# High Sensitivity Mapping of Monoclonal anti-Endorphin Antibody 3-E7 on PEPperCHIP® Microarrays

## Introduction

Peptide microarrays comprise thousands of ligands in a highly miniaturized format with only picomolar amounts of material per spot. An elaborated surface chemistry with a low degree of non-specific protein binding combined with a sensitive detection method is strongly recommended to provide reliable data at high sensitivity. Fluorescence detection of bound material using labeled probes, such as fluorescence labeled secondary antibodies, represents the method of choice for a specific and highly sensitive analysis of peptide microarrays. In order to evaluate the potential of PEPperCHIP® peptide microarrays, an epitope mapping was performed with a peptide library based on Leu-Enkephalin (YGGFL), a pentapeptide with nanomolar affinity to the monoclonal anti-Endorphin antibody 3-E7. In addition, the library was spiked with nano- to micromolar ligands as positive controls to investigate the detection limit of PEPperCHIP® peptide microarrays in line with immunoassays.[1]

## Material and Methods

Microarray Content: The microarray covered a full permutation scan of epitope YGGFL (substitution of all positions with 20 amino acids) including eight additional positive controls (see table 1). Peptides were synthesized on chip from the C-to the N-terminus and linked with a neutral $(GS)_n$ linker (n =1; n =5) to the PEPperCHIP® glass slide coating. Each peptide was printed in duplicates, and the starting sequence YGGFL ten times. Peptide spots were separated in a checkerboard pattern by glycine spots as negative controls. Each array was framed by FLAG (DYKDDDDKGG, 90 spots) and HA (YPYDVPDYAG, 72 spots) control peptides.

Immunoassay Protocol:

Standard buffer: PBS, pH 7.4 + 0.05 % Tween20

Blocking buffer: Rockland blocking buffer MB-070

Incubation buffer: Standard buffer with 10 % blocking buffer

Sample: Monoclonal mouse anti-Endorphin antibody, β, clone 3-E7 (MAB5276, Millipore); incubation for 16 h at 4°C and shaking at 500 rpm at a concentration of 10 µg/ml

Secondary antibody: Goat-anti-mouse IgG (H+L) IRDye680, 1:5000

Post staining: Monoclonal anti-HA (12CA5)-LL Atto 680 (1:1000), monoclonal anti-FLAG (M2)-FluoProbes752 (1:1000)

Scanner: Odyssey Imaging System, LI-COR Biosciences

[1] S.E. Cwirla et al., *Peptides on phage: A vast library of peptides for identifying ligands*, PNAS **87**, 6378, (1990)

## Results

After 30 min incubation in blocking buffer, the microarray was incubated with the secondary antibody to analyze background interactions with the peptide probes on the chip. No background interactions that could interfere with the main assays were observed (data not shown). Subsequently, the array was incubated for 16h at 4°C with the monoclonal anti-Endorphin antibody 3-E7 followed by 30 min staining with the IRDye680 labeled secondary anti-mouse antibody. Read-out with an Odyssey Imaging System at a resolution of 21 µm and a scanning intensity of 6 shows clear and high intensity spots in a checkerboard pattern; the glycine spacer spots as negative controls were not stained (Fig. 1, left). Subsequent staining of Flag and HA control peptides that frame the arrays gave rise to high and homogeneous spot intensities at a scanning intensity of 6/6 in green/red (Fig. 1, right). Quantification of spot intensities and peptide annotation were done with PepSlide® Analyzer. The intensities of selected peptides including the additional positive controls synthesized without and with a $(GS)_5$ spacer were correlated with published affinity data and listed in table 1.

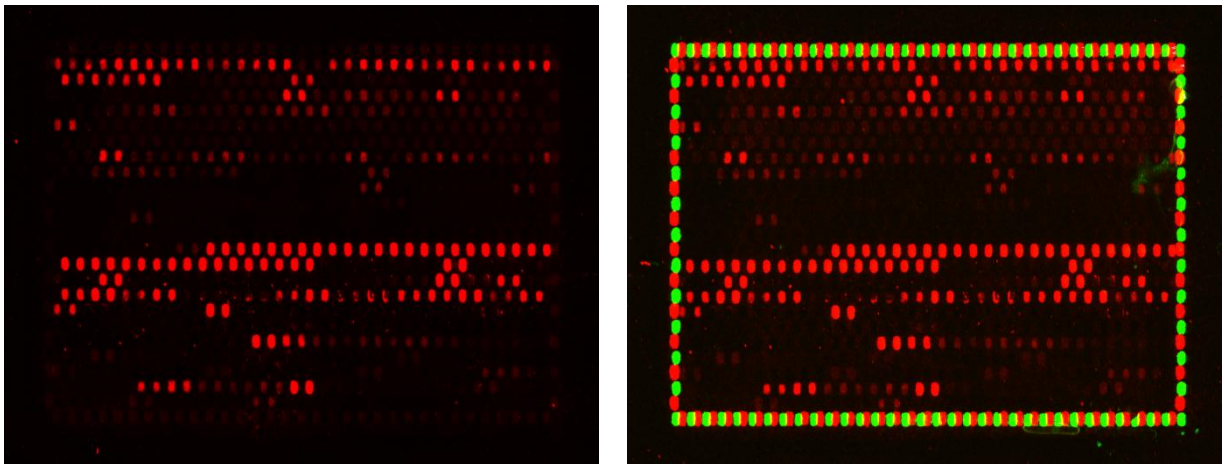

**Fig. 1:** Fluorescence intensities on a Leu-Enkephalin based peptide library after staining with anti-ß-Endorphin antibody 3-E7, detection with a secondary anti-mouse IgG IRDye 680 antibody (left) and post staining of HA and FLAG control peptides (right).

| Peptide No. | Sequence | $IC_{50}$ (µm) [1)] | Fluorescence Intensity (no spacer) | Fluorescence Intensity $(GS)_5$ spacer |
|---|---|---|---|---|
| 1 | YGGFL | 0.007 | 17,780 | 44,284 |
| 2 | YGGF | 0.19 | 19,480 | 38,787 |
| 3 | YGFWGM | 0.35 | 1,497 | 4,345 |
| 4 | YGGFPD | 2.3 | 19,283 | 40,021 |
| 5 | YGGL | 3.8 | 2,255 | 541 |
| 6 | YGGWAG | 7.8 | 664 | 0 |
| 7 | YGNWTY | 7.8 | 2,884 | 0 |
| 8 | YAGFAQ | 8.3 | 1,956 | 4,894 |
| 9 | YGFL | 28 | 2,492 | 0 |
| - | G | - | 101.6 | - |

**Table 1:** Peptide sequences, affinities and spot intensities of selected peptides in comparison with the glycine spacer spots. Sequences and $IC_{50}$ values were taken from reference 1.

## Discussion and Conclusion

The experiment provided a comprehensive data set at excellent signal-to-noise ratios. Among the positive controls, non-surprisingly the high affinity peptides YGGFL and YGGF (peptides 1 + 2) clearly showed up with highest spot intensities, followed by the low micromolar ligand YGGFPD (peptide 4). Increased signals were observed by presenting these peptides on an elongated spacer. YGFWGM (peptide 3) with a reported affinity of 0.35 µM to the 3-E7 antibody exhibited a rather low spot intensity without spacer, but gave a clearer response when coupled to the $(GS)_5$ spacer. A similar effect was observed with YAGFAQ (peptide 8). The higher spot intensities of some GS linked peptides could be likely explained by a better accessibility to the 3-E7 antibody.

Remaining positive controls 5, 6, 7 and 9 with reported affinities between 2 and 28 µM were only detected at very weak spot intensities, regardless of the immobilization strategy. Data quantification of the YGGFL permutation scan further revealed a number of additional 3-E7 ligands with unknown affinities at strong to medium intensities.

The epitope mapping study using a PEPperCHIP® peptide microarray positively demonstrates the potential of PEPperPRINT´s microarray platform and superior glass slide coatings. Peptides could reliably be identified with high sensitivity up to apparent affinities in the submicromolar range, allowing the high throughput analysis of even weaker peptide protein interactions as required for the characterization of early immune responses upon infection, autoimmunity or oncogenesis, the optimization of antibody epitopes, in-depth evaluation of various adjuvants in vaccine development or the identification of affinity ligands to purify antigen-specific antibodies from polyclonal sera.