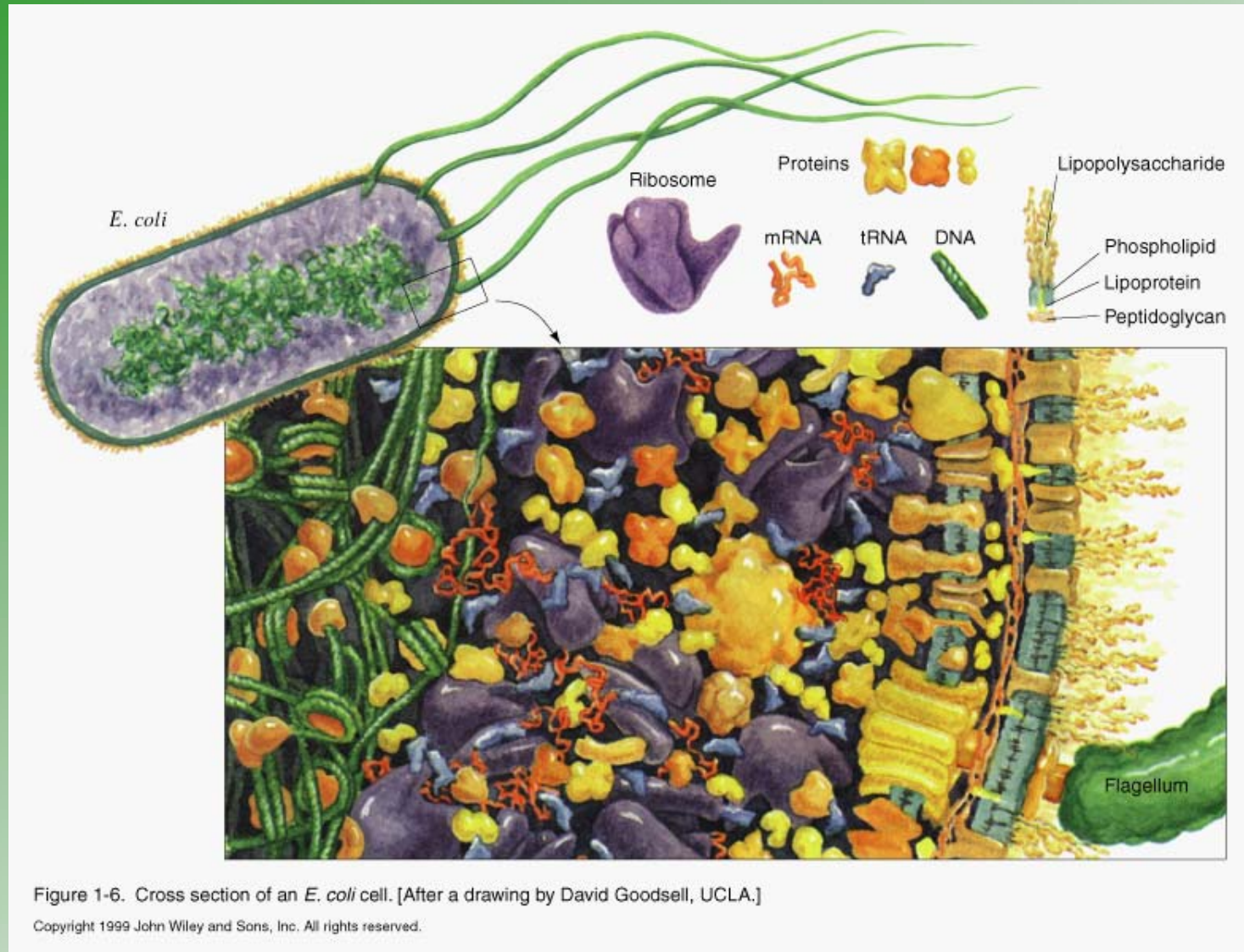


Physics 177

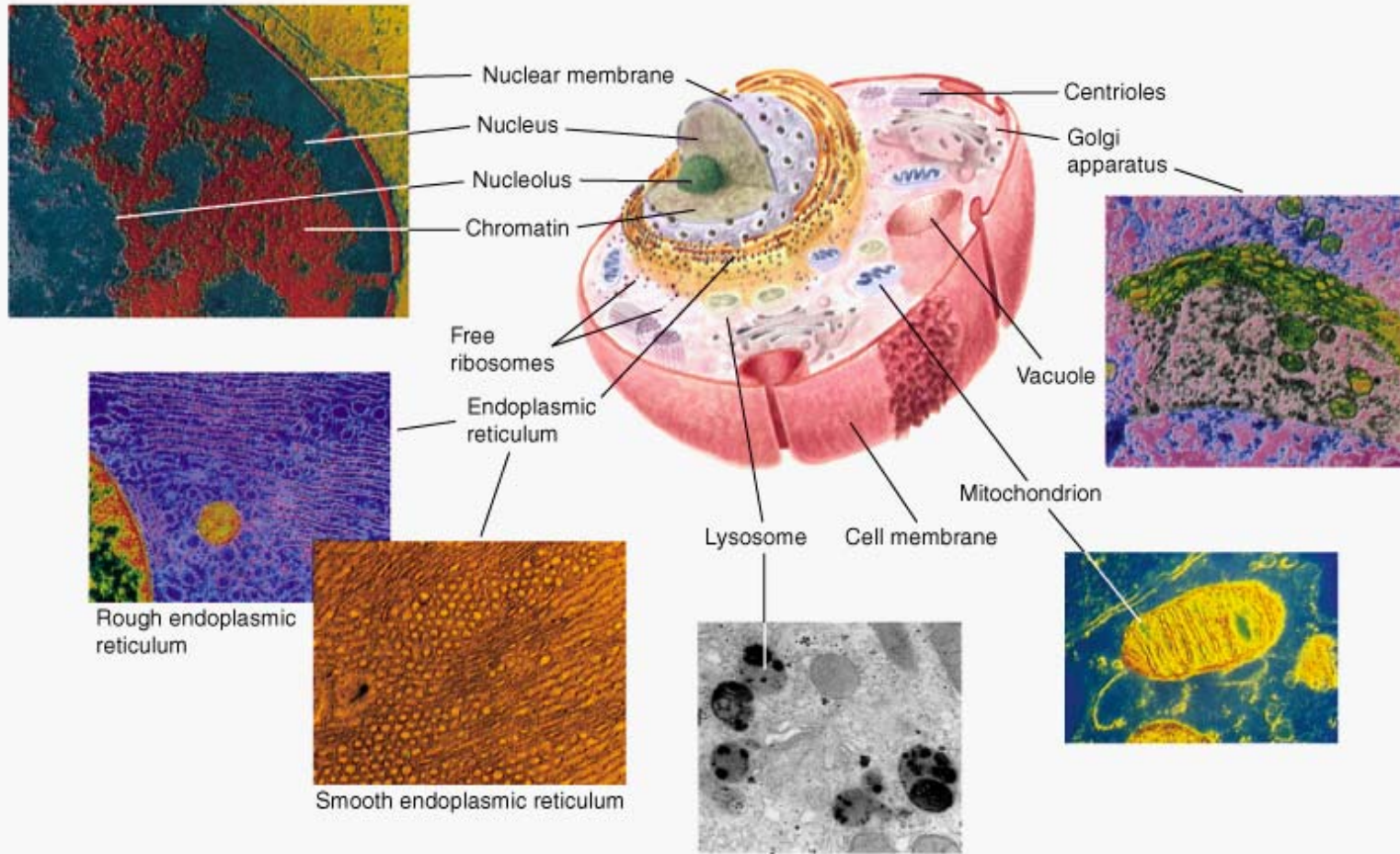
Biophysics

Professor Carlos Bustamante

A Bacterial Cell



An Mammalian Cell



Nucleus: Tektoff-RM, CNRI/Photo Researchers; rough endoplasmic reticulum and Golgi apparatus: Secchi-Lecaque/Roussel-UCLAF/CNRI/Photo Researchers; smooth endoplasmic reticulum: David M. Phillips/Visuals Unlimited; mitochondrion: CNRI/Photo Researchers; lysosome: Biophoto Associates/Photo Researchers. Copyright 1999 John Wiley and Sons, Inc. All rights reserved.

Biological Systems and Biochemistry

Complexity:

The parts that carry out these functions in biological systems are there for a reason:

All biological systems must:

- Extract, store and transform energy
- Self-replicate (store and preserve the information from one generation to the next)
- Must be highly structured (complex macromolecular assemblies)
- Adapt to changing conditions: control of function and evolution

The strategy of biochemical studies is ...

Divide and Conquer

The title 'Divide and Conquer' is written in a large, red, serif font. Below the main text, the words 'Divide and Conquer' are repeated in a smaller, gold, cursive font, creating a shadow effect.

Take things apart and put them back together to determine:

- What are the parts
- How these parts fit together
- How do they work

Hierarchy of Complexity

Animals and Plants as Organisms



Specialized Organs



Specialized Tissues



Cells



Organelles



Macromolecular Assemblies



Macromolecules



Simplest Molecular Building Blocks

Components of a Living Cell

How many components are needed to build a living cell?

The genome project has started to provide answers to what is the minimal complexity, compatible with the living state...

The simplest cell...

Mycoplasma genitalium

- 468 genes only
- Intercellular parasite
- Can be cultivated in enriched medium

By comparison...

E. coli (Eubacterium)

- ~ 4289 genes

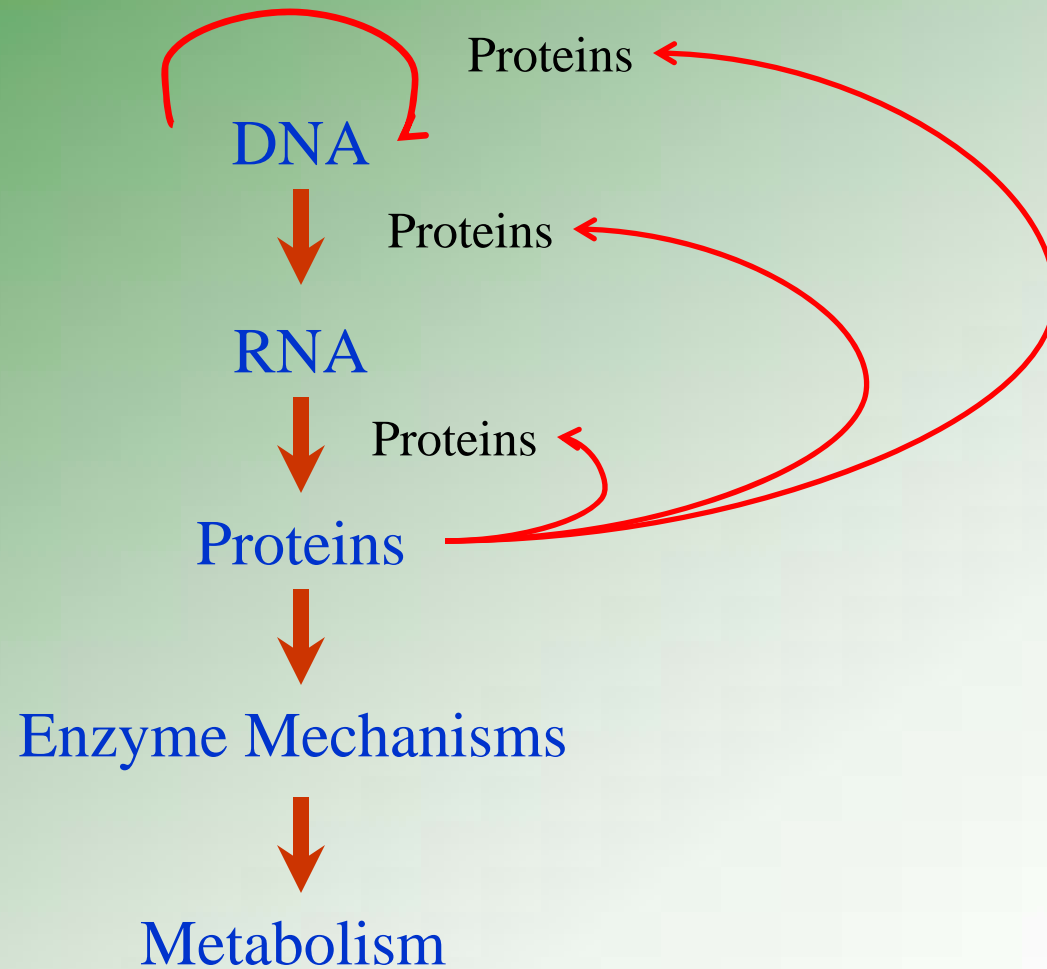
Yeast (*Saccharomyces cerevisiae*)

- ~ 6300 genes

Humans...

- Estimates: 30,000 – 35,000 genes (10,000 are purely regulatory)
- Complexity grows exponentially with the number of parts in machinery

The Central Dogma



Biophysics

We seek a quantitative description of biological processes and phenomena. A description founded on fundamental physical laws.

- Molecular Biophysics
- Cellular Biophysics
- Organismal or Systems Biophysics

Biophysics at Berkeley...

Thermodynamics Review

1. Formalism to keep track (accounting) of energies
2. Predict if a process is spontaneous or not
3. How much useful energy can be obtained from a chemical rxn as it proceeds from:

Initial Conditions \longrightarrow Equilibrium

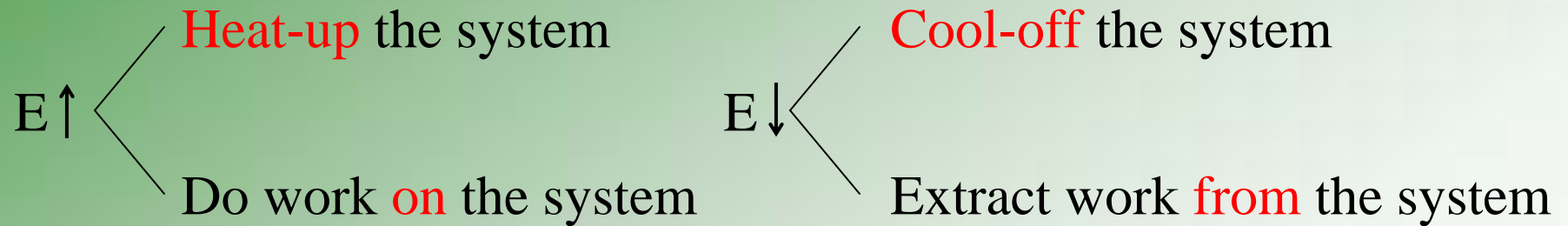
The laws...

Thermodynamics

First Law:

Energy conservation

Internal energy (E).- Total energy content of a system. It can be changed by exchanging heat or work with the system:



$$\Delta E = q + w$$

$$w \begin{cases} -P\Delta V \\ w' \end{cases}$$

Thermodynamics

A useful concept is: **ENTHALPY (H)**

$$H = E + PV$$

$$\Delta H = q_p - \cancel{P\Delta V} + \overset{0}{\cancel{w'}} + \cancel{P\Delta V} + \overset{0}{\cancel{V\Delta P}}$$

$\underbrace{\hspace{10em}}_{\Delta E}$

At constant pressure...

**Only P-V work involved... $w' = 0$
(as in most biological systems)**

So...

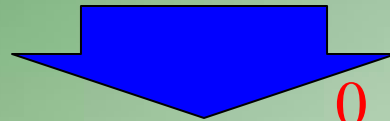
$$\Delta H = q_p$$

At constant pressure, the enthalpy change in a process is equal to amount of heat exchanged in the process by the system.

Thermodynamics

We have...

$$H = E + PV$$



$$\Delta H = \Delta E + \cancel{P\Delta V} + \cancel{V\Delta P} \quad \text{in biological systems} \quad \begin{cases} \Delta P = 0 \\ \Delta V \approx 0 \end{cases}$$

$$\Delta H \cong \Delta E$$

at $\Delta P = 0$ and since $\Delta V \approx 0$

Q: How is this energy stored in the system?

- A:** 1) As **kinetic energy** of the molecules. In *isothermal* ($\Delta T = 0$) processes this kinetic energy does not change.
- 2) As **energy stored in chemical bonds and interactions**. This “potential” energy could be released or increased in chemical reactions

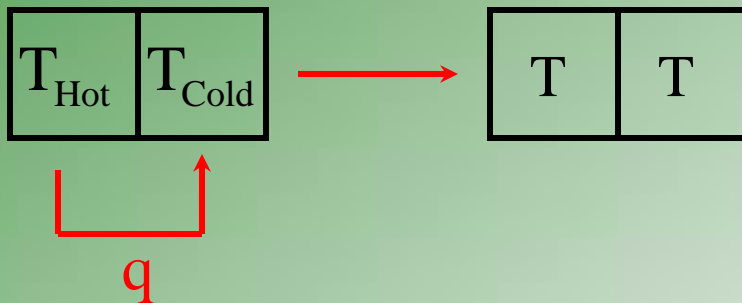
Thermodynamics

Second Law:

Entropy and Disorder

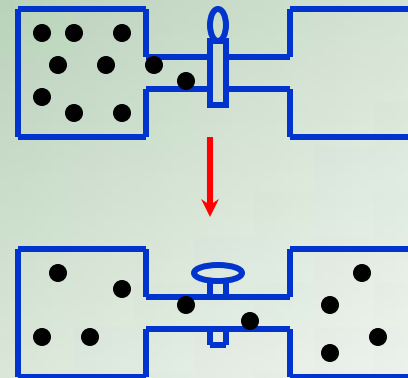
Energy conservation is **not** a criterion to decide if a process will occur or not:

Examples...



$$\Delta E = \Delta H = 0$$

This rxn occurs in one direction and not in the opposite



these processes occur because the final state (with $T = T$ & $P = P$) are **the most probable states of these systems**

Let us study a simpler case...

tossing 4 coins

Thermodynamics

All permutations of tossing 4 coins...

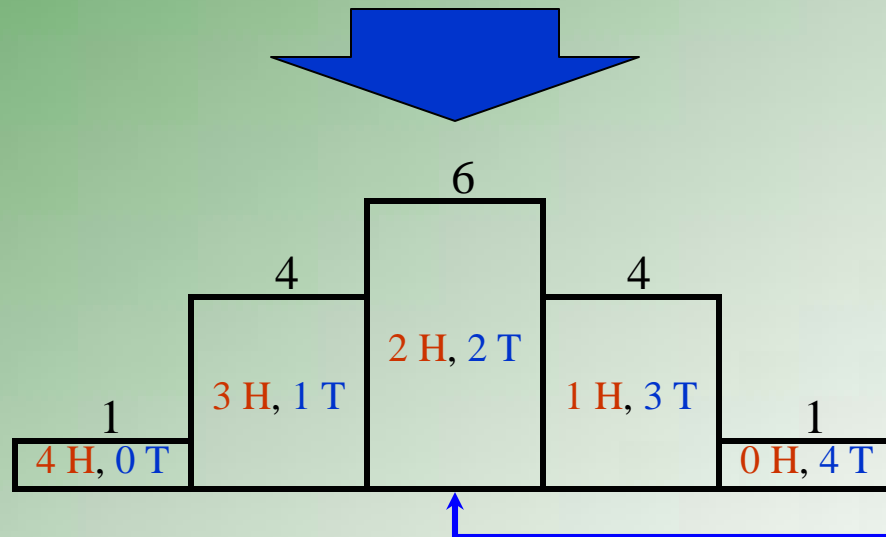
Macroscopic states...

- 1 way to obtain 4 heads
- 4 ways to obtain 3 heads, 1 tail
- 6 ways to obtain 2 heads, 2 tails
- 4 ways to obtain 1 head, 3 tails
- 1 way to obtain 4 tails

Microscopic states...

H T T H
H H T T
H T H T
T H H T
T T H H
T H T H

$$6 = \frac{4!}{2!2!}$$



The most probable state is also the most disordered

Thermodynamics

In this case we see that $\Delta H = 0$,
i.e.:

there is not exchange of heat between the system and its surroundings, (the system is isolated) yet, there is an unequivocal answer as to which is the **most probable** result of the experiment

The most probable state of the system is also the most disordered, i.e. ability to predict the microscopic outcome is the poorest.

Thermodynamics

A measure of how disordered is the final state is **also** a measure of how probable it is:

$$P_{2H, 2T} = \frac{6}{16}$$

Entropy provides that measure (Boltzmann)...

For Avogadro number's of molecules...

$$S \equiv k_B \ln W$$

Molecular Entropy

Boltzmann Constant

Number of microscopic ways in which a particular outcome (macroscopic state) can be attained

$$S = \underbrace{(N_{\text{Avogadro}} k_B)}_{R \text{ (gas constant)}} \ln W$$

Therefore: the most probable outcome maximizes entropy of isolated systems

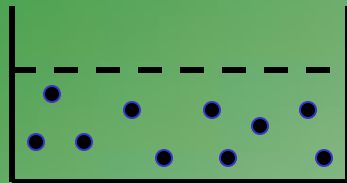
Criterion for Spontaneity:

$$\Delta S > 0 \text{ (spontaneous)}$$

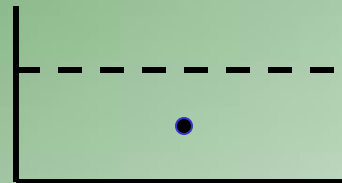
$$\Delta S < 0 \text{ (non-spontaneous)}$$

Thermodynamics

Entropy of Dilution...



1 M Sucrose



0.1 M Sucrose

$\Delta H = 0$:
no interaction among
sucrose molecules to
begin with

For 1 M sucrose: [water] = 55M

55 slots $\left\{ \begin{array}{l} 54 - \text{water} \\ 1 - \text{sucrose} \end{array} \right.$

$W = \#$ of ways to arrange 1 slot
among possible 55 slots = 55

For 0.1 M sucrose:

550 slots $\left\{ \begin{array}{l} \sim 550 - \text{water} \\ 1 - \text{sucrose} \end{array} \right.$

$W = \#$ of ways to arrange 1 slot
among possible 550 slots = 550

Thermodynamics

In general...

$$W_e \sim \frac{1}{\text{Conc.}}$$

$$\Delta S_{\text{dilution}} = S_{\text{final}} - S_{\text{initial}} = R \ln\left(\frac{1}{C_{\text{final}}}\right) - R \ln\left(\frac{1}{C_{\text{initial}}}\right)$$

$$\Delta S_{\text{dilution}} = R \ln\left(\frac{C_{\text{initial}}}{C_{\text{final}}}\right)$$

For the previous sucrose example...

$$\Delta S_{1 \rightarrow 0.1} = R \ln 10 \approx 4.57 \text{ cal/mol } ^\circ\text{K}$$

Thermodynamics

The macroscopic (thermodynamic) definition of entropy:

$$dS = dq_{\text{rev}}/T$$

i.e., for a system undergoing a change from an initial state A to a final state B, the change in entropy is calculated using the heat exchanged by the system between these two states when the process is carried out reversibly.

Thermodynamics

$$\Delta S = \int_{initial}^{final} \frac{dq_{rev}}{T} \quad (\text{Carried through a reversible path})$$

$$\Delta S = \int_{initial}^{final} \frac{C_P}{T} dT \quad (\text{If process occurs at constant pressure})$$

$$\Delta S = \int_{initial}^{final} \frac{C_V}{T} dT \quad (\text{If process occurs at constant volume})$$

Spontaneity Criteria

In these equations, the equal sign applies for reversible processes. The inequalities apply for irreversible, spontaneous, processes :

$$\Delta S(\text{system}) + \Delta S(\text{surroundings}) \geq 0$$

$$\Delta S(\text{isolated system}) \geq 0$$

Thermodynamics

Free-energy...

- Provides a way to determine spontaneity whether system is isolated or not
- Combining enthalpic and entropic changes

$$\Delta G \equiv \Delta H - T\Delta S \quad (\text{Gibbs free energy})$$

What are the criteria for spontaneity?

Take the case of $\Delta H = 0$:

$$\Delta G = - T\Delta S$$

$$\downarrow \\ < 0$$

$$\downarrow \\ > 0$$

$\Delta G > 0 \rightarrow$ non-spontaneous process

$\Delta G < 0 \rightarrow$ spontaneous process

$\Delta G = 0 \rightarrow$ process at equilibrium

Thermodynamics

Free energy and chemical equilibrium...

Consider this rxn:



Suppose we mix arbitrary concentrations of products and reactants...

- These are not equilibrium concentrations
- Reaction will proceed in search of equilibrium
- What is the ΔG is associated with this search and finding?:

$$\Delta G = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]}$$

ΔG° is the **Standard Free Energy** of reaction

i.e. ΔG when A, B, C, D are mixed in their standard state:
Biochemistry: **1M, 25°C, pH = 7.0**

$$\Delta G_{\text{Rxn}} = \Delta G^\circ + RT \ln \frac{1 \times 1}{1 \times 1}$$

$$\Delta G_{\text{Rxn}} = \Delta G^\circ$$

Thermodynamics

Now... Suppose we start with equilibrium concentrations:

Reaction will not proceed forward or backward...

$$\Delta G_{\text{Rxn}} = 0$$

Then...

$$0 = \Delta G^{\circ} + RT \ln \frac{[C]_{\text{eq}} [D]_{\text{eq}}}{[A]_{\text{eq}} [B]_{\text{eq}}}$$

$$\Delta G^{\circ} = -RT \ln \frac{[C]_{\text{eq}} [D]_{\text{eq}}}{[A]_{\text{eq}} [B]_{\text{eq}}}$$

$$\Delta G^{\circ} = -RT \ln K_{\text{eq}}$$

Rearranging

$$K_{\text{eq}} = e^{-\frac{\Delta G^{\circ}}{RT}}$$

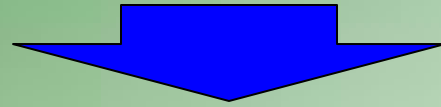
$$K_{\text{eq}} = e^{-\frac{(\Delta H^{\circ} - T\Delta S^{\circ})}{RT}}$$

$$K_{\text{eq}} = \left(e^{-\frac{\Delta H^{\circ}}{RT}} \right) \left(e^{+\frac{\Delta S^{\circ}}{R}} \right)$$

Thermodynamics

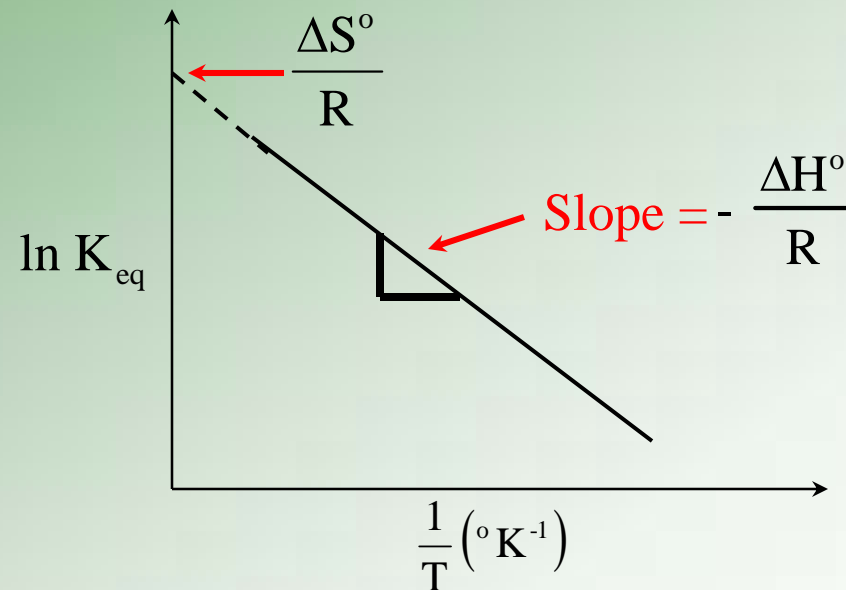
Graph:

$$\ln \left[K_{\text{eq}} = \left(e^{-\frac{\Delta H^\circ}{RT}} \right) \left(e^{+\frac{\Delta S^\circ}{R}} \right) \right]$$



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

Van't Hoff Plot



Thermodynamics

Summary: in chemical processes

ΔH°

- 1) Change in potential energy stored in bonds and interactions
- 2) Accounts for T-dependence of K_{eq}
- 3) Reflects: #, type, and quality of bonds
- 4) If $\Delta H^\circ < 0$: $\uparrow T \Rightarrow K_{eq} \downarrow$
If $\Delta H^\circ > 0$: $\uparrow T \Rightarrow K_{eq} \uparrow$

ΔS°

- 1) Measure of disorder
 $S = R \ln (\# \text{ of microscopic ways of macroscopic states can be attained})$
- 2) T-independent contribution to K_{eq}
- 3) Reflects order-disorder in bonding, conformational flexibility, solvation
- 4) $\Delta S^\circ \uparrow \Rightarrow K_{eq} \uparrow$
Rxn is favored

Thermodynamics

Examples:

Consider the Reaction...



$$[A]_{\text{initial}} = 1\text{M}$$

$$[B]_{\text{initial}} = 10^{-5}\text{M}$$

$$K_{\text{eq}} = 1000$$

Free energy change
when products and
reactants are present at
standard conditions

$$\Delta G^{\circ} = -RT \ln K_{\text{eq}}$$

$$\Delta G^{\circ} = - \left(1.98 \frac{\text{cal}}{\text{mol K}} \right) (298 \text{ K}) \ln (1000)$$

$$\Delta G^{\circ} = -4.076 \frac{\text{Kcal}}{\text{mol}} \leftarrow \text{Spontaneous rxn}$$

How about ΔG_{Rxn} ...

$$\Delta G_{\text{Rxn}} = \Delta G^{\circ} + RT \ln \frac{[B]}{[A]}$$

$$\Delta G_{\text{Rxn}} = - \left(4.076 \frac{\text{Kcal}}{\text{mol}} \right) + \left(1.98 \times 10^{-3} \frac{\text{Kcal}}{\text{mol K}} \right) (298 \text{ K}) \ln \frac{10^{-5}}{1}$$

$$\Delta G_{\text{Rxn}} = -10.9 \frac{\text{Kcal}}{\text{mol}} \leftarrow \text{Even more spontaneous}$$

Thermodynamics

Another question... What are $[A]_{\text{eq}}$ and $[B]_{\text{eq}}$?

$$[A] + [B] = 1 + 10^{-5} \approx 1\text{M}$$

$$[A] = 1 - [B]$$

$$K_{\text{eq}} = \frac{[B]_{\text{eq}}}{[A]_{\text{eq}}} = 1000$$

$$[B]_{\text{eq}} = 1000 (1 - [B]_{\text{eq}})$$

$$1001 [B]_{\text{eq}} = 1000$$

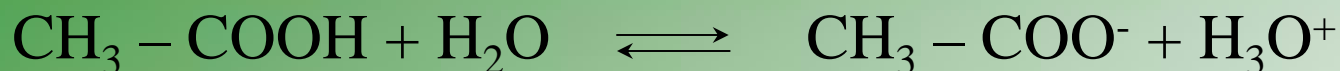
$$[B]_{\text{eq}} = \frac{1000}{1001} = 0.999\text{M} \approx 1\text{M}$$

$$[A]_{\text{eq}} = 0.001\text{M}$$

Thermodynamics

Another Example... Acetic Acid Dissociation

$$\Delta H^\circ = 0$$



Creation of **charges** \Rightarrow Requires ion solvation
 \Rightarrow Organizes H_2O around ions

At 1M concentration, this is entropically unfavorable:

$$K_{\text{eq}} \sim 10^{-5}$$

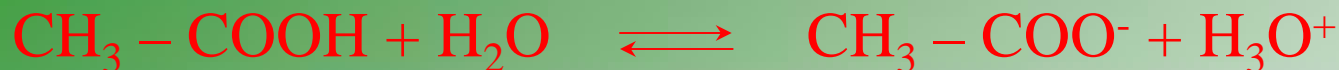
$$K_{\text{eq}} = \frac{[\text{CH}_3 - \text{COO}^-][\text{H}_3\text{O}^+]}{[\text{CH}_3 - \text{COOH}]} \sim 10^{-5}$$

If $[\text{CH}_3 - \text{COOH}]_{\text{total}} \sim 10^{-5} \rightarrow 50\% \text{ ionized}$

Percent ionization is concentration dependent. We can favor the forward rxn (ionization) by diluting the mixture

If $[\text{CH}_3 - \text{COOH}]_{\text{total}} \sim 10^{-8} \rightarrow 90\% \text{ ionized}$

Thermodynamics

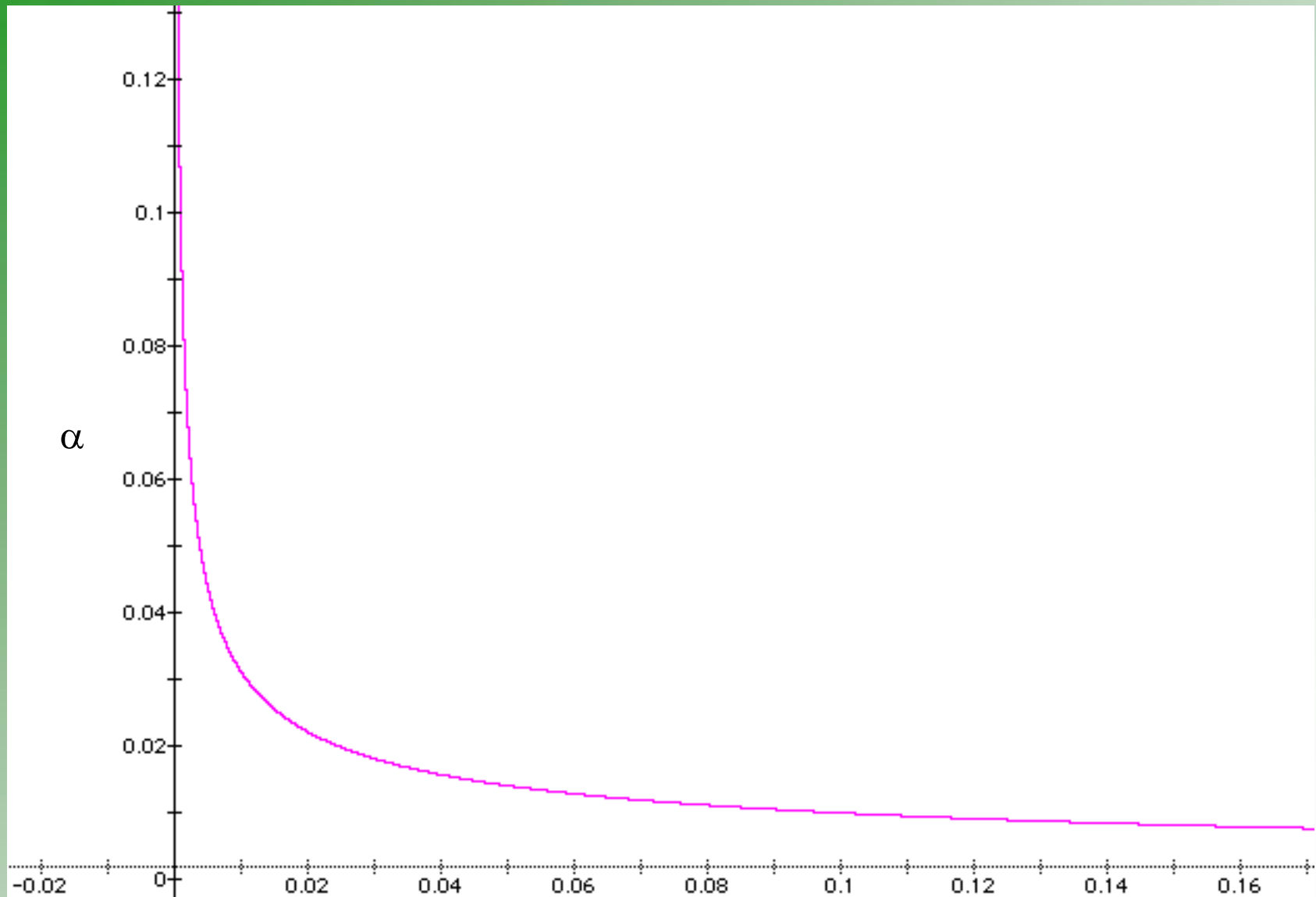


$$K_{\text{eq}} = \frac{[\text{CH}_3 - \text{COO}^-][\text{H}_3\text{O}^+]}{[\text{CH}_3 - \text{COOH}]} = \frac{\frac{[\text{CH}_3 - \text{COO}^-][\text{H}_3\text{O}^+]}{[\text{CH}_3 - \text{COOH}]_{\text{T}}^2}}{\frac{[\text{CH}_3 - \text{COOH}]_{\text{T}} - [\text{CH}_3 - \text{COO}^-]}{[\text{CH}_3 - \text{COOH}]_{\text{T}}^2}}$$

$$K_{\text{eq}} = \frac{\alpha^2 [\text{CH}_3 - \text{COOH}]_{\text{T}}}{1 - \alpha} \quad \text{with} \quad \alpha \equiv \frac{[\text{CH}_3 - \text{COO}^-]}{[\text{CH}_3 - \text{COOH}]_{\text{T}}}$$

$$\text{and } \alpha = \frac{-K_{\text{eq}} + \sqrt{K_{\text{eq}}^2 + 4[\text{CH}_3 - \text{COOH}]_{\text{T}} K_{\text{eq}}}}{2[\text{CH}_3 - \text{COOH}]_{\text{T}}}$$

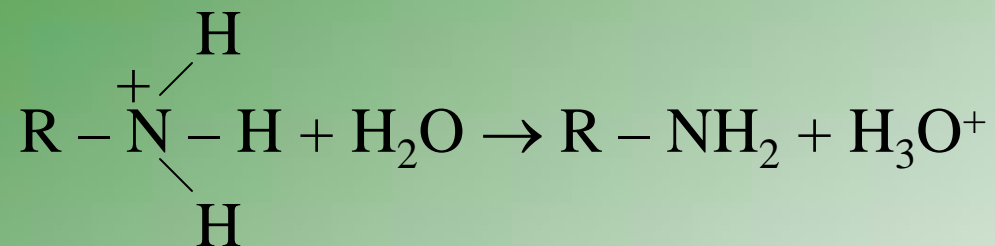
Thermodynamics



$\text{CH}_3\text{-COOH total}$

Thermodynamics

Third Example... Amine Reactions

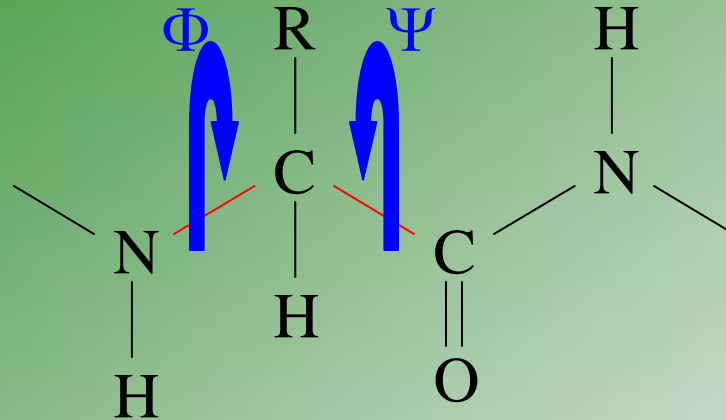


$$\Delta S^\circ \approx 0$$

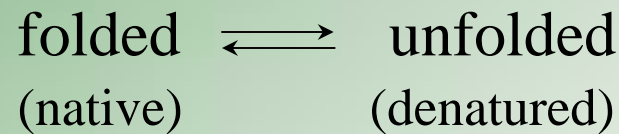
not favorable

$$\Delta H^\circ = 14 \frac{\text{Kcal}}{\text{mol}}$$
$$K_{\text{eq}} = 10^{-10}$$

Backbone Conformational Flexibility



For the process...



$$\Delta S_{\text{backbone conf.}}^{\circ} = R \ln \frac{W_{\text{unfolded}}}{W_{\text{folded}}}$$

How many ways to form the unfolded state?...

Backbone Conformational Flexibility

degrees of freedom = 2 $\begin{cases} \Phi \\ \Psi \end{cases}$

Assume **2 possible values** for each degree of freedom. Then...

Total of 4 ^{conformational isomers}/_{residue}

For 100 amino acids...

$4^{100} \sim 10^{60}$ conformations

These results do not take into account **excluded volume effects**.

When these effects are considered the number of accessible configurations for the chain is quite a bit smaller...

$W_{\text{unfolded}} \sim 10^{16}$ conformations

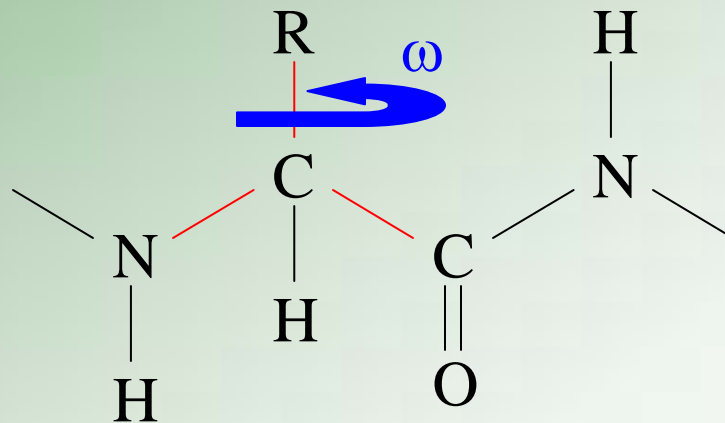
Backbone Conformational Flexibility

Thermodynamic considerations...

$$\begin{aligned}\Delta S_{\text{backbone conf.}}^{\circ} &= R \ln 10^{16} \\ &\cong 1.987 \times 16 \times 2.303 \\ &= 73 \frac{\text{cal}}{\text{mol K}}\end{aligned}$$

$$\Delta G_{\text{backbone conf.}}^{\circ} = -T\Delta S^{\circ} = -22 \frac{\text{Kcal}}{\text{mol}} \text{ at } 25^{\circ}\text{C}$$

In addition other degrees of freedom may be quite important, for example...



We will see this later in more detail

Ionization of Water

- Water is the silent, most important component in the cell
- Its properties influence the behavior and properties of all other components in the cell.

Here we concern ourselves with its ionization properties:



$$K_{\text{eq}} = \frac{[\text{H}_3\text{O}^+][\text{OH}^-]}{[\text{H}_2\text{O}]}$$

Since in the cell, $[\text{H}_2\text{O}] \sim 55\text{M}$, and ionization is very weak, then $[\text{H}_2\text{O}] \sim \text{constant}$, so we can define...

$$K_{\text{w}} = [\text{H}_3\text{O}^+][\text{OH}^-]$$

“the ionic
product of
water”

Ionization of Water

From the previous equation...

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-]$$

$$K_w = 10^{-14} \quad \text{For pure water...}$$

$$[\text{H}^+] = [\text{H}_3\text{O}^+] = [\text{OH}^-] = 10^{-7} \text{ M}$$

i.e. in a neutral soln: $[\text{H}_3\text{O}^+] = 10^{-7} \text{ M}$ $[\text{OH}^-] = 10^{-7} \text{ M}$

The overall **acidity** of the medium greatly affects many biochemical reactions, because **most biological components** can function either as **bases** or **acids**.

A measure of acidity is given by the pH scale, defined as...

$$\text{pH} = \log_{10} \frac{1}{[\text{H}_3\text{O}^+]} = -\log [\text{H}_3\text{O}^+]$$

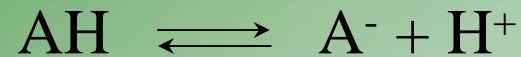
So, in fact for
pure water:

$$\text{pH} = \log_{10} \frac{1}{10^{-7}} = 7$$

Weak Acids and Bases

All biological acids and bases belong to this category

Consider acetic acid...



The **Dissociation Constant**...

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{AH}]}$$

rearrange...

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{AH}]}$$

**Henderson-
Hasselbalch
equation**

where, $\text{pK}_a = -\log K_a$

Weak Acids and Bases

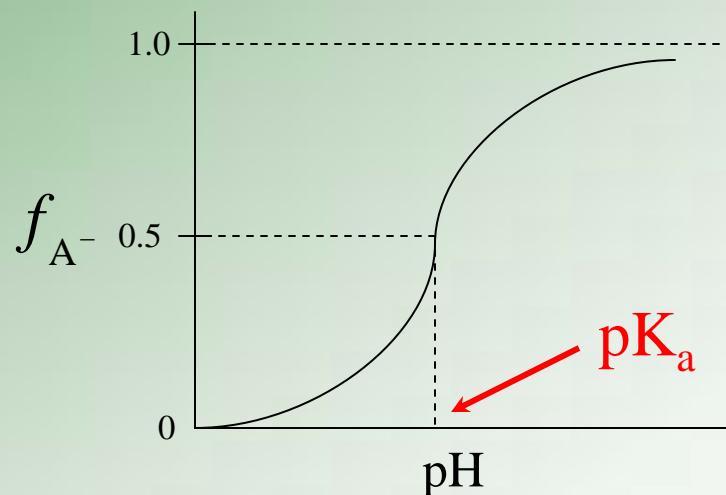
Fraction of deprotonated acid is...

$$f_{A^-} = \frac{[A^-]}{[A^-] + [AH]}$$

$$\text{Also... } f_{AH} = 1 - f_{A^-}$$

So, we can re-write the Henderson-Hasselbalch equation

$$\text{pH} = \text{pK}_a + \log \frac{f_{A^-}}{1 - f_{A^-}}$$



i.e. pK_a is the pH at which the acid is 50% ionized

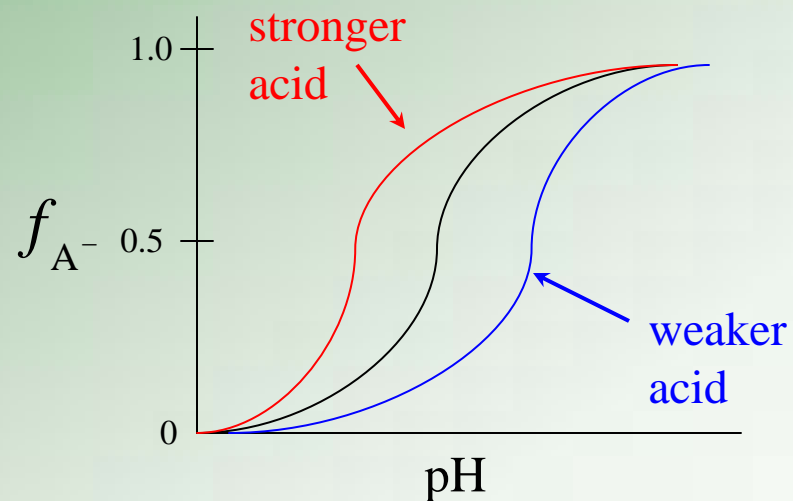
Weak Acids and Bases

Based on the previous page...

$$\text{If...} \left\{ \begin{array}{l} \text{pH} = \text{pK}_a + 1 ; f_{\text{A}^-} = \frac{10}{11} \approx 90\% \\ \text{pH} = \text{pK}_a - 1 ; f_{\text{A}^-} \approx 9\% \\ \text{pH} = \text{pK}_a - 2 ; f_{\text{A}^-} \approx 0.9\%, \text{ etc.} \end{array} \right.$$

$$\text{pH} = \text{pK}_a + \log \frac{f_{\text{A}^-}}{1 - f_{\text{A}^-}}$$

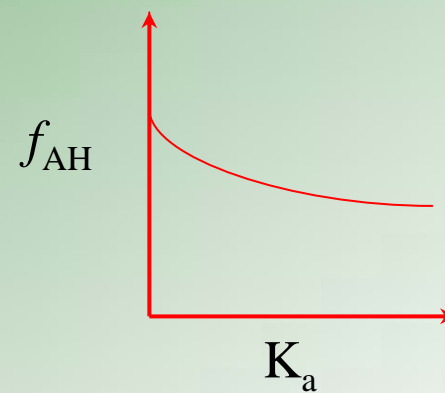
Moreover... the lower the pK_a , the stronger the acid



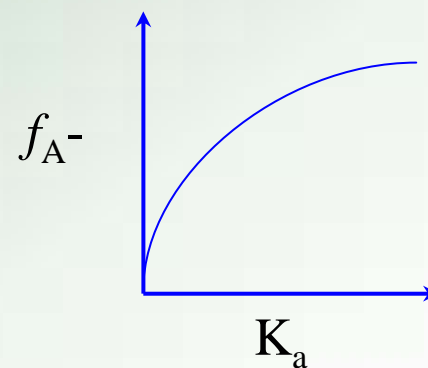
Weak Acids and Bases

Some useful relationships...

$$f_{\text{AH}} = \frac{\text{AH}}{\text{A}^- + \text{AH}} = \frac{[\text{H}^+]}{K_a + [\text{H}^+]}$$

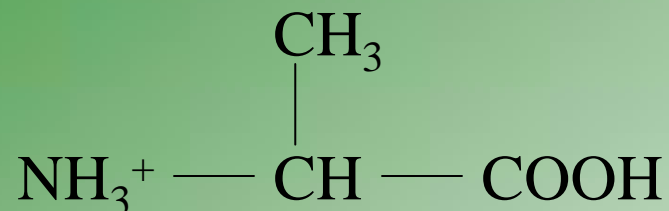


$$f_{\text{A}^-} = \frac{\text{A}^-}{\text{A}^- + \text{AH}} = \frac{K_a}{K_a + [\text{H}^+]}$$

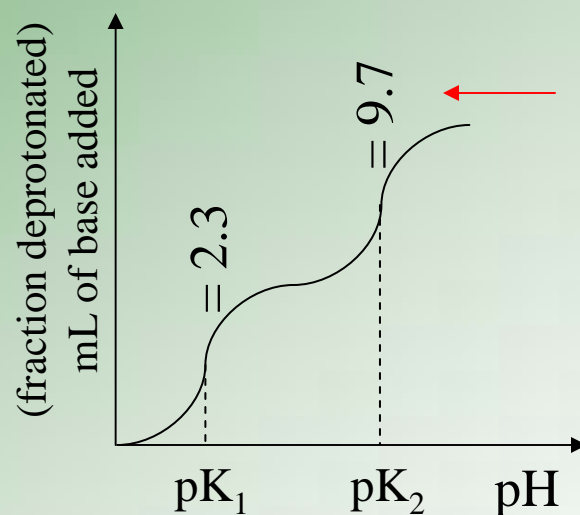


Multiple Acid-Base Equilibria

Consider **Alanine**...



Titrate a solution of ala, using a gas electrode (pH meter), and a buret to add a strong base of known concentration:

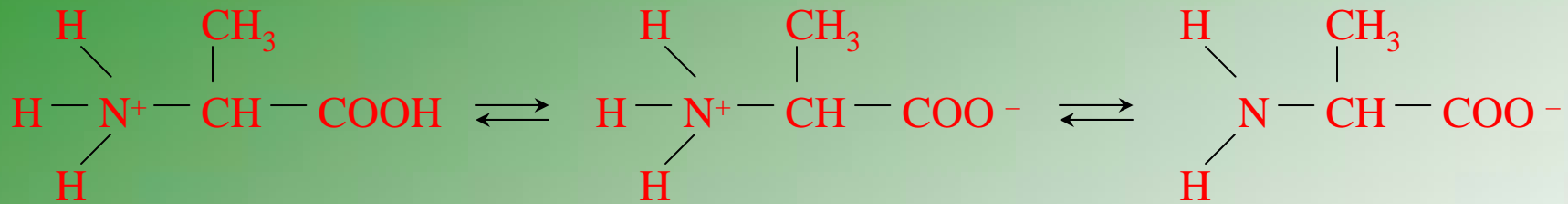


← Please correct in your notes

Macroscopic experiment shows 2 inflection points (2 pKs)

Multiple Acid-Base Equilibria

As we vary the pH of the solution from low to high:



Cation

Zwitterion

Anion

So, in fact the two inflection points seen correspond to the deprotonation of the carboxylic group (at low pH) and then to the deprotonation of the amine group (at high pH).

So, How can we estimate the fraction of these different species in solution?

If we assume that the ionization of a given group is independent of the state of ionization of the others, then...

Multiple Acid-Base Equilibria

$$f_{+HAH^-} = f_{COOH} \times f_{NH_3^+} = \left(\frac{H^+}{K_{a1} + H^+} \right) \left(\frac{H^+}{K_{a2} + H^+} \right)$$

$$f_{+HA^-} = f_{COO^-} \times f_{NH_3^+} = \left(\frac{K_{a1}}{K_{a1} + H^+} \right) \left(\frac{H^+}{K_{a2} + H^+} \right)$$

$$f_{AH} = f_{COOH} \times f_{NH_2} = \left(\frac{H^+}{K_{a1} + H^+} \right) \left(\frac{K_{a2}}{K_{a2} + H^+} \right)$$

$$f_{A^-} = f_{COO^-} \times f_{NH_2} = \left(\frac{K_{a1}}{K_{a1} + H^+} \right) \left(\frac{K_{a2}}{K_{a2} + H^+} \right)$$

$$f_{+HAH^-} + f_{+HA^-} + f_{AH} + f_{A^-} = 1$$