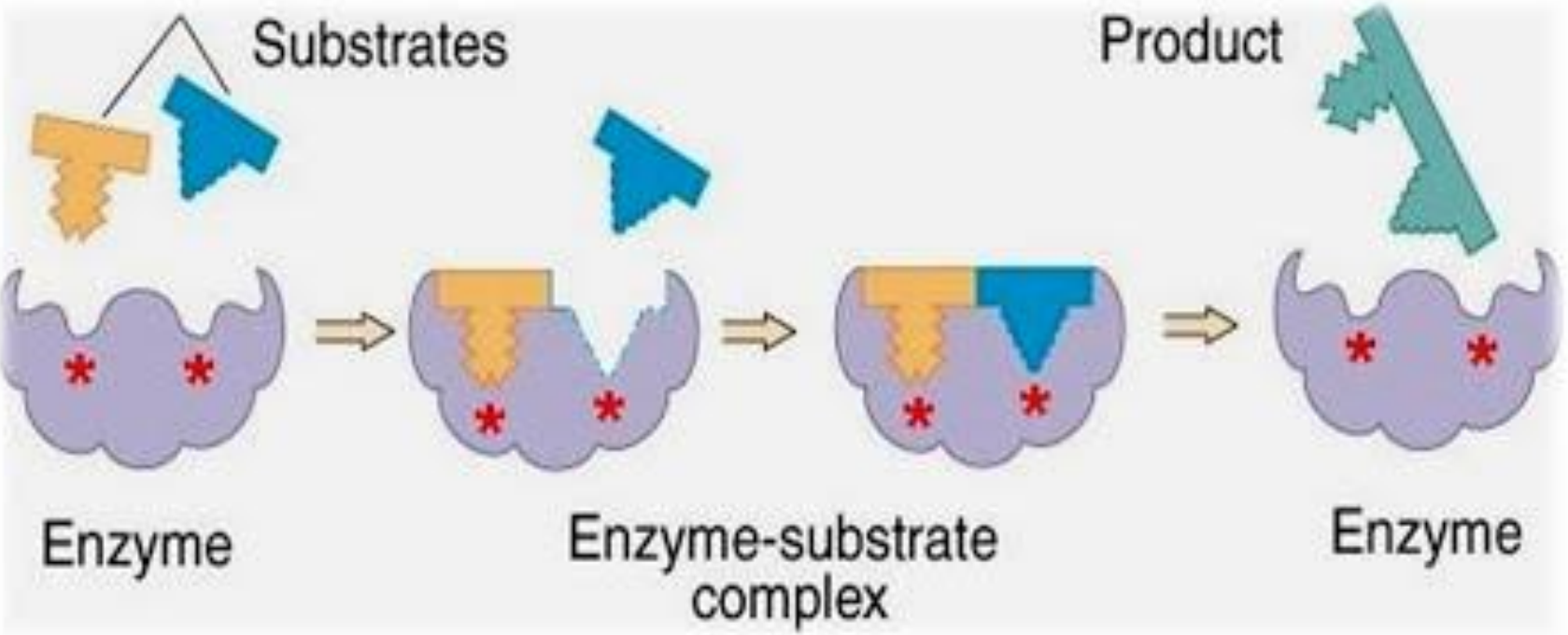
Enzyme catalysis



What Are Enzymes?

- Enzymes are biological catalysts.
- Increase reaction rates without being used up
  - ✓ Effective in small amounts, very efficient
  - ✓ Do not affect reaction equilibrium
  - ✓ High degree of specificity
  - ✓ Extraordinary catalytic power
  - ✓ Function in aqueous solutions under very mild conditions of temperature and pH

Enzymes are central to every biochemical process

- Catalyze hundreds of step wise reactions:

1. that degrade nutrient molecules.
2. make biological macromolecules from simple precursors.
3. conserve and transform chemical energy.
4. regulatory enzymes (metabolic pathways).

- Importance:

✓ Diseases (inheritable genetic disorders)
  – Deficiency, absence or excessive activity.
✓ Diagnosis

Most Enzymes Are Proteins

- Exceptions: catalytic RNA molecules.
- Structures of protein enzymes are essential to their catalytic activity.
- molecular weights: 12,000 to more than 1 million.

Enzyme requirement

- Some enzymes do **not** **require** chemical component for activity.

- Some enzymes **require** chemical component:
  - Cofactors – either one or more inorganic ions.
  - Coenzymes – complex organic or metalloorganic molecules.
  - Some enzymes require both.

Enzymes requiring chemical component

- **Holoenzyme**:

A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions (cofactor).

- **prosthetic group:**

A coenzyme or metal ion (cofactor) that is very tightly (covalently bound) to the enzyme.

- **Apoenzyme (Apoprotein):**

The protein part of holoenzyme.

Enzymes requiring chemical component

- Some enzymes require chemical component:
  - ✓ Cofactor (inorganic ions): Fe2+, Mg2+, or Zn2+

Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Enzymes requiring chemical component

- Some enzymes require chemical component:
  - ✓ Cofactor: inorganic ions: Fe2+, Mg2+, or Zn2+
  - ✓ Coenzyme: complex organic or metalloorganic molecule.

Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups*

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Enzymes are divided into six classes, each with subclasses, based on the type of reaction catalyzed.

- Commission number (E.C. number), e.g. 2.7.1.1
- Trivial name is more commonly used.

International Classification of Enzymes*

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group-transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

Enzymes: six classes

Group	Reaction catalyzed	Typical reaction	Enzyme example(s) with trivial name
EC 1 *Oxidoreductases*	To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another	AH + B → A + BH (reduced) A + O → AO (oxidized)	Dehydrogenase, oxidase
EC 2 *Transferases*	Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group	AB + C → A + BC	Transaminase, kinase
EC 3 *Hydrolases*	Formation of two products from a substrate by hydrolysis	AB + H_2O → AOH + BH	Lipase, amylase, peptidase
EC 4 *Lyases*	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	RCOCOOH → RCOH + CO_2 or [X-A-B-Y] → [A=B + X-Y]	Decarboxylase
EC 5 *Isomerases*	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	AB → BA	Isomerase, mutase
EC 6 *Ligases*	Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP	X + Y+ ATP → XY + ADP + Pi	Synthetase

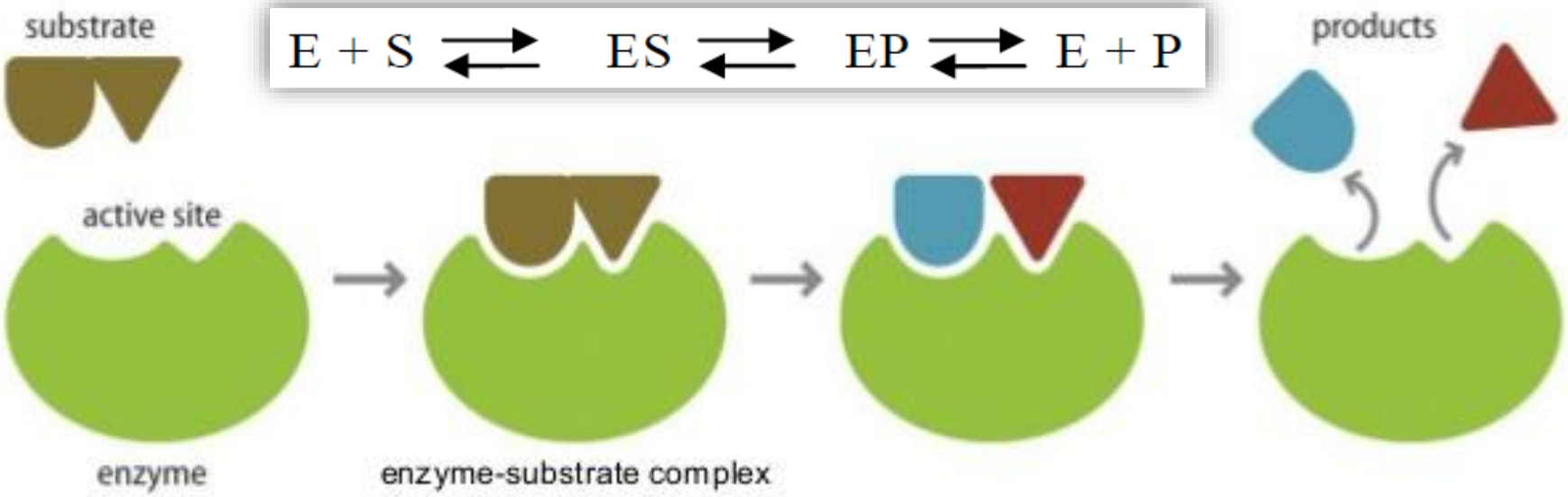
Enzymes: Example

ATP + D-glucose → ADP + D-glucose 6-phosphate

- **Common name:** hexokinase
- **E.C. number**: 2.7.1.1
  - 2: class name (transferases)
  - 7: subclass (phosphotransferases)
  - 1: phosphotransferase with –OH as acceptor
  - 1: D-glucose is the phosphoryl group acceptor

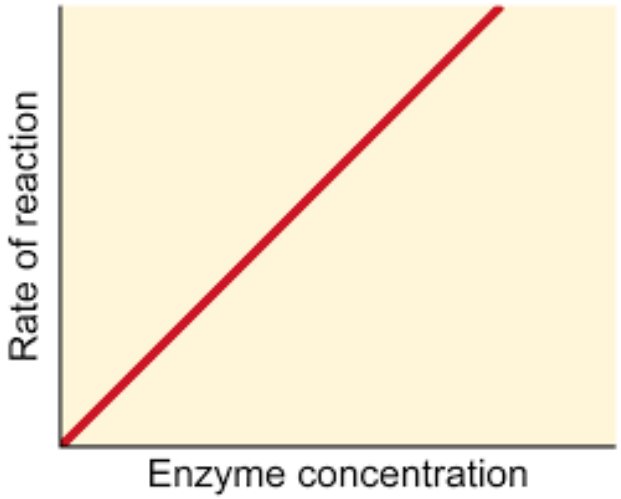
How enzymes work

- Enzymes catalyze chemical reactions that do not normally proceed under conditions such as neutral pH, mild temperature, and aqueous solvent.
- The site of catalytic activity on the enzyme is known as the *active site*.
- The molecule that binds to the active site and is acted upon by the enzyme is called the ***substrate***
- The two together form what is known as the *enzyme-substrate complex*
- The function of an enzyme is to increase the rate of a chemical reaction without affecting its equilibrium.
- Therefore, enzymes don't make more product, they just make product faster.

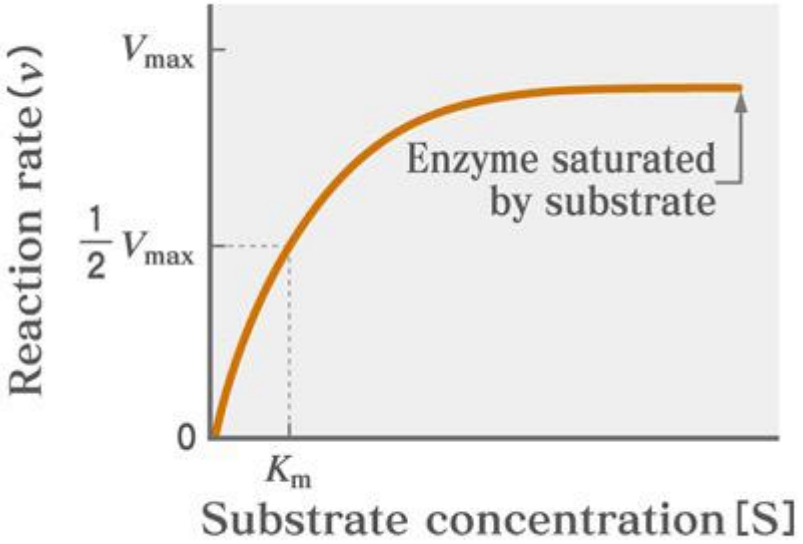


Factors affecting enzymatic activity

1. Enzyme concentration



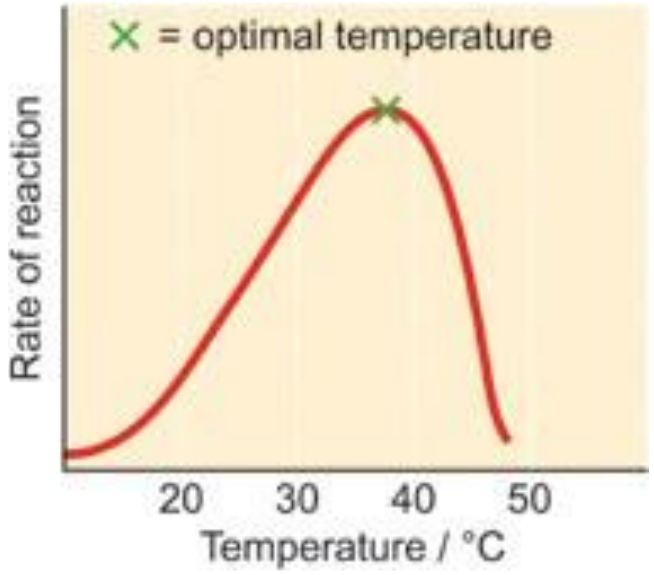
2. Substrate concentration



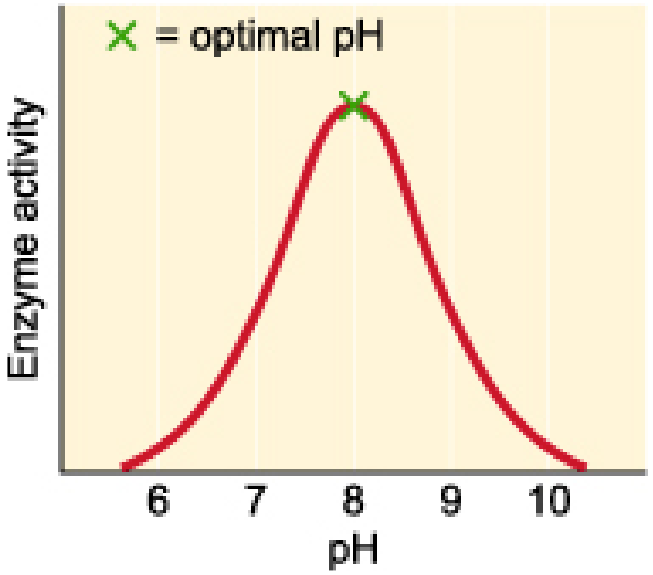
- **Enzyme concentration**:
  Increasing enzyme concentration will speed up the reaction, as long as there is substrate available to bind to.
- Once all of the enzymes have bound, any substrate increase will have no effect on the rate of reaction, as the available enzymes will be saturated.

Factors affecting enzymatic activity

3. Temperature

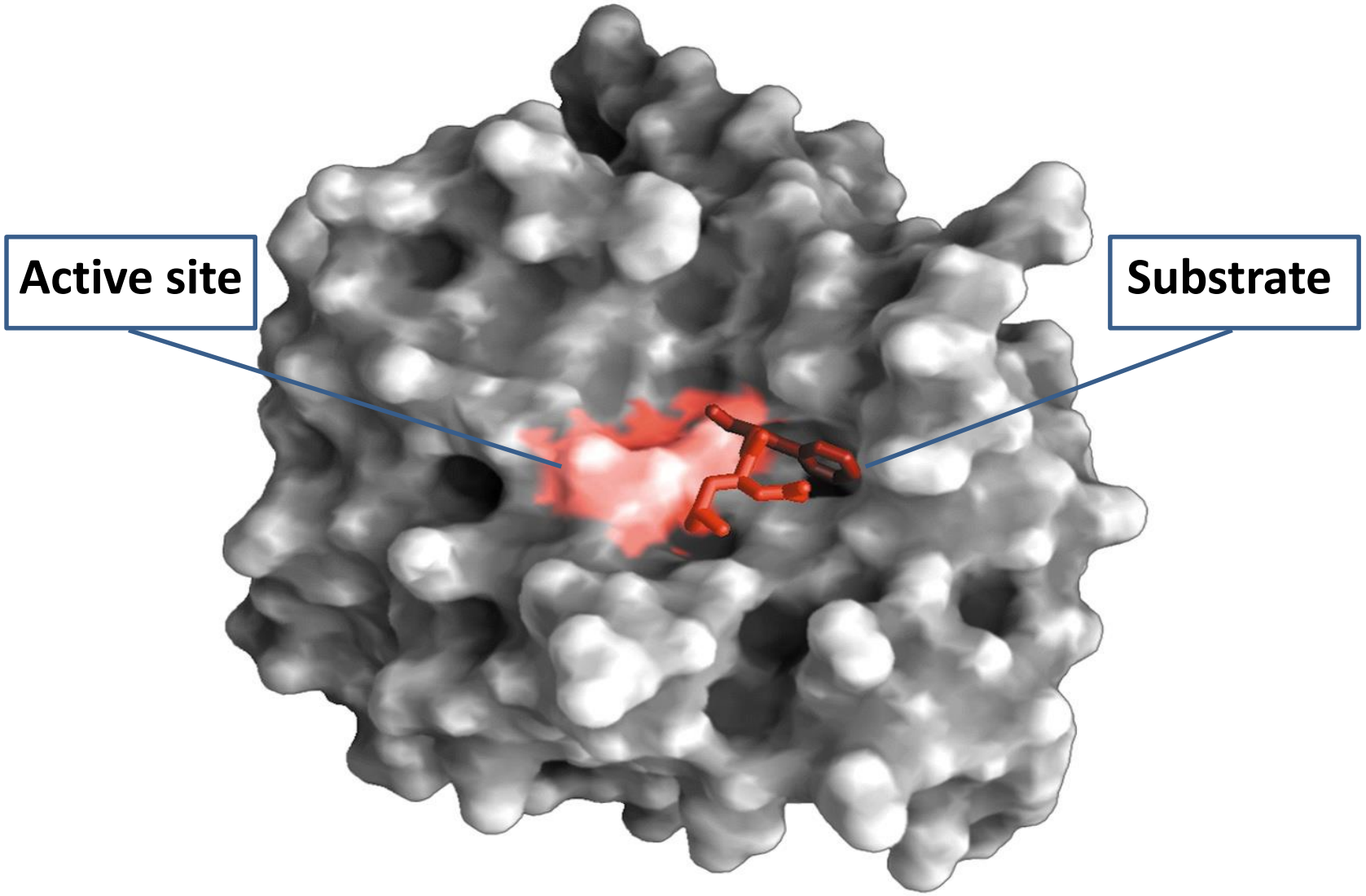


4. pH



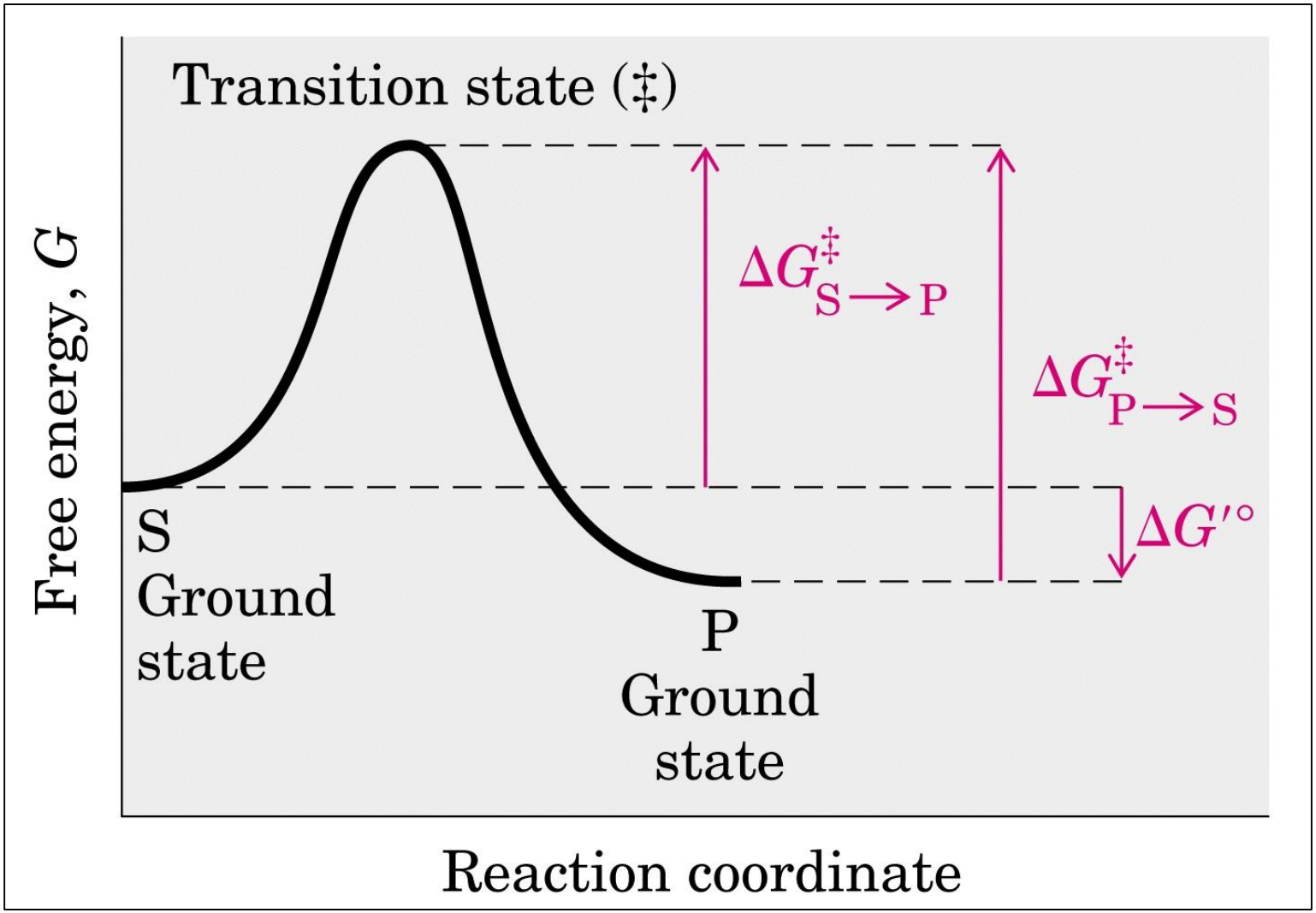
Enzymes show maximal catalytic activity at a characteristic pH and Temperature.

Chymotrypsin: Binding of a **substrate** to an enzyme at the **active site**



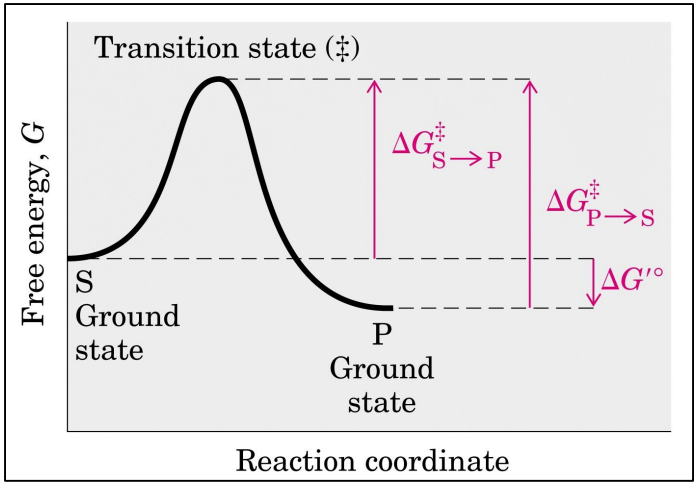
Reaction coordinate diagram for a chemical reaction

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



Reaction coordinate diagram for a chemical reaction

- **Enzymes Affect Reaction Rates, Not Equilibria**
  - ✓ The function of a catalyst is to increase the rate of the reaction.
  - ✓ Catalysts do not affect the equilibrium.



- **Transition state**:

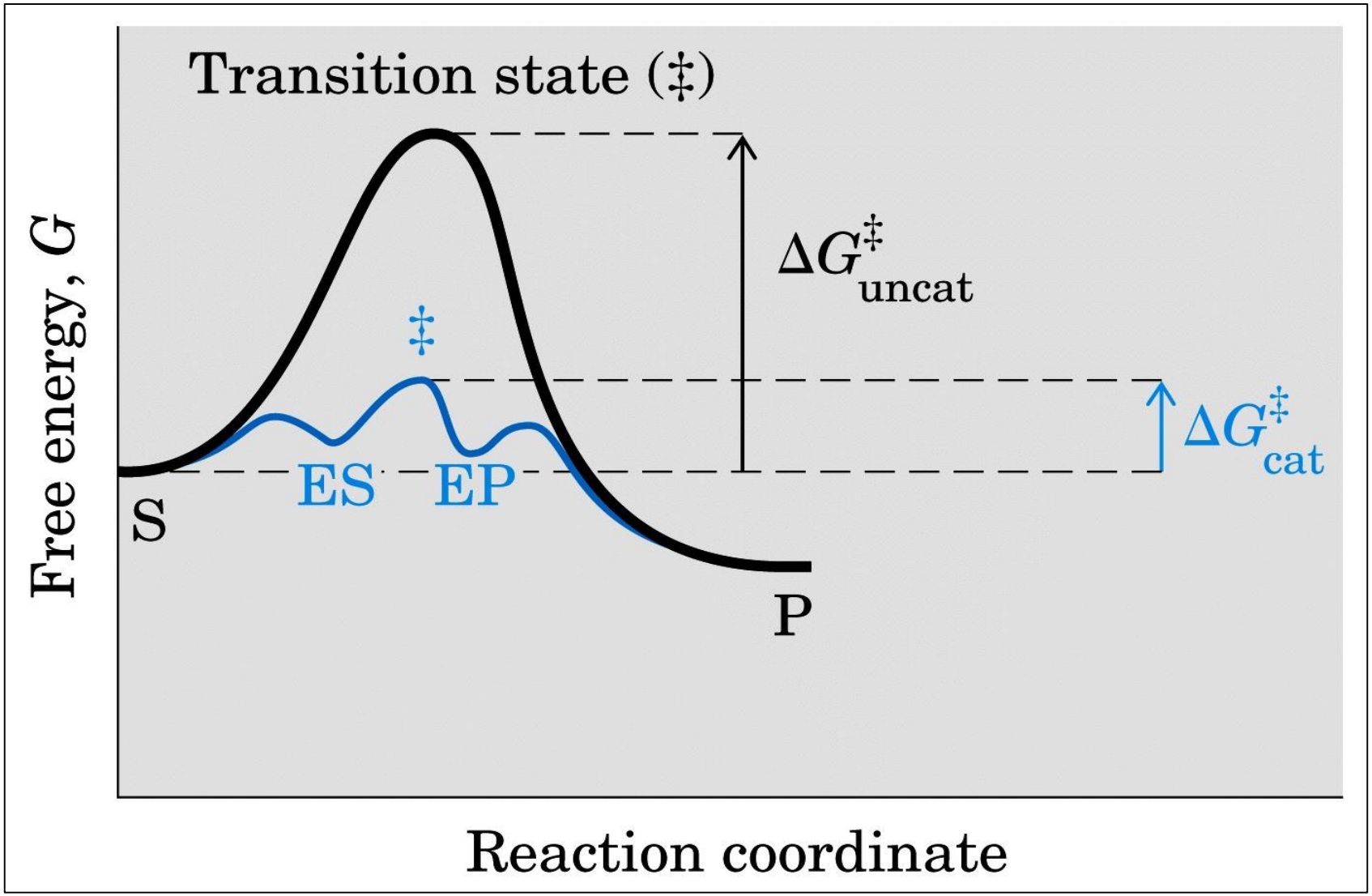
The molecules must be raised to a higher energy level.

- **Activation energy**:

The difference between the energy levels of the ground state and the transition state.

- The free-energy change is expressed as *ΔGo,* **standard free energy change.**
- *ΔGo'* for the reaction is negative and the equilibrium favors P. Since the free energy of the ground state of P is lower than that of S.

Reaction coordinate diagram comparing **enzyme-catalyzed** reaction and **uncatalyzed** reaction

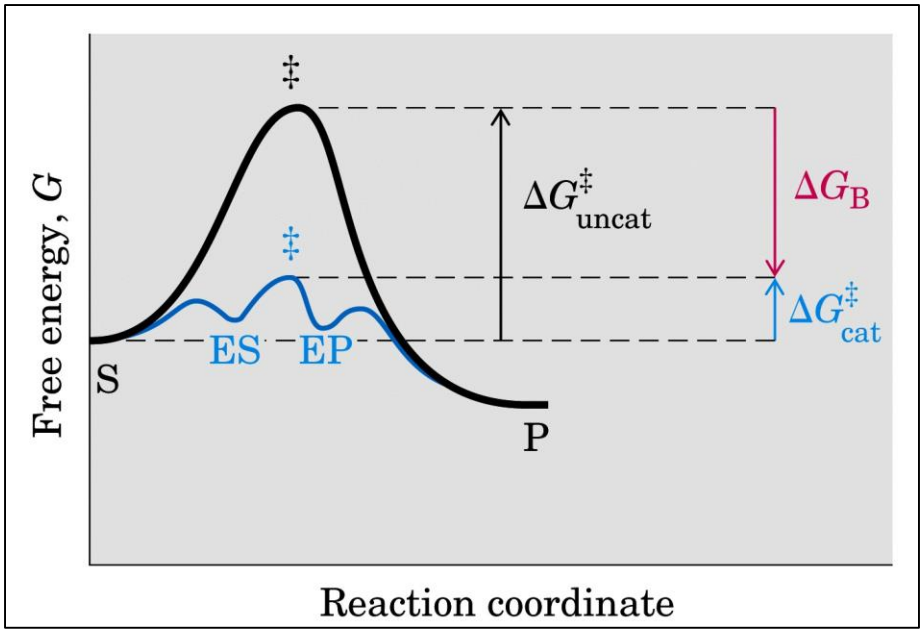


Enzymes

- Slow reactions face **significant activation barriers** ($\Delta G‡$) that must be surmounted during the reaction !
- **How do enzymes catalyze the chemical reaction?**

  OR increase the rate of a reaction?

➢ Catalysts enhance reaction rates (k) by <u>lowering activation energies</u> (ΔG‡). ???



Activation energies are **energy barriers** to chemical reactions

- The rate at which a molecule undergoes a particular reaction decreases as the activation barrier for that reaction increases.
- Crucial to life itself ???
- Without energy barriers →
  complex macromolecules would revert to much simpler molecular forms.

Enzyme activation vs. inhibition

Enzyme activation

- **Activators**:
  - compounds that increase enzyme activity.
  - Positive modifiers of enzyme activity.
- Usually metal ions:
  - Hexokinase (Mg^{+2})
  - Alcohol dehydrogenase (Zn^{+2})
  - Xanthine oxidase (Fe^{+3}, Mo^{+4})
- **Removal** of these metal ions results in partial or total loss of enzymatic activity.
- **Restoration** of lost metal ions regains the lost activity.

Enzyme activation vs. inhibition

Enzyme inhibition

- **Inhibitors**:
  - Compounds that decrease enzyme activity.
  - Reversible or Irreversible:

1. Reversible inhibitors:

✓ interact with an enzyme via noncovalent associations.

✓ bind to and can dissociate from the enzyme.

2. Irreversible inhibitors (inactivators):

✓ interact with an enzyme via covalent associations.

Enzyme activation vs. inhibition

Enzyme inhibition

1. Reversible inhibitors:

- ✓ Effect of inhibitor may be reversed.
- ✓ They are often:
  - structural analogs of substrates or products.
  - used as drugs to slow down a specific enzyme.

2. Irreversible inhibitors:

- ✓ One inhibitor molecule can permanently shut off one enzyme molecule.
- ✓ They are often powerful toxins but also may be used as drugs (e.g. aspirin inactivates cyclooxygenase)

Classes of Inhibition

Two real, one hypothetical

- **Competitive inhibition**

Inhibitor (I) binds only to E, not to ES.

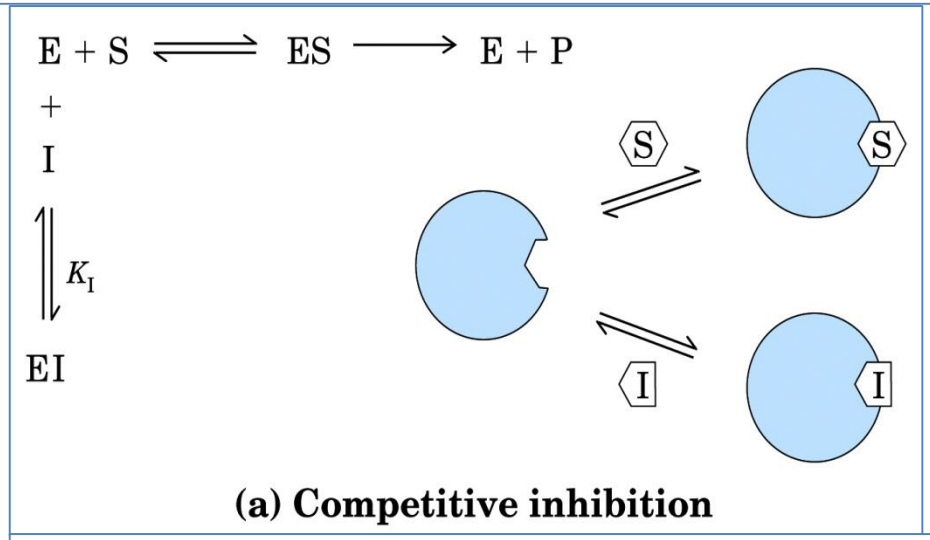
- **Noncompetitive inhibition**

Inhibitor (I) binds either to E and/or to ES.

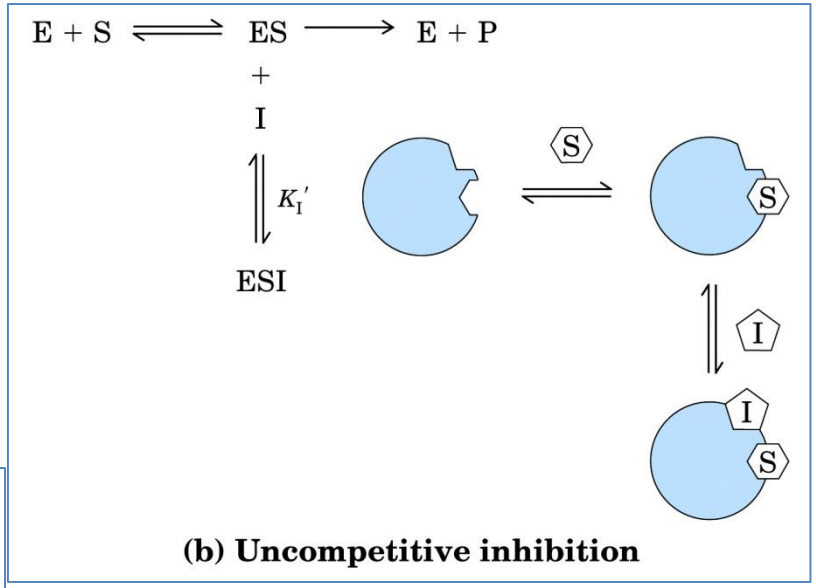
- Uncompetitive inhibition

Inhibitor (I) binds only to ES, not to E.

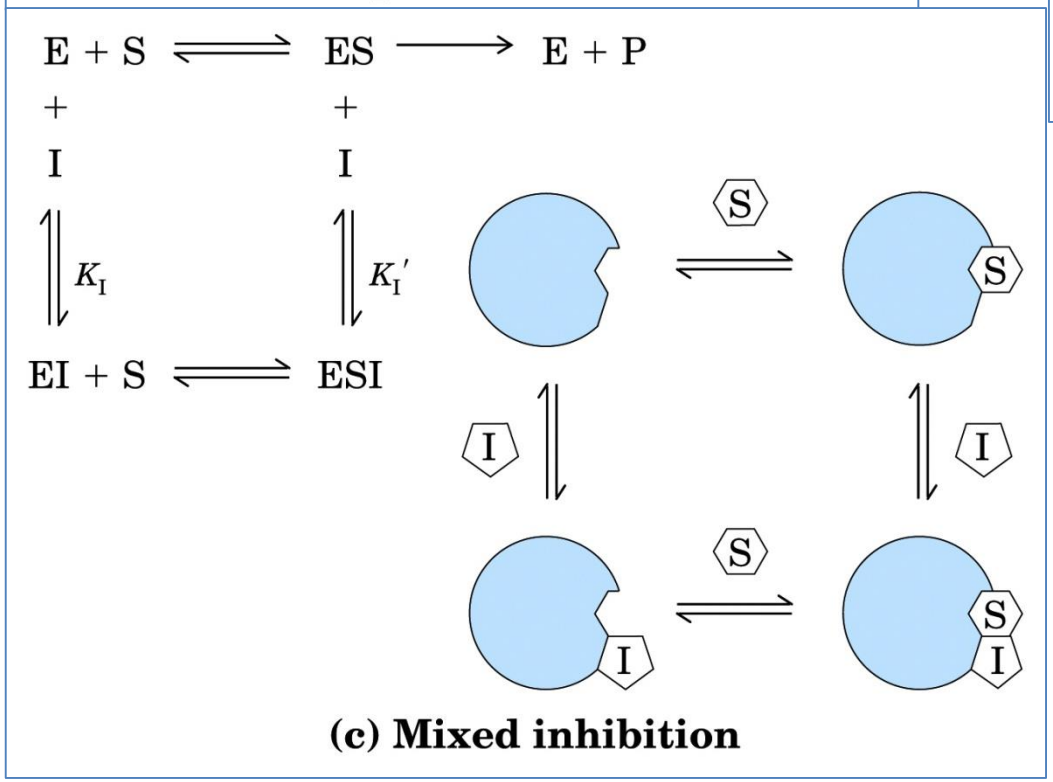
Three types of reversible inhibition



(a) Competitive inhibition



(b) Uncompetitive inhibition



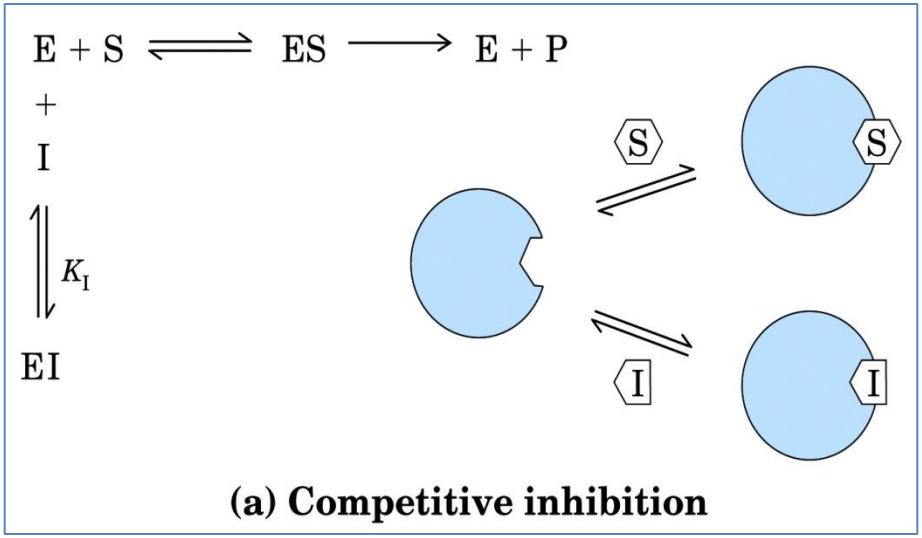
(c) Mixed inhibition

Classes of Inhibition

Two real, one hypothetical

- **Competitive inhibition**

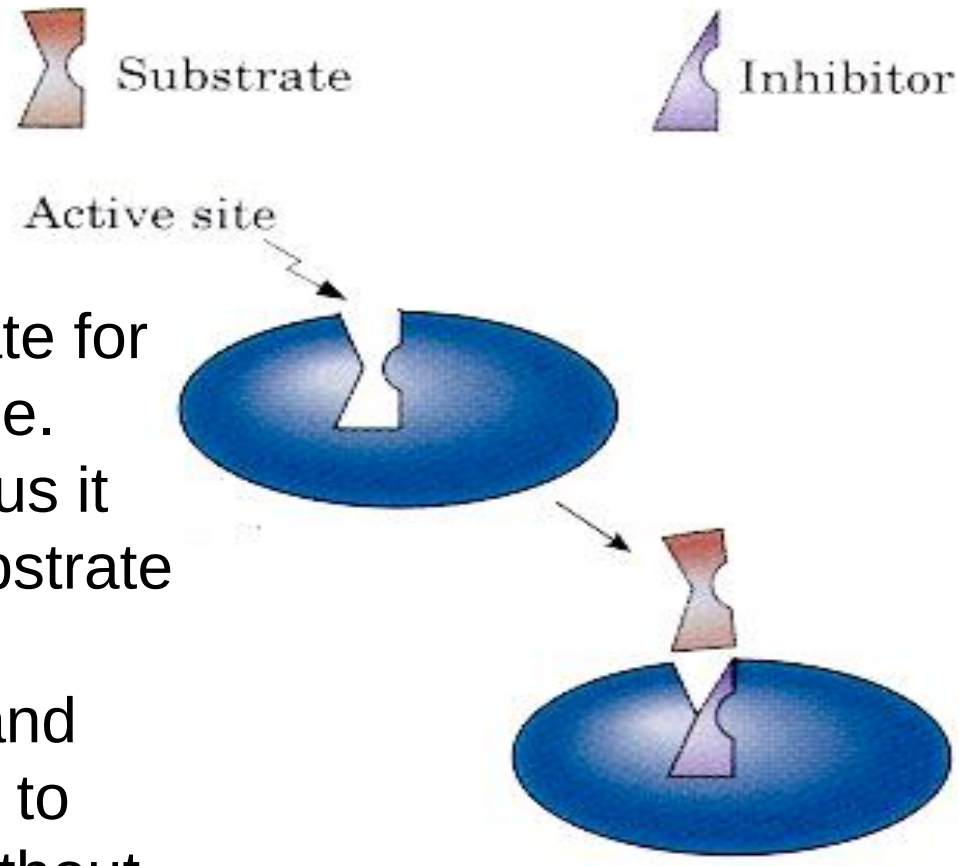
Inhibitor (I) binds only to E, not to ES.



(a) Competitive inhibition

Competitive Inhibitor

- Competes with the substrate for the active site of an enzyme.
- Occupies the active site thus it prevents binding of the substrate to the enzyme.
- Resembles the substrate and combines with the enzyme to form an **EI** complex, but without leading to catalysis.
- Can be reversed by adding more substrate ?



Enzyme mistakes inhibitor for substrate

Competitive inhibitor

A medical therapy based on competition at the active site is used to treat patients who have ingested methanol.

- methanol —**alcohol dehydrogenase**→ formaldehyde

- ethanol —**alcohol dehydrogenase**→ acetaldehyde

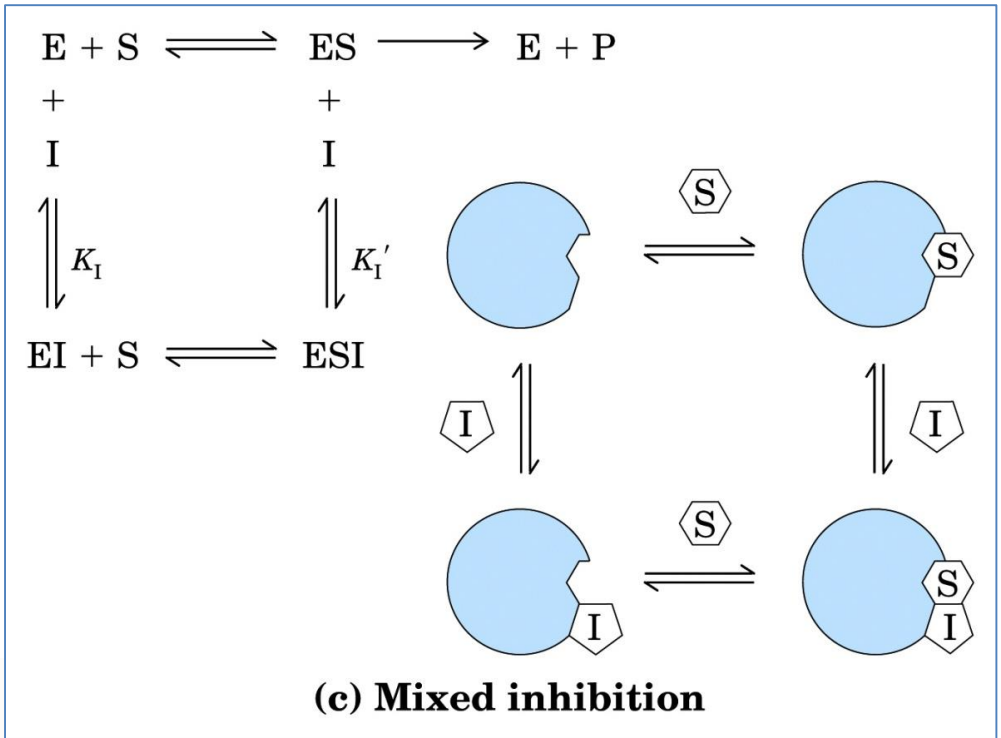
Classes of Inhibition

- **Competitive inhibition**

Inhibitor (I) binds only to E, not to ES.

- **Noncompetitive inhibition**

Inhibitor (I) binds either to E and/or to ES.



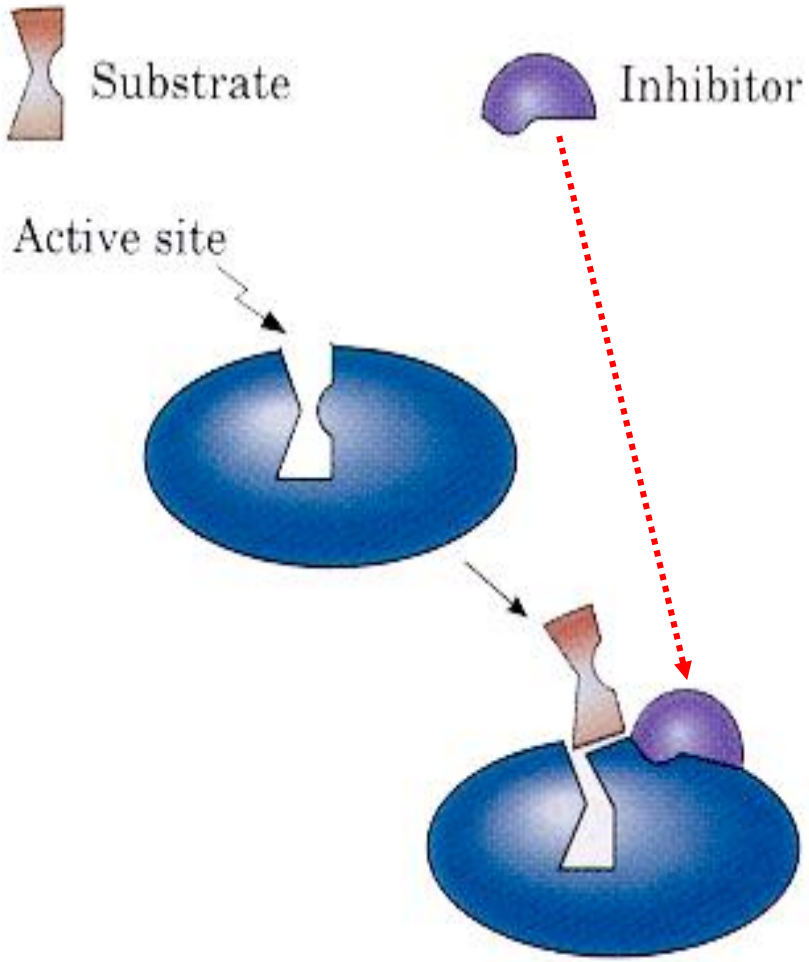
(c) Mixed inhibition

Non competitive Inhibition

•A noncompetitive inhibitor can bind to an enzyme in many ways:

• If it binds somewhere on the surface of the enzyme, it causes a change in the tertiary structure.

•The substrate is inhibited because it can't get into the enzyme.



Classes of Inhibition

Two real, one hypothetical

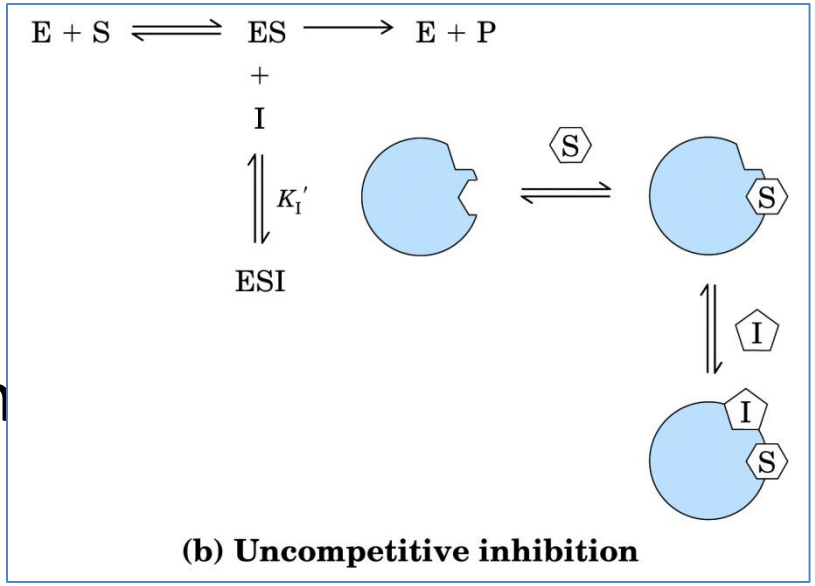
- **Competitive inhibition**

Inhibitor (I) binds only to E, not

- **Noncompetitive inhibition**

Inhibitor (I) binds either to E an

- Uncompetitive inhibition



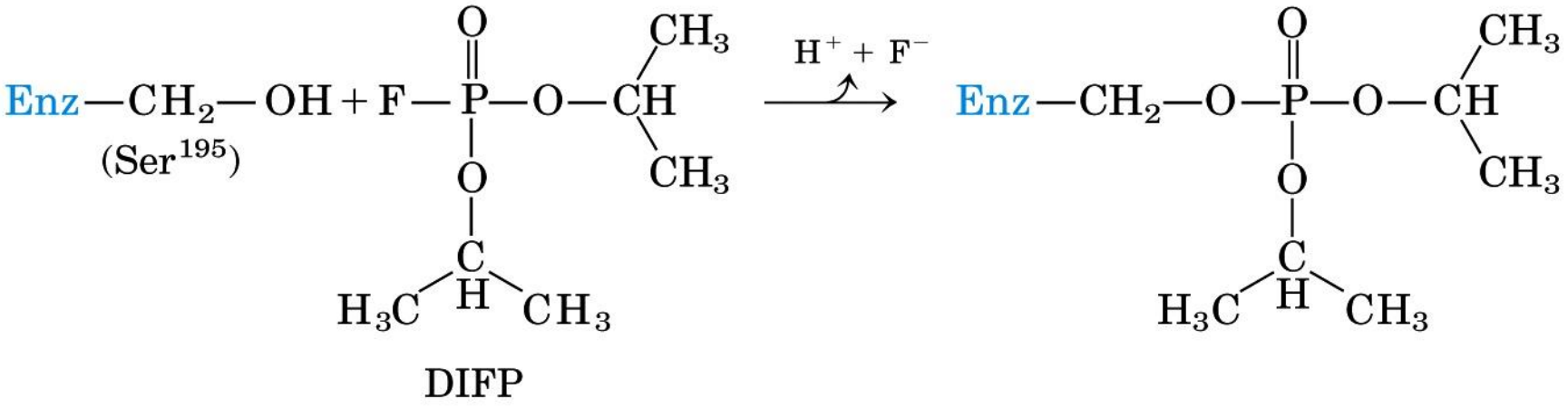
(b) Uncompetitive inhibition

Inhibitor (I) binds only to ES, not to E.

This is a hypothetical case that has never been documented for a real enzyme, but which makes a useful contrast to competitive inhibition

Irreversible inhibition

Irreversible inhibitors:
inhibitors that bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme's activity,
or form a particularly stable noncovalent association.



DIFP

DIFP (Diisopropylfluorophosphate):

- nerve gas.
- an irreversible inhibitor of acetylcholinesterase.

Regulatory Enzymes

Classes:

1. **Allosteric enzymes:**

- Affected by reversible noncovalent binding of allosteric modulators.

2. **Nonallosteric/covalent enzymes:**

- Regulated by reversible covalent modification.

3. **Regulatory protein binding enzymes**

- Stimulated or inhibited by the binding of separate regulatory proteins.

4. **Proteolytically activated enzymes**

- Activated by the removal of some segments of their polypeptide sequence by proteolytic cleavage.

Allosteric enzymes

- Function through reversible, noncovalent binding of allosteric effectors.
- **Allosteric effectors (allosteric modulators):** regulatory compounds **(**small metabolites or cofactors).
- In addition to active sites, allosteric enzymes generally have one or more regulatory or allosteric sites for binding the modulator.

Allosteric Enzymes Undergo Conformational Changes in Response to Modulator Binding

- The modulator for allosteric enzyme:
- may be inhibitory or stimulatory.
- Is the substrate itself or molecule other than the substrate

- Regulatory Enzymes:

1. Homotropic:

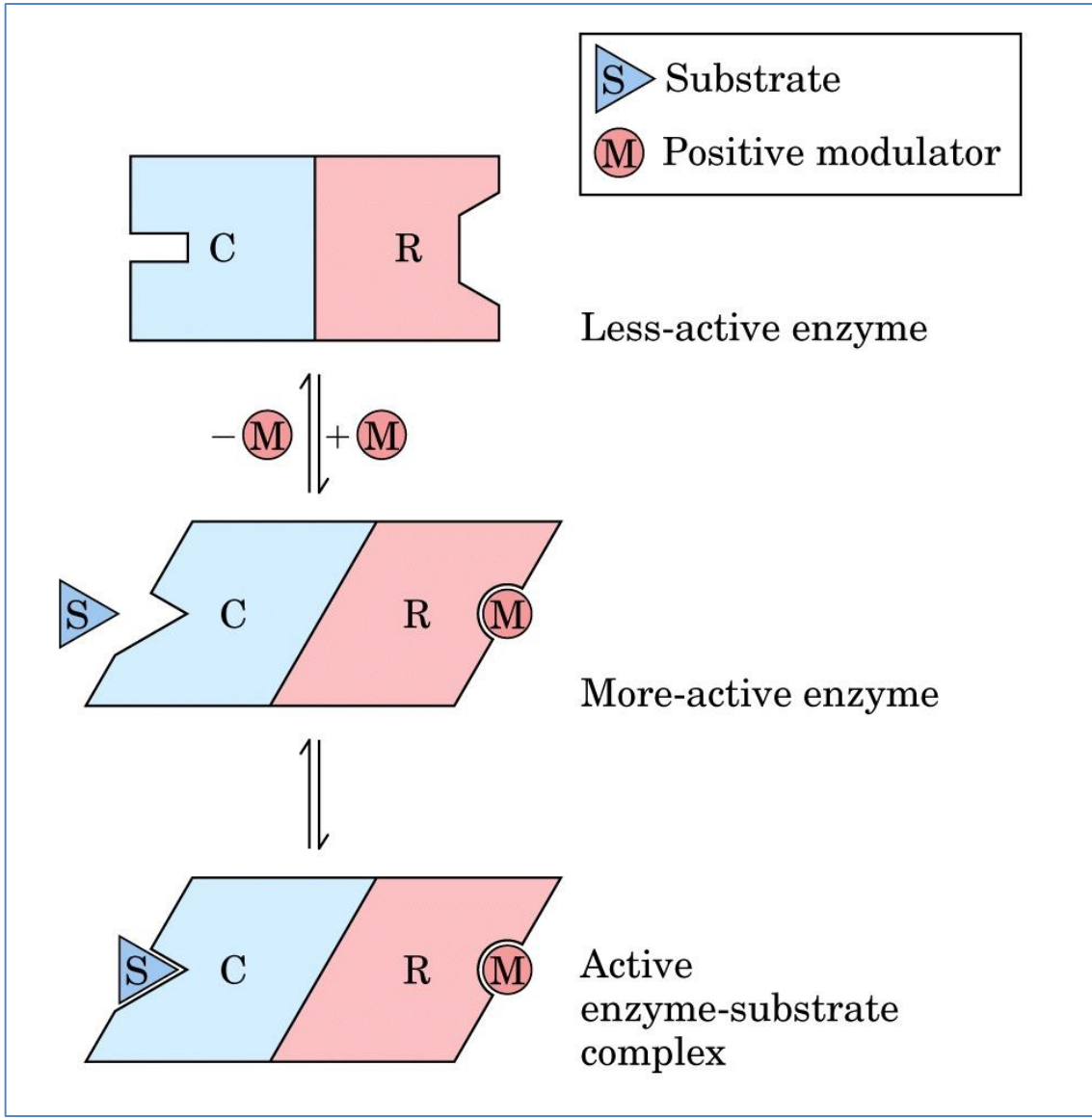
regulatory enzymes for which substrate and modulator are identical.

2. Heterotropic:

When the modulator is a molecule other than the substrate.

Subunit interactions in an allosteric enzyme and interactions with inhibitors and activators

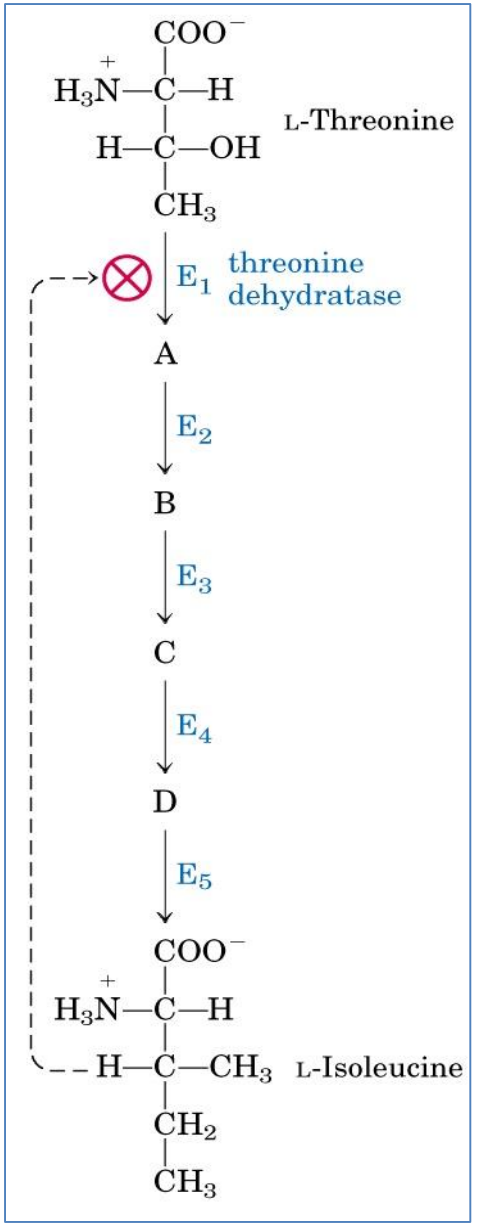
- enzyme's active site is specific for its substrate.
- Regulatory site is specific for its modulator.



Feedback inhibition

In many pathways a regulated step is catalyzed by an allosteric enzyme:

The **regulatory enzyme** is specifically inhibited by the end product of the pathway whenever the concentration of the end product exceeds the cell's requirements.



Feedback inhibition

Pentose phosphate pathway Regulation