

# Crystallization process development: Peptide crystallization

Solving the challenges of peptide crystallization yields downstream rewards.

## A scalable solution: Are peptides worth the extra effort?

Peptides play an increasingly important role in drug discovery with their potential to offer therapeutic agents beyond traditional hormone replacement therapies. In addition to therapeutic agents, peptides are often subcomponents in other drug substances. Regardless of the application, mastering peptide crystallization opens possibilities for large-scale processing in pharma applications.

The molecular complexity of peptides may have kept them out of development pipelines in the past, but innovations in synthesis and purification have supported their recent momentum as alternatives to small molecules for addressing unmet medical needs. Still, peptides present a particularly challenging class of molecules in crystallization process development. Success of scale-up crystallizations relies on a robust crystallization process to control the thermodynamic and kinetic factors that impact the solid form.

Given the difficulty of peptide crystallization, why do companies invest the time and resources into pursuing a crystalline form of their peptide? Having a crystalline solid form offers clear advantages compared to their amorphous counterparts. Not only does it allow full structural determination, but it also confers benefits in processing of the material. Crystalline products tend to be less hygroscopic than amorphous materials, aiding in further processing. Significant impurity rejection and a greater ease of residual solvent removal is afforded through crystallization, resulting in a higher purity product. Furthermore, peptide crystallization offers a significant cost advantage over column chromatography methods for isolation and purification of the desired solid form.

Obtaining a pure crystalline form of any API is exacting work, and peptides are a special case within this area of chemistry. Before scientists can consistently produce a stable solid form, they must have an in-depth understanding of the thermodynamic and kinetic processes that drive the crystallization for a specific peptide. Only then can they manipulate various critical process parameters to yield the optimal result. Cambrex employs world class experts in the understanding of crystal formation and thermodynamics, allowing us to develop custom scalable crystallization processes for peptides.



David Booth, Ph.D.  
Team Leader, Crystallization

## There is no substitute for expertise.

Peptide crystallization introduces greater complexity than smaller molecules.

Each peptide requires unique insight to optimize individual conditions.

### About Cambrex

**Cambrex is a leading global contract development and manufacturing organization (CDMO) that provides comprehensive analytical and IND enabling services, as well as drug substance development and manufacturing across the entire drug lifecycle.**

With over 40 years of experience and a team of 2,000 experts servicing global clients from North America and Europe, Cambrex is a trusted partner in branded and generic markets for API development and manufacturing.

Our combined experience ensures that we conduct the right experiments and modify parameters to achieve the optimal crystalline form. Whether it is performed during formation of an API or as a means to purify intermediates, achieving control of nucleation and crystal growth is critical to designing a successful peptide crystallization process.

### Milestones to success: 3 stages of strategy

For peptide crystallization to be successful, the fine tuning of several process conditions is critical. Typically, operating ranges for peptide crystallization are much tighter than for small molecules, so the design and assessment of initial screening can make or break scale-up efforts.

Cambrex meets 3 milestones during our overall strategy to deliver crystallized peptides. Solubility is evaluated with respect to solvents, pH, temperature, counterion inclusion and excipients. This serves a dual purpose of finding conditions under which the peptide is soluble and discovering the optimal conditions for later crystallization. High throughput screening followed by small-scale crystallization trials are then carried out to locate experimental conditions that allow for crystallization of the free peptide or its salt form. A wide range of conditions are considered, in order to avoid gelation, aggregation, amorphous solids and liquid crystals. Achieving single crystal growth during these first steps allows structural analysis and provides material for seeding protocols.

Next, the team looks for scalable elements of the process. At this point, they assess the critical process parameters at the 100mL to 1L scale. The conditions are adjusted to achieve the best yield, with the highest purity and efficiency in filtration, in a minimal reaction volume. At the same time, they must define the operating range for each of the critical process factors to deliver a controlled and scalable crystallization.

The third milestone involves technology transfer to ensure a successful crystallization process in the client's facility. Our experts transfer the process to achieve consistent results in the plant using data and analysis from hands-on experiments as well as state-of-the-art modeling software.

### Significant uplift in purity: Preferred conditions

Solubility of the peptide can be affected by several factors, including pH, counterion inclusion, temperature and solvent selection. Once solubility curves are established, a screening array can be designed to further

#### Crystallization monitoring technology



Process Analytical Tools (PAT) allow in-situ monitoring of crystallization.

define crystallization conditions and obtain first crystals for analysis.

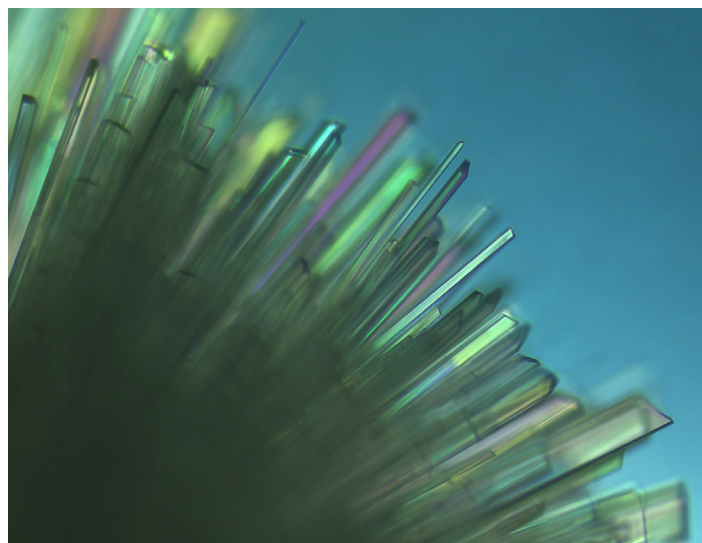
In the case discussed here, the team started with an amorphous lyophilized solid with 89% w/w purity that had low stability at high pH and sensitivity to light and heat. The goal was to increase purity to greater than 98% w/w. An array of experimental conditions investigated various organic solvents, pH levels, peptide concentrations, and multiple temperatures. For this peptide, the most effective crystallization occurred with low starting pH, using an organic solvent precipitant, with counterion inclusion and a temperature of 20°C. After these steps of optimization, a purity uplift to 97% w/w was observed from the initial crystallization.

The team then set out to determine the critical crystallization process parameters to ensure optimal operating ranges during scale-up. Using controlled laboratory reactors equipped with an online FBRM probe, the team measured several parameters in-situ, including



temperature, cooling profiles, precipitant addition rate and volume, changes in concentration, aging periods, effect of seeding, counterion concentrations and mixing effects. Cambrex exceeds industry standards in online monitoring using next generation technology. The Blaze Metrics 3-in-1 PAT tool allows sophisticated real-time monitoring of particle shape, size and polymorphic form. It also allows Cambrex to offer clients reduced set-up time with a single probe, while providing state-of-the-art insight into particle and process behavior.

In this case, the team determined several parameters that were deemed most critical for successful crystallization of the studied peptide. Follow-up experiments separately investigated conditions including peptide concentration, aqueous to organic solvent ratio, counterion concentration and solvent addition rate under various pH conditions and temperatures. They also demonstrated that changes in peptide concentration had little effect on the crystallization yield or purity. While seeding was not required to obtain the final crystalline material, the team's analysis showed that it resulted in an increased particle size, improving the filtration rate.



Next, the team performed washing and drying studies to ensure that the crystalline nature of the peptide would be maintained upon isolation. This can be a critical step during scale-up to avoid deliquescence of the peptide solid on the filter. In this case, a range of organic/aqueous solvent mixtures were tested for optimal washing, and it was determined that organic solvent without an aqueous component allowed a final flowable solid to be isolated, avoiding loss of material as an oil residue to the filter.

With these parameters in place, the process was carried out in a 1L reactor, and the team was able to successfully produce a purified, flowable solid with a purity increase

from 89% w/w to 99.6% w/w. Success at the 1L scale allowed method transfer to begin at the client site, with full support during the process to ensure a smooth transfer and consistent replication in production of the crystalline solid form.

### Process design innovation: No substitute for expertise

While crystallization efforts are traditionally associated with smaller molecules, the step-wise approach discussed here is a successful strategy to tackle the design of a peptide crystallization process, adding to Cambrex's command in this space of drug development. The increased understanding provided by these studies is a powerful tool for our clients to assess the potential of their peptide and to reveal its viability as a candidate for crystallization. Knowing whether the properties of a specific crystallized peptide will offer advantages over the amorphous material can prevent wasted scale-up efforts.

Achieving control of crystal nucleation and growth through in-depth analysis of critical process parameters is key to being able to transfer a bench-scale process to meet clinical demand. This example highlights that strategic design principles coupled with leading experts and technology can lead to successful scale-up of even the most stubborn peptide crystallizations. We leverage the best minds in the industry to replicate laboratory-scale crystallization in large-scale reactors, helping our clients achieve clinical delivery timelines.

### We'll cross that bridge when we come to it.

Even the most stubborn molecules are no match for our expertise.

We leverage the right tools with the best experts to bridge success from the bench to the plant.