Part II => PROTEINS and ENZYMES

§2.5 ENZYME CATALYSIS
§2.5a Enzyme Properties
§2.5b Transition State Theory
§2.5c Catalytic Mechanisms
Section 2.5a: Enzyme Properties
Enzymes are biological catalysts—they are almost exclusively proteins though some RNAs (ribozymes) also serve as biological catalysts (eg mRNA translation).

- Enzymes differ from chemical catalysts in reaction rate, reaction conditions, reaction specificity, and regulatory control.

- The unique physical and chemical properties of the active site limit an enzyme’s activity to specific substrates and reactions.

- Enzymes may also require cofactors for their catalytic activity.
Differences Between Enzymes and Catalysts

Enzymes differ from ordinary chemical catalysts in the following ways:

(1) **Higher Reaction Rates:**
   Enzymes catalyze biological reactions at rates that are typically several orders of magnitude greater than their chemical counterparts.

(2) **Milder Reaction Conditions:**
   Enzymatically-catalyzed reactions occur under relatively mild temperatures (typically between 0-50°C), close to neutral pH (typically between 6-8), and around atmospheric pressure (~1 atm)—in contrast, chemical catalysts require elevated temperatures and pressures and extremes of pH.

(3) **Greater Reaction Specificity:**
   Enzymes display a remarkable degree of **substrate specificity** compared to their chemical counterparts.

(4) **Greater Regulatory Control:**
   Catalytic activities of many enzymes are tightly regulated by **allosteric modulators**, covalent modifications, and feedback loops.
# Enzyme Classification: Six Major Classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Catalysis</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidoreductase</strong></td>
<td>Reduction-oxidation (redox) reactions</td>
<td>Alcohol dehydrogenase—reduction of acetaldehyde to ethanol (fermentation)</td>
</tr>
<tr>
<td><strong>Transferase</strong></td>
<td>Intermolecular transfer of functional groups</td>
<td>Hexokinase—phosphorylation of glucose to glucose-6-phosphate (glycolysis)</td>
</tr>
<tr>
<td><strong>Isomerase</strong></td>
<td>Intramolecular group rearrangement</td>
<td>Phosphoglucoisomerase—isomerization of glucose-6-phosphate to fructose-6-phosphate (glycolysis)</td>
</tr>
<tr>
<td><strong>Hydrolase</strong></td>
<td>Bond cleavage with H$_2$O</td>
<td>VHR phosphatase—dephosphorylation of ERK kinases (cellular signaling)</td>
</tr>
<tr>
<td><strong>Lyase</strong></td>
<td>Group elimination to generate double bonds</td>
<td>Enolase—dehydration of phosphoglycerate to phosphoenol pyruvate (glycolysis)</td>
</tr>
<tr>
<td><strong>Ligase</strong></td>
<td>Bond formation to generate larger molecules</td>
<td>DNA ligase—joining together of single-stranded DNA strands (DNA replication/repair)</td>
</tr>
</tbody>
</table>
Catalytic Power of Enzymes

- Enzymatically-catalyzed reactions are typically \(10^6\) to \(10^{12}\) times faster than their uncatalyzed counterparts due to their high specificity.

- Enzyme specificity is largely defined by the shape/chemistry (geometric specificity) and chirality/asymmetry (stereospecificity) of interacting surfaces of both the enzyme and substrate.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Nonenzymatic Reaction Rate ((s^{-1}))</th>
<th>Enzymatic Reaction Rate ((s^{-1}))</th>
<th>Rate Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic anhydrase</td>
<td>(1.3 \times 10^{-1})</td>
<td>(1 \times 10^6)</td>
<td>(7.7 \times 10^6)</td>
</tr>
<tr>
<td>Chorismate mutase</td>
<td>(2.6 \times 10^{-5})</td>
<td>50</td>
<td>(1.9 \times 10^6)</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>(4.3 \times 10^{-6})</td>
<td>4300</td>
<td>(1.0 \times 10^9)</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>(3.0 \times 10^{-9})</td>
<td>578</td>
<td>(1.9 \times 10^{11})</td>
</tr>
<tr>
<td>AMP nucleosidase</td>
<td>(1.0 \times 10^{-11})</td>
<td>60</td>
<td>(6.0 \times 10^{12})</td>
</tr>
<tr>
<td>Staphylococcal nuclease</td>
<td>(1.7 \times 10^{-13})</td>
<td>95</td>
<td>(5.6 \times 10^{14})</td>
</tr>
</tbody>
</table>
Enzyme Specificity: Geometric Specificity

Geometric specificity is concerned with the shape and the amino acid functional groups lining the interacting surfaces of both the enzyme and substrate:

- Substrate binding cleft within the enzyme active site is **geometrically complementary** to the shape of the substrate.

- Functional groups lining the active site or substrate binding pocket within the enzyme are **electronically complementary** to those on the surface of the substrate.

- Such complementation of shape and functional groups results in the **optimization of intermolecular forces** such as van der Waals and ionic interactions between the enzyme and the substrate.

- Hydrophobic sidechains
- Acidic/basic sidechains
- Hydrogen bonding between $\delta^+$ and $\delta^-$ polarized pairs
Stereospecificity is concerned with chirality or asymmetry (e.g., spatial orientation) of substrates due to the fact that enzymes (composed of L-amino acids) themselves are chiral or harbor asymmetric active sites:

- Enzymes can only accommodate the substrate in an asymmetric manner.

- Thus, enzymes catalyze not only chiral but also prochiral (can become chiral in a single step!) substrates in a highly stereospecific manner—e.g., citrate binds to the enzyme active site asymmetrically via three-point attachment.

- This is due to the fact that while the two CH$_2$COO$^-$ groups on citrate are chemically-equivalent, they occupy two distinct spatial positions relative to OH and COO$^-$ groups—only one of these two CH$_2$COO$^-$ groups can therefore undergo catalysis!
While enzyme stereospecificity is difficult to compromise, many enzymes are highly promiscuous with respect to the geometric requirements—eg chymotrypsin

- Chymotrypsin can catalyze hydrolysis of both the peptide/amide bond and the chemically-related ester bond
- Many enzymes require small non-protein helper molecules called “cofactors”

- Such cofactors can be divided into TWO major categories:
  1. Metal ions
  2. Coenzymes

- Proteins/enzymes that require cofactors for their action can also be classified according to whether they lack (apoprotein/apoenzyme) or harbor (holoprotein/holoenzyme) their cognate cofactor(s)
Enzyme Cofactors: Metal Ions

- Metals ions such as $\text{Cu}^{2+}$, $\text{Fe}^{2+}$, $\text{Fe}^{3+}$, $\text{Mg}^{2+}$, $\text{Mn}^{2+}$, $\text{Mo}^{2+}$, $\text{Ni}^{2+}$ and $\text{Zn}^{2+}$ are commonly employed as cofactors in biology.

- With rare exceptions, all protein kinases require a divalent metal ion as a cofactor—with $\text{Mg}^{2+}$ being the most common!

- Metal ions are usually tightly and permanently bound to enzymes via the coordinate covalent bonding and are only released upon their denaturation.

- Such metal ions are also referred to as “trace elements”—an essential component of dietary intake.

Phosphorylation of glucose to glucose-6-phosphate by hexokinase using $\text{Mg}^{2+}$ as a metal-ion-cofactor.
Enzyme Cofactors: Coenzymes

Coenzymes are usually organic or organonometallic compounds that are further subdivided into two categories:

1. **Cosubstrates**—they bind to enzymes only **TRANSIENTLY**—they step “on” and “off”, or dissociate and re-associate, during each catalytic cycle

2. **Prosthetic groups**—they are **PERMANENTLY** attached (non-covalently or covalently) to enzymes so as to constitute an integral component of the host protein—they never dissociate off the enzyme and only do so under harsh treatments such as protein denaturation

But, be aware that:

(a) Designations such as cofactors, coenzymes and prosthetic groups are loosely applied in the literature—rigid boundaries mentioned above are rarely exercised

(b) Many coenzymes can serve as a “cosubstrate” in one enzyme but as a “prosthetic group” in another!
Cosubstrates include organic and organometallic compounds such as:

- **NAD** Nicotinamide adenine dinucleotide
- **NADP** Nicotinamide adenine dinucleotide phosphate
- **CoA** Coenzyme A
Prosthetic groups include organic and organometallic compounds such as:

- **FAD**: Flavin adenine dinucleotide
- **FMN**: Flavin mononucleotide
- **LPA**: Lipoic acid (Lipoamide)
- **TPP**: Thiamine pyrophosphate

Dehydrogenation of succinate to fumarate by SDH—FAD is covalently attached to SDH and does not dissociate off during the catalytic cycle but rather it is regenerated “in situ” via the action of a bulk pool of coenzyme Q.
Exercise 2.5a

- What properties distinguish enzymes from other catalysts?
- Describe how different enzymes are classified and named.
- What factors influence an enzyme’s substrate specificity?
- Why are cofactors required for some enzymatic reactions?
- What is the relationship between cofactors, coenzymes, cosubstrates, and prosthetic groups?
Section 2.5b: Transition State Theory
Synopsis 2.5b

- A chemical reaction proceeds through a maximum potential energy barrier along the reaction coordinate—ie it requires activation energy or the free energy of activation ($\Delta G^\ddagger$)

- The molecular structure corresponding to this maximum potential energy point is referred to as the “transition state”—in the physical sense, the transition state can be envisioned as a transient pseudo-intermediate in the reaction with the maximum potential energy.

- Transition state (TS) theory postulates that enzymes catalyze biological reactions by virtue of their ability to lower $\Delta G^\ddagger$ of the transition state—but without affecting the overall free energy change ($\Delta G$) associated with a reaction.

- In particular, the TS theory is concerned with the overall reaction rates (kinetics) and energies (thermodynamics) associated with the transition state(s) in a chemical reaction.

- TS theory thus offers a powerful tool to understand how enzymes work in qualitative terms.
Consider the following reaction proceeding via a transient intermediate $X$:

$$A + B \leftrightharpoons X^\dagger \leftrightharpoons P + Q$$

A plot of free energy ($G$) versus reaction coordinate—a time variable—for the above reaction yields the so-called “reaction profile” or “reaction coordinate diagram”.

Intermediate $X$ is called the “transition state” if it lies at the highest point (maximum $G$) on the reaction profile.

In symbolic terms, the transition state and parameters associated with it are indicated by the “double dagger ($\dagger$)” notation—expressed as a superscript.

Thus, $X^\dagger$ represents the physical intermediate that equates to the transition state and $\Delta G^\dagger$ is the free energy associated with it—what does $\Delta G^\dagger$ mean?!
- \( \Delta G^\ddagger \) is the difference between the free energies of ground states (reactants A and B) and the transition state \( X^\ddagger \)—it is the so-called “free energy of activation”

- In lay terms, \( \Delta G^\ddagger \) is the minimum thermal energy that the reactants must possess in order to overcome the “thermodynamic barrier” for the reaction to proceed—it is the “spark” needed to ignite the fuel!

- Thus, lower the \( \Delta G^\ddagger \) the greater the likelihood that a biological reaction will ensue—or simply put, reaction will proceed faster (it will occur over seconds rather than days or weeks!)

- But, how do we lower \( \Delta G^\ddagger \)? This is where our catalytic workhorses step in—enzymes speed up biological reactions by simply lowering \( \Delta G^\ddagger \)
The dependence of the rate of a reaction \( (k) \) on temperature is given by the simplified Arrhenius equation (cf the more complex Eyring equation):

\[
k = A \cdot \exp\left(-\frac{\Delta G^\ddagger}{RT}\right)
\]

where
- \( A \) = Pre-exponential factor (s\(^{-1}\))
- \( R \) = Universal molar gas constant (cal/mol/K)
- \( T \) = Absolute temperature (K)
- \( \Delta G^\ddagger \) = Free energy of activation (cal/mol)

While the Arrhenius equation assumes quasi-equilibrium (a crude approximation to true thermodynamic equilibrium!) between \( X^\ddagger \) and the reactants, it nonetheless reaffirms the notion that lowering \( \Delta G^\ddagger \) will make the reaction go faster.

This is indeed the basis of all catalysts—chemical and biological alike—in that they speed up reactions by simply lowering the free energy of the transition state or \( \Delta G^\ddagger \).

Thus, greater the reduction in \( \Delta G^\ddagger \) (\( \Delta \Delta G^\ddagger_{\text{cat}} \)) the faster the reaction will proceed but without affecting the overall free energy change (\( \Delta G \)).
Exercise 2.5b

- Sketch and label various parts for reaction profiles with and without a catalyst

- What is $\Delta G^\ddagger$?

- What is the relationship between $\Delta G$ and $\Delta G^\ddagger$? Which of these parameters are altered by enzymes?
Section 2.5c: Catalytic Mechanisms
- **Enzymes accelerate reactions** by bringing reacting groups together and orienting them for reaction so as to stabilize the transition state

- **Transition state stabilization** can significantly lower the activation energy for a reaction

- To accomplish this feat, **the active sites of enzymes are usually clustered with charged and polar residues**
Catalytic Mechanisms: Key Players

In enzymes, **His** reigns supreme in mediating acid-base catalysis due to its sidechain pK value being close to neutral pH—thus being able to serve both as an acid and base—**Asp/Glu** acidic residues also participate to varying degrees in mediating acid-base catalysis.

**Ser/Cys/Tyr** polar residues primarily participate in mediating nucleophilic catalysis.

**Arg/Lys** basic residues are usually involved in substrate stabilization.

Active sites of many enzymes usually harbor a cluster of such charged and polar residues so as to enable them to participate in catalysis of their bound substrates in a highly concerted fashion.

Such clustering can also modulate the pK values (by as much as several pH units) of active site residues so as to enable them to overcome unfavorable charge-charge interactions—alteration of pK is key to enzyme catalysis.

### Catalytically Important Amino Acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Residue</th>
<th>pK&lt;sub&gt;R&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>10.5</td>
</tr>
<tr>
<td><strong>Arginine</strong></td>
<td>Arg</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Histidine</strong></td>
<td>His</td>
<td>6.0</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>Asp</td>
<td>3.9</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Glu</td>
<td>4.1</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>13.0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>10.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>8.5</td>
</tr>
</tbody>
</table>

**Acid-Base Catalysis**

**Nucleophilic Catalysis**
Because ionization (such as protonation or deprotonation) of charged and polar residues mediating enzyme catalysis is highly sensitive to pH, most enzymes are active only within a narrow pH range—displaying a classical bell-shaped curve.

- Mechanisms employed by enzymes to catalyze their substrates can be classified into three major categories—enzymes usually employ TWO or more of these in a concerted fashion:
  1. Acid-Base Catalysis
  2. Nucleophilic Catalysis
  3. Metal Catalysis
(1) Acid-Base Catalysis: Acid Catalysis

**Keto-enol tautomerization**

- An acid (HA) is a proton donor (or electron acceptor)

- In acid catalysis, a proton is usually donated to the substrate—eg from HA to the carbonyl group to catalyze keto-enol tautomerization—this facilitates the formation of a carbanion-like transition state (TS) by lowering its activation energy

- Without such acid catalysis mediated by HA, the formation of TS (the square brackets indicate its transient stability) would be energetically unfavorable and thus a very slow process

- TS subsequently decays into the enol form by releasing one of the methyl protons to the bulk water

- HA is regenerated by virtue of the ability of its conjugate base (A⁻) to accept a proton from the bulk water
A base (B) is a proton acceptor (or electron donor).

In base catalysis, a proton is usually stripped or abstracted from the substrate—eg from the methyl group to B to catalyze keto-enol tautomerization—this facilitates the formation of a carbanion-like transition state (TS) by lowering its activation energy in a manner akin to acid catalysis.

TS subsequently decays into the enol form by virtue of the ability of carbonyl group to take up a proton from the bulk water.

B is regenerated by virtue of the ability of its conjugate acid (BH⁺) to donate a proton to the bulk water.
Pancreatic ribonuclease A (RNase A) catalyzes endo-cleavage of RNAs.

- pH dependence of RNase A activity suggests the involvement of two ionizable residues with pK values around 5.5 and 6.5—what would be the most likely suspects?! His residues—why?

- Further experimental interrogation confirms that His12 and His119 are essential for catalysis—what catalytic role could they be playing?
(1) Acid-Base Catalysis: RNAse A Catalysis

(1) His12 acts as a general base and abstracts a proton from an 2′-OH group within RNA, thereby promoting its nucleophilic attack on the adjacent 3′-phosphoester bond—acting in concert with His12, His119 serves as a general acid and aids the cleavage of 5′-phosphoester bond coupled with the formation of a 2′-3′-cyclic nucleotide intermediate.

(2) Reversing their roles, His119 now acts as a general base and abstracts a proton from water so as to promote its nucleophilic attack on the phosphate moiety of the 2′-3′-cyclic nucleotide—while His12 serves as a general acid and aids the hydrolysis of cyclic phosphodiester bond resulting in the release of 3′-phosphonucleotide.

What catalytic mechanism(s) does RNAse A employ? Two of the three:
- Acid-base catalysis
- Nucleophilic catalysis
(1) Nucleophilic catalysis (also called covalent catalysis) involves nucleophilic attack by an enzyme (e.g., N atom of pyridine) on an electrophilic center on the substrate (e.g., carbonyl C atom of ester)—resulting in the formation of a transient enzyme-substrate covalent intermediate coupled with the release of an outgoing group (e.g., ROH) aided by an acid (H⁺).

(2) In the covalent intermediate, what was originally the nucleophilic atom (e.g., N atom of pyridine) now withdraws electrons from what originally served as the electrophilic center on the substrate (e.g., carbonyl C atom of ester)—resulting in the release of enzyme and substrate aided by a base (e.g., H₂O).
(2) Nucleophilic Catalysis: Functional Groups

**Nucleophiles**

- \( R\dot{O}H \) \( \Leftrightarrow \) \( \dot{R}O: \) + H\(^+\) Hydroxyl group
- \( \dot{R}SH \) \( \Leftrightarrow \) \( \dot{R}S: \) + H\(^+\) Sulfhydryl group
- \( RJH_3^+ \) \( \Leftrightarrow \) \( RJNH_2 \) + H\(^+\) Amino group
- [Imidazole structure] + H\(^+\) Imidazole group

**Electrophiles**

- H\(^+\) Protons
- \( Mn^+ \) Metal ions
- [Carbonyl carbon atom] Carboxyl carbon atom
- [Cationic imine (Schiff base)] Cationic imine (Schiff base)
- VHR (Vaccinia H1-Related) is a protein phosphatase that dephosphorylates tyrosine and/or serine residues in ERK (extracellular-regulated kinase) family of protein kinases.

- Experimental investigations suggest that the D92/C124/R130 catalytic triad conspires in a concerted fashion to dephosphorylate phosphotyrosine (pY) residue in ERK (pERK)—so as to inactivate it in playing any further role in cellular signaling.

- How does the D92/C124/R130 catalytic triad accomplish this feat? Presumably via nucleophilic catalysis—but, how exactly? And what is the role of H123?
(2) Nucleophilic Catalysis: VHR Catalysis

1. After stabilization of pY moiety of ERK via hydrogen bonding with the guanidino group of R130 within the VHR active site, nucleophilic attack of the thiolate anion of C124 on the phosphate group of pY results in the formation of a transient phospho-enzyme covalent intermediate coupled with concomitant release of dephosphorylated ERK—aided by the donation of a proton from D92 acting as a general acid.

2. Reversing its role, D92 now serves as a general base and accepts a proton from a water molecule and the resulting hydroxyl group attacks the phosphate atom within the phospho-enzyme intermediate to eliminate inorganic phosphate and regenerate a thiolate anion of C124 for the next cycle.

What catalytic mechanism(s) does VHR employ? Two of the three:
- Nucleophilic catalysis
- Acid-base catalysis
Given that the pK value of the –SH group of cysteine is about 8.5, C124 ought to be largely protonated @ pH 7.4—so how does the thiolate anion form? Recall that neighboring residues can modulate pK values of sidechain groups of amino acids by several pH units.

Indeed, C124 is preceded by H123 such that its imidazole sidechain orchestrates an hydrogen bonding network in and around the vicinity of C124 that effectively results in the abstraction of a proton from the –SH group, thereby facilitating the formation of a thiolate anion under physiological conditions.

Since its sidechain orients away from the catalytic center in the direction opposite to that of C124, H123 cannot directly abstract the proton from –SH group of C124.

pH dependence of VHR catalytic activity yields a pK value of around 5.5 for C124—a whopping reduction of 3 pH units!

In the related bacterial Yersinia protein tyrosine phosphatase (YopH), the pK value of catalytically-equivalent cysteine to C124 is even more astounding at 4.5!
(3) Metal Catalysis: General Features

- Many enzymes, called metalloenzymes, require metal ions for catalysis

- Such metal ions include $\text{Cu}^{2+}$, $\text{Fe}^{2+}$, $\text{Fe}^{3+}$, $\text{Mg}^{2+}$, $\text{Mn}^{2+}$, $\text{Mo}^{2+}$, $\text{Ni}^{2+}$ and $\text{Zn}^{2+}$

- Metal ions are usually tightly and permanently bound to enzymes via coordinate covalent bonding and are only released upon their denaturation

- Metal ions mediate enzyme catalysis in four major ways:
  
  (a) **Substrate orientation**—metal ions bind to substrates to orient them properly for catalytic reaction

  (b) **Electrostatic stabilization**—metal ions electrostatically stabilize or shield negative charges on bound substrates

  (c) **Redox reactions**—metal ions promote oxidation-reduction of substrates by virtue of their ability to undergo a change in their oxidation state

  (d) **pK reduction**—coordination of metal ions (acting as Lewis acids—electron acceptors!) to water reduces its pK value from 14 to 7, thereby allowing it act as a strong nucleophile ($\text{HO}^-$) under physiological settings
**Carbonic anhydrase (CAH)** catalyzes the conversion of CO$_2$ to bicarbonate (HCO$_3^-$):

$$\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+$$

- CAH thus plays a key role in the **maintenance of pH** and the transport of CO$_2$ produced during respiration to the lungs (recall §2.4)

- CAH is a metalloenzyme with a bound Zn$^{2+}$ ion—tetrahedrally coordinated via three histidine residues (H94/H96/H119) with water transiently acting as a fourth ligand (not shown)—what is the role of H64?
(3) Metal Catalysis: CAH Catalysis

Im = Imidazole ring of one of the three histidine ligands (H94/H96/H119) of Zn$^{2+}$ within CAH

(1) Zn$^{2+}$ ion, acting as a Lewis acid, coordinates with H$_2$O (as a fourth ligand) resulting in the hyperpolarization of its O-H bonds—H64 preys upon such hyperpolarization of water and abstracts one of its protons acting as a general base, thereby generating the OH$^{-}$ nucleophile—the subsequent release of proton into solution by H64 (acting as a general acid) regenerates it for the next catalytic cycle

(2) OH$^{-}$ nucleophile attacks the electrophilic center of CO$_2$, thereby resulting in the formation of a zinc-bicarbonate intermediate

(3) Zinc-bicarbonate intermediate is subsequently hydrolyzed by H$_2$O (acting as a general base) to release HCO$_3^{-}$ and regenerate water-bound Zn$^{2+}$ ion for the next catalytic cycle

What catalytic mechanism(s) does CAH employ? All three:
- Metal catalysis
- Nucleophilic catalysis
- Acid-base catalysis
Exercise 2.5c

- Describe how protein functional groups can act as acid and base catalysts. How is it possible for a single amino acid side chain to function as both?

- Explain how nucleophiles function as covalent catalysts. Which amino acids are good at this?

- List the ways in which metal ions participate in catalysis.