CHE 361
Bioprocess Engineering

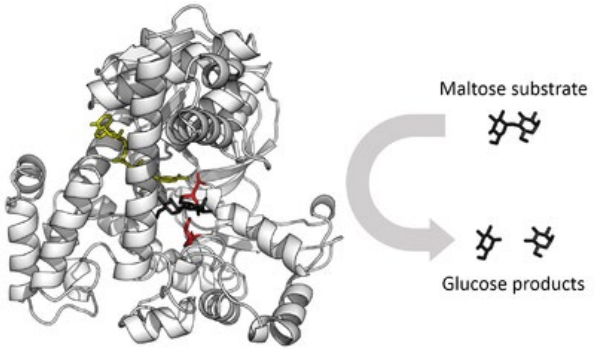
Lecture 3: Introduction to Enzyme Catalysis

Outline

- Enzymatic Reaction Fundamentals
- Enzyme Nomenclature
- Michaelis-Menten Kinetics
- Enzymatic Reaction Inhibition

Enzymatic Reaction Fundamentals

An enzyme is a protein (or protein-like molecule) **that acts as a (bio)-catalyst** on a substrate (reactant molecule) to chemically transform it at a greatly accelerated rate (usually 10^3 to 10^{17} times faster than the uncatalyzed rate).



The enzyme *glucosidase* converts sugar *maltose* to two *glucose* sugars (https://en.wikipedia.org/wiki/Enzyme)

- Enzymes are present in small quantities and are not consumed during the course of the reaction
- Without enzymes, essential biological reactions would not take place at a rate necessary to sustain life
- Enzymes are perfectly specific: a given enzyme can catalyze only one reaction

The **enzyme** molecule is **analogous to a heterogeneous catalytic surface**. It also contains active sites.

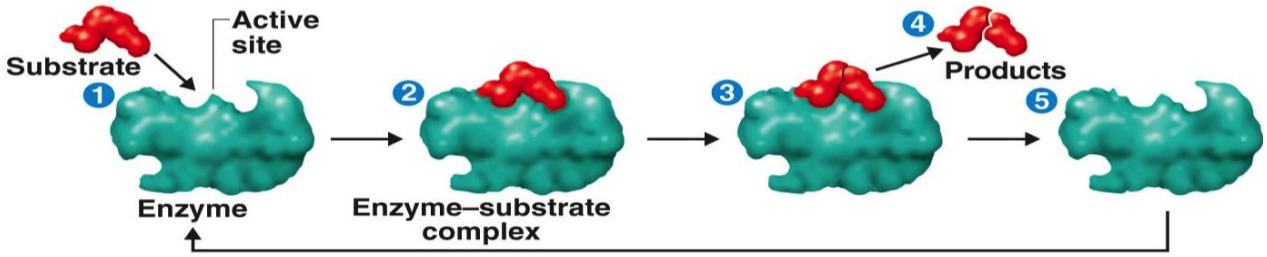
Enzymatic Catalysis Advantages

Why to use enzymes instead of conventional organic synthesis?

- Environmentally friendly process
- Reaction conditions are mild
- Catalytic efficiency is high
- Selectivity is superior
- Broad substrate range, i.e., the ability to catalyze the same reaction (e.g., attack the same functional group) with a variety of substrates

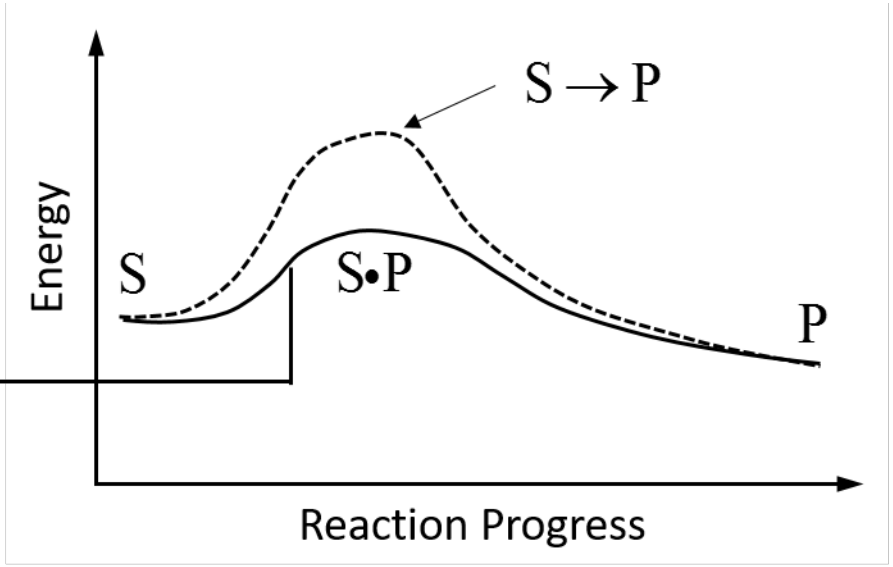
Enzyme-Substrate Interaction

Enzymes must bind their substrates first in order to catalyze a reaction. Enzymes are very specific as to what substrates they bind. Specificity is achieved by binding pockets that have complementary shape.



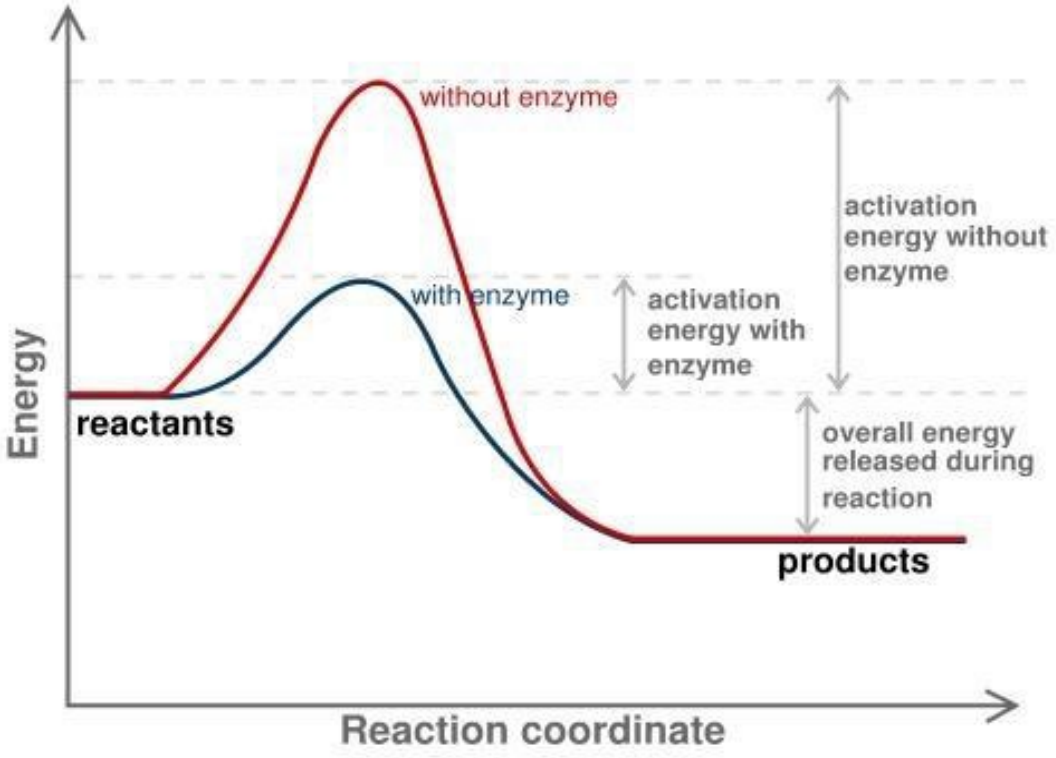
Enzymes provide an alternative reaction pathways that requires a lower activation energy.

$$S + E \rightleftarrows E{\bullet}S \rightarrow E + P$$



Activation Energy

Enzymes increase the rate of a biochemical reactions by lowering the activation energy necessary for the reaction to proceed.



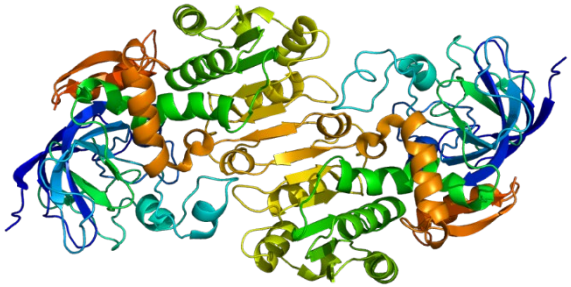
Enzyme Nomenclature

- Enzymes are classified according to the reaction being catalyzed. There are six classes (EC 1-6):

Oxidoreductases (EC 1): catalyze the transfer of hydrogen or oxygen atoms or electrons between substrates (also called oxidases, dehydrogenases, or reductases). Since these are redox reactions, an electron donor/acceptor is required to complete the reaction.

$$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$$

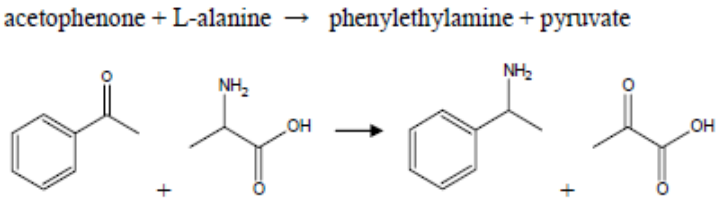
Example: **Alcohol dehydrogenases** (ADH) (EC 1.1.1.1) facilitate the interconversion between alcohols and aldehydes or ketones with the reduction of nicotinamide adenine dinucleotide (NAD+ to NADH). In humans and many other animals, they serve to break down alcohols that otherwise are toxic.



Enzyme Nomenclature (Cont'd)

Transferases (EC 2): catalyze group transfer reactions (excluding oxidoreductases which transfer hydrogen or oxygen) by the transfer of specific functional groups (e.g., methyl or glycosyl group) from one molecule (called the donor) to another (called the acceptor).

Example: **Transaminases** (EC 2.6.1.x) catalyze the exchange of an amine group (NH_2) and a keto group (=O) between an amino acid and an α-keto acid.

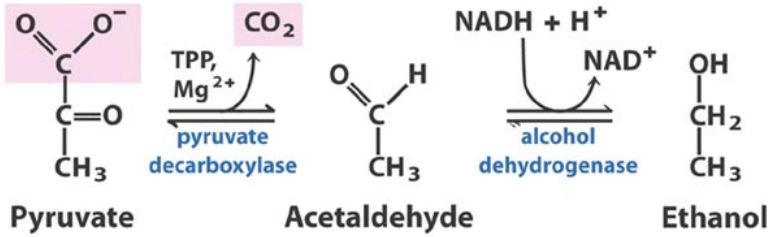


Hydrolases (EC 3): catalyze hydrolytic reactions. Includes lipases, esterases, nitrilases, peptidases/proteases:

$$\text{A-B} + \text{H}_2\text{O} \rightarrow \text{A-OH} + \text{B-H}$$

Lyases (EC 4): catalyze non-hydrolytic removal/addition of functional groups from/to substrates. Includes decarboxylases and aldolases in the removal direction, and synthases in the addition direction.

Example: **Pyruvate decarboxylase** (EC 4.1.1.1) catalyzes the decarboxylation of pyruvic acid to acetaldehyde and CO_2 participating in ethanol production via fermentation (in yeast).



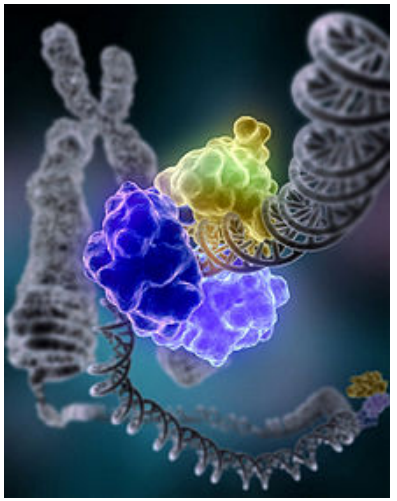
Enzyme Nomenclature (Cont'd)

Isomerases (EC 5): catalyze isomerization reactions, i.e., convert a molecule from one isomer to another. Isomerases can facilitate intramolecular rearrangements in which bonds are either broken and formed.

Example: **UDP-glucose 4-epimerase** (EC 5.1.3.2) performs the final step in the Leloir pathway of galactose metabolism, catalyzing the reversible conversion of UDP-galactose to UDP-glucose.

Ligases (EC 6): catalyze the synthesis of various bonds (mostly C-X, but also phosphoric ester and nitrogen-metal), coupled with the breakdown of energy-containing substrates (e.g., ATP). Ligases join two large molecules by forming a new chemical bond.

Example: **DNA ligase** (EC 6.5.1.1) facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond. DNA ligase is used in both DNA repair and DNA replication.

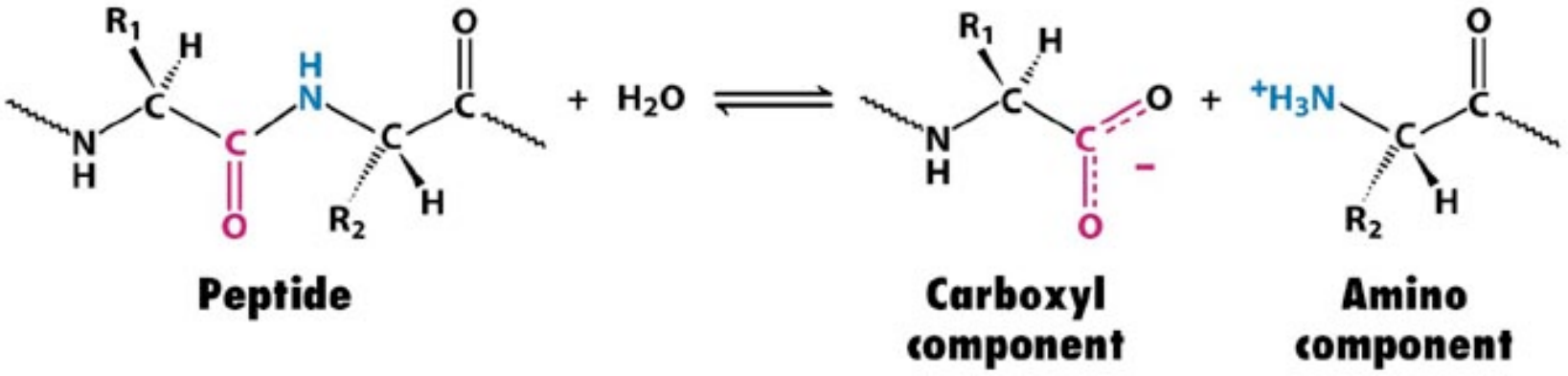


Specificity of Enzymes

Enzymes are very specific for

- substrate acted upon
- reaction catalyzed

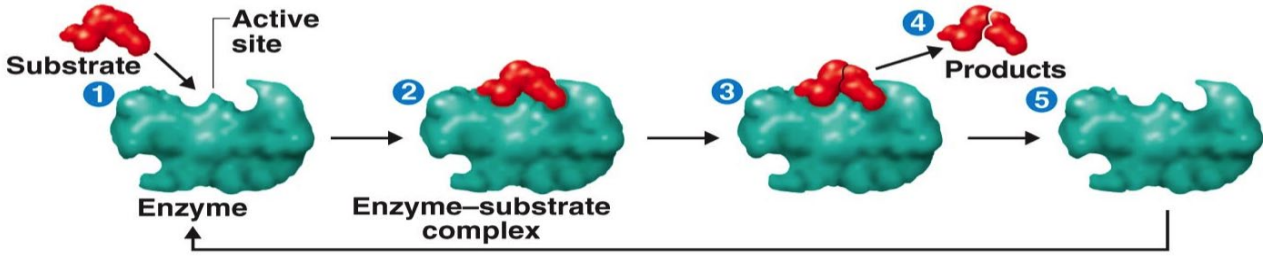
Example: Proteases, a sub-class of hydrolases, catalyze hydrolysis of peptide bonds:



Specificity of Enzymes (Cont'd)

Substrate specificity is due to the precise interaction of an enzyme with a substrate:

- Enzyme active sites have 3D structure
- For enzymatic catalysis to occur, a substrate has to be properly oriented and to bind to an enzyme

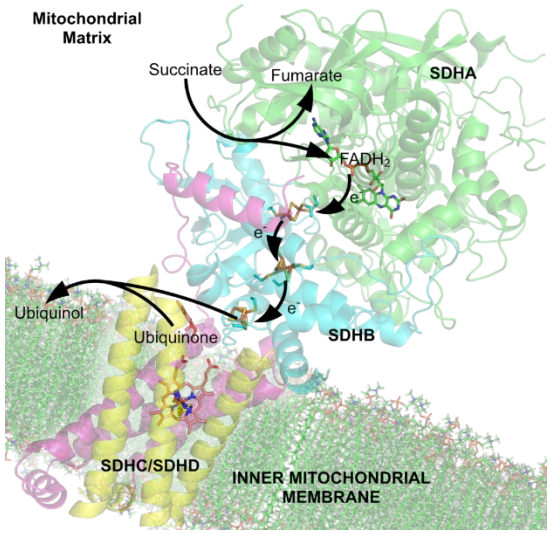


Cofactors

Some enzymes need cofactors:

- Cofactors can be organic molecules or metal ions
- Cofactors can be considered "helper molecules" that "assist" in biochemical transformations

Example:
The **succinate dehydrogenase** complex showing several cofactors, including flavin, iron-sulfur centers, and heme



https://en.wikipedia.org/wiki/Cofactor_(biochemistry)

Michaelis-Menten Kinetics

General mechanism of an enzyme-catalyzed reaction:

$$S + E \rightleftarrows E\bullet S \rightarrow E + P$$

$$S + E \xrightarrow{k_1} E\bullet S$$

$$E\bullet S \xrightarrow{k_2} E + S$$

$$E\bullet S \xrightarrow{k_3} P + E$$

(1) The net rate of disappearance of substrate (S):

$$-r_S = k_1[E][S] - k_2[E\bullet S]$$

(2) Formation of the enzyme-substrate complex:

$$r_{E\bullet S} = k_1[E][S] - (k_2 + k_3)[E\bullet S]$$

(3) Using PSSH and enzyme conservation ($[E_t] = [E] + [ES]$):

$$r_P = -r_S = \frac{k_{cat}[E_t][S]}{[S] + K_M}$$

Michaelis-Menten Kinetics (Cont'd)

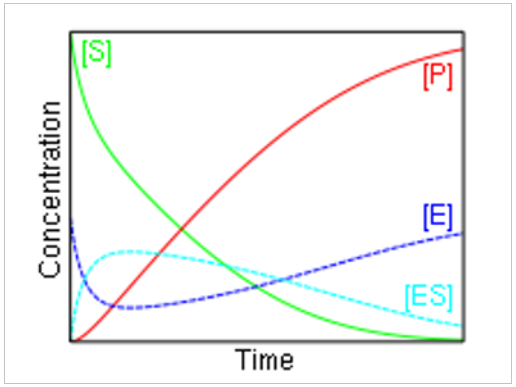
$$S + E \rightleftarrows E{\bullet}S \rightarrow E + P$$

$$-r_S = \frac{V_{max}[S]}{K_M + [S]}$$

k_{cat} – turnover number

K_M – Michaelis constant

$V_{max} = k_{cat}[E_t]$

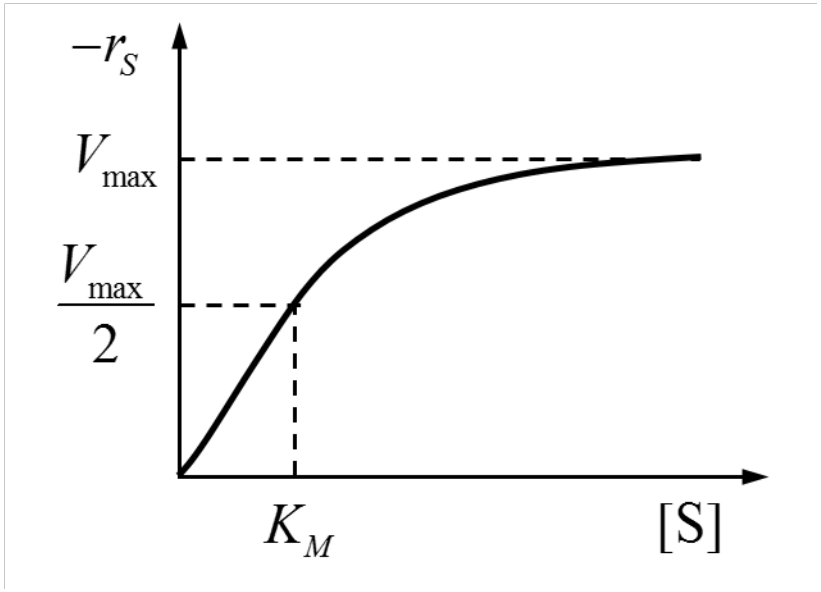


*** At low substrate concentration:**

$$[S] \ll K_M \qquad -r_S \approx \frac{V_{max}[S]}{K_M}$$

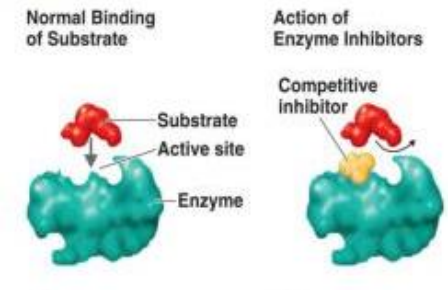
*** At high substrate concentration:**

$$[S] \gg K_M \qquad -r_S \approx V_{max}$$



Enzymatic Reaction Inhibition

An i**nhibitor** is a molecule that interacts with an enzyme and prevents the enzyme from catalyzing its specific reaction, i.e. **deactivates the enzyme**.



The three most common types of *reversible* inhibition occurring in enzymatic reactions are ***competitive***, ***uncompetitive***, and ***noncompetitive***.

Competitive – substrate and inhibitor are similar molecules competing for the same active site on the enzyme

Uncompetitive – the inhibitor deactivates the enzyme-substrate complex

Noncompetitive – the enzyme contains at least two different sites; the substrate attaches to only one type of site, and the inhibitor attached only to the other to deactivate the enzyme

Enzymatic Reaction Inhibition (Cont'd)

Competitive Inhibition:

$$S + E \xrightarrow{k_1} E\bullet S$$
$$E\bullet S \xrightarrow{k_2} E + S$$
$$E\bullet S \xrightarrow{k_3} P + E$$
$$I + E \xrightarrow{k_4} E\bullet I$$
$$E\bullet I \xrightarrow{k_5} E + I$$

$$-r_S = \frac{V_{\max}[S]}{[S] + K_M\left(1 + \frac{[I]}{K_I}\right)}$$

Uncompetitive Inhibition:

$$S + E \xrightarrow{k_1} E\bullet S$$
$$E\bullet S \xrightarrow{k_2} E + S$$
$$E\bullet S \xrightarrow{k_3} P + E$$
$$I + E\bullet S \xrightarrow{k_4} E\bullet I\bullet S$$
$$E\bullet I\bullet S \xrightarrow{k_5} E\bullet S + I$$

$$-r_S = \frac{V_{\max}[S]}{K_M + [S]\left(1 + \frac{[I]}{K_I}\right)}$$

$$K_I = \frac{k_5}{k_4}$$