AI-Powered Antibody Discovery

Unlocking High-Throughput Biology and Drug Discovery

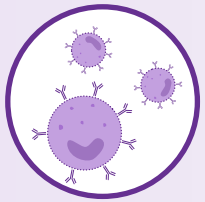
May 2023

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

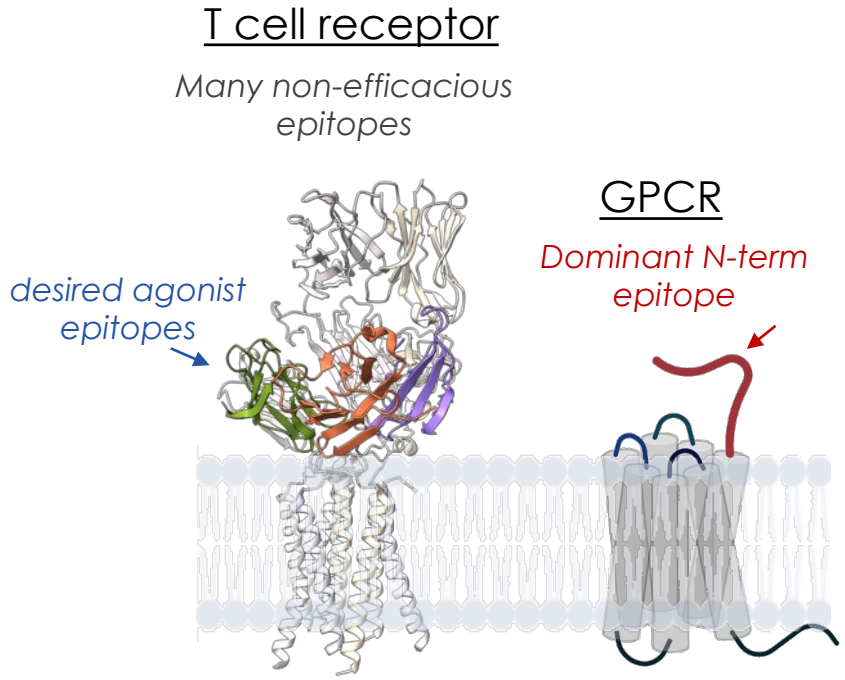
Multiple validations with difficult targets and MoAs

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]

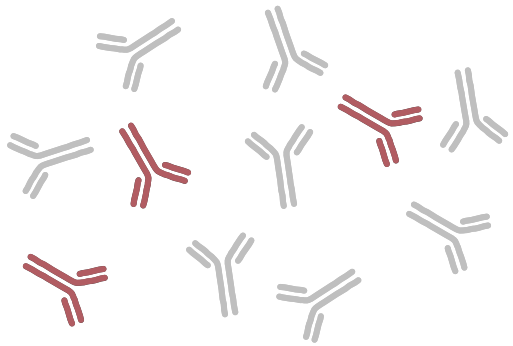


(1) Wicker *et al.*, *Eur. J. Immunol.* (1984)14, p.447
(2) Victora *et al.*, *Cell* (2015) 163, p.545
(3) Nakra *et al.*, *J. Immunol.* (2000) 164, p.5615
(4) Trkulja *et al.*, *Sci. Adv.* (2021) 7:16, p.eabe6397
(5) Sesterhenn *et al.*, *Science* (2020) 368, p. eaay5051

Engineered Epitopes Focus Antibody Repertoires On Desired Binding Sites

1

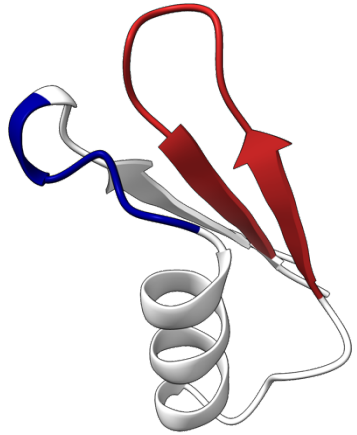
Naïve in vivo or in vitro antibody library



epitope-specific Ab

2

Focus library with engineered epitopes

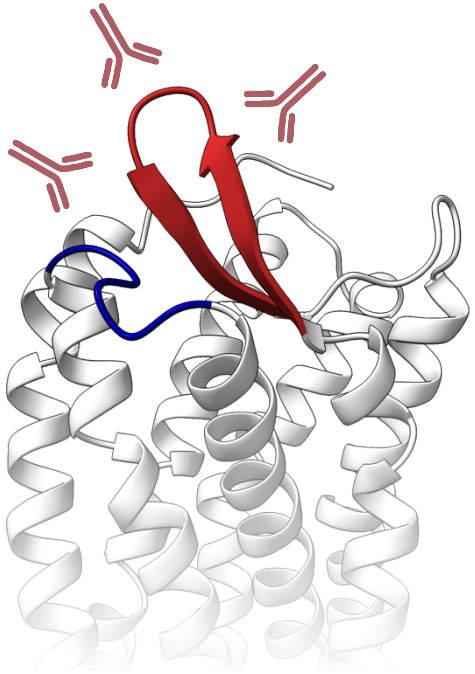


epitope

de novo scaffold

3

Efficient discovery of epitope-specific Abs

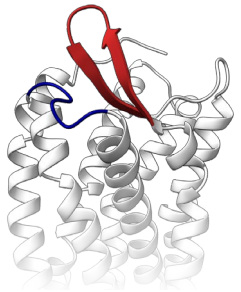


full length target

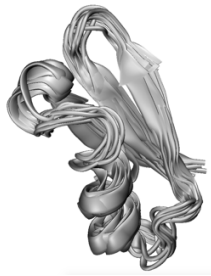
AI-Engine Optimizes Engineered Epitope Structure, Stability, and Solubility

Engineered Epitope Design Objectives

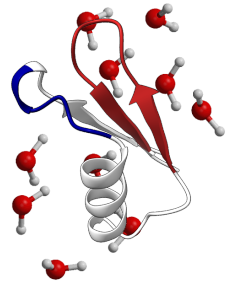
1. **Match Structure to Target**



2. **Refine for Greater Stability**



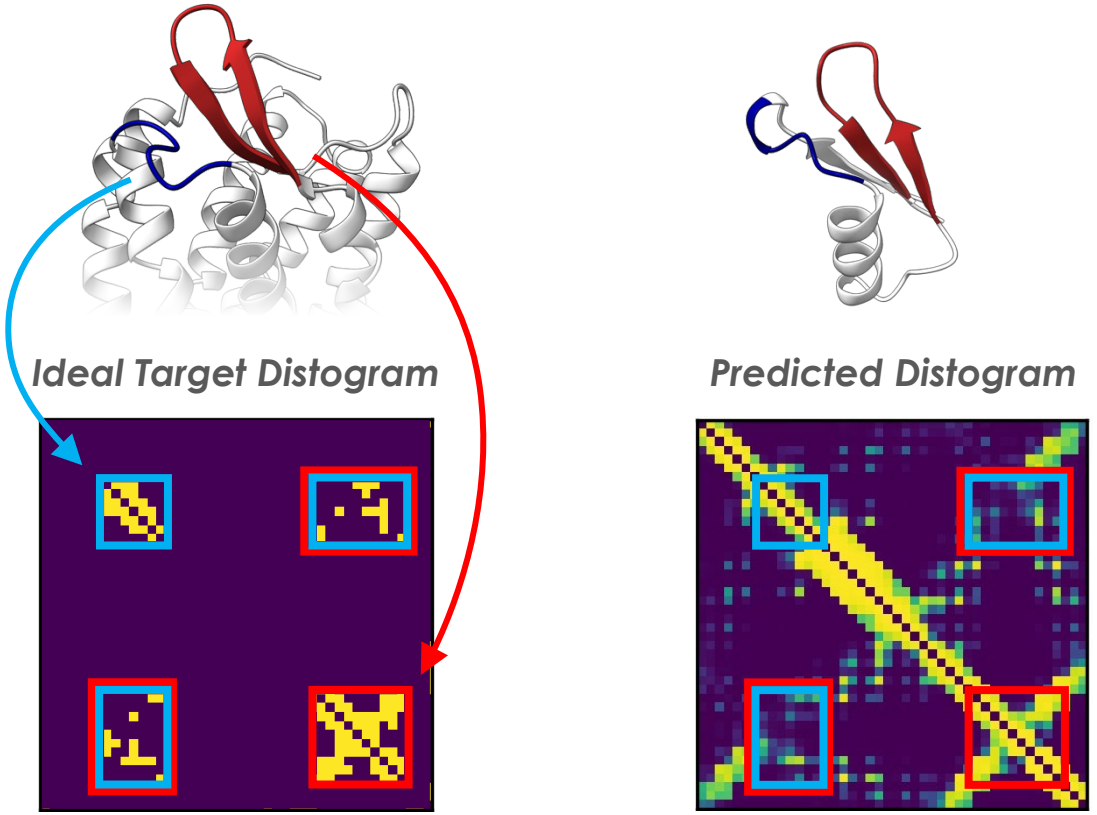
3. **Optimize for Water Solubility**



AI Discovery Engine

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

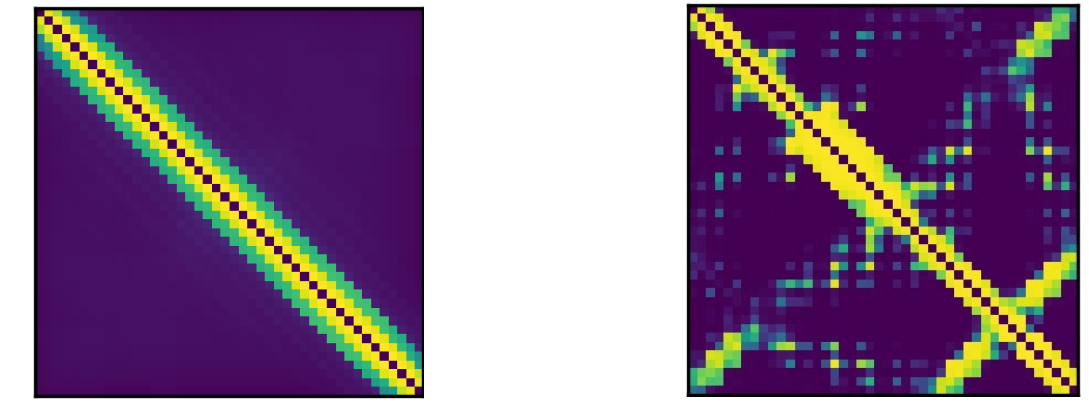
Loss Term #1



Minimize Cross-Entropy between engineered & target epitope residues

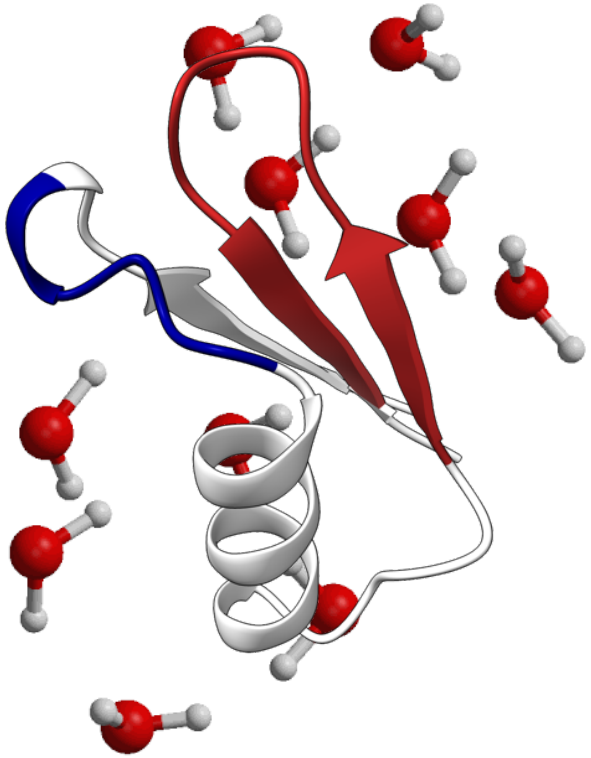
Loss Term #2

Average predicted distograms across random sequence space



Maximize KL-Divergence between unstructured coil and engineered epitope

Multi-Loss Function Optimizes Engineered Epitope Solubility



Loss Term #3

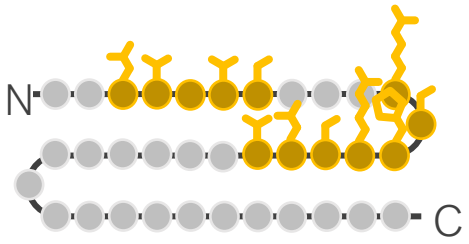
Amino Acid Hydropathies

I: 4.5	V: 4.2	L: 3.8	F: 2.8
C: 2.5	M: 1.9	A: 1.8	G: -0.4
T: -0.7	S: -0.8	W: -0.9	Y: -1.3
P: -1.6	H: -3.2	E: -3.5	Q: -3.5
D: -3.5	N: -3.5	K: -3.9	R: -4.5

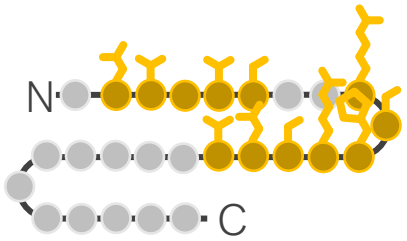
Average hydropathy is minimized

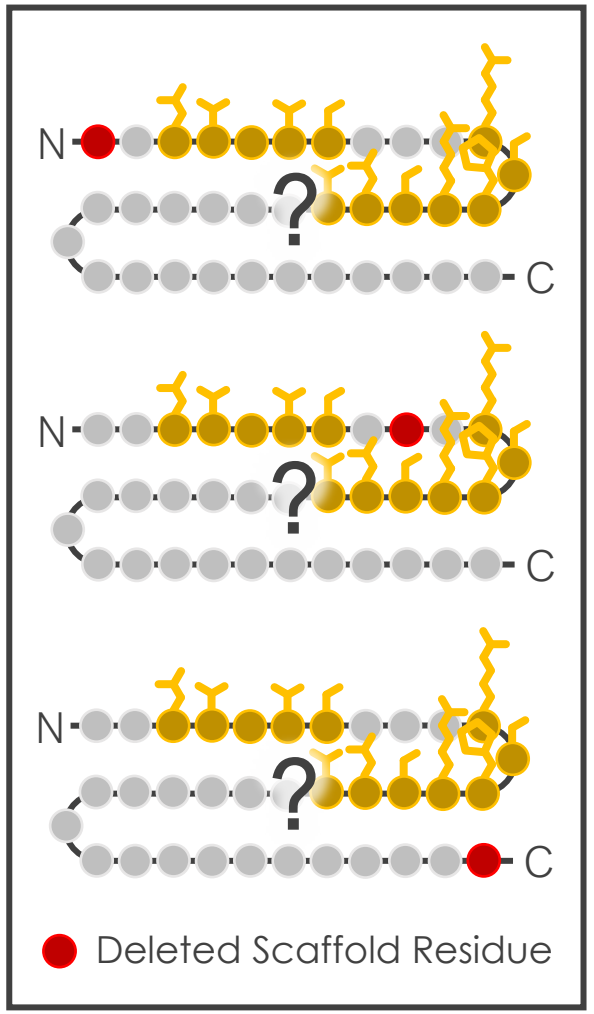
Engineered Epitopes are Further Optimized by Maximizing the Epitope-to-Scaffold Ratio to Reduce Scaffold-Specific Antibodies

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



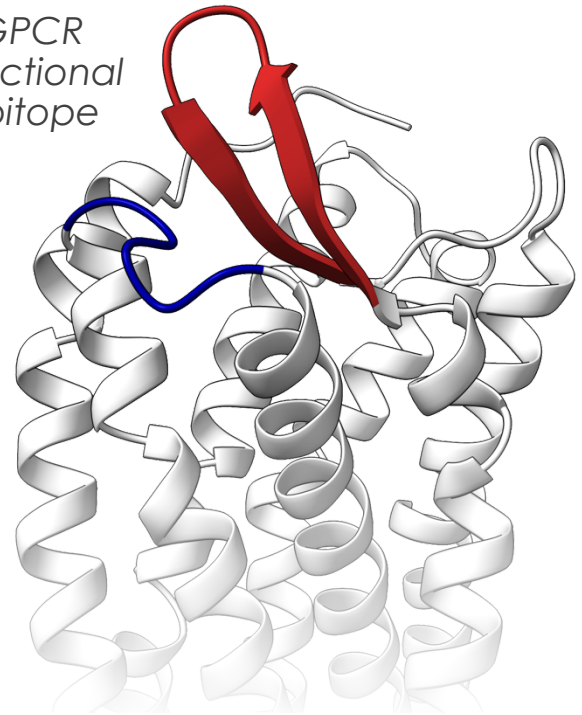
Optimized Topology = $\frac{\#\text{Epitope residues}}{(\#\text{Scaffold} - \#\text{Deleted})}$





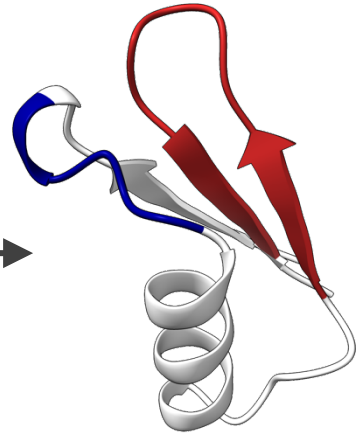
Iteratively trim scaffold residues until epitope destabilizes

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



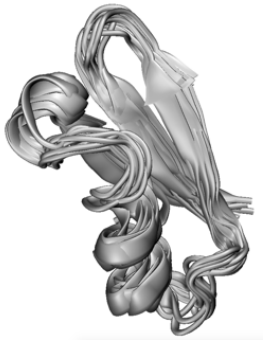
GPCR Junctional Epitope

AI Discovery Engine



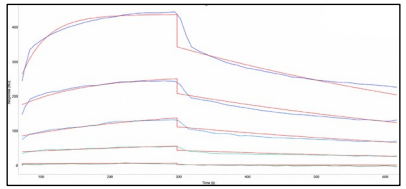
Folding Simulations

e.g. AlphaFold2



Antibody Binding

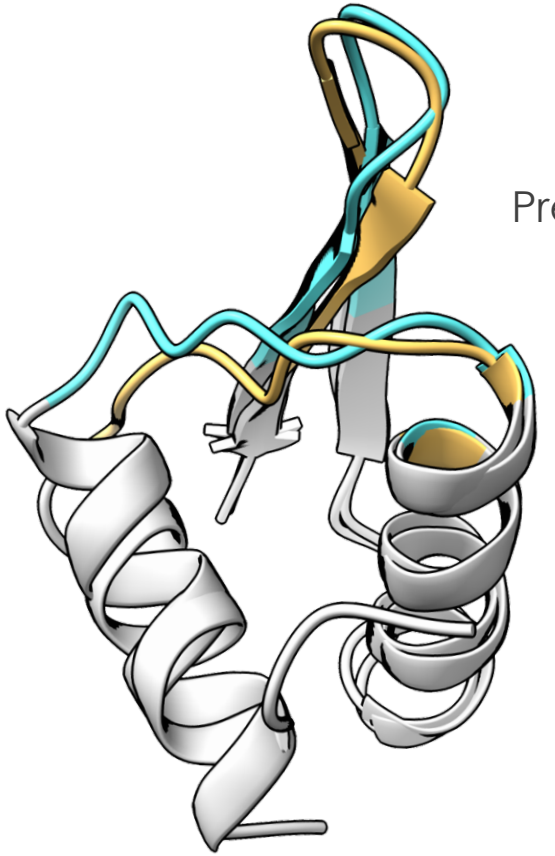
e.g. SPR



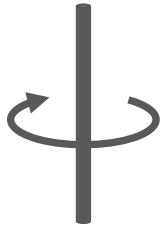
NMR, Melting Temp.

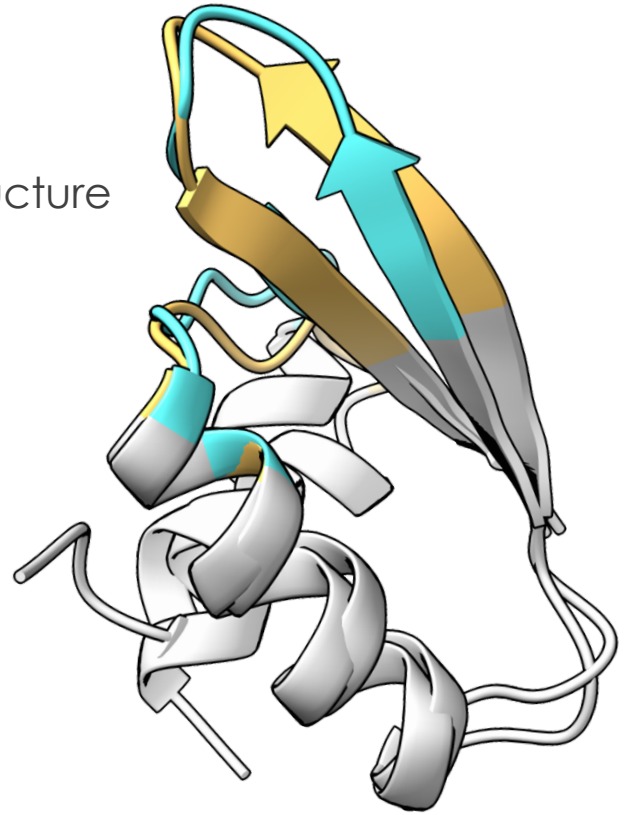
NMR Structure Validates Engineered Epitope Design Engine

NMR Solved Structure
Engineered Epitope Engine

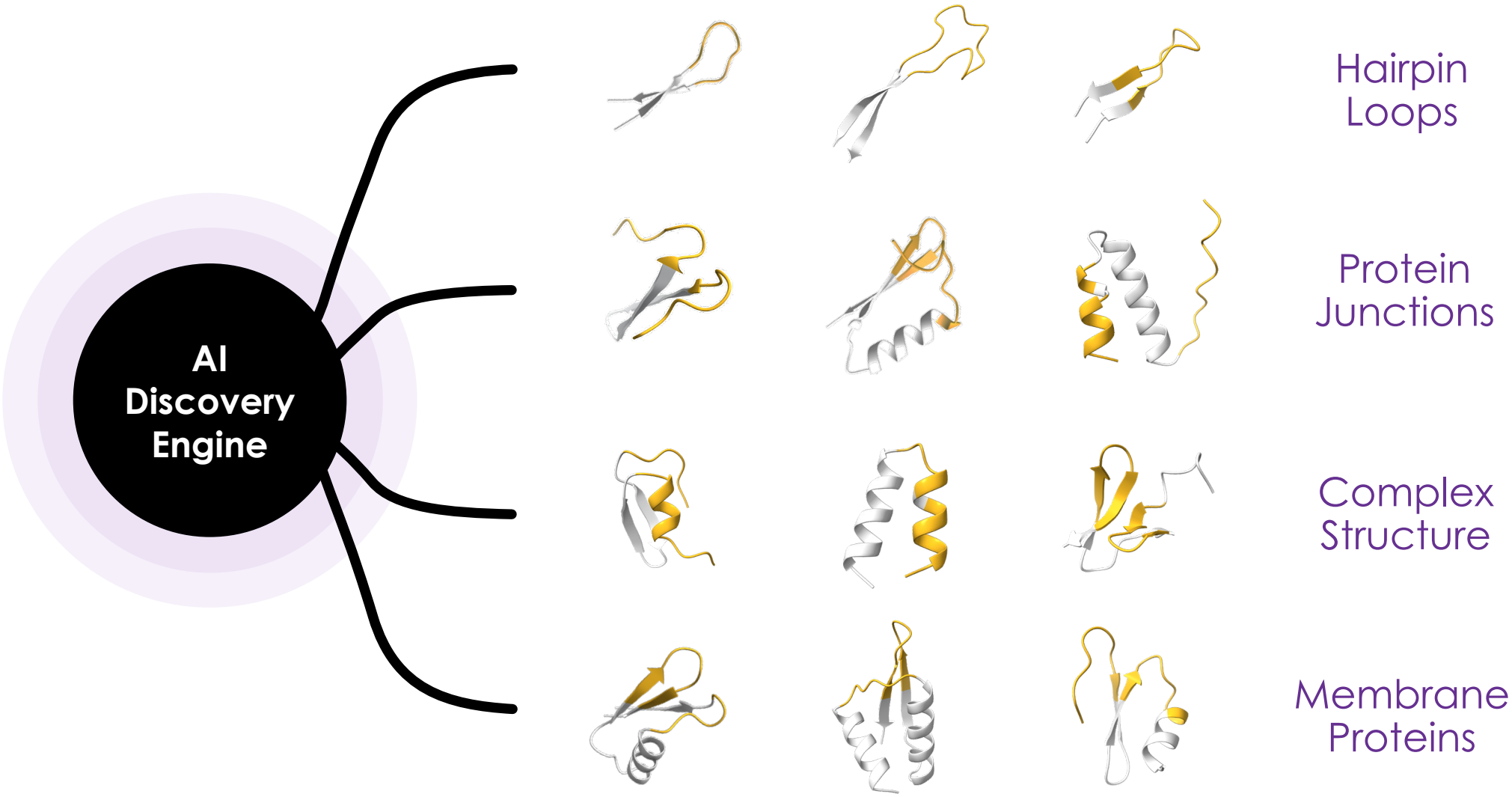


Predicted vs. Actual epitope structure
Backbone RMSD: **1.6 Å**



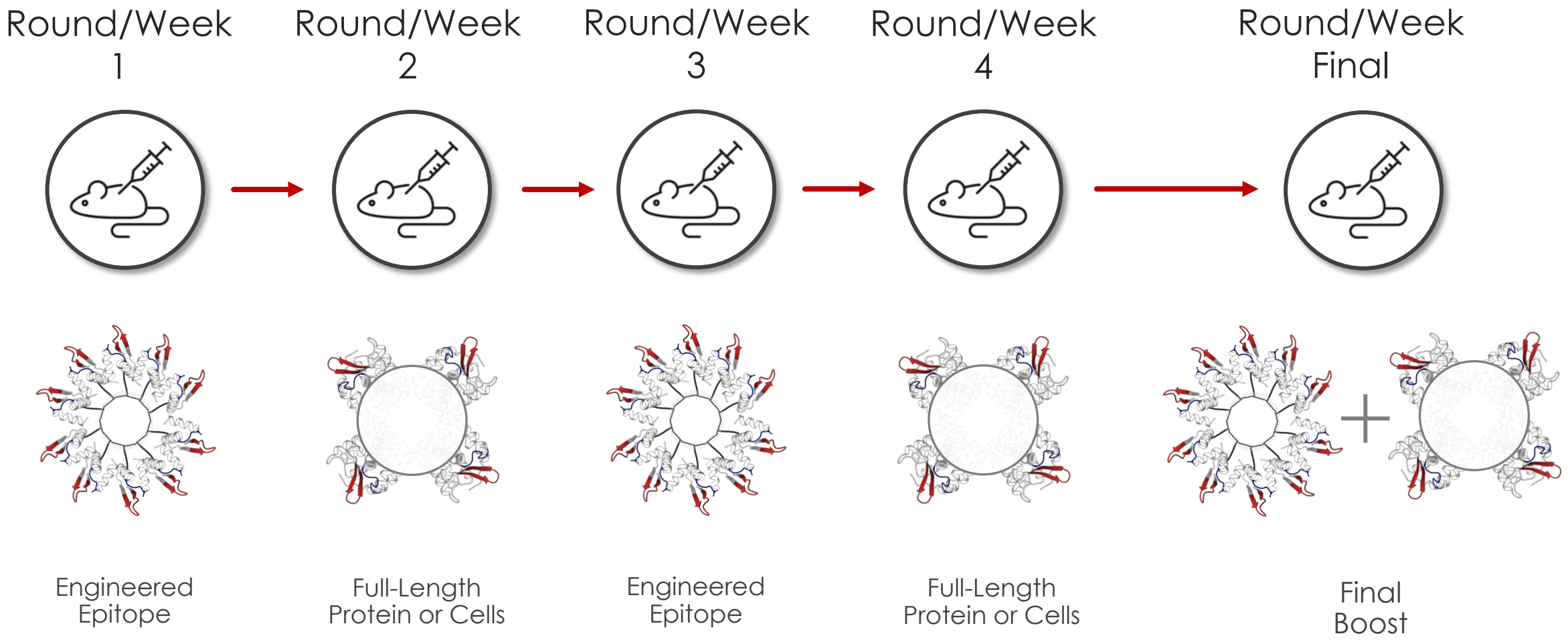


Engineered Epitopes Are Generalizable to a Broad Set of Targets



Engineered Epitopes Steer Immunization and In Vitro Libraries to Target Epitopes

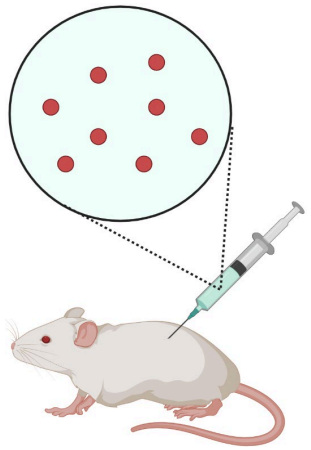
Engineered epitopes alternated with full length protein/cells steers immunizations and in vitro selections while enforcing full length protein and cell binding



Immunized Repertoires Are Cloned and Screened Via Two Tracks

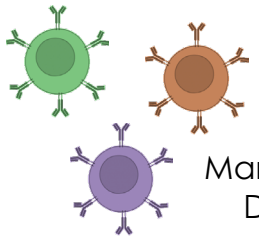
Multi Epitope-Steered Immunization

Engineered epitopes & T cells



Dual-Track Library Display

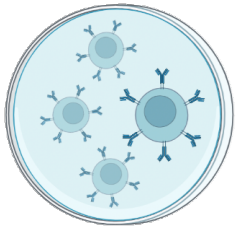
High-throughput Track 1



Mammalian Display

Immunized Repertoire

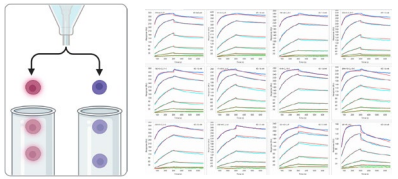
Traditional screen Track 2



Hybridoma

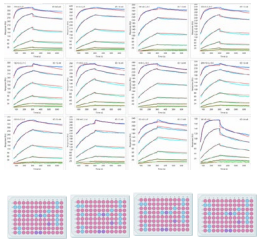
Multi Dimension Screening

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

Library Display

Mammalian

10^6 diversity

High developability

Phage

$>10^{10}$ diversity

High diversity

HT screen to identify hits

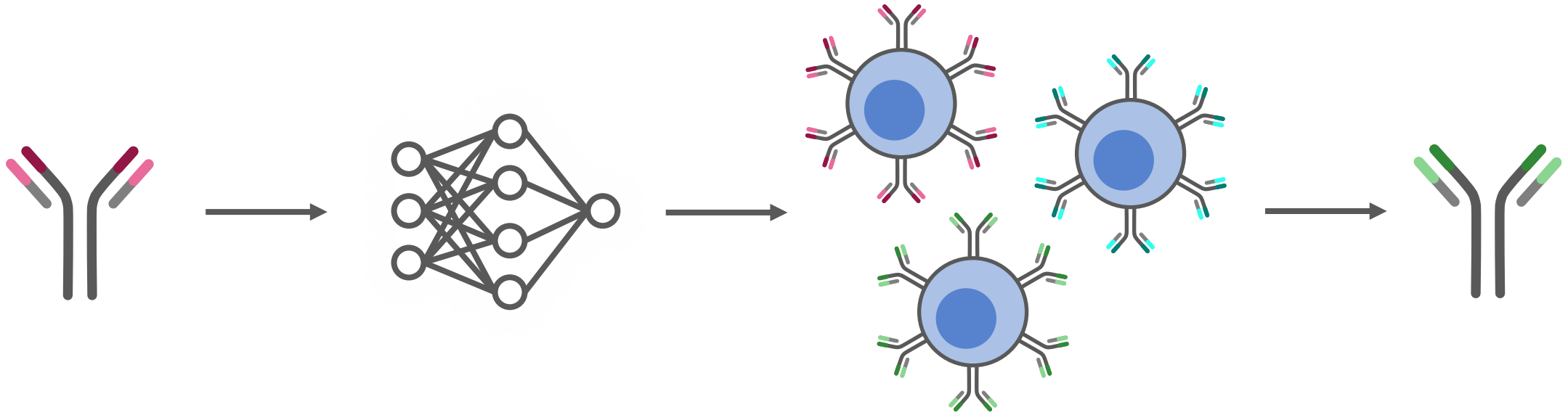
StableHu™ Optimizer Generates Focused Library Diversity Within the Capacity of Mammalian Display

Input Antibody

StableHu Optimizer AI-Engine

Mammalian Display

Output Antibody



Template CDR

Predict library of human CDR variants

HT screen mammalian display CDR library

Optimized antibody with fully human CDRs

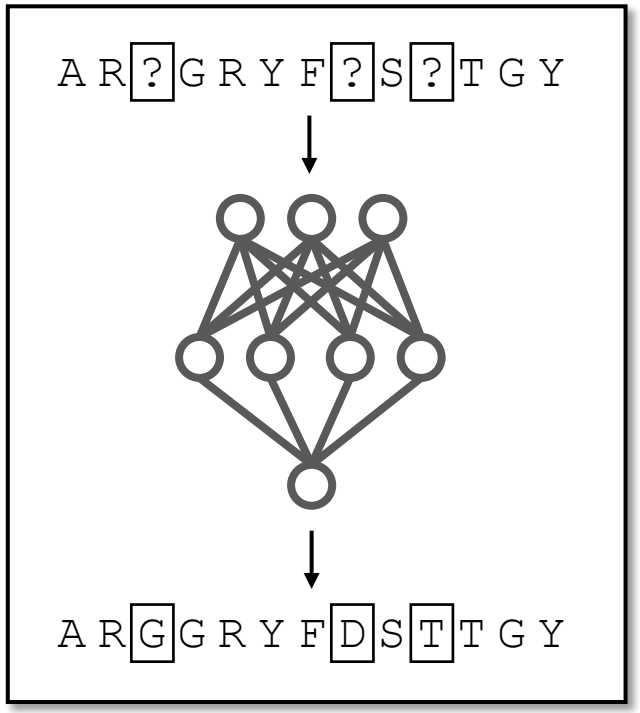
Optimizer AI Model is Trained to Predict Fully Human CDR Sequences

Antibody Database

cAb-Rep & OAS
Hu Ig databases

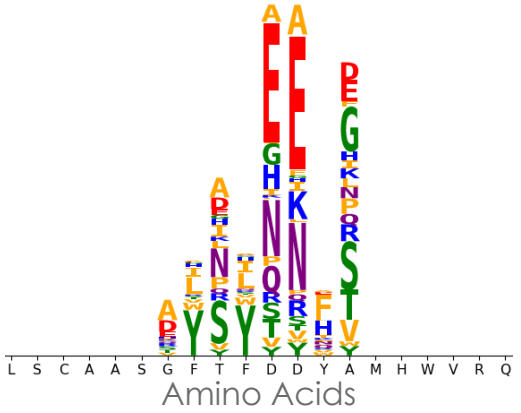
>1 billion curated human antibody sequences

Optimizer AI



AI trained to predict fully human CDR from masked CDR

Trained Model

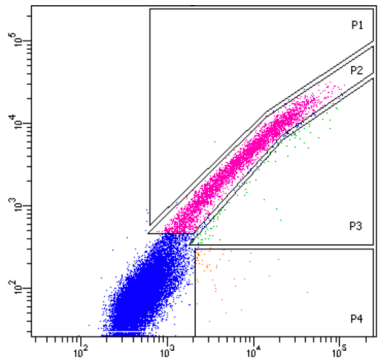


Predict library of fully human CDRs from template CDR

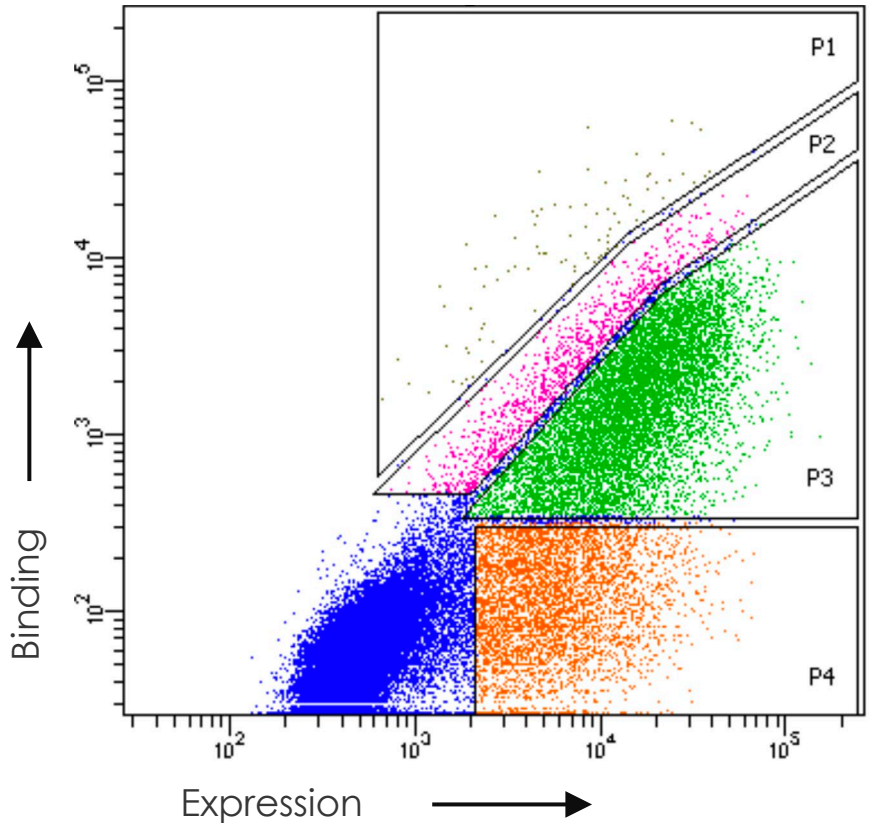
StableHu Library Sorting and NGS Identify Improved Human CDR Variants

Mammalian Display Single-Cell Sorting

Template Ab



StableHu Library



Sorting Bin

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

Sorted Bins
NGS &
HT SPR Screen

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

Mammalian-Display sorted clones →

Scored Sequences

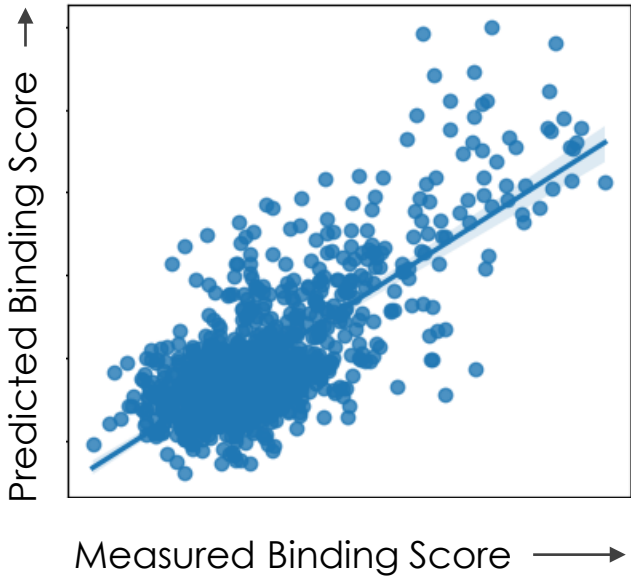
Rank	Sequence	Score
1	QQSYSTGPPT	2.45
2	QQSYGNPPT	2.35
3	QQGYSSPAT	2.32
4	QQSYSEPTT	2.31

⋮

Score: sorting bin and/or affinity

→

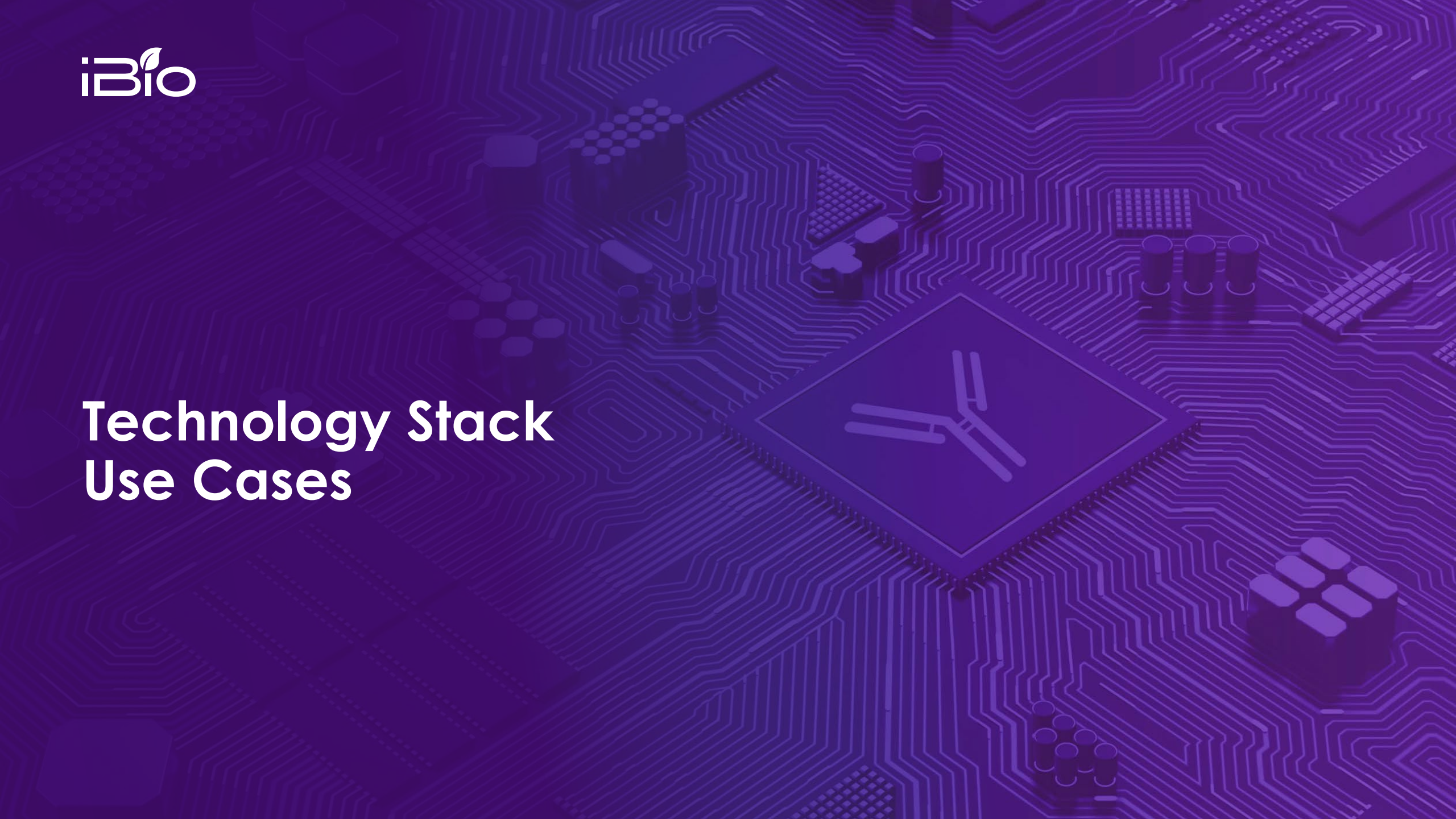
Predictive Model



Train, then test model on hold-out set

→ HT Screen for further optimized variants

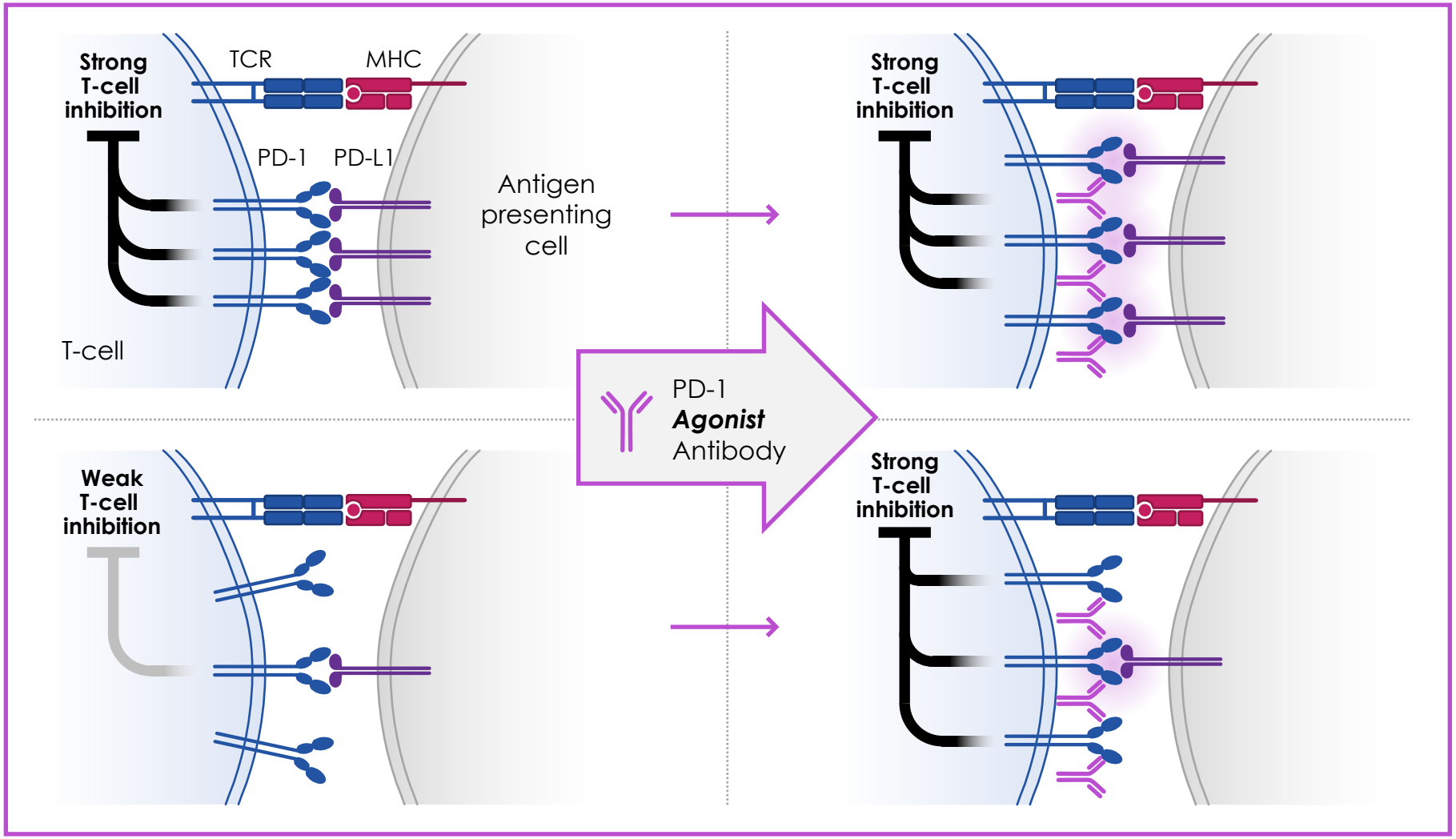
Technology Stack Use Cases



Agonist Epitope

PD-1 Checkpoint Agonist Antibody

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



Autoimmunity

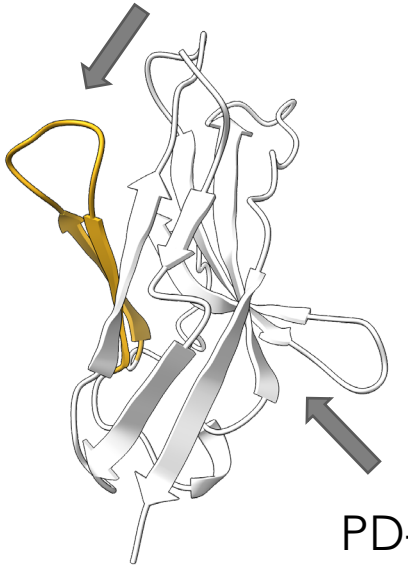
Improved

Healthy Tissue
Low-inflammation preserved

Diseased Tissue
Reduced inflammation

Parallel Paths to PD-1 Agonist Antibody Discovery

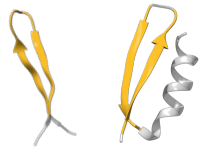
Known PD-1 agonist epitope



PD-L1 binding site

AI Discovery Engine

Engineered Epitope Immunization



AI-Designed Immunogens

Screen Fully Human Naïve Antibody Library

$>10^{10}$ diversity

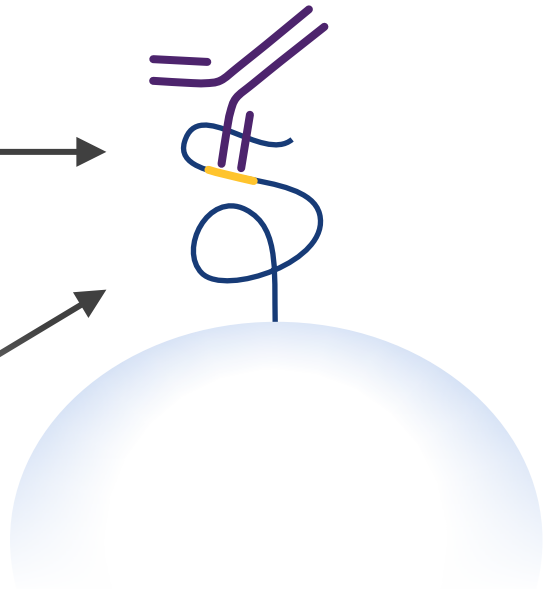
Clinically validated, developable antibody frameworks

Antibody Optimizer

Template Antibody

Optimized Antibody

PD-1 Agonist Antibodies

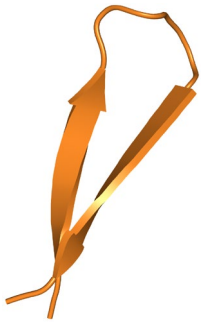


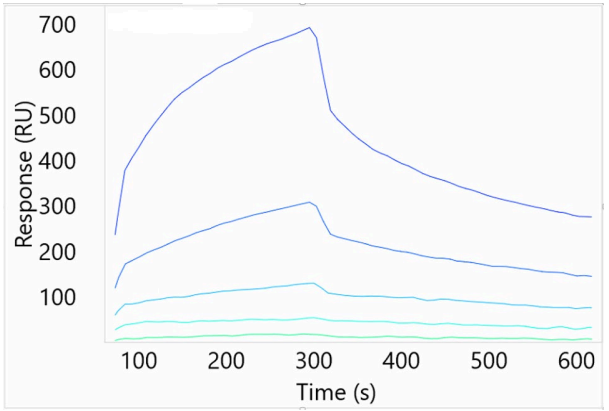
Engineered Epitopes Are Validated By Binding to a Known Antibody or Ligand

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

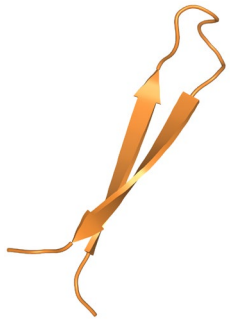
Top PD-1 Agonist Epitope Designs

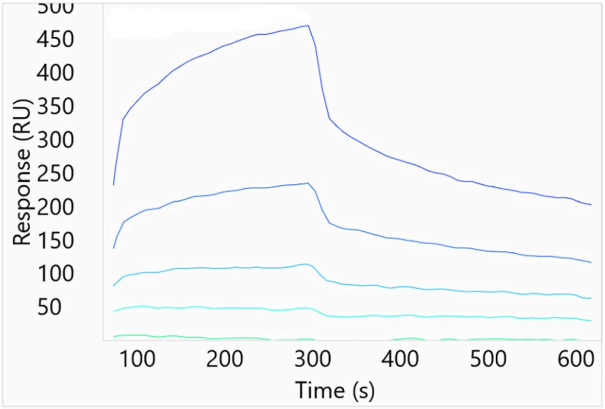
Design 1



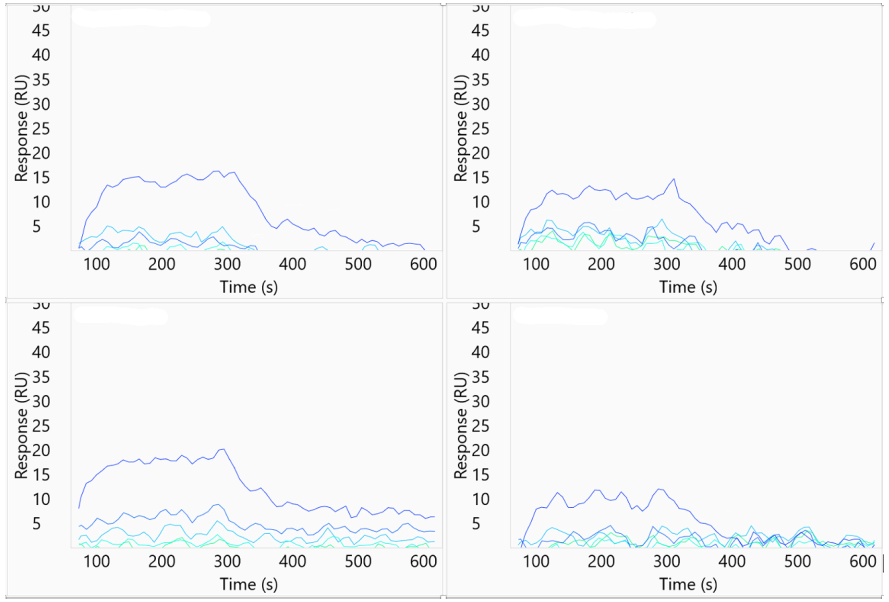


Design 2



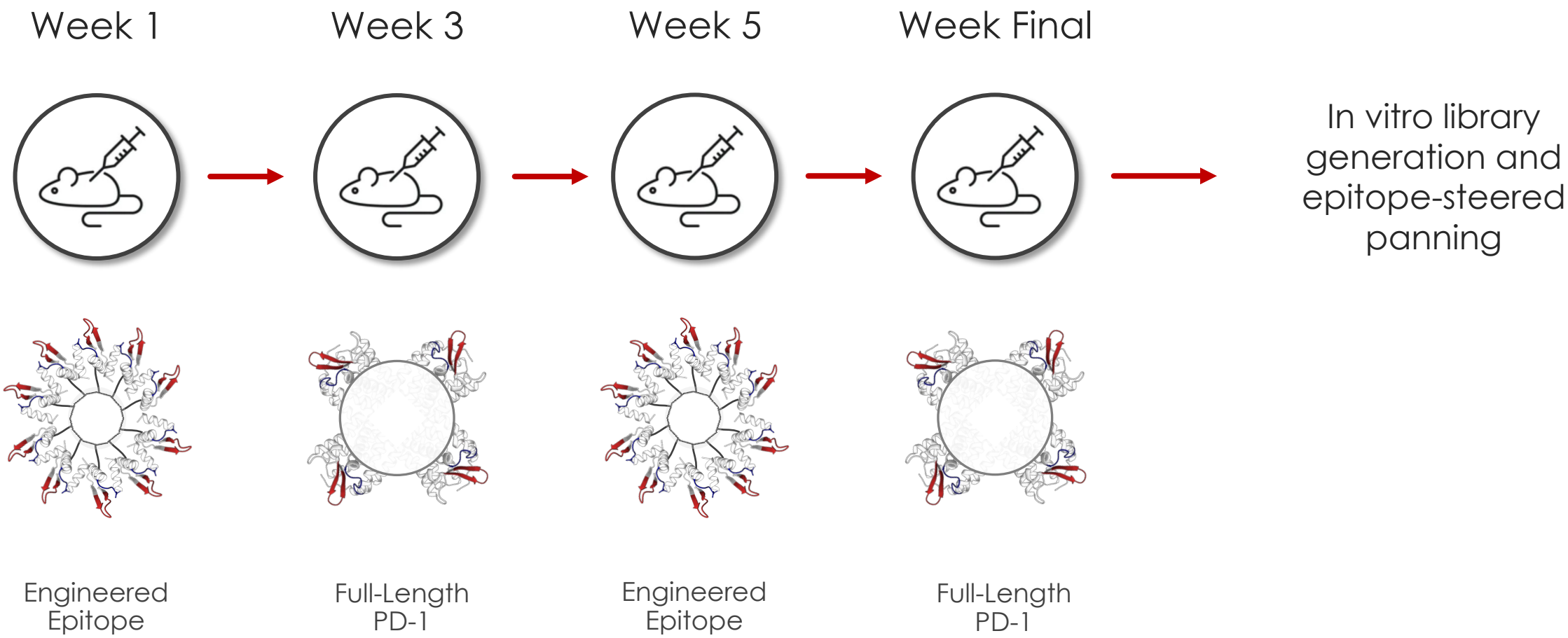


Ineffective Agonist Epitope Designs



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

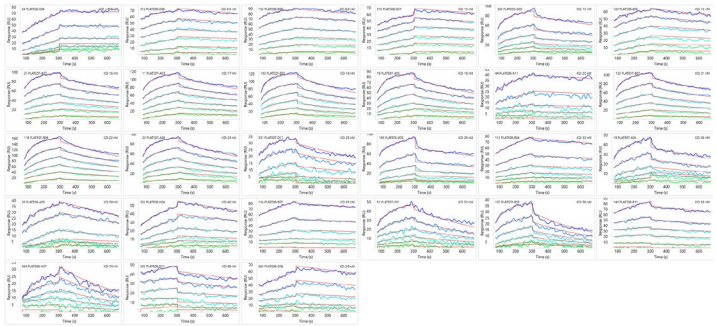


PD-1 Agonist Epitope-Steered Immunization & In Vitro Selection Enriched Towards Non-Antagonist Hits

Epitope-Steered
Mostly non-antagonist hits

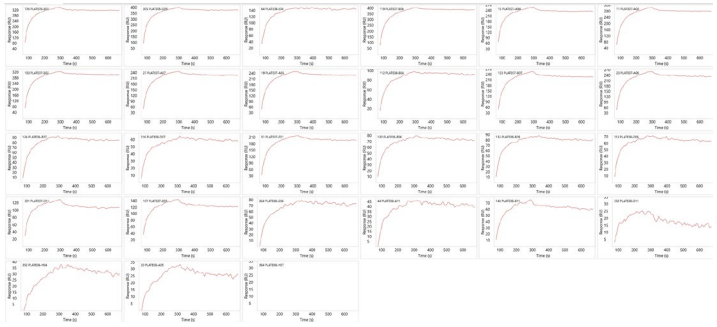
27 PD-1 binding hits
KD: 1 – 80 nM

PD-1 binding HT-SPR



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

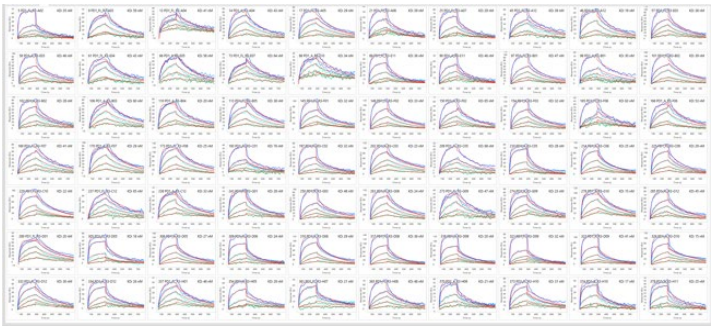
PD-1 antagonist Ab competition HT-SPR



Not Epitope-Steered
All antagonist hits

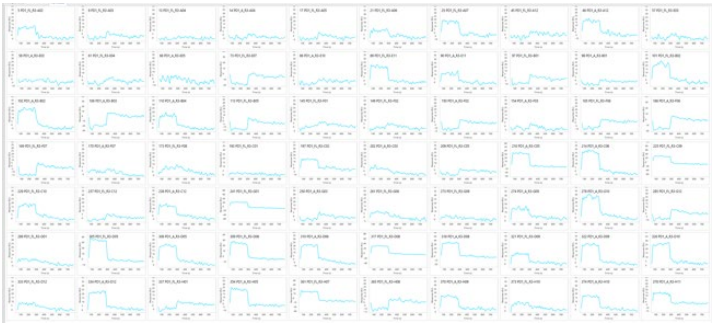
70 PD-1 binding hits
KD: 10 – 80 nM

PD-1 binding HT-SPR



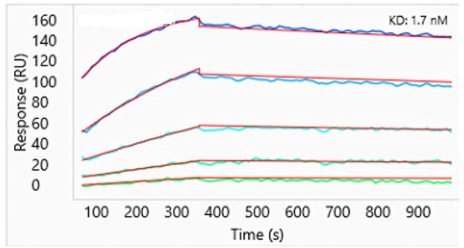
70/70 **do** compete with PD-1 antagonist Ab

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

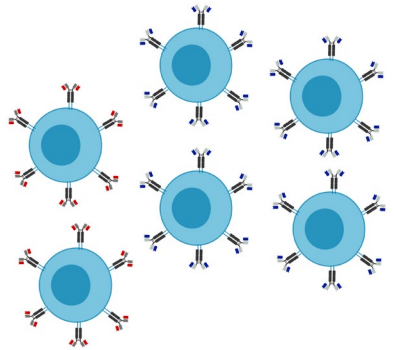
Template Ab Mu CDRs

AI-model predicts human CDRs

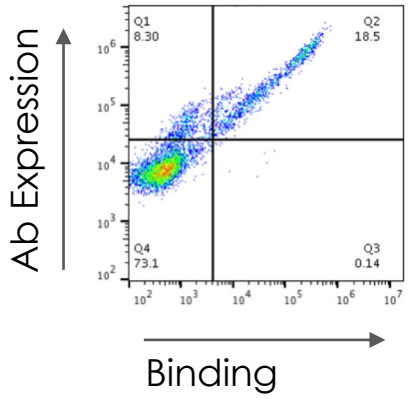
Fully human heavy & light chain CDRs diversity

HCDR1	HCDR2	HCDR3
4000	4000	4000
LCDR1	LCDR2	LCDR3
4000	2162	4000

Per-CDR mammalian display library



Single-cell sorting: binding & expression



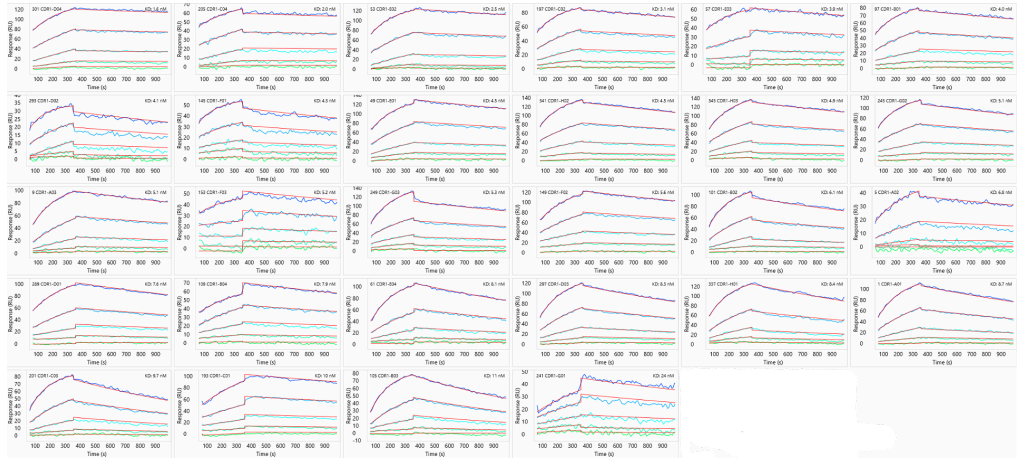
NGS

HT-SPR hit validation and quantitation

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

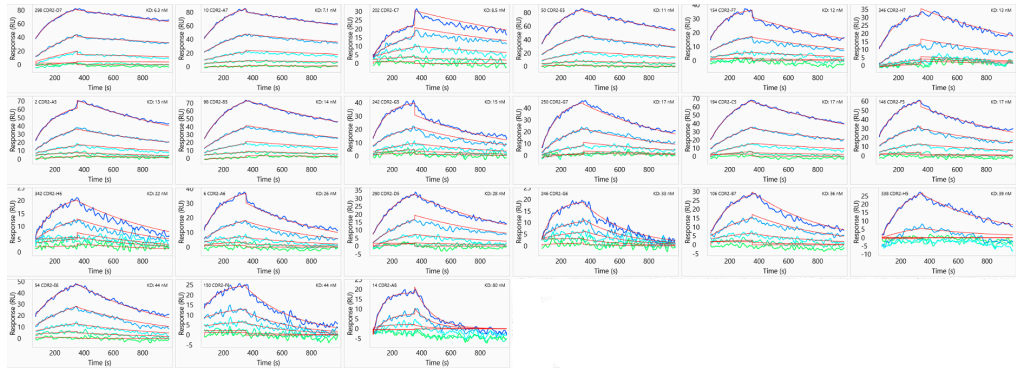
Fully-Human HCDR1:
28 hits

KD: 1.6 - 25 nM



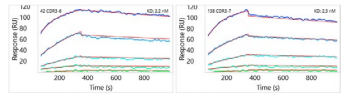
Fully-Human HCDR2:
21 hits

KD: 6.3 - 80 nM



Fully-Human HCDR3:
2 hits

KD: 2.2, 2.3 nM

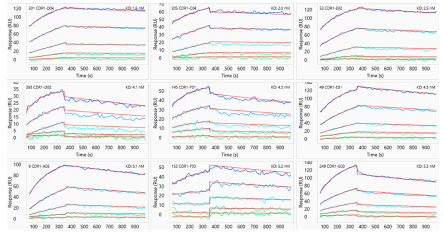


Many fully-human
LCDR1, 2, 3
hits identified

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

Starting with individual fully-human CDR hits

SPR confirmed hits:

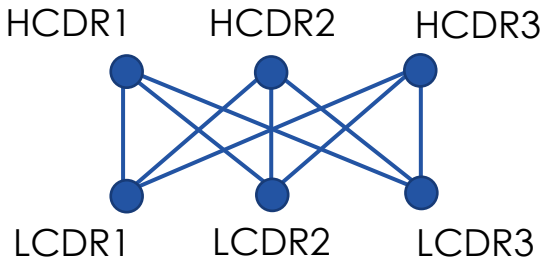


HCDR1,2,3

LCDR1,2,3

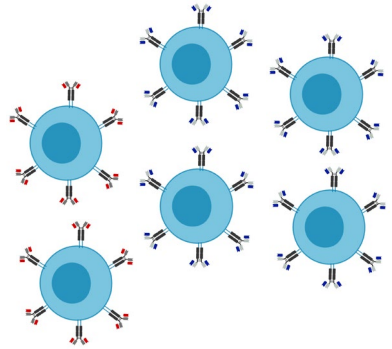
Combine human CDRs

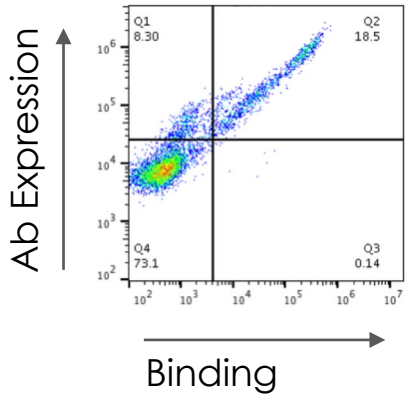
Fully human CDRs combinatorial diversity



Mammalian display library

Single-cell sorting: binding & expression





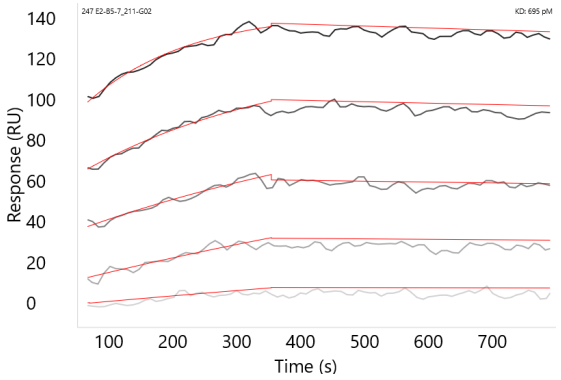
NGS

HT-SPR hit validation and quantitation

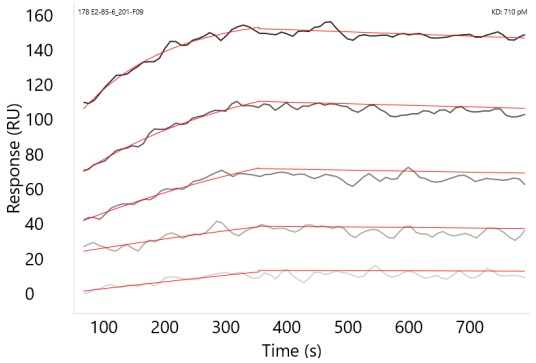
Combining Individual Fully-Human H/L CDR123 Hits Improves Affinity and Humanness

Top Four Fully-Human CDRs StableHu Hits

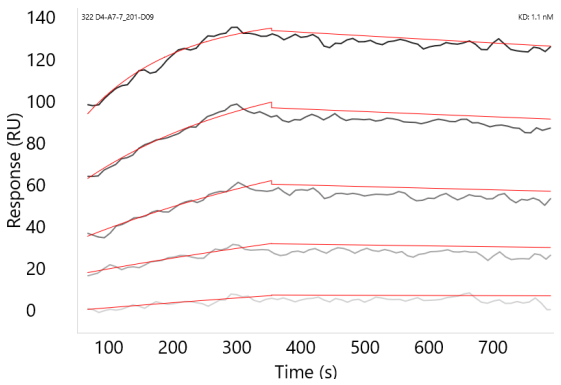
KD = 695 pM



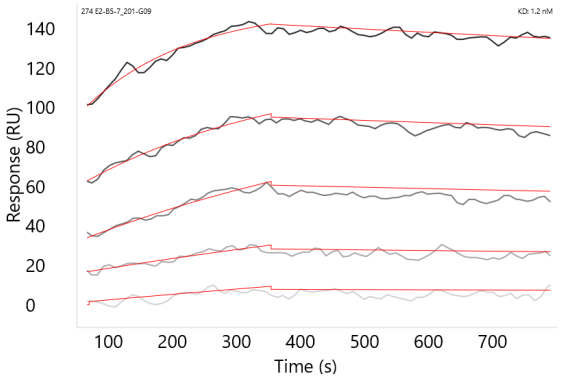
KD = 710 pM



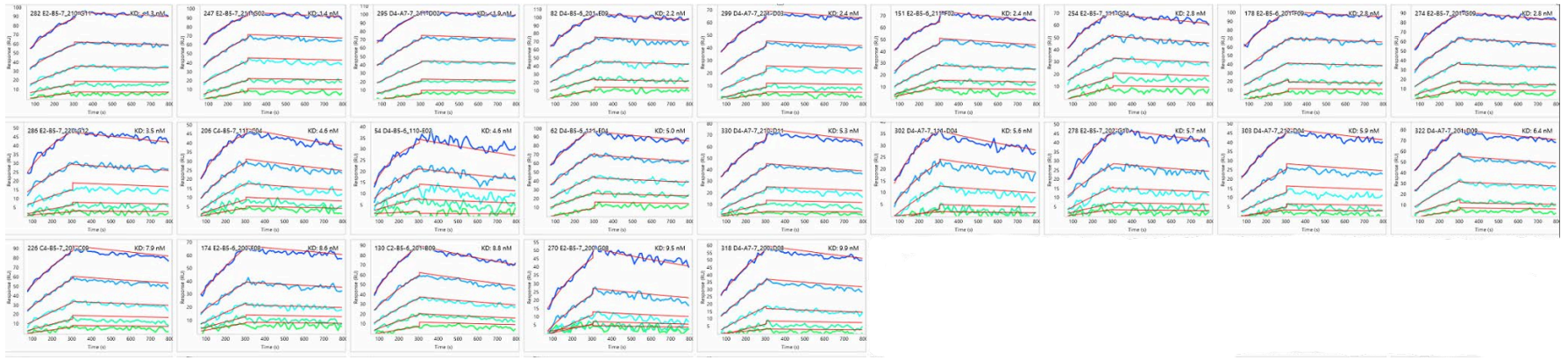
KD = 1.1 nM



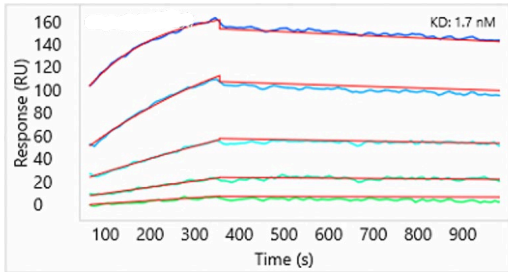
KD = 1.2 nM



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



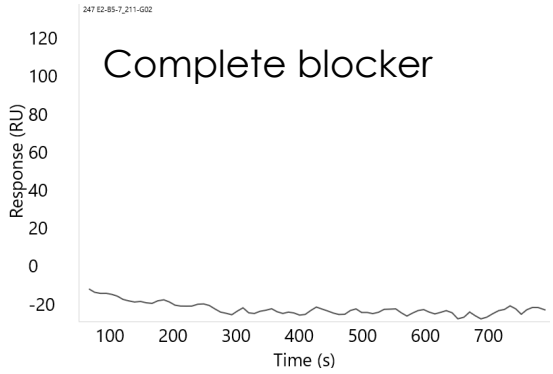
Starting Mu CDRs template

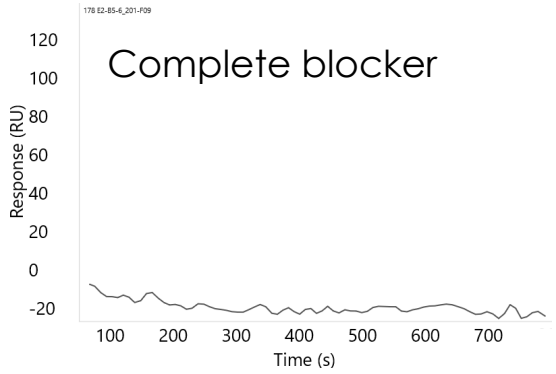


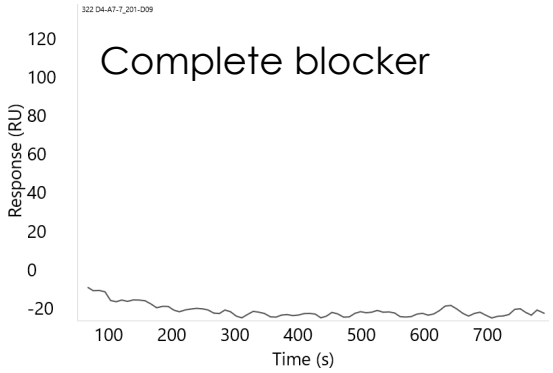
KD = 1.7 nM

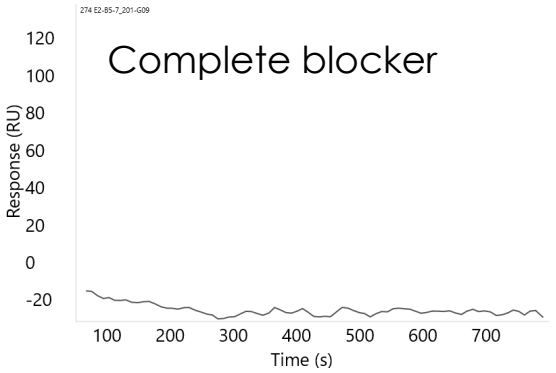
All StableHu Hits Cross-Block Starting Template Antibody With Mu CDRs

Top Four Fully-Human CDRs StableHu Hits



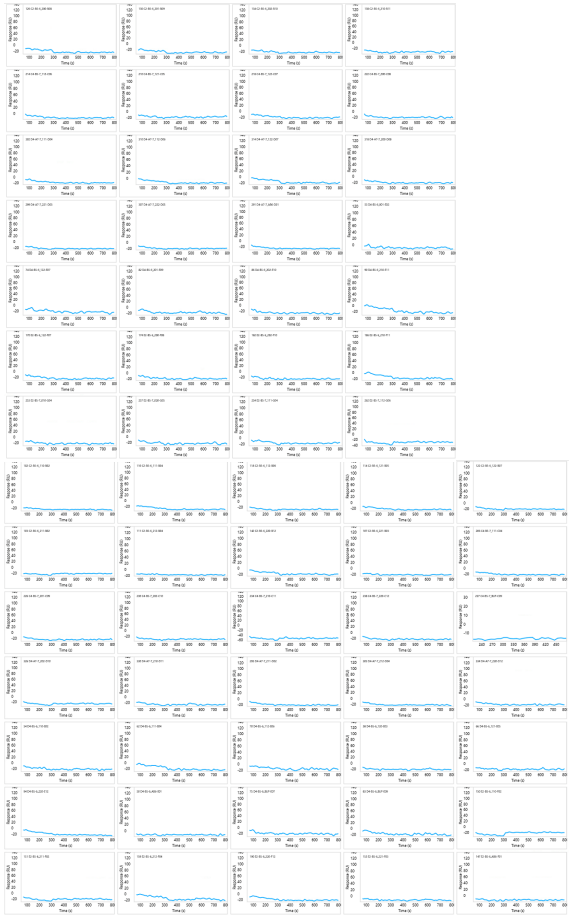




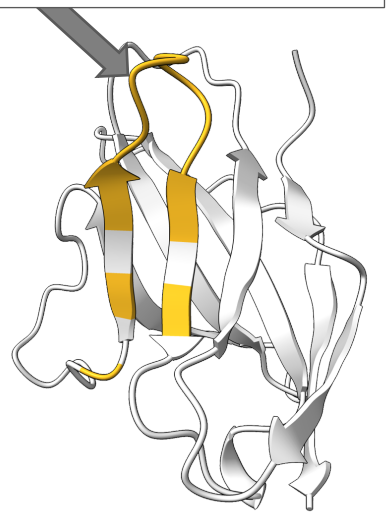


Remaining Fully-Human CDRs StableHu Hits

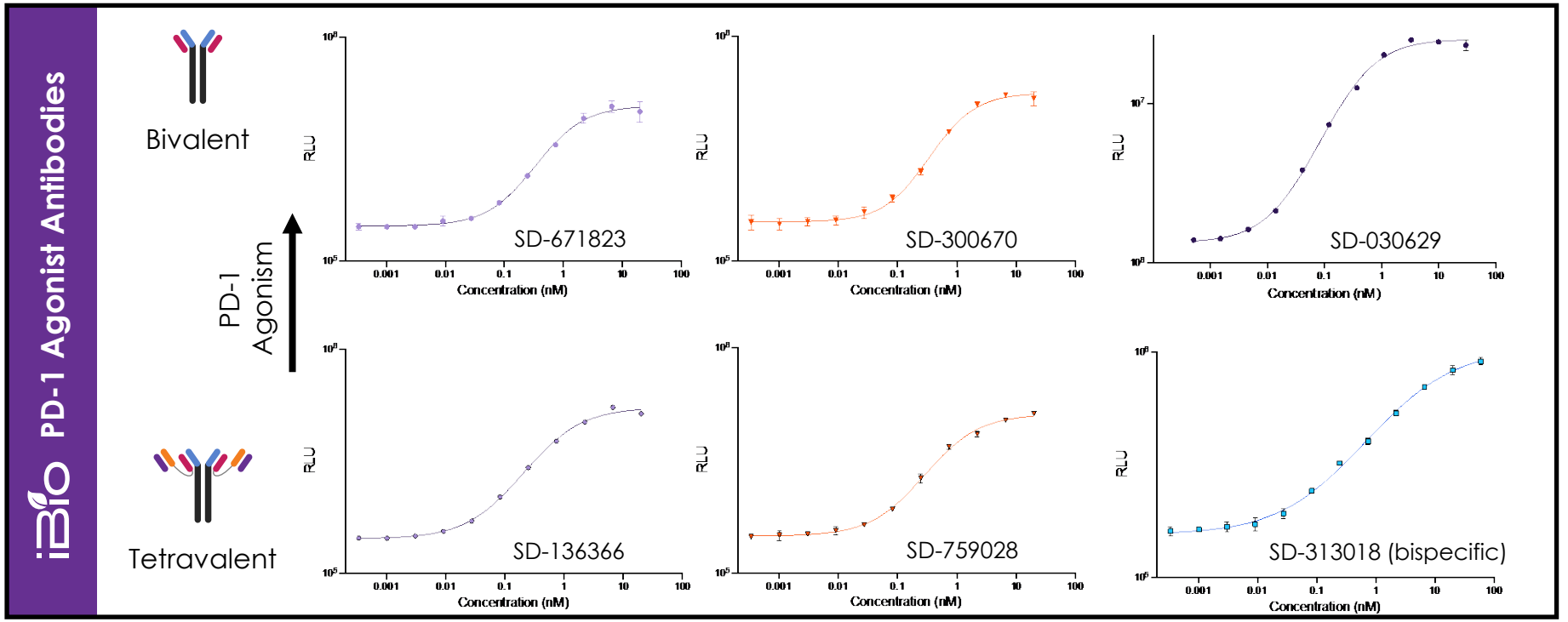
Complete blockers

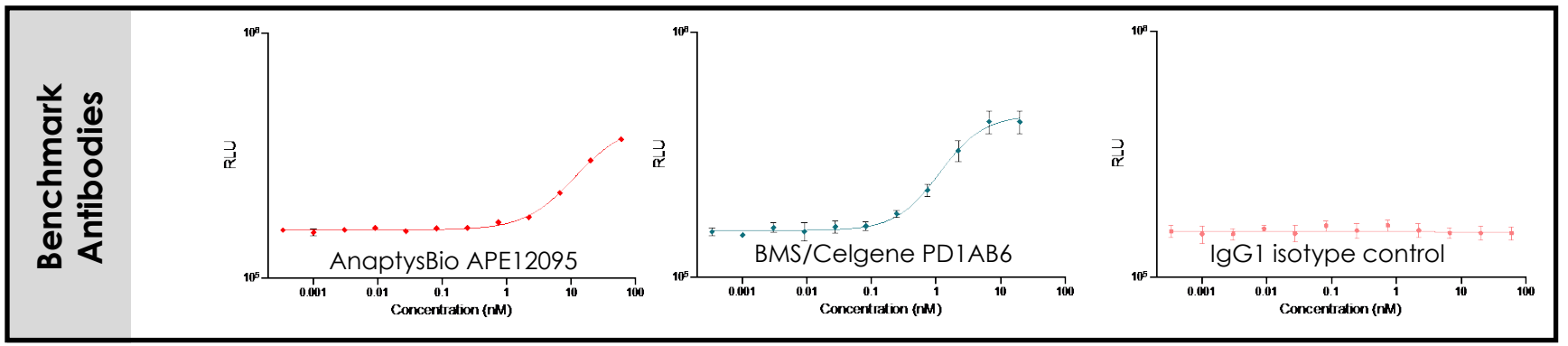


StableHu hits cross-block the template PD-1 agonist antibody



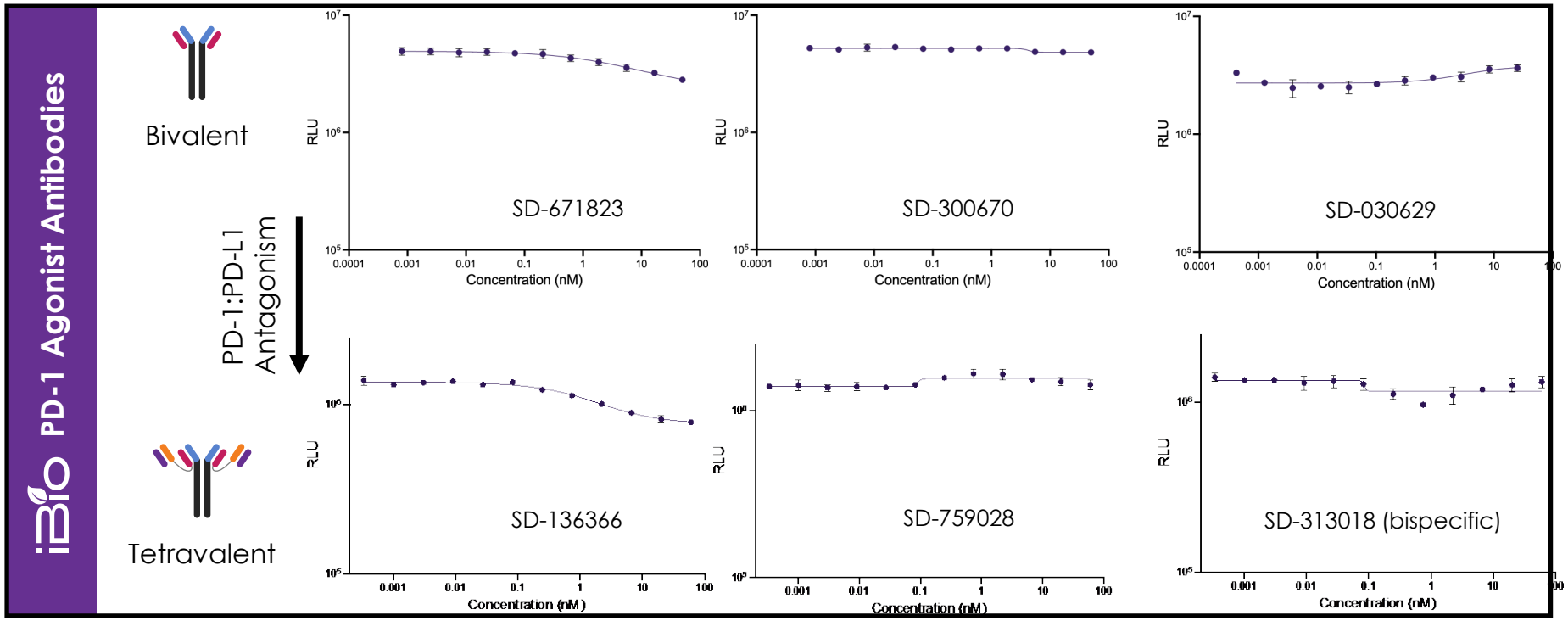
In vitro PD-1 Agonism Equals or Surpasses Benchmarks

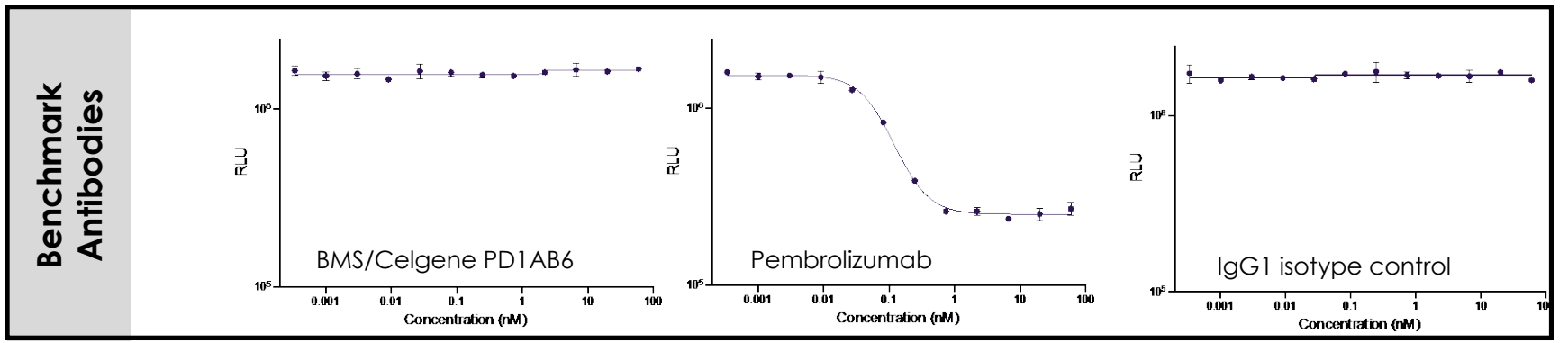




Ab ID	EC50 (nM)
SD-671823	0.88
SD-300670	0.31
SD-030629	0.36
SD-136366	0.28
SD-759028	0.52
SD-313018 (bispecific)	0.30
AnaptysBio APE12095	17.4
BMS/Celgene PD1AB6	0.76
IgG1 isotype control	inactive

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

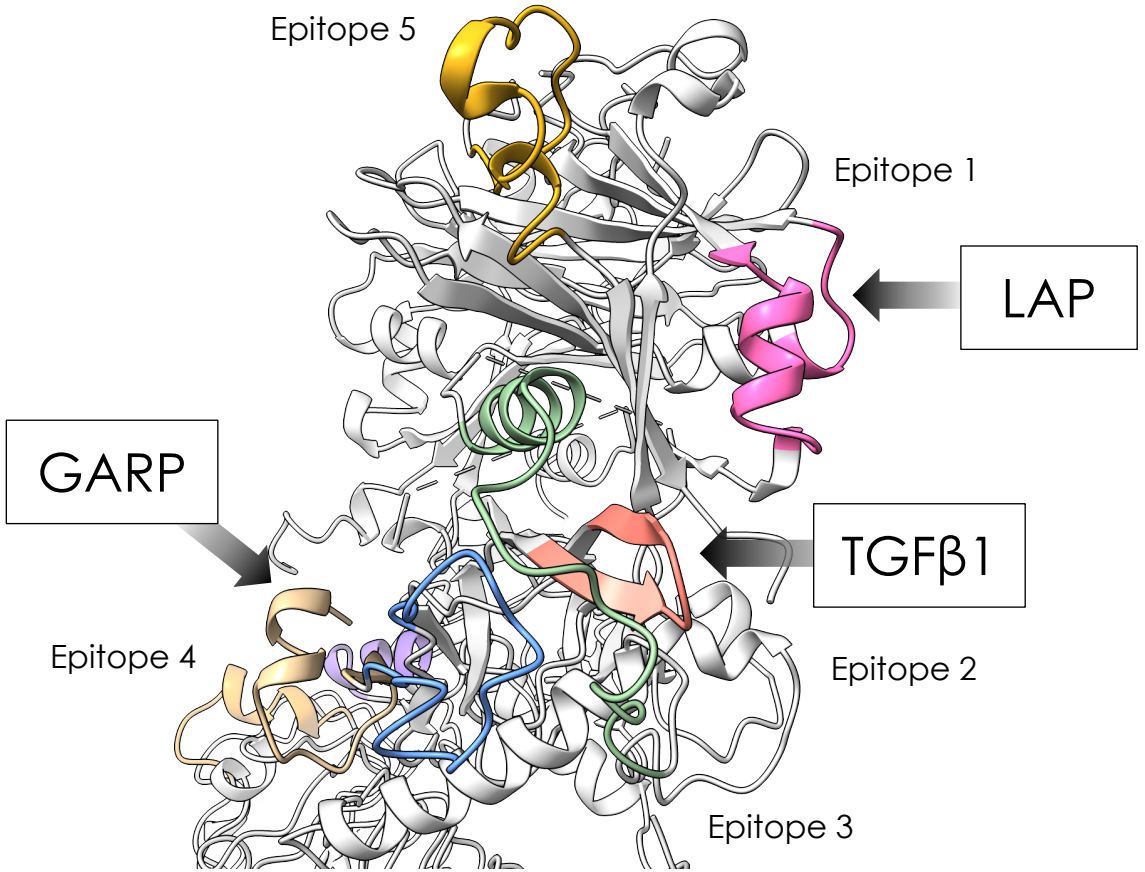
Multi-Protein Junctional Epitope

Latent-TGFβ1 Antibody

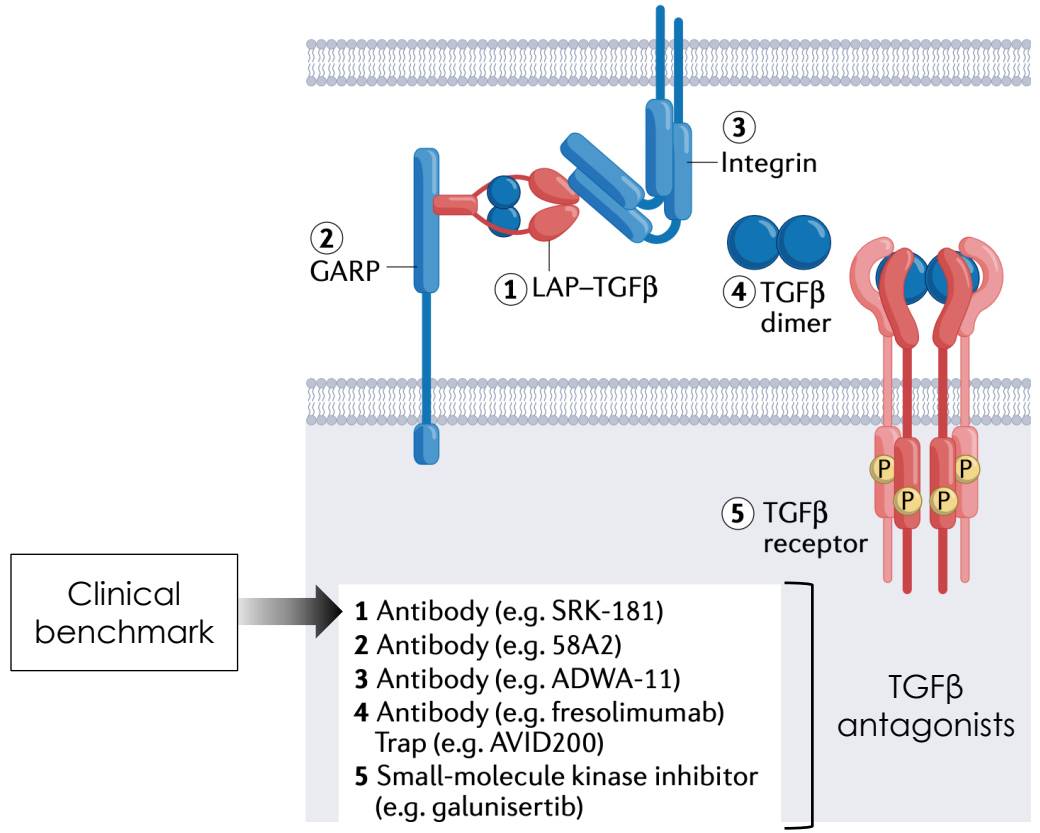
Latent-TGFβ1 Multimeric Complex Regulates TGFβ1 Release and Signaling

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

Latent-TGFβ1 Multimeric Structure



Released TGFβ1 is Immunosuppressive

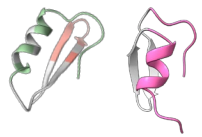


Nat. Rev. Immunol. (2022) 10.1038/s41577-022-00796-z

Parallel Paths to Latent-TGFβ1 Antibody Discovery

AI Discovery Engine

Engineered Epitope Immunization



AI-Designed Immunogens

Screen Fully Human Naïve Antibody Library

$>10^{10}$ diversity

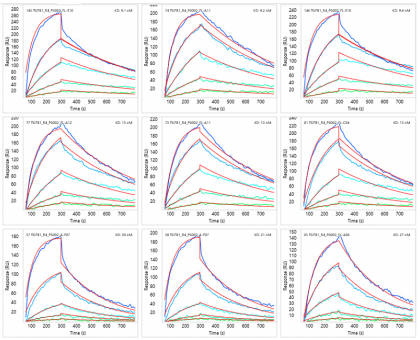
Clinically validated, developable antibody frameworks

Antibody Optimizer

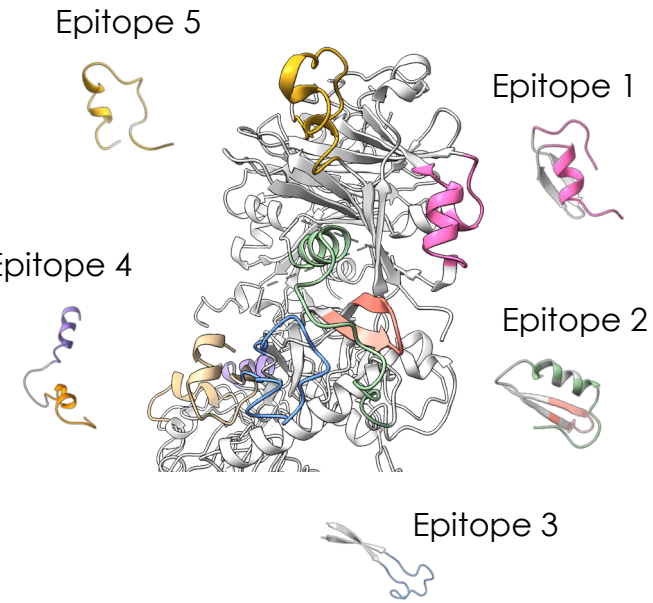
Template Antibody

Optimized Antibody

HT-SPR Screen

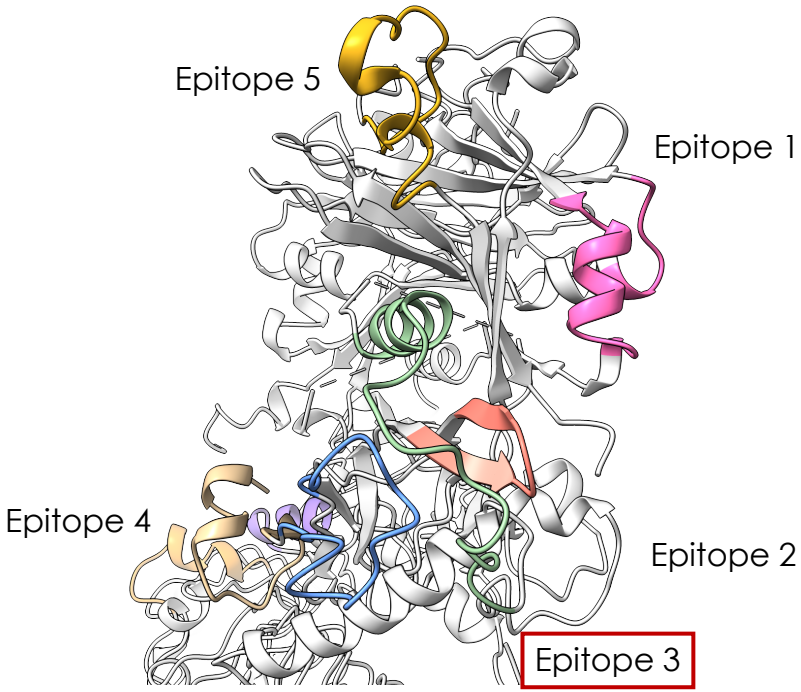


Latent-TGFβ1
Structure & Epitopes

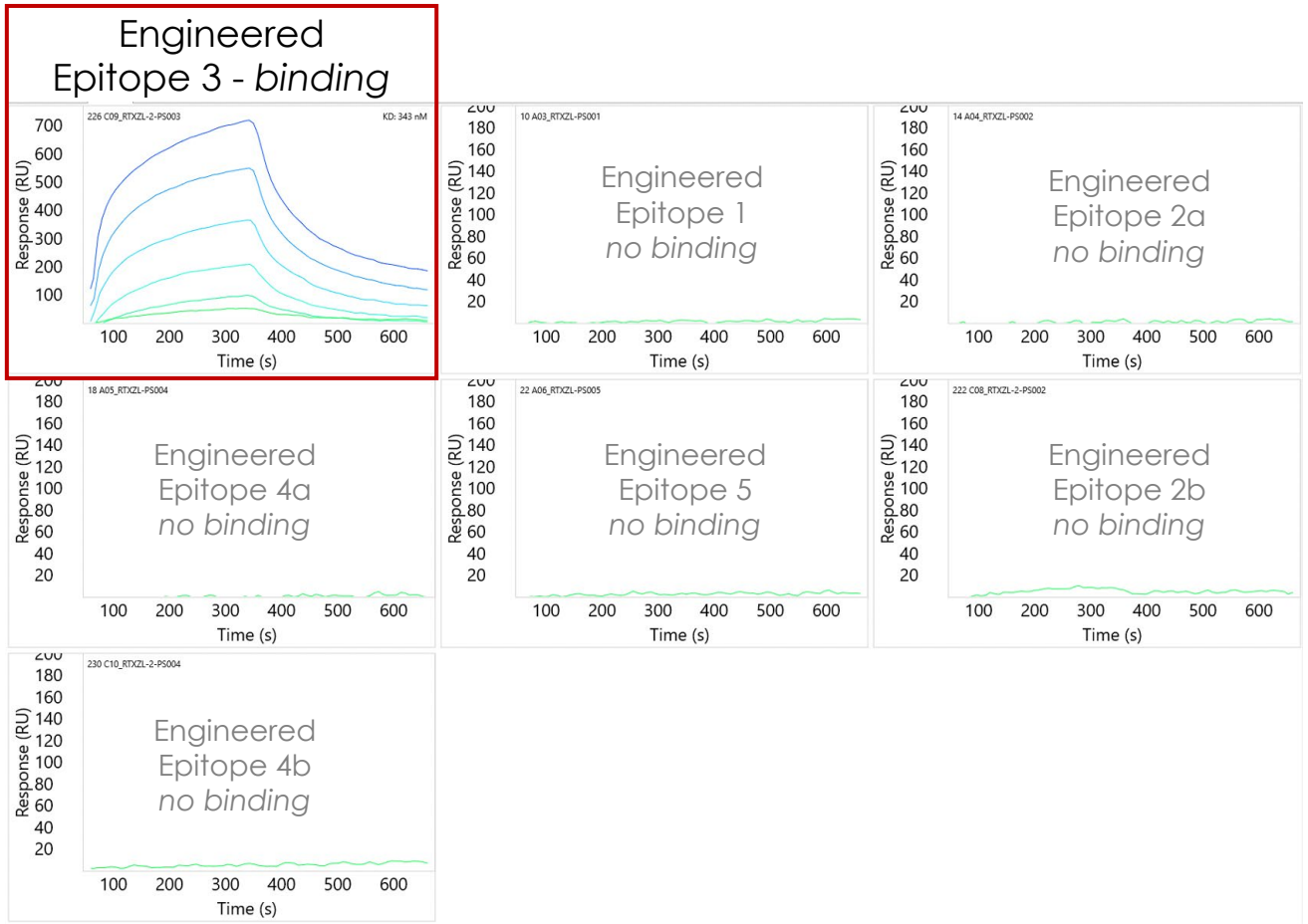


Mapping SRK-181 Benchmark Ab Using Engineered Epitopes

Latent-TGFβ1 Structure & Epitopes

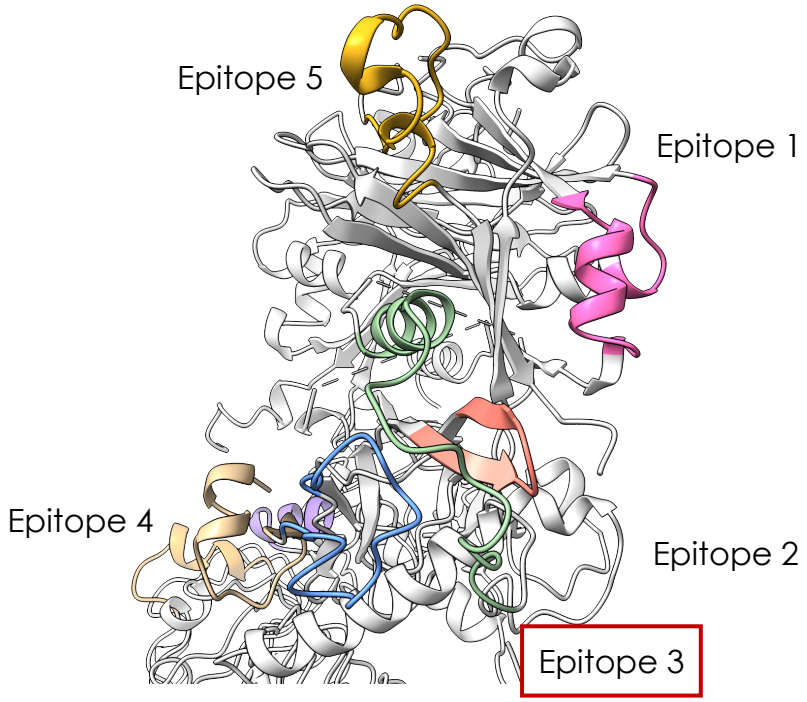


Multiple Engineered Epitopes Binding to the Benchmark Ab

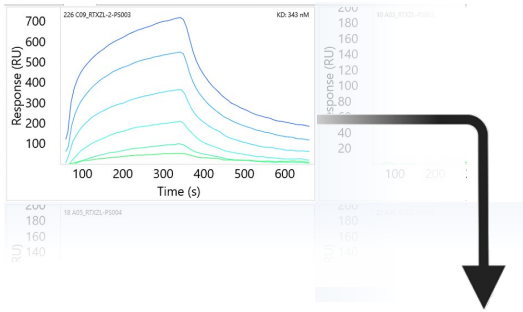


SRK-181 Benchmark HD-X MS Corroborates Engineered Epitope Mapping by SPR

Latent-TGFβ1 Structure

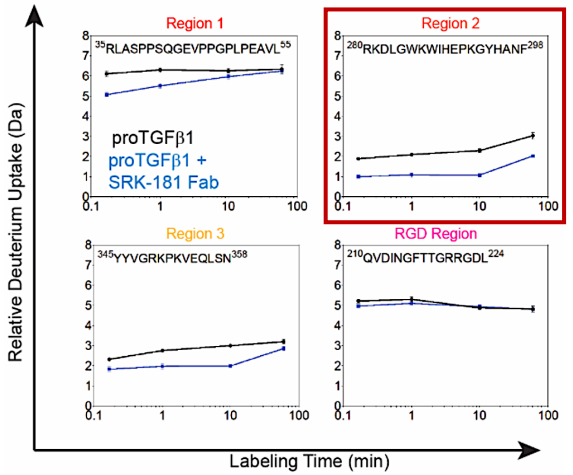


Engineered Epitope 3 SRK-181 SPR



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

SRK-181 HD-X MS

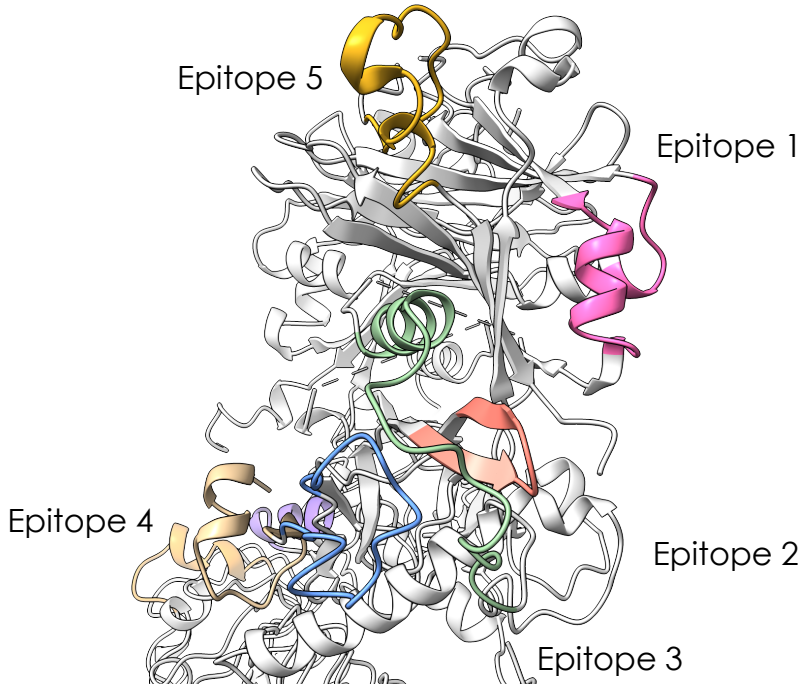


HD-X region corresponding to engineered epitope 3

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

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

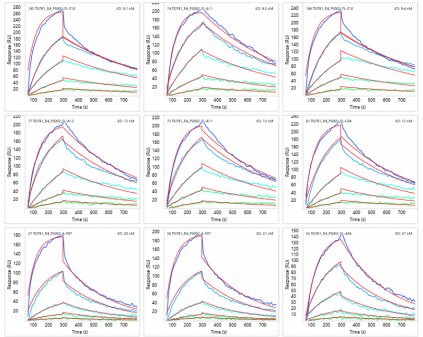
Latent-TGFβ1
Structure & Epitopes



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



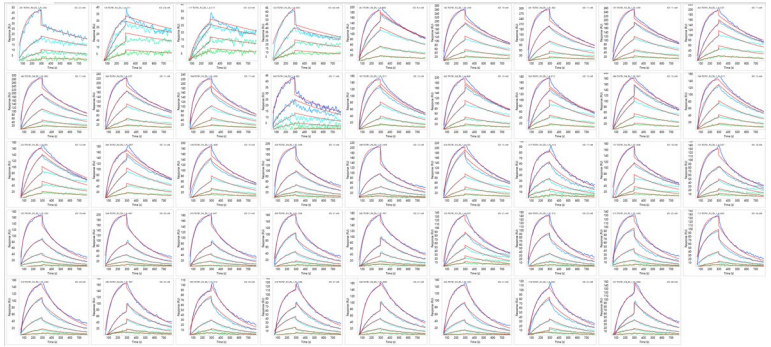
HT-SPR Screen Demonstrates Specificity, Diversity & Affinity of Epitope Steered Selections

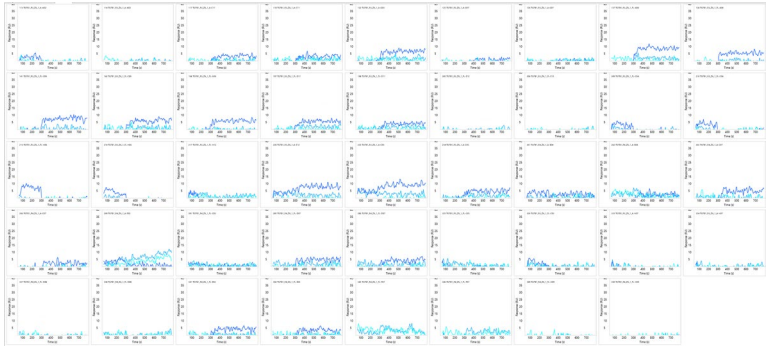
SPR Screen Results

Latent-TGFβ1 (desired target) | TGFβ1 (undesired target)

Epitope 1 steered binders

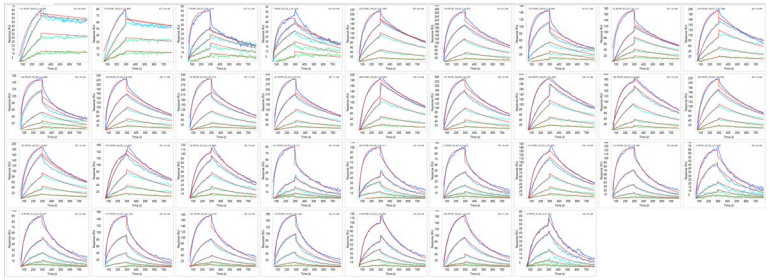
Latent-TGFβ1 specific	44
KD range (nM)	2.5 – 40 nM
TGFβ1 off-target	13

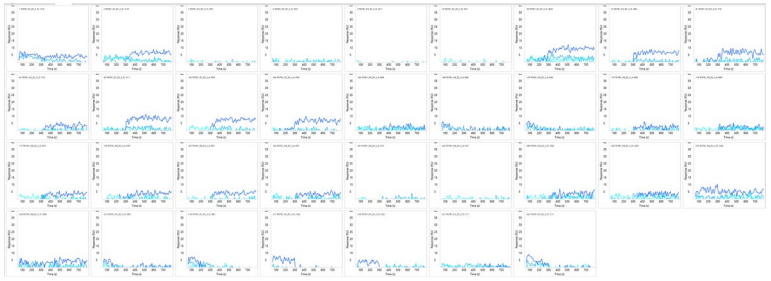




Epitope 2 steered binders

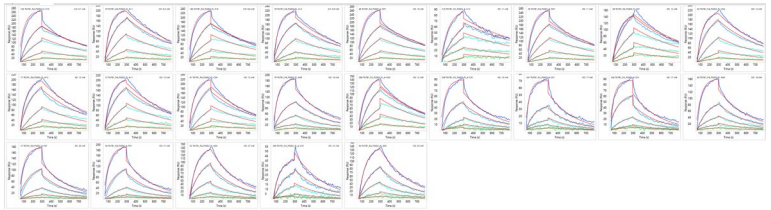
Latent-TGFβ1 specific	34
KD range (nM)	1.0 – 36 nM
TGFβ1 off-target	7

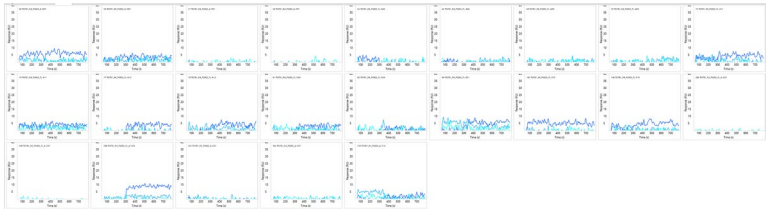




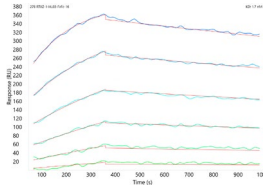
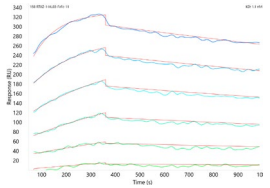
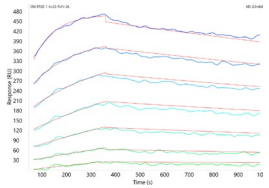
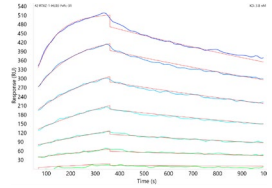
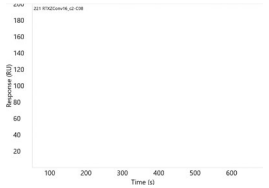
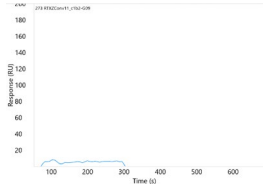
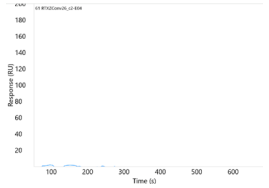
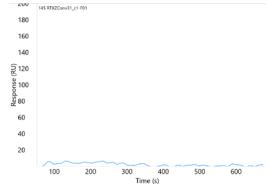
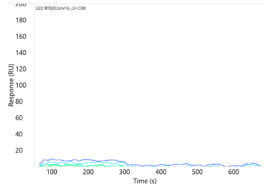
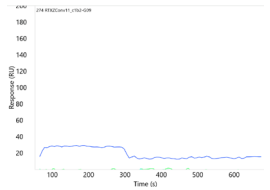
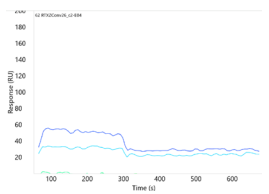
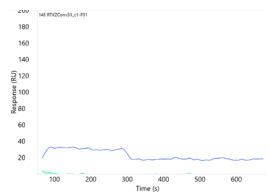
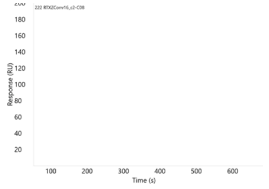
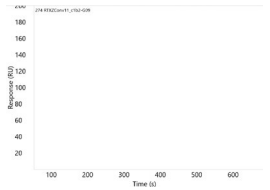
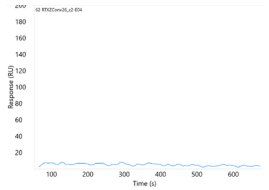
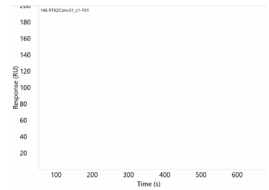
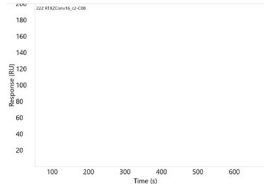
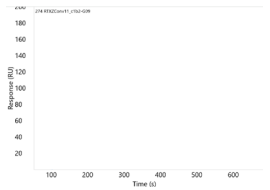
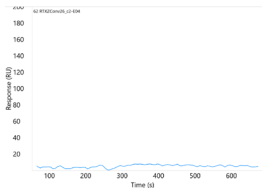
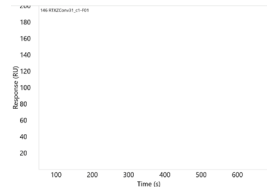
Epitope 3 steered binders

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

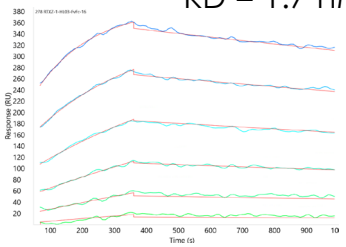
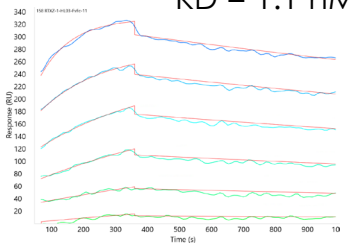
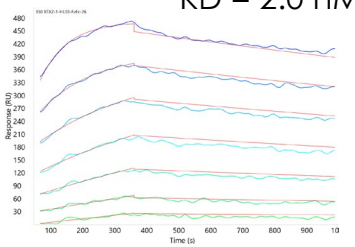
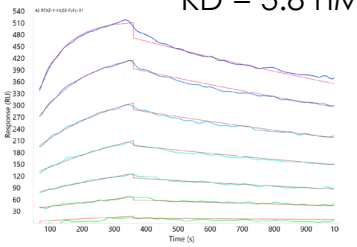
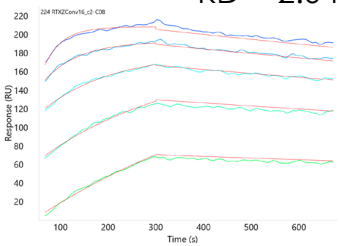
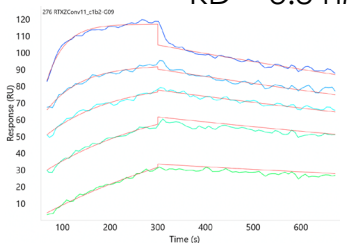
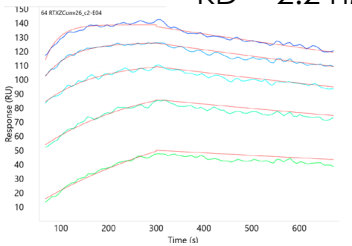
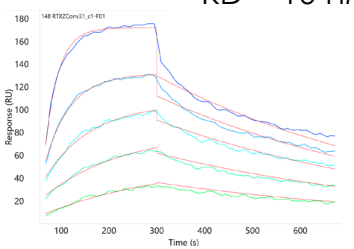
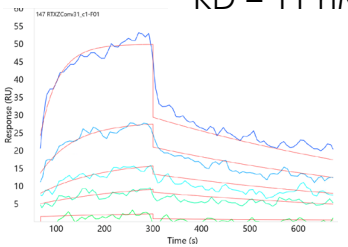




Four Clones Were Identified with Required Affinity and TGFβ Cross-Family Specificity

		Clone: 1	2	3	4
Do bind	Latent-TGFβ1 KD < 5 nM				
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

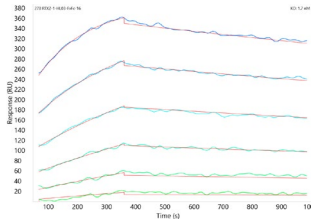
	Clone: 1	2	3	4
Hu Latent-TGFβ1	KD = 1.7 nM 	KD = 1.1 nM 	KD = 2.0 nM 	KD = 3.8 nM 
Cyno Latent-TGFβ1	KD = 2.0 nM 	KD = 3.8 nM 	KD = 2.2 nM 	KD = 10 nM 
Mu Latent-TGFβ1				KD = 11 nM 

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

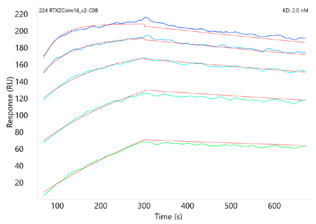
SPR

Does bind:

Hu Latent-TGFβ1
KD = 1.7 nM

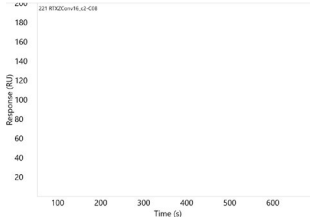


Cyno Latent-TGFβ1
KD = 2.0 nM

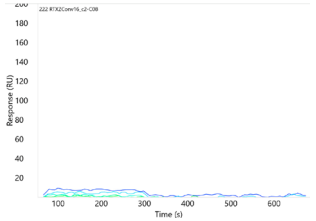


Does not bind:

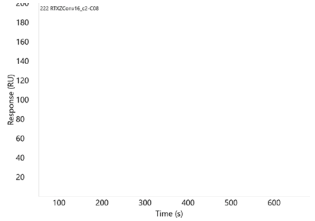
TGFβ1



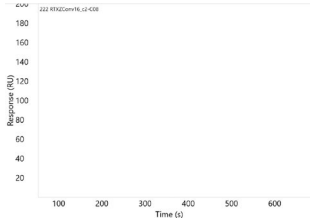
Latent-TGFβ2



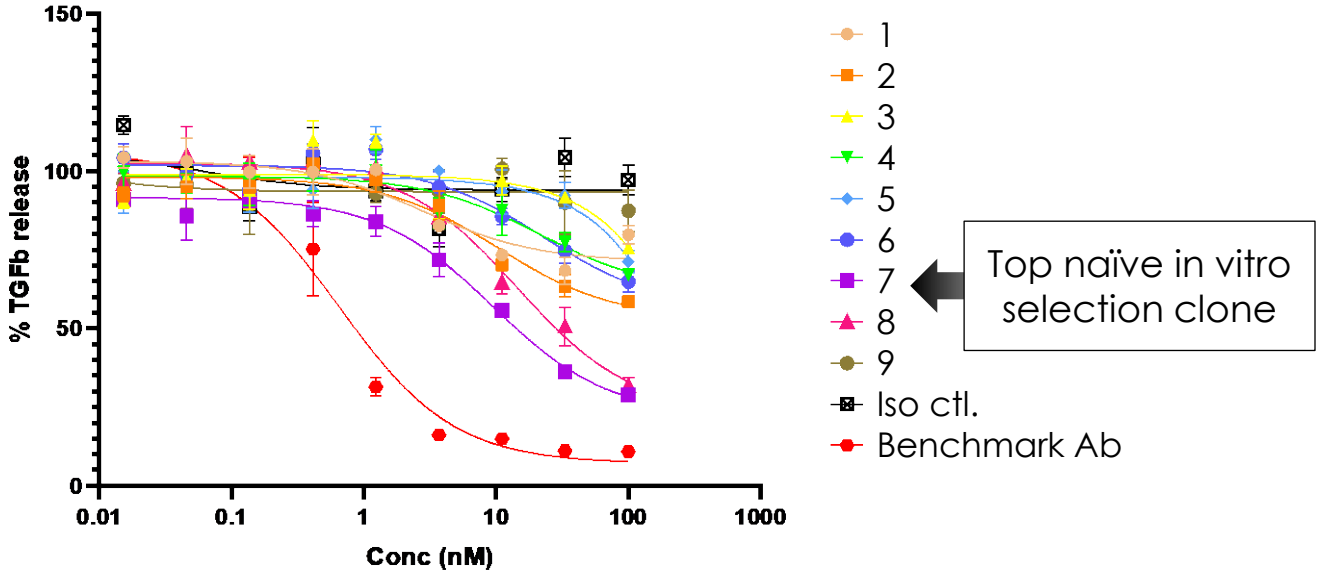
TGFβ2



TGFβ3



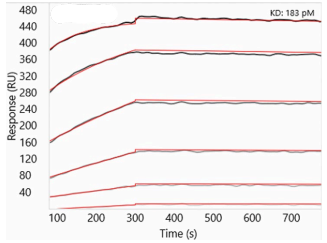
TGFβ1 Inhibition Assay



Clone	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

IC_{50} = 700 pM

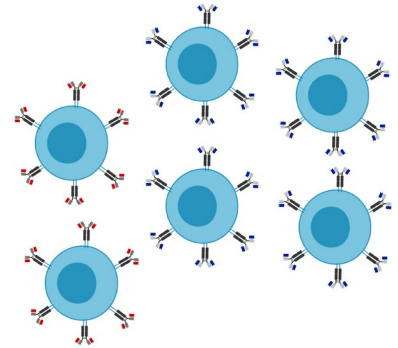
Published Ab template

AI-model predicts human CDRs

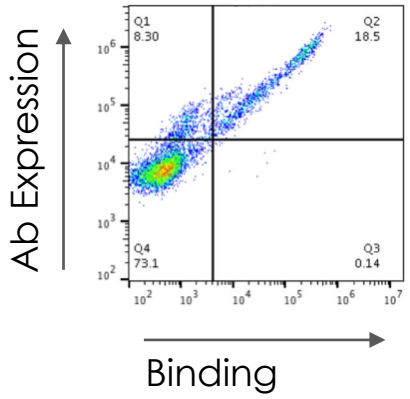
Fully human heavy & light chain CDRs diversity

HCDR1	HCDR2	HCDR3
2000	2000	24000
LCDR1	**LCDR2**	**LCDR3**
2000	1000	2000

Per-CDR mammalian display library



Single-cell sorting: binding & expression



NGS

HT-SPR hit validation and quantitation

StableHu Optimization Identifies Improved Fully-Human CDR Variants

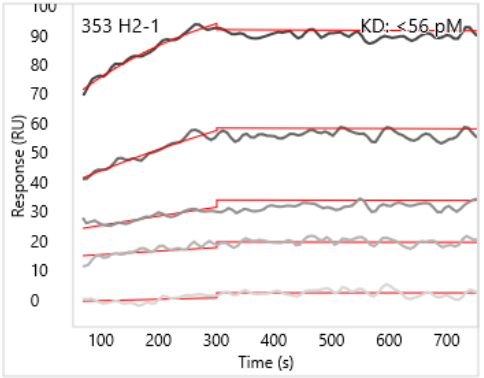
Variant Clones Latent-TGFβ1 SPR

Template clone

Hu Latent-TGFβ1

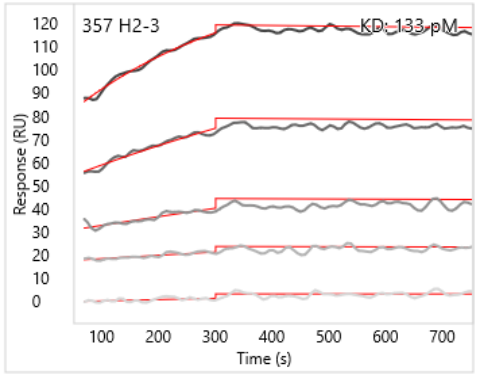
Variant 1

KD = 50 pM

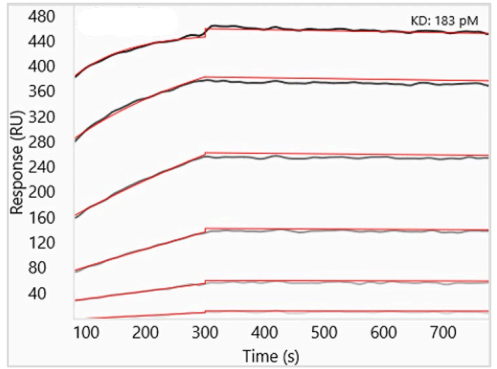


Variant 2

KD = 130 pM

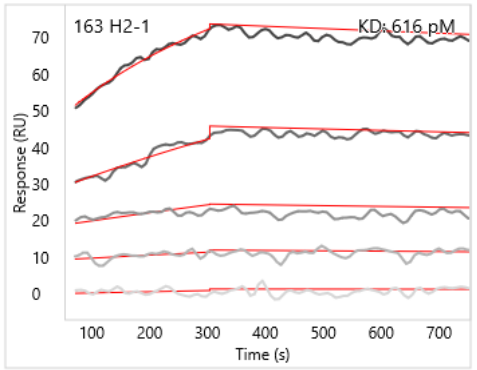


KD = 180 pM

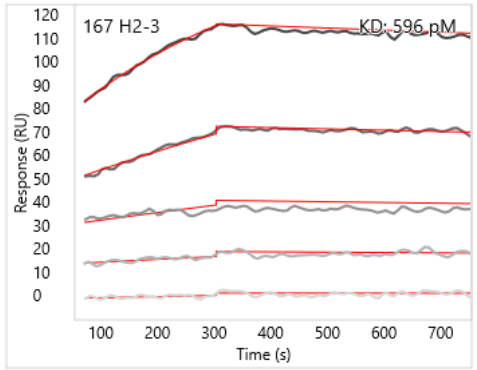


Mu Latent-TGFβ1

KD = 620 pM



KD = 600 pM



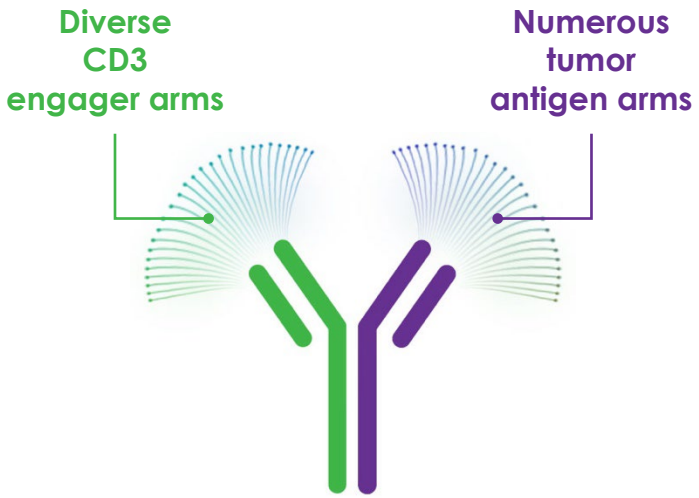
T-Cell Engager Epitope

CD3 Antibody

Key Challenges of CD3 T Cell Engager Discovery

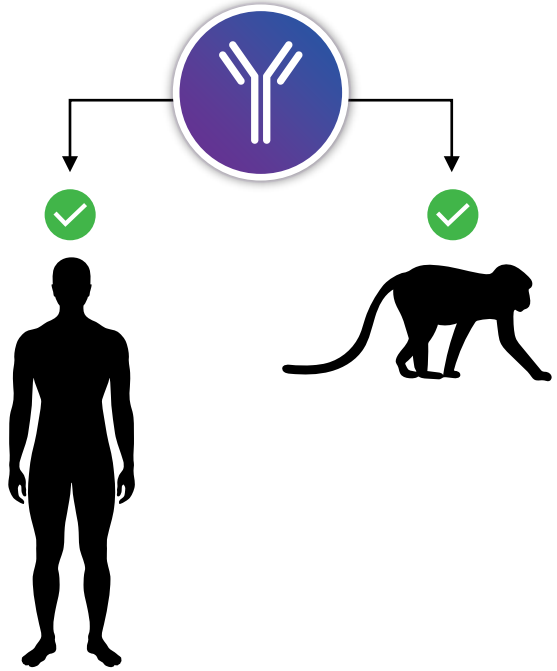
1 Sequence Diversity

Broad CD3 activity for optimized paring with tumor antigen arms



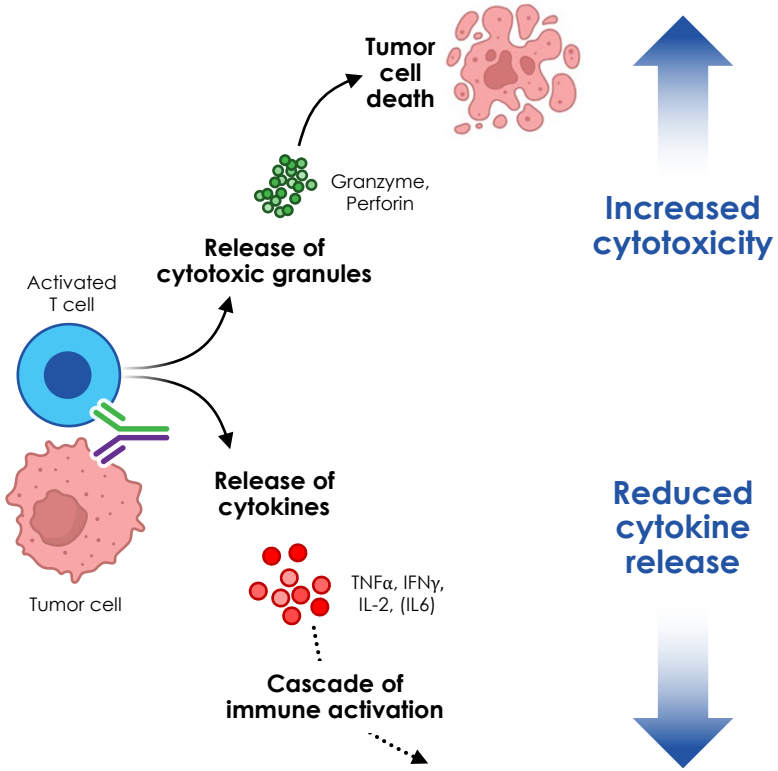
2 Hu-Cyno Cross-Reactivity

Risk reduction via cyno monkey toxicity study compatibility



3 Range of Cytokine Release

Tailored cytokine release for expanded therapeutic window



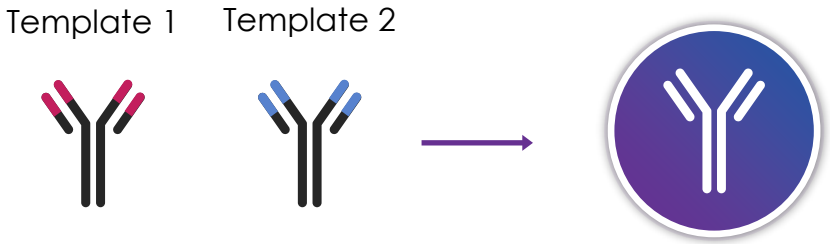
Dual Approaches to a Diverse Panel of Anti-CD3 Antibodies

AI Discovery Engine

Structural-Epitope Immunization & Screening

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

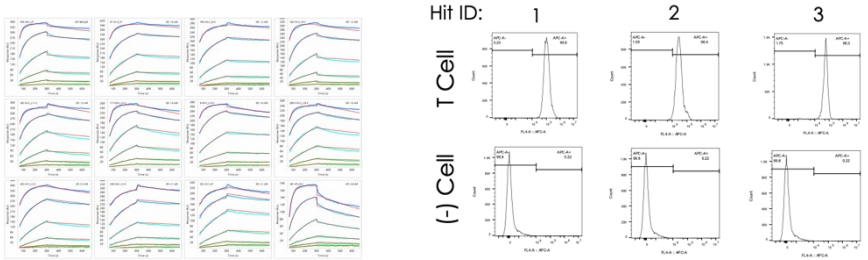
StableHu Optimizer



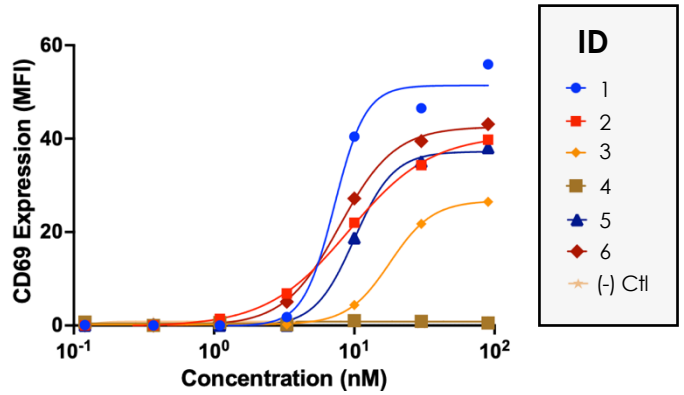
Template Antibodies → Optimized Antibodies

SCREEN

Hu/Cyno CD3 & T Cell Binding



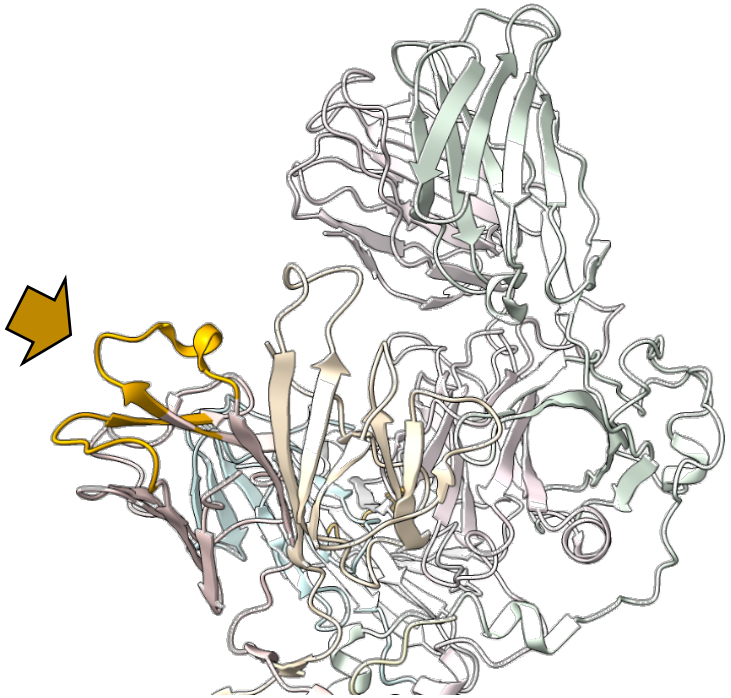
T Cell Activation



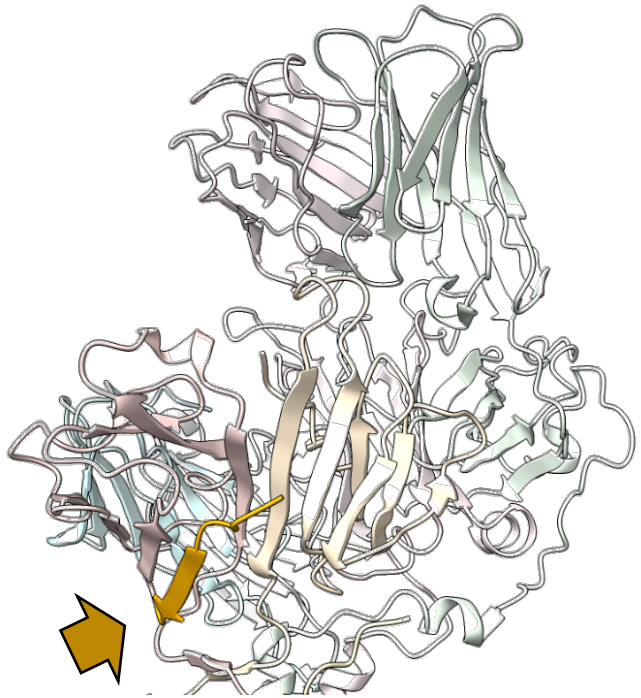
Engineered Epitopes Guide Immunization to TCR-Accessible CD3 Epitopes

CD3 target epitopes in the context of the full TCR

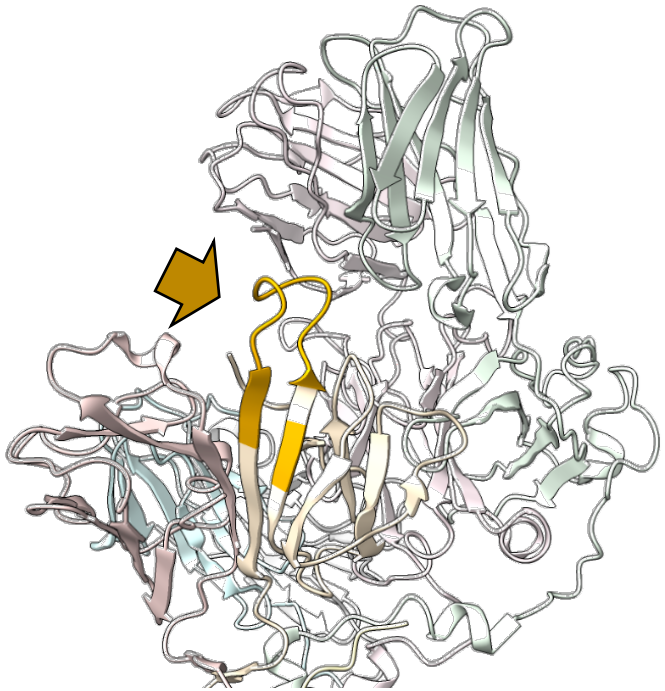
Epitope 1



Epitope 2



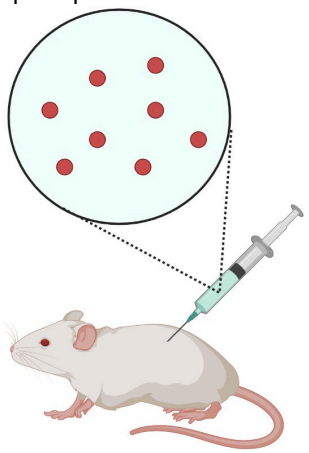
Epitope 3



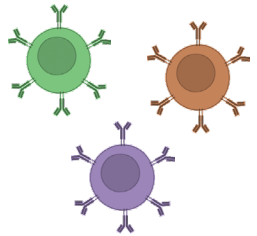
Immunized CD3 Repertoires Were Cloned and Screened in Mammalian Display

Multi Epitope-Steered Immunization

CD3 engineered epitopes & T cells



Mammalian Library Display

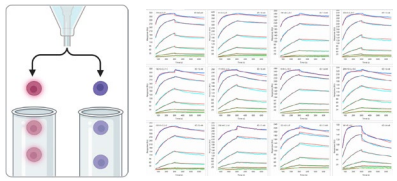


Mammalian Display

Immunized Repertoire

Multi Dimension Screening

FACS, NGS & SPR

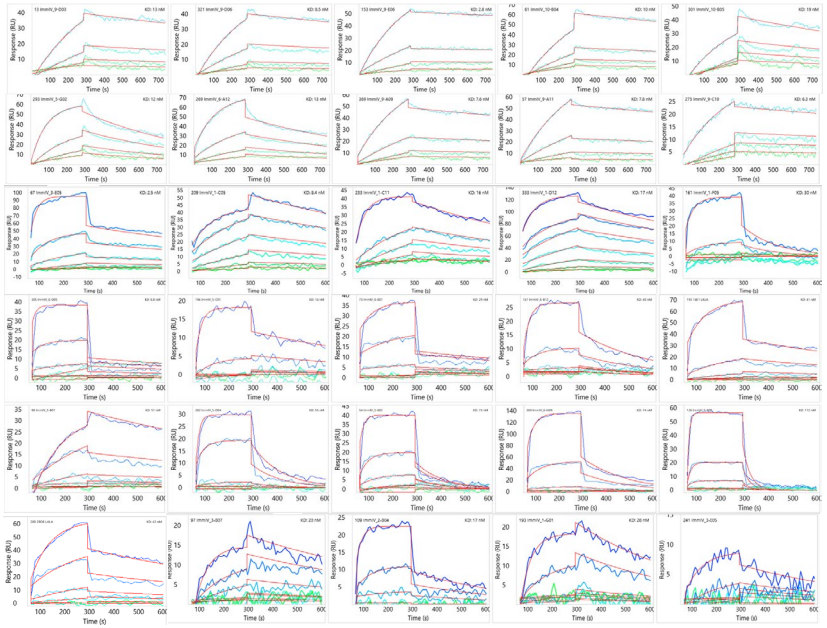


Single-Cell Screen:
Engineered epitopes &
CD3 binding &
Ab expression

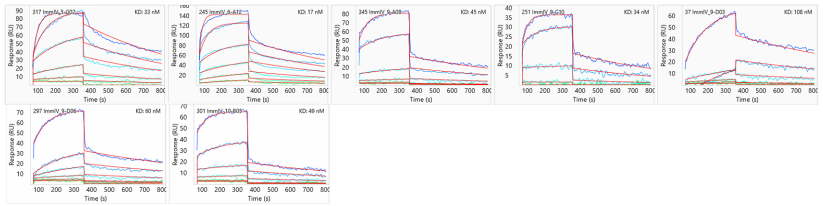
Epitope-Steered Immunization Identifies T Cell Binders – Some With Cyno Cross-Reactivity

HT-SPR Screen
Hu & Cyno CD3 Binding

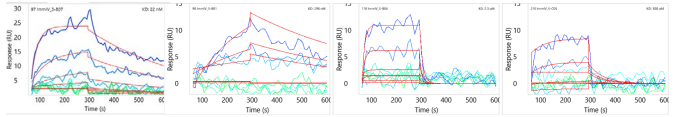
Hu CD3ED
KD: 3 - 100 nM



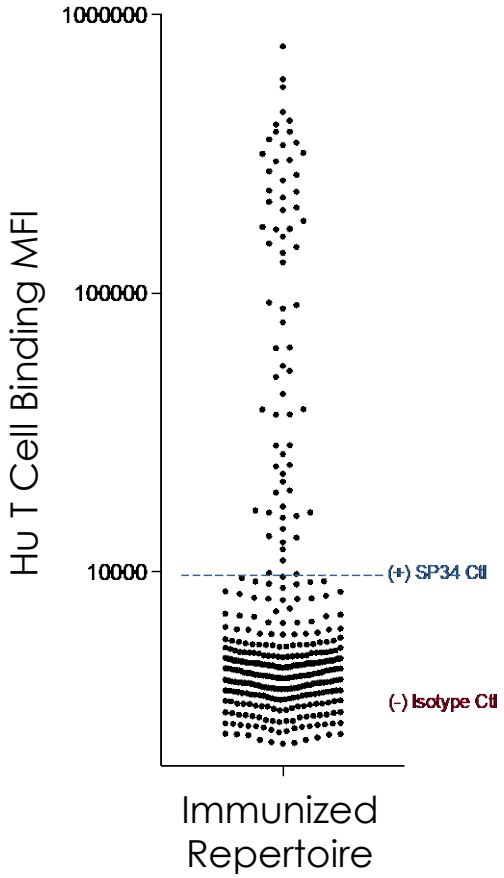
Hu CD3EG
KD: 17 - 100 nM



Cyno CD3ED
KD: 20 - 100+ nM



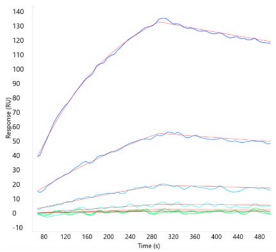
HT-Flow Cytometry Screen
Hu T Cell Binding



StableHu Optimization of Anti-CD3 Template Antibodies

Starting with anti-CD3 Ab template

KD = 27 nM



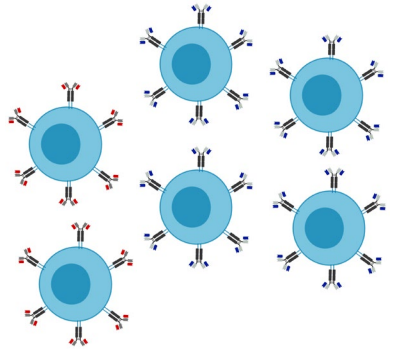
Template Ab

AI-model predicts human CDRs

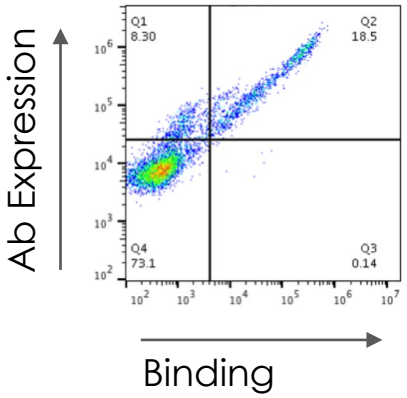
Fully human heavy & light chain CDRs diversity

HCDR1	HCDR2	HCDR3
2000	2000	2000
LCDR1	**LCDR2**	**LCDR3**
2000	1000	2000

Per-CDR mammalian display libraries



Single-cell sorting: binding & expression

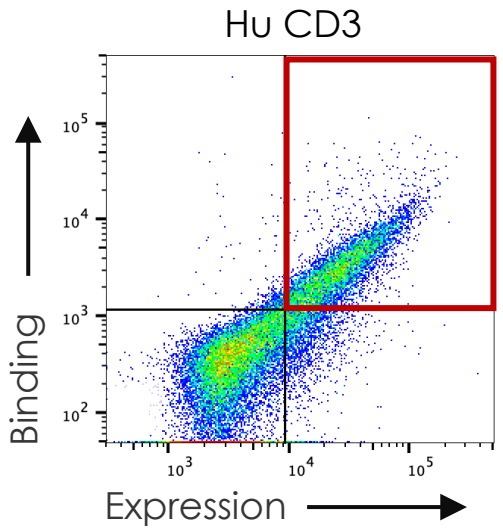


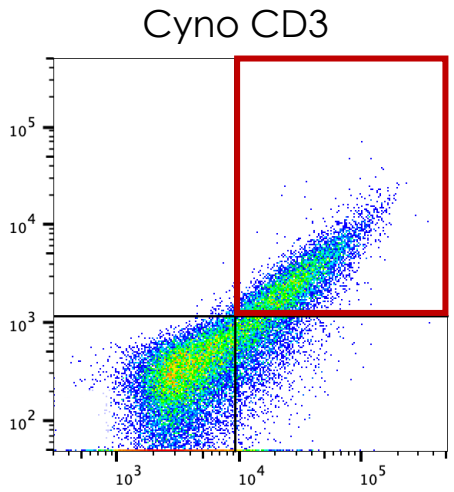
NGS

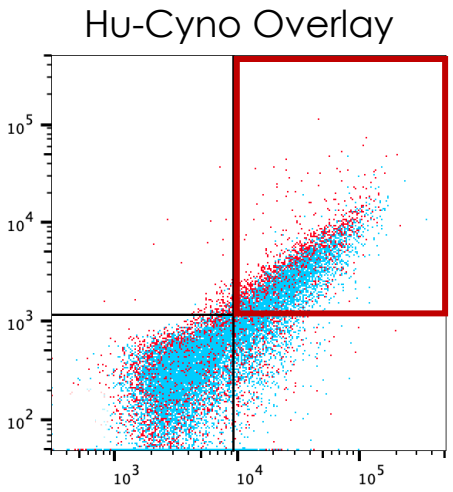
HT-SPR hit validation and quantitation

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

Cell Sorting of Pooled Single CDR Libraries

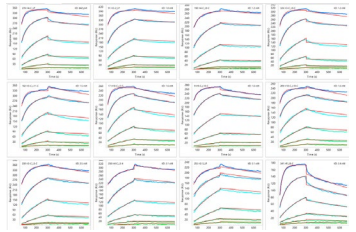




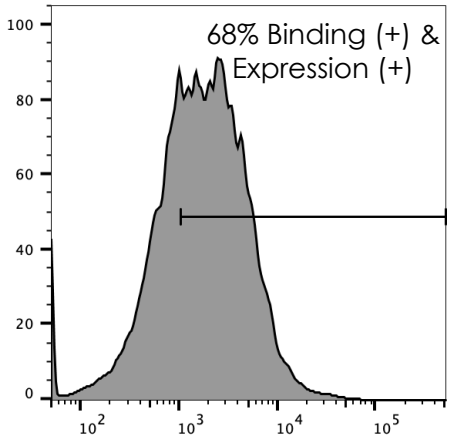


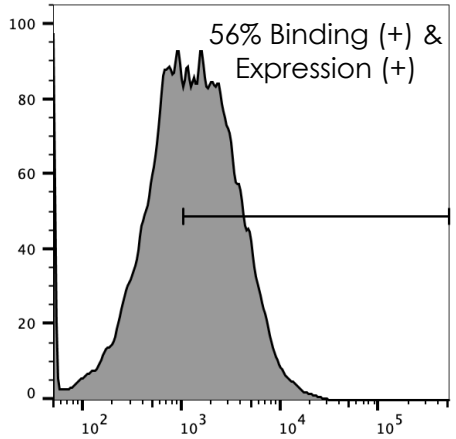
NGS

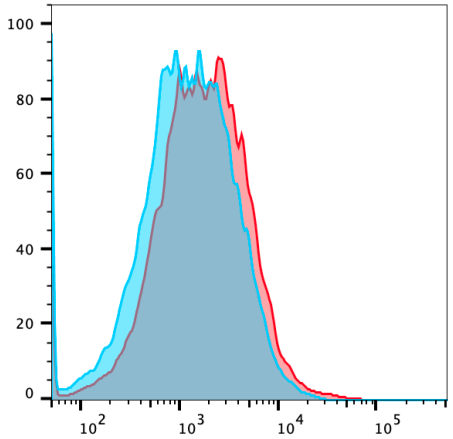
SPR screen



High-expression & binding double-positive distributions







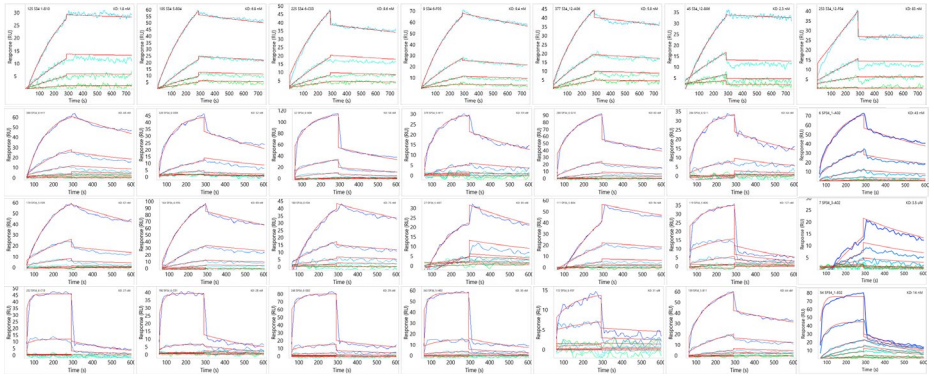
Hu-Cyno CD3 binding distributions significantly overlap

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

HT-SPR Screen
Hu & Cyno CD3 Binding

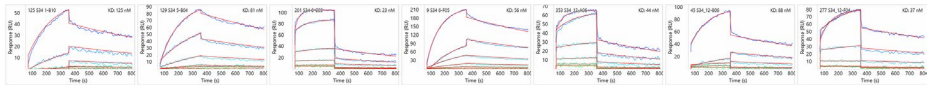
Hu CD3ED

KD: 2 - 100 nM



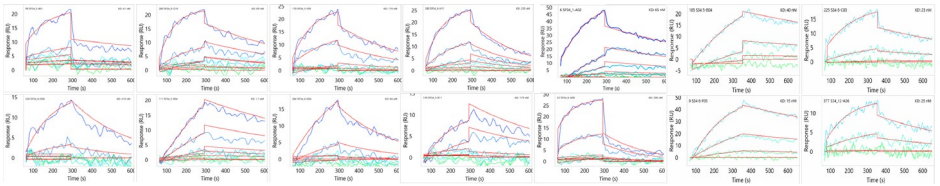
Hu CD3EG

KD: 20 - 100 nM



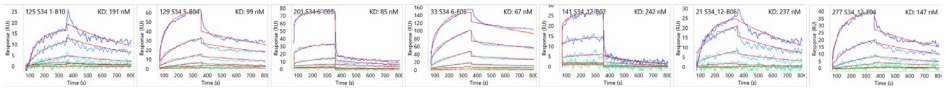
Cyno CD3ED

KD: 15 - 100 nM

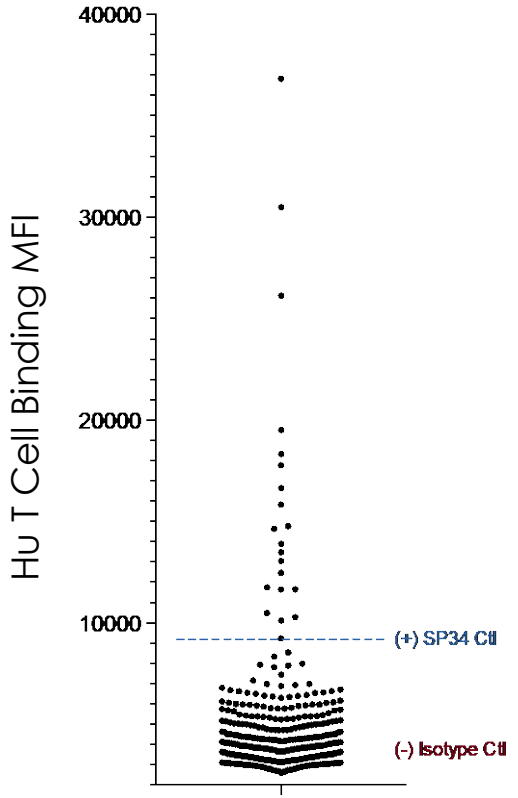


Cyno CD3EG

KD: 67 - 200 nM



HT-Flow Cytometry Screen
Hu T Cell Binding

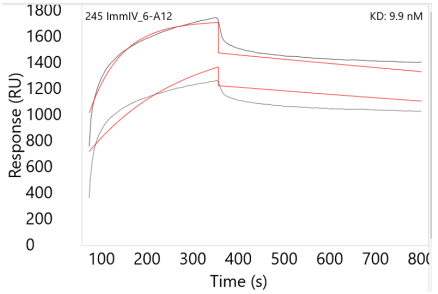


StableHu Sorted
Repertoire

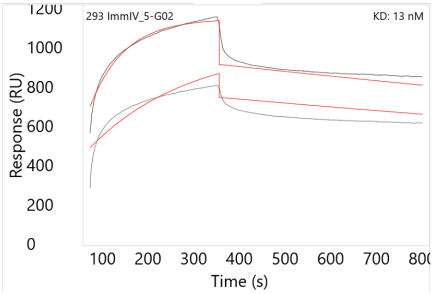
CD3 Antibody Hits – Epitope Mapping by Engineered Epitope SPR

Epitope 1

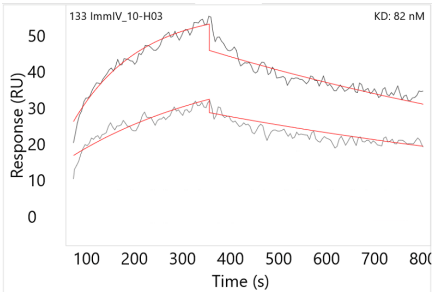
Hit 1

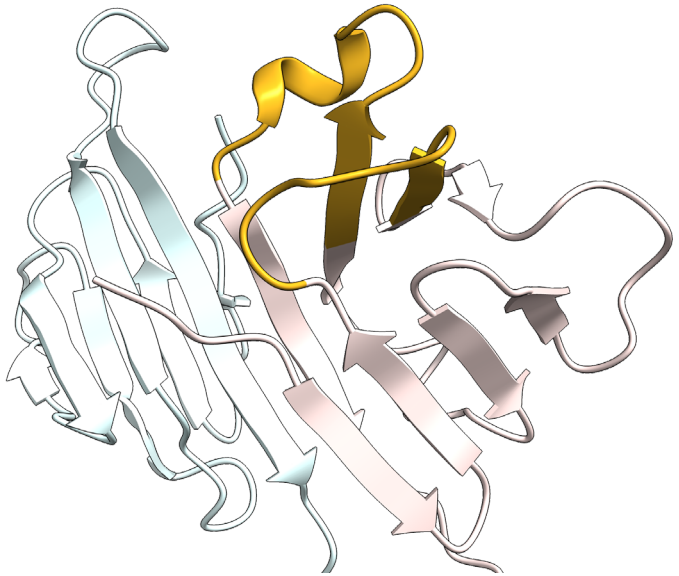


Hit 2



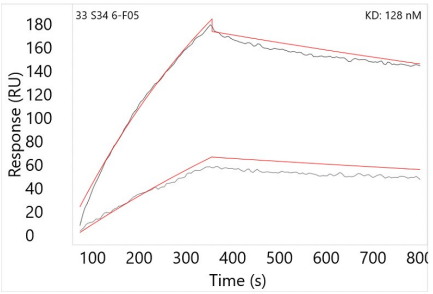
Hit 3

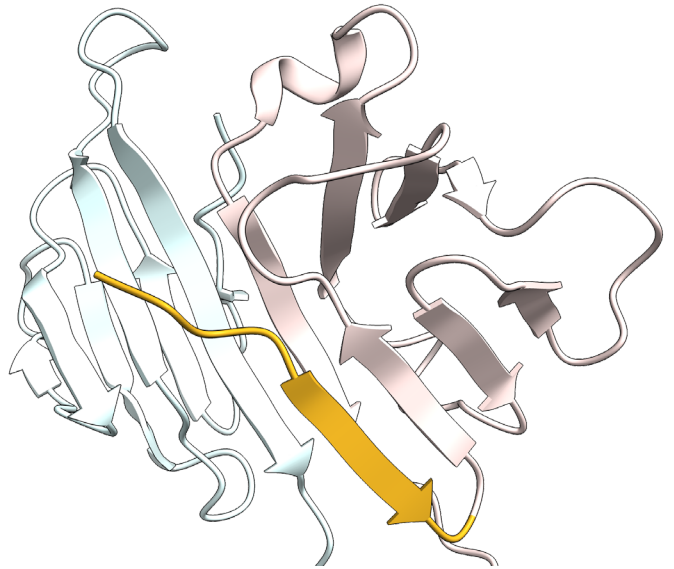




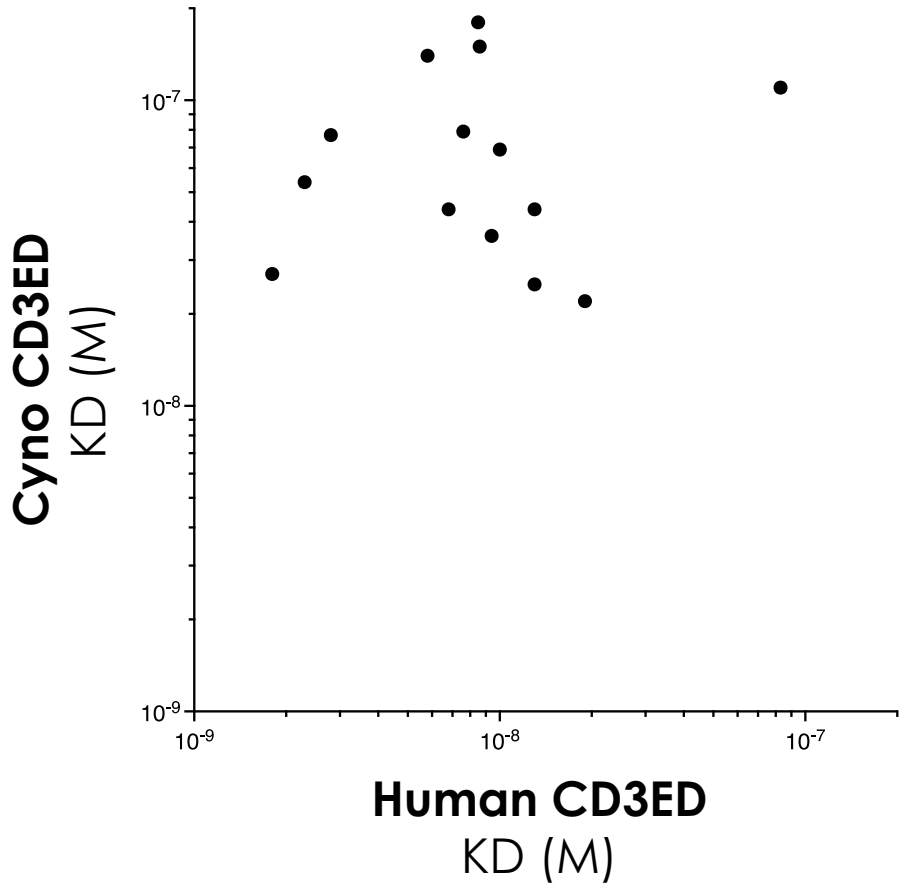
Epitope 2

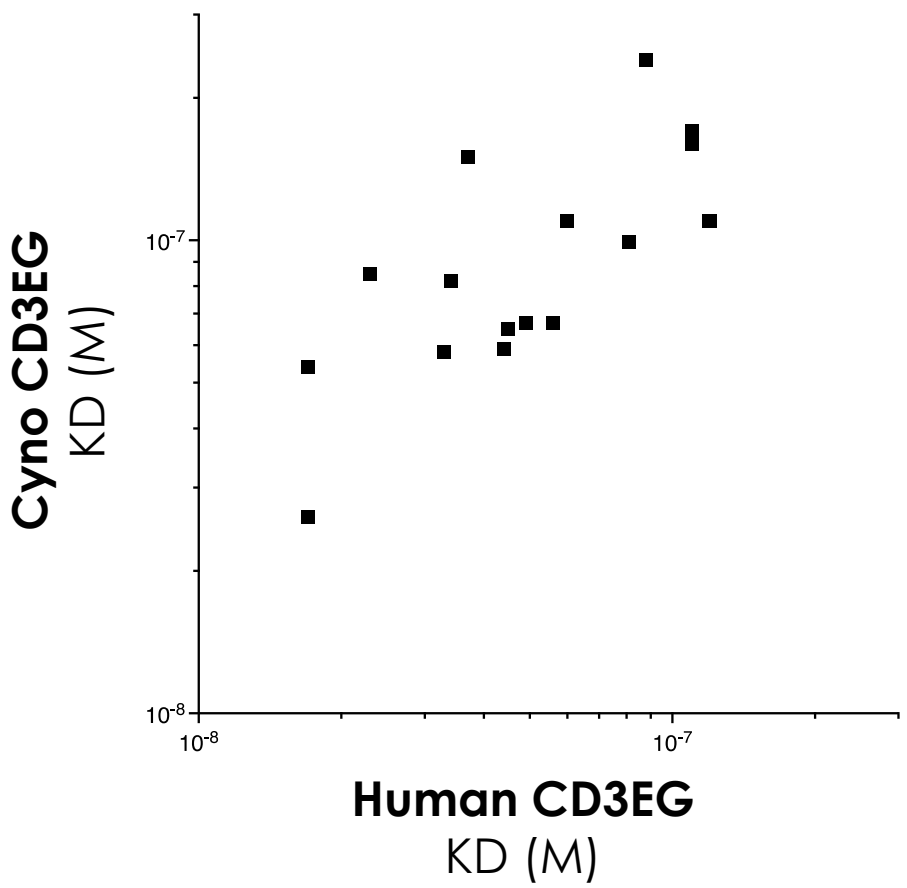
Hit 4





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