A validated bioinformatics tool-set for predicting TCR specificity

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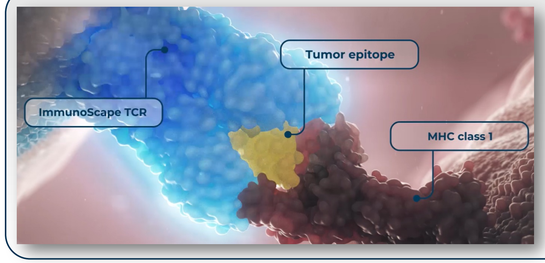
Background



The T-cell receptor (TCR) is a transmembrane receptor on T cells, which is responsible for recognizing foreign antigen derived peptides, presented on infected or abnormal cells by the MHC-I complex (see left). Predicting TCR specificity computationally is a long-standing problem. Many methods leverage large libraries of TCR sequences and cognate epitopes as input for machine learning and predict specificity from sequence alone. A perfect approach would in theory allow to discover new TCR therapeutics at scale. However, methods often focus on the beta chain of TCRs only and/or do generally not perform very well, especially in the case of unseen peptides, i.e., in cases where peptides were not part of the input library. Here we present three in-silico TCR discovery modules that are seamlessly integrated with our wet-lab and *in-vitro* validation platform and show validation examples for each.

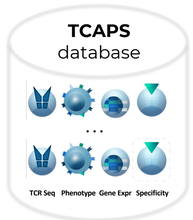
In-silico discovery, seamlessly integrated with a deep immunomics and validation platform

Wetlab discovery

Step 1: Multiplexed CyTOF screening

informs

Step 2: Single-cell VDJ CITE-seq profiling



Multimodal T cell profiles

>80 healthy donors | >130 disease donors

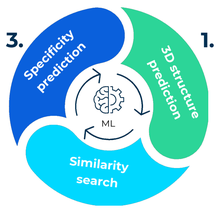
>2.3M T cell profiles | > 330k clonotypes (paired)

+

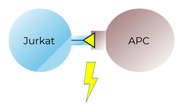
"Unimodal" public data

>420k paired TCR clonotypes ()

Multi-tiered in-silico discovery



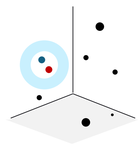
Validation platform



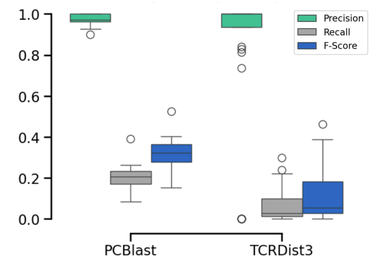
- mRNA electroporation into Jurkat reporter cells expressing candidate TCR
- Evaluation against peptide-pulsed APCs expressing only one HLA allele
- Peptide recognition results in expression of a luciferase reporter gene detected as luminescence readout

Similarity searches for validated TCRs (2)

PCBlast is our alignment-free similarity search method that translates amino acids into physicochemical (PC) property groups (see e.g. Dubchak *et al.* 1995 and 1999). Similarity between two sequences (red & blue dot right) can be measured by e.g. computing the correlation of their embedded features.



PC embedding

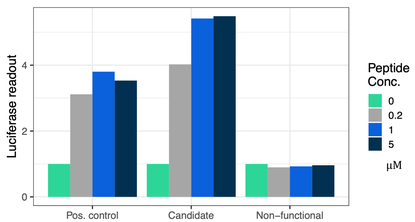


PCBlast performance on TCR-Scapes (Hudson *et al.* 2024) modified to be used for paired TCRs only

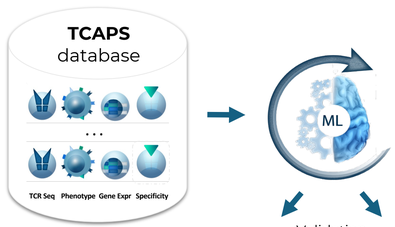
Search pipeline

- Query: any validated TCR
- Query DB: TCAPS + Public data (in total >750k paired TCRs)
- Search engines: PCBlast (left) and TCRDist3 (Mayer-Blackwell *et. al* 2021)

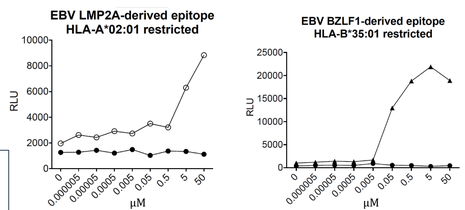
Example: ***In-vitro* validation** of a discovered **MAGE-A10** specific TCR



Specificity predictions via phenotype (3)



ML models are trained on TCAPS' deep phenotypes with measured specificity and applied to those T cells with unknown specificity. A prototype is described in Schmidt *et al.* 2023.

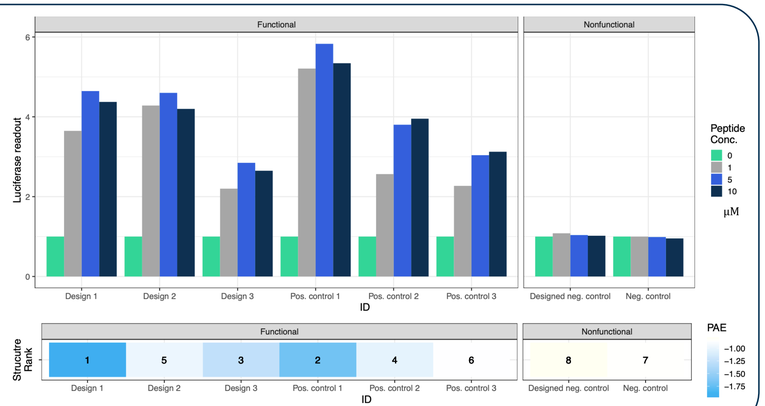


Epitope was not part of our experimental panels!

Neither epitope nor antigen were part of our experimental panels!

3D structure predictions and TCR design (1)

Based on three validated **SF3B1-mut specific TCRs** and one negative control we **computationally designed TCRs**, creating three positive and one negative control TCRs. Control and designed TCRs were evaluated through 3D structure predictions based on TCRDock (Bradley 2021), a TCR-specific extension of AlphaFold2. Structures were ranked based on their normalized PAE values (see bottom). All TCRs were *in-vitro* validated (see barchart for luminescence readout). TCRDock's PAE ranks match *in-vitro* validation results.



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