The Immune Epitope Database Analysis Resource:

MHC class I peptide processing and immunogenicity predictions

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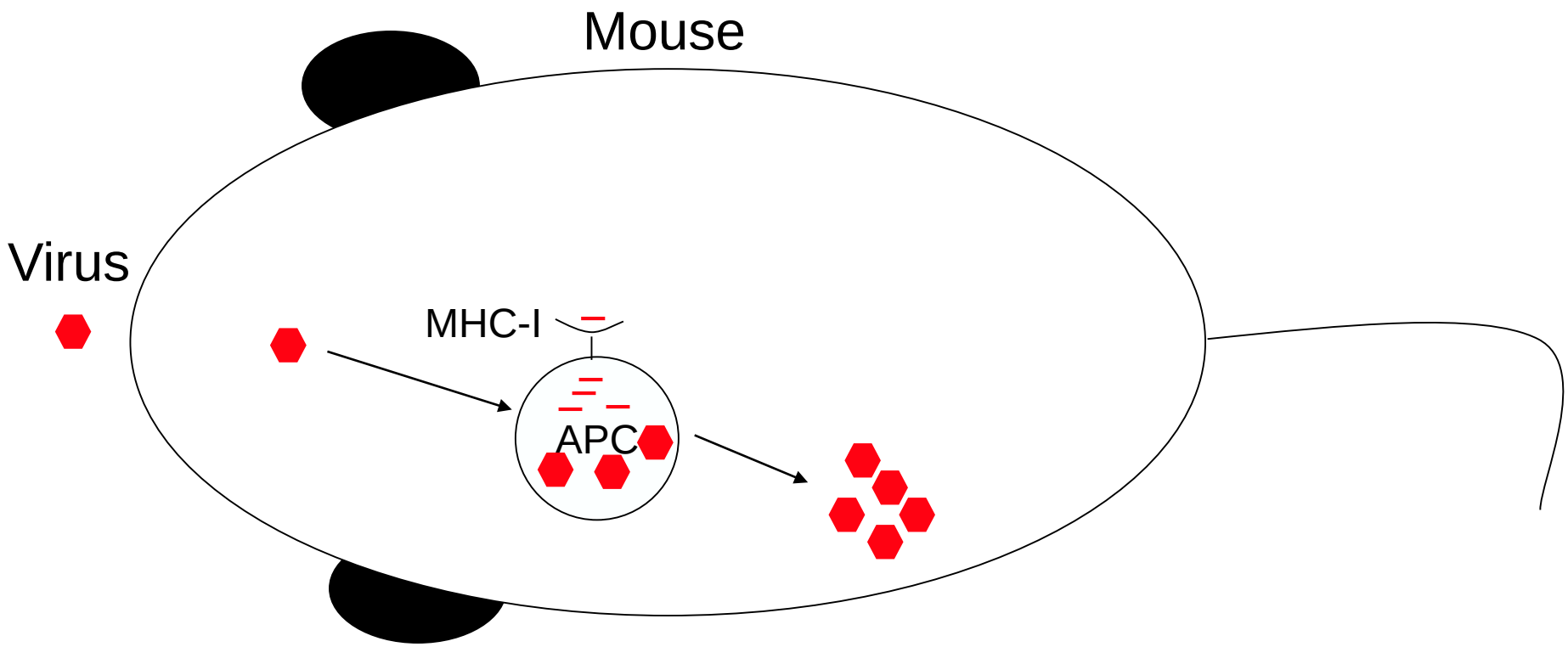
IEDB Workshop

Oct 23, 2018

Outline

- Motivation – Factors apart from MHC binding determine what peptides are T-cell epitopes
- Processing tools in the IEDB
  - Interfaces + Prediction output
  - Performance / Caveats
- Immunogenicity tool
  - Interface + Prediction output
  - Performance / Caveats

CD8+ T cell epitopes in viral infection



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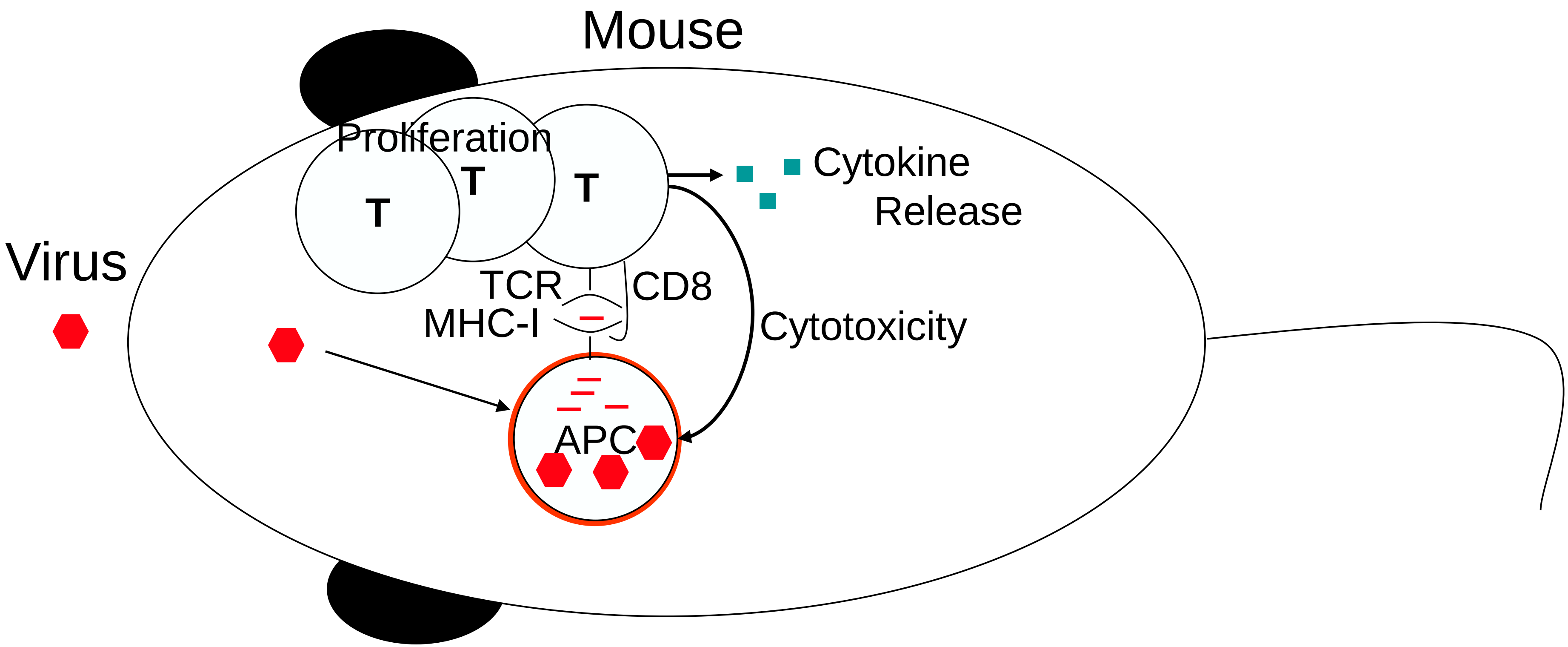
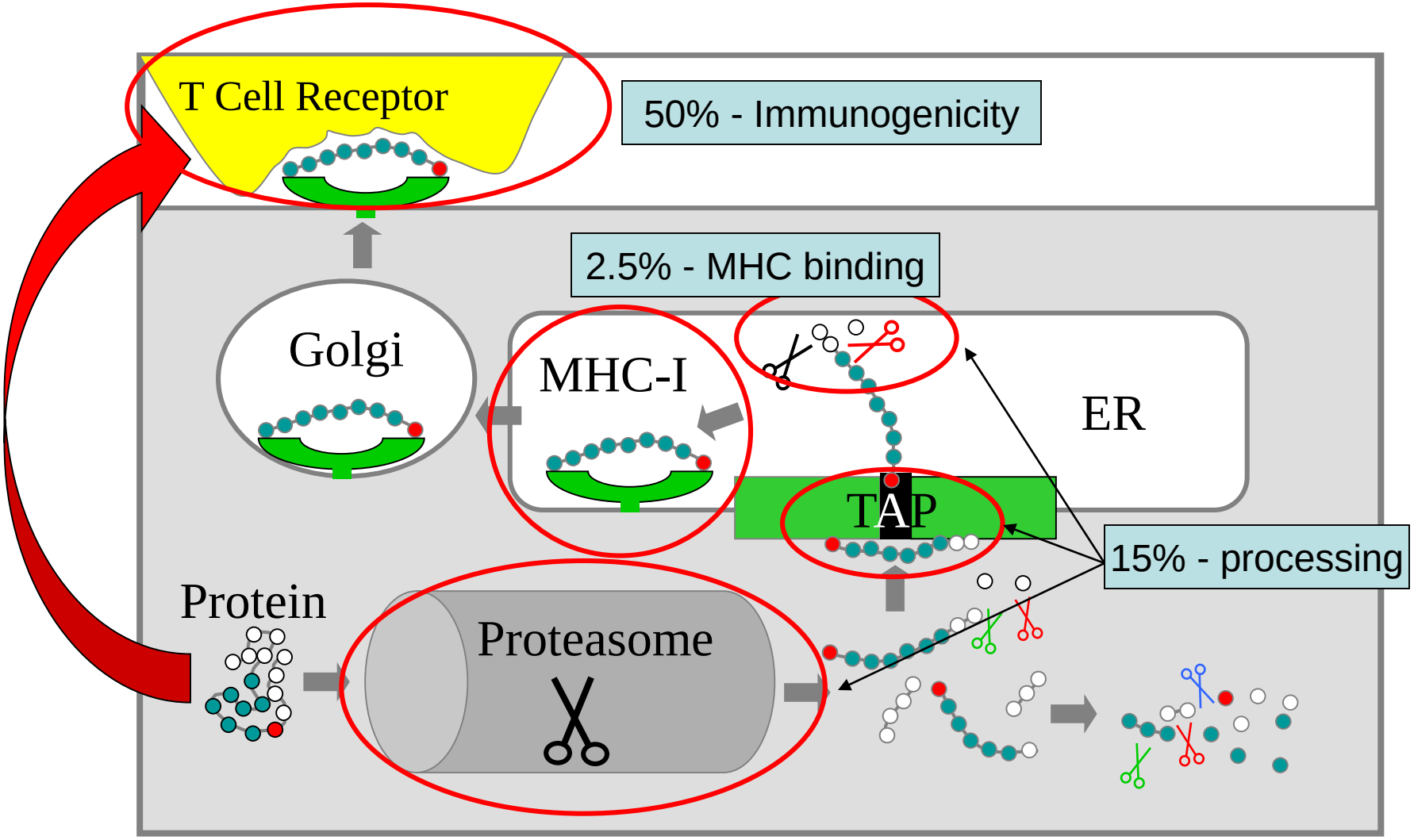


T cell epitope mapping

ORF 1 M G Q I V T M F E A L P H I **I D E V I N I V I** I V L I V I T G I K A V Y N ...
ORF 2 M G L K G P D I Y K G V Y Q F K S V E F D M S H L N L T M P N A C S A N N ...
ORF 3 M H N F C N L T S A F N K K T F D H T L M S I V S S L H L S I D G N S N Y ...
ORF 4 M S A Q S Q C R T F R G R V L D M F R T A F G G K Y M R S G W G W T G S D ...
ORF 5 M H C T Y A G P F G M S R I L L S Q E K T K F F T R R L A G T F T W T L S ...
ORF 6 M K C F G N T A V A K C N V N H D A E F C D M L R L I D Y N K A A L S K F ...
ORF 7 M L M R N H L **L D L M G V P Y C N Y** S K F W Y L E H A K T G E T S V P K C ...

MHC I - Antigen processing and presentation pathway



Peters et al, J Mol Biol 2002, Bioinformatics 2003, J Immunol.2003; CMLS 2005 ; Assarson, J Immunol 2007

Processing + immunogenicity tools available in the IEDB

- 'Combined predictor' – Combines proteasomal cleavage and TAP transport predictions, trained on specific in vitro datasets
- Neural Network based predictors (NetChop, NetCTL)
- MHC-NP: Prediction of peptides naturally processed by the MHC
- Immunogenicity predictor

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MHC-I Processing Predictions

Prediction Method Version	2013-02-22 [Older versions]
Specify Sequence(s)	
Enter protein sequence(s) in FASTA format (Browse for sequences in NCBI)	
Or select file containing sequence(s)	Choose File No file chosen
Choose sequence format	auto detect format
Choose a Prediction Method	
Prediction Method	IEDB recommended Help on prediction method selections
Specify what to make binding predictions for	
MHC source species	human
Show only frequently occuring alleles: Select MHC allele(s)	Allele Length Upload allele file
Proteasomal cleavage prediction	
Specify proteasome type	immuno
TAP transport predictions	
Maximum precursor extension	1
Alpha factor	0.2
Specify Output	
Output format	XHTML table
	Submit Reset

Proteasomal cleavage

Proteasomal cleavage prediction	
Specify proteasome type	immuno
TAP transport predictions	
Maximum precursor extension	1
Alpha factor	0.2

- Proteasomes create the C-terminal end of peptides. The prediction looks for a sequence motive up and downstream of a potential cleavage site
- Cleavage sequence motif was determined based on in vitro protein digests by proteasomes
- Choice between two types of proteasomes with slightly different motif constitutive or immuno (should be default choice)

TAP transport

Proteasomal cleavage prediction	
Specify proteasome type	immuno
TAP transport predictions	
Maximum precursor extension	1
Alpha factor	0.2

- TAP transports peptides into the ER that can be further N-terminally trimmed before binding to MHC.
- The TAP transport efficiency of peptides is sequence dependent, and a motif was derived based on in vitro assays
- The overall TAP transport efficiency of a presented MHC ligand can be the result of a collection of precursors.
- The parameters shown describe that collection. Unless you read the paper and know something about the precursor length distribution, **keep parameters unchanged**

Difference in prediction output

Allele	#	Start	End	PepLength	Sequence	Proteasome Score	TAP Score	MHC Score	Processing Score	Total Score	MHC IC50[nM]
H-2-Kb	1	1	10	10	MGQIVTMFEA	0.91	-0.29	-4.36	0.62	-3.74	22777.84
H-2-Kb	1	2	11	10	GQIVTMFEAL	1.51	0.42	-3.93	1.93	-2.00	8485.76
H-2-Kb	1	3	12	10	QIVTMFEALP	0.65	0.13	-4.49	0.77	-3.72	31246.67
H-2-Kb	1	4	13	10	IVTMFEALPH	0.65	-0.20	-4.11	0.45	-3.67	12949.50
H-2-Kb	1	5	14	10	VTMFEALPHI	1.24	0.28	-3.59	1.52	-2.06	3850.57
H-2-Kb	1	6	15	10	TMFEALPHII	1.06	0.34	-3.52	1.40	-2.11	3273.98
H-2-Kb	1	7	16	10	MFEALPHIID	1.13	-0.75	-4.23	0.37	-3.85	16798.51

- Higher scores = higher efficiency for MHC-I presentation
- MHC binding score = –log10(IC50) (→sign change)
- Combined scores are additive
  - Processing = proteasome + TAP
  - Total = proteasome + TAP + MHC
- Different variance in scores reflects different selectivity
  - Proteasome (1.7) < TAP (2.8) < MHC (6.7)

Caveats / performance of processing predictions

- Processing predictions beat MHC binding predictions when predicting **eluted peptides**
- So far, there is no clear evidence that processing predictions are better at predicting **epitopes**
- Issues are:
  - All data has been derived for *human* proteasome and TAP; most well defined epitopes are mapped in mice (which has different TAP specificity)
  - Eluted peptides may over represent ‘best possible’ ligands, and the difference in processing may not be relevant in practice
- Recommendation: Use MHC binding predictions alone by default. If resources require limiting the number of peptides considered, use total score of processing predictions as an additional filter.

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Additional processing predictions

- NetChop (proteasomal cleavage)
- NetCTL (combines NetChop, TAP transport, NetMHC)
- NetCTLpan (combines NetChop, TAP transport, NetMHCpan)

→ Key difference is the use of NetChop

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NetChop/NetCTL/NetCTLpan

Choose a Prediction Method

Prediction Method	NetCHOP

Specify Sequence(s)

Enter protein equence(s) in FASTA format	>BHB191648|gi:90572034|gb:CY010133|UniProtKB:Q1WPY8|Gene Symbol:M2|Protein Name:Matrix protein 2|Organism:Influenza A Virus A/Canterbury/100/2000|Segment:7|Subtype:H1N1|Host:Human MSLLTEVETPIRNEWGCRCNDSSDPLVVAASIIGIVHLILWIIDRLFSKSIYRIFKHGLKR TEGVPESMREEYREEQQNAVDADDGHFVSIELE >BHB191653|gi:90572040|gb:CY010136|UniProtKB:Q1WPY3|Gene Symbol:NS1|Protein Name:Nonstructural protein
Or select file containing sequence(s)	Choose File No file chosen

Method Specific Options

Method	C term 3.0
Threshold	0.5

Submit

NetChop 3.0

- Predicts C-terminal cleavage based on two approaches
  - *C-term 3.0*: C-terminal residues found for MHC ligands
  - *20S 3.0*: Cleavage sites based on in vitro protein digests
- C-term 3.0 is not truly a proteasome predictor but performs better.
- NetCTL and NetCTLPan use C-term 3.0 by default

References

- Peters et al, JMB 2002 (proteasome)
- Peters et al, J Immunol 2003 (TAP)
- Tenzer et al, CMLS, 2005 (combined)
- Nielsen, Immunogenetics, 2005 (NetChop)
- Larsen, BMC Bioinformatics, 2007 (NetCTL)
- Stranzl, Immunogenetics, 2010 (NetCTLPan)

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MHC-NP: Prediction of Peptides Naturally Processed by the MHC

Developed by: Sébastien Giguère Alexandre Drouin, Alexandre Lacoste, Mario Marchand, Jacques Corbeil and François Laviolette

Specify Sequence(s)

Enter protein sequence(s) in FASTA format (Browse for sequences in NCBI)

Or select file containing sequence(s) — Choose File — No file chosen

Choose sequence format — auto detect format

Specify what to make binding predictions for

Select MHC allele(s)

Allele	Length	
		Upload allele file

H-2-Db
H-2-Kb
HLA-A*02:01
HLA-B*07:02
HLA-B*35:01
HLA-B*44:03
HLA-B*53:01
HLA-B*57:01

Specify Output

Sort peptides by

Output format

Submit Reset

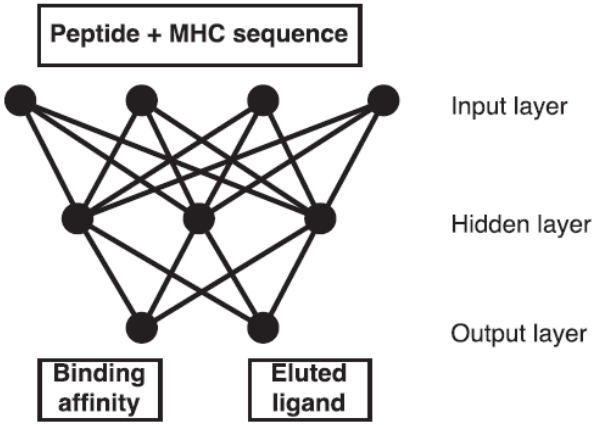
Supported by a contract from the National Institute of Allergy and Infectious Diseases, a component of the National Institutes of Health in the Department of Health and Human Services.

Coming soon: Pan-predictions trained on both binding + eluted ligand data

NetMHCpan-4.0: Improved Peptide–MHC Class I Interaction Predictions Integrating Eluted Ligand and Peptide Binding Affinity Data

Vanessa Jurtz, Sinu Paul, Massimo Andreatta, Paolo Marcatili, Bjoern Peters and Morten Nielsen

J Immunol 2017; 199:3360-3368; Prepublished online 4 October 2017;



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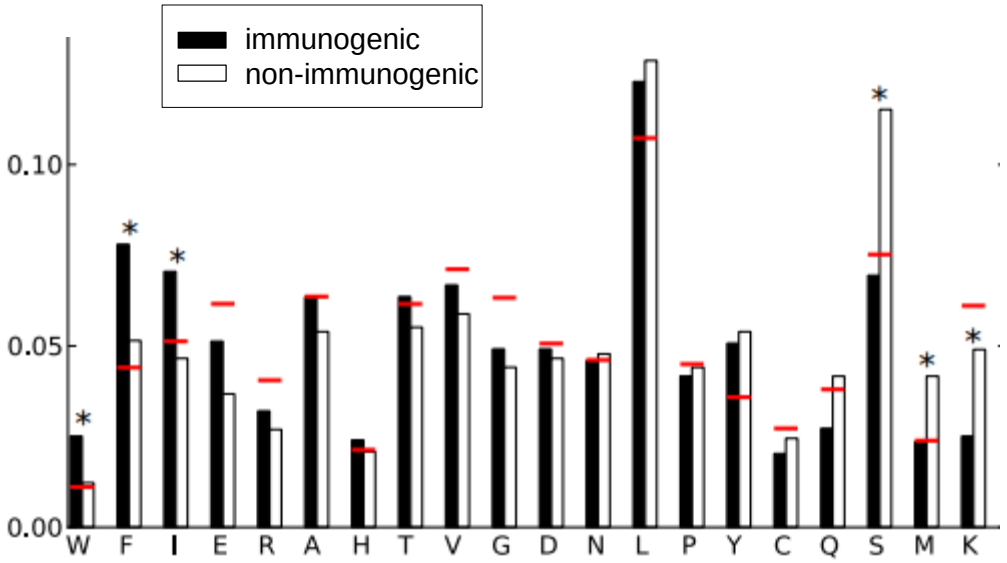
Immunogenicity prediction

- Approach: Assemble two datasets of peptides with similar MHC binding affinity, that are
  1) recognized or
  2) not recognized
  by T cells

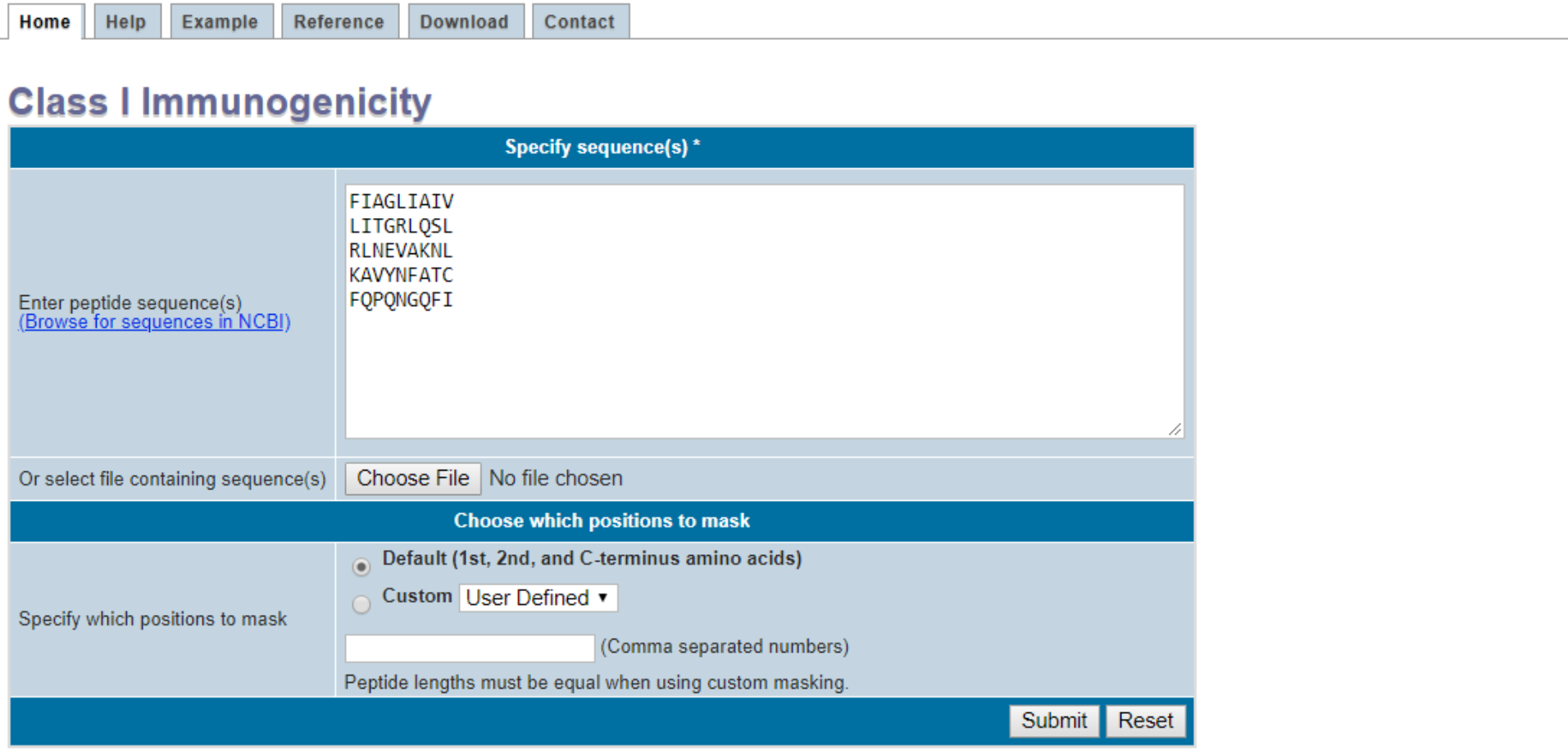
→ Enrichment of W,F,I and depletion of S,M,K in immunogenic peptide

→ Use enrichments to calculate propensity scores



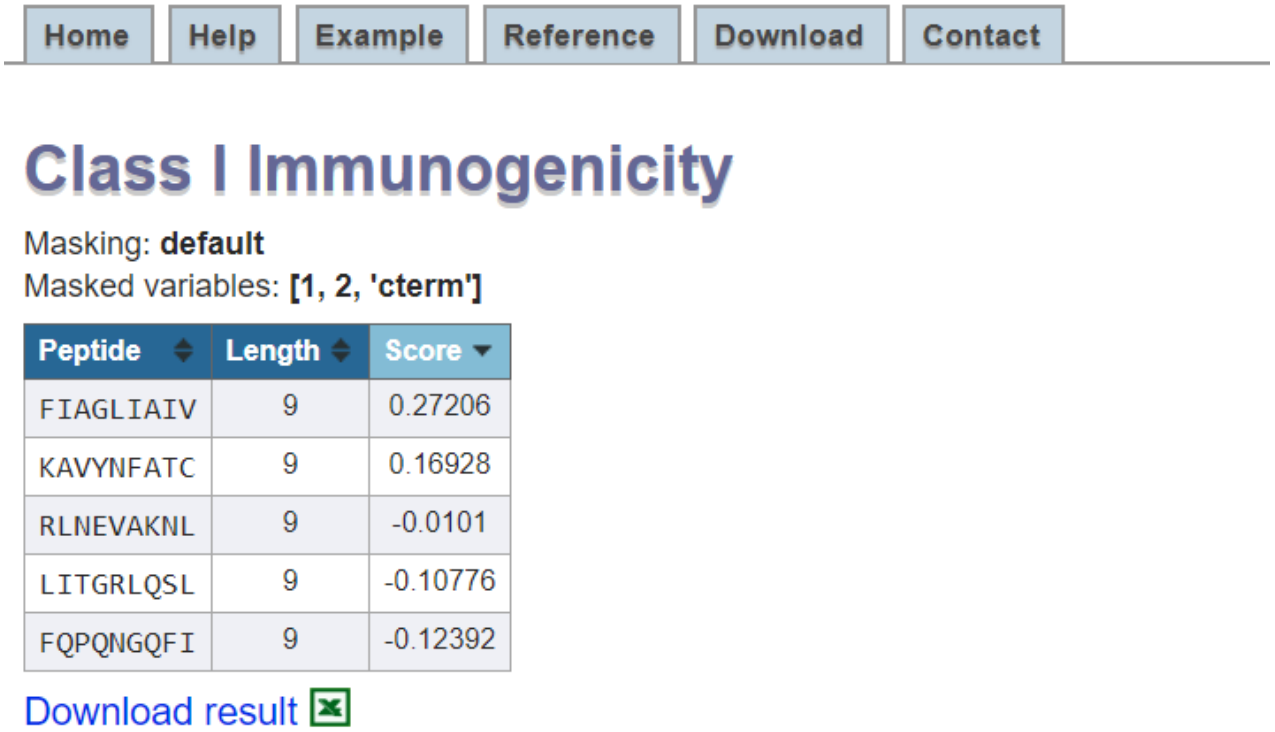
Calis et al, PLoS Comp Biol, 2013

Immunogenicity prediction - interface



- Mask positions that are MHC anchors

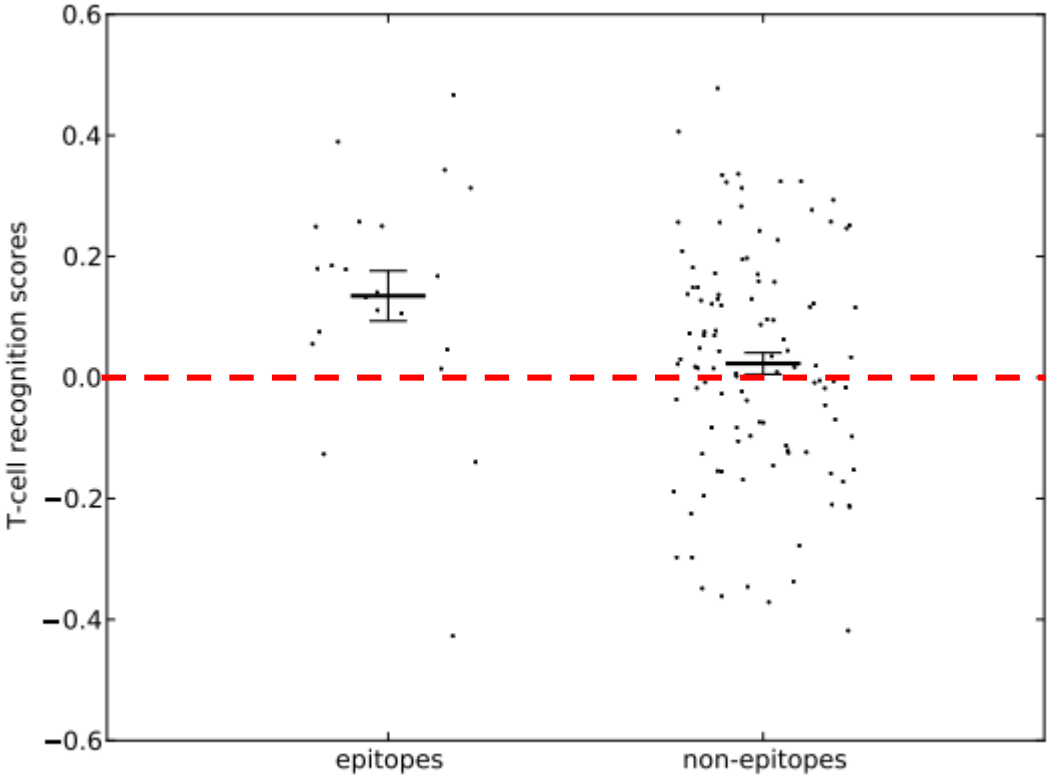
Immunogenicity prediction - output



- Scores are sums of propensity scores at all unmasked predictions
- High scores = peptide is more likely to be immunogenic

Caveats / Prediction performance

- Experimentally, many MHC binding peptides can be immunogenic (~50%)
- Cross validation gave AUC values ~ 0.65. Test on independent blind set gave AUC = 0.69
- Recommendation: Use as filter (cutoff 0) if high specificity is desired. Suggested cutoff is 0.



Questions?