ENZYMES AND COENZYMES

CEA November Lectures 2015

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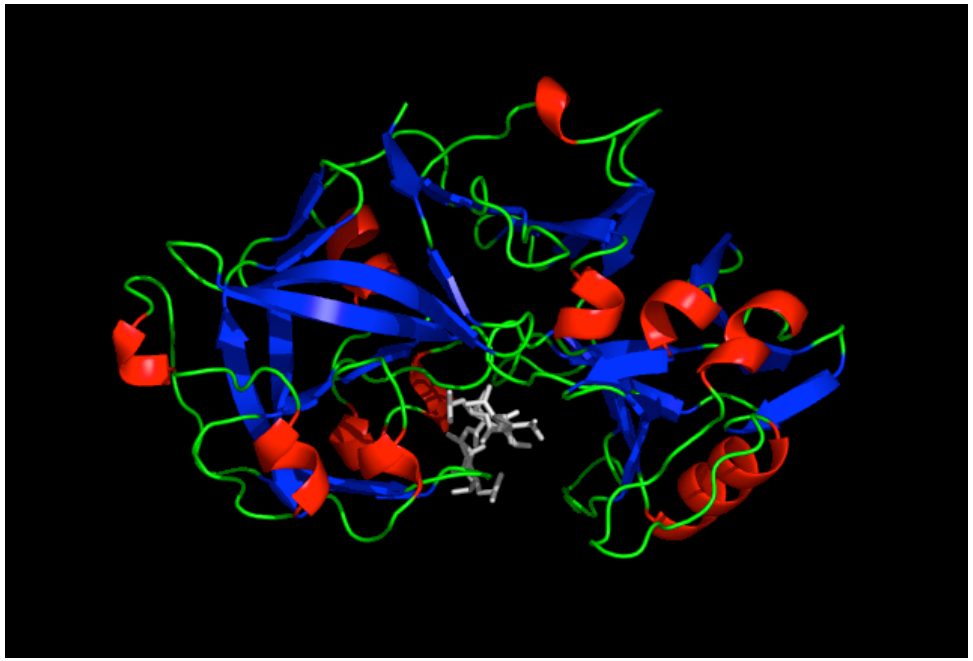
INTRODUCTION

- What is an enzyme (coenzyme)?
- The structure of enzymes
- How enzymes work



What is an enzyme?

Enzymes act as *catalysts* for almost all chemical reactions that occur in all living organisms.

Chemical reactions – the breaking and forming of bonds – are an essential process in living organisms.

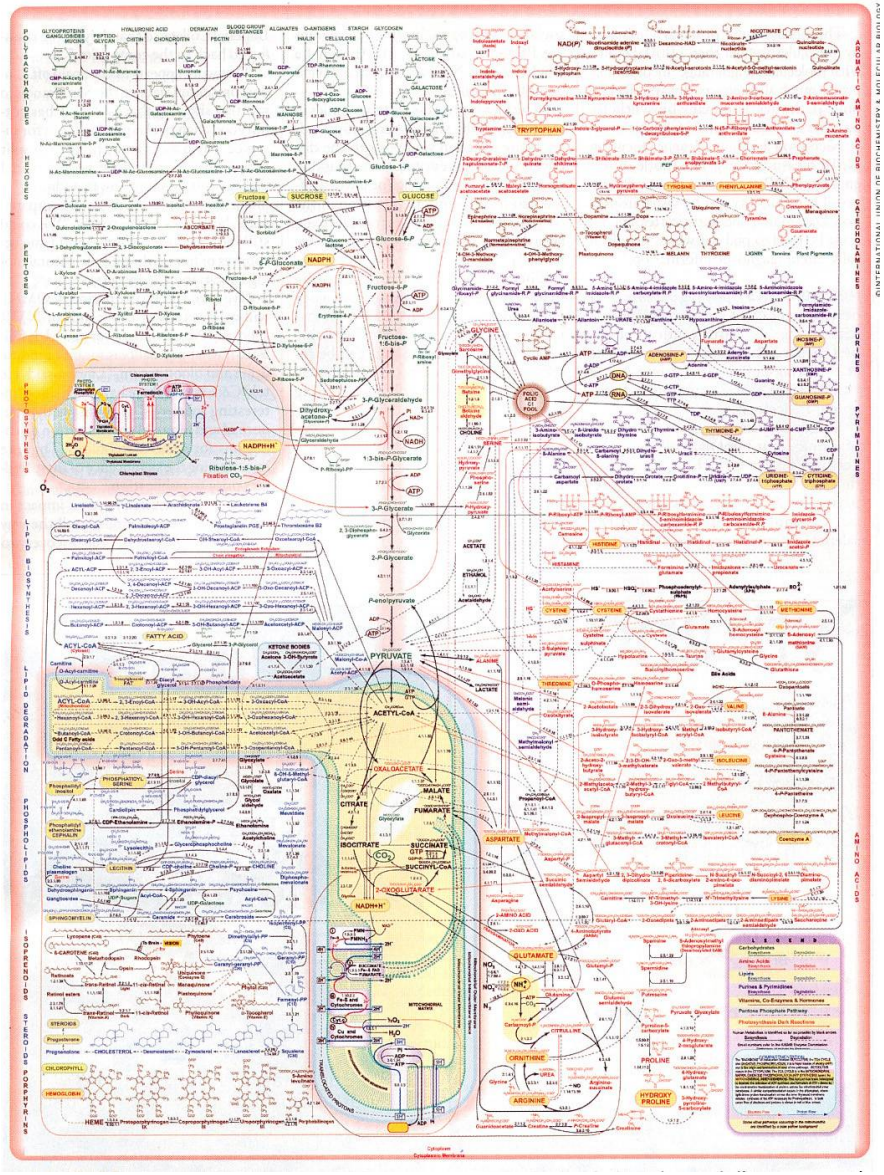
Enzymes allow reactions to occur more efficiently – much faster rate of reaction with less energy input.

The cellular chemical factory

Millions of reactions are occurring per second within a single cell.

The cellular ‘factory’ must be very organized to control such a huge variety of reactions taking place simultaneously.

A typical cell contains thousands of different enzymes, each assigned a specific purpose – to control a particular chemical reaction.



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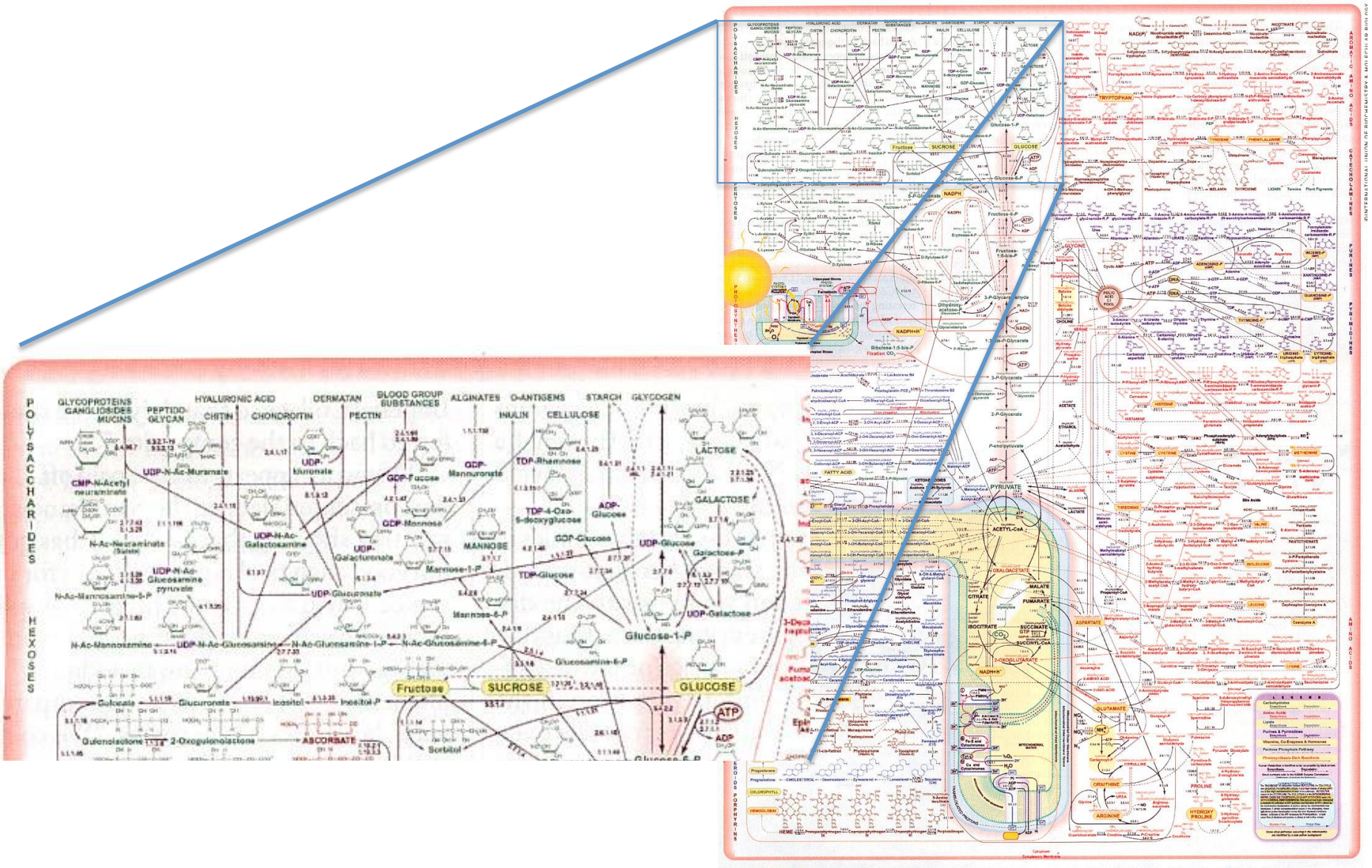
The cellular chemical factory



Characteristics of an enzyme

Enzyme properties:

1) Mild conditions are required for enzyme action

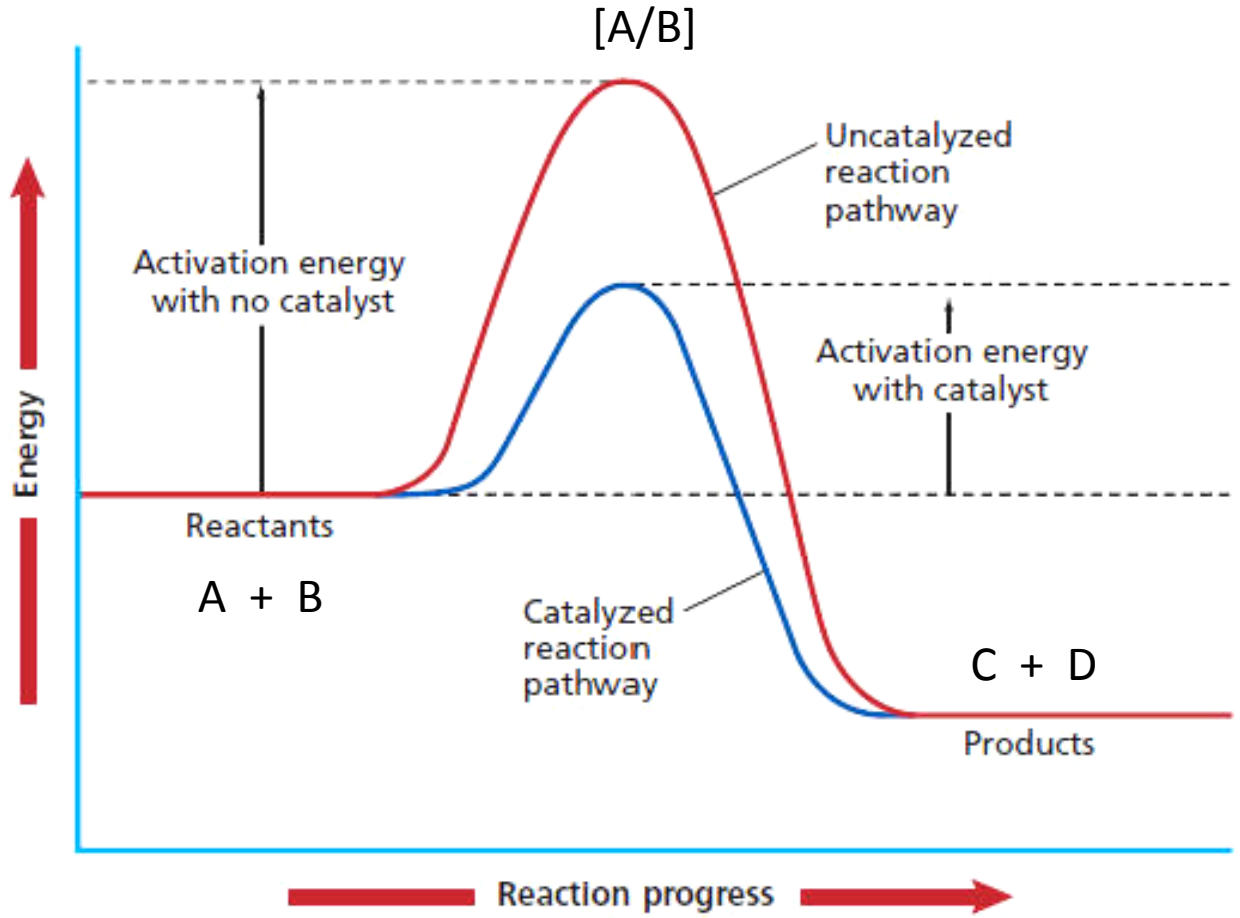
2) They have huge capacity
(very small amounts of enzyme required to produce large amount of product)

3) They usually have a high degree of specificity

4) Their activity can be controlled by substances other than their usual substrates

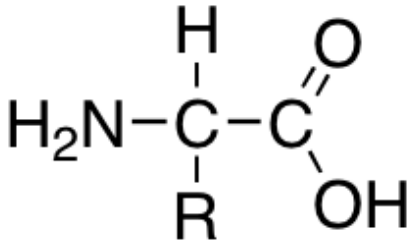
The reaction pathway

- Chemical reactions have an energy barrier to overcome – *activation energy*
- A catalyst will lower the activation energy by providing a different reaction pathway
- Catalyzed reactions will occur more quickly and require milder conditions

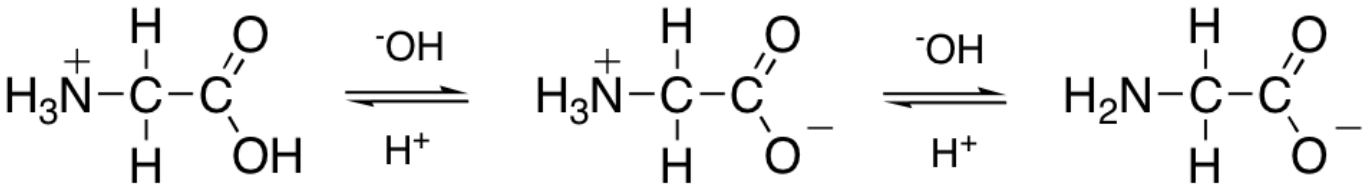


The structure of enzymes

- Amino acids act as the building blocks of enzymes
- Amino acids have the general structure:



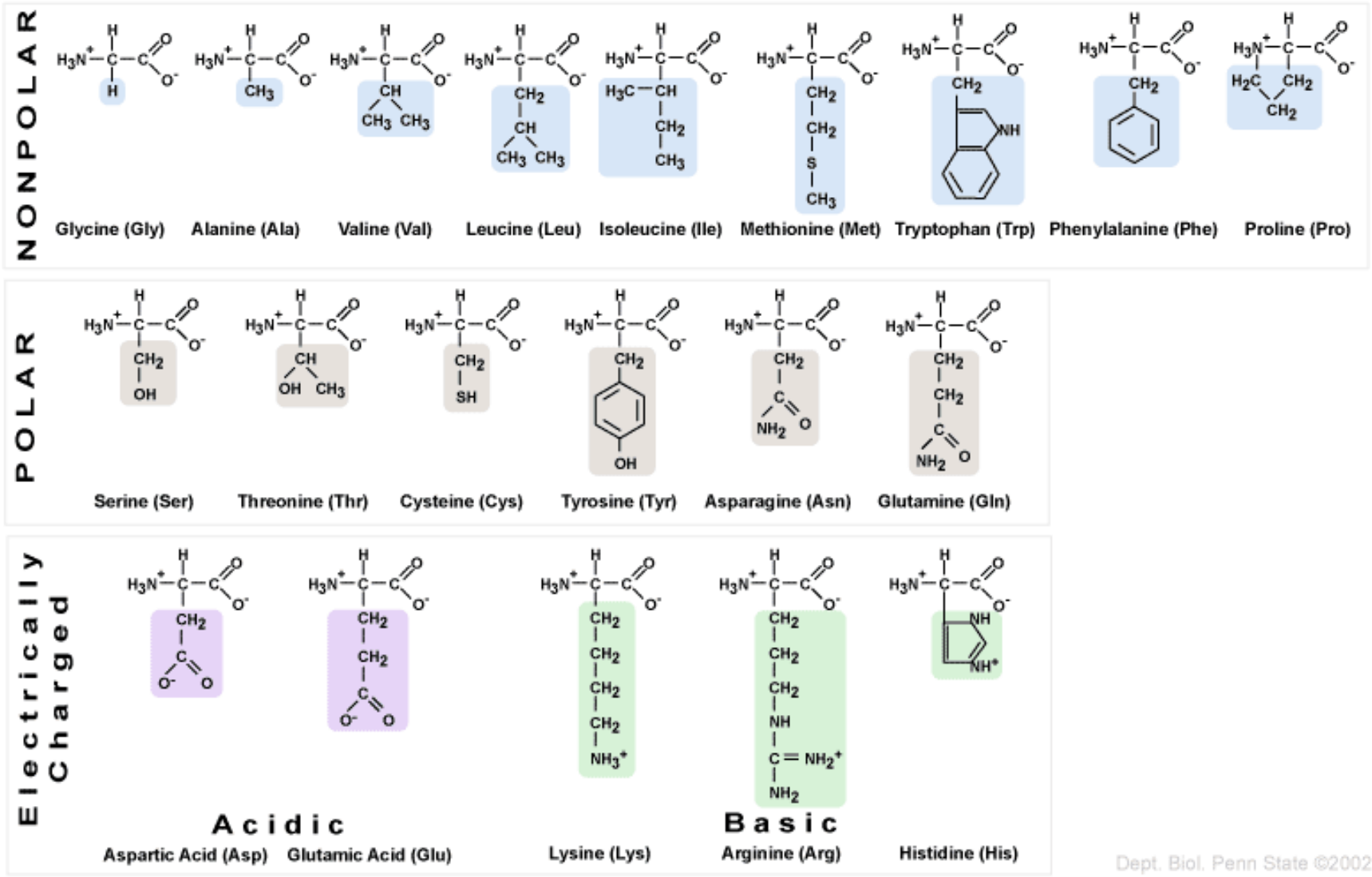
- The simplest amino acid is glycine (R=H)
- At physiological pH amino acids (e.g. glycine) exist as a zwitterions



low pH pH~7 high pH

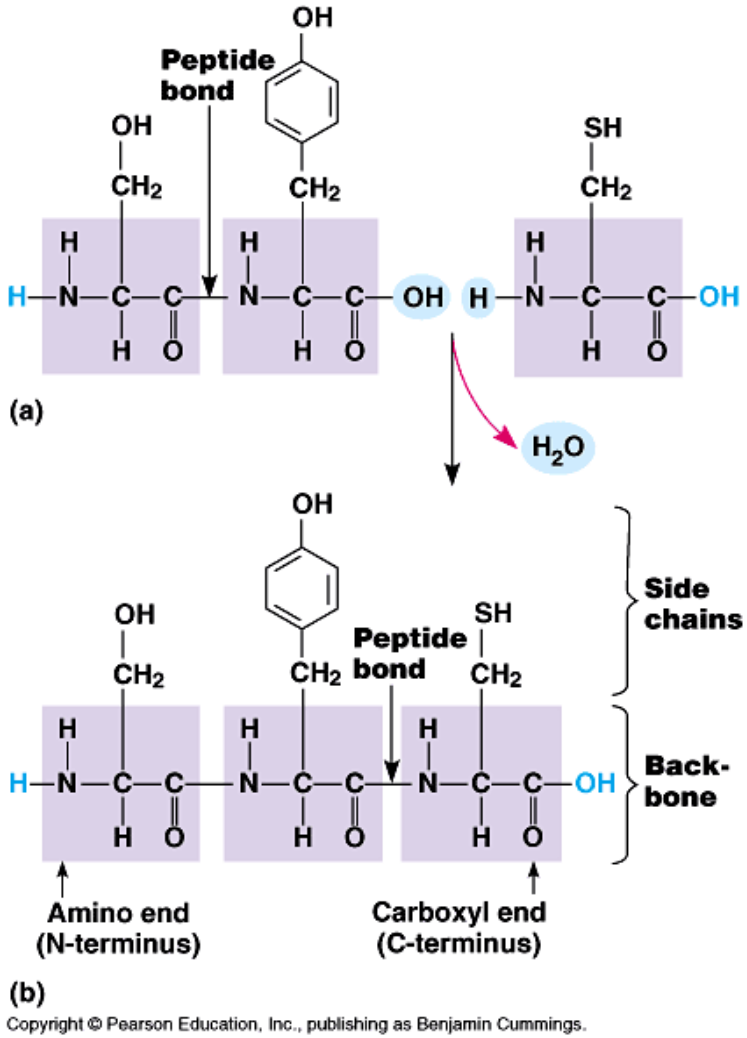
The structure of enzymes

The amino acids:



The structure of enzymes - primary

Amino acids join together by forming peptide bonds:



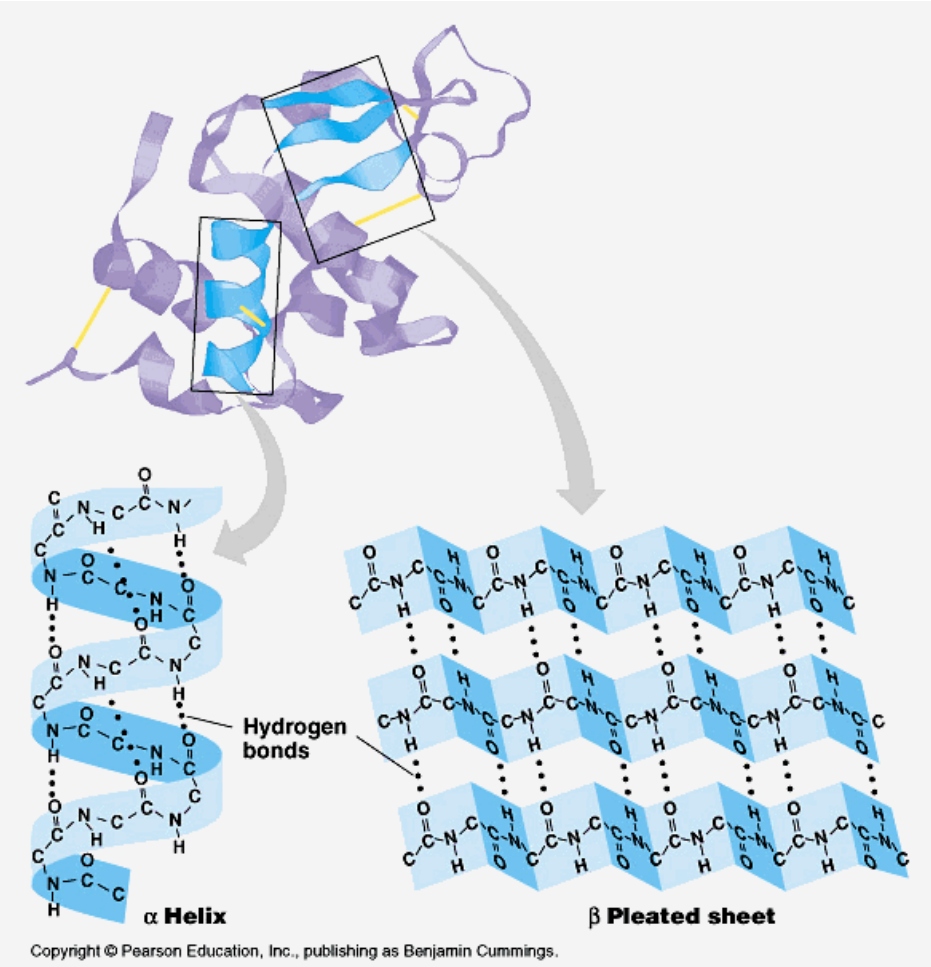
This reaction is an example of a condensation reaction

Short chains of amino acids are referred to as peptides
Long chains of amino acids are referred to as proteins

The completed amino acid chain is the PRIMARY structure of an enzyme

The structure of enzymes - secondary

Amino acid chains (proteins) form some regular structures:



- α-Helix
- β-pleated sheet

Hydrogen bonds can form between carboxyl and amino groups on the peptide backbone

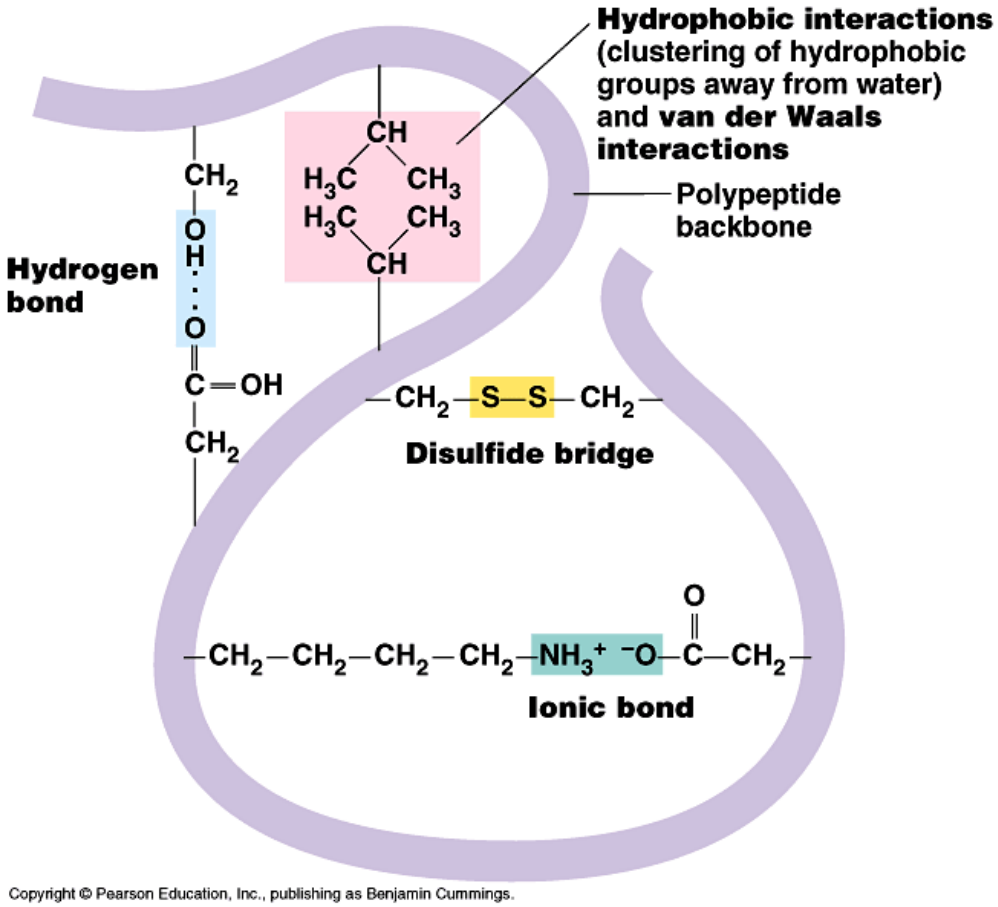
δ+ δ- δ+ δ-

C=O ····· H-N

Folding and coiling due to hydrogen bonding forms the SECONDARY structure of an enzyme

The structure of enzymes - tertiary

The final 3-D structure results from interactions between side chains on the amino acids.



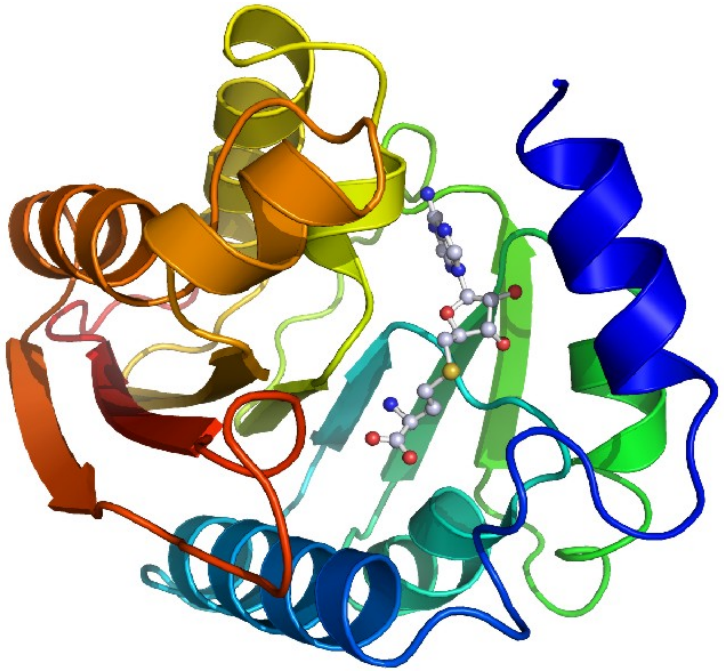
Stabilized by interactions between 'like' groups or 'complementary' groups

Relies on the cumulative effect of <u>many</u> weak interactions.

Folding of a protein into the final 3-D shape is the TERTIARY structure of an enzyme

How do enzymes work?

- Each enzyme has a unique 3-D shape, and includes a surface groove called an active site.
- The enzyme works by binding a specific chemical reactant – the *substrate* – to its active site.
- Favorable interactions between the substrate and enzyme hold the substrate in the active site.

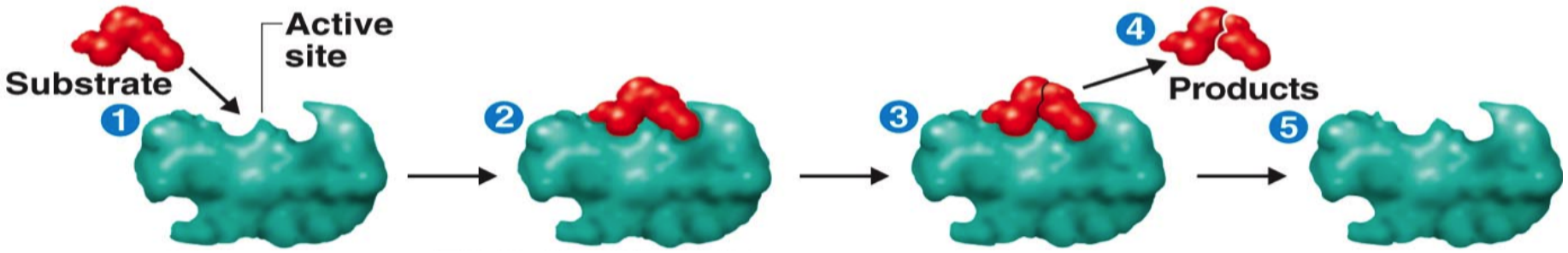


S-adenosyl-L-methionine (SAM) bound to the active site of an enzyme

SAM is used to transfer a methyl group to other molecules

How do enzymes work?

- Favorable interactions between the substrate and enzyme weaken bonds in the substrate allowing a reaction to take place.
- By assisting in the breaking and forming of bonds in the substrate, enzymes act to lower the activation energy of a reaction.
  The result is a *faster* reaction under *milder* conditions.

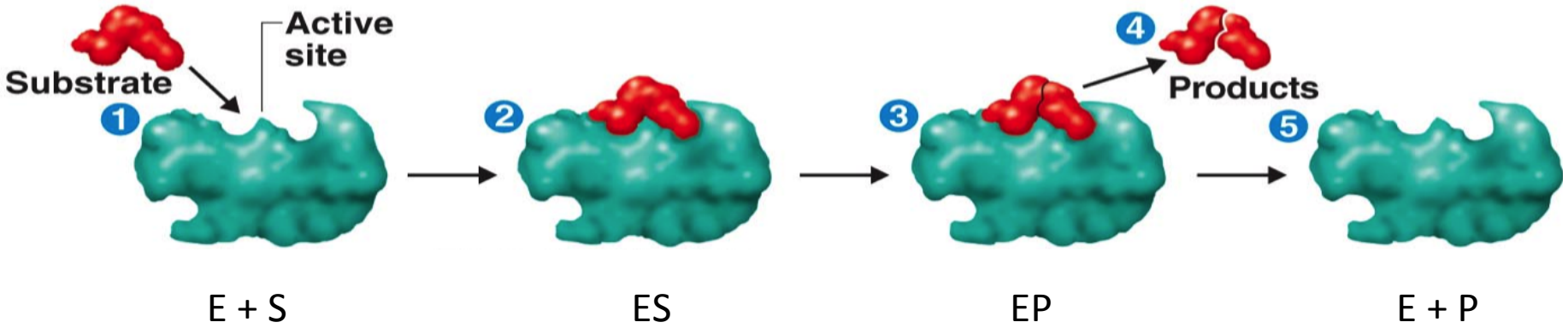


Two models are used to describe the process by which an enzyme acts:

1) 'Lock and Key' Model
2) 'Induced Fit' Model

How do enzymes work?

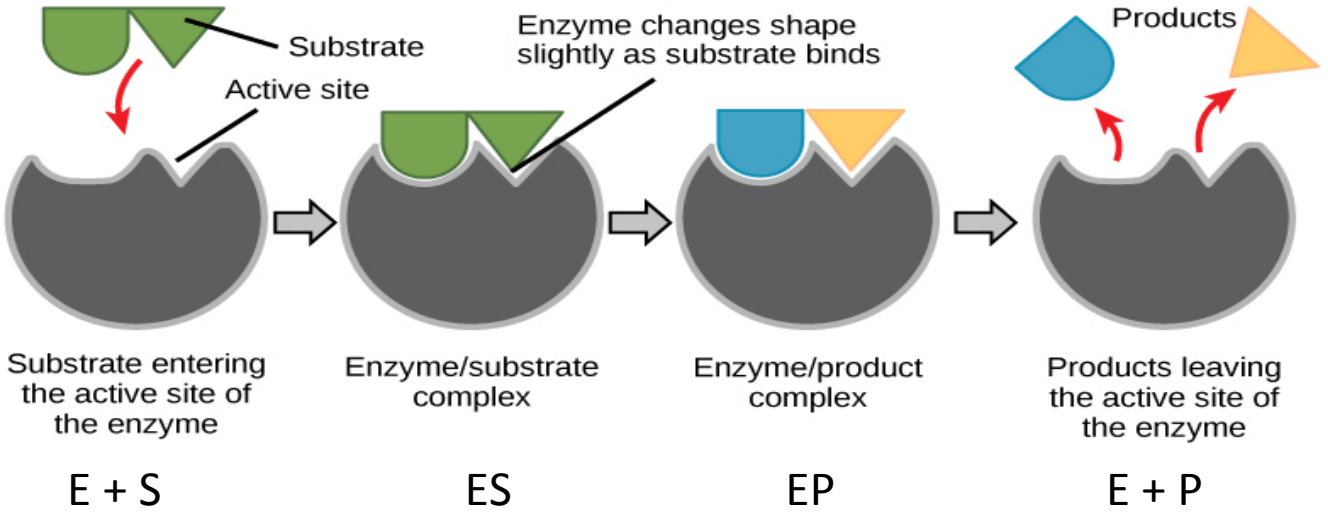
- **'Lock and Key'** Model
- Proposed by Emil Fisher in 1898
- The active site of the unbound enzyme is rigid and complementary in shape to the substrate
- Accounts for the specificity of enzyme-substrate interactions



- But how are some enzymes able to act on a variety of substrates?

How do enzymes work?

- **'Induced Fit'** Model
- Proposed by Daniel Koshland in 1958
- The active site of the unbound enzyme is flexible and can adjust to maximize interactions with the substrate
- As the substrate approaches conformational change takes place
- Accounts for ability of some enzymes to act on a range of substrates



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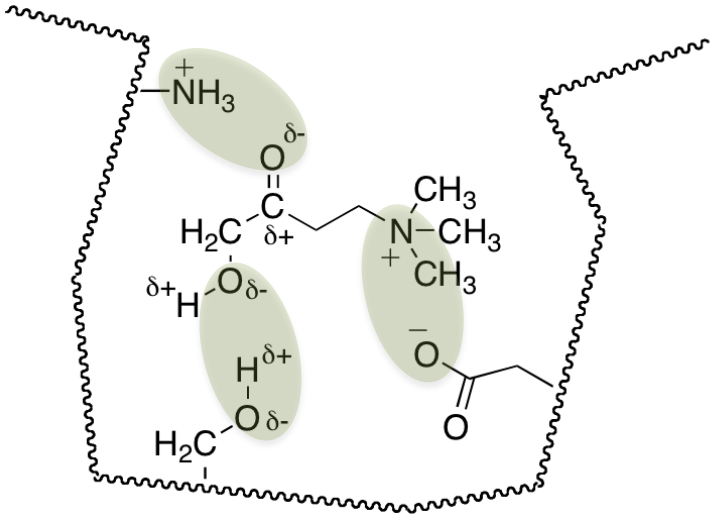
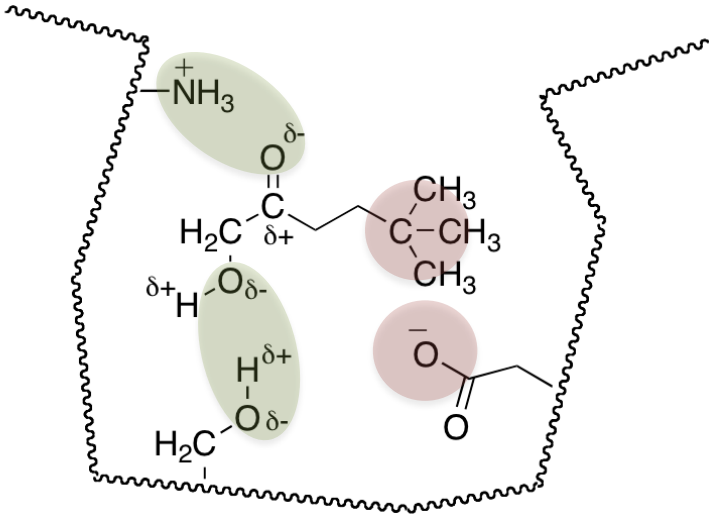
How do enzymes work?

- The active site of an enzyme must not only be the correct size and shape to fit the substrate, it must also have favorable interactions between the substrate and the amino acids in the active site.
- The same interparticle forces that hold the enzyme in its tertiary structure can occur between enzyme and substrate.



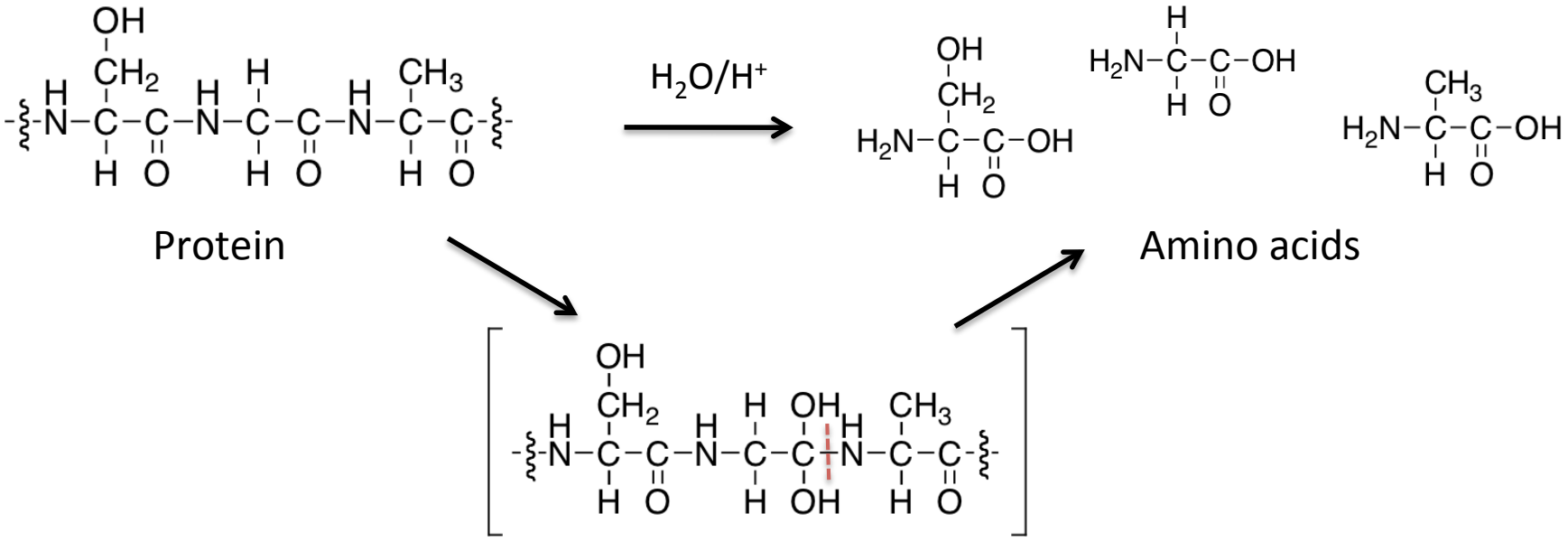
Substrate with correct shape
and favorable interactions



Substrate with correct shape
but mismatched interactions

How do enzymes work?

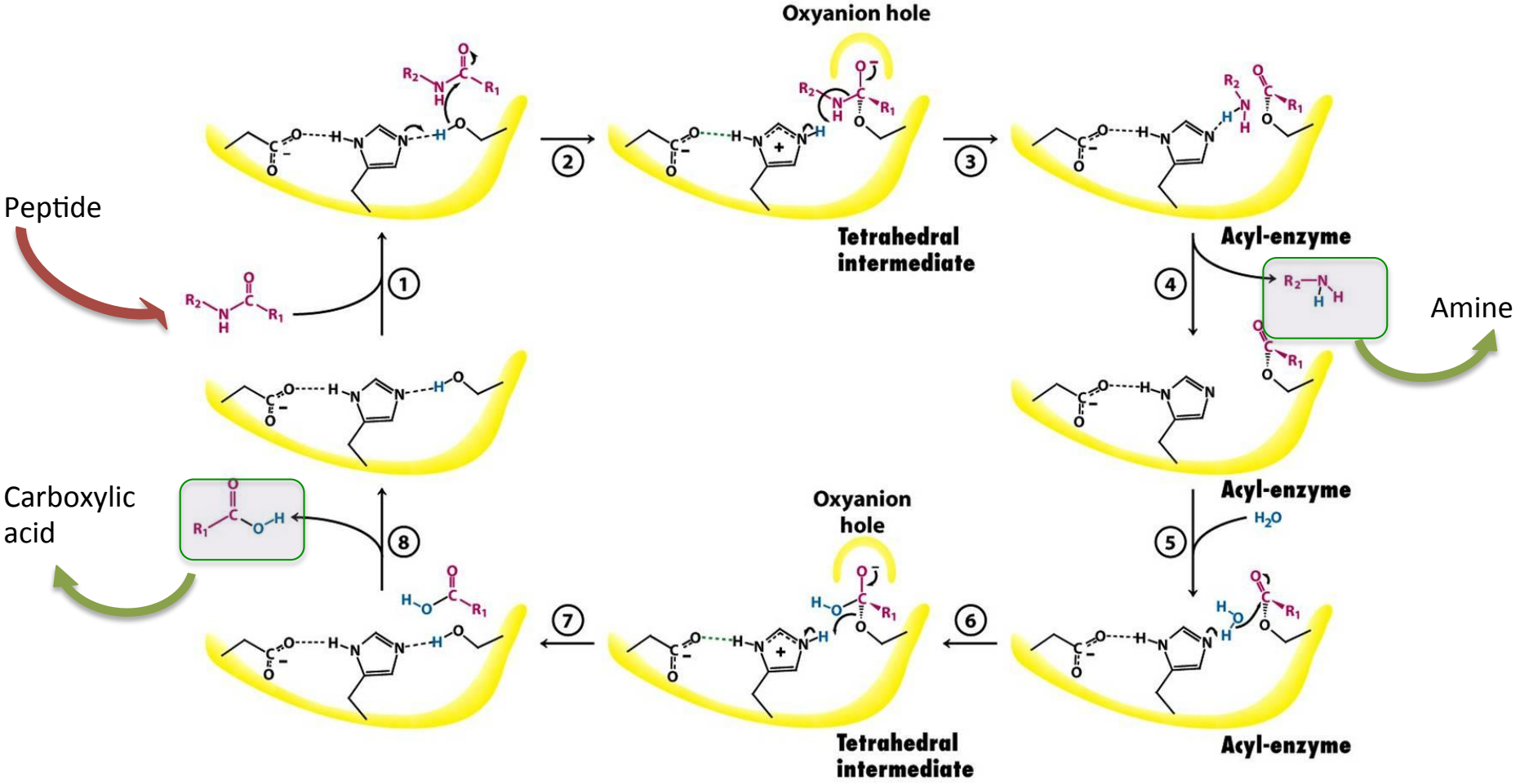
- Breaking the peptide bonds found in dietary proteins provides the raw ingredients (amino acids) to build new proteins.
- The peptide bond can be broken using an acid catalyzed hydrolysis reaction.



- In a 'test-tube' the hydrolysis reaction requires 6 M HCl heated at 110 ^oC for 24 hrs!

How do enzymes work?

- The enzyme *chymotrypsin*, found in the duodenum (pH=8), is able to catalyze hydrolysis of proteins at 37 ^{o}C.

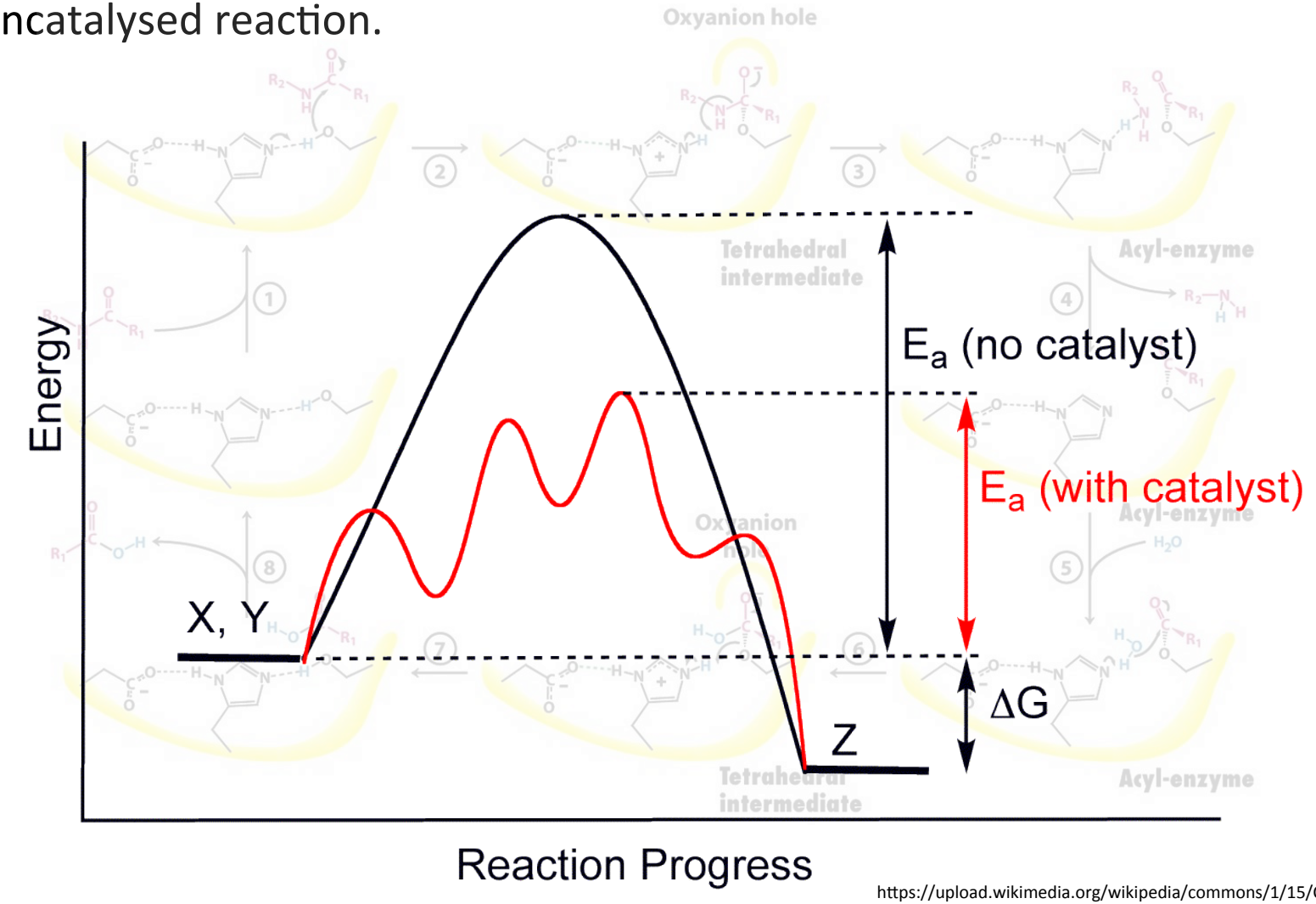


https://upload.wikimedia.org/wikipedia/commons/e/e9/Ch9f8.jpg

- In the stomach (pH=2) *pepsin* performs a similar role.

How do enzymes work?

- An enzyme will often have a series of intermediate transition states (energy barriers) to overcome that are lower in energy compared to the uncatalysed reaction.



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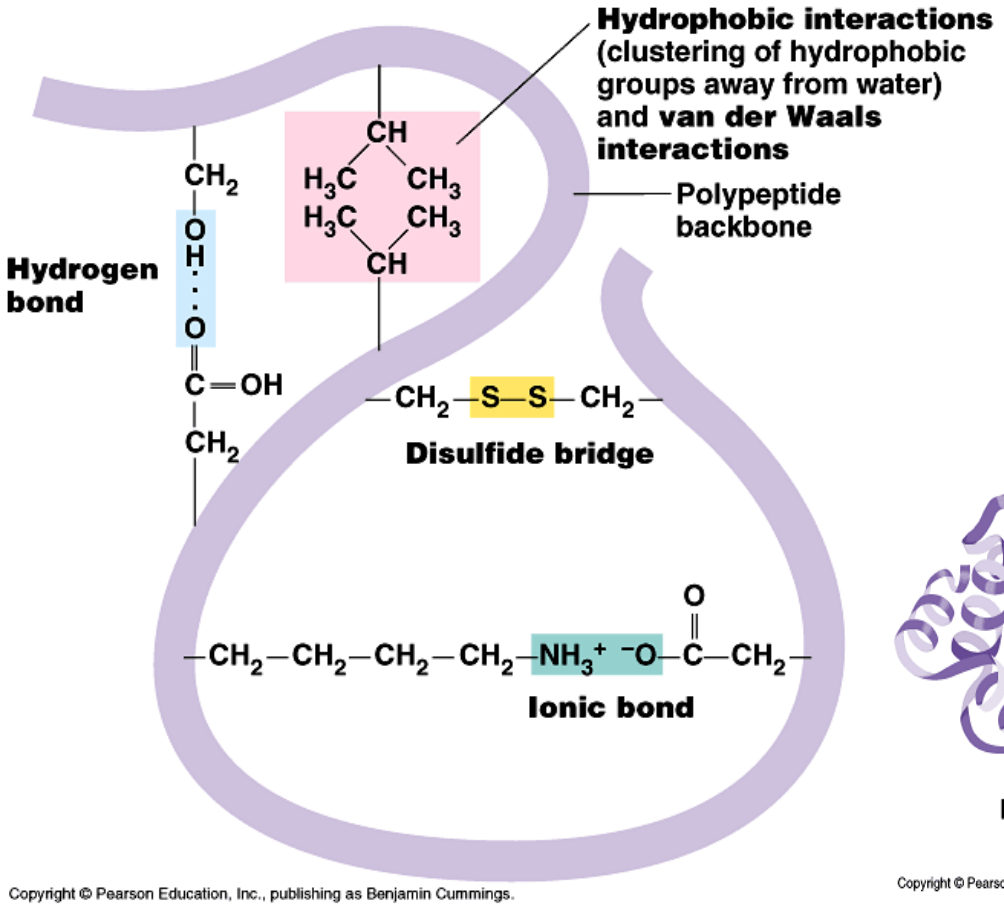
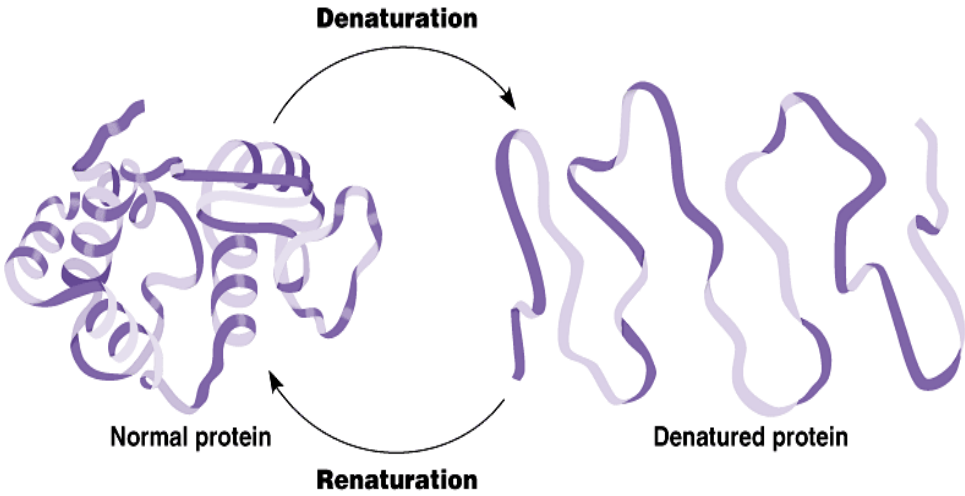
The structure of enzymes

Since the bonding interactions holding a protein in its 3-D shape are relatively weak, enzymes are vulnerable to denaturation



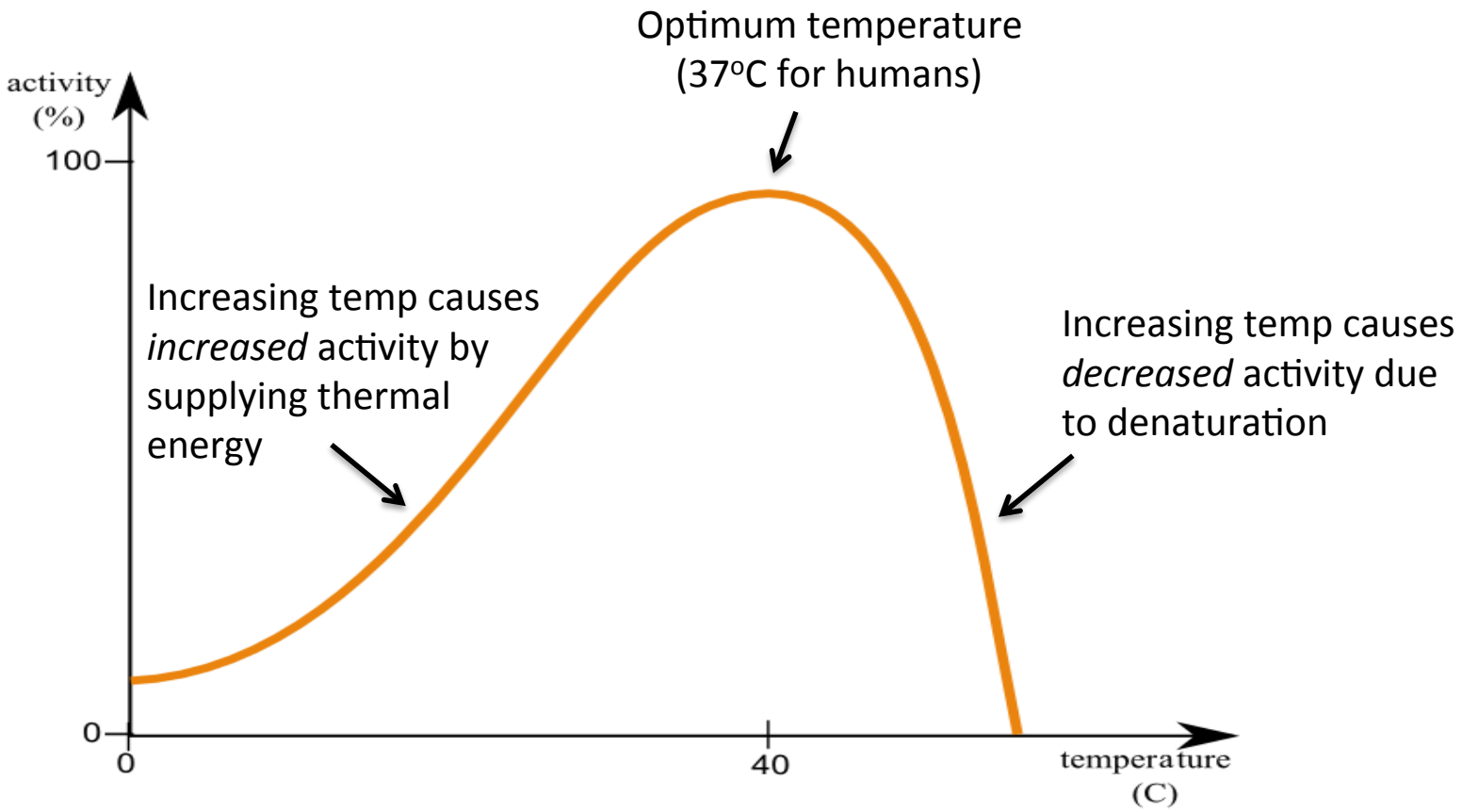
Relative bond strengths:

Covalent bond	50-100 kcal/mol
Ionic bond	3-7 kcal/mol
H-bond	3-7 kcal/mol
van der Waals	1 kcal/mol



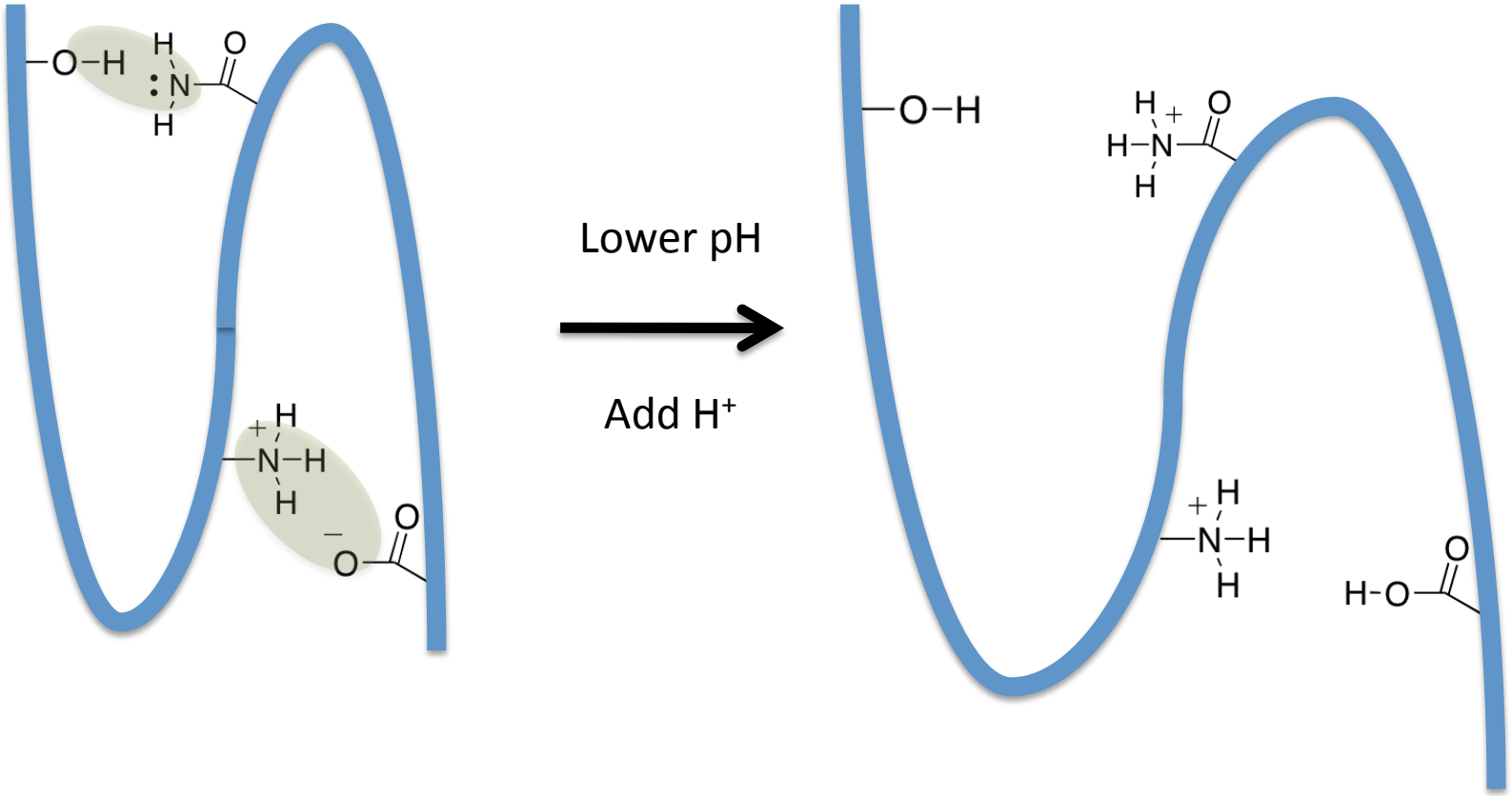
Effect of temperature

- Enzymes have an optimum temperature at which they can operate
- Supplying energy (heat) will break the weak interactions that hold the enzyme in its active shape - denaturation



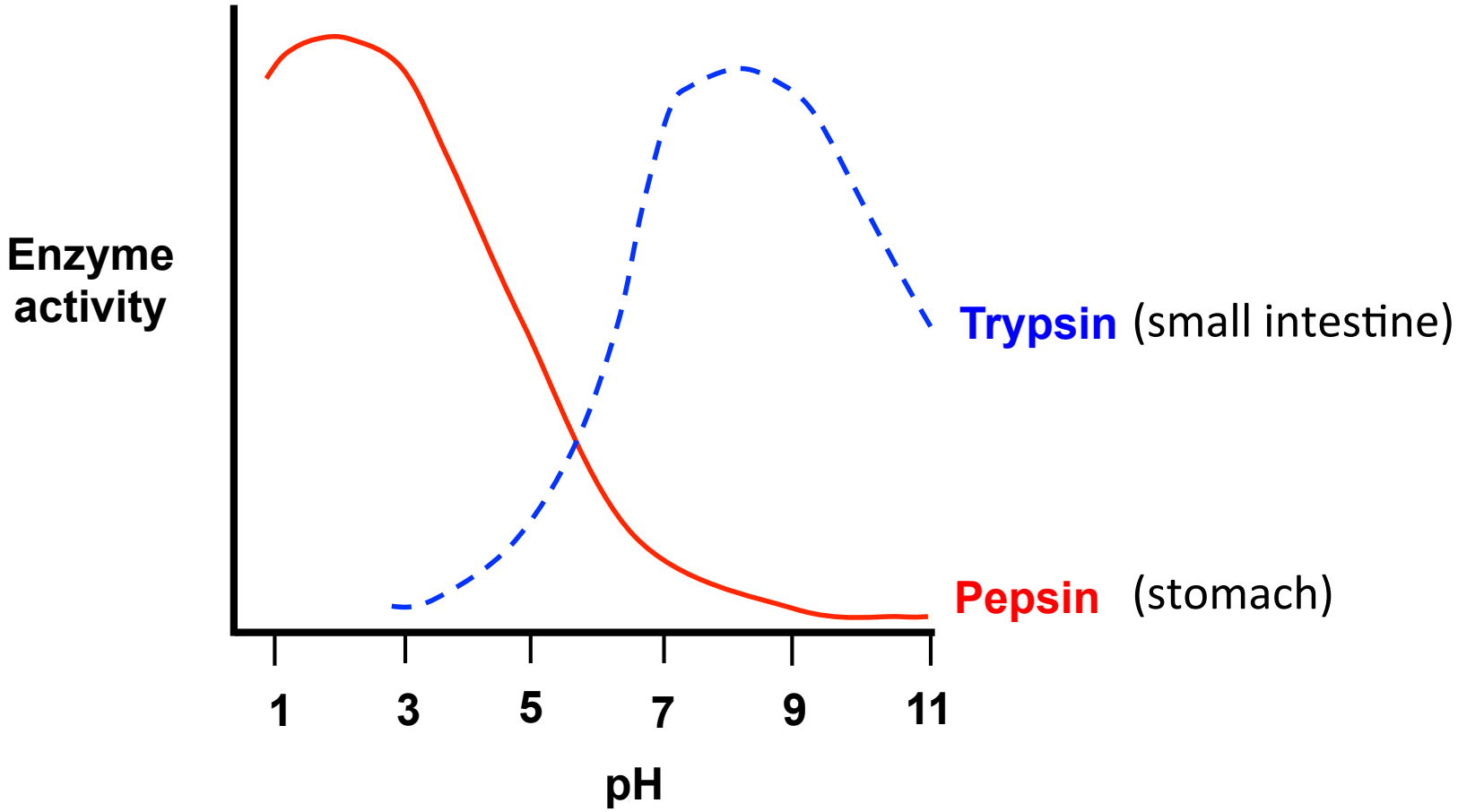
Effect of pH

- Consider what could happen to a peptide chain when pH is lowered (i.e. more acidic environment)
- Protonation of some functional groups can cause disruption to the attractive forces holding the protein in its tertiary structure



Effect of pH

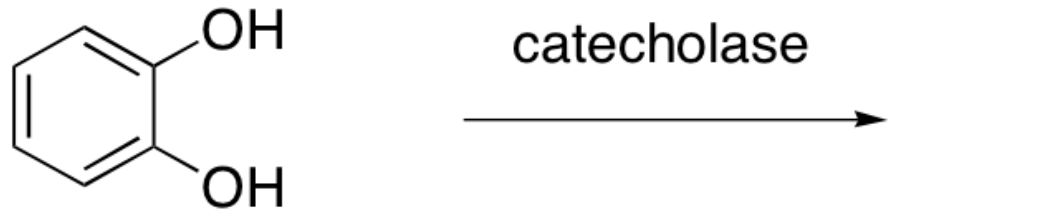
- Enzymes will have an optimal pH range in which they work
- The optimal pH will be different for enzymes that operate in different environments



Enzymes we can see in action!

CATECHOLASE

- Is the enzyme present in many fruits and vegetables that facilitates the discoloration after cutting or bruising.
- Catalyzes the oxidation of catechol (colorless compound) to benzoquinone (brown compound).



catechol
(colorless)

benzoquinone
(brown)

- Adding lemon juice to fruit will slow down the browning process – through denaturation and removal of copper (a *cofactor*).

Enzymes we can see in action!

BROMELAIN

- Is a *protease* present in pineapple, and in its isolated form used as a meat tenderizer.
- A protease is a protein-digesting enzyme. Chymotrypsin, pepsin and trypsin are proteases involved in the human digestive system.

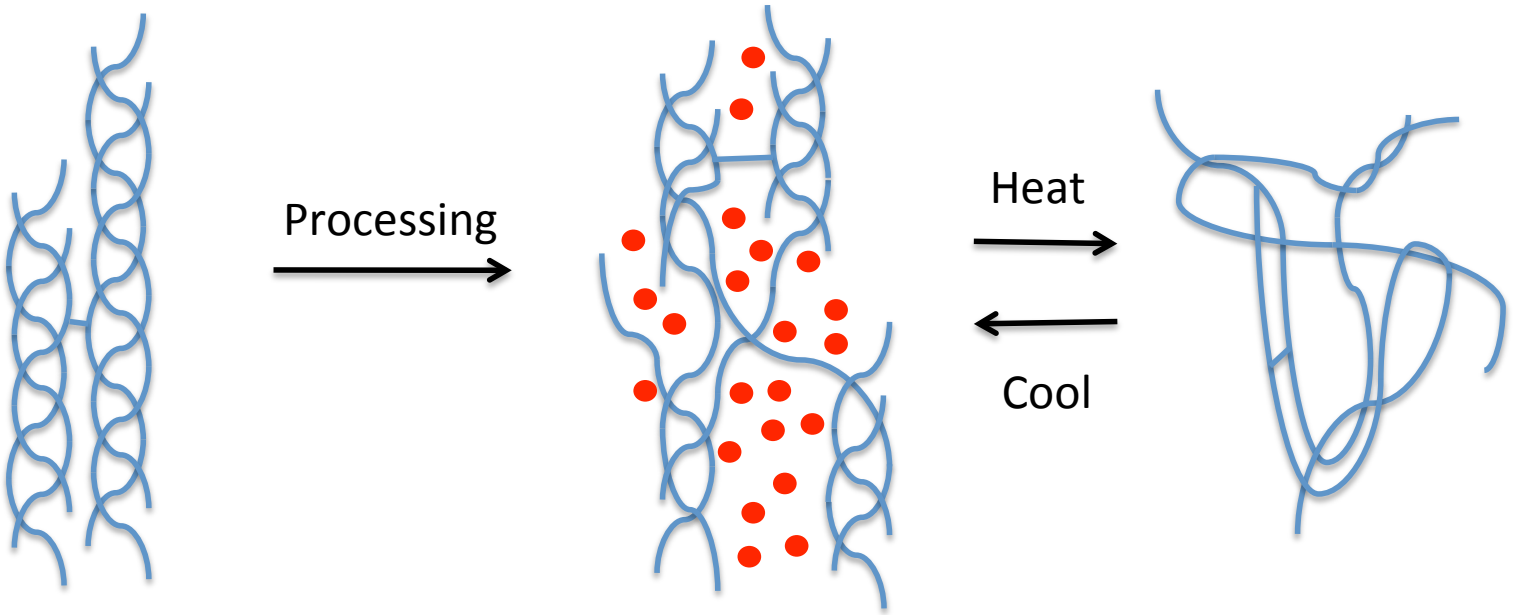
+

= ?

Enzymes we can see in action!

BROMELAIN

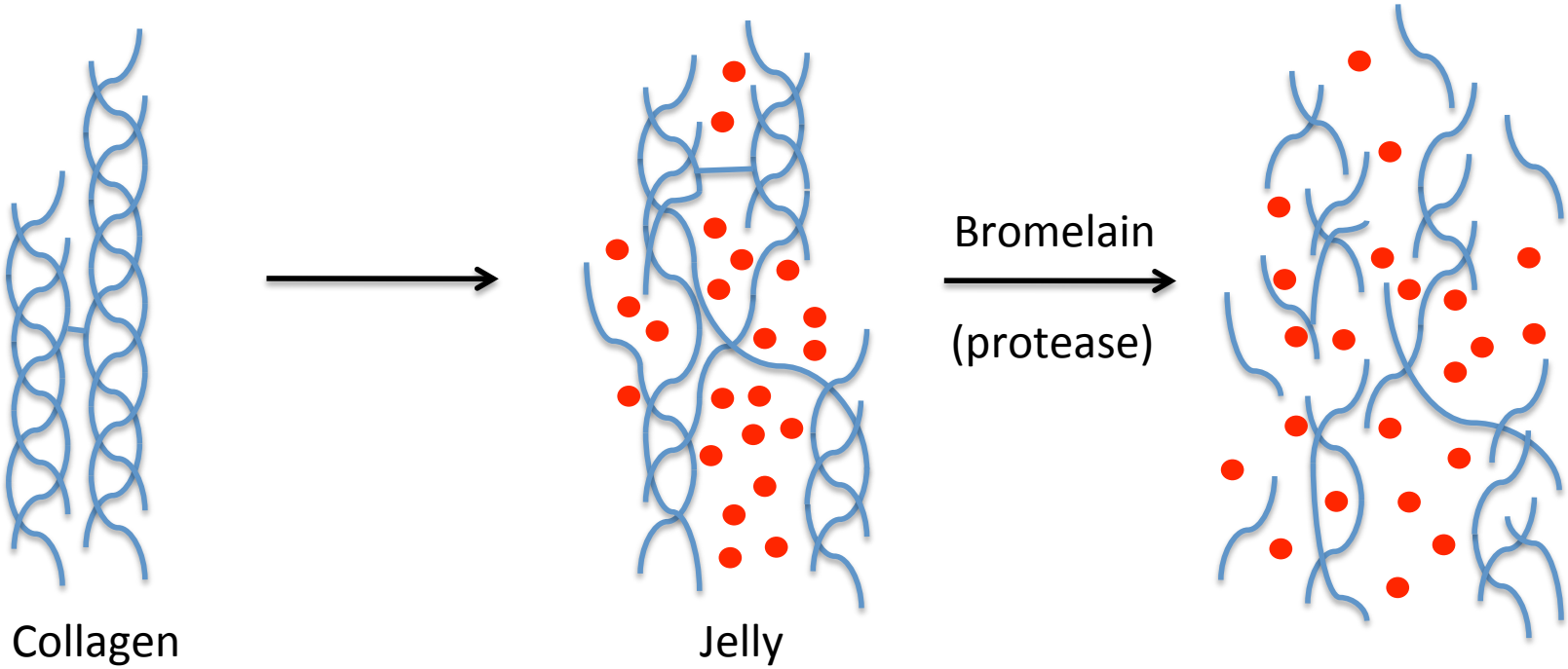
- Gelatine is derived from collagen, a triple-stranded structural protein.
- Upon dissolving in hot water the strands separate and become dispersed in the water.
- When cooled the strands tangle up, with water filling the gaps, forming a gel.



Enzymes we can see in action!

BROMELAIN

- Bromelain is able to break the peptide bonds in the gelatine.
- The resulting peptide chains are shorter and dissolve in the water more easily because there are fewer attractive forces between chains to maintain the more ordered structure of the gel.

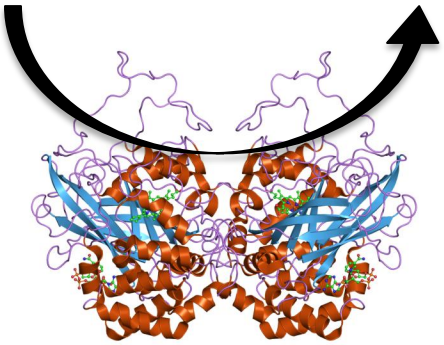


Enzymes we can see in action!

CATALASE

- Is present in most living organisms.
- It is the enzyme that facilitates the decomposition of hydrogen peroxide (a damaging reactive oxygen species) to form water and oxygen.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$



Catalase
(a tetrameric enzyme containing iron as part of the active site)

The action of catalase provides the basis of the 'elephant's toothpaste' demonstration (when performed using yeast)

Enzyme experiments

Resources:
http://www.ableweb.org/volumes/vol-6/10-miller.pdf (Some 'Simple Enzyme Experiments' using a variety of different enzymes including catalase and catecholase)

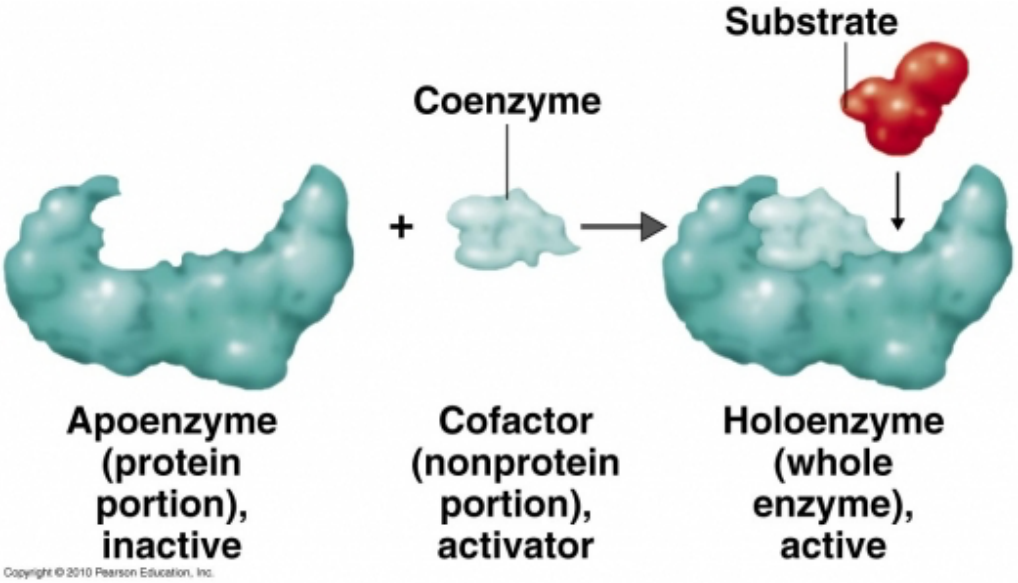
Catalase experiments:
http://www.abc.net.au/science/surfingscientist/demonstrations/ (A good source for the 'elephant's toothpaste' experiment using yeast. Many other variations of elephant's toothpaste are available online)

Catalase activity in potato (and other foods) and the effects of pH and temperature can be observed and measured easily. Many variations of this experiment are available online.

Catecholase and bromelain experiments:
Many variations of these experiment are available online.

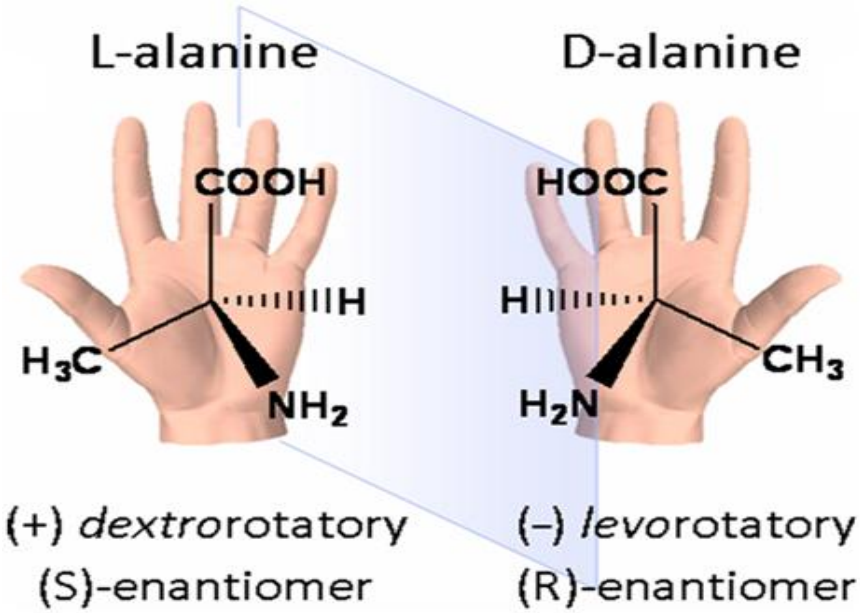
Coenzymes

- A *coenzyme* is a 'substance' (usually an organic molecule) required to assist enzyme activity
- A coenzyme will bind to a protein to form an active enzyme
- Coenzymes often help by carrying a group of atoms to the active site which are then transferred to the substrate
- Most vitamins act as coenzymes



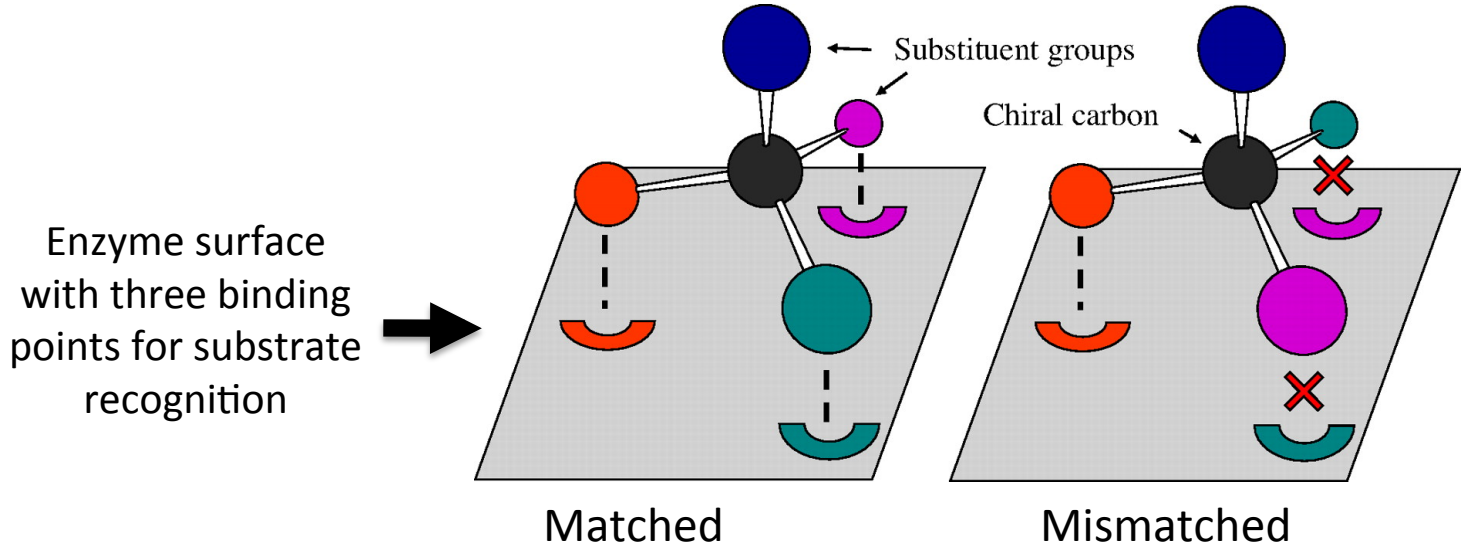
Enzymes and chirality

- Some objects and their mirror images are non-superimposable (just like your left and right hands).
- When a molecule cannot be superimposed on its mirror image the molecule is described as *chiral*.
- This situation occurs when a carbon atom is attached to four different groups: **Amino acids, that are used to build enzymes, are chiral.**
- In Nature only one of the mirror-image forms of the amino acids exists naturally!



Enzymes and chirality

- As a result of the 'chiral' nature of enzymes, only molecules that have the correct chirality will be able to interact with an enzyme.

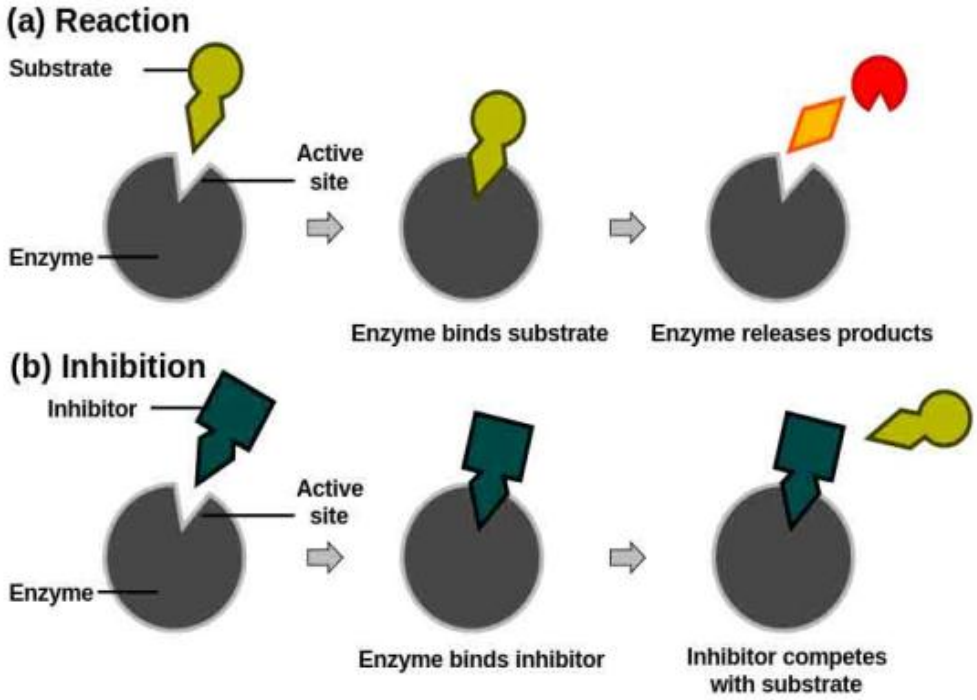


- Many molecules involved in human metabolism are chiral – including sugars, peptides/proteins, DNA, hormones/steroids.
- Medicines that are chiral must be made as a single 'mirror image' form to avoid undesired side-effects.

Drugs and enzymes

- Some drugs exert their activity by inhibiting enzymes.
- Two ways in which this can happen are competitive inhibition and non-competitive inhibition.

A <u>competitive inhibitor</u> acts by blocking the site where the usual substrate binds.



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Drugs and enzymes

- Some drugs exert their activity by inhibiting enzymes.
- Two ways in which this can happen are competitive inhibition and non-competitive inhibition.

A <u>non-competitive inhibitor</u> acts by binding at a site other than the active site causing a change in the active site.