Cloning, expression, purification, and biophysical characterization of SSB from pathogenic bacteria

Week 7: Crystallization of SSB protein

Introduction – The first step in the determination of an X-ray crystal structure of the SSB protein is to obtain crystals. The crystals also need to diffract to sufficient quality to enable structure determination.

In this class, we will form SSB-DNA complexes and setup hanging-drop crystal trays and 'hope' to obtain crystals.

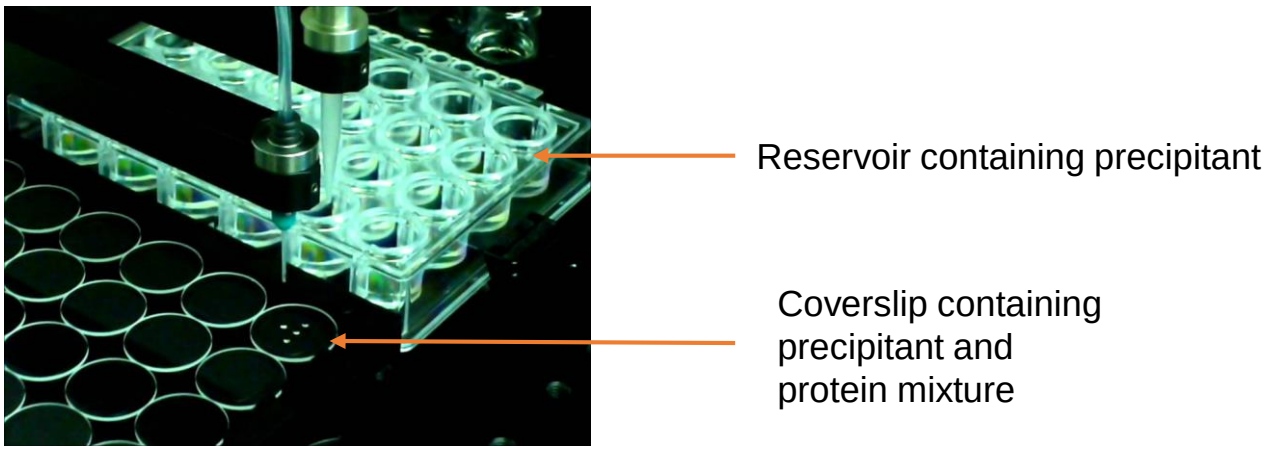
Here is a list of requirements for successful protein crystallization:

a. *Pure (homogenous) protein*. When you purified the SSB protein and checked it using SDS-PAGE, you obtained clean protein.

b. *Large quantities of the SSB protein*. Milligram quantities of the protein are often required for protein crystallization. Most of you have between 75-200 uM SSB protein (tetramer concentrations). We will further concentrate the protein solutions to a final SSB:DNA concentration of 100 uM:101 uM before setting up the trays.

What happens during crystallization?

a. You start with a crystallization plate which has a reservoir to to hold your 'precipitant' solution. You will add 500 ul of the precipitant solution into the reservoir. You also have a coverslip where you will place a small volume of your protein solution (often 1-3 ul) and mix it with a small volume of the precipitant solution.



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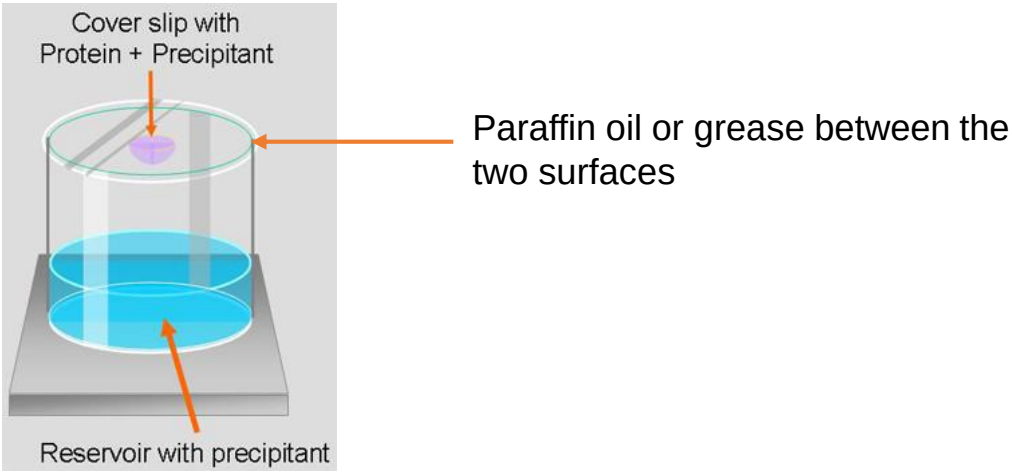
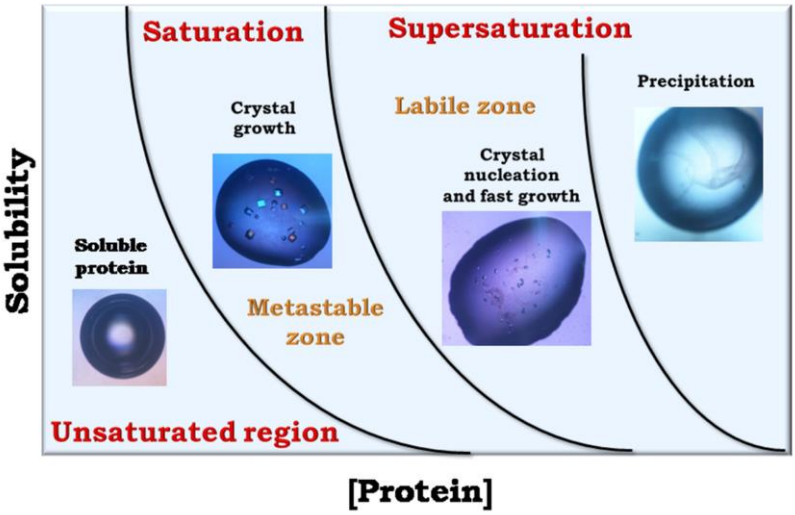
What happens during crystallization (contd)?

b. You then take the cover slip and place it over the reservoir and create a sealed chamber by putting paraffin oil between the two surfaces.



c. Within the chamber, in the protein drop on the cover slide, the concentration of the precipitant is lower compared to the reservoir. **Vapor Diffusion** now starts to occur, slowly over time, and the concentration of the protein in the drop slowly increases. This summarizes the process of setting up a crystallization tray.

d. **Protein solubility curve**.



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What happens during crystallization (contd)?

d. **Protein solubility curve**. As the protein in the drop undergoes a change in concentration, its solubility profile will gradually change. One can broadly classify these solubility regions into:

1. *The Unsaturated* Region, where the protein is soluble.
2. *The Saturation Region*, where the protein is in a metastable zone and crystal growth occurs.
3. *The Supersaturation Region,* where the crystals are labile and crystal nucleation and growth are fast. Here, the crystals are often not suitable to obtain good diffraction.
4. *Precipitation Region,* where instead of forming crystals, the protein precipitates.

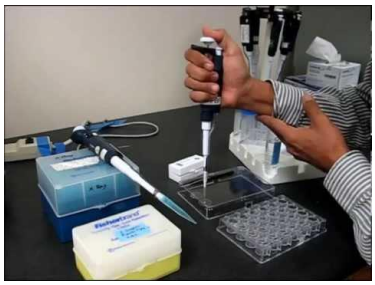
The trick with protein crystallization is finding the optimal precipitant composition, concentration and temperature where the protein is in a metastable zone.

Trial and Error: There is NO secret recipe to obtain the crystal structure of a novel protein. One has to try out hundreds of conditions to obtain crystal hits, which then have to be optimized to get large enough crystals that can diffract well. *If you do not have to go through this iterative process of crystallization, consider yourself very, very, very, very lucky!!*

Robot versus Hand Trays:

Crystal Setup using a Robot

Crystal Setup Where you are the Robot



Step 1- Form the SSB-DNA complex

a. We will be crystallizing the SSB-(dT)35 complex in Buffer $T^{0.2}$
Mix a 500 ul reaction with the following concentration (100 uM SSB and 101 uM dT35) in Buffer $T^{0.2}$ (20 mM Tris-Cl, pH8.3, 0.1 mM EDTA and 200 mM NaCl).

b. Dialyze the mixture overnight (at 4 deg C) against 1 L of Buffer $T^{0.2}$.

c. Next day, setup the trays. In the cover slip, setup a 2ul:2ul and 3ul:1ul sample:precipitant drops in your cover slip.

d. Place a layer of paraffin oil over you're the mouth of the reservoir and gently invert the coverslip over the reservoir.

e. Continue to do this for all the 24 wells in your plate.

f. Gently place the tray in the 20 deg C incubator.

g. Look at your plates during next class and the following week to see if any crystals have formed.

A few images of protein crystals