MONASH University

Medicine, Nursing and Health Sciences

Immunoproteomics: New sources of protein with functional consequences from this alternative peptidome

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From the proteome to the (immuno)peptidome

- The proteome is relatively easily studied using well established qualitative and quantitative methodologies.

- Upon infection and during tumorigenesis changes in the cellular proteome are apparent; e.g. Viral antigen expression

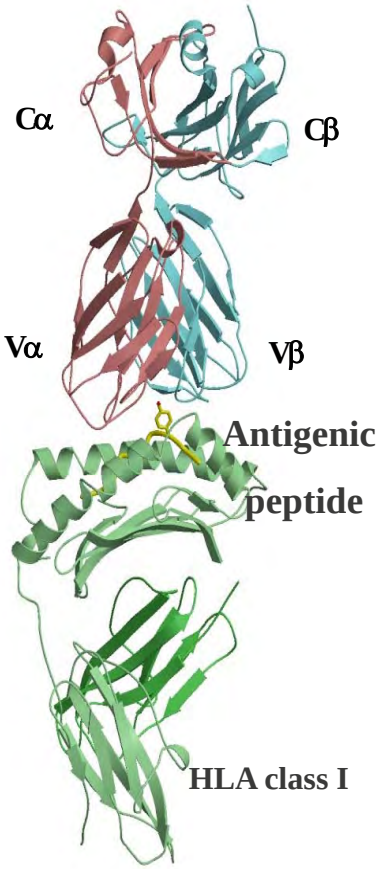
- But how is this information revealed to the immune system?

  = Molecular dissection of antigen presentation

Immune System Provides Continual Surveillance

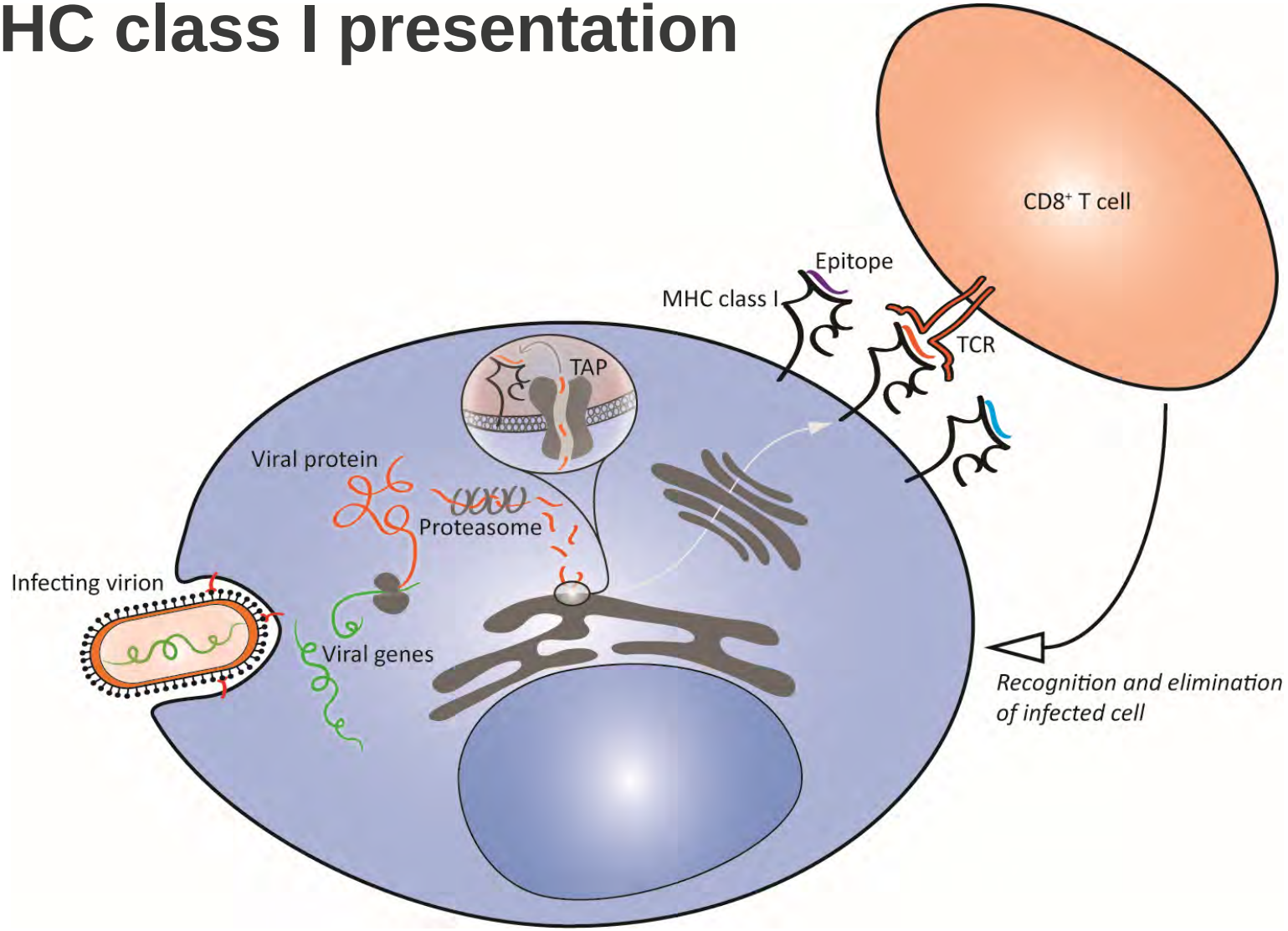
- Major Histocompatibility Complex (MHC) proteins display peptides on the cell surface
  - Cells degrade proteins into peptide fragments by diverse, non-specific proteolytic processes
  - MHC molecules bind to selected peptide fragments, and the MHC-peptide complexes are transported to the cell surface
- T cells continually scrutinize the MHC-peptide complexes displayed on cells throughout the body
  - Changes alert the immune system

Immunopeptidome



- Immunopeptidome is the collection of peptides bound to MHC molecules of interest
- Immunopeptidome plays a key role in immune defense
- We have developed a reliable method to map the immunopeptidome
- Due to the nature of the peptides they require special methods and analytical tools

MHC class I presentation



Immunopeptidomics (Purcell Lab, Monash University)

- Study of HLA-bound peptides
  - Characterisation of constitutive and infected cell immunopeptidomes
  - Influence and HLA polymorphism
  - Influence of the environment and drugs
  - Quantitation of antigen expression and epitope presentation (MRM, SWATH-MS)

What can the immunopeptidome reveal about protein expression in the cell?

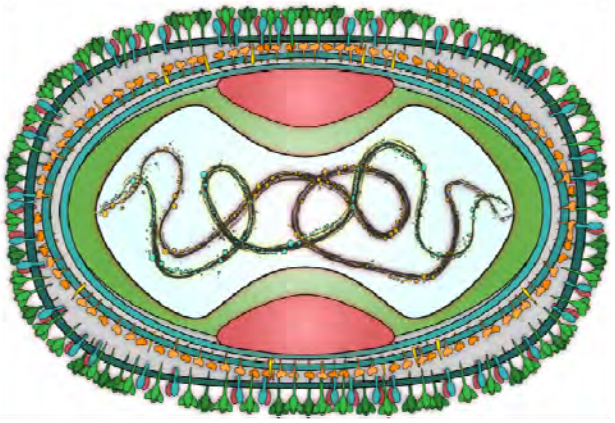
- Often a disconnect between protein abundance and representation in the immunopeptidome (4 orders of magnitude)
- Source of MHC peptides is impacted upon by bias towards DRiPs and pioneer strand synthesis
- SNPs are well represented in the immunopeptidome
- Peptides resulting from protein splicing are represented in the immunopeptidome
- Immune signatures can reveal the existence of proteins (e.g. SEREX approach identified the CT antigens

Vaccinia virus

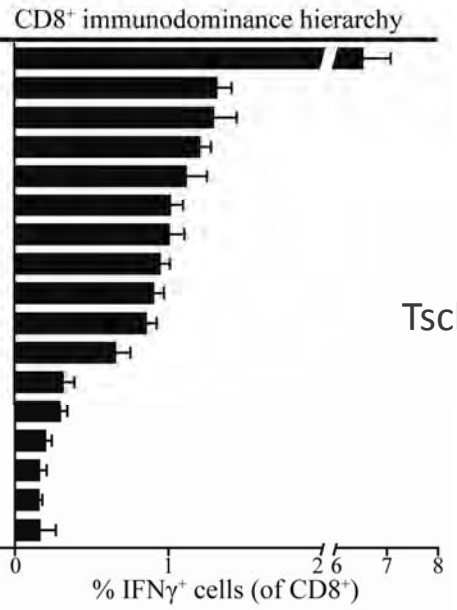
Large, complex dsDNA virus
Used to eradicate world of smallpox
(remains a bioterrorist threat)

VACV seeing potential as a viral vector for gene/epitope delivery for vaccine strategies

Established immunodominance hierarchy



Epitope	Sequence	IC_{50} (nM)	Function	Time
$B8_{20-27}$	TSYKFESV	K^b, 1.1	immune modulator	E
$A8_{189-196}$	ITYRFYLI	K^b, 6.2	viral transcription factor	IE
$A3_{270-277}$	KSYNYMLL	K^b, 2.7	virion core protein	L
$A23_{297-305}$	IGMFNLTFI	D^b, 0.34	viral transcription factor	E
$A47_{171-180}$	YAHINALEYI	D^b, nd	unknown	IE
$C4_{125-132}$	LNFRFENV	K^b, 0.84	unknown	E/L
$B2_{54-62}$	YSQVNKRYI	D^b, 32	unknown	IE
$K3_{6-15}$	YSLPNAGDVI	D^b, 8.4	immune modulator	IE
$A47_{138-146}$	AAFEFINSL	K^b, 0.61	unknown	IE
$L2_{53-61}$	VIYIFTVRL	K^b, 0.85	unknown	IE
$J3_{289-296}$	SIFRFLNI	K^b, 1.2	viral RNA pol component	E/L
$A8_{70-77}$	IHYLFRCV	K^b, 6.8	viral transcription factor	IE
$A42_{88-96}$	YAPVSPIVI	D^b, 1.4	profilin homologue	L
$N2_{60-68}$	FLMMNKDEL	D^b, 6.6	modifies host transcription	IE
$G8_{34-41}$	LMYIFAAL	K^b, 0.86	viral transcription factor	L
$A19_{47-55}$	VSLDYINTM	K^b, 0.94	unknown	L
$A47_{93-101}$	TMMINPFMI	D^b, 7.9	unknown	IE

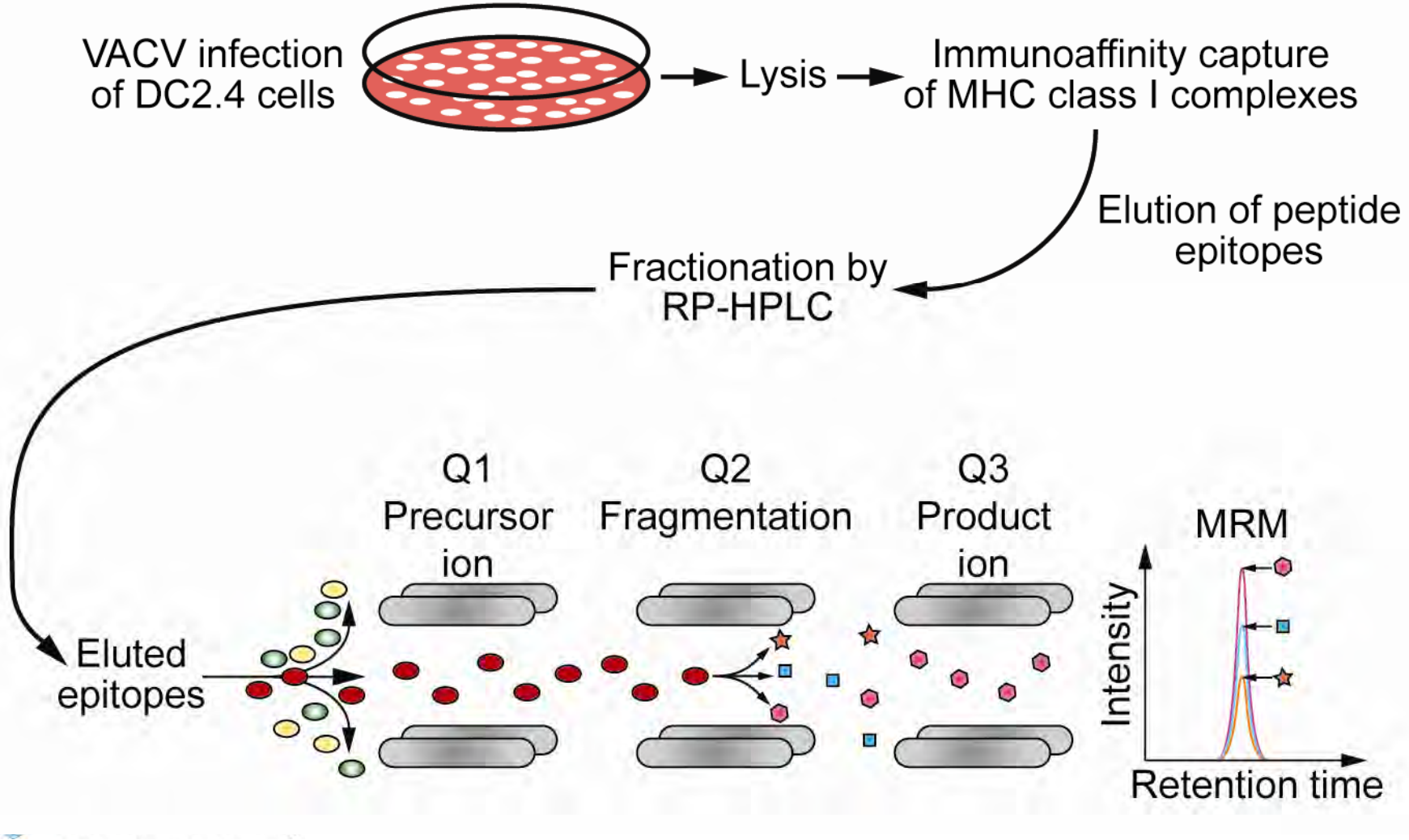


Tscharke *et al*, *J Exp Med*; 2005

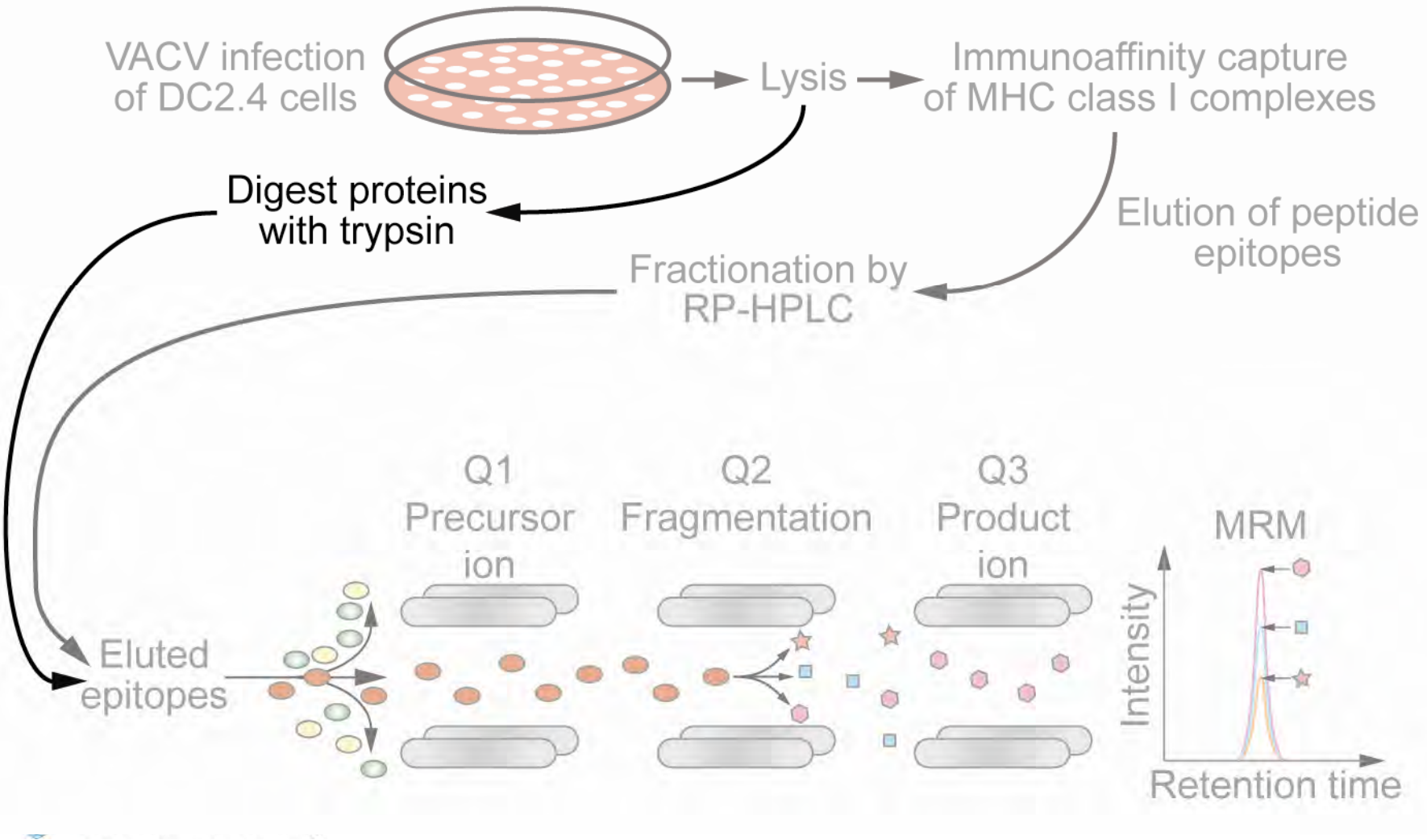
and unpublished

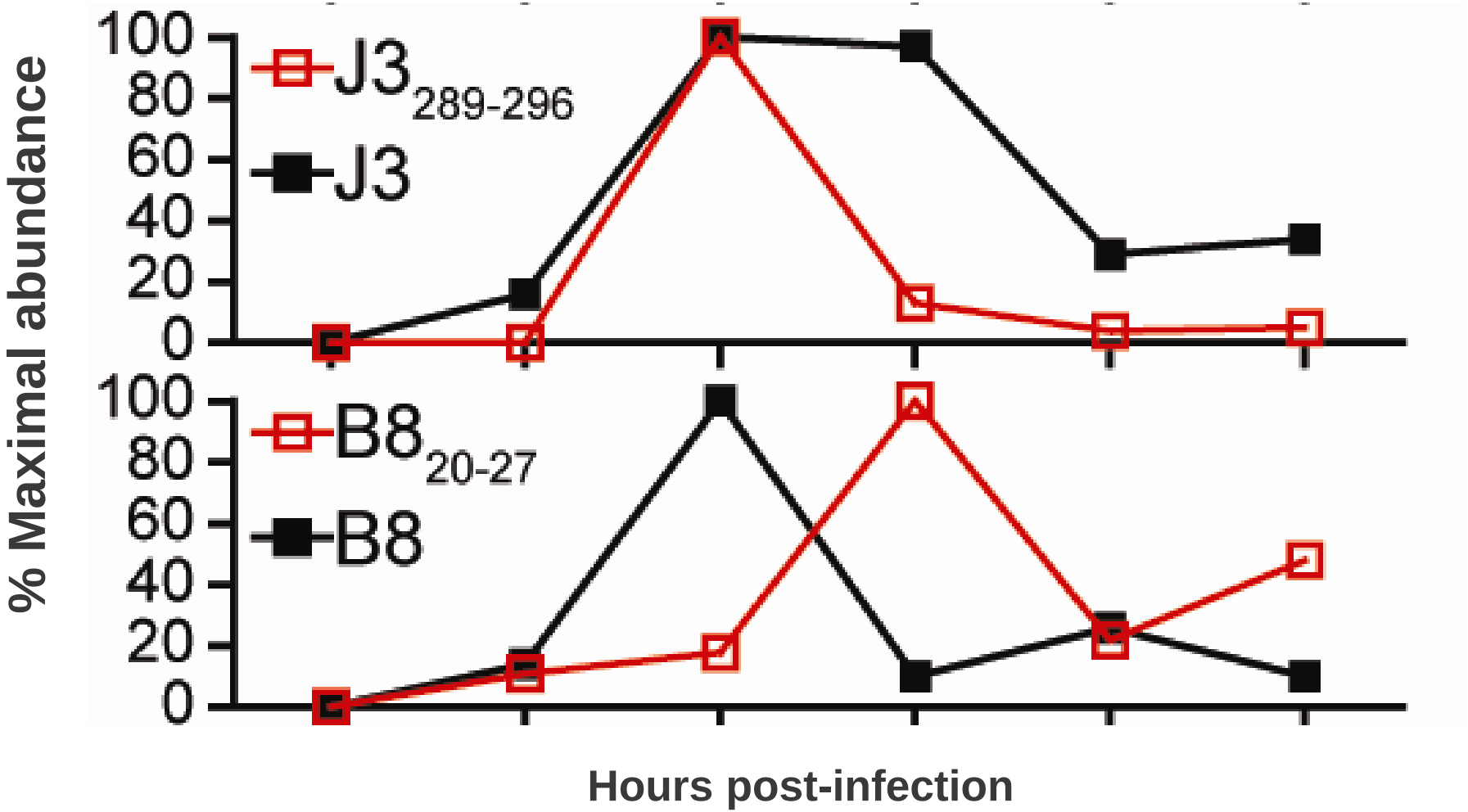
Strategy

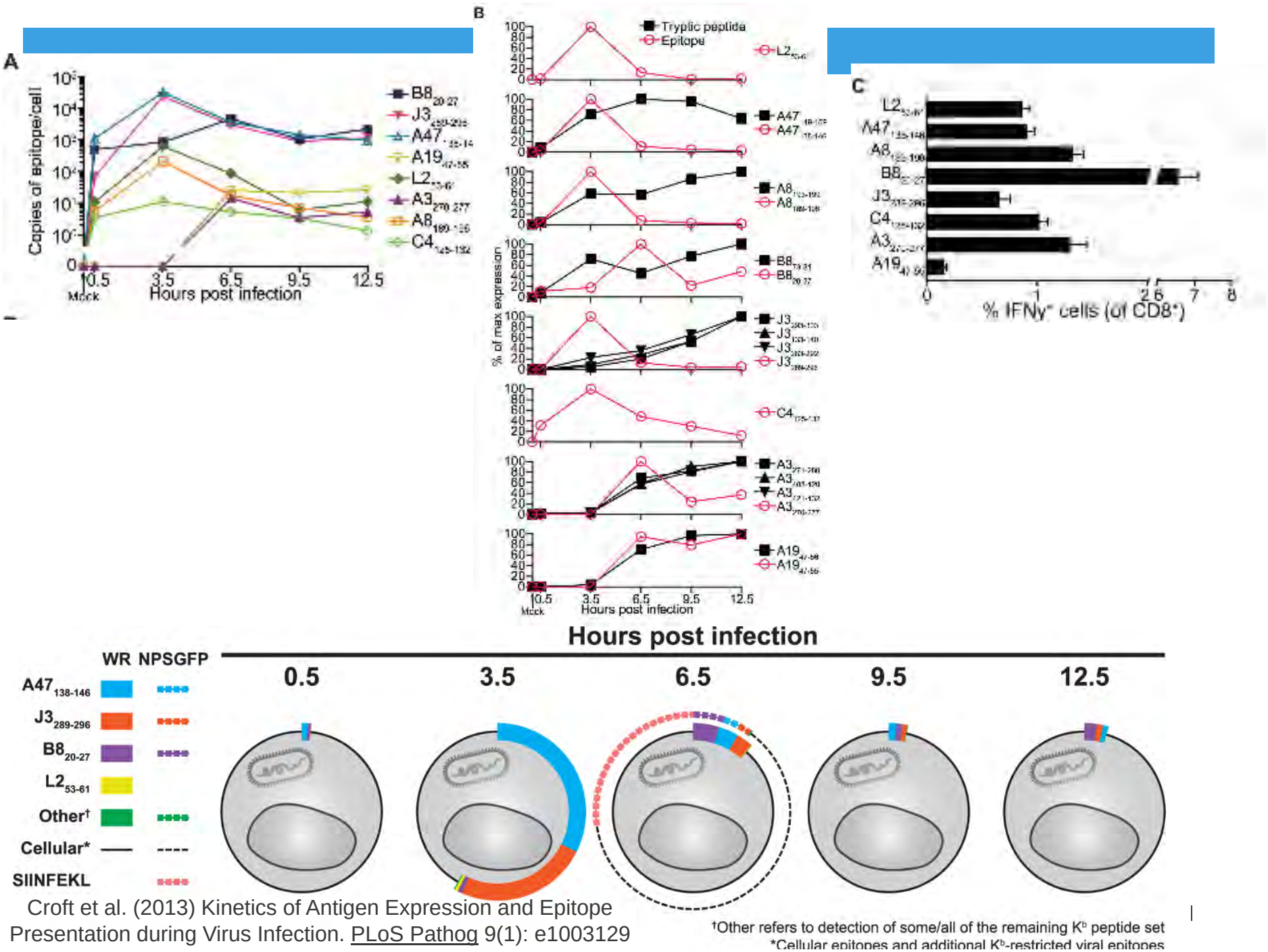
MHC epitope elution and multiple reaction monitoring



Correlating protein and epitope expression

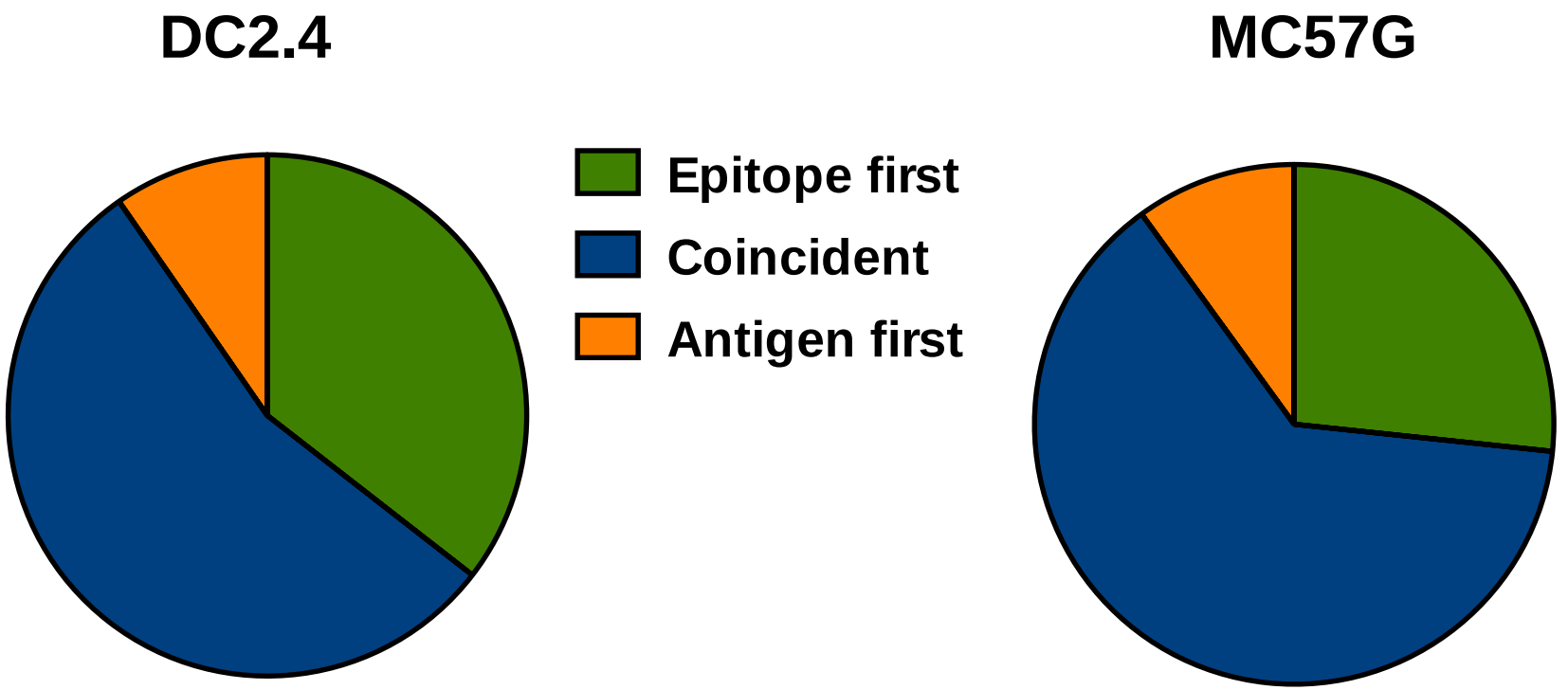



% Maximal abundance
100
80
60
40
20
0
J3289-296
J3
100
80
60
40
20
0
B820-27
B8
Hours post-infection



Croft et al. (2013) Kinetics of Antigen Expression and Epitope Presentation during Virus Infection. PLoS Pathog 9(1): e1003129

Relative kinetics: Antigen Vs Epitope

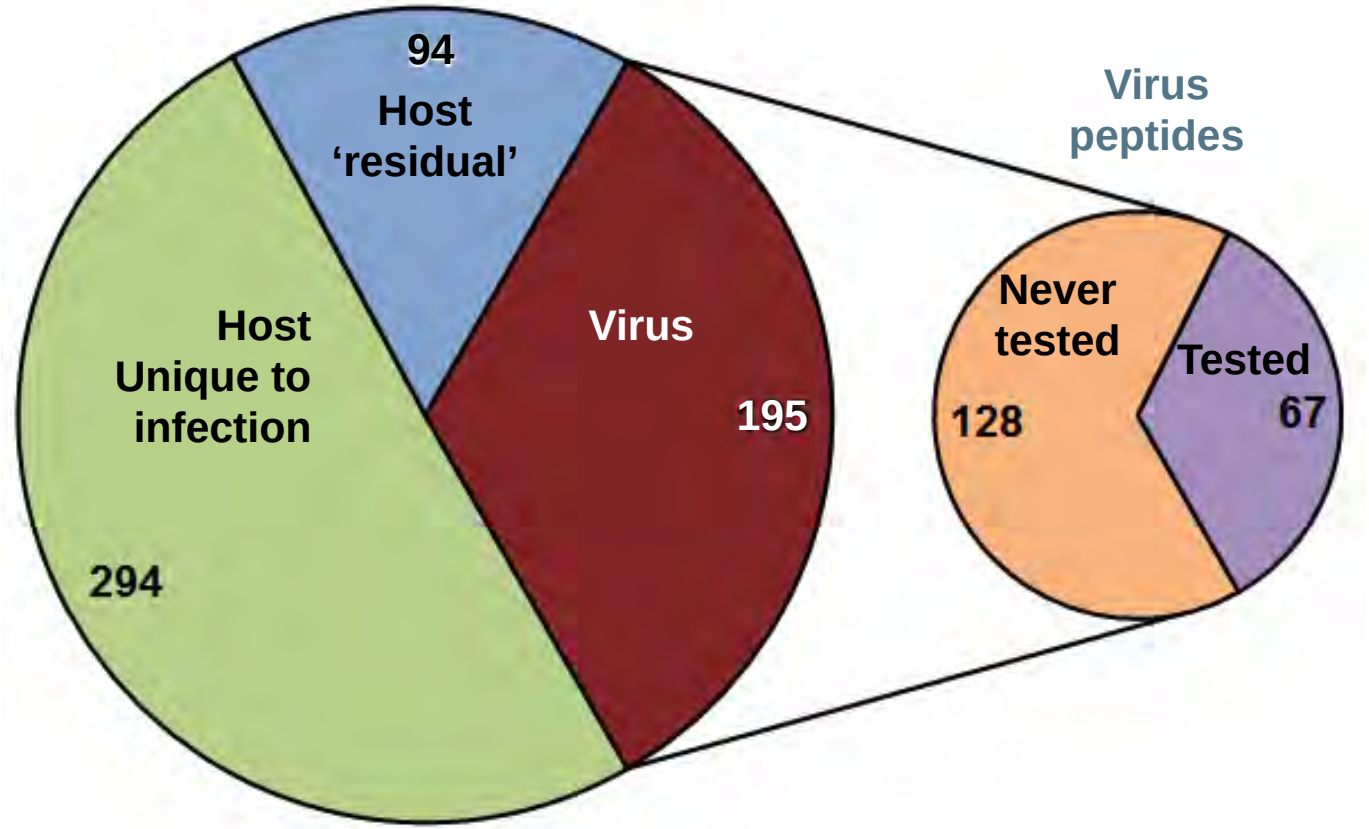


- 42 epitope / antigen pairs
- 90% of the time epitope appears with or earlier than antigen

LC-MS/MS detects many new presented peptides

- **Used unbiased LC-MS/MS approach**
  - › **Finds everything presented on MHC-I**

All peptides presented (6 hrs after infection)



Current in house database of >400,000 MHC peptides

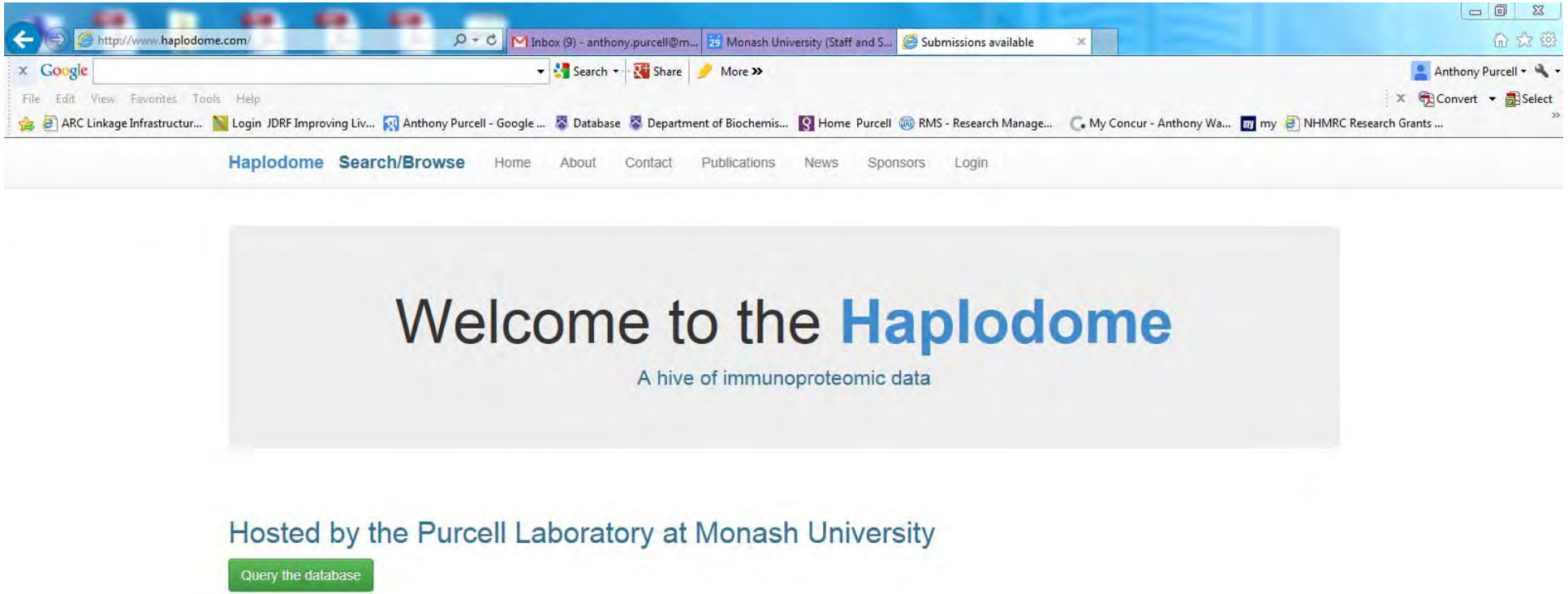
DRB1*0101 (DR1)
DRB1*0401 (DR4)
DRB1*0402
DRB1*0403
DRB1*0404
DRB1*0405
DRB1*0406
DRB1*0407
DRB1*0408
DRB1*0409
DRB1*0410
DRB1*0411
DRB1*0413
DRB1*0414
DRB1*0417
DRB1*0419
DRB1*0420
DRB1*0421
DRB1*03:01/DRB3:01:01 (DR3/DR52)
DRB1*1501/DRB5*01:01 (DR15/DR51)
DRB1*04:01/DRB4*1:03 (DR4/DR53)
DRB1*1201/DRB3*02:01 (DR12/DR52)

DQA1*0501/DQB1*02:01 (DQ2)
DQA1*0101/DQB1*0501 (DQ5)
DQA1*01:02/DQB1*0602 (DQ6)
DQA1*0301/DQB1*0302 (DQ8)
DQA1*0505/DQB1*0301 (DQ7.5)
DQA1*0301/DQB1*0201 (DQ trans)
DA11*0501/DQB1*0302 (DQ trans)
0501/0201 – 0301/0302 (DQ2/8 het)

DPA1*01:03/DPB1*04:01
DPA1*01/DPB1*03:01

A*01:01
A*01:01/B*08:01/C*07:01
A*02:01/B*15:01/C*03:04
A*0201
A*0301/B*0702/C*0702
A*0301
A*1501
B*1502
B*1801
A*2401
A*3101
B*5601
B*5602
B*5701
B*5703
B*5801
B*2705
B*3501
B*4402
B*4403
Cw4
Cw1

Mouse:
H2-K^d
H2-K^b
H2-D^b
H2-IA^{g7}
H2-Ia^b
H2-D^d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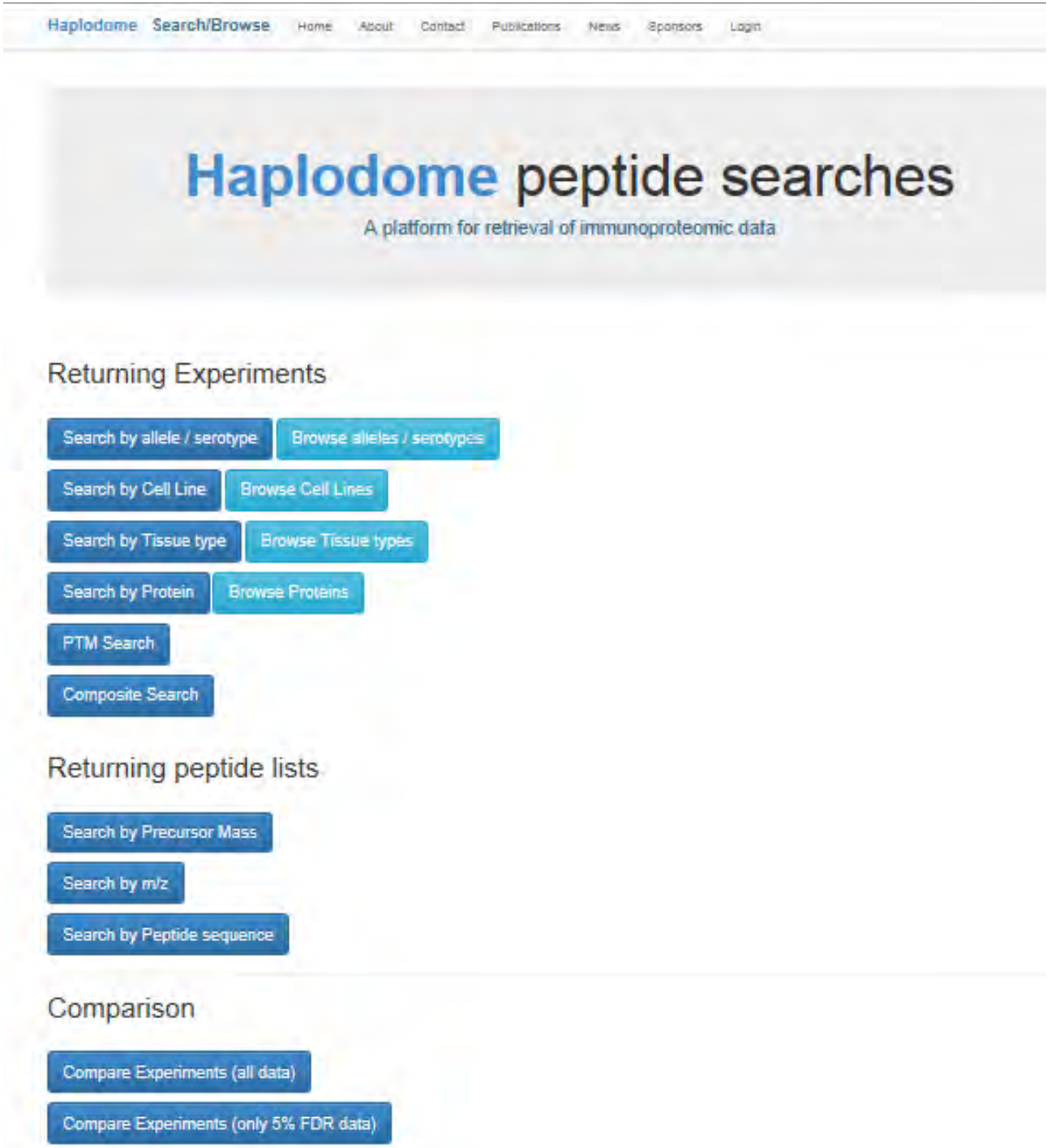
Welcome to the Haplodome
A hive of immunoproteomic data
Hosted by the Purcell Laboratory at Monash University
Query the database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A platform for retrieval of immunoproteomic data
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Browse alleles / serotypes
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Browse Cell Lines
Search by Tissue type
Browse Tissue types
Search by Protein
Browse Proteins
PTM Search
Composite Search
Returning peptide lists
Search by Precursor Mass
Search by m/z
Search by Peptide sequence
Comparison
Compare Experiments (all data)
Compare Experiments (only 5% FDR data)

Conclusions

- Immunopeptidome can reveal both novel and “missing” proteins
- Cases where no protein is detectable but immune responses can be directed towards these species – short lived, DRiPs, intractable to normal proteomic workflows, difficult proteins
- Inflammation, infection and autoimmunity are different and these states induce a different subset of proteins