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Peptide microarrays as a novel high-throughput tool for mapping HLA class II-peptide interactions: a case study on HCMV epitopes

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INNOVATIVE STRENGTH AND APPLICATIONS

High-density peptide microarrays provide a scalable, high-throughput platform for mapping peptide–HLA interactions and identifying T-cell epitopes with functional relevance. Applications include selection of peptides effectively presented by HLA class II subtypes, discovery of T-cell epitope candidates for immunotherapy and vaccine design, and assessment of potential T-cell–dependent anti-drug antibody (ADA) responses.

BACKGROUND

The identification of pathogen-derived peptides presented by human leukocyte antigens (HLA) is crucial for the development of targeted immunotherapies. The specificity of HLA–peptide binding determines which epitopes elicit T-cell responses and contributes to inter-individual differences in immune reactivity. However, the high polymorphism of HLA molecules and the vast diversity of pathogen peptides make systematic identification difficult. While computational models can predict HLA class II binding, their accuracy remains limited, emphasizing the need for novel high-throughput epitope discovery approaches.

PROJECT DESCRIPTION

We applied high-density peptide microarrays to systematically map peptide interactions with HLA class II, focusing on human cytomegalovirus (HCMV) epitopes restricted by HLA-DRB1*1101. The microarrays contained 4,327 overlapping 15-mer peptides covering 24 HCMV proteins. Peptide-HLA binding was detected using biotinylated HLA monomers and fluorescent streptavidin. Selected peptides were further analyzed with FluoroSpot assay to assess their capacity to stimulate T-cell responses. This approach enables high-throughput identification of HLA class II binders as T-cell epitope candidates.

METHODS

We used the PEPperCHIP® Peptide Microarray platform technology to create overlapping peptide sequences (15 aa with an overlap of 13 aa) of 24 different HCMV protein antigens. The resultant peptide microarray contained a total of 4,327 different peptides, printed in duplicates on glass slides.

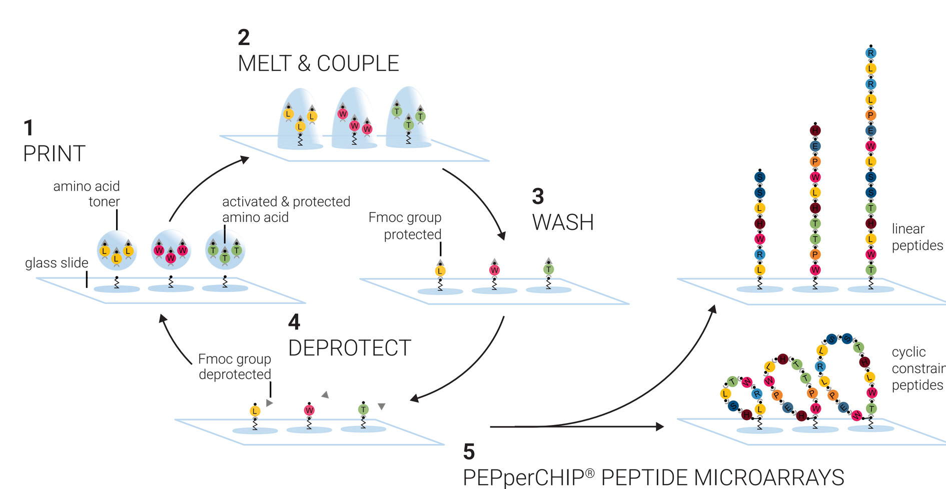


Figure 1. The PEPperCHIP® Peptide Microarray platform technology

In the binding assay the peptide microarrays were incubated with biotinylated HLA class II monomers (HLA-DRB1*1101) and fluorescently labelled streptavidin (**Fig.2**). To verify the specificity of binding competitive binding assays were conducted. Additionally, data were compared with published binding affinities of tested peptides to HLA molecules.

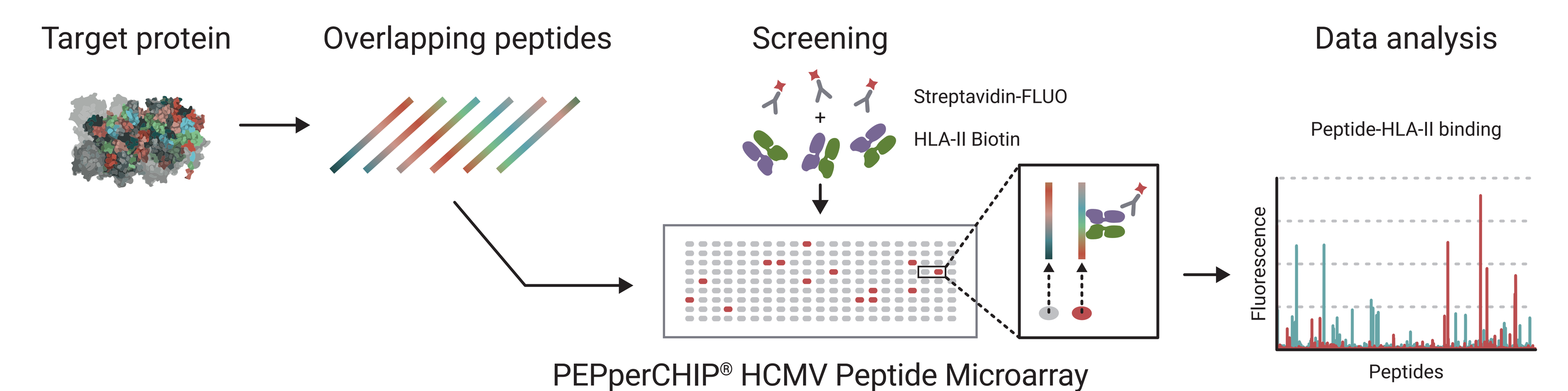


Figure 2. HLA-peptide binding assay with the PEPperCHIP® HCMV Peptide Microarray

RESULTS

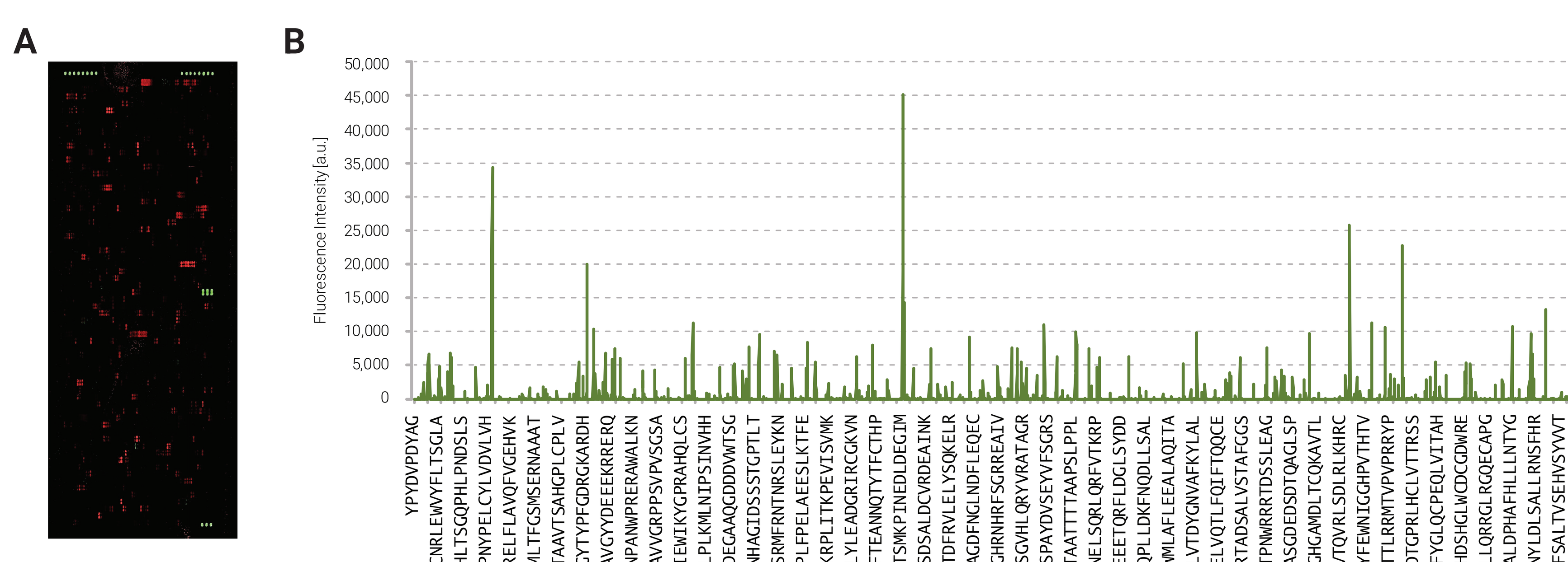


Figure 3A shows the scanned microarray. After the staining procedure, binding of HLA-DRB1*1101 monomers to an immobilized peptide results in fluorescent spots that can be further analysed to receive fluorescent intensities and the assignment to the bound peptide (**Fig. 3B**) and associated proteins.

Protein	UniProtID	Nb of binders	Seqeunce of best binder	FI
Tegument protein UL99	P13200	12	PRPDTPRTPRQKKIS	6788
Major capsid protein UL86	P16729	30	HYRNLVAVLRLVTRI	52
Protein UL40	P16780	0	RTLILTVGLLCMRIR	1175
Large structural phosphoprotein UL32	P08318	44	RALVSAVILAKMSVR	0083
Orf UL153	Q68408	9	LYMGSRRVPRRPRYT	11279
65 kDa matrix phosphoprotein UL83	P06725	17	ATACTSGVMTRGRLK	5235
Immediate-early protein 2 IE2	P19893	18	KPRKTTRPFKVIIKP	9601
Immediate-early protein 1 IE1	P13202	11	MVRHRIKEHMLKKYT	8452
Protein UL130	P16772	12	GAHMVPKQTKLLRFV	8024
Envelope protein UL131A	Q8AZ45	3	FRRQNRRGGTNKRTT	2857
Envelope glycoprotein B UL55	P06473	24	HTFKVRVYQKVLTFR	
Protein UL36	P16767	4	GFMRYQLIVLIGQRG	1355
Protein UL28	P16847	17	GFIRQGSFWFRCPRR	11048
Deneddylase UL48	P16785	35	RKDLSGSKHGGKKKP	10013
Capsid-binding protein UL94	P16800	29	YQGHYGVNHVHRRRR	9713
Protein UL103	P16734	6	RHTCLQLVARFFFRL	849
Membrane glycoprotein US3	P09712	4	VVLVLLTVGVSARLR	11284
Tegument protein US24	P09700	15	FRMGLLKMVFRHRRF	2770
Protein HHRF4	P09705	20	RGNCTAPKRTYPRRL	5487
Orf UL151	Q68405	3	QGSRTRRPIPPILQR	2902
Envelope glycoprotein H	P12824	15	ITSLVRLVYILSKQN	13311

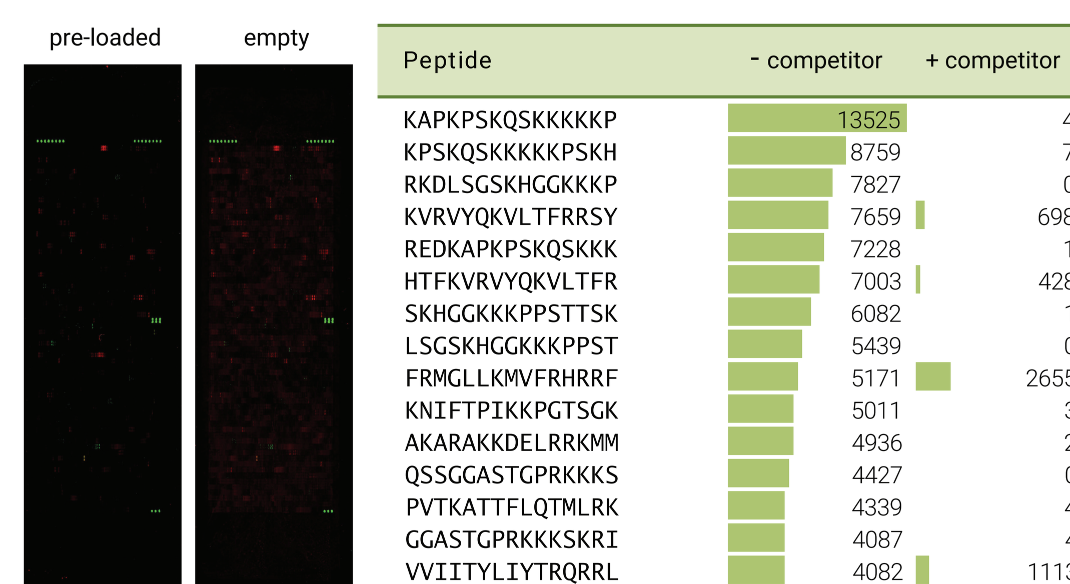
In the following, signals (FI) above 500 were defined as positive and further analyzed. From the 24 (4327 peptides) proteins included on the microarray, 21 proteins contained peptide binders of HLA-DRB1*1101.

Table 1 depicts the number of HLA-DRB1*110-bound peptides per protein and the sequence of the best binder.

To compare the results of the new microarray-based approach to established biochemical peptide/HLA affinity measurements we utilized published data of Braendstrup et al. (PLoS One. 2014, PMID: 24760079) on the binding affinity of HCMV peptides of immediate-early protein 2 (IE2) to HLA-DRB1*1101.

Figure 4 shows the matching data. The peptides that were identified as non-binders (a very low affinity of over 1000nM) by Braenstrup et al. showed no signal on the microarray-based binding assay. The peptides with a high affinity to HLA-DRB1*1101 were also identified as binders with the microarray-based assay.

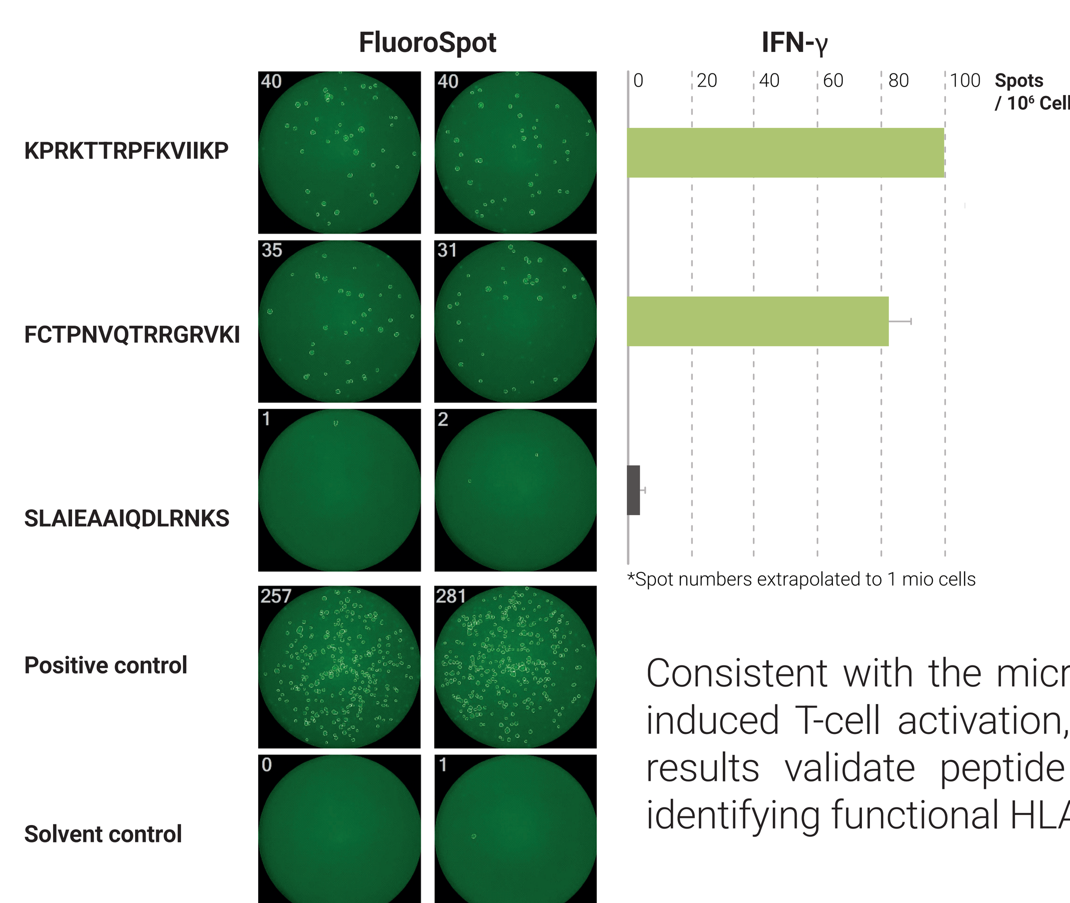
Braendstrup et al., PLoS One. 2014 Apr 23 PMID: 24760079				Microarray data PEPperPRINT	
Protein	Position	Sequence	Affinity assay	Sequence	FI
IE2	151-165	KTTRPFKVIIKPPVP	binder	ATPELSPRKKPRKTT	4806
				ELSPRKKPRKTTRPF	4650
				PRKKPRKTTRPFKVI	8241
				KPRKTTRPFKVIIKP	9599
				KTTRPFKVIIKPPVP	1103
				RPFKVIIKPPVPPAP	0
				KVIIKPPVPPAPIML	0
				IKPPVPPAPIMLPLI	0
IE2	156-170	FKVIIKPPVPPAPIM	binder	PRKKPRKTTRPFKVI	8241
				KPRKTTRPFKVIIKP	9599
				KTTRPFKVIIKPPVP	1103
				RPFKVIIKPPVPPAP	0
				KVIIKPPVPPAPIML	0
				IKPPVPPAPIMLPLI	0
				PVPPAPIMLPLIKQE	0
				PAPIMLPLIKQEDIK	0
IE2	408-422	KGIQIIYTRNHEVKS	binder	IKACKTMQVNNKGIQ	1277
				CKTMQVNNKGIQIIY	0
				MQVNNKGIQIIYTRN	2237
				NNKGIQIIYTRNHEV	0
				GIQIIYTRNHEVKSE	0
				IIYTRNHEVKSEVDA	0
				TRNHEVKSEVDAVRC	0
				HEVKSEVDAVRCRLG	0
IE2	438-452	ALSTPFLMEHTMPVT	non-binder	VRCRLGTMCNLALST	0
				RLGTMCNLALSTPFL	0
				TMCNLALSTPFLMEH	0
				NLALSTPFLMEHTMP	0
				LSTPFLMEHTMPVTH	0
				PFLMEHTMPVTHPPE	0
				MEHTMPVTHPPEVAQ	0
				TMPVTHPPEVAQRTA	0
IE2	443-457	FLMEHTMPVTHPPEV	non-binder	TMCNLALSTPFLMEH	0
				NLALSTPFLMEHTMP	0
				LSTPFLMEHTMPVTH	0
				PFLMEHTMPVTHPPE	0
				MEHTMPVTHPPEVAQ	0
				TMPVTHPPEVAQRTA	0
				VTHPPEVAQRTADAC	0
				PPEVAQRTADACNEG	0



Peptide	- competitor	+ competitor
KAPKPSKQSKKKKKP	13525	4
KPSKQSKKKKKPSKH	8759	7
RKDLSGSKHGGKKKP	7827	0
KVRVYQKVLTFRRSY	7659	698
REDKAPKPSKQSKKK	7228	1
HTFKVRVYQKVLTFR	7003	428
SKHGGKKKPPSTTSK	6082	1
LSGSKHGGKKKPPST	5439	0
FRMGLLKMVFRHRRF	5171	2655
KNIFTPIKKPGTSGK	5011	3
AKARAKKDELRRKMM	4936	2
QSSGGASTGPRKKKS	4427	0
PVTKATTFLQTMLRK	4339	4
GGASTGPRKKKSKRI	4087	4
VVIITYLIYTRQRRL	4082	1113

To verify the specificity of binding, competitive binding assays with high affinity binders were conducted. **Figure 5** depicts the scans (left) of the binding assay with and without the competitor peptide and the associated quantification. The results suggest a specificity of the HLA-peptide binding.

Figure 6. To evaluate the T-cell activating potential of HLA-bound peptides, FluoroSpot assays were performed using HLA-DRB1*11:01-typed PBMCs and selected peptides. Representative well images and corresponding quantifications show that microarray-identified binders (KPRKTTRPFKVIIKP, FCTPNVQTRRGRVKI) induced IFN-γ production in T-cells, whereas the tested non-binder (SLAIEAAIQDLRNKS) did not.



Assay setup:

- PBMCs from a HLA-DRB1*1101 typed individual
- 400,000 cells per well, done in duplicates
- Concentration of peptides: 30µg/ml
- Positive control: Control peptide pool

Consistent with the microarray results, HLA-DRB1*1101 binders induced T-cell activation, whereas the non-binder did not.These results validate peptide microarrays as an effective tool for identifying functional HLA class II epitopes.

CONCLUSION

Our study demonstrates that high-density peptide microarrays can systematically identify HLA class II binders and functional T-cell epitopes, complementing computational predictions and accelerating epitope discovery for immunotherapies, vaccine development, and T-cell-dependent anti-drug antibody (ADA) assessment. This approach provides an efficient, scalable tool for immune system analysis and supports the development of targeted interventions against infectious diseases.

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