Chromatography Advisor #5

Process Proteomics Explained

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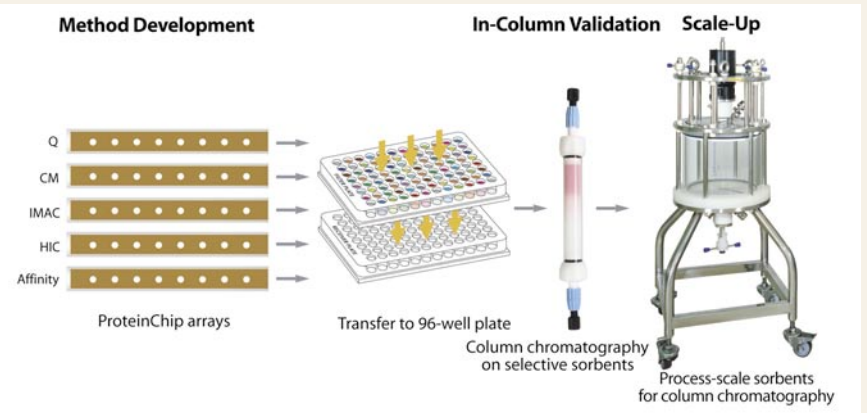
Proteomics — the measurement and characterization of proteins — has become standard in developing novel protein therapies. Robust, reliable analytical methods for targeting and tracking disparate elements of protein samples and quantifying them in final products are critical to manufacturing biopharmaceuticals. However, current methods are time-consuming and complex. *Process proteomics* is a new approach to pharmaceutical, vaccine, and diagnostic molecule process development. This integrated technology can significantly reduce biopharmaceutical development times and costs — shortening the period from research laboratory to large-scale production.

Advances in technology to produce protein-based drugs have accelerated the search for novel protein therapeutic candidates. But practical and cost-effective expression of proteins in amounts that allow for their characterization, evaluation, and scale-up to production presents many difficulties.

State-of-the-art methods for producing protein-based drug therapies rely on cloning, expression, and purification systems that take place in automated configurations across multiple wells. Yet these methods can be complex, time-consuming, and expensive and may hinder preliminary assessments of a protein's potential. Information about target protein integrity and impurities cannot be gathered in real time because these methods are incapable of direct analysis of crude samples.

Figure 1: Surface chemistry continuum



Product Focus: All protein products

Process Focus: Product and process development

Who Should Read: Process and purification engineers

Keywords: Proteomics, protein arrays, purification, automation

Level: Basic

Speeding Up Process Development

Process proteomics is a comprehensive new approach that enables protein optimization and analysis to be performed directly from crude samples. This technology harnesses the power of a ProteinChip system to use chromatographic arrays that capture proteins from biological samples, eliminating contaminating proteins, viruses, nucleic acids, and other impurities. It provides direct qualitative and quantitative data about the composition of a sample.

Process proteomics can reduce protein purification cycle times from several months to days. It offers a method of efficient protein expression and rapid purification steps that can meet market demands for increased speed and productivity in biopharmaceutical development.

Benefits

- Capture proteins from crude fermentation or cell culture feedstreams.
- Analyze samples in a fraction of the time required by conventional methods.
- Require very low sample volumes (10-400 µL)
- Enable direct transfer to chromatography sorbents for scale-up.

The technology is based on retentate chromatography SELDI mass spectrometry (RC-SELDI-MS), a technique that mirrors all aspects of protein production at a small-scale, for fermentation and cell culture optimization, purification development, and product analysis. SELDI refers to *surface-enhanced laser desorption ionization*, a process of selectively retaining proteins on a functionalized surface. It can be used for analysis of samples that are incompatible with other liquid-based chromatography techniques and for traditional mass spectrometry.

On-Chip Purification

The ProteinChip system has two components: ProteinChip arrays and a ProteinChip reader. ProteinChip arrays

use chromatography surfaces to capture proteins from biological samples. The arrays contain multiple "spots," each of which has been modified with a chemical functional group typical of those on chromatography sorbents: anion-exchange, cation-exchange, reverse-phase, and immobilized metal-ion chromatography (IMAC). Because all spots on an array carry the same functional group, multiple separations under different binding, washing, and elution conditions can take place simultaneously.

When biological mixtures are applied to an array, proteins and peptides bind to the various spots. Components of a sample that do not bind can be rinsed from the array in a processing unit. The sample can be further purified by washing individual spots with appropriate buffers to selectively desorb bound components. Target proteins and any impure components that remain bound are referred to as the "retentate."

Arrays save time and effort by developing chromatography purification conditions on chips, eliminating the need for separate fraction analysis.

A Powerful Analytical Tool

The ProteinChip reader is the partnering technology to the ProteinChip arrays. It is a specially designed mass spectrometer that can provide direct qualitative analysis of sample purity for a retentate as well as quantitative analysis of its concentration levels. The analysis relies on time-of-flight-based molecular weight analysis to identify the biochemical constitution of a substance. This information can be used for process optimization and measurement of product specifications. Peaks correspond with molecular weights of the retentate components in a mass spectrum analysis. The reader has a molecular mass range from below one to over 500 kDs.

Analysis requires only small amounts of material — as little as a few microliters — and can be completed in a few minutes per sample. Evaluating the molecular weight pattern of retained proteins can help determine the best chromatographic approach to a given protein purification process. The most efficient binding chemistries and separation conditions for a single step or combination of chromatographic strategies can be determined in a matter of hours. In conventional methods, proteins must be bound together and then eluted from sorbent beads before they can be detected and analyzed, with each combination of conditions often requiring a dedicated run using time, material, and precious amounts of product sample.

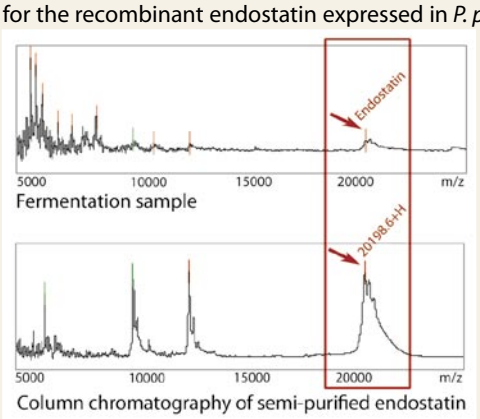
Once a separation method determined by process proteomics has been transferred to traditional column chromatography, the reader can be used to track target proteins and remaining impurities through the final stages in a purification process. Real-time analysis can incorporate multiple protein expression samples at faster rates with greater accuracy than traditional methods. The analysis is extremely precise with sensitivity ranges from one to 50 femtamoles per protein.

The reader can screen various chemical functionalities with precision and speed, lending power to the protein therapy development process. Whereas conventional methods analyze one chromatography chemistry at a time, this system can screen multiple chemistries simultaneously. That reduces purification and development times from several weeks to days and gives a significant head-start to release-assay development.

Rapid growth continues in the biotechnology separations market. Process proteomics can accelerate research and development processes by streamlining and improving each step of protein purification and optimization.

Rapid Process Development of Endostatin from *Pichia pastoris*

As illustrated in Figure 2, a range of ProteinChip arrays (reverse-phase, anion-exchange, and cation-exchange) were used to quickly determine capture conditions for the recombinant endostatin expressed in *P. pastoris,* with its molecular weight confirmed and compared with a reference endostatin sample. The CM ion-exchange chemistry was determined to be the best capture candidate, and the binding pH was selected. Protein elution conditions were also determined on the CM ProteinChip array. Chromatography conditions were successfully transferred to a column with appropriate sorbents, yielding an endostatin purity of greater than 90% for this capture step (**1**).



Protein Array Technology

Protein arrays are rapidly becoming a leading technology for biopharmaceutical research.

The Technology: "Protein arrays are solid-phase ligand binding assay systems using immobilized proteins on surfaces which include glass, membranes, microtiter wells, mass spectrometer plates, and beads or other particles. The assays are highly parallel (multiplexed) and often miniaturized (microarrays and protein chips)" (**2**). The advantages of protein arrays are that they are automatable, highly sensitive, and economical, and produce a large amount of data with a single experiment.

Its Applications: The technology is currently being applied in four major areas: diagnostics, proteomics, protein functional analysis, and selection of antibodies from libraries for use in capture arrays.

More information about protein arrays can be found at the Genomic Solutions Protein Arrays Resource Page: www.functionalgenomics.org.uk/sections/resources/protein_arrays.htm.

References

1 Shiloach J, et al. Endostatin Capture from *Pichia pastoris* Culture in a Fluidized Bed: From On-Chip Process Optimization to Application. *J. Chromatogr. B* 790(1) 2003: 327–336.

2 Taussig M. Protein Arrays Resource Page. *Genomic Solutions*, 8 September 2003; www.functionalgenomics.org.uk/sections/resources/protein_arrays.htm.

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