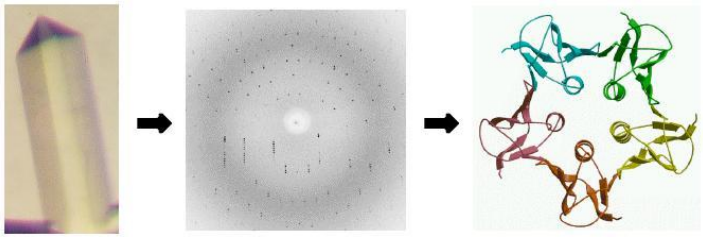
METHODS OF PROTEIN CRYSTALLIZATION AND CRYSTAL GROWTH



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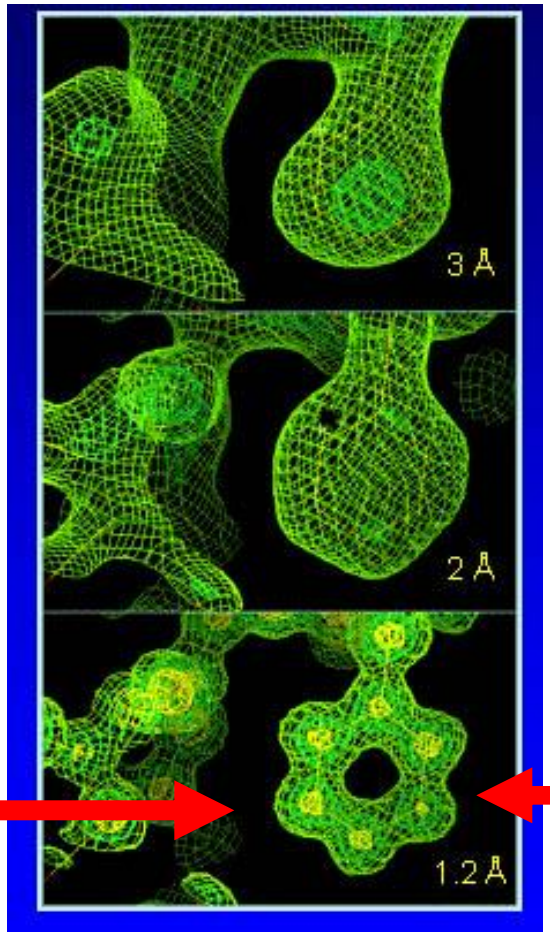
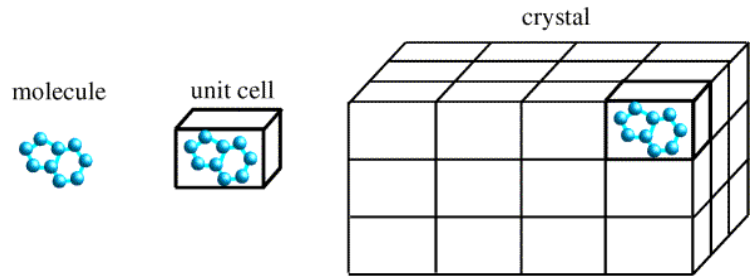
abel.moreno@mac.com

What does crystal quality mean?





High quality single crystals

Ultrapure protein samples

A chronology of protein crystal growth

PROTEINS OR FAMILY	Time period (year)	Investigator or research group
Hemoglobins	1840 - 1855	Hunefeld, Reichert, Leydig, Kohlker, Budge, Funke, Lehmann
Excelsin (from Brazil nut)	1858	Maschke
Globulins	1880 - 1894	Rithausen, Grubler, Osborne
Hen egg albumins	1890 - 1899	Hofmister, Hopkins and Pincu, Wichman,
Horse serem albumin	1915	Gurber, Sørensen
Concanavalins A & B	1919	Summer
Urease	1926	Summer
Insulin	1927	Abel et al.
Tripsin and trypsinogen, trypsin inhibitor	1930's	Northrop et al.
Pepsin and pepsinogen	1930's	Northrop et al.
Chymotrypsin & chymotrypsinogen	1930's	Northrop et al.
Ribonuclease	1930's	Northrop et al.
Hexokinase	1930's	Northrop et al.
Diptheria toxin	1930's	Northrop et al.
Pepsin	1934	Diffracted these crystals, this was done by Bernal J. D. & Crowfoot, D.
β-Lactalbumin	1934	Palmer
Carboxypeptidase	1935	Anson
Catalase	1937	Summer and Dounce
Ferritin	1937	Laufberger
GPDH	1939	Baranowski
Lysozyme	1939	Alderton & Fevold
Human Haemoglobin crystallisation & resolution	1959	Perutz and Kendrew

Adapted from reference: A. McPherson J. Cryst. Growth 110 (1991) 1-10

Protein crystallization what for?

At the beginning (latter half of the 19th century):

1. It provided a means for the purification of specific proteins.
2. It served as a demonstration that a protein had been purified.
3. It was an interesting laboratory curiosity.

Between 1900 and 1940

1. Emphasis was on enzymes to prove properties and nature of catalytic macromolecules.
2. Protein crystallisation for X-ray diffraction.

In the 1980's and up to now

1. Due to the development of the recombinant DNA technology permitted researchers, for the first time, to prepare ample amounts of otherwise rare and elusive proteins.
2. Structural biologists would like to describe all living systems, and the materials they produce, in molecular and even atomic terms.

Biological Macromolecules

- **The most important biomacromolecules for living organisms are:**

- **A) PROTEINS**
- **B) NUCLEIC ACIDS**
- **C) POLYSACHARIDES**
- **D) LIPIDS***

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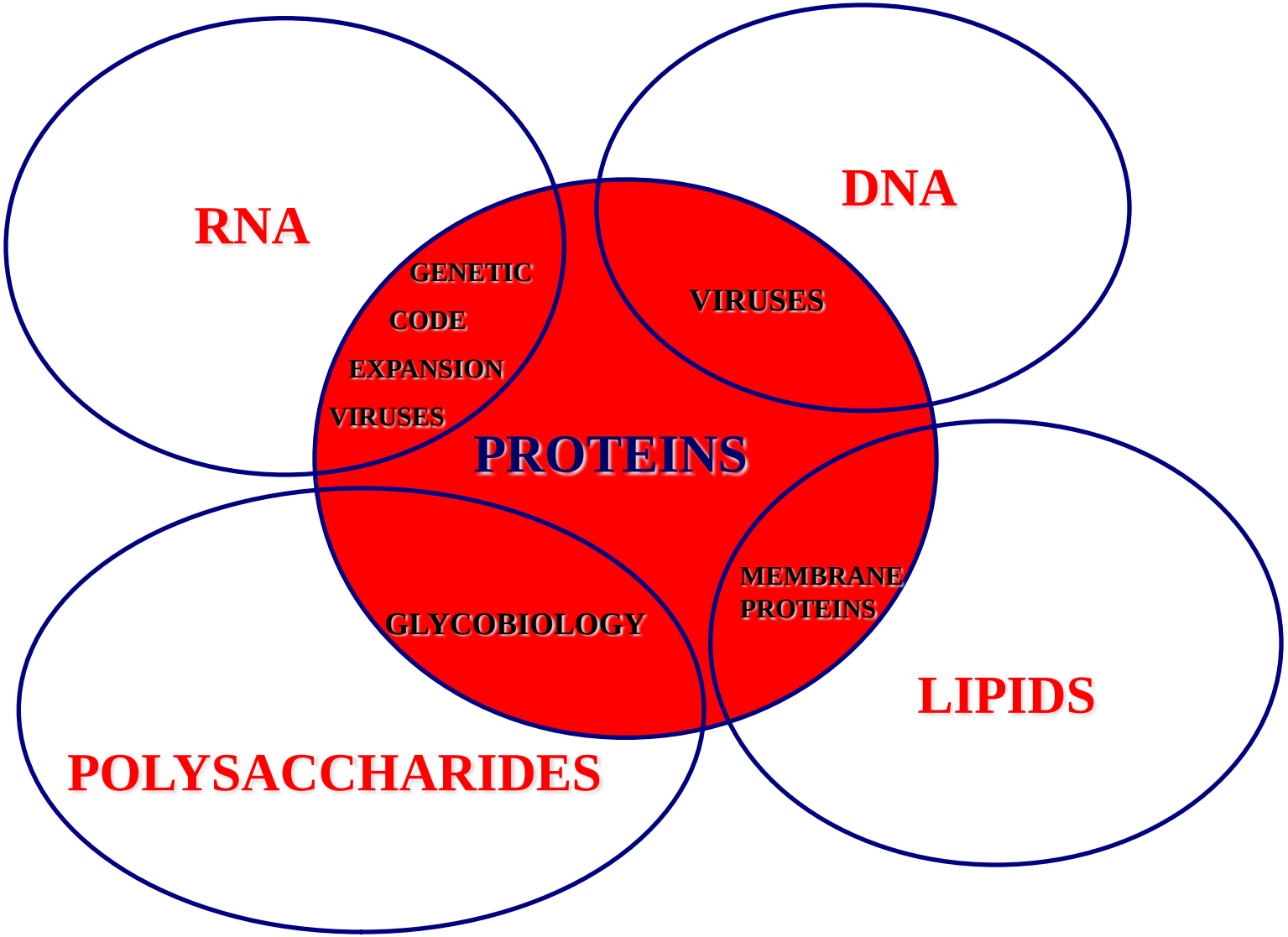

RNA
DNA
GENETIC
CODE
EXPANSION
VIRUSES
VIRUSES
PROTEINS
MEMBRANE
PROTEINS
GLYCOBIOLOGY
LIPIDS
POLYSACCHARIDES

CLASSICAL CRYSTAL GROWTH METHODS

1) Hanging-drop
2) Sitting-drop
3) Batch

SOLID STATE SYNTHESIS & HYDROTHERMAL METHODS

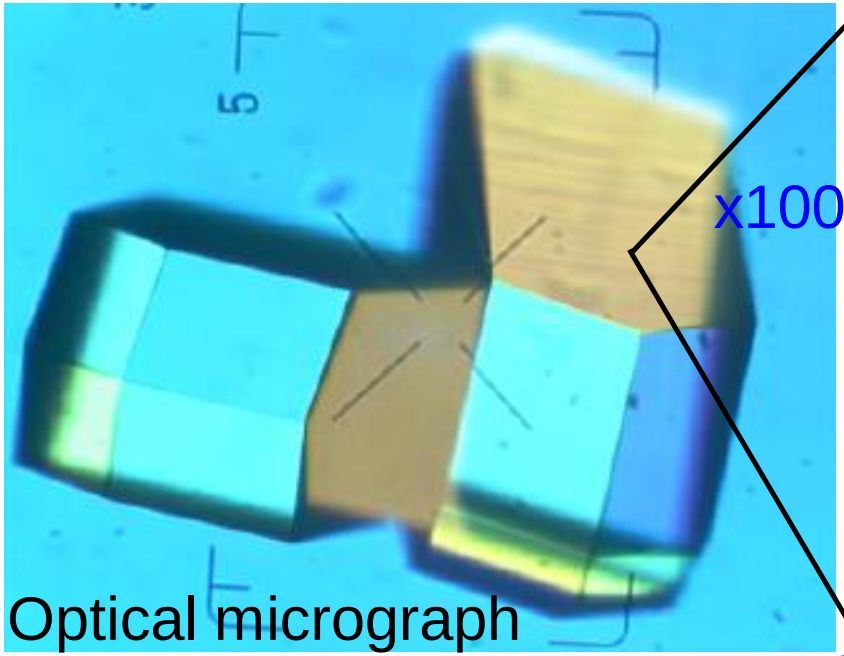
Crystal growth in solution

a) Classical methods in small droplets.
b) Small growth cells and nano-droplets
c) ROBOTS for highthrougput nano-crystallization

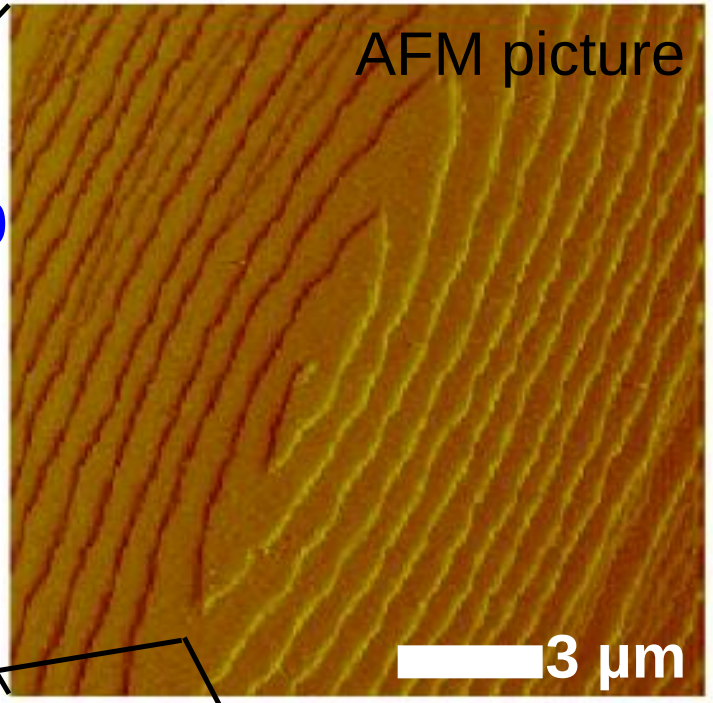
HIPERGRAVITY

BIOMINERALIZATION and GEL METHODS

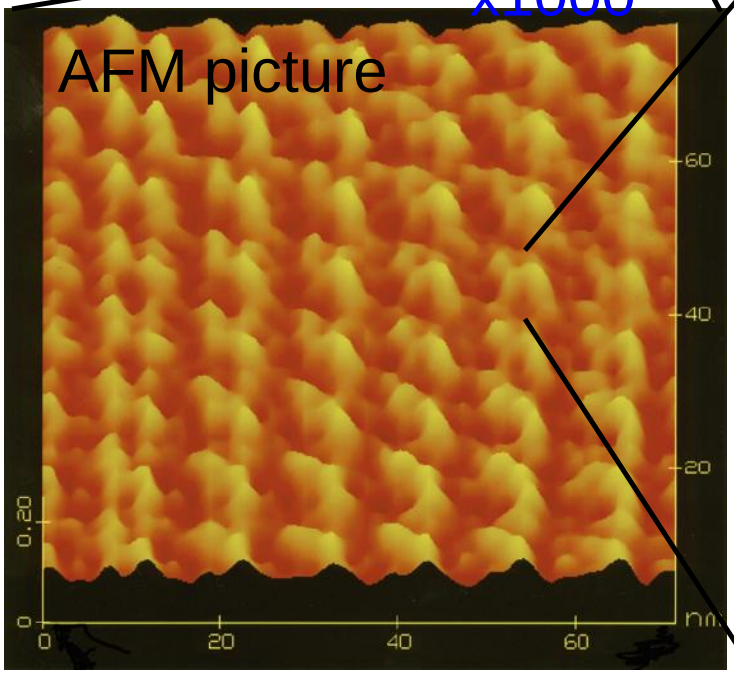
MICROGRAVITY

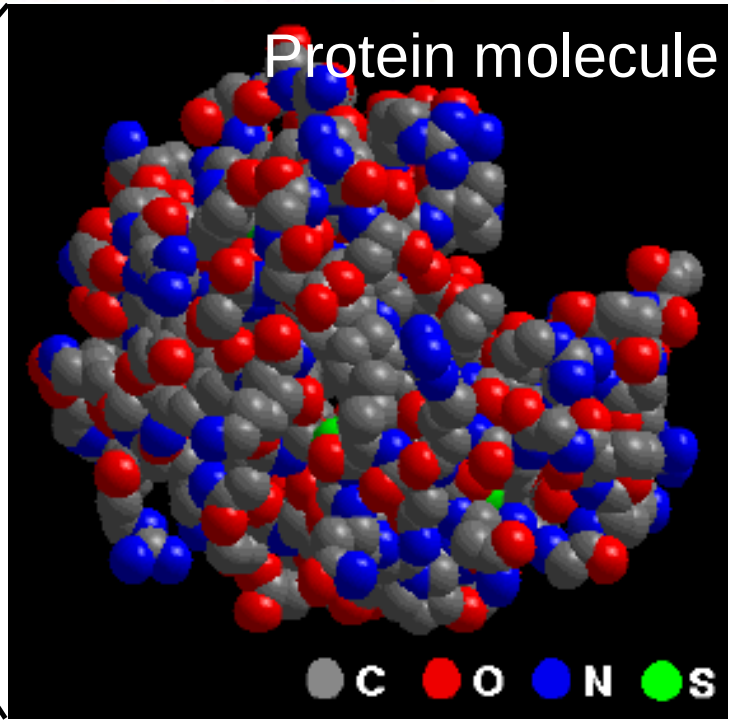


x1000



x1000

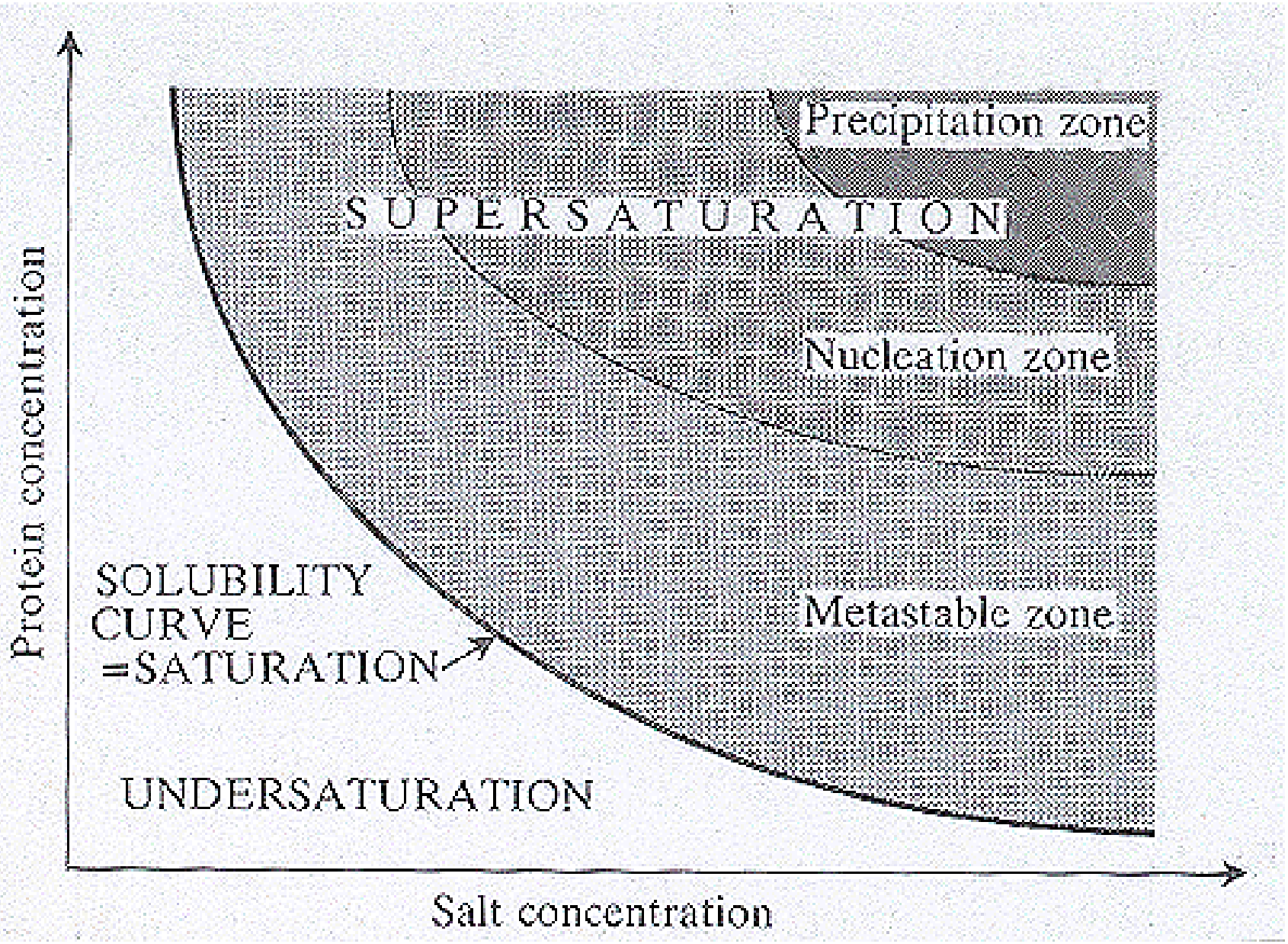




Hen egg-white lysozyme

Protein Crystallisation

- We have to consider two aspects:
- 1) The Thermodynamics of the process related to the solubility problem.
- 2) The Kinetics of the process related to nucleation phenomena and crystal growth methods.


Precipitation zone
SUPERSATURATION
Nucleation zone
Metastable zone
SOLUBILITY
CURVE
=SATURATION
UNDERSATURATION
Protein concentration
Salt concentration

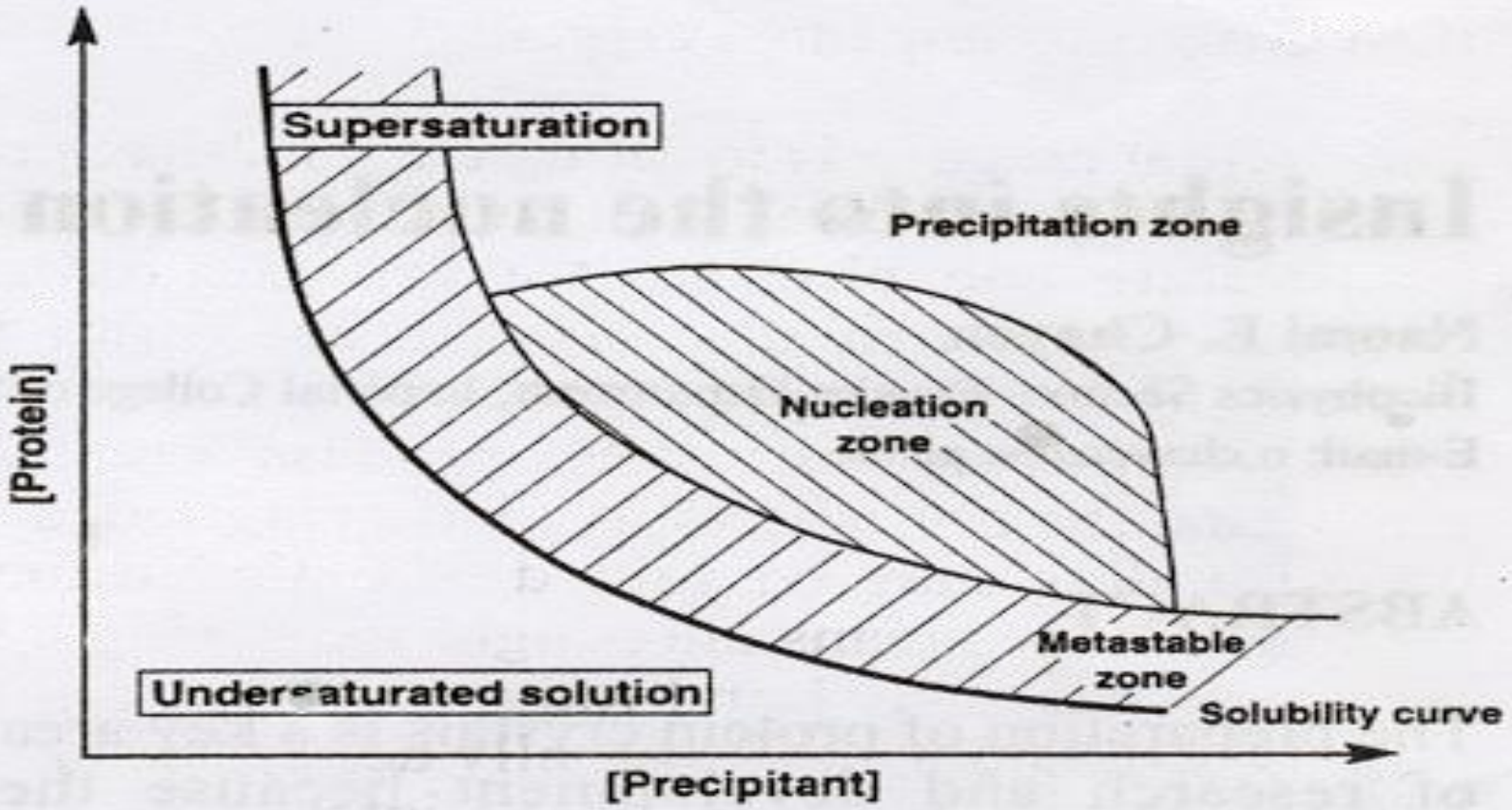


Figure 1: Illustration of a protein crystallization phase diagram based on variation in protein and precipitant concentrations.

Phase diagram for protein crystallisation

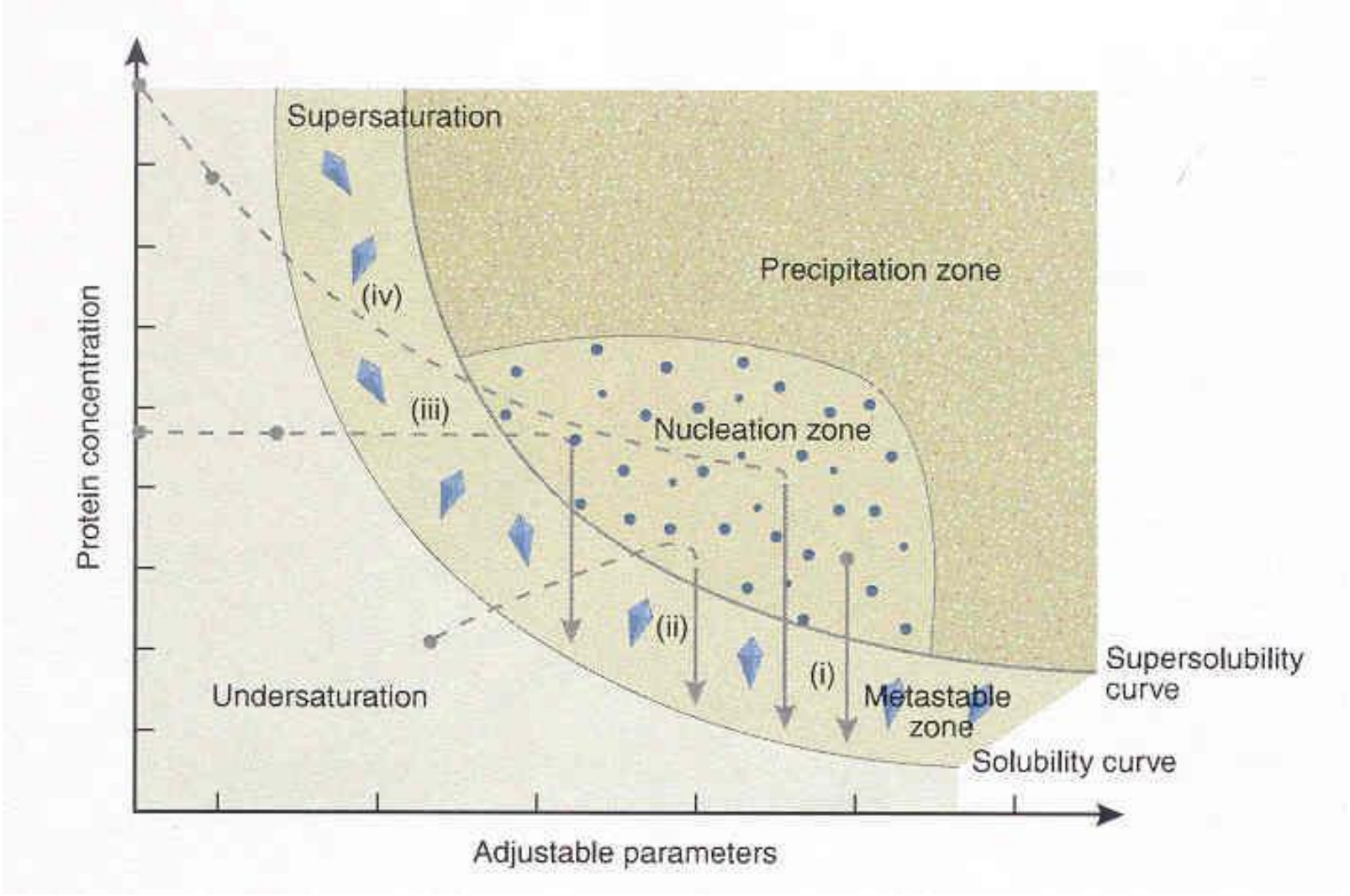
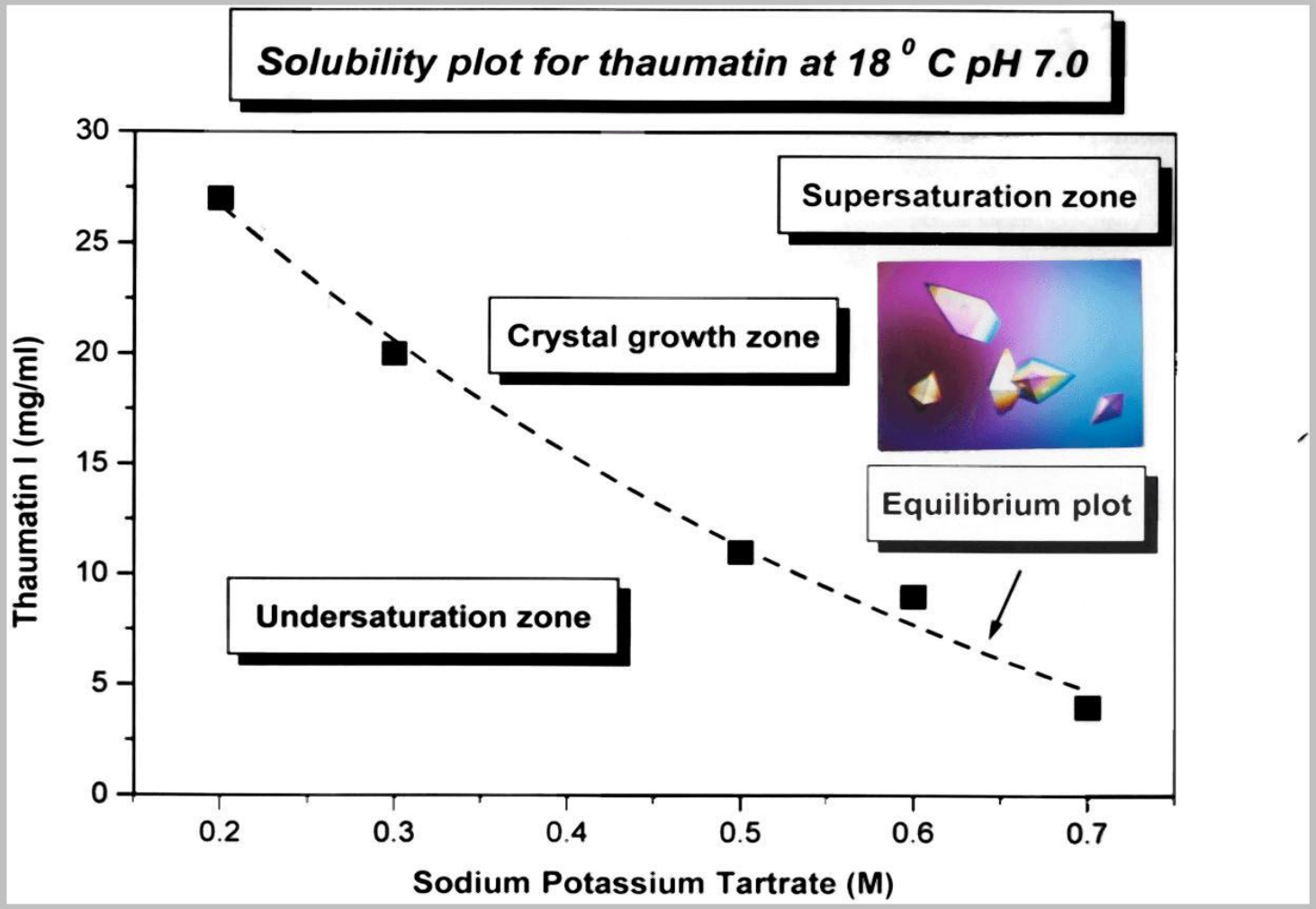
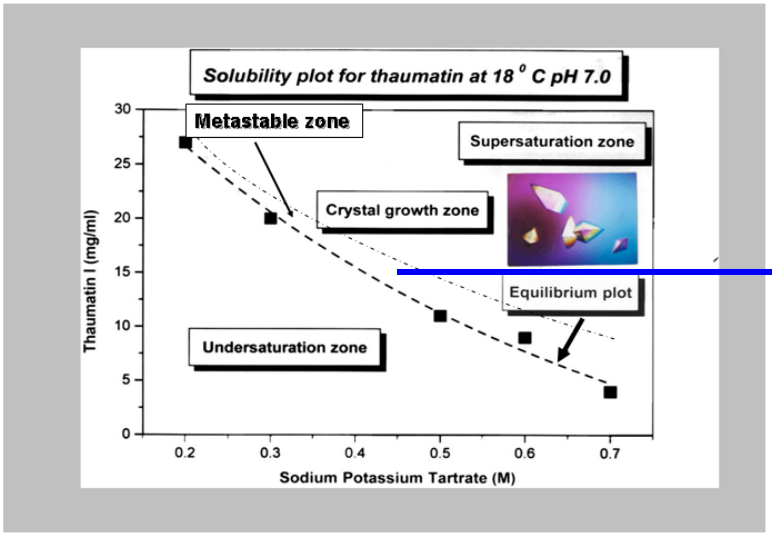


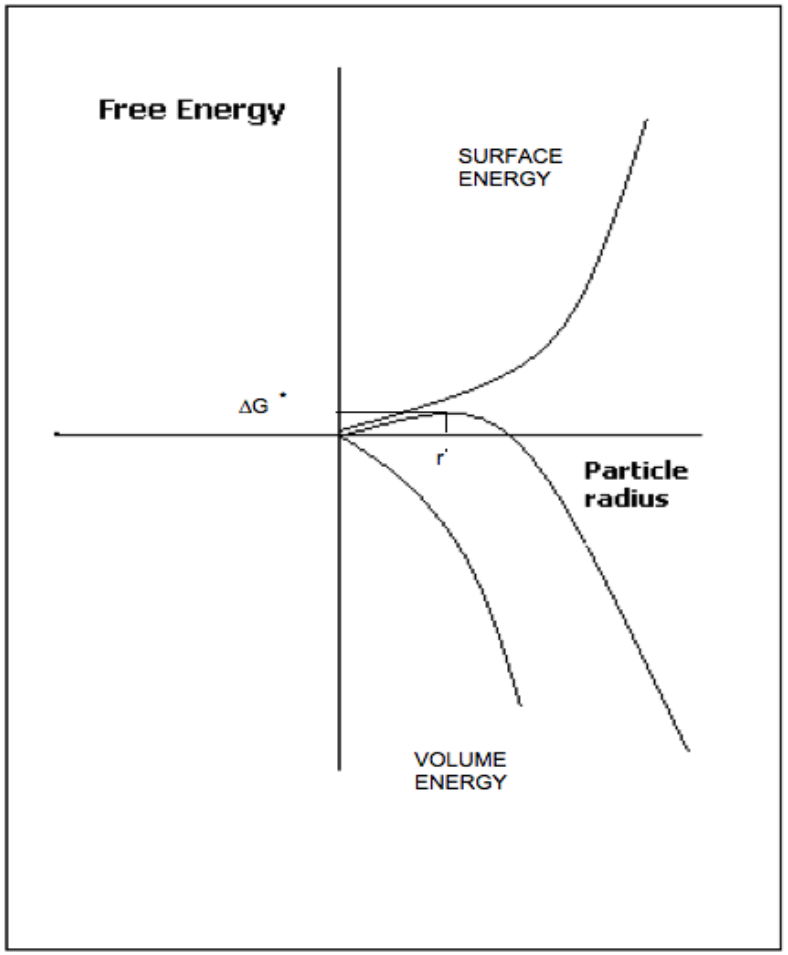
Figure pathways: i) microbatch, ii) vapour diffusion, iii) dialysis, iv) FID

Reference: Chayen, N. & Saridakis, E. Nature 5, No. 2 (2008) 147-153


Solubility plot for thaumatin at 18 ^{0} C pH 7.0
Supersaturation zone
Crystal growth zone
Equilibrium plot
Undersaturation zone
Thaumatin I (mg/ml)
Sodium Potassium Tartrate (M)
0
5
10
15
20
25
30
0.2
0.3
0.4
0.5
0.6
0.7

Separating nucleation and crystal growth

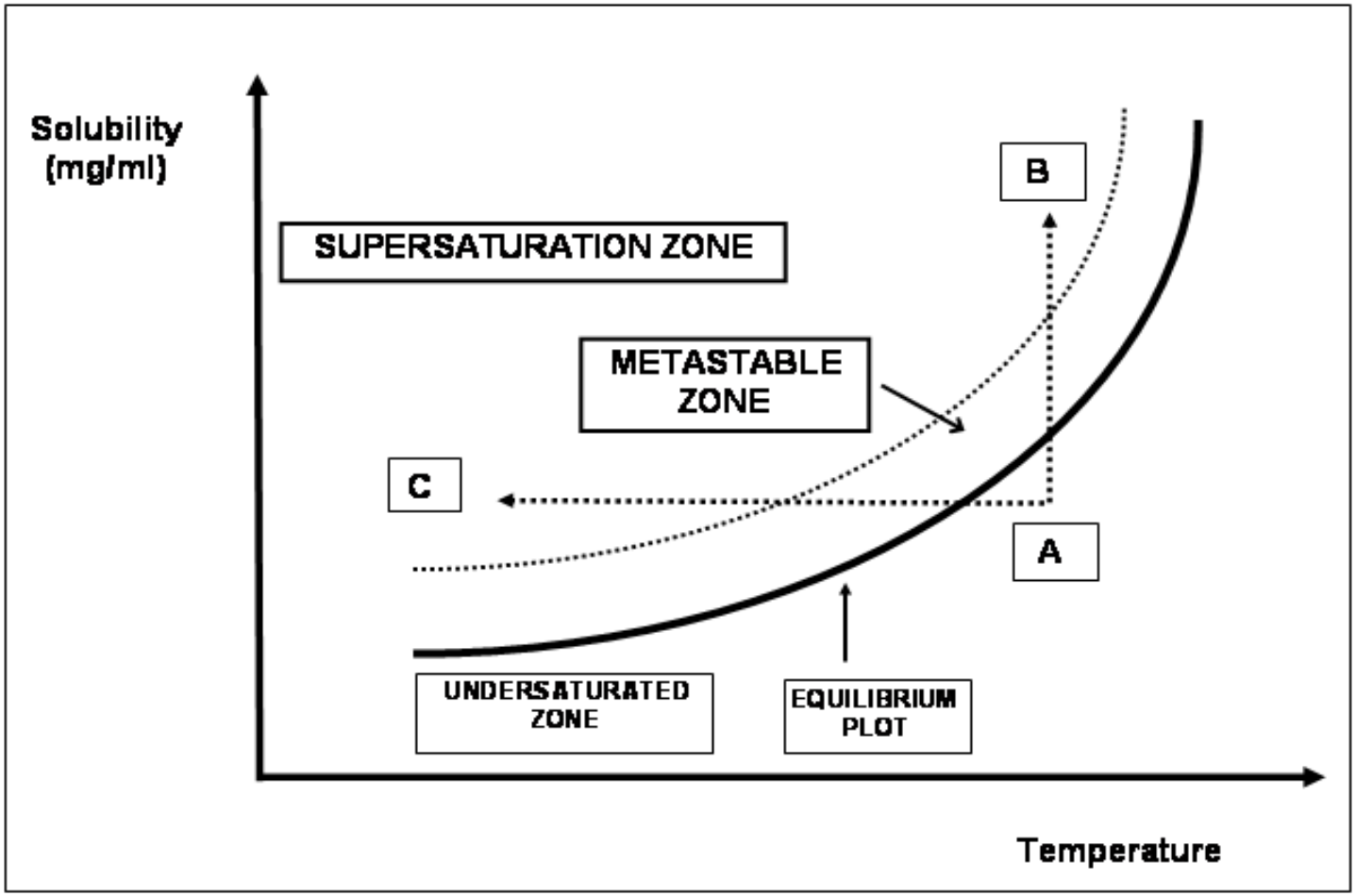


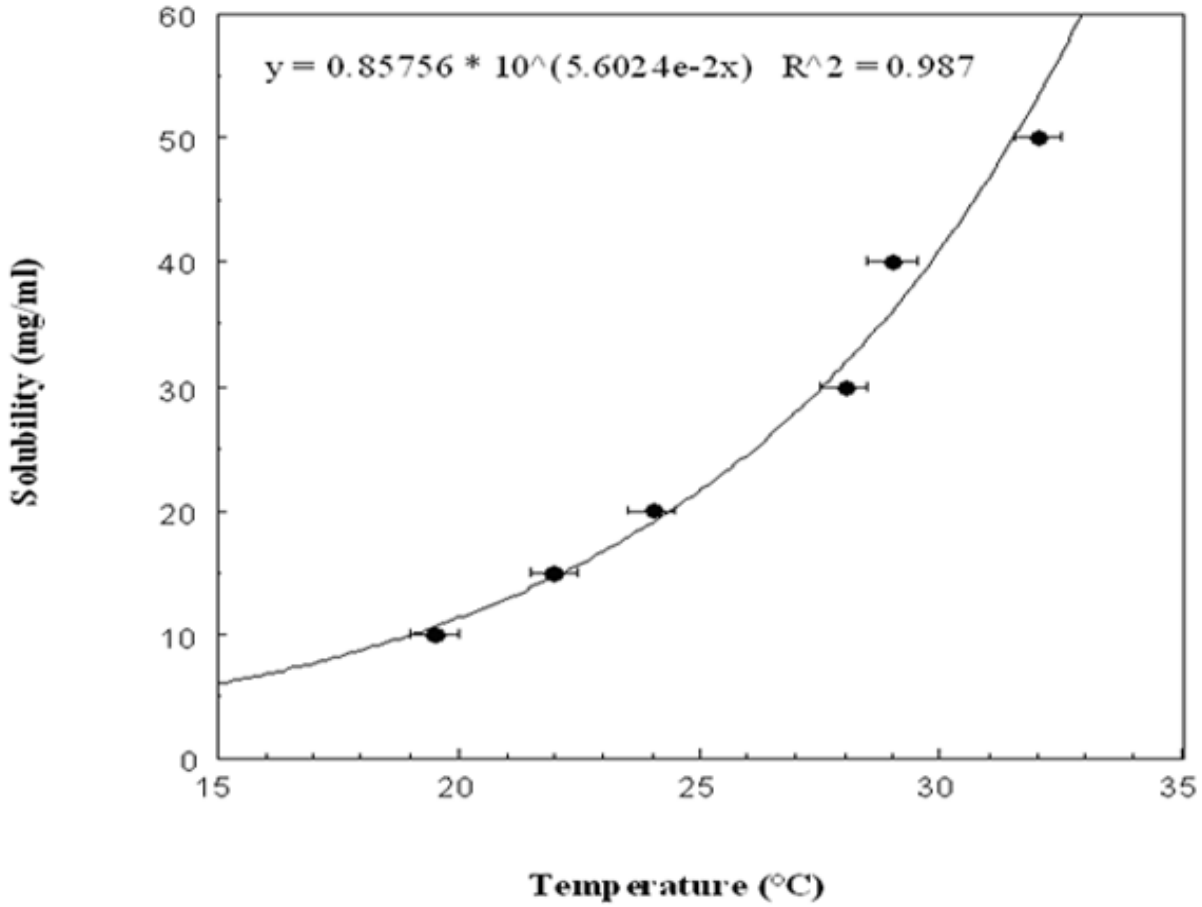


What are the Chemical and Physical parameters to be taken into account?

Remember that Crystallization process involves two separate processes: nucleation and growth, which are rarely unconnected.

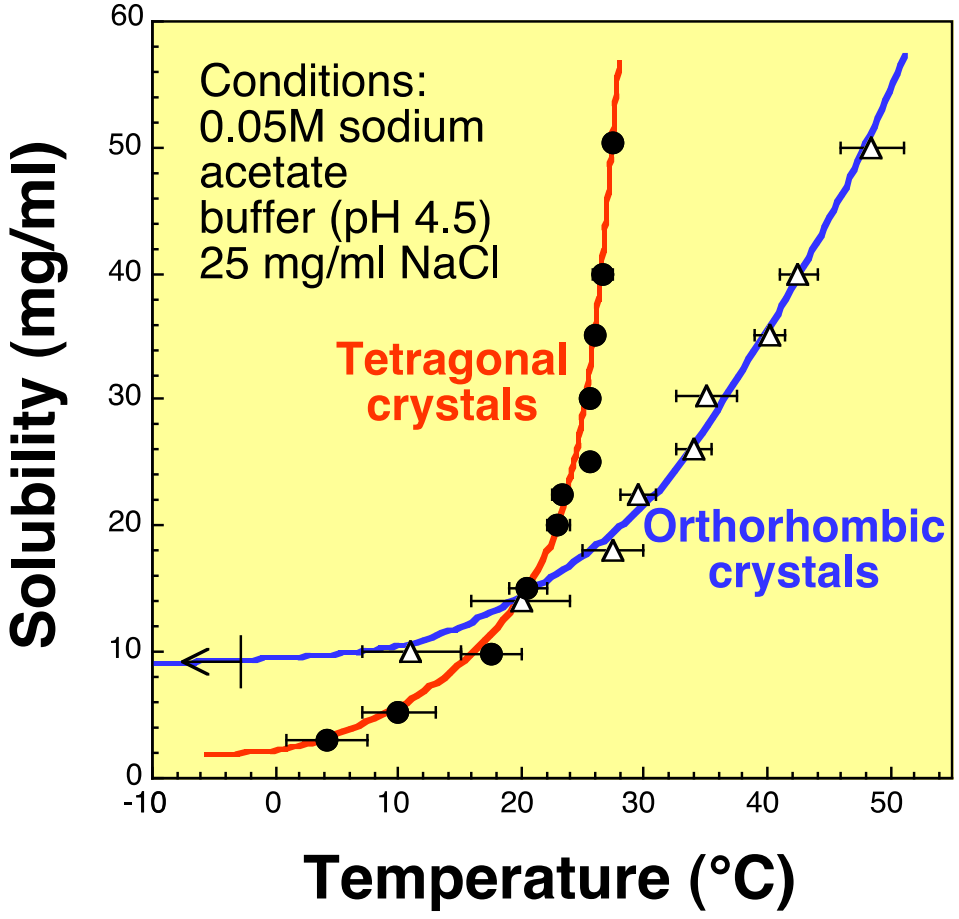
Solubility versus Temperature




y = 0.85756 * 10^(5.6024e-2x) R^2 = 0.987
Solubility (mg/ml)
Temperature (°C)
0
10
20
30
40
50
60
15
20
25
30
35

Solubility curves of lysozyme crystals

Interferometry can be a strong tool to determine solubility curves of protein crystals.



Advantages

1) **Quick** (60-100 min): 5-10 times faster than other methods.

2) **Small sample volume** (min. 10μl)

3) Applicable for a **mestable phase**

4) Including both **growth & Dissolution** processes

5) **Small impurity effect:** including dissolution process

6) **In-situ observation**

Disadvantages

Following items are necessary:

1) Temperature dependency of the solubility

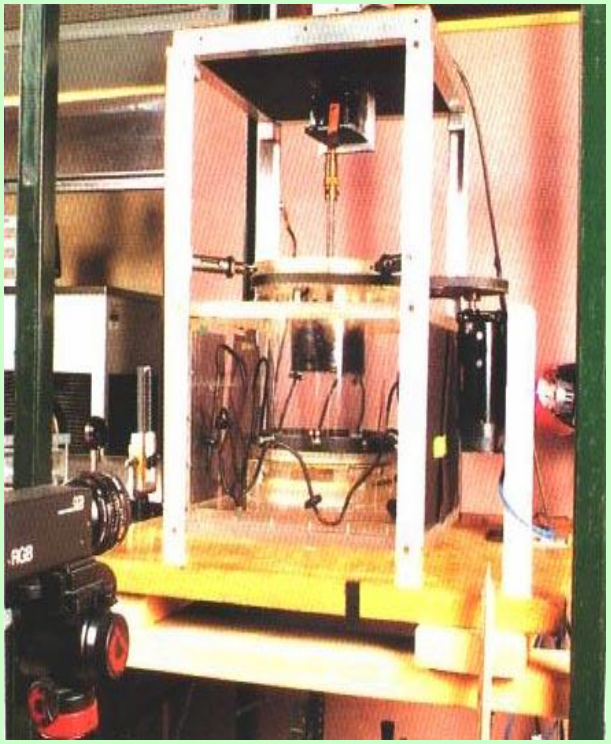
2) Size of crystals (> 100 μm) (cluster of small crystals: OK)

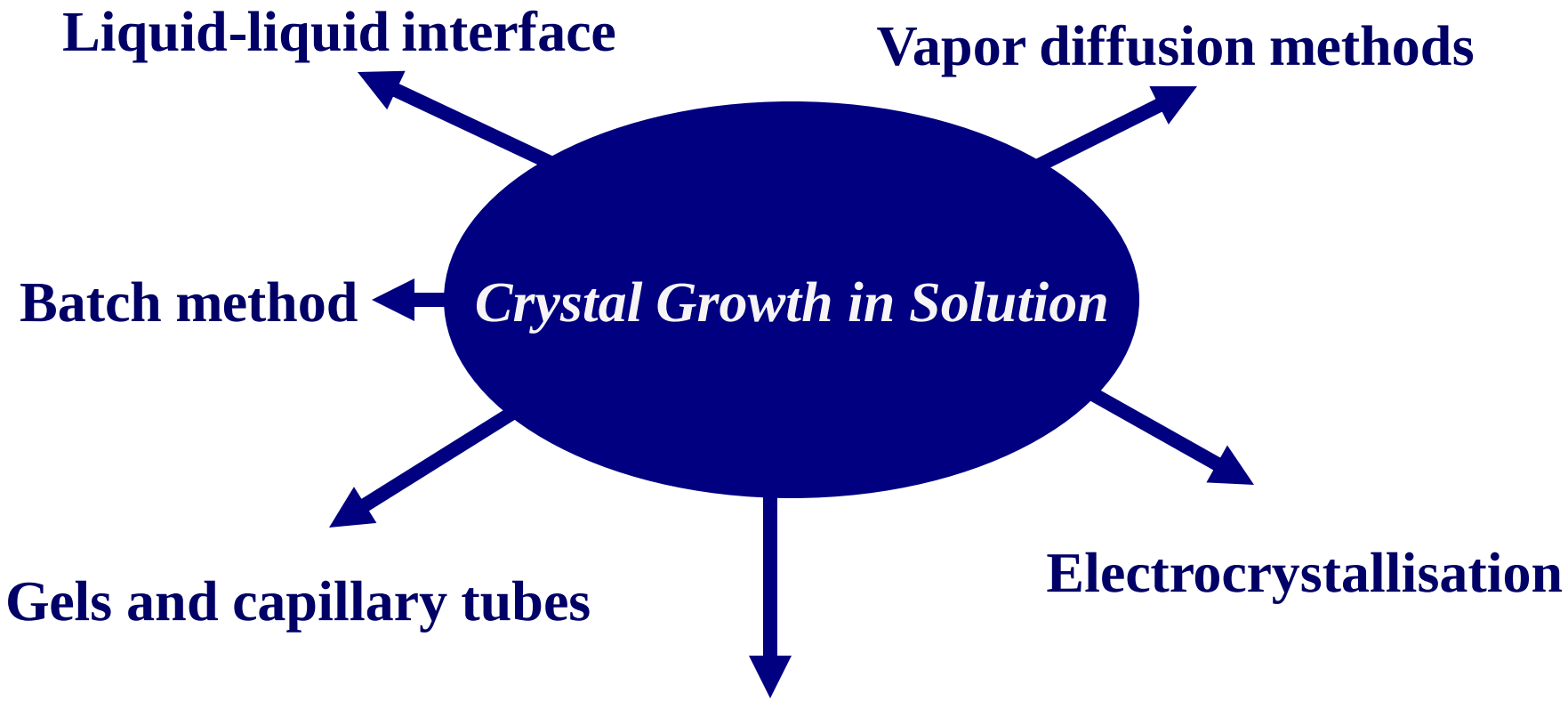
3) Transparent solution

Crystal Growth Methods

- **INORGANICS (small molecular weight):**

- **From melts as the Czochralski Process**
- **By solid State Synthesis: using furnaces and crucibles at high temperature**
- **In μ-gravity or hyper-gravity conditions**




Liquid-liquid interface
Vapor diffusion methods
Batch method
Crystal Growth in Solution
Gels and capillary tubes
Electrocrystallisation
Natural-crystal-growth: biomineralization phenomena

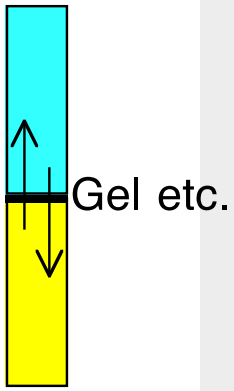
ROBOTICS for the New Millennium

This robot is valid only for screening the crystallisation conditions, and optimization, but not for crystal quality enhancement!!!

Methods & tracks of supersaturation

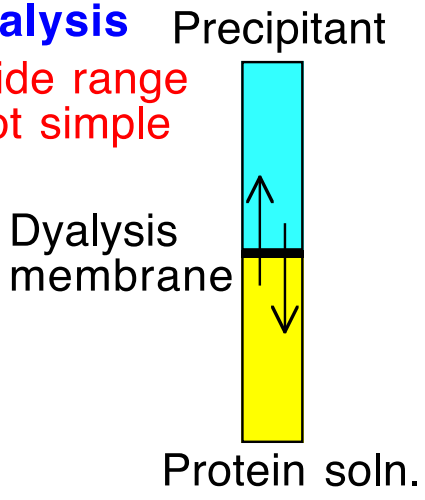
Liquid-liquid diffusion

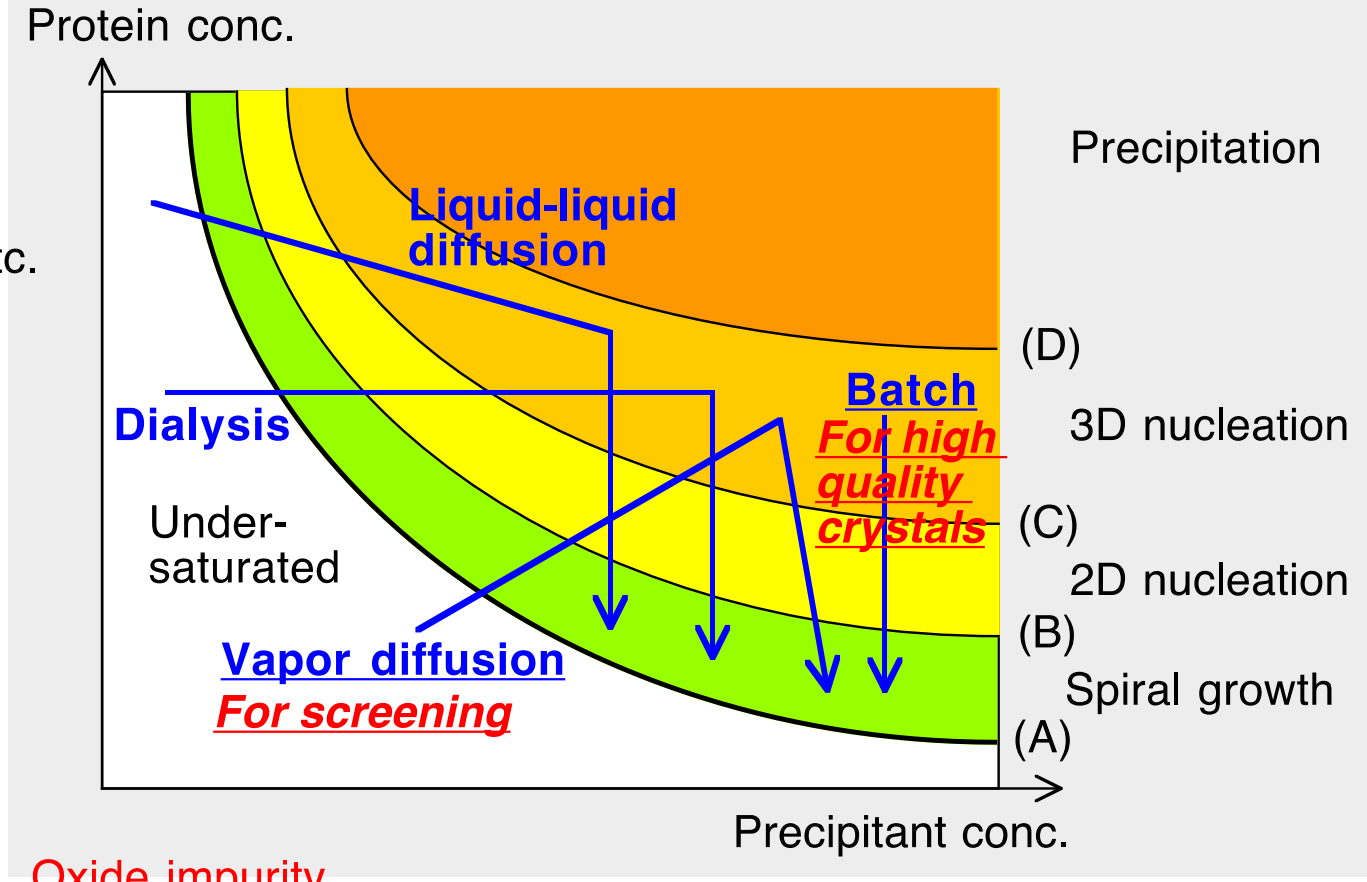
Wide range
Not simple

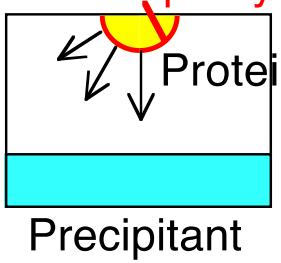


Dialysis

Wide range
Not simple

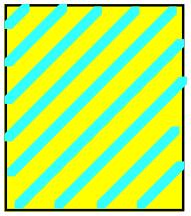






Vapor diffusion

Wide range & simple, but low quality

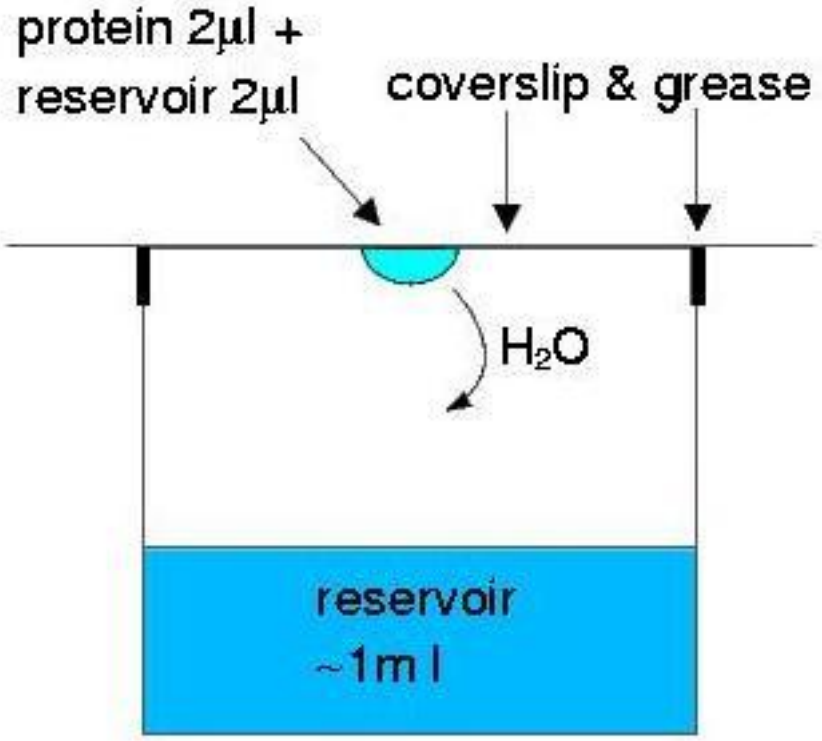


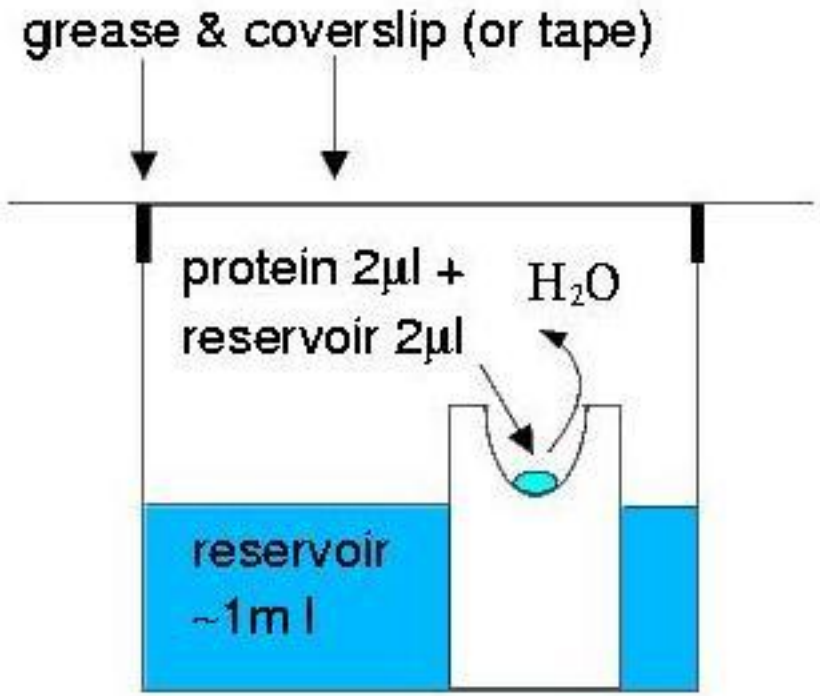
Batch

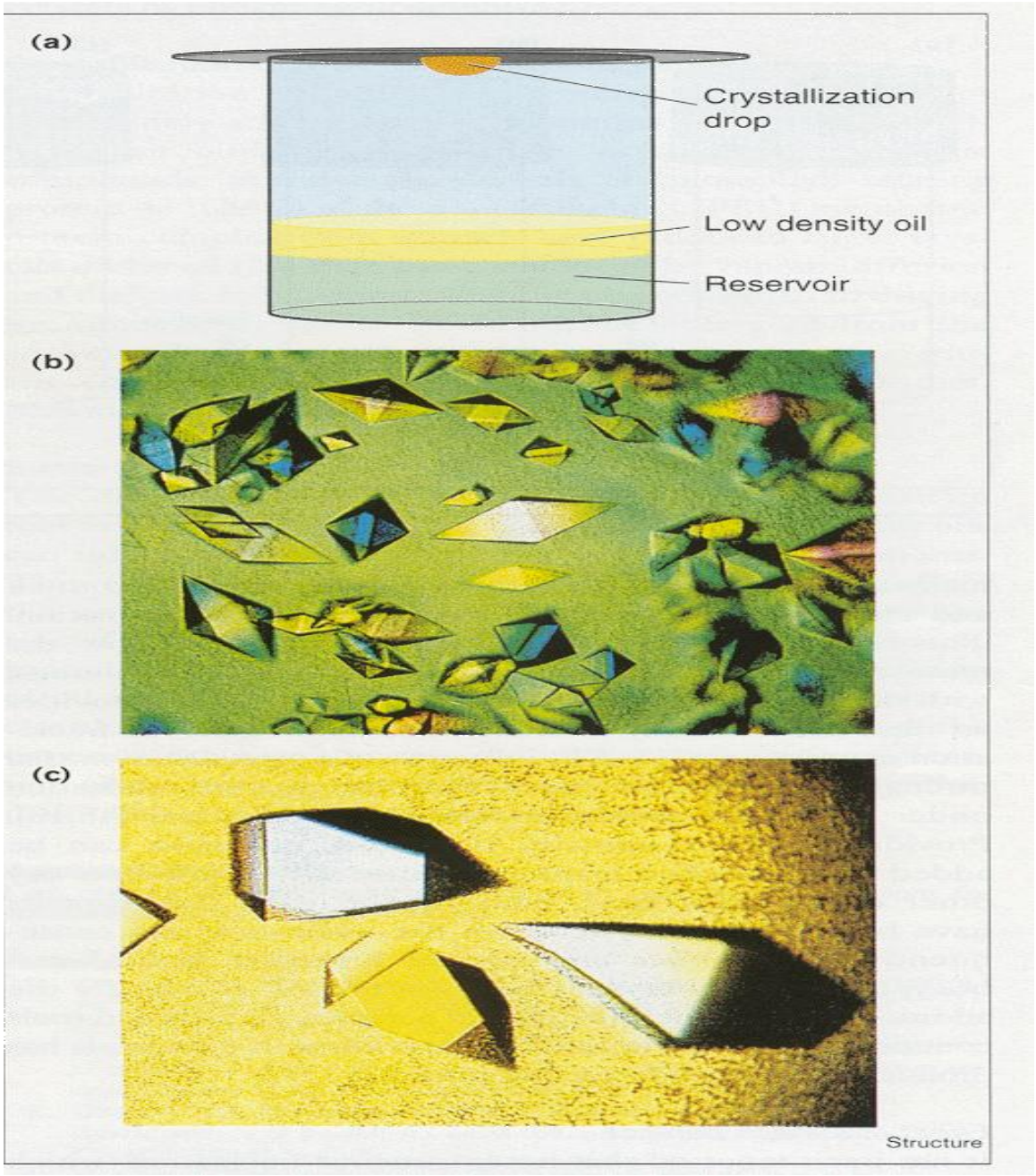
Easy to control
Low efficiency in screening

Mixture of protein-precipitant soln.

Hanging & Sitting- drop Method



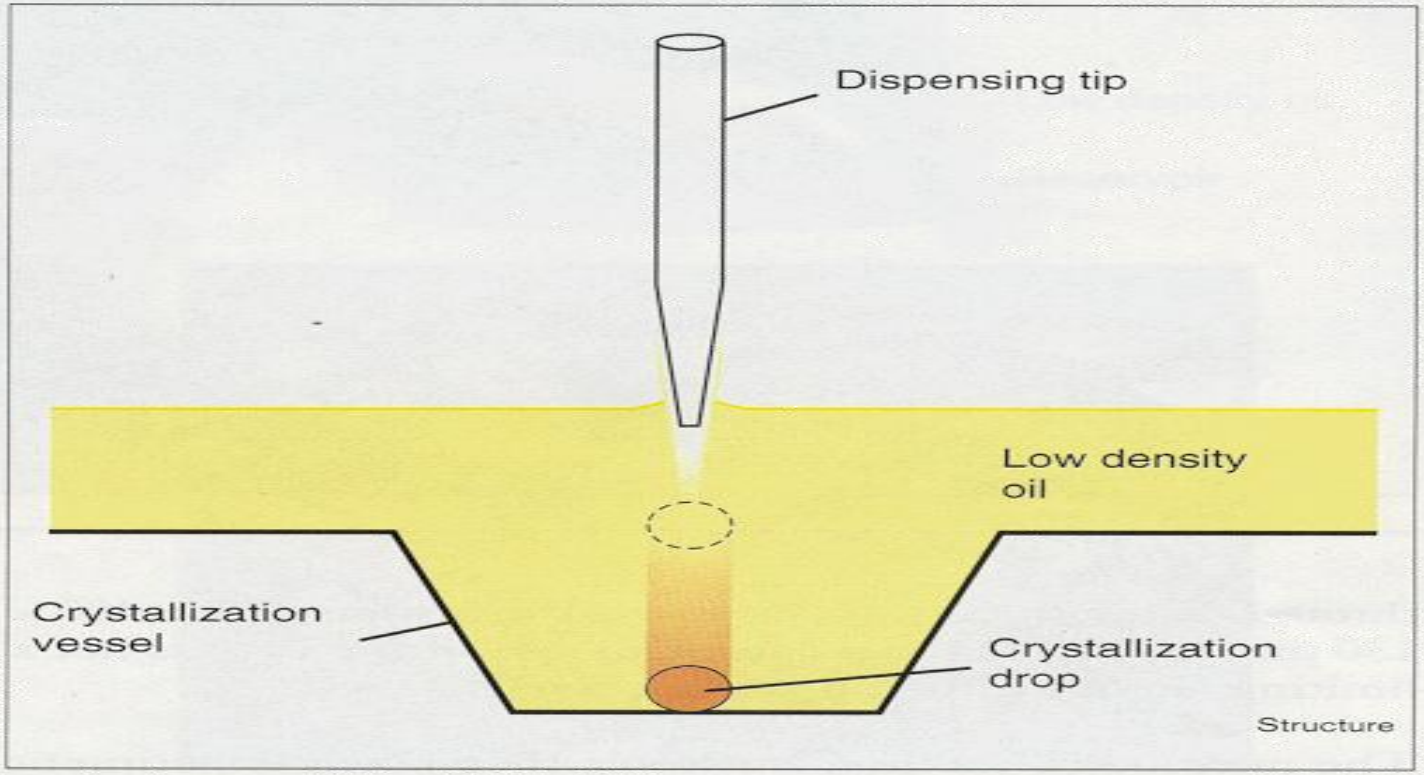




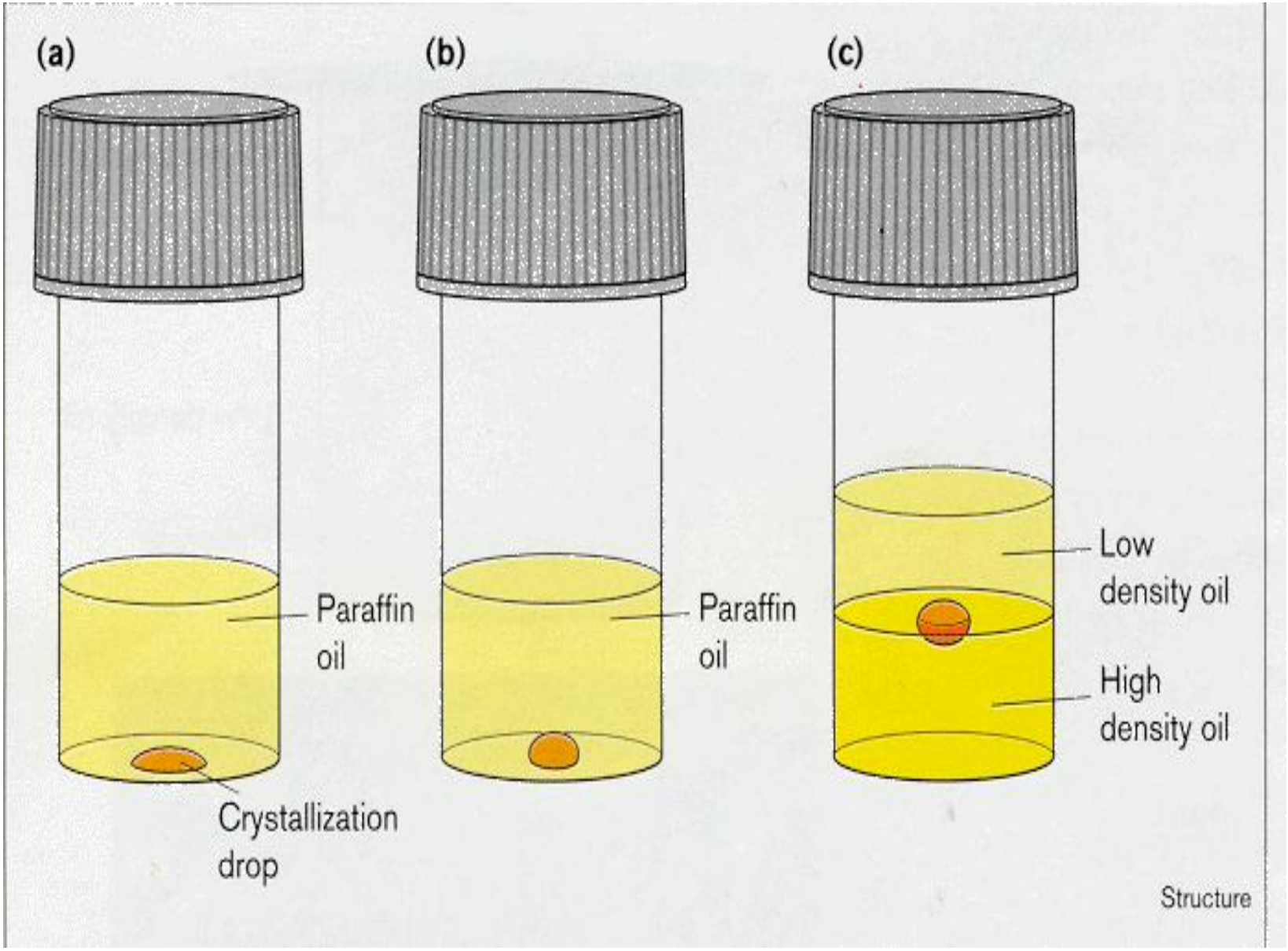
Structure

The batch crystallisation method

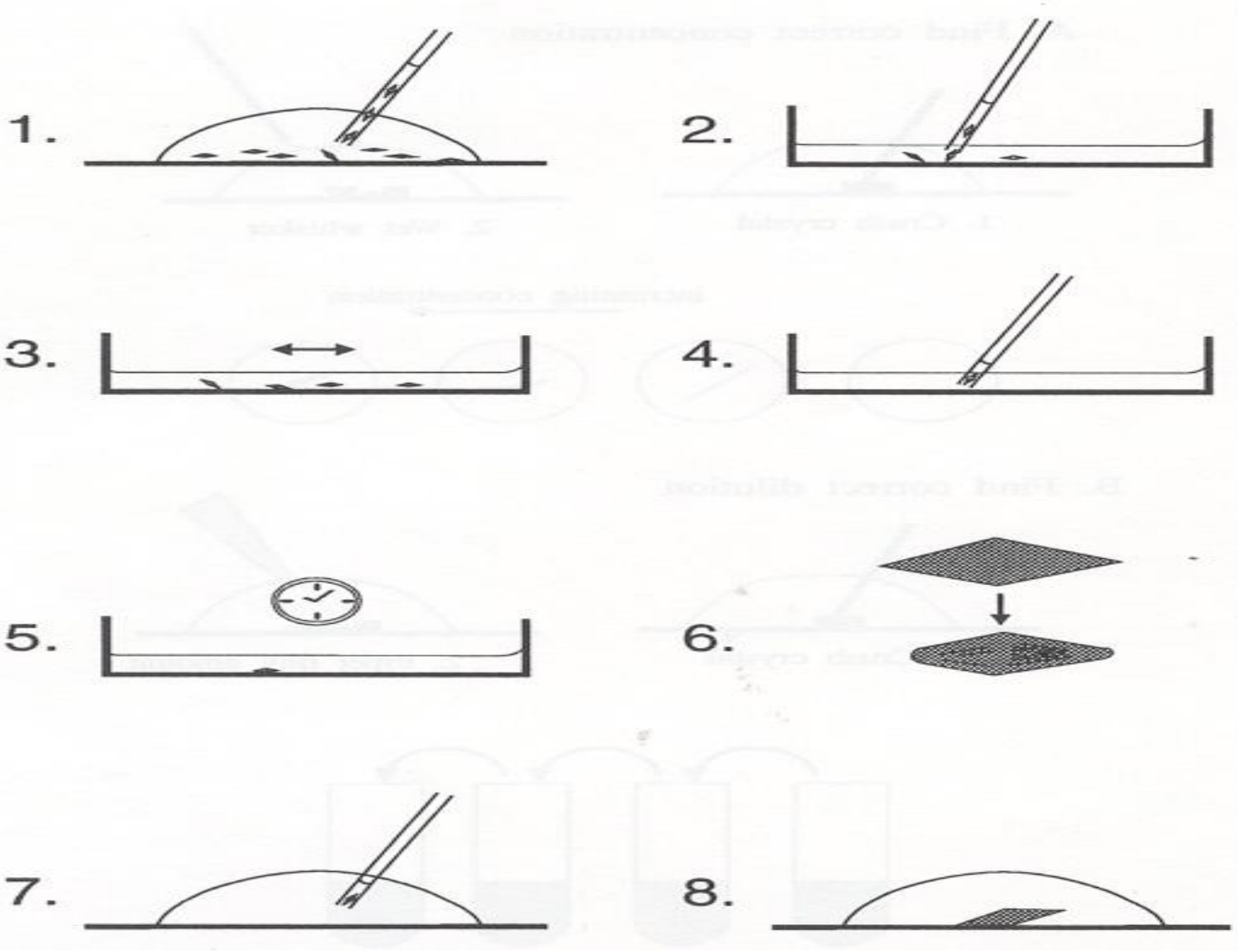
Figure 1



The mechanism of dispensing a crystallization trial under oil. A crystallization drop is dispensed into a container, under the surface of a layer of oil. The dashed circle represents the initial position of the drop at the time of dispensing. As the dispensing tip is withdrawn from the oil, the aqueous drop detaches from it and sinks to the bottom of the vessel.


(a)
(b)
(c)
Paraffin oil
Paraffin oil
Low density oil
High density oil
Crystallization drop
Structure

MACROSEEDING METHOD



MICROSEEDING METHOD

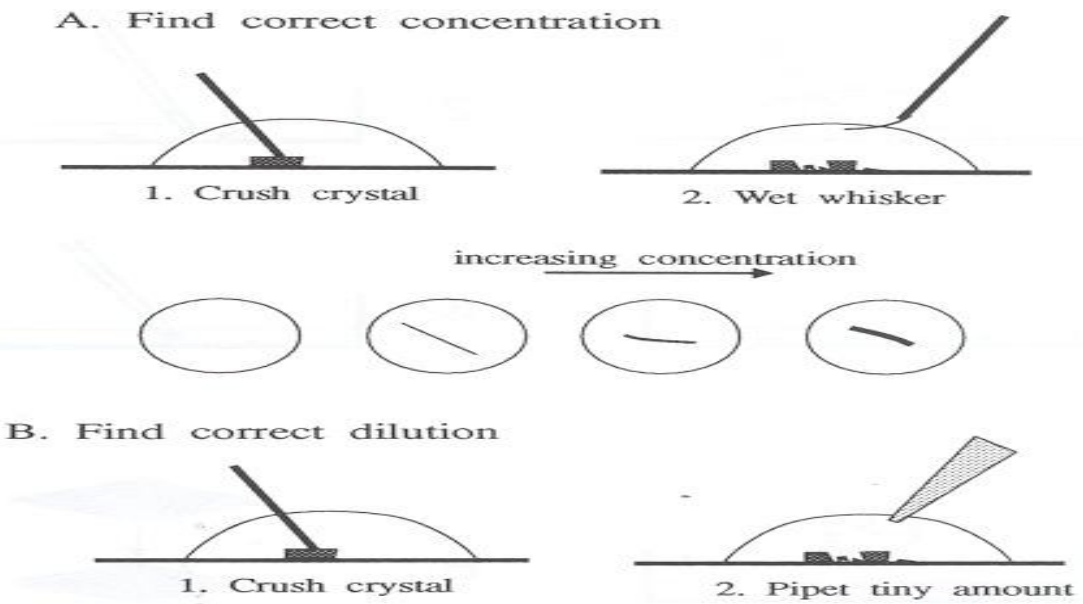
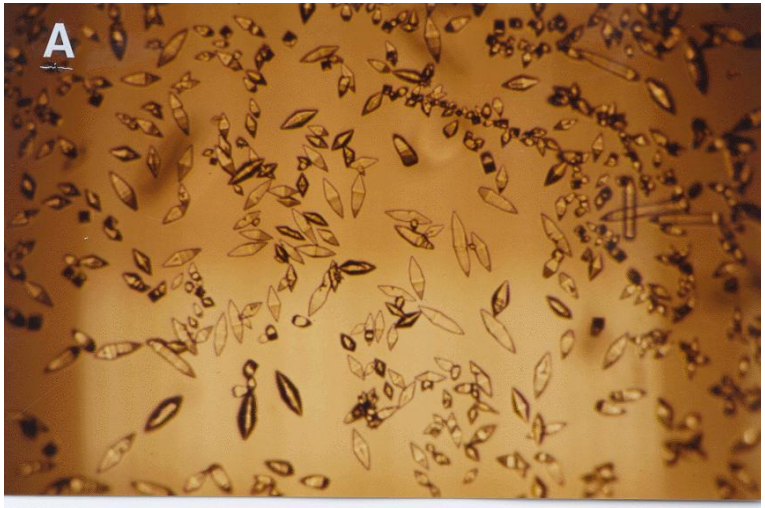
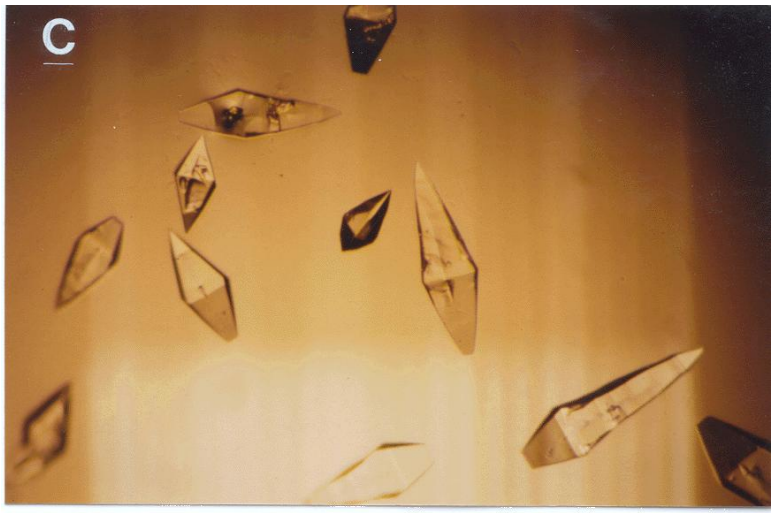


FIG. 1.6 Microseeding techniques.

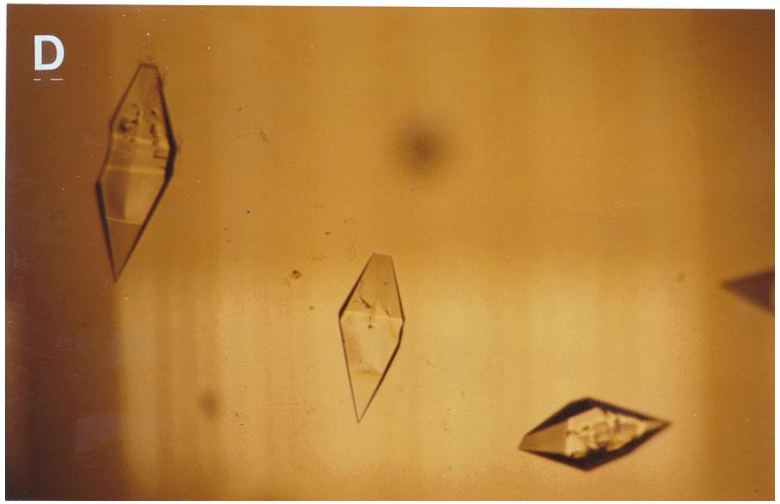
Non popular micro-seeding



Dilutions: A) 1:2, B) 1:10



Dilutions: c) 1:50, d) 1:100



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