Keywords for this lecture: Supersaturation, nucleation, aggregation, precipitation, precipitate, phase diagram

Precipitate, precipitation: a solid state of the *protein.*

Precipitant: *a chemical entity* (usually a salt or polymer) used to drive the protein out of solution.

1. Crystallization: it's all about the water of solvation

Regardless of what method you use or what chemical conditions you choose, the ultimate goal is to to remove the water of solvation so that the protein molecules are forced make contacts with each other. If these contacts are favorable, a crystal will form. So, just like at salt drying pools (shown here), you want to create controlled dehydration in your experimental drop.

2. You need two things

To crystallize a salt, a protein, or any molecule you require two things: **Supersaturation and nucleation.** The molecule must be in a supersaturated state so that nucleation can occur. **Supersaturation**: a state in which there is more of the molecule dissolved than what the normal limits of solubility allow (at a given temperature or pressure). Example of a supersaturated metastable solution from real life: sugar syrup!

3. Controlled dehydration

So why don't we just boil the protein and let it cool down to get it supersaturated? With proteins we must use GENTLE methods to create supersaturation. The three most common in protein crystallization are:

- salts (they compete with the protein for water molecules)
- polymers (e.g., polyethylene glycol; these work by volume of exclusion)
- small molecular weight alcohols (e.g., MPD, isopropanol) These change the dielectric constant of the solvent. Water has a dielectric constant of 80, which is very high.

These three can be used alone or in combination with each other. For example, a common condition is: 30% polyethylene glycol, 0.2 M NaCl, 0.1 M buffer

4. Nucleation only happens in supersaturated solutions.

"The large supersaturation is required to overcome the activation energy barrier which exists when forming the crystal. This barrier represents the free energy required to create the small microscopic cluster of proteins-the nucleus-from which the crystal will eventually grow". N. Ahserie Methods 34 (2004) 266-272

Nucleation: the formation of a critical nucleus.

5. The phase diagram

"The phase diagram is a map which represents the state of a material as a function of ambient conditions e.g., temperature and concentration".(Asherie, ibid.) Remember that these are only *models.* To my knowledge a complete phase diagram has only been done for two proteins (lysozyme and crystallin).

The phase diagrams can look differently, depending on the protein solubility. Three examples are shown here.

6. Three parts of the phase diagram: undersaturated, saturated, supersaturated

7. The solubility line

The thick black line (c eq) is the equilibrium concentration (the solubility). This is the solublity curve. It will vary with the solution conditions (Asherie, 2004).

The solubility limit/line is "an unambiguous description of the equilibrium between solution and crystal. At the solubility limit, the crystal loses protein molecules at the same rate at which protein molecules rejoin the crystal-the system is at equilibrium." (Neerie, ibid.)

The supersaturated region can be divided into three zones, but these zones (phase boundaries) are determined by the kinetics of the experiment. These zones are not "fixed", but will shift depending on the kinetics.

labile zone: nucleation is very slow, maybe not in your lifetime. However, a crystal will grow here, but not nucleate (within any reasonable amount of time). Seeding experiments exploit this labile zone (more on this tomorrow). A small seed crystal is placed in the labile zone and it will grow, bypassing the long wait for spontaneous nucleation.

metastable zone: spontaneous nucleation can occur here, if the energy barrier is surmounted. Remember though that nucleation is *always* a stochastic (random) event. It can never be guaranteed to happen. You can only adjust the kinetic parameters to favor its occurrence, but never guarantee it.

precipitation zone: unfavorable for crystal formation "because the aggregates and precipitates form faster than the crystals". The precipitate is a solid state of the protein that we can see in the microscope, but disordered. On the submicroscopic scale, it is usually referred to as aggregation. We want the ordered form, i.e., crystals, not precipitate.

Aggregation: the assembly of proteins into amorphous clusters. It is a kinetic phenomenon. Too fast kinetics and you get aggregation, rather than ordered nuclei.