


**Foundations of  
Biochemistry**


**Chapter 6: Enzyme Principles and Biotechnical  
Applications**

 **Western Oregon**  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.


**TOGETHER WE  
SUCCEED**

Overall, in Chapter 6 we will be learning some basic enzyme principles to get us started on our journey of Enzyme activity and regulation.



## Major Topics

- [6.1 The Nature and Classification of Enzymes](#)
- [6.2 Enzyme Names and Classification](#)
- [6.3 Enzyme Structure and Substrate Binding](#)
- [6.4 Enzymes and Reaction Equilibrium](#)
- [6.5 Properties and Mechanisms of Enzyme Action](#)
- [6.6 Enzymes are Affected by pH and Temperature](#)
- [6.7 Enzymes are Sensitive to Inhibitors](#)
  
- [6.8 Allosteric Regulators and the Control of Enzyme Activity](#)
- [6.9 Origin, Purification, and Uses of Enzymes](#)
  
- [6.10 Industrial Enzymology](#)

 **Western Oregon**  
UNIVERSITY


If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE  
SUCCEED**

There are quite a few sections in this chapter, but as you will see, many are quite short!

**Foundations of  
Biochemistry**

**Chapter 6.1 The Nature and Classification of  
Enzymes**

 **Western Oregon**  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

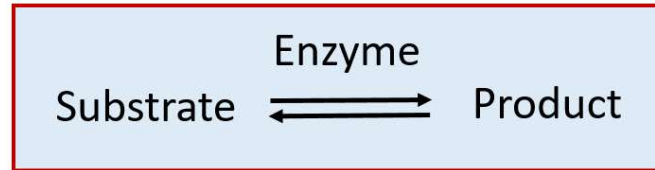
**TOGETHER WE  
SUCCEED**

In this first section, we will cover the nature and classification of enzymes



## The Catalytic Constant

- $K_{\text{cat}}$  = turnover rate or frequency




Western Oregon  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.


TOGETHER WE  
SUCCEED

As catalysts, enzymes are only required in very low concentrations, and they speed up reactions without themselves being consumed during the reaction. We usually describe enzymes as being capable of catalyzing the conversion of substrate molecules into product molecules. And this property is described by the constant called  $K_{\text{cat}}$  or the turnover rate or frequency. This catalytic constant represents the number of substrate molecules that can be converted to product by a single enzyme molecule per unit time (usually per minute or per second).



# $K_{cat}$

Enzyme	Turnover rate (mole product s <sup>-1</sup> mole enzyme <sup>-1</sup> )
Carbonic anhydrase	600 000
Catalase	93 000
β-galactosidase	200
Chymotrypsin	100
Tyrosinase	1



**Western Oregon**  
UNIVERSITY


If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

TOGETHER WE  
SUCCEED

As well as being highly potent catalysts, enzymes also possess remarkable specificity in that they generally catalyze the conversion of only one type (or at most a range of similar types) of substrate molecule into product molecules. Some enzymes demonstrate group specificity. For example, alkaline phosphatase (an enzyme that is commonly encountered in first-year laboratory sessions on enzyme kinetics) can remove a phosphate group from a variety of substrates. Other enzymes demonstrate much higher specificity, which is described as absolute specificity. For example, glucose oxidase shows almost total specificity for its substrate, β-D-glucose, and virtually no activity with any other monosaccharides. Each enzyme has a specific catalytic rate for each substrate that it can catalyze


# Foundations of Biochemistry

## Chapter 6.2 Enzyme Names and Classification

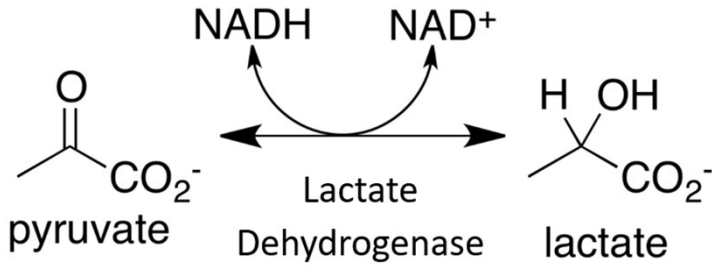
 **Western Oregon**  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or [ods@wou.edu](mailto:ods@wou.edu) at least 72 hours in advance.


**TOGETHER WE SUCCEED**

 **Naming Enzymes**

- '-ase'



pyruvate  $\xrightleftharpoons[\text{Lactate Dehydrogenase}]{\text{NADH} \rightarrow \text{NAD}^+}$  lactate

 **Western Oregon UNIVERSITY**

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE SUCCEED**

Enzymes typically have common names (often called 'trivial names') which refer to the reaction that they catalyze, with the suffix '-ase' (e.g. oxidase, dehydrogenase, carboxylase), although individual proteolytic enzymes generally have the suffix '-in' (e.g. trypsin, chymotrypsin, papain). This is because these were some of the earliest enzymes discovered, and they were named before the '-ase' enzyme classification system was put into place. In the early 1960s the Enzyme Commission came up with a layered enzyme classification system. In this system, each enzyme is given a specific number. For example, Lactate Dehydrogenase, shown here has the EC number 1.1.1.27. Each number refers to a classification level that gets more detailed and detailed at each step. Let's use Lactate Dehydrogenase as an example. First, you may recognize from your experience with organic chemistry that lactate dehydrogenase is mediating a redox reaction. In this case the reduction reaction is shown in the forward direction and the oxidation reaction in the reverse. Recall that ketones can be reduced to secondary alcohols (and/or secondary alcohols can be oxidized to a ketone, in the reverse reaction). Note that Lactate Dehydrogenase can go either way depending on the circumstance...ie levels of product and reactant.



## EC Primary Classification

First EC digit	Enzyme class	Reaction type
1.	Oxidoreductases	Oxidation/reduction
2.	Transferases	Atom/group transfer (excluding other classes)
3.	Hydrolases	Hydrolysis
4.	Lyases	Group removal (excluding 3.)
5.	Isomerases	Isomerization
6.	Ligases	Joining of molecules linked to the breakage of a pyrophosphate bond




Western Oregon  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

TOGETHER WE  
SUCCEED

The first number in the system refers to the primary classification system. So if we look carefully, we can see that oxidoreductase enzymes are listed at #1, and the first digit in the Lactate Dehydrogenase E.C. number is 1.



 **Second Level EC Number**


CC(=O)C(=O)[O-]
 $\xrightleftharpoons[\text{NAD}^+]{\text{NADH}}$ 
C[C@@H](O)C(=O)[O-]

pyruvate Lactate Dehydrogenase lactate

---

**Oxidoreductases:** **Hydrogen or electron donor**  
**second EC digit**

1.	Alcohol (CHOH)
2.	Aldehyde or ketone (C=O)
3.	—CH—CH—
4.	Primary amine (CHNH <sub>2</sub> or CHNH <sub>2</sub> <sup>+</sup> )
5.	Secondary amine (CHNH)
6.	NADH or NADPH (when another redox catalyst is the acceptor)


**Western Oregon UNIVERSITY**

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE SUCCEED**

Each type of enzyme class then has sublevels that it is divided into, based on the types of substrates and reactants involved in the reactions. For oxidoreductase enzymes, the second level of classification corresponds with the electron donor molecule in the reaction. In the case of lactate dehydrogenase, this is the alcohol. As the oxidation reaction is shown in the reverse direction. So first, you would need to identify the oxidation reaction and then look to see what the reactant for the oxidation reaction is...Within the numbering system, alcohol is listed as #1. So the first two levels of classification for Lactate Dehydrogenase is 1.1


**Third Level EC Number**

Oxidoreductases: third EC digit	Hydrogen or electron acceptor
1.	NAD <sup>+</sup> or NADP <sup>+</sup>
2.	Fe <sup>3+</sup> (e.g. cytochromes)
3.	O <sub>2</sub>
4.	Other

**Western Oregon UNIVERSITY**  
 If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.


TOGETHER WE SUCCEED

For oxidoreductase enzymes, the third level of classification refers to the electron acceptor in the oxidation reaction. In the case of Lactate Dehydrogenase, NAD<sup>+</sup> is the electron acceptor. Recall that we noted previously that electrons and hydrogens usually move together in biological oxidation/reduction reactions. So if you follow the hydrogens, you are also following the electrons. If a molecule loses electrons (and the associated hydrogens) it becomes oxidized. If it gains electrons (and the associated hydrogens) it is reduced. If we look at the classification scheme for Lactate Dehydrogenase, we can see that the third position is also 1! So it is 1.1.1. The last digit for Lactate Dehydrogenase is 27, as it is the 27<sup>th</sup> enzyme to be characterized in this class. So its full classification is: 1.1.1.27. For this class, you will only need to be familiar with the first classification level.



## EC Primary Classification

First EC digit	Enzyme class	Reaction type
1.	Oxidoreductases	Oxidation/reduction
2.	Transferases	Atom/group transfer (excluding other classes)
3.	Hydrolases	Hydrolysis
4.	Lyases	Group removal (excluding 3.)
5.	Isomerases	Isomerization
6.	Ligases	Joining of molecules linked to the breakage of a pyrophosphate bond


**Western Oregon UNIVERSITY**

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE SUCCEED**

We will systematically visit these different types of reactions throughout both this term and next term. You should be able to place an enzyme into its proper primary class. So on this list, you are already familiar now with two classes...the oxidoreductases that we just looked at, and the hydrolase reactions. Can you think of any dehydration/hydrolysis reactions that we have already looked at? Hopefully, you jumped up and down and shouted, yes! Protein synthesis...you can identify these reactions with the consumption or formation of water as one of the reactants or products, respectively. You've also heard about another one of these, although we didn't look at the reaction in detail. The DNA Ligase enzyme that fuses the backbone of DNA together during those cloning reactions that we talked about. This enzyme also is active during DNA replication and falls in the ligase class of enzymes. Ligases join two things together to form one thing. This differs from the hydrolase enzymes in that water is not involved in the process. Ligases often use the energy of ATP hydrolysis to mediate the reaction.

ATP catalyzes the reaction for DNA ligase to repair/restore DNA or RNA strands.

The diagram illustrates the DNA ligase reaction in three stages:

- Stage 1:** A DNA double strand with a nick is shown. The top strand has a 3'-OH group and a 5'-phosphate group. A yellow DNA ligase enzyme is positioned above the nick. ATP is converted to AMP and  $P_i$ .
- Stage 2:** The DNA ligase enzyme is bound to the 5'-phosphate group of the top strand, forming a covalent bond with it. The 3'-OH group of the top strand is now positioned to attack the 5'-phosphate group of the bottom strand.
- Stage 3:** The 3'-OH group of the top strand has successfully attacked the 5'-phosphate group of the bottom strand, forming a phosphodiester bond. The DNA ligase enzyme is released, and AMP is released from the 5'-phosphate group.


Image from: [SwtsOlitude](https://www.swinsolitude.com)

**Western Oregon UNIVERSITY**

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE SUCCEED**

Here is the DNA Ligase reaction.

 DNA Ligase

ATP catalyzes the reaction for DNA ligase to repair/restore DNA or RNA strands.

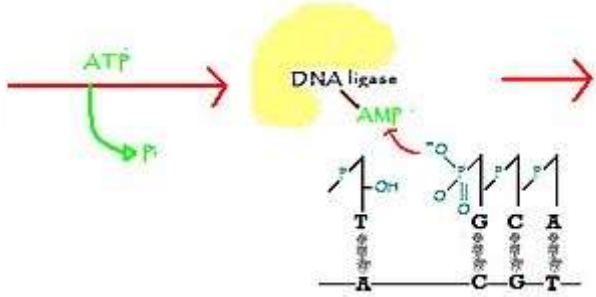



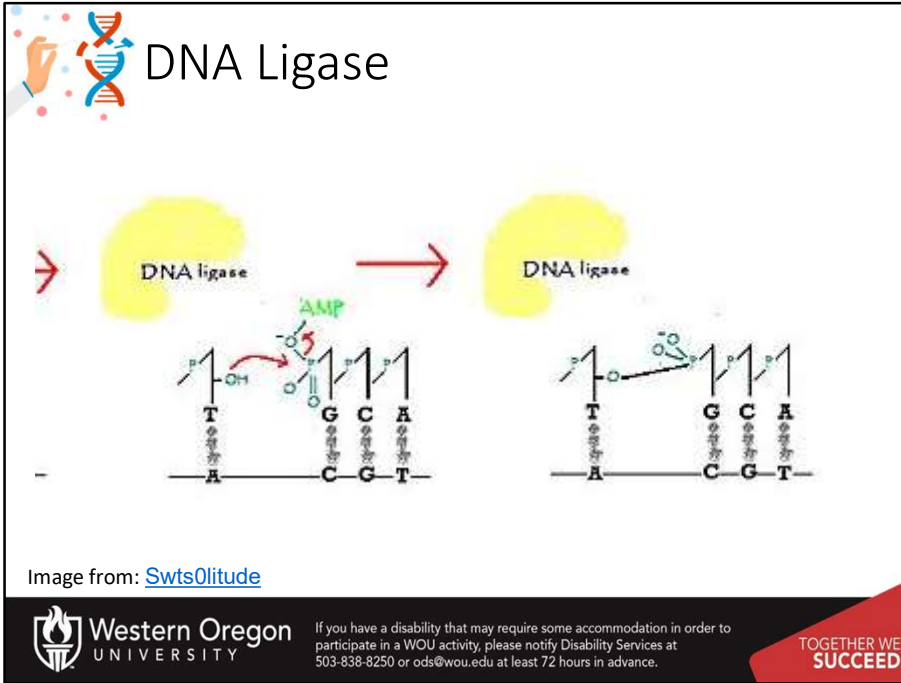
Image from: [SwtsOlitude](https://www.swinsolitude.com)

 **Western Oregon**  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE SUCCEED**

Here is the DNA Ligase reaction. In the first part of the reaction, ATP is cleaved and the monophosphate is attached covalently to the enzyme. An oxygen from the phosphate group of the broken link in the DNA backbone, can then mediate nucleophilic attack on the AMP bound to the enzyme, causing it to release from the enzyme and covalently join with the phosphate group.



In the second part of the reaction, the AMP bound to the phosphate group can then serve as a leaving group. In this part of the reaction, the alcohol oxygen from the 3'-hydroxyl group mediates nucleophilic attack on the phosphorous of the 5' phosphate group. The AMP bond is cleaved, releasing AMP and the sugar-phosphate backbone is resealed.



## EC Primary Classification

First EC digit	Enzyme class	Reaction type
1.	Oxidoreductases	Oxidation/reduction
2.	Transferases	Atom/group transfer (excluding other classes)
3.	Hydrolases	Hydrolysis
4.	Lyases	Group removal (excluding 3.)
5.	Isomerases	Isomerization
6.	Ligases	Joining of molecules linked to the breakage of a pyrophosphate bond

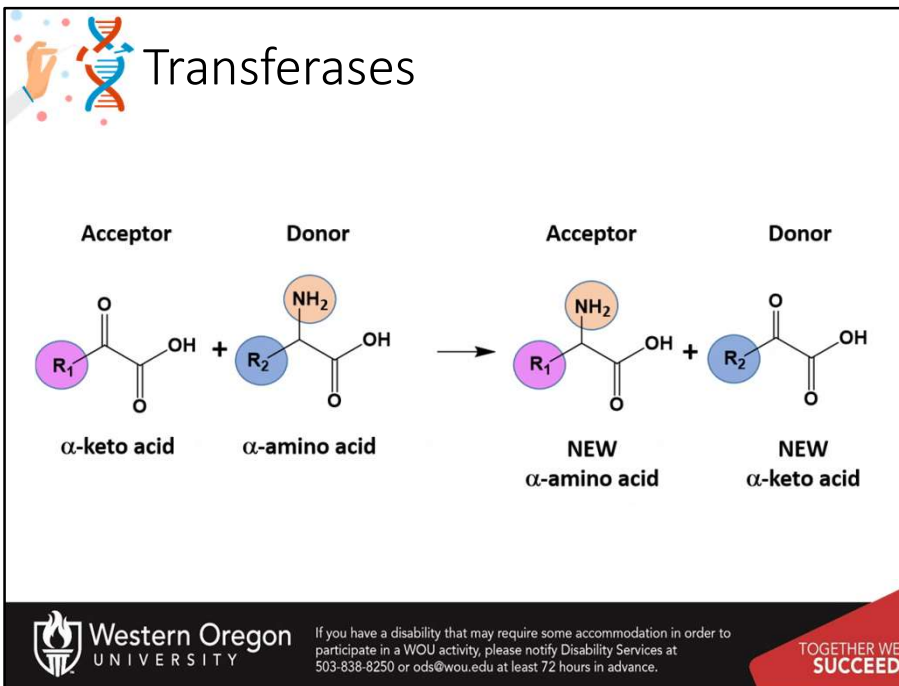


Western Oregon  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

TOGETHER WE  
SUCCEED

Now we've looked at three classes...let's see an example of a transferase



Transferases mediate **group transfer reactions**. In **group transfer reactions**, a functional group will be transferred from one molecule that serves as the donor molecule to another molecule that will be the acceptor molecule. The transfer of an amine functional group from one molecule to another is common example of this type of reaction and is shown here.





## EC Primary Classification

First EC digit	Enzyme class	Reaction type
1.	Oxidoreductases	Oxidation/reduction
2.	Transferases	Atom/group transfer (excluding other classes)
3.	Hydrolases	Hydrolysis
4.	Lyases	Group removal (excluding 3.)
5.	Isomerases	Isomerization
6.	Ligases	Joining of molecules linked to the breakage of a pyrophosphate bond

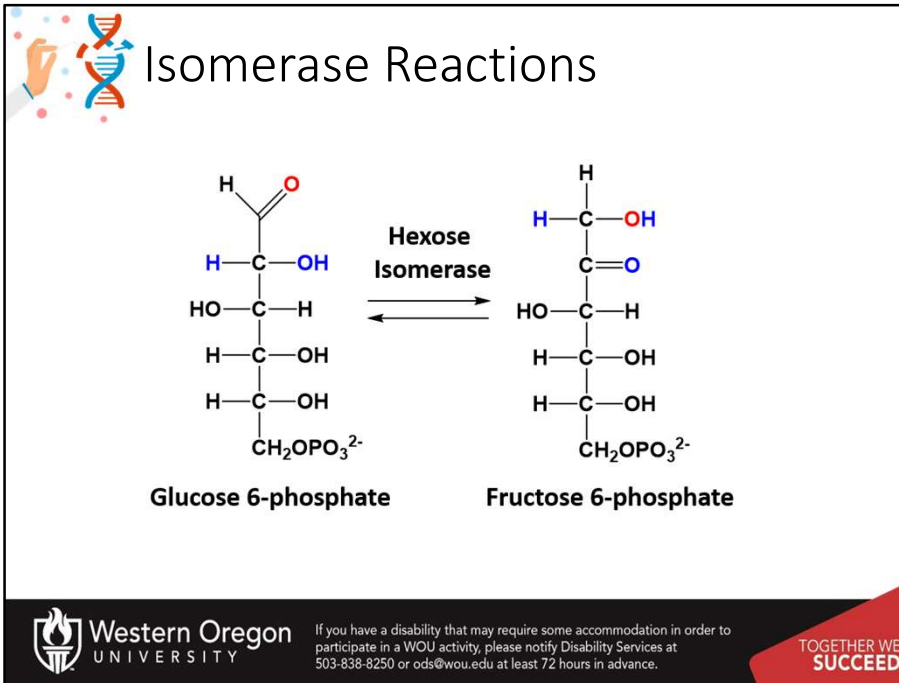


Western Oregon  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

TOGETHER WE  
SUCCEED

let's see an example of an isomerase next...



In **isomerization reactions** a single molecule is rearranged such that it retains the same molecular formula but now has a different bonding order of the atoms forming a structural or stereoisomer. The conversion of glucose 6-phosphate to fructose 6-phosphate is a good example of an isomerization reaction



## EC Primary Classification

First EC digit	Enzyme class	Reaction type
1.	Oxidoreductases	Oxidation/reduction
2.	Transferases	Atom/group transfer (excluding other classes)
3.	Hydrolases	Hydrolysis
4.	Lyases	Group removal (excluding 3.)
5.	Isomerases	Isomerization
6.	Ligases	Joining of molecules linked to the breakage of a pyrophosphate bond




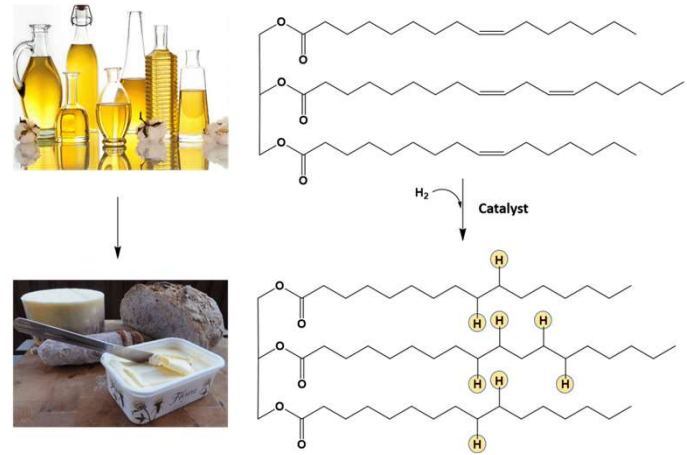
Western Oregon  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

TOGETHER WE  
SUCCEED

The last class we need to take a look at are the lyases.

 Lyase Reactions



Western Oregon UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

TOGETHER WE SUCCEED

Lyase reactions mediate the formation and removal of carbon-carbon double bonds within a molecule. Now you may notice that when the double bond is removed, that new hydrogen atoms are added to the molecule...yes, if you were caught up thinking about our oxidoreductase story, you might be saying to yourself...hold up...are electrons also moving with those hydrogens? Is this also an oxidoreductase reaction? And you would be correct!! Yes, lyase reactions are also redox reactions.

**EC Primary Classification**

First EC digit	Enzyme class	Reaction type
1.	Oxidoreductases	Oxidation/reduction $A + B \leftrightarrow C + D$
2.	<del>X</del> Transferases	Atom/group transfer (excluding other classes)
3.	<del>X</del> Hydrolases	Hydrolysis
4.	Lyases	Group removal (excluding 3.) $A \leftrightarrow B + C$
5.	Isomerases	Isomerization $A \leftrightarrow B$
6.	Ligases	Joining of molecules linked to the breakage of a pyrophosphate bond $X$

The diagram includes handwritten annotations: a bracket above the table, a checkmark next to class 1, 'X' marks next to classes 2, 3, and 6, and chemical equations  $A + B \leftrightarrow C + D$ ,  $A \leftrightarrow B + C$ , and  $A \leftrightarrow B$  with arrows pointing to their respective rows.


**Western Oregon UNIVERSITY**  
If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE SUCCEED**

Even though lyase reactions are technically also oxidoreductase reactions, they have a special classification of their own. Class 4. This is mainly due to the type of redox reactions that are classified in EC 1. These tend to have the formula of:  $A + B = C + D$ ; or two reactants form two products. Hydrolases and transferases also follow this reaction style. Lyase reactions differ, in that their major formula is:  $A = B + C$  where one molecule is converted to two or two into one in the reverse direction. Ligases also follow this type of formula. Isomerases follow the formula  $A = B$  or only 1 substrate and 1 product. Anyway, this difference in formula type is the main reason why lyases are classified as their own enzyme class, rather than with the other oxidoreductase enzymes.

**Foundations of  
Biochemistry**

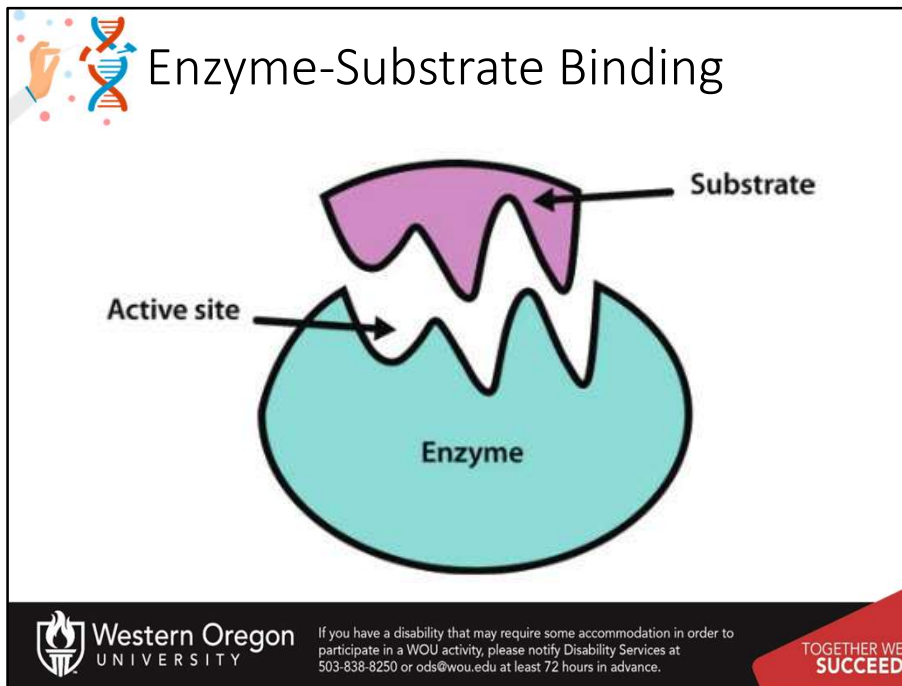
**Chapter 6.3 Enzyme Structure and Substrate  
Binding**

 **Western Oregon**  
UNIVERSITY

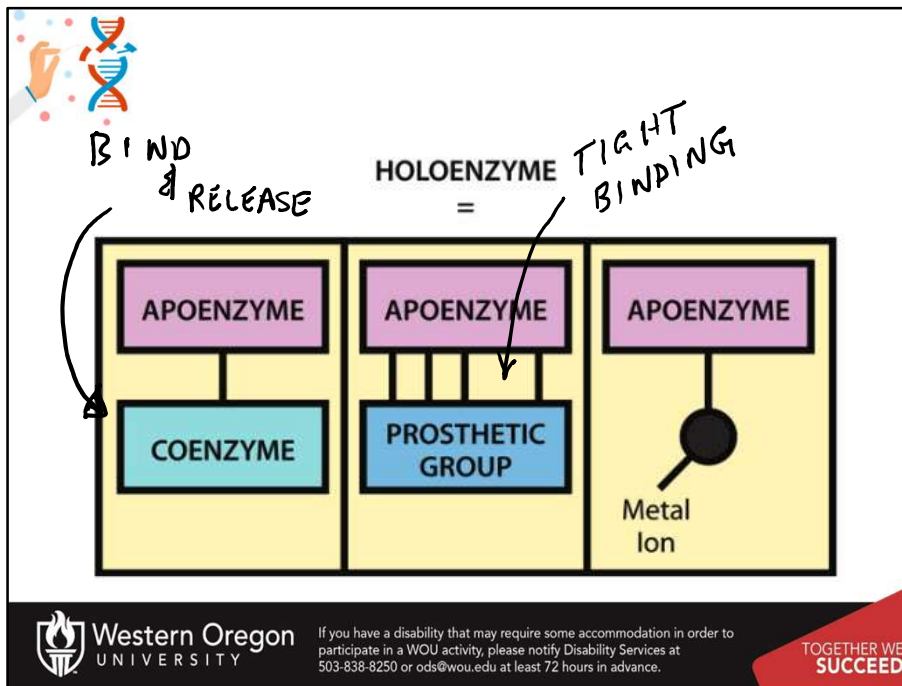
If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE  
SUCCEED**

In this section, we will define some general features of enzymes.



Amino acid-based enzymes are globular proteins that range in size from less than 100 to more than 2,000 amino acid residues. These amino acids can be arranged as one or more polypeptide chains that are folded and bent to form a specific three-dimensional structure, incorporating a small area known as the active site, where the substrate actually binds. The active site may well involve only a small number (less than 10) of the constituent amino acids. It is the shape and charge properties of the active site that enable it to bind to a single type of substrate molecule, so that the enzyme is able to demonstrate considerable specificity in its catalytic activity.




Some enzymes require a cofactor to be functional. This can be a small organic molecule or a metal ion that helps mediate the reaction. Small organic molecules that can bind and be released from the enzyme are called coenzymes. If the small organic molecules bind really tightly to the enzyme they are called prosthetic groups. When the enzyme does not contain its cofactor, it is called an apoenzyme. With the cofactor it is called the holoenzyme.



**Foundations of  
Biochemistry**


**Chapter 6.4 Enzymes and Reaction Equilibrium**

 **Western Oregon**  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.


**TOGETHER WE  
SUCCEED**

This section provides an introduction to the energetics of enzyme reactions.



## Enzyme and Reaction Rates

$$S \rightarrow P$$
$$S \rightleftharpoons P$$
$$K_{\text{eq}} = \frac{\text{Product concentration at end point}}{\text{Substrate concentration at end point}} = \frac{0.5}{0.5} = 1$$

 **Western Oregon**  
UNIVERSITY

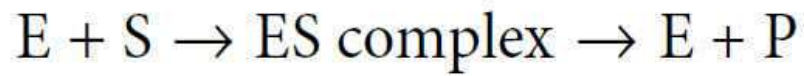
If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

**TOGETHER WE  
SUCCEED**

Recall that enzymes enhance reaction rates, however, they do not change the equilibrium of the reaction or the spontaneity. These are determined by the intrinsic properties of the substrate and products. Recall that  $K$  equilibrium is determined by a ratio of the products over the substrates.



## Enzymes for Complexes with their Substrates

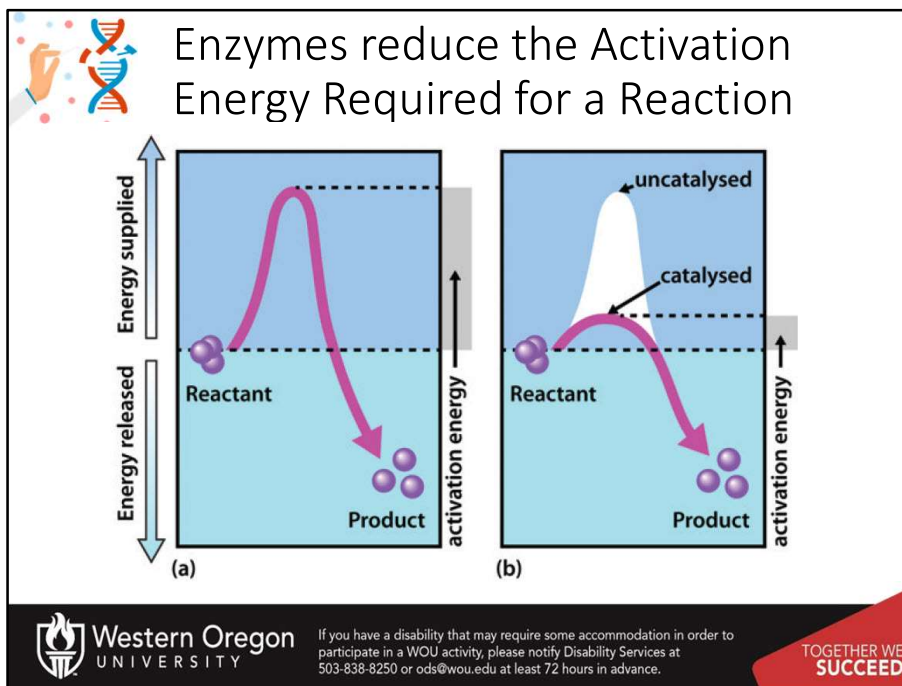


Western Oregon  
UNIVERSITY

If you have a disability that may require some accommodation in order to participate in a WOU activity, please notify Disability Services at 503-838-8250 or ods@wou.edu at least 72 hours in advance.

TOGETHER WE  
SUCCEED

Enzymes form complexes with their substrates during the reaction. The ES complex makes the reaction more favorable. Once the reaction has occurred and the product is formed, the product dissociates from the enzyme.



In terms of energetics, reactions can be either exergonic (releasing energy) or endergonic (consuming energy). The reaction shown is exergonic (or the energy of the products is much lower than the energy of the reactants causing a release of energy during the reaction).

Enzymes will not affect the overall change in Gibbs Free Energy. What enzymes do, is reduce the activation energy required to start the reaction process. The second energy graph shows the reduction of the transition state energy when the enzyme is present. Ultimately, enzymes make the transition state of the reaction more likely to occur. Note, however, this cannot make an endergonic reaction exergonic. In the next section, we will begin to introduce enzyme kinetics.