



AI-Powered Antibody Discovery

Unlocking High-Throughput Biology and Drug Discovery

May 2023



Antibody Discovery Tech Stack: Precision-Targeted Antibodies with Lower Downstream Risk

1

Engineered Epitope

Design Engine

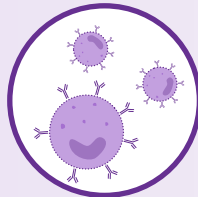


- Patented* epitope engineering
- AI-engineered epitope preserves target structure

2

Human Diversity

Antibody Library



- Human antibody diversity
- Clinically validated frameworks
- Benchmarked vs. competitive libraries

3

StableHu™

Antibody Optimizer



- Functional antibody enriched mammalian-display library
- Faster human sequence and optimization vs. traditional methods



**Multiple validations
with difficult targets
and MoAs**



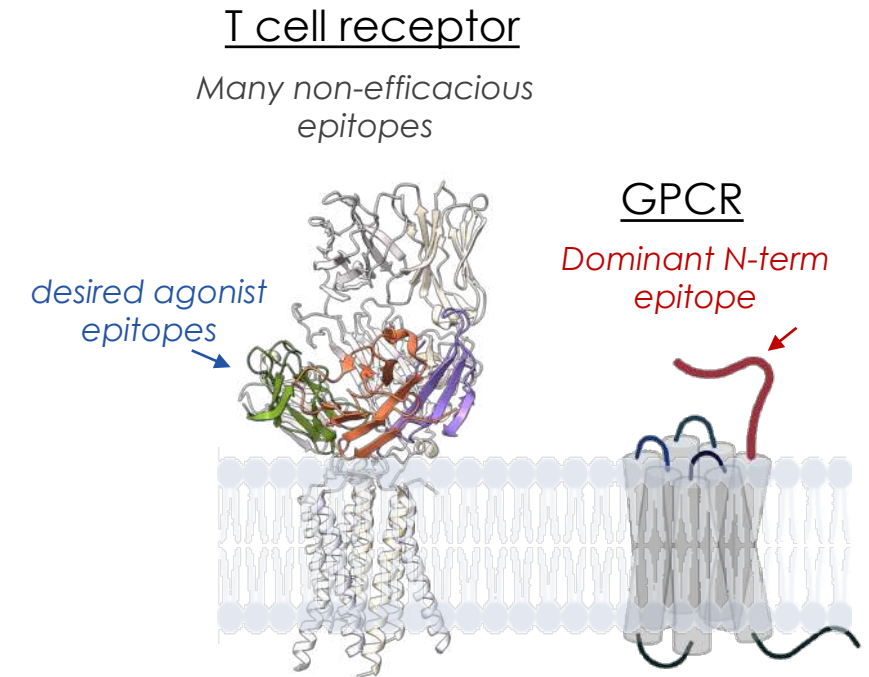


Epitope-Targeted Antibody Discovery

Therapeutic Antibody Efficacy Depends Heavily on the Epitope

Epitope-specific antibody discovery is hindered by:

- Dominant-epitope, low/no efficacy antibodies inundate traditional discovery approaches^(1, 2, 3)
- Low/zero discovery yield for high-value, challenging therapeutic epitopes⁽⁴⁾
- Limited availability of epitope-stabilizing immunogen scaffolds for epitope grafting⁽⁵⁾



(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

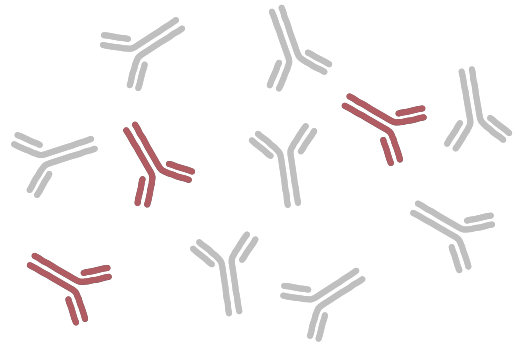
(4) Trkulja et al., *Sci. Adv.* (2021) 7:16, p.eabe6397

(5) Sesterhenn et al., *Science* (2020) 368, p. eaay5051

Engineered Epitopes Focus Antibody Repertoires On Desired Binding Sites

1

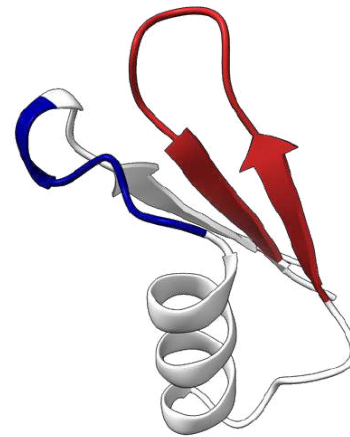
Naïve in vivo or in vitro antibody library



■ epitope-specific Ab

2

Focus library with engineered epitopes

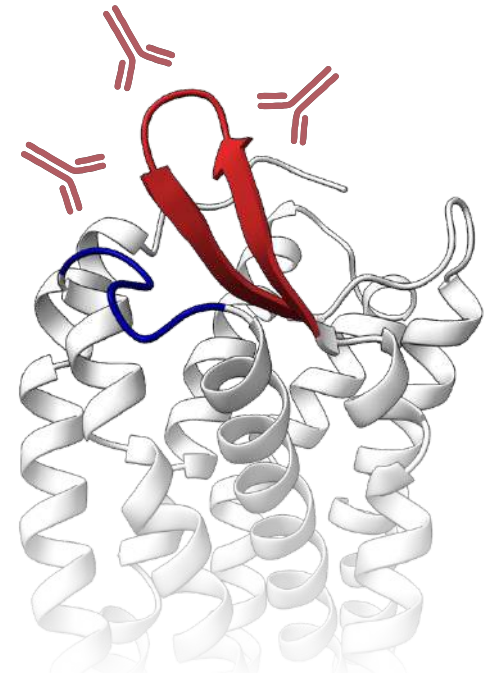


■ epitope

■ de novo scaffold

3

Efficient discovery of epitope-specific Abs



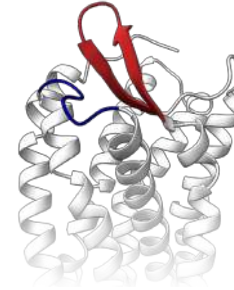
full length target

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

Engineered
Epitope
Design
Objectives

1

Match Structure
to Target



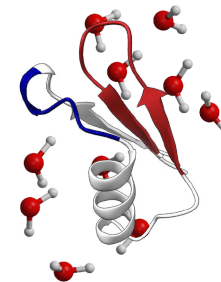
2

Refine for
Greater Stability



3

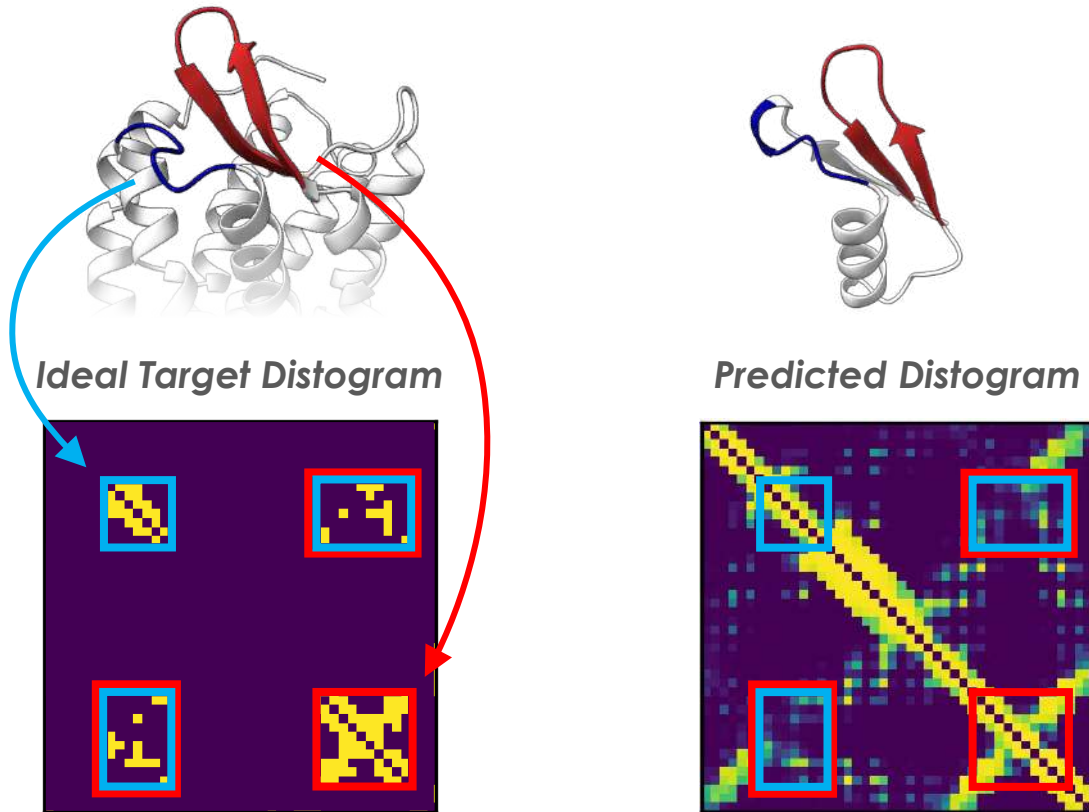
Optimize for
Water Solubility



AI
Discovery
Engine

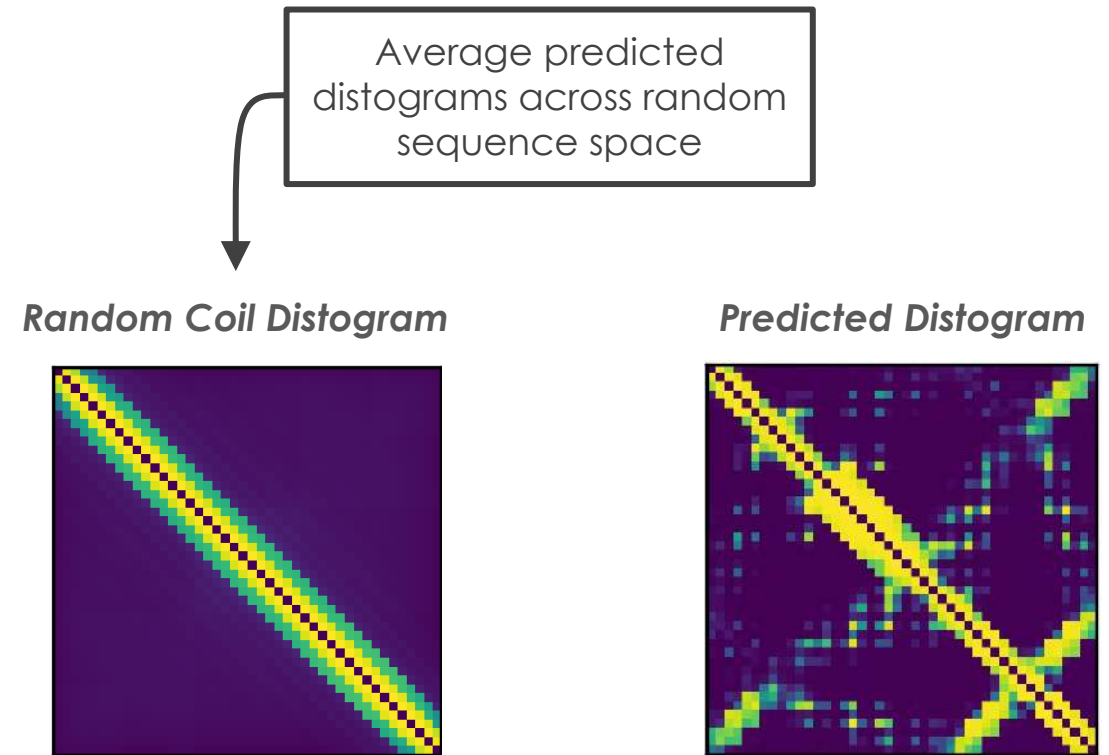
Multi-Loss Function Enforces Engineered Epitope Structure Match to Target and Overall Stability

Loss Term #1



Minimize Cross-Entropy between engineered & target epitope residues

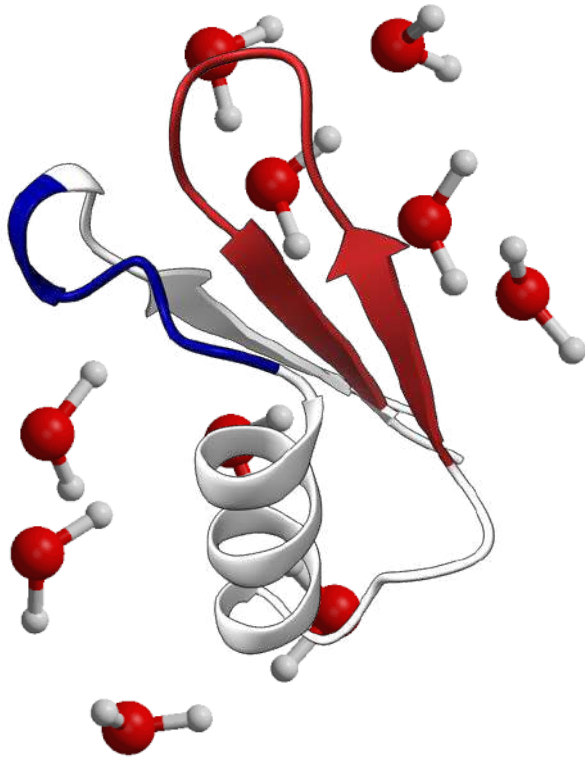
Loss Term #2



Maximize KL-Divergence between unstructured coil and engineered epitope

Multi-Loss Function Optimizes Engineered Epitope Solubility

Loss Term #3

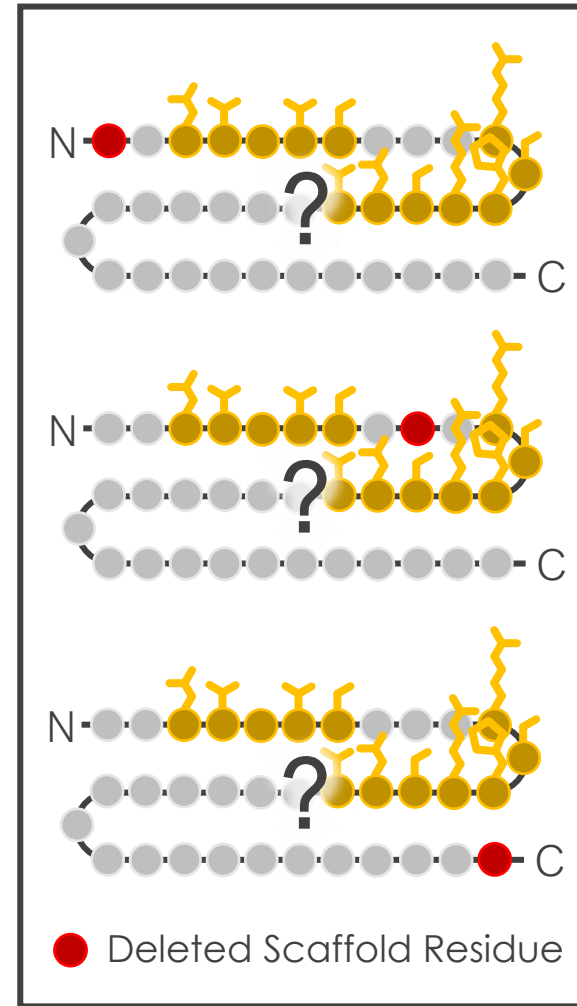
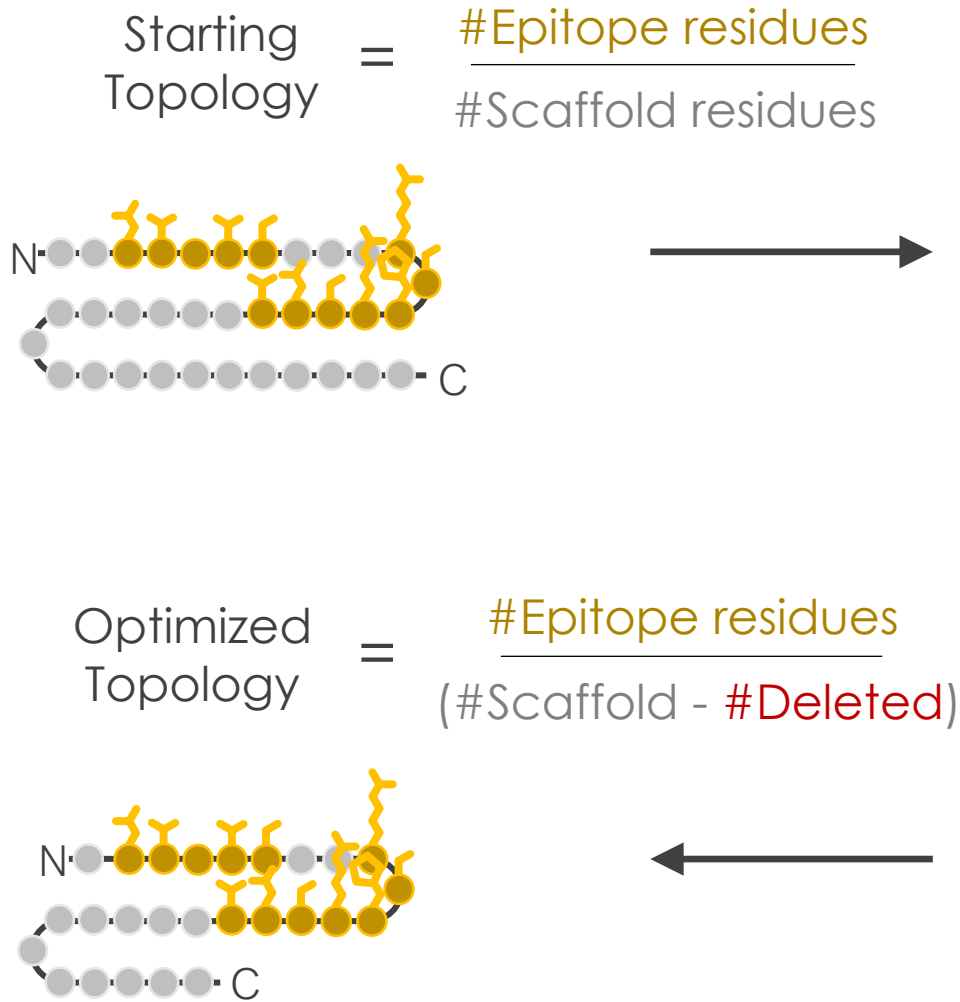


Amino Acid Hydropathies

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

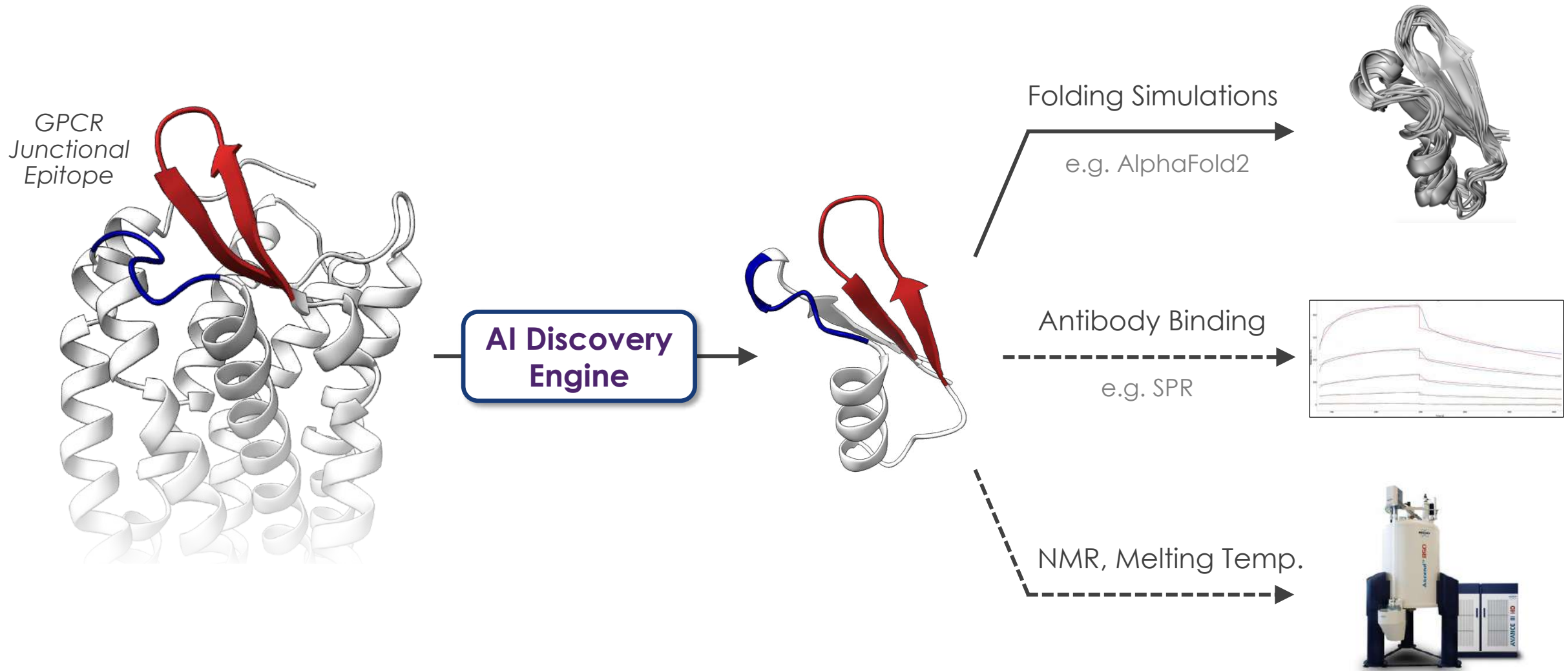
Average hydropathy is minimized

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

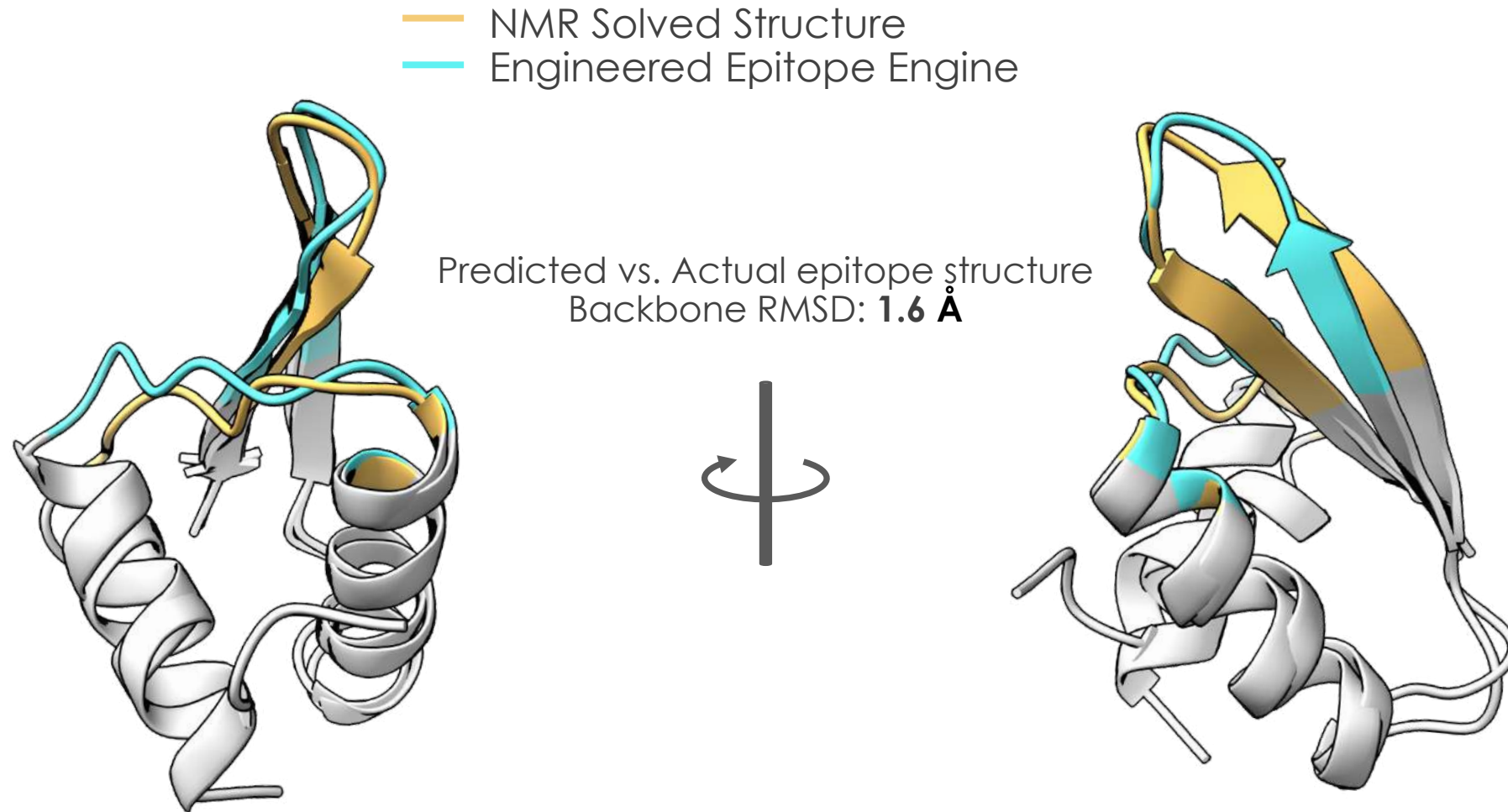


Iteratively trim scaffold residues until epitope destabilizes

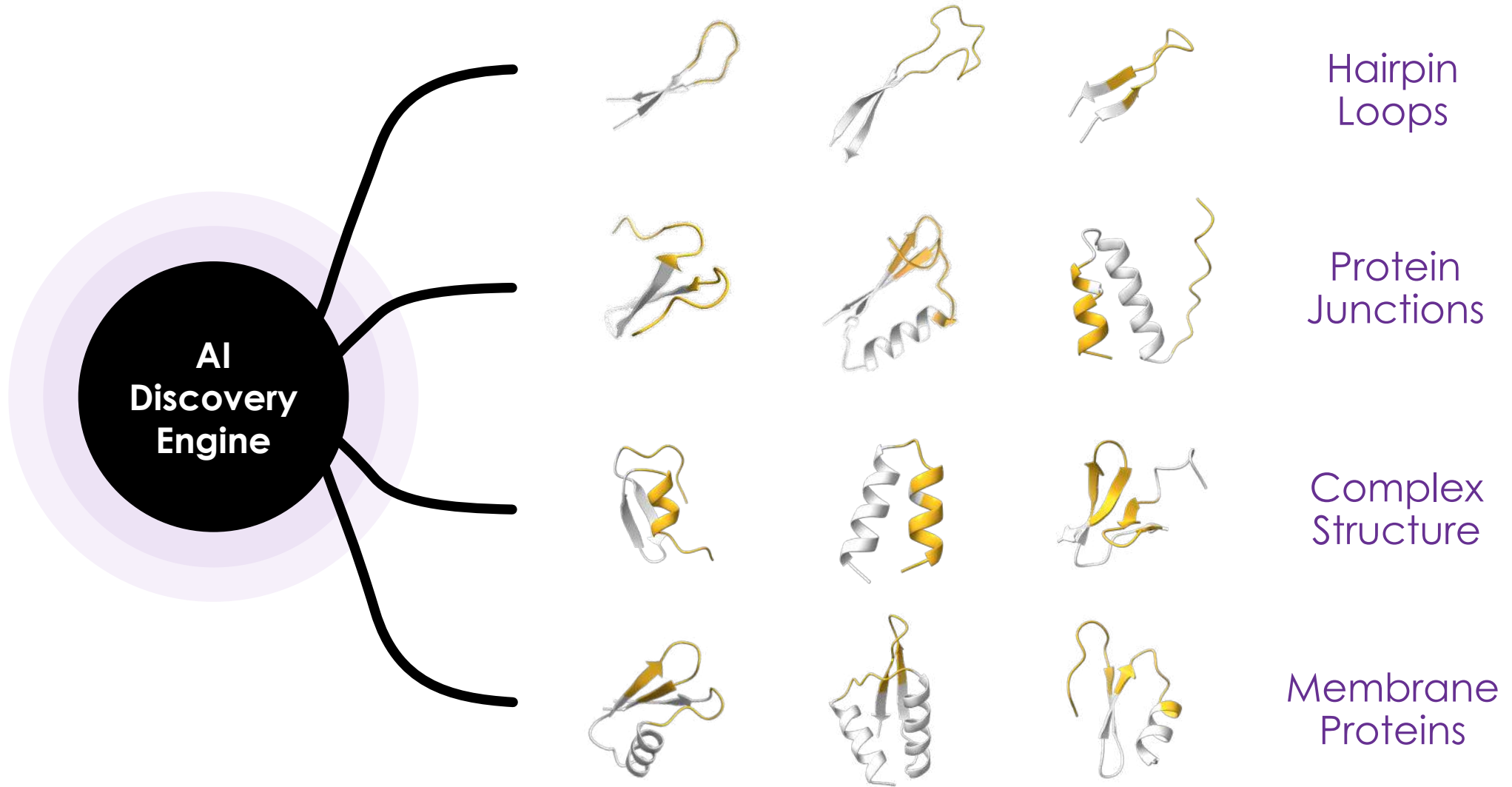
Engineered Epitopes are Designed with the AI-Engine and Cross Validated with Folding Simulations, Binding Measurements, T_m , and NMR



NMR Structure Validates Engineered Epitope Design Engine

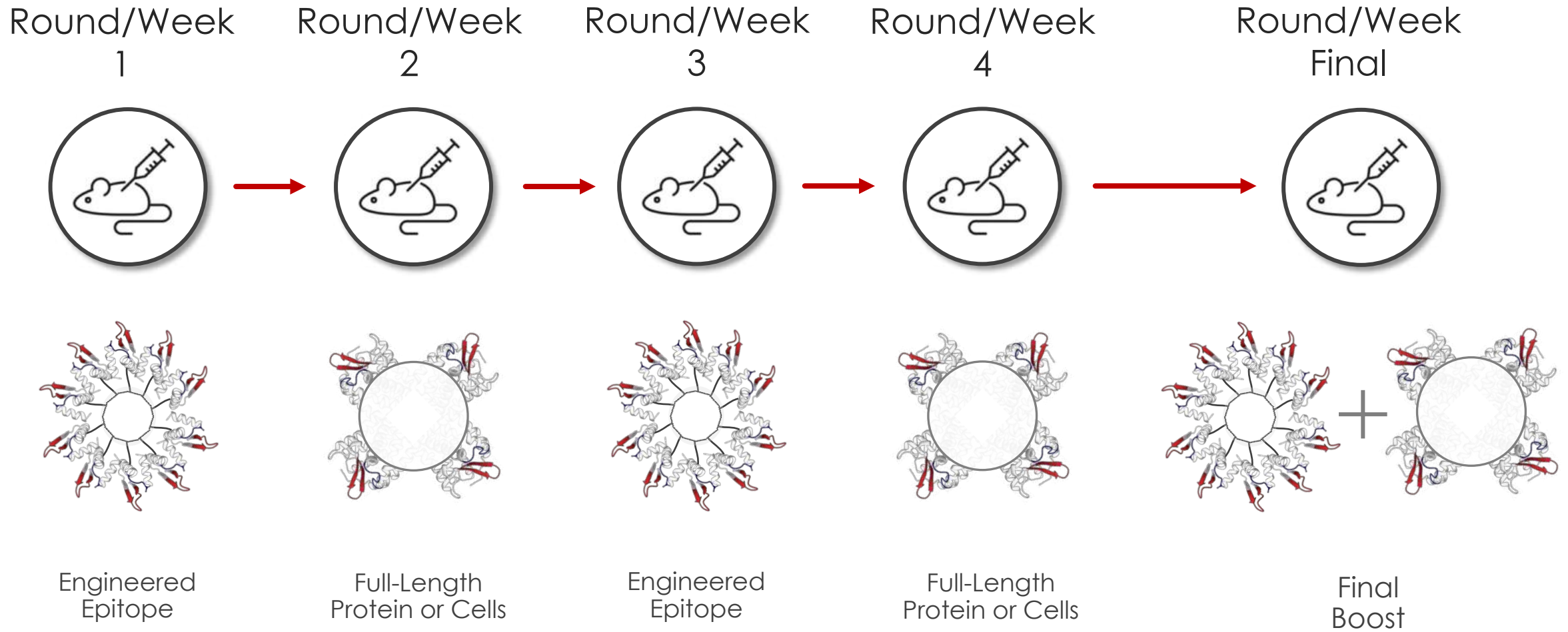


Engineered Epitopes Are Generalizable to a Broad Set of Targets



Engineered Epitopes Steer Immunization and In Vitro Libraries to Target Epitopes

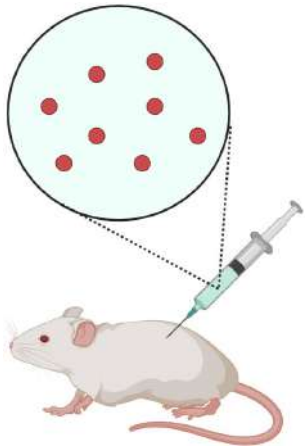
Engineered epitopes alternated with full length protein/cells steers immunizations and in vitro selections while enforcing full length protein and cell binding



Immunized Repertoires Are Cloned and Screened Via Two Tracks

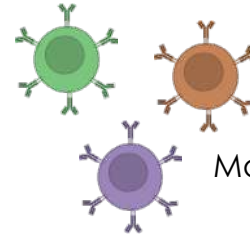
Multi Epitope-Steered Immunization

Engineered epitopes & T cells



Dual-Track Library Display

High-throughput Track 1

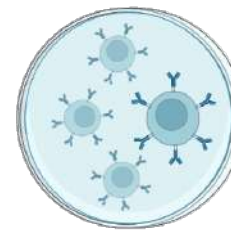


Mammalian Display



Immunized Repertoire

Traditional screen Track 2

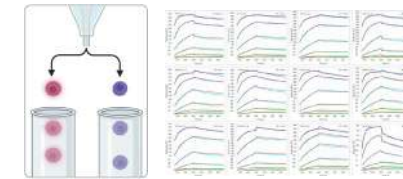


Hybridoma



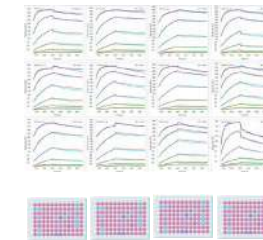
Multi Dimension Screening

FACS, NGS & SPR



Engineered epitopes & target binding & Ab expression

SPR & ELISA



Engineered epitopes & target binding



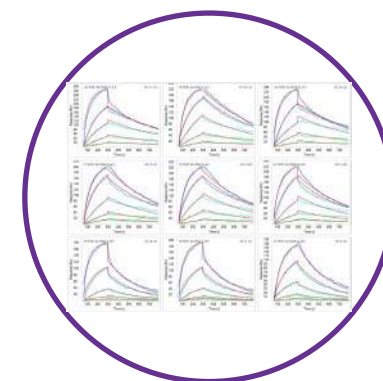
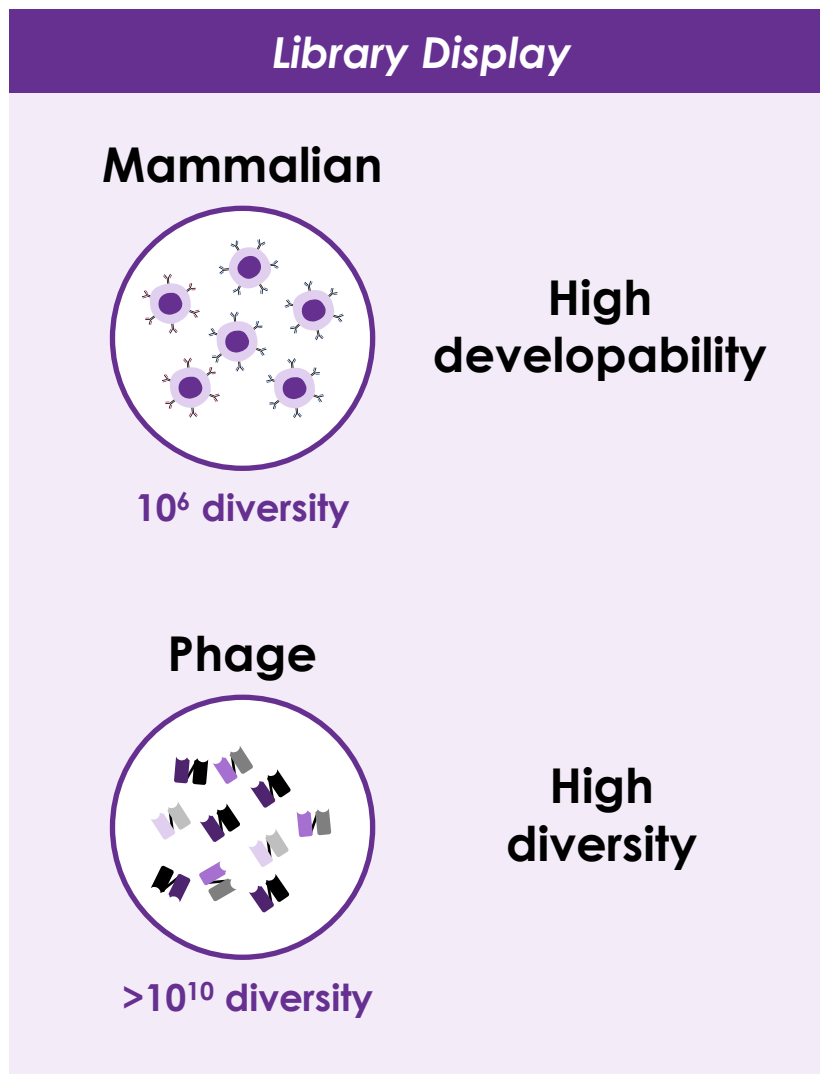
High Developability, Human Diversity Antibody Libraries

Naïve In Vitro Library Uses Human Diversity to Minimize Immunogenicity Risk

Learn diversity from
cAb-Rep & OAS Hu
Ig databases

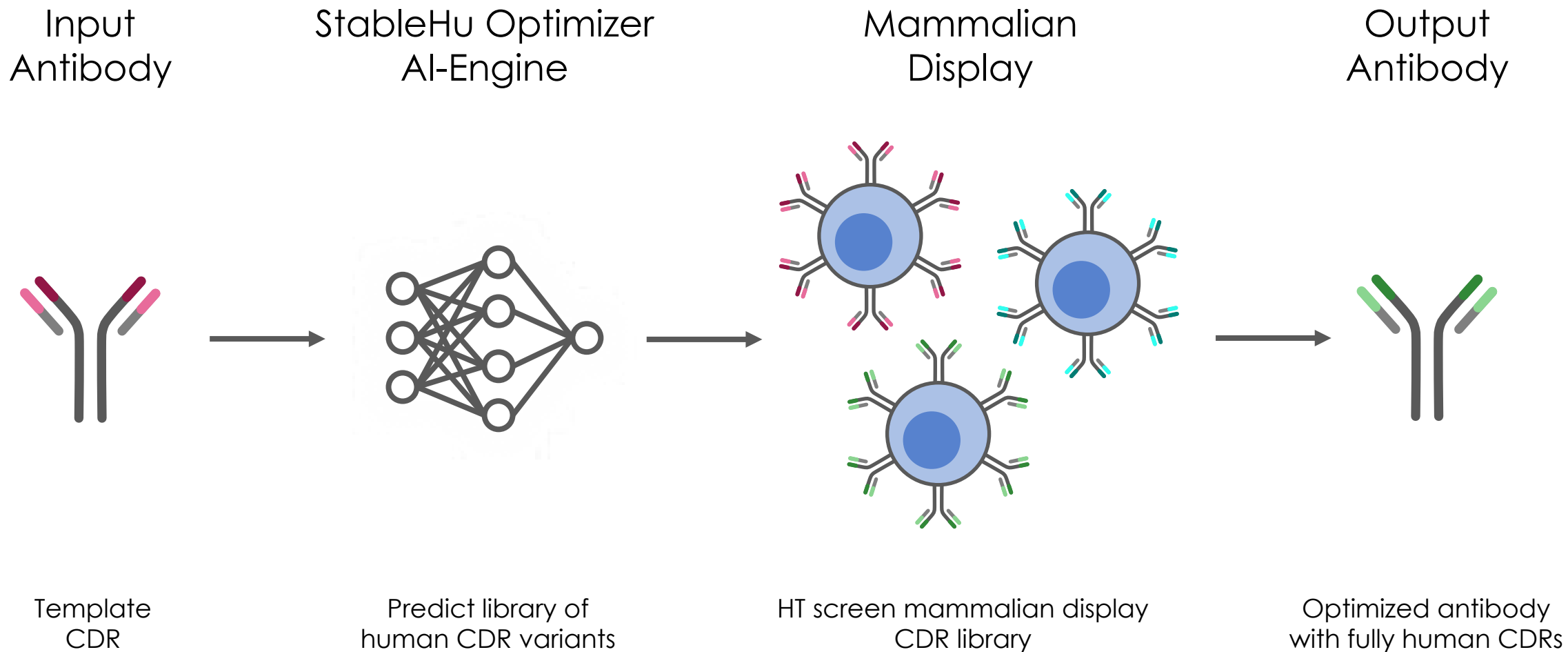


Extract CDR
sequences for
clinically validated
frameworks



HT screen to
identify hits

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



Optimizer AI Model is Trained to Predict Fully Human CDR Sequences

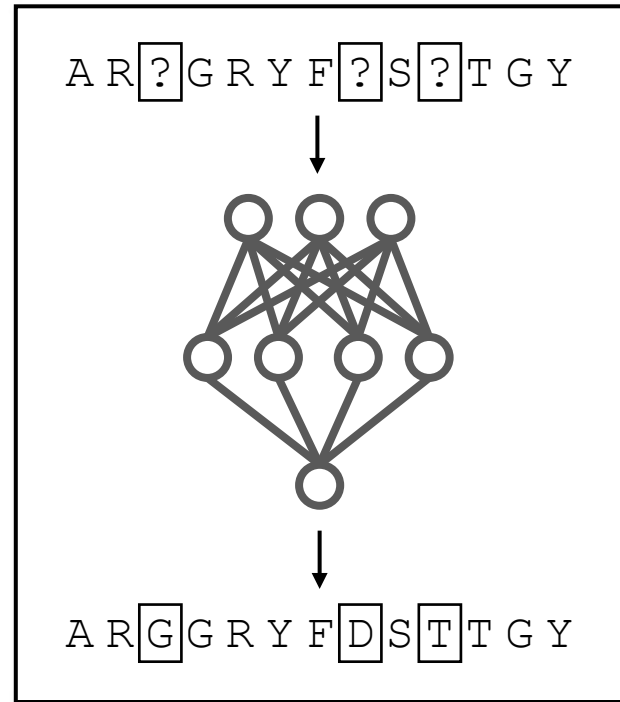
Antibody Database

cAb-Rep & OAS
Hu Ig databases



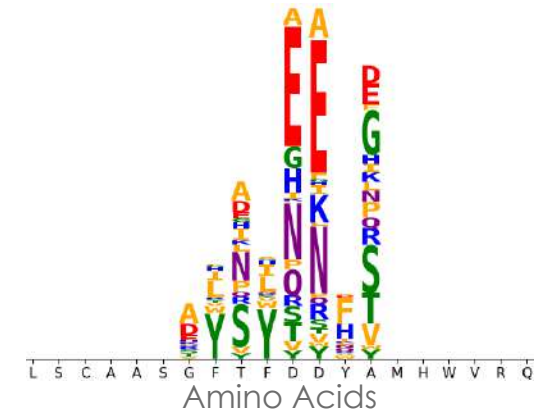
>1 billion curated
human antibody
sequences

Optimizer AI



AI trained to predict
fully human CDR from masked CDR

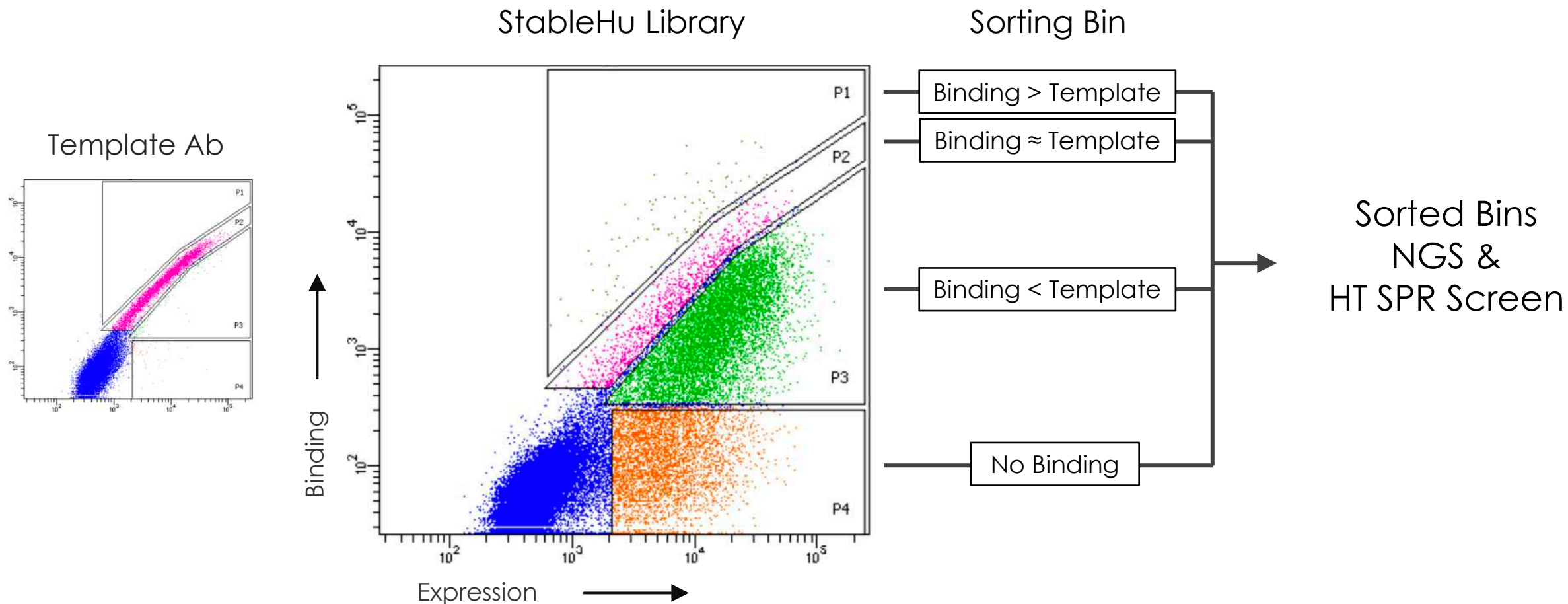
Trained Model



Predict library of fully
human CDRs from
template CDR

StableHu Library Sorting and NGS Identify Improved Human CDR Variants

Mammalian Display Single-Cell Sorting



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

Scored Sequences

Predictive Model

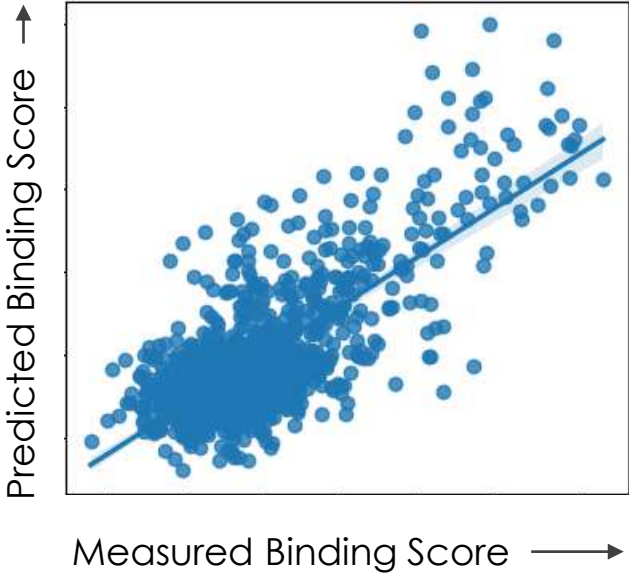
Mammalian-Display
sorted clones



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



Train, then test model on hold-out set



HT Screen
for further
optimized
variants

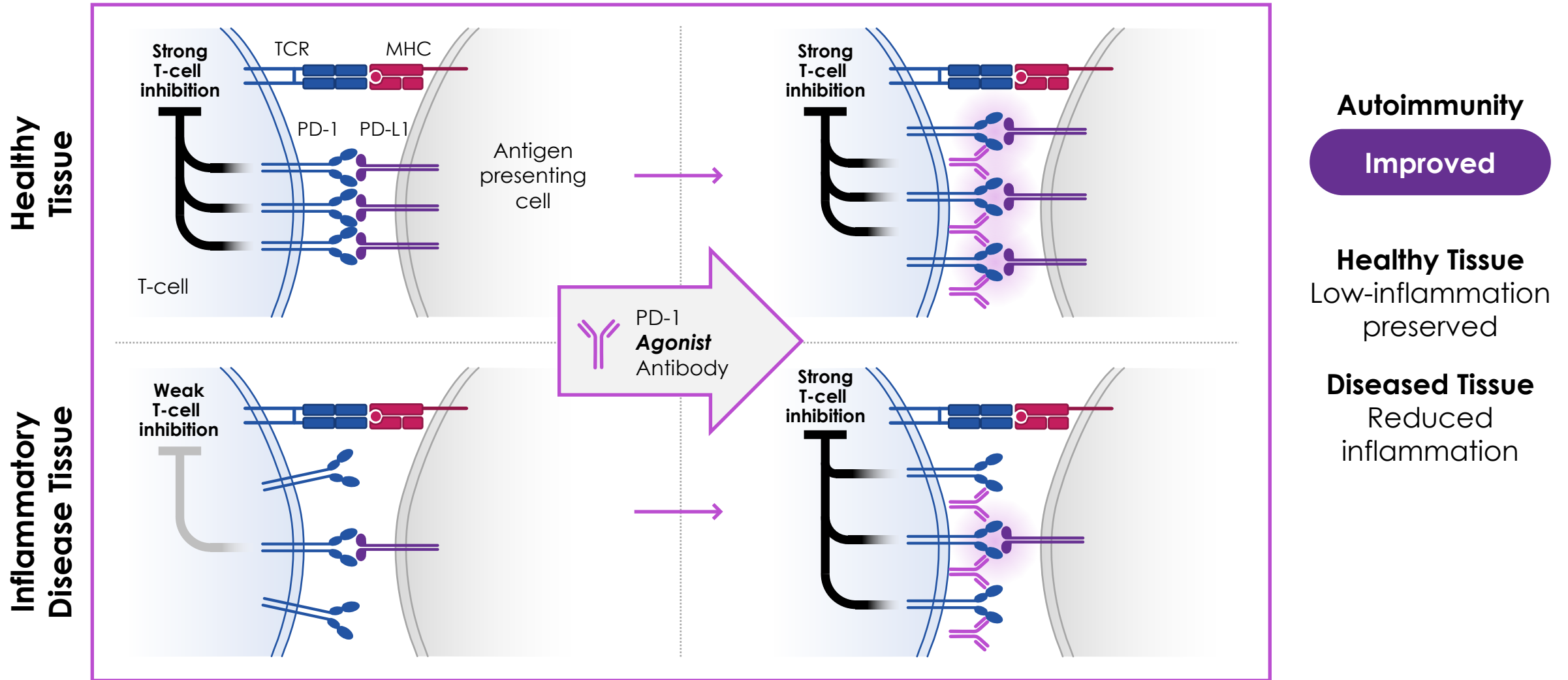


Technology Stack Use Cases

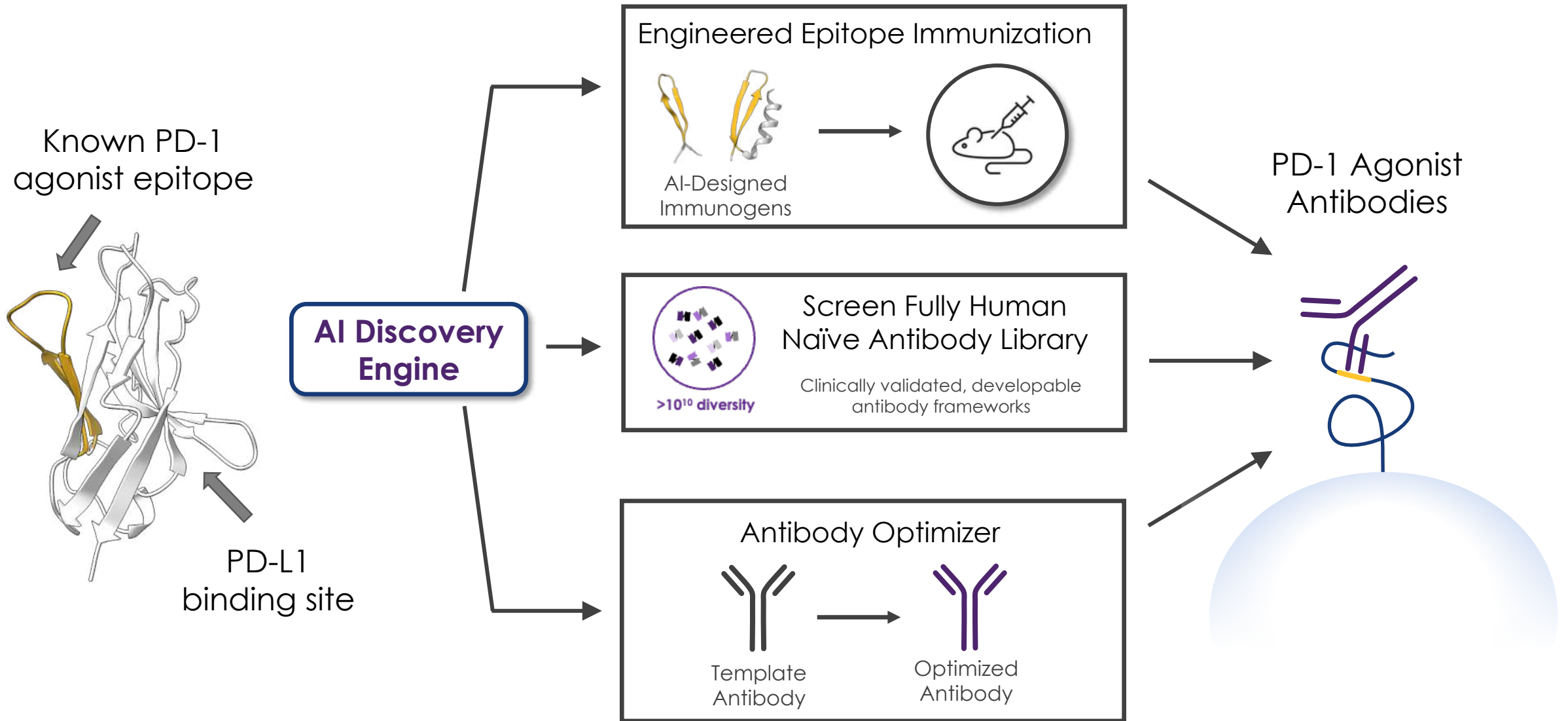
Agonist Epitope

PD-1 Checkpoint Agonist Antibody

Agonizing PD-1 Without Blocking PD-L1 Restores Activated T-Cell Suppression



Parallel Paths to PD-1 Agonist Antibody Discovery



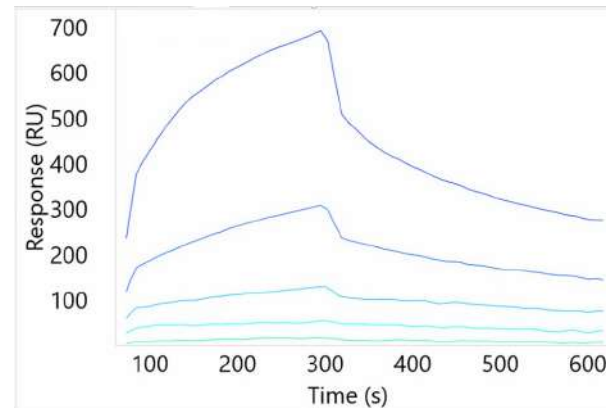
Engineered Epitopes Are Validated By Binding to a Known Antibody or Ligand

Benchmark PD-1 Agonist Ab SPR vs. Engineered Epitope Designs

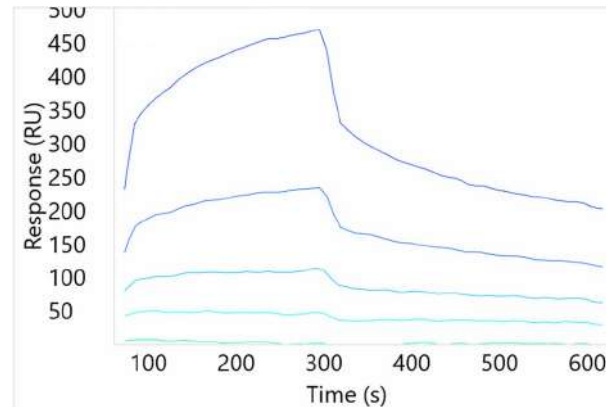
Design 1



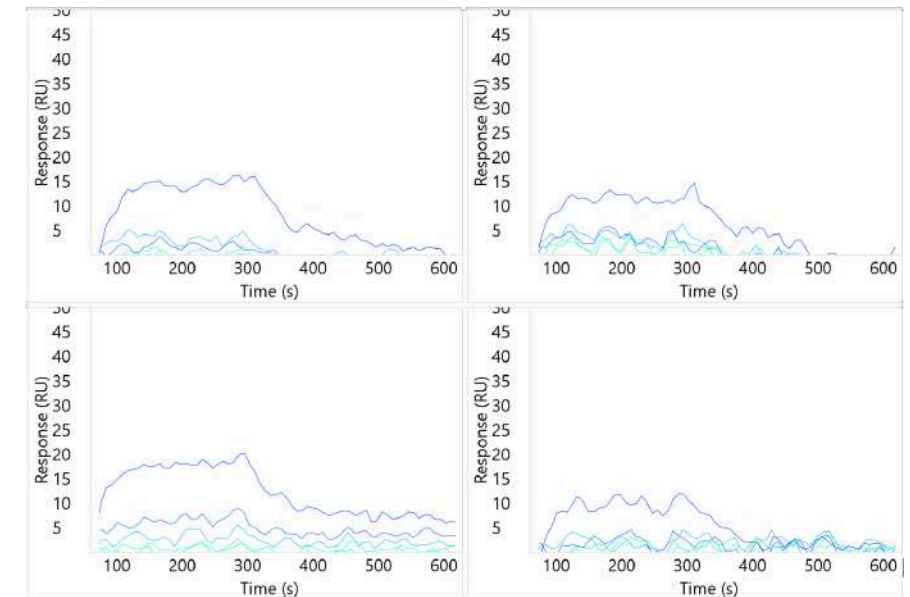
Top PD-1 Agonist Epitope Designs



Design 2

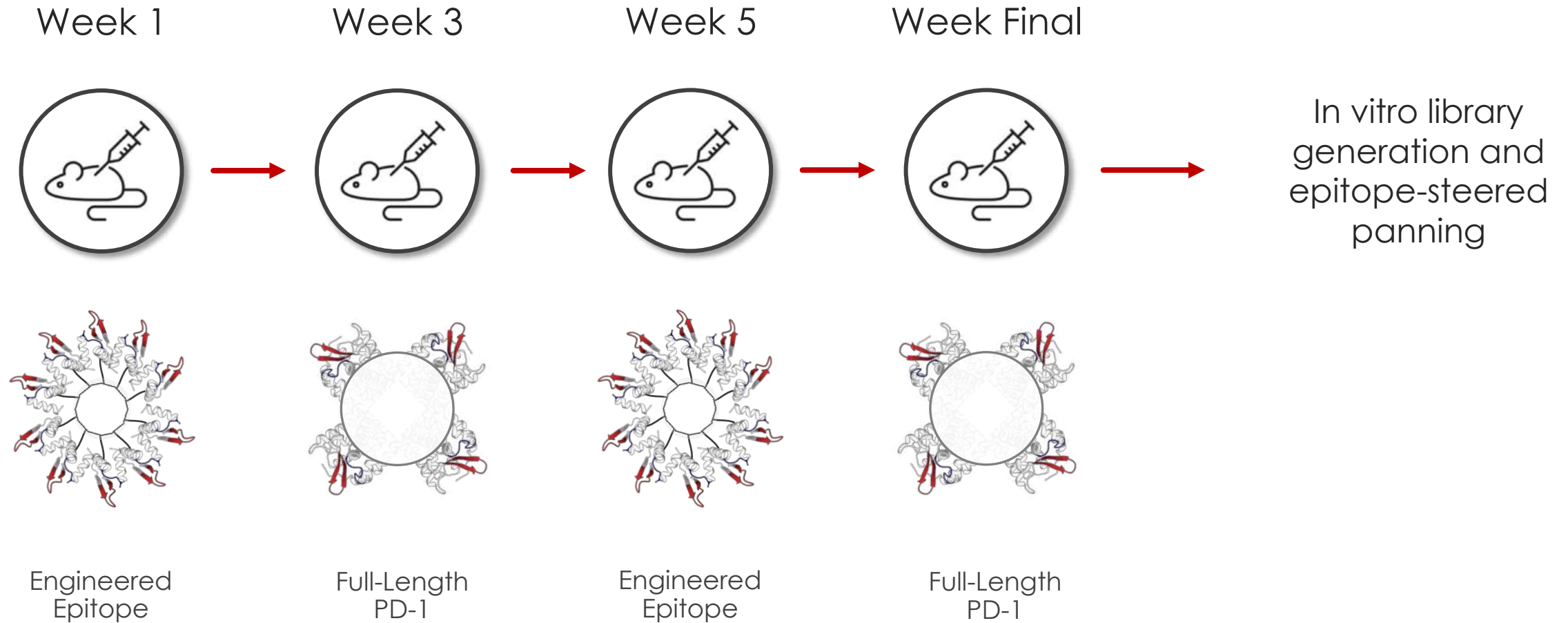


Ineffective Agonist Epitope Designs



PD-1 Agonist Engineered Epitope Steered Immunization and In Vitro Libraries

Engineered epitope alternates with full length PD-1 to enforce full length PD-1 binding

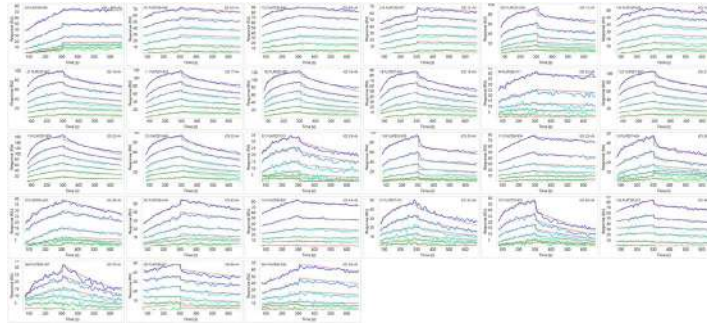


PD-1 Agonist Epitope-Steered Immunization & In Vitro Selection Enriched Towards Non-Antagonist Hits

Epitope-Steered
Mostly non-antagonist hits

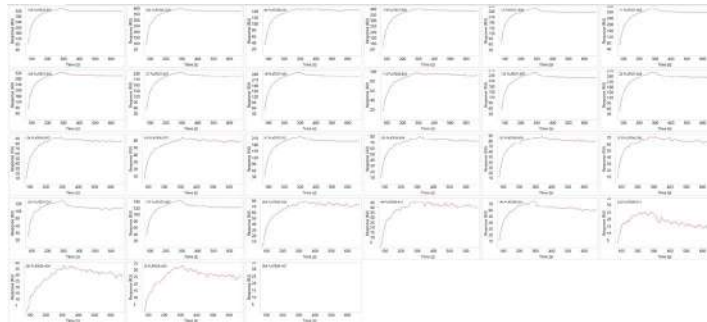
PD-1 binding HT-SPR

27 PD-1
binding hits
KD: 1 – 80 nM



PD-1 antagonist Ab competition HT-SPR

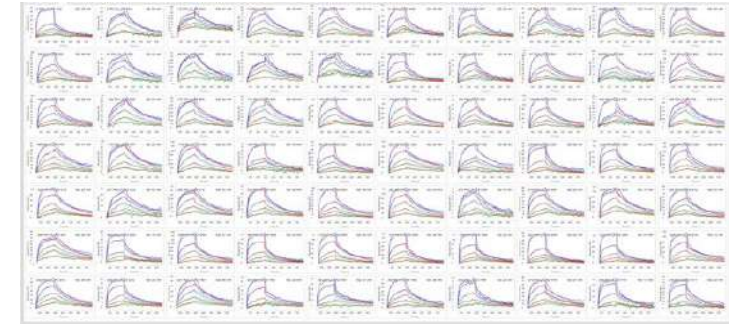
26/27 **do not**
compete with PD-1
antagonist Ab



Not Epitope-Steered
All antagonist hits

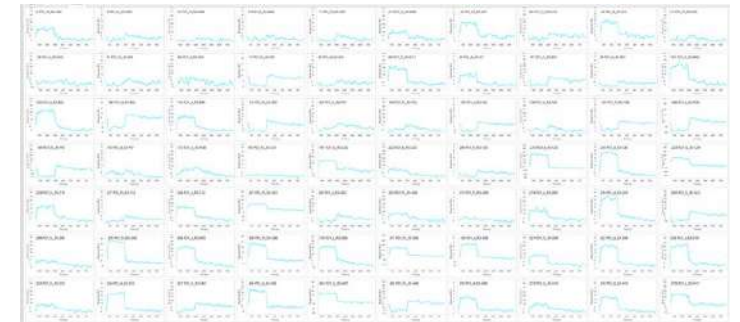
PD-1 binding HT-SPR

70 PD-1
binding hits
KD: 10 – 80 nM



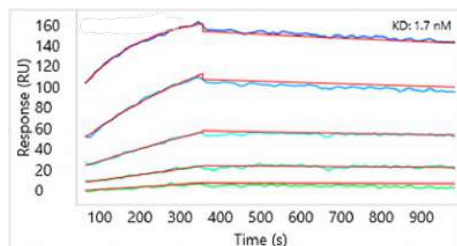
PD-1 antagonist Ab competition HT-SPR

70/70 **do**
compete with PD-1
antagonist Ab



StableHu Optimization of a Template PD-1 Agonist Clone with Murine CDRs

Starting with PD-1 agonist
murine CDRs template



KD = 1.7 nM

Template Ab
Mu CDRs



AI-model predicts
human CDRs

HCDR1

4000

HCDR2

4000

HCDR3

4000

LCDR1

4000

LCDR2

2162

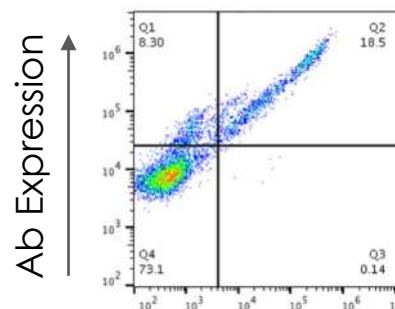
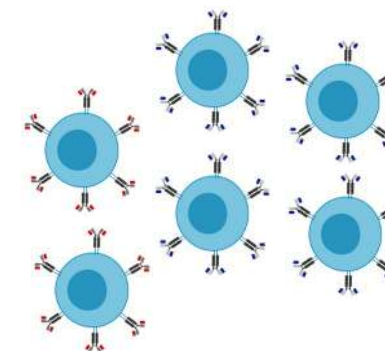
LCDR3

4000

Per-CDR
mammalian
display library



Single-cell sorting:
binding & expression



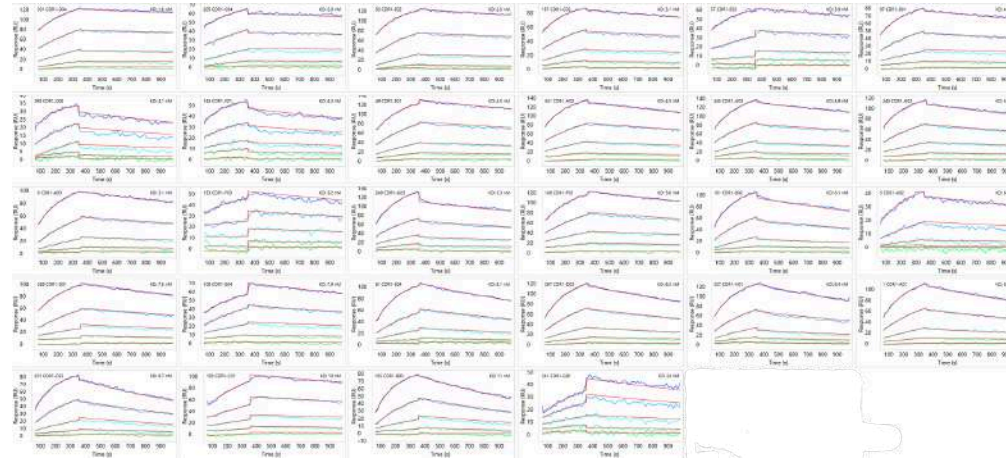
NGS

HT-SPR hit validation
and quantitation

HT-SPR Screen of StableHu Cell Sorts Identifies Fully-Human CDRs That Replace Template Murine CDRs

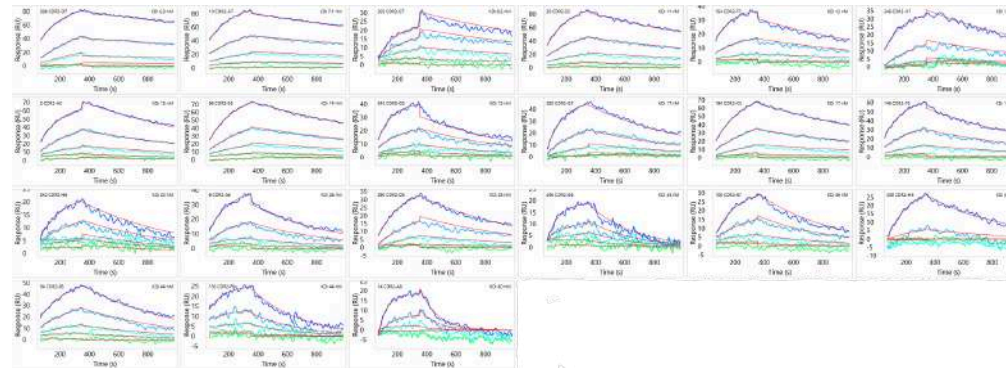
KD: 1.6 - 25 nM

Fully-Human HCDR1:
28 hits



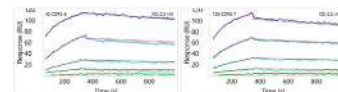
KD: 6.3 - 80 nM

Fully-Human HCDR2:
21 hits



Fully-Human HCDR3:
2 hits

KD: 2.2, 2.3 nM

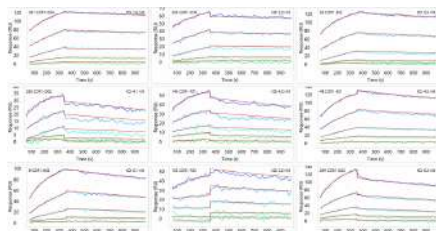


Many fully-human
LCDR1, 2, 3
hits identified

Individual CDR Hits Are Combined to Build Fully-Human Combinatorial Libraries

Starting with individual
fully-human CDR hits

SPR confirmed hits:

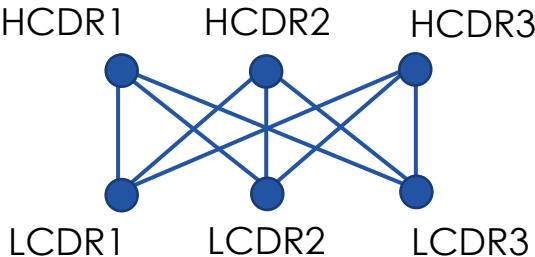


HCDR1,2,3
LCDR1,2,3

Combine
human CDRs



Fully human CDRs
combinatorial diversity

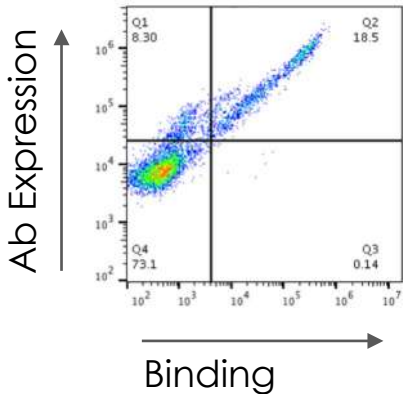


Mammalian
display library

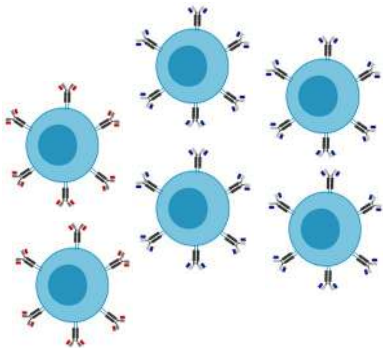


HT-SPR hit validation
and quantitation

NGS



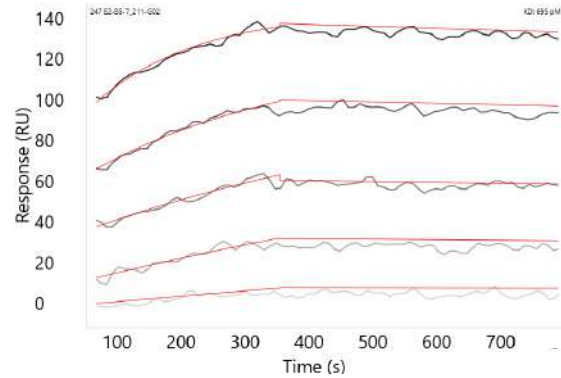
Single-cell sorting:
binding & expression



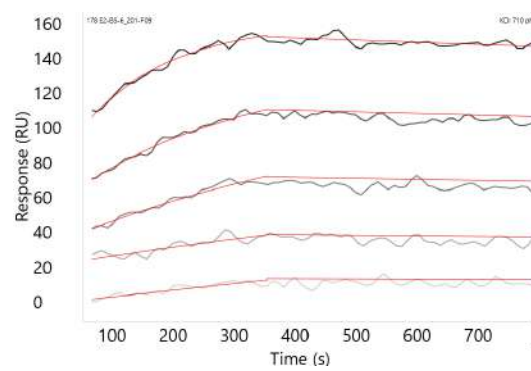
Combining Individual Fully-Human H/L CDR123 Hits Improves Affinity and Humanness

Top Four Fully-Human CDRs StableHu Hits

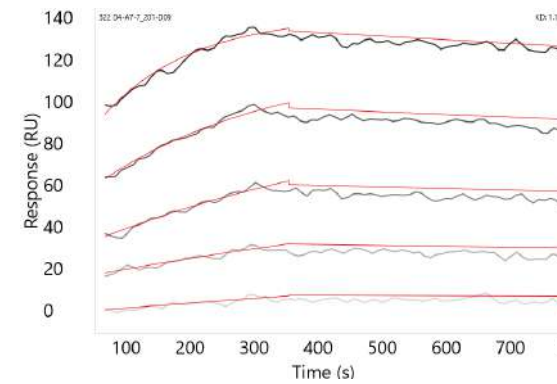
KD = 695 pM



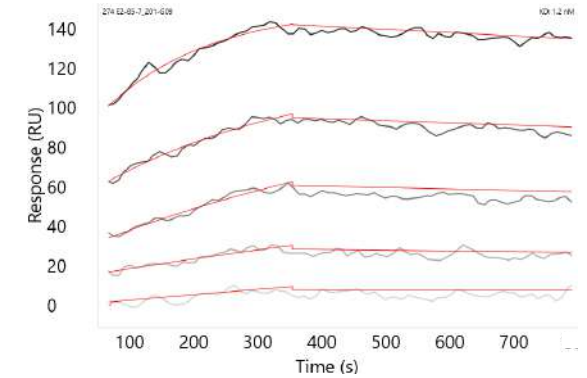
KD = 710 pM



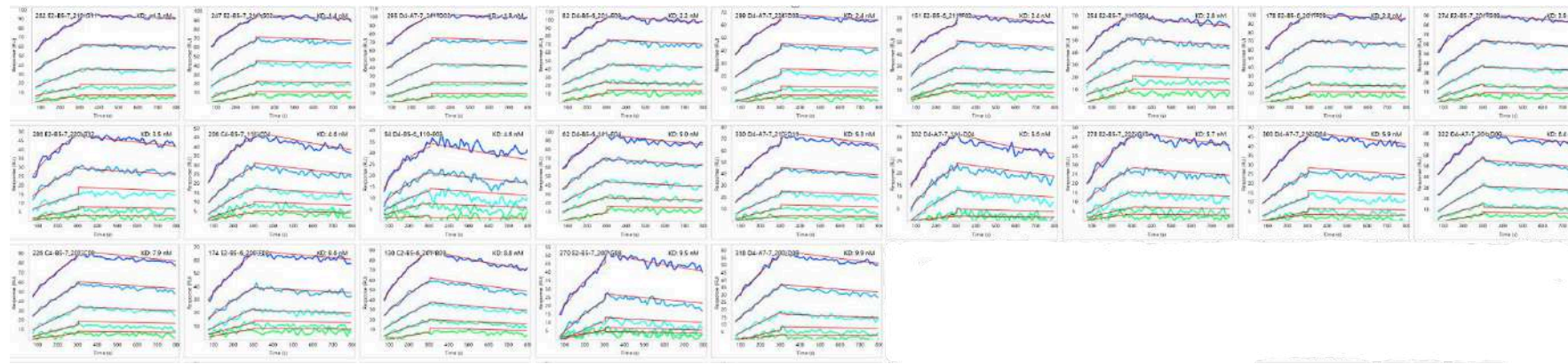
KD = 1.1 nM



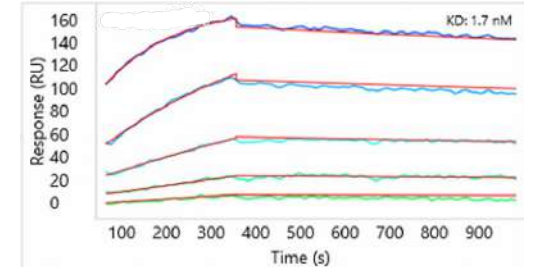
KD = 1.2 nM



23 Additional Fully-Human CDRs StableHu Hits with KD < 10 nM



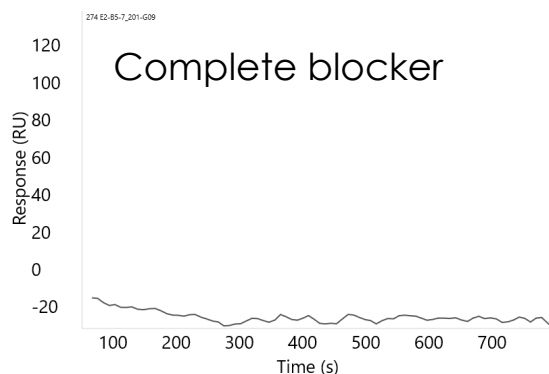
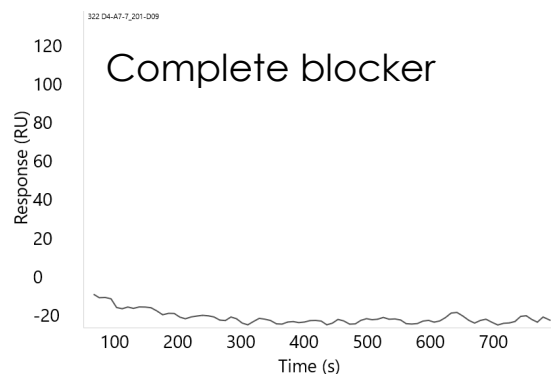
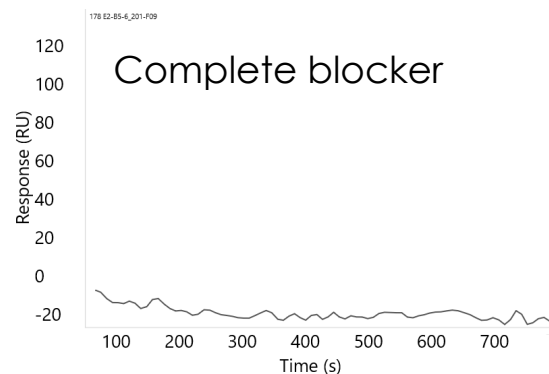
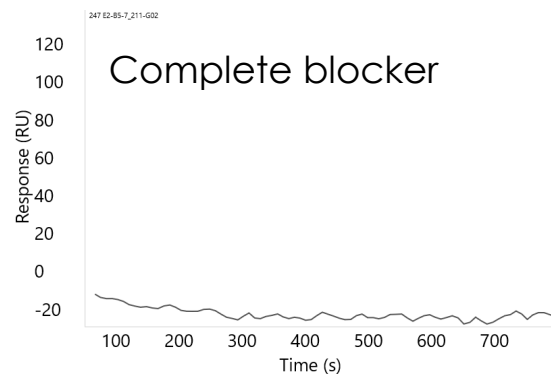
Starting Mu CDRs template



KD = 1.7 nM

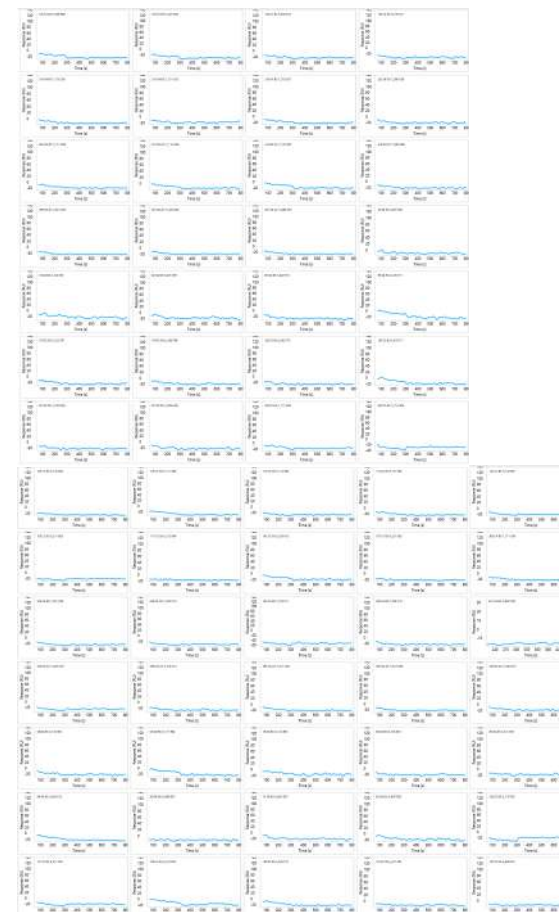
All StableHu Hits Cross-Block Starting Template Antibody With Mu CDRs

Top Four Fully-Human CDRs StableHu Hits

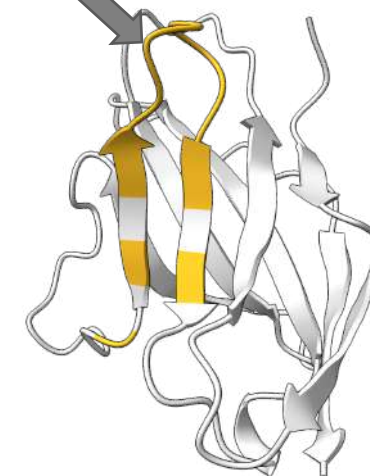


Remaining Fully-Human CDRs StableHu Hits

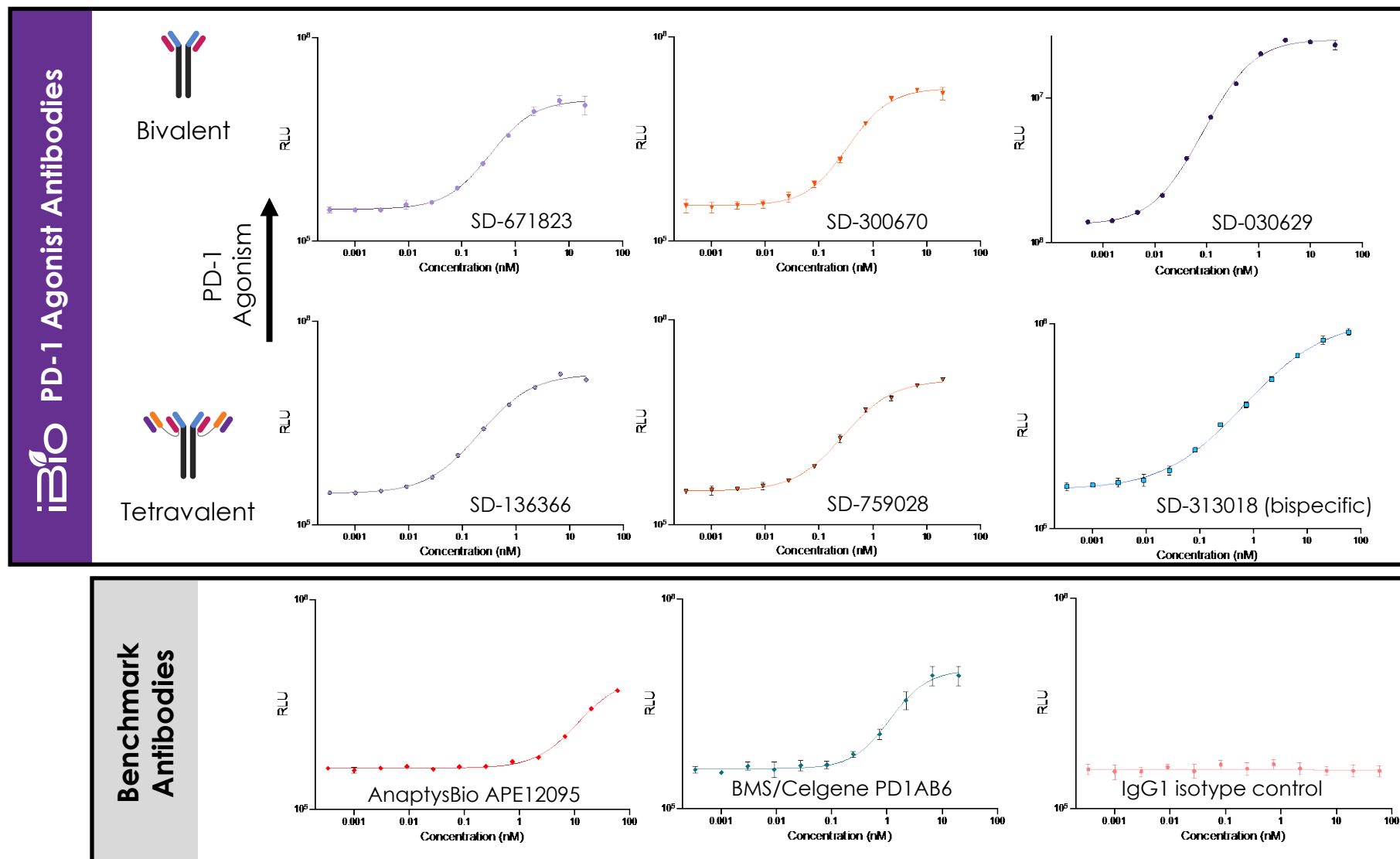
Complete blockers



StableHu hits cross-block the template PD-1 agonist antibody

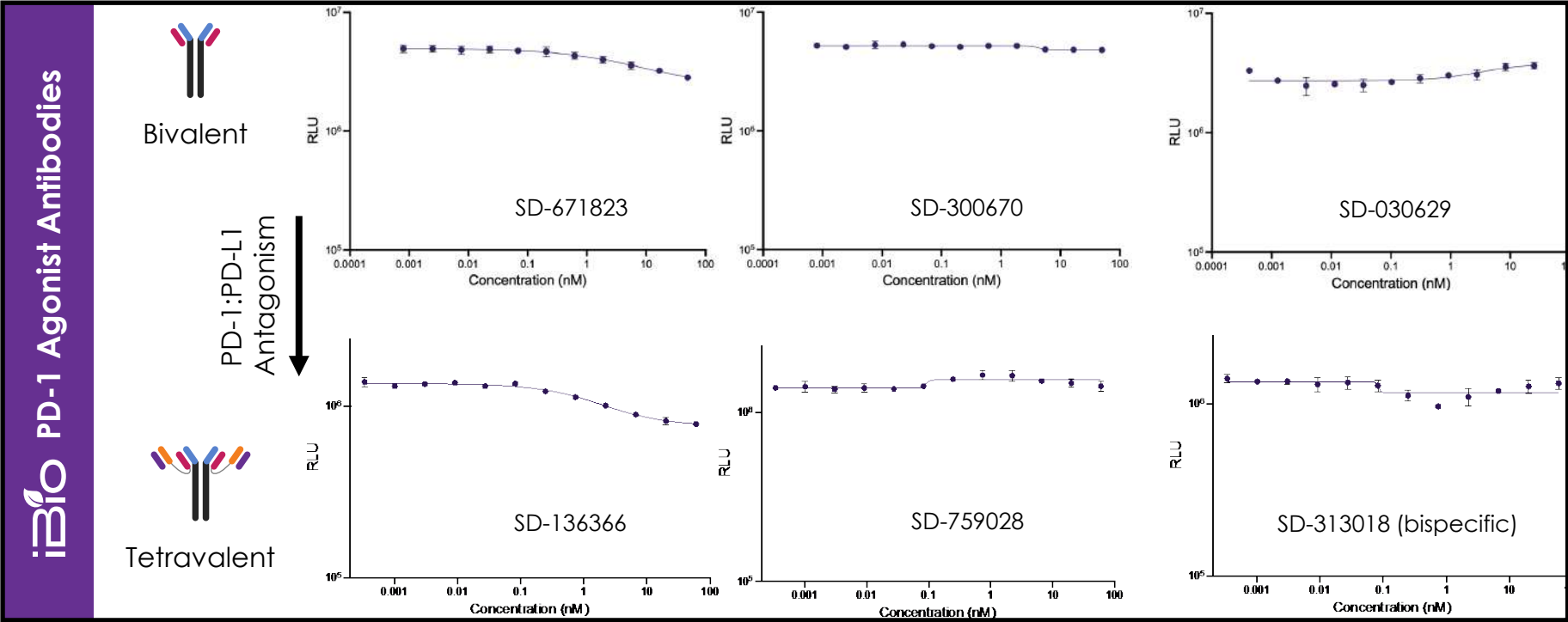


In vitro PD-1 Agonism Equals or Surpasses Benchmarks

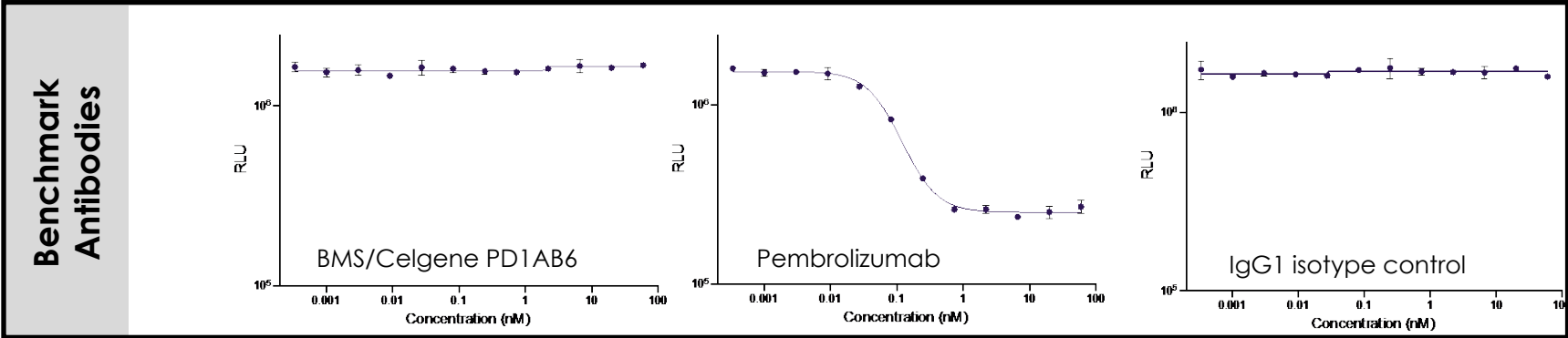


Ab ID	EC50 (nM)
SD-671823	0.88
SD-300670	0.31
SD-030629	0.36
SD-136366	0.28
SD-759028	0.52
SD-313018 (bispecific)	0.30
AnaptysBio APE12095	17.4
BMS/Celgene PD1AB6	0.76
IgG1 isotype control	inactive

PD-1 Agonist Antibodies Are Not PD-1:PD-L1 Antagonists



Ab ID	IC50 (nM)
SD-671823	inactive
SD-300670	inactive
SD-030629	inactive
SD-136366	inactive
SD-759028	inactive
SD-313018 (bispecific)	inactive
BMS/Celgene PD1AB6	inactive
Pembrolizumab	0.20
IgG1 isotype control	inactive



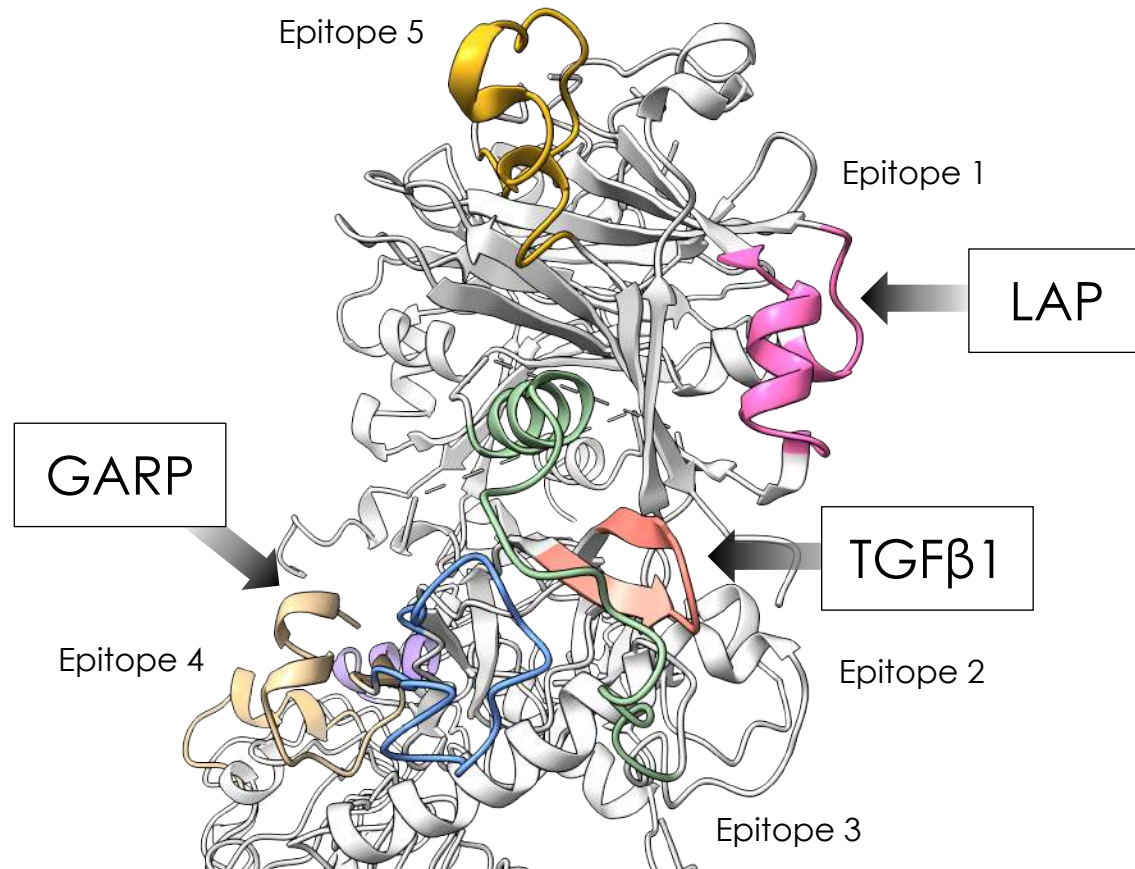
Multi-Protein Junctional Epitope

Latent-TGF β 1 Antibody

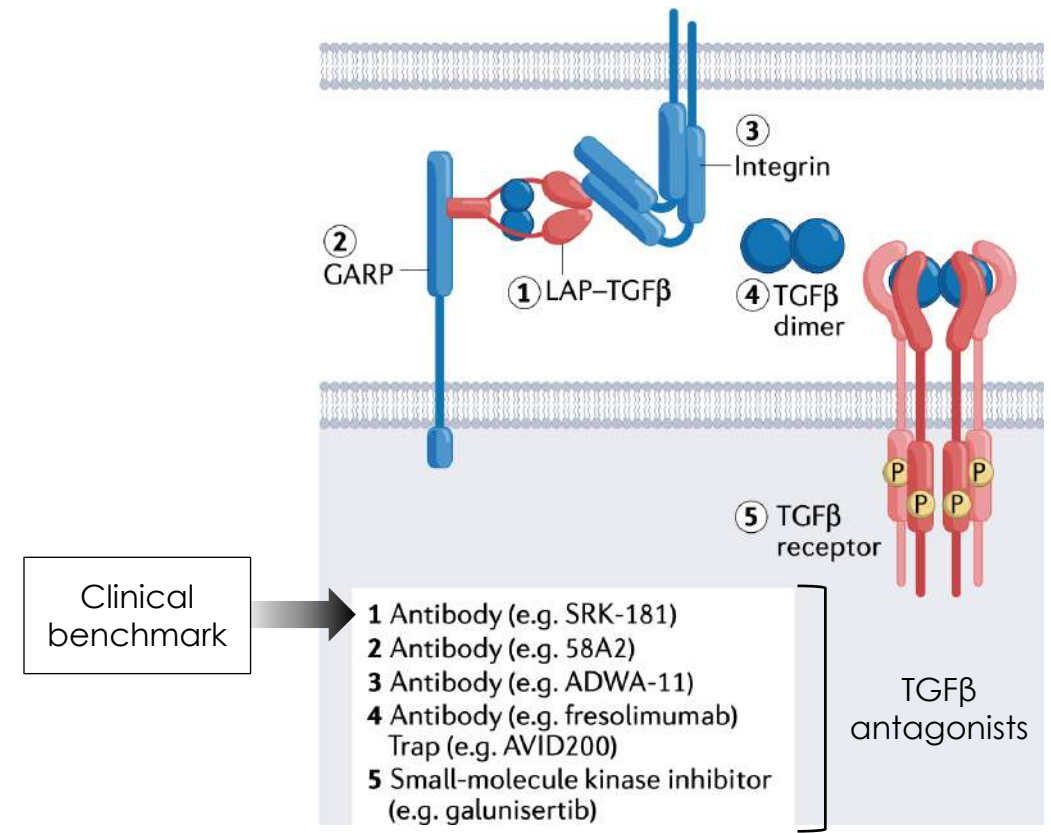
Latent-TGFβ1 Multimeric Complex Regulates TGFβ1 Release and Signaling

Multiple engineered epitopes were used to explore per-epitope TGFβ1-release antagonist potency

Latent-TGFβ1 Multimeric Structure

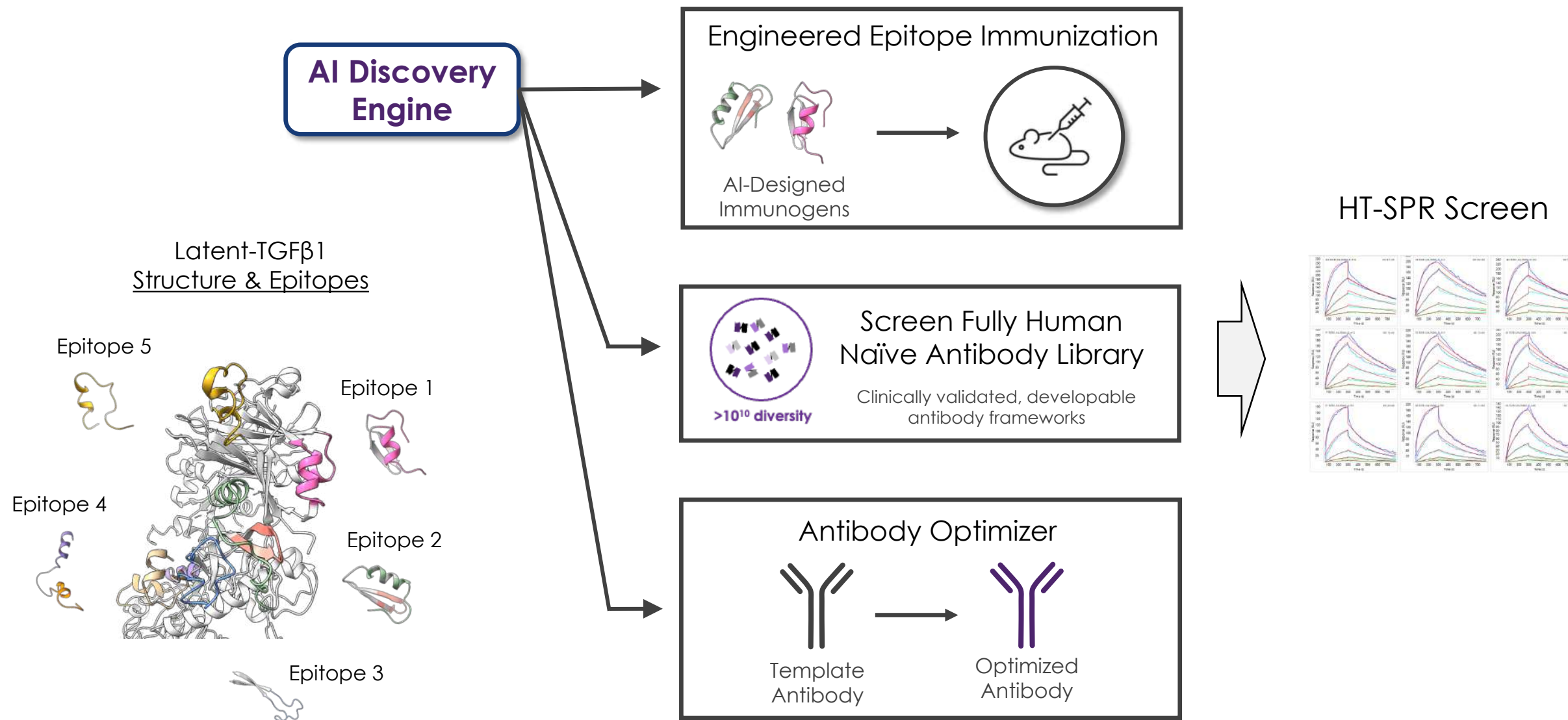


Released TGFβ1 is Immunosuppressive



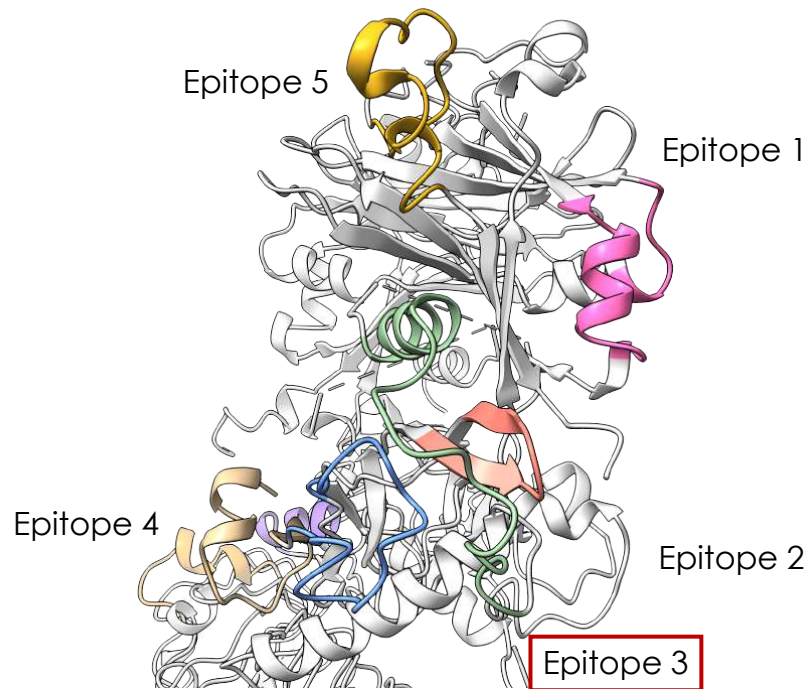
Nat. Rev. Immunol. (2022) 10.1038/s41577-022-00796-z

Parallel Paths to Latent-TGFβ1 Antibody Discovery

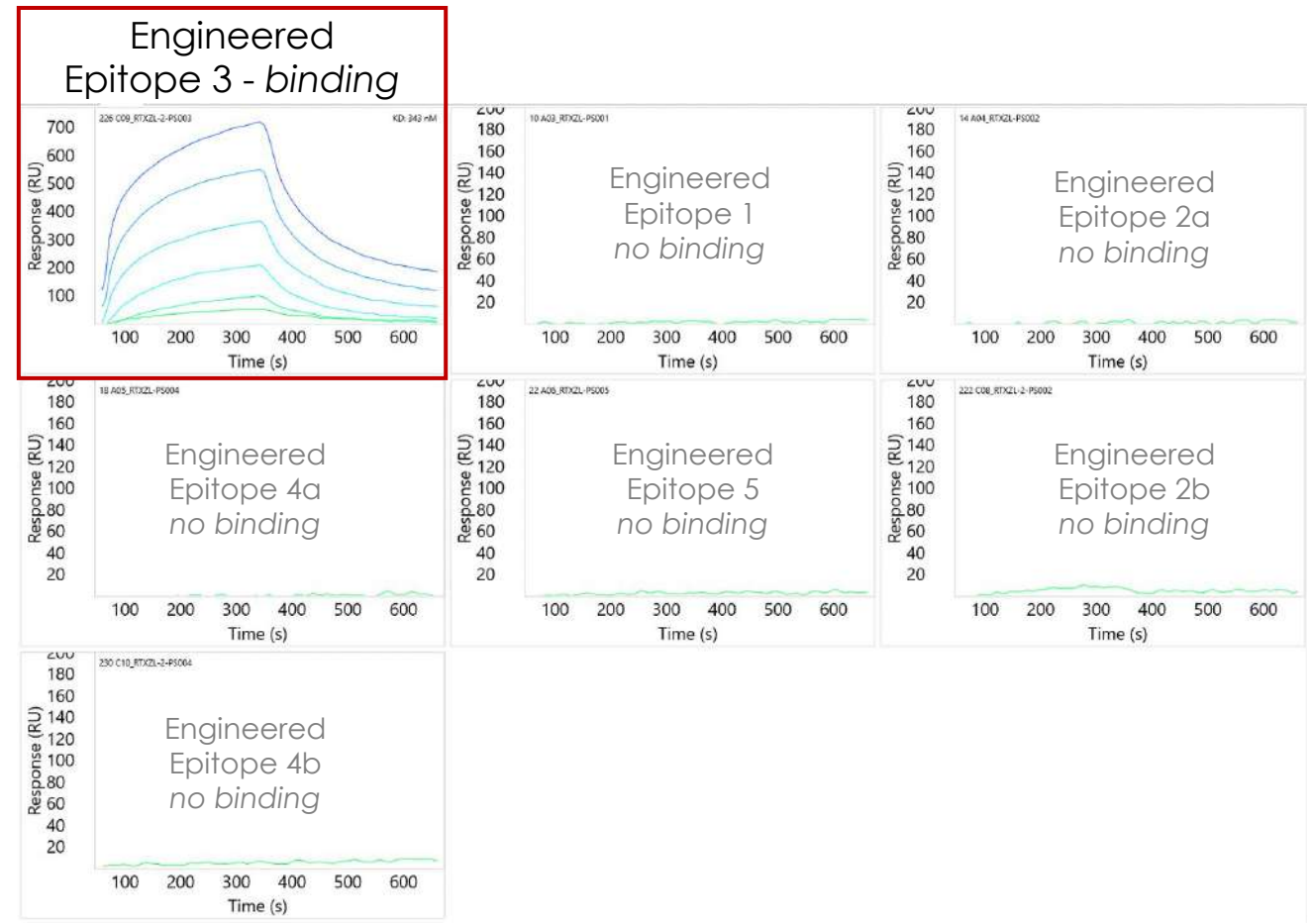


Mapping SRK-181 Benchmark Ab Using Engineered Epitopes

Latent-TGFβ1 Structure & Epitopes

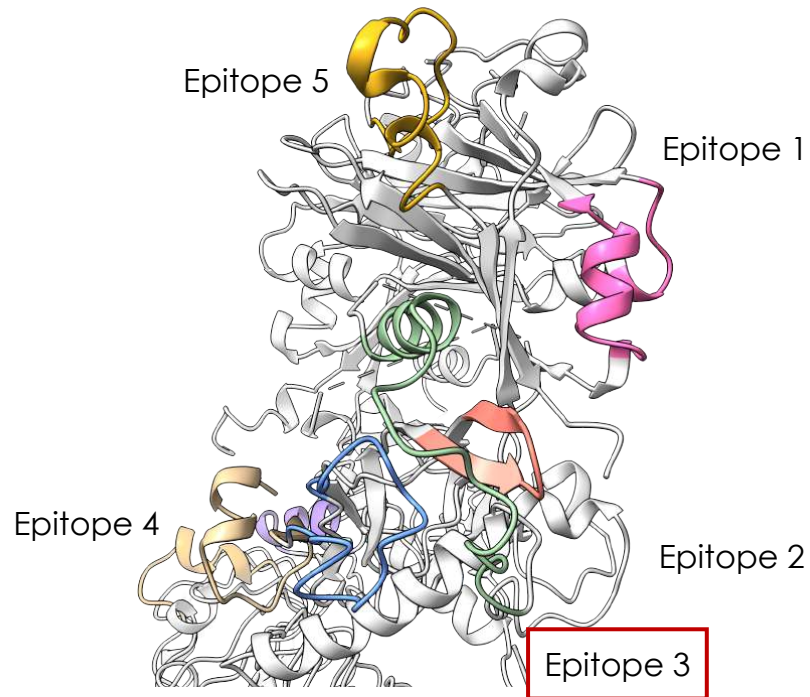


Multiple Engineered Epitopes Binding to the Benchmark Ab

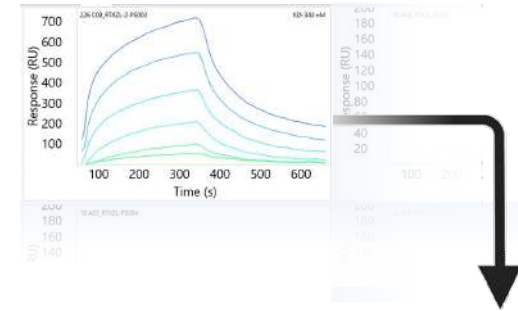


SRK-181 Benchmark HD-X MS Corroborates Engineered Epitope Mapping by SPR

Latent-TGFβ1 Structure

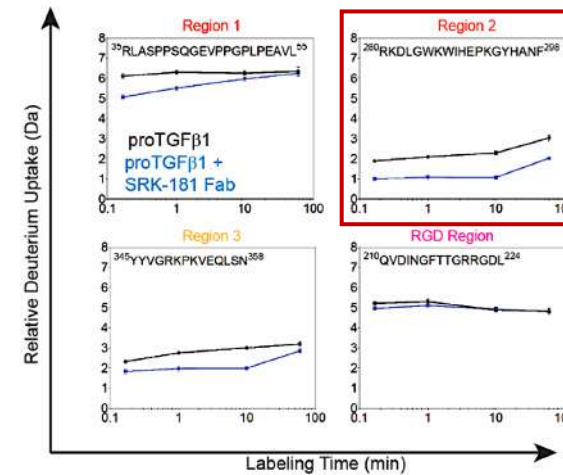


Engineered Epitope 3 SRK-181 SPR



Top binding engineered epitope maps to key binding region identified by HD-X MS

SRK-181 HD-X MS



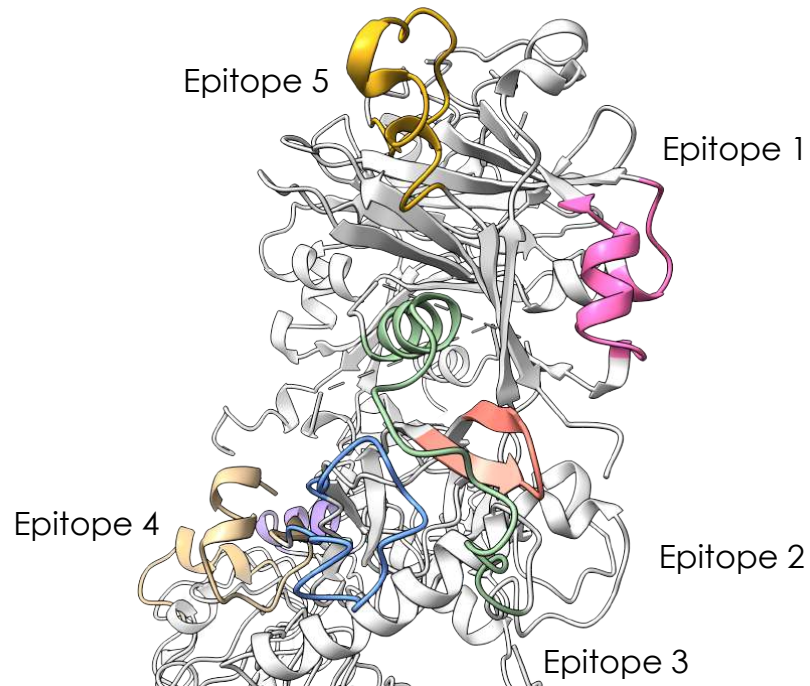
HD-X region corresponding to engineered epitope 3

Sci. Transl. Med. (2020) 10.1126/scitranslmed.aay8456

Epitope-Steered Naïve In Vitro Selection Was One Path to Latent-TGFβ1 Clones

Latent-TGFβ1 Structure & Epitopes

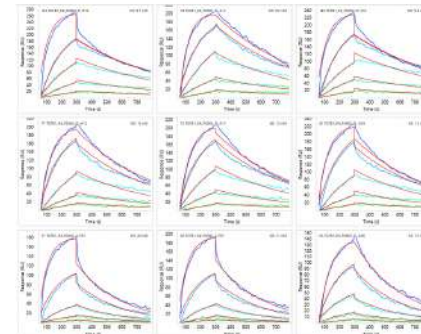
Multiple engineered epitopes were used during rounds of phage library panning



Round 1	Round 2	Round 3
LTGFβ1	LTGFβ1	Engineered Epitope
LTGFβ1	Engineered Epitope	LTGFβ1



HT-SPR
Screen



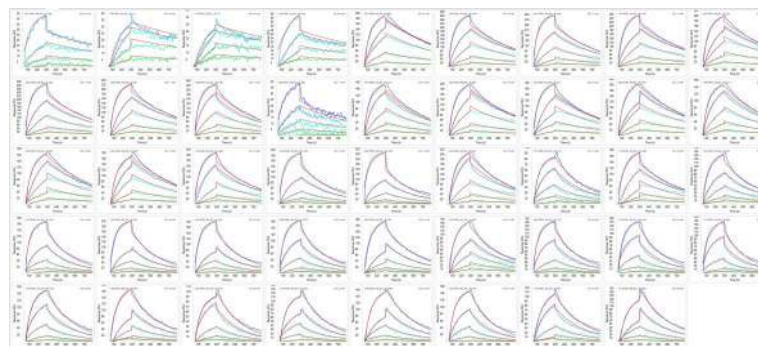
HT-SPR Screen Demonstrates Specificity, Diversity & Affinity of Epitope Steered Selections

SPR Screen Results

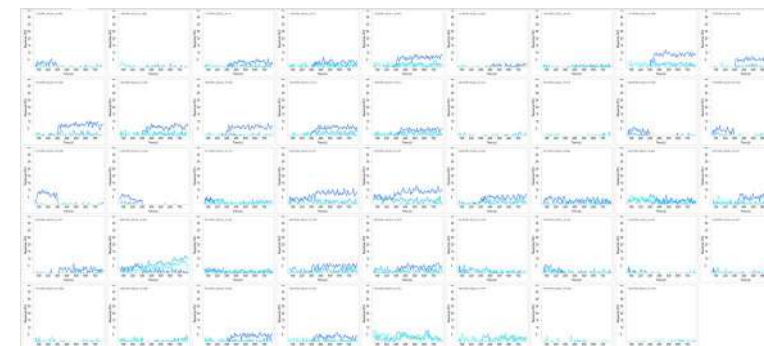
Epitope 1 steered binders

Latent-TGFβ1 specific	44
KD range (nM)	2.5 – 40 nM
TGFβ1 off-target	13

Latent-TGFβ1 (desired target)

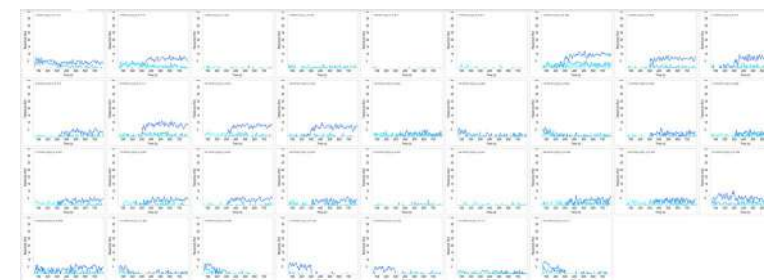
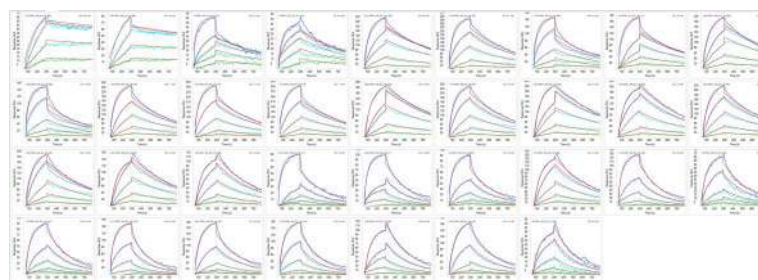


TGFβ1 (undesired target)



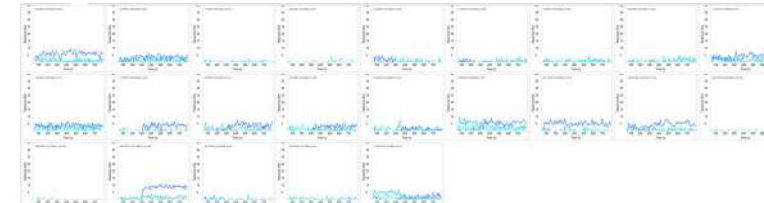
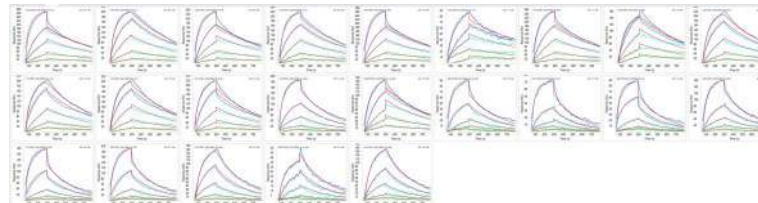
Epitope 2 steered binders

Latent-TGFβ1 specific	34
KD range (nM)	1.0 – 36 nM
TGFβ1 off-target	7



Epitope 3 steered binders

Latent-TGFβ1 specific	23
KD range (nM)	9.0 – 29 nM
TGFβ1 off-target	5

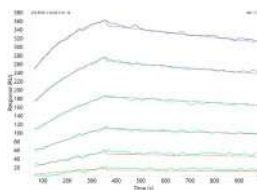


Four Clones Were Identified with Required Affinity and TGFβ Cross-Family Specificity

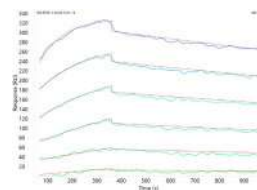
Do bind

Latent-TGFβ1
KD < 5 nM

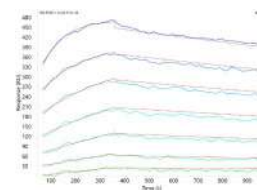
Clone: 1



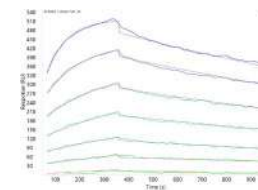
2



3



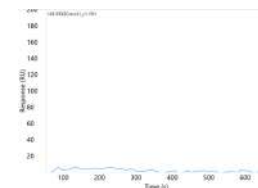
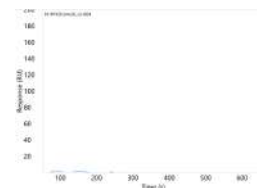
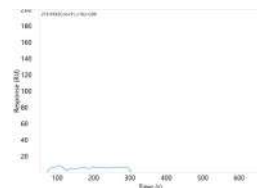
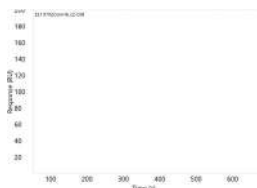
4



AND

Do Not bind

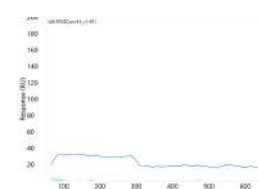
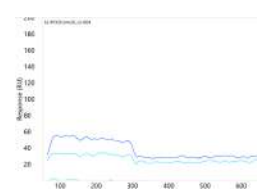
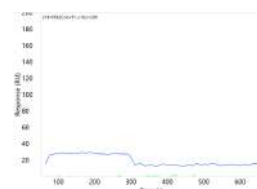
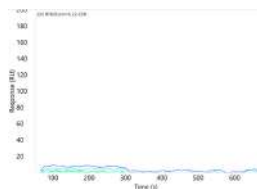
TGFβ1



AND

Do Not bind

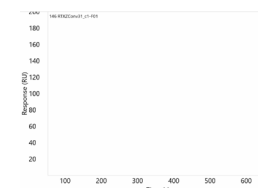
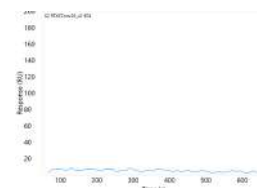
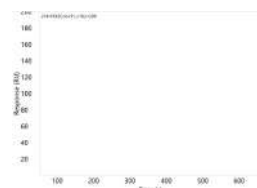
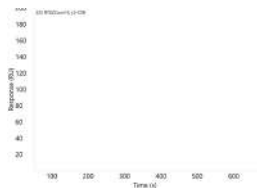
Latent-TGFβ2



AND

Do Not bind

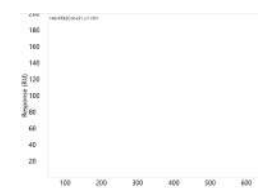
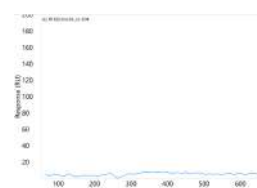
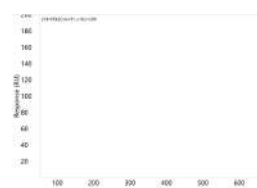
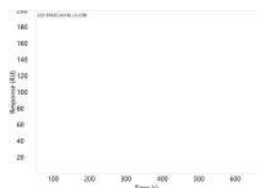
TGFβ2



AND

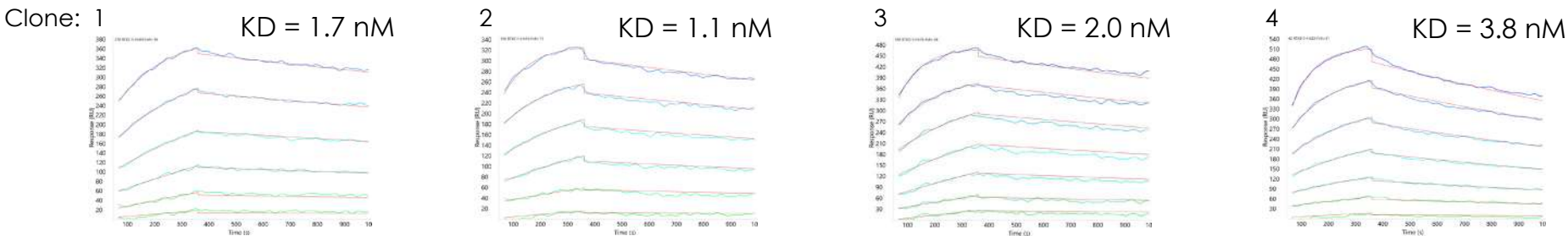
Do Not bind

TGFβ3

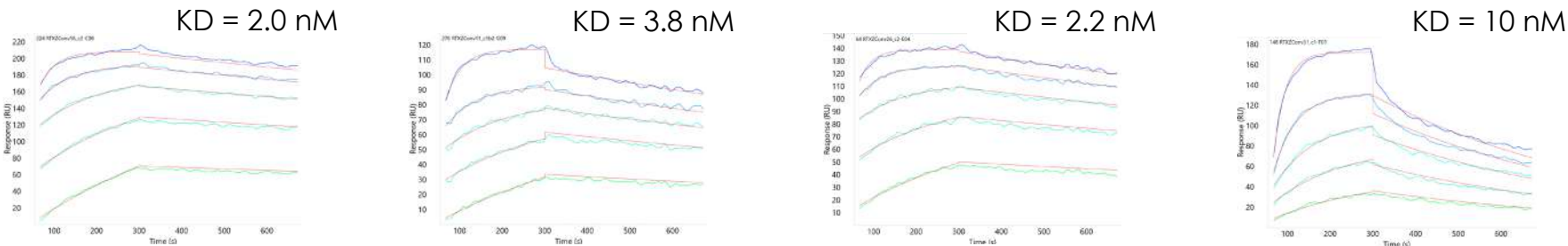


4/4 Latent-TGFβ1 Specific Clones are Hu-Cyno Cross-Reactive – 1/4 is Mu Cross-Reactive

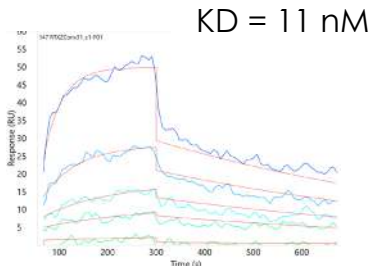
Hu
Latent-TGFβ1



Cyno
Latent-TGFβ1



Mu
Latent-TGFβ1

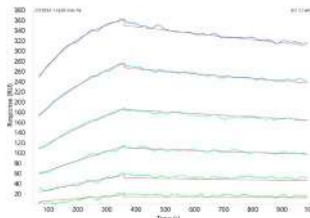


Top Naïve In Vitro Selection Clone Met All Affinity, Specificity and Potency Criteria

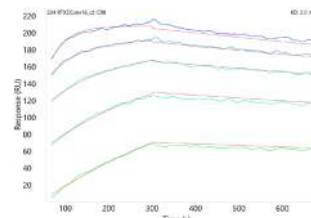
SPR

Does bind:

Hu Latent-TGFβ1
KD = 1.7 nM

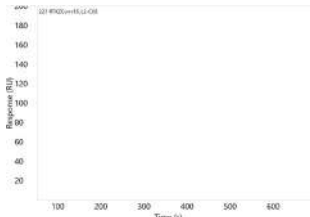


Cyno Latent-TGFβ1
KD = 2.0 nM

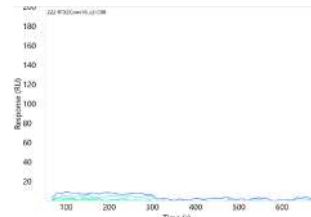


Does not bind:

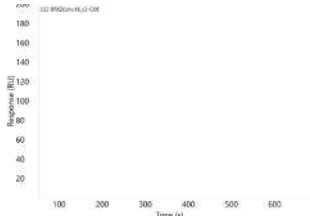
TGFβ1



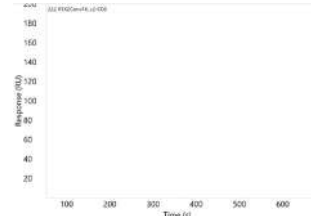
Latent-TGFβ2



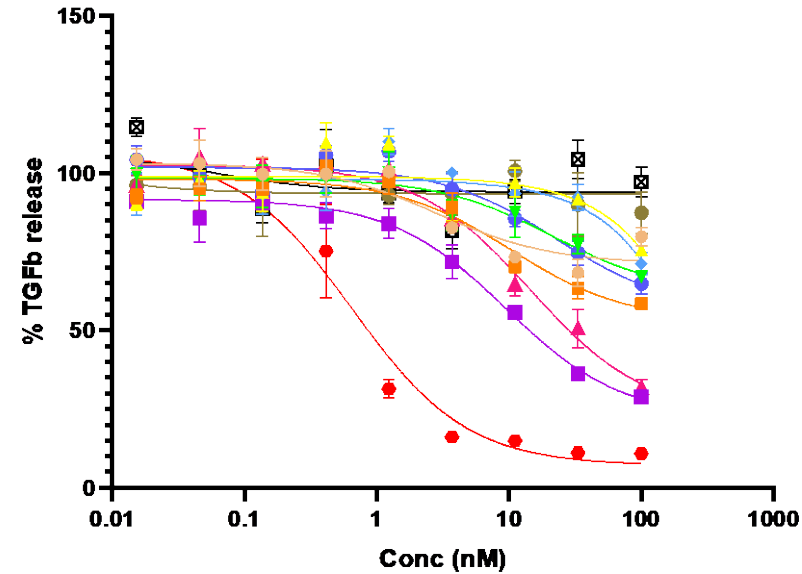
TGFβ2



TGFβ3



TGFβ1 Inhibition Assay



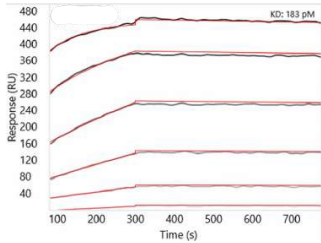
Clone ID

- 1
- 2
- 3
- 4
- 5
- 6
- 7 ← Top naïve in vitro selection clone
- 8
- 9
- Iso ctl.
- Benchmark Ab

Clone	IC ₅₀
Top naïve clone #7	9.5 nM
Benchmark Ab	0.7 nM

StableHu Optimization of an Anti-Latent-TGFβ1 Benchmark Ab

Starting with published benchmark CDRs template



KD = 180 pM
IC₅₀ = 700 pM

Published Ab template

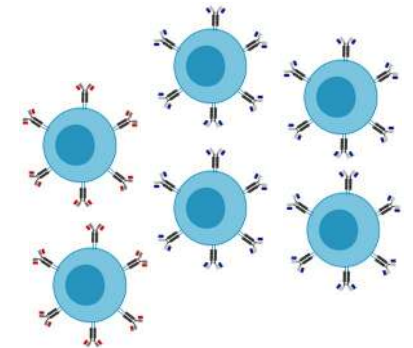


AI-model predicts human CDRs

<u>HCDR1</u>	<u>HCDR2</u>	<u>HCDR3</u>
2000	2000	24000
<u>LCDR1</u>	<u>LCDR2</u>	<u>LCDR3</u>
2000	1000	2000

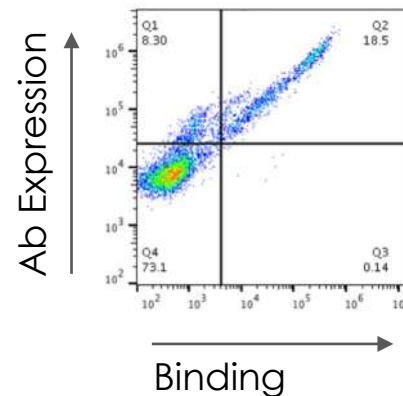
Per-CDR
mammalian
display library

Single-cell sorting:
binding & expression



HT-SPR hit validation
and quantitation

NGS



StableHu Optimization Identifies Improved Fully-Human CDR Variants

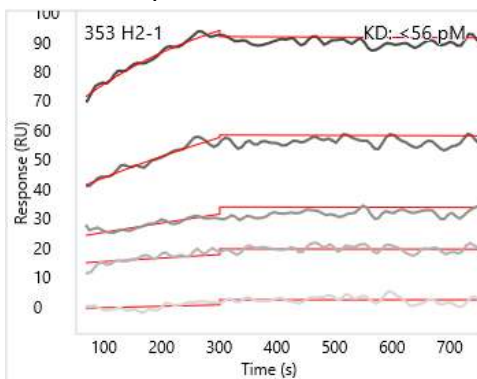
Variant Clones Latent-TGFβ1 SPR

Template clone

Hu
Latent-TGFβ1

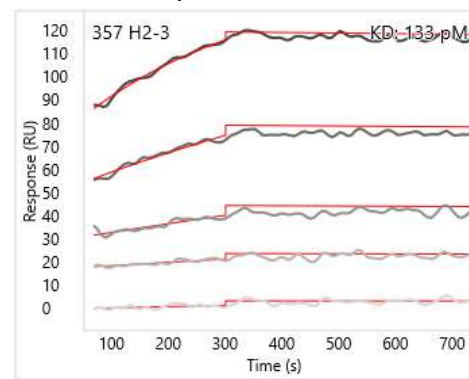
Variant 1

KD = 50 pM

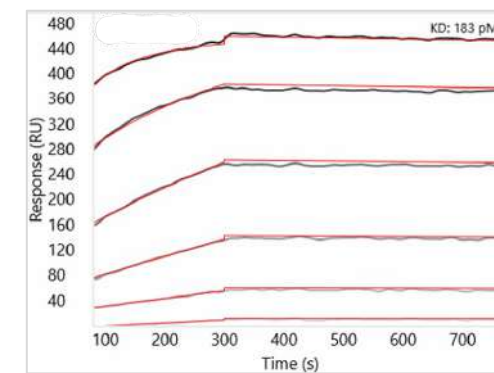


Variant 2

KD = 130 pM

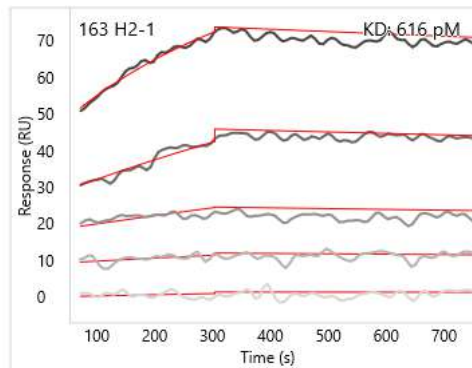


KD = 180 pM

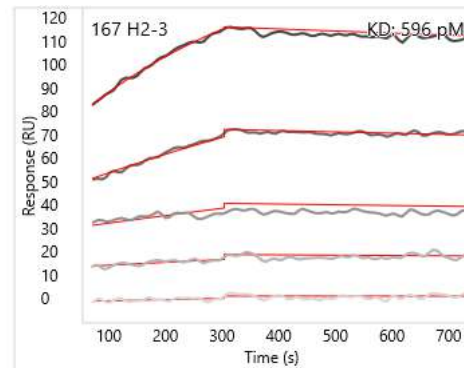


Mu
Latent-TGFβ1

KD = 620 pM



KD = 600 pM



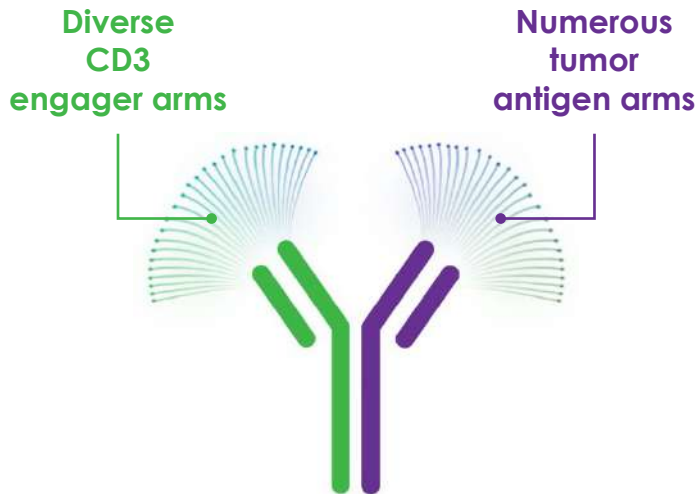
T-Cell Engager Epitope

CD3 Antibody

Key Challenges of CD3 T Cell Engager Discovery

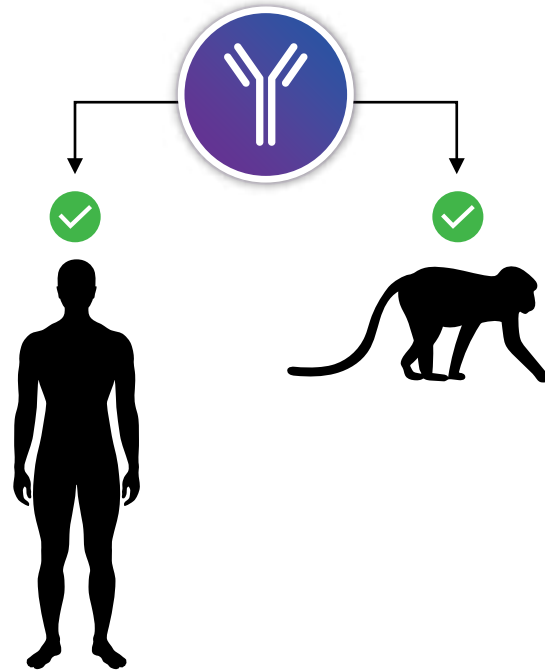
1 Sequence Diversity

Broad CD3 activity for optimized pairing with tumor antigen arms



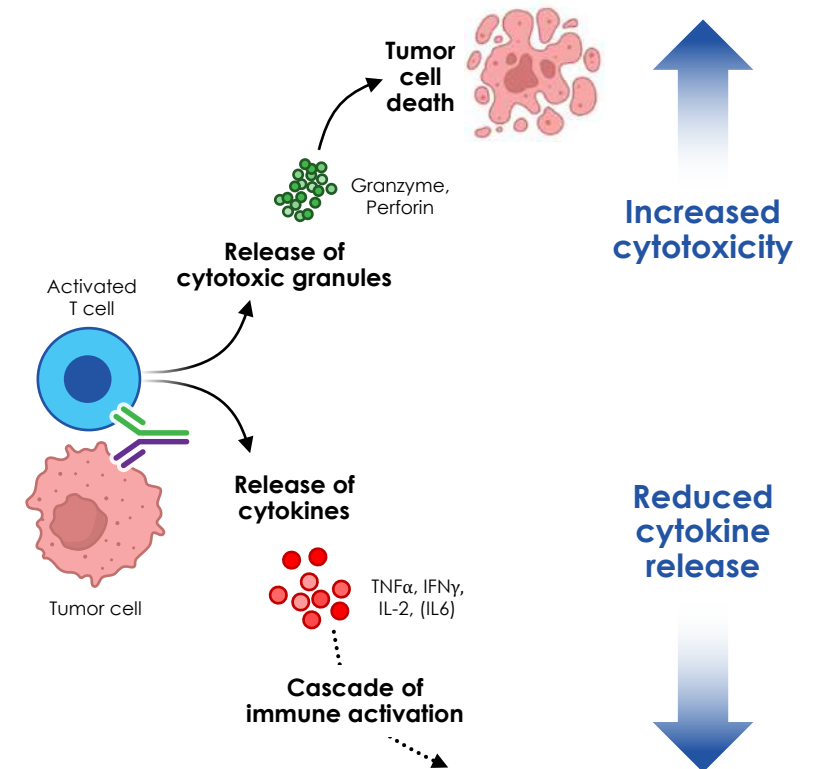
2 Hu-Cyno Cross-Reactivity

Risk reduction via cyno monkey toxicity study compatibility



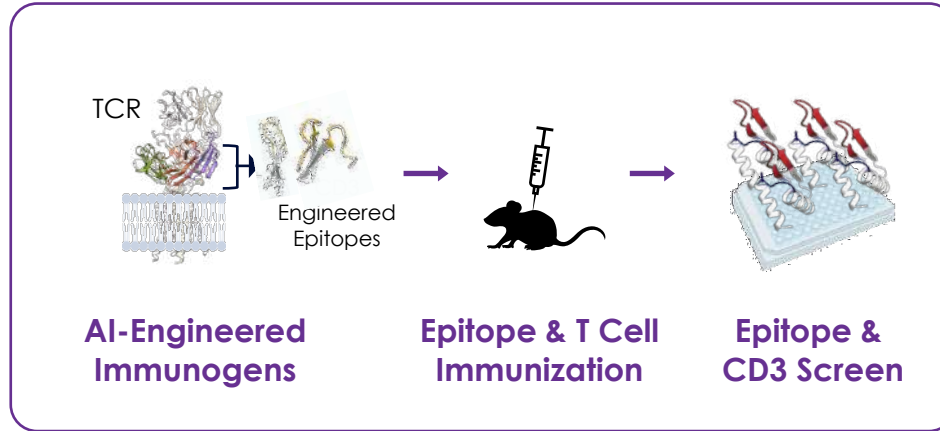
3 Range of Cytokine Release

Tailored cytokine release for expanded therapeutic window



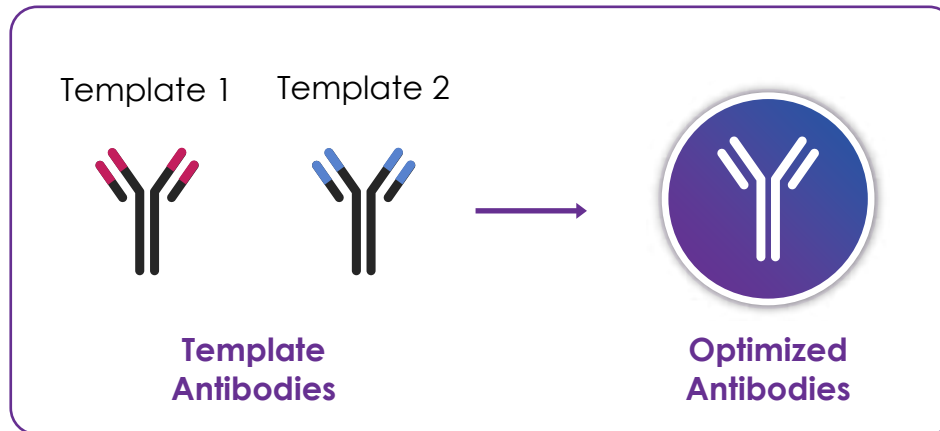
Dual Approaches to a Diverse Panel of Anti-CD3 Antibodies

Structural-Epitope Immunization & Screening



AI Discovery Engine

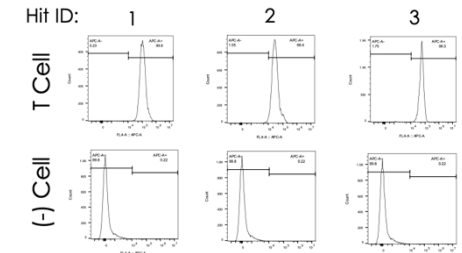
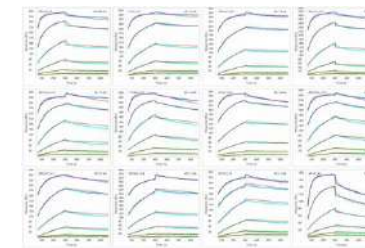
StableHu Optimizer



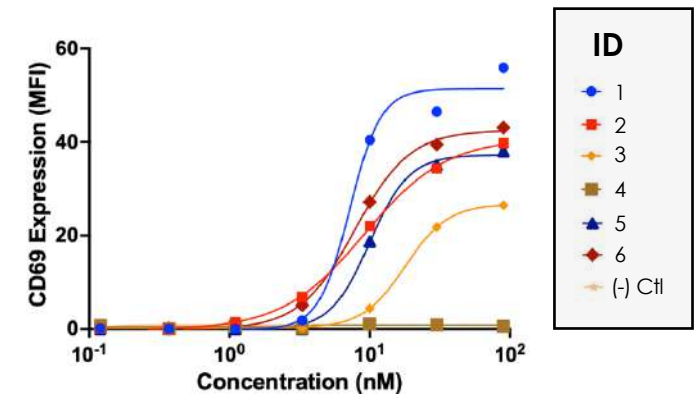
SCREEN

Hu/Cyno CD3 & T Cell

Binding



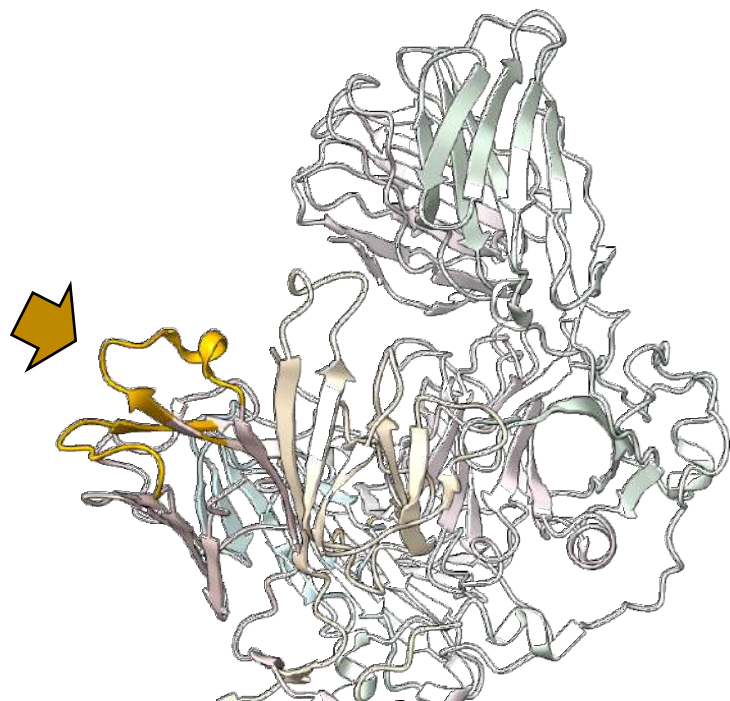
T Cell Activation



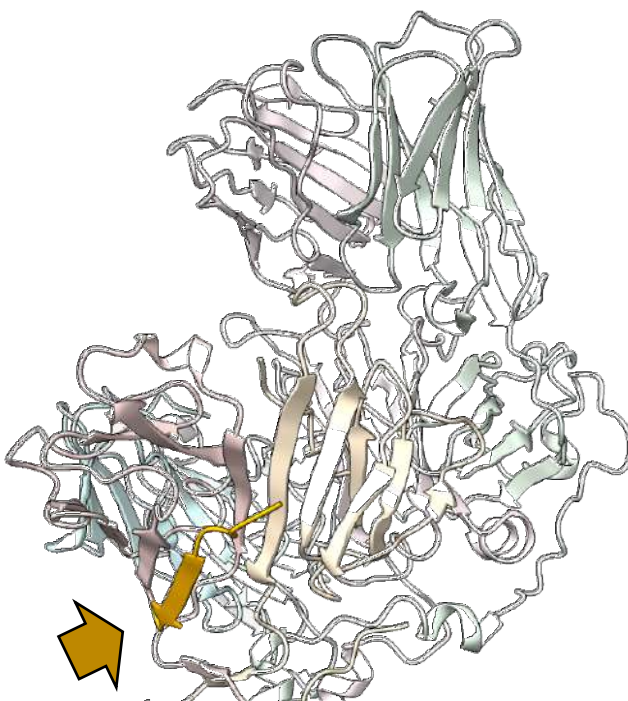
Engineered Epitopes Guide Immunization to TCR-Accessible CD3 Epitopes

CD3 target epitopes in the context of the full TCR

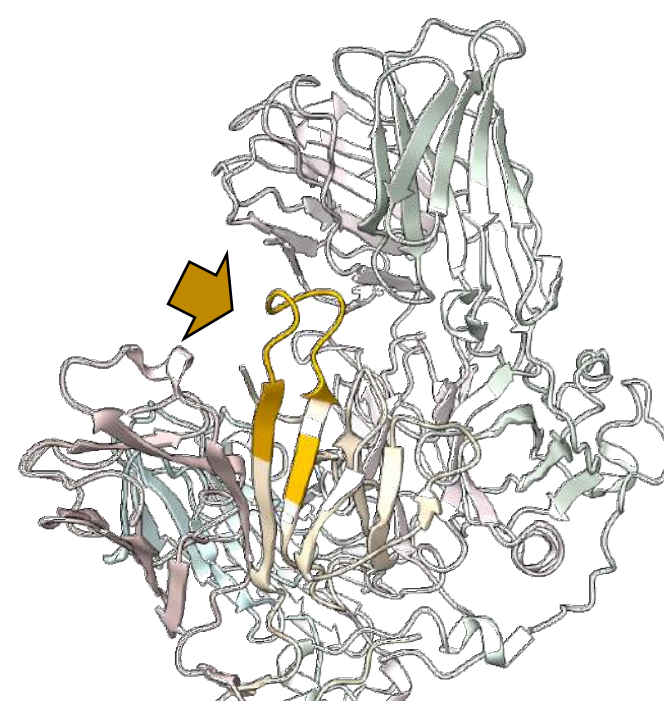
Epitope 1



Epitope 2



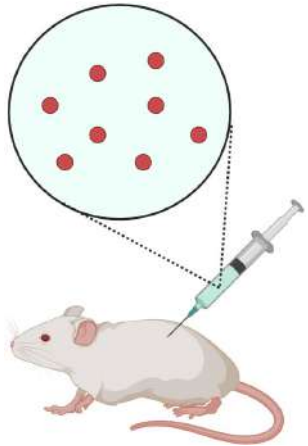
Epitope 3



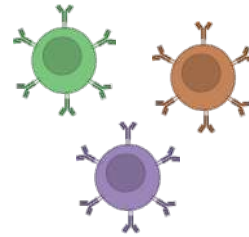
Immunized CD3 Repertoires Were Cloned and Screened in Mammalian Display

Multi Epitope-Steered Immunization

CD3 engineered epitopes & T cells



Mammalian Library Display



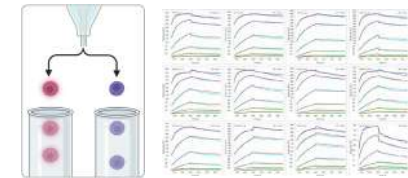
Mammalian Display

Immunized Repertoire



Multi Dimension Screening

FACS, NGS & SPR



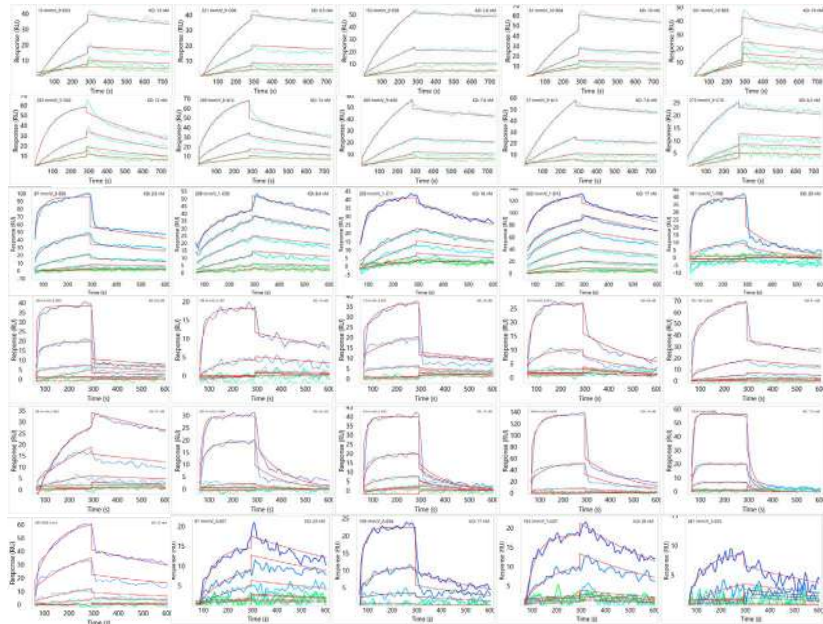
Single-Cell Screen:
Engineered epitopes &
CD3 binding &
Ab expression

Epitope-Steered Immunization Identifies T Cell Binders – Some With Cyno Cross-Reactivity

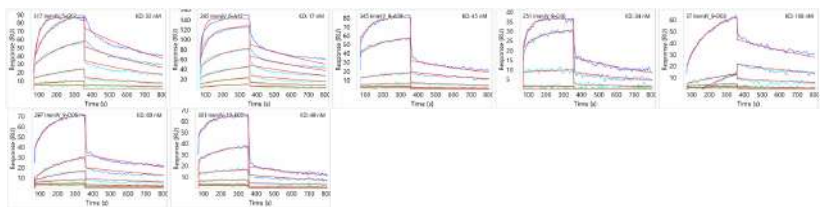
HT-SPR Screen
Hu & Cyno CD3 Binding

HT-Flow Cytometry Screen
Hu T Cell Binding

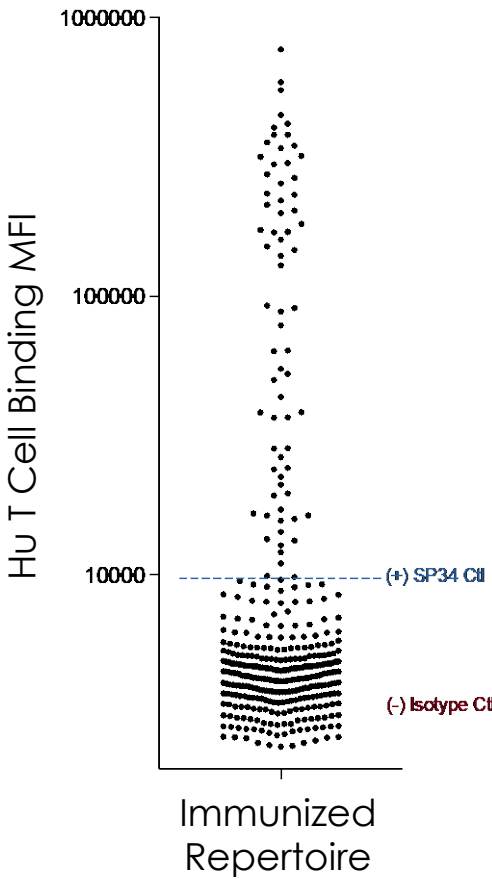
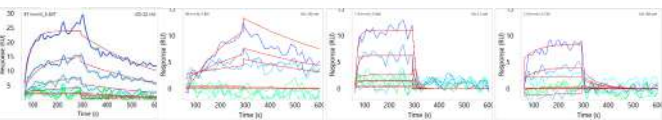
Hu CD3ED
KD: 3 - 100 nM



Hu CD3EG
KD: 17 - 100 nM



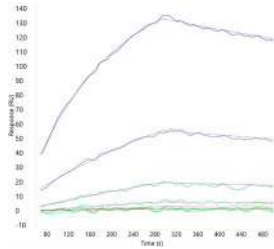
Cyno CD3ED
KD: 20 - 100+ nM



StableHu Optimization of Anti-CD3 Template Antibodies

Starting with anti-CD3 Ab
template

KD =
27 nM



Template Ab



AI-model predicts
human CDRs

HCDR1

2000

HCDR2

2000

HCDR3

2000

LCDR1

2000

LCDR2

1000

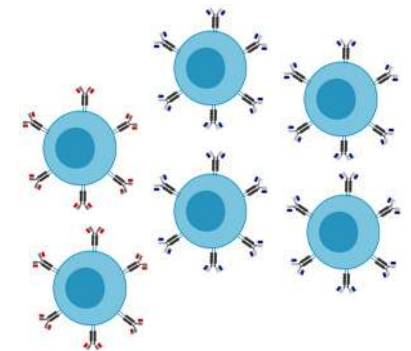
LCDR3

2000

Per-CDR
mammalian
display libraries

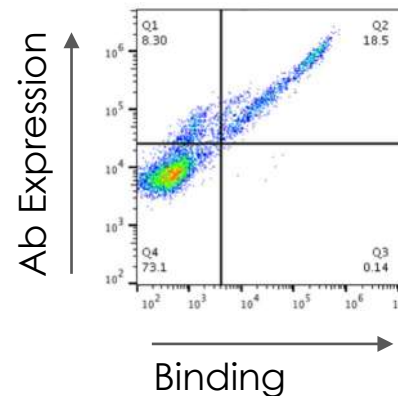


Single-cell sorting:
binding & expression



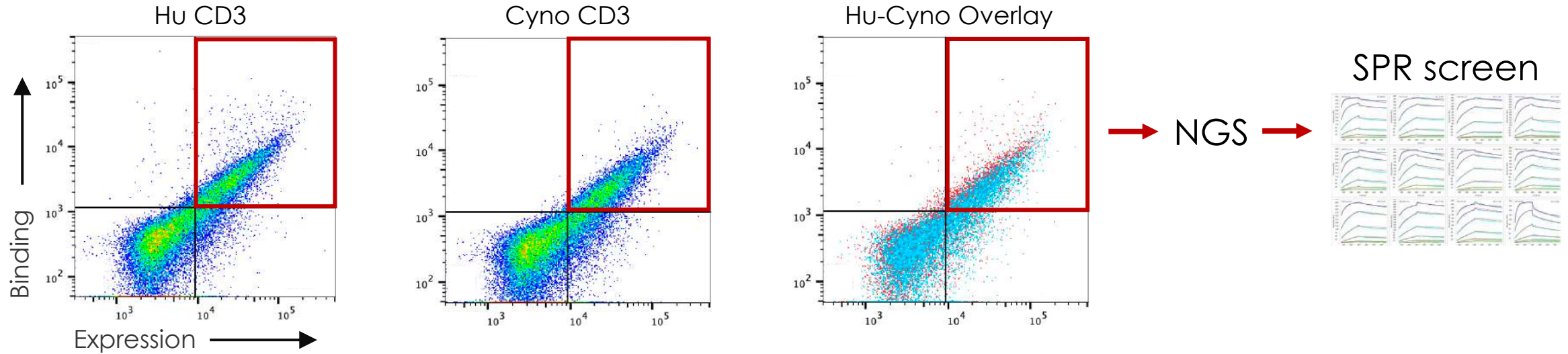
HT-SPR hit validation
and quantitation

NGS

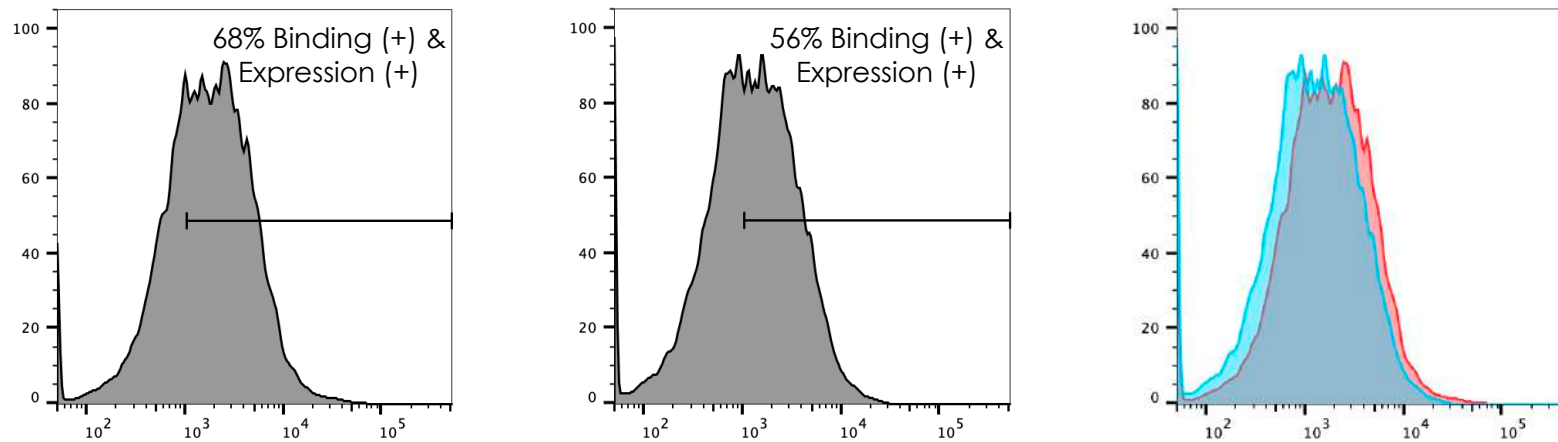


StableHu Generates Hu-Cyno Cross-Reactivity Library from Anti-CD3 Template

Cell Sorting of Pooled Single CDR Libraries



High-expression & binding double-positive distributions

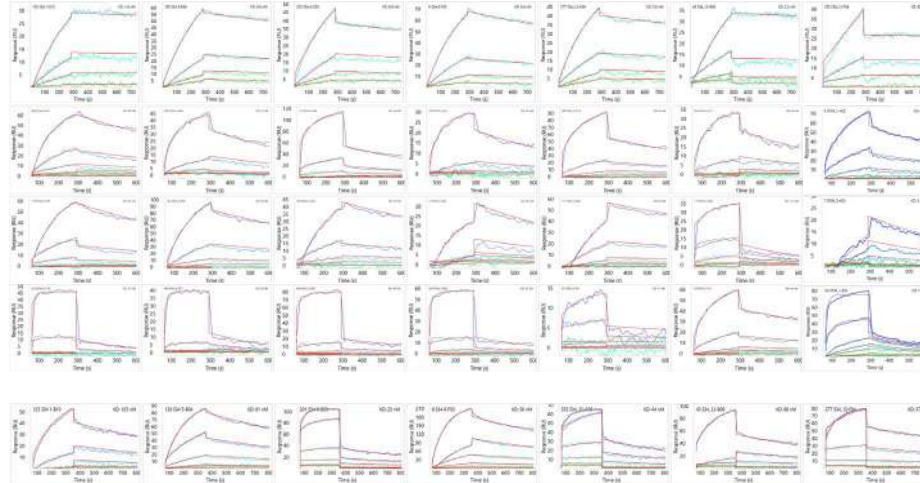


Hu-Cyno CD3 binding distributions significantly overlap

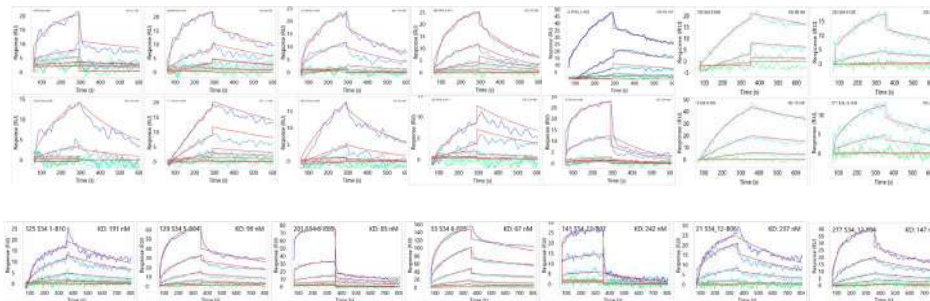
StableHu Identifies T Cell Binders – Some With Cyno Cross-Reactivity

HT-SPR Screen Hu & Cyno CD3 Binding

Hu CD3ED
KD: 2 - 100 nM



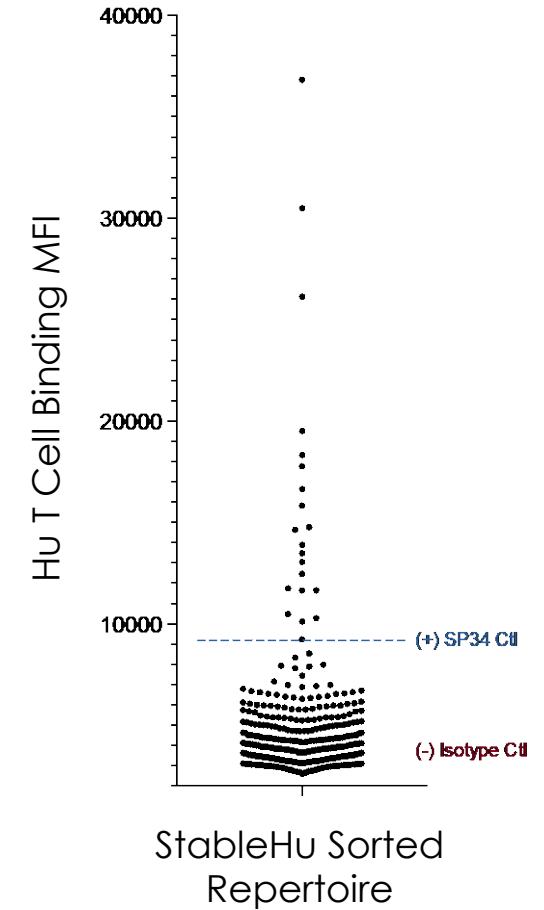
Hu CD3EG
KD: 20 - 100 nM



Cyno CD3ED
KD: 15 - 100 nM

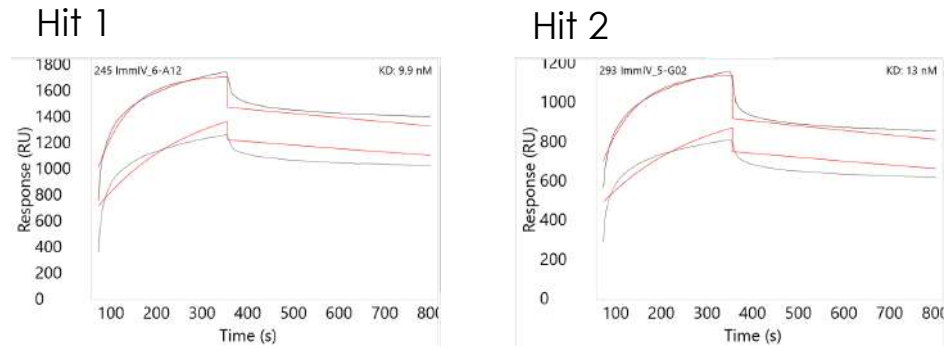
Cyno CD3EG
KD: 67 - 200 nM

HT-Flow Cytometry Screen Hu T Cell Binding

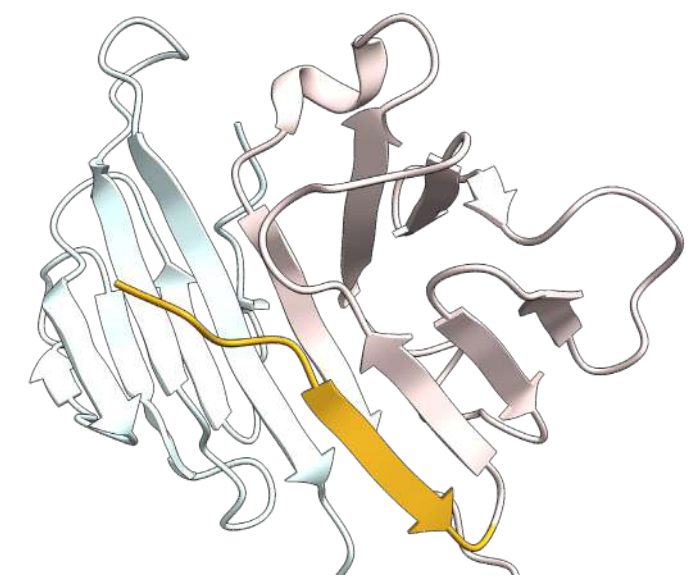
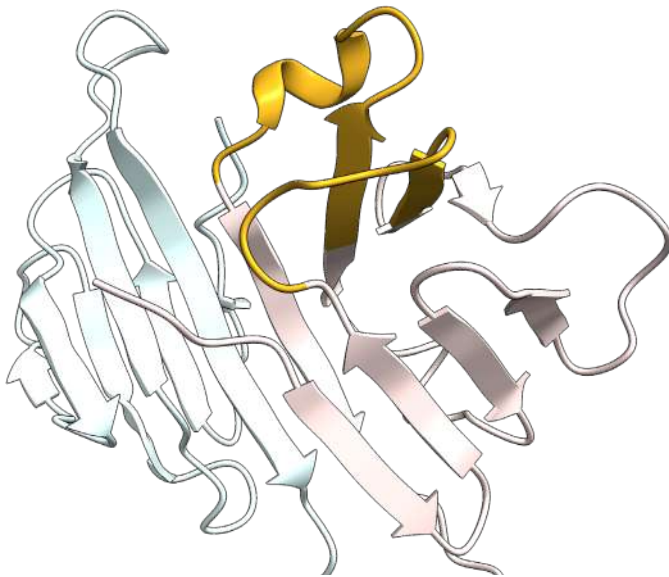
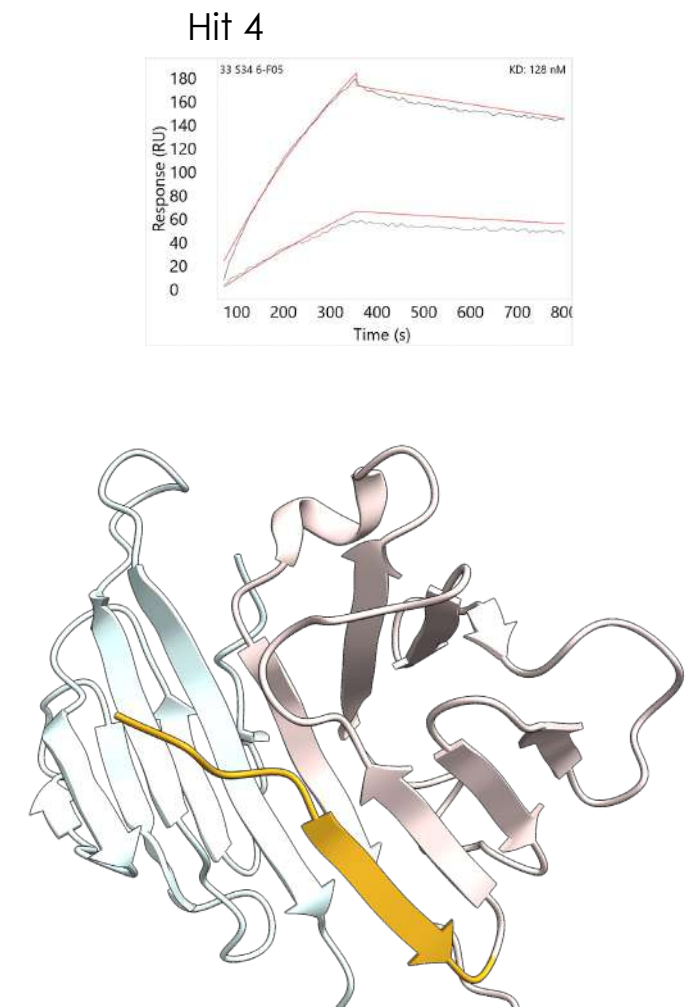


CD3 Antibody Hits – Epitope Mapping by Engineered Epitope SPR

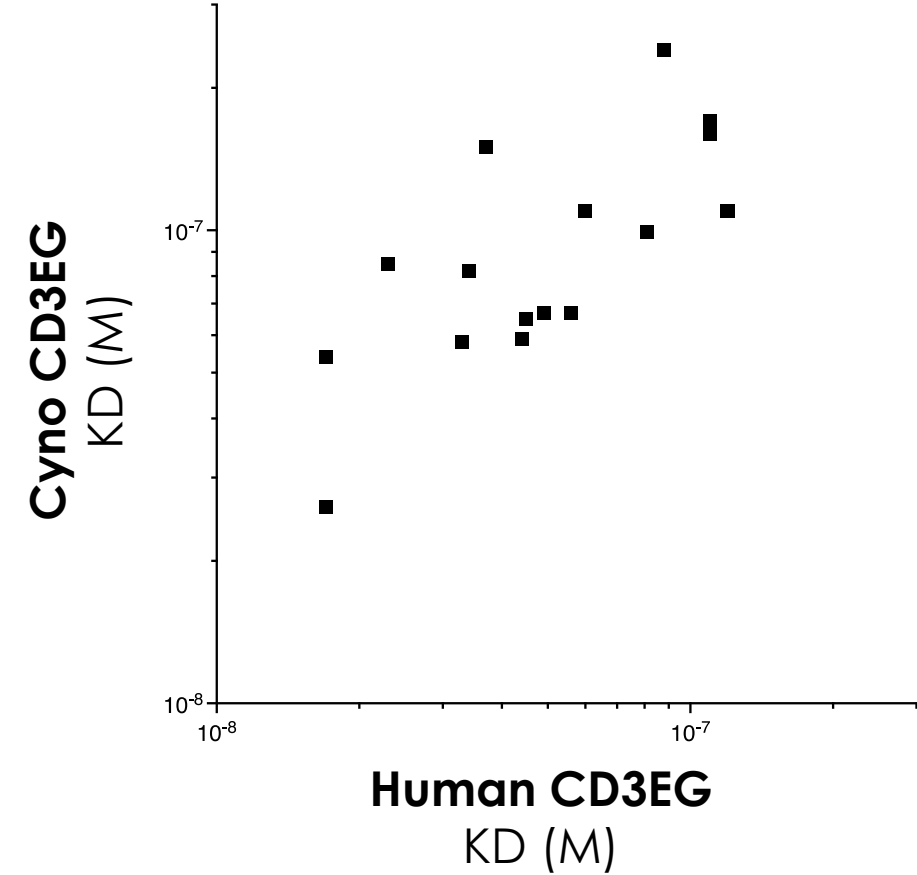
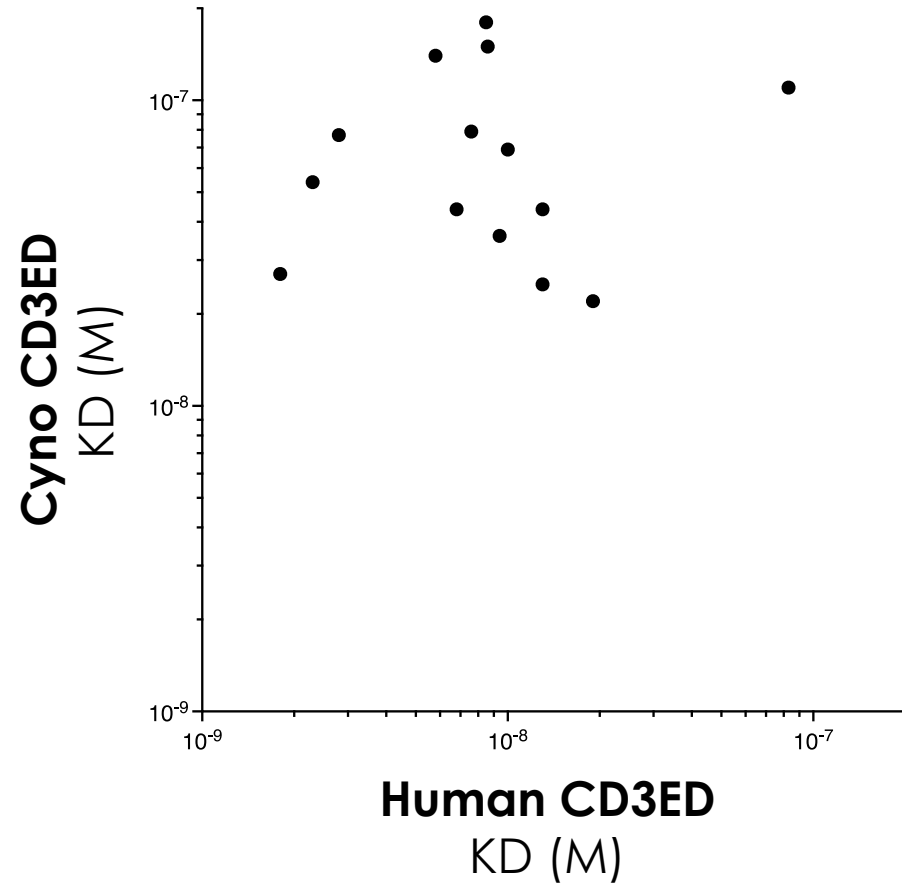
Epitope 1



Epitope 2



Diverse Hu-Cyno CD3 Cross-Reactive Antibodies Identified from Multiple Library and Screening Tracks



Conclusions

AI Combined with HT-Screening Can Efficiently Discover Traditionally-Challenging Antibodies

AI-engineered epitope steering facilitates next-gen antibody targets:

- Challenging targets and MOAs
- Per-epitope target biology exploration

AI-generated fully-human antibody libraries reduce downstream risks:

- Improved sequence humanness
- Broad sequence and activity hit set from a template

HT-screening with SPR and flow cytometry enhances AI development:

- Kinetic & affinity dimensions for AI model training and hit selection
- Data scale for AI model refinement & development

Thank You

iBIO
San Diego
team

