Production of high-density peptide arrays

Frank Breitling and Alexander Nesterov-Müller from the Karlsruhe Institute of Technology (KIT) are working on the development of a second-generation peptide laser printer and a peptide chip printer based on computer chips. This development will enable the low-cost production of high-density peptide arrays consisting of up to one million peptides as well as opening up completely new areas of application.

Peptides consist of chains of amino acids, which can be synthesised on chips in a similar way to the oligonucleotide microarrays that have revolutionised genomics research. Such peptide arrays are of great importance for diagnostics and drug discovery in the biotechnology and pharmaceutical industries as well as for basic research, in particular in the fields of immunology and proteomics. The cost of 1€ per peptide for the production of peptides is still extraordinarily high. In order to improve the cost efficiency of array printing, the Chip-based Peptide Libraries workgroup at the German Cancer Research Center (DKFZ) in Heidelberg has developed a laser printing method for the production of peptide chips. The method has been awarded numerous prizes and has also led to the establishment of the company PEPperPRINT GmbH which offers arrays at around 13 cents per peptide (figures as of 2010, see BIOPRO article "PEPperPRINT wins award for its peptide chips" published on 6th August 2009). The novelty of this printing method is based on the application of amino acids, which are the building blocks of peptides, not as a solution, but as solid, electrically chargeable particles like those used for toner powder in electrophotography or xerography.

PD Dr. Frank Breitling

Dr. Frank Breitling and Dr. Alexander Nesterov-Müller, who are based at the Karlsruhe Institute of Technology (KIT), are working on the development of novel chip printers for the inexpensive production of high-density peptide arrays consisting of between 500,000 and one million arbitrary peptides. These chip printers have the potential to be used in a broad range of new areas of application.

What are arrays consisting of several thousand peptides used for? There is a huge variety of combinations of peptides that can theoretically be printed on such arrays. From the 20 amino acids that naturally occur in proteins, it is possible to produce 20^{10}, i.e. around 10 trillions (10^{13}) of different decameric peptides (chains of 10 amino acids). Although this theoretical number is limited by the amino acids' physical and chemical properties, the number of decameric peptides is nevertheless unimaginably high.

PD Dr. Alexander Nesterov-Müller
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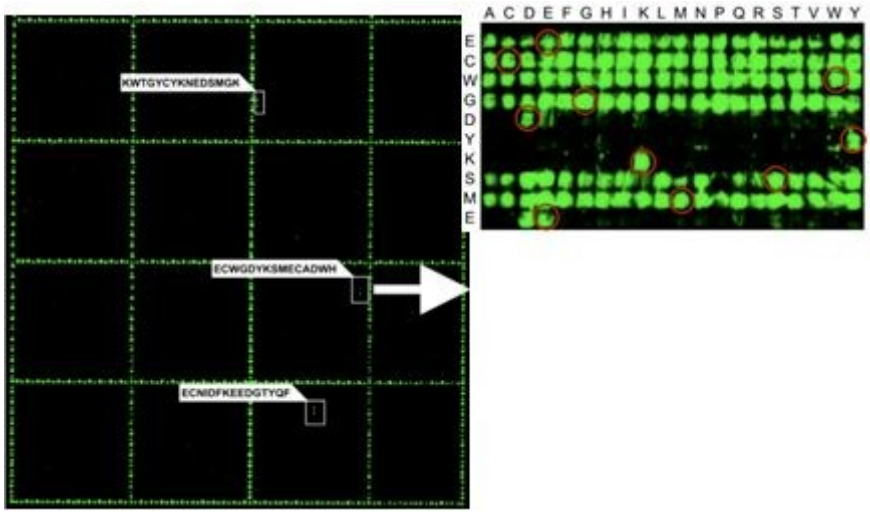
Thanks to the copies that it makes of gene segments, different recombination mechanisms and somatic hypermutation, the human immune system has also created the conditions for the selection of a huge number of possible antibodies (and hence peptide patterns). Immunological research is one of the fields where the new generation of high-density peptide arrays can be applied. Therefore, the majority of projects listed on the "Peptide Arrays and Antibody Libraries" research group website at the Institute of Microstructure Technology (IMT) at the Karlsruhe Institute of Technology involve the application of this innovative technology for elucidating immunological issues.

The PEPLASER



Array with 10,000 arbitrarily selected peptide double spots stained with a Flag M2 antibody. The insert shows a highly efficient peptide binder with the NNNNDYKNND/E binding motive detected in two screening rounds.
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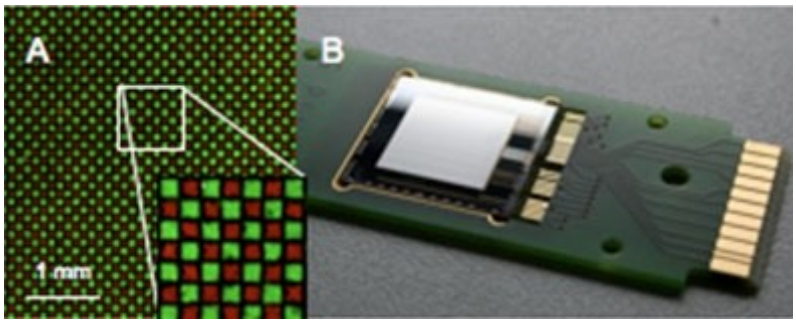
The researchers from Karlsruhe are pursuing two different strategies to produce chips with a high peptide density. The first strategy, i.e. the development of a second-generation peptide laser printer, is part of the PEPLASER project funded under the 7th EU Framework Programme. This cooperative project, which is coordinated by the Karlsruhe Institute of Technology, brings together scientific and industrial partners from numerous European countries. The engineering part of the project is being carried out by the company KMS Automation GMBH headquartered in the city of Schramberg-Waldmössingen in Baden-Württemberg.

This device is expected to make it possible to increase the number of individual peptide spots performed from 0.5 million to over 10 million per month. The Heidelberg-based company PEPperPRINT GmbH will commercialise the novel particle-based peptide arrays and the company AKAtech GmbH will market the new laser scanner. In addition, in order to create a market for high complexity peptide arrays, the researchers will also work on paradigmatic scientific applications. One of these applications, which is being worked on by Dr. Breitling and his team at the IMT, aims at enabling a comprehensive readout of the different antibodies (in concentrations > 5 µg/ml) that patrol the serum of malaria patients. The goal is to pinpoint which antibodies and binding peptides correlate with malaria and have the potential to mediate protection against the disease. The researchers have already been able to show that the method works.

Chip printer



Combinatorial synthesis of a peptide array with a computer chip. Left: Pixel electrodes revealing a staining pattern caused by two different antibodies; right: plate with bonded chip.

In addition to the PEPLASER project, which is based on a laser printing method developed at the DKFZ and PEPperPRINT, the second project focuses on the development of a chip printer that enables the synthesis of peptide arrays using a computer chip. The project is being coordinated by Dr. Nesterov-Müller who did his doctorate at the University of Heidelberg on the "Targeted deposition of amino acid particles for the combinatorial synthesis of peptide arrays on a chip". This chip printer uses a CMOS chip (complementary metal oxide semiconductor chip) whose surface is covered with an array of pixel electrodes used to address the amino acid particles and subsequent combinatorial peptide synthesis.

This chip-based method has already been used to immobilise 40,000 peptides per square centimetre. In cooperation with Prof. Michael Hausmann at the Kirchhoff Institute of Physics at the University of Heidelberg and the Institute of Printing Machines and Printing Methods at the University of Darmstad led by Prof. Dr. Edgar Dörsam, Nesterov-Müller's team is working on the development of a chip printer for the routine production of high-density peptide arrays. The cooperative project aims to make it possible to synthesise up to one million high-quality, low-cost arbitrary peptides on a glass

slide. Dr. Nesterov-Müller's project entitled "Arrays of protein-like molecules with the help of magnetic particles" won the "Molecular Bionics" category in the Biotechnology and Medical Technology competition run by the Baden-Württemberg Ministry of Science, Research and the Arts in January 2011.

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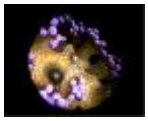
Article

02-May-2011
EJ
BioRN

The article is part of the following dossiers



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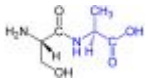

Peptides – diverse molecules of life