Enzyme Fundamentals: Introduction, Classification, Substrate Specificity & Regulation

CHEM 420 – Principles of Biochemistry
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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

Example of EC Classification: Carboxypeptidase A
EC 3.4.17.1

3: enzyme major class = hydrolase
4: subclass of hydrolase = peptide hydrolase
17: sub-subclass = metallocarboxypeptidase (carboxypeptidase A has a Zn^{2+} ion bound in its active site)
1: arbitrarily assigned serial number in its sub-subclass

Some terminology:

Cofactors: non-protein components required by an enzyme for activity; can be inorganic (ions) or organic

An organic cofactor = **coenzyme**

Cofactors/coenzymes can be strongly bound by the protein or reversibly associate with the protein.
Strongly bound cofactor = **prosthetic group**

holoenzyme = enzyme + all cofactors required for activity

apoenzyme = enzyme (protein) only

Coenzymes and their vitamin precursors

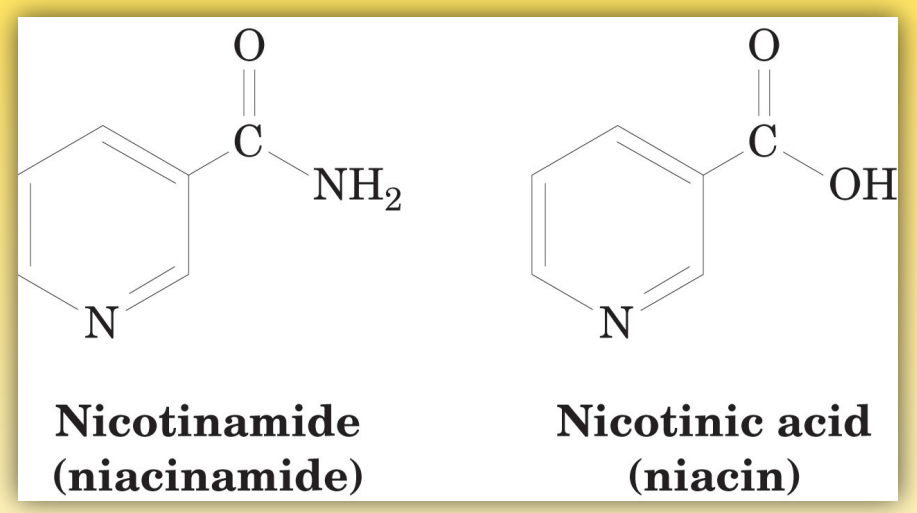
Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	*a*
Cobalamin (B_{12})	Cobalamin (B_{12}) coenzymes	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellagra
Pantothenate	Coenzyme A	*a*
Pyridoxine (B_6)	Pyridoxal phosphate	*a*
Riboflavin (B_2)	Flavin coenzymes	*a*
Thiamine (B_1)	Thiamine pyrophosphate	Beriberi

[a]No specific name; deficiency in humans is rare or unobserved.

Common coenzymes and the enzyme reactions they mediate

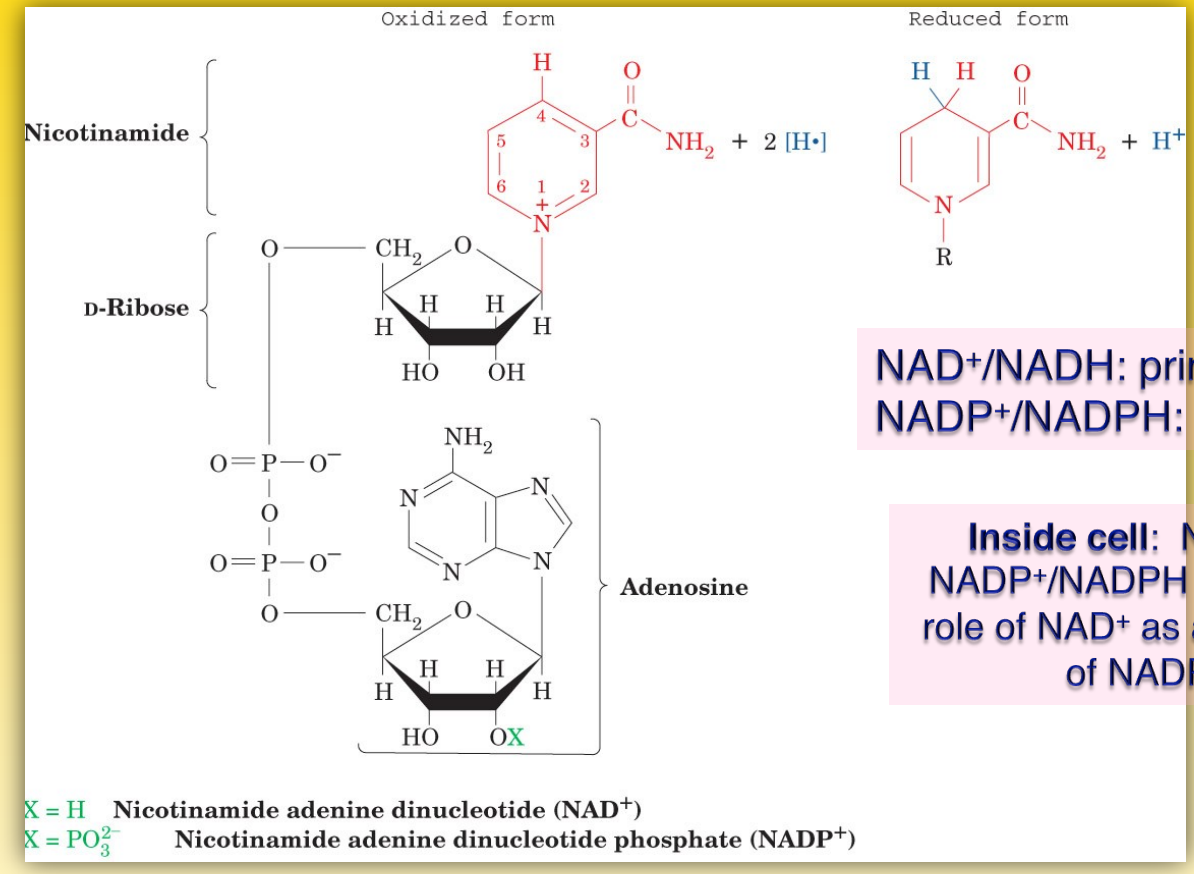
Coenzyme	Reaction Mediated
Biotin	Carboxylation
Cobalamin (B_{12}) coenzymes	Alkylation
Coenzyme A	Acyl transfer
Flavin coenzymes	Oxidation–reduction
Lipoic acid	Acyl transfer
Nicotinamide coenzymes	Oxidation–reduction
Pyridoxal phosphate	Amino group transfer
Tetrahydrofolate	One-carbon group transfer
Thiamine pyrophosphate	Aldehyde transfer

Structures of vitamins: nicotinamide and nicotinic acid



Nicotinamide (niacinamide)

Nicotinic acid (niacin)



NAD+/NADH: primarily catabolic, mitochondrial
NADP+/NADPH: primarily anabolic, cytosolic

Inside cell: NAD+/NADH ratio is high, and NADP+/NADPH ratio is low; consistent with the role of NAD+ as an oxidizing agent, and the role of NADPH as a reducing agent

Structures and reactions of coenzymes: nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+): 2-electron redox reactions

In a 2-electron, 2-proton redox reaction, NAD^+ ($NADP^+$) accommodates two electrons and one proton during reduction of the nicotinamide ring; the second proton is released into solution.

In its reduced form, the C4 carbon of the nicotinamide ring is prochiral. Either the pro-*R* or pro-*S* site is involved in the redox reaction, depending on the dehydrogenase. This selectivity can be determined by conducting reactions with deuterated substrates and/or deuterated NADH.

Common coenzymes and the reactions they mediate

Coenzyme	Reaction Mediated
Biotin	Carboxylation
Cobalamin (B_{12}) coenzymes	Alkylation
Coenzyme A	Acyl transfer
Flavin coenzymes	Oxidation–reduction
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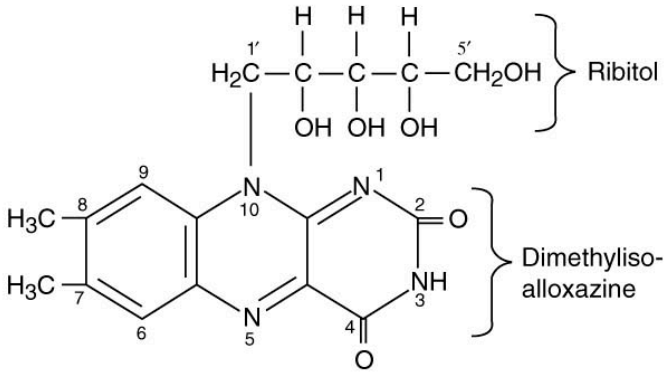
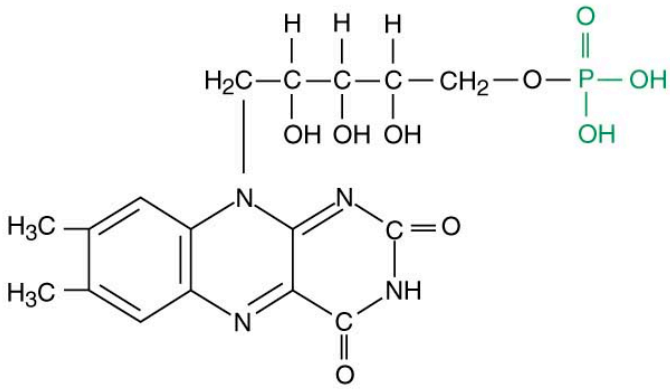


Riboflavin

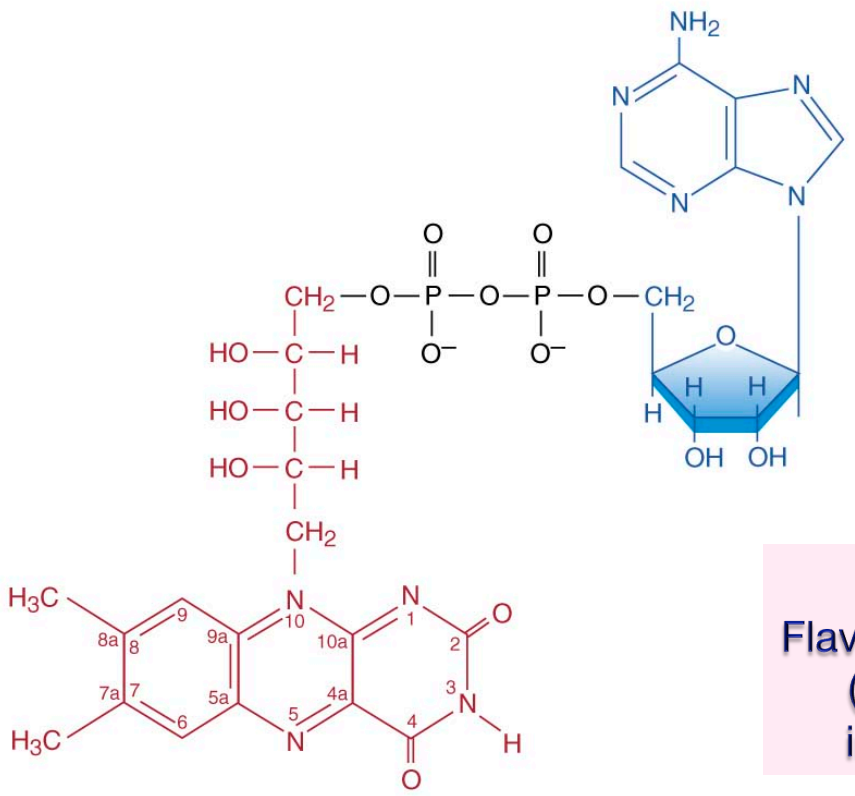


Flavin mononucleotide (FMN)

Figure 10.32. Riboflavin and flavin mononucleotide.

Vitamin B_2

Coenzyme:
Flavin mononucleotide (**FMN**) (oxidized form); also exists in its reduced form, **$FMNH_2$**

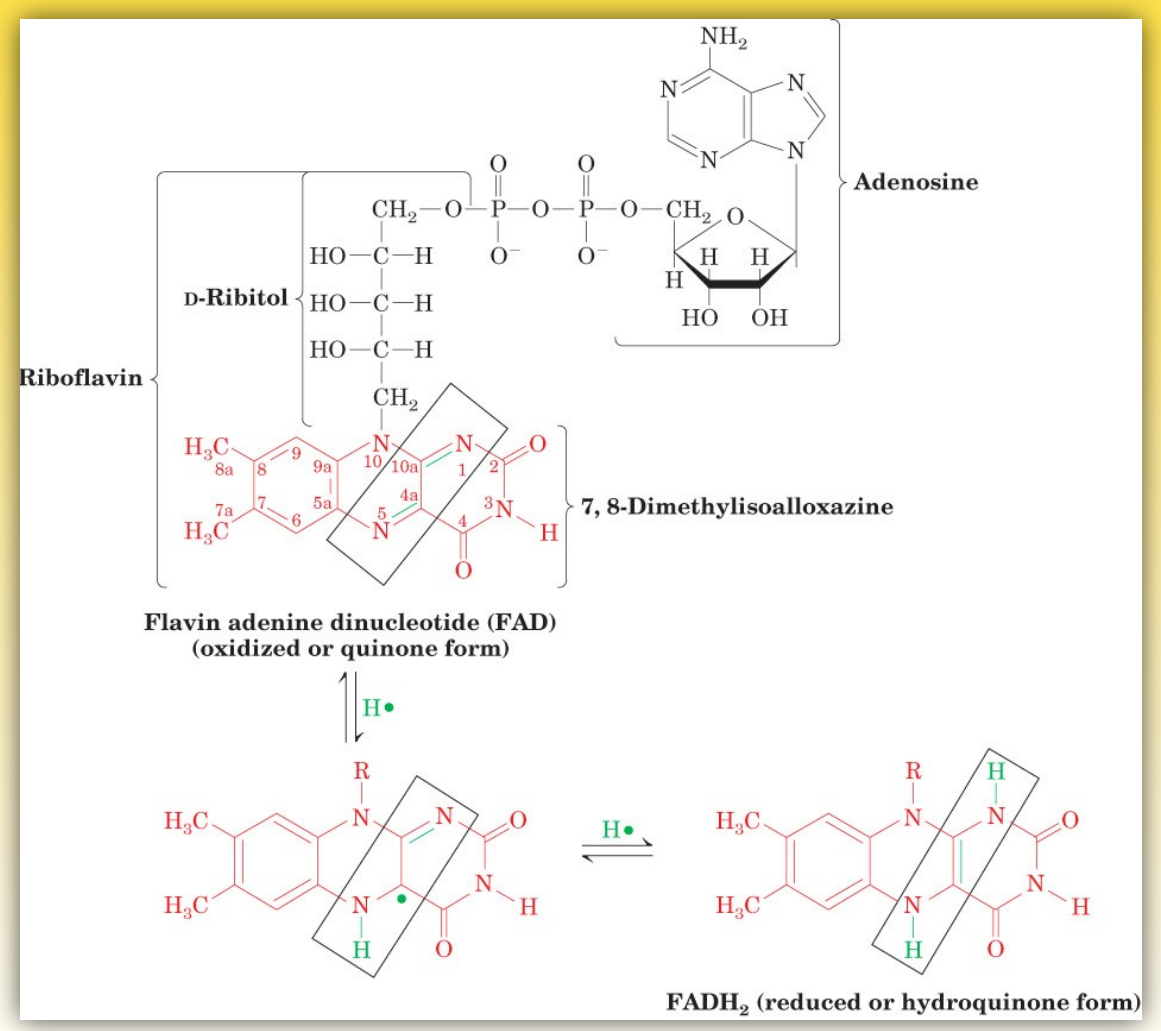


Flavin adenine dinucleotide (FAD)

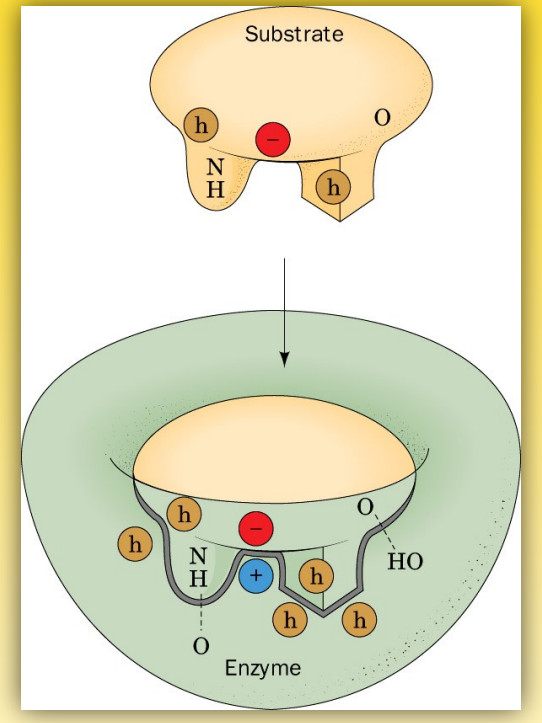
Figure 10.33. Flavin adenine dinucleotide (FAD).

Coenzyme:
Flavin adenine dinucleotide (**FAD**) (oxidized form); also exists in its reduced form, **$FADH_2$**

The molecular formula and reactions of the coenzyme, flavin adenine dinucleotide (FAD), showing both $1e^-$ and $2e^-$ reduction products



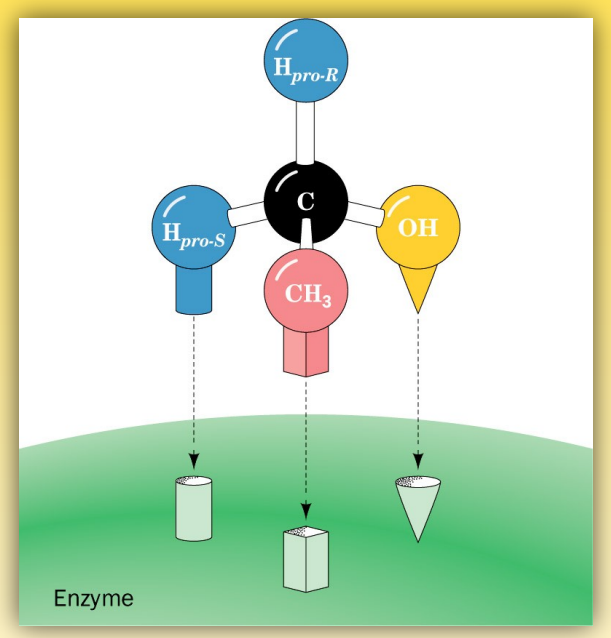
However, unlike the NAD^+ system, the FMN/FAD coenzymes can undergo one electron-one proton reduction to form a stable species (radical).



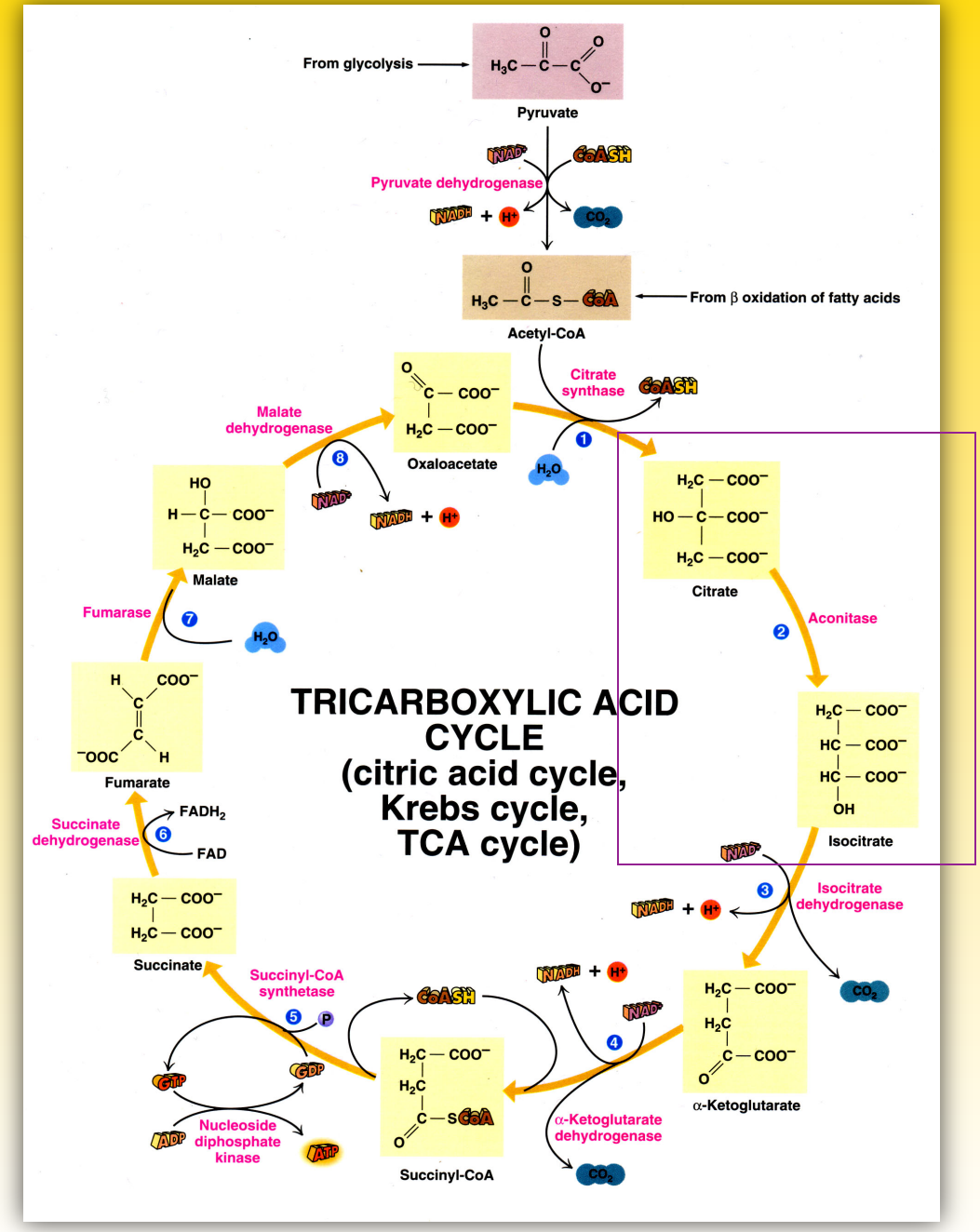
Complementary electrostatic, hydrophobic (h) and H-bonding interactions at the binding interface

An enzyme-substrate complex: geometric and physical complementarity between the enzyme active site and the substrate; also applies to other types of binding, e.g., protein-protein

Dealing with prochiral centers in substrates: ethanol, citric acid



Prochiral differentiation in a chiral protein binding site: distinguishing between the pro-*R* and pro-*S* hydrogens in prochiral ethanol


From glycolysis
Pyruvate
Pyruvate dehydrogenase
NAD+
CoASH
NADH + H+
CO2
Acetyl-CoA
From β oxidation of fatty acids
Citrate synthase
Oxaloacetate
Malate dehydrogenase
Malate
Fumarase
Fumarate
Succinate dehydrogenase
FADH2
FAD
Succinate
Succinyl-CoA synthetase
GTP
GDP
Nucleoside diphosphate kinase
ADP
ATP
Succinyl-CoA
α-Ketoglutarate dehydrogenase
α-Ketoglutarate
Isocitrate dehydrogenase
Isocitrate
Aconitase
Citrate
TRICARBOXYLIC ACID CYCLE
(citric acid cycle, Krebs cycle, TCA cycle)

Protein phosphorylation: a major form of reversible post-translational covalent modification that affects enzyme structure and activity

- Introduction of negative charge: often results in major conformational change
- Introduction of a new binding site

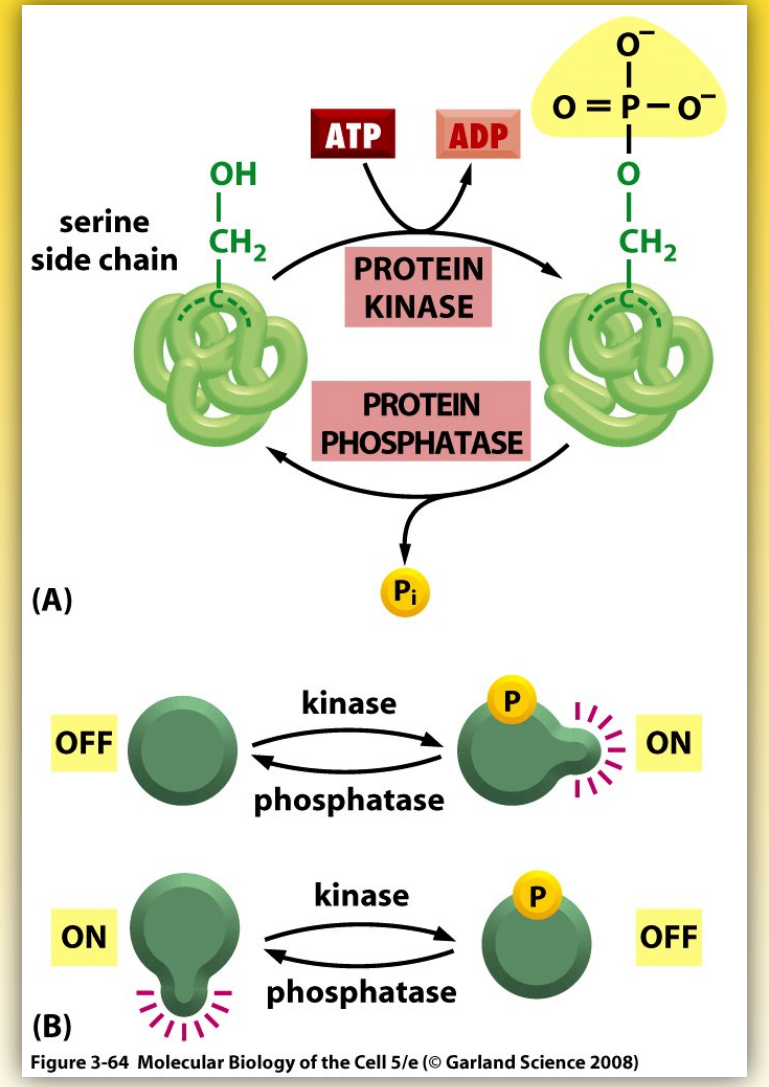


Figure 3-64 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Enzyme-catalyzed phosphorylation and dephosphorylation of a protein (serine phosphomonoester shown) by protein kinases and phosphatases; other phosphorylation sites on proteins include threonine and tyrosine

Correlating the state of enzyme phosphorylation with enzyme activity

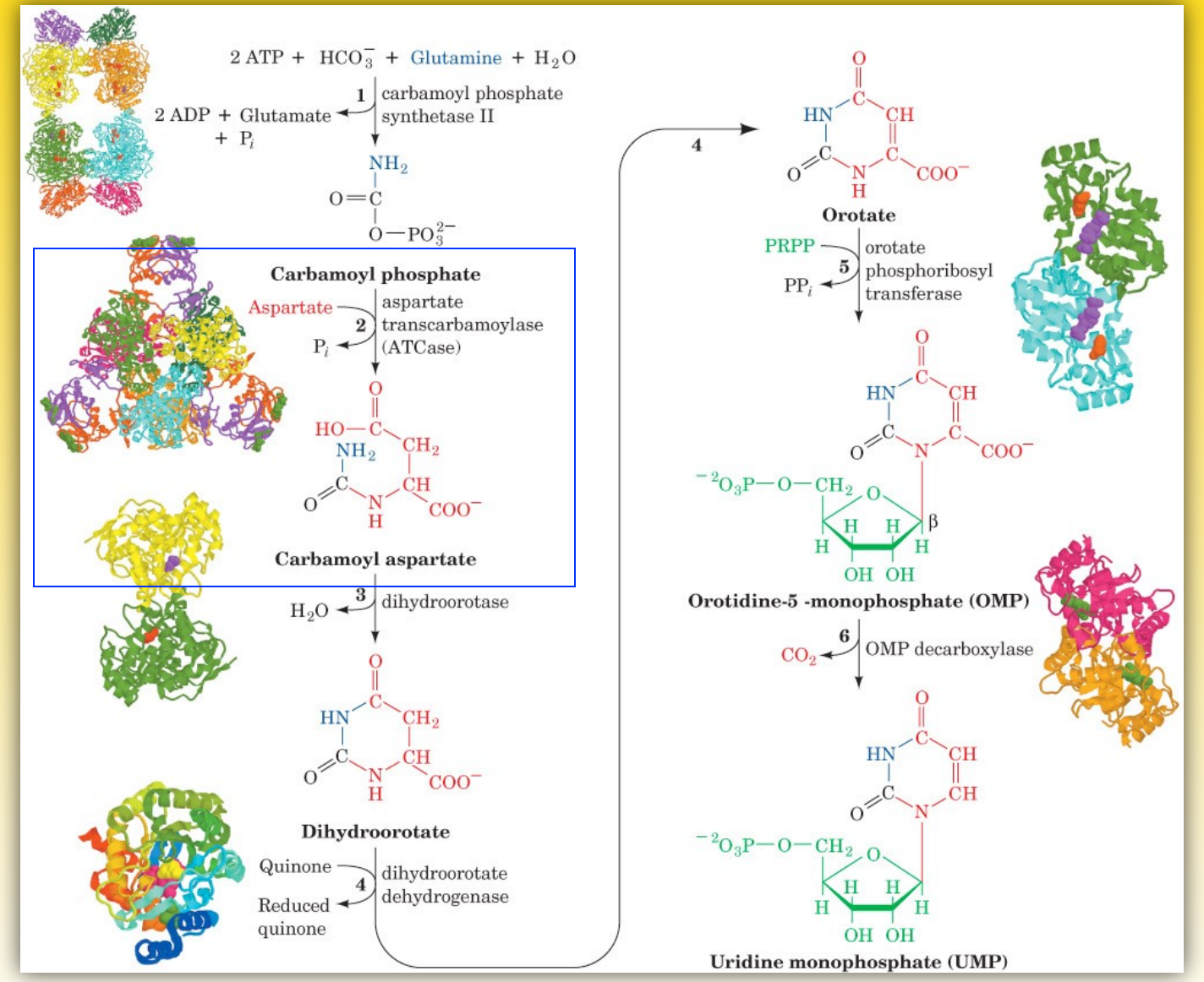
Other types of covalent modification of proteins/enzymes

Table 3–3 Some Molecules Covalently Attached to Proteins Regulate Protein Function

MODIFYING GROUP	SOME PROMINENT FUNCTIONS
Phosphate on Ser, Thr, or Tyr	Drives the assembly of a protein into larger complexes (see Figure 15–19).
Methyl on Lys	Helps to creates histone code in chromatin through forming either mono-, di-, or tri-methyl lysine (see Figure 4–38).
Acetyl on Lys	Helps to creates histone code in chromatin (see Figure 4–38).
Palmityl group on Cys	This fatty acid addition drives protein association with membranes (see Figure 10–20).
N-acetylglucosamine on Ser or Thr	Controls enzyme activity and gene expression in glucose homeostasis.
Ubiquitin on Lys	Monoubiquitin addition regulates the transport of membrane proteins in vesicles (see Figure 13–58).
	A polyubiquitin chain targets a protein for degradation (see Figure 3–79).

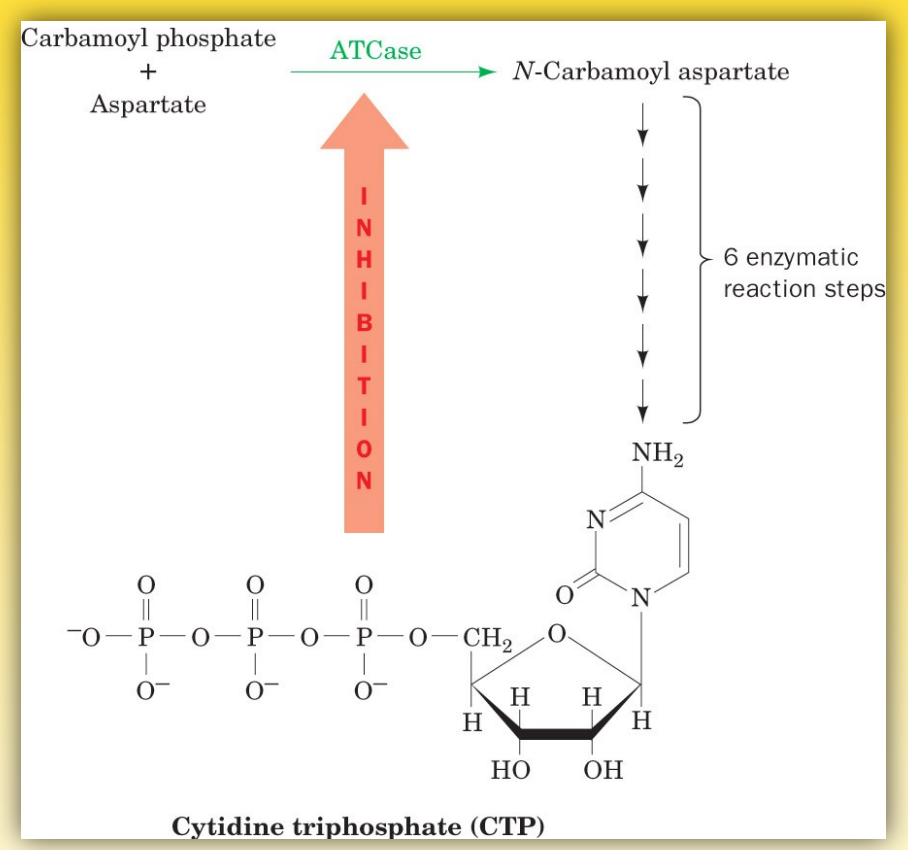
Ubiquitin is a 76 amino acid polypeptide; there are at least 10 other ubiquitin-related proteins, such as SUMO, that modify proteins in similar ways.

Enzyme catalysis



Metabolic pathway for the *de novo* synthesis of UMP; role of ATCase

Allosteric regulation of an enzyme

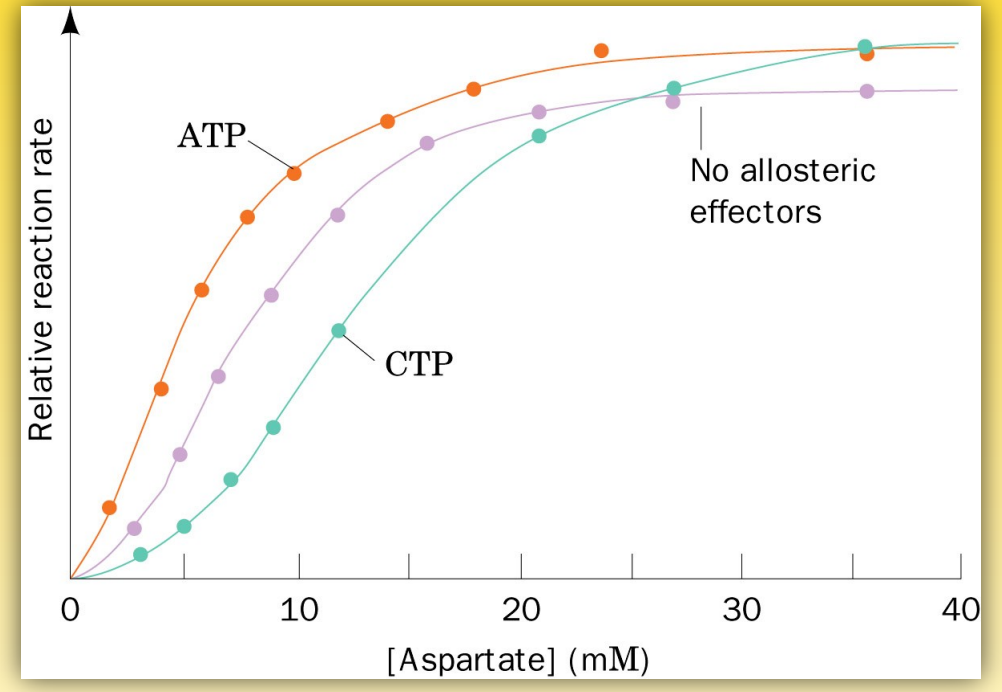


ATCase = aspartate transcarbamoylase (300 kD): two sets of catalytic trimers and three sets of regulatory dimers (total of 12 subunits)

Regulatory enzymes often catalyze the initial reaction of a metabolic pathway.

Schematic representation of the UTP/CTP biosynthesis pathway: end-product inhibition (feedback)

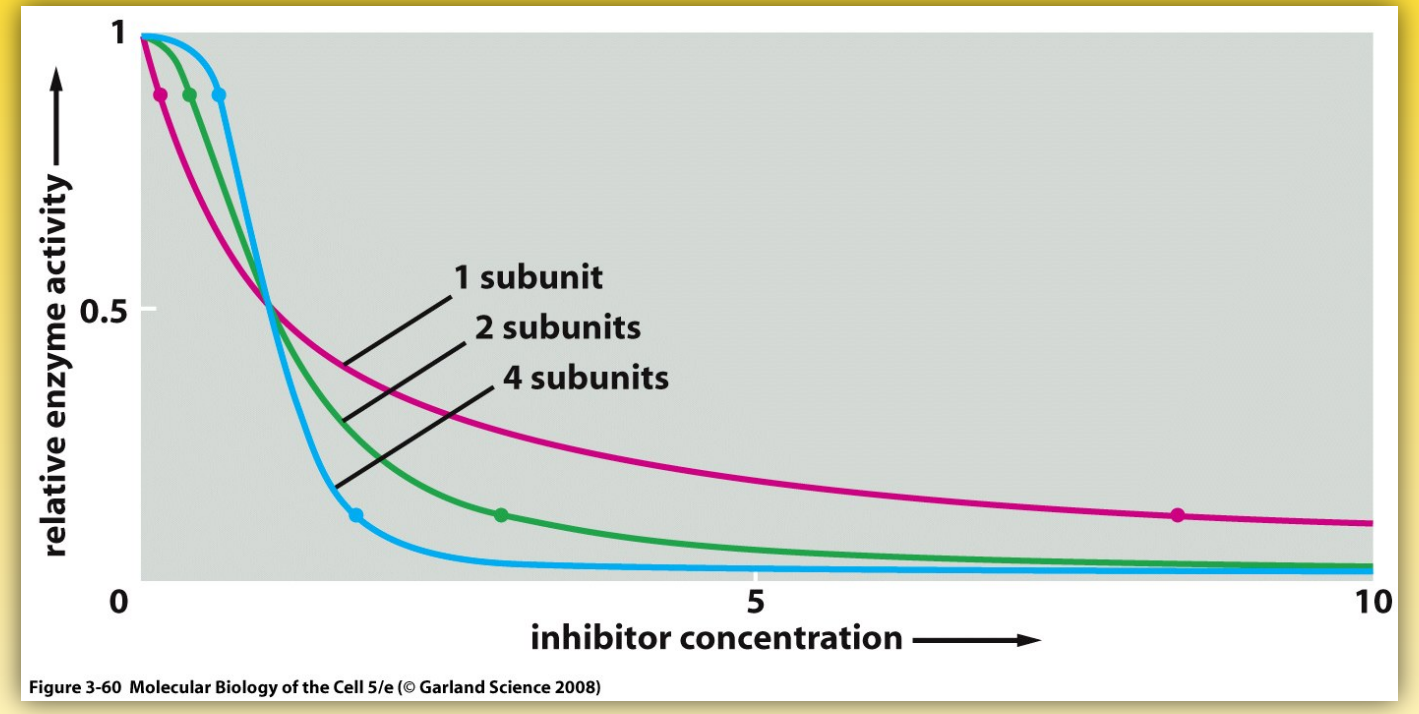
ATCase catalyzes a two-substrate reaction to give a single product.



Rate *vs* [S] curves are sigmoidal: cooperativity (allosterism)

The rate of the reaction catalyzed by ATCase as a function of aspartate concentration: allosteric regulation of enzymic activity

ATP = positive effector; CTP = negative effector



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General principle. Enzyme activity versus the concentration of inhibitory ligand for a single-subunit enzyme and multi-subunit allosteric enzymes. Dots show drop from 90% to 10% activity. Note switch-like behavior of the multi-subunit allosteric enzymes.

Rate accelerations caused by five different enzymes:
Accelerations range from 10^9 to 10^{23}

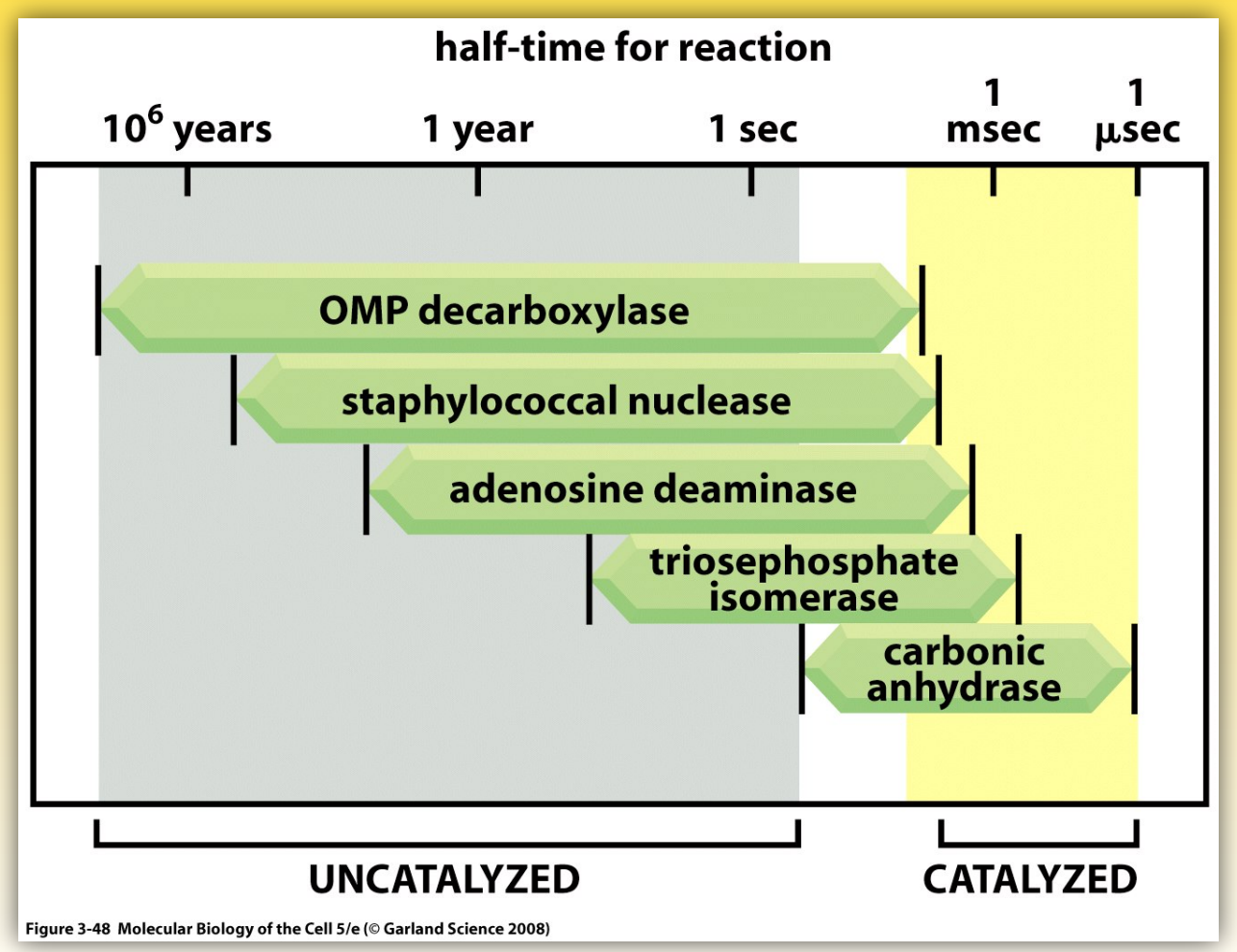

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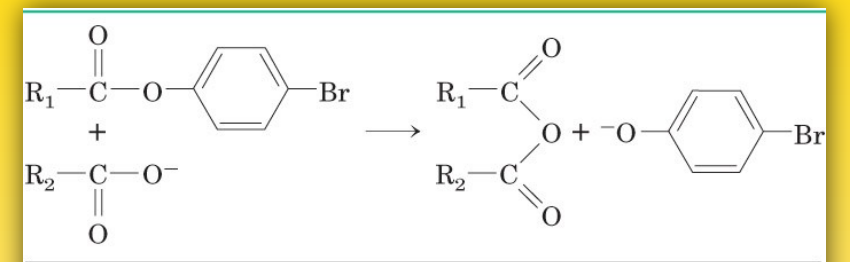
TABLE 1.2 Examples of Enzymatic Rate Acceleration

Enzyme	Nonenzymatic rate k_{non} (s^{-1})	Enzymatic rate k_{cat} (s^{-1})	Rate acceleration k_{cat}/k_{non}
Cyclophilin[a]	2.8×10^{-2}	1.3×10^{4}	4.6×10^{5}
Carbonic anhydrase[a]	1.3×10^{-1}	10^{6}	7.7×10^{6}
Chorismate mutase[a]	2.6×10^{-5}	50	1.9×10^{6}
Chymotrypsin[b]	4×10^{-9}	4×10^{-2}	10^{7}
Triosephosphate Isomerase[b]	6×10^{-7}	2×10^{3}	3×10^{9}
Fumarase[b]	2×10^{-8}	2×10^{3}	10^{11}
Ketosteroid Isomerase[a]	1.7×10^{-7}	6.6×10^{4}	3.9×10^{11}
Carboxypeptidase A[a]	3×10^{-9}	578	1.9×10^{11}
Adenosine Deaminase[a]	1.8×10^{-10}	370	2.1×10^{12}
Urease[b]	3×10^{-10}	3×10^{4}	10^{14}
Alkaline Phosphatase[b]	10^{-15}	10^{2}	10^{17}
Orotidine 5′-Phosphate Decarboxylase[a]	2.8×10^{-16}	39	1.4×10^{17}

[a] Taken from Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90.

[b] Taken from Horton, H. R.; Moran, L. A.; Ochs, R. S.; Rawn, J. D.; Scrimgeour, K. G. *Principles of Biochemistry,* Neil Patterson: Englewood Cliffs, NJ, 1993.



Reactants[a]		Relative Rate Constant
CH_3COO Br + CH_3COO^-	ϕ	1.0
COOϕBr, COO^-		$\sim 1 \times 10^3$
COO Br, COO^-	ϕ	$\sim 2.3 \times 10^5$
O, COO Br, COO^-	ϕ	$\sim 8 \times 10^7$

[a]Curved arrows indicate rotational degrees of freedom.

Source: Bruice, T.C. and Lightstone, F.C., *Acc. Chem. Res.* **32,** 127 (1999).

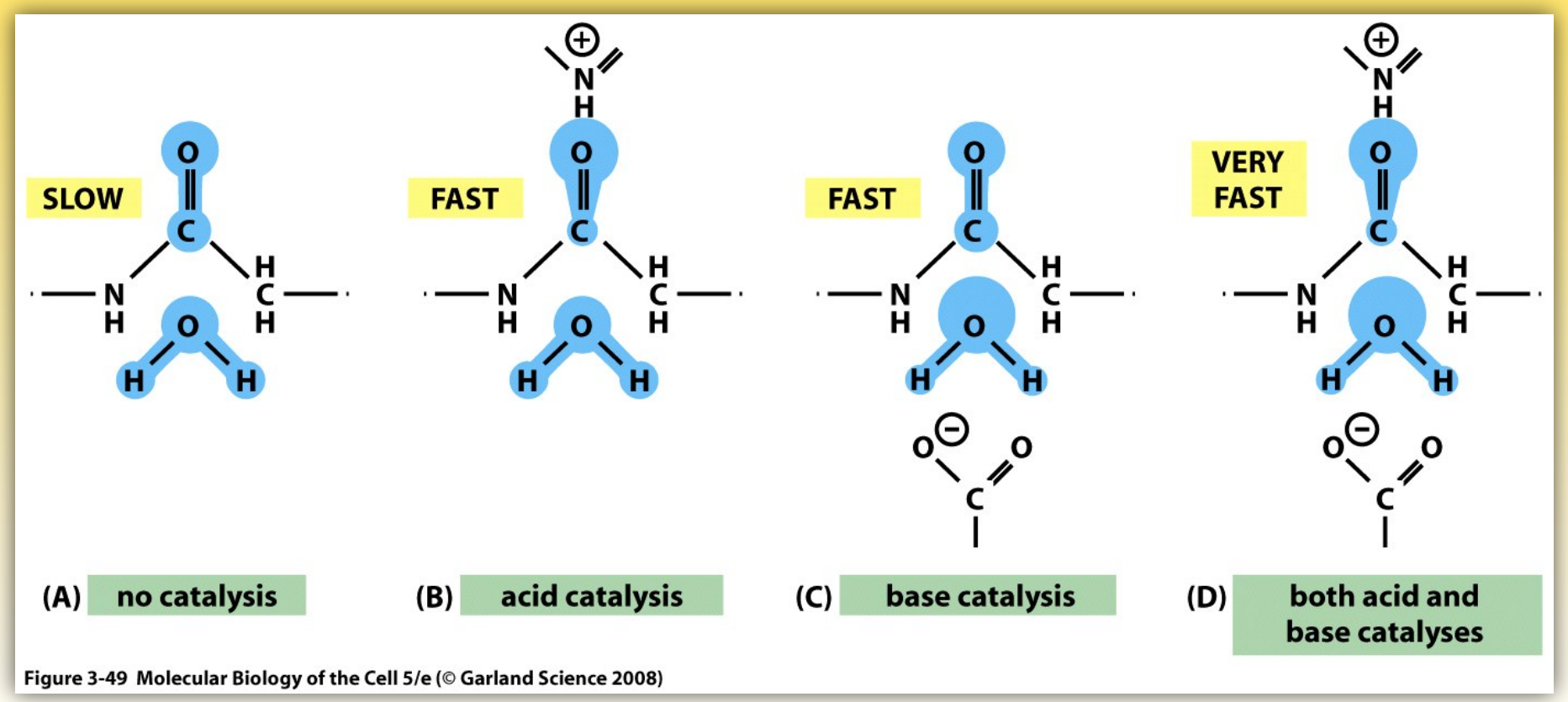
Relative rates of anhydride formation (bimolecular and intramolecular) for esters possessing different degrees of motional freedom

Factors responsible for rate enhancements by enzymes

Proximity/orientation effects on non-enzymic reaction rates; model for similar effects in enzyme binding sites

Factors responsible for rate enhancements by enzymes

Acid and base catalysis in enzymes: peptide (amide) bond hydrolysis



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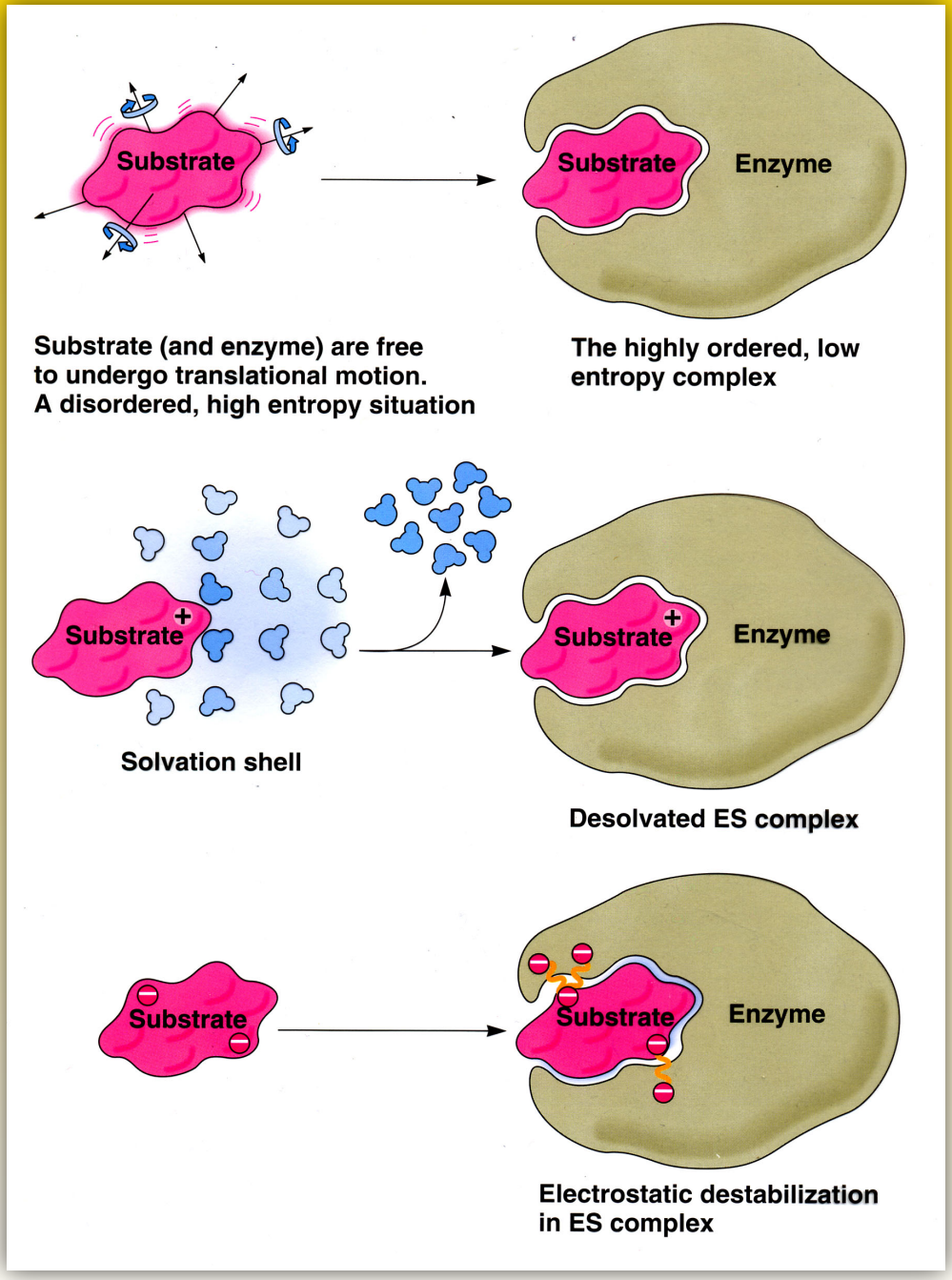
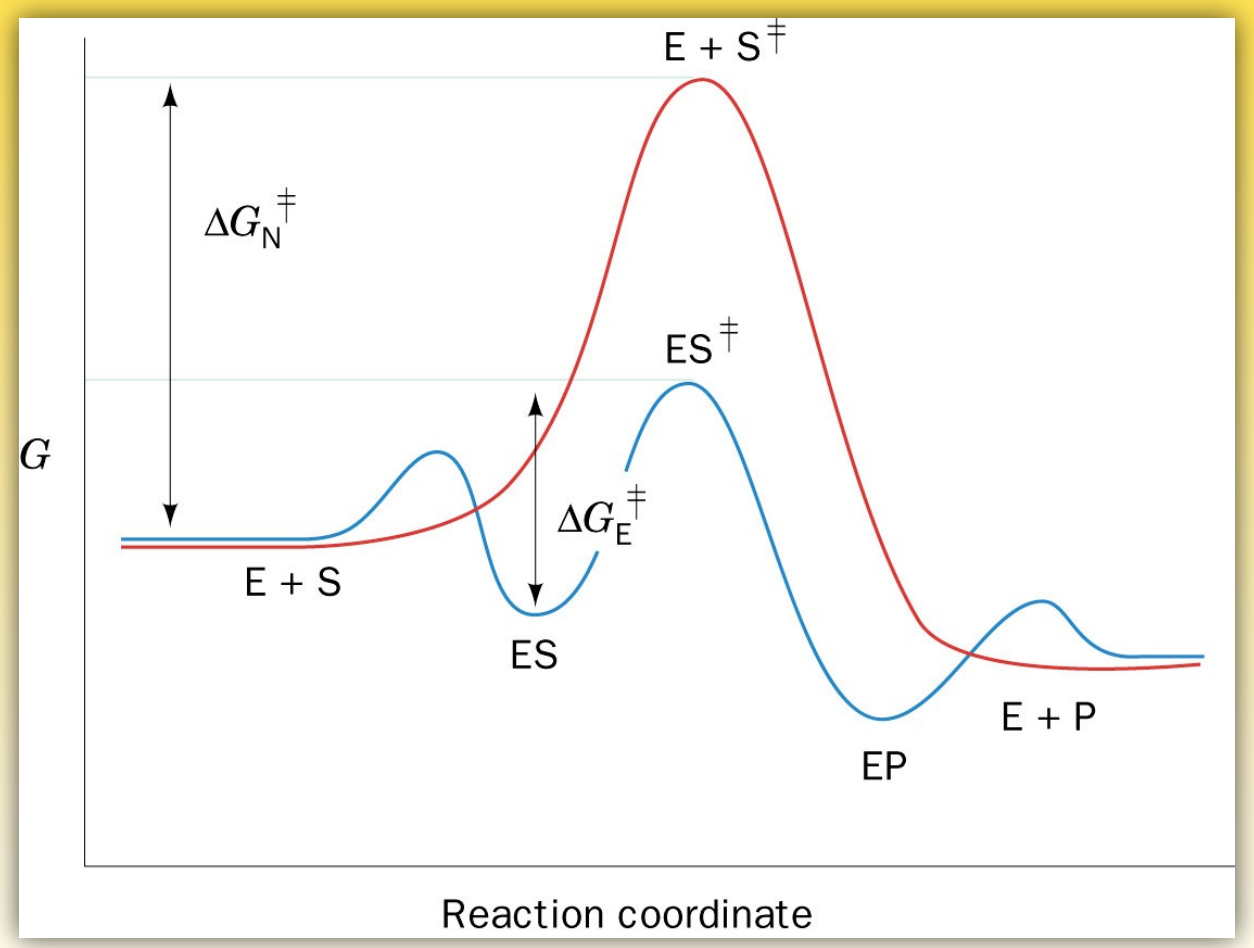


Enthalpic and entropic factors affecting the strength of enzyme-substrate binding

Reaction coordinate diagrams for a hypothetical enzyme-catalyzed reaction (single substrate, *blue*; corresponding uncatalyzed reaction, *red*).



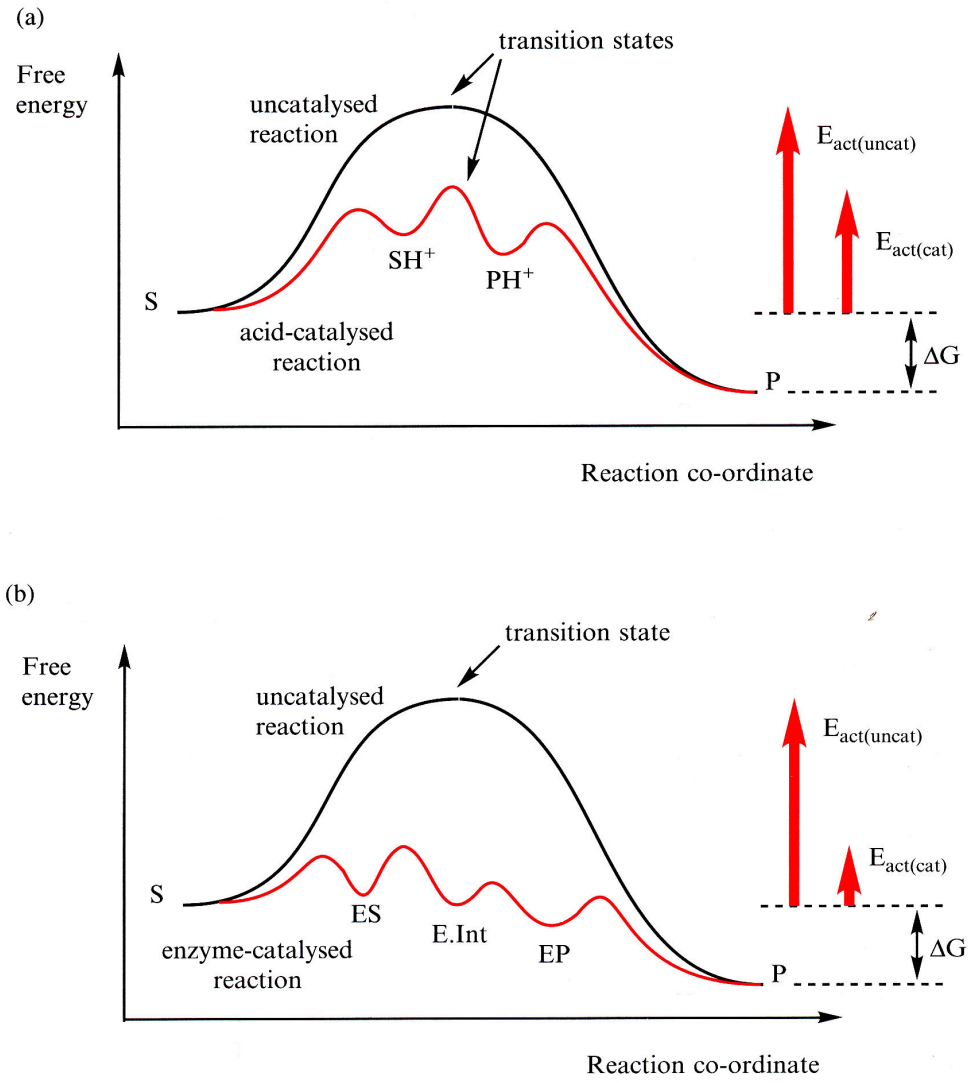


Figure 3.4 Free energy profiles for (a) an acid-catalysed reaction and (b) an enzyme-catalysed reaction which converts substrate S to product P.

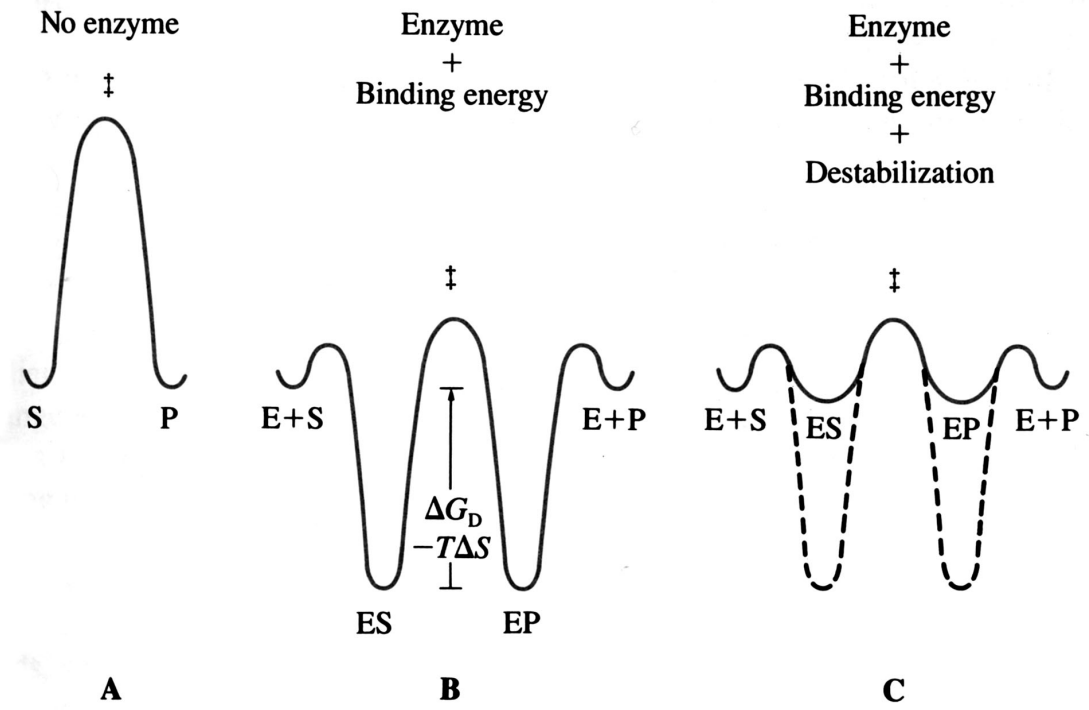
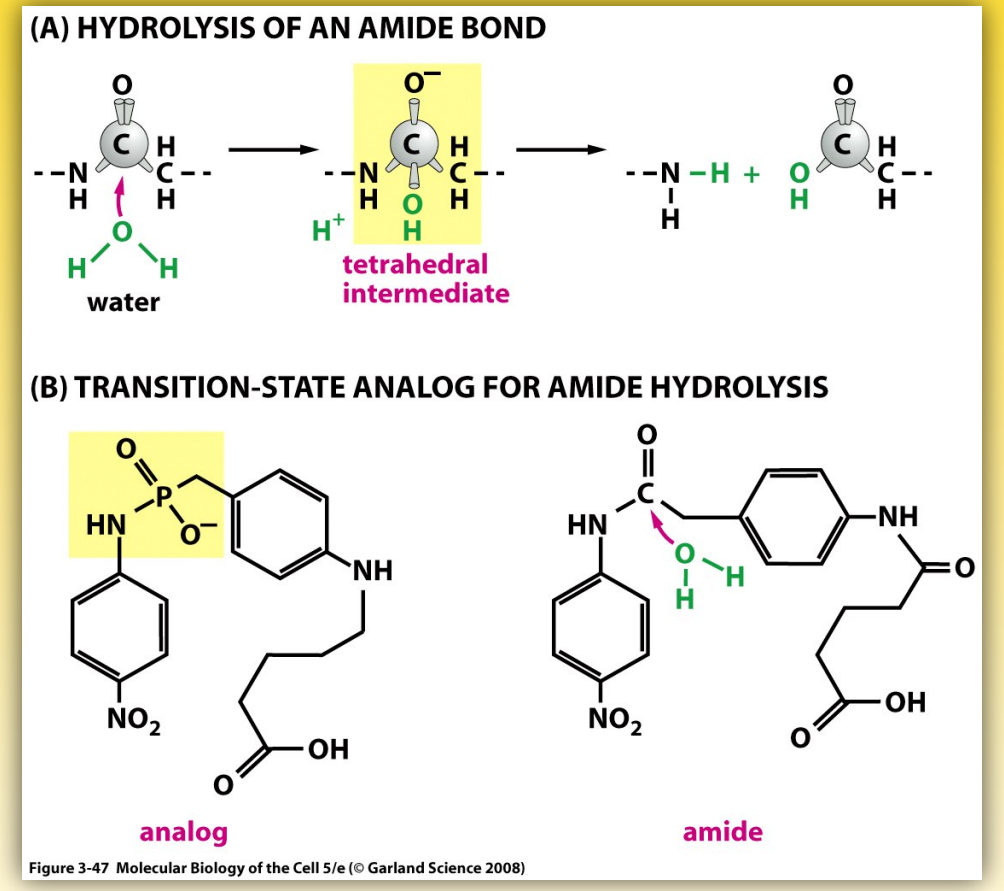


Figure 5-11

If an enzyme stabilizes the E·S complex and the transition state for the reaction of S by the same amount, there is no catalysis, because the barrier for the reaction of E·S (B) is the same as the barrier for the reaction of S (A). The enzyme becomes an effective catalyst when it stabilizes the transition state much more than the E·S and E·P complexes (C). This is brought about by destabilization mechanisms (ΔG_D) and loss of entropy ($-T\Delta S$) in the E·S and E·P complexes.

For catalysis to occur, the enzyme must bind the transition state more tightly than the substrate.



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Generating catalytic antibodies: antibody generation against a molecule that *mimics the putative transition state* of amide hydrolysis.

Summary of common mechanisms of enzyme catalysis

- acid-base catalysis
- covalent catalysis
- metal ion catalysis
- electrostatic catalysis
- catalysis via proximity/orientation effects
- catalysis via preferential transition state binding