Comparison of High Resolution PEPperMAP® Epitope Mappings with Low Resolution Epitope Mappings

Introduction

For peptide microarray-based epitope mappings, an antigen sequence is usually translated into overlapping peptides. In order to save costs, many commercial suppliers frequently choose reduced peptide-peptide overlap to e.g. 10 or 11 amino acids with 15mer peptides. The peptide-peptide overlap, however, plays an important role for epitope prediction. A reduced peptide-peptide overlap hampers the exact identification of conserved core motifs, the unambiguous itemization of adjacent epitopes in polyclonal samples, or the discrimination of non-specific binders from real epitopes.

PEPperPRINT's proprietary laser printer-based on-chip synthesis is capable to routinely translate antigens into peptide microarrays with maximum overlap (14 amino acids with 15mer peptides) providing the maximum amount of epitope coverage and prediction at reasonable costs. Therefore, PEPperMAP® Epitope Mappings are routinely performed with PEPperCHIP® Peptide Microarrays based on any given antigen translated into overlapping peptides with a maximum peptide-peptide overlap.

The scheme in Fig. 1 highlights how the peptide-peptide overlap affects the number of peptides and data points based on a hypothetical 25 amino acid antigen elongated by C- and N-terminal GS linkers. Based on 15mer peptides and a peptide-peptide overlap of 14 amino acids, the hypothetical 8mer epitope EFYLYLAA (red dotted box) is covered by 8 adjacent peptides (orange box) with detailed and clear information on the epitope length and the conserved core motif.

Based on 15mer peptides and a peptide-peptide overlap of only 11 amino acids, however, the hypothetical 8mer epitope EFYLYLAA (red dotted

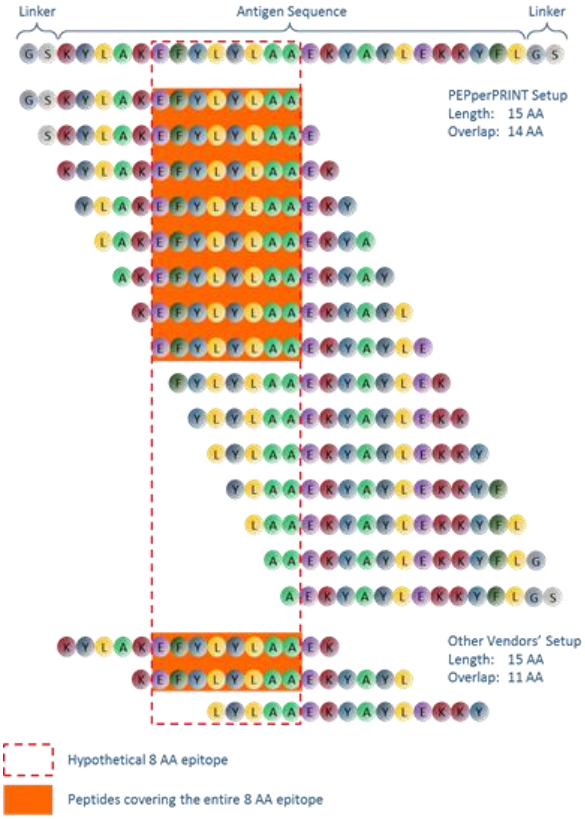

Fig. 1: In the PEPperMAP® epitope mapping setup, the antigen sequence translated into overlapping 15mer peptides with maximum overlap of 14 amino acids. A hypothetical 8mer epitope (red dotted box) is then covered by 8 peptides (orange box). A reduced overlap of 11 amino acids results in only 3 overlapping peptides, of which only two contain the full epitope: the actual epitope length and the conserved core motif cannot be unambiguously determined.

box) is only covered by 2 adjacent peptides (orange box) with unclear epitope boundaries, since both adjacent peptides contain the hypothetical epitope sequence in the middle.

In summary, PEPperMAP® high resolution epitope mappings with a maximum peptide-peptide overlap allow:

- the exact identification of conserved core motifs
- a detailed analysis of epitope length
- unambiguous itemization of adjacent epitopes in polyclonal samples
- discrimination of unspecific binders from real epitopes

In order to demonstrate the influence of the peptide-peptide overlap on epitope mapping and prediction, we generated peptide microarrays with the antigen CENPA translated into overlapping peptides. The systemic sclerosis antigen consists of 140 amino acids that were further elongated by neutral GSGSGSG linkers at the N- and C-terminus to avoid truncated peptides. Based on 15mer peptides, we selected a maximum peptide-peptide overlap of 14 amino acids resulting in 140 different peptides per array, and compared it with a reduced overlap of 11 amino acids (36 different peptides per array). The resulting peptide microarrays were assayed with a systemic sclerosis patient serum to investigate the prediction of CENPA autoantibody epitopes in relation of the peptide-peptide overlap.

Results

Epitope mappings of a human systemic sclerosis patient serum (SSc1) were carried out against antigen CENPA translated into 15mer peptides with peptide-peptide overlaps of 14 (high resolution epitope mapping) and 11 amino acids (low resolution epitope mapping). The peptide microarrays with the antigen-derived peptides were incubated with the human serum SSc1 (1:5000) followed by staining with the secondary goat anti-human IgG(H+L) conj. DyLight680 antibody and by read-out with a LI-COR Odyssey Imaging System. The quantification of spot intensities and peptide annotation were performed with PepSlide® Analyzer.

On both arrays, we observed strong and well-defined interactions with clear epitope-like spot patterns formed by rows of adjacent peptides (Fig. 2).

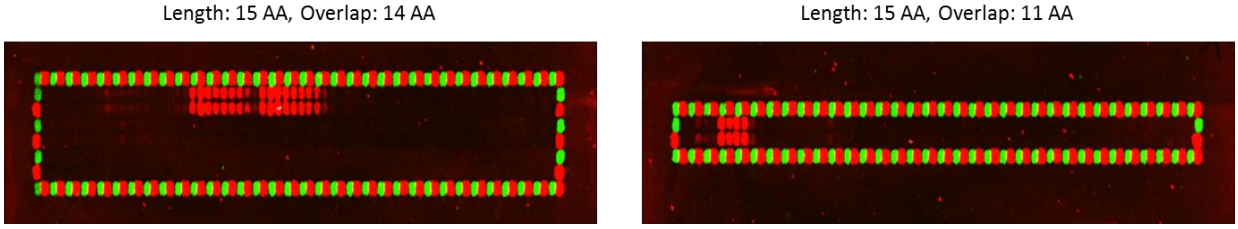

Fig. 2: Peptide arrays were assayed with human serum SSc1 (1:5000) followed by staining with the secondary antibody and data read-out. Final staining of the HA and Flag control peptides framing the peptide arrays gave rise to the expected and well-defined spot pattern and validated the overall peptide microarray integrity.

In accordance with the microarray scan, the intensity plot clearly revealed two N-terminal CENPA epitopes PTPTPGPS and RGPSLGAS with the maximum peptide-peptide overlap of 14 amino acids (Fig. 3). The large overlap enables a clear separation of the two adjacent epitopes from each other.

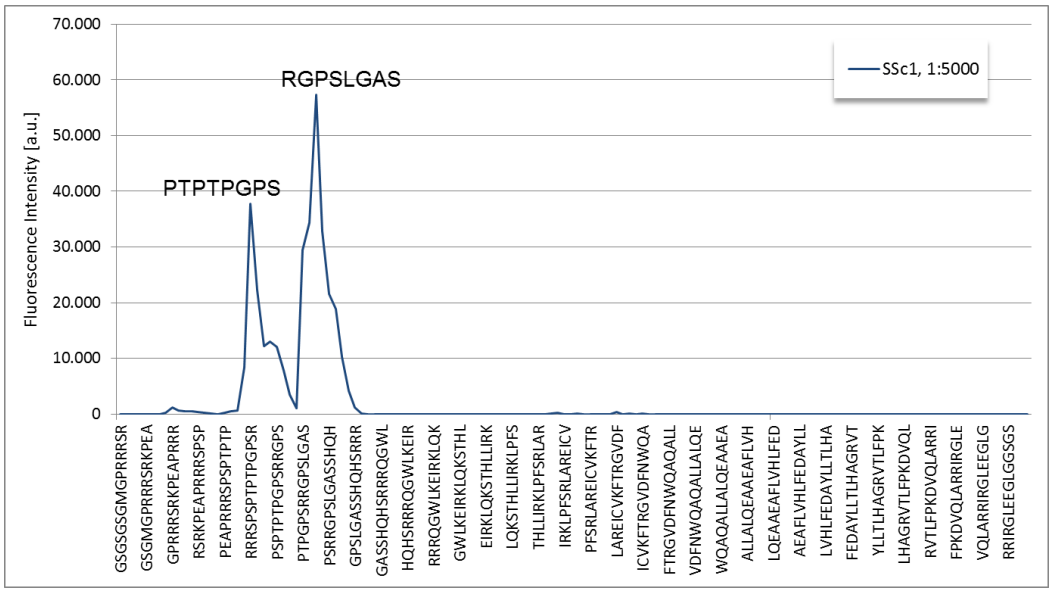

Fig. 3: The intensity plot of the CENPA peptides highlights the very strong response of serum SSc1 with spot intensities close to the limit of the dynamic range of the microarray scanner. Due to the maximum peptide-peptide overlap, we clearly observed two different, well-separated epitopes PTPTPGPS and RGPSLGAS close to the N-terminus of CENPA.

With a reduced peptide-peptide overlap of only 11 amino acids, only one signal with an apparent single epitope PTP was observed. Only the curve shape in the intensity plot hints at the presence of two different epitopes, which cannot be discriminated due to reduced peptide-peptide overlap (Fig. 4).

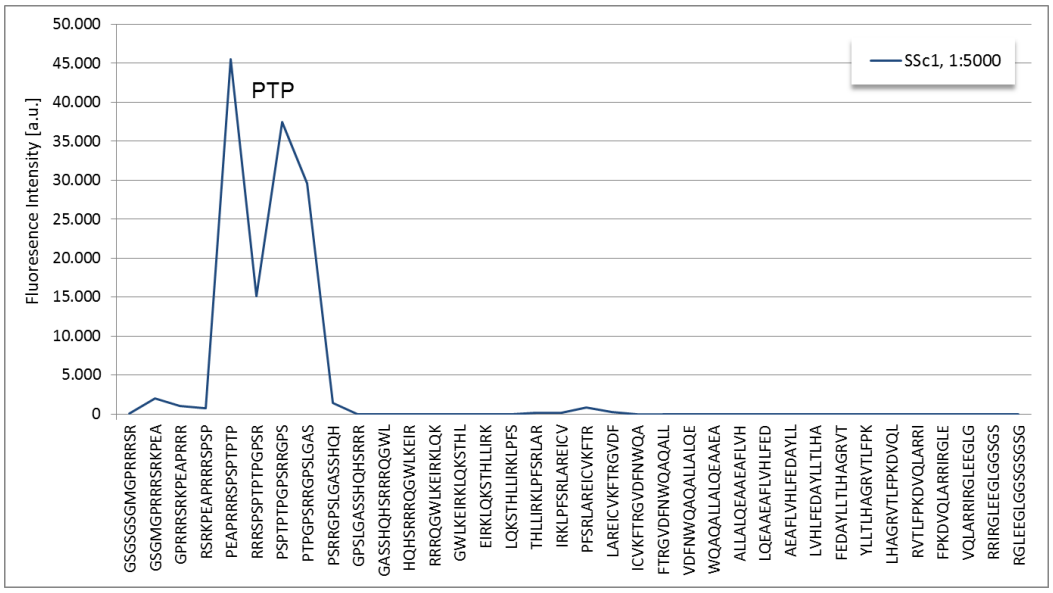

Fig. 4: In contrast to the high resolution epitope mapping with a maximum overlap of 14 amino acids shown in Fig. 3, the low resolution setup with a reduced overlap of only 11 amino acids results in one signal in the intensity plot with an apparent single epitope PTP; the two adjacent epitopes cannot be separated any further.

Conclusion

The autoimmune epitope mapping of human systemic sclerosis patient serum SSc1 was carried out against antigen CENPA translated into 15mer peptides with a maximum peptide-peptide overlap of 14 amino acids as well as with a reduced peptide-peptide overlap of 11 amino acids. Applying the maximum peptide-peptide overlap, serum SSc1 showed a strong response with excellent signal-to-noise ratios against the two N-terminal CENPA-epitopes PTPTPGPS and RGPSLGAS. With low resolution epitope mapping and a peptide-peptide overlap of only 11 amino acids, we observed only one signal with an apparent single epitope PTP.

The data clearly reveal that a maximum peptide-peptide overlap is highly recommended for the itemization of adjacent epitopes. The exact identification of conserved core motifs and the determination of the actual epitope length demonstrate the advantages of PEPperPRINT's approach to high resolution epitope mapping.

For more information, please download the full report from our website:
http://www.pepperprint.com/science/application-notes/