Lect#10: Enzyme Mechanisms II

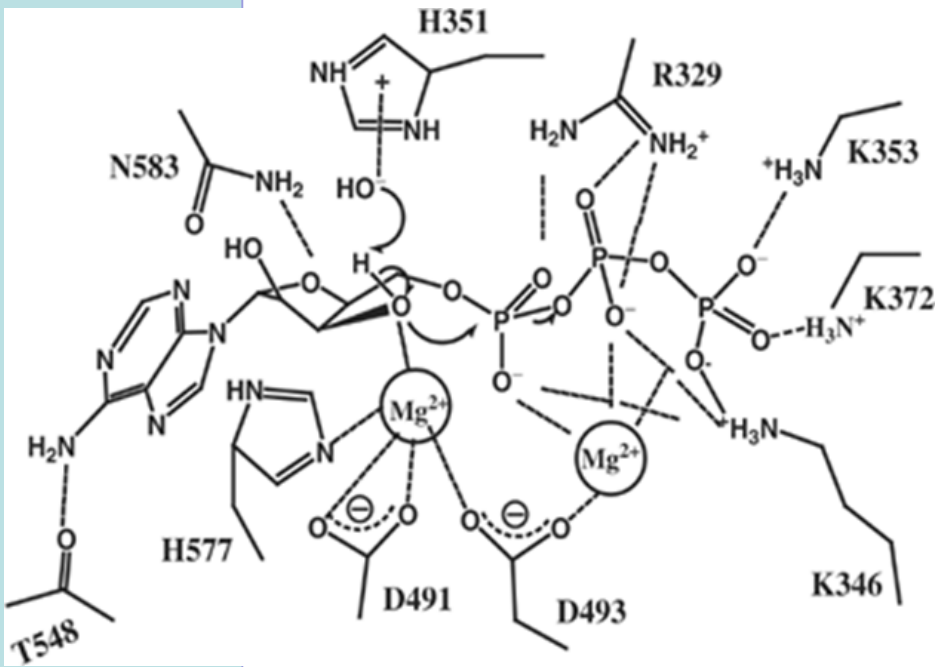
(5) Covalent catalysis
- amino transferases

(6) Metal ion catalysis
- thermolysin
- pyruvate kinase

(7) Electrostatic catalysis
- active site residue location
- computational chemistry
- site-directed mutagenesis

(8) Low barrier H-bonds
- role in catalysis
- catalytic triad

(9) Structural flexibility
- hexokinase
- control of reaction environment



(5) Covalent Catalysis

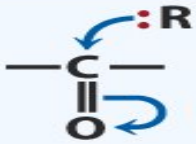
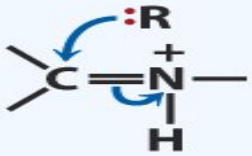
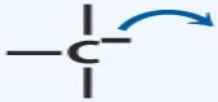
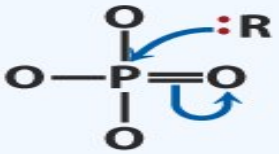
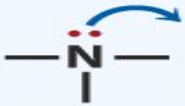
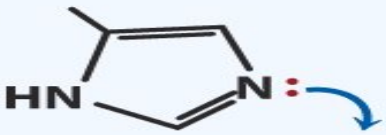
- A transient covalent bond between the enzyme and the substrate
- Changes the reaction pathway
  - uncatalyzed:

$$A — B \xrightarrow{H_2O} A + B$$

  - catalyzed (X = catalyst):

$$A — B + X: \rightarrow A — X + B \xrightarrow{H_2O} A + X: + B$$

- Requires a nucleophile on the enzyme
  - can be a reactive serine, thiolate, amine, or carboxylate

Nucleophiles	Electrophiles
 Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)	 Carbon atom of a carbonyl group (the more electronegative oxygen of the carbonyl group pulls electrons away from the carbon)
 Negatively charged sulfhydryl	 Pronated imine group (activated for nucleophilic attack at the carbon by protonation of the imine)
 Carbanion	 Phosphorus of a phosphate group
 Uncharged amine group	 Proton
 Imidazole	
 Hydroxide ion	

L7:F6-23

- **Covalent catalysis** can have **two main** stages

  (1) nucleophilic reaction between the catalyst and the substrate to form a covalent bond (acylation)

  (2) elimination of the catalyst (deacylation) —reverse of stage 1

Covalent catalysis

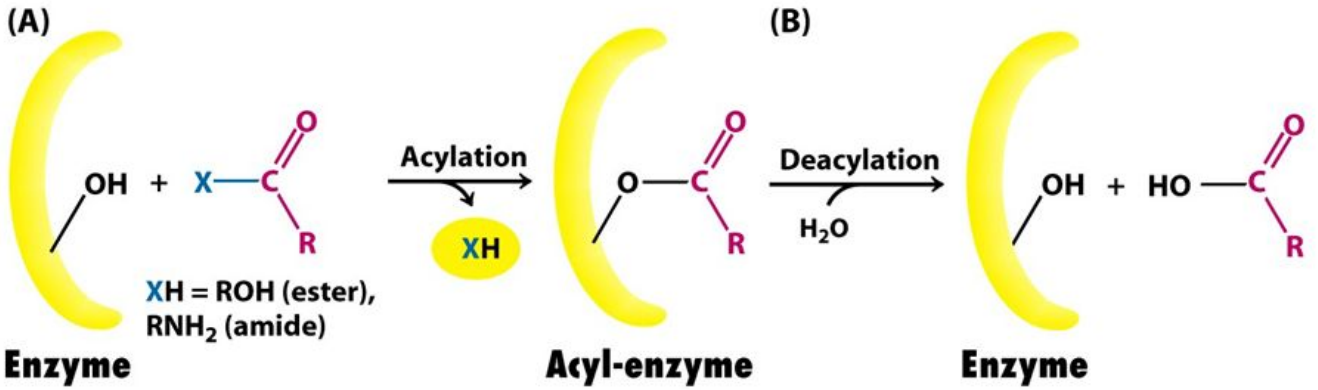


Figure 9-5
Biochemistry, Sixth Edition

Two stages

•the more stable the covalent bond formed, the less easily it can decompose in the final steps of a reaction

•a good covalent catalyst must combine properties of high nucleophilicity with the ability to form a good leaving group

- His, Cys, Asp, Glu, and Lys
- coenzyme pyridoxal phosphate

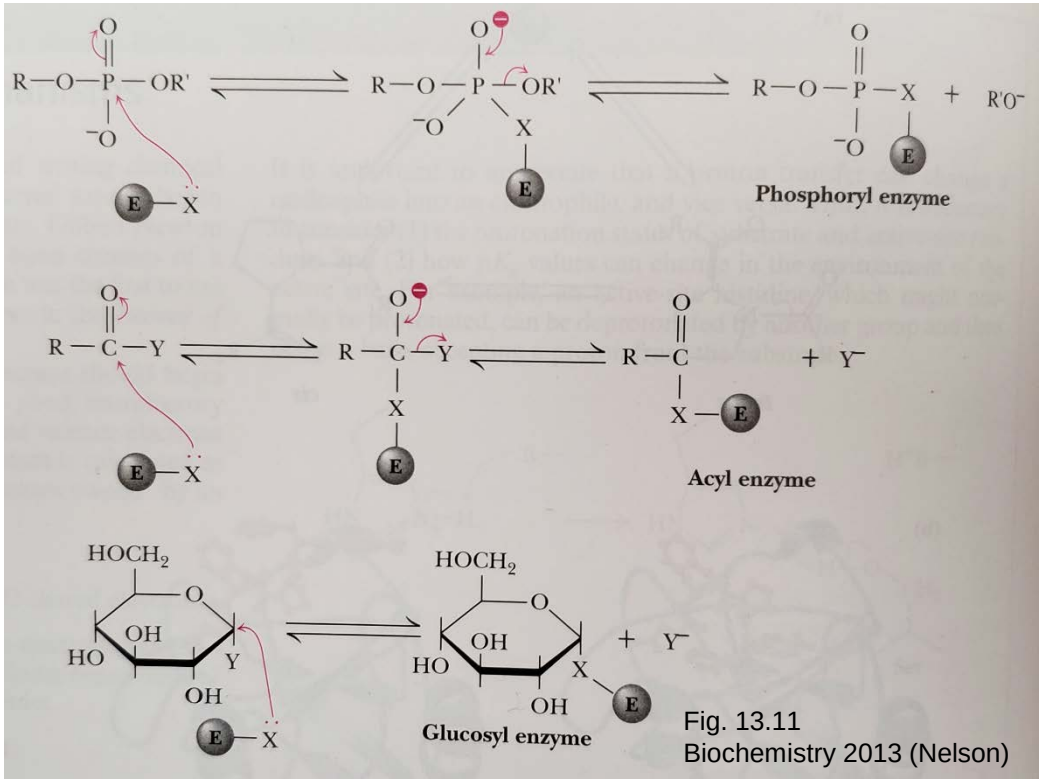
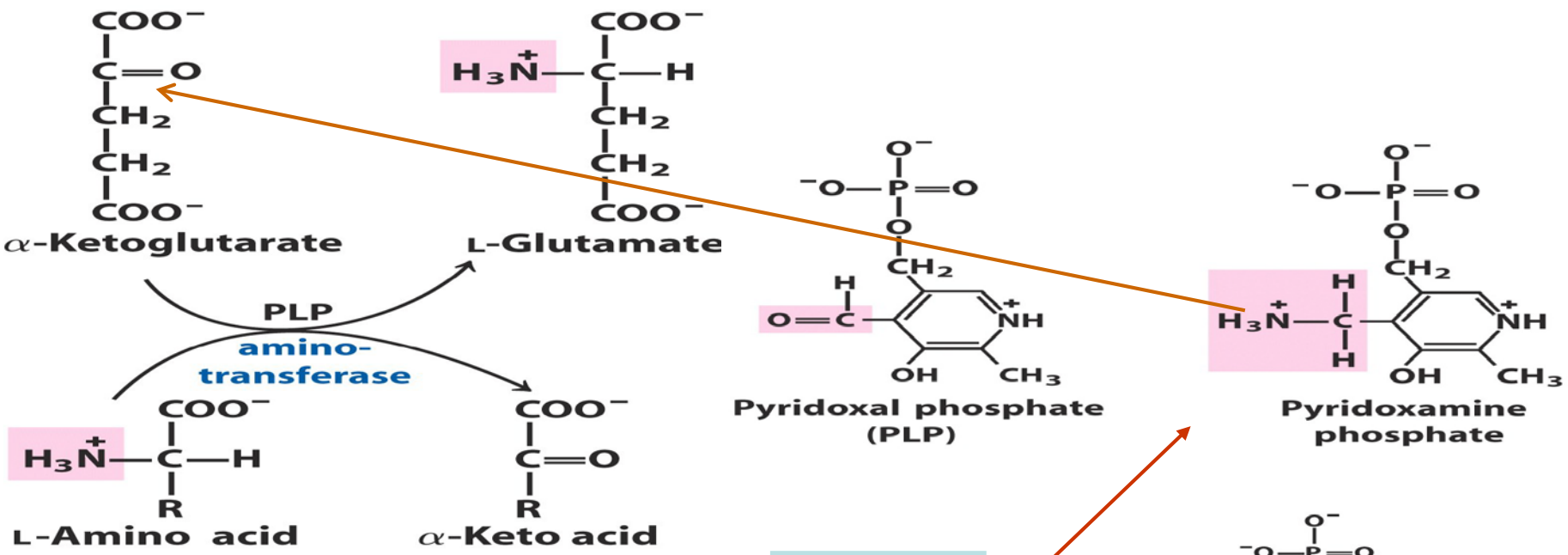


Fig. 13.11
Biochemistry 2013 (Nelson)

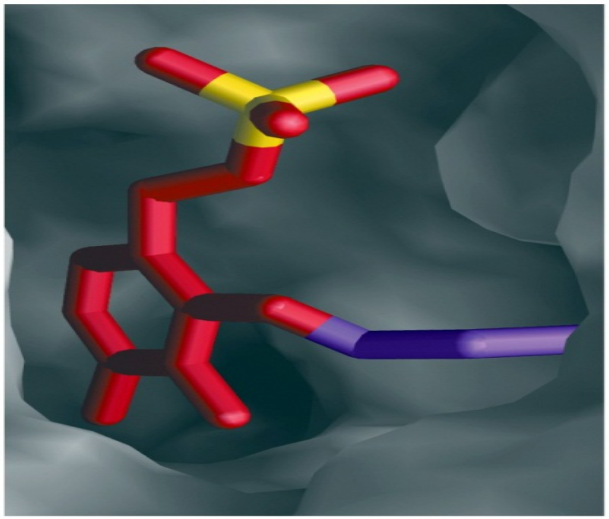
- Enzyme nucleophiles attack electrophilic centers in substrates forming covalently chemically bonded intermediates
- Electrophiles in substrates include:
  - Phosphoryl, acyl, and glycosyl groups

Covalent catalysis example: Aminotransferases

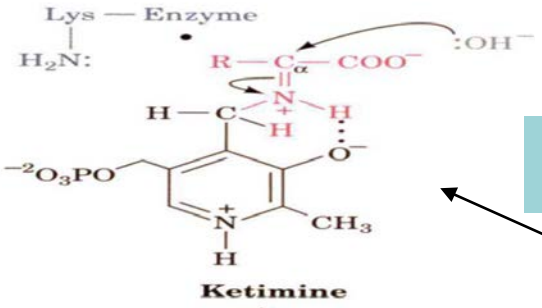


L6:F18-4

Hydrolyzed
by OH-

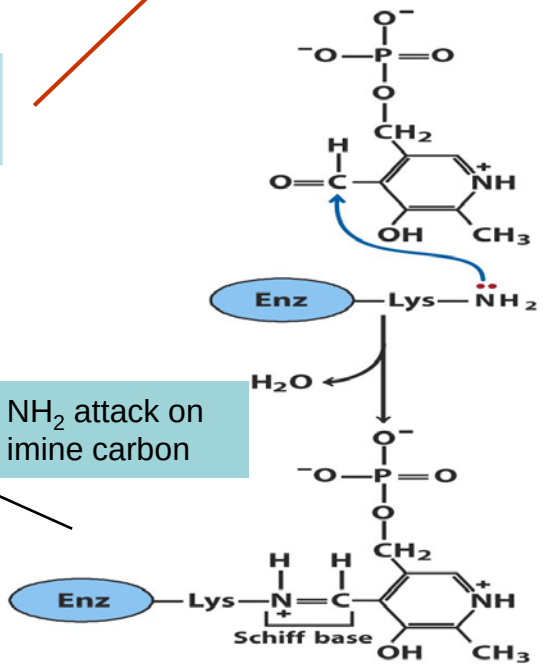


L6:F18-5



V&V:F26-2

NH_2 attack on
imine carbon

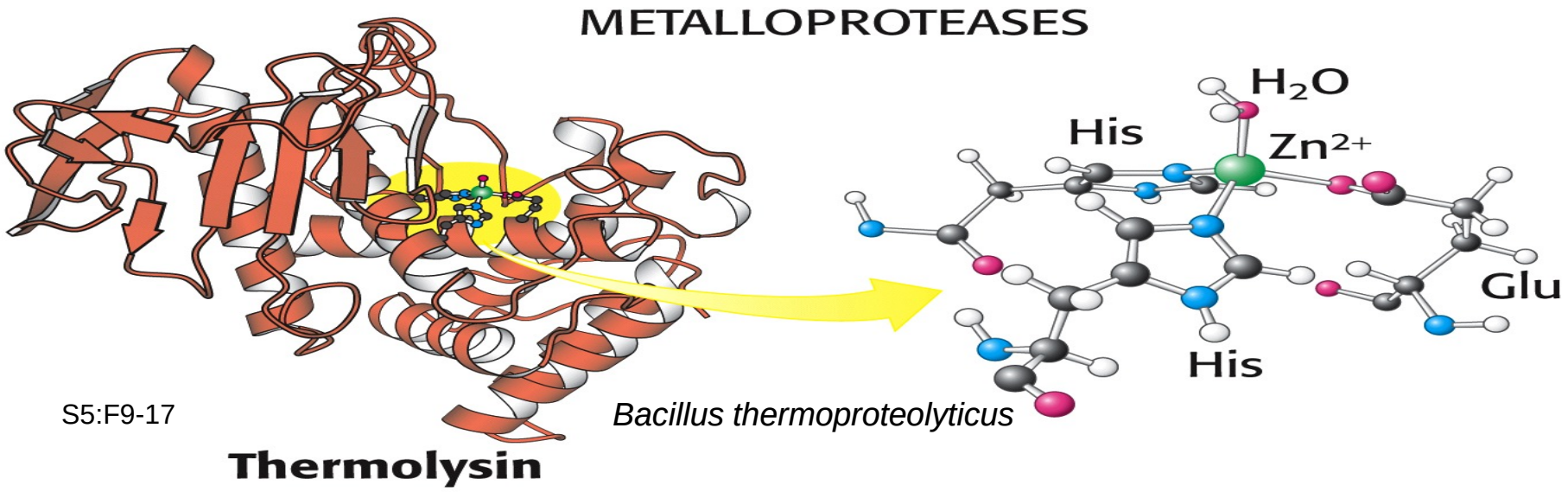


(6) Metal ion catalysis

- nearly one-third of all known enzymes require the presence of metal ions
- enzymes that bind the metal tightly are known as **metalloenzymes**
  - tightly bound metal ion cofactors such as: Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, or Co^{2+}
  - Metals usually held by **co-ordinate covalent** bonds to amino acid residues or bound to a prosthetic group
  - Metals are bound with high affinity (K_d = nanomolar)
- enzymes that bind metal ions more weakly are **metal-activated enzymes**
  - usually do not keep the metal ion bound but release it during the catalytic cycle

Thermolysin is a thermostable zinc endopeptidase (metalloenzyme)

- Contains a single Zn^{2+} and 4 Ca^{2+}
- Zn^{2+} has tetrahedral co-ordination with 3 ligands (2 His and 1 Glu side chain)
  - Fourth ligand is H_2O
- Zn^{2+} plays 2 roles
  - (1) polarizes C=O of the substrate
  - (2) facilitates deprotonation of the H_2O nucleophile



Thermolysin Reaction

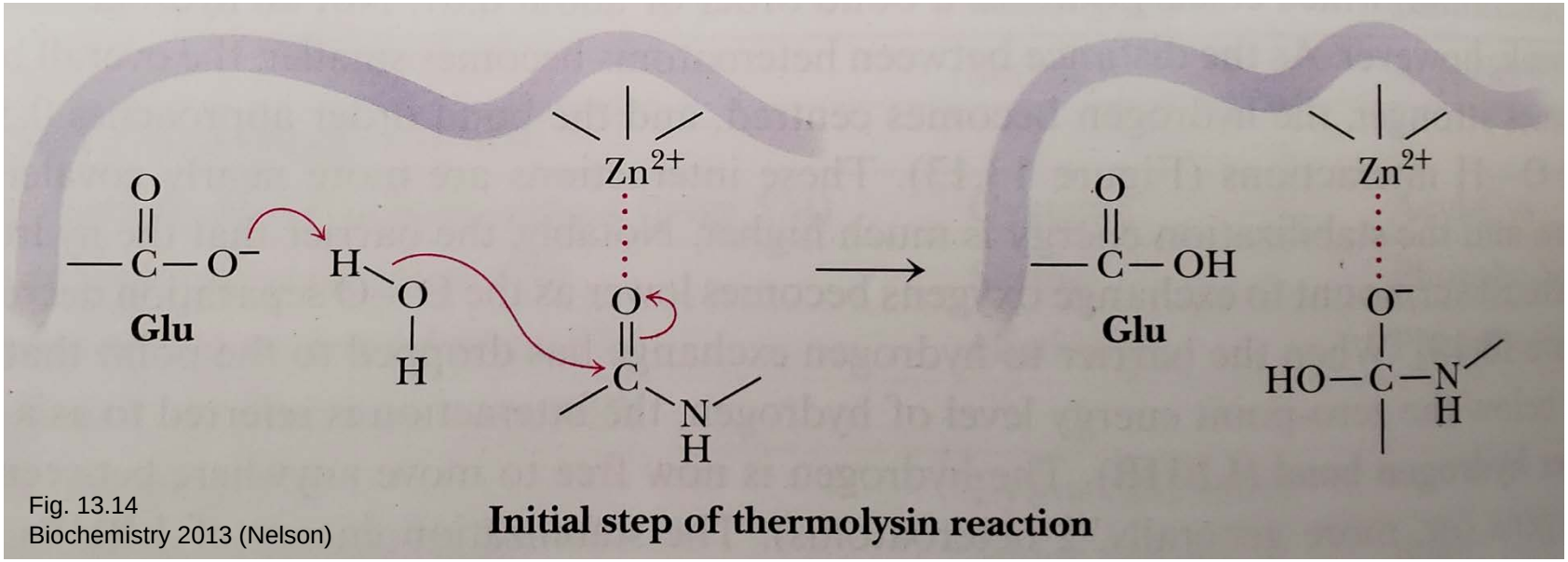


Fig. 13.14
Biochemistry 2013 (Nelson)

Initial step of thermolysin reaction

- Zn^{2+} ion stabilizes the build-up of negative charge on the peptide carbonyl group
- Glutamate residue deprotonates H_2O
- Promotes OH^- attack on the carbonyl carbon

Metal-activated enzymes

- loosely bind metal ions such as: Na^+, K^+, Mg^{2+}, or Ca^{2+} (K_d = micromolar)
- e.g., Mg^{2+} bound with ATP in kinases

- metal ions participate in **catalysis** in three ways:
- (1) binding to substrates to orient them properly
- (2) mediating oxidation-reduction reactions through reversible changes in metal ion's oxidation states
- (3) electrostatically stabilizing or shielding negative charges

- In several kinases, the true substrate is **not** ATP but Mg^{2+}-ATP

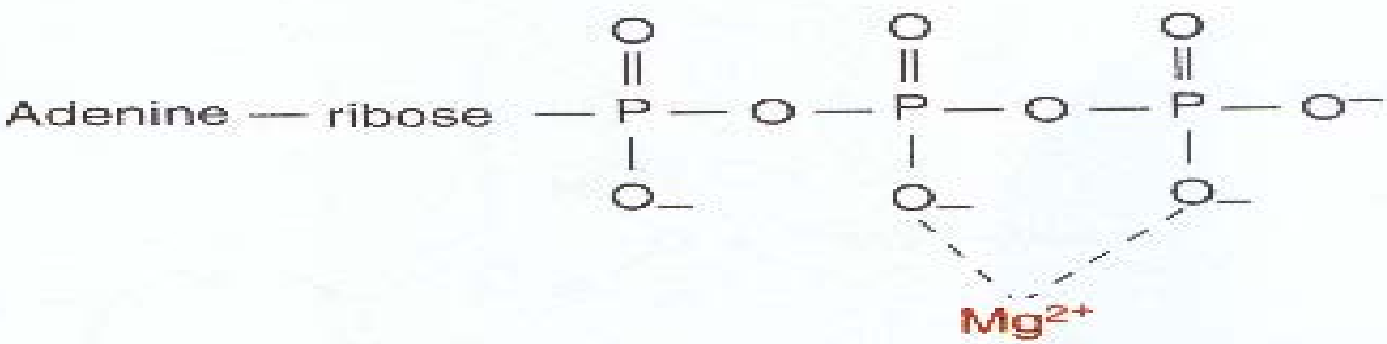
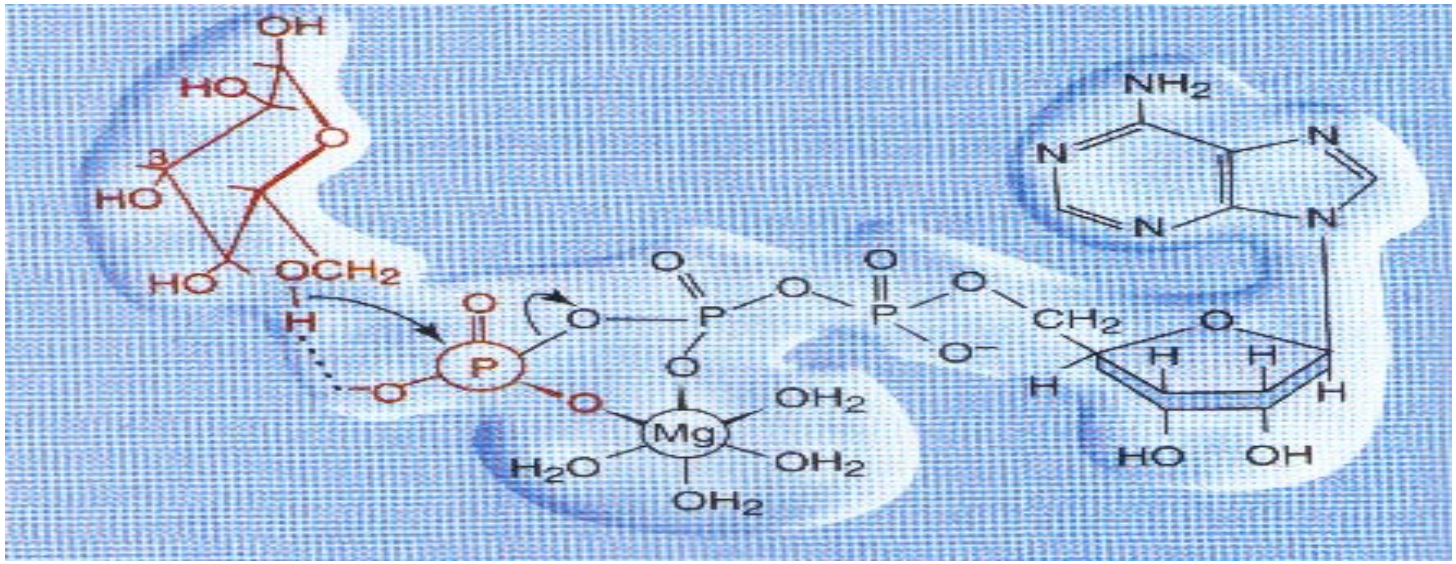


FIGURE 10.26
Structure of Mg^{2+}–ATP.

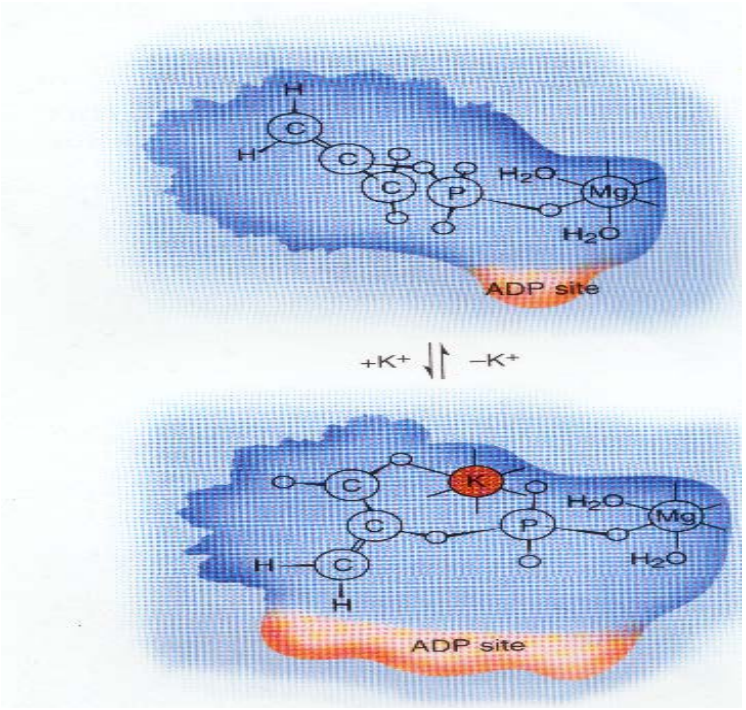
Devlin5:F10-26

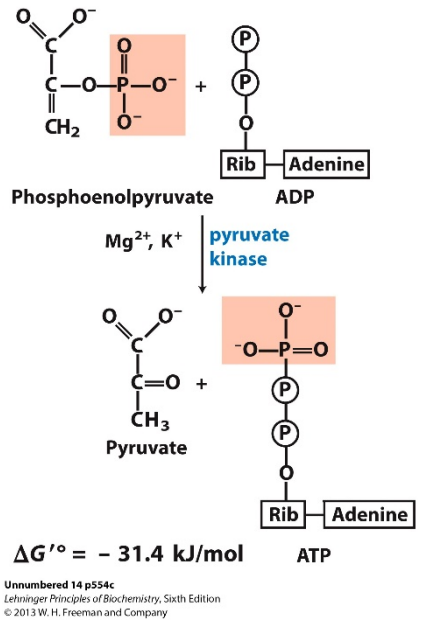


Devlin5:F10-27

- Mg^{2+} doesn't interact directly with enzyme but may neutralize the negative charge density on ATP
  - Ternary complexes of this conformation are called "**substrate-bridged**" complexes

Devlin5:F10-28





- Metals may bind directly to the substrate to stabilize it in the active conformation or to induce the formation of a binding site
- In pyruvate kinase, K^+ induces an initial conformational change that is necessary for ternary complex formation
- Upon substrate binding, K^+ induces a 2^{nd} conformational change to the catalytically active complex
- K^+ is <u>passive</u> in catalytic mechanism of pyruvate kinase **but is essential**

(7) Electrostatic catalysis

•binding of substrate by the enzyme excludes H_2O from active site

•local dielectric constant of the active site resembles that in an organic solvent

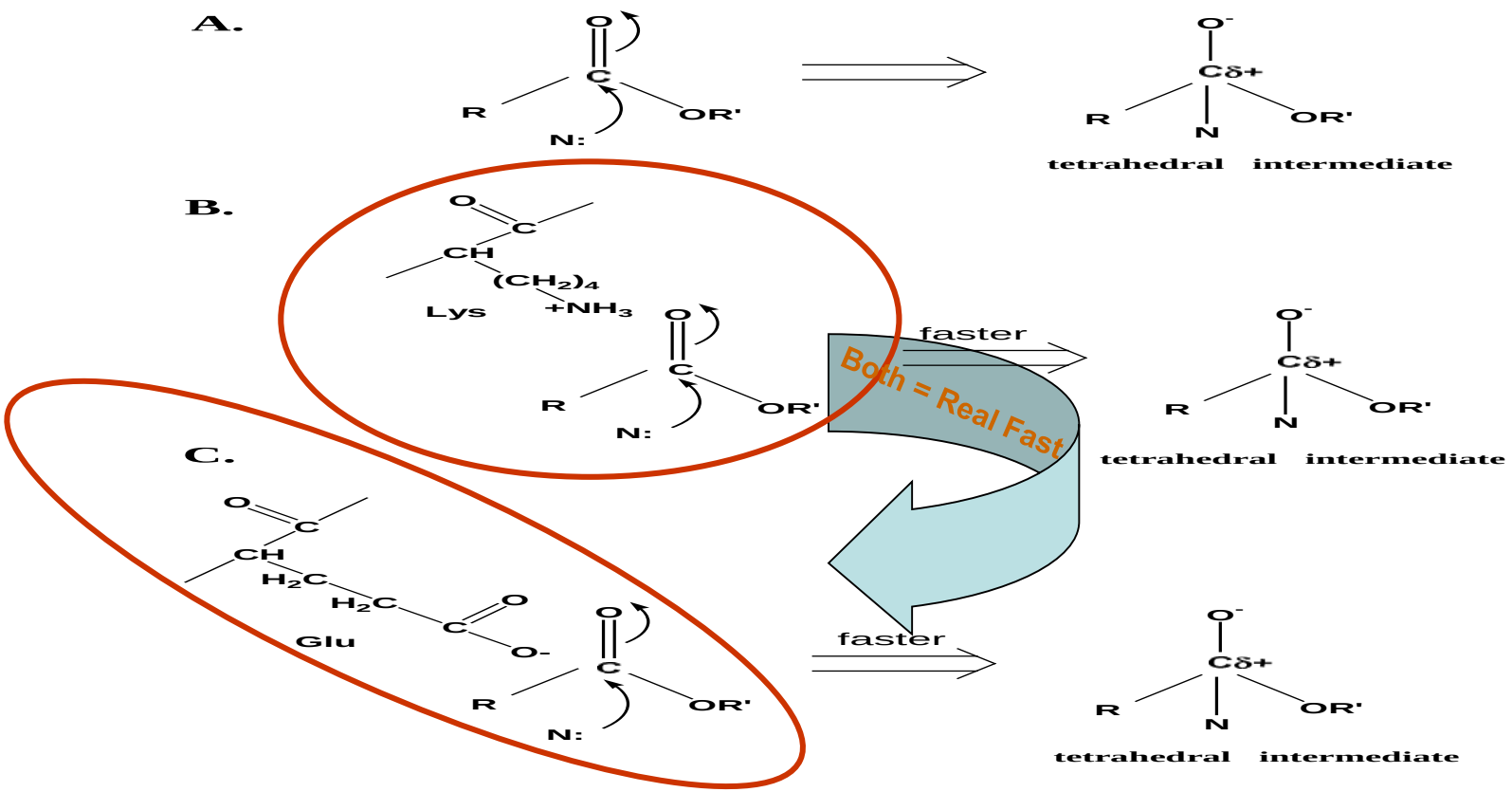
•enzymes act by stabilizing the distribution of electrical charge in transition states

•If transition state carries an electric charge absent in the ground state then the enzyme active site will include a chemical group with an opposite charge

•Stabilizes the transition state via electrostatic interactions

•Lys or Arg located near the oxygen atom of a carbonyl group would favour the formation of the tetrahedral intermediate, even if there were no transfer of a proton from the charged species to the oxygen

•A fixed negative charge in the region of the nucleophile would have a similar effect



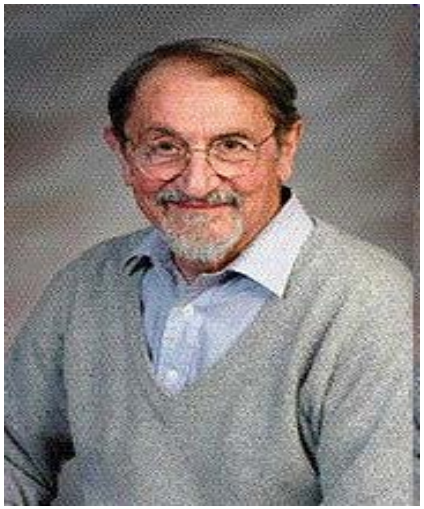
• as a reacting substrate changes into a **transition state**, the charges on its atoms interact with the charges on all of the other atoms in the surrounding protein, and also with the charges on any nearby water molecules

•the energy difference between the initial state and the transition state thus depends critically on the **details** of the protein structure

•**Computational Approach**

- combined with a wealth of structural information from X-ray crystallography and other biophysical studies
- it possible to calculate the contributions that various components of an enzyme's active site make to the activation free energy ($\Delta G^{\ddagger}$), and to predict quantitatively how $\Delta G^{\ddagger}$ might be altered by modifications of the protein

•can be tested experimentally by modifying the gene that encodes the protein (site-directed mutagenesis)



Prof Martin Karplus
Nobel Prize Chemistry 2013

Prof Michael Smith
Nobel Prize Chemistry 1993

(8) Low barrier H-bonds

-when the barrier to H-exchange has dropped to the zero point energy level for hydrogen

- Typical strength for an H-bond is 10 – 30 kJ/mol

  - •For **O-H-O** H-bond the O-O separation is typically 2.8Å and is **relatively weak electrostatic interaction**

  - •The H is firmly linked to one of the oxygens at a distance of 1Å and distance to the other oxygen is 1.8Å

  - •Bond order of the O----H bond is 0.07

  - •Not all H-bonds are weak: as the distance between the heteroatoms decreases, the hydrogen becomes

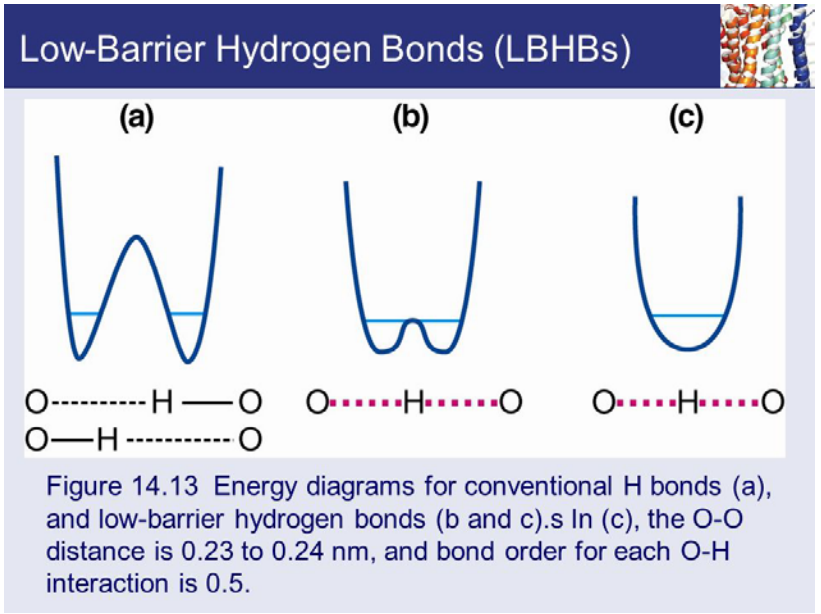
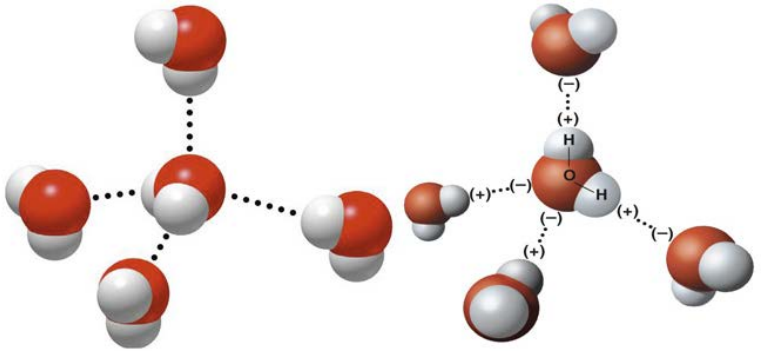


Figure 14.13 Energy diagrams for conventional H bonds (a), and low-barrier hydrogen bonds (b and c).s In (c), the O-O distance is 0.23 to 0.24 nm, and bond order for each O-H interaction is 0.5.



The Low Barrier Hydrogen Bond in Enzymatic Catalysis*

W. Wallace Cleland[‡§], Perry A. Frey[‡] and John A. Gerlt[¶]
[‡]Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53705 and
[¶]Department of Biochemistry, University of Illinois, Urbana, Illinois 61801

October 2, 1998 *J. Biol. Chem.* **273**, 25529-25532.

centered and the **bond order approaches 0.5** for both O-H interactions

•Importantly: the energy barrier that the H-atom must surmount to exchange oxygens becomes **lower** as the O-O separation decreases

•When the barrier to H-exchange becomes the **zero point** energy level for hydrogen the interaction is referred to as a **low-barrier H-bond (LBHB)**

•The hydrogen is now free to move anywhere between the two oxygens (or heteroatoms)

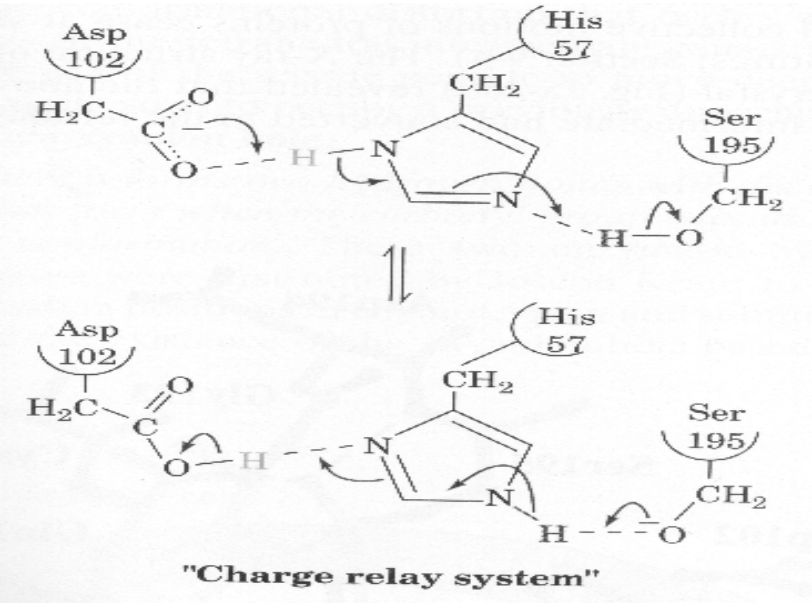
•The stabilization energy of LBHBs may approach 60- 100 kJ/mol in solution

•LBHBs require matched pK's for the two electronegative atoms that share the hydrogen

How may LBHBs affect enzyme catalysis?

•A weak H-bond in an enzyme ground state may become a LBHB in a transient intermediate or in the **transition-state** for the reaction

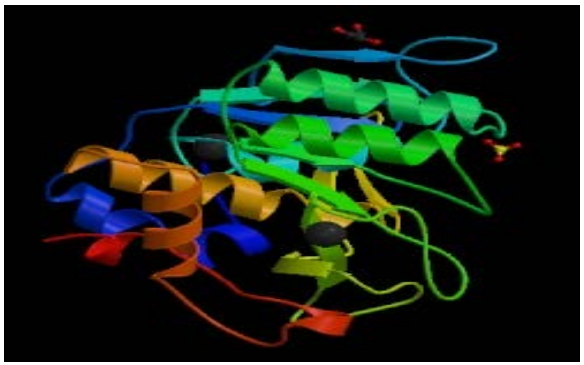
•Energy released in forming the LBHB may be used to help the reaction by lowering the activation barrier for the reaction



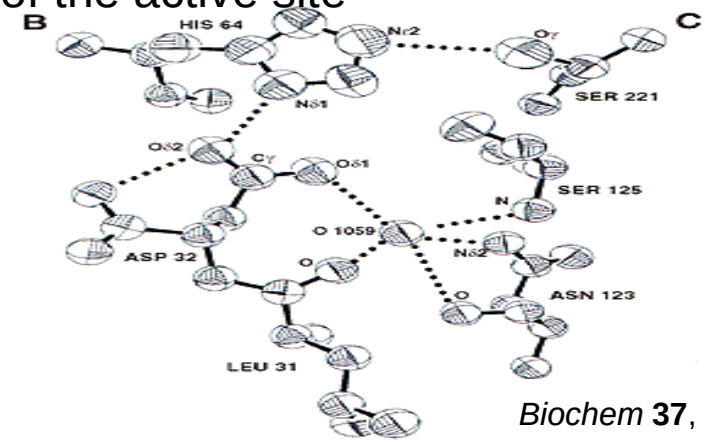
V&V:pg 526

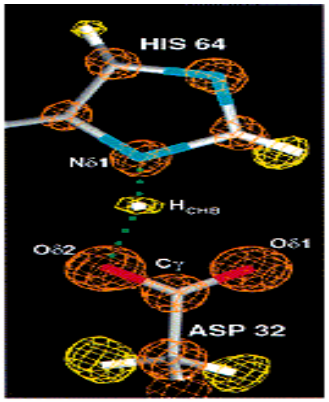
- Previously postulated that Asp-102 polarized His-57 which assisted to abstract a proton from Ser-195
- Converts a weakly nucleophilic (—CH_2OH) group to a highly nucleophilic alkoxide ion (— CH_2O^-)
- Anionic charge of Asp-102 believed to be transferred via a tautomeric shift of His-57 to Ser-195
- <u>Problem:</u> alkoxide ion (pK > 15) has far greater proton affinity than His-57 (pK = 7)

- W.W. Cleland and others explained the reaction by LBHBs
- In the transition state the pKs for His-57(64) and Asp-102(32) are nearly equal in the **anhydrous environment** of the active site



Bacillus subtilisin 0.78 Å X-ray structure

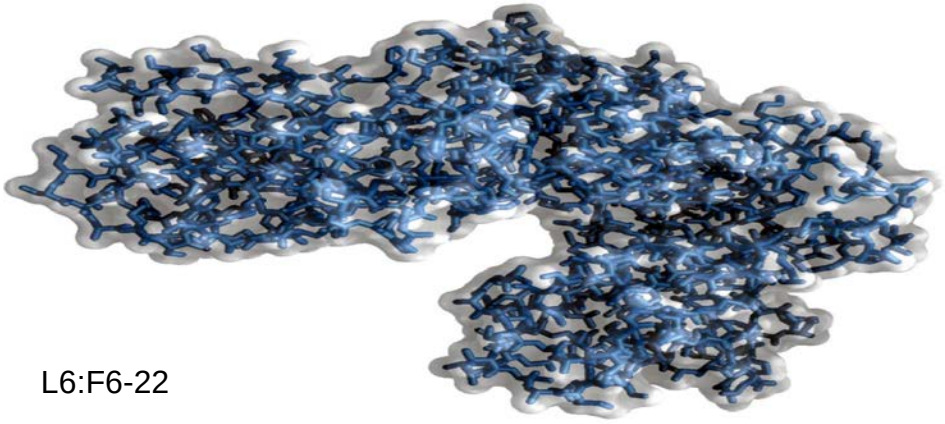


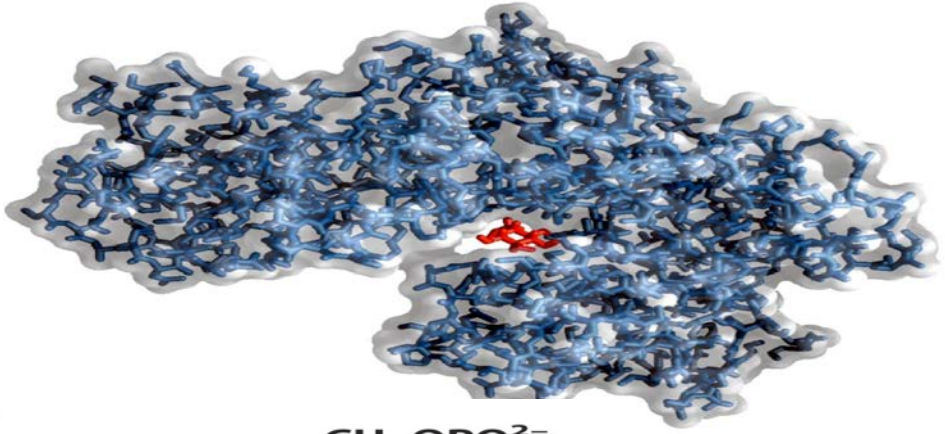


Biochem **37**, 13446-52 (1998)

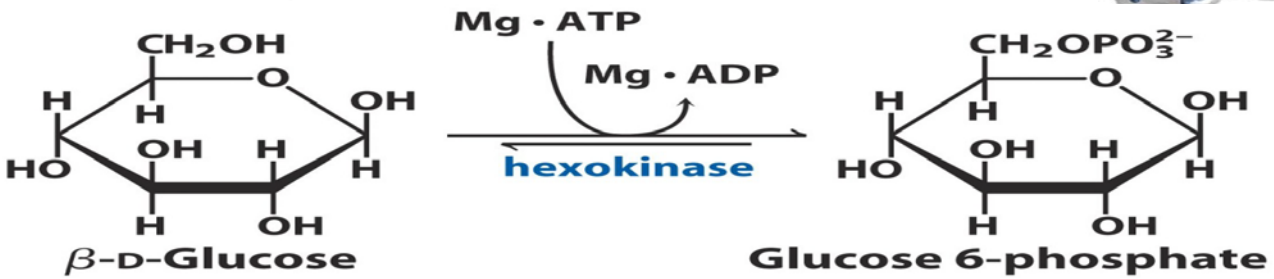
(9) Structural Flexibility and Enzyme Dynamics

• some enzymes undergo major structural rearrangements when they bind substrates or inhibitors





L6:F6-22



• hexokinase binds glucose, it undergoes a structural organization that brings together the elements of the active site

• the enzyme literally closes like a set of jaws around the substrate referred to as an **induced fit**

•rearrangement of the protein pulls the hydrophobic part of the substrate out of the aqueous soln by surrounding it with nonpolar portions of the protein

Advantages:

(1) maximizes the favorable entropy change associated with removing a hydrophobic molecule from H_2O

(2) allows enzyme to intensify the electrostatic effects to promote the formation of the transition state

(3) the substrate is forced to respond to the directed electrostatic fields from the enzyme's functional groups, instead of the disordered fields from the solvent

(4) structural change explains the high specificity of some enzymatic reactions

- hexokinase undergoes a structural change upon binding glucose which promotes the binding of the ATP substrate
- ATP doesn't bind unless glucose is already present in the catalytic site

- if ATP binds in the absence of glucose, the enzyme may catalyze the transfer of phosphate from ATP to water, resulting in a wasteful loss of ATP: create heat ≡ futile cycle

• 5 carbon sugar, xylose (stereochemically similar to glucose but 1 carbon shorter) binds to hexokinase but in a position where it can not be phosphorylated

- Addition of xylose increases the rate of ATP hydrolysis
- Hexokinase is "tricked" into phosphorylating H_2O

$ATP + H_2O \rightarrow ADP + P_i$

- Enzyme function is controlled by local and global motions
  - Protein dynamic timescales overlap with those associated with catalysis
  - Ligand diffusion into the binding site
  - Mediates induced fit of the protein to the ligand
  - Changes physical properties of the binding site
  - Optimizes quantum phenomena

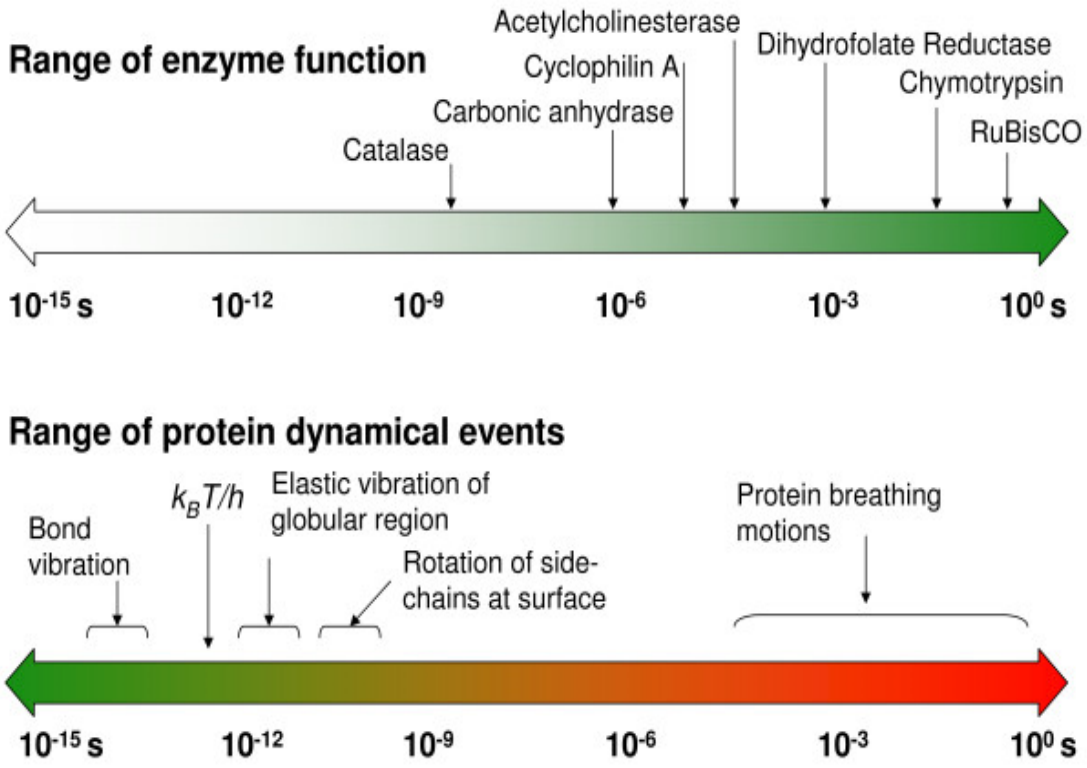


Fig. 9.31 Proteins 2018

TABLE 11.3 Timescale of protein motions related to catalysis

Motion	Approximate Time Scale (seconds)
Bond vibration	10^{-13} to 10^{-14}
Proton transfer	10^{-12}
Hydrogen bonding	10^{-11} to 10^{-12}
Elastic vibration of globular region	10^{-11} to 10^{-12}
Sugar repuckering	10^{-9} to 10^{-12}
Rotation of side chains at surface	10^{-10} to 10^{-11}
Torsional libration of buried groups	10^{-9} to 10^{-11}
Hinge bending at domain interfaces	10^{-7} to 10^{-11}
Water structure reorganization	10^{-8}
Helix-coil breakdown/ formation	10^{-7} to 10^{-8}
Allosteric transitions	1 to 10^{-5}
Local denaturation	10 to 10^{-5}
Rotation of medium-sized side chains in interior	1 to 10^{-4}

Data from *Science* 301:1196–1202 (2003), S. J. Benkovic and S. Hammes-Schiffer, A perspective on enzyme catalysis.