Purification process optimisation used to be a long drawn-out, iterative process – often based on the art of prior experience tempered with a knowledge of the chemistry of the molecule of interest. Robotics and the advent of chromatography media – presented in a form whereby behaviour can be studied directly in real time by time-of-flight mass spectroscopy – has permitted the screening of multiple media, binding and elution conditions in a matter of hours, rather than weeks or months.

This article demonstrates how the screening of purification conditions for a molecule of known therapeutic value but less well-understood purification protocols can be achieved in less than two days, together with data on performance under production conditions directly predicted by the optimisation study.

PROCESS PROTEOMICS

Proteomics – the measurement and characterisation 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. Process proteomics, a new approach to protein purification and analysis, is an integrated technology that can significantly reduce biopharmaceutical development time and costs – speeding the journey from research laboratory to large-scale production.

Advances in technology for the production of protein-based drugs has accelerated the search for novel protein therapeutic candidates. But the practical and cost-effective expression of proteins in amounts large enough to allow for their characterisation and evaluation, and finally for their scale-up to production-scale, presents many difficulties.

State-of-the-art methods for producing candidate protein-based drug therapies tend to rely on cloning, expression and purification systems that can take place in automated configurations using robotics based on multi-well culture and filter plates. Not accessible to all, these early discovery techniques were often implemented on a manual basis. Such methods can be complex, time-consuming and expensive, and may hinder preliminary assessment of a protein's potential. Information about target protein integrity and impurities cannot be gathered in real time because 'traditional' methods (even those encompassing high-end HPLC and techniques such as MALDI-TOF) are not capable of direct analysis of crude samples.

During the early stages of protein production, choice of appropriate protein expression systems (bacterial, yeast, insect or mammalian) and growth conditions are crucial. Insufficient real-time information about protein expression efficiency, protein integrity and the presence of deleterious contaminants can slow down the development process and create problems in downstream production.

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

The technology can meet the challenges of developing efficient protein expression and rapid purification steps, meeting market demands for increased speed and productivity in biopharmaceutical development. In fact, use
of the technology can reduce protein purification cycle times from several months to a matter of days. Process proteomics is based on Retentate Chromatography SELDI Mass Spectrometry (RC-SELDI-MS), a process that facilitates all aspects of protein production – from fermentation and cell culture optimisation to purification development and product analysis. SELDI refers to surface enhanced laser desorption/ionisation, a process of selectively retaining proteins on a functionalised surface; it can be used for the analysis of samples that are incompatible with liquid-based chromatography techniques, and other mass spectrometry technologies.

**ProteinChip Arrays**

The ProteinChip system has two components: ProteinChip Arrays and the ProteinChip Reader. As shown in Figure 1, 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 (for example, anion exchange, cation exchange, reverse phase and immobilised metal-ion chromatography, or IMAC). Surfaces can also easily be prepared with specific functionalities for ligand-binding, receptor assays, antibody-antigen interactions and a host of other techniques. Because all spots on an array carry the same functional group, multiple separations and binding and washing conditions can take place at the same time. For example, each of the 8 spots on an array can be presented with the crude sample at 8 different pH values to study the best pH to achieve binding of the target protein.

When biological mixtures are applied to the arrays, proteins, peptides and other biological molecules bind to the various spots according to the functionality of the matrix, pH and ionic condition, in exactly the same way that these molecules would bind (or not) to a traditional chromatographic sorbent. 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 impurity components that remain bound to the array are referred to as the ‘retentate’. The material that has bound can then be subsequently eluted from the chip and is directly studied using the time-of-flight mass spectrometer; this quantifies 100 readings from each spot and reads 96 spots in about 10 minutes. (The chips are arranged in exactly the same format as 96-well plates – that is, 12 chips of 8 spots).

The technique can be coupled to work done in 96-well plates. For example, for subclone selection, cells can be cultured for a very short period in 96-well culture plates and then directly filtered off; the culture fluid is then applied directly to chips with chemistry appropriate for the target molecule, and the results obtained in an hour or less to allow determination of which cells are the highest producers. For IgG, the sensitivity of the method is of the order of 2 femtomole. All of this can be done robotically; use of a 96-well plate format for the chips, the culture and for separating the cells means that standard robots already available in many development laboratories can be employed (1).

Another use of the technique coupled to multi-well plates is to directly screen other process steps that may not normally be resolvable in near-real time. Studying the effects on samples of different filter materials is one simple example; the most powerful application, however, involves the screening of any available chromatography sorbent. In this case, around 90µL of sorbent is placed into each well of a standard filter plate. 96 replicates can then be run – either directly comparing different sorbents, or comparing different conditions for binding and elution – with the flow through and eluates from the 96-well sorbent-containing plates being captured by ‘Normal Phase’ chips. All this takes a matter of hours, including complete analysis of the results.

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

**THE ProteinChip Reader**

The ProteinChip Reader is the partnering technology to 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 differences in molecular weight to identify the biochemical constitution.
of the substance; this can then be used for process optimisation 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 1 to 500 kilodaltons, and readily separates very small differences in molecular weight. In one application, differences between expression and purification of N-terminal and C-terminal proteins with a $\Delta$ of just 200 daltons were able to be easily determined (2).

The analysis requires only small amounts of material – as little as a few microlitres – and can be completed in a few minutes per run. Evaluating the molecular weight pattern of retained proteins can help to determine the best chromatographic approach for a given protein purification process. The system facilitates evaluation by not only providing a traditional mass spec trace (Figure 2), but also converting this to a ‘gel view’ that looks like the electrophoresis gel that most researchers are more familiar with. 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 days.

With conventional methods, the sample must be applied to a small column and the full chromatographic procedure carried out – with detection using UV giving very little real information about the composition of the sample. After separation, the target proteins must be eluted from the sorbent beads before they can be detected and analysed – a further processing step that may involve HPLC, or in fact, mass spec. The RC-SELDI-MS system combines everything into a single, information-rich procedure.

The optimal conditions for binding and elution determined by the ProteinChip system can be directly used – usually with no modification – to process the ‘real’ samples or product on laboratory- and production-scale chromatography equipment. Purification conditions for a recombinant endostatin were determined this way and scaled up faultlessly – the entire study only taking two days (3).

Once a separation sample has been transferred to traditional column chromatography, the ProteinChip Reader can be used to track proteins, as well as remaining impurities, through the final stages in the purification process. This is the benefit of information-rich technology. No other technique optimises purification so readily – not only for the target molecule, but also simultaneously tracking where all of the other components are going in the process.

The system is used to improve the production of a monoclonal antibody by determining not only which impurities are still present, but also elucidating the best conditions for getting rid of them in the remaining steps of the purification process. In this example, we see not only the Protein-A that is leached from a preceding affinity step but also other co-eluting proteins; the readings from the chips indicate the conditions under which these contaminants can be removed.

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 analyse 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 a matter of days.

There continues to be rapid growth in the biotechnology separations market. Process proteomics can accelerate the research and development by streamlining and improving each step of the protein purification and optimisation processes.

The author can be contacted at ian_sellick@pall.com

References