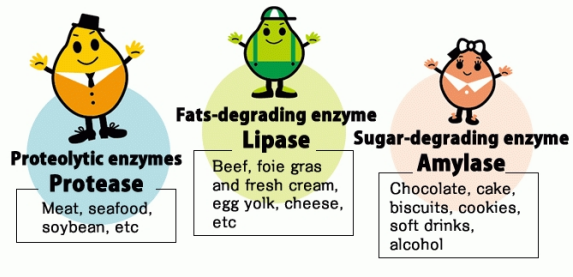
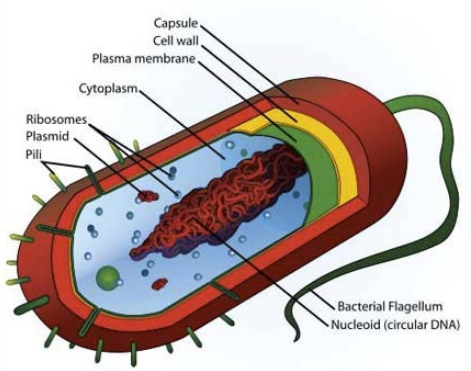
Lecture #1: Introduction and History of Enzymes

(1) Historical Aspects
(2) Discovery of enzymes
(3) Chemistry of enzymes
(4) Enzymes as proteins
(5) Function and importance
(6) Enzymes in Biotechnology
- Aspartic proteases
- Pectinases
- Detergents
  - Proteases
  - Lipases
  - Amylases
  - Cellulases
- Sucrases



Lecture #1: Introduction and History of Enzymes

1. Historical Aspects



public domain image via Wikipedia Creative Commons

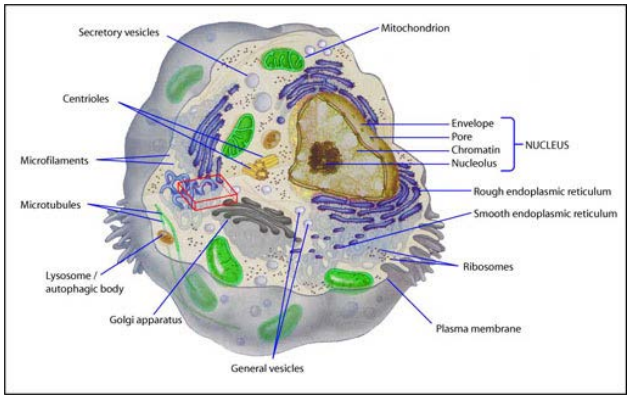
Enzymes are catalysts: increase the rate of reaction without undergoing change

-1 reaction: 1 or more enzymes

-*E. coli*--3000 enzymes

"**Proteomics**"-large scale study of proteins (structure and function)

-eukaryotic cell--50,000 enzymes



"Enzyme"--means "in yeast" (Greek)

-used by man--fermentation of alcohol and cheese

2. Discovery of enzymes

-enzyme first used in 1878 by W. Kühne while working with trypsin from pancreatic juices

-yeast were organized systems capable of fermentation

-1897 E. Büchner showed that yeast extracts could also ferment sugar to alcohol

-dispelled the doctrine of "vitalism": '*living organisms are fundamentally different from non-living entities*'

-the term enzyme (in yeast) adopted and now refers to **biological catalysts produced by living organisms**

Willhelm Kühne, German Physiologist

Science Museum/Science & Society Picture Library

Eduard Buchner, 1860–1917

Unnumbered 6 p188a
Lehninger Principles of Biochemistry, Seventh Edition

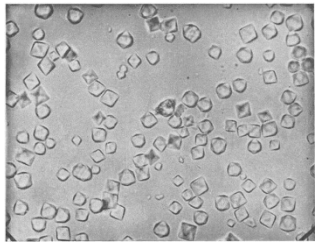
Nobel Prize in Chemistry in 1907

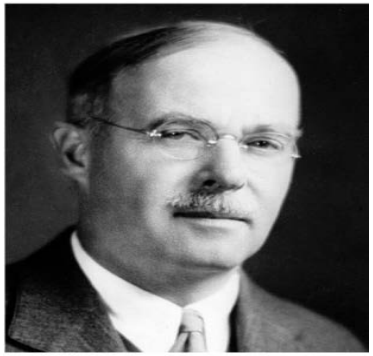
-extracts of yeast could ferment sugar to alcohol

3. Characterization of Enzymes as Proteins

•attempts to purify enzymes began in early 1900's

•crystallization of urease by Sumner in 1926
  – **enzyme consisted of protein!**

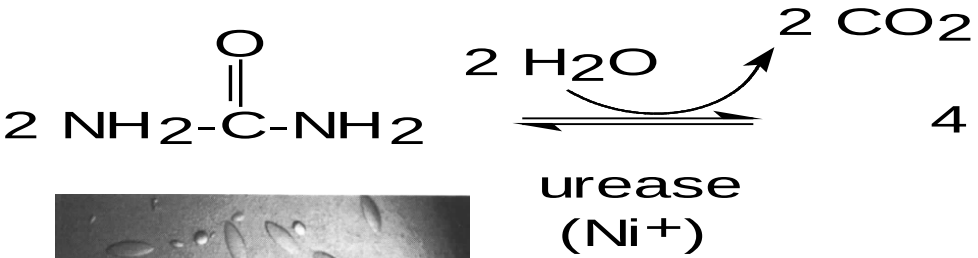


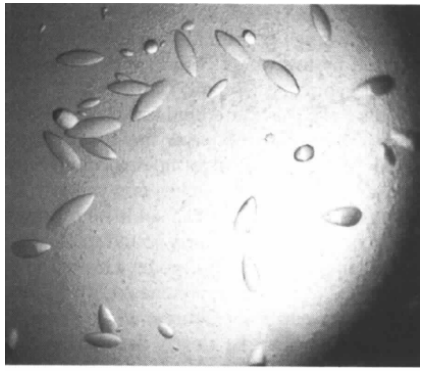


James Sumner
1887–1955

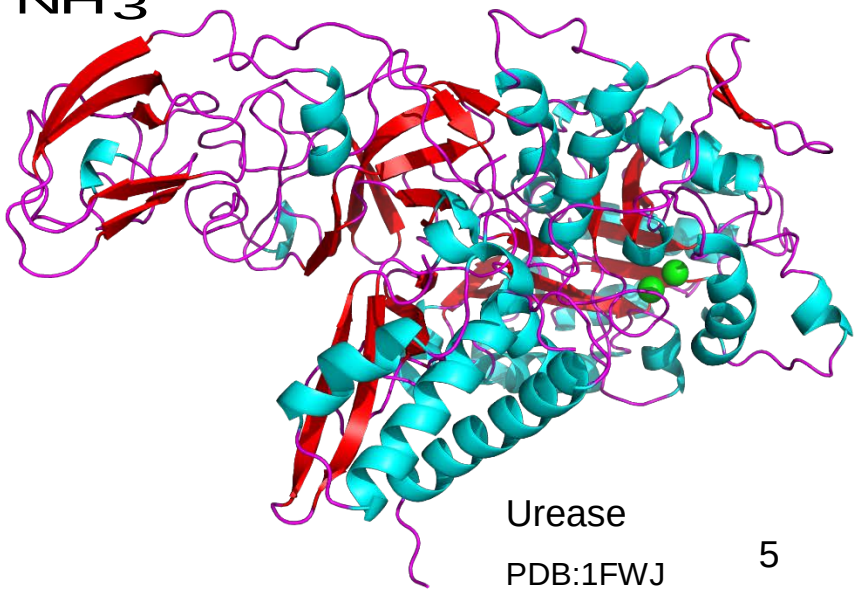
Nobel Prize in Chemistry 1946

• urease catalyzes the hydrolysis of urea to form CO_2 and NH_3





- Crystal structure was not determined until 1990's (50 years later)!

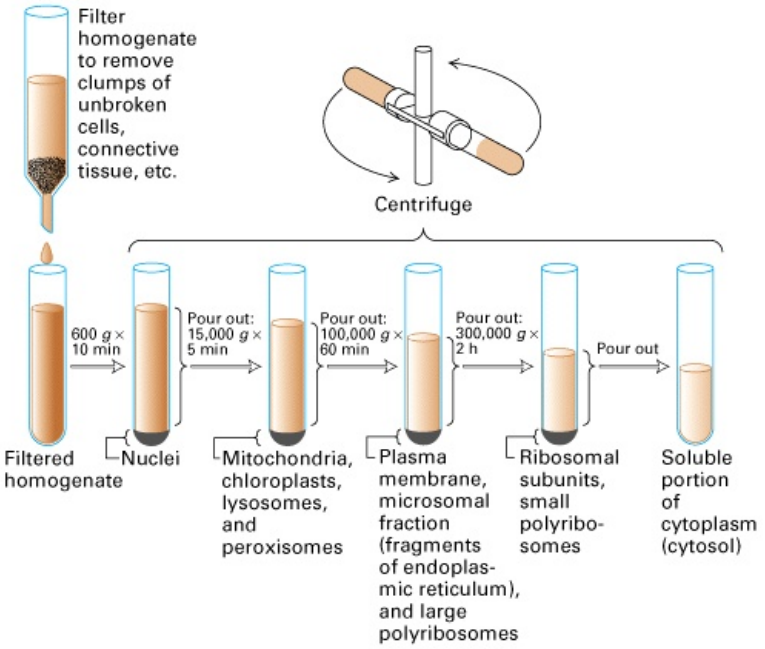


Urease
PDB:1FWJ

Theo Svedberg

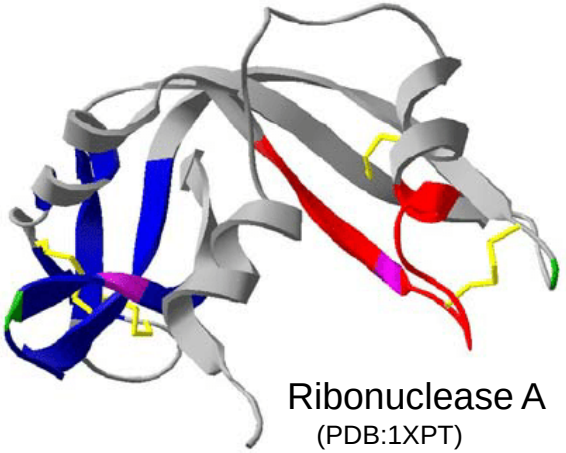
Nobel Prize in Chemistry in 1926

- ultracentrifugation by T. Svedberg showed large mass of enzymes as proteins
- (M_r of 10^4 - 10^7 D) for enzymes
- each enzyme is a homogeneous population of proteins

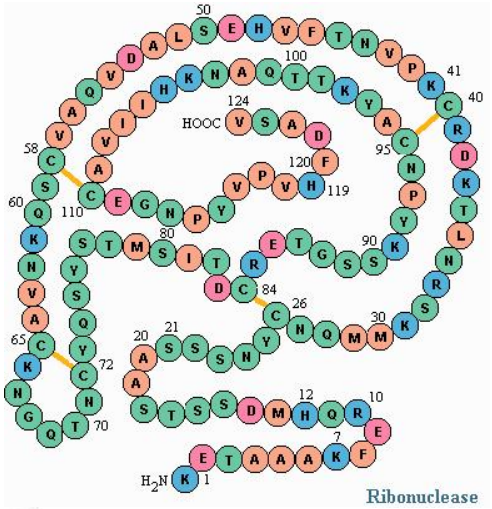


4. Chemistry of enzymes

- first required an understanding of their structure
- Ribonuclease A
  - large positive surface charge (pI = 8.6)
  - very polar with little nonpolar core
  - required 4 disulfide bonds to stabilize the structure
  - 1st amino acid sequence (RNase A) obtained in 1960
    - 1st enzyme synthesized in 1969 (chemically)
    - Synthesized enzyme (124 residues) was low purity and low activity



Ribonuclease A
(PDB:1XPT)



- 1st 3D structure of an enzyme (X-ray crystal structure) of lysozyme in 1965
- some knowledge of their mechanism of action (Fischer's lock & key model - 1894) by detailed kinetic investigation

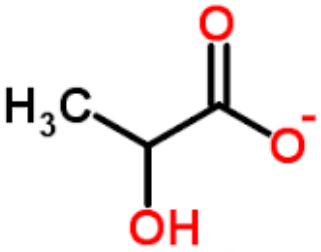
Lysozyme
(PDB:253L)

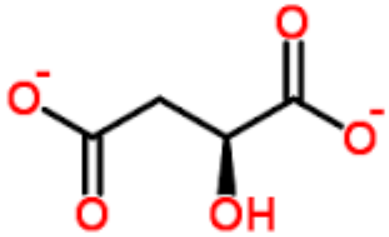
• present day can easily synthesize proteins by **recombinant DNA methodology**

• manipulate the structure using **site-directed mutagenesis** to examine important sections of the enzyme

Lactate dehydrogenase from a parasitic protist

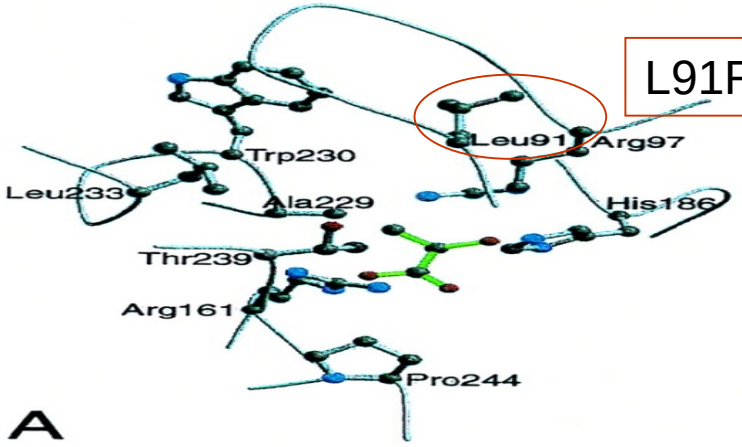
lactate dehydrogenase → malate dehydrogenase

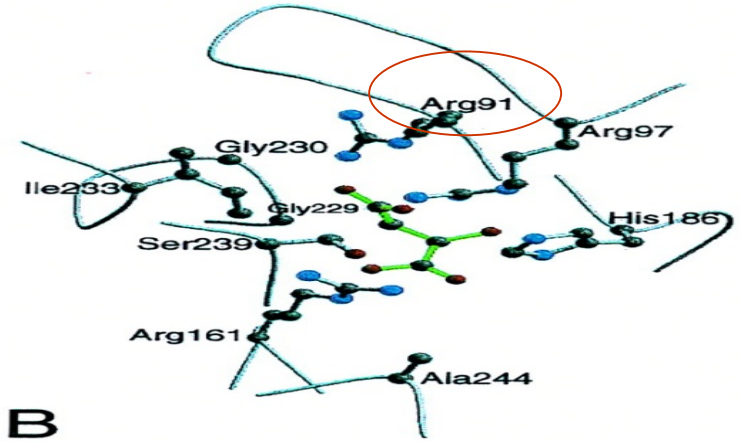




Trichomonas

L91R





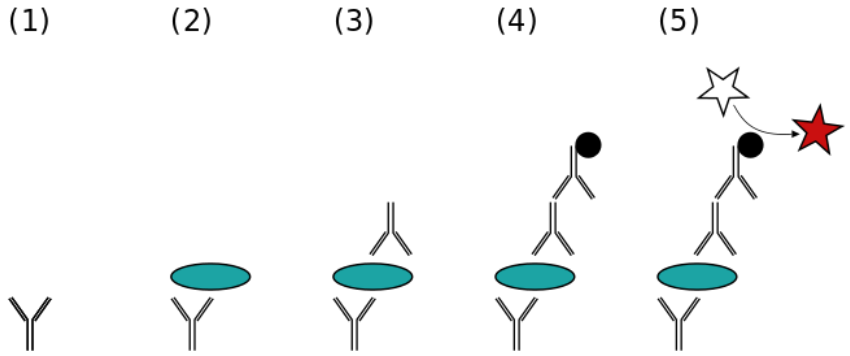
Wu, Gang et al. (1999) Proc. Natl. Acad. Sci. USA 96, 6285-6290

5. Function and importance

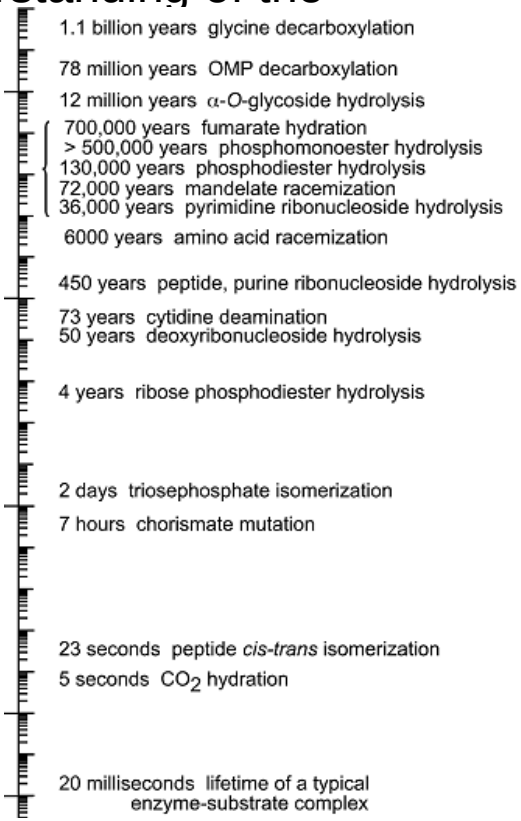
- enzymes are **biological catalysts**
  - Catalytic “nanomachines”
- **primary function:** to catalyze **all metabolic processes** within a cell

- an understanding of enzymes therefore leads to an understanding of the **relationships and control** of cellular function

  - *Enzymes are necessary because:*
    - *(1) reactions must be biologically friendly,*
    - *(2) reactions must be specific,*
    - *(3) reactions must have very high reaction rates*
- enzymes are also used as analytical reagents, eg. ELISA



A sandwich ELISA. (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.



Genetic Disorders

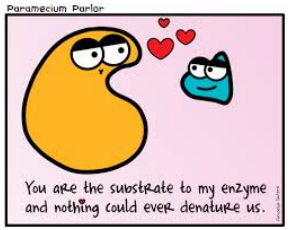
- many human genetic disorders are **caused** by a **defective enzyme**

TABLE 18-2 Some Human Genetic Disorders Affecting Amino Acid Catabolism

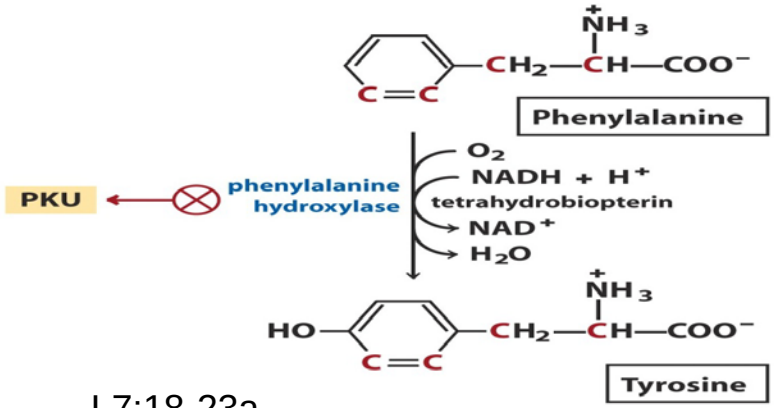
Medical condition	Approximate incidence (per 100,000 births)	Defective process	Defective enzyme	Symptoms and effects
Albinism	<3	Melanin synthesis from tyrosine	Tyrosine 3-monooxygenase (tyrosinase)	Lack of pigmentation; white hair, pink skin
Alkaptonuria	<0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine; late-developing arthritis
Argininemia	<0.5	Urea synthesis	Arginase	Intellectual disability
Argininosuccinic acidemia	<1.5	Urea synthesis	Argininosuccinase	Vomiting; convulsions
Carbamoyl phosphate synthetase I deficiency	<0.5	Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy; convulsions; early death
Homocystinuria	<0.5	Methionine degradation	Cystathionine *β*-synthase	Faulty bone development; Intellectual disability
Maple syrup urine disease (branchedchain ketoaciduria)	<0.4	Isoleucine, leucine, and valine degradation	Branched-chain *α*-keto acid dehydrogenase complex	Vomiting; convulsions; Intellectual disability; early death
Methylmalonic acidemia	<0.5	Conversion of propionyl-CoA to succinyl-CoA	Methyl-malonyl-CoA mutase	Vomiting; convulsions; Intellectual disability; early death
Phenylketonuria	<8	Conversion of phenylalanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; Intellectual disability

Lehninger 7th ed (2017)

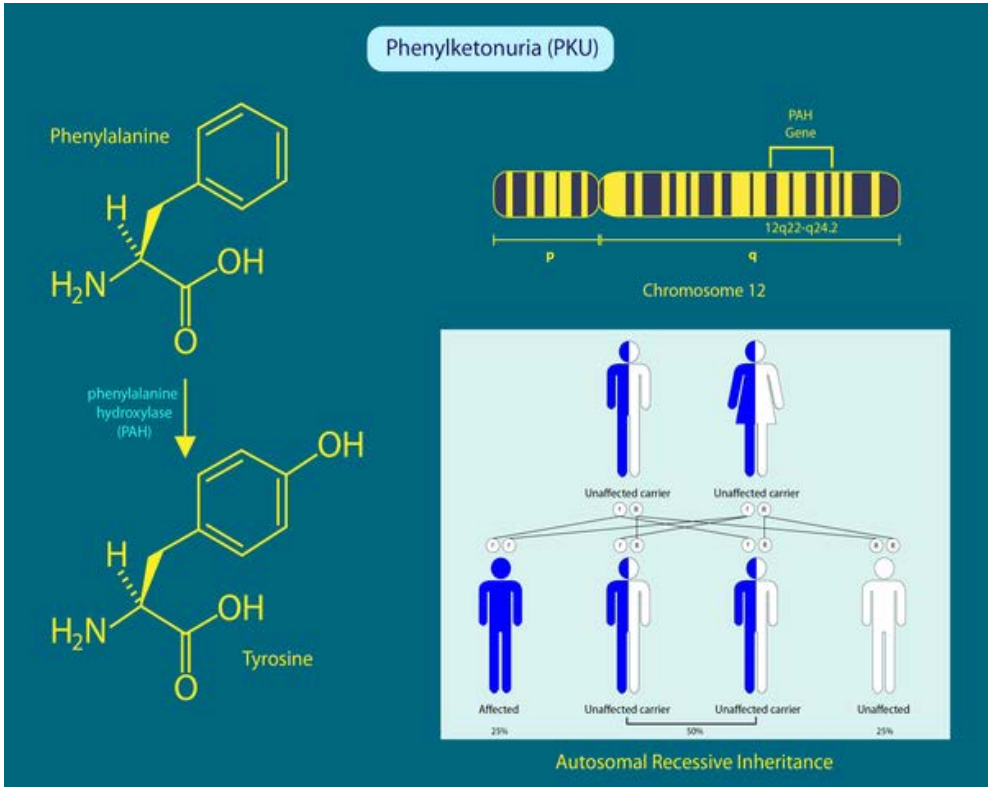
Phenylketonuria (PKU)

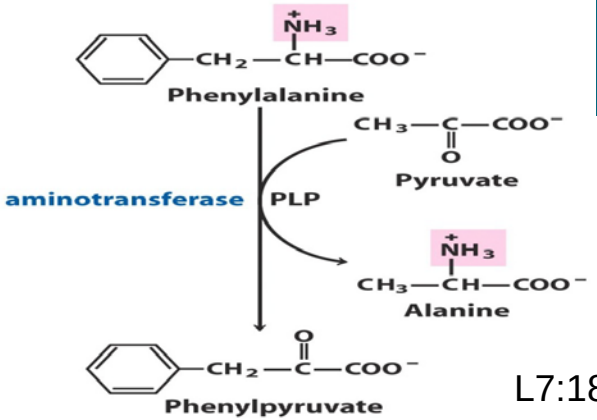


- caused by a genetic defect in **phenylalanine hydroxylase** (1st enzyme in catabolism of Phe)



L7:18-23a





L7:18-25a

•Much of the phenylpyruvate is decarboxylated or reduced

•Phe and its metabolites compete with other amino acids for transport across the blood-brain barrier ⇒ impairs brain development in infants ⇒ **severe mental disabilities (permanent)!**

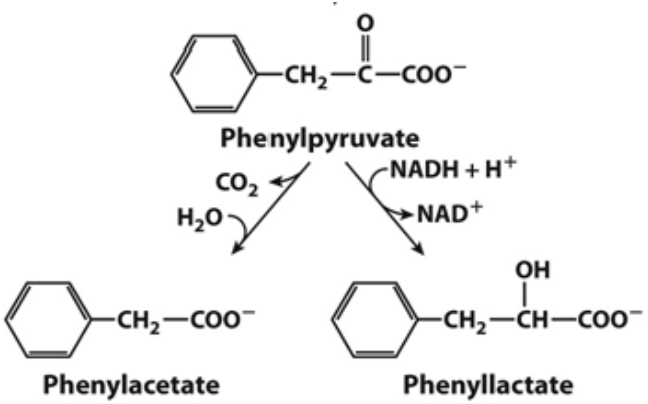


Figure 18-25
Lehninger Principles of Biochemistry, Seventh Edition

•**Test-**blood sample taken from heel of baby
•(2-3 days after birth) and test for phenylhydroxylase activity in blood


The blood of a two week-old infant is collected for a phenylketonuria, or PKU, screening
https://en.wikipedia.org/wiki/Neonatal_heel_prick

•**Treatment-**dietary control to ensure that only enough Phe and Tyr are consumed for protein synthesis needs
  –People with PKU need to avoid various high-protein foods, including:
    - Milk and cheese
    - Eggs
    - Nuts
    - Soybeans
    - Beans
    - Chicken, pork or beef
    - Fish
    - Peas
    - beer

6. Enzymes in Biotechnology/Industry

- **Catalyzing the production of required materials**
  - Examples:

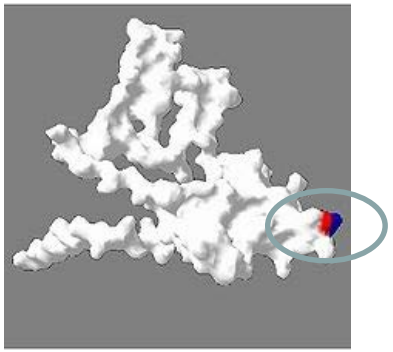
Sector	Enzymes	Applications
Pharmaceuticals	Nitrile hydratase, transaminase, monoamine oxidase, lipase, penicillin acylase	Synthesis of intermediates for production of active pharmaceutical ingredients
Food Processing	Trypsin, amylase, glucose isomerase, papain, pectinase	Conversion of starch to glucose, production of high fructose corn syrup, production of prebiotics, debittering of fruit juice
Detergent	Protease, lipase, amylase, cellulase	Stain removal, removal of fats and oils, color retention,
Biofuels	Lipase, cellulase, xylanase	Production of fatty acid methyl esters, decomposition of lignocellulotic material for bioethanol production
Paper and Pulp	Lipase, cellulase, xylanase	Removal of lignin for improved bleaching, improvement in fiber properties

- **Catalyzing the production of required materials**

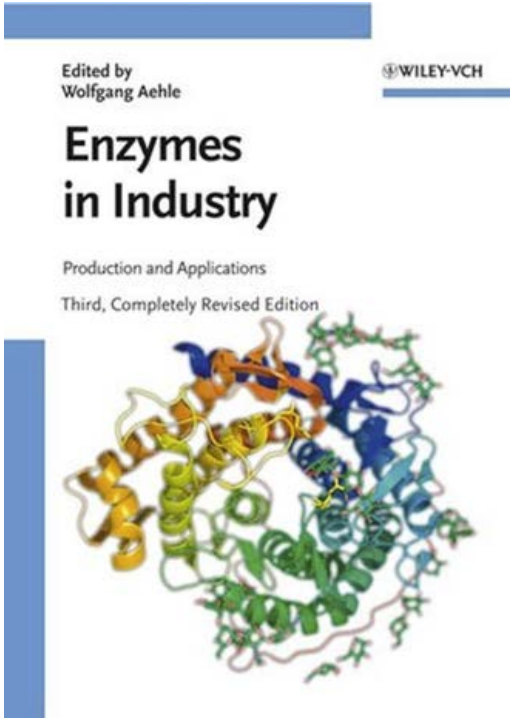
  - In the pharmaceutical industry, several enzymes are used to produce active ingredients with high specificity
  - These enzyme catalyze various chemical reactions
  - Some have been engineered for improved activity/stability
  - Examples:

Drug	Disease or Condition	Enzyme	Catalyzed Reaction	Company
Cymbalta	Depression	Ketoreductase	C=O ⟶ C–OH	Codexis
Lipitor	High cholesterol	Halohydrin dehalogenase	C–Cl ⟶ C–C≡N	Codexis
Januvia	Diabetes	Transaminase	C=O ⟶ C–NH_2	Codexis
Lyrica	Epilepsy	Esterase	COO–Et ⟶ COOH	Pfizer
Tekturna	Hypertension	Esterase	COO–Me ⟶ COOH	DSM

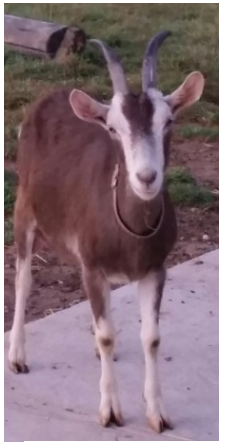
- **Aspartic proteases** (AP) used in cheese making
  - •APs hydrolyse the Phe^{105}-Met^{106} of bovine κ-casein splitting the protein into the hydrophobic and hydrophilic parts
    - natural source: rennet from young calves
    - Recombinant source: bovine chymosin from *E. coli*
  - •Coagulation of casein micelles due to destabilization from proteolysis



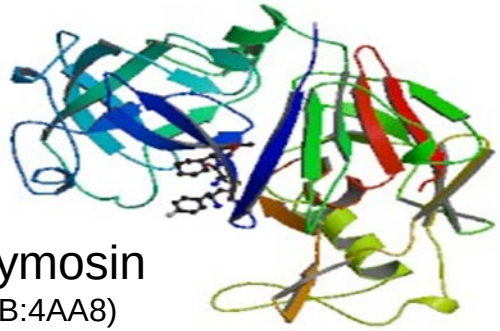
κ-casein with Phe^{105}-Met^{106} bond



Goat cheese

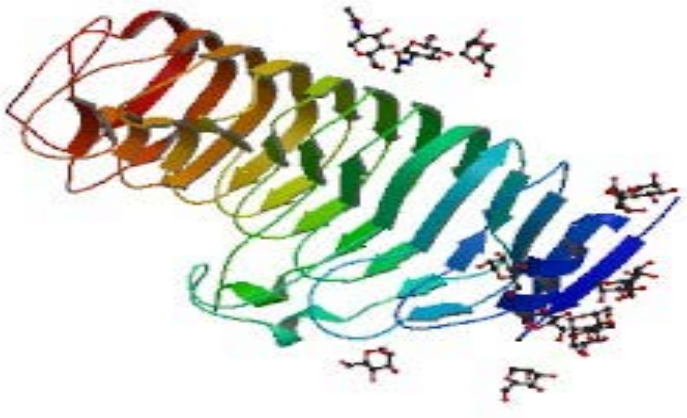


Pepper



Chymosin
(PDB:4AA8)

•**Pectinases** (P) degrade complex polysaccharides of plant cell walls which clarifies fruit juices and lowers the viscosity for drinking



polygalacturonase from *Aspergillus aculeatus*

•**Enzymes in household detergents**

- Enzymes break down stains and dirt into soluble and smaller fragments which aid household detergents in the solubilization process
- Involves proteases, amylases, and lipases that have been genetically engineered for greater thermal, pH and detergent stability

Proteases

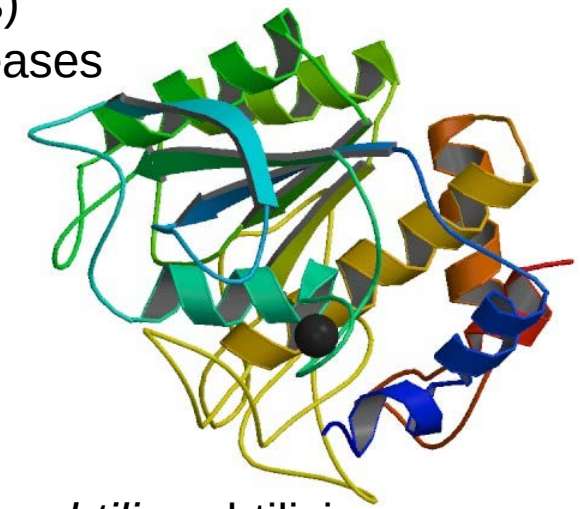
-remove protein stains (grass, blood, egg and human sweat)

-organic stains adhere to textile fibres preventing the water-detergent systems from removing pigments and street dirt

-proteases hydrolyse proteins and break them down into more soluble polypeptides or free amino acids

Biotouch™ Detergent Proteases (Ab Enzymes)—tailored detergent enzyme solutions

- Effective at lower temp (20 – 40°C)
- Work well even “in soft” water
- Improved enzyme stability (stable even after 18 weeks)
- Subtilisins, trypsin and chymotrypsin-like, metalloproteases



B. subtilis subtilisin

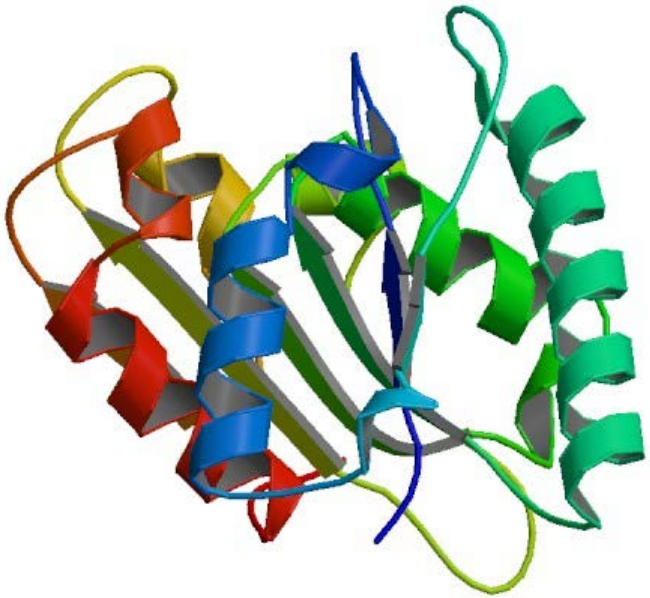
PDB:1SBC

Lipases

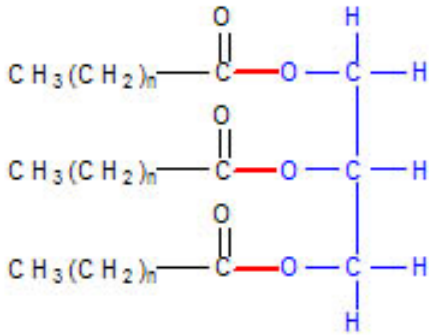
-the trend towards lower washing temperatures has made the removal of grease spots a **big problem**

-lipases remove fatty stains such as fats, butter, salad oil, sauces and the tough stains on collars and cuffs

-break ester bonds in triglycerides and phospholipids
and produce more soluble fatty acids



B. subtilis lipase
PDB:3D2A



triglyceride

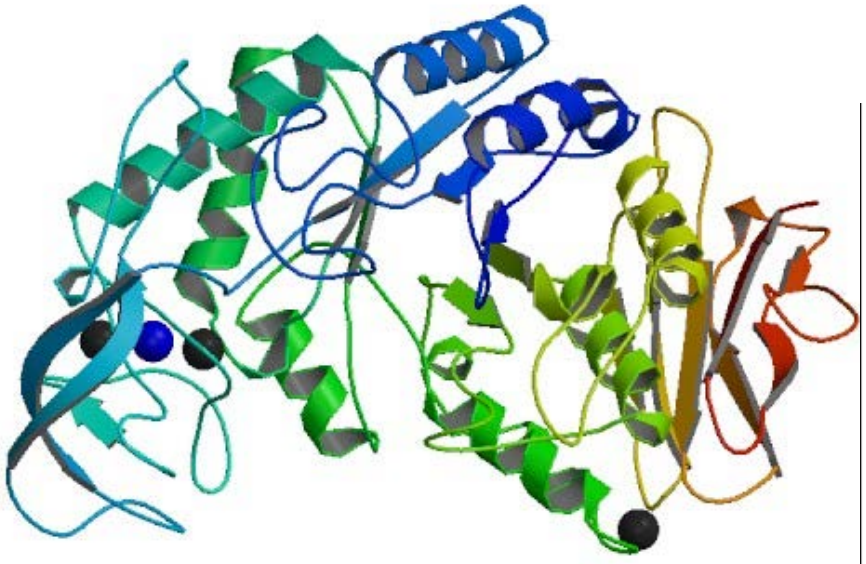
Amylases

- remove residues of starch-based foods (potatoes, spaghetti, custards, gravies and chocolate)

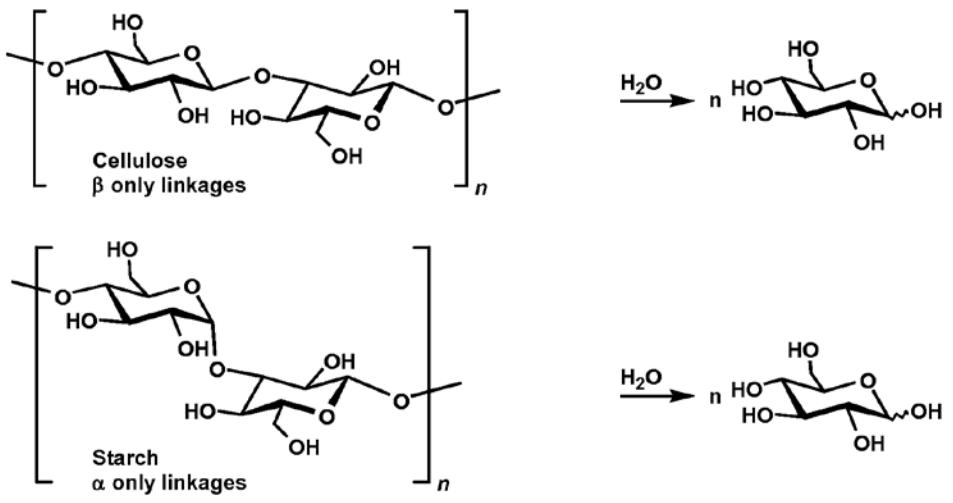
-breakdown complex carbohydrates into short-chain (soluble sugars)

-α-amylases, β-amylases

-<u>application</u>: used for cleaning stains from clothes and dishes



B. licheniformis α-amylase
PDB:1BLI



Cellulases

- Cotton fibrils are degraded by cellulases restoring a smooth surface to the fibre and restoring the garment to its original color
- Softening-the enzyme has a softening effect on the fabric, probably due to the removal of the microfibrils
- Soil removal -some dirt particles are trapped in the network of microfibrils and are released when the microfibrils are removed by cellulases

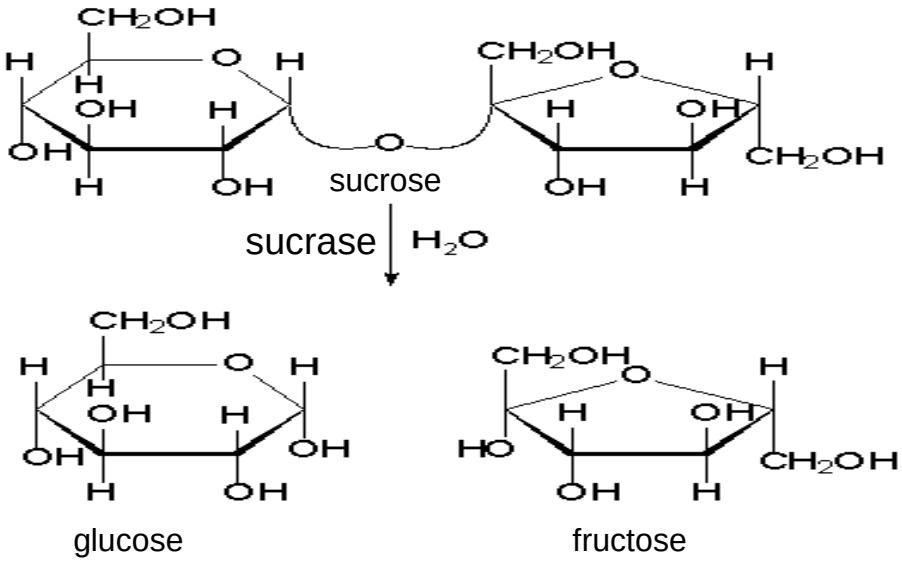
S. Lividans endoglucanase PDB:1NLR

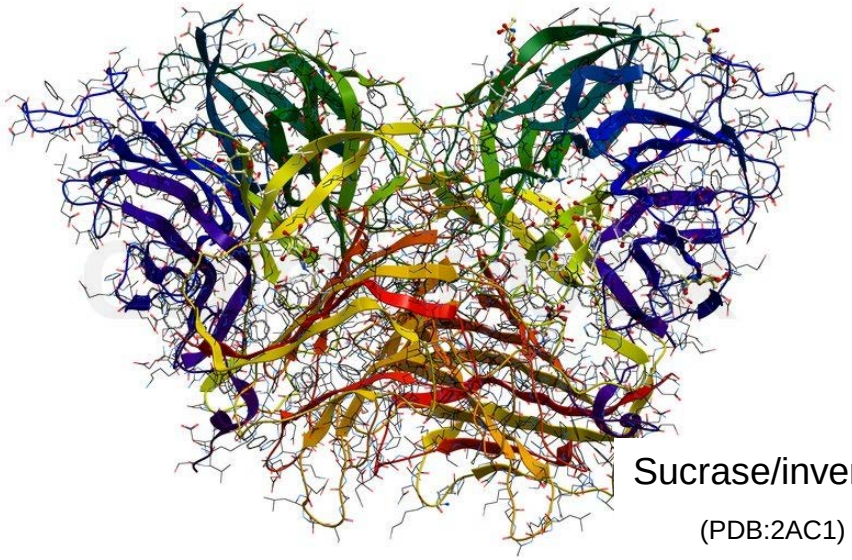
Denim finishing

- denim jeans are treated with cellulases
- Cellulases partly hydrolyze the surface of the denim fiber causing the release of indigo dye to give a random, faded pattern

Sucrase

- catalyzes the hydrolysis of sucrose





Sucrase/invertase

(PDB:2AC1)

How do they get the Caramilk into the Caramilk™ bar?

• use solid sucrose caramilk centre with a miniscule (fmol) of **sucrase** ⇒ converts sucrose to fructose + glucose (more soluble and much sweeter than sucrose-Merrill hypothesis)