TAO: Twist Antibody Optimization of an Anti-PD-1 Antibody

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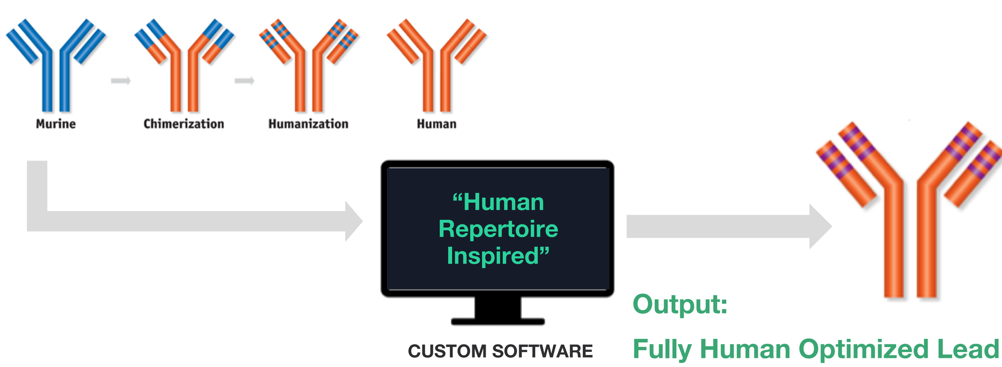
ABSTRACT

The antibody humanization and affinity maturation process often requires mutagenesis in the CDR and framework regions by using NNK/NNS/TRIM randomization schemes. These schemes, however, generate a large amount of unwanted residues and create downstream manufacturability liabilities.

We used the Twist Antibody Optimization platform (TAO), a custom algorithm to explore the CDR mutation space from reference sequence as well as the closest germline. Only natural LCDR1-3 and HCDR1-2 sequences identified from an NGS-derived human antibody database, combined with single amino acid scans of HCDR3, are incorporated into the final library. Twist's silicon-based DNA synthesis allows the rapid and precise synthesis of antibody libraries that avoid non-functional sequence strings, amino acid liabilities, and existing commercial CDR spaces. The libraries also lower the risk of immunogenicity for drug discovery pipelines by printing exact sequence strings that match the natural CDR repertoire.

We applied the TAO platform to optimize a low-affinity antibody to human PD-1. Mutations made from both the parent and nearest germline sequence were checked against an NGS database from 12 human donors while also suppressing manufacturability liabilities. The resulting library was assembled into a phage-displayed scFv library and panned for binding across several different conditions. ELISA-positive hits were directly reformatted and converted to full IgGs for testing on both soluble protein and PD-1-overexpressing CHO cells. Several variants demonstrated 50-100x improved monovalent binding affinities, comparable to those from commercial anti-PD-1 antibodies, while maintaining on-cell binding and retaining the original antibody-binding epitope.

NATURAL REPERTOIRE-BASED OPTIMIZATION LIBRARY



Heavy Chain — 1 1 0 0 1 0

Germ	d1	d2	V	FW	CDR	H/L	FW1	SDR1	FW2	SDR2	FW3	SDR3	FW4
donor IGHV1-18	0	0	97.1%	97.6%	95.6%	100%	EVQLVQSGAEVKKPGASVKVSCKASG QVQLVQSGAEVKKPGASVKVSCKASG	YRFTSYGIS YTFTSYGIS	WVRQAPGQGLEW WVRQAPGQGLEW	MGWISAYNGNTNYA MGWISAYNGNTNYA	QKLQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC QKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYC	ARDADYSSGSGY ------------	WGQGTLVTVSS WGQGTLVTVSS

Light Chain — 0 0 1 1 1 6 1

Germ	d1	d2	V	FW	CDR	H/L	FW1	SDR1	FW2	SDR2	FW3	SDR3	FW4
donor IGLV3-25	0	0	96%	96.3%	94.4%	94.7%	---LTQP-PSVSVSPGQTARITC ---LTQP-PSVSVSPGQTARITC	SGDALPKQYAY SGDALPKQYAY	WYQQKPGQAPVMVIY WYQQKPGQAPVLVIY	KDTERPS KDSERPS	GIPERFSGSSSGTKVTLTISGVQAEDEADYYC GIPERFSGSSSGTTVTLTISGVQAEDEADYYC	QSADNSITYRV LSADSSGTWV	FGGGTKVT-- FGGGTKLT--

TAO CRITERIA

natural-fitness-scan	distance 1, 2, or 3 (default 1)
min_subjects	min number of people with CDR (default 2)
suppress-liabilities	default yes
explore-germline	distance 1, 2, or 3 (default 1)
amino-scan=H,D,E	charge and feature scan
custom_cdrs=mycdrs.txt include	custom CDRs that the user can force
perform_h3_scan=0	single amino acid scan of H3 (default yes)

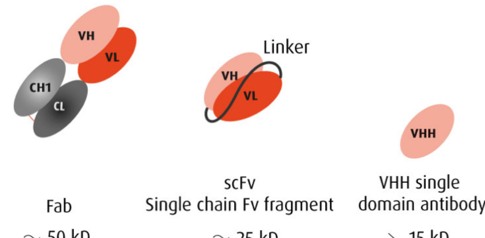
Uses NGS database from 12 people

Leveraging Twist DNA Synthesis Capabilities for Rapid Antibody Optimization
Using oligo pools to generate synthetic libraries offers significant advantages over using NNS/NNK and TRIM-based approaches. Starting with the parental antibody sequence and the closest germline sequence, mutational space is explored using natural LCDR1-3 and HCDR1-2 sequences derived from NGS data from 12 human donors.

Technology	Full Control of Amino Acid Distribution	No Stop Codons or Cys	Lack of Out-of-Frame Mutations	No Liability Dipeptide Motifs	Match Natural CDR Repertoire or Contain Sequence Motifs
NNS/NNK					
TRIM	✓	✓	✓		
Twist Oligo Pools	✓	✓	✓	✓	✓

Twist's long oligo synthesis technology can synthesize pools of DNA that are optimized during the design phase to maximize antibody library quality. Liabilities such as isomerization, cleavage, deamidation, and glycosylation sites are removed and exact sequences are explicitly encoded in the oligo pools. The synthetic oligos can then be assembled as VH-VL scFv and electroporated into *E. coli* TG1 cells. The fully assembled phage library as well as the selection outputs are then analyzed by NGS to determine library diversity and track enrichment of PD-1 binding antibodies.

Assembly into other antibody fragment formats, such as Fab and VHH single domain antibodies are also possible with the TAO platform.



TWIST BIOPHARMA'S ANTIBODY OPTIMIZATION AND DISCOVERY WORKFLOW

A. Library Generation

1 Input antibody sequence into software
Option to use human germline or parent frameworks

```
Scan natural diversity for IGHV1-18, max SHM=3 min subjects=2
H1      406 liability free local to ref YRFTSYGIS
H2      114 liability free local to ref GWISAYNGNTNYA
H3      0 liability free local to ref CARDADYSSGSGYW
H1      561 liability free local to germline IGHV1-18
H2      114 liability free local to germline IGHV1-18
Scan natural diversity for IGLV3-25, max SHM=3 min subjects=2
L1      164 liability free local to ref SGDALPKQYAY
L2      105 liability free local to ref KDTERPS
L3      41 liability free local to ref CQSADNSITYRVF
L1      164 liability free local to germline IGLV3-25
L2      105 liability free local to germline IGLV3-25

IGHV1-18     QVQLVQSGAEVKKPGASVKVSCKASG     968     WVRQAPGQGLEWM   229    QKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYY      267     WGQGTLVTVS
IGLV3-25     SYELMQPPSVSVSPGQTARITC  329     WYQQKPGQAPVLVIY 211     GIPERFSGSSSGTTVTLTISGVQAEDEADYY 42      FGGGTKLTVL
CDR Diversity, final:
CDR-H1: 968
CDR-H2: 229
CDR-H3: 267
CDR-L1: 329
CDR-L2: 211
CDR-L3: 42

Library Diversity:
Heavy chain:    5.9e+07
Light chain:    2.9e+06
Library:        1.7e+14
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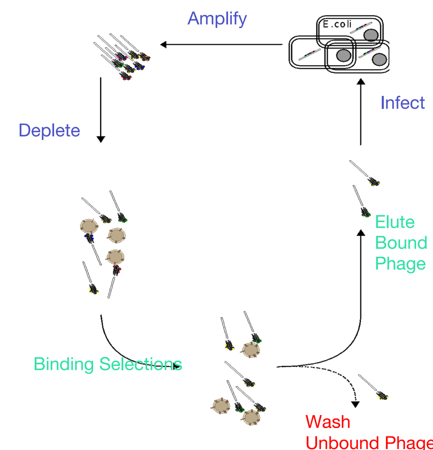
2 Order oligo pools & template DNA

3 Synthesize and screen library against target

Heavy- and light-chain mutational space is derived from the parent sequence and the closest germline sequence. All CDR sequences must be represented in two or more individuals from the NGS database. While the PD-1 TAO library was derived from a human parental sequence, non-human antibodies can be humanized while optimizing the CDR sequences.

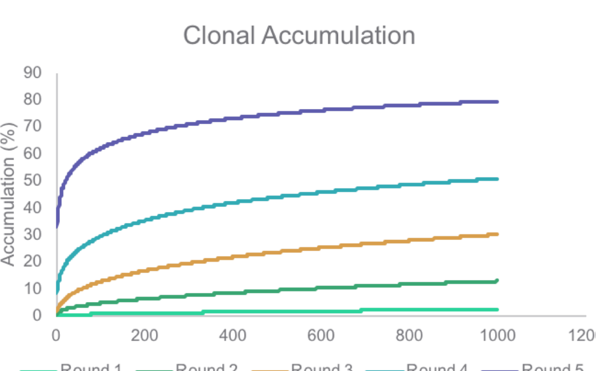
B. Bead-Based Selections

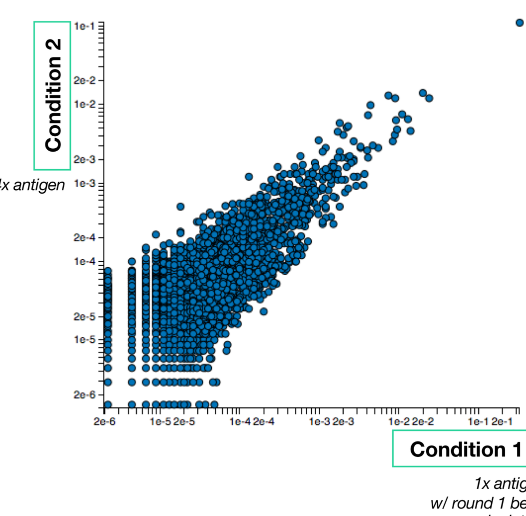


C-terminal biotinylated PD-1 antigen is bound to streptavidin-coated magnetic beads for five rounds of selection. Bead-binding variants are depleted between each round. Stringency of selection is increased with each round, and enrichment ratios track on-target binding.

C. ELISA and Next-Generation Sequencing

Two parallel methods are applied for hit identification – traditional phage ELISAs and deep-sequencing on Illumina MiSeq platforms to identify PD-1-specific binders. ELISAs allow direct identification of binders, while NGS allows selection of enriched clones that do not show up in the initial ELISA screening. All screens are conducted in 384-well plates and are automated using HighRes robotics to increase throughput and ensure assay reproducibility.

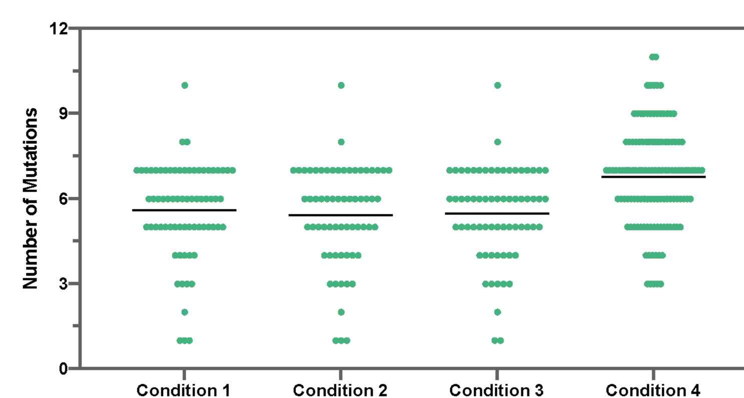




The data at left were obtained after five rounds of selection, completed with three different initial selection conditions. Clone enrichment was tracked through each successive round by NGS. Sequences enriched for off-target or background binders were removed.

Sequence analysis showed that the vast majority of enriched clones (>95%) for binding to PD-1 were equally captured across the different selection conditions, demonstrating the robustness of the PD-1 TAO antibody library.

A fourth, low-stringency selection was also performed. NGS data showed that the mutation rate differs from the high-stringency selections. Clones identified from these selections had lower overall binding affinity in both scFv and IgG formats.

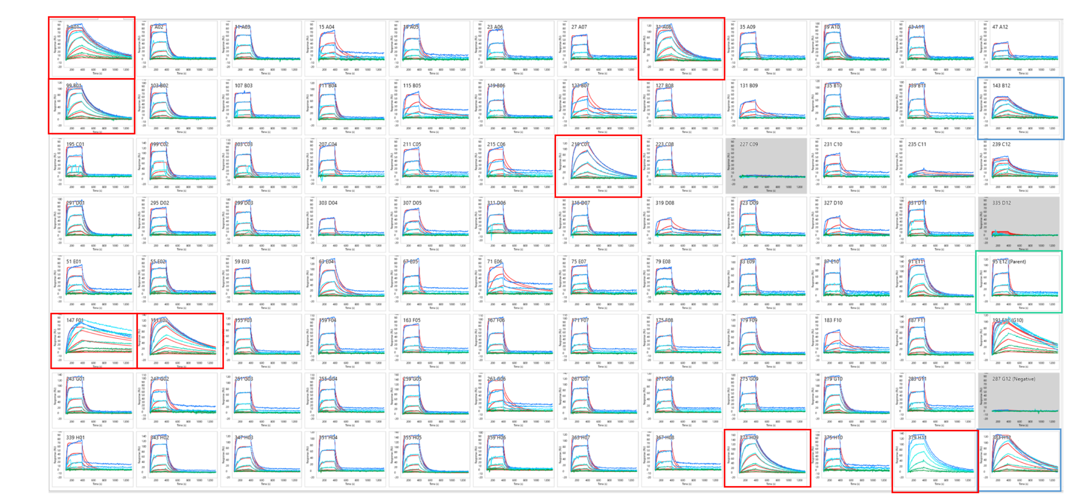


D. High-Throughput IgG Characterization

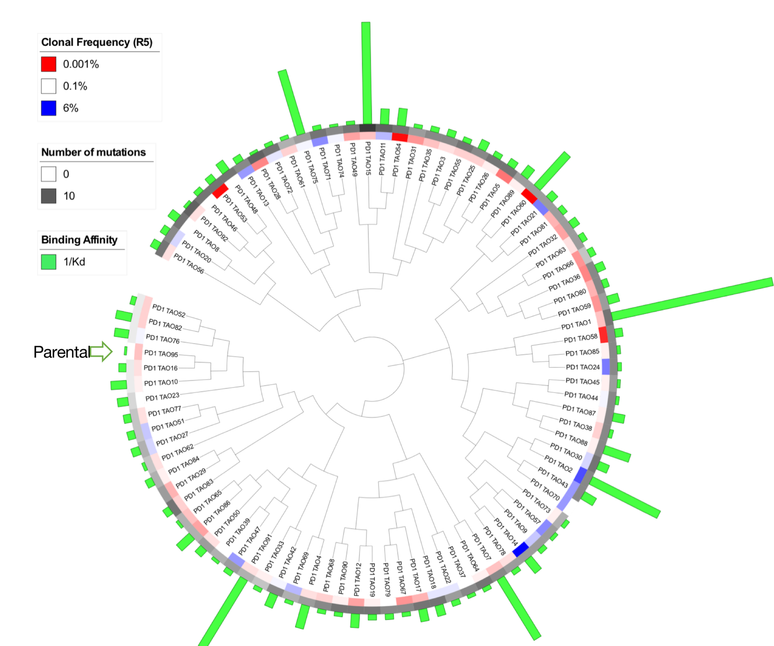
Twist's DNA synthesis platform enables high-throughput gene conversion from scFv to IgG in 3 weeks. Clones are transiently transfected in ExpiCHO and then purified by Kingfisher and Hamilton automation decks. Yield and purity are confirmed by Perkin Elmer Labchip and analytical HPLC.

Twist Clonal Gene Synthesis

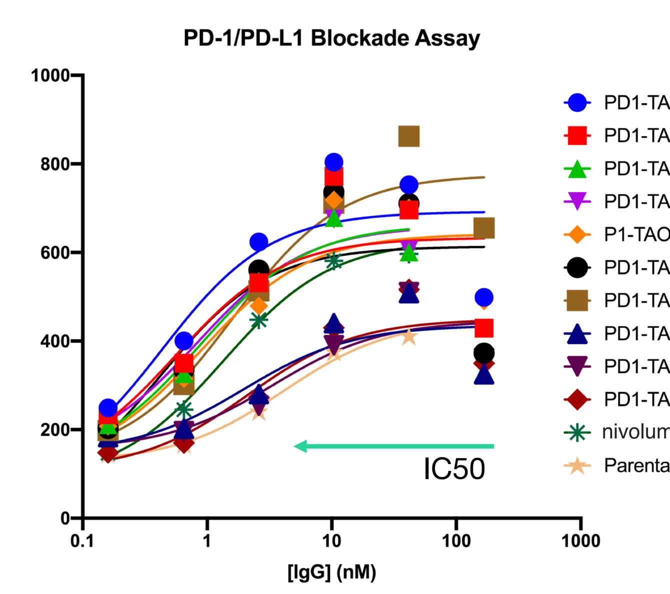
To fully leverage our high-throughput IgG production capabilities, the Carterra LSA system quickly assessed the binding affinity and epitope binning of >170 IgG variants.



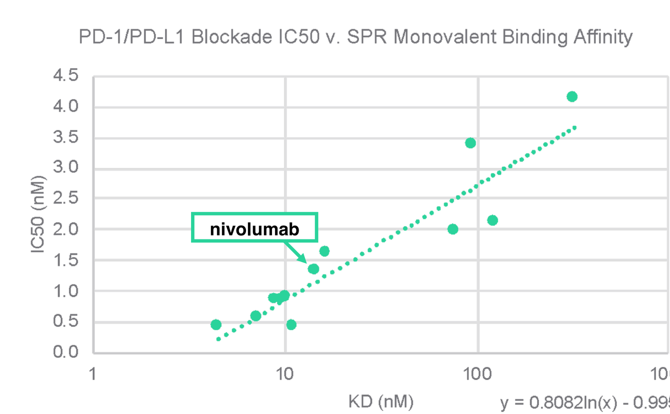
TAO-optimized IgGs demonstrate 100x improvement in monovalent binding affinity compared to the parental sequence. The PD1_TAO1 clone bound to PD-1 with a K_D of **4.52 nM**, while others show binding affinities of <10 nM. These high-affinity binders each contain unique CDRH3 and are not clustered by sequence lineage.



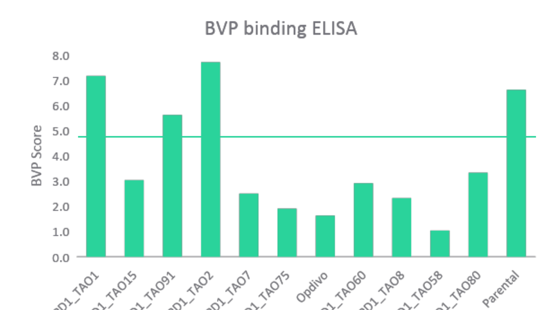
E. Functional and Developability Assays



TAO-optimized IgGs were tested for functional blocking of the PD-1/PD-L1 interaction. High-affinity variants demonstrated improved IC50 compared to the wild-type as well as commercial anti-PD1 antibodies, such as nivolumab. IC50 and monovalent binding affinity were highly correlative.
All binders also retained binding to cyno PD-1.



Several high-affinity IgGs demonstrated low polyspecificity scores, as measured by BVP binding ELISAs. Additionally, IgGs are tested on Unchained UNCLE machines for Tm and Tagg, as well as analytical HPLC.



SUMMARY

The Twist Antibody Optimization platform enables high-throughput, high-quality biophysical characterization of high-affinity, functional humanized antibodies. Several of these antibodies show improved affinity, functionality, and developability relative to approved commercial anti-PD1 biologics. **Twist Biopharma has also developed a GPCR-focused phage display library that has demonstrated rapid identification of functional, high-affinity antibodies.**