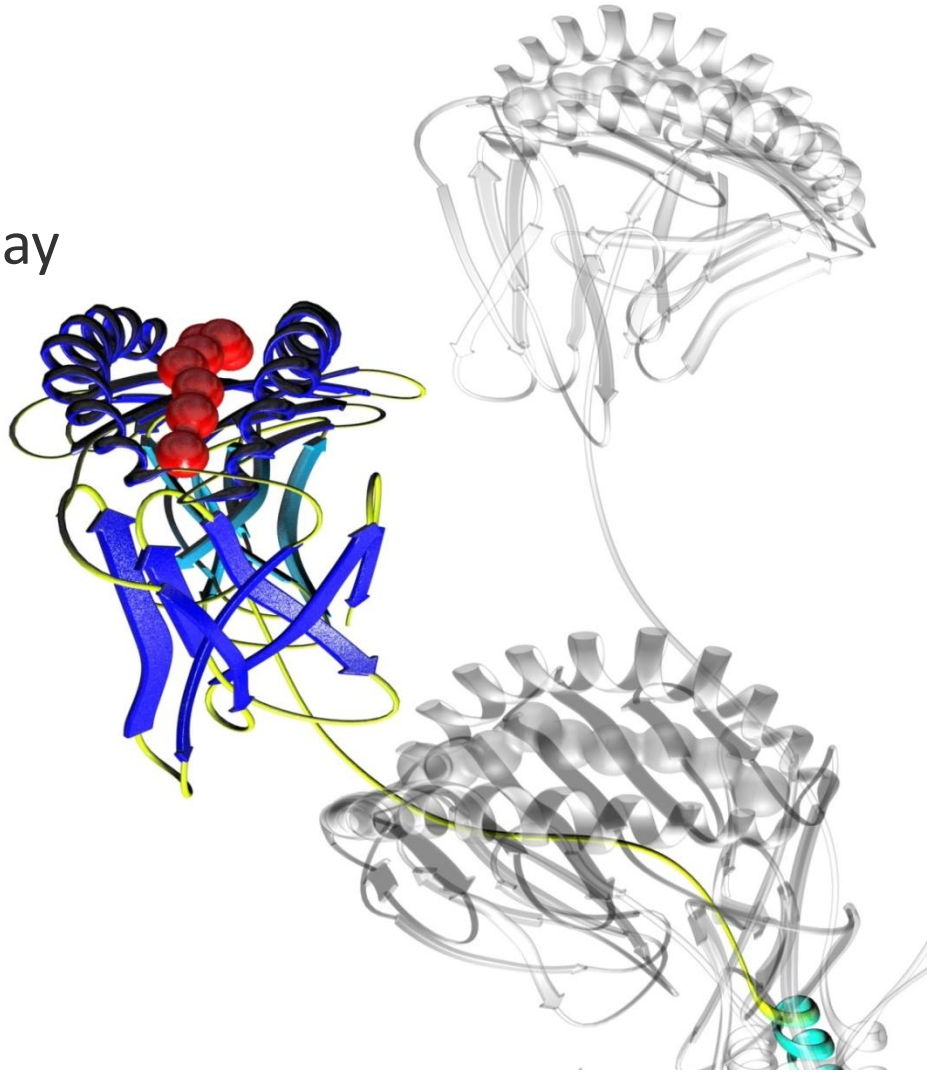
Mastering Immunity

ProPresent™

Antigen Presentation Assay



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Background

Options to explore T cell epitopes

- Prediction (fast, inexpensive, accuracy?, does not confirm epitope)
- Proliferation assays (well suited for naïve responses)
- Functional assays: ELISPOT or ICS (often used for recall responses)
- HLA binding assays (narrows selection, clarifies mode of action)

➢ *In vitro* assays for identifying epitopes typically use synthetic peptides

❖ **But does the antigen get presented?**

In order to be a T cell epitope

1. A peptide must be processed, bind to MHC and be **presented** by antigen presenting cells

2. The peptide-MHC complex must be recognizable by T cells actually present in the donor

In order find a presented peptide

- Elute it from MHC molecules on the antigen presenting cell

- Sequence it by mass spectrometry

Historic use of the peptide elution technique

- Most often used for Class I MHC : identification of tumor antigens
- Lesser extent for Class II: fewer publications
- Potentially powerful technique, but was very cumbersome

Key problems

Technically challenging and resource intensive

- Potentially billions of cells required per donor
- Several donors covering a representative set of HLA
- Involved cell culture / recovery protocol
- Complicated and expensive LC-MS/MS setup
- Analysis of very large datasets

That's why we do it for you!

- ProPresent™ directly measures MHC-peptide presentation on DCs cultured with protein of interest
- Cellular *in vitro* assay, carried out on a set of HLA typed donor samples provided by ProImmune
- The only commercial service for this purpose
- Currently available for HLA-DR; other *loci* in development
- Rapid service: e.g. 2-3 antigens can be tested on 10 donors each in just **6** weeks

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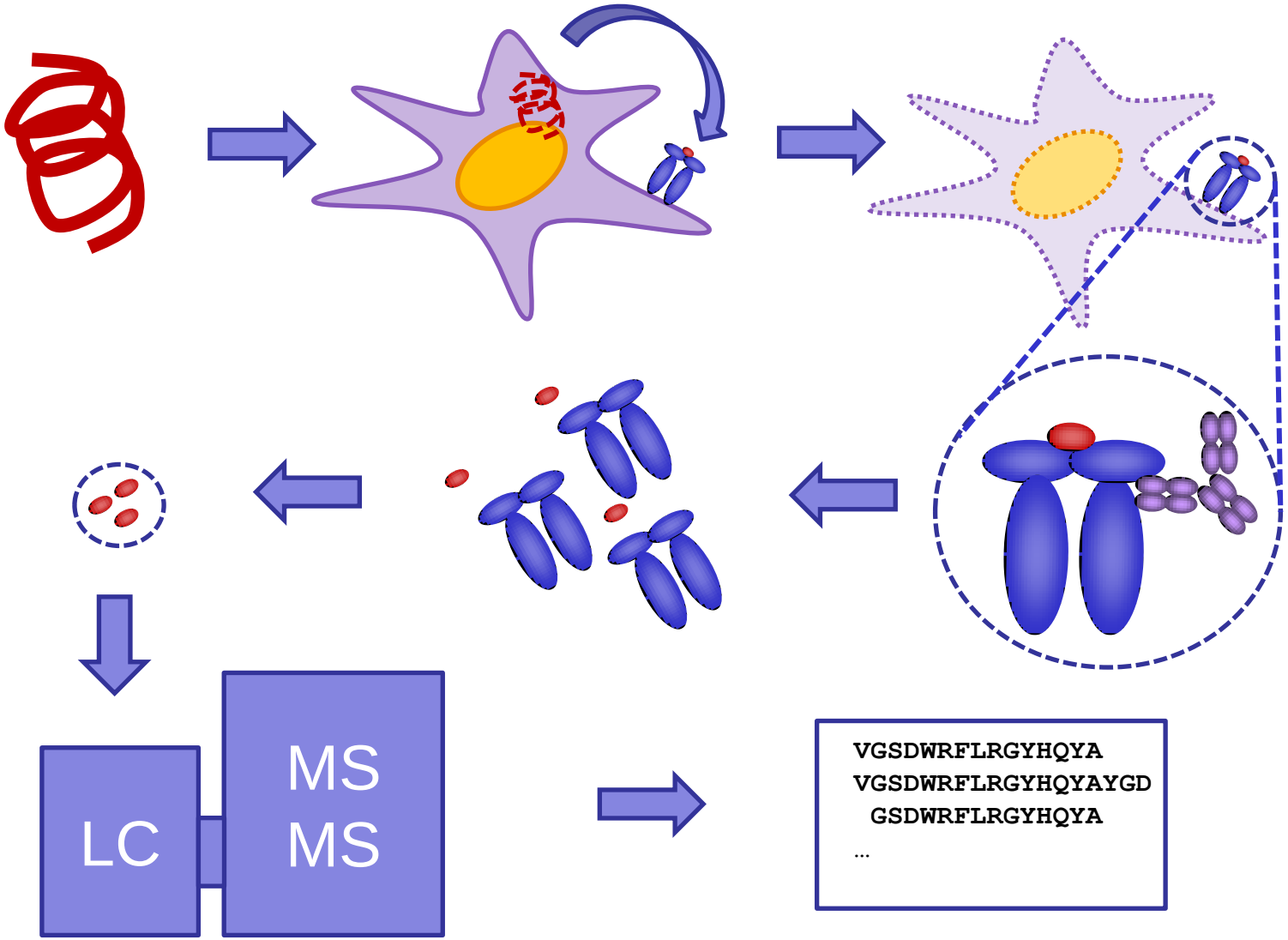
Applications of ProPresent

- Final confirmation that sequences can be presented
- Key tool for understanding immunogenicity
  - Evaluating the impact of protein modifications
  - Assessing similarities / differences between compounds
  - Impact of allelic variants of proteins (can be very important for replacement factors)
  - Key data contributing to understanding of mode of action in vaccines

ProPresent Method

- 10 fully tissue typed donors from ProImmune's tissue bank, matched to global population
- Generate immature monocyte derived DCs in culture
- Load with antigen(s) of interest
- Lyse cells
- Recover HLA-DR complexes
- Elute peptides
- Apply recovered eluent to sequencing (tandem) mass spectrometry

ProPresent workflow



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Routine QC Steps

- High resolution HLA typing of buffy coats
- Routine viability / phenotypic testing of cells by flow cytometry
- MHC recovery
- Peptide recovery
- Number of peptide hits obtained overall

Specific QC/Analysis Steps

- 5% confidence based exclusion search
- Match to actual protein sequence of antigen
- Peptides have the correct range of lengths with a distribution max at approximately 15 aa
- Anchor matching of peptides against donor HLA
- Detect housekeeping proteins every time

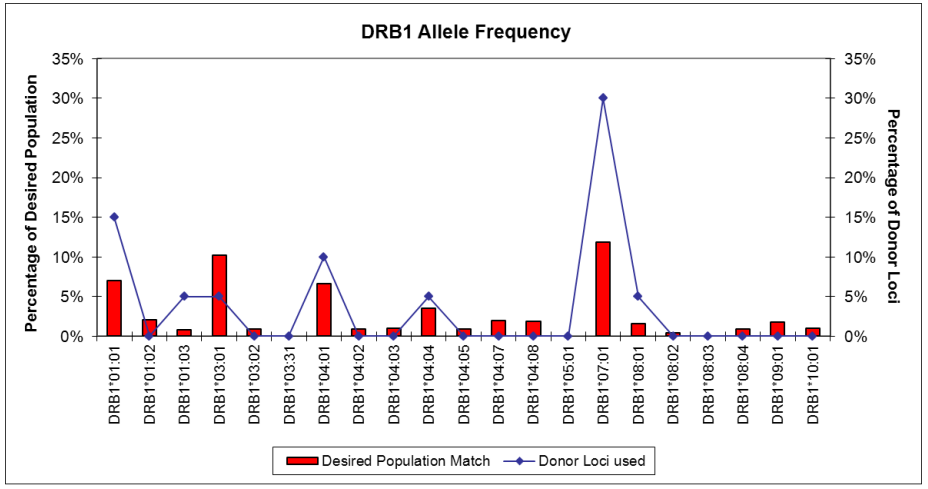
Case Study:
Keyhole Limpet Hemocyanin (KLH)

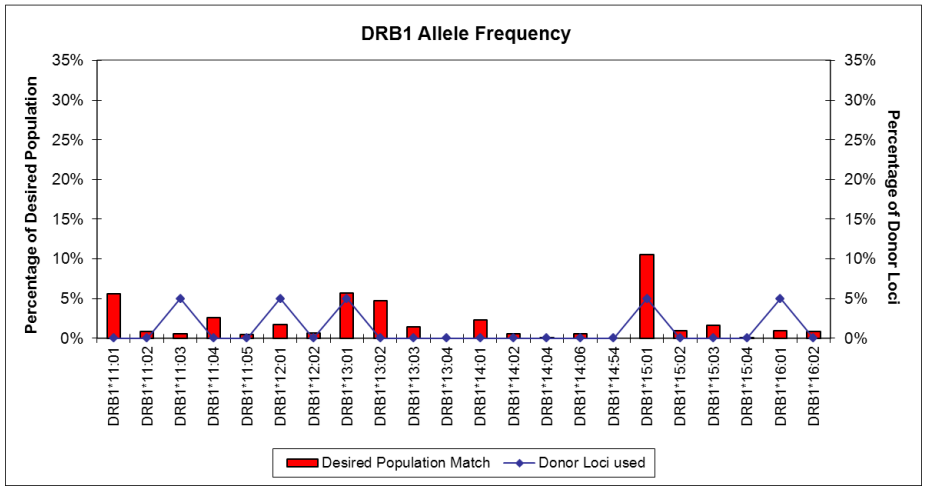
- Large multi-subunit protein (450 kDa to 13 MDa range) purified from giant keyhole limpet
- Used extensively as carrier protein, e.g. for conjugation of peptides for antibody production purposes
- Immunogen causing particularly strong CD4+ T cell response
- KLH-1 and KLH-2 isoforms present in most preparations
- Numerous epitopes thought to induce immune response *in vivo*

➢ Analyzed by ProPresent on 10 donors

Allele Matching to Global Distribution

Donor DRB1 Allele Frequency Distribution vs. Distribution in the Global Population





Donor DRB1 and DRB2

Donor ID	DRB1_1	DRB1_2
1	*03:01	*07:01
2	*12:01	*08:01
3	*01:01	*07:01
4	*01:01	*15:01
5	*04:01	*16:01
6	*01:03	*01:01
7	*11:03	*13:01
8	*04:04	*07:01
9	*07:01	*07:01
10	*04:01	*07:01

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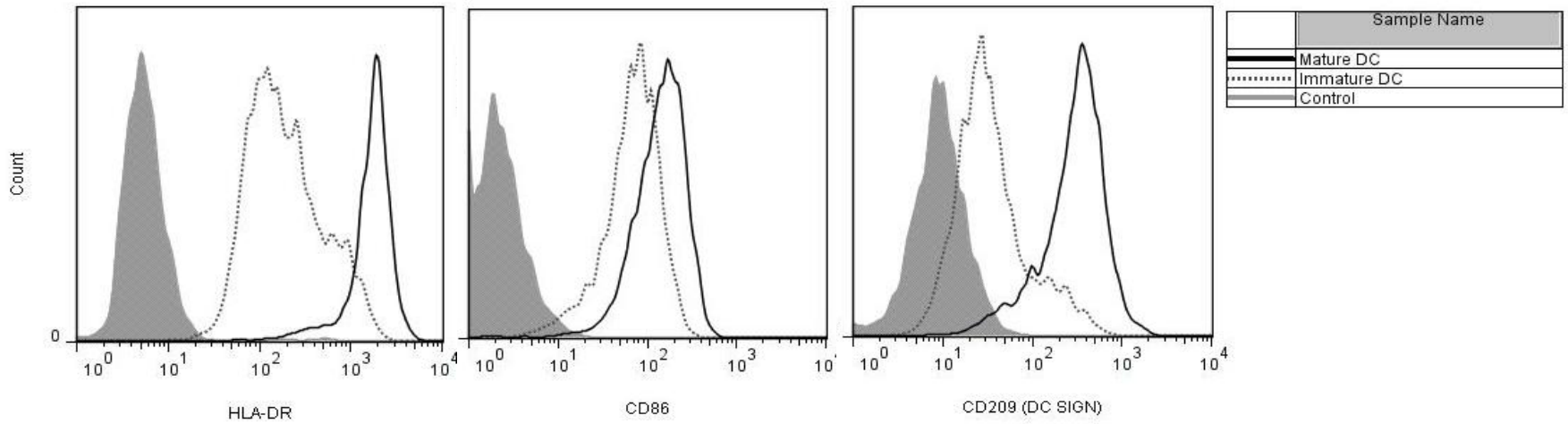
QC of Dendritic Cells: Expression of key markers



HLA-DR **CD86** **CD209 (DC-SIGN)**

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Detection of Control Proteins

Donor ID	CLIP	LAMP-3	TFRC	FcER2/FcGR2	Apolipoprotein B	ITGAM
1	Yes	Yes	Yes	Yes	Yes	Yes
2	No	No	Yes	No	Yes	Yes
3	No	Yes	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	Yes	Yes	Yes
5	No	Yes	Yes	Yes	Yes	No
6	Yes	Yes	Yes	Yes	Yes	Yes
7	No	Yes	Yes	Yes	Yes	Yes
8	No	Yes	Yes	yes	Yes	Yes
9	Yes	No	Yes	No	Yes	Yes
10	No	Yes	Yes	Yes	Yes	Yes

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Results: Identification of KLH Peptides

Donor	DRB1_1	DRB1_2	Sequence	Protein Domain	Amino Acid Start/End	Expect Value
1	*03:01	*07:01	LPSLINDATYFNSRSQTFDPNPF	KLH-2	1372-1394	0.091
			SLINDATYFNSRSQTFDPNPF	KLH-2	1374-1393	0.021
			INDATYFNSRSQTFDPNPF	KLH-2	1376-1394	0.0038
2	*12:01	*08:01	SSDEVLALEKALDD*	KLH-2	32-45	0.000078
			SSDEVLALEKALDDLQ	KLH-2	32-47	0.001
			SSDEVLALEKALDDLQQ	KLH-2	32-48	0.025
			VGDNFFLKYEAFDL	KLH-2	1226-1239	0.023
			VGDNFFLKYEAFDLNG	KLH-2	1226-1241	0.047
			VGDNFFLKYEAFDLNGG	KLH-2	1226-1242	0.06
			YDDTFTIKVHIKDIAG	KLH-2	2058-2073	0.039

Nested sequences are identified by dashed (----) lines between nested sets for each donor sample
Peptides marked with * have detectable modifications
Expect values ≤ 0.05 are indicative of peptide identity; currently accepted stringency criterion
Expect values <0.3 are indicative of peptide homology: expect values of ≥0.05 are indicated by shaded areas
False Discovery Rates are < 1% for ProPresent™

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Allele Association Anchor Analysis

Protein	Amino Acid Start-Stop	Unique Peptides	DRB1* Alleles Present with Detected Peptide						Likely Allele Association Based on known Anchors		
KLH-1	905-923	DENEMPWAYDRVFKYDITE	*13:01	*11:03					*11:03		
KLH-1	913-929	YDRVFKYDITEKLHDLK	*04:01	*11:03	*13:01				*04:01		
KLH-1	1006-1023	IENDGTYESIAKFHGSPG	*07:01						*07:01		
KLH-1	2468-2483	DPINHNAFTHSNAKPTD	*01:01	*07:01					*01:01		
KLH-1	2659-2677	NDESHGGYEHIAGFHGYPN	*01:01	*07:01					*01:01		
KLH-1	2876-2894	VNNDDFTRENSLPNAVVDS	*04:01	*15:01					*04:01		
KLH-2	31-48	SSDEVLALEKALDDLQQ	*12:01	*08:01					*12:01	*08:01	
KLH-2	39-56	LEKALDDLQQDDSNQGYQ	*04:01	*16:01					*04:01		
KLH-2	50-66	DSNQGYQAIAGYHGVPT	*01:01	*07:01					*01:01		
KLH-2	141-156	DNSWYRADITFLNKKTS	*04:01	*16:01					*04:01		
KLH-2	454-471	AEETYSLRKAMERFQNDK	*01:01	*15:01					*15:01		
KLH-2	463-479	AMERFQNDKSVDGYQAT	*04:01	*16:01					*04:01		
KLH-2	1207-1224	YDRVYKYEITQQLHDLDL	*01:01	*07:01	*15:01	*04:01			*01:01	*07:01	*15:01
KLH-2	1226-1242	VGDNFFLKYEAFDLNGG	*12:01	*08:01					*12:01		
KLH-2	1372-1394	LPSLINDATYFNSRSQTFDPNPF	*03:01	*07:01	*01:01	*04:01			*07:01	*01:01	
KLH-2	1773-1790	VGLPYWDWLKPQSALPDL	*01:01	*15:01					*01:01	*15:01	
KLH-2	1774-1790	LPYWDWLKPQSALPDL	*01:01	*07:01	*15:01	*04:01	*16:01	*01:03	*04:01	*07:01	*01:01
KLH-2	2058-2073	YDDTFTIKVHIKDIAG	*12:01	*08:01					*12:01		
KLH-2	2226-2243	AKGYIKSEDAYTVRDPQD	*01:01	*07:01	*15:01				*01:01	*07:01	*15:01
KLH-2	2591-2606	HRLFVKQMEDALAAHG	*04:04	*07:01					*07:01		

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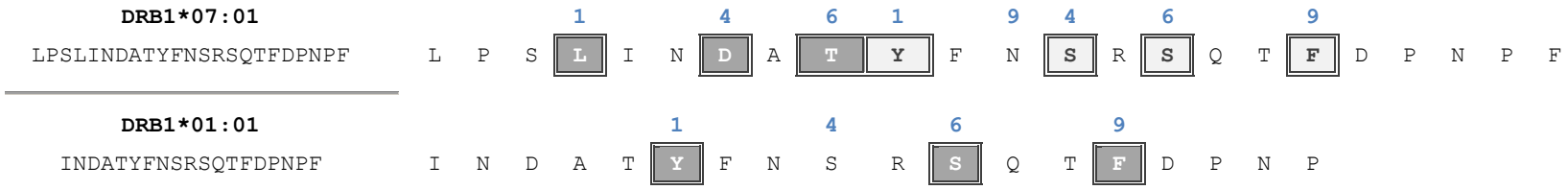
Anchor Sequence Identification



Highlighted regions correspond to potential anchor residues

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Case Study Conclusions

- Significant number of key new peptides identified for KLH with high confidence
- Assay very repeatable and reproducible in its control features
- Proliferation assays could confirm whether these are T cell epitopes

ProPresent Conclusions

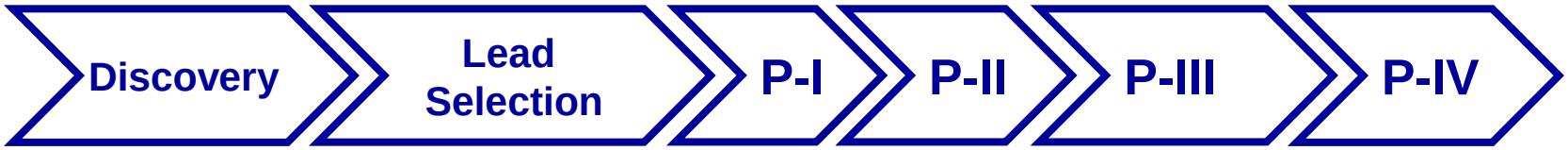
- Unique service – only available at ProImmune
- Rapid way to identify sequences of key relevance for the immunogenicity of target protein
- Can answer otherwise confounding questions in a clear and decisive way
- Represents a key element in profiling any protein based product candidate that should be considered for inclusion in any product file

Master Immunity with ProImmune

A modular approach

- ProArray Ultra™
- ProImmune Reveal®
- ProPresent™
- Pro5® Pentamers
- GLP/GCP Monitoring
- typeHLA™
- thinkpeptides®

delivers what you need when you need it



throughout your product life cycle

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