

The background of the slide is a photograph of an industrial facility, likely a refinery or chemical plant. It features a complex network of large, silver-colored metal pipes and structures. The pipes are arranged in various directions, some running horizontally and others vertically. The sky is a clear, bright blue with some light, wispy clouds. The overall scene is brightly lit, suggesting a sunny day.

# ΔΙΕΡΓΑΣΙΕΣ ΔΙΑΧΩΡΙΣΜΟΥ

## Μάθημα 2ο

Ακαδημαϊκό έτος 2017-2018

**Solid phase** : mostly rigid structure

**Disadvantage** : formation of solid particles is a rather slow process, and to reach an acceptable production rate large vessels are generally needed.

**Advantage**: The rigid structure of the solid phase impedes the incorporation of foreign substances or solvent molecules, and **in only one separation step a pure solid product is obtained.**



# Crystallization

Often used as a generic term for:

- Evaporative
- Cooling crystallization
- Precipitation
- Melt crystallization.

Considerable differences between the four types of crystallization as far as the processing method and the corresponding equipment are concerned.



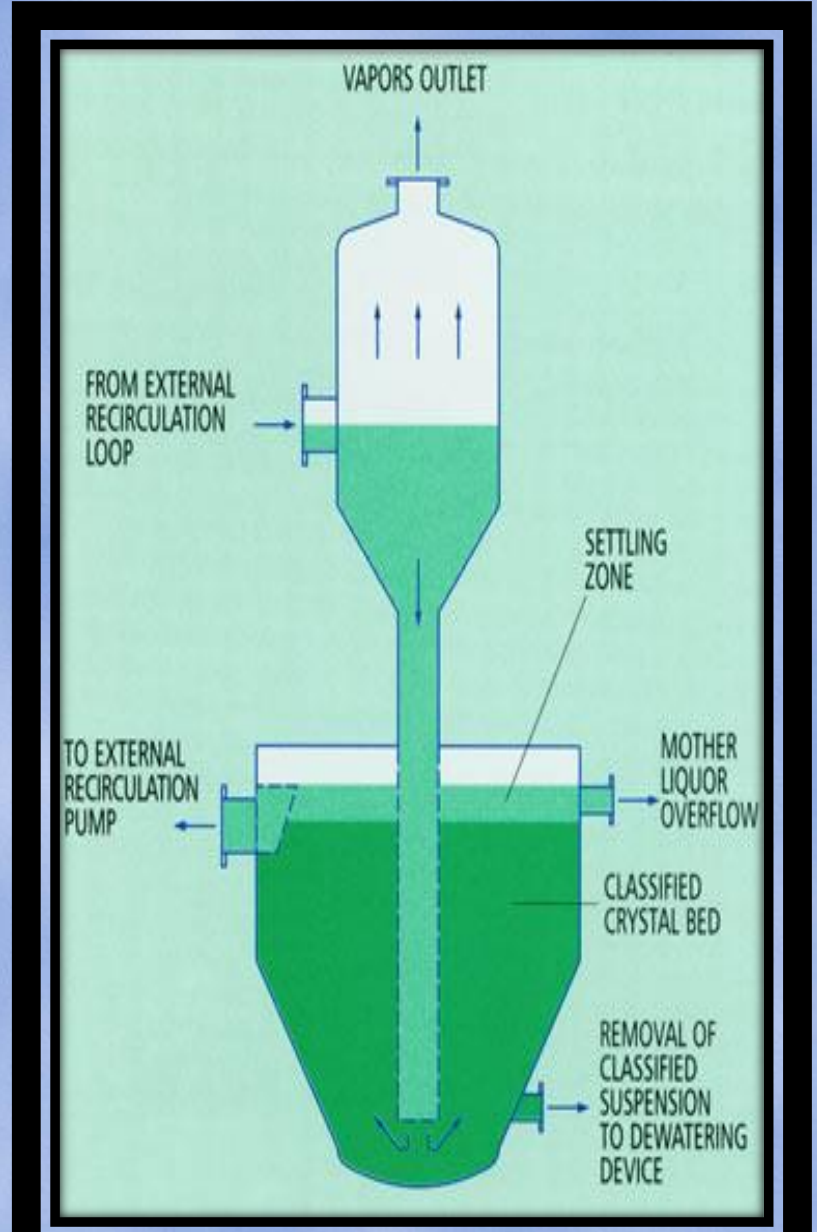
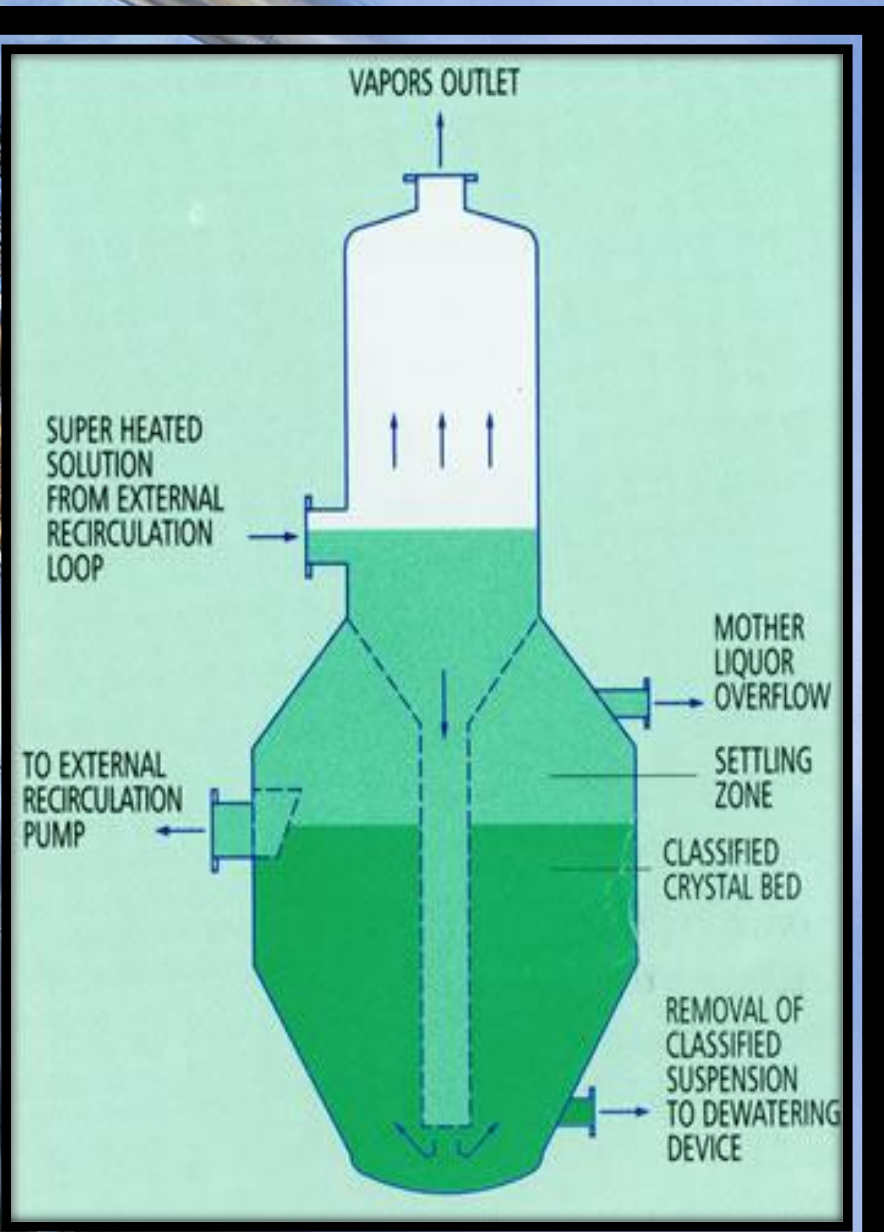
The background of the slide features a photograph of an industrial distillation column and associated piping. The column is a tall, cylindrical structure with a complex network of pipes and ladders. The scene is set against a clear blue sky with some light clouds. The overall image has a slightly grainy, high-contrast appearance.

## Evaporative Crystallization

Evaporative crystallization is usually a process that is conducted under vacuum. This process is chosen when solubility of the solute is nearly independent of temperature.

Special scaling problems are not a serious problem as long as boiling on the heater surface is avoided

# OSLO CRYSTALLIZER



# Crystallization

**Precipitation:** the separation of the solid phase is achieved by mixing two feed streams that are either two reactants or a solvent containing the solute and an antisolvent.

Hydrodynamics of the process play a predominant role in precipitation with regard to the properties of the obtained product.

**Melt crystallization:** production of a pure product is by re-melting the solid phase to obtain the final product.

Applications: ultra-purification of organic compounds  
production of pure water (concentration technique).



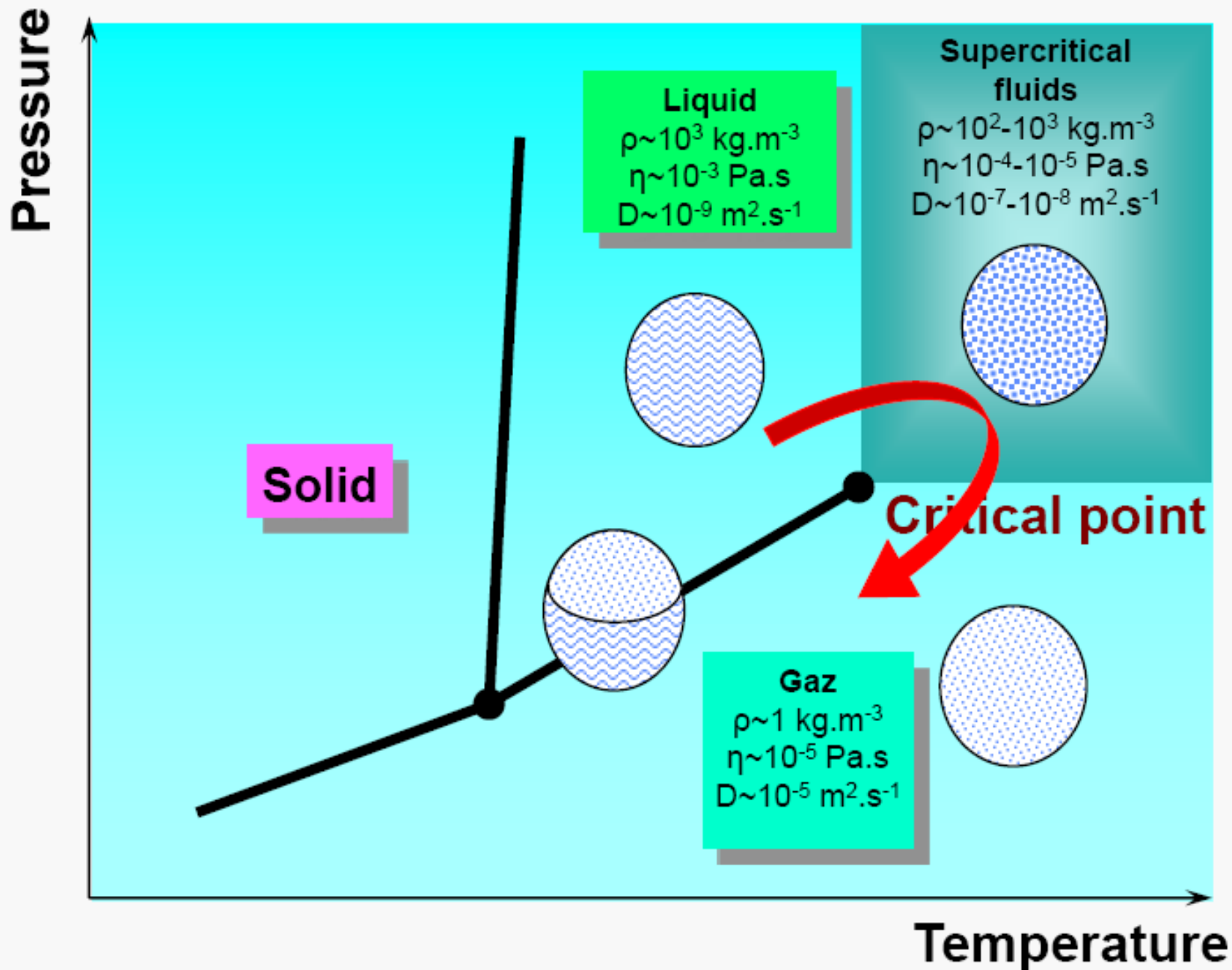
# Cooling Crystallization

- ❖ Crystallization is based on the principles of solubility: compounds (solutes) tend to be more soluble in hot liquids (solvents) than they are in cold liquids.
- ❖ If a saturated hot solution is allowed to cool, the solute is no longer soluble in the solvent and forms crystals of pure compound.
- ❖ Impurities are excluded from the growing crystals and the pure solid crystals can be separated from the dissolved impurities by filtration.

# Crystallization

- **Supercritical crystallization:**
- mostly with condensed  $\text{CO}_2$ , because of its benign properties compared to organic solvents.
- Condensed  $\text{CO}_2$  can be used either as a solvent or as an anti-solvent, and specifically adapted processes and equipment have been developed for these high pressure crystallization techniques.






# Importance of Supercritical Fluid

- Liquids have solubilizing nature
- Gases have diffusivity and compressibility / expandable nature.
- Supercritical fluids
- SCFs offer:
  - ✓ Liquid-like density and solubilizing capacity
  - ✓ Gas-like viscosity, compressibility and diffusivityallowing for good mixing and mass transfer hence labeled as fluids.

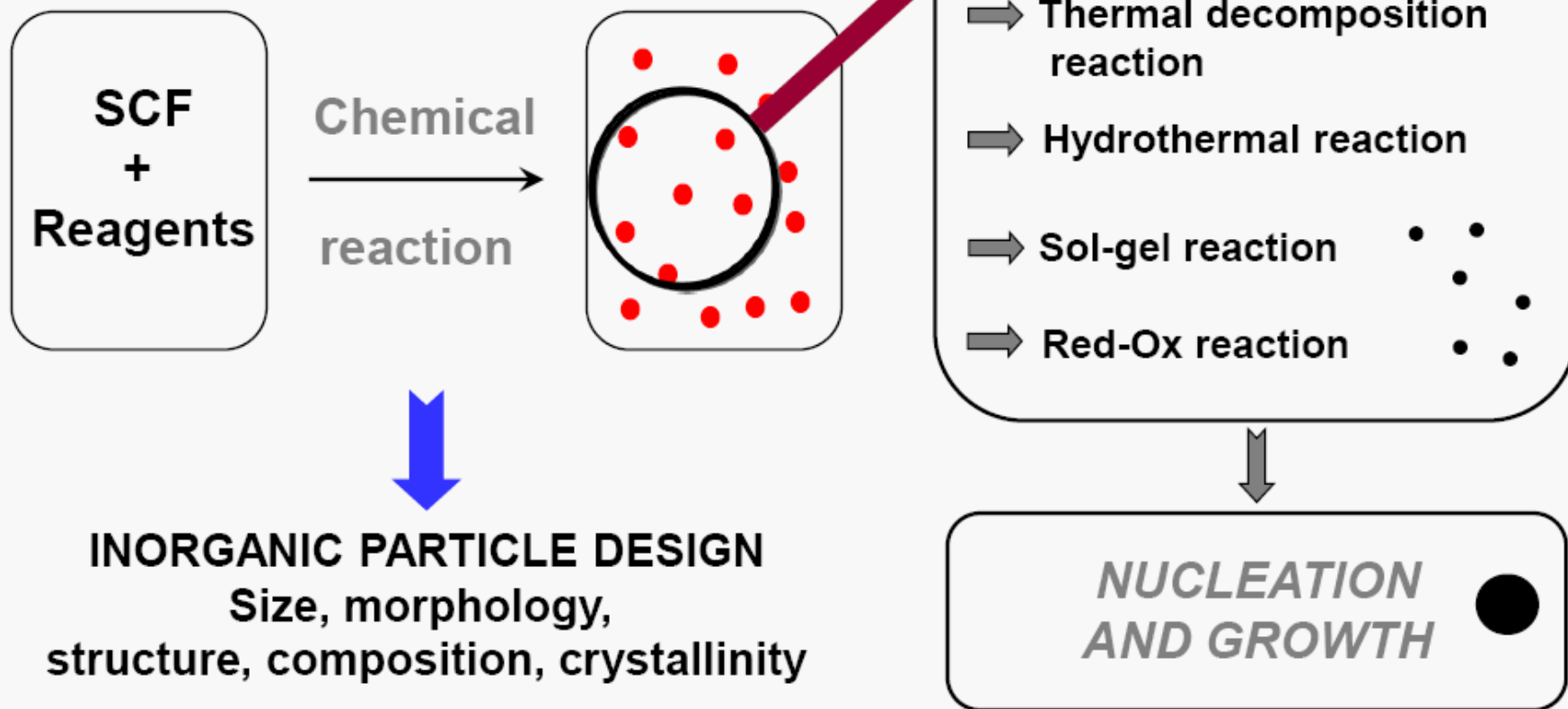


## Key Features

- SCFT offers tremendous potential, as it is safe, inexpensive, eco-friendly, non-toxic and economical.
  - With SCFs at hand, there is no need of any organic solvents.
  - Solvation capacity of SCF  $\propto$  fluid density.
  - Sensitive to small changes in operating conditions.
  - Operating conditions of low temperature and pressure make SCFs attractive for pharmaceutical research, especially thermolabile materials.
- 



## Particle design based on chemical transformation



**SCFs → UNCONVENTIONAL REACTION MEDIA WITH TUNEABLE PROPERTIES FOR A GREEN CHEMISTRY**

# Critical properties of commonly used SCFs

Fluid	Critical Temperature ( $^{\circ}\text{C}$ )	Critical Pressure (bar)
Ethylene	9.4	50.4
Trimethoflurane	26.3	48.6
Chlorotrifluoromethane	29.0	38.7
<b>Carbon dioxide</b>	<b>31.1</b>	<b>73.8</b>
Ethane	32.4	48.8
Propylene	91.9	46.0
Propane	96.8	42.5
Ammonia	132.5	113.5
n-Pentane	196.7	33.7
Trichloromethane	198.2	44.1

## Ideal Properties of CO<sub>2</sub>


- Low critical temperature (31.1° C)
- Moderate critical pressure of 73 bar
- Non-flammable
- Non-toxic
- Miscible with variety of organic solvents
- Recoverable after processing
- Diffuses faster than conventional liquid solvents
- Generally Recognized As Safe (GRAS) status
- Approved by FDA for use in food and pharmaceutical operations
- Eco-friendly
- Inexpensive



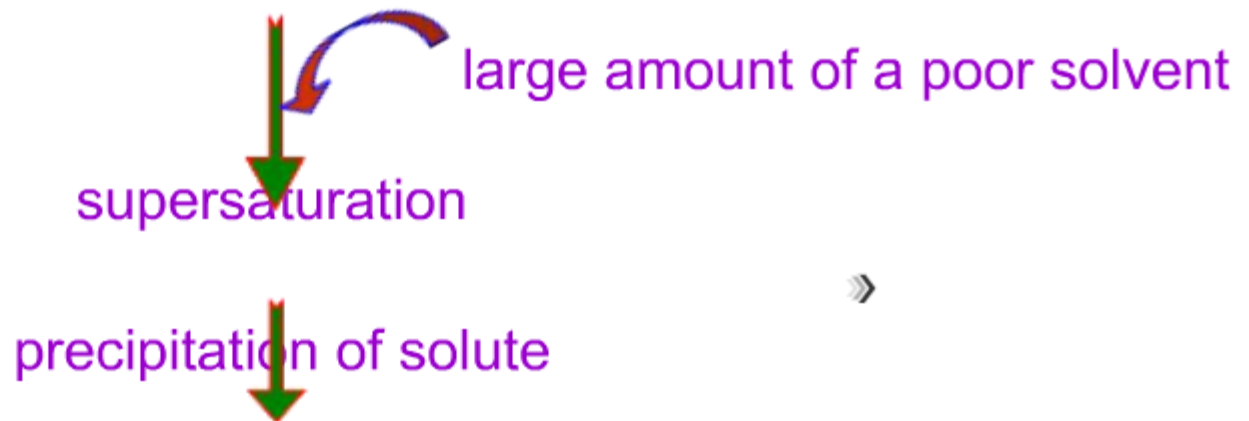
## Co-solvent

- Polar or non-polar miscible solvent (1% to 5%).
- Purpose: To modify the polarity and solvent strength of the SCF.  
E.g., Methanol, Ethanol, Acetone & Dimethyl sulfoxide (DMSO).
- Mechanisms:
  - ✓ Hydrogen bonding
  - ✓ Complex formation
  - ✓ Dipole interactions
  - ✓ Solvent / co-solvent / solute interactions

# Anti Solvent Processes:

- Substances that are not soluble in SCFs . . . 
- Anti solvent process
- Principle: Salting out technique

Dissolution of solid material in a suitable solvent



# Rapid Expansion of Supercritical Solutions (RESS) Or Supercritical Fluid Nucleation (SFN)

Rapid expansion



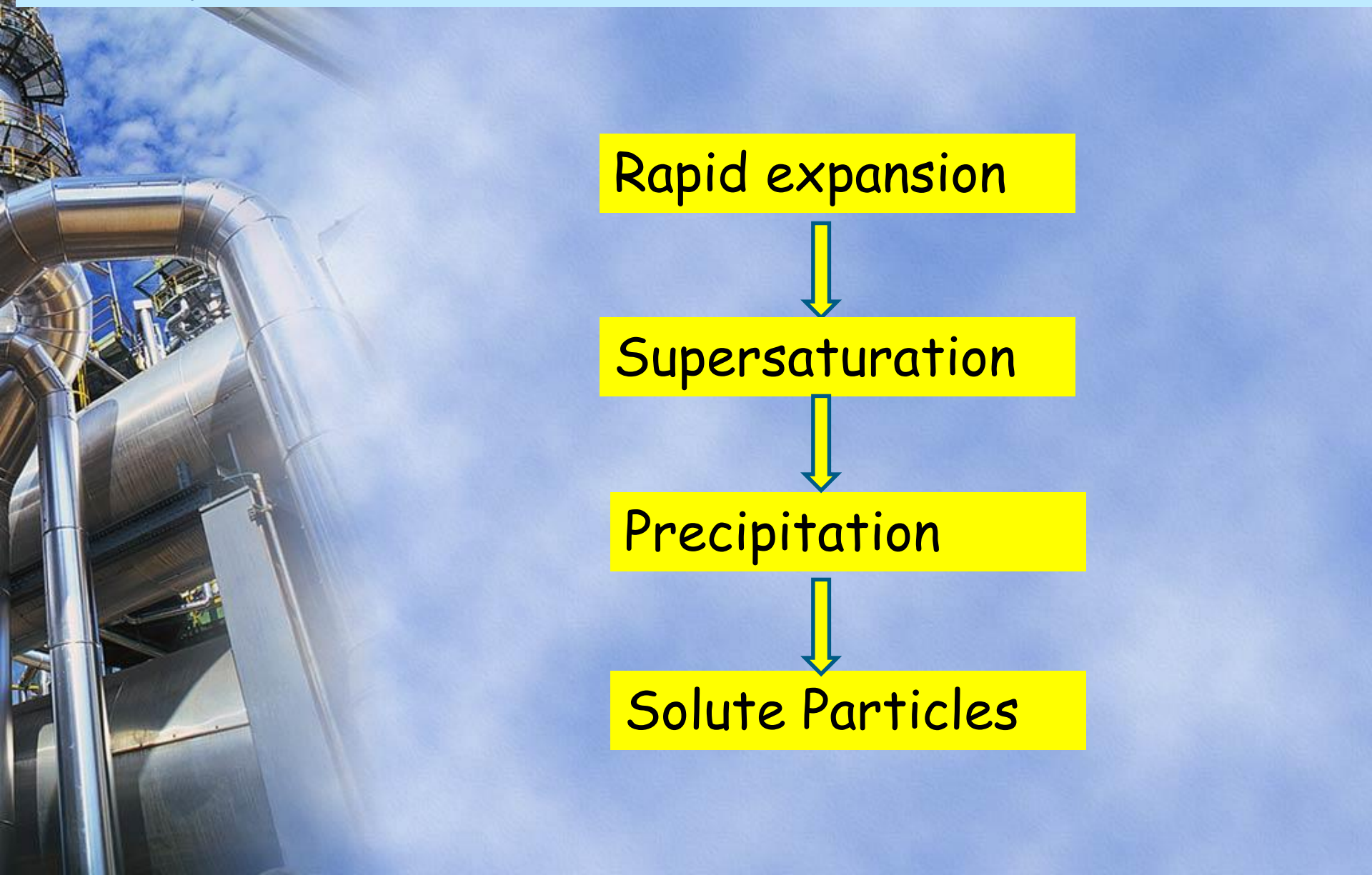
Supersaturation



Precipitation

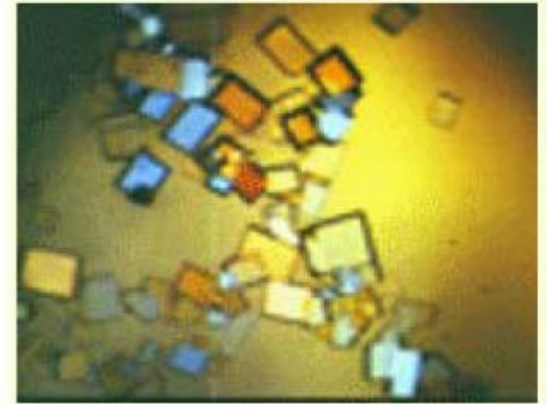
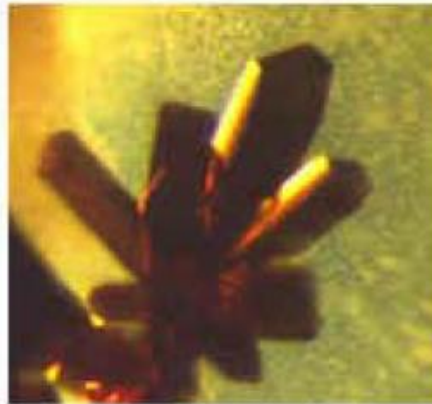


Solute Particles



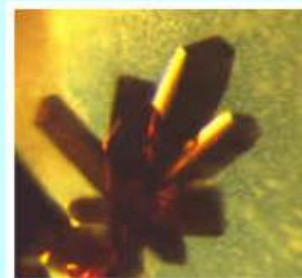


# I. Protein crystals

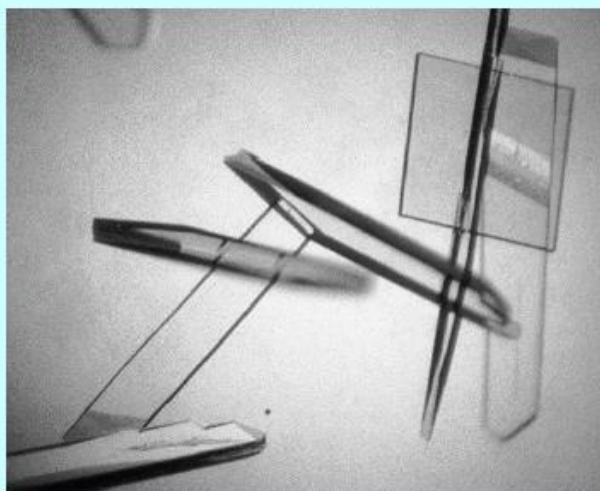




## II. Protein crystals



Sperm whale myoglobin

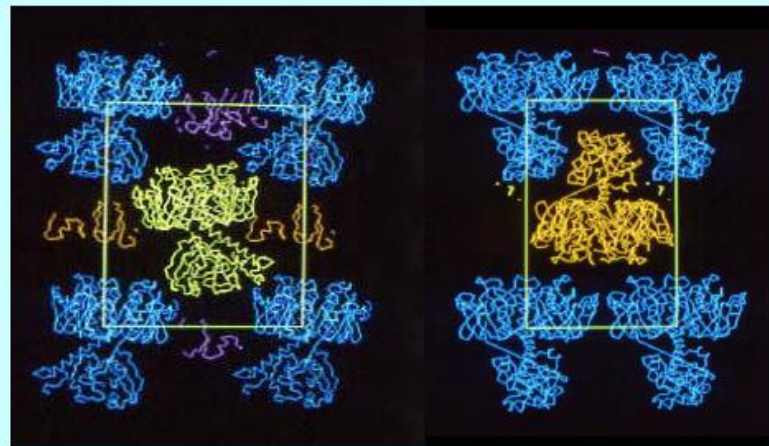
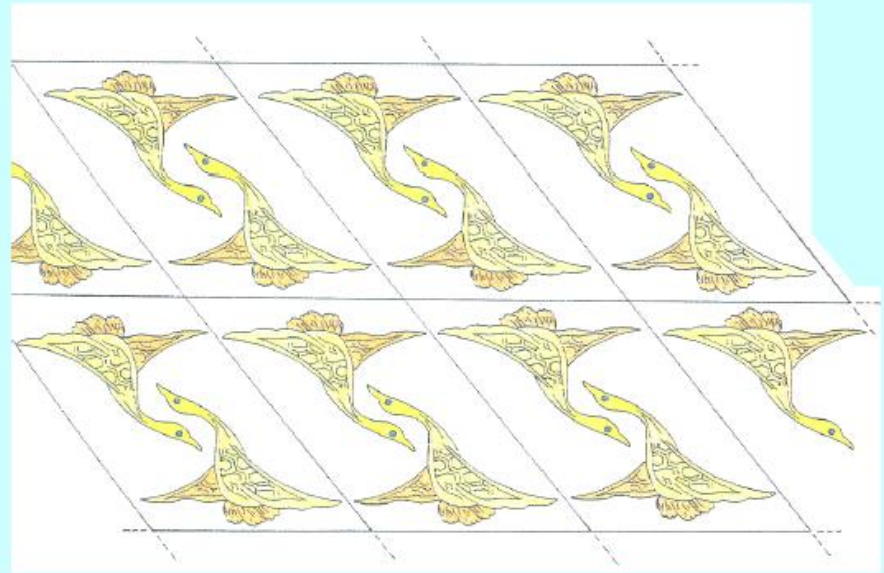


See Hampton Research: <http://www.hamptonresearch.com>) their Photo Gallery.



# Protein crystals

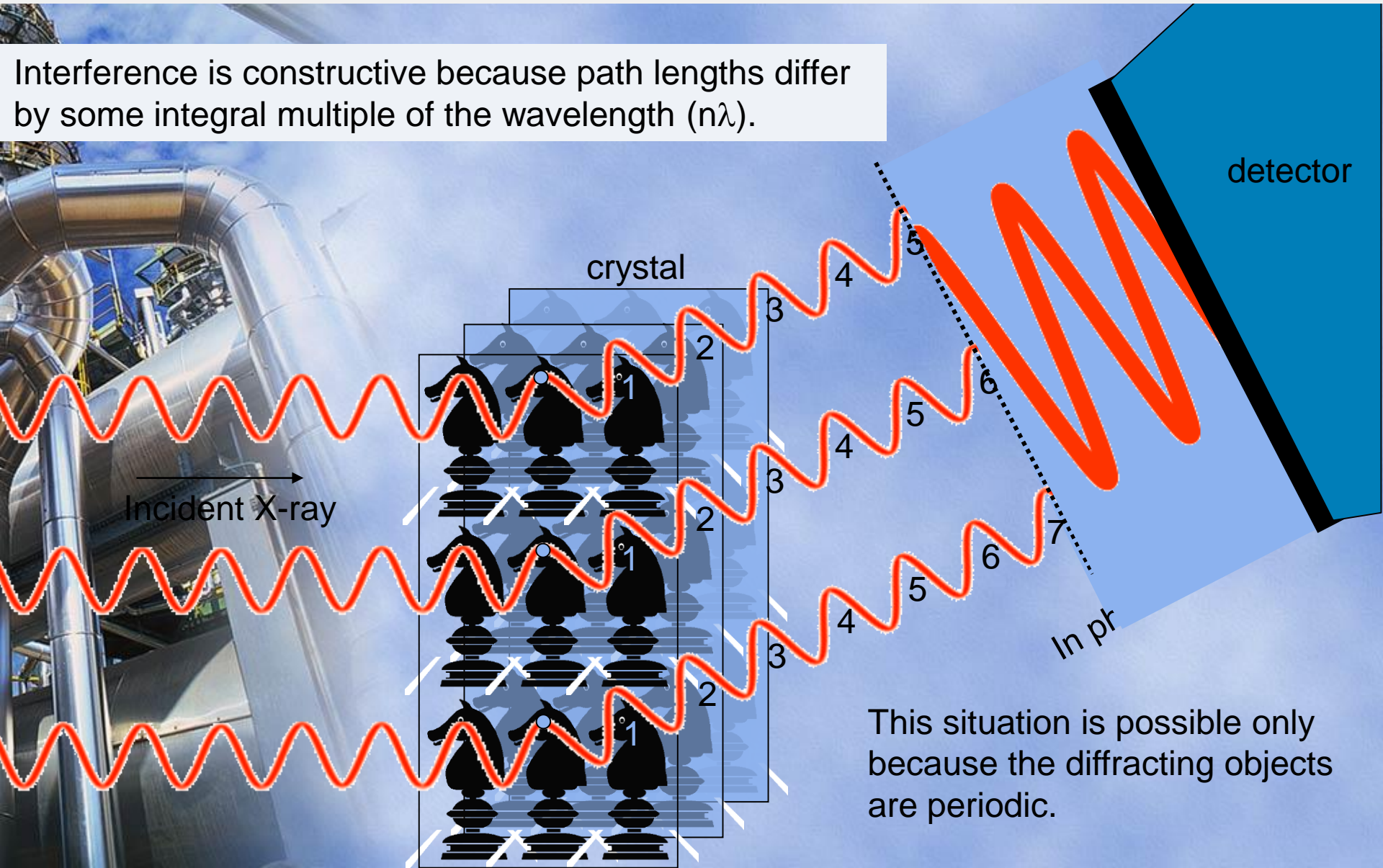
- Regular arrays of protein molecules
- Few crystal contacts
- Protein crystals contain active protein
- Enzyme turnover
- Ligand binding



Example of crystal packing

# When a crystal is ordered, strong diffraction results from constructive interference of photons.

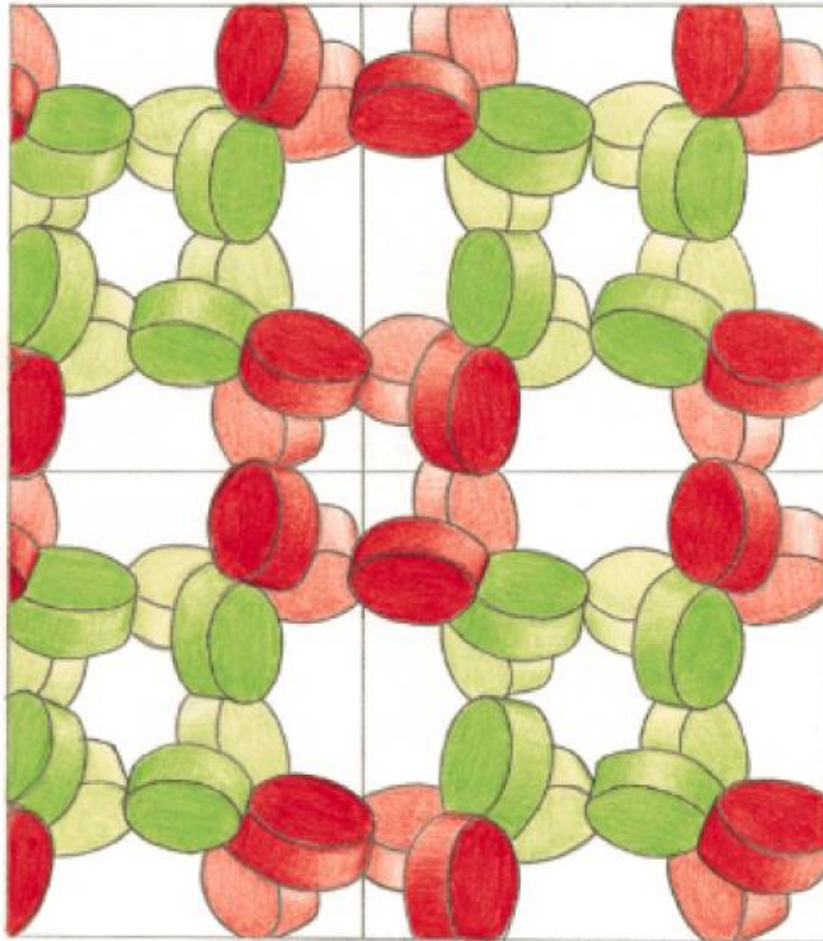
Interference is constructive because path lengths differ by some integral multiple of the wavelength ( $n\lambda$ ).



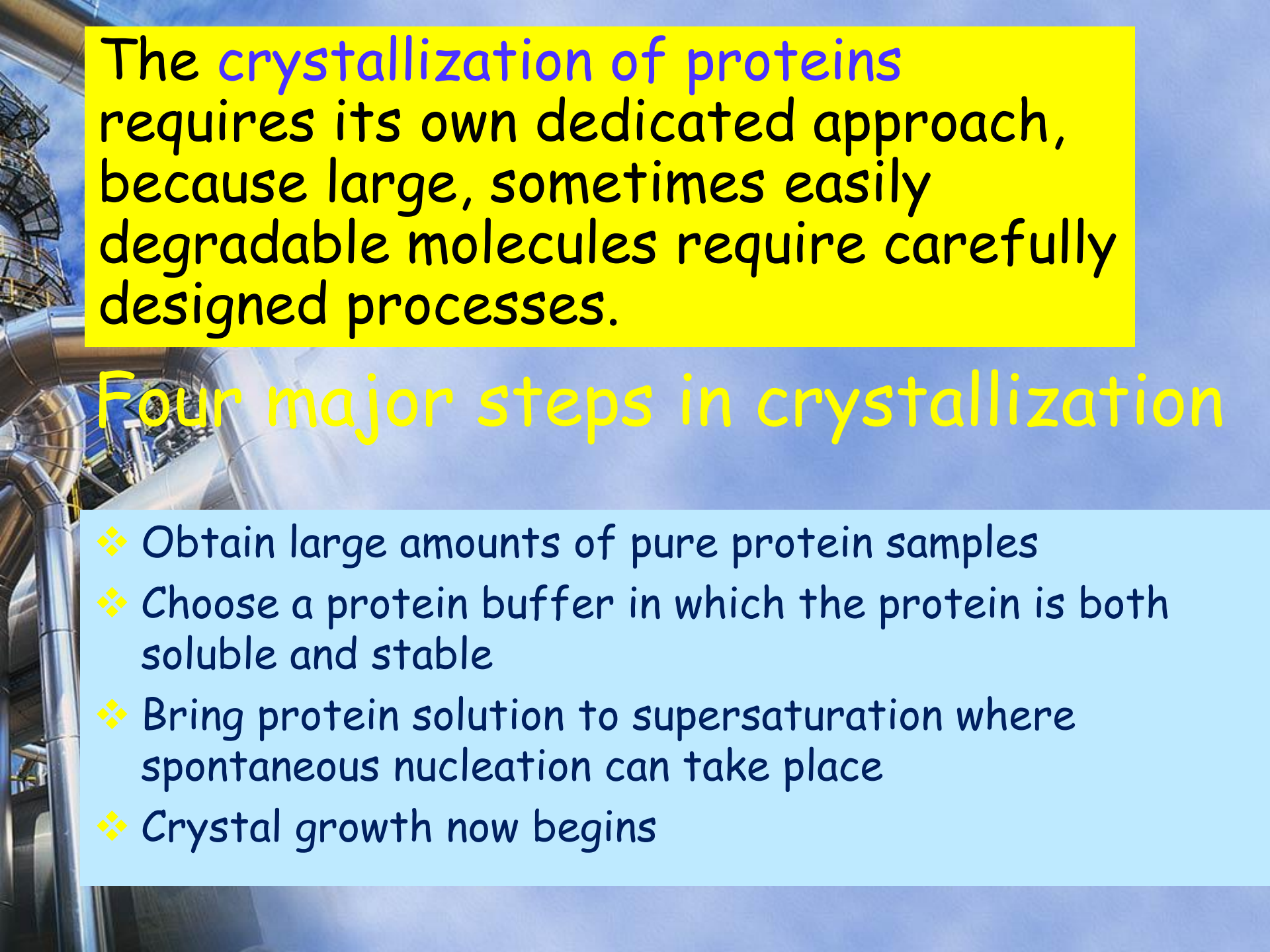
This situation is possible only because the diffracting objects are periodic.



# Protein crystals contain lots of solvent



typically 30 to 70%  
solvent by volume  
'Wet crystals'

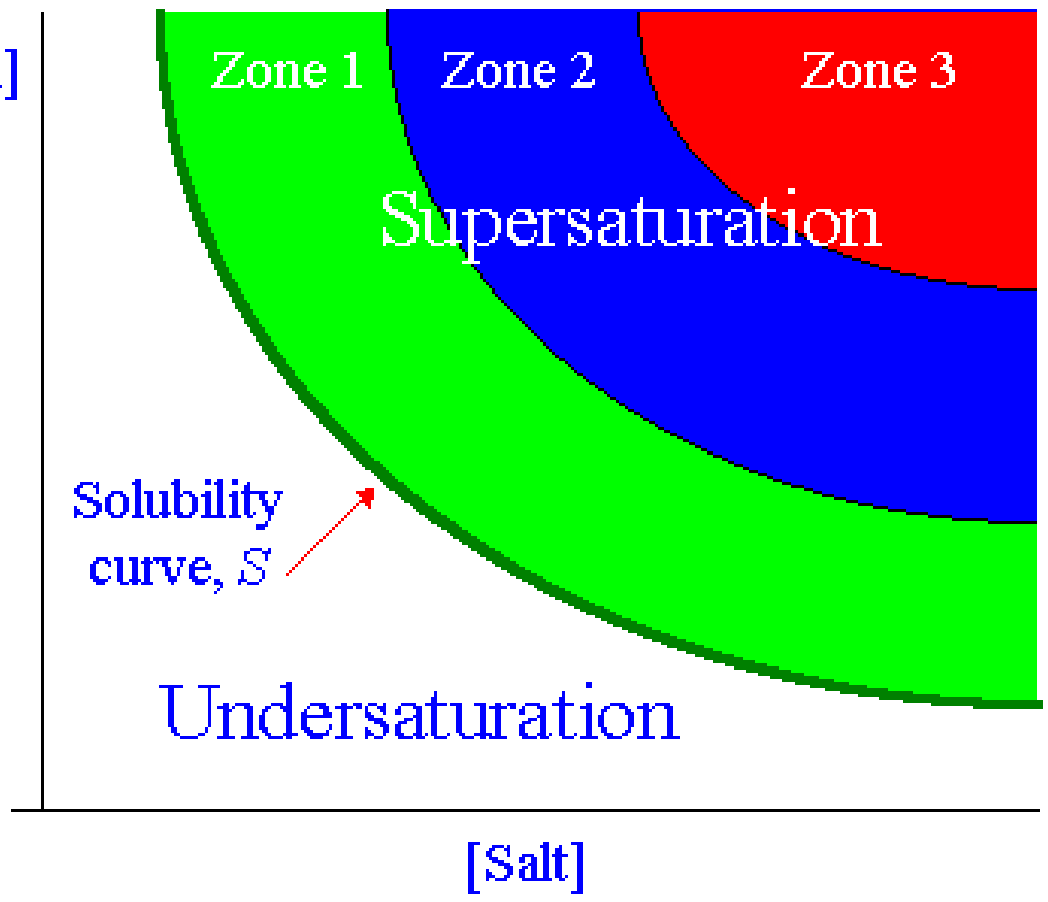


The crystallization of proteins requires its own dedicated approach, because large, sometimes easily degradable molecules require carefully designed processes.

## Four major steps in crystallization

- ❖ Obtain large amounts of pure protein samples
- ❖ Choose a protein buffer in which the protein is both soluble and stable
- ❖ Bring protein solution to supersaturation where spontaneous nucleation can take place
- ❖ Crystal growth now begins

[Protein]



Below  $S$  - no ppt

Zone 1 -  
Metastable  
rare nucleation  
sustains growth  
(seed)

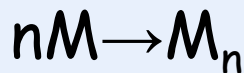
Zone 2 - Nucleation  
crystals grow

Zone 3 -  
Precipitation



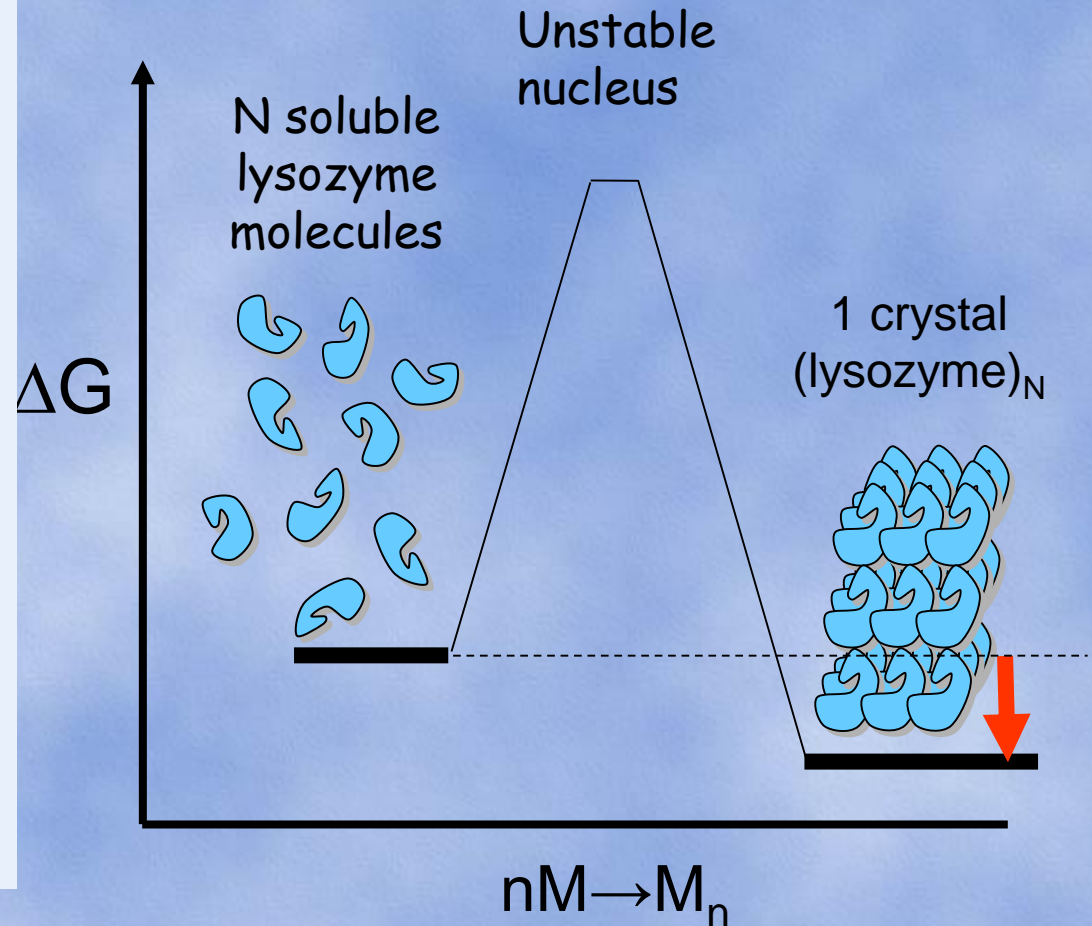
# Increase [protein] to favor crystallization

Increasing the monomer concentration  $[M]$  pushes the equilibrium toward the product.



$$\Delta G = \Delta G^\circ + RT \ln( [M_n] / [M]^n )$$

**Lesson:** To crystallize a protein, you need to increase its concentration to exceed its solubility (by 3x). Force the monomer out of solution and into the crystal. Supersaturate!

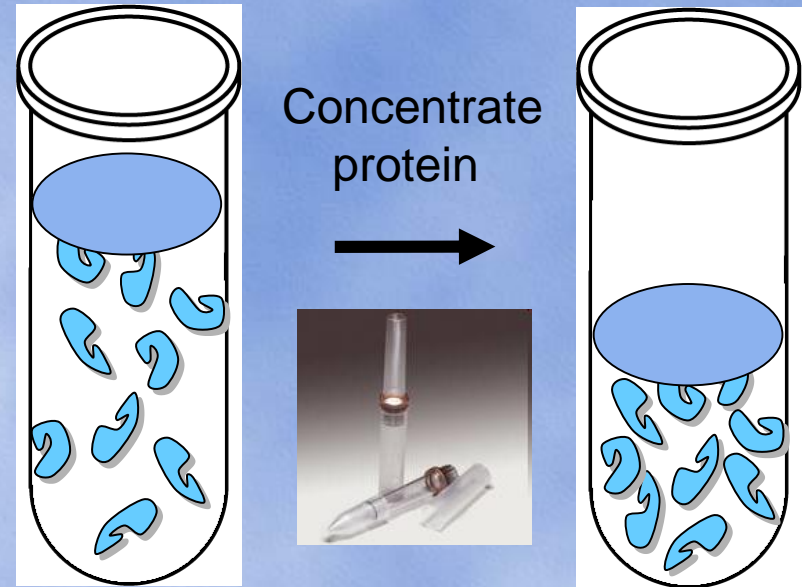




# Three steps to achieve supersaturation.

## 1) Maximize concentration of purified protein

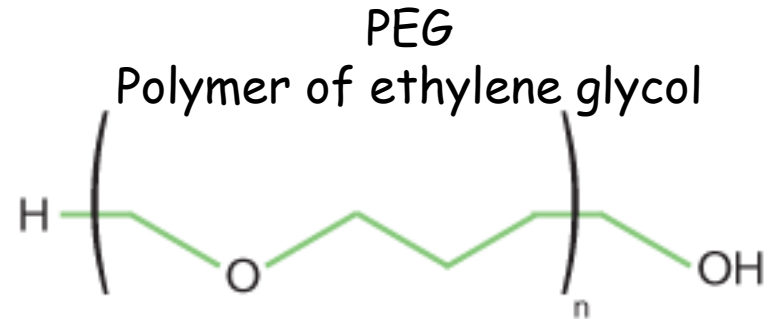
- Centricon-centrifugal force
- Amicon-pressure
- Vacuum dialysis
- Dialysis against high molecular weight PEG
- Ion exchange.
- Slow! Avoid precipitation. Co-solvent or low salt to maintain native state.



# Three steps to achieve supersaturation.

## 2) Add a precipitating agent

- Polyethylene glycol
  - PEG 8000
  - PEG 4000
- High salt concentration
  - $(\text{NH}_4)_2\text{SO}_4$
  - $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$
- Polyethylene glycol
- Small organics
  - ethanol
  - Methylpentanediol (MPD)



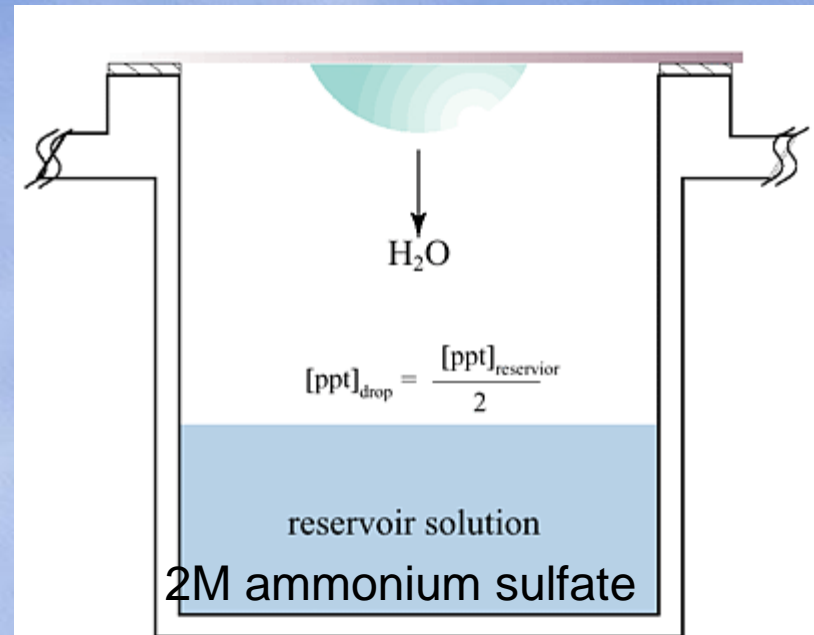
Precipitating agents monopolize water molecules, driving proteins to neutralize their surface charges by interacting with one another. It can lead to (1) amorphous precipitate or (2) crystals.

# Three steps to achieve supersaturation.

## 3) Allow vapor diffusion to dehydrate the protein solution

- Hanging drop vapor diffusion
- Sitting drop vapor diffusion
- Dialysis
- Liquid-liquid interface diffusion

Drop =  $\frac{1}{2}$  protein +  $\frac{1}{2}$  reservoir



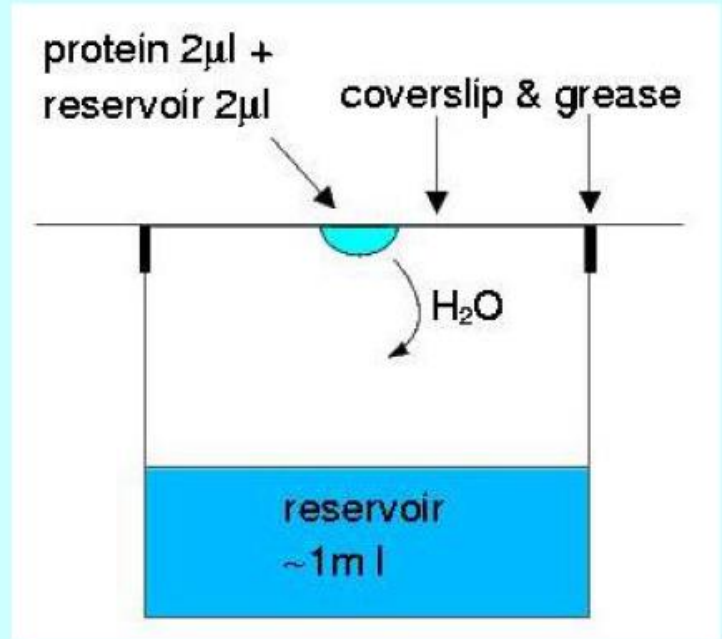
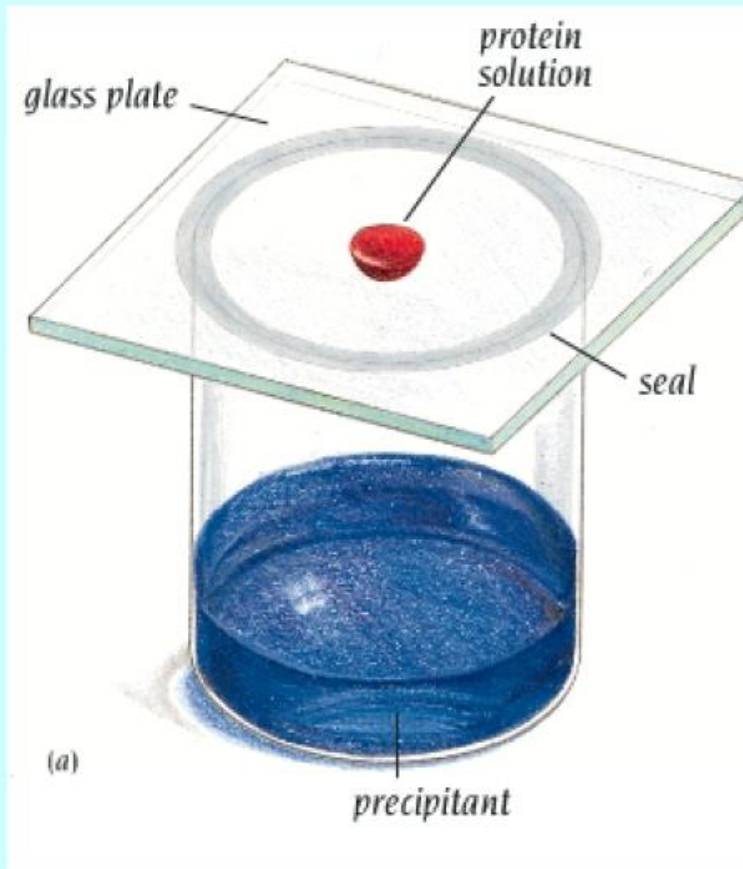
Note: Ammonium sulfate concentration is 2M in reservoir and only 1M in the drop.

With time, water will vaporize from the drop and condense in the reservoir in order to balance the salt concentration.—  
**SUPERSATURATION is achieved!**



# Protein crystallization

'Hanging drop':



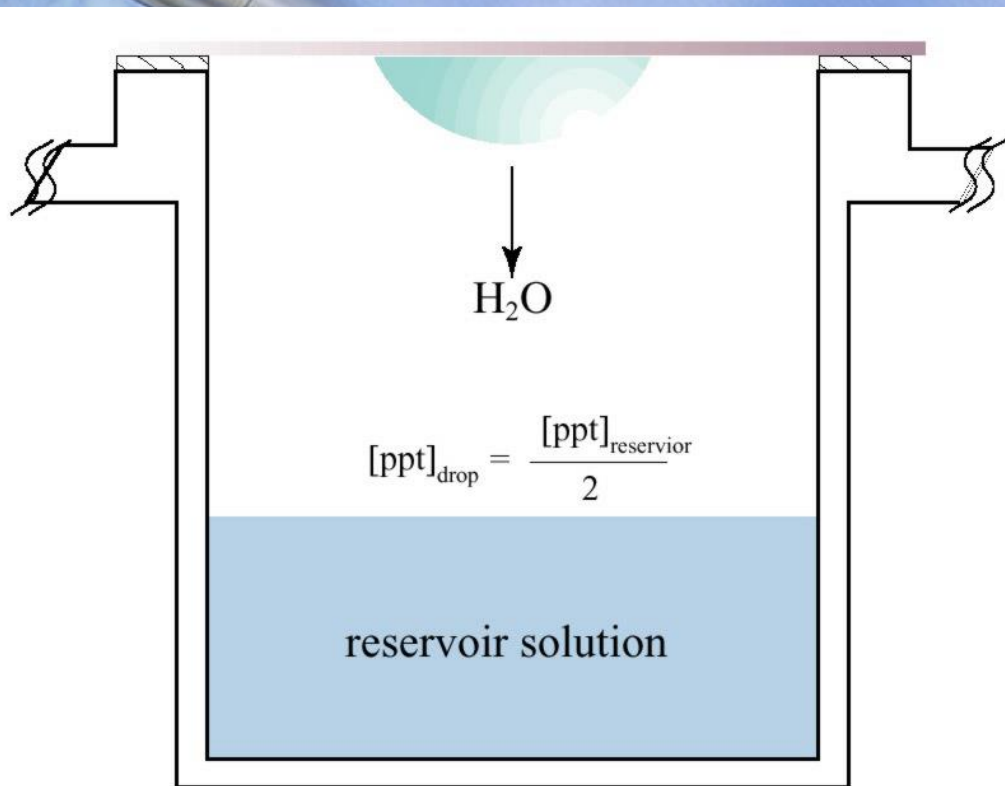
**Example:**

**Protein: 10mg/ml  
in 10 mM Tris buffer, pH7.5**

**Reservoir solution:  
2M ammonium sulphate  
in 100mM citrate buffer, pH5.5**



# Hanging Drop Vapor Diffusion

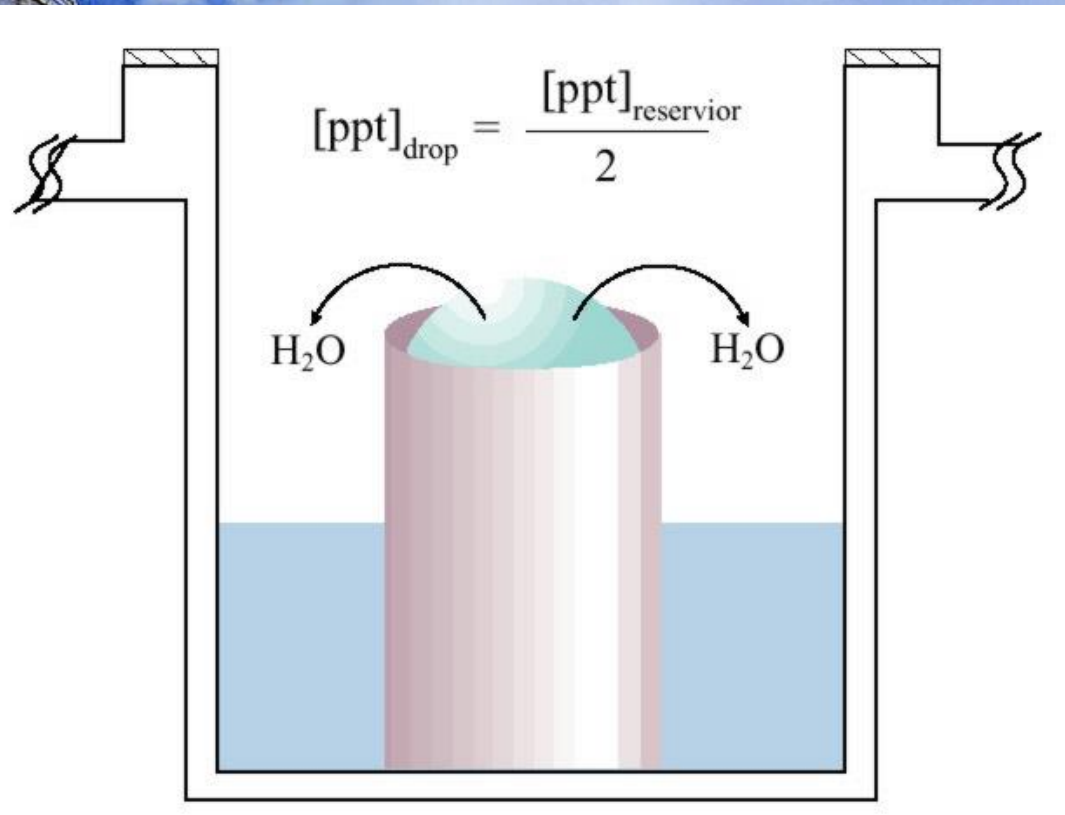


Most popular method among protein crystallographers.

1. Crystal screen buffer is the well solution (0.5 - 1 mL)
2. Drop (on siliconized glass cover slip) is 1/2 protein solution, 1/2 crystal screen buffer (6-10  $\mu\text{L}$ ). So, the concentration of precipitant in the drop is 1/2 the concentration in the well.
3. Cover slip is inverted over the top of the well and sealed with vacuum grease (airtight).

4. The precipitant concentration in the drop will equilibrate with the precipitant concentration in the well via vapor diffusion.

## Sitting Drop Vapor Diffusion

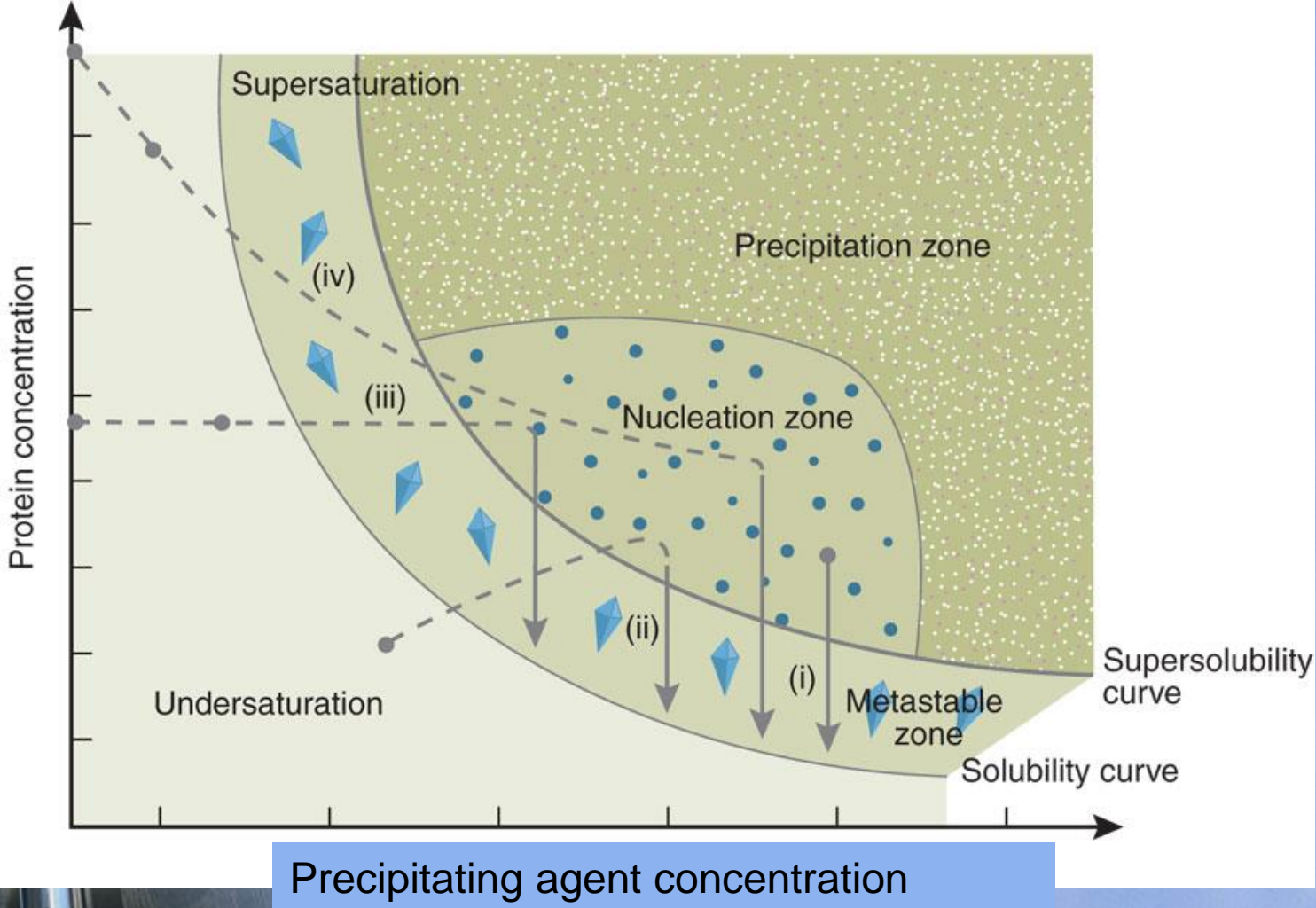


Same basic principles as in hanging drop method, except the drop containing your sample sits on a bridge within the well. This allows for a larger sample size (20 - 40  $\mu\text{L}$ ), however protein is frequently precious to the crystallographer, so there isn't that much demand for a larger sample size.

# Naomi E Chayen & Emmanuel Saridakis

Nature Methods - 5, 147 - 153 (2008)Published

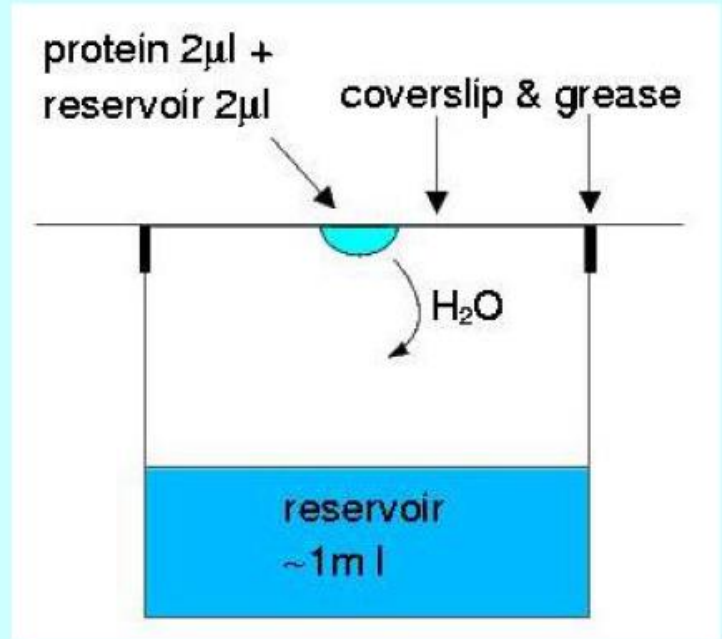
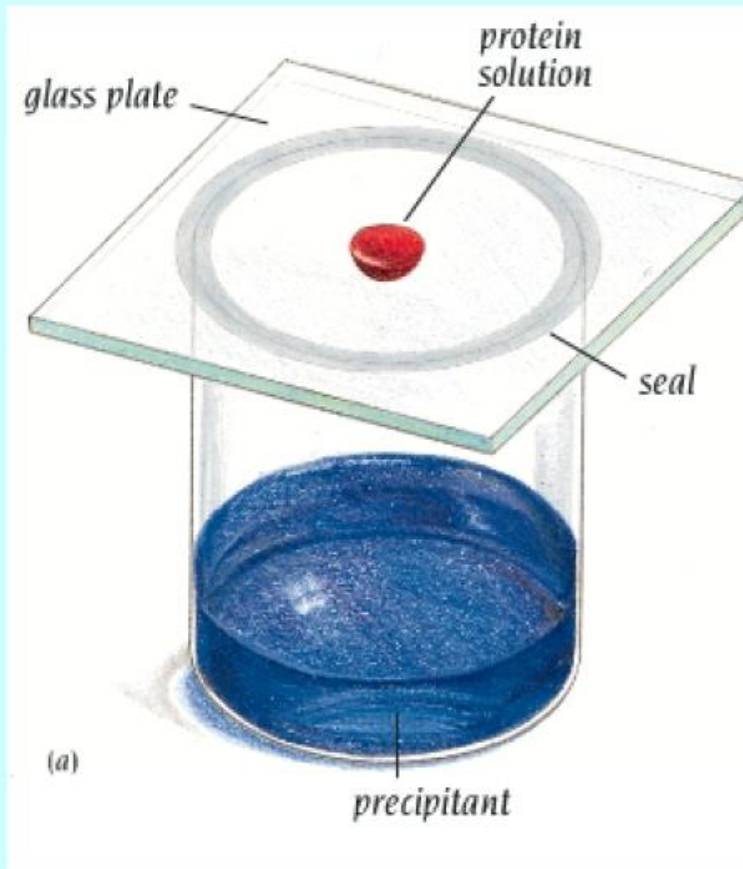
online: 30 January 2008; | doi:10.1038/nmeth.f.203





# Protein crystallization

'Hanging drop':



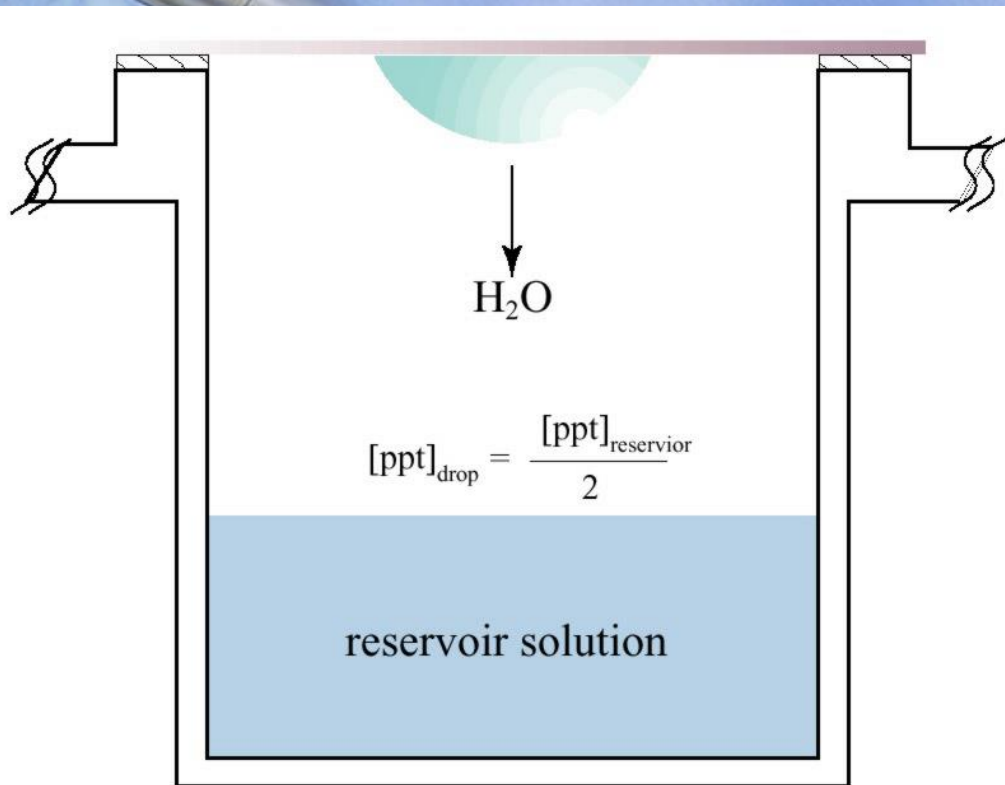
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# Hanging Drop Vapor Diffusion



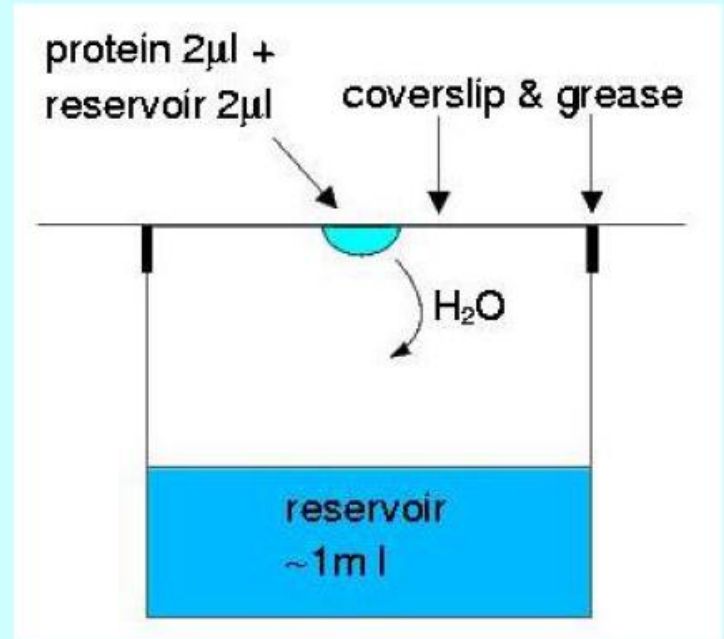
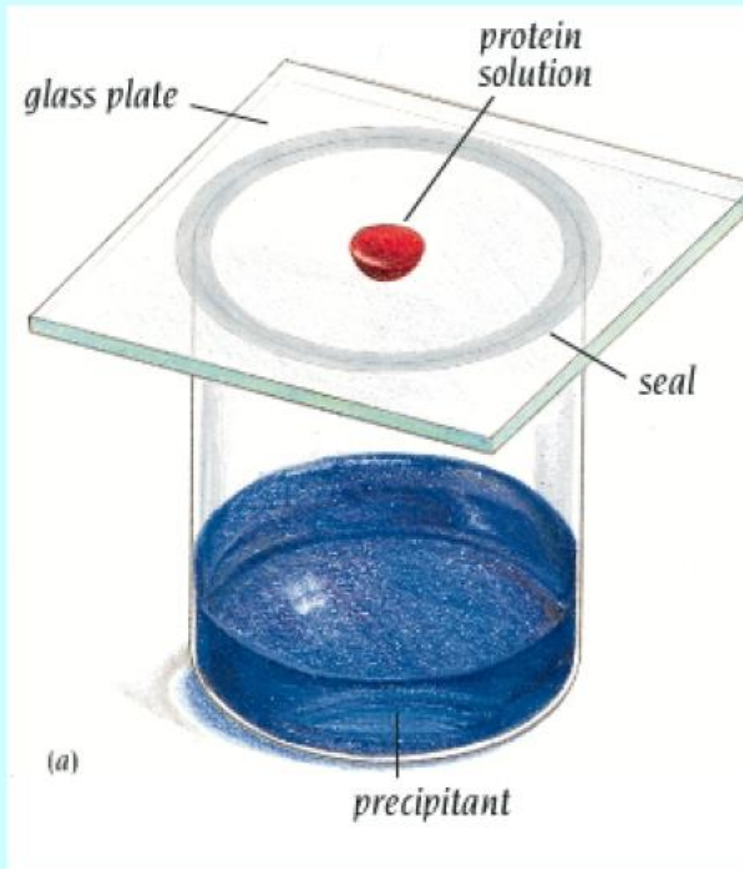
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# Protein crystallization

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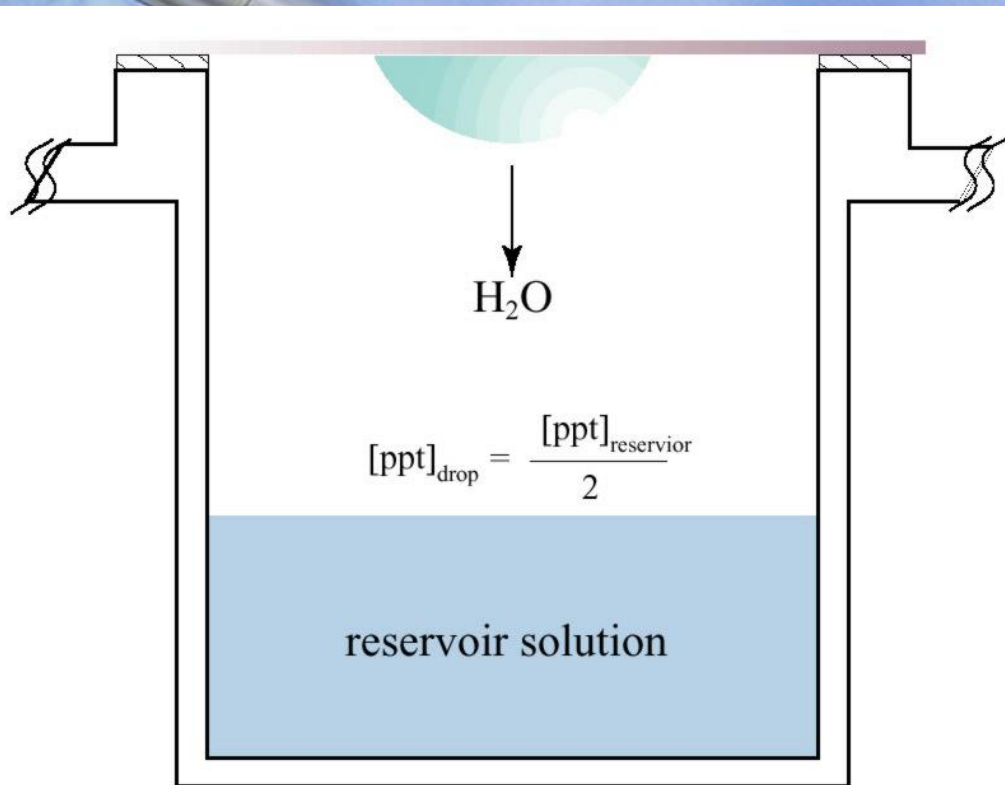


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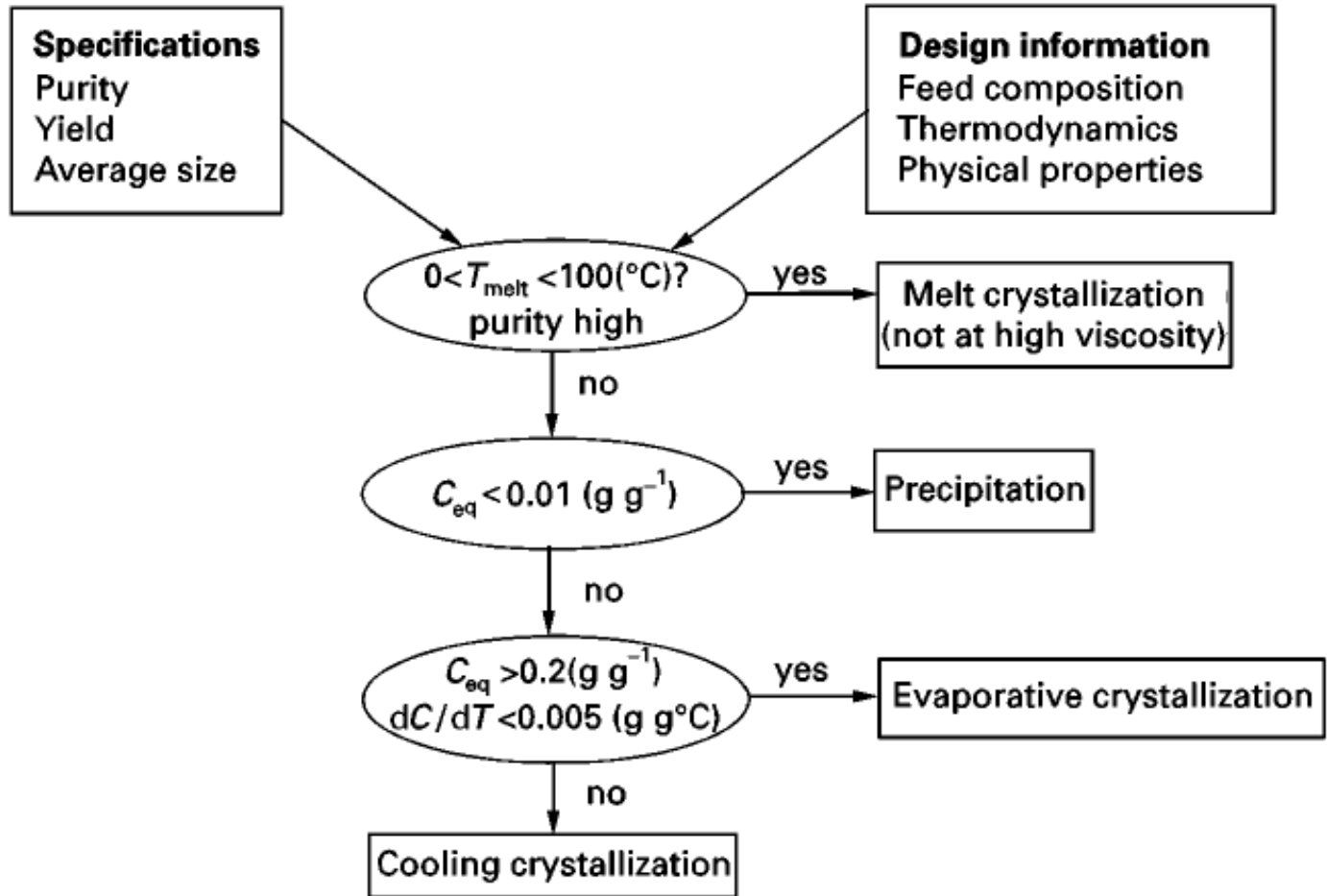


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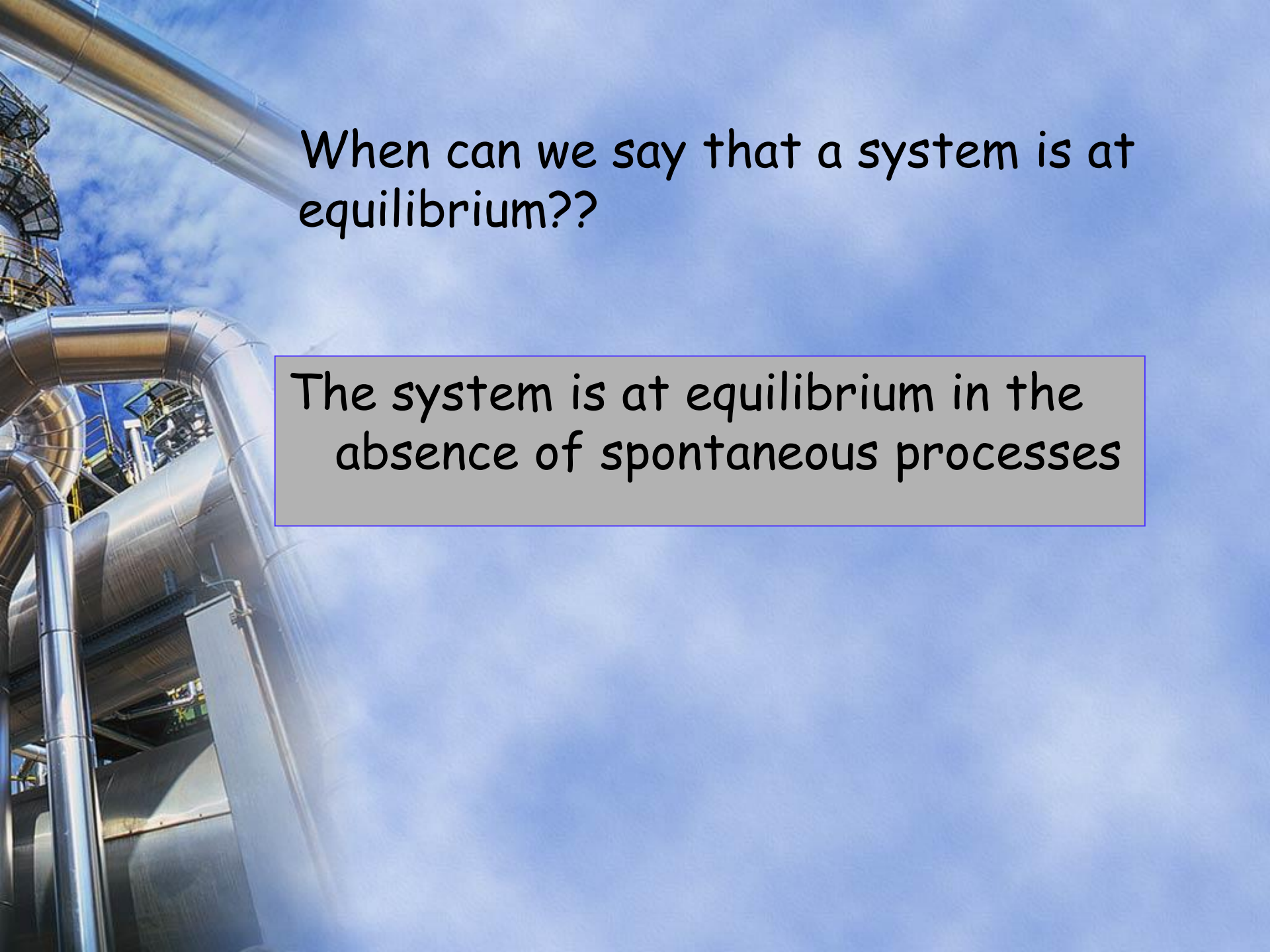




Decision diagram for choosing the method of crystallization.

# Crystallization Thermodynamics

- The crystallization method is selected on the basis of **Thermodynamics** taking into account the physicochemical parameters both of the crystallizing substance and of the solvent based on the desired purity of the end product
- $\epsilon$ -caprolactam: 99,999% raw material for the production of NYLON-66 by polymerization



When can we say that a system is at equilibrium??

The system is at equilibrium in the absence of spontaneous processes



The background of the slide is a photograph of an industrial facility, likely a refinery or chemical plant. It features a complex network of large, silver-colored metal pipes and structures. The pipes are arranged in various orientations, some running horizontally and others vertically. The sky is a clear, bright blue with some light, wispy clouds. The overall scene is brightly lit, suggesting a sunny day.

# Temperature

- The system should be at the same temperature as its surroundings
- The temperature should be uniformly distributed
- **Steady state should not be confused with equilibrium**

## Steady state

$$\left( \frac{\delta T}{\delta x} \right) \neq 0 \quad \left( \frac{\delta T}{\delta t} \right) = 0$$

At steady state, there are different temperatures at different points but the system does not change with time.



# Equilibrium

$$\left( \frac{\delta T}{\delta x} \right) = 0 \quad \left( \frac{\delta T}{\delta t} \right) = 0$$

At equilibrium the temperature is the same throughout the bulk of the system and it does not change with time





# Energy

- Mechanical energy cannot be fully converted into another form of mechanical energy
- It may be converted into heat with a friction process
- The opposite, i.e. the full conversion of heat into energy through friction **is not possible!**



## Immobile parts

- A system at equilibrium, does not have moving parts because in real systems *motion is associated with friction*, which is **irreversible**



A photograph of an industrial facility, likely a refinery or chemical plant, featuring large, shiny metal pipes and structures against a bright blue sky with scattered white clouds. The pipes are arranged in a complex, curved pattern, and some scaffolding is visible in the background.

## Constant pressure

- In the absence of gravity, electrostatic, magnetic, osmotic or other type of surface forces the system should be at a state of uniform pressure
- If not, the pressure differences (gradients), result in motion





# Lack of electric current flow

- Electric current flowing through a resistance causes its heating
- Electric current is converted into heat
- The process is **irreversible**



# Phase equilibria

- Definitions
  - Gas - substance in gas state
  - Vapor - gas at temperature lower than the critical
  - Increasing pressure it may be liquefied



A photograph of an industrial distillation column and associated piping, set against a blue sky with light clouds. The pipes are made of polished metal and curve through the scene. The distillation column is visible on the left side of the image.

# Liquid-Vapor Equilibria

- Water (liquid) -water vapour equilibria
- The rates in the two directions (departure of molecules from the liquid-return of the gas molecules for the liquid) become equal
- Evaporation = condensation
- Vapour pressure of a liquid = Pressure of the vapours



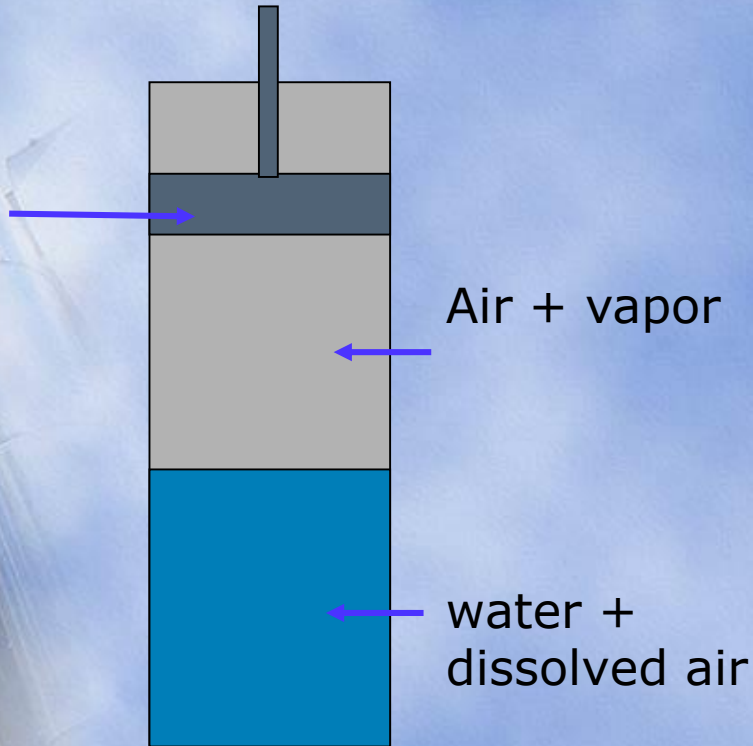
A photograph of an industrial distillation column with a complex network of large, shiny metal pipes. The scene is set against a clear blue sky with some light clouds. The pipes are arranged in a way that suggests a multi-stage distillation process.

## If the system is not at equilibrium

- Spontaneous boiling transferring mass to the vapour phase until equilibrium is reached, or...
- Vapour condenses until the pressure of the gas phase becomes equal to the vapour pressure of the liquid.

# A more complex system involving air

Frictionless  
piston



Air dissolves  
in water and  
vapour in the  
gas phase



## Air-water composition at equilibrium 20 °C ,1 atm

	Gas Phase	Liquid Phase
<b>Water</b> mol fraction	0.023	.999985
<b>Oxygen</b> mol fraction	0.205	$5 \times 10^{-6}$
<b>Nitrogen</b> mol fraction	0.772	$10 \times 10^{-6}$
<b>Totals</b>	1.0	1.0

The composition of gas and liquid phases is different



# What happens during temperature changes??

- More liquid is vaporized and is transferred in vapour phase.
- Less gas is dissolved in the liquid



# Increase of complexity

- For a pure substance the composition of the two phases is the same (100%)
- The addition of more components results in the differentiation of the composition of the two phases
- Chemical engineers take advantage of this differentiation in separation processes





It is the basis of the distillation columns, extraction, drying and crystallization processes



A photograph of an industrial facility, likely a refinery or chemical plant, featuring large, shiny metal pipes and structures. The scene is set against a bright blue sky with scattered white clouds. The pipes are arranged in a complex, curved pattern, and some scaffolding is visible in the background.

Is it possible to predict the composition of each of the phases in a system consisting of several components??

- Raoult's Law
- Henry's Law

## Raoult's Law - Partial Pressure

$$P_i = y_i P$$

$P_i$  partial pressure of component  $i$

$y_i$  mole fraction of component  $i$  in the gas

$P$  Total gas pressure



# Raoult's Law - Vapour partial pressure

$$P_i = x_i P_i^0 = y_i P \quad \text{At equilibrium}$$

$P_i$  partial vapour pressure of component  $i$

$x_i$  mole fraction of component  $i$  in the liquid phase

$P_i^0$  vapour pressure of pure component  $i$



# Raoult's Law

$$y_i P = x_i P_i^0$$

and hence for a multicomponent system

$$P = \frac{\sum x_i P_i^0}{\sum y_i}$$

$$P = \sum x_i P_i^0$$

# Fugacity

$$y_i P = x_i P_i^0$$

Partial pressure

Partial vapour pressure

Fugacity of gas

Fugacity of the liquid

Refers to ideal gases and ideal solutions



A photograph of an industrial facility with large, shiny metal pipes and structures against a blue sky with light clouds. The pipes are arranged in a complex network, with some curving upwards and others running horizontally. The background shows a clear blue sky with some wispy clouds.

# Henry's Law

- Used for gases above their respective critical temperature
- e.g. dissolution of  $O_2$  in water
  - $O_2$  is not a liquid at room temperature, It is not possible to use Raoult's Law
  - We do not have a vapour pressure. We have thus to use a "pseudo" vapour pressure, defined as Henry's law constant



# Henry's Law

- Henry's law coincides with Raoult's law. The difference is in the fact that Henry's law constant replaces for the vapour pressure

$$y_i P = x_i H_i$$

A photograph of an industrial facility, likely a refinery or chemical plant, featuring large, shiny metal pipes and towers against a clear blue sky. The pipes are arranged in a complex network, with some curving upwards and others running horizontally. The background shows a bright blue sky with a few wispy clouds.

## Which equation should we use?

- Raoult's law refers to vapour-liquid equilibria
- Henry's law refers to gas-liquid equilibria
  - Gases do not dissolve extensively in liquids



# Problem solution

- In a system, water is at equilibrium with air, pressure 1 atm

$$y_{\text{water}}P = x_{\text{water}}P_{\text{water}}^0$$

Raoult's Law

$$y_{\text{oxygen}}P = x_{\text{oxygen}}H_{\text{oxygen}}$$

Henry's law

$$y_{\text{nitrogen}}P = x_{\text{nitrogen}}H_{\text{nitrogen}}$$

Henry's Law

Known: Total pressure  
 $P = 1$  atm, vapour  
pressure of water,  
Henry's constant for  
nitrogen and oxygen.

3 equations and 6 unknown!!!



## 3 more equations...

- The sum of the mole fractions of all components in vapour is 1
- The sum of the mole fractions of all components in the gas is 1
- The O:N ratio

$$y_{water} + y_{oxygen} + y_{nitrogen} = 1$$

$$x_{water} + x_{oxygen} + x_{nitrogen} = 1$$

$$\frac{y_{oxygen}}{y_{nitrogen}} = \frac{0.21}{0.79} = 0.266$$

A photograph of a complex industrial system of large, shiny metal pipes and structures against a bright blue sky with light clouds. The pipes are arranged in a vertical and horizontal pattern, with some curving upwards. The lighting is bright, creating strong highlights and shadows on the metallic surfaces.

# Solution of the system of equations

- Excel worksheets
- MATLAB
- Pocket calculators
- Pencil, paper and brain..



# Use and limitations of Raoult's and Henry's laws

1. In dilute solutions, Raoult's law is possibly valid for the solvent.
2. In case both the solvent and the solute are similar, Raoult's law is valid for the entire concentration range.
3. If the solvent and the solute interact chemically, Raoult's law **is no more** valid.



A photograph of an industrial facility with large, shiny metal pipes and structures against a clear blue sky. The pipes are arranged in a complex network, with some curving upwards and others running horizontally. The background shows more industrial equipment and a clear sky.

## Use and limitations of Raoult's and Henry's laws

4. Henry's law is valid for most of the gases except for the case they interact chemically with the solvent.
5. Henry's law is valid for liquids practically immiscible with water (very low solubility).
6. Henry's law may be used for solvents other than water.

## Use and limitations of Raoult's and Henry's laws

7. It is possible to use a "fudge factor" (συντελεστή μαγειρέματος), known as **activity coefficient**, to correct for deviations from ideal behaviour.