Nucleic Acid Hybridization → Why do we care? Hybridizations: Northerns, Southerns

PCR primers!

TAACGAG
+
ATTGCTC
→
TAACGAG
ATTGCTC

T A A C G G
‖ + ‖ + ‖ + ‖‖‖ + ‖‖‖ + ‖‖‖
A T T G C C

Can we add the energetics of each base pair?

Experiment: CCXGG / CCYGG Measure thermo for each possibility

Compare $\frac{A}{T}$, $\frac{T}{A}$, $\frac{G}{C}$, $\frac{C}{G}$

Simple → expect $\frac{A}{T} = \frac{T}{A}$ and $\frac{G}{C} = \frac{C}{G}$

Not true. Why?

G···C A T
C···G T A

Neighbor interactions

(kind of like solvent effects)

Nevertheless, lots of programs use this…

So instead, measure CCXZGG / GGQWCC varying XZ pairs.

$$\Delta G^\circ = \Delta G^\circ(\text{CG or AT initiation}) + \Delta G^\circ\left(\frac{CC}{GG}\right) + \Delta G^\circ\left(\frac{CX}{GQ}\right) + \Delta G^\circ\left(\frac{XZ}{QW}\right) + \cdots$$

Data in Table 4.3

Calculate theoretical ΔG and then K

Similar calc's for ΔH, ΔS

EXAMINE

1) Why ΔG° (xx initiation) special?

loss of translational entropy

2) Measure not just ΔG°, but ΔH° and ΔS°
this allows calculation at other temperatures
(Assuming ΔH° and ΔS° independent of temperature, which is OK for DNA)

3) Not discussed in book → what's really of practical interest here is melting temperature

ΔG has a dependence on temperature

How would we calculate T_m?

T_m = temperature at which DNA is half melted

ie. $ss \longrightarrow ds$ $\quad K_{eq_{T_m}} = \frac{1}{1}$

Which means $\Delta G_{T_m} = ? = 0$

$$\Delta G_{T_m} = 0 = \Delta H - T_m \Delta S$$

$$\therefore T_m = \frac{\Delta H}{\Delta S}$$

Simple analyses use X° per AT pair
and Y° per GC pair

But there is no theoretical basis for
T_m being directly proportional to # pairs,
or even to simple ΔG, ΔH, etc. !

The simple calculators only work for a limited length/range of oligo's.

In the text, it they talk about how you simply add energetics for nearest neighbors, and that "it doesn't matter which base pair is formed first."

← Class explain.

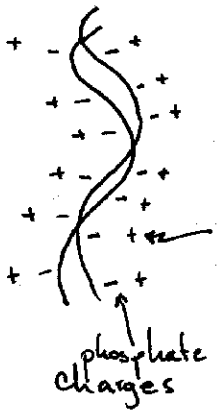
New Thought → DNA in aqueous solution

~~Ideal~~ Non-ideality

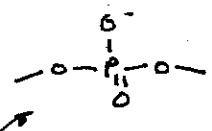
Interactions with salts

Calculation story → strands fly apart!

Duplex DNA



Na^+ ions are bound up by DNA



Let $\gamma \equiv$ effective fraction of each $P^{\ominus}$ neutralized by Na^+
(Complete 1:1 assoc would yield $\gamma = 1$)

Typical values $\gamma = 0.88$

Binding of a protein liberates n Na^+ ions

P = [DNA phosphates]
L = [ligand/protein]

$$P + L \rightleftharpoons PL + (n\gamma)Na^+$$

$$K = \frac{a_{PL} \cdot (a_{Na^+})^{n\gamma}}{a_P \cdot a_L} \approx \frac{[PL][Na^+]^{n\gamma}}{[P][L]}$$

$$K_{obs} = \frac{[PL]}{[P][L]} \quad \text{so} \quad K = [Na^+]^{n\gamma} K_{obs}$$

Look at dependence of K_{obs} on $[Na^+]$

$$\log K_{obs} = \log K - n\gamma \log [Na^+]$$

Partial Molal Quantities

$$\frac{\partial X}{\partial n} \qquad X = G, H, E, S, A$$

$$\mu_A = \bar{G}_A = \left(\frac{\partial G}{\partial n_A}\right)_{T,P,\, n_j \neq n_a}$$

How does free energy change as we change the # moles of component A?

Partial molal volumes

How does volume change as # moles of X changes?

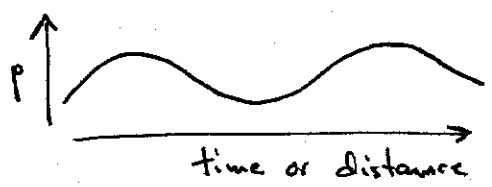
$$V = n_1\left(\frac{\partial V}{\partial n_1}\right) + n_2\left(\frac{\partial V}{\partial n_2}\right) + \cdots$$

$$MgSO_4 \rightleftharpoons Mg^{2+}(aq) + SO_4^{2-}(aq)$$

has a large change in volume associated with the reaction going to the right (decreases, I think — why?)

Increase pressure ⇒ shifts equilibrium (as we saw before)

Sound wave



As pressure peak goes by, equilibrium shifts. As trough follows, equilibrium shifts in opposite direction.

Effect: dampens the sound wave

* SONAR *