Enzyme Specificity

- Each enzyme has an active site.
  - Substrates have to fit (geometry)
  - Substrates have to bind (affinity)
    - H-bonds, electrostatics, hydrophobicity
  - Substrates have to react
    - bonds to be broken or formed have to have proper reactivity
    - Substances that fit and bind but don't react are inhibitors

Number	Classification	Biochemical Properties
1.	Oxidoreductases	Act on many chemical groupings to add or remove hydrogen atoms.
2.	Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules.
3.	Hydrolases	Add water across a bond, hydrolyzing it.
4.	Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds.
5.	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others.
6.	Ligases	Catalyze reactions in which two chemical groups are joined (or **ligated) with the use of energy from ATP.**

The binding site is specific for a restricted set of ligands

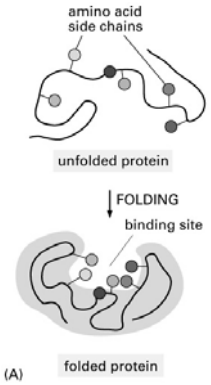
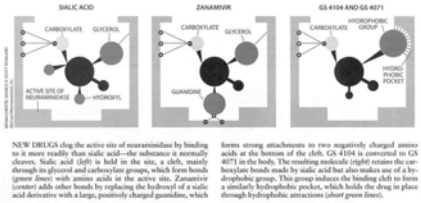


Figure 3–38 part 1 of 2. Molecular Biology of the Cell, 4th Edition

Influenza can be treated with neuraminidase inhibitors



NEW DRUGS clog the active site of neuraminidase by binding to it more readily than sialic acid—the substance it normally cleaves. Sialic acid (*left*) is held in the site, a cleft, mainly through its glycerol and carboxylate groups, which form bonds (*green lines*) with amino acids in the active site. Zanamivir (*center*) adds other bonds by replacing the hydroxyl of a sialic acid derivative with a large, positively charged guanidine, which forms strong attachments to two negatively charged amino acids at the bottom of the cleft. GS 4104 is converted to GS 4071 in the body. The resulting molecule (*right*) retains the carboxylate bonds made by sialic acid but also makes use of a hydrophobic group. This group induces the binding cleft to form a similarly hydrophobic pocket, which holds the drug in place through hydrophobic attractions (*short green lines*).

Binding sites in enzymes often contain reactive amino acids

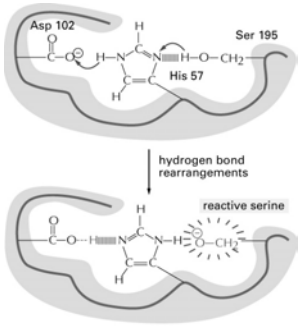


Figure 3–39. Molecular Biology of the Cell, 4th Edition.

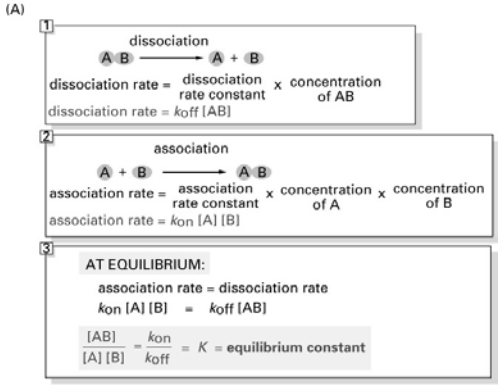


Figure 3–44 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

(B)

The relationship between free-energy differences and equilibrium constants

equilibrium constant $\frac{[AB]}{[A][B]} = K$ (liters/mole)	free energy of AB minus free energy of A + B (kcal/mole)
1	0
10	−1.4
10^2	−2.8
10^3	−4.3
10^4	−5.7
10^5	−7.1
10^6	−8.6
10^7	−10.0
10^8	−11.4
10^9	−12.9
10^{10}	−14.2
10^{11}	−15.6

(C)

Consider 1000 molecules of A and 1000 molecules of B in a eucaryotic cell. The concentration of both will be about 10^{-9} M.

If the equilibrium constant (K) for A + B ⇌ AB is 10^{10}, then at equilibrium there will be

270	270	730
A molecules	B molecules	AB molecules

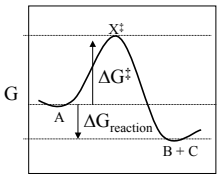
If the equilibrium constant is a little weaker at 10^8, which represents a loss of 2.8 kcal/mole of binding energy from the example above, or 2–3 fewer hydrogen bonds, then there will be

915	915	85
A molecules	B molecules	AB molecules

Figure 3–44 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Activation Energy $\Delta G^{\ddagger}$

- The transition state is defined as the most unstable intermediate in the reaction pathway between reactants and products
- $A \Leftrightarrow X^{\ddagger} \longrightarrow B + C$
- The difference in energy between A and $X^{\ddagger}$ is the activation energy
- The higher the activation energy the more slowly the reaction will proceed



Relationship between Rate and $\Delta G^{\ddagger}$

- We can approximate the relative concentrations of A and $X^{\ddagger}$ by an equilibrium distribution
- $K^{\ddagger} = X^{\ddagger} / A$ which can be rearranged to give $X^{\ddagger} = K^{\ddagger} A$
- The rate of formation of B and C can be given in terms of $X^{\ddagger}$
- rate = k $X^{\ddagger}$ substituting the pre-equilibrium expression rate = k $K^{\ddagger}$ A
- The relationship between free energy and the equilibrium constant $\Delta G = -RT \ln K$
- leads to the substitution of exp $(-\Delta G^{\ddagger} / RT)$ for $K^{\ddagger}$
- rate = k $(e^{-\Delta G^{\ddagger} / RT})$ A

Enzymes accelerate chemical reactions by lowering the activation energy

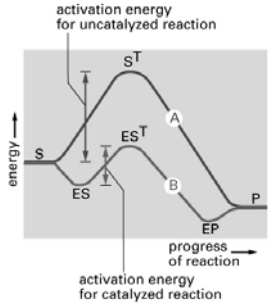


Figure 3–46. Molecular Biology of the Cell, 4th Edition

Enzyme changes the substrate into a product $S \xrightarrow{E} P$

This change is achieved through formation of a ES complex $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$

Through rearrangements and simplifications, the reaction rate can be expressed i a simple formula: Michaelis-Menten $v = \frac{V_{max}[S]}{K_m + [S]}$

When v = Vmax/2 , Km = [S]

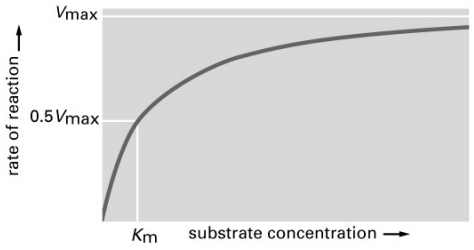


Figure 3–45. Molecular Biology of the Cell, 4th Edition.

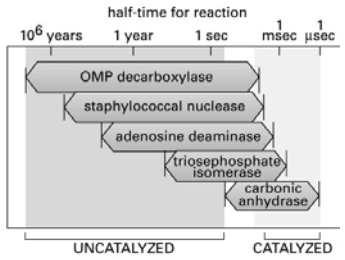


Figure 3–48. Molecular Biology of the Cell, 4th Edition.

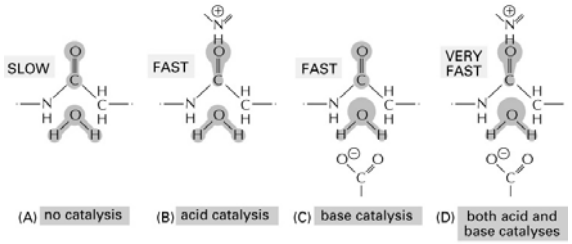


Figure 3–49. Molecular Biology of the Cell, 4th Edition.

Hydrolysis of polysaccharides by lysozyme

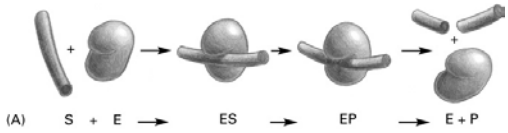


Figure 3–50 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

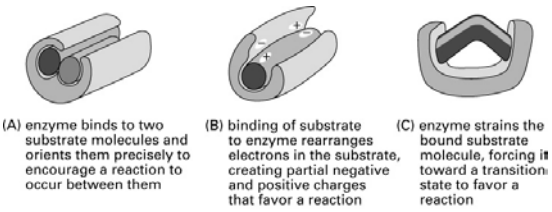


Figure 3–52. Molecular Biology of the Cell, 4th Edition.

Enzymes in the same pathway often form large complexes

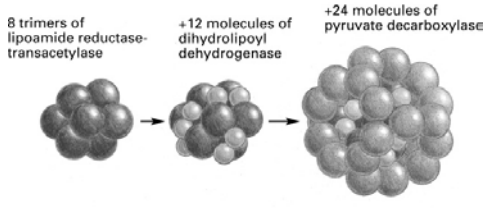


Figure 3–54. Molecular Biology of the Cell, 4th Edition.

Negative feedback regulation

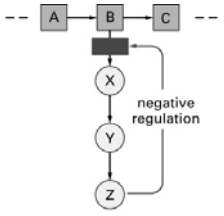


Figure 3–55. Molecular Biology of the Cell, 4th Edition

Positive allosteric regulation

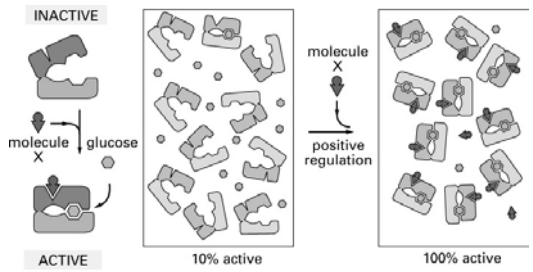


Figure 3–57. Molecular Biology of the Cell, 4th Edition.

Negative allosteric regulation

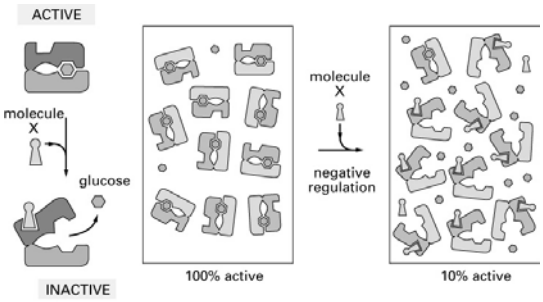


Figure 3–58. Molecular Biology of the Cell, 4th Edition.

Multisubunit allosteric enzymes are easier to regulate

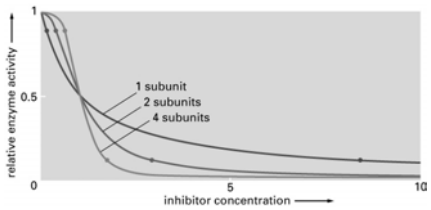


Figure 3–59. Molecular Biology of the Cell, 4th Edition.

The conformation of one subunit influences the other

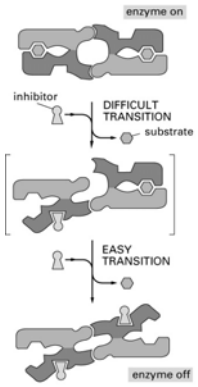
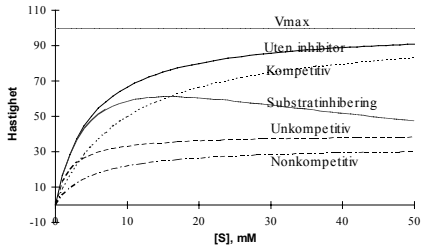
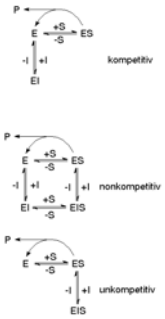
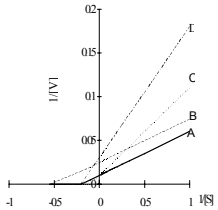


Figure 3–60. Molecular Biology of the Cell, 4th Edition.

Reaksjonshastigheten som funksjon av tiden i nærvær av forskjellige typer inhibitorer.







A – uten inhibitor
B – unkompetitiv inhibitor
C - kompetitiv inhibitor
D – nonkompetitiv inhibitor

The binding properties of proteins and catalytic activity of enzymes can be regulated by phosphorylation by kinases

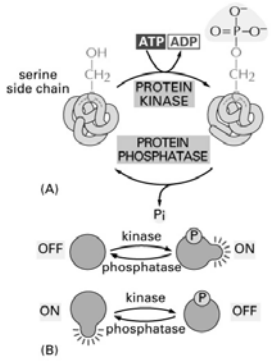


Figure 3–63. Molecular Biology of the Cell, 4th Edition.

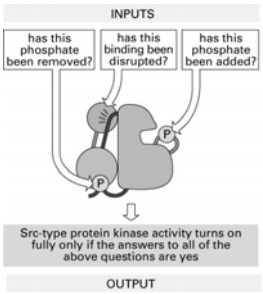


Figure 3–69. Molecular Biology of The Cell, 4th Edition.

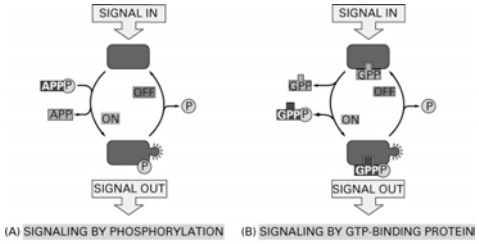
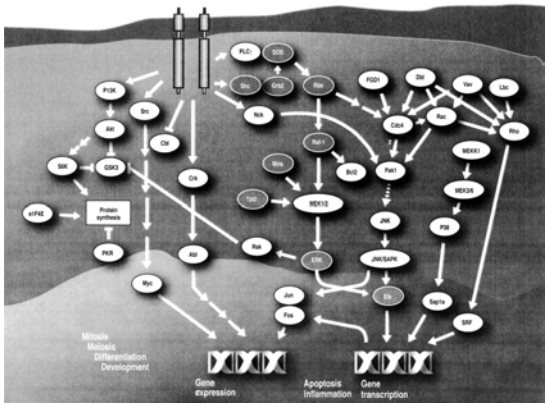


Figure 15–17. Molecular Biology of the Cell, 4th Edition.



Kinase/phosphatase inhibitors potential drugs

- The bacterially derived drug rapamycin (also known as sirolimus) specifically inhibits TOR (Target Of Rapamycin), resulting in reduced cell growth, a reduced rate of cell cycle progression, and a reduced rate of proliferation . As a result, rapamycin analogs, such as CCI-779 and RAD001, are currently being tested in clinical trials for efficacy against a variety of human tumors

Rapamycin does not directly inhibit phosphorylation

- Collectively, these data suggest that *in vivo*, rapamycin does not directly inhibit the kinase activity of mTOR. Rather, rapamycin likely acts by altering the composition of multiprotein mTOR complexes to hinder the integration of critical upstream regulatory signals or the accessibility of the kinase to downstream substrates

- **Selective protein kinase C inhibitors and their applications.**

  Shen GX.

  Department of Internal Medicine, University of Manitoba, Canada. gshen@ms.umanitoba.ca

  Protein kinase C (PKC) represents a family of phospholipid-dependent serine/threonine kinase. PKC was detected in almost all types of cells and tissues in the body. The activation of PKC is involved in the signal regulation of many physiological and pathological processes. PKC has multiple isoforms (alpha, betaI, betaII, gamma delta, epsilon, eta, theta, xi, iota and micro). PKC-mediated cellular processes are tissue- and isoform-specific. Investigations on selective or isoform-specific PKC inhibitors have attracted great attention during last two decades. Recent studies demonstrated that LY333531, a PKC-beta-specific inhibitor, reduced the development of diabetic vascular complications in animal models and prevented hyperglycemia-induced impairment of endothelial-dependent vasodilation in healthy subjects

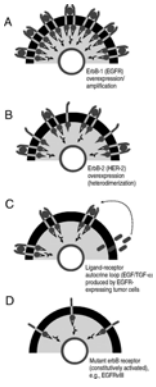


Figure 2. Mechanisms of increased ErbB receptor activation. *A) Overexpression or amplification of ErbB-1 receptor, increasing the potency of responses to available ligands. B) Overexpression of ErbB-2, resulting in preferential formation of ErbB-2 heterodimers with other ErbB receptors or autoactivated ErbB-2 homodimers. C) Ligand-receptor autocrine loop in which both receptor and ligand are produced by the same cell. D) Mutations in receptors, resulting in their constitutive activation, such as occurs with EGFRvIII. EGF = epidermal growth factor; EGFR = EGF receptor; TGF- = transforming growth factor . (From* Rowinsky EK. *Targeting signal transduction: the erbB receptor family as a target for therapeutic development against cancer. In: Horizons in Cancer Therapeutics: from Bench to Bedside. Meniscus Education Institute, West Conshohocken, PA. 2001;2:3–36.) Adapted with permission.*

- **Clinical experience with the HER1/EGFR tyrosine kinase inhibitor erlotinib.**

  Sandler A.

  Division of Hematology/Oncology, Vanderbilt University, Medical Center, Nashville, Tennessee, USA.

  In phase I trials in healthy volunteers and patients with refractory cancers, erlotinib (Tarceva) was well tolerated and showed activity against non-small-cell lung cancer and other tumors. The dose identified for further clinical development was 150 mg/d; at this dose, erlotinib achieves high exposure, with maximum concentrations greater than 2,000 ng/mL and 24-hour area under the concentration-time cure greater than 35,000 ng.h/L. In a phase II trial in 57 patients with previously treated advanced non-small-cell lung cancer, erlotinib treatment produced an objective response rate of 12.3% and a stable disease rate of 38.6%, with median duration of response of 19.6 weeks; median overall survival was 8.4 months and 1-year survival was 40%, with 9 patients remaining alive over follow-up of greater than 18 months

Human liver aldehyde oxidase: inhibition by 239 drugs.

Obach RS, Huynh P, Allen MC, Beedham C.

Pfizer Global Research and Development, Groton Laboratories, Groton, CT 06340, USA.

The authors tested 239 frequently used drugs and other compounds for their potential to inhibit the drug-metabolizing enzyme, aldehyde oxidase, in human liver cytosol. A sensitive, moderate throughput HPLC-MS assay was developed for 1-phthalazinone, the aldehyde oxidase-catalyzed product of phthalazine oxidation. Inhibition of this activity was examined for the 239 drugs and other compounds of interest at a test concentration of 50 microM. **Thirty-six compounds** exhibited greater than 80% inhibition and were further examined for measurement of IC50. The most potent inhibitor observed was the selective estrogen receptor modulator, raloxifene (IC50=2.9 nM), and tamoxifen, estradiol, and ethinyl estradiol were also potent inhibitors.