Molecular Cell Biology A

"Protein function and regulation"

BIOX24ZL

Tuesdays 9-10:30

Ray LC

Protein binding sites are highly specific for its ligand.

- Protein binding sites are specific for certain ligands via noncovalent interactions, like glove
- Binding site restricts access to water which bond to each other in network
- Site reactive by having polar groups together
- Evolutionary tracing to identify seq regions critical to function that are invariant in evolution, usually found in binding sites

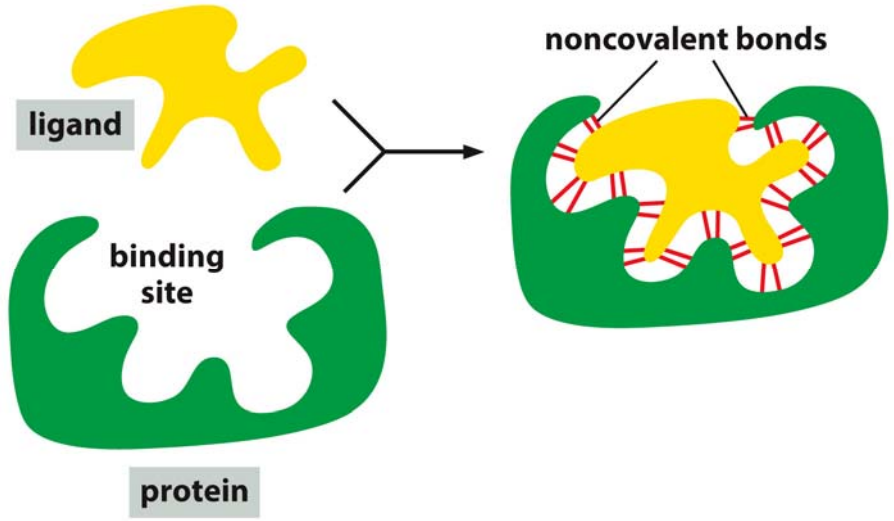
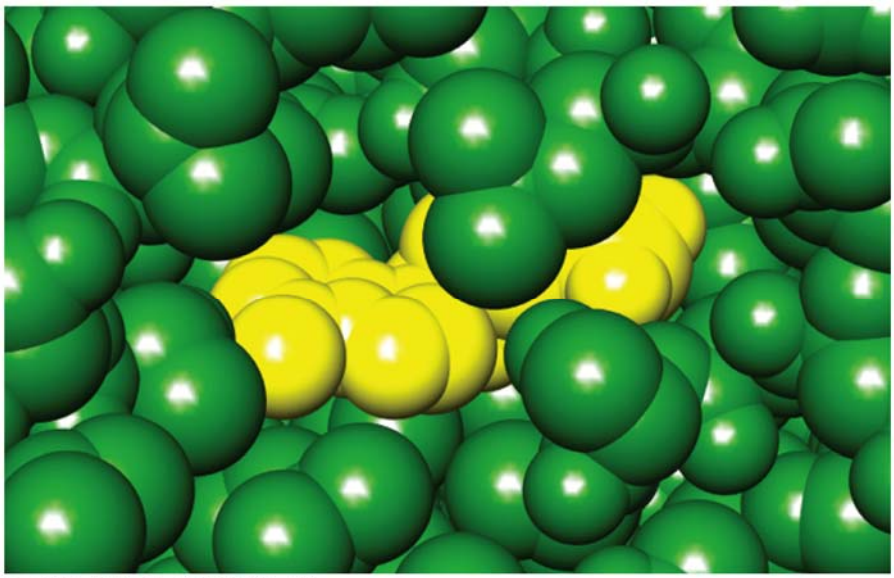


Figure 3-37a Molecular Biology of the Cell 6e (© Garland Science 2015)



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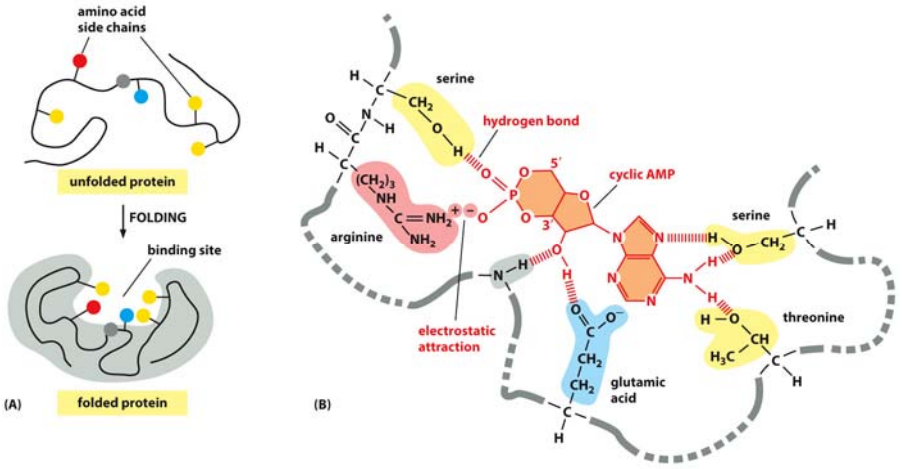

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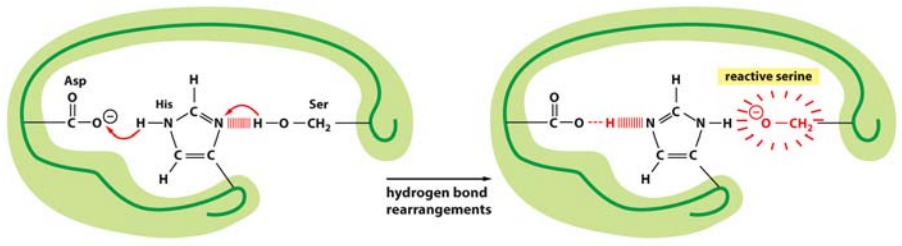

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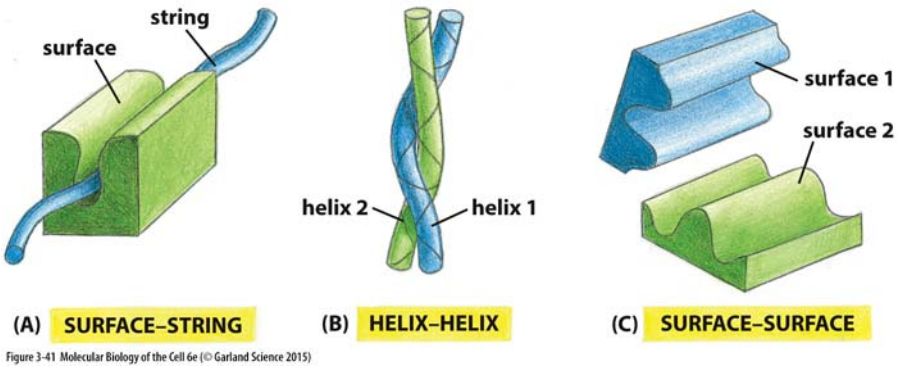
Three main types of protein binding characterize protein interactions.

- Extended loop such as phosphorylated group
- Coiled coil of one helix with another
- Matching of surfaces, many bonds, tight
- Antibodies binding to antigens with two identical sites: example of specific loops
- Equilibrium constant K measures strength of binding (in L/mol): steady state when binding per second is same as unbinding



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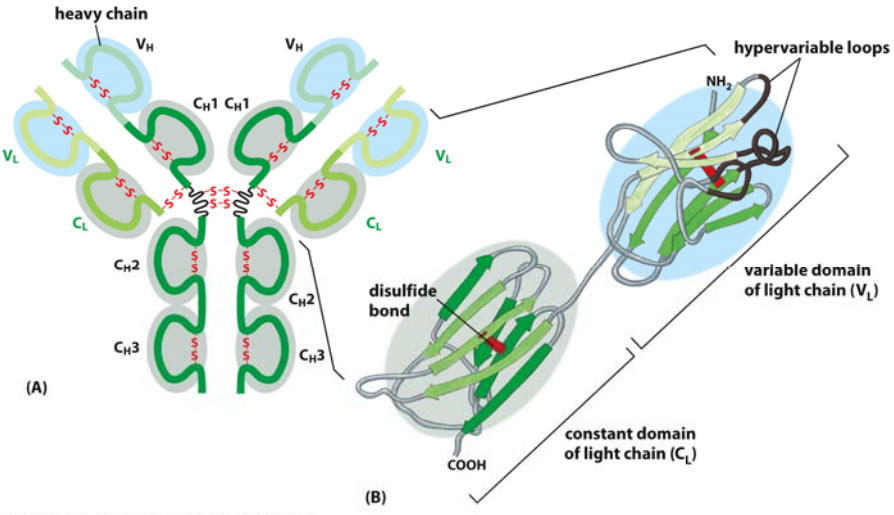

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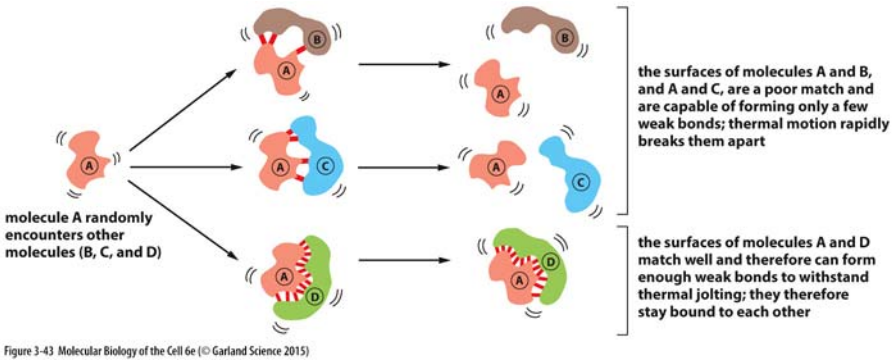

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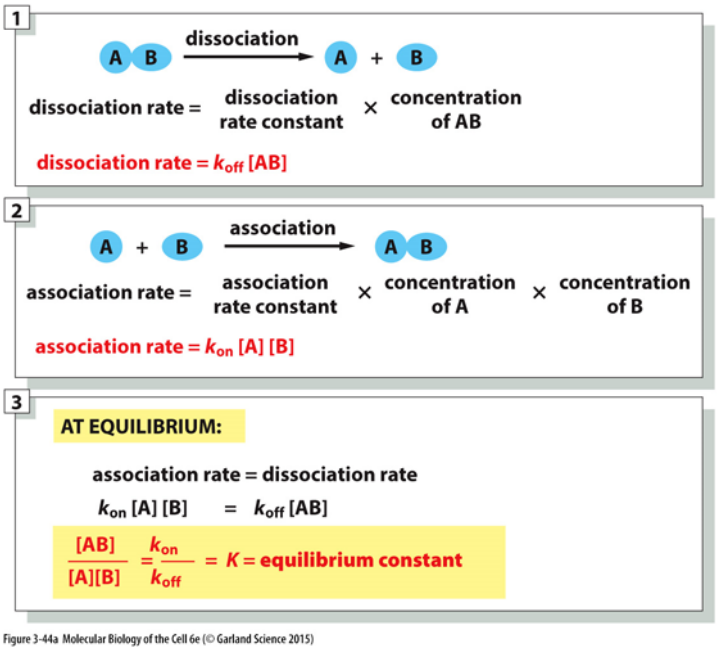


Figure 3-44a Molecular Biology of the Cell 6e (© Garland Science 2015)

Enzymes are proteins that catalyze reactions: substrates -> products.

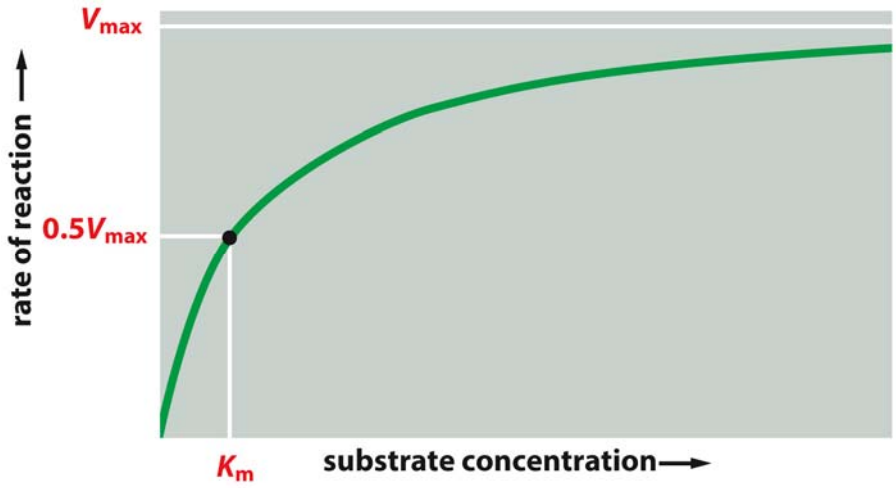
- E + S -> ES -> EP -> E + P
- Vmax = rate of reaction when fully saturated
- Vmax / enzyme conc = turnover number = Kcat
- Km = substrate conc at 0.5Vmax, low Km means tight binding, high Km weak binding
- Increases local substracte conc
- Lowers activation energy to transition state by binding tightly to the transition state
- As acid (proton donor) and base (proton accept)

TABLE 3–1 Some Common Types of Enzymes

Enzyme	Reaction catalyzed
Hydrolases	General term for enzymes that catalyze a hydrolytic cleavage reaction; *nucleases* and *proteases* are more specific names for subclasses of these enzymes
Nucleases	Break down nucleic acids by hydrolyzing bonds between nucleotides. *Endo–* and *exonucleases* cleave nucleic acids *within* and *from the ends of* the polynucleotide chains, respectively
Proteases	Break down proteins by hydrolyzing bonds between amino acids
Synthases	Synthesize molecules in anabolic reactions by condensing two smaller molecules together
Ligases	Join together (ligate) two molecules in an energy-dependent process. DNA ligase, for example, joins two DNA molecules together end-to-end through phosphodiester bonds
Isomerases	Catalyze the rearrangement of bonds within a single molecule
Polymerases	Catalyze polymerization reactions such as the synthesis of DNA and RNA
Kinases	Catalyze the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins
Phosphatases	Catalyze the hydrolytic removal of a phosphate group from a molecule
Oxido-Reductases	General name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often more specifically named *oxidases*, *reductases*, or *dehydrogenases*
ATPases	Hydrolyze ATP. Many proteins with a wide range of roles have an energy-harnessing ATPase activity as part of their function; for example, motor proteins such as *myosin* and membrane transport proteins such as the *sodium–potassium pump*
GTPases	Hydrolyze GTP. A large family of GTP-binding proteins are GTPases with central roles in the regulation of cell processes

Enzyme names typically end in "-ase," with the exception of some enzymes, such as pepsin, trypsin, thrombin, and lysozyme, that were discovered and named before the convention became generally accepted at the end of the nineteenth century. The common name of an enzyme usually indicates the substrate or product and the nature of the reaction catalyzed. For example, citrate synthase catalyzes the synthesis of citrate by a reaction between acetyl CoA and oxaloacetate.

Table 3-1 Molecular Biology of the Cell 6e (© Garland Science 2015)



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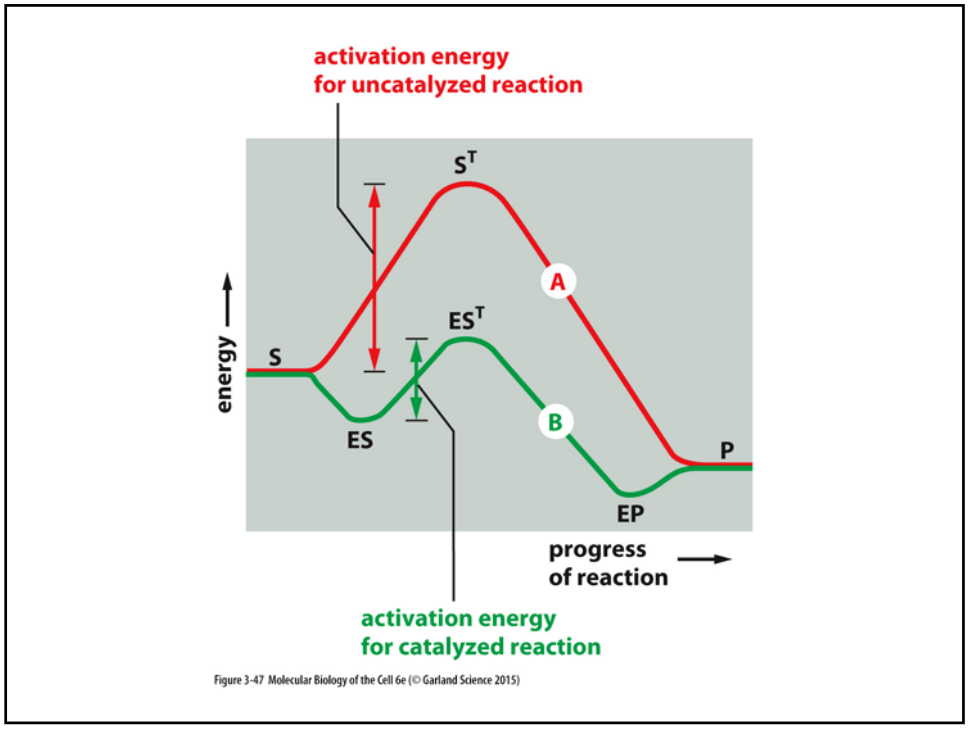

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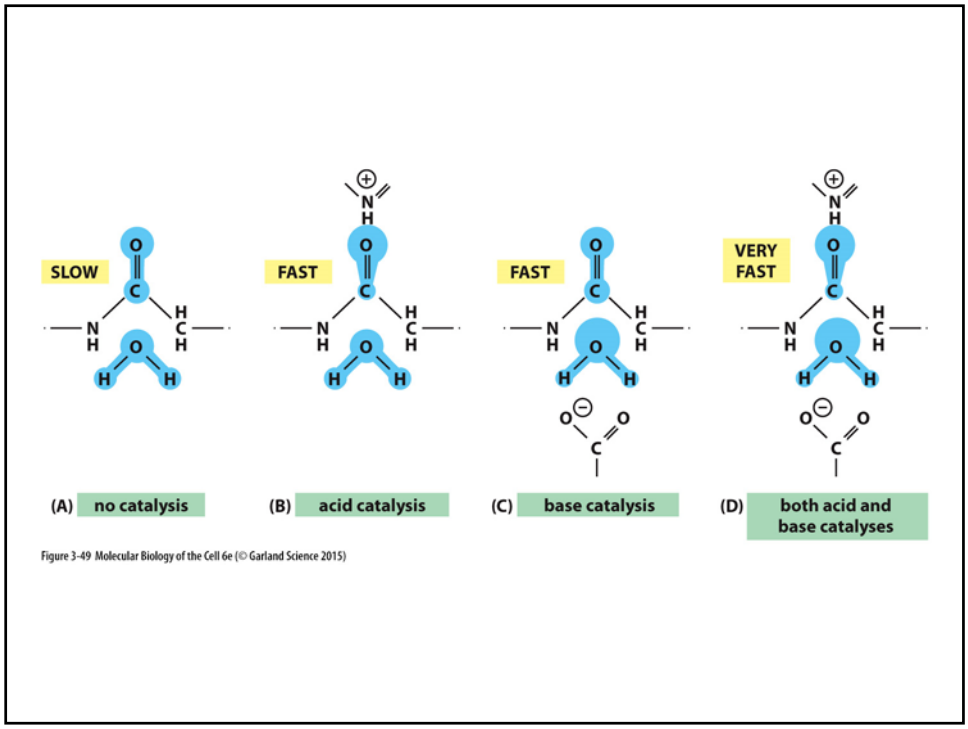


Figure 3-49 Molecular Biology of the Cell 6e (© Garland Science 2015)

STEADY-STATE ENZYME KINETICS

Many enzymes have only one substrate, which they bind and then process to produce products according to the scheme outlined in Figure 3–50A. In this case, the reaction is written as

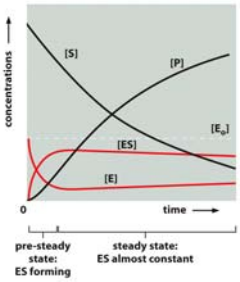
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

Here we have assumed that the reverse reaction, in which E + P recombine to form EP and then ES, occurs so rarely that we can ignore it. In this case, EP need not be represented, and we can express the rate of the reaction—known as its velocity, V, as

$$V = k_{cat}[ES]$$

where [ES] is the concentration of the enzyme–substrate complex, and k_{cat} is the turnover number, a rate constant that has a value equal to the number of substrate molecules processed per enzyme molecule each second.

But how does the value of [ES] relate to the concentrations that we know directly, which are the total concentration of the enzyme, $[E_0]$, and the concentration of the substrate, [S]? When enzyme and substrate are first mixed, the concentration [ES] will rise rapidly from zero to a so-called steady-state level, as illustrated below.



Panel 3-2 (Part 2) Molecular Biology of the Cell 6e (© Garland Science 2015)

At this steady state, [ES] is nearly constant, so that

$$\text{rate of ES breakdown } k_{-1}[ES] + k_{cat}[ES] = \text{rate of ES formation } k_1[E][S]$$

or, since the concentration of the free enzyme, [E], is equal to $[E_0] - [ES]$,

$$[ES] = \left(\frac{k_1}{k_{-1} + k_{cat}}\right)[E][S] = \left(\frac{k_1}{k_{-1} + k_{cat}}\right)\left([E_0] - [ES]\right)[S]$$

Rearranging, and defining the constant K_m as

$$\frac{k_{-1} + k_{cat}}{k_1}$$

we get

$$[ES] = \frac{[E_0][S]}{K_m + [S]}$$

or, remembering that $V = k_{cat}[ES]$, we obtain the famous Michaelis–Menten equation

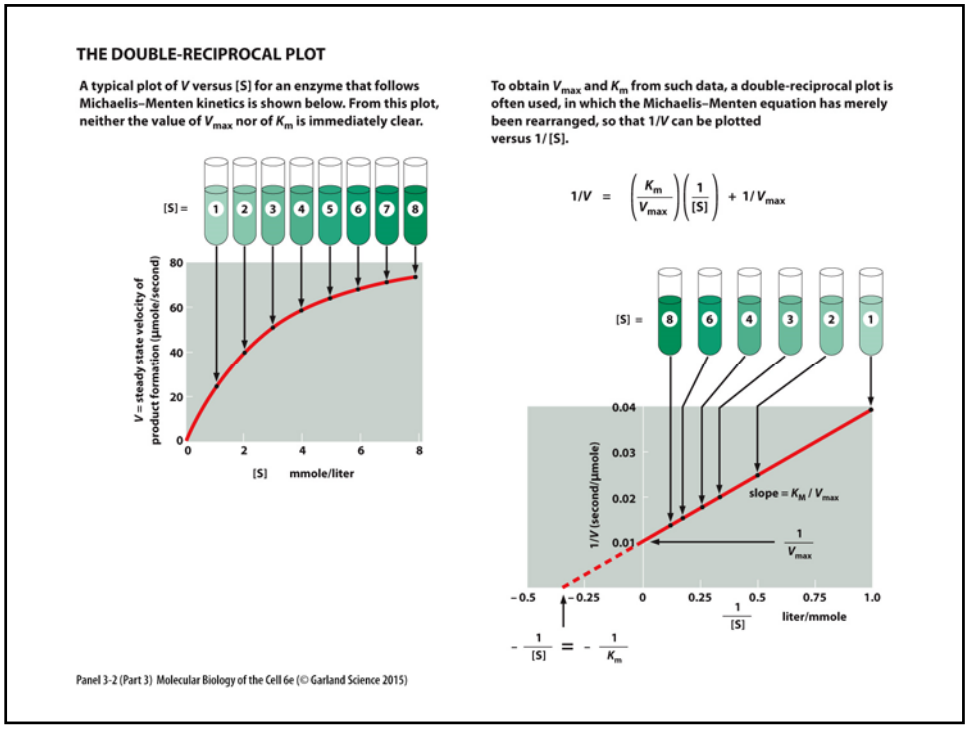
$$V = \frac{k_{cat}[E_0][S]}{K_m + [S]}$$

As [S] is increased to higher and higher levels, essentially all of the enzyme will be bound to substrate at steady state; at this point, a maximum rate of reaction, V_{max}, will be reached where $V = V_{max} = k_{cat}[E_0]$. Thus, it is convenient to rewrite the Michaelis–Menten equation as

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

Enzyme kinetics can be understood most easily at steady state.

- Define ratio of rates Km = (K- + Kcat)/K1,
- And rate of reaction as V = Kcat[ES],
- Then Km[ES] = [E0][S] – [ES][S],
- And [ES](Km + [S]) = [E0][S],
- And V = Kcat[E0][S] / (Km + [S]),
- But Vmax = Kcat[E0] when all enzymes work,
- So V = Vmax[S] / (Km + [S]),
- Notice: when [S] large, Km << [S], V = Vmax

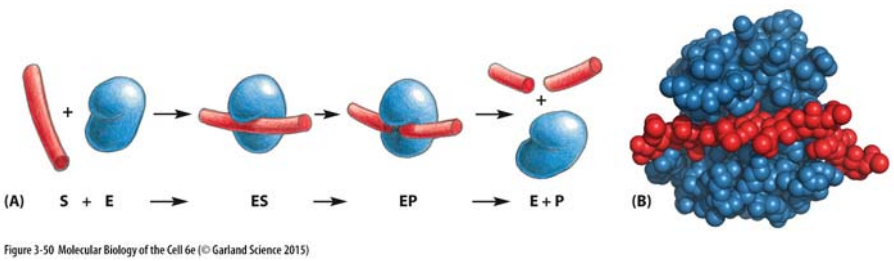


Enzyme kinetics can be understood most easily at steady state.

- Get Vmax from when 1/[S] = 0 (i.e. [S] infinite)
- Get Km (mmol per liter) from when 1/V = 0,
- Or V = Vmax/2, then 2 = Km/[S] + 1, Km = [S]
- Small Km tight binding V curve vs [S] steeper
- If Kcat is much smaller than K- then Km is dissociation constant K-/K1, or [E][S]/[ES]
- When [S] << Km then [E] = [E0],
  V = Kcat/Km[E][S], Kcat/Km rate constant for reaction of E + S, measures enzyme effectiveness

Lysozyme catalyzes cleavage of bacteria polysaccharides by hydrolysis.

- Activation energy usually high for hydrolysis
- Lysozyme activate site long groove holds 6 linked sugars cut 2 using two acidic chains
- Aspartate covalent bond to C1, other side glutamate donates proton to neighbor oxygen
- Attracted by negative glutamate, water reacts with C1 and releases aspartate back to normal
- Enzymes can use conformational strategies, stablizes transition state over stable form



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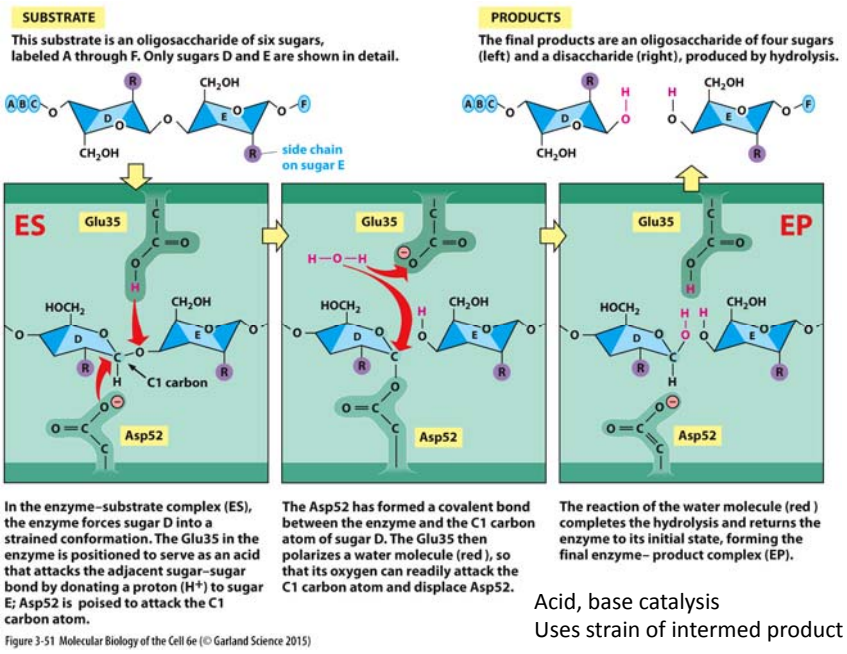
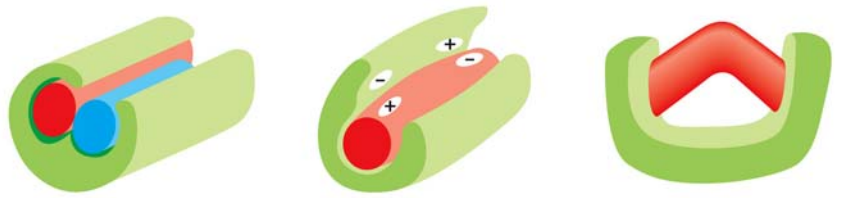


Figure 3-51 Molecular Biology of the Cell 6e (© Garland Science 2015)

Acid, base catalysis
Uses strain of intermed product


(A) enzyme binds to two substrate molecules and orients them precisely to encourage a reaction to occur between them

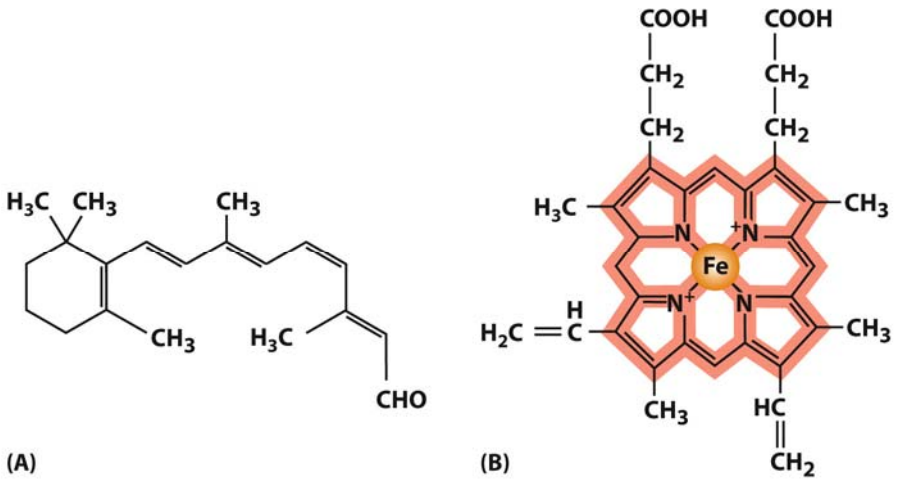
(B) binding of substrate to enzyme rearranges electrons in the substrate, creating partial negative and positive charges that favor a reaction

(C) enzyme strains the bound substrate molecule, forcing it toward a transition s tate to favor a reaction

Figure 3-52 Molecular Biology of the Cell 6e (© Garland Science 2015)

Enzyme evolved accessories and mechanisms for speeding reactions.

- Enzymes often need outside molecules, e.g. rhodopsin with retinal (from vit A), iron for hemoglobin, coenzymes biotin (move COO-)
- Efficient enzymes are only diffusion limited
- Molecular tunnels connect two active sites to link unstable intermediates preventing escape
- Multienzyme complex to channel intermed from one enzyme directly to another in path
- Membrane segregation (euk) reduce diffusion



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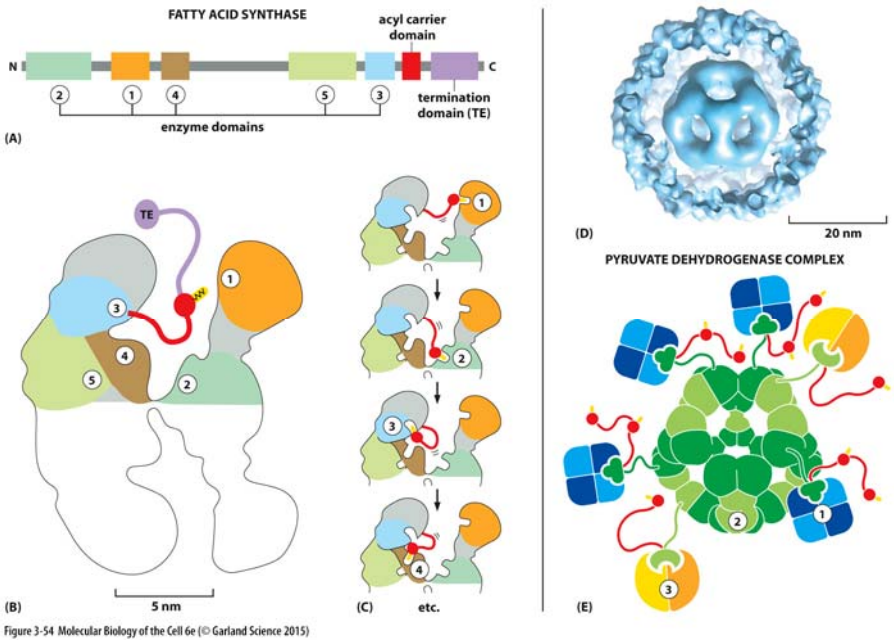


Figure 3-54 Molecular Biology of the Cell 6e (© Garland Science 2015)

Team work.

The turnover number for an enzyme is equivalent to the number of substrate molecules processed per second per enzyme molecule. To a test tube containing a 100 mM concentration of its substrate, you have added an enzyme at a final concentration of 10 µM, and have measured the rate of the reaction to be approximately 500 µM/sec. If the K_m for the binding of the enzyme to this substrate is about 100 mM, what is the turnover number?

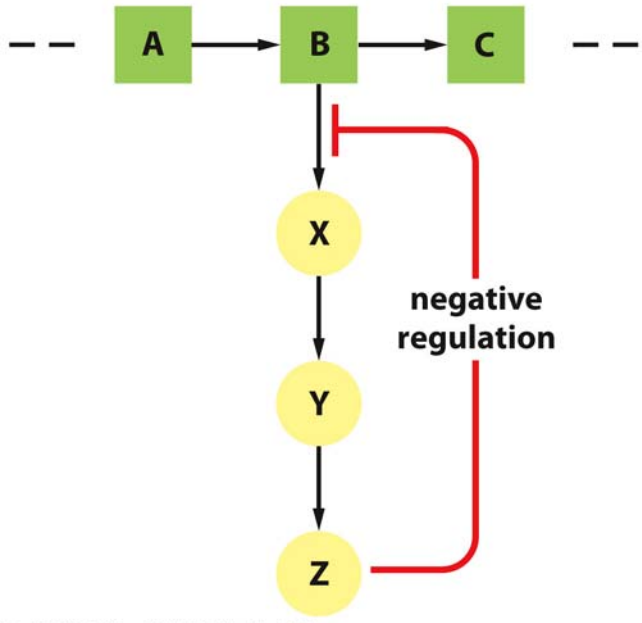
- A. 500
- B. 10,000
- C. 1000
- D. 100
- E. 5000

Enzymes can catalyze cellular reactions through various mechanisms. Which of the following statements is NOT true regarding enzymes?

- A. They can provide the chemical groups necessary for simultaneous acid and base catalysis.
- B. They have a higher affinity for the transition state of the substrate than for its stable form.
- C. They can form covalent bonds with the substrate during catalysis.
- D. They accelerate a cellular reaction by destabilizing the transition state.
- E. They can strain a substrate to force it toward a specific transition state.

Enzymes are regulated by the cell most directly by feedback

- Enzyme regulation of expression, confinement destruction, reversible change in activity
- Feedback neg inhibition, positive feedback
- Allosteric control: separate regulatory site bind causes conformational change (conc dep)
- Linkage of ligand binding can lead to positive (both prefer same conformation) or negative (each prefer diff conformation) regulation



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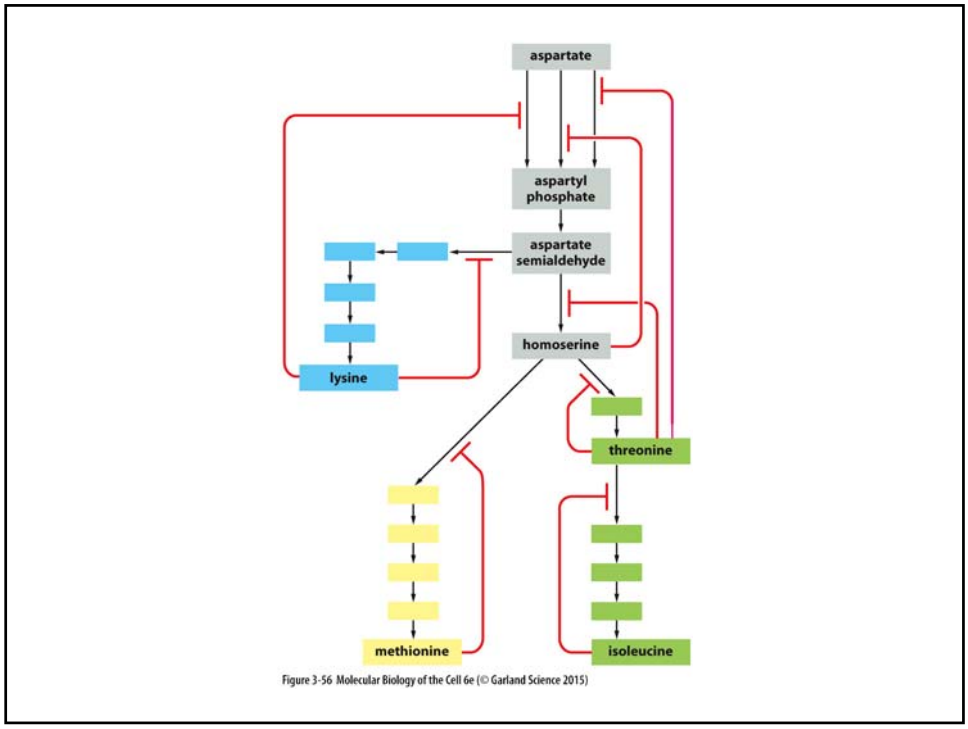

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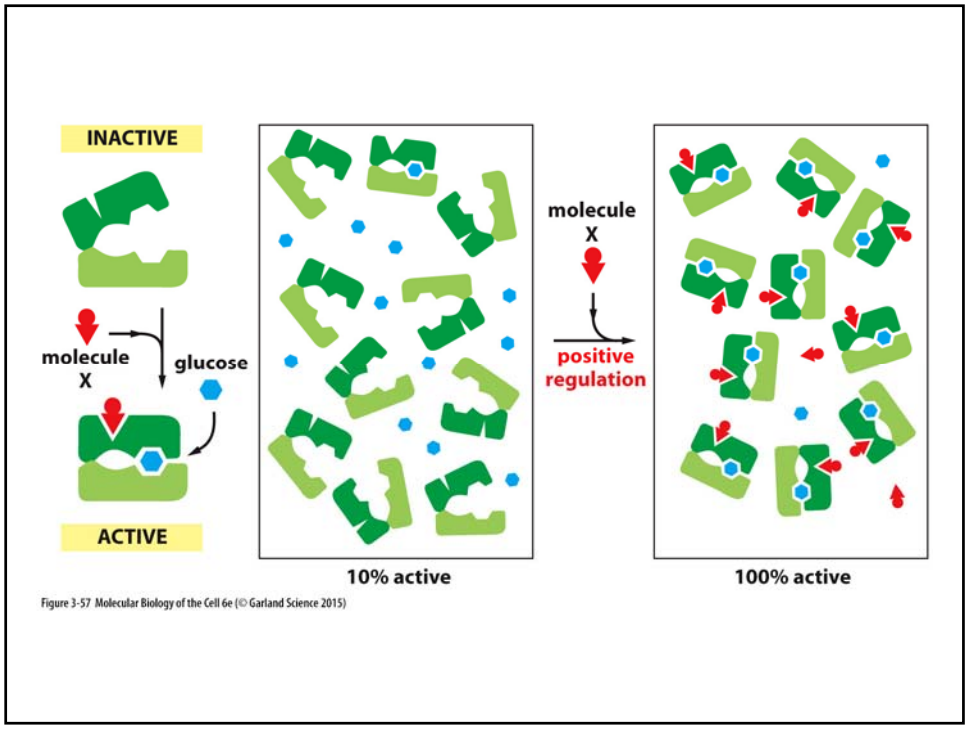

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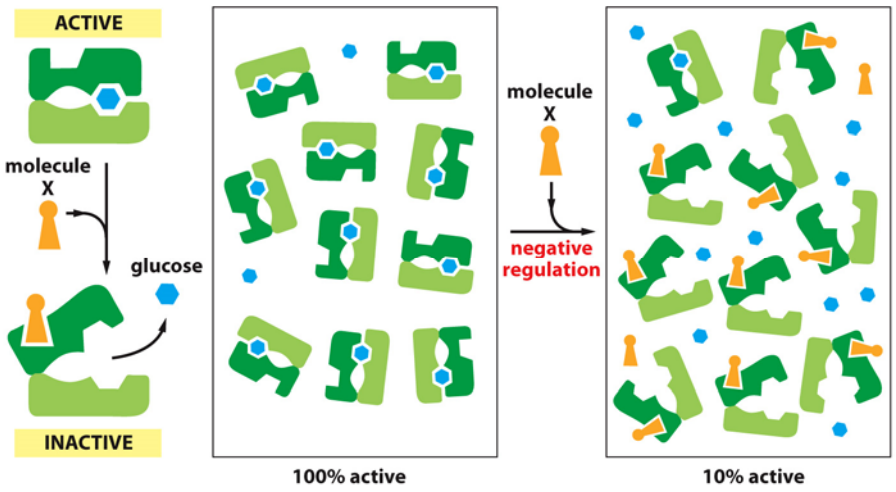
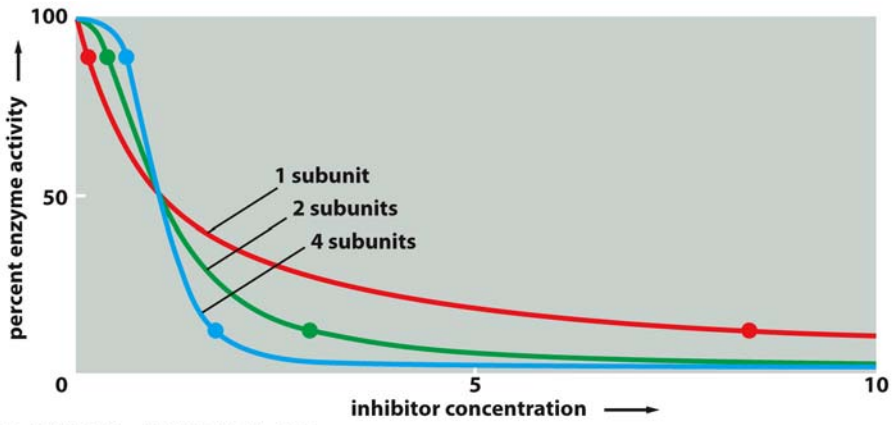


Figure 3-58 Molecular Biology of the Cell 6e (© Garland Science 2015)

Phosphorylation and cooperativity modulate enzyme function.

- Cooperative enzymes for symmetric structs so that allosteric molec lead to coop changes
- Phosphorylation (-2) attract positively charged AAs, make binding site recognized by others
- ATP coupled kinases can activate or inactivate, phosphatases remove such modification
- Cyclin-dependent kinases cdk activity requires binding of cyclin, threonine phosphorylation, tyrosine dephosphorylation -> cell cycle step
- Src tyr kinase: remove C-phos, activate SH3



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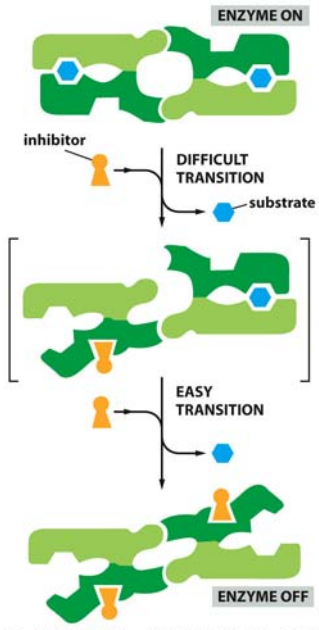

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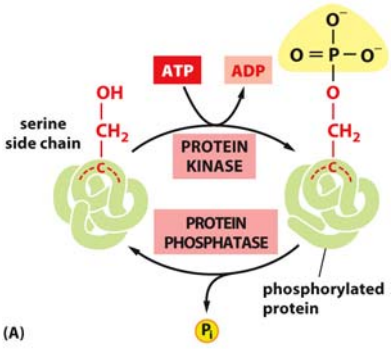

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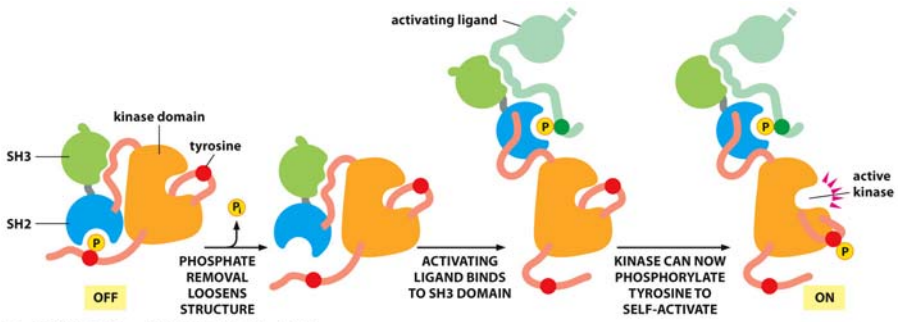

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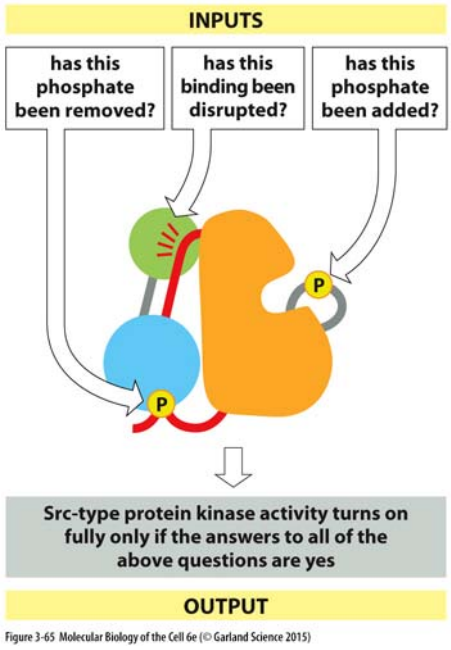
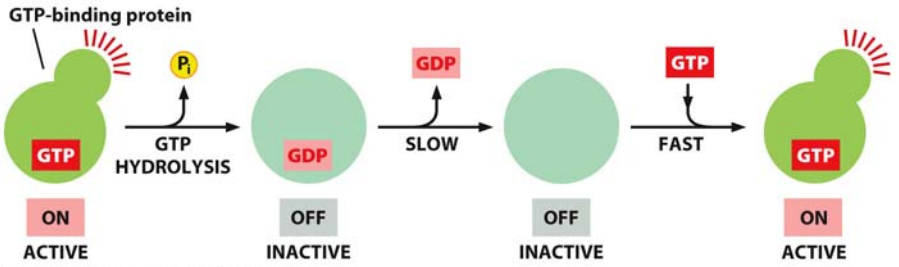


Figure 3-65 Molecular Biology of the Cell 6e (© Garland Science 2015)

Protein-bound GTP hydrolyses can be used to regulate protein activity.

- Phosphate on GDP inactive -> GTP active form, movement of protein subunits
- Growth factor -> GTPase *Ras* -> phosph others
- GTPase Activating Protein (GAP) hydrolyze *Ras* GTP to GDP (inactivate), Guanine nucleotide Exchange Factor (GEF) releases GDP from *Ras* which immediately gets a new GTP (activate)
- Ex EF-Tu-GTP tightly binds tRNA, ribosome hydrolyses to GDP -> switch helix detach -> AA



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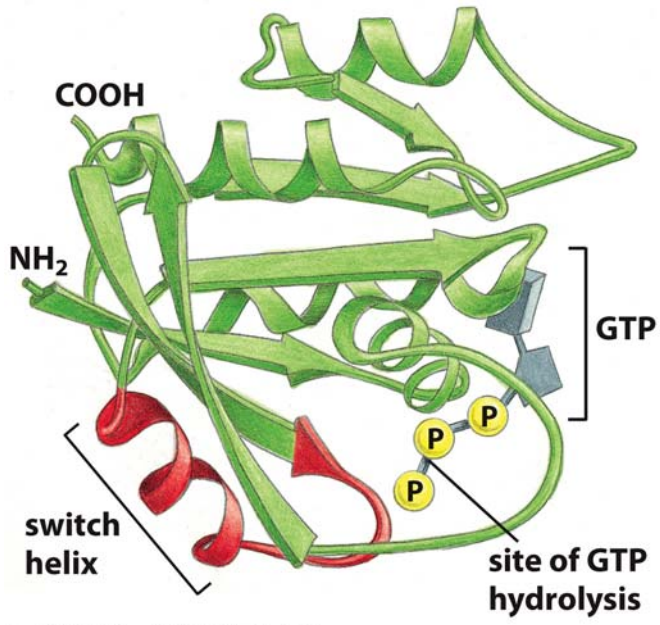

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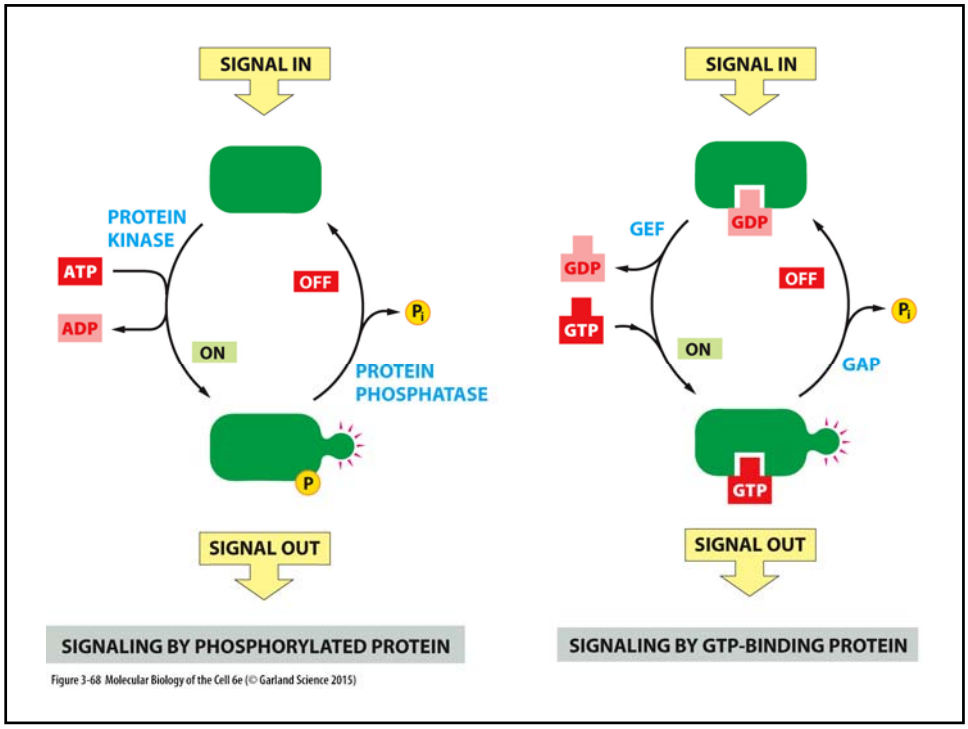

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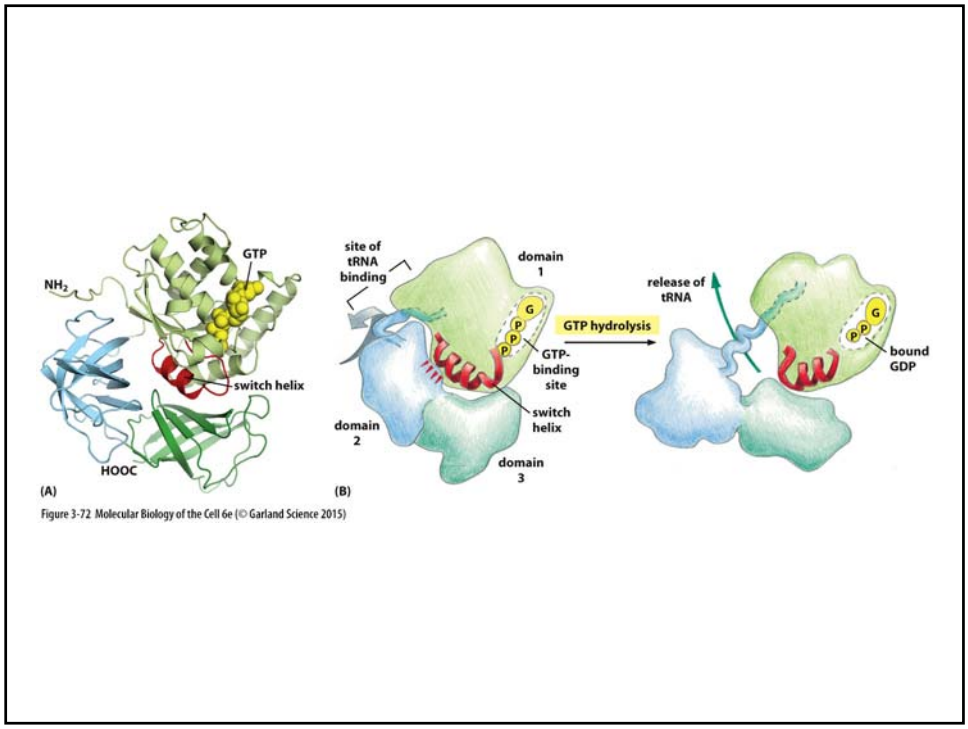
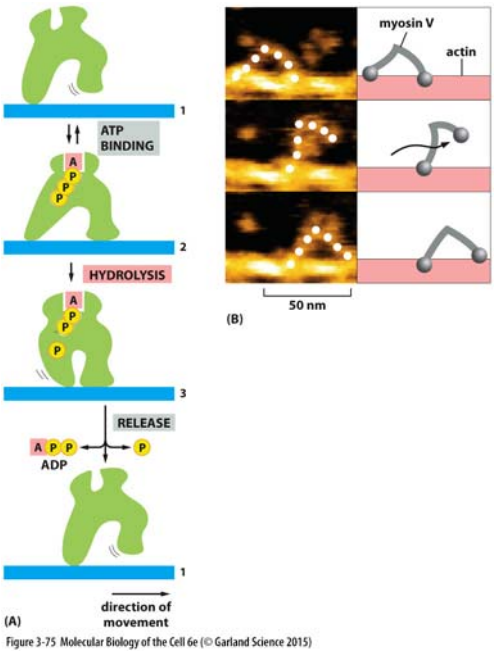


Figure 3-72 Molecular Biology of the Cell 6e (© Garland Science 2015)

Diversity of proteins are regulated by phosphorylation and scaffolding.

- Motor proteins couple ATP hydrolysis to irreversible conformational changes -> move
- ABC transporter pumps out hydrophobic molec out using 2ATP hydrolysis
- SCF ubiquitin ligase C end E2 ubiquitin-conjugating, other end substrate F-box arm, add ubiquitin to lysines mark for destruction
- Scaffolding proteins (e.g. cullin) concentrate reactions to specific locations w/o membranes



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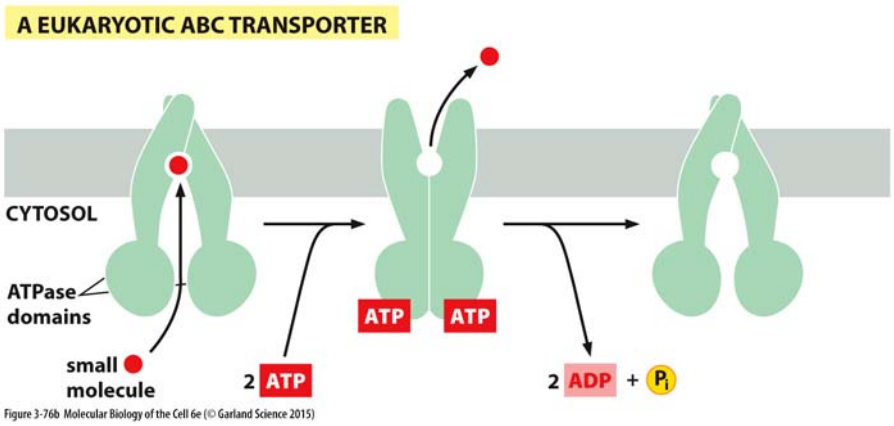


Figure 3-76b Molecular Biology of the Cell 6e (© Garland Science 2015)

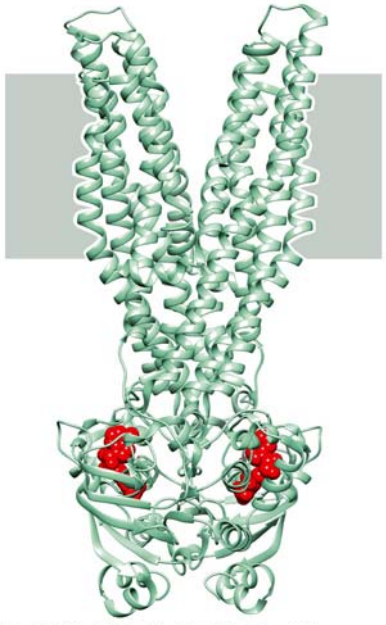


Figure 3-76c Molecular Biology of the Cell 6e (© Garland Science 2015)

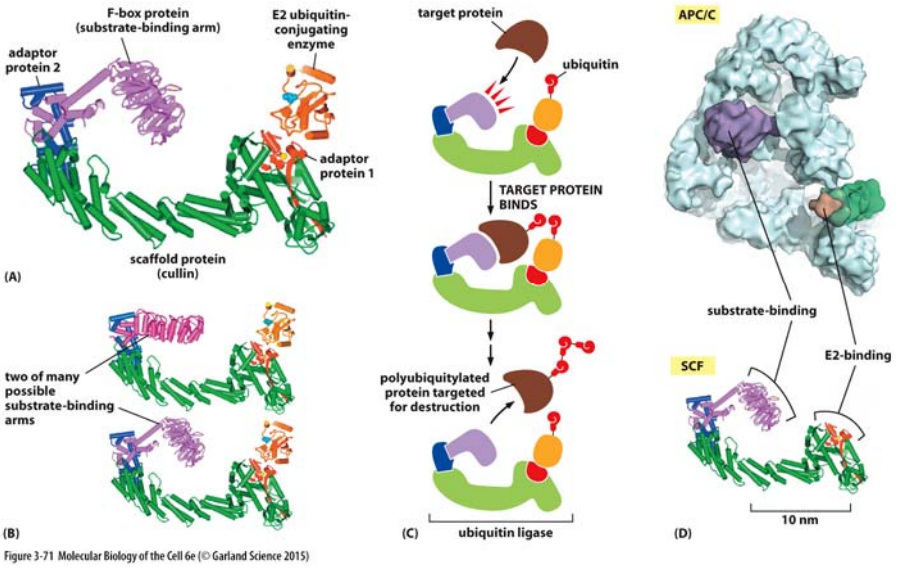


Figure 3-71 Molecular Biology of the Cell 6e (© Garland Science 2015)

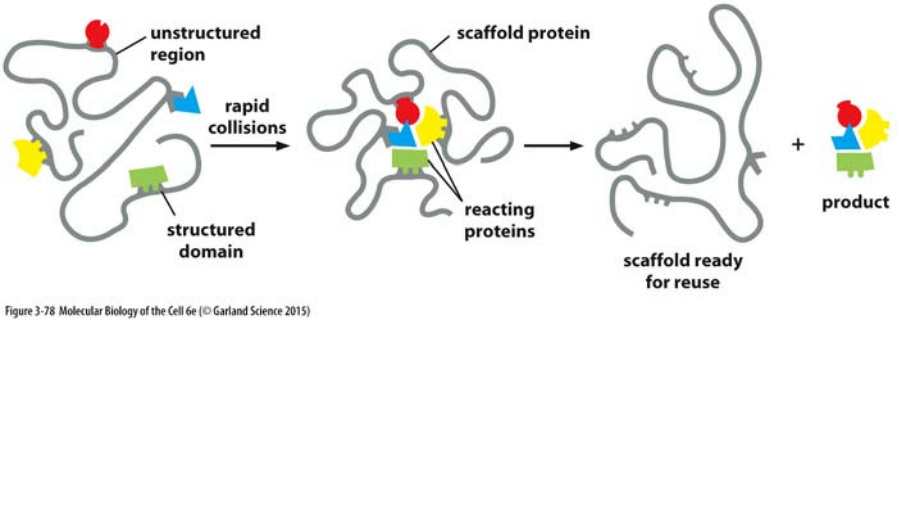


Figure 3-78 Molecular Biology of the Cell 6e (© Garland Science 2015)

Computational protein analysis provides understanding of complexity.

- Covalent modifications of proteins at multiple sites form combinatorial code of regulation
- Protein interaction networks: proteomics
- Direct binding inferred from yeast 2 hybrid
- Same protein can be used in two complexes with vastly different function
- Cross-species validation: humans with model organisms

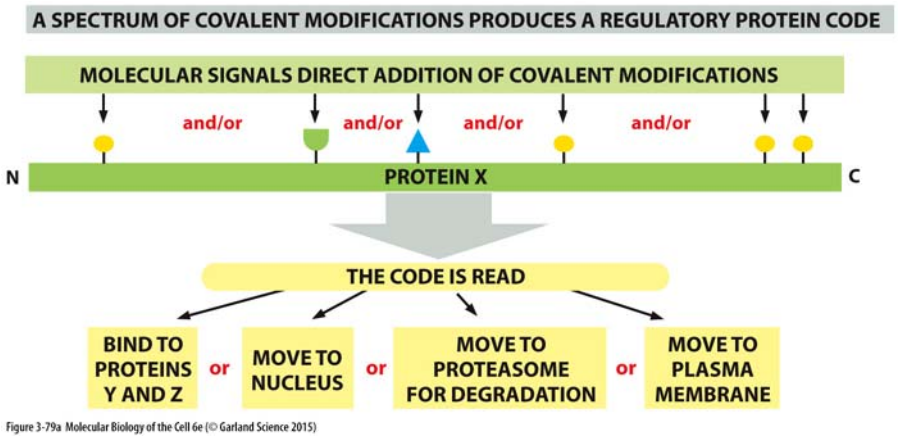


Figure 3-79a Molecular Biology of the Cell 6e (© Garland Science 2015)

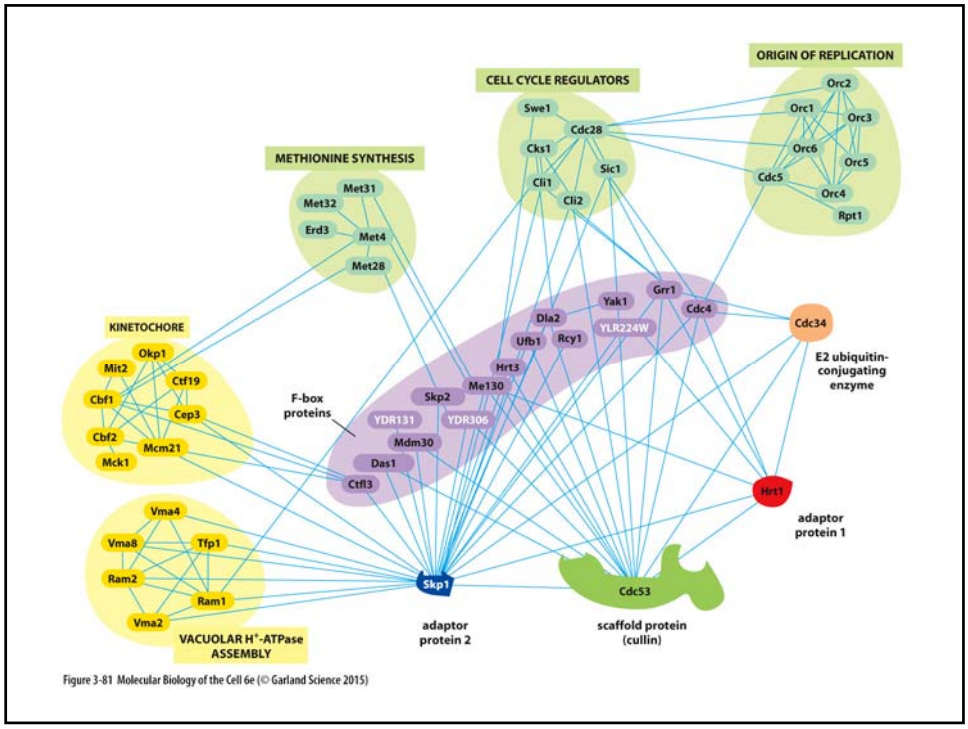


Figure 3-81 Molecular Biology of the Cell 6e (© Garland Science 2015)

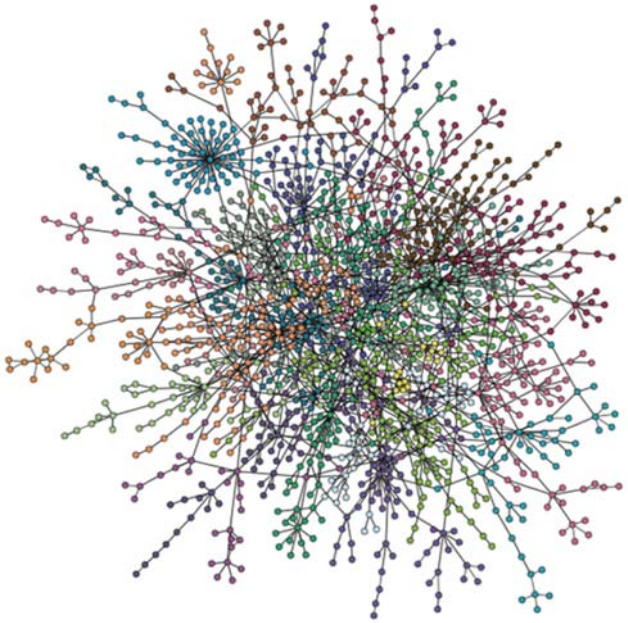
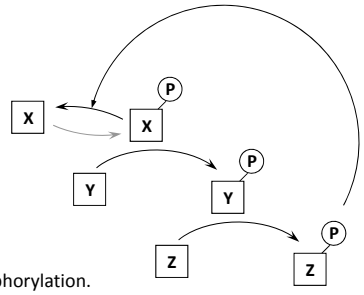


Figure 3-80 Molecular Biology of the Cell 6e (© Garland Science 2015)

Team work.

In the following schematic diagram of a simple signaling pathway, protein Z regulates the activity of protein X, which is an upstream protein kinase, through a negative feedback loop. Which of the following better describes protein Z?



- A. It is a protein kinase that is activated by phosphorylation.
- B. It is a protein kinase that is inactivated by phosphorylation.
- C. It is a protein that can act both as a kinase and as a phosphatase.
- D. It is a protein phosphatase that is inactivated by phosphorylation.
- E. It is a protein phosphatase that is activated by phosphorylation.