ENZYMES

(The most important class of proteins on Earth)

Enzymes

*$t_{½} = 0.693 / k_{un}$(1st order)
$k_{un} = 0.693/t_{½}$

General Properties of Enzymes

1. Enhance reaction rates
2. Mild reaction conditions
3. Reaction specificity
4. Regulated activity

Rate Enhancement by Enzymes

Enzyme	Nonenzymatic half-life	* Uncatalyzed rate (k_{un} s^{-1})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}
AMP nucleosidase	69,000 years	1.0×10^{-11}
Carboxypeptidase A	7.3 years	3.0×10^{-9}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}
Chorismate mutase	7.4 hours	2.6×10^{-5}
Carbonic anhydrase	5 seconds	1.3×10^{-1}

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.

Enzymes

General Properties of Enzymes

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$k_{un} = 0.693/t_{½}$

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Rate Enhancement by Enzymes

Enzyme	Nonenzymatic half-life	* Uncatalyzed rate (k_{un} s^{-1})	Catalyzed rate (k_{cat} s^{-1})	Rate enhancement (k_{cat} s^{-1}/k_{un} s^{-1})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

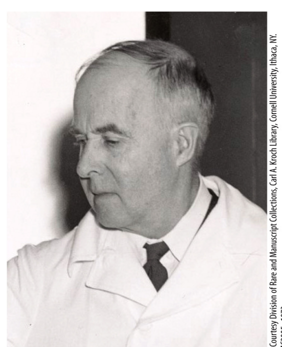
Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.

Enzymes

Pioneers

Eduard Buchner, 1860–1917

Unnumbered 6 p188a
Lehninger Principles of Biochemistry, Seventh Edition



James Sumner, 1887–1955

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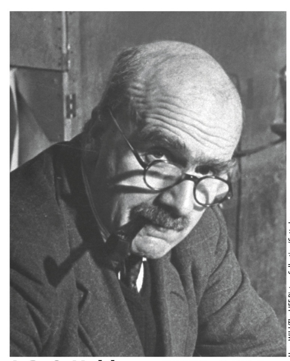
Lehninger Principles of Biochemistry, Seventh Edition



J. B. S. Haldane, 1892–1964

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Lehninger Principles of Biochemistry, Seventh Edition

Buchner and his brother Hans (of the funnel fame) were first to separate biological catalysis (fermentation) from living cells by trying to make jam using yeast extracts as a preservative, which they called a zyme. Enzyme was derived from "in das zyme."

Sumner was the first to isolate and crystalize an enzyme, urease. It was all protein, therefore enzymes=protein

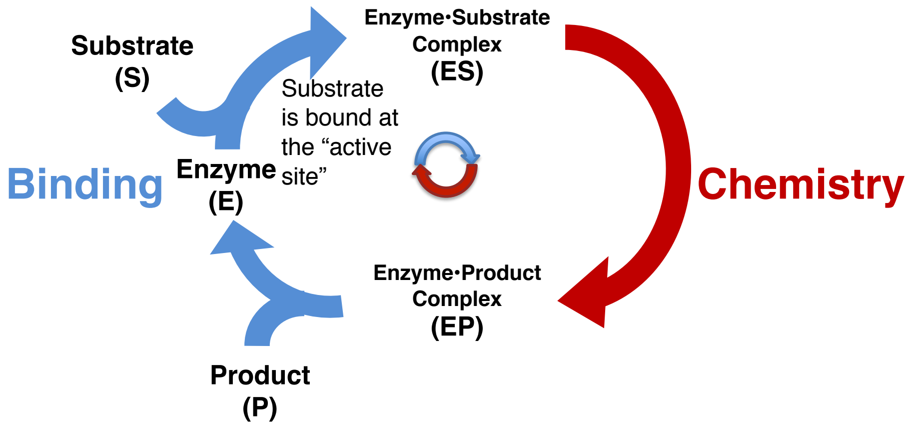
Haldane was the first really theorize how enzymes worked.

Enzymes

You can write out the catalysis as a series of reactions: $E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$

But, this is better understood as a CATALYTIC CYCLE

Divide the problem into two parts: Binding and Catalysis



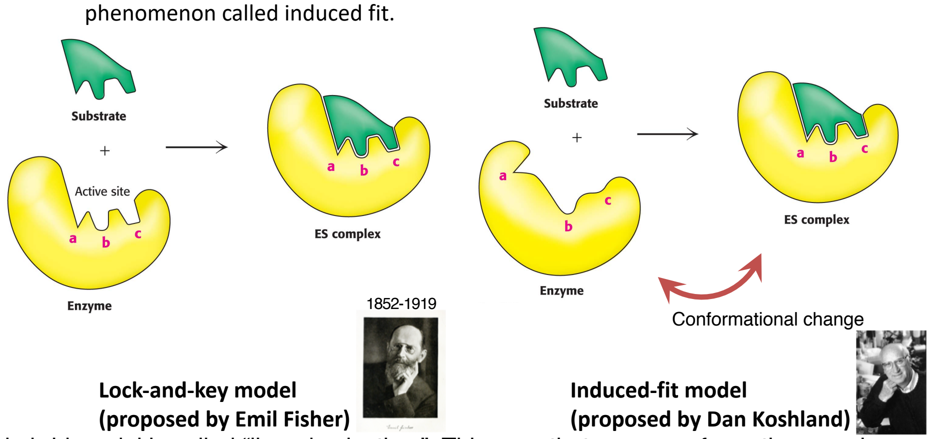
- The rate that an individual enzyme can go around this cycle is called the “turnover number”
- The turnover number is like a rate constant for catalysis, or k_{cat}

Enzymes

BINDING: Formation of the ES-complex

Most Enzymes do not interact with their substrates like a lock and key.

Rather, the enzyme changes shape upon substrate binding, a phenomenon called induced fit.



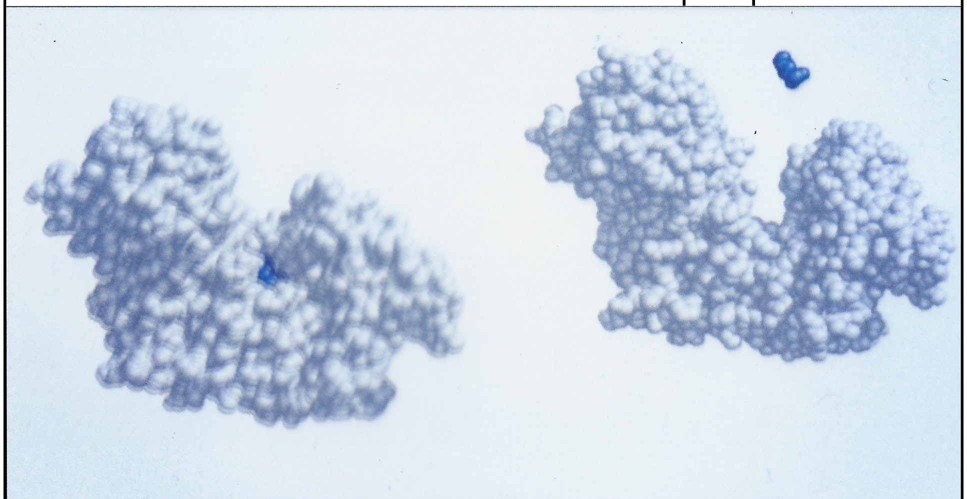
A hybrid model is called “ligand selective.” This says that many conformations are in equilibrium in solution and the substrate binds one best, pulling equilibrium.

1920-2007

Enzymes

BINDING: First demonstration of Induced Fit

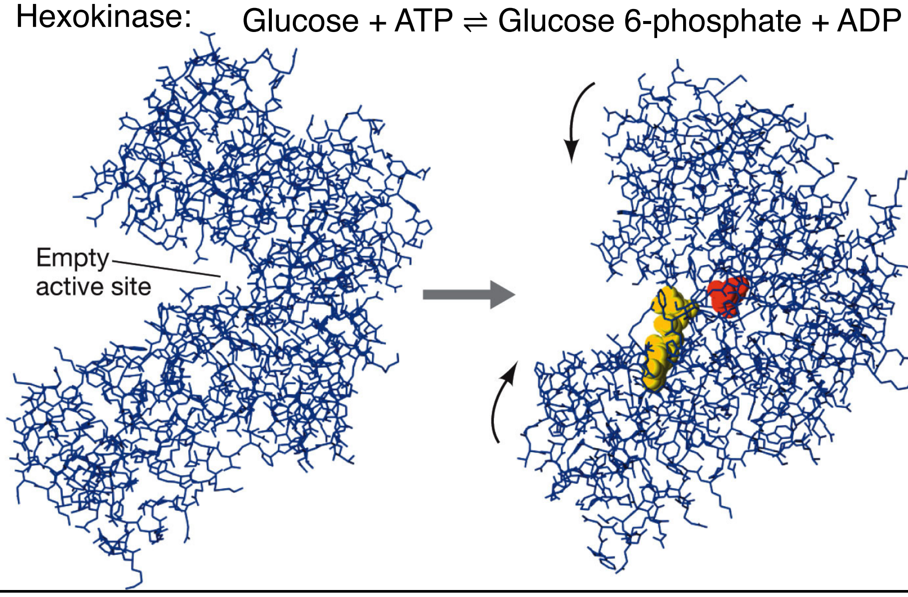
Hexokinase: Glucose + ATP ⇌ Glucose 6-phosphate + ADP



Hexokinase•Glc 6-P | Hexokinase

Enzymes

Hexokinase: Glucose + ATP ⇌ Glucose 6-phosphate + ADP



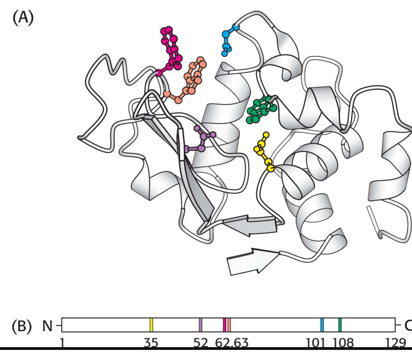
Enzymes

The Formation of an Enzyme–Substrate Complex Is the First Step in Enzymatic Catalysis

Enzymes bring substrates together to form an enzyme-substrate complex on a particular region of the enzyme called the active site.

ES-complex

The interaction of the enzyme and substrates at the active site promotes catalysis. The protein fold brings residues important for Binding & Catalysis to the Active Site.



A schematic representation of the primary structure of lysozyme shows that the active site is composed of residues that come from different parts of the polypeptide chain.

Enzymes

Enzyme-Substrate Complex Drives Selectivity

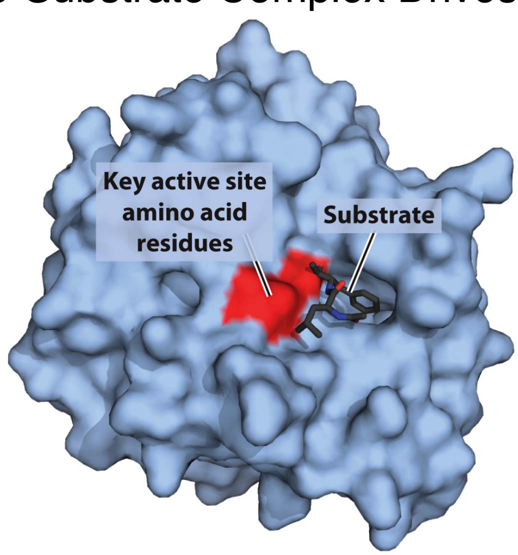


Figure 6-1

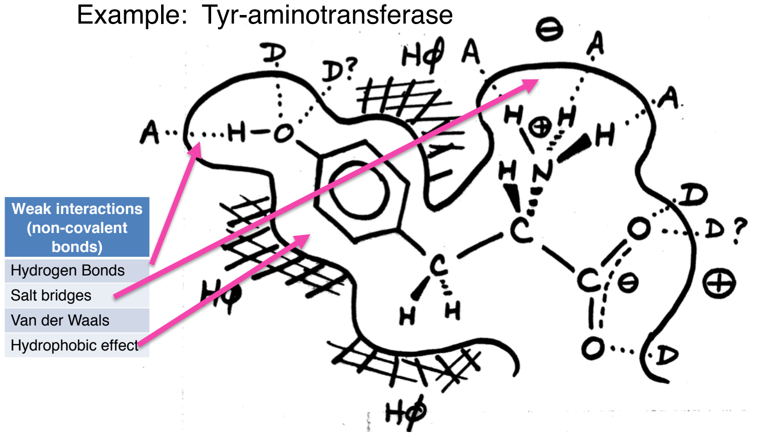
Chymotrypsin with bound substrate

(PDB ID 7GCH to just observe the interactions of substrate and enzyme)

Enzymes

BINDING: great example of complementarity

Example: Tyr-aminotransferase



This kind of complementarity is not restricted to ES complex: interactions of receptor-ligand, protein-protein, protein-nucleic acid, etc.

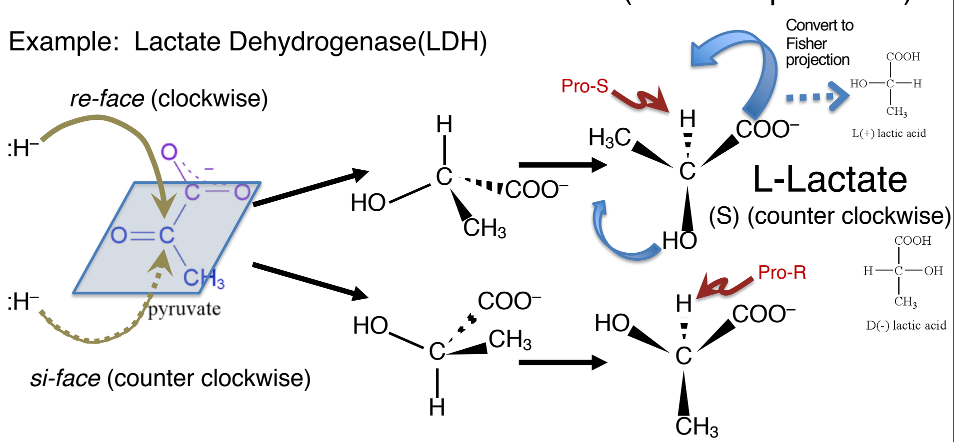
Notice that this kind of complementarity allows for binding L-Tyr much better than D-Tyr.

Enzymes

BINDING: Enzymes are stereo-selective

……even for molecules that are not chiral (so-called pro-chiral)

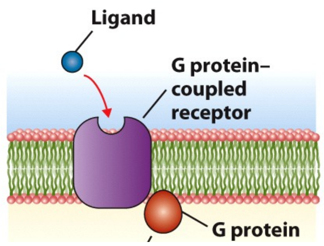
Example: Lactate Dehydrogenase(LDH)

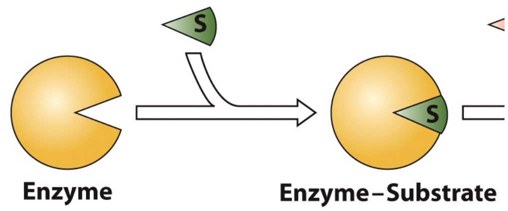


The hydride (:H⁻)(from NADH) can add to the sp² carbonyl carbon from the top (on the *re-face*) or the bottom (*si-face*). But, it can only do ONE.

Enzymes

Binding affinities: examples





- Binding of receptor and ligand
- Receptors can be soluble or membrane-bound
- Similar to binding of enzyme and substrate

How do we measure the degree of the affinity?

Enzymes

Receptor-Ligand binding

$$\mathrm{R} + \mathrm{L} \underset{k_{-1}^{A}}{\overset{k_{1}^{A}}{\rightleftharpoons}} \mathrm{R{\cdot}L}$$

Association reaction

$$\mathrm{R{\cdot}L} \underset{k_{-1}^{D}}{\overset{k_{1}^{D}}{\rightleftharpoons}} \mathrm{R} + \mathrm{L}$$

Dissociation reaction

$k_1^{D} = k_{-1}^{A}$, etc.

$$K_D = \frac{[R][L]}{[R \bullet L]}$$

What are the units of a dissociation constant? M

How about an association constant? M^{-1}

How can we express how TIGHT a ligand binds to a protein? K_D

How can we **measure** K_D?

Enzymes: Receptor-Ligand binding

Define fraction of R bound to a ligand = Y
Define R_T = total of all species of R

$Y = [R\bullet L] / ([R]+[R\bullet L])$

Use K_{eq} to calculate expression for free R

$$K_D = \frac{[R][L]}{[R\bullet L]}$$

$[R] = K_D[R\bullet L] / [L]$

Substitute for free R

$Y = [R\bullet L] / (K_D[R\bullet L] / [L]+[R\bullet L])$
$\div[R\bullet L] / [R\bullet L]$

$Y = 1/(K_D/[L] + 1)$
$\times [L] / [L]$

$$R_T = R + R{\cdot}L$$

$$Y = \frac{[R\bullet L]}{[R]_T}$$

$$Y = \frac{[L]}{K_D + [L]}$$

Equation for hyperbola → y = x/(b+x)

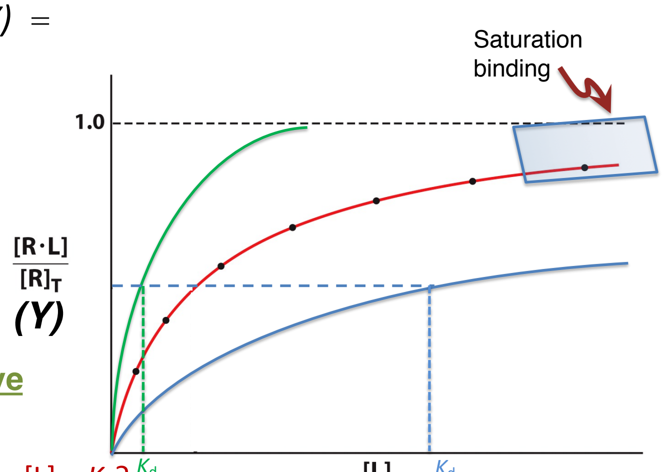
Graph [L] vs fraction of receptor that is bound (Y)

Enzymes: Receptor-Ligand binding

$$Y = \frac{[L]}{K_D + [L]}$$

hyperbola → y = x/(b+x)

Fraction bound (*Y*) = $\frac{[R\bullet L]}{[R]_T}$



Hyperbolic Curve

NOTE: what is Y when $[L] = K_d$?