Driving Antibody Optimization with Broad-Space and Structure-Guided Platforms: Tumbler™ and Targeted Engineering

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Antibodies are increasingly used in drug development due to their ability to target a wide range of antigens. However, many antibody-based drug candidates require additional engineering for optimal therapeutic efficacy in humans. This can include affinity maturation, humanization, pH engineering, enhancement of stability, half life, and other developability parameters. Achieving this broad range of objectives requires a diverse set of technologies and approaches, each tailored to address specific challenges in antibody design and optimization.

Here, we introduce two flexible and complementary approaches to antibody optimization: Tumbler™ and Targeted Engineering. While both rely on rational CDR-focused mutational design for library generation, they differ in their design principles and engineering capabilities. Tumbler enables multi-parameter optimization by exploring a broader sequence space, allowing simultaneous improvement across several molecular attributes, such as affinity maturation, humanization, and induction of cross-binding. In contrast, Targeted Engineering generates libraries tailored to a predefined optimization goal, focusing on obtaining the best possible molecules for specific target characteristics. Both approaches then interrogate their respective libraries with selection pressure to discover optimized clones. Together, Tumbler and Targeted Engineering provide a powerful, unified optimization platform: one that accelerates early multi-parameter improvements and subsequently refines candidates through targeted, goal-specific optimization.

Comparison of Antibody Engineering Platforms at FairJourney Bio

	Tumbler™	Targeted Engineering

Design principles	• **Customizable:** Start with one or several starting molecules, which can originate from various sources including animal immunizations or phage display campaigns
	  • Build one custom library of up to $1x10^9$ variants of the original antibody
	• **Developable**: Tumbler Library uses CDR shuffling to interrogate both near and far sequence space without compromising developability
	  • Combines liability-free custom CDR variants + SuperHuman2.0 or Cosmic CDRs, grafted into a fully germline, human framework
		• One design per antibody and one library per CDR, covering theoretical diversity – combination of all libraries after two rounds of selection
		• Randomization of each CDR position based on natural diversity and structural data (Germline structure, cryo-EM)
		• Controlled number of mutations per sequence
		• Compatible with antibodies from all sources and formats

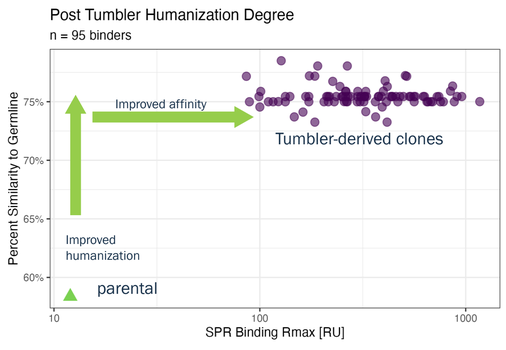
Engineering capabilities	• Flexible and multiparameter: enables simultaneous affinity maturation, humanization, cross-binding induction, pH engineering, and more • Enhances freedom to operate by producing clones dissimilar to parental sequence	• Powerful affinity maturation, pH dependency and cross-species engineering. Developability optimization if combined with mammalian display
Selection platform	• Phage display or phage combined with yeast display	• Phage display or phage combined with mammalian display
Timeline	• 3-6 months	• 6 months

Results

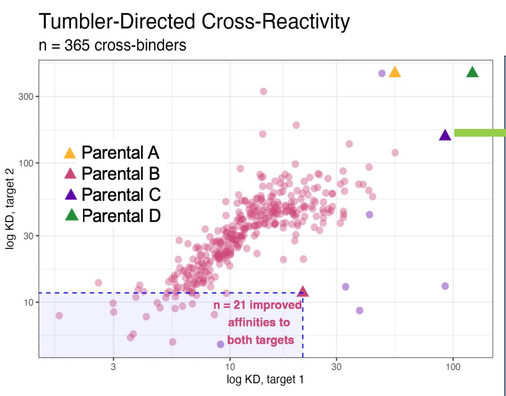
Curating Hits with Tumbler

Affinity Maturation + Humanization

Tumbler optimizes leads originating from nonhuman sources. Through framework grafting, CDR shuffling, and panning, we expand a developable selection of hits with a greatly reduced risk of immunogenicity in humans.
The plot below illustrates the degree of humanization and off-rate of the original parental sequence, synthetic graft containing parental CDRs and our germline framework, and final optimized clones. With Tumbler, we retained the humanness of a graft, and optimized binding from micromolar to nanomolar affinities.



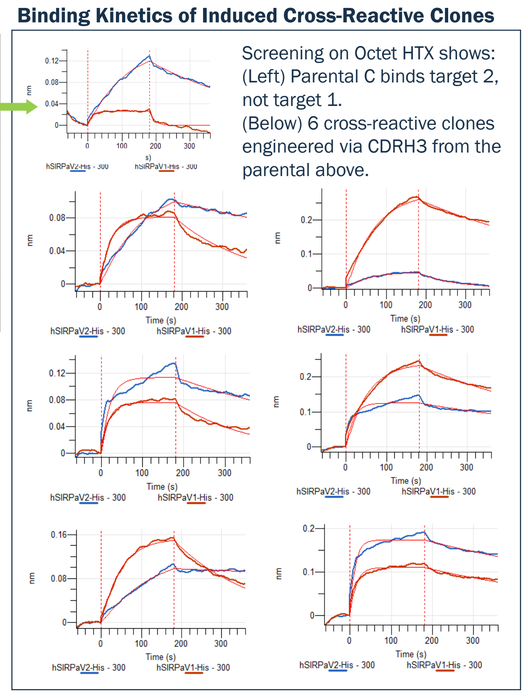
Unique panning strategies enrich binders that accomplish multi-parameter engineering objectives:



Affinity Maturation + Cross-Reactivity

Utilizing a library built with four parental clones, one panning arm focused on inducing and maintaining cross-reactivity to a variant of the original target. Here we identified 365 novel, cross-reactive clones generated through the Tumbler process. 21 of these binders had improved affinities to both targets compared to the parental cross-binding clone. For 6 of these binders, cross-reactivity was induced from a parental CDRH3 which originally bound to only target 2. We can therefore leverage library real-estate and subsequent panning to elucidate which parental(s) are conducive to the panning goal.

Binding Kinetics of Induced Cross-Reactive Clones



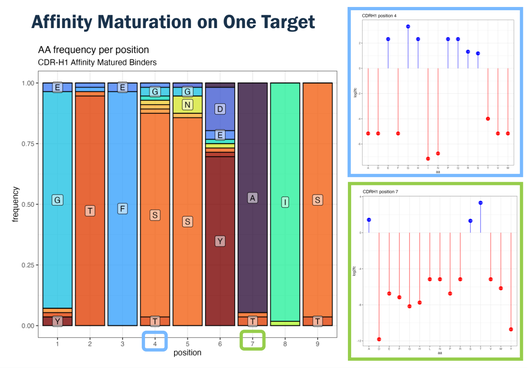
Screening on Octet HTX shows:
(Left) Parental C binds target 2, not target 1.
(Below) 6 cross-reactive clones engineered via CDRH3 from the parental above.

Sequence-Activity Relationship

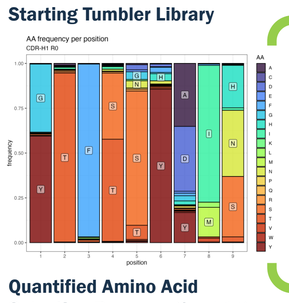
Examining sequence-activity relationships guides future development and engineering decisions of candidate clones.

Positional Weight Matrices
Comparing the preferences of certain amino acids at R0 (below), affinity matured (top right), and cross-reactive (bottom right) binders at each CDR provides specific insights into conserved and mutable residues.

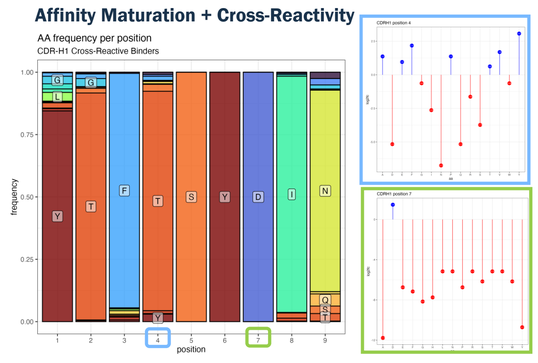
Affinity Maturation on One Target



Starting Tumbler Library



Affinity Maturation + Cross-Reactivity



Quantified Amino Acid Selection Pressure (far right)
The selection pressure of each amino acid in CDR-H1 Positions 4 and 7 is visualized as log2 foldchange of binders compared to the starting Tumbler Library. While position 4 tolerates higher variability across both targets, we also observe consolidation of specific and distinct residues between Affinity Matured and Affinity Matured + Cross-Reactive clones.

Precision Engineering

Targeted Engineering utilizes rational library design based on natural diversity and germline structure for each CDR, followed by phage panning selection, to improve affinity towards both human and cyno antigens:

Affinity Maturation on Human & Cyno Target

The sequence of a parental antibody was used as starting point for CDR randomization. Phage display libraries were generated by introducing a controlled number of mutations in each CDR (each CDR was changed separately). Affinity-driven phage display selections were performed both on human and cyno target, using the generated libraires. The best variants from each library were combined in new sequences, to further improve the affinity.

102 different clones with mutations in a single CDR identified, all with same epitope bin as initial clone

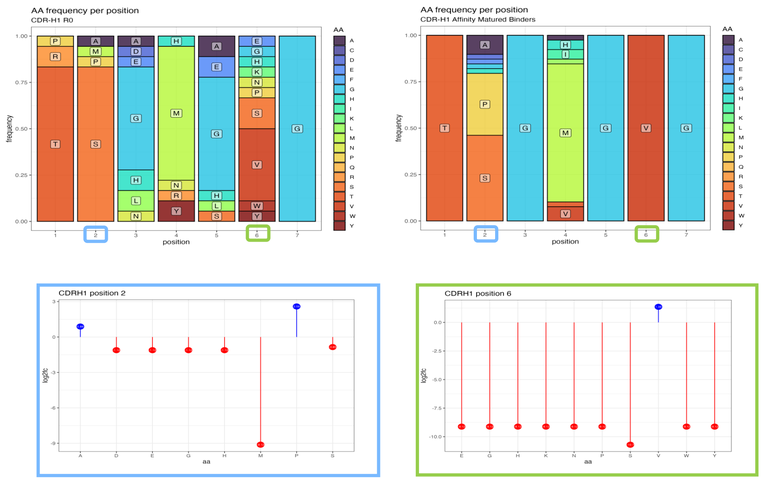
99 clones that displayed binding to cell-expressed target

63 clones with slower off-rate compared to parental clone

20 off-rate top clones with mutations in only one CDR selected for further characterization

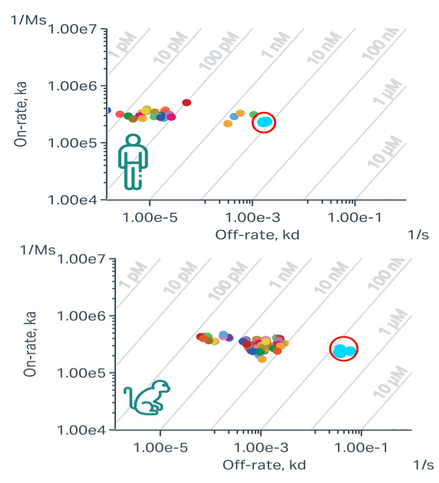
288 variants combining best mutations on each CDR produced and characterized

Preference for Specific Amino Acids in Affinity Matured Variants



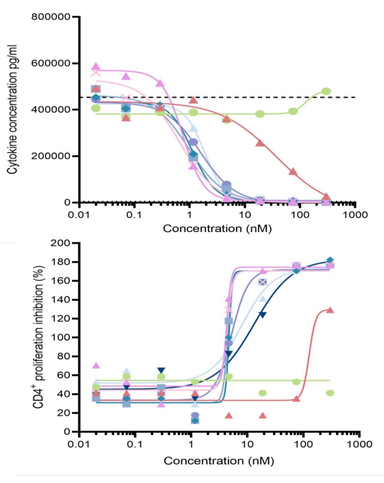
Comparison of amino acid frequency at each CDR-H1 position in the original library (left) and in affinity-matured clones (right). Position 2 was prone to mutation, while position 6 completely reverted to the WT residue.

1000-fold Affinity Improvement



Isoaffinity plots obtained for affinity matured variants. Red circle highlights the parental antibody. Improvement in affinity was obtained mainly through a slower off-rate, for both human and cyno antigens.

10-fold Improvement in Functional Assay



Inhibition of cytokine release and of T cell proliferation upon incubation with affinity matured variants, in comparison to parental antibody (red). Green line represents isotype control.

Future Directions

- Seamless integration: as both technologies are flexible and somewhat interchangeable, we can combine both approaches to suit a variety of antibody engineering goals
- Enhance freedom to operate by interrogating far sequence space and identifying flexible residues, to further expand paratope diversity with Tumbler
- Develop a library-free approach for multi-parameter antibody optimization, using cryo-EM and SAR Analysis to guide rational variant design
- Run Tumbler in Fab format, and Targeted Engineering with Yeast Display

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