MACQUARIE University

Enzymes 1:
Intro and Enzyme Kinetics

Shoba Ranganathan

Applied Biosciences

T: 02 9850 6262; E: shoba.ranganathan@mq.edu.au

Objectives

- What are enzymes?
  - Different types of enzymes
  - Cofactors
- Energetics of enzyme-catalysed reactions
- Free energy as the driving force
  - How to overcome unfavourable energetics
- Enzyme kinetics
  - Experimental determination of kinetic constants.

Textbook Chapter 8

Enzymes: Protein catalysts

A catalyst

- increases the rate or velocity of a chemical reaction without itself being changed in the overall process
- accelerates the approach to equilibrium for a given reaction without changing the thermodynamic favourability of the reaction
- lowers the energy barrier (activation energy of the transition state) in the process of converting a substrate into a product

Enzymes are agents of **metabolic change** – name from 1878!

- Enzymes cannot catalyse reactions that are energetically unfavourable
- A catalyst is something that reduces the activation energy of a reaction and hence accelerates the reaction rate
  - e.g. Carbonic acid formation

    $CO_2 + H_2O \leftrightarrow H_2CO_3$
  - This reaction proceeds **10 million times** faster in the presence of the enzyme (carbonic anhydrase)

Enzyme catalysis

- Reaction rates (i.e. products formed/sec) are 10^6 to 10^{12} times faster with enzymes

TABLE 11-1 Catalytic Power of Some Enzymes

Enzyme	Nonenzymatic Reaction Rate (s^{-1})	Enzymatic Reaction Rate (s^{-1})	Rate Enhancement
Carbonic anhydrase	1.3×10^{-1}	1×10^{6}	7.7×10^{6}
Chorismate mutase	2.6×10^{-5}	50	1.9×10^{6}
Triose phosphate isomerase	4.3×10^{-6}	4300	1.0×10^{9}
Carboxypeptidase A	3.0×10^{-9}	578	1.9×10^{11}
AMP nucleosidase	1.0×10^{-11}	60	6.0×10^{12}
Staphylococcal nuclease	1.7×10^{-13}	95	5.6×10^{14}

Source: Radzicka, A. and Wolfenden, R., *Science* 267, 91 (1995).

- Milder reaction conditions: T < 100°C, P = 1 atm and pH almost neutral.
- Greater specificity: in both substrates and products.
- Capacity for regulation: allosteric and covalent modifications and the amount of enzyme synthesized.

Enzymes are classified by the type of reaction they catalyse

- Kinase: transfers a phosphate from ATP to another molecule
- Alcohol dehydrogenase: oxidation of alcohols
- Common name, which comes from the name of the substrate followed by the suffix "-ase"
  - The enzyme, **glucose-6-phosphatase** hydrolyses the phosphate group from glucose-6-phosphate

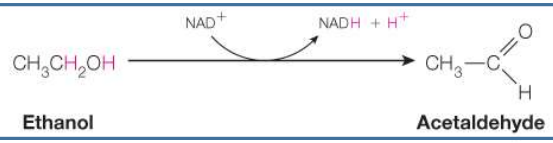
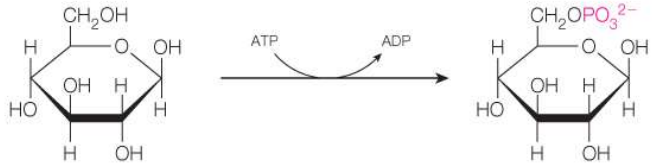
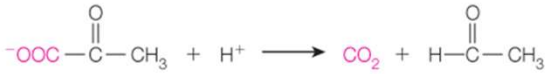
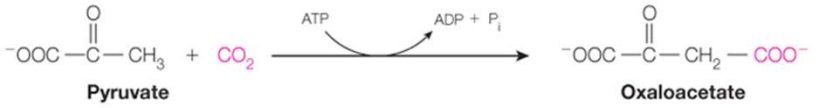
TABLE 11-2 Enzyme Classification According to Reaction Type

Classification	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization
6. Ligases	Bond formation coupled with ATP hydrolysis

Table 11-2

Major Classes of Enzymes

TABLE 8.1 Examples of each of the major classes of enzymes

Class	Example (reaction type)	Reaction Catalyzed
1. Oxidoreductases	Alcohol dehydrogenase (oxidation with NAD^+)	 Ethanol — Acetaldehyde
2. Transferases	Hexokinase (phosphorylation)	 D-Glucose — D-Glucose-6-phosphate
3. Hydrolases	Carboxypeptidase A (peptide bond cleavage)	 C-terminus of polypeptide — Shortened polypeptide — C-terminal residue
4. Lyases	Pyruvate decarboxylase (decarboxylation)	 Pyruvate — Acetaldehyde
5. Isomerases	Maleate isomerase (*cis–trans* isomerization)	 Maleate — Fumarate
6. Ligases	Pyruvate carboxylase (carboxylation)	 Pyruvate — Oxaloacetate

MACQUARIE University

Enzymes are both substrate- and stereo-specific

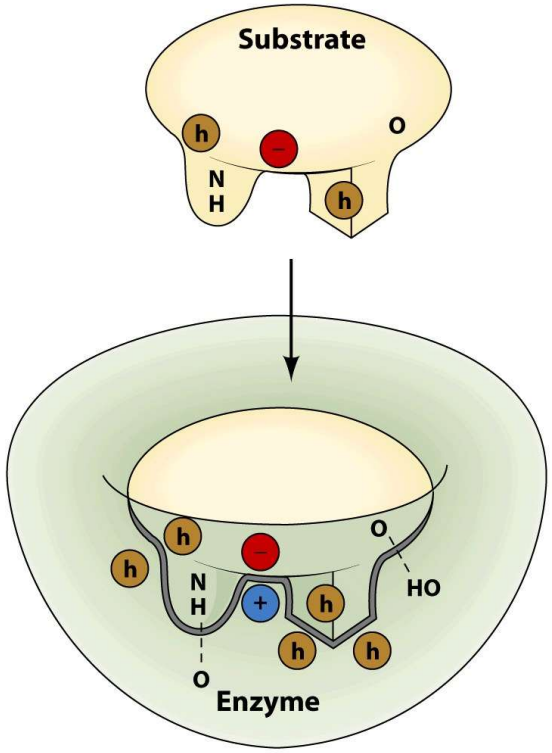


Figure 11-1

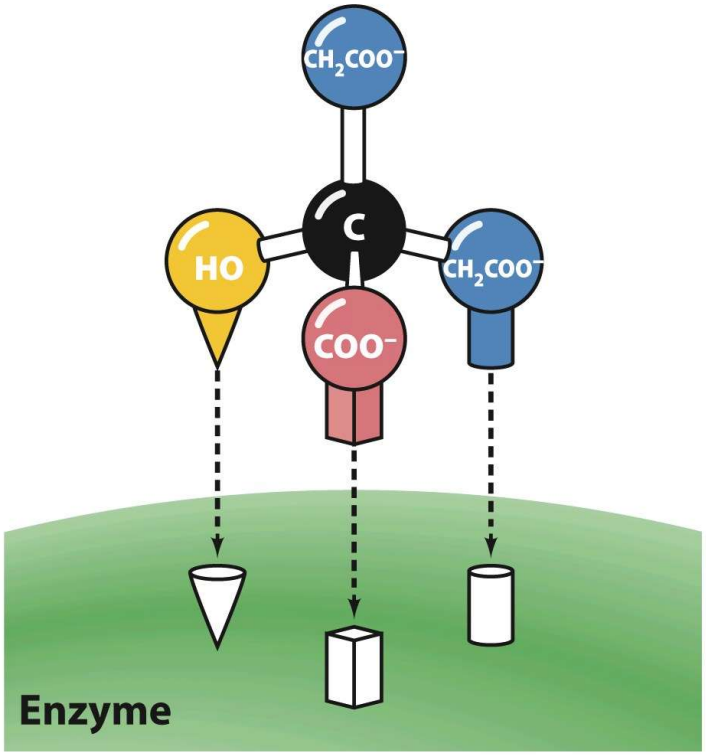
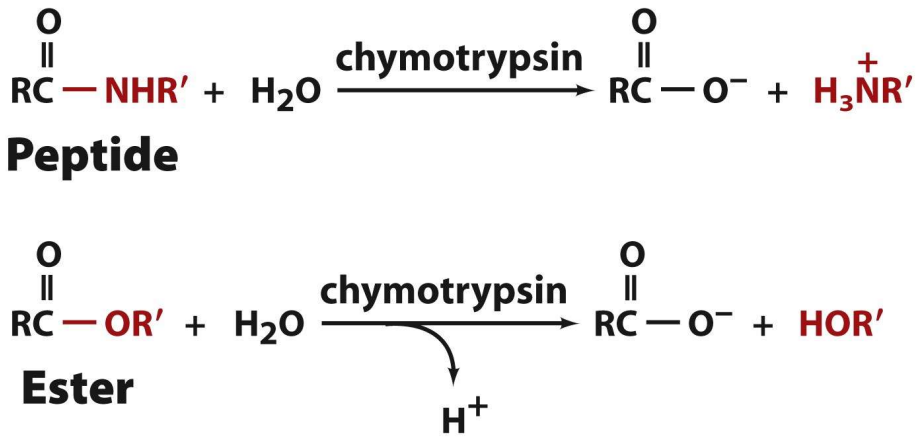


Figure 11-2

Enzymes are substrate specific

- Some enzymes will work on related molecules, although not at maximum efficiency:
  - main role of chymotrypsin is peptide bond hydrolysis
  - Less efficient in hydrolysing synthetic esters!



Some enzymes require cofactors

- For efficient catalytic activity, enzymes often use **ions or small molecules**
  - These are bound to enzymes, but not irreversibly changed during catalysis.
- These ions or molecules are called coenzymes or cofactors.
- Many vitamins come under this category.

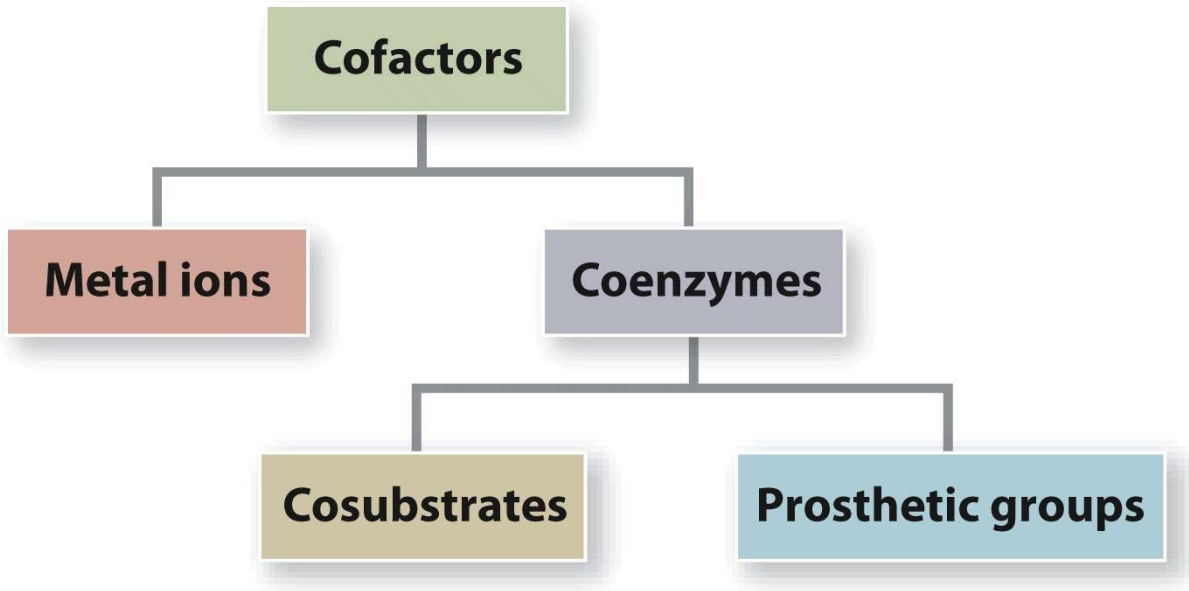


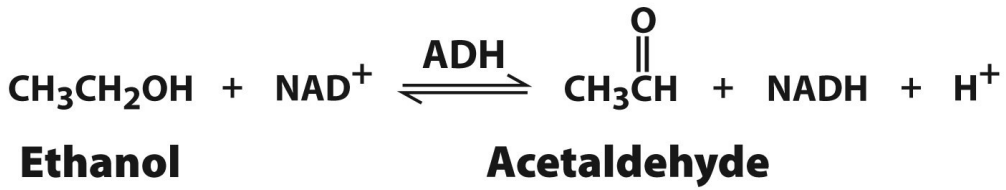
Figure 11-3

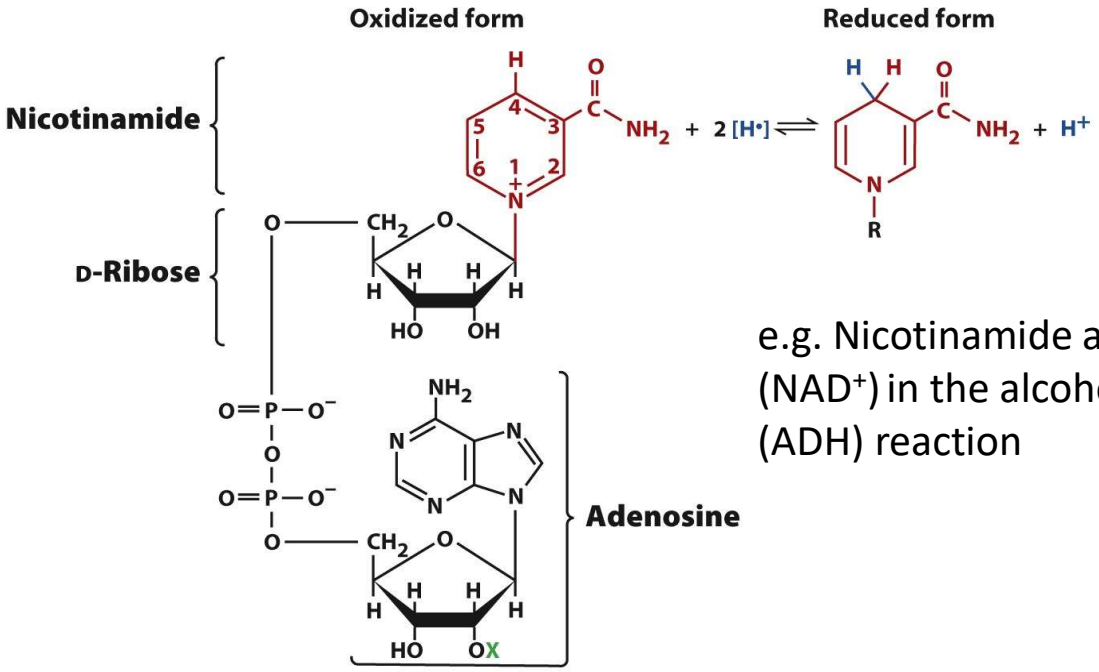
Vitamins provide coenzymes essential for function

TABLE 8.3 Some important coenzymes and related vitamins

Vitamin	Coenzyme	Reactions involving the coenzyme
Thiamine (vitamin B_1)	Thiamine pyrophosphate	Activation and transfer of aldehydes
Riboflavin (vitamin B_2)	Flavin mononucleotide; flavin adenine dinucleotide	Oxidation-reduction
Niacin (vitamin B_3)	Nicotinamide adenine dinucleotide; nicotinamide adenine dinucleotide phosphate	Oxidation-reduction
Pantothenic acid (vitamin B_5)	Coenzyme A	Acyl group activation and transfer
Pyridoxine (vitamin B_6)	Pyridoxal phosphate	Various reactions involving amino acid activation
Biotin (vitamin B_7)	Biotin	CO_2 activation and transfer
Lipoic acid	Lipoamide	Acyl group activation; oxidation-reduction
Folic acid (vitamin B_9)	Tetrahydrofolate	Activation and transfer of single-carbon functional groups
Vitamin B_{12}	Adenosyl cobalamin; methyl cobalamin	Isomerizations and methyl group transfers

Some cofactors function as co-substrates





e.g. Nicotinamide adenine dinucleotide (NAD^+) in the alcohol dehydrogenase (ADH) reaction

MACQUARIE University

Metal Ions in Enzymes

TABLE 8.4 Metals and trace elements important as enzymatic cofactors

Metal	Example of Enzyme	Role of Metal
Fe	Cytochrome oxidase	Oxidation–reduction
Cu	Ascorbic acid oxidase	Oxidation–reduction
Zn	Alcohol dehydrogenase	Helps bind NAD^+
Mn	Histidine ammonia lyase	Aids in catalysis by electron withdrawal
Co	Glutamate mutase	Co is part of cobalamin coenzyme
Ni	Urease	Catalytic site
Mo	Xanthine oxidase	Oxidation–reduction
V	Nitrate reductase	Oxidation–reduction
Se	Glutathione peroxidase	Replaces S in one cysteine in active site
Mg	Many kinases	Helps bind ATP

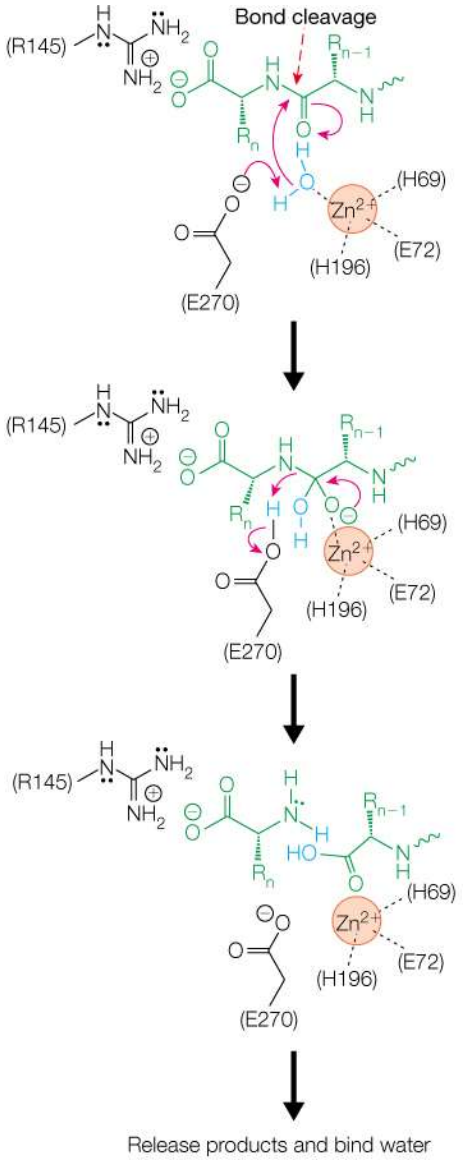


FIGURE 8.19 The mechanism of the protease carboxypeptidase A.

Apo and holo enzymes

- Some enzymes are inactive without the bound cofactor
  - **Apoenzyme (inactive)**
- The active form of the enzyme has the cofactor bound in its specific binding site
  - **Holoenzyme (active)**
- Coenzymes may be chemically changed by the enzymatic reactions they are part of – but they **must be regenerated** for the enzyme activity to continue.

Transition State and Reaction Rate

- The rate of a chemical reaction depends on at least one of the following:
  1) the order of the reaction;
  2) the concentrations of the reactants and products;
  3) the temperature;
  4) the value of the rate constant
- Whether a chemical reaction is favorable depends on the **free energy difference** (ΔG) between the initial state and the final state (must be negative to occur)

- *G*, the **Gibbs free energy** (or just "free energy") is related to the enthalpy ("heat"): *H*, the entropy ("disorder"): *S* and the absolute temperature: T

  $G = H - TS$, and

  $\mathbf{\Delta G = \Delta H - T\Delta S}$

- ΔG represents that portion of total energy change that is available to do useful work at constant temperature (T) and pressure (P)

TABLE 3.2 Free energy rules

If ΔG is . . .	Free energy is . . .	The process is . . .	
Negative	Available to do work	Thermodynamically favorable (and the reverse process is unfavorable)	An exergonic process
Zero	Zero	Reversible; the system is at equilibrium	An equilibrium process
Positive	Required to do work	Thermodynamically unfavorable (and the reverse process is favorable)	An endergonic process

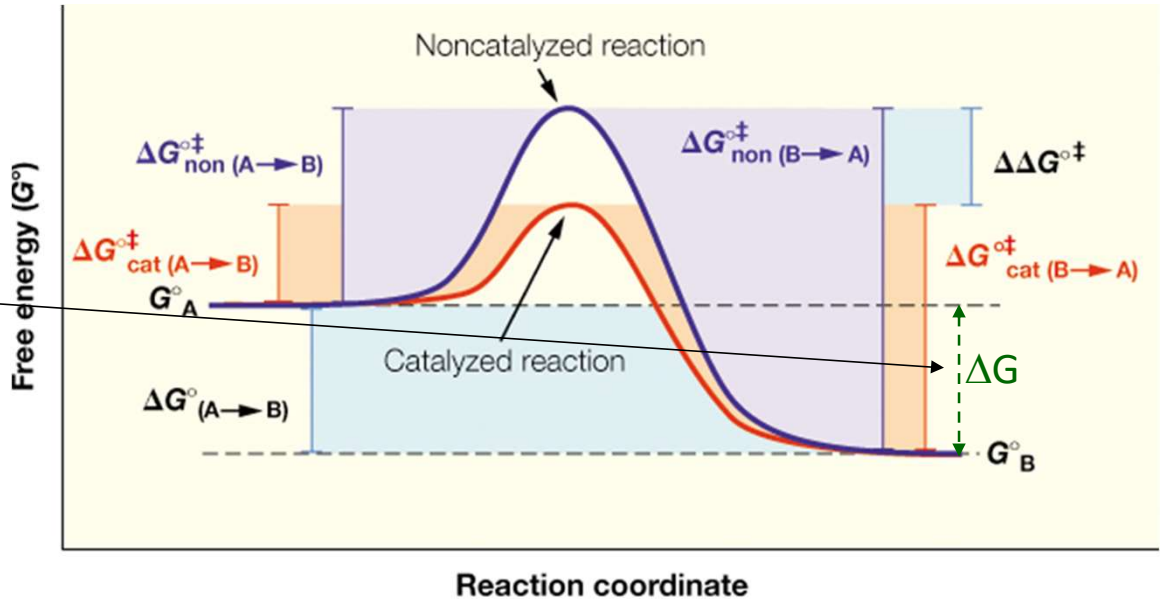
Transition State and Reaction Rate

- The rate of a chemical reaction depends on at least one of the following:
  1) the order of the reaction;
  2) the concentrations of the reactants and products;
  3) the temperature;
  4) the value of the rate constant
- Whether a chemical reaction is favorable depends on the **free energy difference** (ΔG) between the initial state and the final state (must be negative to occur)
- However, to determine the rate of a chemical reaction, not only its initial state and its final state have to be considered, but also its **transition state**
- The transition state is an additional energy barrier to convert reactants into products (and vice versa)
- The transition state has more free energy in comparison to that of the reactant or product; thus, it is the least stable state during the progress of a chemical reaction

Enzymes lower activation energy of the catalyzed reaction

- Enzymes stabilize **the transition state** and lower the activation energy to achieve rate enhancement
- However, they cannot change the ΔG of the reaction.
  - ➢ΔG < 0: spontaneous reaction
  - ➢ΔG > 0: reverse reaction is spontaneous

➢Actual velocity of the reaction depends on kinetics



Enzymes preferentially bind the transition state ($S^{\ddagger}$) of the reaction

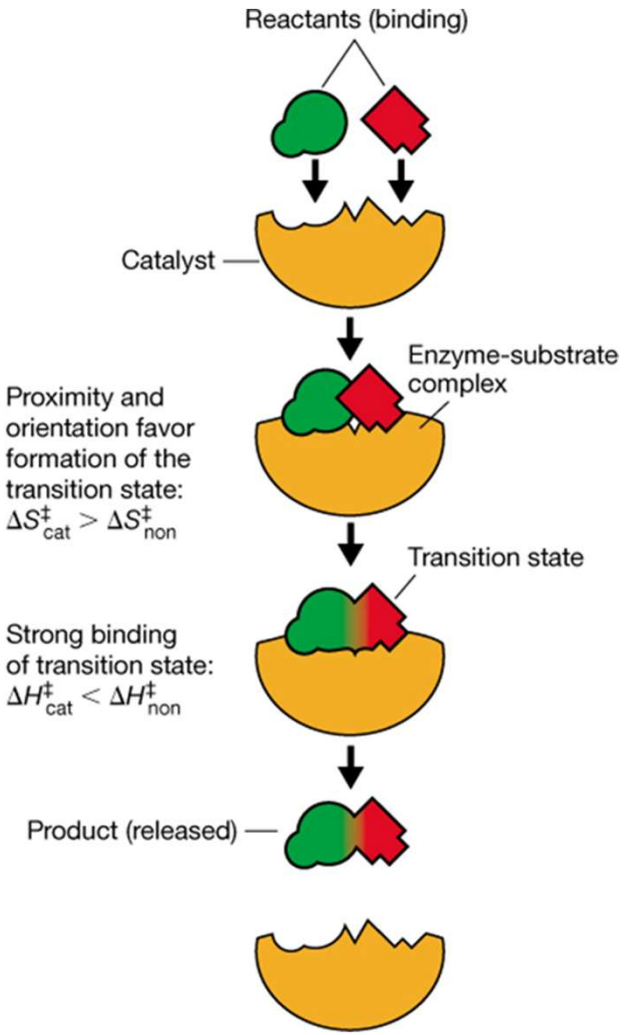


FIGURE 8.6 Entropic and enthalpic factors in catalysis.

- The **transition state** of the reaction binds with <u>greater affinity</u> to the enzyme than the substrates or the products.
- **Transition state analogues** are often used as inhibitors in industry and in drug design

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

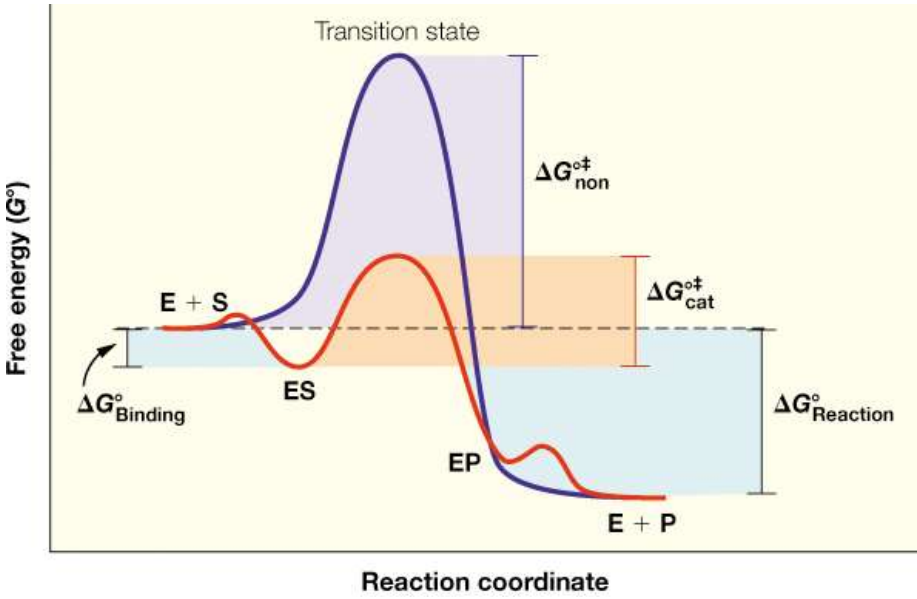
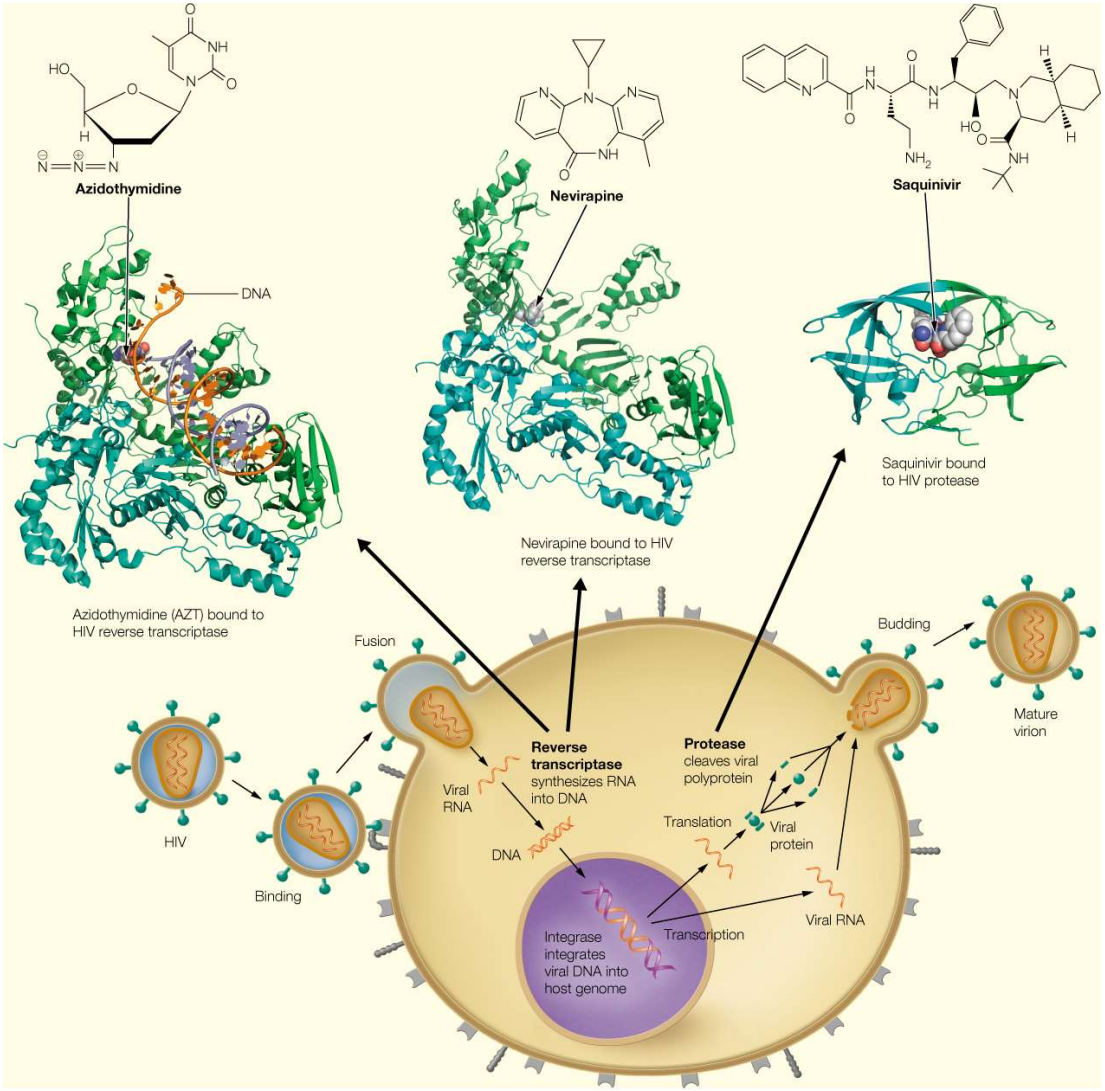


FIGURE 8.10 Reaction coordinate diagram for a simple enzyme-catalyzed reaction.

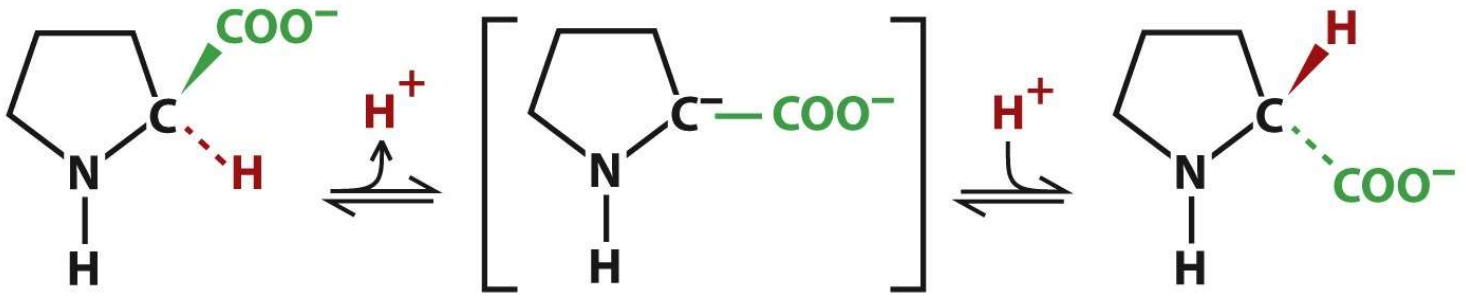
Enzymes and Cell Biology

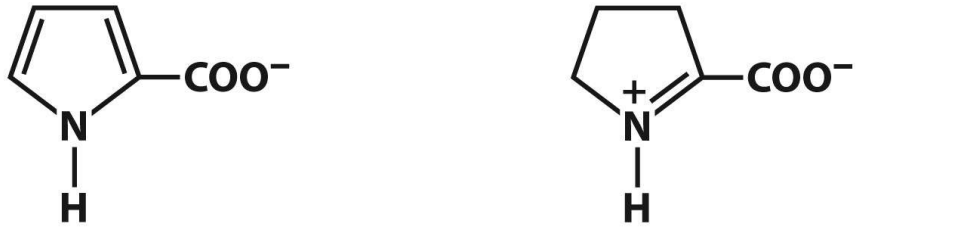
Enzyme inhibitors as drugs

- Many different therapeutic drugs have been developed to treat HIV infection.
- The three shown here—azidothymidine (AZT), nevirapine, and saquinivir—all have different modes of action against two enzymes critical to the HIV life cycle.
- AZT is a substrate analog that causes premature DNA chain termination.
- Nevirapine distorts the enzyme active site and thereby inhibits reverse transcription.
- Saquinivir blocks the active site of the HIV protease.



Transition state analogs are enzyme inhibitors: e.g. proline racemase *(Clostridium sticklandii)*





Enzymes Summary

- Enzymes form six classes, based on the reactions they catalyse.
- Enzymes are substrate specific.
- Some enzymes need metal ion cofactors or organic coenzymes (derived from vitamins) to function.
- Enzymes accelerate reactions by lowering activation energy.
  - do **not** change the free energy of the reaction
  - bind preferentially to the **transition state**

Why does a reaction occur?

- A general reaction is:

$$\underset{\text{initial state (reactants)}}{A + B} \rightleftharpoons \underset{\text{final state (products)}}{C + D}$$

- Free energy of the reaction at constant T and P is:
  - $\Delta G = \Delta H - T\,\Delta S$
    - ΔG is **free energy** change (final state – initial state)
    - ΔH is **enthalpy** change (i.e. heat transfer)
    - T is temperature (in K, Kelvin)
    - ΔS is **entropy** change (a measure of randomness, increasing for spontaneous processes)
  - If ΔG is 0, the reaction is at equilibrium.
  - If ΔG is negative, the reaction occurs spontaneously.
  - If ΔG is positive, the reaction does not occur spontaneously.

Free energy for a reaction

- A general reaction is:

$$\underset{\text{initial state (reactants)}}{A + B} \underset{}{\overset{K_{eq}}{\rightleftharpoons}} \underset{\text{final state (products)}}{C + D}$$

- Standard free energy of the reaction:
  - $\Delta G^o = \Sigma\, \Delta G^o$ (products) - $\Sigma\, \Delta G^o$ (reactants)
- ΔG versus $\Delta G^{o'}$
  - To calculate the free energy change for reactions not at standard state, use

$$\Delta G = \Delta G^{o'} + RT \ln ([C][D]/[A][B])$$
$$= \Delta G^{o'} + RT \ln (K_{eq})$$

Free energy and equilibrium contant

- For general reaction,

  with equilibrium constant K_{eq}

  A + B $\rightleftharpoons$ C + D

  initial state (reactants) — final state (products)

$\Delta G^{o'} = - RT \ln ([C][D]/[A][B])$

$\Delta G^{o'} = - RT \ln (K_{eq})$

$\Delta G^{o'} < 2.73$; $K_{eq} = 10^2$

- reaction goes to completion

Table 2.5
Relationship between ΔG° and K_{eq} (at 25°C)

ΔG° (kcal/mole)[a]	K_{eq}
−6.82	10^5
−5.46	10^4
−4.09	10^3
−2.73 (-11.2 kJ/mole)	10^2
−1.36	10
0	1
1.36	10^{-1}
2.73	10^{-2}
4.09	10^{-3}
5.46	10^{-4}
6.82	10^{-5}

Biological systems perform work

- **Mechanical work**
  - muscle contraction, flagella rotation, chromosome movement
- **Concentration and electrical work**
  - movement of ions and molecules across membranes, osmotic changes, etc.
- **Synthetic work**
  - changes in chemical bonds

All these actions require **energy**

- Usually from the hydrolysis of high energy phosphate bonds (**ATP**, **ADP**)
  - Also from **reduced coenzymes**

Biological systems can perform energetically unfavorable work

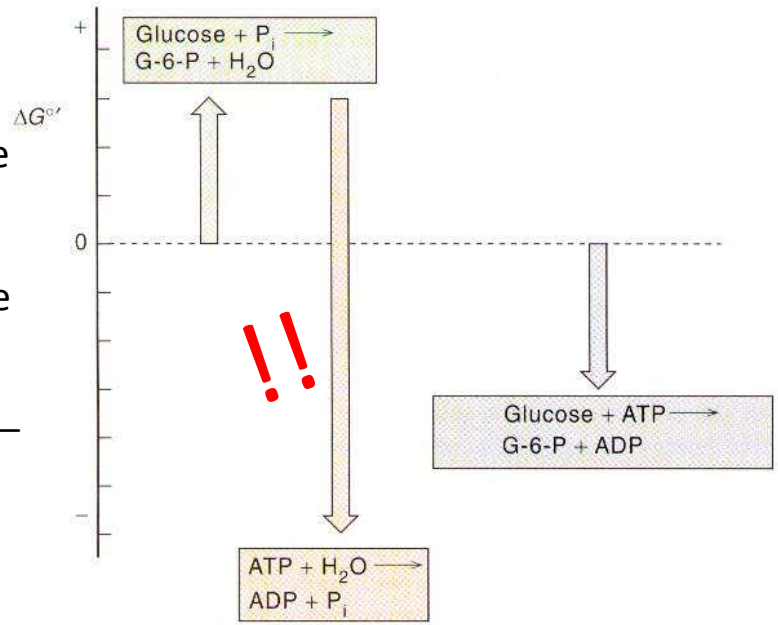
- By coupling an energetically favourable reaction with an unfavourable one:

Glucose + P_i $\rightleftharpoons$ glucose-6-phosphate $\Delta G^{o'}$ = +13.8 kJ/mole

ATP + H_2O $\rightleftharpoons$ ADP + P_i + H^+ $\Delta G^{o'}$ = -32.2 kJ/mole

---

ATP + Glucose $\rightleftharpoons$ ADP + Glu-6-phosphate $\Delta G^{o'}$ = -18.4 kJ/mole



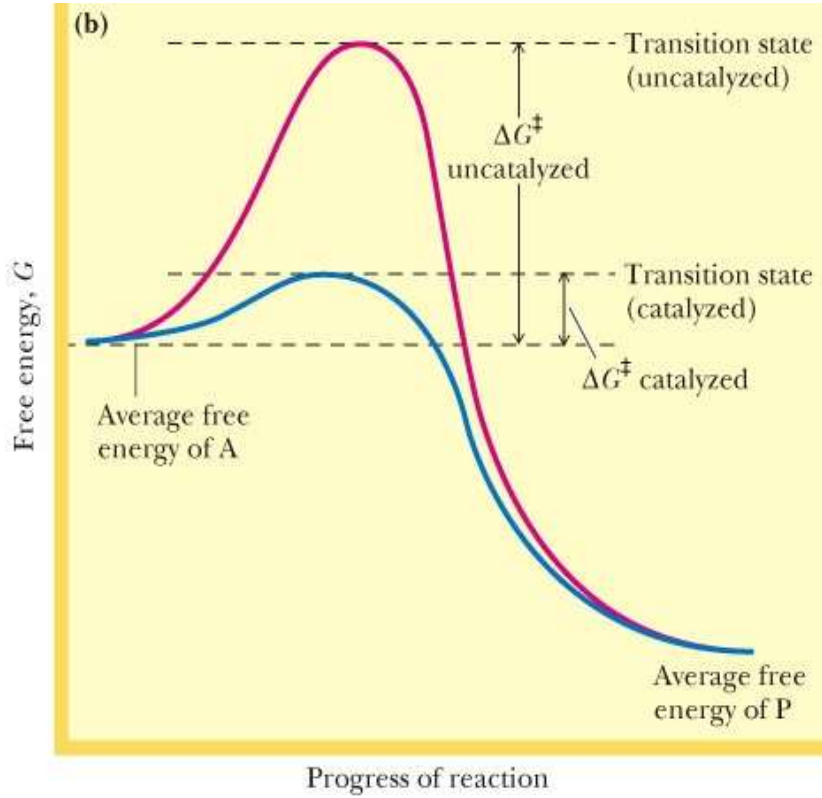
Enzymes are catalysts

- Enzymes speed up the reaction rate by decreasing the activation energy
  - Uncatalysed:

    $S \leftrightarrow P$

    substrate to product
  - Catalysed:

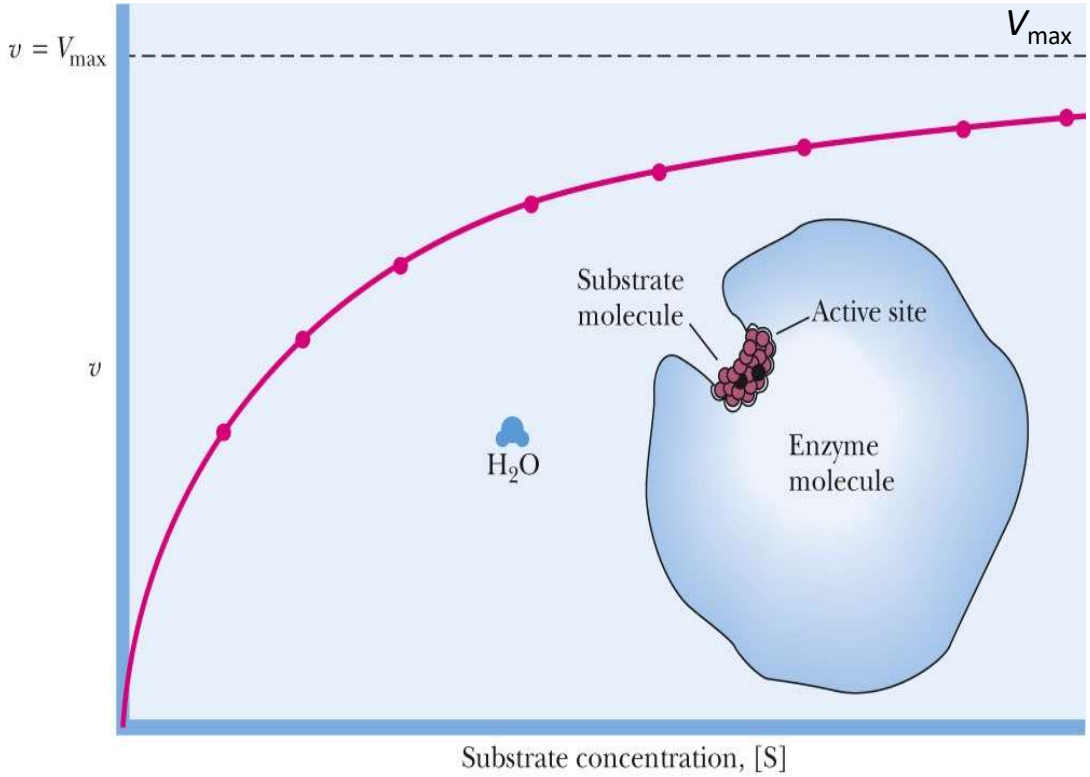
    $E + S \leftrightarrow ES \rightarrow E + P$



Rate of the reaction

- Substrate S forms product P.
- The rate of the reaction (v, "velocity") is the rate of P formation.
- v in an uncatalysed reaction is dependent on the starting concentration [S]
  - If we plot the rate vs. the starting concentration of [S] we ideally get a straight line (known as a first order reaction)
- For enzyme catalysed, reactions, v is also dependent on the enzyme concentration [E] (second order reaction)
  - so if we plot v vs. the starting concentration of [S] we get a hyperbolic curve that reaches a maximum

Rate of the reaction with increasing substrate concentration [S]: Michaelis-Menten kinetics



Concentrations vs. time

- Time-dependent changes in concentrations of S, P, E and ES.
- Only a small quantity of E is needed
- E quickly forms ES, so that [E] ~ [ES]
- v is usually measured at constant [ES]

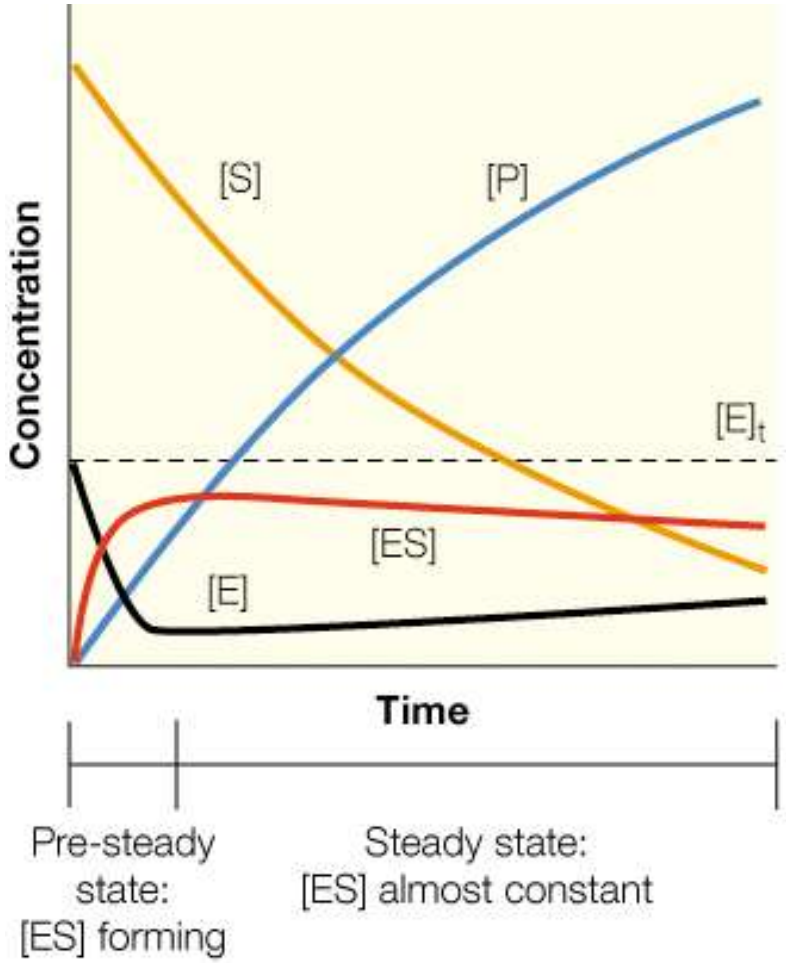


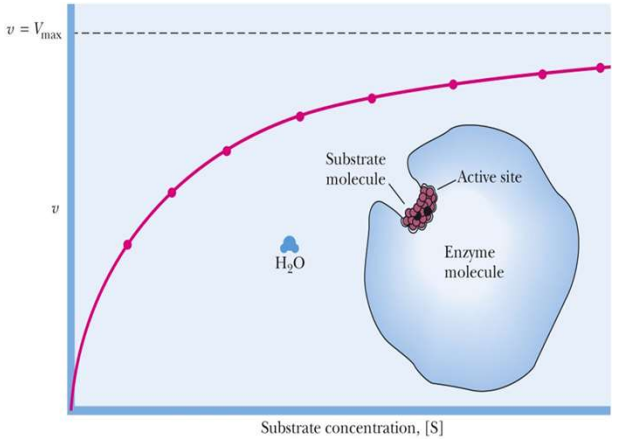
FIGURE 8.20 The steady state in enzyme kinetics.

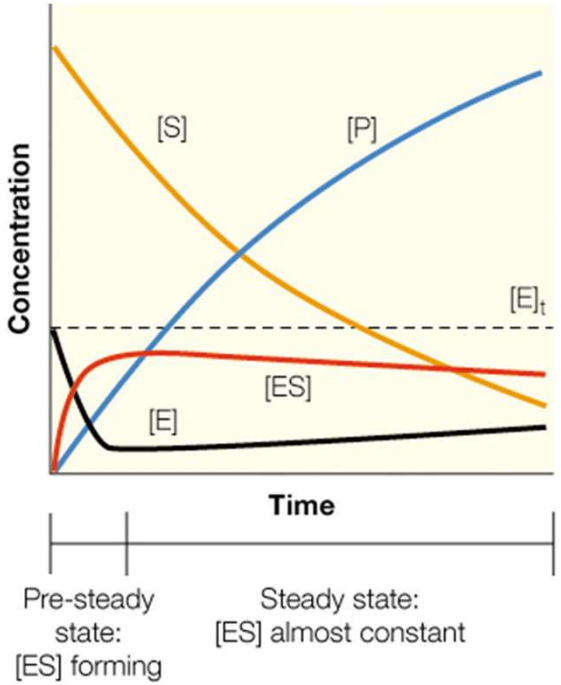
Analysis of enzyme activity – initial velocity and steady state kinetics

- [ES] remains constant for the duration of the steady state
- **The Michaelis-Menten equation** (1913!) describes the hyperbolic graph for v vs. [S].

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

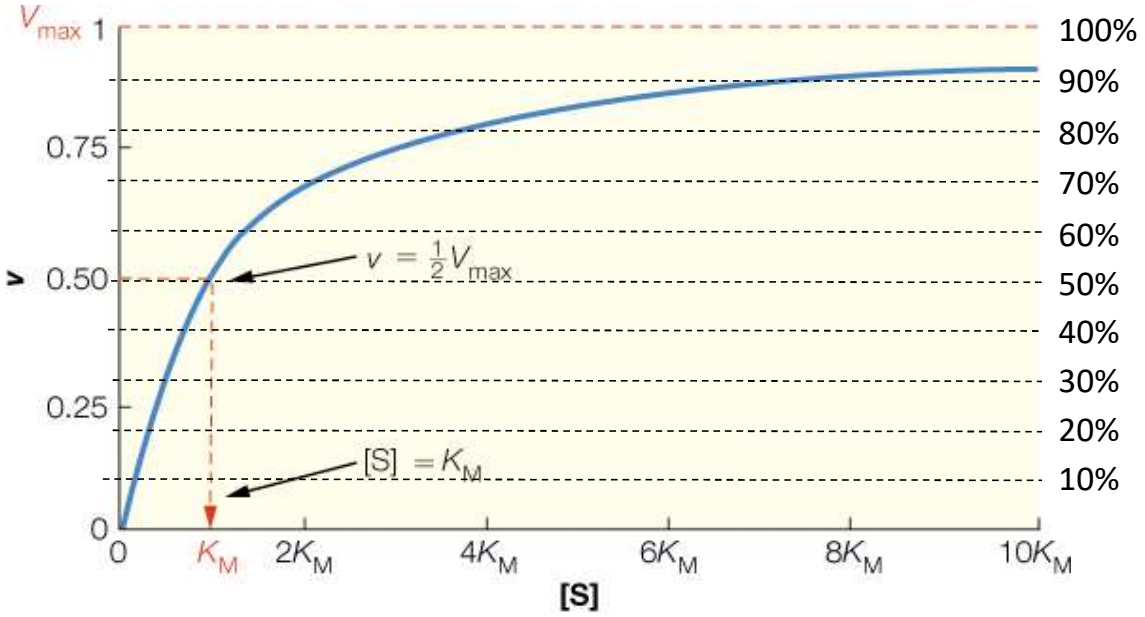
  - V_{max} is the maximum velocity at infinite substrate concentration.
  - K_m is the Michaelis constant (units mol.L^{-1})





Michaelis-Menten constants: K_m and the maximum rate, V_{max}

When [S] = K_m, $v = V_{max}/2$ or *vice versa*.



Kinetic data provides values for K_m and V_{max} : Double-Reciprocal (Lineweaver-Burk) Plot

- Rearranging the Michaelis-Menten relationship, we get a straight line equation:

$$\frac{1}{v} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

- This means that we can determine V_{max} and K_m graphically by plotting $(1/v)$ *vs.* $(1/[S])$
- **Lineweaver-Burk double reciprocal plot** (1934) (**Prac 5**)

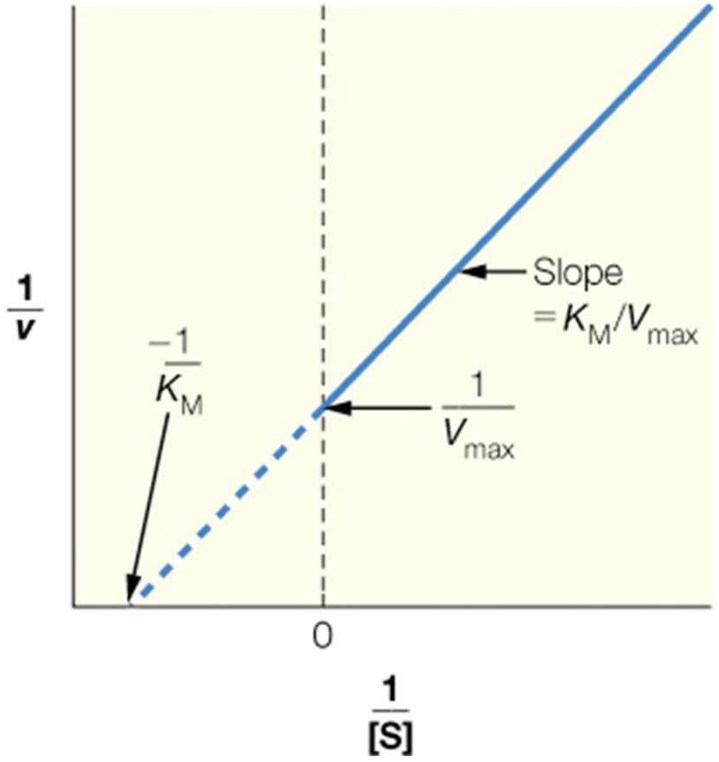


FIGURE 8.23 A Lineweaver–Burk plot.

The Michaelis constant, K_m

- K_m = [S] when $v = V_{max}/2$
  - Units of $mol.L^{-1}$
- **Inversely** related to the **affinity** of a particular substrate for an enzyme
  - **Small K_m** = less substrate required for high reaction rate = **great affinity**
  - **High K_m** = more substrate required for high reaction rate = **low affinity**

The catalytic constant, k_{cat}

- enzyme property that measures its **internal speed**
- $k_{cat} = V_{max} / [E]$
  - Turnover number – the number of times a given site can turn substrate into product per second
  - Measures how **rapidly** the enzyme can operate
  - It has the unit s^{-1} (Specific activity is k_{cat} /mg of E)

The enzyme specificity constant

- k_{cat} / K_m
  - It has the units $L.mol^{-1}s^{-1}$
  - Measures enzyme efficiency
  - Determines which is the best substrate and how rapidly an enzyme will work at low [S]

Enzyme Kinetic Parameters

TABLE 8.5 Michaelis–Menten parameters for selected enzymes, arranged in order of increasing efficiency as measured by k_{cat}/K_M

Enzyme	Reaction Catalyzed	K_M(mol/L)	$k_{cat}(s^{-1})$	$k_{cat}/K_M[(mol/L)^{-1}s^{-1}]$
Chymotrypsin	Ac–Phe–Ala $\xrightarrow{H_2O}$ Ac–Phe + Ala	1.5×10^{-2}	0.14	9.3
Pepsin	Phe–Gly $\xrightarrow{H_2O}$ Phe + Gly	3×10^{-4}	0.5	1.7×10^{3}
Tyrosyl-tRNA synthetase	Tyrosine + tRNA $\longrightarrow$ tyrosyl-tRNA	9×10^{-4}	7.6	8.4×10^{3}
Ribonuclease	Cytidine 2′, 3′ cyclic phosphate $\xrightarrow{H_2O}$ cytidine 3′-phosphate	7.9×10^{-3}	7.9×10^{2}	1.0×10^{5}
Carbonic anhydrase	$HCO_3^- + H^+ \longrightarrow H_2O + CO_2$	2.6×10^{-2}	4×10^{5}	1.5×10^{7}
Fumarase	Fumarate $\xrightarrow{H_2O}$ malate	5×10^{-6}	8×10^{2}	1.6×10^{8}

- K_m is unique for each enzyme-substrate pair.
- A low K_m value means the enzyme achieves maximal catalytic efficiency at low substrate concentrations.
- The larger the k_{cat} value, the greater the amount of product.

MACQUARIE University

Enzyme Kinetics Summary

- ΔG determines if a reaction will occur:
  - $\Delta G = 0$, the reaction is at equilibrium
  - $\Delta G < 0$, the reaction occurs spontaneously
  - $\Delta G > 0$, the reaction does not occur spontaneously.

- When $\Delta G > 0$, this reaction can be powered by coupling it with a reaction that has $\Delta G < 0$ (such as ATP → ADP + P_i)

- Michaelis constant, K_M for a particular enzyme reaction = [substrate] when $v = V_{max}/2$.

- An enzyme's overall catalytic efficiency is expressed as k_{cat}/K_M.

- A Lineweaver–Burk plot can be used to experimentally plot kinetic data and calculate values for K_M and V_{max} from the linear graph.