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The crystallization of lysozyme

Theoretical part:

Structural biology is an interdisciplinary research field, which studies the molecular structure and dynamics of biological macromolecules. For many decades, **X-ray crystallography** has been the predominant structural method, since it achieved the resolutions incomparably higher than any other structural methods. Nowadays, **cryogenic electron microscopy** has overtaken this prominent role because of the numerous advantages over X-ray crystallography. Cryo-EM removes the need for crystals, requires less sample and can tolerate some impurities. These considerations permit the study of diverse and large complexes. Because of improved hardware and software, the resolution provided by cryo-EM is getting closer to those provided by X-ray crystallography. Moreover, cryo-EM structures include large molecular complexes up to the whole structures in the cell **(cryo-EM tomography)**.

In 2017, the Nobel Prize in Chemistry was awarded to Jacques Dubochet, Joachim Frank and Richard Henderson for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution.

Protein crystallization

During this task, you will become familiar with protein crystallization, which has been mentioned as the first and most demanding step in X-ray crystallography. The preparation of pure (> 90%) and homogenous protein of high concentration (> 10 mg/mL) is crucial as well as the determination of the best precipitant/crystallization condition. The precipitant is usually chosen and optimized based on results of crystallization screens when hundreds of conditions

are tested. The relationship between protein and precipitant concentration is depicted as the **phase diagram**. The popular method for the protein crystallization is **the hanging drop vapor diffusion technique**.

Phase diagram of protein crystallization. The supersaturated region is divided into a metastable and unstable zone. The metastable region consists of a crystal growth and nucleation zone. In the crystal growth zone only transient nuclei are formed that do not reach the critical size. If the supersaturation proceeds, the nucleation zone is reached, where nuclei can achieve a critical size and become stable (nucleation; the black arrow pointing to the nucleation zone). As the nuclei become larger and crystals start to appear, the protein concentration in the solution is decreased and the system reaches the crystal growth zone again (the black arrow pointing downwards).

The hanging drop vapor diffusion method. A drop composed of a mixture of protein and precipitant solutions (both diluted by each other) is placed in vapor equilibration with a liquid reservoir of precipitant solution. To achieve equilibrium, water vapor leaves the drop and eventually ends up in the reservoir. As water leaves the drop, the sample undergoes an increase in relative supersaturation. Both the protein and precipitant increase in concentration. Equilibration is reached when the precipitant concentration in the drop is approximately the same as that in the reservoir.

Practical part:

A/ Lysozyme crystallization

1/ The assistant provides you with 1 M sodium acetate, pH 4.5, and 1 M sodium chloride, from which you must prepare **10 mL of the condition A** (0.6 M sodium chloride, 0.1 M sodium acetate, pH 4.5) and **10 mL of the condition B** (0.9 M sodium chloride, 0.1 M sodium acetate, pH 4.5).

2/ The assistant provides you with **50 mg/mL of lysozyme** in 0.02 M sodium acetate, pH 4.6.

3/ Prepare the crystallization plate with **3 µL-hanging drops of the 1:1, 1:2 and 2:1 ratios of protein/precipitant**.

3/ Check the hanging drops **every day** under the microscope.