

## It is a well-known fact that F&T negatively impacts product quantity and quality

In respect of the marketing of large volumes of protein bulk drug substance (BDS), F&T processes have become essential for safe storage and distribution. The total production capacity can be improved to its maximum by means of optimized F&T processing. Plus, it guarantees safe global distribution. Safe storage of deep frozen high value proteins extends stability of valued APIs.

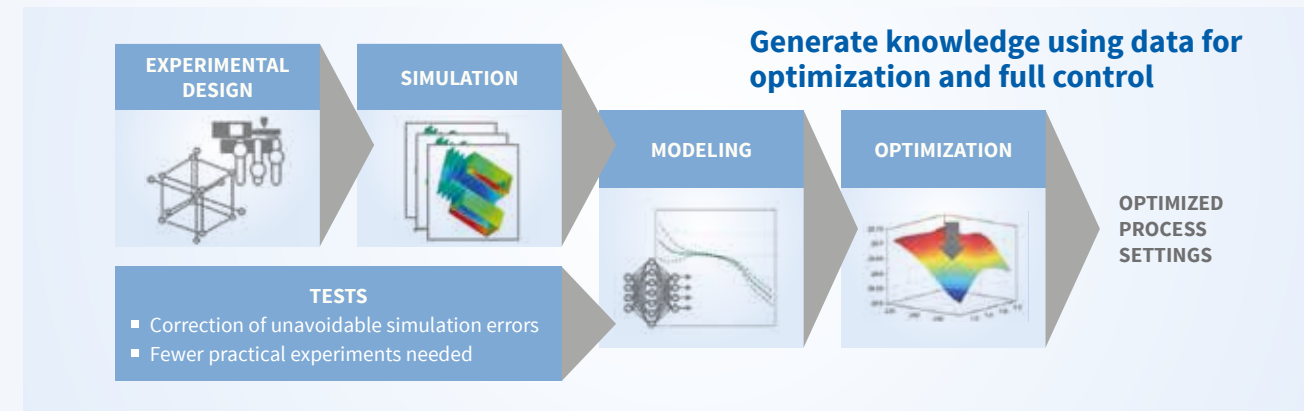
Freezing reduces risks from mechanical stress during transport and minimizes the possibility of microbial growth. However, the risk of freezing-induced aggregation, caused by cryoconcentration, crystallization of solutes or buffer salts, pH changes, and ice water interface-induced denaturation, influences the success with this unit operation. The systematic knowledge-based freezing process for each individual formulation is a must for meeting the QbD approach. There is the need to consider, proof and analyze all parameters carefully to avoid freezing-induced quality losses.

**The FDA provides an advisory paper for freezing and thawing limits and requirements.**

**They conclude that:**

- there is a need to define "fast" and "slow" freeze conditions;
- increasing the rate of freezing mainly decreases the product loss up to a certain point;
- the thawing method is not less important than the freezing method in terms of product quality;
- process conditions differ for individual product formulation.

*(FDA, Mark Weinstein, PhD.)*

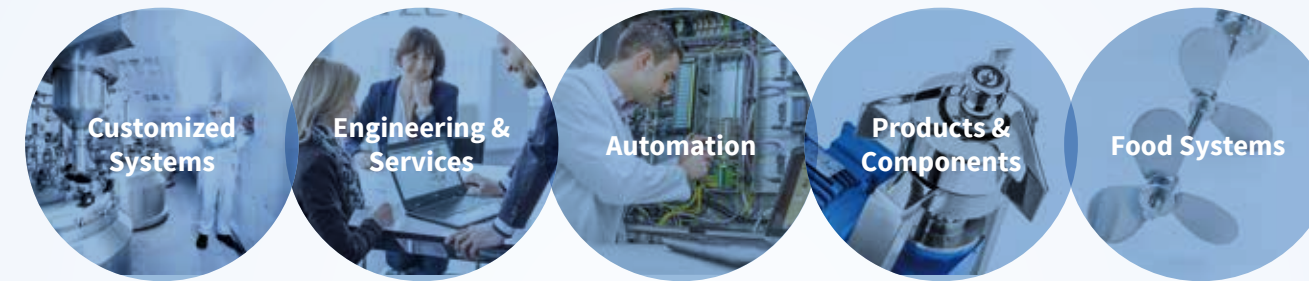


**For further information on Freeze & Thaw Services please contact:**

ZETA Biopharma, Research & Development  
 Dipl.-Ing. Birgit Pittermann (Head of R&D)  
 e-mail: birgit.pittermann@zeta.com  
 www.zeta.com

## INNOVATIVE SOLUTIONS FOR OUR CUSTOMERS

### EVOLUTION OF TECHNOLOGY



#### ZETA Business Activities

- Bioreactors & Fermentation Systems
- Downstream Systems
- Preparation Systems
- CIP/SIP Systems
- Magnetic Agitators
- Freeze & Thaw Systems
- Engineering
- Automation

#### Customer Benefits

- Deep process understanding
- GMP FDA Compliance
- Super-Skid Design
- Focus on sterility
- High process reliability
- Scale-up capabilities
- Experience in complex biologics
- Customized process systems

#### ZETA Biopharma

Zetaplatz 1, 8501 Lieboch/Graz, AUSTRIA  
 Phone: + 43 3136 90 100, E-Mail: info@zeta.com

## FREEZE & THAW SERVICES

Systematic knowledge-based process development for ideal transport and storage of proteins.



Biopharmaceutical Proteins are usually most stable in frozen form. Therefore, freezing and thawing (F&T) are important steps to stabilize the protein drug before fill-and-finish for storage and transportation. Debates regarding effects to purity, activity, safety, and efficacy of the finished product are arising with special respect to product quality risk. The F&T process itself means stress to the product. Cryoconcentration and molecular interactions during F&T process may cause significant product loss. Mechanical stress and phase interaction may even overlay these effects.

Negative impacts on product quality differ for each formulation, thus proper understanding of interdependencies are essential!

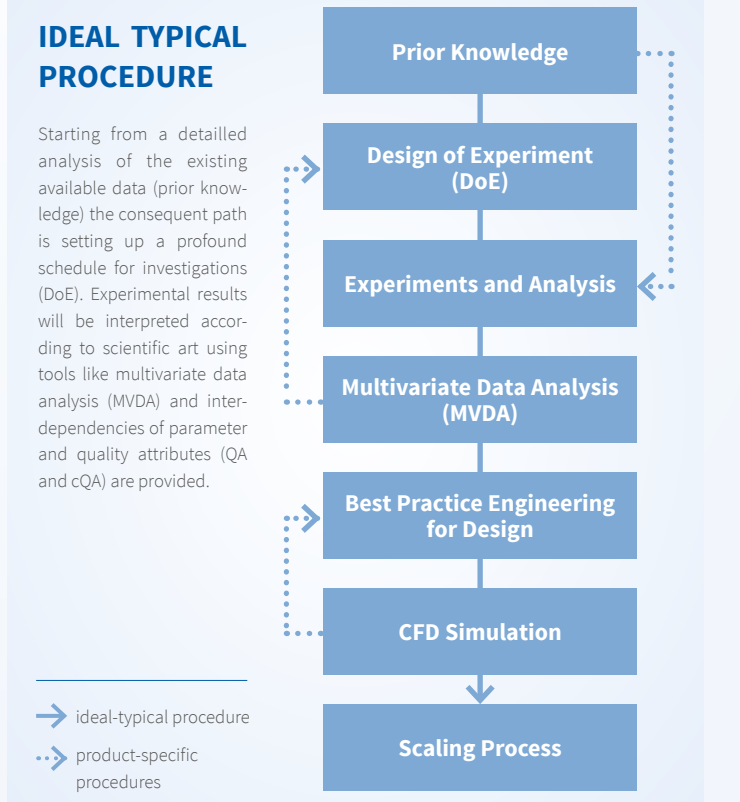
Setting-up a mechanistic process design for F&T increases the product quality and serves the QbD approach. Regulatory bodies such as the FDA require details of the F&T cycles for product registration.

#### Advantages for the customer:

- Best in practice risk control
- Best in process product quality
- Reduction of product losses

#### IDEAL TYPICAL PROCEDURE

Starting from a detailed analysis of the existing available data (prior knowledge) the consequent path is setting up a profound schedule for investigations (DoE). Experimental results will be interpreted according to scientific art using tools like multivariate data analysis (MVDA) and interdependencies of parameter and quality attributes (QA and cQA) are provided.





ZETA LabFreeze



## STARTER SET

Find suitable formulation buffer for F&T

### STARTING POINT:

- The product formulation is not finalized.
- There is the need to find a suitable formulation buffer for freezing & thawing.

### STRATEGY:

The starter service includes F&T studies with a previously determined number of test runs and analysis methods to identify the buffer conditions at various freezing ramps and freezing temperatures.

### BENEFITS OF THE FREEZE SERVICES:

Test runs in a small scale are best suited for identifying the buffer behavior in F&T cycles. The best buffer composition can then be specified as a result.

### STARTER SET AT A GLANCE



**Study on relevant buffer systems, relevant prior data**

**Output:** Establish Prior knowledge as a starting point



**Numbering of experiments, determination of experimental set-up**

**Output:** Design of Experiment (DoE)



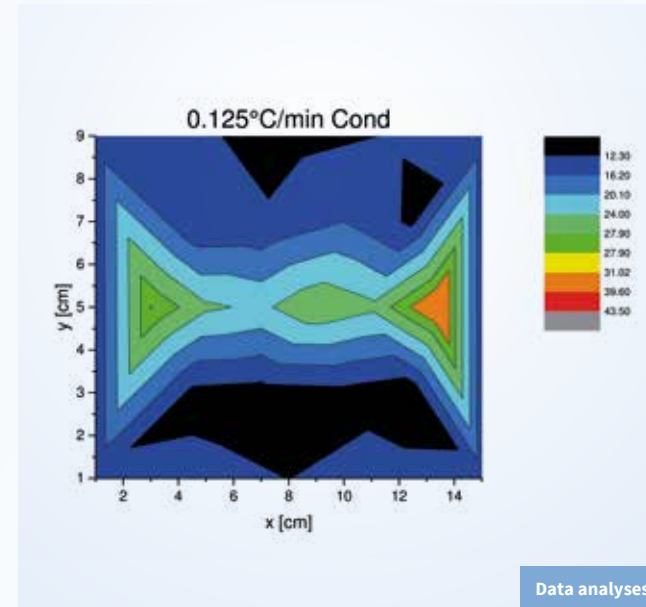
**Performance of at least 7 F&T runs, sampling of liquid & ice phase, analysis**

**Output:** Cryoconcentration profile of buffer



**Calculation of phase transition 2D**

**Output:** Scalable process information



Data analyses

## INTERMEDIATE SERVICES

Develop knowledge on basic product behavior during F&T in formulation buffer

### STARTING POINT:

- The product formulation is finalized, but the freezing process is still unknown.
- There is a need to develop knowledge on basic product behavior during F&T in formulation buffer.

### STRATEGY:

The intermediate services offer scientifically approved methods in order to investigate F&T processes in respect to freezing temperatures and F&T ramps. The target is to determine the best homogenous freezing scenario.

### BENEFITS OF THE FREEZE SERVICES:

The systematic process analysis provides knowledge about product behavior during F&T. It is the basis for the correlation of critical process parameters (cPP) and critical quality attributes (cQA).



### INTERMEDIATE SERVICES AT A GLANCE



**Study on relevant existing data**

**Output:** Establish prior knowledge as a starting point



**Numbering of experiments, determination of experimental set-up**

**Output:** Design of Experiment (DoE)



**ZETA performance of at least 7 F&T runs, sampling of liquid & ice phase, analysis**

- Output:**
- Cryoconcentration profile of product, changes in buffer system as pH, conductivity
  - Dependencies of cPP and cQA



**Calculation of phase transition 2D**

**Output:** Scalable process information



**CFD Simulation support of cryoeffects, model verification**

- Output:**
- Valid prediction of cryoeffects 2D
  - Transferable models



**Geometrical definition of system, heat balances, ice formation distances**

**Output:** Scalable process information



**Multivariate Data Analysis in order to identify interdependencies of Process design to Product quality**

**Output:** Profound dependencies of process to Product Quality – basis for design space



Pilot scale system

## ADVANCED SERVICES

F&T process optimization and scale-up

### STARTING POINT:

- The product formulation buffer is finalized and the F&T process is known.
- There is a need to optimize F&T process and develop scale-up.

### STRATEGY:

The advanced services provide the definition of a 3D F&F process. The target is to define scale-up and process parameter for technology transfer to any scale.

### BENEFITS OF THE FREEZE SERVICES:

A 3D system provides systematic and holistic process understanding. Results of the F&T cycles are valid process data and transferable to any scale.



### ADVANCED SERVICES AT A GLANCE



**Study on relevant existing data**

**Output:** Establish prior knowledge as a starting point



**Identification of scalable process parameter**

**Output:** Process transfer to 3D pilot scale



**ZETA numbering of experiments, determination of experimental set-up**

**Output:** Design of Experiment (DoE)



**Performance of at least 7 F&T runs, sampling of liquid & ice phase, analysis**

- Output:**
- Cryoconcentration effects 3D
  - Dependencies of cPP and cQA



**CFD simulation of cryoeffects, model verification**

- Output:**
- Valid prediction of cryoeffects 3D
  - Transferable models



**Geometrical definition of system, heat balances, ice formation distances**

**Output:** Scalable process information



**Multivariate Data Analysis in order to identify interdependencies of process design to product quality**

**Output:** Profound dependencies of process to product quality – basis for design space