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Preparative Protein Crystallization from Impure Sources:
Incorporation of Impurities into the Crystals

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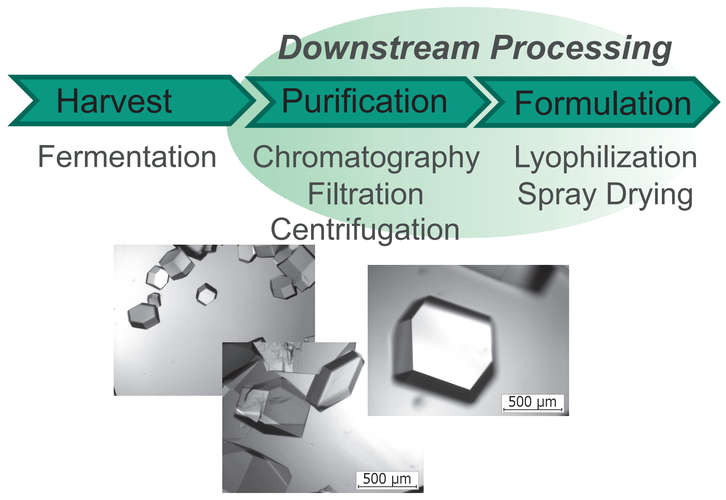
Introduction

Motivation

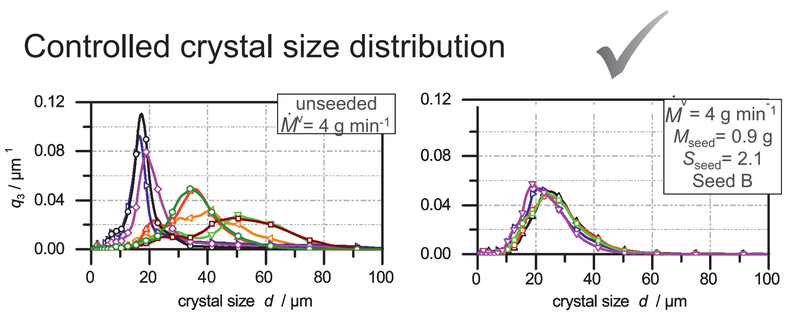
Crystallization as an alternative to Conventional Processes



Objectives

Reproducible and Controlled Protein Crystallization

Controlled crystal size distribution



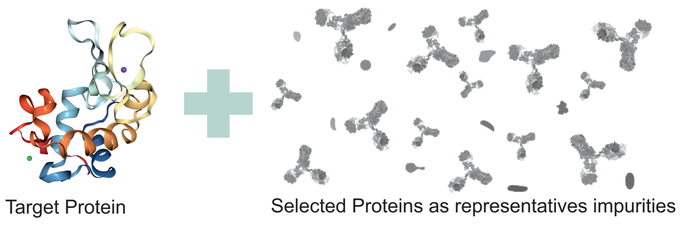
High process yield

High crystal purity

Further research is still required

Research Question

Process Yield and Crystal Purity in a Multicomponent Protein Mixture...



...How extensive is the incorporation of impurities into the protein crystals?

...Can the incorporation of impurities be avoided?

Research Proposal

Systematic Studies of the Distribution Coefficient

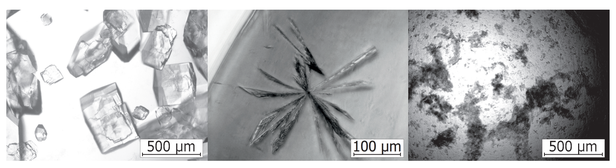
$$k_i = \frac{x_i^S}{x_{i,0}}$$

i: impurity, S: solid phase

I. Experimental Studies for Different Model Systems

Binary protein mixtures having different similarity degree e.g:

- *Mutant variations*
- *Molecular weight*



II. Solution Composition and System Conditions

Impurity content in the feed, pH and temperature

III. Process Operation

Vacuum Evaporative Crystallization

Evaporation rate, stirring speed

Methodology

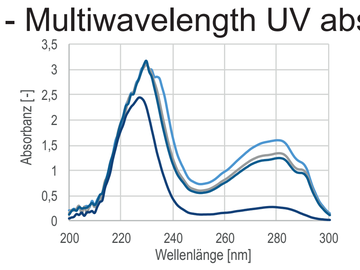
Model Substances

	System I	System II	System III
Target Protein	**Lysozyme (LSZ)**	**Lysozyme (LSZ)**	**Alcohol Dyhidrogenase (LbADH-WT)**
Impurity	**Bovine Serum Albumin (BSA)**	**β-Lactoglobulin (β–lgB)**	**Alcohol Dyhidrogenase (LbADH-Mutant)**

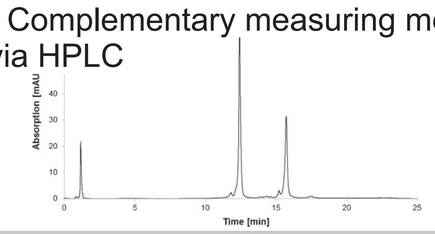
Analytics

- Multiwavelength UV absorption spectra

Protein concentration:

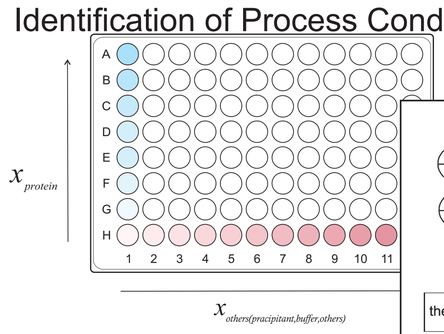


- Complementary measuring method via HPLC



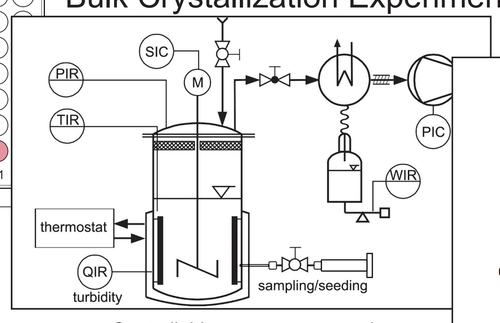
Experimental Approach

Identification of Process Conditions



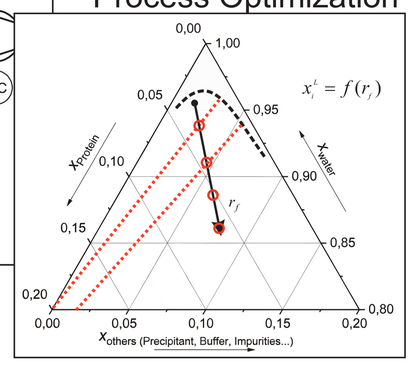
Determination of protein phase diagram by microbatch experiments

Bulk Crystallization Experiments



Controllable vacuum evaporative crystallization

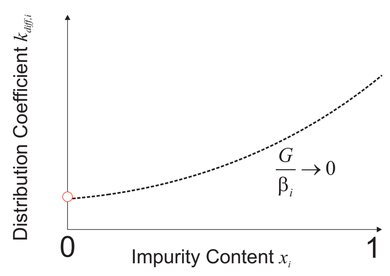
Process Optimization



Process conditions are modified to obtain crystals with less impurity content

Expected Results

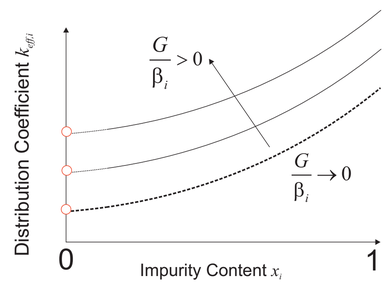
Distribution Coefficient Determination



In Equilibrium

$$k_i^* = \left(\frac{x_i^s}{x_i^L}\right)^* \Bigg|_{\frac{G}{\beta_i}\to 0}$$

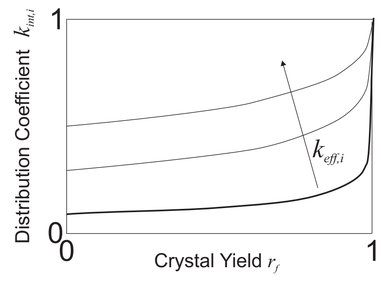
„Solution interaction ratio“



Dynamic

$$k_{i,eff} = \frac{x_i^S}{x_i^L}\Bigg|_{\frac{G}{\beta_i}>0} = k_{i,eff} = \frac{k_i^*}{k_i^* + (1-k_i^*)\,e^{\left(\frac{-G}{\beta_i}\right)}}$$

Crystal growth rate
Mass transfer rate

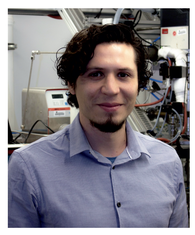


Integral

$$k_{int,i} = \frac{1-\left(1-r_f\right)^{keff,i}}{r_f}$$

Advance of the crystallization
i.e. Impurity content increases

Contact



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