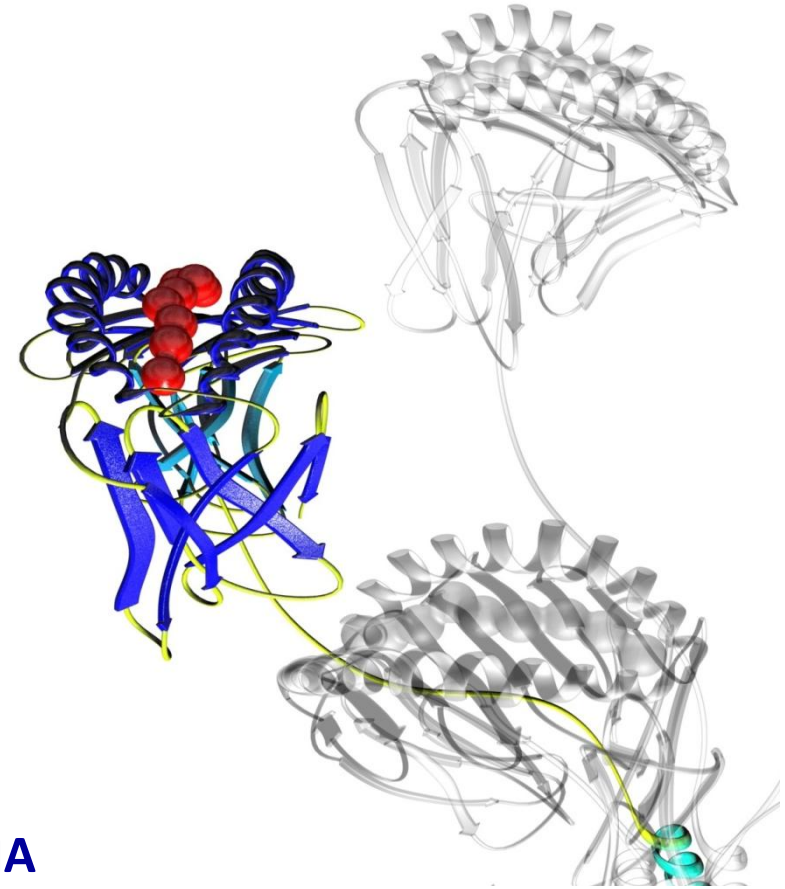
REVEAL™ Immunogenicity System: An integrated suite of *in vitro* assays

Dr. Jeremy Fry



The British Consulate-General, Boston, MA
September 12-13, 2011

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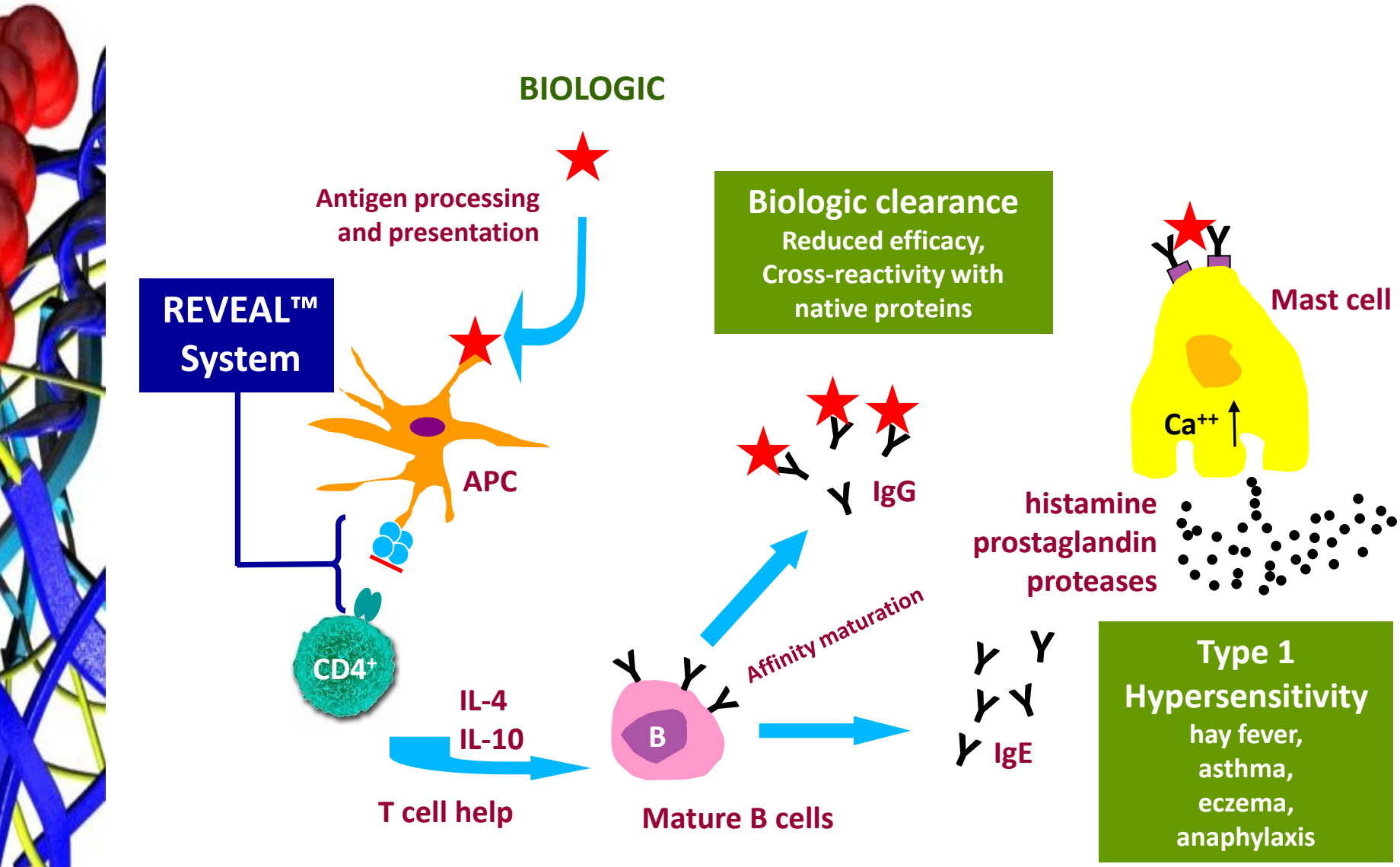
Immunogenicity of Biologics

- New biologics or consumer products contain novel protein content
- Unwanted immunogenicity can result in:
  - loss of biologic efficacy
  - altered pharmaco-kinetics
  - cross-reactive immune responses leading to serious adverse events
- Understanding potential unwanted immune responses to protein content is vital at the earliest development opportunity

Immunogenicity of Biologics

- **Intrinsic factors**
  - Protein sequence: T and B cell epitopes
- **Extrinsic factors**
  - Immune system status: hyper/hypo-responsive
  - Formulation
  - Frequency and route of administration / exposure



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How can antigenicity be predicted?

- **T cell epitopes are the key**
  - T cell epitopes by themselves do not cause, but are required, to drive clinical immunogenicity
    - *Antigen-specific CD4⁺ T cells recognize short protein-derived peptides presented by HLA class II molecules*
    - *Activated CD4⁺ T cells then provide B cell help to induce high affinity antibody responses*
- **B cell epitopes**
  - Anti-drug Antibodies (ADA) are directed towards B cell epitopes on biologics, but they can only mature in their affinity with the help of CD4⁺ T cells

T cell Epitope Antigenicity Profiling

- **Best practice for immunogenicity risk management**
- **Lead Ranking**
  - T cell epitope content
  - Evaluate the population impact of antigenicity (based on establishing a detailed knowledge of HLA-restriction of epitopes and the frequency of HLA alleles in the target population)
- **Pre-clinical Lead selection**
- **Lead optimization**
  - Identify regions suitable for re-engineering to reduce immunogenicity while maintaining efficacy
- **Strengthen regulatory position**
- **Create new relevant intellectual property**

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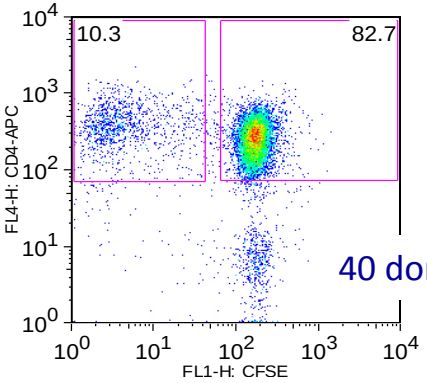
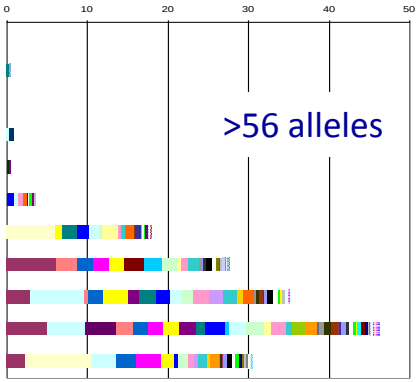
ProImmune REVEAL™ Immunogenicity System

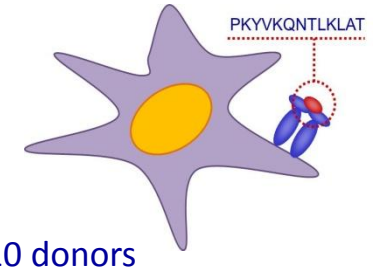
Naïve T cell / DC-T cell Proliferation assays



HLA class II Binding assays



ProPresent™ Antigen Presentation Assays



10 donors
LC-MS/MS

Identify & rank naturally processed T cell epitopes

Compare whole protein antigenicity

Lead selection / engineering

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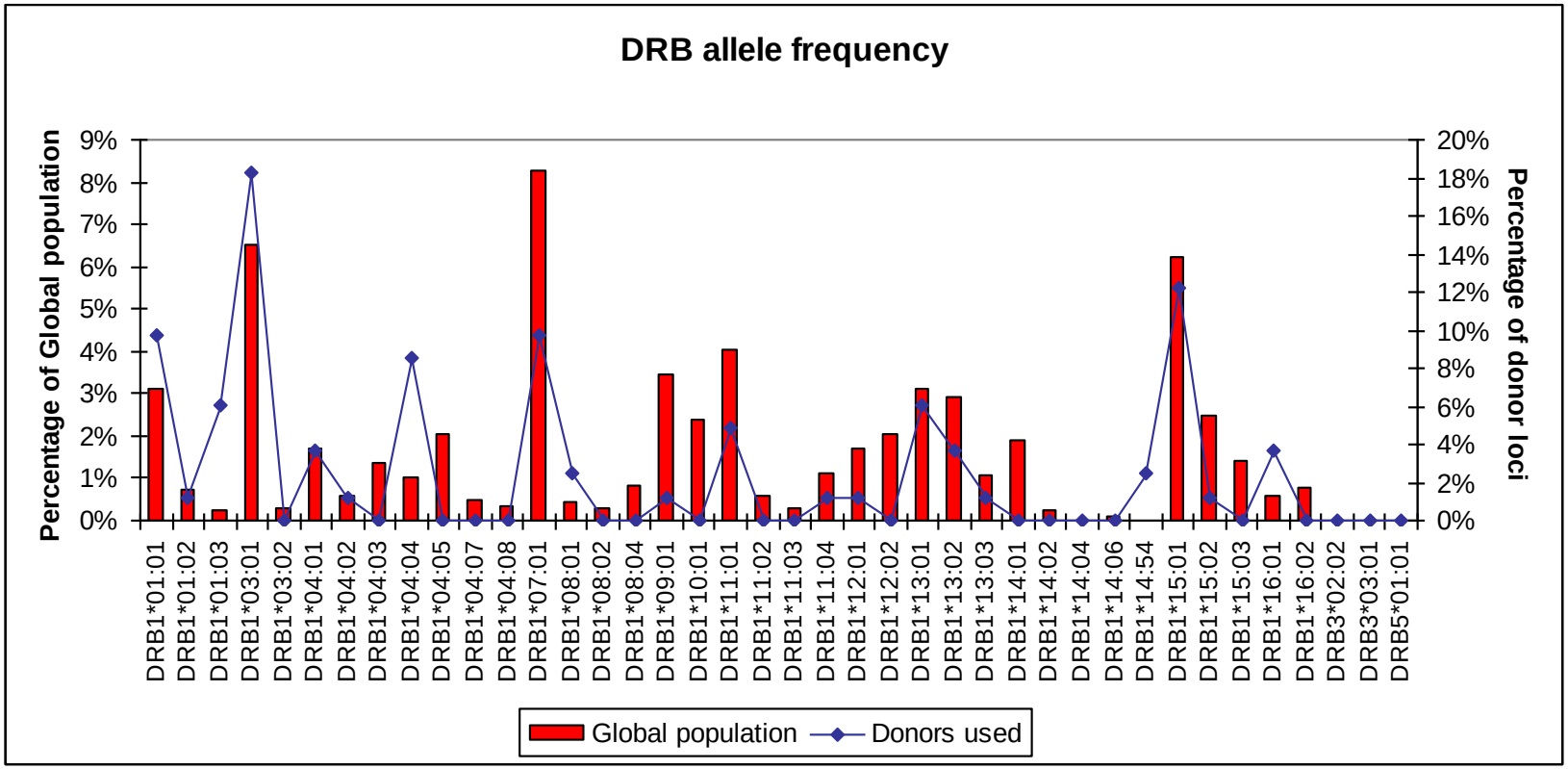
Primary Naïve T cell Assays

- **CD8⁺ depleted** PBMC from 20-50 x HLA-typed individuals representing tissue type distribution in the general population
- HLA-DR, -DP and –DQ; specific HLA distributions can be requested



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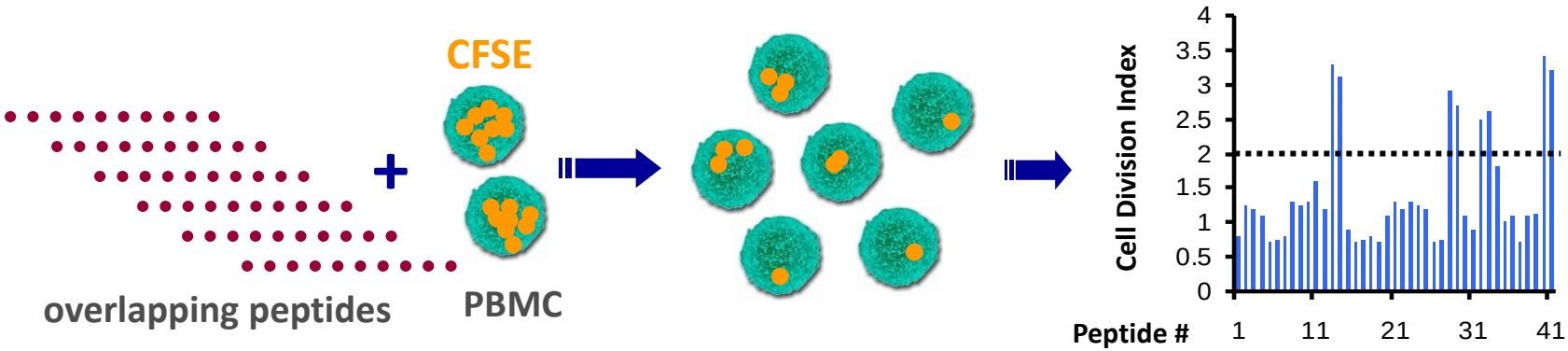
Primary Naïve T cell Assays

- Generate overlapping peptides for protein of interest: 15-mers offset by 3 amino acids
- Co-culture of PBMC with synthetic peptides
- T cell proliferation is measured over 7 day period by ultra-sensitive CFSE flow cytometry assay in sextuplicate analysis



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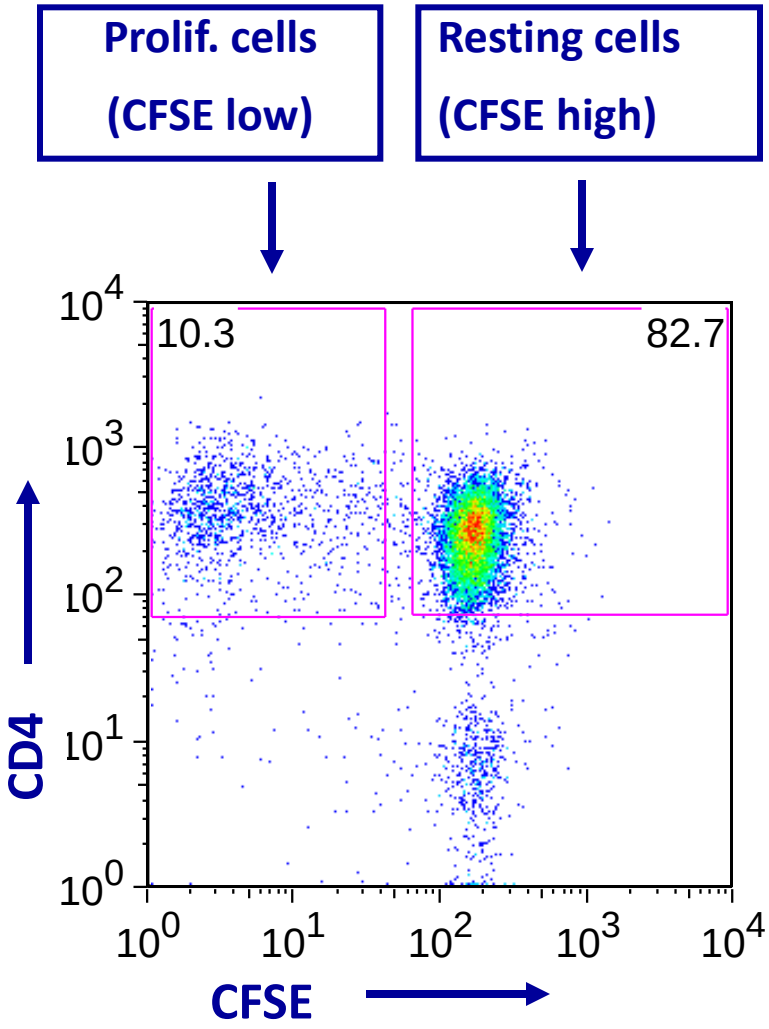
CFSE T cell Proliferation Assays

- Flow cytometry assay that measures only live $CD4^+$ cells
- Significantly more sensitive than ^3H-thymidine incorporation assays
- % $CD4^+$ proliferating cells can be accurately determined
- Allows detailed phenotyping of T cell responses
- Allows downstream monitoring and back-mapping of immune responses in clinical trials



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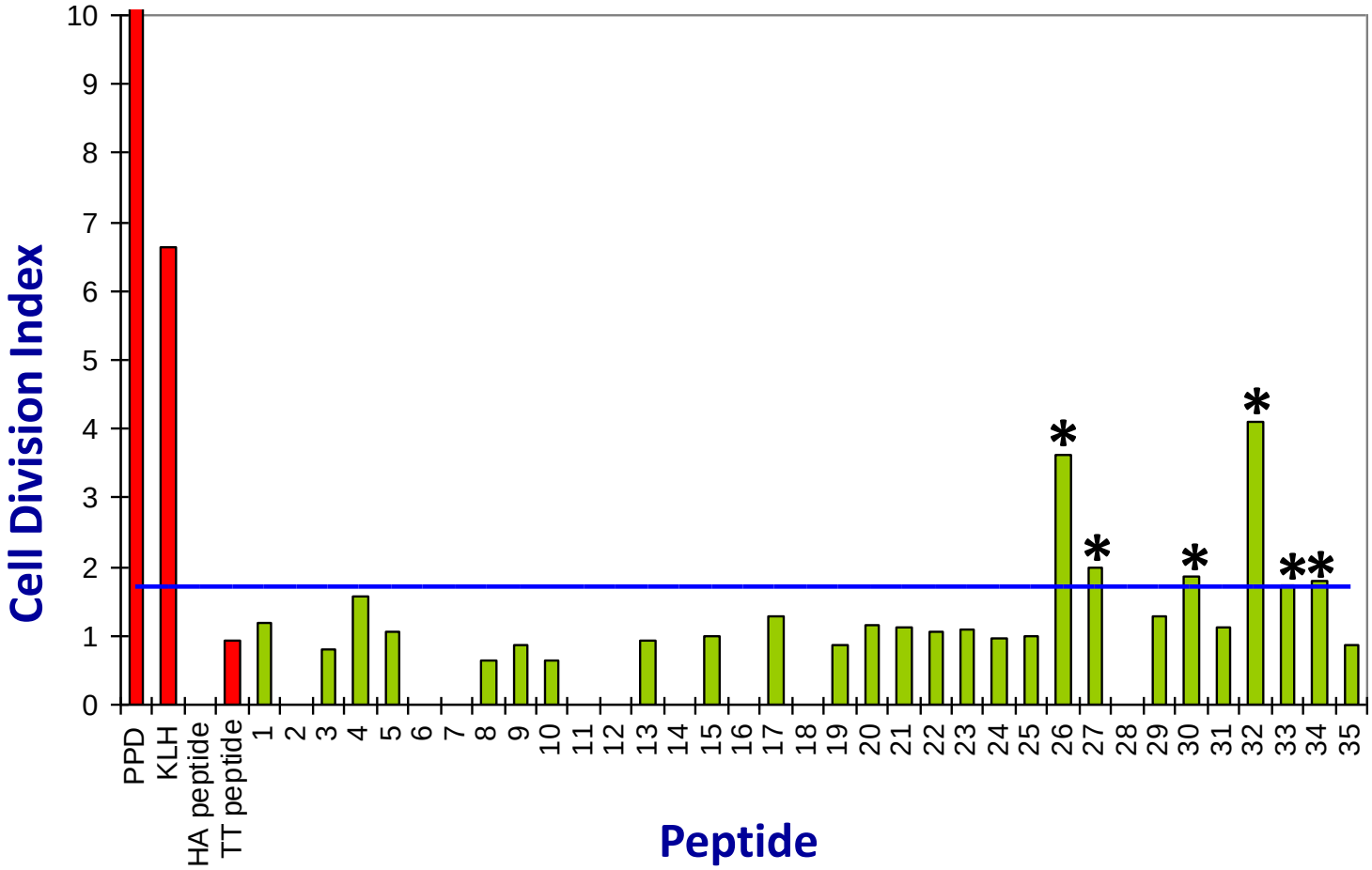
T cell epitope map example: SINGLE DONOR

***6 x Positive peptides: peptides 26, 27, 30, 32, 33, 34**

CDI>1.7; SD=2



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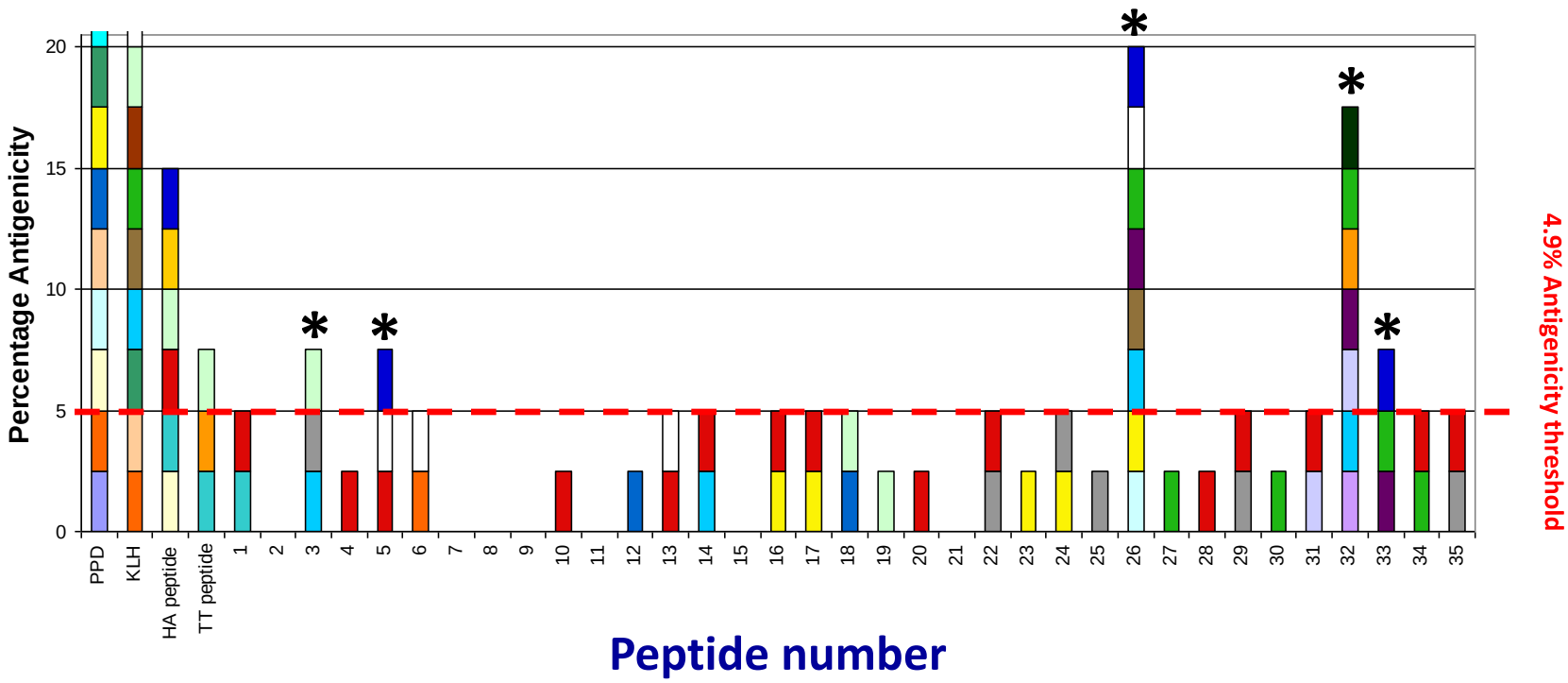
T cell epitope map example: ALL DONORS

*** 5 x Confirmed Epitopes:**
Peptides 3, 5, 26, 32

CDI >1.7; SD=2; n=41



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REVEAL™ HLA-peptide Binding Assays

- High-throughput physical HLA-peptide binding assays
  - Eliminate inaccuracies associated with *in silico* approaches
  - Broadest coverage of HLA-DR, -DP and -DQ alleles world-wide
  - >90% global coverage
  - Rapidly identifies the precise HLA restriction of T cell epitopes
  - Proven to rapidly identify T cell epitopes from proteins of interest
  - Applicable to wanted and un-wanted immune responses
- Understand immunogenicity risk in specific target populations, especially with HLA-linked diseases
- Assess the impact of mutations on HLA-binding

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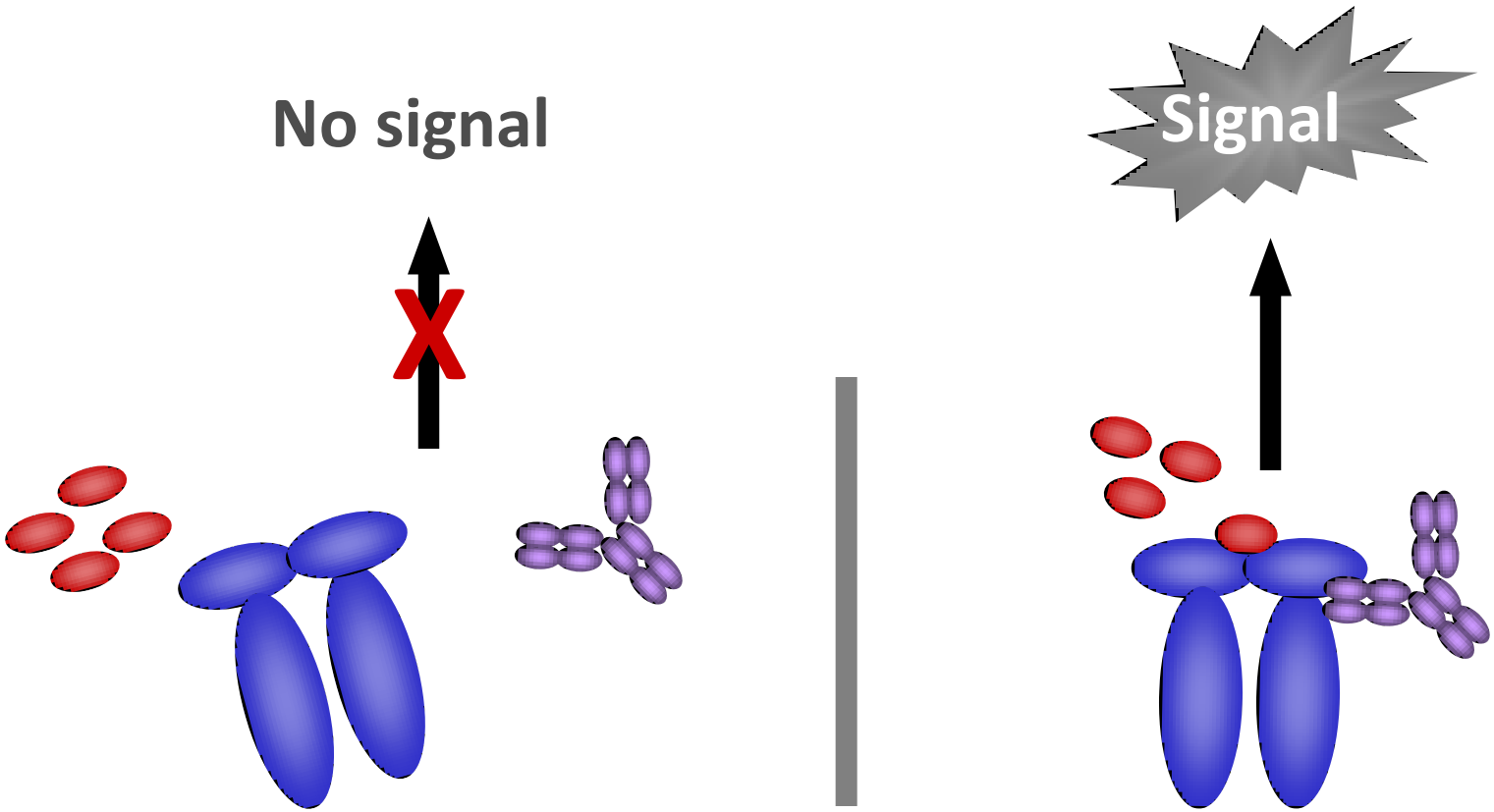
ProImmune REVEAL™ HLA Binding Assay



2 components:

MHC-peptide Binding Assay

MHC-peptide Stability Assay (24h, 37˚C)

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ProImmune REVEAL™ MHC Alleles

Class I

A*0101	DPA1*0103 + DPB1*0101
A*0201	DPA1*0103 + DPB1*0201
A*0301	DPA1*0103 + DPB1*0301
A*1101	DPA1*0103 + DPB1*0401
A*2402	DPA1*0103 + DPB1*0402
A*2902	DPA1*0103 + DPB1*0501
B*0702	DPA1*0201 + DPB1*0101
B*0801	DPA1*0201 + DPB1*0201
B*1402	DPA1*0201 + DPB1*0301
B*1501	DPA1*0201 + DPB1*0401
B*2705	DPA1*0201 + DPB1*0402
B*3501	DPA1*0201 + DPB1*0501
B*4001	DPA1*0201 + DPB1*0601
H-2Kb	DPA1*0201 + DPB1*0901
H-2Db	DPA1*0201 + DPB1*1101
H-2Ld	DPA1*0201 + DPB1*1301
H-2Kd	DPA1*0201 + DPB1*1401
H-2Dd	DPA1*0201 + DPB1*1501
Mamu-A*01	DPA1*0201 + DPB1*1701
Mamu-A*02	

Class II

DRA1*0101 + DRB1*0101	DRA1*0101 + DRB1*1502	
DRA1*0101 + DRB1*1501	DRA1*0101 + DRB1*1503	
DRA1*0101 + DRB1*0301	DRA1*0101 + DRB1*1601	
DRA1*0101 + DRB1*0401	DRA1*0101 + DRB1*1602	
DRA1*0101 + DRB1*1101	DRA1*0101 + DRB3*0202	
DRA1*0101 + DRB1*1301	DRA1*0101 + DRB3*0301	
DRA1*0101 + DRB1*0701	DRA1*0101 + DRB5*0101	
DRA1*0101 + DRB1*0102	DQA1*0101 + DQB1*0501	
DRA1*0101 + DRB1*0402	DQA1*0501 + DQB1*0301	
DRA1*0101 + DRB1*0404	DQA1*0102 + DQB1*0502	
DRA1*0101 + DRB1*0405	DQA1*0102 + DQB1*0602	
DRA1*0101 + DRB1*0407	DQA1*0301 + DQB1*0302	
DRA1*0101 + DRB1*0408	DQA1*0102 + DQB1*0604	
DRA1*0101 + DRB1*0804	DQA1*0501 + DQB1*0201	
DRA1*0101 + DRB1*0901	DQA1*0201 + DQB1*0202	
DRA1*0101 + DRB1*1001	DQA1*0301 + DQB1*0301	
DRA1*0101 + DRB1*1102	DQA1*0201 + DQB1*0303	
DRA1*0101 + DRB1*1103	DQA1*0303 + DQB1*0303	
DRA1*0101 + DRB1*1104	H-2IAb	
	H-2IAd	

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REVEAL™ Immunogenicity Case Study 1

- **Remicade® (infliximab)**
  - Chimeric monoclonal IgG1 antibody (human constant and murine variable regions)
  - Binds to soluble and transmembrane forms of TNFα and inhibits binding of TNFa with its receptors
  - Indication: RA, Crohn's disease, Ulcerative Colitis, Ankylosing spondilitis
  - **Immunogenicity:** 10-50% of patients develop low titer neutralizing Abs against (depending on indication)
- **Humira® (adalimumab)**
  - IgG1 antibody with human derived heavy and light chain variable regions and human IgG1:k constant regions generated by phage-display
  - **Immunogenicity:** 5%-20% of patients develop low titer neutralizing Abs (depending on indication)

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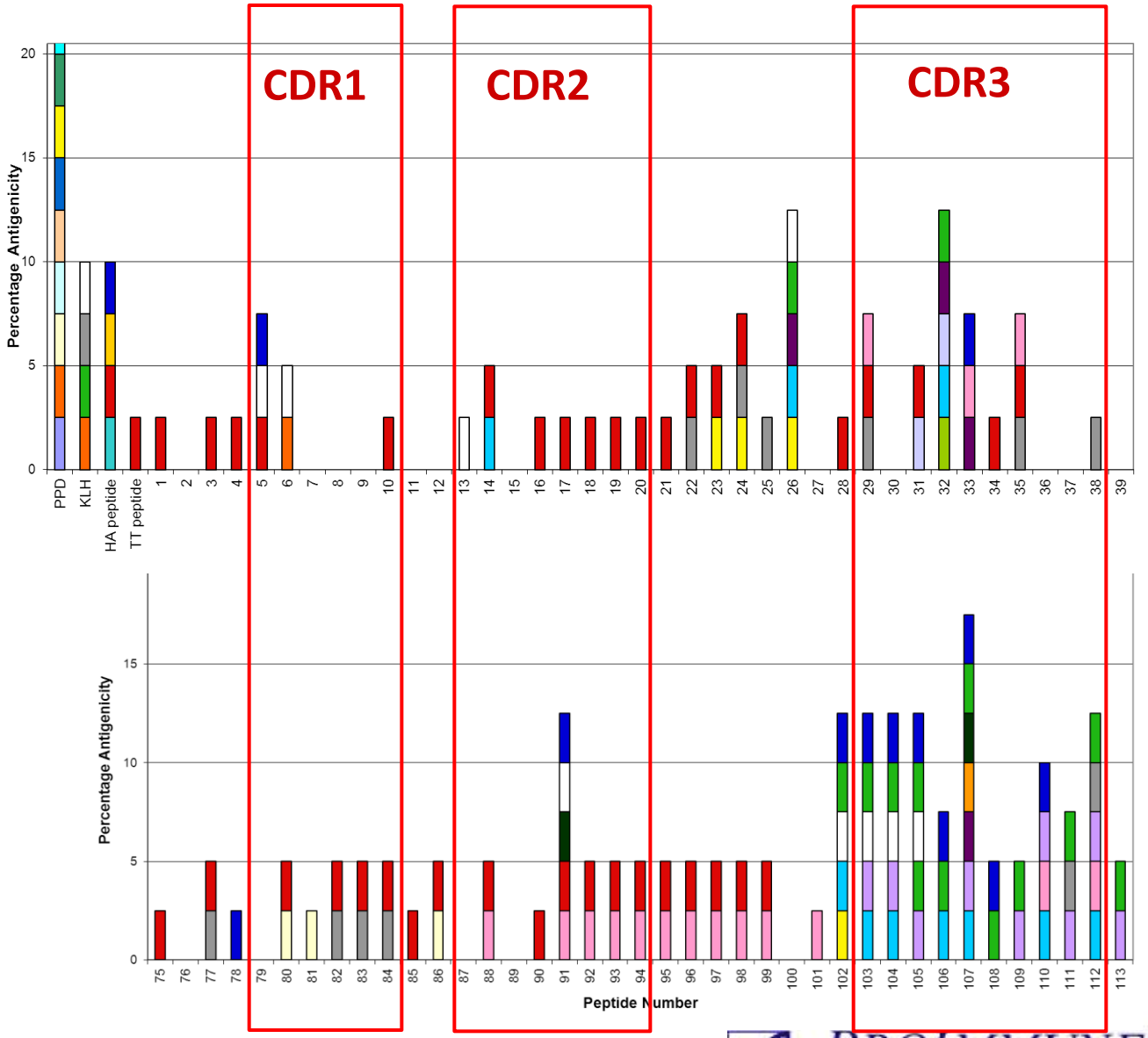
T cell Proliferation – Heavy Chain

Remicade®
Heavy Chain



Humira®
Heavy Chain

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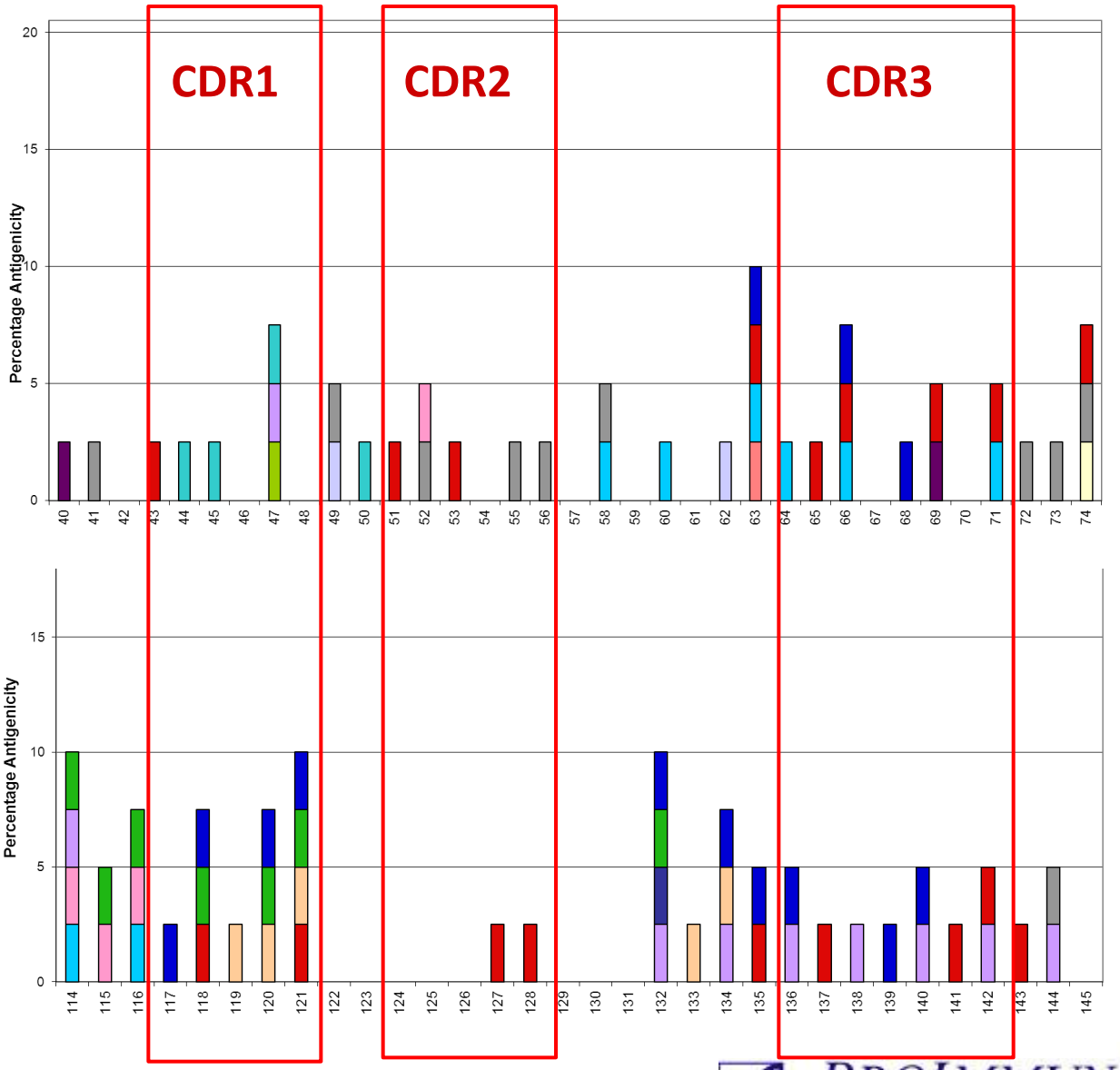
T cell Proliferation – Light Chain

Remicade®
Light Chain



Humira®
Light Chain

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REVEAL™ Binding Assay

DRA*01:01;DRB1*11:01					
Peptide I.D.	MHC-Binding Score at 0 h	MHC-Binding Score at 24 h	Graphical Representation of REVEAL™ Score	Stability Index	Graphical Representation of Stability Index
1. AKNSLYLQMNSLRAE	88.7	82.7		106.4	
2. SLYLQMNSLRAEDTA	99.3	72.5		52.5	
3. LQMNSLRAEDTAVYY	2.8	1.8		1.0	
4. NSLRAEDTAVYYCAK	4.2	0.0		0.0	
5. RAEDTAVYYCAKVSY	38.1	0.3		1.3	
6. AEDTAVYYCAKVSYL	36.6	0.6		1.5	
7. TAVYYCAKVSYLSTA	46.2	10.9		5.3	
8. YYCAKVSYLSTASSL	45.2	18.7		8.5	
9. AKVSYLSTASSLDYW	34.3	7.1		3.6	
10. SYLSTASSLDYWGQG	2.8	0.0		0.0	
11. STASSLDYWGQGTLV	0.0	0.0		0.0	
12. SSLDYWGQGTLVTVS	6.8	0.0		0.0	
13. SLDYWGQGTLVTVSS	13.7	0.0		0.0	
14. DIQMTQSPSSLSASV	34.8	0.4		1.3	
15. MTQSPSSLSASVGDR	0.0	0.0		0.0	
16. SPSSLSASVGDRVTI	0.0	0.0		0.0	
17. SLSASVGDRVTITCR	0.0	0.0		0.0	
18. ASVGDRVTITCRASQ	26.7	1.3		1.5	
19. GDRVTITCRASQGIR	38.9	9.6		4.6	
20. VTITCRASQGIRNYL	13.7	2.7		1.4	
Positive Control	100.0 +/- 6.6	94.0 +/- 13.0		114.9 +/- 12.6	
Intermediate Control	13.8 +/- 3.5	13.8 +/- 3.2		16.6 +/- 4.1	
			20 40 60 80	6 120	
				Stability Guide:	LOW HIGH VERY HIGH

Representative analysis of a single allele (DRB1*11:01) from the C-terminus of Humira® heavy chain (incorporating the CDR3 region) and N-terminus of the light chain

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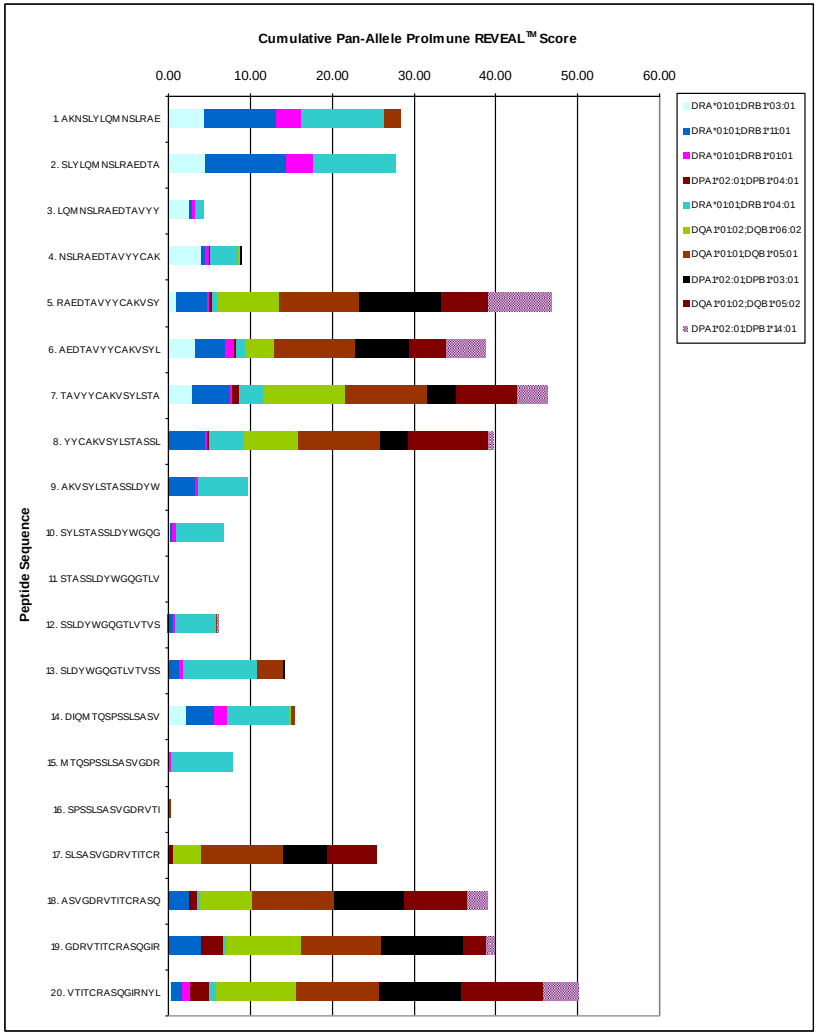
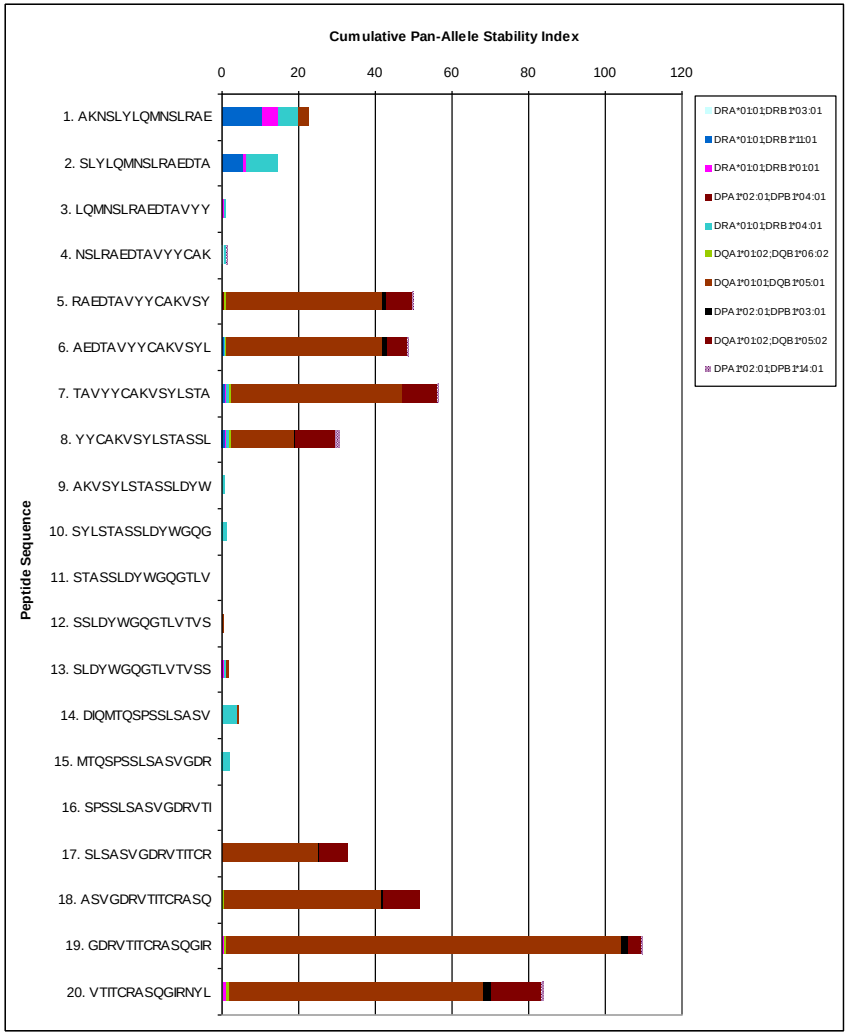
REVEAL™ Binding Assay



REVEAL™ Stability Assay



Representative analysis (Binding and Stability assay) of peptides from C-terminus of Humira® heavy chain (incorporating the CDR3 region) and N-terminus of the light chain against a sub-set of 10 x HLA class II alleles

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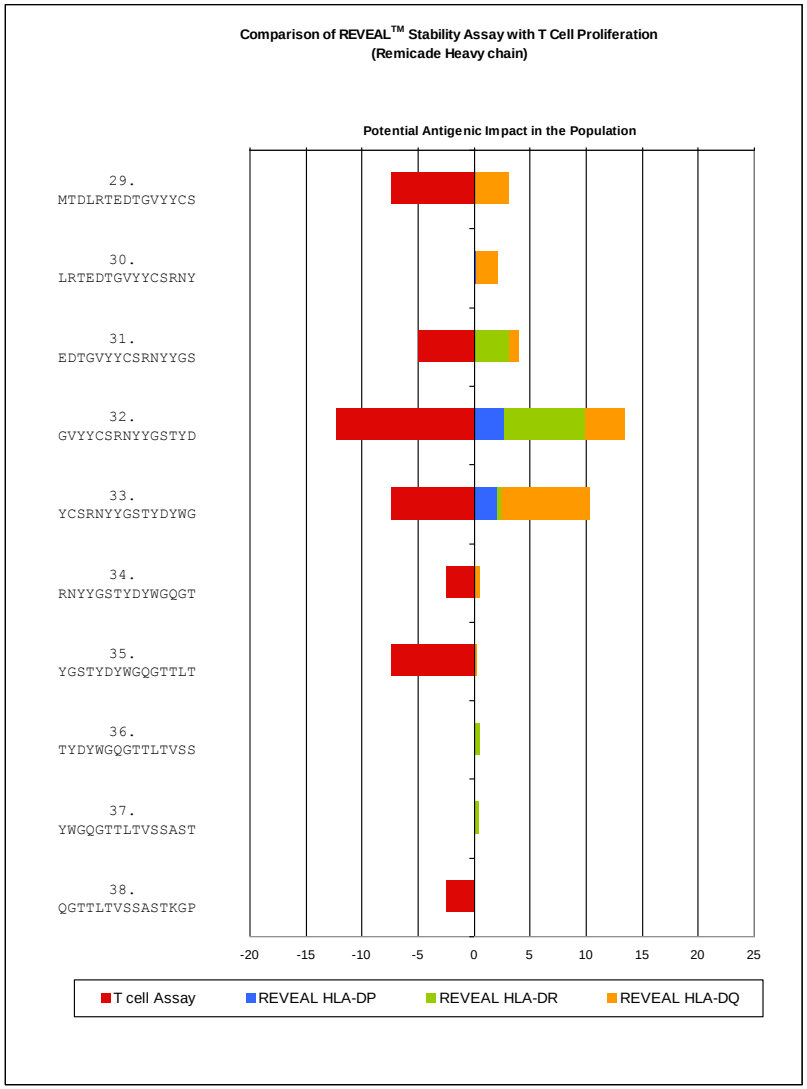
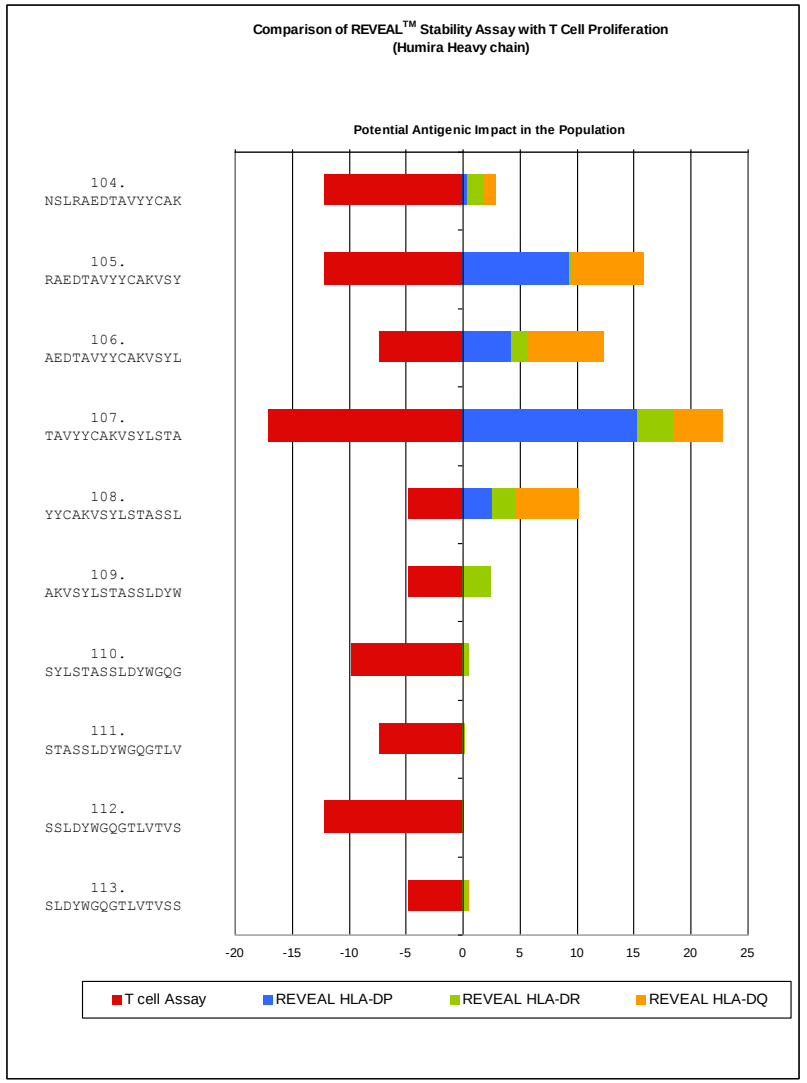
Remicade Heavy chain



Humira Heavy chain



T cell assay **HLA-DP** **HLA-DR** **HLA-DQ**

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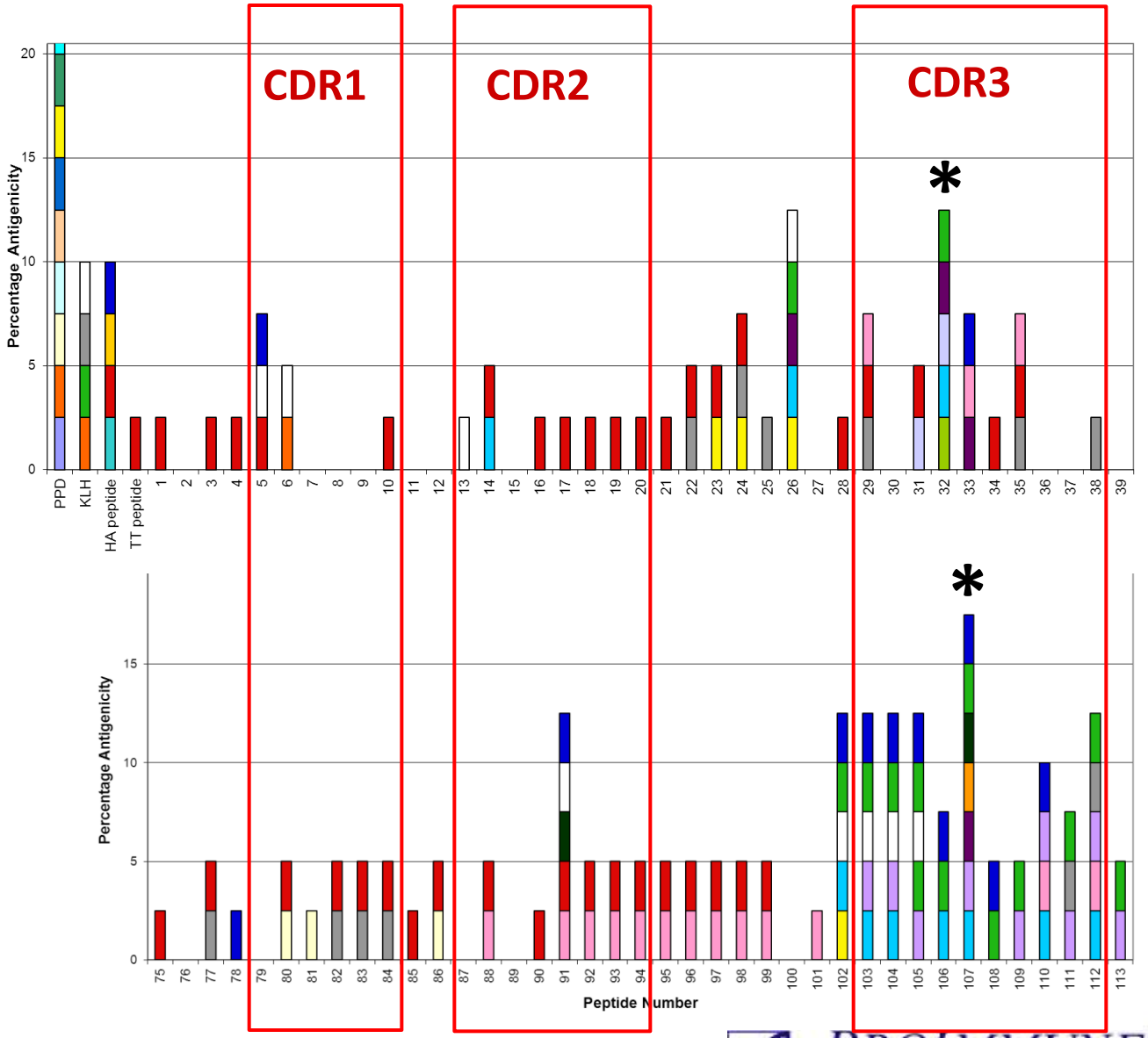
T cell Proliferation – Heavy Chain

Remicade®
Heavy Chain



Humira®
Heavy Chain

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HLA restriction of functional T cell epitopes

Analysis of Peptide 32 (GVYYCSRNYYGSTYD) from Remicade® Heavy Chain CDR3

Donor ID	DRB1		DQB1		DPB1	
D387	*13:01	*15:01	*06:03	*06:02	*13:01	*01:01
D400	*03:01	*16:01	*02:01	*05:02	*04:01	*04:01
D401	*04:04	*15:01	*06:02	*03:02	*04:01	*04:01
D415	*03:01	*07:01	*02:02	*02:01	*04:01	*02:01
D426	*04:01	*13:02	*03:02	*06:05	*04:02	*10:01

Analysis of Peptide 107 (TAVYYCAKVSYLSTA) from Humira® Heavy Chain CDR3

Donor ID	DRB1		DQB1		DPB1	
D393	*03:01	*04:04	*02:01	*03:02	*02:02	*03:01
D400	*03:01	*16:01	*02:01	*05:02	*04:01	*04:01
D407	*01:01	*03:01	*02:01	*05:01	*04:01	*04:02
D413	*01:03	*01:01	*05:01	*05:01	*04:01	*04:02
D415	*03:01	*07:01	*02:02	*02:01	*04:01	*02:01
D424	*01:03	*15:01	*05:01	*06:02	*04:01	*04:01
D430	*01:01	*11:04	*03:01	*05:01	*04:01	*04:02

Key

Red – No Binding to MHC

Green – Stable Binding

Yellow – Weak affinity binding

Grey – Untested / Unavailable

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ProImmune REVEAL™ Case Study 2

Biodefense project (DSTL, UK govt.)

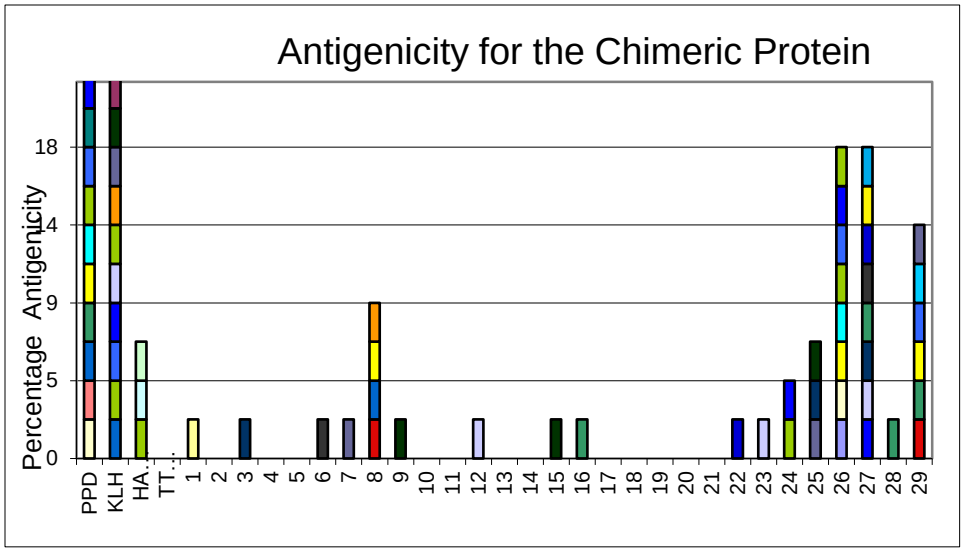
- mAb for passive immunisation
- Standard humanization / assess impact on antigenicity

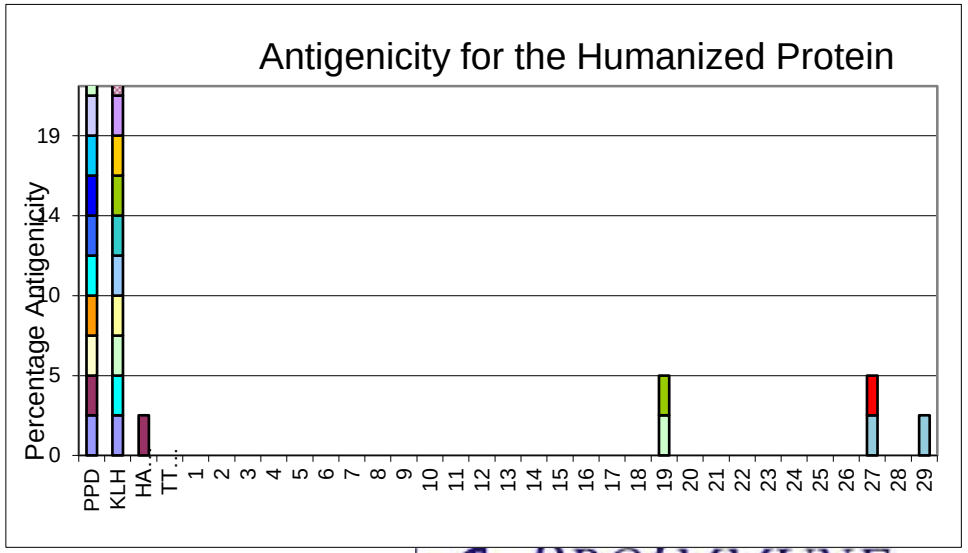
Result:

- T cell antigenicity all but eliminated from humanized protein
- Control responses unchanged

➢ Humanization a success; but this is not always the case

➢ Requires confirmation





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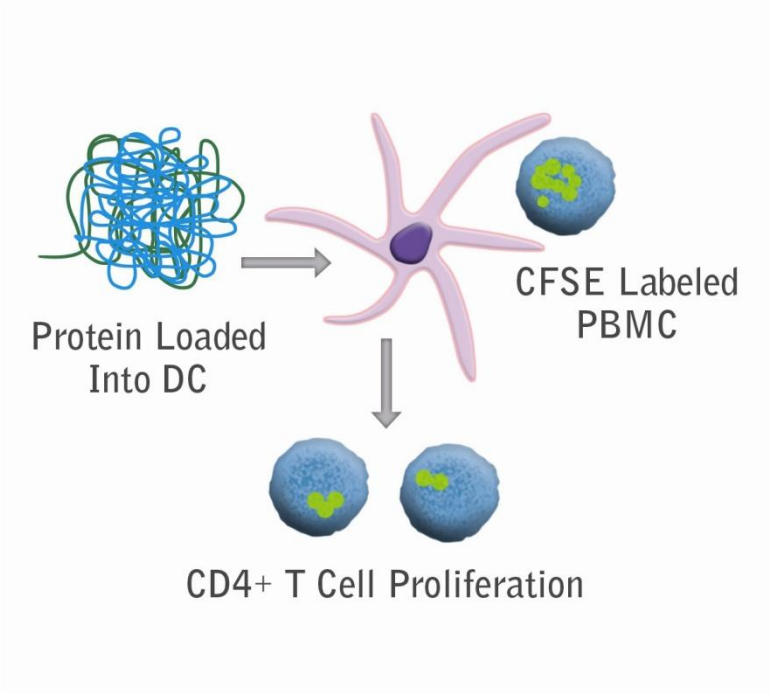
DC-T cell assays

- Donor panel of 20-50 x HLA-typed individuals representing tissue type distribution in the general population
- Dendritic Cells (DCs) are generated from monocytes in culture and loaded with protein antigen of interest.
- Activated loaded DCs are used to challenge T cells leading to measurement of T cell proliferation



- ✓ Relative antigenicity of different drug candidates can be compared directly
- ✓ Donor assay interference due to immunomodulation by candidate drug is avoided
- ✓ Rapid: typical completion in just 6-8 weeks

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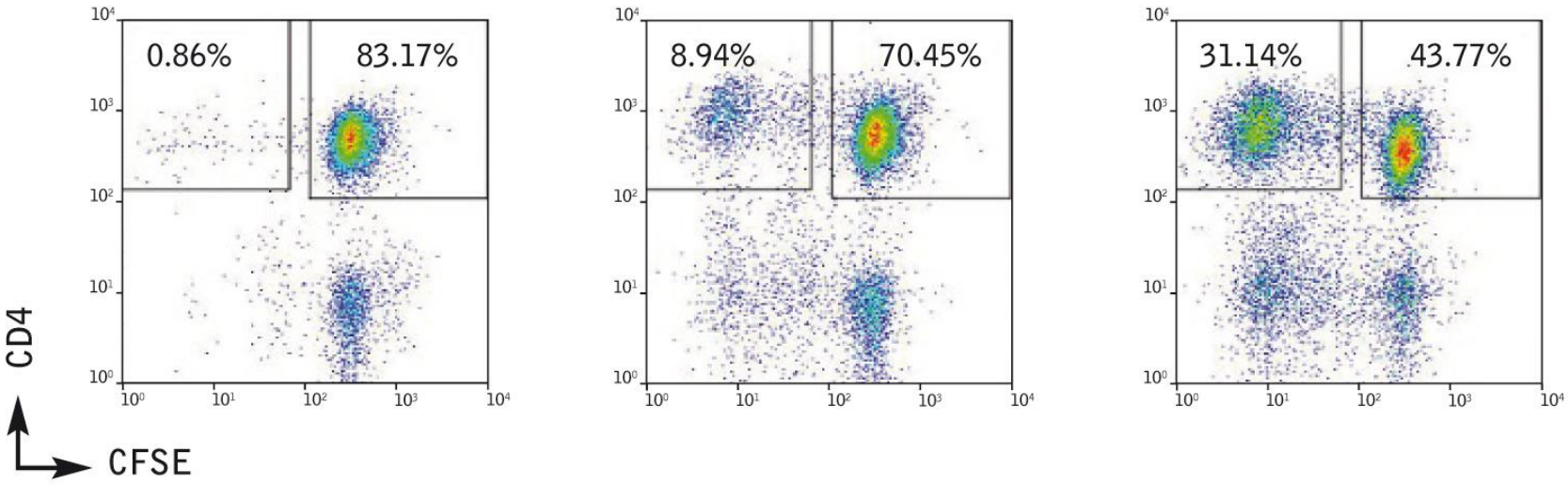
Whole protein antigenicity assessment: DC-T cell assay



Unstimulated cells

T cells stimulated with DCs loaded with Remicade

T cells stimulated with DCs loaded with control (PPD)

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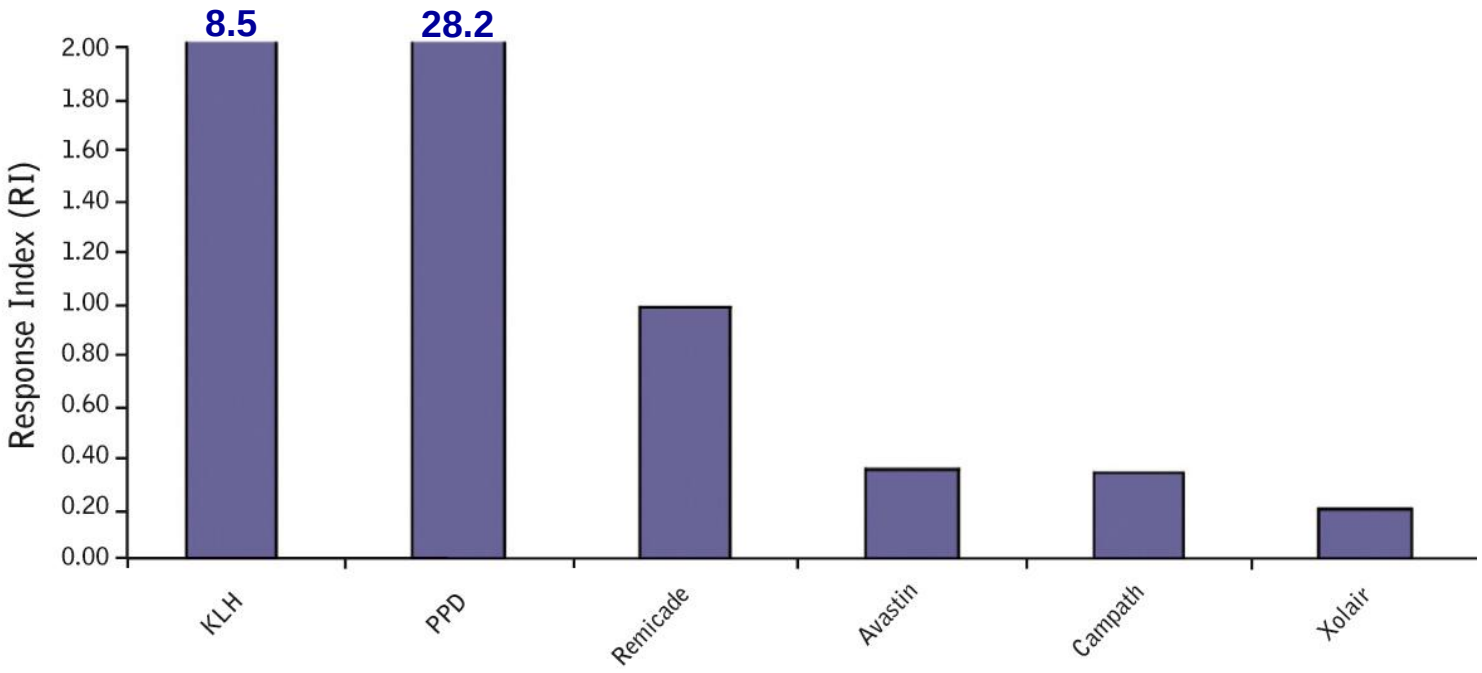
Whole protein antigenicity assessment: DC-T cell assay



Response Index = (% donors responding) x (average strength of response) / 100

- **Measure of relative T cell epitope content**
- **Direct comparison of biotherapeutic candidates for antigenicity**
- **Assessment of post-translational modifications**

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ProImmune REVEAL™ Immunogenicity System

- **Powerful technology**
  - Unique physical MHC-peptide binding assays combined with CFSE T cell proliferation assays
  - Flexible platform with unrivalled data quality
  - Widely adopted by most leading pharma
- **Integrated approach**
  - T cell *and* B cell epitope determination
  - Epitope mapping and whole protein analysis for immunogenicity risk assessment
- **Rapid**
  - Results delivered in 6-8 weeks to guarantee fast decision-making
- **Experts**
  - Embedded corporate immunology expertise

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ProArray Ultra™

Scale :: Flexibility :: Precision :: Power

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ProArray Ultra™

- **Standard microarrays**
  - artificial peptide presentations
  - inherent physiochemical properties of peptides
  - leads to potential erroneous results
- **ProArray Ultra™ overcomes such limitations**
  - Peptide presentation is more physiologically relevant
  - Peptide/antibody interactions may be assessed as a purified solution, or within a complex mixture (e.g. plasma / serum)
  - Biological interactions between peptides and antibodies are accurately measured

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ProArray Ultra™ Detail

- **Highly sensitive**
  - Significantly improved dynamic range, limit of detection and CV compared to optimized ECL ELISA
  - Performance comparable to MSD but significantly more flexible in array design
  - Significant reduction of artefacts in peptide/protein spotting compared to existing technologies
- **Flexible**
  - As low as 1µl sample required for analysis
  - >30,000 protein or peptide ligands per array
  - protein or peptide can be immobilized
  - 1 - 24 replicate sub-arrays; up to 24 different samples/dilutions per array

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ProArray Ultra™ Detail

- **Rapid**
  - Delivering entire proteomics/immune-discovery projects in 6-8 weeks.
- **Comprehensive**
  - Assay projects delivered as a turnkey service, including full dilution range, data analysis and reporting
  - Moves early stage discovery and analytical assay development onto a single platform

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ProArray Ultra™
Key Applications

Single platform for HTS to analytical validation/deployment

- Mapping of monoclonal antibody binding sites
- Determination of specificity of anti-drug antibodies
- Scan entire pathogen proteomes for epitopes
- Batch release testing: vaccines, biologics, etc.
- Biomarker discovery / validation / tracking

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ProArray Ultra™ Layout

24 Sub-Arrays:			8 Sub-Arrays:	4 Sub-Arrays:	2 Sub-Arrays:	1 Array:
293 / 146	293 / 146	293 / 146	1181 / 590	2845 / 1422	6205 / 3102	12733 / 6366
293 / 146	293 / 146	293 / 146	1181 / 590			
293 / 146	293 / 146	293 / 146	1181 / 590	2845 / 1422		
293 / 146	293 / 146	293 / 146	1181 / 590			
293 / 146	293 / 146	293 / 146	1181 / 590	2845 / 1422	6205 / 3102	
293 / 146	293 / 146	293 / 146	1181 / 590			
293 / 146	293 / 146	293 / 146	1181 / 590	2845 / 1422		
293 / 146	293 / 146	293 / 146	1181 / 590			

Feature numbers are shown for triplicate / sextuplicate technical replicates in each sub-array

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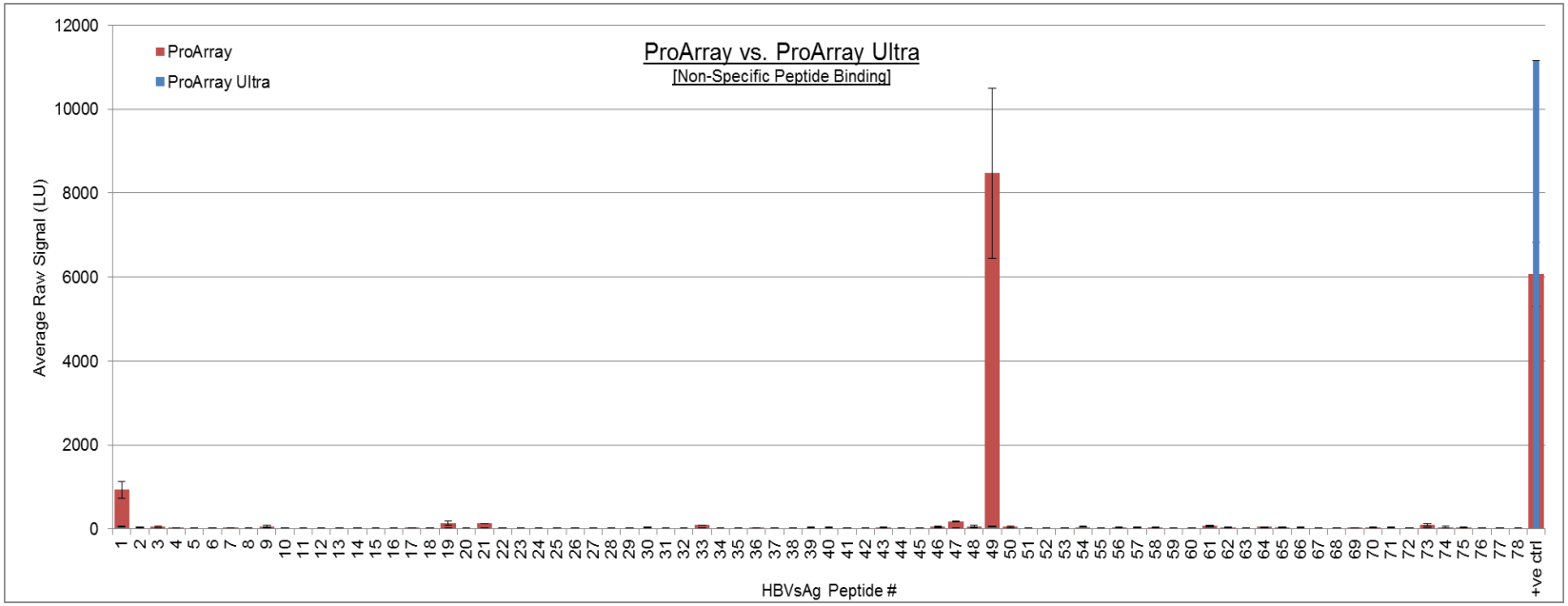
ProArray vs. ProArray Ultra™

Non-Specific Binding



A direct comparison of identical peptide libraries incubated in the absence of serum sample (negative control); differences in assay system output/detection are immediately highlighted by the inherently non-specific binding issues associated with standard ProArray

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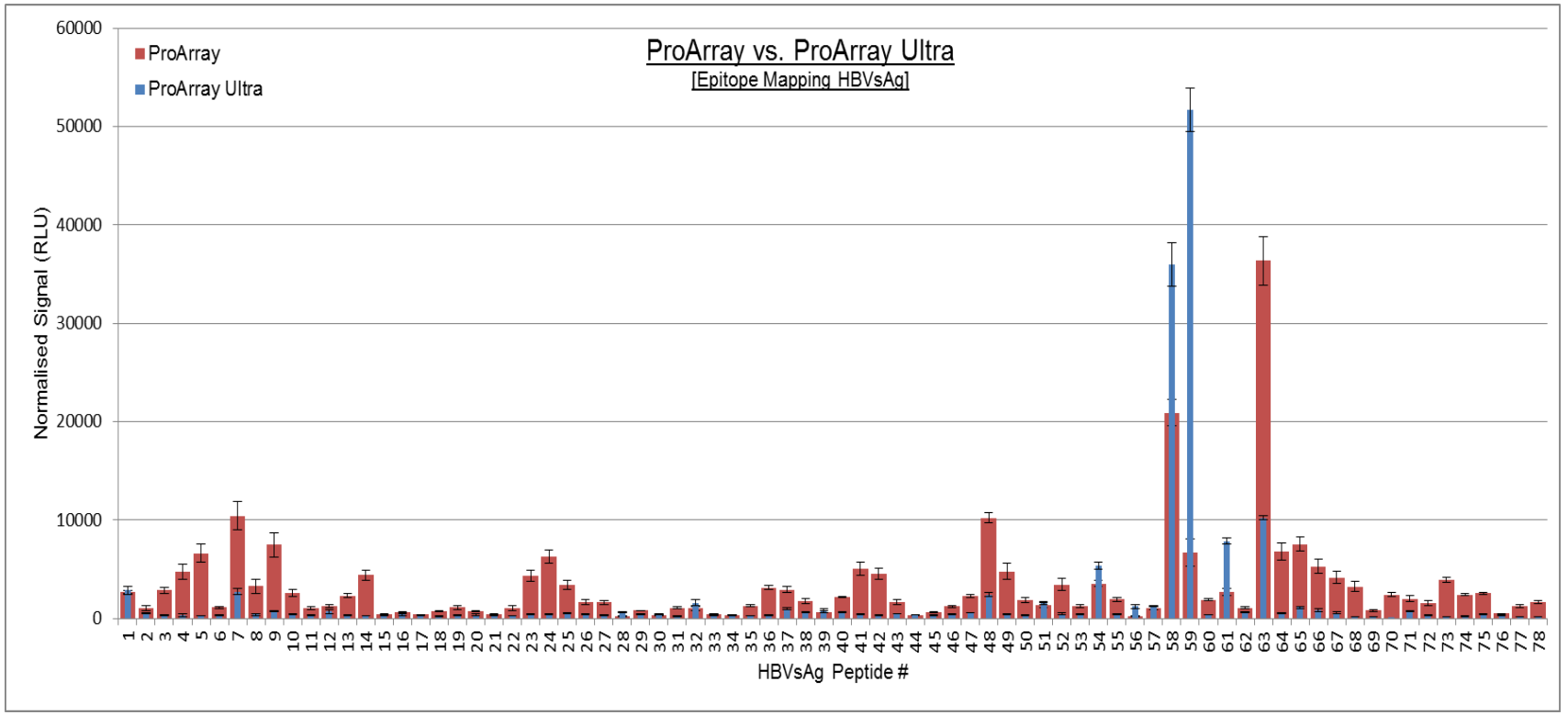
ProArray vs. ProArray Ultra™

System Differences



A direct comparison of identical peptide libraries for HBVsAg incubated with the same (positive) serum sample;

Differences in assay system output/detection, in particular background signal levels are substantively reduced with the ProArray Ultra™ system

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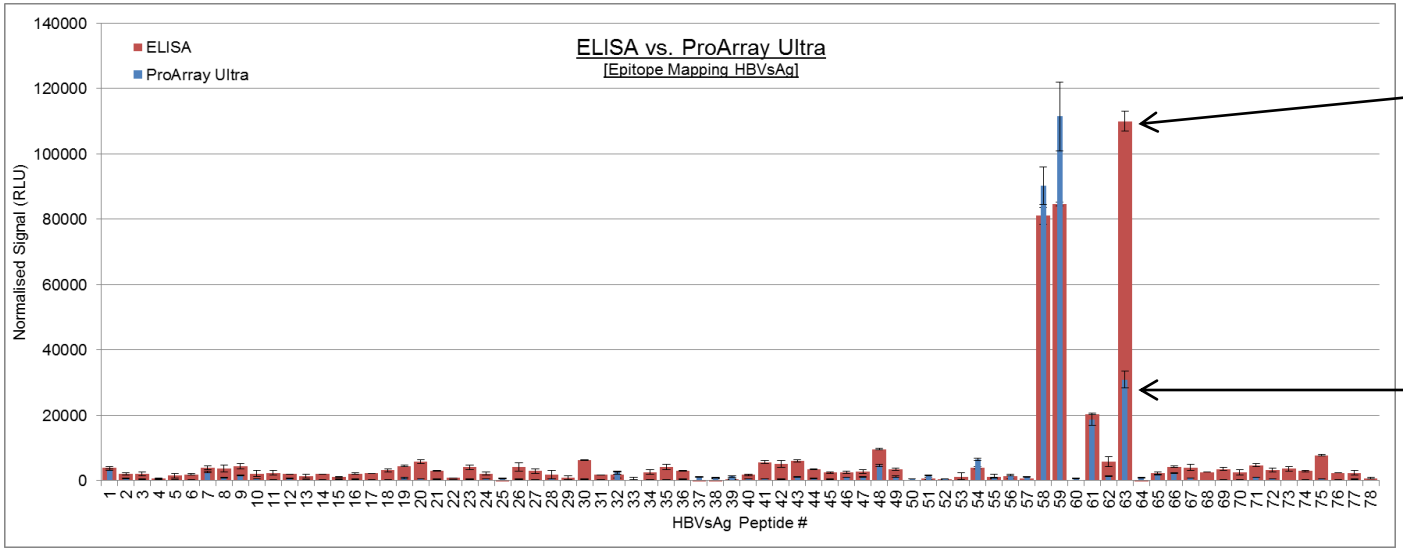
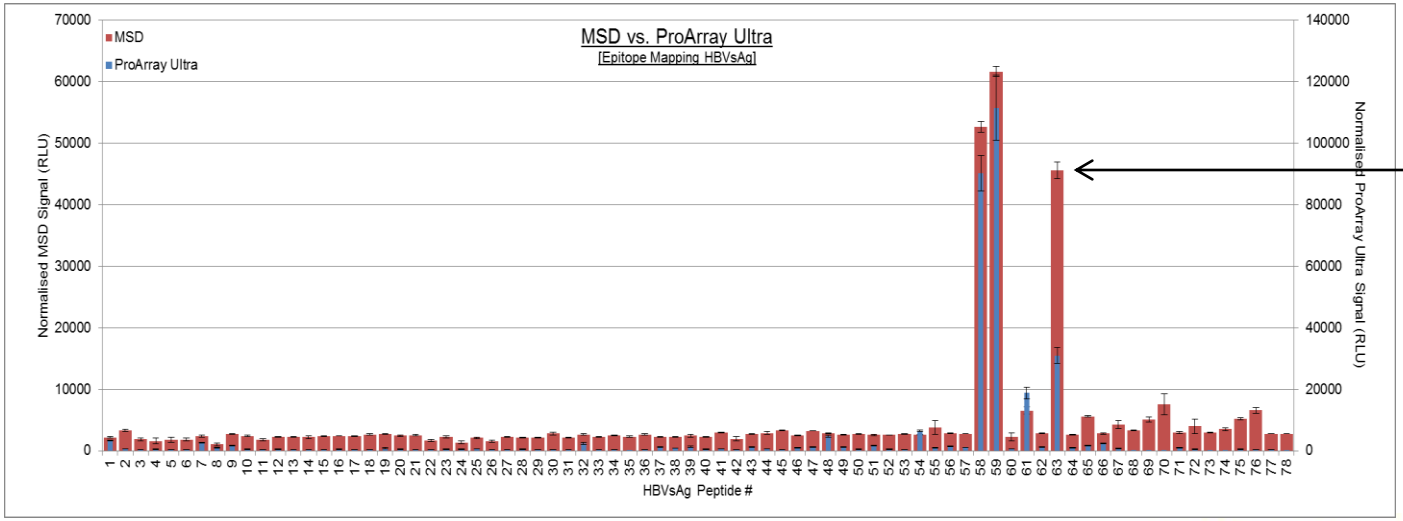
ProArray Ultra™ vs. ECL ELISA & MSD



~63x background

~45x background



~18x background

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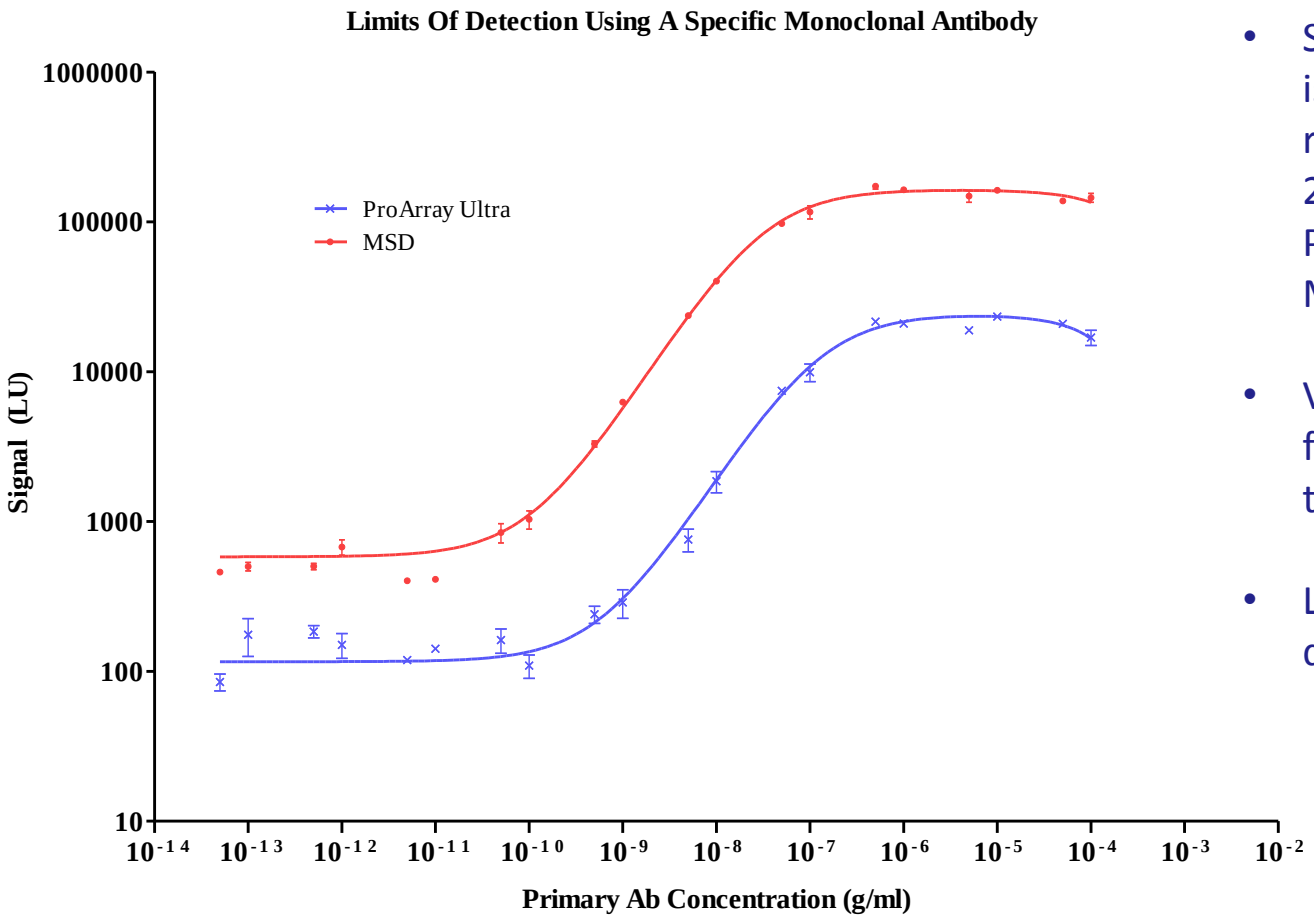
Dynamic Range and LOD



- Saturation curve/profile is obtained with a linear range of approximately 2.5 logs for both ProArray Ultra™ and MSD
- Very low signal variation for both technologies in terms of signal detected
- Limit of physical detection (~0.5ng/ml)

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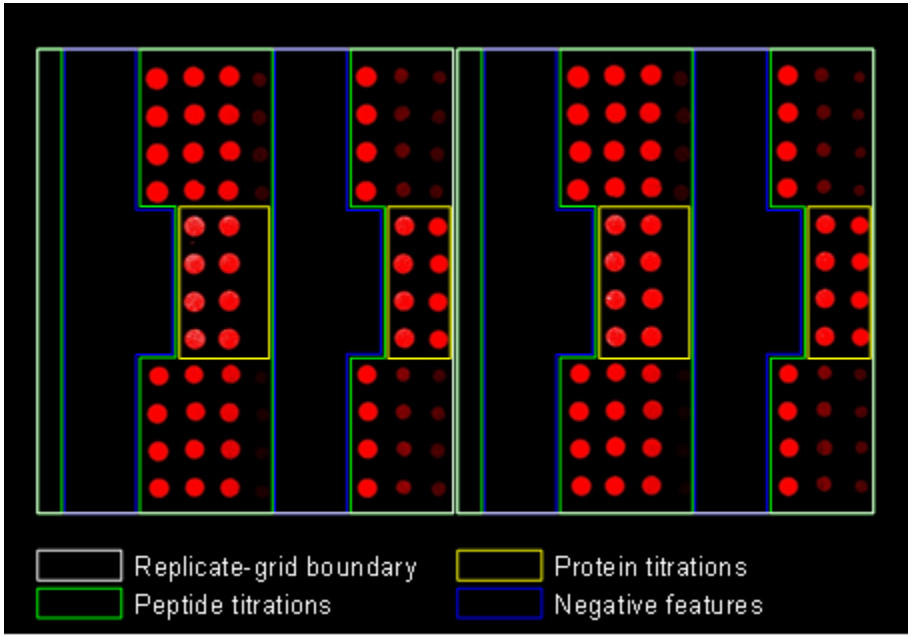
ProArray Ultra™ Spot Quality



ProArray Ultra™ peptide array imaged at 5µm resolution.
Peptide and protein titrations, spotted in quadruplicate rows, in duplicate sub-grids, detected with purified antibody.

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ProArray Ultra™

- a combined protein and peptide array platform
- providing unrivalled scalability and flexibility
- exceeding the signal quality and performance of optimized ECL ELISA assays

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ProImmune Summary

- Your strategic partner that fully understands **Immune Responses** - unrivalled technical competence
- Products and services based on the best new technologies
- Integrated assay platforms saving you a material amount of time, risk and money

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