Protein Crystallization: Basic Approach

1-Protein characterization

To start a protein crystallographic project we need crystals and for this we need to crystallize the protein. However, prior to starting the crystallization, we first need to purify the protein in relatively large quantities (few milligrams). High purity and homogeneity of the sample are crucial for the crystallization to be successful. For crystallization, first of all we need a protein of high purity in a homogeneous, low polydispersity state. These characteristics are crucial for the success of the project, and partially can be controlled by us. Purity is determined by standard laboratory techniques, while the presence of aggregates or polydispersity can be determined by methods such as **dynamic light scattering** (DLS) or **small-angle X-ray scattering** (SAXS). If it is the first time the protein has been purified, it may also be a good idea to run **CD spectroscopy** prior to crystallization to make sure that the protein is correctly folded and active (also using activity measurements). This can be true even for highly soluble proteins, which may be soluble and monodisperse even in an unfolded state. Another parameter, which needs to be controlled is the stability of the protein in different buffers and in the presence of various ligands or so called additives. A screening, for example following the method developed by the SGC and published in Nature Protocols (https://www.ncbi.nlm.nih.gov/pubmed/17853878), will help us to choose the most optimal conditions for the protein to be stable. These may include the type of buffer, presence of salts, co-factors, etc. One should keep in mind that proper and

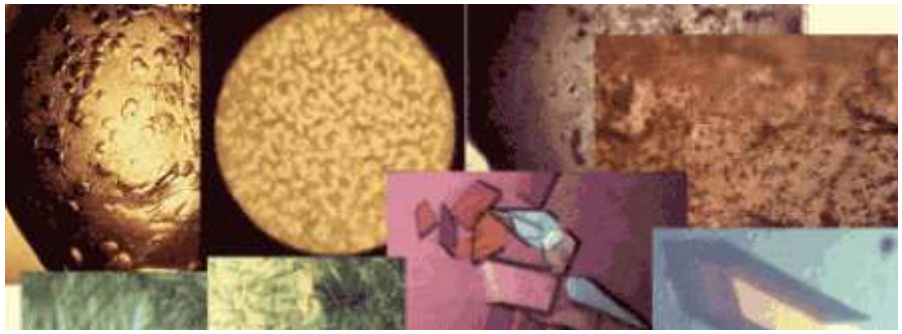
detailed characterization of a protein preparation in crystallography is much more important than in biochemical experiments, when often partial purity and the activity of the protein in question are taken as sufficient criteria for the quality of the preparation.

2-Protein crystallization

The laws of physical chemistry and thermodynamics control the process of crystallization. Thermodynamically, protein crystallization is not very different from the crystallization of NaCl. In both cases, we need to bring the solution into a supersaturated state after which the salt or the protein will hopefully start to crystallize. However, protein crystallization methods are very different. In the case of NaCl supersaturation may be achieved by first preparing a saturated solution of the salt at some high temperature (for example, 40 deg Celsius) and then leaving it at room temperature for some time. Since at room temperature the solubility of the salt is lower than at 40 deg, the concentration we got at 40 deg will be much higher than the solubility limit at room temperature. At room temperature the solution will be in a thermodynamically metastable state. The result is that after a short while salt crystals will be found at the bottom of the glass. In the case of proteins, heating is not a method to use, proteins may quickly denature at high temperatures (unless it is a protein from a thermophilic organism). We are helped by the fact that protein solubility depends on many factors and not only on temperature. Among these factors is the concentration and type of salt present in the buffer, the pH of the buffer, the presence of possible co-factors, etc.

Depending on the protein, different crystallization methods may be used to bring the solution into supersaturation, normally through a gradual decrease of the solubility of the protein. The most common way to reduce protein solubility for crystallization is by the addition of so-called precipitants. A precipitant binds water molecules, essentially competing with the protein for water, thus reducing water availability, which mimics higher protein concentration. Popular precipitants include polyethylene glycol and ammonium sulfate, probably the most widely used, but there are many other precipitants. When precipitant concentration is gradually increased, for example by using the method of vapor diffusion, the amount of solvent available for the protein is decreased, which in turn may lead to protein precipitation, or if the conditions are correct, to crystallization of the protein.

The pictures show examples of what may happen in a protein crystallization drop. For details see Terese Bergfors site on protein crystallization.



There are many empirical rules and different **protein crystallization methods**, many of which have been developed from experience, while others were developed during the recent years, much due to the efforts of structural genomics consortia, like SGC (http://www.thesgc.org/).

A general problem in protein crystallization is that the crystallization condition, which includes a combination of a right pH, ionic strength, temperature, protein concentration, the presence of various salts, ligands or additives, the type of precipitant and the actual crystallization method to use (hanging drop, sitting drop, dialysis, etc.), are practically impossible to predict in advance. For example, a ligand, which may be a co-factor, a substrate analogue or an inhibitor, may contribute substantially to the stabilization of the protein and increase its chances to crystallize.The solution to this problem was introduced around 20 years ago, with the introduction of crystallization screening methods. In this case, a number of pre-made solutions with different precipitants, buffers, salt concentrations, etc., are tested to reveal a special "beneficial" conditions, which may yield crystals. Commercial screens, like those from Hampton Research (http://hamptonresearch.com/Default.aspx) or Molecular Dimensions (http://www.moleculardimensions.com/default.asp), may be used. A protein crystallization experiment is set up with all these solutions, for example by using the hanging-drop variant of the method of vapor diffusion. The drops are checked periodically for the presence of crystals. Crystals may grow within few hours, few days or even weeks. When crystals are obtained in these screens, the crystallization conditions usually need to be further optimized to obtain larger single crystals suitable for X-ray diffraction experiments. In the following page (../../Experimental/Experimental/crystallization-tools.html) we will continue with protein crystallization method and discuss some of the techniques and tools.
