Bioinformatics for Precision Medicine

Prediction of T cell epitopes

DTU

Agenda for today

Day 4: Neo epitope prediction and vaccine development (on-line)

Where: Zoom link

Program:

9.00 - 9.30: MHC binding and T cell epitopes (Carolina Barra Quaglia)

Exercise: Making Sequence logos

10.00 - 10.30: MHC binding predictions (Carolina Barra Quaglia)

Exercise: Calculate the weights of a neural network

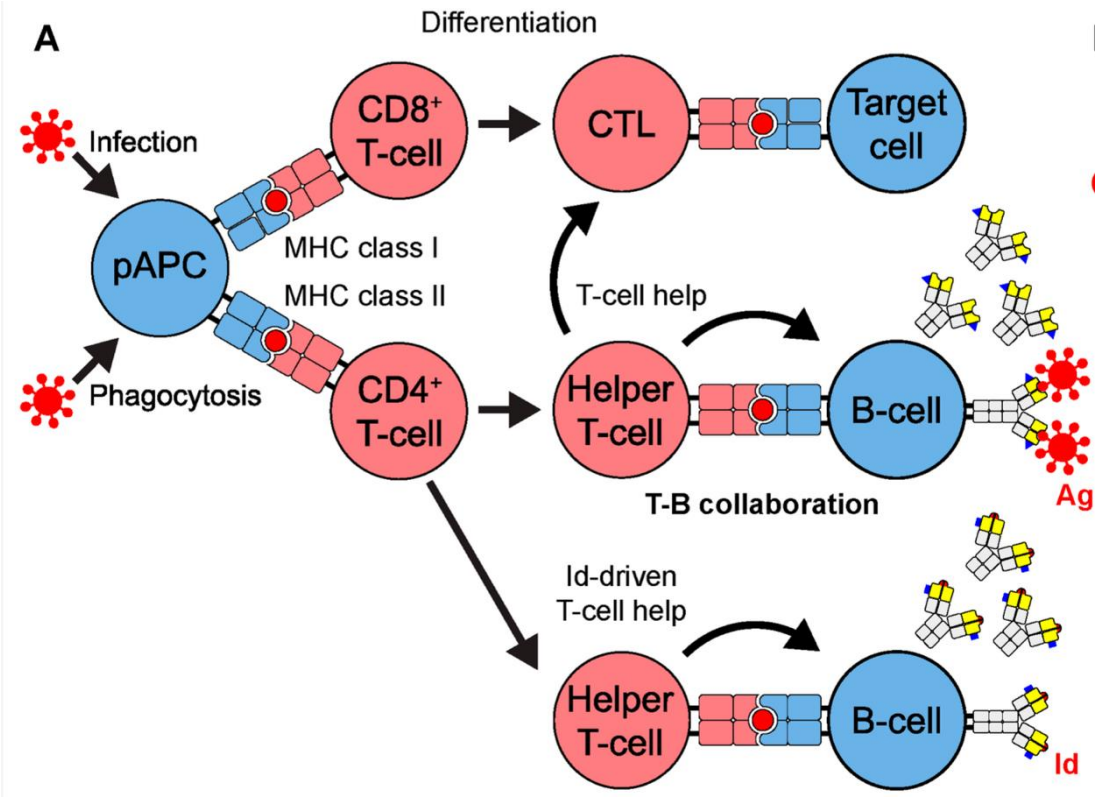
11.00 - 11.30: MHC binding predictions and medical applications (Carolina Barra Quaglia)

Exercise: IEDB database search

12.00 - 14.00: Lunch + Exercise T cell Epitopes prediction **(time off-line)**

14.00 - 15.00: Walk-through Exercise T cell Epitopes prediction (Carolina Barra Quaglia)

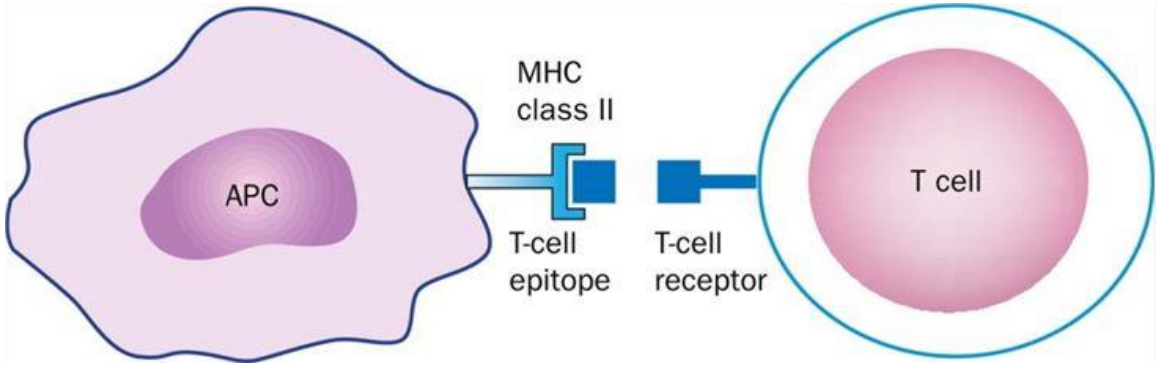
First of all a few notes on T and B cell epitopes



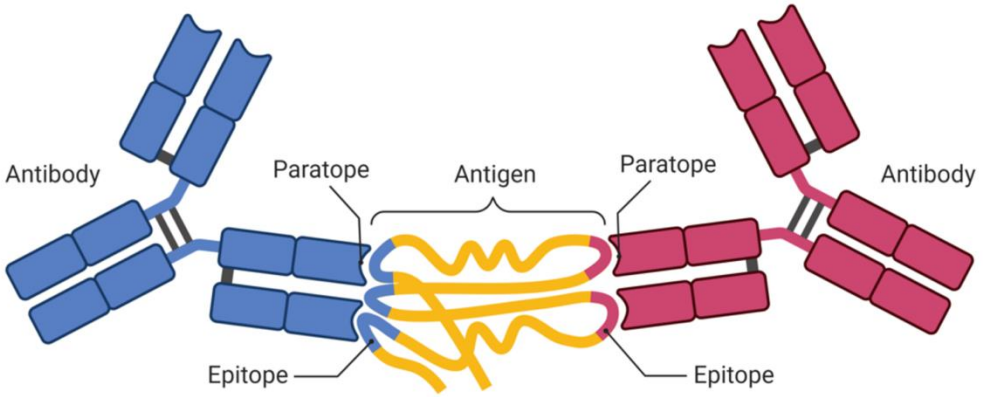
https://doi.org/10.3390/v15051186

First of all a few notes on T and B cell epitopes

T cell epitopes

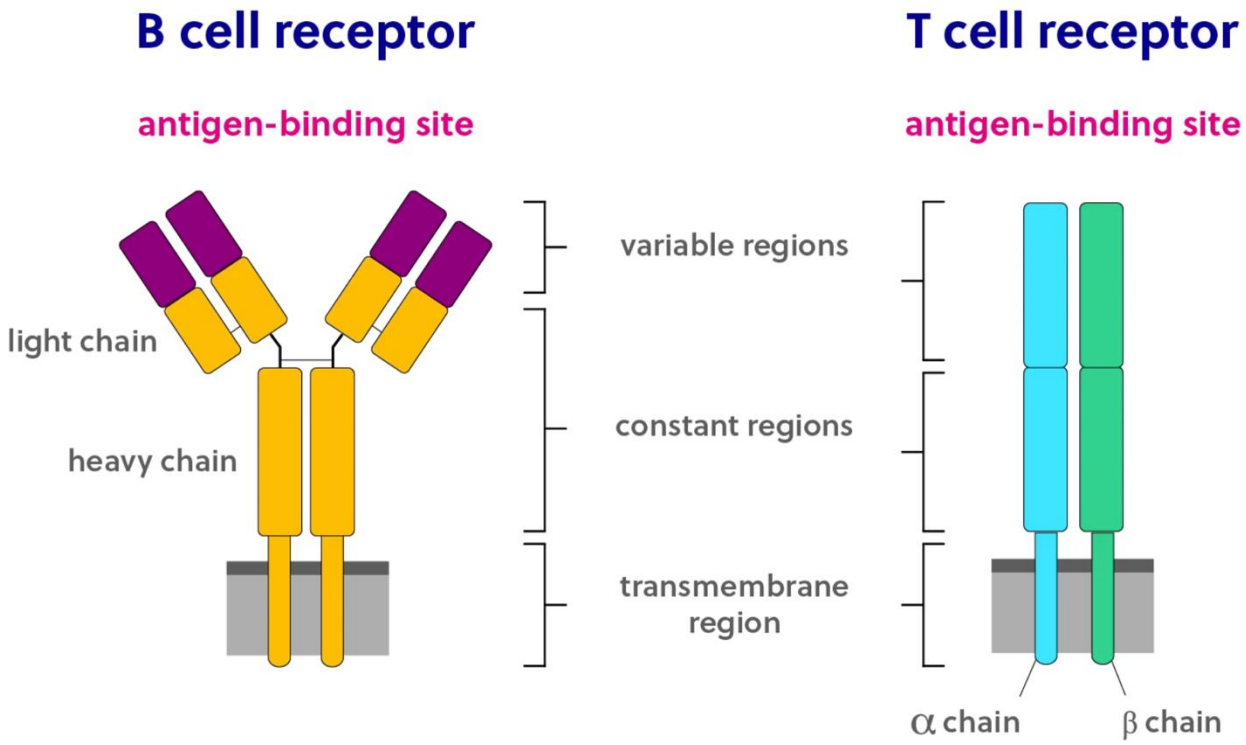


B cell epitopes



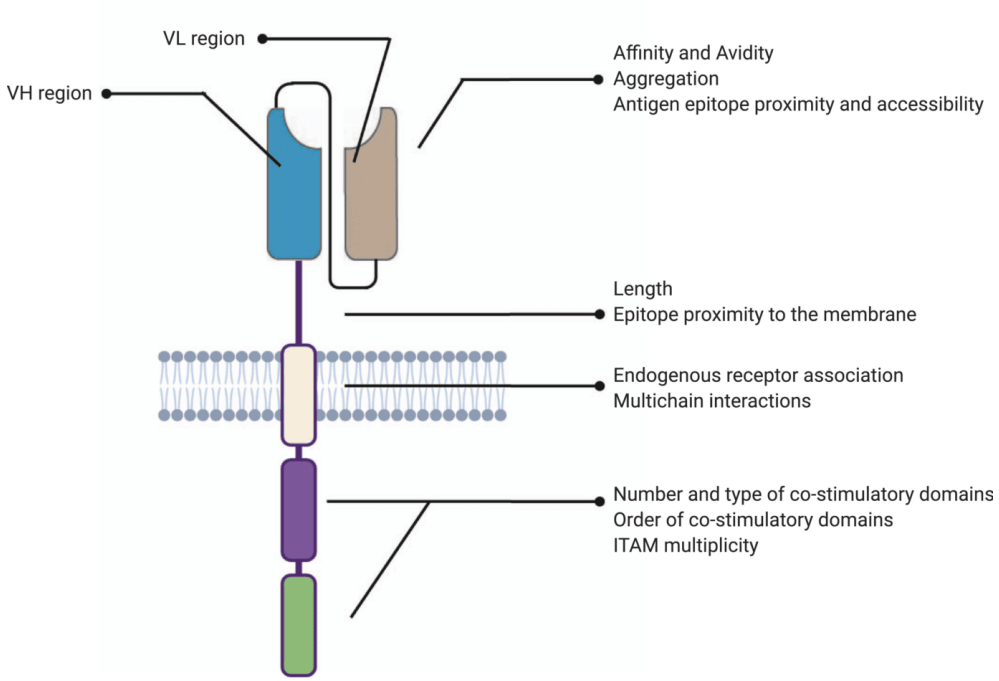
https://www.creative-biolabs.com/cremap-t-cell-epitope-discovery-service.html

First of all a few notes on T and B cell epitopes



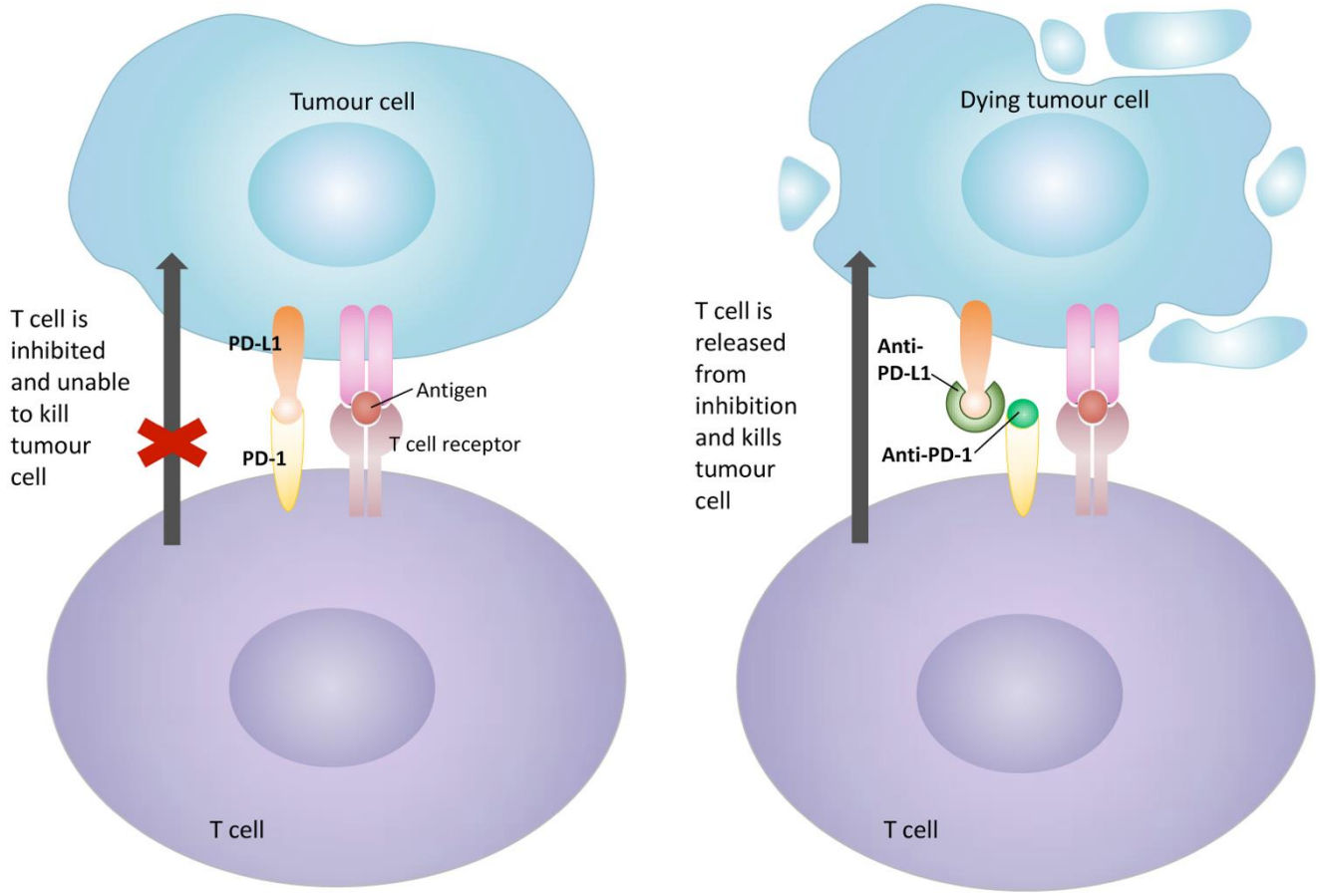
First of all a few notes on T and B cell epitopes

CAR – T cell receptor



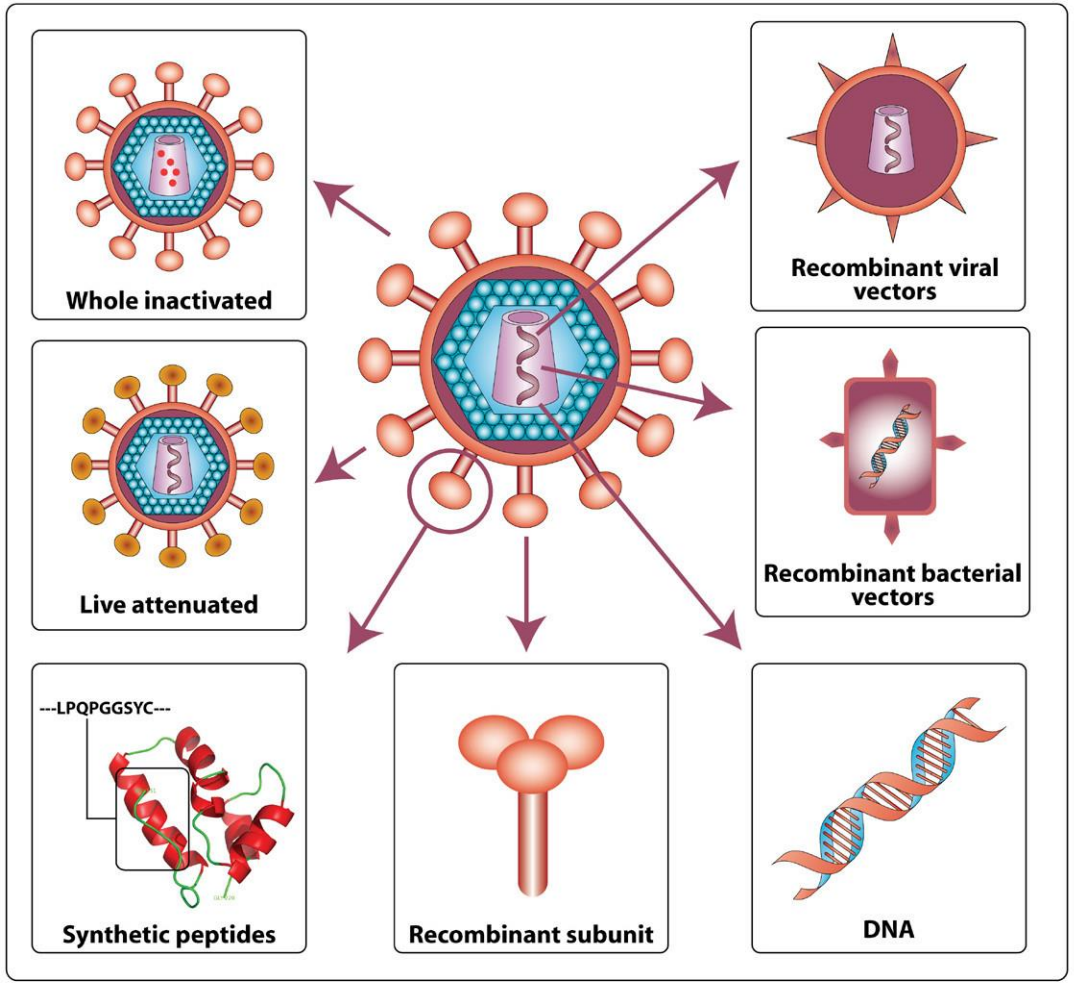
Why are we interested on T cell epitopes?

Cancer immunotherapeutics



Credit: Dr Koh Shimin Grace, Department of Paediatrics, NUS

Peptide vaccines



MHC binding predictions, a historical overview - From a few to all in a decade

Morten Nielsen
Immunoinformatics and Machine learning
Bioinformatics Section
DTU Health Tech

- 1994-1997, Bimas HLA-A2, B27 motif, SYFPEITHI
- **2003, NetMHC-1.0**

HLA-A0204, H-2Kk

- **2007, NetMHCII-1.0**

Prediction for prevalent HLA-DR molecules

- **2007, NetMHCpan-1.0, 2008 NetMHCIIpan-1.0**

Pan-specific prediction for any HLA-I and HLA-DR molecule with known protein sequence

…

- **2020, NetMHCpan-4.1 and NetMHCIIpan-4.0**

Pan-specific prediction to any MHC molecule with known protein sequence. Predictions of binding for 8-13mer peptides. Pan-specific predictions to any HLA class II molecule (DR, DP and DQ) with known protein sequence. Integration of multi-allele MS immunopeptidomics data

MHC binding motifs and information content

Learning Objectives

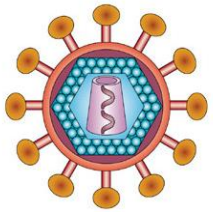
Upon completion of this lecture you should be able to:

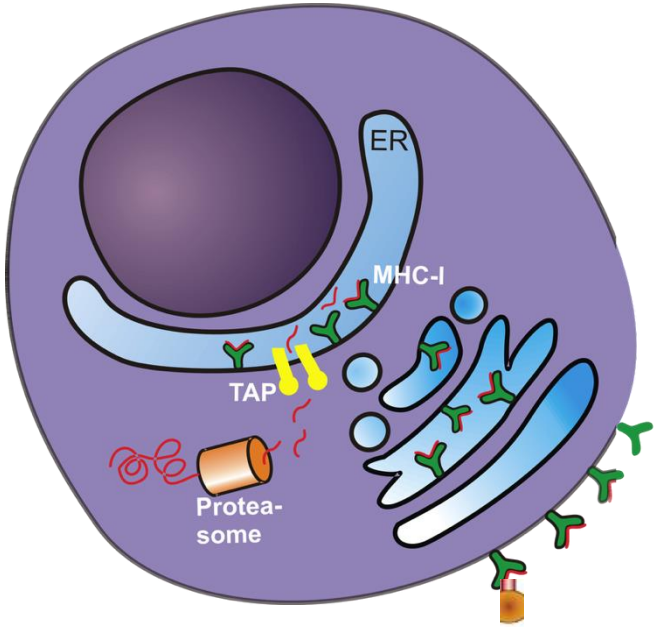
- Describe the process of MHC binding peptides and T cell epitopes
- Interpret and generate binding motifs by the construction of sequence logos
- Understand the concepts of weight matrix construction

What defines a T cell epitope?

- Antigen Processing (MHC-I: Proteasomal cleavage, TAP;

  MHC-II: cathepsins)
- MHC binding
- MHC:peptide complex stability
- T cell repertoire
- T cell recognition
- Source protein abundance, cellular location and function
- ???





MHC class I antigen presentation pathway

1/200 peptides make to the surface

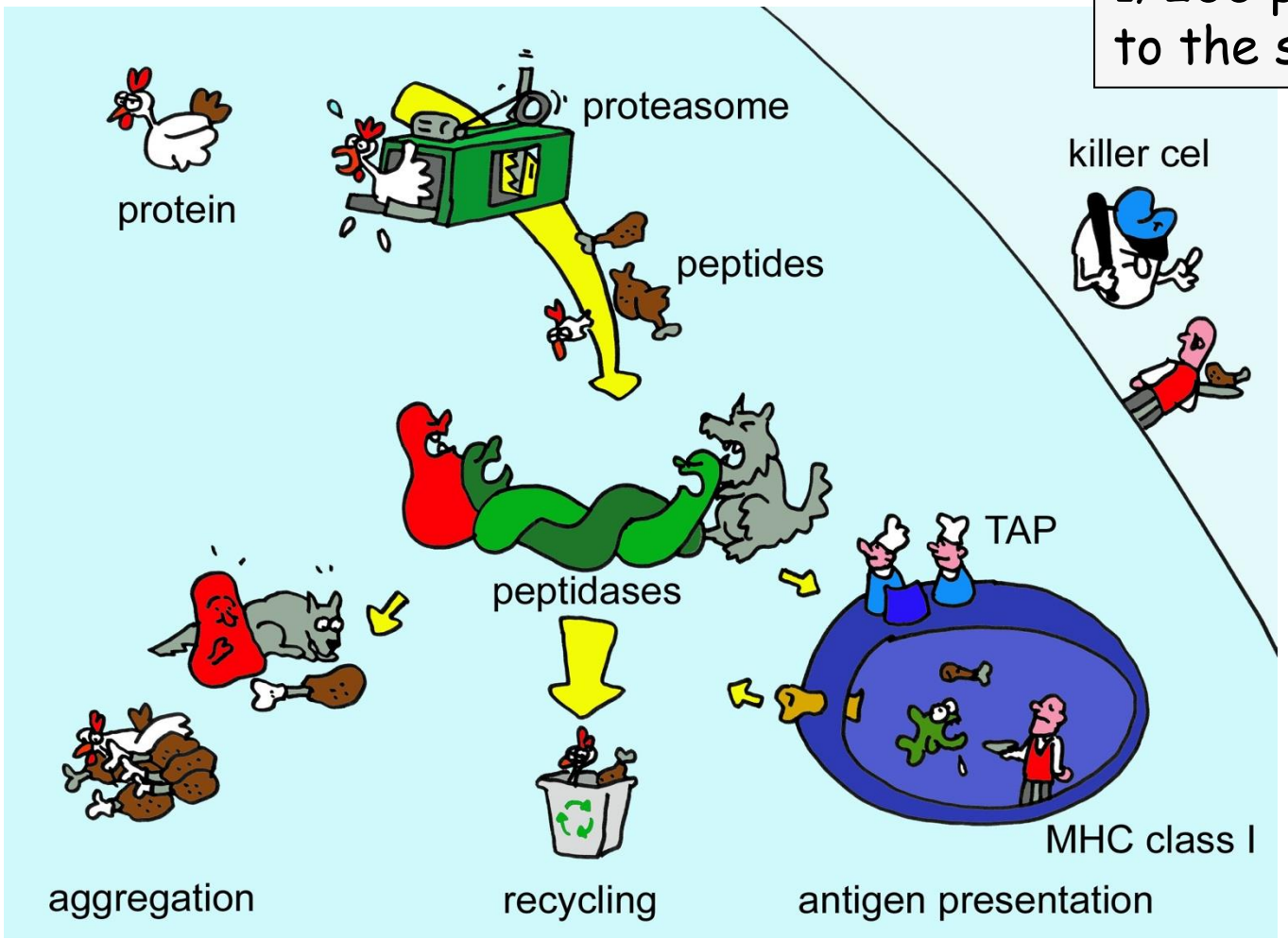
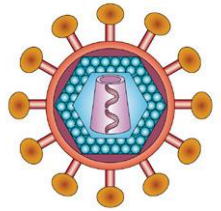


Figure by Eric A.J. Reits

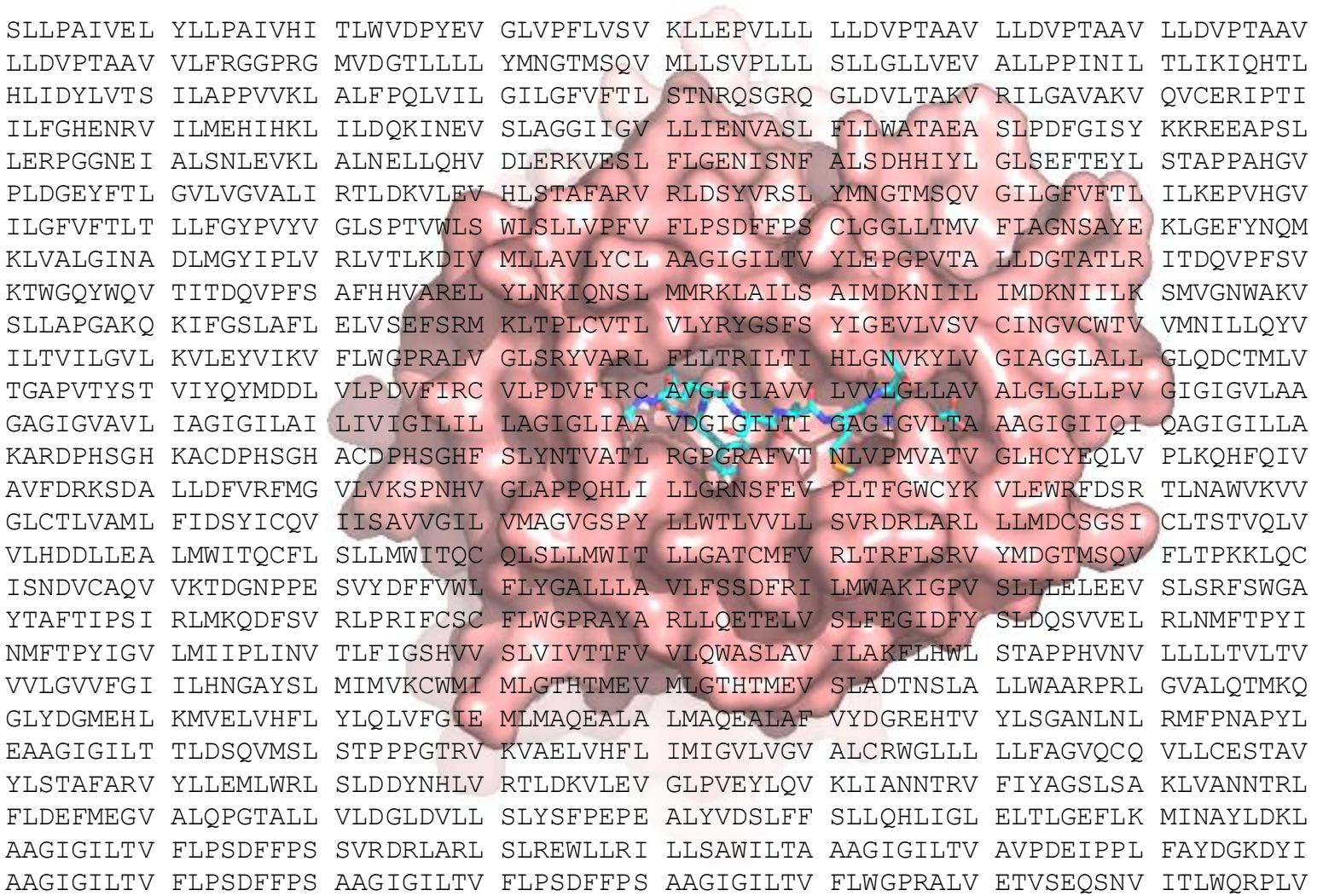
The challenge of identifying the target of T cells



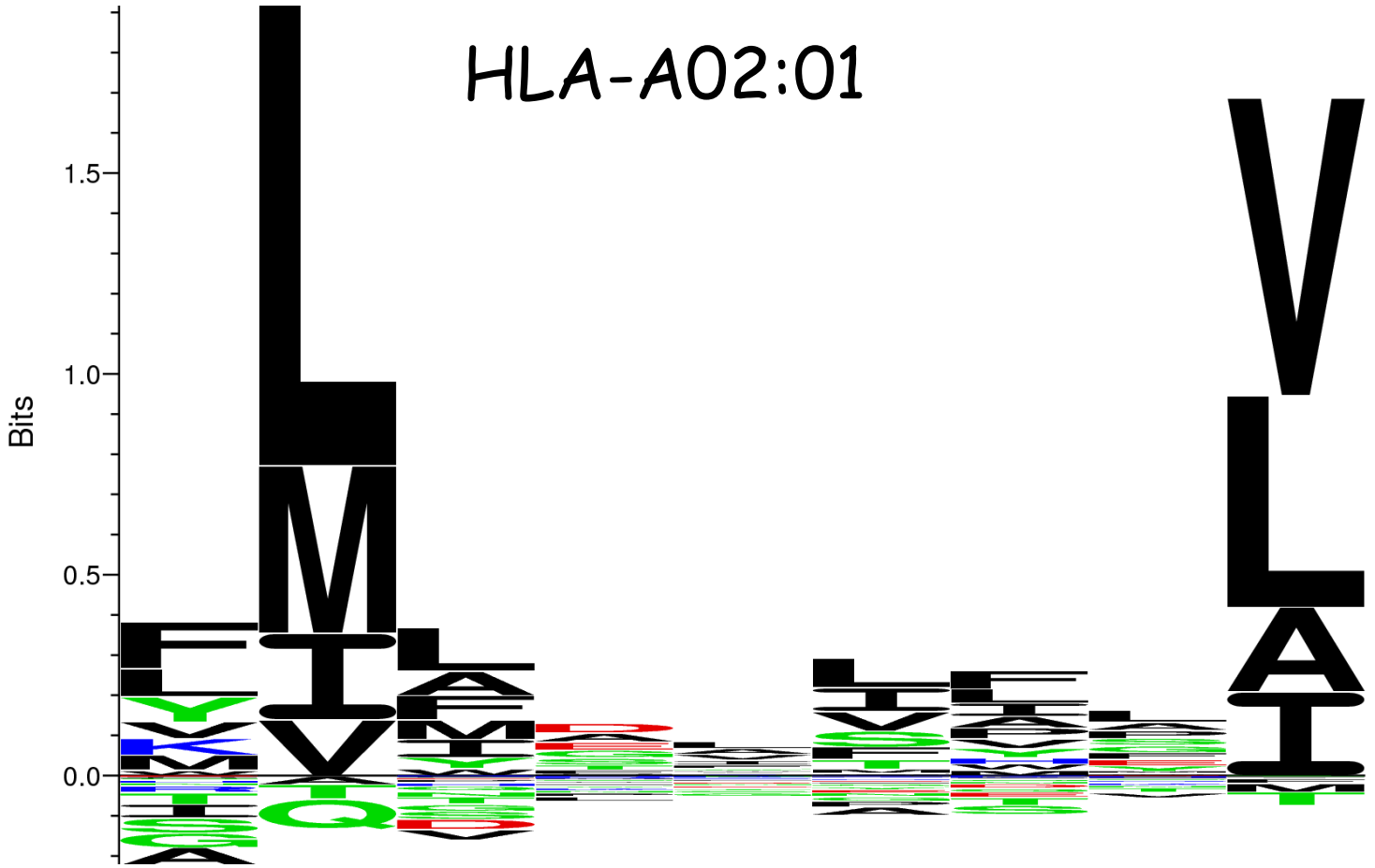
- A pathogen encodes ~10,000 unique peptides
- In a given population, we have ~25-50 different prevalent HLA-I alleles
- This gives all-together ~ 500,000 different HLA:peptide combinations
- Only 10-20 (<0.002%) of these are immunogenic in a given individual

Can we understand why this is, and more importantly can we learn the rules and predict these immunogenic peptides?

Sequence information and binding motifs



HLA binding motifs



https://services.healthtech.dtu.dk/service.php?Seq2Logo-2.0

Sequence Information

- Say that a peptide must have L at P_2 in order to bind, and that A, F, W, and Y are found at P_1. Which position has most information?
- How many questions do I need to ask to tell if a peptide binds looking at only P_1 or P_2?

ALAKAAAAM
ALAKAAAAN
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ALAKAAAAT
ALAKAAAAV
WLNERPILT
YLLGFVFTM
FLNAWVKVV
YLNEPVLLL
ALVPFIVSV

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- P1: 4 questions (at most)
- P2: 1 question (L or not)
- P2 has the most information

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- Calculate p_a at each position
- Entropy

$$S = -\sum_a p_a \log(p_a)$$

- Information content

$$I = \log(20) + \sum_a p_a \log(p_a)$$

- Conserved positions
  - P_L=1, $P_{!L}$=0 => S=0, I=log(20)
- Mutable positions
  - P_{aa}=1/20 => S=log(20), I=0

Information content

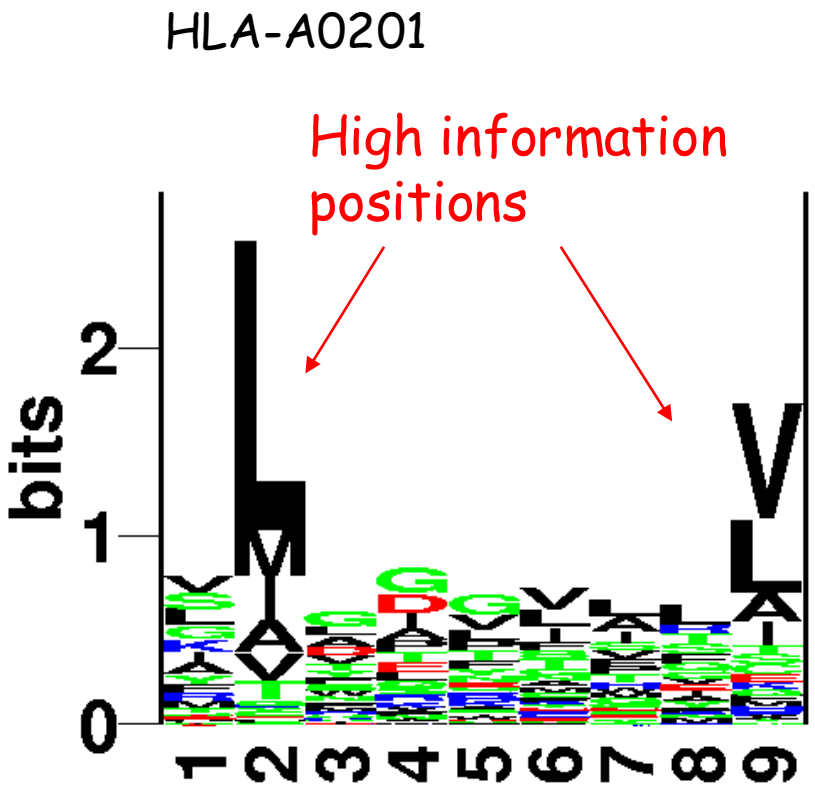
	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	S	I
1	0.10	0.06	0.01	0.02	0.01	0.02	0.02	0.09	0.01	0.07	0.11	0.06	0.04	0.08	0.01	0.11	0.03	0.01	0.05	0.08	3.96	0.37
2	0.07	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.08	0.59	0.01	0.07	0.01	0.00	0.01	0.06	0.00	0.01	0.08	2.16	2.16
3	0.08	0.03	0.05	0.10	0.02	0.02	0.01	0.12	0.02	0.03	0.12	0.01	0.03	0.05	0.06	0.06	0.04	0.04	0.04	0.07	4.06	0.26
4	0.07	0.04	0.02	0.11	0.01	0.04	0.08	0.15	0.01	0.10	0.04	0.03	0.01	0.02	0.09	0.07	0.04	0.02	0.00	0.05	3.87	0.45
5	0.04	0.04	0.04	0.04	0.01	0.04	0.05	0.16	0.04	0.02	0.08	0.04	0.01	0.06	0.10	0.02	0.06	0.02	0.05	0.09	4.04	0.28
6	0.04	0.03	0.03	0.01	0.02	0.03	0.03	0.04	0.02	0.14	0.13	0.02	0.03	0.07	0.03	0.05	0.08	0.01	0.03	0.15	3.92	0.40
7	0.14	0.01	0.03	0.03	0.02	0.03	0.04	0.03	0.05	0.07	0.15	0.01	0.03	0.07	0.06	0.07	0.04	0.03	0.02	0.08	3.98	0.34
8	0.05	0.09	0.04	0.01	0.01	0.05	0.07	0.05	0.02	0.04	0.14	0.04	0.02	0.05	0.05	0.08	0.10	0.01	0.04	0.03	4.04	0.28
9	0.07	0.01	0.00	0.00	0.02	0.02	0.02	0.01	0.01	0.08	0.26	0.01	0.01	0.02	0.00	0.04	0.02	0.00	0.01	0.38	2.78	1.55

$$S = -\sum_a p_a \log(p_a)$$

$$I = \log(20) + \sum_a p_a \log(p_a)$$

Sequence logos

- Height of a column equal to I
- Relative height of a letter is p
- Highly useful tool to visualize sequence motifs

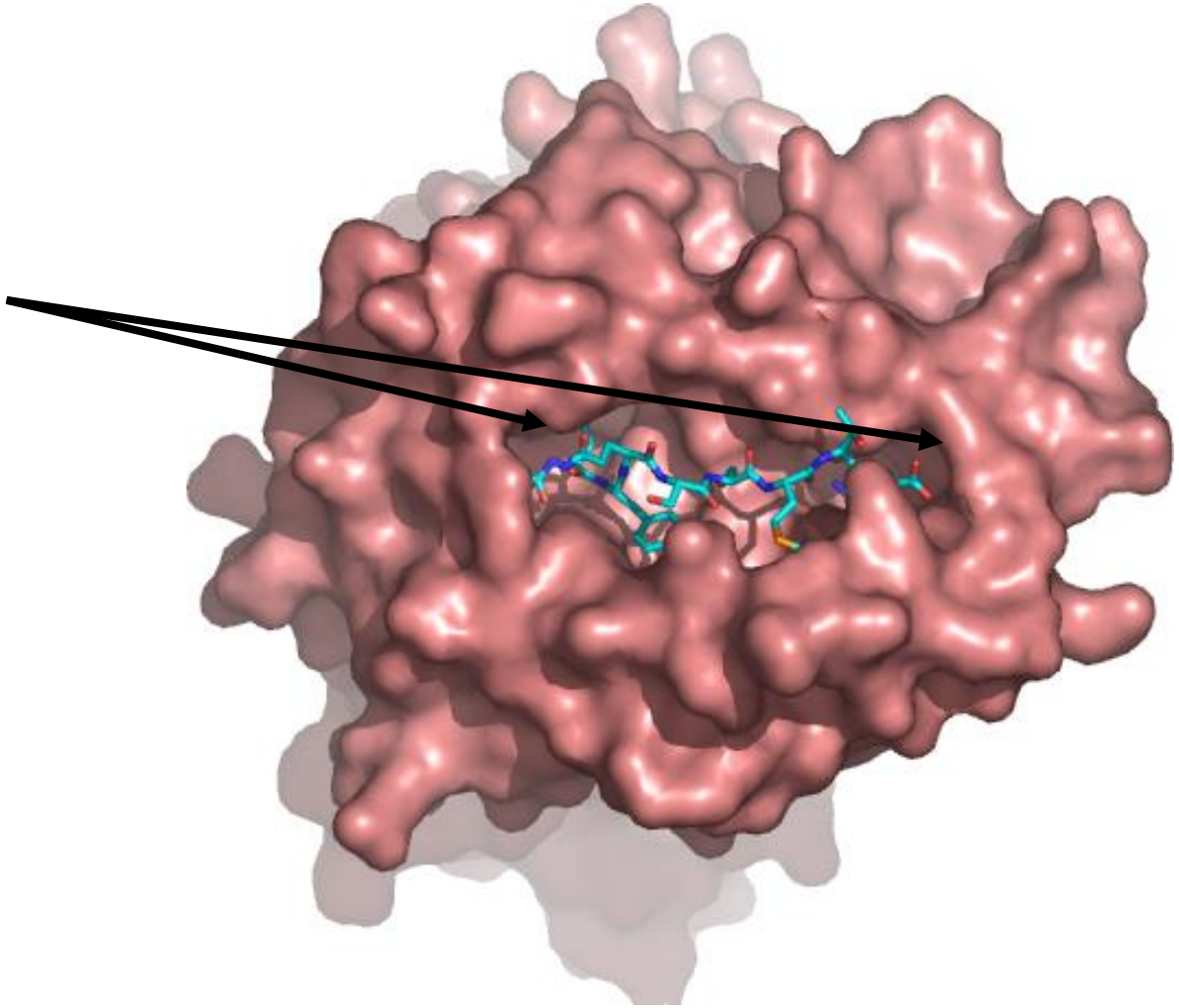


http://www.cbs.dtu.dk/~gorodkin/appl/plogo.html

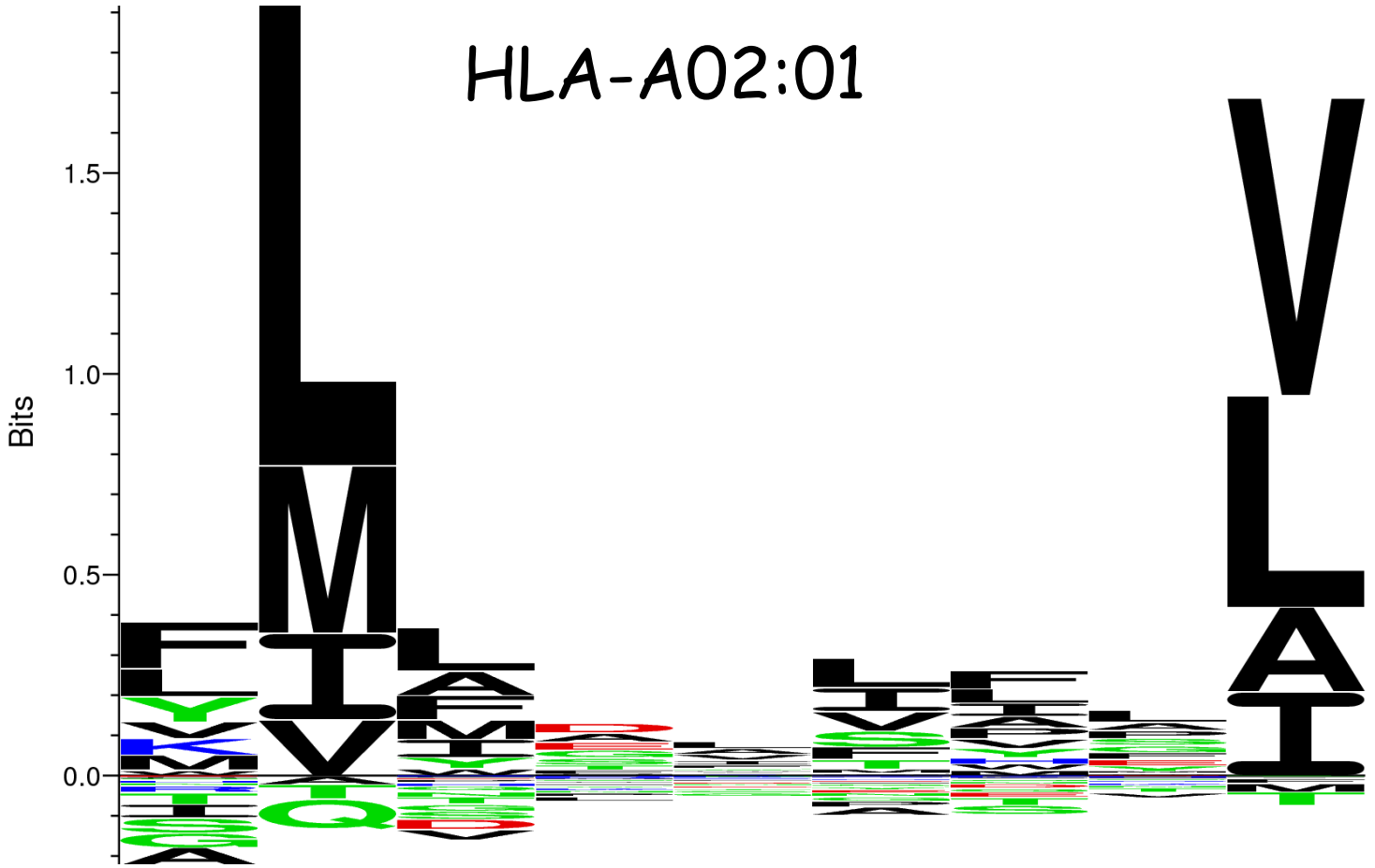
Now, do it yourself!

Binding Motif of MHC class I with peptide

Anchor positions



HLA binding motifs



https://services.healthtech.dtu.dk/service.php?Seq2Logo-2.0

Characterizing a binding motif from small data sets

10 MHC restricted peptides

ALAKAAAAM
ALAKAAAAN
ALAKAAAAR
ALAKAAAAT
ALAKAAAAV
GMNERPILT
GILGFVFTM
TLNAWVKVV
KLNEPVLLL
AVVPFIVSV

What can we learn?

A at P1 favors binding?
I is not allowed at P9?

Characterizing a binding motif from small data sets

ALAKAAAAM
ALAKAAAAN
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ALAKAAAAV
GMNERPILT
GILGFVFTM
TLNAWVKVV
KLNEPVLLL
AVVPFIVSV

Sequence weighting

- Poor or biased sampling of sequence space
- Example P1

$P_A = 2/6$
$P_G = 2/6$
$P_T = P_K = 1/6$
$P_C = P_D = \ldots P_V = 0$

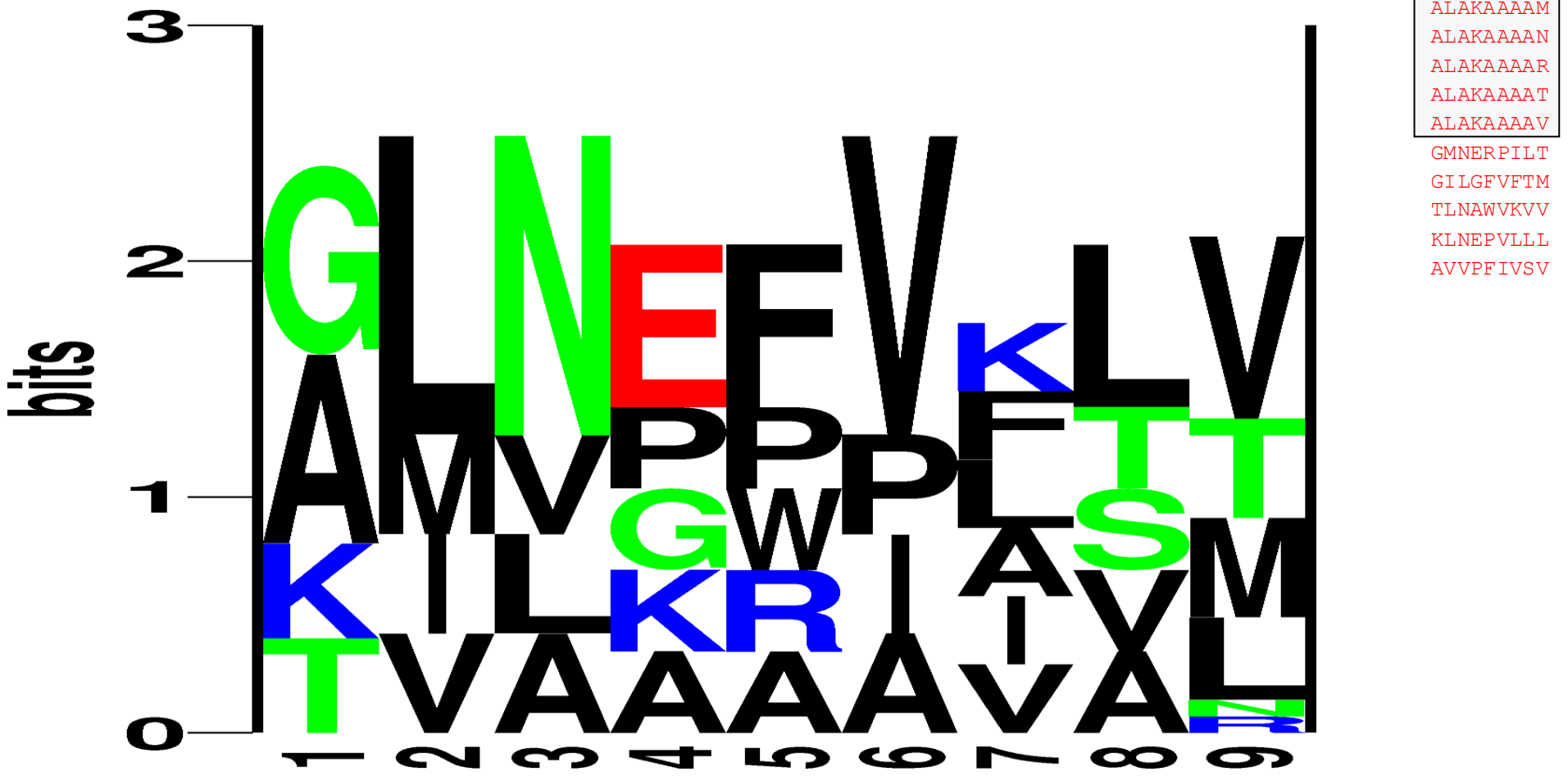
ALAKAAAAM
ALAKAAAAN
ALAKAAAAR
ALAKAAAAT
ALAKAAAAV

} Similar sequences Weight 1/5

GMNERPILT
GILGFVFTM
TLNAWVKVV
KLNEPVLLL
AVVPFIVSV

RLLDDTPEV 84 nM
GLLGNVSTV 23 nM
ALAKAAAAL 309 nM

Sequence weighting



ALAKAAAAM
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KLNEPVLLL
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Pseudo counts

- I is not found at position P9. Does this mean that I is forbidden (P(I)=0)?

- No! Use Blosum substitution matrix to estimate pseudo frequency of I at P9

The Blosum (substitution frequency) matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	0.29	0.03	0.03	0.03	0.02	0.03	0.04	0.08	0.01	0.04	0.06	0.04	0.02	0.02	0.03	0.09	0.05	0.01	0.02	0.07
R	0.04	0.34	0.04	0.03	0.01	0.05	0.05	0.03	0.02	0.02	0.05	0.12	0.02	0.02	0.02	0.04	0.03	0.01	0.02	0.03
N	0.04	0.04	0.32	0.08	0.01	0.03	0.05	0.07	0.03	0.02	0.03	0.05	0.01	0.02	0.02	0.07	0.05	0.00	0.02	0.03
D	0.04	0.03	0.07	0.40	0.01	0.03	0.09	0.05	0.02	0.02	0.03	0.04	0.01	0.01	0.02	0.05	0.04	0.00	0.01	0.02
C	0.07	0.02	0.02	0.02	0.48	0.01	0.02	0.03	0.01	0.04	0.07	0.02	0.02	0.02	0.02	0.04	0.04	0.00	0.01	0.06
Q	0.06	0.07	0.04	0.05	0.01	0.21	0.10	0.04	0.03	0.03	0.05	0.09	0.02	0.01	0.02	0.06	0.04	0.01	0.02	0.04
E	0.06	0.05	0.04	0.09	0.01	0.06	0.30	0.04	0.03	0.02	0.04	0.08	0.01	0.02	0.03	0.06	0.04	0.01	0.02	0.03
G	0.08	0.02	0.04	0.03	0.01	0.02	0.03	0.51	0.01	0.02	0.03	0.03	0.01	0.02	0.02	0.05	0.03	0.01	0.01	0.02
H	0.04	0.05	0.05	0.04	0.01	0.04	0.05	0.04	0.35	0.02	0.04	0.05	0.02	0.03	0.02	0.04	0.03	0.01	0.06	0.02
I	0.05	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.27	0.17	0.02	0.04	0.04	0.01	0.03	0.04	0.01	0.02	0.18
L	0.04	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.12	0.38	0.03	0.05	0.05	0.01	0.02	0.03	0.01	0.02	0.10
K	0.06	0.11	0.04	0.04	0.01	0.05	0.07	0.04	0.02	0.03	0.04	0.28	0.02	0.02	0.03	0.05	0.04	0.01	0.02	0.03
M	0.05	0.03	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.10	0.20	0.04	0.16	0.05	0.02	0.04	0.04	0.01	0.02	0.09
F	0.03	0.02	0.02	0.02	0.01	0.01	0.02	0.03	0.02	0.06	0.11	0.02	0.03	0.39	0.01	0.03	0.03	0.02	0.09	0.06
P	0.06	0.03	0.02	0.03	0.01	0.02	0.04	0.04	0.01	0.03	0.04	0.04	0.01	0.01	0.49	0.04	0.04	0.00	0.01	0.03
S	0.11	0.04	0.05	0.05	0.02	0.03	0.05	0.07	0.02	0.03	0.04	0.05	0.02	0.02	0.03	0.22	0.08	0.01	0.02	0.04
T	0.07	0.04	0.04	0.04	0.02	0.03	0.04	0.04	0.01	0.05	0.07	0.05	0.02	0.02	0.03	0.09	0.25	0.01	0.02	0.07
W	0.03	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.03	0.05	0.02	0.02	0.06	0.01	0.02	0.02	0.49	0.07	0.03
Y	0.04	0.03	0.02	0.02	0.01	0.02	0.03	0.02	0.05	0.04	0.07	0.03	0.02	0.13	0.02	0.03	0.03	0.03	0.32	0.05
V	0.07	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.16	0.13	0.03	0.03	0.04	0.02	0.03	0.05	0.01	0.02	0.27

Some amino acids are highly conserved (i.e. C), some have a high change of mutation (i.e. I)

Pseudo count estimation

- Calculate observed amino acids frequencies f_a
- Pseudo frequency for amino acid b
- Example $g_b = \sum_a f_a \cdot q_{b|a}$

ALAKAAAAM
ALAKAAAAN
ALAKAAAAR
ALAKAAAAT
ALAKAAAAV
GMNERPILT
GILGFVFTM
TLNAWVKVV
KLNEPVLLL
AVVPFIVSV

$$g_I = 0.2 \cdot q_{I|M} + 0.1 \cdot q_{I|R} + \ldots + 0.3 \cdot q_{I|V} + 0.1 \cdot q_{I|L}$$
$$g_I = 0.2 \cdot 0.1 + 0.1 \cdot 0.02 + \ldots + 0.3 \cdot 0.16 + 0.1 \cdot 0.12 = 0.094$$

Weight on pseudo count

- Example

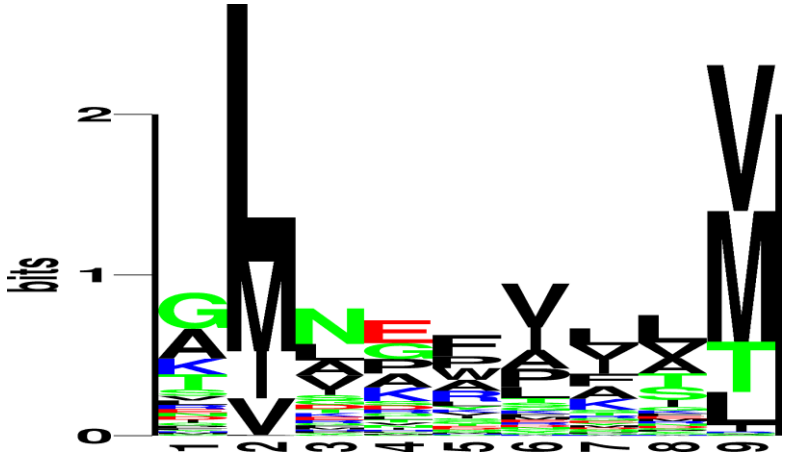
$$p_a = \frac{\alpha \cdot f_a + \beta \cdot g_a}{\alpha + \beta}$$

- If α large, p ≈ f and only the observed data defines the motif
- If α small, p ≈ g and the pseudo counts (or prior) defines the motif
- β is [50-200] normally

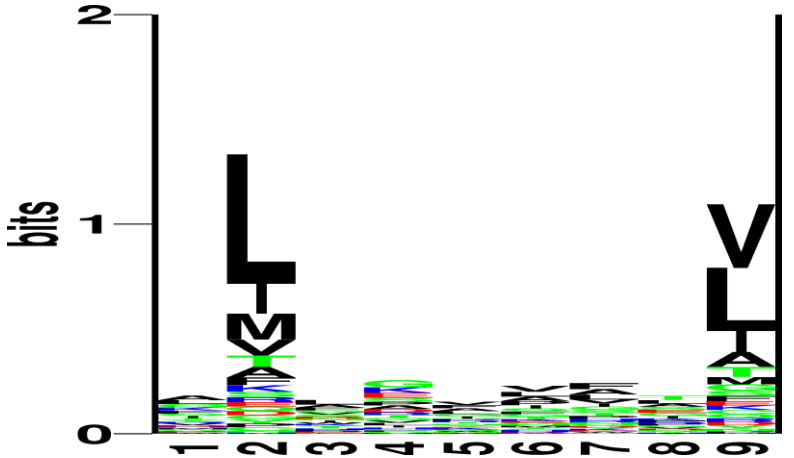
ALAKAAAAM
ALAKAAAAN
ALAKAAAAR
ALAKAAAAT
ALAKAAAAV
GMNERPILT
GILGFVFTM
TLNAWVKVV
KLNEPVLLL
AVVPFIVSV

Position specific weighting

- We know that positions 2 and 9 are anchor positions for most MHC binding motifs
  - Increase weight on high information positions



- Motif found on large data set



Weight matrices

- Estimated amino acid frequencies from alignment

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	0.08	0.06	0.02	0.03	0.02	0.02	0.03	0.08	0.02	0.08	0.11	0.06	0.04	0.06	0.02	0.09	0.04	0.01	0.04	0.08
2	0.04	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.11	0.44	0.02	0.06	0.03	0.01	0.02	0.05	0.00	0.01	0.10
3	0.08	0.04	0.05	0.07	0.02	0.03	0.03	0.08	0.02	0.05	0.11	0.03	0.03	0.06	0.04	0.06	0.05	0.03	0.05	0.07
4	0.08	0.05	0.03	0.10	0.01	0.05	0.08	0.13	0.01	0.05	0.06	0.05	0.01	0.03	0.08	0.06	0.04	0.02	0.01	0.05
5	0.06	0.04	0.05	0.03	0.01	0.04	0.05	0.11	0.03	0.04	0.09	0.04	0.02	0.06	0.06	0.04	0.05	0.02	0.05	0.08
6	0.06	0.03	0.03	0.03	0.03	0.03	0.04	0.06	0.02	0.10	0.14	0.04	0.03	0.05	0.04	0.06	0.06	0.01	0.03	0.13
7	0.10	0.02	0.04	0.04	0.02	0.03	0.04	0.05	0.04	0.08	0.12	0.02	0.03	0.06	0.07	0.06	0.05	0.03	0.03	0.08
8	0.05	0.07	0.04	0.03	0.01	0.04	0.06	0.06	0.03	0.06	0.13	0.06	0.02	0.05	0.04	0.08	0.07	0.01	0.04	0.05
9	0.08	0.02	0.01	0.01	0.02	0.02	0.03	0.02	0.01	0.10	0.23	0.03	0.02	0.04	0.01	0.04	0.04	0.00	0.02	0.25

- What do the numbers mean?
  - P2(V)>P2(M). Does this mean that V enables binding more than M.
  - In nature not all amino acids are found equally often
    - In nature V is found more often than M, so we must somehow rescale with the background
    - $q_M = 0.025$, $q_V = 0.073$
    - Finding 7% V is hence not significant, but 7% M highly significant

Weight matrices

- A weight matrix is given as

  $W_{ij} = \log(p_{ij}/q_j)$

  - where i is a position in the motif, and j an amino acid. q_j is the background frequency for amino acid j

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	0.6	0.4	-3.5	-2.4	-0.4	-1.9	-2.7	0.3	-1.1	1.0	0.3	0.0	1.4	1.2	-2.7	1.4	-1.2	-2.0	1.1	0.7
2	-1.6	-6.6	-6.5	-5.4	-2.5	-4.0	-4.7	-3.7	-6.3	1.0	5.1	-3.7	3.1	-4.2	-4.3	-4.2	-0.2	-5.9	-3.8	0.4
3	0.2	-1.3	0.1	1.5	0.0	-1.8	-3.3	0.4	0.5	-1.0	0.3	-2.5	1.2	1.0	-0.1	-0.3	-0.5	3.4	1.6	0.0
4	-0.1	-0.1	-2.0	2.0	-1.6	0.5	0.8	2.0	-3.3	0.1	-1.7	-1.0	-2.2	-1.6	1.7	-0.6	-0.2	1.3	-6.8	-0.7
5	-1.6	-0.1	0.1	-2.2	-1.2	0.4	-0.5	1.9	1.2	-2.2	-0.5	-1.3	-2.2	1.7	1.2	-2.5	-0.1	1.7	1.5	1.0
6	-0.7	-1.4	-1.0	-2.3	1.1	-1.3	-1.4	-0.2	-1.0	1.8	0.8	-1.9	0.2	1.0	-0.4	-0.6	0.4	-0.5	-0.0	2.1
7	1.1	-3.8	-0.2	-1.3	1.3	-0.3	-1.3	-1.4	2.1	0.6	0.7	-5.0	1.1	0.9	1.3	-0.5	-0.9	2.9	-0.4	0.5
8	-2.2	1.0	-0.8	-2.9	-1.4	0.4	0.1	-0.4	0.2	-0.0	1.1	-0.5	-0.5	0.7	-0.3	0.8	0.8	-0.7	1.3	-1.1
9	-0.2	-3.5	-6.1	-4.5	0.7	-0.8	-2.5	-4.0	-2.6	0.9	2.8	-3.0	-1.8	-1.4	-6.2	-1.9	-1.6	-4.9	-1.6	4.5

Scoring a sequence to a weight matrix

- Score sequences to weight matrix by looking up and adding L values from the matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	0.6	0.4	-3.5	-2.4	-0.4	-1.9	-2.7	0.3	-1.1	1.0	0.3	0.0	1.4	1.2	-2.7	1.4	-1.2	-2.0	1.1	0.7
2	-1.6	-6.6	-6.5	-5.4	-2.5	-4.0	-4.7	-3.7	-6.3	1.0	5.1	-3.7	3.1	-4.2	-4.3	-4.2	-0.2	-5.9	-3.8	0.4
3	0.2	-1.3	0.1	1.5	0.0	-1.8	-3.3	0.4	0.5	-1.0	0.3	-2.5	1.2	1.0	-0.1	-0.3	-0.5	3.4	1.6	0.0
4	-0.1	-0.1	-2.0	2.0	-1.6	0.5	0.8	2.0	-3.3	0.1	-1.7	-1.0	-2.2	-1.6	1.7	-0.6	-0.2	1.3	-6.8	-0.7
5	-1.6	-0.1	0.1	-2.2	-1.2	0.4	-0.5	1.9	1.2	-2.2	-0.5	-1.3	-2.2	1.7	1.2	-2.5	-0.1	1.7	1.5	1.0
6	-0.7	-1.4	-1.0	-2.3	1.1	-1.3	-1.4	-0.2	-1.0	1.8	0.8	-1.9	0.2	1.0	-0.4	-0.6	0.4	-0.5	-0.0	2.1
7	1.1	-3.8	-0.2	-1.3	1.3	-0.3	-1.3	-1.4	2.1	0.6	0.7	-5.0	1.1	0.9	1.3	-0.5	-0.9	2.9	-0.4	0.5
8	-2.2	1.0	-0.8	-2.9	-1.4	0.4	0.1	-0.4	0.2	-0.0	1.1	-0.5	-0.5	0.7	-0.3	0.8	0.8	-0.7	1.3	-1.1
9	-0.2	-3.5	-6.1	-4.5	0.7	-0.8	-2.5	-4.0	-2.6	0.9	2.8	-3.0	-1.8	-1.4	-6.2	-1.9	-1.6	-4.9	-1.6	4.5

RLLDDTPEV

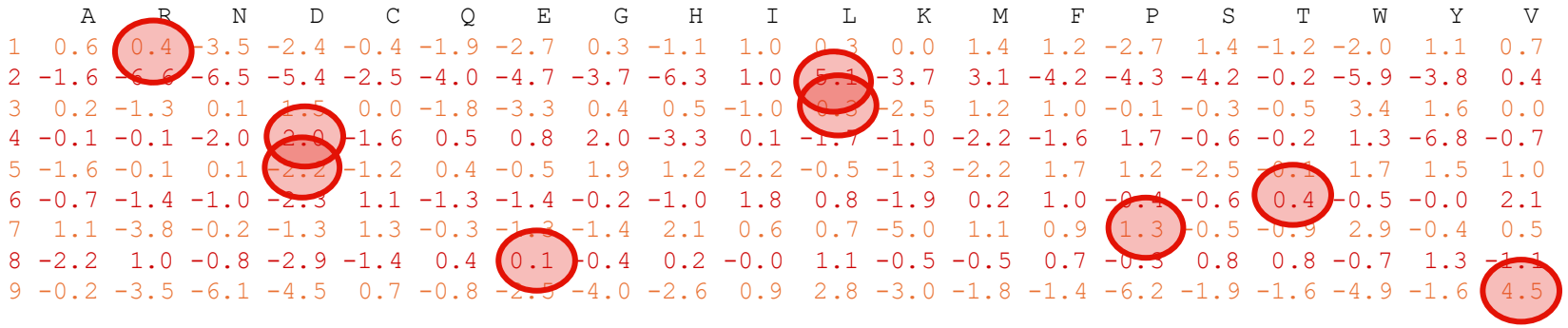
GLLGNVSTV

ALAKAAAAL

Which peptide is most likely to bind?
Which peptide second?

Scoring a sequence to a weight matrix

- Score sequences to weight matrix by looking up and adding L values from the matrix



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	0.6	0.4	-3.5	-2.4	-0.4	-1.9	-2.7	0.3	-1.1	1.0	0.3	0.0	1.4	1.2	-2.7	1.4	-1.2	-2.0	1.1	0.7
2	-1.6	-6.6	-6.5	-5.4	-2.5	-4.0	-4.7	-3.7	-6.3	1.0	5.1	-3.7	3.1	-4.2	-4.3	-4.2	-0.2	-5.9	-3.8	0.4
3	0.2	-1.3	0.1	1.5	0.0	-1.8	-3.3	0.4	0.5	-1.0	0.3	-2.5	1.2	1.0	-0.1	-0.3	-0.5	3.4	1.6	0.0
4	-0.1	-0.1	-2.0	2.0	-1.6	0.5	0.8	2.0	-3.3	0.1	-1.7	-1.0	-2.2	-1.6	1.7	-0.6	-0.2	1.3	-6.8	-0.7
5	-1.6	-0.1	0.1	-2.2	-1.2	0.4	-0.5	1.9	1.2	-2.2	-0.5	-1.3	-2.2	1.7	1.2	-2.5	-0.1	1.7	1.5	1.0
6	-0.7	-1.4	-1.0	-2.3	1.1	-1.3	-1.4	-0.2	-1.0	1.8	0.8	-1.9	0.2	1.0	-0.4	-0.6	0.4	-0.5	-0.0	2.1
7	1.1	-3.8	-0.2	-1.3	1.3	-0.3	-1.3	-1.4	2.1	0.6	0.7	-5.0	1.1	0.9	1.3	-0.5	-0.9	2.9	-0.4	0.5
8	-2.2	1.0	-0.8	-2.9	-1.4	0.4	0.1	-0.4	0.2	-0.0	1.1	-0.5	-0.5	0.7	-0.3	0.8	0.8	-0.7	1.3	-1.1
9	-0.2	-3.5	-6.1	-4.5	0.7	-0.8	-2.5	-4.0	-2.6	0.9	2.8	-3.0	-1.8	-1.4	-6.2	-1.9	-1.6	-4.9	-1.6	4.5

RLLDDTPEV	11.9	84nM
GLLGNVSTV	14.7	23nM
ALAKAAAAL	4.3	309nM

Which peptide is most likely to bind?
Which peptide second?

Neural networks and MHC binding predictions

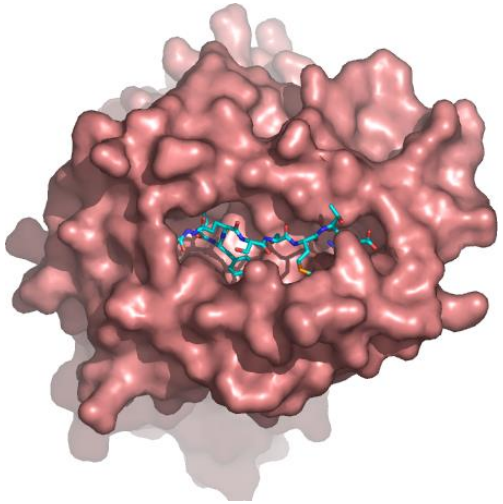
Learning Objectives

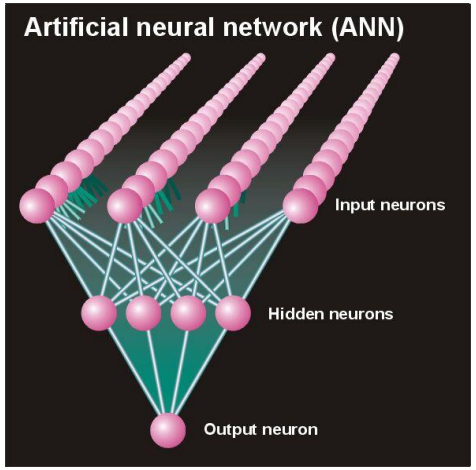
Upon completion of this module the student should be able to:

- Summarize the limitations of linear functions such as weight matrices
- Identify and interpret the components of a neural network and calculate its weights
- Discuss the challenges of model fitting: overfitting
- Discuss the solutions to overfitting: test-set model, cross-validation

Is there anything beyond weight matrices

- The effect on the binding affinity of having a given amino acid at one position can be influenced by the amino acids at other positions in the peptide (sequence correlations).
    - Two adjacent amino acids may for example compete for the space in a pocket in the MHC molecule.
- Artificial neural networks (ANN) are ideally suited to take such correlations into account





HUMAN

Higher order sequence correlations

Neural networks can learn higher order correlations!

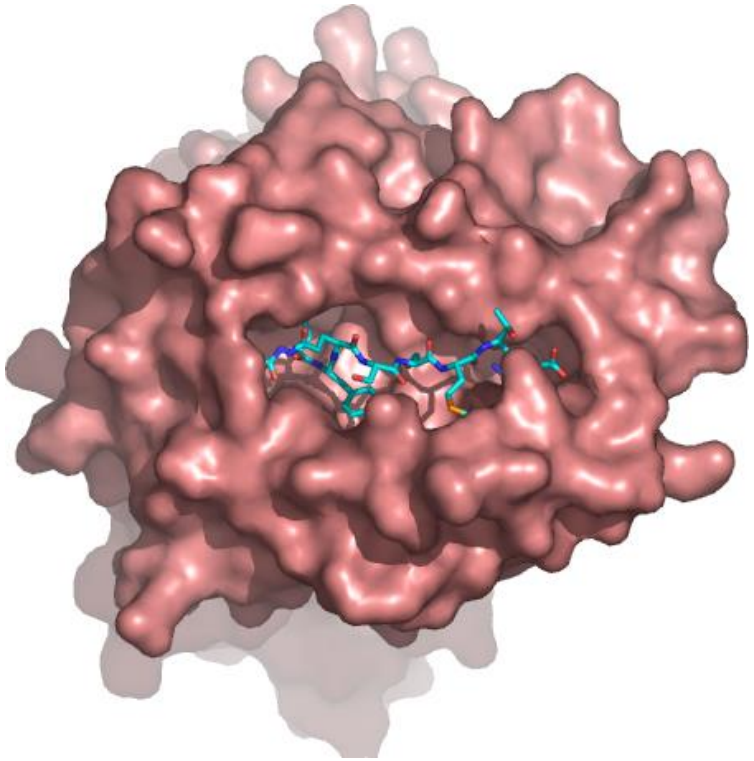
- What does this mean?

Say that the peptide needs one and only one large amino acid in the positions P3 and P4 to fill the binding cleft

How would you formulate this to test if a peptide can bind?

S S => 0
L S => 1
S L => 1
L L => 0

No linear function can learn this (XOR) pattern



Linear functions (like PSSM's) cannot learn higher order signals

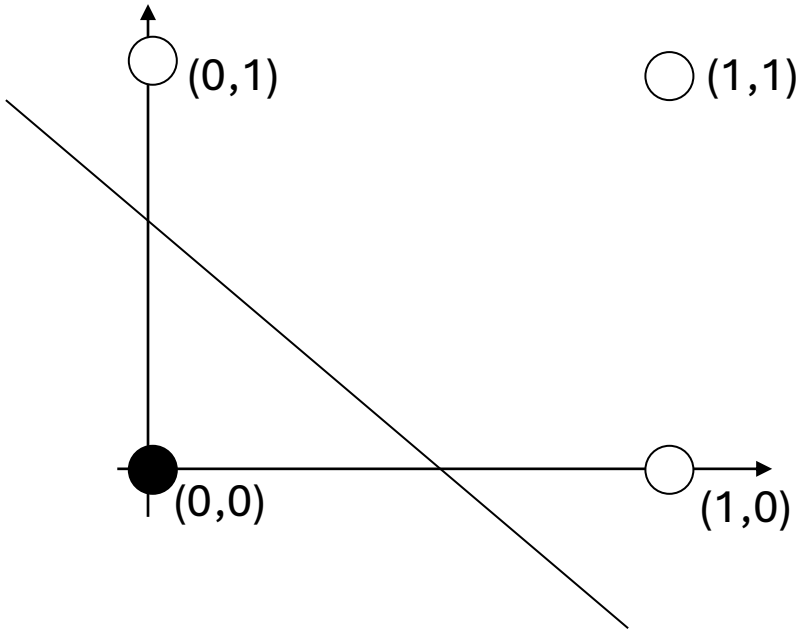
XOR function:

0 0 => 0

1 0 => 1

0 1 => 1

1 1 => 0



Linear functions (like PSSM's) cannot learn higher order signals

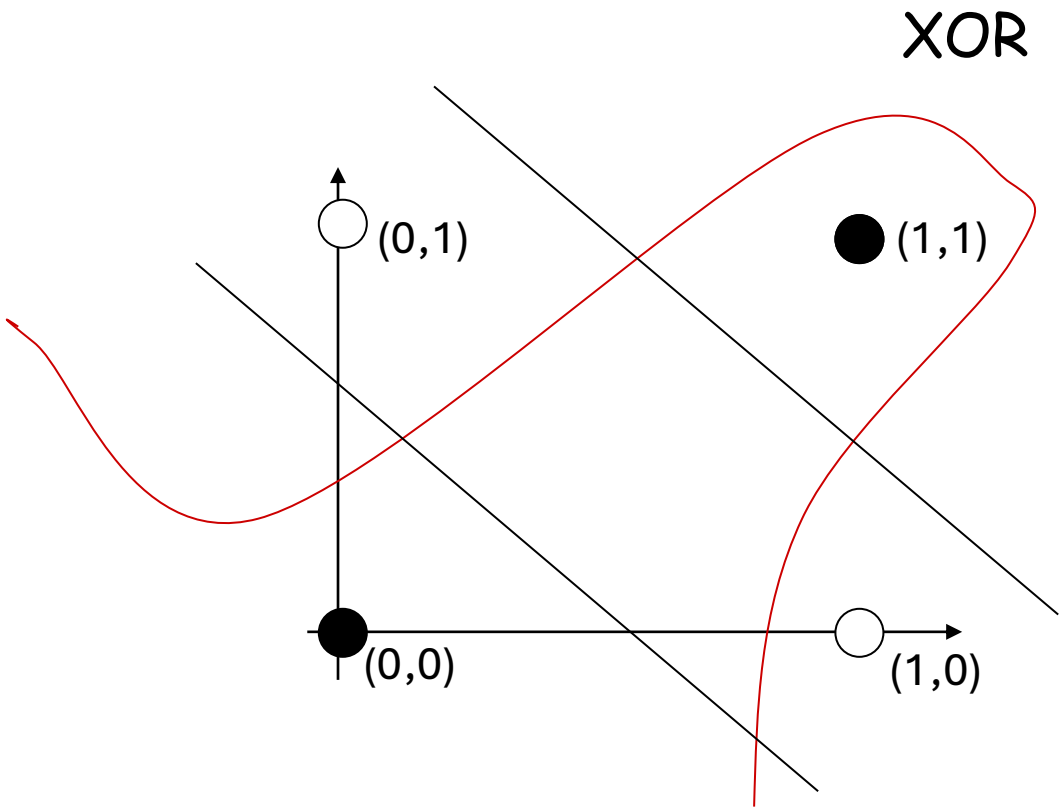
XOR function:

0 0 => 0

1 0 => 1

0 1 => 1

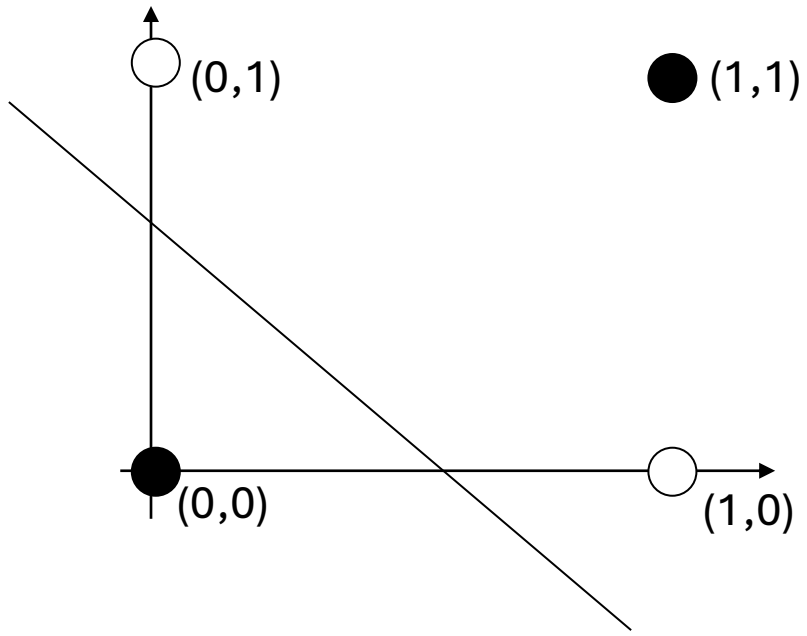
1 1 => 0



No linear function can separate the points

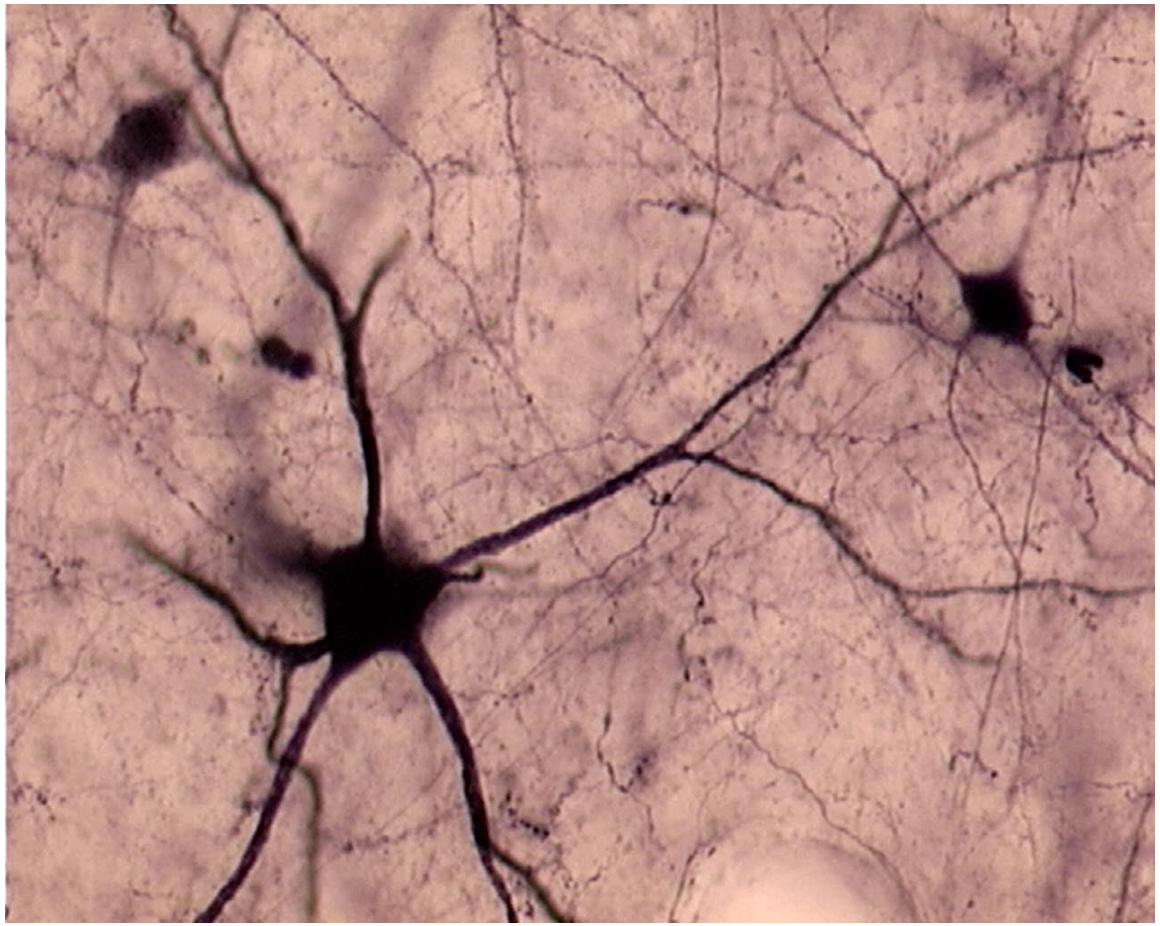
Error estimates

XOR	Predict	Error
0 0 => 0	0	0
1 0 => 1	1	0
0 1 => 1	1	0
1 1 => 0	1	1

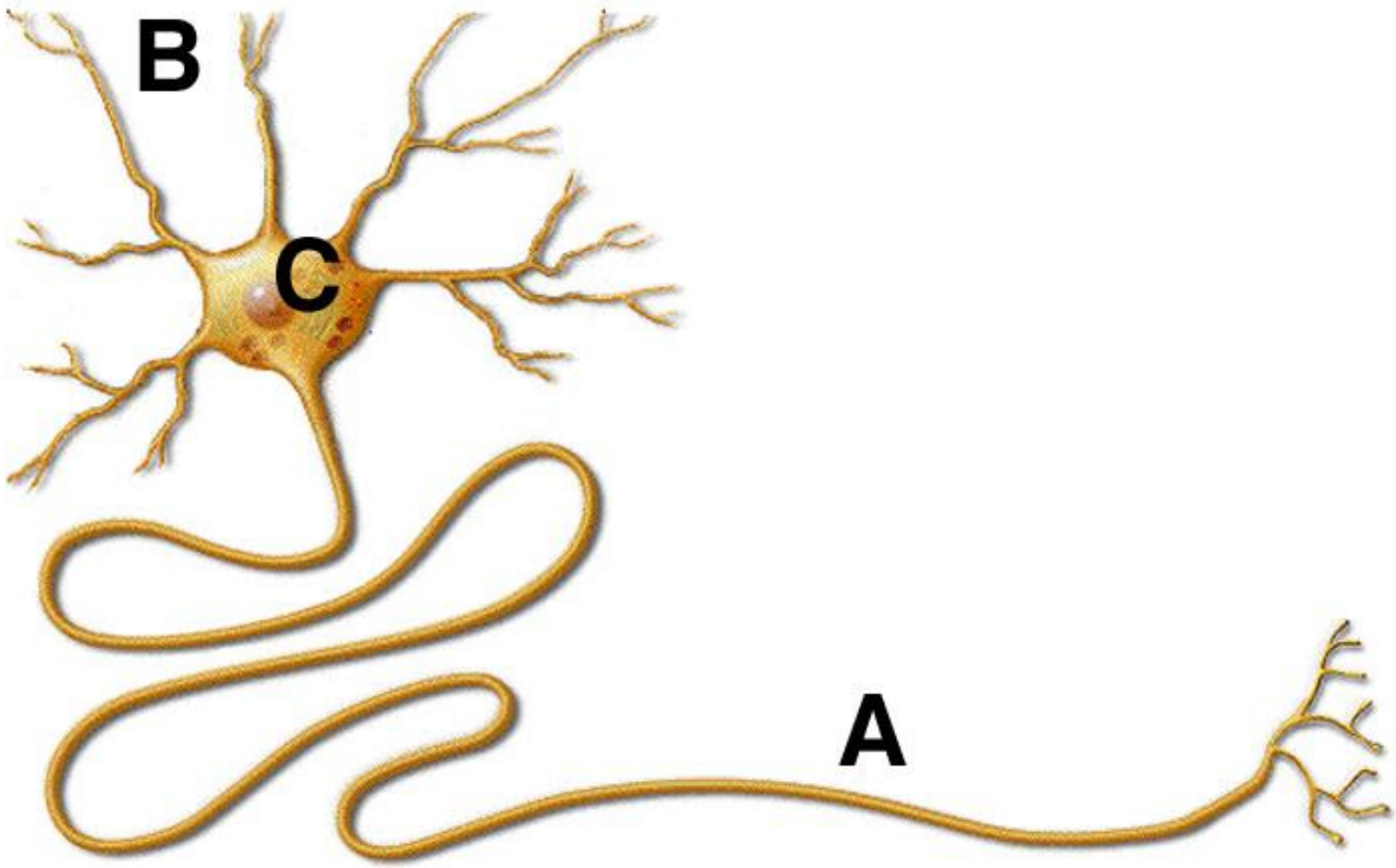


Mean error: 1/4

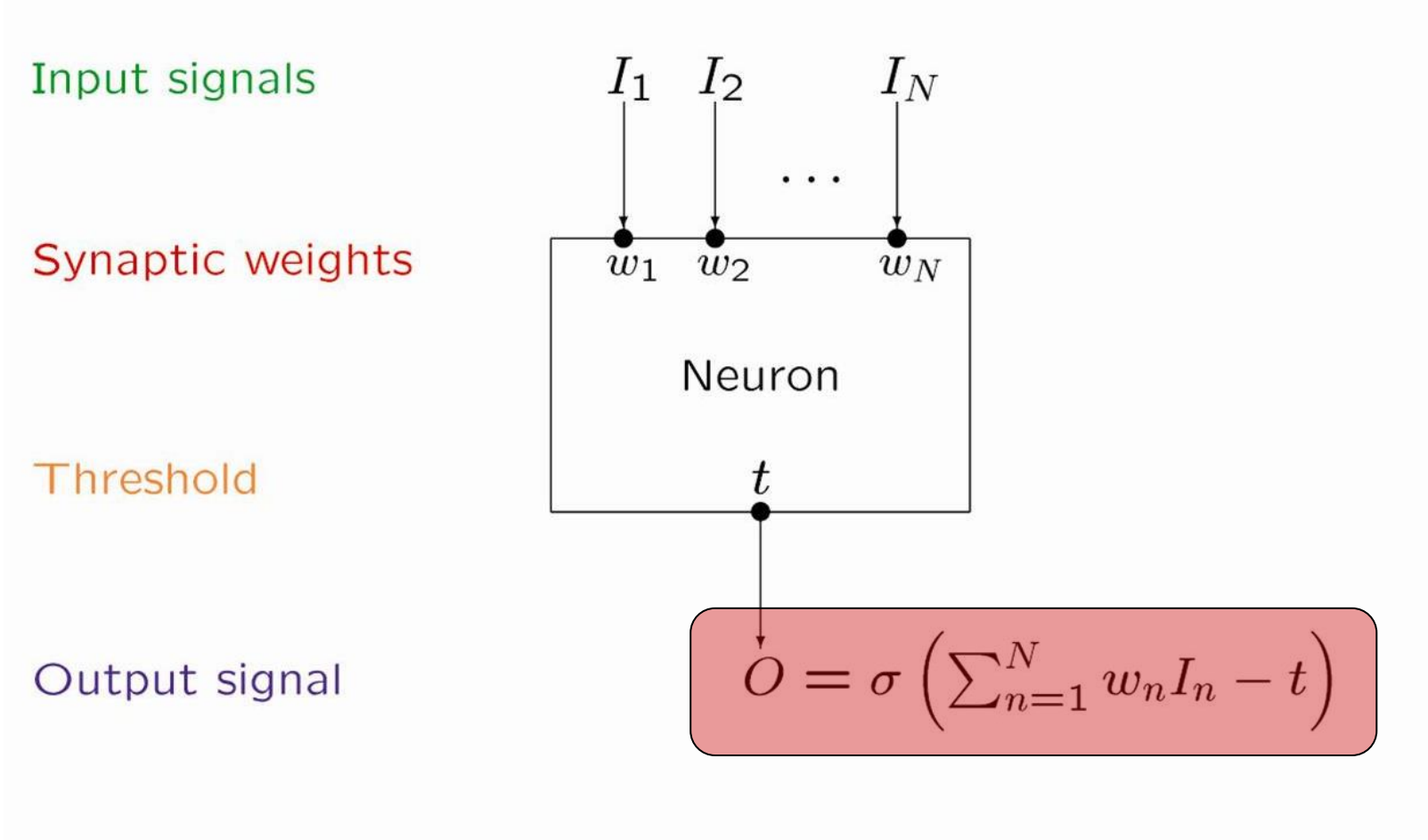
Biological neural network



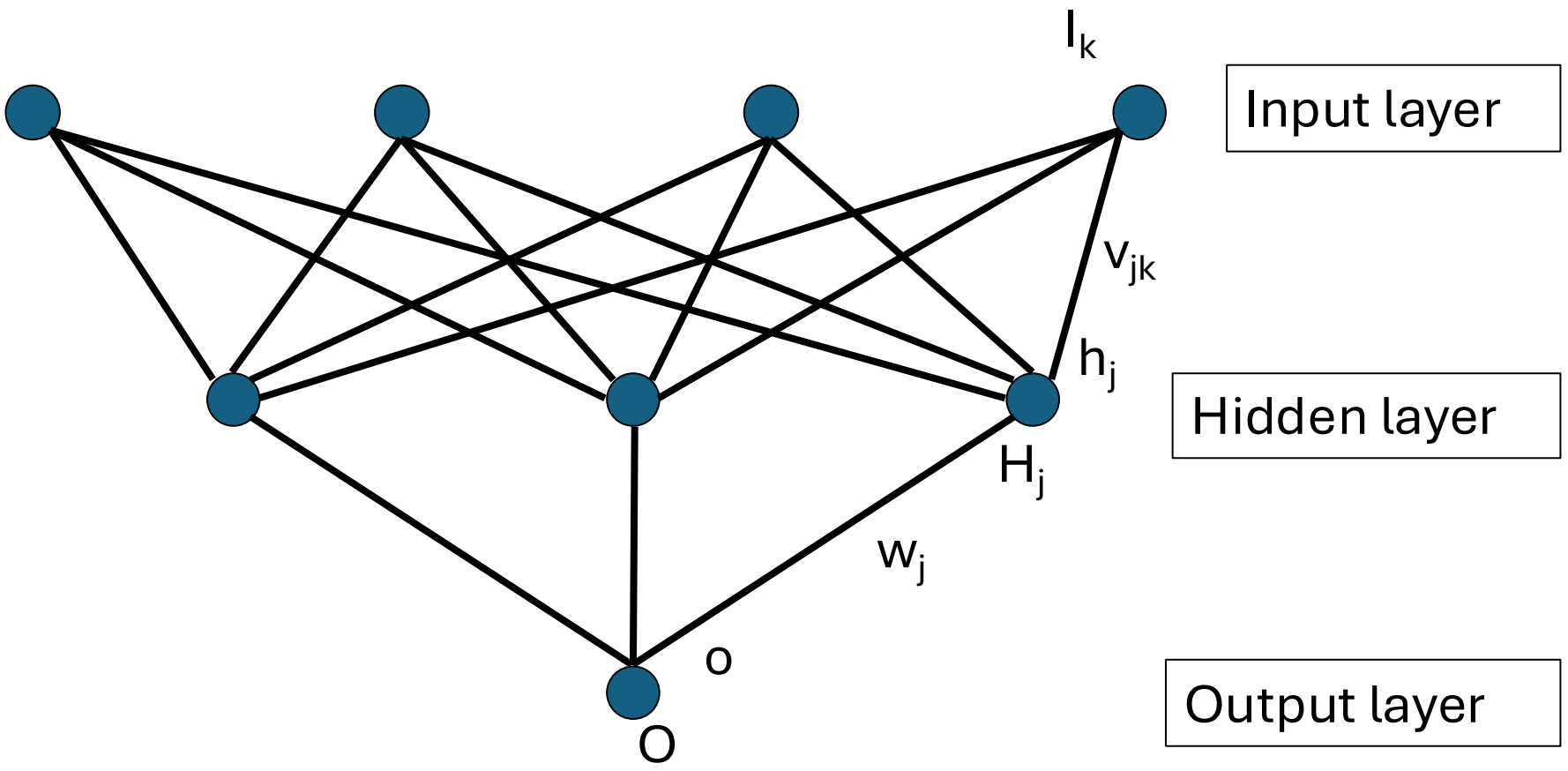
Biological neuron structure



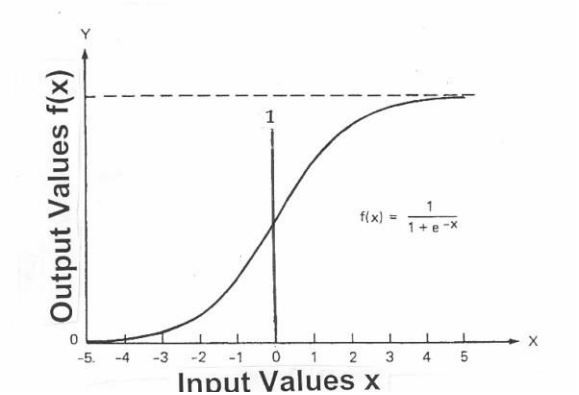
Artificial neural networks



Artificial neural network architecture

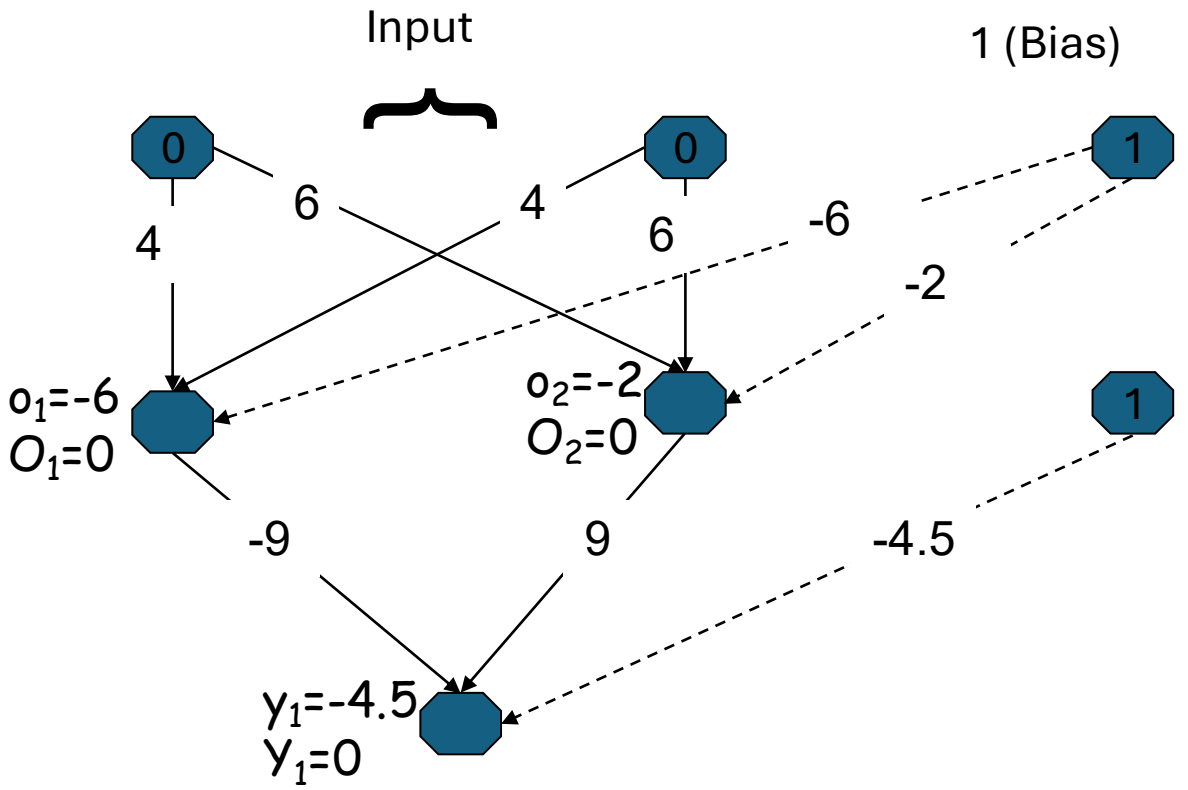


Neural networks: How does it work?



$$O = \frac{1}{1 + \exp(-o)}$$

$$o = \sum_i x_i w_i$$

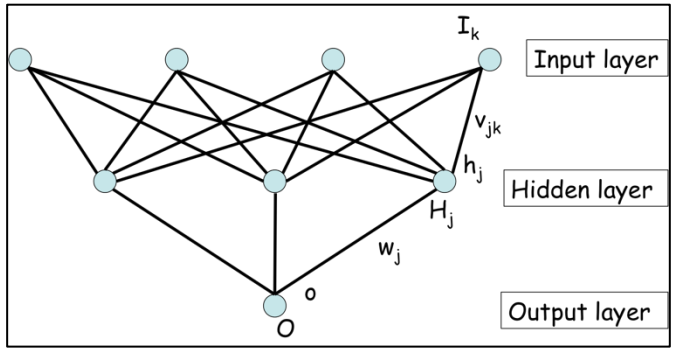


Now, do it yourself!

Data interpretation (fitting mathematical models)

```
AADFPGIAR 0.085
AAVDLSHFL 0.169
FTFDLTALK 0.085
WVWDTWPLA 0.085
TMMRHRREL 0.085
LLPYPIAGC 0.085
LMFSTSAYL 0.735
KLNENIIRF 0.536
MRVLHLDLK 0.085
GLICGLRQL 0.196
FEFILRYGD 0.085
EFVSANLAM 0.085
RAAHRRQSV 0.085
SPLHVFVAV 0.085
RTFGKLPYR 0.085
GSLFTEQAF 0.197
SYGNANVSF 0.349
CSEVPQSGY 0.085
GSFDRDLLY 0.085
```

Artificial neural networks,
Support vector machines,
Similarity kernel,
Regression, ..

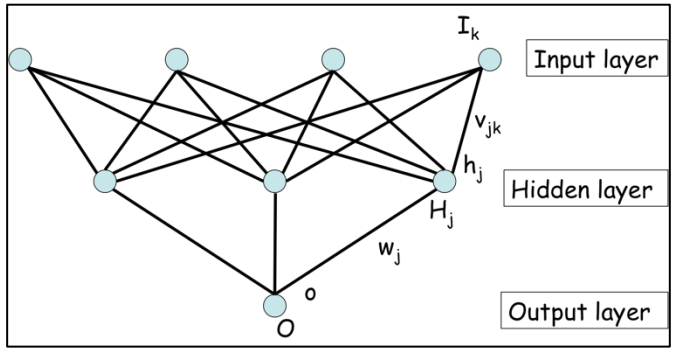


Machine learning

Data interpretation (fitting mathematical models)

```
AADFPGIAR 0.085
AAVDLSHFL 0.169
FTFDLTALK 0.085
WVWDTWPLA 0.085
TMMRHRREL 0.085
LLPYPIAGC 0.085
LMFSTSAYL 0.735
KLNENIIRF 0.536
MRVLHLDLK 0.085
GLICGLRQL 0.196
FEFILRYGD 0.085
EFVSANLAM 0.085
RAAHRRQSV 0.085
SPLHVFVAV 0.085
RTFGKLPYR 0.085
GSLFTEQAF 0.197
SYGNANVSF 0.349
CSEVPQSGY 0.085
GSFDRDLLY 0.085
```

Artificial neural networks,
Support vector machines,
Similarity kernel,
Regression, ..



Machine learning

```
0.036
0.227
0.131
0.147
0.338
0.082
0.713
0.467
0.044
0.239
0.032
0.162
0.126
0.050
0.087
0.392
0.181
0.169
0.187
```

Training an ANN: Identify weights to get lowest error

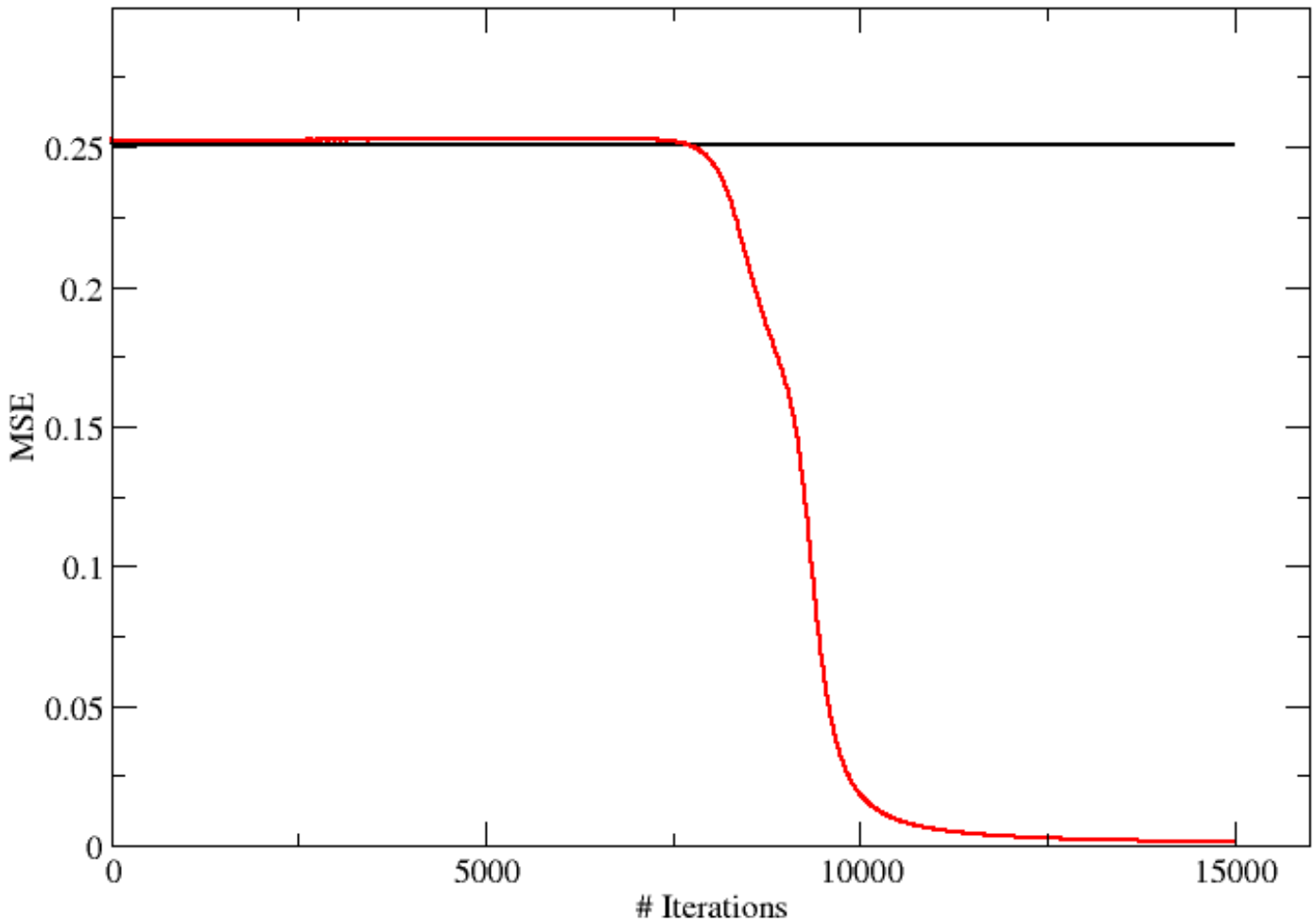
```
AAAKTPVIV 0.033693
AADFPGIAR 0.084687
ALVARAAVL 0.139013
FILIFNIIV 0.891622
IMDQVPFSV 0.727865
DEFLKVPEW 0.084687
DEWECTRDD 0.084687
LLFLGVVFL 0.638438
LVFIKPPLI 0.630086
KVDDTFYYV 0.669121
FVDFVIHGL 0.864383
TMDPSVRVL 0.654552
YGPDVEVNV 0.084687
MTAEDMLTV 0.755627
MMVILPDKI 0.530313
APTGDLPRA 0.080705
SLTECPTFL 1.000000
```

$$w_i^{'} = w_i + \Delta w_i$$

$$\Delta w_i = -\varepsilon \cdot \frac{\partial E}{\partial w_i}$$

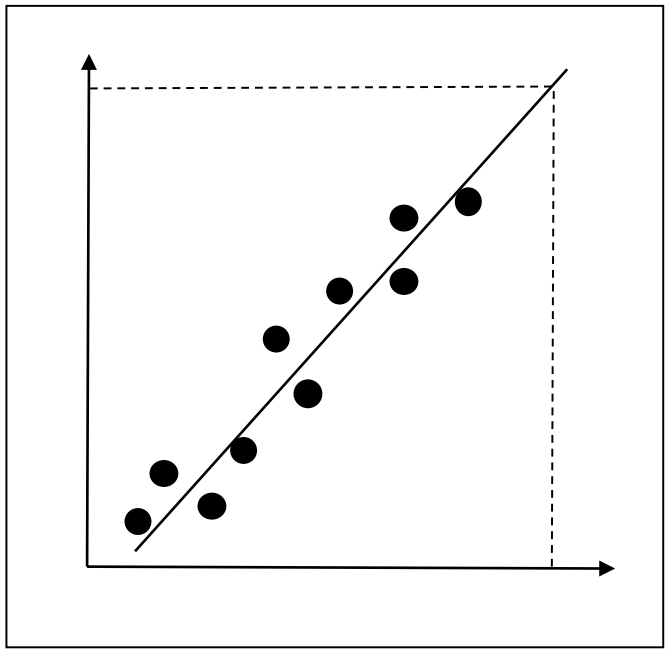
$$E = \frac{1}{2} \cdot (O - t)^2$$

Neural networks and the XOR function



Demo: https://playground.tensorflow.org/

How to train a method?
A simple statistical method: Linear regression



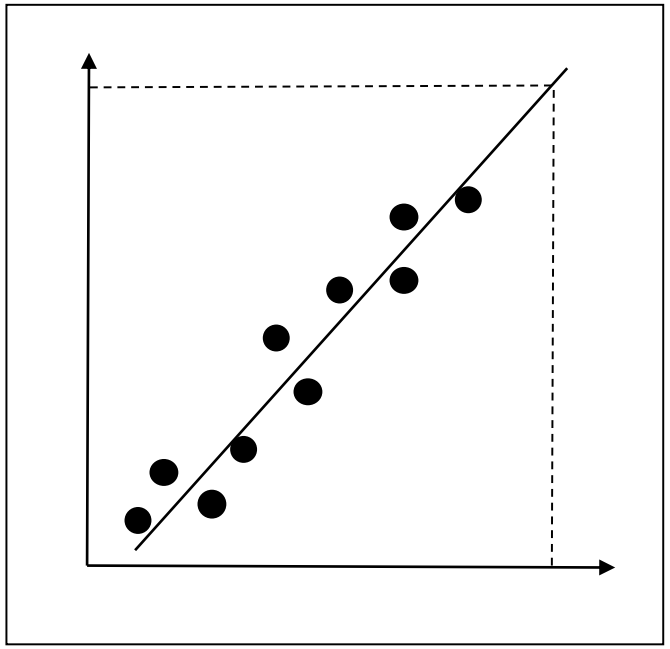
Observations (*training data*): a set of *x* values (*input*) and *y* values (*output*).

Model: $y = ax + b$ (2 *parameters*, which are estimated from the training data)

Prediction: Use the model to calculate a *y* value for a *new x* value

Note: the model does not fit the observations exactly. Can we do better than this?

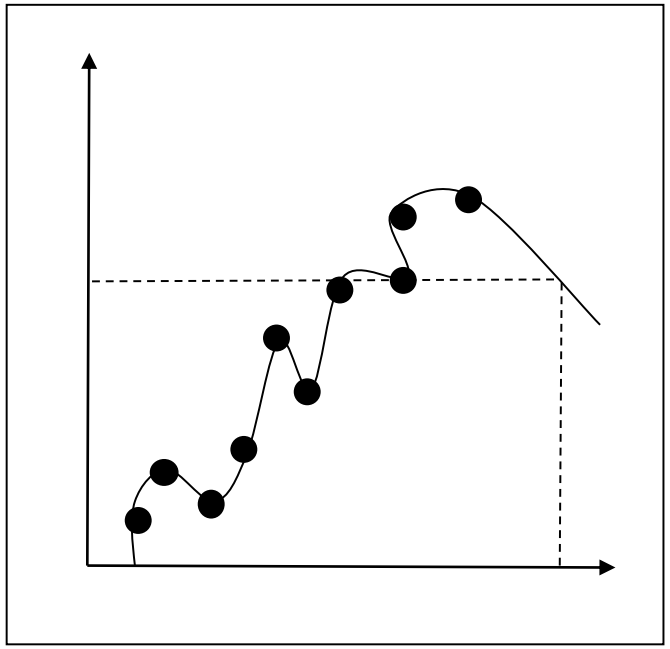
Overfitting



$y = ax + b$

2 parameter model

Good description, poor fit



$y = ax^6+bx^5+cx^4+dx^3+ex^2+fx+g$

7 parameter model

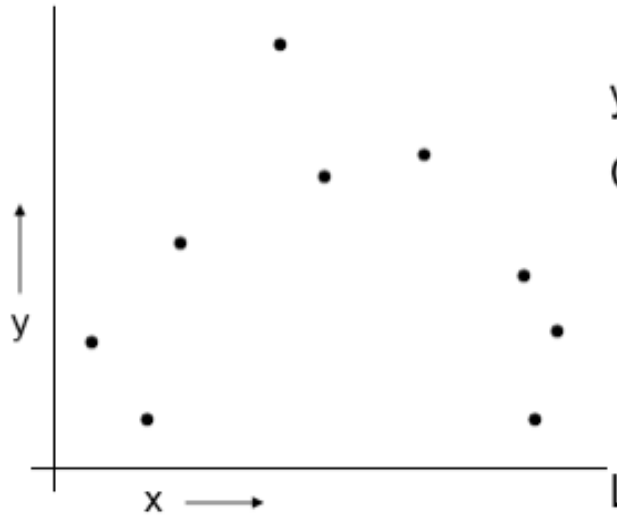
Poor description, good fit

Note: It is *not interesting* that a model can fit its observations (training data) exactly.

To function as a prediction method, a model must be able to *generalize*, i.e. produce sensible output on *new* data.

How to estimate parameters for prediction?

A Regression Problem



y = f(x) + noise

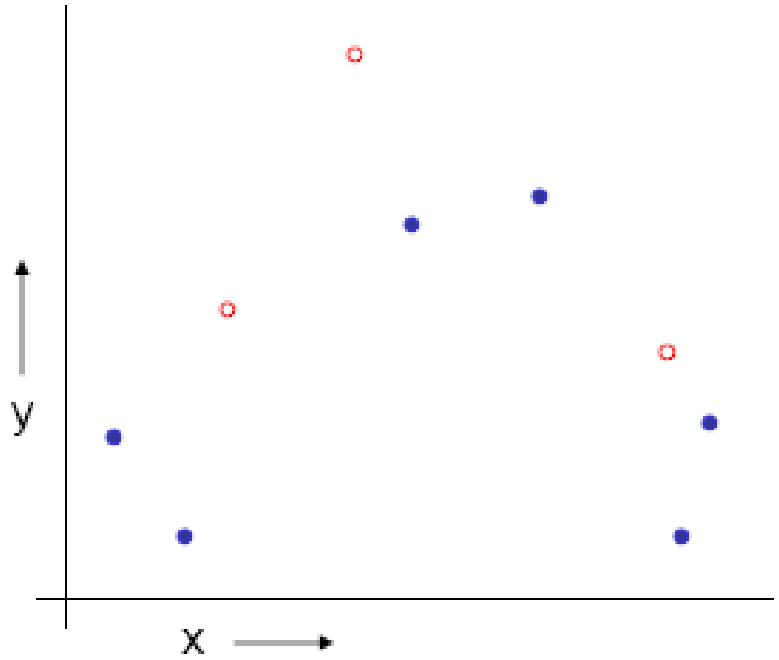
Can we learn f from this data?

Let's consider three methods…

Model selection

Linear Regression Quadratic Regression Join-the-dots

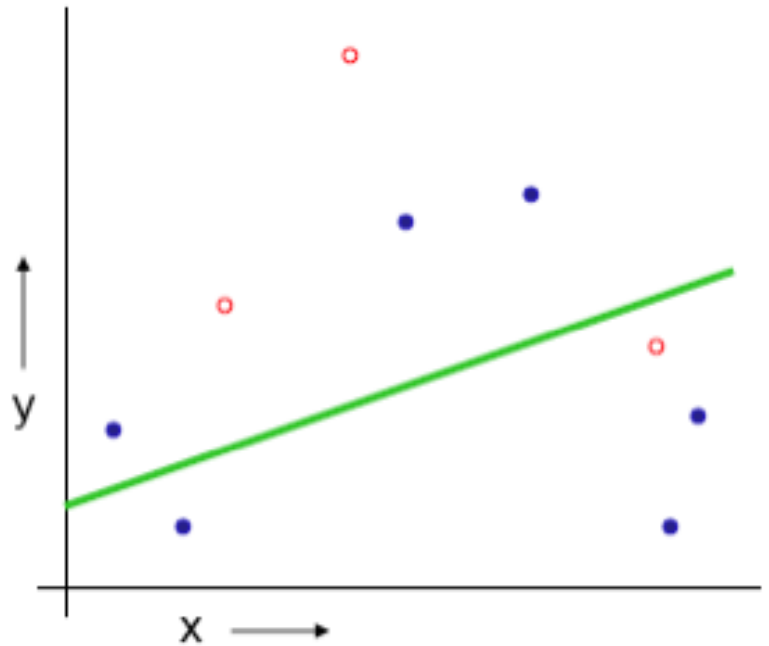
The test set method



1. Randomly choose 30% of the data to be in a test set

2. The remainder is a training set

The test set method



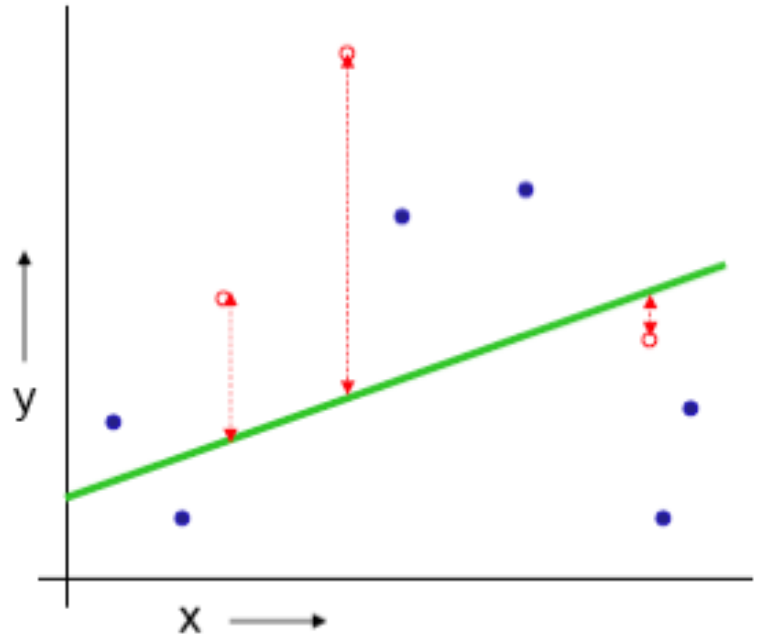
(Linear regression example)

1. Randomly choose 30% of the data to be in a test set

2. The remainder is a training set

3. Perform your regression on the training set

The test set method



(Linear regression example)

Mean Squared Error = 2.4

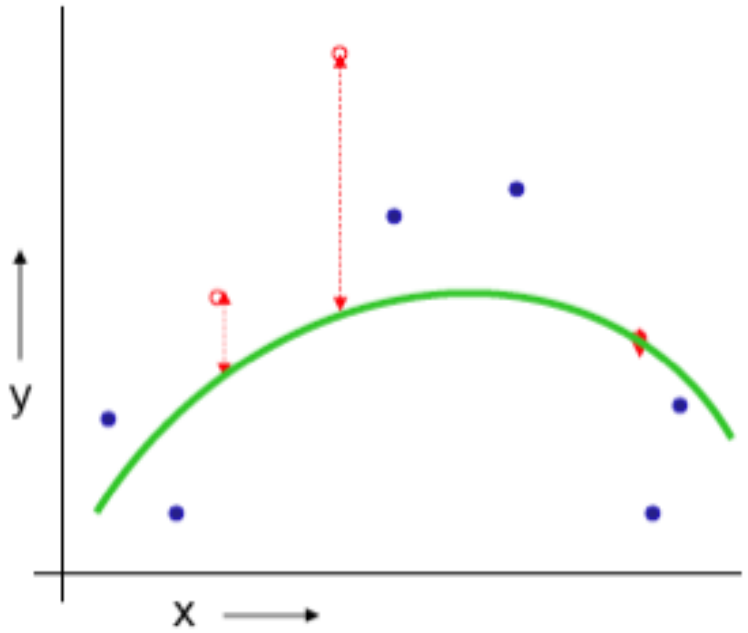
1. Randomly choose 30% of the data to be in a test set

2. The remainder is a training set

3. Perform your regression on the training set

4. Estimate your future performance with the test set

The test set method



(Quadratic regression example)

Mean Squared Error = 0.9

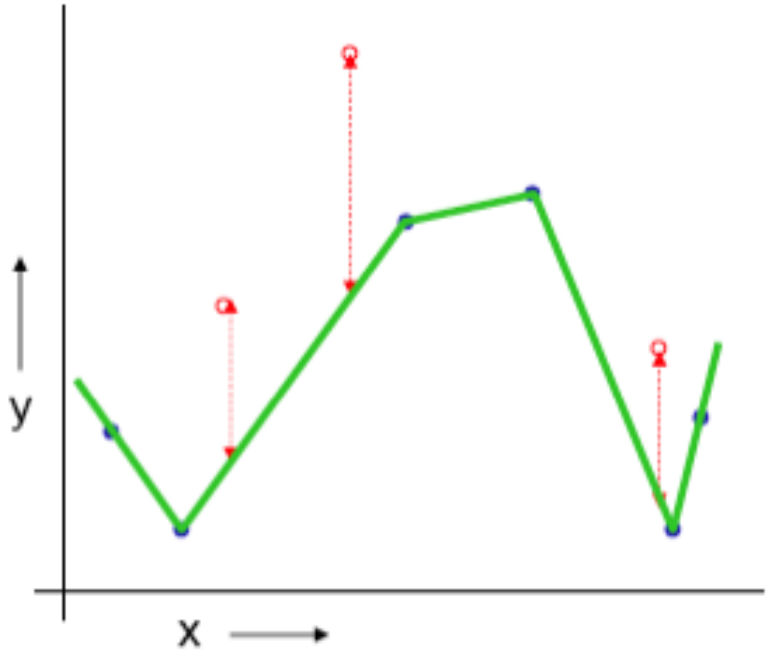
1. Randomly choose 30% of the data to be in a test set

2. The remainder is a training set

3. Perform your regression on the training set

4. Estimate your future performance with the test set

The test set method



(Join the dots example)

Mean Squared Error = 2.2

1. Randomly choose 30% of the data to be in a test set

2. The remainder is a training set

3. Perform your regression on the training set

4. Estimate your future performance with the test set

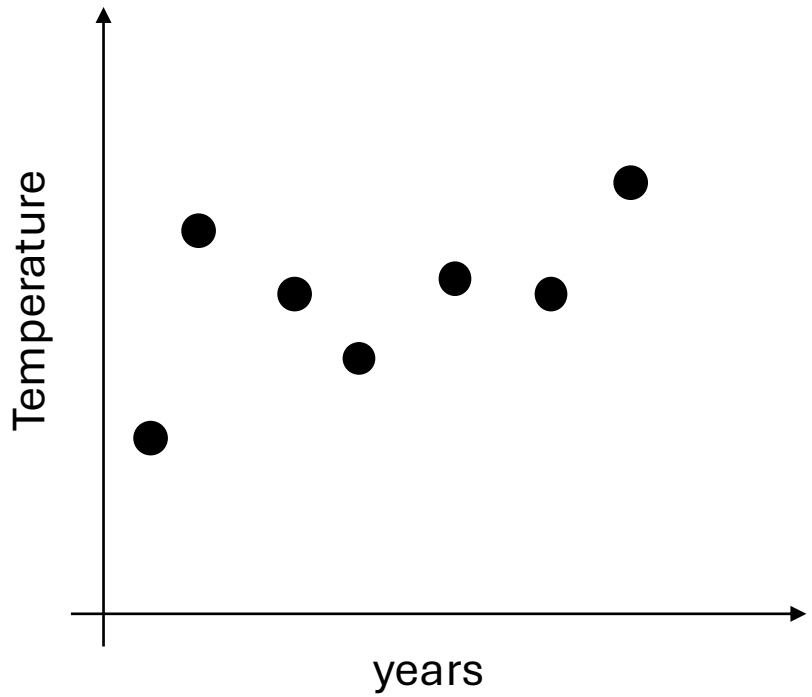
So quadratic function is best

Neural network training

- A Network contains a very large set of parameters
    - A network with 5 hidden neurons predicting binding for 9meric peptides has more than 9x20x5=900 weights

- Overfitting is a problem

- Stop training when test performance is optimal



Neural network training

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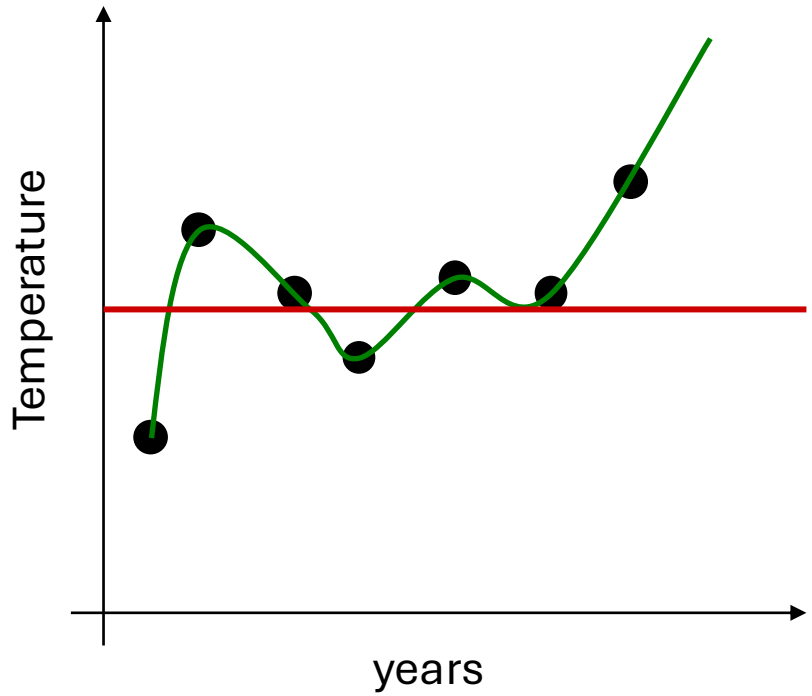
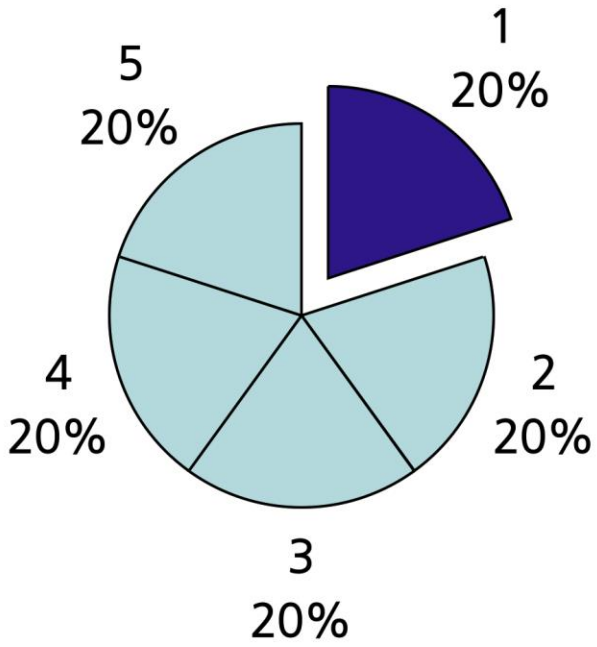
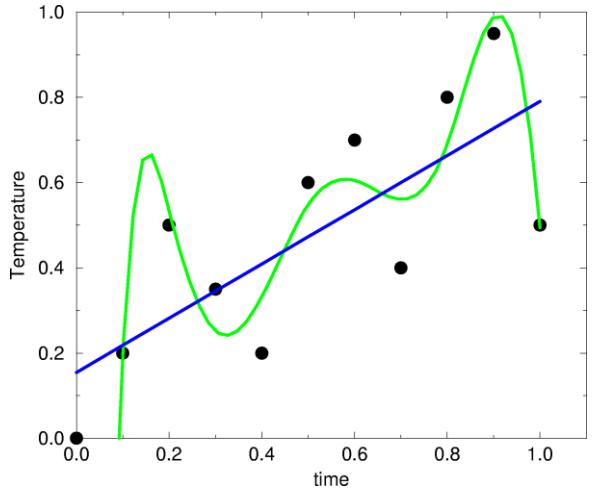


Neural network training. Cross validation

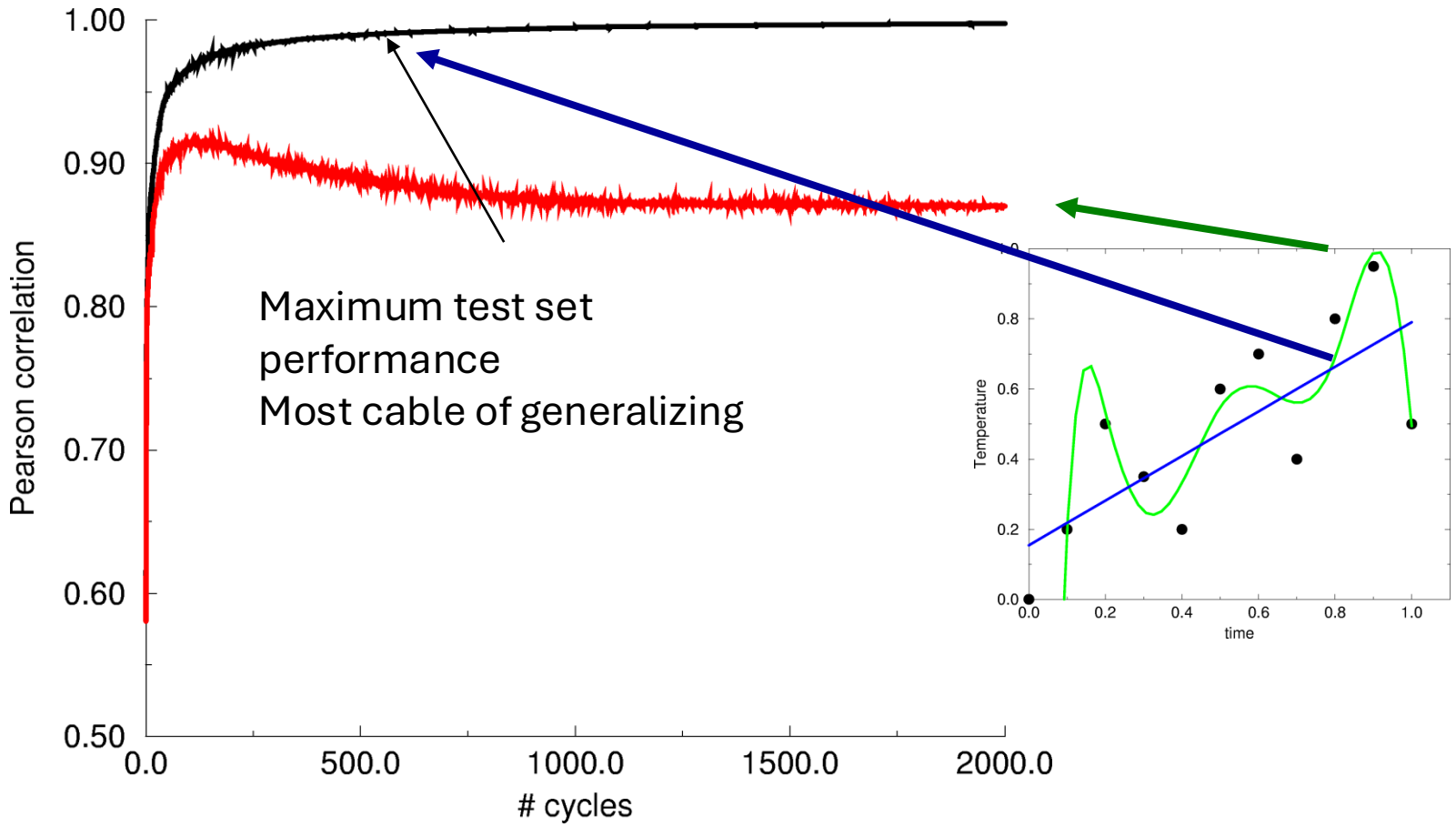
Cross validation

Train on 4/5 of data
Test on 1/5
=>
Produce 5 different neural networks each with a different prediction focus

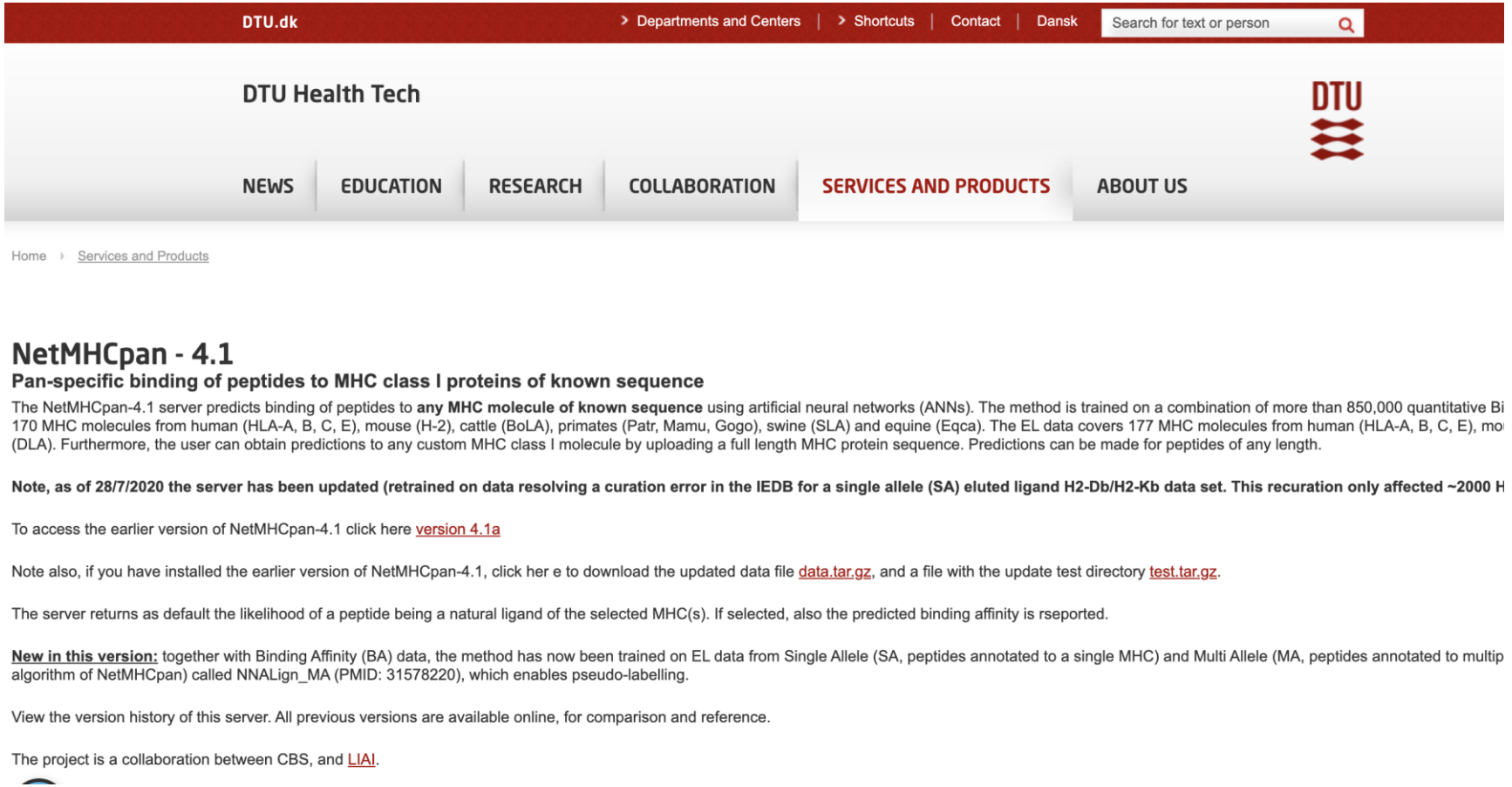




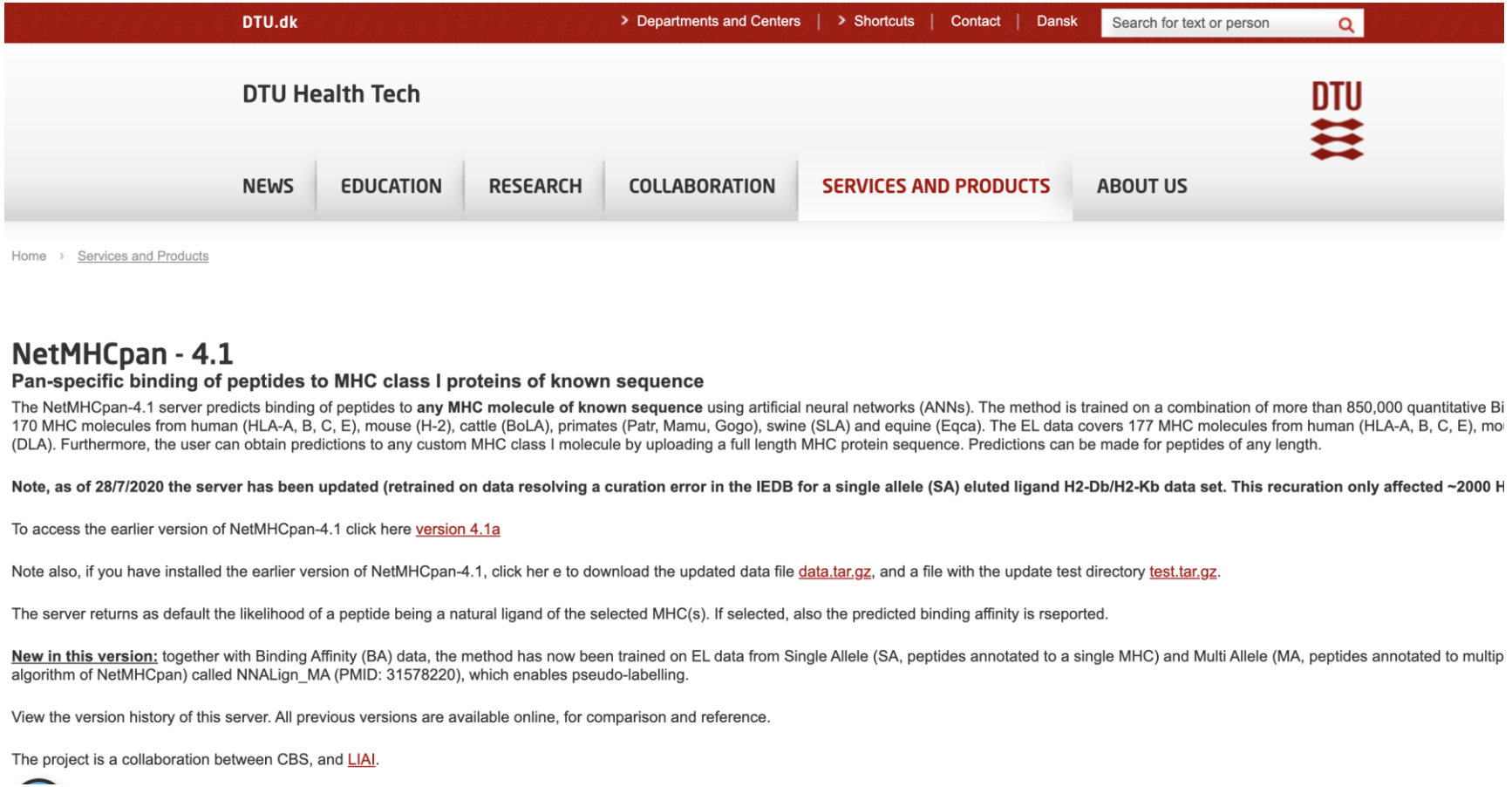
Neural network training curve



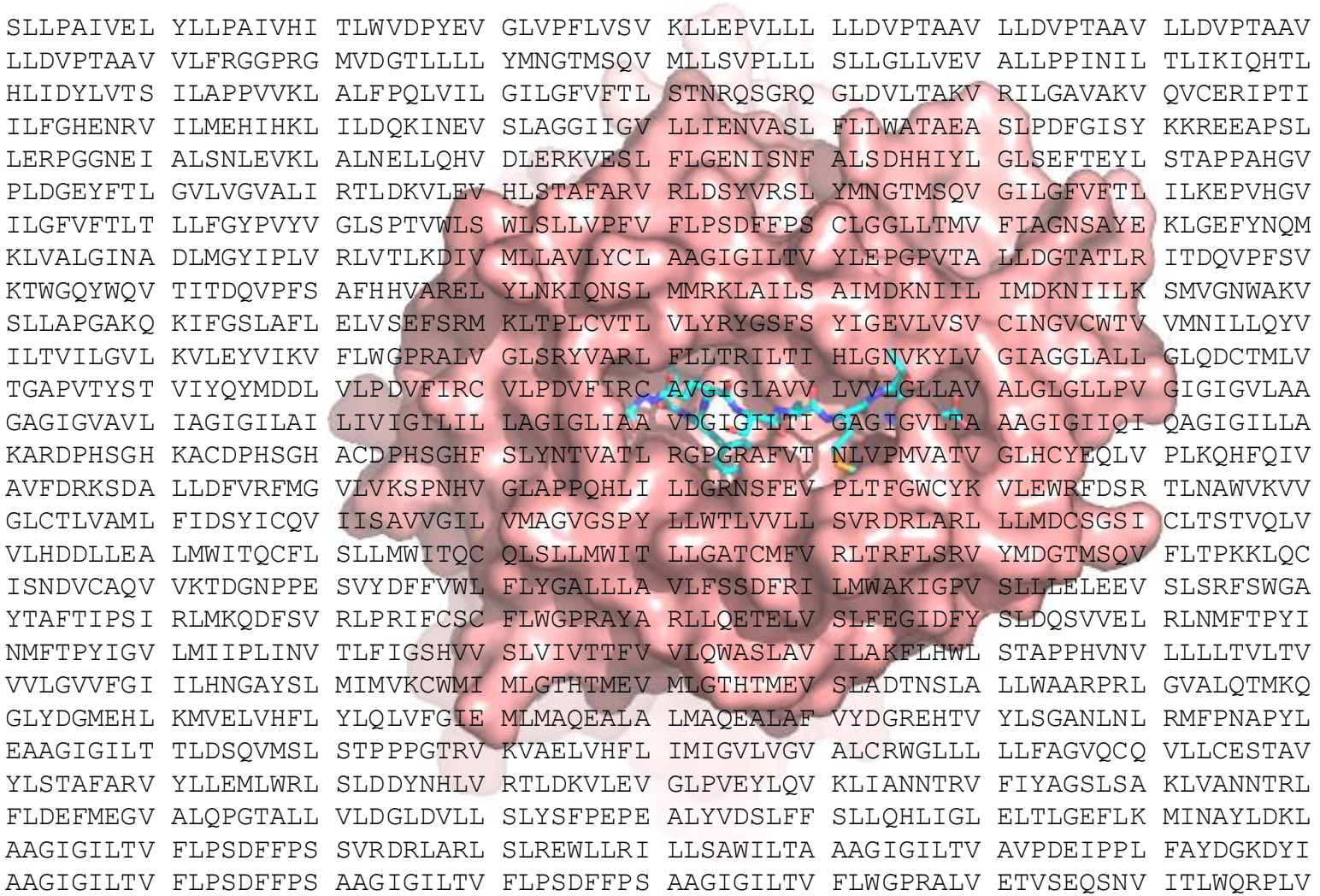
NetMHC services.healthtech.dtu.dk/service.php?NetMHCpan-4.1



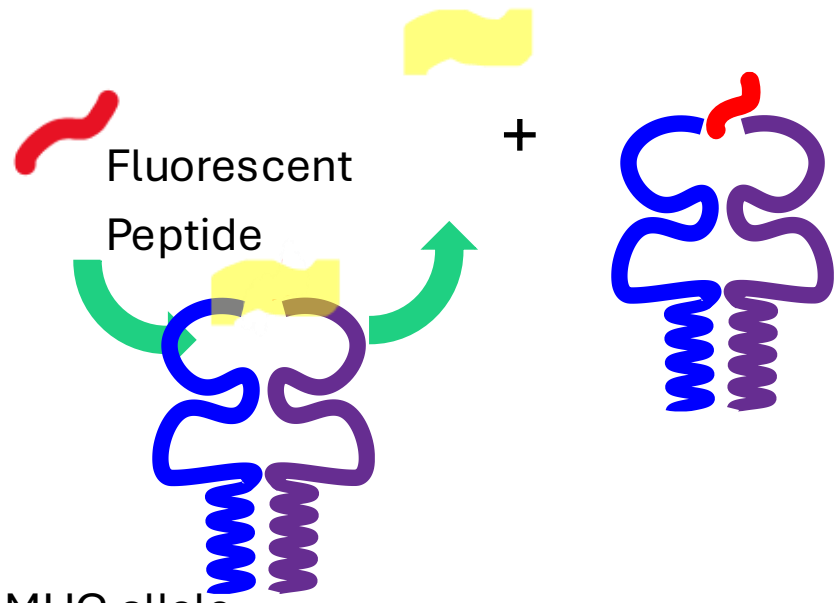
NetMHC services.healthtech.dtu.dk/service.php?NetMHCpan-4.1

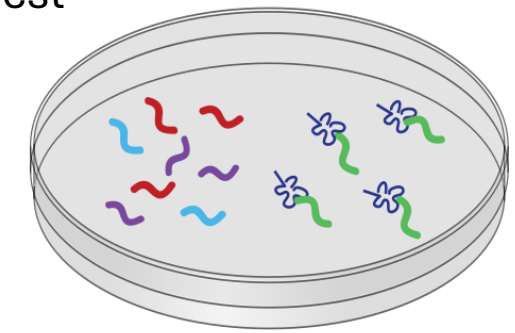


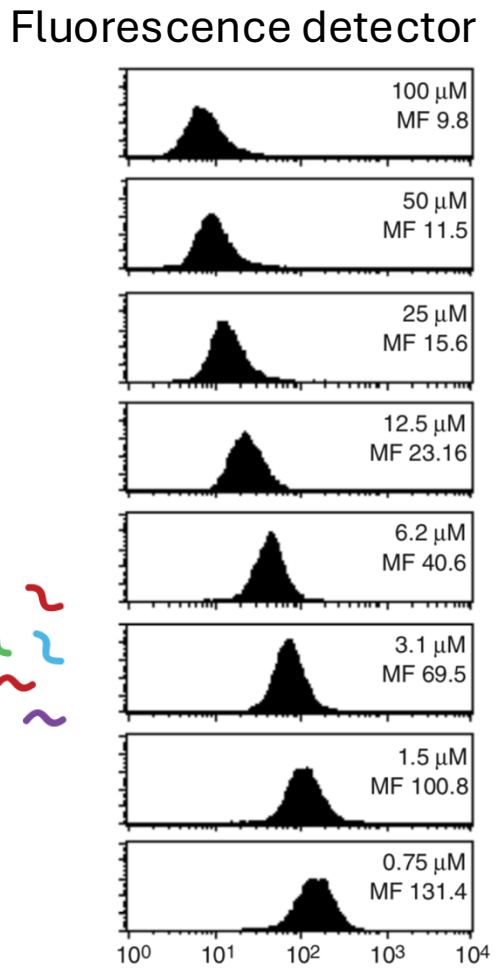
Where is the data coming from?



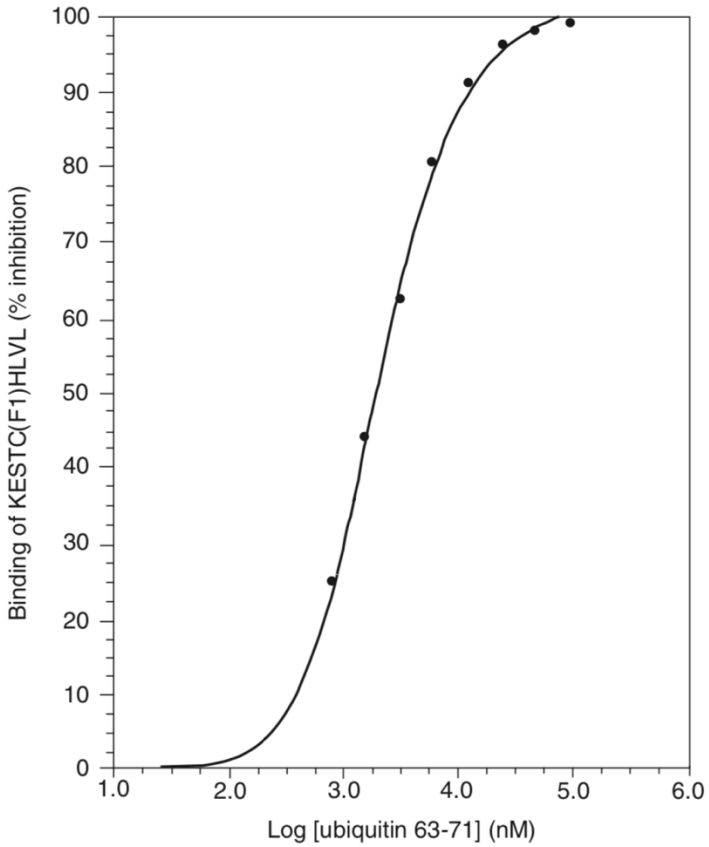
Binding affinity assay





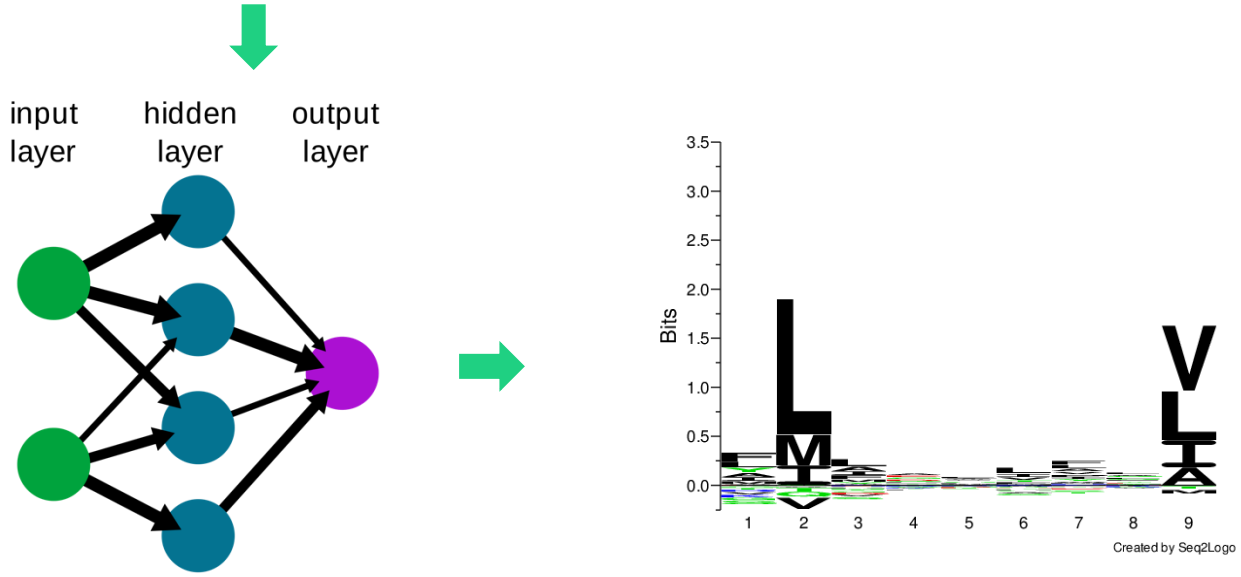


IC50



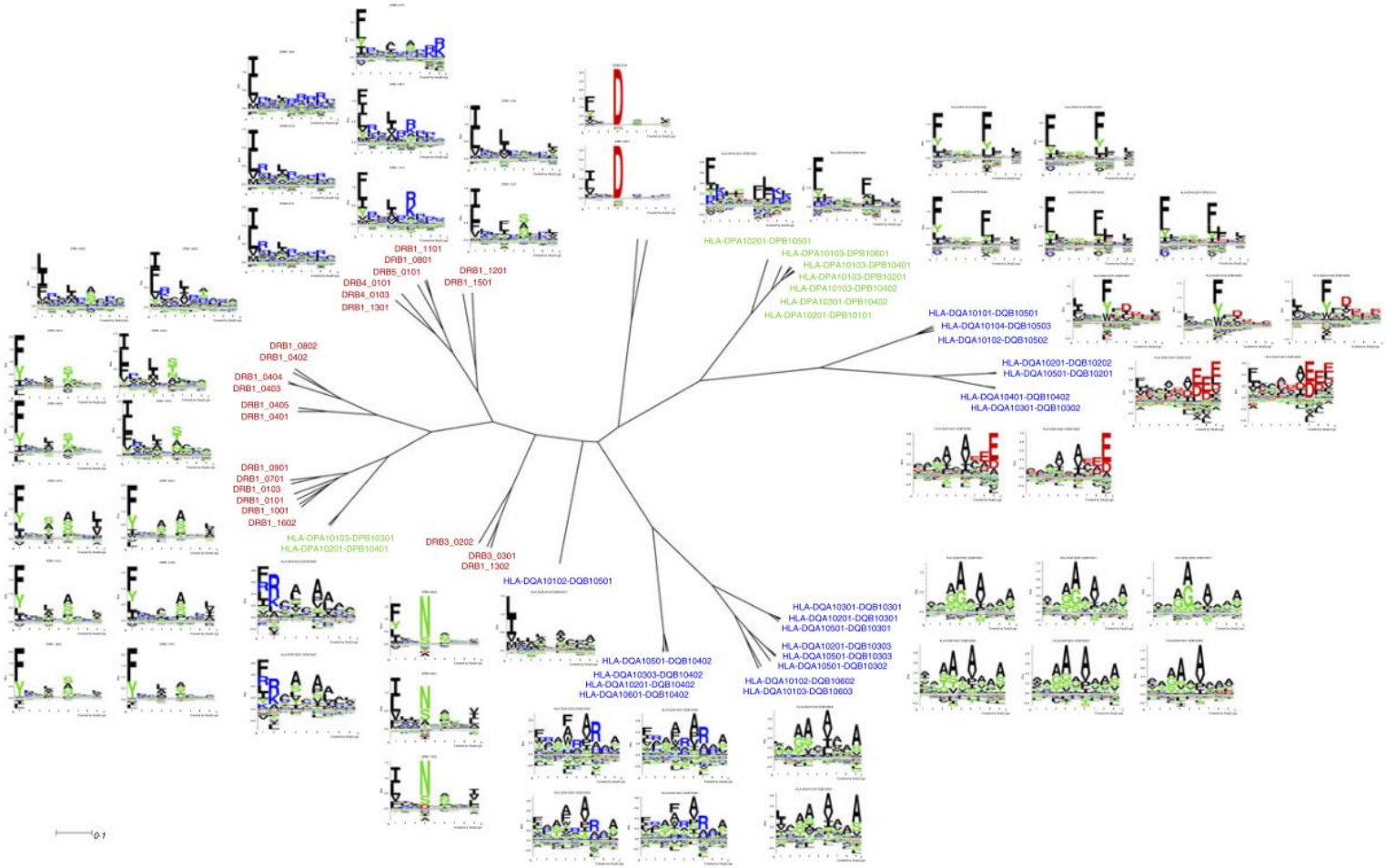
First MHC predictors used binding affinity data

```
FMIDWILDA YFAMYGEKVAHTHVDTLYVRYHYYTWAVLAYTWY 0.89 A0201
FMIDWILDA YFAMYQENMAHTDANTLYIIYRDYTWVARVYRGY 0.08 A0101
DSDGSFFLY YFAMYGEKVAHTHVDTLYVRYHYYTWAVLAYTWY 0.08 A0201
DSDGSFFLY YFAMYQENMAHTDANTLYIIYRDYTWVARVYRGY 0.85 A0101
```



NetMHCIIpan-3.2 predicts binding affinity to MHC-II

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE



services.healthtech.dtu.dk/service.php?NetMHCIIpan-3.2

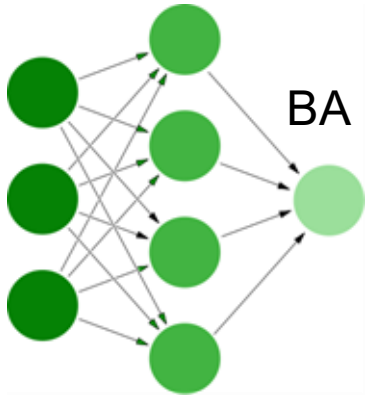
Jensen KK, et al. *Immunology*, 2017

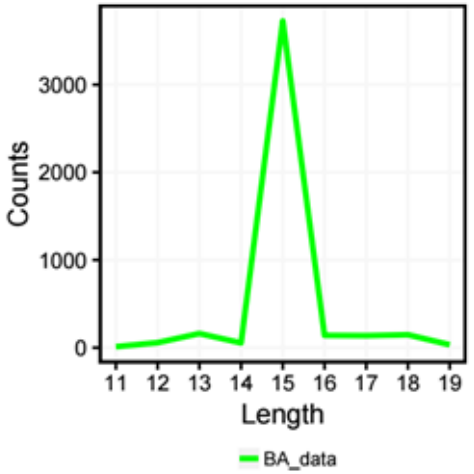
Challenges of binding affinity data

ALAKAAAAM
ALAKAAAAN
ALAKAAAAR
ALAKAAAAT
ALAKAAAAV
GMNERPILT
GILGFVFTM
TLNAWVKVV
KLNEPVLLL
AVVPFIVSV

} Similar sequences Weight 1/5

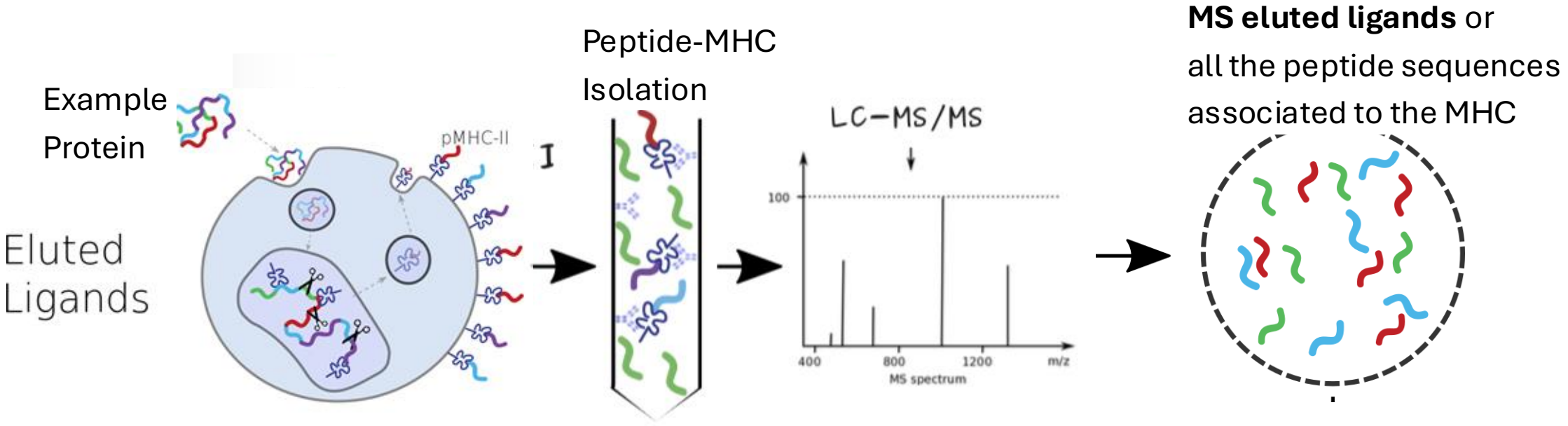
AAYKLAYKTAEGATP	0.208682
RMMEYGTTMVSYQLV	0.305733
VDKCLELAEYLYNII	0.0
PRYFNQLSTGLDMDN	0.357937
TFSSEIRVGDELLER	0.376583
LSGHAFGAMAKKGDE	0.63762
RPQVPLRPMTYKGAF	0.576544
INAGFKAALAAAAGV	0.84466
IVGILLVLMAVVLAS	0.0
TTLAEMSTPEAT	0.18153
LAATVLLGCTSAKVH	0.588207
FIKVRQYDQILIEIL	0.397951
VSGLSIGTGRAMLGT	0.0
GGLLMSRKHKWKLSL	0.0





- Biased sequences -> Alanine
- Over-representation of paradigmatic HLA alleles A*02:01, DRB1*01:01
- Artificial length distribution -> 15mers
- NetMHCpan was used to “design” new synthetic peptides and later feedback loop

Mass spectrometry assay

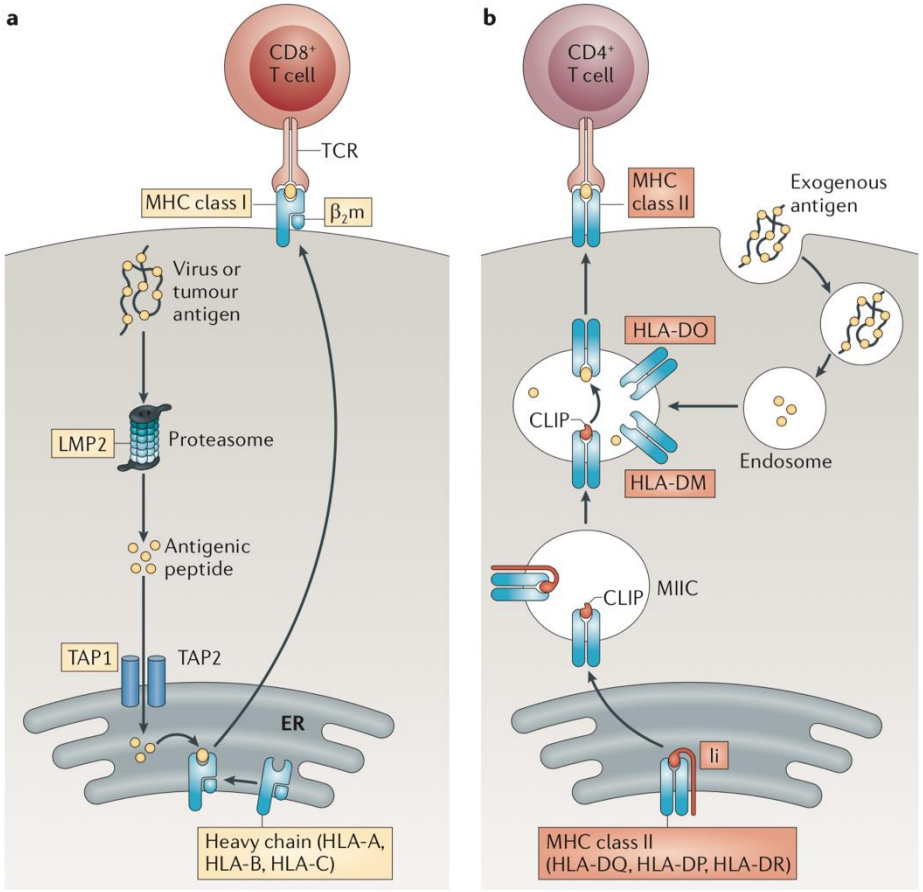


MS eluted ligands or all the peptide sequences associated to the MHC

MHC Typing or which MHC alleles are expressed by this particular cell

Barra C, et al. ***Proteomics***. 2018 Jun;18(12):e1700252.

MS contain antigen processing and natural presentation information

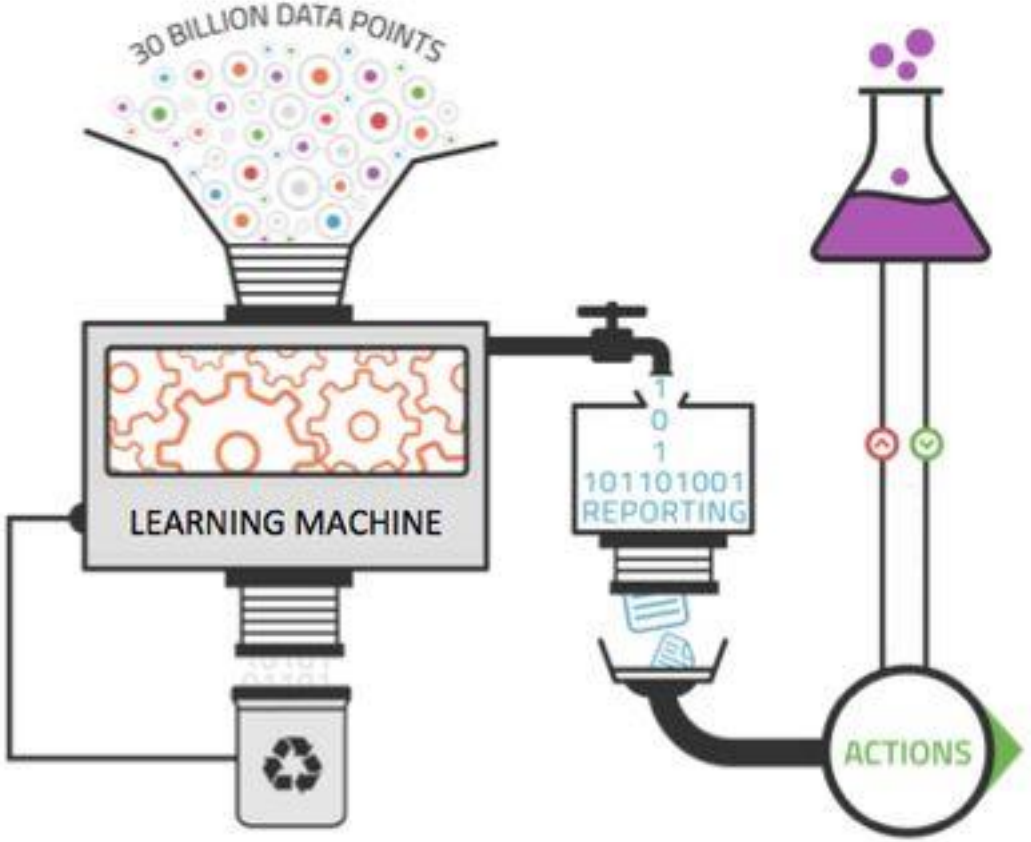


From MacMillan Publishers Ltd 2012. ***Nat. Rev. Imm.*** Vol 12

MS becomes high-throughput > immunopeptidomics

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

Game changers on immuno-informatics



Now the data follows a natural length distribution

AADTVTQFDNVRLWLG	1
AADVVLIRNDLLD	1
AADVVLIRNDLLDVV	1
AAEAAASHTGTLTGS	0
AAEAIETEAKKRGWWVK	0
DTFENMGPATKKY	0
FENMWEFFPATKKY	0
AADLPQLVGHDVPGAVL	0
AADPGATNTDLVGD	0
AADPQFVTVAL	0
AADPQWVPTETDV	0
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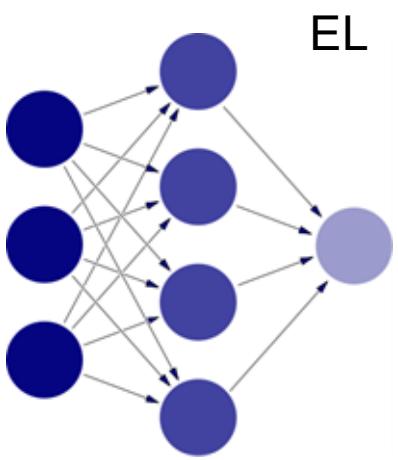
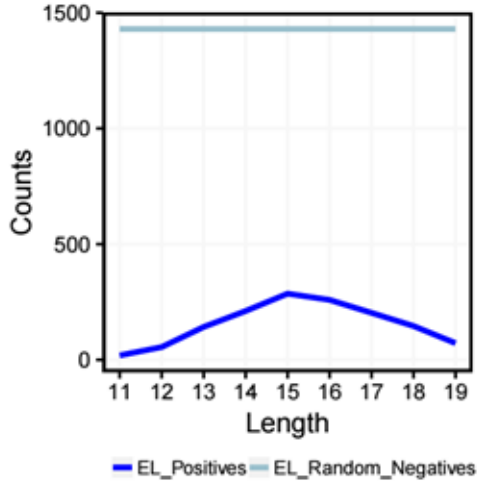
AADTRLDDPRSFS	0

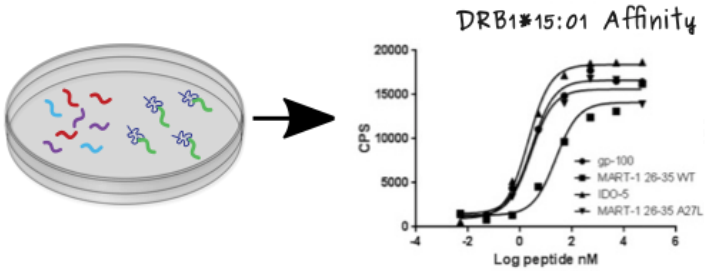




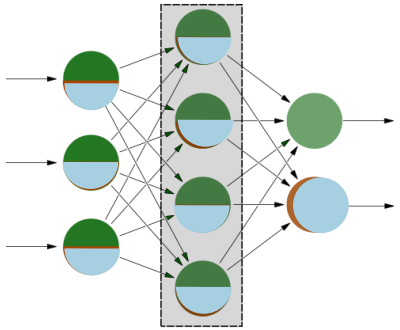
Barra C, et al. ***Proteomics.*** 2018 Jun;18(12):e1700252.

MS eluted ligands integration to MHC-II predictors

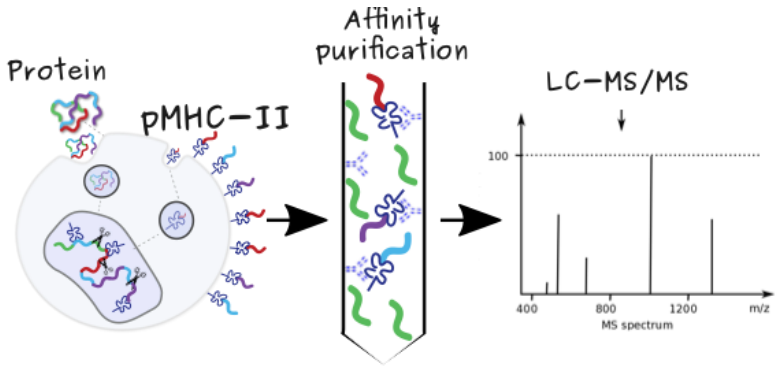
Peptides from binding affinity measurements

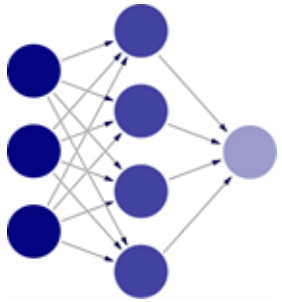


NNAlign-2outputs



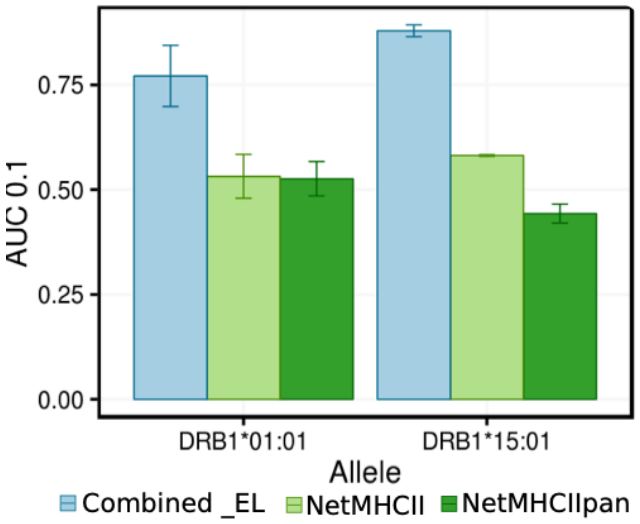
Peptides from mass spectrometry eluted ligands





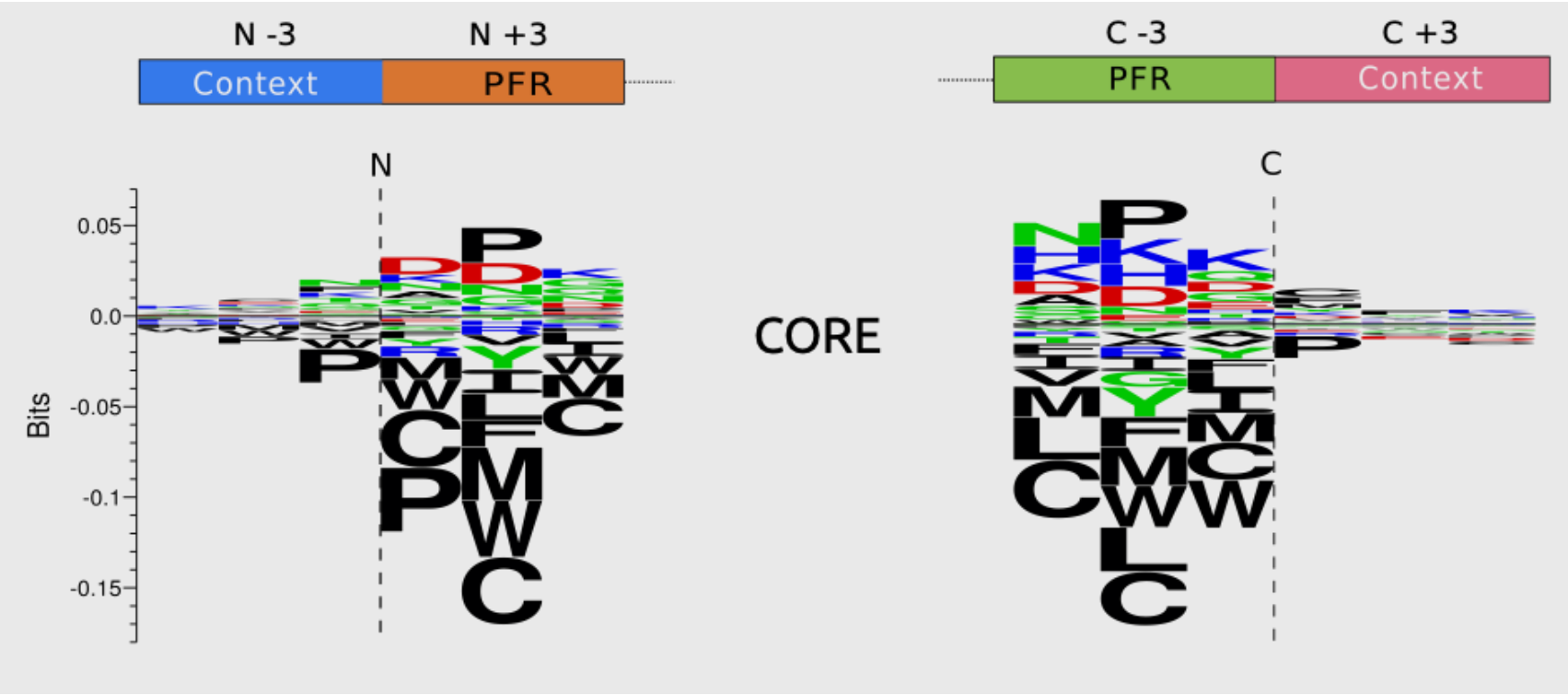
Eluted ligands

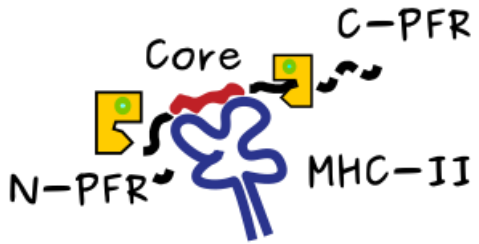
Evaluation dataset



Barra C, et al. *Genome Medicine, 2017*

Footprints of antigen processing in MHC class II MS data





Barra C, et al. *Genome Medicine, 2017*

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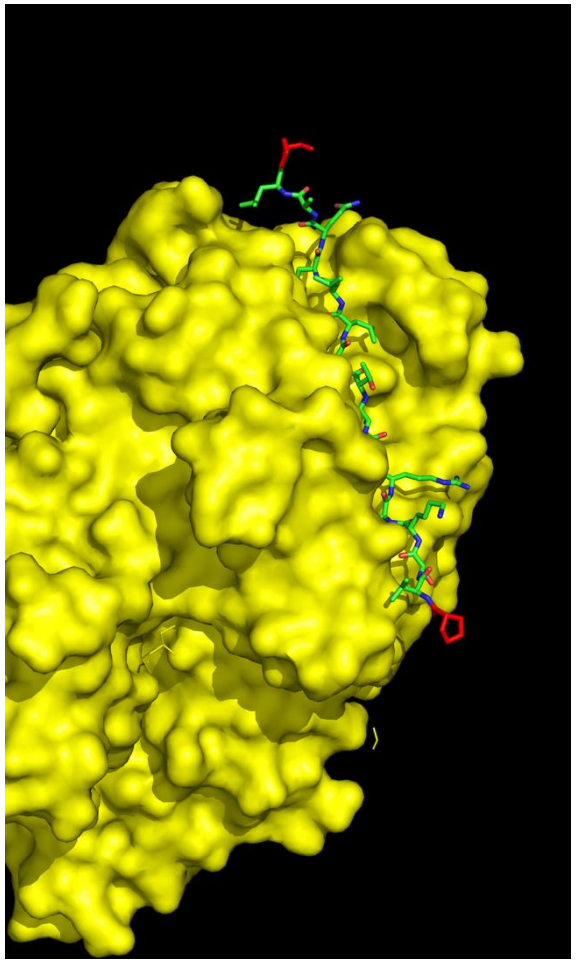
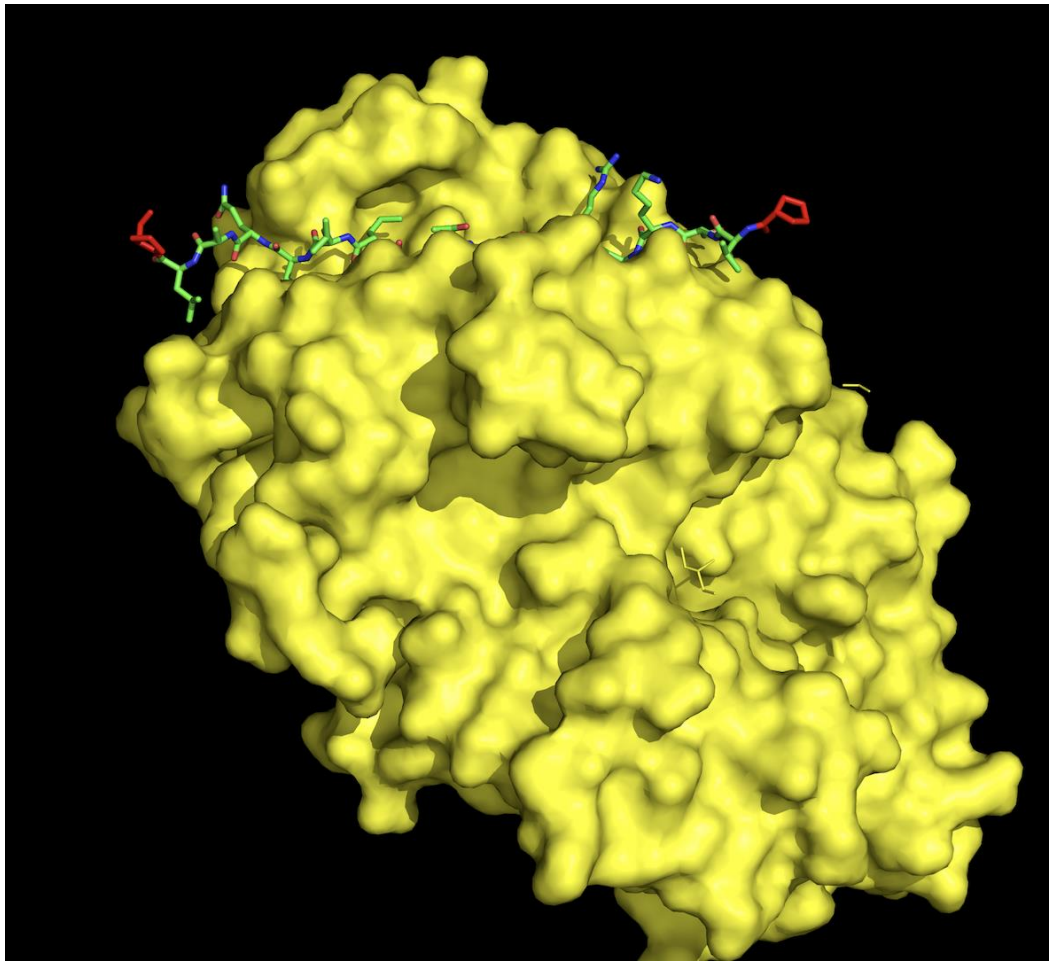
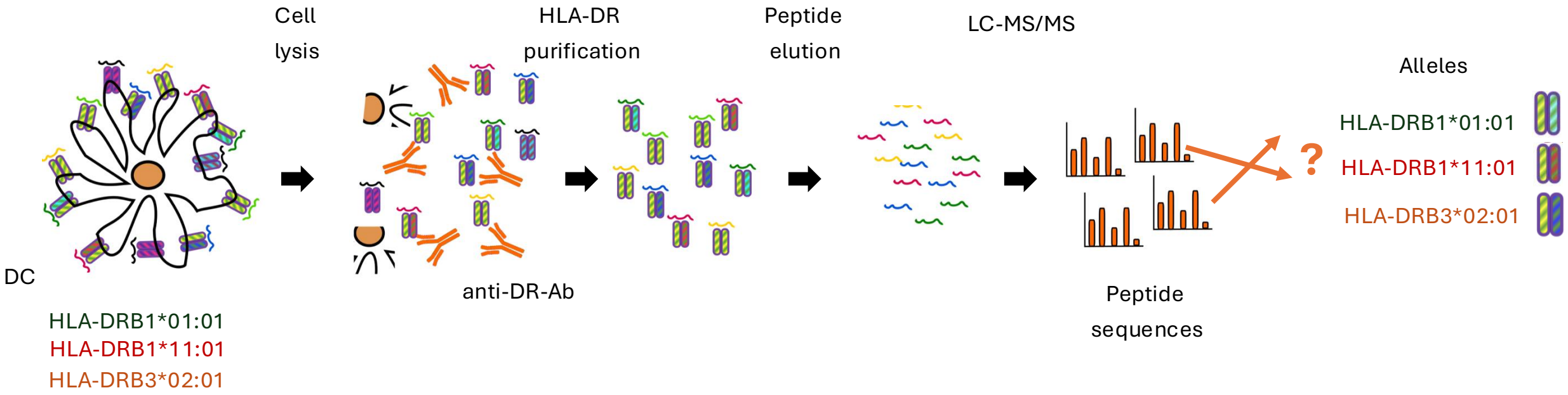




Challenges on immunopeptidomics

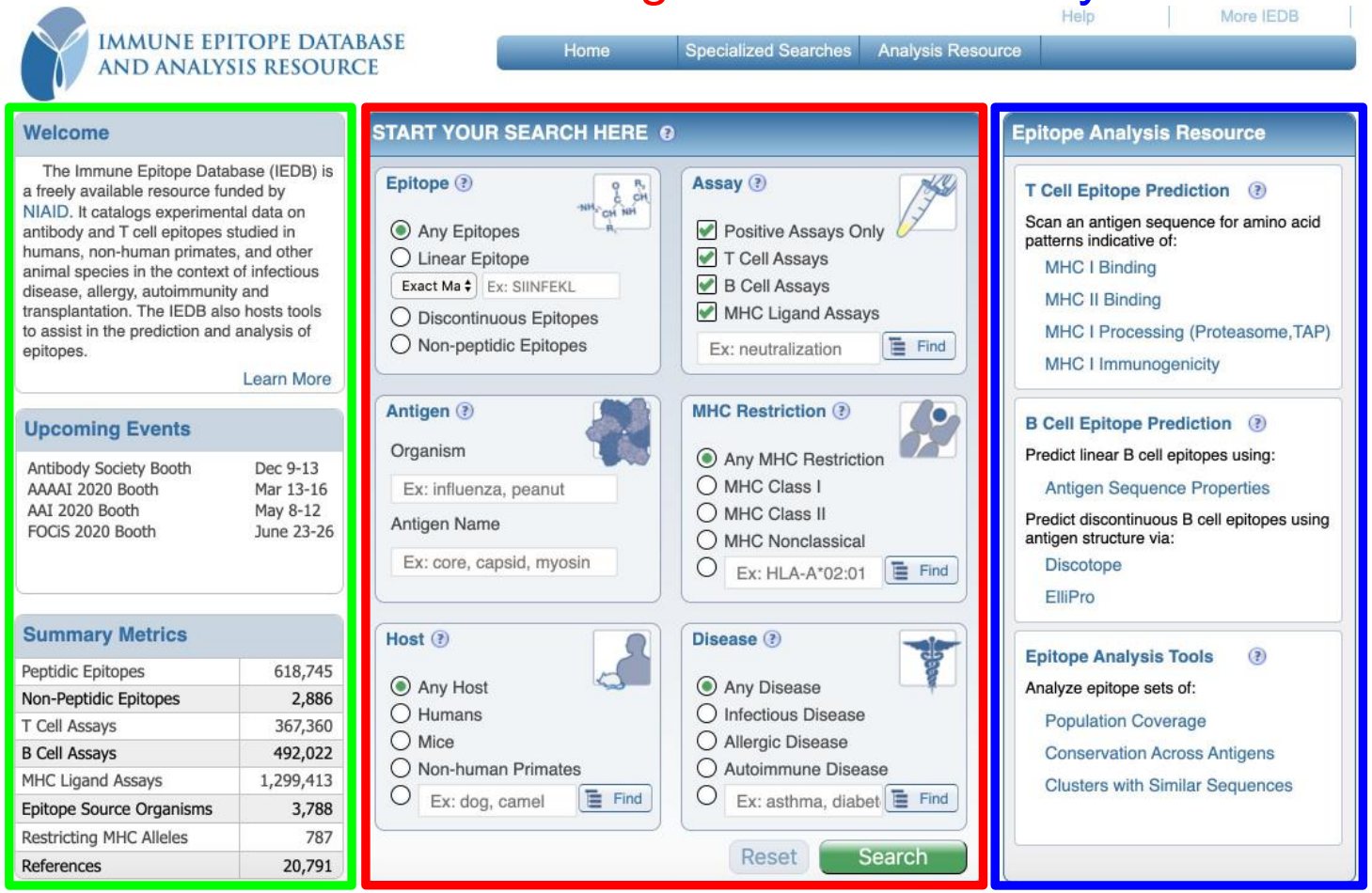


What is the IEDB?

- Database of experimentally-derived epitope assay results
- Maintained and curated by experts
- Data from literature and submissions
    - ~ 1 Million Epitopes
    - ~ 4 Million Assay Results
    - ~ 22K References
- Graphical User Interface
- Analysis Resource - Prediction Tools

IEDB Home Page

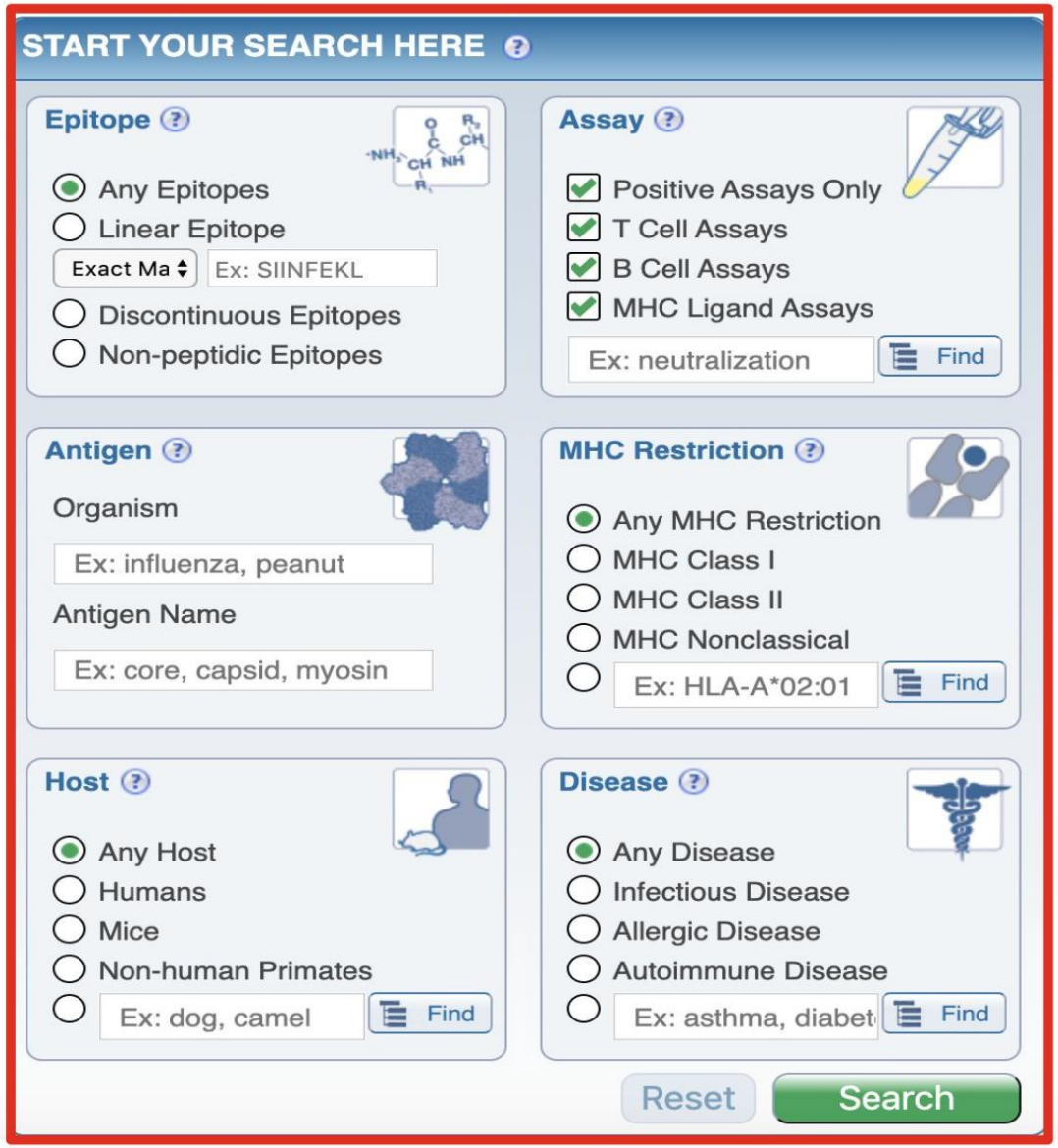
Intro+Stats Home Page Search Analysis Resource



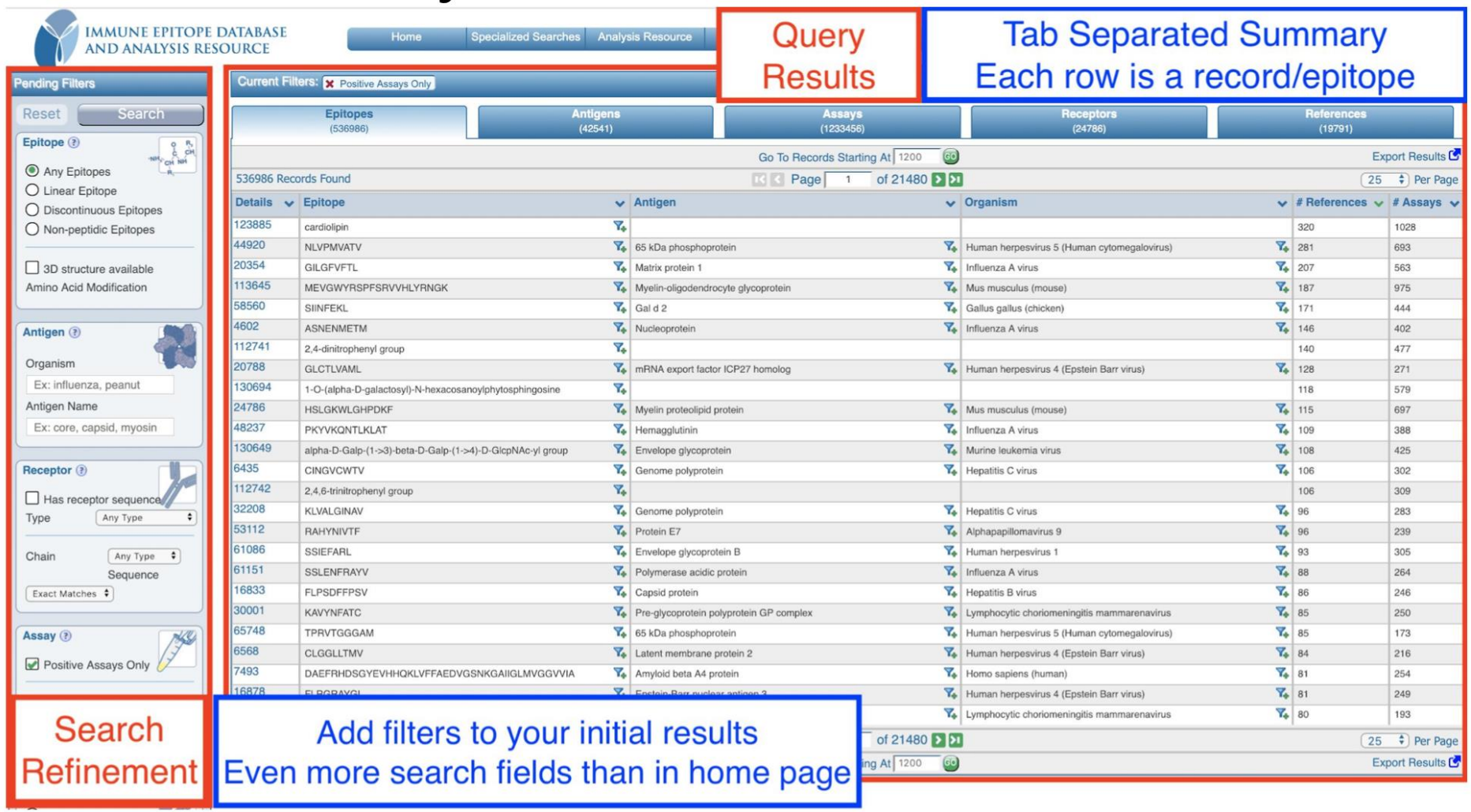
Home Page Search

- Epitope Sequence
- Antigen
- Host
- Assay
- MHC
- Disease

Positive epitopes by default



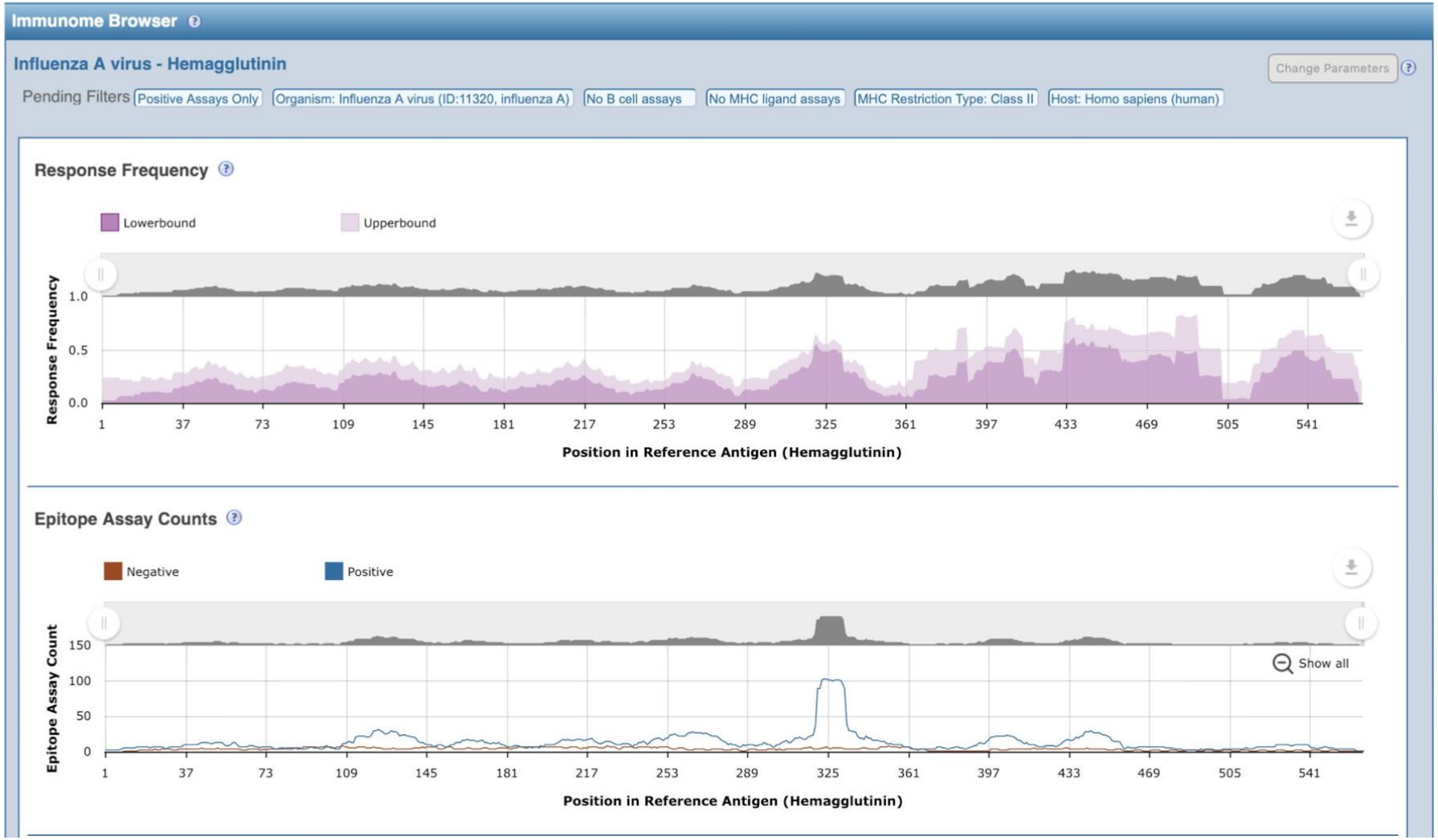
Results Summary



Results Summary - Refined - Immunome Browser

Immunome Browser

- Visual Representation of epitopes, mapped onto antigen sequence



Now, explore it yourself!

Links

Detailed Course Program