

# One-semester GOB

## Part 4: Biochemistry

Part 1: atoms and compounds  
Part 2: organic chemistry  
Part 3: aqueous solutions  
Part 4: biochemistry

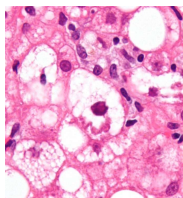
## 3 ingredients to support life

1. Heredity
2. Cell structure
3. Driving unfavorable reactions

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What makes a human cell different from a bacterial cell?



What makes a brain cell different from a liver cell?

What makes a normal cell different from a cancer cell?

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## Amino acids and proteins

- Proteins are the biomolecules that “do everything” in our bodies.
- Amino acids are the building blocks of proteins
- Proteins function as follows:
  - They transport oxygen in the blood.
  - They are the primary components of skin and muscle.
  - They work as defense mechanisms against infection.
  - They serve as biological catalysts called enzymes.
  - They control the metabolism of hormones.

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How does the cell know what proteins to make?

## The Central Dogma

(of molecular biology)



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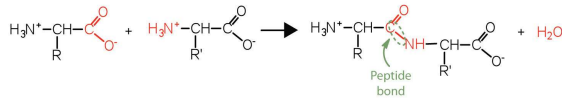
## Amino acid chemistry in a nut shell

- Every amino acid contains two common functional groups, an **amine** and a **carboxylic acid**, bonded to a central carbon atom known as the  **$\alpha$  carbon**.
- Amino acids undergo a condensation reaction forming a **peptide bond** between the carboxylic acid of one amino acid and the amine of the next. This is called the **backbone structure**.
- Aside from these common features, each of the **20 natural amino acids** such as phenylalanine or glutamine carries a unique side chain attached to the alpha carbon. The side chains determine structure and function.

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## Protein Formation

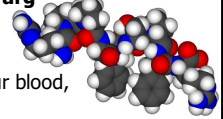
- When two amino acids condense, a dipeptide is formed. A protein is a string of amino acids.
- The carboxylate ion ( $\text{COO}^-$ ) of one amino acid reacts with the protonated amine ( $\text{NH}_3^+$ ) of a second amino acid.
- A water molecule is lost and an amide bond is formed between the two amino acids.



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## Protein sequence

- Structures are always written from N-terminus to C-terminus.
- Bradykinin is a nine-amino acid peptide  
**arg-pro-gly-phe-ser-pro-phe-arg**

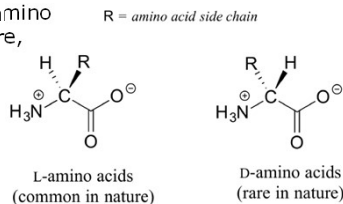


- This peptide lowers the pressure of our blood, and has other roles.
- If we reverse the order of amino acids, the resulting peptide is not bradykinin, and does not share its properties.
- arg-phe-pro-ser-phe-gly-pro-pro-arg**
- Database of human proteins:  
<http://www.ncbi.nlm.nih.gov/protein>

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## Amino acids are chiral

- The **L-amino acids** are the building blocks for proteins. Some D-amino acids occur in nature, but not in proteins.

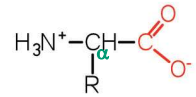


- As a consequence, **proteins** (including enzymes and receptors) **are chiral**.

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## Amino acid chemistry in a nut shell

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- Aside from these common features, each of the **20 natural amino acids** such as phenylalanine or glutamine carries a unique side chain attached to the alpha carbon. The side chains determine structure and function.



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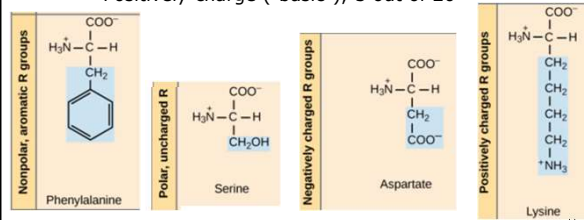
## Side chains (R groups)

- The side chain gives each amino acid its unique identity and characteristics.
- Twenty amino acids are found in most proteins.
- We have studied most of the functional groups of the side chains
  - Alkanes
  - Aromatics
  - Alcohols
  - Carboxylic acids
  - Some new functional groups
    - Sulfur-containing: Thioethers, Thiols
    - Nitrogen-containing: Amines, Amides

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## Polarity and charge of side chains

- The functional groups divide the amino acids into the following four categories:
  - Nonpolar, 9 out of 20
  - Polar but not charged, 6 out of 20
  - Negatively charged ("acidic"), 2 out of 20
  - Positively charged ("basic"), 3 out of 20



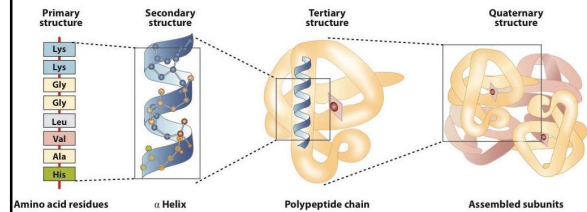
## Essential amino acids

- Val, Leu, Ile, Phe, Met, Trp, Thr, His, Lys, Arg
- essential in children, but not adults: Arg, His
- Nonessential amino acids can be synthesized in the body from essential amino acids.
- Proteins that contain all the essential amino acids are called complete proteins. Soybeans and most proteins found in animal products are complete proteins.
- Some plant proteins like those in rice and beans are incomplete proteins because they lack one or more essential amino acid. Complete proteins can be obtained by combining foods, e.g. rice and beans (mexican cuisine does that).

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## Structure: atoms in 3D-space

- Proteins adopt a specific structure in their functional (native) state
- We use four levels to describe protein structure:



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## Primary Structure

- The primary structure is the order in which the amino acids are joined together by peptide bonds that forms the backbone from N-terminus to C-terminus. The amino acid side chains are substituents to this backbone.
- For example, the eight amino acid peptide angiotensin II, which is involved in normal blood pressure regulation in humans, has the following amino acid sequence:  
Asp—Arg—Val—Tyr—Ile—His—Pro—Phe
- Any other order of amino acids in this peptide would result in a peptide that would not function as angiotensin II.

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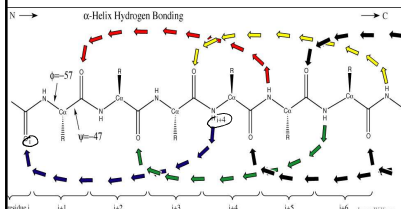
## Secondary Structure

- The secondary structure of a protein describes repeating patterns of structure within the three-dimensional structure of a protein.
- The three most common secondary structures are:
  - Alpha helix ( $\alpha$  helix)
  - Beta-pleated sheet ( $\beta$ -pleated sheet)
  - Turn
- Secondary structure is defined by the path of the main chain and by the hydrogen bonds of the main chain

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## Alpha helix

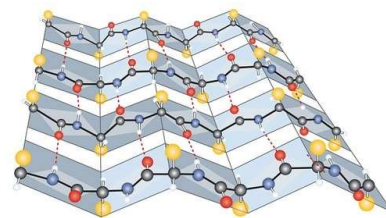
- Hydrogen bond from amide hydrogen to carbonyl four residues ahead
- Side chains stick out in all directions



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## Beta sheet

- The  $\beta$ -pleated sheet is an extended structure in which segments of the protein chain (strands) align to form a zigzag structure.
- Hydrogen bonds from one strand to the next
- Side chains stick out above and below the sheet



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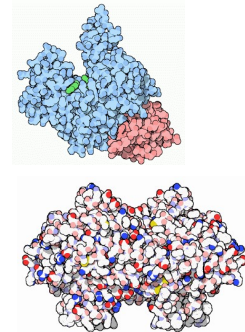
## Tertiary Structure

- The tertiary structure is the three-dimensional structure of the protein.
- It involves twisting and folding of the polypeptide chain
- The main reason for folding is because water prefers not to bind to hydrophobic side chains
  - The nonpolar amino side chains end up in the interior of the protein away from the aqueous environment.
  - The polar side chains appear on the surface of the protein since they are attracted to the aqueous surroundings.

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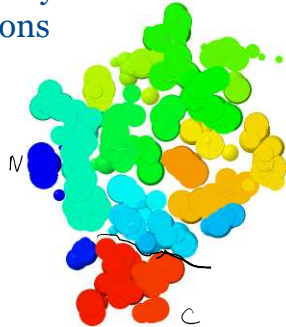
## Proteins fold into a unique structure

- Actin/profilin (1hlu)
- Cyclooxygenase



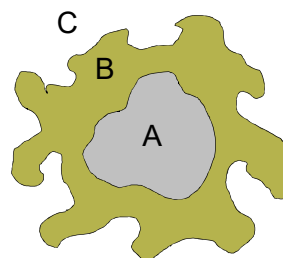
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Tight packing excludes water and allows many weak non-covalent interactions



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## Proteins: Inside and outside



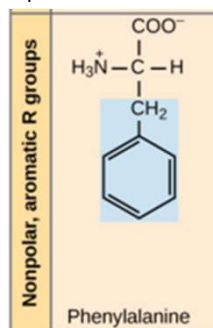
A: Hydrophobic core  
B: Protein surface  
C: Water (solvent)

Slice through a globular protein

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## Nonpolar

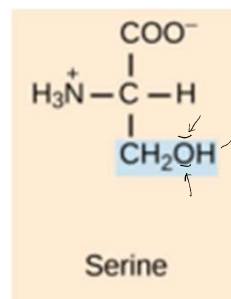
- Form the hydrophobic core of the protein



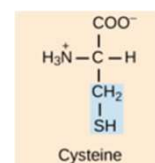
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## Polar

- Found at the surface of the protein



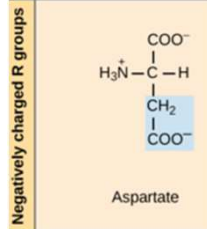
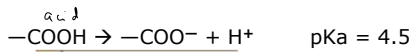
- Special case cysteine:
  - Forms disulfide bridges and becomes non-polar:  
 $-\text{SH} + \text{HS}- \rightarrow -\text{S}-\text{S}-$



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## Negatively charged

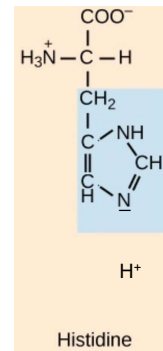
- Called acidic side chains, but occur as the conjugate base
- At which pH is Asp neutral?



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## Positively charged

- Lys and Arg are almost always positively charged
- Histidine is a special case: pKa = 6.5, easy to add or remove proton at near neutral pH
  - Often found in active sites of enzymes

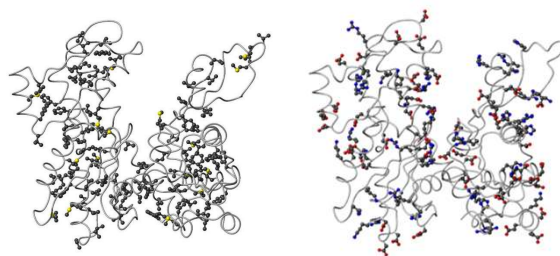


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## Hydrophobic core vs. surface

Hydrophobic: Phe, Leu, Val, Ile, Met

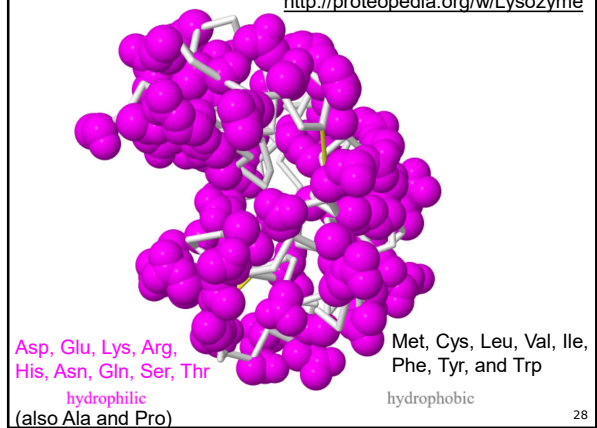
Charged: Arg, Lys, His, Asp, Glu



Actin PDB-ID 1HLU

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<http://proteopedia.org/w/Lysozyme>



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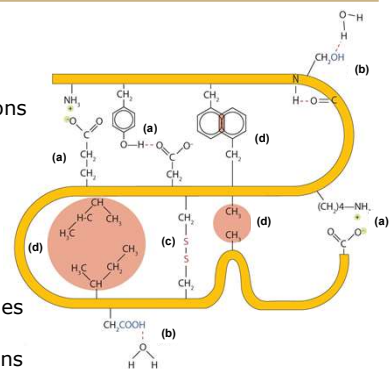
## Native and denatured state

- Native state: the functional conformation found inside the cell (and under conditions similar to cellular ones)
- A protein has a defined conformation in the native state: protein molecules with the same sequence have the same shape, same residues close to each other, same properties
- Denatured state: non-functional conformations (many different ones) found under conditions different from those inside the cell

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## Forces holding proteins together

- H-bonds and ionic interactions within the protein
- H-bonds between the protein and aqueous environment
- Disulfide bridges
- Hydrophobic core interactions



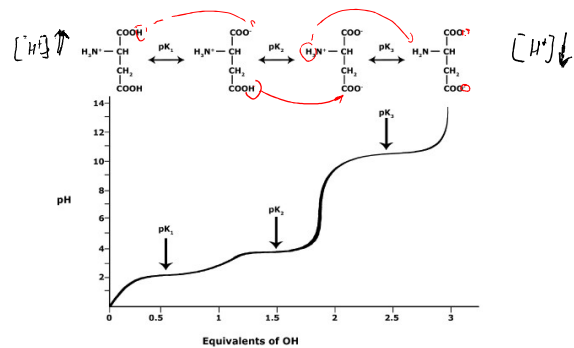
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## Denaturation of Proteins

- Denaturation is a process that disrupts secondary, tertiary, and quaternary structures, but not primary structure
- Denatured protein are inactive**
- Frying an egg is an example of denaturation by heat. Increased temperature makes atoms move faster, disrupting the orderly protein structure
- Other denaturing agents:
  - pH (alters side chain charges)
  - Reductants (disulfide bonds)
  - Soap (breaks hydrophobic core)
  - Mechanical agitation

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## Different charges at different pH values



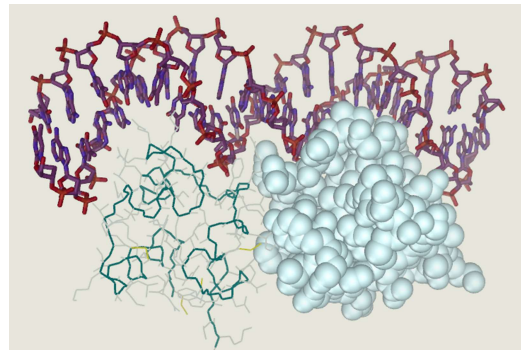
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## Protein function: “activity”

- Proteins bind** molecules, including other proteins, small molecules, DNA, RNA and carbohydrates
- Proteins catalyze reactions** and thus determine what gets made and broken down in the body. Proteins with catalytic activity are called enzymes
- Protein undergo conformational change** in response to binding, allowing them to act as molecular machines with hinges and levers (e.g. muscle action or molecular pumps), and to process information (“if glucose levels are high and there is insulin in the blood, ...”)

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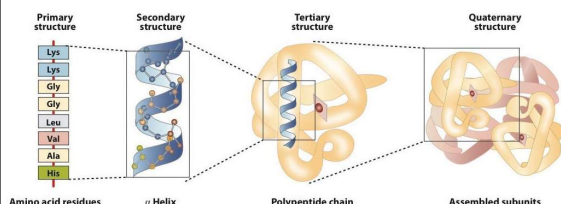
## Proteins bind ... DNA (gene regulation)



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## Proteins bind ... protein (e.g. hemoglobin)

- Quaternary structure: protein chains bind to each other to form functional protein



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## Quaternary Structure

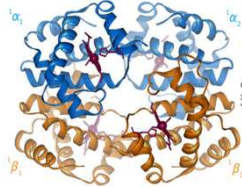
- Not all proteins have a quaternary structure.
- The quaternary structure is two or more polypeptide chains interacting to form a biologically active protein.
- Hemoglobin, an oxygen transport protein, is an example of a protein with a quaternary structure.
  - It consists of four polypeptide chains or subunits.
  - It has two identical alpha subunits and two identical beta subunits.
  - All four subunits must be present for the protein to function as an oxygen carrier.

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## Hemoglobin

- Hemoglobin transports oxygen in blood.
- It is composed of two alpha subunits and two beta subunits held together by hydrogen bonds, London forces, and salt bridges.
- Each subunit contains heme, a nonprotein part (or prosthetic group) that binds oxygen.
- When  $O_2$  binds to heme, a conformational change occurs, i.e. the shape of hemoglobin changes.



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## Antibodies—Your Body's Defense System

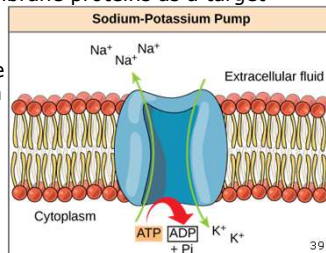
- Antibodies, also known as immunoglobulins, are produced in our bodies when a foreign agent like bacteria enters.
- The foreign agent recognized by **antibodies** is known as an **antigen**.
- Antibodies consists of four polypeptide chains held together by disulfide bonds and intermolecular forces.
- Antibodies are "Y" shaped. Antigens bind at the top of each arm of the Y. The top of each Y has a unique primary structure for a particular antigen.



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## Integral Membrane Proteins

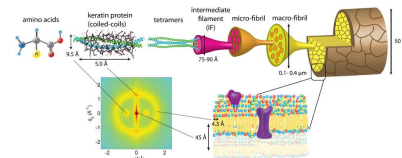
- Integral membrane proteins span the nonpolar region of a cell membrane
- They have roles in signaling (receptors), transport (channels and pumps), and metabolism
- Most drugs have membrane proteins as a target
- An important integral membrane protein involved in electrolyte balance is the sodium potassium pump ( $Na^+/K^+$  ATPase).



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## Globular vs. fibrous proteins

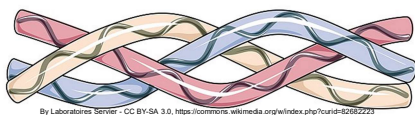
- Proteins are classified into groups based on their three-dimensional shape.
  - Globular proteins are compact, spherical structures that are soluble in an aqueous environment. Myoglobin, which stores oxygen in muscle, is an example.
  - Fibrous proteins are long, threadlike structures that have high helical content. Keratins, found in hair, nails, the scales of reptiles, and collagen, are examples.



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## Collagen

- Collagen is the most abundant protein in the body. One-third of the bodies protein is collagen.
- It is found in connective tissue like cartilage, skin, blood vessels, and tendons.
- A special quaternary structure called a triple helix forms the fibrous structure of collagen.

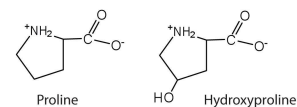


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## Non-standard amino acid: hydroxyproline

- Collagen primarily contains the amino acids glycine, proline, alanine, and hydroxyproline.

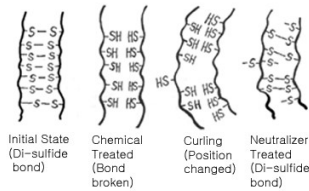


- Hydrogen bonds between the polypeptide chains in collagen are formed through the hydroxyl group on hydroxyproline.
- Proline is converted to hydroxyproline with the aid of Vitamin C. A deficiency of Vitamin C causes scurvy, which is a collagen malformation disease. Scurvy can be reversed with a vitamin C diet.

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## Disulfides and hairstyling

- Ammonium thioglycolate, which reduces (breaks) disulfide bonds, and hydrogen peroxide (reforms disulfide bonds) are two chemical agents used in hairstyling.



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## Schedule for this and next week

	Monday	Wednesday	Friday
Morning 9:20 am	Lecture	Review	By appt.
Afternoon	12:30 lab 1:15 lab 2:00 lab	12:30 lab 1:15 lab 2:00 lab	By appt.

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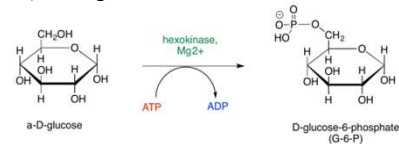
## Enzymes

- biologically active proteins that accelerate the breakdown of food that is eaten, and many other reactions in the body.
- biological catalysts: They accelerate reactions, but are not consumed or changed in reactions.
- change the rate of reaction (kinetics), not the equilibrium constant (thermodynamics).
- have complex, three-dimensional structures
- have specific surface chemistry for catalysis of a given reaction in the aqueous environment of the cell
- Enzymes in 5 minutes:  
<https://pdb101.rcsb.org/learn/videos/how-enzymes-work>

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## Substrates

- Consider hexokinase, an enzyme whose job is to transfer a phosphate group from the high energy molecule, adenosine triphosphate, ATP, to D-glucose.



- In this equation, the enzyme name is written above or below the reaction arrow.
- The phosphate group is sometimes represented by a P in a circle.

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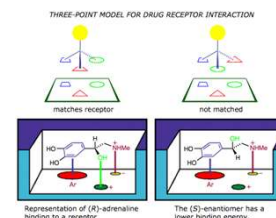
## Active Site

- The location on the surface where the reaction takes place
- When in its proper three-dimensional shape, hexokinase has an indentation on one side of the structure.
- This indentation is known as the active site, and it is lined with amino acid side chains.
- The active site is the functional part of an enzyme where catalysis occurs.

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## Substrate specificity

- Glucose, the reactant for hexokinase, fits snugly in the active site. In an enzyme reaction, the reactant is called the substrate.
- Enzymes have specific substrates, a property known as substrate specificity. For example, the active site of hexokinase reacts with glucose, but will not react with ribose.
- Enzymes are specific for one enantiomer of the substrate.



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## Cofactors and prosthetic groups

- Some enzymes, like hexokinase, have non-protein helpers. Two categories of helpers are as follows:
  - Cofactors are common substances that take part in the reaction, such as ATP.
  - Prosthetic groups are non-aminoacid components of an enzyme, such as heme in hemoglobin

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## Enzyme catalysis is multistep

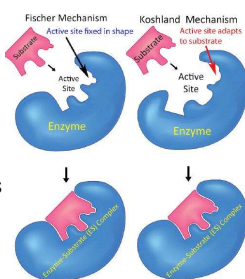
- A substrate is drawn into the active site by intermolecular attractions like hydrogen bonding.
- Hydrogen bonding orients the substrate properly within the active site.
- The initial interaction of the enzyme with the substrate is called the enzyme-substrate complex (ES). This complex forms prior to catalysis.
- In a second step, the bound substrate reacts



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## Lock and key vs. induced fit

In the Lock-and-key model, the active site is thought to be a rigid, inflexible shape that is an exact complement to the shape of the substrate.



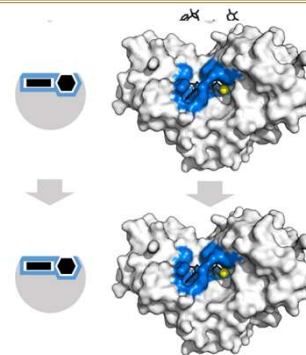
In the induced-fit model, the active site undergoes a conformational change, adjusting to the shape of the substrate when the substrate interacts with the enzyme.

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## Induced fit for hexokinase

- A good example of an induced-fit model is when hexokinase and glucose form an enzyme-substrate complex as shown.

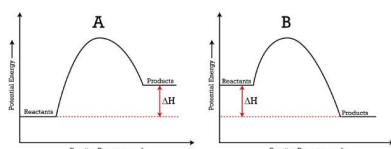
(substrates are ATP and glucose)



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## Thermodynamics of chemical reactions

- some bonds are formed and some are broken, and in the process, the amount of energy changes.

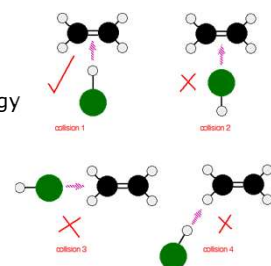


- Equilibrium is on the side with lower free energy
- Enzymes don't (can't) change equilibrium

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## Kinetics of chemical reactions

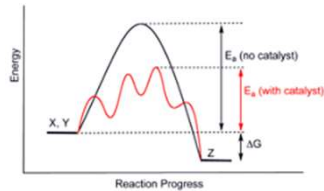
- Reactant molecules have to collide. Number of collisions increases with temperature and with concentration of reactants
- Activation energy is required to break bonds
- Enzymes help to bring substrates together and to lower activation energy
- Both factors increase reaction rates



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## Enzyme action in a nut shell

- An enzyme-catalyzed reaction increases the rate of a reaction by forming ES and lowering activation energy

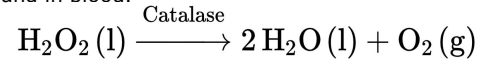


- Activation energy is lower, equilibrium is the same

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## Another example enzyme: Catalase

- Consider the addition of hydrogen peroxide to a cut. The area bubbles considerably because oxygen is produced by the enzyme catalase found in blood.



- A high concentration of oxygen is produced at the wound site that kills germs.
- Without catalase, this reaction occurs very slowly (peroxide is stable in a bottle).

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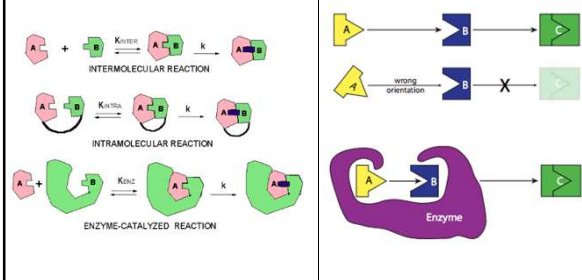
## Enzyme mechanism

- Anything that reduces the activation energy will speed up the reaction, e.g.
  - Bringing reactants together: proximity
  - Changing the "local pH"
  - Changing the charges of the reactants

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## Proximity and Orientation

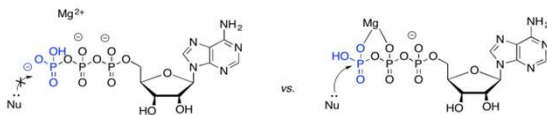
- The reacting molecules are in close proximity to each other, and the functional groups are in the correct orientation



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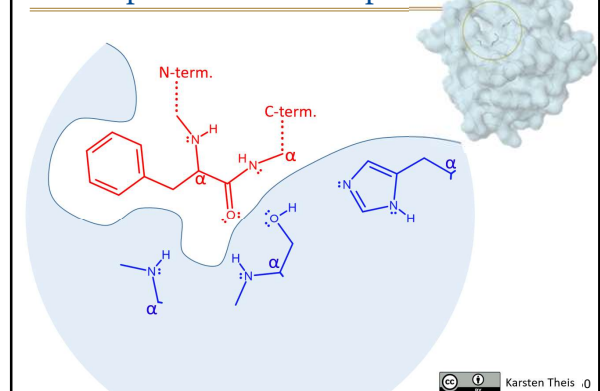
## Metal catalysis

E.g. formation of glucose-6-phosphate by hexokinase  
The negatively charged phosphates of ATP are unreactive because negatively charged reactants will not approach.  $\text{Mg}^{2+}$  (a coenzyme) holds ATP in one area of the active site and helps to reduce the repulse ionic interactions.



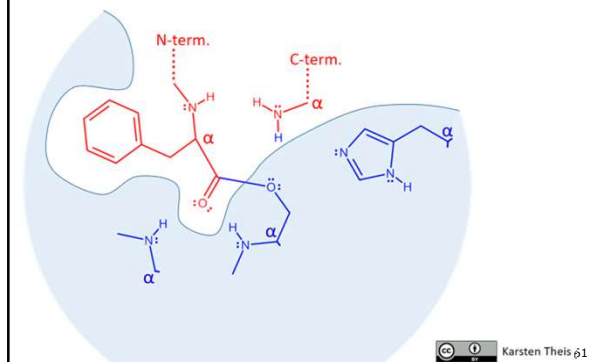
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## Serine proteases: ES complex.



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## Covalent catalysis



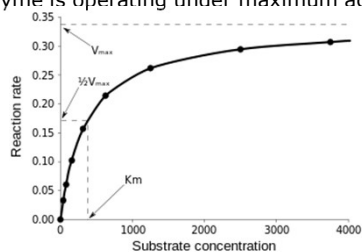
## What determines enzyme activity?

- Enzyme concentration
- Substrate concentration
- Inhibitors and activators
- pH
- Temperature

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## Enzymes get saturated

- Increasing substrate concentration above a certain concentration will not affect the rate of the reaction.
- A condition known as saturated state is when an enzyme is operating under maximum activity.



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## pH and reaction rate

- Changes in pH will also affect the nature of the amino acid side chains in the active site.
- The optimum pH for enzymes is based on the pH of the organ they are active in

Enzyme	Optimal pH	Enzyme	Optimal pH
Lipase (pancreas)	8.0	Invertase	4.5
Lipase (stomach)	4.0 - 5.0	Maltase	6.1 - 6.8
Lipase (castor oil)	4.7	Amylase (pancreas)	6.7 - 7.0
Pepsin	1.5 - 1.6	Amylase (malt)	4.6 - 5.2
Trypsin	7.8 - 8.7	Catalase	7.0
Urease	7.0		

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## Temperature and reaction rate

- Enzymes have an optimum temperature at which they are most active.
- The optimum temperature for most human enzymes is normal body temperature, 37 °C.
- Above optimum temperature, enzymes lose activity due to disruption of intermolecular forces stabilizing the tertiary structure. At high temperatures, enzymes denature, which modifies the active site.
- At low temperatures, enzyme activity is low due to a lack of energy for the reaction to occur. Food is stored in a refrigerator or freezer to slow spoilage brought on by enzymes.

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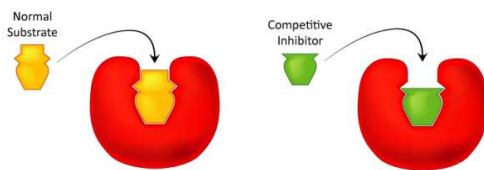
## Enzyme inhibition

- If allowed to sit untouched, the flesh of sliced apples will turn brown by a process known as oxidation, catalyzed by an enzyme.
- If lemon juice is sprinkled on the sliced apple, the lemon juice will inhibit the formation of this brown color by changing the pH of the environment of the enzyme.
- Enzyme reactions are affected by reaction conditions such as substrate concentration, pH, temperature, and the presence of inhibitors.

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## Competitive inhibition

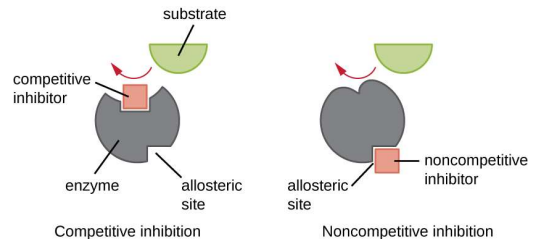
- As long as an inhibitor remains in the active site, the enzyme cannot react with the substrate to form product.
- Adding high concentrations of substrate displaces the inhibitor, allowing the reaction to go forward even in the presence of inhibitor



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## Non-competitive inhibition

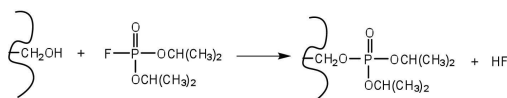
- Binding to a site distant to the active site
- Conformational change slows down the enzyme



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## Irreversible (covalent) inhibition

- Heavy metals like silver, mercury, and lead are examples of irreversible inhibitors.
- Even if these poisons are flushed out of the system, the enzymes stay inhibited
- Some inhibitor look like a substrate, but bind covalently to the active site (suicide inhibitor)



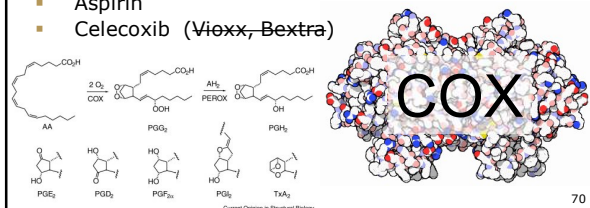
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## How are drugs developed?

Target: the enzyme the drug acts on  
Lead compound: "the first idea"  
Drug: the compound used for treatment

Examples: anti-inflammatory agents

- Aspirin
- Celecoxib (Vioxx, Bextra)



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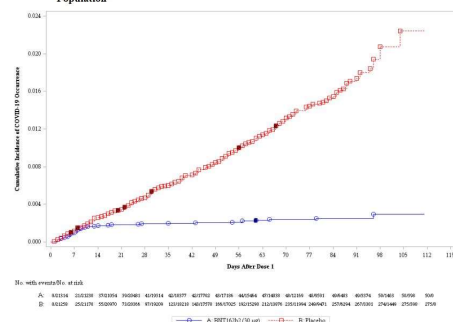
## What types of drugs exist?

- Infectious diseases and cancer: drugs that kill cells by interfering with vital function
- Metabolic or regulatory disease: inhibitors that shut down pathways, effectors that upregulate pathways
- Juvenile diabetes, malnutrition: provide insulin or the missing nutrient, respectively

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## Vaccines – giving antigens to get antibodies

Figure 13 Cumulative Incidence Curves for the First COVID-19 Occurrence After Dose 1 – Dose 1 All-Available Efficacy Population



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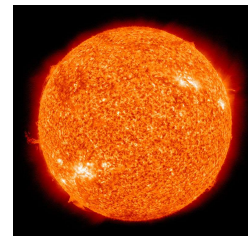
## Free energy $\Delta G$

- Without free energy, nothing changes
- "Free" means available for doing work, not in the sense being available for free.
- Free energy is consumed when we build things, lift things, speed up things
- Once free energy is converted into heat, we can't convert it back to free energy
- Energy is conserved, but free energy can be lost
- We don't have an energy crisis, we have a free energy crisis
- Reaction proceed in the direction that lowers free energy

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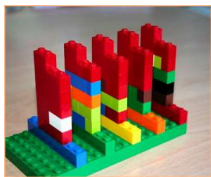
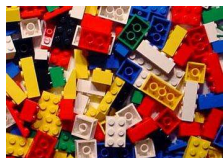
## Sources of free energy

- Primary
  - Sun (nuclear reaction)
- Secondary
  - Wind, Oil, Coal, Braking
- Most convenient
  - Electricity



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## Structure from building blocks



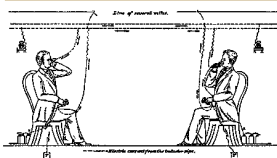
75

## Going up



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## Communication



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## Free energy storage



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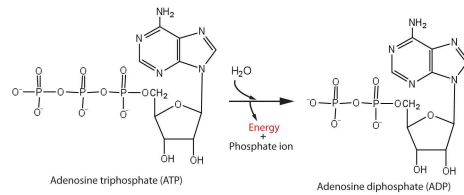
## Free energy storage in our body

- Long term (need oxygen to utilize)
  - Fat
  - Starch
- Short term
  - ATP (need water to utilize)
  - NADH (need oxygen to utilize)

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## ATP + H<sub>2</sub>O

- Hydrolysis is slow in the absence of catalysts
- Hydrolysis is a downhill reaction



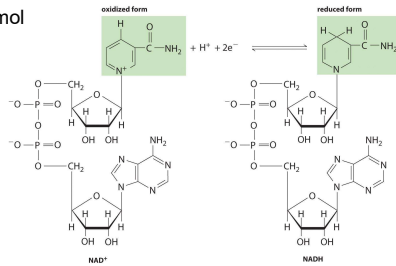
$$\Delta G = -50 \text{ kJ/mol}$$

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## NADH + O<sub>2</sub>

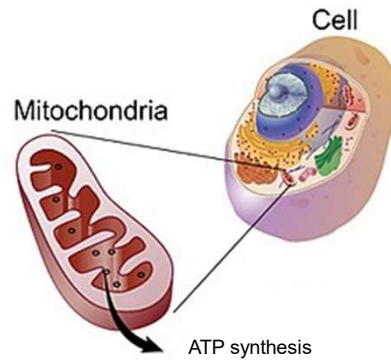
- NADH oxidation is slow in the absence of catalyst
- NADH oxidation by oxygen is a downhill reaction
- $\text{NADH} + \frac{1}{2} \text{O}_2 + \text{H}^+ \rightarrow \text{NAD}^+ + \text{H}_2\text{O}$

$$\Delta G = -220 \text{ kJ/mol}$$



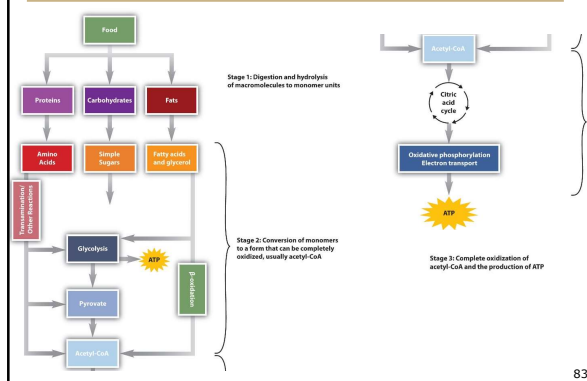
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## Mitochondria: ATP synthesis



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## Overview of metabolism to capture $\Delta G$



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## Overview of how ATP is made

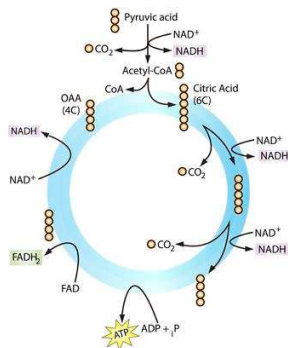
- ATP synthase: makes ATP from ADP and phosphate (uphill reaction) using free energy from a "proton gradient" across a membrane
- Electron transfer chain: builds up proton gradient (uphill reaction) using free energy from oxidizing NADH with oxygen
- Citric acid cycle: reduces NAD<sup>+</sup> to NADH by oxidizing food to CO<sub>2</sub>

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## Citric Acid Cycle— Net reaction

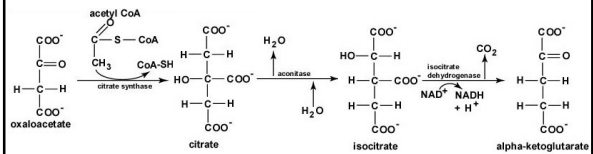
- Acetic acid is oxidized to  $\text{CO}_2$ .  $\text{NAD}^+$  and  $\text{FAD}$  are reduced to  $\text{NADH}$  and  $\text{FADH}_2$ , and  $\text{GTP}$  is made from  $\text{GDP}$  and phosphate in this series of reactions.



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## Citric Acid Cycle—Details

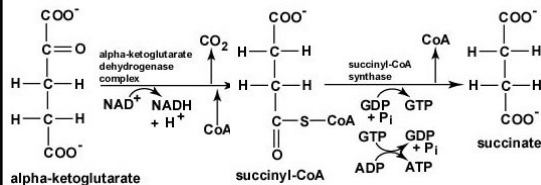
Redox  
Hydrolysis/Condensation  
Cofactor CoA



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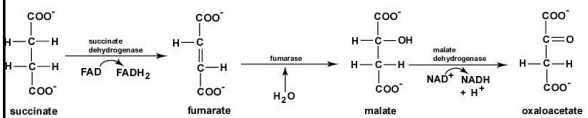
## Citric Acid Cycle—Details

Redox  
Coupled reaction



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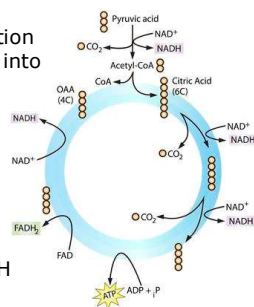
## Citric Acid Cycle—Details



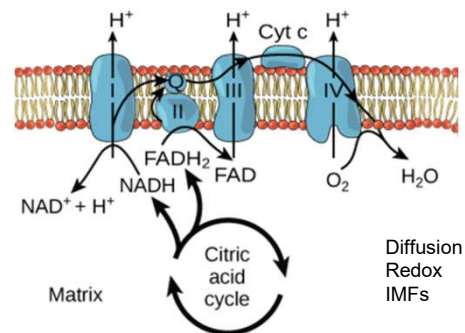
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## Citric Acid Cycle— Free energy balance

- Oxidizing acetic acid with oxygen ("burning it") has a free energy change of 873 kJ/mol
- Instead of running this reaction in a single step, it is broken into multiple steps
- Some of these steps are redox reactions, oxidizing the metabolites and reducing  $\text{NAD}^+$  to  $\text{NADH}$
- Not quite enough for 4  $\text{NADH}$



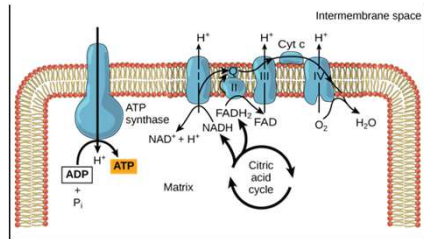
## Electron Transport Chain



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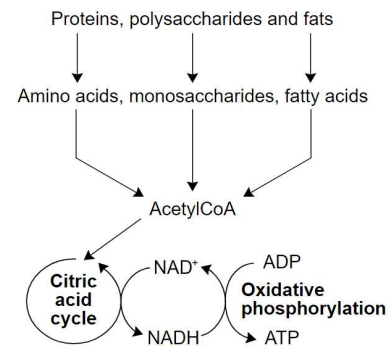
## Proton pumps

- The movement of protons from an area of high concentration of protons to an area of fewer protons releases energy that drives ATP synthase.
- $\text{ADP} + \text{P}_i + \text{free energy} \rightarrow \text{ATP}$



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## Grand Overview



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