Introduction to Bioproducts and Bioseparations

Bioproducts

- **Definition**
  - Chemical substance or combinations of chemical substances made by living things
- **Types**
  - From small molecules to whole cells
- **Value of bioproducts**
  - Depending on their activities
    - Methanol : solvent
    - Penicillin : antibacterial activity
    - Taxol : anticancer activity
- **Separation methods**
  - Depending on the nature of the product
- **The goals of bioseparation**
  - Activity > purity, yield

Production Levels vs. Prices of Bioproducts

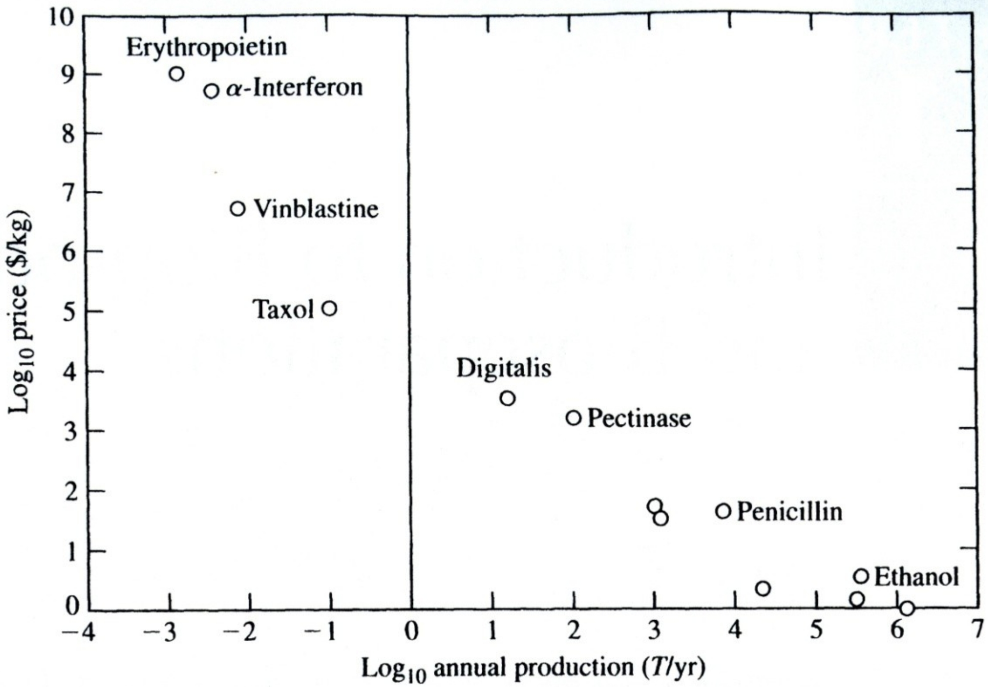


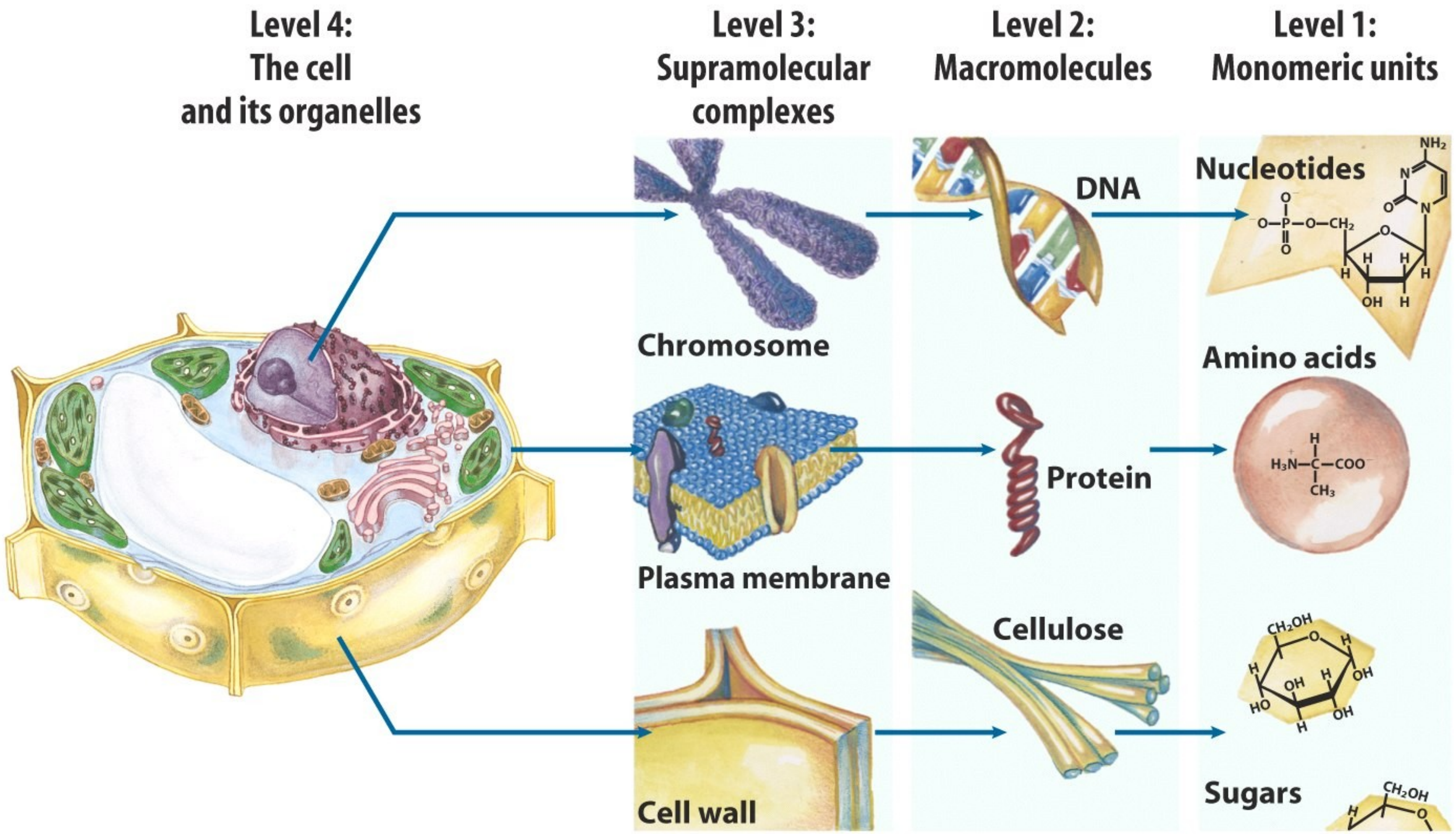
Figure 1.1 World production levels and prices of bioproducts, showing the inverse relationship between price and production. (Data from sources in the early 1990s time frame.)

Biotechnology Product Forecasts

TABLE 1.1
Summary of U.S. Biotechnology Product Forecasts (Million of 2000 Dollars) by Key Market Sectors: 2000-2010

Key sectors	Sales Forecasts ($ million)			Annual Growth, 2000-2010(%)
	2000	2005	2010	
Human therapeutics	$11,700	20,600	36,300	12
Human diagnostics	2,500	3,700	5,400	8
Agriculture	980	2,250	5,100	18
Specialty chemicals	550	1,170	2,400	16
Nonmedical diagnostics	320	480	700	8
Totals	16,050	28,200	49,900	12

Cells Build Supramolecular Structures



Classification of bioproducts

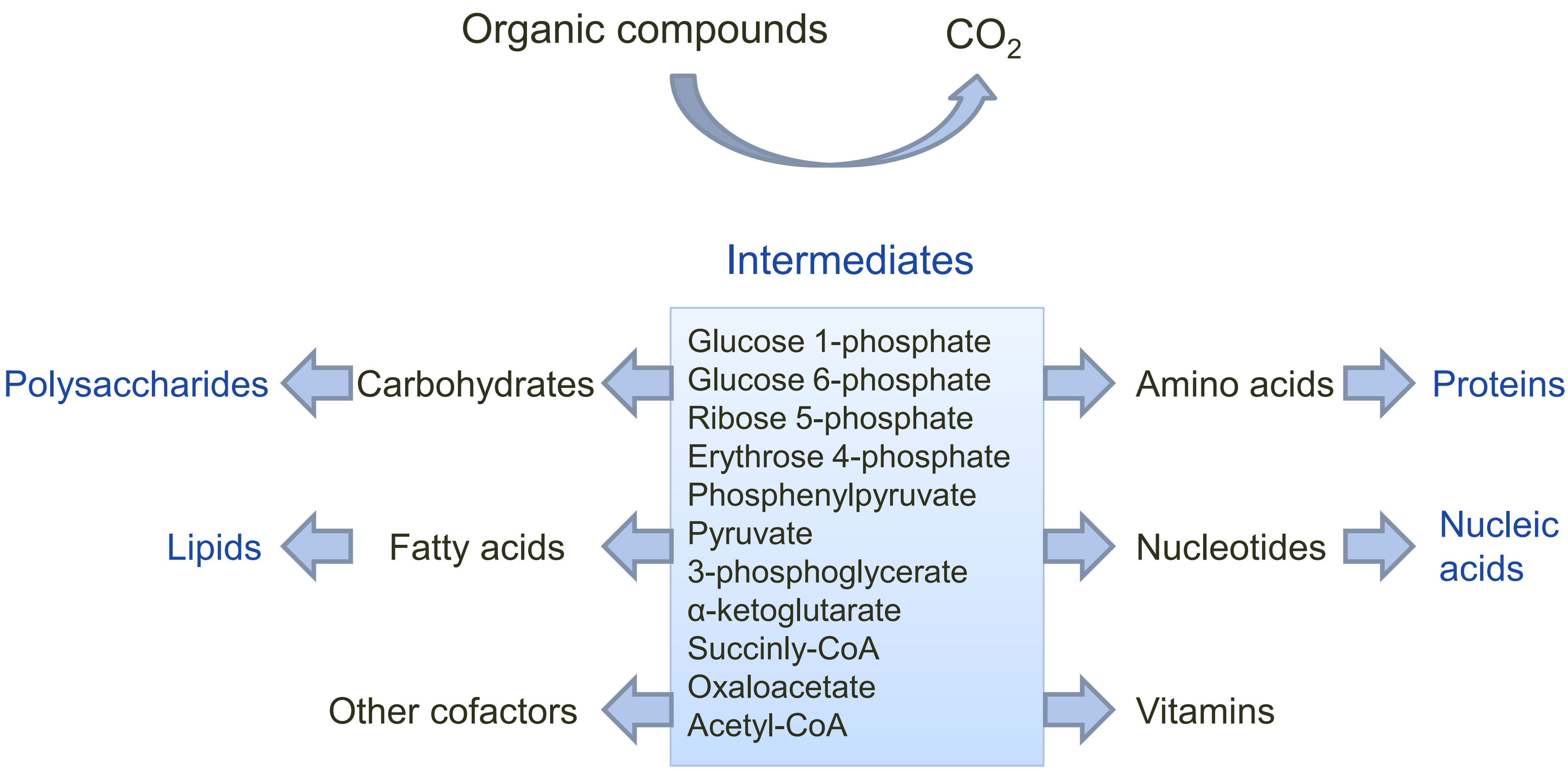
Bioproduct	Examples	MW (Da)	Typical Radius	Separation Methods
Small molecules	Sugars	200-600	0.5 nm	Extraction
	Amino acids	60-200	0.5 nm	
	Vitamins	300-600	1-2 nm	
	Organic acids	30-300	0.5 nm	
Large molecules	Proteins	10^3-10^6	3-10 nm	Adsoprtion
	Polysaccharides	10^4-10^7	4-20 nm	
	Nucleic acids	10^3-10^{10}	2-1000 nm	
Particles	Ribosomes		25 nm	Sedimentation or filtration
	Viruses		100 nm	
	Bacteria		1 μm	
	Organelles		1 μm	
	Yeast cells		4 μm	
	Animal cells		5-100 μm	

1.1 Small Molecules

Small Molecules

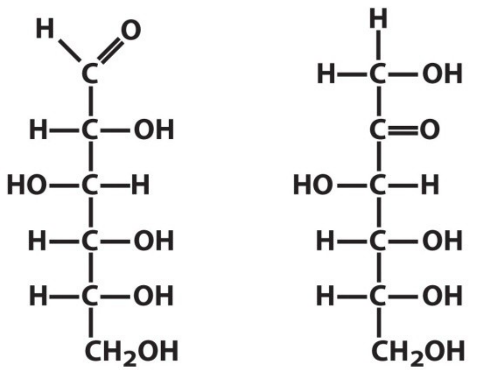
- **Primary metabolites**
  - Metabolites formed during the primary growth phase
    - Sugars
      - Monosaccharide
      - Disaccharide
    - Organic alcohols, acids, and ketones
      - Produced by anaerobic fermentation of microorganisms
      - Ethanol, isopropanol, acetone, acetic acid, lactic acid
    - Vitamins
      - Vitamin B, C
      - Vitamin A, D, E, K
    - Amino acids
    - Lipids
- **Secondary metabolites**
  - Metabolites produced near the beginning of the stationary phase
    - Antibiotics
    - Dyes

Biosynthetic Pathways in Heterotrophs



Sugars

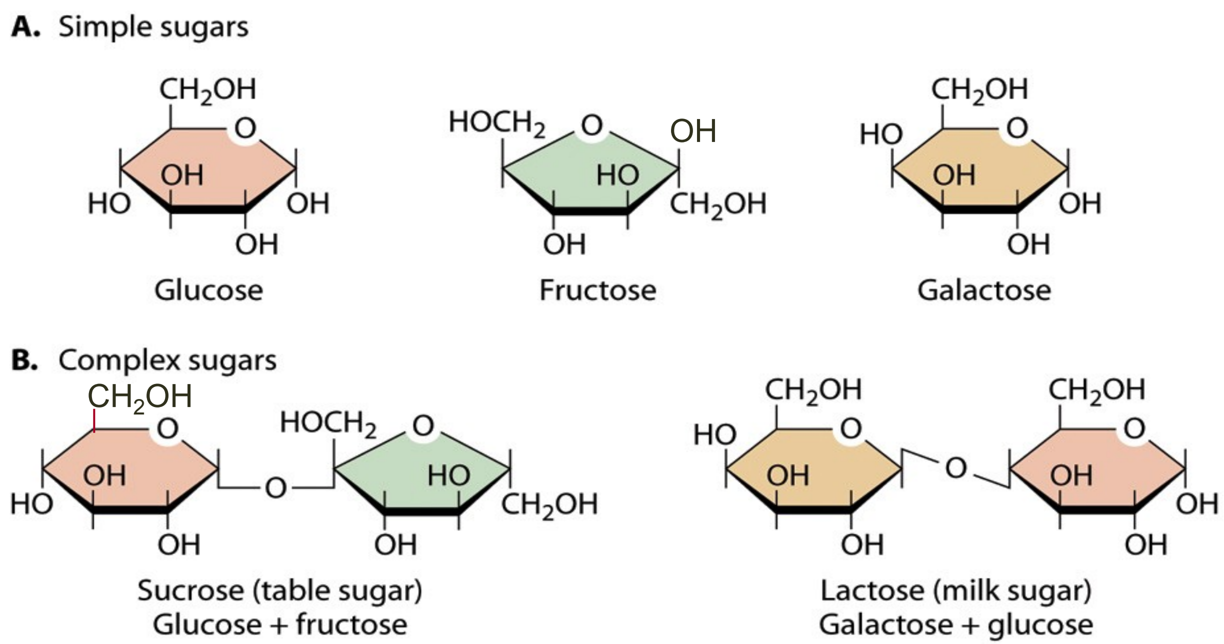
- **Structure**
  - Polyhydroxy aldehydes or ketones
  - C:H:O = 1:2:1
- **Classes of carbohydrates**
  - Simple sugars (monosaccharide)
    - 3 to 7 carbons
    - Aldose: aldehyde group, e.g) glucose, galactose
    - Ketose: ketone , e.g) fructose
  - Disaccharides
    - Simple sugars linked by glycosidic bond
      - Sucrose: glucose (α1↔2β) fructose
      - Lactose : galactose (β1→4) glucose
      - Maltose : glucose (α1 → 4) glucose
  - Oligosaccharides
  - Polysaccharides



D-Glucose, an aldohexose

D-Fructose, a ketohexose

Sugars

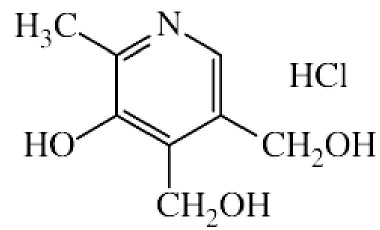


Glc (α1↔2β)Fru

Gal (β1→4)Glc

Vitamins

- **Vitamin B, C**

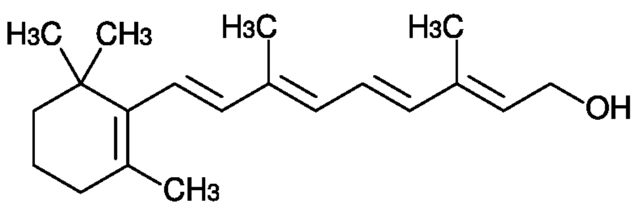


Vitamin B6 (Pyridoxine)

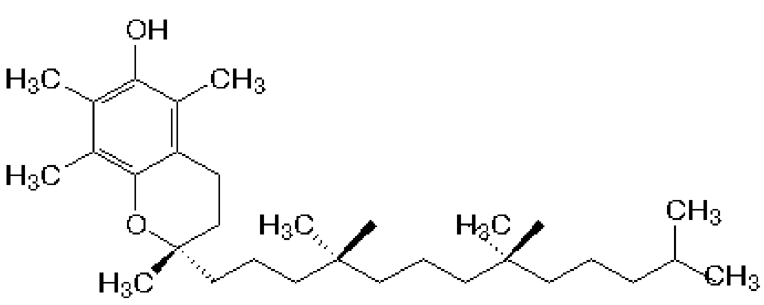
[redacted]

Vitamin C (ascorbic acid)

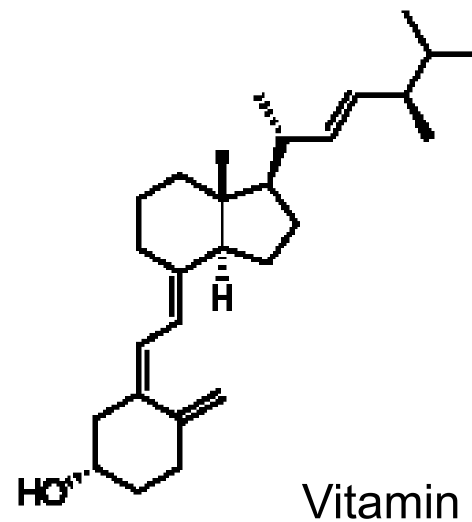
- **Vitamin A, D, E, K**



Vitamin A (retinol)



Vitamin E (α-tocopherol)



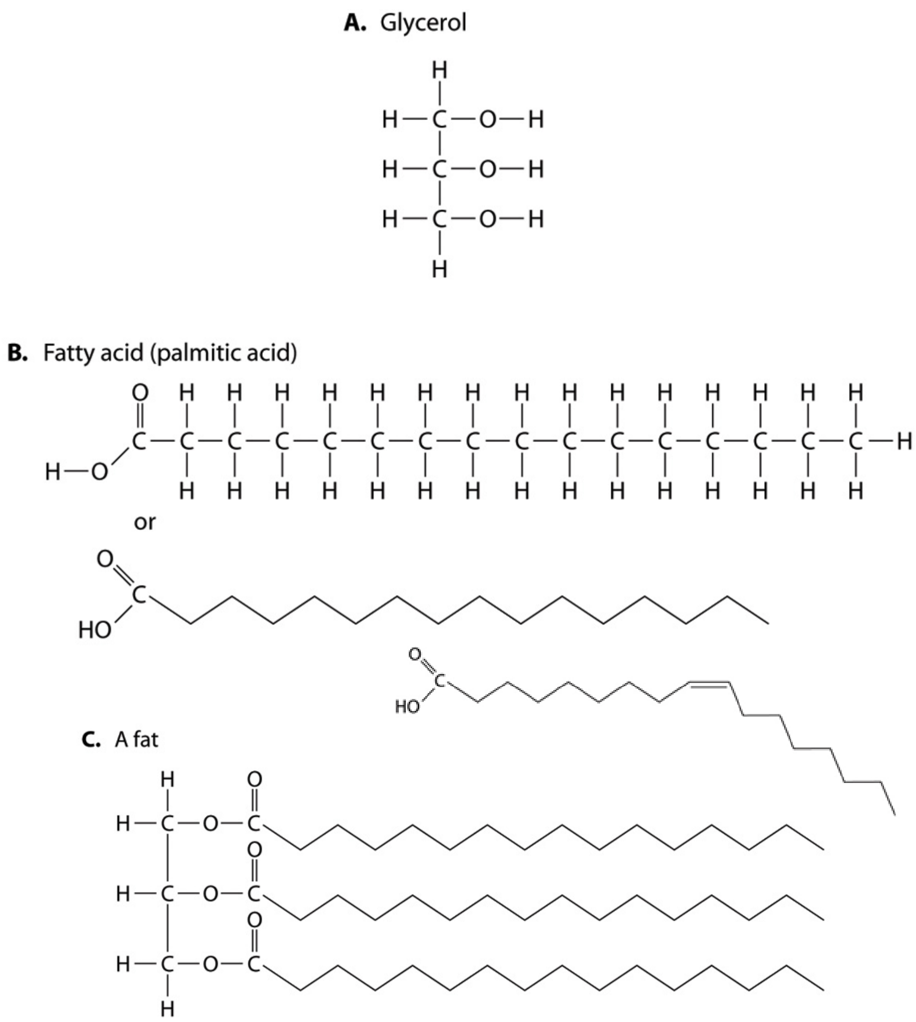
Vitamin D_2

Lipids

- **Characteristics**
  - Water insolubility
  - Diverse structures
- **Functions**
  - Energy storage : high energy C-H, C-C bonds
    - Fats : fatty acid + glycerol
  - Components of biological membrane
    - Phospholipids
    - Cholesterol
  - Signals
    - Steroid hormones
  - Enzyme cofactors
    - Vitamins

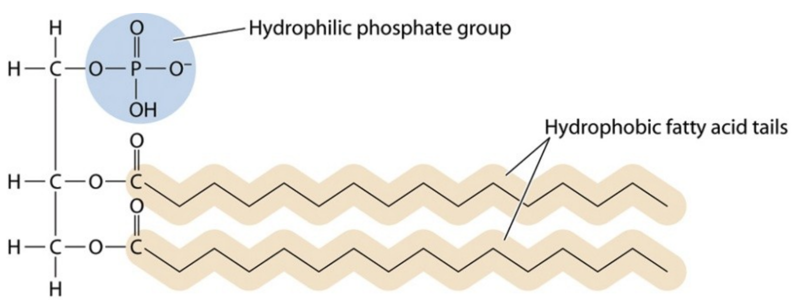
Lipids-1: Fats

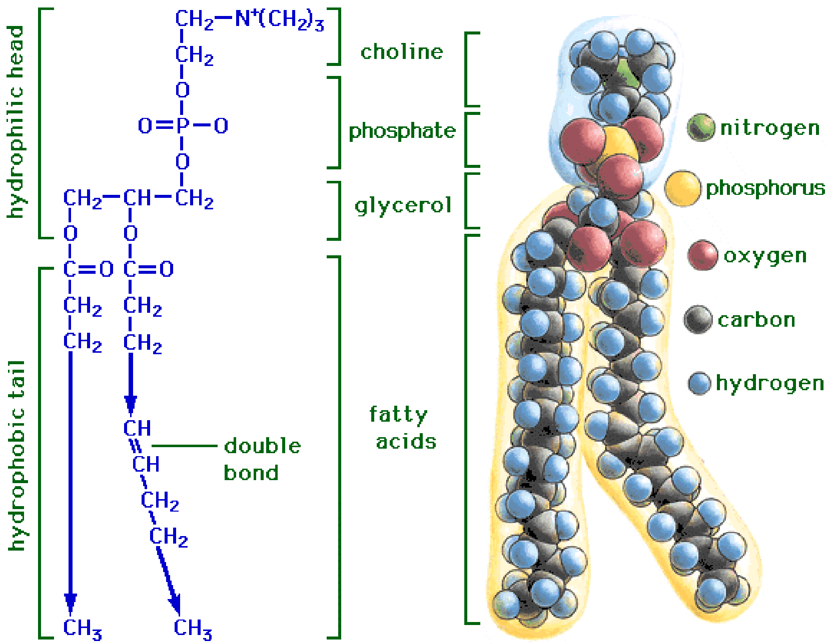
- **Structure**
  - Glycerol + 3 fatty acids linked by ester linkage
  - Fatty acids
    - Saturated fatty acids
      - Tight packing → High melting point
      - Solid at room temperature
    - Unsaturated fatty acids
      - More than one cis-double bond → Low melting point
      - Lliquid at room temperature
- **Function**
  - Energy storage
- **Other names**
  - Triacylglycerol, triglycerides, neutral fats
  - Oil : liquid at room temperature



Lipids-2: Phospholipids

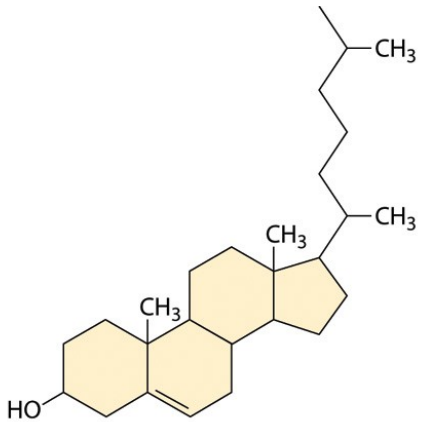
- **Structure**
  - Glycerol backbone
  - Two fatty acids (hydrophobic)+ phosphate (hydrophilic)
  - Additional small polar molecules bound to phosphate group
- **Function**
  - Major component of cellular membrane



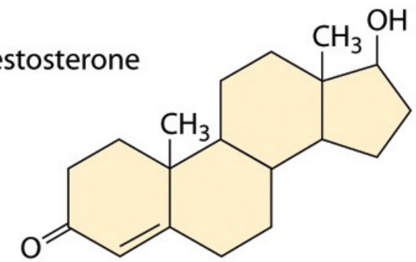


Lipids-3: Sterols

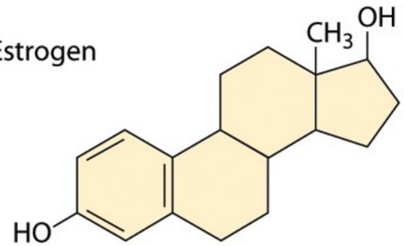
- **Structure**
  - Cyclic hydrocarbon compounds
  - Planar and rigid rings
    - 3 with C_6 and 1 with C_5
- **Cholesterol**
  - Component of animal cell membranes
    - Decrease membrane fluidity
  - Starting material for steroid hormones and bile synthesis



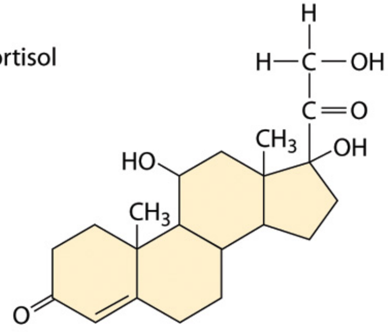
A. Testosterone



B. Estrogen

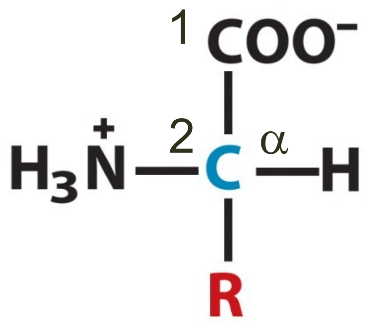


C. Cortisol

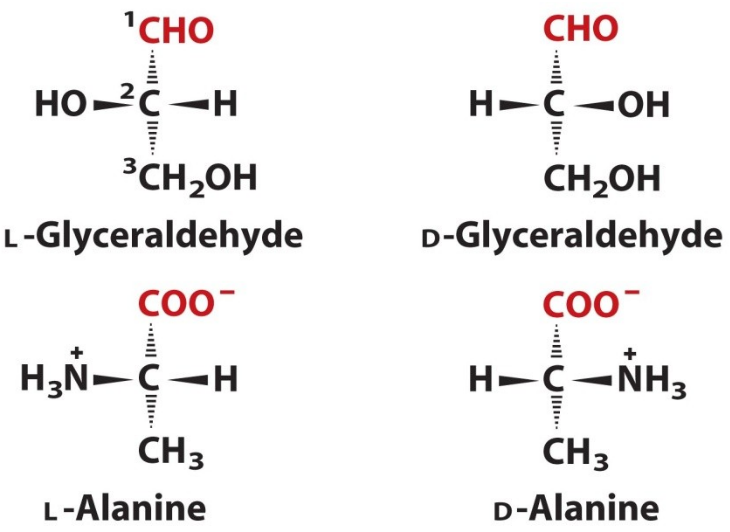


Amino Acids

- **20 Residues**
- **Numbering of carbons**
  - 1,2,3... from C of COO^-
  - α , β, γ… from C bonded to NH_3^+ and COO^-

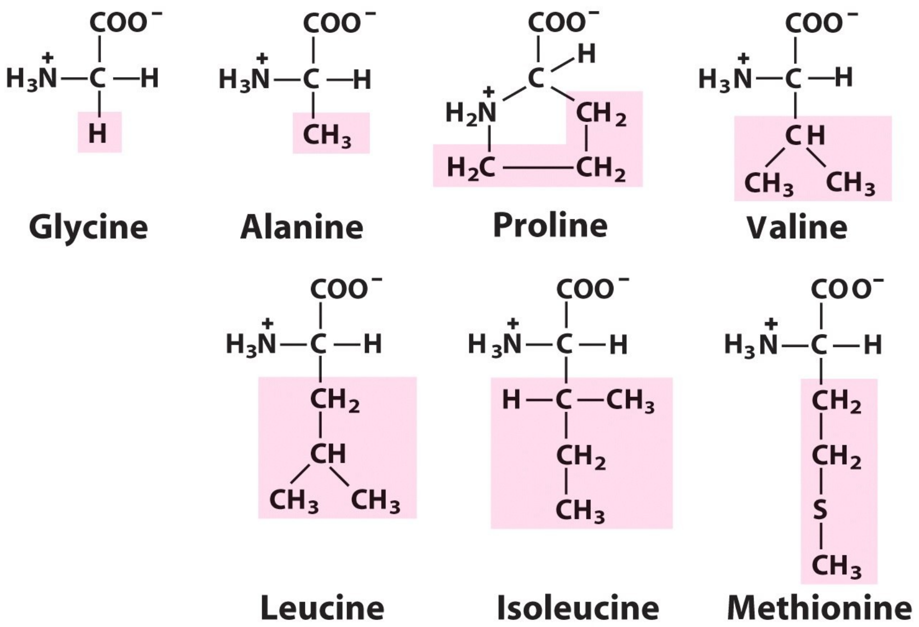


- **Absolute configuration of amino acids**
  - L-amino acids in biological system

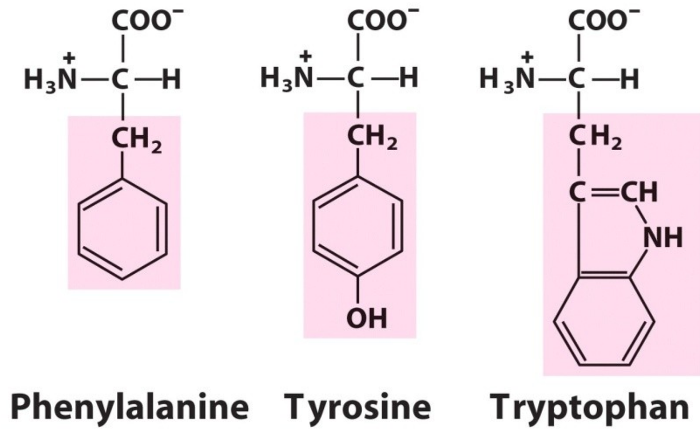


Classification of Amino Acids

Nonpolar, aliphatic R groups

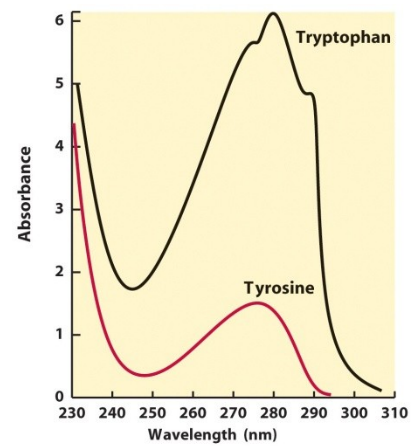


Aromatic R groups

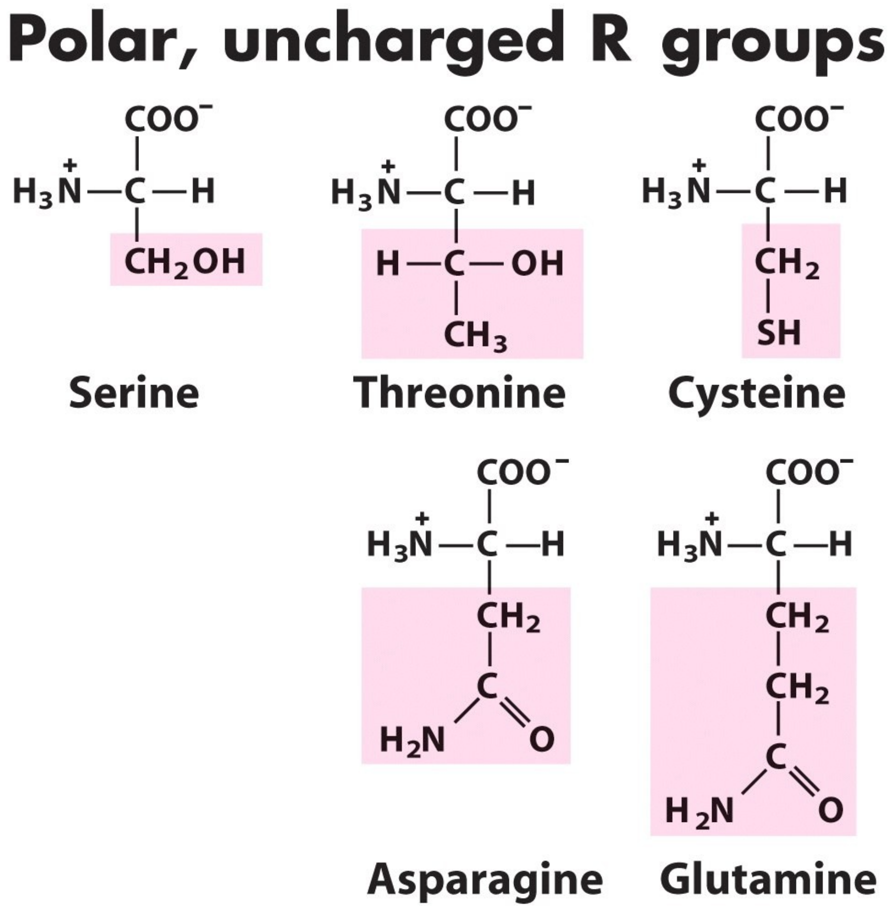


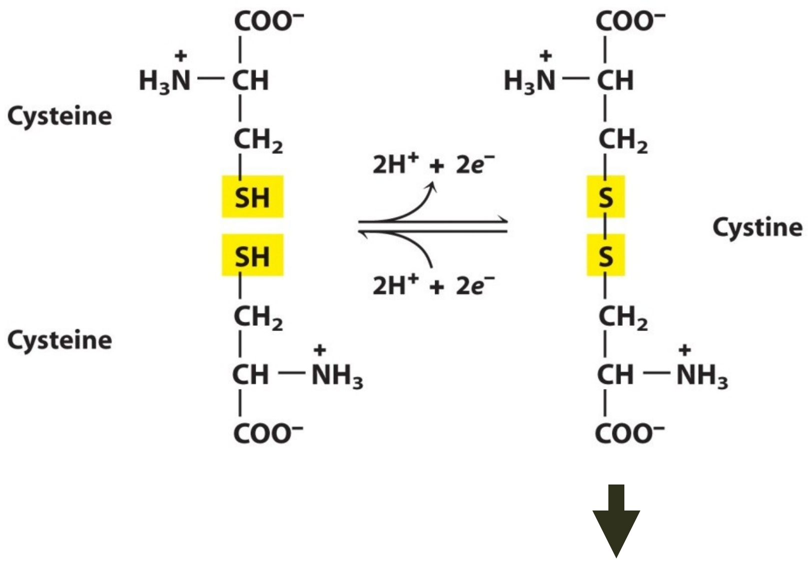
Nonpolar

UV absorption at 280 nm



Classification of Amino Acids



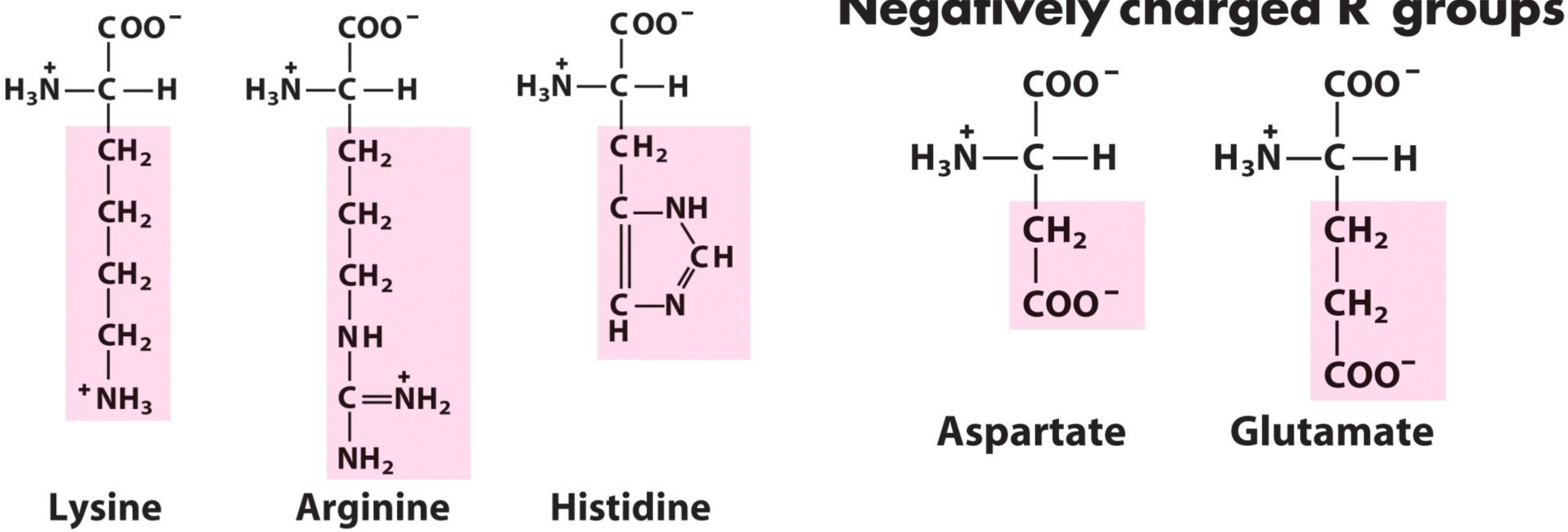


- Nonpolar
- Structural role

Classification of Amino Acids

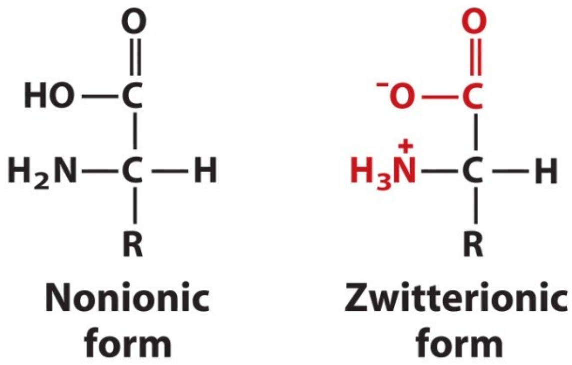
Positively charged R groups

Negatively charged R groups



Amino Acids as Acids and Bases

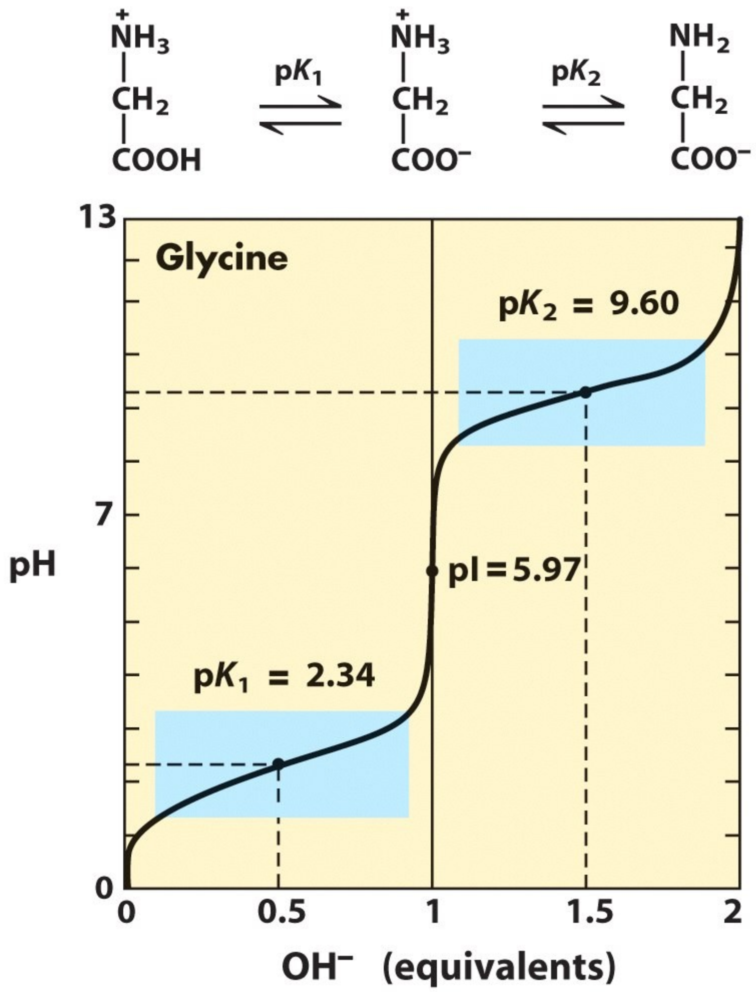
- **Zwitterion**
  - Acts as either an acid or a base
  - Ampholytes
    - Amphoteric substances
    - Substances with dual nature



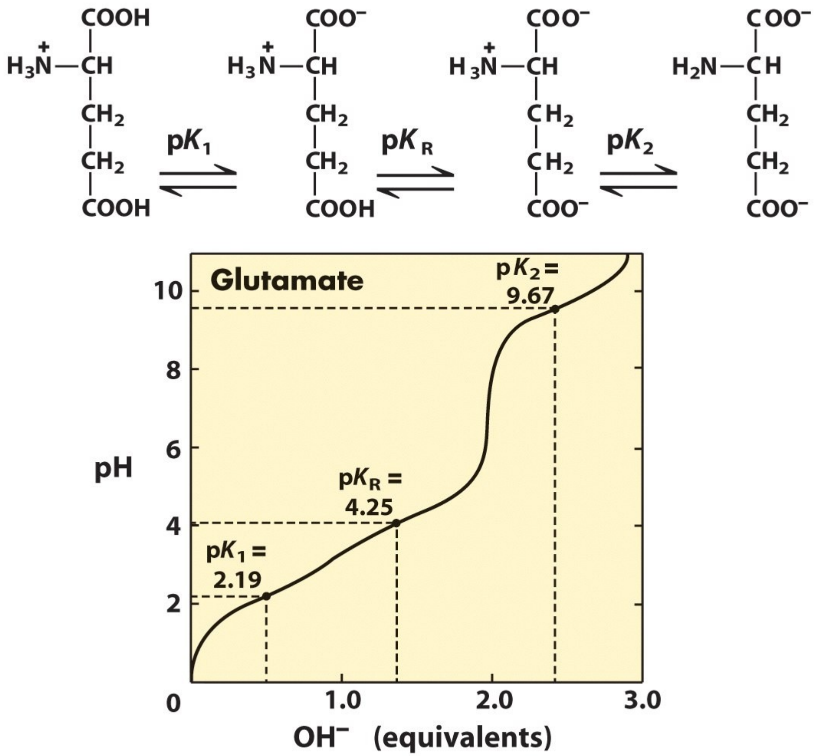
- **Amino acid is a diprotic acid**

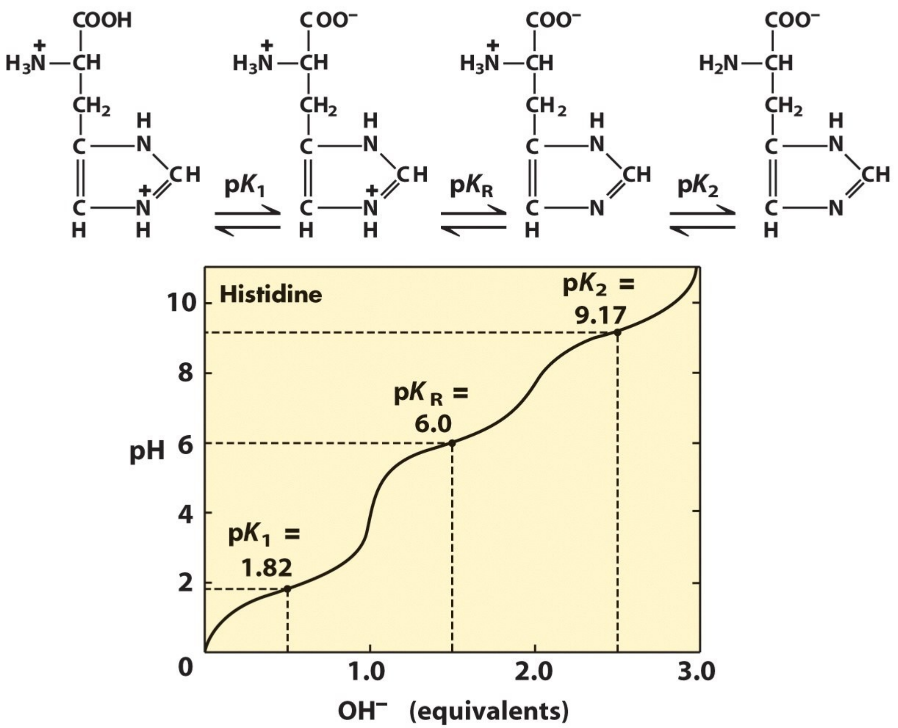
Titration of Amino Acids

- **Two p*Ka* and two buffering regions**
- **pI: ioselectric point or isoelectric pH**
  - The point with zero electric charge
  - Above pI : negative charge
  - Below pI : positive charge
- **pI = (pK_1 + pK_2)/2 = 5.92**



Amino Acids with Ionizable R Group





- pI = $(pK_1 + pK_R)/2 = 3.22$
- pI = $(pK_R + pK_2)/2 = 7.59$

Commercial Uses of Small Molecules

Table 6.1 Chemcials currently produced by microbial metabolism of glucose and their industrial applications

Chemical	Microbial source	Industrial uses
Ethanol	*Saccharomyces*	Industrial solvent, fuel, beverages
Acetic acid	*Acetobacter*	Industrial solvent, rubber, plastics, food acidulant[a] (vinegar)
Citric acid	*Aspergillus*	Food, pharmaceuticals, cosmetics, detergents
Gluconic acid	*Aspergillus*	Pharmaceuticals, food, detergent
Glycerol	*Saccharomyces*	Solvent, cosmetic preparations, soaps, antifreezes
Isopropanol	*Clostridium*	Industrial solvent, cosmetic preparations, antifreeze, inks
Acetone	*Clostridium*	Industrial solvent, intermediate in many chemical synthesis reactions
Lactic acid	*Lactobacillus, Streptococcus*	Food acidulant, fruit juice, soft drinks, dyeing, leather treatment, pharmaceuticals, plastics
Butanol	*Clostridium*	Industrial solvent, intermediate in many chemical synthesis reactions
Fumaric acid	*Rhizopus*	Intermediate in synthesis of synthetic resins, dyeing, acidulant, antioxidant
Succinic acid	*Rhizopus*	Manufacture of lacquers, dyes, and esters for perfumes
Malic acid	*Aspergillus*	Perfumes
Tartaric acid	*Acetobacter*	Acidulant, tanning, commercial esters for lacquers, printing
Itaconic acid	*Aspergillus*	Textiles, paper manufacture, paint

[a]An acidulant is a substance added to food or beverages to lower pH and to impart a tart, acid taste.

Table 6.2 Useful products from microbial metabolic pathways other than glucose metabolism

Type of product	Examples	Applications
Amino acid	Glutamic acid, phenylalanine, aspartic acid, lysine	Nutritional supplements, flavor enhancers, sweeteners
Carbohydrate	Dextran, xanthan gum	Food emulsifiers and thickeners, oil recovery
Vitamin	B_{12}, riboflavin, β-carotene	Nutritional supplements, pigments
Metabolic enzyme	Proteases, amylases, lipases	Detergents, sweeteners, brewing, cheese making, textiles, leather softening
Nucleotide	Guanosine, inosine	Flavor enhancers

Secondary Metabolites

- **Production of secondary metabolites**
  - At or near the beginning of the stationary phase
  - Primary metabolites are the raw material for the synthesis of secondary metabolites
- **Usage**
  - Antibiotics
  - Anticancer drug
  - Immunosuppressant

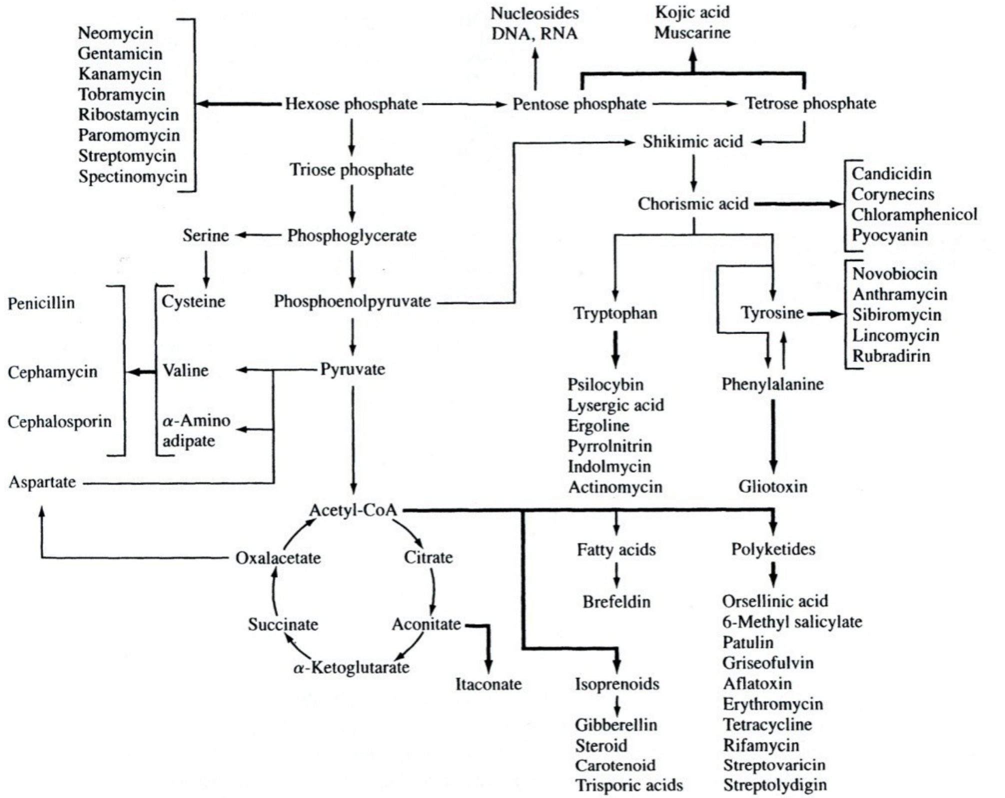
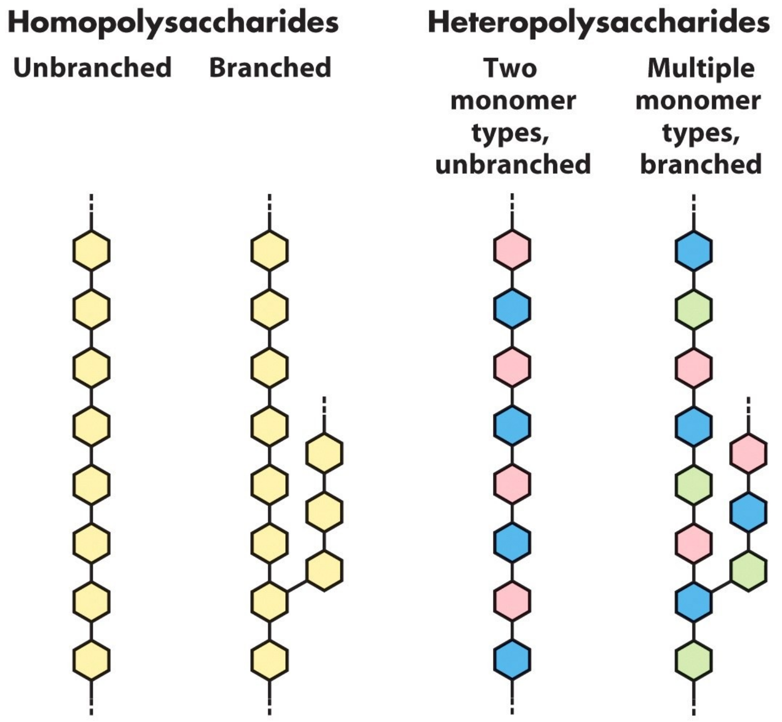


Figure 1.10 General routes of biosynthesis of secondary metabolites. The heavy arrows signify enzyme-catalyzed reaction sequences, and the products at the end of these arrows are secondary metabolites.

1.2 Macromolecules

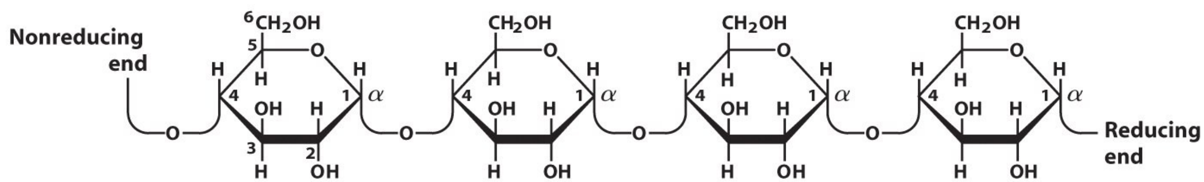
Polysaccharides (Glycan)

- **Types of polysaccharide**
  - Homopolysaccharides
    - Contain single type of monomer
    - Storage of monomer : glycogen, starch
    - Structural elements : cellulose, chitin
  - Heteropolysaccharides
    - Contain two or more types of monomer
    - Extracellular support
      - Bacterial cell wall
      - Extracellular matrix of animal
- **Synthesis of polysaccharides**
  - Enzymatic polymerization without template
  - No specific stopping point

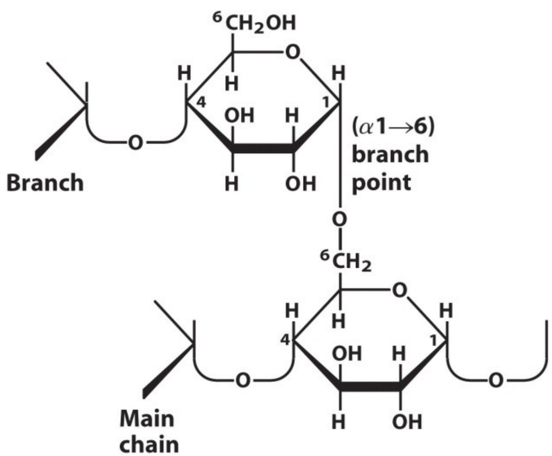


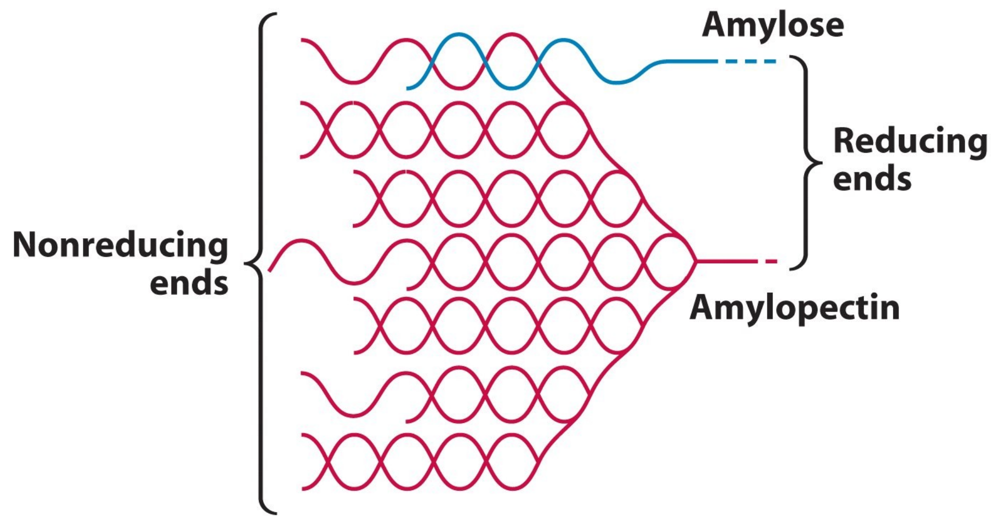
Homopolysaccharides as Stored Forms of Fuel

- **Starch**
  - Amylose
    - D-glucose connected by α1→4 linkages
  - Amylopectin
    - α1→4 chains with α1→6 branches



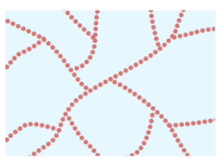
amylose

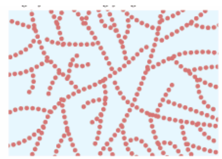


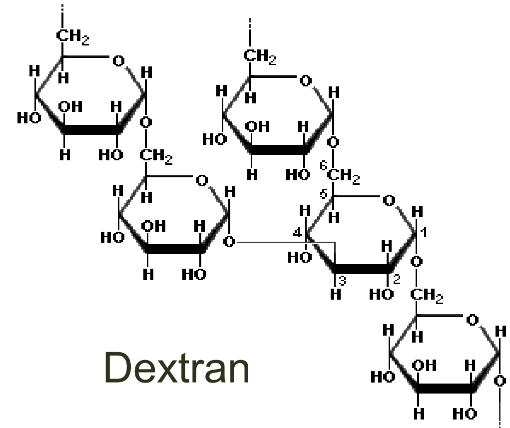


Homopolysaccharides as Stored Forms of Fuel

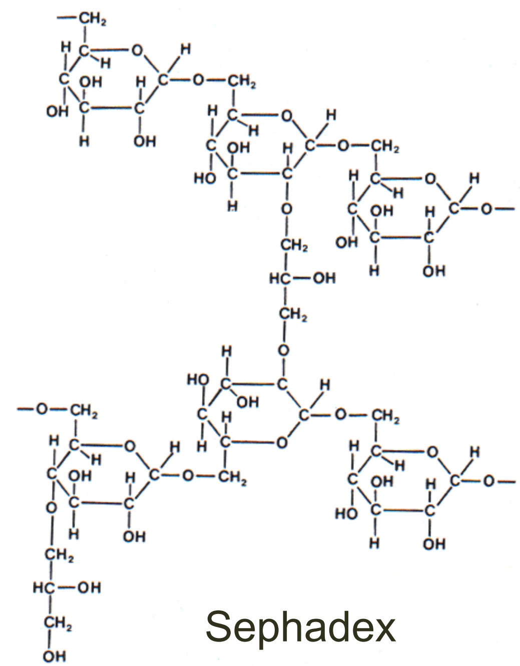
- **Glycogen**
  - $\alpha 1 \rightarrow 4$ chains with extensive $\alpha 1 \rightarrow 6$ branches
    - Branches every 8 to 12 residues
    - More compact than starch
  - Stored in liver (7% of wet weight) and skeletal muscle


Starch


Glycogen

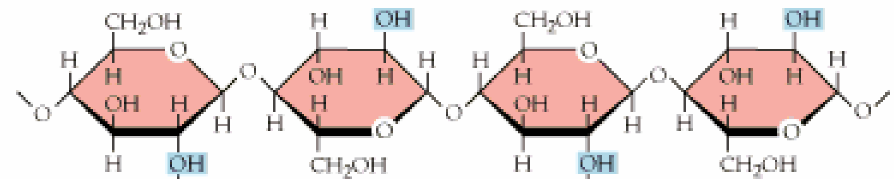

Dextran

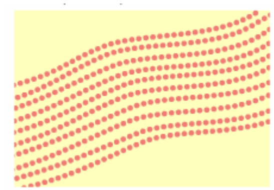
- **Dextrans**
  - Bacterial or yeast polysaccharides
    - e.g. dental plaque
  - $\alpha 1 \rightarrow 6$ chains and $\alpha 1 \rightarrow 3$ branches, some $\alpha 1 \rightarrow 2$ or $\alpha 1 \rightarrow 4$ branches
  - Synthetic dextran
    - Used for fractionation of protein by size exclusion chromatography
    - e.g. Sephadex


Sephadex

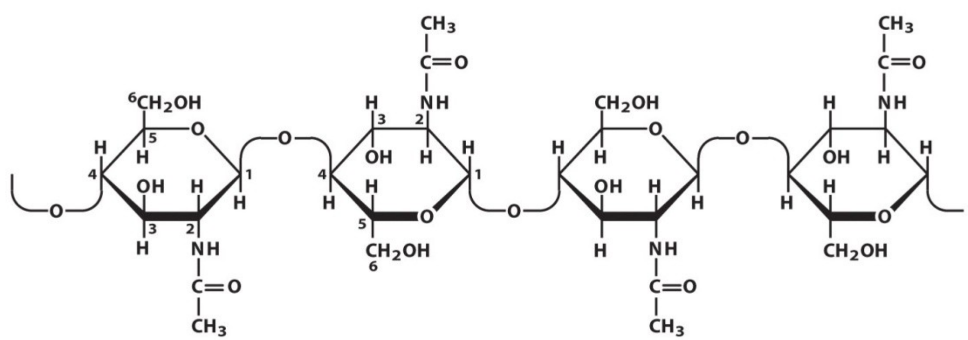
Homopolysaccharides Playing Structural Roles

- **Cellulose**
  - Component of plant cell wall
  - Linear D-glucose chains connected by β1→4 linkages
    - 10,000 to 15,000 glc units





- **Chitin**
  - Exoskeleton of arthropods
  - Linear N-acetylglucosamine chains connected by β1→4 linkages

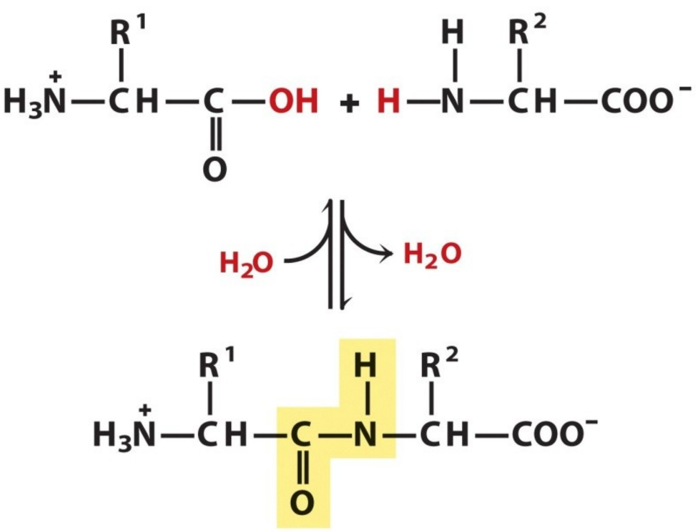


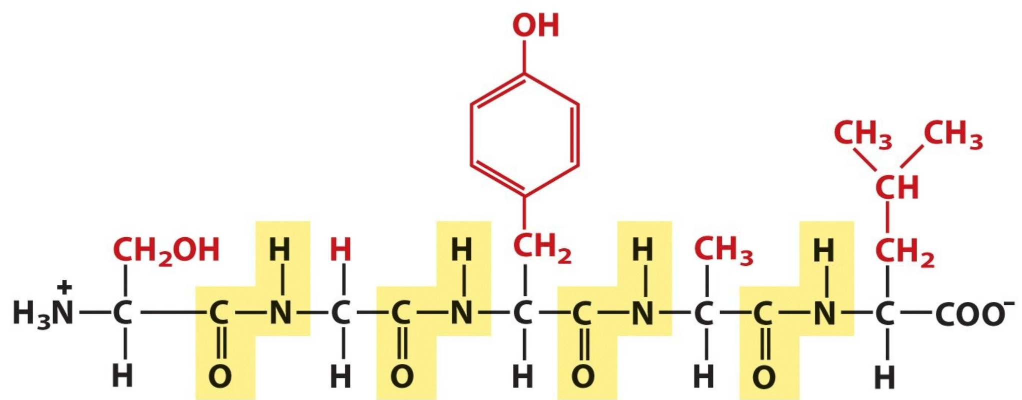
Uses of Polysaccharides

Name	Sources	Uses
Starch	Potatoes, corn	Food, clothing
Cellulose	Wood, cotton	Paper, clothing
Carrageenan	Mycophyta (algae)	Food
Agar	Mycophyta	Microbiology, food
Dextran	Corn	Food, medicine
Agarose	Mycophyta	Biochemistry
Xanthan gum	X.campestris	Food, Industrial chemicals, oil field drilling

Peptides and Proteins

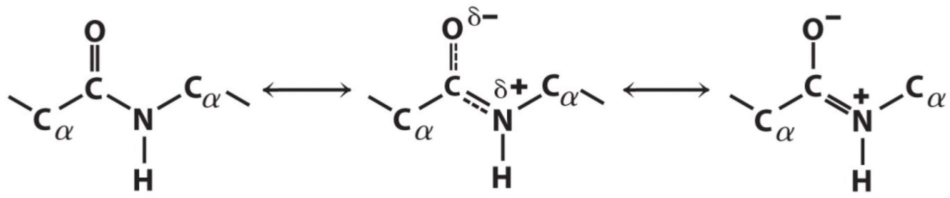
- **Peptide bond**
  - Dehydration reaction
- **Polypeptide vs. protein**
  - Polypeptide: M_r<10,000
- **Amino-terminal (N-terminal)**
- **Carboxyl-terminal (C-terminal)**

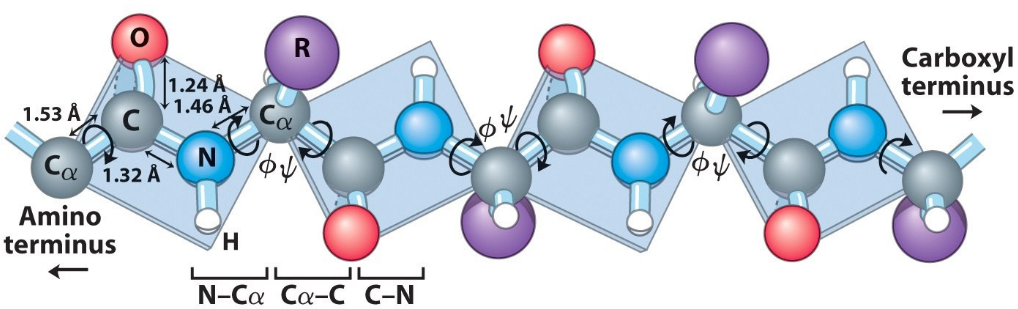




The Peptide Bond is Rigid and Planar

- **Double bond character of peptide bond**
  - Resonance between the carbonyl oxygen and the amide nitrogen
  - 6 atoms of the peptide group lie in a single plane
  - No free rotation of peptide C-N bond (trans)
- **Rotation of peptide chain**
  - ϕ : rotation angle of N-Cα
  - ψ : rotation angle of Cα-C

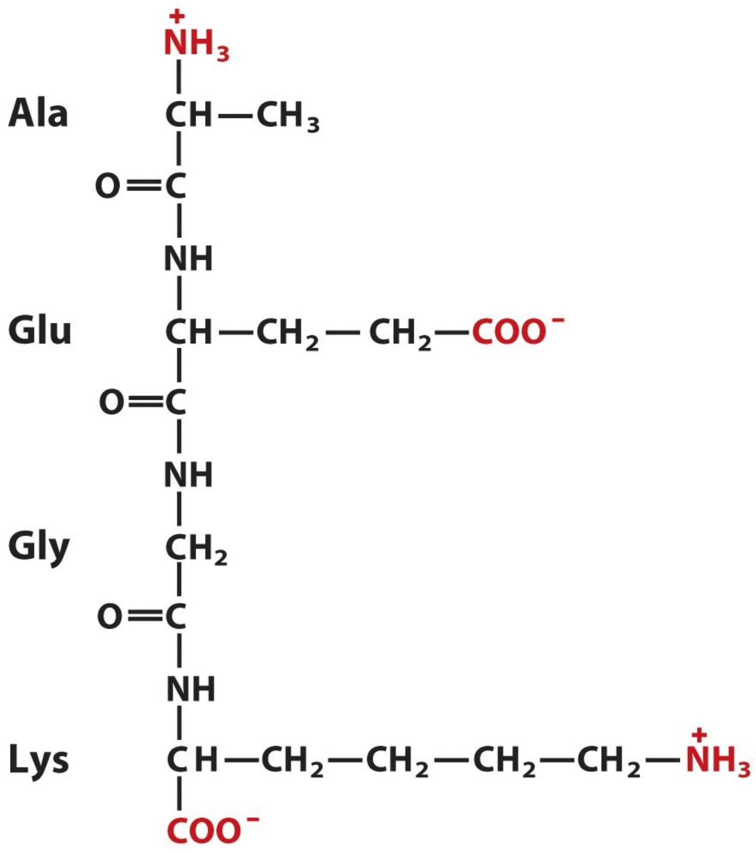




ϕ, ψ = 180 (or -180)

Ionization of Peptide

- **Ionization of peptide**
  - One free α-amino group
  - One free α-carboxyl group
  - Inonizable R groups
- **pK_a of R groups in peptide**
  - Different from pK_a of free amino acid



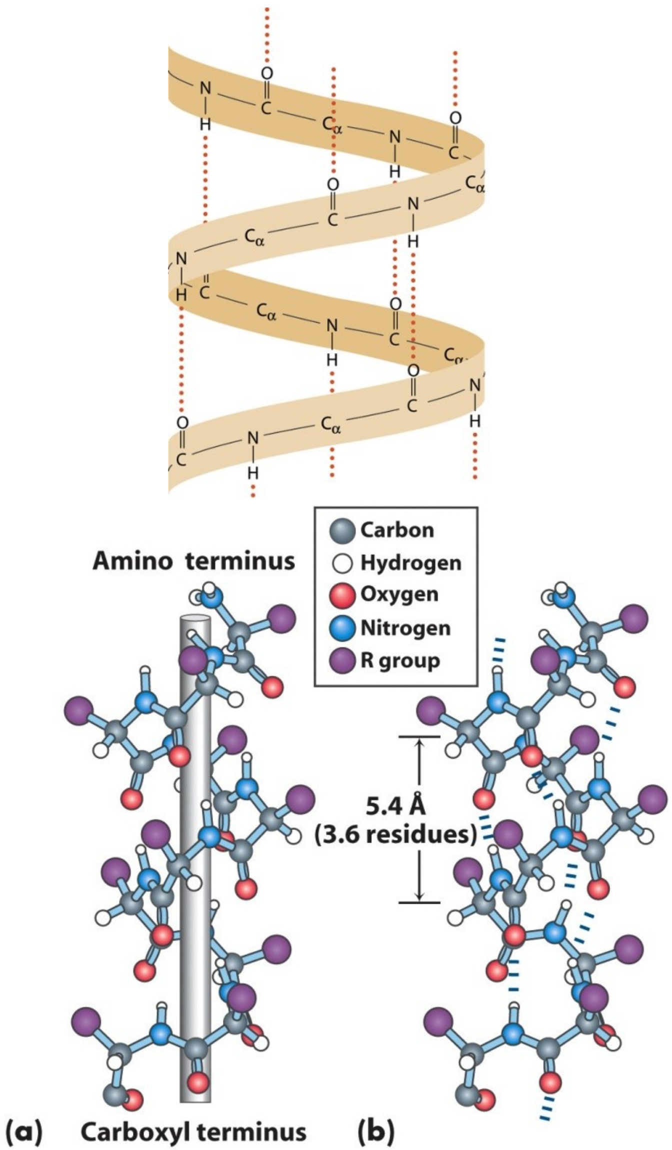
Biologically Active Peptides and Polypeptides

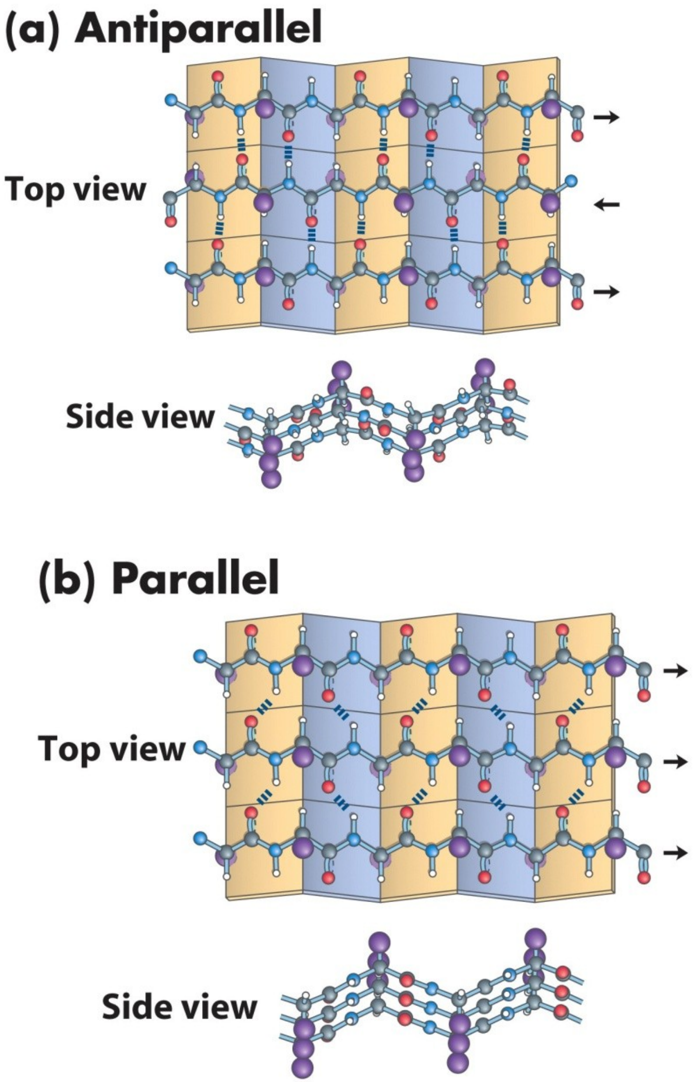
- **Size**
  - Small peptide
    - Vertebrate hormones
      - Oxytocin (9), thyrotropin-releasing factor (3), insulin (30 + 21)
    - Antibiotics
  - Large proteins
    - Titin: vertebrate muscle protein (27,000 a.a.)
  - Most of the proteins
    - < 2,000 a.a.
- **Oligomeric status**
  - Single polypeptide chain
  - Multisubunit proteins
    - Oligomeric : at least two subunits are identical
- **Calculation of the number of amino acid residues**
  - M_r / 110
    - Average M_r of 20 a.a. : 138
    - Average M_r of protein a.a : 128
    - Removal of water during peptide bond formation : 128 -18 =110

Protein Structure

- **Primary Structure**
  - The sequence of amino acid
- **Secondary structure**
  - Local conformation of polypeptide
    - α helix, β sheet : 60% of the polypeptide chain
    - Random coils and β- turn
  - α helix
    - Hydrogen bond between carbonyl O (n) and amid H (n+4)
    - Right-handed helix
    - One turn: 5.4 Å along the axis, 3.6 amino acids
    - Side chains point outward
  - β sheet, β-pleated sheet
    - Hydrogen bonding between adjacent β strands
    - Parallel
    - Antiparallel

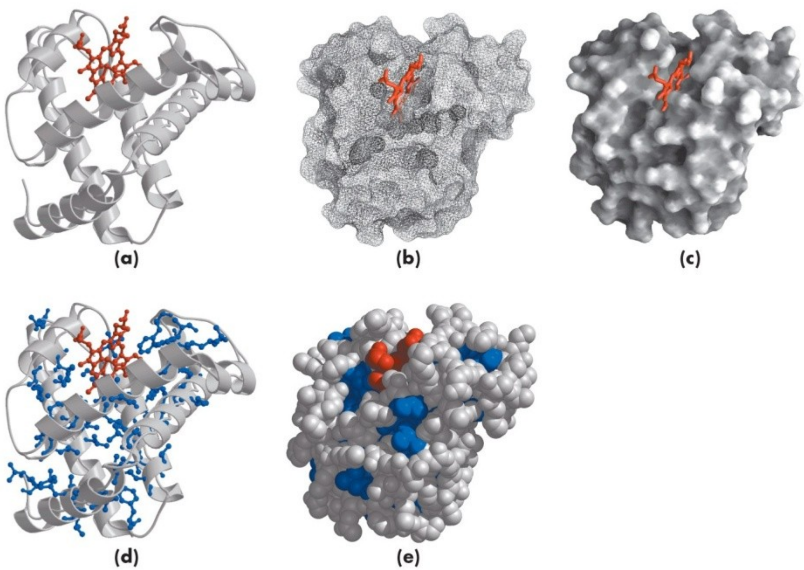
Protein Secondary Structure

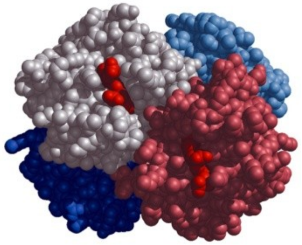




Higher Protein Structure

- **Tertiary structure**
  - Overall 3D arrangement of all atoms in a protein
  - Tendency to have the lowest Gibbs free energy (highest stability)
    - Noncovalent interactions
      - Maximum hydrogen bonding within the protein
      - Hydrophobic interaction
        - » Hydrophobic residues are buried in the protein interior
      - Ionic interactions (salt bridge)
    - Disulfide bonds
- **Quaternary structure**
  - Arrangement of protein subunits
    - Hemoglobin
      - two α chains and two β chains





Conjugated Proteins

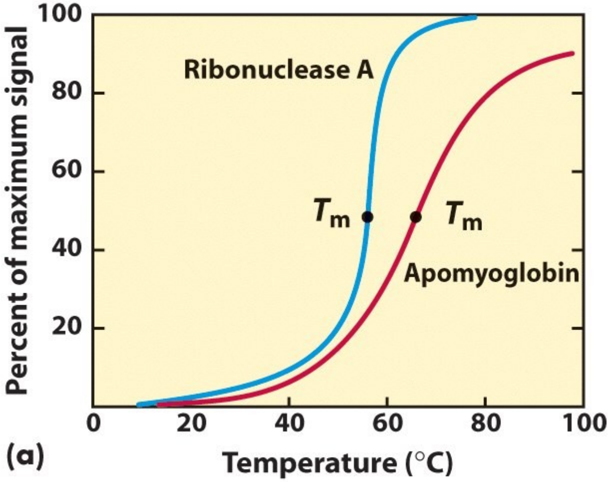
- **Conjugated proteins**
  - Proteins with permanently associated chemical components
  - Prosthetic group
    - Non-amino acid part of a conjugated protein

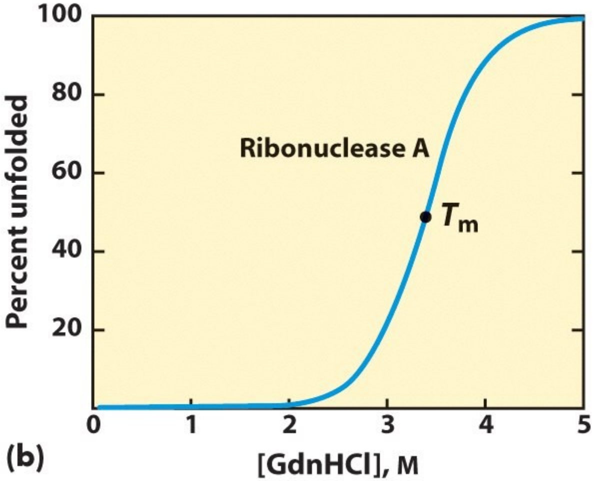
TABLE 3–4 Conjugated Proteins

Class	*Prosthetic group*	*Example*
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

Protein Denaturation

- **Denaturation**
  - A loss of three-dimensional structure sufficient to cause loss of function
    - Not necessarily means complete unfolding or random conformations
  - Abrupt unfolding over a narrow temperature range
    - Cooperative unfolding process
- **Denaturing agents**
  - Heat
    - Affect weak interactions (H bonds)
  - pH
    - Alternation of the protein net charge
    - Electrostatic repulsion, disruption of H bonds
  - Organic solvents (alcohol, acetone), urea, guanidine HCl, detergents
    - Disruption of hydrophobic interactions
  - Sear





The pH effect on Enzyme Activity

- **Mild pH change**
  - Mild inactivation or denaturation

- **At extremes pH**
  - Hydrolysis
    - 6 M HCl (pH 0.8)
    - 4 M NaOH (pH 14.6)

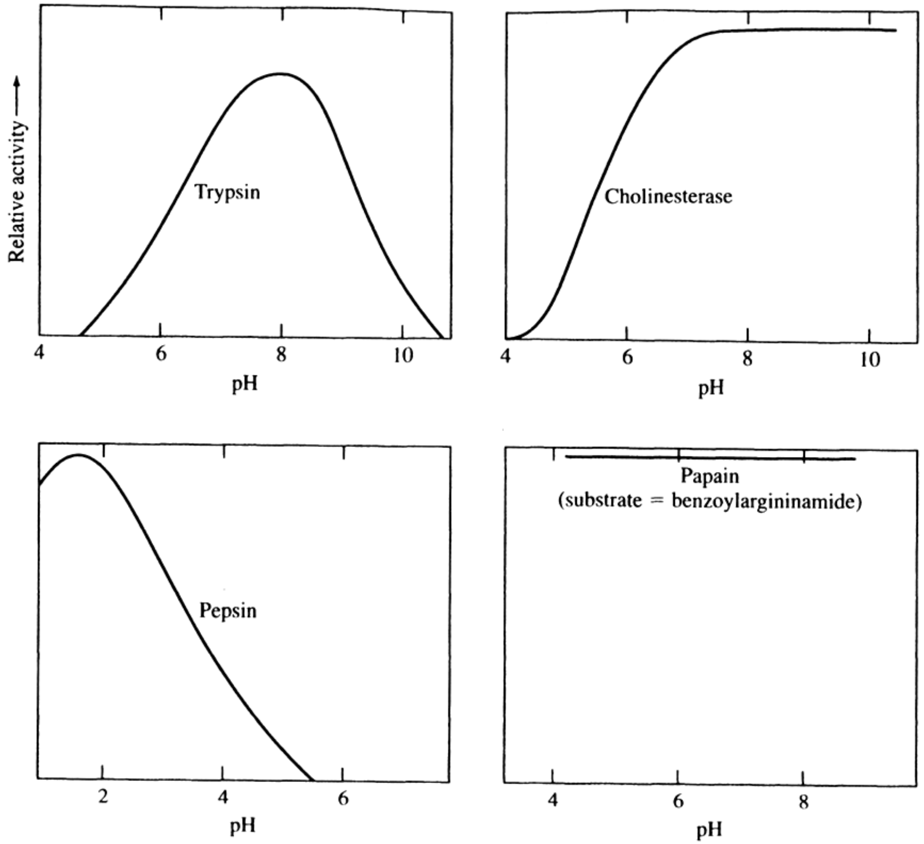
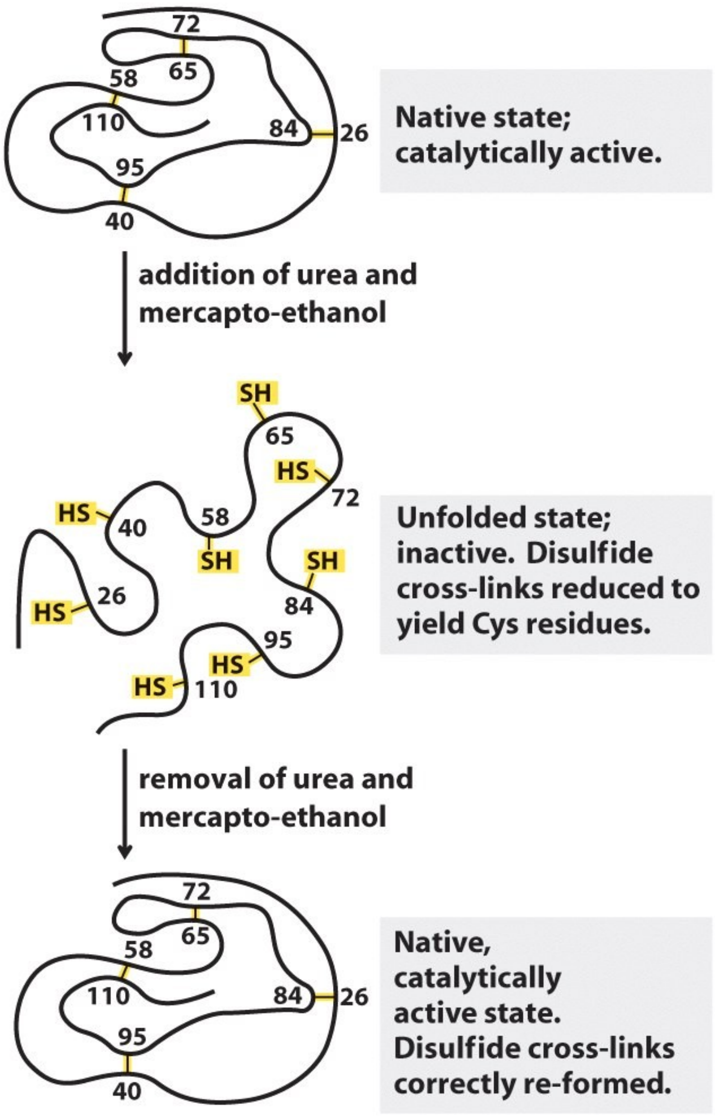


Figure 1.19 The effect of pH on the relative activity of the enzymes trypsin, cholinestrase, pepsin, and papain. (Data from A. L. Lehninger, *Biochemistry*, 2nd ed., p. 196, Worth, New York, 1975.)

Amino Acid Sequenc Determines Tertiary Structure

- **Renaturation**
  - Reversal of denaturation
- **Amino acid sequence contains all the information required to protein folding**
  - First experimental evidence by Christian Anfinsen (1950s)
    - Denaturation of ribonuclease with urea and reducing agent
    - Spontaneous refolding to an active form upon removal of the denaturing reagents



Classification of Proteins According to Function

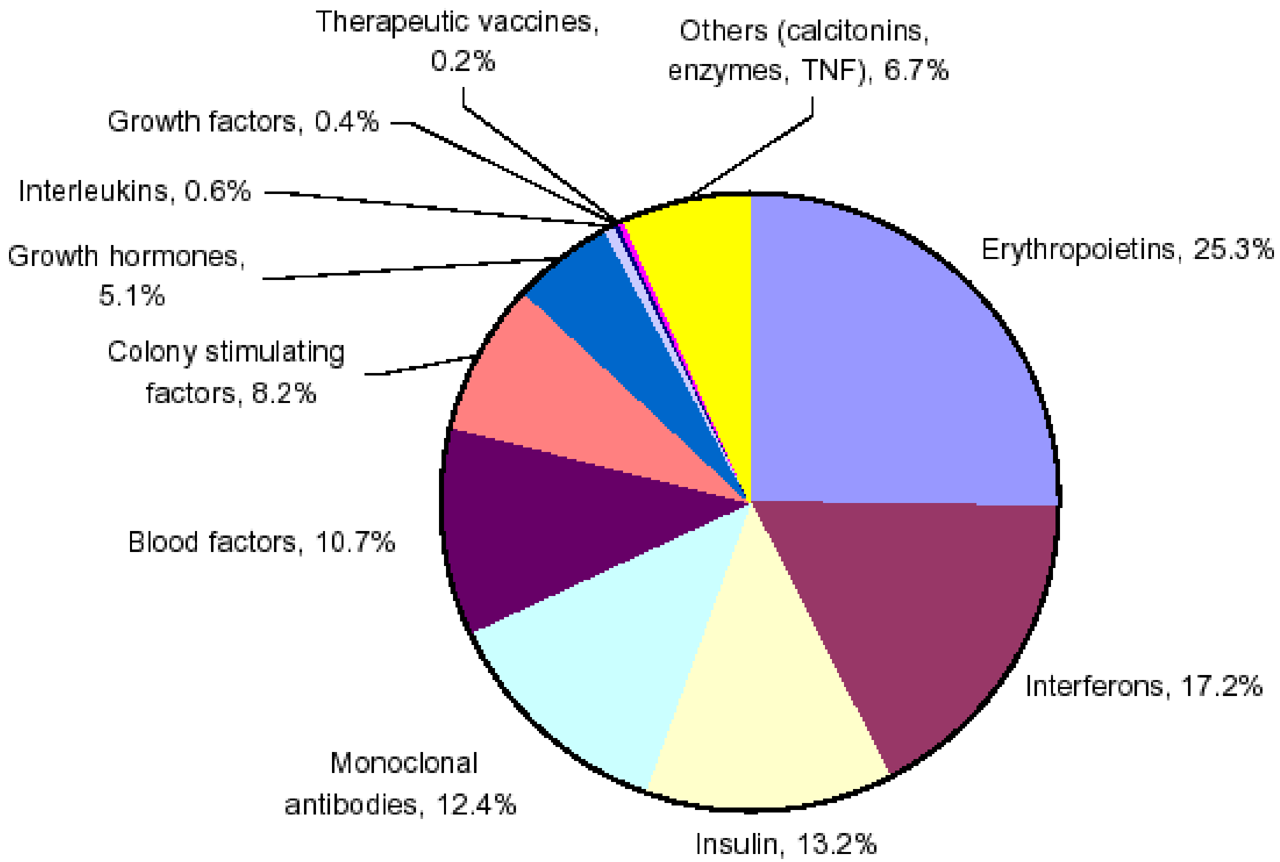
Class	Examples	Class	Examples
Enzymes	Ribonuclease	Structural	Keratin(skin)
	Trypsin		Fibroin(silk)
	Urokinase		Collagen(tendons, ligaments)
	DNApolymerase		Elastin(joints)
	Cellulase		Proteoglycans(cell walks)
Transport proteins	Hemoglobin		Immunoglobulin(antibodies)
	Serum albumin	Defense	Venom proteins
	Myoglobin		Ricin
	B_1-Lipoprotein		Interferon
Nutrient, storage, protein	Ovalbumin		Insulin
	Casein	Regulatory	Growth hormone
Contractile or motile	Actin		Lymphokines/ cytokines
	Myosin		Protein kinases(signal transduction)
	Tubulin		DNA binding repressors and activators
			Soybean trypsin inhibitor
		Inhibitors	Plasminogen activator inhibitor
			HIV protease inhibitor

Production of recombinant proteins

- **Protein drugs**
  - Anticoagulant : Factor VIII, Factor IX
  - Hormones: Insulin, Human growth factors
  - Hematopoietic growth factors: EPO,GM-CSF
  - Interferon and interleukins: IFN-α, β, γ, IL-2
- **Enzymes**
  - DNase I, Restriction enzymes, Lipase, Protease
- **Antibodies**
- **Vaccines**

Commercial Uses of Proteins

- Generated by recombinant DNA technology
- Global therapeutic protein market
  - 2002 sales: $33,340m
  - 24% increase on 2001



The 10 Leading Biotechnology Proteins

Protein	Activity/Use
Erythropoietin	Red blood cell growth
Colony- stimulating fator	White blood cell growth
Insulin	Diabetes
α-Interferon	Anticancer, infections
Vaccine against Hepatitis B	Hepatitis B
Glucocerebrosidase	Genetic deficiency
Tissue plasminogen activitor	Heart attack/stroke
Growth hormone (somatotropin)	Growth deficiencies
GPIIb/IIa antibody	Prevents blood clots
Interferon β-1a	Multiple sclerosis

In order of worldwide sales in 1997[a]

1.3 Introduction to Bioseparations

Biochemical Engineering

- **Upstream engineering**
  - Fermentation
- **Downstream engineering**
  - Purification or bioseparation
    - Series of steps
      - – Unit operations
        - » Sedimentation, adsorption, drying etc.
    - Important to maintain product stability
      - – Temperature, pH, concentration

Stages of Downstream Processing

Stage	Objective(s)	Typical Unit Operations
Separation of insolubles	Remove or collect cells, cell debris, or other particulates Reduce volume (depends on unit operation)	Filtration, sedimentation, extraction, adsoption
Isolation of product	Remove materials having properties widely different from those desired in product Reduce volume (depends on unit operation)	Extraction, adsorption, ultrafiltration, precipitation
Purification	Remove remaining impurities which are similar to the desired product in chemical and physical properties	Chromatography, affinity methods, crystallization, fractional precipitation
Polishing	Remove liquids Convert the product to crystalline form	Drying, crystallization

Basic Principles of Engineering Analysis

- **The purpose of engineering analysis**
  - To determine how much
  - To determine how fast
- **Components of engineering analysis**
  - Material balance
    - Accumulation = inflow – outflow + amount produced – amount consumed
  - Equilibrium
    - Equilibrium constant (K_{eq}) of chemical reaction

      $A + B \leftrightarrow C$, $K_{eq} = [C]/[A][B]$
    - Partition coefficient of extraction

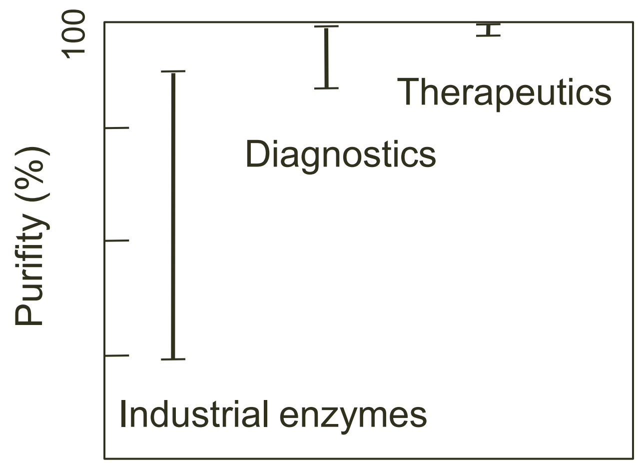
      K = y/x

      y: concentration of a separand in the extract phase

      x: concentration of a separand in the raffinate phase
  - Flux relationships
    - Flux = coefficient x driving force
      - Flux : units flowing/unit area, unit time
      - Driving force : a gradient down which units flow
      - Coefficient : Permeability or the inverse of a resistance

Determination of Process and Product Quality

- **Purify**
  - Amount of product / (amount of product + amount of total impurities)
  - Different requirement of purities depending on the products



- **Fold purification**
  - Purity after a process / purity before the process
- **Specific activity**
  - Units of biological activity / mass
- **Yield**
  - Amount of product produced / amount of product in feed