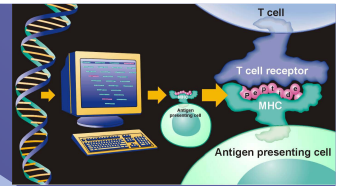
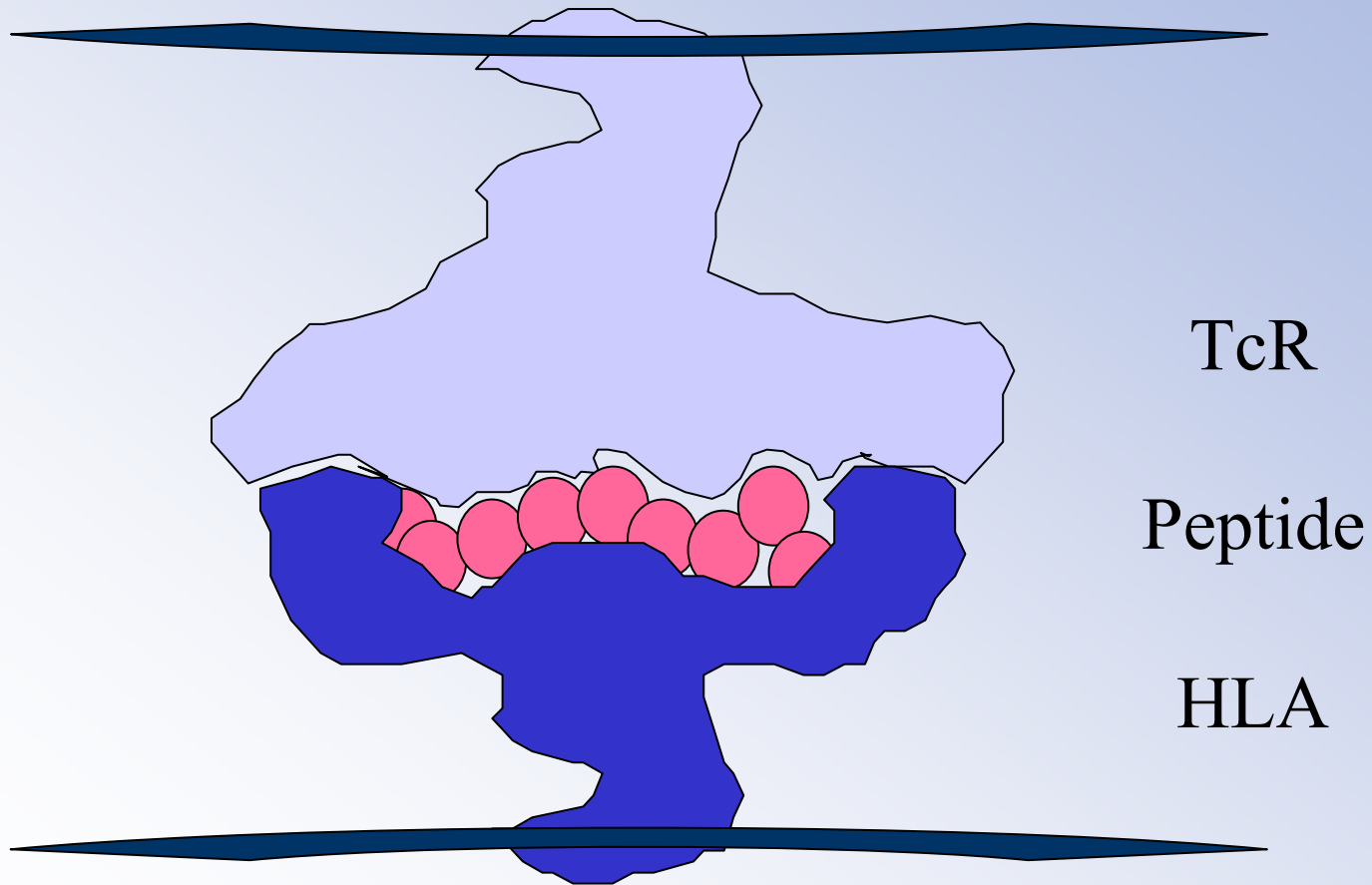


Large-scale T cell Epitope Discovery tools

Professor Soren Buus
University of Copenhagen

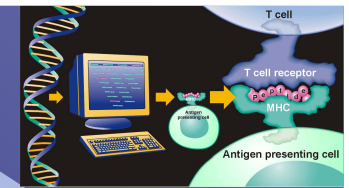
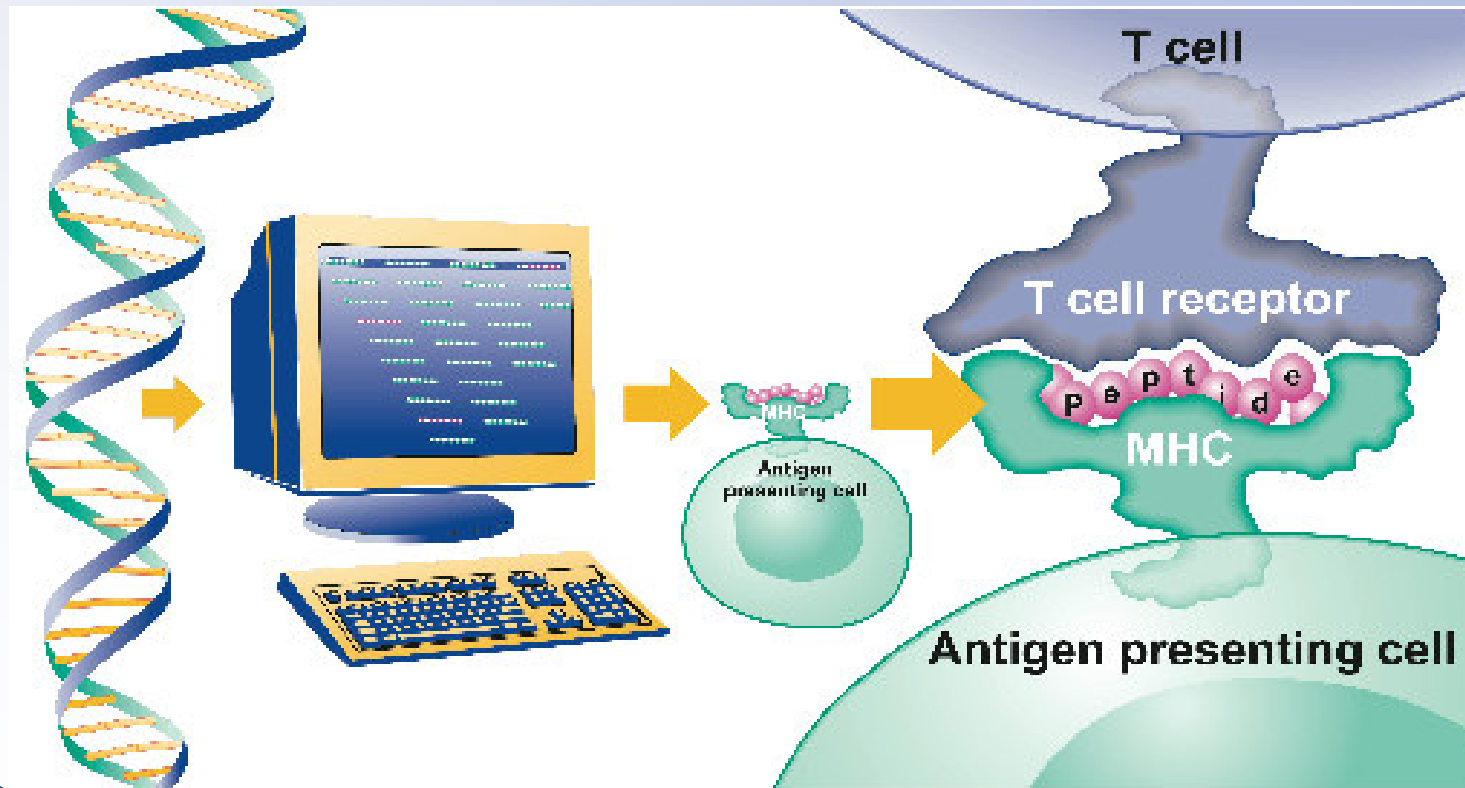


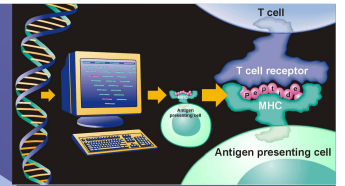
Peptides - prime targets of immune recognition



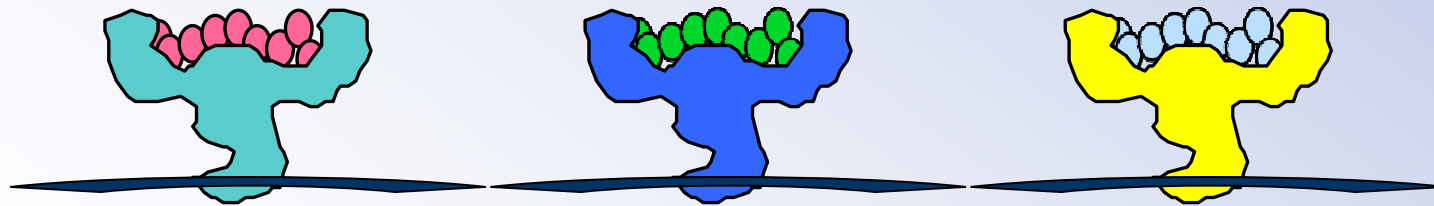
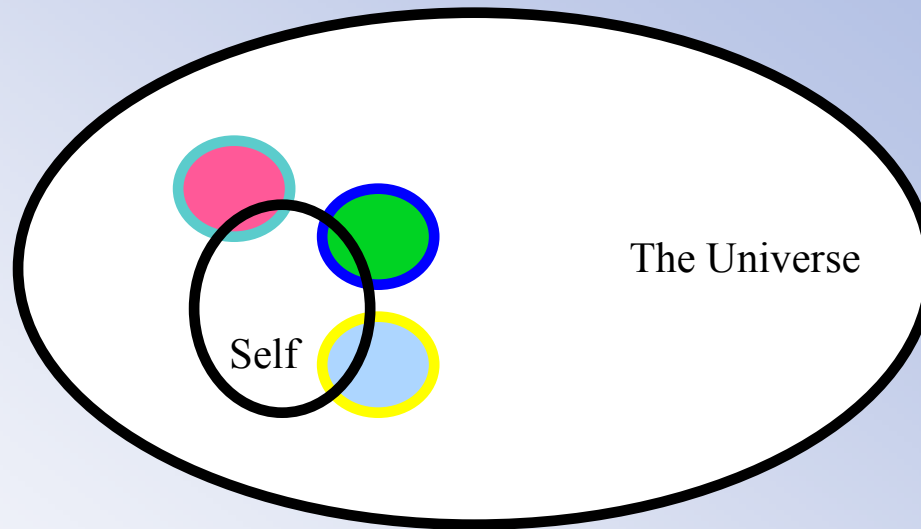
Scientific rationale

- T cells read-off the primary protein sequence (peptides)
- Determine how are proteins handled, peptides are generated & selected
- You should be able to exploit the primary sequence of genomics



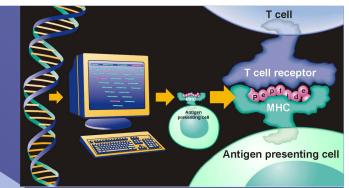


HLA polymorphism and immune specificity



- and there are > 2250 registered HLA class I molecules and > 3000 different HLA class II molecules

HLA polymorphism



The IMGT/HLA Sequence Database currently contains more than 2250 HLA class I proteins

	Oct 2009	Oct 2007	Oct 2005	Oct 2002
HLA-A	681	486	325	224
HLA-B	1165	817	626	446
HLA-C	431	263	165	103
Total	2277	1566	1089	773

Source: <http://www.anthonynolan.com/HIG/index.html>

HLA polymorphism

- and an even greater combinatorial class II polymorphism

2 HLA-DRA

595 HLA-DRB

25 HLA-DQA1

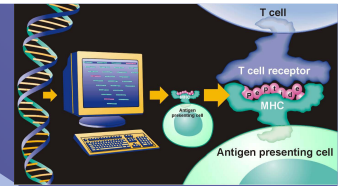
72 HLA-DQB1

16 HLA-DPA1

118 HLA-DPB1

(as of September 2009)

Source: <http://www.anthonynolan.com/HIG/index.html>



HLA recognizes peptide motifs

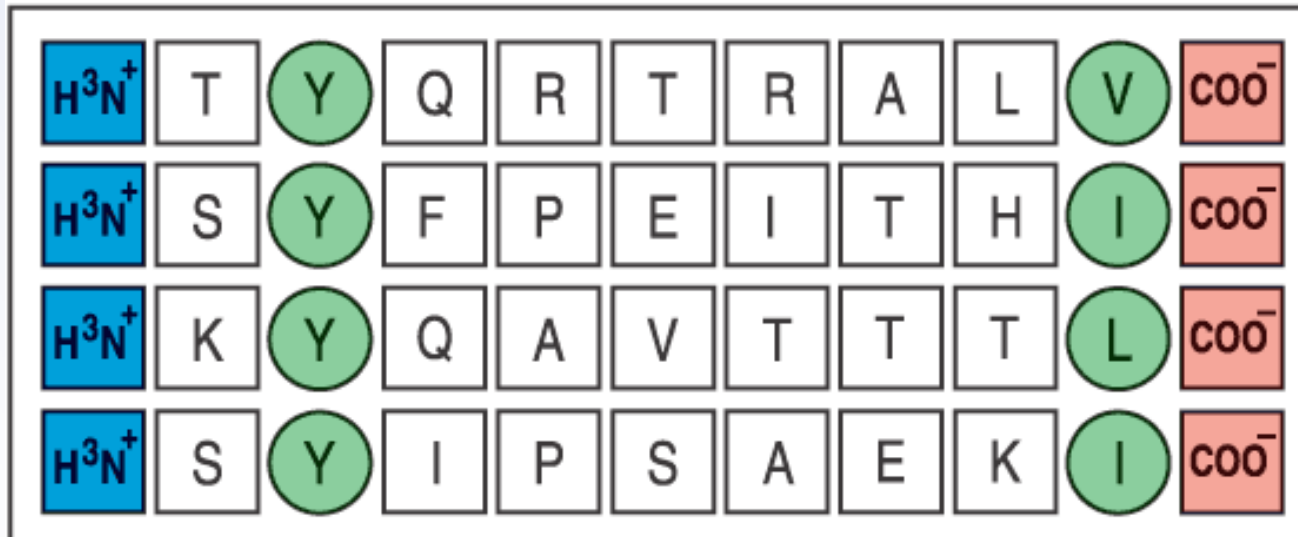
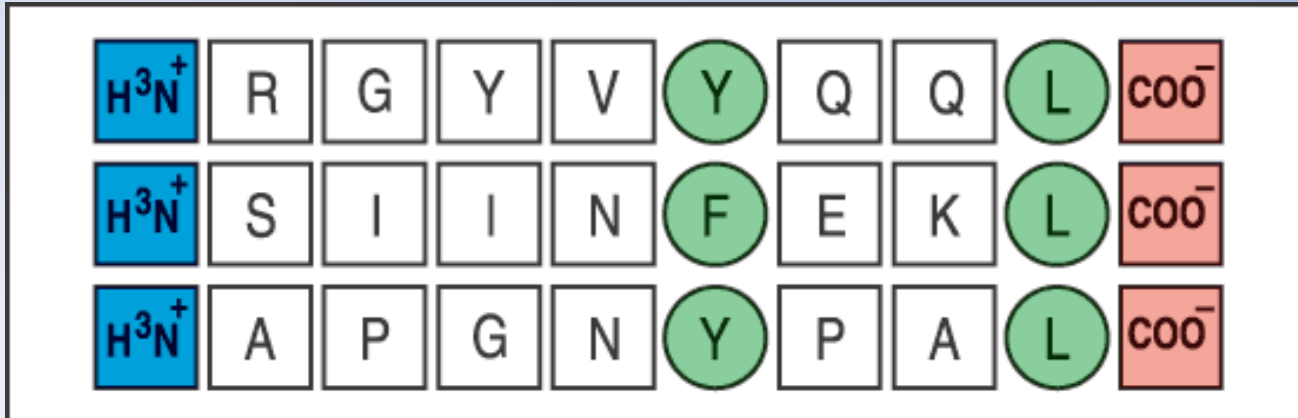
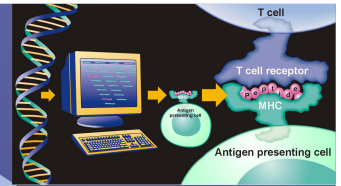


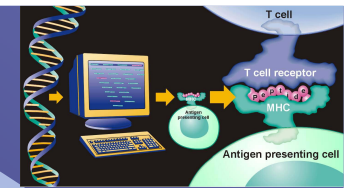
Fig 3.24 © 2001 Garland Science

... but peptide motifs are not easy to interpret

GILKSILKV
S**Q**YDPKELL
TLTRGQNTV
SLYNTIATL**Y**
QVNDVLHSV
KMYEYVFKG
RLGPGKISV
TLKPGTMSV
TMDPSVRVL
FLDDASNS**A**
TVYPKTHYV
SLYNTVATL**Y**
ALDWIGERL
KLTEEIIKL
LLLTLLATV
SLADQLIHL
KLADMSI**Y**C
FMYEDALK**S**
IMALKQAGL
RLQDGEPYL
LMAEDLANV
ILHRLAPWI

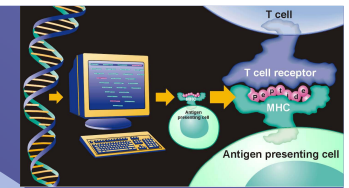
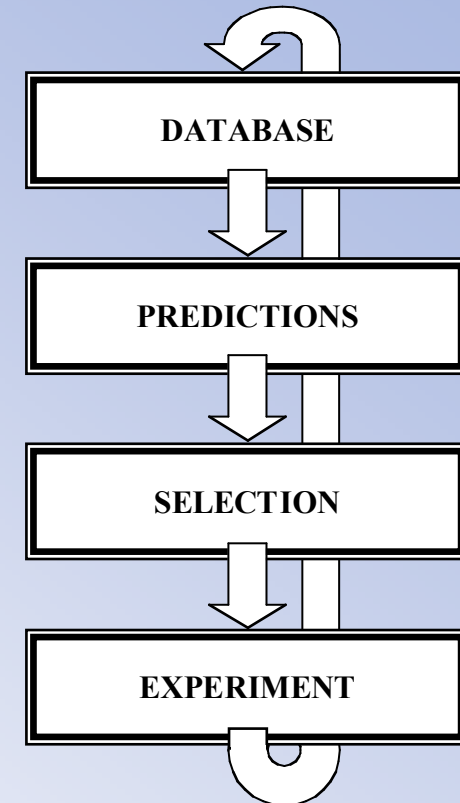
PLNEGIMAV
NISLPLYTV
TFWMSHEV
YMLWNSWL**S**
TVVPLMAFL
YVAAWKAKV
LLKDLMPFV
FLKDVMESM
WLPSGGTLV
W**Q**NLAWAGV
KLAEIFQ**P**F
SIFFDYMAI
ALAGNHWHV
YLLLT**T**NGT
ALQARGHVV
RMWEFLDRL
YVDIIGLSV
FVAEGDALV
YMHGSIHEV
MTSERTLAV
IMGAVLIWV
SLYNTIAVL**Y**

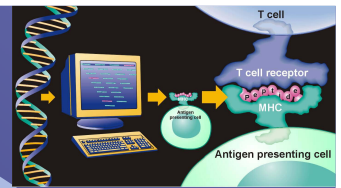
LLPEQLLKI
YIIPCILIL



Technical approach

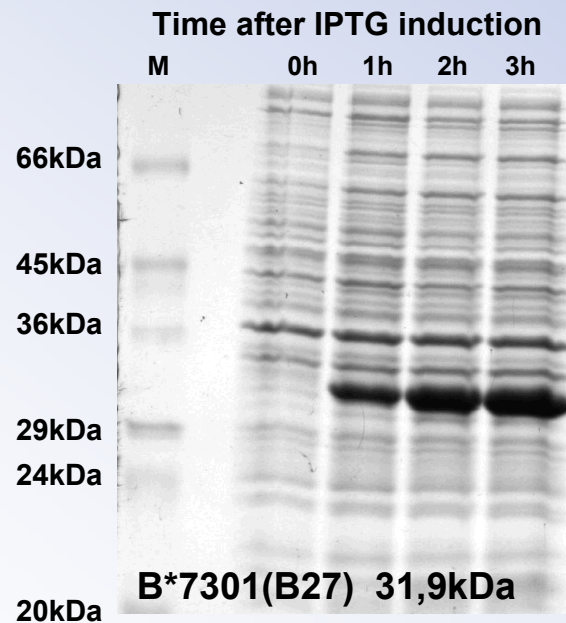
- Generate functional recombinant HLA molecules
- Establish quantitative assay of antigen processing and presentation
- Generate data representing these events
- Develop prediction tools
- Select new data point for experimentation
- Iterate this integrated biochemistry/bioinformatics loop

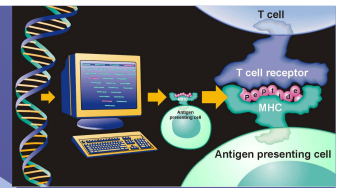




Protein expression

- 2L fed-batch fermentor
 - IPTG induction at $OD_{600} = 25$
 - Harvest inclusion bodies through cell-disrupter
 - Wash inclusion bodies
 - Extract inclusion bodies in 8M urea





Purification of "empty" HLA-I heavy chains

Fermentation

Urea extraction

DNA precipitation

Immobilized Metal Affinity Chromatography

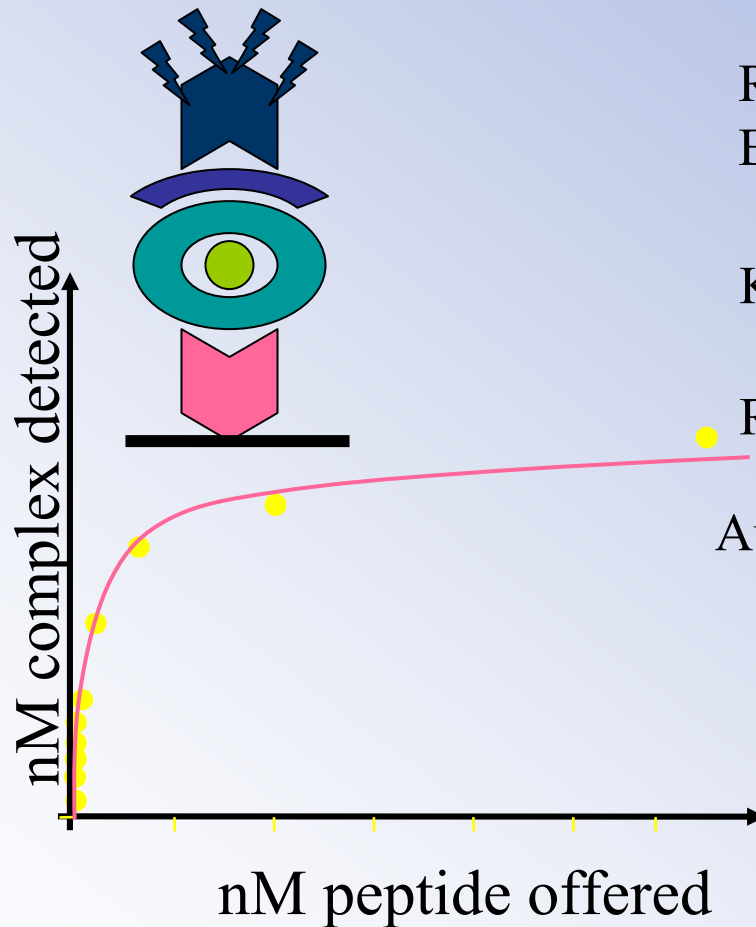
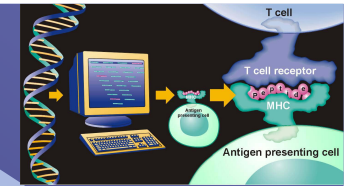
Hydrophobic Interaction Chromatography

Gel Filtration

Biotinylation test

Freezer storage

ELISA driven assay - EpiLISA



Concentrations of complexes generated are plotted as a function of the concentration of peptide offered

Results are expressed as:

B_{MAX} : Amount of detected complex including 95% confidence interval

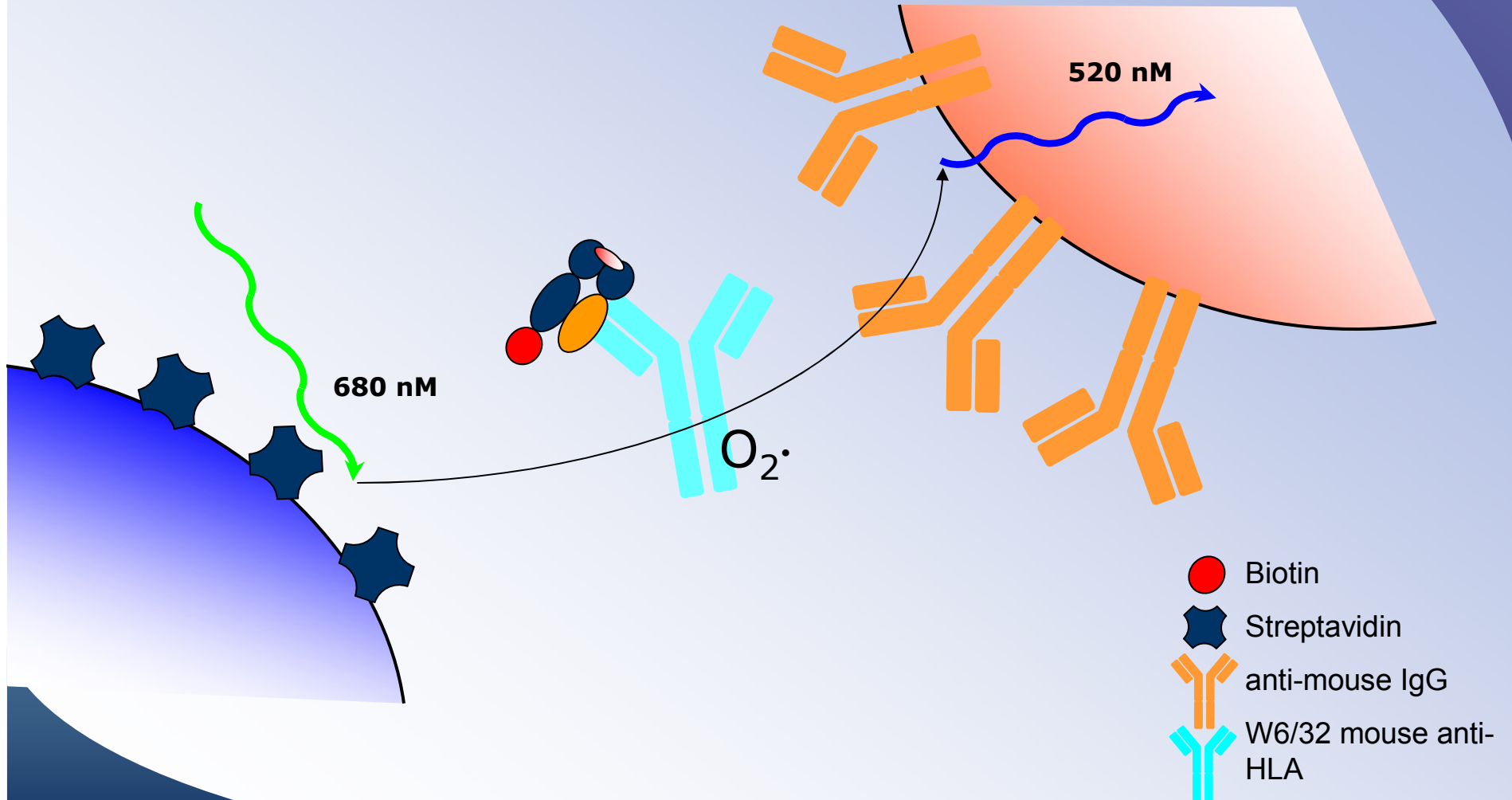
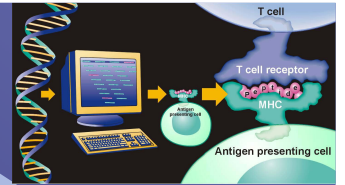
K_D : Peptide affinity including 95% confidence interval

R^2 : Precision

Automated, 384 format

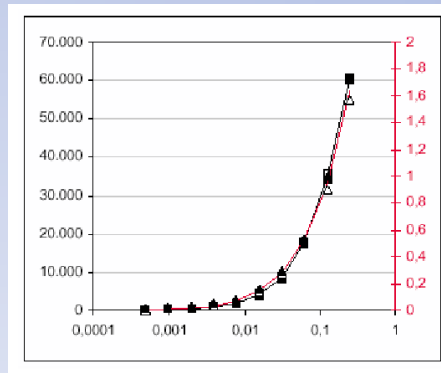
Sylvester-Hvid, C. *et al.*,
Tissue Antigens (2002) 59:251

AlphaScreen®

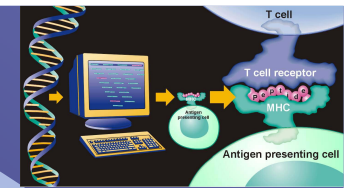
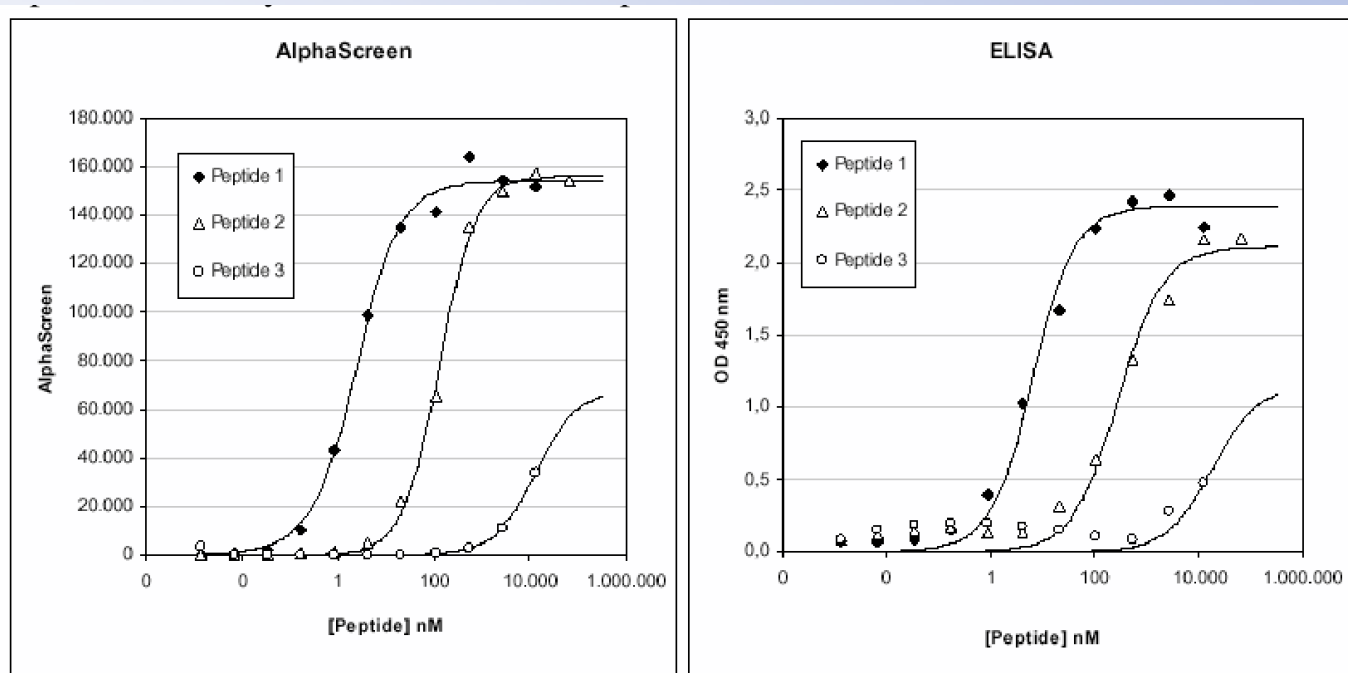


AlphaScreen® - high throughput

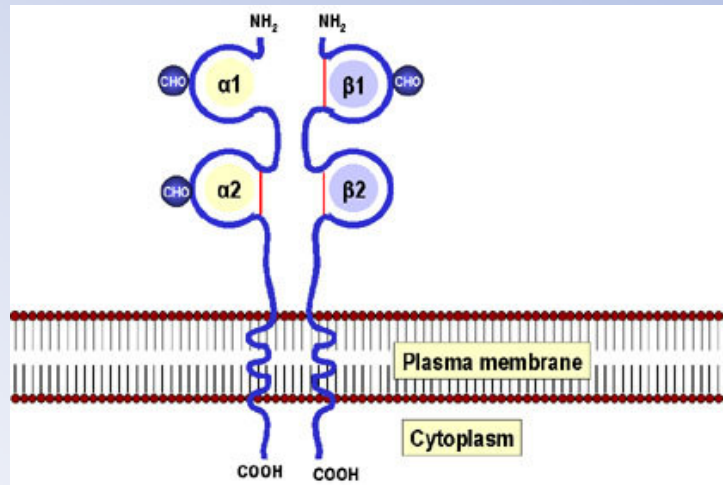
Dose-response



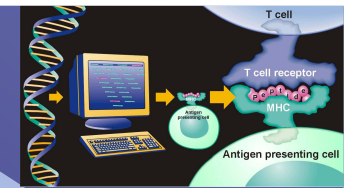
Binding isotherms



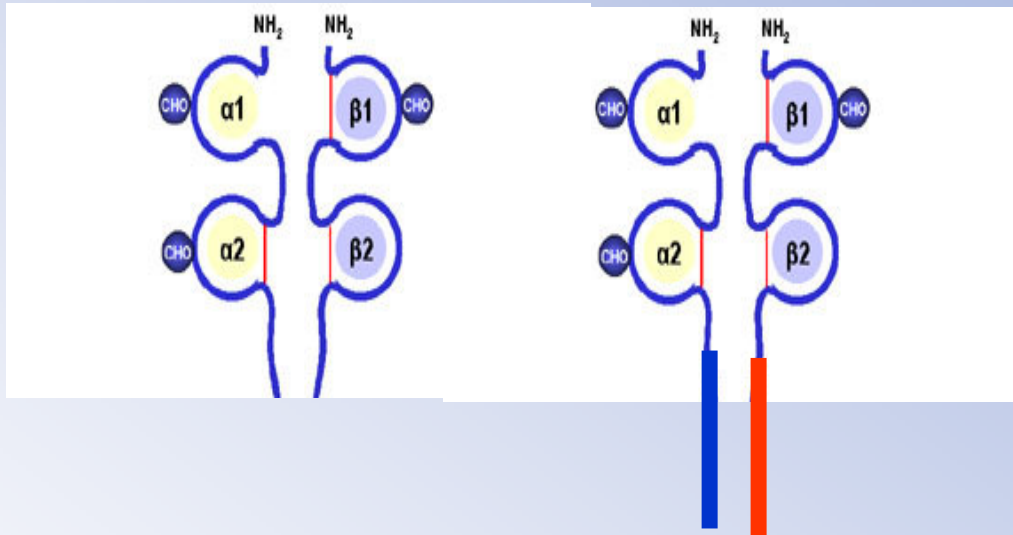
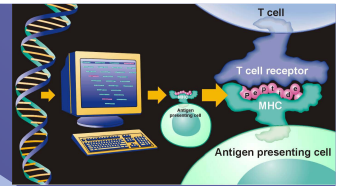
HLA class II molecules



- Molecules produced in mammalian cells:
- Laborious, low yields
- Assay: Pre-occupied binding cleft
- Transmembrane segment: Detergents



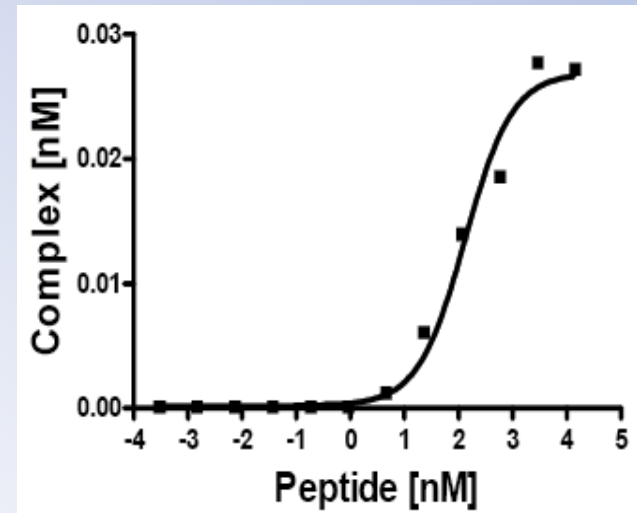
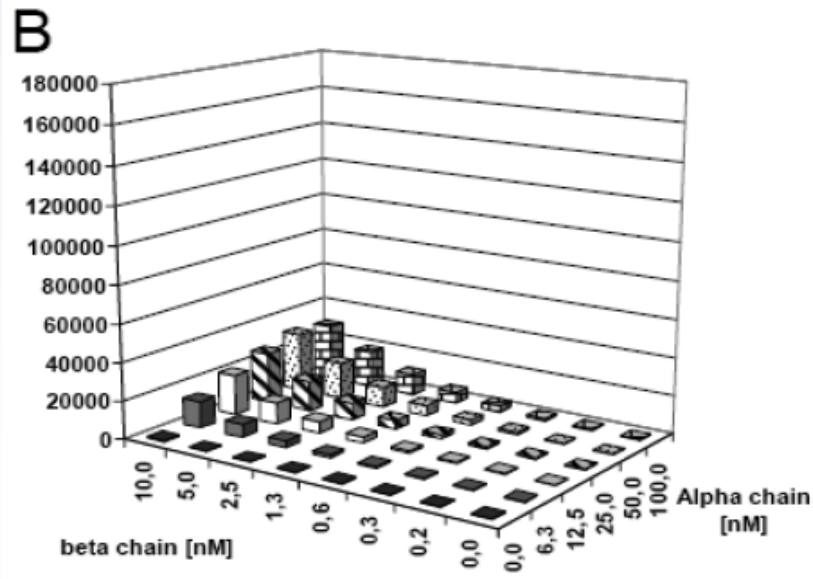
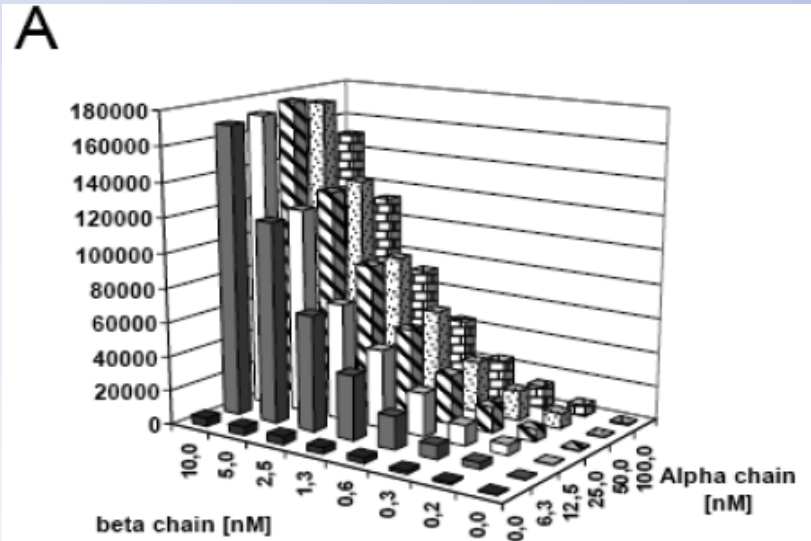
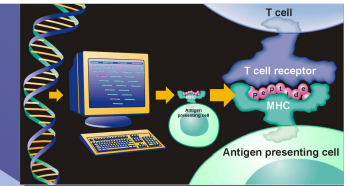
HLA class II molecules



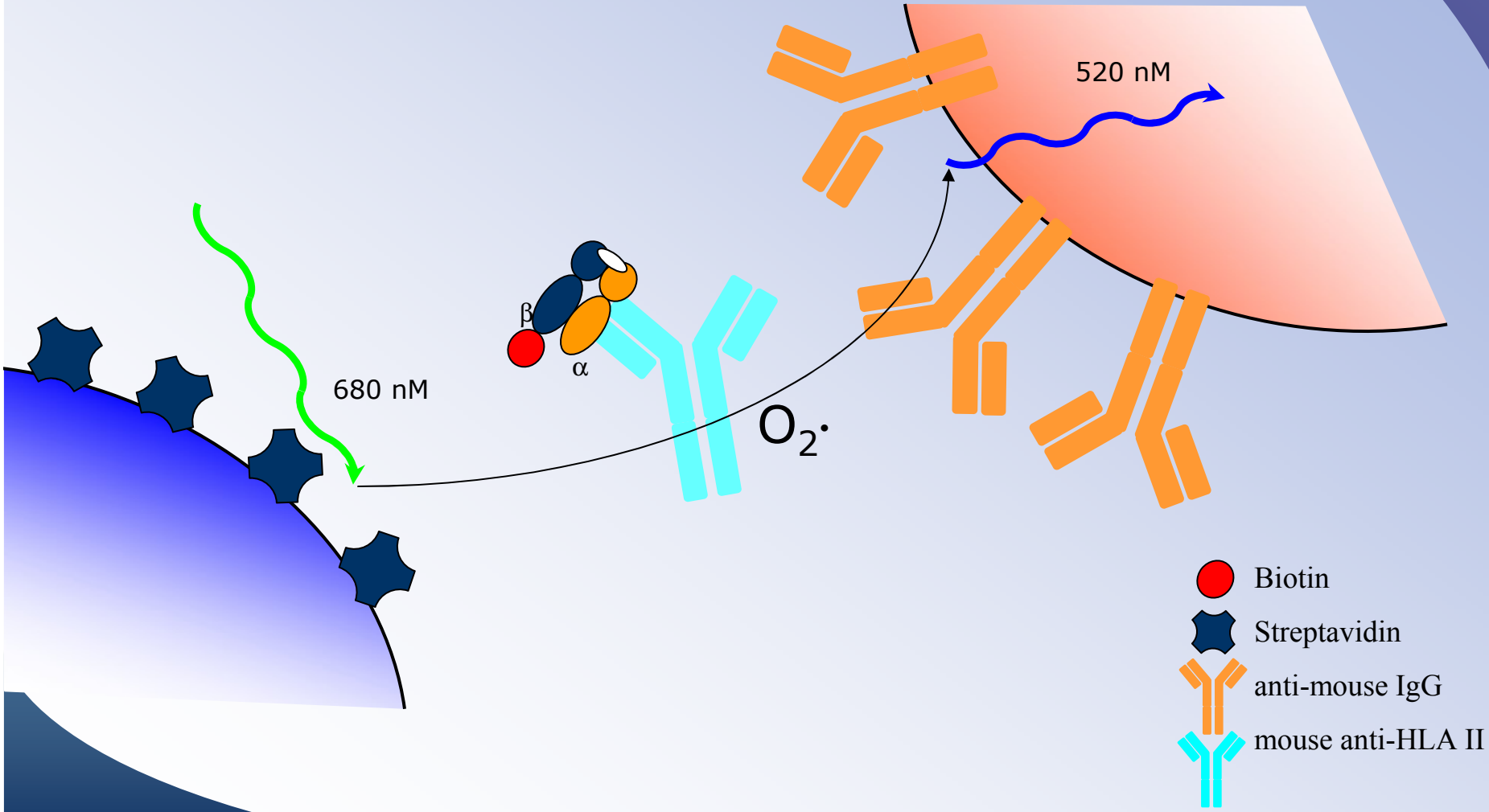
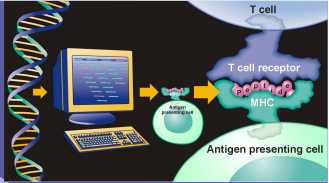
Leucine zipper (LZ)
Jun/Fos

- Transmembrane segment, important for chain interaction. Leucine zipper
- Soluble expression e.g. in *Drosophilla*
- Higher yields but still: laborious and preoccupied cleft

HLA class II molecules

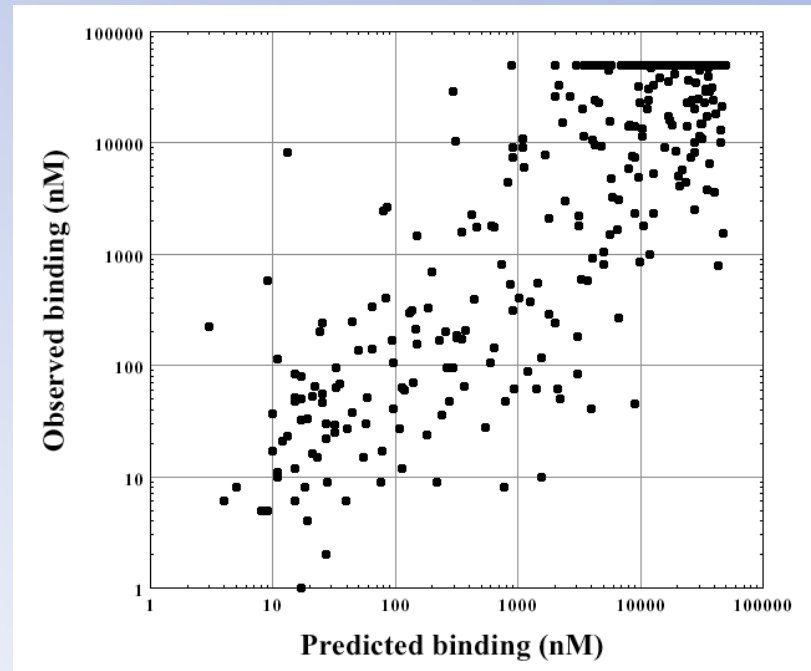
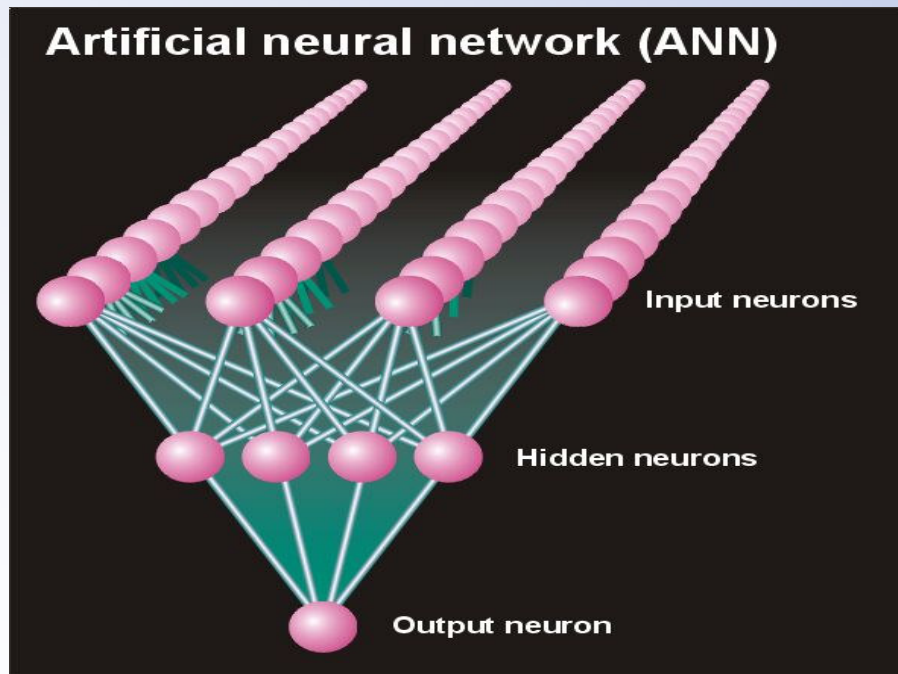
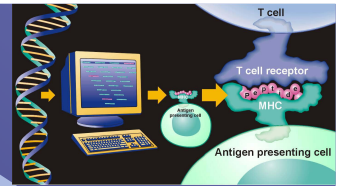


High throughput MHC class II assay



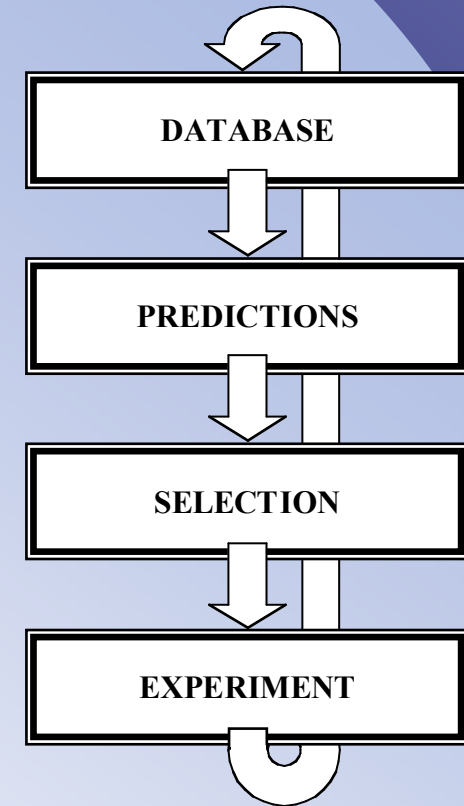
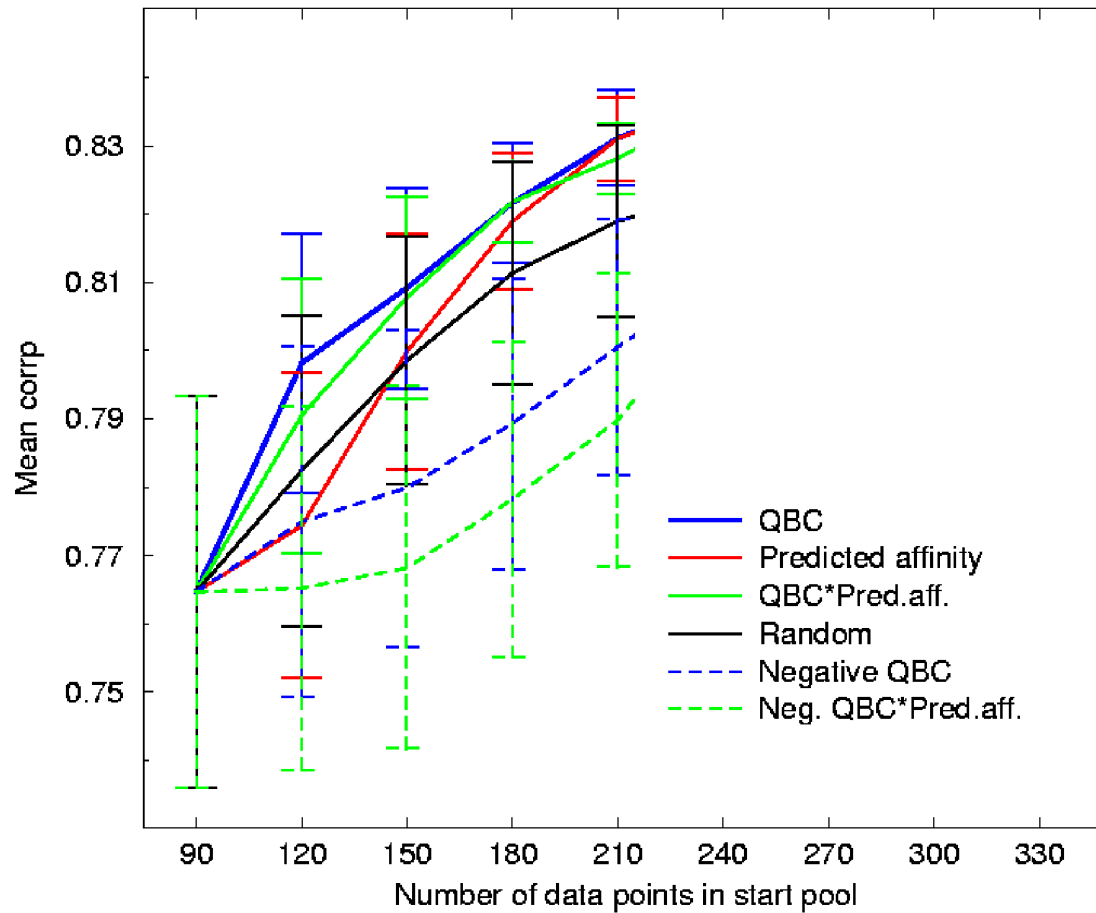
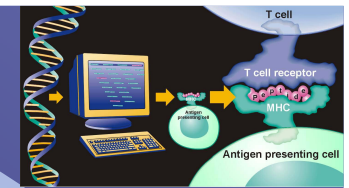
Predictions: e.g. Artificial Neural Networks (ANN)

- Simple and extended motifs searches are of limited sensitivity, ignores correlated effects
- Artificial Neural Networks incorporate correlated effects and are more accurate

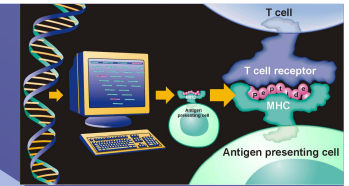


Buus, S. *et al.*,
Tissue Antigens (2003) 62:378

QBC efficiently selects complementing data points



Public web service: NetMHC



NetMHC 3.0 Server

http://www.cbs.dtu.dk/services/NetMHC/

NCI BRB Workshops PLoS Google Yahoo! News (1482) Lib \$ MHC Radio Events 3 ZYB Vejr Biz

CENTER FOR RIBIOMOLOGICAL SEQUENCE ANALYSIS CBS	EVENTS	NEWS	RESEARCH GROUPS	CBS PREDICTION SERVERS	CBS DATA SETS	PUBLICATIONS	BIOINFORMATICS EDUCATION PROGRAM
	STAFF	CONTACT	ABOUT CBS	INTERNAL	CBS BIOINFORMATICS TOOLS	CBS COURSES	OTHER BIOINFORMATICS LINKS

CBS >> [CBS Prediction Servers](#) >> NetMHC

NetMHC 3.0 Server

NEW UPDATED VERSION!!! [Previous version](#)

NetMHC 3.0 server predicts binding of peptides to a number of different HLA alleles using artificial neural networks (ANNs) and weight matrices.

View the [version history](#) of this server. All the previous versions are available on line, for comparison and reference.

Predictions can be obtained for 12 human supertypes, and 120 individual human alleles using ANNs and weight matrices (ungapped HMMs). Furthermore 12 animal (Monkey and Mouse) allele predictions are available. ANNs have been trained for 43 different Human MHC (HLA) alleles representing all 12 HLA A and B Supertypes as defined by Lund et al. (2004). Weight matrices are generated using an ungapped HMM approach as described in Nielsen et al. (2004) with data from the [SYFPEITHI](#) database.

For ANN prediction values are given in nM IC50 values. For weight matrices prediction values are given as a fitness score, so that a high fitness score correlates to strong binding.

For both ANN and weight matrix predictions strong and weak binding peptides are indicated in the output. In the selection window for HLA alleles, the recommended allele for each HLA supertype is indicated.

The project is a collaboration between CBS and [IMM](#).

[Instructions](#) [Output format](#) [Article abstract](#)

SUBMISSION

Paste a single sequence or several sequences in [FASTA](#) format into the field below:

Submit a file in [FASTA](#) format directly from your local disk:

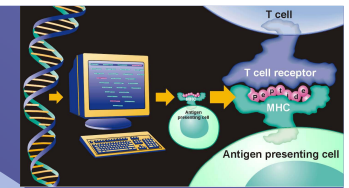
Choose File no file selected

9-11mer predictions

MHC Allele Peptide length

<http://www.cbs.dtu.dk/services/NetMHC/>

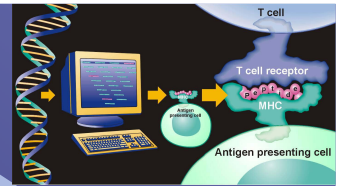
Coverage of HLA polymorphism



	Binding data	Members covered	Member with >50
HLA-A	37940	52 of 548	27
HLA-B	13294	84 of 936	30
HLA-C	187	15 of 300	2
HLA-E	6	1 of 3	0
HLA-F	0	0 of 4	0
HLA-G	30	1 of 14	0
HLA-DR	9562	340 of 556	26
HLA-DQ	441	11 of 1840?	5
HLA-DP	163	9 of 1725?	2

Publicly available peptide binding data in IEDB, SUFPEITHI etc showing number of data points, how many HLA-x are covered out of the registered polymorphism (as of Nov 2008) and the number of molecules populated with >50 datapoints.

Extent of HLA polymorphism



B0807 B4804 B0710 B1513 A6817 B5130 A0204 B3503 A2415 B0740 B3929 A0250 B5204 A2420 B1804 B3523 B3502 A3202 B0802 A3601 B4047
A6601 A0268 B0817 B5002 B5602 B3811 B4810 A0103 B1530 B4415 A3111 B7803 A6804 B3520 B3528 A2610 A6802 A2404 A7406 B0744 B3701
B4058 B1803 B1527 B3801 A6826 B5606 B0725 B5603 A0110 B1586 A3205 A0212 B3511 A2603 B5120 A0251 A3106 A6801 B5135 B1567 B4012
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B2728 A3404 A6820 A3107 A2430 A0235 A2914 B1301 B4004 A2620 B1573 A0259 B0804 B1548 A2616 B5401 B0707 A2453 A2609 B3554 A0245
B4411 A0220 B1510 A2433 B5512 B5306 B1540 B5114 B3934 B5510 B1521 B0810 B5137 B3932 B4802 B4044 B3709 B3915 B2729 B3810 A0238
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Coverage of HLA polymorphism



B1513

B3811

A3106

B3912

B5102

A3107

B3709

A2314

A7411

A0216

A3108

A2405

B4052

B4408

B4426

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B5901

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B1515

A3015

B4422

A0273

B4403

B5207

B3514

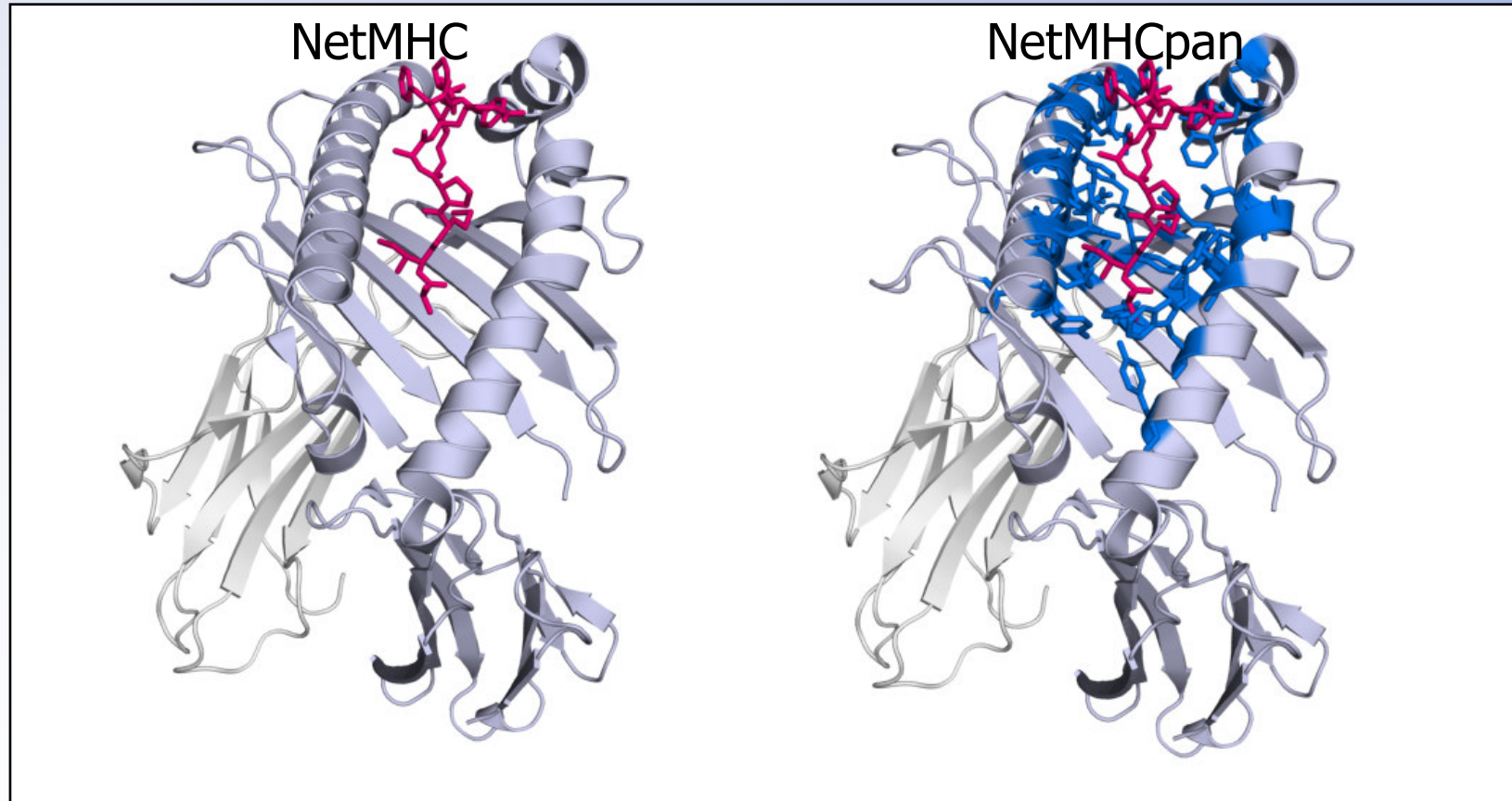
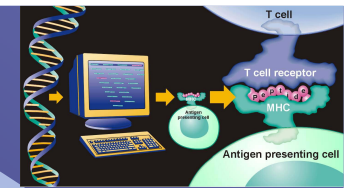
B1578

2nd Open Scientific EIP Symposium, Leiden

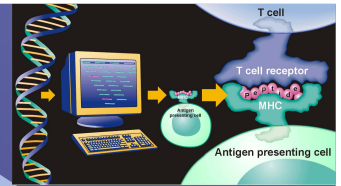
A6824

18NOV09

NetMHCpan - encompassing polymorphism

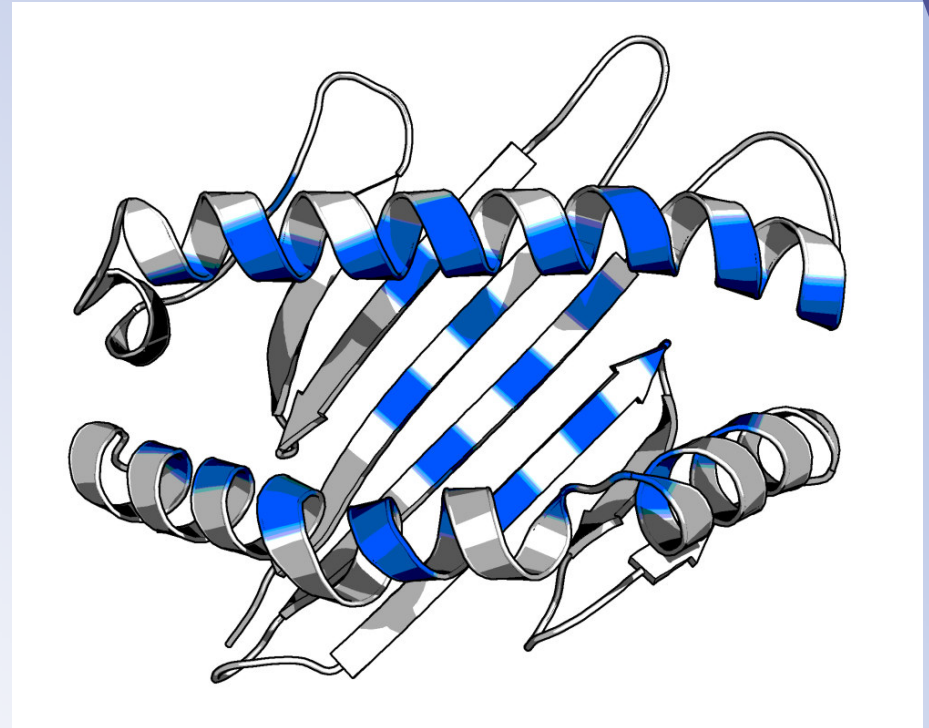


A Method for Quantitative Predictions of Peptide Binding to Any HLA-A and -B Locus Protein of Known Sequence, Nielsen et al. PLoS ONE 2:e796, August 2007

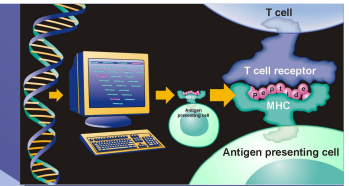


Pan-specific method

- Include polymorphic residues in potential contact with the bound peptide
- The contact residues are defined as being within 4.0 Å of the peptide in any of a representative set of HLA-A and -B structures with nonamer peptides.
- Only polymorphic residues from A, B, and C alleles are included
- Pseudo-sequence consisting of 34 amino acid residues.



HLA pseudo sequence

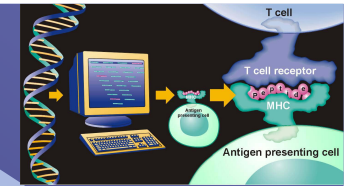


>HLA-XXXX

MRVMAPRTLILLLSGALALTETWACSHSMK**Y****F**TSVSRPGRGEPRFISVGYVDDTQFVRF
DSDAASPRGEPRAPWVEQEGPEYWDRETQKYKRQAQTDRVSLRNLRGYYNQSEAGSHTLQ
WMCGCDLGPDRLLRGYDQYAYDGKDYIALNEDLRSWTAADTAAQITQRKWEAAREAEQR
RAYLEGTCVEWLRRYLENGKETLQPAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLT
WQWDGEDQTQDTELVETRPAGDGTFOKWAAMVPSGEEQRYTCHVQHEGLPEPLTLRWEP
SSOPTIPIVGIVAGLAVLAVLAVLGAVVAVVMCRRKSSGGKGGSCSQAASSNSAQSDES
LIACKA

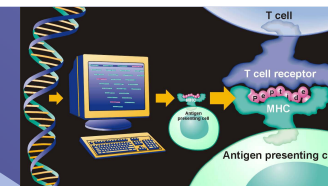
AXXX YFSGYREKYRQTDVSNLYLWCDYYTWAERAYTWY

Training example



Peptide	Amino acids of HLA pockets	HLA	Aff
VVLQQHSIA	YFAVLTWYGEKVHTHVDTLVRYHY	A0201	0.131751
SQVSFQQPL	YFAVLTWYGEKVHTHVDTLVRYHY	A0201	0.487500
SQCQAIHNV	YFAVLTWYGEKVHTHVDTLVRYHY	A0201	0.364186
LQQSTYQLV	YFAVLTWYGEKVHTHVDTLVRYHY	A0201	0.582749
LQPFLQPQL	YFAVLTWYGEKVHTHVDTLVRYHY	A0201	0.206700
VLAGLLGNV	YFAVLTWYGEKVHTHVDTLVRYHY	A0201	0.727865
VLAGLLGNV	YFAVWTWYGEKVHTHVDTLLRYHY	A0202	0.706274
VLAGLLGNV	YFAEWTWYGEKVHTHVDTLVRYHY	A0203	1.000000
VLAGLLGNV	YYAVLTWYGEKVHTHVDTLVRYHY	A0206	0.682619
VLAGLLGNV	YYAVWTWYRNNVQTDVDTLIRYHY	A6802	0.407855
.....			

Validation details

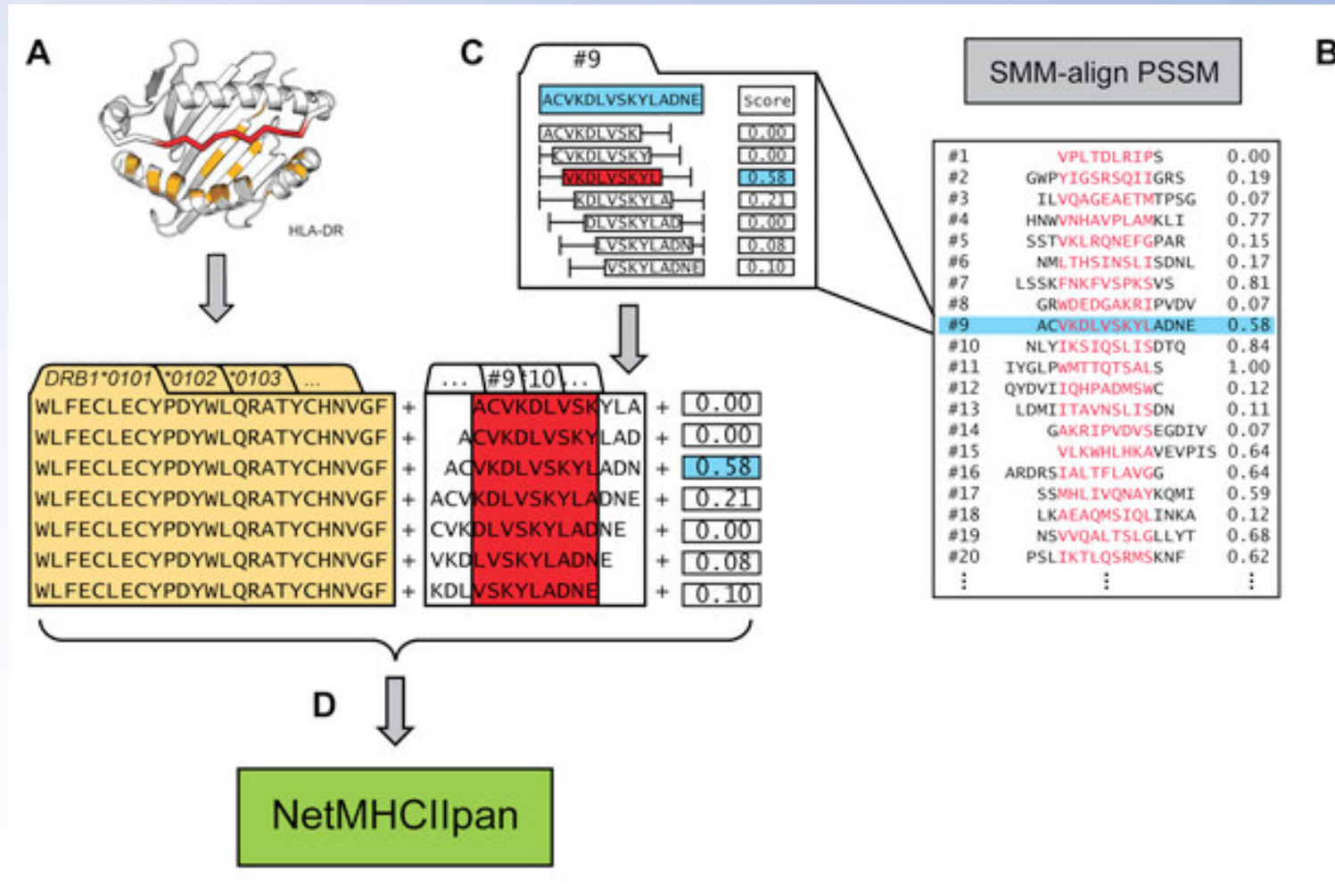
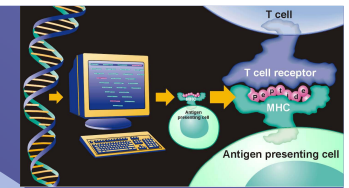


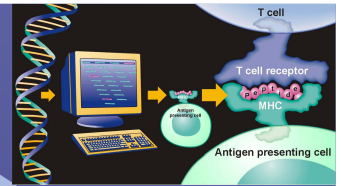
MHC allele	A*8001
Sequence	K_D -value (μ M)
HSNASTLLY	<0,001
KVDWNQFTY	<0,001
WMSNGTWN Y	<0,001
LTAHYCFLY	0.001
GMFSWNLAY	0.003
LVFLGPGLY	0.006
MTDVDLNY Y	0.010
VIAAIHNAY	0.036
SMIYFFHHY	1.454
LMDHWRGYK	16.543
LSNFGYPGY	non
YTIGIGAFY	N/A
VSM DQLASY	N/A

MHC allele	A*7401
Sequence	K_D -value (μ M)
RVYHLTWLR	0.001
TTMGWLF LK	0.001
MMHEFFGPR	0.003
KTYAPLAFR	0.003
HMMKRMSYR	0.004
KVNNH L FHR	0.010
MTMFV T ASK	0.012
MAMSN YLLR	0.014
MVAGRTPFK	0.063
IVFAFH FYR	0.188
SVYFWW LNR	0.402
MTSPYRMNR	N/A

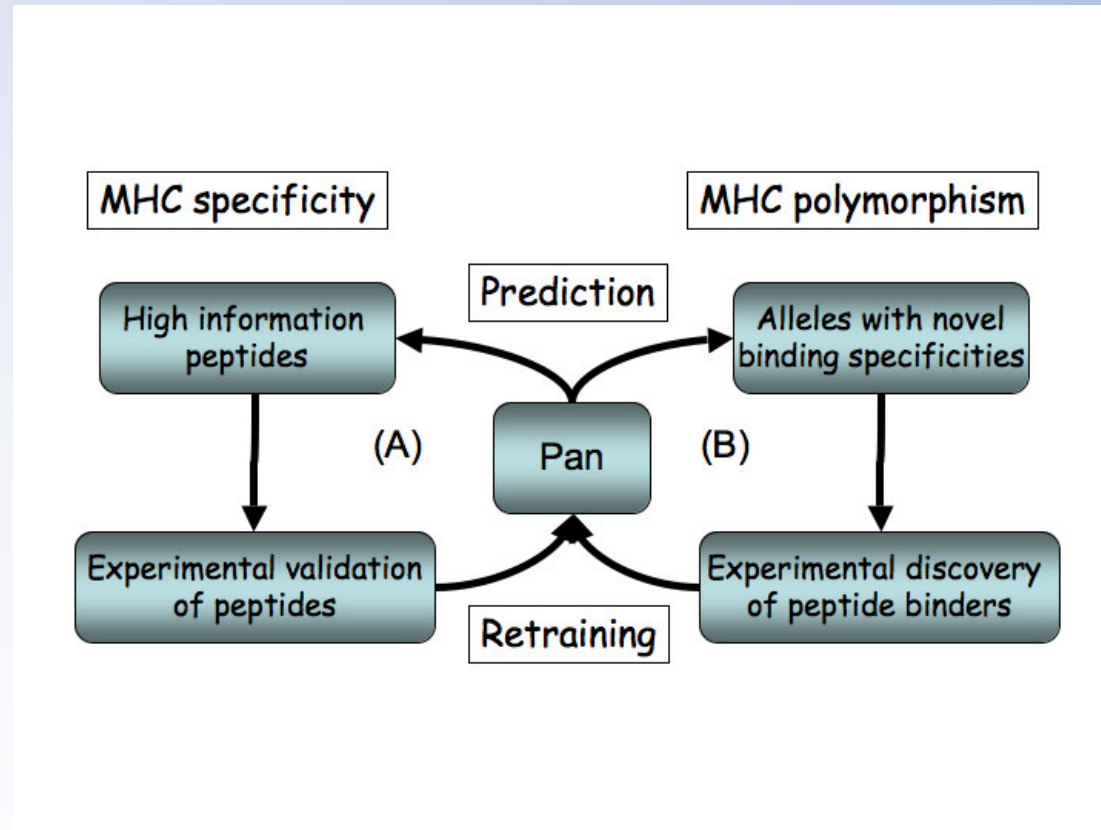
MHC allele	B*5101
Sequence	K_D -value (μ M)
MPVWLPIVI	0.151
FPIQDFP I I	0.156
MAMGILHTI	0.168
LANYAFFAI	0.230
MPYAAHDPI	0.364
MPSSYAAGM	0.863
LPDLPTTTI	0.922
MAW ERGPAL	0.934
YPGFGEHLI	1.889
FPMIIGSEL	2.127
LPAOGLIEF	11.254

- and now also for class II

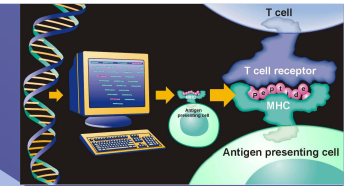




Informatics-guided development



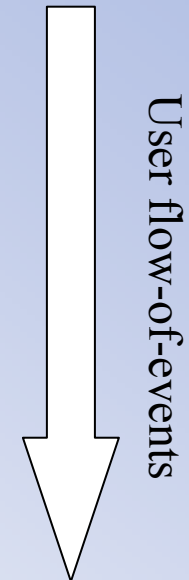
Shared resources used

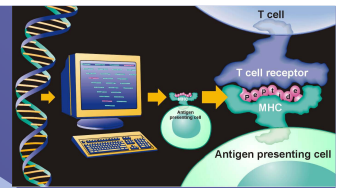


USE BIOINFORMATICS RESOURCE TO IDENTIFY EPITOPES

USE BIOCHEMICAL TOOLS TO VALIDATE EPITOPES

- Access artificial neural networks (HLA, proteasome etc) through web-services
- Apply ANN to own/public genomic/proteomic data
- Select peptides of interest
- Order peptides
- Order recombinant HLA class I molecules
- Apply protocols and reagents/standards to validate predictions





Public web service: NetMHC

NetMHC 3.0 Server

http://www.cbs.dtu.dk/services/NetMHC/ ANN SMM ARB

NCI BRB Workshops PLoS Google Yahoo! News (1482) Lib \$ MHC Radio Events 3 ZYB Vejr Biz

CENTER FOR RIBBON CALCULATION ANALYSIS CBS	EVENTS	NEWS	RESEARCH GROUPS	CBS PREDICTION SERVERS	CBS DATA SETS	PUBLICATIONS	BIOINFORMATICS EDUCATION PROGRAM
	STAFF	CONTACT	ABOUT CBS	INTERNAL	CBS BIOINFORMATICS TOOLS	CBS COURSES	OTHER BIOINFORMATICS LINKS

CBS >> [CBS Prediction Servers](#) >> NetMHC

NetMHC 3.0 Server

NEW UPDATED VERSION!!! [Previous version](#)

NetMHC 3.0 server predicts binding of peptides to a number of different HLA alleles using artificial neural networks (ANNs) and weight matrices.

View the [version history](#) of this server. All the previous versions are available on line, for comparison and reference.

Predictions can be obtained for 12 human supertypes, and 120 individual human alleles using ANNs and weight matrices (ungapped HMMs). Furthermore 12 animal (Monkey and Mouse) allele predictions are available. ANNs have been trained for 43 different Human MHC (HLA) alleles representing all 12 HLA A and B Supertypes as defined by Lund et al. (2004). Weight matrices are generated using an ungapped HMM approach as described in Nielsen et al. (2004) with data from the [SYFPEITHI](#) database.

For ANN prediction values are given in nM IC50 values. For weight matrices prediction values are given as a fitness score, so that a high fitness score correlates to strong binding.

For both ANN and weight matrix predictions strong and weak binding peptides are indicated in the output. In the selection window for HLA alleles, the recommended allele for each HLA supertype is indicated.

The project is a collaboration between CBS and [IMM1](#).

[Instructions](#) [Output format](#) [Article abstract](#)

SUBMISSION

Paste a single sequence or several sequences in [FASTA](#) format into the field below:

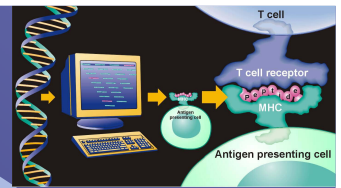
Submit a file in [FASTA](#) format directly from your local disk:

Choose File

<http://www.cbs.dtu.dk/services/NetMHC/>

9-11mer predictions

MHC Allele Peptide length



Other public web services

Immunological features

BepiPred

Linear B-cell epitopes.

DiscoTope

Discontinuous B-cell epitopes.

NetChop **WS**

Proteasomal cleavages (MHC ligands).

NetCTL **WS**

Integrated class I antigen presentation.

NetMHC

Binding of peptides to MHC class I alleles.

NetMHCII

Binding of peptides to MHC class II alleles.

NetMHCpan

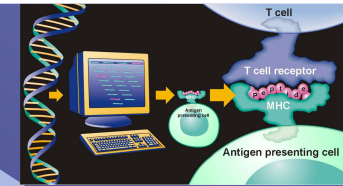
Pan-specific binding of peptides to MHC class I alleles of known sequence.

VDJsolver

Analysis of human immunoglobulin VDJ recombination.

<http://www.cbs.dtu.dk/services/>

Other public web services



Immune Epitope Database and Analysis Resource

http://www.immuneepitope.org/home.do

Yahoo! News Lib \$ Veluxfondene 2800Vejr NCI BRB Workshops PLoS Google Yahoo! News Lib \$ MHC Ra

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

HOME | QUERY | RESOURCES | ACCOUNT | SUPPORT | ABOUT IEDB

The Immune Epitope Database and Analysis Resource (IEDB)

Welcome to the Immune Epitope Database and Analysis Resource (IEDB). The IEDB is a project hosted by scientists at the [La Jolla Institute for Allergy and Immunology \(LIAI\)](#), with support from the [National Institute of Allergy and Infectious Diseases \(NIAID\)](#), a part of the [National Institutes of Health \(NIH\)](#), and [Department of Health and Human Services \(HHS\)](#). While not strictly limited, the current focus is presenting information that facilitates the dissemination of immune epitope information, the generation of new research tools, diagnostic techniques, vaccines and therapeutics for emerging and re-emerging diseases.

The IEDB contains data related to antibody and T cell epitopes for humans, non-human primates, rodents, and other animal species. Curation of data relating to [NIAID Category A, B, and C priority pathogens](#) (including Influenza) and [NIAID Emerging and Re-emerging infectious diseases](#) is complete through June 2007. Curation of Malaria, Hepatitis B, Clostridium tetani, Leishmania, and Candida albicans is current through June 2007. Present efforts include herpesviruses and allergen epitopes. Curation of autoimmune epitopes will start in second quarter 2008.

The database also contains MHC binding data from a variety of different antigenic sources and immune epitope data from the FIMM (Brusic), HLA Ligand

Quick Links

- Perform a Simple Query
- Perform an Advanced Query
- Browse Records by Allele
- Browse Records by Species
- Browse Records by 3D Structure
- Analysis Tools
- Links
- Forums
- Tour the IEDB
- Register
- Feedback

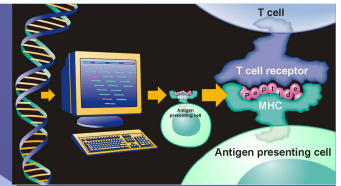
News / Updates

- 2006 Annual Workshop Summary
- Workshop report on B cell epitope prediction tools
- Ab and T cell epitopes of influenza A virus, knowledge and opportunities
Supplemental information
- 2006 Annual Compendium
- A tool developer resource - Benchmarking MHC-I binding predictions

References:	4826
Records:	111334
Distinct Structures:	77003
Distinct Epitopes:	36242

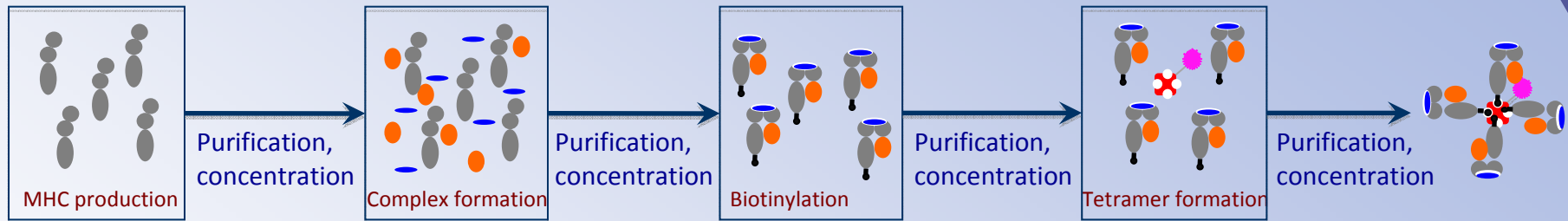
Add IEDB Search to your Browser

IEDB: <http://www.immuneepitopes.org>

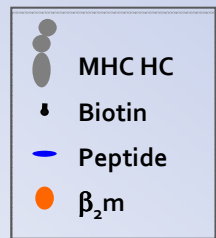
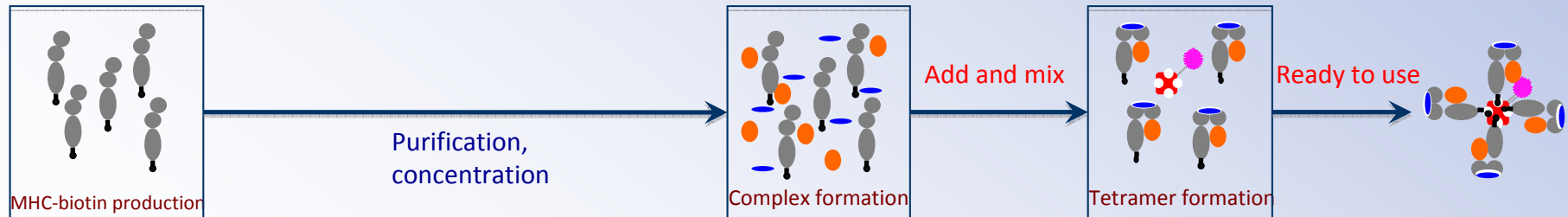


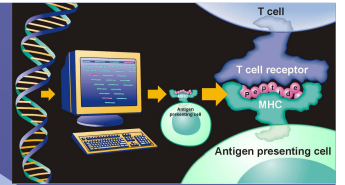
Tetramer production

Traditional protocol (John Altman):

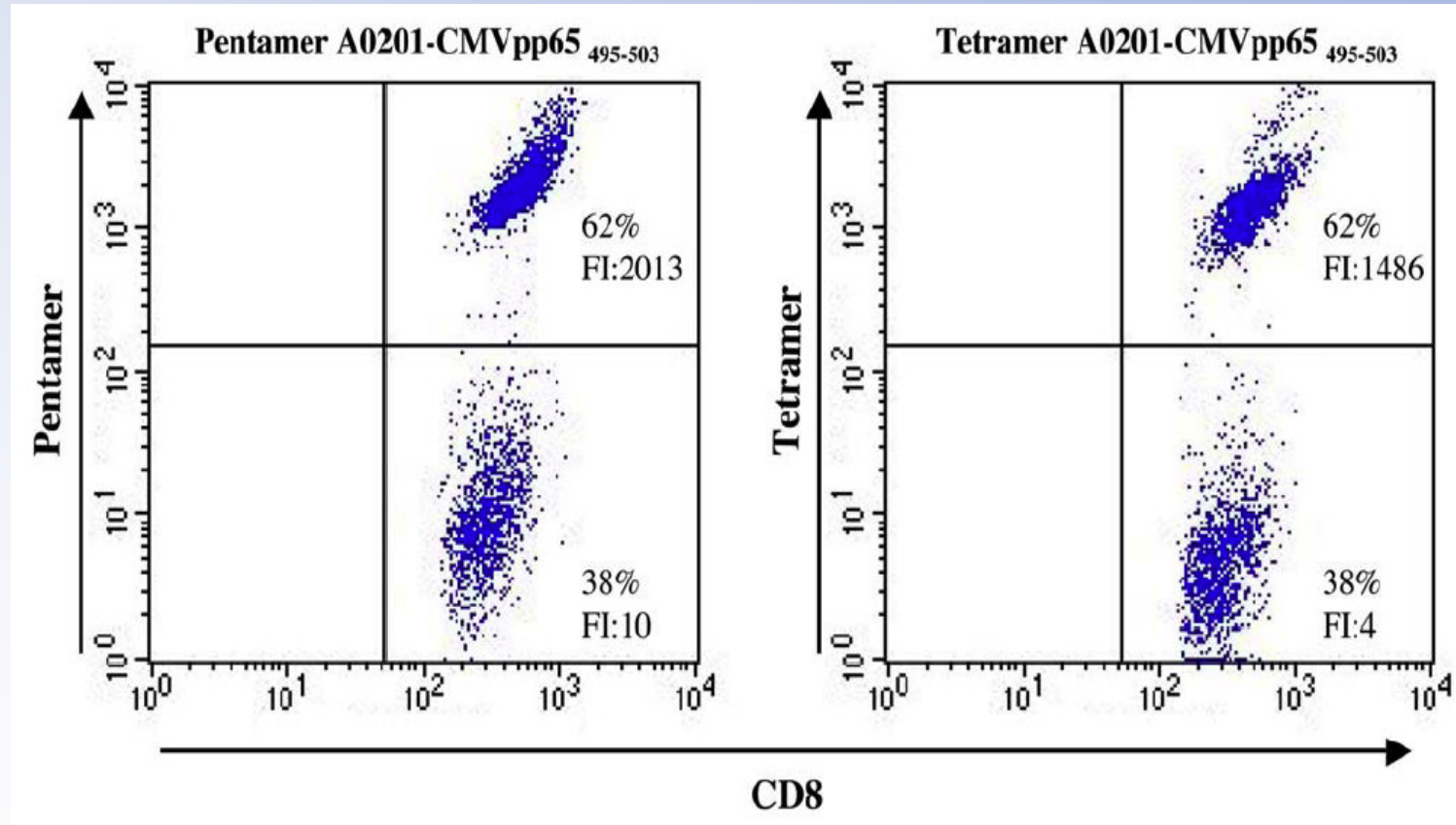


Our “One pot, mix and read” protocol:

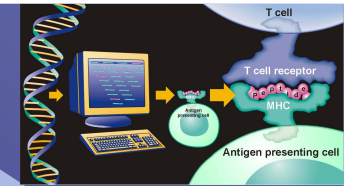




One-pot, mix-and-read tetramers



The Human MHC project



- Department of Medical Microbiology and Immunology, University of Copenhagen
 - Kasper Lamberth, Mikkel Harndahl, Sune Justesen, Michael Madsen, Gustav Roder, Anette Stryhn, Lotte Nielsen, Mingjun Wang, Mogens H Claesson & Soren Buus
- Center for Biological Sequence Analysis, Technical University of Denmark
 - Morten Nielsen, Claus Lundegaard, Thomas Blicher, Anne Møllgaard, Pernille Haste Andersen, Hao Zhang, Soren Brunak, & Ole Lund
- Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences
 - Jette Kastrup, Michael Gajhede
- La Jolla Institute of Allergy and Immunology
 - Björn Peters, Huynh-Hoa Buip, Ward Fleri, Stephen Wilson, John Sidney, Alessandro Sette
- Fred Hutchinson Cancer Research Center
 - Effie W Petersdorf, John A Hansen