IIT Guwahati Lecture 32

Course BT 631

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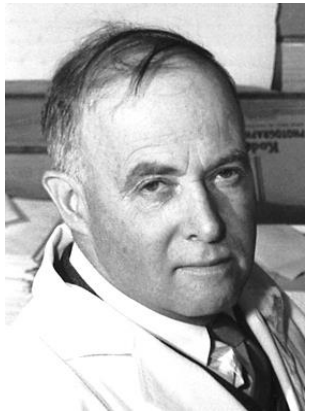
Protein Structure, Function and Crystallography

Protein Crystallisation

The first protein, *Urease* was crystallized by James Sumner in 1926



James Batcheller Sumner (Nov, 1887 – Aug, 1955)

and was followed by crystallization of *Pepsin* in 1930 by John Northrop.

John Howard Northrop (July 5, 1891 – May 27, 1987)

Protein Crystallisation

One of the slowest steps in protein crystallography is the production of protein crystals.

Methods employed in crystal production rely on the ordered precipitation of proteins.

- **Crystallization requires the formation of large (with dimensions greater than 0.1 mm along each axis) and stable crystals with sufficient long-range order (translational periodicity) to diffract the X-rays.**
- **To form a crystal, the protein molecules assemble into a periodic lattice from super-saturated solutions. This involves starting with solution of pure protein at a concentration between 0.5 and 200 mg/ml and adding reagents that reduce, the protein solubility close to the point of precipitation.**
- **The protein molecules must separate from the solution and self-assemble into periodic crystal lattice.**
- **The proteins are concentrated to a suitable high concentration. The average protein concentration extracted from PDB data is around 14 mg/ml. There are many examples of successful crystallization in the low concentration (mg/ml) range.**

Protein Crystallisation

- Reagents that reduce the protein solubility called, precipitants, are added to a concentrated protein preparation.
- Once the solubility limit of the protein is exceeded, the solution becomes supersaturated and a metastable state is created in which system is not in equilibrium.
- Once the activation barrier towards equilibrium is overcome by spontaneous nucleation or external creation of nucleation sites, excess protein comes out of the solution and a protein rich phase (crystal) will form.

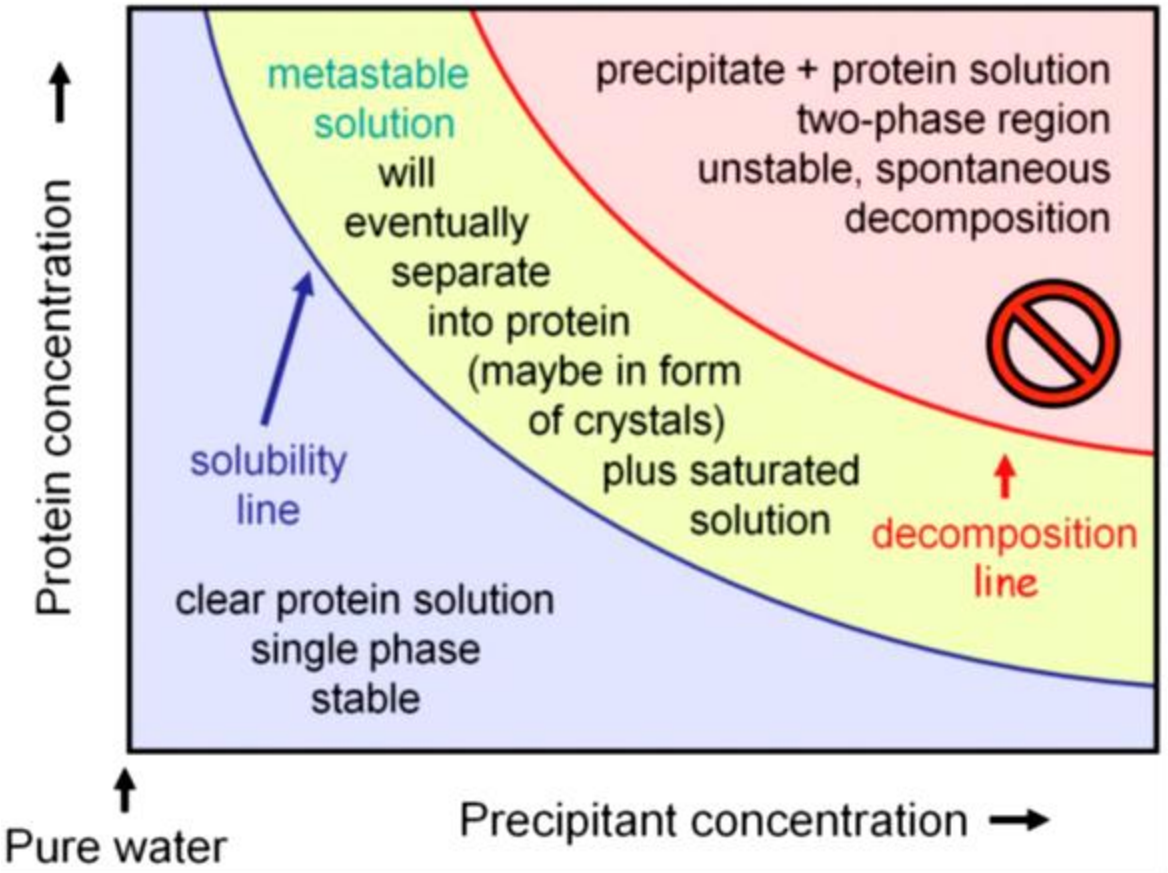


Fig. Solubility phase diagram at a given temperature. The solubility line separates the region that contains as single phase- the protein solution from the two phase region that contains protein and saturated protein solution in thermodynamic equilibrium.

Protein Crystallisation

- It is not possible to predict the type of precipitants and conditions which will produce well diffracting crystals. Therefore, many trials using different precipitants and concentration at several pHs and temperatures are done.
- Once a suitable single crystal is observed, it is harvested using special nylon fibre loops under a microscope and mounted on a diffractometer. To protect the crystal from radiation damage the crystals are cooled by liquid nitrogen.
- Prior to this cooling step, the cryo-protection step is required. The crystals are soaked in Cryo-buffer containing a suitable cryo-protectant. e.g. Glycerol or Polyethylene glycol (PEG).

Protein Crystallisation

Interactions in Protein Crystals

- In protein crystal only a limited number of interactions that form a network, keep the molecules connected.
- Intermolecular forces that exist between molecules in protein crystals are mainly dipole-dipole interactions, hydrogen bonds, salt bridges and van der Walls contacts.
- Also these interactions need to take place at specific locations on the protein surface to form ordered self-assemblies.
- The interactions between irregular and flexible protein molecules are weak and sparse thus a protein crystal will not be hard and robust.

Protein Crystallisation

Different Forms of Protein Crystals

- It is usual to find different forms of crystals under different crystallisation conditions. However, it may be also possible that multiple forms of crystals are formed in the same crystallisation drop.
- Different forms of crystals exhibit different diffraction quality and it is advisable to test all the forms.