



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

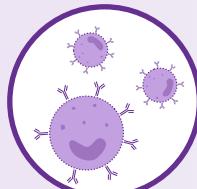


- Patented* epitope engineering
- AI-engineered epitope preserves target structure

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



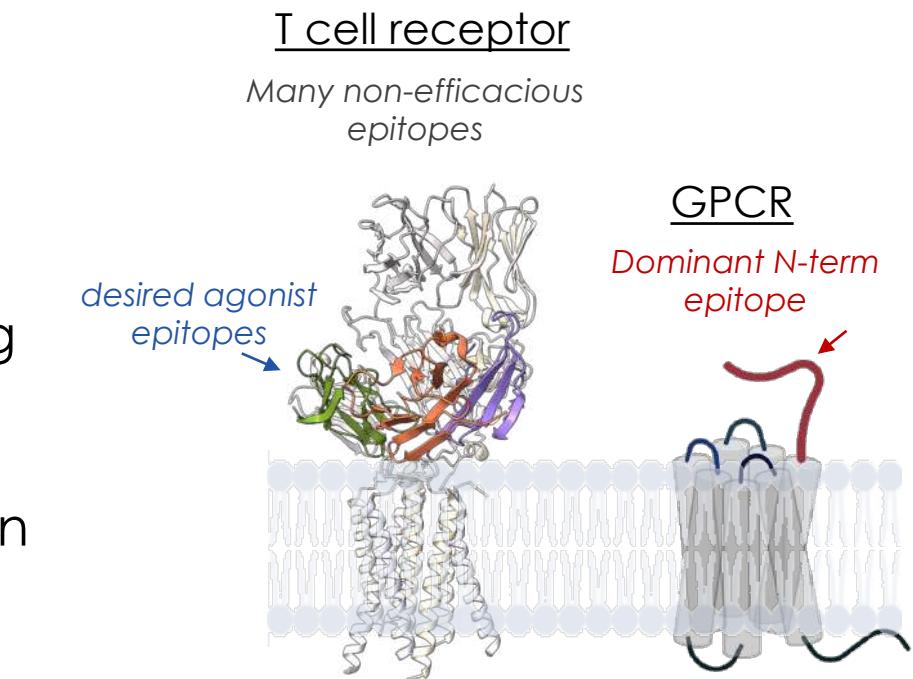


Epitope-Targeted Antibody Discovery

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

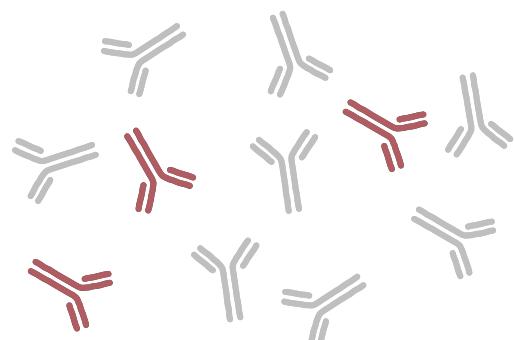
(4) Trkulja et al., Sci. Adv. (2021) 7:16, p.eabe6397

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

Engineered Epitopes Focus Antibody Repertoires On Desired Binding Sites

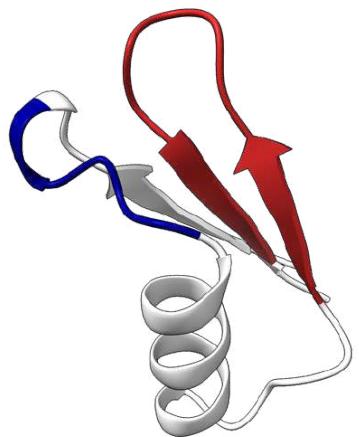
1

Naïve in vivo or in vitro antibody library



2

Focus library with engineered epitopes

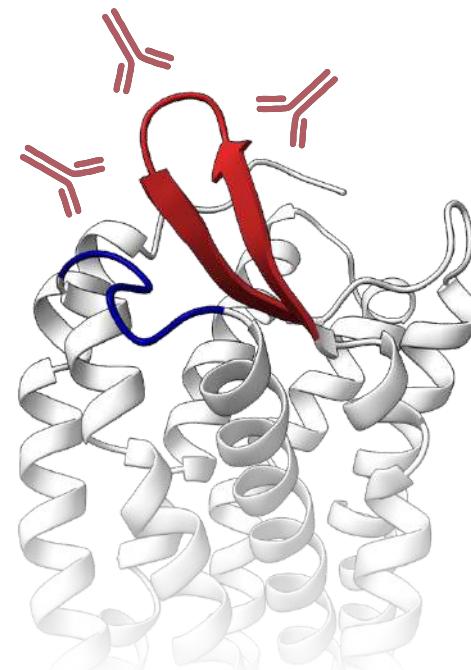


■ epitope-specific Ab

■ epitope
■ *de novo* scaffold

3

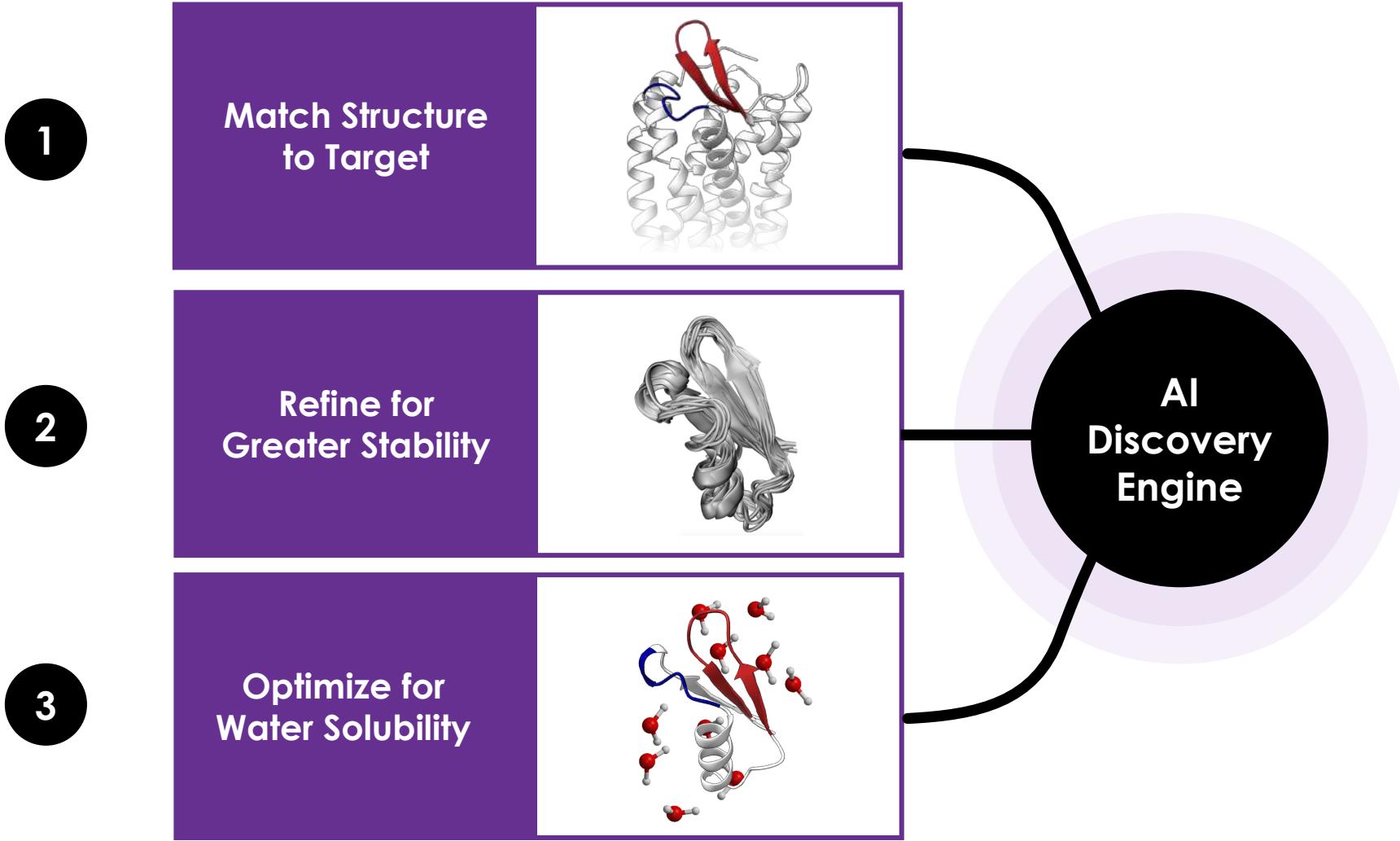
Efficient discovery of epitope-specific Abs



full length target

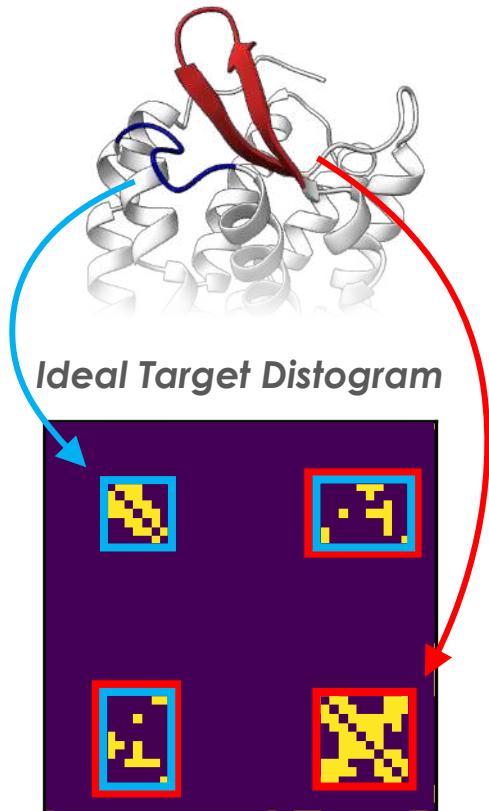
AI-Engine Optimizes Engineered Epitope Structure, Stability, and Solubility

Engineered
Epitope
Design
Objectives

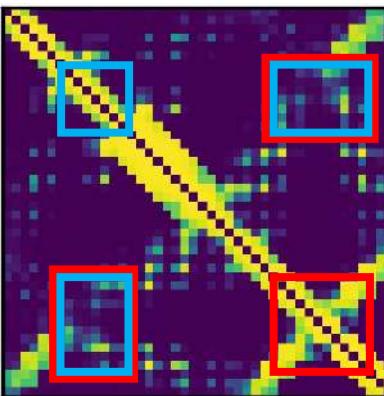


Multi-Loss Function Enforces Engineered Epitope Structure Match to Target and Overall Stability

Loss Term #1



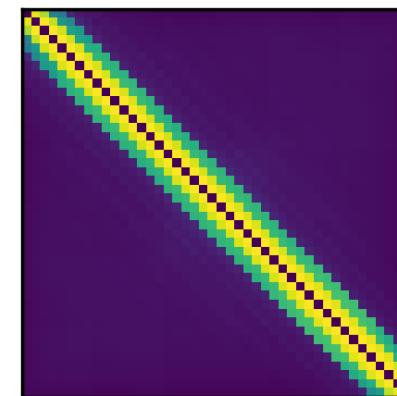
Predicted Distogram



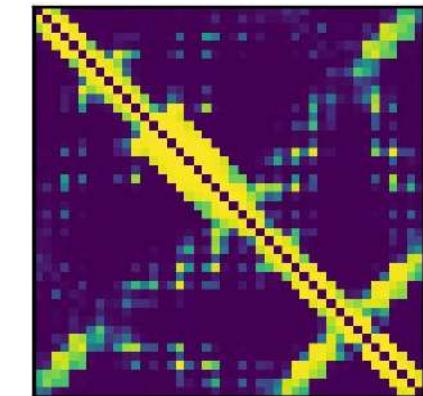
Loss Term #2

Average predicted distograms across random sequence space

Random Coil Distogram



Predicted Distogram

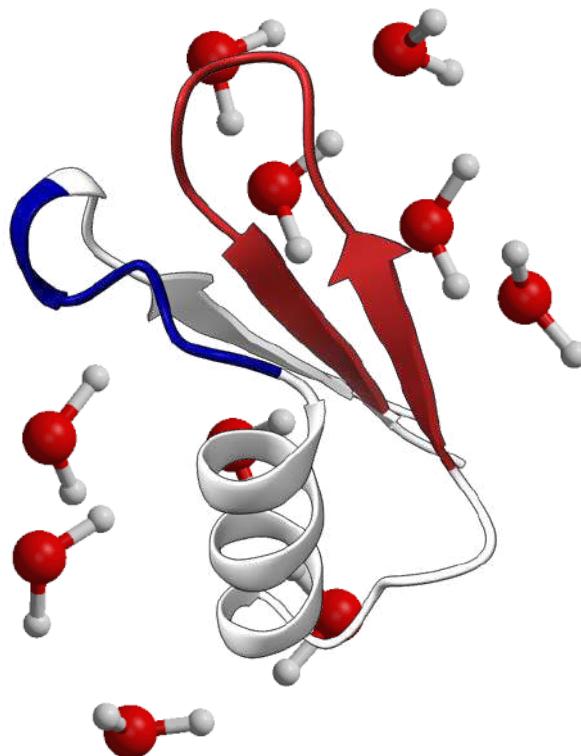


Minimize Cross-Entropy between engineered & target epitope residues

Maximize KL-Divergence between unstructured coil and engineered epitope

Multi-Loss Function Optimizes Engineered Epitope Solubility

Loss Term #3

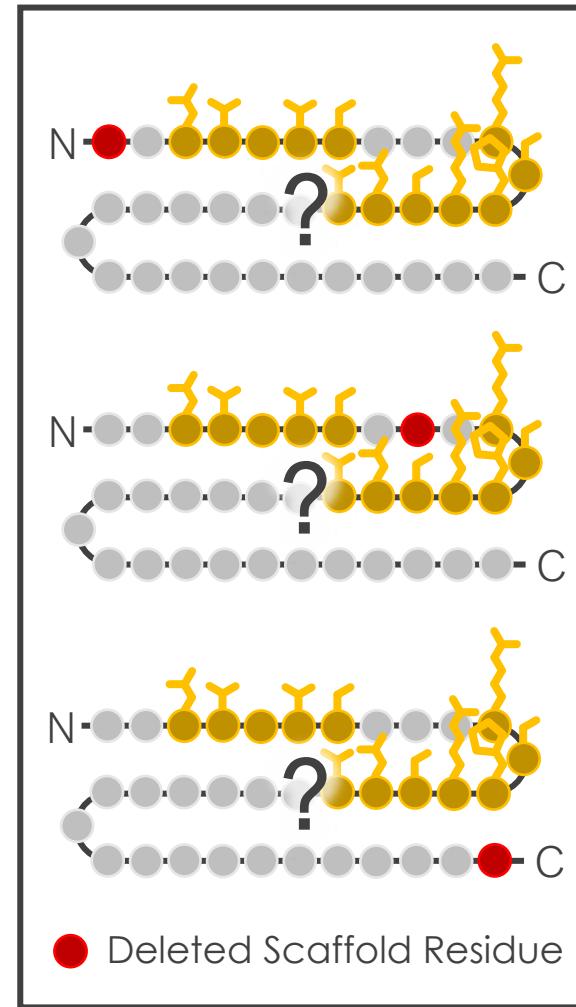
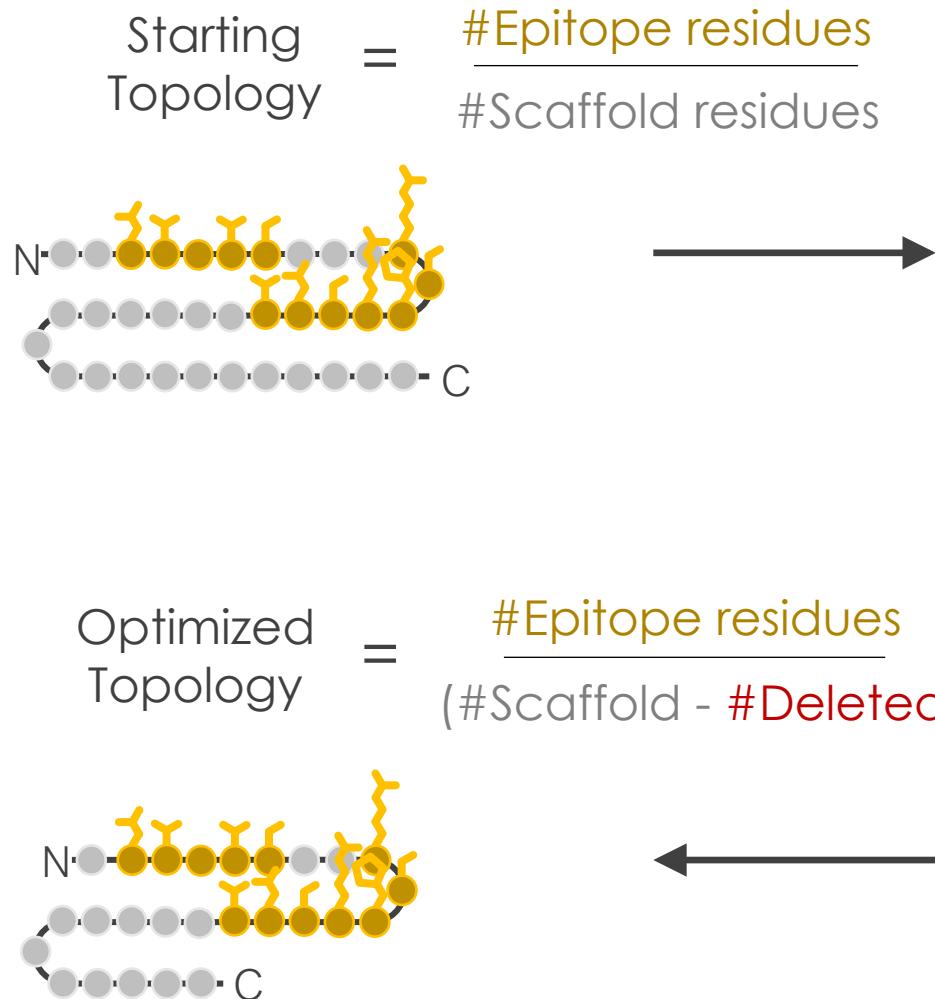


Amino Acid Hydropathies

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

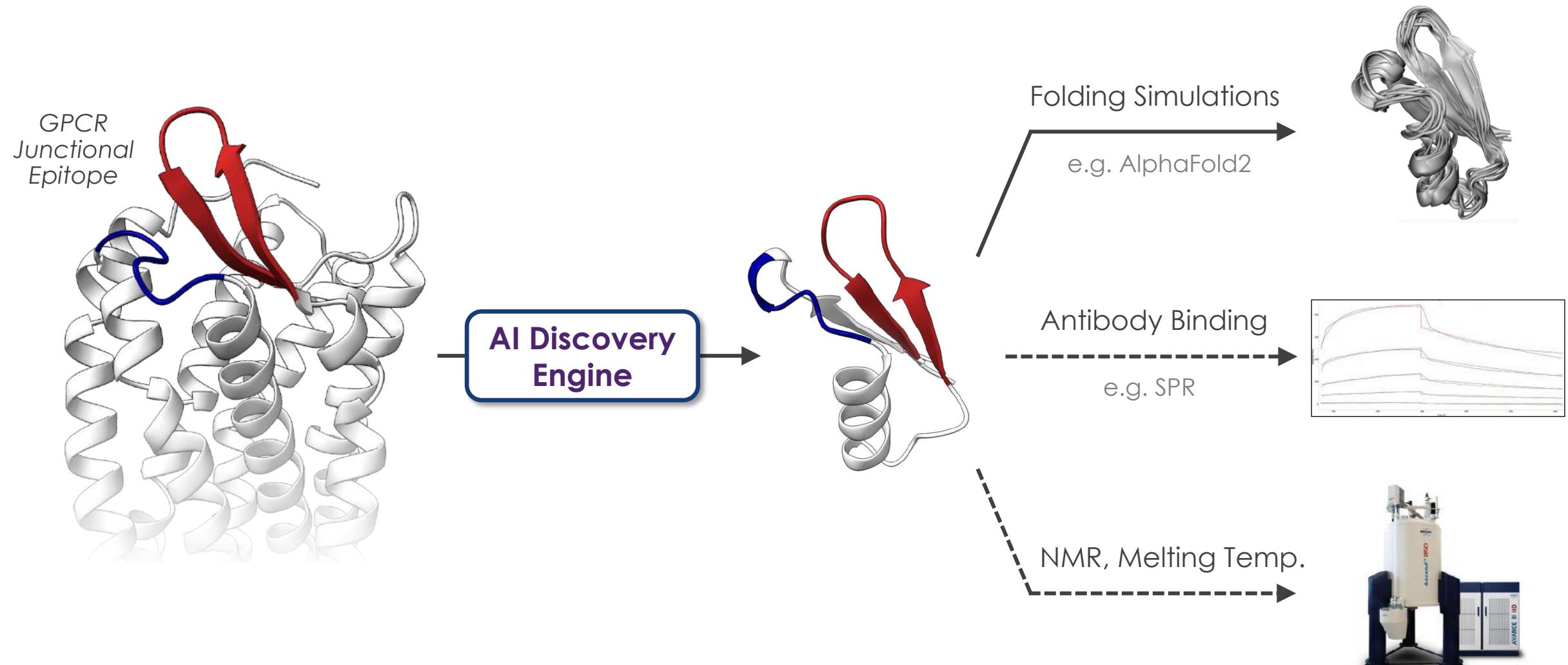
Average hydropathy is minimized

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



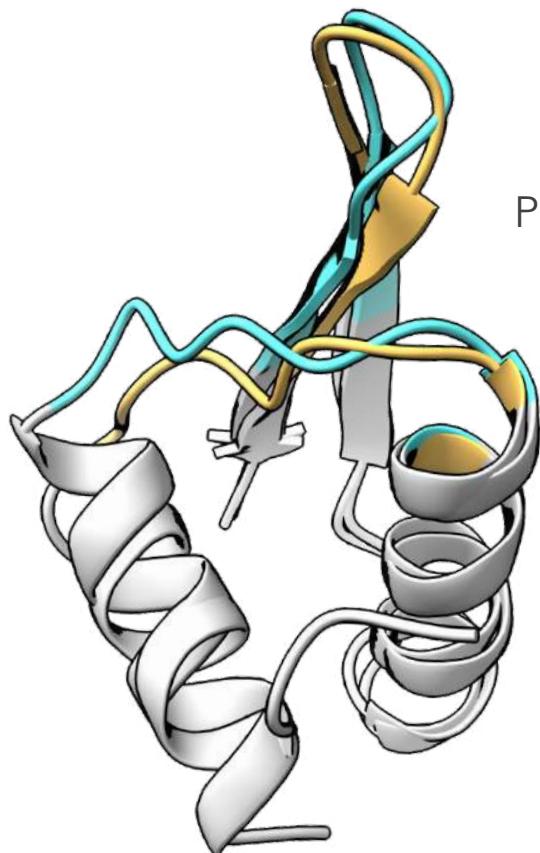
Iteratively trim scaffold residues until epitope destabilizes

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

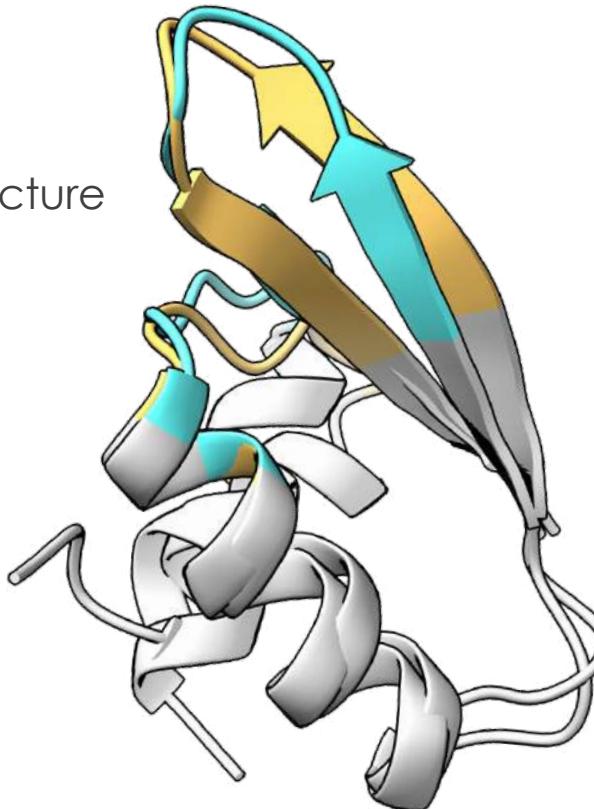
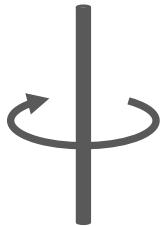


NMR Structure Validates Engineered Epitope Design Engine

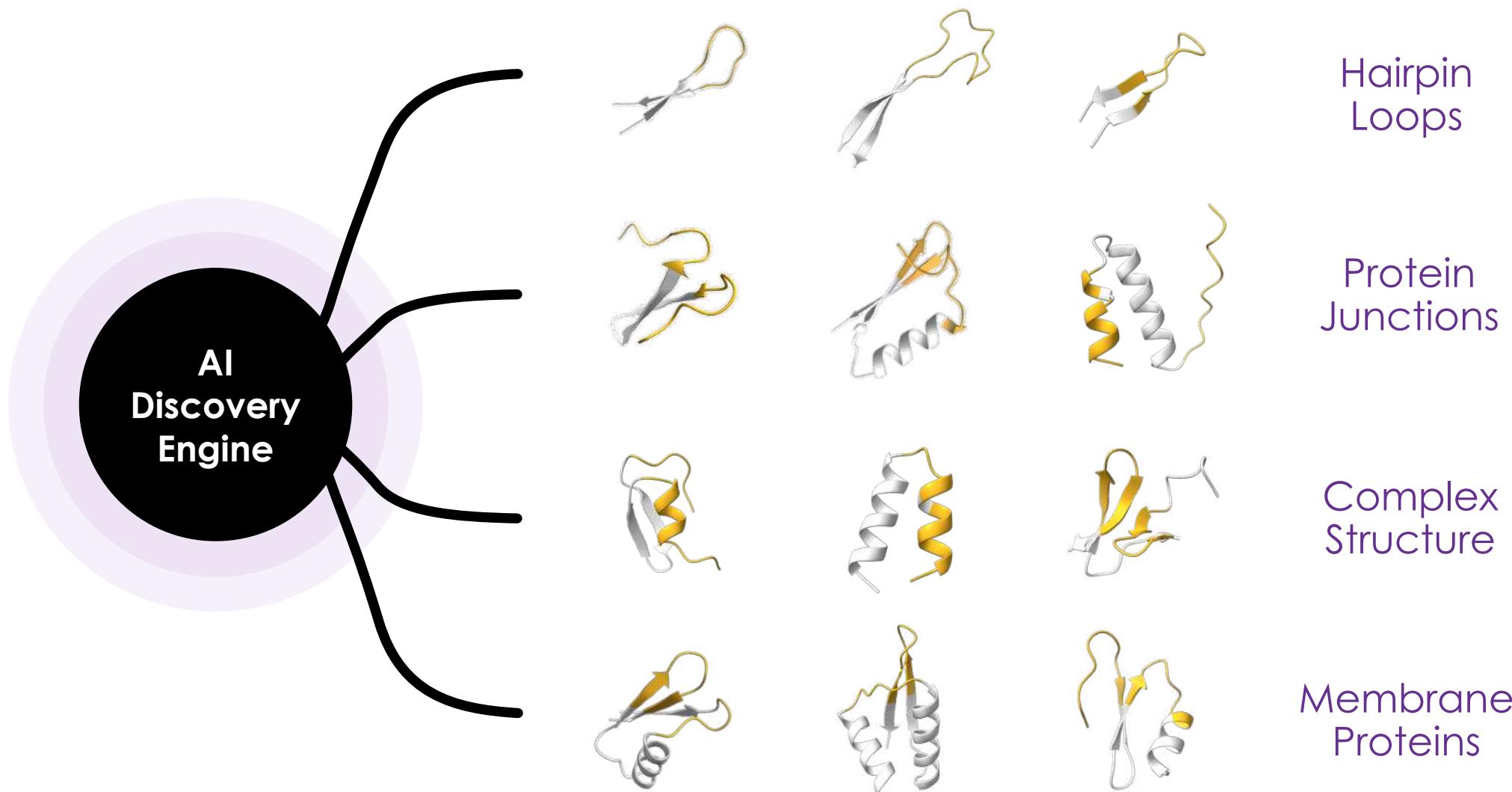
— NMR Solved Structure
— Engineered Epitope Engine



Predicted vs. Actual epitope structure
Backbone RMSD: **1.6 Å**

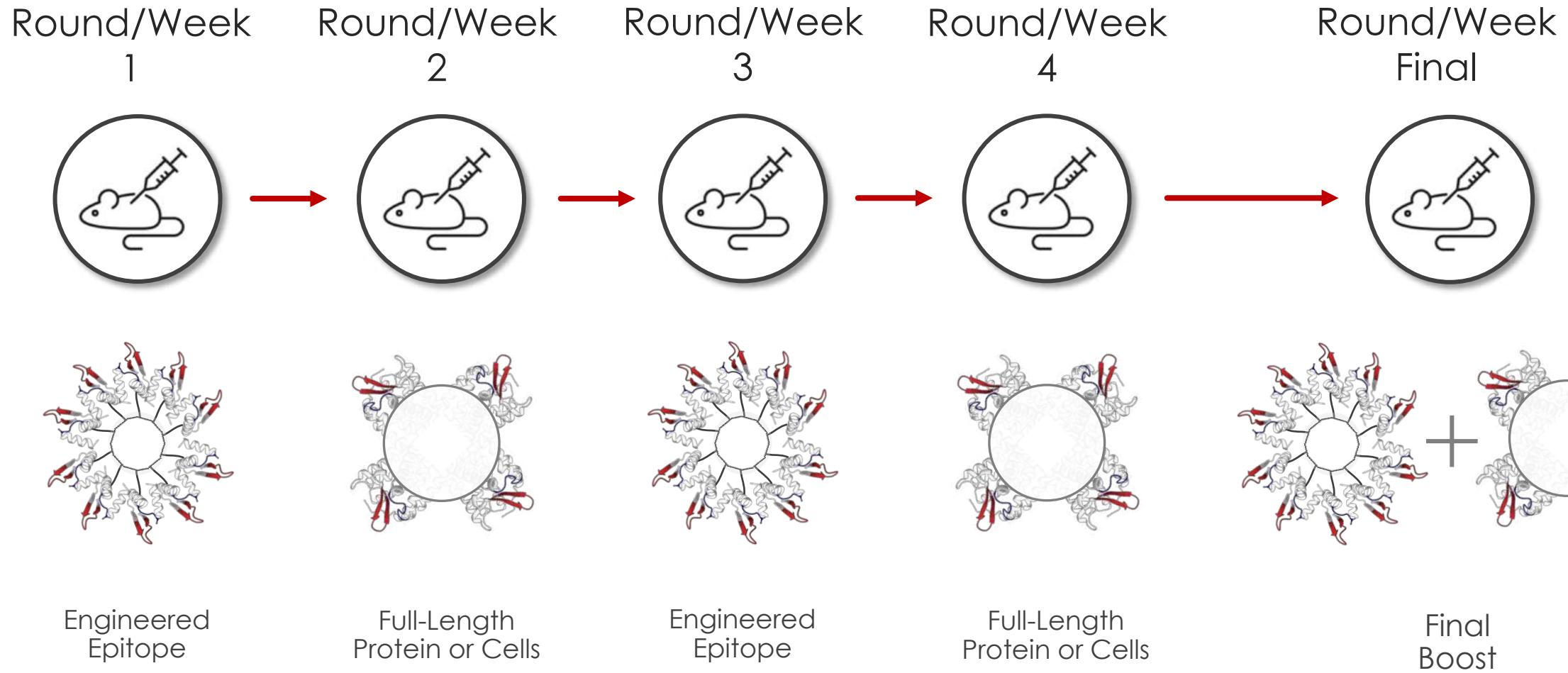


Engineered Epitopes Are Generalizable to a Broad Set of Targets



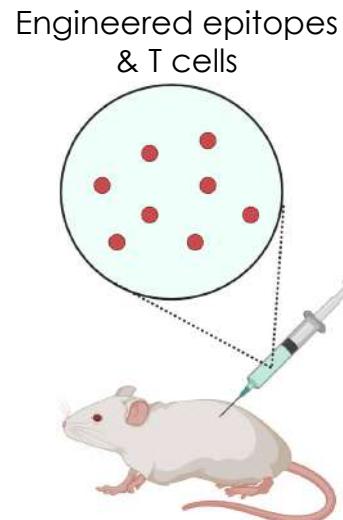
Engineered Epitopes Steer Immunization and In Vitro Libraries to Target Epitopes

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

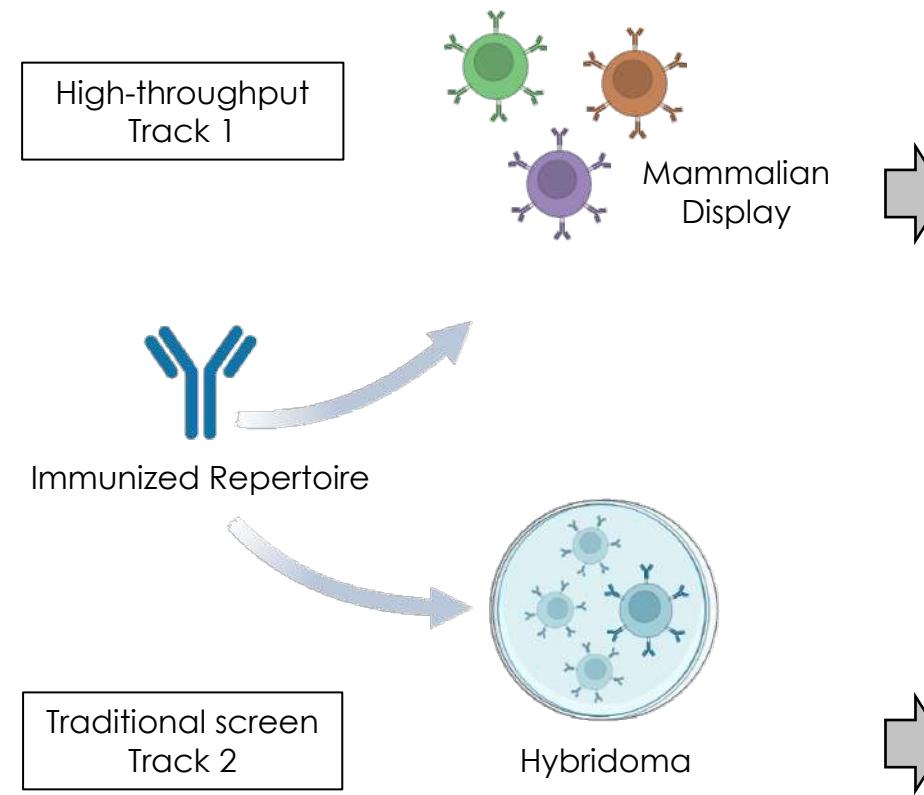


Immunized Repertoires Are Cloned and Screened Via Two Tracks

Multi Epitope-Steered Immunization

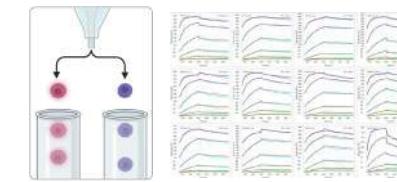


Dual-Track Library Display



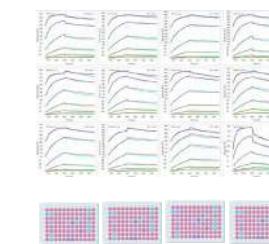
Multi Dimension Screening

FACS, NGS & SPR



Engineered epitopes & target binding & Ab expression

SPR & ELISA



Engineered epitopes & target binding

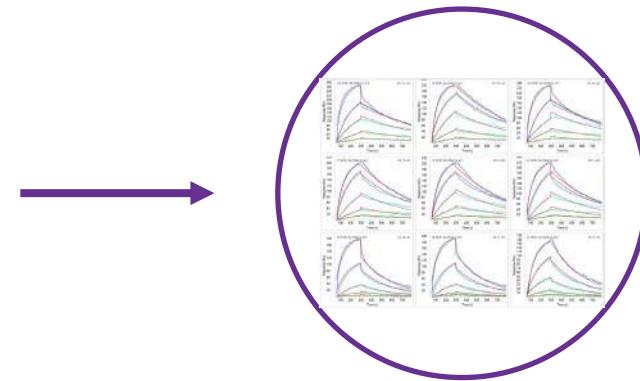
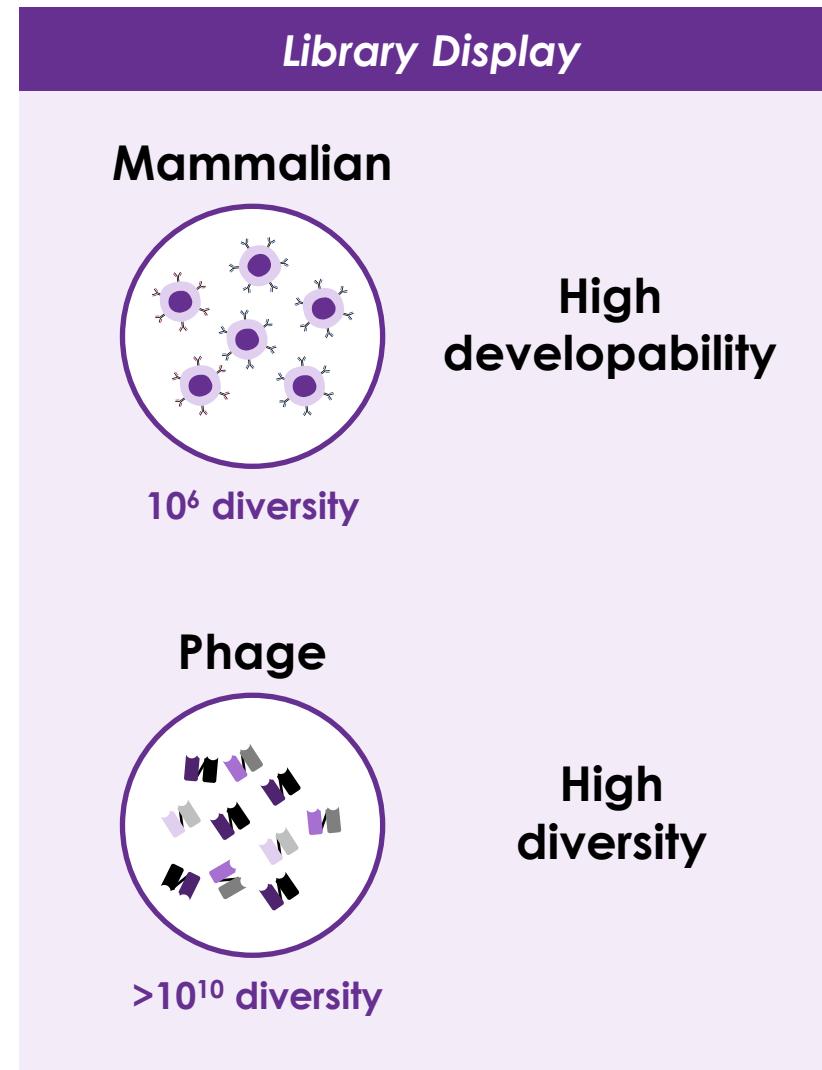
High Developability, Human Diversity Antibody Libraries

Naïve In Vitro Library Uses Human Diversity to Minimize Immunogenicity Risk

Learn diversity from
cAb-Rep & OAS Hu
Ig databases

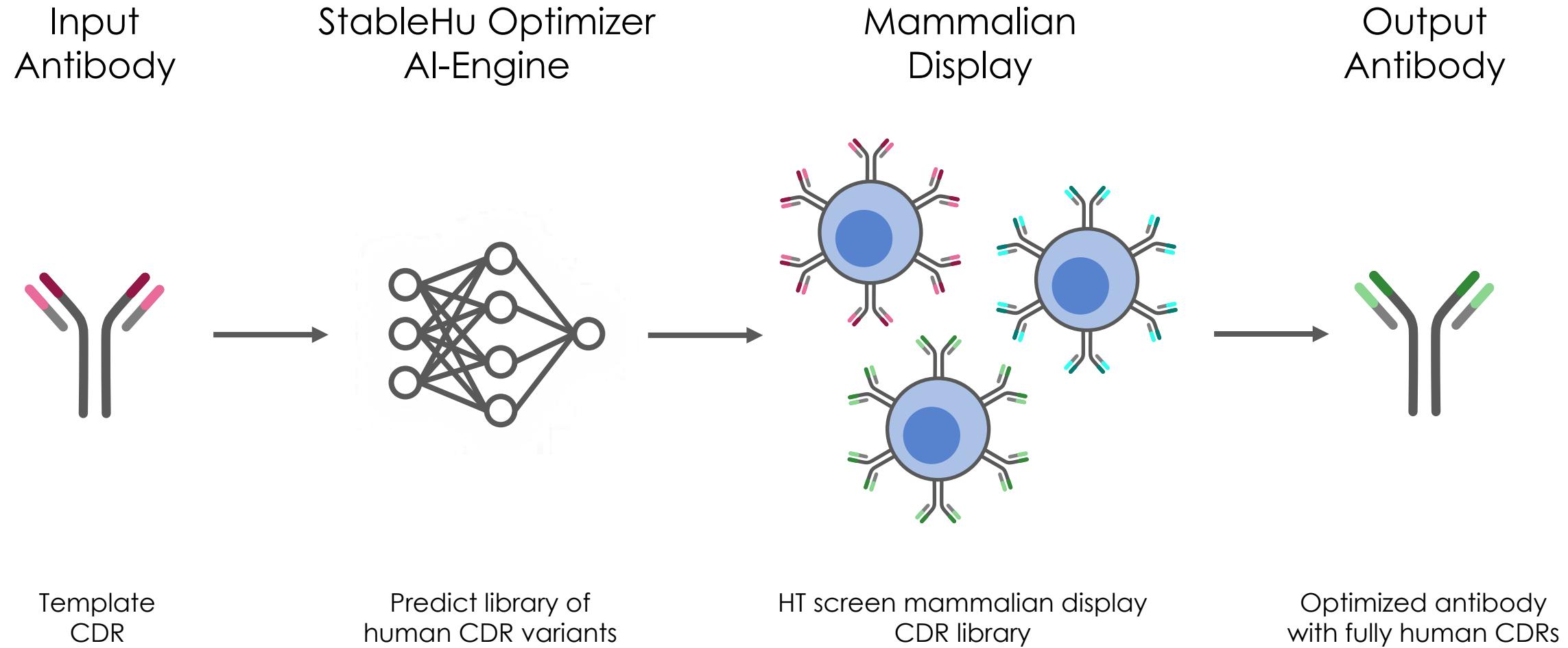


Extract CDR
sequences for
clinically validated
frameworks



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

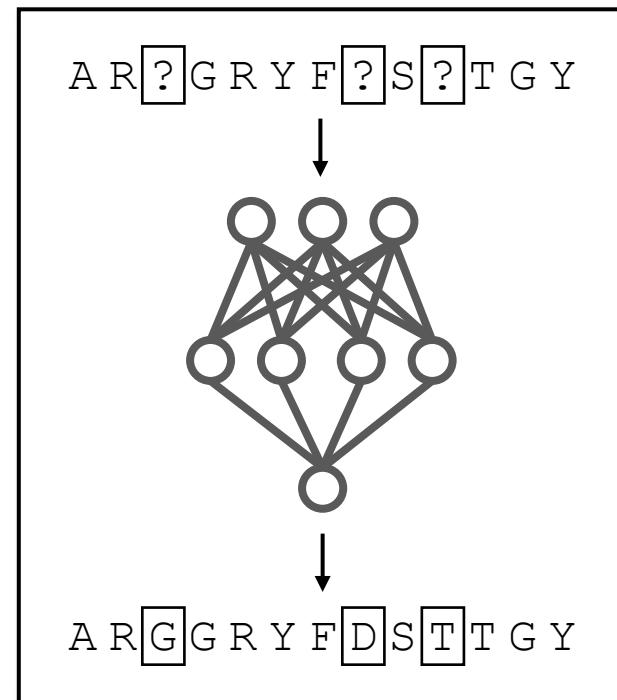
Antibody Database

cAb-Rep & OAS
Hu Ig databases



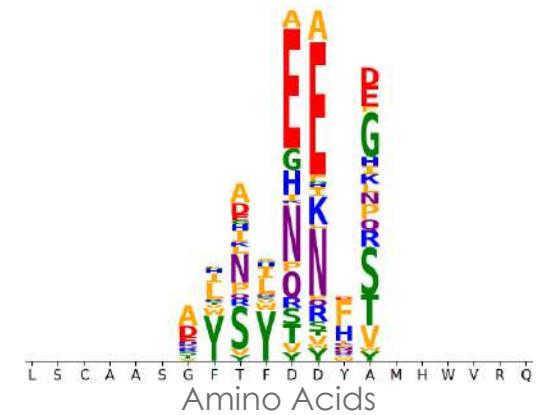
>1 billion curated
human antibody
sequences

Optimizer AI



AI trained to predict
fully human CDR from masked CDR

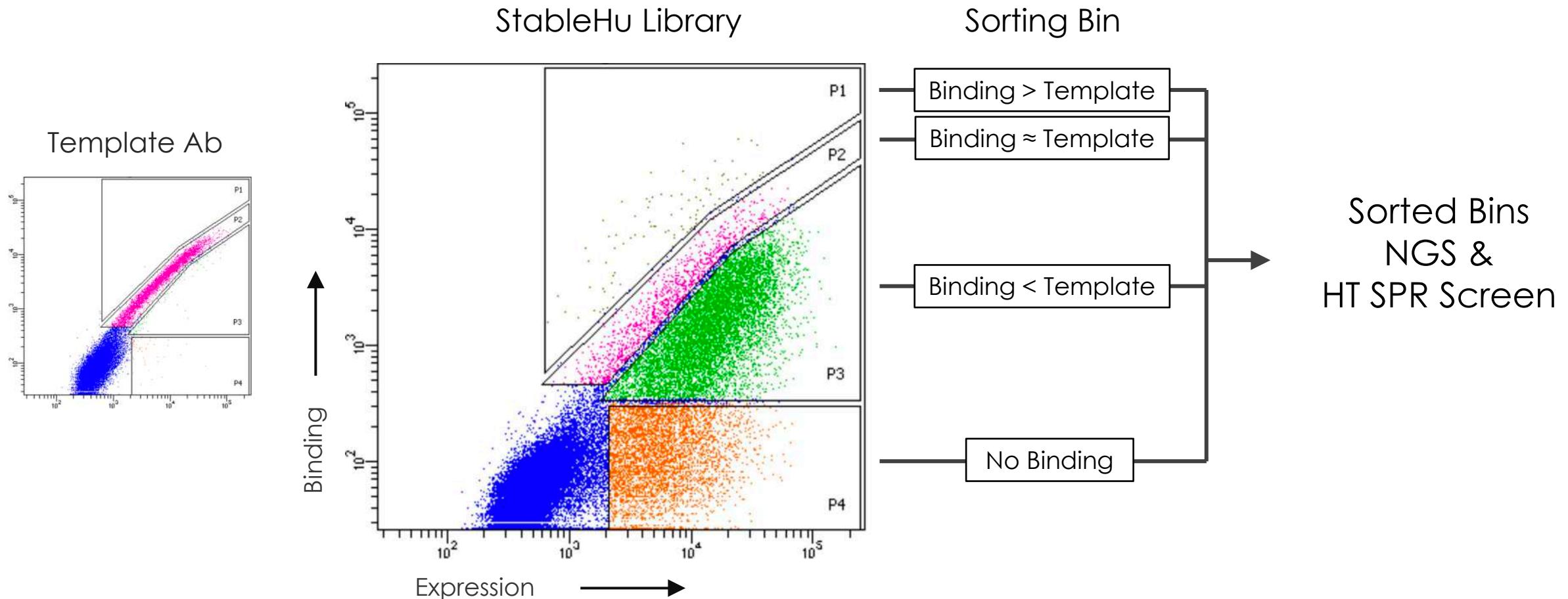
Trained Model



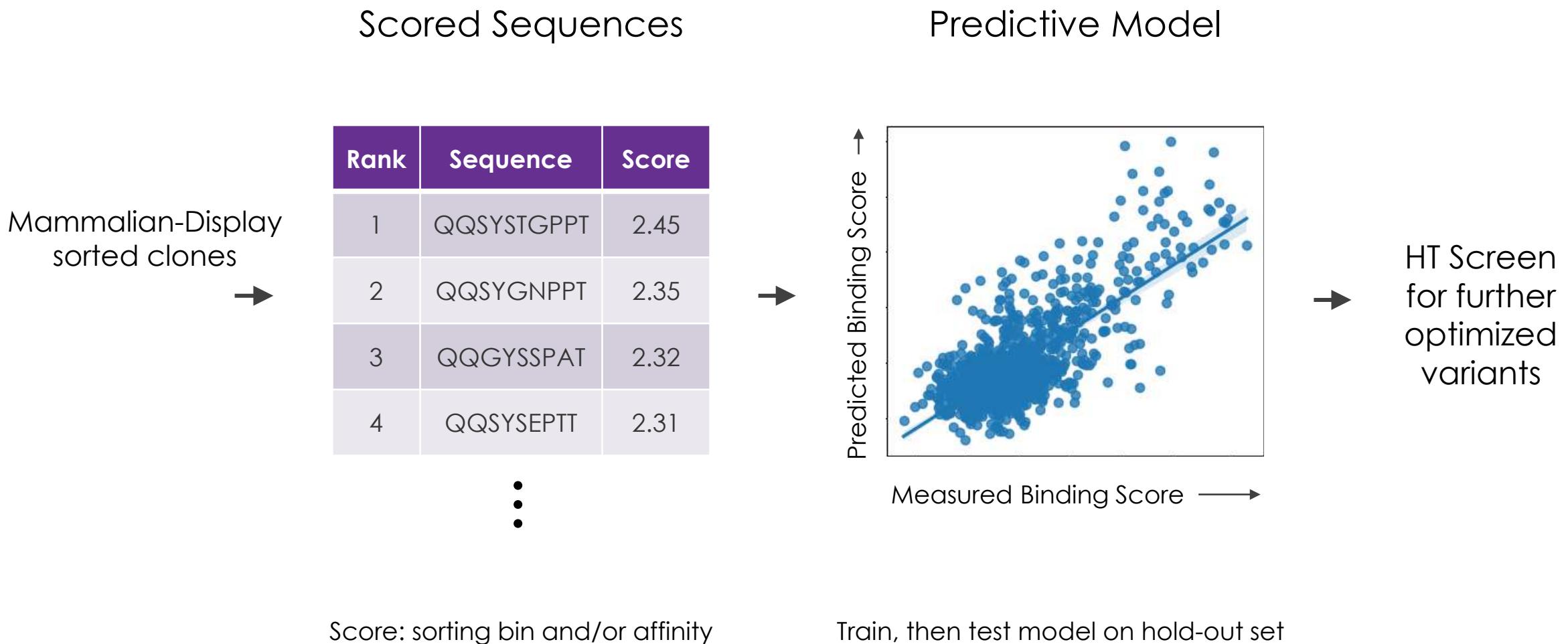
Predict library of fully
human CDRs from
template CDR

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



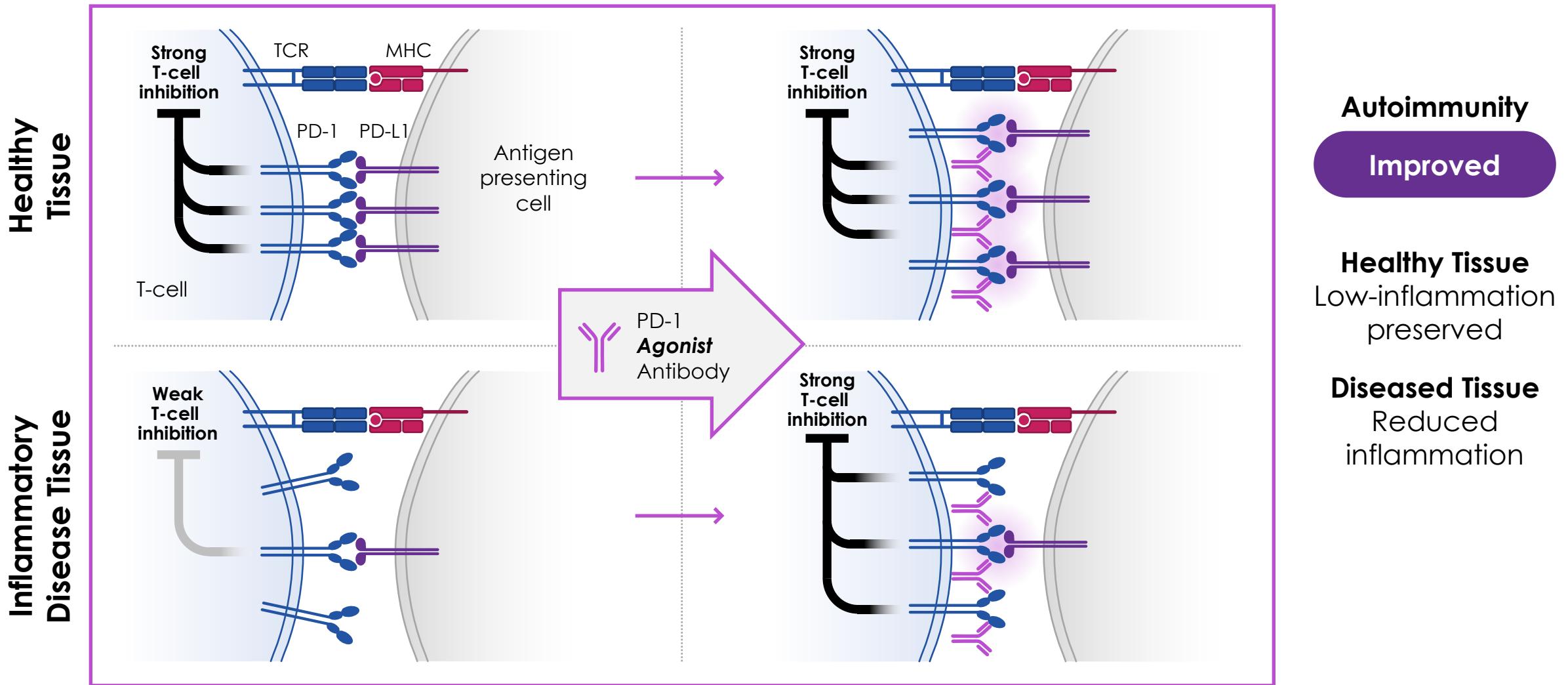


Technology Stack Use Cases

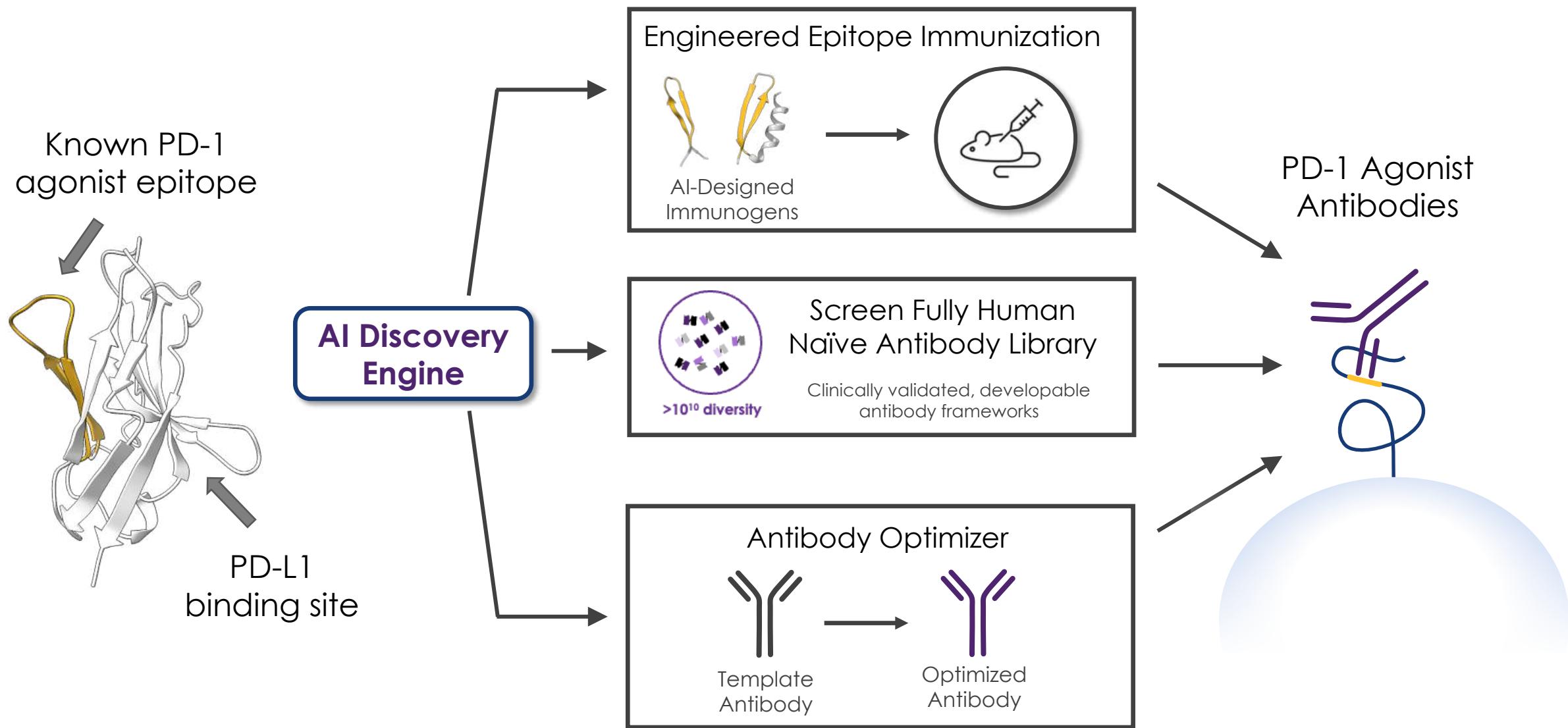
Agonist Epitope

PD-1 Checkpoint Agonist Antibody

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

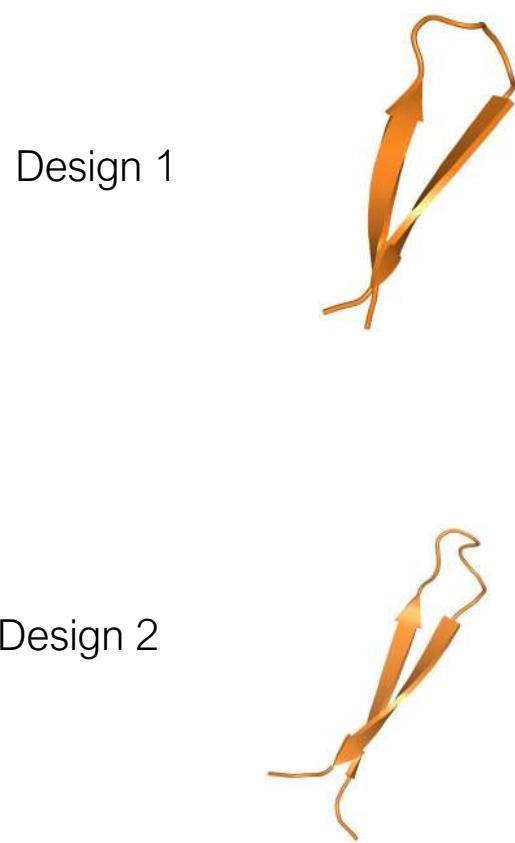


Parallel Paths to PD-1 Agonist Antibody Discovery

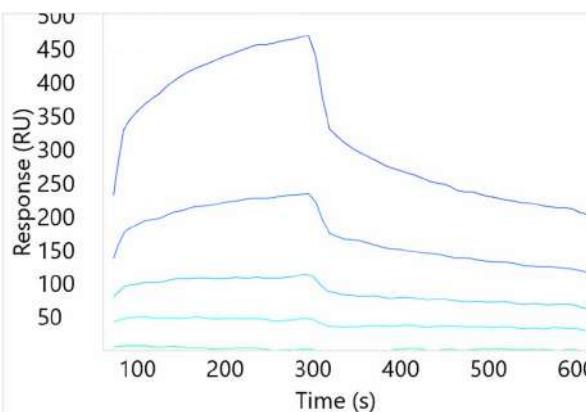
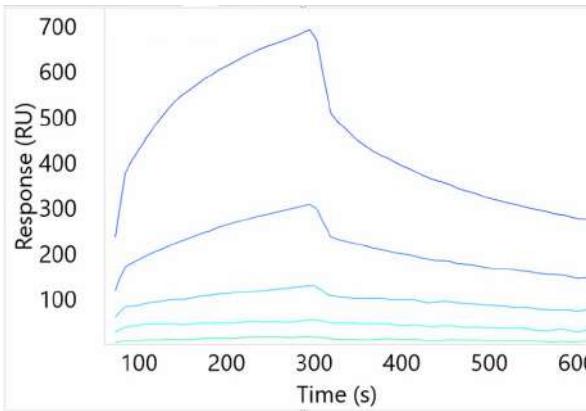


Engineered Epitopes Are Validated By Binding to a Known Antibody or Ligand

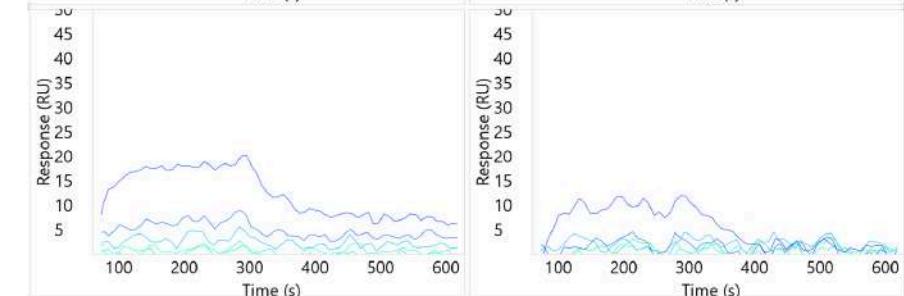
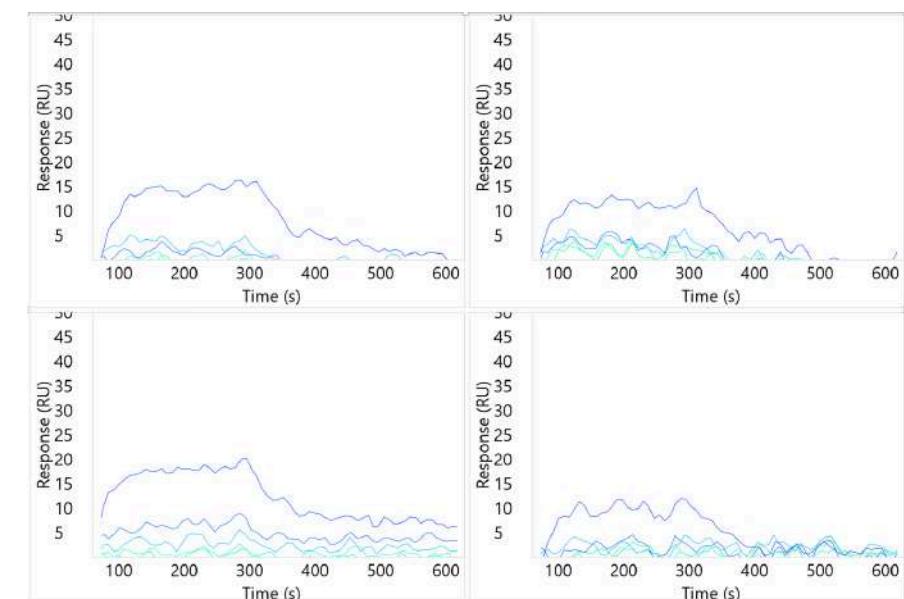
Benchmark PD-1 Agonist Ab SPR vs. Engineered Epitope Designs



Top PD-1 Agonist Epitope Designs



Ineffective Agonist Epitope Designs



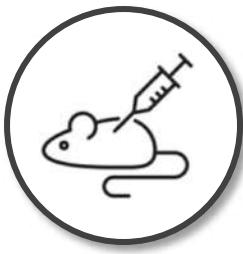
PD-1 Agonist Engineered Epitope Steered Immunization and In Vitro Libraries

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

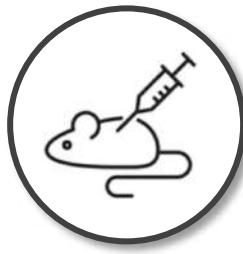
Week 1



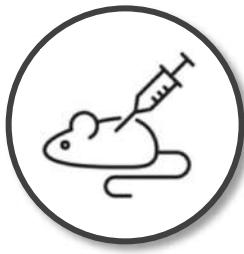
Week 3



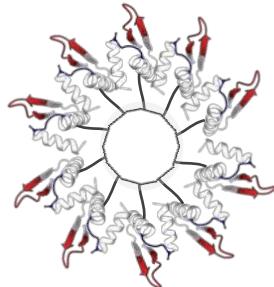
Week 5



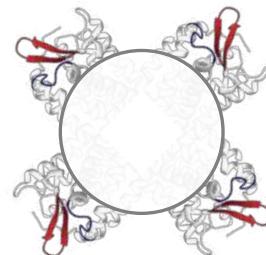
Week Final



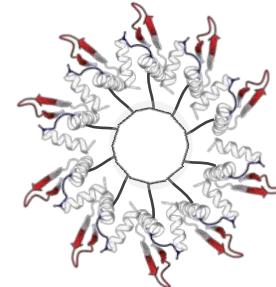
In vitro library generation and epitope-steered panning



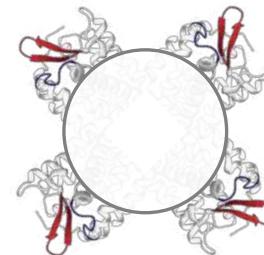
Engineered Epitope



Full-Length PD-1



Engineered Epitope



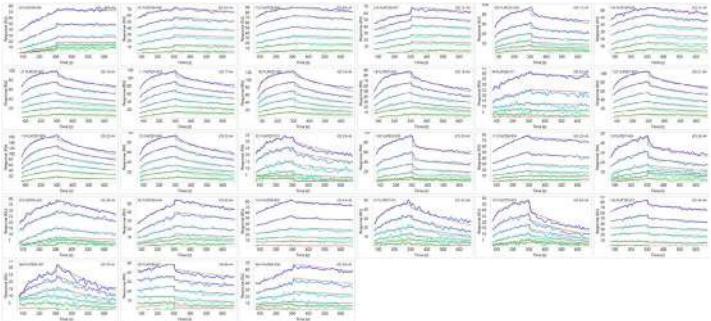
Full-Length PD-1

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

Epitope-Steered
Mostly non-antagonist hits

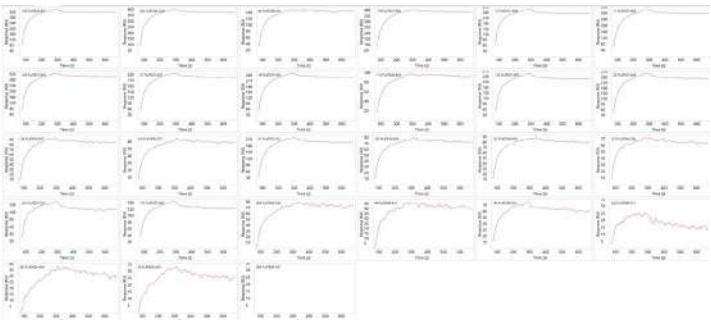
27 PD-1
binding hits
KD: 1 – 80 nM

PD-1 binding HT-SPR



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

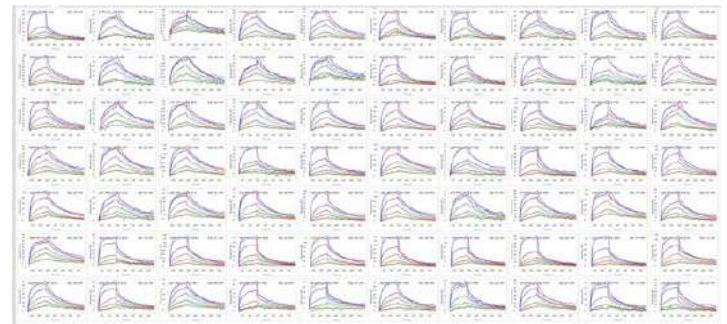
PD-1 antagonist Ab competition HT-SPR



Not Epitope-Steered
All antagonist hits

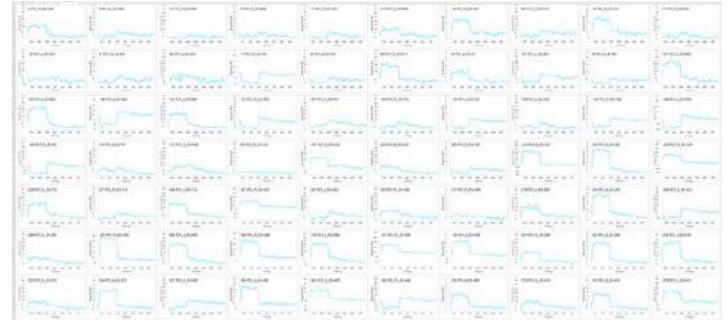
70 PD-1
binding hits
KD: 10 – 80 nM

PD-1 binding HT-SPR



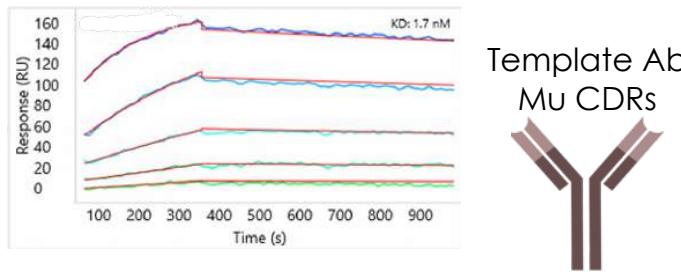
70/70 **do**
compete with PD-1
antagonist Ab

PD-1 antagonist Ab competition HT-SPR



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

Starting with PD-1 agonist
murine CDRs template



KD = 1.7 nM

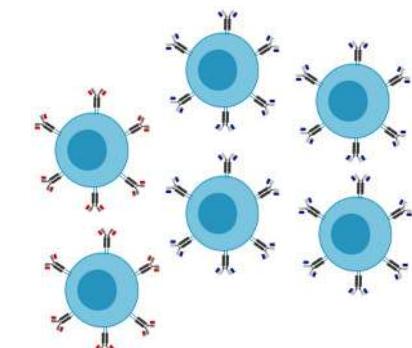
Fully human heavy &
light chain CDRs diversity

<u>HCDR1</u>	<u>HCDR2</u>	<u>HCDR3</u>
4000	4000	4000
<u>LCDR1</u>	<u>LCDR2</u>	<u>LCDR3</u>
4000	2162	4000

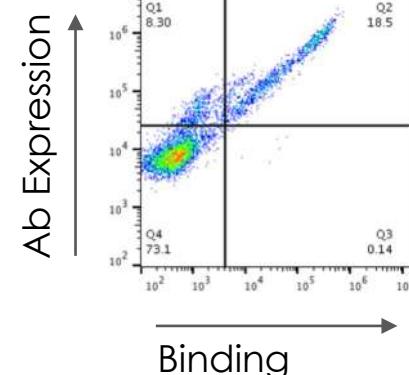
AI-model predicts
human CDRs



Per-CDR
mammalian
display library



Single-cell sorting:
binding & expression



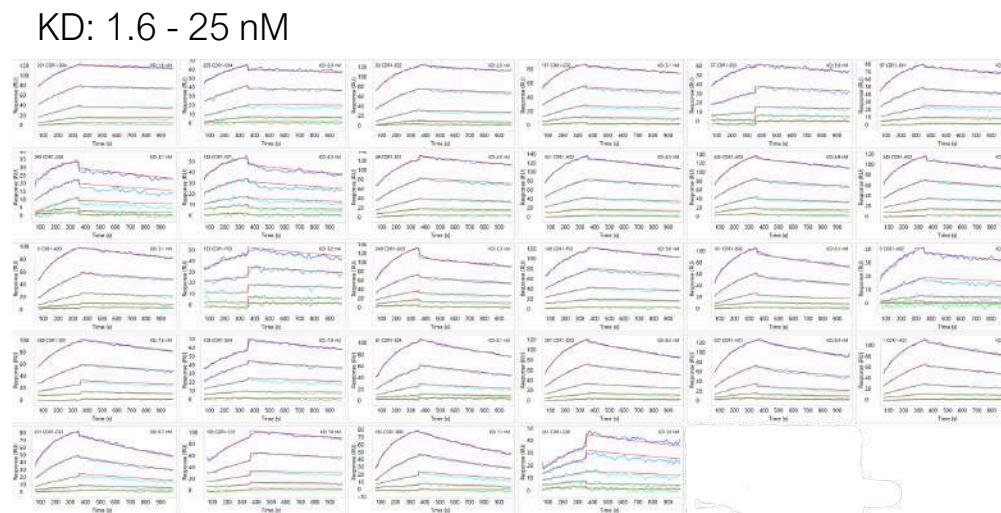
HT-SPR hit validation
and quantitation

NGS

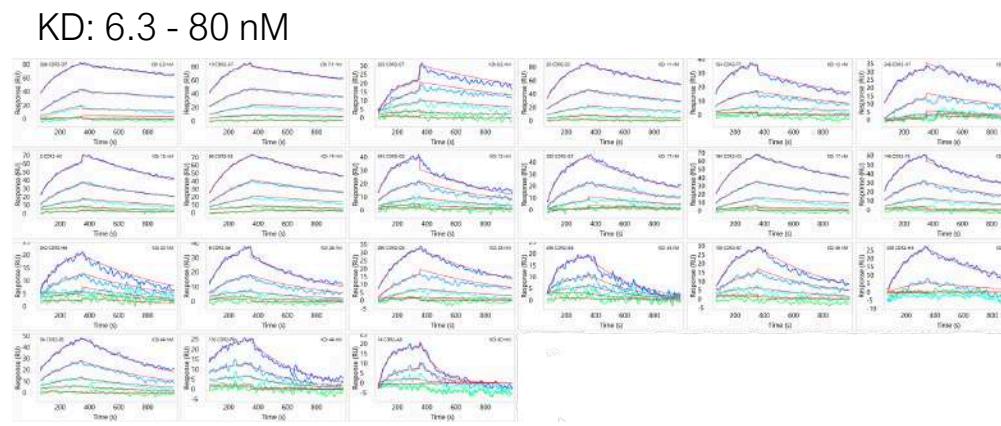


HT-SPR Screen of StableHu Cell Sorts Identifies Fully-Human CDRs That Replace Template Murine CDRs

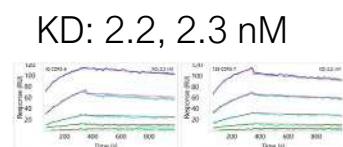
Fully-Human HCDR1:
28 hits



Fully-Human HCDR2:
21 hits



Fully-Human HCDR3:
2 hits

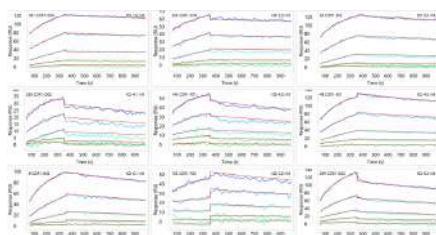


Many fully-human
LCDR1, 2, 3
hits identified

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

Starting with individual
fully-human CDR hits

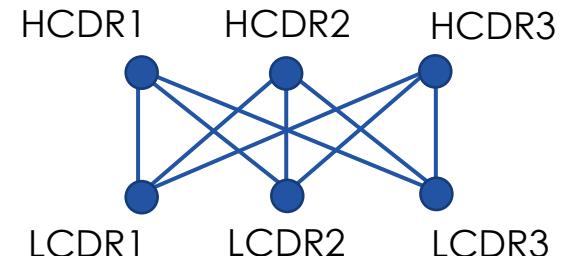
SPR confirmed hits:



HCDR1,2,3
LCDR1,2,3

Combine
human CDRs

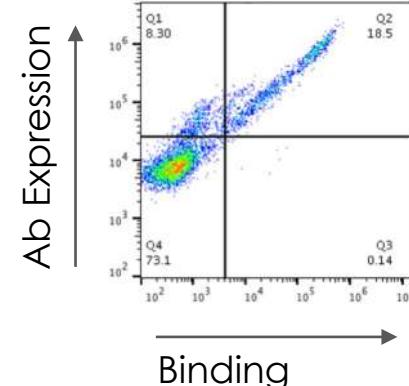
Fully human CDRs
combinatorial diversity



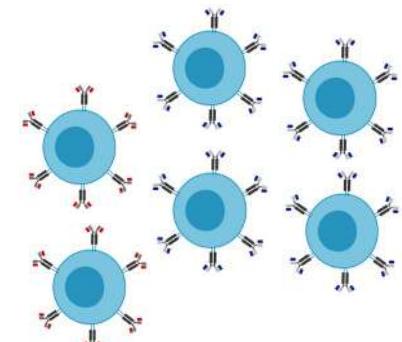
Mammalian
display library

HT-SPR hit validation
and quantitation

NGS



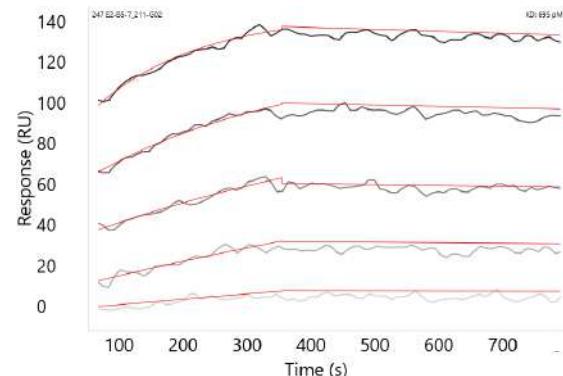
Single-cell sorting:
binding & expression



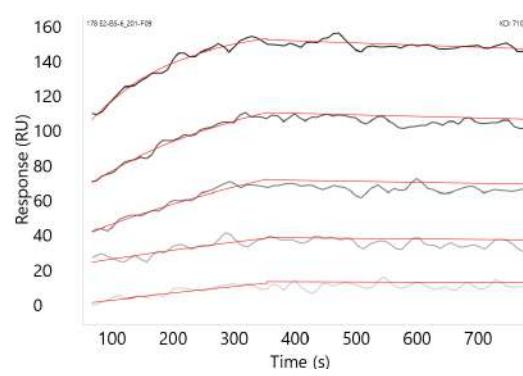
Combining Individual Fully-Human H/L CDR123 Hits Improves Affinity and Humanness

Top Four Fully-Human CDRs StableHu Hits

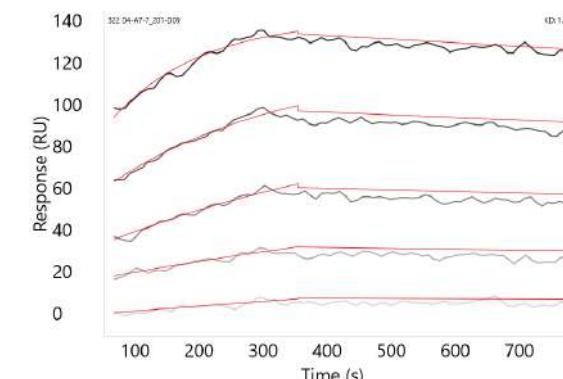
KD = 695 pM



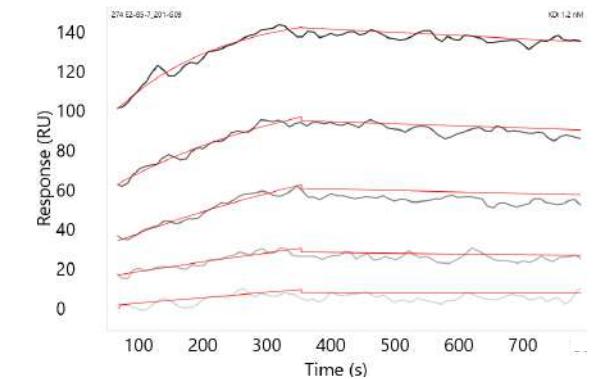
KD = 710 pM



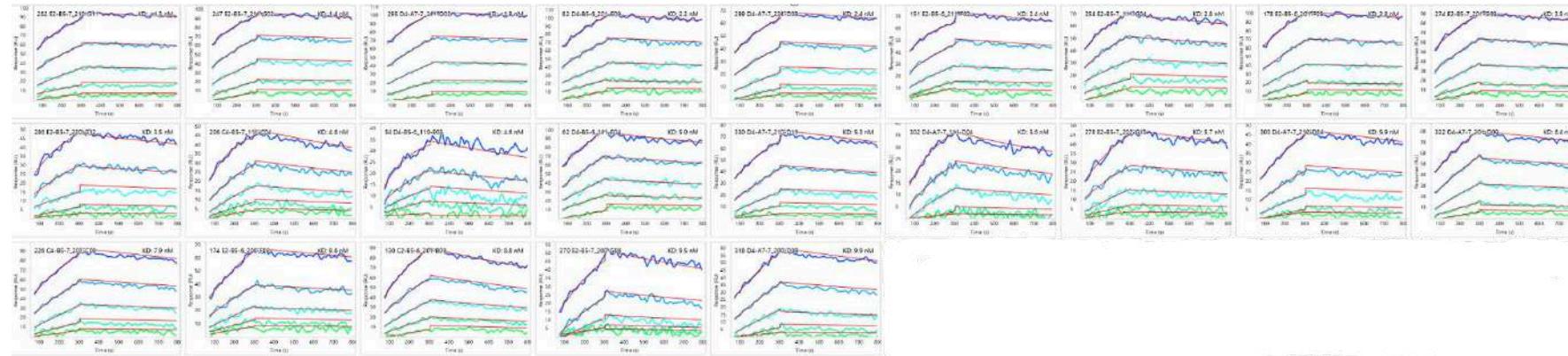
KD = 1.1 nM



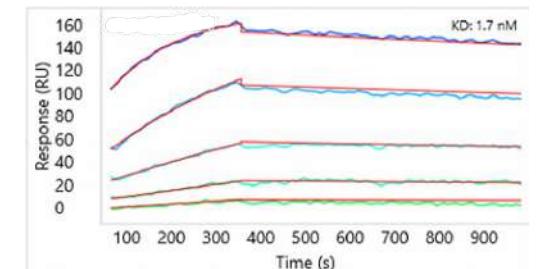
KD = 1.2 nM



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



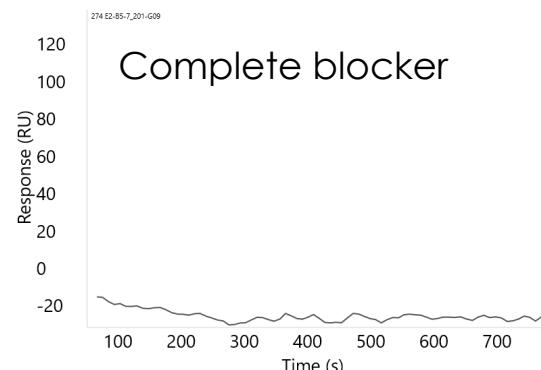
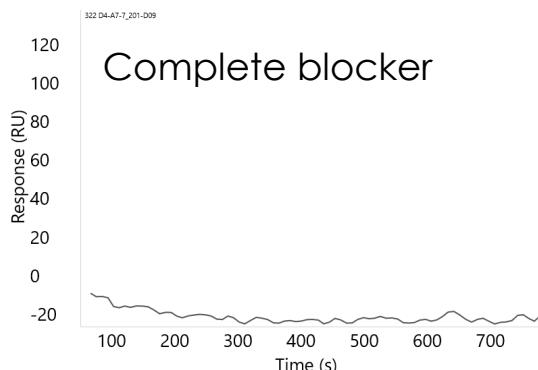
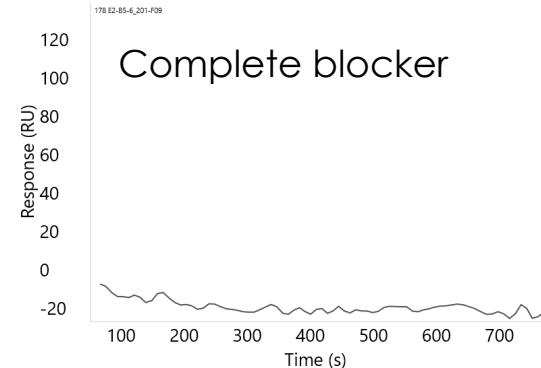
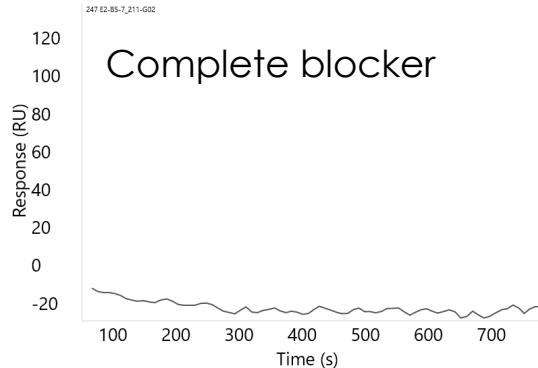
Starting Mu CDRs template



KD = 1.7 nM

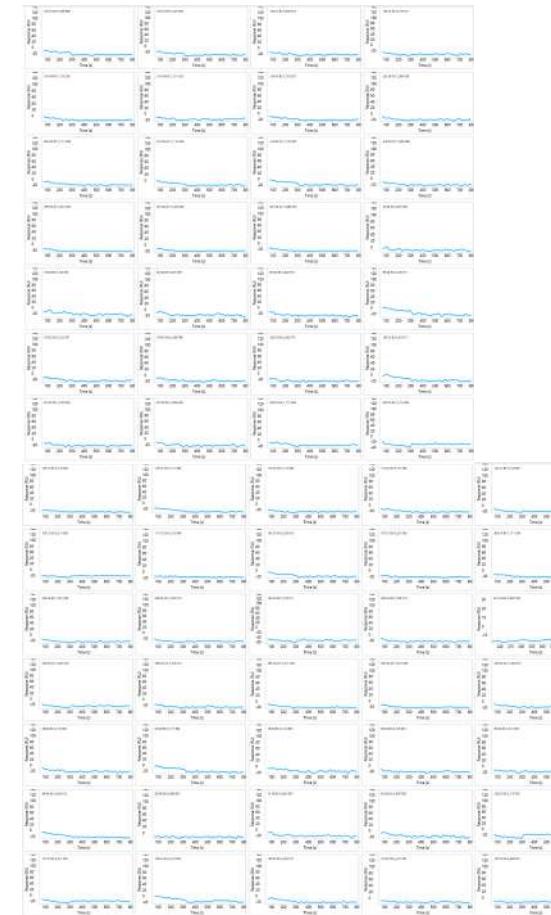
All StableHu Hits Cross-Block Starting Template Antibody With Mu CDRs

Top Four Fully-Human CDRs StableHu Hits

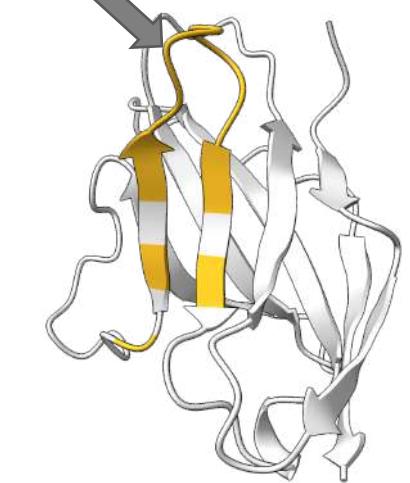


Remaining Fully-Human CDRs StableHu Hits

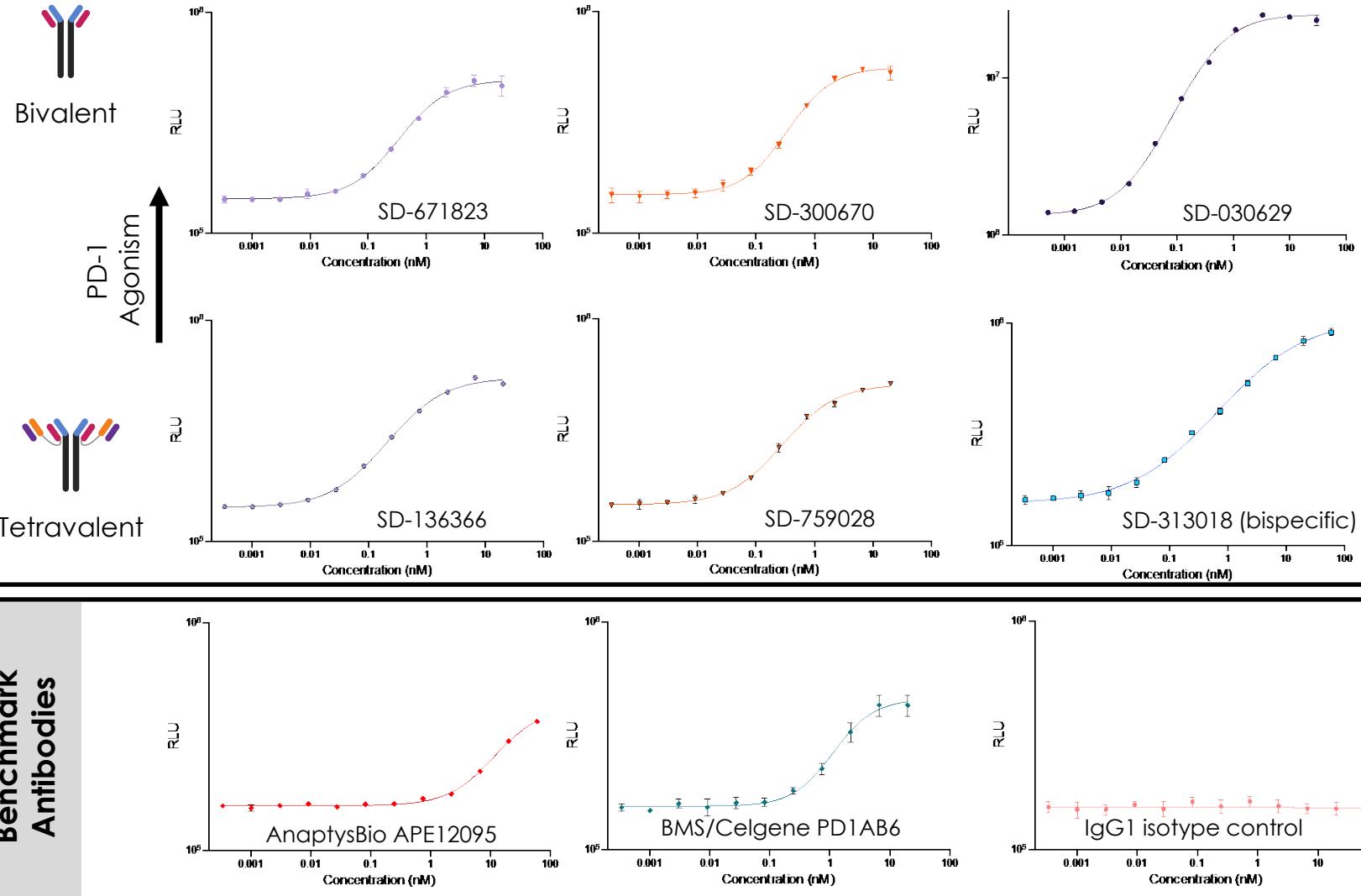
Complete blockers



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

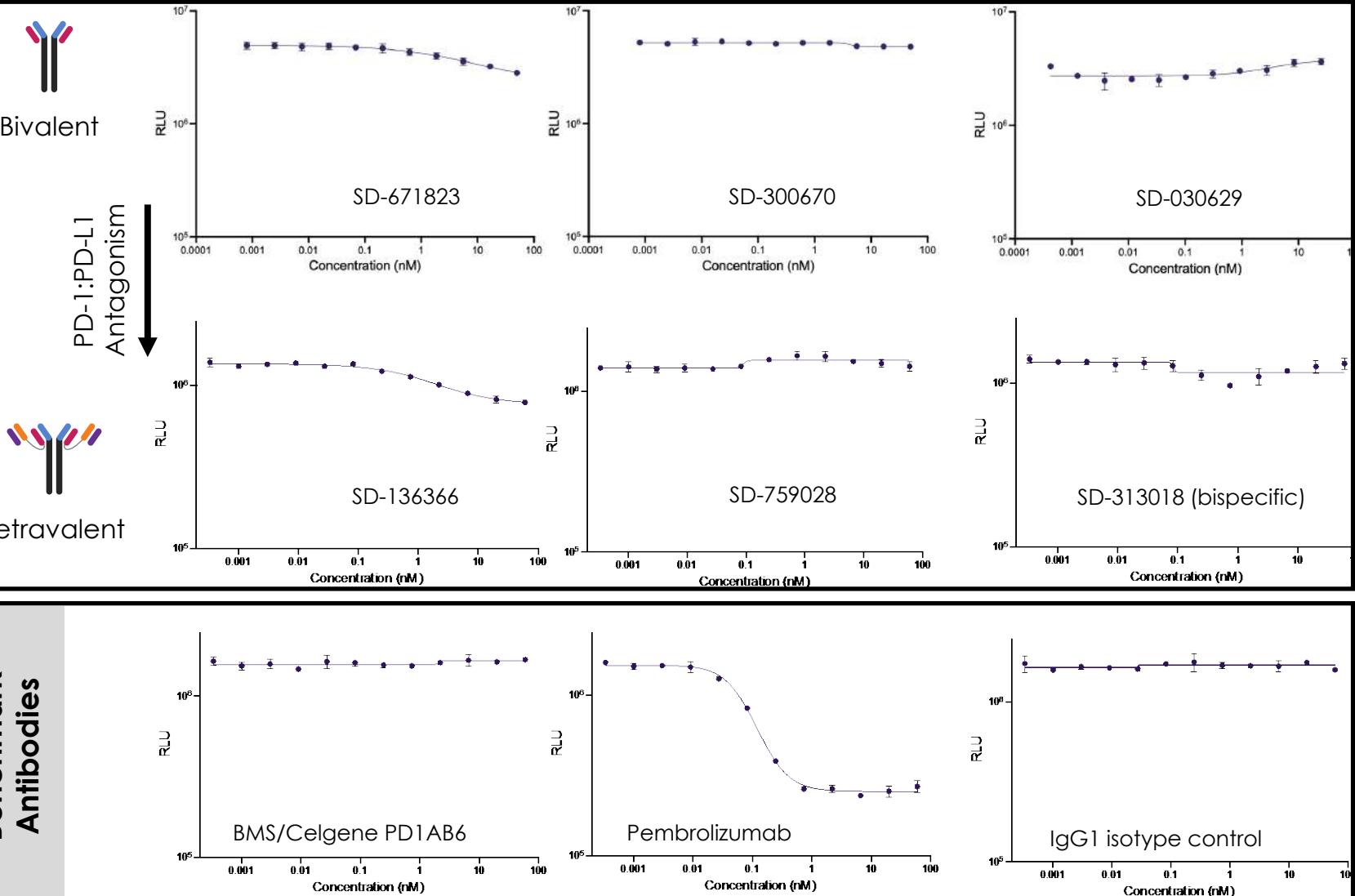


In vitro PD-1 Agonism Equals or Surpasses Benchmarks



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

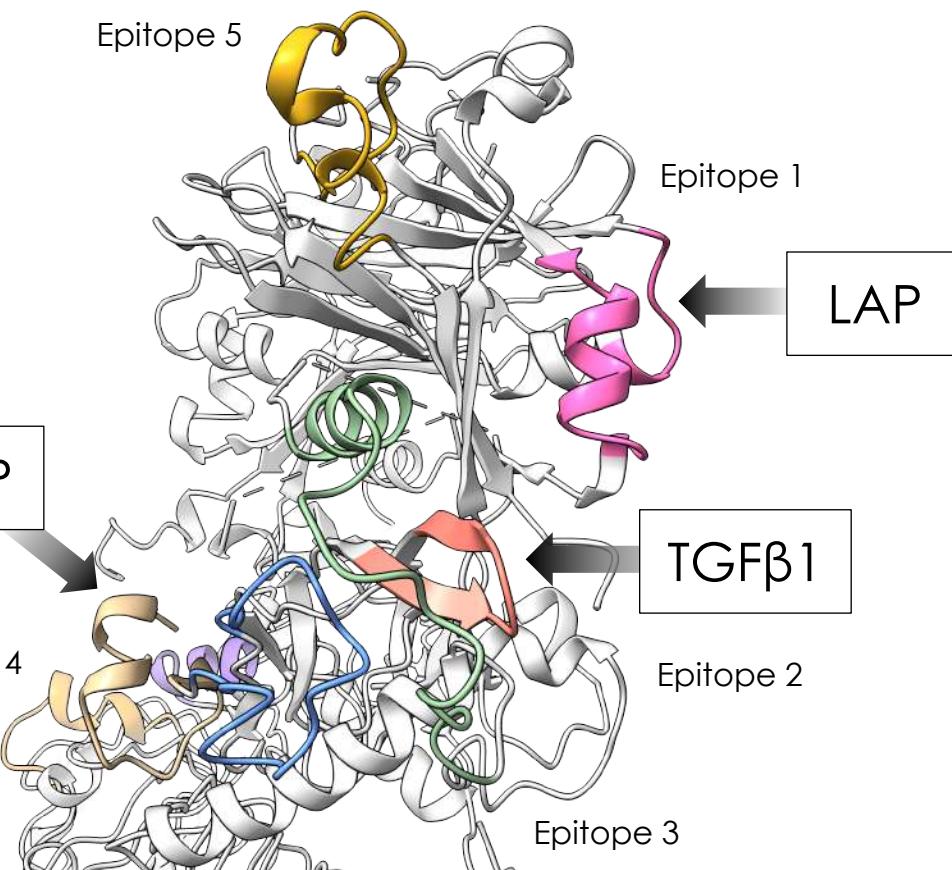
Multi-Protein Junctional Epitope

Latent-TGF β 1 Antibody

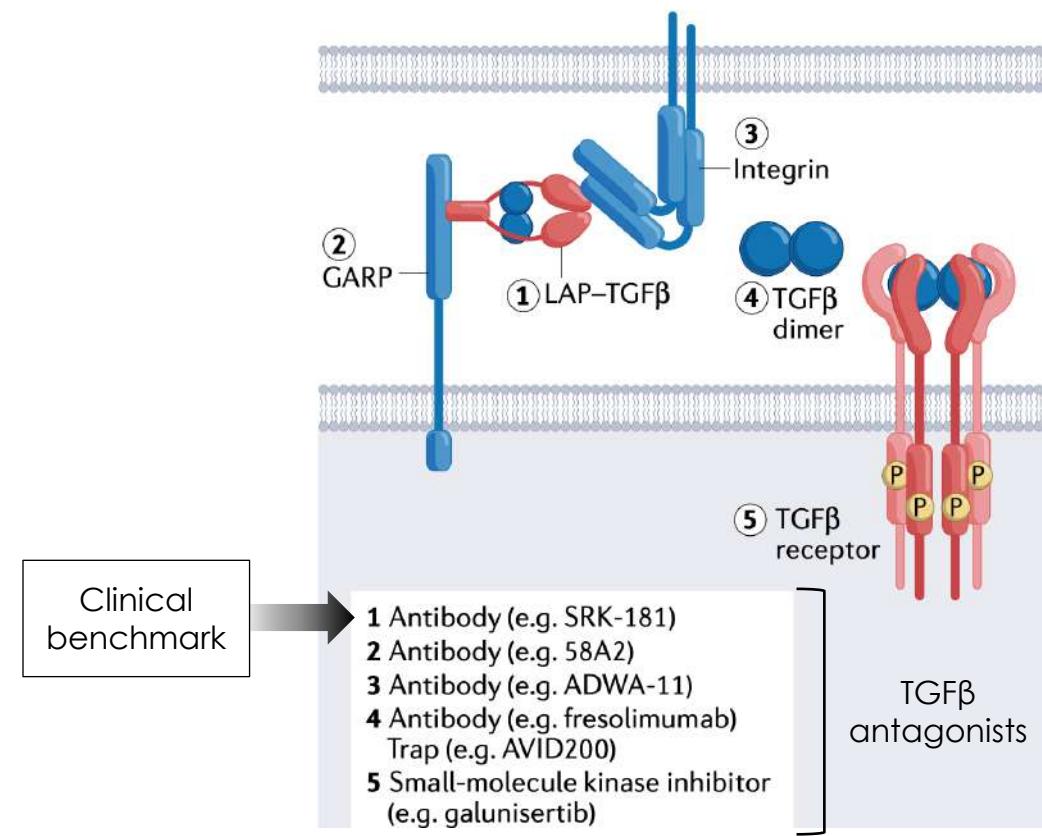
Latent-TGF β 1 Multimeric Complex Regulates TGF β 1 Release and Signaling

Multiple engineered epitopes were used to explore per-epitope TGF β 1-release antagonist potency

Latent-TGF β 1 Multimeric Structure

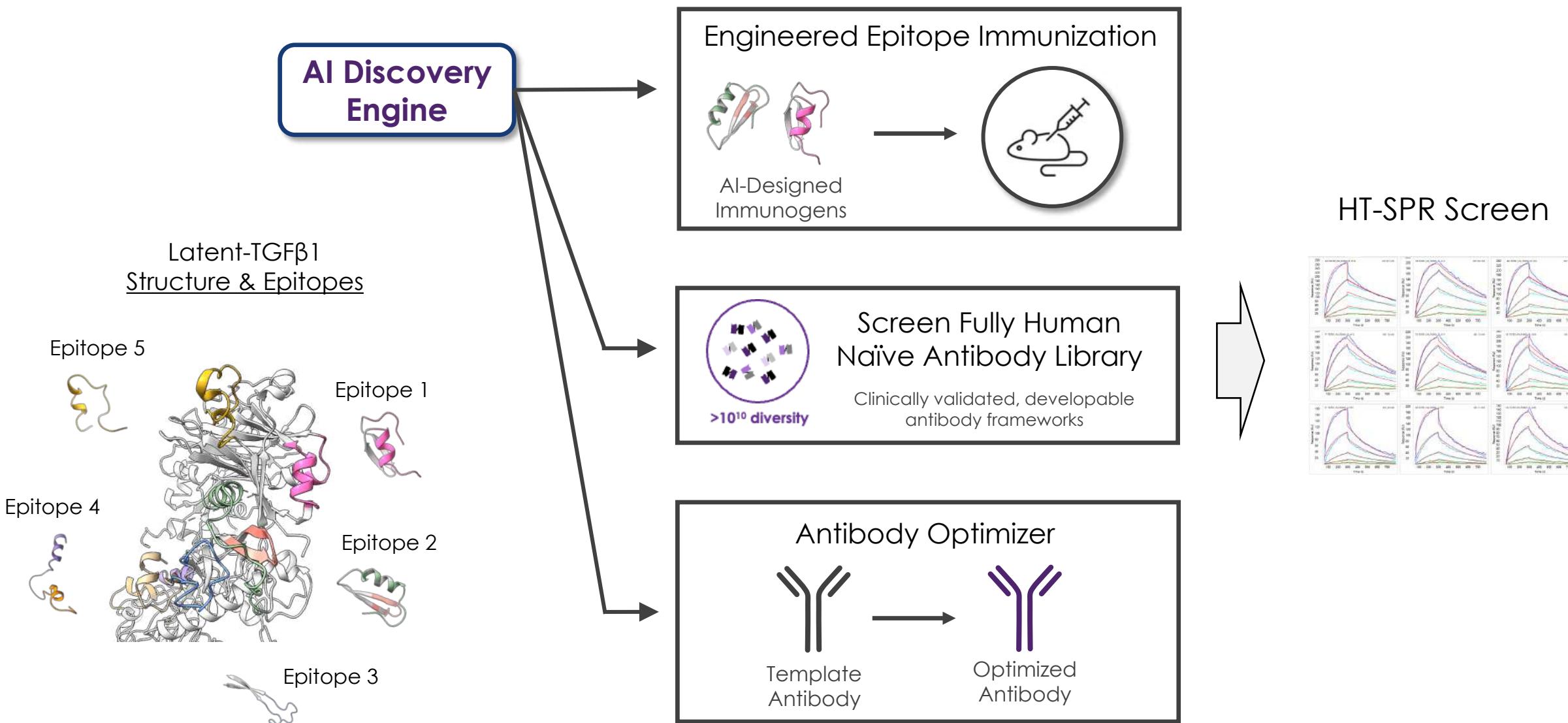


Released TGF β 1 is Immunosuppressive



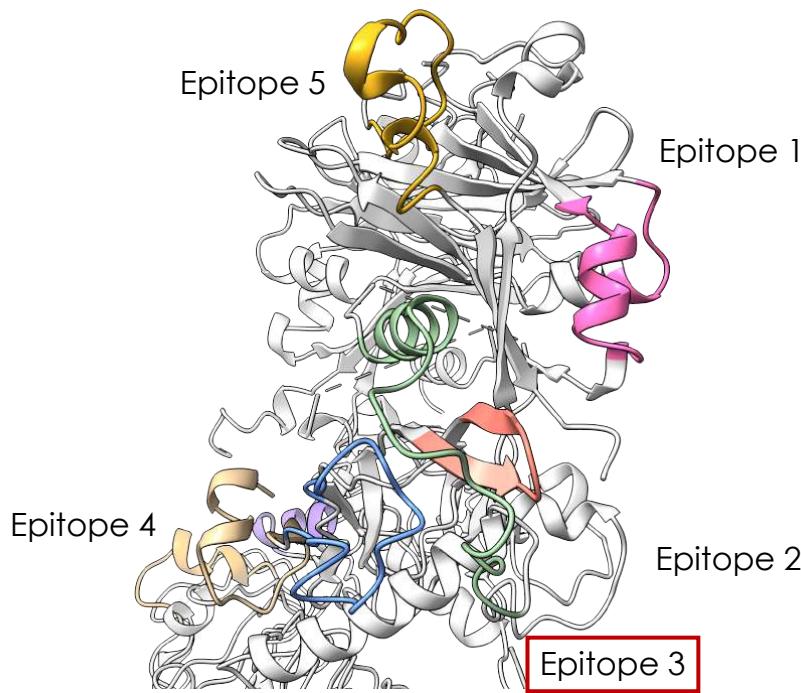
Nat. Rev. Immunol. (2022) 10.1038/s41577-022-00796-z

Parallel Paths to Latent-TGF β 1 Antibody Discovery

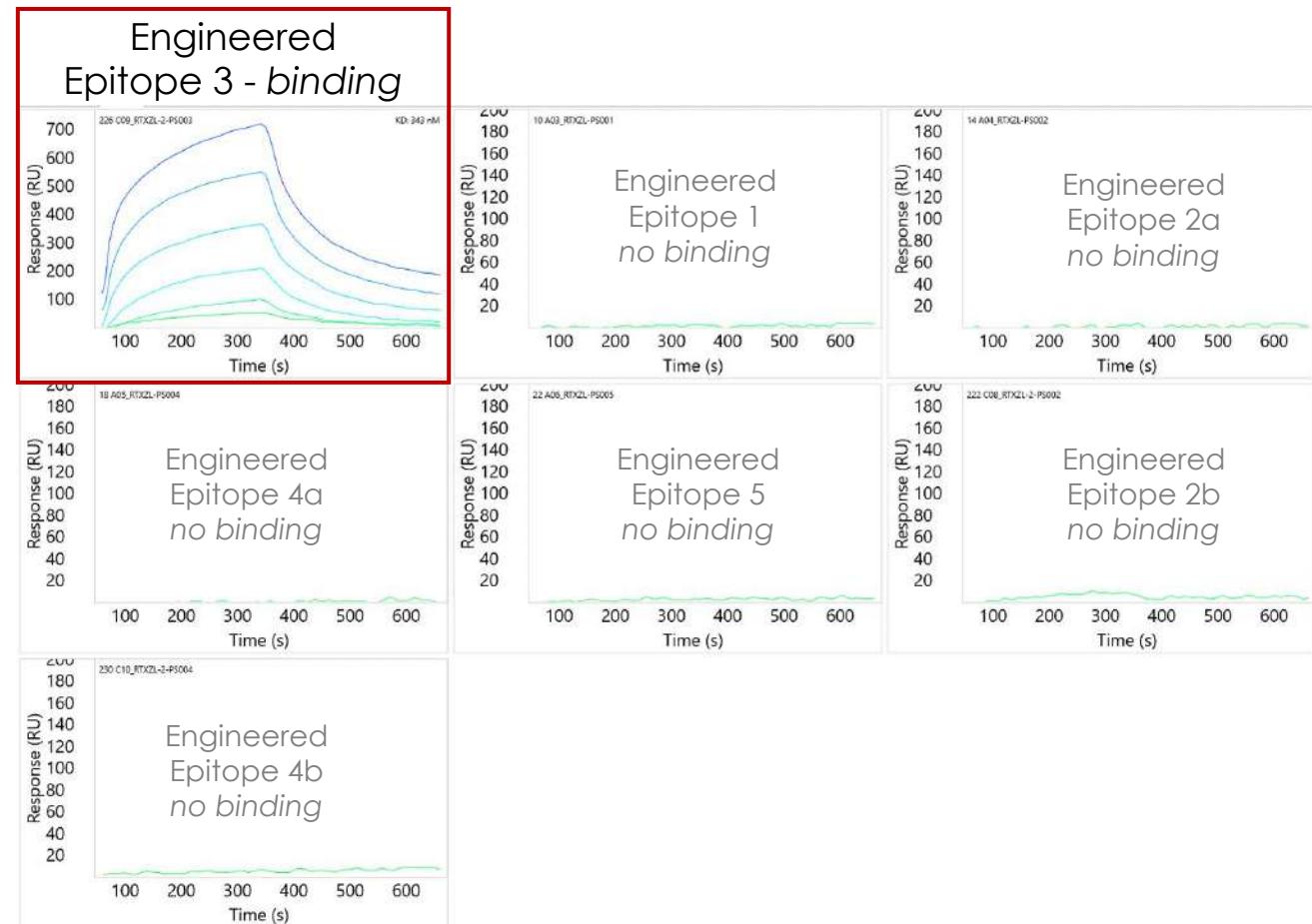


Mapping SRK-181 Benchmark Ab Using Engineered Epitopes

Latent-TGF β 1 Structure & Epitopes

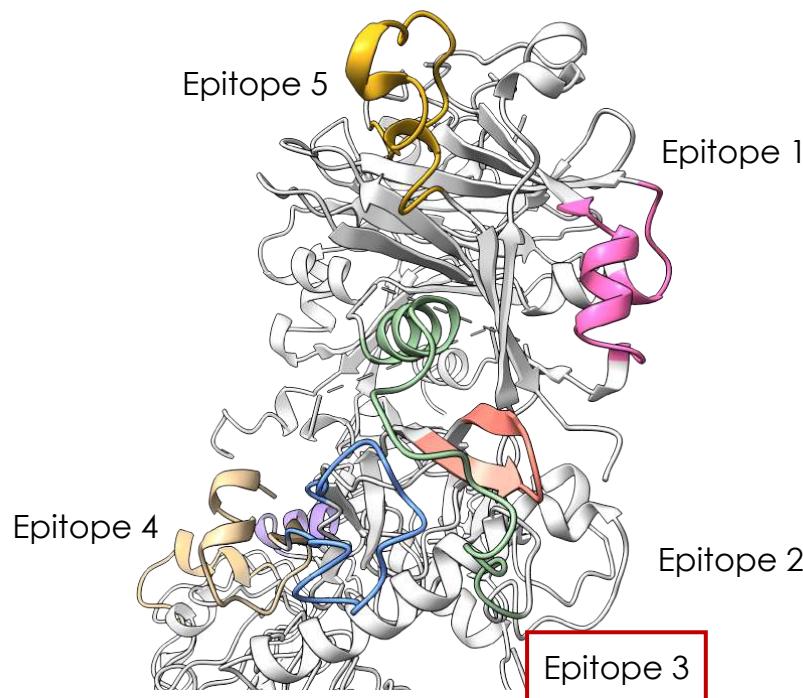


Multiple Engineered Epitopes Binding to the Benchmark Ab

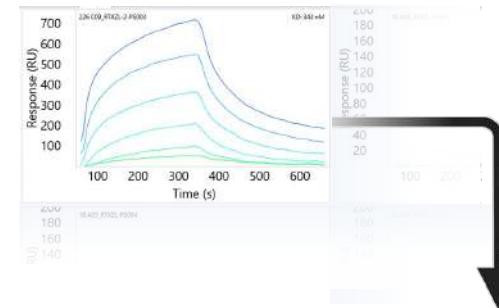


SRK-181 Benchmark HD-X MS Corroborates Engineered Epitope Mapping by SPR

Latent-TGF β 1 Structure

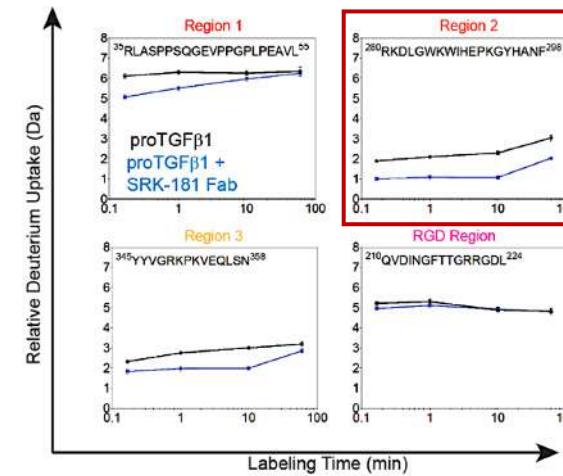


Engineered Epitope 3 SRK-181 SPR



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

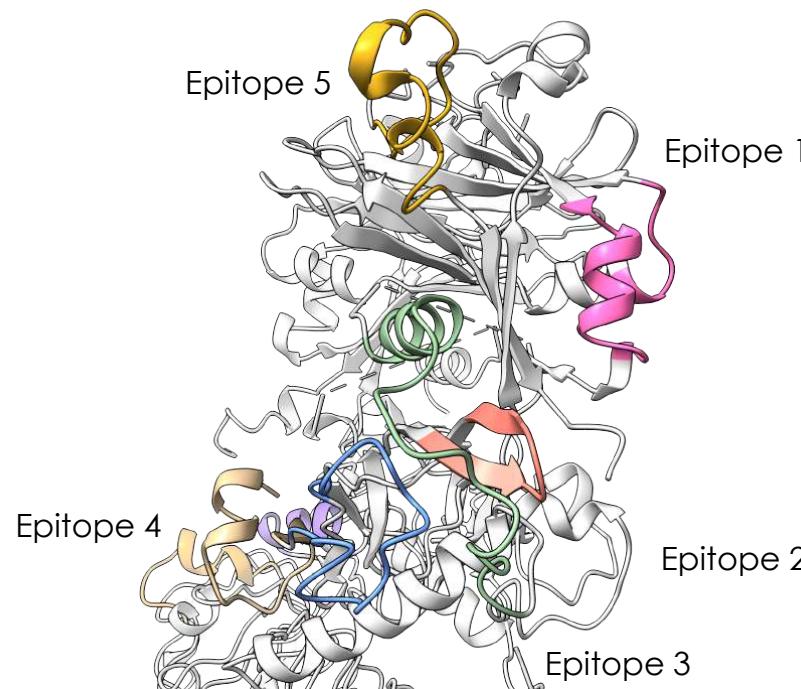
SRK-181 HD-X MS



HD-X region corresponding to engineered epitope 3

Epitope-Steered Naïve In Vitro Selection Was One Path to Latent-TGF β 1 Clones

Latent-TGF β 1 Structure & Epitopes

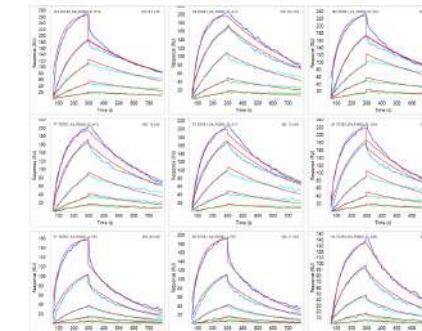


Multiple engineered epitopes were used during rounds of phage library panning

Round 1	Round 2	Round 3
LTGF β 1	LTGF β 1	Engineered Epitope
LTGF β 1	Engineered Epitope	LTGF β 1



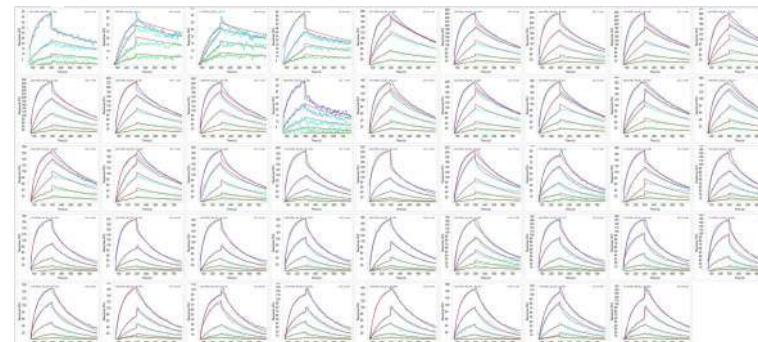
HT-SPR Screen



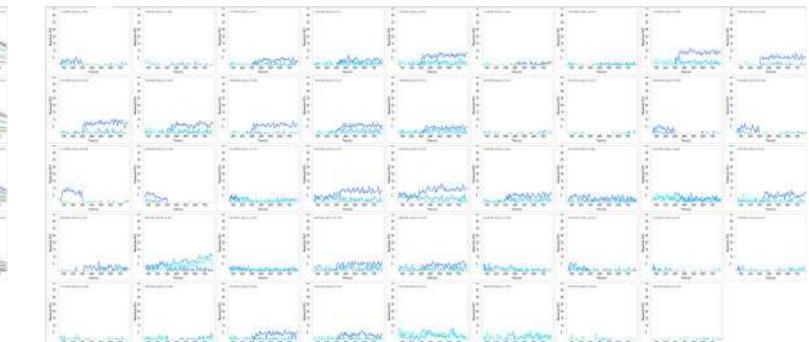
HT-SPR Screen Demonstrates Specificity, Diversity & Affinity of Epitope Steered Selections

SPR Screen Results

Latent-TGF β 1 (desired target)

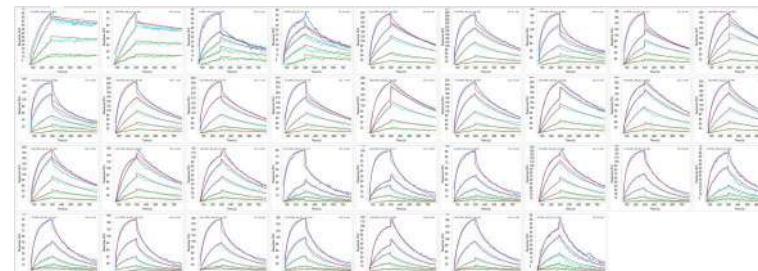


TGF β 1 (undesired target)



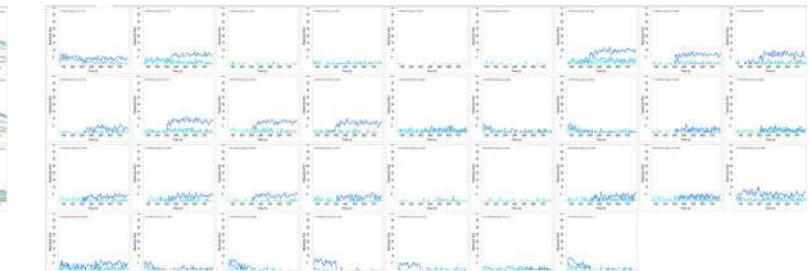
Epitope 1 steered binders

Latent-TGF β 1 specific	44
KD range (nM)	2.5 – 40 nM
TGF β 1 off-target	13



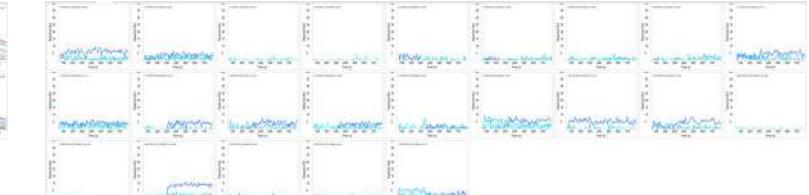
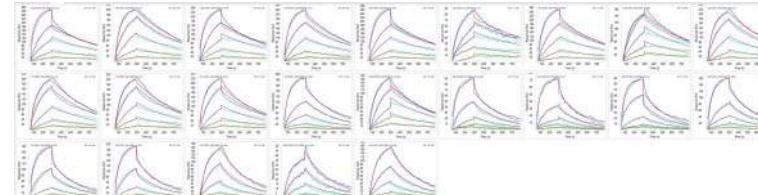
Epitope 2 steered binders

Latent-TGF β 1 specific	34
KD range (nM)	1.0 – 36 nM
TGF β 1 off-target	7



Epitope 3 steered binders

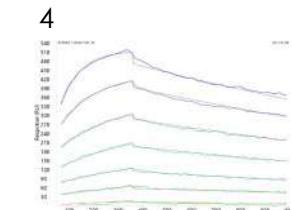
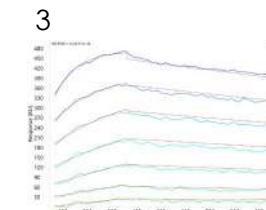
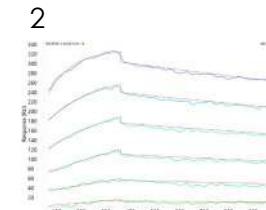
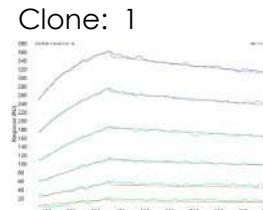
Latent-TGF β 1 specific	23
KD range (nM)	9.0 – 29 nM
TGF β 1 off-target	5



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

Do bind

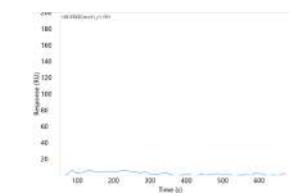
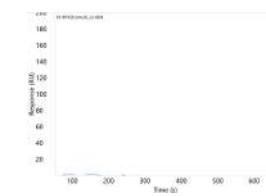
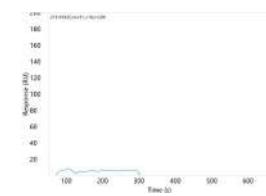
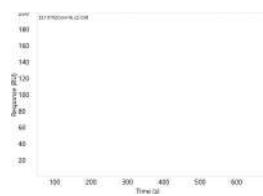
Latent-TGF β 1
KD < 5 nM



AND

Do Not bind

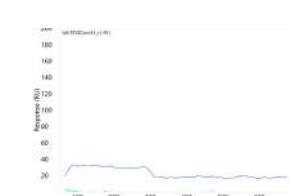
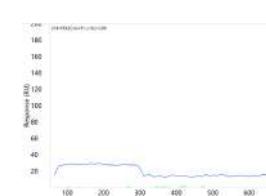
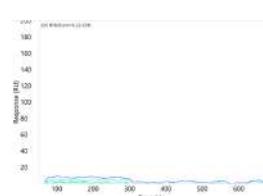
TGF β 1



AND

Do Not bind

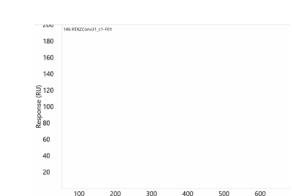
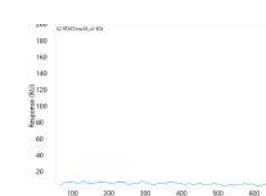
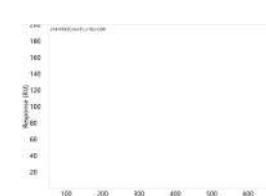
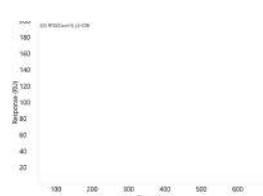
Latent-TGF β 2



AND

Do Not bind

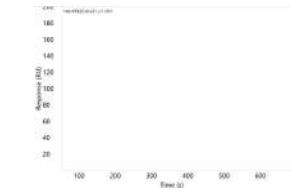
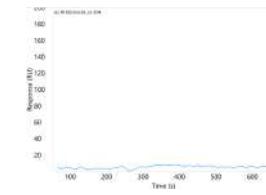
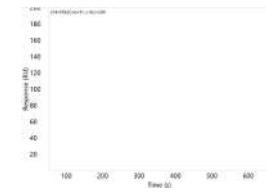
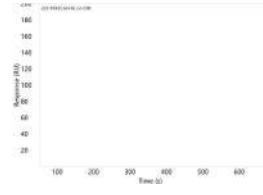
TGF β 2



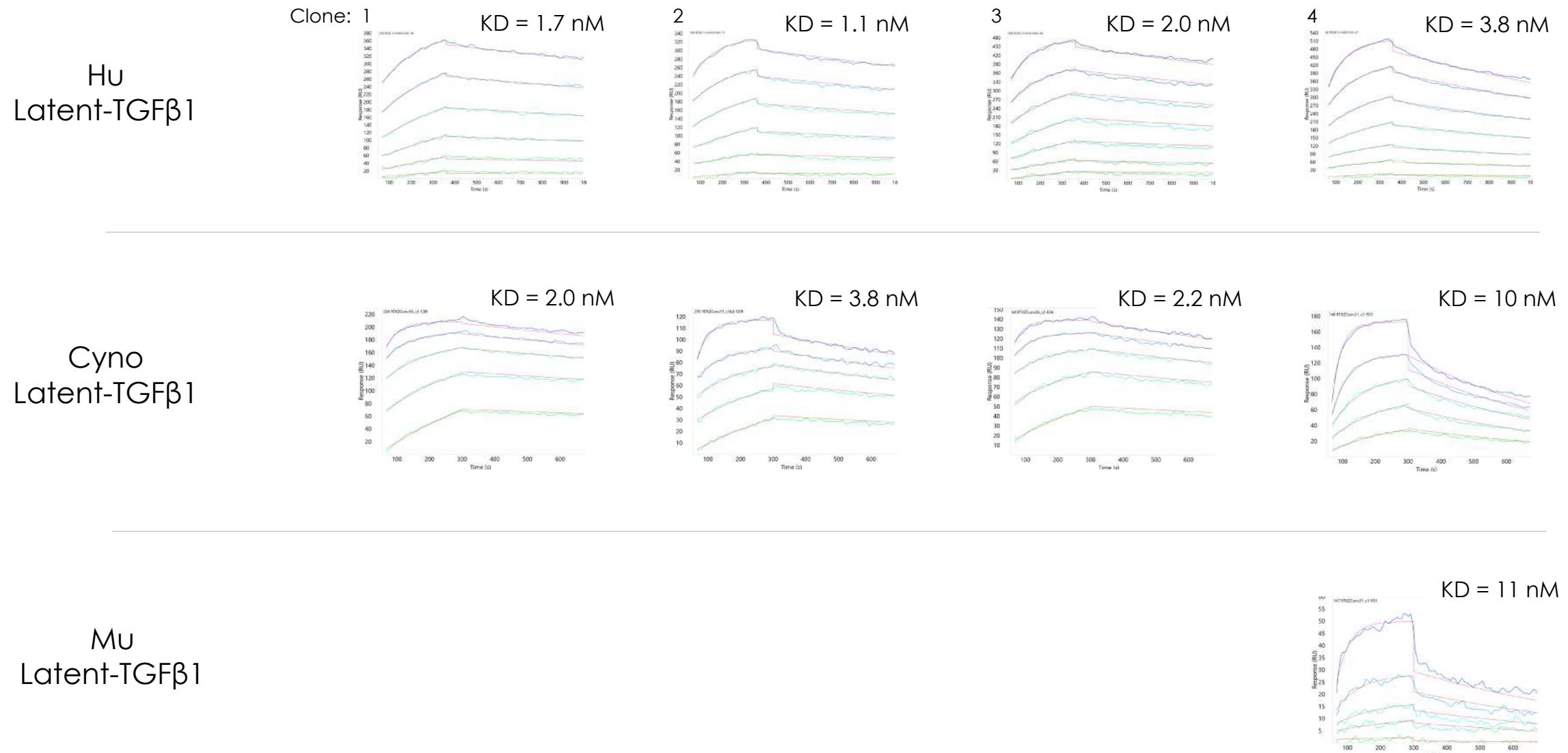
AND

Do Not bind

TGF β 3



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

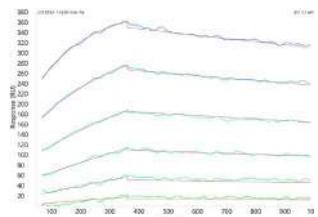


Top Naïve In Vitro Selection Clone Met All Affinity, Specificity and Potency Criteria

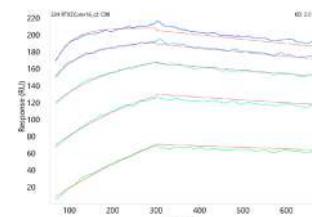
SPR

Does bind:

Hu Latent-TGF β 1
KD = 1.7 nM

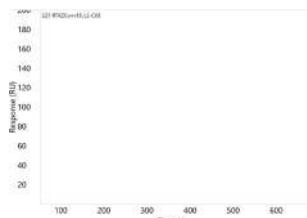


Cyno Latent-TGF β 1
KD = 2.0 nM

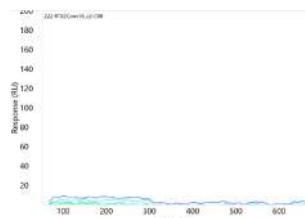


Does not bind:

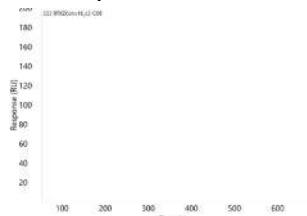
TGF β 1



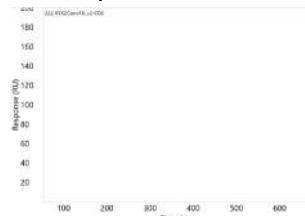
Latent-TGF β 2



TGF β 2



TGF β 3

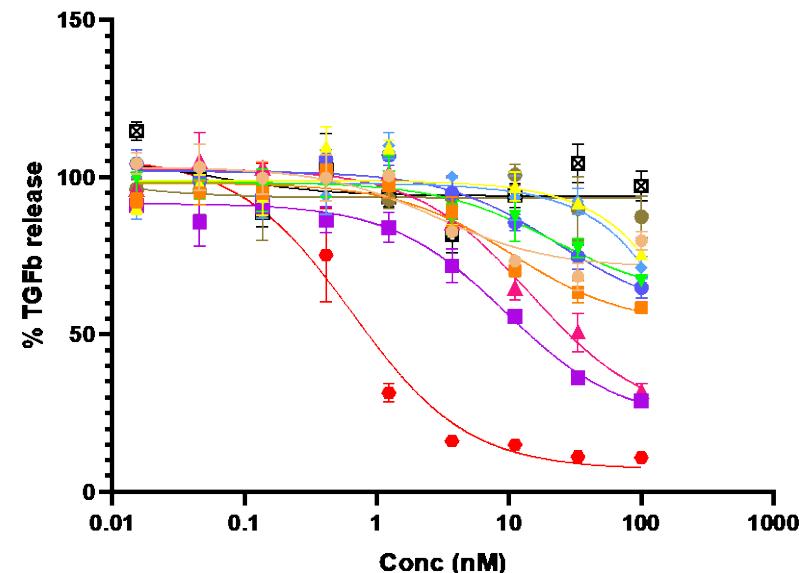


TGF β 1 Inhibition Assay

Clone ID

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- Iso ctrl.
- Benchmark Ab

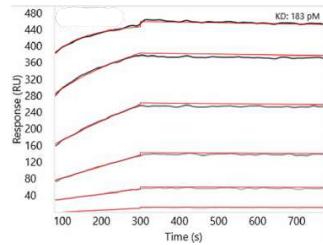
Top naïve in vitro selection clone



Clone	IC_{50}
Top naïve clone #7	9.5 nM
Benchmark Ab	0.7 nM

StableHu Optimization of an Anti-Latent-TGF β 1 Benchmark Ab

Starting with published
benchmark CDRs template



Published Ab
template



KD = 180 pM
 IC_{50} = 700 pM

AI-model predicts
human CDRs

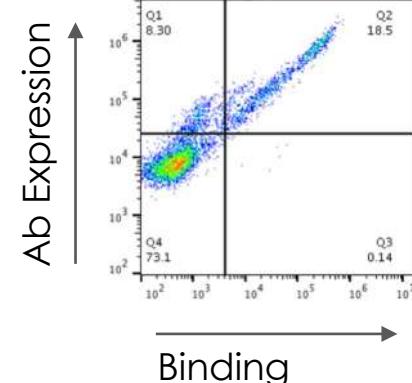
Fully human heavy &
light chain CDRs diversity

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

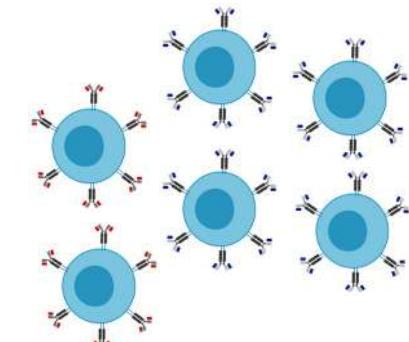
Per-CDR
mammalian
display library

HT-SPR hit validation
and quantitation

NGS



Single-cell sorting:
binding & expression



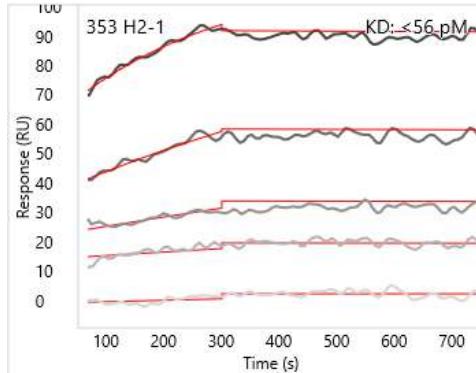
StableHu Optimization Identifies Improved Fully-Human CDR Variants

Variant Clones Latent-TGF β 1 SPR

Hu
Latent-TGF β 1

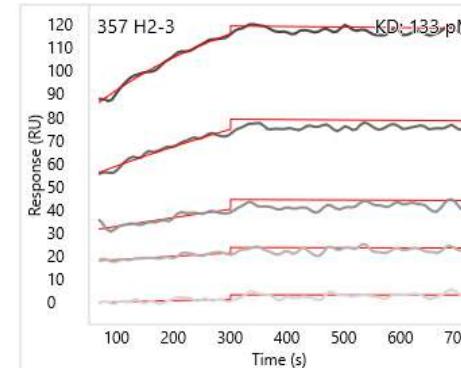
Variant 1

KD = 50 pM



Variant 2

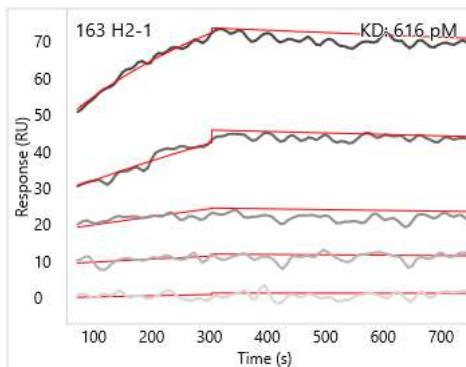
KD = 130 pM



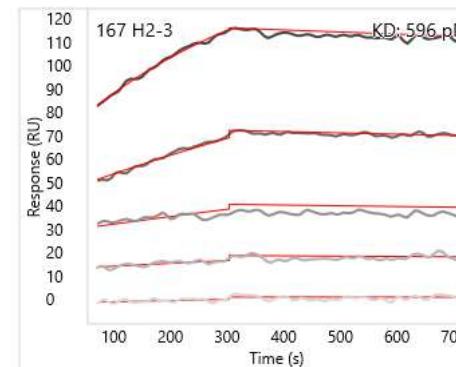
Template clone

Mu
Latent-TGF β 1

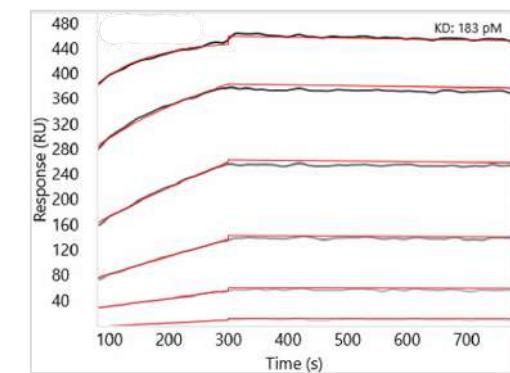
KD = 620 pM



KD = 600 pM



KD = 180 pM



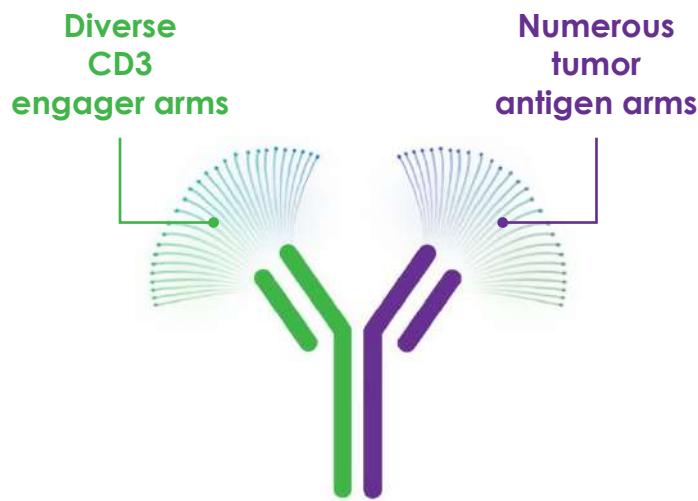
T-Cell Engager Epitope

CD3 Antibody

Key Challenges of CD3 T Cell Engager Discovery

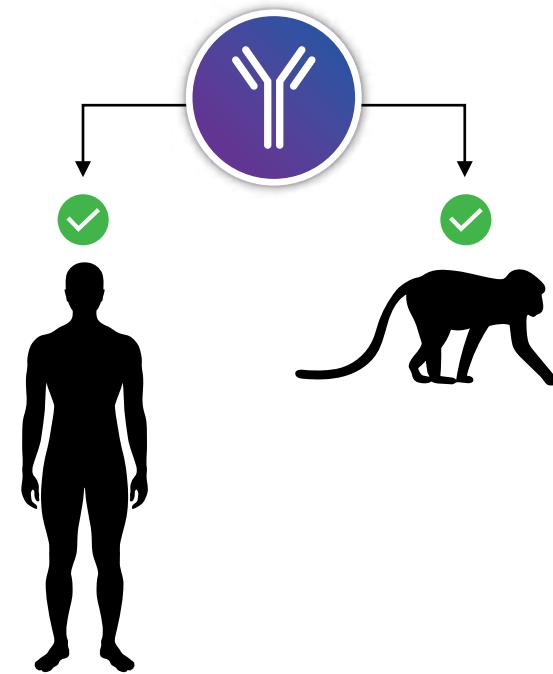
1 Sequence Diversity

Broad CD3 activity for optimized paring with tumor antigen arms



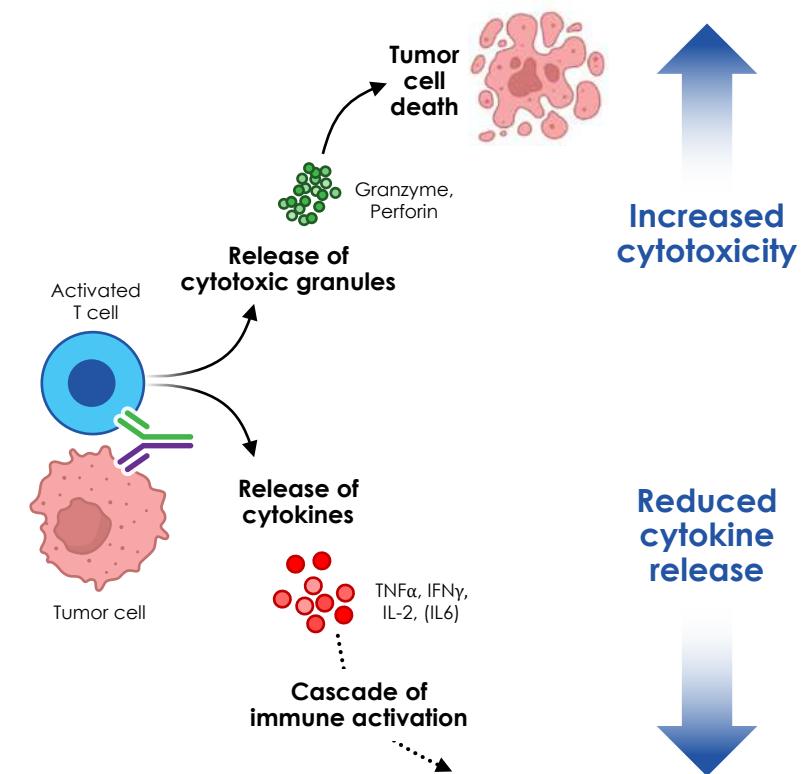
2 Hu-Cyno Cross-Reactivity

Risk reduction via cyno monkey toxicity study compatibility

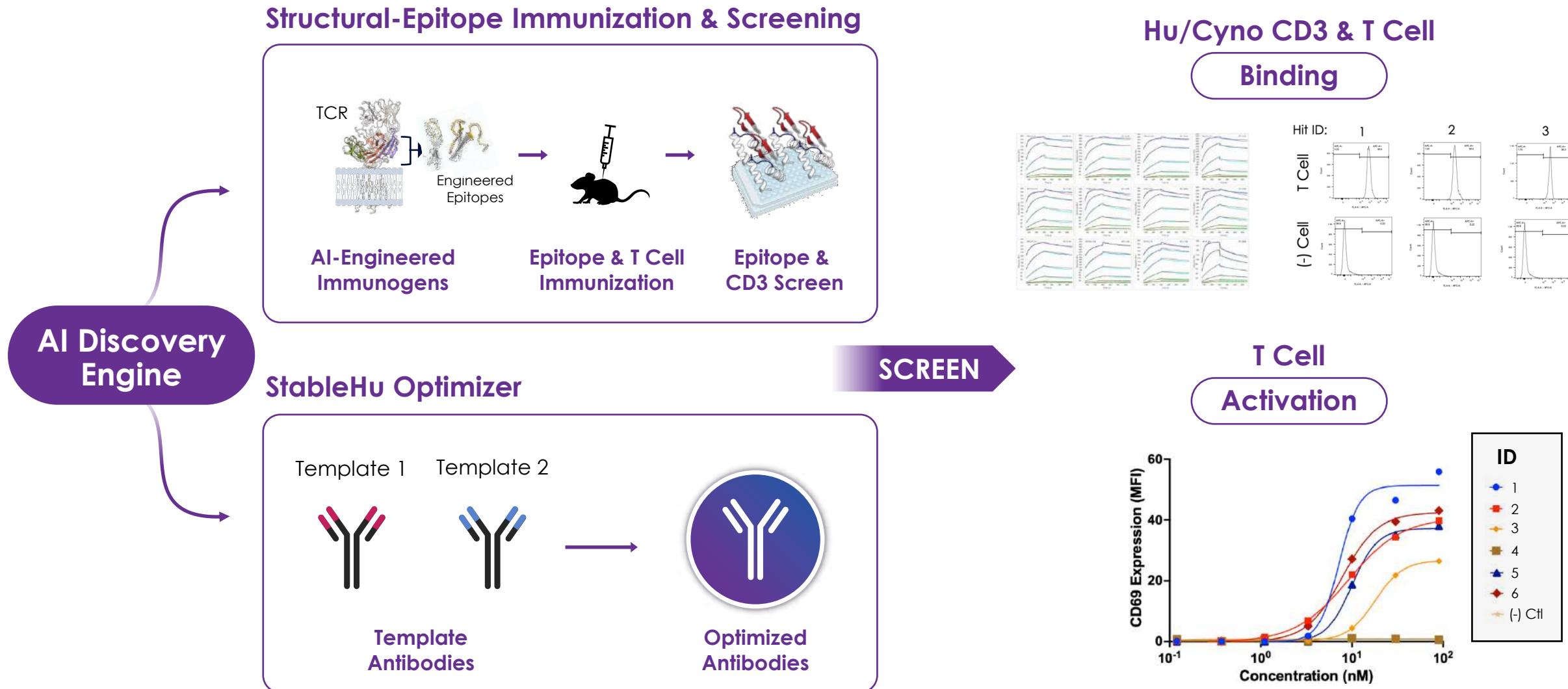


3 Range of Cytokine Release

Tailored cytokine release for expanded therapeutic window



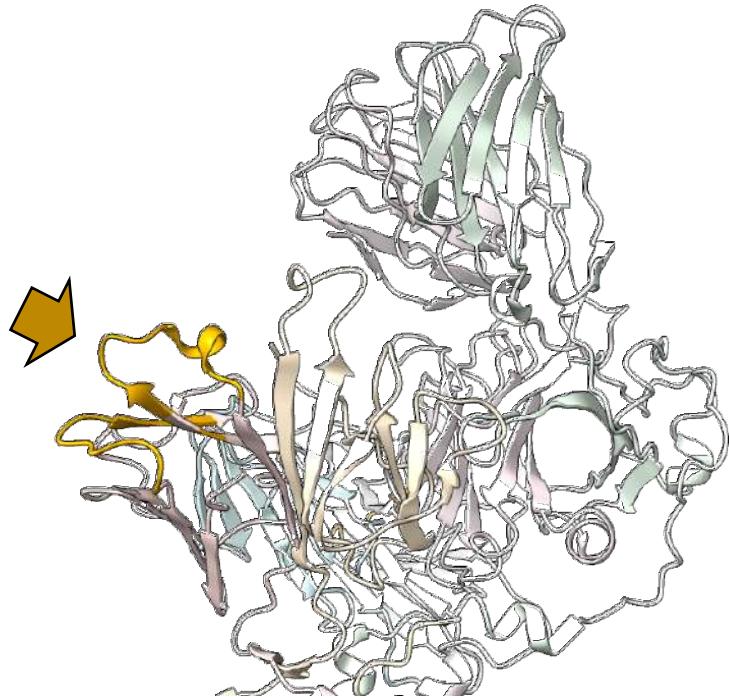
Dual Approaches to a Diverse Panel of Anti-CD3 Antibodies



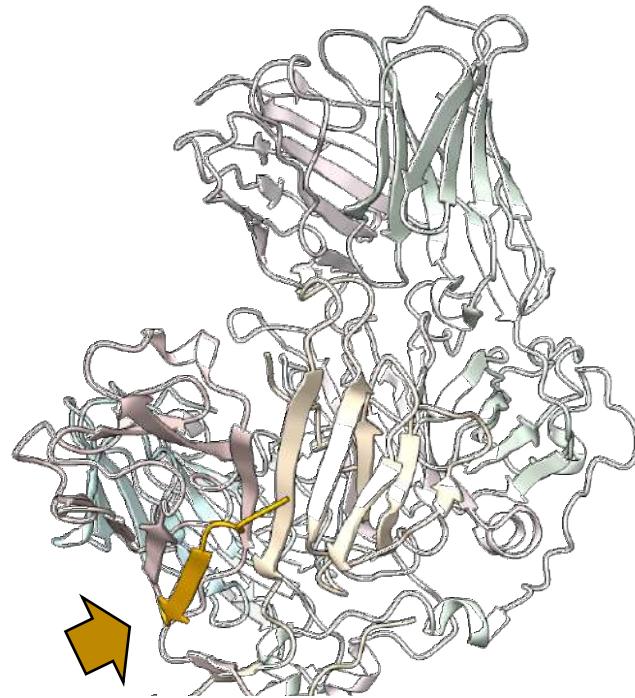
Engineered Epitopes Guide Immunization to TCR-Accessible CD3 Epitopes

CD3 target epitopes in the context of the full TCR

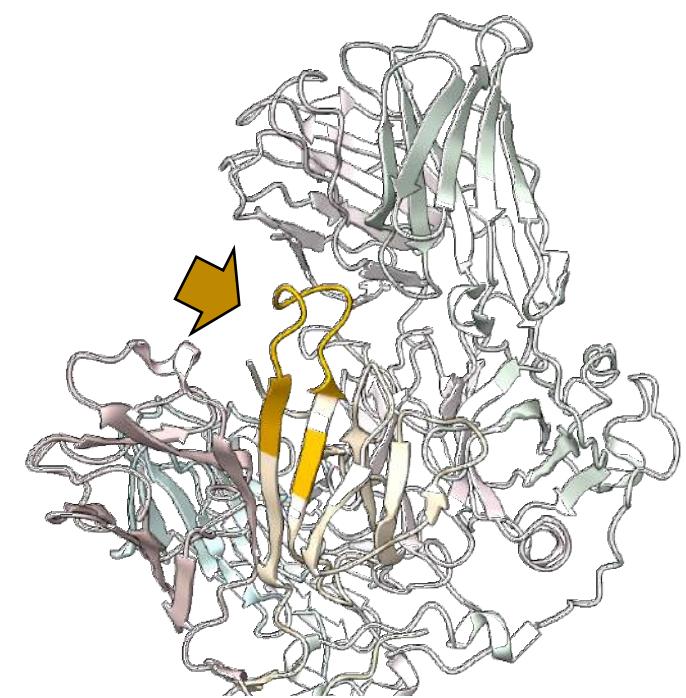
Epitope 1



Epitope 2

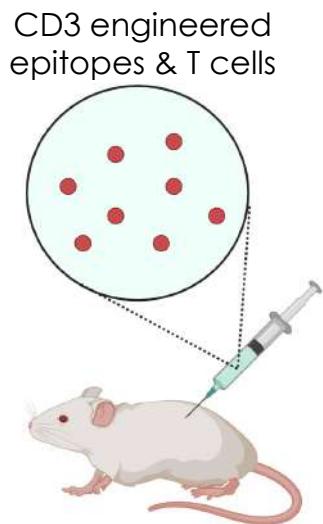


Epitope 3

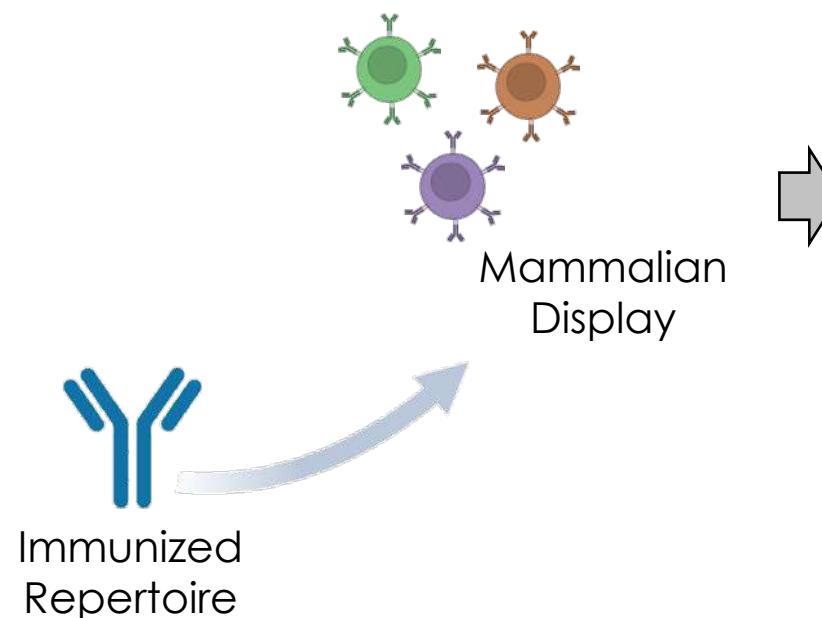


Immunized CD3 Repertoires Were Cloned and Screened in Mammalian Display

Multi Epitope-Steered Immunization

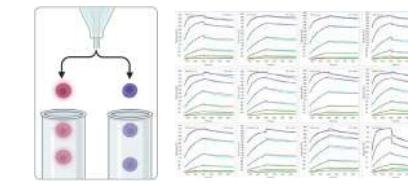


Mammalian Library Display



Multi Dimension Screening

FACS, NGS & SPR



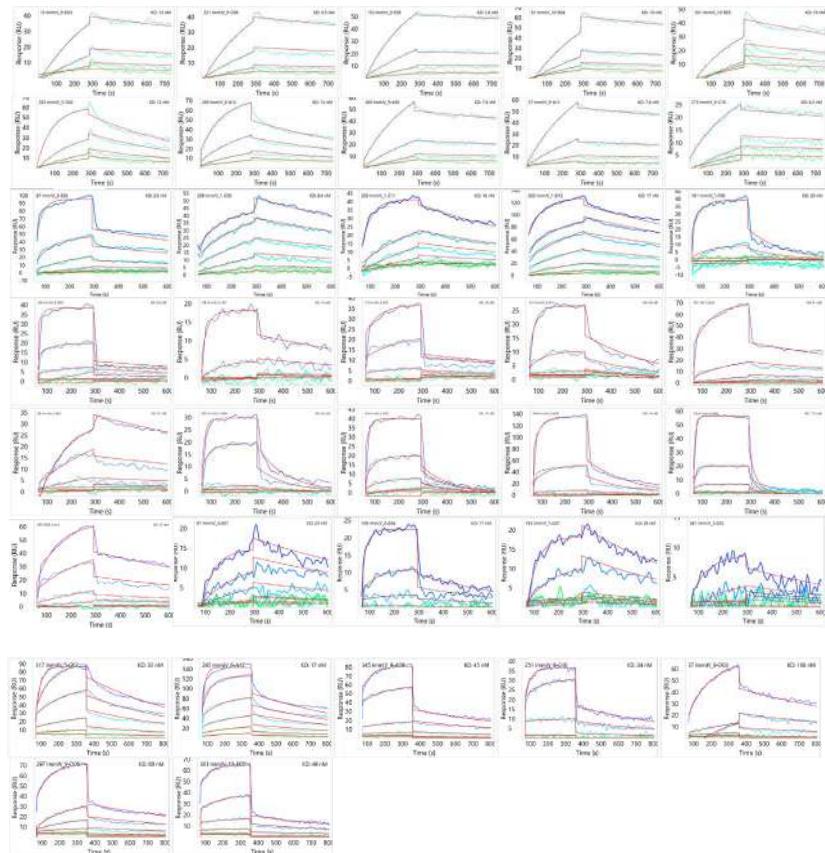
Single-Cell Screen:
Engineered epitopes &
CD3 binding &
Ab expression

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

HT-SPR Screen Hu & Cyno CD3 Binding

Hu CD3ED

KD: 3 - 100 nM



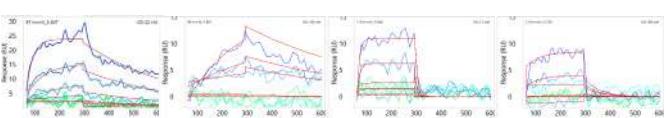
Hu CD3EG

KD: 17 - 100 nM

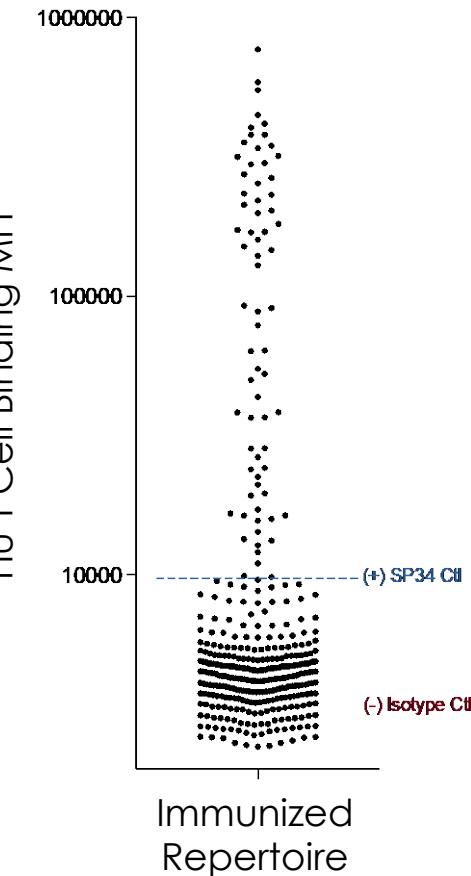


Cyno CD3ED

KD: 20 - 100+ nM

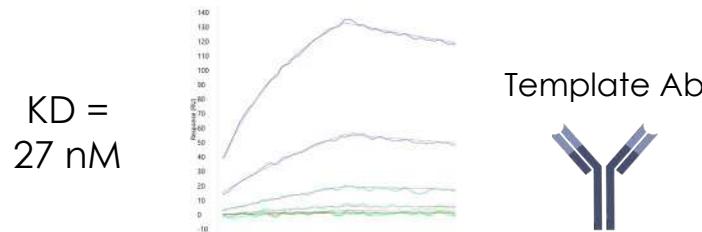


HT-Flow Cytometry Screen Hu T Cell Binding



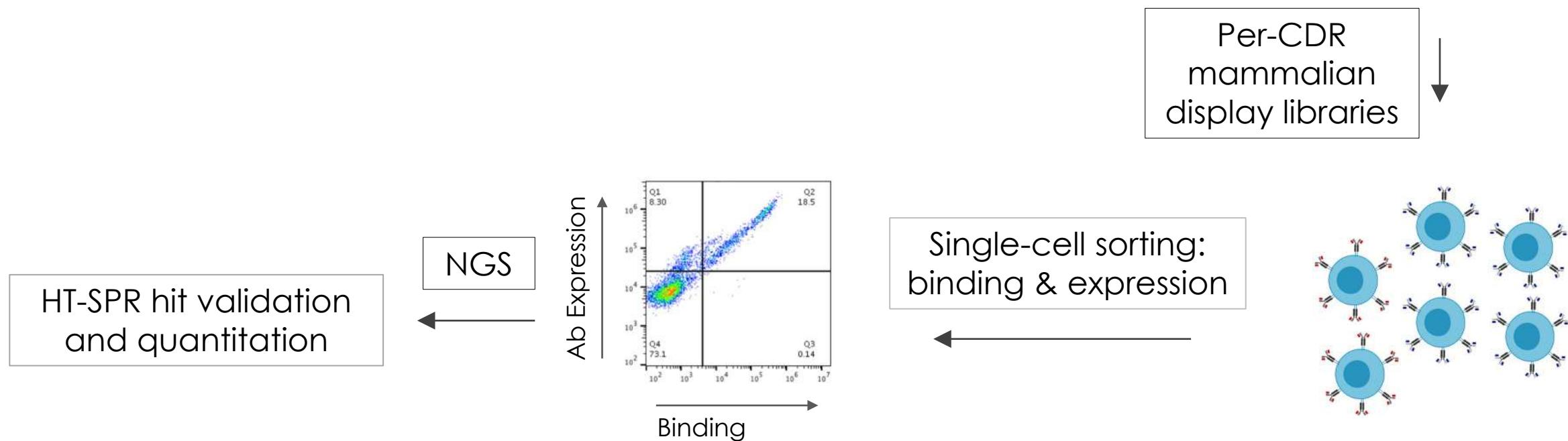
StableHu Optimization of Anti-CD3 Template Antibodies

Starting with anti-CD3 Ab template



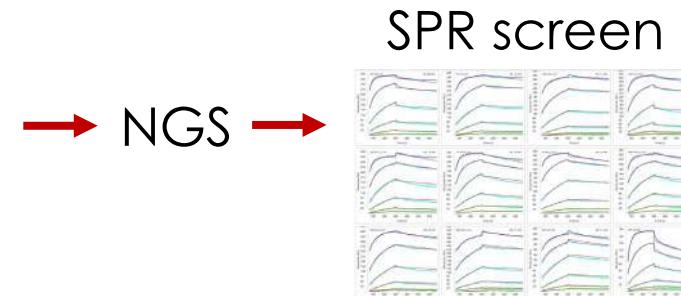
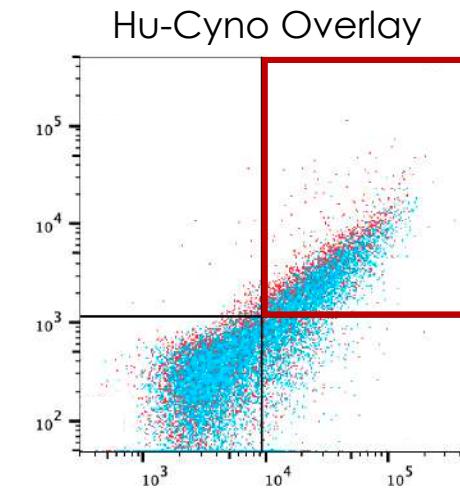
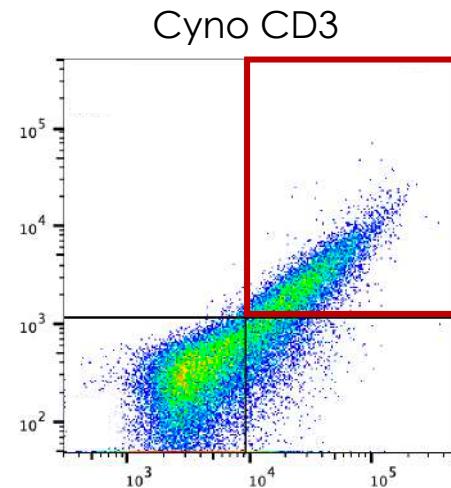
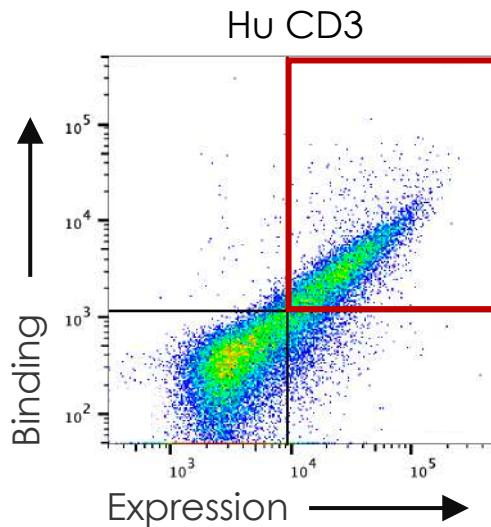
Fully human heavy & light chain CDRs diversity

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

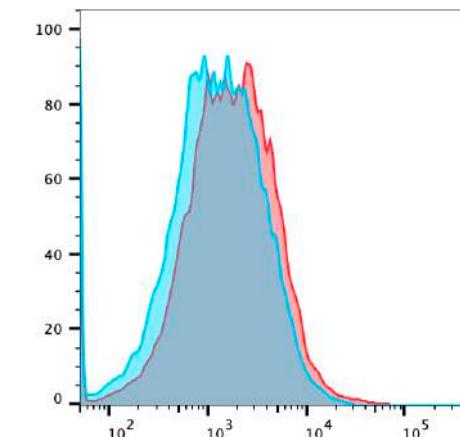
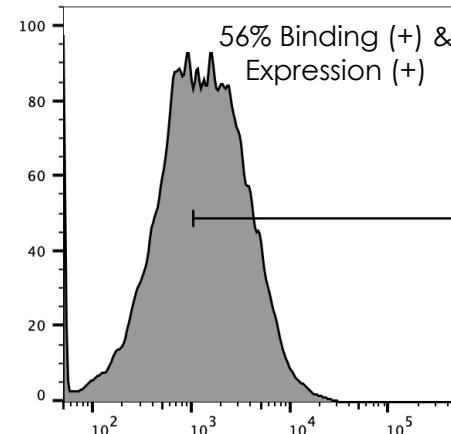
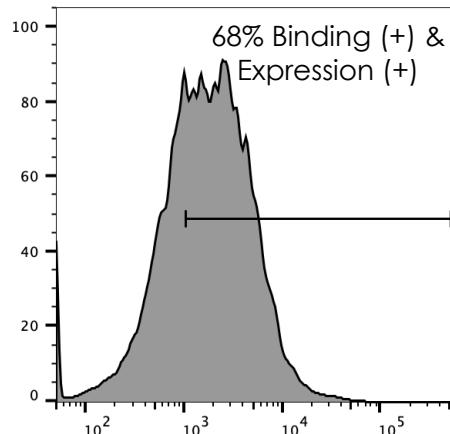


StableHu Generates Hu-Cyno Cross-Reactivity Library from Anti-CD3 Template

Cell Sorting of Pooled Single CDR Libraries



High-expression & binding double-positive distributions

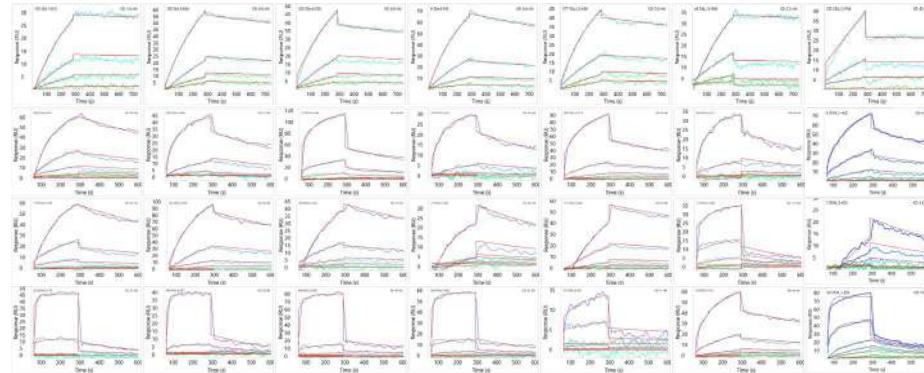


StableHu Identifies T Cell Binders – Some With Cyno Cross-Reactivity

HT-SPR Screen Hu & Cyno CD3 Binding

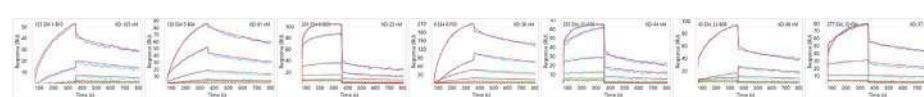
Hu CD3ED

KD: 2 - 100 nM



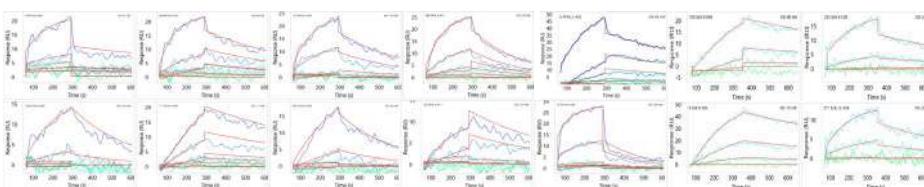
Hu CD3EG

KD: 20 - 100 nM



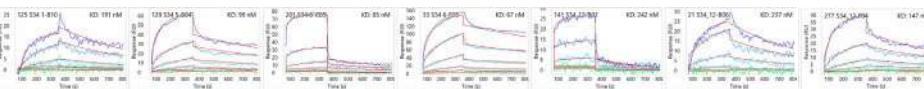
Cyno CD3ED

KD: 15 - 100 nM

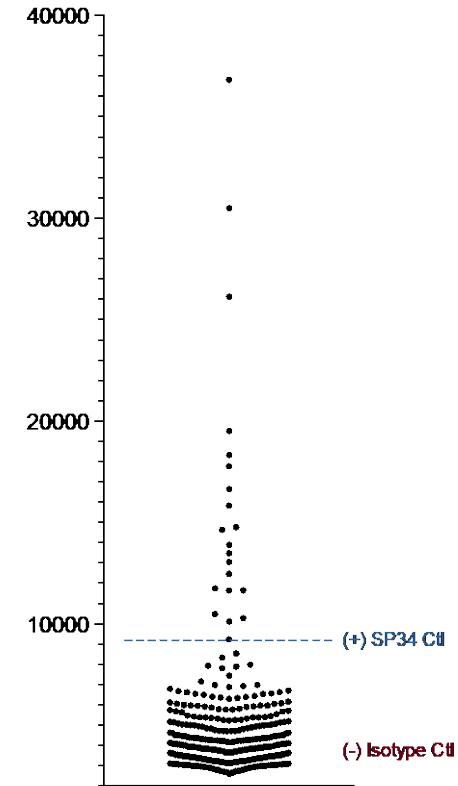


Cyno CD3EG

KD: 67 - 200 nM



HT-Flow Cytometry Screen Hu T Cell Binding

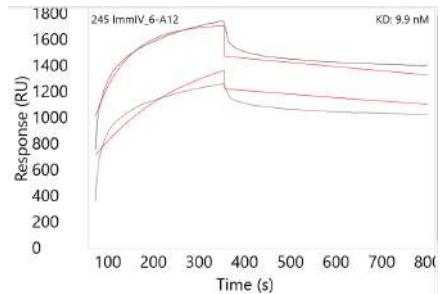


StableHu Sorted
Repertoire

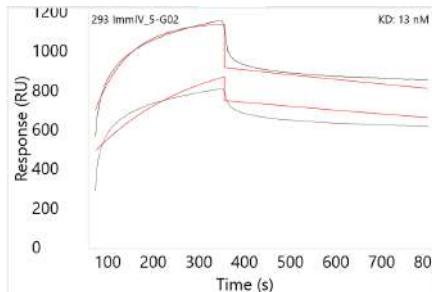
CD3 Antibody Hits – Epitope Mapping by Engineered Epitope SPR

Epitope 1

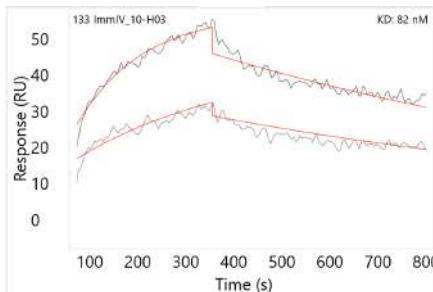
Hit 1



Hit 2

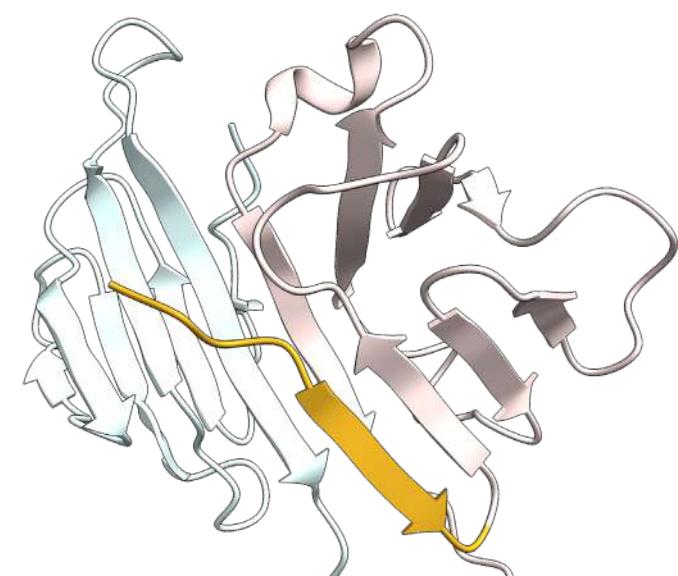
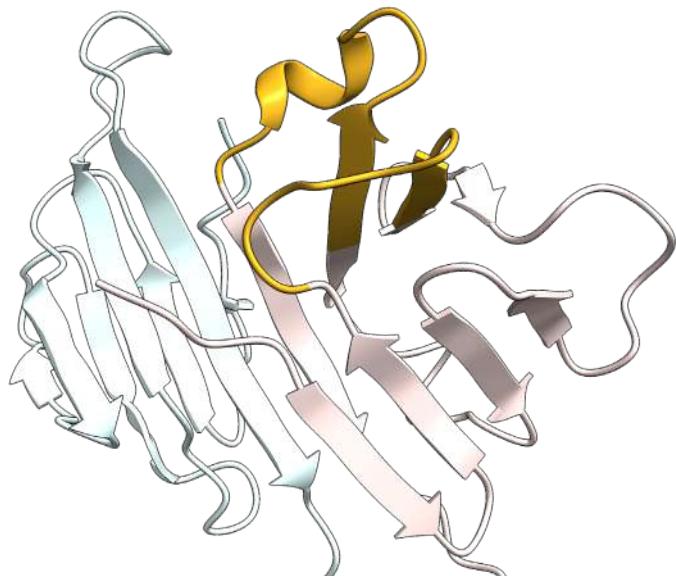
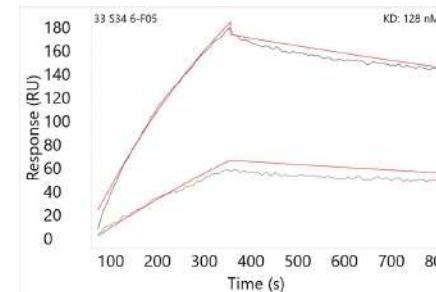


Hit 3

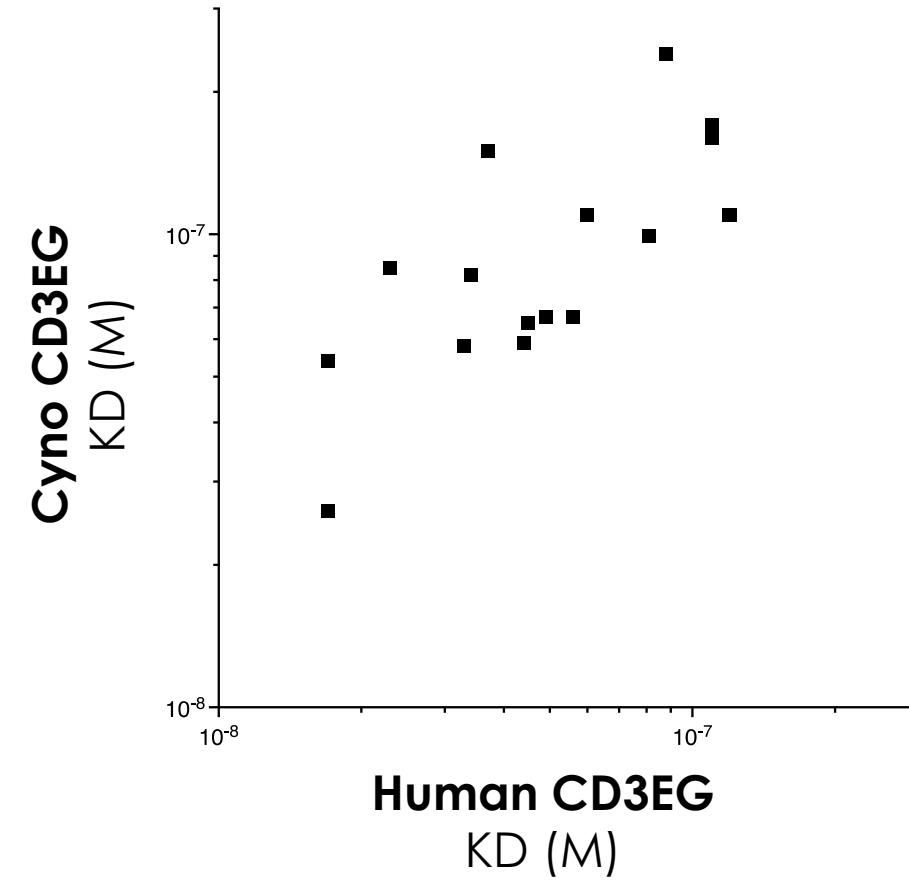
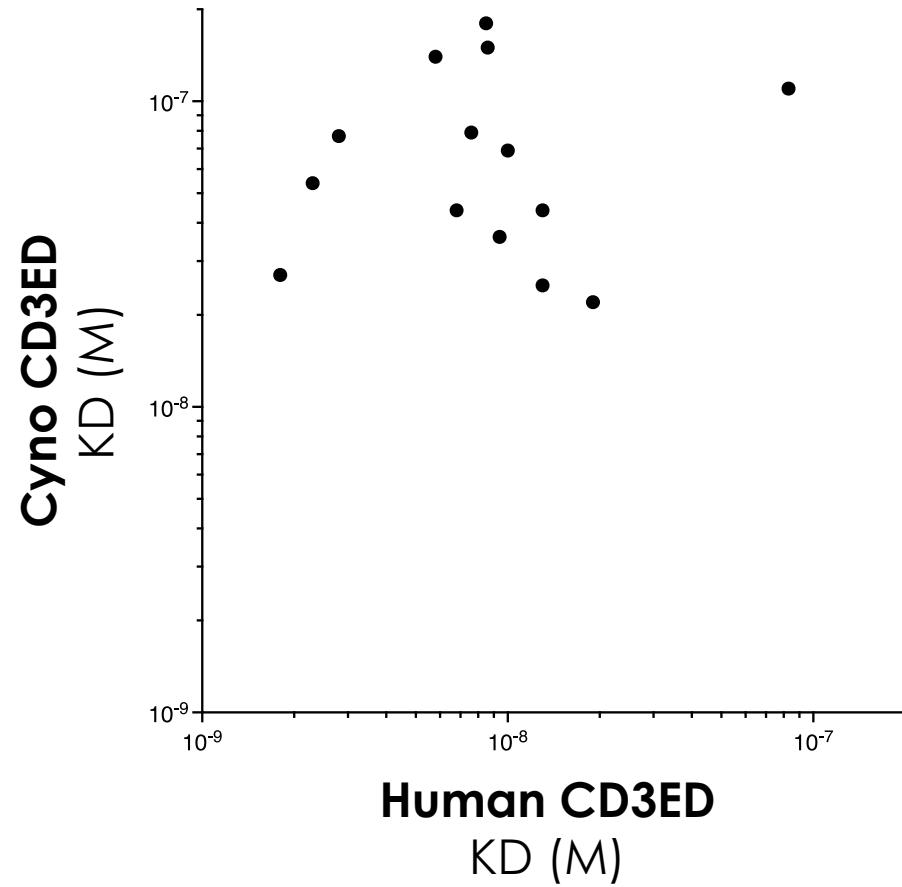


Epitope 2

Hit 4



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

