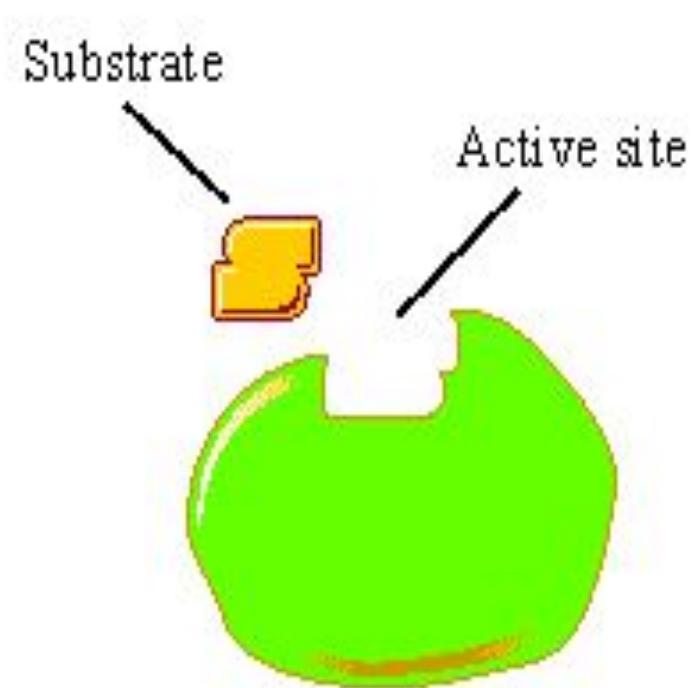
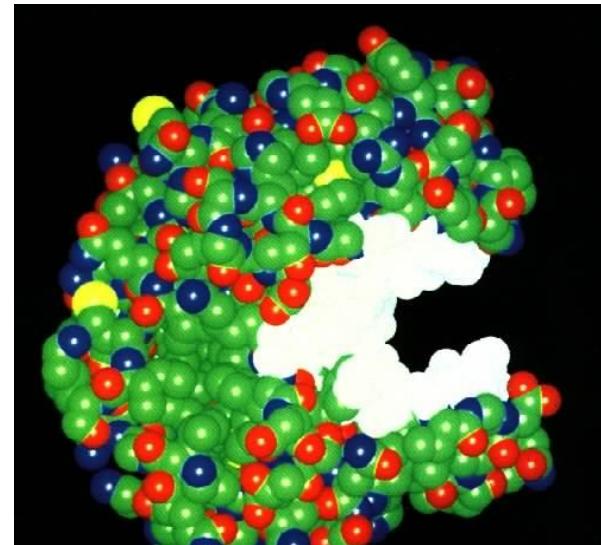


Unit III

- Enzymes
- Proteases
- Carbonic anhydrase, Restriction enzymes, and Nucleoside monophosphate kinases
- Photosynthesis
- Biological Energy production

What Are Enzymes?

- Most enzymes are **Proteins** (tertiary and quaternary structures)
- Act as **Catalyst** to accelerates a reaction
- Not permanently changed in the process
- Are specific for what they will catalyze
- Are **Reusable**
- End in **-ase**
 - Sucrase*
 - Lactase*
 - Maltase*



Why Enzymes?

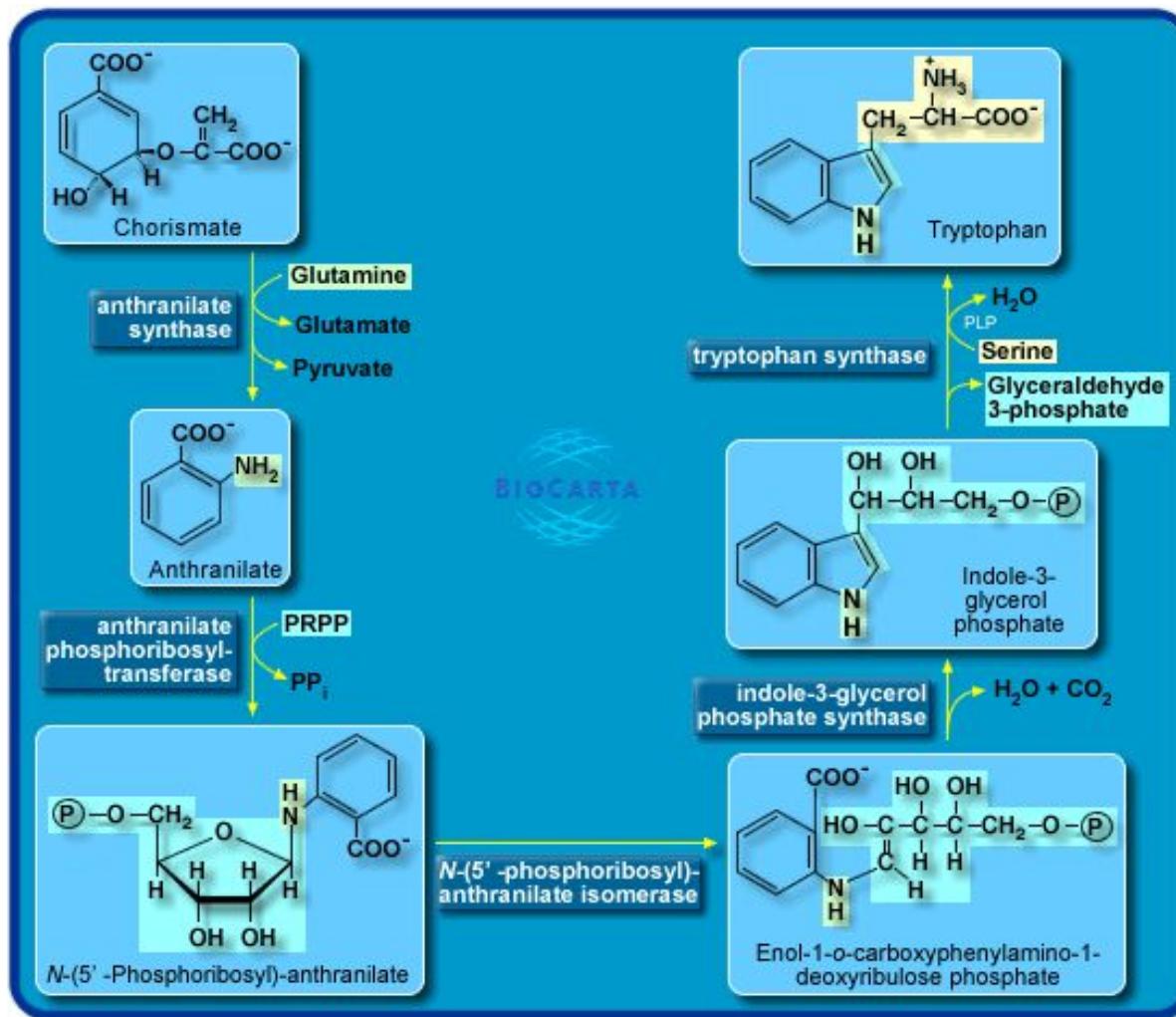
- Natural catalysts

- Speed: 10^{16} over un-catalyzed rates!

- Specificity: only the desired reaction occurs

- Permit reactions under mild conditions

Metabolic Pathways



The metabolic pathway that produces tryptophan – an amino acid. This is one of hundreds of metabolic pathways essential for life.

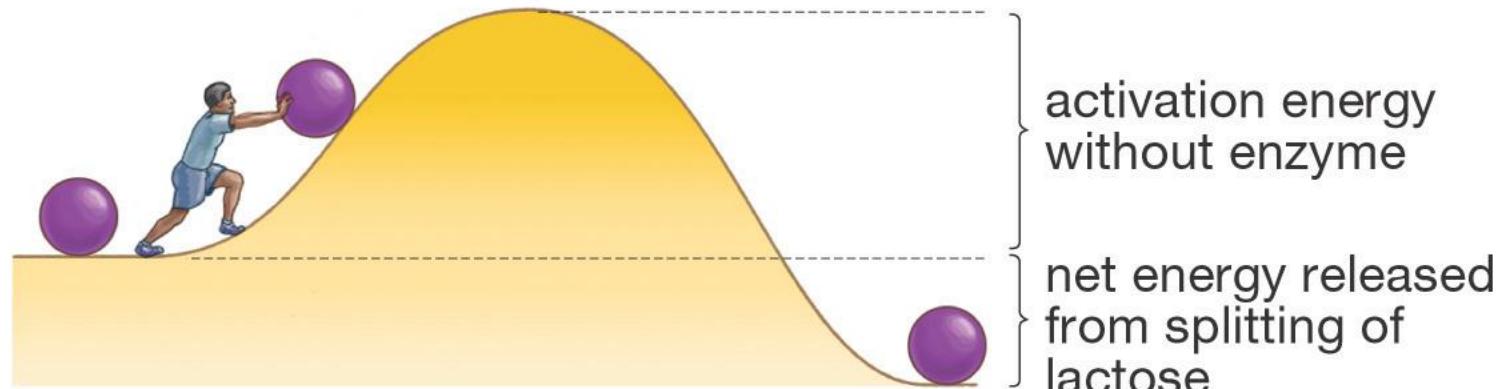
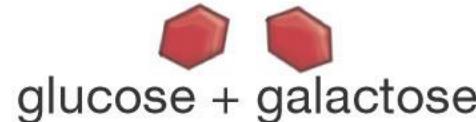
Enzyme Deficiency and Health



Most genetic disorders are due to a deficiency in enzyme function.

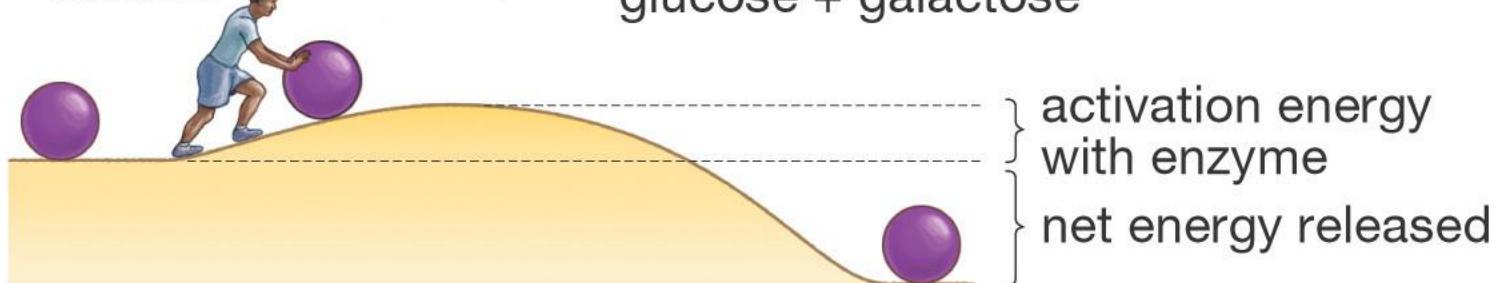
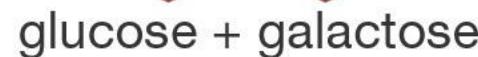
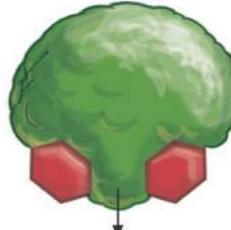
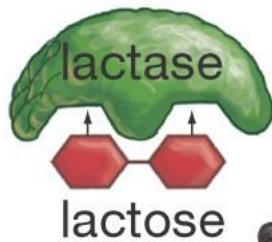
This archival photo shows three children with the enzyme (phenylalanine hydroxylase) deficiency that causes phenylketonuria.

(a) Without enzyme

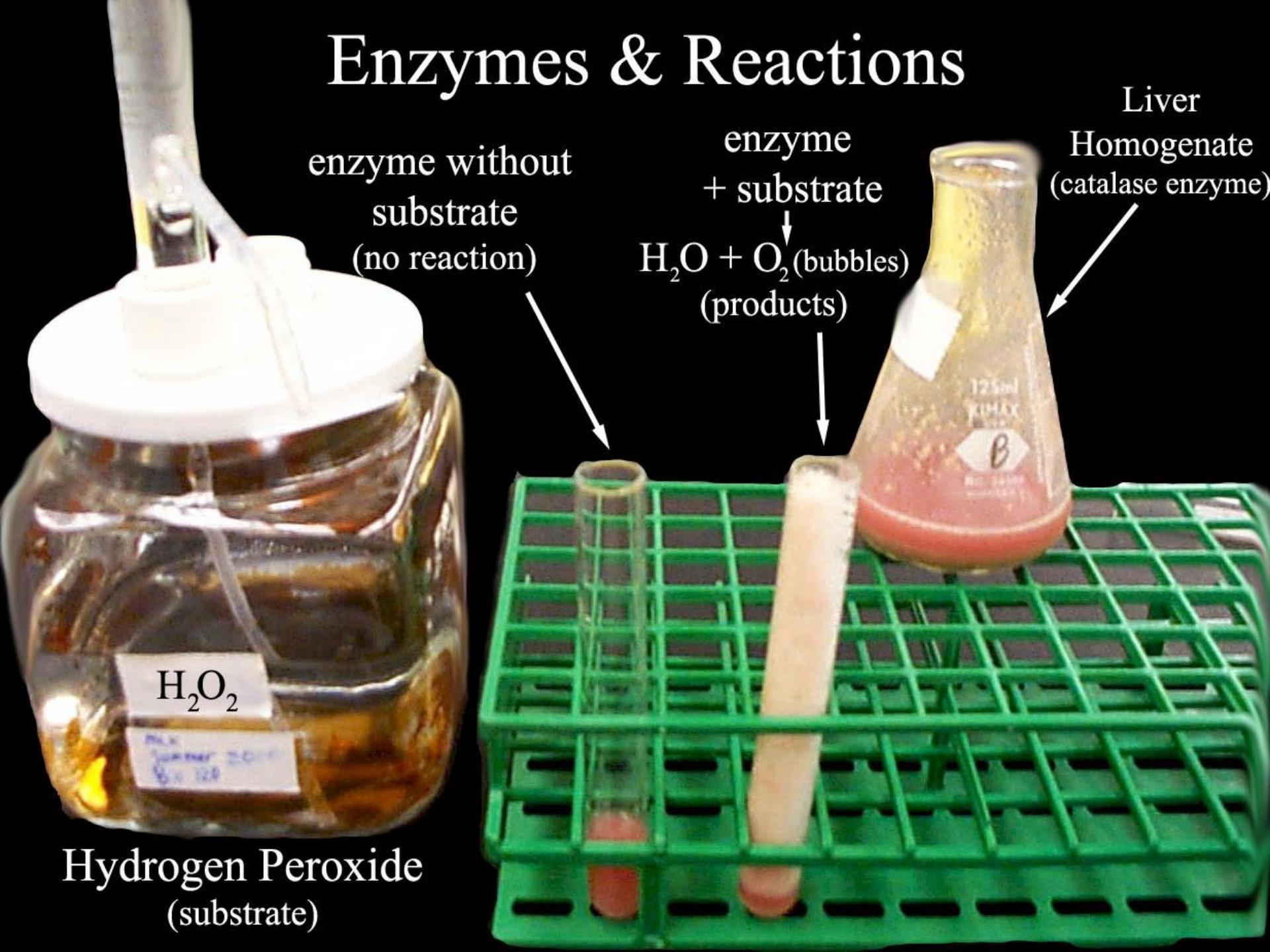


**Enzymes
work by
weakening
bonds
which
lowers
activation
energy**

(b) With enzyme



Enzymes & Reactions

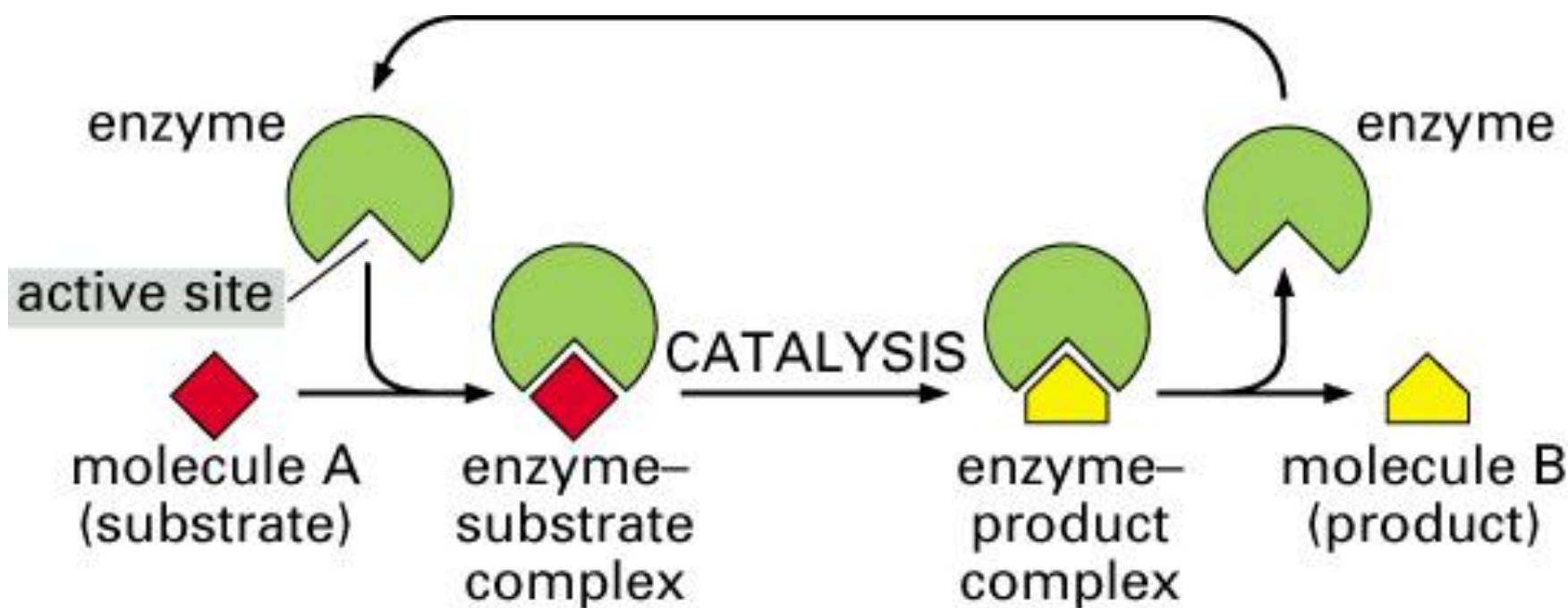


Enzyme Action: Lock and Key Model

Conventionally we say the enzyme (E) acts on the substrate (S) to yield products (P)



Since E is a catalyst it remains unchanged at the end of the reaction



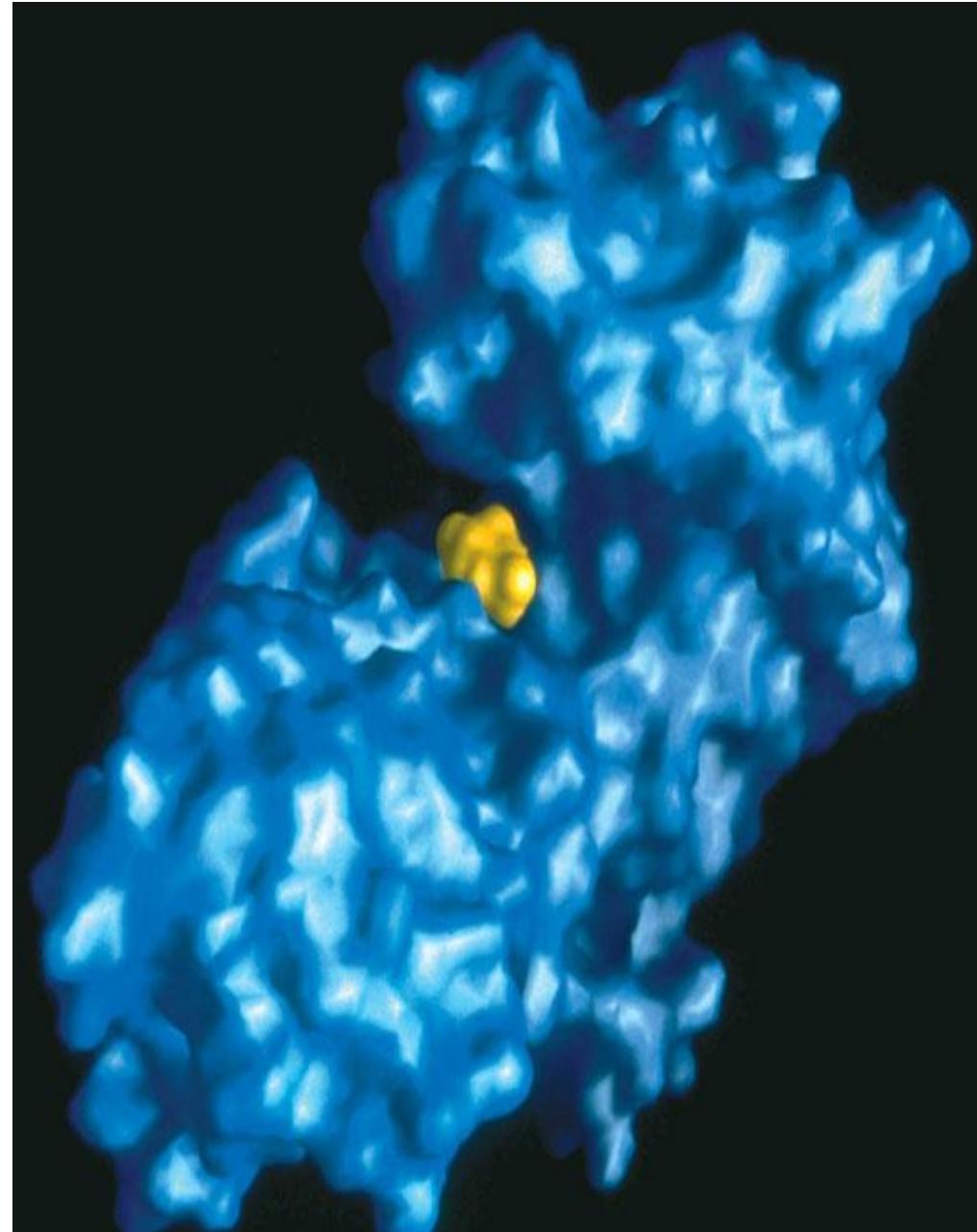
The active site

Typically a pocket or groove on the surface of the protein into which the substrate fits.

The specificity of an enzyme –fit between the active site and that of the substrate.

Enzyme changes shape –tighter induced fit, bringing chemical groups in position to catalyze the reaction.

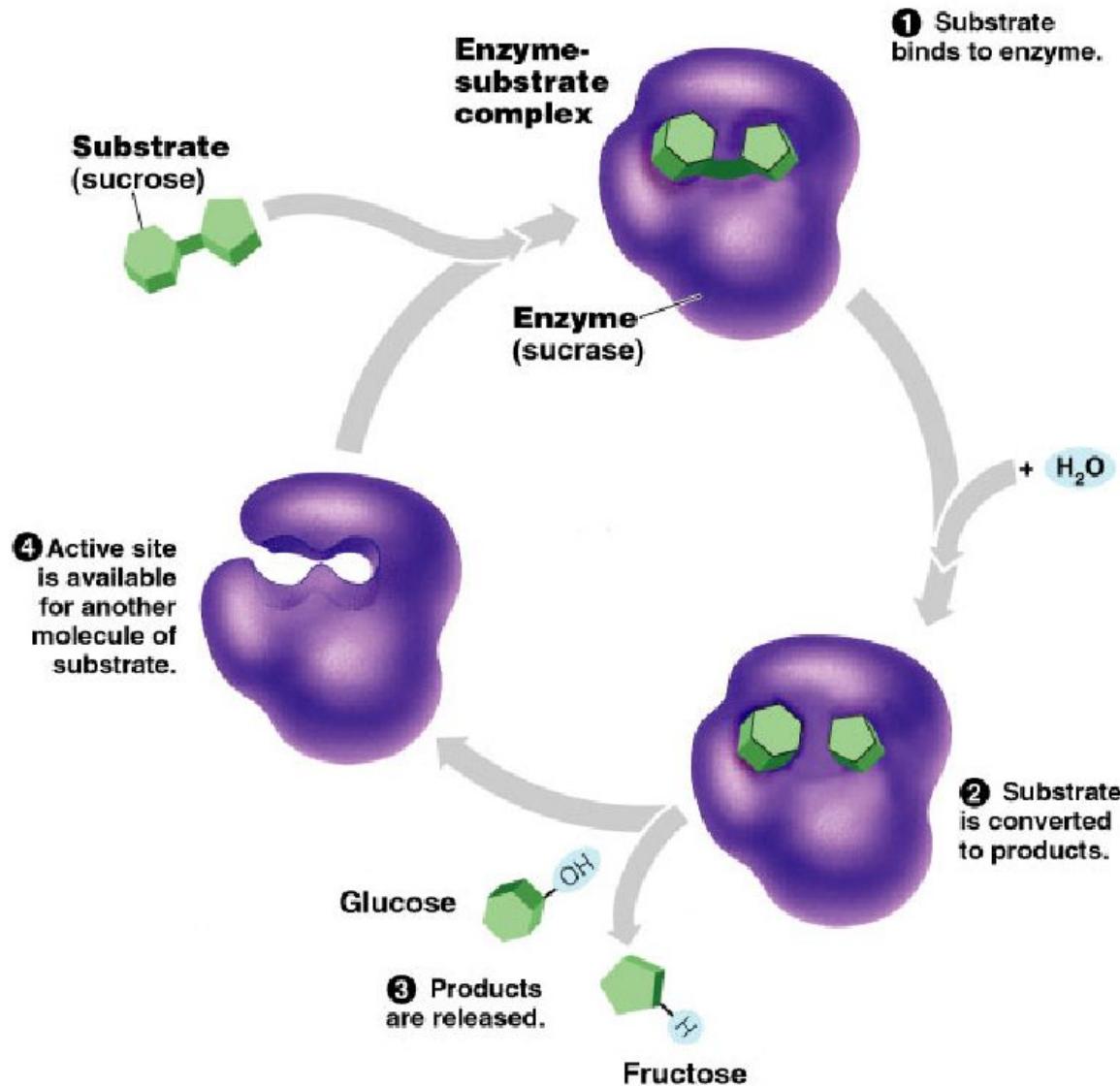
Hexokinase, an enzyme (blue), binding its substrate, glucose (yellow).



Specificity

Enzymes selectively recognize proper substrates over other molecules

Specificity is controlled by structure – the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield



What Affects Enzyme Activity?

- Three factors:
 1. Environmental Conditions
 2. Cofactors and Coenzymes
 3. Enzyme Inhibitors

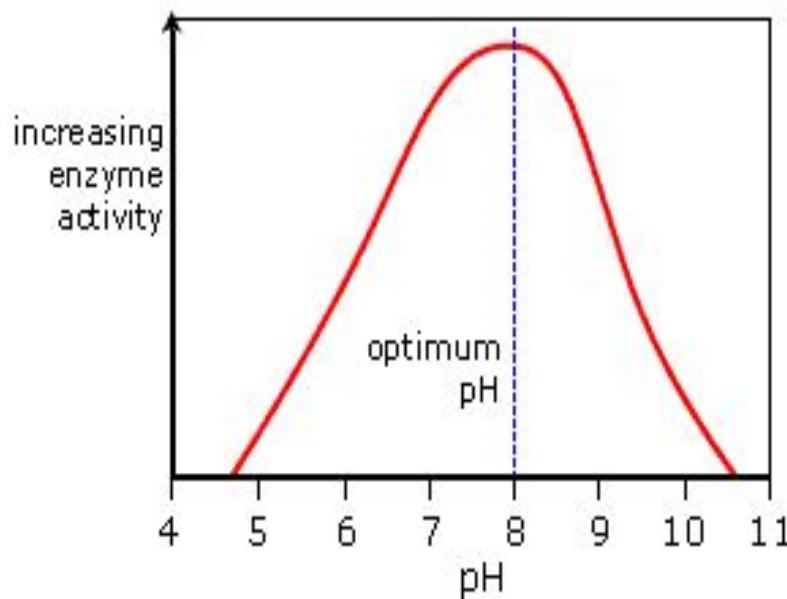
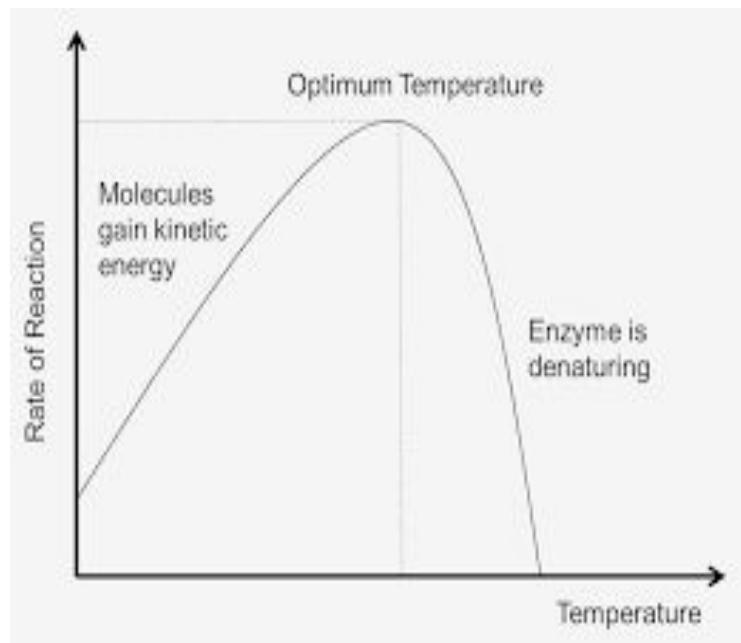
1. Environmental Conditions:

1. Extreme Temperature are the most dangerous
 - high temps may denature (unfold) the enzyme.
2. pH (most like 6 - 8 pH near neutral)
3. Ionic concentration (salt ions)

2. Cofactors and Coenzymes

- Inorganic substances (zinc, iron) and vitamins (respectively) are sometimes need for proper enzymatic activity.

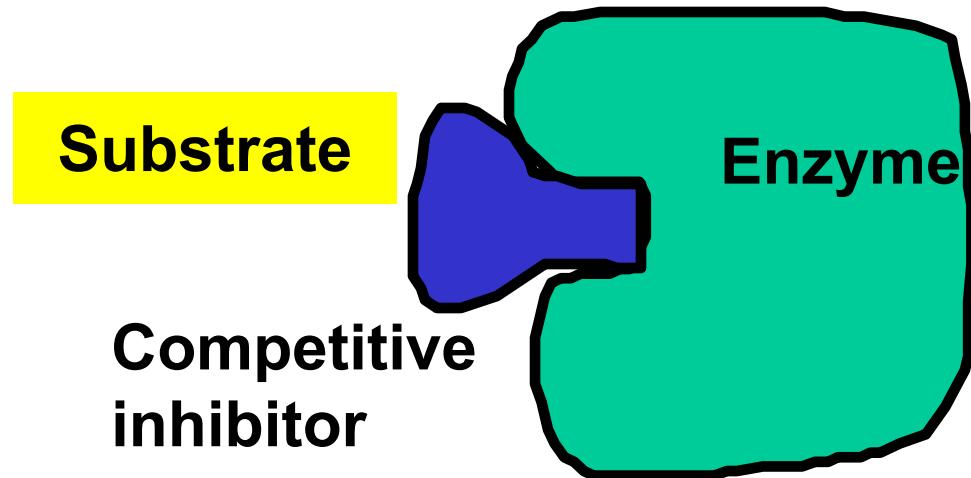
Example: Iron must be present in the quaternary structure - hemoglobin in order for it to pick up oxygen.



3. Enzyme Inhibitors

Two Types:

a. **Competitive inhibitors:** are chemicals that resemble an enzyme's normal substrate and compete with it for the active site.



b. **Noncompetitive inhibitors:** Inhibitors that do not enter the active site, but bind to another part of the enzyme causing the enzyme to change its shape, which in turn alters the active site.



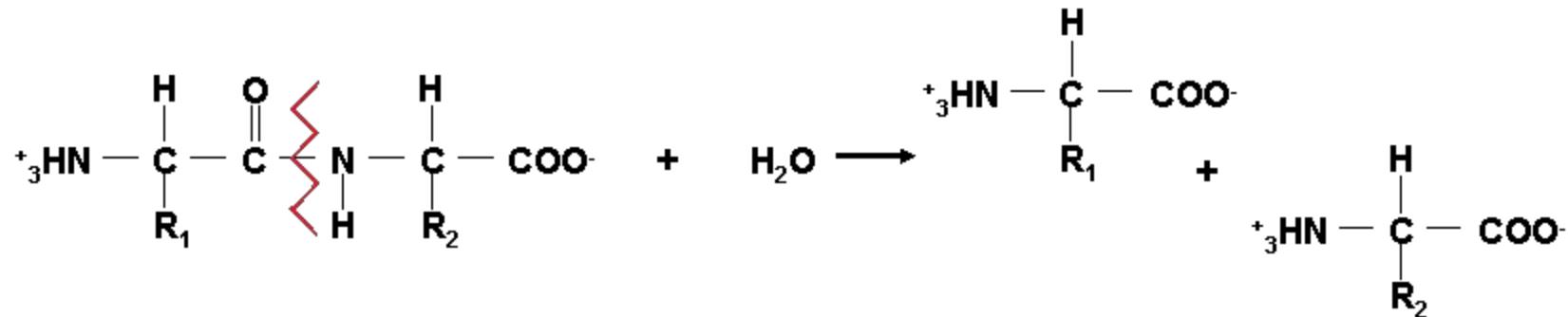
Catalytic Mechanisms

- Acid-Base catalysis
 - Enzyme side chains act as proton donors and acceptors.
- Covalent Catalysis
 - Powerful nucleophilic side chain forms an unstable covalent bond to the substrate.
- Metal ion catalysis
 - By serving as an electrophilic catalyst, generating a nucleophile, and increasing the binding energy between E and S
- Catalysis by approximation
 - When two substrates involved, the reaction rate is enhanced by bringing them together on the active binding site of E.

Proteases

Proteases

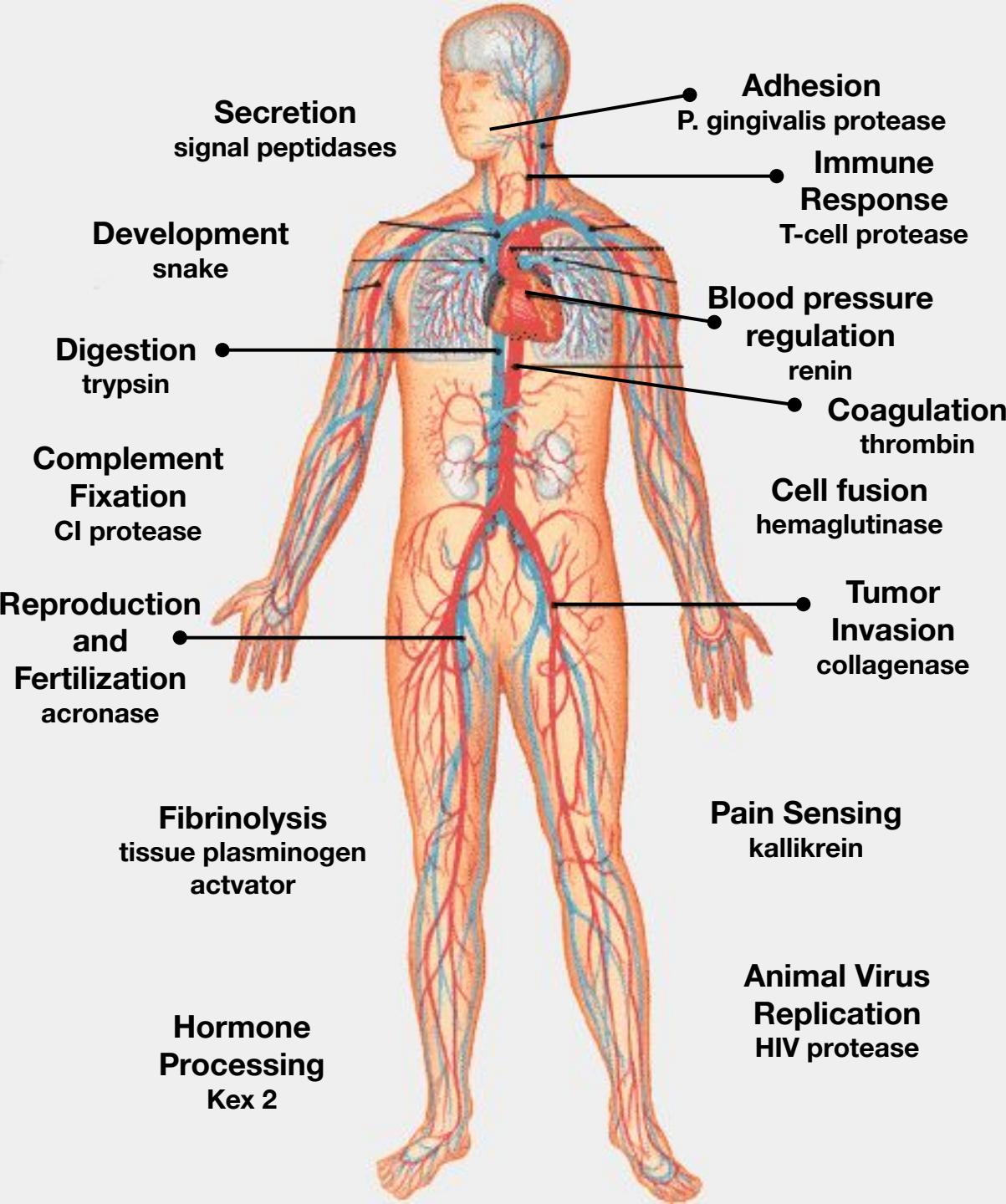
What do proteases do?



Proteases cleave proteins by a hydrolysis reaction – the addition of a molecule of water to a peptide bond

Mechanistic Sets of Proteases

set	feature	inhibitor	examples	function
Serine protease	active site serine	fluorophosphates	trypsin	digestion
	thrombin		blood coagulation	
	plasmin		lysis of blood clots	
	coccoonase		mechanical	
	subtilisin		digestion	
	acrosin		sperm penetration	
Cysteine protease	active site cysteine	iodoacetate	papain	digestion
	strept. proteinase		digestion	
	cathepsin B		intracell. digestion	
Acid protease	acidic pH optimum	diazoketones	pepsin	digestion
	chymosin		milk coagulation	
Metalloproteases	Zn ²⁺ , Zn ²⁺ , Ca ²⁺	o-phenanthroline	carboxypeptidase thermolysin	digestion
		o-phenanthroline		digestion



6 Broad Categories

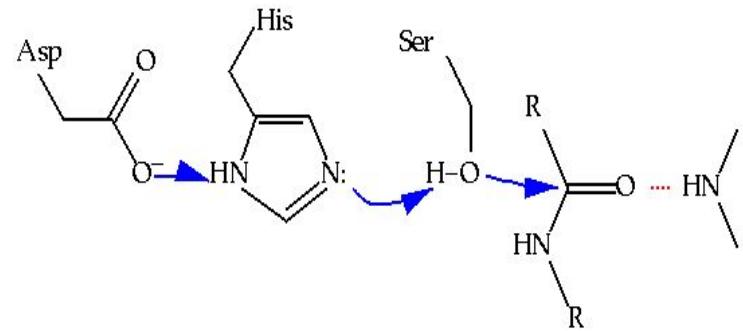
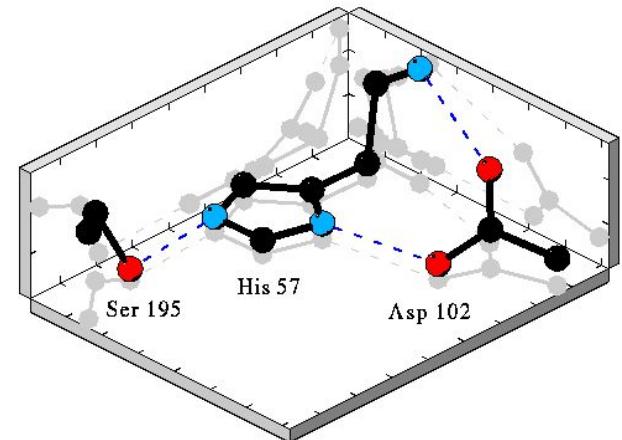
<u>Function</u>	<u>Protease</u>
Nutrition	trypsin, subtilisin, α -lytic protease
Invasion	matrix metallo proteases
Evasion	IgA protease
Adhesion	P. gingivalis protease
Processing	signal peptidase, viral proteases, proteosome
Signaling	caspases, granzymes

Catalytic triad

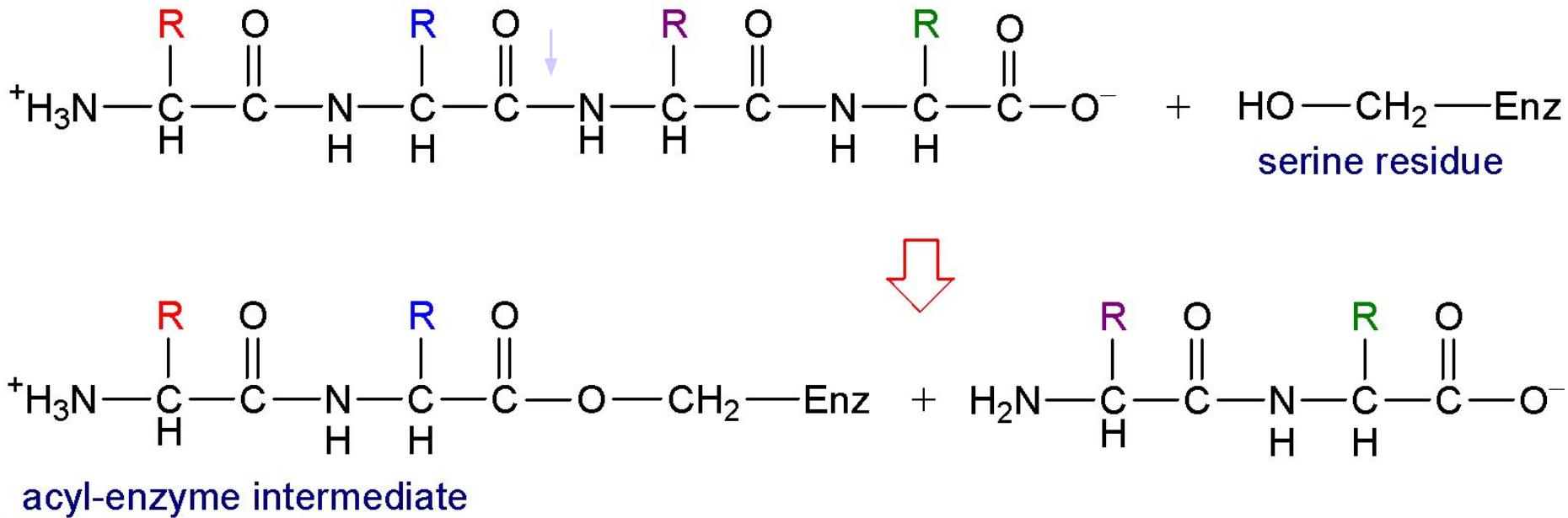
A **catalytic triad** is a set of three coordinated amino acids that can be found in the active site of some enzymes.

The active site in each serine protease includes a **serine** residue, a **histidine** residue, & an **aspartate** residue.

During attack of the serine hydroxyl oxygen, a proton is transferred from the serine hydroxyl to the imidazole ring of the histidine, as the adjacent aspartate carboxyl is H-bonded to the histidine.



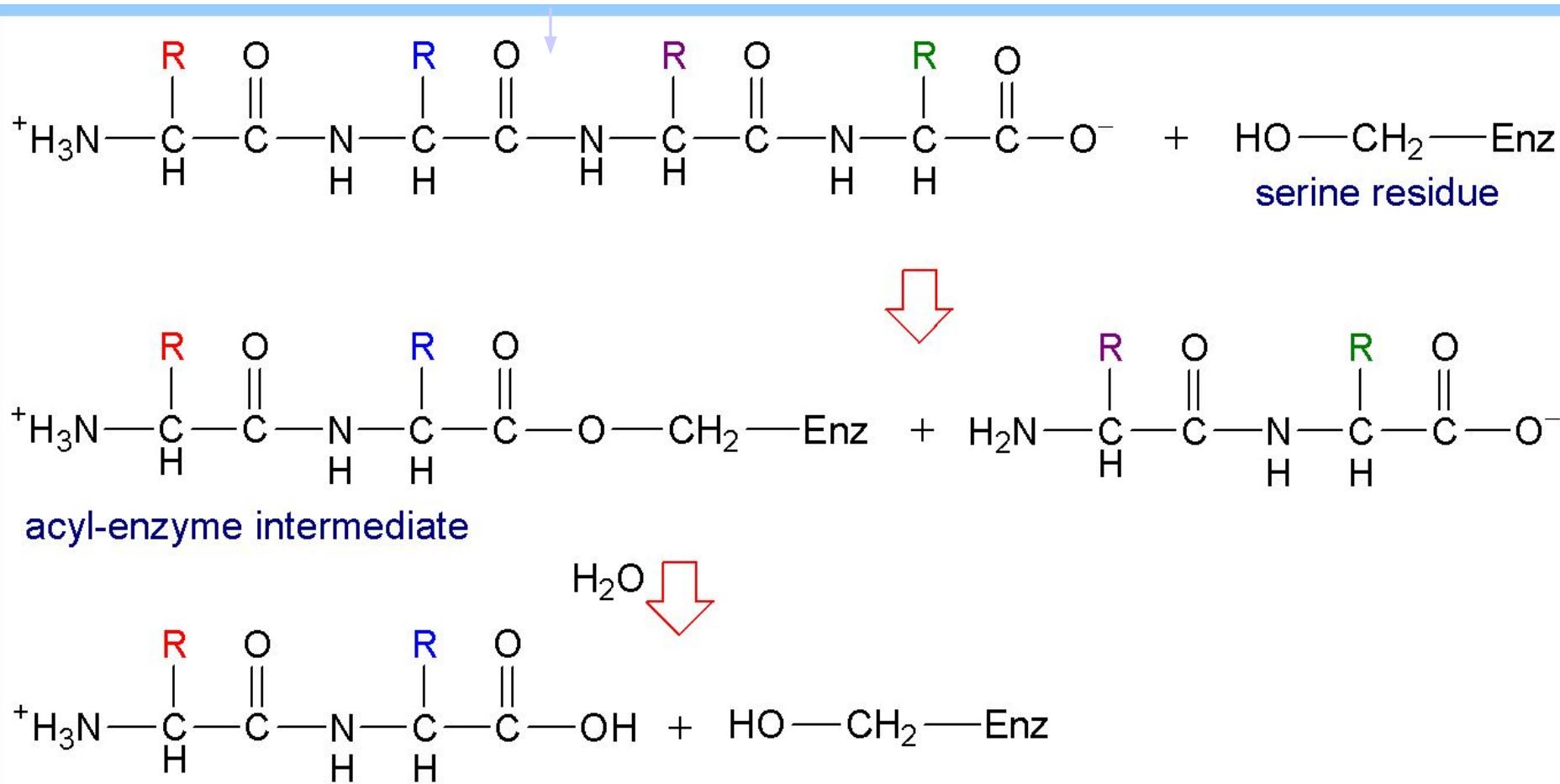
Catalytic Mechanism



During catalysis, there is nucleophilic attack of the **hydroxyl O** of a **serine** residue of the protease on the carbonyl **C** of the peptide bond that is to be cleaved.

An **acyl-enzyme intermediate** is transiently formed.

In this diagram a small peptide is shown being cleaved, while the usual substrate would be a larger polypeptide.



Hydrolysis of the ester linkage yields the second peptide product.

- The serine has an -OH group that is able to act as a nucleophile, attacking the carbonyl carbon of the scissile peptide bond of the substrate.

(Nucleophile: Any electron rich species that can donate electron to form a bond.

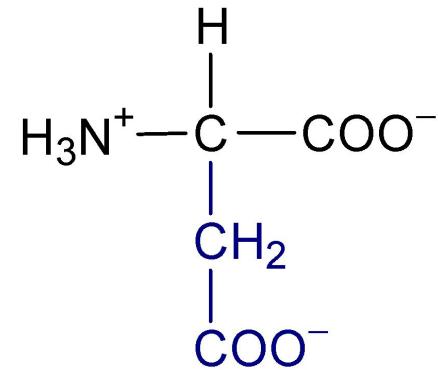
Scissile Bond: A covalent bond that can be broken by an enzyme)

- A pair of electrons on the nitrogen of the histidine has the ability to accept the hydrogen from the serine -OH group, thus coordinating the attack of the peptide bond.
- The carboxyl group (-OH group) on the aspartic acid in turn hydrogen bonds with the histidine, making the nitrogen atom mentioned above much more electronegative.

Aspartate proteases include

- the digestive enzyme **pepsin**
- Some proteases found in **lysosomes**
- the kidney enzyme **renin**
- HIV-protease.**

aspartate (Asp)



Two aspartate residues participate in **acid/base catalysis** at the active site.

Use an activated water molecule bound to one or more **aspartate** residues for catalysis of their peptide substrates

In the initial reaction, one aspartate accepts a proton from an active site **H₂O**, which attacks the carbonyl carbon (C=O) of the peptide linkage.

Simultaneously, the other aspartate donates a proton to the oxygen of the peptide carbonyl group.

Zinc proteases (metalloproteases) include:

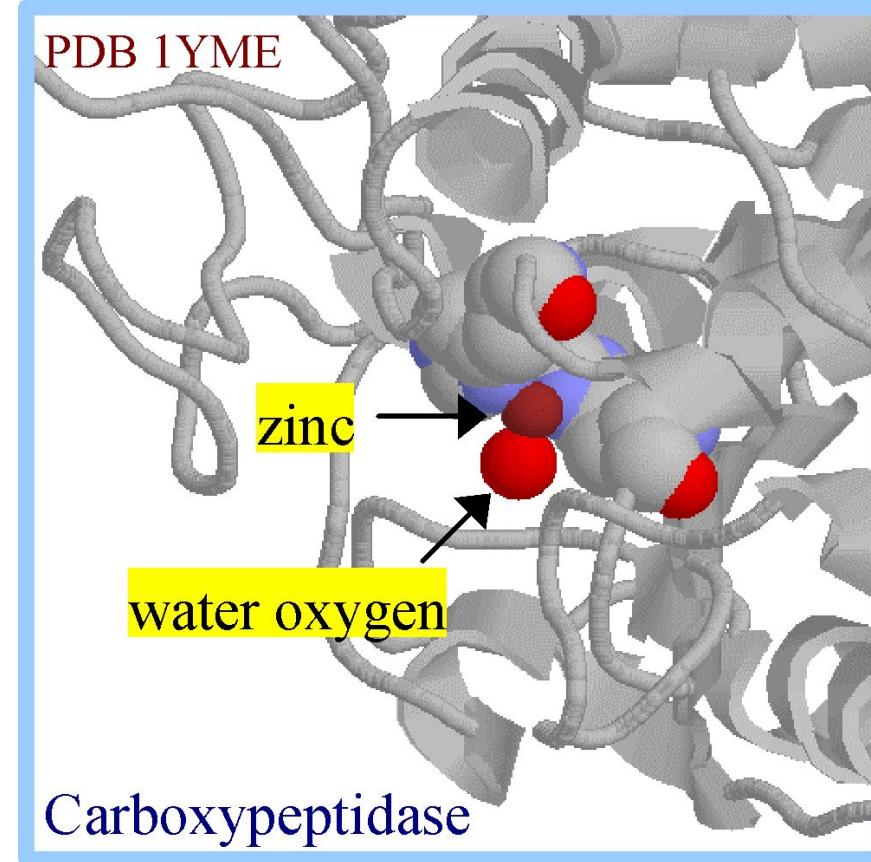
- ◆ digestive enzymes **carboxypeptidases**
- ◆ **matrix metalloproteases (MMPs)**, secreted by cells
- ◆ one **lysosomal** protease.

Some MMPs (e.g., collagenase) are involved in **degradation of extracellular matrix** during tissue remodeling.

Some MMPs have roles in cell **signaling** relating to their ability to release cytokines or growth factors from the cell surface by **cleavage** of membrane-bound **pre-proteins**.

A zinc-binding motif at the active site of a metalloprotease includes two **His** residues whose imidazole side-chains are ligands to the **Zn⁺⁺**.

Colors in Carboxypeptidase image at right: **Zn**, **N**, **O**.

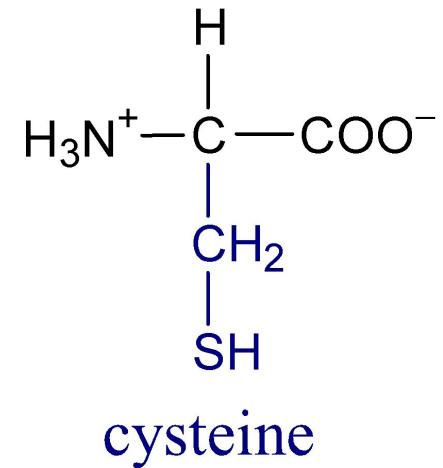


During catalysis, the **Zn⁺⁺** promotes nucleophilic attack on the carbonyl carbon by the oxygen atom of a **water** molecule at the active site.

An active site **base** (Glu in Carboxypeptidase) facilitates this reaction by extracting H⁺ from the attacking H₂O.

Cysteine proteases have a catalytic mechanism that involves a cysteine **sulphydryl** group.

Deprotonation of the cysteine SH by an adjacent His residue is followed by nucleophilic **attack of the cysteine S** on the peptide carbonyl carbon.



Cysteine proteases:

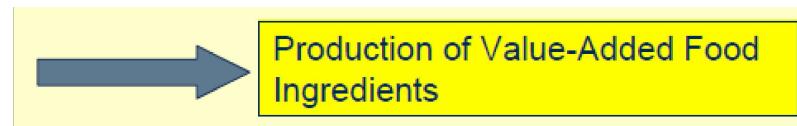
- ◆ **Papain** is a well-studied **plant** cysteine protease.
- ◆ **Cathepsins** are a large family of **lysosomal** cysteine proteases, with varied substrate specificities.
- ◆ **Caspases** are cysteine proteases involved in activation & implementation of **apoptosis** (programmed cell death).
Caspases get their name from the fact that they cleave on the carboxyl side of an aspartate residue.
- ◆ **Calpains** are **Ca⁺⁺-activated** cysteine proteases that cleave intracellular proteins involved in cell motility & adhesion.
They regulate processes such as cell migration and wound healing.

Protease Applications in Food Processing

- **Basic rationale:** Proteases are a powerful tool for modifying the properties of food proteins.
 - Improved digestibility
 - Improved solubility
 - Modified functional properties: emulsification, fat-binding, water-binding, foaming properties, gel strength, whipping properties, etc.
 - Improved flavor & palatability
 - Improved processing: viscosity reduction, improved drying, etc.

Protease Applications in Food Processing

- Proteases are also used in a wide range of foods & food processing applications.
- Dairy: milk coagulation, flavor development
- Baking: gluten development
- Fish & seafood processing: fishmeals, enhanced oil recovery, aquaculture
- Animal protein processing: improved digestibility, reduced allergenicity, improved flavor, meat tenderization
- Plant protein processing: improved functionality & processing, generation of bio-active peptides.
- Yeast hydrolysis: flavor compounds.



Nonfood Protease Applications

- Medicine
- Pharmacology & drug manufacture
- Laundry & dishwashing detergents (#1)
- Hard surface cleaning formulations
- Contact lens cleaning formulations
- Waste treatment
- Industrial applications
- Fermentation (fuel EtOH, etc.)
- Chondroitin & heparin production
- Animal feed additives
- Digestive supplements

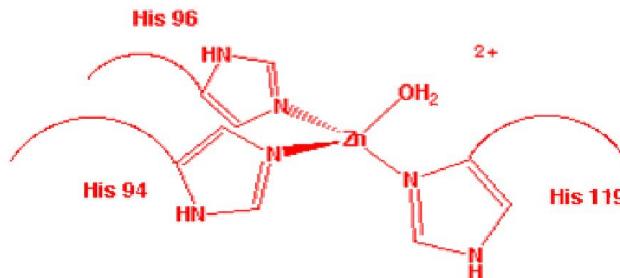


Carbonic anhydrase

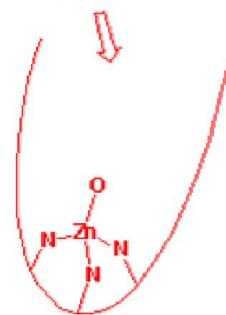
- Carbonic anhydrase, a zinc metallo-enzyme, is widespread throughout the bacterial, plant and animal kingdoms. It is generally considered one of the most efficient enzymes known as it catalyzes the reversible interconversion between CO_2 and the bicarbonate ion.

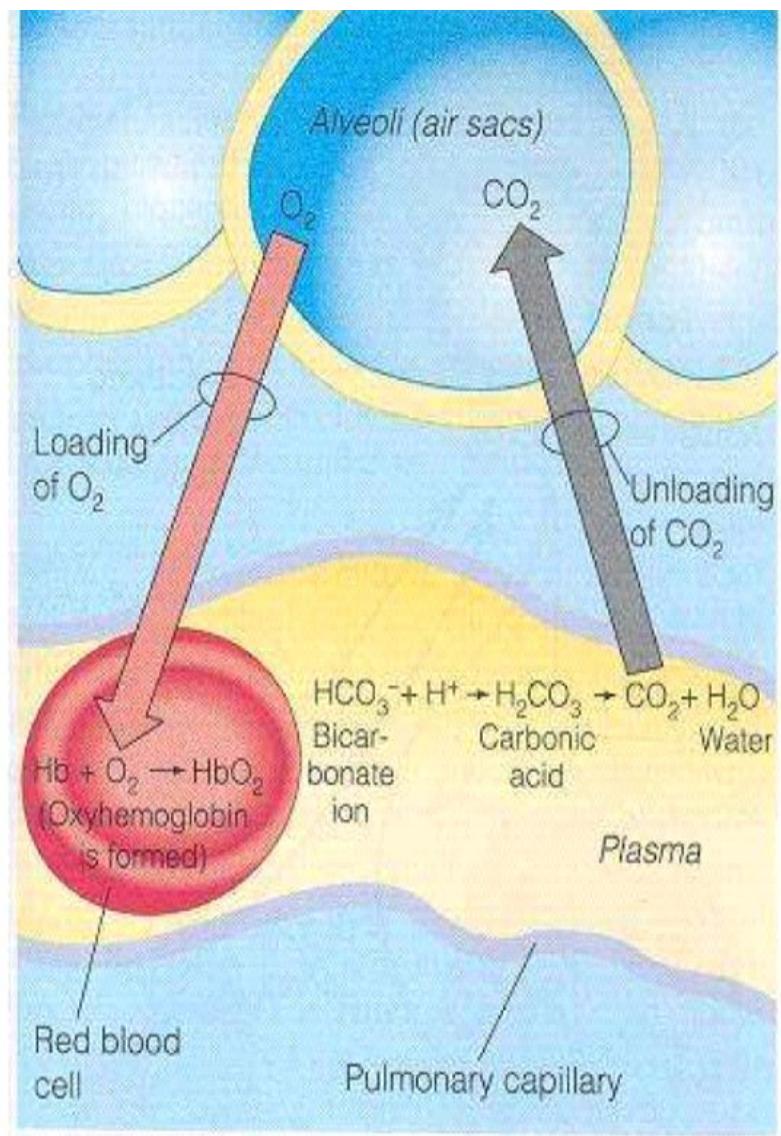
Catalytic Mechanism

The zinc coordination sphere of the "resting" enzyme

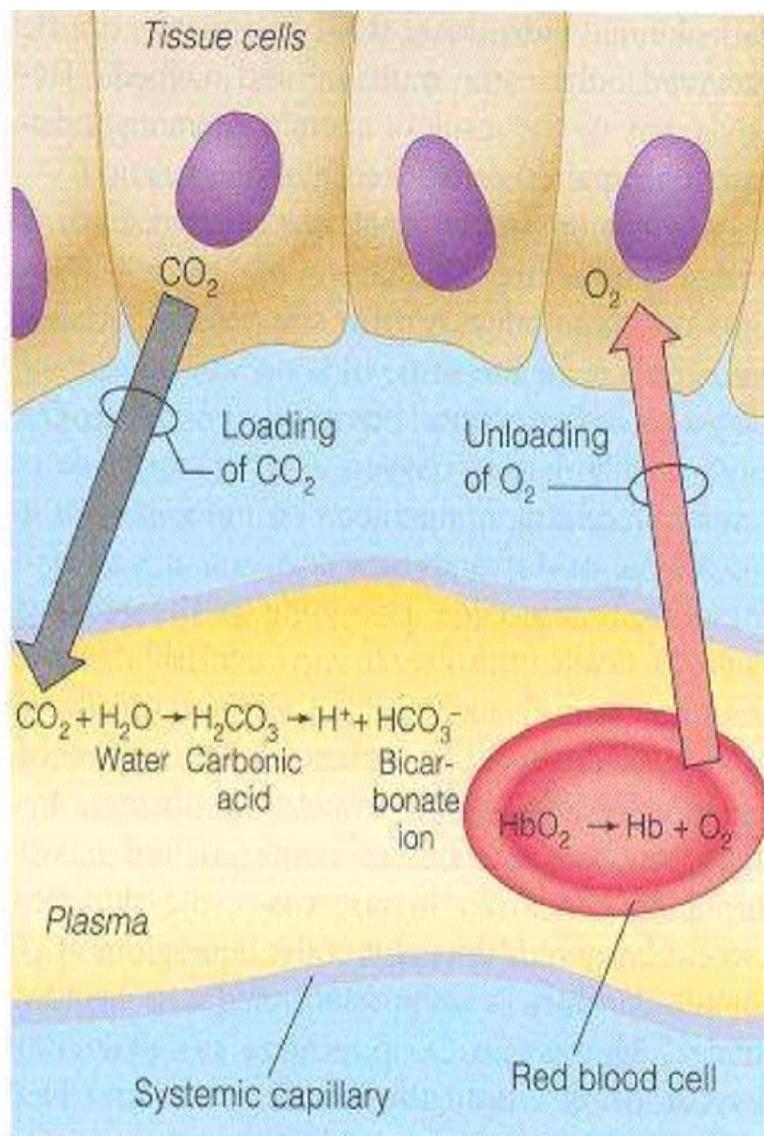


The enzyme "cleft"





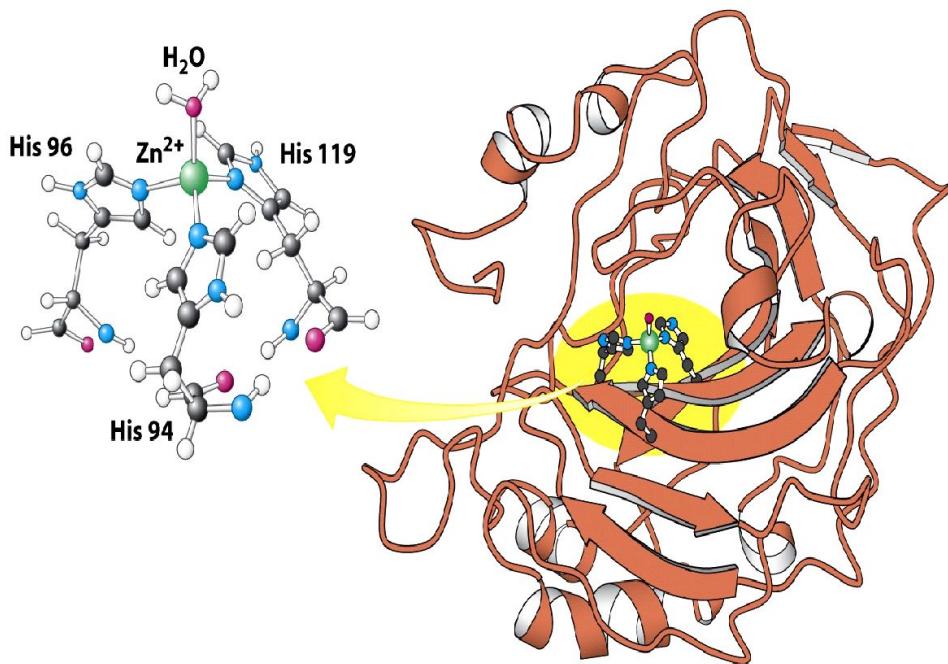
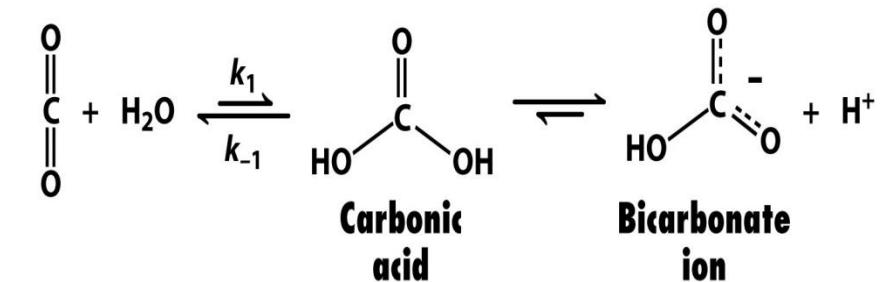
(a)



(b)

Diagrammatic representation of the major means of oxygen (O_2) and carbon dioxide (CO_2) loading and unloading in the body.

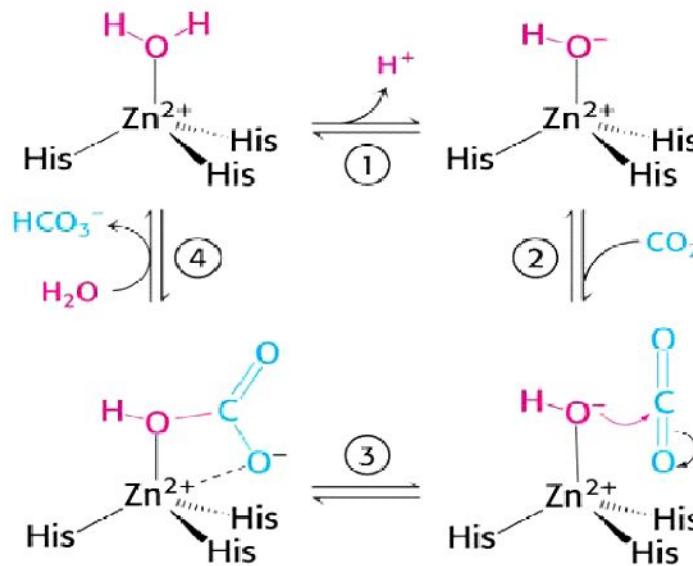
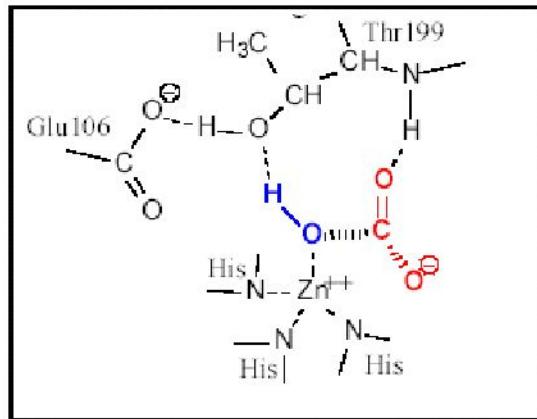
- 1) carbon dioxide
 - a major end product of aerobic metabolism
- 2) carbonic anhydrase as Zn^{2+} metalloenzyme
 - positive charge: strong but kinetically labile bonds
 - more than one oxidation state
 - Zn^{+2} : four or more ligands
 - three histidines + water
- 3) Zinc activation of water



convergent evolution for generation zinc-based active sites

- α -carbonic anhydrase: one conserved histidine
- β -carbonic anhydrase: three conserved histidines
- γ -carbonic anhydrase: trimeric, zinc sites at the interfaces

Reaction Mechanism



- (1) $Zn(II)$ assists in the formation of a nucleophile (hydroxide) with a pK_a of near 7.0
- (2) CO_2 binds to enzyme's active site and is positioned to react with hydroxide
- (3) Hydroxide attacks carbon dioxide converting it to the bicarbonate ion
- (4) Water molecule binds to active site, thus releasing bicarbonate product

Climate Change

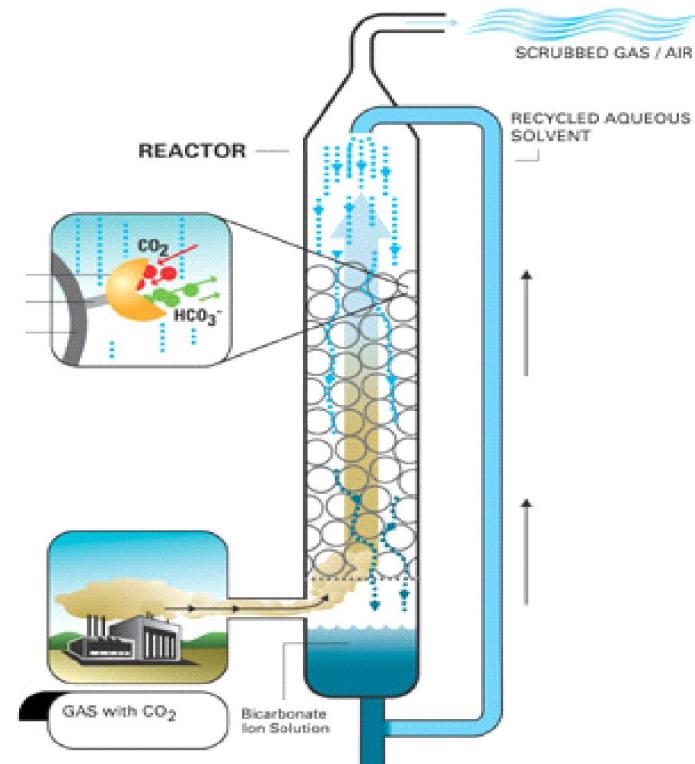
Nature does capture CO₂

- **One more type of air pollution**
 1. Toxic gas pollution (acid rain)
 - Industries, local impacts
 2. Ozone layer depletion (CFC)
 - Industries and households, regional impacts
 3. Air temperature is rising (Greenhouse gases)
 - Industries, transports, agriculture, homes, individuals
 - Global impacts on nature and humans
- **Greenhouse Gases (GHG)**
 - Carbon dioxyde (CO₂)
 - Methane (CH₄)

- **Forests inhale atmospheric CO₂**
 - Not enough trees for man-made CO₂
- **CO₂ capture in human muscles**
 - Cellular CO₂ transformed into bicarbonate
 - Carbonic anhydrase enzyme
- **A new industrial bio-catalyst**
 - Genetically engineered enzyme
 - Allows industrial CO₂ capture

CO₂ Solution Inc. offers an innovative alternative

- CO₂ capture at a large municipal waste incinerator (Quebec City)
- CO₂ Solution's Industrial prototype
 - 5 weeks non-stop, 7/24
 - 50% CO₂ capture
 - Permanent sequestration
- Sound basis for larger scale-up



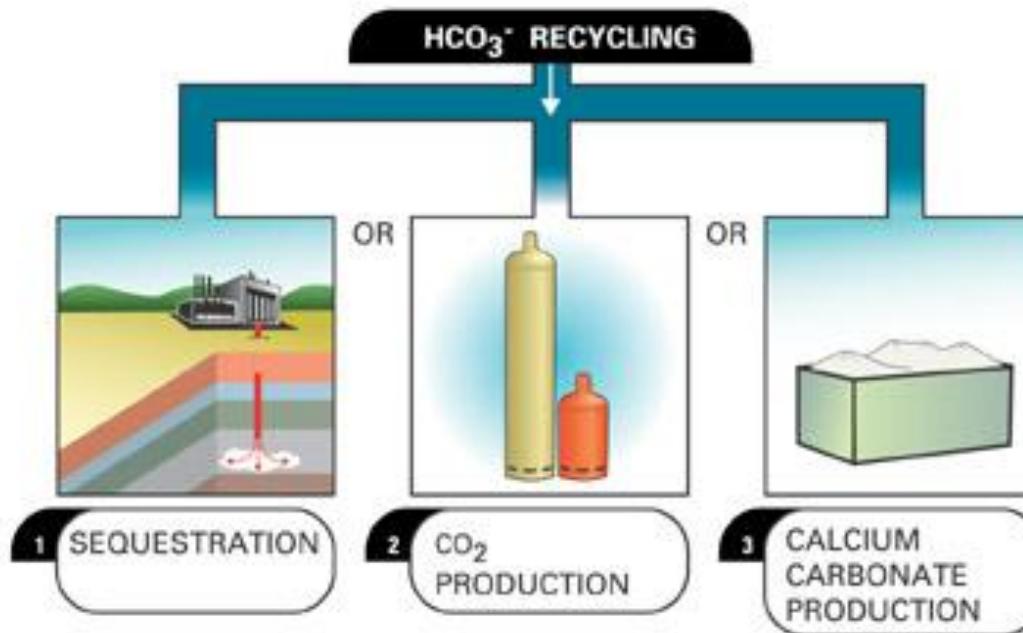
- CO₂ gas is converted into an unharful aqueous by-product, inside the reactor
- Low energy process
- Captured Carbon can be recycled

Sustainable Development

- CO_2 Solution's bio-catalysis treatment unit:
 - instantaneously converts harmful CO_2 gas
 - into unharful bicarbonate ion (HCO_3^{--}) solution

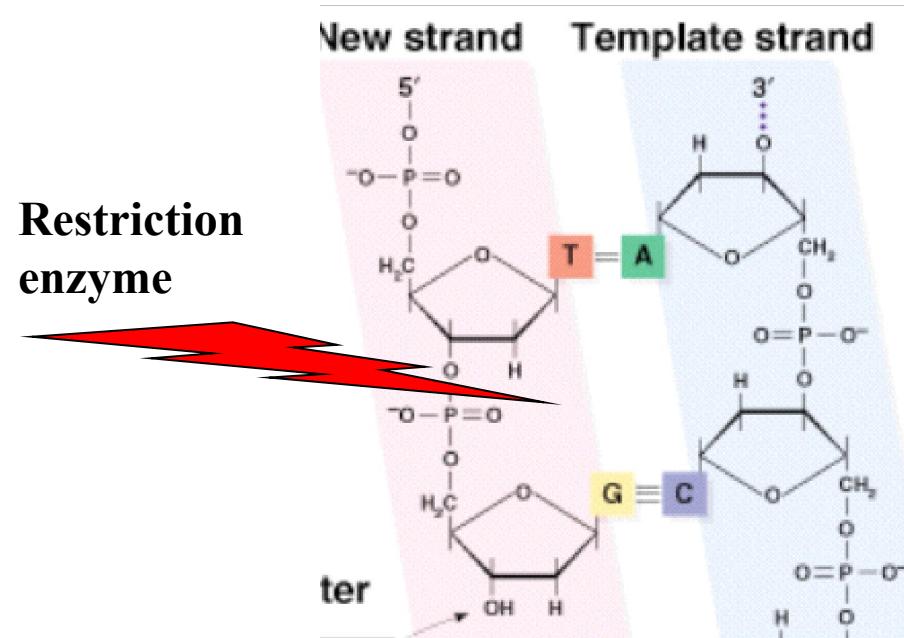
Offers flexible alternatives for CO_2 sequestration

Fossil carbon for energy production can be reused for chemicals



Restriction enzymes

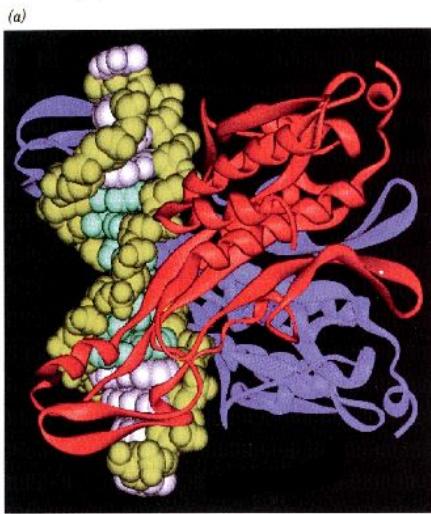
- Recognizes specific base sequences in double-helical DNA and cleave, at specific places, both strands of a duplex containing the recognized sequences.
- Restriction enzymes recognize specific bases pair sequences in DNA called restriction sites and cleave the DNA by hydrolyzing the phosphodiester bond.
- Cut occurs between the 3' carbon of the first nucleotide and the phosphate of the next nucleotide.
- Restriction fragment ends have 5' phosphates & 3' hydroxyls.



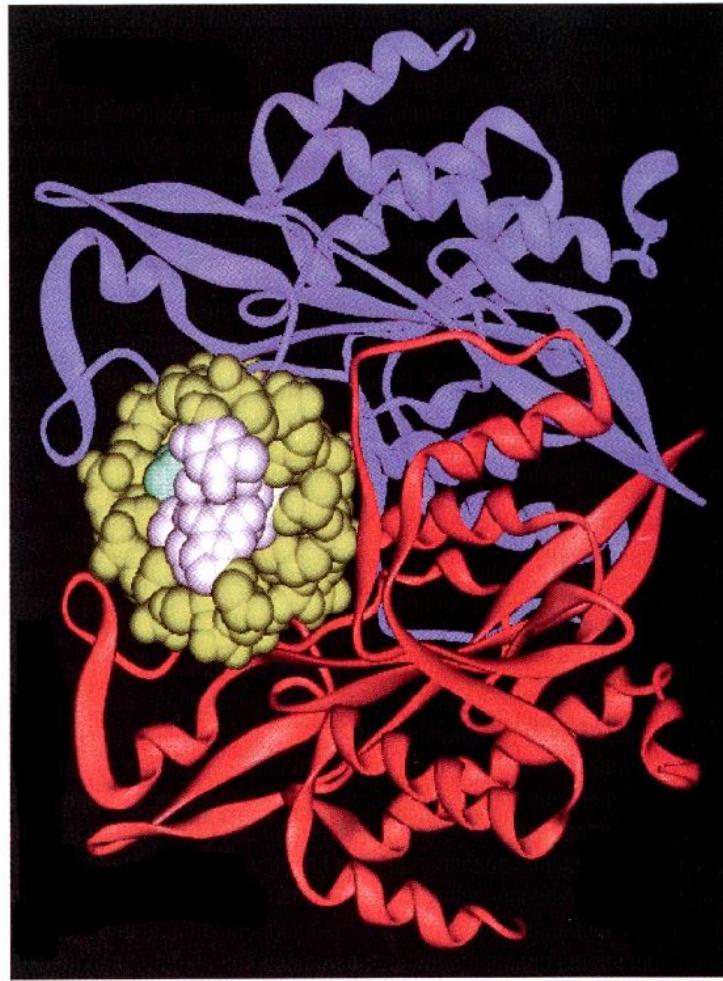
- Most restriction enzymes occur naturally in bacteria.
- Protect bacteria against viruses by cutting up viral DNA.
- Bacteria protects their DNA by modifying possible restriction sites (methylation).
- More than 400 restriction enzymes have been isolated.
- Names typically begin with 3 italicized letters.
 - Enzyme Source
 - *Eco*RI *E. coli* RY13
 - *Hind*III *Haemophilus influenzae* Rd
 - *Bam*HI *Bacillus amyloliquefaciens* H
- Many restriction sites are palindromes of 4-, 6-, or 8-base pairs.
- Short restriction site sequences occur more frequently in the genome than longer restriction site sequences, e.g., $(1/4)n$.

Structure of a restriction endonuclease

Restriction enzymes are usually dimers of identical subunits, analogous to the symmetry of their binding sites in DNA.



b)

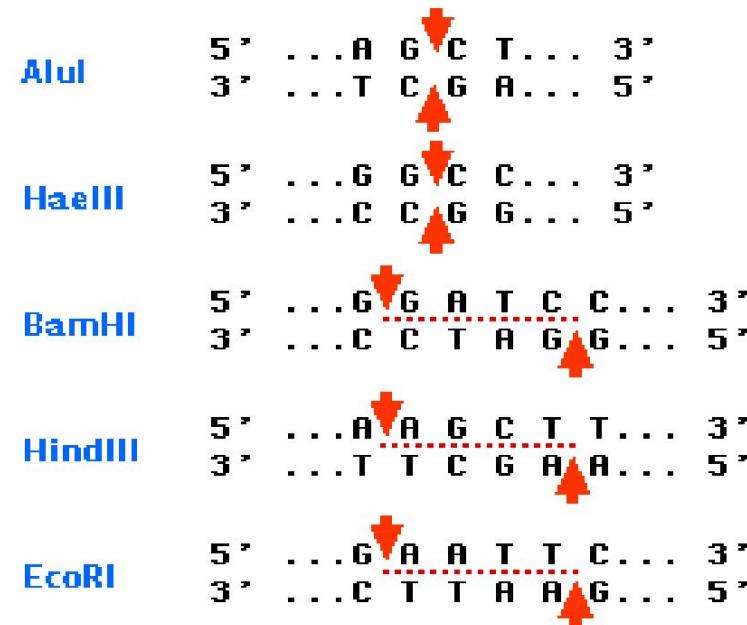


Patterns of DNA cutting by restriction enzymes

Base pairing between overhangs with complementary sequences enables two fragments to be joined by another enzyme DNA Ligase.

A sticky end fragment can be ligated not only to fragment from which it was originally cleaved, but also to any other fragment with a compatible sticky end.

The result is a molecule of recombinant DNA (rDNA)



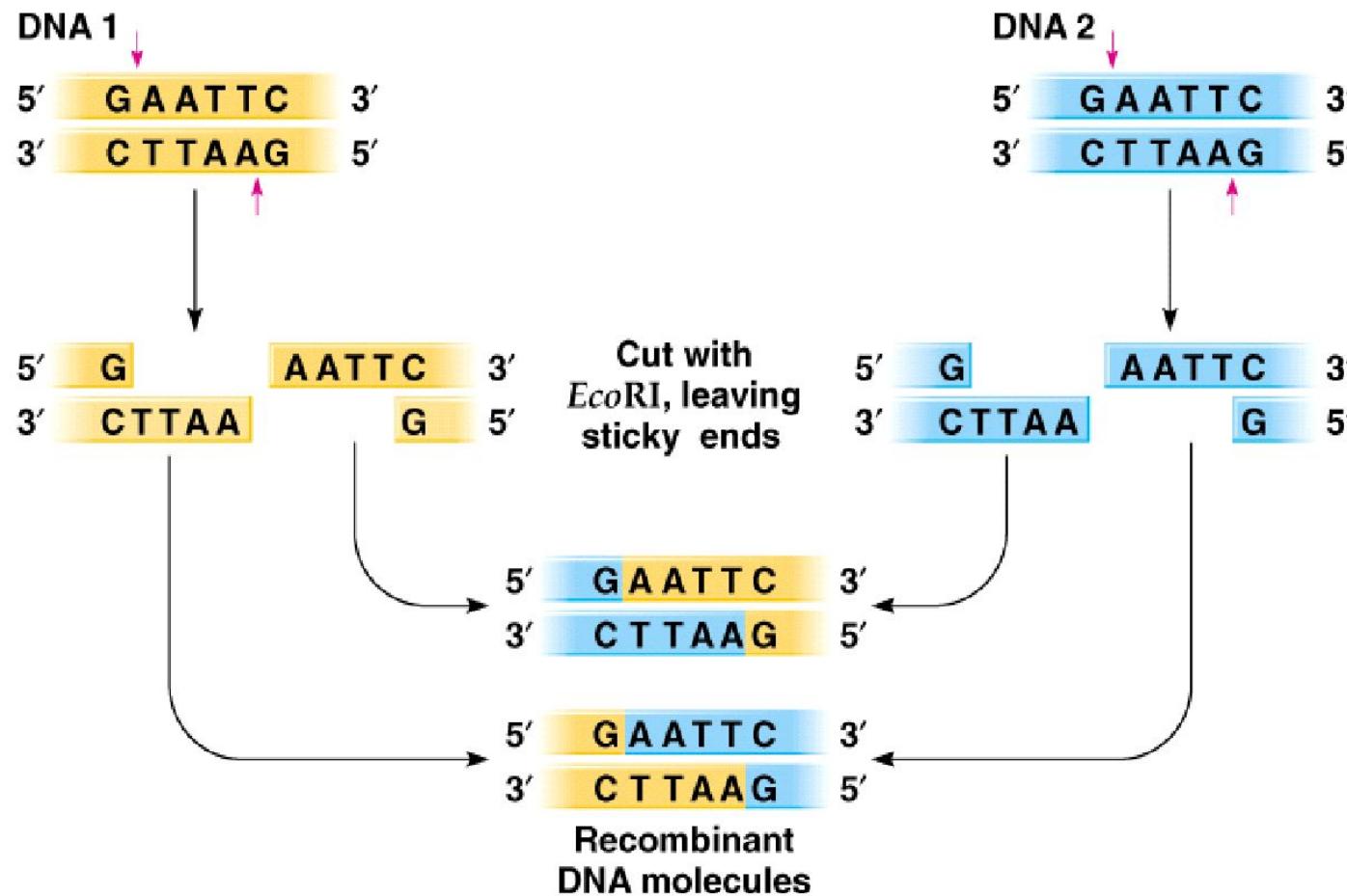
AluI and **HaeIII** produce blunt ends

BamHI **HindIII** and **EcoRI** produce "sticky" ends

Recombinant DNA:

DNA molecules constructed *in vitro*, consisting of DNA from 2 or more sources (i.e. cloning vector with foreign DNA inserted)

Cut and ligate 2 DNAs with *Eco*RI ---> recombinant DNA



Applications of Recombinant DNA technologies

- pharmaceutical products
 - insulin – cheaper and safer compared to animal insulin
 - vaccine sub-unit (against hepatitis B) – safer since will not be infected by pathogens
 - DNA of vaccines against malaria, influenza etc.
- gene therapy
 - replacing defective or missing gene with normal gene using adeno~ and retrovirus as vector
- gene silencing
 - known as RNA interference (RNAi) using dsRNA called short interfering RNA (siRNA) that target specific gene (mRNA) and degrade it
- human genome project
 - 3 billion human genome nucleotides have been sequenced
 - 20,000 – 25,000 genes
 - < 2% encodes function products, 98% intron, transposon (repeating sequence) etc
 - may provide diagnostics and treatments

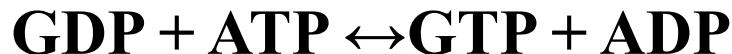
Purine synthesis

Purine monophosphates must be converted to the triphosphate forms before they can be utilized in DNA synthesis. This is done in two steps.

1. The nucleoside monophosphate kinase class of enzymes phosphorylates the monophosphate nucleotides.



2. The nucleoside diphosphate kinases add another phosphate group to the nucleotide diphosphates



Nucleoside monophosphate kinase (NMP kinase)

1) NMP + ATP (or NTP) \rightleftharpoons NDP + ADP (or NDP)

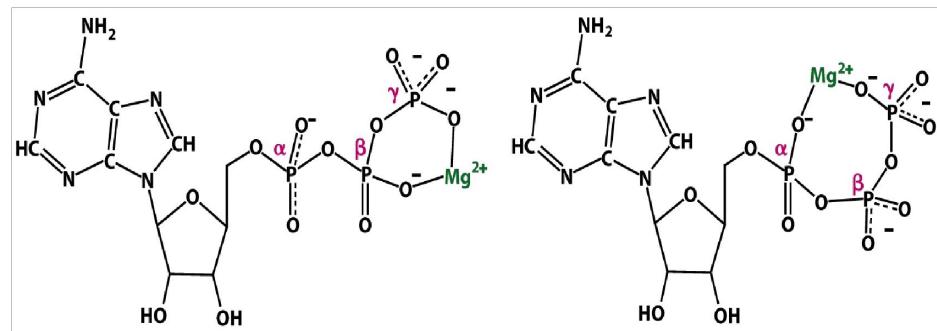
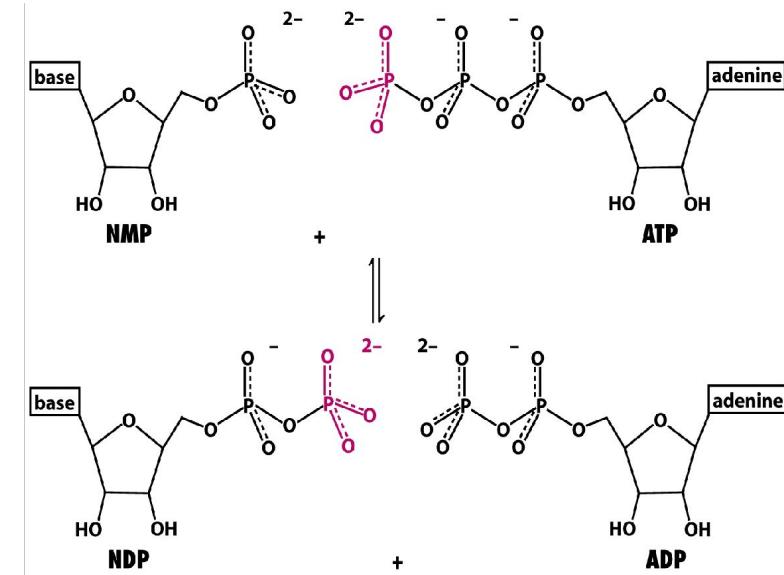
- transfer of a phosphoryl group from ATP to NMP, not to water
- induced fit

2) P-loop structure

- conserved NTP-binding domain: central β sheet surrounded by α helices
- a loop between the first β strand and the first α helix: Gly-X-X-X-X-Gly-Lys
- present in a wide variety of nucleotide-binding proteins

3) magnesium (or manganese) complex of NTP

- requirement of divalent metal ions, Mg²⁺ or Mn²⁺
- neutralization of some negative charges
- well-defined conformations of NTP by coordination: α and β , β and γ , or α and γ .
- more binding energy by interaction with NTP-Mg²⁺ complex

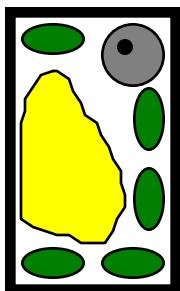


Photosynthesis

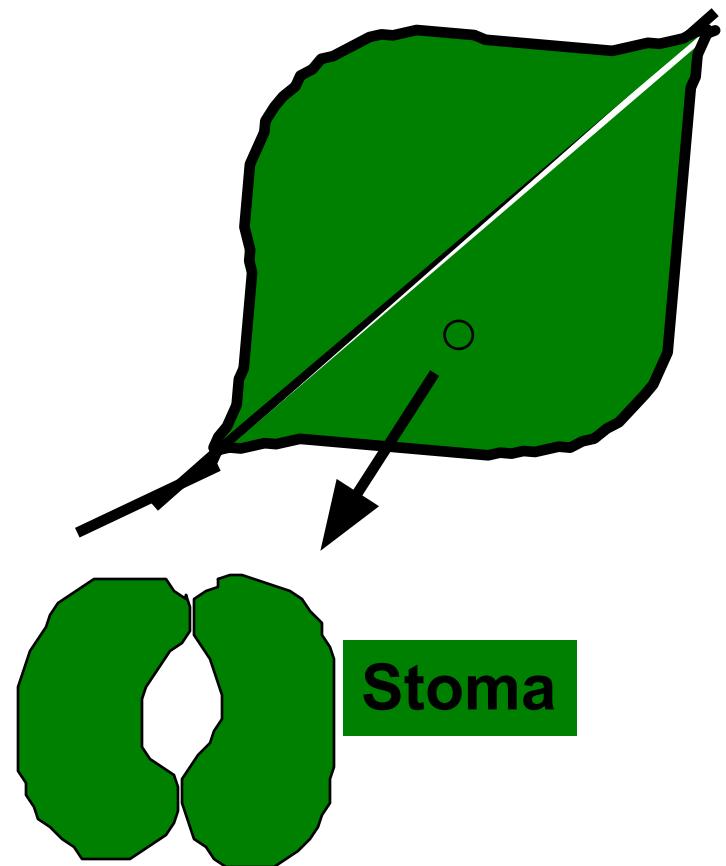
- Autotrophs – produce their own food (glucose) **Plants**
- Process called photosynthesis
- Mainly occurs in the leaves:
 - a. stoma - pores
 - b. mesophyll cells



Mesophyll
Cell →

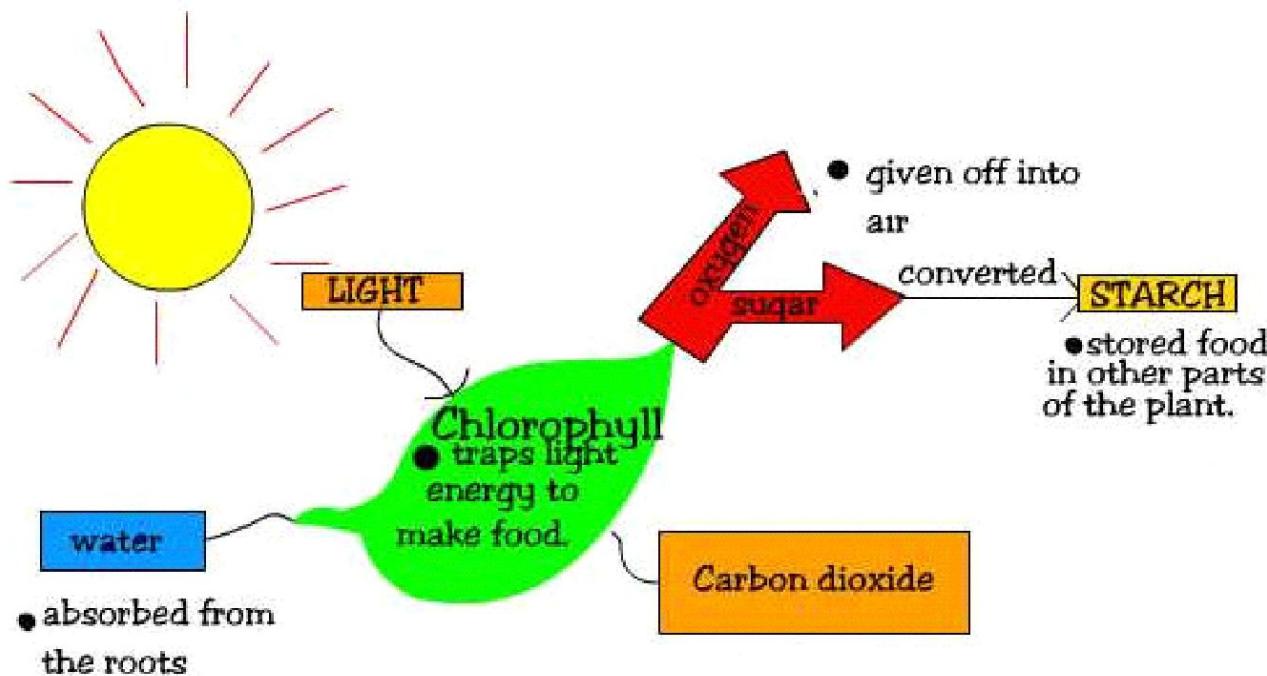
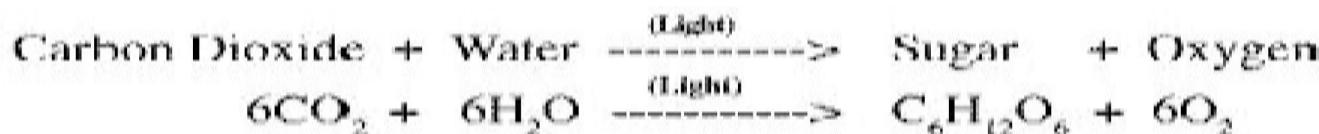
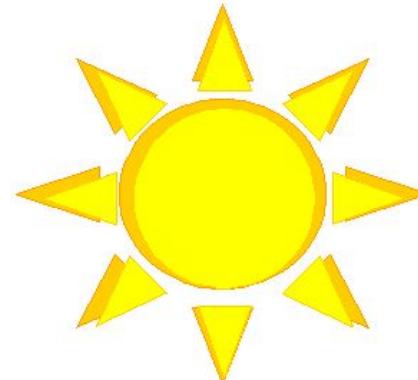


Chloroplast



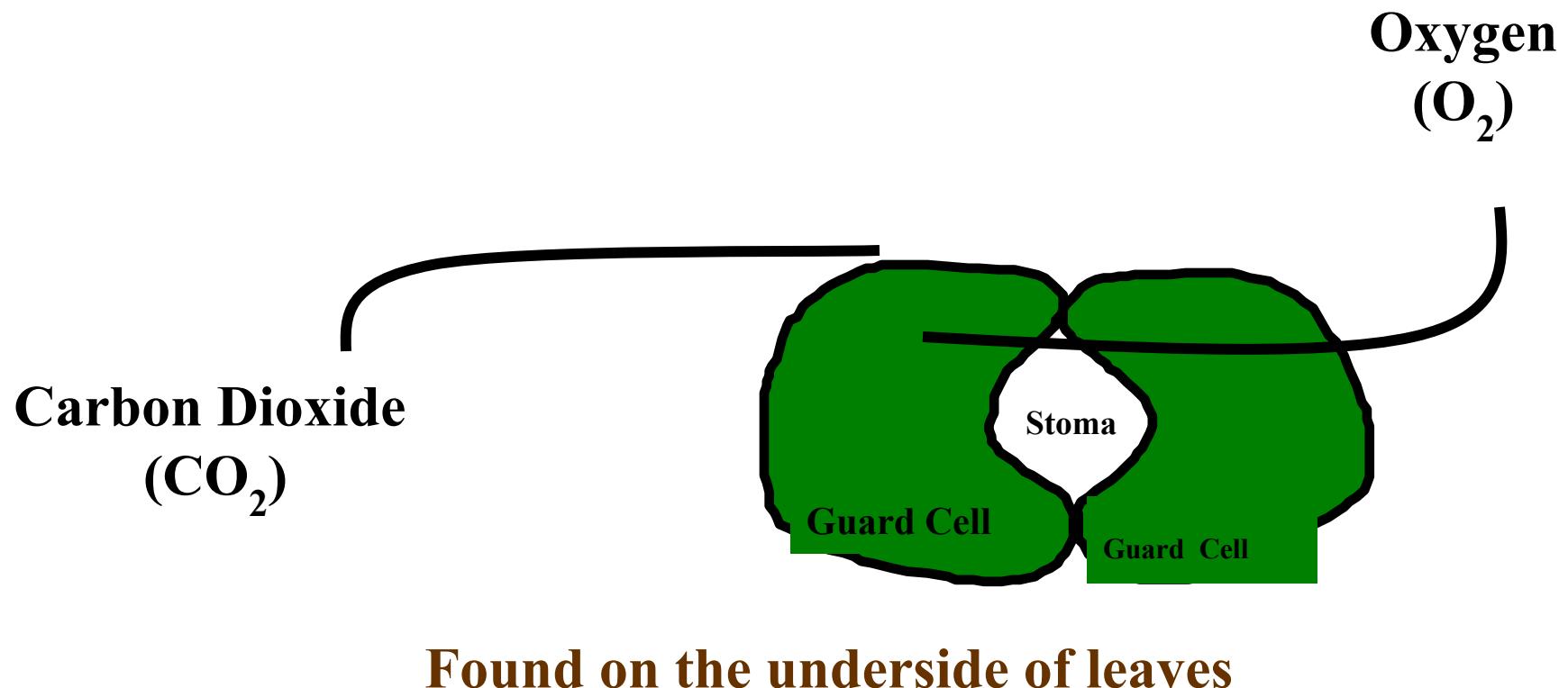
Photosynthesis

- Involves the Use Of light Energy to convert Water (H_2O) and Carbon Dioxide (CO_2) into Oxygen (O_2) and High Energy Carbohydrates (sugars, e.g. Glucose) & Starches



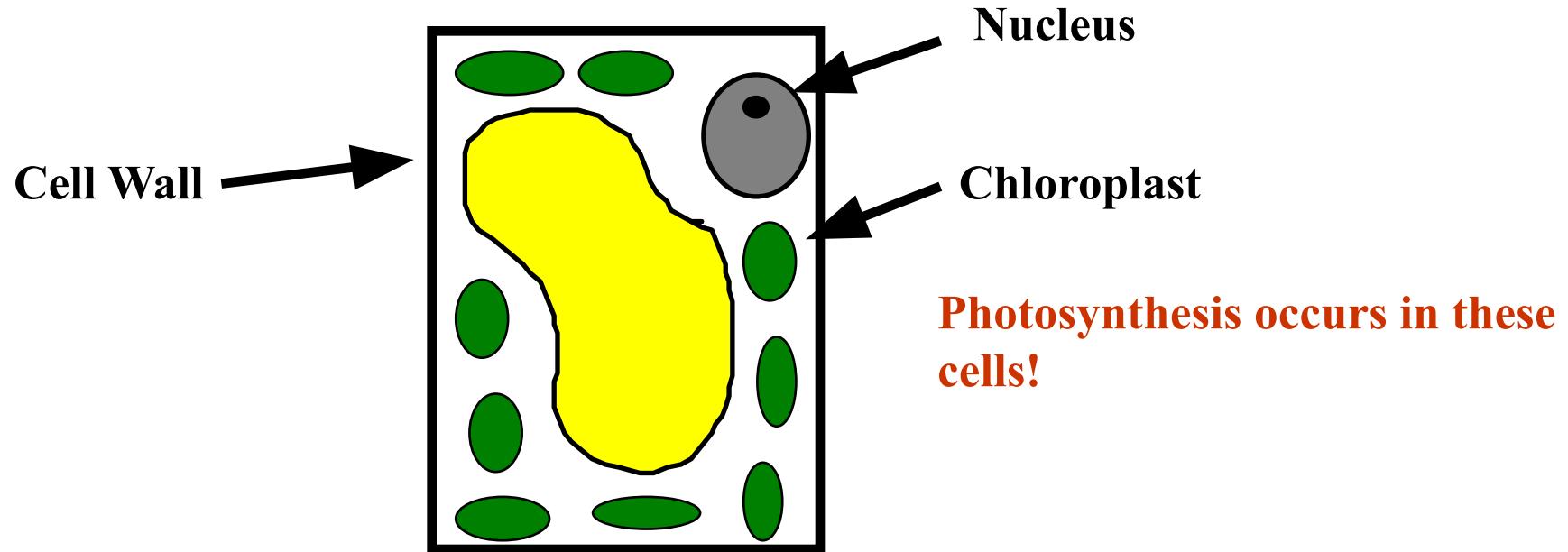
Stomata (stoma)

Pores in a plant's cuticle through which water vapor and gases (CO_2 & O_2) are exchanged between the plant and the atmosphere.



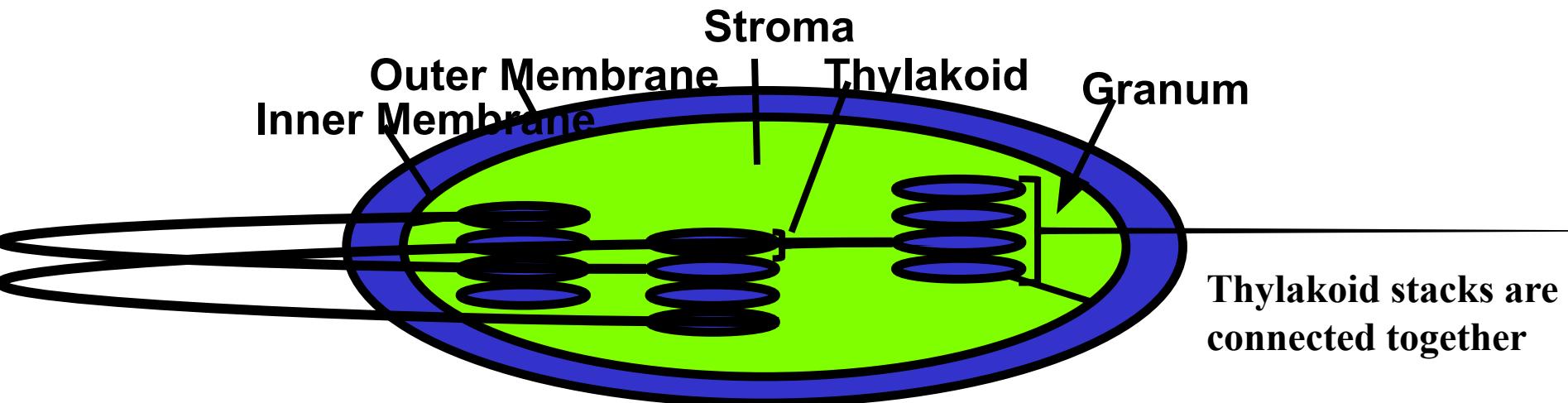
Found on the underside of leaves

Mesophyll Cell of Leaf

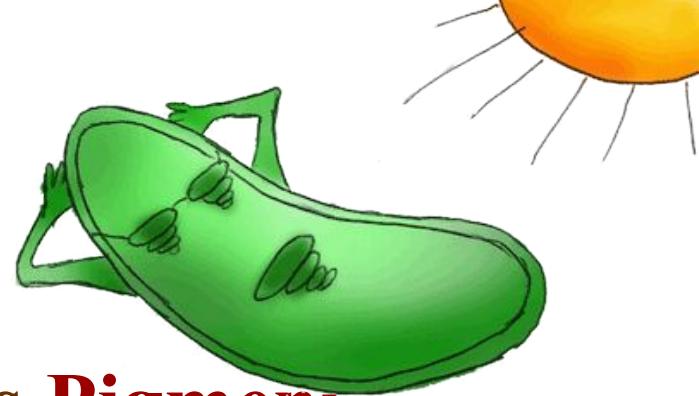


Chloroplast

Organelle where photosynthesis takes place.



Chlorophyll



- In addition to water, carbon dioxide, and light energy, photosynthesis requires **Pigments**
- Chlorophyll is the primary light-absorbing pigment in autotrophs
- Chlorophyll is found inside **chloroplasts**
- Located in the **thylakoid membranes**
- Chlorophyll have **Mg⁺** in the center
- Chlorophyll pigments harvest energy (photons) by absorbing certain wavelengths (**blue-420 nm and red-660 nm** are most important)
- Plants are **green** because the **green wavelength is reflected**, not absorbed.

Two Parts of Photosynthesis

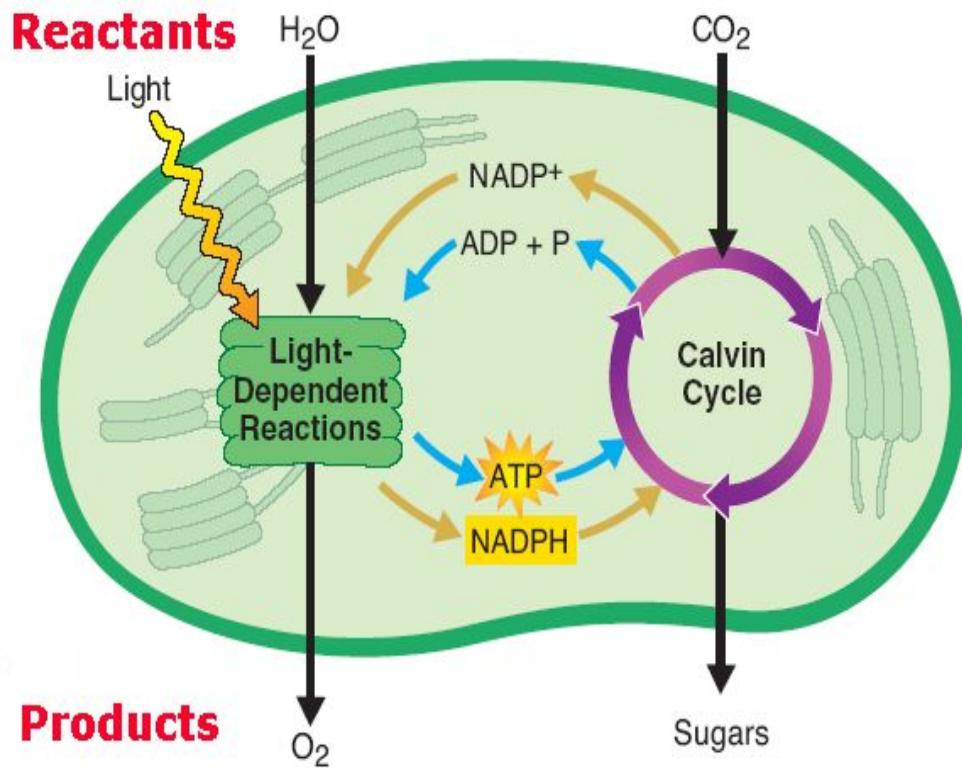
Two reactions make up photosynthesis:

1. Light Reaction or Light-dependent Reaction

- Produces energy from solar power (photons) in the form of ATP and NADPH.
- Occurs in Thylakoid membranes

2. Calvin Cycle or Light-independent Reaction

- Also called Carbon Fixation or C₃ Fixation
 - 3-carbon molecule called Ribulose Biphosphate (RuBP) is used to regenerate the Calvin cycle
- Uses energy (ATP and NADPH) from light reaction to make sugar (glucose).
- Occurs in Stroma



- Amount of available water
- Temperature
- Amount of available light energy



Biological Energy production

Bioenergetics

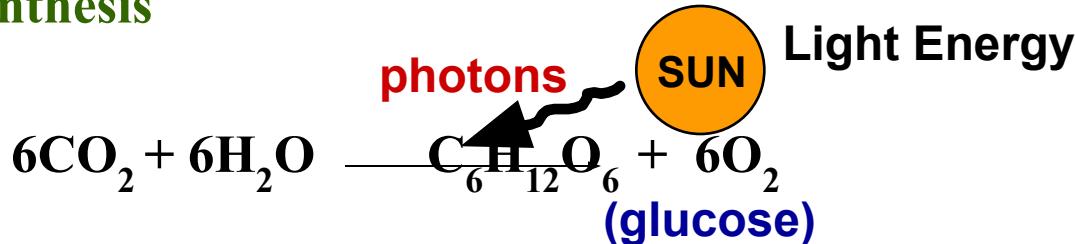
- The study of energy in living systems (environments) and the organisms (plants and animals) that utilize them
- Energy: Required by all organisms
 - May be Kinetic or Potential energy
- Kinetic Energy: Energy of Motion
 - Heat and light energy are examples
- Potential Energy: Energy of position
 - Includes energy stored in chemical bonds



Two Types of Energy Reactions

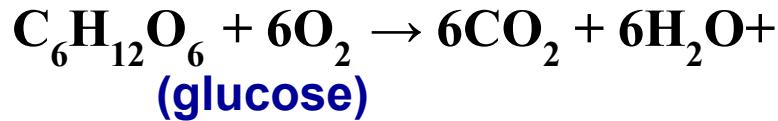
- Endergonic Reactions:

- Chemical reaction that requires a net input of energy.
 - Photosynthesis



- Exergonic Reactions :

- Chemical reactions that releases energy
 - Cellular Respiration



Two Types of Metabolism

- Anabolic Pathways

- Metabolic reactions, which consume energy (endergonic), to build complicated molecules from simpler compounds

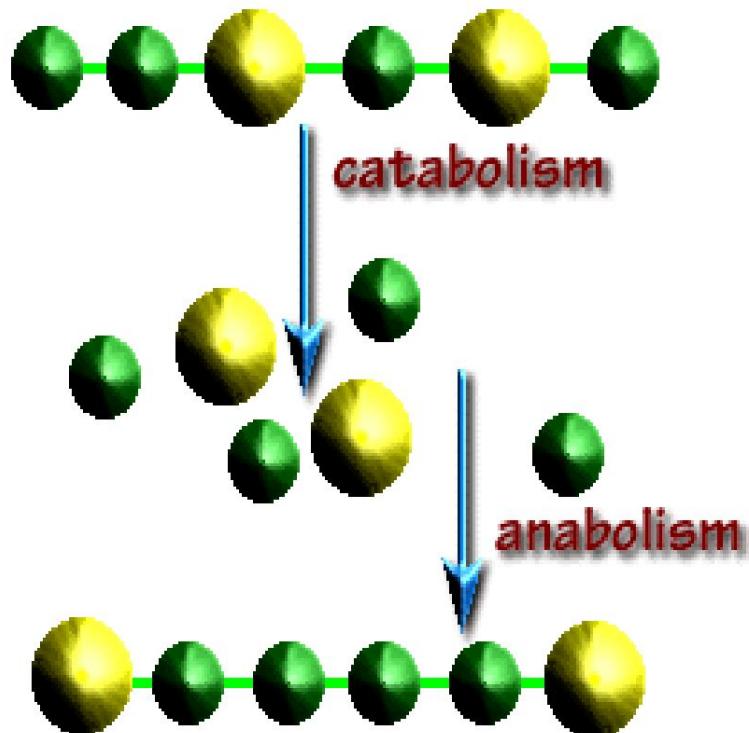
- Photosynthesis

- Catabolic Pathways

- Metabolic reactions which release energy (exergonic) by breaking down complex molecules in simpler compounds

- Cellular Respiration

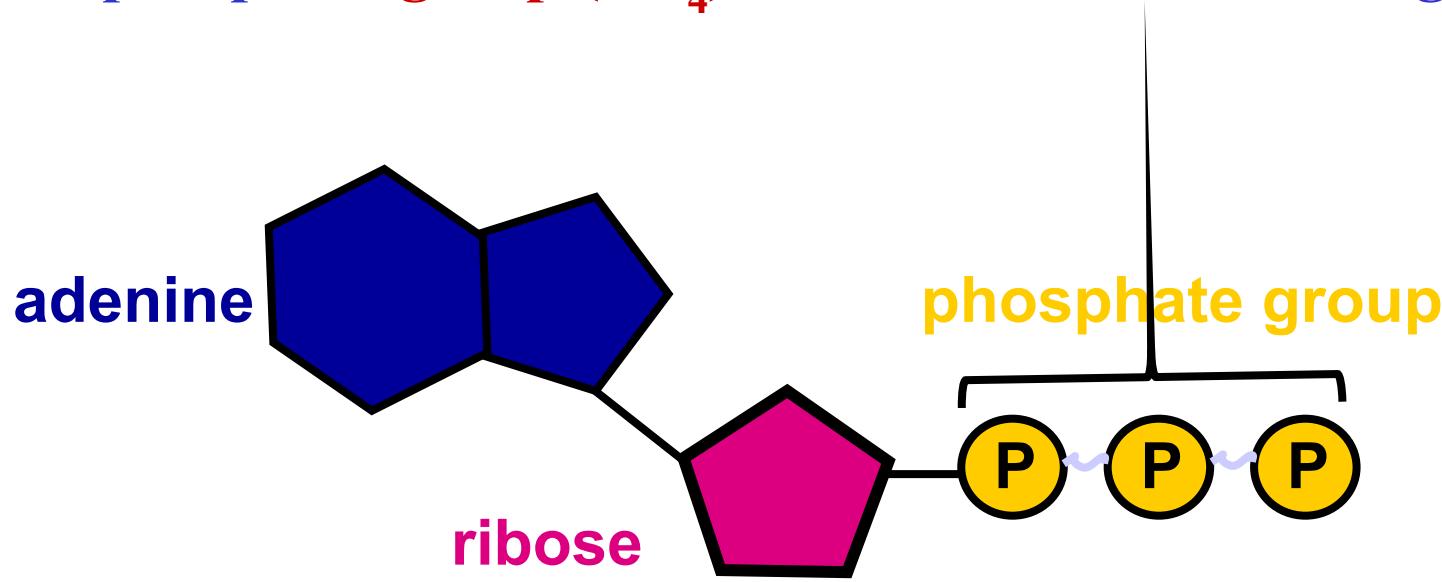
Metabolism
breaking down and building up



Cellular Energy-ATP

- Components:
 1. adenine: nitrogenous base
 2. ribose: five carbon sugar
 3. phosphate group: chain of 3

Three phosphate groups-(two with high energy bonds
Last phosphate group (PO_4) contains the MOST energy



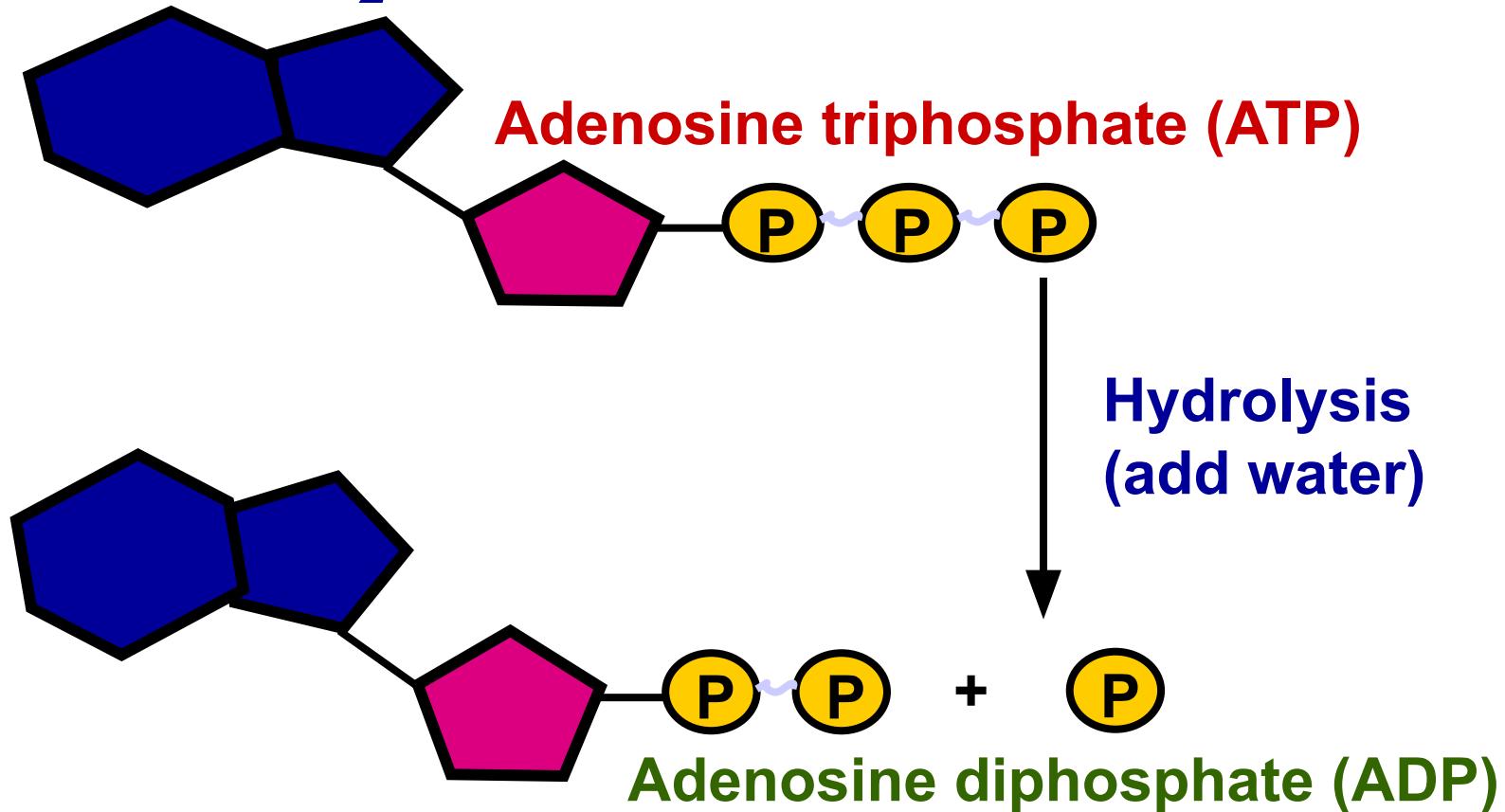
How does ATP work ?

- Organisms use enzymes to break down energy-rich glucose to release its potential energy
- This energy is trapped and stored in the form of adenosine triphosphate(ATP)
- It is estimated that each cell will generate and consume approximately 10,000,000 molecules of ATP per second

ATP - LIKE A RECHARGEABLE BATTERY

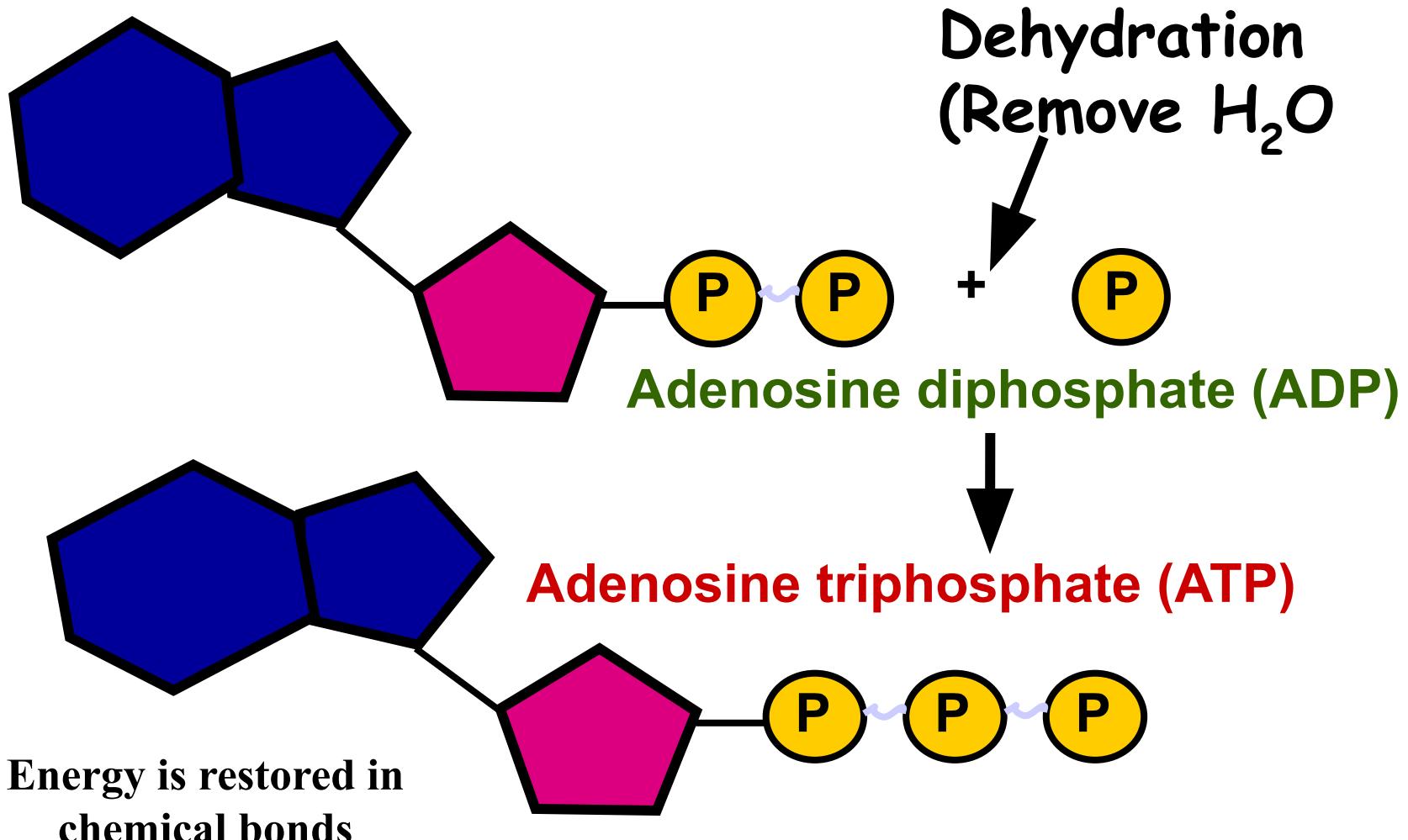
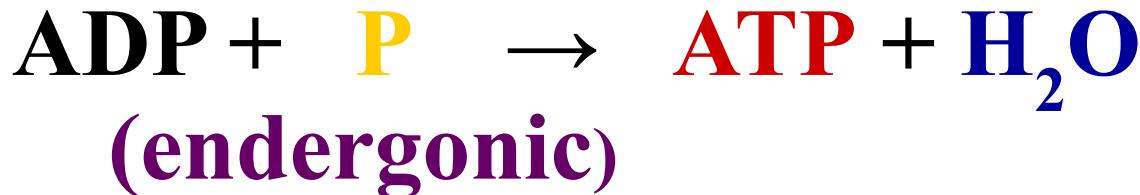


Hydrolysis of ATP

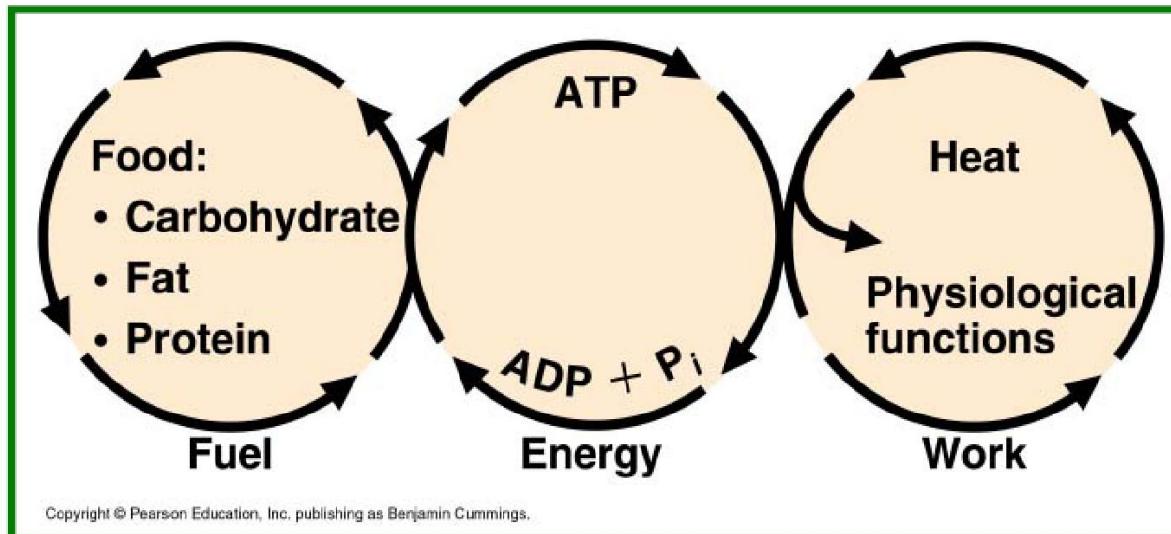


Energy is used by cells

Dehydration of ATP



Biological Energy Cycle



Biological Energy Cycle

- ***Cellular Respiration*** - The process by which cells transfer energy from food to ATP in a stepwise series of reactions; relies heavily upon the use of oxygen
- ***Anaerobic*** - In the absence of, not requiring, nor utilizing oxygen
- ***Aerobic*** - In the presence of, requiring, or utilizing oxygen

