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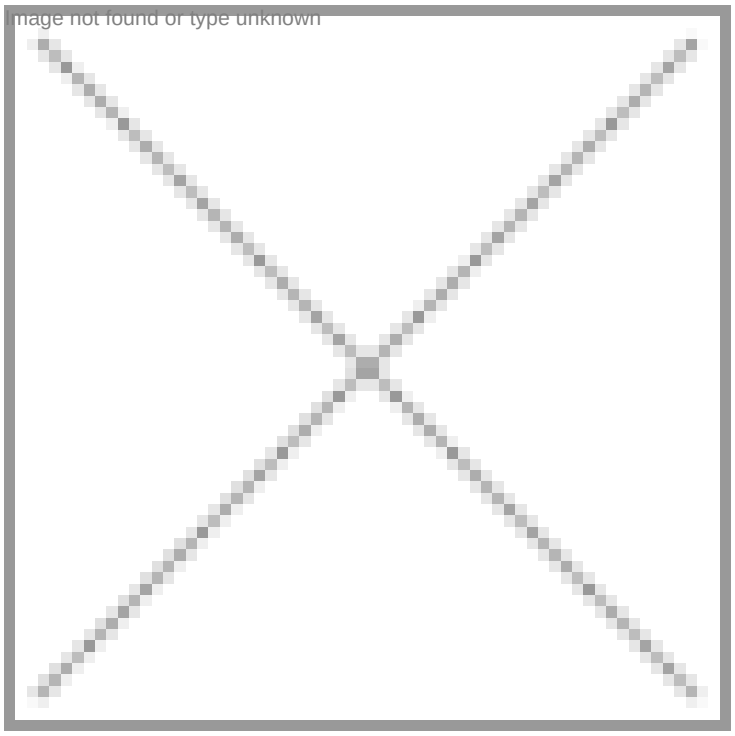
"Process Proteomics" Explained

07 March 2007 | News



Chromatography Advisor # 5

"Process Proteomics" Explained

Proteomics- the measurement and characterization of proteins-has become the standard for developing novel protein therapies. Critical to the manufacture of these biopharmaceuticals are robust, reliable analytical methods for targeting and tracking disparate elements of protein samples and quantifying them in the final product. But current methods are time-consuming and complex. This advisor takes a look at Process Proteomics, a new approach to pharmaceutical, vaccine and diagnostic molecule process development. This integrated technology can significantly reduce biopharmaceutical development time and costs-speeding the journey from research laboratory to large-scale production.

Advances in the technology to produce protein-based drugs have accelerated the search for novel pro- tein therapeutic candidates. But the practical and cost-effective expression of proteins in amounts large enough to allow for their characterization and evaluation, and, finally, for the 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 not capable of direct analysis of crude samples.

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 to capture proteins from biological samples, eliminating contaminating proteins, viruses, nucleic acids, and other impurities; and provide direct qualitative and quantitative data about the composition of the sample.

Benefits of ProteinChip Technology

Capture of proteins from crude fermentation or cell culture feedstreams

Rapid analysis of samples in a fraction of the time compared to conventional methods

Very low sample volume requirements (10-400 µL)

Enables direct transfer to chromatography sorbents for scale-up

The technology behind Process Proteomics can reduce protein purification cycle time from several months to a matter of days. It offers a method of efficient protein expression and rapid purification steps, and therefore can meet market demands for increased speed and productivity in biopharmaceutical development.

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

ProteinChip Arrays: 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 (e.g., anion exchange, cation exchange, reverse phase, and immobilized metal-ion chromatography, or IMAC). Because all spots on an array carry the same functional group, multiple separations under different binding, washing and elution conditions can take place at the same time.

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

In this manner, the ProteinChip Arrays save time and effort by developing chromatography purification conditions on-chip, thus eliminating the need for separate fraction analysis.

ProteinChip Reader: Powerful Analytical Tool

The ProteinChip Reader is the partnering technology to the ProteinChip Arrays. A specially designed mass spectrometer, the reader can provide direct qualitative analysis of the sample purity of the 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 the substance, which can be used for process optimization and measurement of product specifications. In the mass spectrum analysis, peaks correspond to the molecular weights of the retentate components. The instrument has a molecular mass range of below one to over 500 kilodaltons.

The 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 to determine the best chromatographic approach to a given protein purification process. The most efficient binding chemistries and separation conditions for a single step or a combination of chromatographic strategies can be determined in a matter of hours. In conventional methods, the proteins must be bound together and then eluted from the sorbent beads before they can be detected and analyzed, with each combination of conditions often requiring a dedicated run, using time, material and, importantly, precious product sample.

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

The reader provides the ability to screen various chemical functionalities with precision and speed, lending power to the protein therapy development process. While conventional methods analyze one chromatography chemistry at a time, the ProteinChip system can screen multiple chemistries simultaneously. This can reduce purification and development time from several weeks to only days, and additionally gives a significant head-start to release assay development.

There continues to be rapid growth in the biotechnology separations market. Process Proteomics can accelerate the R&D process by streamlining and improving each step of the protein purification and optimization.

Rapid Process Development of endostatin from Pichia pastoris

As illustrated, a range of ProteinChip arrays (Reverse phase, Anion, 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 to the reference endostatin sample.

The CM ion-exchange chemistry was determined to be the best capture candidate, and the binding pH 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 percent for this capture step. (Journal of Chromatography B, 790, 327-336, (2003), Shiloach, J., Santambien, P., Trinh, L., Schapman, A., Boschetti, E.)

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Note: This article has earlier appeared in Bioprocess International.