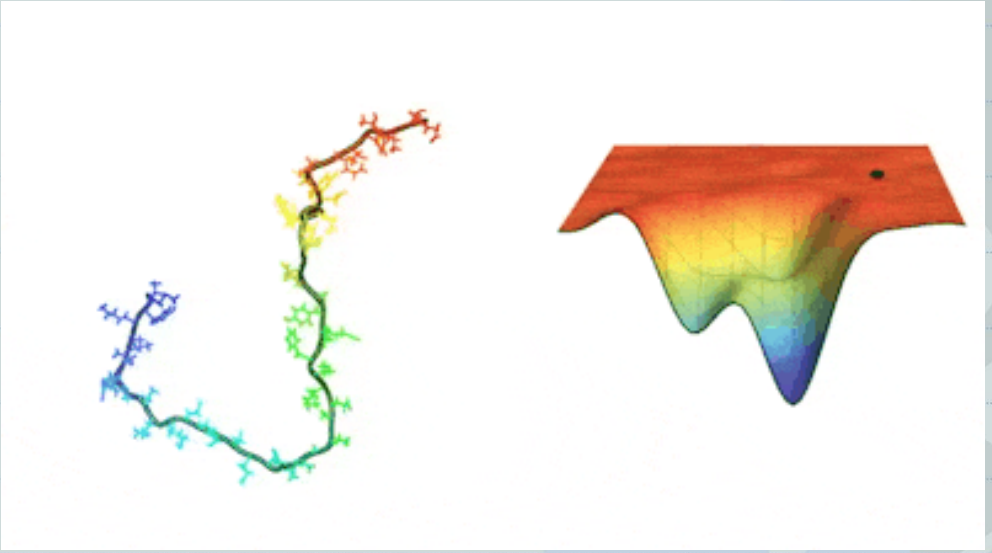
LECTURE 13

Protein Folding
Enzyme Kinetics

How do protein fold perfectly each time?



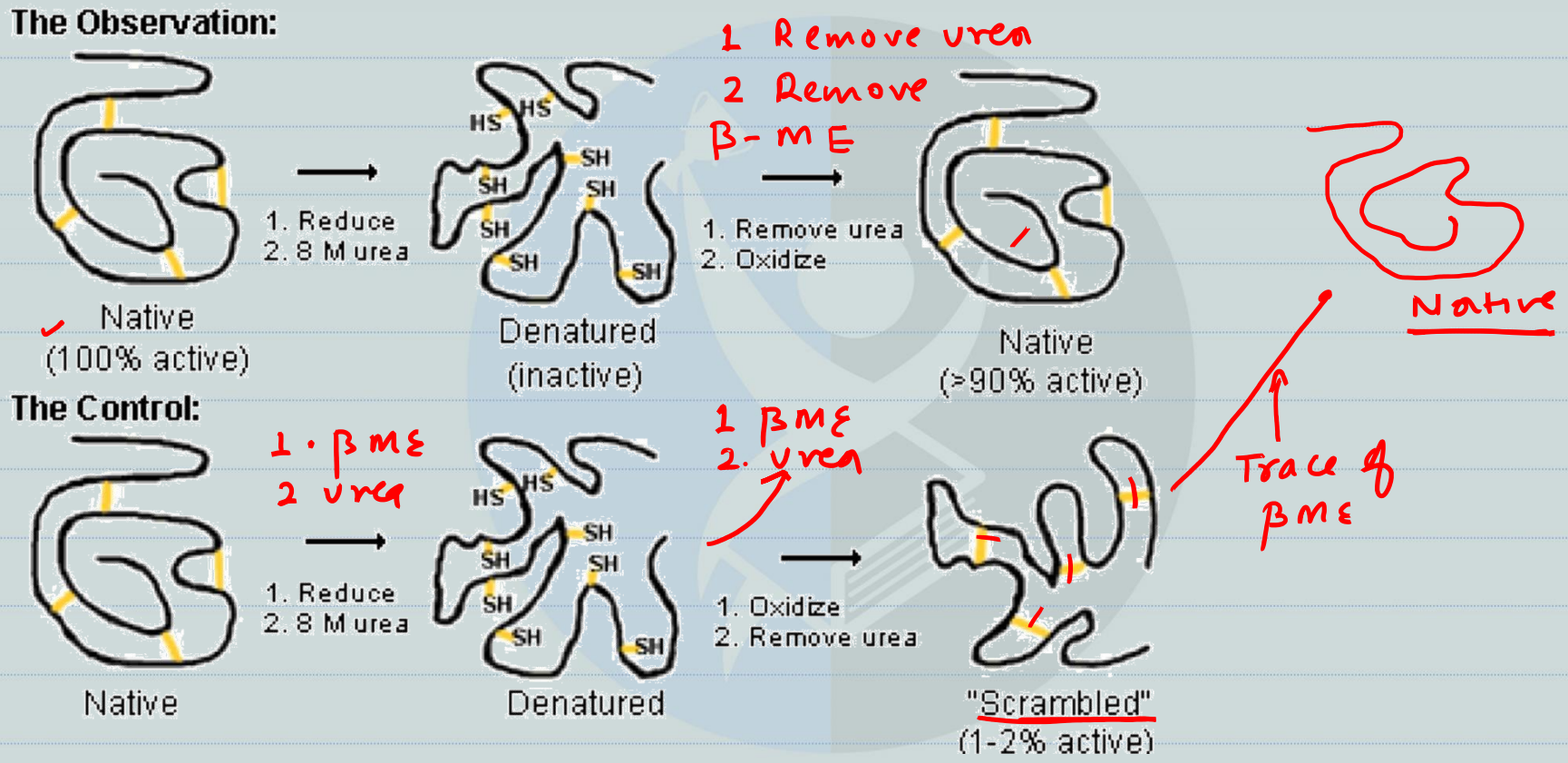
$2^0 \longrightarrow 3^0$

unstable state — High energy

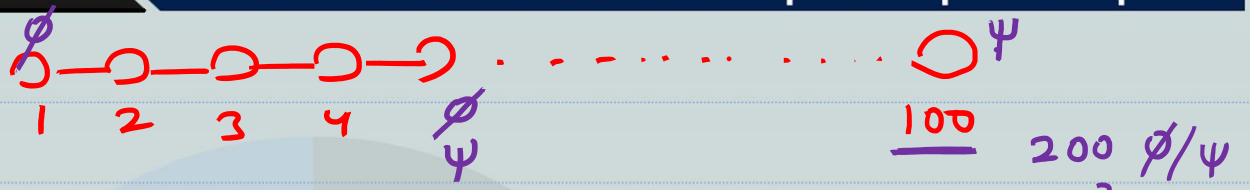
ΔG = −ve
Spontaneous

Stable state — Low energy

Reversible Denaturation of Ribonuclease A: Christian Anfinsen



Levinthal's paradox



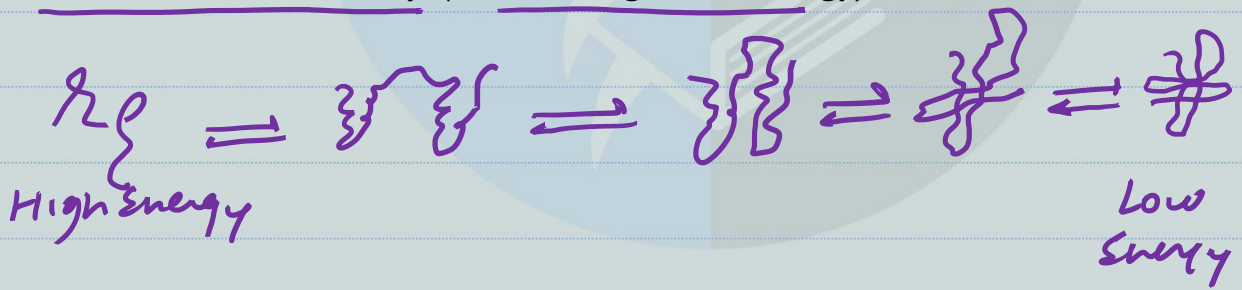
If a protein with 100 amino acids, it would have 99 peptide bonds and 198 considerations for ϕ and ψ angles.

ϕ → 3 -20, +20, -60
ψ → 3

If each of these had only three conformations, that would result in 3^{198} different possible folding.

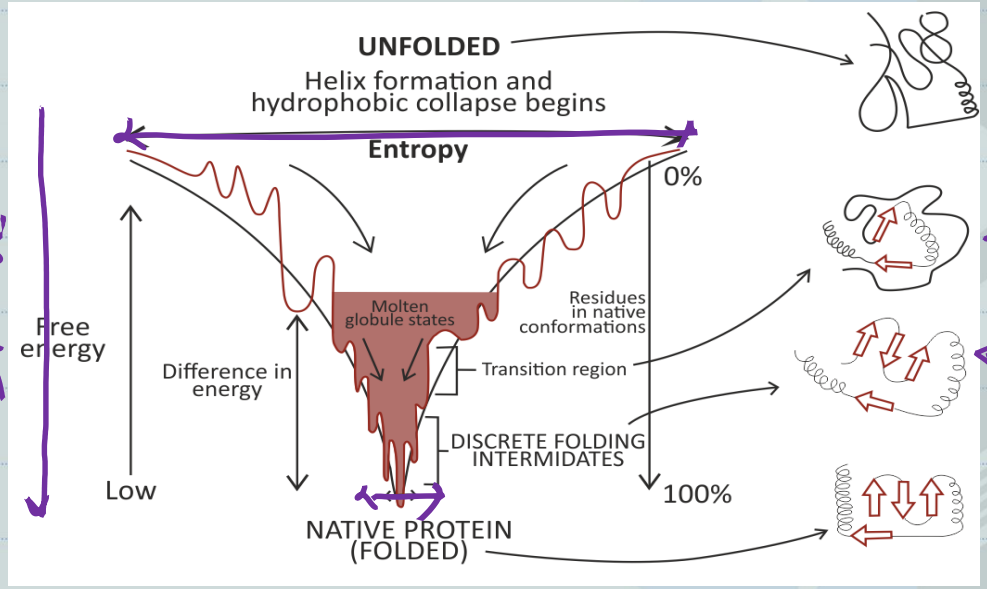
Conformation = Possibility
Bonds - rotation
$= 3^{198}$ = ...

Levinthal suggested, *proteins must fold by some sort of ordered pathway or set of pathways in which the approach to the native state is accompanied by sharply increasing conformational stability (decreasing free energy).*



Energy Landscape Theory (Folding Funnel):

Polypeptides fold via a series of conformational adjustments that reduce their free energy and entropy until the native state is reached



High Energy, High Entropy
High Enthalpy

← Molten globule

← Transition intermediate

← Native folded state
Low Free energy
Low Entropy, low Enthalp

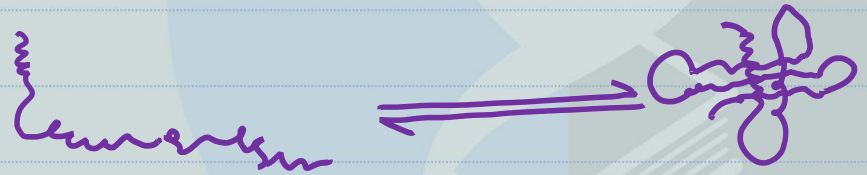
Single largest contribution to the stability of a folded protein is ΔS (solvent) for the nonpolar residues.

Techniques to Monitor Protein Folding

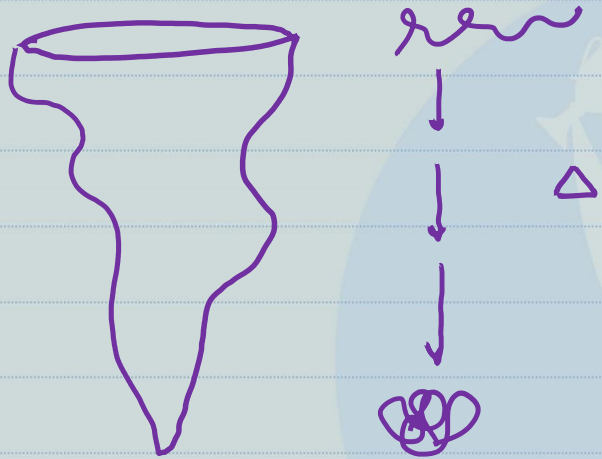
(1) **Circular dichroism (CD)** spectroscopy

(2) **Pulsed HD exchange** followed by 2D-NMR spectroscopy or mass spectrometry

(3) **Fluorescence resonance energy transfer (FRET).**



Thermodynamics of protein folding



Yes Yes

$\Delta G = -ve$, $\Delta H = -ve$, $\Delta S = -ve$ (NO)

Hydrophobic interaction → Although entropy of protein is decreasing but there is large increase in entropy of solvent (H_2O)

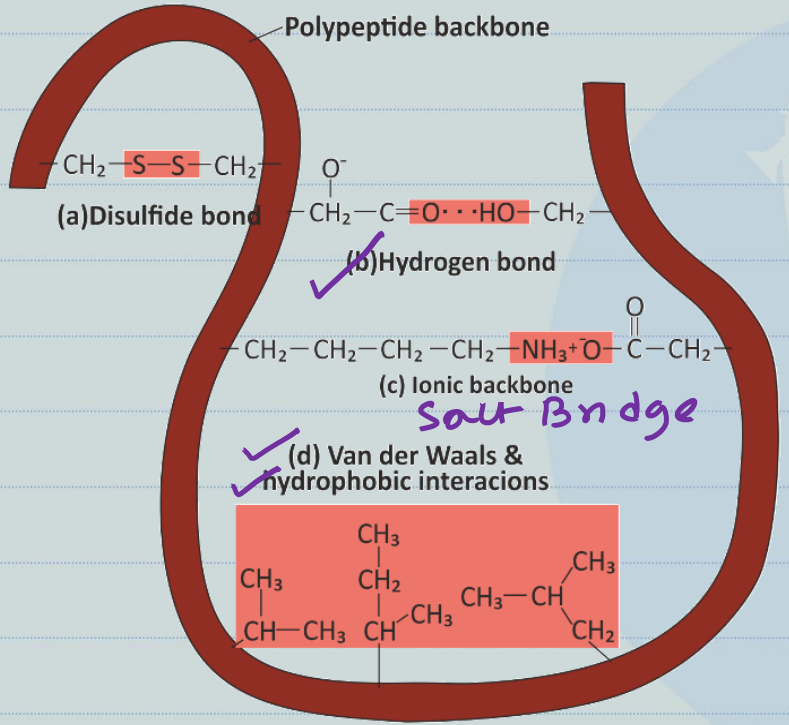
- $\Delta G^0 = \Delta H^0 - T\,\Delta S^0$

Spontaneous $T < \Delta H / \Delta S$

- $\Delta G^0 = -2.303\,RT \log K_{eq}$

K_{eq} = Folded / unfolded

PROTEIN STABILITY



3D structure
- Tertiary structure or Quaternary structure

- Non-covalent interaction
  - Hydrogen bond
  - Salt Bridge (Ionic)
  - Hydrophobic
  - Vanderwall's

May be
- covalent bond
  - Disulphide bond

Conditions for denaturation

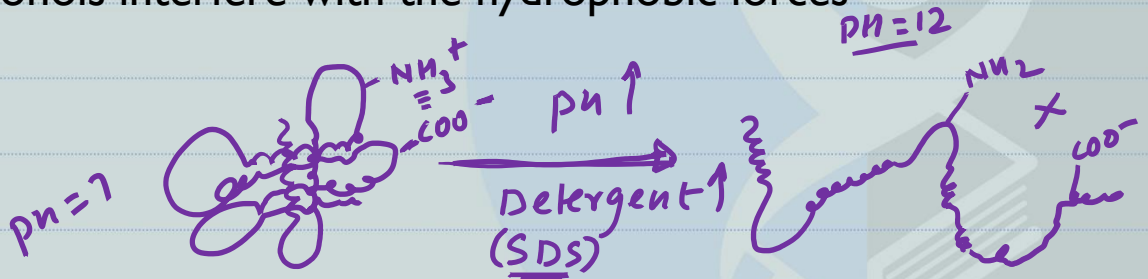
Urea ↑ : Disrupt Hydrogen Bonds
Temp^{r} ↑ : Disrupt all weak interactions

pH variations: alter the ionization states of amino acid side chain

Detergents: hydrophobically associate with the nonpolar residues

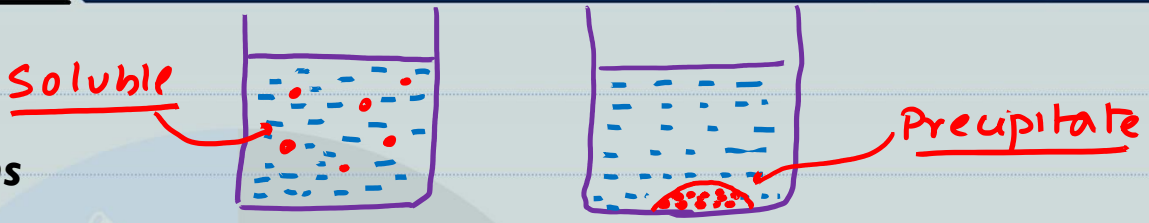
(non-polar)

High concentrations of water-soluble organic substances such as aliphatic alcohols interfere with the hydrophobic forces



- Hydrophobic Interaction
- Octanol
- Butanol

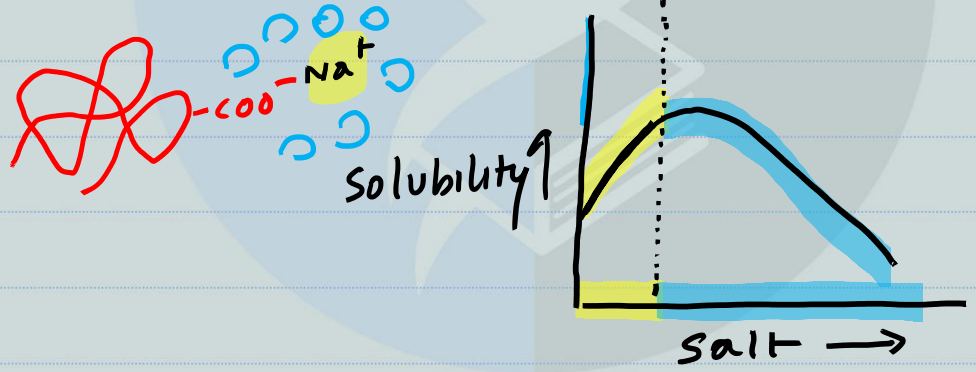
SOLUBILITIES OF PROTEINS



A. *Effects of Salt Concentrations*

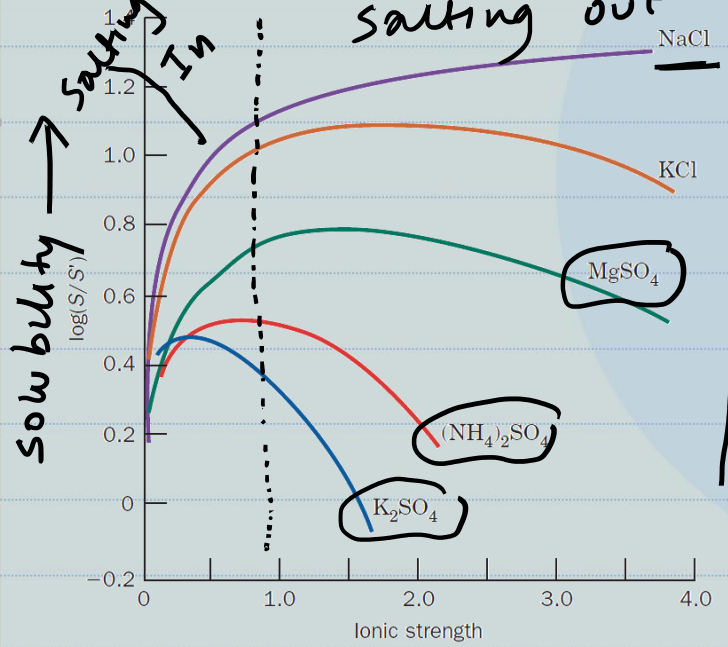
Salting in: *The solubility of a protein at low ionic strength generally increases with the salt concentration*

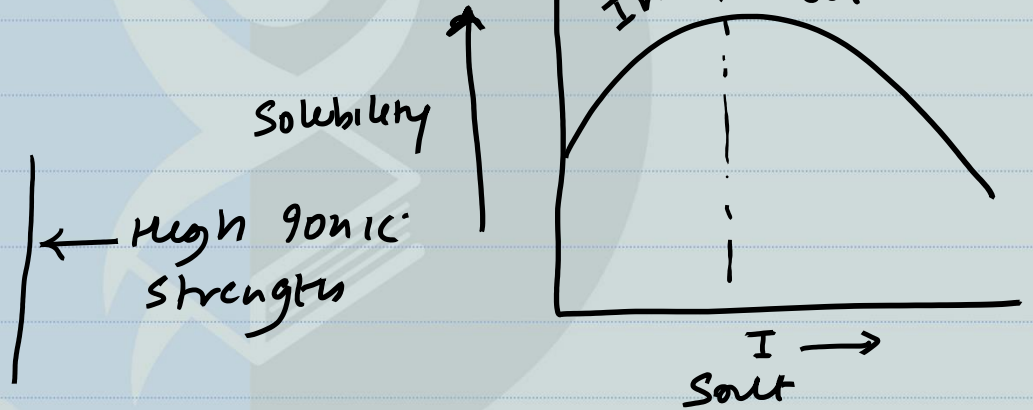
Low ionic strength of salt = solubility = ↑



Salting out:

- The solvent's activity is decreased.
- Solute–solute interactions become stronger than solute-solvent interactions and the solute precipitates.
- Ammonium sulfate, Potassium sulfate, magnesium sulfate





B. Effects of pH

$pI = pH$ where charge is zero

Solubility minimum

Iso-electric precipation

$pH < pI$

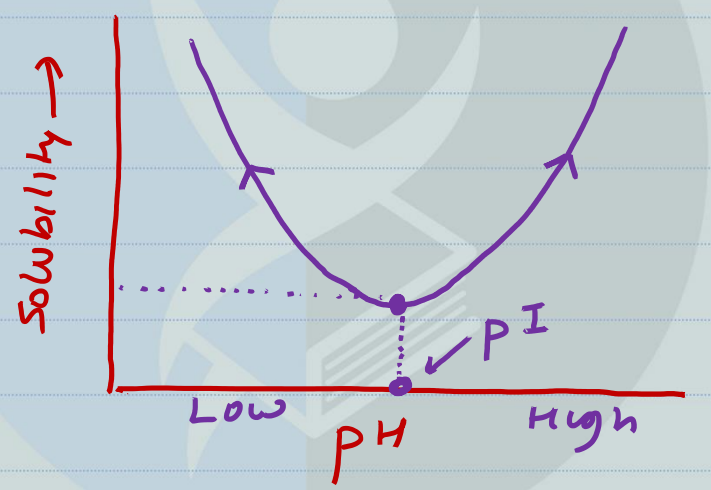
Protein = ⊕ charge

Solubility = ↑

$pH > pI$

protein = ⊖ charge

solubity = ↑



C. *Effects of Organic Solvents*

Precipitant : Low dielectric constant

D ↓ = solubility ↓

✓ Acetone and ethanol

Solvent: High dielectric constant

D ↑ = solubility ↑

✓ Dimethyl sulfoxide (DMSO) or *N,N*-dimethylformamide (DMF)

✓ Glycerol — Hydration of proteins

CHEMICAL KINETICS



Reaction Kinetics

1. Rate of a Reaction : Amount of substrate converted into product per unit time

or

velocity or Speed

Substrate $\longrightarrow$ Product

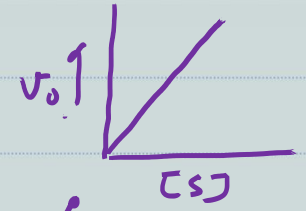
C

$$\boxed{\text{Rate}} = \frac{dC}{dt} = \frac{\text{Change in conc}^{r}}{\text{Change in time}} = \frac{M}{\text{Sec}}$$

- M/sec or M/minute.

Factors affecting Rate of a Reaction

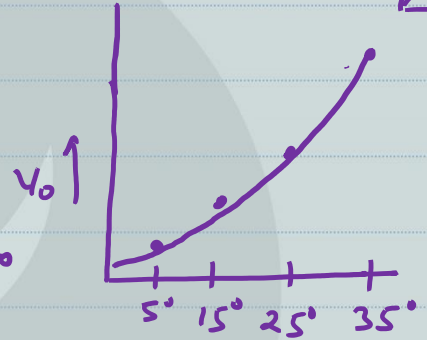
1. Rate $\propto$ substrate concentration



2. Rate $\propto$ Temperature

$\theta_{10} = 2$ (10°c, 10°c, 10°c)

Temp^r	5°	15°	25°	35°	45°
V_0	1	2	4	8	16



3. Radiation $\longrightarrow$ Rate = ↑
   - Sunlight, UV, α, β, γ, X-Ray
     - $E = h\nu$

4. Rate of reaction $\propto$ $\frac{\text{Surface area}}{\text{Volume Ratio}}$

Small size particles = SA/V = ↑ Rate = ↑

large size particles = SA/V = ↓ Rate = ↓

5 Rate $\propto$ catalyst (enzyme)

↓

- Lower activation energy

2. Molecularity of a reaction

Number of molecules reacting in an elementary step.

Molecularity

A → Product
Glucose-6-P ⟷ Fructose-6-P — 1

A + B → Product
Glucose + ATP → Glucose-6-P + ADP — 2

A + B + C → Product
PEP + ADP + iP → Pyruvate + ATP — 3

3. Order of reaction:

How **concentration** of the **substrate(s)** affects the rate of reaction

$$A + B + C \longrightarrow \text{Product}$$

$$x \quad x \quad x$$

- Single [s] = First order
  Two [s] = Second order
  Three [s] = Third order
  None of [s] = Zero order

First order reaction: Rate of reaction depends on conc^{n} of single substrate.

$$\underset{C}{[A]} \longrightarrow \text{Product}$$

$$\frac{dC}{dt} \propto C$$

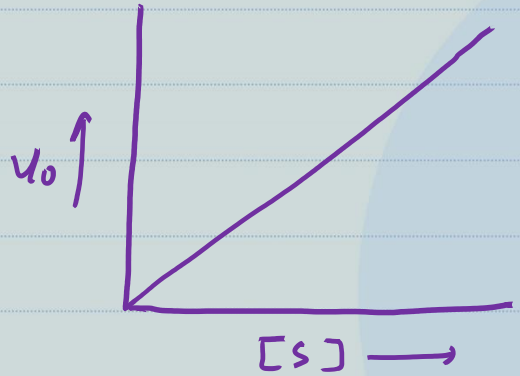
K = first order rate constant

$$\frac{dC}{dt} = K \cdot C$$

Unit of first order rate constant:

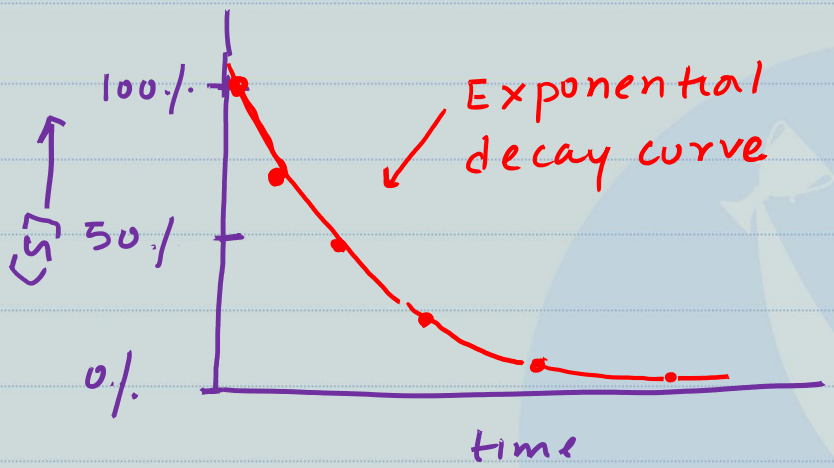
$$\frac{1}{\cancel{M}} \cdot \frac{\cancel{M}}{sec} = K = \frac{1}{sec} = sec^{-1}$$

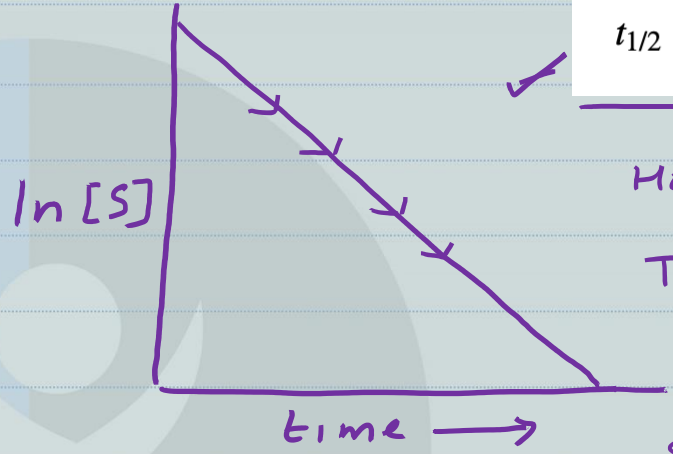
Rate v/s Substrate



Rate $\propto$ [S]

Concentration v/s time





$$t_{1/2} = \frac{\ln 2}{k} \approx \frac{0.693}{k}$$

Half life

Time duration in which concn of substrate reduces to half

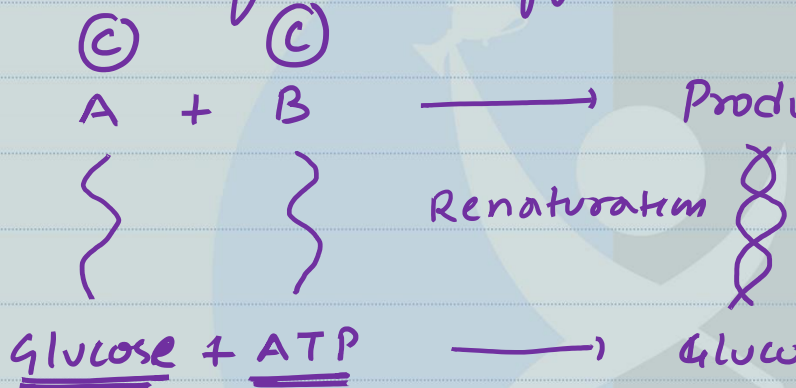
$$T_{1/2} = \frac{\ln 2}{K} = \frac{0.693}{K}$$

(4) Radioactivity

G6P ⟷ F6P

For straight line plot ln[A] with time

Second order reaction: Rate of reaction depends on concentration of two different substrates.



$$\frac{dc}{dt} \propto c \cdot c$$

$$\frac{dc}{dt} \propto c^2$$

$$\frac{dc}{dt} = k \cdot c^2$$

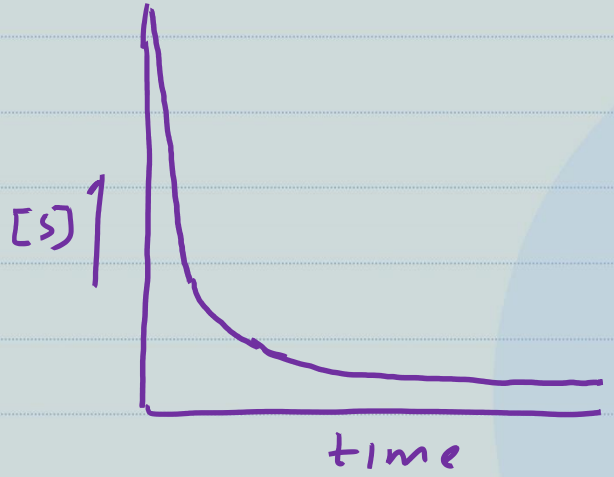
k = 2nd order rate constant

Unit of second order rate constant:

$$\frac{1}{M^2} \frac{M}{sec} = k = \frac{1}{M} \cdot \frac{1}{sec}$$

$$M^{-1} \quad sec^{-1}$$

Concentration v/s time





Substrate concⁿ

For straight line plot 1/[A] with time

Zero order reaction:

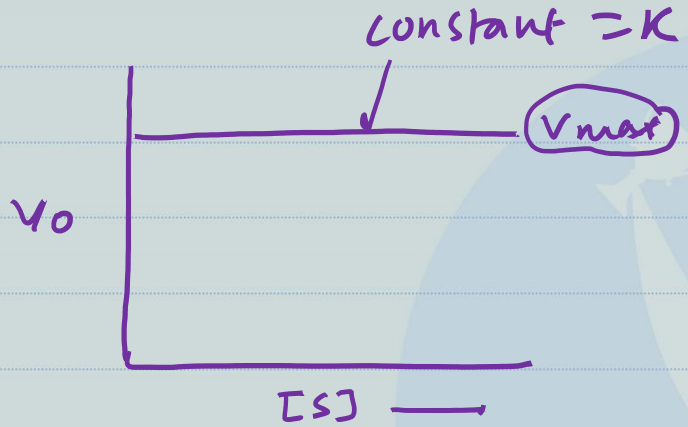
The rate does not vary with the increase or decrease in the concentration of the reactants



$$\frac{M}{sec} = \frac{dc}{dt} = \boxed{K}$$

Unit of zero order rate constant: M/sec or $M\ sec^{-1}$

Velocity versus substrate

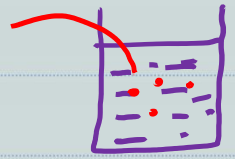


- when substrate concᵛ is very high



Concentration versus time

Colligative properties of solution :



Properties of solutions that depend upon the concentration of solute molecules or ions, but not upon the identity of the solute.

↳ mw, size, charge

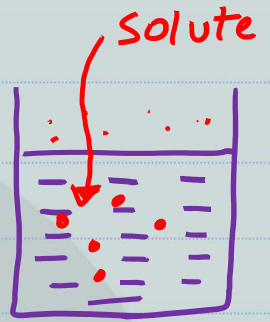
Solutes alter the colligative properties of aqueous solutions by lowering the effective concentration of water.

Solute + Solvent (H_2O) = 1 L solution

↑ ↓ Fixed

Colligative properties include

- Decrease in Vapour pressure = ↓
- Increase in Boiling point = ↑
- Decrease in Freezing point = ↓
- Increase in Osmotic pressure = ↑



BP = 100°C
FP = 0°C
O.P = 0

Depends on (i.c)

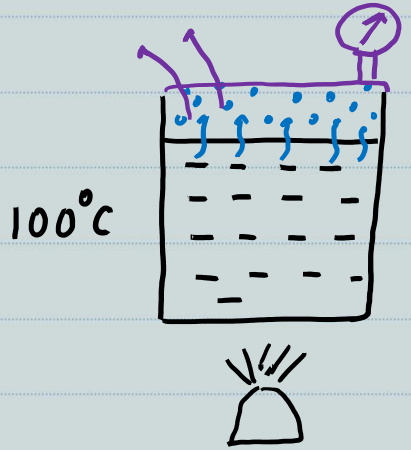
i = Degree of ionization

c = Conc^{r} of solute (M)

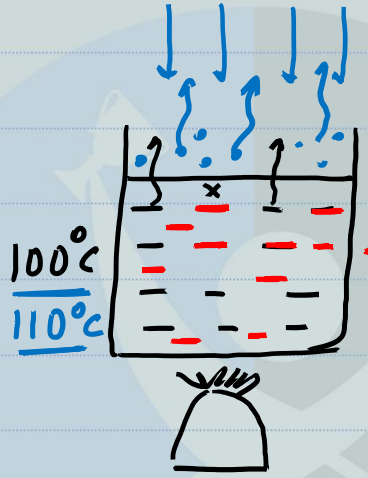
Covalent bonds i = 1 Glucose, Proline

Salts NaCl i = 2, $CaCl_2$ i = 3

- Decrease in Vapour pressure and increase in Boiling point



1 atm Pressure



← 1 atm pressure

Vapour pressure = ↓ = 0.9

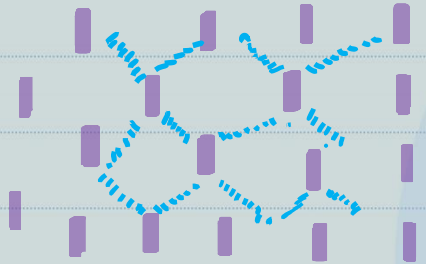
= 1.0

← Non-volatile Solute (Glucose)

Boiling point 100°C

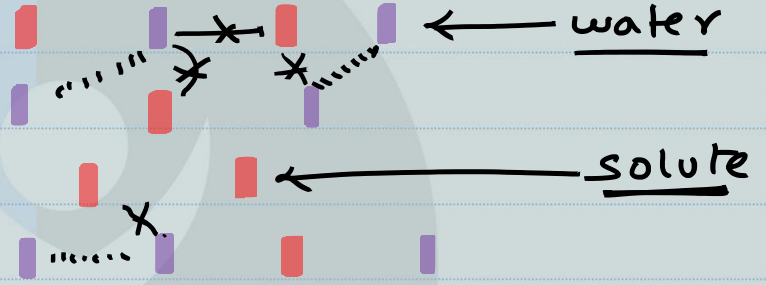
- Decrease in Freezing point

0°C



0°C

- Ice
- All 4- H- bonds are formed



0°C → Ice → No liquid water

freeze

Lower temp^{r}

Increase in Osmotic pressure (π)

The pressure exerted on a solution to prevent osmosis (inflow) with the help of semi-permeable membrane

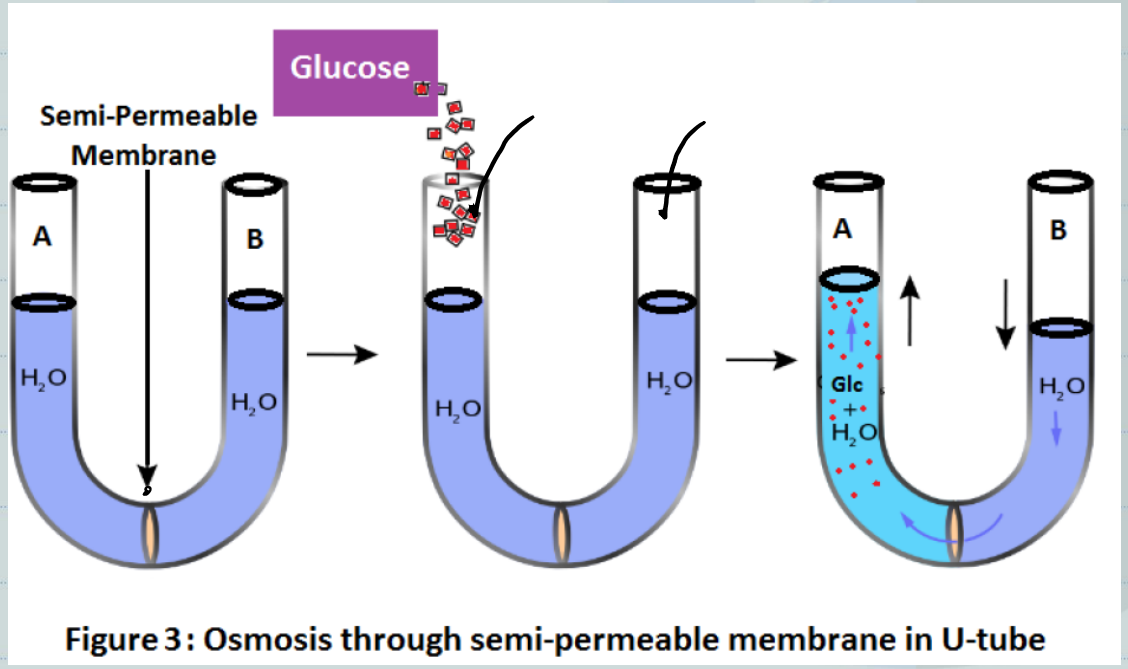


Figure 3: Osmosis through semi-permeable membrane in U-tube

$$\pi = i \cdot C \cdot RT$$

0.1 M glucose $i = 1$ = 0.1 M glycogen $i = 1$

0.1 M glucose < 0.1 M NaCl

$i \times C$

$1 \times 0.1 = 0.1$

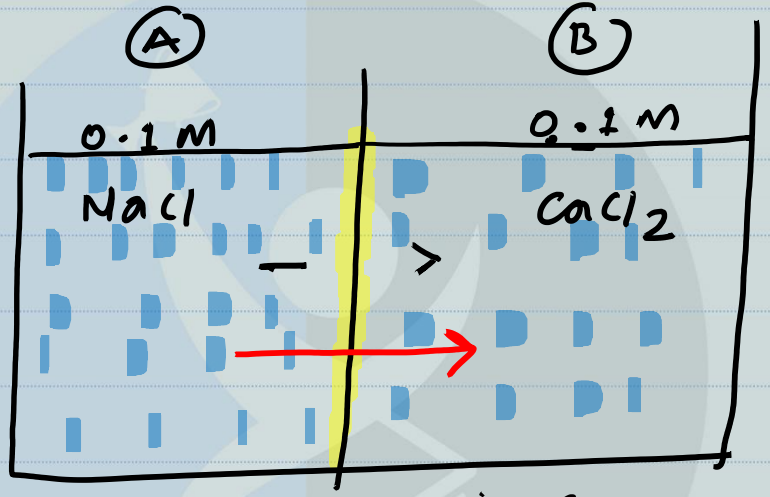
$2 \times 0.1 = 0.2$

Osmotic pressure

$\Pi = i\,c\,RT$

$NaCl \longrightarrow Na^{+} + Cl^{-}$ $i = 2$

$CaCl_2 \longrightarrow 1Ca^{2+} + 2Cl^{-}$ $i = 3$



$i \cdot c$ $= i \cdot c$

2×0.1 $= 3 \times 0.1$

$= 0.2$ $= 0.3$

Hypotonic Hypertonic

(↑) Hyper water Hypo water (↓)

HAPPY LEARNING

THANKS

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