



Starting with: $G_{tot} = \sum_i n_i \mu_i$

Derivative: $dG_{tot} = \sum_i n_i d\mu_i + \sum_i \mu_i dn_i$

Another expression of dG : $dG = Vdp - sdT + \sum_i \mu_i dn_i$

Set the two expressions of dG equal to one another: $\sum_i n_i d\mu_i + \sum_i \mu_i dn_i = Vdp - sdT + \sum_i \mu_i dn_i$

constant T and P: $Vdp - sdT = 0$

$\sum_i n_i d\mu_i = 0$

When solute conc. is not high:

$$\text{at equilibrium } \mu_{w,eq}(T, P + \pi, a_w) = \mu_{w,ext}(T, P)$$

$$\mu_{w,ext}(T, P + \pi, a_w) = \mu_w(T, P) + RT \ln a_w + \int_{a_w}^{\infty} \left(\frac{\partial \mu_w}{\partial a_w} \right) da_w$$

$$\text{The activity of water } (a_w) \text{ can be estimated by its mole fraction in solution: } a_w = \frac{n_w}{n_{tot}}$$

$$\text{which leads to: } RT \ln a_w + \bar{V}_w \pi = 0 \quad \text{where } \left(\frac{\partial \mu_w}{\partial a_w} \right)_{T, P} = \bar{V}_w = 55.5 \text{ liter/mol}$$

This equation can be simplified if we assume ideal behavior (no molecular interactions and zero molecular volume) and a low concentration of solute:

$$\frac{\pi}{RT} = \sum_i c_i \quad \pi = RTC_s$$

This is for an "ideal" case where the molecular volumes and any interactions are ignored.

c_i = Solute concentration in M, ions and cations must be treated separately, giving concentration of each.

Osmotic pressure in reality: very non-ideal behavior of high concentrations of solutes

$$c_s = \frac{\pi}{RT} \quad \text{ideal behavior}$$

Define: Osmolarity, $OsM = \varphi c_s = \frac{\pi}{RT}$ non-ideal behavior

φc_s effective concentration of solute
 φ deviation from ideal behavior
 φ is one way to measure the activity coefficient (γ)

Deviations from ideal are due to:
1) Interactions between the solute and water
or
2) Aggregation of the solute
or
3) Molecular volume of the solute excluding other solute molecules (excluded volume effect)

osmolarity of the cytoplasm of cells is 300 to 400 mOsm

Cells with rigid walls (plant cells, bacterial cells) can withstand positive osmotic pressure without bursting

For *E. coli*:

$$\text{turgor pressure} = (\pi^{cyto} - \pi^{ext}) \approx 3 \text{ atm}$$

PS: low osmolarity (0.4M)

Tonicity adjusts this value to include only those solutes that cannot pass through the membrane one refers to hypotonic and hypertonic solutions only referring to non-permeable solutes.

E. coli Peptidoglycan layer (cell wall) relatively rigid

1. Membrane allows water to freely pass
2. Permeability to small molecules is mostly controlled by protein-dependent transport systems **Active transport**: use energy to accumulate solutes in the cytoplasm
3. Osmolarity is dependent on the sum of activities of all ions, solutes etc.

2.1 Low conc (< 0.2 M, glucose, MOPS, minimal salts medium)

osmolyte K+ Activity ≈ 0.14 M

K+ is also present in the cytoplasm that is NOT osmotically active - due to binding to DNA, RNA

Add 0.5 M NaCl to growth medium (Water out, K+ in)

1. Loss of water from cytoplasm → cytoplasmic volume decreases plasmolysis → cytoplasm separates from the cell wall
2. Cell growth rate decreases in proportion to loss of cytoplasmic water
3. K+ is actively transported in, to counterbalance high NaCl on outside.
4. Lose turgor pressure (0)

proline and betaine are present in the growth medium (1 mM), these are actively transported in response to hypertonic shock have a higher φ than K+ (K+ does not go up as much), so cytoplasmic volume does not shrink as much and cell growth is faster

High concentrations of salts also demonstrate non-ideal behavior and can also stabilize or denature macromolecules

At concentrations below about 0.2 M salts influence biopolymers by affecting electrostatic interactions The Debye-Hückel theory is a good description

2.2 higher salt concentrations (> 0.5 M)

- Some ions can bind to the polypeptide directly (causes protein denaturation)
- Some ions prefer to interact with water and stabilize proteins effects determined by effective charge density of the ion: smaller ionic radius/larger charge on the ion kosmotrope increases T_m | chaotropic decreases T_m

$SO_4^{2-} < CH_3COO^-$, $Cl^- < Br^- < NO_3^- < I^- < CC_2CO_2^- < SCN^-$

structure makers kosmotropes (Stabilizers) structure breakers chaotropes (Denaturants)

1. Decrease hydrocarbon solubility in water
2. Salt-out proteins e.g. $(NH_4)_2SO_4$
3. Stabilize ordered structures DNA helix, α -helix
4. Protein subunit association

Increase hydrocarbon solubility in water
Salt-in protein, etc (chaotropic agent)
denature biopolymer (random coil)
Protein subunit dissociation

Kosmotropes interact strongly with water
Multivalent ions are all kosmotropes

Chaotropes interact weakly with water
NaF: solubility 1 M
KF: solubility 15.9 M

Reason: A salt consisting of an anion and cation that have similar interaction strength with water tends to be less soluble and to stick together as an ion pair in solution.

Major intracellular anions are **kosmotropes** phosphates, carboxylates, sulfates

Major intracellular cations are **chaotropes** potassium (not sodium) protein nitrogenous ions (histidine, arginine, lysine) kosmotrope/chaotrope pairing optimizes solubility and solvent-separated ions

Nucleoside: Attaching a nucleobase to a ribose (or deoxyribose) ring Nucleotide: Attaching one or more phosphate groups to a nucleoside

Bicyclic purines: Adenine (A) Guanine (G)

Monocyclic pyrimidines: Cytosine (C) Thymine (T, R=H) Uracil (U, R=H)

Examples of modified bases found in tRNA: 1-methyladenine (m¹A) 7-methylguanine (m⁷G) 3-methylcytosine (m³C) pseudouracil (Ψ)

Phosphodiester Bond

DNA is polymorphic Different helix geometry in fibers depending on G+C content and humidity

% Relative Humidity vs % (G+C)

DNA melting double strand ⇌ single strand (steep melting curves) Narrow temperature range of the transition means a large value of ΔH_{melt}

Length makes a big difference for ds to ss transition (oligo A): (oligo U)

Increasing length → Increasing T_m | Increasing ΔH_{melt}

1. By calorimetry (ds to ss): normalize values per basepair (favor ds) $\Delta H^\circ \sim +7 \text{ kcal/mol basepair}$ (favor ss) $\Delta S^\circ \sim +22 \text{ cal/K} \cdot \text{mol}^{-1} \text{ base pair}$

2. By van't Hoff analysis ΔH° values are ~ 300 times larger

$\Delta H^\circ \text{ van't Hoff} \sim 300 \times \Delta H^\circ_{\text{cal}} \text{ (per nt)}$

Cooperative unit for DNA melting is a few hundred basepairs

Cartoon version

Calorimetry was used to determine the heat of reaction $\Delta G^\circ = \Delta H - T \Delta S$ Delta of enthalpy is unfavorable for the formation of supercoils, but the change in entropy favors supercoil formation despite the loss of configurational entropy

Study transitions between known forms - monitor changes optically, e.g., D (absorbance)

Use 2-state theory when applicable

Obtain ΔH° , ΔS° , ΔG° , T_m

van't Hoff enthalpy calorimetric enthalpy

Parameters to be considered

- stacking: nearest neighbor interactions
- hydrogen bonding
- hydrophobic effect
- cooperative unit for "melting" transitions

Stacking $\pi - \pi$ interactions primarily due to van der Waals interaction plus electrostatic interactions between the stacked basepairs.

- Can explain 36 o twist between basepairs in B-DNA
- Can explain some sequence-dependent conformation (twist, roll angles)

hypochromism - lower absorption due to stacking

- Ultraviolet absorption spectra

Mononucleotides

Can use this to monitor transitions:

Single-strand stacked ↔ Single-strand unstacked
Double-strand ↔ Single-strand

Dependence on GC content Short (~ 50 bp) double-stranded DNA: $T_m = 40 C_x (\# G/C \text{ nucleotides}) + 20 C_x (\# A/T \text{ nucleotides})$

Very dependence on sequence poly(dAT)_n has a different T_m than does poly(dA) • poly(dT)

A + B ⇌ AB

$K = \frac{[AB]}{[A][B]}$

Total strand concentration: $C_t = [A] + [B]$

Fraction double strand: $[AB] = [a]C_t/2$

$[A] = [B] = (1 - a)C_t/2$

Assume there is an equimolar mixture

$K = \frac{[AB]}{[A][B]} = \frac{2a}{(1-a)^2 C_t} = e^{-\frac{\Delta G^\circ}{RT}}$

vary this

$\Delta G^\circ = \frac{\Delta H^\circ - \Delta S^\circ}{T}$

since $\Delta G^\circ = -RT \ln K$

vary this

$\frac{1}{T_m} = \frac{R \ln(C_t/4)}{\Delta H^\circ} + \frac{\Delta S^\circ}{\Delta H^\circ}$

Measure this

$\text{Slope} = -\frac{\Delta H^\circ}{R}$

$y\text{-intercept} = \frac{\Delta S^\circ}{R}$

$\Delta H_{\text{melt}} = -RT_m = \frac{2(0.5)}{(0.5)^2 C_t} = \frac{4}{C_t} = e^{-\frac{\Delta G^\circ}{RT_m}}$

The temperature at which $a=0.5$ (50% of the strands are melted) varies with C_t

$\alpha = 0.5 \quad T = T_m$

$K = \frac{2(0.5)}{(0.5)^2 C_t} = \frac{4}{C_t} = e^{-\frac{\Delta G^\circ}{RT_m}}$

vary this

$\Delta G^\circ = -R \ln K = \frac{\Delta H^\circ}{T} - \Delta S^\circ$

therefore

$\Delta G^\circ = -\frac{\Delta H^\circ}{T} + \Delta S^\circ$

$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R}$

$y\text{-intercept} = \frac{\Delta S^\circ}{R}$

T_m is concentration dependent

Slope + Intercept of line give ΔH° , ΔS°

the broad temp range means there is a small ΔH_{melt} for Single-strand Single-strand (pi stack) vs Double-strand (pi stack + H-bond)

Single-strand stacking is basepair dependent

π-Ho favors stacking, π-S favors unstacked π-G + 1.7 (ApA Poly A, Stacked) to ~0.9 (UpU Poly U, unstacked) kcal/mol (25°C)

Non-cooperative

1. ApA is similar to polyA for π-Ho . π-S (per nucleotide)
2. Calorimetric π-Ho (per nucleotide) similar to van't Hoff π-Ho