High-Density Peptide Microarrays for Serum Biomarker Discovery

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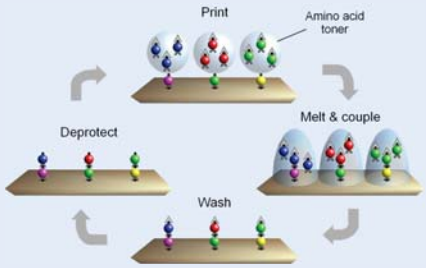
Background Technology



Combinatorial peptide synthesis with amino acid toner particles

PEPperPRINT's unique peptide microarray platform is based on a completely new approach for combinatorial synthesis: Instead of using e.g. standard spotting or lithographic technologies, we print amino acid toner particles with a custom 24-color laser printer. In less than a minute, a layer of a defined amino acid toner particle pattern is printed with micro resolution onto a coated glass slide (see sketch on left). After printing, the toner particles are melted by heating to release the embedded amino acids with high spatial resolution. The released amino acids can now couple to the functionalized glass slide or previously coupled amino acids. In accordance with solid phase Fmoc chemistry, a cycle of synthesis is completed by washing and cleavage of the N-terminal Fmoc protection group. This combinatorial cycle of synthesis is repeated until the intended peptide length of the microarray is reached. By routine double coupling steps, the coupling efficiency of the peptide laser printing process is close to 99%.

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

Peptide Laser Printer

The identification of early biomarkers for severe diseases by noninvasive methods is highly important but demanding. High-density peptide microarrays are a very efficient tool for antibody biomarker discovery from serum (see case studies below). For the first time, PEPperPRINT's platform technology enables the generation of high-density peptide microarrays with a unique content flexibility in the most cost-effective manner.

Infectious Disease Research

Diagnostic tests for infectious diseases are often based on the analysis of serum antibodies directed against pathogen antigens. PEPperPRINT developed solutions for infectious disease research that allow highly multiplexed screenings of pathogen-specific serum antibodies and the discovery of new prognostic markers on the epitope level.

Example: Identification of Zika virus epitopes in patient sera

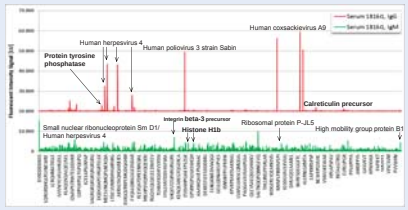
- Goal: Identification of Zika virus epitopes in patient sera, differentiation between patient groups
- Microarray setup: Zika virus genome polyprotein translated into 2,744 overlapping peptides
- Samples: Zika patient sera from Brazil (pregnancy, microcephaly, neurozika, dengue negative sera)
- Outcome: Common IgG epitopes (red, IgM in green) in dengue negative, pregnancy and microcephaly cases

Autoimmune Disease Research

Autoimmune diseases are based on immune responses against healthy cells and tissues of an individual, and result from pathogenic autoantibodies or self-reactive T-cells. We developed a number of autoimmune-related peptide microarrays to pinpoint and profile every possible autoantibody response throughout an entire patient population.

Example: PEPperCHIP® Autoimmune Epitope Microarray

- Goal: Identification of IgG and IgM autoantibody profiles in patient serum
- Microarray setup: 4,287 linear autoimmune epitopes of the Immune Epitope Database
- Samples: Serum of 56 year old female diabetes mellitus type 1 patient (1:500, 3 µl serum)
- Outcome: IgG response against diabetes protein tyrosine phosphatase, IgG and IgM responses against Sjögren's syndrome epitopes



The PEPperCHIP® Autoimmune Epitope Microarray was assayed with human serum 181641 (1:500) followed by staining with the secondary anti-IgG (red) and anti-IgM (green) antibodies and data read-out with a microarray scanner. Data quantification resulted in intensity plots for the IgG (red) and IgM (green) antibody profiles. For a better data overview, the baselines of the intensity plots were leveled.

Microarray Scan

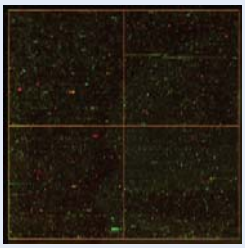
Bridging infectious and autoimmune diseases: The Human Epitome Microarray

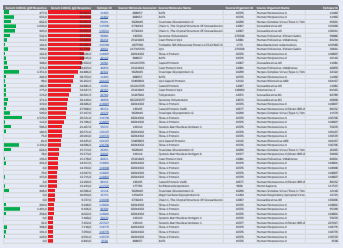
For the very first time, the new PEPperCHIP® Human Epitome Microarray covers all linear human B-cell epitopes of the Immune Epitope Database. The 28,895 different peptides of the PEPperCHIP® Human Epitome Microarray are based on 23,163 database epitopes of 2,542 different proteins and 468 different organisms related to infectious diseases, autoimmune diseases, allergies, cancer and vaccines.

Applications:

- Epitome-wide antibody screening
- Differentiation between IgG and IgM or IgA response profiles
- High-throughput serum biomarker discovery for autoimmune and infectious diseases as well as cancer
- Identification of prognostic epitopes for IVD and Cdx development
- Patient stratification
- Investigation of links between autoimmune and infectious diseases

Case study: IgG and IgM screening with clinical serum of SLE patient



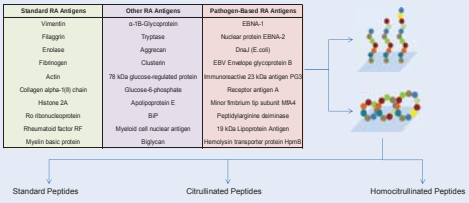


The PEPperCHIP® Human Epitome Microarray was assayed with the serum of a patient with SLE followed by IgG and IgM read-out. The top IgG responses were directed against epitopes of EBV, polio virus and herpes simplex virus. The strongest IgG response was against an autoantigen was directed against Ro ribonucleoprotein , a known antigen for SLE and Sjögren's syndrome. Moreover, we observed a number of IgM responses against calreticulin epitopes that are also correlated with SLE and coeliac disease.

Peptide Microarrays for CDx development: The SeroRA Library

- Application: Patient stratification, serum-based IVD and CDx development for rheumatoid arthritis
- Library setup: >100,000 linear and cyclic constrained peptides with citrulline and homocitrulline variants
- Antigen coverage: Epitope-wide coverage of standard autoantigens like Vimentin, Fillagrin, Enolase or Fibrinogen, new autoantigens (BiP, α-1B-Glycoprotein) and pathogen-derived antigens like EBNA-1 or DnaJ

Standard RA Antigens	Other RA Antigens	Pathogen-Based RA Antigens
Vimentin	α-1B-Glycoprotein	EBNA-1
Filaggrin	Tryptase	Nuclear protein EBNA-2
Enolase	Aggrecan	DnaJ (E.coli)
Fibrinogen	Clusterin	EBV Envelope glycoprotein B
Actin	78 kDa glucose-regulated protein	Immunoreactive 23 kDa antigen P23
Collagen alpha-1(II) chain	Glucose-6-phosphate	Receptor antigen A
Histone 2A	Apolipoprotein E	Minor fimbrium tip subunit MR/4
Ro ribonucleoprotein	BiP	Peptidylarginine deiminase
Rheumatoid factor RF	Myeloid cell nuclear antigen	19 kDa Lipoprotein Antigen
Myelin basic protein	Biglycan	Hemolysin transporter protein HpmB



- Largest RA peptide library based on hundreds of antigens
- First comprehensive library with homocitrulline
- More than 100,000 linear and cyclic constrained peptides
- Higher epitope specificity than protein-based assays
- Closes gap for anti-CCP, anti-MCV and RF negative RA

Summary

The SeroRA Library is the world's biggest library for the isotype-specific analysis of antibody responses in RA patients. It is based on more than 100,000 peptides from hundreds of autoantigens and pathogen antigens and includes not only the native peptides, but also the corresponding citrullinated and homo-citrullinated peptides in linear and cyclic constrained form. The SeroRA library enables the identification of new prognostic RA peptide markers or marker combinations with higher sensitivity than standard tests for IVD and CDx development.

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