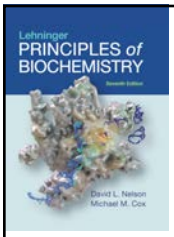



# ENZYMES

**(The most important class of proteins on Earth)**

Lecture 12-part II (10/5/20)

**OUTLINE**

**ENZYMES: Binding & Catalysis**

A. General

B. Catalytic cycle; turnover number = $k_{cat}$

1. Binding
   a. Models
   **b. How?**
   **c. How tight?** – Binding curves
      i. Hyperbolic –saturation
      ii. Sigmoidal –cooperativity in saturation
2. Catalysis

# Enzymes

General Properties of Enzymes

*$t_{½} = 0.693 / k_{un(1^{st}\ order)}$
$k_{un} = 0.693/t_{½}$

1. Enhance reaction rates
2. Mild reaction conditions
3. Reaction specificity
4. Regulated activity

## Rate Enhancement by Enzymes

| Enzyme | Nonenzymatic half-life | * Uncatalyzed rate ($k_{un}$ $s^{-1}$) |
|---|---|---|
| OMP decarboxylase | 78,000,000 years | $2.8 \times 10^{-16}$ |
| Staphylococcal nuclease | 130,000 years | $1.7 \times 10^{-13}$ |
| AMP nucleosidase | 69,000 years | $1.0 \times 10^{-11}$ |
| Carboxypeptidase A | 7.3 years | $3.0 \times 10^{-9}$ |
| Ketosteroid isomerase | 7 weeks | $1.7 \times 10^{-7}$ |
| Triose phosphate isomerase | 1.9 days | $4.3 \times 10^{-6}$ |
| Chorismate mutase | 7.4 hours | $2.6 \times 10^{-5}$ |
| Carbonic anhydrase | 5 seconds | $1.3 \times 10^{-1}$ |

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.

# Enzymes

General Properties of Enzymes

*$t_{½} = 0.693 / k_{un(1^{st}\ order)}$
$k_{un} = 0.693/t_{½}$

1. Enhance reaction rates
2. Mild reaction conditions
3. Reaction specificity
4. Regulated activity

## Rate Enhancement by Enzymes

| Enzyme | Nonenzymatic half-life | * Uncatalyzed rate ($k_{un}$ $s^{-1}$) | Catalyzed rate ($k_{cat}$ $s^{-1}$) | Rate enhancement ($k_{cat}$ $s^{-1}/k_{un}$ $s^{-1}$) |
|---|---|---|---|---|
| OMP decarboxylase | 78,000,000 years | $2.8 \times 10^{-16}$ | 39 | $1.4 \times 10^{17}$ |
| Staphylococcal nuclease | 130,000 years | $1.7 \times 10^{-13}$ | 95 | $5.6 \times 10^{14}$ |
| AMP nucleosidase | 69,000 years | $1.0 \times 10^{-11}$ | 60 | $6.0 \times 10^{12}$ |
| Carboxypeptidase A | 7.3 years | $3.0 \times 10^{-9}$ | 578 | $1.9 \times 10^{11}$ |
| Ketosteroid isomerase | 7 weeks | $1.7 \times 10^{-7}$ | 66,000 | $3.9 \times 10^{11}$ |
| Triose phosphate isomerase | 1.9 days | $4.3 \times 10^{-6}$ | 4,300 | $1.0 \times 10^{9}$ |
| Chorismate mutase | 7.4 hours | $2.6 \times 10^{-5}$ | 50 | $1.9 \times 10^{6}$ |
| Carbonic anhydrase | 5 seconds | $1.3 \times 10^{-1}$ | $1 \times 10^{6}$ | $7.7 \times 10^{6}$ |

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.

# Enzymes

## Pioneers

Eduard Buchner, 1860–1917

James Sumner, 1887–1955

J. B. S. Haldane, 1892–1964

Buchner and his brother Hans (of the funnel fame) was first to separate biological catalysis (fermentation) from living cells by trying to make jam using yeast extracts as a preservative, which they called a zyme. Enzyme was derived from "in das zyme."

Sumner was the first to isolate and crystalize an enzyme, urease. It was all protein, therefore enzymes=protein

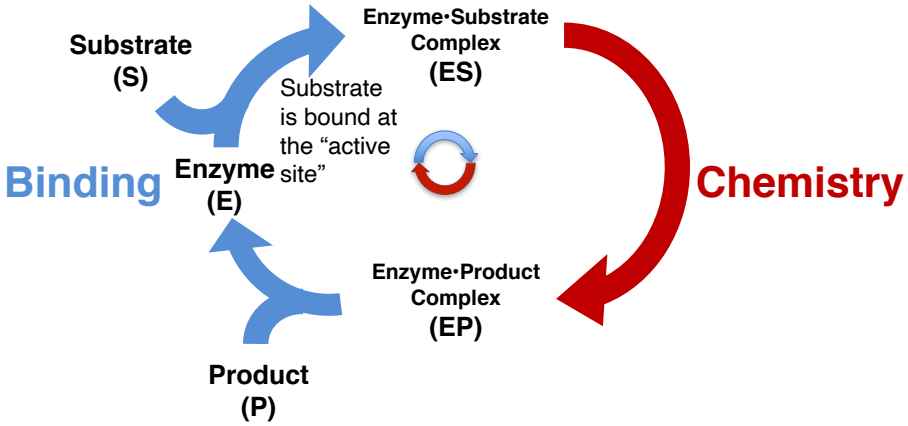
Haldane was the first really theorize how enzymes worked.

# Enzymes

You can write out the catalysis as a series of reactions:
But, this is better understood as a CATALYTIC CYCLE

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

Divide the problem into two parts: Binding and Catalysis



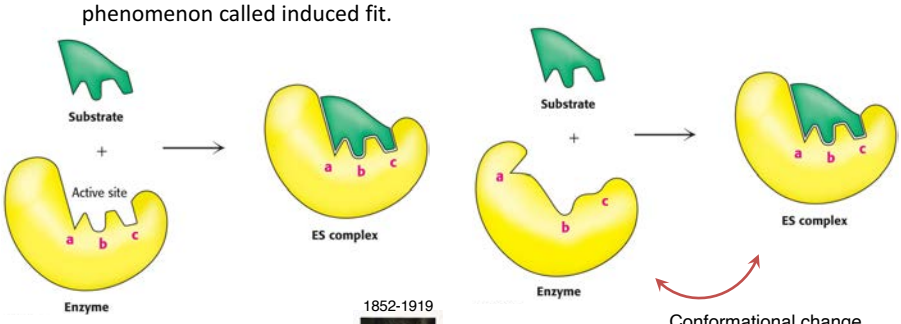

- The rate that an individual enzyme can go around this cycle is called the "turnover number"
- The turnover number is like a rate constant for catalysis, or $k_{cat}$

# Enzymes

## BINDING: Formation of the ES-complex

Most Enzymes do not interact with their substrates like a lock and key.

Rather, the enzyme changes shape upon substrate binding, a phenomenon called induced fit.




1852-1919

**Lock-and-key model (proposed by Emil Fisher)**

**Induced-fit model (proposed by Dan Koshland)**

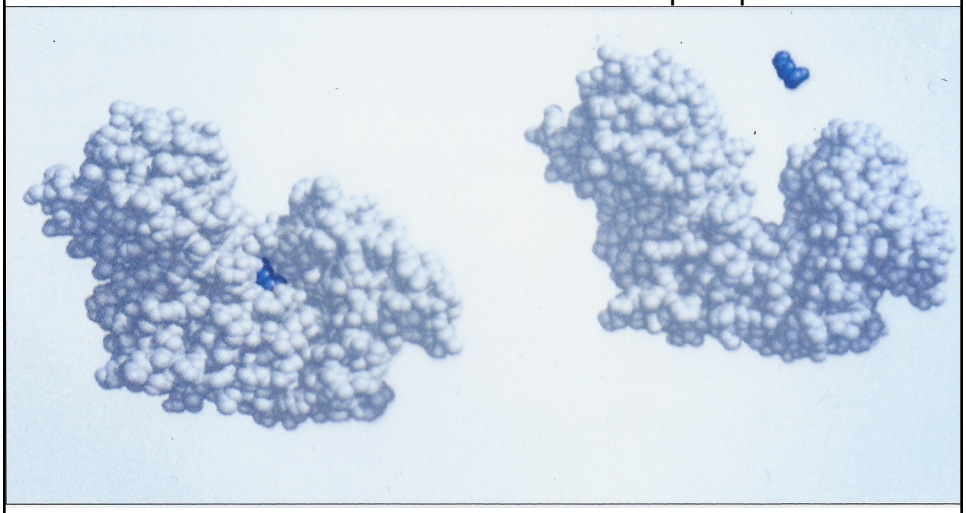
A hybrid model is called "ligand selective." This says that many conformations are in equilibrium in solution and the substrate binds one best, pulling equilibrium.

1920-2007

# Enzymes

## BINDING: First demonstration of Induced Fit

Hexokinase: Glucose + ATP ⇌ Glucose 6-phosphate + ADP

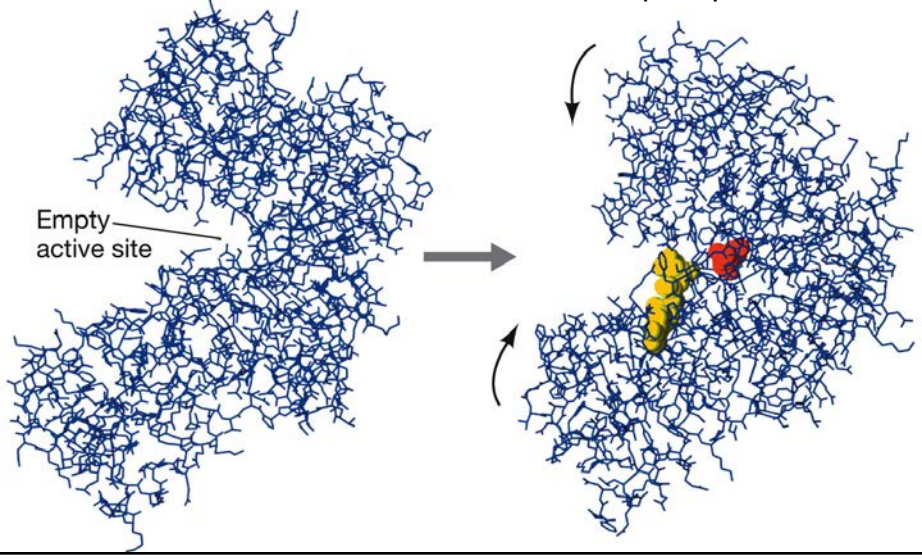


Hexokinase•Glc 6-P

Hexokinase

# Enzymes

Hexokinase: Glucose + ATP ⇌ Glucose 6-phosphate + ADP

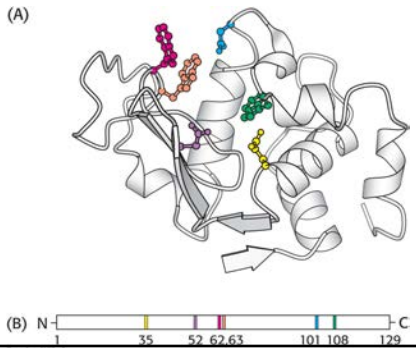



# Enzymes

## The Formation of an Enzyme–Substrate Complex Is the First Step in Enzymatic Catalysis

Enzymes bring substrates together to form an enzyme-substrate complex on a particular region of the enzyme called the active site. ES-complex

The interaction of the enzyme and substrates at the active site promotes catalysis. The protein fold brings residues important for Binding & Catalysis to the Active Site.




A schematic representation of the primary structure of lysozyme shows that the active site is composed of residues that come from different parts of the polypeptide chain.

# Enzymes

## Enzyme-Substrate Complex Drives Selectivity

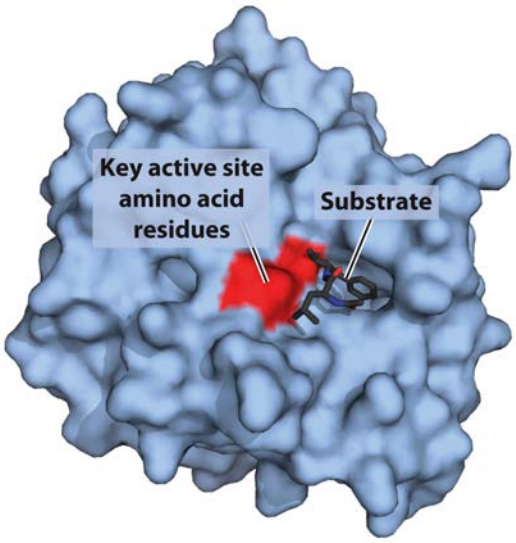


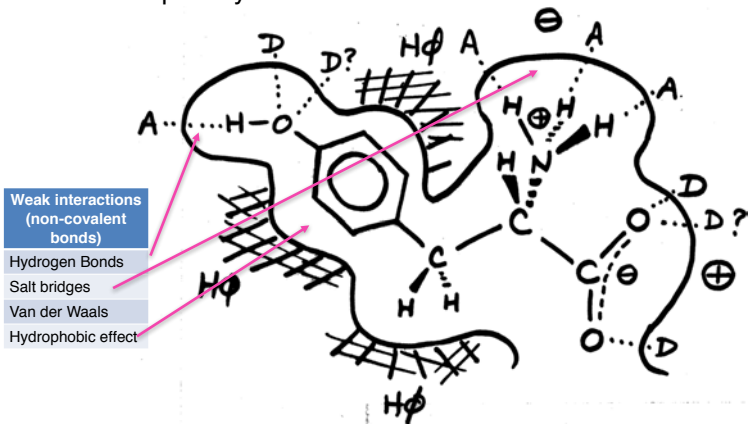

Figure 6-1

Chymotrypsin with bound substrate
(PDB ID 7GCH to just observe the interactions of substrate and enzyme)

# Enzymes

BINDING: great example of complementarity

Example: Tyr-aminotransferase



| Weak interactions (non-covalent bonds) |
|---|
| Hydrogen Bonds |
| Salt bridges |
| Van der Waals |
| Hydrophobic effect |

This kind of complementarity is not restricted to ES complex: interactions of receptor-ligand, protein-protein, protein-nucleic acid, etc.

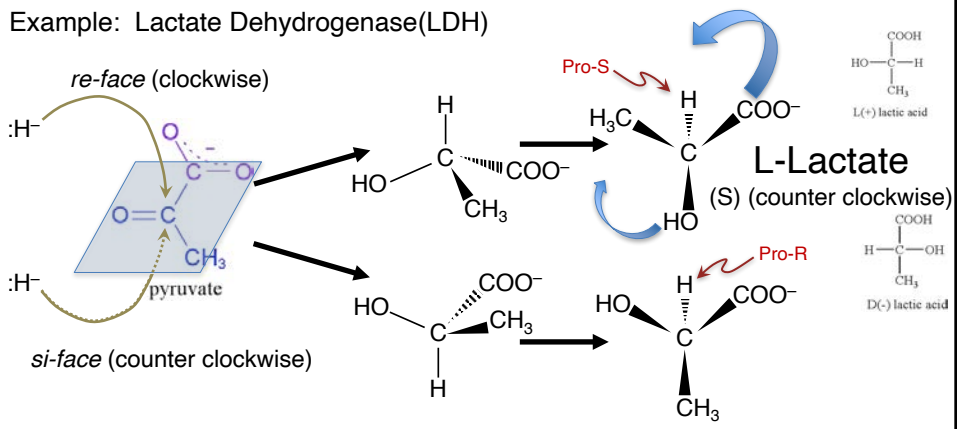
Notice that this kind of complementarity allows for binding L-Tyr much better than D-Tyr.

# Enzymes

BINDING: Enzymes are stereo-selective

……even for molecules that are not chiral (so-called pro-chiral)

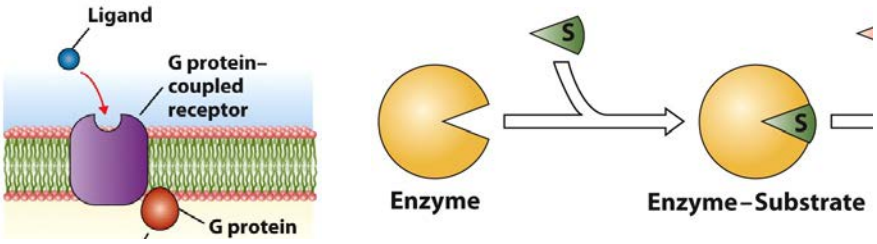
Example: Lactate Dehydrogenase(LDH)




The hydride (:H⁻)(from NADH) can add to the $sp^2$ carbonyl carbon from the top (on the *re-face*) or the bottom (*si-face*). But, it can only do ONE.

# Enzymes

## Binding affinities: examples




- Binding of receptor and ligand
- Receptors can be soluble or membrane-bound
- Similar to binding of enzyme and substrate

How do we measure the degree of the affinity?

# Enzymes

## Receptor-Ligand binding

$$R + L \underset{k^A_{-1}}{\overset{k^A_1}{\rightleftharpoons}} R{\bullet}L$$ Association reaction

$$R{\bullet}L \underset{k^D_{-1}}{\overset{k^D_1}{\rightleftharpoons}} R + L$$ Dissociation reaction

$k^D_1 = k^A_{-1}$, etc.

$$K_D = \frac{[R][L]}{[R \bullet L]}$$

What are the units of a dissociation constant? $\frac{M}{}$

How about an association constant? $\frac{}{M^{-1}}$

How can we express how TIGHT a ligand binds to a protein? $K_D$

How can we **measure** how TIGHT a ligand binds to a protein?

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# Enzymes: Receptor-Ligand binding

Define fraction of R bound to a ligand = Y
Define $R_T$ = total of all species of R

$Y = [R{\bullet}L] / ([R]+[R{\bullet}L])$

Use $K_{eq}$ to calculate expression for free R

$$K_D = \frac{[R][L]}{[R \bullet L]}$$

$[R] = K_D[R{\bullet}L] / [L]$

Substitute for free R

$Y = [R{\bullet}L] / (K_D[R{\bullet}L] / [L]+[R{\bullet}L])$
$\div [R{\bullet}L] / [R{\bullet}L]$

$Y = 1/(K_D/[L] + 1)$
$\times [L] / [L]$

$$R_T = R + R{\bullet}L$$

$$Y = \frac{[R \bullet L]}{[R]_T}$$

$$Y = \frac{[L]}{K_D + [L]}$$

Equation for hyperbola → y = x/(b+x)

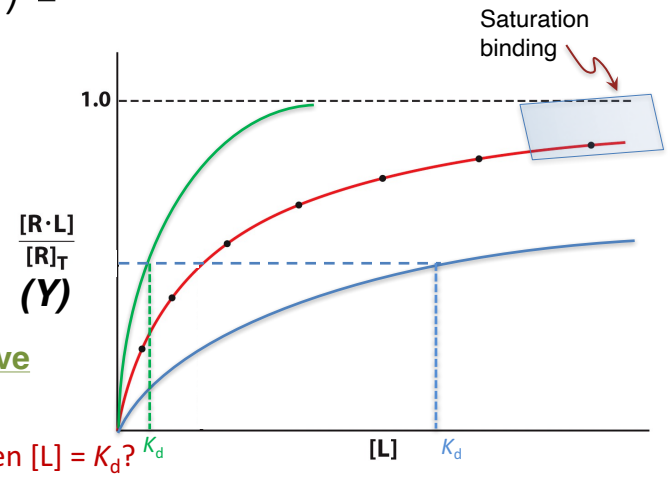
Graph [L] vs fraction of receptor that is bound (Y)

# Enzymes: Receptor-Ligand binding

$$Y = \frac{[L]}{K_D + [L]}$$

hyperbola → y = x/(b+x)

*Fraction bound (Y)* = $\frac{[R \bullet L]}{[R]_T}$




Hyperbolic Curve

NOTE: what is Y when [L] = $K_d$?

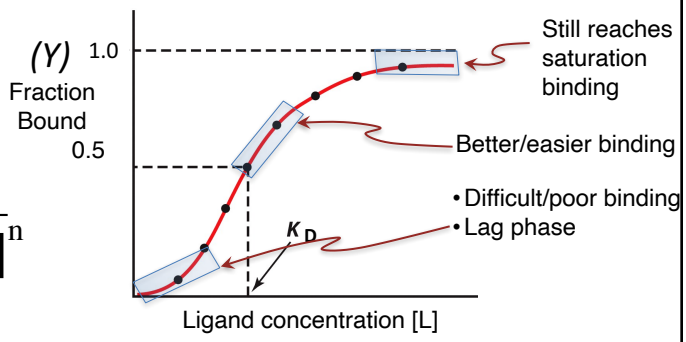
# Enzymes: Receptor-Ligand binding

## Cooperative Binding: Multiple binding sites

$$R{\cdot}L_n \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R + nL$$

Multiple sites on R

$$Y = \frac{[L]^n}{K_D + [L]^n}$$




Sigmoidal Curve

These curves (hyperbolic & sigmoidal) are called binding curves or "isotherms."

- Binding of a ligand at one site can affect the binding at other sites.

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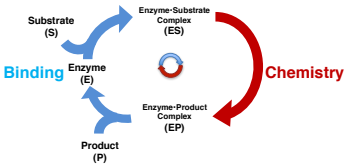
# Enzymes




Now that we have some concept of binding, the first important part of the catalytic cycle, lets discuss the second part of the cycle:

## Catalysis

Four introductory aspects to Enzyme Catalysis:

1) Rate enhancement
2) How do you measure catalysis (enzyme activity)
3) What is the relationship between reaction rate and [E]?
4) Enzyme nomenclature
    a. reaction
    b. helpers
    c. enzymes

# Enzymes

## Catalysis

1) Rate Enhancement by Enzymes

Rate enhancement $(k_{cat}\ s^{-1}/k_{un}\ s^{-1})$

This ratio is sometimes called Proficiency

| TABLE 6-5 | Some Rate Enhancements Produced by Enzymes |
|---|---|
| Cyclophilin | $10^5$ |
| Carbonic anhydrase | $10^7$ |
| Triose phosphate isomerase | $10^9$ |
| Carboxypeptidase A | $10^{11}$ |
| Phosphoglucomutase | $10^{12}$ |
| Succinyl-CoA transferase | $10^{13}$ |
| Urease | $10^{14}$ |
| Orotidine monophosphate (OMP) decarboxylase | $10^{17}$ |
| Uroporphyrinogen decarboxylase | $2.5 \times 10^{24}$ |

# Enzymes

## Catalysis

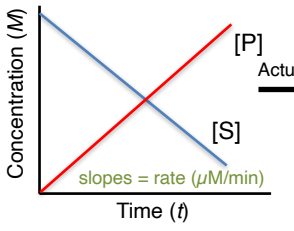
2) How do you measure enzyme activity?

$$S \rightleftharpoons P$$

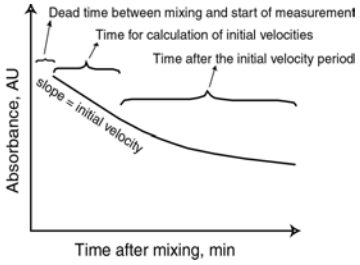
For this, you need an ASSAY.
You can either measure the disappearance of S or the appearance of P
(or couple to faster reactions that measure these indirectly; called a coupled assay)

For example: LDH for pyruvate + NADH ⇌ D-lactate + $NAD^+$
Which of these, S or P, can you measure?
You can measure the loss of NADH or the appearance of $NAD^+$

Rate ($v$) = d[P]/dt
Rate ($v$) = -d[S]/dt



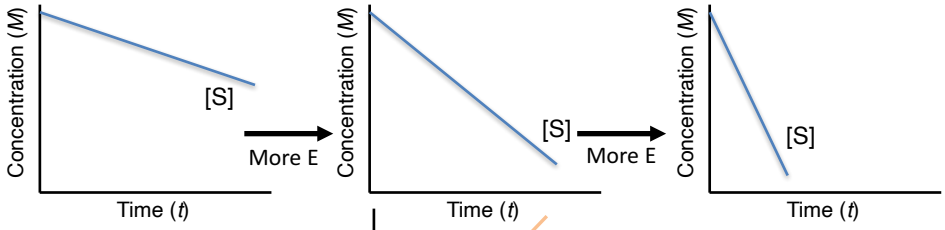

Actually….



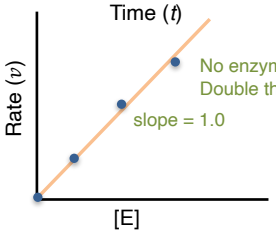

# Enzymes

## Catalysis

3) What is the relationship between reaction rate and [E]?




Rate ($v$) = $k_{cat}$ Δ[S]
Rate ($v$) = $k_{cat}$[E]
($\mu M \cdot sec^{-1}$) ($sec^{-1}$) ($\mu M$)




- The turnover number ($k_{cat}$) is the number of catalytic cycles/unit time per enzyme active site.
- Range of values from <1 to $10^7$ $sec^{-1}$

Said to be "Diffusion Controlled"

| Enzyme | Catalyzed rate ($k_{cat}$ $s^{-1}$) |
|---|---|
| OMP decarboxylase | 39 |
| Staphylococcal nuclease | 95 |
| AMP nucleosidase | 60 |
| Carboxypeptidase A | 578 |
| Ketosteroid isomerase | 66,000 |
| Triose phosphate isomerase | 4,300 |
| Chorismate mutase | 50 |
| Carbonic anhydrase | $1 \times 10^6$ |