THE JENNER INSTITUTE
DEVELOPING INNOVATIVE VACCINES

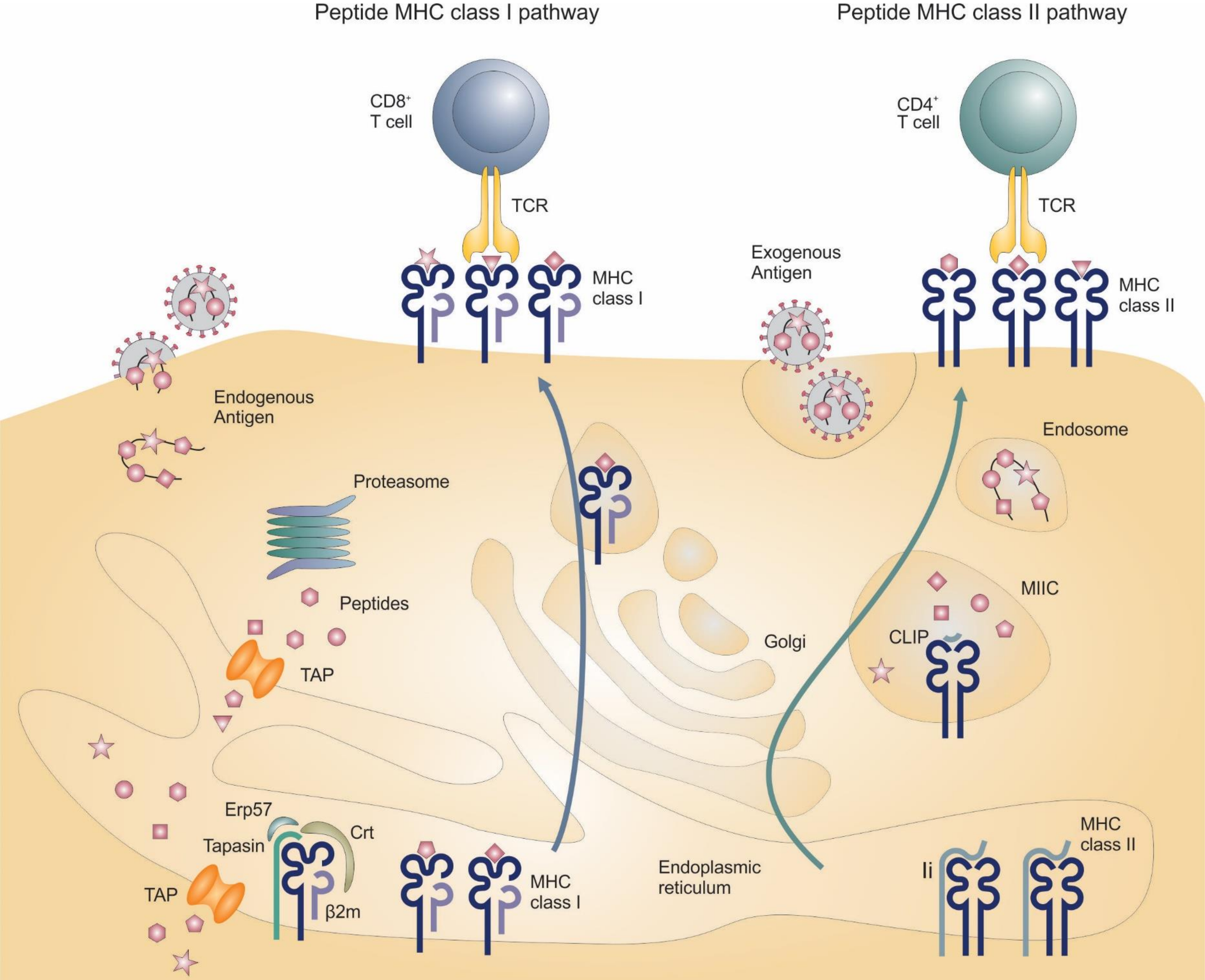
UNIVERSITY OF OXFORD

HIDI DAY
1/10/2018

IMMUNOPEPTIDOMICS

Dr. Nicola Ternette
The Jenner Institute
nicola.ternette@ndm.ox.ac.uk

Immunopeptidomics: Analysis of MHC ligands by nUPLC-MS



Immunopeptidomics Group

TripleTof 5600
AB SCIEX

Fusion Lumos
Thermo Scientific

Dr Robert Parker

Dr Annalisa Nicastri

Dr Yoanna Ariosa

Xu Peng

TDI MS laboratory led by Benedikt Kessler

HUPO- Human Immunopeptidome Project (HIPP)

Jacques Neefjes
(Leiden University Medical Centre, Leiden, The Netherlands)

Stefan Stevanovic
(University of Tubingen, Tubingen, Germany)

Etienne Caron
(ETH, Zurich, Switzerland)

Nathan Croft
(Monash University, Melbourne, Australia)

Albert Heck
(University of Utrecht, Utercht, The Netherlands)

Markus Mueller
(Swiss Institute of Bioinformatics, Lausanne, Switzerland)

Alessandro Sette
(La Jolla Institute for Allergy and Immunology, La Jolla, USA)

Lennart Martens
(Ghent University, Ghent, Belgium)

HUPO HIPP SUMMER SCHOOL

1ST HUPO HUMAN IMMUNO-PEPTIDOME PROJECT (HIPP) SUMMER SCHOOL

ORGANIZED BY:

MICHAL BASSANI-STERNBERG
(Ludwig Institute, Lausanne, Switzerland)

MIGUEL MARCILLA
(Spanish National Biotechnology Centre, Madrid, Spain)

FABIO MARINO
(Ludwig Institute, Lausanne, Switzerland)

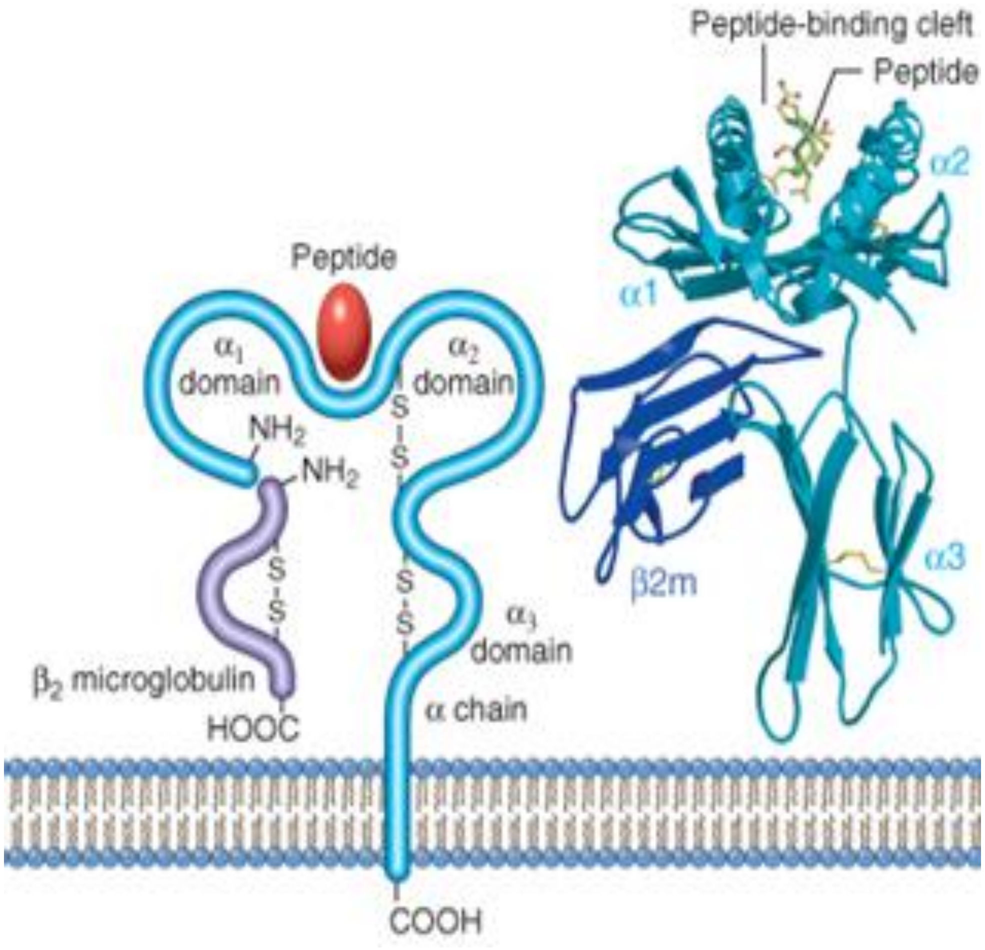
STEFAN TENZER
(University of Mainz, Mainz, Germany)

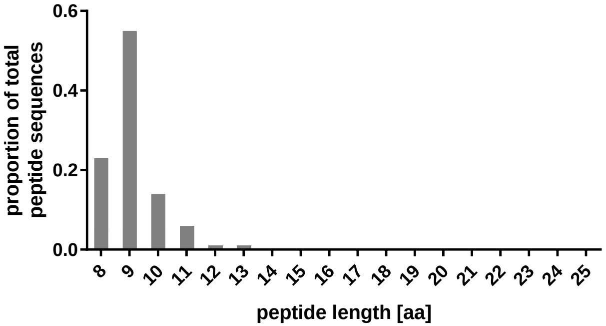
NICOLA TERNETTE
(University of Oxford, Oxford, UK)

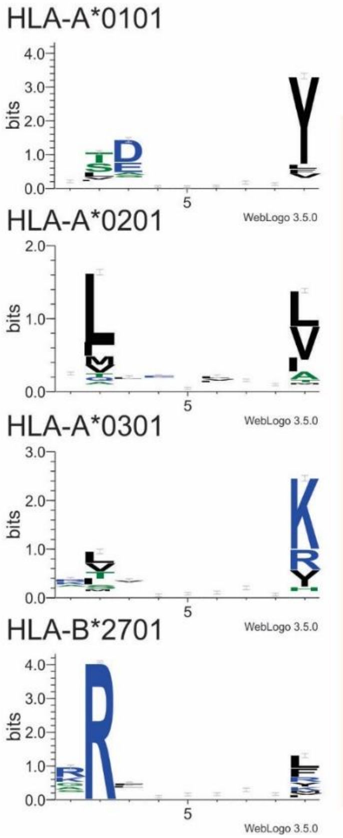
SEPTEMBER 10-13, 2018

THE SPANISH NATIONAL BIOTECHNOLOGY CENTRE (CNB) AND THE STUDENT RESIDENCE (CSIC) IN MADRID 2018

MHC class I

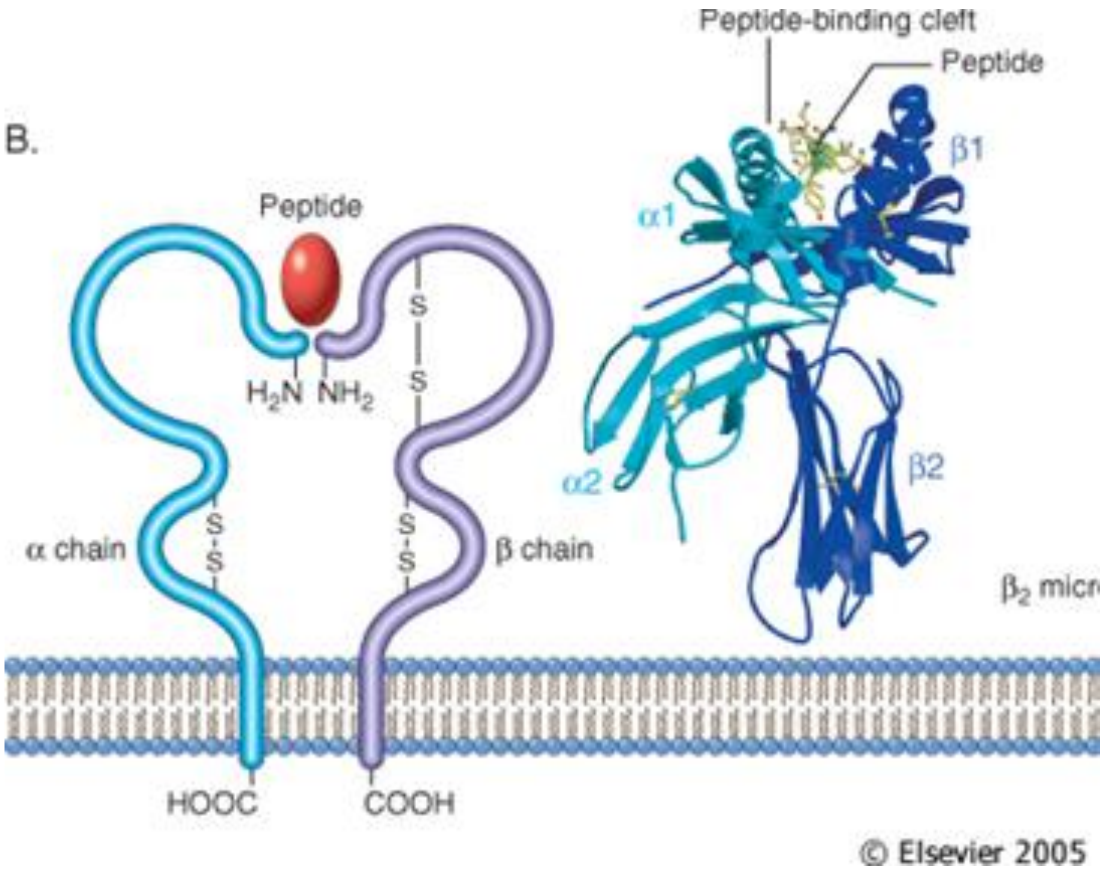




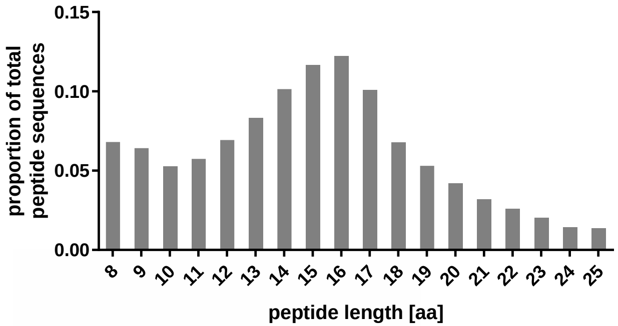


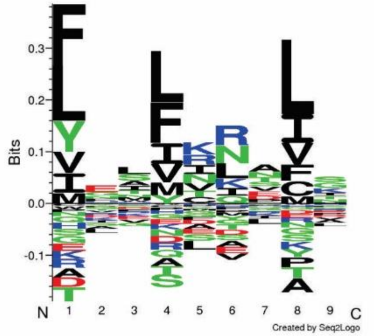
http://intranet.tdmu.edu.ua/data/kafedra/internal/patologanatom/classes_stud/en/med/lik/ptn/Pathomorphology/3/04_Pathomorph_immune_syst.htm

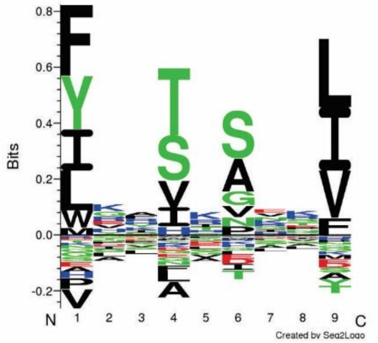
MHC class II



© Elsevier 2005

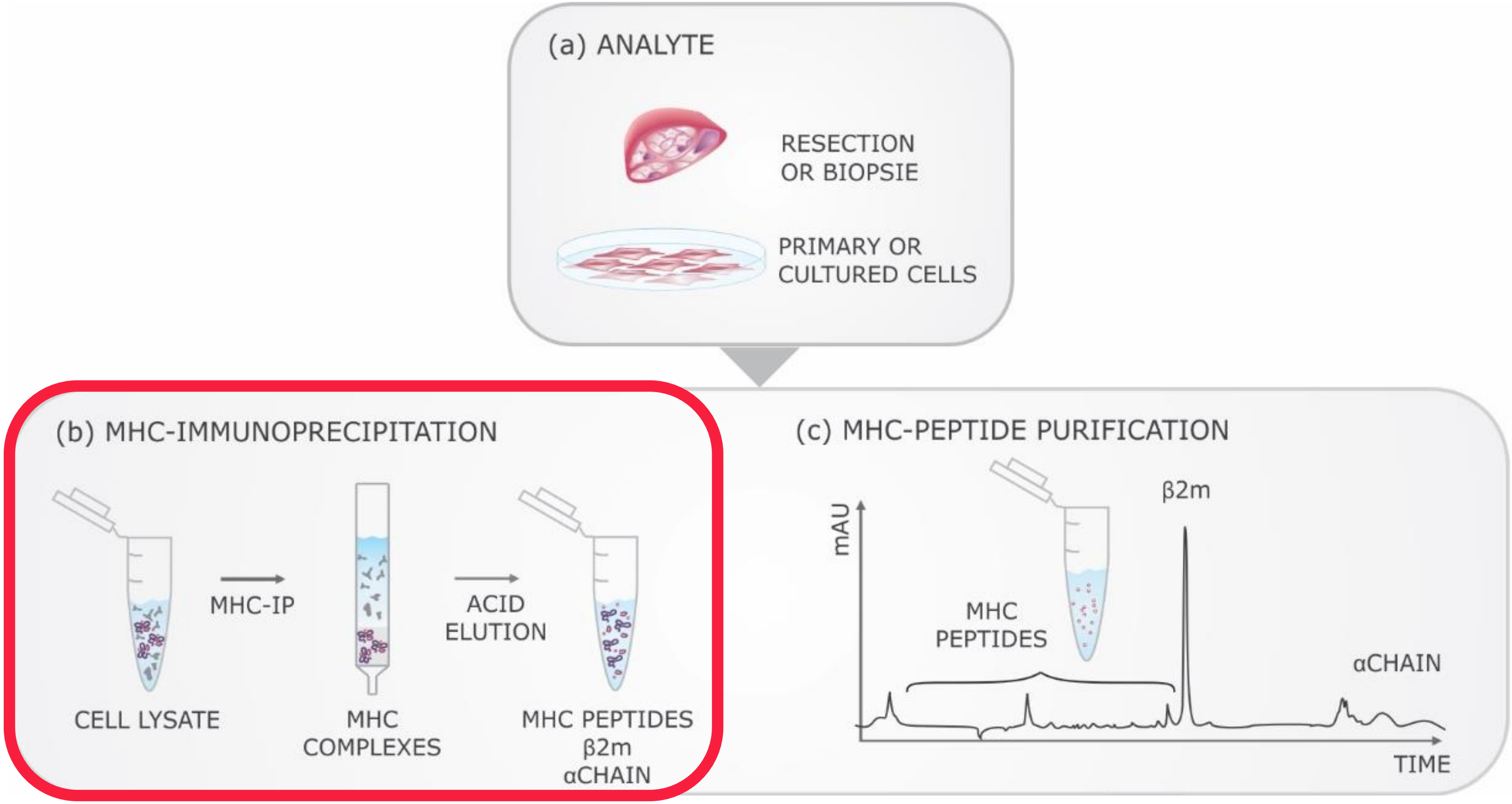




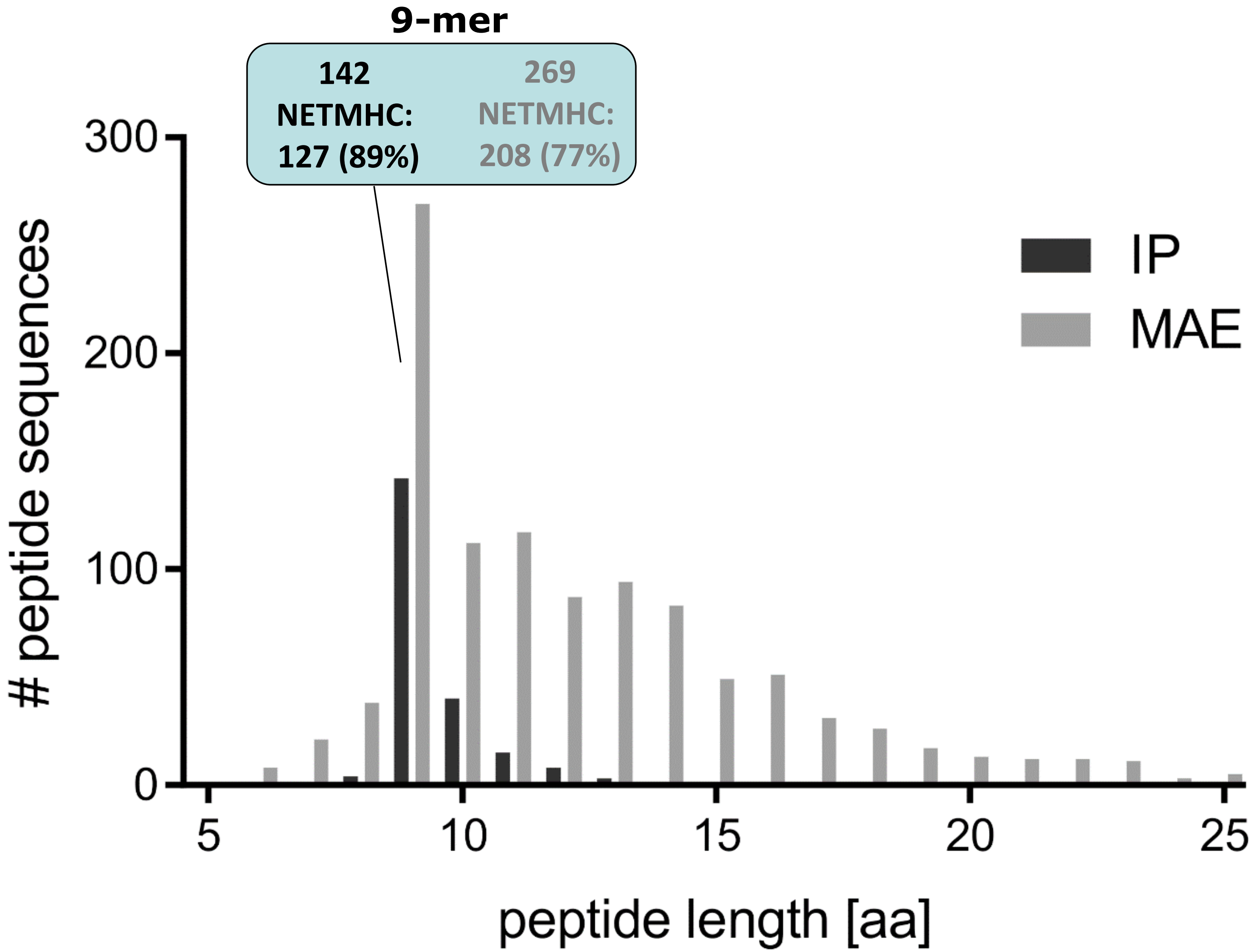


http://intranet.tdmu.edu.ua/data/kafedra/internal/patologanatom/classes_stud/en/med/lik/ptn/Pathomorphology/3/04_Pathomorph_immune_syst.htm

HLA-associated peptide purification - Specificity



Mild acid elution vs Immunoprecipitation



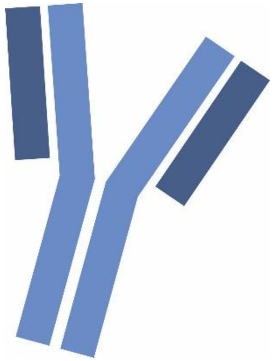
9-mer

142	269
NETMHC:	NETMHC:
127 (89%)	208 (77%)

Antibodies

Human

Hybridoma Line	Type	Antigen	ATCC	sepharose
W6/32	IgG$_{2a}$	HLA class I ABCDE	ATCC® HB-95	A/G
L243	IgG$_{2a}$	HLA DR (Ia)	ATCC® HB-55	A/G
IVD12	IgG$_{1}$	HLA DQw3	ATCC® HB-144	G
B7/21	IgG$_{3}$	HLA DP	na	G
IVA12	IgG$_{1}$	HLA DR, DQ, DP	ATCC® HB-145	G
MA2.1	IgG$_{1}$	HLA A2, B17	ATCC® HB-54	G
ME1	IgG$_{1}$	HLA B7, Bw22, B27	ATCC® HB-119	G
DT9	IgG$_{2b\kappa}$	HLA C (HLA E)	na	A/G



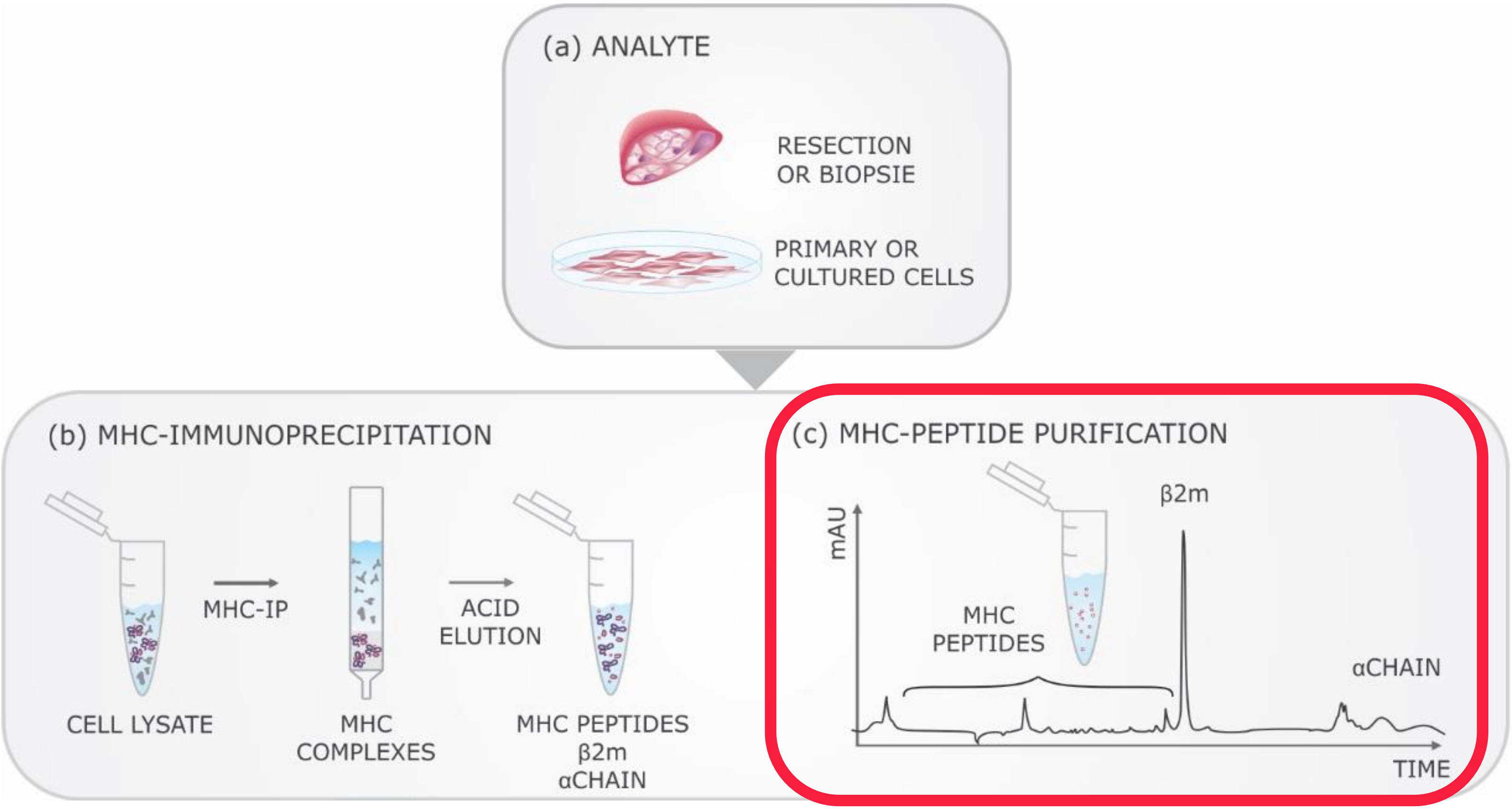
Mouse

Hybridoma Line	Type	Antigen	ATCC	protein
HB-51	IgG$_{2a}$	H-2 Kb, Db	ATCC® HB-51	G
M5/114.15.2	(rat) IgG$_{2b}$	I-A b, I-A d, I-A q, I-E d, I-E k	ATCC® TIB-120™	G

1-5 mg antibody required per experiment!

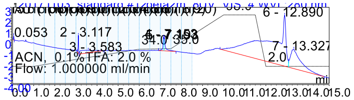
Sofron et al. 2016. Eur. J. Immunol. 46: 319–328 DOI: 10.1002/eji.201545930

HLA-associated peptide purification - Purity

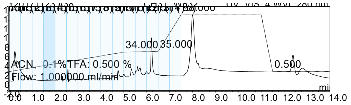


Separation of peptides from larger complex components

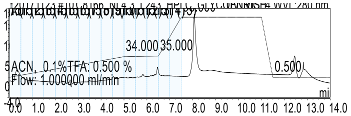
beta2m



class I



class II



Starting Material and Yield

Discovery Experiments (DDA)

Table: Peptide yield from various starting material

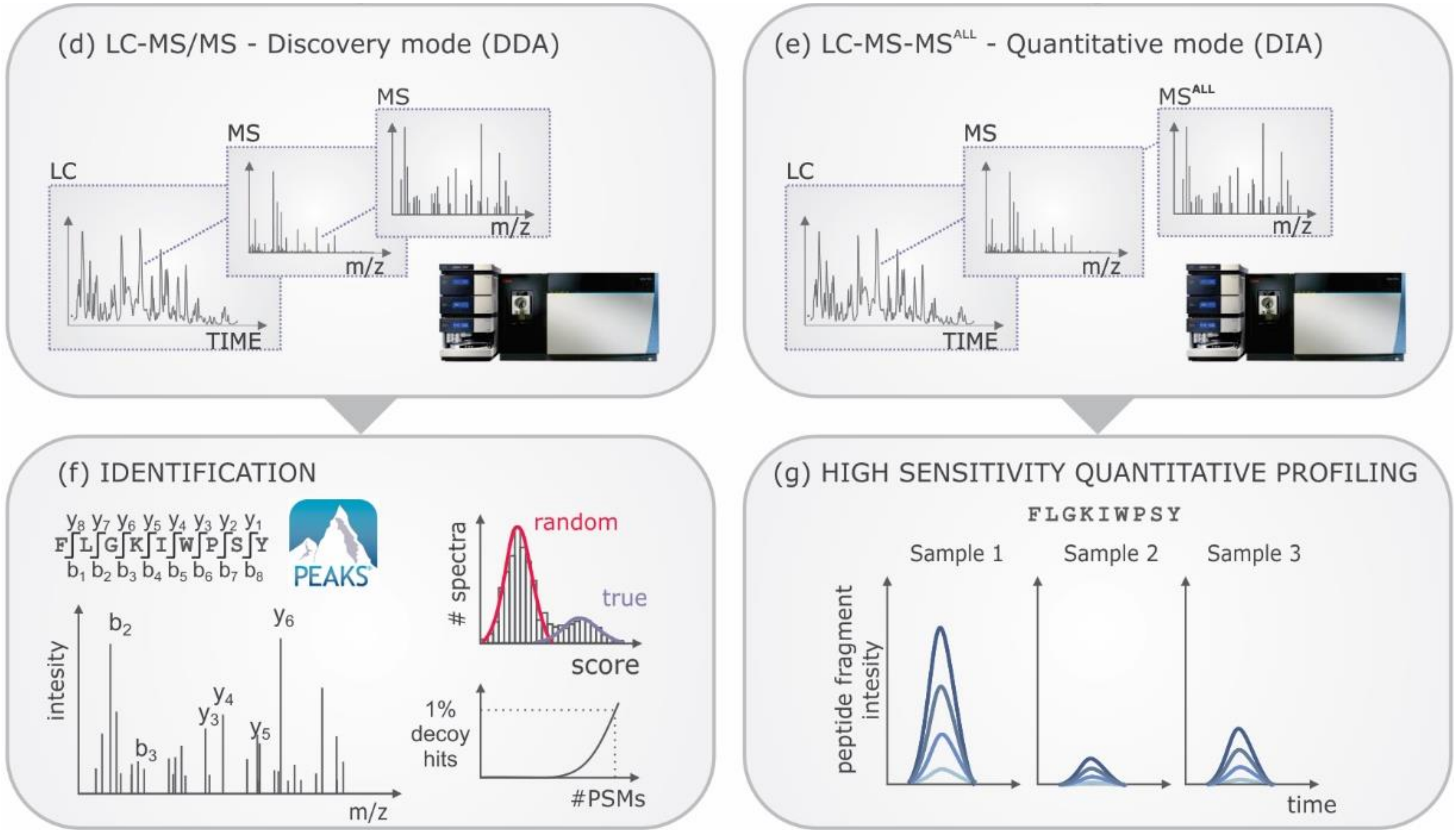
Cell number	Approx. size	Protein [mg]	Peptide sequences
$5 \cdot 10^6$	1 x 1 x 5 mm (5 mm^3)	1 mg	<100
$5 \cdot 10^7$	5 x 5 x 1 mm (25 mm^3)	5 mg	>1,500
$5 \cdot 10^8$	5 x 5 x 10 mm (250 mm^3)	50 mg	~10,000
$2 \cdot 10^9$	10 x 10 x 10 mm (1000 mm^3)	200 mg	15,000

www.wikipedia.com

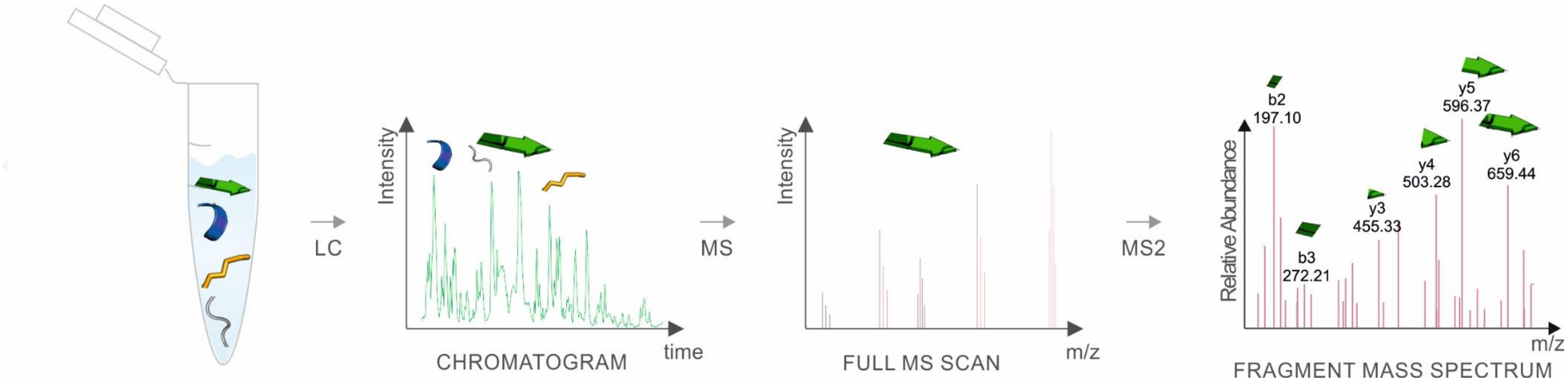
Estimated amount of MHC molecules per cell: 100,000

$5 \cdot 10^8$ peptide molecules equivalent to 1 femtomol of peptide

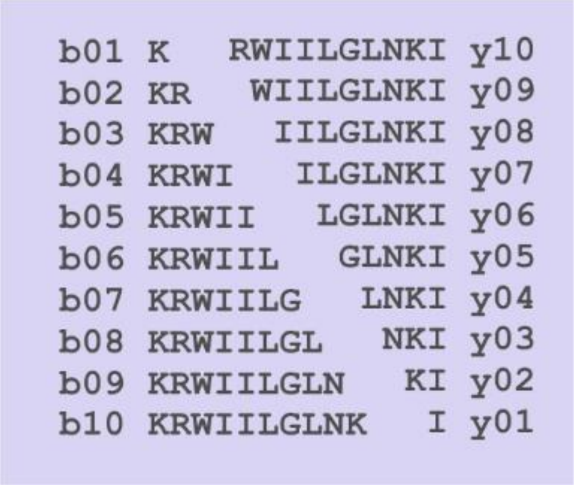
HLA-associated peptide profiling by nUPLC/MS^2



Peptide Sequencing using nUPLC-MS



K R W I I L G L **N** K I



b01	K	RWIILGLNKI	y10
b02	KR	WIILGLNKI	y09
b03	KRW	IILGLNKI	y08
b04	KRWI	ILGLNKI	y07
b05	KRWII	LGLNKI	y06
b06	KRWIIL	GLNKI	y05
b07	KRWIILG	LNKI	y04
b08	KRWIILGL	NKI	y03
b09	KRWIILGLN	KI	y02
b10	KRWIILGLNK	I	y01

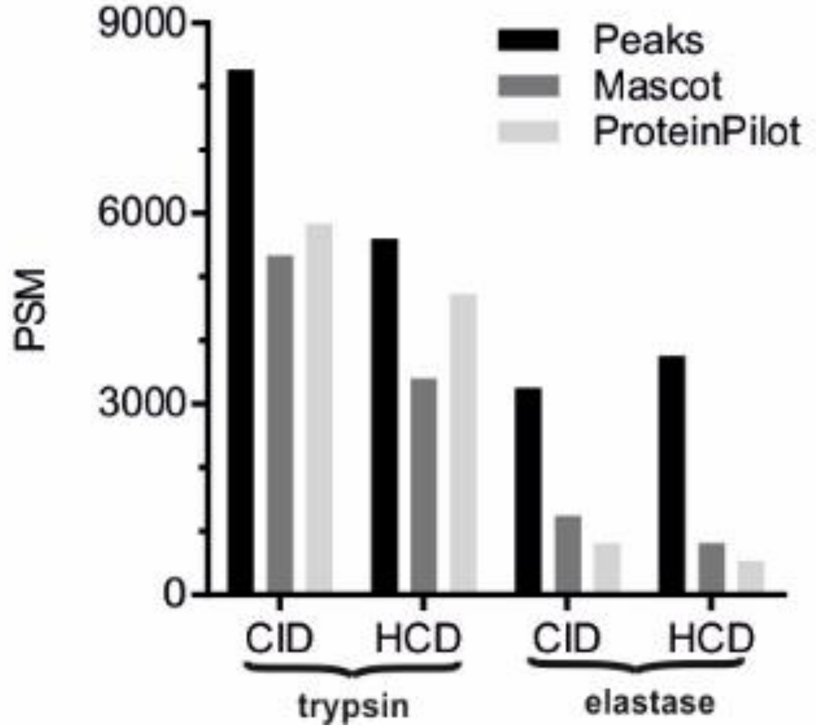
Software evaluation

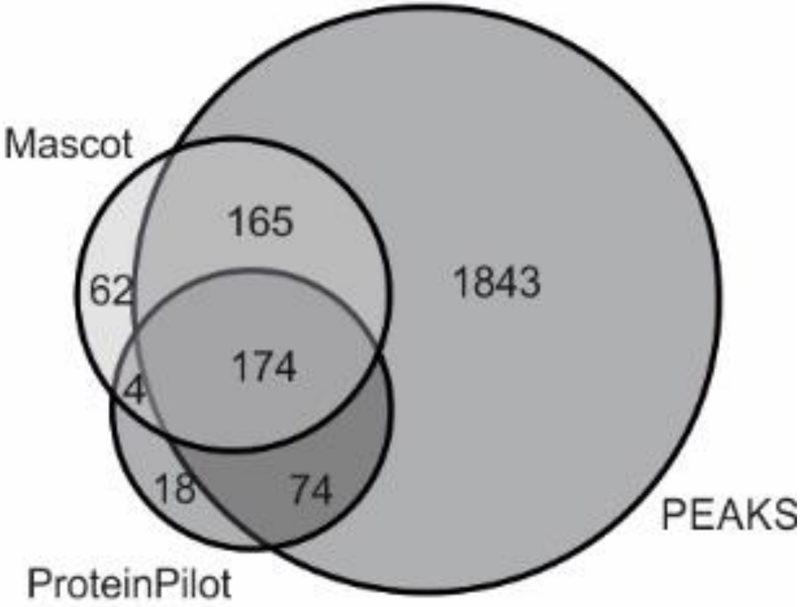
CID: low resolution, high sensitivity;
HCD: high resolution, lower sensitivity

MATRIX SCIENCE Mascot

AB SCIEX ProteinPilot™

PEAKS® Complete Software for Proteomics



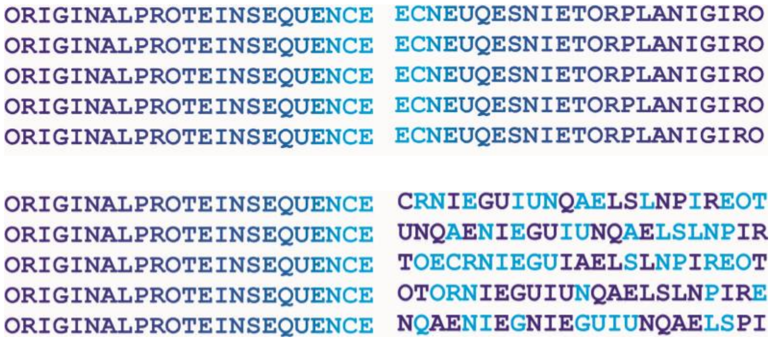


Validation

Decoy database search

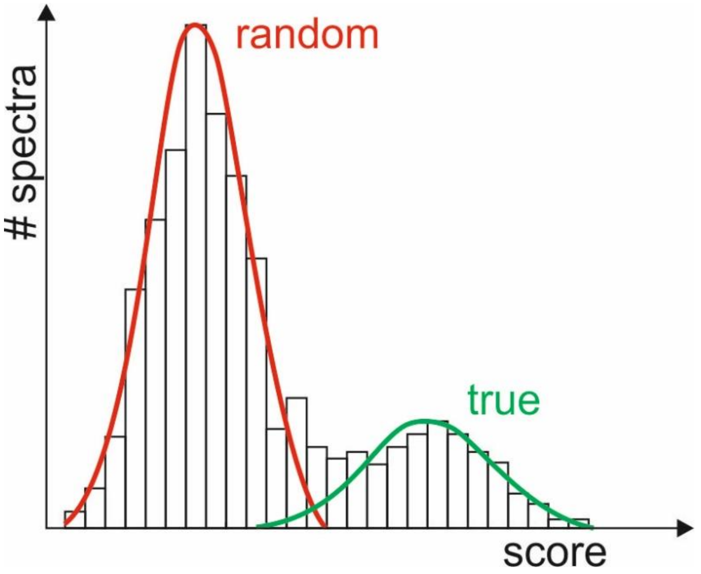
PeptideProphet

Target **Decoy**



ORIGINALPROTEINSEQUENCE ECNEUQESNIETORPLANIGIRO
ORIGINALPROTEINSEQUENCE ECNEUQESNIETORPLANIGIRO
ORIGINALPROTEINSEQUENCE ECNEUQESNIETORPLANIGIRO
ORIGINALPROTEINSEQUENCE ECNEUQESNIETORPLANIGIRO
ORIGINALPROTEINSEQUENCE ECNEUQESNIETORPLANIGIRO

ORIGINALPROTEINSEQUENCE CRNIEGUIUNQAELSLNPIREOT
ORIGINALPROTEINSEQUENCE UNQAENIEGUIUNQAELSLNPIR
ORIGINALPROTEINSEQUENCE TOECRNIEGUIAELSLNPIREOT
ORIGINALPROTEINSEQUENCE OTORNIEGUIUNQAELSLNPIRE
ORIGINALPROTEINSEQUENCE NQAENIEGNIEGUIUNQAELSPI

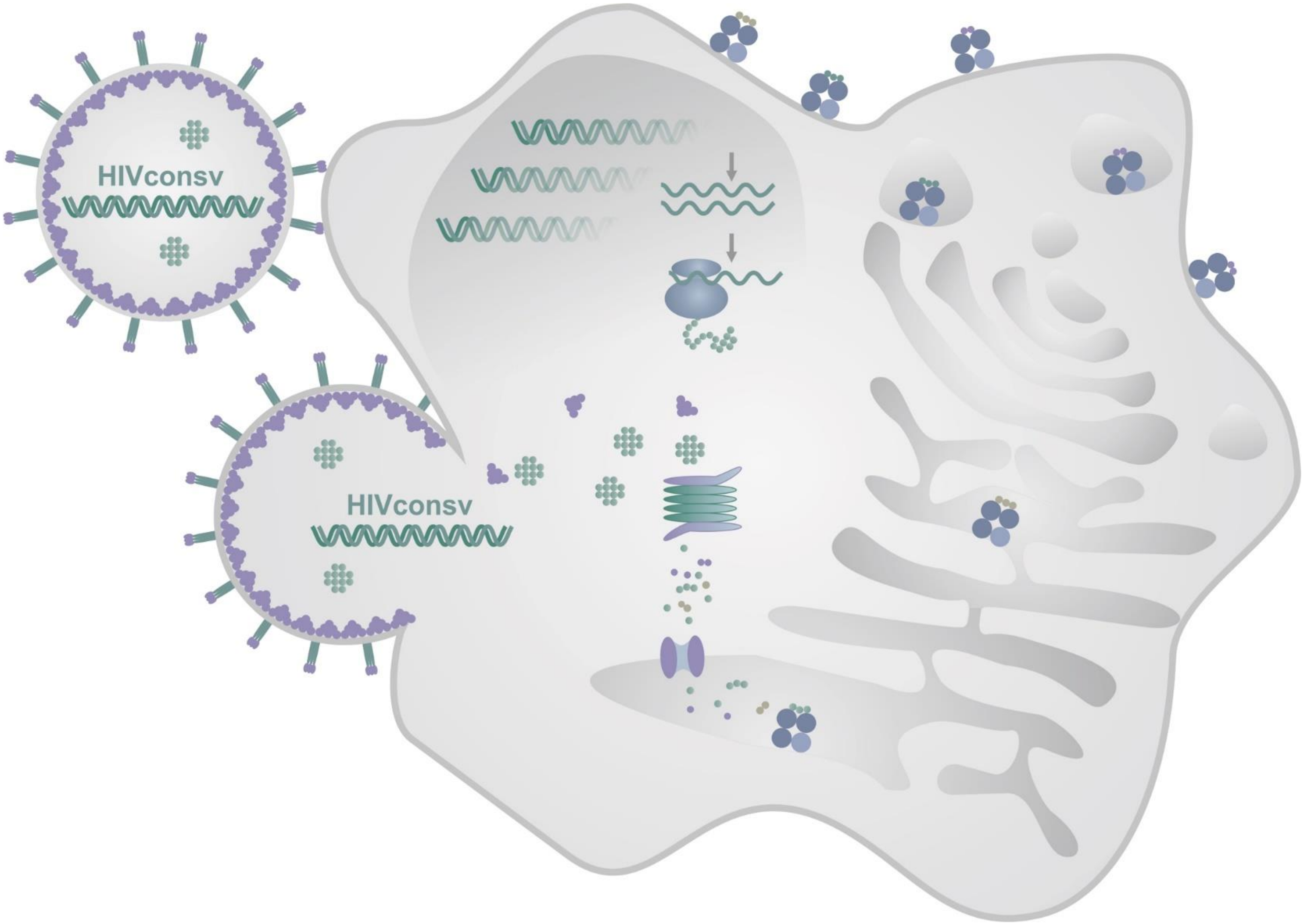


Threshold-based filtering **Probability-based filtering**

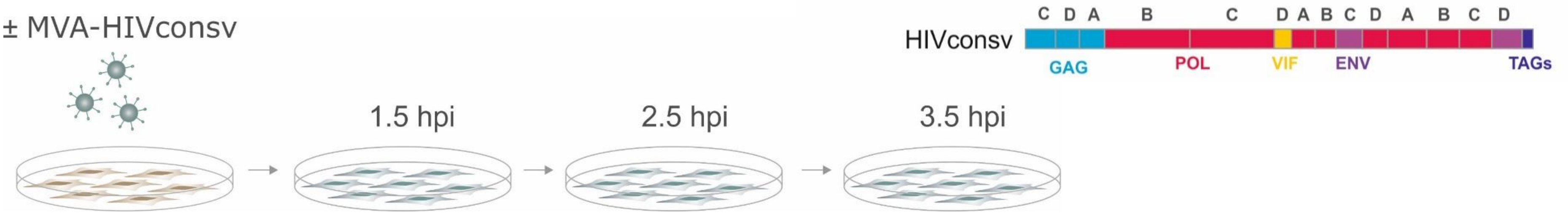
Keller *et al.* Anal.Chem. 2002. Empirical Statistical Model To Estimate the Accuracy of Peptide Identifications Made by MS/MS and Database Search

DATA EXAMPLE I

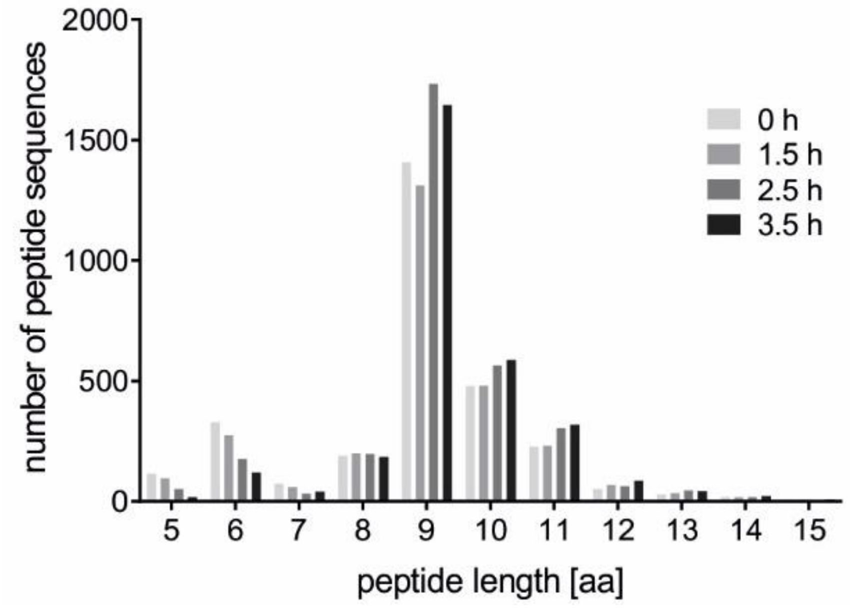
Characterisation of Antigen Presentation of Viral Vector Vaccines



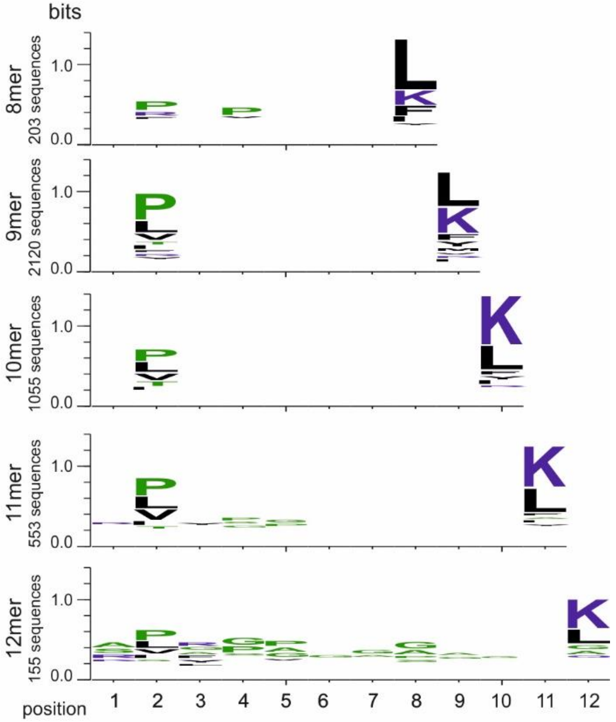
MHC peptide presentation in MVA-HIVconsv infection



sample	proteins	peptides			
	total	total	human	MVA	HIVconsv
0 h	1884	2951	2947	4	0
1.5 h	1829	2814	2777	36	1
2.5 h	2012	3217	3151	62	4
3.5 h	1946	3103	3025	73	5
all	3516	6358	6253	98	7

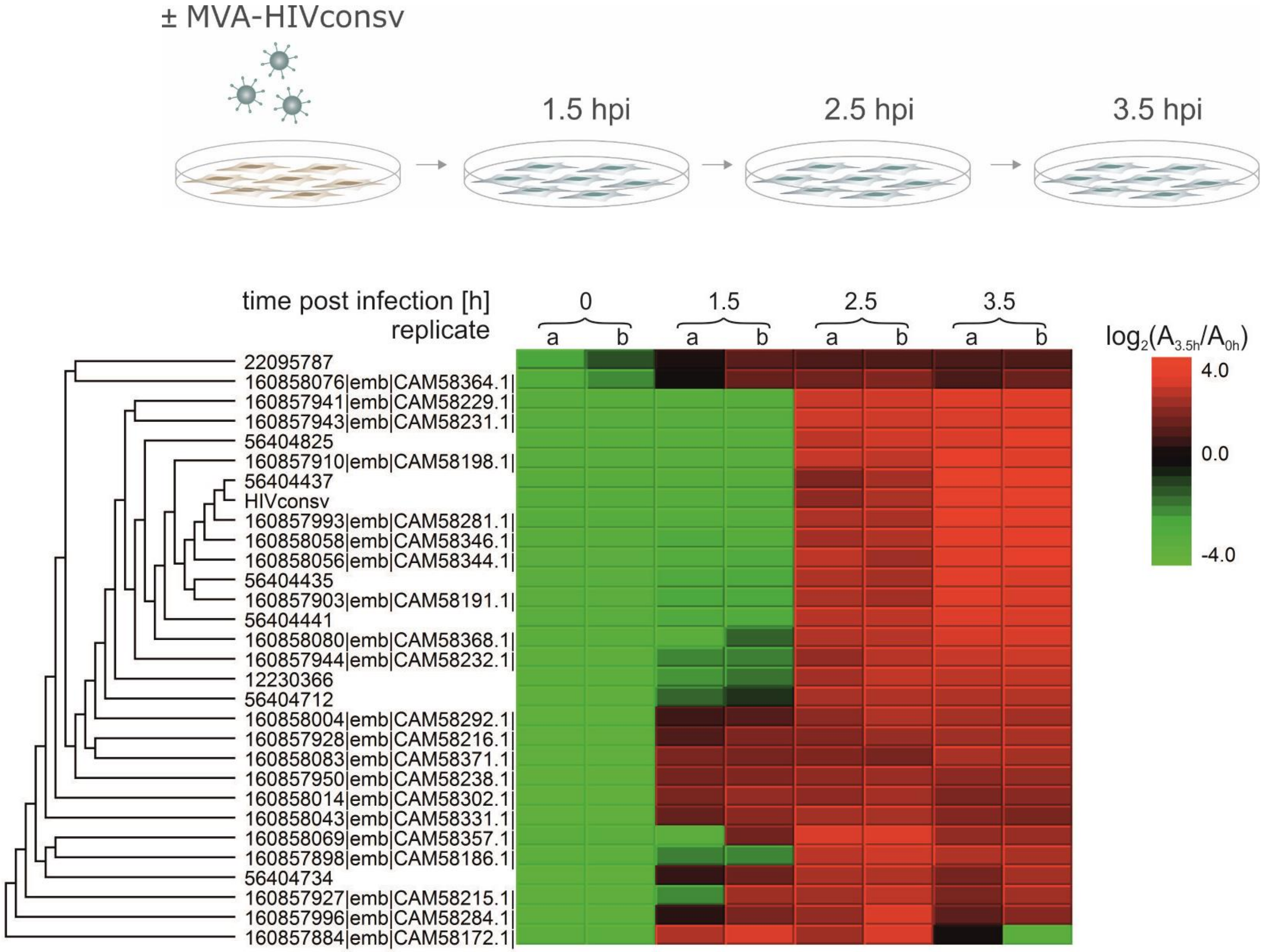


position	1	2	3	4	5	6	7	8	9
A*03:01		VLM							KYF
A*03:01		VLM							KYF
B*07:02		P							L
B*35:03		P							LF
C*04:01		YFP							LFM
C*07:02									LYF



Ternette *et al.* JVI 2015

Dynamics of MHC peptide presentation in MVA.HIVconsv infection



Ternette *et al.* JVI 2015

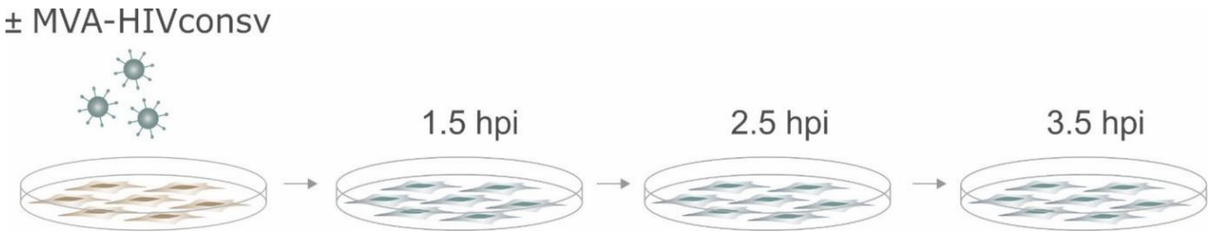
HIVconsv peptides identified in MVA-HIVconsv transfected cells

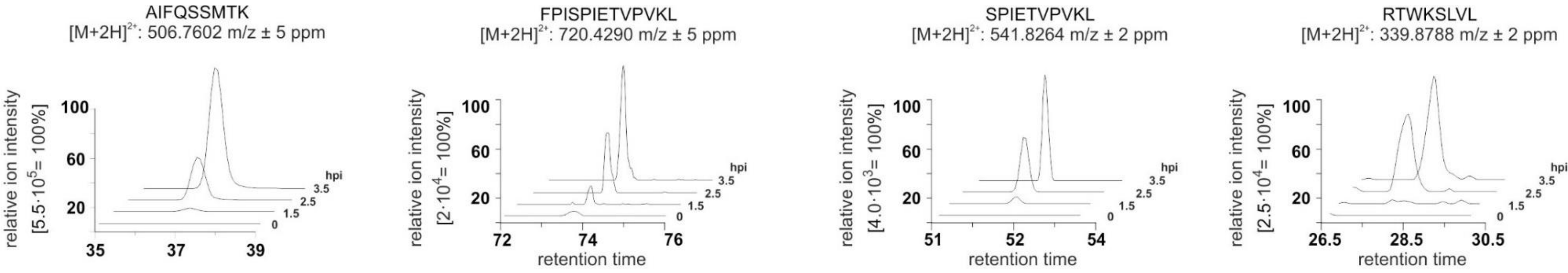
Sequence	length [number of amino acids]	Position in HXB2 (amino acid)	Position in HIVconsv (amino acid)	Previously reported in LANL-HSD	HLA supertype (allele)	max. score [-10lgP]	time-point post infection [h]
YKRWIILGLNK	11	Gag 262-272	58-68	KRWIILGLNK	A2 (A*02:01)	15.30	1.5
KRWIILGLNK	10	Gag 263-272	59-68	KRWIILGLNK	A2 (A*02:01)	16.35	2.5
IYKRWIILGLNK	12	Gag 261-272	57-68	KRWIILGLNK	A2 (A*02:01)	15.32	3.5
IILGLNK	7	Gag 266-272		KRWIILGLNK	na	14.82	2.5/3.5
FPISPIETVPVKL	13	Pol 155-167	194-206	SPIETVPVKL	B7 (B*81:01)	56.52	3.5/6
SPIETVPVKL	10	Pol 158-167	197-206	SPIETVPVKL	B7 (B*81:01)	41.33	2.5/3.5/6
AIFQSSMTK	9	Pol 313-321	351-360	AIFQSSMTK	A3 (A*03:01)	47.95	2.5/3.5/6
KIWPS-RWKPK[a]	10	Gag/Pol	131-140	na	na	19.25	6
RTWKSLVK	8	Vif 19-26	412-419	RIRTWKSLVK	A3 (A*03:01)	28.89	6
KLTP-WVPAHK[a]	10	Env/Pol	518-527	na	na	40.52	6
RKGGIGGYSAG	11	Pol 902-912	663-673	KRKGGIGGYSAGERI	B27	14.82	6

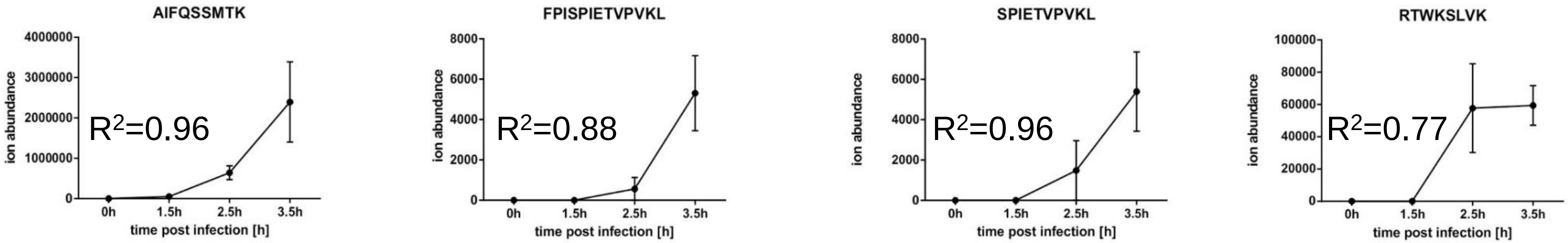
a – Peptides spanning a junction (-) between two conserved regions in the HIVconsv immunogen, thus creating a novel epitope not present on HIV-1-infected cells.

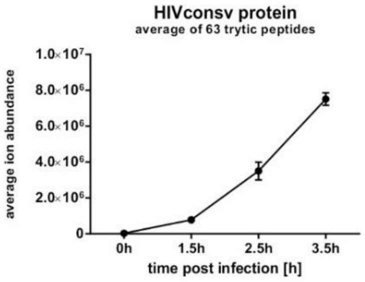
Ternette *et al.* JVI 2015

HIVconsv epitope presentation correlates with intracellular protein abundance









HIVconsv PROTEIN abundance

Ternette *et al.* JVI 2015

DATA EXAMPLE II
Triple negative breast cancer (TNBC)

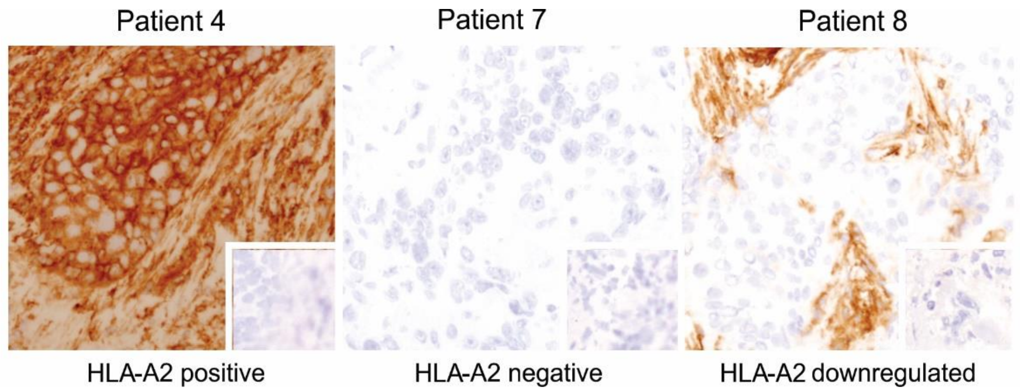


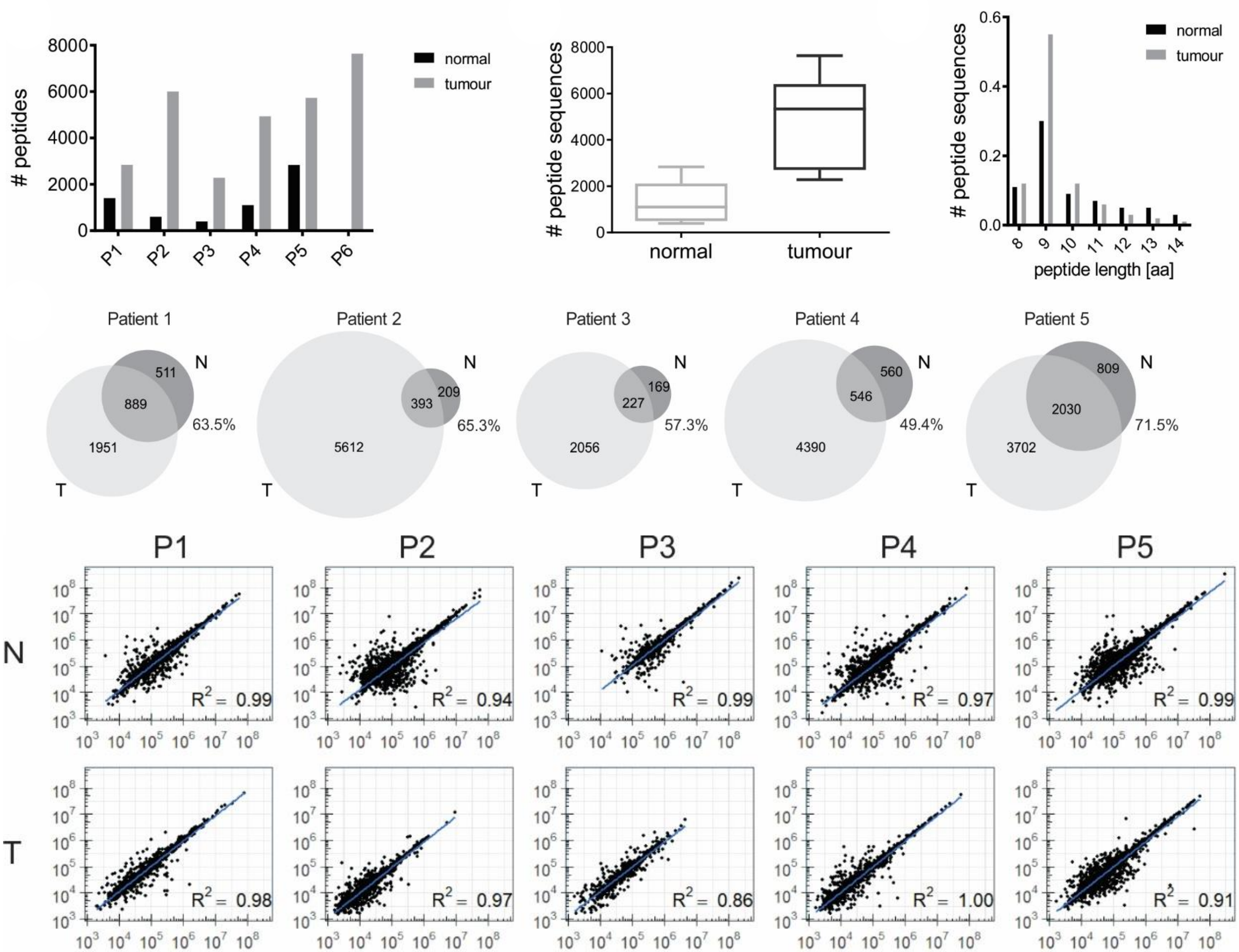
Table 1. Patient characteristics

Patient ID	Age	Sex	Diagnosis	HLA-A2 staining
1	53	F	Ductal NST	+
2	70	F	Pure special type - Basal	+
3	50	F	Ductal / NST	+
4	65	F	Ductal / NST	+
5	31	F	Ductal / NST	+
6	50	F	Ductal / NST	+
7	88	F	Ductal / NST	-
8	71	F	Ductal / NST	+
9	76	F	Ductal / NST	+
10	38	F	Ductal / NST	-
11	46	F	Ductal / NST	-
12	61	F	Classic Basal with Squamous areas	-
13	88	F	Ductal/Metaplastic Mixed	-
14	56	F	Ductal / NST	+
15	60	F	Ductal / NST	-

* NST, no-specific type; N/A, not applicable

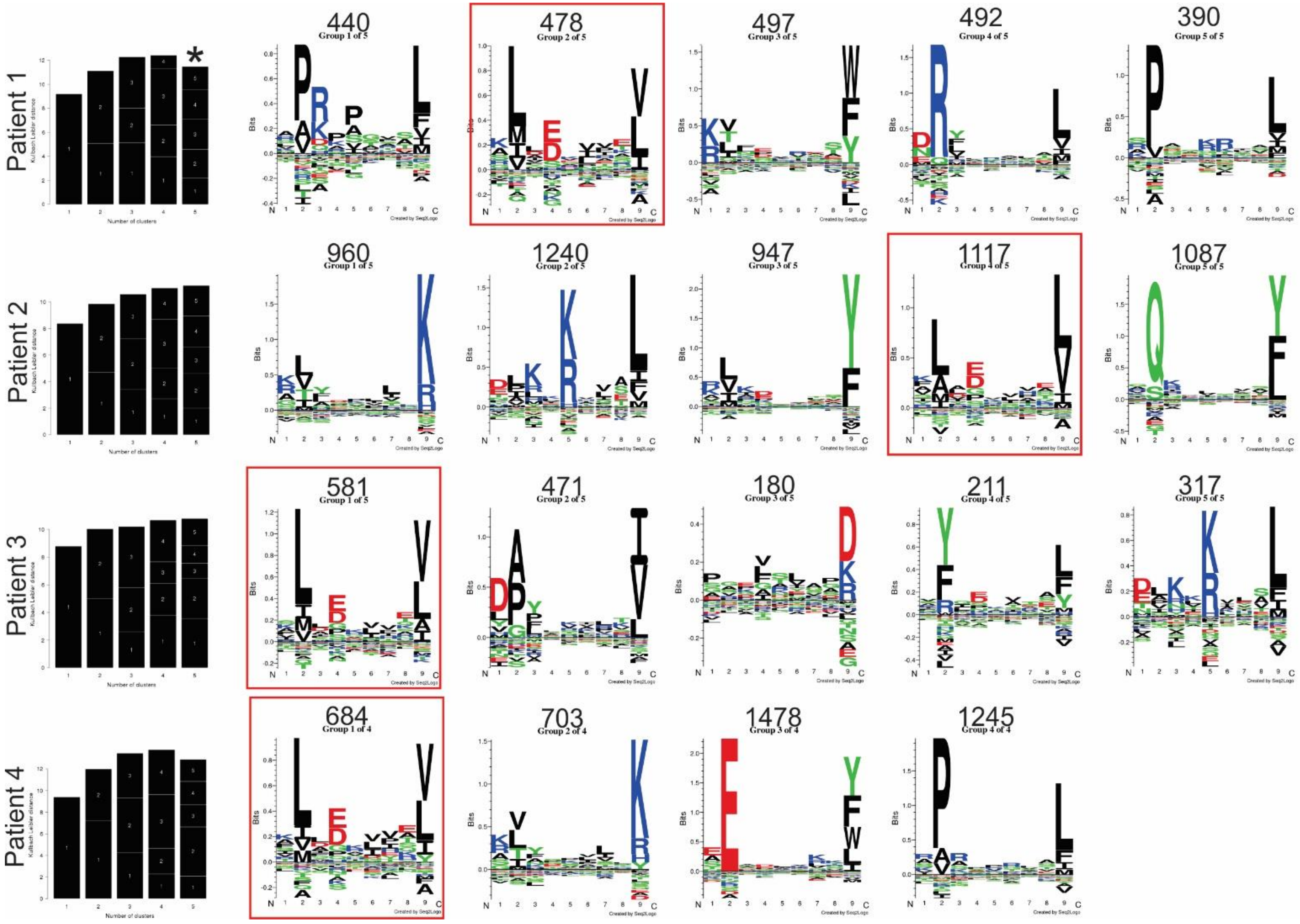
Ternette *et al.* Proteomics 2018

Triple negative breast cancer (TNBC)



Ternette *et al.* Proteomics 2018

A2-binding peptides are returned by Gibbs clustering



Ternette *et al.* Proteomics 2018

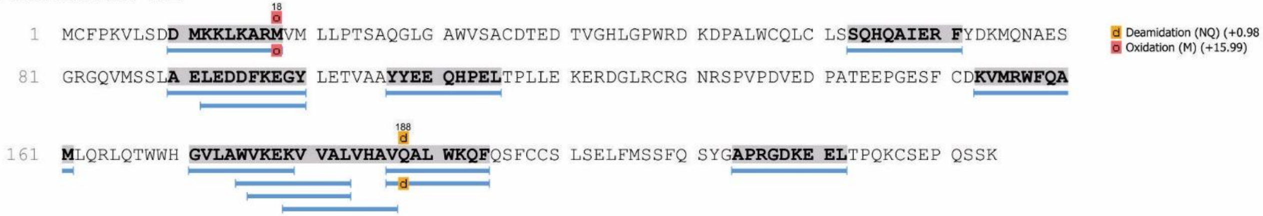
Shortlisting Protein Antigens in a cohort study: aTaCC

average **T**umour**−a**ssociated **C**ohort **C**overage

$$\text{aTaCC} = \frac{1}{P * M} \sum_{i=1}^{P} \sum_{j=1}^{N} l_{ij}$$

N: number of non-overlapping peptides presented by each patient
l: number of non-overlapping amino acids
M: length of the protein in amino acids
P: number of patients

Interleukin-32

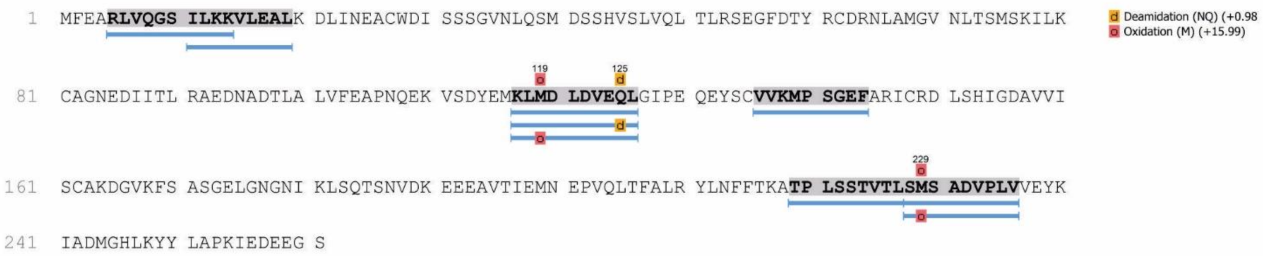


1 MCFPKVLSDD MKKLKARMVM LLPTSAQGLG AWVSACDTED TVGHLGPWRD KDPALWCQLC LSSQHQAIER FYDKMQNAES
81 GRGQVMSSLA ELEDDFKEGY LETVAAYYEE QHPELTPLLE KERDGLRCRG NRSPVPDVED PATEEPGESF CDKVMRWFQA
161 MLQRLQTWWH GVLAWVKEKV VALVHAVQAL WKQFQSFCCS LSELFMSSFQ SYGAPRGDKE ELTPQKCSEP QSSK

Syntenin-1

1 MSLYPSLEDL KVDKVIQAQT AFSANPANPA ILSEASAPIP HDGNLYPRLY PELSQYMGLS LNEEEIRANV AVVSGAPLQG
81 QLVARPSSIN YMVAPVTGND VGIRRAEIKQ GIREVILCKD QDGKIGLRLK SIDNGIFVQL VQANSPASLV GLRFGDQVLQ
161 INGENCAGWS SDKAHKVLKQ AFGEKITMTI RDRPFERTIT MHKDSTGHVG FIFKNGKITS IVKDSSAARN GLLTEHNICE
241 INGQNVIGLK DSQIADILST SGTVVTITIM PAFIFEHIIK RMAPSIMKSL MDHTIPEV

Proliferating cell nuclear antigen (PCNA)



1 MFEARLVQGS ILKKVLEALK DLINEACWDI SSSGVNLQSM DSSHVSLVQL TLRSEGFDTY RCDRNLAMGV NLTSMSKILK
81 CAGNEDIITL RAEDNADTLA LVFEAPNQEK VSDYEMKLMD LDVEQLGIPE QEYSCVVKMP SGEFARICRD LSHIGDAVVI
161 SCAKDGVKFS ASGELGNGNI KLSQTSNVDK EEEAVTIEMN EPVQLTFALR YLNFFTKATP LSSTVTLSMS ADVPLVVEYK
241 IADMGHLKYY LAPKIEDEEG S

Ternette *et al.* Proteomics 2018

Applications: Antigen Discovery Cancer Immunotherapy