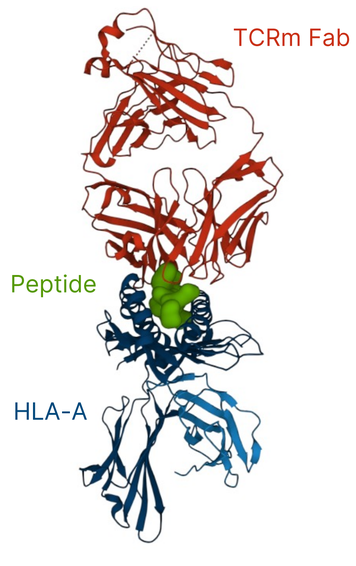
TCR mimetic antibody discovery, characterization, and optimization enabled by the *AlphaSeq* platform

Emily Engelhart, PhD[1]; Davis Goodnight, PhD[1]; Randolph Lopez, PhD[1]; David Younger, PhD[1]

[1]A-Alpha Bio, Inc. Seattle, WA

Problem: T Cell Receptor mimetic (TCRm) antibodies combine a TCR's recognition of intracellular targets with the favorable drug properties of monoclonal antibodies.

The primary challenge for engineering TCRm antibodies is specificity for the peptide.



PDB: 3GJF

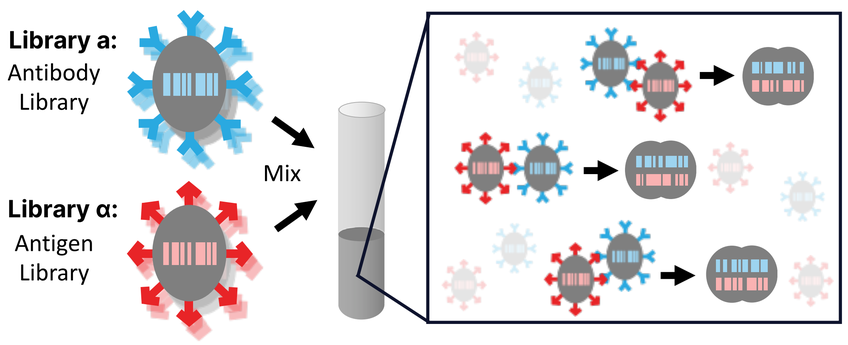
AlphaSeq addresses the primary TCRm challenge:

Broadening the discovery funnel – Library-on-library binding measurements of diverse TCRm antibodies and likely off-target pMHC complexes enables rapid screening for possible off-target binding for up to thousands of antibody candidates.

Detecting weak interactions – The *AlphaSeq* platform has a wide dynamic range, allowing for detection of pM to µM interactions in one assay. This sensitivity enables the discovery and characterization of weak on- and off-target interactions.

Optimizing affinity and specificity – On and off-target binding data from *AlphaSeq* is used to train multi-parameter machine learning (ML) models for affinity and specificity optimization.

The ***AlphaSeq*** platform uses a modified yeast surface display system and a next-generation sequencing readout to quantitatively map millions of protein-protein interactions at a library-on-library scale.



Antibody and antigen yeast display libraries are built and mixed

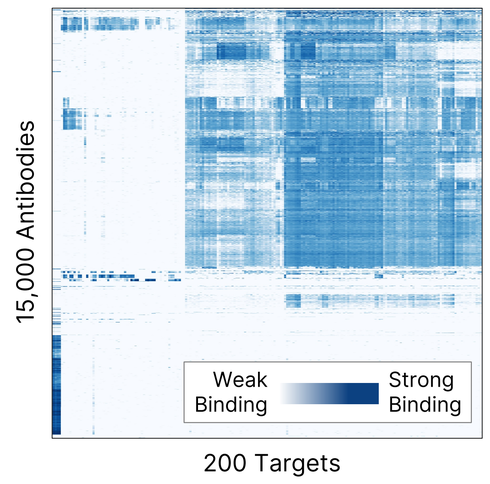
Interactions between antibodies and antigens drive cellular fusion

Cellular fusions are counted with NGS, giving a quantitative readout

The *AlphaSeq* platform is well suited for discovering and optimizing antibodies that require specificity and/or cross-reactivity, since quantitative binding to multiple on- and off-targets are measured at once, as in the example to the right.

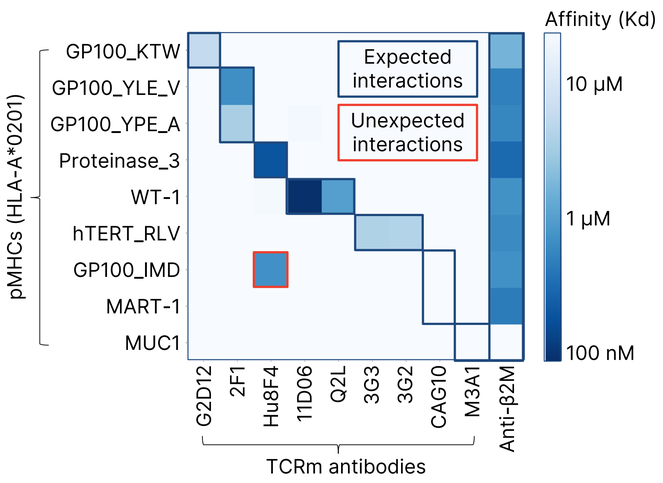
Antibodies with favorable binding profiles are further optimized for affinity, specificity, cross-reactivity, and developability in subsequent ML-guided *AlphaSeq* iterations.

Example Network: 3M total PPIs measured



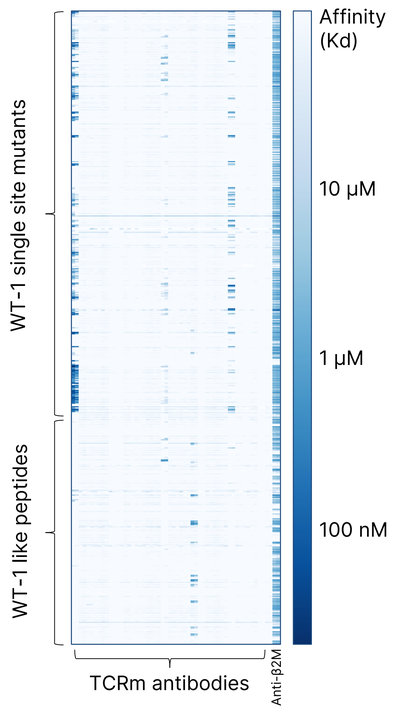
Validating pMHC-TCRm interactions in *AlphaSeq*

1 Can we use *AlphaSeq* to characterize known pMHC-TCRm interactions?



- Multiple pMHC–TCRm interactions validate with strong and specific binding over a wide affinity range.
- Putative off-target interactions are identified for further characterization.
- An anti-β2M antibody recognizes folded pMHC complex and is used to discriminate true negatives from unfolded pMHC.

Characterizing TCRm antibody specificity profiles

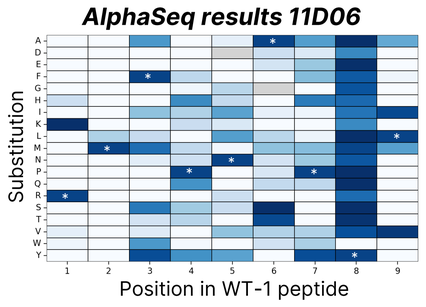


From a single *AlphaSeq* assay, we characterized 27 TCRm antibody specificity profiles against a library of 162 WT-1 single mutants & 100 WT-1-like peptides.

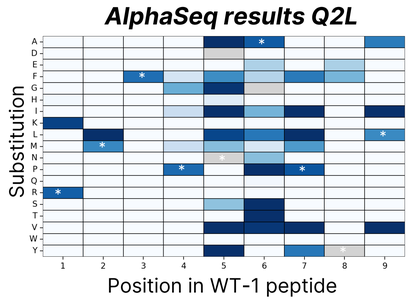
2 Can *AlphaSeq* recapitulate known pMHC-WT-1/11D06 TCRm antibody binding profiles?

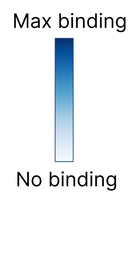
3 How do different TCRm antibodies engage the same pMHC peptide?

AlphaSeq results 11D06

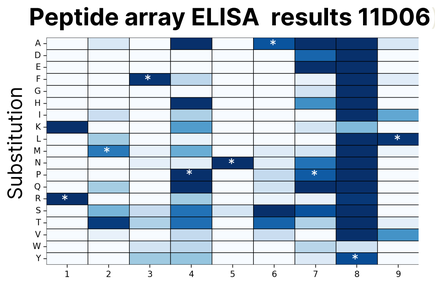


AlphaSeq results Q2L





Peptide array ELISA results 11D06

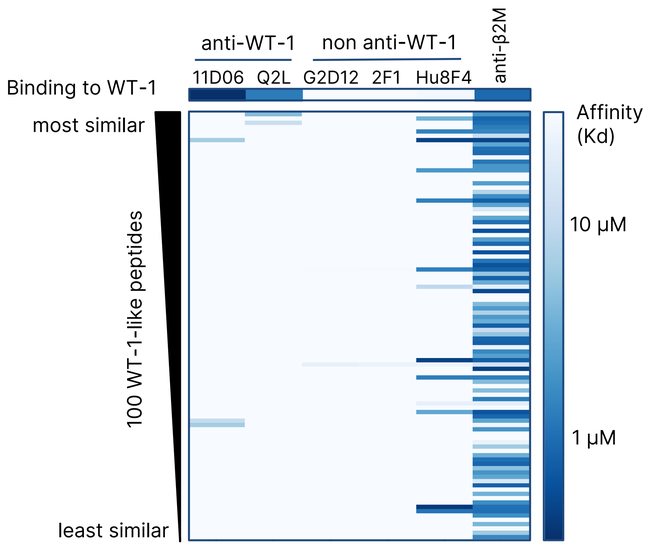


* wild-type positions

- We show a strong agreement between peptide array ELISA results from Augsberger et al., *Blood* 2021 and *AlphaSeq* results.
- Different TCRm antibody specificity profiles were revealed for 11D06 and Q2L. For example, only 11D06 is highly tolerant to substitutions at position 8.

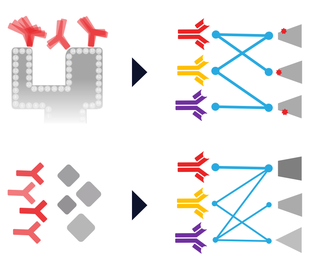
Screening TCRm antibodies for likely off-target binding

4 Can we use *AlphaSeq* to screen for predicted off-target binding?

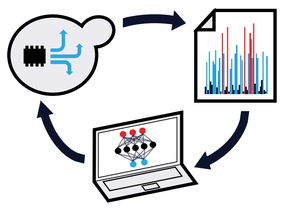


- 100 WT-1-like peptides from the human proteome were computationally identified using a published algorithm (Yarmakovich et al., *Nature* 2021) and off-target binding was tested for two anti-WT-1 antibodies.
- WT-1-targeting TCRm antibodies bind different subsets of predicted off-target peptides.
- 2 out of 3 non anti-WT antibodies do not bind WT-1 like peptides, whereas Hu8F4 shows significant non-specificity.
- Specificity profiling with *AlphaSeq* identified off-target binding and enables antibody prioritization and specificity optimization.

Conclusions & next steps



AlphaSeq is a synthetic biology platform for measuring millions of protein-protein interactions with high quantitative resolution. For TCRm antibodies, where peptide specificity is the primary challenge, we measure binding between a library of candidate TCRm antibodies and a large panel of possible off-target pMHCs to rapidly identify potential liabilities and optimize for specificity.



Antibody optimization for affinity and specificity will be performed with iterations of AlphaSeq and **AlphaBind**, an ML platform that learns the relationship between antibody sequence and binding profile to predict new antibody sequences expected to have desirable binding properties.