Review

1. Hemoglobin: How Hb carries the dual activities of binding and releasing? Cooperativity, Two states: T and R, BPG, pH, CO_2, infant Hb vs adult's.

2. Tools to study proteins: Western Blot, NMR, ELISA, Chromatography, Protein sequencing, Immunoprecipitation

3. Enzyme: Catalytic Mechanisms, lysozyme

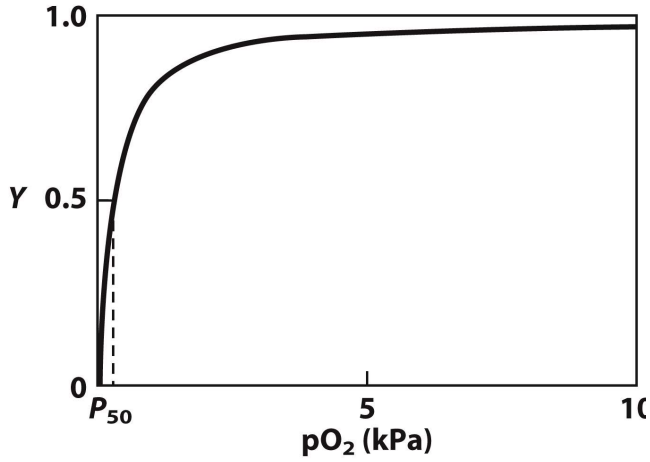
4. Enzyme kinetics: Michaelis-Menten Kinetics, meaning of K_m and K_{cat}, competitive, non-competitive and mixed inhibitors

5. Nucleic acids: DNA, RNA, structures, names, base paring, genetic codes, mutagenesis

6. Tools to study genes: Gene editing, recombinant DNA, RNAi, PCR, sequencing

Could Myoglobin Transport O_2?

- pO_2 in lungs is about 13 kPa (1 mmHg = 133.32 Pa)
- pO_2 in tissues is about 4 kPa



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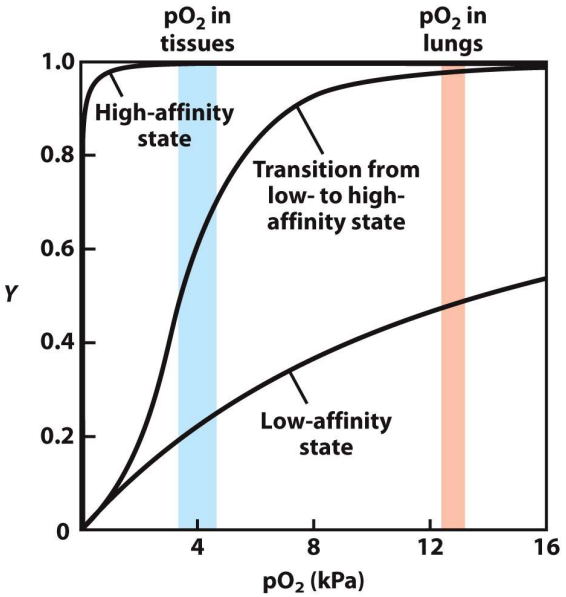

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- Cooperativity
- Two states
- BPG
- pH
- CO_2

cooperativity: multiple binding sites with mutual interactions.
positive (sigmoidal binding curve) and negative cooperativity

Two States of Hb

- T = tense state
  - more interactions
  - more stable
  - lower affinity for O_2
- R = relaxed state
  - fewer Interactions
  - more flexible
  - higher affinity for O_2
- O_2 binding triggers a **T** → **R** conformational change.
- Conformational change from the T state to the R state involves breaking ion pairs between the α1-β2 interface.

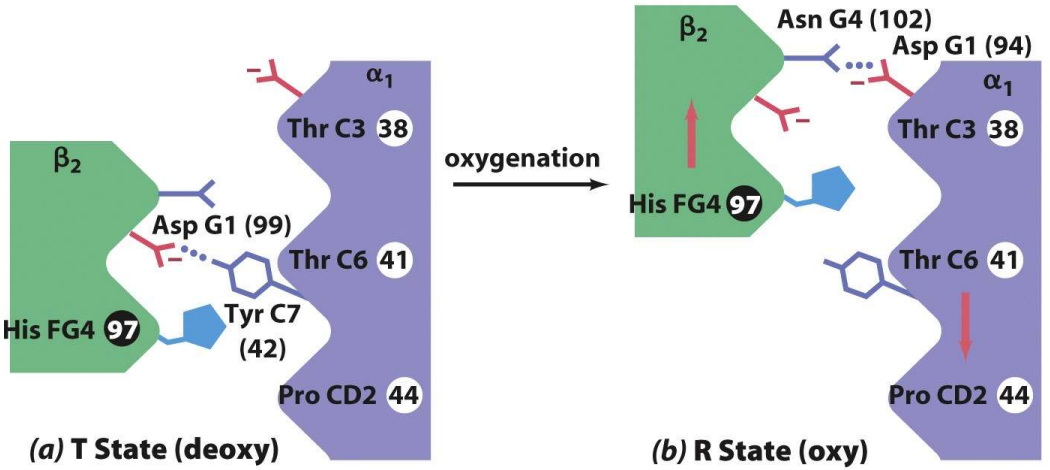
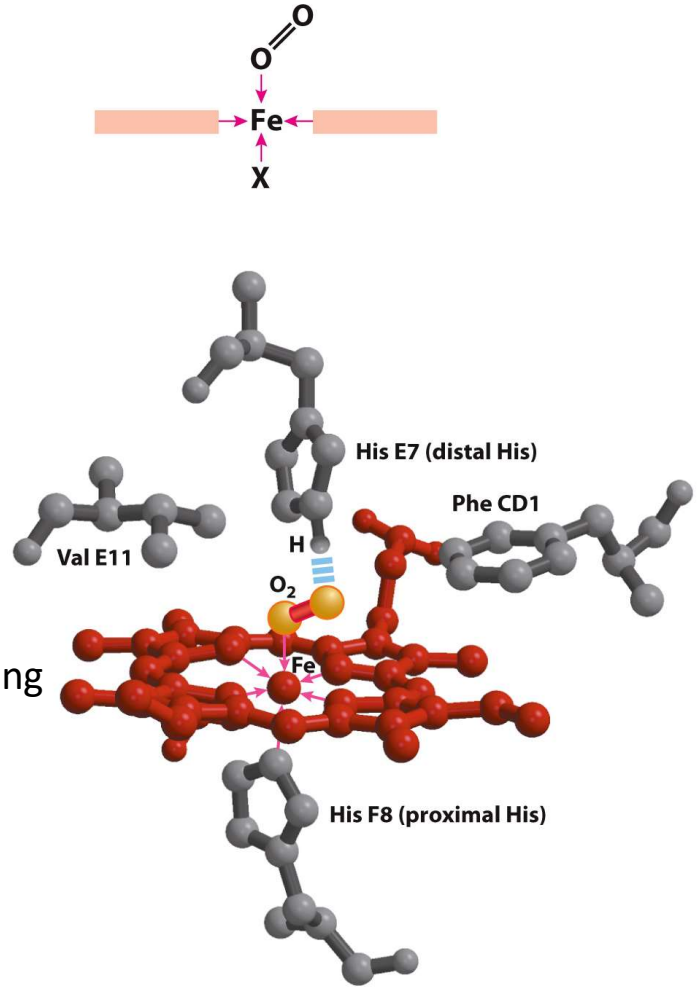


Figure 7-9 part 2

O_2 binding to heme causes shift from T to R



porphyrin ring

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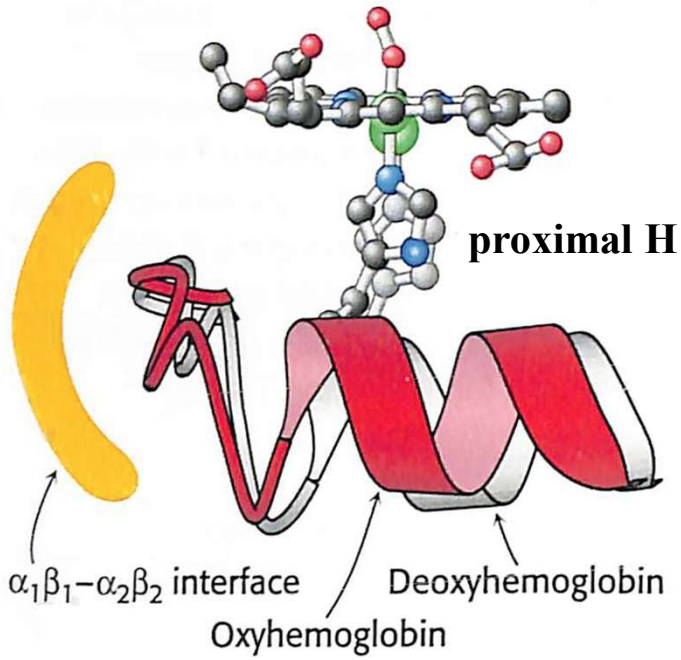

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pH Effect on O_2 Binding to Hemoglobin – Bohr effect

- Actively metabolizing tissues (7.4 vs 7.2) generate H^+, lowering the pH of the blood near the tissues relative to the lungs (catalyzed by carbonic anhydrase).

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

- Hb Affinity for oxygen depends on the pH.
  - H^+ binds to Hb and stabilizes the T state.
    - protonates His146, which then forms a salt bridge with Asp94
    - leads to the release of O_2 (in the tissues)
- The pH difference between lungs and metabolic tissues increases efficiency of the O_2 transport: Bohr effect

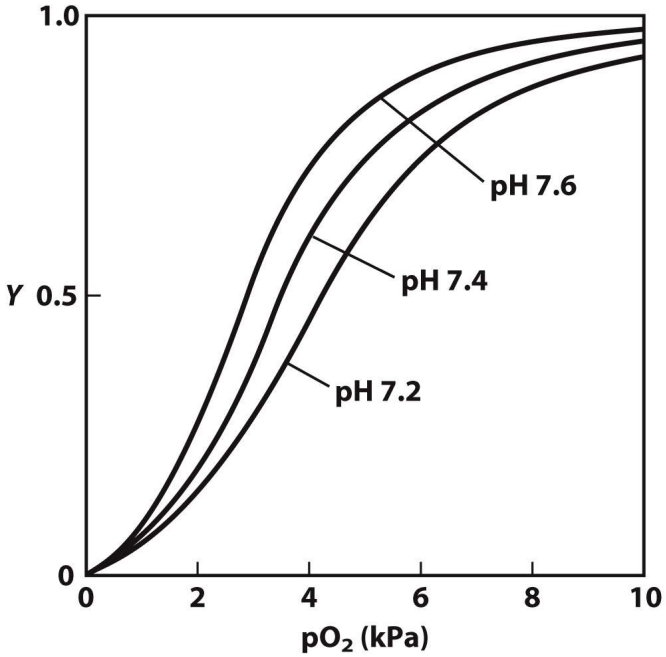

Figure 5-16
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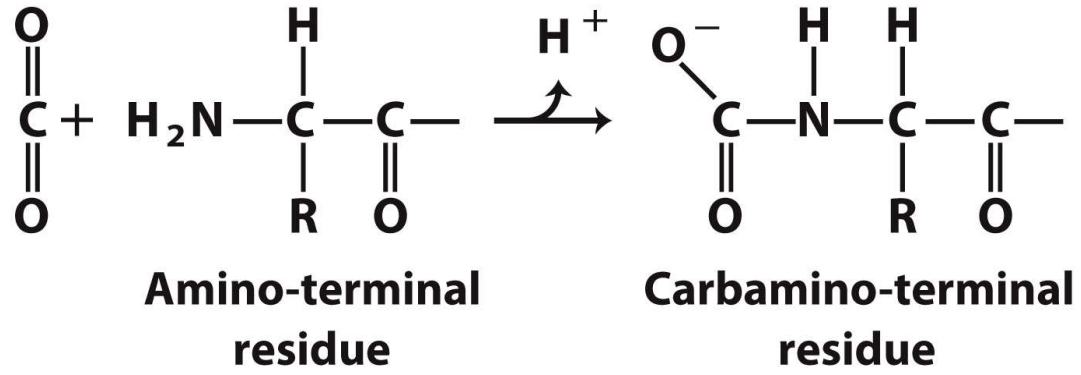
Hemoglobin and CO_2 Export

- CO_2 is produced by metabolism in tissues and must be exported.
- 15–20% of CO_2 is exported in the form of a carbamate on the amino terminal residues of each of the polypeptide subunits.



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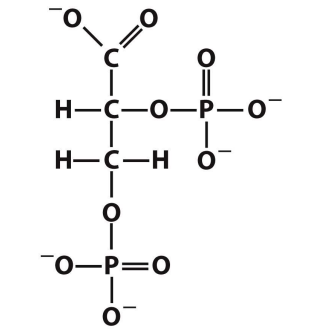
- Notice:
  - The formation of a carbamate yields a proton that can contribute to the Bohr effect.
  - The carbamate forms additional salt bridges, stabilizing the T state.

2,3-BPG Binds to central cavity of Hb

Adaptation to altitude

- Negative regulator – Present at mM concentrations in erythrocytes: an intermediate in glycolysis
- Small negatively charged molecule, binds to the positively charged central cavity of Hb and stabilizes the T states



2,3-Bisphosphoglycerate

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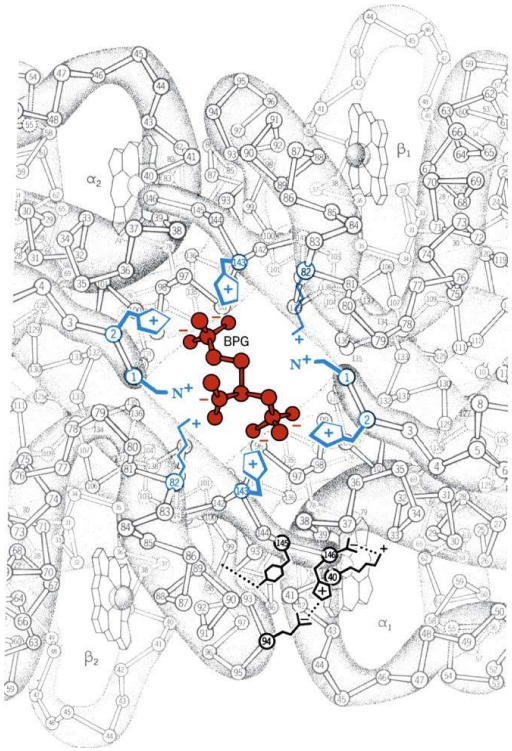

Figure 7-14
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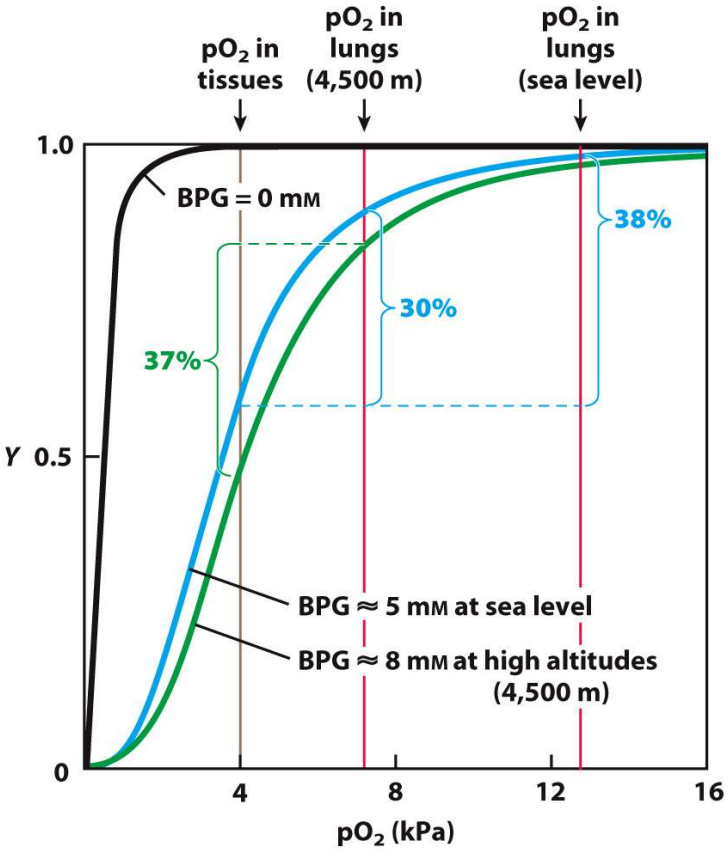

Figure 5-17
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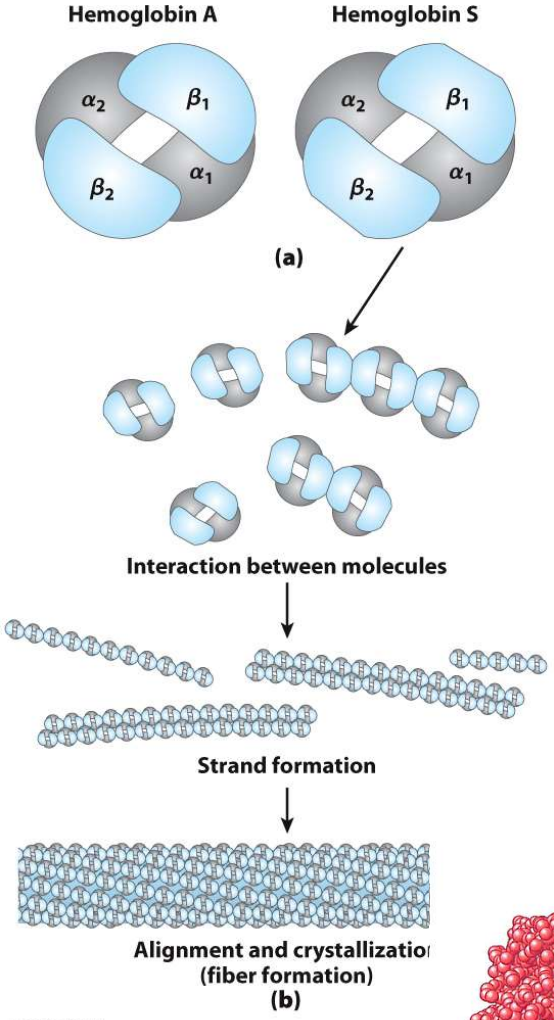
Sickle-Cell Anemia

- Glu6 → Val in the β chain
- The new Val side chain can bind to a different Hb molecule to form a strand similar to the amyloidgenic proteins.
- This sickles the red blood cells.
- Untreated homozygous individuals generally die in childhood.
- Heterozygous individuals exhibit a resistance to malaria.



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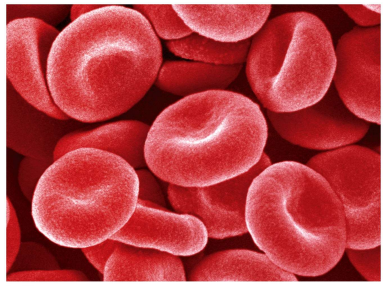

Figure 7-17a
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Figure 7-17b
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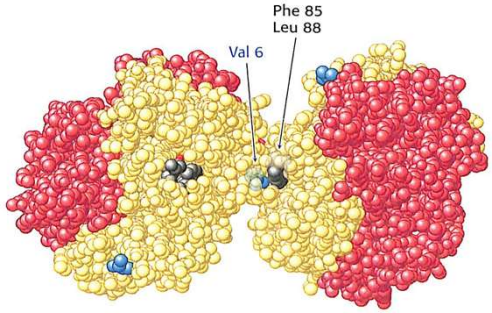
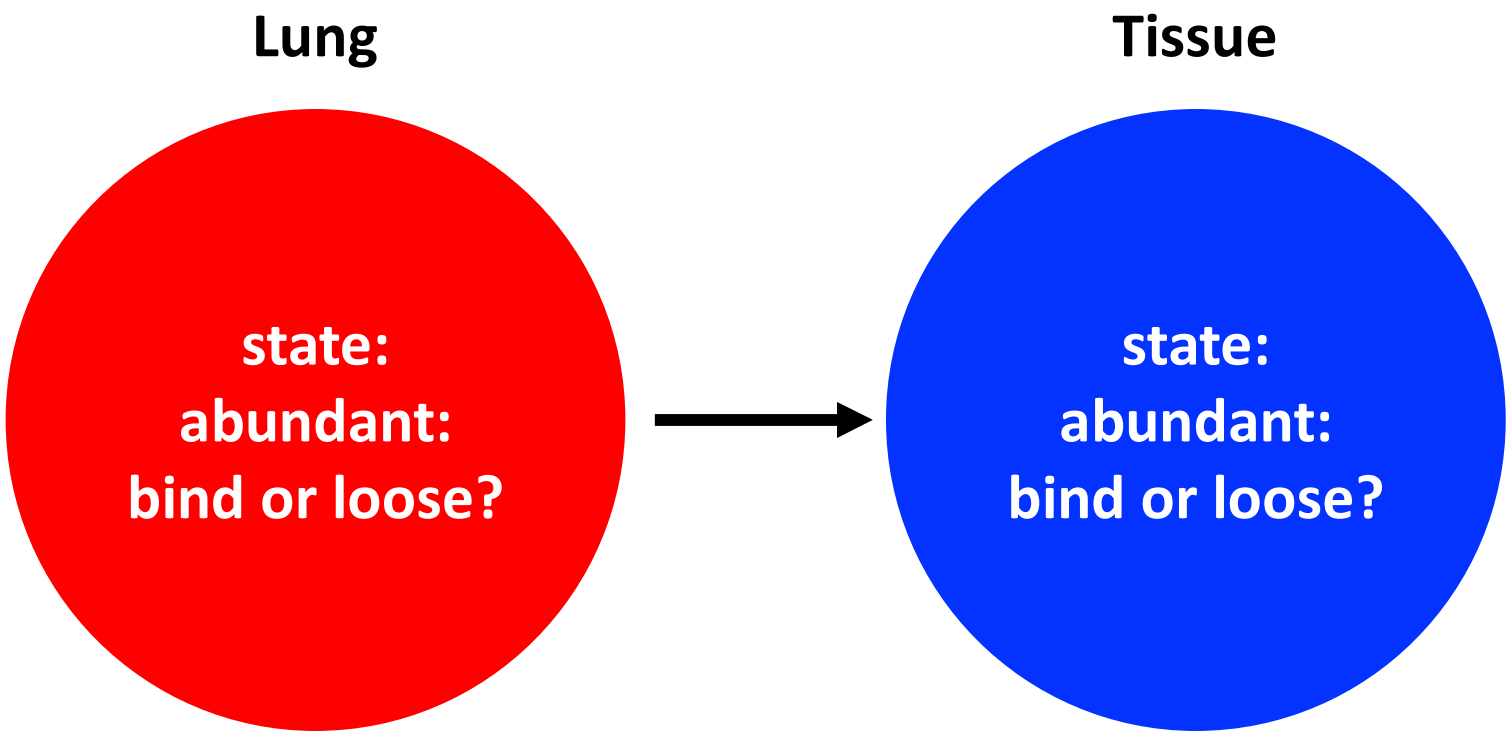
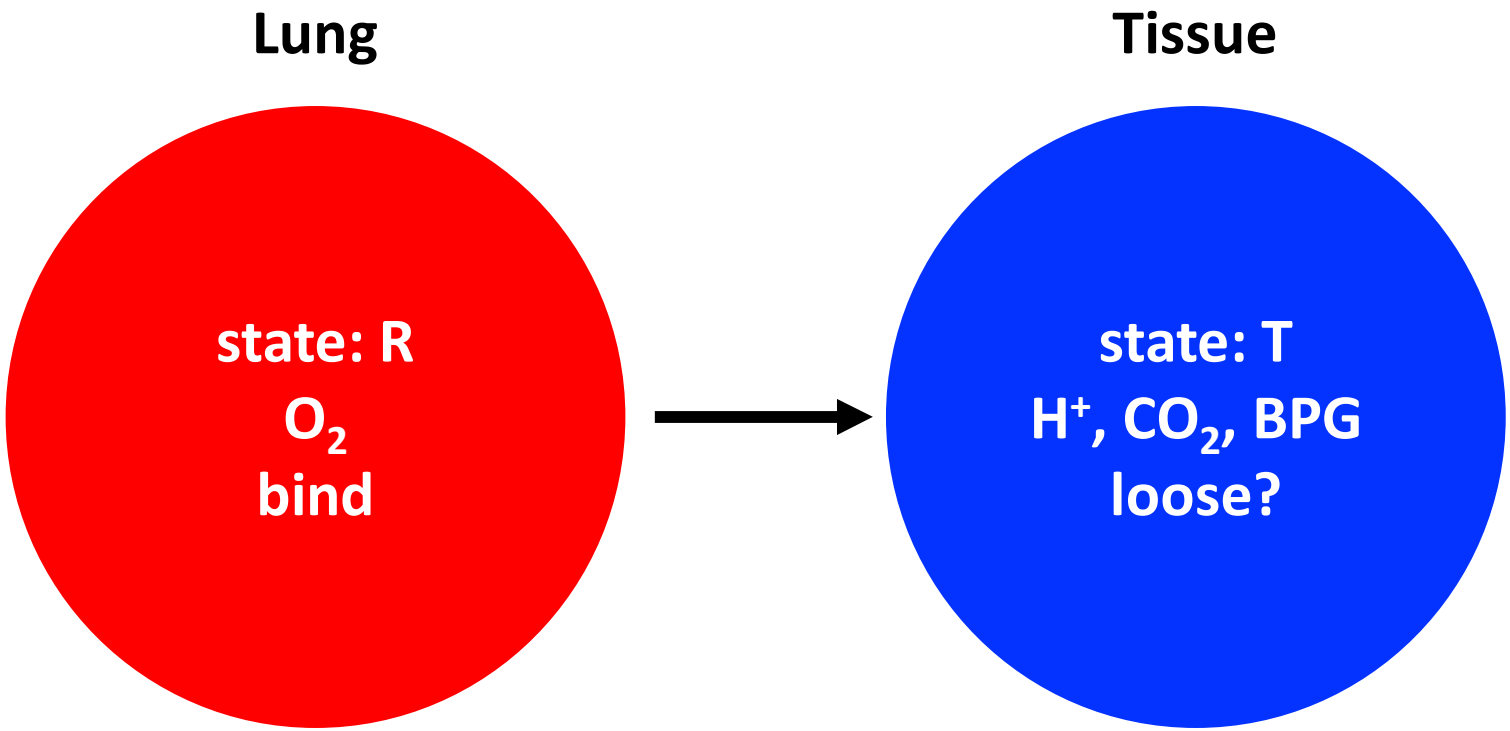



Lung
Tissue
state:
abundant:
bind or loose?
state:
abundant:
bind or loose?


Lung
Tissue
state: R
O_2
bind
state: T
H^+, CO_2, BPG
loose?

Tools to Study Protein

Tools	Purposes
Western Blot	to detect
NMR	to learn the structure + function
ELISA	to measure activity
Chromatography	to separate
Protein Sequencing	to identify

General Properties of Enzymes

- Enzymes differ from ordinary chemical catalysts in reaction rate, reaction conditions, reaction specificity, and control.
- The unique physical and chemical properties of the active site limit an enzyme's activity to specific substrates and reactions.
- Some enzymes require metal ions or organic cofactors.

Catalytic Mechanisms

1. Amino acid side chains that can donate or accept protons can participate in chemical reactions as acid or base catalysts: ***Acid-base catalysis***
2. Nucleophilic groups can catalyze reactions through the transient formation of covalent bonds with the substrate: ***Covalent catalysis***
3. In metal ion catalysis, the unique electronic properties of the metal ion facilitate the reaction: ***Metal ion catalysis***
4. Enzymes accelerate reactions by bringing reacting groups together and orienting them for reaction: ***Proximity and orientation effects***
5. Transition state stabilization can significantly lower the activation energy for a reaction: ***Preferential binding of the transition state complex***

Lysozyme

- Model building indicates that binding to lysozyme distorts the substrate sugar residue.
- Lysozyme's active site Asp and Glu residues promote substrate hydrolysis by acid–base catalysis, covalent catalysis, and stabilization of an oxonium ion transition state.

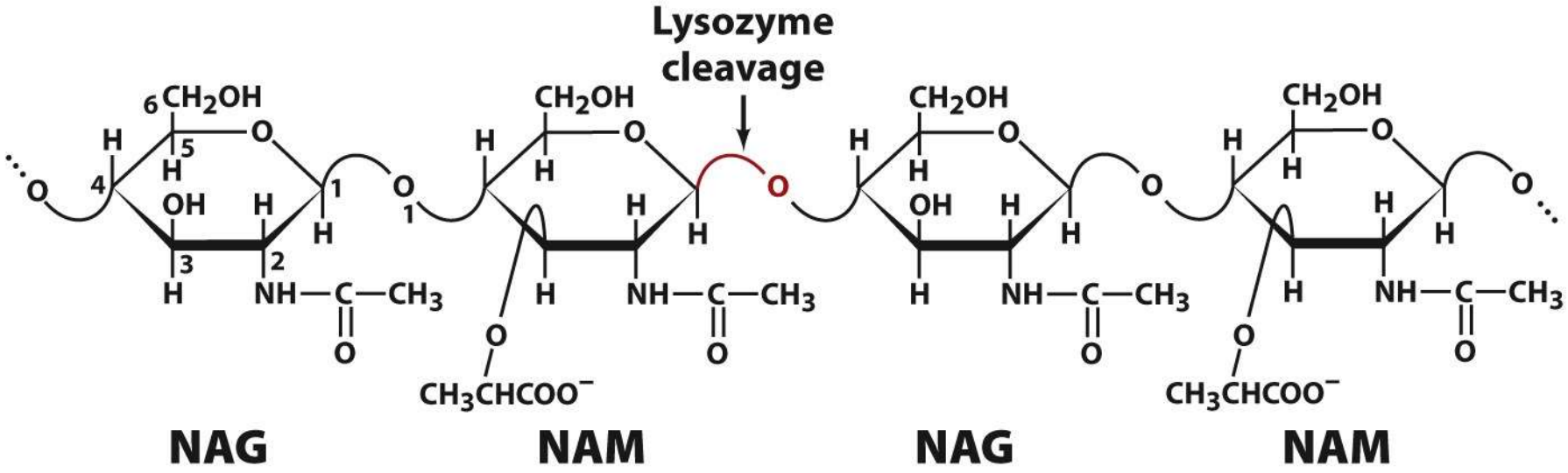
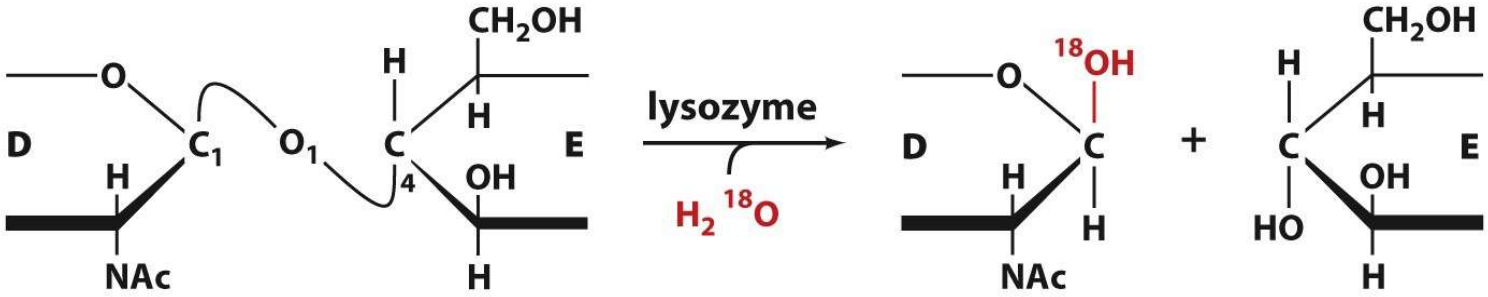


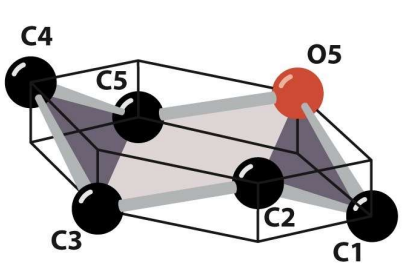
Figure 11-16

Identification of Lysozyme Cleavage Site

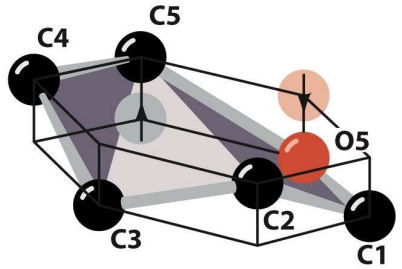


- Destroys bacterial cell walls (peptidoglycan)
- Hydrolyzing the β (1→4) glycosidic linkages from *N*-acetylmuramic acid (NAM) to *N*-acetylglucosamine (NAG)
- Also hydrolyzes β (1→4)-linked poly(NAG) (=chitin)
- Bactericidal agent or helps dispose of killed bacteria
- Hen egg white (HEW) lysozyme is the most studied.

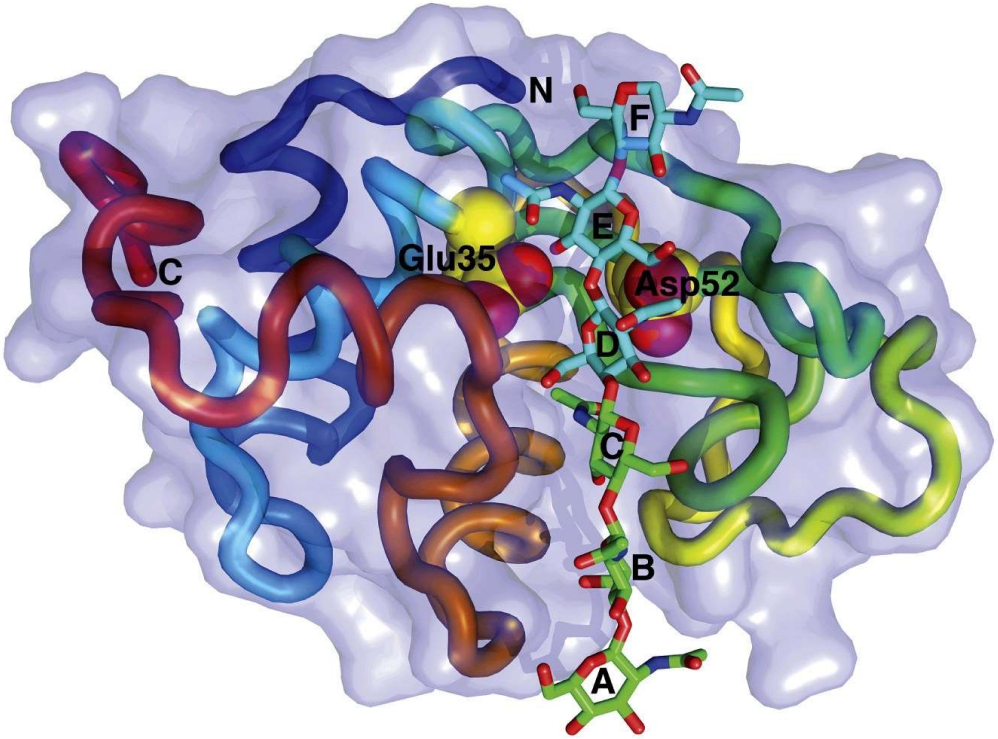
Lysozyme-Substrate Interactions



Chair conformation



Half-chair conformation



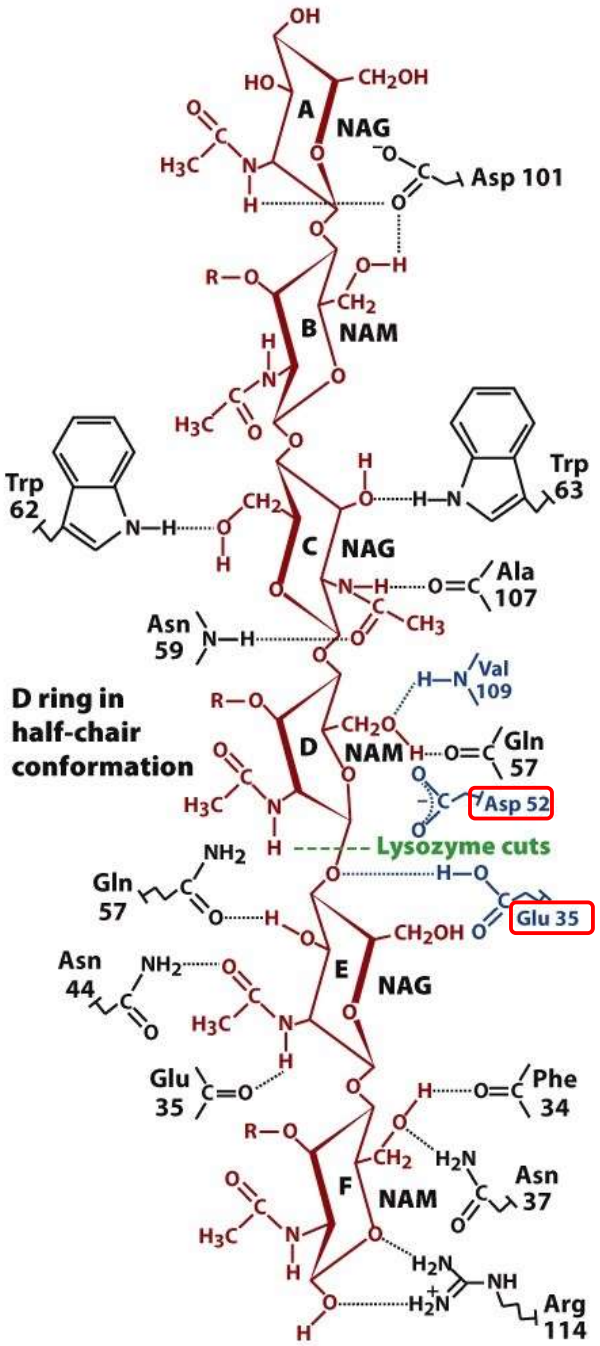
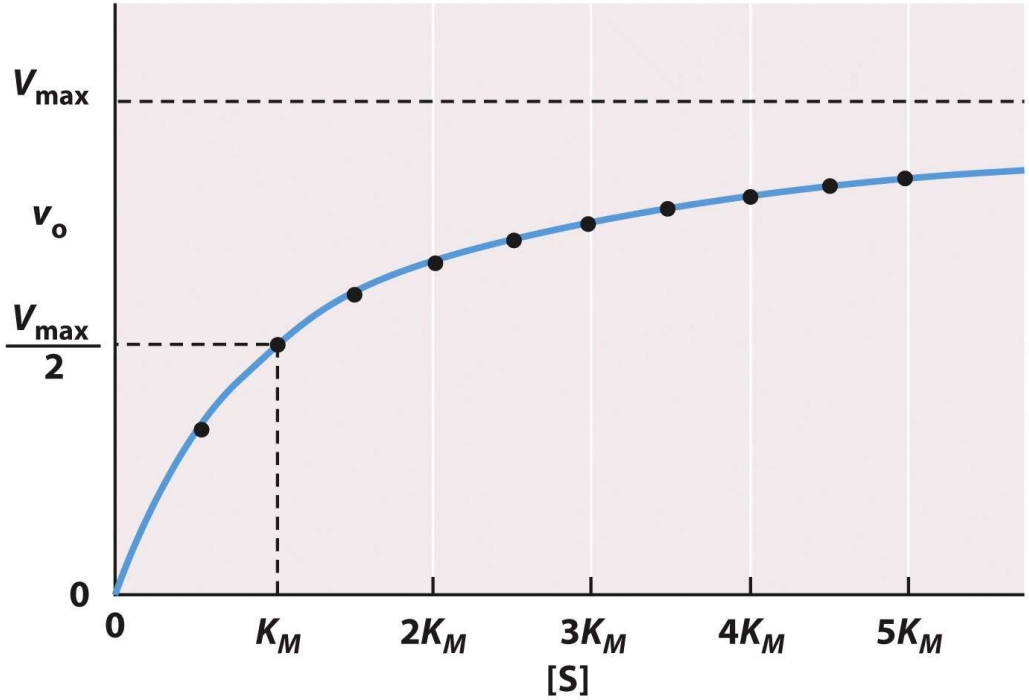


Figure 11-19

Michaelis-Menten Kinetics

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



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https://www.amazon.co.jp/Fundamentals-Biochemistry-Binder-Ready-Version/dp/1118918436/ref=dp_ob_title_bk 2019/6/10

Three assumptions

1. ES is a necessary intermediate step
2. k_{-2} is negligible due to small [P]
3. Steady state: [ES] is a constant independent on [S] or [P]

M-M equation

1.Finding K_M
$[E][S]/[ES] = (k_{-1} + k_2)/k_1$

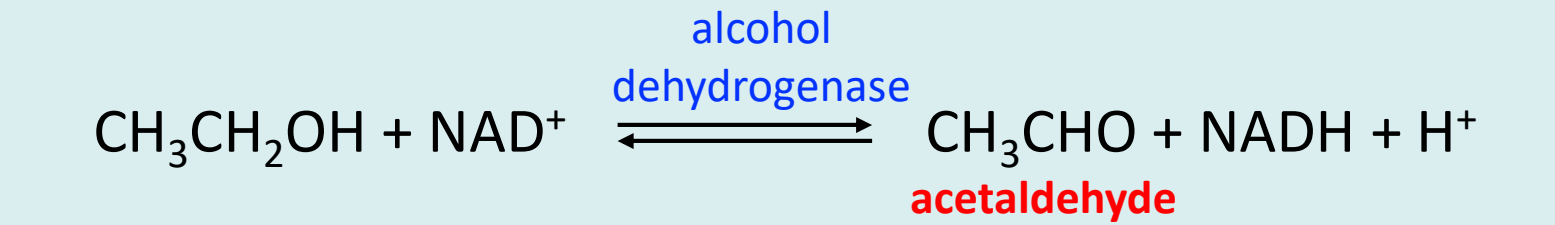
2.Introduce $[E]_T$
$[E] = [E]_T - [ES]$

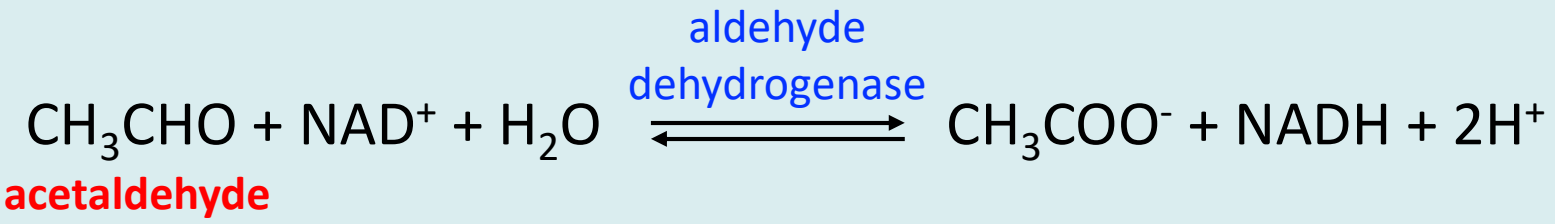
3. Introduce $V_0 = k_2[ES]$

4. Find V_{max} when $[ES] = [E]_T$

Meaning of K_M

The physiological consequence of K_M in alcohol sensitivity





There are two forms of the AD: a low K_M mitochondrial form and a high K_M cytoplasmic form. What happen to the susceptible people?

$$\mathbf{K_M = [E][S]/[ES] = (k_{-1} + k_2)/k_1}$$

Double-Reciprocal (Lineweaver-Burk) Plot

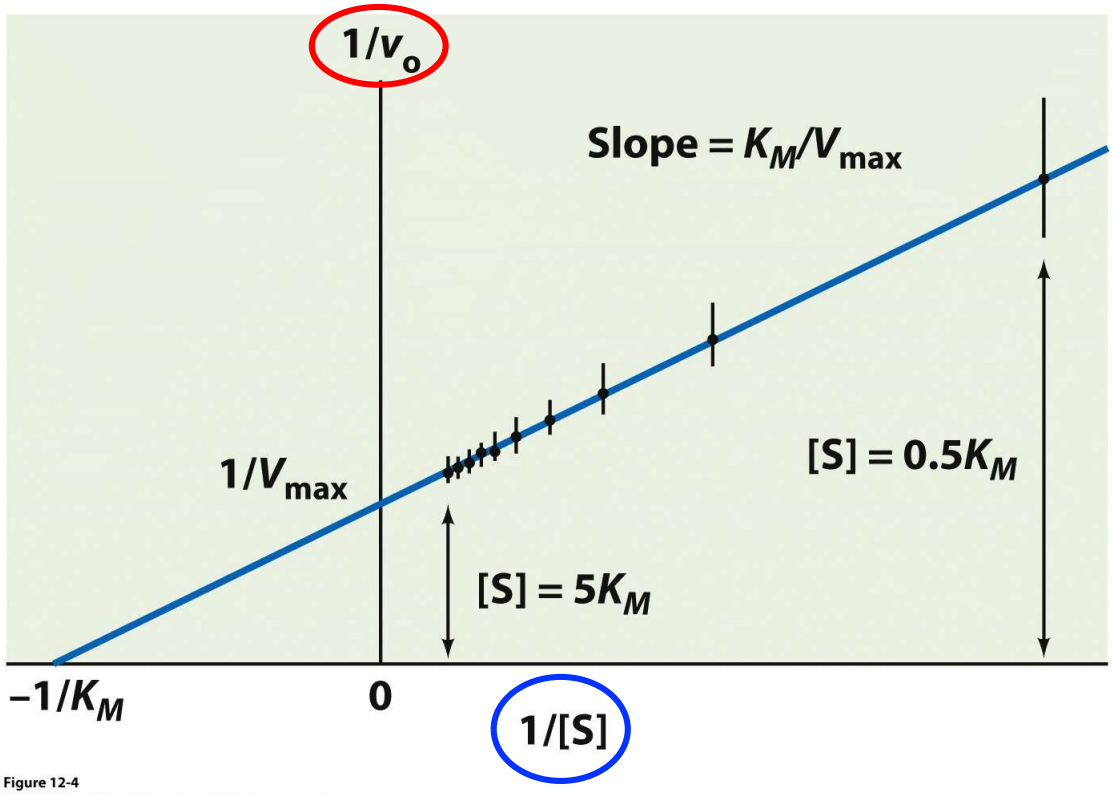


Figure 12-4

double-reciprocal of MM equation

$V_0 = V_{max}\,[S]/[S] + K_M$

→ $1/V_0 = K_M/V_{max} \bullet 1/[S] + 1/V_{max}$

Enzyme Kinetic Parameters

a measure of catalytic efficiency

TABLE 12-1 The Values of K_M, k_{cat}, and k_{cat}/K_M for Some Enzymes and Substrates

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Acetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

Vmax: The maximal rate reveals the **turnover number** of an enzyme which is the number of substrate molecules converted into product by **an** enzyme molecule in a unit time when the enzyme is fully saturated with substrate = k_{cat} (= k_2 when the V is maximum)

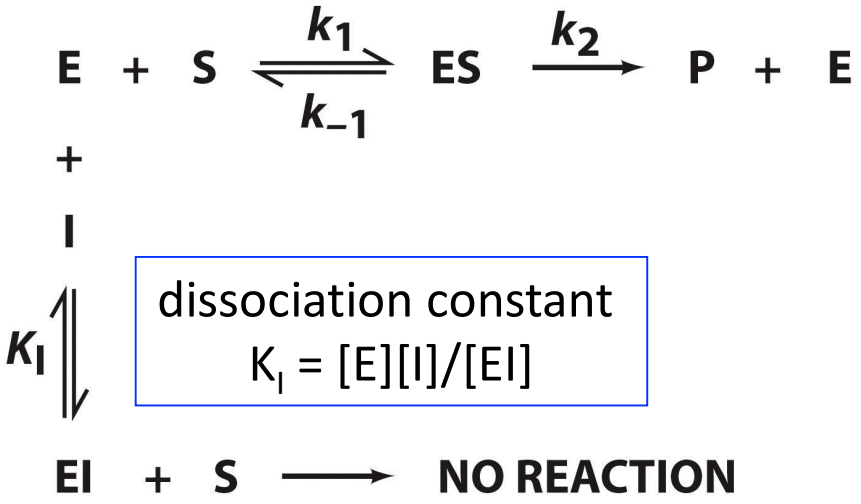
$$V_{max} = k_{cat}[E]_T$$

Q: a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when the enzyme is fully saturated with substrate. What is the k_{cat}?

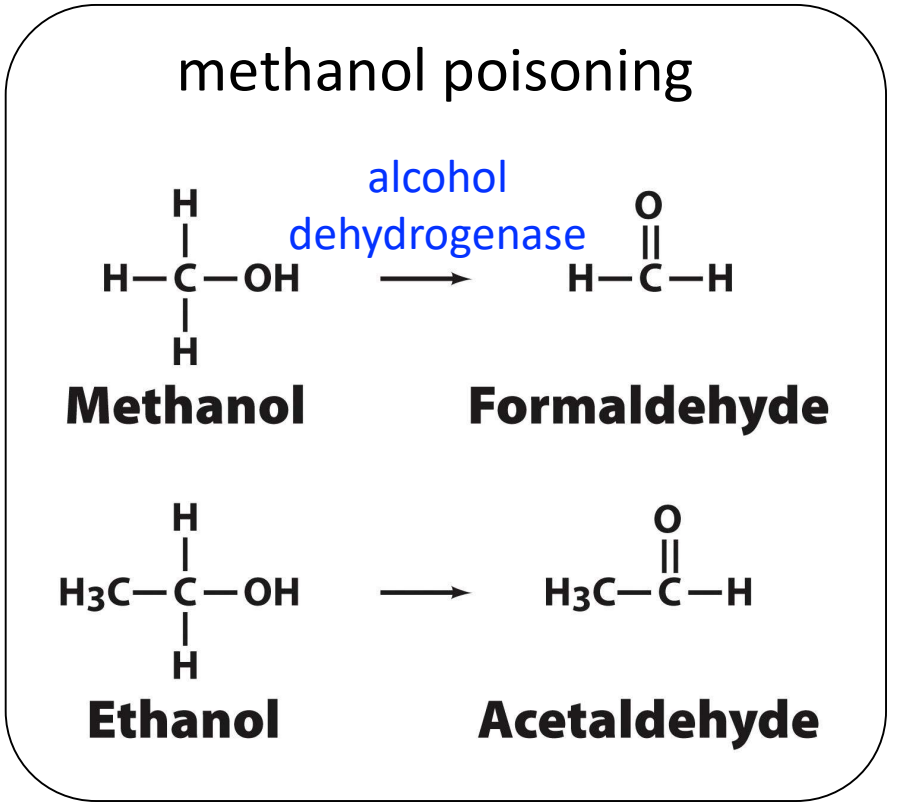
Competitive Enzyme Inhibition

- Inhibitors mimic the substrate: compete for the same site.
- The inhibition can be overcome by adding more substrate.
- Often act as drugs: e.g. Statins

$$K_M = [E][S]/[ES] = (k_{-1} + k_2)/k_1$$



methanol poisoning



Competitive Enzyme Inhibition

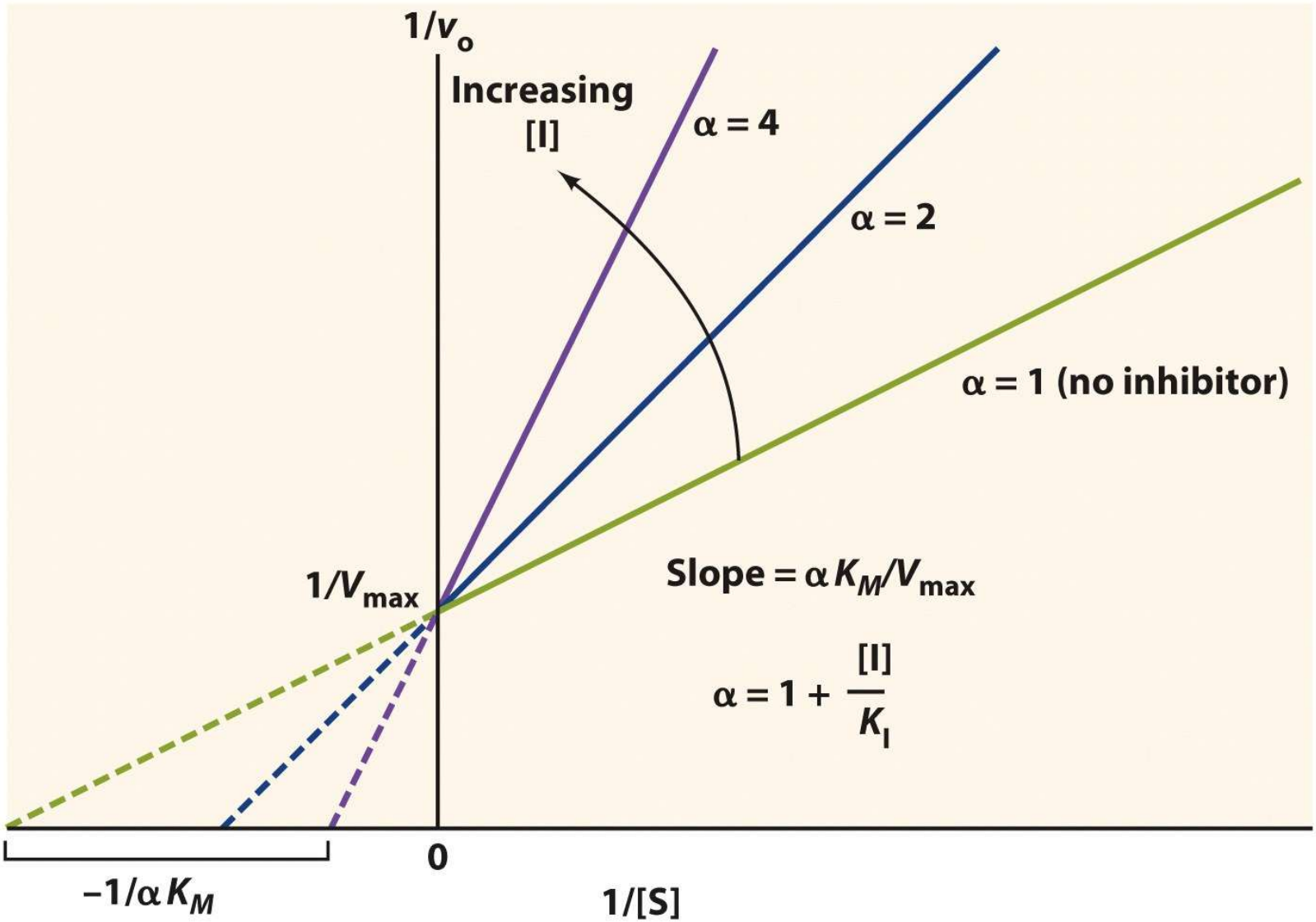
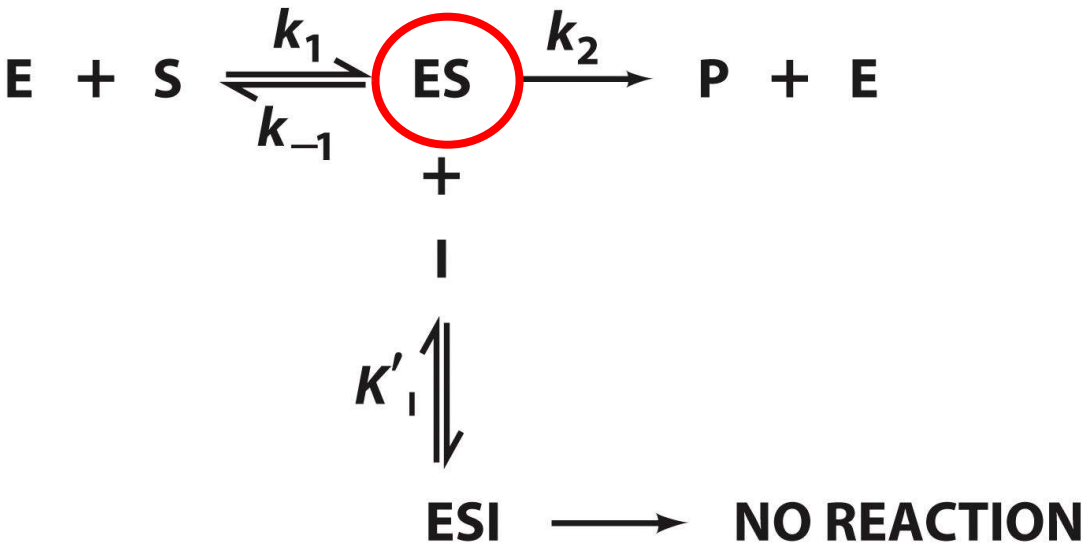


Figure 12-8

Uncompetitive Enzyme Inhibition



ESI: Enzyme-Substrate-Inhibitor complex: a certain portion of ESI always exists, thus decreases V_{max} – as if some enzymes are kidnapped in ES form!

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

$$K_M = (k_{-1} + k_2)/k_1$$

Uncompetitive Enzyme Inhibition

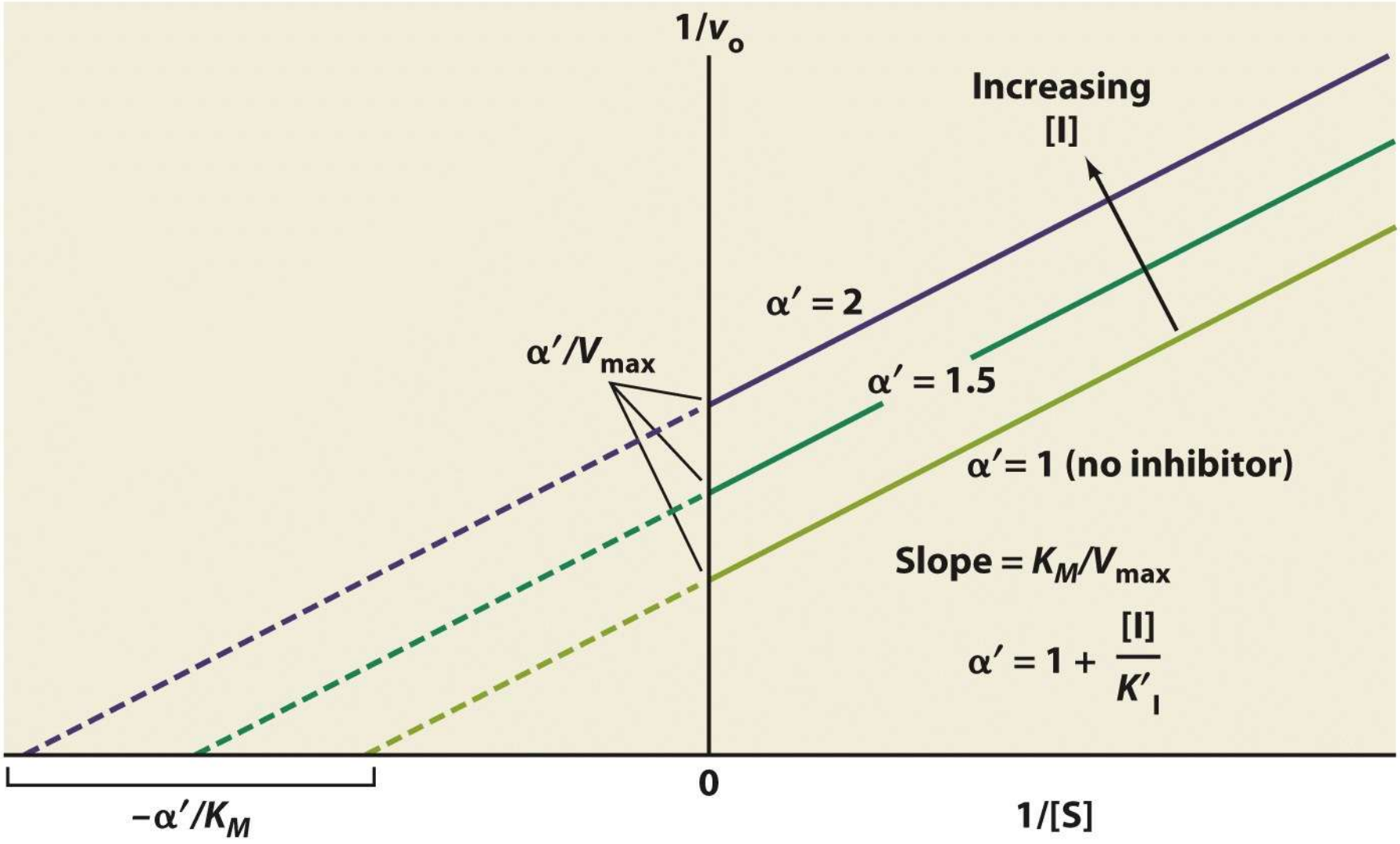
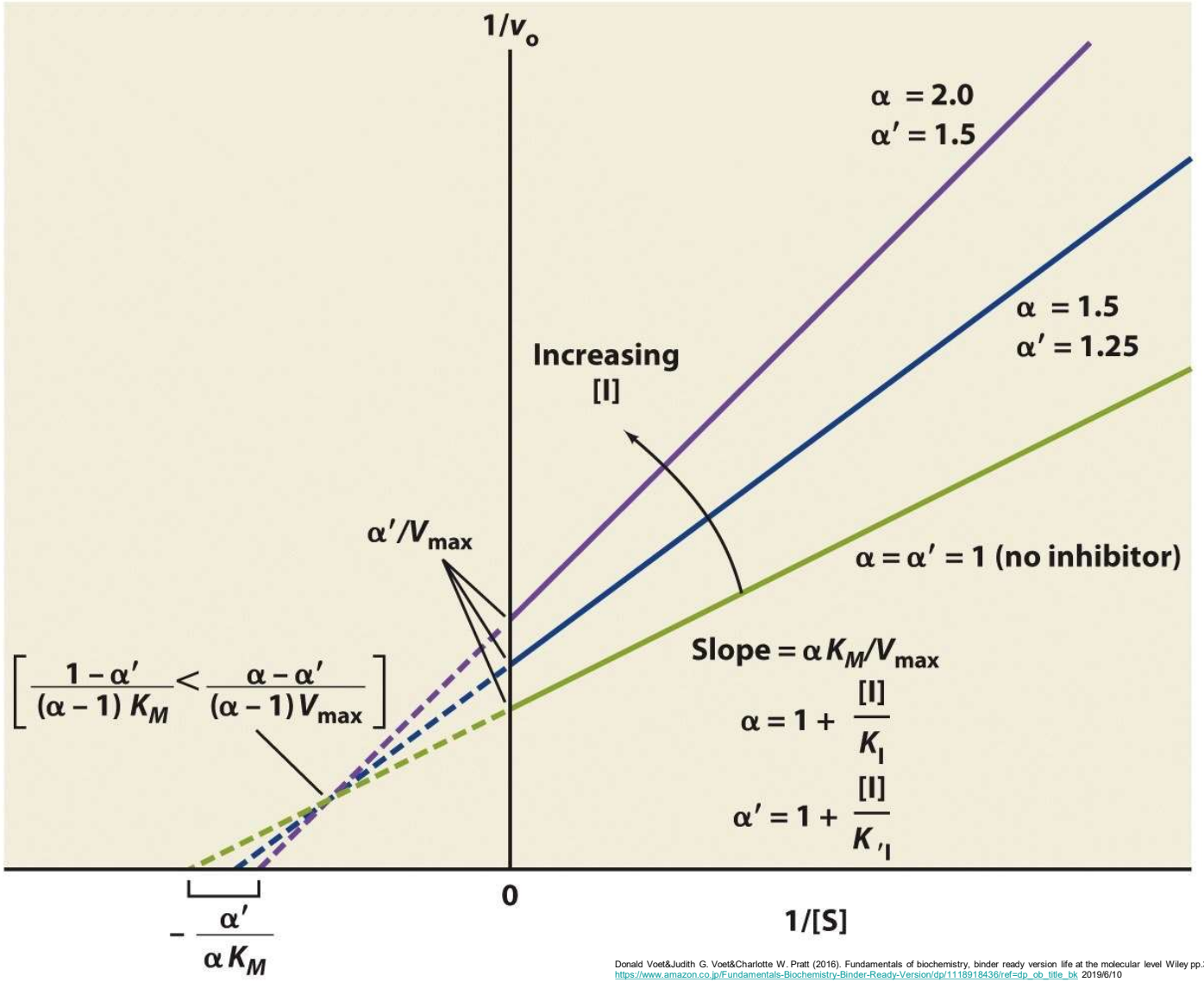


Figure 12-9

Mixed and Noncompetitive Enzyme Inhibition



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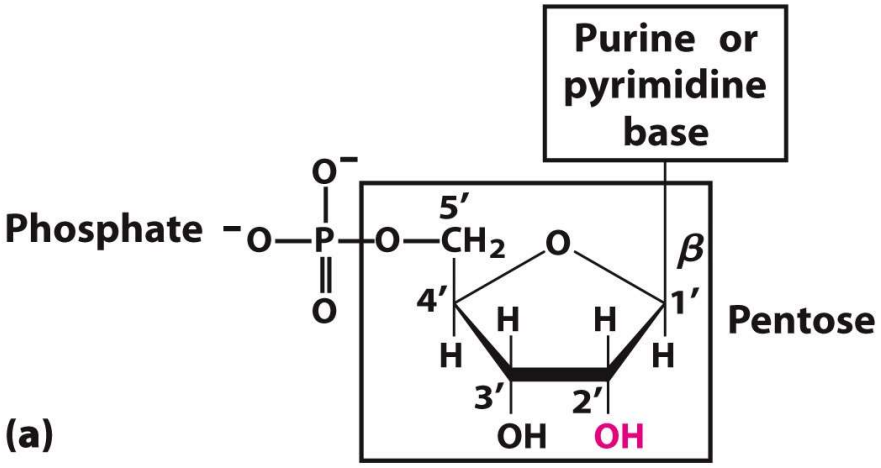
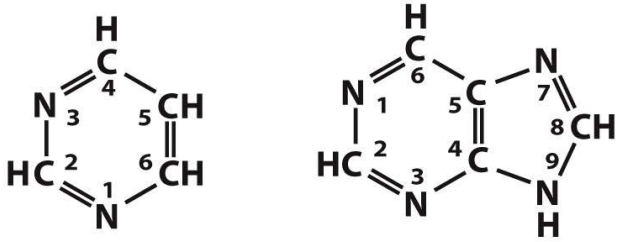
https://www.amazon.co.jp/Fundamentals-Biochemistry-Binder-Ready-Version/dp/1118918436/ref=dp_ob_title_bk 2019/6/10

Nucleotides and Nucleosides

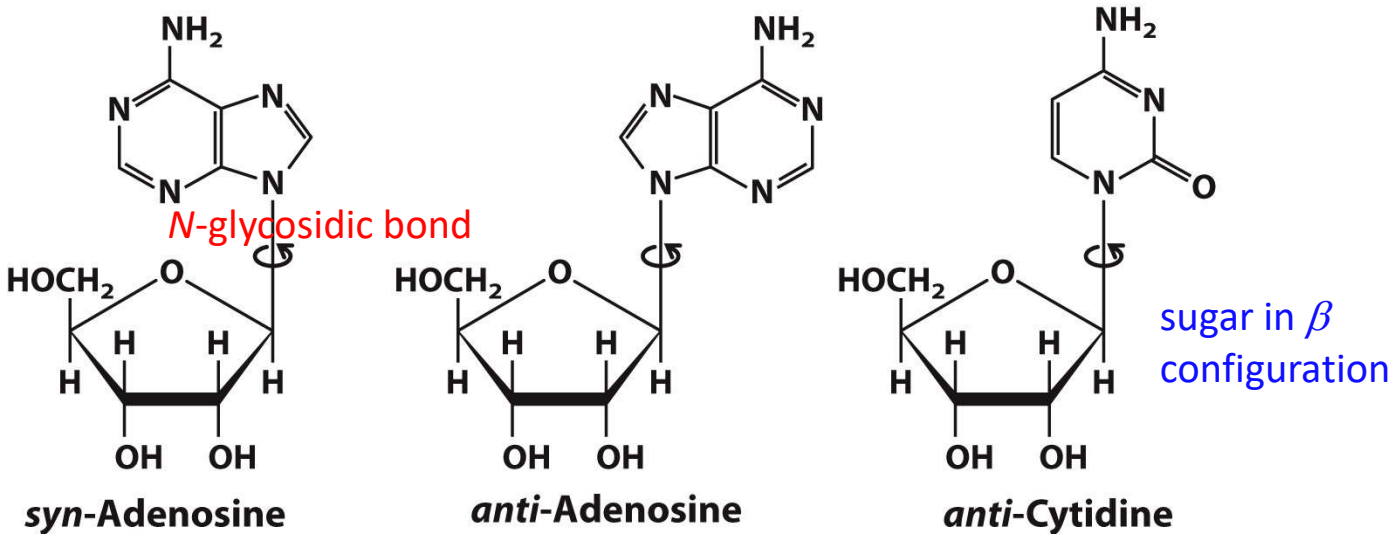
- Nucleotide =
  - nitrogeneous base
  - pentose
  - phosphate
- Nucleoside =
  - nitrogeneous base
  - pentose
- Carbon AND nitrogen atoms on the nitrogenous base are numbered in cyclic format.
- Carbons of the pentose are designated N' to alleviate confusion.





Conformation around *N*-Glycosidic Bond

- Relatively free rotation can occur around the *N*-glycosidic bond in free nucleotides.



- Angle near 0° corresponds to *syn* conformation.
- Angle near 180° corresponds to *anti* conformation.
- Anticonformation is found in normal B-DNA.

Polynucleotides

- Covalent bonds are formed via phosphodiester linkages.
  - negatively charged backbone
- DNA backbone is fairly stable.
  - DNA from mammoths?
  - Hydrolysis accelerated by enzymes (DNAse)
- RNA backbone is unstable.
  - In water, RNA lasts for a few years.
  - In cells, mRNA is degraded in a few hours.
- Linear polymers
  - no branching or cross-links
- Directionality
  - The 5’ end is different from the 3’ end.
  - We read the sequence from 5’ to 3’.

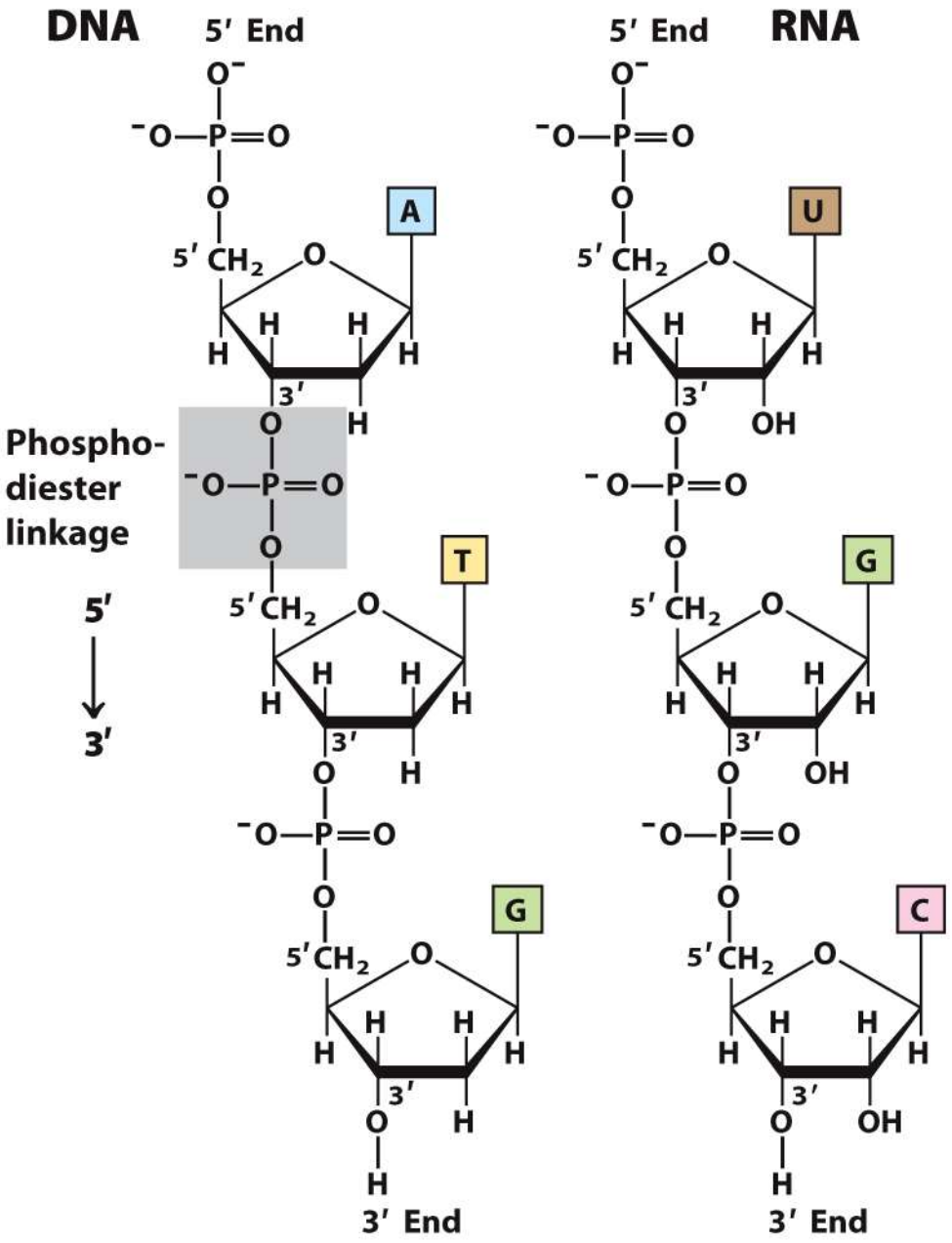


Figure 8-7
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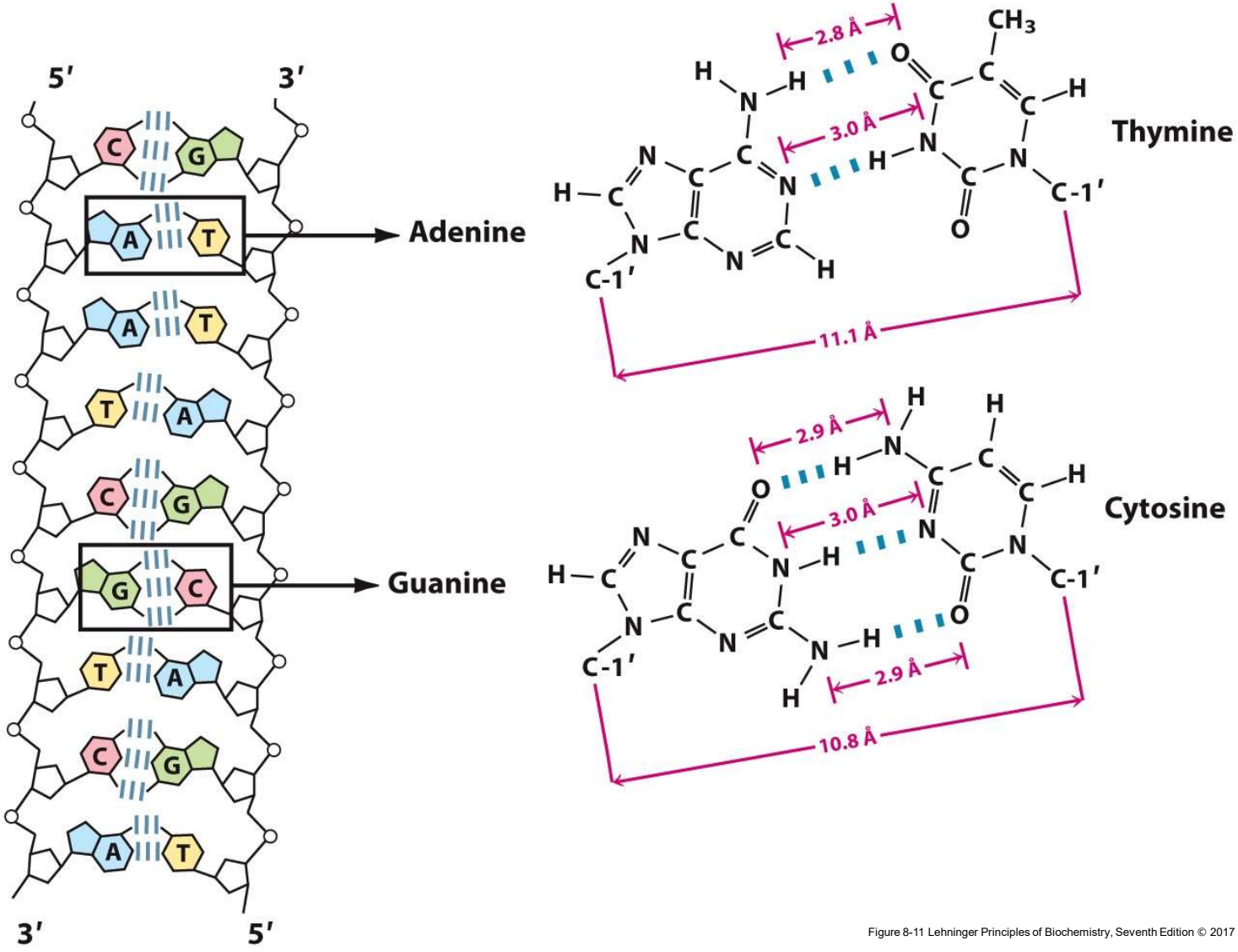
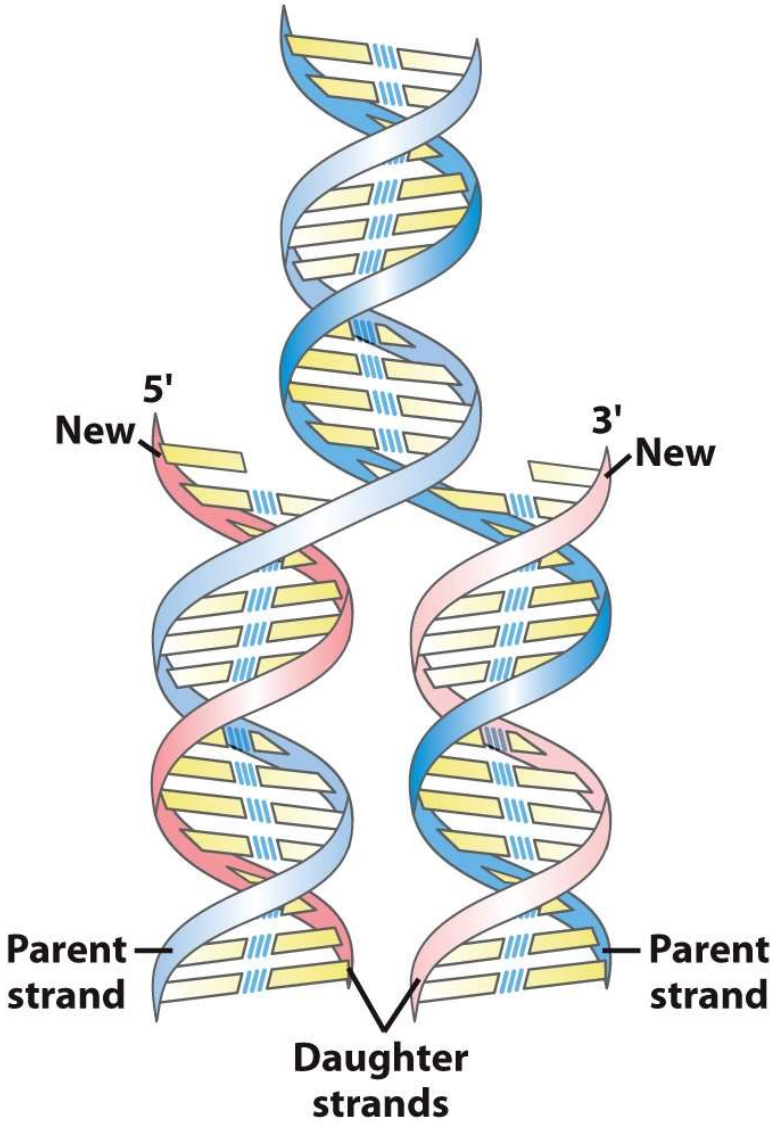
AT and GC Base Pairs



Two chains are complementary and run antiparallel (5’ to 3’).

Replication of Genetic Code



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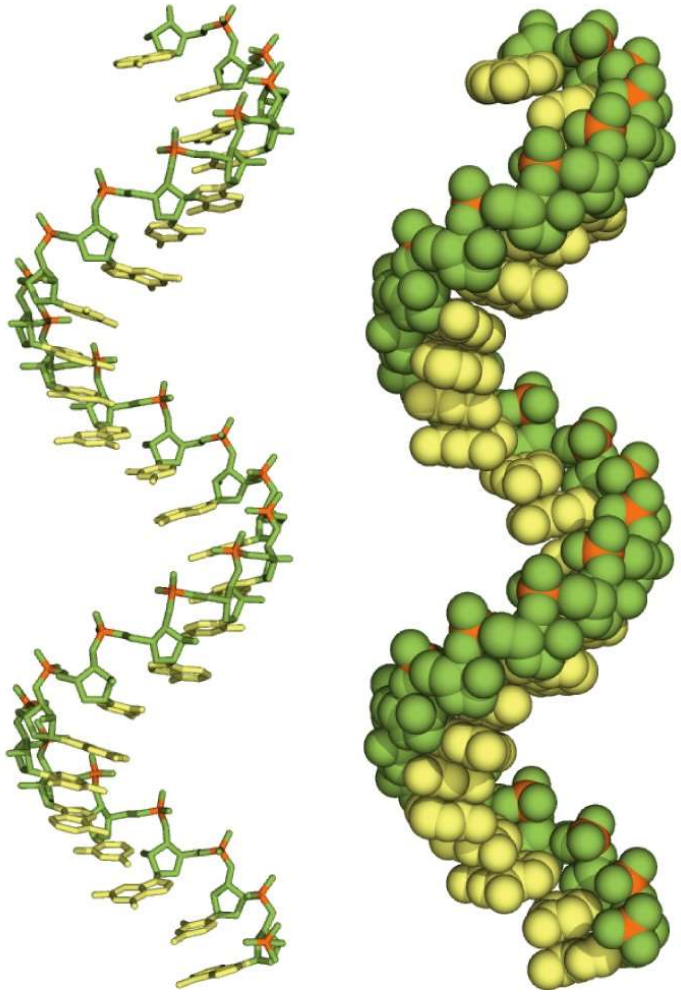
- Strand separation occurs first.
- Each strand serves as a template for the synthesis of a new strand.
- Synthesis is catalyzed by enzymes known as DNA polymerases.
- A newly made DNA molecule has one daughter strand and one parent strand.

Messenger RNA: Code Carrier for the Sequence of Proteins

- Is synthesized using DNA template and generally occurs as a single strand
- Contains ribose instead of deoxyribose
- Contains uracil instead of thymine
- One mRNA may code for more than one protein
- Together with transfer RNA (tRNA), transfers genetic information from DNA to proteins



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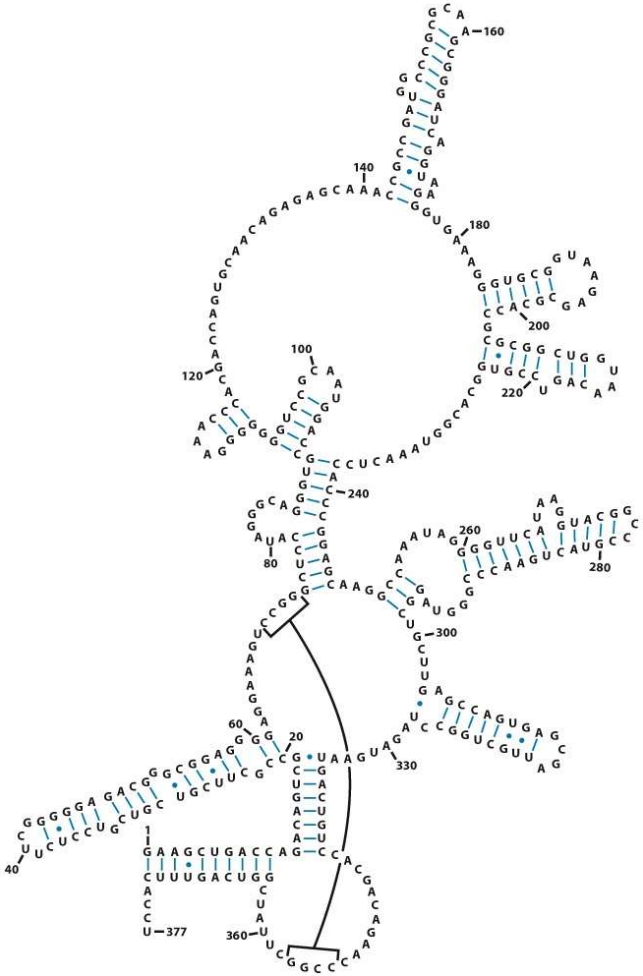
Complex Structures of RNA

M1 RNA component of the enzyme RNase P of *E. coli*



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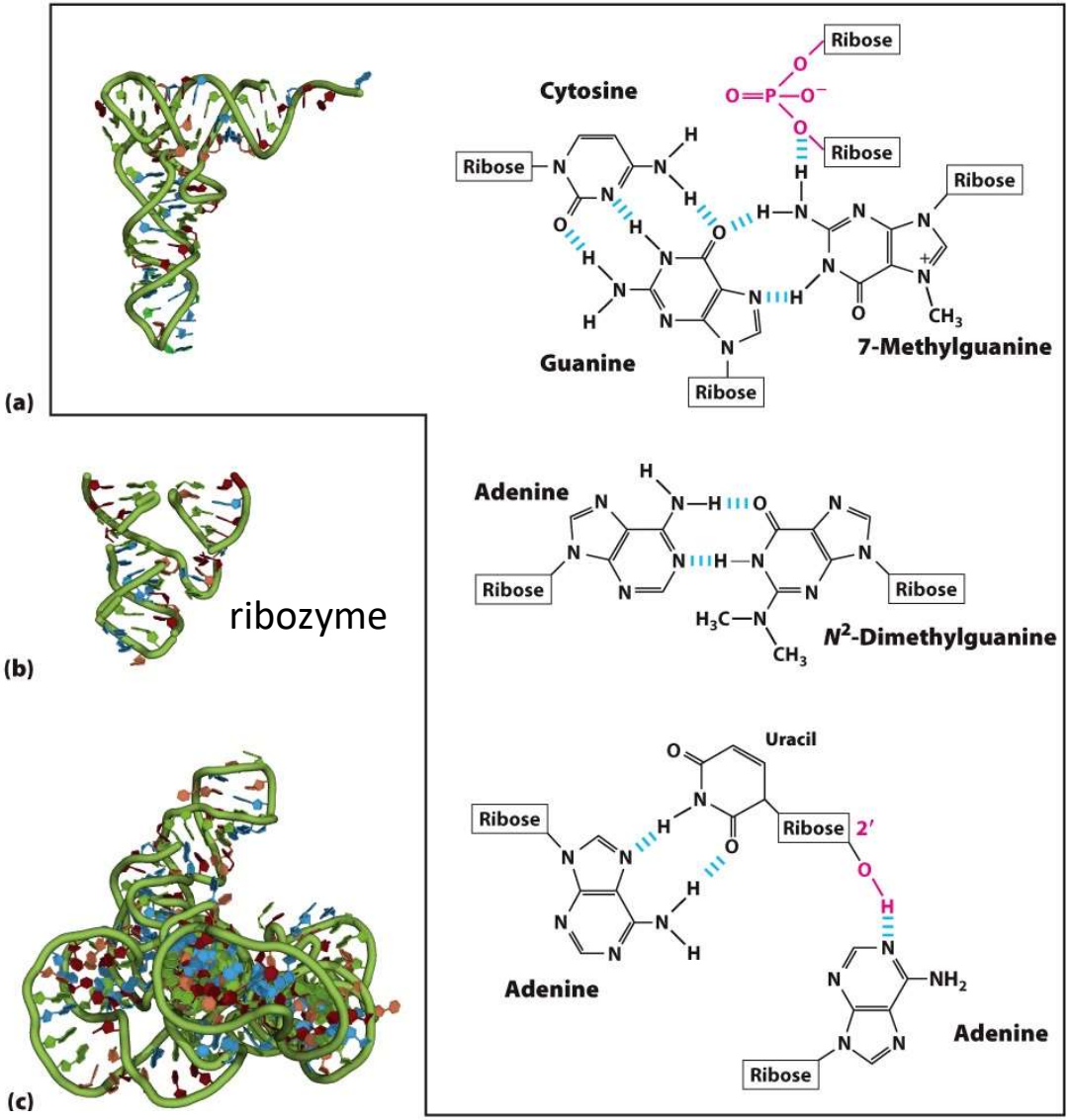
phenylalanine tRNA



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DNA Denaturation

- Covalent bonds remain intact.
  - Genetic code remains intact.
- Hydrogen bonds are broken.
  - Two strands separate.
- Base stacking is lost
  - UV absorbance increases.
- Denaturation can be induced by high temperature (melting), or change in pH.
- Denaturation may be reversible: annealing.

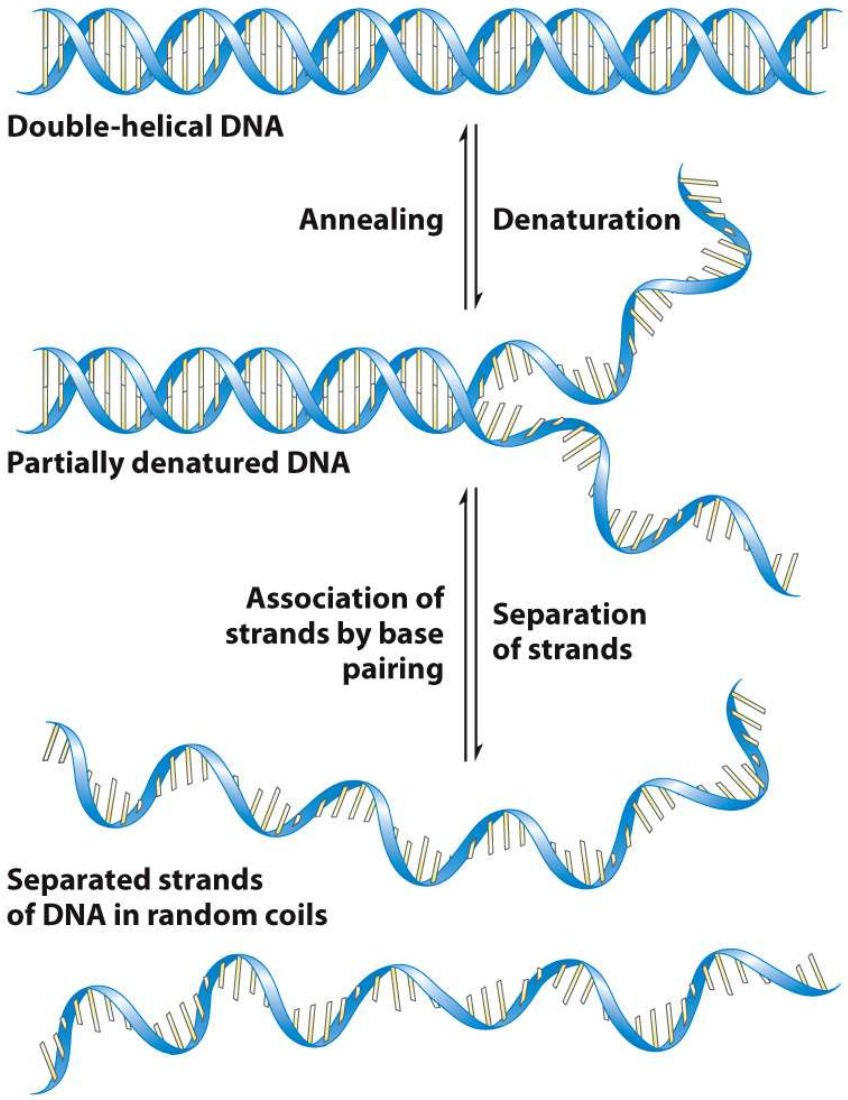

Figure 8-26
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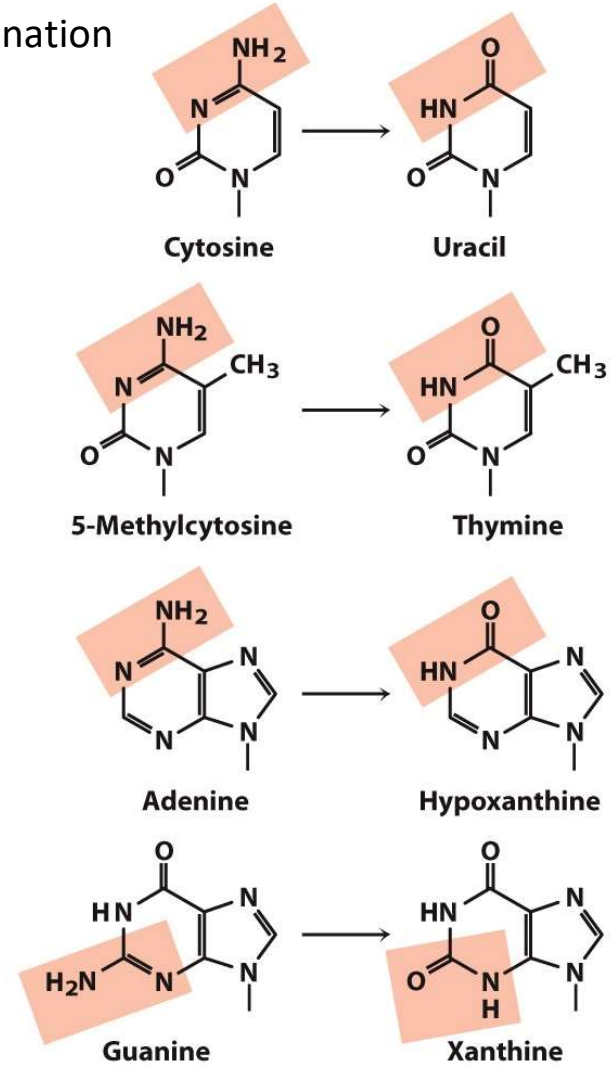
Spontaneous Mutagenesis

100 C → U events/day

Deamination



10,000 purines lost/day

Depurination

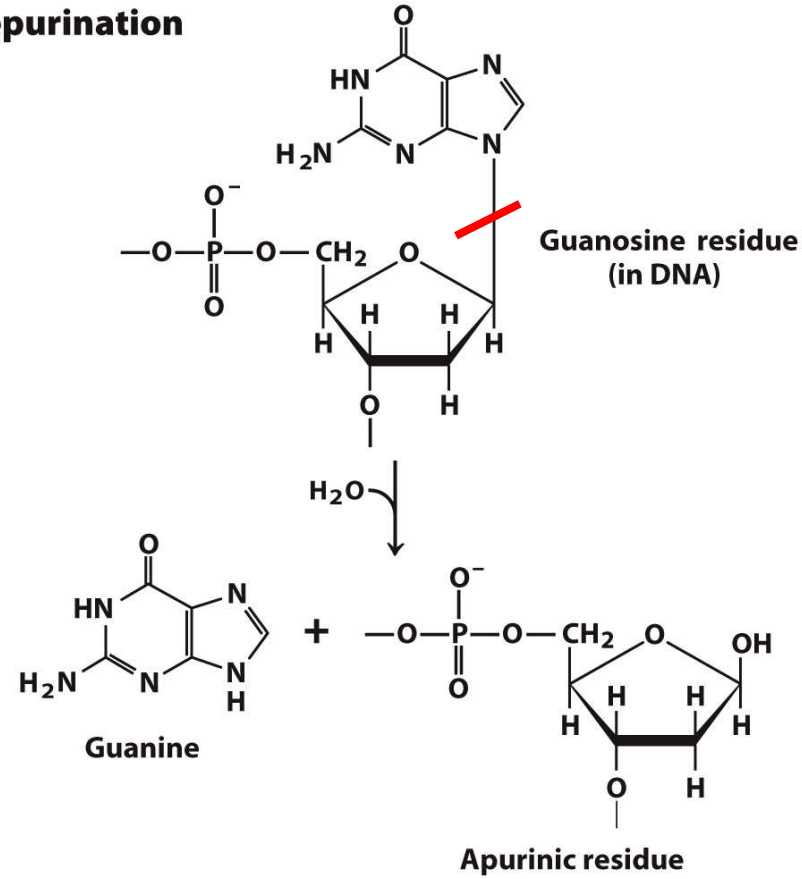


Figure 8-29 (a) (b) Lehninger Principles of Biochemistry, Seventh Edition © 2017 W.H.Freeman and Company

Oxidative & Chemical Mutagenesis

- Oxidative damage: hydroxylation of guanine
  - mitochondrial DNA is most susceptible
- Chemical alkylation: methylation of guanine
- Cells have mechanisms to correct most of these modifications.

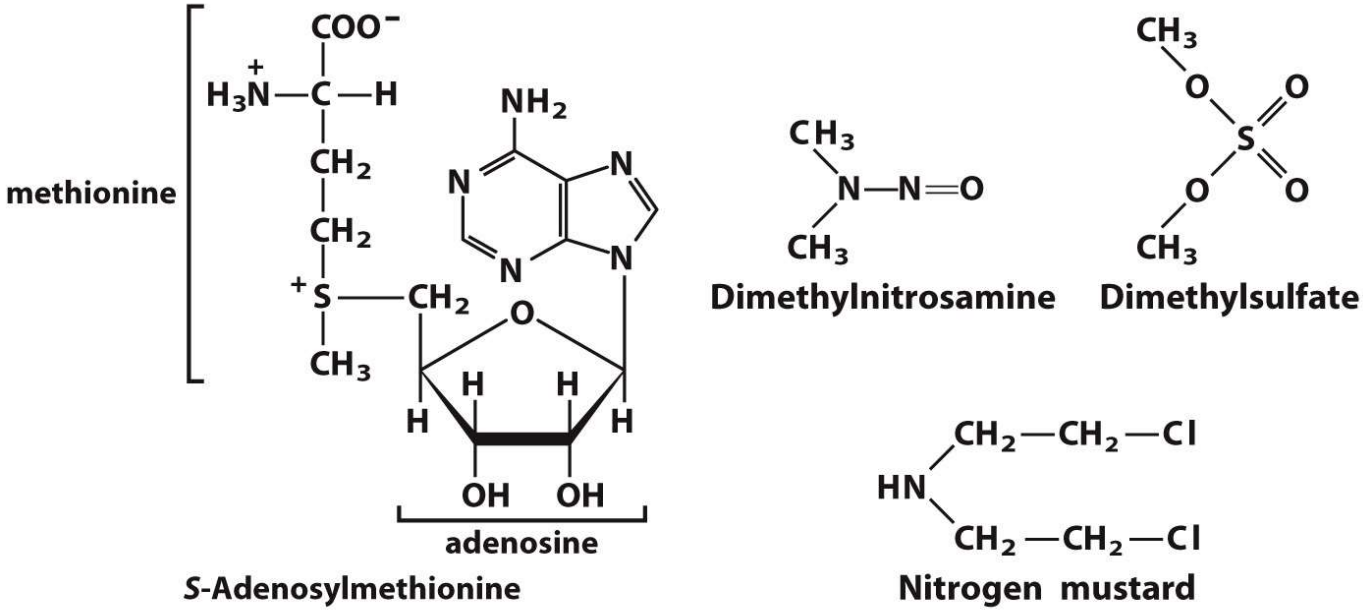


Figure 8-31(b) Lehninger Principles of Biochemistry, Seventh Edition © 2017 W.H.Freeman and Company

Radiation-Induced Mutagenesis

- UV light induces dimerization of pyrimidines; this may be the main mechanism for skin cancers.
- Ionizing radiation (x rays and γ rays) causes ring opening and strand breaking. These are difficult to fix.
- Cells can repair some of these modifications, but others cause mutations. Accumulation of mutations is linked to aging and carcinogenesis.

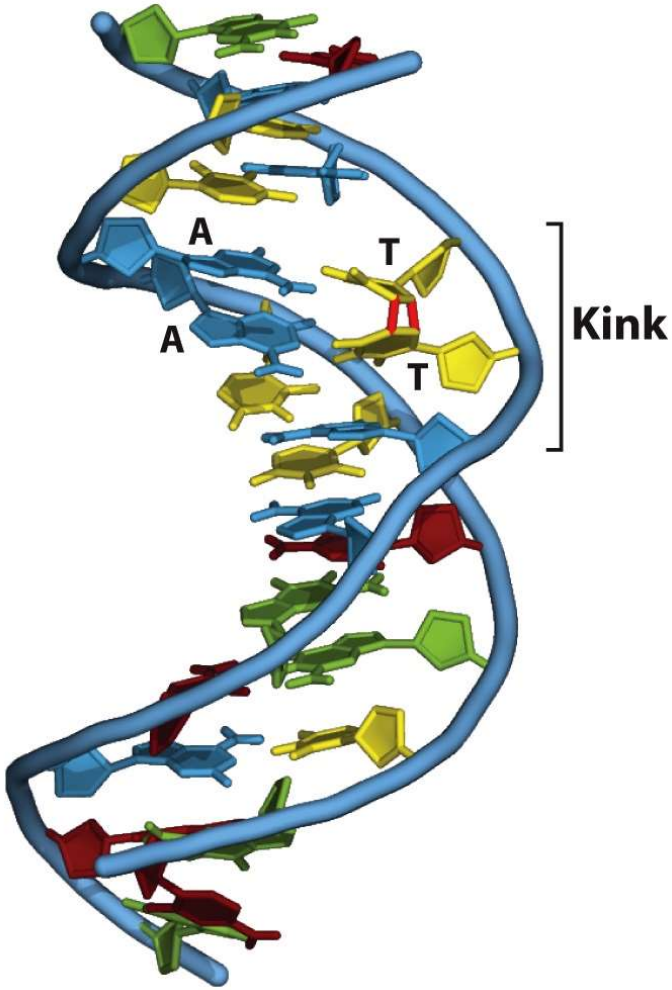


Figure 8-30(b) Lehninger Principles of Biochemistry, Seventh Edition © 2017 W.H.Freeman and Company

Formation of pyrimidine dimers by UV

Tools to Study Genes

Tools	Purposes
gene editing	to modify
Recombinant DNA	to clone or amplify
RNA interference	to inhibit
PCR	to amplify or detect
DNA sequencing	to identify