Modular approaches for diverse molecules: Reinventing smart bioprocessing

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As biopharma companies develop growing numbers of biologic therapeutics and drug budgets are squeezed worldwide, there is an increasing pressure on manufacturers to find more efficient and effective production processes. To reach this goal, Rentschler Biopharma, an independent and family-owned contract manufacturing organization (CMO) based in Laupheim, Germany, is using an approach it calls “smart bioprocessing” to create scalable manufacturing processes rapidly and efficiently.

Manufacturing biologic drugs: Upstream and downstream

Monoclonal antibodies have become one of the most commonly manufactured complex biologic molecules by CMOs. There are also growing demands for hard-to-produce biologics, including fusion proteins, bispecific antibodies, and antibody-drug conjugates to meet specific therapeutic needs. In this burgeoning area of complex molecules, cost-effective manufacturing is critically important. This is particularly true for cost-sensitive applications such as biosimilars and for molecules used to treat rare and orphan indications, where market size is limited. Small companies and startups with financial constraints also look for lower-cost support to be able to move rapidly through proof-of-concept studies to support deals.

To ensure that their manufacturing methods are as efficient and cost-effective as possible, CMOs look to optimize production and purification using platform processes, continuous processing, and process intensification. Many factors need to be considered when developing a manufacturing process, particularly for a complex biologic (Fig 1).

The first step in development is molecule design. This includes adapting the building blocks of the molecule — for instance, to improve the glycosylation sites or using protein engineering to enforce heterodimerization of bispecific antibodies.

The next step involves the generation of the cell line, which begins with a choice of the mammalian host, such as hamster, mouse, or human cells. The decision depends on the type of protein to be produced, as these hosts differ in their ability to generate glycosylation. The DNA coding for the target protein must then be integrated into the cell genome, and recombinant cells are selected primarily according to expression levels, a process that traditionally takes around 18 weeks. Rentschler Biopharma has developed a process that uses site-directed integration in Chinese hamster ovary (CHO) cells to speed up the transfection process. This, along with fluorescence-activated cell sorting (FACS), halves the cell line development timeline, cutting it to around nine weeks.

One key factor in the economics of protein manufacturing is the level of protein produced (titer). In the early days of protein manufacturing in the 1990s, 0.1 g per liter was an acceptable level. Current commercial manufacturing capacities are more commonly around 5 g per liter, though levels of 10 g per liter or more can be
achieved. Routes to increasing titers include cell line engineering, growing cells in higher densities (which requires higher levels of nutrients and oxygen), and changes in processes, such as moving from batch to continuous perfusion processes.

The final step, downstream processing, is harvesting the proteins and removing any process- and product-related impurities, such as host cell protein, DNA, protein A, fragments, aggregates, and undesired isoforms.

The application of “smart bioprocessing”

The drivers of manufacturing therapeutic proteins are upstream productivity, protein quality, and overall cost. The aim of smart bioprocessing is to use bioinformatics, lab-scale processing, and analytics to create better process designs that can be verified before they are scaled up to clinical trial and commercial-scale production.

The smart bioprocessing approach is modular and iterative (Fig 2). Analysis after each step provides more information that can be fed into the next step, and steps can be repeated to refine the entire production process.

The aim with “smart bioprocessing” is to front-load the critical analytics steps and solve as many issues up front as possible before moving into the small-scale and design steps. Once the initial small-scale production begins, the developers can look for issues in the production or purification process and find ways to solve them before moving up to a larger scale. By gathering theoretical and experimental information as early as possible and then ironing out problems at a small scale, it is possible to verify that the process works before committing to larger-scale production.

The end goal is to create a robust and reproducible good manufacturing practice (GMP)-ready process that meets the criteria for overall yield, achieves the critical quality attributes, and creates a final downstream processing specification ready to scale up for production.

Applying bioinformatics: Assessing product properties

In silico bioinformatics allow process developers to analyze biologic molecules and predict how they will act in given situations. Screening and modifications at this stage mean the molecule can...
be optimized to refine post-translational modifications (PTMs) or to eliminate protein aggregation and therefore increase yield.

Examples of physicochemical properties that can be predicted using bioinformatics:

- Isoelectric point (pI)
- Charge distribution and hydrophobicity
- Aggregation-prone regions
- PTM sites
- Protease sites
- Immunogenicity

Companies will need training and expertise to make the most of bioinformatics and in silico screening, but the hurdles for this are much lower than they have ever been, with resources and applications available online or alternatively as desktop solutions. In that context, it is important to remain aware of security and confidentiality issues.

Small-scale analytics: Exploring stability

Once the physicochemical molecular properties are assessed using bioinformatics and the product is expressed in a cell line, explorative capture studies follow. The preliminarily purified protein is then evaluated for its stability at different temperatures and how it copes with the steps used in virus removal/inactivation, which include low pH, or the use of organic solvents, and detergents.

It is also important to look at the mechanical stability of the protein during stirring, agitation, and shaking, as these will be part of the normal production and purification steps. This practical combination of analytics steps allows confirmation that in silico predictions work in the real world.

Design: Process development

The process design stage includes putting together a lab-scale bioreactor and looking at increasing the level of expression of the protein to as high a titer as possible. However, besides expression levels, product quality must also be considered. Several variables can be changed and evaluated in the bioreactor to improve the yield, including:

- Cell culture mode — batch, fed-batch, or continuous perfusion
- Length of culture time
- Cell density
- Oxygen levels
- Media type
- Nutrient levels and feeding strategy
- Temperature

The process development steps are iterative, with the process developers working at a small scale and modifying these variables individually. Analysis of the protein’s quantity, purity, and quality attributes shows the impact on the final process, and each change brings an optimized design a step closer.

Verification: Testing and evaluation

The final test is confirming the feasibility of the process, including confirming the purification steps and finalizing the downstream specification. For this, the proteins must reach the appropriate purity and quality targets, and the process must be GMP-ready and economically viable.

Achieving the benefits of smart bioprocessing

A modular approach to molecule optimization and process development will save money, time, and effort. An ideal manufacturing development process would begin at the stage of molecule design to create a molecule that is optimized, for example, with low propensity for aggregation, high stability, and high expression. However, particularly for CMOs developing a manufacturing process on behalf of a client, this is not always possible. Pharmaceutical companies using this approach begin the process much earlier, which saves time and costs in manufacturing. Yet, CMOs often enter an existing process and then have to refine the process as tightly as possible to suit the molecule. In the future, it would be more efficient for CMOs to work with companies at an earlier stage to collaborate over the optimization of the biologic or even help to design the molecule from the beginning.

Overall, the aim of smart bioprocessing is to use bioinformatics, lab-scale processing, and analytics to create better process designs that can be verified before they are scaled up to clinical trial and commercial-scale production. Companies should start by carrying out as much analysis in silico as possible up front to identify potential problems and eliminate them by redesigning the biologic, if possible. Then, they would verify the predictions from the in silico analysis in small-scale studies. It is crucial to identify the challenges properly either by in silico or experimental approaches to be able to react accordingly in the process design.