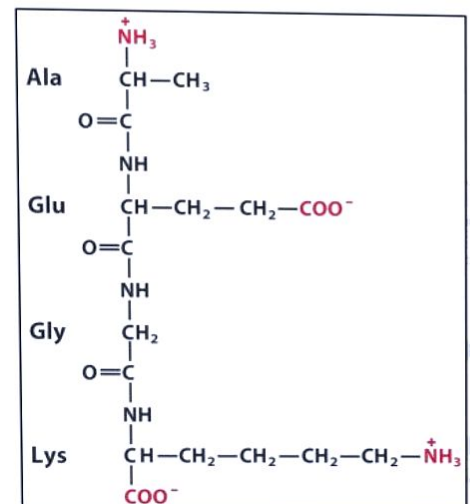


# Enzymes are proteins

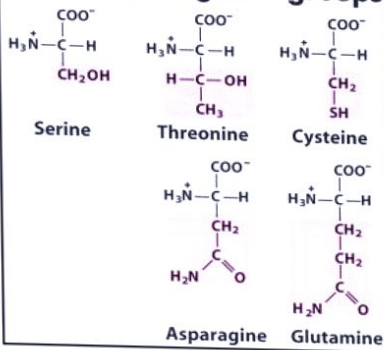
- In general, a protein is a chain of **amino acids** (aa) covalently linked (when the chain is short ~5-10 aa, the protein is often called oligopeptide/polypeptide or simply peptide)
- Thousands of different proteins are built with the same ubiquitous set of 20 amino acids (the protein “alphabet”)
- Some proteins have structural roles (e.g. actin in the muscles), other have catalytic (chemical-reaction-making) activity and are called **enzymes**

- A polypeptide with 4 amino acids (Ala-Glu-Gly-Lys)
- The electrically charged groups are shown in red
- In a longer protein the electrically charged lateral groups can line a pocket of the enzyme 3D structure to generate an active reaction site (see following slides)

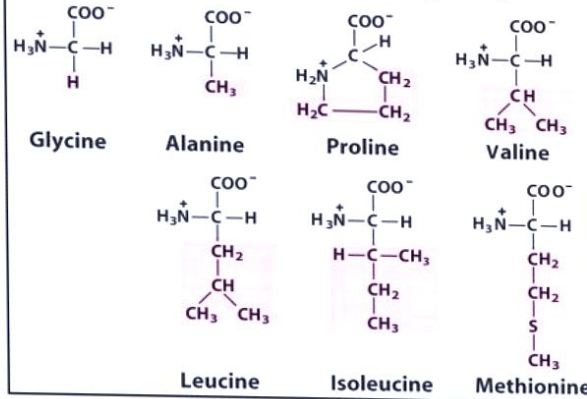


# 20 amino acids

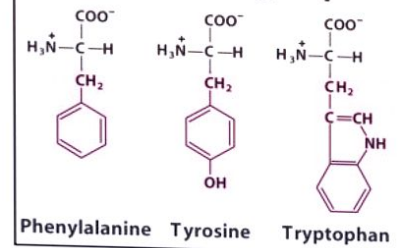
## Polar, uncharged R groups



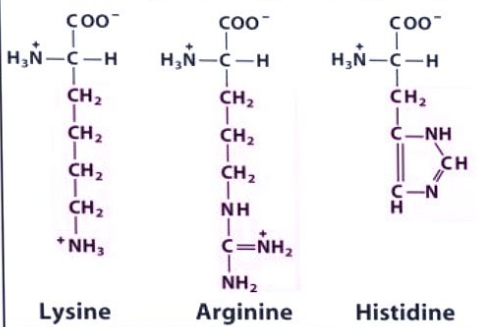
## Nonpolar, aliphatic R groups



## Aromatic R groups



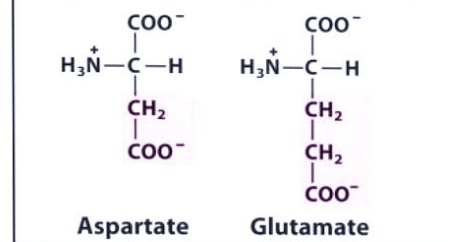
## Positively charged R groups



## The 1 letter and 3 letters codes

G	Glycine	Gly	P	Proline	Pro
A	Alanine	Ala	V	Valine	Val
L	Leucine	Leu	I	Isoleucine	Ile
M	Methionine	Met	C	Cysteine	Cys
F	Phenylalanine	Phe	Y	Tyrosine	Tyr
W	Tryptophan	Trp	H	Histidine	His
K	Lysine	Lys	R	Arginine	Arg
Q	Glutamine	Gln	N	Asparagine	Asn
E	Glutamic Acid	Glu	D	Aspartic Acid	Asp
S	Serine	Ser	T	Threonine	Thr

## Negatively charged R groups



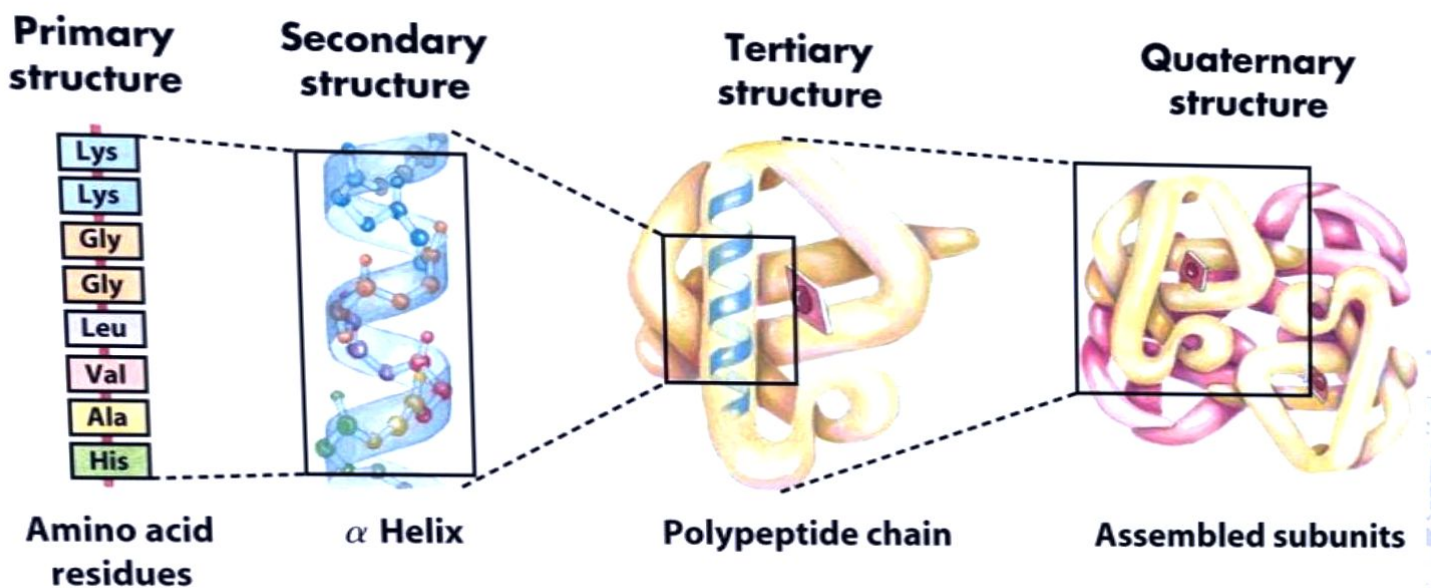
# Proteins come in all shapes and sizes

**TABLE 3-2** Molecular Data on Some Proteins

	<i>Molecular weight</i>	<i>Number of residues</i>	<i>Number of polypeptide chains</i>
Cytochrome c (human)	13,000	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (chicken egg white)	13,930	129	1
Myoglobin (equine heart)	16,890	153	1
Chymotrypsin (bovine pancreas)	21,600	241	3
Chymotrypsinogen (bovine)	22,000	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	68,500	609	1
Hexokinase (yeast)	102,000	972	2
RNA polymerase ( <i>E. coli</i> )	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase ( <i>E. coli</i> )	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

# Enzymes 3D structure

- Enzymes are proteins and their activities depends on the 3D structure of the amino acids that compose them (note: also some RNAs have catalytic activity but they won't be covered in this course)



7

Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.



# PDB - Database of protein structures

- PDB (RCSB Protein Data Bank)
- <http://www.rcsb.org/pdb/home/home.do>
- How is the data collected? Every 3D protein sequence has to be deposited in PDB before publication in reviewed journals

A MEMBER OF THE **PDB**

An Information Portal to Biological Macromolecular Structures

As of Tuesday Dec 19, 2006 there are 40749 Structures | PDB Statistics

PDB ID or keyword Author  **SEARCH** | Advanced Search

Structure Summary **Biology & Chemistry** Materials & Methods Sequence Details Geometry


Learn more: [M] 1HTB

<b>Title</b>	CRYSTALLIZATION OF HUMAN BETA3 ALCOHOL DEHYDROGENASE (10 MG/ML) IN 100 MM SODIUM PHOSPHATE (PH 7.5), 7.5 MM NAD+ AND 1 MM 4-IODOPYRAZOLE AT 25 C		
<b>Authors</b>	Hurley, T.D., Davis, G.J.		
<b>Primary Citation</b>	Davis, G.J., Bosron, W.F., Stone, C.L., Owusu-Dekyi, K., Hurley, T.D. X-ray structure of human beta3beta3 alcohol dehydrogenase. The contribution of ionic interactions to coenzyme binding. <i>J.Biol.Chem.</i> v271 pp.17057-17061, 1996 [Abstract]		
<b>History</b>	Deposition	1995-08-10	Release 1995-12-07
<b>Experimental Method</b>	Type	X-RAY DIFFRACTION Data [ EDS ]	
<b>Parameters</b>	Resolution[Å]	R-Value	R-Free
	2.40	0.175 (obs)	0.243
			Space Group
			P 1
<b>Unit Cell</b>	Length [Å]	a	54.03
		b	44.43
		c	92.74
	Angles [°]	alpha	92.71
		beta	103.17
		gamma	69.15

*Molecular*

**Images and Visualization**

Biological Molecule / Asymmetric Unit

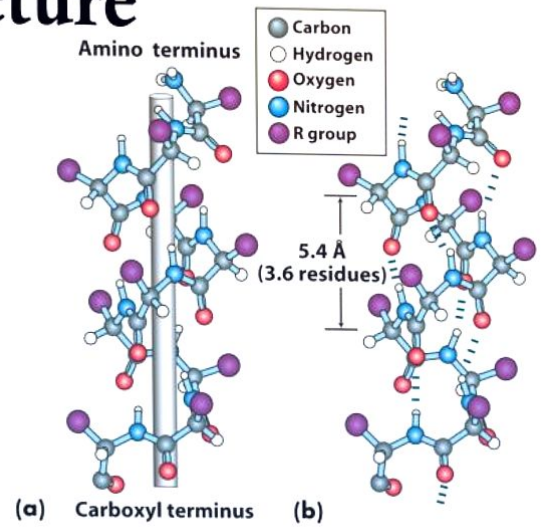
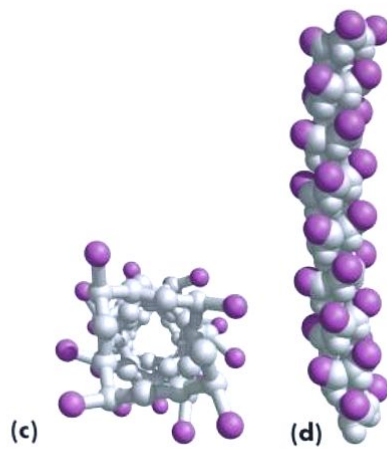


**Display Options**

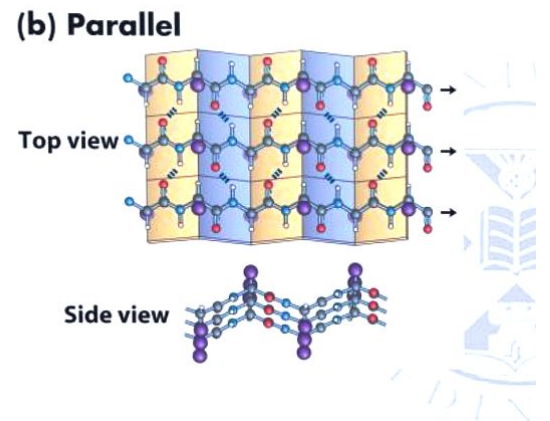
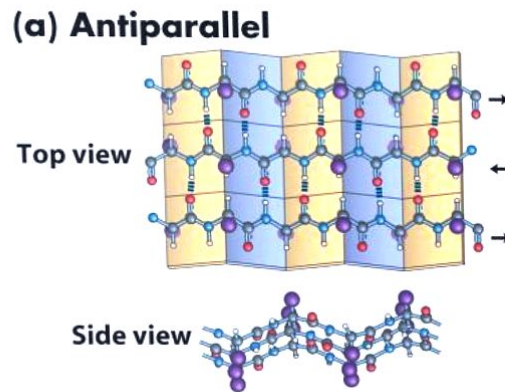
- King
- Jmol
- WebMol
- Protein Workshop
- QuickPDB
- All Images

# Secondary structure

- Common secondary structures are **alpha helixes**  
(here in different graphical representations)



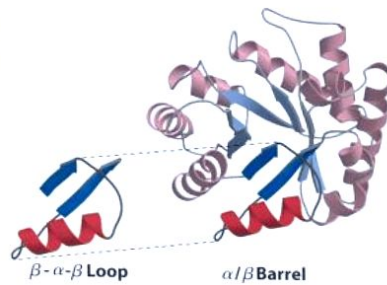
- And **beta sheets**





# Structural domains

- Recurring motifs (here the beta-alpha-beta loop) are the basis for protein structural classification



- Enzymes with similar sequences and structural domains / motifs are classified in the same **protein family**

█	PDB identifier
█	Fold
█	Superfamily
█	Family
█	Protein
█	Species

### All $\alpha$

█ 1AO6	█ 1BCF
█ Serum albumin	█ Ferritin-like
█ Serum albumin	█ Ferritin-like
█ Serum albumin	█ Ferritin
█ Serum albumin	█ Bacterioferritin (cytochrome
█ Human ( <i>Homo sapiens</i> )	█ <i>Escherichia coli</i>

### All $\beta$

█ 1HOE	█ 1LXA
█ $\alpha$ -Amylase inhibitor	█ Single-stranded left-handed $\beta$ helix
█ $\alpha$ -Amylase inhibitor	█ Trimeric LpxA-like enzymes
█ $\alpha$ -Amylase inhibitor	█ UDP <i>N</i> -acetylglucosamine acyltransferase
█ HOE-467A	█ UDP <i>N</i> -acetylglucosamine acyltransferase
█ <i>Streptomyces tendae</i> 4158	█ <i>Escherichia coli</i>



# Interpro: the protein families database

- **InterPro** is a database of protein families, domains and functional sites
- Identifiable features found in known proteins can also be scanned against unknown protein sequences
- *(here an example of domain common to enzymes that use iron as cofactor to cut an hydrogen atom from an alcohol).*

InterPro incorporates data from other databases



## InterPro IPR001670 Iron-containing alcohol dehydrogenase

<b>Matches</b> ?	Overview: <a href="#">sorted by AC</a> , <a href="#">sorted by name</a> , <a href="#">of known struc</a>
	Detailed: <a href="#">sorted by AC</a> , <a href="#">sorted by name</a> , <a href="#">of known struc</a>
	Table: <a href="#">For all matching proteins, of known structure</a>
<a href="#">Architectures</a>	
<b>Accession</b> ?	IPR001670 Fe_ADH Matches: 1309 proteins
<b>Type</b> ?	Domain
<b>Signatures</b> ?	Database ID Name Proteins
	<a href="#">Pfam</a> <a href="#">PF00465</a> Fe-ADH 1293
	<a href="#">PROSITE pattern</a> <a href="#">PS00060</a> ADH_IRON_2 589
	<a href="#">PROSITE pattern</a> <a href="#">PS00913</a> ADH_IRON_1 842

### Example proteins

<a href="#">Q05239</a>	Probable NADH-dependent butanol dehydrogenase 1 (EC 1.1.1.-)	
<a href="#">P10127</a>	Alcohol dehydrogenase 4 (EC 1.1.1.1) (Alcohol dehydrogenase IV)	
<a href="#">P74246</a>	Hypothetical oxidoreductase slr1167 (EC 1.1.-)	
<a href="#">Q09669</a>	Alcohol dehydrogenase 4 (EC 1.1.1.1) (Alcohol dehydrogenase IV)	
<a href="#">Q24803</a>	Aldehyde-alcohol dehydrogenase 2 [Includes: Alcohol dehydrogenase (EC 1.1.1.1) (Al	
<a href="#">More proteins</a>		
<a href="#">IPR001670</a>	Iron-containing alcohol dehydrogenase	
<a href="#">IPR012079</a>	Bifunctional aldehyde/alcohol dehydrogenase	
ModBase		

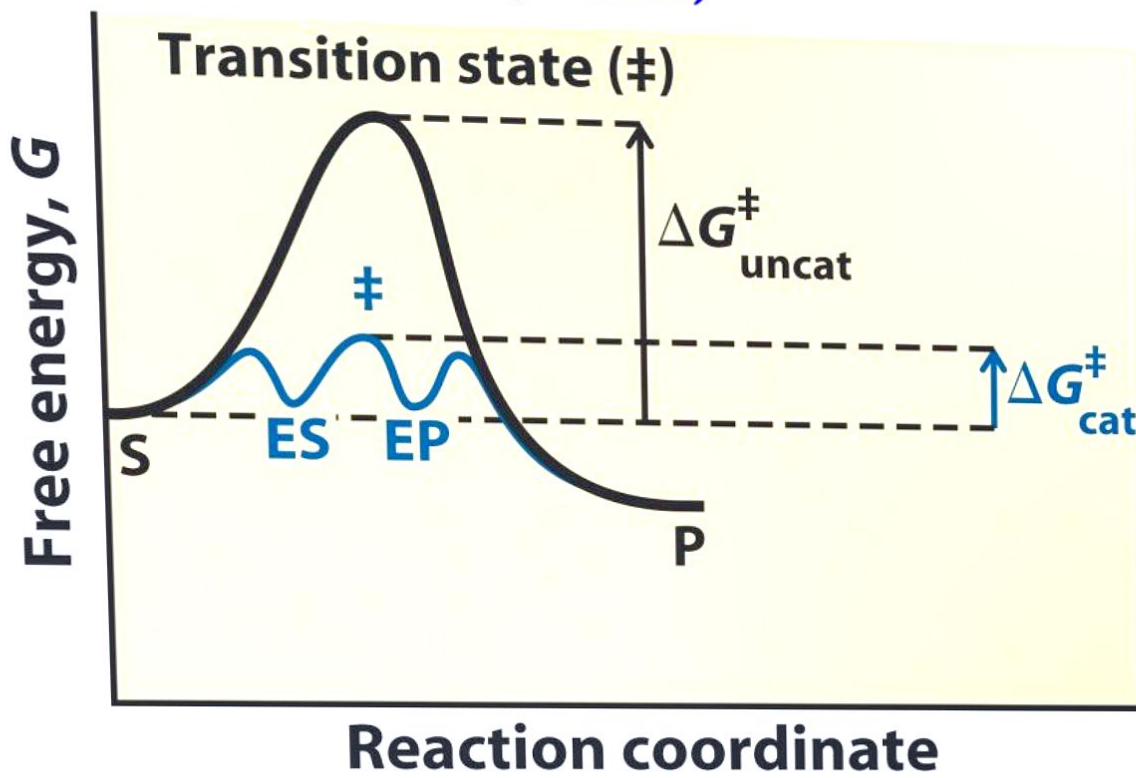


# How enzymes work

- **IMPORTANT:** enzymes **do NOT** and **cannot** affect the equilibrium / free energy difference of a reaction
- Enzymes enhance the reaction rates (molecules produced per second) by lowering the activation energy of the transition state  $\Delta G_{\text{cat}}^{\ddagger}$



# Transition state energy changed by an enzyme (in blue)

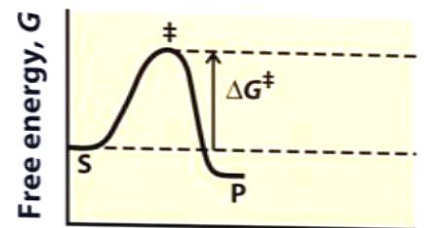


Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.

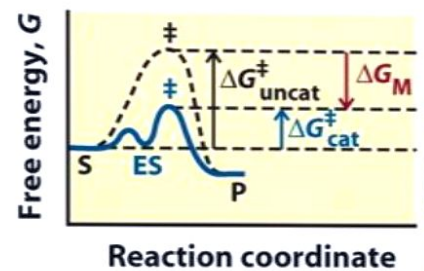
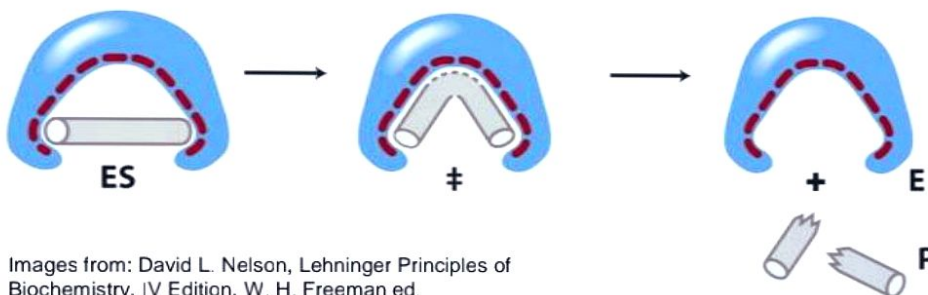


# The enzyme action on the transition state

## (a) No enzyme



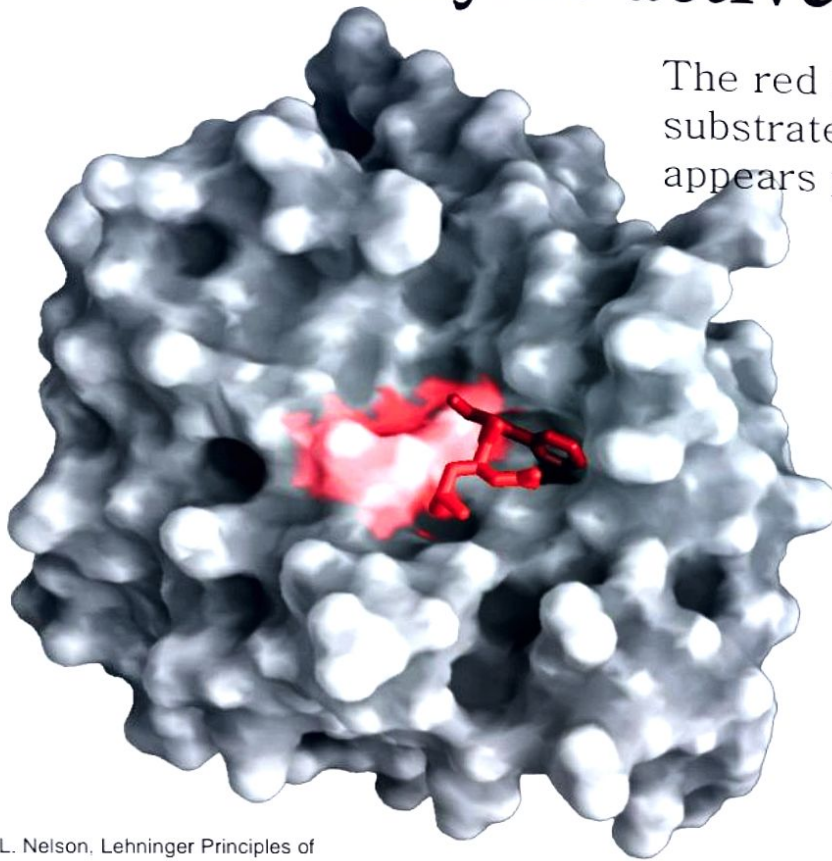
## (c) Enzyme complementary to transition state



14 Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.

## The enzyme active site

The red molecule is the substrate, the active site appears painted in red

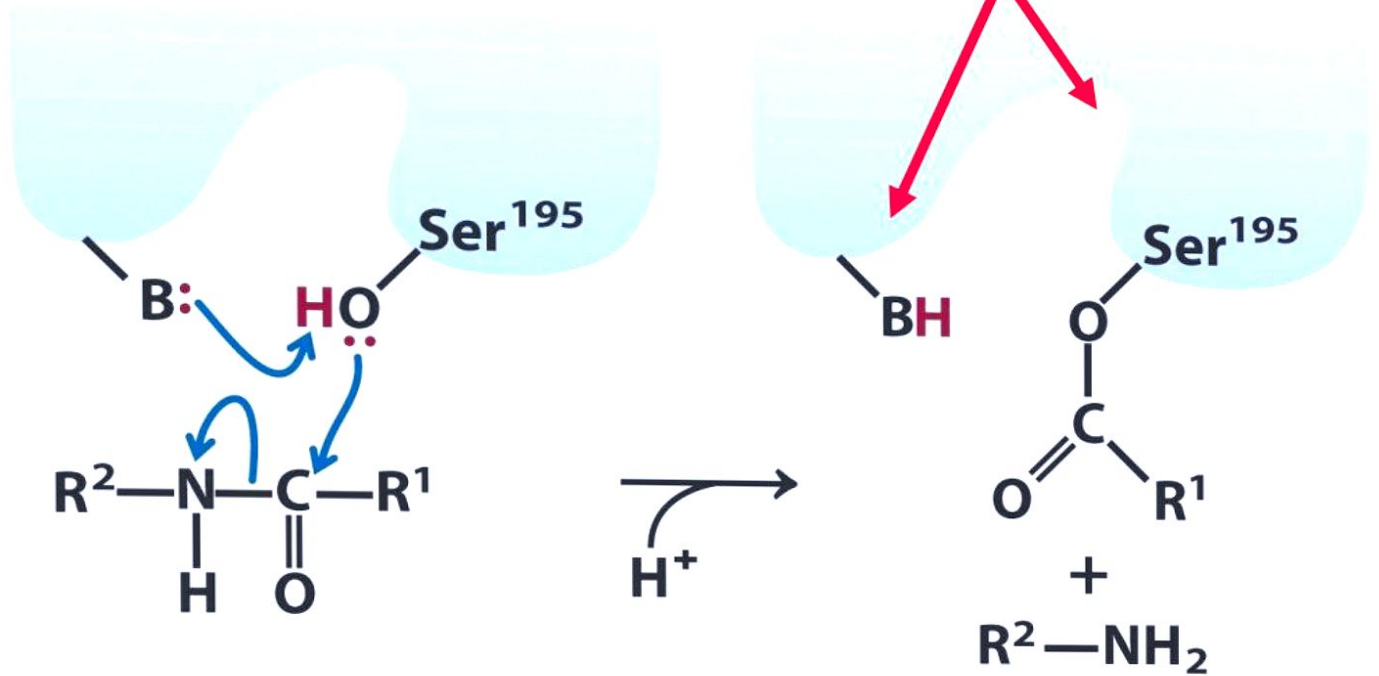


From: David L. Nelson, Lehninger Principles of  
15 Biochemistry, IV Edition, W. H. Freeman ed.





# Enzyme: Chymotrypsin



You can see a flash demo of this enzymatic reaction on:  
<http://bcs.whfreeman.com/lehninger/> clicking on → Chapter 6: Enzymes →  
 16 mechanism animation → fig 6.21 mechanism for chymotrypsin

Images from: David L. Nelson, Lehninger Principles of Biochemistry, IV Edition, W. H. Freeman ed.

## E.C. Enzyme nomenclature

- Enzymes are named depending on the reaction they catalyse.
- Examples of enzyme groups are:
  - EC 1 - Oxidoreductases
  - EC 2 - Transferases
  - EC 3 - Hydrolases
  - EC 4 - Lyases
  - EC 5 - Isomerases
  - EC 6 - Ligases



# An example of E.C. enzyme nomenclature

## EC 3. Hydrolases

- EC 3.1 Hydrolases Acting on Ester Bonds
  - EC 3.1.3 Phosphoric Monoester Hydrolases
    - EC 3.1.3.1 alkaline phosphatase
    - EC 3.1.3.2 acid phosphatase
    - EC 3.1.3.3 phosphoserine phosphatase
    - EC 3.1.3.4 phosphatidate phosphatase
    - EC 3.1.3.5 5'-nucleotidase
    - EC 3.1.3.6 3'-nucleotidase
    - EC 3.1.3.7 3'(2'),5'-bisphosphate nucleotidase
    - EC 3.1.3.8 3-phytase
    - EC 3.1.3.9 glucose-6-phosphatase
    - EC 3.1.3.10 glucose-1-phosphatase
    - **EC 3.1.3.11 fructose-bisphosphatase**



# Example: fructose-bisphosphatase EC 3.1.3.11

From the IUBMB (International Union of Biochemistry and Molecular Biology) web site:

<http://www.chem.qmul.ac.uk/iubmb/enzyme/>

one can reach a record for the enzyme: EC 3.1.3.11 (fructose-bisphosphatase):

<http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/1/3/11.html>

The site also contains the reaction diagram shown here:

<http://www.chem.qmul.ac.uk/iubmb/enzyme/reaction/polysacc/Calvin2.html>

The reaction involves the hydrolysis (separation) of a phosphate group here represented by  $P_i$

The "P" representing the phosphate group that appears on the top region of the D-fructose-1,6-bisphosphate substrate molecule has disappeared after the reaction, and the product is now called D-fructose 6-phosphate (the phosphate group in position 1 is no more there).

