L4 Enzyme functional nature

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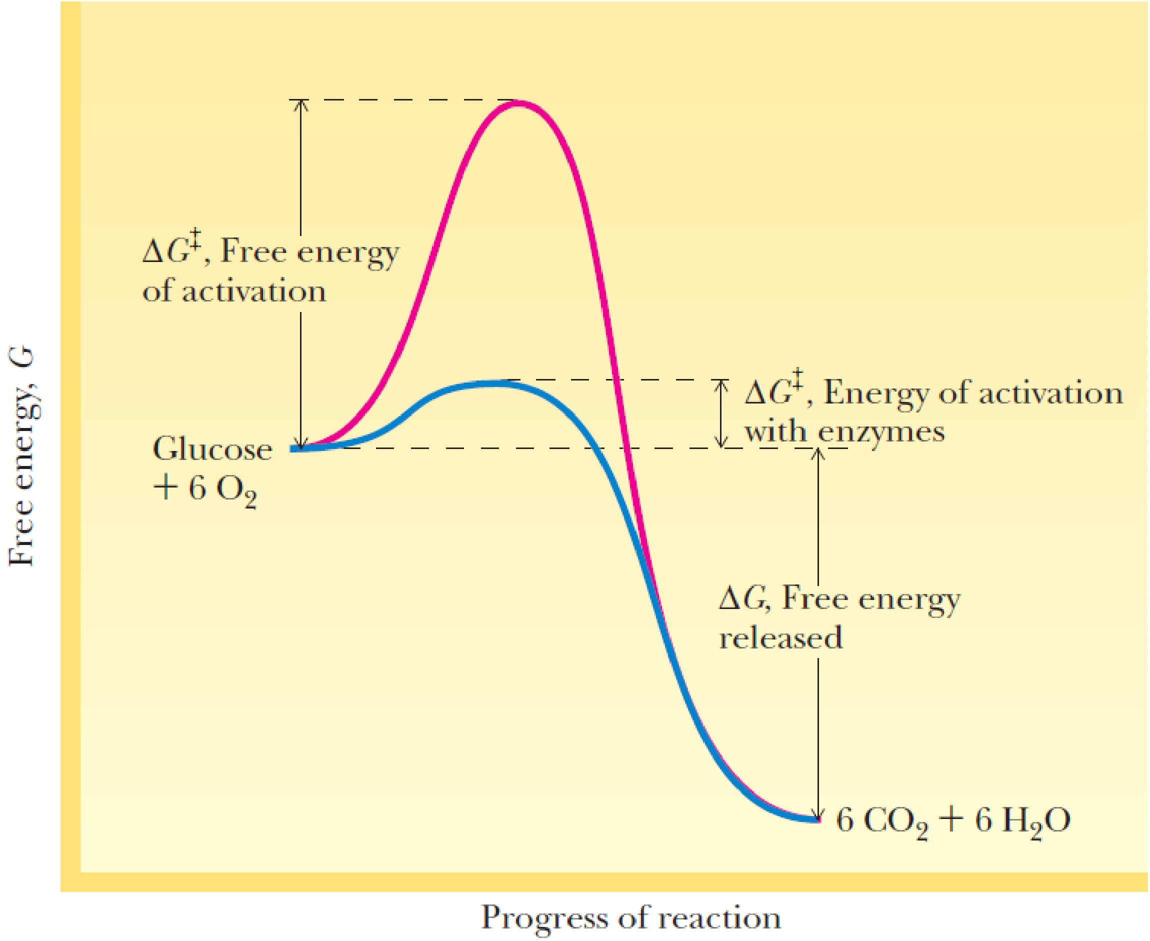
Enzymes are catalyst,
That accelerate chemical reactions

Mostly enzymes are proteins,
some RNA enzymes also exist



Enzyme catalytic power

TABLE 14.1 A Comparison of Enzyme-Catalyzed Reactions and Their Uncatalyzed Counterparts

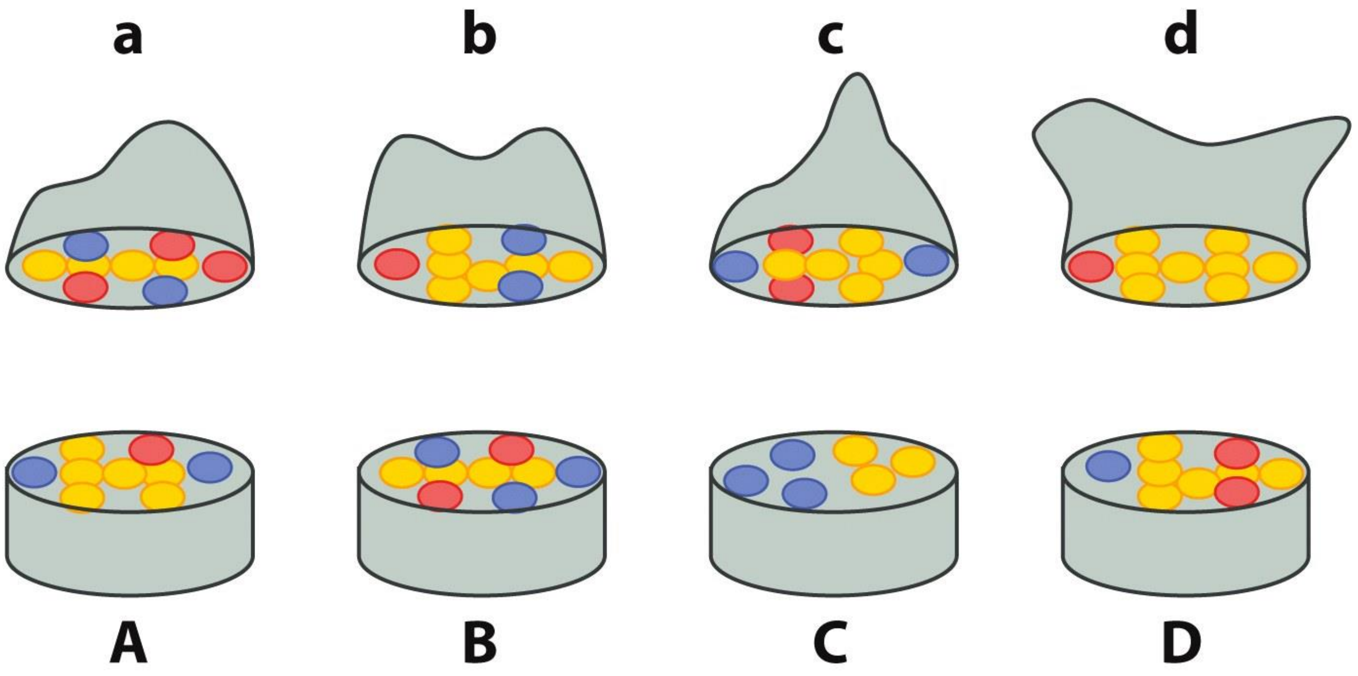
Reaction	Enzyme	Uncatalyzed Rate, v_u (sec^{-1})	Catalyzed Rate, v_e (sec^{-1})	v_e/v_u
Fructose-1,6-bisP $\longrightarrow$ fructose-6-P + P_i	Fructose-1,6-bisphosphatase	2×10^{-20}	21	1.05×10^{21}
$(Glucose)_n + H_2O \longrightarrow (glucose)_{n-2}$ + maltose	β-amylase	1.9×10^{-15}	1.4×10^{3}	7.2×10^{17}
DNA, RNA cleavage	Staphylococcal nuclease	7×10^{-16}	95	1.4×10^{17}
$CH_3{-}O{-}PO_3^{2-} + H_2O \longrightarrow CH_3OH + HPO_4^{2-}$	Alkaline phosphatase	1×10^{-15}	14	1.4×10^{16}
$H_2N{-}C({=}O){-}NH_2 + 2\,H_2O + H^+ \longrightarrow 2\,NH_4^+ + HCO_3^-$	Urease	3×10^{-10}	3×10^{4}	1×10^{14}
$R{-}C({=}O){-}O{-}CH_2CH_3 + H_2O \longrightarrow RCOOH + HOCH_2CH_3$	Chymotrypsin	1×10^{-10}	1×10^{2}	1×10^{12}
Glucose + ATP $\longrightarrow$ Glucose-6-P + ADP	Hexokinase	$<1 \times 10^{-13}$	1.3×10^{-3}	$>1.3 \times 10^{10}$
$CH_3CH_2OH + NAD^+ \longrightarrow CH_3C({=}O)H + NADH + H^+$	Alcohol dehydrogenase	$<6 \times 10^{-12}$	2.7×10^{-5}	$>4.5 \times 10^{6}$
$CO_2 + H_2O \longrightarrow HCO_3^- + H^+$	Carbonic anhydrase	10^{-2}	10^{5}	1×10^{7}
Creatine + ATP $\longrightarrow$ Cr-P + ADP	Creatine kinase	$<3 \times 10^{-9}$	4×10^{-5}	$>1.33 \times 10^{4}$

Adapted from Koshland, D., 1956. Molecular geometry in enzyme action. *Journal of Cellular Comparative Physiology*, Supp. 1, **47**:217; and Wolfenden, R., 2006. Degrees of difficulty of water-consuming reactions in the absence of enzymes. *Chemical Reviews* **106**:3379–3396.

Enzyme specificity

Enzyme specificity:

- Molecular recognition based on shape and charge of the molecule at atomic level
- Even chiral groups will be distinguished



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Yellow- hydrophobic
Blue – positively charged
Red – negatively charged

Enzyme cofactors and coenzymes

Cofactors: Are non protein components that are required for function of an Enzyme
e.g., Ca2+ ions

Prosthetic group: Organic molecule cofactor, tightly bound to enzyme. Eg., Haem group

Coenzyme: Organic molecule cofactor. They are chemically changed in course of enzymatic reaction Eg., NADH

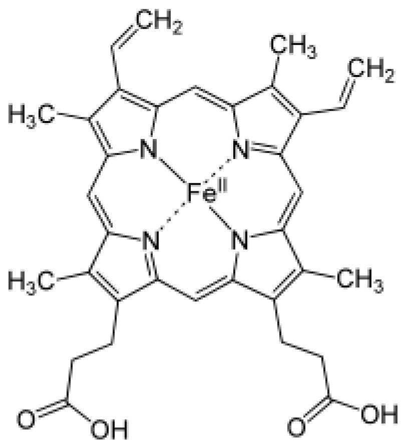
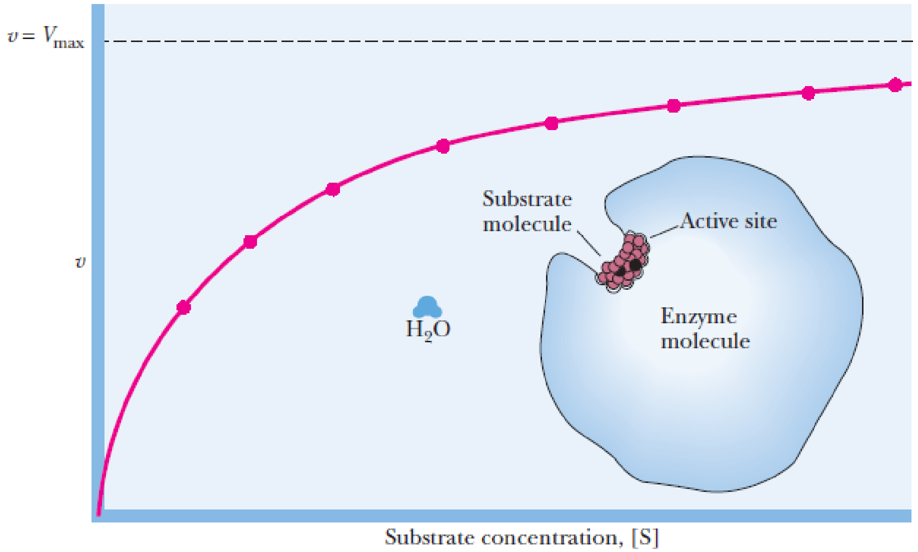


TABLE 13.2 Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated

Metal Ions and Some Enzymes That Require Them		Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups		
Metal Ion	**Enzyme**	**Coenzyme**	**Entity Transferred**	**Representative Enzymes Using Coenzymes**
Fe^{2+} or Fe^{3+}	Cytochrome oxidase	Thiamine pyrophosphate (TPP)	Aldehydes	Pyruvate dehydrogenase
	Catalase	Flavin adenine dinucleotide (FAD)	Hydrogen atoms	Succinate dehydrogenase
	Peroxidase	Nicotinamide adenine dinucleotide (NAD)	Hydride ion (:H^-)	Alcohol dehydrogenase
Cu^{2+}	Cytochrome oxidase			
Zn^{2+}	DNA polymerase	Coenzyme A (CoA)	Acyl groups	Acetyl-CoA carboxylase
	Carbonic anhydrase	Pyridoxal phosphate (PLP)	Amino groups	Aspartate aminotransferase
	Alcohol dehydrogenase			
Mg^{2+}	Hexokinase	5′-Deoxyadenosylcobalamin (vitamin B_{12})	H atoms and alkyl groups	Methylmalonyl-CoA mutase
	Glucose-6-phosphatase			
Mn^{2+}	Arginase	Biotin (biocytin)	CO_2	Propionyl-CoA carboxylase
K^+	Pyruvate kinase (also requires Mg^{2+})	Tetrahydrofolate (THF)	Other one-carbon groups, such as formyl and methyl groups	Thymidylate synthase
Ni^{2+}	Urease			
Mo	Nitrate reductase			
Se	Glutathione peroxidase			

Enzyme kinetics



Michaelis–Menten equation

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

$$k_2 = \frac{V_{max}}{[E_T]} = k_{cat}$$

TABLE 13.3 K_m Values for Some Enzymes

Enzyme	Substrate	K_m (mM)
Carbonic anhydrase	CO_2	12
Chymotrypsin	*N*-Benzoyltyrosinamide	2.5
	Acetyl-L-tryptophanamide	5
	N-Formyltyrosinamide	12
	N-Acetyltyrosinamide	32
	Glycyltyrosinamide	122
Hexokinase	Glucose	0.15
	Fructose	1.5
β-Galactosidase	[illegible]	[illegible]

TABLE 13.4 Values of k_{cat} (Turnover Number) for Some Enzymes

Enzyme	k_{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

Catalytic efficiency of an Enzyme

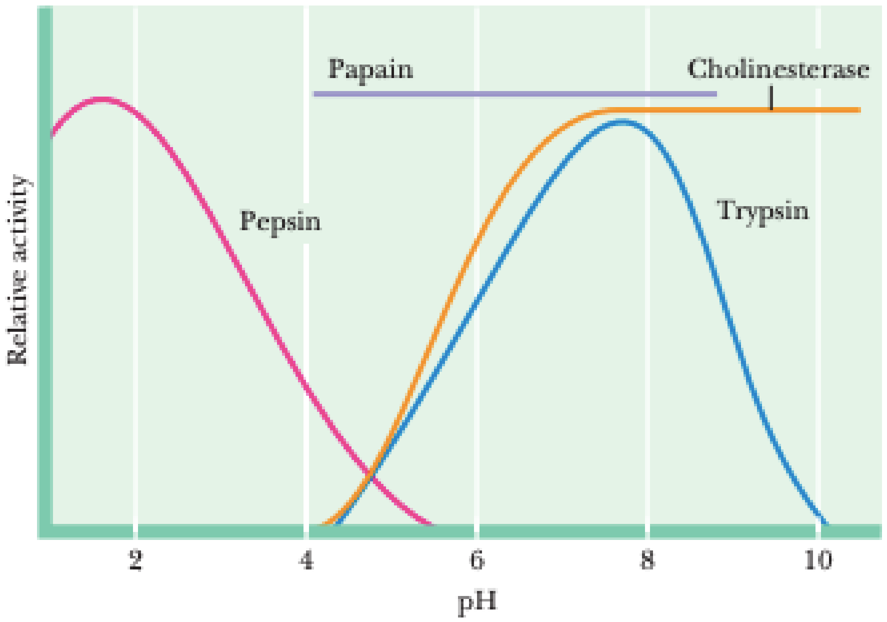
$$k_2 = \frac{V_{max}}{[E_T]} = k_{cat}$$

TABLE 13.5 Enzymes Whose k_{cat}/K_m Approaches the Diffusion-Controlled Rate of Association with Substrate

Enzyme	Substrate	k_{cat} (sec^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}\ sec^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	0.012	8.3×10^7
	HCO_3^-	4×10^5	0.026	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	800	5×10^{-6}	1.6×10^8
	Malate	900	2.5×10^{-5}	3.6×10^7
Triosephosphate isomerase	Glyceraldehyde-3-phosphate*	4.3×10^3	1.8×10^{-5}	2.4×10^8
β-Lactamase	Benzylpenicillin	2×10^3	2×10^{-5}	1×10^8

*K_m for glyceraldehyde-3-phosphate is calculated on the basis that only 3.8% of the substrate in solution is unhydrated and therefore reactive with the enzyme.
Adapted from Fersht, A., 1985. *Enzyme Structure and Mechanism,* 2nd ed. New York: W. H. Freeman.

Enzyme activity as function of pH



Optimum pH of Some Enzymes	
Enzyme	**Optimum pH**
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

A decrease in pH (more acidic) causes an increase in the concentration of hydrogen ions (H+) in solution, which can cause the ionization of acidic functional groups such as carboxyl groups (-COOH) resulting in the formation of carboxylate groups (-COO-). This can cause the enzyme to lose its activity by altering its shape and function. Similarly, an increase in pH (more basic) can cause the ionization of basic functional groups such as amino groups (-NH3+) and this can also affect the enzyme's activity.

Enzyme activity as function of temperature

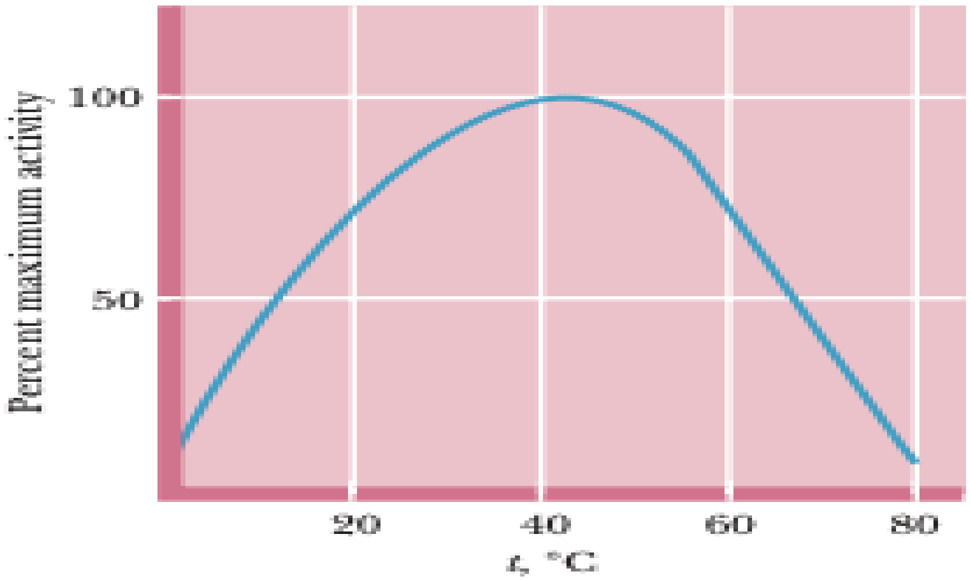
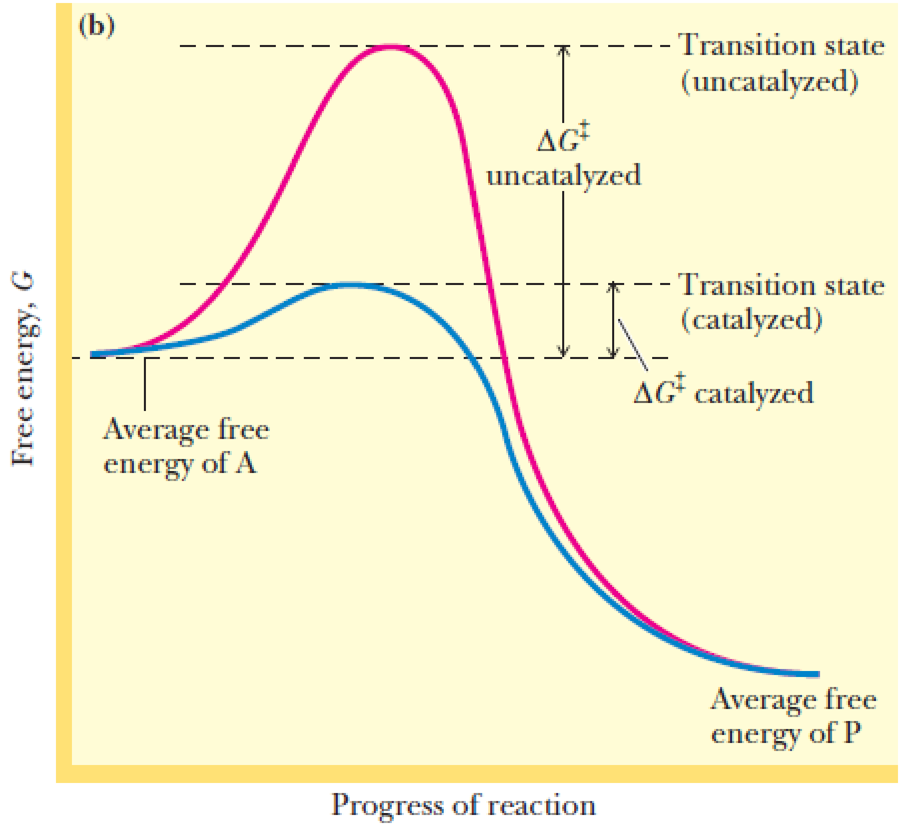
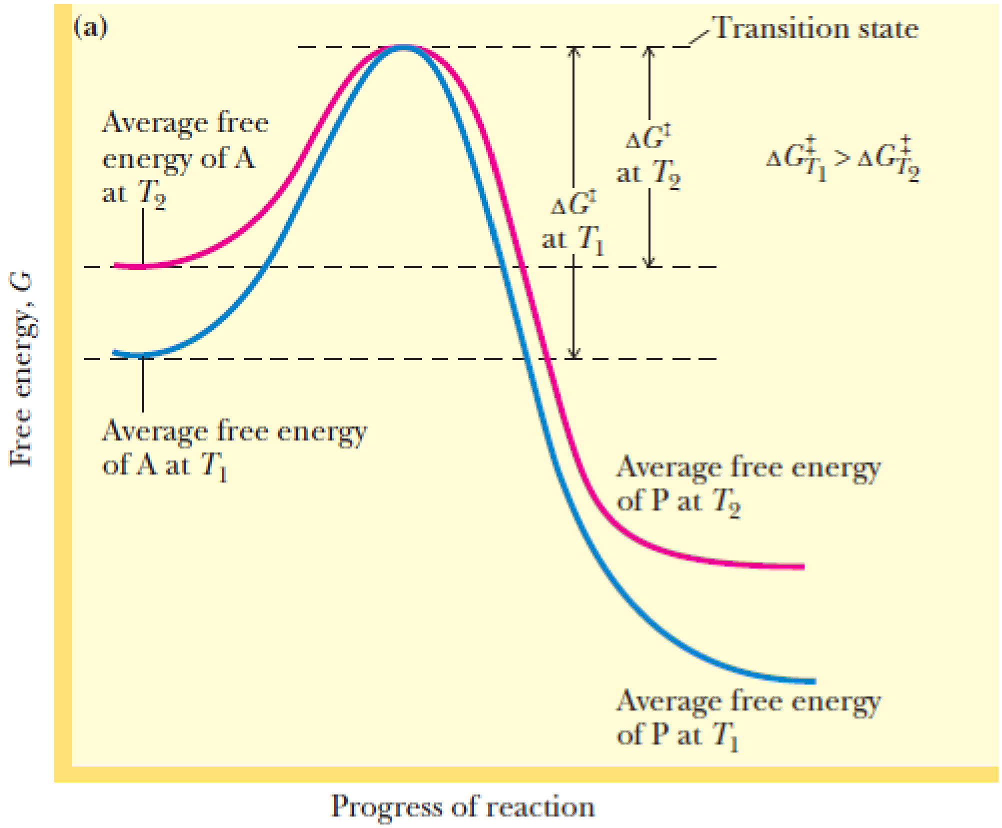


FIGURE 13.12 The effect of temperature on enzyme activity.

Enzyme thermodynamics

Mechanism :Catalysts Lower the Free Energy of Activation for a Reaction





Gibbs free energy

- Thermodynamics: changes in free energy, entropy, …

$$\Delta G = \Delta H - T\cdot\Delta S$$
$$\Delta G = (\Delta U + P\cdot\Delta V) - T\cdot\Delta S$$

- For nearly all biochemical reactions ΔV is small and ΔH is almost equal to ΔU

- Hence, we can write:

$$\boxed{\Delta G = \Delta U - T\cdot\Delta S}$$

If ΔG is negative

Energy was released, products are simpler, greater entropy (2nd Law of Thermodynamics)

Exergonic / exothermic reaction (spontaneous)

If ΔG is positive

Energy input, product more complex, energy needed to go against 2nd Law

Endergonic / endothermic (non-spontaneous)

The Enthalpic term

- Changes in bonding
  - van der Waals
  - Hydrogen bonding
  - Charge interactions

The Entropic term

- Changes the arrangement of the solvent or counterions
- Reflects the degrees of freedom
- Rotational & Translational changes

Protein folding

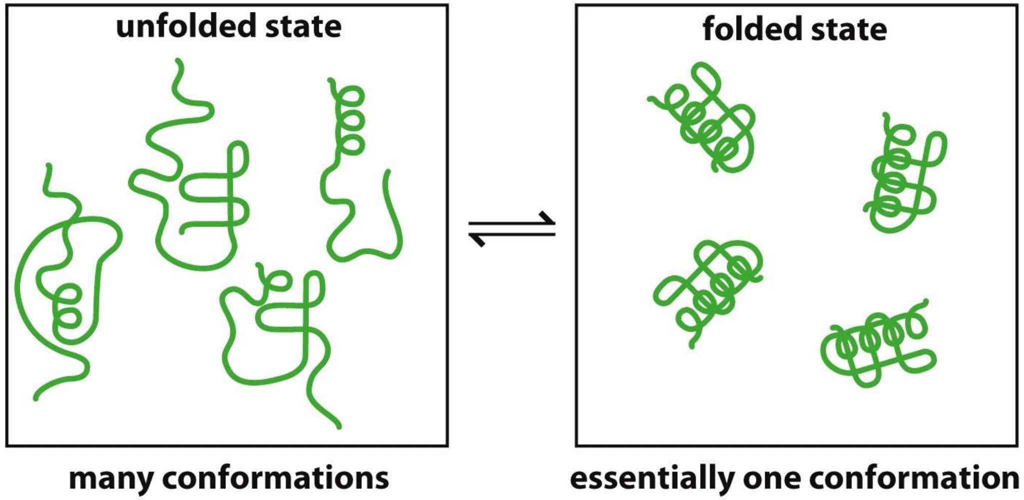


Figure 10.13 The Molecules of Life (© Garland Science 2013)

Protein folding is a spontaneous process?

$\Delta G < 0$???

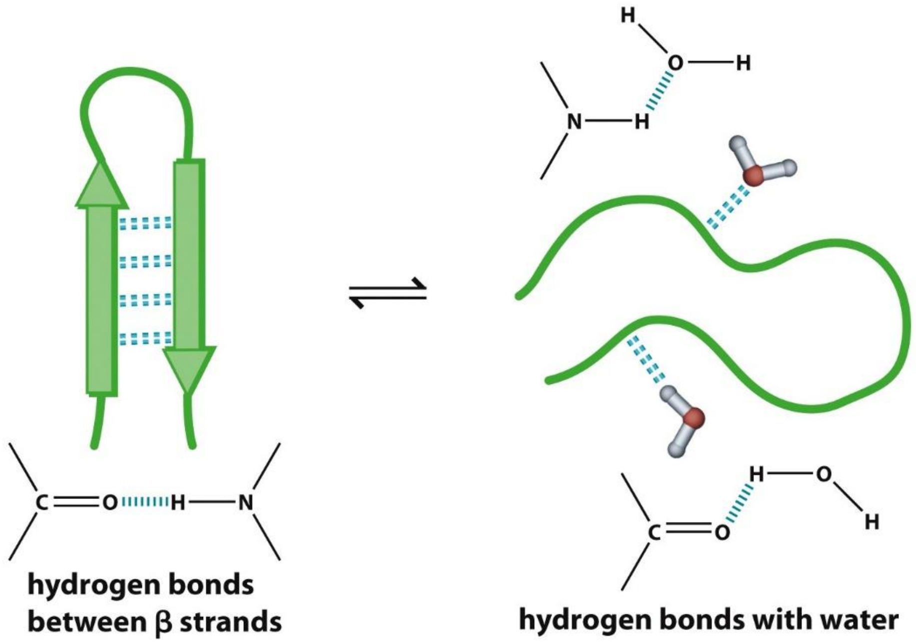
Protein folding

(U) Unfolded $\rightleftharpoons$ Folded (F)

$$K_{folding} = \frac{(F)}{(U)} = \frac{1}{K_{unfolding}}$$

$$\Delta G^\circ_{unfolding} = \Delta H^\circ - T\,\Delta S^\circ$$

Enthalpy change in protein folding



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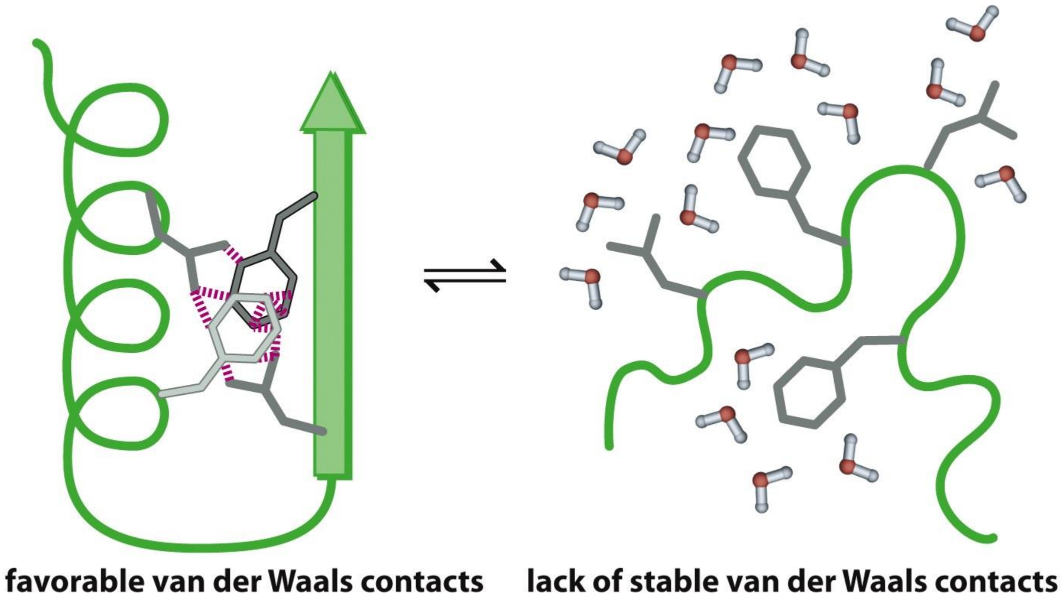
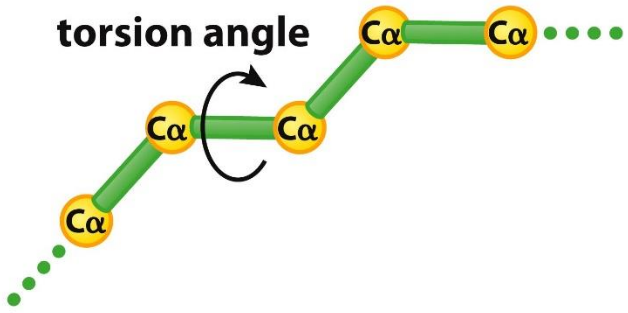


Figure 10.15 The Molecules of Life (© Garland Science 2013)

Entropy change in protein folding



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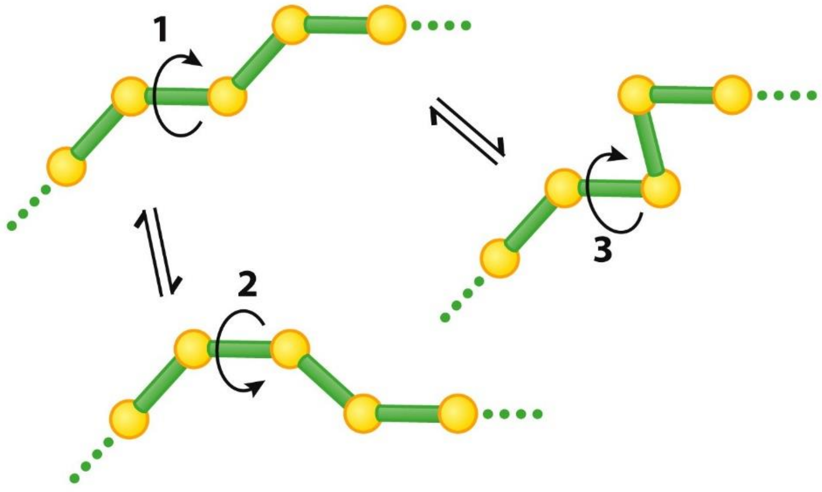
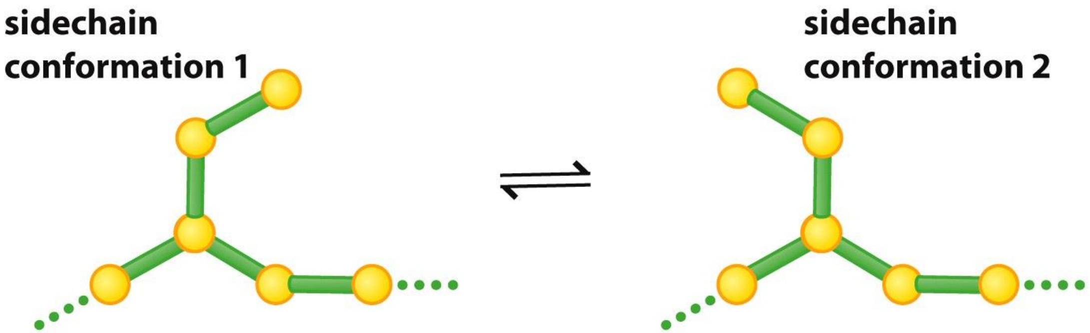


Figure 10.19 The Molecules of Life (© Garland Science 2013)



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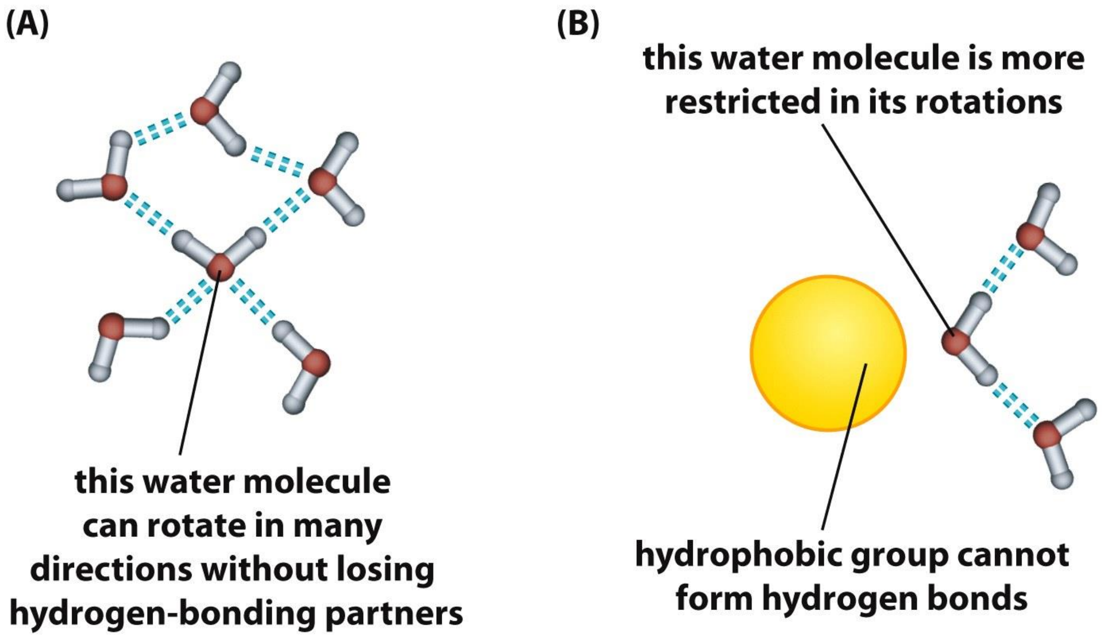
Entropy contribution from water



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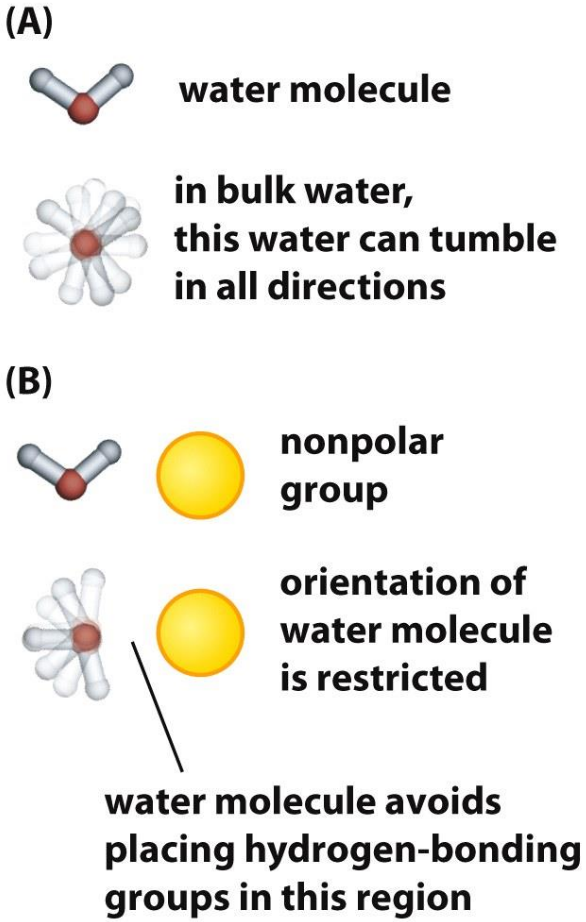

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Protein folding

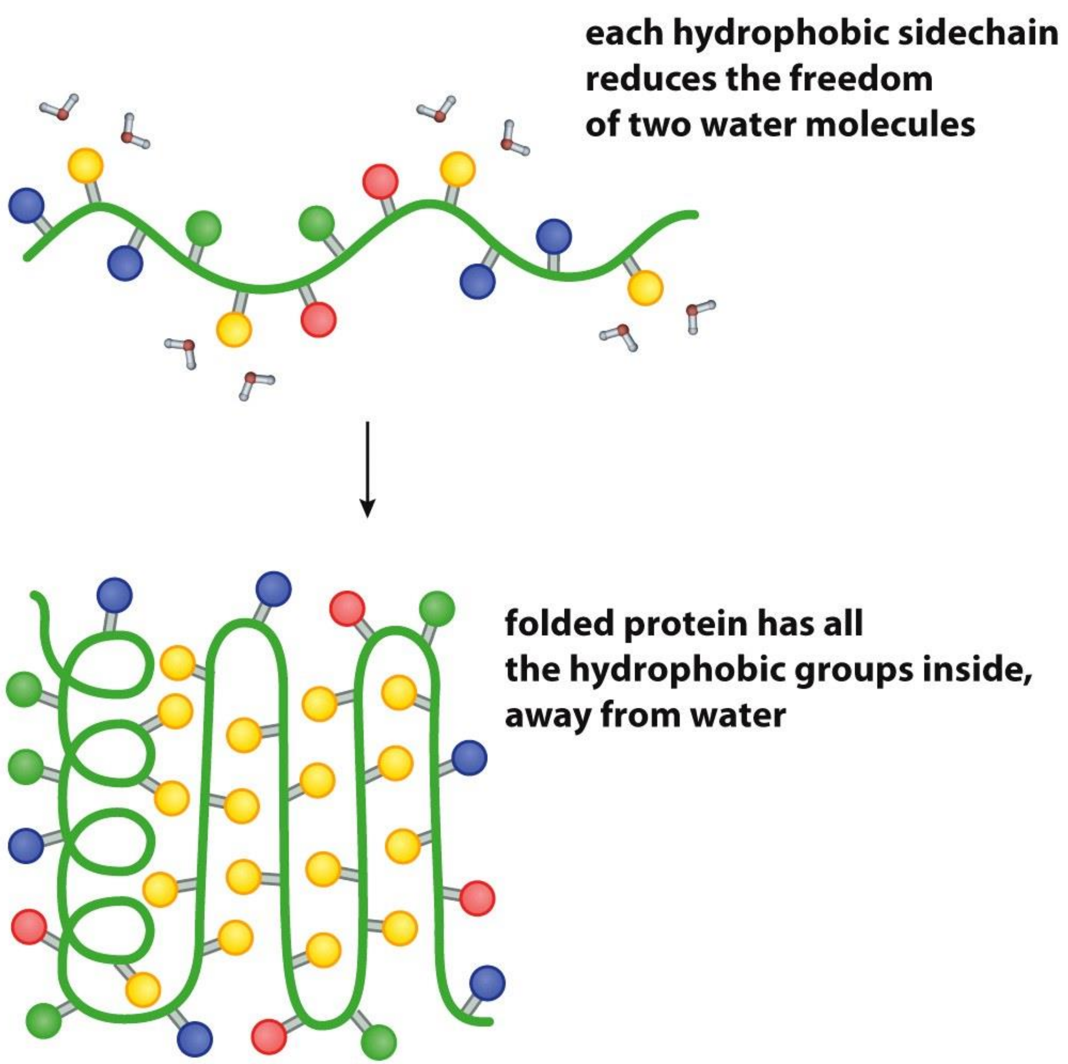
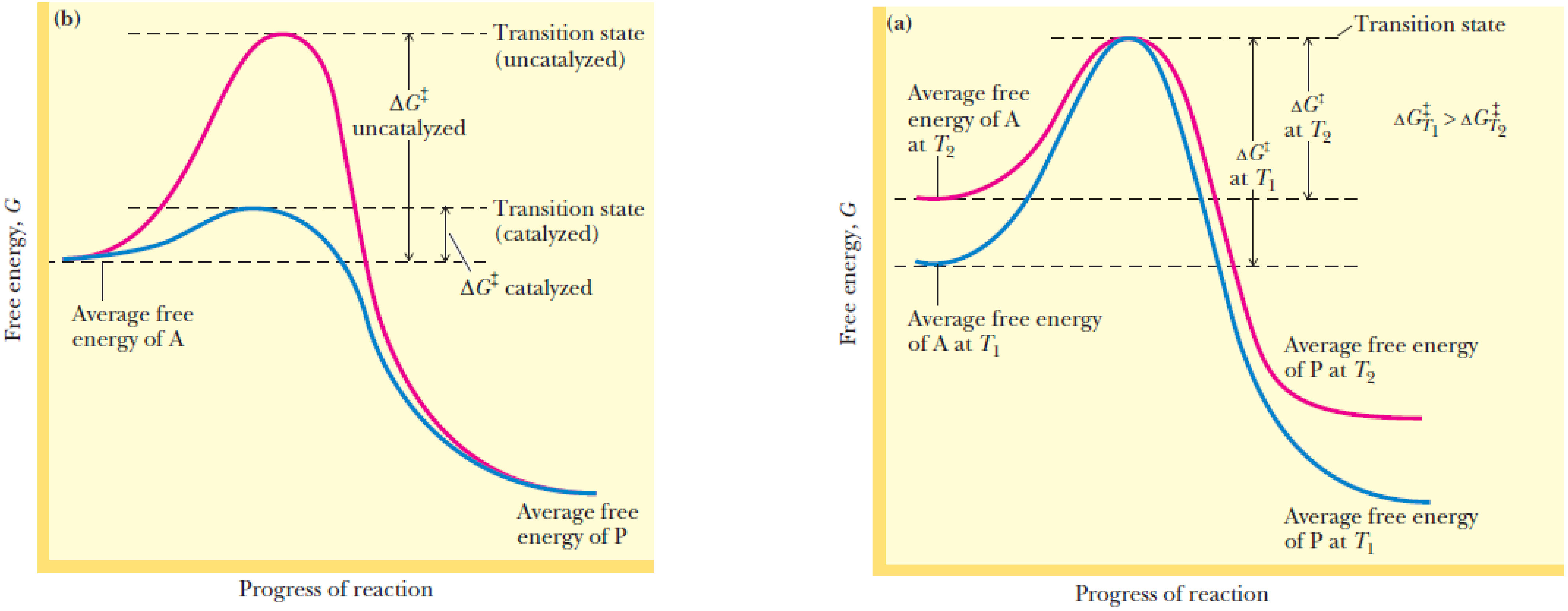


Figure 10.23 The Molecules of Life (© Garland Science 2013)

Enzyme mechanism

Mechanism :Catalysts Lower the Free Energy of Activation for a Reaction



Thank you