Opening the Black Box: Biological Interpretation of Neural Network-Based HLA-DR Peptide Binding Predictions through Systematic Mutational Analysis

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Purpose

In HLA-DR peptide presentation, the HLA binding groove interacts directly with a peptide's 9-amino-acid peptide "core". Binding specificity is primarily determined by core "anchor" residues (typically core positions 1, 4, 6, and 9). Binding affinity can be predicted using a neural-network binding predictor (Frost), which is incorporated in the PIRCHE-T2 algorithm. This study assesses the Frost binding predictor and its consistency with the reported anchors of peptide-HLA-DR binding, to assess how it captures HLA's biological function.

Methods

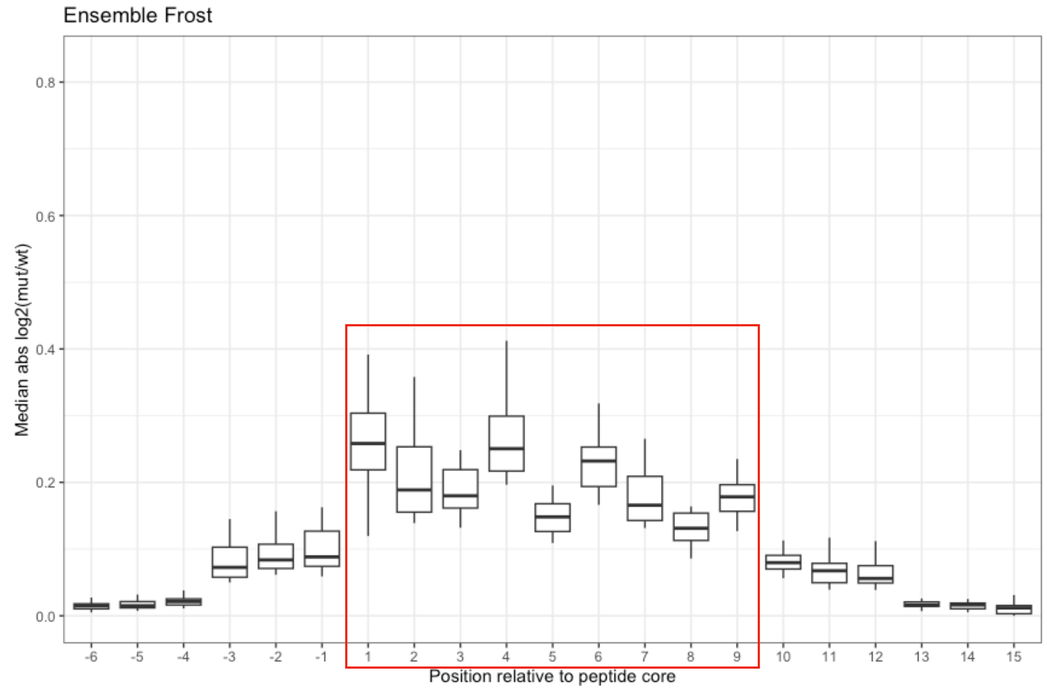
- **20 HLA-DR presenters** with high population representation were selected from IEDB.
- **50 reference peptides** per presenter with high predicted affinity were extracted from IEDB.
- **Mutational Scanning:** Generated all single-amino acid "mutation" variants per peptide. Total = **286,000 peptide-HLA** pairs.
- **Binding Prediction:** Predicted **affinity** and **core** location for "mutated" pHLAs
- **Core Mapping:** **Mutations** mapped to location on the predicted core.
- Median absolute log **differences in binding affinity, stratified by core positions**



A view into the binding groove of a representative **HLA-DRB*15:01 (dark green)** with a bound myelin **peptide (light green)**. The **peptide "anchor" positions (purple)** sit deep within the binding groove, and make contact with ~25 amino acids (gold) of the DR beta chain. The DR alpha chain is semi-transparent in the foreground. (PDB: 1BX2)

Results

- **Anchor Positions:** Mutations at **anchor positions** (1, 4, 6, and 9) showed **highest differences in predicted binding value** compared to non-anchor positions.
- **Flanking Regions:** Substitutions within the 9-mer binding core (red) had a stronger effect on affinity than substitutions in peptide-flanking regions (-3, -2, -1, 10, 11, 12).



- **"Black box"** Frost predictions are **biologically interpretable:** Neural-network predictions can reveal the interactions between the binding groove and residues within the peptide which are critical to binding.

Conclusion

The Frost predictor independently identifies the peptide anchor positions as the drivers of HLA-DR binding affinity, showing a correlation of neural network predictions with our understanding of the biochemical interactions of peptide-HLA binding. This demonstrates the interpretability of the underlying prediction model, and "illuminates the black box" of artificial neural networks.

Disclosures: MN, BM, and BG are employees of PIRCHE AG, publishing the TxPredictor® web service.

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