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

<table>
<thead>
<tr>
<th>Number</th>
<th>Classification</th>
<th>Biochemical Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oxidoreductases</td>
<td>Add or remove hydrogen atoms from a bond</td>
</tr>
<tr>
<td>2.</td>
<td>Transmitters</td>
<td>Transfer functional groups between donor and acceptor molecules. Kinases are</td>
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<tr>
<td></td>
<td></td>
<td>involved in transferring phosphate from ATP to other molecules</td>
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<tr>
<td>3.</td>
<td>Hydrolases</td>
<td>Add water across a bond, hydrolysing it</td>
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<tr>
<td>4.</td>
<td>Lyases</td>
<td>Add or remove a chemical group, or transfer one element to another</td>
</tr>
<tr>
<td>5.</td>
<td>Isomerases</td>
<td>Carry out isomerizations, move reactive units of chemically identical groups</td>
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<tr>
<td>6.</td>
<td>Ligases</td>
<td>Ligase reactions in which two chemical groups are joined (ligated) with the use of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>energy from ATP</td>
</tr>
</tbody>
</table>

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](image)

Influenza can be treated with neuraminidase inhibitors

![Figure 3-38 part 2 of 2. Molecular Biology of the Cell, 4th Edition](image)

Binding sites in enzymes often contain reactive amino acids

![Figure 3-36. Molecular Biology of the Cell, 4th Edition.](image)
Activation Energy $\Delta G^\ddagger$

- The transition state is defined as the most unstable intermediate in the reaction pathway between reactants and products.
- $A \Leftrightarrow X^\ddagger \rightarrow B + C$
- 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 $X^\ddagger$.
- $\text{rate} = k \times X^\ddagger$, substituting the pre-equilibrium expression $\text{rate} = k K^\ddagger A$.
- The relationship between free energy and the equilibrium constant $\Delta G = -RT \ln K$.
- $\text{leads to the substitution of } \exp\left(-\frac{\Delta G^\ddagger}{RT}\right) \text{ for } K^\ddagger$.
- $\text{rate} = k \left(\exp\left(-\frac{\Delta G^\ddagger}{RT}\right) \right) A$.

Enzymes accelerate chemical reactions by lowering the activation energy.

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Enzyme changes the substrate into a product

$S \rightarrow P$

This change is achieved through formation of an ES complex: $S + E \rightarrow ES \rightarrow E + P$.

Through rearrangements and simplifications, the reaction rate can be expressed in a simple formula: Michaelis-Menten

When $v = V_{\text{max}}/2$, $K_m = [S]$. 

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Figure 3-46, Molecular Biology of the Cell, 4th Edition.
Hydrolysis of polysaccharides by lysozyme

Enzymes in the same pathway often form large complexes

Negative feedback regulation
Positive allosteric regulation

Negative allosteric regulation

Multisubunit allosteric enzymes are easier to regulate

The conformation of one subunit influences the other

Reaksjonshastigheten som funksjon av tiden i nærvar av forskjellige typer inhibitorer.
The binding properties of proteins and catalytic activity of enzymes can be regulated by phosphorylation by kinases.

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.
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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, beta1, beta2, gamma, delta, epsilon, theta, 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 vasodilatation in healthy subjects.

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 curve 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, Berdham 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. 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. 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