Nano-scale Biochip for Rapid Separation and Mass Analysis of Protein-Peptide Mixture

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The Cellular Informatics Team has developed, in cooperation with NEC Corporation, the basic technology for the "high-throughput virtual 2D mapping system of proteins" using nano-scale biochip and mass spectrometer. This technique can be effective to the rapid detection of proteins and peptides characteristic to diseases such as cancer, etc., and is applicable to prompt diagnosis. This research was supported by the NEDO's "Project for Developing Biotechnology IT Integration Equipment", which is a part of the ongoing Focus 21 project under the initiative of the METI (Ministry of Economy, Trade and Industry).

This successfully developed system consists of an isoelectric focusing chip that separates proteins and peptides, and a MALDI-TOF mass spectrometer. The system operates as follows:

1) A protein-peptide mixture flows through the on-chip channels, and components are rapidly separated, or fractionated, by isoelectric focusing.
2) The fractionated sample is fixed on the chip by drying.
3) The whole chip is introduced into the mass spectrometer and the on-chip channel is directly laser irradiated.
4) A sample ionized by laser irradiation is introduced directly into the mass spectrometer for detection of the fractionated proteins and peptides and for profiling disease-specific proteins/peptides.

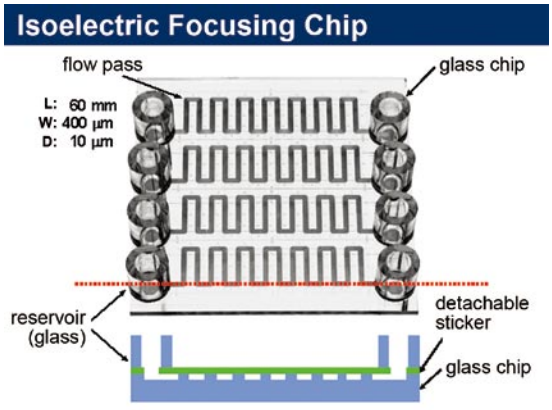
Direct sample introduction eliminates the need to transfer fractionated samples to separate vessels, preventing the loss of precious protein and peptide contained in analyte solution and thus enabling rapid and exhaustive analysis, as well as substantially reducing the analysis time and the number of work operations. The whole process, from introduction of the sample to the chip to completion of mass analysis, requires only approx. 70 minutes. The combination of two separation methods, e.g. isoelectric point (pI) separation and mass-to-charge (m/z) separation using MS, provides essentially the same effect as a virtual 2-dimensional separation.

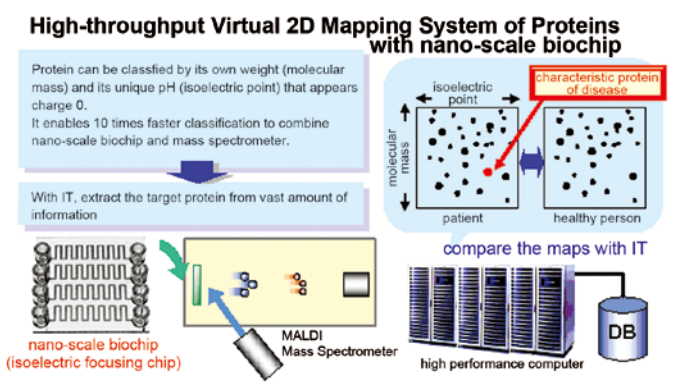
With the human genome sequence determined, proteome analysis has become the focus of attention as a promising post-genome analysis, and it has been the active target of much ongoing research. The target of proteome analysis, analyzing proteins that are built from genome information, is expected to have fundamental applications in genome drug discovery and tailor-made medical treatment. It is well known that the proteins inside tissues are different in each individual and organ, and that protein expression can vary significantly if an individual contracts a disease.

Proteome analysis is aimed at analyzing a varied group of proteins in organs and tissues. The first step of the analysis is separating the components of a protein mixture extracted from tissues and cells. Conventionally, a separation method, typically 2D-electrophoresis, is used to separate each protein from the mixture, and the separated protein spot is then physically cut out of the gel matrix. This is followed by enzymatic digestion and mass analysis for protein identification. Conventional methods, however, have the disadvantages of requiring long analysis times and large amounts of analyte. The new method we present here offers the promise of rapid analyses requiring only a tiny quantity of analyte, thus eliminating multiple disadvantages associated with conventional methods.

Reference

*1 Fujita, M., *et al*.: "High-throughput and High-resolution Two-dimensional Mapping of pI and m/z Using Microchip and MALDI-TOFMS", *International Symposium on MicroScale Bioseparations*, New Orleans (2005).





(The original figures: courtesy of NEC Corporation)