Leveraging synthetic Library of Libraries to enable effective antibody discovery against high-impact targets

Marisa L. Yang, Tom Z. Yuan, Hector Franco, Nathan Raynard, Mouna Villalta, Crystal Safavi, Eric Kwan, Linya Wang, Sean Peterson, Ana Lujan, Fumiko Axelrod, Hoa Giang, Aaron Sato

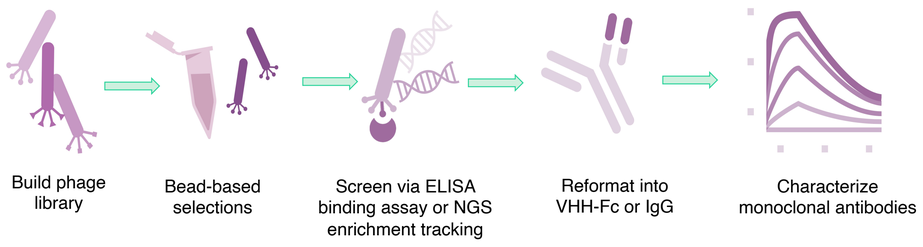
TWIST BIOPHARMA

I. INTRODUCTION

Using Twist's high-throughput DNA synthesis platform, multiple high diversity synthetic antibody libraries have been built to serve as powerful tools for antibody discovery. Leveraging these highly diverse libraries, we have been able to identify high-affinity antibody candidates for a variety of targets.

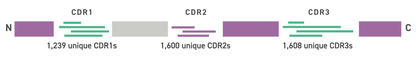
Here we demonstrate the antibody discovery process for 6 cytokine or immunomodulatory protein targets. This process spans constructing synthetic antibody libraries, using phage display to pan libraries against biotinylated protein targets, and screening for lead candidates through ELISA binding assays and NGS enrichment tracking. Utilizing a high-throughput approach, candidates are reformatted into full length IgG or VHH-Fc; the resulting monoclonal antibodies are then validated through SPR and cell-based assays.

Many high affinity candidates with ranges of picomolar to nanomolar binding were identified for the six targets, demonstrating the efficacy of this discovery process.



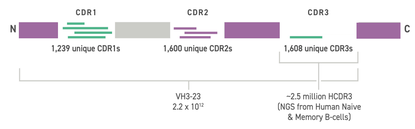
II. LIBRARIES

A. VHH hShuffle



- Created by shuffling thousands of natural llama CDR sequences
- Partially humanized DP-47 framework
- 3.2 x 10^9 actual diversity

B. VHH Hyperimmune (VHH HI)

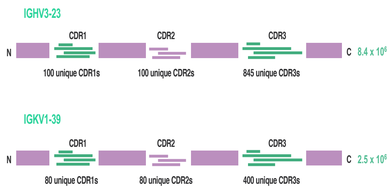


- Created by shuffling millions of natural llama and human CDR sequences
- Natural llama CDR1 & CDR2 sequences
- 2.5 million human HCDR3 sequences
- Partially humanized DP-47 framework
- 1 x 10^{10} actual diversity

C. AI Hypermutated ScFv

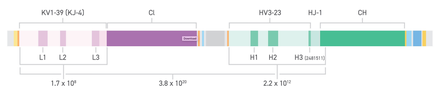
- Created by deep learning algorithm, generating antibody repertoires resembling natural B cell receptor hypermutation & recombination
- Built on 4 combinations of heavy and light chain human germlines.
- 1 x 10^9 actual diversity

D. Ancestral ScFv



- Created by shuffling unique CDRs from thousands of human, monkey, and alpaca antibodies
- Fully human germline framework
- 1 x 10^9 actual diversity

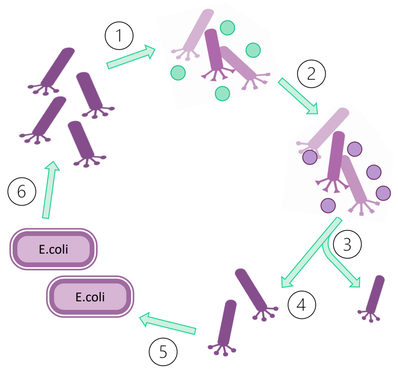
E. Hyperimmune Fab (HI Fab)



- Created from shuffling CDRs from human naïve and memory B-cell receptor sequences
- 2.5 million human HCDR3 sequences
- 1 x 10^{10} actual diversity

III. SELECTIONS

1. Phage pools were incubated with streptavidin beads as a depletion step if applicable
2. Depleted phage pools were incubated with biotinylated protein bound to streptavidin beads
3. Beads were washed with PBST to rid of unbound phage
4. Beads were incubated with trypsin to elute phage bound to beads.
5. Eluted phage was infected into TG1 E.coli cells
6. Infected cells were grown on solid media and rescued with M13K07 helper phage to amplify output.



200nM antigen
No depletion
3x PBST wash

→ 100nM antigen
Bead depletion
4x PBST wash

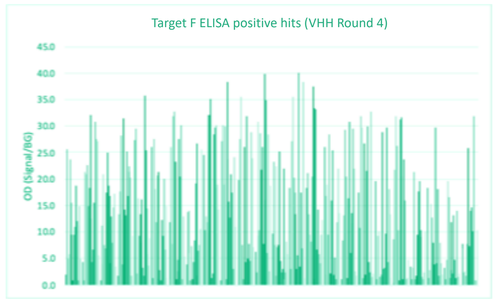
→ 50nM antigen
Bead depletion
5x PBST wash

→ 20-50nM antigen
Bead depletion
6x PBST wash

IV. SCREENING & REFORMATTING

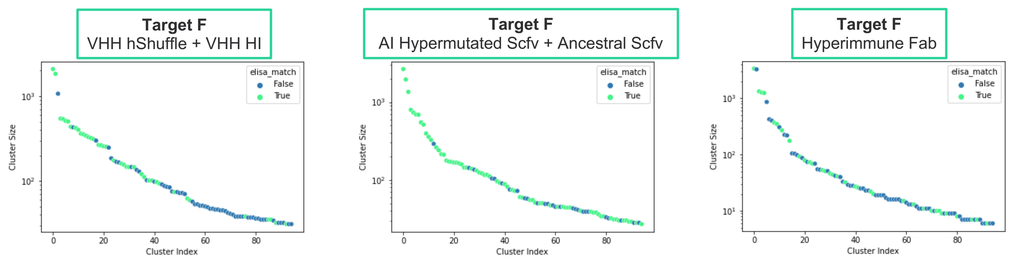
A. ELISA binding assay

Clones from the Round 3 and Round 4 panning output of each library pool were tested. Blocked phage supernatant was incubated with antigen-coated 384-well plates, then incubated with Anti-M13 secondary HRP and developed with TMB. Clones with signals at least 3x over background were considered positive clones.



B. NGS enrichment tracking

Next-generation sequencing (NGS) was performed on Round 3 and Round 4 panning output pools for all targets, generating 10,000 reads per pool. Sequences were assigned to clusters based on Levenshtein distance (≤ 3). The most predominant sequences, one from each of the top 95 enriched clusters, were selected for reformatting into VHH-Fc or IgG.



Reformatting into VHH-Fc & IgG

Clones were reformatted into VHH-Fc or IgG format, then synthesized using Twist's high-throughput synthesis platform. DNA was scaled up and transfected into HEK293 cells. Expressed protein was then harvested and purified.

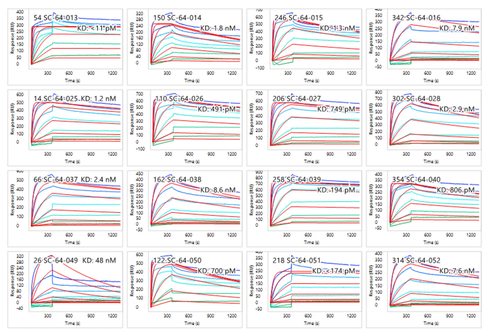
	VHH		Scfv		Fab	
	ELISA	NGS	ELISA	NGS	ELISA	NGS
Target A	42	75	4	92	1	68
Target B	35	79	22	67	7	89
Target C	18	95	48	61	4	93
Target D	93	95	91	95	23	95
Target E	57	95	35	95	20	95
Target F	60	95	65	95	31	95

Table 1. Quantity of unique clone sequences selected for reformatting, derived from ELISA positive clones or NGS enrichment tracking

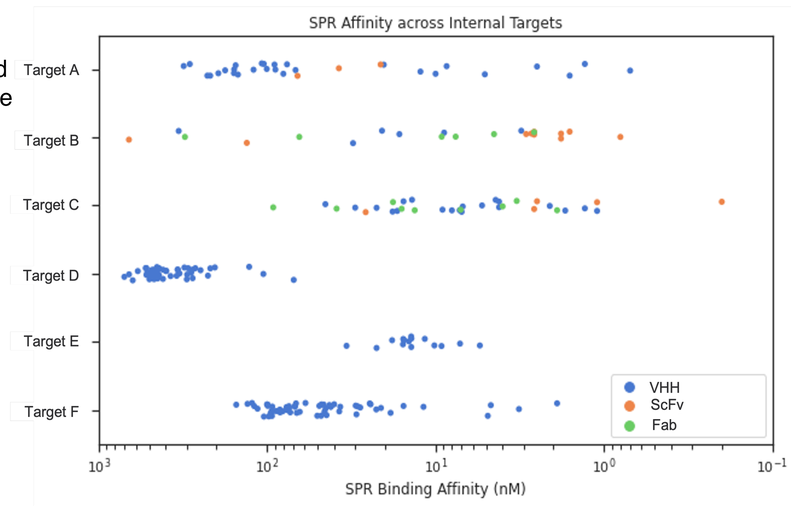
V. BINDING ASSAYS

A. SPR

Following reformatting into VHH-Fc and IgG, purified antibodies were evaluated for binding affinity with the Carterra LSA instrument.



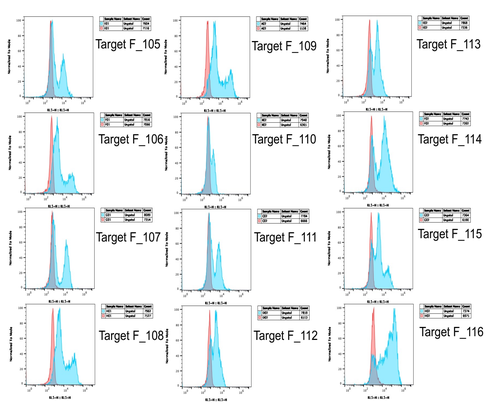
SPR binding profile of Target F reformatted candidate antibodies against Target F.

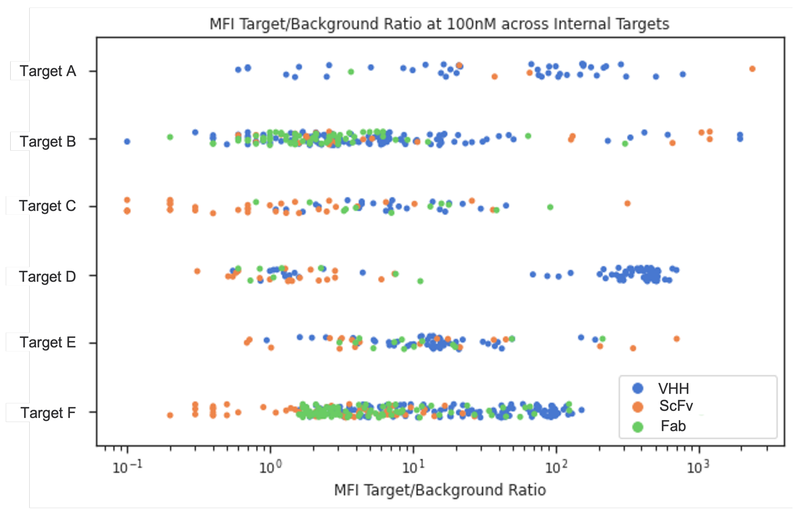


B. Cell-based binding assays

Purified antibodies were incubated with HEK293 cells transiently transfected with relevant target protein DNA, then with APC-conjugated Goat anti-human IgG and run on the iQue instrument to assess cell-binding performance.

Binders were subsequently assessed again through FACs titration assays. Selected hits will undergo various functional assays to further examine performance.





VI. SUMMARY