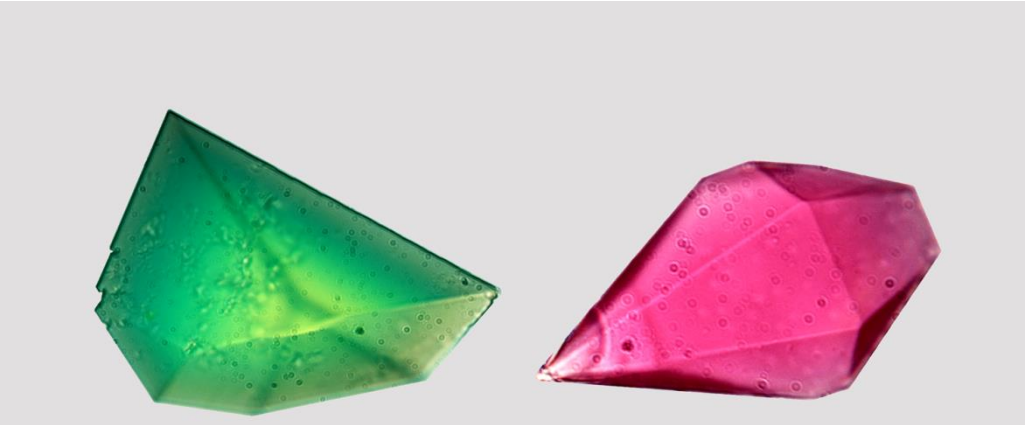
Biophysics Lab Course

ulm university universität ulm

Protein Crystallization

An introduction to the art of protein crystallization

Subject: Crystallization strategies, vapour diffusion techniques, supersaturation, nucleation



Experiment:

The protein lysozyme is crystallized with a vapor diffusion technique, here the hanging drop method. Buffers with different pH and precipitant concentrations are used on a 24 well plate to find optimal crystallization conditions. The success for the different solvent conditions is characterized by size and number of grown crystals in the respective wells..

1. Introduction

The knowledge about the exact molecular structure of a protein is a first prerequisite to understand the function of the protein on a molecular basis. It, however, reflects only the static structure, but not the dynamics of the protein, which are essential for the function of the protein. On the other hand crystal structure of the protein in most cases is a basic fundament for further investigations. To achieve a high degree of accuracy of the structure, good crystals are needed.

It is not enough to dissolve the proteins in water and wait until the solvent starts to evaporate so that the protein concentration will increase slowly till finally crystals will form, like in a procedure that everyone has surely more or less successfully tested with sugar or salt solutions.

2. Basics of Protein Crystallization

In the field of protein crystallization one has to face severe problems. First of all a very pure protein solution is a prerequisite for the successful crystallization of biological macromolecules.

The second challenging problem is to overcome the so called nucleation barrier. In general, nuclei can form only in supersaturated solutions.

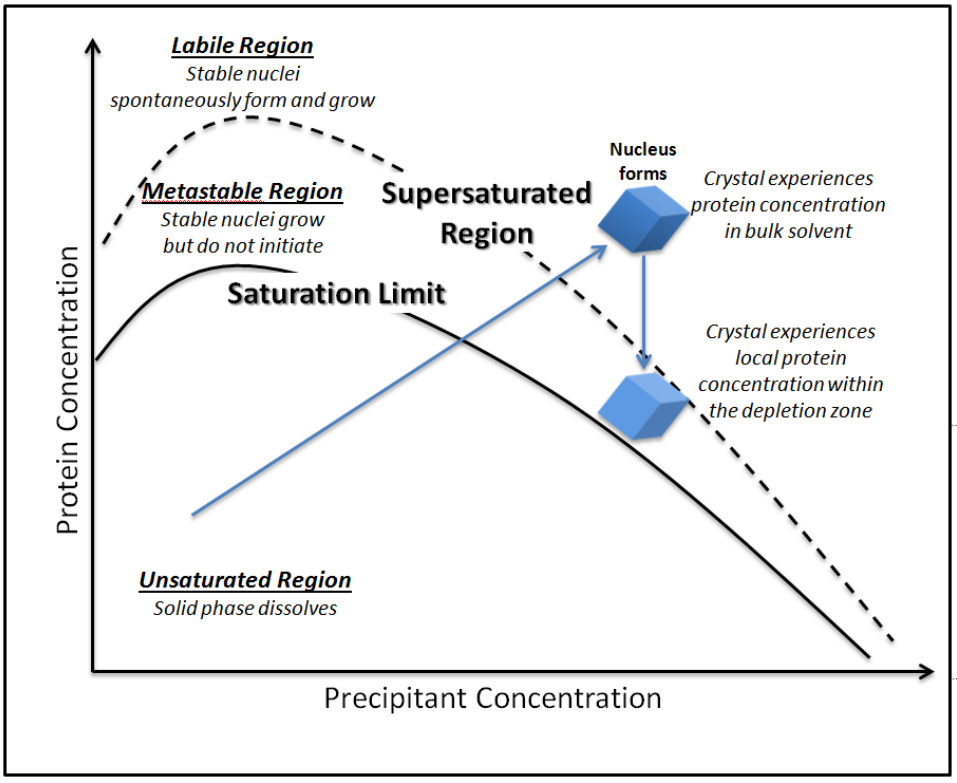


Figure 1 Phase diagram for the description of protein crystallization (figure based on McPherson, 1999). The arrows mark the change of concentrations with time during the process

However in this regime, the crystals grow in an undesired manner, as preferentially many small crystals are formed. To grow large crystals, the solution should be kept in the metastable region. Although in this region no crystal nuclei are formed.

To overcome this dilemma, the so called “vapour diffusion techniques” were developed (see Figure 2). In this technique a droplet containing purified protein, buffer and precipitant is in a sealed chamber together with a much larger reservoir of the same buffer containing a higher concentration of precipitant. Due to the difference in the precipitant concentration in the protein droplet and reservoir, transport of water molecules in the direction of the reservoir occurrs (arrows in Figure 2). The precipitant concentration in the drop increases and at some point the nucleation barrier is exceeded. It is the key to the formation of crystal nuclei. As a consequence, the protein concentration decreased in the drop, and ideally it approaches the metastable region (see arrow in Figure 1). In this region no new crystal nuclei are formed and the existing nuclei can continue to grow slowly. It causes formation of a few large crystals. There are two common methods for crystallization by vapour diffusion: the "sitting drop" and the "hanging-drop" method (see Figure 2).

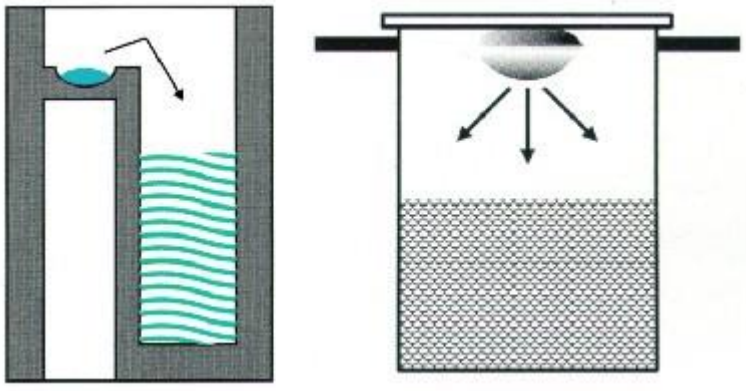


Figure 2 The vapor diffusion crystallization techniques: sitting drop (left side) and hanging drop (right side) (figure adapted from McPherson, 1999).

Another important method for protein crystallization is the "batch crystallization", in which a solution is prepared so that the protein concentration is already in the nucleation region (see Figure 1). By putting the protein solution drop under an

isolating layer of oil, the crystallization takes place under defined and constant conditions (no increase of the precipitating agent concentration with time).
The "microbatch under oil" is however in principle more comparable with the vapour diffusion method, at least in the use of water permeable oils, such as silicone oil. Drops of protein solution and precipitating agent solution are pipetted together under an oil layer and then stored at 18°C. The only difference in between vapour diffusion and microbatch under oil is that the removal of water is not stopping at a certain concentration (water concentration in buffer in reservoir), as it is in the case of the hanging and sitting drop methods. The drop can therefore completely dry out. The rate of water transport through the oil may be influenced by the addition of various other oils. The addition of waterproof paraffin oil slows down the diffusion of water molecules significantly.

3. Experimental procedure

The formation of lysozyme crystals using the hanging drop method, under different pH conditions and precipitant concentrations (here NaCl), will be investigated.

Materials:

Lysozyme (20 mg/ml) in H2O
20% NaCl solution
Distilled water

Buffer solutions:

250mM sodium acetate, pH 4.0
250mM sodium acetate, pH 4.4
250mM sodium acetate, pH 4.8
250mM sodium acetate, pH 5.2

Preparation:

1) Prepare the plate rows (1 per group): label the plate with the date, your group number and names; label each well according to the attached diagram
2) Pipette proper buffers into the wells according to attached diagram
3) put ring of silicone grease around the individual wells
4) Pipette 1 µl of protein solution on the cover slip.
5) Pipette 1 µl of the buffer from the respective reservoir into the protein droplet.
6) Turn the cover slip (→ hanging drop). Be careful, so the drop does not slip out of the middle!
7) Seal the reservoir with the cover slip.
8) Label the well plate and store it at room temperature.

Observation and Characterization:

1) Observation for ~ 6h: Are crystals already visible?
   If yes, in which wells?

2) Characterization of all wells (in tabular form) after 48 hours:
   Notate number and size of the crystals in every well, including photographic documentation of the best crystals.
   (Program: FutureWinJoe, use comparative lenght-scale!)

Literature: McPherson, A. (1999). Crystallization of Biological Macromolecules. New York: Cold Spring Harbor Laboratory Press.

JBS Proteinkristallisations Starter Kit

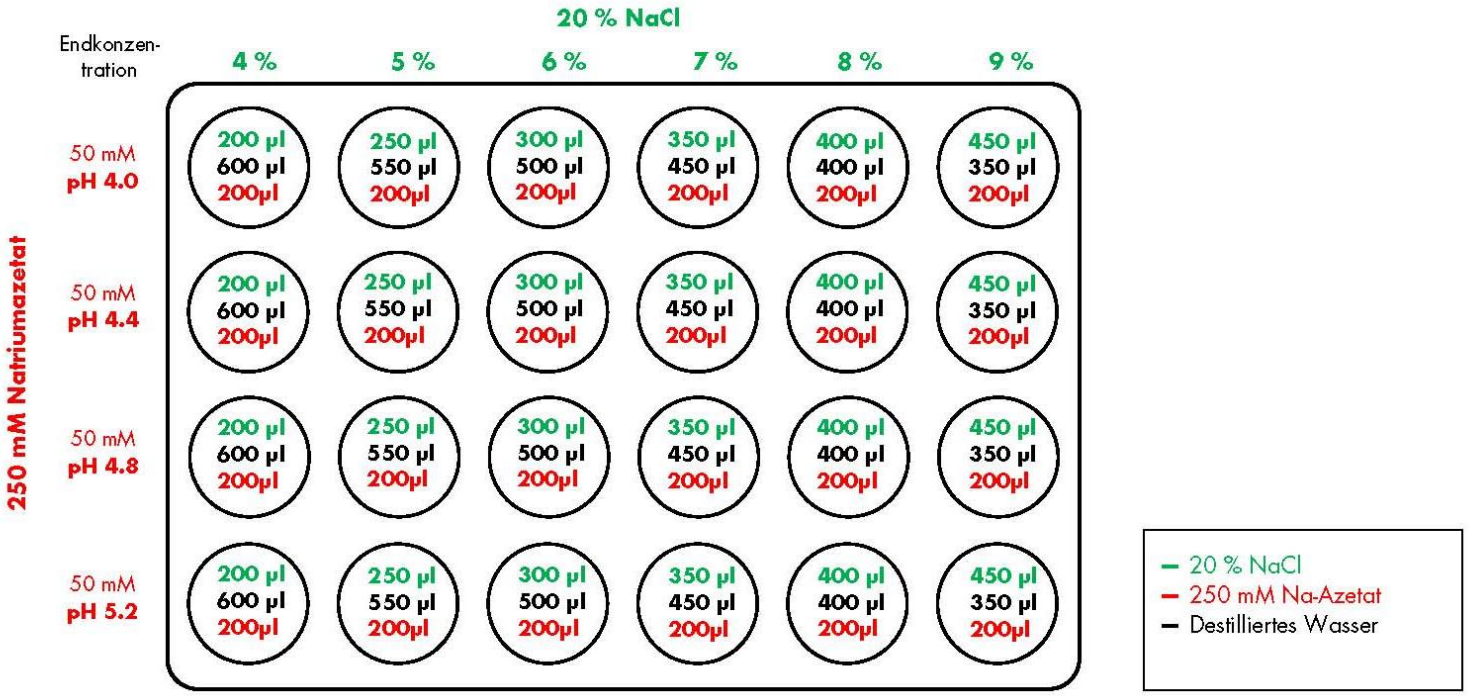


Abb. 4: Pipettierschema für das Hanging-Drop-Experiment