SINE2020 General Assembly

WP 6 Macromolecular Crystallogenesis

Progress report from Julich

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Parma, 04.06.2018

Outline:

1 Vapour diffusion studies on Streptavidin with Biotin

2 Temporal evolution of the crystallization process of lysozyme with small angle scattering techniques

3 Temporal evolution of the crystallization process of lysozyme by means of Neutron diffraction

4 In-situ macroseeding crystallization apparatus

1 Vapour diffusion studies on Streptavidin with Biotin

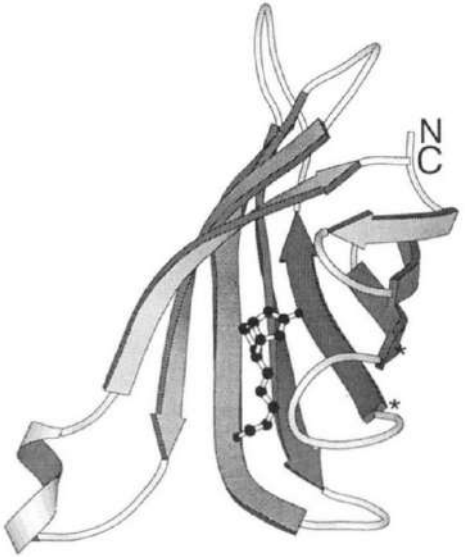
1. Vapour diffusion studies on Streptavidin with Biotin

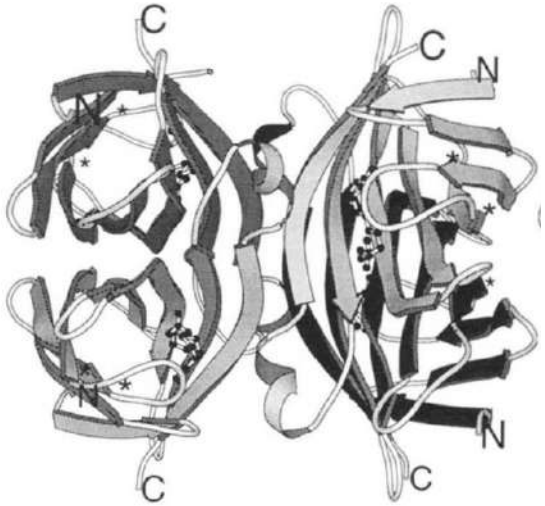
Homotetrameric protein (52.8 kDa)

Each monomer of the protein binds one molecules of Biotin (Vitamin H) non-covalently

$K_d \approx 10^{-14}$ mol/L

One of the strongest non-covalent interaction known





222 symmetry

The tetramer can be considered as a dimer of a dimer

What is the origin of the binding energy?

Tryptophan contact (Trp79,Trp92,Trp Trp 120):

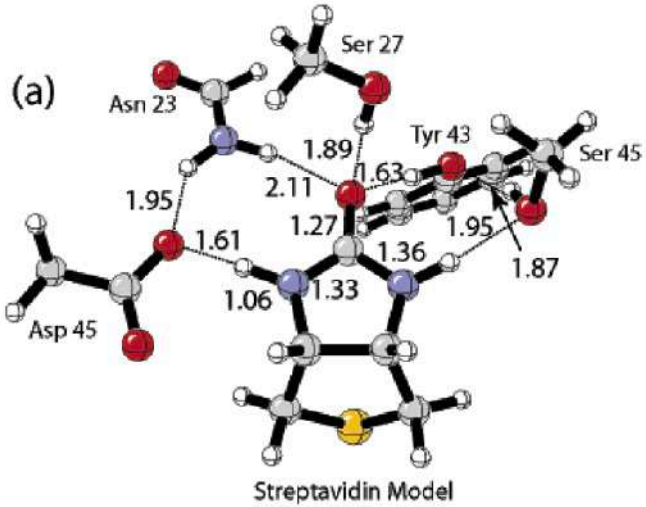
- Hydrophobic interaction
- Van der Waals interaction

J|A|C|S
ARTICLES
Published on Web 04/07/2007

The Origins of Femtomolar Protein–Ligand Binding: Hydrogen-Bond Cooperativity and Desolvation Energetics in the Biotin–(Strept)Avidin Binding Site

Jason DeChancie and K. N. Houk*

Specific hydrogen bonding interaction:
Asn23, Tyr 43, Ser27,Ser45, Asp45, Ser88, Asp128



Streptavidin Model

Asp45 key residue

Neutron crystallography to obtain information on the exact hydrogen pattern

1. Vapour diffusion studies on Streptavidin with Biotin

Hanging drop

- Streptavidin + Biotin at constant concentration of 26.6 mg/ml
- Buffer solution:10 mM Tris/DCl pD=7.5 with 100 mM NaCl added
- Different PEG concentration (10%-16%) in reservoir solution
- Reservoir solution: 100mM MES/NaOD, pH=6.0
- T=20°C

Best PEG concentration condition: 14 to 16 % PEG

1. Vapour diffusion studies on Streptavidin with Biotin

Hanging drop

- Fixed PEG concentration at 14%
- Protein concentration (23.9-45)mg/ml
- 1:1 mixture of reservoir and protein solution
- T=20°C

Best results: protein concentration in the range (30.5-35.5) mg/ml

1. Sitting drop method results:

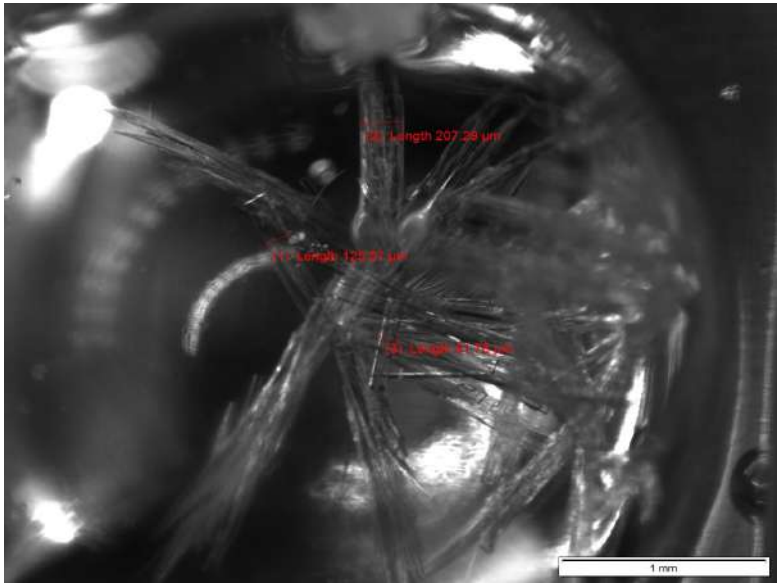
Condition 1:

Fixed concentration: 14% PEG

- Protein concentration:30 mg/ml
- Buffer: 10 mM Tris/DCl @ 7.5pD
- Salt concentration: 60, 70, 80,90 mM NaCl
- Reservoir solution:100 mM MES @ 6.0 pD
- T=20°C

Best results: 80mM NaCl concentration

Crystal volume: 1 x 0.15 x 0.15 mm^3



1. Sitting drop method results:

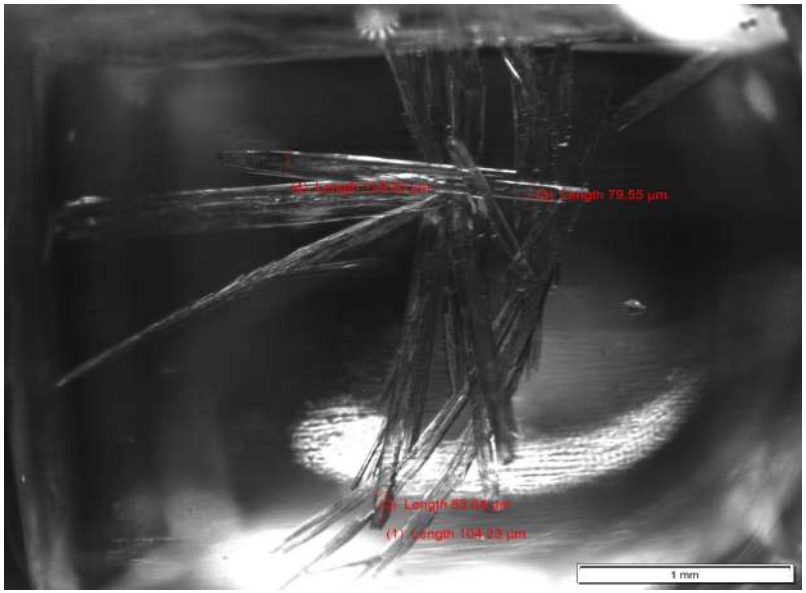
Condition 2:

Fixed concentration: 16% PEG

- Protein concentration:30 mg/ml
- Buffer: 10 mM Tris/DCl @ 7.5pD
- Salt concentration: 60, 70, 80,90 mM NaCl
- Reservoir solution:100 mM MES @ 6.0 pD
- T=20°C

Best results: 90mM NaCl concentration

Crystal volume: 1 x 0.13 x 0.13 mm^3



2. Temporal evolution of the crystallization process of lysozyme with small angle scattering techniques

CRYSTAL GROWTH & DESIGN

Article

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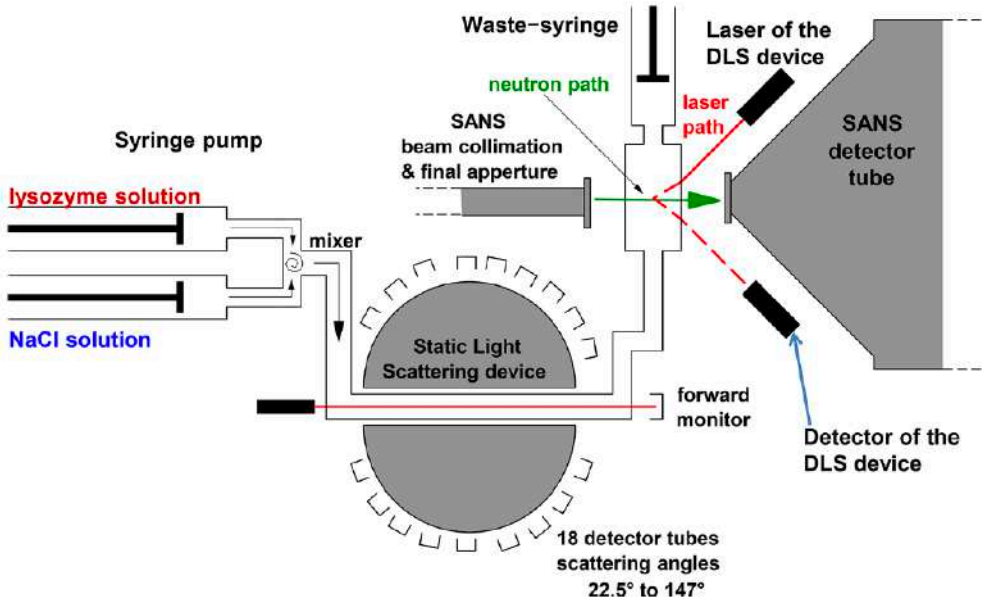
Crossover from a Linear to a Branched Growth Regime in the Crystallization of Lysozyme

R. J. Heigl,[†] M. Longo,[†] J. Stellbrink,[‡] A. Radulescu,[†] R. Schweins,[§] and T. E. Schrader*[,†]

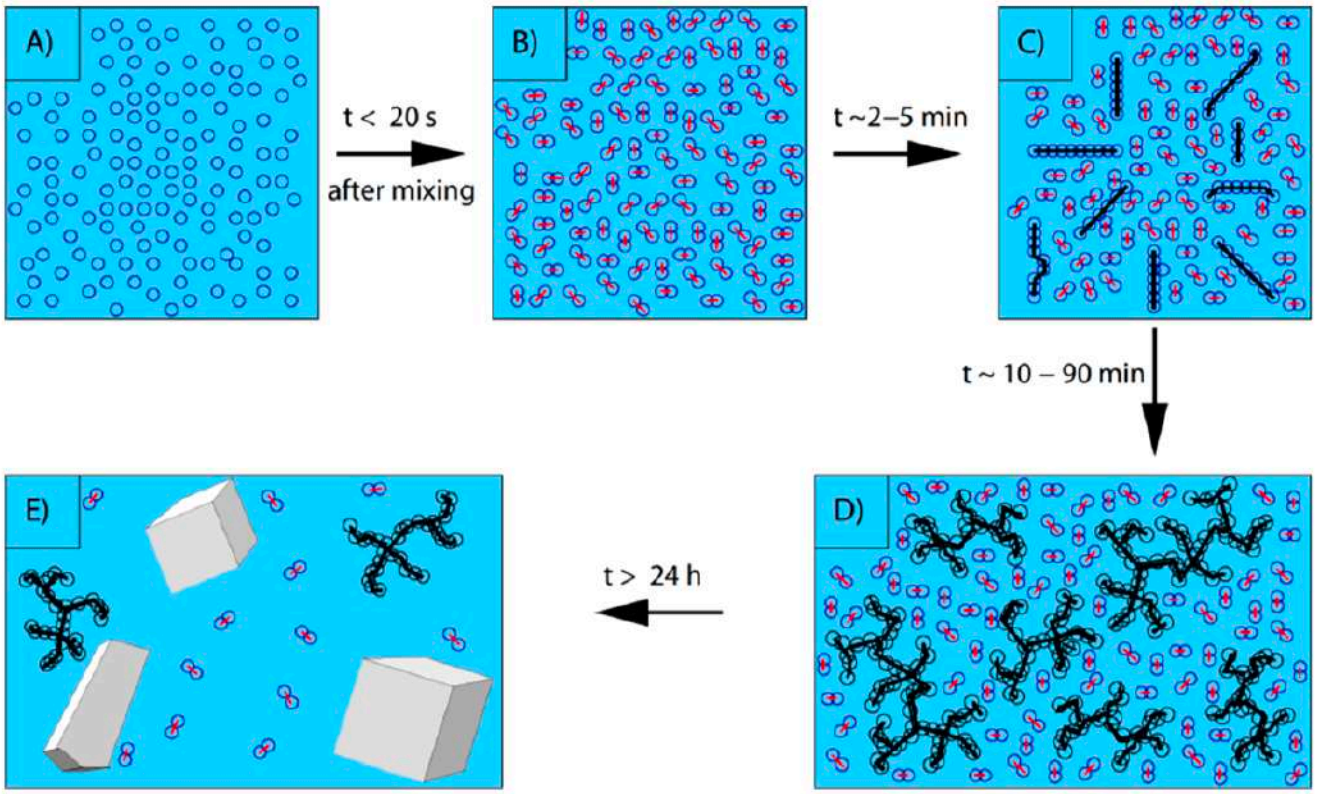
DLCA (Diffusion Limited Cluster-Cluster Aggregation)

$$E_B << K_B T$$

Every collision results in aggregation



2. Temporal evolution of the crystallization process of lysozyme with small angle scattering techniques



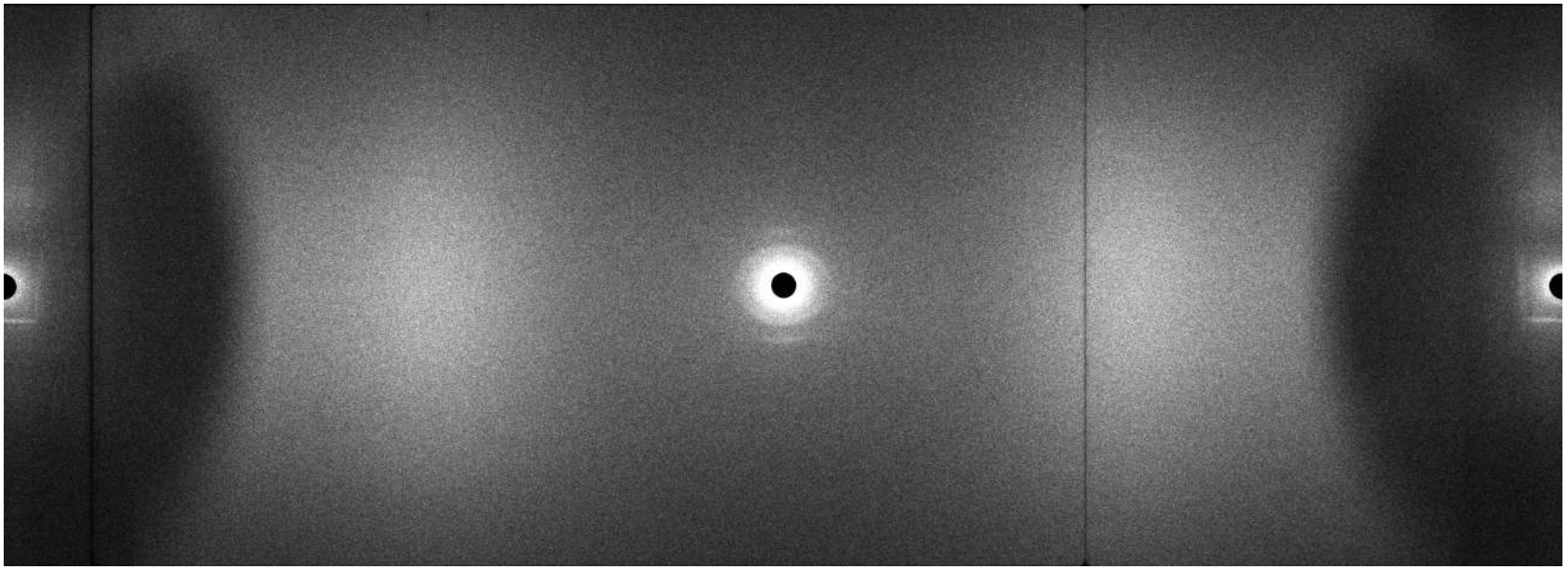
The crystal growth is due to not only by the addition of small dimers, but also of large fractal aggregates.

4. Temporal evolution of the crystallization of Lysozyme with neutron diffraction @ BIODIFF (FRM II,Garching)

Temporal evolution of the crystallization of Lysozyme with neutron diffraction @ BIODIFF (FRM II,Garching)

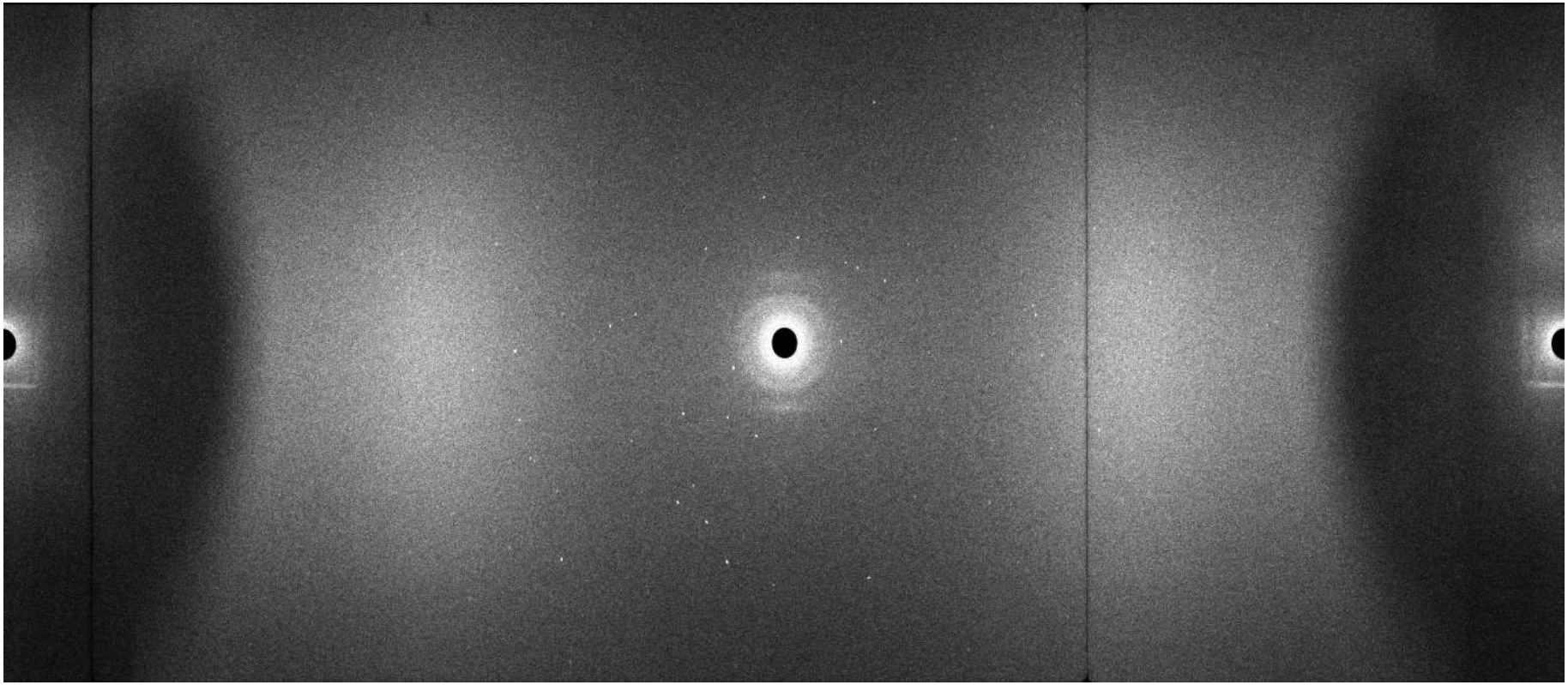
Goal: study of the origin and evolution of Bragg Peaks during the crystallization process of Hen-egg White Lysozyme

No diffraction pattern at the beginning of the experiment: protein solution!



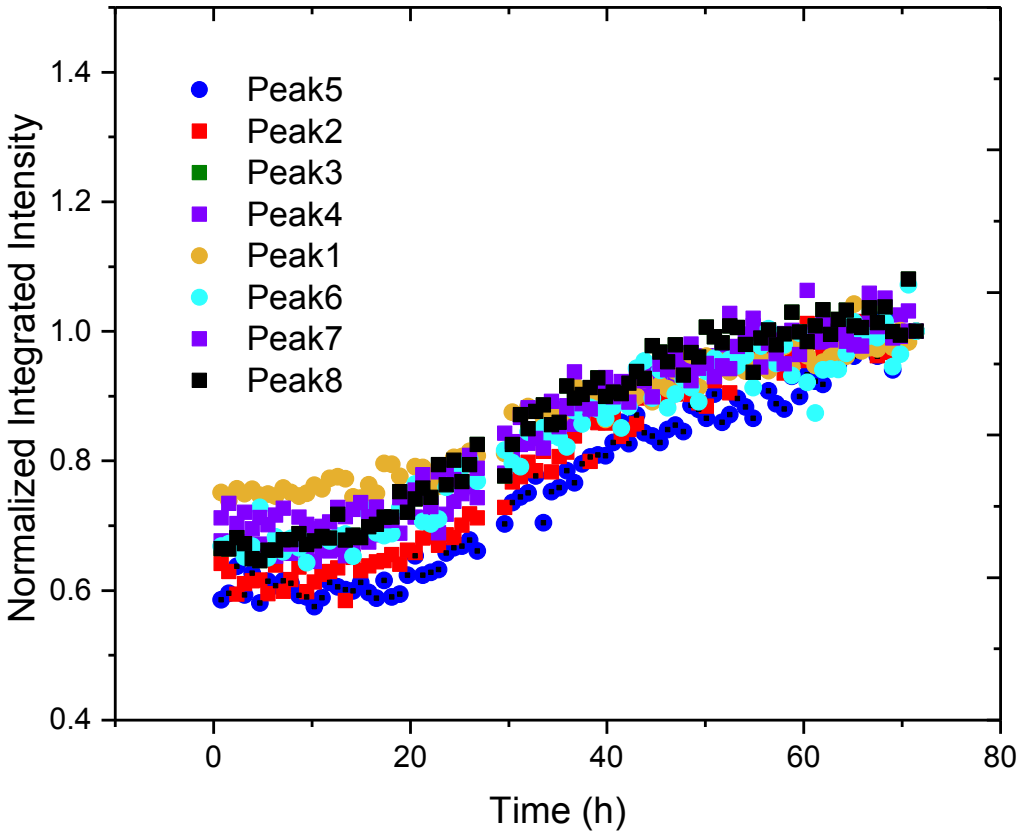
Temporal evolution of the crystallization of Lysozyme with neutron diffraction @ BIODIFF

Diffraction pattern after 70 hours:



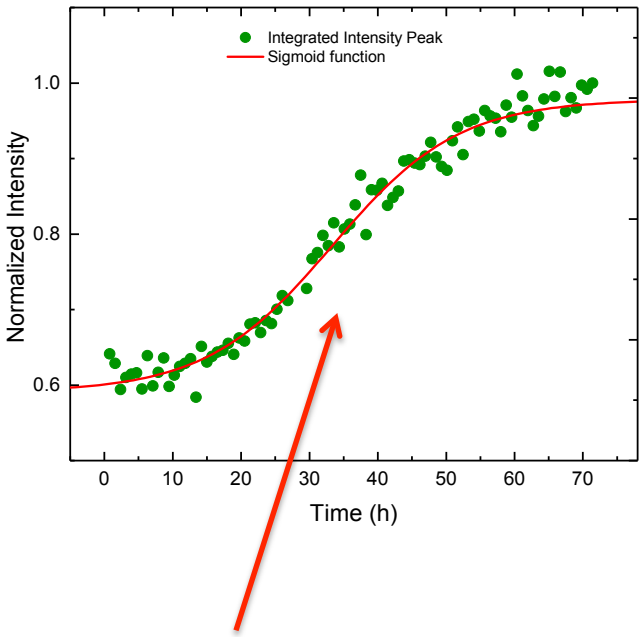
Temporal evolution of the crystallization of Lysozyme with neutron diffraction @ BIODIFF

Integrated intensity of the Bragg peaks as a function of the time:

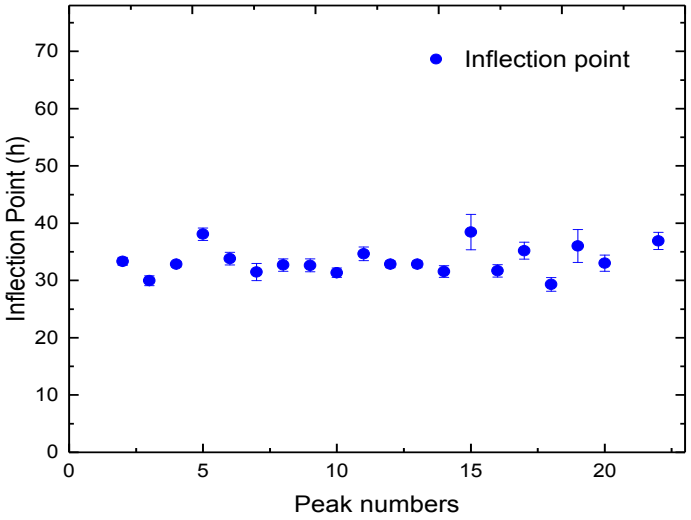


Temporal evolution of the crystallization of Lysozyme with neutron diffraction @ BIODIFF

The normalized intensity peak are well fitted by a sigmoid function:



Inflection point



Isotropic growth of the crystal

4. In-situ macroseeding crystallization apparatus

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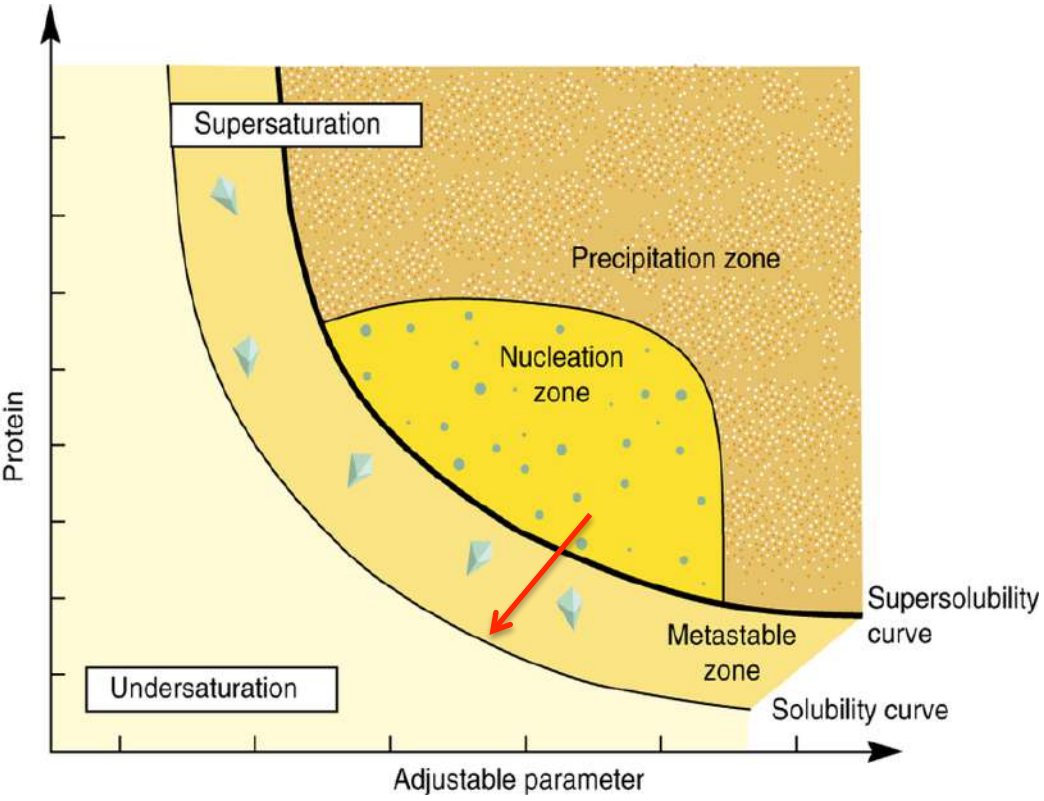


Nucleation:

Transition from a disordered state to an ordered one

How?

High level of supersaturation

Growth of large crystal:

Increasing of the crystal size

How?

Lower level of supersaturation

Separation of Nucleation and Growth

How?

Nucleated crystals are introduces as “seeds” in new drop at lower level of supersaturation.

Microseeding:

Transfer of submicroscopic seeds: too small to be distinguished

Disavantages:

It is not possible to control the number of transferred seeds

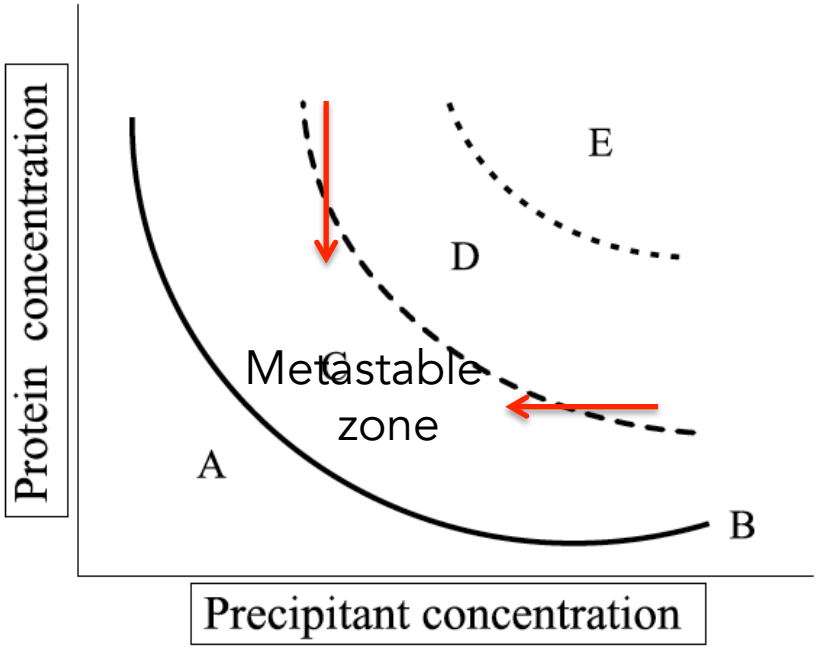
Macroseeding:

Transfer of single crystal

Disavantages:

The moving of the crystal seed is most likely not easy

How do we reach the Metastable zone from the nucleation zone?



$C_{PROTEIN}$=k Solubility^{-1}

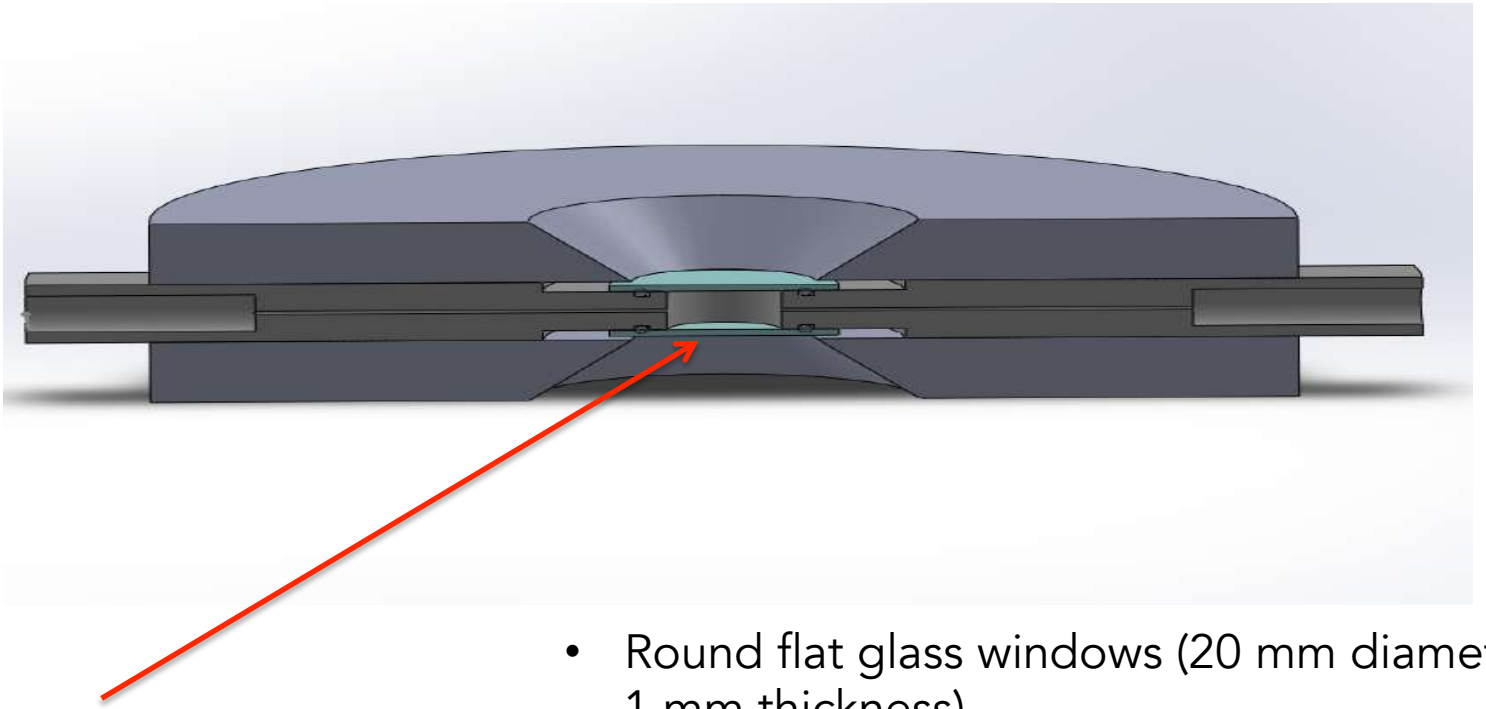
C_{PEG}=k Solubility^{-1}

- Lower the $C_{PROTEIN}$ and/or $C_{PRECIPITANT}$
- Seed in a completely different mother liquor

Seed without moving the seeds: in-situ seeding

In-situ macroseeding apparatus:

Change the crystallization condition without move the crystal:

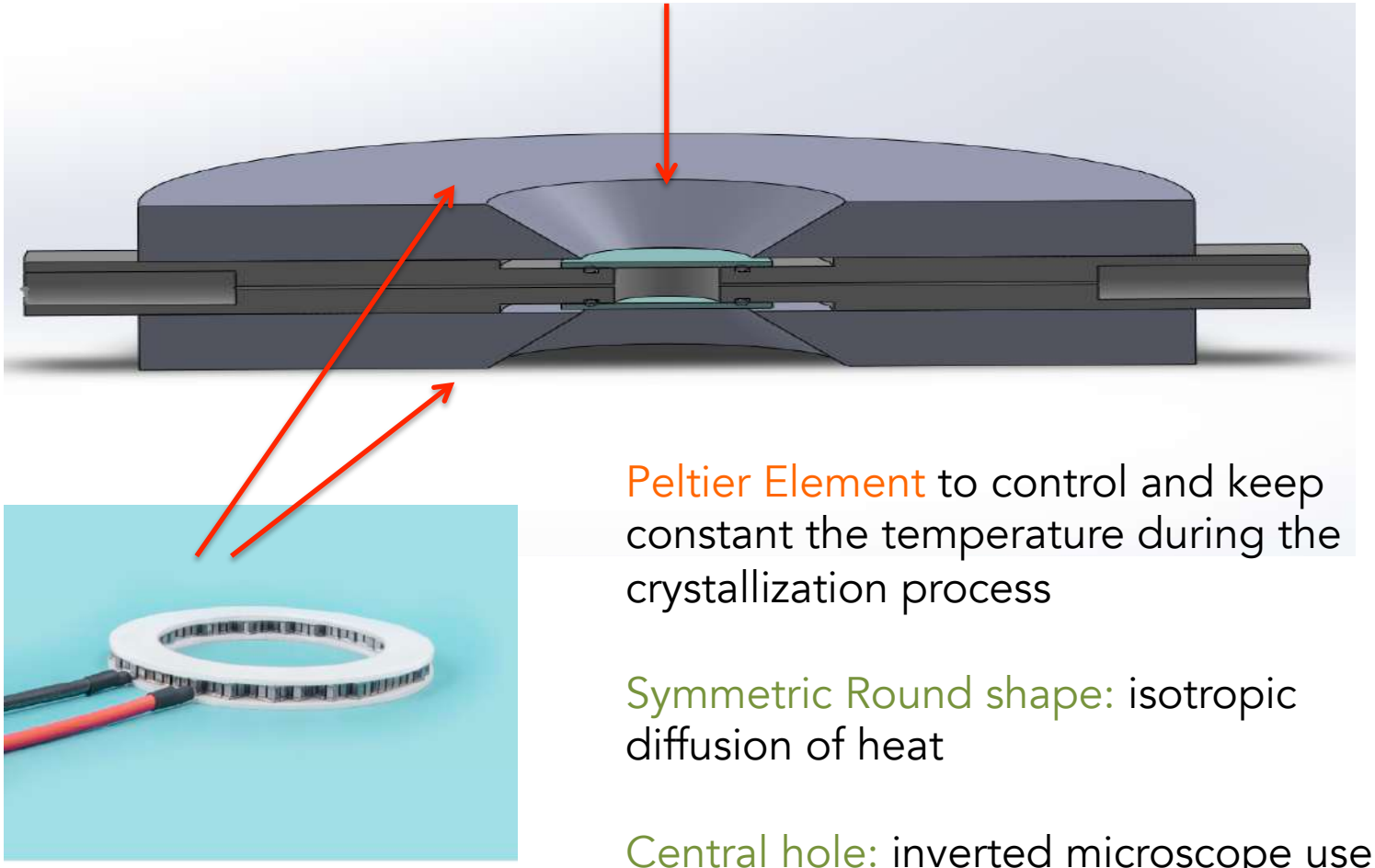


- Crystallization chamber

Internal volume ≈ 0.5 ml^3

- Round flat glass windows (20 mm diameter and 1 mm thickness)
- O-ring
- Internal spacer built by a 3-D printer

Inverted microscope to visualize the crystal during the growth

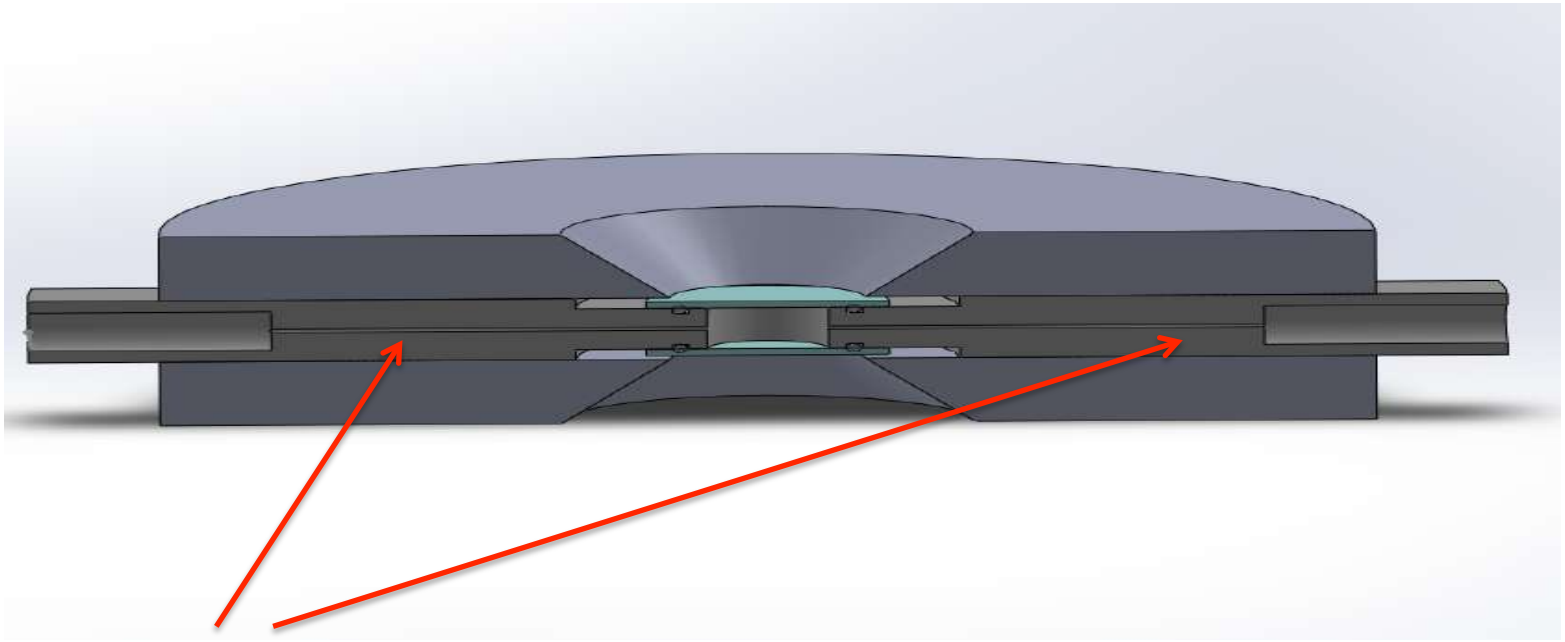


Peltier Element to control and keep constant the temperature during the crystallization process

Symmetric Round shape: isotropic diffusion of heat

Central hole: inverted microscope use

In-situ macroseeding apparatus:

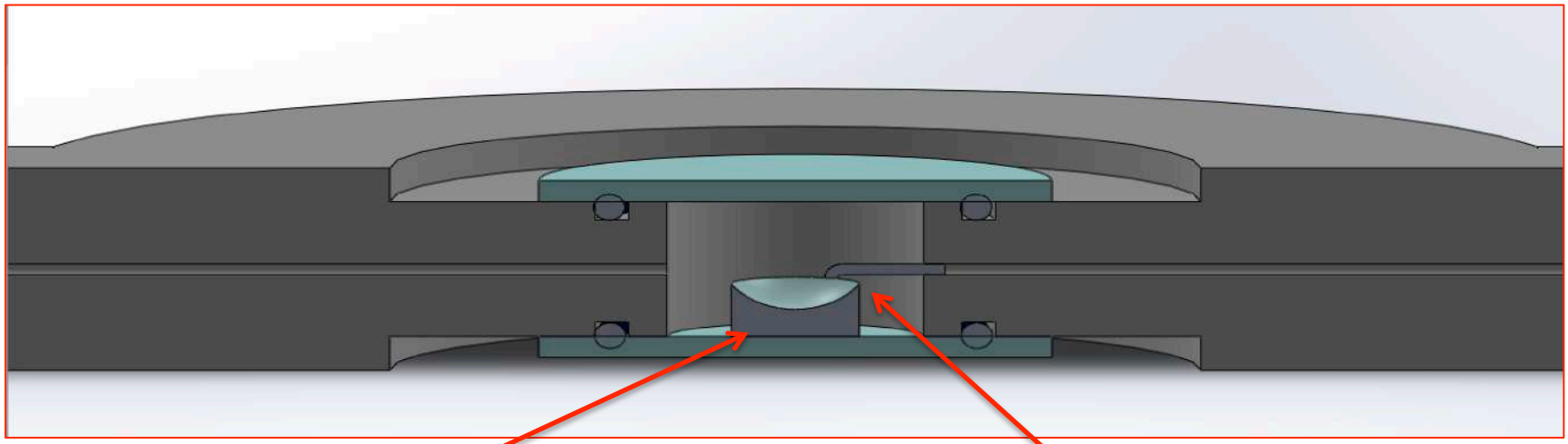


The exchange of the mother liquor is allowed by means of two capillarity built in the spacer

Prevent osmotic shock:

Continuous variation from solution 1 to solution 2 with a slow gradient

How can we avoid microconvective flux?



Sitting drop bridge in the crystallization chamber

Micro-pipe to change the drop Crystallization condition e.g. more protein
(3D built)

Powerful flexibility of the set-up due to the 3D printing option

Thanks for the attention!