Part II
Theoretical background on Protein Crystallography
Chapter 5

Protein crystallization

5.1 Crystallization techniques

Like salts, proteins can form crystals if their concentration in solution exceeds their solubility. The interaction between protein molecules, however, is much weaker than for small (especially ionic) molecules, and crystallization is a rare event compared to disordered aggregation. Finding appropriate conditions is one of the bottlenecks towards obtaining a crystal structure. The phase diagram in protein crystallization is a schematic representation of how protein and precipitate concentration are related. Protein crystals are formed in supersaturated solutions. Low protein and/or precipitate concentrations will cause undersaturation that will not produce protein crystals (Figure 5.1).

![Image of the phase diagram in protein crystallization]

**Figure 5.1:** The Phase Diagram in Protein Crystallization
The red line that separates undersaturated conditions from supersaturated is known as the solubility curve. A benefit of determining the solubility curve is that it can help guide you when analyzing your crystal growth conditions. A crystallization setup that is undersaturated or in the metastable zone will appear clear, however, the latter has the possibility of crystal growth if seeded.

The phase diagram is often broken down into 4 distinct zones one of which undersaturated we have already covered. Precipitation is when the protein comes out of solution as an aggregate and therefore is not useful for crystallographic studies. The labile zone (or nucleation zone) is important since this is where crystal nucleation and initial growth occur. As the crystal forms the protein concentration will be depleted causing one to move from the labile to metastable zone (Figure 5.1).

**Vapour diffusion** Because of its simple set-up, crystallisation trials for proteins are often carried out by the vapour diffusion method. Thereby a small volume of protein solution is brought into a closed system with a large reservoir solution. A concentration difference between the reservoir and the sample causes vapour diffusion between the two solutions until the vapour pressure in the system is at equilibrium. Hence the change of conditions in the protein solution can bring about the precipitation of the protein. Under the right conditions this happens by the formation of crystals; in most cases, however, by amorphous aggregation. In practice, 1–10 μl of the purified protein is mixed with the reservoir solution which contains a precipitant at concentration. If the ratio is 1:1, both protein and precipitant concentration are halved upon mixing and will return to the initial concentration at
equilibrium. But due to the presence of the precipitant the solubility of the protein can now be lower so that it precipitates. One can vary the mixing ratio and even have different constituents for the reservoir solution and the solution the protein is mixed with (56).

The two most common vapour diffusion techniques are the sitting drop and the hanging drop method. In the sitting drop method the protein-precipitant mixture is placed onto a small depression or bridge on top of the reservoir. In the hanging drop method, the protein sample is prepared on a cover slip that is turned upside-down before sealing the well, as illustrated in Figure 5.2.

![Crystallization methods](image)

**Figure 5.2:** Crystallization methods. Crystallisation by vapour diffusion is based on equilibration via the gas phase so that only volatile compounds can interchange. The two most common methods are the sitting (left) and hanging drop (right).

**Screening** Whether or not crystals form depends on a large manifold of parameters and there is no or very limited a prior information about which ones to choose. The following parameters (and more) can influence crystallisation:

- **nature and concentration of precipitant**
- **protein concentration**
• composition of protein and reservoir solutions, i.e., pH, ionic strength etc.
• temperature

Screening kits mostly scan dependencies on chemicals, either in a broad, random manner (matrix screens) or systematically around certain values (grid screens, e.g. pH vs. ionic strength).