

The Role of Hydrophobicity in Peptide-MHC Binding^{*}

Arnav Solanki¹[0000–0003–4039–2814], Marc Riedel^{1,5}[0000–0002–3318–346X],
James Cornette², Julia Udell^{1,3}[0000–0003–3225–733X], Ishaan Koratkar⁴, and
George Vasmatazis³[0000–0002–3401–3497]

¹ University of Minnesota, Minneapolis, MN 55455, USA

{solan053,mriedel,udell1008}@umn.edu

² Iowa State University, Ames, IA 50011, USA

cornette@iastate.edu

³ Center for Individualized Medicine, Biomarker Discovery Group, Mayo Clinic,
Rochester, MN 55902, USA

Vasmatazis.George@mayo.edu

⁴ Eagan High School, Eagan, MN 55123, USA

ishaan@koratkar.com

⁵ Corresponding Author

Abstract. Major Histocompatibility Complex (MHC) Class I molecules provide a pathway for cells to present endogenous peptides to the immune system, allowing it to distinguish healthy cells from those infected by pathogens. Software tools based on neural networks such as NetMHC and NetMHCpan predict whether peptides will bind to variants of MHC molecules. These tools are trained with experimental data, consisting of the amino acid sequence of peptides and their observed binding strength. Such tools generally do not explicitly consider hydrophobicity, a significant biochemical factor relevant to peptide binding. It was observed that these tools predict that some highly hydrophobic peptides will be strong binders, which biochemical factors suggest is incorrect. This paper investigates the correlation of the hydrophobicity of 9-mer peptides with their predicted binding strength to the MHC variant HLA-A*0201 for these software tools. Two studies were performed, one using the data that the neural networks were trained on and the other using a sample of the human proteome. A significant bias within NetMHC-4.0 towards predicting highly hydrophobic peptides as strong binders was observed in both studies. This suggests that hydrophobicity should be included in the training data of the neural networks. Retraining the neural networks with such biochemical annotations of hydrophobicity could increase the accuracy of their predictions, increasing their impact in applications such as vaccine design and neoantigen identification.

Keywords: MHC Class I · Peptide · Machine Learning · Neural Networks.

^{*} Supported by NSF Grant 2036064

1 Introduction

The Human Leukocyte Antigen (HLA) gene system encodes cell-surface proteins that play a key role in the immune system. HLA proteins of Major Histocompatibility Complex (MHC) Class I allow nucleated cells to present peptides from within the cell. In these cells, endogenous proteins are eventually broken down into small peptides, 8-15 amino acids long, by the proteasome. These antigens are then trafficked to and loaded onto MHC Class I molecules. If sufficient binding affinity is achieved then a stable peptide-MHC (pMHC) complex is formed and transported to the cell surface. Self-peptides, antigens encoded in the human proteome, and foreign peptides, derived from pathogenic proteins, can thus be presented. By surveilling these extracellular pMHCs, CD8⁺ T-cells can distinguish normal cells from pathogen-infected cells, and kill the latter.

The mechanics of peptide binding are specific to a given MHC variant. The HLA genes are among the most diverse in the human population [9]. Thus the set of all antigens presented by a person’s MHCs, labelled as their *immunopeptidome*, is unique and determines the capacity of their immune system. Since the immune response of a person to, for instance, a viral infection like COVID-19 is dependent on whether the foreign antigens presented by their MHCs are distinguishable from self-peptides, understanding and predicting pMHC binding is an important topic. In this paper, we have focused on NetMHC-4.0 [2] and NetMHCpan-4.1 [25], two state-of-the-art neural network based methods that predict pMHC binding. Both software tools have been applied in predicting cancer immune escape mechanisms [17], checkpoint blockade immunotherapy for tumors [16], and identifying COVID-19 T-cell response targets [10].

While these tools provide valuable pMHC predictions, they do not model pMHC binding at the molecular level or capture the entire antigen presentation pathway’s effects. Hydrophobicity is a measure of how repulsive a molecule is to water, often a consequence of nonpolarity. It plays a vital role in protein binding – for example, the MHC molecule HLA-A*0201 (A2) contains hydrophobic binding pockets that bind to correspondingly hydrophobic amino acids. Historically, immunopeptidomes have been predicted by modelling the interaction of the MHC binding pocket and peptide, particularly focusing on biochemical attributes such as sidechain conformations, solvation energies, electrostatic interactions, and hydrophobicity [32,30]. However with improved computing power, larger datasets, and the need for interpolation due to the high polymorphism in MHC Class I alleles [21], artificial intelligence based methods have become popular over such mechanistic means of prediction. As NetMHC-4.0 and NetMHCpan-4.1 are trained with sequence data and binding scores only, they lack the means of modelling these biochemical attributes. Other software tools such as ANN-Hydro [6] have utilized hydrophobicity in their immunogenic predictions, but do not predict binding affinity and are outperformed by NetMHCpan [18]. In our use of NetMHC-4.0 we observed a prevalence of highly hydrophobic peptides in the predicted A2 immunopeptidome. We found this unintuitive, since peptides in which all amino acids are hydrophobes would not dissolve in the aqueous cytosol within the cell and would thus likely not be available for binding with the

MHC. We therefore sought to investigate the possibility that these tools were over-estimating binding scores for such hydrophobic peptides. We conducted two analyses on both NetMHC-4.0 and NetMHCpan-4.1, one using training data and the other using a sample of the human proteome, to investigate the correlation of predicted strong binders and hydrophobicity. We present our results and highlight the unintended bias within NetMHC-4.0 for predicting highly hydrophobic peptides as strong binders.

2 Methods

NetMHC and NetMHCpan allow users to input a list of peptides or whole proteins, and test the binding of all peptides within a chosen MHC molecule. Both tools return an adjusted score between 0 (for non binders) and 1 (for strong binders) for all peptides. A notable distinction between the two is that NetMHC is limited to predicting binding for MHC variants it is trained on, i.e. curated MHCs. In contrast, NetMHCpan is capable of interpolating predictions for uncurated MHCs if users provide the MHC amino acid sequence. This is achieved through the integration of MHC sequence as a data feature in training, and by a larger training dataset generated using a sophisticated machine learning method called NNAlign_MA [1]. NetMHCpan-4.1 consists of an ensemble of 50 neural networks, each with hidden layers containing 55 and 66 neurons, that were trained using 5-fold cross validation. NetMHC-4.0 consists of 20 neural networks, each with a single hidden layer of 5 neurons, that were trained using a nested 5-fold cross validation approach [2].

2.1 Data Mining

NetMHC-4.0 was trained on CD8⁺ epitope binding affinity (BA) data from the Immune Epitope Database. This data provides binding scores for peptides to single allele MHCs, with a score that is scaled between 0 and 1 that measures how strongly the peptide binds. NetMHCpan-4.1 was trained on BA data and additional eluted ligand (EL) data from mass spectrometry experiments from multiple sources [25]. The EL data includes multi-allele information that was deconvoluted into single allele datapoints using NNAlign_MA. EL score is binary (either 0 or 1) since it checks if a peptide is present in a MHC’s immunopeptidome. The training data for NetMHCpan-4.1 is provided [here](#).

This cumulative dataset contained more than 13 million pMHC data points, that we filtered down to the 52569 9-mers interacting with HLA-A02:01 (A2) and labelled as set TRN. 9-mers were the most frequent length of antigens in human immunopeptidomes, and A2 was the most frequent MHC in the training dataset. The distribution of all binding scores in TRN is shown in Fig. 1. Please note that Fig. 1 contains two distinct graphs, the second being independently sorted to visualize the cumulative distribution, as discussed in the caption. All peptides from TRN were fed into NetMHC-4.0 to obtain their predicted BA scores, and

then filtered for strong binders predicted by the tool’s default 0.5% rank threshold. This set of predicted strong binding peptides by NetMHC-4.0 was labelled as NSB (NetMHC Strong Binders). Similarly, the strong binders predicted by NetMHCpan-4.1 from TRN based on their EL scores were compiled into the set PSB (NetMHCPan Strong Binders).

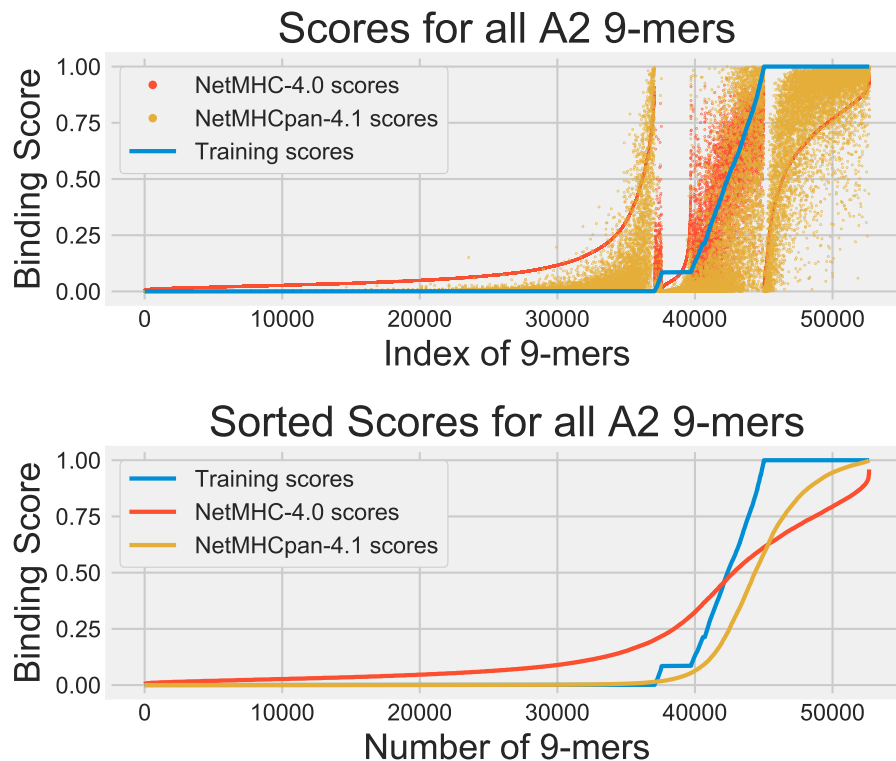


Fig. 1. Binding scores for all A2 9-mers in the NetMHCpan-4.1 training set TRN in blue, NetMHC-4.0 Binding Affinity predicted scores in red, and NetMHCpan-4.1 Eluted Ligand predicted scores in yellow. The top graph has been sorted on the training data, and for each peptide index the NetMHC, NetMHCpan, and training scores are plotted at that x coordinate. The Pearson correlation coefficient between the training scores and NetMHC-4.0 was 0.8492, and between the training scores and NetMHCpan-4.1 was 0.863. In the bottom graph, each plot of scores was independently sorted to demonstrate their cumulative distributions. Note that here the order of peptides is not conserved across the 3 plots in the bottom graph.

From the scores shown in the second graph of Fig. 1, it was clear that the pMHC binding data for A2 9-mers fitted a mostly binary data classification problem, since only 15% peptides had a training score not equal to 0 or to 1.

This was mostly due to the addition of EL data which provided a binary “yes” or “no” answer to whether a given peptide was found attached to A2 through mass spectroscopy. NetMHC-4.0 predicted scores, shown in red, were mostly located in between the extremes of 0 and 1 due to the smaller training data consisting of only BA assay data. It seemed that NetMHCpan-4.1, shown in yellow, was much better at estimating non-binders (scores of 0), and fitted the S-curve transition more tightly than NetMHC-4.0. This was reflected by the correlation coefficients calculated in Fig. 1. However, neither neural network gave a definitive score of 1 to strong binders; they both use a rank based percentile threshold to determine which peptides can be classified as strong binders.

We measured the lowest binding score in NSB and PSB as 0.659 and 0.419 respectively – i.e. all strong binders predicted by NetMHC-4.0 and NetMHCpan-4.1 had binding scores greater than or equal to these thresholds, respectively. We then filtered for all peptides in TRN that had experimental binding scores greater than or equal to 0.659 into set NTF (NetMHC Threshold Filtering) and those greater than or equal to 0.419 into set PTF (NetMHCpan Threshold Filtering). Here, NTF contained all training peptides whose experimentally determined binding scores would classify them as strong binders according to NetMHC-4.0, and likewise for PTF and NetMHCpan-4.1.

Lastly, we gathered the protein sequences for all reviewed human proteins from Uniprot [7], and randomly sampled 100 of them to create a set of 50804 9-mers that we labelled as SHP (Sampled Human Proteome). These peptides were also passed through NetMHC-4.0 and NetMHCpan-4.1, and the resulting list of strong binders were filtered into sets NHB (NetMHC Human Binders) and PHB (NetMHCpan Human Binders). Refer to Fig. 2 to see the distributions of these predicted scores; note that there are no training data readily available for SHP.

The datasets TRN, NSB, PSB, NTF, and PTF were used for analyzing the performance of both neural networks on training data, while the sets SHP, NHB, and PHB were used for investigating the performance upon the human proteome.

2.2 Hydrophobicity

Hydrophobicity scales assign hydrophobicity values to single amino acids. They are designed so the hydrophobicity of long peptides or protein chains can be estimated by simply linearly adding up the scores of their constituent amino acids. While scales such as Kyte-Doolittle [14], Cornette [8], and Hopp-Woods [11] are commonly used, we settled on the Moon scale [20] for calculating hydrophobicity in our analyses. This newer scale differs from the scales listed above in that it specifically focuses on the sidechain hydrophobicity and polarity of single amino acids. Unlike the other scales, which are well suited for protein folding problems that do not correlate with sidechain hydrophobicity [19], the Moon scale is more representative of how small peptides would behave in an aqueous solution. The scale ranks the 20 amino acids in decreasing order of hydrophobicity as follows: F (1.43), L (1.26), I (1.15), P (1.13), Y (0.94), V (0.80), M (0.79), W (0.63), A (0.46), C (0.24), E (-0.27), G (-0.30), T (-0.33), S (-0.35), D (-0.85), Q (-0.88), N (-1.08), R (-1.19), H (-1.65), K (-1.93). For any given 9-mer, we calculated its

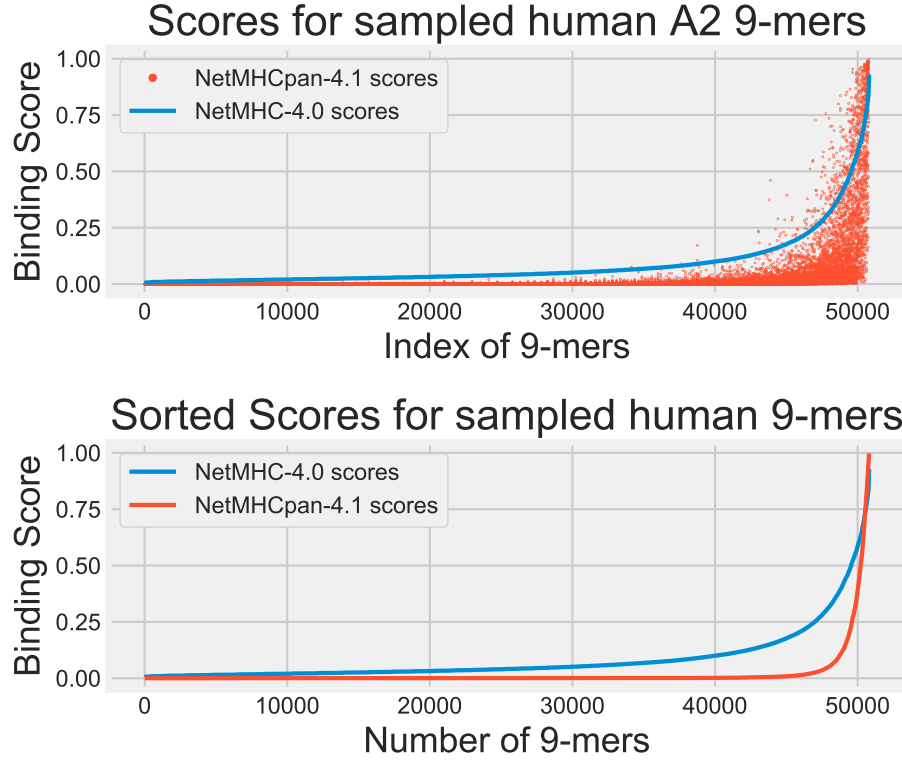


Fig. 2. Predicted binding scores for all 9-mers in the 100 sampled human proteins in SHP, according to NetMHC-4.0 in blue, and NetMHCpan-4.1 in red. In the top graph, the sequence of peptides is conserved for both sequences and sorted by NetMHC scores. In the bottom graph, both sequences are independently sorted and the sequence of peptides is not conserved across both sequences.

total hydrophobicity by adding up the values for each of its 9 amino acids as reported by the scale. For a given set of peptides, we measured the mean and standard deviation of the hydrophobicity scores of all peptides in it. Refer to Tables 1 and 2 for these measurements.

2.3 Hydrophobicity Filtering

An additional filter we applied was for peptides that were entirely hydrophobic. For this, we only accepted peptides from TRN and SHP that had all 9 amino acids with a Moon hydrophobicity score greater than 0.46 (i.e. that of Alanine). This meant that the resulting sets of peptides were made entirely of Phenylalanine, Leucine, Isoleucine, Proline, Tyrosine, Valine, Methionine, and Tryptophan – highly hydrophobic and nonpolar amino acids. For TRN, only 55 such peptides were found. The training scores and predicted scores for these are shown in Fig. 3.

Table 1. Hydrophobicity values for the training data analysis

Set of Peptides	Size of Set	Mean Hydrophobicity	Standard Deviation
TRN	52659	0.902	3.063
NTF	9268	2.794	2.502
PTF	10763	2.857	2.527
NSB	6498	3.458	2.364
PSB	8863	2.756	2.426

Table 2. Hydrophobicity values for the human proteome analysis

Set of Peptides	Size of Set	Mean Hydrophobicity	Standard Deviation
SHP	50804	0.052	3.212
NHB	486	4.519	2.515
PHB	940	2.789	2.645

While the training data in blue showed non-binders, strong binders, and some in between, NetMHC predicted no decisive non-binders and instead seemed to model a uniform distribution. In contrast, NetMHCpan clearly identified non-binders and was notably more conservative in assigning scores greater than 0.419 – it identified fewer strong binders than NetMHC did with its threshold of 0.659 and matched the training scores better with that threshold.

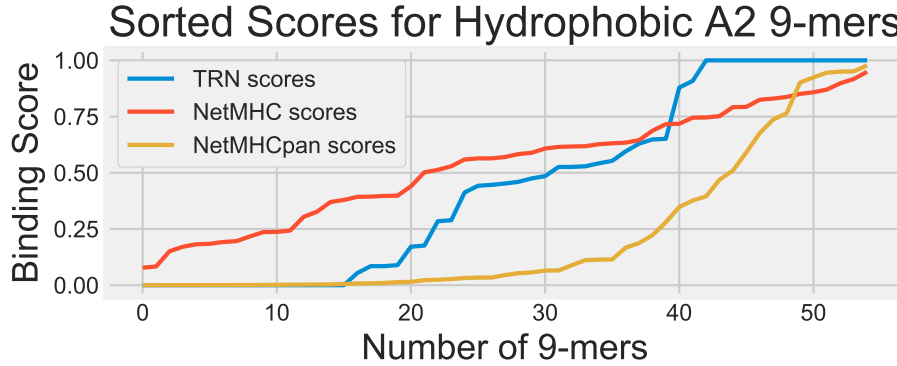


Fig. 3. Binding scores for all highly hydrophobic 9-mers in TRN in blue, and the predicted scores by NetMHC-4.0 in red and NetMHCpan-4.1 in yellow. All of the 3 plots were independently sorted to demonstrate their distributions. Peptides were considered hydrophobic if all their amino acids were more hydrophobic than Alanine.

For SHP, 33 hydrophobic 9-mers were found. Their predicted binding scores by both neural networks are shown in Fig. 4. Once again, the NetMHC scores in blue appeared almost linear and seemed to be uniformly distributed, while

NetMHCpan in red clearly identified lots of non-binders, and fewer strong binders (about 3).

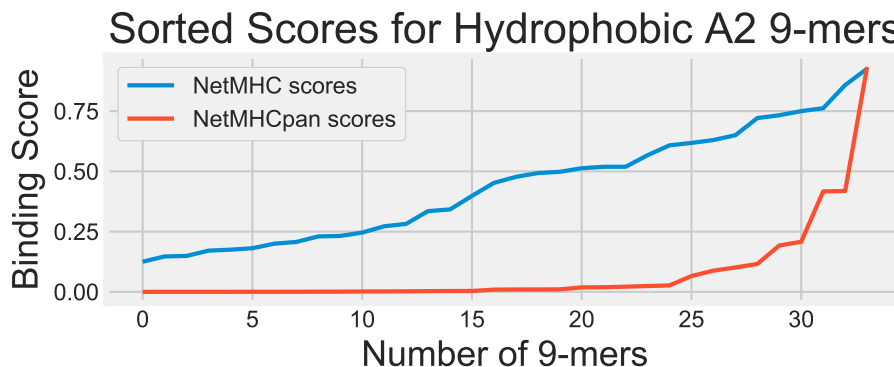


Fig. 4. Predicted Binding scores for all highly hydrophobic 9-mers in SHP, with NetMHC-4.0 in blue, and NetMHCpan-4.1 in red. Both plots were independently sorted to demonstrate their distributions. Peptides were considered hydrophobic if all their amino acids were more hydrophobic than Alanine.

2.4 2 Sample t-Test

For any 2 given sets of sampled numbers, the 2 Sample t-Test allows for comparing their means. Given an arbitrary set S_i with mean μ_i , standard deviation σ_i , and sample size n_i , the t-statistic for two sets S_i and S_j can be computed as

$$t_{i,j} = \frac{(\mu_i - \mu_j)}{\sqrt{(\sigma_i^2/n_i) + (\sigma_j^2/n_j)}}.$$

For all our named sets, we conducted a cross-set 2 sample t-Test using [Python's scipy.stats package](#) to determine how likely shifts in the means of hydrophobicity scores for sets could be due to random sampling. This computer package also calculated p-values, enumerating the probability of the two compared sets having unequal means purely by chance, from the t-statistic.

3 Results

Consider the histograms of the hydrophobicity scores of all peptides in the datasets TRN, PTF, and PSB shown in Fig. 5. The 52,659 peptides in TRN model a gaussian distribution centered at mean hydrophobicity of 0.9. The other two sets containing high binders according to NetMHCpan, PTF and PSB, shift to the right

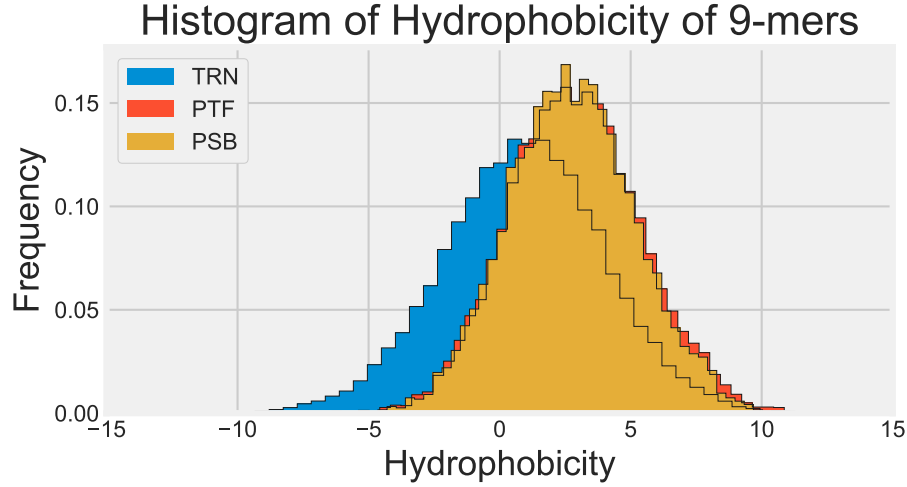


Fig. 5. Histogram of the Hydrophobicity scores (on the x-axis) for sets TRN (in blue), PTF (in red), and PSB (in yellow). Note how PTF and PSB are similar distributions. Refer to section 2.2 for details.

with new means at 2.8 and 2.7 respectively. The shift towards more hydrophobic 9-mers is not unexpected – as the authors of NetMHC [222] depict in the A2 logos [here](#), locations 2 and 9 in the A2 immunopeptidome 9-mers strongly favor amino acids such as Leucine, Methionine, Valine, and Isoleucine. The reservation of these 2 locations with these hydrophobic amino acids corresponds roughly to 2.0 shift in Moon hydrophobicity. The two sets possess comparable means and standard deviations, visually and quantitatively as shown in Table 1.

In contrast, let us now focus on how NetMHC performed in a similar analysis. In Fig. 6, the histograms for TRN, NTF, and NSB are shown. The set NTF in red, consisting of peptides with experimentally measured binding scores greater than 0.659, is centered at a mean of 2.8. However set NSB in yellow, containing peptides that NetMHC predicted as strong binders, is offset to the right with a mean of 3.4. This shift in the distribution of NSB points out an increase in hydrophobicity of 9-mers that bind to A2. That is, NetMHC predicts the A2 immunopeptidome to be more hydrophobic than the experimental data, or even NetMHCpan’s predictions, suggest.

Looking at the histograms of the SHP, NHB, and PHB – i.e. the human proteome sampled 9-mers and the strong binders predicted by the neural networks from them – this shift in hydrophobicity increases. In Fig. 7, the SHP distribution is centered at about 0 (SHP and TRN do not share the same mean, which we suspect is due to the Moon hydrophobicity scale being normalized on human proteins). As in Fig. 5, the strong binders predicted by NetMHCpan are slightly hydrophobic, resulting in a shift in the mean to 2.8 (similar to PTF and PSB). However, the strong binders predicted by NetMHC in the human proteome are

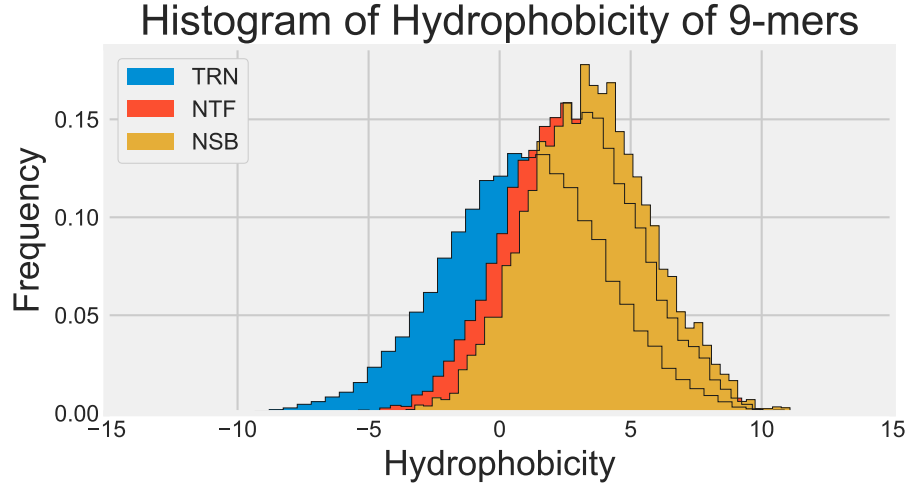


Fig. 6. Histogram of the Hydrophobicity scores (on the x-axis) for sets TRN (in blue), NTF (in red), and NSB (in yellow). Clearly, NTF and NSB do not align, with NSB shifted towards being more hydrophobic.

much more hydrophobic, with a shifted mean at 4.5. The gain in hydrophobicity from SHP to NHB implied by this shift is even larger than the shift observed in Fig. 6. Once again NetMHC overestimates how hydrophobic the A2 immunopeptidome is, and performs worse in the human proteome evaluation compared to the training data.

The cross set T-test tested the equivalence of two given sets with p-values. The smaller the p-value, the more likely the two sets have unequal means. We clustered the sets based on the p-values from the cross set t-test. The clustering criteria were: 1) two sets chosen from separate clusters should have a p-value lower than 0.001; and 2) a set should have a p-value greater than 0.001 with at least one set in its cluster. We obtained the following 5 clusters of sets: (TRN), (SHP), (NTF, PTF, PSB, PHB), (NSB), and (NHB). The first two clusters cover the sets that were put in to the neural networks, TRN and SHP. The third cluster includes NetMHCpan’s predicted immunopeptidomes and the experimentally observed immunopeptidome. The largest observed p-value in this cluster was 0.961 between NTF and PHB. The fourth and fifth clusters cover the set of predicted strong binders according to NetMHC for the training data analysis and the human proteome analysis respectively. The clusters signify how similar sets within them are, and how different they are to sets outside that cluster. As the NSB and NHB sets occupying their own clusters, the t-test highlights that NetMHC’s predictions do not match up with the experimental immunopeptidomes and with NetMHCpan’s predictions. These different analyses confirm the increased hydrophobicity of strong binding 9-mers from NetMHC’s prediction, and expose an unintended bias in the neural network’s performance compared to NetMHCpan.

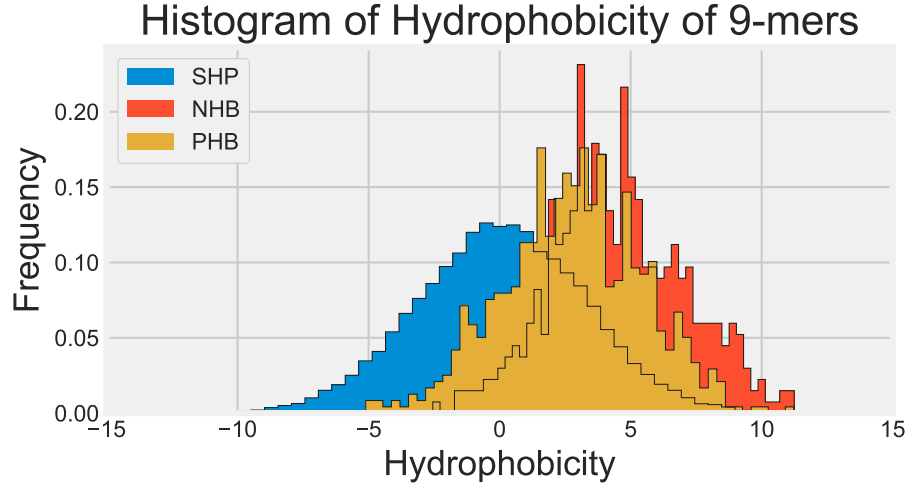


Fig. 7. Histogram of the Hydrophobicity scores (on the x-axis) for sets **SHP** (in blue), **NHB** (in red), and **PHB** (in yellow). Clearly, **NHB** and **PHB** do not align, with **NHB** shifted towards being more hydrophobic.

4 Conclusion

Imagine a toy example of a hydrophilic box filled with water, containing a single HLA-A*0201 protein and a completely hydrophobic 9-mer of LMIPFFILL. The peptide would be repelled by the aqueous medium and latch itself to the A2 protein. Now consider the cell interior, where the highly hydrophobic 9-mer would be repelled by the cytosol and stick to whatever mildly hydrophobic surface it finds nearby. This 9-mer would no longer be trafficked to any MHC for binding, and would not be presented as an antigen on the cell surface despite the 2nd and 9th amino acids highly favoring A2 binding. This cherry-picked peptide was a non-binding peptide (EL score of 0) in the NetMHCpan-4.1 training dataset but was predicted as a strong binder by NetMHC-4.0 for A2 (BA score of 0.792). In general, we do not expect completely hydrophobic antigens to populate any MHC’s immunopeptidome. This example illustrates how the MHC antigen presentation pathway do not support NetMHC’s prediction. Coupled with our observations in Section 3 we conclude that NetMHC has a statistically significant bias towards predicting hydrophobic peptides as strong binders to A2. NetMHC may provide accurate binding affinity predictions, but does not correctly reflect the composition of the A2 immunopeptidome with regards to hydrophobicity. This bias suggests a false positive prediction problem, and limits the utility of NetMHC in applications such as vaccine design [28] and neoantigen identification [15].

In contrast, NetMHCpan-4.1 does not show a similar bias, potentially due to its larger training data set and the use of MHC amino acid sequence as a data

feature of the neural network. The MHC sequence could be allowing the neural network to infer and model the binding mechanics of the A2 binding pockets. Furthermore, eluted ligand data might allow NetMHCpan to capture aspects of the entire antigen presentation pathway instead of estimating pMHC binding strength alone. Lastly, the generated negative data in the training data [1] could be lowering the predicted scores for hydrophobic non binders.

Changes could be implemented in future iterations of NetMHC to address this bias, such as:

- Augmenting training data to include information on hydrophobicity of constituent amino acids. This would entail adding an extra dimension or feature to the training data that stores hydrophobicity scores. We recommend the Moon Hydrophobicity scale for this purpose.
- Incorporating better negative data in training, and properly populating the training dataset with more peptides from the human proteome. Note the offset mean hydrophobicity of TRN compared to SHP in Tables 1 and 2, suggesting that the current training data does not accurately represent the human proteome.
- Designing a post-processing filter that can separate out false positives based on hydrophobicity calculations.

Our emphasis is not on reverse engineering a neural network or trying to divine molecular information from predicted values. Instead, we are highlighting the importance of biochemical attributes pertinent to pMHC binding and cellular machinery. A more insightful neural network, like NetMHCpan-4.1, will avoid false positives and will potentially allow for better performance and greater impact in applications. In future work, we will focus on identifying more significant structural and mechanistic attributes that pose hurdles for AI-based methods. We are developing a structural prediction tool capable of predicting peptide binding with uncurated MHC molecules.

References

1. Alvarez, B., Reynisson, B., Barra, C., Buus, S., Ternette, N., Connelley, T., Andreatta, M., Nielsen, M.: NNAlign-MA; MHC peptidome deconvolution for accurate MHC binding motif characterization and improved T-cell epitope predictions. *Molecular & Cellular Proteomics* **18**(12), 2459–2477 (2019)
2. Andreatta, M., Nielsen, M.: Gapped sequence alignment using artificial neural networks: application to the MHC class I system. *Bioinformatics* **32**(4), 511–517 (2016)
3. Bassani-Sternberg, M., Pletscher-Frankild, S., Jensen, L.J., Mann, M.: Mass spectrometry of human leukocyte antigen class I peptidomes reveals strong effects of protein abundance and turnover on antigen presentation. *Molecular & Cellular Proteomics* **14**(3), 658–673 (2015)
4. Bonsack, M., Hoppe, S., Winter, J., Tichy, D., Zeller, C., Küpper, M.D., Schitter, E.C., Blatnik, R., Riemer, A.B.: Performance evaluation of MHC class-I binding prediction tools based on an experimentally validated MHC-peptide binding data set. *Cancer immunology research* **7**(5), 719–736 (2019)

5. Calis, J.J., Maybeno, M., Greenbaum, J.A., Weiskopf, D., De Silva, A.D., Sette, A., Kesmir, C., Peters, B.: Properties of MHC class I presented peptides that enhance immunogenicity. *PLoS computational biology* **9**(10), e1003266 (2013)
6. Chowell, D., Krishna, S., Becker, P.D., Cocita, C., Shu, J., Tan, X., Greenberg, P.D., Klavinskis, L.S., Blattman, J.N., Anderson, K.S.: TCR contact residue hydrophobicity is a hallmark of immunogenic CD8+ T cell epitopes. *Proceedings of the National Academy of Sciences* **112**(14), E1754–E1762 (2015)
7. Consortium, U.: UniProt: a worldwide hub of protein knowledge. *Nucleic acids research* **47**(D1), D506–D515 (2019)
8. Cornette, J.L., Cease, K.B., Margalit, H., Spouge, J.L., Berzofsky, J.A., DeLisi, C.: Hydrophobicity scales and computational techniques for detecting amphipathic structures in proteins. *Journal of molecular biology* **195**(3), 659–685 (1987)
9. Gourraud, P.A., Khankhanian, P., Cereb, N., Yang, S.Y., Feolo, M., Maiers, M., D. Rioux, J., Hauser, S., Oksenberg, J.: HLA diversity in the 1000 genomes dataset. *PloS one* **9**(7), e97282 (2014)
10. Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jadi, R.S., et al.: Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* **181**(7), 1489–1501 (2020)
11. Hopp, T.P., Woods, K.R.: A computer program for predicting protein antigenic determinants. *Molecular immunology* **20**(4), 483–489 (1983)
12. Huang, L., Kuhls, M.C., Eisenlohr, L.C.: Hydrophobicity as a driver of MHC class I antigen processing. *The EMBO journal* **30**(8), 1634–1644 (2011)
13. Jurtz, V., Paul, S., Andreatta, M., Marcatili, P., Peters, B., Nielsen, M.: NetMHCpan-4.0: improved peptide–MHC class I interaction predictions integrating eluted ligand and peptide binding affinity data. *The Journal of Immunology* **199**(9), 3360–3368 (2017)
14. Kyte, J., Doolittle, R.F.: A simple method for displaying the hydropathic character of a protein. *Journal of molecular biology* **157**(1), 105–132 (1982)
15. Lancaster, E.M., Jablons, D., Kratz, J.R.: Applications of next-generation sequencing in neoantigen prediction and cancer vaccine development. *Genetic testing and molecular biomarkers* **24**(2), 59–66 (2020)
16. Łuksza, M., Riaz, N., Makarov, V., Balachandran, V.P., Hellmann, M.D., Solovyov, A., Rizvi, N.A., Merghoub, T., Levine, A.J., Chan, T.A., et al.: A neoantigen fitness model predicts tumour response to checkpoint blockade immunotherapy. *Nature* **551**(7681), 517–520 (2017)
17. McGranahan, N., Rosenthal, R., Hiley, C.T., Rowan, A.J., Watkins, T.B., Wilson, G.A., Birkbak, N.J., Veeriah, S., Van Loo, P., Herrero, J., et al.: Allele-specific HLA loss and immune escape in lung cancer evolution. *Cell* **171**(6), 1259–1271 (2017)
18. Mei, S., Li, F., Leier, A., Marquez-Lago, T.T., Giam, K., Croft, N.P., Akutsu, T., Smith, A.I., Li, J., Rossjohn, J., et al.: A comprehensive review and performance evaluation of bioinformatics tools for HLA class I peptide-binding prediction. *Briefings in bioinformatics* **21**(4), 1119–1135 (2020)
19. Monera, O.D., Sereda, T.J., Zhou, N.E., Kay, C.M., Hodges, R.S.: Relationship of sidechain hydrophobicity and α -helical propensity on the stability of the single-stranded amphipathic α -helix. *Journal of peptide science: an official publication of the European Peptide Society* **1**(5), 319–329 (1995)
20. Moon, C.P., Fleming, K.G.: Side-chain hydrophobicity scale derived from trans-membrane protein folding into lipid bilayers. *Proceedings of the National Academy of Sciences* **108**(25), 10174–10177 (2011)

21. Nielsen, M., Andreatta, M., Peters, B., Buus, S.: Immunoinformatics: predicting peptide–MHC binding. *Annual Review of Biomedical Data Science* **3**, 191–215 (2020)
22. Nielsen, M., Lundegaard, C., Worning, P., Lauemøller, S.L., Lamberth, K., Buus, S., Brunak, S., Lund, O.: Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. *Protein Science* **12**(5), 1007–1017 (2003)
23. Paul, S., Grifoni, A., Peters, B., Sette, A.: Major histocompatibility complex binding, eluted ligands, and immunogenicity: benchmark testing and predictions. *Frontiers in immunology* **10**, 3151 (2020)
24. Peters, C., Elofsson, A.: Why is the biological hydrophobicity scale more accurate than earlier experimental hydrophobicity scales? *Proteins: Structure, Function, and Bioinformatics* **82**(9), 2190–2198 (2014)
25. Reynisson, B., Alvarez, B., Paul, S., Peters, B., Nielsen, M.: NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. *Nucleic acids research* **48**(W1), W449–W454 (2020)
26. Sarkizova, S., Klaeger, S., Le, P.M., Li, L.W., Oliveira, G., Keshishian, H., Hartigan, C.R., Zhang, W., Braun, D.A., Ligon, K.L., et al.: A large peptidome dataset improves HLA class I epitope prediction across most of the human population. *Nature biotechnology* **38**(2), 199–209 (2020)
27. Schmidt, J., Guillaume, P., Dojcinovic, D., Karbach, J., Coukos, G., Luescher, I.: In silico and cell-based analyses reveal strong divergence between prediction and observation of T-cell-recognized tumor antigen T-cell epitopes. *Journal of Biological Chemistry* **292**(28), 11840–11849 (2017)
28. Schubert, B., Brachvogel, H.P., Jürges, C., Kohlbacher, O.: EpiToolKit—a web-based workbench for vaccine design. *Bioinformatics* **31**(13), 2211–2213 (2015)
29. Simm, S., Einloft, J., Mirus, O., Schleiff, E.: 50 years of amino acid hydrophobicity scales: revisiting the capacity for peptide classification. *Biological research* **49**(1), 1–19 (2016)
30. Vasmatzis, G., Zhang, C., Cornette, J.L., DeLisi, C.: Computational determination of side chain specificity for pockets in class I MHC molecules. *Molecular immunology* **33**(16), 1231–1239 (1996)
31. Wieczorek, M., Abualrous, E.T., Sticht, J., Álvaro-Benito, M., Stolzenberg, S., Noé, F., Freund, C.: Major histocompatibility complex (mhc) class I and MHC class II proteins: conformational plasticity in antigen presentation. *Frontiers in immunology* **8**, 292 (2017)
32. Zhang, C., Vasmatzis, G., Cornette, J.L., DeLisi, C.: Determination of atomic desolvation energies from the structures of crystallized proteins. *Journal of molecular biology* **267**(3), 707–726 (1997)
33. Zhang, Y.H., Xing, Z., Liu, C., Wang, S., Huang, T., Cai, Y.D., Kong, X.: Identification of the core regulators of the HLA I-peptide binding process. *Scientific reports* **7**(1), 1–11 (2017)