

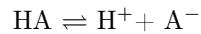
CHEM 153A Week 2

Aidan Jan

January 17, 2025

Ionization Constants

- The tendency for any acid (HA) to lose a proton and form its conjugate base (A^-) is defined by the equilibrium constant (K_{eq}) for the reversible reaction



for which

$$K_{eq} = \frac{[H^+][A^-]}{[HA]} = K_a$$

Acid Strength Comparison: K_a and pK_a Values of Common Acids

No.	Acid	K_a	pK_a
1	Hydroiodic acid (HI)	3.16×10^9	-9.5
2	Hydrobromic acid (HBr)	1.0×10^9	-9
3	Hydrochloric acid (HCl)	1.0×10^6	-6
4	Sulfuric acid (H_2SO_4)	1.0×10^3	-3
5	Hydronium ion (H_3O^+)	55	-1.74
6	Nitric acid (HNO_3)	28.2	-1.45
7	Trifluoroacetic acid (CF_3COOH)	5.62×10^{-1}	0.25
8	Oxalic acid ($HOOC-COOH$)	5.37×10^{-2}	1.27
9	Acetic acid (CH_3COOH)	1.75×10^{-5}	4.76

pK_a

- pK_a = analogous to pH and defined by the equation

$$pK_a = \log \frac{1}{K_a} = -\log K_a$$

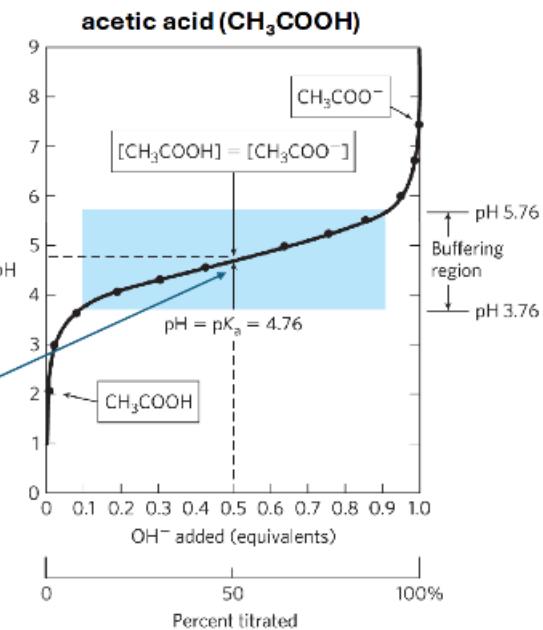
- the stronger the tendency to dissociate a proton, the stronger the acid and the lower its pK_a
- pK_a can be determined experimentally

Titration Curves Reveal the pK_a of Weak Acids

- titration curve = a plot of pH against the amount of OH equivalents added

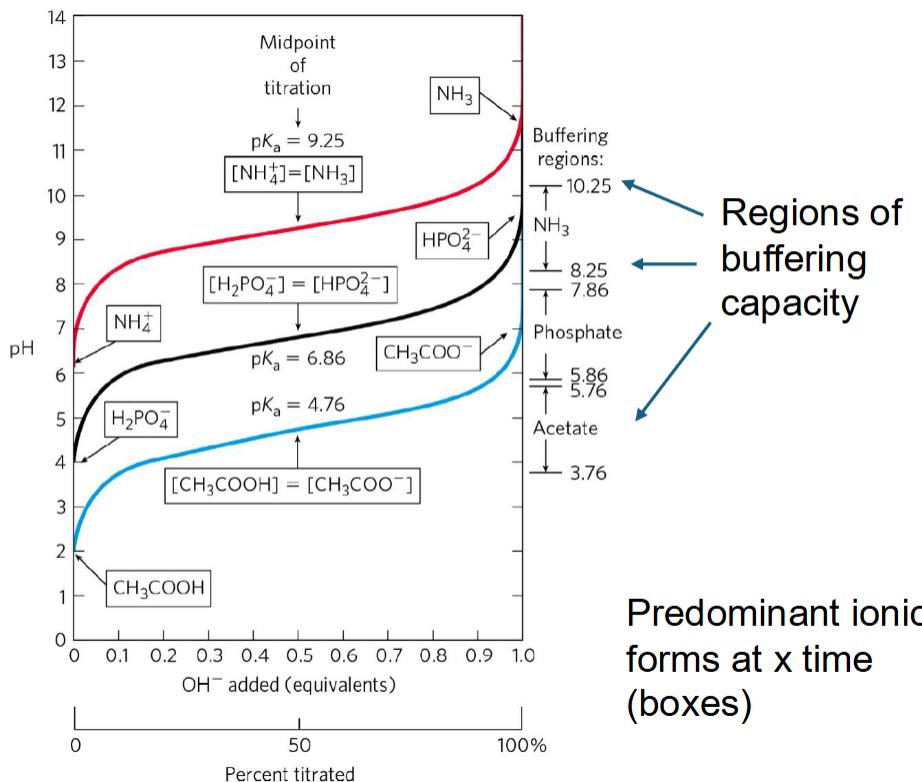
The NaOH is added in small increments until the acid is consumed (neutralized)

- at the midpoint, the pH of the equimolar solution = the pK_a of acetic acid



Comparison of the Titration Curves of Three Weak Acids

- a weak acid and its anion - a conjugate acid-base pair - can act as a buffer
- Titration curves for acetic acid, dihydrogen phosphate, and ammonium are shown below
- Conjugate acid-base pairs are effective buffers between approximately 10% and 90% neutralization of the proton donor species



Predominant ionic forms at x time (boxes)

Buffers are Mixtures of Weak Acids and Their Conjugate Bases

- buffers = aqueous systems that tend to resist changes in pH when small amounts of acid (H^+) or base (OH^-) are added
- a buffer system consists of a weak acid (the proton donor) and its conjugate acid (the proton acceptor)
- The **buffering region** is the flat zone of a titration curve (see above)
 - the boundaries of a buffer system are $\text{pH} = \text{p}K_a \pm 1$ (so acetic acid buffer range is 3.76-5.76)

The buffering capacity is strongest when the ratio of $[\text{HA}]$ to $[\text{A}^-]$ is close to 1:1. This occurs at the $\text{p}K_a$ of the weak acid, where half of the weak acid is dissociated.

If the ratio of acid to base (or base to acid) becomes too large - greater than 10:1 or less than 1:10 - the buffer's capacity to neutralize added acids or bases weakens significantly

The Henderson-Hasselbalch Equation Relates pH, $\text{p}K_a$, and Buffer Concentration

- **Henderson-Hasselbalch equation** = describes the shape of the titration curve of any weak acid

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

- Equation only works within the buffer region, outside of this it starts becoming inaccurate

Primary Uses of the Henderson-Hasselbalch Equation

1. Calculating pH of Buffers
 - Predicts pH based on acid/base ratios
2. Designing Buffers
 - Helps create buffers with a desired pH by adjusting the acid-base ratio.
3. Estimating $\text{p}K_a$
 - Can determine the $\text{p}K_a$ of weak acids and bases experimentally

Deriving the Henderson-Hasselbalch Equation (not needed for exam)

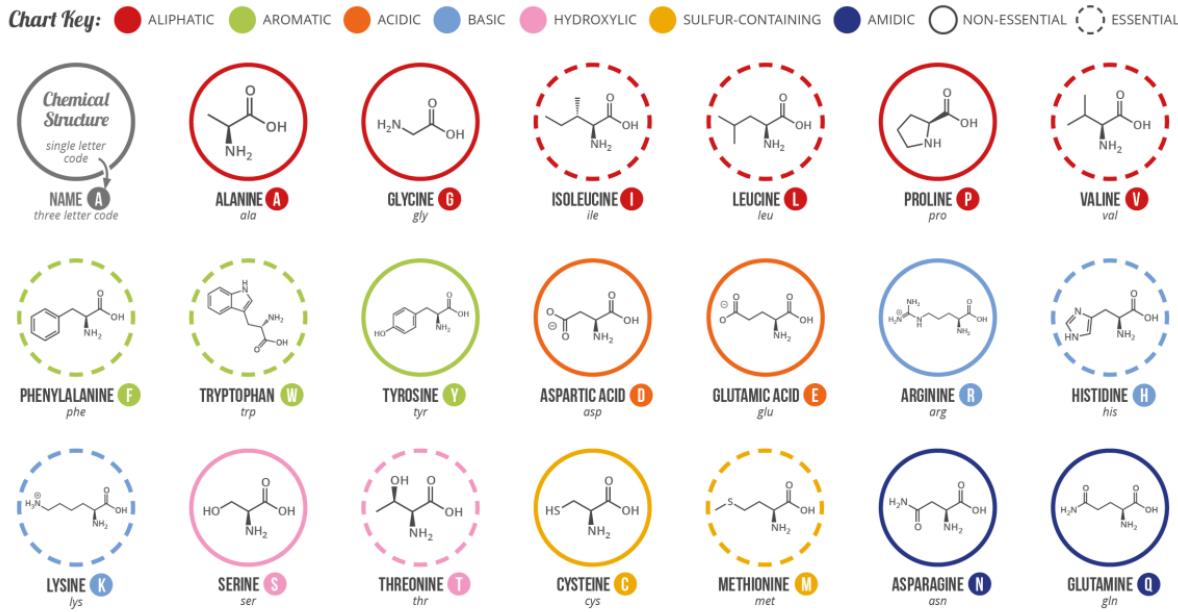
$$\begin{aligned} K_a &= \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \\ [\text{H}^+] &= K_a \cdot \frac{[\text{HA}]}{[\text{A}^-]} \\ -\log[\text{H}^+] &= -\log K_a - \log \frac{[\text{HA}]}{[\text{A}^-]} \\ \text{pH} &= \text{p}K_a - \log \frac{[\text{HA}]}{[\text{A}^-]} \\ \text{pH} &= \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \end{aligned}$$

Amino Acids

- In every living organism, proteins are constructed from a common set of 20 amino acids*
- Each amino acid has a side chain with distinctive chemical properties. Amino acids may be regarded as the alphabet in which the language of protein structure is written.

A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.



Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes *asx* (B) and *glx* (Z) are respectively used.

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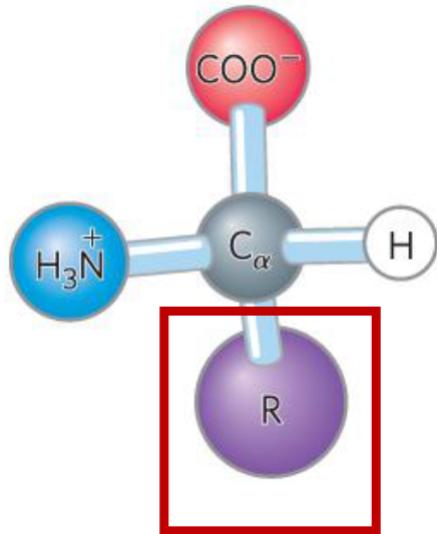


Amino Acids Share Common Structural Features

- α carbon and four substituents
- α carbon is the **chiral center** (except in Glycine, which is not chiral)
- Tetrahedral

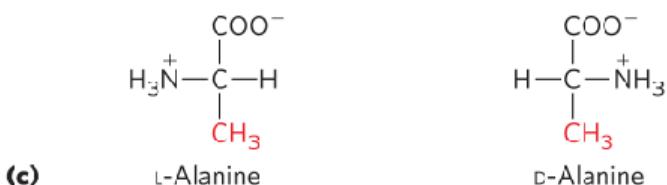
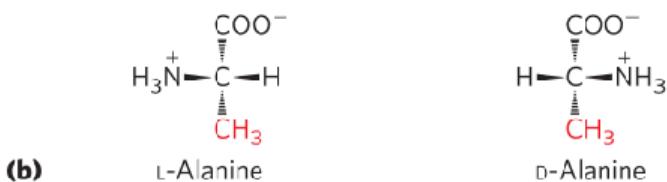
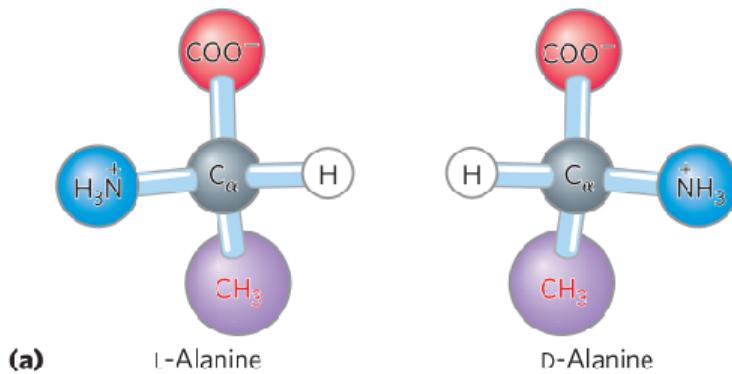
The four substituents are:

- a carboxyl group
- an amino group
- a hydrogen atom
- an **R group** (a side chain unique to each amino acid)
 - Glycine has a second hydrogen atom instead of an R group.



The Amino Acid Residues in Proteins are L Stereoisomers

- Two possible stereoisomers = **enantiomers**
- **optically active** = polarize light is rotated in different directions by enantiomers (Glycine is the exception)
- D, L system specifies **absolute configuration**



Amino Acids can be classified by the R Group

There are five main classes:

- Nonpolar, aliphatic (7)
- Aromatic (3)
- Polar, uncharged (5)
- Positively charged, Basic (3)
- Negatively charged, Acidic (2)

Nonpolar, Aliphatic R Groups

The **hydrophobic effect** stabilizes protein structure

- Glycine
- Alanine
- Proline
- Valine
- Leucine
- Isoleucine
- Methionine

Aromatic R Groups

R groups absorb UV light at 270-280 nm, and can contribute to the hydrophobic effect.

- Phenylalanine
- Tyrosine
- Tryptophan

Polar, Uncharged R Groups

R groups can **form hydrogen bonds**, and Cysteine can **form disulfide bonds**

- Serine
- Threonine
- Cysteine
- Asparagine
- Glutamine

Positively Charged R Groups

Have significant positive charge at pH 7.0.

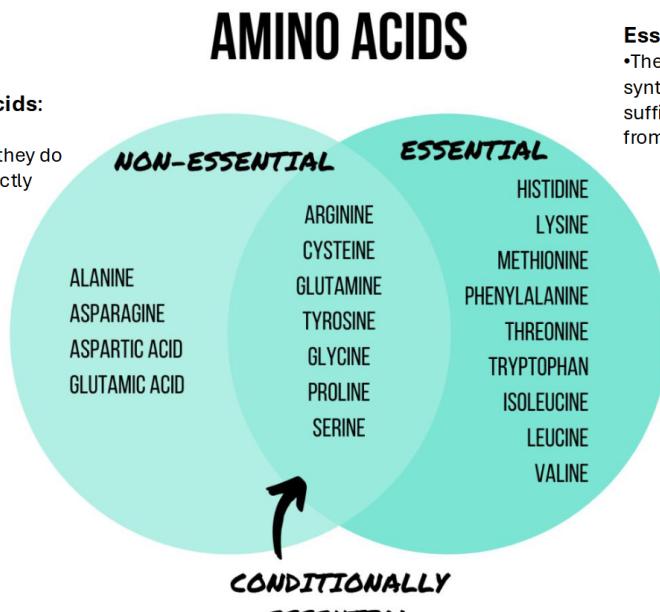
- Lysine
- Arginine
- Histidine

Negatively Charged R Groups

Have net negative charge at pH 7.0.

- Aspartate
- Glutamate

Essential Amino Acids



Non-Essential Amino Acids:

- These amino acids can be synthesized by the body, so they do not need to be obtained directly through the diet

Essential Amino Acids:

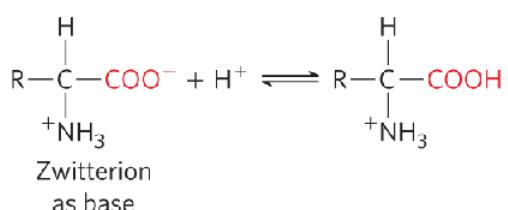
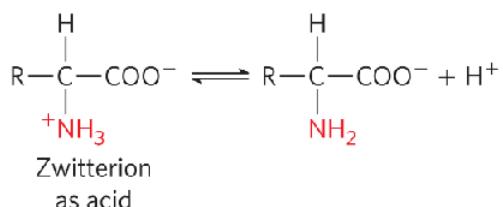
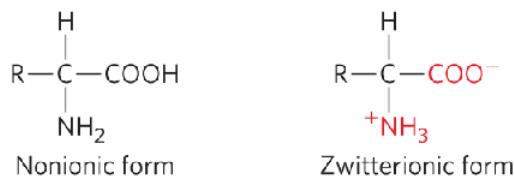
- These amino acids cannot be synthesized by the human body in sufficient amounts and must be obtained from the diet

Conditionally Essential Amino Acids:

- Under normal conditions, these amino acids are synthesized by the body, but during periods of illness, stress, or growth, their production might not meet the body's demands, and they must be supplemented through the diet

Amino Acids can act as Acids or Bases

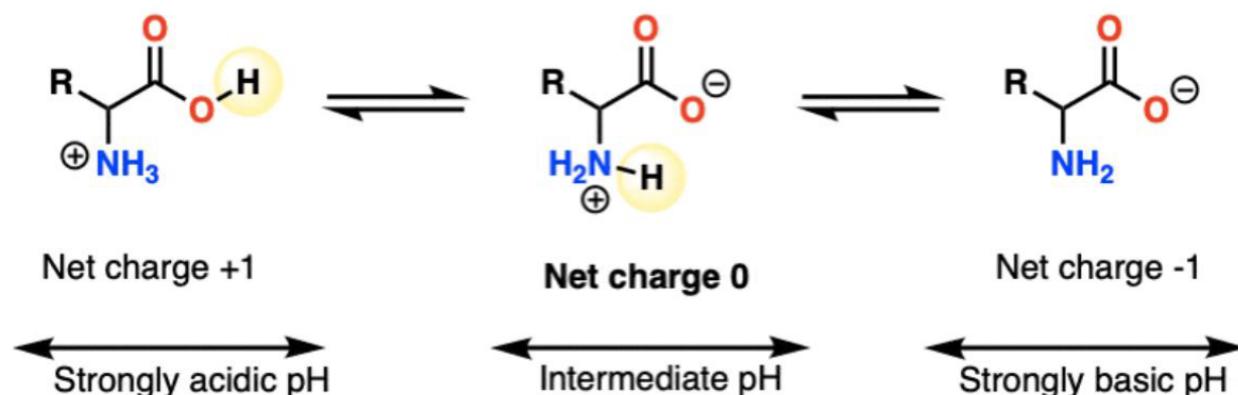
- Amino acids are **acids**. They are also **bases** containing an amino group.
- The term **amphoteric** is often used to describe amino acids, meaning that they are capable of acting as both acids and bases
- **zwitterion** occurs at neutral pH.



The pH-dependent structures of a typical amino acid

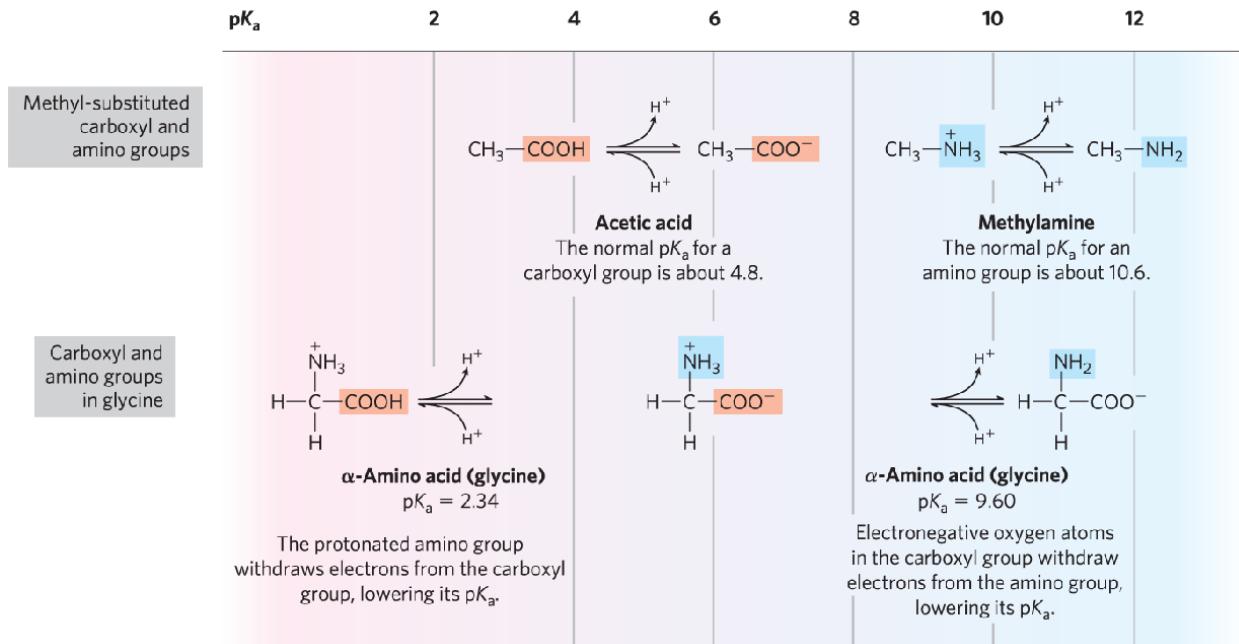
For a typical amino acid with a neutral sidechain **R**:

- the positively charged form (+1) dominates at low pH.
- the zwitterionic (neutral) form dominates at intermediate pH, and
- the negatively charged form (-1) dominates at high pH.



Effect of the Chemical Environment on pK_a

- α -carboxyl group is more acidic than in carboxylic acids
- α -amino group is less basic than in amines



Structures (and pK_a) values of selected amino acids

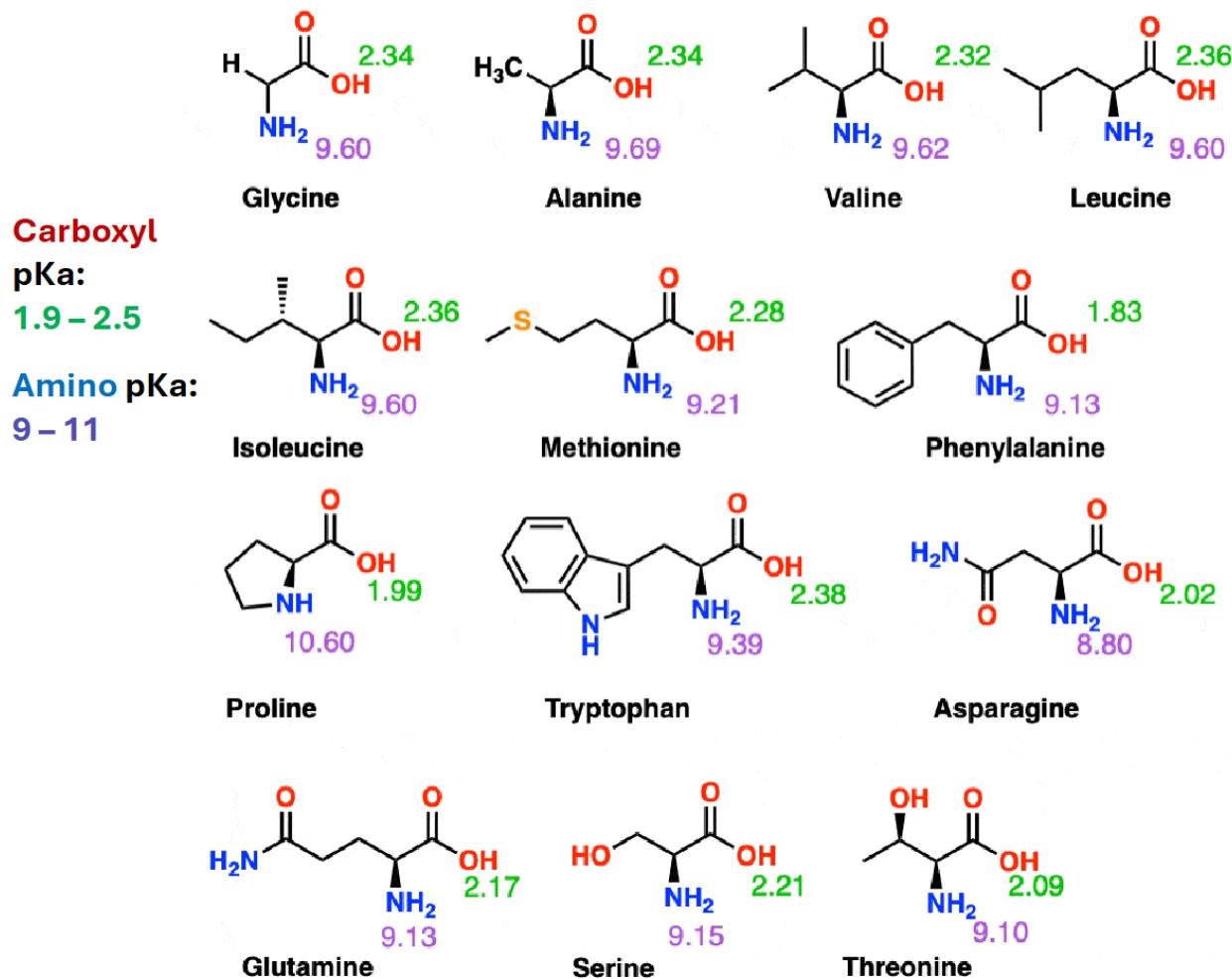


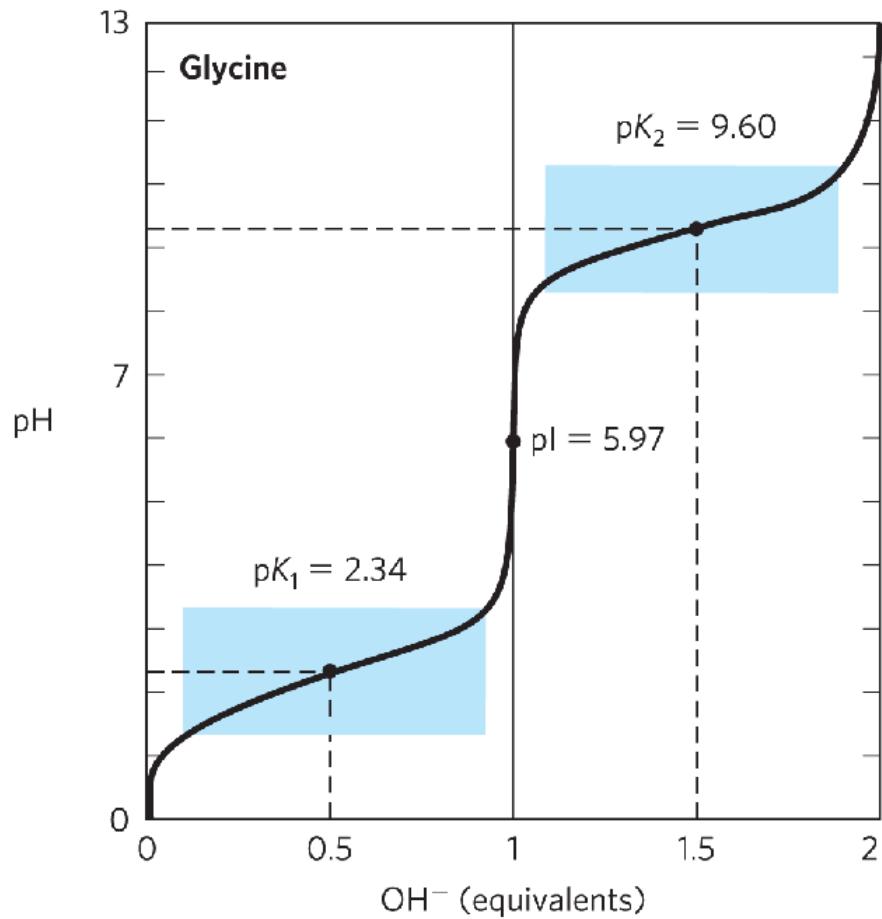
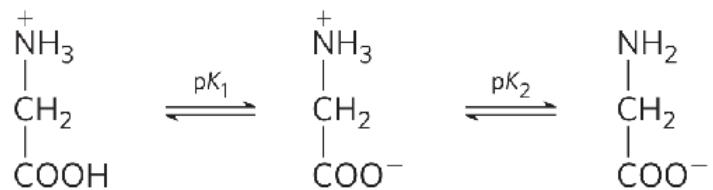
Table of Amino Acid pK_a s

Functional Group	pK_a
COO^- -terminus	3.5
$^+\text{NH}_3$ -terminus	8.5
$\alpha\text{-COO}^-$ (free amino acid)	2
$\alpha\text{-}^+\text{NH}_3$ (free amino acid)	9.5
Aspartate R group	3.9
Glutamate R group	4.3
Histidine R group	6
Cysteine R group	8.3
Tyrosine R group	10
Lysine R group	10.8
Arginine R group	12.5

Titration of Amino Acids

- Cation \rightleftharpoons zwitterion \rightleftharpoons anion
- $-\text{COOH}$ (carboxyl) has an acidic pK_a (pK_1)

- $-\text{NH}_3^+$ (amino) has a basic $\text{p}K_a$ ($\text{p}K_2$)
- the pH at which the net electric charge is zero is the **isoelectric point (pI)**



This titration curve is a qualitative measure of the $\text{p}K_a$ of each ionizing group.

- shows buffering power
 - flat regions are buffer regions. Glycine has two, one centered at $\text{p}K_1 = 2.34$, the other at $\text{p}K_2 = 9.6$.
 - Buffer regions are highlighted in blue.
- shows relationship between its net charge and the pH of the solution
 - isoelectric point, or pI, can be calculated
- In the above image, glycine is present predominantly as its dipolar form, fully ionized with no net electric charge. At the point ($\text{pH} = 5.97$, 1eq base), glycine has an equal number of positive and negative charges.

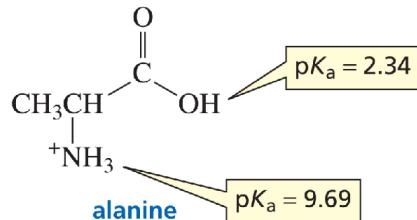
The Isoelectric Point

- The **isoelectric point (pI)** determines the pH at which a molecule carries no net electric charge
- This occurs when the positive and negative charges on the molecule are balanced. For amino acids, the pI is determined by the pK_a values of its ionizable groups, such as the amino ($-NH_3^+$) and carboxyl ($-COOH$) groups, and sometimes the side chain, if it is ionizable.
- for amino acids without ionizable side chains, the isoelectric point (pI) is:

$$pI = \frac{pK_1 + pK_2}{2}$$

- $pH = pI =$ net charge is zero (amino acid least soluble in water, does not migrate in electric field)
- $pH > pI =$ net negative charge
- $pH < pI =$ net positive charge

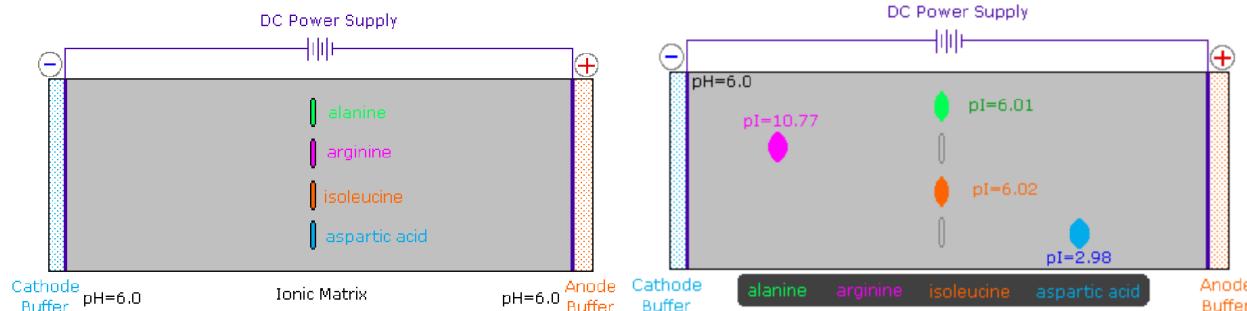
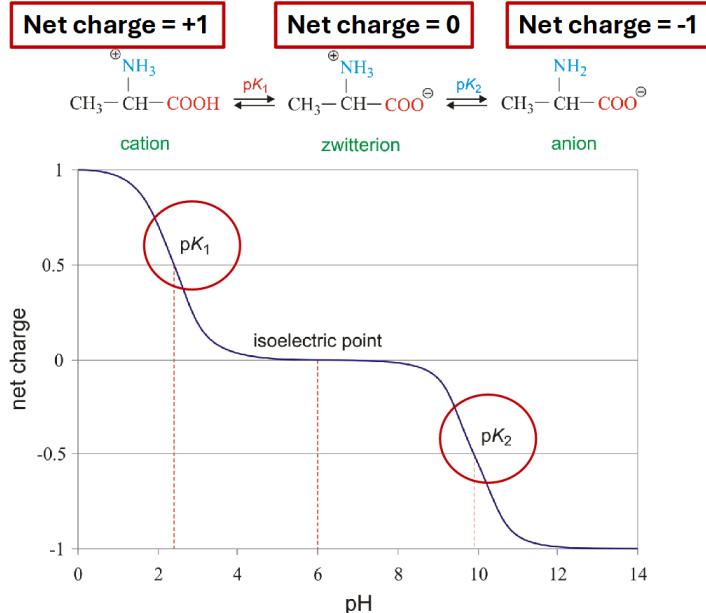
Isoelectric point - Alanine



$$pI = \frac{2.34 + 9.69}{2}$$

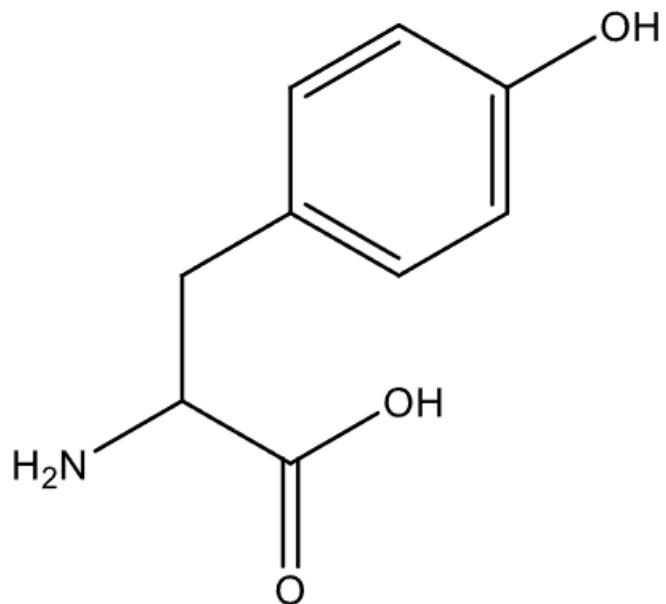
$$pI = 6.015$$

- Not a range, we average the two pK_a s around the point to get one number



Tyrosine at different pH

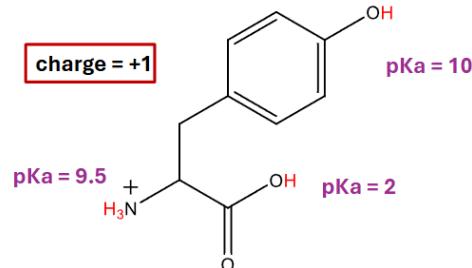
Standard Representation



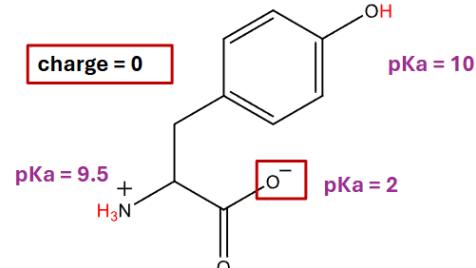
- This is the standard representation of Tyrosine, found in many online sources and textbooks
- It is important to note that **this structure does not exist at any pH!**
- The molecule is made neutral just because, to display its structure.

Representations at different pH

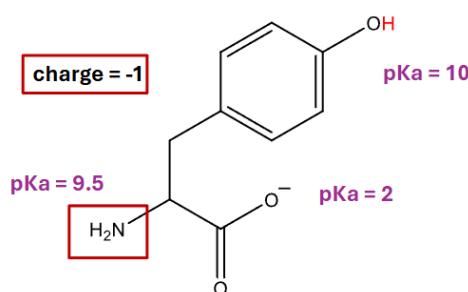
Predominant tyrosine species at pH = 1
(or any pH < 2)



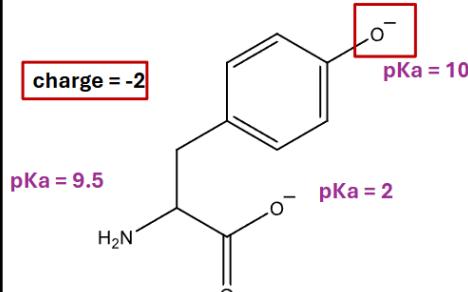
Predominant tyrosine species at pH = 7
(in range $2 < \text{pH} < 9.5$)



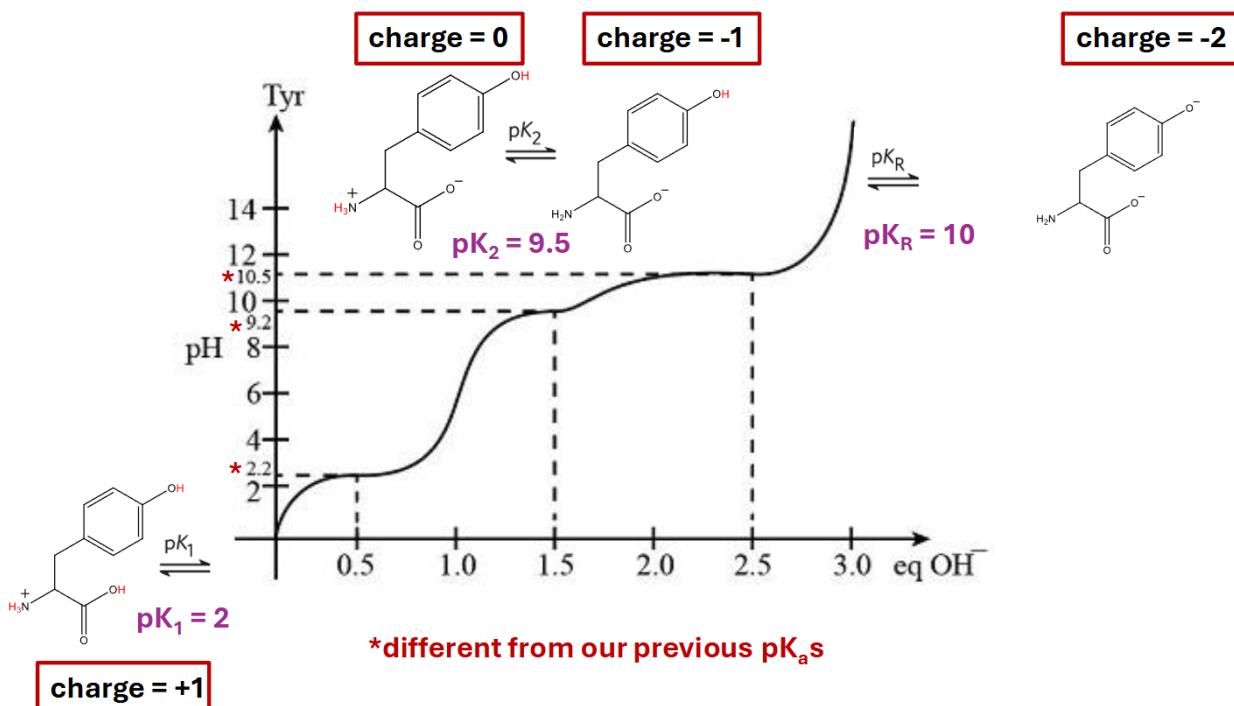
Predominant tyrosine species at pH = 9.75
(in range $9.5 < \text{pH} < 10$)



Predominant tyrosine species at pH = 12
(any pH > 10)

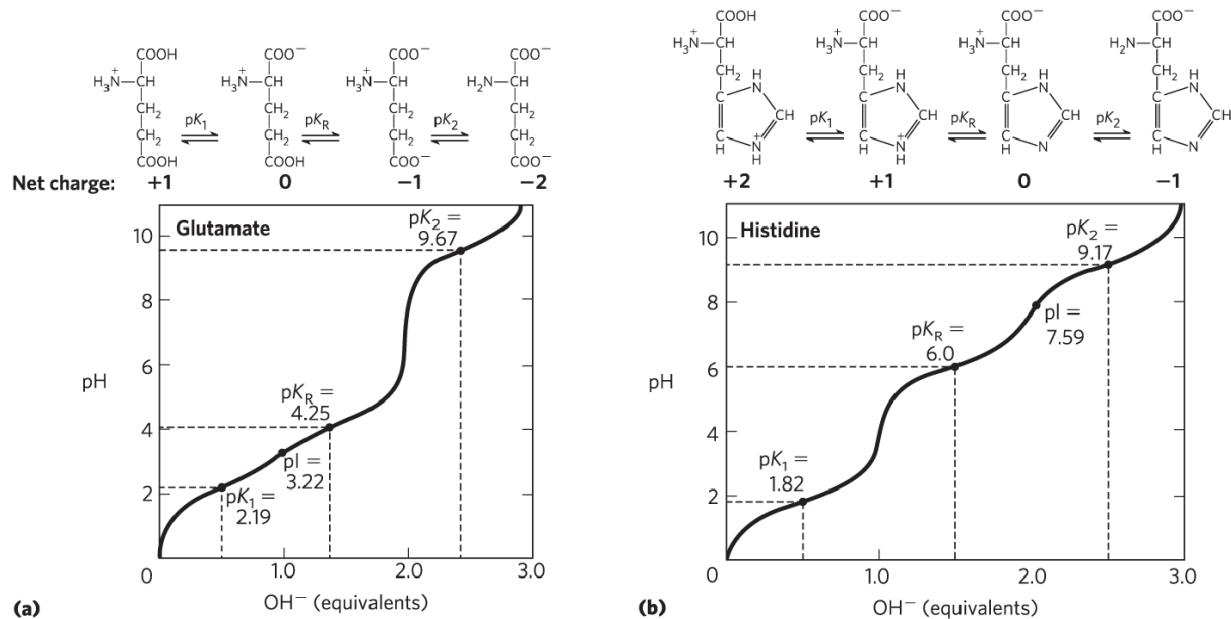


Titration of Tyrosine



- Notice there is a buffer region around each pK_a

Titration of Amino Acids with an Ionizable R Group

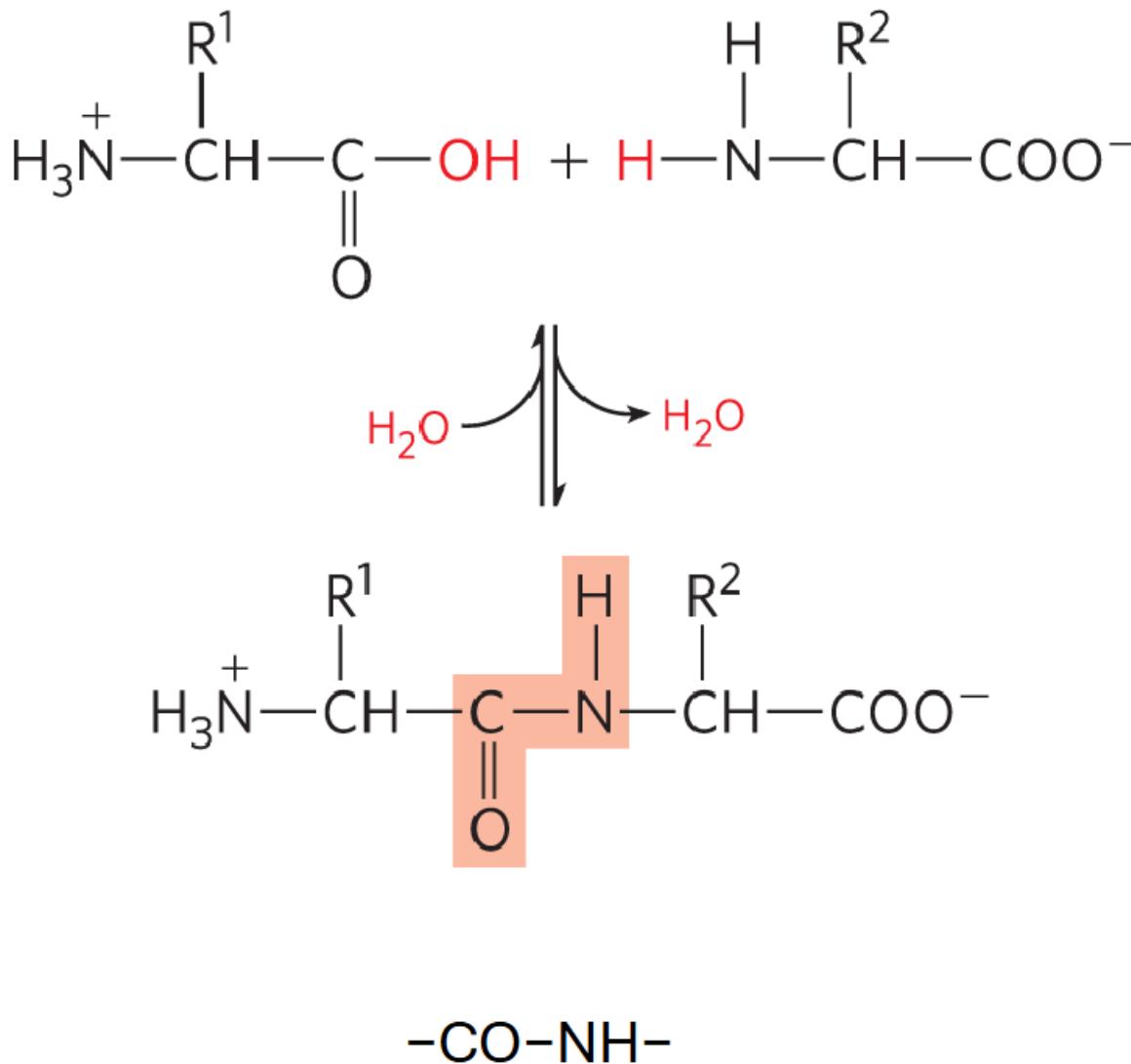


Nelson & Cox, Lehninger Principles of Biochemistry, 8e, © 2021 W.H. Freeman and Company

Peptides and Proteins

In proteins, amino acids are joined in characteristic linear sequences through a common amide linkage, the peptide bond. The amino acid sequence of a protein constitutes its primary structure.

- Peptides are chains of amino acids
- Peptide bond:
 - Covalent
 - formed through **condensation**
 - broken through **hydrolysis**
- The **carboxyl group** of one amino acid loses a hydroxyl group (-OH)
- The **amino group** of the second amino acid loses a hydrogen atom (-H)



Peptide Types by the Number

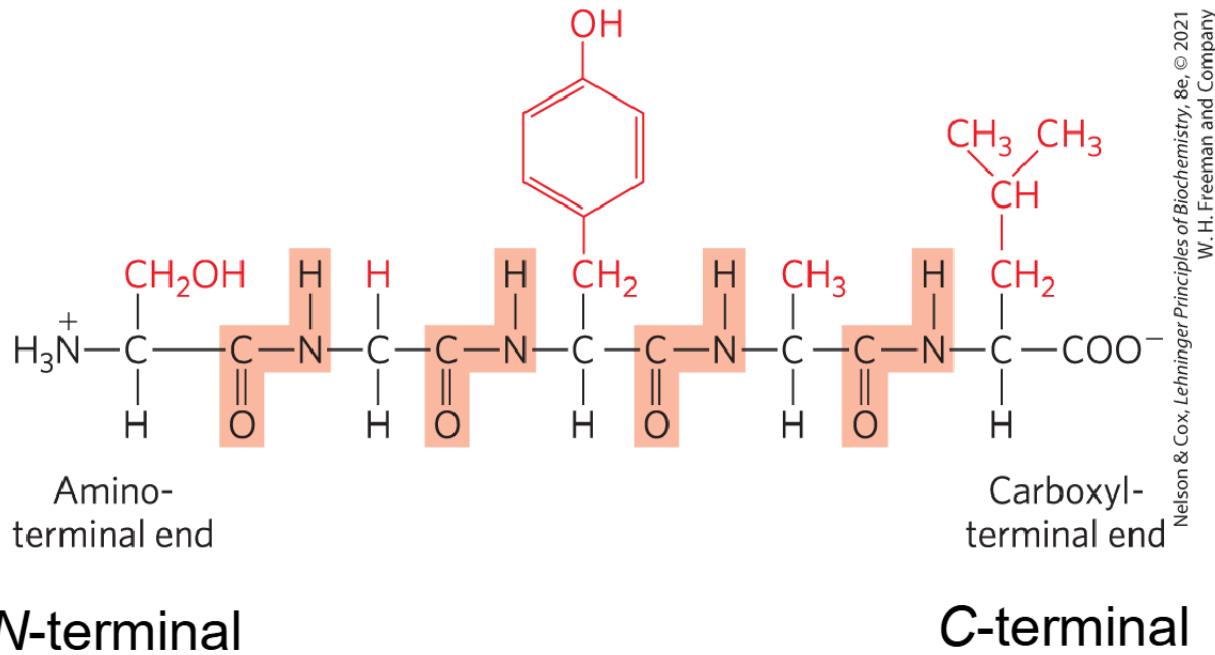
- dipeptide = 2 amino acids, 1 peptide bond
- tripeptide = 3 amino acids, 2 peptide bonds
- **oligopeptide** = a few amino acids
- **polypeptide** = many amino acids, molecular weight < 10 kDa
- **protein** = thousands of amino acids, molecular weight > 10 kDa

Aside: Daltons

- The average molecular weight of an amino acid is 110Da.
- Dalton (Da) is an alternate name for the atomic mass unit, and kilodalton (kDa) is 1000 daltons
- Thus, a protein with a mass of 64kDa has a molecular weight of 64,000 grams per mole

Peptide Terminals

Convention: numbering (and naming) starts from the **amino-terminal residue (N-terminal)**



N-terminal

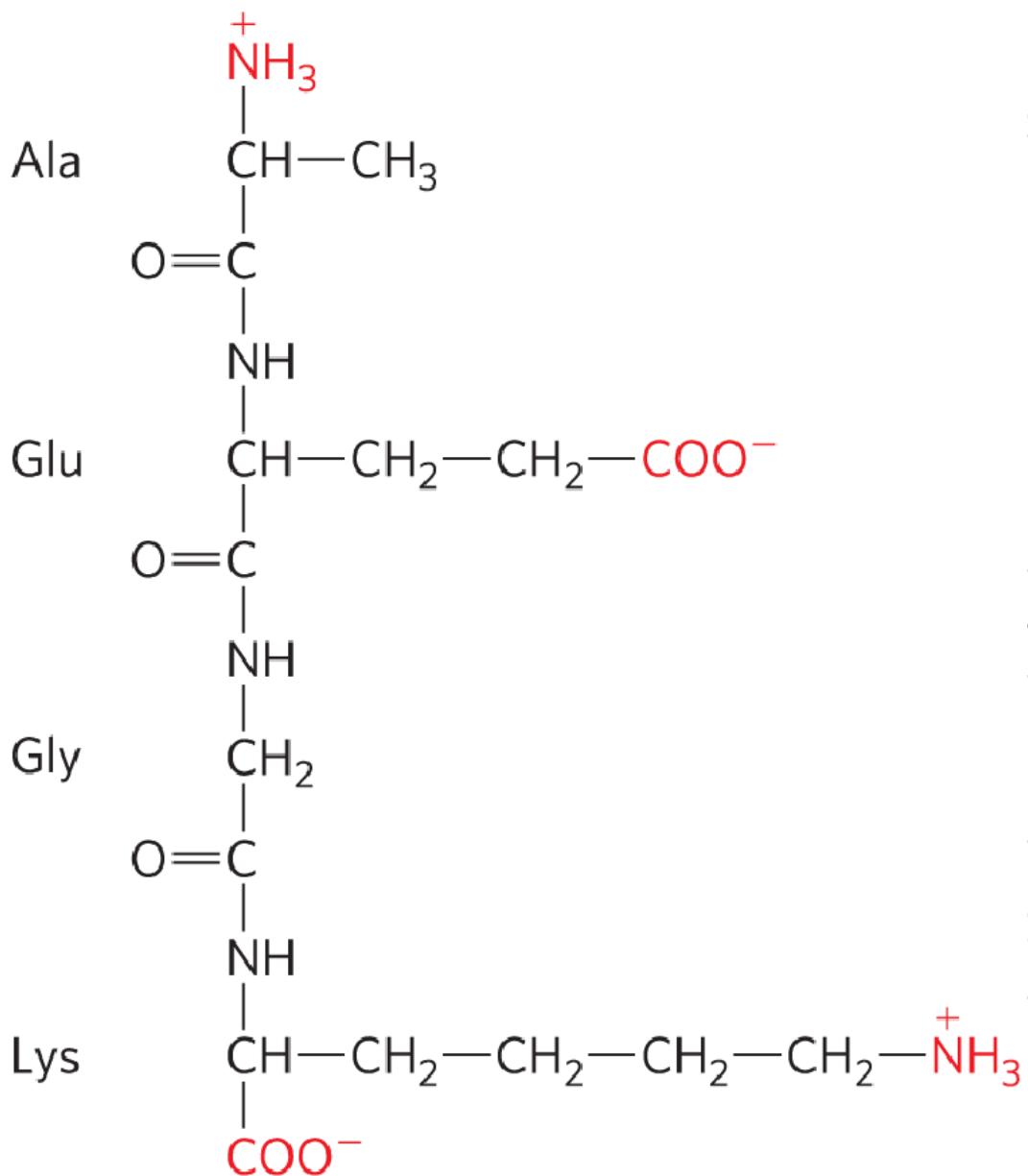
C-terminal

Naming Peptides

- Full amino acid names: serylglycyltyrosylalanyleucine
- Three letter code abbreviations: Ser-Gly-Tyr-Ala-Leu
- One letter code abbreviation: SGYAL

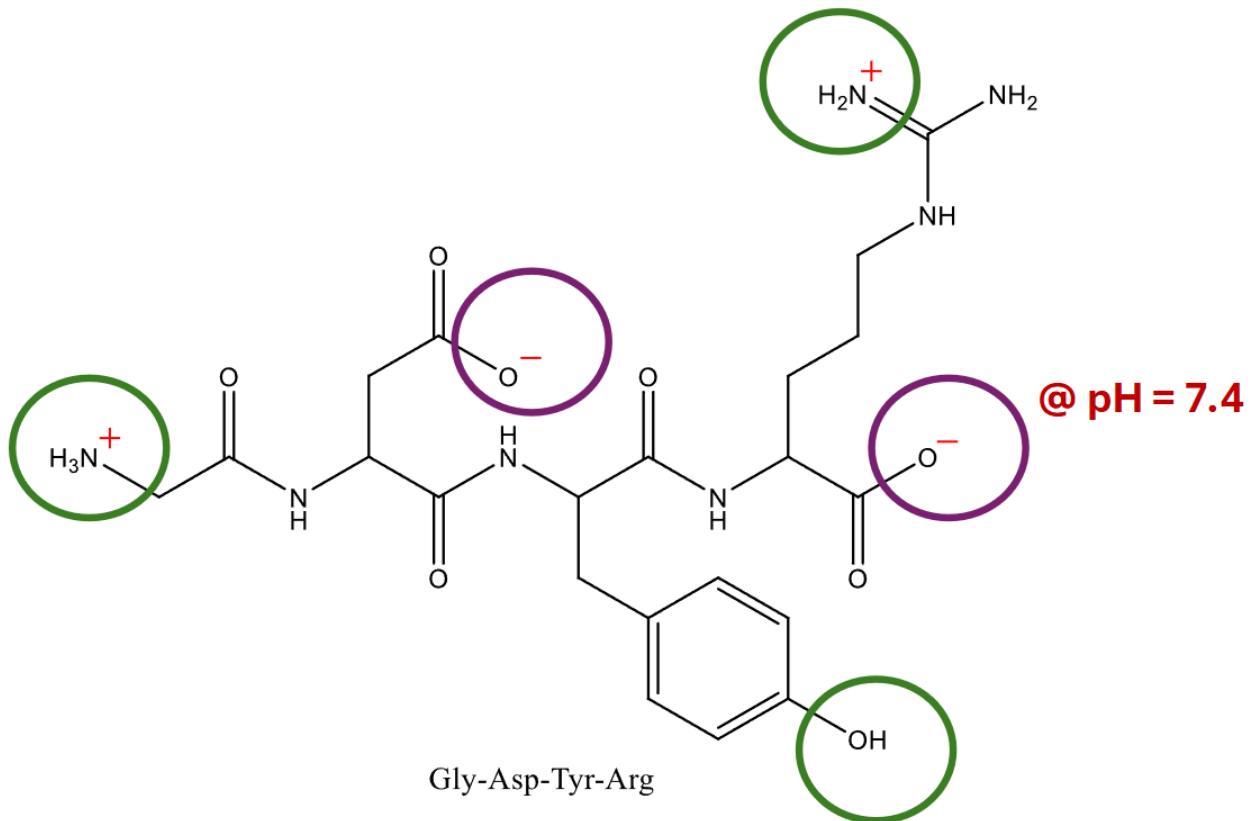
Peptides can be distinguished by their ionization behavior

- Ionizable groups in peptides:
 - one free α -amino group
 - one free α -carboxyl group
 - some R groups



Drawing oligopeptides

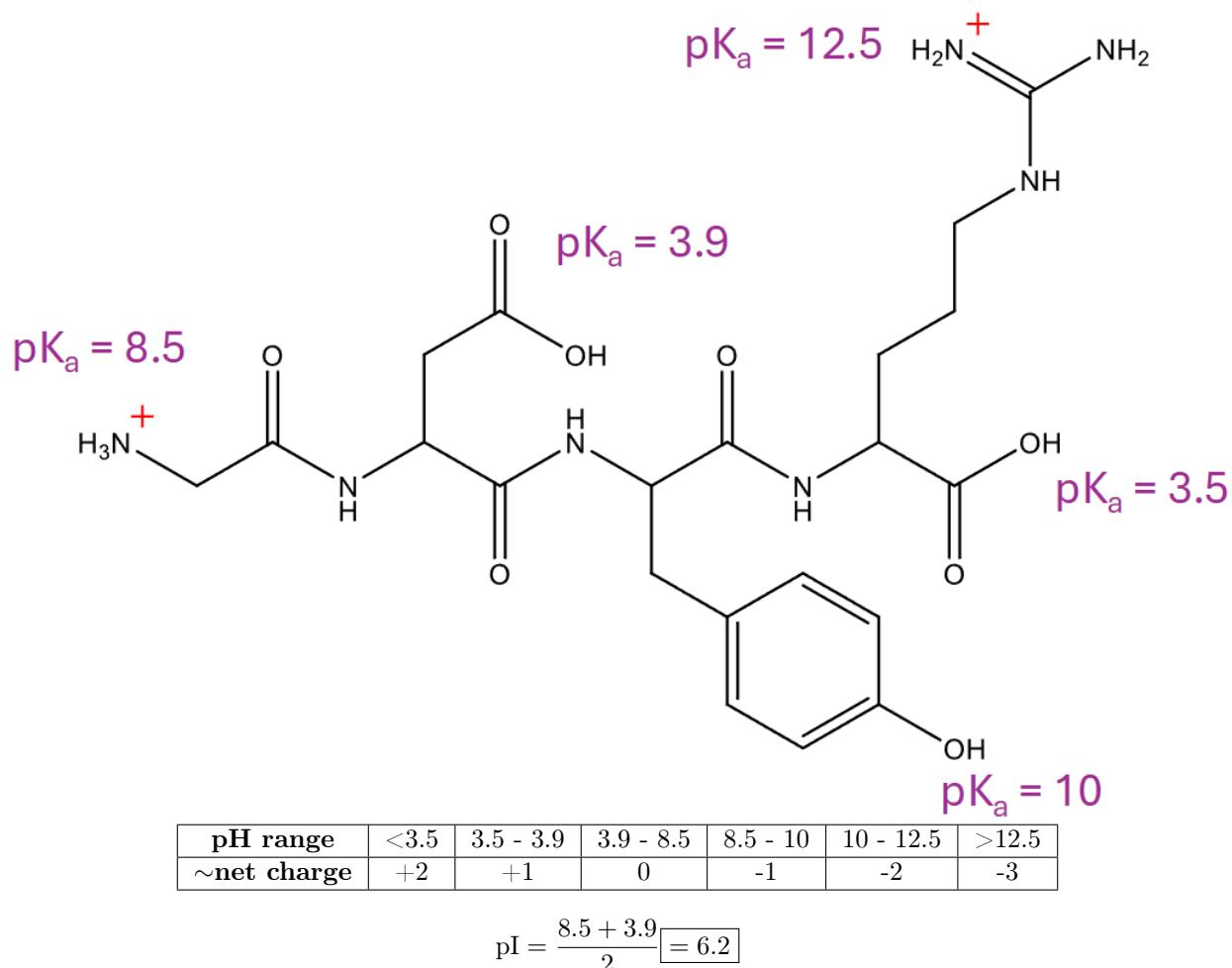
Draw the oligopeptide Gly-Asp-Tyr-Arg at physiological pH



- Refer to the functional group pH table earlier in this document.
- pH < pKa: The molecule is protonated (it holds onto its protons)
- pH > pKa: The molecule is deprotonated (it loses its protons)

Determining pI of peptide

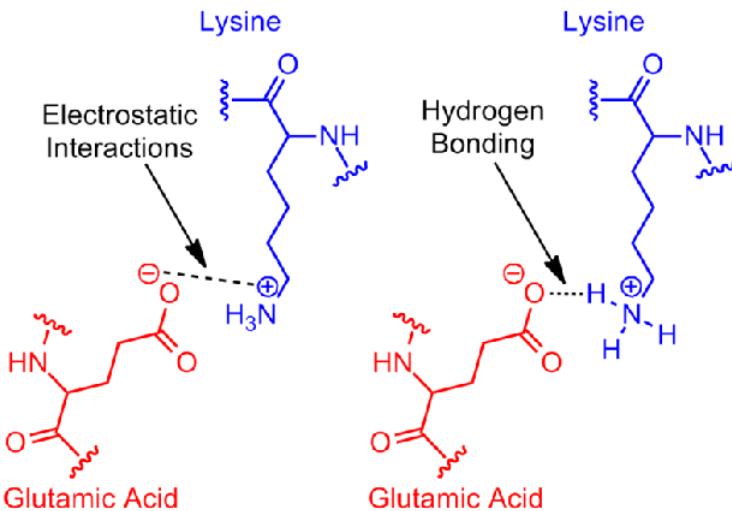
1. Draw the peptide at its most protonated form (low pH)
2. Calculate overall charge
3. Calculate the change in charge as pH rises (noting $pK_{a\delta}$)
4. Use the 2 $pK_{a\delta}$ s surrounding peptide at 0 charge → average



Intermolecular Interactions within Proteins

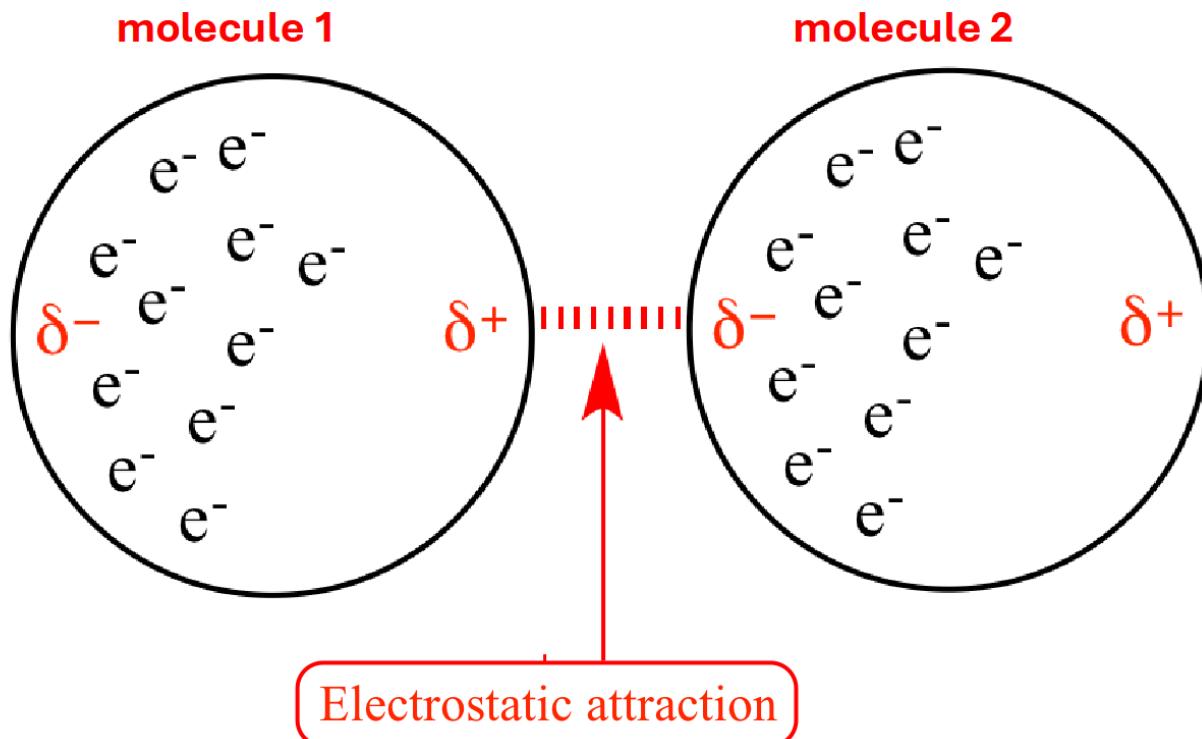
Ionic Bonds

- Strong electrostatic forces of attraction between oppositely charged ions
- Protein structures are formed by the interactions of amino acid side chains
- If an acidic chain and a basic side chain interact (e.g., Glu and Lys) **both an ionic interaction and a hydrogen bond will form**
- This is called a **salt bridge** - but most of the strength of this interaction comes from the opposing charges



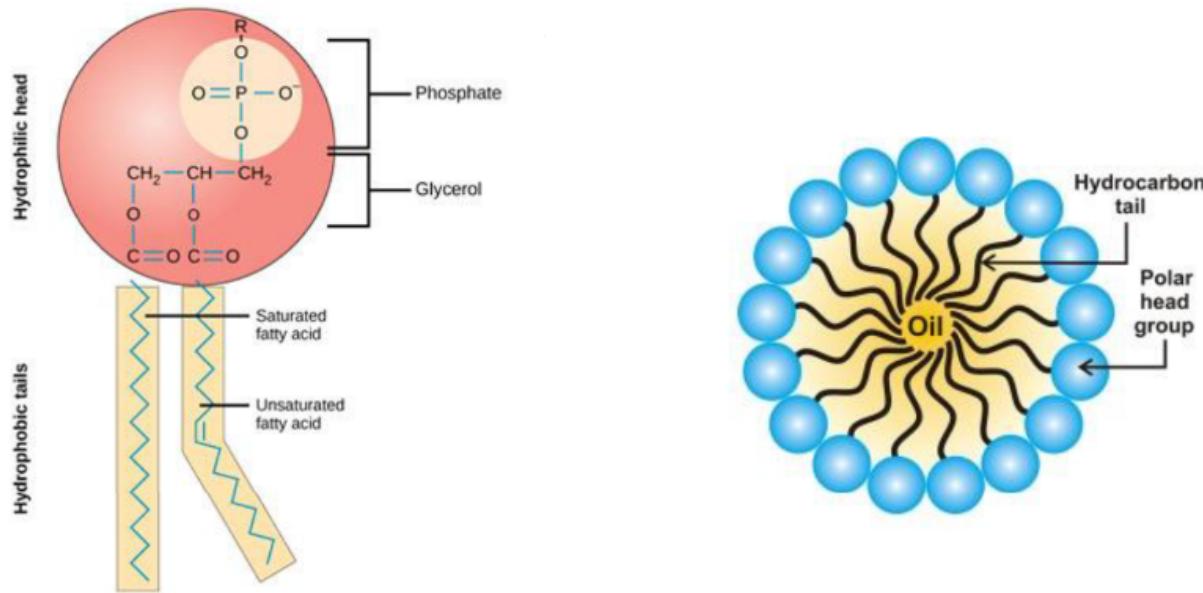
Induced Dipoles - London Dispersion Forces (LDFs)

- The weakest intermolecular force
- The LDF is a temporary attractive force that results when **the electrons in two adjacent atoms occupy positions that make the atoms form temporary dipoles**. This force is sometimes called an induced dipole-induced dipole attraction.
- This is (often) the predominant force between nonpolar molecules
- The London dispersion force is sometimes called a 'Van der Waals force.' Van der Waals force is a general term that describes any attractive intermolecular force between molecules and includes both the London dispersion force and the dipole-dipole force



Micelle - Where are the LDFs?

LDFs are primarily found among the hydrophobic (nonpolar) tails of the amphiphilic molecules (like fatty acids or detergents) that form the core of the micelle.



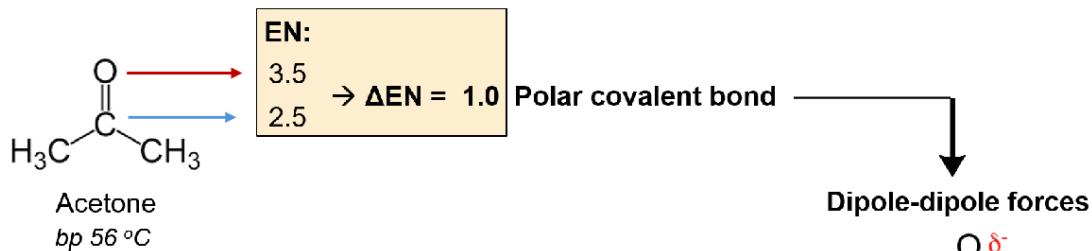
LDFs contribute to the stability of the micelle by keeping the nonpolar tails in close proximity in the micelle's core

Dipole-dipole interactions

Attractive forces that occur between polar molecules, where permanent dipoles are present due to the uneven distribution of electrons

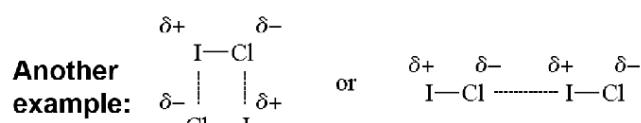
- Dipole-dipole interactions are generally stronger than London dispersion forces but weaker than hydrogen bonds or ionic interactions.

This creates a **polar bond**, where the O : δ^- , and the carbonyl C: δ^+ , resulting in a **permanent dipole** in the molecule

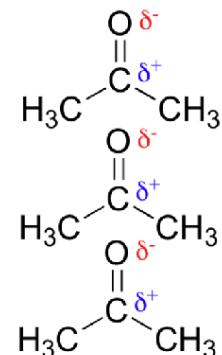


Molecules with polar bonds:

- Have **permanent dipoles** that can interact with neighboring dipoles (**electrostatics** between δ^+/δ^-)

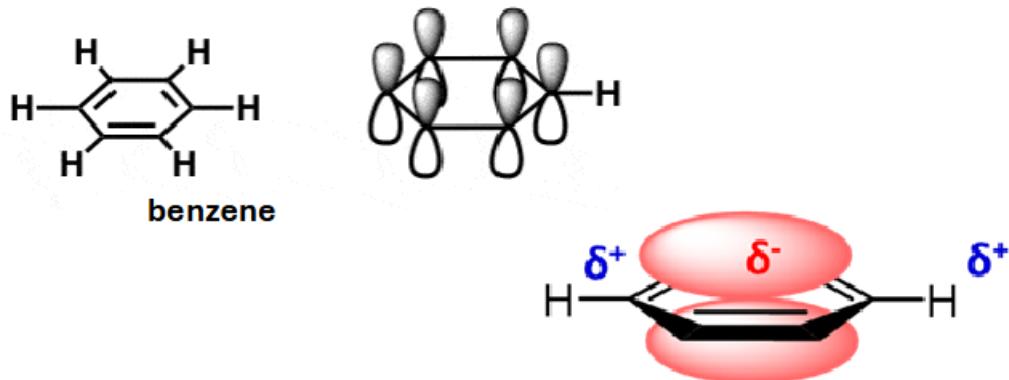


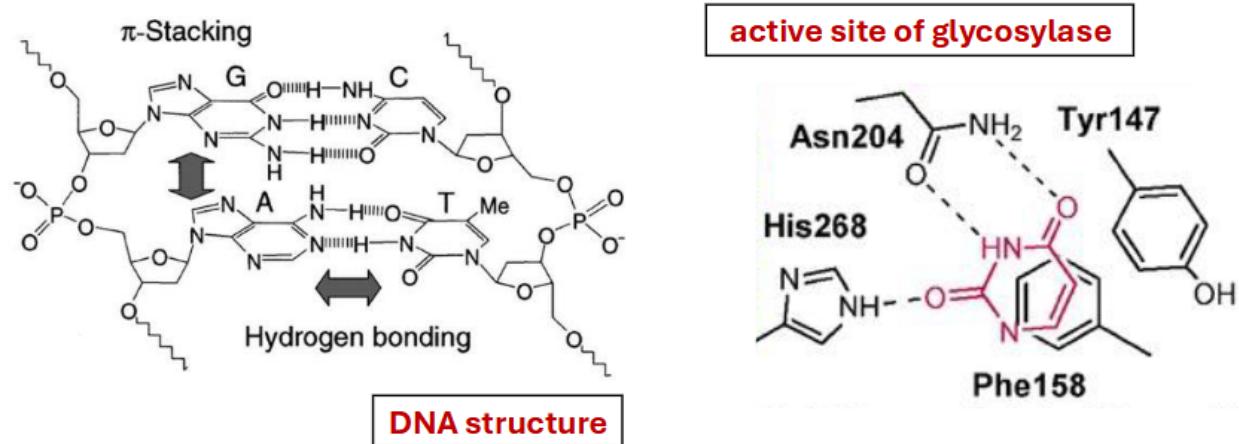
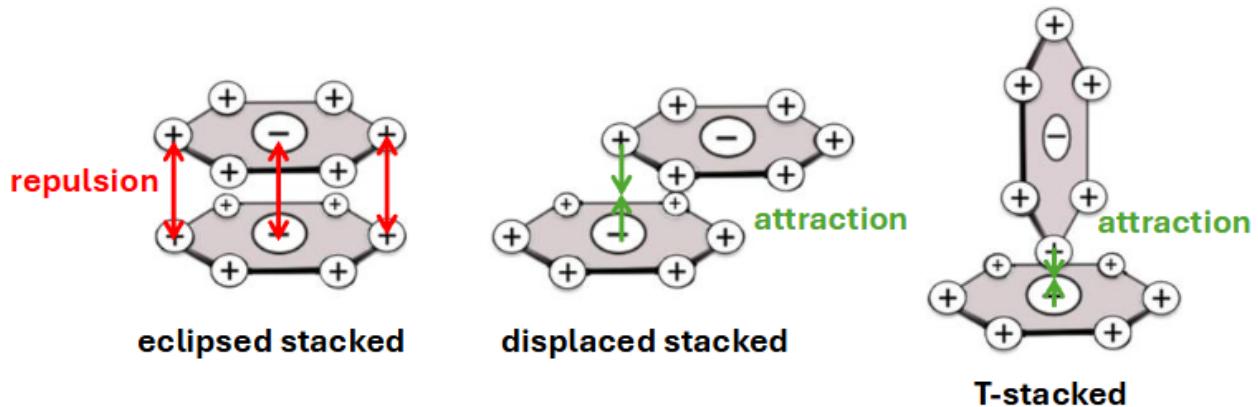
Iodine monochloride



π -stacking interactions

- Non-covalent interactions that occur between aromatic rings, where the delocalized π -electrons in these systems interact with each other.
- Cyclic aromatic compounds have π orbital rings stacked above and below the molecular structure.





Importance of Non-Covalent Interactions

1. Protein Folding and Stability: Hydrogen bonds, hydrophobic interactions, ionic interactions, and van der Waals forces contribute to the folding of proteins into their three-dimensional structures. These forces also help stabilize protein structures, such as maintaining the secondary, tertiary, and quaternary structures
2. DNA Double Helix: Hydrogen bonds between complementary bases (A-T and G-C) and $\pi-\pi$ stacking interactions between the aromatic bases help stabilize the DNA double helix structure
3. Membrane Formation: Hydrophobic interactions between the fatty acid tails of phospholipids drive the formation of lipid bilayers, which form the basic structure of cell membranes
4. Enzyme-Substrate Binding: Play a key role in the binding of enzymes to their substrates or cofactors, allowing for specificity and reversibility in catalysis
5. Molecular Recognition: Participate in the recognition of molecules such as ligands by receptors, or antigens by antibodies, facilitating many biological processes such as signal transduction and immune responses

The Thermodynamic Problem

- Living cells must constantly perform work, such as building complex molecules, transporting substances, and maintaining ion gradients, to stay alive. This requires an understanding of **thermodynamic principles** to predict which processes can occur naturally and how energy flows in biological systems

First Law of Thermodynamics

Energy Conservation: Energy cannot be created or destroyed, only transformed

- ΔG tells us how much energy is available to do work
- ΔH (enthalpy) represents the heat exchanged in a system
- Example: In cellular respiration, glucose releases energy ($\Delta H < 0$) that is used to produce ATP. Energy is transformed but conserved

Second Law of Thermodynamics

Entropy of the universe always increases in spontaneous processes

- ΔS (entropy) is a measure of disorder
- $\Delta G = \Delta H - T\Delta S$: A negative ΔG means a process is spontaneous and aligns with the second law (entropy increases)
- Even if a system's entropy decreases ($\Delta S < 0$), the surroundings must increase entropy for the reaction to be spontaneous
- Example: Protein folding is spontaneous ($\Delta G < 0$) even though it decreases system entropy, because heat is released, increasing the entropy of the surroundings

Third Law of Thermodynamics

As temperature approaches absolute zero, entropy approaches zero

- At very low temperatures, $T\Delta S$ becomes small, and ΔH dominates
- Spontaneous processes at low temperatures: Exothermic reactions ($\Delta H < 0$) are more likely to be spontaneous when entropy is low
- Example: Water freezing is spontaneous at low temperatures because it releases heat ($\Delta H < 0$) even though it decreases disorder ($\Delta S < 0$)

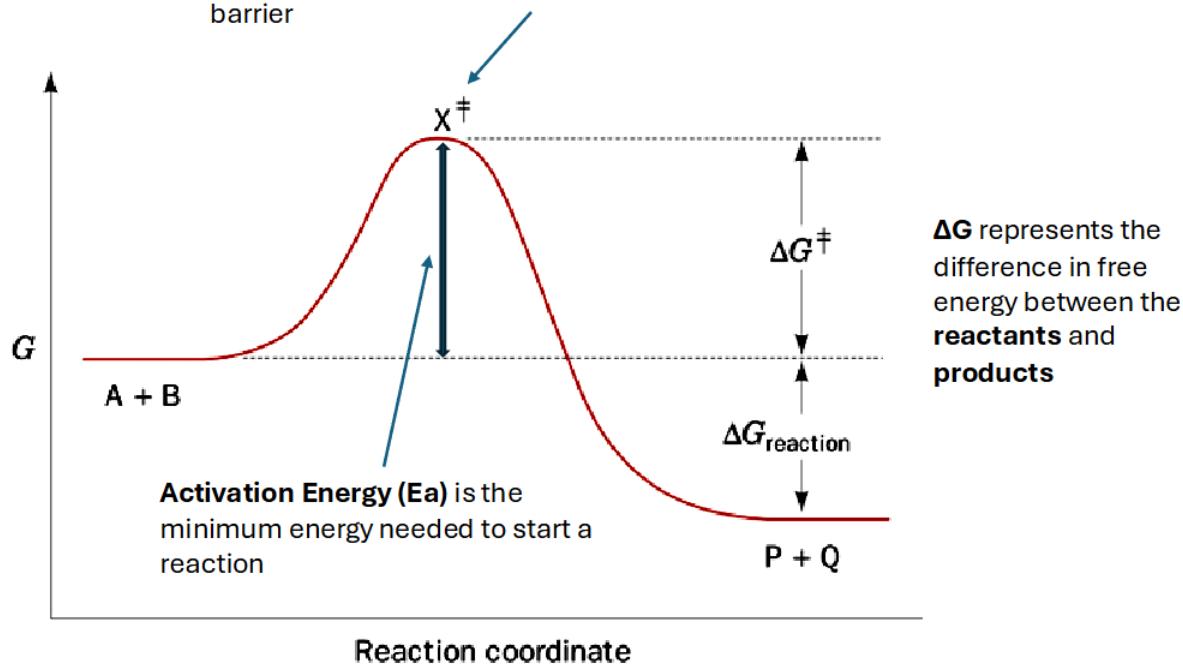
Gibbs Free Energy (G)

- **Gibbs free energy (G)** represents the maximum amount of energy available to perform work in a system. It's the key to understanding how cells manage energy.
- The **Gibbs free energy change (ΔG)** tells us the difference in available free energy between reactants and products. It determines whether a process will occur spontaneously (i.e., without needing an external energy input).
- ΔG determines the spontaneity of a process

Reaction Coordinate Diagram

- A reaction coordinate diagram shows the progress of a reaction vs the overall free energy (G) as it proceeds
 - Energy flowing downhill is favorable, uphill is unfavorable.

Transition state is the highest energy point in the reaction, located at the peak of the activation energy barrier

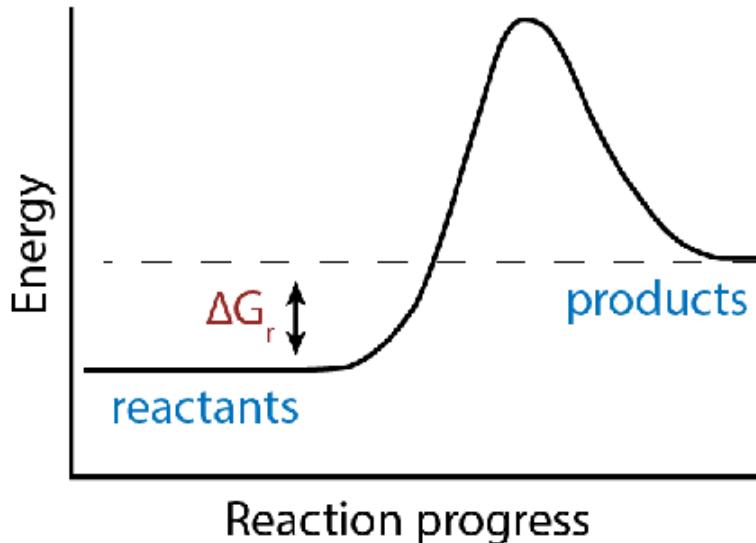


Spontaneity and ΔG : What it Really Means

- If $\Delta G > 0$, the process is non-spontaneous (it requires an input of energy to occur). This type of reaction is called **endergonic**.
 - **Example:** The synthesis of glucose from CO_2 and water during **photosynthesis** is endergonic ($\Delta G > 0$). Plants need energy from sunlight to drive this process because it doesn't happen spontaneously
- **Why:** It makes sense - if the ΔG is positive, how could you get more work out of a system without putting energy into it? In biological systems, cells **couple** endergonic reactions with exergonic ones to make them proceed.

Endergonic reaction

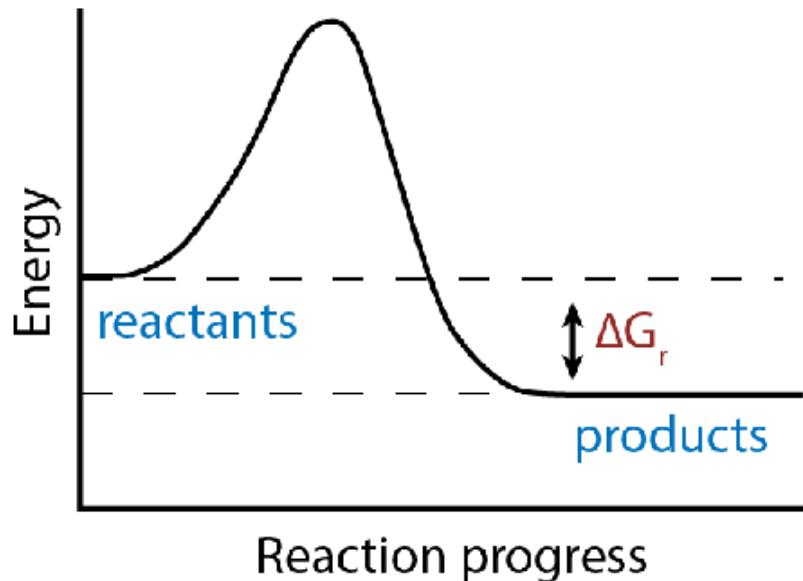
$$\Delta G_r > 0$$



- If $\Delta G < 0$, the process is spontaneous (it can occur naturally without additional energy input). This is called an **exergonic** process.
 - Example: The breakdown of glucose during **cellular respiration** is exergonic ($\Delta G < 0$). When glucose is metabolized into CO_2 and H_2O , energy is released that cells can use to drive other processes (like making ATP).
- Why: Energy is released when bonds in glucose are broken, allowing the system to do work - such as moving ions, powering cellular machinery, or synthesizing molecules. The process moves towards equilibrium, where the system is most stable.

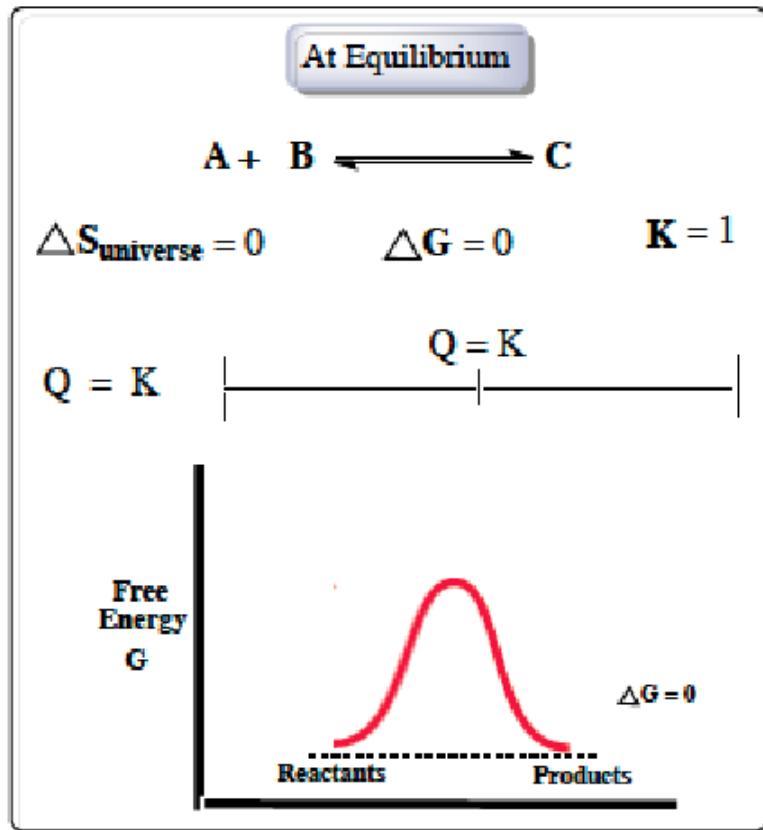
Exergonic reaction

$$\Delta G_r < 0$$



However

- In **biological systems**, **true equilibrium** is rarely reached because cells operate under a **steady state** rather than equilibrium
- In a steady state, the concentrations of molecules like glucose and ATP are kept constant, but continuous input of reactants (like glucose) and removal of products (like CO_2) allow work to be performed
- If equilibrium were reached, the system would be in a **low-energy state**, and no further work could be done. This is why biological systems keep reactions away from equilibrium to continue performing work
- If $\Delta G = 0$, the system is at **equilibrium**, meaning there's no net change in the reactants or products, and no work can be done. This happens when the forward and reverse reactions occur at the same rate.
 - **Example:** Consider **ATP** in equilibrium with **ADP** and inorganic phosphate (P_i) in a cell. If the reaction reaches equilibrium, no energy would be released or consumed, making it impossible for the cell to do any work involving ATP.



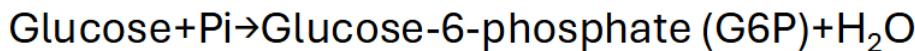
How Cells Use ΔG to Perform Work: Reaction Coupling

- Cells often use **reaction coupling** to make non-spontaneous processes occur. By coupling a reaction with **positive ΔG** (endergonic) to one with **negative ΔG** (exergonic), the overall process can still be spontaneous
 - Example:** The phosphorylation of glucose in the first step of **glycolysis** ($\Delta G > 0$) is coupled with the **hydrolysis of ATP** to ADP and P_i ($\Delta G < 0$). Together, these reactions allow glucose to be phosphorylated and the overall process to move forward with a net negative ΔG
 - Key Insight:** Cells use ATP as sort of "energy currency" to drive many otherwise non-spontaneous reactions. ATP hydrolysis ($\Delta G < 0$) provides the energy necessary to make those reactions happen

Coupled Reactions

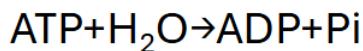
- How does biochemistry pull off an anabolic reaction?
 - By coupling it with an energetically favorable reaction (the classic example is the hydrolysis of ATP)
 - i.e., coupling an endergonic reaction with an exergonic reaction

phosphorylation of glucose

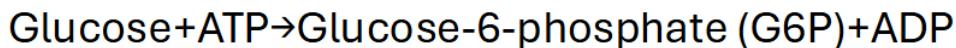


ΔG° for this reaction = **+13.8 kJ/mol** (non-spontaneous, $\Delta G > 0$)

hydrolysis of ATP



ΔG° for this reaction = **-30.5 kJ/mol** (spontaneous, $\Delta G < 0$)



$$\Delta G^\circ_{\text{total}} = (+13.8 \text{ kJ/mol}) + (-30.5 \text{ kJ/mol}) = -16.7 \text{ kJ/mol}$$

spontaneous

Quantifying ΔG - Enthalpy

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

- H, enthalpy is the heat content locked in a system
- ΔH is the change in enthalpy

Equivalent to the difference between bonds/interactions formed and bonds/interactions broken

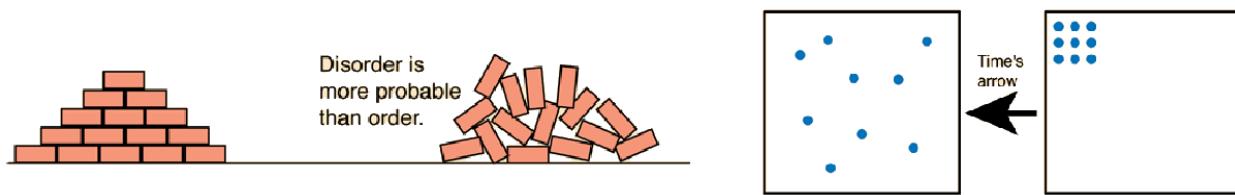
- **Bond formation** typically **releases energy** because when atoms form bonds, they move to a lower energy state. This means that energy is released as the atoms become more stable
- **Bond breaking** generally **requires energy input** because energy must be provided to overcome the stability of the bond and separate the atoms, moving them to a higher energy state

Quantifying ΔG - Entropy

- S, entropy is the degree of disorder in a system
- ΔS is the change in entropy

Equivalent to disorder of products minus disorder of reactants

- Systems tend toward disorder



Why Temperature Matters: ΔG and Temperature Dependency

- ΔG is temperature-dependent, as shown by the equation

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

As temperature (T) increases, the **entropy** term ($T\Delta S$) becomes more significant:

- **Example:** At low temperatures, the **melting of ice** is non-spontaneous ($\Delta G > 0$) because the $T\Delta S$ term is too small to offset the positive ΔH (heat absorption). But as temperature increases, the $T\Delta S$ term becomes large enough to make the overall ΔG negative, and the ice melts spontaneously.
- In biochemistry, temperature can impact reaction spontaneity as well. Consider **protein denaturation**: At high temperatures, proteins unfold because the entropy term ($T\Delta S$) becomes large, overpowering the stabilizing interactions that keep the protein folded.

ΔG under different conditions

- Whether a reaction is spontaneous or not can depend on temperature
 - Entropically driven ($\Delta H > 0, \Delta S > 0$)
 - Enthalpically driven ($\Delta H < 0, \Delta S < 0$)

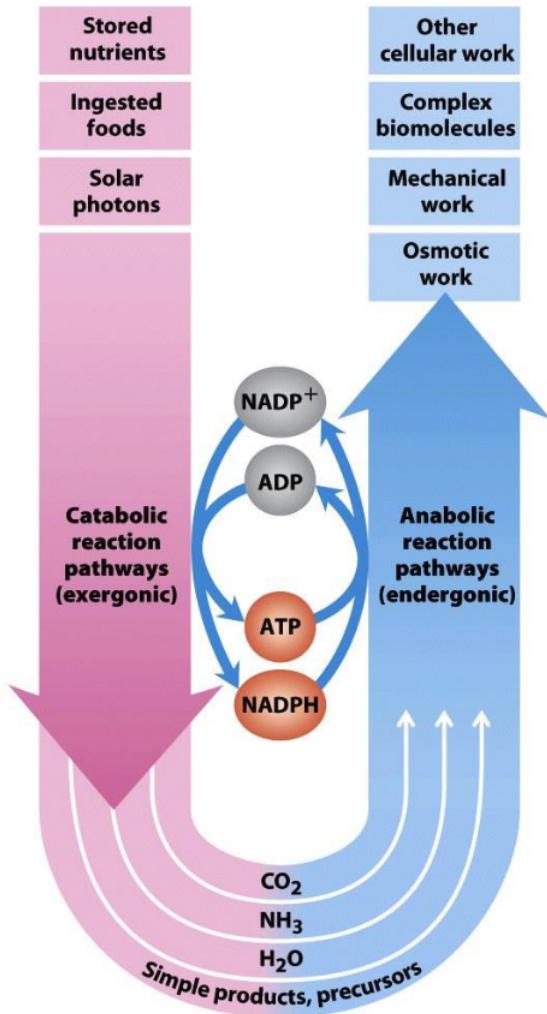
	$\Delta H < 0$	$\Delta H > 0$
$\Delta S > 0$	Spontaneous at all T ($\Delta G < 0$)	Spontaneous at high T (when $T\Delta S$ is large)
$\Delta S < 0$	Spontaneous at low T (when $T\Delta S$ is small)	Non-spontaneous at all T ($\Delta G > 0$)

Key Points

- **Exergonic Reactions ($\Delta G < 0$):** Spontaneous and release energy that can be used to drive work in the cell. Examples include **ATP hydrolysis**, **glucose oxidation**, and **protein folding**
- **Endergonic Reactions ($\Delta G > 0$):** Non-spontaneous and require an input of energy. In cells, they are often coupled with exergonic processes to proceed. Examples include **photosynthesis**, **DNA synthesis**, and **fatty acid synthesis**
- **Coupling of Reactions:** Many cellular processes rely on the coupling of endergonic and exergonic reactions. For example, **glucose phosphorylation** has a positive ΔG (+13.8 kJ/mol), but it proceeds because it is coupled with **ATP hydrolysis** ($\Delta G = -30.5$ kJ/mol), resulting in a net negative ΔG for the overall process
- **Temperature and ΔG :** The values of ΔG can vary depending on temperature, reactant concentrations, and the cellular environment. The **standard free energy change (ΔG°)** assumes standard conditions (1M concentrations, 25°C, 1 atm pressure), but actual ΔG values in cells can differ due to non-standard conditions

Biology and ΔG

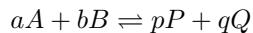
- Generating and maintaining order requires work and energy
- How do living organisms fight against entropy?
- They use **catabolic** pathways to harvest external sources of energy (larger molecules to smaller molecules)
 - e.g., $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + \text{energy}$
- And they use **anabolic** pathways to build up functional biomolecules (smaller molecules to larger molecules)
 - e.g., formation of polypeptides



Chemical Equilibrium

- Another way of viewing the thermodynamic endpoint of a reaction is by using an **equilibrium constant** (K_{eq})
- K_{eq} equals the ideal ratio of products to reactants
- Depending on conditions (like temperature) K_{eq} has different values
- At equilibrium, a reaction is macroscopically static, but microscopically dynamic.
- At equilibrium, the forward rate and reverse rate of the reaction are equivalent
 - i.e., nothing happens

Generic reaction formula:



Reaction quotient Q :

$$Q = \frac{[P]^p [Q]^q}{[A]^a [B]^b}$$

- If $Q < K$: The concentration of products is too low, and the reaction will proceed in the **forward direction** to reach equilibrium. Reaction drives \rightarrow

- If $Q > K$: The concentration of products is too high, and the reaction will proceed in the **reverse direction** to reach equilibrium. Reaction drives \leftarrow
- If $Q = K_{eq}$: The system is already at equilibrium, and no net change will occur in the concentrations of reactants or products.

The reaction quotient (Q) is similar to K_{eq} , but it is used to determine the state of the reaction at any point, not just at equilibrium.

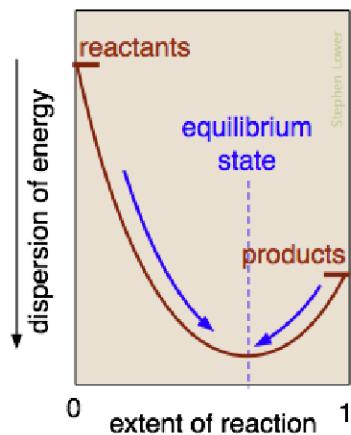
Equilibrium and ΔG

- An equilibrium constant (K_{eq}) and ΔG both deal with the final thermodynamic state of a system
 - As such there are several equations that describe the relationship between them
- ΔG° is the standard free energy change (relates directly to K_{eq})
- ΔG is the free energy change for a given moment ($\Delta G = 0$ at equilibrium)
 - $\Delta G < 0$, reaction moves towards products (same as $Q < K$)
 - $\Delta G > 0$, reaction moves towards reactants (same as $Q > K$)

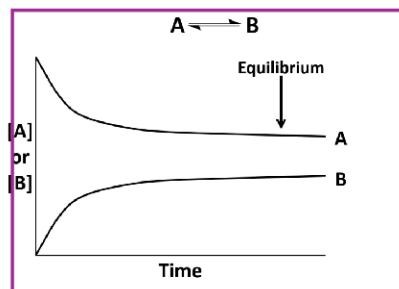
$$\begin{aligned}\Delta G &= \Delta G^\circ + RT \ln Q \\ \Delta G^\circ &= -RT \ln K \\ \Delta G &= RT \ln \frac{Q}{K}\end{aligned}$$

Chemical Equilibrium

Chemical equilibrium is the state in a reversible chemical reaction where the **rate of the forward reaction** equals the **rate of the reverse reaction**. At equilibrium, the concentrations of the reactants and products remain constant over time, though the reaction continues to occur in both directions.



Essentially, there is no net change in the amounts of reactants and products, as the system has reached a balance



Equilibrium vs. Steady state

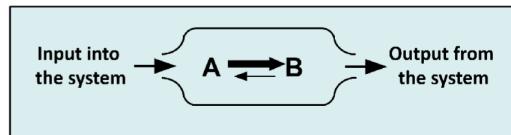
Equilibrium

- Time invariant concentrations
- Forward and reverse rates are equal
- No differences between environment and system (equally disordered)



Steady State

- Hypothetically, time invariant concentrations
- Input into the system is equivalent to output from the system
- System is in higher state of order than surroundings



- **Equilibrium:** A state where the system is at its lowest energy, no net change occurs, and the system is stable with no energy flow. Common in **closed systems** and **reversible reactions**.
- **Steady State:** A dynamic state where concentrations of substances are constant but maintained by continuous flows of energy and matter. This occurs in **open systems** and is typical of **living systems** like metabolism, which require continuous energy input to sustain life.

Key Characteristics of Steady State

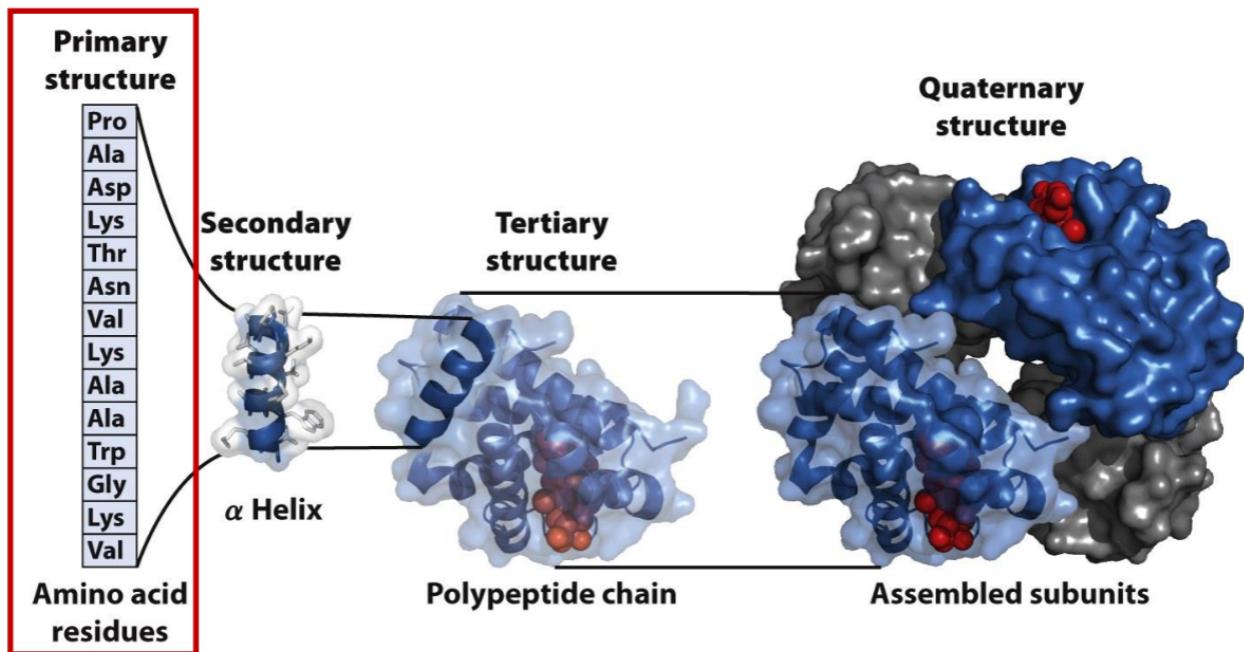
- **Dynamic Process:** Even though the concentrations or amounts of certain substances remain constant, the system is not static. Processes such as reactions or flows are continuously happening
- **Input and Output:** Steady state requires continuous input and output of materials and energy to maintain constant conditions
- **Energy Requirement:** Many steady-state systems, especially in biology, require a constant energy supply to maintain steady conditions. For example, maintaining ATP levels in cells requires continuous metabolic activity

Body Temperature Regulation

- **What happens:** The human body maintains a constant internal temperature of about **37°C** (98.6°F), despite changes in the external environment (e.g., cold or hot weather)
- **Steady state:** The body is constantly producing heat (through metabolism) and losing heat (through sweating, radiation, etc.). Even though these processes are dynamic, the **internal temperature** remains steady

The internal temperature is kept constant, but energy (in the form of heat) is continuously produced and lost. This makes it a steady state.

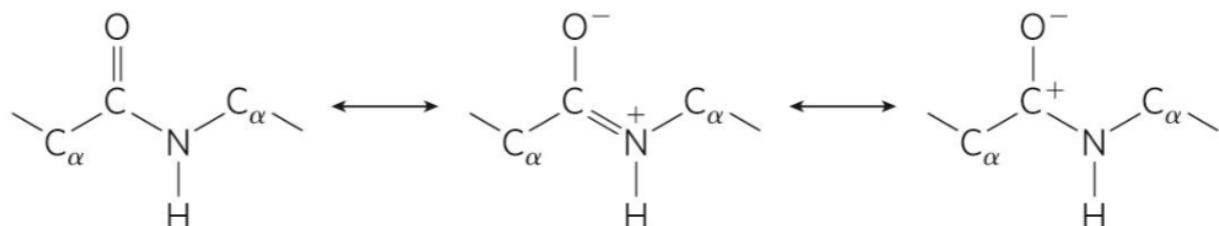
Protein Structure



- Proteins are formed of chains of amino acids (peptides).
- Review the section Peptides and Proteins above.

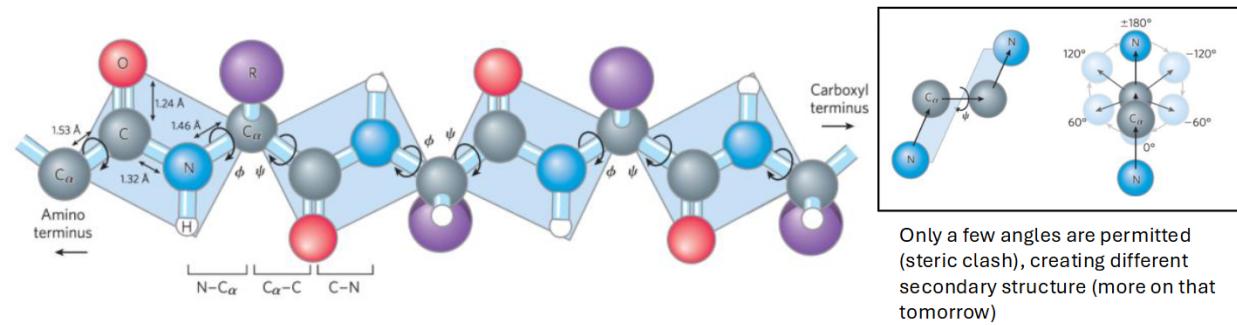
The Peptide Bond is Rigid and Planar

- 3 covalent bonds separate α carbons of adjacent amino acid residues: $C_\alpha—C—N—C_\alpha$
- double-bond character due to resonance between the carbonyl oxygen and the amide nitrogen
- partial negative charge and partial positive charge sets up a small electric dipole



Polypeptide Chains - Primary Structure

- 3 covalent bonds separate the α carbons of adjacent amino acid residues: $C_\alpha—C—N—C_\alpha$
 - The N- C_α bond and the C_α -C bond can be described with dihedral angles phi Φ and psi Ψ (why are we ignoring C-N? See below.)



Why are we ignoring C-N?

- The **C-N bond** in a polypeptide chain is typically **ignored** when considering protein folding and secondary structure formation because it has **partial double-bond character**. This is due to **resonance** between the lone pair of electrons on nitrogen and the adjacent carbonyl group ($\text{C}=\text{O}$). This resonance restricts rotation around the **C-N bond**, making it **planar and rigid**, and thus not as flexible as the **N-C_α** (ϕ , Φ) and **C_α-C** (ψ , Ψ) bonds, which allow for more rotational freedom.
- The C-N bond is an ω (omega) bond.

Dihedral Angles Define Peptide Conformations

There are 3 dihedral angles:

- ϕ (phi) = between -180 and +180 degrees
- ψ (psi) = between -180 and +180 degrees
- ω (omega) = -180 and +180 degrees for trans
 - The peptide bond is almost always (99.6% of the time) in the trans configuration, constraining ω to a value of $-180^\circ < \omega < +180^\circ$. For a rare cis peptide bond, $\omega = 0^\circ$.

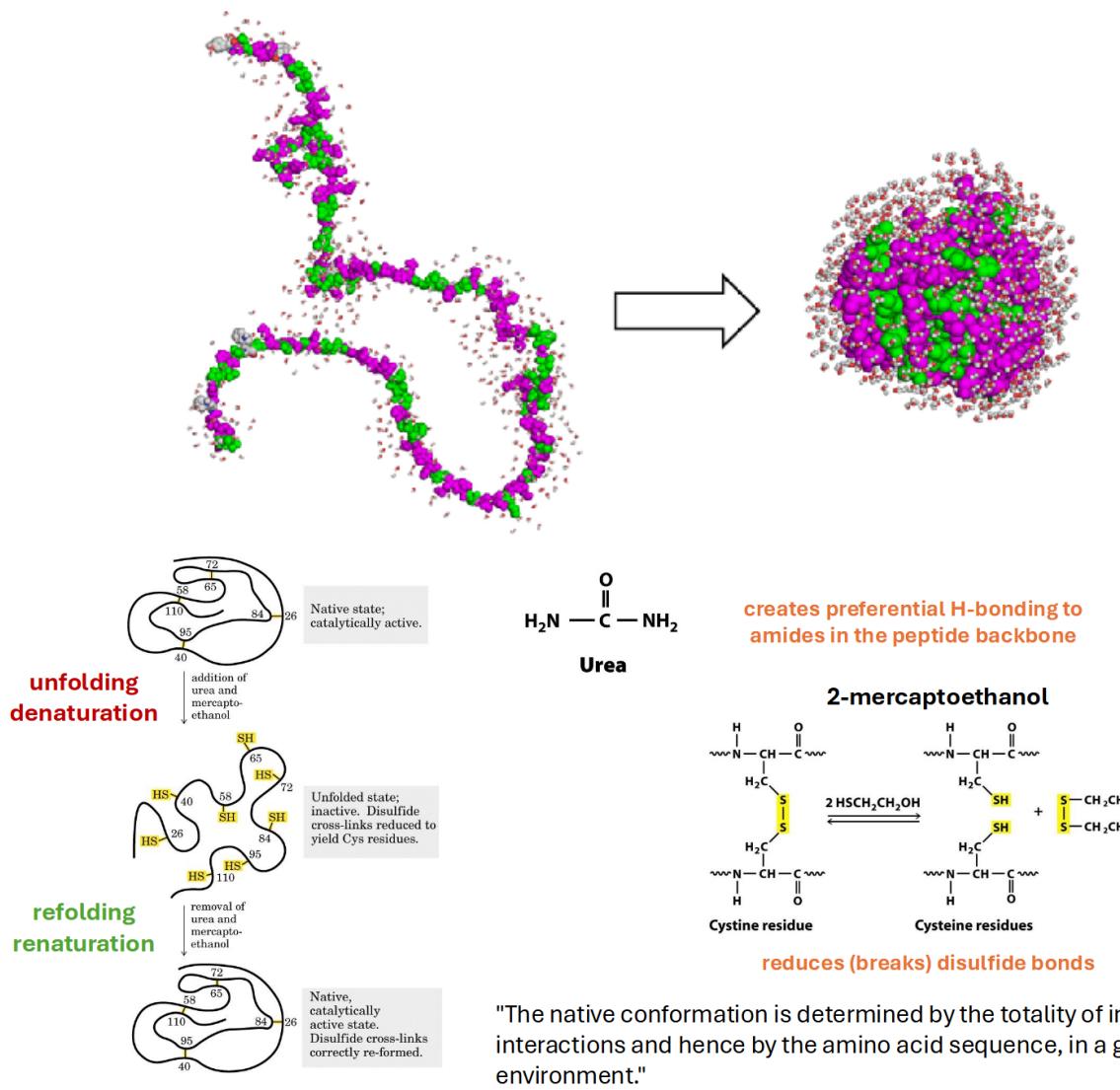
Prohibited Conformations

Many ϕ (phi) and ψ (psi) values are prohibited by steric interference

- For example, ϕ and ψ cannot both be 0 degrees.

How does a loose polypeptide chain become a functional biomolecule?

- Christian Anfinsen demonstrated that proteins could spontaneously refold after disruption.
- Anfinsen's Theorem:**
 - The information determining the tertiary structure of a protein is present in its sequence
 - i.e., going from protein chain to functional structure requires no external input.

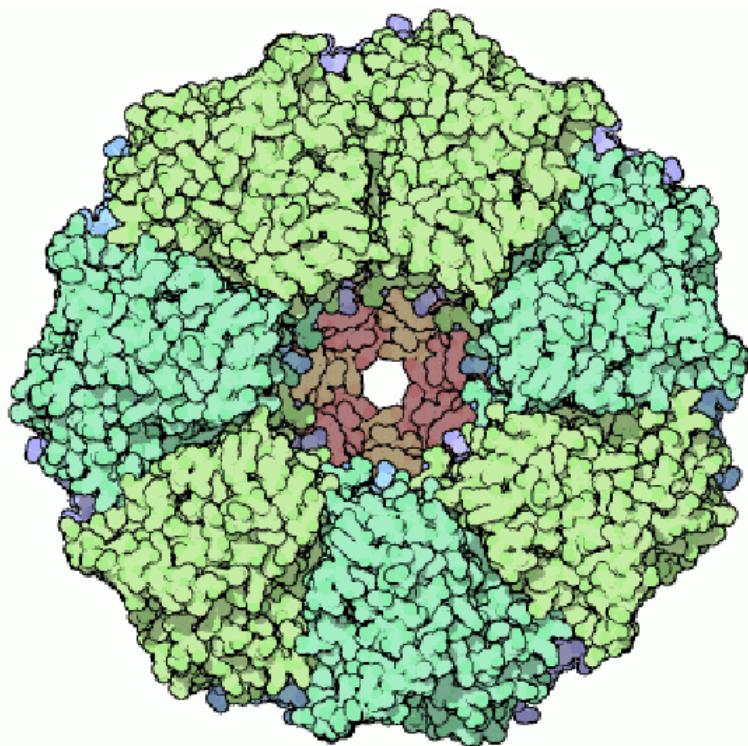


"The native conformation is determined by the totality of interatomic interactions and hence by the amino acid sequence, in a given environment."

- Christian Anfinsen, 1972

Challenges to Anfinsen

This is a **chaperone**.



- Chaperone proteins help assist other proteins in their folding (or re-folding)
- Often expressed under certain stress conditions, like heat shock
- While chaperones contest a hard-line Anfinsen dogma, the broader point still stands - proteins fold based on the thermodynamics of their sequence
- Besides the assembly of protein complexes and de novo folding of nascent polypeptides, chaperones play a role in protein translocation across membranes, stabilization of protein-protein interactions, and ribosome biogenesis. Regardless of their exact function, different chaperones provide assistance in the same assignment: **proteins have to maintain their designated function in the right place at the right time**

More Questions - Levinthal's Paradox

- Other experiments like Anfinsen's raised more questions
 - Denatured proteins refold in 0.1-1000 seconds
 - Take a hypothetical protein with 100 amino acids
 - Due to allowed rotations, amino acids can have 3 conformations
 - That's roughly 3^{100} possibilities ($\approx 5 \times 10^{47}$)
- If the protein can visit one conformation every picosecond (10^{-12} s), searching every possibility would take $5 \times 10^{47} \times 10^{-12}$ seconds, or 1.6×10^{28} years.