Module 13 Assignment

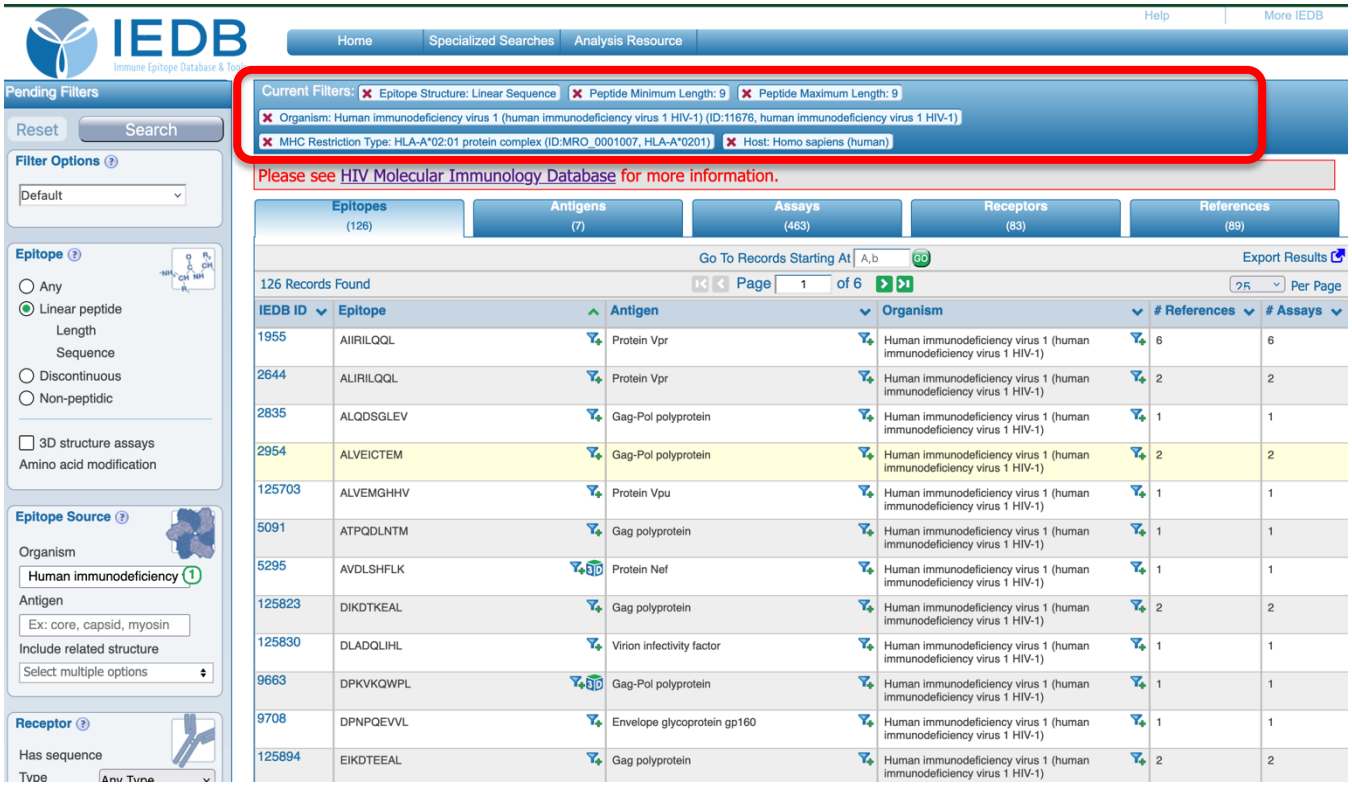
585.751.81 Immunoengineering

1. Read the following paper on models of T cell activation: https://www.nature.com/articles/nri3728 at least up to "Extensions of phenotypic models" on page 623. Describe each of the five possible models for T cell activation listed in the paper (in 2-3 sentences each). Which model best describes T cell activation and why? (50 points)
   The paper describes t5 models for T cell activation:
   - **Occupancy model**: also known as affinity model, proposes that T cell activation is proportional to the number of T-cell receptors (TCRs) occupied by peptide-MHC (pMHC) complexes. This model states that TCRs become signaling-competent immediately upon pMHC binding. This model predicts that pMHC potency (EC_{50}) correlates directly with the dissociation time and the TCR-pMHC dissociation constant (Kd) and that the maximum response (Emax) is independent from the binding parameters which contradicts experimental data.
   - **Kinetic proofreading model** explains how T cells discriminate between ligands based on the dissociation time of the ligand-receptor interaction. It proposes that T cell activation is proportional to the fraction of TCRs that remain bound by pMHC complexes long enough to undergo biochemical modifications, like tyrosine phosphorylation, to reach a signaling-competent state. The delay between pMHC binding and TCR signaling, enables T cells to discern between pMHC complexes based on their dissociation times from the TCR, with the prediction that longer binding times lead to greater T cell activation, which is supported by observed correlations between EC_{50} and Kd.
   - **Kinetic proofreading with limited signaling model:** both the kinetic proofreading model and serial triggering models start with the same biochemical assumptions but differ in what triggers T cell activation. In serial triggering models, each TCR can signal only once per pMHC binding event, limiting continuous activation, particularly from pMHC complexes that dissociate slowly. The kinetic proofreading with limited signaling suggests that TCRs can only signal for a limited time after they become active, necessitating multiple pMHCs to bind sequentially for sustained T cell activation. Because of this limited time for signaling, even at high concentrations of pMHCs, each complex can only activate TCRs for a short period. This results in an optimal dissociation time for effective T cell activation, where pMHCs with too long dissociation times fail to sustain activation because they remain bound to non-signaling TCRs.
   - **Kinetic proofreading with sustained signaling model** allows signaling-competent TCRs to sustain signaling even after pMHC unbinding. This sustained signaling capability is supported by experimental evidence suggesting that TCRs, along with their associated signaling complexes, can remain active until they are either dephosphorylated by phosphatases or internalized by the cell. This model modifies the dynamics of T cell activation by allowing pMHC complexes with various dissociation times to produce maximal signaling at high concentrations, without the need for serial binding, thus allowing an optimal dissociation time that varies with pMHC concentration.
   - **Kinetic proofreading with negative feedback model** is an extension of the kinetic proofreading model and introduces a mechanism to regulate the activation of TCRs through negative feedback loops involving phosphorylation. This negative feedback is

mediated by phosphatases like SHP1 and other phosphatases. Such feedback results in T cell activation showing an optimal response function to the pMHC dose, which is modulated by the dissociation time between TCR and pMHC.

2. You are studying HIV and want to identify viral epitopes that may be recognized by CD8+ T cells to kill HIV-infected CD4+ T cells. Use the following database (http://www.iedb.org/home_v3.php) to search for linear peptide epitopes from the organism Human immunodeficiency virus 1 (the more common type of the HIV virus) that bind to HLA-A*02:01 (the most common HLA-A allele in humans) in human hosts. Once you submit the search, change the linear peptide length to 9 amino acids (as most HLA molecules have a strong preference for binding 9mers) on the left-hand side. Export your results, pick the first 50 epitopes in your search results and input them into the netMHC artificial neural network prediction program using the PEPTIDE format (https://services.healthtech.dtu.dk/service.php?NetMHC-4.0). For this problem, look at the binding affinity of your peptides to HLA-A*02:01 allele in the HLA-A species/loci. (50 points)

    a. Please list/provide a screenshot of the peptides that you tested in the software and list the peptides that were predicted to be strong binders to the HLA molecule (those labeled “SB”). (15 points)



IEDB search for linear peptides of length 9mers from Human immunodeficiency virus 1 that bind to HLA-A*02:01

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# NetMHC version 4.0

# Input is in PEPTIDE format
# Rank Threshold for Strong binding peptides   0.500
# Rank Threshold for Weak binding peptides   2.000
-----------------------------------------------------------------------------------------------------------------------------
 pos          HLA      peptide         Core Offset  I_pos  I_len  D_pos  D_len        iCore        Identity 1-log50k(aff) Affinity(nM)    %Rank  BindLevel
-----------------------------------------------------------------------------------------------------------------------------
   0    HLA-A0201    AIIRILQQL    AIIRILQQL      0      0      0      0      0    AIIRILQQL        PEPLIST         0.383       793.35     3.50
   0    HLA-A0201    ALIRILQQL    ALIRILQQL      0      0      0      0      0    ALIRILQQL        PEPLIST         0.591        83.87     0.90 <= WB
   0    HLA-A0201    ALQDSGLEV    ALQDSGLEV      0      0      0      0      0    ALQDSGLEV        PEPLIST         0.686        29.75     0.40 <= SB
   0    HLA-A0201    ATPQDLNTM    ATPQDLNTM      0      0      0      0      0    ATPQDLNTM        PEPLIST         0.113     14771.97    21.00
   0    HLA-A0201    AVDLSHFLK    AVDLSHFLK      0      0      0      0      0    AVDLSHFLK        PEPLIST         0.071     23295.88    33.00
   0    HLA-A0201    DPKVKQWPL    DPKVKQWPL      0      0      0      0      0    DPKVKQWPL        PEPLIST         0.034     34473.86    60.00
   0    HLA-A0201    DPNPQEVVL    DPNPQEVVL      0      0      0      0      0    DPNPQEVVL        PEPLIST         0.027     37415.11    75.00
   0    HLA-A0201    ELHPDKWTV    ELHPDKWTV      0      0      0      0      0    ELHPDKWTV        PEPLIST         0.387       758.61     3.50
   0    HLA-A0201    ELRSLYNTV    ELRSLYNTV      0      0      0      0      0    ELRSLYNTV        PEPLIST         0.187      6589.51    12.00
   0    HLA-A0201    EPIVGAETF    EPIVGAETF      0      0      0      0      0    EPIVGAETF        PEPLIST         0.046     30464.55    48.00
   0    HLA-A0201    EVIPMFSAL    EVIPMFSAL      0      0      0      0      0    EVIPMFSAL        PEPLIST         0.163      8567.42    14.00
   0    HLA-A0201    FPVRPQVPL    FPVRPQVPL      0      0      0      0      0    FPVRPQVPL        PEPLIST         0.088     19342.74    26.00
   0    HLA-A0201    FPVTPQVPL    FPVTPQVPL      0      0      0      0      0    FPVTPQVPL        PEPLIST         0.110     15206.25    21.00
   0    HLA-A0201    GPGHKARVL    GPGHKARVL      0      0      0      0      0    GPGHKARVL        PEPLIST         0.024     38633.61    80.00
   0    HLA-A0201    GSEELRSLY    GSEELRSLY      0      0      0      0      0    GSEELRSLY        PEPLIST         0.037     33626.54    60.00
   0    HLA-A0201    HLEGKVILV    HLEGKVILV      0      0      0      0      0    HLEGKVILV        PEPLIST         0.490       248.06     1.90 <= WB
   0    HLA-A0201    HPDIVIYQY    HPDIVIYQY      0      0      0      0      0    HPDIVIYQY        PEPLIST         0.068     23882.63    34.00
   0    HLA-A0201    ILKEPVHGV    ILKEPVHGV      0      0      0      0      0    ILKEPVHGV        PEPLIST         0.574        99.95     1.10 <= WB
   0    HLA-A0201    ILLEPVHGV    ILLEPVHGV      0      0      0      0      0    ILLEPVHGV        PEPLIST         0.852         4.95     0.03 <= SB
   0    HLA-A0201    IPLTEEAEL    IPLTEEAEL      0      0      0      0      0    IPLTEEAEL        PEPLIST         0.054     27961.78    42.00
   0    HLA-A0201    IPRRIRQGL    IPRRIRQGL      0      0      0      0      0    IPRRIRQGL        PEPLIST         0.035     34355.45    60.00
   0    HLA-A0201    IVGAETFYV    IVGAETFYV      0      0      0      0      0    IVGAETFYV        PEPLIST         0.704        24.61     0.40 <= SB
   0    HLA-A0201    KAACWWAGI    KAACWWAGI      0      0      0      0      0    KAACWWAGI        PEPLIST         0.370       911.12     4.00
   0    HLA-A0201    KLTPLCVTL    KLTPLCVTL      0      0      0      0      0    KLTPLCVTL        PEPLIST         0.634        52.70     0.70 <= WB
   0    HLA-A0201    KLVGKLNWA    KLVGKLNWA      0      0      0      0      0    KLVGKLNWA        PEPLIST         0.563       112.62     1.10 <= WB
   0    HLA-A0201    LLNATDIAV    LLNATDIAV      0      0      0      0      0    LLNATDIAV        PEPLIST         0.677        32.88     0.50 <= SB
   0    HLA-A0201    LLQLTVWGI    LLQLTVWGI      0      0      0      0      0    LLQLTVWGI        PEPLIST         0.605        71.44     0.80 <= WB
   0    HLA-A0201    LLWKGEGAV    LLWKGEGAV      0      0      0      0      0    LLWKGEGAV        PEPLIST         0.605        71.44     0.80 <= WB
   0    HLA-A0201    LTFGWCFKL    LTFGWCFKL      0      0      0      0      0    LTFGWCFKL        PEPLIST         0.621        60.45     0.70 <= WB
   0    HLA-A0201    LVGPTPVNI    LVGPTPVNI      0      0      0      0      0    LVGPTPVNI        PEPLIST         0.225      4389.37     9.00
   0    HLA-A0201    MTNNPPIPV    MTNNPPIPV      0      0      0      0      0    MTNNPPIPV        PEPLIST         0.485       262.89     1.90 <= WB
   0    HLA-A0201    NANPDCKTI    NANPDCKTI      0      0      0      0      0    NANPDCKTI        PEPLIST         0.059     26303.06    38.00
   0    HLA-A0201    NPDIVIYQY    NPDIVIYQY      0      0      0      0      0    NPDIVIYQY        PEPLIST         0.066     24360.48    34.00
   0    HLA-A0201    NSSKVSQNY    NSSKVSQNY      0      0      0      0      0    NSSKVSQNY        PEPLIST         0.036     33741.71    60.00
   0    HLA-A0201    PPIPVGDIY    PPIPVGDIY      0      0      0      0      0    PPIPVGDIY        PEPLIST         0.033     35127.12    65.00
   0    HLA-A0201    RAIEAQQHL    RAIEAQQHL      0      0      0      0      0    RAIEAQQHL        PEPLIST         0.176      7430.63    13.00
   0    HLA-A0201    RAMASDFNL    RAMASDFNL      0      0      0      0      0    RAMASDFNL        PEPLIST         0.444       408.75     2.50
   0    HLA-A0201    RIKQIINMW    RIKQIINMW      0      0      0      0      0    RIKQIINMW        PEPLIST         0.073     22633.75    31.00
   0    HLA-A0201    RILQQLLFI    RILQQLLFI      0      0      0      0      0    RILQQLLFI        PEPLIST         0.543       140.54     1.30 <= WB
   0    HLA-A0201    RLVNGSLAL    RLVNGSLAL      0      0      0      0      0    RLVNGSLAL        PEPLIST         0.609        68.79     0.80 <= WB
   0    HLA-A0201    RMYSPISIL    RMYSPISIL      0      0      0      0      0    RMYSPISIL        PEPLIST         0.567       108.49     1.10 <= WB
   0    HLA-A0201    RPIVSTQLL    RPIVSTQLL      0      0      0      0      0    RPIVSTQLL        PEPLIST         0.081     20814.80    29.00
   0    HLA-A0201    RPMTYKAAL    RPMTYKAAL      0      0      0      0      0    RPMTYKAAL        PEPLIST         0.071     23270.69    32.00
   0    HLA-A0201    SLYNTIAVL    SLYNTIAVL      0      0      0      0      0    SLYNTIAVL        PEPLIST         0.637        50.52     0.60 <= WB
   0    HLA-A0201    SLYNTVATL    SLYNTVATL      0      0      0      0      0    SLYNTVATL        PEPLIST         0.632        53.76     0.70 <= WB
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netMHC 50 first binding affinity results

b. Are there any features in common between the peptides listed as “strong binders”? If so, what are those features and why are they conserved between the peptides? If you do not see any features in common or only have 1-2 strong binding peptides, answer the question more generally: what common features would you expect to see between peptides that bind strongly to a given HLA/MHC allele? (20 points)

Looking at the list of epitopes which express a strong binding affinity with HLA-A*02:01 returned by the tool netHMC, we see a pattern of specific amino acids recurring at identical positions across the 9-mer sequences. In the color-coded visualizations of duplicate residues within each position, below, we see that a high frequency of leucine (L) at positions 2 and 9, followed by valine (V) at position 9. Research by Vadim Karnaukov et al., support these findings (Fig. 1). Leucine and valine are hydrophobic amino acids, which may suggest the importance of hydrophobic interactions in the binding process. This also, indicates that these positions are key features for strong binding to HLA-A*02:01, possibly because they are the residues that fit into the binding groove of the HLA molecule.
These features are conserved because they contribute to the stability and specificity of the peptide-HLA complex, the peptide needs to bind strongly to the HLA molecule and present its key residues to the T cell receptor in an optimal orientation to trigger an effective immune response.

Peptide	1	2	3	4	5	6	7	8	9
ALVEICTEM	A	L	V	E	I	C	T	E	M
ILLEPVHGV	I	L	L	E	P	V	H	G	V
IVGAETFYV	I	V	G	A	E	T	F	Y	V
LLNATDIAV	L	L	N	A	T	D	I	A	V
TLTSCNTSV	T	L	T	S	C	N	T	S	V
VLAEAMSQV	V	L	A	E	A	M	S	Q	V
WLWYIKIFI	W	L	W	Y	I	K	I	F	I
YTAFTIPSI	Y	T	A	F	T	I	P	S	I
ALVEMGHHV	A	L	V	E	M	G	H	H	V
GLADQLIHI	G	L	A	D	Q	L	I	H	I
GLADQLIHL	G	L	A	D	Q	L	I	H	L
GLADQLIHM	G	L	A	D	Q	L	I	H	M
NLADQLIHL	N	L	A	D	Q	L	I	H	L
SLADQLIHL	S	L	A	D	Q	L	I	H	L
SLVKHHMYV	S	L	V	K	H	H	M	Y	V
VLYCVHQRV	V	L	Y	C	V	H	Q	R	V
SLFNTVATL	S	L	F	N	T	V	A	T	L
SLFNAVATL	S	L	F	N	A	V	A	T	L
SLFNTIATL	S	L	F	N	T	I	A	T	L
SLFNTVATV	S	L	F	N	T	V	A	T	V
SLFNTVVTL	S	L	F	N	T	V	V	T	L
SLFNAVAVL	S	L	F	N	A	V	A	V	L
SLFNAVVTL	S	L	F	N	A	V	V	T	L
SLFNTIAVL	S	L	F	N	T	I	A	V	L
SLYNAIATL	S	L	Y	N	A	I	A	T	L
SLYNAVATL	S	L	Y	N	A	V	A	T	L
SLYNAVVTL	S	L	Y	N	A	V	V	T	L
SLYNSVATL	S	L	Y	N	S	V	A	T	L
SLYNTIATL	S	L	Y	N	T	I	A	T	L
SLYNTVVTL	S	L	Y	N	T	V	V	T	L
YTAFTIPSV	Y	T	A	F	T	I	I	S	V
IIIGALVGV	I	I	I	G	A	L	V	G	V

Color-coded duplicates within each column

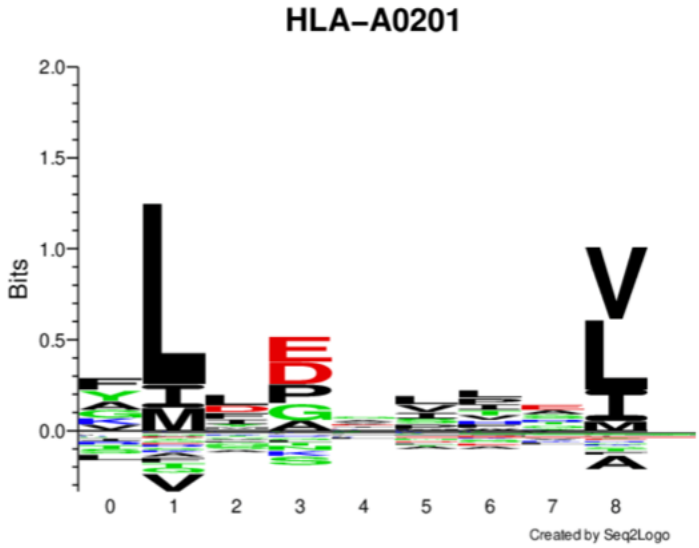

Figure 1 - Sequence Logo – Ref: [1]

c. Describe (in no more than a few sentences) how netMHC could be utilized in an immunoengineering context. (15 points)

Mapping the binding sites (or epitopes) of antibodies and their target antigens is critical for understanding their mechanism of action. In addition, understanding the motion and dynamics of the antigen in response to binding provides additional insight to advance therapeutic candidates, maximize efficacy, and reduce adverse immune reactions. Epitope identification is costly and time-consuming as it requires experimental screening of large arrays of potential epitope candidates. NetHMC could be utilized in understanding disease condition, immune monitoring, developing diagnosis assays, and designing epitope-based vaccines.
In the development of personalized medicine, netHMC could help in identifying neoantigens in individual tumors, enabling the design of tailored immunotherapies that target specific cancer mutations.

[1] V. Karnaukhov *et al.*, “HLA binding of self-peptides is biased towards proteins with specific molecular functions,” *bioRxiv*, p. 2021.02.16.431395, 2021, doi: 10.1101/2021.02.16.431395