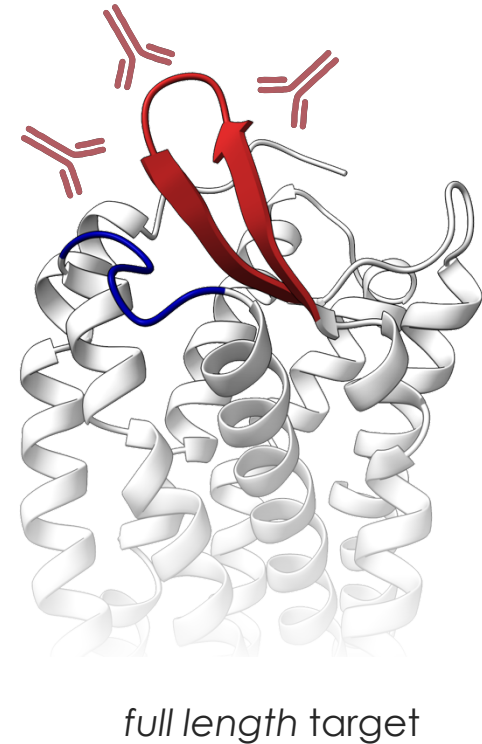**1**

Naïve in vivo or in vitro antibody library

epitope-specific Ab

epitope-specific Ab

epitope-specific Ab

2

Focus library with engineered epitopes

epitope

epitope

de novo scaffold

3

Efficient discovery of epitope-specific Abs

full length target

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

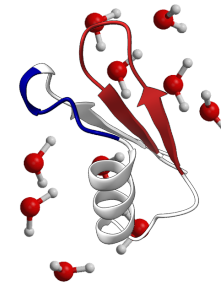Engineered Epitope Design Objectives

1 Match Structure to Target
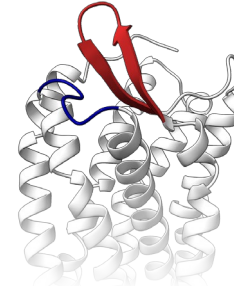
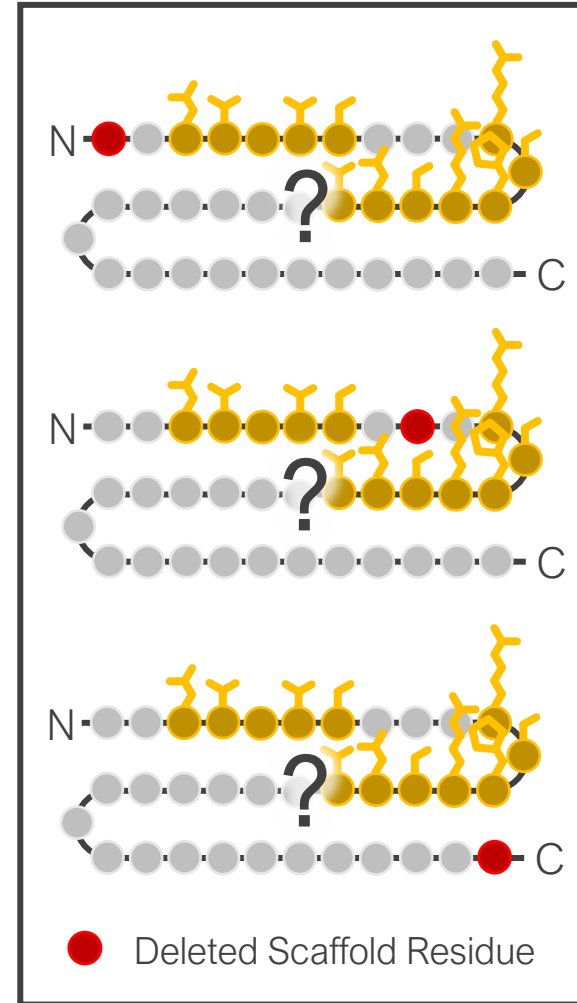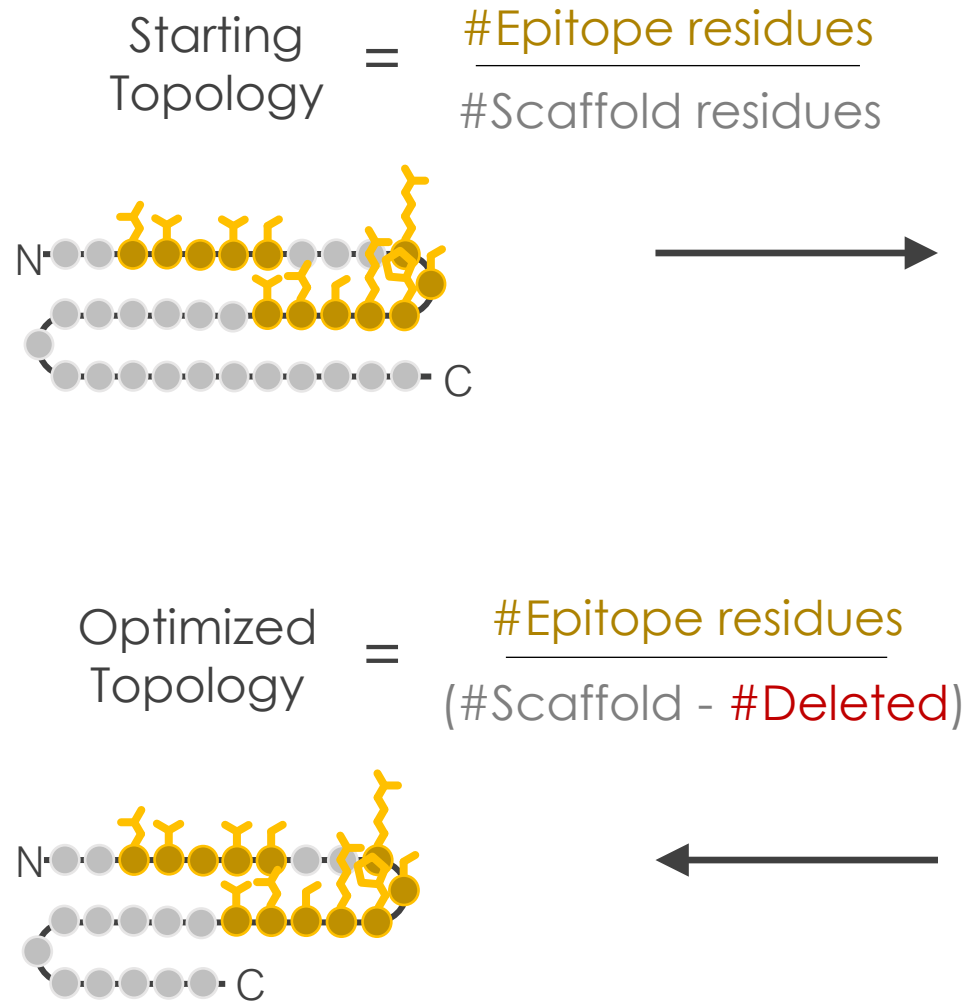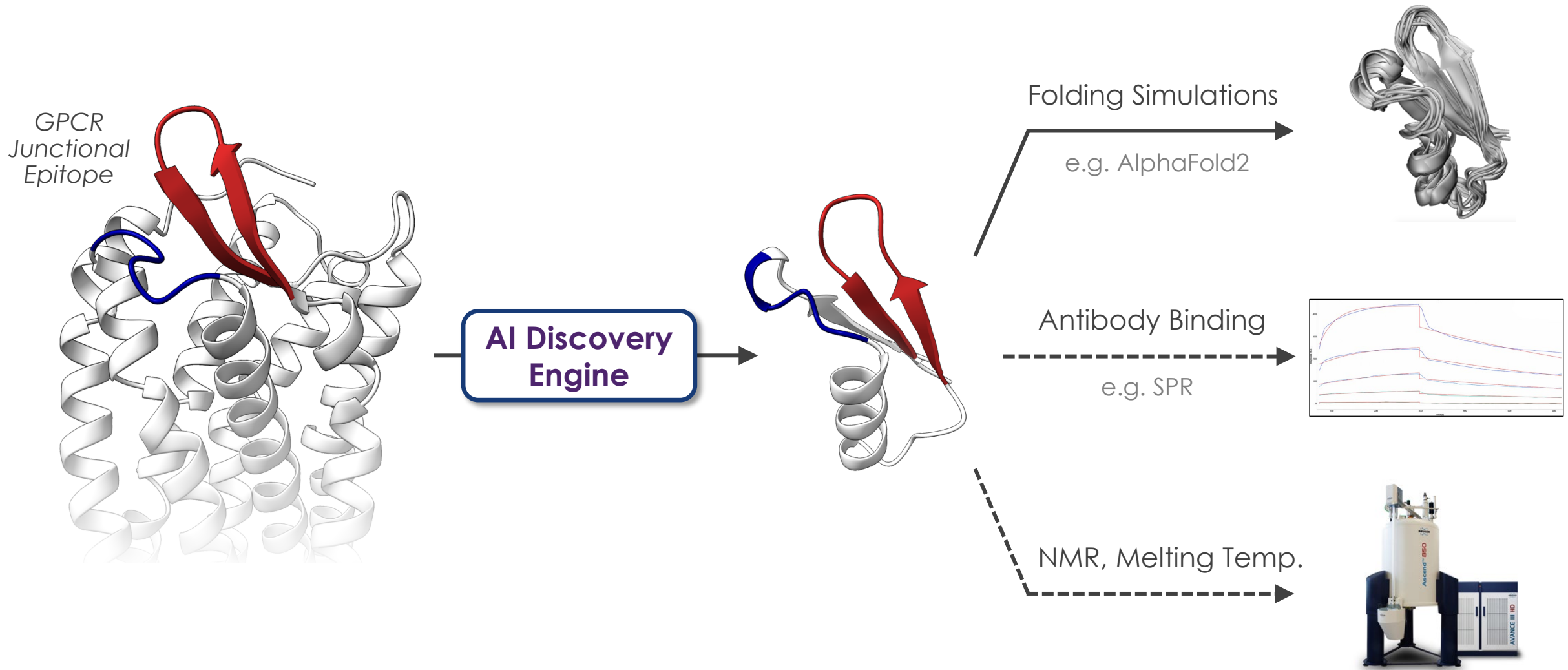**2** Refine for Greater Stability

3 Optimize for Water Solubility

AI Discovery Engine

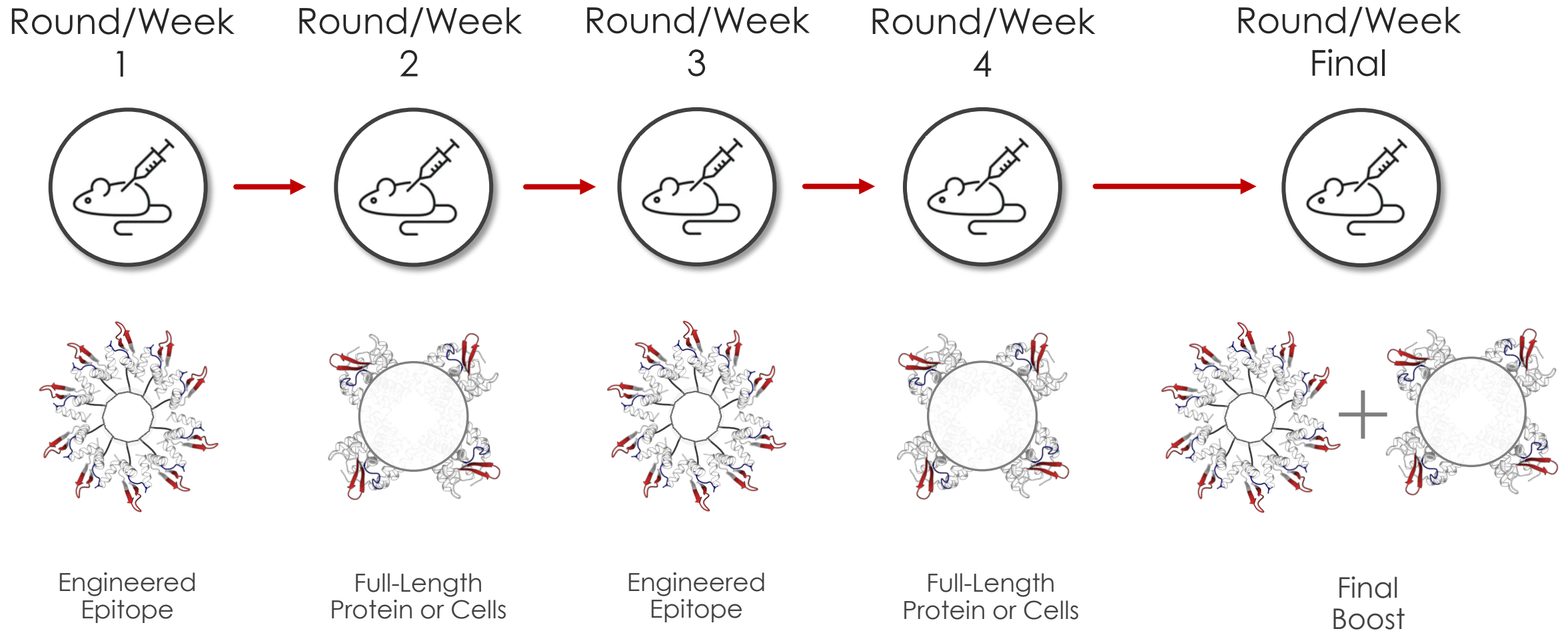# Engineered Epitopes are Further Optimized to Minimize Designed Scaffold

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

Deleted Scaffold Residue

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

GPCR Junctional Epitope

AI Discovery Engine

Folding Simulations

e.g. AlphaFold2

Antibody Binding

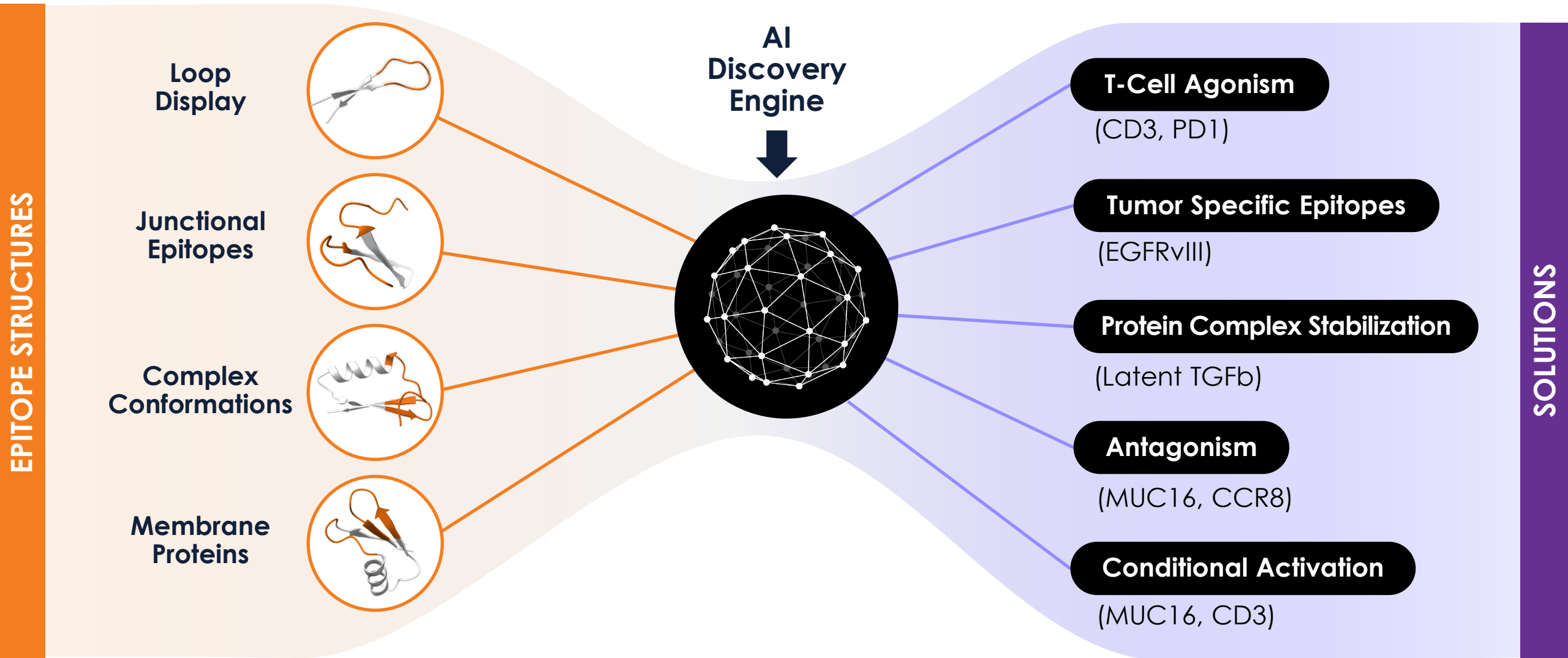e.g. SPR

NMR, Melting Temp.

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

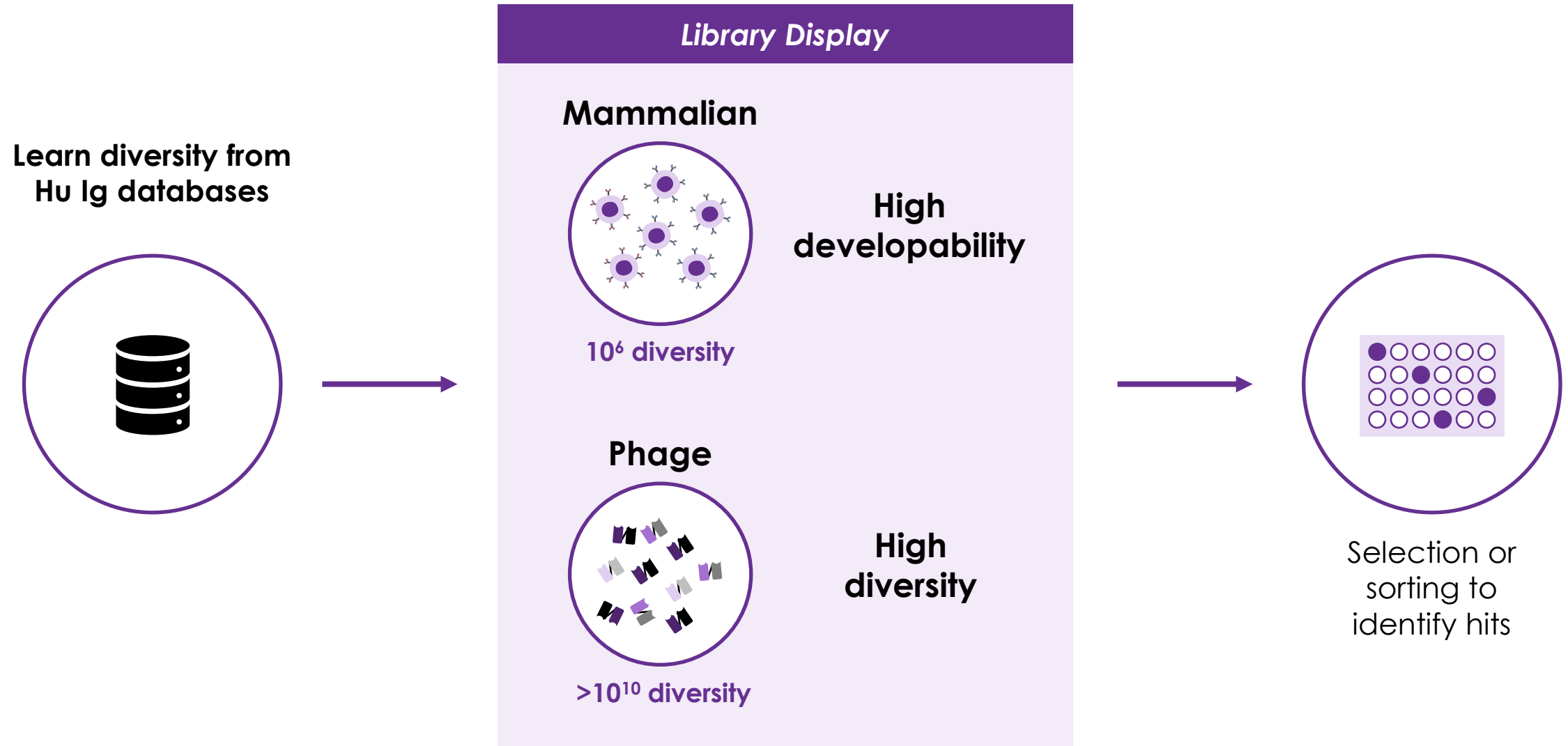Engineered epitopes alternated with full length native target protein and/or cells

Round/Week 1 — Engineered Epitope

Round/Week 2 — Full-Length Protein or Cells

Round/Week 3 — Engineered Epitope

Round/Week 4 — Full-Length Protein or Cells

Round/Week Final — Final Boost

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

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 Diversity for Mammalian Display

Input Antibody	StableHu Optimizer AI-Engine	Mammalian Display	Output Antibody
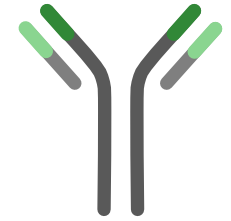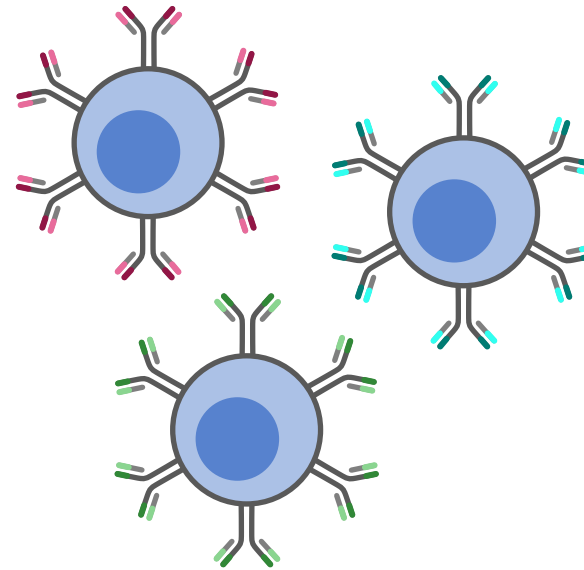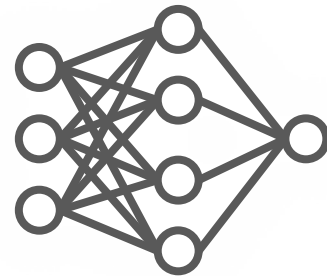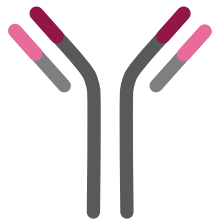
Template CDR	Predict library of human CDR variants	Single-cell screen CDR library	Optimized antibody with fully human CDRs

Optimizer AI Model is Trained to Predict Fully Human CDR Sequences

Antibody Database

Optimizer AI

Trained Model

cAb-Rep & OAS
Hu Ig databases

>1 billion curated
human antibody
sequences

A R ? G R Y F ? S ? T G Y

A R G G R Y F D S T T G Y
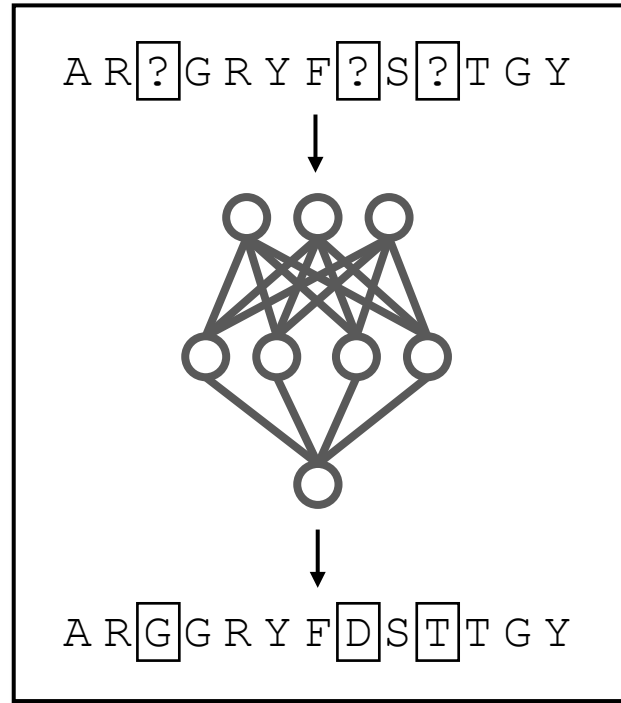
AI trained to predict
fully human CDR from masked CDR
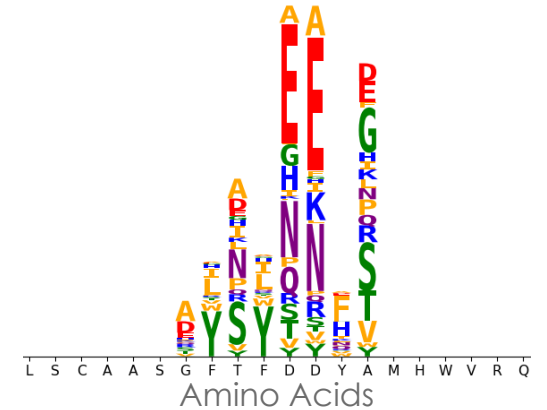
Predict library of fully
human CDRs from
template CDR

StableHu Library Sorting and NGS Identify Improved Human CDR Variants

Mammalian Display Single-Cell Sorting

StableHu Library   Sorting Bin

Template Ab

Binding

Expression

| Binding > Template |
| Binding ≈ Template |
| Binding < Template |
| No Binding |

Sorted Bins
NGS &
HT SPR Screen
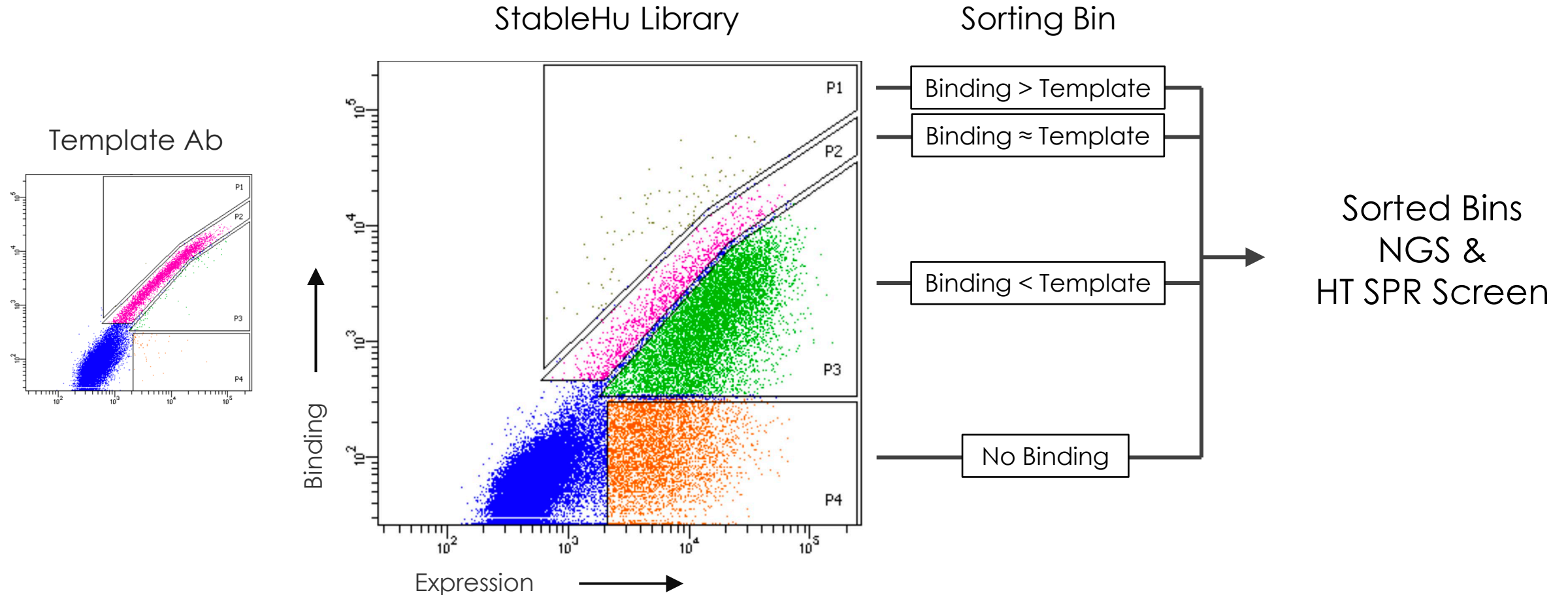
CD3 T Cell Engager Arm

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

Diverse CD3 engager arms

Numerous tumor antigen arms
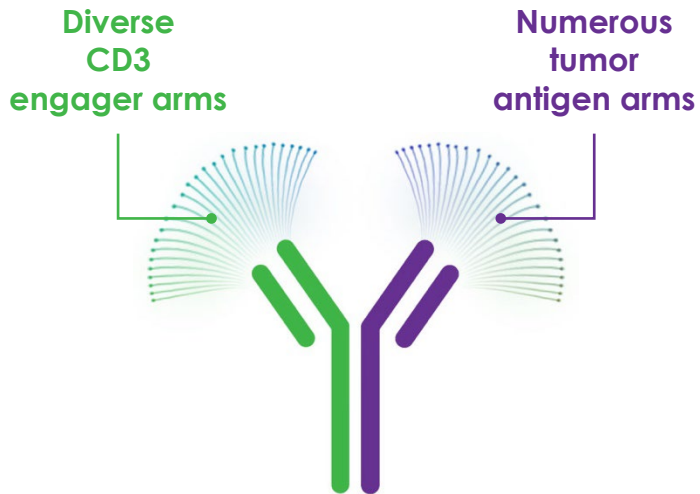
2 **Hu-Cyno Cross-Reactivity**

Risk reduction via cyno monkey toxicity study compatibility
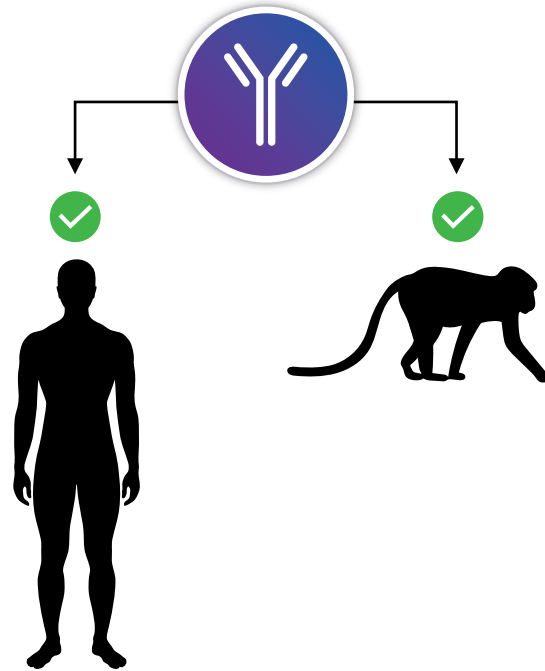
3 **Range of Cytokine Release**

Tailored cytokine release for expanded therapeutic window

Tumor cell death

Granzyme, Perforin

Release of cytotoxic granules

Activated T Cell

Increased cytotoxicity

Release of cytokines

Tumor cell

TNFα, IFNγ, IL-2, (IL6)

Cascade of immune activation

Reduced cytokine release
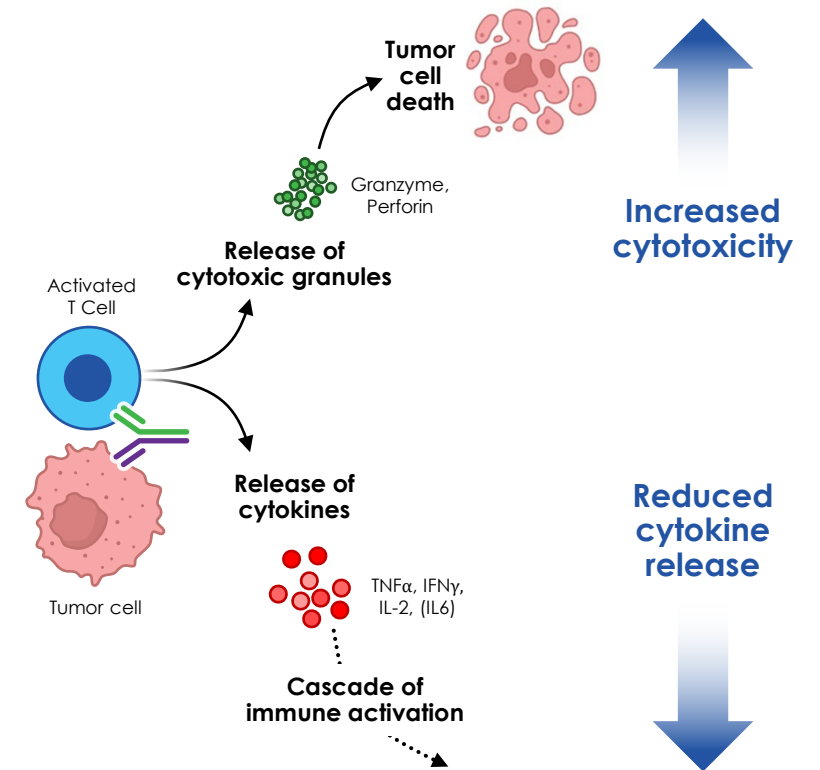
Dual Approaches to a Diverse Panel of Anti-CD3 Antibodies

AI Discovery Engine

Engineered-Epitope Immunization & Screening

TCR

Engineered Epitopes

AI-Engineered Immunogens

Epitope & T Cell Immunization

Epitope & CD3 Screen

StableHu Optimizer

Template Antibody

Optimized Antibodies
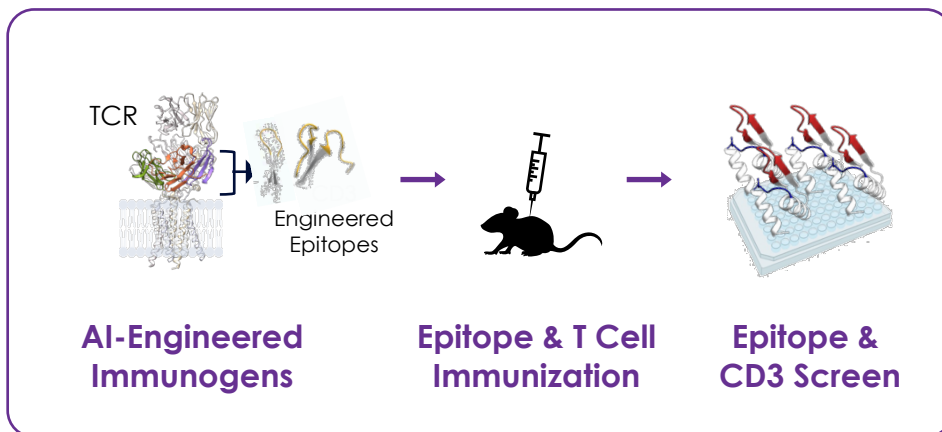
SCREEN

Hu-Cyno CD3 & T Cell

Binding

Hit ID: 1 2 3

T Cell

(-) Cell

T Cell

Activation

CD69 Expression (MFI)

Concentration (nM)

ID
- 1
- 2
- 3
- 4
- 5
- 6
- (-) Ctl
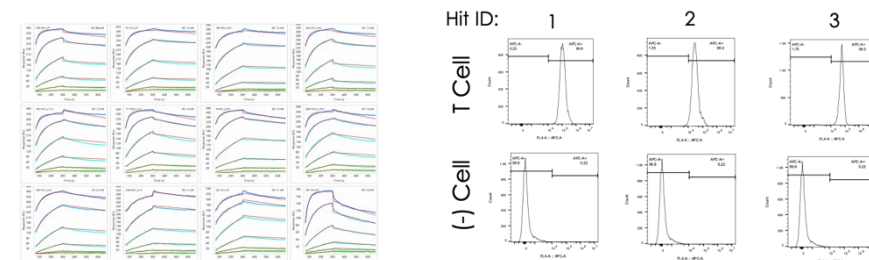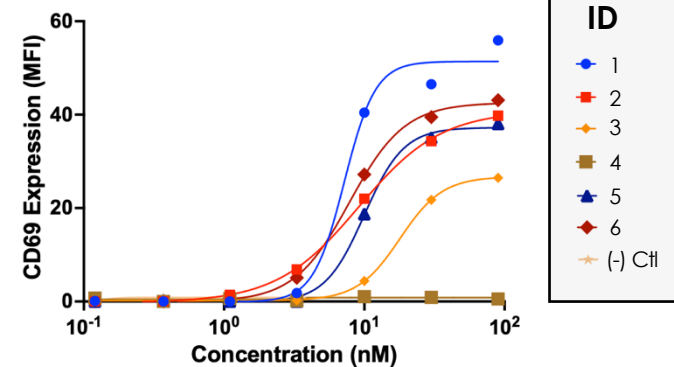
Epitope Engineering for TCR Accessibility & Hu-Cyno Cross-Reactivity

CD3 target epitopes in the context of the full TCR
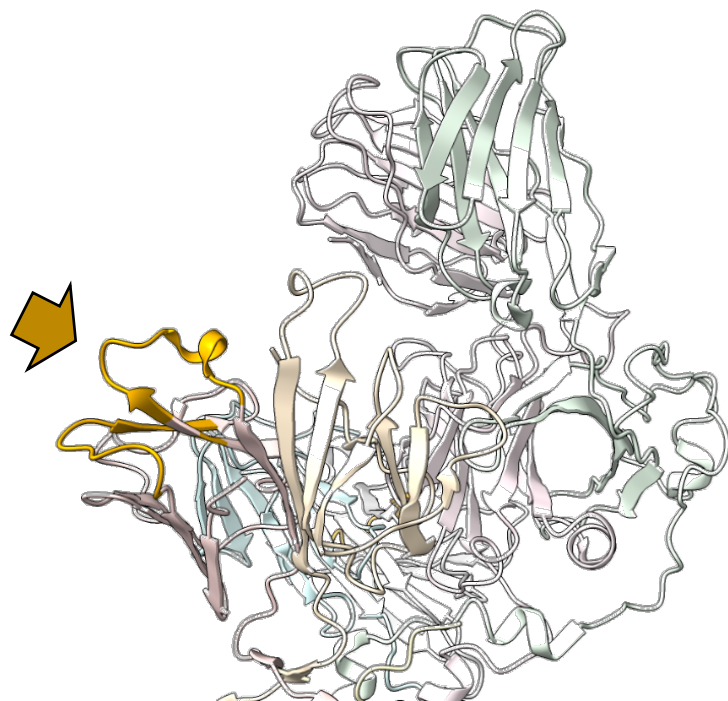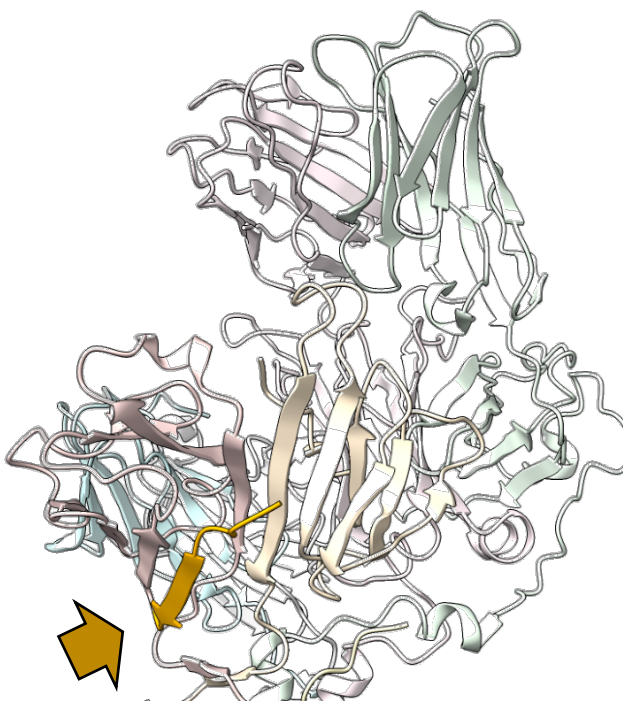
Epitope 1 | Epitope 2 | Epitope 3
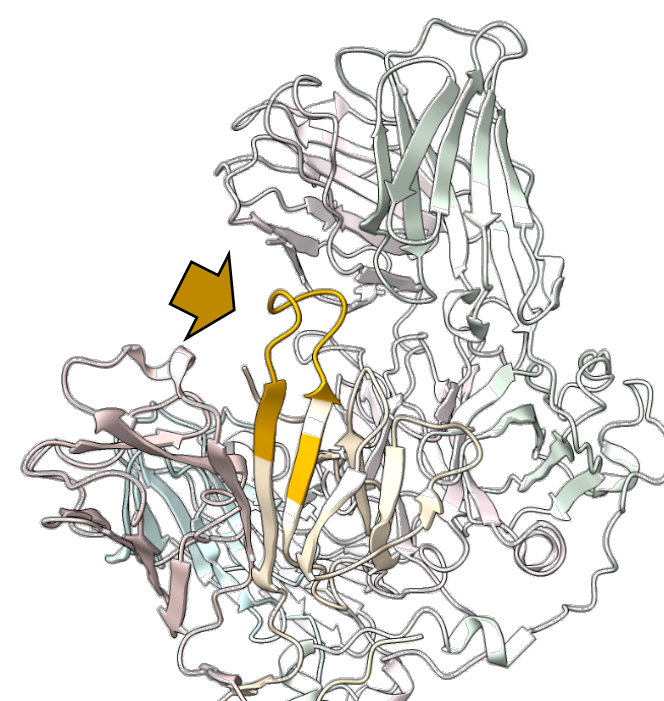
Immunized CD3 Repertoires Were Cloned & Screened in Mammalian Display

1. Epitope-Steered Immunization

Prime   Boost   Final

epitope nanoparticle   T cells   T 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. Hu-Cyno CD3 binding
3. Ab expression
4. Poly-Specificity

NGS, SPR, In vitro activity
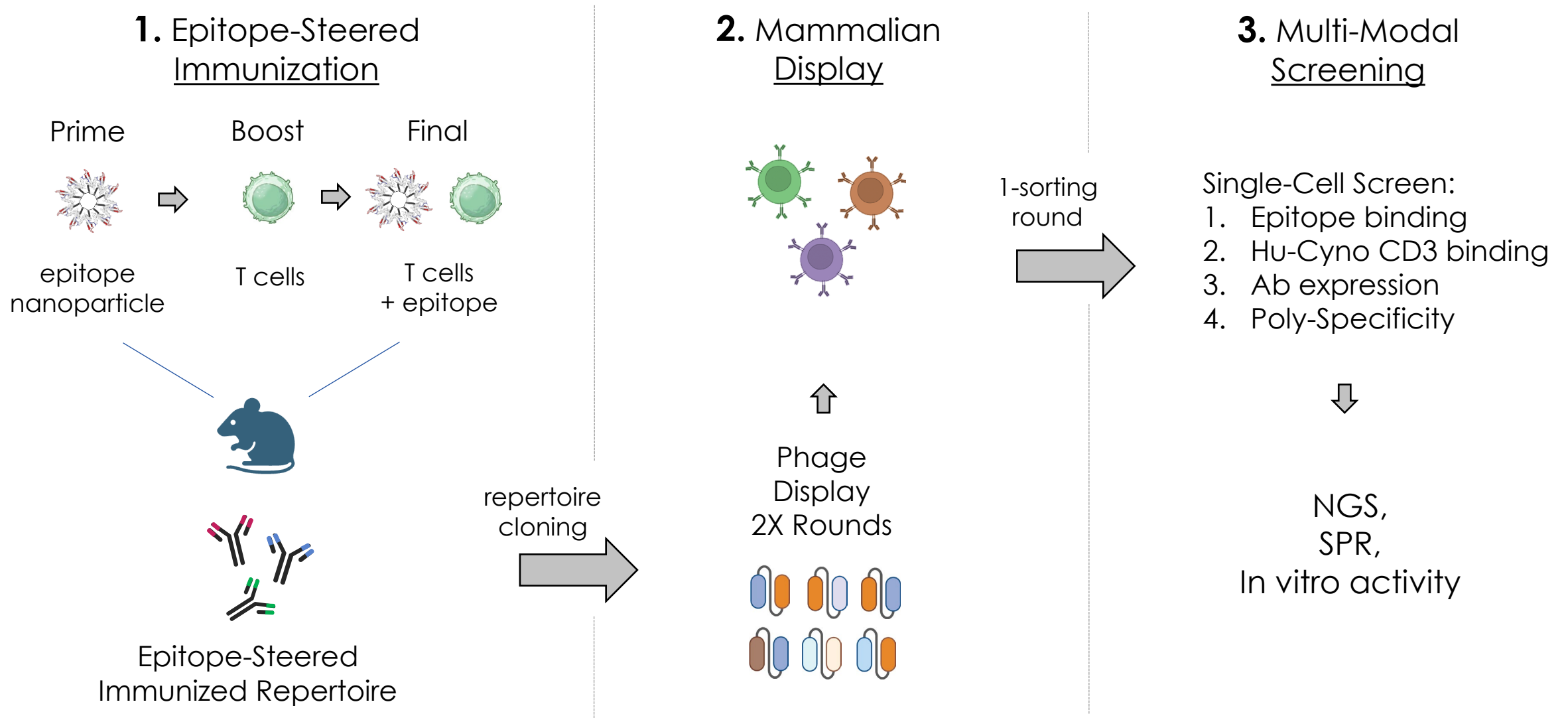
Mammalian Cell Sorting for Hu-Cyno CD3 Binding & Enhanced Expression
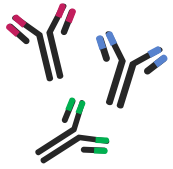
Epitope-Steered Immunized Repertoire

Mammalian Display

repertoire cloning

Phage Display 2X Rounds

2 libraries

cell sorting

VL–**VH**

VH–*VL*

VL–**VH** scFv-Fc library

VH–*VL* scFv-Fc library
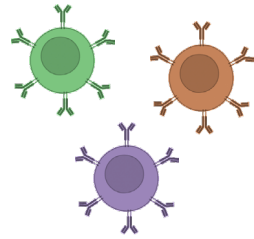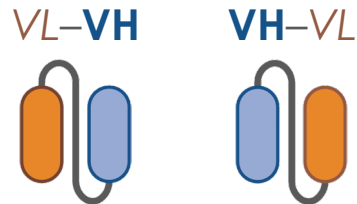
CD3 Binding
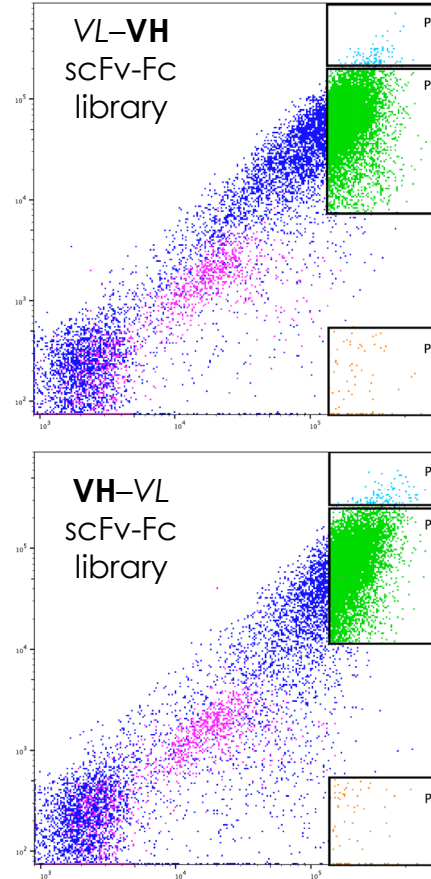
Antibody Expression

■ 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 Hu-Cyno CD3 10^4 Affinity Range Binders

Human vs Cyno CD3ED HT-SPR Affinity
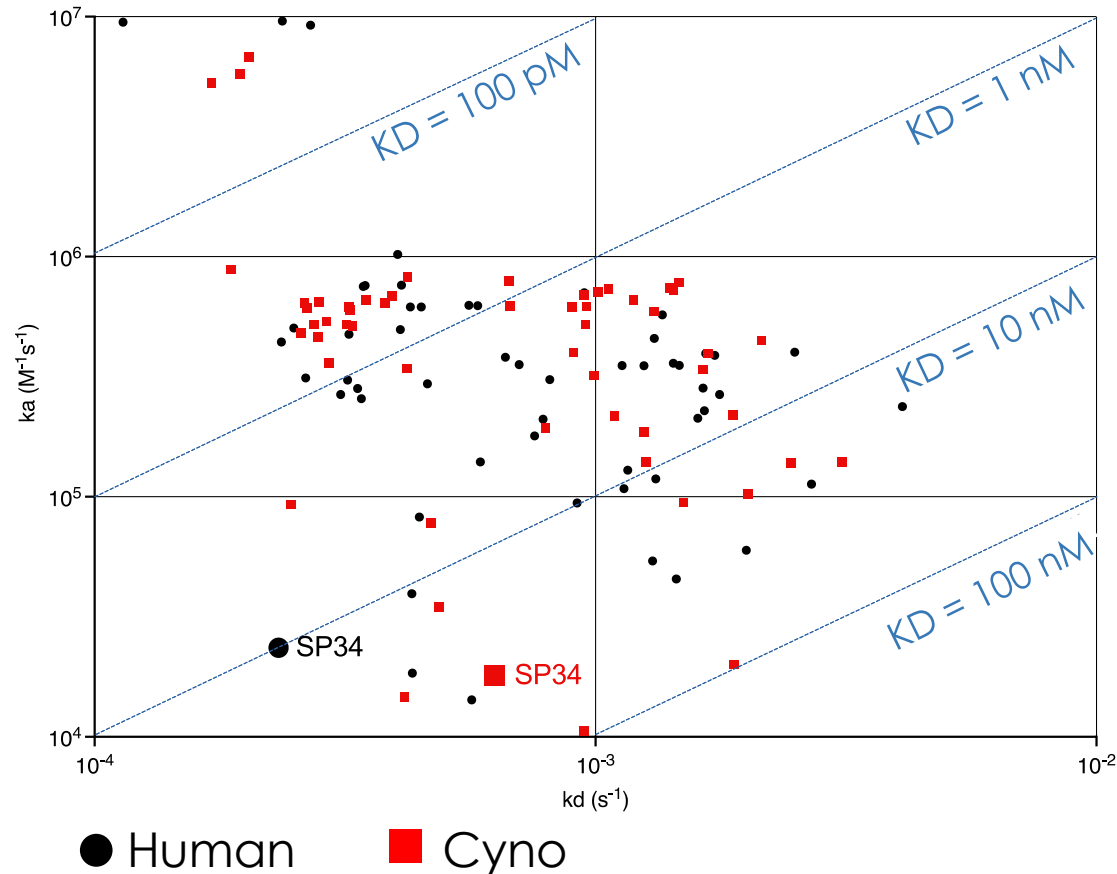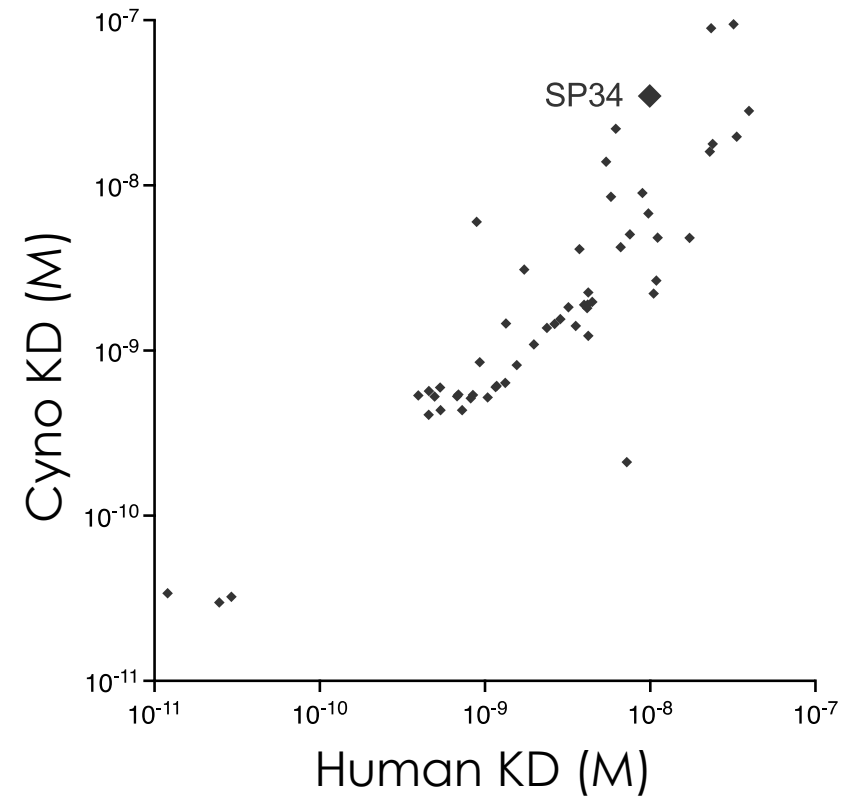
39/54 = 72% Human-Cyno CD3 Cross-Reactive Hits Bind Engineered Epitopes

Human **CD3ED**, **Epitopes 1, 2** HT-SPR Affinity
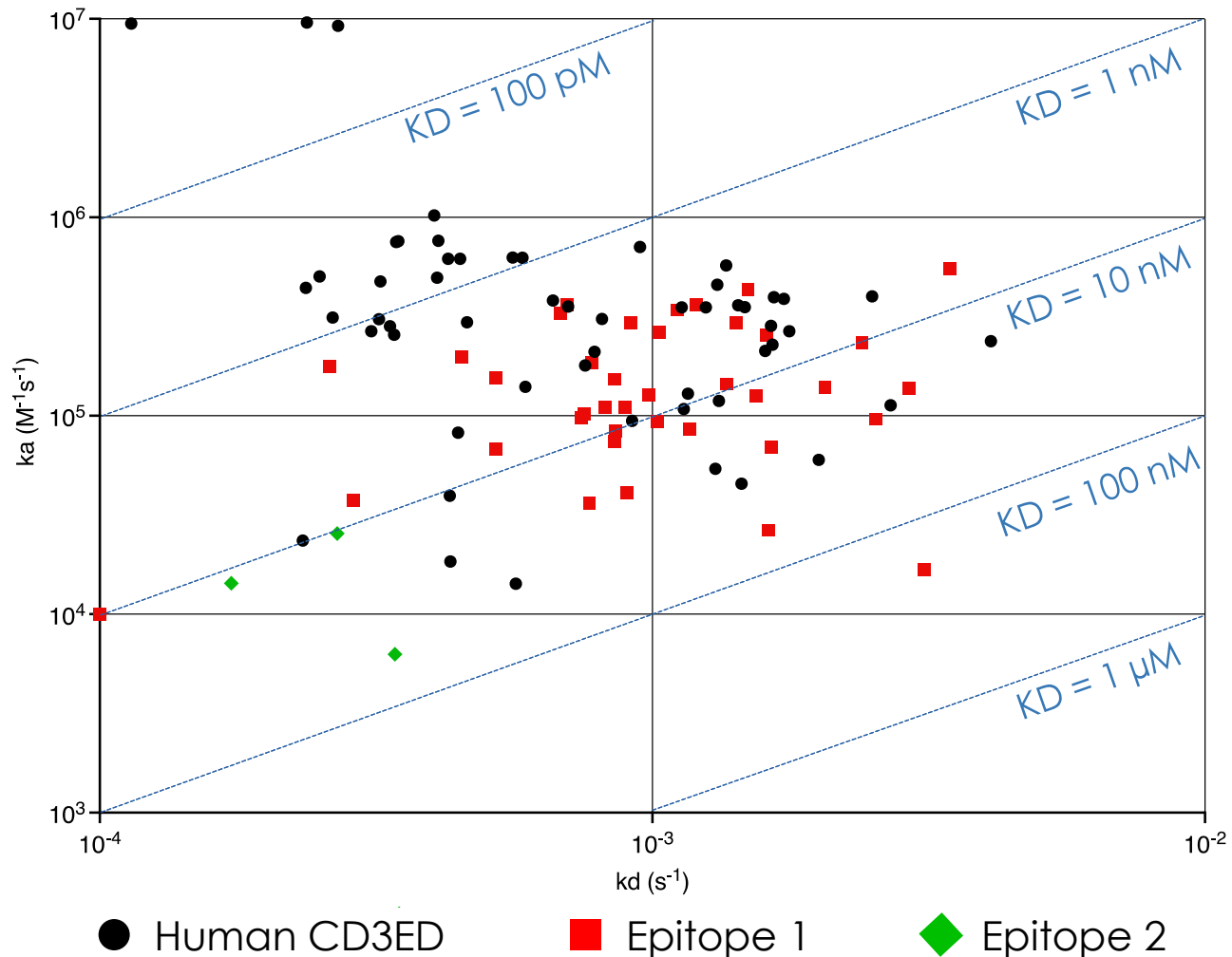
- All engineered-epitopes identified epitope-specific antibodies

- Epitopes 1 & 2 identified Hu + Cyno cross-reactive antibodies meeting affinity threshold of KD ≤ 100 nM

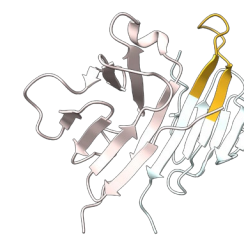- Epitope 1 is the most productive, potentially due to high accessibility
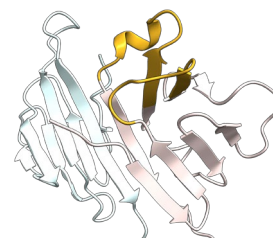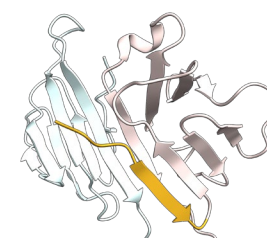
Epitope 1   Epitope 2   Epitope 3

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

Representative Subset of T Cell Binding Hits
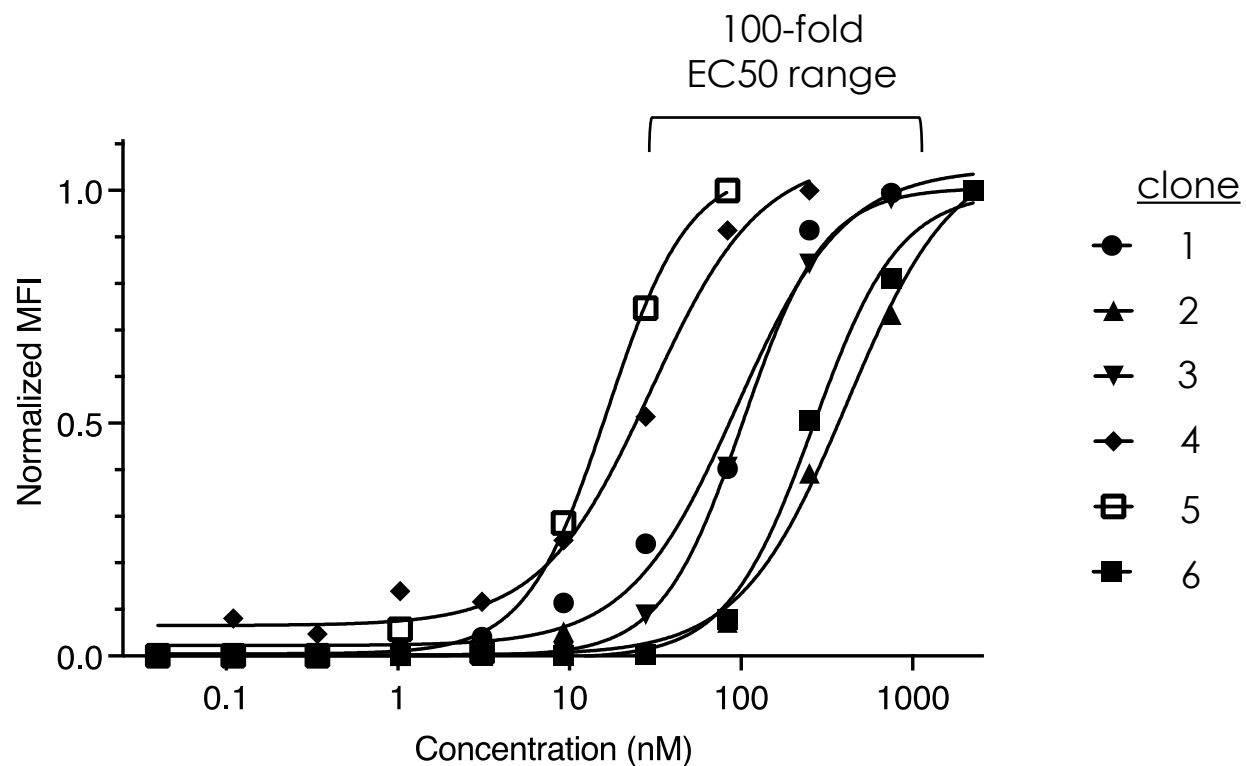
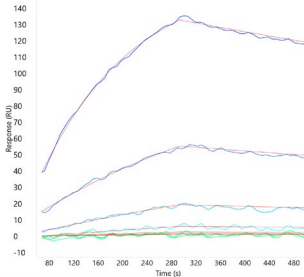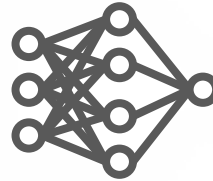T Cell Binding Hit Panel
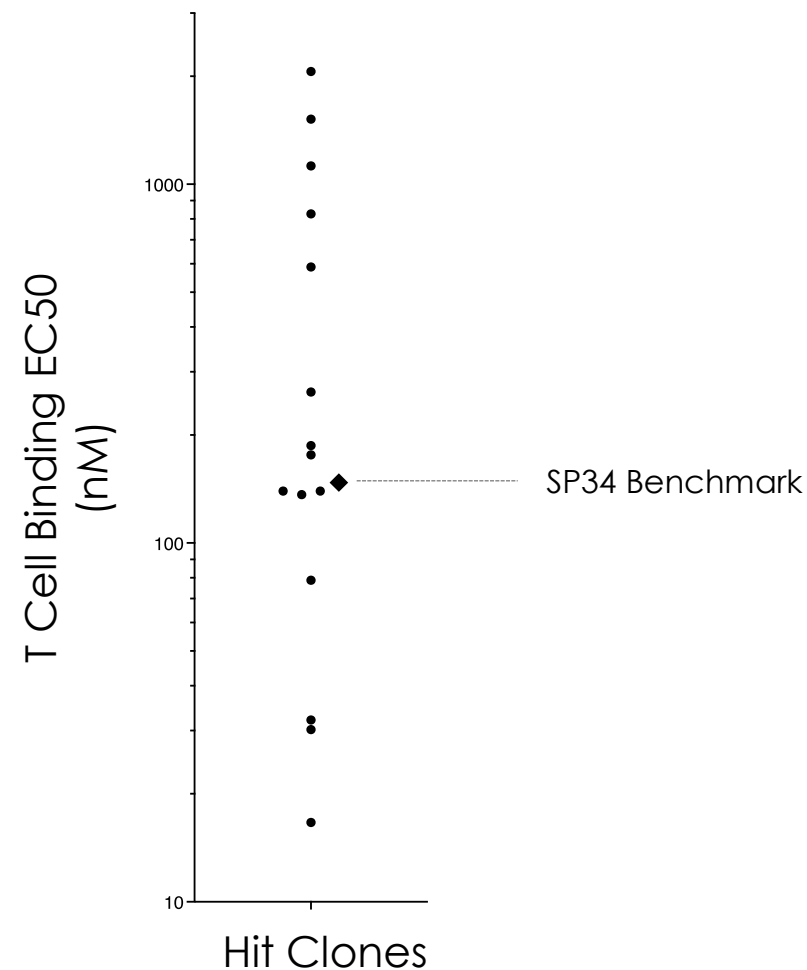
Anti-CD3 Template Antibody Human Diversification with StableHu AI
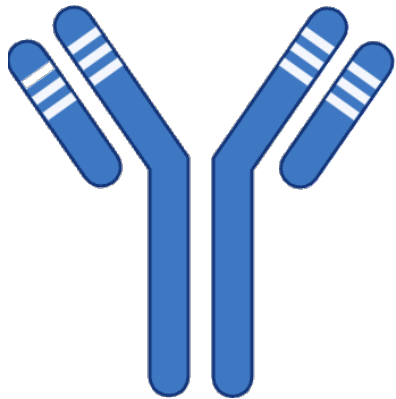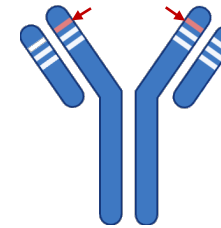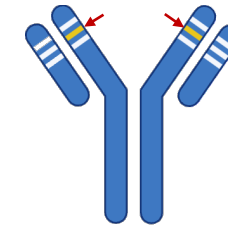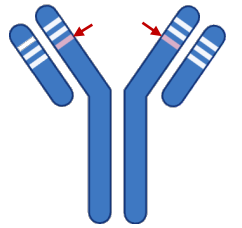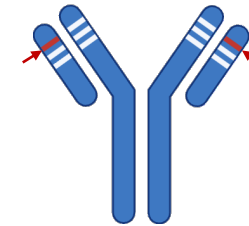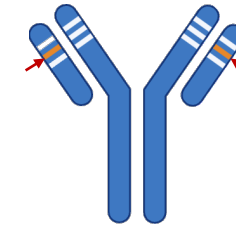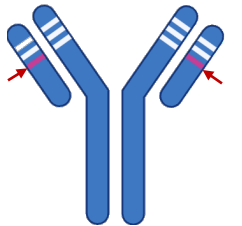
StableHu Screening Identifies 7 Hu-Cyno CD3 100-Fold Affinity Range Binders

Dual-Track Discovery Identifies 22 Hits That Activate T Cells Across a 10^3 Range

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

Expanding Anti-CD3 Hit Diversity with Functional Mammalian Display Screen
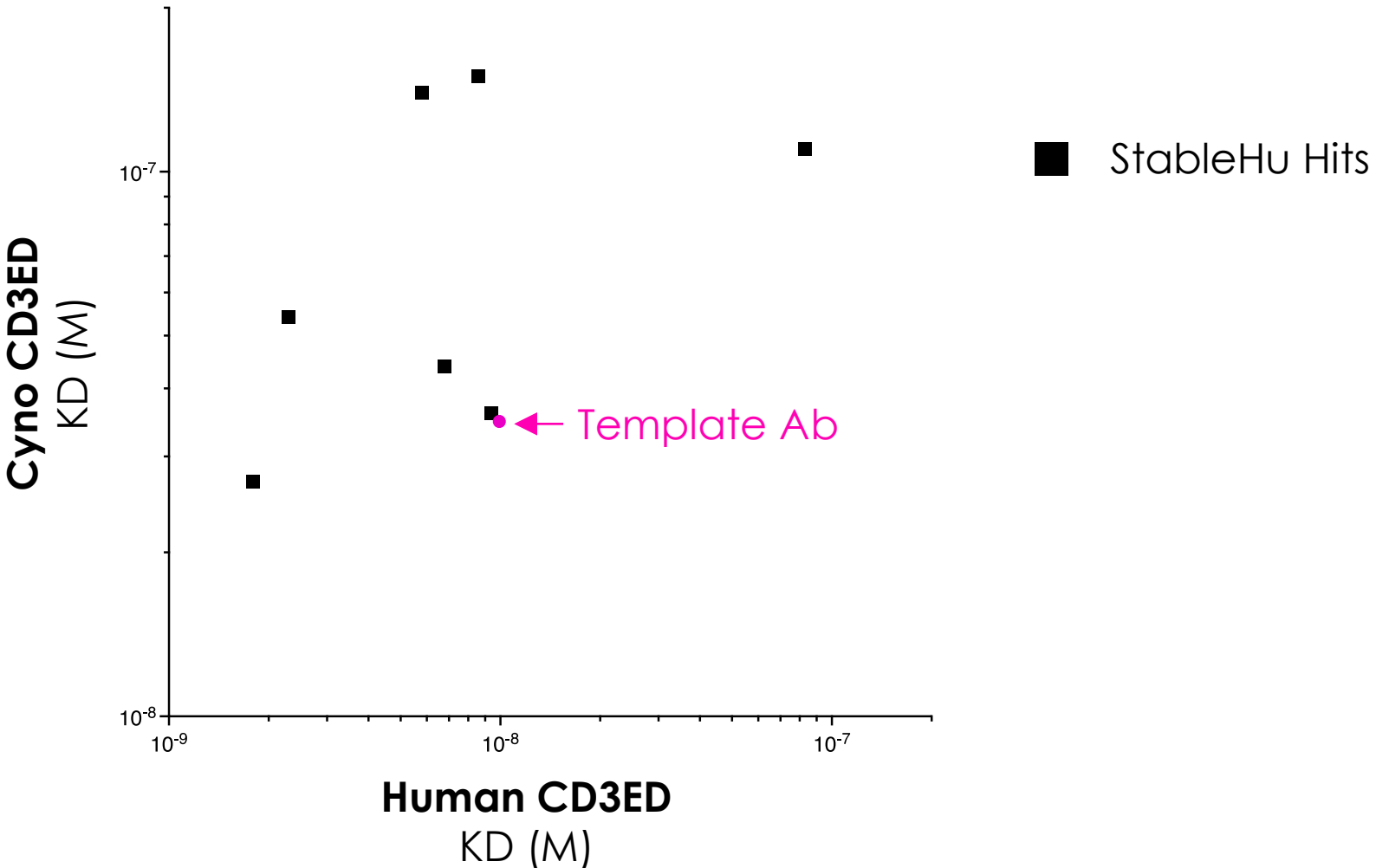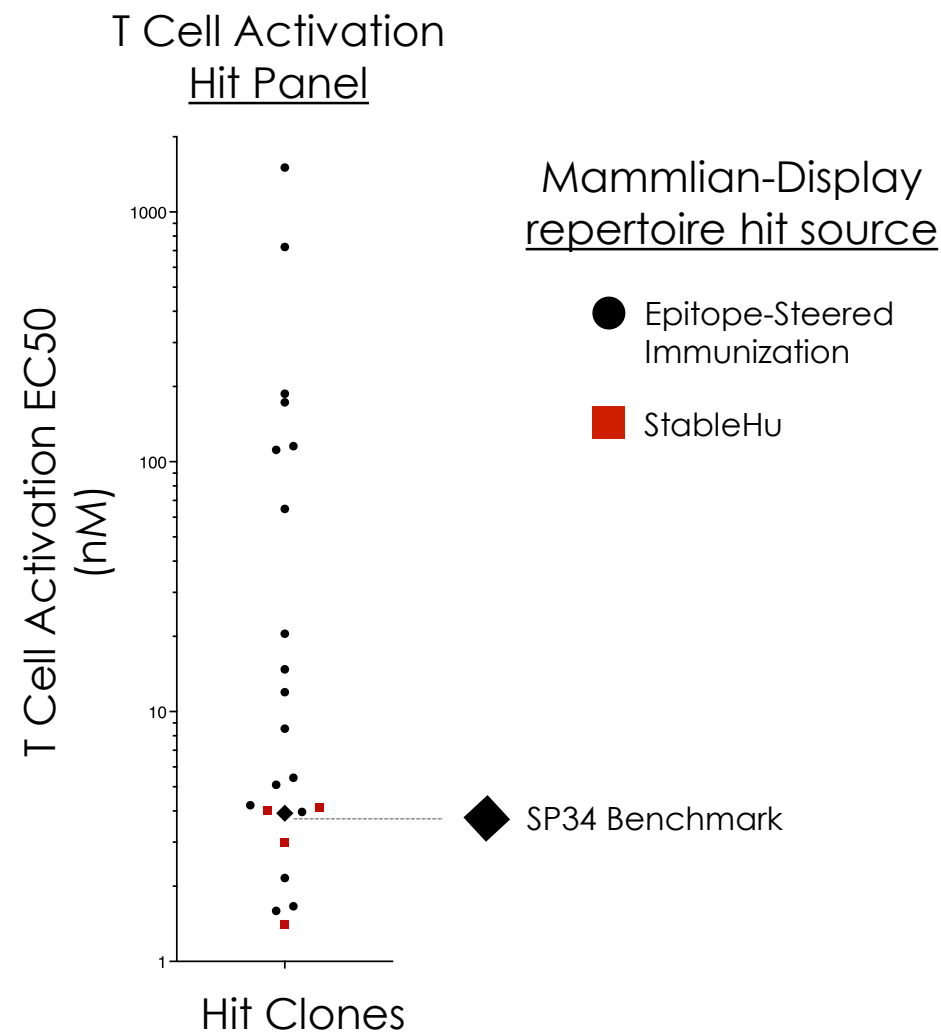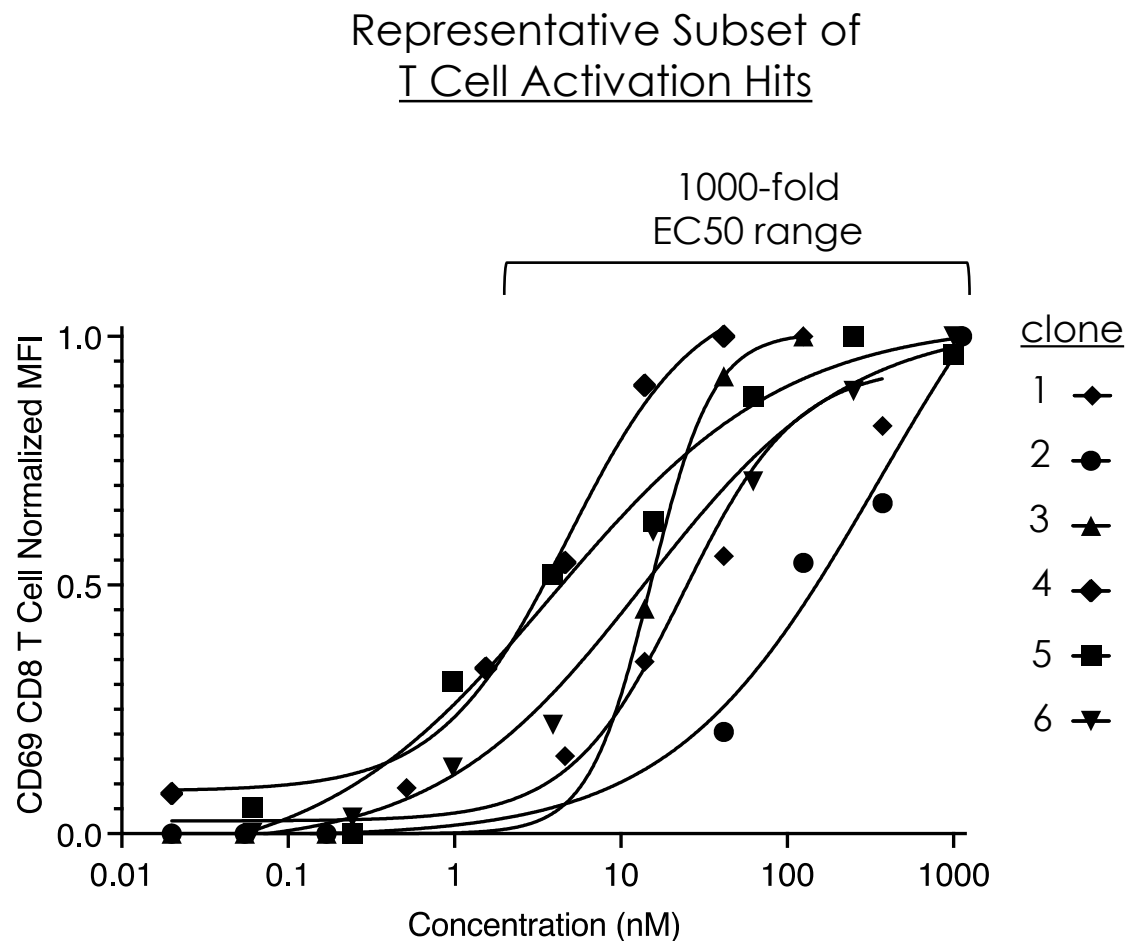
Microwell T Cell reporter screen with mammalian display library cuts weeks off CD3 agonist discovery time

Epitope-Steered Immunized Repertoire
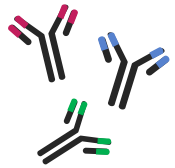
Mammalian Display
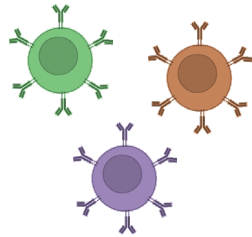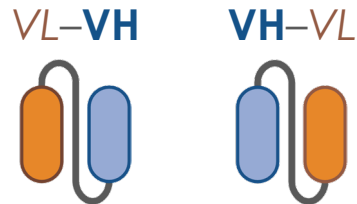
Large-Scale Functional Screen

50K-200K wells

Assay: CD3 activation NFAT-GFP (green)

repertoire cloning

Phage Display 2X Rounds

2 libraries

VL–VH   VH–VL

CD3 activation reporter cell assay

CD3 agonist library hit

Reporter cells

Library cells
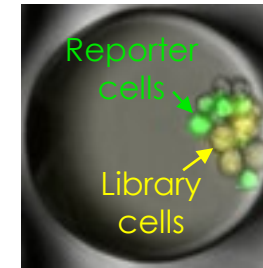
Positive Control: Soluble SP34

Reporter cells
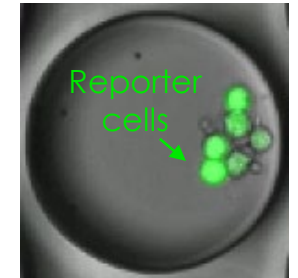
Sequence

Tumor Associated Antigen Arm

Non-Shed Epitope Anti-MUC16 Antibody

MUC16 Is Overexpressed and Shed by Tumor Cells

N-terminal and tandem repeat (TR) domains that are shed

MUC16 epitope shedding

Tumor associated epitope that is not shed

Ovarian cancer cells

Shedding eliminates the epitope and creates an antigen sink for most MUC16 antibodies

Antibodies that bind the non-shed domain maintain activity

Potential dual MOA: Binding this epitope inhibits the cadherin pathway and cancer EMT*

*npj Precision Oncology (2023)7:74

O-glycosylation   N-glycosylation

Discovery Tracks Were Steered to a MUC16 Epitope that Avoids Shedding

MUC16

Non-shed Aglycosylated Epitope

AI Discovery Engine

MUC16 Engineered Epitope

Epitope-Steered Immunization & Screening

Weeks 1–2	Weeks 3–4	Week 5
Engineered Epitope Nanoparticle	MUC16 Expressing Cells	Engineered Epitope + Cells
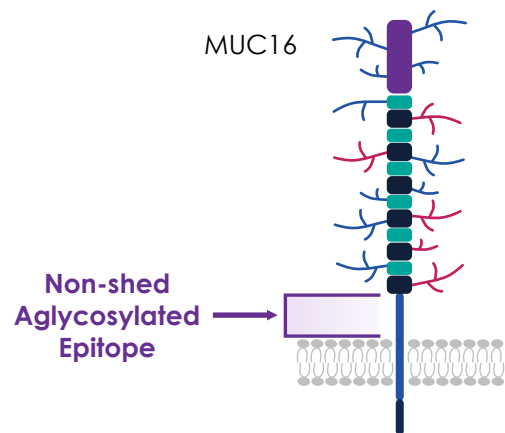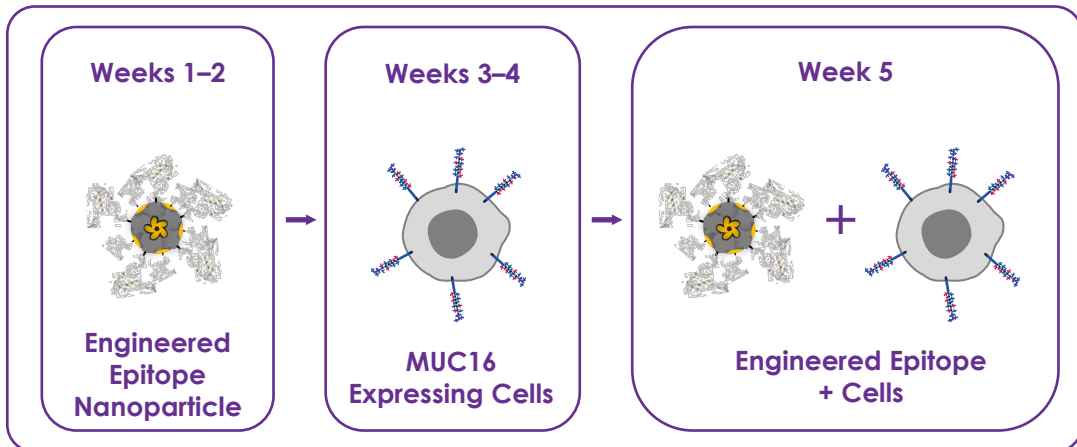
Epitope-Steered Naïve In Vitro Selection

Round 1	Round 2
MUC16 Engineered Epitope	MUC16 ECD
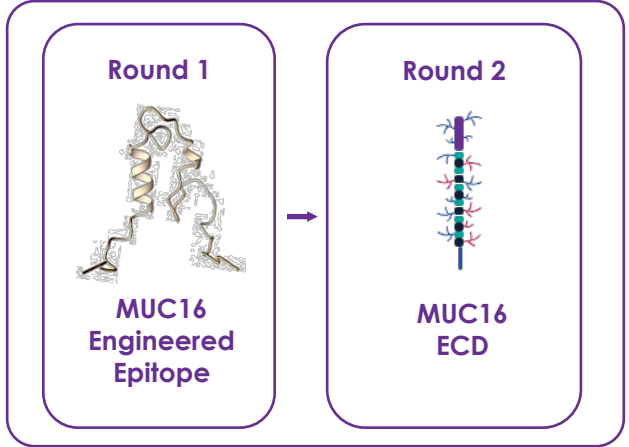
Immunized MUC16 Repertoires Were Cloned and Screened in Mammalian Display

1. Epitope-Steered
Immunization

Prime   Boost   Final

epitope
nanoparticle

MUC16
cells

cells
+ epitope

Epitope-Steered
Immunized
Repertoire

repertoire
cloning

2. Mammalian
Display

1-sorting
round

Phage
Display
2X Rounds

3. Multi-Modal
Screening

Single-Cell Screen:
1. Epitope binding
2. MUC16 binding
3. Ab expression
4. Poly-Specificity

NGS,
SPR,
In vitro activity
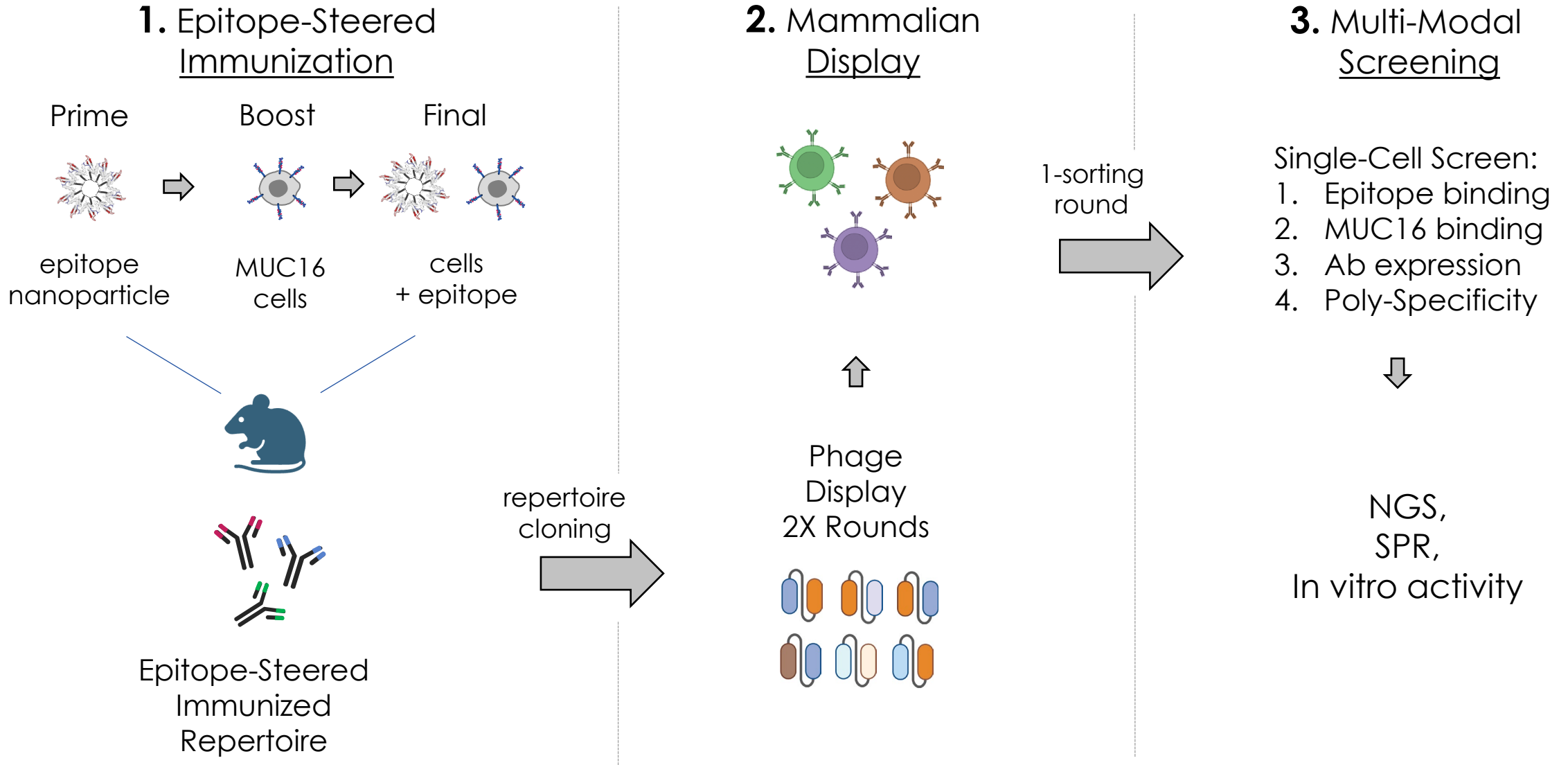
Mammalian Cell Sorting for MUC16 Epitope Binding & Enhanced Expression

Epitope-Steered Immunized Repertoire

repertoire cloning

Mammalian Display

Phage Display 2X Rounds

cell sorting

MUC16 Epitope Binding

Antibody Expression

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

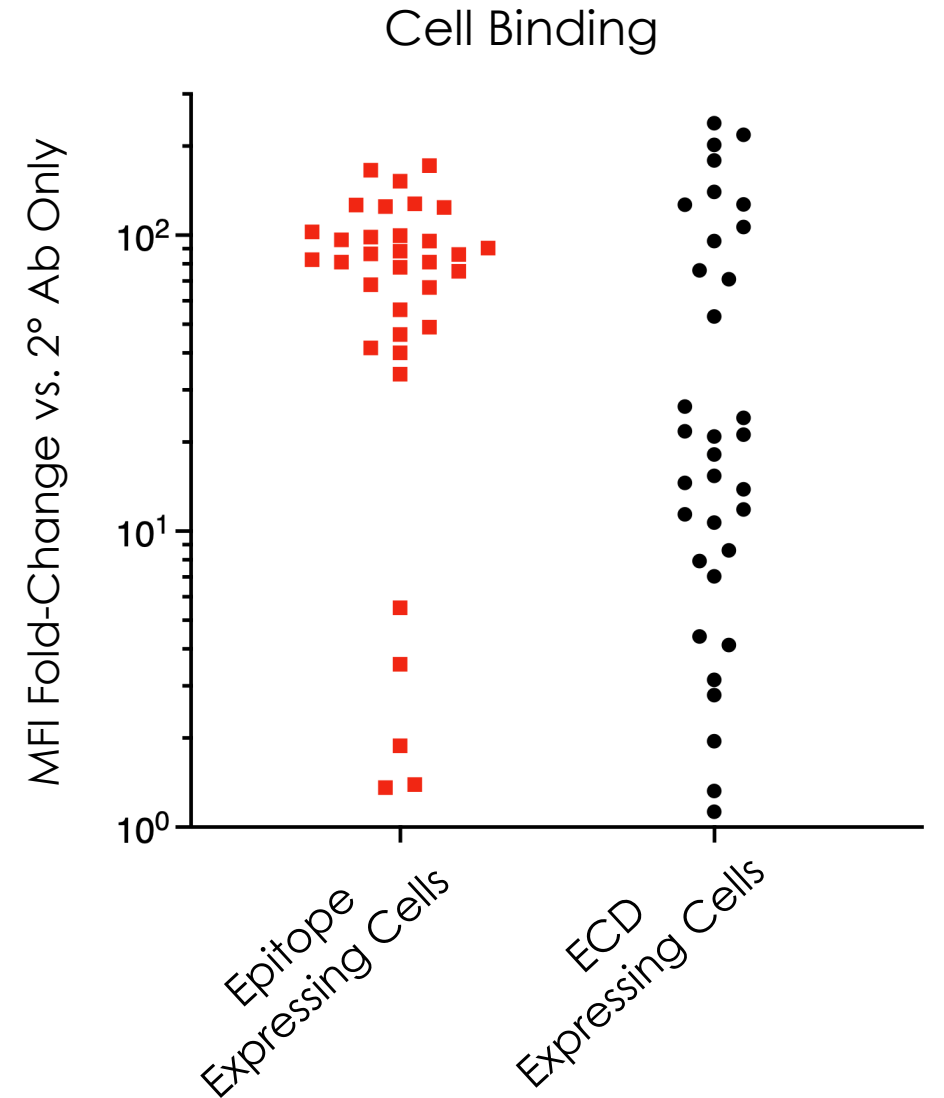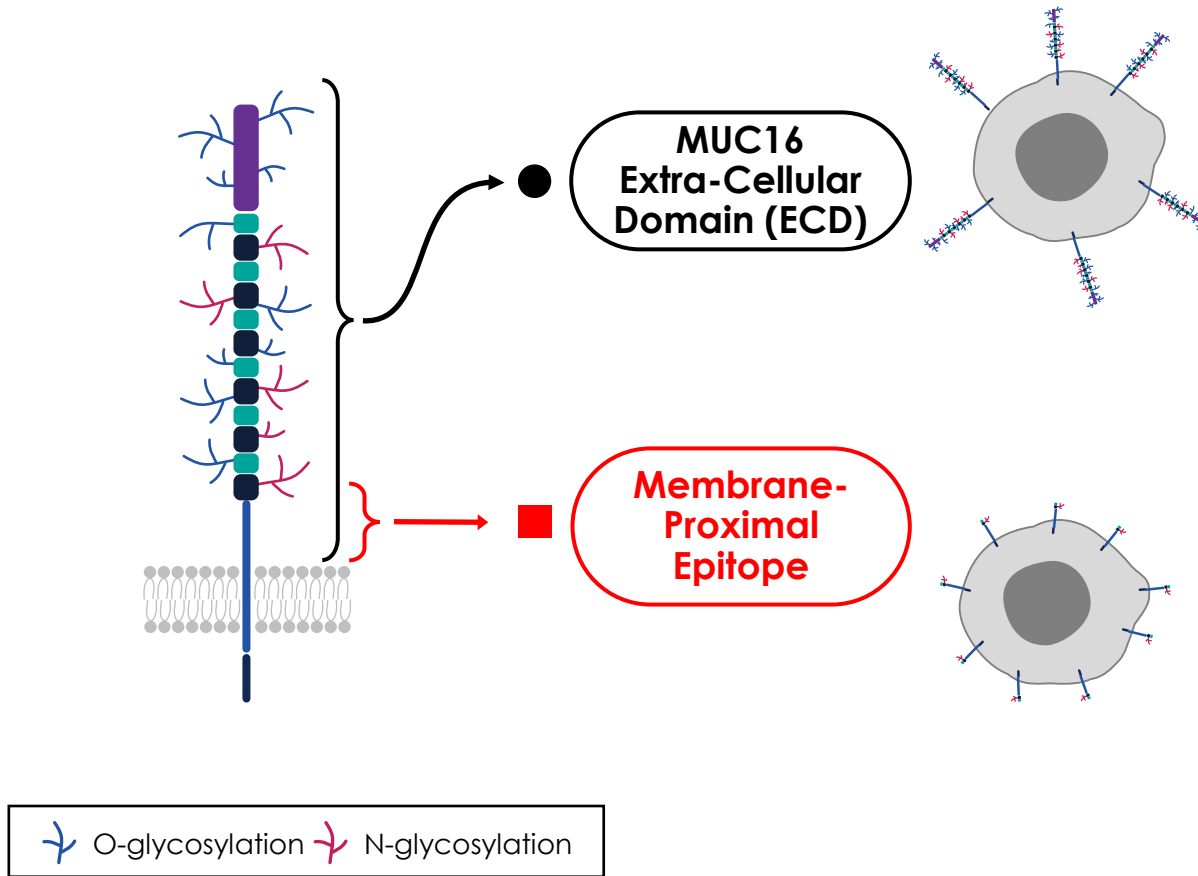P1 NGS Enrichment = (P1 Clone Count) / (P3 Clone Count)
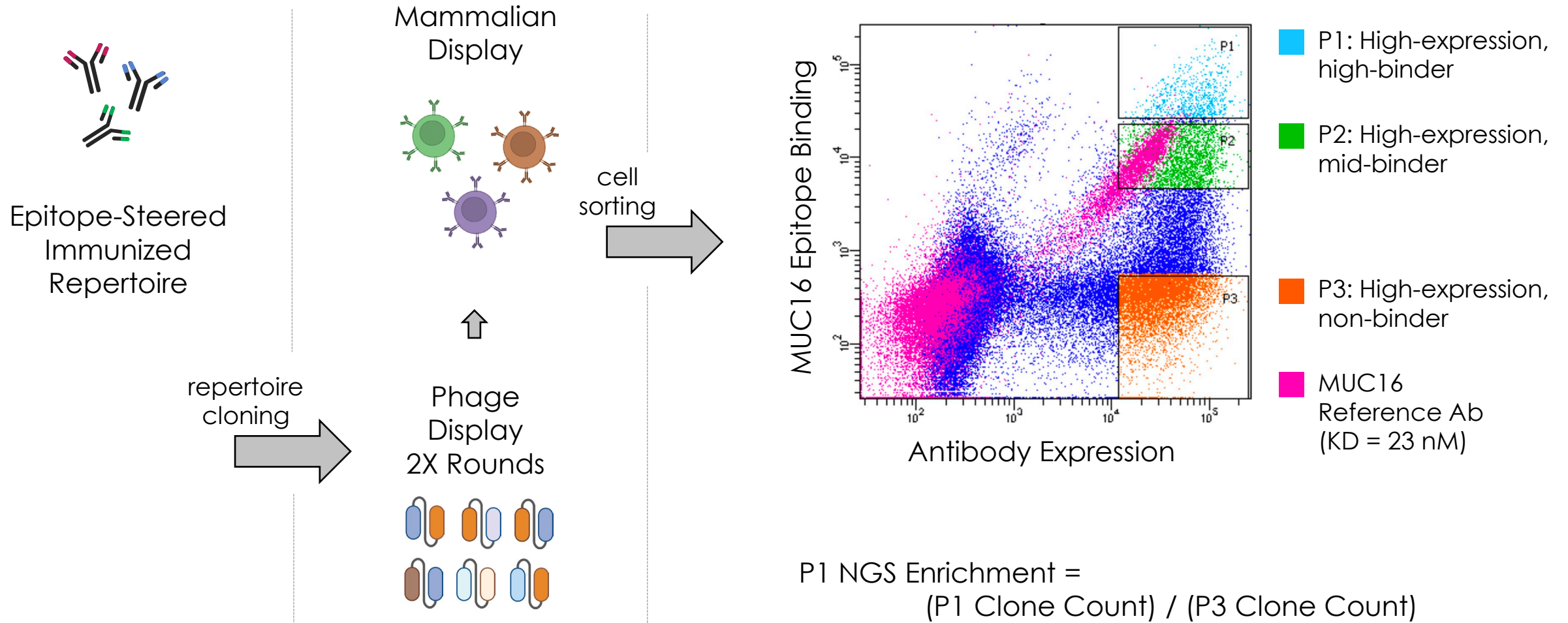
Dual-Track Discovery Identifies 34 Hits that Bind the MUC16 Epitope and ECD
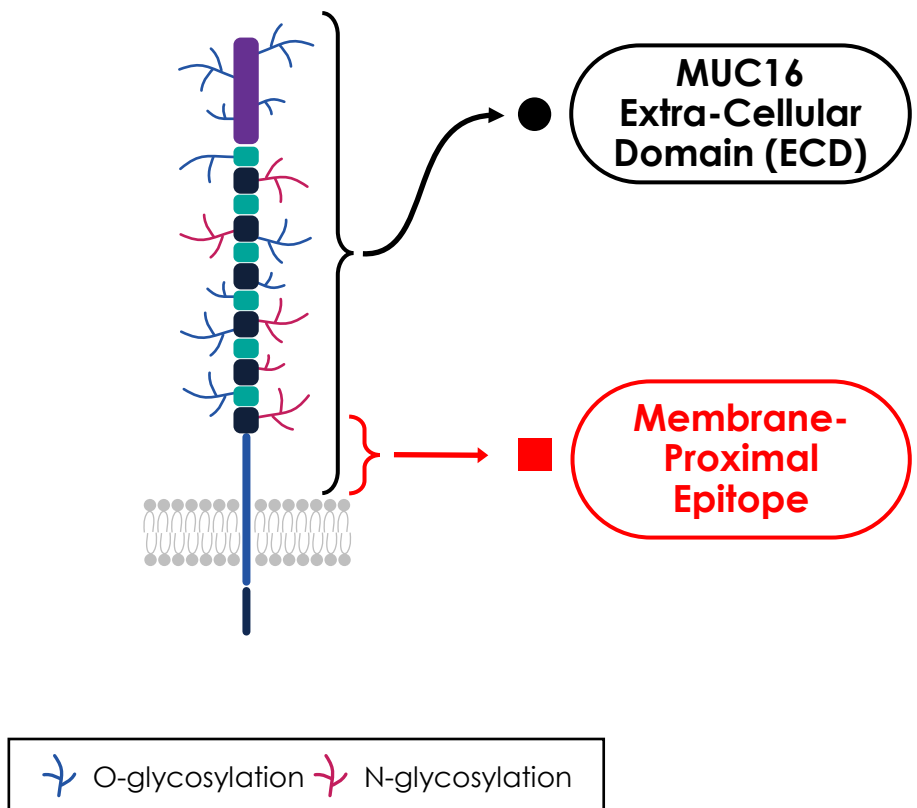
ECD and Epitope HT-SPR Iso-Affinity
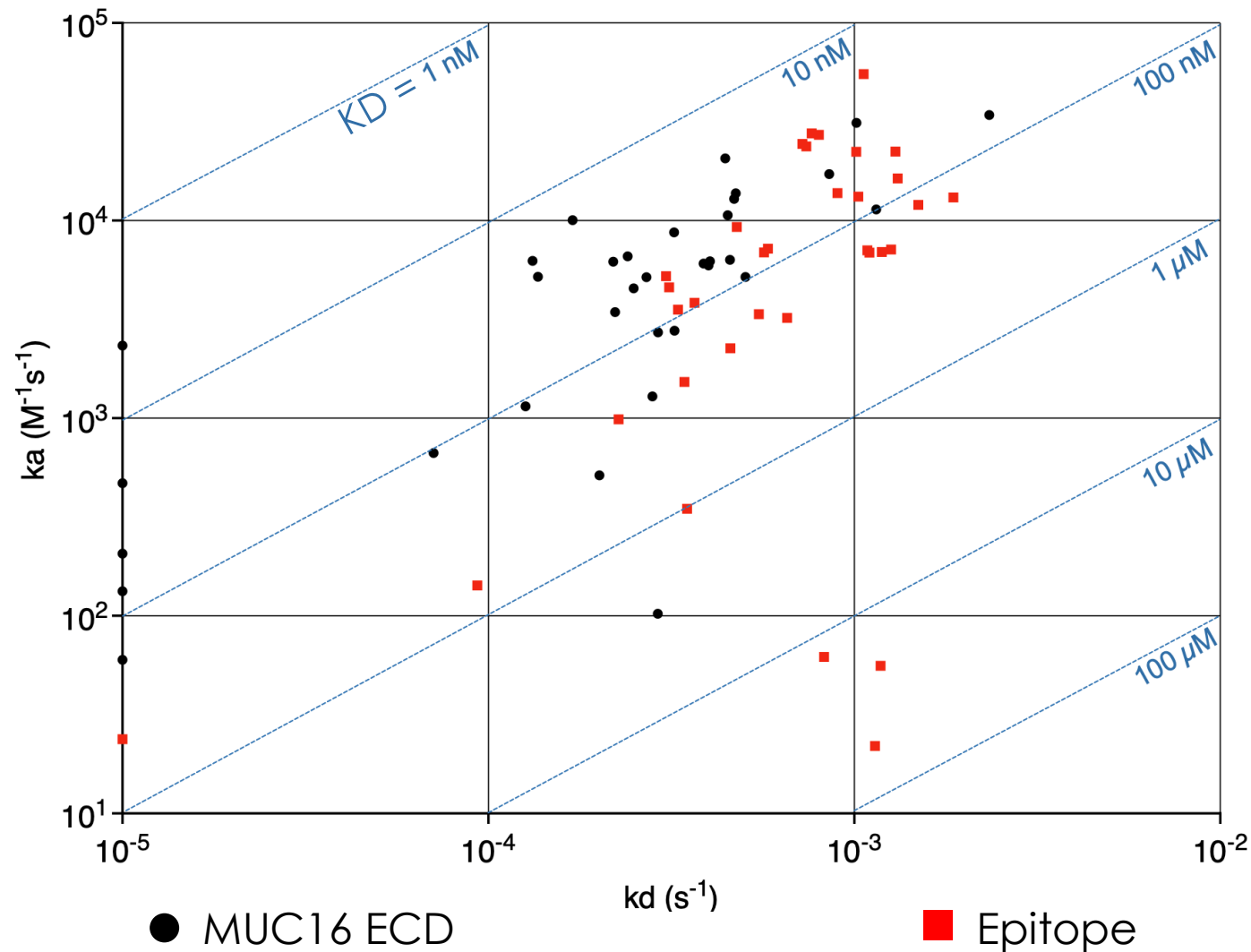
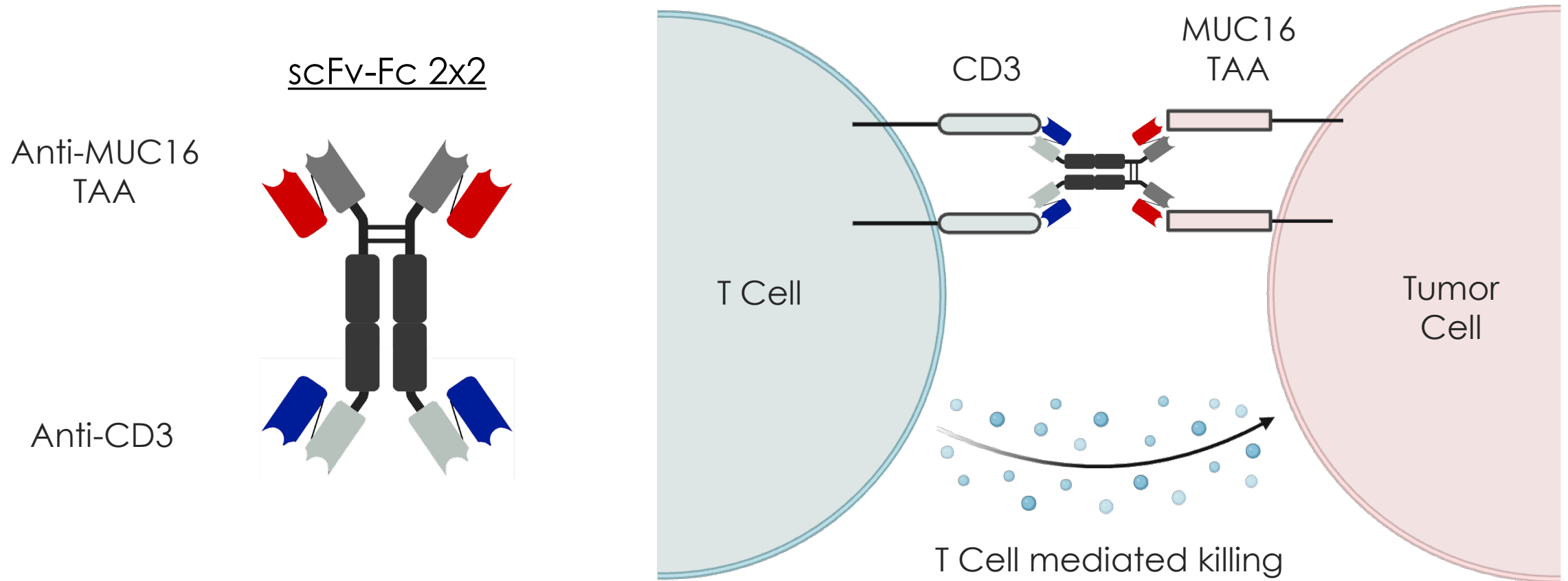# 34/34 Hits Bind MUC16 Membrane-Proximal Epitope and ECD Expressing Cells

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

CD3

MUC16 TAA

T Cell

Tumor Cell

T Cell mediated killing

2X2 Anti-CD3 X MUC16 T Cell Engagers Kill OVCAR-3 Ovarian Cancer Cells in PBMCs
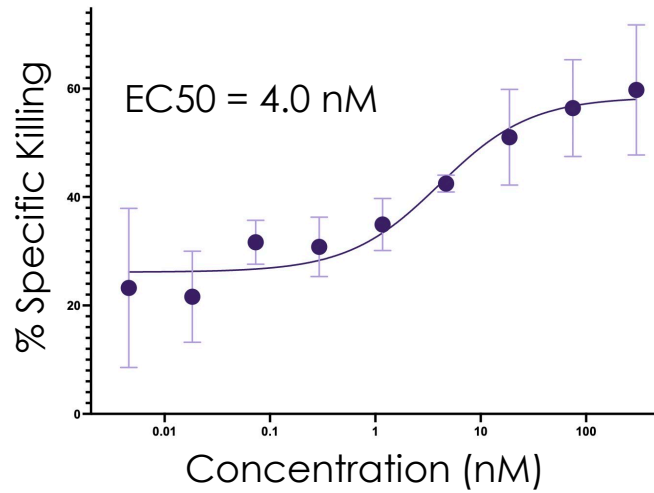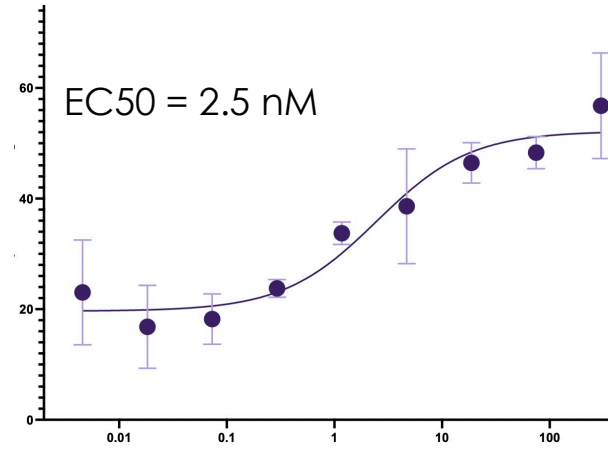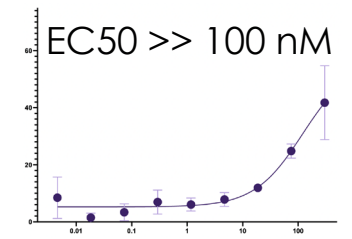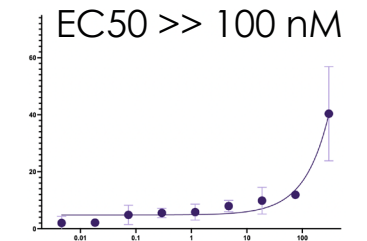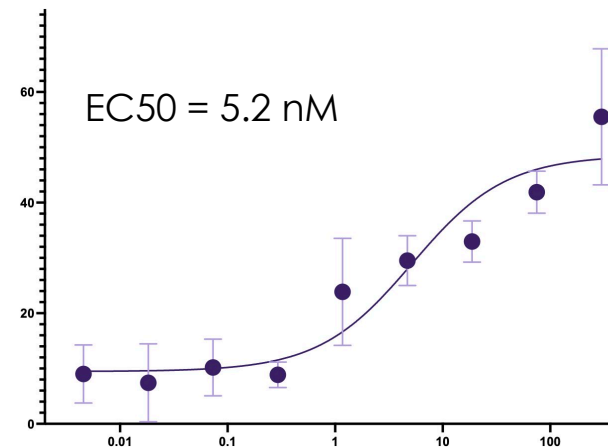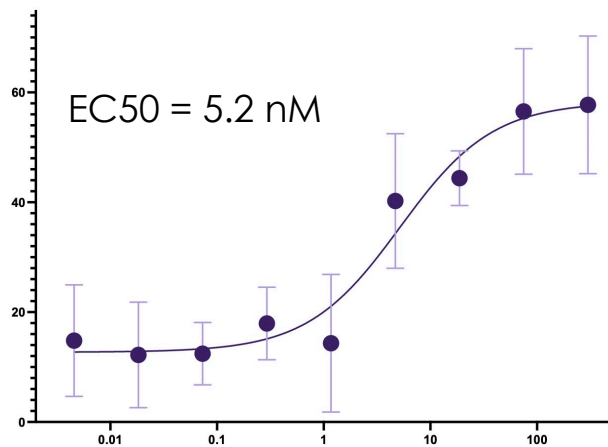
Epitope-Targeted & Conditionally-Activated Anti-CD3 X MUC16

On-Target & On-Tissue T Cell Engager

Conditionally-Activated Antibodies Minimize On-Target, Off-Tissue Risks
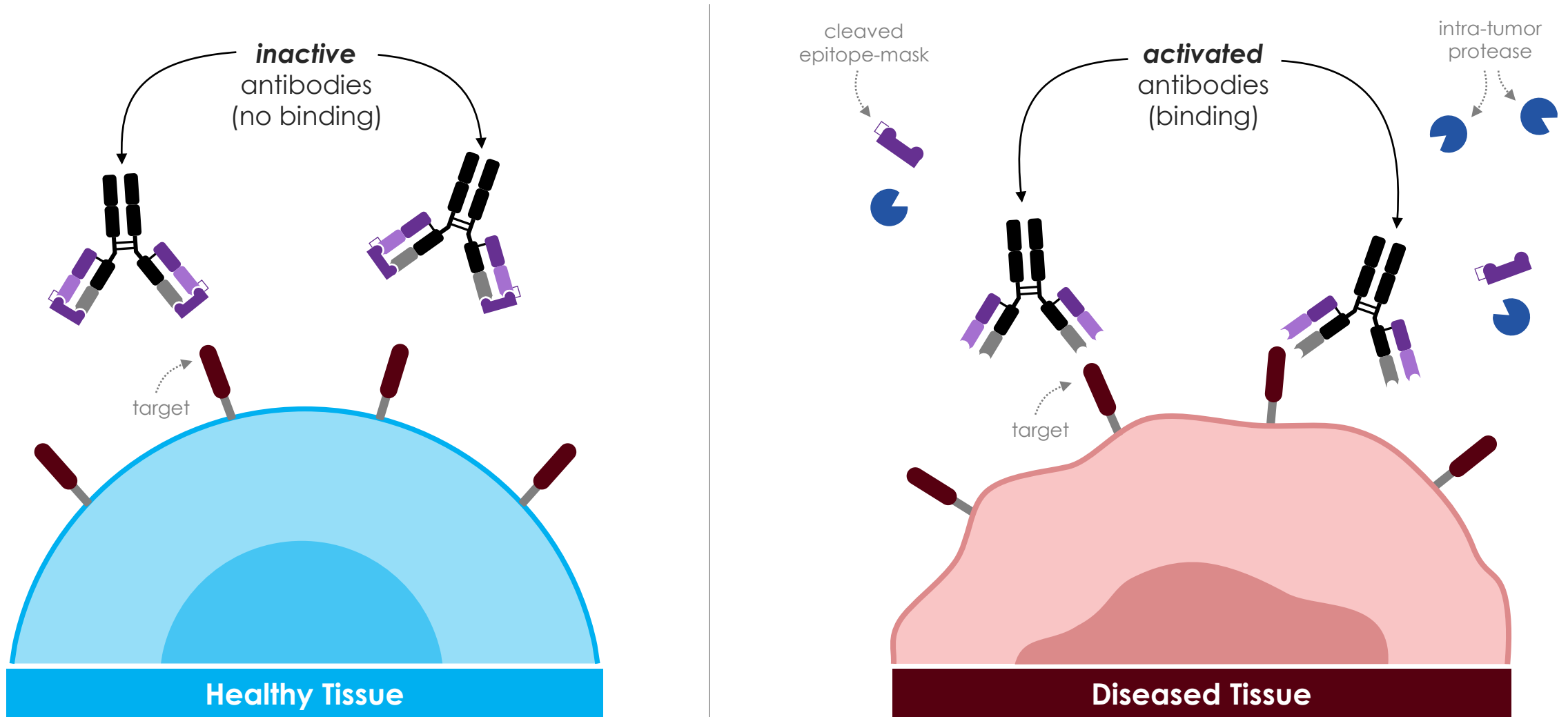
Efficient, Single-Cycle Discovery of Conditionally-Activated Antibodies

1 Naïve in vivo or in vitro antibody library

epitope-specific Ab

epitope-specific Ab

epitope-specific Ab

2 Focus library with engineered epitopes

epitope

epitope

de novo scaffold
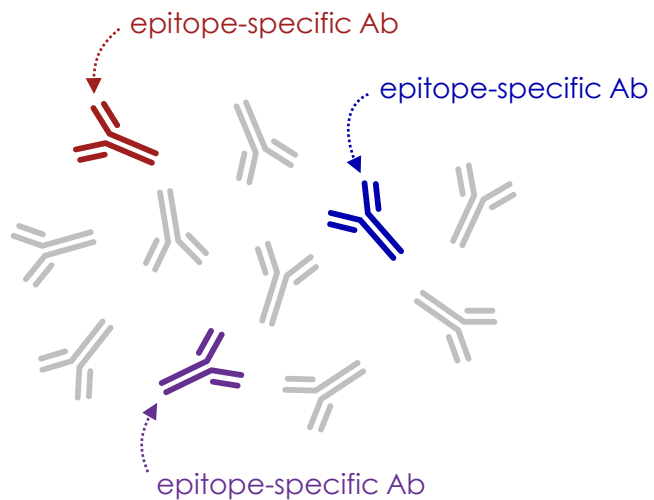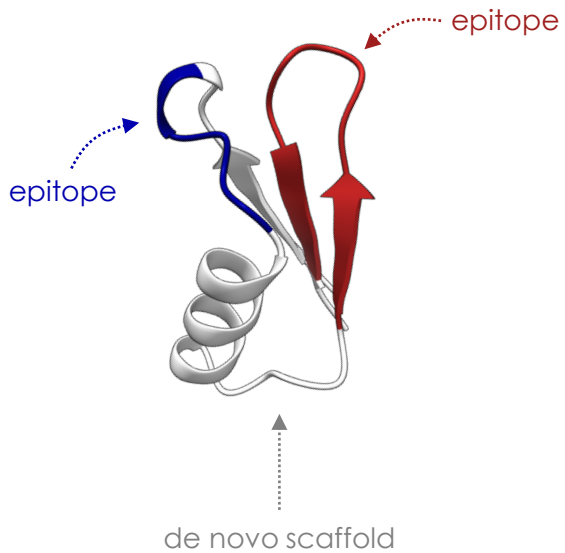
3 Engineered-epitope conditionally-activated Ab

epitope + cleavable linker

target

tumor microenvironment cleavage and Ab activation
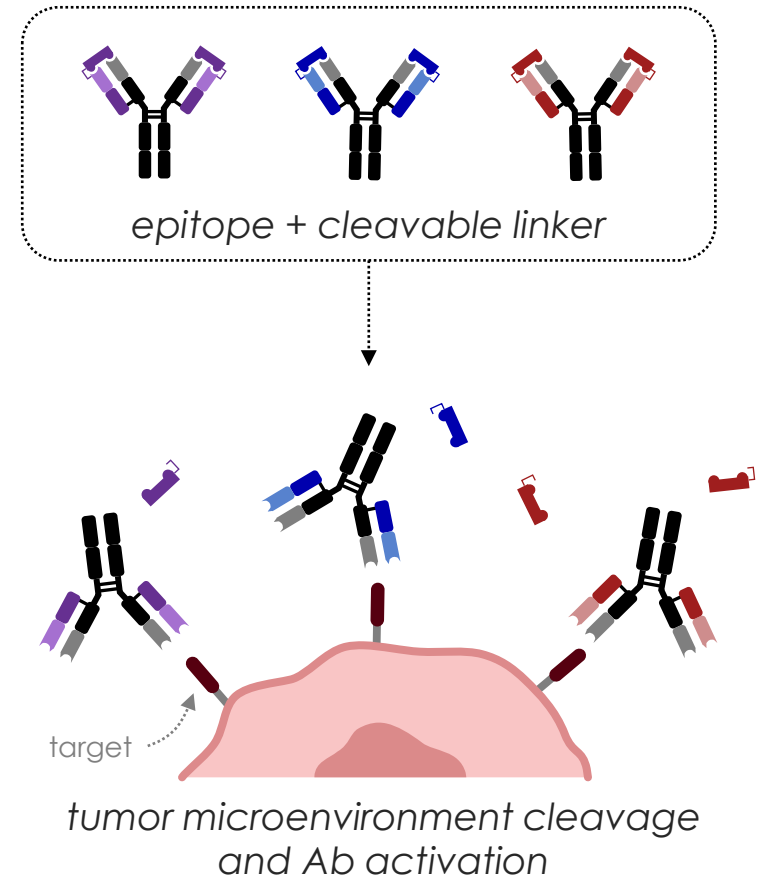
Engineered Epitope Mask Conditionally Activates MUC16 and CD3 Hits

Engineered Epitope
Mask Intact
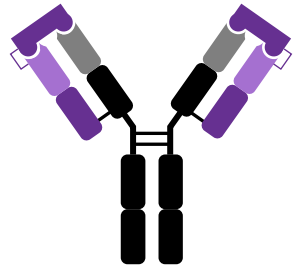
Mask Cleavage

MMP
protease
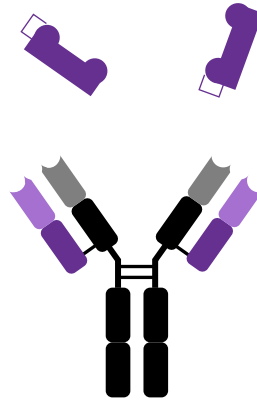
Inactive
antibody

Active
antibody

No Mask

Mask (−MMP9)

Mask (+MMP9)

Anti-MUC16 hit

MUC16 Binding Response

time (s)

Anti-CD3 hit

CD3 Binding Response
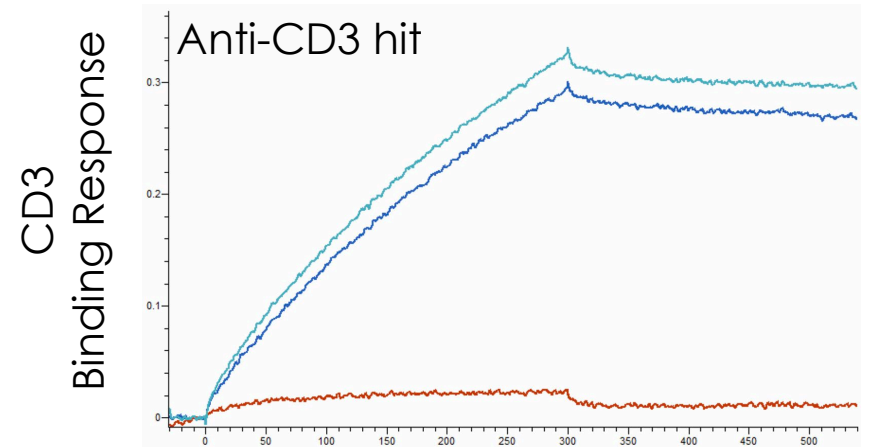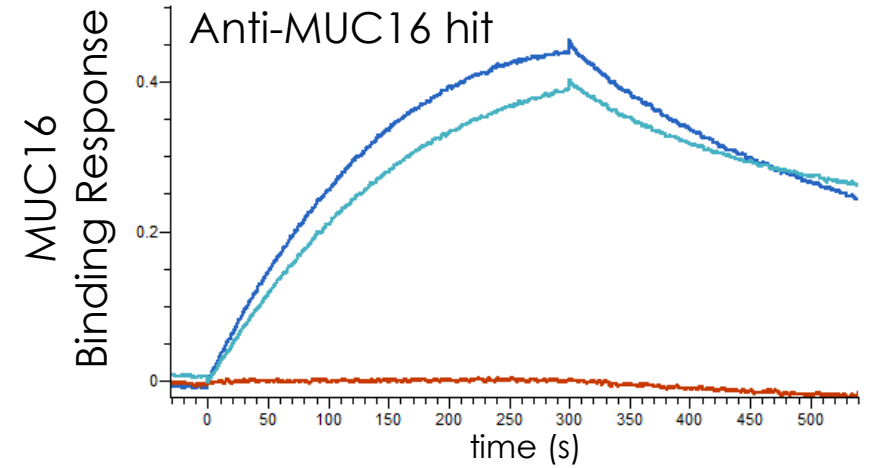
Conclusions

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

Epitope-Steering Enhances Difficult Target and MOA Discovery Productivity

Epitope Engineering Engine*

Engineered Epitope Steering

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

2
Epitope-specific antigens that steer discovery to intended epitopes
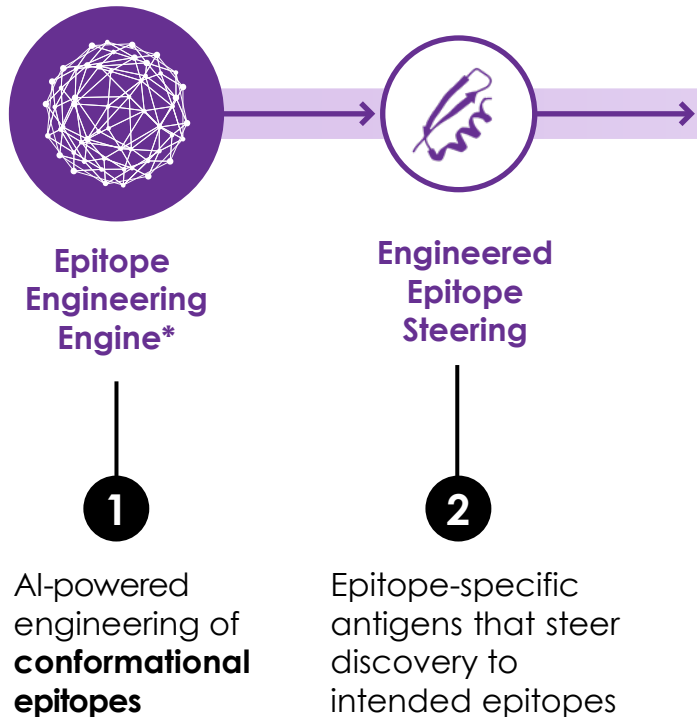
- Strategically steer antibody discovery to intended epitopes, including difficult targets

- Investigate multiple epitopes to reveal per-epitope difficult mode-of-action activity

- Single-cycle antibody-mask discovery for on-target & on-tissue activation

Mammalian-Display Selects for Developability – Including Advanced Formats

Epitope Engineering Engine*

Engineered Epitope Steering

Proprietary Antibody Library

StableHu Antibody Optimizer

EngageTx™

ShieldTx™
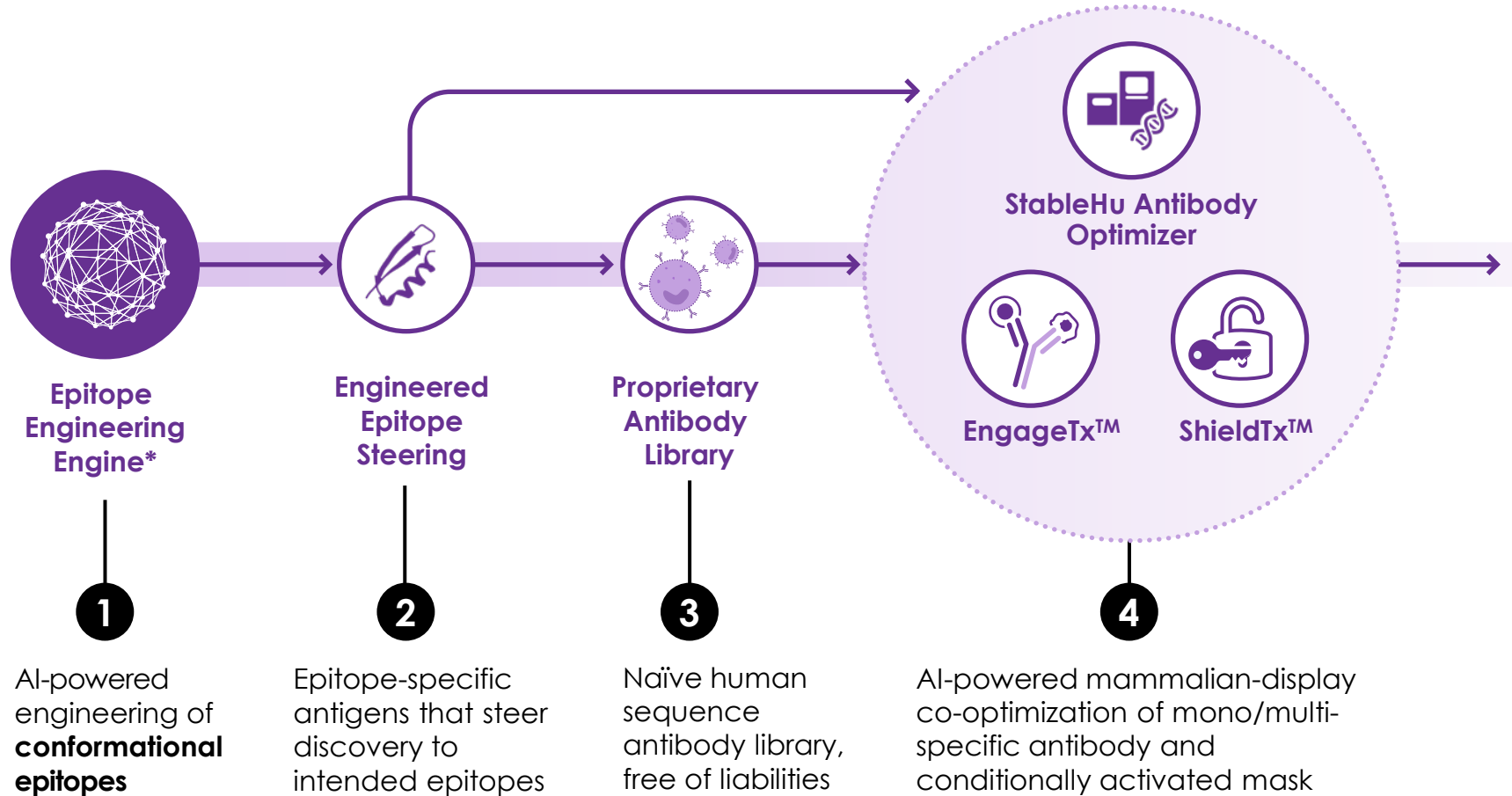
• High developability bi-specific masked antibodies

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

Non-confidential

Epitope-Steering and Mammalian-Display Tackle Discovery Challenges

Epitope Engineering Engine*

Engineered Epitope Steering

Proprietary Antibody Library

StableHu Antibody Optimizer

EngageTx™

ShieldTx™

Optimized Antibody Leads

On-epitope, On-tissue Clinical Candidate

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

5 Evaluated & ranked in translational disease models

Data feedback improves tech stack

Thanks to the iBio Scientific Team!

Cody Moore
Alex Taguchi

Primary contributors

Martin Brenner

Matt Greving

Dillon Phan

Cory Schwartz

Domyoung Kim

Matt Dent

Tom Hsu

Tam Phuong

Jenny Le

John Chen