IIT Guwahati Lecture 33

Course BT 631

Protein Structure, Function and Crystallography

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Protein Crystallisation Cocktails

Crystallisation cocktails

- The crystallisation cocktails function to reduce solubility of protein to the point of super-saturation.
- The reagents present in crystallisation cocktails are divided in different classes: precipitant, buffer, additive, detergent and any other specific reagent.
- A set of cocktails for crystallisation screening contains combination of components from different classes at varying concentrations.

Protein Crystallisation Cocktails

Precipitants

Salt precipitants

Salts are the most common precipitants in use. At low concentrations salts increase solubility (salt-in) but at higher concentrations they reduce the solubility (salt-out). At high concentration salts also act as cryo-protectants. e.g. Ammonium sulfate, Sodium Chloride etc.

Organic precipitants

Other common precipitants include organic polyalcohols and polyethylene glycols (PEGs). They compete for the water molecules around the protein molecules causing proteins to interact with each other. ***Fortunately, the side effect of using PEGs is that they also act as cryo-protectants.***

Low molecular weight alcohols and ketones *e.g.* propyl alcohol, acetone are also used as precipitants. They act by lowering the dielectric constants, hence polarity of the solvent. However, mostly these are volatile and hard to maintain their concentration, therefore **2-methyl-2, 4-pentanediol (MPD)** is a very popular reagent which is not volatile.

Protein Crystallisation Cocktails

Buffers

The function of buffers in crystallization cocktails is to establish a certain pH and provide a local charge distribution on the protein. Thus, the buffer should have higher concentration so that the crystallization cocktail can derive the pH of the crystallization drop.

Additives, detergents and cofactors

Additives are the wide variety of reagents which facilitate the crystallization. The additives such as β-mercaptoethanol (that prevents oxidation of cysteine), heavy metal chelating agents like EDTA (Ethylenediamine-tetraacetic acid) and EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid) are also used.

Physiologically or biochemically important small molecules like cofactors, ligands, substrate, metal ions may be added to crystallization cocktails as stabilizing agents.

Additives act by

i) promoting the intermolecular contacts by divalent metal ions,

ii) stabilising a protein by changing its aggregation state with a detergent or

iii) changing the dipole moment of solution with alcohols.

Protein Crystallisation

Crystallisation techniques

The molecular mechanism behind the crystallisation process is complex, but the setup for crystallisation is simple in which, a buffered protein solution is combined in 1:1 ratio with the crystallisation cocktail and placed in a closed system.

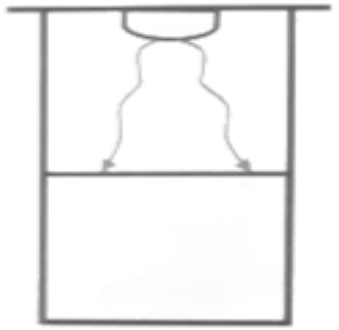
Various crystallisation techniques have been developed and are used depending on the application e.g. initial screening, optimisation, harvesting and ease of automation.

Vapour diffusion techniques rely on the presence of precipitant in a reservoir that absorbs water from the crystallisation drop to drive the system into super-saturated state.

Protein Crystallisation

Hanging drop vapour diffusion

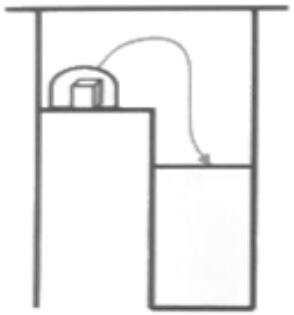
In this technique a drop of protein solution is placed on a siliconised glass cover slip and mixed with equal amount of crystallisation solution from the reservoir. The cover slip is flipped over the reservoir and sealed with grease. The water vapour diffuses from the hanging drop into the reservoir containing the double concentration of precipitant.



The classic: hanging-drop vapor diffusion

Sitting drop vapour diffusion

In this technique the crystallisation drop sits on a shelf surrounded by the crystallisation solution. *The hanging drop method is used commonly in manual setups while sitting drop method is used in automated setups*.



The variant: sitting-drop vapor diffusion

Protein Crystallisation

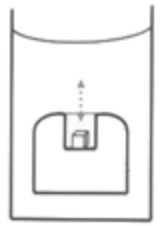
Microbatch crystallisation

In this technique the protein solution is mixed with the crystallisation cocktail and pipetted onto the surface of oil covered microtiter plate. The drop sinks to the bottom. The oil used is impermeable oils and precipitant concentrations are higher compared to vapour diffusion.

Micro-batch under oil

Micro-dialysis crystallisation

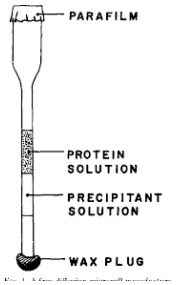
In this technique the small volume of protein solution is placed in enclosed container separated from precipitant by a dialysis membrane which is held by an O-ring in a groove at the bottom of well. The membrane allows water and small molecules to be exchanged but restricts the protein molecules. Thus dialysis is used in combination with high concentration of precipitant to reach super-saturation. This method is suitable for achieving large crystals.



Micro-dialysis

Free interface diffusion crystallisation

In this technique the protein and precipitant are brought in contact in a narrow vessel but without pre mixing and allowing equilibration against each other by diffusion only.



Free-interface diffusion

Protein Crystallisation

Nucleation

Spontaneous nucleation is rare at low super-saturation and takes a long time. However, the crystals obtained are usually large. While at high levels of super-saturation, simultaneous nucleation occurs and tiny microcrystals, unsuitable for diffraction are formed. The most commonly employed means to strike a balance between nucleation and crystal growth is the introduction of nucleation through seeding. Two general methods for seeding are:

Micro-seeding

Crushed crystal fragments of not well formed crystals, the initially formed crystal clusters or particulate matter like ground glass can be introduced in the crystallization drop.

Macro-seeding

In macro-seeding once already formed, but a small crystal is transferred to a new crystallization drop of identical reagents to induce the crystal to grow big. The underlying rationale is that if the crystal has stopped growing due to lack of material a fresh solution may allow further growth.

Protein Crystallisation

Soaking and co-crystallization

When the aim of a structure determination is to describe the interaction of ligand and the active site of protein, the soaking and co crystallization is done to introduce the ligands in the binding site.

Soaking

In this method the ligands or small molecules are introduced into drop containing already grown crystals. Diffusion takes the small molecules through the solvent channels into the crystal. Some ligands however, destroy the crystal upon soaking and thus co-crystallization is employed.

Co-crystallisation

The protein is incubated with small molecules or the ligands before setting up the crystallization drop.

Protein Crystallisation

Automation of crystallisation experiments

- Automated crystallisation setup has become possible due to the availability of crystallisation robots that can perform high speed, low volume and accurate pipetting, cocktail mixing and plate shuffling.
- The most significant task that these robots achieve is the miniaturisation of crystallisation drop such that drops of around 100 nl can be dispensed reliably.

Protein Crystallisation (Automation)

Protein Crystallisation

Crystallization is performed in temperature controlled rooms or kept under vibration free incubators.

Crystals appear over a period of 4-10 days with exception from few hours to over 6 months for certain proteins.