Chip printer

YUN CHIEN CHENG[1], FELIX LÖFFLER[3], KAI KÖNIG[3], ALEXANDER NESTEROV[2]*, EDGAR DÖRSAM[1]*, FRANK BREITLING[2]*
[1]Institute of Printing Science and Technology
Darmstadt University of Technology
Magdalenenstr. 2; 64289 Darmstadt
[2]Institute for Microstructure Technology
Karlsruhe Institute of Technology
Hermann-von-Helmholtz-Platz 1; 76344 Eggenstein-Leopoldshafen
[3]Department of chip based peptide libraries
German Cancer Research Center
INF580; 69120 Heidelberg
GERMANY
Frank.Breitling@KIT.edu, Alexander.Nesterov@KIT.edu, doersam@idd.tu-darmstadt.de
www.fzk.de/fzk/idcplg?IdcService=FZK&node=0046&document=ID_007703&lang=en; www.idd.tu-darmstadt.de/idd/team/leitung/doersam/doersam.de.jsp
* these authors contributed equally

Abstract: - Lithographic methods allow for the combinatorial synthesis of >50.000 oligonucleotides per cm^2, which revolutionized the field of genomics. High density peptide arrays promise to advance the field of proteomics in a similar way, but until recently were not available. This is mainly due to the monomer-by-monomer repeated consecutive coupling of 20 different amino acids associated with lithography, which adds up to an excessive number of coupling cycles. A combinatorial synthesis based on electrically charged solid amino acid particles resolves this problem. Here we report the manufacturing of a "chip printer" that could be used to consecutively address the different charged particles to a solid support, where, when completed, the whole layer of solid amino acid particles is melted at once. This frees hitherto immobilized amino acids to couple all 20 different amino acids in one single coupling reaction to the support. The method should allow for the synthesis of very high-density peptide arrays.

Key-Words: - chip printer, peptide array, chip, combinatorial synthesis

1 Introduction

The Nobel prize awarded solid phase peptide synthesis was invented more than forty years ago, when Merrifield consecutively coupled amino acid monomers to a growing peptide chain immobilized on a solid support [1]. His approach allowed to incubate the growing oligomer chain with excess monomers, which drives the coupling reaction near completeness, and, thereby, results into repetitive coupling yields that routinely exceed 95% during peptide synthesis. Since the growing peptide products remain tethered to the support throughout synthesis, all the non-reacted monomers simply are washed away. These two basic principles are the prerequisite for the cost efficient synthesis of peptides. In order to manufacture many different peptides in parallel, Ronald Frank parallelized the Merrifield synthesis by adding not one amino acid to the support, but instead patterning the 20 different activated amino acid derivatives as small droplets on a flat two dimensional surface. There, they react with the solid support, with each droplet defining a small reaction sphere. Consecutively printed layers result in the parallel growth of many different peptide chains, whereby the number of different oligomers is only dependent on the achievable miniaturization of individual spots [2].

2 Problem Formulation

Ronald Frank's SPOT synthesis still dominates the field because over the years it earned a reputation of reliability and wide applicability. High peptide densities of *in situ* synthesized peptides that exceed 25 peptides per cm^2, however, are difficult to obtain with this method, mainly due to the difficult handling of tiny droplets that tend to evaporate or spread over the array's surface.

Another method that is widely used for the combinatorial synthesis of oligomer arrays is the lithographic synthesis. It is successfully used for the synthesis of very high-density oligonucleotide arrays, with a density that today exceeds 100.000 oligonucleotides per cm^2 [3]. However, this method has a peptide-specific drawback as shown in Fig. 1 [4]. First, a lithographic mask defines very small areas to be irradiated by light (Fig. 1a). Irradiation removes a photolabile transient protection group at the end of

oligomer chains, but only in those areas selected by the lithographic mask (Fig. 1b). The whole array then is incubated with a solution of activated monomer that reacts with the deprotected oilgomer (Fig. 1c), and non-reacted monomer is washed away (Fig. 1d). This whole process is repeated until one whole layer of monomers has been added to the array, i.e. further areas are defined with other lithographic masks, further monomers couple to deprotected oligomers, and eventually all the different monomers have been coupled to the support to add one layer of monomers to the growing oligomer chain. Then, the whole procedure starts again until finally, e.g. 10 layers of monomers have been coupled to generate an array of 10meric oligomers.

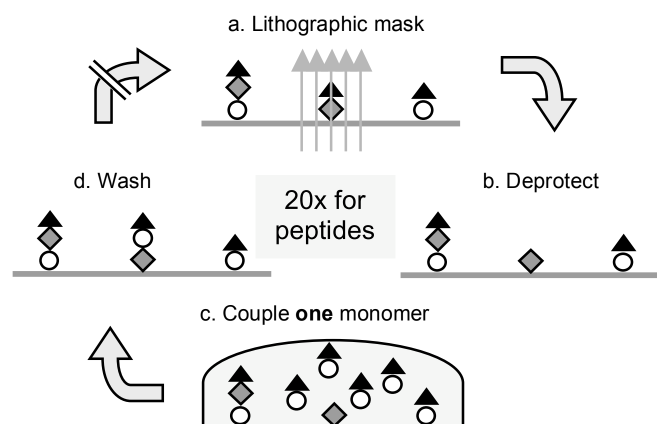

Fig. 1 Lithographic synthesis method

The consecutive addition of monomers intrinsic to the lithographic procedure results in many more coupling cycles when compared to the printing methods, where all the monomers of one layer are added in parallel (400 *vs.* 20 coupling cycles for an array of 20meric peptides). This drawback is acceptable for oligonucleotide synthesis with its 4 different monomers, but it severely reduces the quality of synthesized peptides with its 20 different amino acid monomers.

3 Problem Solution

Recently we developed a method for the combinatorial synthesis of peptide arrays that is based on 20 different solid amino acid particles [5]. In a normal laser printer small solid toner particles are triboelectrically charged (by mild friction), e.g. by grinding the particles against a rubber foam drum inside the toner cartridge. Depending on the material, this procedure strongly charges the toner particles, which for state of the art toner particles comes close to electrical breakdown in air [6]. Due to these charges toner particles can be moved within electrical fields, whereby, e.g. the electrical fields of individual pixel electrodes of a computer chip direct amino acid particles to very small synthesis areas (Fig. 2a). By simply switching the voltage to other pixel patterns (Fig. 2b), different amino acids embedded into particles are consecutively added to the chip's surface until, finally, the whole layer of amino acids is melted to start the coupling reaction. This procedure achieves a similar miniaturization of discrete synthesis sites, but it avoids the many consecutive coupling steps intrinsic to lithographic synthesis. Moreover, the very reactive amino acid derivatives are nearly perfectly shielded from decay inside solid particles until the coupling reaction is induced by melting the particles, which is another reason for the high-quality peptide arrays delivered by this method [6]. Currently, the miniaturization achieved by the particle-based combinatorial synthesis method stands at 40.000 peptides per cm^2 (Fig. 2) [7].

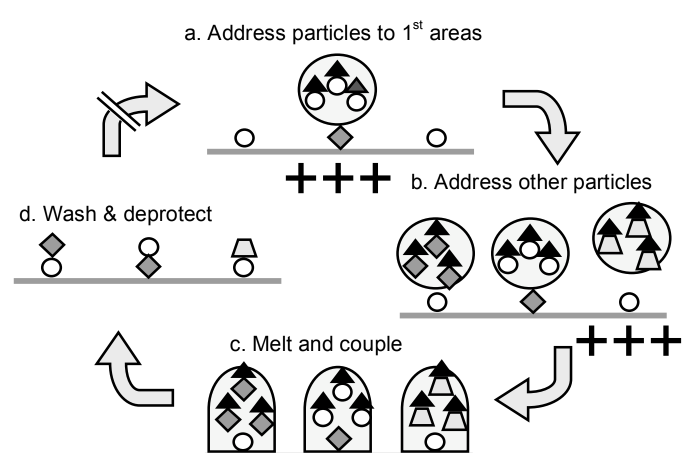

Fig. 2 Particle based synthesis method

In this publication, we describe a variant procedure of our particle based synthesis method that should add even more flexibility to the method, and, in addition, saves costs. We constructed a "chip printer" (Figs. 3 & 4) that picks up particles to defined pixel areas, and, in addition, "prints" these amino acid particles in high resolution to a support that is suitable for peptide synthesis [8].

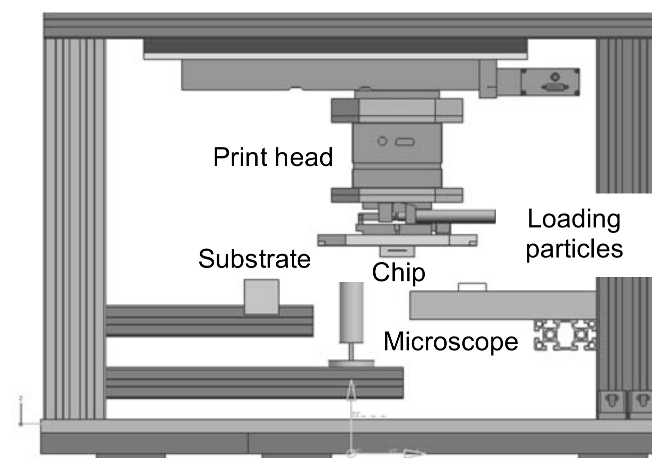

Fig. 3 Chip printer, schematic drawing

In order to closely and parallel align the chip's pixels with the substrate, where synthesis of peptides ensues after the printing of particles, we mounted the chip on a print head that is adjustable in x, y, and z directions (±0.1µm; ±5µrad).

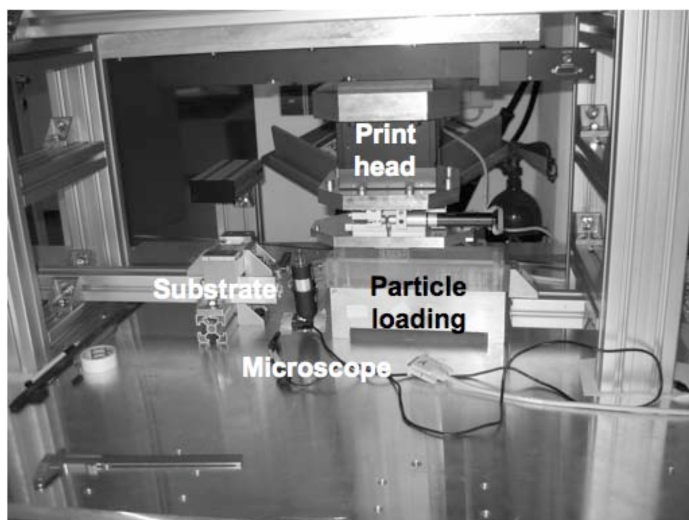

Fig. 4 Chip printer

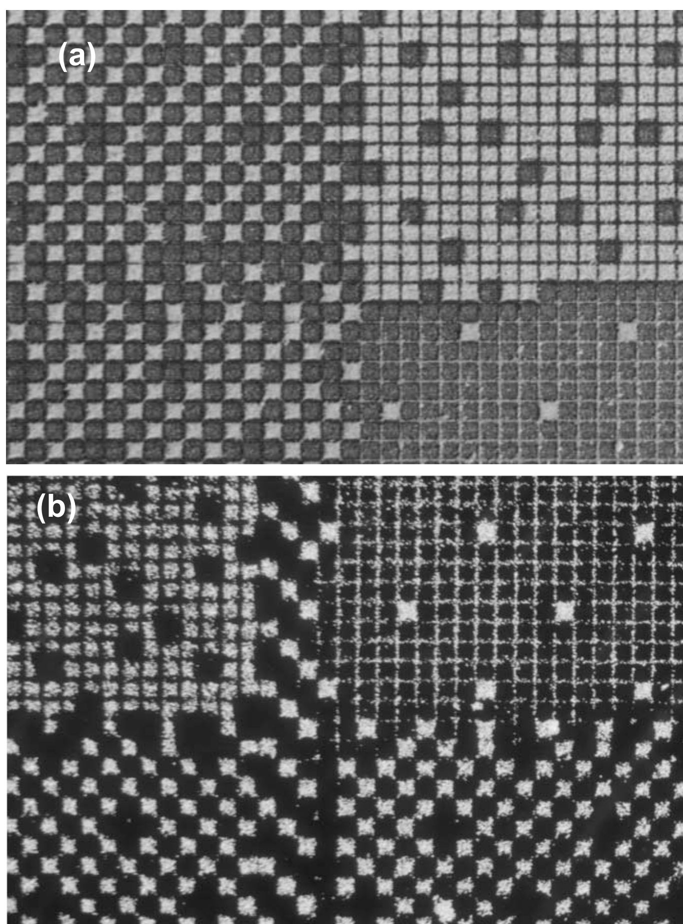

Fig. 5 Amino acid particles selectively deposited on a chip (a); amino acid particles printed to a glass slide (b)

The chip mounted on the print head was then moved in x-direction to pick up particles on selected pixels that were switched on voltage (Fig. 5a). Then, the chip was moved to the "substrate position" and the particles were printed by a strong external electrical field (1kV per mm) due to additional positive voltage applied to the opposite side of the substrate (Fig. 5b).

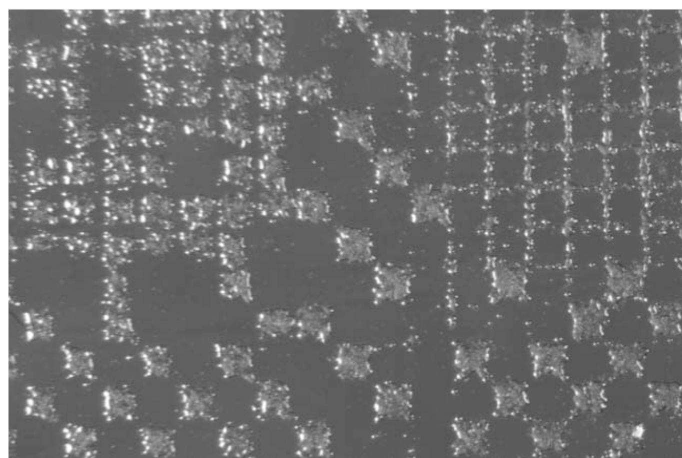

Fig. 6 Melted amino acid particles

Finally, we melted the particles (Fig. 6) to induce the coupling reaction to free amino groups on the PEG grafted glass slide [8], blocked non-reacted free amino groups with a large excess of acetic anhydride, removed the Fmoc protecting group, and stained the surface coupled amino acids with bromophenole blue (Fig. 7).

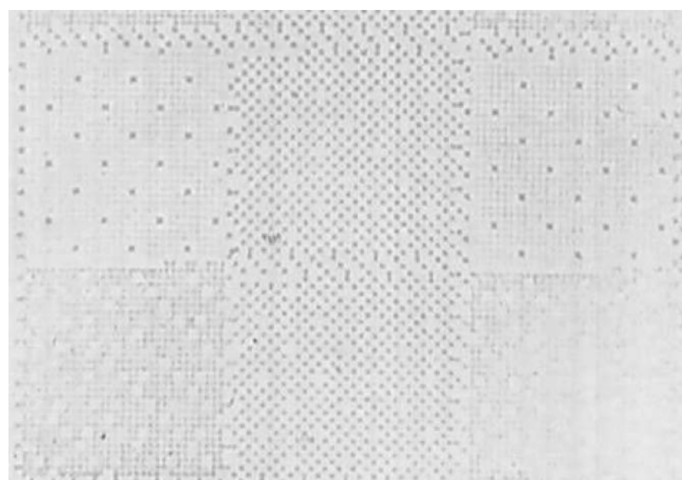

Fig. 7 Amino acids coupled to selected areas on the glass slide

4 Conclusion

Particle-based synthesis introduces a novel concept into combinatorial synthesis: a reactive monomer that is "canned" into particles and sent as postal packages to different addresses where the monomer is freed by melting. The resulting oily droplets don't creep on the surface, which allows for densely spaced reaction spheres. Thereby, the combinatorial synthesis of very high density peptide arrays is feasible. The solid particle

matrix shields reactive chemicals from each other and from their environment, and thus contributes robustness, long term storage, and easy handling.
The chip printer, which we report here, adds flexibility and saves costs. Within the next months, we will assemble the machine depicted in Fig. 4 together with a particle deposition unit, and add an automated quality control system that reads out the particle pattern on the chip's surface.
These features together with the reduced number of coupling cycles intrinsic to all printing methods should bring affordable high density peptide arrays into laboratories. These might have a similar impact as high density oligonucleotide arrays had: a panel of peptides that comprehensively diagnoses a patient's or even a population's antibody response should bring clues to hitherto enigmatic diseases; and an easy screen for (better) binding molecules might have a big impact on novel therapeutics. Beyond the life sciences, a screen for peptidic semiconductors or catalysts might be feasible.

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