A Ceiling Crystallization Kit for Optimizing the Quality of Protein Crystals

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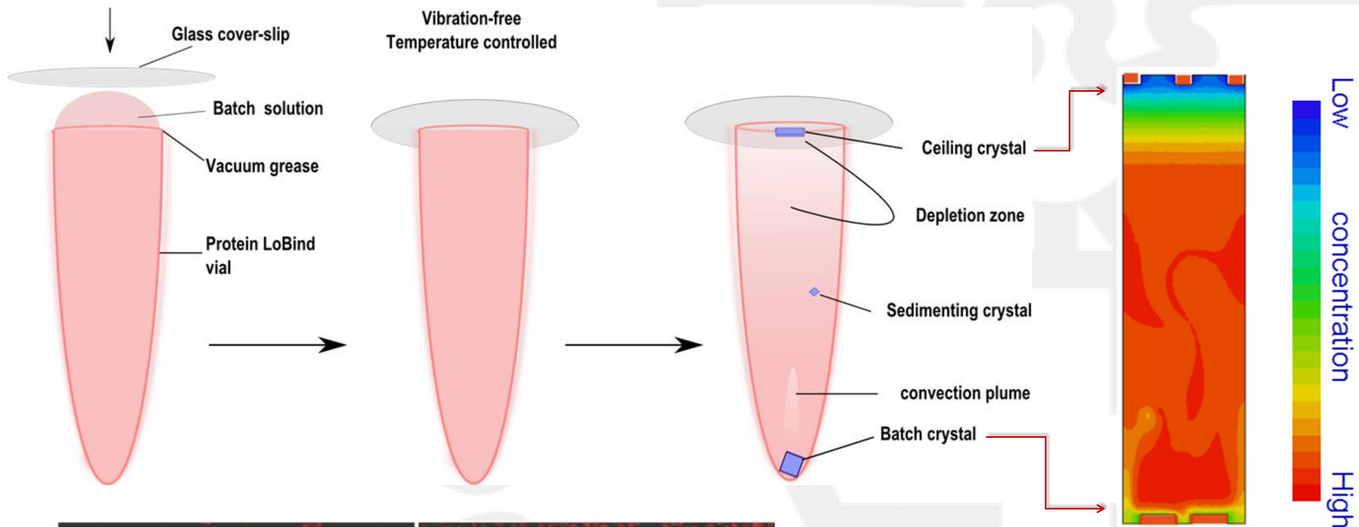
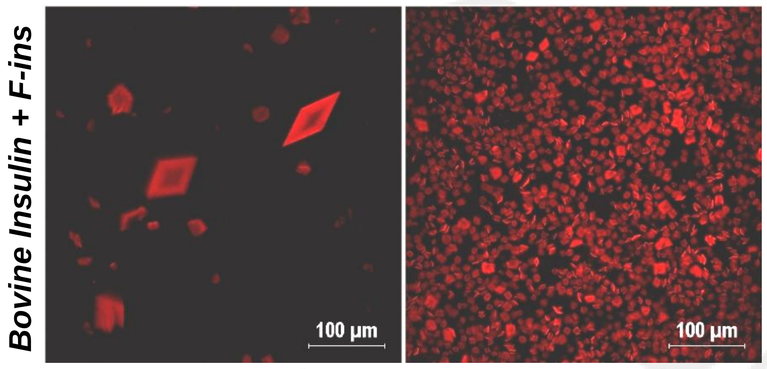
Introduction

The growth of high quality single crystals, yielding diffraction to the highest possible X-ray resolution, remains a bottleneck for macromolecular crystallography. Mass transport has a high impact on the final quality.

We have shown that diffusion-limited crystal growth can be effectuated using the **Ceiling Crystallization Method**. This method is simple, easily applicable and provides an entirely convection-free crystallization environment [1-2], similar to that in microgravity.

In order to make this method available to a large community, we have designed a convenient growth cell using a clamped rubber O-ring for an air-tight sealing, which also facilitates microscopic inspection of the growing crystals and afterwards their fishing and cryo-cooling.

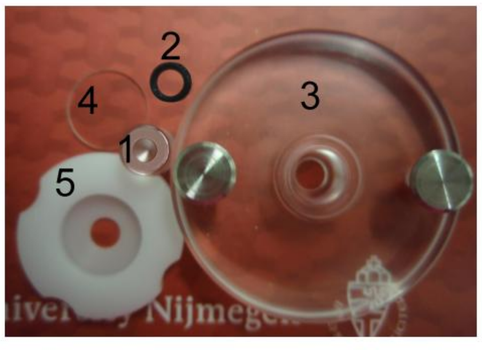


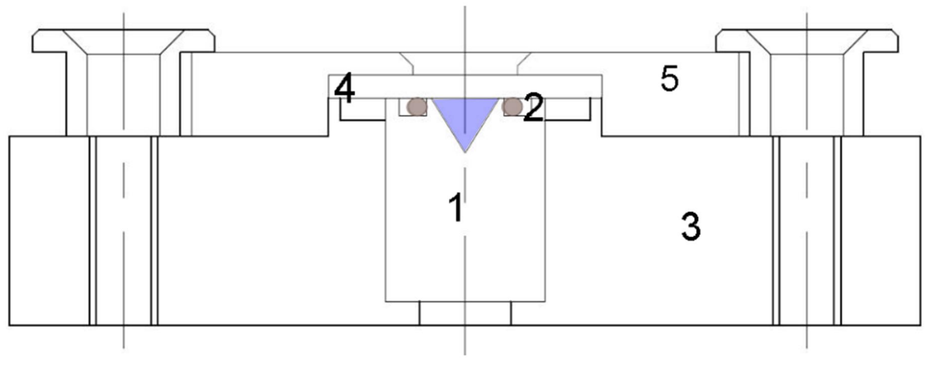


Ceiling (1.53 Å) *Batch (1.94 Å)*

Ceiling crystals grow through diffusive mass transport while batch crystals suffer from convection

Ceiling Crystallization Kit



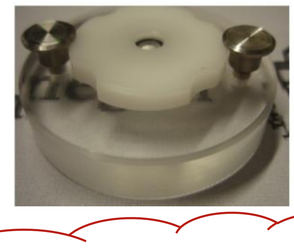


The ceiling kit consists of a growth vial (1), rubber ring (2), mounting plate (3), cover slip (4) and Teflon clamping plate (5).

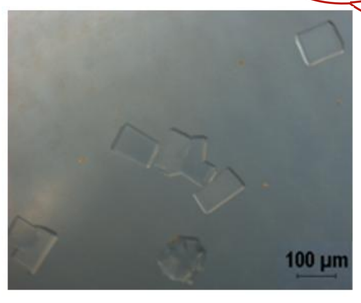
The crystallization solution fills the wedge-shaped volume in the growth cell. The protein crystals should grow underneath the cover slip.

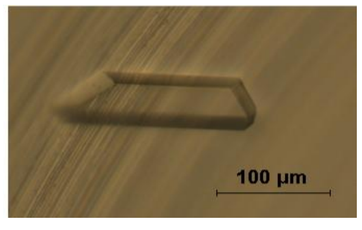
Application

1. Prepare the crystallization solution using the same protocol for setting (micro-)batch crystallization.
2. Insert a clean vial into the mounting plate and position a rubber O-ring into its respective groove.
3. Insert a thick glass cover slip into inner side of the clamping plate.
4. Overfill the vial with the crystallization solution and directly cover it with the clamping plate.



Protein crystals grow to sufficient size for X-ray diffraction experiments





Outlook

In our view, ceiling crystallization is not a replacement for the currently used screening methods. But once the appropriate conditions are found, the ceiling method could improve the crystal final quality, because of its convection-free crystallization environment . We hope that this kit will encourage the crystallization community to use the ceiling method and we encourage researchers to contact us for discussing the application of this hardware.

1. A. Adawy, E. Rebuffet, S. Tornroth-Horsefield, W.J. DeGrip, W.J.P. van Enckevort and E. Vlieg, *Cryst Growth Des, 2013,* ***13, 775−781.***
2. A. Adawy, K. Marks, W. J. de Grip, W. Van Enckevort and E. Vlieg, *CrystEngComm, 2013,* ***15, 2275-2286.***