

IDENTIFY THE PRODUCTION STRATEGY BEST SUITED FOR YOUR PROTEIN OR PEPTIDE

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Proteins and peptides are used across a wide array of drug products thanks to their important biologic impact to human health. In *Novel Approaches and Strategies for Biologics, Vaccines and Cancer Therapies*, Andrew Buchanan and Jefferson Revell described the appeal of these agents: “Proteins and peptides have a significant and rapidly expanding role as medicines due to their high affinity, specificity, low toxicity, and ability to alter protein-protein interactions.”¹ Thus, these therapies offer the opportunity to treat patients with fewer negative side effects. As their usage diversifies across the industry, drug developers and contract development and manufacturing organizations (CDMOs) are assessing how best to manufacture them.

Depending on the type of protein or peptide being manufactured, there are several strategies worth considering, including chemical synthesis, microbial/mammalian fermentation, and hybrid strategies that combine multiple approaches. Identifying the best manufacturing method largely depends on the scale, budget, and intended molecule. Beyond that, as the pharma industry strives to design greener processes, sustainability is another major factor. Manufacturers are reviewing whether common processes generate high quantities of solvent waste, and if so, are there greener alternatives?

All factors considered, it's a highly specific choice dependent on a developer's targets. If you're a drug sponsor hoping to identify a protein or peptide manufacturing system that maximizes yield, minimizes waste, and remains budget friendly, consider the pros and cons of the different protein and peptide manufacturing strategies available, including the following.

I. Solid-Phase Synthesis: A Small-Scale Production Method for Short Peptides

Solid-phase synthesis is a popular method for producing peptides; in fact, it is sometimes considered the quickest platform for short peptide manufacturing since *E. coli* or other fermentation methods often require peptide-specific development work for both producer strain and upstream/downstream processing. It is also typically the best option for incorporating non-natural amino acids,

or modifications, often used to generate pharmaceutical peptides.¹ Ultimately, whether solid-phase synthesis is the best fit for producing your peptide depends on the length, scale, and goals of your project.

Producing peptides at a large scale via solid-phase synthesis requires high amounts of hazardous reagents and solvents to ensure appropriate coupling and protection/deprotection of the amino acids and high yields. Not only are these raw materials costly as they increase in quantity, but the process also ceases to be environmentally friendly at large scales. Despite this limitation, solid-phase synthesis continues to be an excellent choice for small-scale peptide production, and various technologies to address the drawback of solid-phase synthesis are emerging.²

II. *E. coli* Expression: An Affordable Choice for Protein Production

E. coli is a microbial expression system commonly used to manufacture proteins. It was the first system used to express recombinant proteins and remains a popular platform thanks to its speed of growth, ease of use in the upstream, low cost, and relative scalability.

In terms of challenges or disadvantages, the *E. coli* expression system may accumulate proteins inside the cell, which often form insoluble inclusion bodies that

require harvest of the cell, cell disruption, inclusion body collection, solubilization of the inclusion body, refolding, and purification. Another challenge may be high endotoxin levels since *E. coli* is a gram-negative bacterium. These require a complex downstream process, the yield of which could be low for specific proteins. *E. coli* processes might also struggle to successfully make peptides due to proteolytic activities inside the cell. Overall, the advantages must be weighed alongside the challenges to determine whether this is the best process to manufacture your specific protein.

III. CORYNEX®:

A Scalable Platform to Secrete Antibody Mimetics, Peptides, Enzymes, and More

Ajinomoto Bio-Pharma Services' CORYNEX® platform is a microbial secretory production system for proteins and peptides that has demonstrated the ability to successfully produce single-domain antibodies known as VHHs, antibody mimetics, enzymes, and peptides. The platform uses a gram positive, non-sporulating soil bacterium, *Corynebacterium glutamicum*, which has historically been used to make a wide variety of amino acids and, more recently, proteins and peptides.

CORYNEX®'s *C. glutamicum* secretion system lends itself to high titer and purity as well as no endotoxins, making it a strong alternative to *E. coli* for VHHs and antibody mimetics. It offers higher yield, ease of purification, and ultimately reduced production time and cost. The secretion system has demonstrated the ability to secrete various soluble, correctly folded, and biologically active proteins. Another advantage of CORYNEX® is that it does not produce proteases in the culture media and thus it allows you to collect your protein or peptide directly from the culture media, ensuring your product can be easily recovered at a large scale without suffering from protein degradation. Together, these factors help minimize complexity of the downstream process. Overall, CORYNEX® contributes to major production cost savings, thanks to the minimal column steps required to achieve a high-quality yield.

Leveraging secretion without proteolytic activities is also relevant to peptide production, and from a sustainability perspective, CORYNEX® allows you to significantly reduce the quantity of solvents used when compared to solid-phase synthesis. Its high scalability makes it a reasonable stepping stone when scaling up from small-scale, solid-phase synthesis. When compared to traditional synthesis methods, CORYNEX® offers increased productivity and cost-saving opportunities alongside environmentally friendly processes.

If your peptide contains non-natural amino acids, Ajinomoto offers an approach to support this: CORYNEX® can be leveraged in concert with solid-phase synthesis to reduce the overall amount of solvents needed in your process. In this approach, the fragment containing natural amino acids is produced via CORYNEX®, while the fragment containing non-natural amino acids is produced using chemical synthesis. They are then combined by condensation or a native chemical ligation (NCL) reaction. This hybrid strategy is a viable option for long peptides including non-natural amino acids.

For example, CORYNEX® can produce a fragment incorporated with a BocLys, while leaving a native Lys in a specific position. The N-terminal amino group can be also protected by genetically fusing a special peptide tag sequence. Thanks to the incorporation of the protection group on the BocLys and the N-terminus, only the free Lys can be modified. By conducting a deprotection reaction of the Boc group and N-terminal tag cleavage, a site-specifically modified fragment can be obtained. In another example, one can incorporate AzF to covalently and site-specifically attach a molecule such as a half-life extension moiety onto the protein via click chemistry.

Despite misconceptions that CORYNEX® is used only for research, it has been used to scale up and manufacture GMP material to support Phase 1 and Phase 2 clinical trials. If the CORYNEX® platform appeals to you as an option for producing your protein or peptide, Ajinomoto can rapidly conduct streamlined expression trials to assess compatibility.

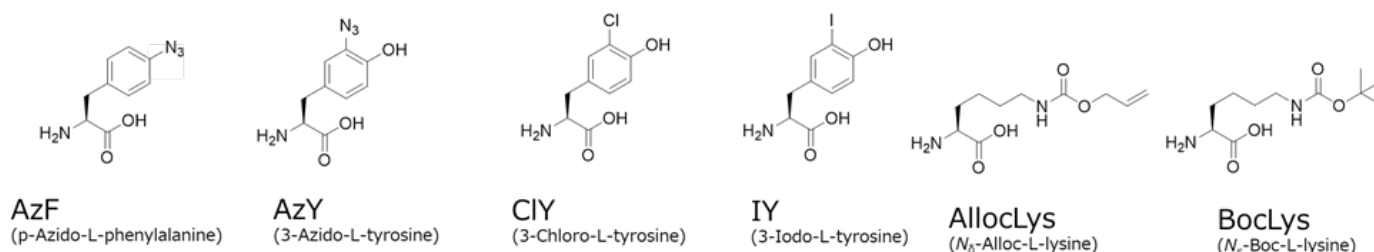


Fig. 1 – Non-natural amino acids incorporated by Ajinomoto's CORYNEX® platform.

Choose the Process to Meet Your Goals

While there are a wide range of possibilities for protein and peptide manufacture, each method comes with its own unique advantages and challenges.

To ensure you select the best strategy, work with a manufacturing partner to identify the process that will result in the best yields for your protein or peptide type, accommodate your manufacturing scale, and achieve your sustainability objectives.

Whether you're considering one technique at a small scale or a hybrid of multiple processes as you scale up production, an experienced CDMO will help identify the process or processes that will bring your therapeutic to patients as efficiently as possible.

Resources

1. Andrew Buchanan, Jefferson D. Revell, Chapter 8 - Novel Therapeutic Proteins and Peptides, Editor(s): Manmohan Singh, Maya Salnikova, *Novel Approaches and Strategies for Biologics, Vaccines and Cancer Therapies*, Academic Press, 2015, Pages 171-197, ISBN 9780124166035, <https://doi.org/10.1016/B978-0-12-416603-5.00008-0>
2. Rossino, G., Marchese, E., Galli, G., Verde, F., Finizio, M., Serra, M., Linciano, P., & Collina, S. (2023). Peptides as Therapeutic Agents: Challenges and Opportunities in the Green Transition Era. *Molecules (Basel, Switzerland)*, 28(20), 7165. <https://doi.org/10.3390/molecules28207165>

About the Author

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Ajinomoto Bio-Pharma Services is a fully integrated contract development and manufacturing organization, with sites in Belgium, United States, Japan, and India, providing comprehensive process development services, cGMP manufacturing, and drug product fill finish services of small molecule and biologic APIs and intermediates. We offer a broad range of innovative platforms and capabilities to rapidly scale from clinical and pilot programs to commercial quantities, including: CORYNEX® protein expression technology, oligonucleotide synthesis, antibody drug conjugations (ADC), high potency APIs (HPAPI), biocatalysis, continuous flow manufacturing, and more. Ajinomoto Bio-Pharma Services provides a high level of quality and service to meet our clients' needs.