High-content peptide microarrays for the identification of new cancer and autoimmune biomarkers as well as antigen and epitope discovery

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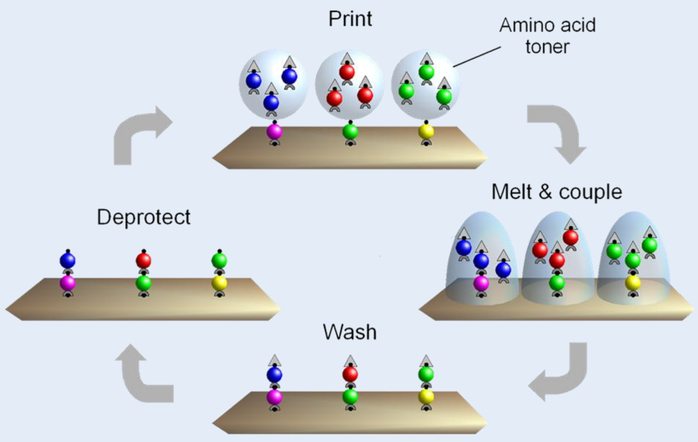
Background Technology



Combinatorial synthesis with amino acid toner particles

High-content PEPperCHIP® peptide microarrays were *in-situ* synthesized by digital laser printing on standard glass slides. The printer accommodates 20 amino acid cartridges instead of the 4 usual color cartridges. The cartridges contain microparticles with the activated amino acid embedded in the toner material. After printing, all microparticles are melted releasing the activated amino acid to initiate coupling. The cycle of synthesis is completed when excessive monomers are washed away and the Fmoc protection group is removed. The benefits of this technique are a unique digital flexibility in terms of peptide content, a high spot density with up to 9.000 features per glass slide as well as a significantly reduced material consumption enabling lower cost solutions.

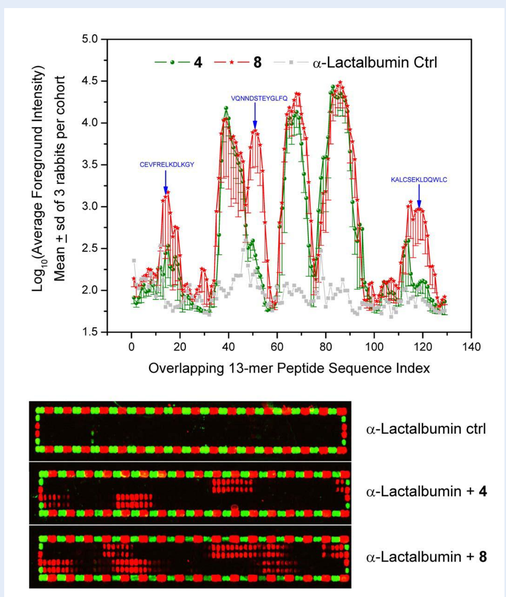
The identification of early biomarkers for severe diseases by noninvasive methods is very important but demanding. In this regard high-content peptide microarrays are a very efficient tool for antibody biomarker discovery from sera, as demonstrated by several case studies.

Bayer *et al.* Science 2007, Stadler *et al.* Angew Chem Int Ed Engl. 2008

Epitope Mapping

The aim of the project was the investigation of humoral immune responses after immunization with different adjuvants stimulating TLR-7 and/or TLR-8. Therefore, rabbits were immunized with bovine α-lactalbumin alone or in combination with adjuvants **4** or **8**. The antigen sequence of α-lactalbumin was translated into 13mer peptides with a peptide-peptide overlap of 12 amino acids in duplicates. Four PEPperCHIP® peptide microarrays with 129 overlapping peptides were printed on each glass slide. Each array was framed by HA and Flag control peptides. The arrays were hybridized with rabbit serum, stained with labeled secondary antibody and analyzed using a microarray scanner as well as PepSlide Analyzer® software (http://www.sicasys.de/).

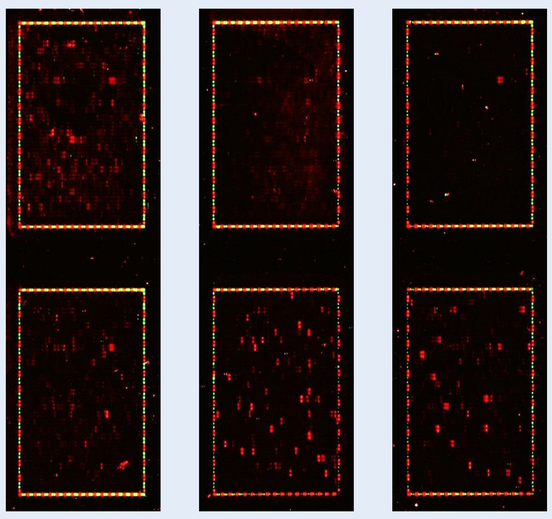
Linear epitope mapping clearly showed that the dendrimer **8** induced immunoreactivity to more contiguous peptide epitopes along the amino acid sequence of α-lactalbumin than compound **4**. Accordingly, immunization with **8** as adjuvant extends the immunoreactivity profile of the humoral response to encompass longer stretches of the amino acid sequence of the protein antigen.



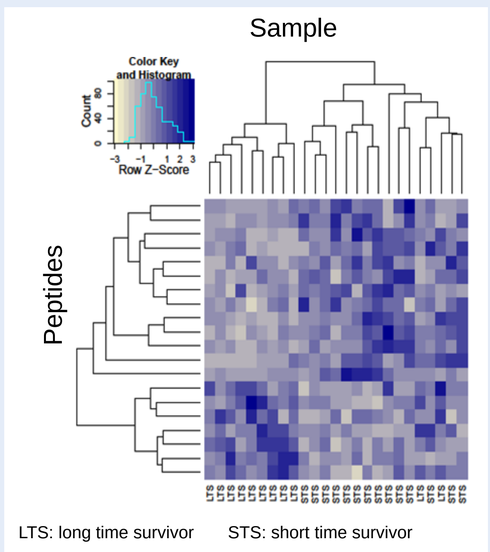
Results published by Shukla *et al.* PLoS One 2012

Discovery of prognostic markers in tumor patients

In a collaboration with the Heidelberg University Hospital we investigated serum profiles of glioblastoma patients and compared long vs. short time survivors with the aim to identify new prognostic markers. The corresponding PEPperCHIP® peptide microarrays covered 6 tumor antigens translated into 13mer peptides with peptide-peptide overlap of 12 aa. With two array copies per microarray we initially tested 25 serum samples of both patient groups and identified 70 peptide candidates of statistical significance for further evaluation. Based on this outcome the identification of prognostic tumor markers is presently ongoing and extended to 160 serum samples.



Patient 1/2 Patient 3/4 Patient 5/6



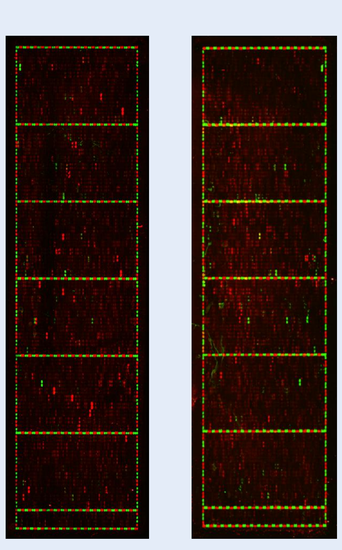
LTS: long time survivor STS: short time survivor

Discovery of new markers for autoimmune diseases

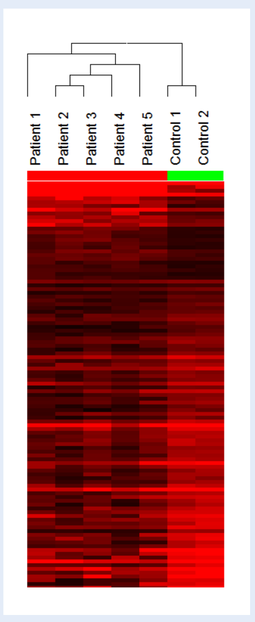
PEPperCHIP® Signature Microarrays

Since a multitude of autoantibody antigens are simply unknown, it is difficult to identify new serum biomarkers for such autoimmune diseases. In a collaboration with a French University we used PEPperCHIP® signature microarrays containing 4,092 random 15mer peptides to screen for peptides and peptide patterns of statistical significance in patient and control sera of an undisclosed autoimmune disease.

SUMO (Statistical Utility for Microarray and Omics data) software was used for statistical analysis based on total intensity and quantile data normalization as well as and unpaired two-class t-test to detect differently regulated peptides. We found 45 peptides with p-value below 0.001. 26 of these peptides were strongly recognized by antibodies of autoimmune patients, but not by antibodies of healthy controls. These peptides are now validated as new autoimmune biomarkers.



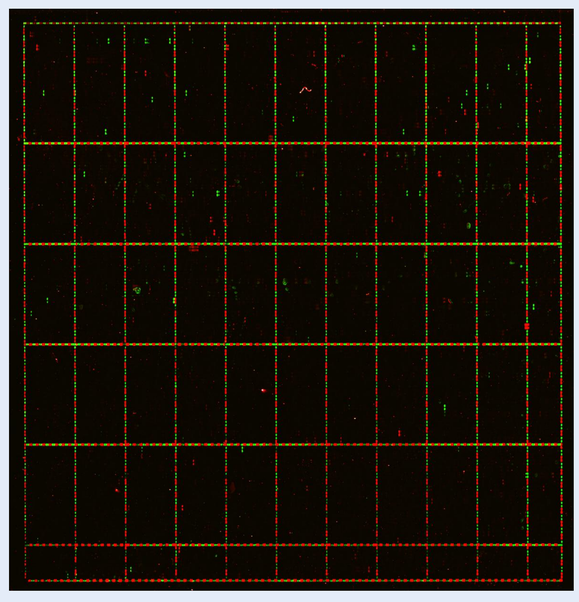
Patient Control



Large Scale PEPperCHIP® Discovery Arrays

Recent studies have shown that autoantibodies play a role in the pathogenesis of dilated cardiomyopathy (DCM). We wanted to identify new cardio vascular proteins that serve as targets for autoantibodies in DCM patients. To this end, we used a large scale PEPperCHIP® Discovery Array containing 26.364 different 15mer peptides. derived from 168 antigens associated with cardiovascular diseases. The arrays were incubated with pooled sera of 6 different patient groups vs. 2 pooled control sera.

We observed clearly differential antibody responses with all samples. All peptides above a pre-defined threshold were used for software-assisted epitope identification. These epitopes as well as the top 100 peptides of each patient group were used to identify active cardiovascular antigens for all samples that provide a basis for upcoming assays with a focus on a narrowed peptide and antigen collection.



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