A peptide MHC II stability assay
-part of the Immunitrack platform

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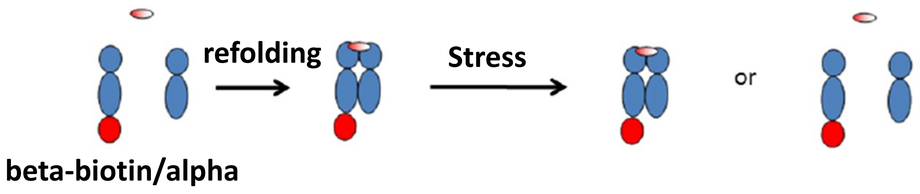
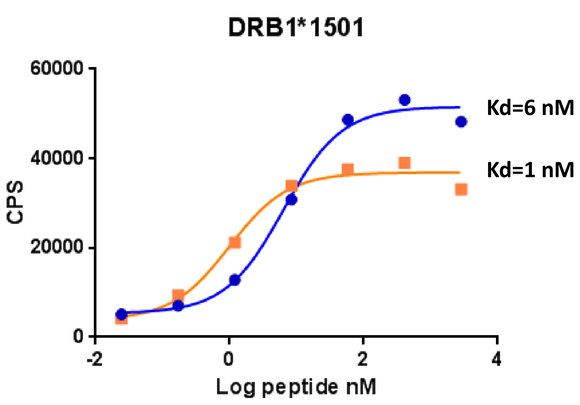
Background: Immunitrack is a spin-out from the University of Copenhagen that builds on a decade of research in major histocompability complex (MHC) peptide interactions. Our molecules have formed the basis of several large NIH and EU grants. High throughput peptide MHC affinity assays have generated data that have been used to develop best in class *in silico* prediction tools such as netMHC I and II. More than 100.000 peptide MHC (pMHC) Kd measurements deposited in the Immune Epitope Data Base (EIDB) comes from these assays. The molecules have also been successfully used as tetramers for the staining of CD4 and CD8 T cells.

Problem: Potential CD4 T cell epitopes can be predicted by in silico methods or measured in vitro by peptide MHC II affinity assays. For MHC I it was shown that stability is a better predictor of immunogenicity than affinity. Current pMHC II assays or *in silico* methods do not address stability, making them over-predictive and less useful for immunogenicity assessment. Immunitrack has developed a high throughput pMHC II stability assay that removes most of the false positives or unstable pMHC II complexes enabling us to identify true CD4 T cell epitopes with a higher accuracy.

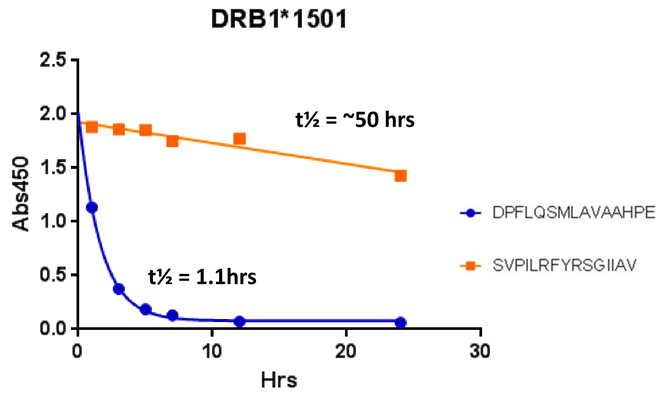
Stability assay



Affinity

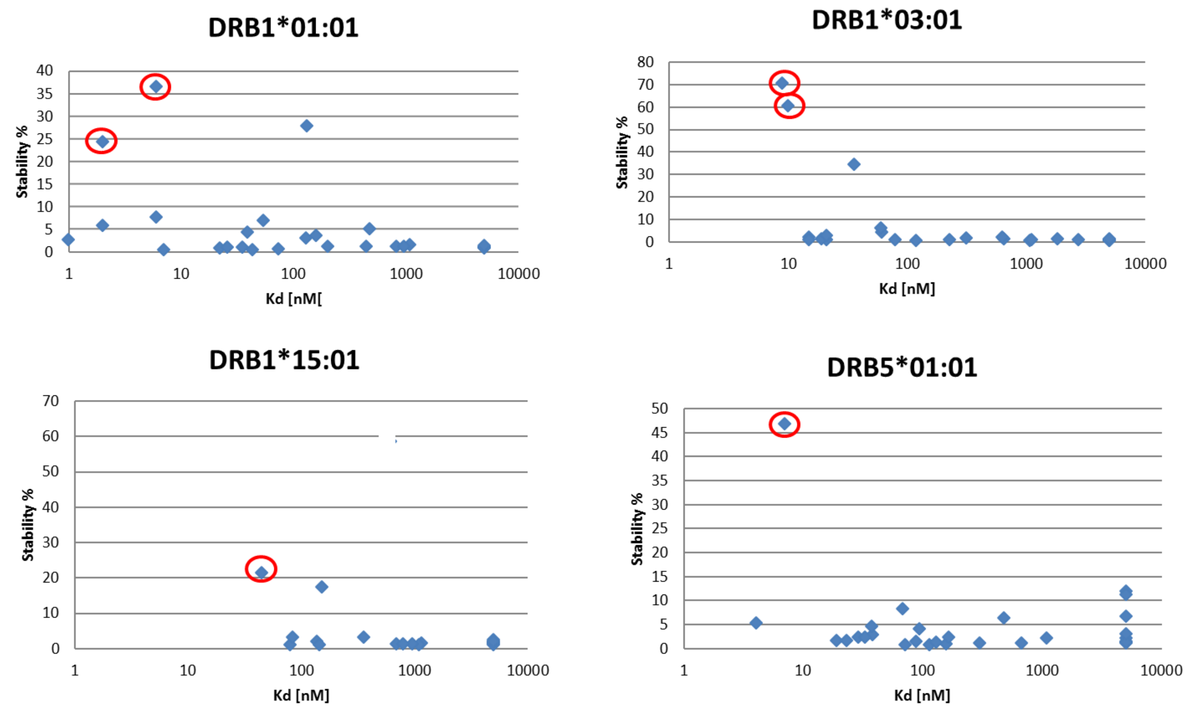


Stability



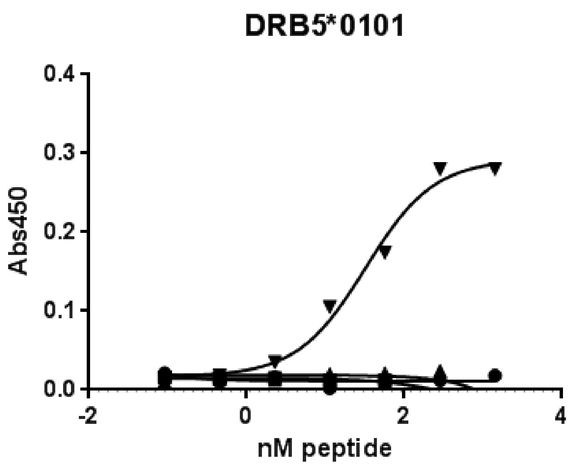
Affinity: Two EBV derived peptides with equal affinity to DRB1*1501. Stability assay shows large differences in their half-lives. The peptide with the long half-live (SVPILRFYRSGIIAV) is a known T cell epitope.

Affinity vs. Stability CMV T-Cell epitopes



Affinity (x axis, nM) and stability (y axis) of CMV derived peptides. Most high affinity peptides (Kd<50nM) are relatively unstable. True T cell epitopes, confirmed by ELISPOT and MHC II Tetramer analysis are marked with red circles. Stability appears as a better indicator of immunogenicity than affinity

Immunogenicity of a Factor VII analogue

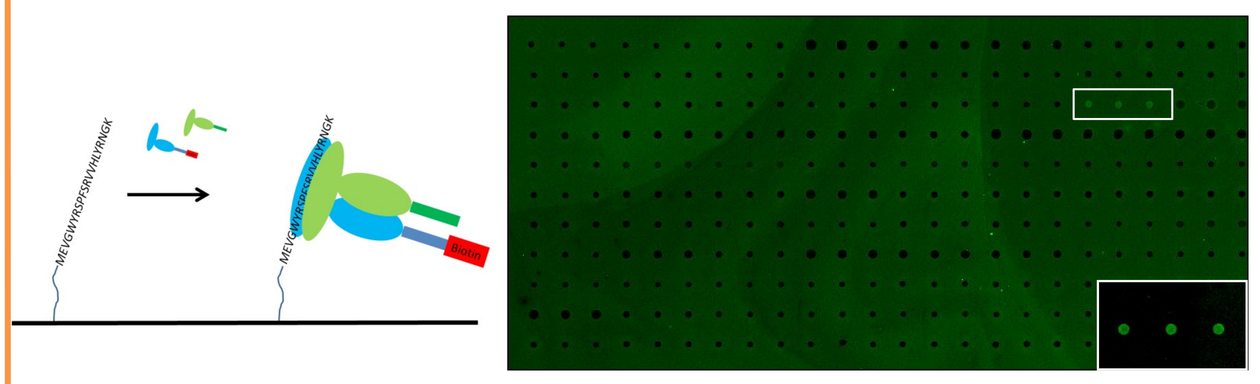


wtFVII
GQLLDRGATALELMVLNVPRLMTQD
GQLLDRGATAL**V**L**Q**VLNVPRLMTQD
Vatreptacog

- 1 F-VII WT
- 1B F-VII E->V
- 2 F-VII WT
- 2B F-VII M->Q

Vatreptacoq alfa, a Factor VII analogue with 3 mutations was discontinued in a ph3 clinical trial due to immunogenicity. Retrospect analysis showed that M298Q mutation led to formation of a neo-epitope on DRB5*0101 (Kd 32nM)

MHC II peptide array

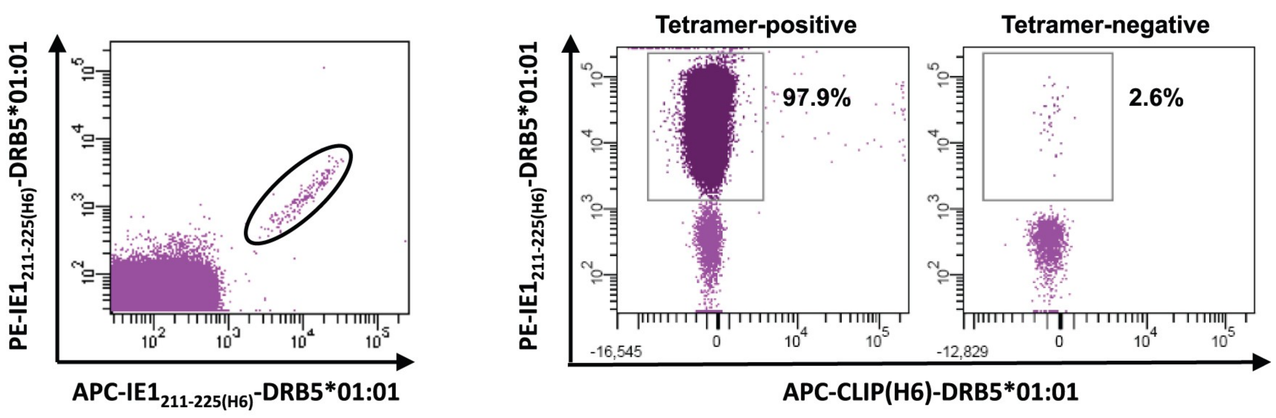


Commercial peptide array displaying 96 overlapping 15-mers from from p53 was incubated with DRB1*1501 (large array) and DRB3*0101 (small insert) and detected with cy3 labelled DR specific antibody. Both analysis reveals the same peptide

MHC II tetramers

A ***Ex vivo***

B **Sorted *in vitro* cultured cells**



A: Double staining and sorting ex vivo of 1000 PBMC´s positive for DRB5*0101/$IE_{211-225}$. B: After 14 days of culture, cells are analyzed by relevant and irrelevant tetramer revealing 98% positive CD4 T cells*.

See more at www.immunitrack.com

*MHC Class II Tetramers Made from Isolated Recombinant α and β Chains Refolded with Affinity-Tagged Peptides, PLOS One September 2013 | Volume 8 | Issue 9
Peter Braendstrup , Sune Justesen , Thomas Østerbye, Lise Lotte Bruun Nielsen, Roberto Mallone, Lars Vindeløv, Anette Stryhn, Søren Buus