The World's Leading Global Single-Source Platform From Concept To Commercialization

Display Technology for Antibody/Protein Discovery and Optimization

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Global Solution Provider

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Outline

01 WuXi Biologics Display Platforms

02 Case studies of Lead Identification

03 Case studies of Lead Optimization
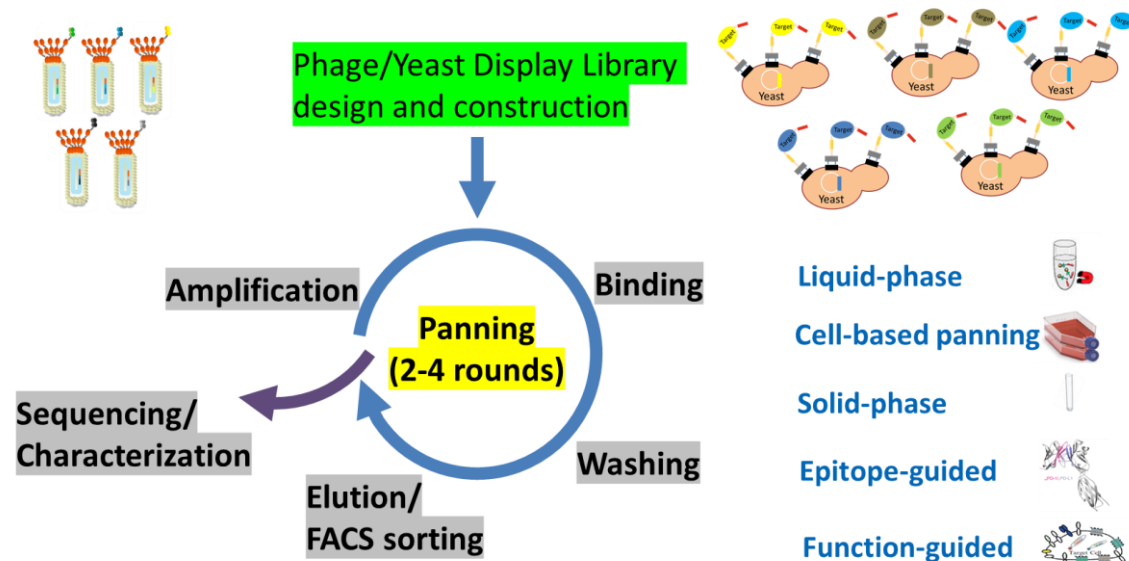
04 Summary

- **Major platforms: Phage, Yeast**

- **Major Applications: Lead Identification, Lead Optimization**

  - **Lead Identification: VHH, *scFv*, Fab, IgG, TCR, Peptide, Cytokine receptor, *etc***

  - **Lead Optimization: Affinity Maturation, pH dependent engineering, Tm, *etc***

# WuXi Bio Display Library summary table for lead identification

Species	Resource	Display format	Library size	# of project delivered	Time line for Lead Identification (Week)
Human	Native	scFv	10^{11}	50+	4-8 for Native and Synthetic;
	Synthetic	Fab	10^{12}		
Alpaca/Llama	Native	VHH	10^{11}	100+	14-20 starting from immunization
	Humanized Synthetic	VHH	10^{11}		
	Immunization	VHH	10^{8-9}		
Rodent/Rabbit	Immunization	scFv/Fab	10^{8-9}	~ 10	

Example of High Quality library construction: Native Single Domain Antibody VHH Libraries
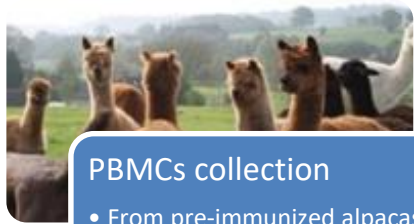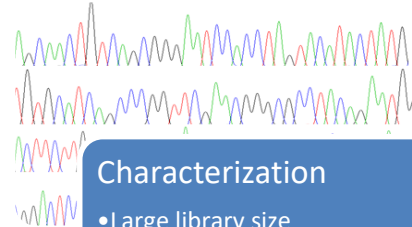

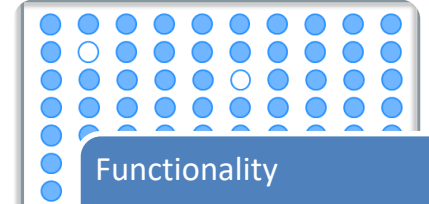**PBMCs collection**
- From pre-immunized alpacas
- 100+ animals
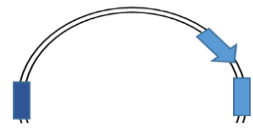
Optimization
- Proprietary primers for broad coverage
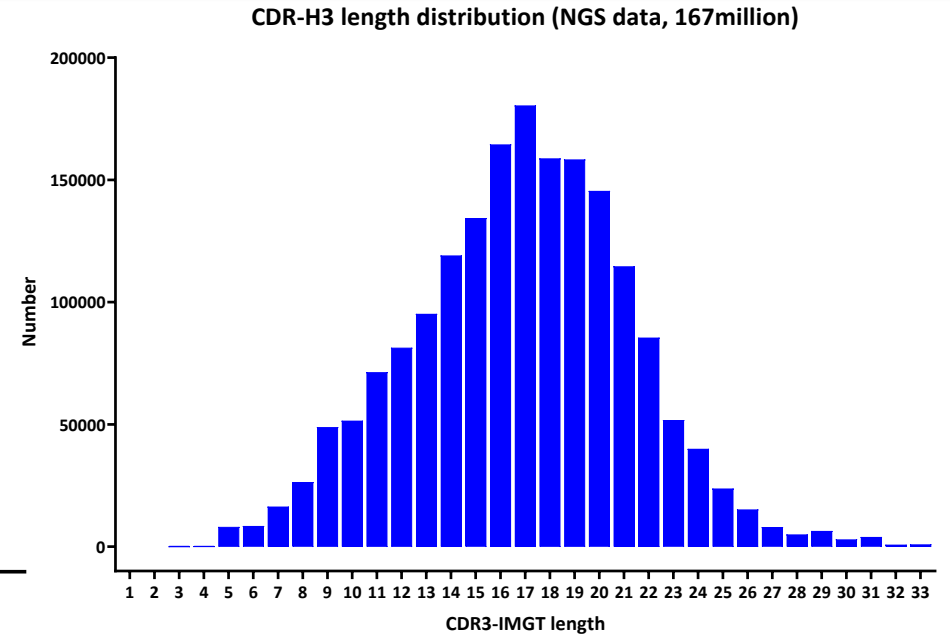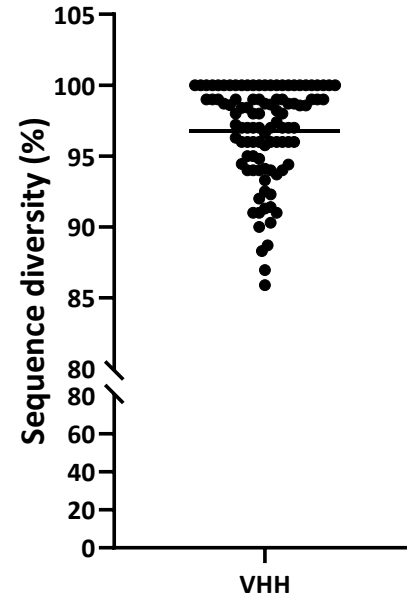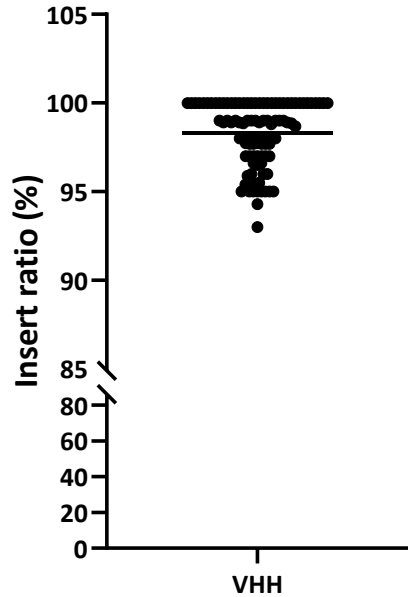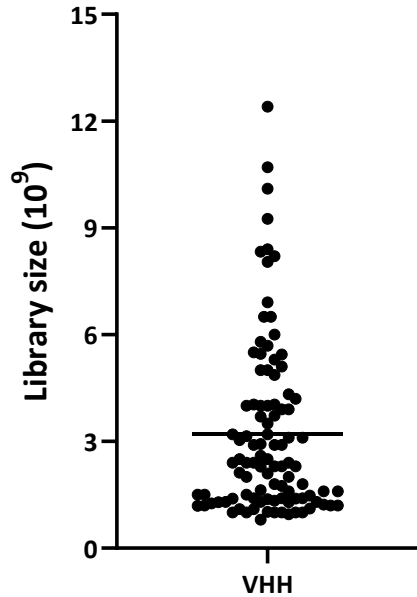- Optimized RT-PCR conditions
- Highly efficient transformation

Characterization
- Large library size
- High insertion rate
- High diversity with in-frame sequences

Functionality
- High expression rate
- Confirmed display on phage

CDR-H3 length distribution (NGS data, 167million)

Example of High Quality library: Humanized synthetic VHH Libraries

❑ **Library Advantages:**

➢ **Fast: no immunization, no limit to antigen types, no humanization, target to binders in 4-6 weeks**

➢ **Good developability: high expression, high stability**

➢ **Can be purified by Protein A**

❑ **WuXi Bio strength:**

➢ **Expertise knowledge for protein design**

➢ **Accumulated extensive experience from hundred of VHH projects for high frequency and high stable germline**
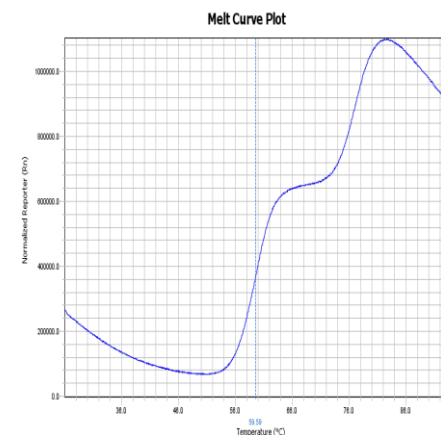
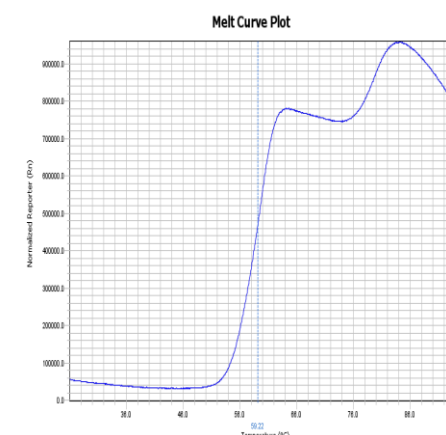# Humanized VHH Synthetic Library QC
Thermal Stability (Tm1)

Protein Name	Tm1 (℃)
Clone 1	68.0
Clone 2	59.6
Clone 3	60.1
Clone 4	62.1
Clone 5	62.0
Clone 6	58.5

■ The library is designed based on high thermal stability scaffolds.
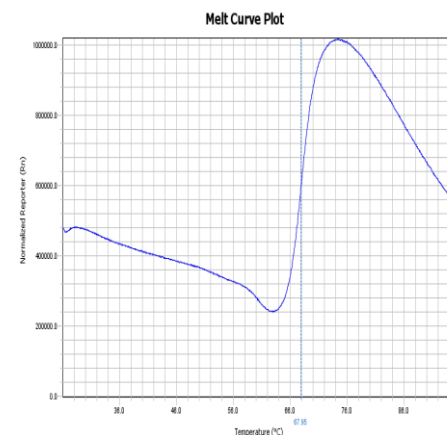
Clone 1

Clone 2

Clone 3

Clone 4

Clone 5

Clone 6

Humanized VHH Synthetic Library QC
Expression Yield in E.coli

No	Protein Name	MW (kD)	PI	Yield (mg/L) by Protein A
1	Clone 1	15.40	6.31	115.38
2	Clone 2	15.50	6.31	48.82
3	Clone 3	15.50	6.31	31.15
4	Clone 4	15.40	6.31	77.61
5	Clone 5	15.70	6.31	40.79
6	Clone 6	15.30	6.66	22.52

Gel info: Genscript SurePAGE™, Bis-Tris, 4-12%
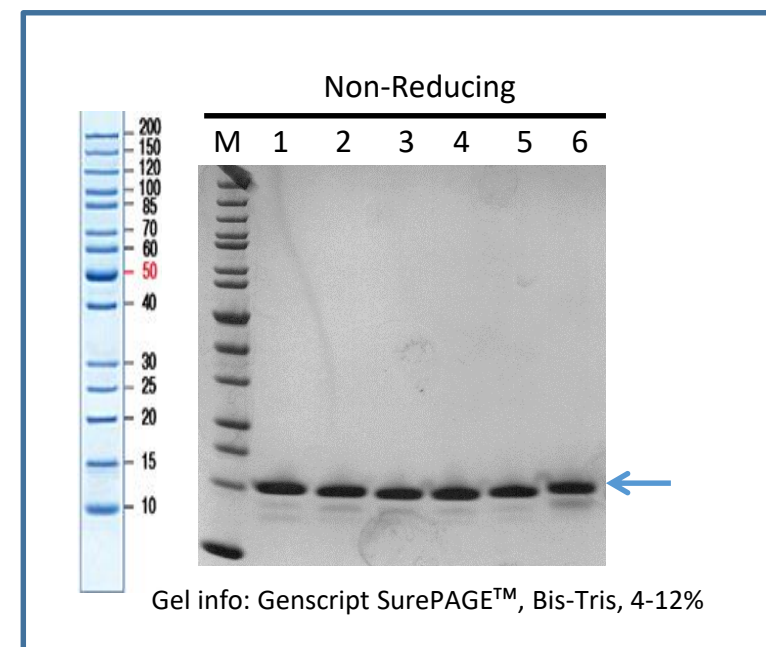
- **VHH molecules from VHH synthetic library can be purified by Protein A.**
- **Good expression yield in *E.coli*, ranges from 23 mg/L to 115 mg/L.**

➢ **VHH immune library case study-GPCR**

➢ **VHH Native library case study**

➢ **Humanized VHH Synthetic library case study**

➢ **Human Native scFv library case study**

➢ **Human Synthetic Fab library case study**

➢ **Peptide phage display case study**

➢ **Peptide yeast display case study**

VHH immune library case study-GPCR

- GPCR, a seven-pass transmembrane protein family, is an important and major drug target with more than one third approved drugs targeting for this family.

- Small molecule and peptide as major drugs targeting GPCRs. However, due to small size, specificity, affinity and pharmacokinetics (PK) is not as good as those properties from mAb.

- Purification of GPCR in native conformation is very challenging.

- GPCR has limited epitopes that make antibody discovery very challenging.

- **Client request**: Specific binding to target GPCR, different epitope as compared from BMKs, can be used for Cyno PK study.

- Via **proprietary DNA vector and cell line immunization strategy**, we immunized alpacas.
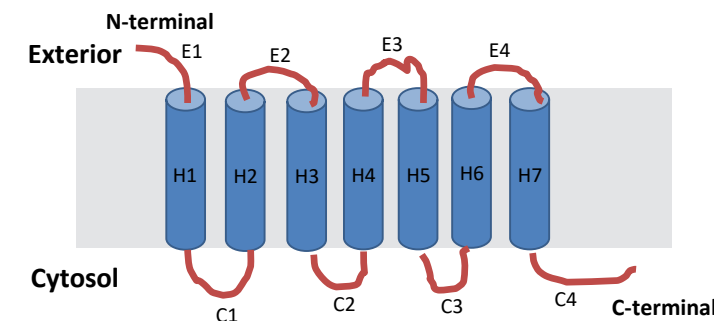
Agent type targeting GPCR

Antibody 0.1% (5)
Peptide 32.1% (1303)
Small molecule 67.8% (2753)
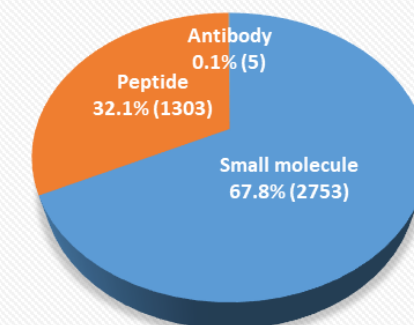
■ Small molecule ■ Peptide ■ Antibody

Data from IUPHAR's Guide to Pharmacology database
doi.org/10.1038/s41392-020-00435-w

GPCR specific binding characterization summary from FACS

| Antibody	Binding affinity range (M) to specific human tumor cell	Binding affinity range (M) to specific cyno 293 cell line	Binding affinity range (M) to negative human tumor cell	Binding affinity range (M) to human empty 293 cell
WuXi Bio lead 1	**10^{-10}**	**10^{-9}**	No binding	No binding
WuXi Bio lead 2	**10^{-9}**	**10^{-9}**	No binding	No binding
BMK	10^{-9}	No binding	No binding	No binding
Human IgG1	No binding	No binding	No binding	No binding

➢**Client request satisfied**: Specific binding to target GPCR, different epitope as compared from BMKs, can be used for Cyno PK study. **Patent filing** is underway for TCE and CAR-T application.

➢ VHH Native library case study list

|	Project 1 *(human Pro panning)*	Project 2 *(human Pro panning)*	Project 3 *(human Pro panning)*	Project 4 *(cell panning, human 4-pass membrane Pro)*	Project 5 *(human Pro panning)*	Project 6 *(human Pro panning)*
Sequence identity between target/ camelid protein	100%	100%	76%	90%	84%	73%
Positive clone rate	45.2%	29.1%	14.9%	13.5%	87.7%	90.9%
Unique sequence rate	10.6%	19.4%	12.0%	1.7%	7.0%	7.3%
Binding affinity range (M)	10^{-13}	10^{-11}	10^{-7}	10^{-9}	10^{-11}	10^{-11}

Humanized VHH Synthetic library case study

FACS binding on human target X engineered cells
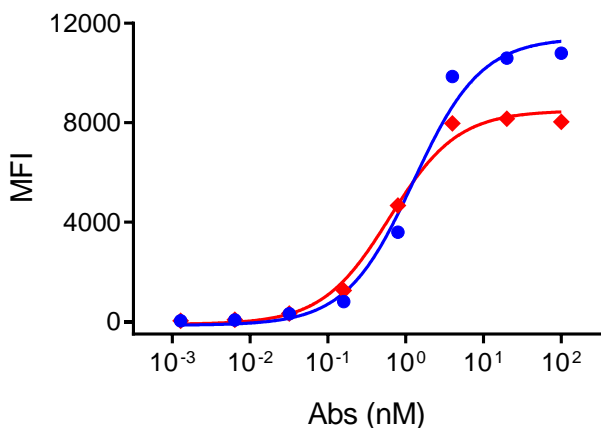
ELISA binding on Cyno target X protein
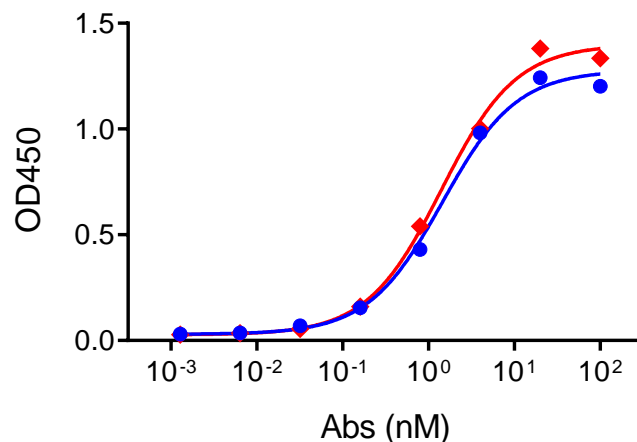
Internalization on target X engineered cells

- Benchmark Ab
- WuXi Bio lead
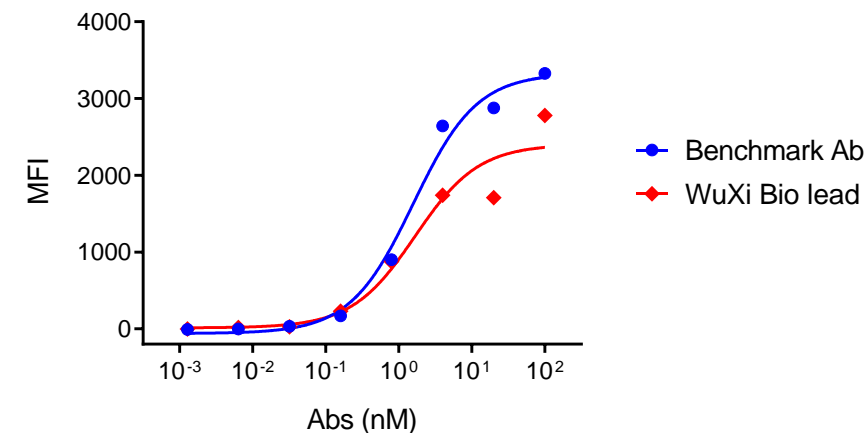
Ab	Human target X binding FACS EC50 (nM)	Cyno target X binding ELISA EC50 (nM)	Internalization EC50 (nM)	Yield (mg/L)	DSF Tm1
Benchmark Ab	1.3	1.5	1.6	NA	NA
WuXi Bio lead	0.6	1.4	1.7	490	63.4°C

➢ **WuXi Bio lead: screened from humanized VHH synthetic library.**

➢ **It showed similar binding affinity to human target X compared with Benchmark Ab and can be effectively internalized.**

➢ **It showed good yield and thermostability.**

# Human Native scFv library case study-Multi-pass membrane protein

- ✓ **47.8% specific binding positive rate** from single selection campaign, **high affinity binders were efficiently enriched.**
- ✓ **Superior affinity as compared to BMK, no need to do affinity maturation.**
- ✓ **Efficient in vivo tumor killing as compared to BMK for ADC format.**
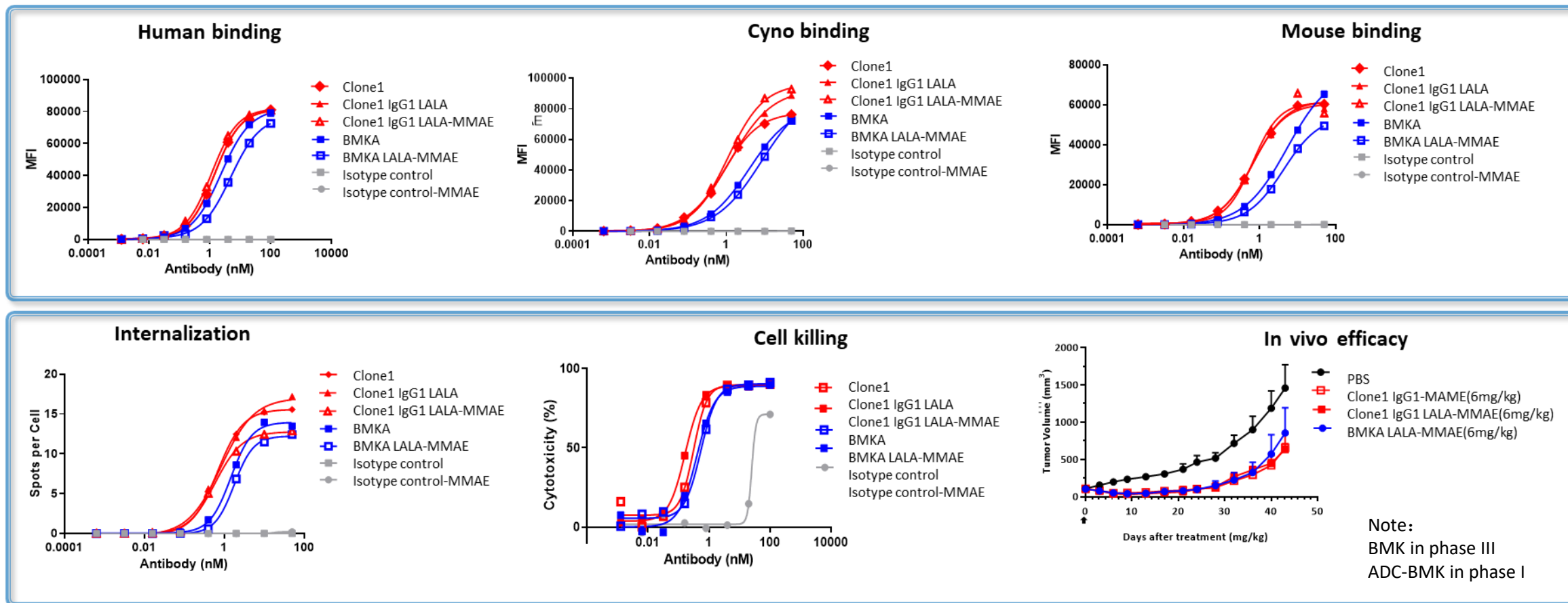
Note：
BMK in phase III
ADC-BMK in phase I

➤ Human Native scFv library case study-Peptide and Small molecule

➤ **Small Peptide: ~2 KDa**
➤ **The WuXi Biologics candidates show strong binding activity to peptide.**
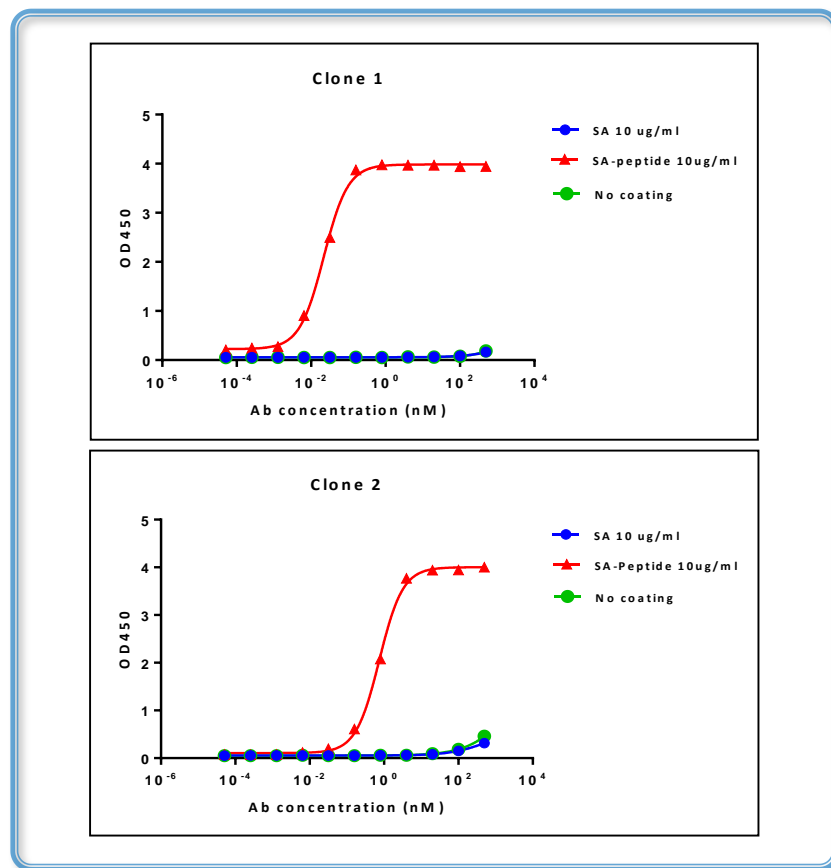
➤ **Small molecule compound: ~0.75KDa**
➤ **14 unique sequences from single selection campaign, broad sequence diversity.**
➤ **The WuXi Biologics candidates show binding activity to target compound.**
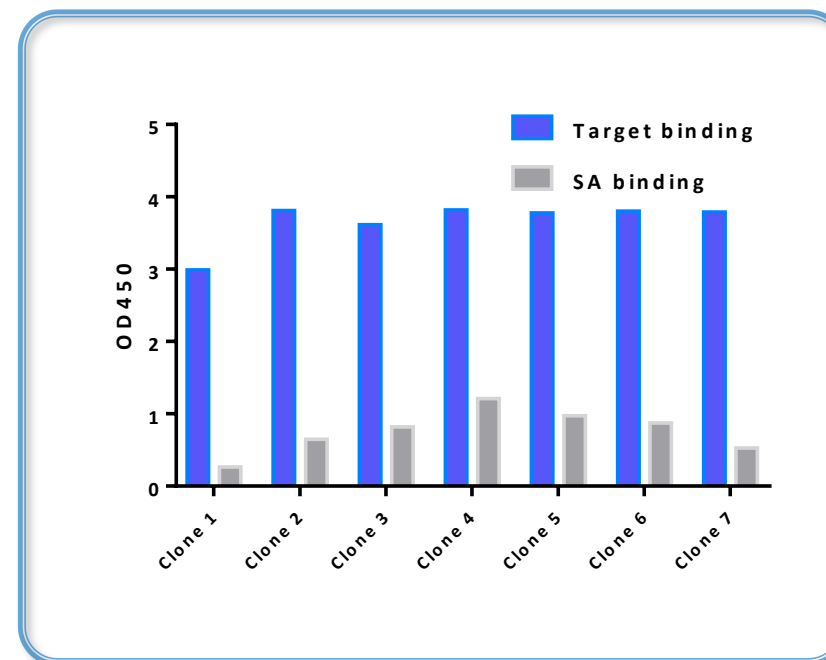
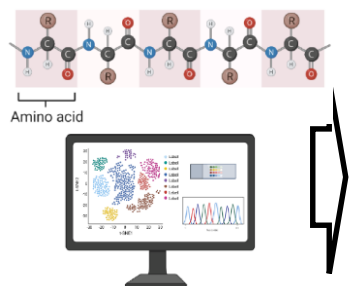# Human Synthetic Fab library case study list

Project No.	Human binding positive rate	Human/mouse/ cyno cross binding rate	Unique sequence	Antigen type
1	89%	100%	12	Protein complex via cell panning
2	22%	NA	10	Protein
3	57%	100%	5	Protein
4	18%	83%	8	Protein epitope specific panning
5	24%	NA	32	GPCR via peptide and cell panning

Binding to human target
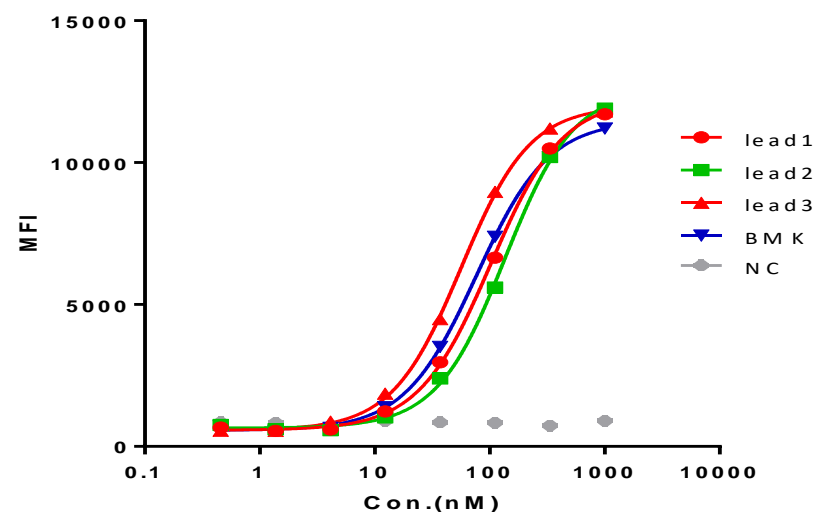
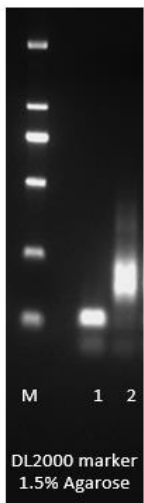# ➢ **Peptide phage display case study**
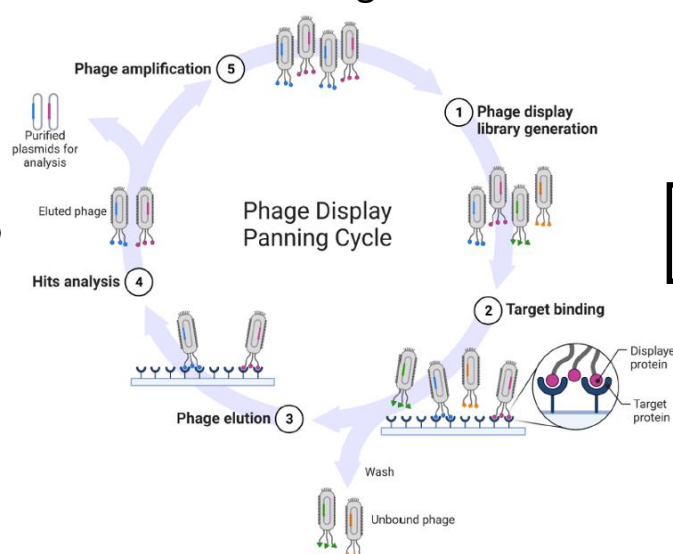

CADD/AIDD based library design

Library construction

Panning

NGS

Hits verification
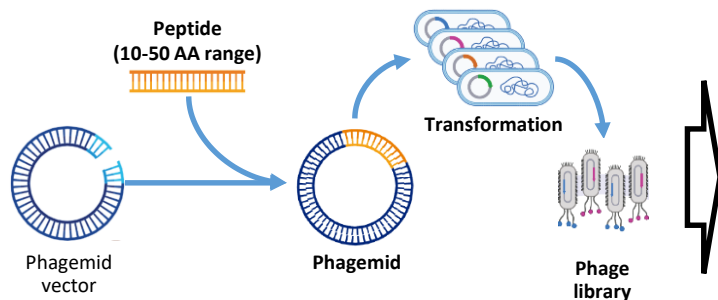
Screening

Peptide library data

Library name	library size	Insertion rate
Peptide library	4.0E+09	100%

Peptide library QC

	Sample	Comments
1	Peptide library PCR	Smear is consistent with designed size
2	Primers only	\
M		DL2000 marker
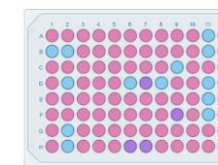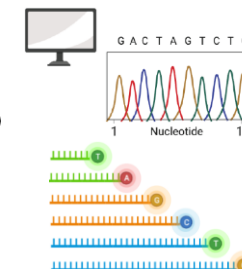
Hits verification with capture ELISA

ELISA data for target A (Tumor-related)

- Negative control
- Positive hit 1
- Positive hit 2
- Positive hit 3
- Positive hit 4
- Positive hit 5
- Positive hit 7
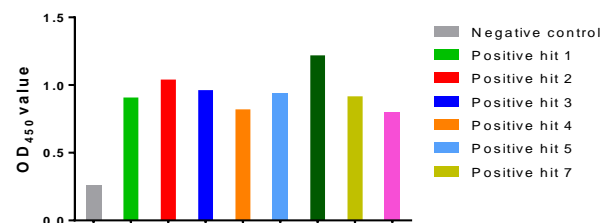
ELISA data for target B (Cytokine related)
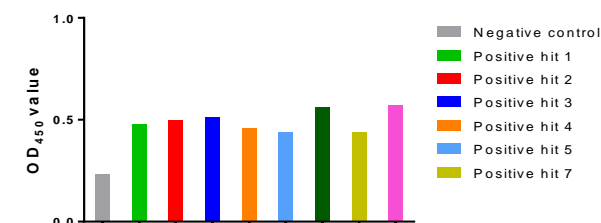
- Negative control
- Positive hit 1
- Positive hit 2
- Positive hit 3
- Positive hit 4
- Positive hit 5
- Positive hit 7

➤ Peptide yeast display case study

CADD/AIDD

Amino acid

Library construction

Peptide

cMyc

Aga2p

Aga1p

Yeast surface

Yeast
library

Yeast
display

Yeast
display
vector

Sorting with Biotinylated
Antigen

4 Rounds sorting

Screening/Sequencing

BD Accuri™ C6 Plus
Flow Cytometer
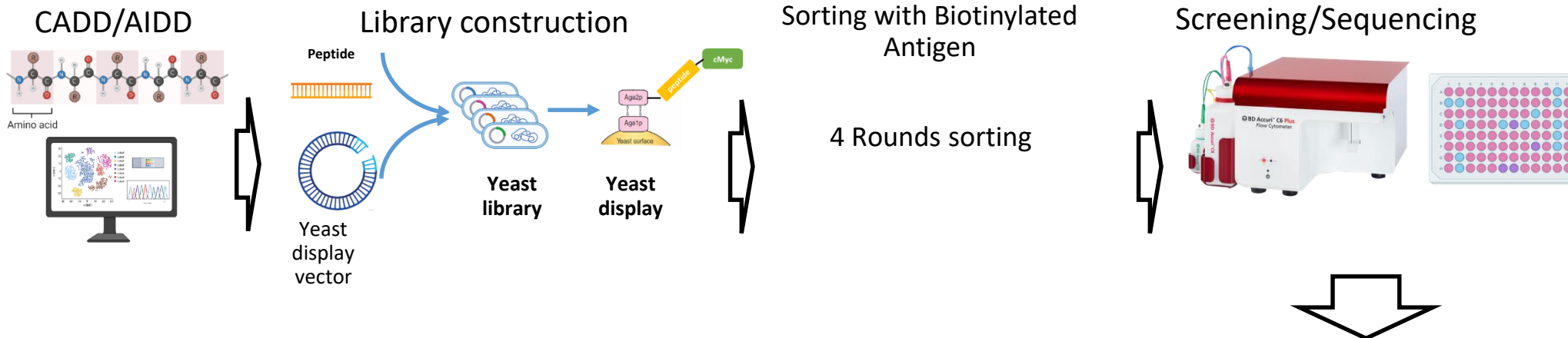
Peptide library QC

Sample name	Library size	Expression rate	Sequence analysis
Library	4E+08	95.5%	89.1%

Sorting

12.4% P4

2.7% P4

Round 1

3.3% P4

Round 2

46.0% P4
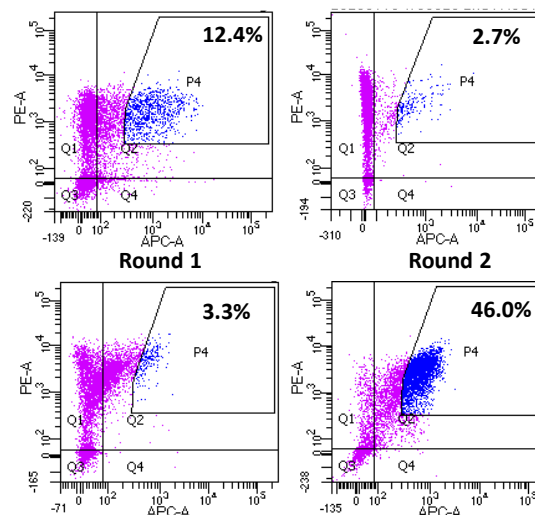
Hits verification with FACS

| Group Number	Sample Name	MFI by 40nM Ag1
1	Negative control	300
1	BMK	1900
2	Positive hit1	5700
3	Positive hit2	5500
4	Positive hit3	5400
5	Positive hit4	5200
6	Positive hit5	5200
7	Positive hit6	5100
8	Positive hit7	5000

Case studies of Lead Optimization

- ➢ **Strategy and summary**

- ➢ **VHH affinity maturation case study**

- ➢ **scFv affinity maturation case study**

- ➢ **Fab affinity maturation case study based on yeast display**

- ➢ **pH dependent antibody engineering case study**

➤ **Strategy and summary**
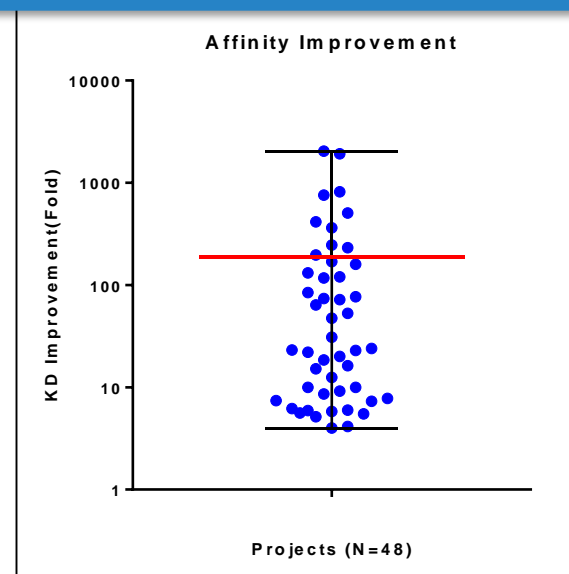
Multi-site tailored mutant library

CDR1   CDR2   CDR3
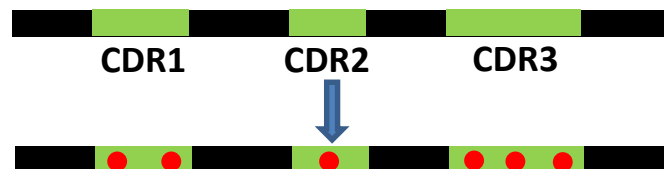
Sequencing of mutant library
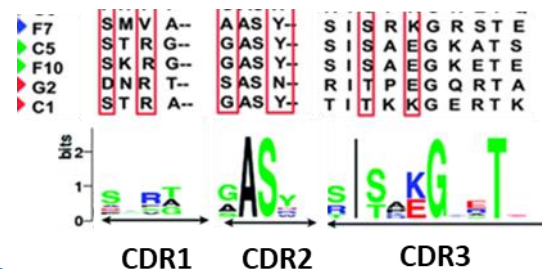
- Parsimonious mutagenesis screening of hot-spot
- Design and construct hot-spot combinatorial library with tailored diversity in each position
- Large-diversity space (up to 10^9 library size) and high-throughput panning/screening
- Simultaneous removal of PTM risks
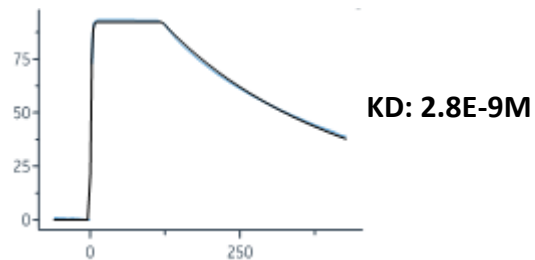- 6-10 weeks timeline

High average improved affinity

Case 1: parental VHH
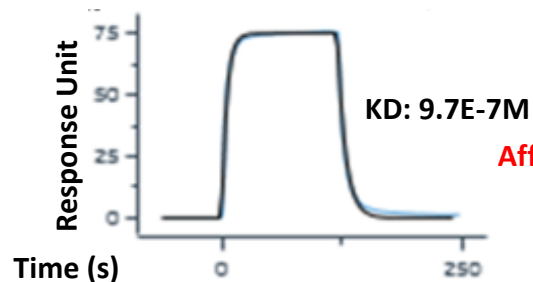
Case 1: VHH after affinity maturation

KD: 9.7E-7M

KD: 2.8E-9M

Affinity 346 fold

Case 2: parental VHH
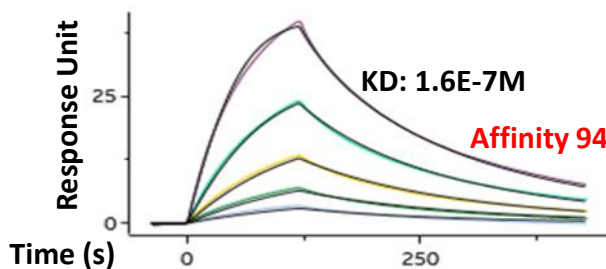
Case 2: VHH after affinity maturation

KD: 1.6E-7M

KD: 1.7E-10M
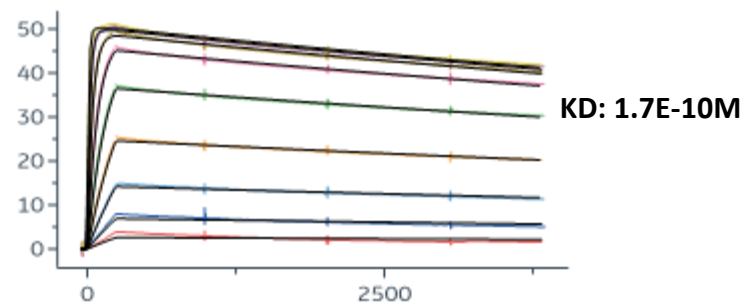
Affinity 941 fold

➤ scFv affinity maturation case study

Improvement validated by SPR

Candidate   **Lead**
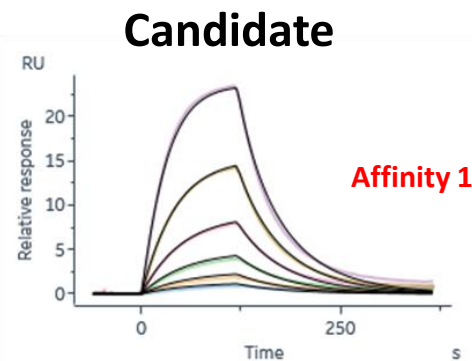
Human antigen
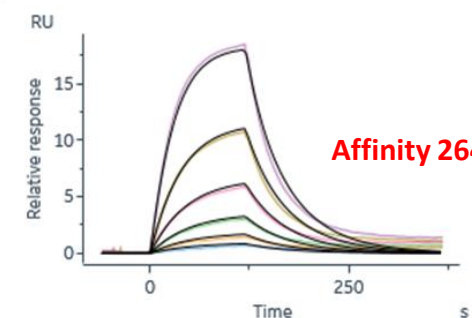
Affinity 1800 fold ⬆

Human Ag	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
Candidate	7.35E+06	3.59E-02	4.88E-09
Lead	3.70E+06	<1.00E-05*	<2.70E-12

Mouse antigen

Affinity 2640 fold ⬆

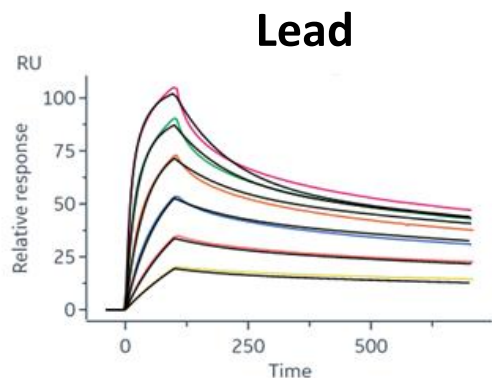| Mouse Ag	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
Candidate	5.94E+06	3.38E-02	5.69E-09
Lead	4.65E+06	<1.00E-05*	<2.15E-12

➤ scFv affinity maturation case study

- High affinity improvement, **4622** fold on mouse antigen.
- Increase cross-species specificity. Narrow the difference between mouse and human binding from **4696** fold to **6** fold.
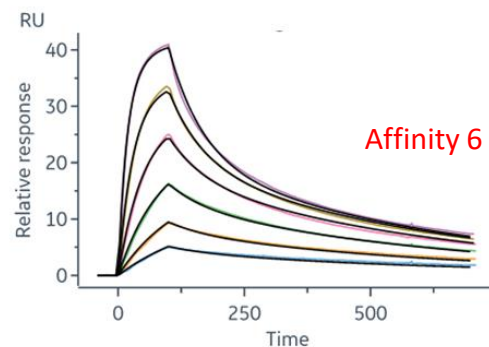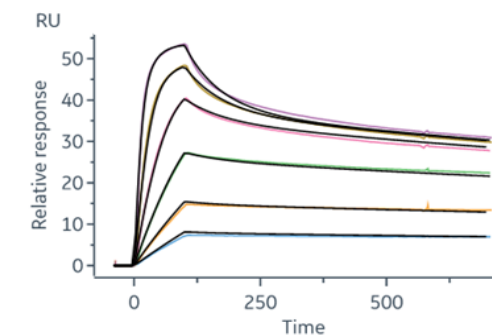
Improvement validated by SPR

Candidate | Lead
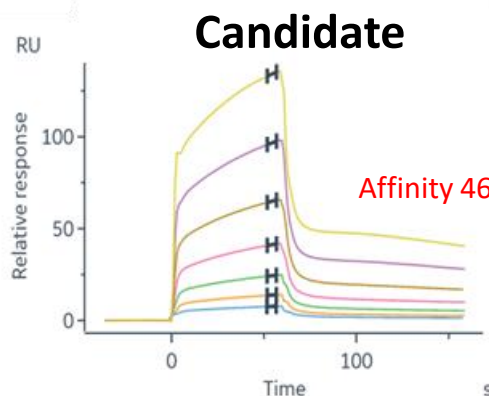
Mouse antigen — Affinity 4622 fold

Human antigen — Affinity 6 fold

Mouse Ag	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
Candidate	NA		5.87E-07
Lead	9.62E+06	1.23E-03	**1.27E-10**

Human Ag	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
Candidate	2.51E+07	3.14E-03	1.25E-10
Lead	3.31E+07	6.82E-04	**2.06E-11**

➢ Fab affinity maturation case study based on yeast display

Library construction and QC (2-3 weeks)

Design and construction of multi-point mutagenesis
yeast display library

Yeast Display

cMyc

Aga2p

Aga1p

Yeast surface
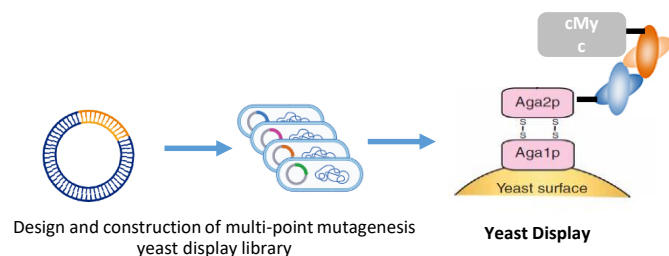
➡ **5 Rounds sorting** ➡

Screening/Sequencing (2-3 weeks)
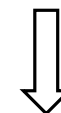
Fab mutant library QC

Sample name	Library size	Expression rate	Sequence analysis
Library	7.7E+07	97.7%	97.7%

Sorting

Round 1 — 4.5%
Round 2 — 1.8%
Round 3 — 2.0%
Round 4 — 7.7%
Round 5 — 30.2%

Hits verification with FACS

Leads KD-FACS by Ag1

Legend: Lead1, Lead2, Wild type, Positive control
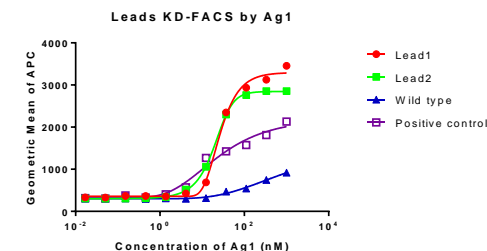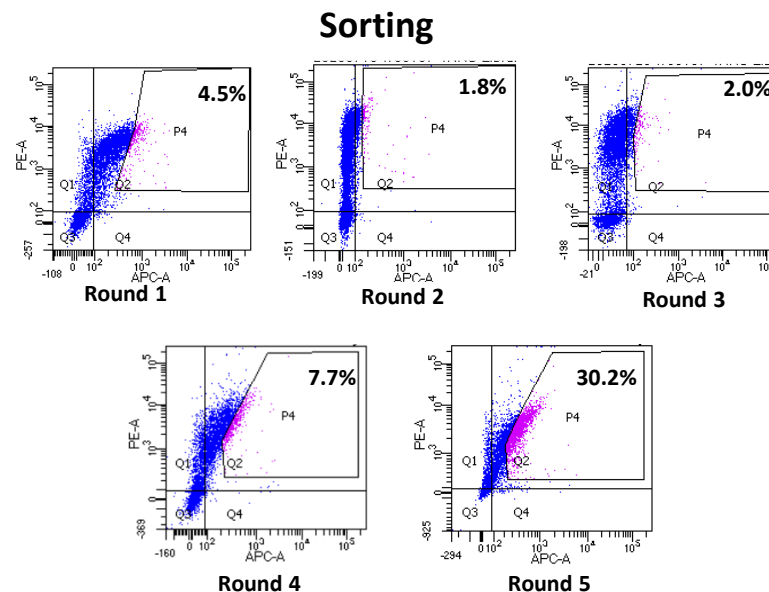
Sample Name	Ag1	
	Bmax	**EC50 (nM)**
Lead 1	3400	27
Lead 2	2800	20
Wild type	900	NA
Positive control	2100	NA

➢ pH dependent antibody engineering case study

pH Engineering

Mutagenesis → Sequence Validation → Primary Screen → Double confirm and mutation combination → Secondary Screen → Leaders Validation

Case Study

Before
After
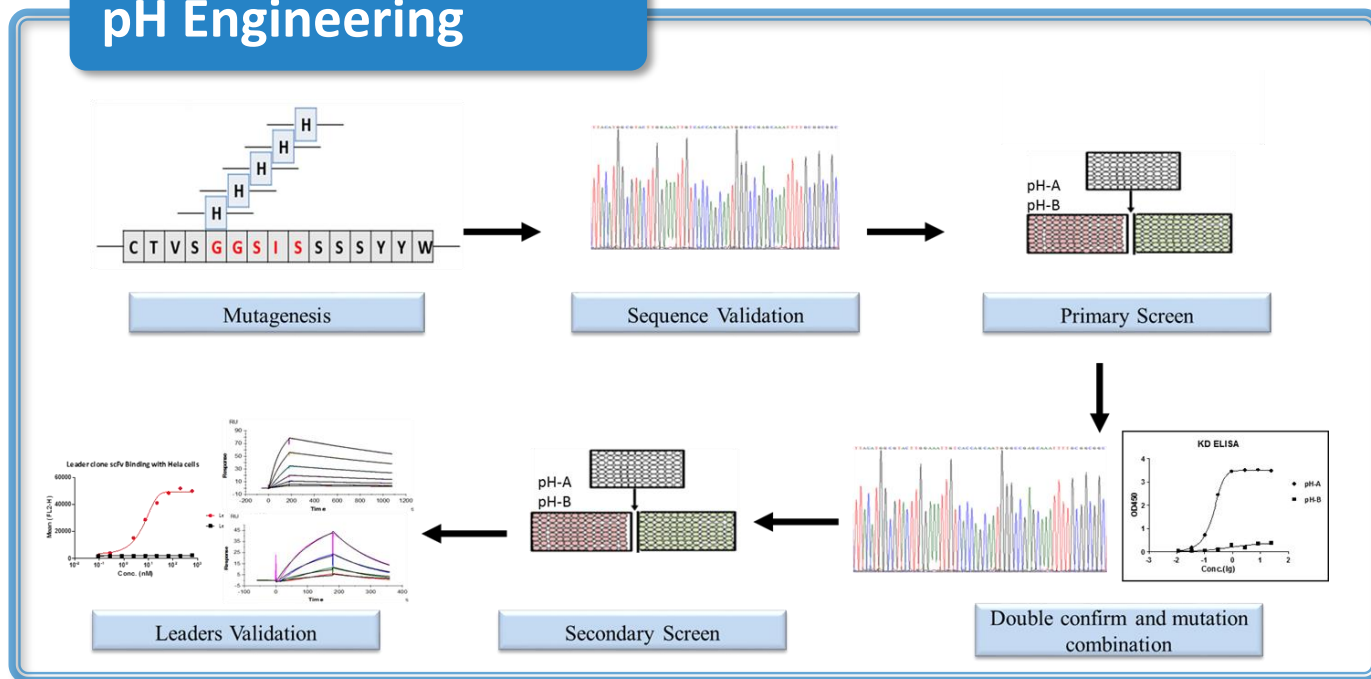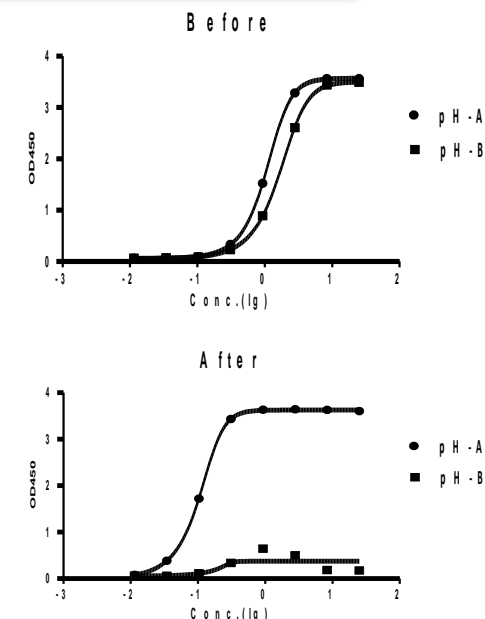
➢ **Histidine and charged residues based library design and construction**
➢ **Fast, cover all the V region positions, High-throughput screening without purification**
➢ **~3 months turn over rate**

➤ **For lead identification:** WuXi Biologics Display platform can generate leads towards diverse targets with tailored epitope and affinity.

➤ **For lead optimization:** WuXi Biologics Display platform can optimize leads in diverse formats for improved affinity, conditional properties (pH-dependent, and other request) and developability.

Thank you!

Acknowledgement: Clients' permission to share their excellent cases

WuXi Biologics Vision

"Every drug can be made and every disease can be treated" by building an open-access platform with the most comprehensive capabilities and technologies in the global biologics industry.

Learn More

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