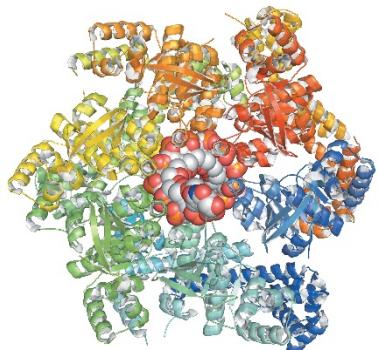


biochemistry



Reginald H. Garrett | Charles M. Grisham

SUPPLIMENTARY



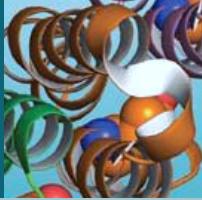
Reginald H. Garrett  
Charles M. Grisham

[www.cengage.com/chemistry/garrett](http://www.cengage.com/chemistry/garrett)

# Chapter 13

# Enzyme Kinetics

# Outline



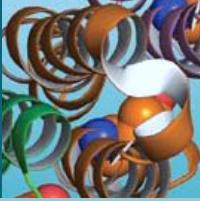
- Characteristic features of enzymes
- Classification of enzymes
- Equations that define the kinetics (i.e., rates) of enzyme-catalyzed reactions
  - Michaelis-Menten Rapid Equilibrium Equation
  - Briggs-Haldane Steady State Equation
- Meaning of  $K_m$ , the Michaelis constant, and turnover number
- Measurement of Enzyme Efficiency
- Experimental determination of  $K_m$

# What Characteristic Features Define Enzymes?



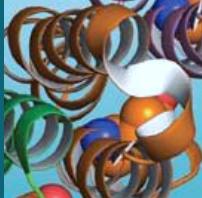
- Enzymes can accelerate reactions as much as  $10^{21}$  over uncatalyzed rates!
- Urease is a good example:
  - Catalyzed rate:  $3 \times 10^4$ /sec
  - Uncatalyzed rate:  $3 \times 10^{-10}$ /sec
  - Ratio is  $1 \times 10^{14}$  !

# What Characteristic Features Define Enzymes?



- Enzymes endow cells with the remarkable capacity to exert kinetic control over thermodynamic potentiality
- Enzymes are the agents of metabolic function

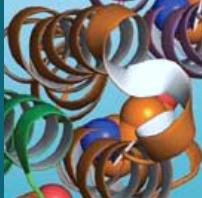
# Enzyme Classification by Enzyme Commission (EC) Table 13.1



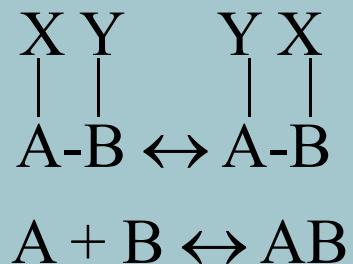
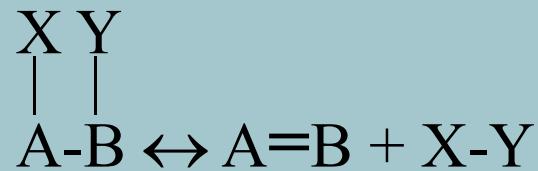
E.C. Number	Systematic Name and Subclasses
1	Oxidoreductases (oxidation-reduction reactions)
1.1	Acting on CH—OH group of donors
1.1.1	With NAD or NADP as acceptor
1.1.3	With O <sub>2</sub> as acceptor
1.2	Acting on the >C=O group of donors
1.2.3	With O <sub>2</sub> as acceptor
1.3	Acting on the CH—CH group of donors
1.3.1	With NAD or NADP as acceptor
2	Transferases (transfer of functional groups)
2.1	Transferring C-1 groups
2.1.1	Methyltransferases
2.1.2	Hydroxymethyltransferases and formyltransferases
2.1.3	Carboxyltransferases and carbamoyltransferases
2.2	Transferring aldehydic or ketonic residues
2.3	Acyltransferases
2.4	Glycosyltransferases
2.6	Transferring N-containing groups
2.6.1	Aminotransferases
2.7	Transferring P-containing groups
2.7.1	With an alcohol group as acceptor
3	Hydrolases (hydrolysis reactions)
3.1	Cleaving ester linkage
3.1.1	Carboxylic ester hydrolases
3.1.3	Phosphoric monoester hydrolases
3.1.4	Phosphoric diester hydrolases



# Enzyme Classification by Enzyme Commission (EC) Table 13.1

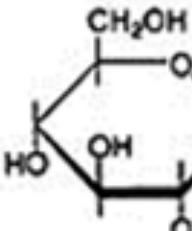
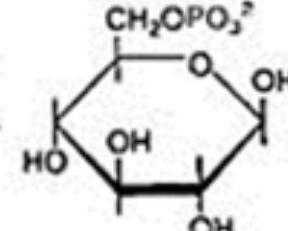


E.C. Number	Systematic Name and Subclasses
4	<i>Lyases</i> (bond cleavage by means other than hydrolysis or oxidation)
4.1	C—C lyases
4.1.1	Carboxy lyases
4.1.2	Aldehyde lyases
4.2	C—O lyases
4.2.1	Hydrolases
4.3	C—N lyases
4.3.1	Ammonia lyases
5	<i>Isomerases</i> (isomerization reactions)
5.1	Racemases and epimerases
5.1.3	Acting on carbohydrates
5.2	<i>Cis-trans</i> isomerases
6	<i>Ligases</i> (formation of bonds with ATP cleavage)
6.1	Forming C—O bonds
6.1.1	Amino acid–RNA ligases
6.2	Forming C—S bonds
6.3	Forming C—N bonds
6.4	Forming C—C bonds
6.4.1	Carboxylases





# Examples of Each Enzyme Class

Class	Example (Reaction Type)	Reaction Catalyzed	
1. Oxidoreductases	Alcohol dehydrogenase (EC 1.1.1.1) (oxidation with NAD <sup>+</sup> )	$\text{CH}_3\text{CH}_2\text{OH} \xrightarrow{\text{NAD}^+} \text{CH}_3-\text{C}(=\text{O})\text{H}$ Ethanol	NADH + H <sup>+</sup>
2. Transferases	Glucokinase (EC 2.7.1.2) (phosphorylation)	$\text{CH}_2\text{OH}$ 	$\text{ATP} \rightarrow \text{ADP}$ $\text{CH}_2\text{OPO}_4^{2-}$ 
3. Hydrolases	Carboxypeptidase A (EC 3.4.17.1) (peptide bond cleavage)	$\begin{array}{c} \text{R}_{n-1} \quad \text{O} \\   \quad    \\ -\text{N}-\text{C} - \text{C} = \text{N}-\text{C}-\text{COO} \\   \quad   \quad   \quad   \\ \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \end{array}$ C-terminal of polypeptide	$\text{H}_2\text{O} \rightarrow$ $\begin{array}{c} \text{R}_{n-1} \quad \text{R}_n \\   \quad   \\ -\text{N}-\text{C} - \text{COO}^- + \text{H}_3\text{N} - \text{C} - \text{COO}^- \\   \quad   \\ \text{H} \quad \text{H} \end{array}$ Shortened polypeptide      C-terminal residue

# Examples of Each Enzyme Class



4. lyases - addition to double bonds or reverse

6. Ligases - formation of bonds with cleavage of ATP or similar molecule

# Sec 13.7 RNA Molecules That Are Catalytic Have Been Termed “Ribozymes”



The peptidyl transferase of the ribosome is a ribozyme

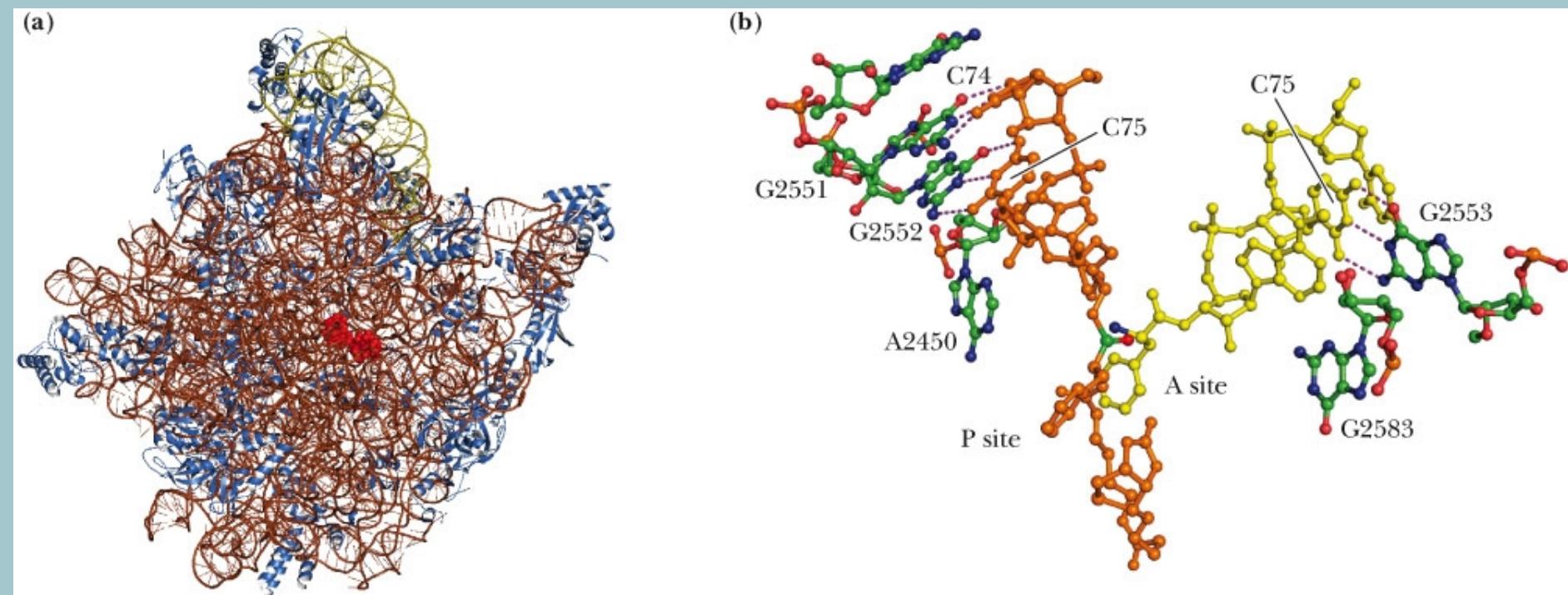


Figure 13.26 (a) The 50S subunit from *H. marismortui*. (b) The aminoacyl-tRNA (yellow) and the peptidyl-tRNA (orange) in the peptidyl transferase active site.

## Sec 13.7 RNA Molecules That Are Catalytic Have Been Termed “Ribozymes”

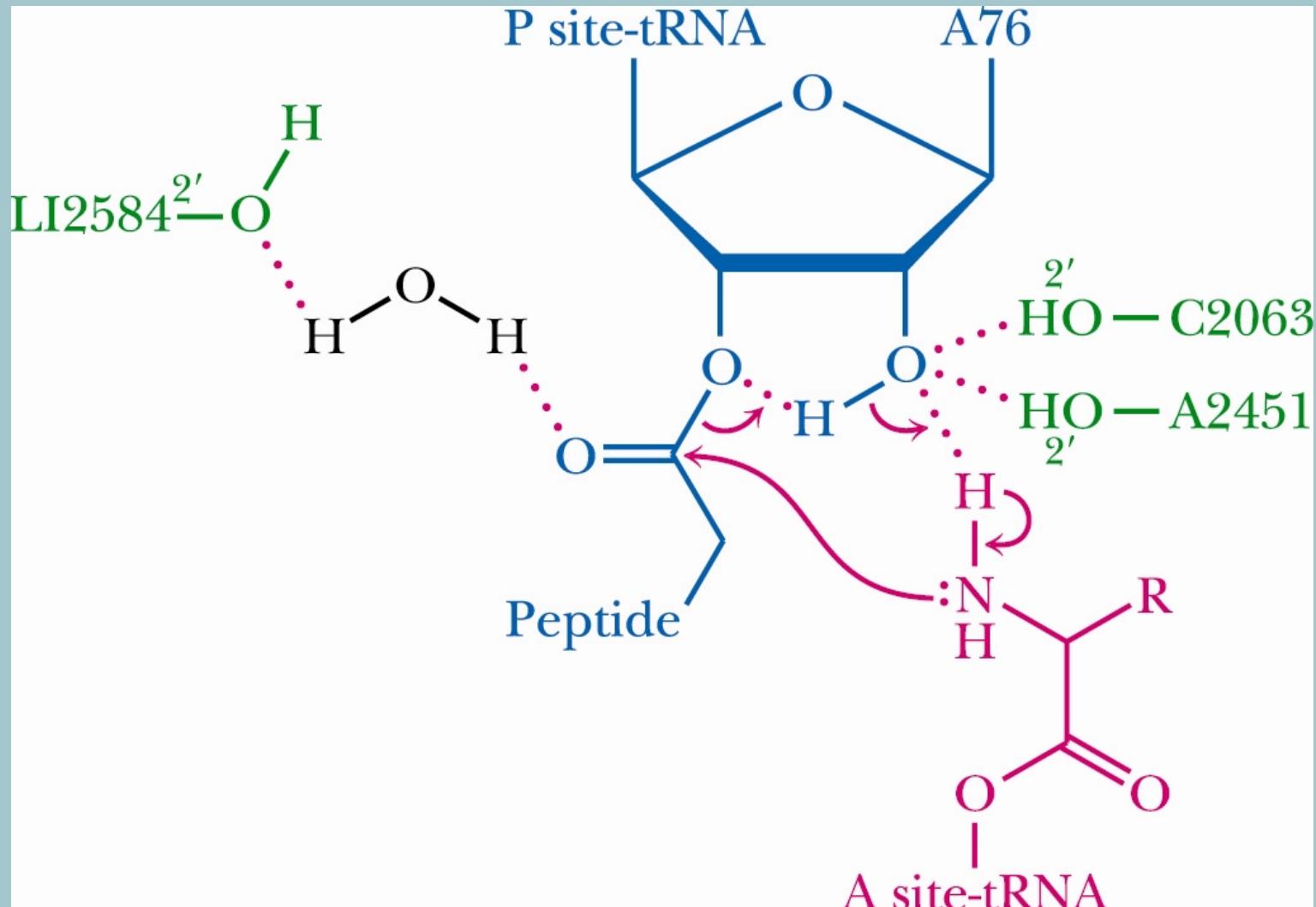
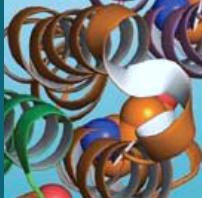


Figure 13.27 The peptidyl transferase reaction.

Std. chemical Rx



At equilibrium

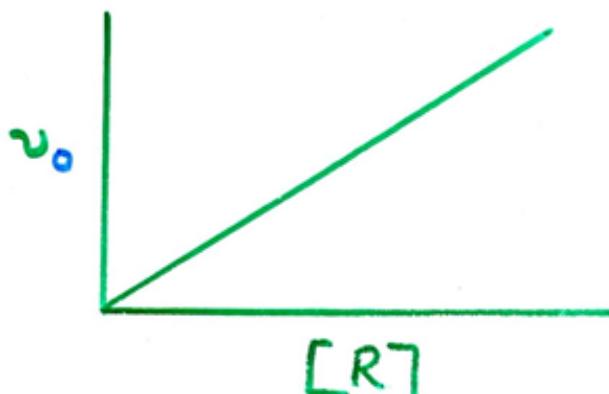
$$k_1 [R]_{eq} = k_{-1} [P]_{eq}$$

$$\frac{k_1}{k_{-1}} = \frac{[P]_{eq}}{[R]_{eq}} = K_{eq}$$

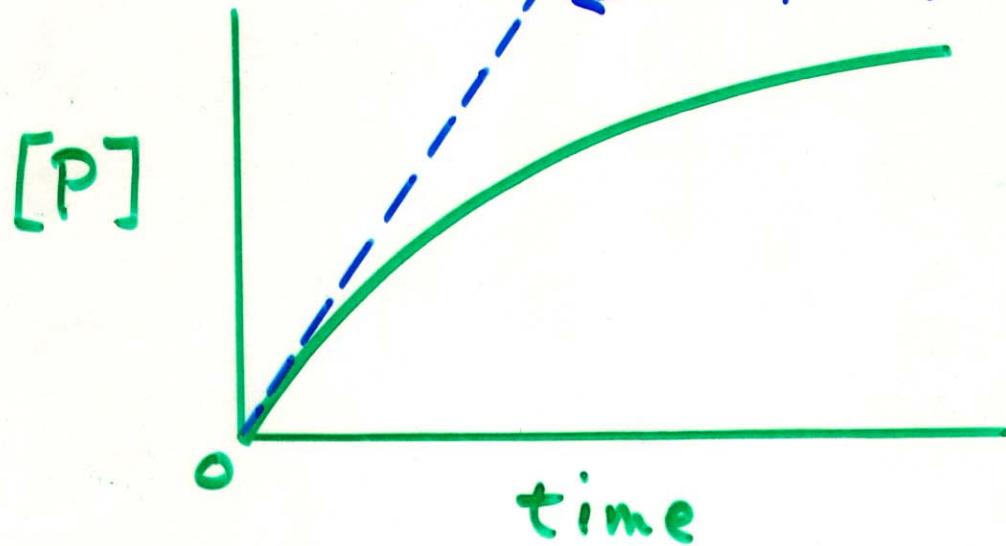
At any point during a reaction

$$v = k_1 [R] - k_{-1} [P]$$

$$v_0 = k_1 [R]$$



$v_0$  = initial velocity  $\Rightarrow$   
slope of tangent





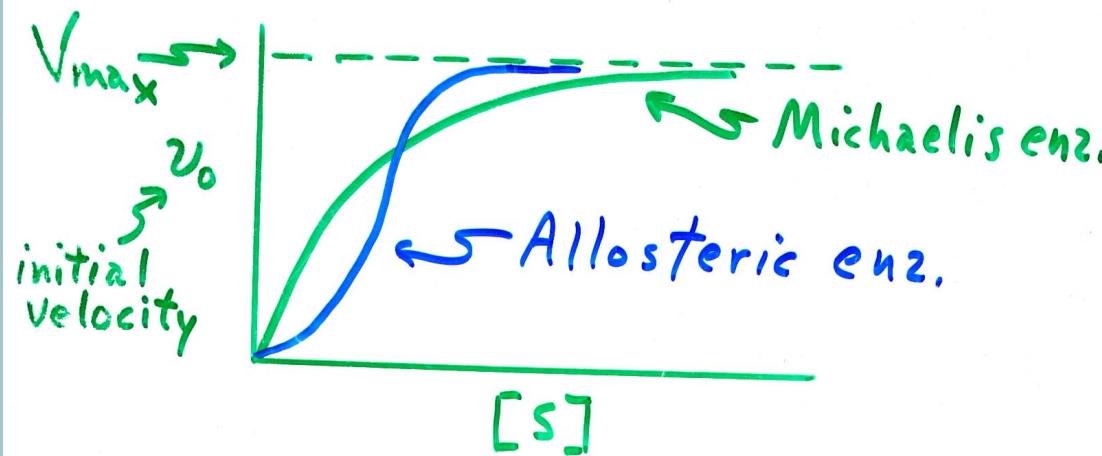
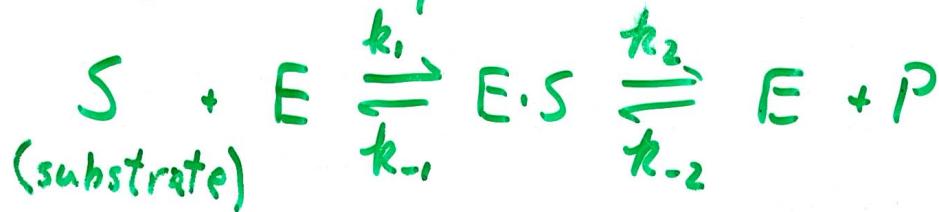
Leonor Michaelis  
1875–1949



Maud Menten  
1879–1960

You can read an interesting synopsis of Menten's career at  
[http://chemheritage.org/women\\_chemistry/body/menten.html](http://chemheritage.org/women_chemistry/body/menten.html)

Enzyme-catalyzed Rx



$v([S])$  in Michaelis-Menten Eg.

Initial derivation

based on Rapid Equilibrium assumption, i.e.

$$k_1 \& k_{-1} \gg k_2$$

For  $v(s)$ , the following eqs. hold

$$1. \quad v_0 = k_2 [E \cdot S]$$

$$2. \quad [E]_{\text{total}} = [E]_{\text{free}} + [E \cdot S]$$

$$3. \quad K_{\text{eq}} = \frac{[E]_f [S]}{[E \cdot S]} = \frac{k_{-1}}{k_1} = K_S$$

#3 results from rapid equilibrium:

$$[S][E]k_1 = [E \cdot S]k_{-1}$$

$$\frac{[S][E]}{[E \cdot S]} = \frac{k_{-1}}{k_1} = K_S = \frac{1}{K_A}$$

If Eqs 1-3 are combined

$$v_0 = \frac{k_2 [E]_f [S]}{K_S + [S]}$$

$$= \frac{V_{\text{max}} [S]}{K_S + [S]}$$

$$\therefore \frac{v_0}{V_{\max}} = \frac{[S]}{K_S + [S]}$$

where  $V_{\max} = k_2 [E]_{\text{total}}$

Rapid equil. means  $k_2$  is rate limiting for  $R_x$

This rate limiting const. is termed the catalytic rate const.

i.e.,

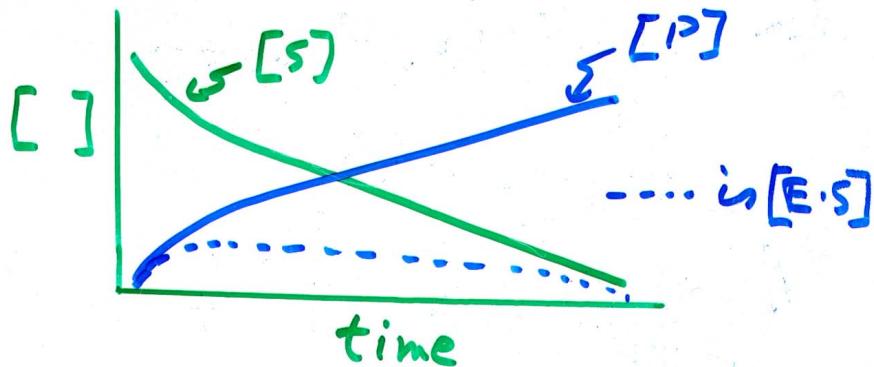
$$k_2 = k_{\text{cat}}$$

---

Briggs & Haldane generalized  $v(S)$  by assuming steady state instead of rapid equilibrium

i.e. rate of E·S formation =  
rate of its breakdown

$$\frac{d [E \cdot S]}{dt} \approx 0$$



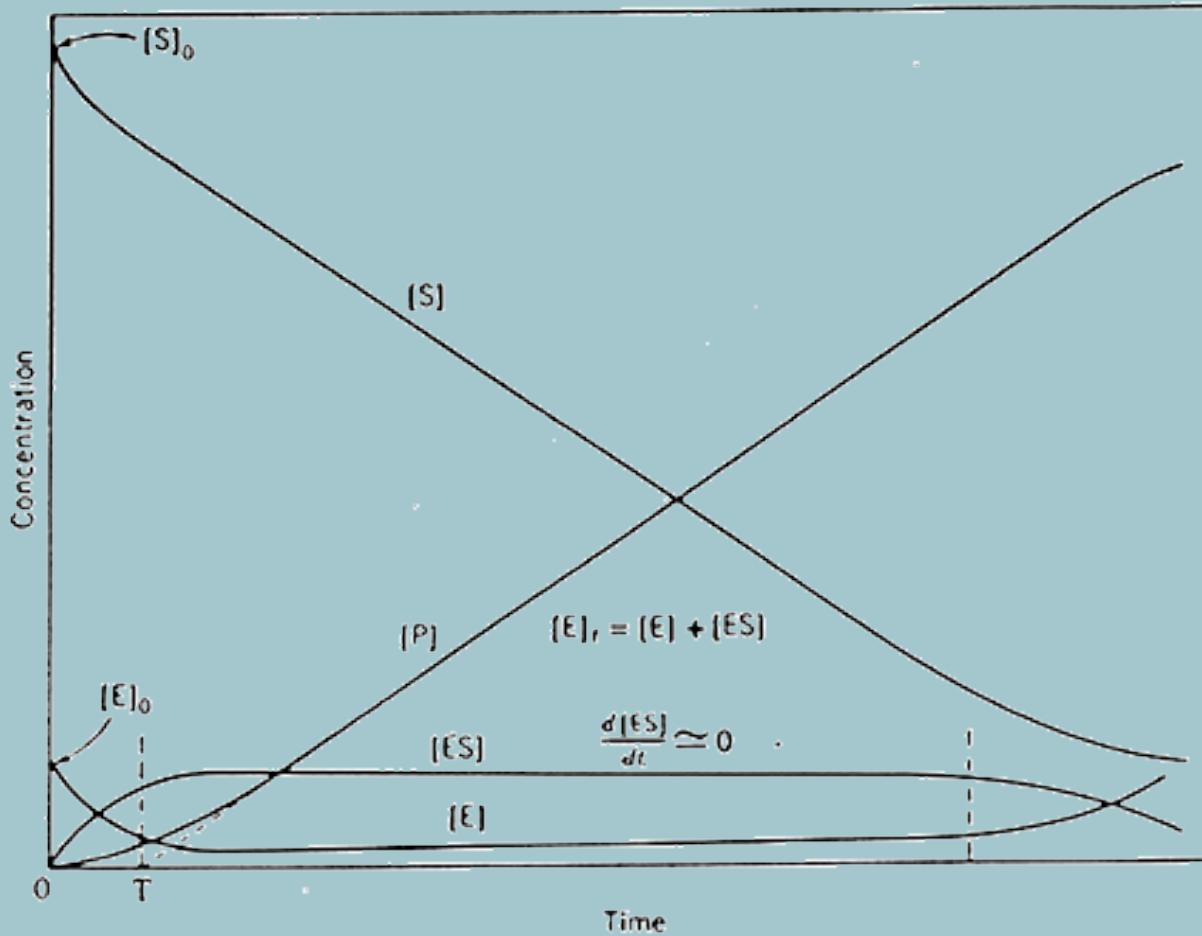
$$\therefore [S][E] k_1 = [E \cdot S] (k_2 + k_{-1})$$

Eg 4.  $\frac{[S][E]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$

Combining eqs. 1, 2 & 4

$$\frac{v_0}{V_{max}} = \frac{[S]}{K_m + [S]}$$

# Time Course for Reaction



Progress curve for a catalyzed reaction where the initial reactant (substrate) concentration,  $[S]_0$ , is significantly greater than the initial enzyme concentration,  $[E]_0$ .

As the ratio of  $[S]_0/[E]_0$  increases, the steady-state region accounts for an increasing fraction of the total time.  
T represents the presteady-state time interval.

Ways to think of  $K_m$

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

If  $k_2 \ll k_{-1}$ ,

$$K_m \approx \frac{k_{-1}}{k_1} = K_s$$

Also, when  $v_o = \frac{1}{2} V_{max}$

$$\frac{v_o}{V_{max}} = \frac{1}{2} = \frac{[S]}{K_m + [S]}$$

$$\therefore K_m = [S]$$

Way to think of  $k_2$

In most cases,

$$k_2 = k_{cat}$$

which is the catalytic rate const.

Also Known as turnover number

---

Basis for enzyme assay:

$$v_0 = \frac{k_2 [E]_t [S]}{K_m + [S]}$$

If  $[S]$  is essentially constant,

$$v_0 \propto [E]_t$$

Ideally  $[S]$  should be several times  $K_m$  for best assay.

$k_{cat}/K_m$  measures enzyme efficiency

If  $[S] \ll K_m$

$$v_0 = \frac{k_{cat} [E]_t [S]}{K_m + \cancel{[S]}}$$

$$v_0 = \frac{k_{cat}}{K_m} [E]_t [S]$$

$k_{cat}/K_m$  becomes a 2<sup>nd</sup> order rate const.

Also, since

$$\frac{k_{cat}}{K_m} = \frac{k_{cat}}{\frac{k_{-1} + k_{cat}}{k_1}}$$

If  $k_{-1} \ll k_{cat}$

$$\frac{k_{cat}}{K_m} \approx k_1$$

$\therefore k_1$  sets maximum, which is limited by diffusion to  $\sim 10^9 / M \cdot \text{sec}$

**TABLE 13.3**  $K_m$  Values for Some Enzymes

Enzyme	Substrate	$K_m$ (mM)
Carbonic anhydrase	$\text{CO}_2$	12
Chymotrypsin	<i>N</i> -Benzoyltyrosinamide	2.5
	Acetyl-L-tryptophanamide	5
	<i>N</i> -Formyltyrosinamide	12
	<i>N</i> -Acetyltyrosinamide	32
	Glycyltyrosinamide	122
Hexokinase	Glucose	0.15
	Fructose	1.5
$\beta$ -Galactosidase	Lactose	4
Glutamate dehydrogenase	$\text{NH}_4^+$	57
	Glutamate	0.12
	$\alpha$ -Ketoglutarate	2
	$\text{NAD}^+$	0.025
	NADH	0.018
Aspartate aminotransferase	Aspartate	0.9
	$\alpha$ -Ketoglutarate	0.1
	Oxaloacetate	0.04
	Glutamate	4
Threonine deaminase	Threonine	5
Arginyl-tRNA synthetase	Arginine	0.003
	tRNA <sup>Arg</sup>	0.0004
	ATP	0.3
Pyruvate carboxylase	$\text{HCO}_3^-$	1.0
	Pyruvate	0.4
	ATP	0.06
Penicillinase	Benzylpenicillin	0.05
Lysozyme	Hexa- <i>N</i> -acetylglucosamine	0.006

# The Turnover Number Defines the Maximal Activity of One Active Site

**TABLE 13.4**

Values of  $k_{\text{cat}}$  (Turnover Number) for Some Enzymes

Enzyme	$k_{\text{cat}}$ (sec $^{-1}$ )
Catalase	40,000,000
Carbonic anhydrase	1,000,000
Acetylcholinesterase	14,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

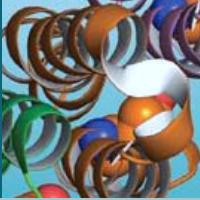
# The Ratio $k_{\text{cat}}/K_m$ Defines the Catalytic Efficiency of an Enzyme

**TABLE 13.5**

Enzymes Whose  $k_{\text{cat}}/K_m$  Approaches the Diffusion-Controlled Rate of Association with Substrate

Enzyme	Substrate	$k_{\text{cat}}$ (sec $^{-1}$ )	$K_m$ (M)	$k_{\text{cat}}/K_m$ (M $^{-1}$ sec $^{-1}$ )
Acetylcholinesterase	Acetylcholine	$1.4 \times 10^4$	$9 \times 10^{-5}$	$1.6 \times 10^8$
Carbonic anhydrase	CO <sub>2</sub>	$1 \times 10^6$	0.012	$8.3 \times 10^7$
	HCO <sub>3</sub> <sup>-</sup>	$4 \times 10^5$	0.026	$1.5 \times 10^7$
Catalase	H <sub>2</sub> O <sub>2</sub>	$4 \times 10^7$	1.1	$4 \times 10^7$
Crotonase	Crotonyl-CoA	$5.7 \times 10^3$	$2 \times 10^{-5}$	$2.8 \times 10^8$
Fumarase	Fumarate	800	$5 \times 10^{-6}$	$1.6 \times 10^8$
	Malate	900	$2.5 \times 10^{-5}$	$3.6 \times 10^7$
Triosephosphate isomerase	Glyceraldehyde-3-phosphate*	$4.3 \times 10^3$	$1.8 \times 10^{-5}$	$2.4 \times 10^8$
$\beta$ -Lactamase	Benzylpenicillin	$2 \times 10^3$	$2 \times 10^{-5}$	$1 \times 10^8$

# The Ratio $k_{\text{cat}}/K_m$ Measures the Substrate Specificity of an Enzyme



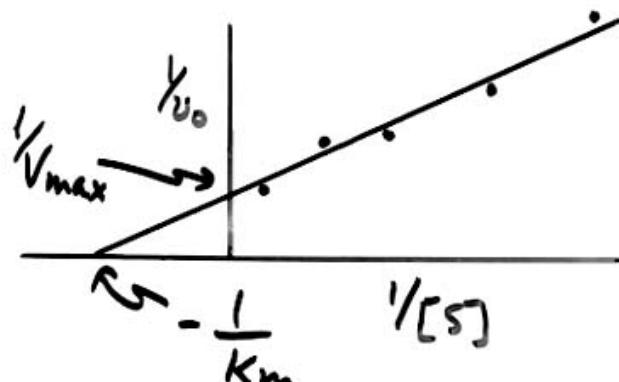
Preference of chymotrypsin for different N-acetyl amino acid methyl esters, as measured by  $k_{\text{cat}}/K_m$

Amino Acid in Ester	$k_{\text{cat}}/K_M$ ((mol/L) $^{-1}$ s $^{-1}$ )
Glycine	0.13
Norvaline	$3 \times 10^2$
Norleucine	$3 \times 10^3$
Phenylalanine	$1 \times 10^5$

To determine  $K_m$  value &  $V_{max}$   
 rearrange M-M eq and do  
 Lineweaver-Burk Plot  
 (aka. double reciprocal plot)

$$v_0 = \frac{V_{max} [S]}{K_m + [S]}$$

$$\frac{1}{v_0} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$



$$\text{slope} = K_m / V_{max}$$