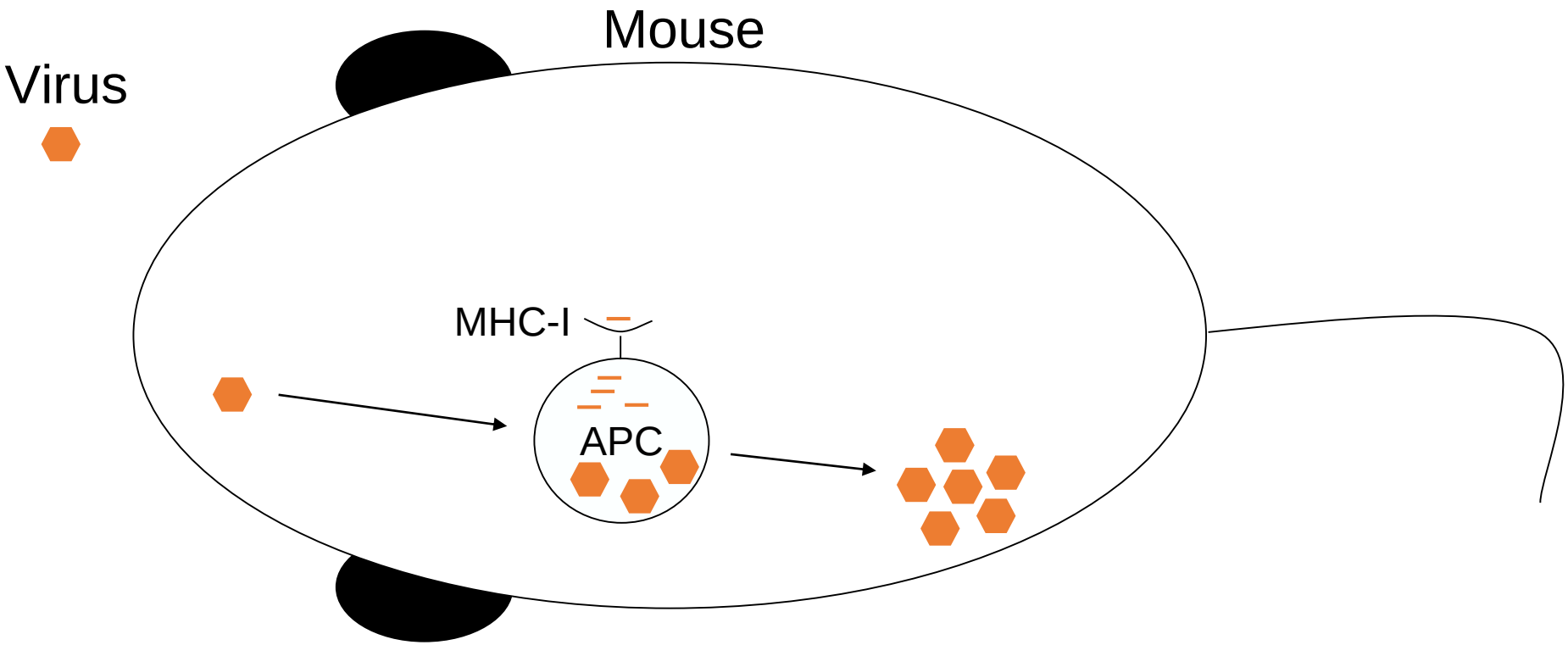

# T Cell processing & immunogenicity predictions

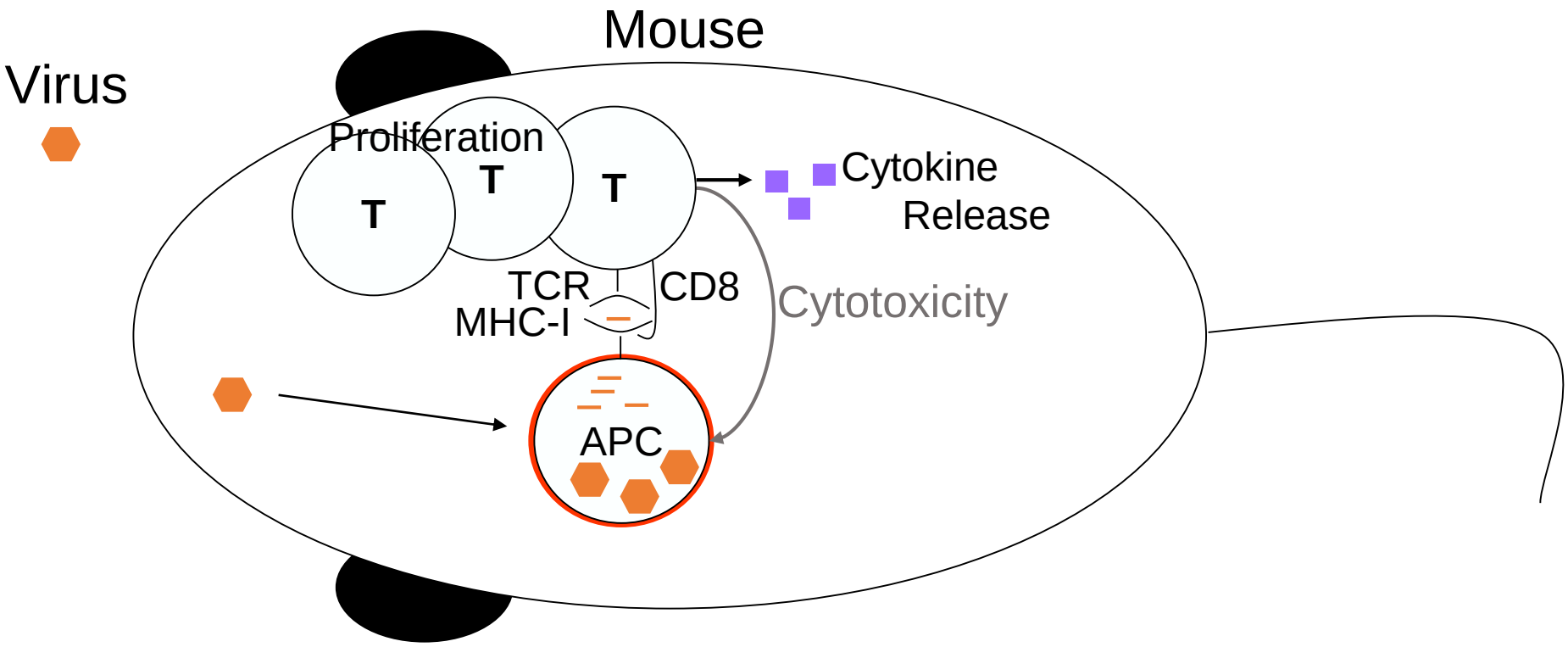
tools.iedb.org

Presented by:
Bjoern Peters, PI
Austin Crinklaw, Research Technician

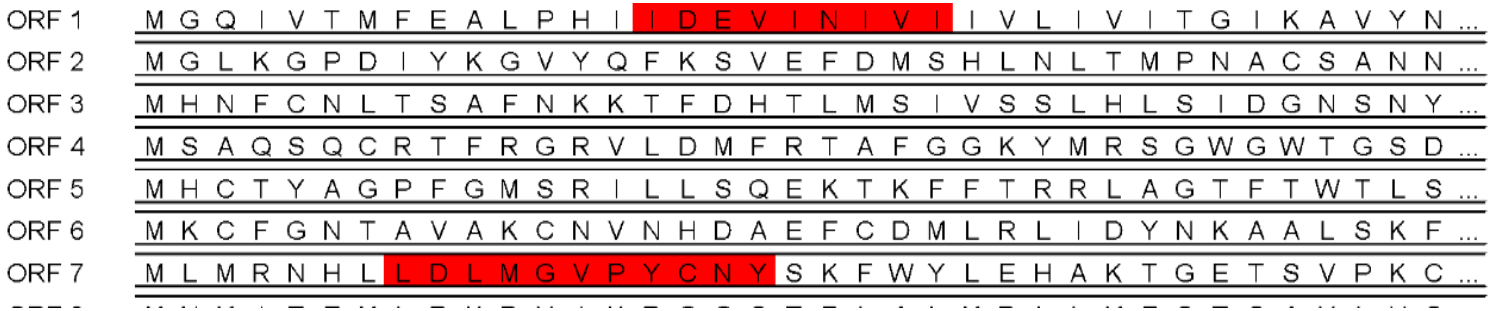
# CD8+ T cell epitopes in viral infection



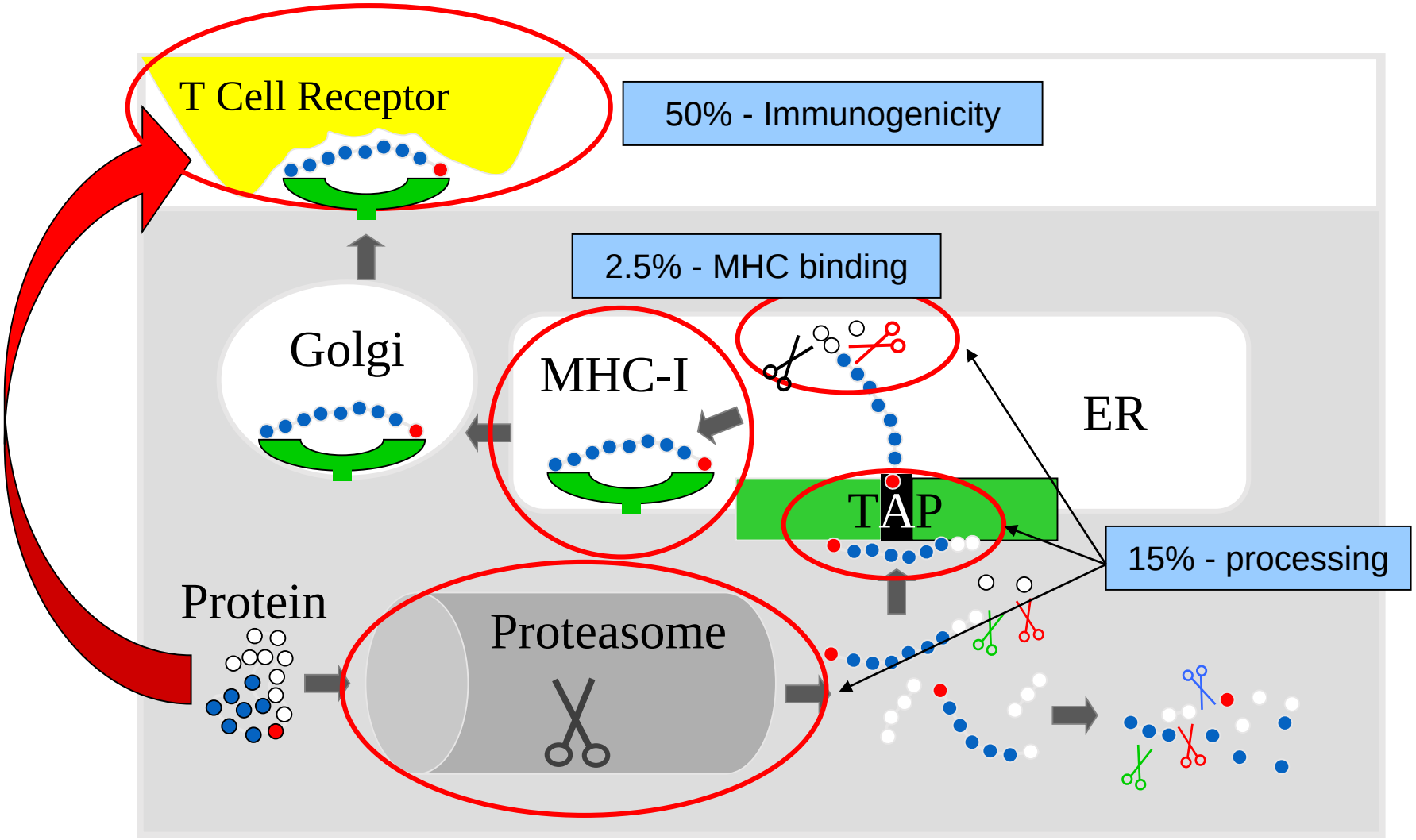
# CD8+ T cell epitopes in viral infection




T cell epitope mapping



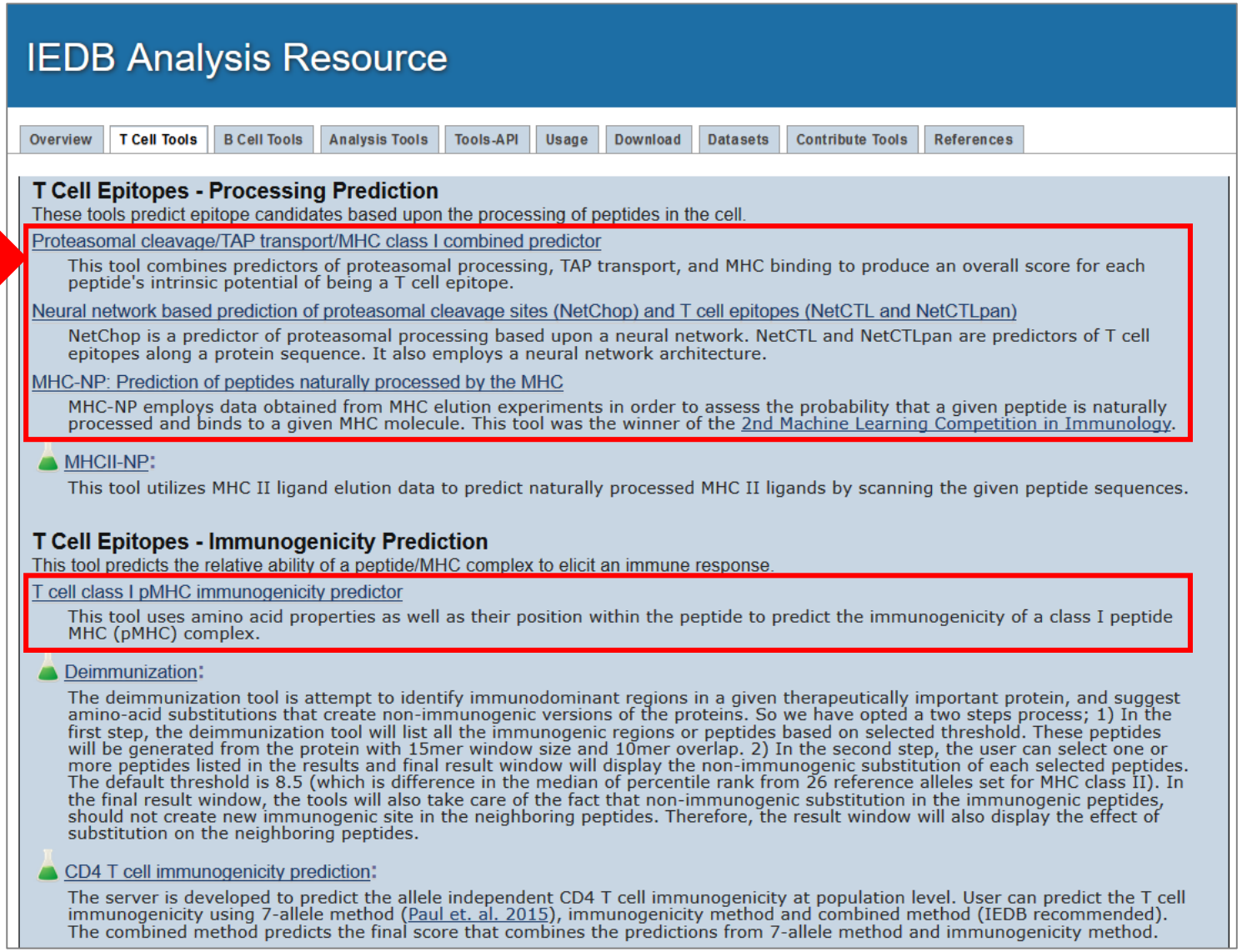
# MHC I - Antigen processing and presentation pathway




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

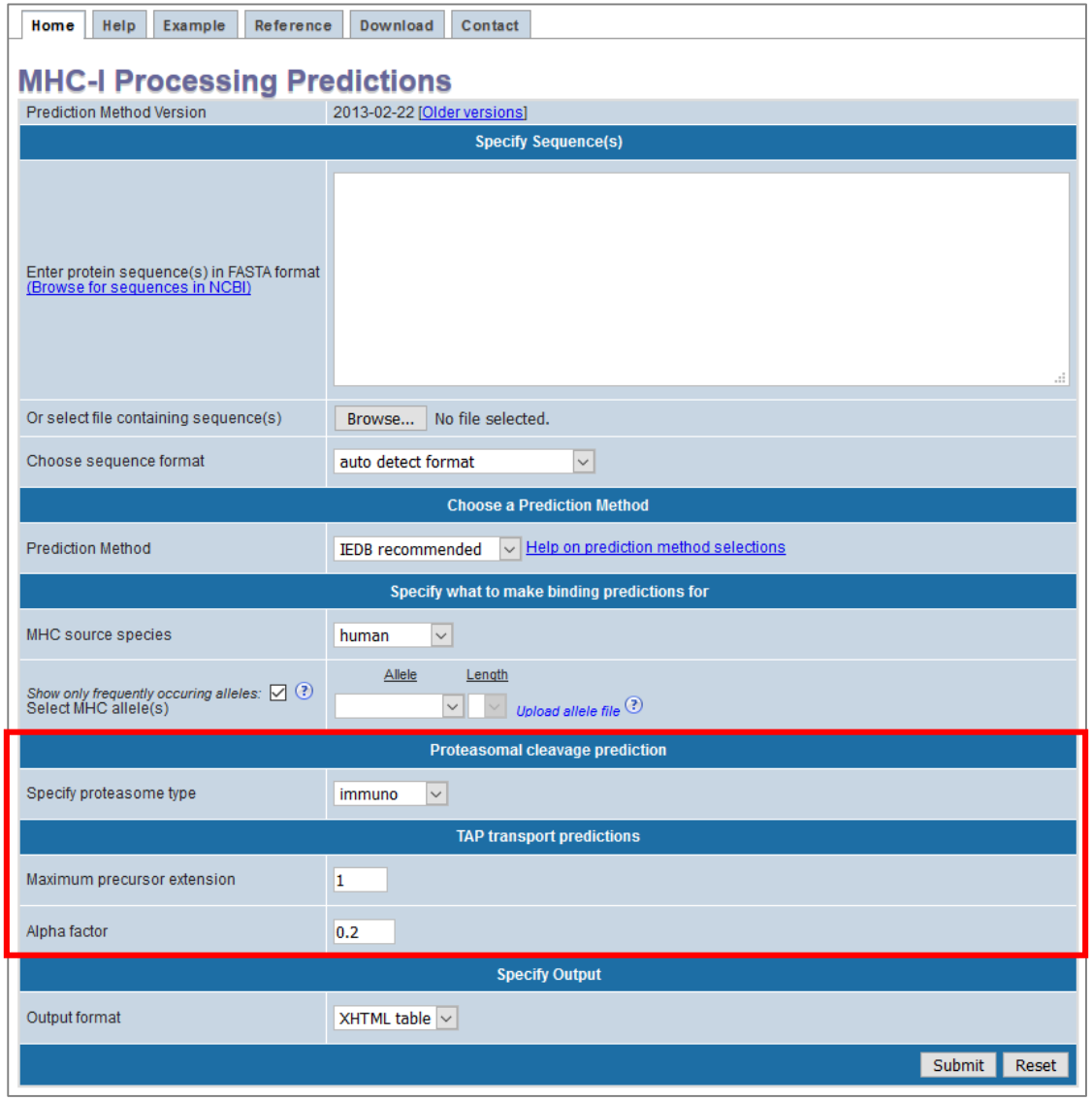
# Class I Processing + immunogenicity tools available in the IEDB

http://tools.iedb.org/main/tcell/



# Class I 'combined predictor'

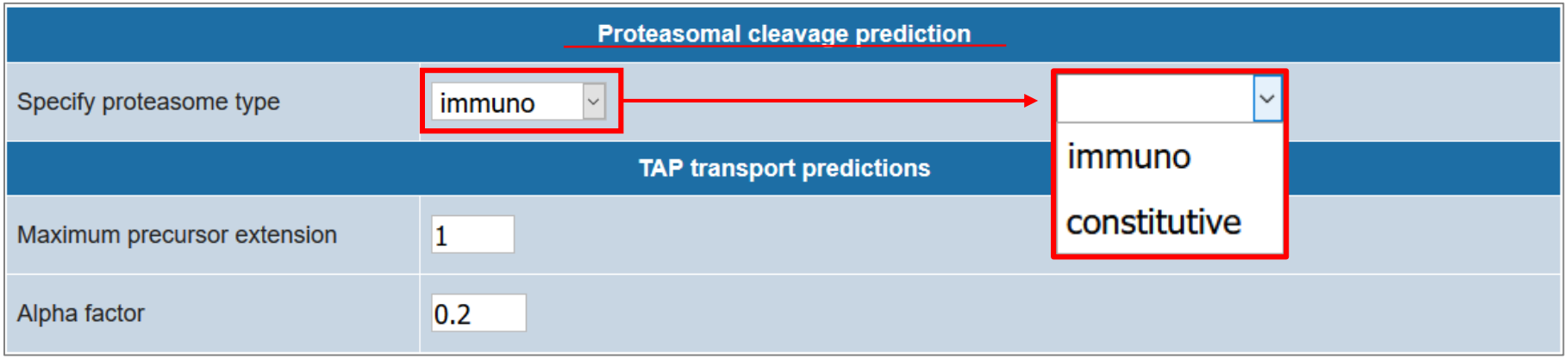
http://tools.iedb.org/processing/




- Combines predictions for:
  - proteasomal cleavage
  - TAP transport
- Trained on specific in vitro datasets

# Proteasomal cleavage

http://tools.iedb.org/processing/




- Proteasomes create the C-terminal end of peptides
- Prediction looks for sequence motive up and downstream of potential cleavage site
- Cleavage sequence motif determined based on in vitro protein digests by proteasomes

# TAP Transport

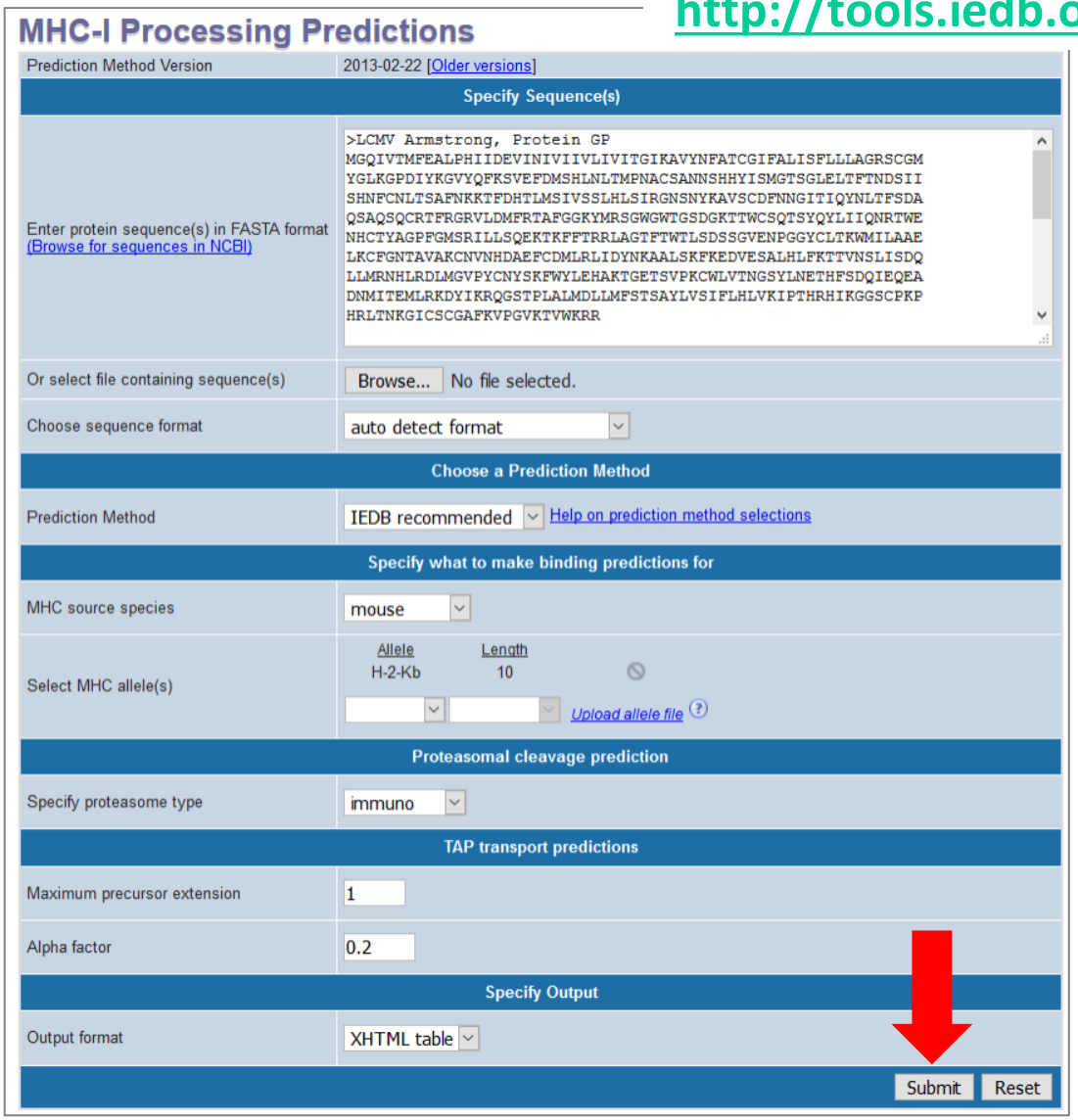
http://tools.iedb.org/processing/

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

- TAP transport efficiency of peptides is sequence dependent; motif derived based on in vitro assays
- Overall TAP transport efficiency of a presented MHC ligand can be result of a collection of precursors
- Unless paper specifically read and details about the precursor length distribution are known, **keep parameters unchanged**

# Class I ‘combined predictor’ - example

http://tools.iedb.org/processing/



# Class I 'combined predictor' - example

## MHC-I Processing Prediction Results

http://tools.iedb.org/processing/

Input Sequences

| # | Name | Sequence |
|---|---|---|
| 1 | LCMV Armstrong, Protein GP | MGQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATCGIFALISFLLLAGRS<br>CGMYGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNACSANNSHHYISMGTSGLELTF<br>TNDSIISHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITI<br>QYNLTFSDAQSAQSQCRTFRGRVLDMFRTAFGGKYMRSGWGWTGSDGKTTWCSQTS<br>YQYLIIQNRTWENHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLSDSSGVEN<br>PGGYCLTKWMILAAELKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKFKEDV<br>ESALHLFKTTVNSLISDQLLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCW<br>LVTNGSYLNETHFSDQIEQEADNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYL<br>VSIFLHLVKIPTHRHIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVWKRR |
| 2 | LCMV Armstrong, Protein NP | MSLSKEVKSFQWTQALRRELQSFTSDVKAAVIKDATNLLNGLDFSEVSNVQRIMRK<br>EKRDDKDLQRLRSLNQTVHSLVDLKSTSKKNVLKVGRLSAEELMSLAADLEKLKAK<br>IMRSERPQASGVYMGNLTTQQLDQRSQILQIVGMRKPQQGASGVVRVWDVKDSSLL<br>NNQFGTMPSLTMACMAKQSQTPLNDVVQALTDLGLLYTVKYPNLNDLERLKDKHPV<br>LGVITEQQSSINISGYNFSLGAAVKAGAALLDGGNMLESILIKPSNSEDLLKAVLG<br>AKRKLNMFVSDQVGDRNPYENILYKVCLSGEGWPYIACRTSIVGRAWENTTIDLTS<br>EKPAVNSPRPAPGAAGPPQVGLSYSQTMLLKDLMGGIDPNAPTWIDIEGRFNDPVE<br>IAIFQPQNGQFIHFYREPVDQKQFKQDSKYSHGMDLADLFNAQPGLTSSVIGALPQ<br>GMVLSCQGSDDIRKLLDSQNRKDIKLIDVEMTREASREYEDKVWDKYGWLCKMHTG<br>IVRDKKKKEITPHCALMDCIIFESASKARLPDLKTVHNILPHDLIFRGPNVVTL |

Prediction method: recommended | High Score = high efficiency

Download result

Citations

| Allele | # | Start | End | Peptide Length | Peptide | Proteasome Score | TAP Score | MHC Score | Processing Score | Total Score | MHC IC50[nM] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| H-2-Kb | 2 | 203 | 212 | 10 | LLYTVKYPNL | 1.79 | 0.50 | -2.01 | 2.28 | 0.27 | 103.5 |
| H-2-Kb | 1 | 116 | 125 | 10 | SIISHNFCNL | 1.51 | 0.46 | -1.90 | 1.97 | 0.08 | 78.6 |
| H-2-Kb | 2 | 7 | 16 | 10 | VKSFQWTQAL | 1.42 | 0.39 | -2.01 | 1.81 | -0.21 | 102.9 |
| H-2-Kb | 2 | 235 | 244 | 10 | INISGYNFSL | 1.53 | 0.41 | -2.18 | 1.95 | -0.23 | 149.7 |
| H-2-Kb | 1 | 35 | 44 | 10 | VYNFATCGIF | 1.42 | 1.27 | -3.12 | 2.68 | -0.44 | 1327 |
| H-2-Kb | 1 | 75 | 84 | 10 | KSVEFDMSHL | 1.50 | 0.47 | -2.45 | 1.96 | -0.49 | 283.6 |
| H-2-Kb | 1 | 369 | 378 | 10 | YCNYSKFWYL | 1.45 | 0.31 | -2.32 | 1.76 | -0.56 | 206.8 |
| H-2-Kb | 1 | 449 | 458 | 10 | VSIFLHLVKI | 1.33 | 0.33 | -2.25 | 1.66 | -0.59 | 178.2 |

# Class I 'combined predictor' - example

http://tools.iedb.org/processing/

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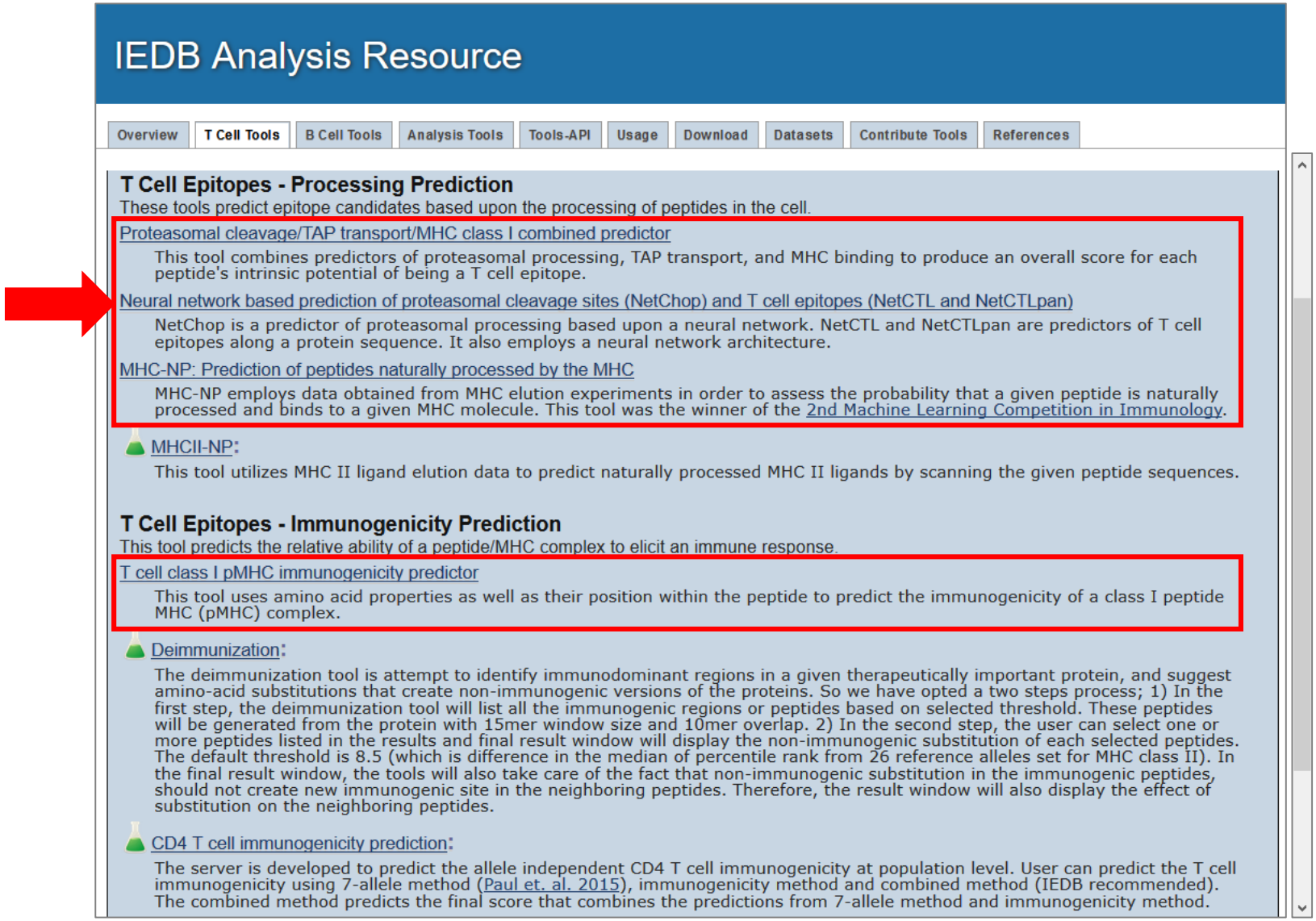
- Higher scores = higher efficiency for MHC-I presentation
- MHC binding score = –log10(IC50) (sign change)
- Combined scores are additive
  - Processing = proteasome + TAP
  - Total = proteasome + TAP + MHC
- Different variance in scores reflects different selectivity

# Caveats / performance of processing predictions

- Processing predictions beat MHC binding predictions when predicting **eluted peptides**
- No clear evidence that processing predictions are better at predicting **epitopes**
- Eluted peptides may over represent ‘best possible’ ligands, and the difference in processing may not be relevant in practice
- Co-evolution of MHC molecules to bind peptides with motifs that are generated by proteasome and TAP means that most high affinity MHC binders are also efficiently processed
- Recommendation: Use MHC binding predictions alone by default
  - If resources require limiting the number of peptides considered, use total score of processing predictions as additional filter

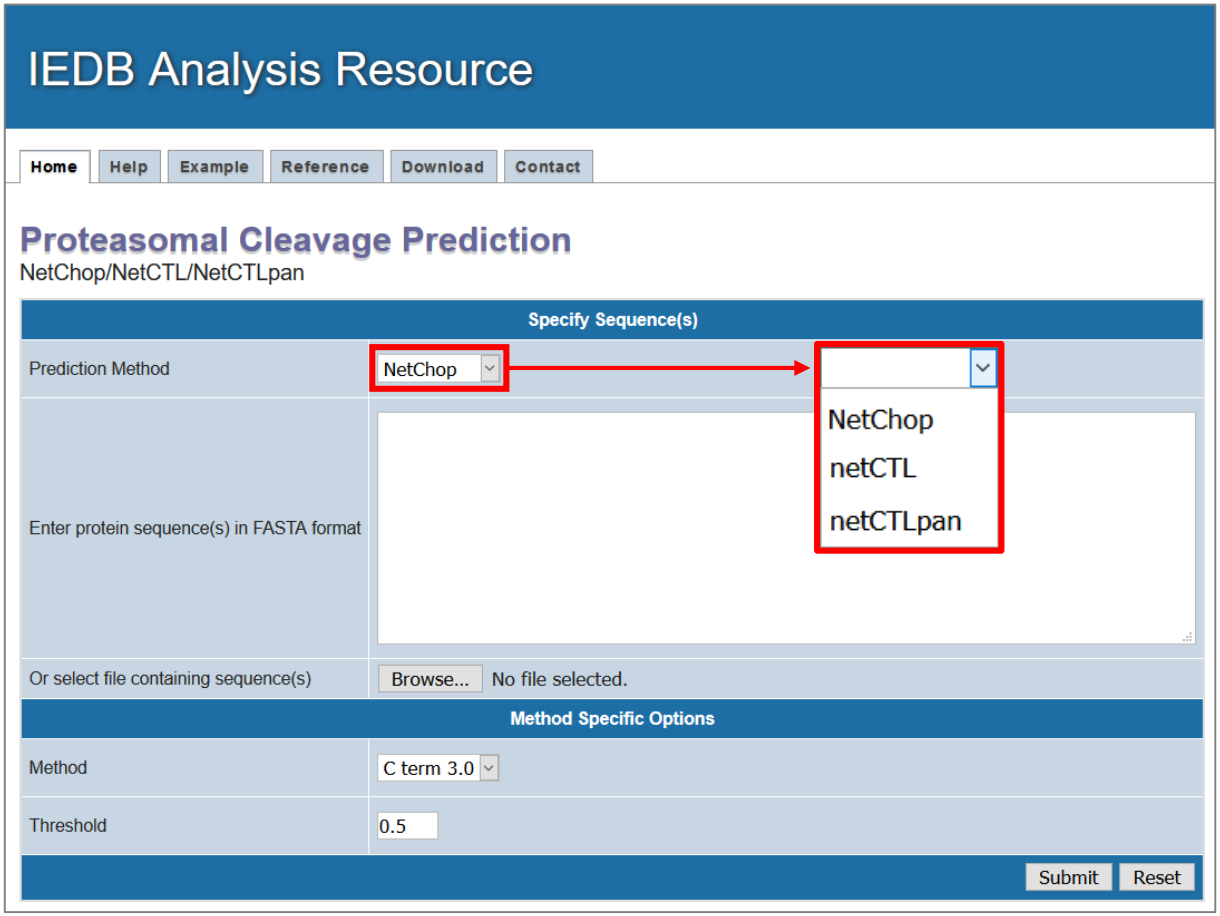
# Class I Processing + immunogenicity tools available in the IEDB

http://tools.iedb.org/main/tcell/



# Neural Network based predictors

http://tools.iedb.org/netchop/




- **NetChop**: proteasomal cleavage
- **NetCTL:** combines NetChop, TAP transport, NetMHC
- **NetCTLpan**: combines NetChop, TAP transport, NetMHCpan

**Key difference is the use of NetChop**

# Class I Processing + immunogenicity tools available in the IEDB

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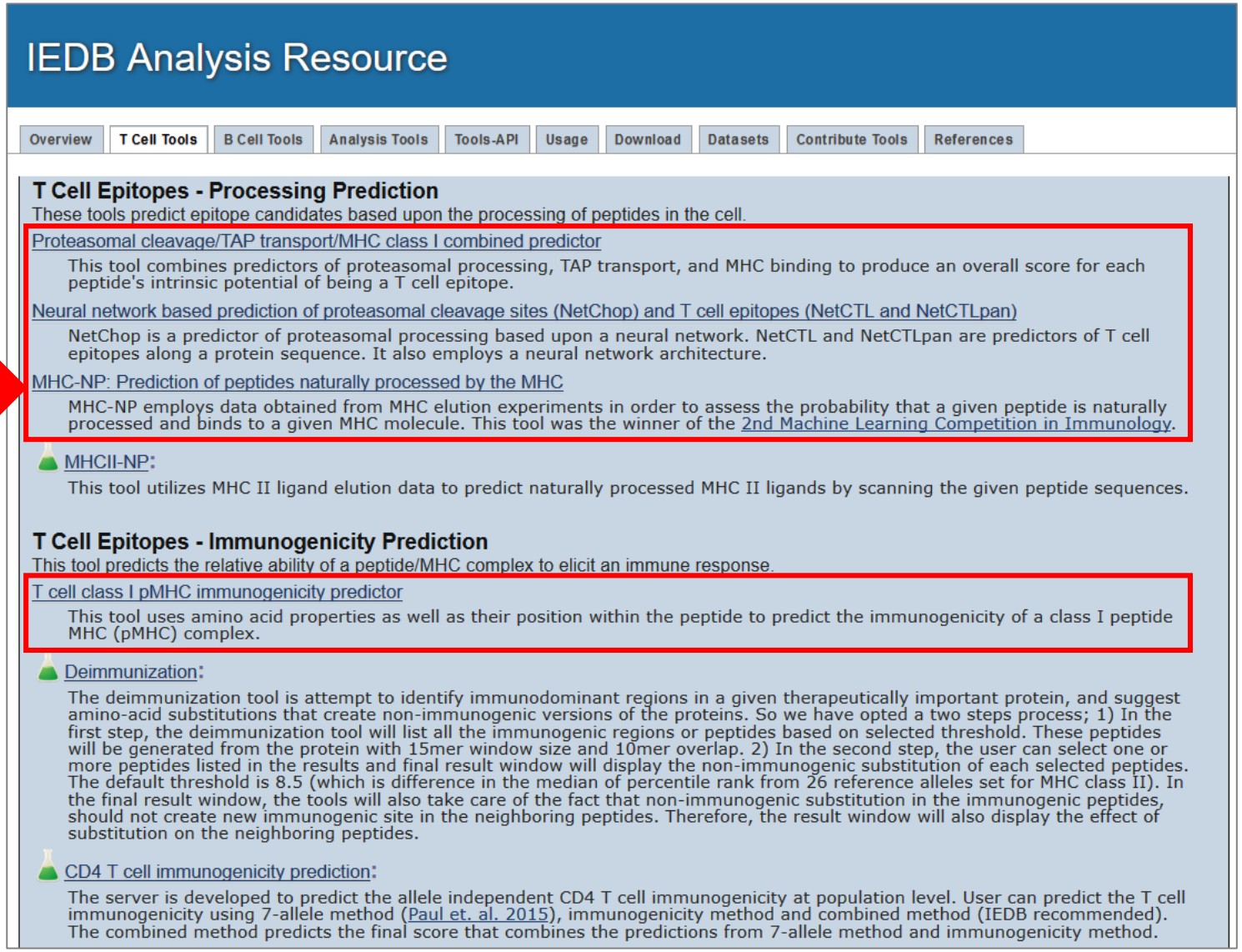
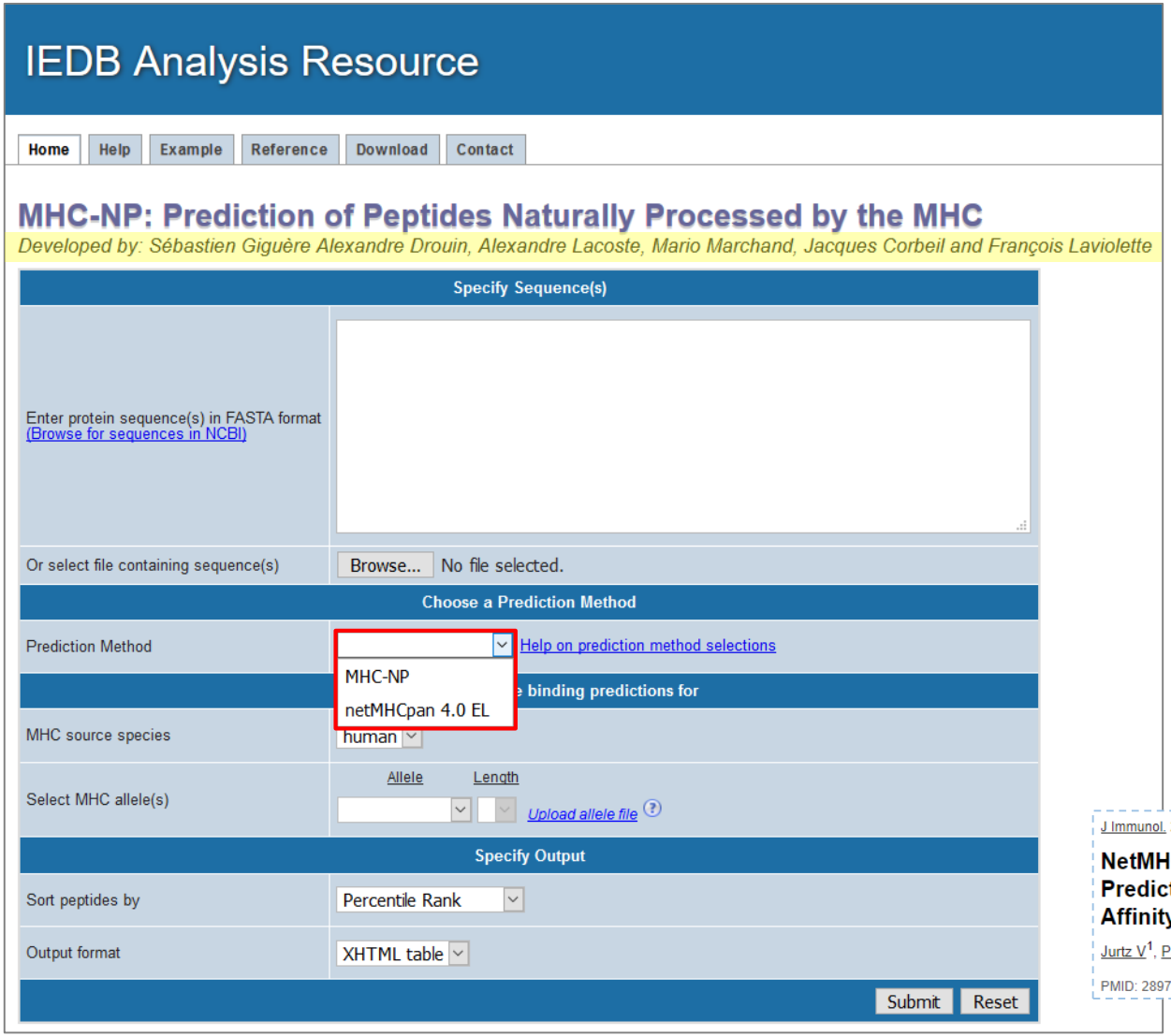


# MHC-NP: Prediction of peptides naturally processed by the MHC

http://tools.iedb.org/mhcnp/




Pan-predictions trained on both binding + eluted ligand data now available!

J Immunol. 2017 Nov 1;199(9):3360-3368. doi: 10.4049/jimmunol.1700893. Epub 2017 Oct 4.

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

Jurtz V[1], Paul S[2], Andreatta M[3], Marcatili P[1], Peters B[2], Nielsen M[4,3].

PMID: 28978689 PMCID: PMC5679736 DOI: 10.4049/jimmunol.1700893

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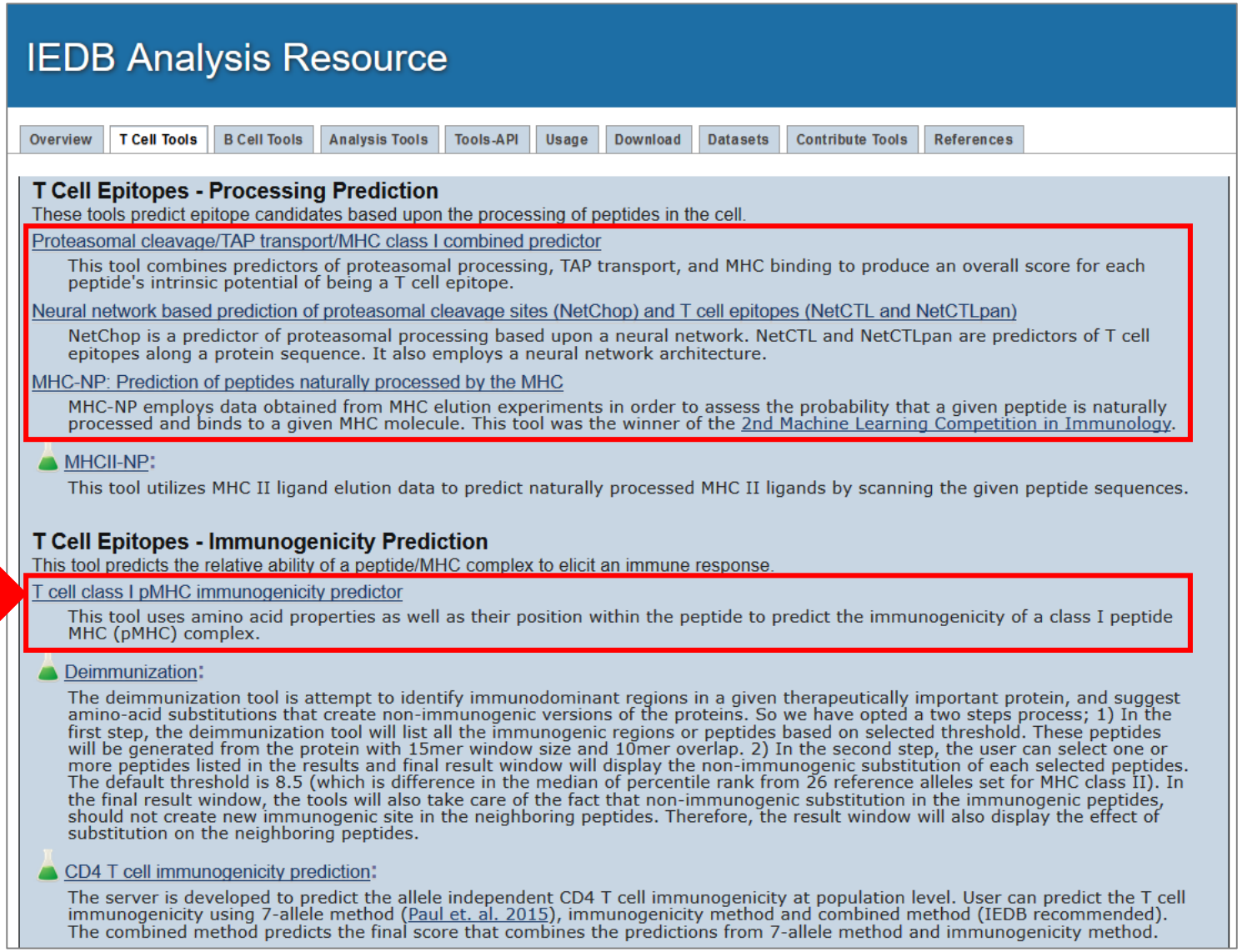
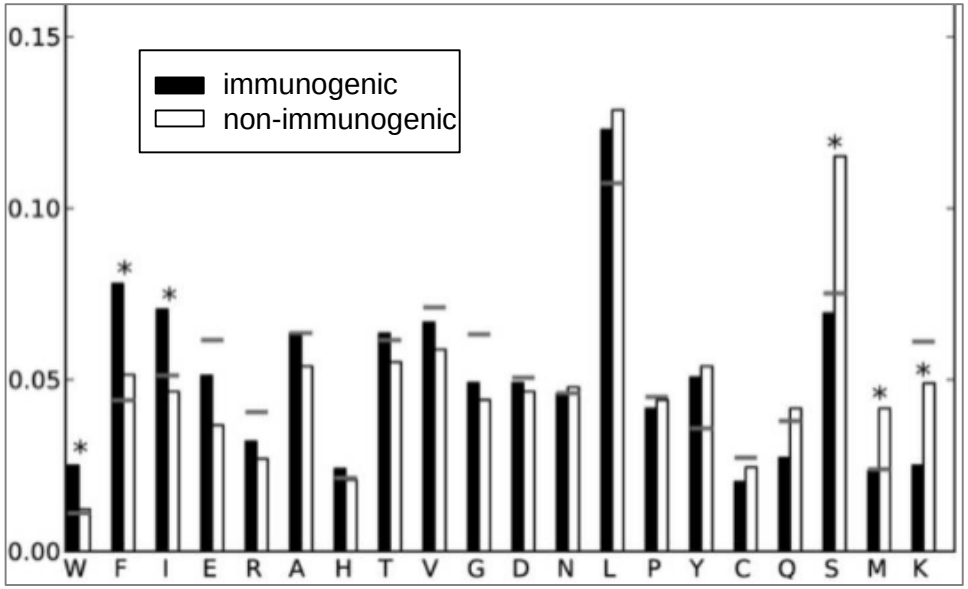


# Class I immunogenicity prediction

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

- Enrichment of W,F,I and depletion of S,M,K in immunogenic peptides

- Use enrichments to calculate propensity scores




PLoS Comput Biol. 2013 Oct;9(10):e1003266. doi: 10.1371/journal.pcbi.1003266. Epub 2013 Oct 24.

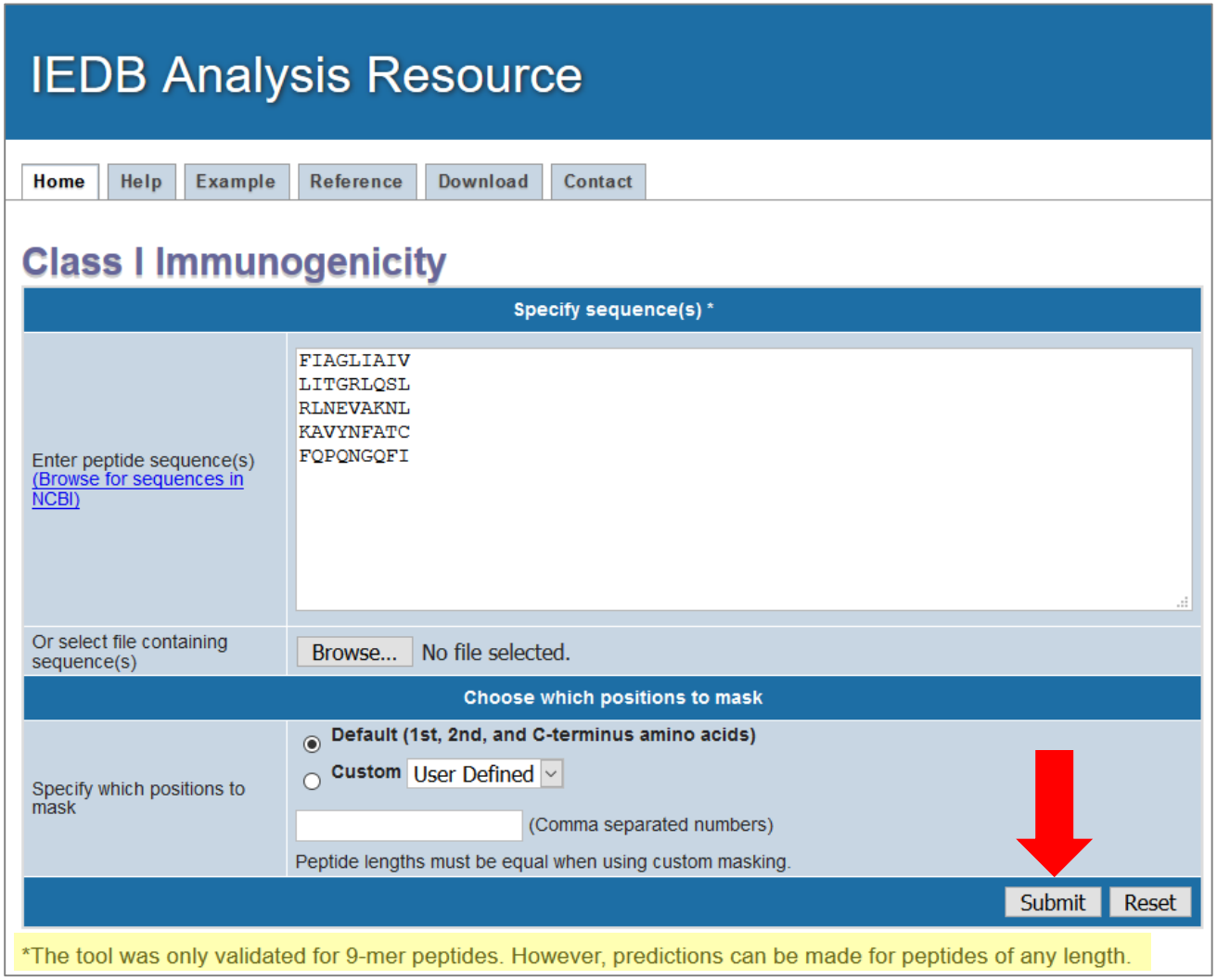
**Properties of MHC class I presented peptides that enhance immunogenicity.**

Calis JJ[1], Maybeno M, Greenbaum JA, Weiskopf D, De Silva AD, Sette A, Keşmir C, Peters B.

PMID: 24204222 PMCID: PMC3808449 DOI: 10.1371/journal.pcbi.1003266

# Class I immunogenicity prediction -example

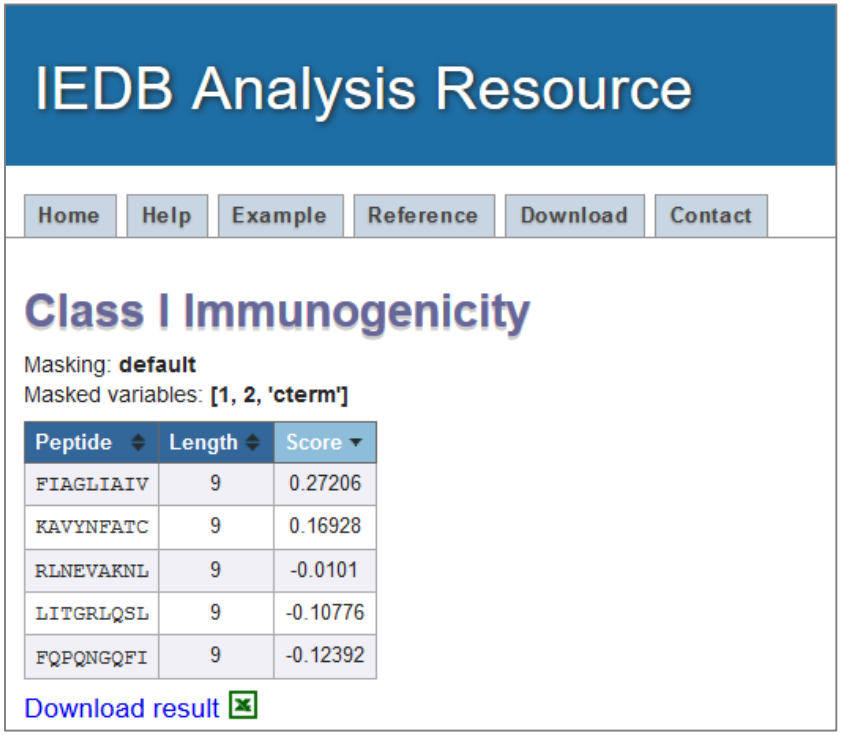
http://tools.iedb.org/immunogenicity/




**Mask positions that are MHC anchors**

# Class I immunogenicity prediction -example

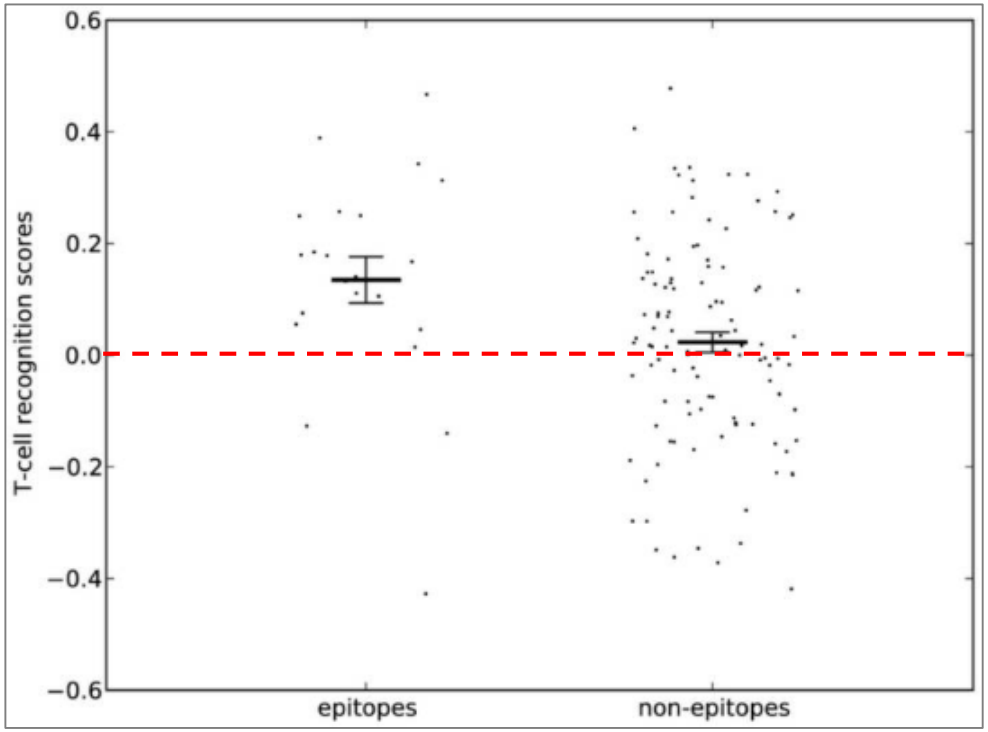
http://tools.iedb.org/immunogenicity/




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

# Class I immunogenicity prediction caveats / performance

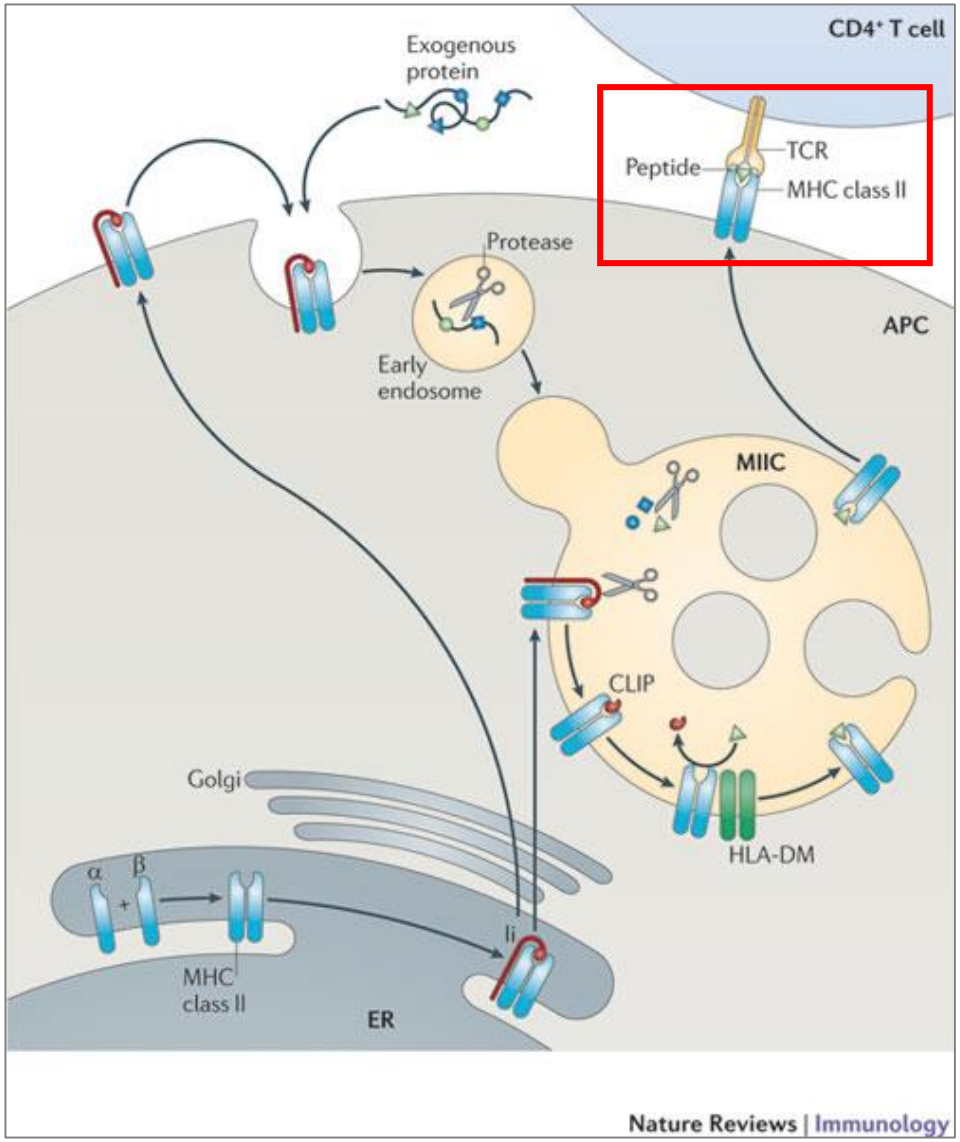
- Experimentally, many MHC binding peptides can be immunogenic (~50%)
- Cross validation gave AUC values ~ 0.65. Test on independent blind set gave AUC = 0.69
- Recommendation: Use as filter (cutoff 0) if high specificity is desired. Suggested cutoff is 0



# Class I Summary

- Processing predictions are better at identifying naturally processed ligands, but have not been shown to be superior in identifying epitopes compared to MHC binding predictions
- Specific processing and immunogenicity predictions are good additional filters if the only goal is to select high likelihood T cell epitopes
- NetMHCPan 4.0 EL scores, which are trained on both MHC binding and ligand elution data are a straightforward replacement of MHC binding predictions, and show some enhanced performance
  - Use these, and consider pairing with immunogenicity scores, when predicting epitope candidates

# CD4 T cell epitopes (MHC class II)



# Class II Processing + immunogenicity tools available in the IEDB

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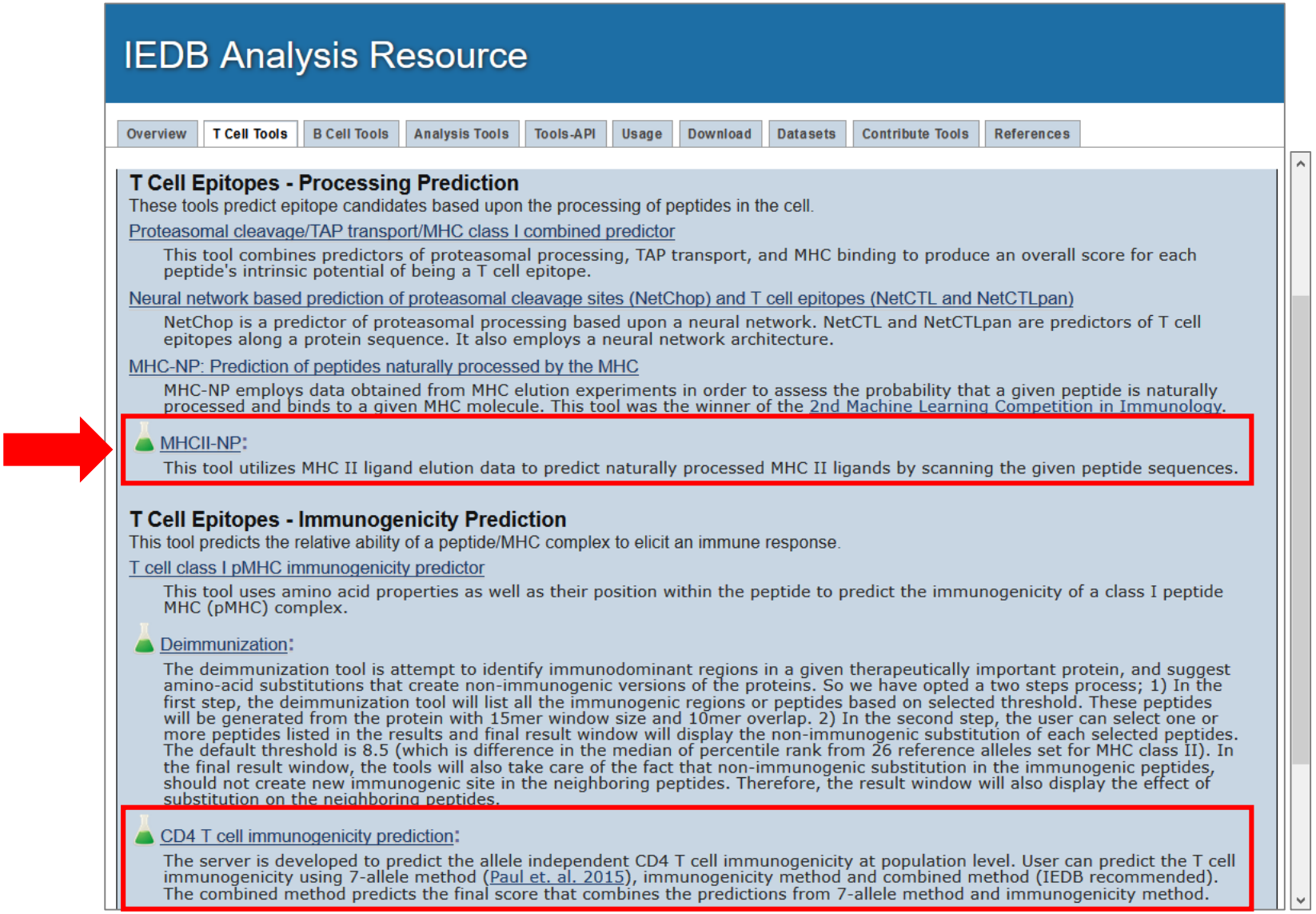
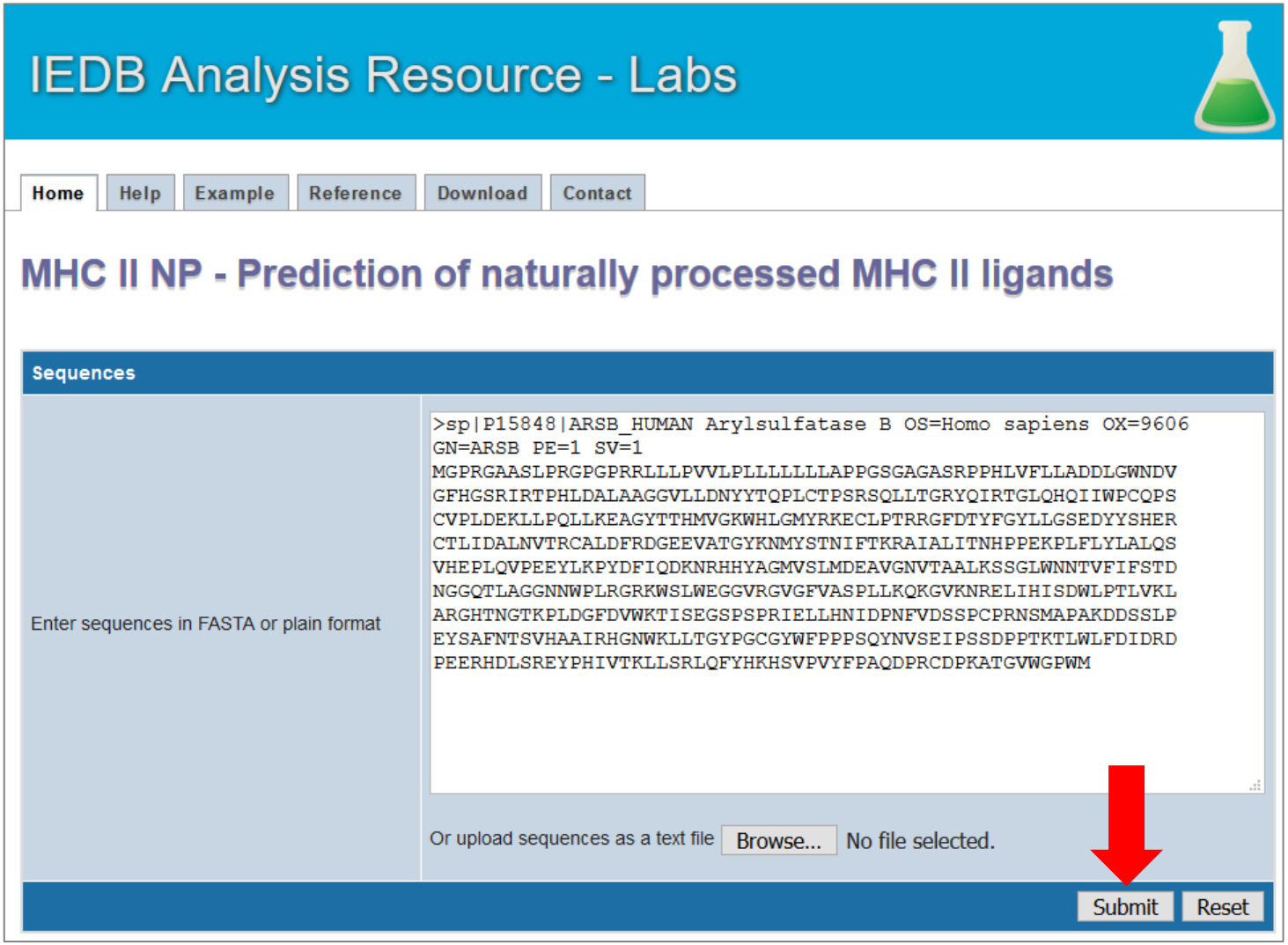


# MHCII-NP

- Predicting the naturally processed peptides for MHC class II
- Based on
  - Cleavage motif analysis at C and N terminal of peptides
  - Ligand elution data derived from IEDB
- Ligand predictions is improved markedly when combining the binding and cleavage motifs
- T cell epitope prediction is not significantly improved

# MHCII-NP - example

http://tools.iedb.org/mhciinp/



# MHCII-NP -example

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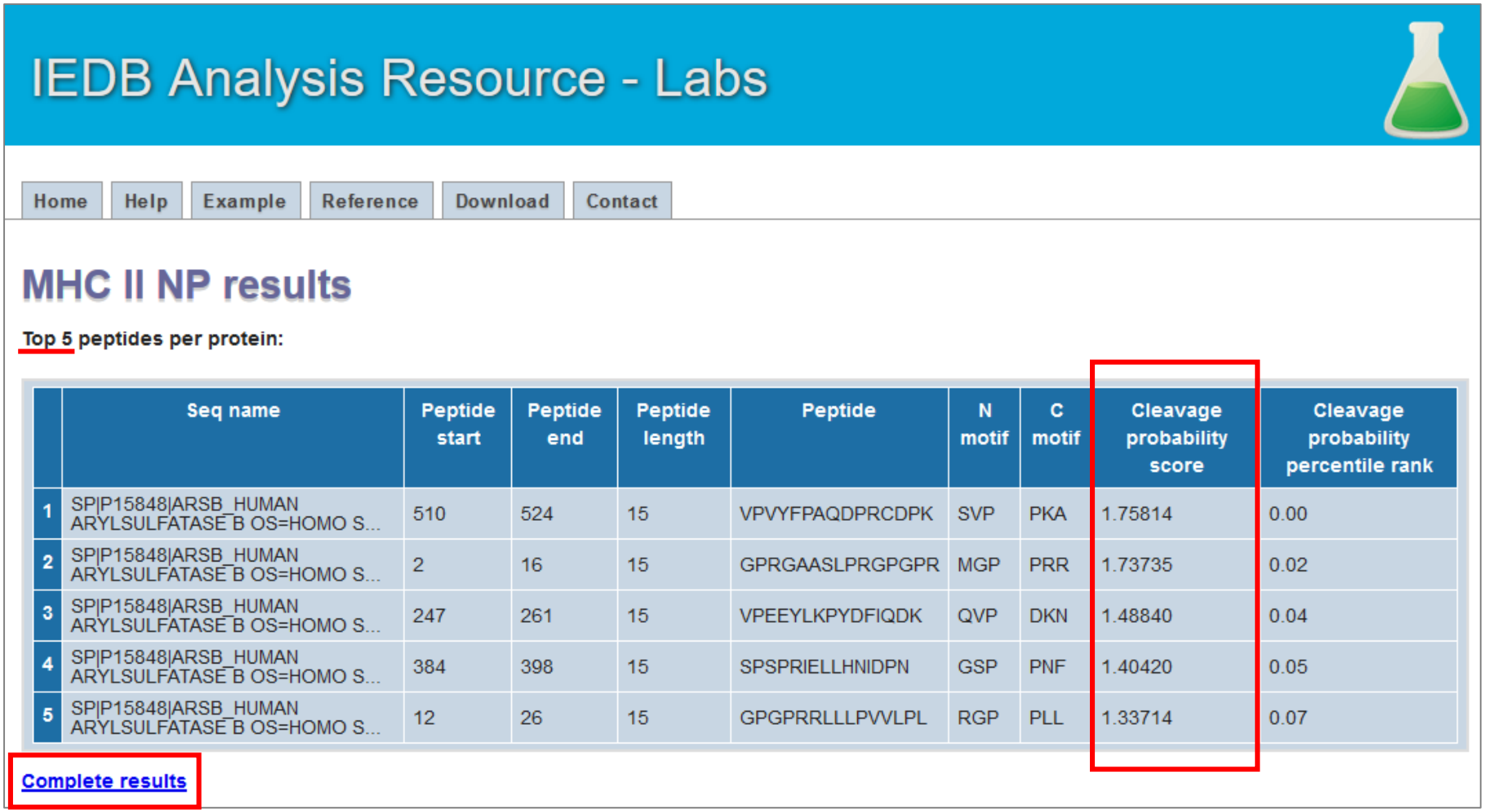



## MHC II NP results

**Top 5 peptides per protein:**

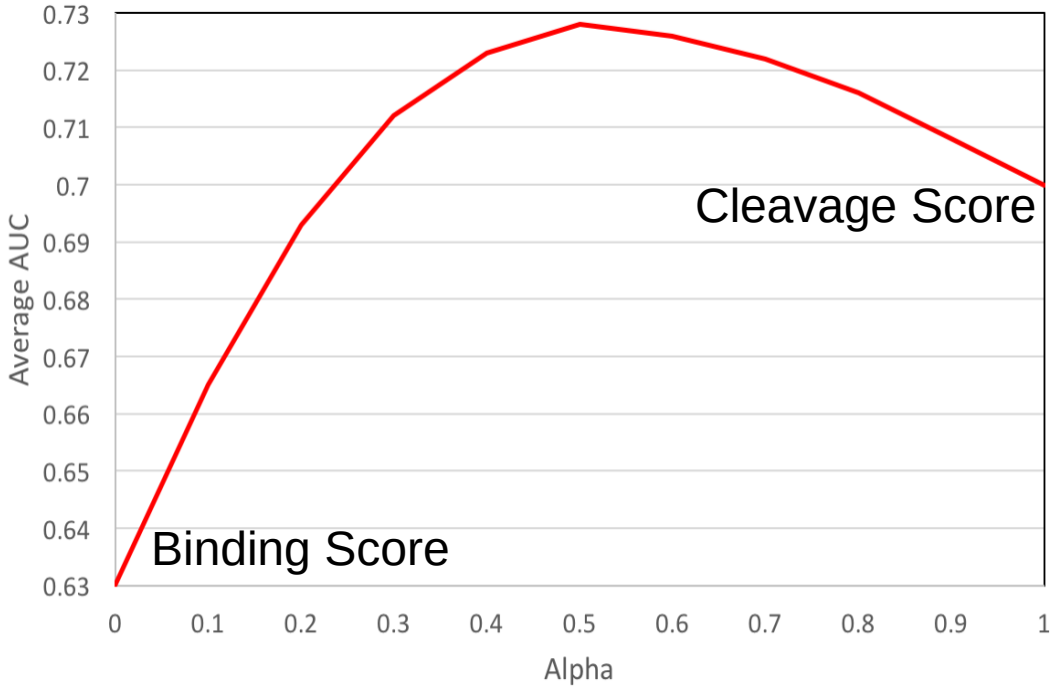
| | Seq name | Peptide start | Peptide end | Peptide length | Peptide | N motif | C motif | Cleavage probability score | Cleavage probability percentile rank |
|---|---|---|---|---|---|---|---|---|---|
| 1 | SP\|P15848\|ARSB_HUMAN ARYLSULFATASE B OS=HOMO S... | 510 | 524 | 15 | VPVYFPAQDPRCDPK | SVP | PKA | 1.75814 | 0.00 |
| 2 | SP\|P15848\|ARSB_HUMAN ARYLSULFATASE B OS=HOMO S... | 2 | 16 | 15 | GPRGAASLPRGPGPR | MGP | PRR | 1.73735 | 0.02 |
| 3 | SP\|P15848\|ARSB_HUMAN ARYLSULFATASE B OS=HOMO S... | 247 | 261 | 15 | VPEEYLKPYDFIQDK | QVP | DKN | 1.48840 | 0.04 |
| 4 | SP\|P15848\|ARSB_HUMAN ARYLSULFATASE B OS=HOMO S... | 384 | 398 | 15 | SPSPRIELLHNIDPN | GSP | PNF | 1.40420 | 0.05 |
| 5 | SP\|P15848\|ARSB_HUMAN ARYLSULFATASE B OS=HOMO S... | 12 | 26 | 15 | GPGPRRLLLPVVLPL | RGP | PLL | 1.33714 | 0.07 |

Complete results

# MHCII-NP scores

- Cleavage Score: Derived from the cleavage motif analysis in ligand elution data
- Binding Score: Derived from HLA binding affinity using 7-allele method (Paul et. al. 2015).

$$Combined\ score = \alpha \times cleavage\ \ probability\ score + (1 - \alpha) \times binding\ \ score$$




Barra et al. Genome Medicine (2018) 10:84
https://doi.org/10.1186/s13073-018-0594-6

Genome Medicine

RESEARCH Open Access

Footprints of antigen processing boost MHC class II natural ligand predictions

Carolina Barra[1*], Bruno Alvarez[1†], Sinu Paul[2], Alessandro Sette[2], Bjoern Peters[2], Massimo Andreatta[1], Søren Buus[3] and Morten Nielsen[1,4*]

Front Immunol. 2018 Aug 6;9:1795. doi: 10.3389/fimmu.2018.01795. eCollection 2018.

**Determination of a Predictive Cleavage Motif for Eluted Major Histocompatibility Complex Class II Ligands.**

Paul S[1], Karosiene E[1], Dhanda SK[1], Jurtz V[2], Edwards L[1], Nielsen M[2,3], Sette A[1,4], Peters B[1,4].

PMID: 30127785 PMCID: PMC6087742 DOI: 10.3389/fimmu.2018.01795

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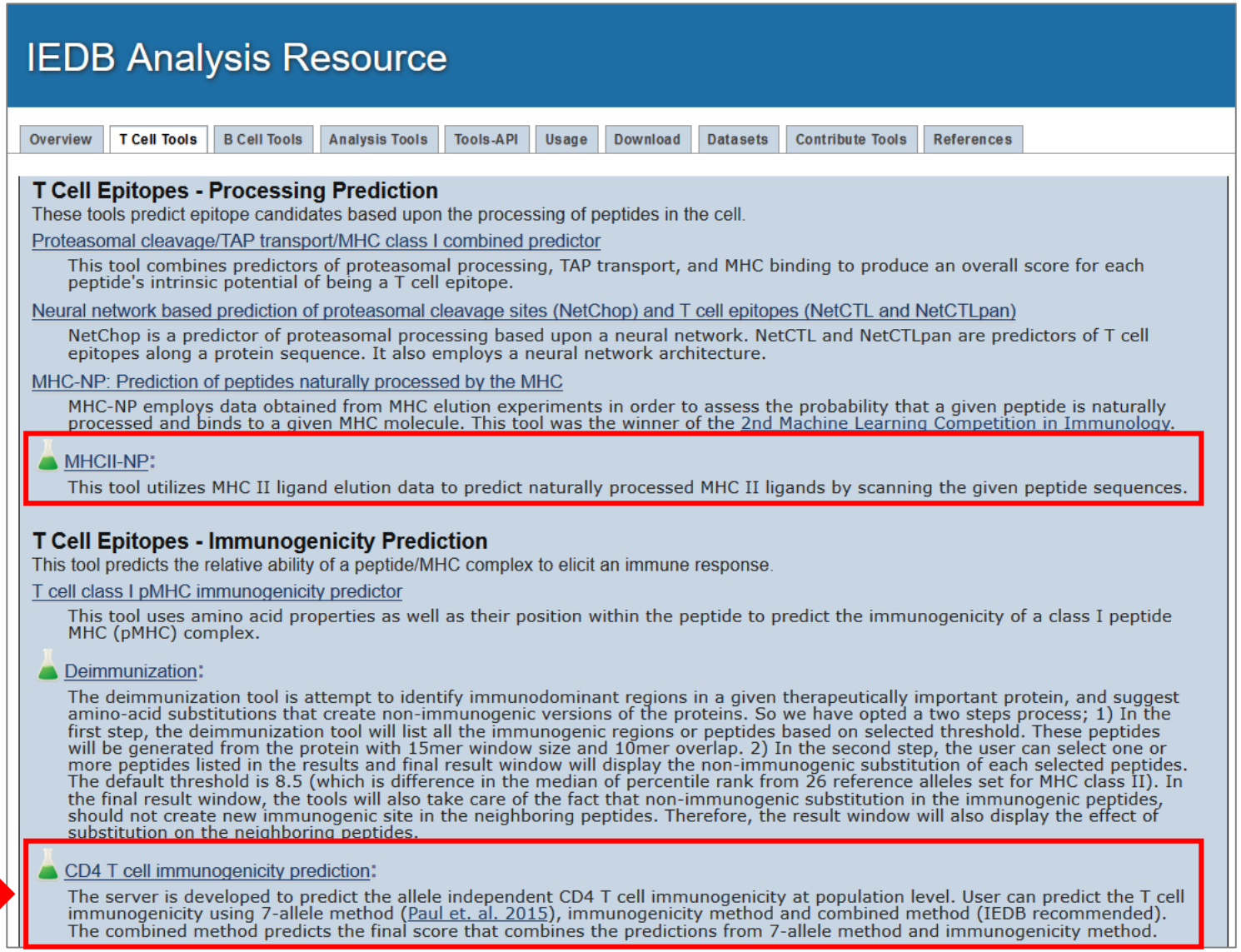


# MHC-II restricted immunogenicity prediction

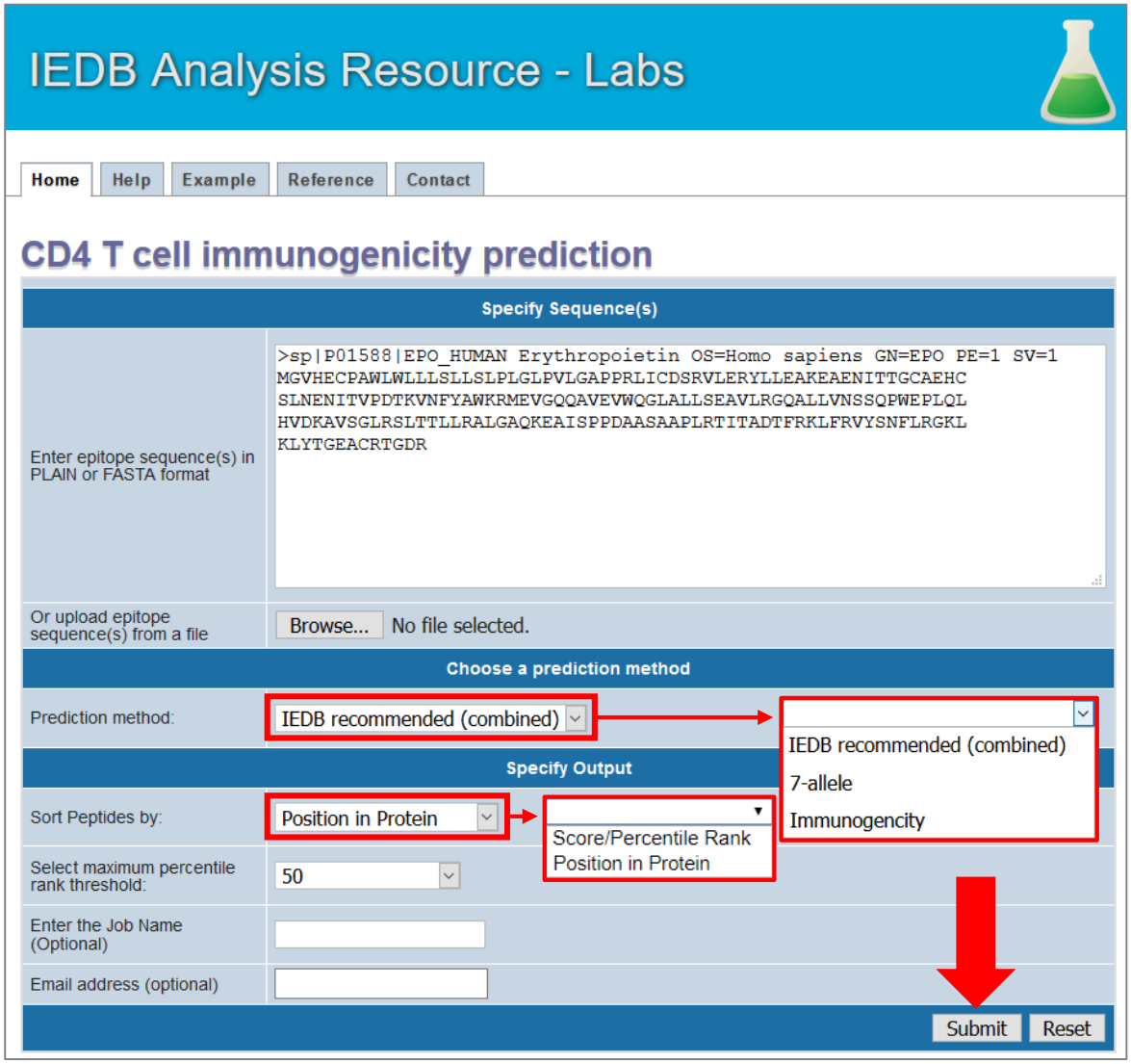
- Extracted datasets of proteins from the IEDB for which overlapping peptides were tested for immunogenicity
- Utilized these datasets to train a Neural Network to learn ‘motifs’ associated with immunogenicity independent of specific MHC alleles expressed
- Resulting score can be combined with ‘7 allele method’ quantifying MHC binding across alleles to predict overall immunogenicity

# Class II immunogenicity prediction

- Based on  Neural network model trained on
  - In house dataset for different antigens tested on different population cohorts
  - Tetramer dataset- derived from IEDB
- Validated on 57 independent studies from different groups across the world
- Implemented three approaches
  - 7-allele method (*Paul et. al. 2015*)
  - Immunogenicity predictions
  - Hybrid approach

# Class II immunogenicity prediction -example

http://tools.iedb.org/CD4episcore/



# Class II immunogenicity prediction - example

http://tools.iedb.org/CD4episcore/

IEDB Analysis Resource - Labs

Home | Help | Example | Reference | Contact

## CD4 Immunogenicity prediction results

**Number of proteins:** 1

**Number of 15mer (overlapping 10mer):** 37

**Threshold :** 50.0%

**Method :** combined

Download result

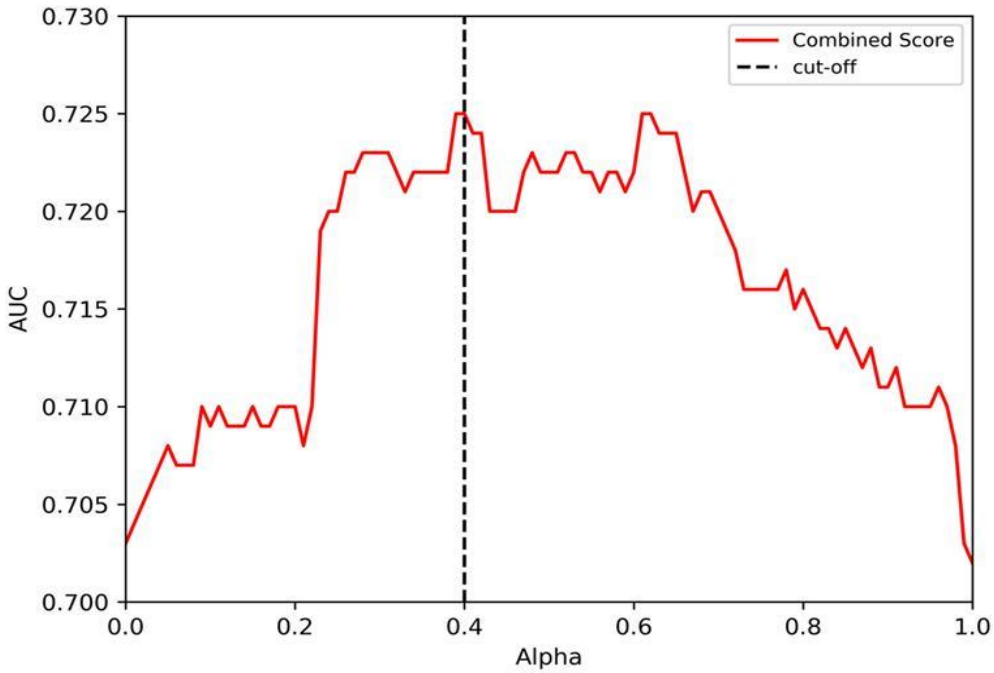
Citations

| Protein Number | Protein Description | Peptide | Start | End | Combined Score | Immunogenicity Score | Peptide core | Median Percentile Rank (7-allele) | HLA-DRB1:03:01 | HLA-DRB1:07:01 | HLA-DRB1:15:01 | HLA-DRB3:01:01 | HLA-DRB3:02:02 | HLA-DRB4:01:01 | HLA-DRB5:01:01 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | sp\|P01588\|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 | WLLLSLLSLPLGLPV | 11 | 25 | 42.16452 | 95.0613 | LLSLLSLPL | 6.9 | 25.0 | 3.2 | 3.6 | 73.0 | 33.0 | 6.9 | 6.5 |
| 1 | sp\|P01588\|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 | TKVNFYAWKRMEVGQ | 71 | 85 | 47.39488 | 67.4872 | TKVNFYAWK | 34.0 | 52.0 | 22.0 | 15.0 | 71.0 | 30.0 | 65.0 | 34.0 |
| 1 | sp\|P01588\|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 | EPLQLHVDKAVSGLR | 116 | 130 | 32.55636 | 43.8909 | LHVDKAVSG | 25.0 | 5.4 | 59.0 | 40.0 | 22.0 | 7.0 | 38.0 | 25.0 |
| 1 | sp\|P01588\|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 | VSGLRSLTTLLRALG | 126 | 140 | 44.95964 | 86.8991 | LTTLLRALG | 17.0 | 12.0 | 17.0 | 9.3 | 70.0 | 20.0 | 20.0 | 1.3 |
| 1 | sp\|P01588\|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 | SLTTLLRALGAQKEA | 131 | 145 | 42.78744 | 69.4686 | LLRALGAQK | 25.0 | 47.0 | 46.0 | 21.0 | 89.0 | 25.0 | 14.0 | 1.6 |
| 1 | sp\|P01588\|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 | PLRTITADTFRKLFR | 156 | 170 | 46.18064 | 85.4516 | LRTITADTF | 20.0 | 6.1 | 47.0 | 40.0 | 8.0 | 20.0 | 20.0 | 20.0 |
| 1 | sp\|P01588\|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 | TADTFRKLFRVYSNF | 161 | 175 | 46.66984 | 44.6746 | FRKLFRVYS | 48.0 | 63.0 | 58.0 | 23.0 | 48.0 | 33.0 | 53.0 | 24.0 |
| 1 | sp\|P01588\|EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 | RKLFRVYSNFLRGKL | 166 | 180 | 13.8966 | 22.4415 | FRVYSNFLR | 8.2 | 53.0 | 8.2 | 0.12 | 27.0 | 4.8 | 26.0 | 1.5 |

# Class II immunogenicity prediction scores

- Immunogenicity Score: Derived from the neural network model trained on Immunogenicity data
- HLA Score: Derived from HLA binding affinity using 7-allele method (Paul et. al. 2015).

$$\text{Combined score} = \alpha \times \text{Imm score} + (1 - \alpha) \times \text{HLA score}.$$

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# Class II Summary

- Similar to MHC class I, enhancement of epitope prediction efficacy is minor compared to using MHC binding predictions alone
- Prediction of naturally eluted ligands is greatly improved with processing predictions
- As of now, recommendation is to stick to allele specific MHC binding predictions (NetMHCPanII), or the 7-allele method for broad populations

→ Both class I and II epitope predictions are constantly being re-evaluated, and these recommendations are subject to change