Validating synthetic Library of Libraries against high-impact targets to enable novel antibody discovery

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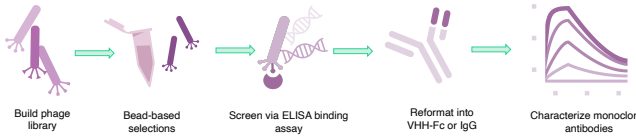
I. INTRODUCTION

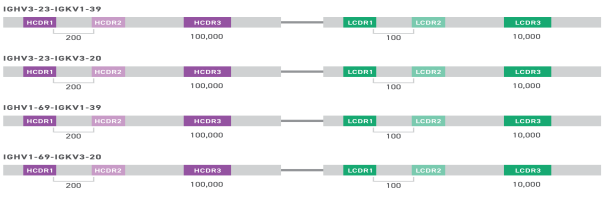
Multiple highly diverse synthetic antibody libraries have been generated using Twist's precise DNA synthesis technology to empower the antibody discovery process. A few of these "Library of Libraries" include the Structural scFv Library, AI Hypermutated scFv Library and hCamel Zero VHH Library. By leveraging these synthetic libraries, a wide range of high-affinity antibody candidates have been identified across multiple protein targets.

In this project, we demonstrate the validation process of constructed synthetic antibody libraries. Phage display was used to pan the libraries against biotinylated protein targets and ELISA binding assays were performed to screen for unique candidates. A high-throughput protein purification process allows candidates to be reformatted into VHH-Fc or IgG, which are then characterized through SPR.



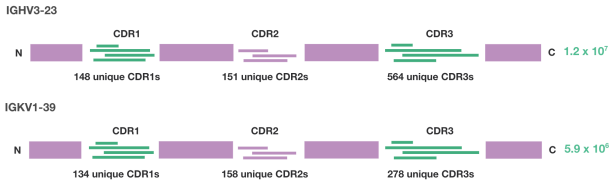
II. LIBRARIES

A. AI Hypermutated scFv



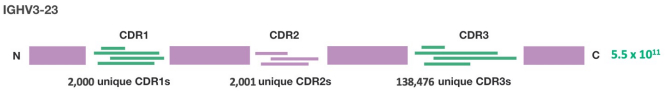
- Harnesses deep machine learning Neural Network to generate antibody repertoires resembling B cell receptor recombination and hypermutation
- Natural CDR sequences were shuffled into two human heavy chain and light chain frameworks with canonical CDR1 and CDR2 linkages maintained
- Designed with 4 combinations of heavy and light chain human germlines
- 1 x 10^9 actual diversity

B. Structural scFv



- Incorporates 6 CDR sequences from about 3,700 antibodies with known crystal structures for increased developability
- CDRs were shuffled into one human heavy chain and one light chain framework
- Manufacturing liabilities such as unpaired cysteines, N-glycosylation, deamination and hydrolysis sites were all eliminated during the design process
- 4x10^9 actual diversity

C. hCamel Zero VHH

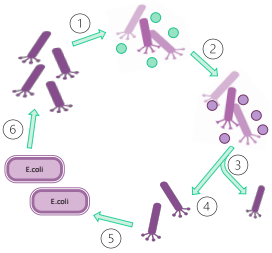


- Includes fully natural camel HCDR1, HCDR2 and HCDR3 sequences to improve folding and display
- Zero additional cysteines to minimize immunogenicity
- 6x10^8 actual diversity

III. IN VITRO DISCOVERY

Selections

1. Biotinylated proteins were incubated with magnetic streptavidin beads and phage library pools were incubated with empty streptavidin beads after the first round to serve as a depletion step.
2. Depleted phage library pools were added to the biotinylated proteins that were now bound to streptavidin beads.
3. Beads were pulled down with a magnet and washed with PBST at varying frequencies to discard of unbound phage.
4. Beads bound with phage were incubated with trypsin to elute phage.
5. Eluted phage was infected into *E.coli* TG1 cells.
6. Infected cells were grown on solid media and rescued with M13K07 helper phage to expand output for PEG precipitation preceding the next round.



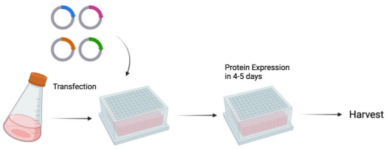
ELISA binding assay

380 clones from the Round 3 and Round 4 panning outputs of each library pool were screened in a 384-well plate format, with background plates included. The plates were incubated with Anti-M13 secondary HRP before being developed with TMB. Clones with target signals at least 3-fold over background (S/B) were considered positive hits and the top 96 hits from each panning round were sequenced by Sanger.

Library	Target	Round	# of clones 3-fold S/B	Unique Sequences
AI Hypermutated scFv	1	R3	162	71
		R4	335	18
	2	R3	96	22
		R4	96	8
		Total:	**689**	**119**
Structural scFv	1	R3	42	40
		R4	196	15
	2	R3	20	14
		R4	97	10
		Total:	**335**	**79**
hCamel Zero VHH	1	R3	178	10
		R4	223	15
	2	R3	62	8
		R4	91	13
		Total:	**554**	**46**

Reformatting into VHH-Fc or IgG

Sequence unique clones were reformatted into VHH-Fc or IgG and DNA was generated using Twist's high-throughput synthesis platform before being transfected into HEK293 cells. Expressed VHH-Fc or IgG were then harvested and purified using a Hamilton liquid handler.

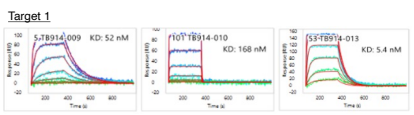


IV. SPR CHARACTERIZATION

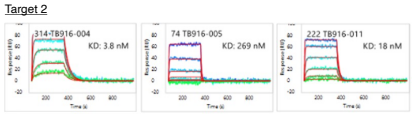
Following reformatting into VHH-Fc or IgG, purified antibodies were evaluated for binding affinity in a capture kinetics experiment with the Carterra LSA instrument. A high diversity of binding profiles was observed for each library against the two protein targets, ranging from triple-, double- and single-digit nanomolar to triple-digit picomolar binders. A snapshot of these moderate to high affinity binders are shown in the sensorgrams below, which were fit with a 1:1 kinetics binding model.

AI Hypermutated scFv mAbs bind to:

Target 1

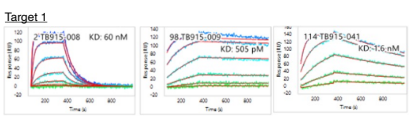


Target 2

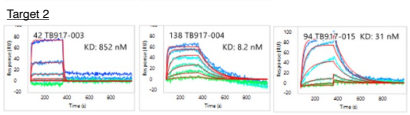


Structural scFv mAbs bind to:

Target 1

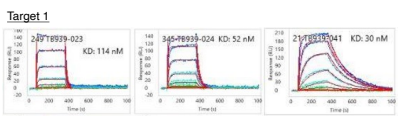


Target 2

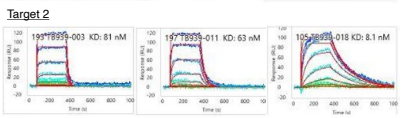


hCamel Zero VHH mAbs bind to:

Target 1



Target 2



V. SUMMARY

Library Name	# of target-specific mAbs	% of target-specific mAbs
AI Hypermutated scFv	79	66.4%
Structural scFv	39	49.4%
hCamel Zero VHH	37	78.7%
Total:	**155**	**63.3%**

Twist's high-throughput technologies in DNA synthesis, phage display, and protein purification have empowered the engineering of diverse synthetic libraries and the production of high avidity antibodies. The synthetic libraries introduced here show a wide diversity of binders in both the nanomolar and picomolar range against high-impact targets. This validates the robust performance and strong potential of these "Library of Libraries" for future antibody discovery in diagnostic and therapeutic applications.