Combinatorial synthesis of peptide arrays with a high resolution on a computer chip

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A novel combinatorial method of synthesizing high resolution peptide libraries based on Merrifield solid phase chemistry has been proposed. In contrary to the standard Merrifield synthesis, the amino acids are incorporated into solid particles. The surface of the computer chip consists of the electrode matrix. The high voltage up to 100 V can be applied to each electrode independently. The switched electrodes attract the triboelectrically charged particles from the aerosol. Using different voltage patterns and different aerosols (20 for each biogenic amino acid), one can realize the addressed combinatorial particle deposition. Amino acids are mobilised simply by heating the particles, which enables the activated amino acids to couple to the support. Repeated coupling cycles (according to Merrifield) with washing and deprotection steps result into the combinatorial synthesis of a peptide array. This method allows for a significant improvement in the state of the art in this field. The peptide synthesis with a spot density up to 40,000 spots/cm² was demonstrated.

These arrays could be used e.g. to screen for differential immune responses towards a pathogen linked to infectious disease or cancer.

**Amino Acid Particles**

<table>
<thead>
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<th>I – Advantages</th>
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<td>1. 10 µm, 0.1 µl</td>
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Solid, “amino acid particles” on the chip (Fig. 1). 40,000 spots/cm². Amino acids are incorporated in a solid matrix Chip by lyophilisation. Heating the particles (melting temperature ~ 70 °C) and crystalization. Amino acids are mobilised by heating the particles and melting of solid matrix.

**II – Generation of particles**

The particle matrix comprises of:

- activated amino acids (Free-a-OpII as reactive component, Fig. 2)
- a polymer (e.g. polyethylene for triboelectric charging and charge storage)
- charge control agents, charge stabilizers and pigments
- a so-called solid matrix (DPF as a higher homologue of DMF, mp~70 °C)

To generate the target particle batch:

A basic particle batch made of the single components is coarse milled after drying and crystallisation. Particle sizes range from 1 to 50 µm are received by use of an air-jet mill (Fig. 3) to narrow the size distribution. Different sieves are utilized.

**Combinatorial Peptide Synthesis on a Chip with Amino Acid Particles**

**I – Chip on the Chip-Board**

Free chip surface for particle deposition from aerosol. The surface of the computer chip consists of the electrode matrix (Fig. 1). The voltage can be applied to each pixel electrode separately.

**II – Combinatorial principle**

The switched electrodes attract the triboelectrically charged particles from the aerosol. Using different voltage patterns and different aerosols (20 for each biogenic amino acid), one can realize the addressed combinatorial particle deposition. Amino acids are mobilised simply by heating the particles, which enables the activated amino acids to couple to the support. Repeated coupling cycles (according to Merrifield) with washing and deprotection steps result into the combinatorial synthesis of a peptide array (Fig. 2).

**Application of Peptide Libraries**

Existing protein and peptide arrays are very expensive, and technical difficulties confine their complexity to a maximum of a few thousand peptides or proteins. Peptide arrays of higher complexity should open a wide field of applications, e.g. efficient screening of patient sera for pathogen-specific antibodies.

For example, a typical bacterial pathogen could be represented by an array comprising some 150,000 overlapping peptides. Arrays of this size could help to build arrays of diagnostic relevance enabling accurate prognosis of fine-tuned diagnosis. With peptide arrays that represent human proteins we might find antibodies typical for acute immune diseases, or auto-antibodies that went of a growing tumour.

**Proof of Principle**

**I – Particle deposition from aerosol**

A chip-board particle pattern on the microelectronic chip (Fig. 1) presented me means of two aerosol chambers. Aerosol chambers (Fig. 3) have several functions: destruction of particle complements, triboelectric charging of particles and their transport into the chip.

**II – Screening of peptides synthesized on the chip**

40,000 spots/cm²

Synthesis and staining of FLAG and HA epitopes (Science 2007, 318, 1888)

**40,000 human proteins**

4,000,000 peptides

With arrays representing human protein sequences we might find auto-antibodies in patients with diseases like Parkinson or we could find antibodies that might even of a growing tumour.