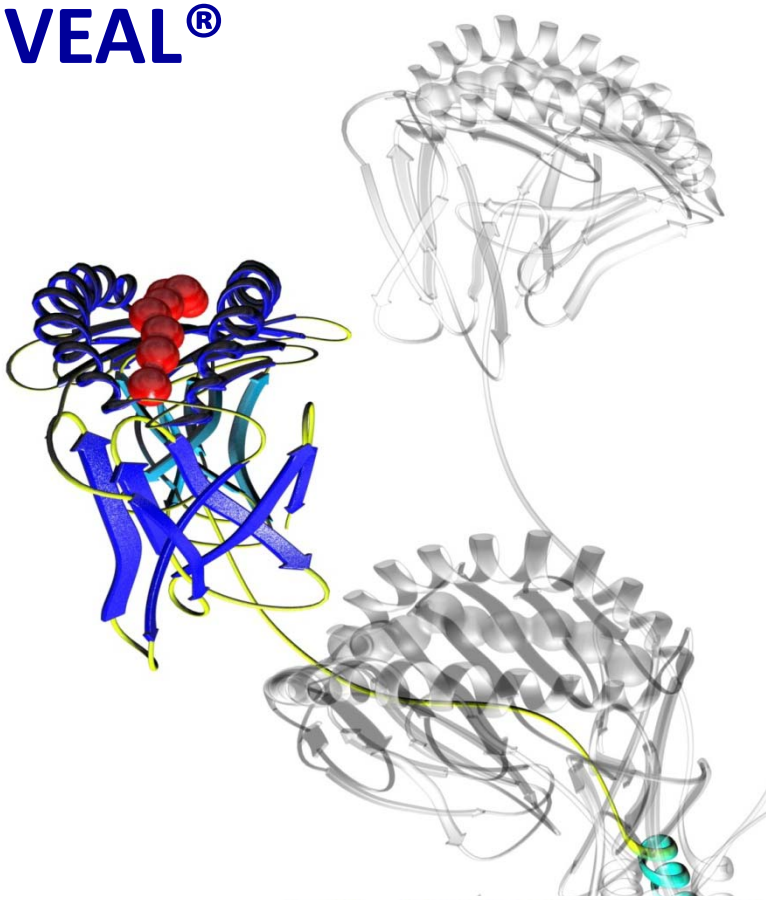
T cell epitope identification in Remicade® and Humira® using the REVEAL® Immunogenicity System



Matthew Frayne

7 February 2012

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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 TNFα 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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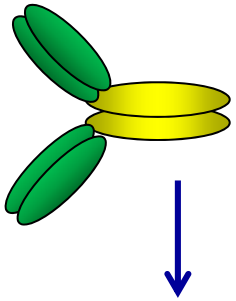
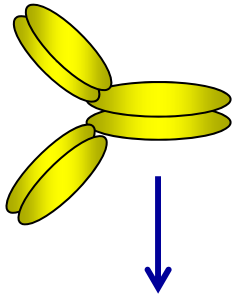
CD4+ T Cell Epitope Discovery and Characterization Strategy



Remicade®



Humira®

Library of overlapping peptides from variable regions

15-mers offset by 3 amino acids

1. **CFSE T cell proliferation assay**
2. ***In vitro* REVEAL® MHC-peptide binding assay**

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CFSE T Cell Proliferation Assays

- **CD8$^+$ depleted** PBMCs from minimum 40 HLA-typed individuals representing tissue type distribution in the global population
- High resolution tissue typed for HLA-DR, -DP and –DQ
- CFSE staining of PBMCs at the start of the assay
- Co-culture of PBMCs with synthetic peptides
- T cell proliferation is measured over 7 day period by CFSE flow cytometry assay in sextuplicate analysis
- **Does the peptide elicit a significant functional response?**

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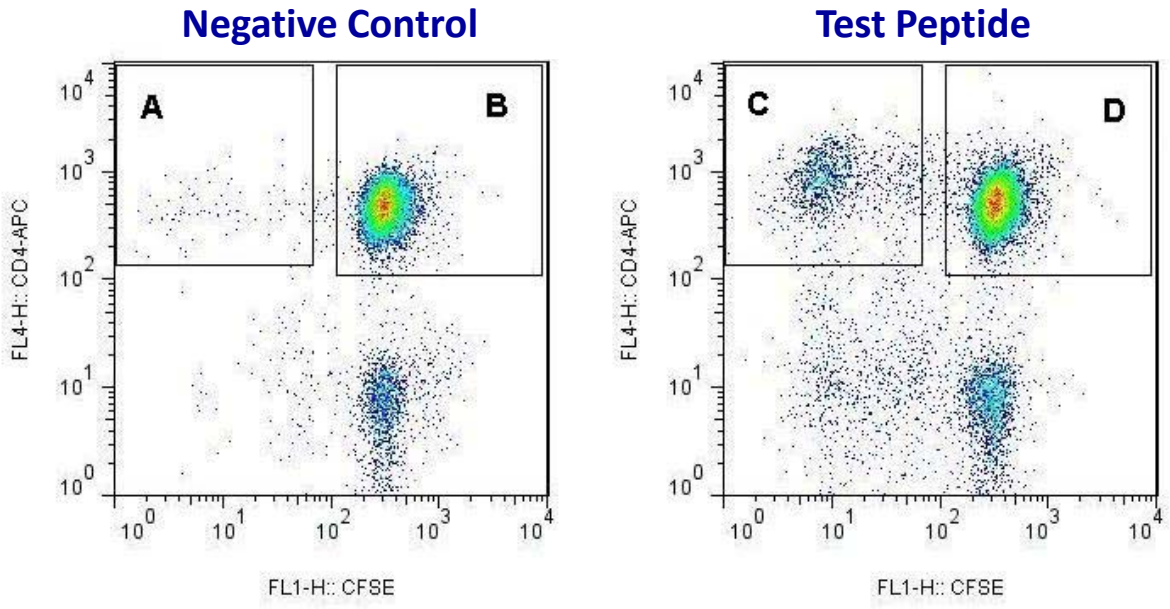
CFSE T Cell Proliferation Assays

- **Flow cytometry assay that measures only live CD4$^+$ cells**



- **Cell Division Index = [C/(C+D)] / [A /(A+B)]**

  % proliferating to peptide / % proliferating in negative control

- **A significant response is determined as $p<0.05$ by ANOVA analysis, CDI>2 and 2 SEM above background**

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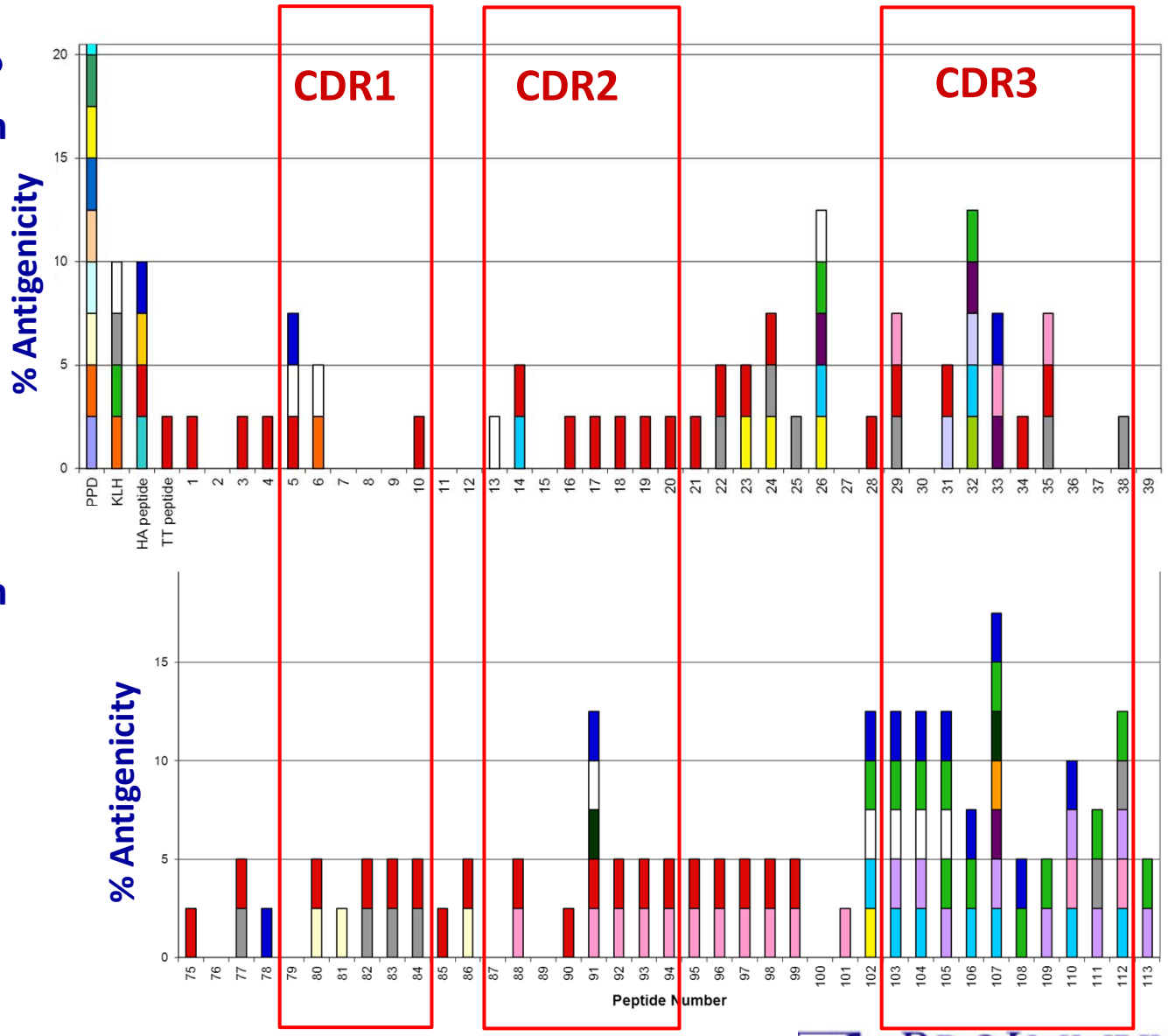
T Cell Proliferation – Heavy Chain

Remicade®
Heavy Chain



Humira®
Heavy Chain

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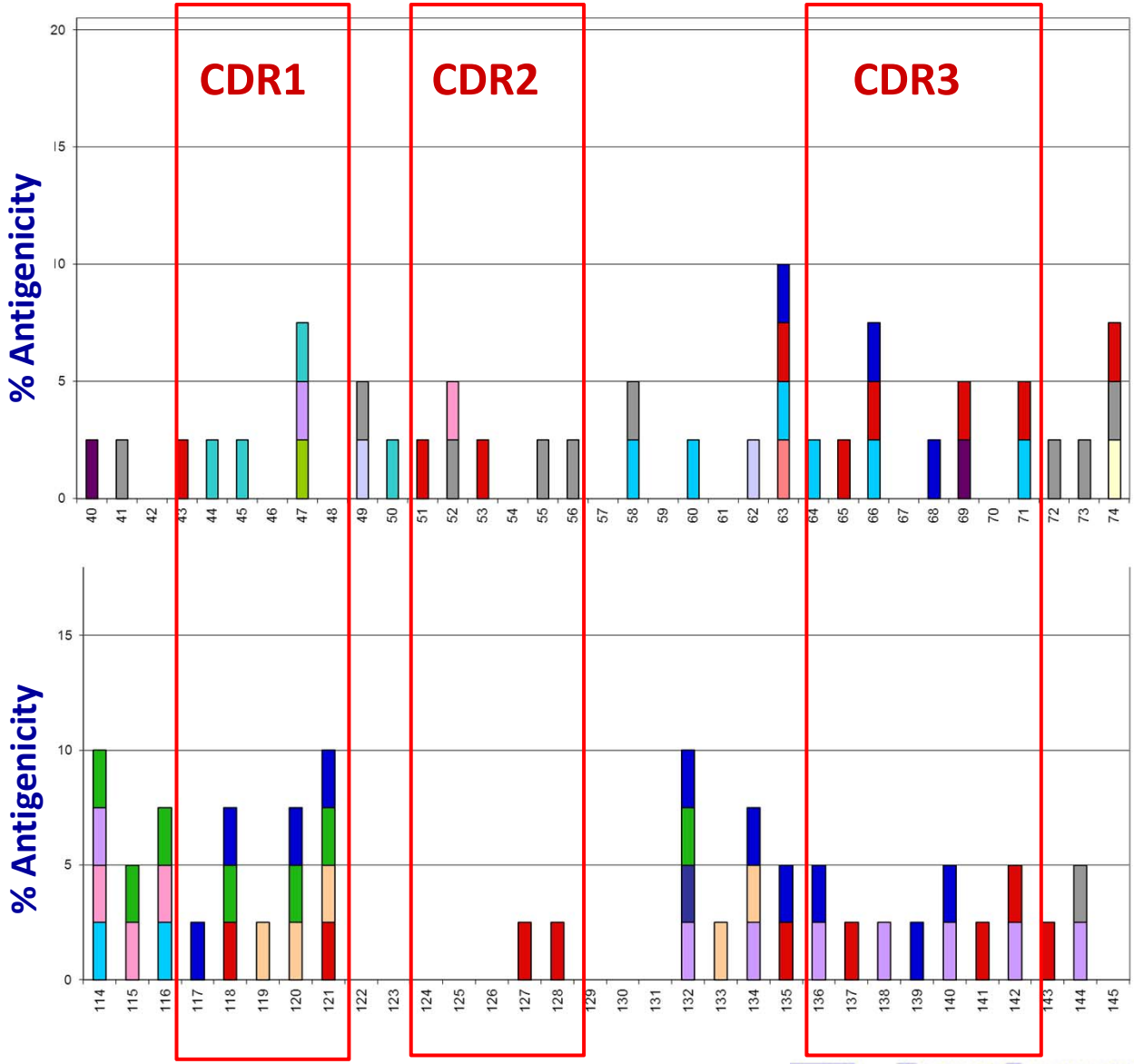
T cell Proliferation – Light Chain

Remicade®
Light Chain



Humira®
Light Chain

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Characterization of HLA-Associations

- A number of epitopes with functional responses identified and characterized from the variable domains of Remicade® and Humira®
- What are the HLA associations of these epitopes?
- What is the global immunogenicity impact of each epitope?

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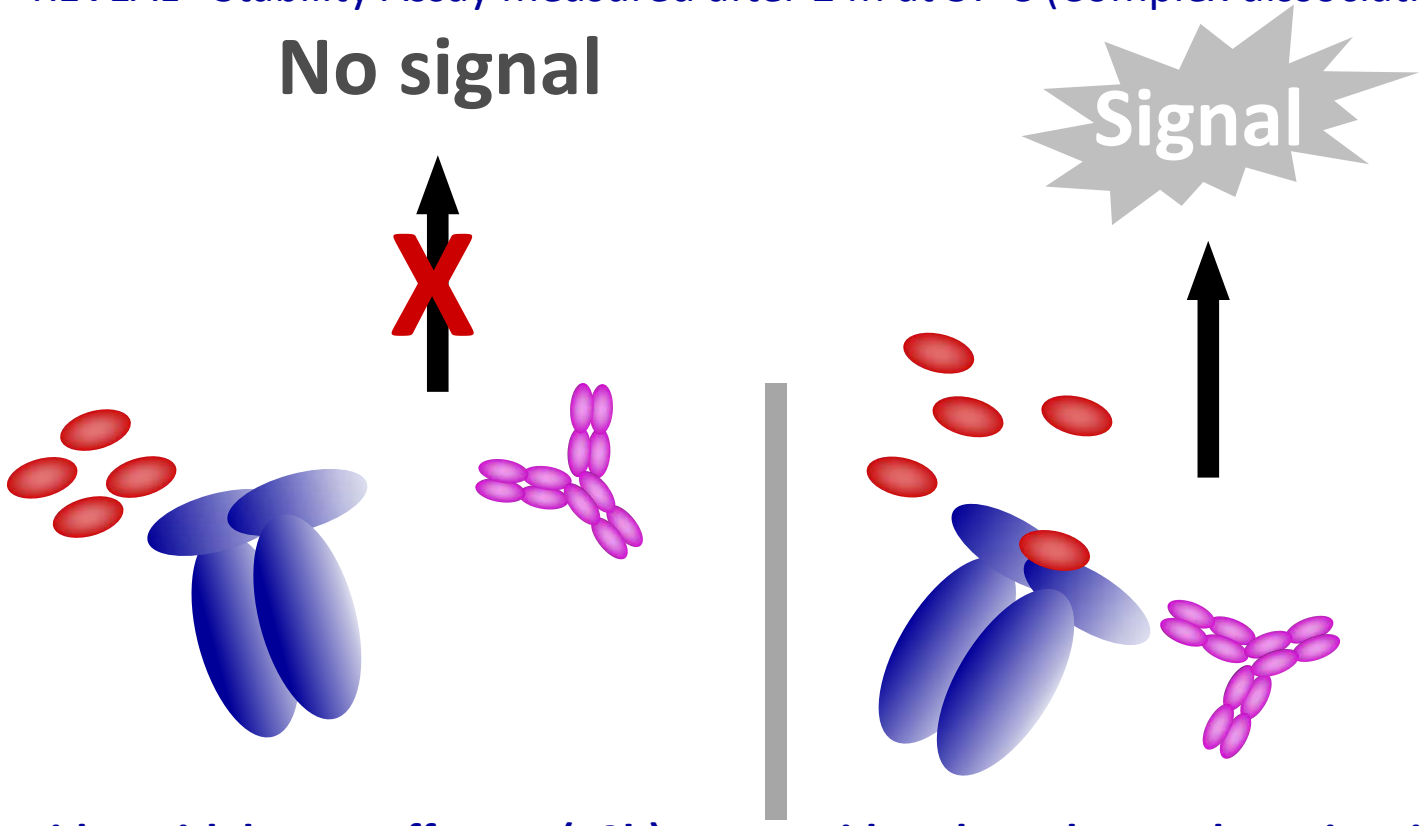
REVEAL® Binding Assay

2 components:

- REVEAL® Binding Assay (Complex formation)
- REVEAL® Stability Assay measured after 24h at 37˚C (Complex dissociation)



Peptides with longer off-rates (>3h) are considered good enough to give rise to immunogenicity*

*Peter *et al.* (2001) *Vaccine* 19: 4121-4129
*Burshtyn and Barber (1993) *J Immunol.* 151: 3082-3093
*van der Burg *et al.* (1996) *J Immunol.* 156: 3308-3314

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REVEAL® MHC Alleles

Class II

DPA1*01:03 + DPB1*01:01
DPA1*01:03 + DPB1*02:01
DPA1*01:03 + DPB1*03:01
DPA1*01:03 + DPB1*04:01
DPA1*01:03 + DPB1*04:02
DPA1*01:03 + DPB1*05:01
DPA1*02:01 + DPB1*01:01
DPA1*02:01 + DPB1*02:01
DPA1*02:01 + DPB1*03:01
DPA1*02:01 + DPB1*04:01
DPA1*02:01 + DPB1*04:02
DPA1*02:01 + DPB1*05:01
DPA1*02:01 + DPB1*06:01
DPA1*02:01 + DPB1*09:01
DPA1*02:01 + DPB1*11:01
DPA1*02:01 + DPB1*13:01
DPA1*02:01 + DPB1*14:01
DPA1*02:01 + DPB1*15:01
DPA1*02:01 + DPB1*17:01

DRA1*01:01 + DRB1*01:01
DRA1*01:01 + DRB1*15:01
DRA1*01:01 + DRB1*03:01
DRA1*01:01 + DRB1*04:01
DRA1*01:01 + DRB1*11:01
DRA1*01:01 + DRB1*13:01
DRA1*01:01 + DRB1*07:01
DRA1*01:01 + DRB1*01:02
DRA1*01:01 + DRB1*04:02
DRA1*01:01 + DRB1*04:04
DRA1*01:01 + DRB1*04:05
DRA1*01:01 + DRB1*04:07
DRA1*01:01 + DRB1*04:08
DRA1*01:01 + DRB1*08:04
DRA1*01:01 + DRB1*09:01
DRA1*01:01 + DRB1*10:01
DRA1*01:01 + DRB1*11:02
DRA1*01:01 + DRB1*11:03
DRA1*01:01 + DRB1*11:04

DRA1*01:01 + DRB1*15:02
DRA1*01:01 + DRB1*15:03
DRA1*01:01 + DRB1*16:01
DRA1*01:01 + DRB1*16:02
DRA1*01:01 + DRB3*02:02
DRA1*01:01 + DRB3*03:01
DRA1*01:01 + DRB5*01:01
DQA1*01:01 + DQB1*05:01
DQA1*05:01 + DQB1*03:01
DQA1*01:02 + DQB1*05:02
DQA1*01:02 + DQB1*06:02
DQA1*03:01 + DQB1*03:02
DQA1*01:02 + DQB1*06:04
DQA1*05:01 + DQB1*02:01
DQA1*02:01 + DQB1*02:02
DQA1*03:01 + DQB1*03:01
DQA1*02:01 + DQB1*03:03
DQA1*03:03 + DQB1*03:03

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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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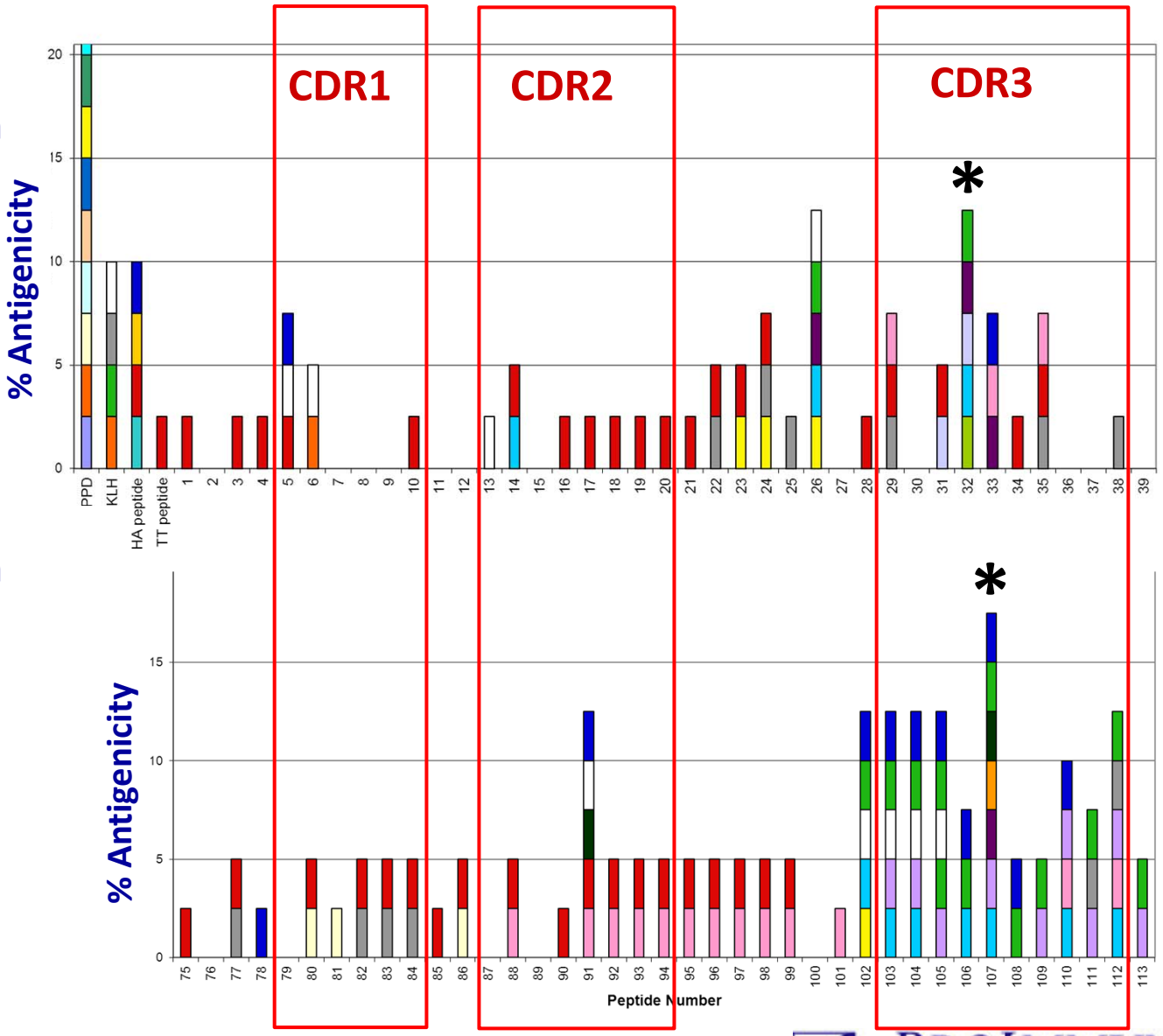
T Cell Proliferation – Heavy Chain

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

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Conclusions

- **Remicade®**
  - Numerous epitopes identified and characterized from the whole variable domain
  - Weak to intermediate strength of proliferative responses
  - Restricted HLA association
- **Humira®**
  - Fewer epitopes, largely restricted to the CDR regions
  - Strong proliferative responses
  - Promiscuous HLA binding

- **Which are the immunodominant epitopes in patients with ADA responses?**
- **Are these peptides processed and presented to the immune system?**

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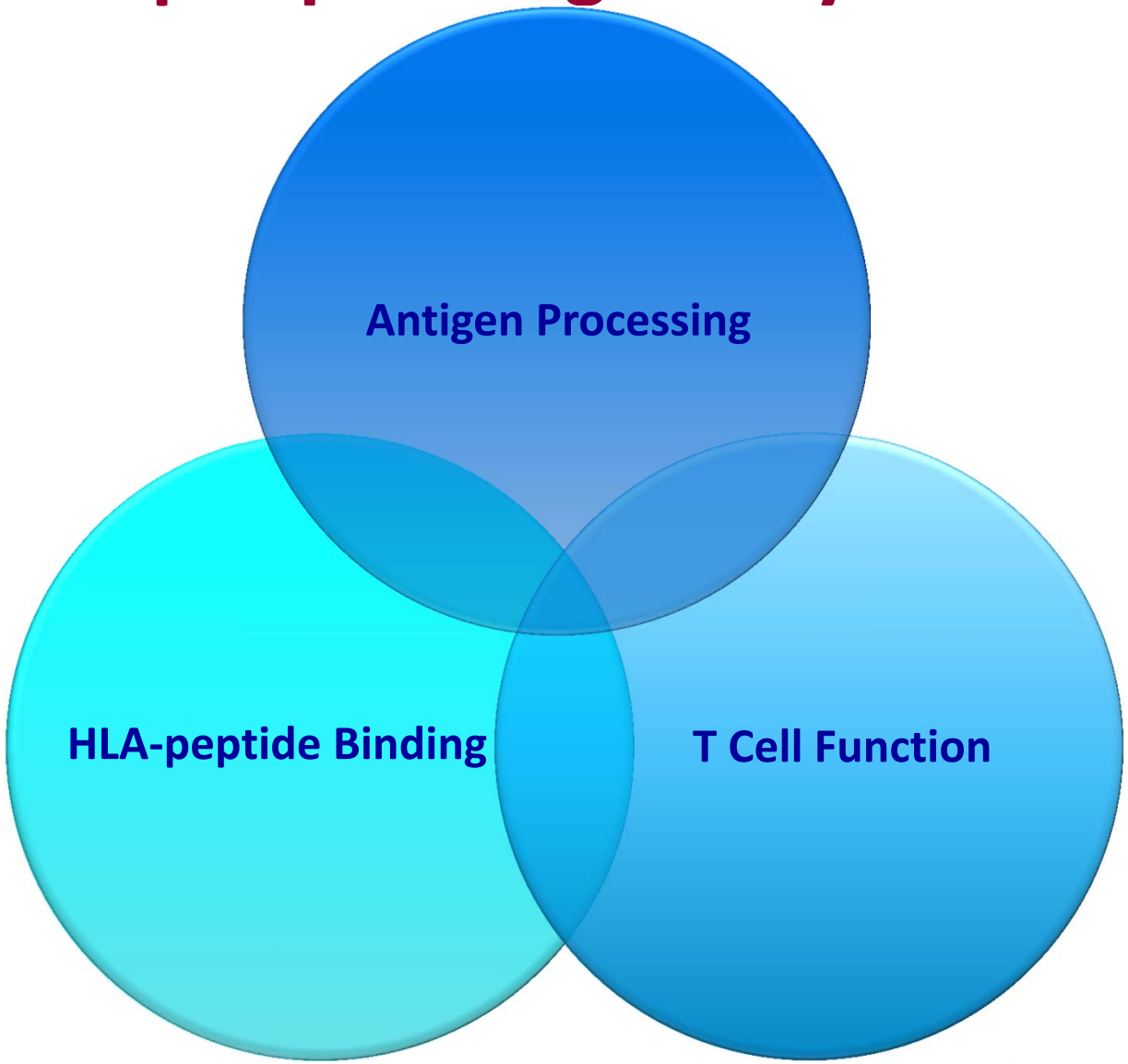
T Cell Epitope Antigenicity Profiling



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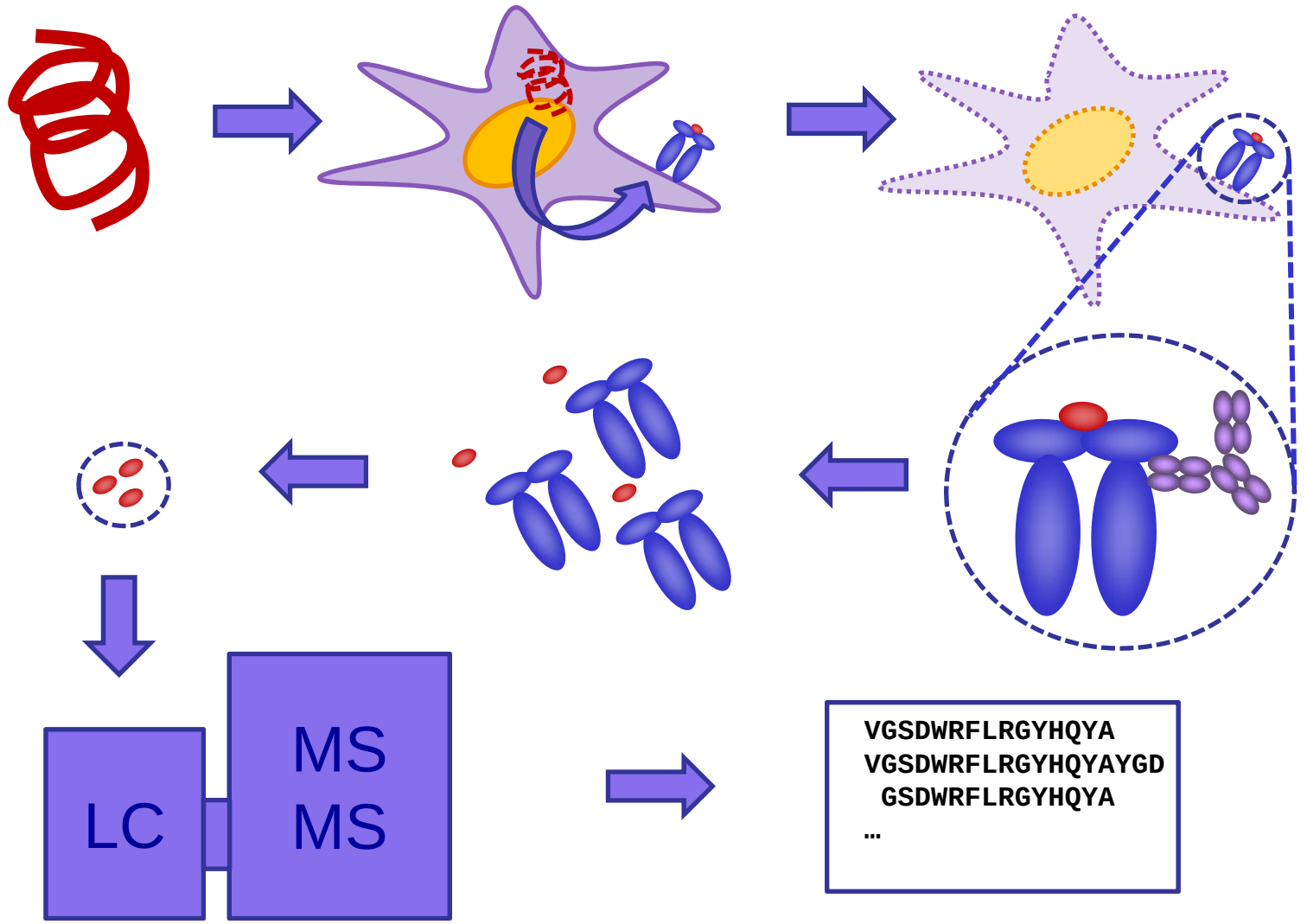
ProPresent® workflow



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Integrated Approaches for Antigenicity Characterization

- **Immunogenicity is a complex issue!**
- **No single approach is perfect**
- **Multiple approaches to aid prediction of clinical immunogenicity and therapeutic outcomes**

- **T Cell Proliferation and REVEAL® Binding Assays**
  - **Detailed epitope identification and characterization**
- **ProPresent**
  - **Whole protein epitope processing and HLA-associated presentation**
  - **Post-translational and formulation analysis**
- **DC-T Proliferation**
  - **Comparison of whole protein antigenicity in Lead Selection**

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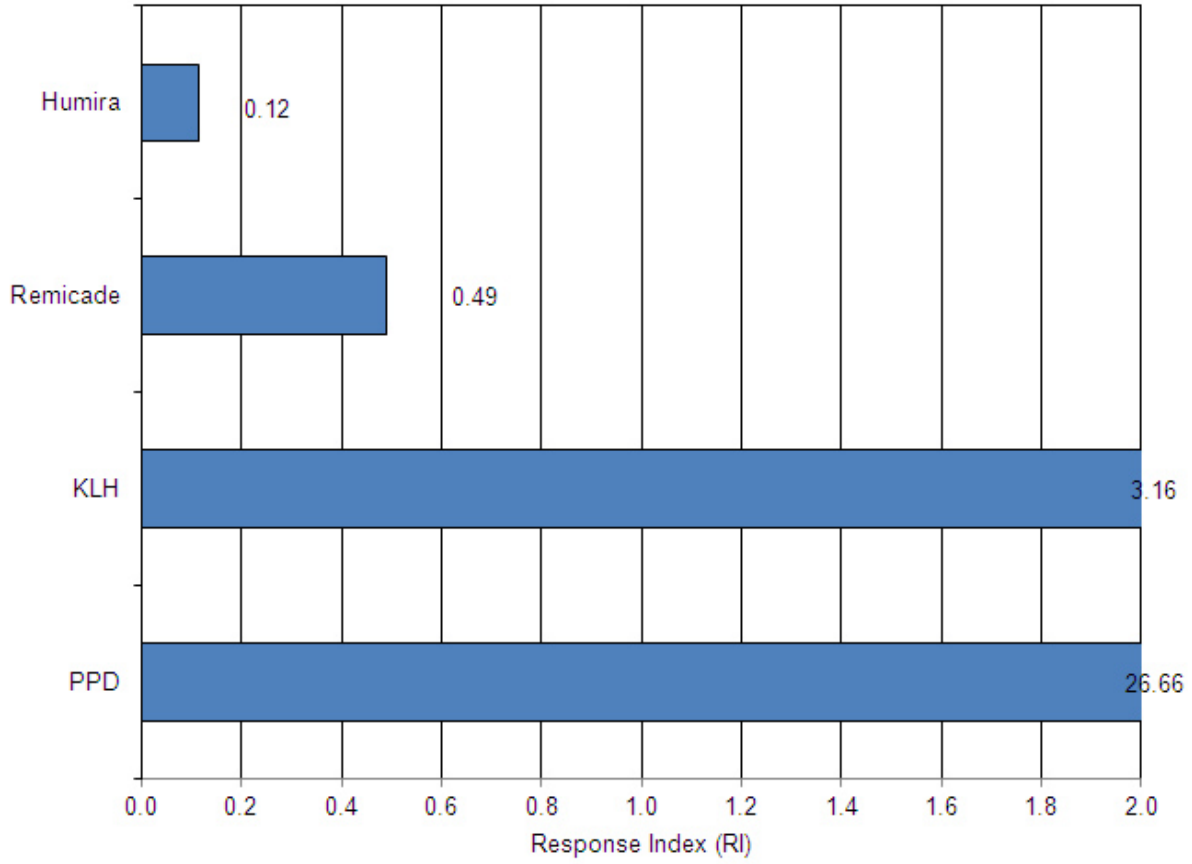
Whole Protein Immunogenicity

- MDDCs generated from human tissue bank
- Pulsed with Remicade® or Humira®
- Co-cultured with autologous PBMCs labelled with CFSE



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