Enzymes:
all enzymes are proteins

catalysts speed up reactions by lowering the activation energy (ΔG‡), NOT by changing the thermodynamics of the reaction (ΔG°)

Stabilization of the transition state
Bringing reactants together in the proper orientation for the reaction to occur
\[
\begin{align*}
\text{H}_3\text{C} \text{O} & \quad \text{O} \quad \text{Br} \\
\text{C}_\text{O} & \quad \text{O} \\
\text{Br} & \quad \text{C}_\text{O} \quad \text{2-} \\
+ & \quad \text{K}_{\text{rel}} = 1 \text{ M}^{-1} \text{ s}^{-1}
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{O} & \quad \text{C}_\text{O} \\
\text{K}_{\text{rel}} & = 220 \text{ s}^{-1} \quad \text{effect molarity} \quad 220 \text{ M}
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{O} & \quad \text{C}_\text{O} \\
\text{K}_{\text{rel}} & = 5 \times 10^4 \text{ s}^{-1} \quad \text{effect molarity} \quad 5 \times 10^4 \text{ M}
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{O} & \quad \text{C}_\text{O} \\
\text{K}_{\text{rel}} & = 2 \times 10^6 \text{ s}^{-1} \quad \text{effect molarity} \quad 2 \times 10^6 \text{ M}
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{O} & \quad \text{C}_\text{O} \\
\text{K}_{\text{rel}} & = 1 \times 10^7 \text{ s}^{-1} \quad \text{effect molarity} \quad 1 \times 10^7 \text{ M}
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{Br} \\
\text{O} & \quad \text{C}_\text{O} \\
\text{K}_{\text{rel}} & = 5 \times 10^4 \text{ s}^{-1}
\end{align*}
\]
Mechanism:

Mechanisms can not be proven absolutely, they can only be disproved (inconsistent with the available data)

Accepted mechanism is based on the preponderance of the available evidence

- consistent with the products- labeling studies
- intuition: consistent with well-known chemistry and widely accepted mechanistic tenets
- consistent with modest changes in the substrate
- kinetic rate expression
- model reactions- easier to study

Proteases: catalyzes the hydrolysis of peptide bonds
Bugg, Chapter 5, pp. 81-98

1. Serine protease Bugg, p. 84
2. Cysteine protease p. 89
3. Aspartyl protease p. 95
4. Zinc (metallo) protease p. 92

- chymotrypsin: cleaves at the C-terminal side of aromatic residues Phe, Tyr, Trp
- trypsin: cleaves at the C-terminal side of basic residues Arg, Lys but not His

Fig. 5.3 Specificity of endopeptidases.
Serine Protease: Chymotrypsin

Catalytic triad of α-chymotrypsin

pdb code: 5CHA
Active site of bovine trypsin with a bound inhibitor


Oxy-anion hole of trypsin

pdb code: 1BTX
**Cysteine Protease**: Papain (212 amino acids) 
active-site cysteine and histidine, overall mechanism is similar to serine proteases 
usually not a digestive enzyme (intracellular)

Structure of papain with a bound inhibitor

pdb code: 1BP4

Bugg, p. 91
**Aspartyl Protease Mechanism:** Renin, HIV protease
Bell shaped pH vs. rate profile: max rate at pH ~ 2.4 indicative of *general acid-general base catalysis.*

![Diagram of Aspartyl Protease Mechanism]

**HIV Protease: an Aspartyl Protease**

Catalytic Asp-25 with a bound inhibitor

![Diagram of HIV Protease with ligand]

pdb code: HIVJ
inhibitor

154
Interaction of the catalytic Asp-25 with the bound inhibitor

Metallo-protease (zinc): carboxypeptidase:
   important catalytic groups: Glu-270, Tyr-248
   Zn ion coordination: Glu-72, His-196, His-69
   General base mechanism

Nucleophilic mechanism (acyl enzyme complex)

Bugg, p. 94
Active site of carboxypeptidase

Phosphate ester hydrolysis
1. Phosphatases: over transfer (hydrolysis) of a phosphate monoester to water
2. Phosphodiesterases: hydrolysis of a phosphodiester to a phosphate monoester and an alcohol
3. Kinases: transfer of the \( \gamma \)-phosphate group of ATP to an acceptor group

Glucose 6-phosphatase

\[
\text{glucose-6-phosphate} \quad \xrightarrow{\text{M}^2+} \quad \text{fructose-1,6-bisphosphate} + \text{H}_2\text{O} \\
\]

Fructose 1,6-bisphosphatase: \( \text{M}^2+ \) dependent

\[
\text{fructose-1,6-bisphosphate} \quad \xrightarrow{\text{M}^2+} \quad \text{fructose-6-phosphate} + \text{P}_i
\]

Alkaline phosphatase: \( \text{M}^2+ \) dependent

Bugg, pp. 102-109
Phosphatases: General acid-base mechanism

Phosphatases: Covalent mechanism

$S_{N2}$ mechanism
Glucose 6-phosphatase: covalent catalysis

Fructose 1,6-bisphosphatase: General acid-base catalysis

Associative vs dissociative mechanism: Chiral phosphate

Use three isotopes of oxygen, $^{16}\text{O}$, $^{17}\text{O}$, $^{18}\text{O}$

Does the observation of stereochemically defined products (retention or inversion of stereochemistry) rule out the dissociative mechanism involving metaphosphate ion?
Tyrosine phosphatase
conserved aspartate, cysteine, and arginine

Phosphodiesterase:
Ribonuclease (RNase): catalyzes the hydrolysis of the phosphodiester bond of RNA.
Catalytic groups are His-12 and His-119

Bell shaped pH vs. rate profile: max rate at pH ~ 6.8 indicative of general acid-general base catalysis. Mechanism requires both an imidazole and imidazolium for catalysis

pKa of hisidine is ~ 6.1
Some Cofactors

**Thiamin Dependent Reactions (Vitamin B₁)**
(Bugg, Chapt. 7, pp. 176-9)

**Pyruvate Decarboxylase**

\[ \text{H}_3\text{C}-\text{C}=-\text{CO}_2\text{H} \xrightarrow{\text{thiamin}} \text{H}_3\text{C}-\text{C}=-\text{C} \xrightarrow{\text{thiamin}} \text{H}_3\text{C}-\text{C}=-\text{CO}_2\text{H} + \text{CO}_2 \]

**Acetolactate synthase**

\[ 2 \text{H}_3\text{C}-\text{C}=-\text{CO}_2\text{H} \xrightarrow{\text{thiamin}} \text{H}_3\text{C}-\text{C}=-\text{C} \xrightarrow{\text{thiamin}} \text{H}_3\text{C}-\text{C}=-\text{CO}_2\text{H} + \text{CO}_2 \]

**Pyruvate dehydrogenase:** pyruvate decarboxylase, dihydrolipoyl transacetylase, dihydrolipoyl dehydrogenase,

\[ \text{H}_3\text{C}-\text{C}=-\text{CO}_2\text{H} + \text{thiamin} + \text{lipoic acid} + \text{CoA-SH} \xrightarrow{\text{thiamin}} \text{Acetyl-CoA} + \text{CO}_2 \]

\[ \text{S} \text{S} \text{N} \text{H} \text{Lys} \xrightarrow{\text{thiamin, CoA-SH}} \text{H}_2\text{C}=-\text{S} \xrightarrow{\text{thiamin, CoA-SH}} \text{S} \text{S} \text{N} \text{H} \text{Lys} \xrightarrow{\text{CoA-SH}} \text{S-CoA} \]
Mechanistic insight into thiamin dependent reactions:
Breslow, R. J. Am. Chem. Soc. 1958, 80, 3719.

Benzoin condensation

Mechanism of pyruvate decarboxylase:

Mechanism of acetolactate synthase:
Pyruvate Dehydrogenase: dihydrolipoyl transacetylase

Transketolase: carbohydrate biosynthesis

D-ribose-5-P
(C5 ketose)

D-ribose-5-P
(C5 aldose)

D-ribose-5-P
(C5 aldose)

D-ribose-5-P
(C5 ketose)

Glyceraldehyde-3-P
(C3 aldose)

Glyceraldehyde-3-P
(C3 aldose)

Sedoheptulose-7-P
(C7 ketose)

Sedoheptulose-7-P
(C7 ketose)

Thiamin-PP
Thiamin acidity and anion stability is special to the \textit{N-alkyl thiazole ring system}: anion is stabilized by three major resonance strictures: two are ylides, one is a carbene.

Nature may have tried the imidazole and oxazole ring systems, but these would not have performed the necessary chemistry.

Pyridoxal Phosphate (PLP) dependent enzymes (Vitamin B$_6$) (Bugg, Chapt. 9, pp 210-221)

Involved in amino acid biosynthesis, metabolism and catabolism
Pyridoxal is often covalently bound to the resting enzyme as a Schiff base to the sidechain of an active site lysine residue.

Decarboxylase:

\[
\begin{align*}
\text{L-DOPA} & \xrightarrow{\text{decarboxylase}} \text{Dopamine} \\
\text{L-DOPA} & \xrightarrow{\text{decarboxylase}} \text{Serotonin}
\end{align*}
\]
Transaminase: amino acid biosynthesis

Stereochemistry of the transamination reaction:
proton transfer is mediated by an active site lysine
Stereochemistry of transamination (aspartate aminotransferase)

Pdb code: 1AJS

Reactions at the β- and γ-carbons of amino acids

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Compounds</th>
</tr>
</thead>
</table>
| Threonine dehydratase | H₂C⁻⁺⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻˓
Threonine Dehydratase

Tryptophan Synthase:
reactivity of indole toward electrophiles:

Similar to the mechanism of threonine dehydratase an electrophilic PAL-amino acid complex is generated
Tryptophan Synthase (con’t):  

Serine hydroxymethyl transferase: one-carbon (methyl) donors in biology  

Bugg, Ch. 5, pp. 220-1

S-adenosyl methionine (SAM)
Methyl groups from:

**SAM**

**Methylene tetrahydrofolate**

Mechanism of serine hydroxymethyl transferase:

Note: this mechanism is not the same as on page 221 of Bugg, which is probably incorrect
Mechanism of methionine $\gamma$-lyase