High Throughput Systems for Peptide Synthesis & Protein Identification

Yoon-Sik Lee

School of Chemical & Biological Engineering, Seoul National University
(e-mail : yslee@snu.ac.kr)

The demand for developing a rapid and effective method for the peptide array synthesis and protein identification on a chip is increasing. We have developed two methods for peptide synthesis on a glass chip surface, spotting method and maskless photolithography method. For effective spot synthesis on a glass surface, we have made spot arrays on a glass surface through patterning with photo-resist and subsequent perfluorination. Then, amino groups were introduced with various silane compounds and polymers on the spots. Firstly, we measured the coupling time and isokinetic ratio of each amino acid on the glass surface by fluorescence labeling, identified the optimal surface conditions, and confirmed the effect of surface grafting on the couplings of each amino acid. Even more, we could identify the effect of spacer length on the interaction between streptavidin and surface-immobilized biotin (Fig. 1).

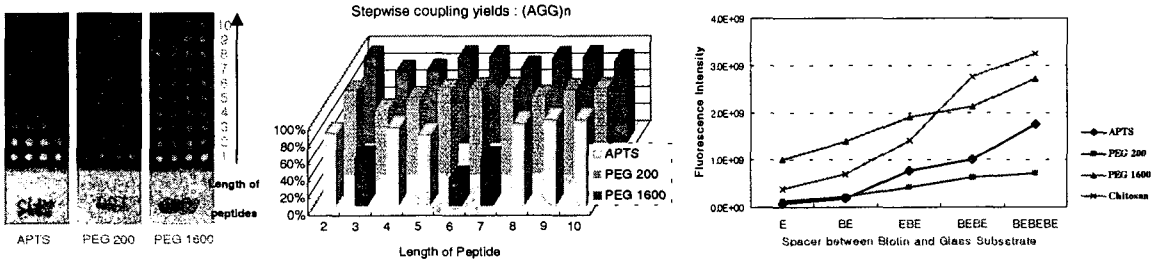

Fig. 1. Stepwise coupling yields *vs.* spacer length on variously modified glass slides.

We can also synthesize various peptides on the glass chip using NVOC-protected amino acid by maskless photolithography method. The NVOC groups on the glass surface were site-specifically cleaved by a UV illuminator (360 nm) using micromirror array system. After removing the NVOC group, we coupled NVOC amino acids successively and synthesized a series of pentapeptides on the chip. After incubating the peptide array chip with Cy3-streptavidin, we obtained different intensities of fluorescence images, which correspond to the binding activities of the peptides to the protein (Fig. 2).

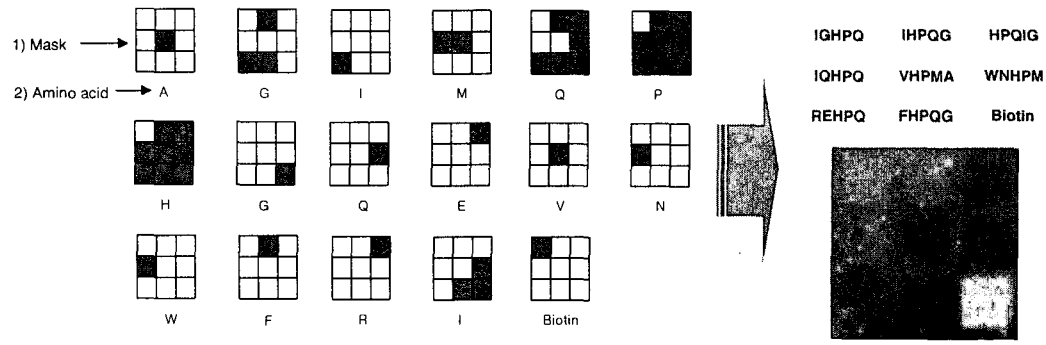

Fig. 2. Scheme of peptide array synthesis on a chip & binding assay of Cy3-streptavidin

As a lab-on-a-chip (LOC) system for protein identification, we have integrated a bead affinity chromatography system, which is based on the photolytic elution method, into a glass-silicon microchip to purify specific target proteins (Fig. 3). To demonstrate the feasibility of this system as a diagnosis chip, RNA Polymerase of Hepatitis C virus (HCV) was selectively captured from patient serum, eluted by UV light exposure, and then analyzed by MALDI-TOF MS.

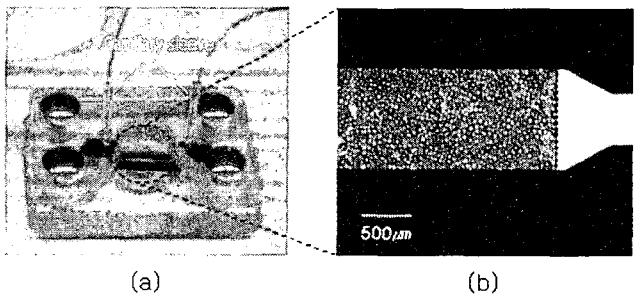

Fig. 3. Micro-affinity purification system: (a) microchip; (b) packed beads in the microchamber.

As another method for massive protein identification, the surface enhanced Raman scattering (SERS) spectroscopic encoded beads were prepared by introducing silver nanoparticles with tagging compounds for high throughput screening. To prepare the encoded beads, ethylene diamine was coupled to the monodispersed carboxyl beads to introduce amine groups, which have strong affinity with the silver nanoparticles, and then Fmoc-Lys(Fmoc)-OH was coupled to the amine groups to amplify the functional groups. Single silver nanoparticles were self-aggregated at the free amine groups of lysine on the bead surface, and the tagging chemical compounds such as 1,2-mercaptotoluene, 1,4-mercaptotoluene and 4-mercaptopyridine were introduced to the silver nanoparticle self-aggregates. We found that the beads were effectively encoded with silver nanoparticles containing the tagging compounds, and that any ligands on the beads can be decoded by SERS.

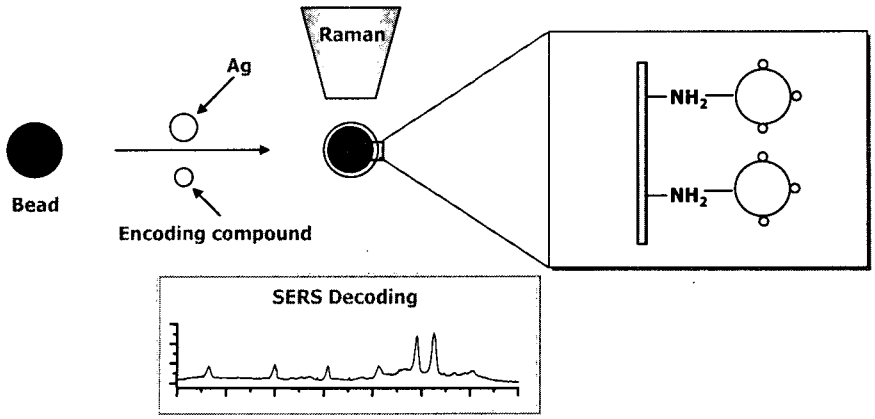

Fig. 4. Preparation of surface enhanced Raman scattering spectroscopic encoded beads for massive protein identification.