Combinatorial Synthesis of Peptide Arrays onto a Microchip

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High-complexity oligonucleotide arrays are combinatorially synthesized by lithographic methods (*1*), localized electrolysis (*2*), or electrophoretic transport of the four different nucleotides (*3*). In all these methods, each of the monomers is coupled layer by layer consecutively to the solid support. Therefore, they all depend on an excessive number of coupling cycles to generate a peptide array from the 20 different amino acid monomers, which explains why peptide arrays lag behind nucleotide arrays in terms of complexity (*4*).

In order to upgrade peptide array density over currently available 22 peptides per cm^2 (*5*) and to avoid an excessive number of coupling cycles, we manufactured 20 different kinds of chargeable amino acid particles that are guided step by step onto a microchip surface by electric field patterns from individual pixel electrodes (Fig. 1, A and E). Because the solid particle matrix effectively "freezes" the activated amino acid derivatives, coupling reaction ensues only when finally a completed layer of all 20 different kinds of amino acid particles is melted at once (Fig. 1, C and F). This releases activated amino acids to diffuse to free amino groups incorporated into the chip's coating (*6*). Thereby, only nine repeated coupling cycles resulted into an array of nonameric peptides, with the density only restrained by the sizes of particles and pixel electrodes (Fig. 1, D and G).

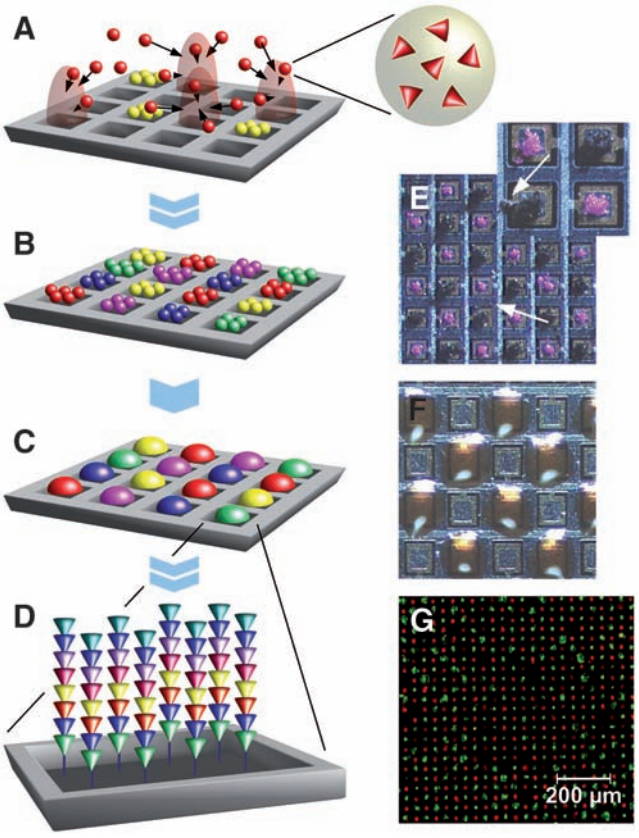

Fig. 1. Particle-based synthesis of peptide arrays. Activated amino acids are embedded within particles that are addressed onto a chip by electrical fields generated by individual pixel electrodes (**A**). A whole layer of consecutively addressed amino acid particles (**B**) is melted at once to induce coupling (**C**). Repetitive cycles generate a peptide array (**D**). Consecutively deposited, unmelted particles stick to the surface because of strong adhesion forces. Arrows point to wrongly deposited particles (**E**). Melted particles delimit individual coupling areas. For better visualization, pixel areas are overloaded (**F**). Particle-based in situ synthesis of chessboard-arranged FLAG (green) and HA epitopes (red) with a density of 40,000 cm^{-2}. Peptides were stained with rabbit antibodies against FLAG and monoclonal mouse antibodies against HA (**G**).

When we consecutively addressed different kinds of commercial color toner particles to microchips manufactured by a standard lithographic process, few wrongly deposited particles were observed (Fig. 1E, arrows), which was also true for our amino acid particles. Strong adhesion forces keep unmelted microparticles sticking to defined addresses even when the pattern of pixels switched on voltage is changed. The amino acid particles mainly comprise OPfp (pentafluorophenyl) esters of Fmoc (9-fluorenylmethoxycarbonyl)-protected amino acids and a higher homolog of standard solvents, for example, the solid diphenyl formamide, which adds the trait of an oily solvent that forms spatially confined reaction cavities when melted (Fig. 1F).

When we compared our particle-based method to standard Merrifield synthesis, we found similar yields of synthesized peptides, no conversion of L to D form amino acids during synthesis, and a rather surprising stability of Fmoc–amino acid–OPfp esters immobilized inside particles (fig. S1). We observed a negligible decay rate of <1% Fmoc–amino acid–OPfp ester per month at room temperature with 19 amino acid particles analyzed, except for Fmoc-Arg-OPfp, with a corresponding decay rate of 5%.

Next, we synthesized an array of peptides Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala [hemaglutinin (HA)] and Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (FLAG) onto the microchip's surface. Peptides were differently labeled with FLAG-and HA-specific antibodies, which revealed an epitope-specific staining pattern with a density of 40,000 peptide spots per cm^2 (Fig. 1G).

In contrast to other methods, the particle-based approach renders the delivery of monomers to individual pixels completely independent from the coupling reaction; that is, we reduced the number of coupling cycles to one per layer. In addition, the independence of particle production, storage, deposition, and coupling reaction allows for rigorous quality control of individual steps. Our method should be especially helpful in the field of proteomics because it allows for the translation of whole proteomes into arrays of overlapping peptides. Such high complexity peptide arrays could be used in diagnosis and biomedical research, for example, to scan the humoral immune response toward a pathogen's proteome.

References and Notes

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Materials and Methods
Figs. S1 and S2
References

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