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Fundamentals of Biochemistry

Second Edition

Chapter 5:

Proteins: Primary Structure

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Unlimited variation in protein structure and function

$20x20x20\ldots\ldots = 20^{100} = 1.27x10^{130}$

Table 5-1 Compositions of Some Proteins

Protein	Amino Acid Residues	Subunits	Polypeptide Molecular Mass (*D*)
Proteinase inhibitor III (bitter gourd)	30	1	3,427
Cytochrome *c* (human)	104	1	11,617
Myoglobin (horse)	153	1	16,951
Interferon-γ (rabbit)	288	2	33,842
Chorismate mutase (*Bacillus subtilis*)	381	3	43,551
Triose phosphate isomerase (*E. coli*)	510	2	53,944
Hemoglobin (human)	574	4	61,986
RNA polymerase (bacteriophage T7)	883	1	98,885
Nucleoside diphosphate kinase (*Dictyostelium discoideum*)	930	6	100,764
Pyruvate decarboxylase (yeast)	2,252	4	245,456
Glutamine synthetase (*E. coli*)	5,616	12	621,264
Titin (human)	26,926	1	2,993,428

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The primary structure of insulin: synthesized as a polypeptide chain and cleaved

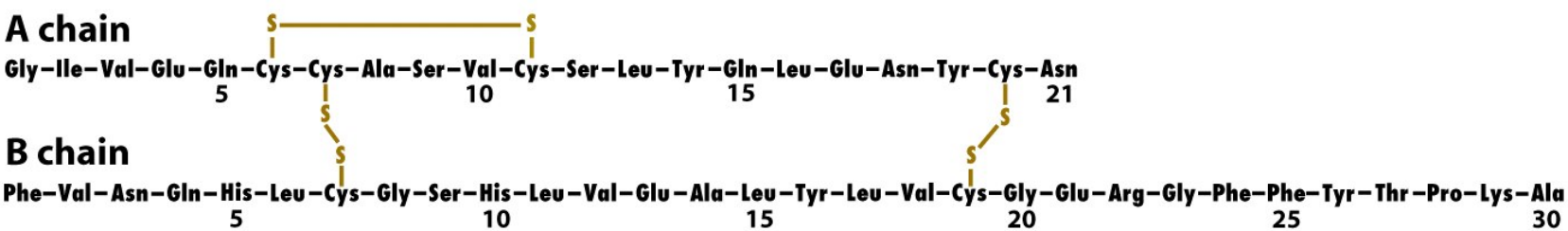

Figure 5-1 Fundamentals of Biochemistry, 2/e
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Some restrictions in protein
Size: lower limit, around 40 residues; upper limit, many hundreds
Amino acid composition: most abundant (L, A, G, S, V, E) & least abundant (W, C, M, H)
Nonpolypeptide components

Protein purification and analysis

Purification is mandatory for studying macromolecules
The purification principle is universal to other molecules

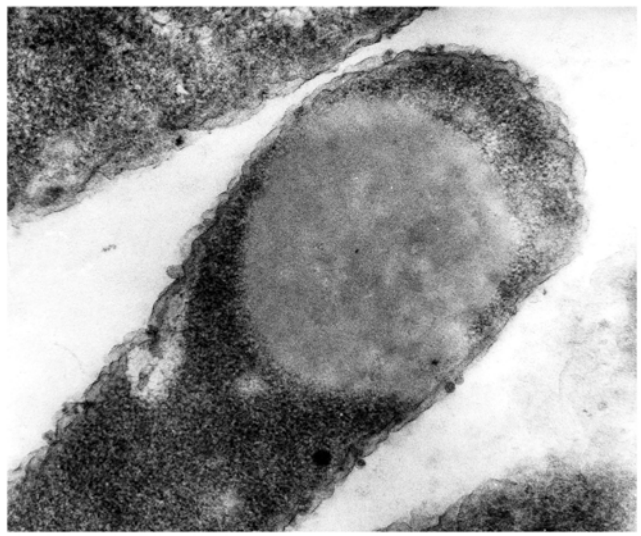
Figure 5-2 Fundamentals of Biochemistry, 2/e

A. General approach

Native proteins & recombinant proteins
Intracellular & extracellular
Soluble protein & membrane protein

Stabilizing protein

pH, temperature, degradative enzymes, adsorption to surface, storage

Assaying proteins

Assay: quantitative detection method
catalysis reactions: measure substrate, product, cofactor
    colorimetric,
    coupled enzymatic reaction
Immunoassays: using antibody
    RIA
    ELISA

Absorption spectroscopy
    Beer-Lambert law: $A = \log (I_o/I) = \varepsilon cl$

ELISA

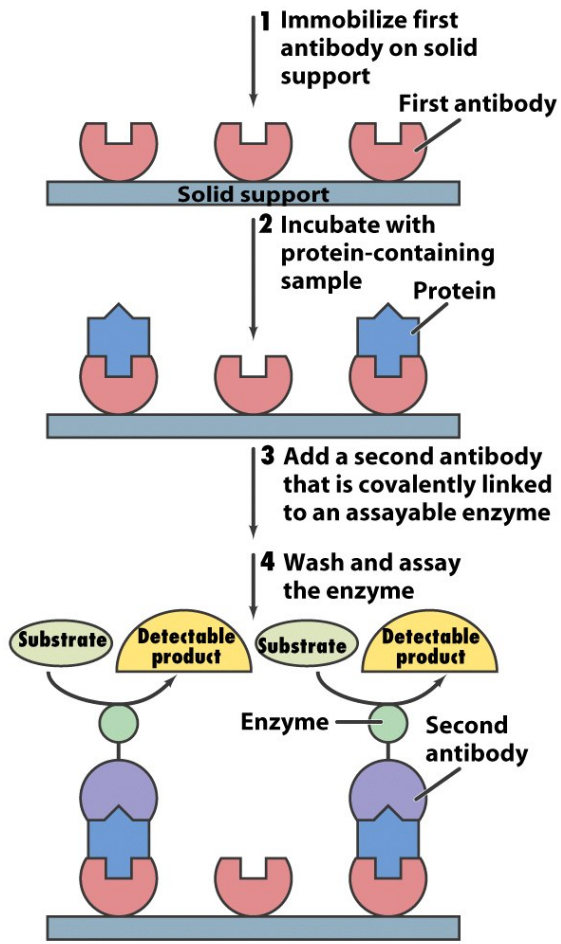

Figure 5-3 Fundamentals of Biochemistry, 2/e
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Absorption spectrum
- Chromophore

Protein quantitation
- UV
- Bradford assay

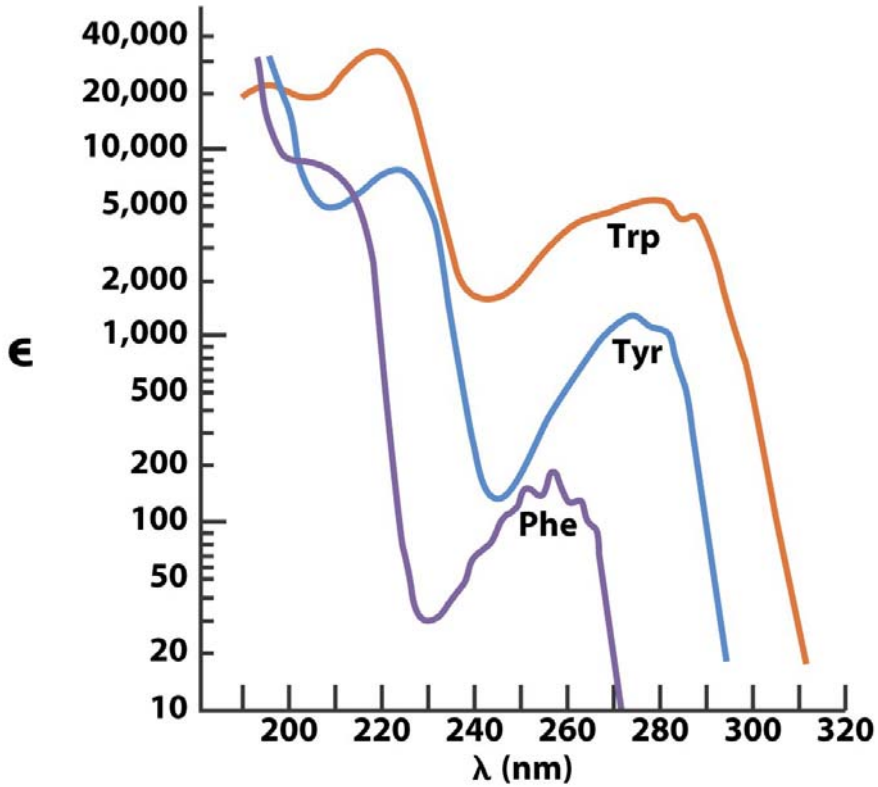

Figure 5-4 Fundamentals of Biochemistry, 2/e
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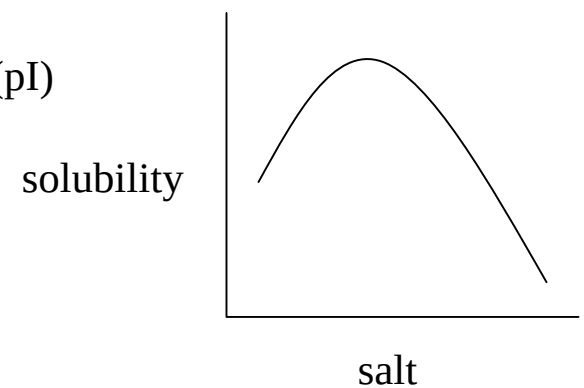
UV absorption spectrum

Separation techniques (fractionation procedures)

Protein characteristic	purification procedure
Solubility	salting out
Ionic charge	ion exchange chromatography
	electrophoresis
	isoelectric focusing
Polarity	hydrophobic interaction chromatography
	gel filtration chromatography
	SDS-PAGE
	ultracentrifugation
	ultrafiltration
Binding specificity	affinity chromatography

Protein solubility

- Salting in
- Salting out
- Isoelectric point (pI)



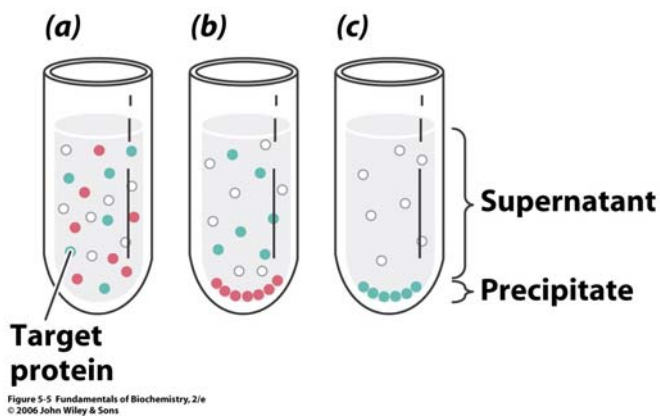


Table 5-2 Isoelectric Points of Several Common Proteins

Protein	p*I*
Pepsin	<1.0
Ovalbumin (hen)	4.6
Serum albumin (human)	4.9
Tropomyosin	5.1
Insulin (bovine)	5.4
Fibrinogen (human)	5.8
γ-Globulin (human)	6.6
Collagen	6.6
Myoglobin (horse)	7.0
Hemoglobin (human)	7.1
Ribonuclease A (bovine)	9.4
Cytochrome *c* (horse)	10.6
Histone (bovine)	10.8
Lysozyme (hen)	11.0
Salmine (salmon)	12.1

Table 5-2 Fundamentals of Biochemistry, 2/e
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Chromatography

Mobile phase & stationary phase (matrix)

Matrix type: paper, gel, HPLC

Ion exchange chromatography: electric charge

Anion exchangers: DEAE

Cation exchangers: CM

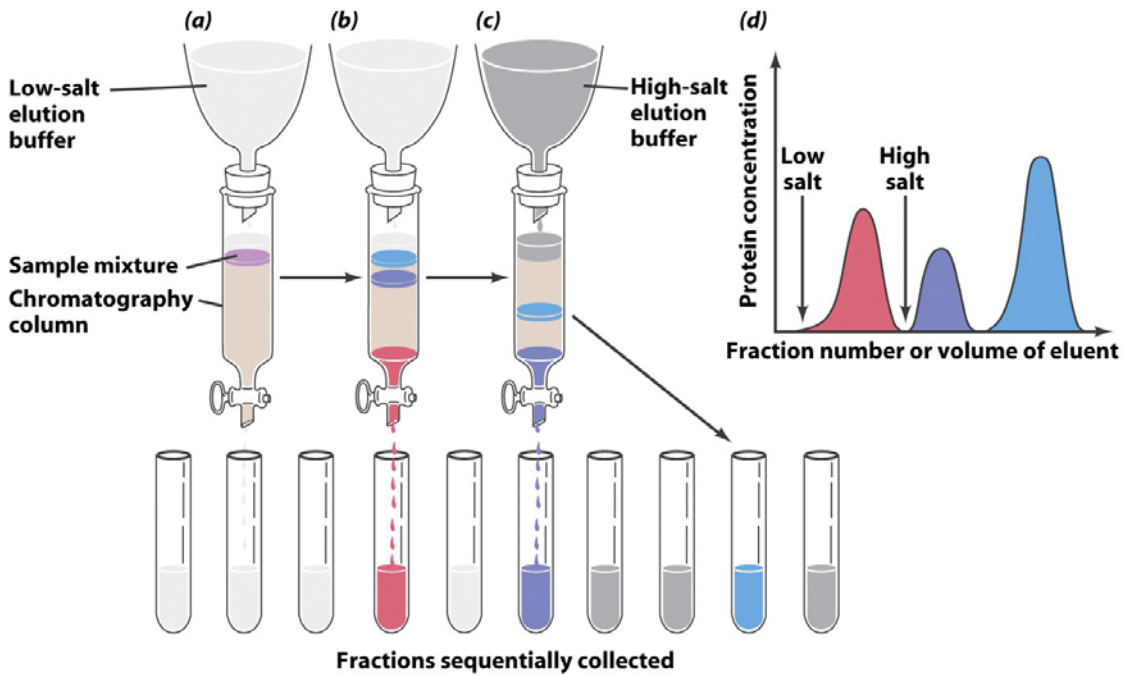

Figure 5-6 Fundamentals of Biochemistry, 2/e

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Hydrophobic interaction chromatography: hydrophobicity
phenyl, octyl (C8), C18, etc

Gel filtration chromatography: molecular sieve: size & shape

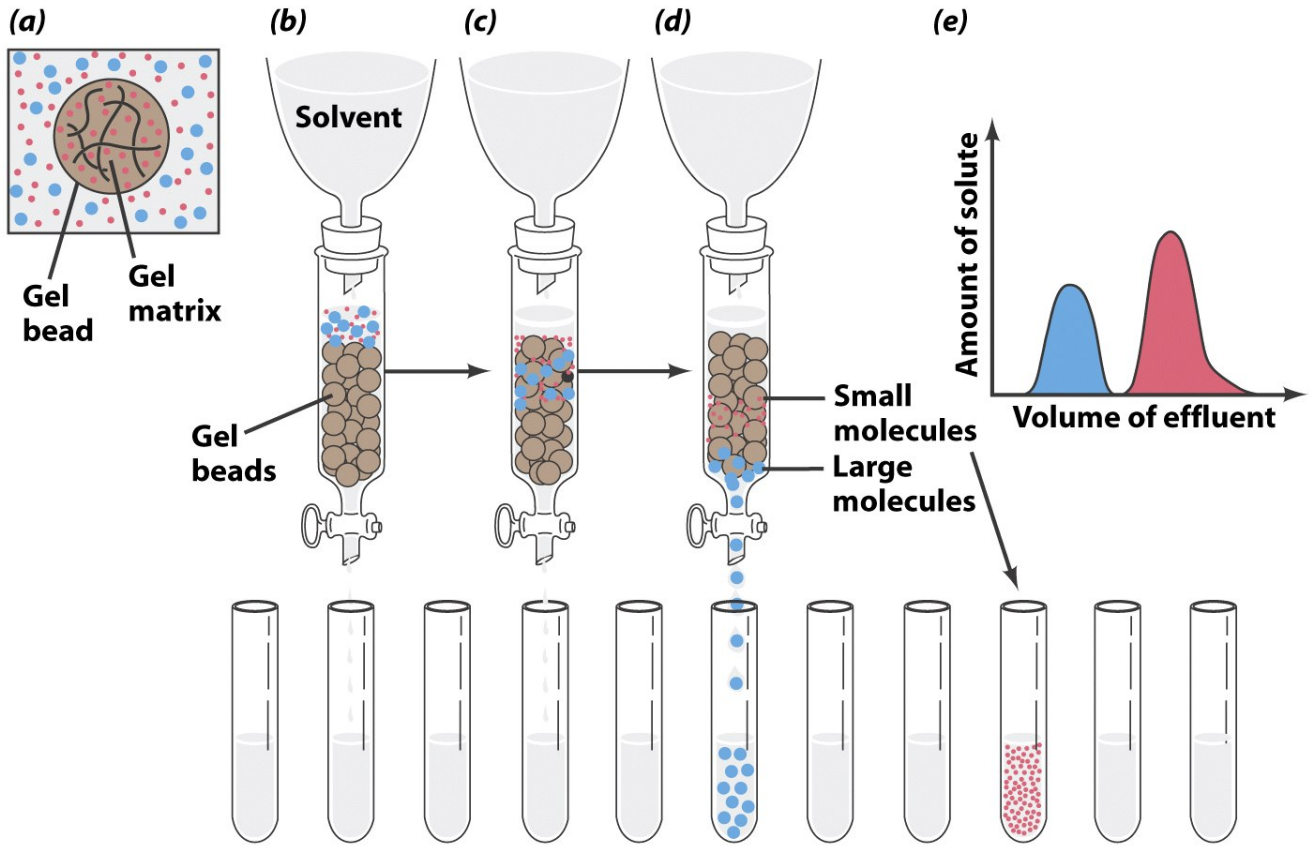

Figure 5-7 Fundamentals of Biochemistry, 2/e
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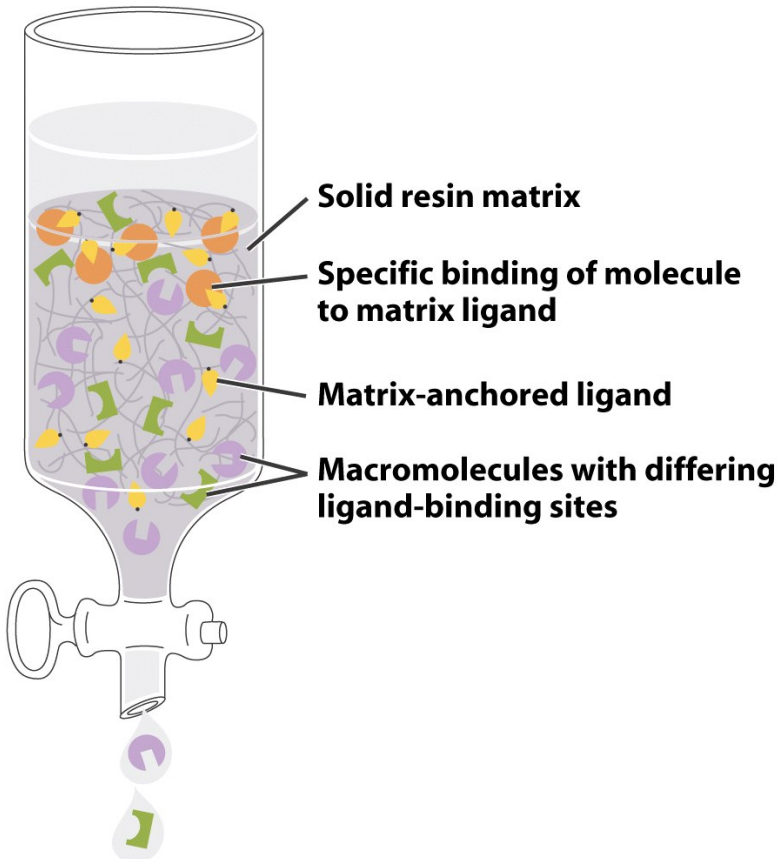
Affinity chromatography

Spacer

Ligand: substrate, inhibitor, etc

Immunoaffinity chromatography

Metal chelate affinity chromatography



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Electrophoresis

Depends on size, shape, electric charge

Polyacrylamide
Agarose
paper

SDS-PAGE

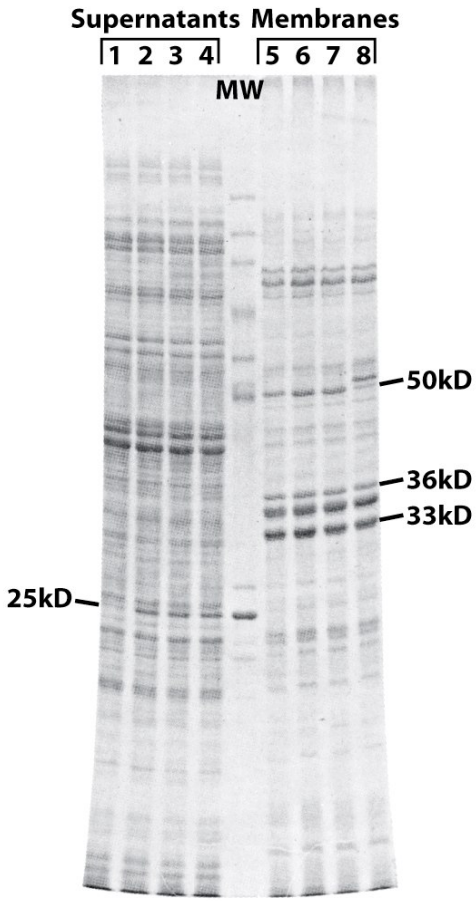
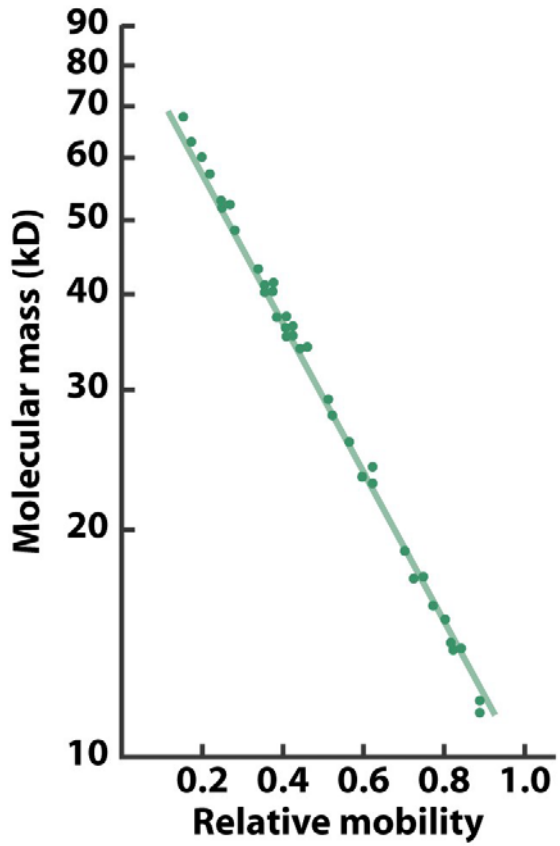


Figure 5-9 Fundamentals of Biochemistry, 2/e



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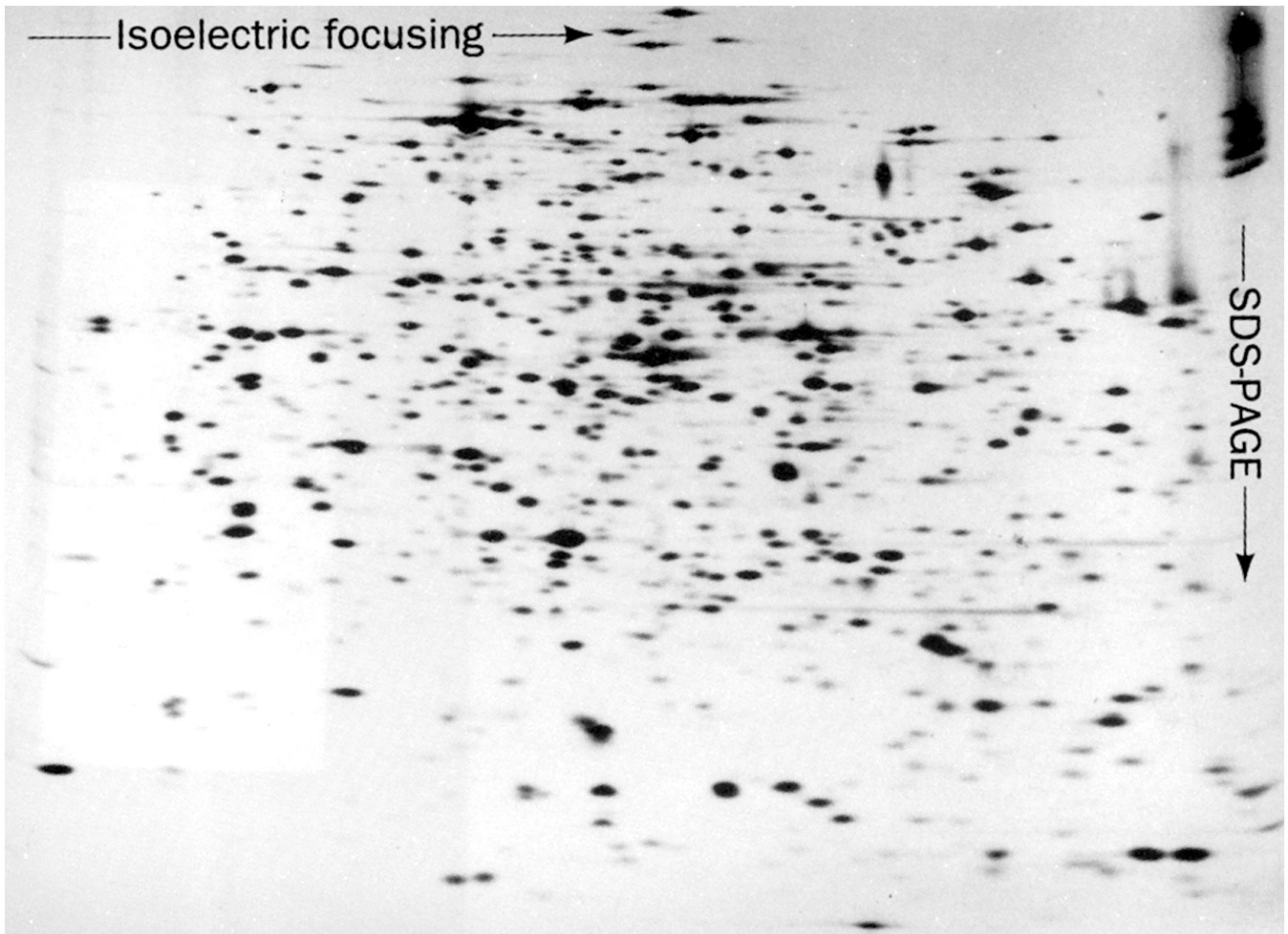
IEF & 2D-PAGE



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Ultracentrifugation

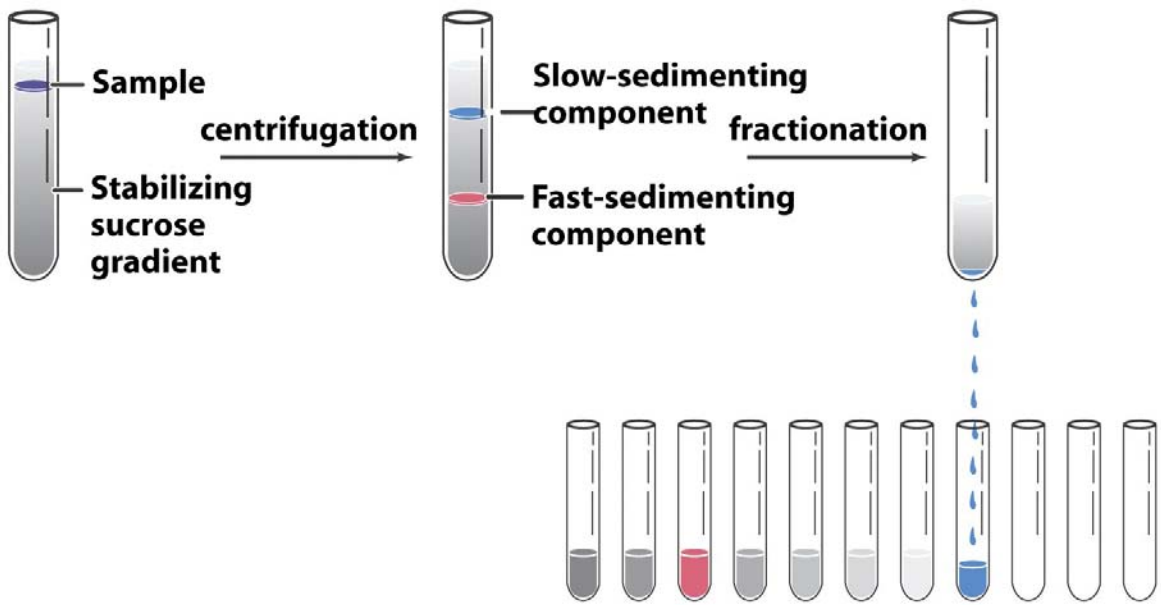
Sedimentation rate: depends on mass, shape, density of the solution

Svedbergs (S) units: (10^{-13} s)

40S + 60S = 80S (not 100S)

Zonal ultracentrifugation: premade density gradient

Equilibrium density gradient centrifugation: CsCl



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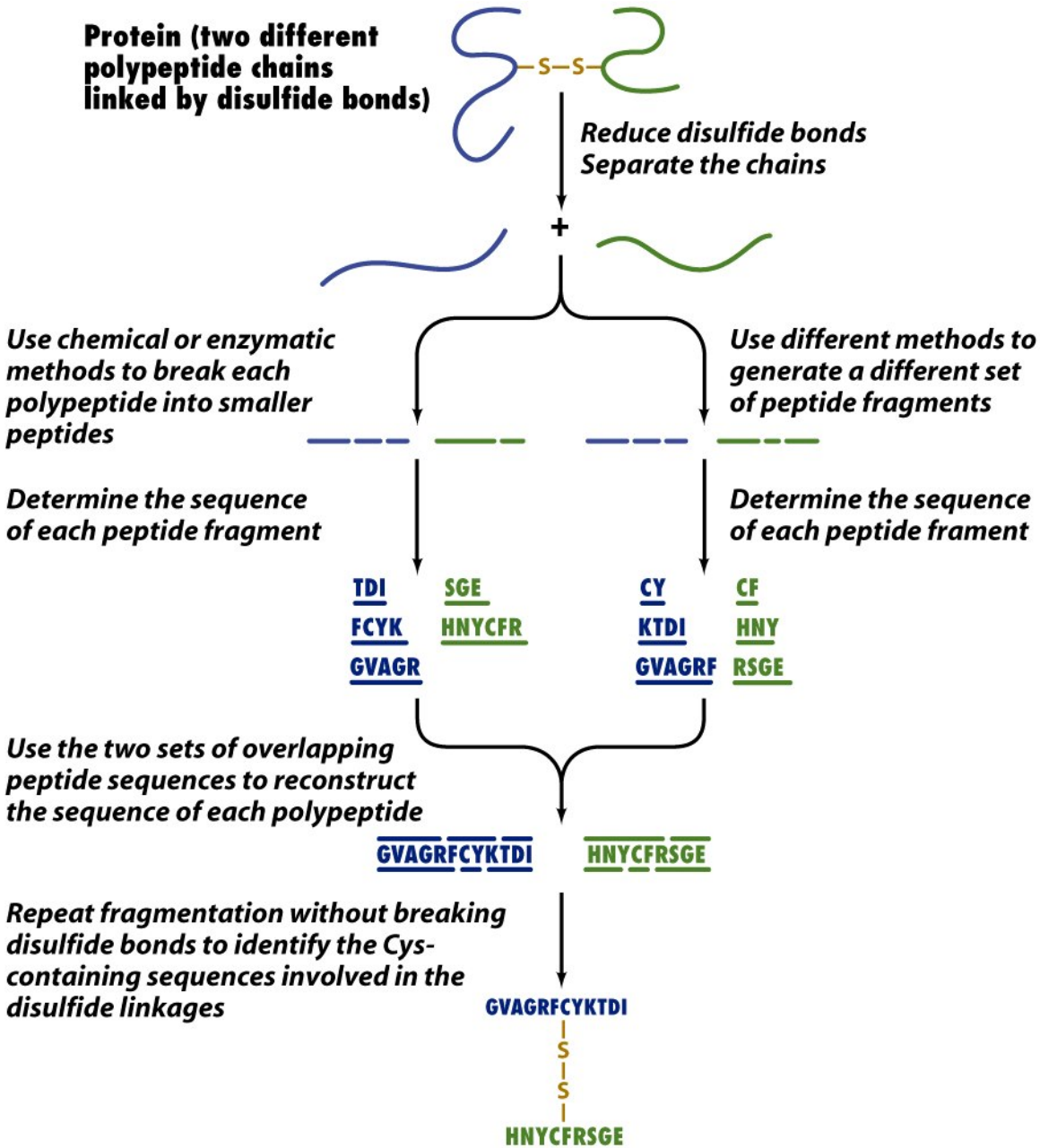
Protein sequencing



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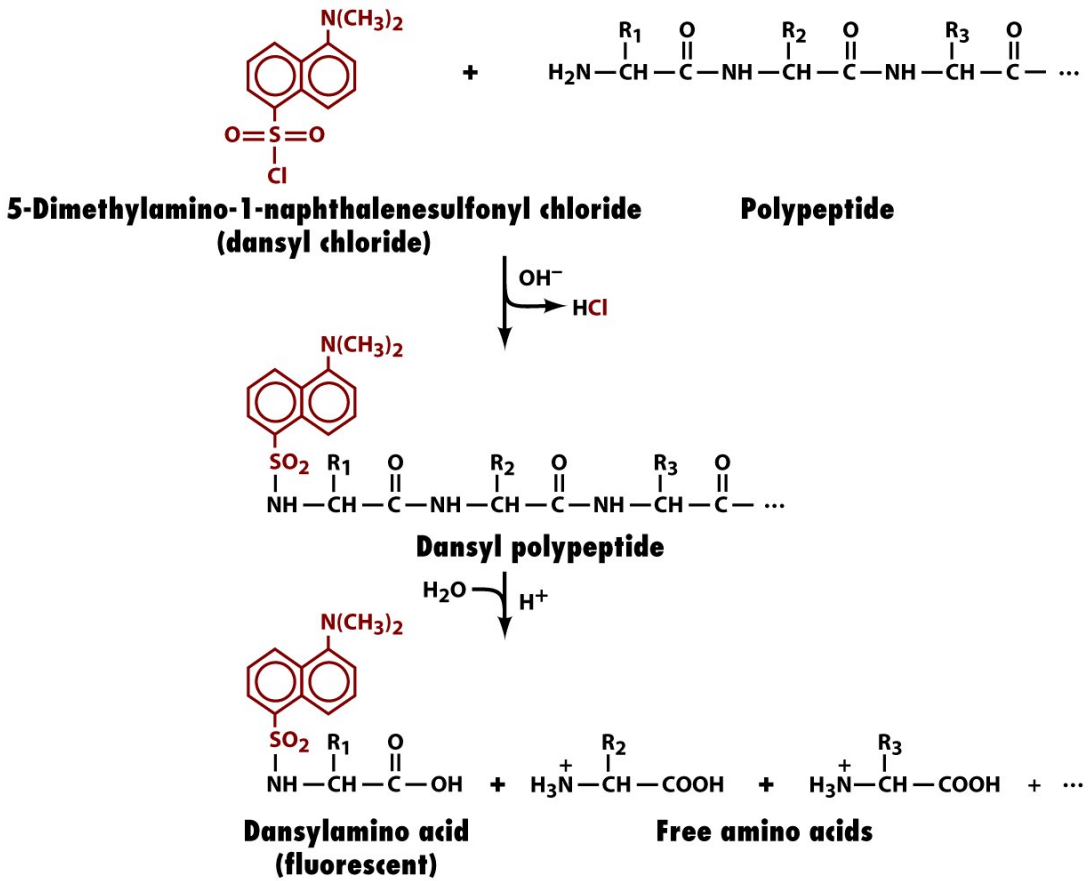
N-terminal sequencing



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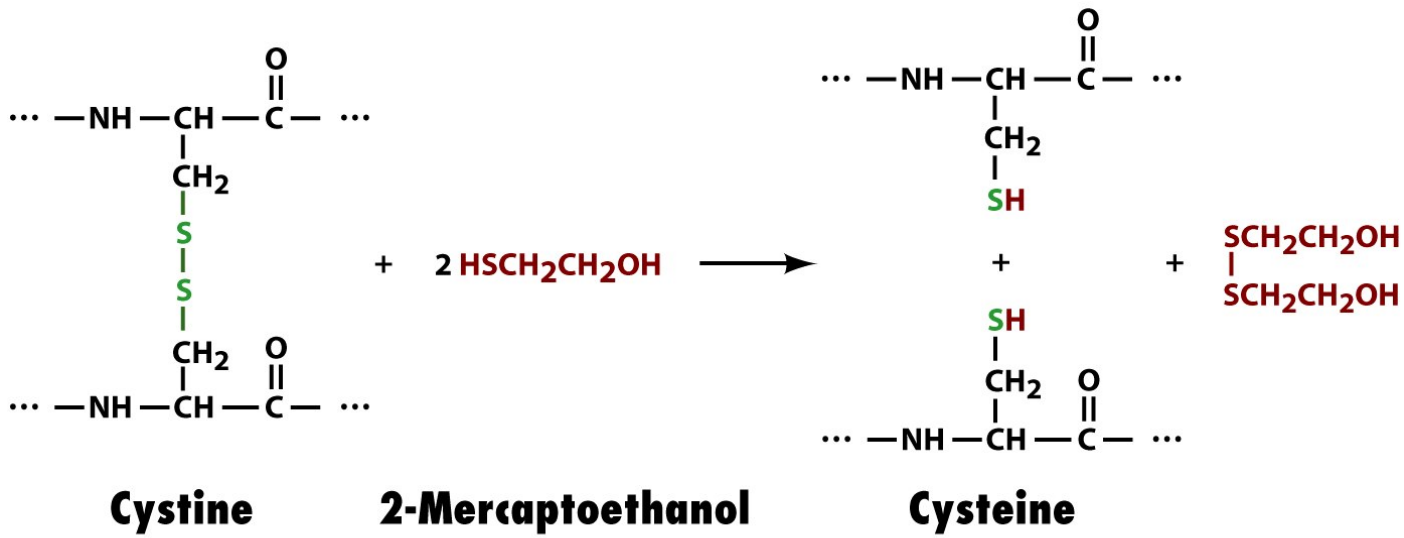
Disulfide bond cleavage



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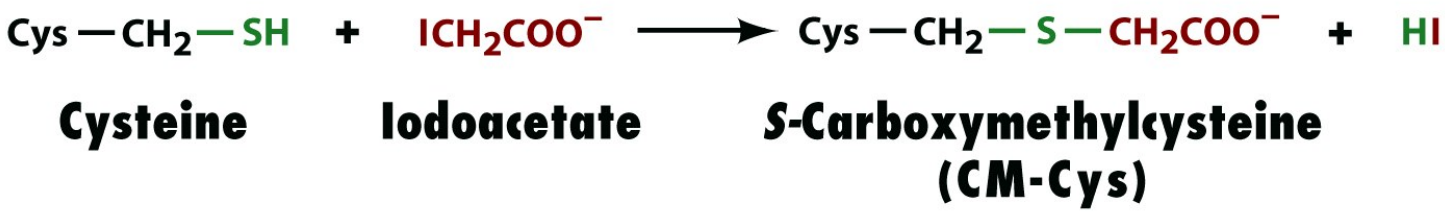

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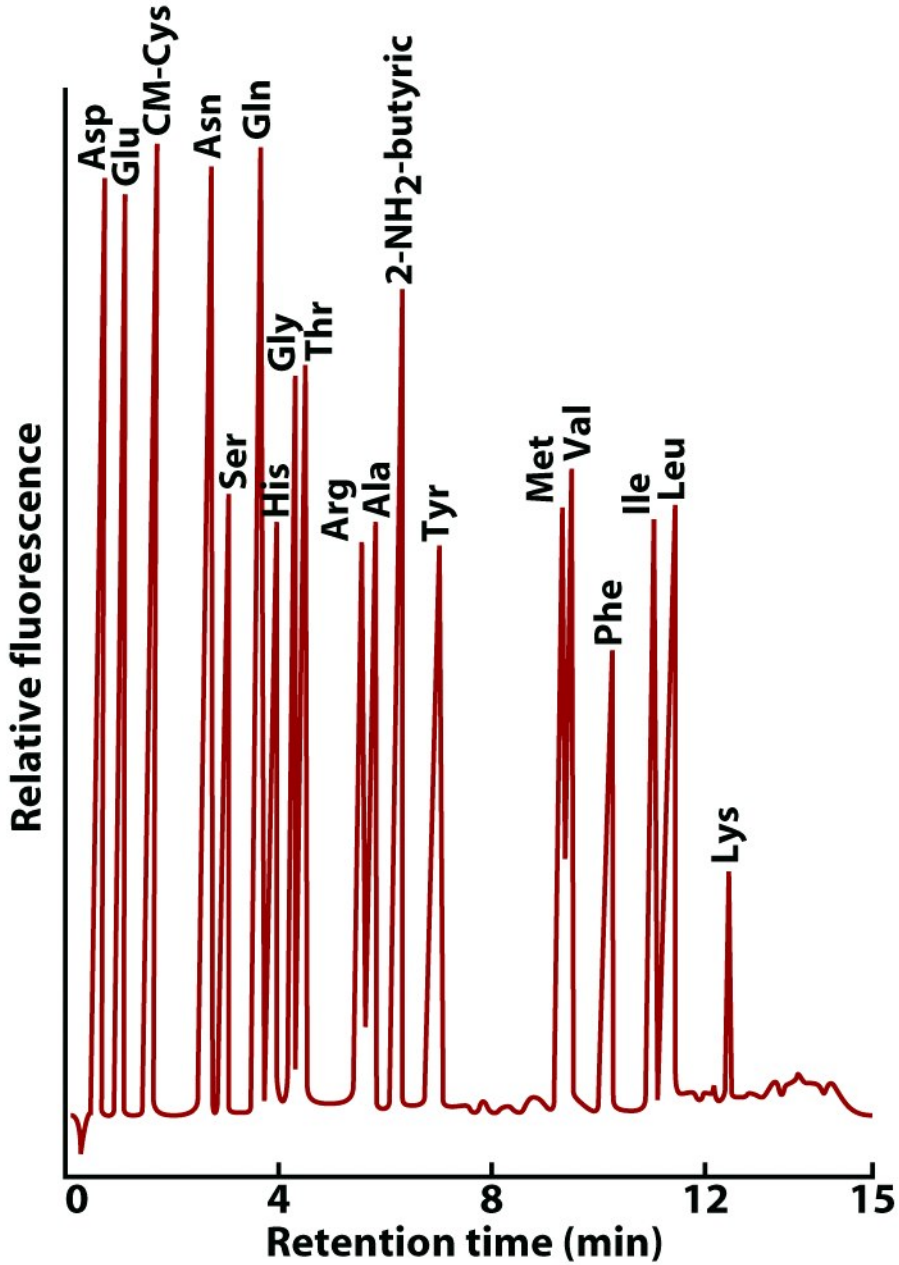
Amino acid composition

- Hydrolysis
- Derivatization
- HPLC



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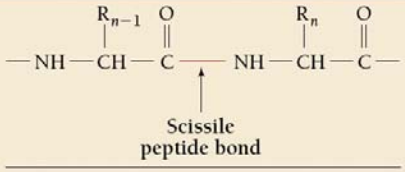
Polypeptide cleavage

- Enzymatic
- Chemical

Table 5-3 Specificities of Various Endopeptidases



Enzyme	Source	Specificity	Comments
Trypsin	Bovine pancreas	R_{n-1} = positively charged residues: Arg, Lys; $R_n \neq$ Pro	Highly specific
Chymotrypsin	Bovine pancreas	R_{n-1} = bulky hydrophobic residues: Phe, Trp, Tyr; $R_n \neq$ Pro	Cleaves more slowly for R_{n-1} = Asn, His, Met, Leu
Elastase	Bovine pancreas	R_{n-1} = small neutral residues: Ala, Gly, Ser, Val; $R_n \neq$ Pro	
Thermolysin	*Bacillus thermoproteolyticus*	R_n = Ile, Met, Phe, Trp, Tyr, Val; $R_{n-1} \neq$ Pro	Occasionally cleaves at R_n = Ala, Asp, His, Thr; heat stable
Pepsin	Bovine gastric mucosa	R_n = Leu, Phe, Trp, Tyr; $R_{n-1} \neq$ Pro	Also others; quite nonspecific; pH optimum = 2
Endopeptidase V8	*Staphylococcus aureus*	R_{n-1} = Glu	

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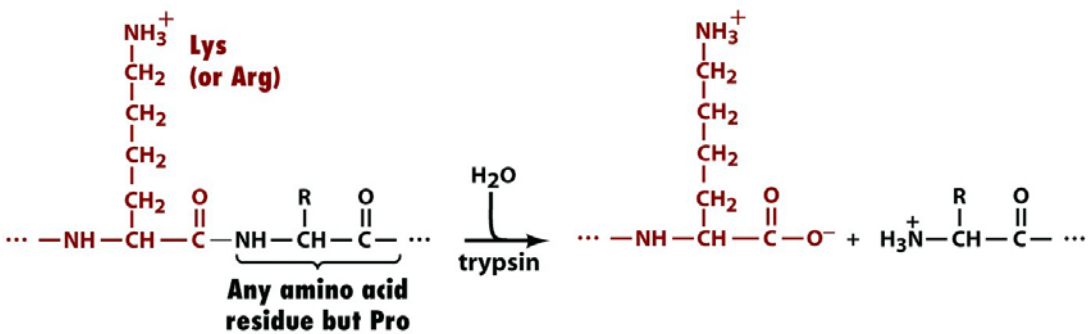

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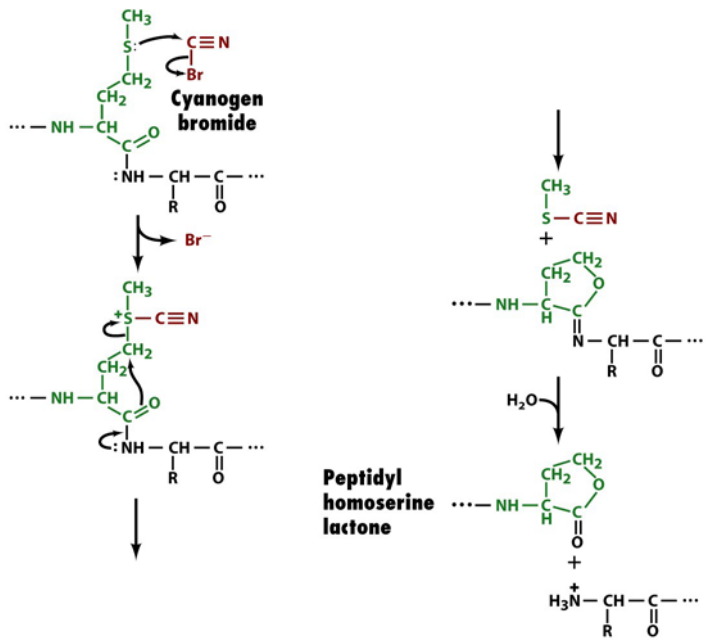

Figure 5-16 Fundamentals of Biochemistry, 2/e
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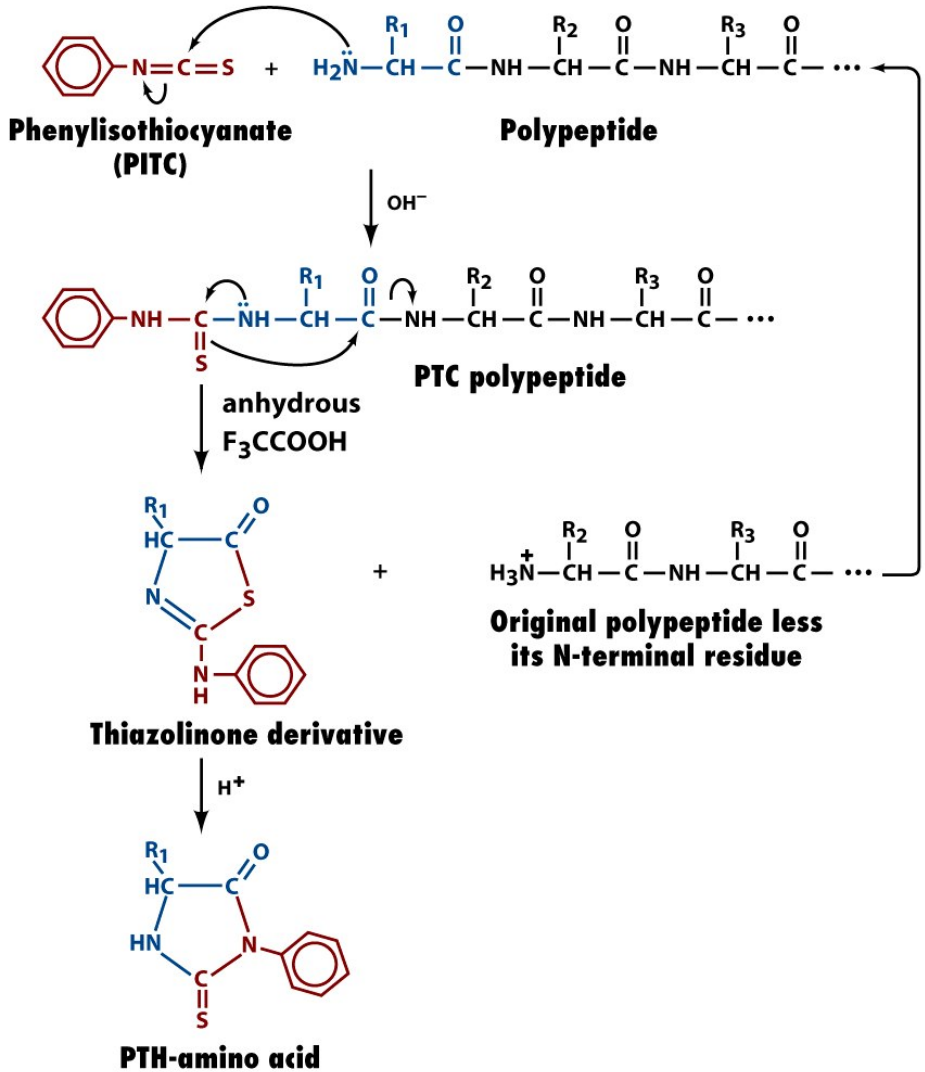
Edman degradation



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Sequencing by mass spectrometry

Mass-to-charge ratio (m/z) for ions in the gas phase

Electron spray ionization (ESI)

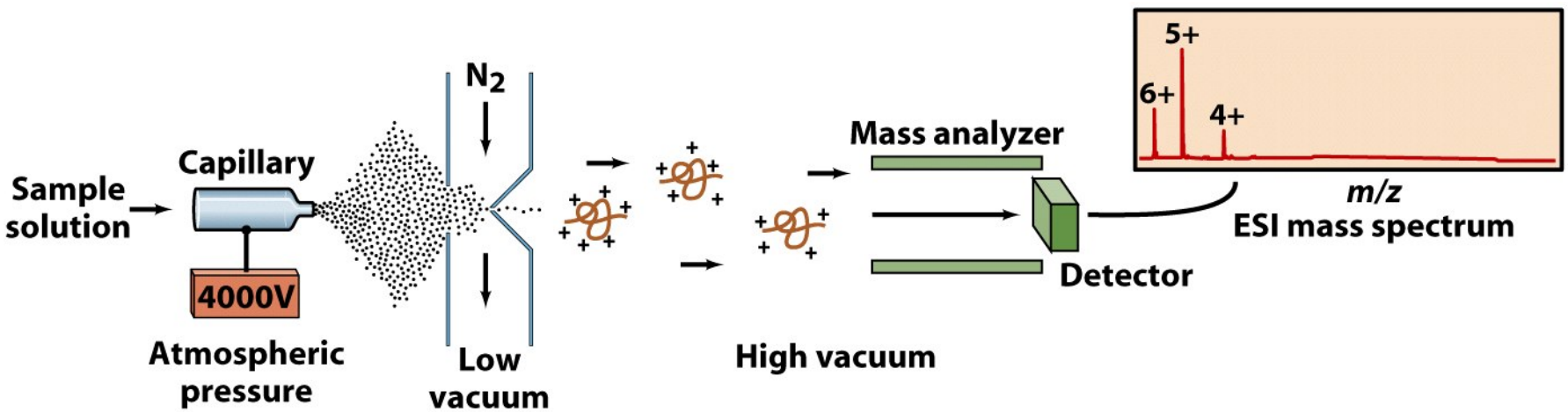

Figure 5-18a Fundamentals of Biochemistry, 2/e

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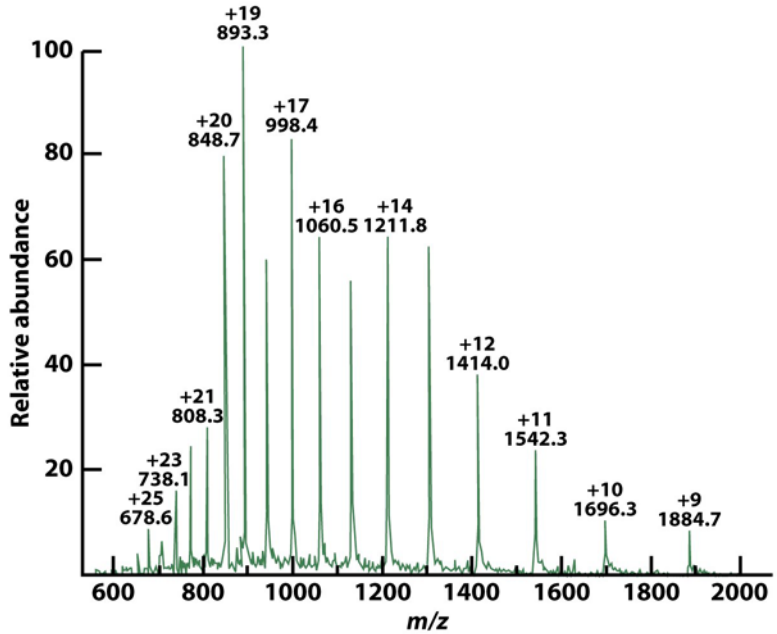

Figure 5-18b Fundamentals of Biochemistry, 2/e
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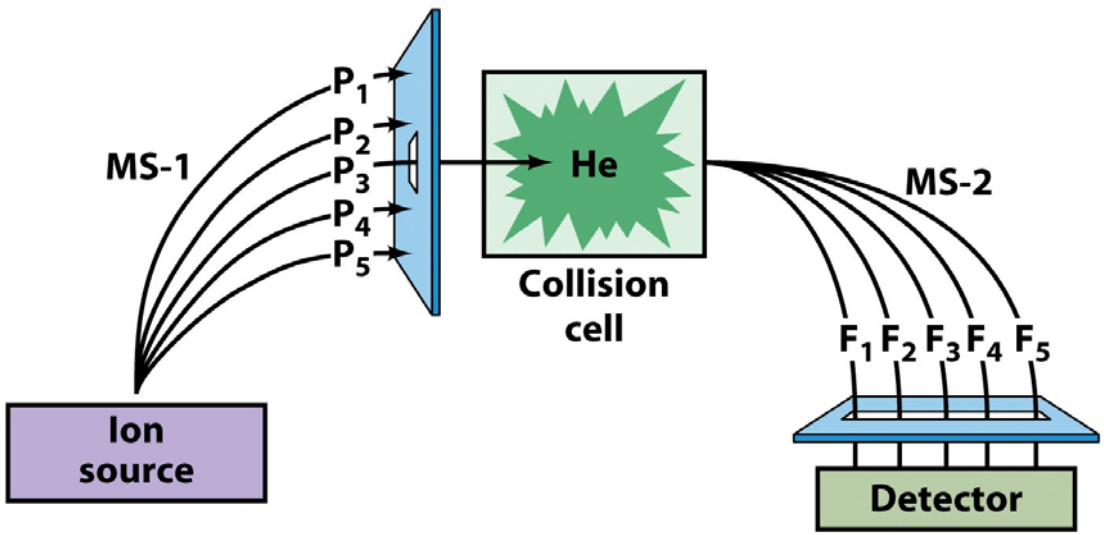

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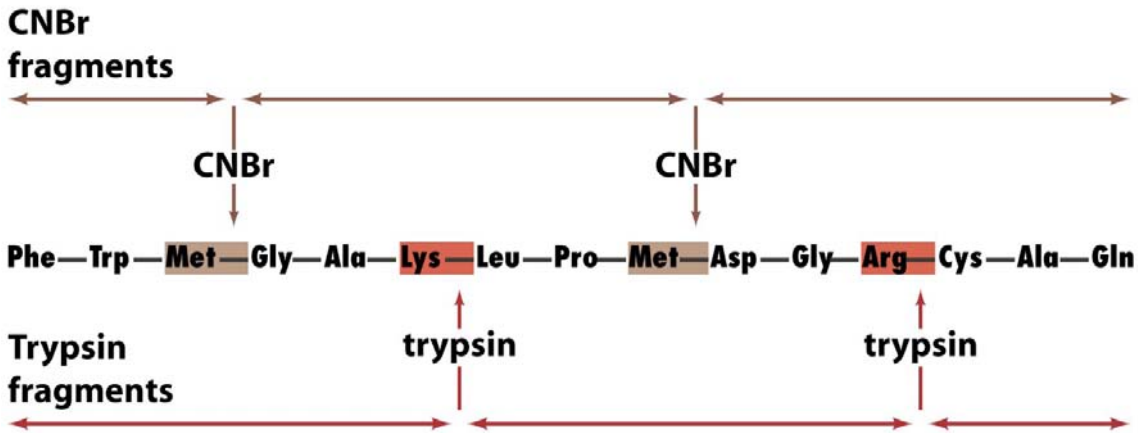

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Final step in protein sequencing

Generating overlapping fragments
Determining the positions of disulfide bonds

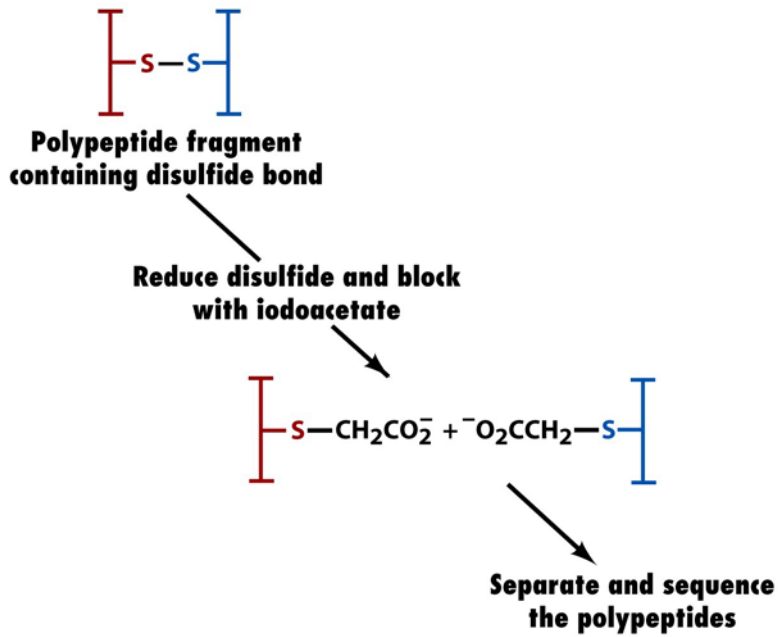

Figure 5-21 Fundamentals of Biochemistry, 2/e
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Homology search

Table 5-4 Internet Addresses for the Major Protein and DNA Sequence Data Banks

Data Banks Containing Protein Sequences

ExPASy Molecular Biology Server (Swiss-Prot): http://au.expasy.org
Protein Information Resource (PIR): http://pir.georgetown.edu/
Protein Research Foundation (PRF): http://www4.prf.or.jp/
UniProt: http://www.ebi.uniprot.org/

Data Banks Containing Gene Sequences

GenBank: http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html
European Bioinformatics Institute (EBI): http://srs.ebi.ac.uk/
DBGET/Integrated Database Retrieval System: http://www.genome.ad.jp/dbget

Table 5-4 Fundamentals of Biochemistry, 2/e
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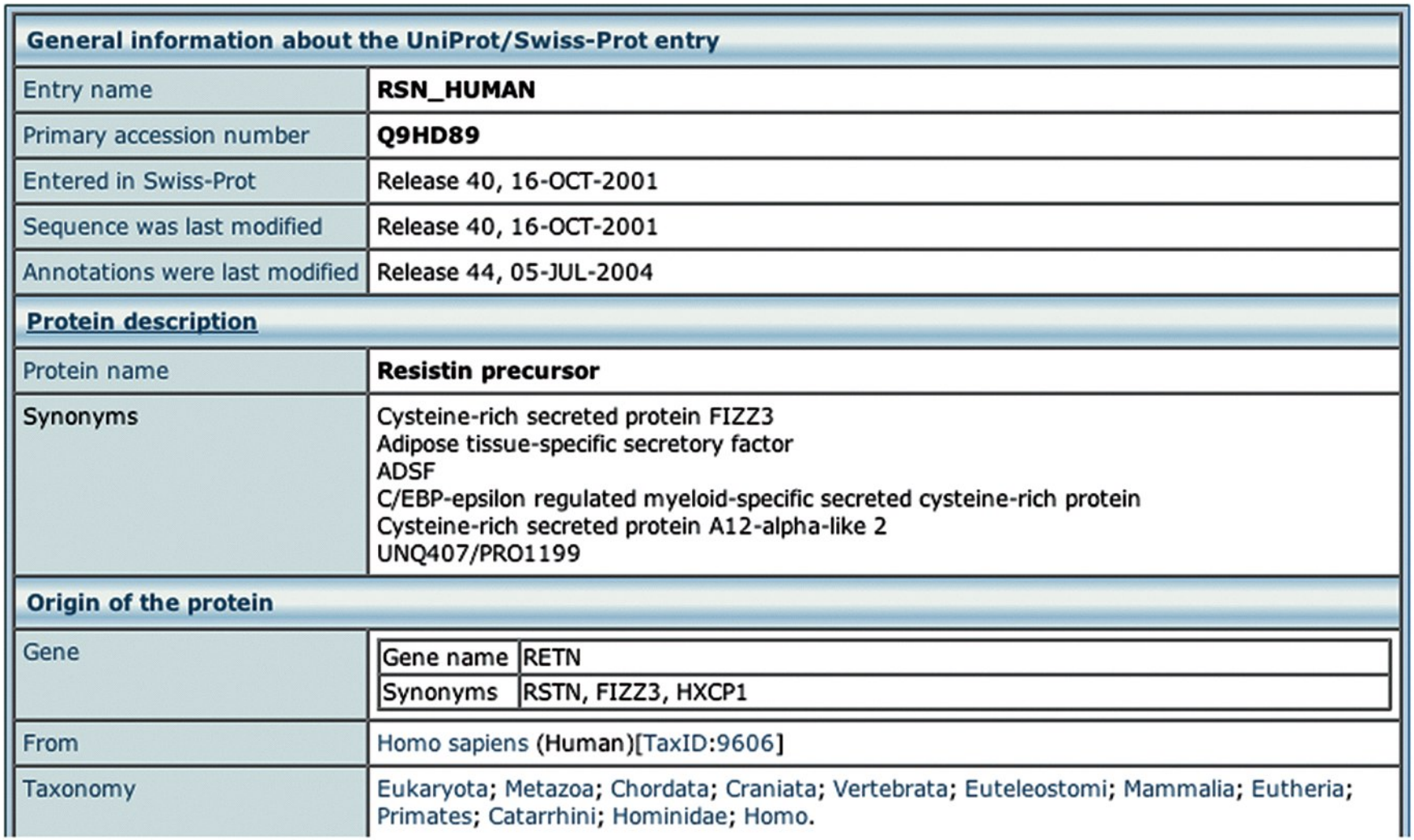


General information about the UniProt/Swiss-Prot entry	
Entry name	**RSN_HUMAN**
Primary accession number	**Q9HD89**
Entered in Swiss-Prot	Release 40, 16-OCT-2001
Sequence was last modified	Release 40, 16-OCT-2001
Annotations were last modified	Release 44, 05-JUL-2004
Protein description	
Protein name	**Resistin precursor**
Synonyms	Cysteine-rich secreted protein FIZZ3 Adipose tissue-specific secretory factor ADSF C/EBP-epsilon regulated myeloid-specific secreted cysteine-rich protein Cysteine-rich secreted protein A12-alpha-like 2 UNQ407/PRO1199
Origin of the protein	
Gene	Gene name: RETN Synonyms: RSTN, FIZZ3, HXCP1
From	Homo sapiens (Human)[TaxID:9606]
Taxonomy	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

Figure 5-22 Fundamentals of Biochemistry, 2/e

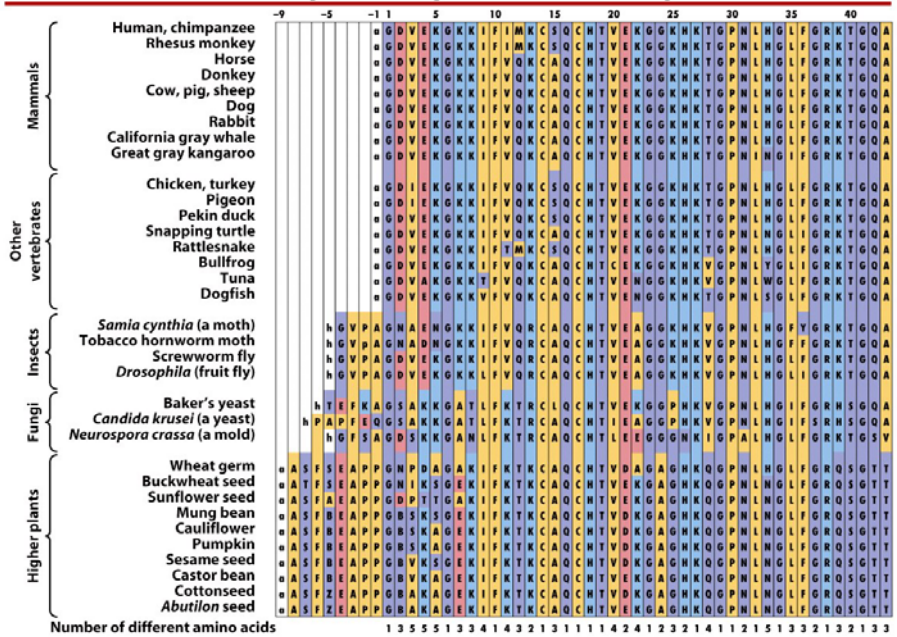
Protein evolution

Protein sequence evolution

Sequence comparison

- Invariant residue
- Conservatively substituted
- Hypervariable
- Neutral drift

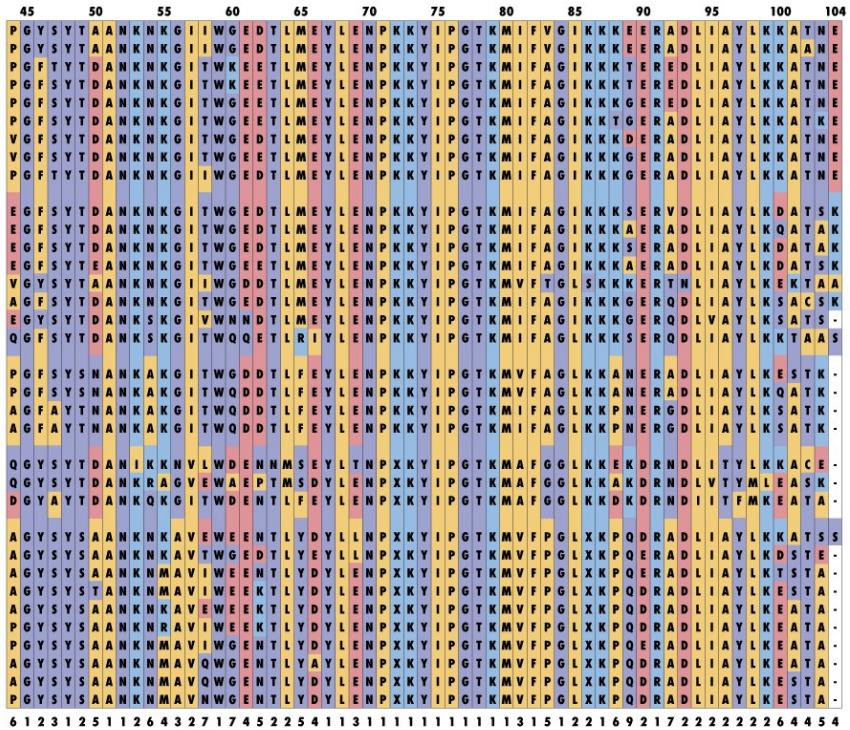
TABLE 5-5 Amino Acid Sequences of Cytochrome *c* from 38 species[a]



[a]The amino acid side chains have been shaded according to their polarity characteristics so that an invariant or conservatively substituted residue is identified by a vertical band of a single color. The letter a at the beginning of the chain indicates that the N-terminal amino group is acetylated; an h indi-cates that the acetyl group is absent.

Source: After Dickerson, R.E., *Sci. Am.* 226(4); 58–72 (1972), with corrections from Dickerson, R.E., and Timkovich, R., *in* Boyer, P.D. (Ed.), *The Enzymes* (3rd ed.), Vol. 11, pp. 421–422, Academic Press (1975). Table copyrighted © by Irving Geis.

Table 5-5 part 1 Fundamentals of Biochemistry, 2/e



Hydrophilic, acidic: D Asp, E Glu

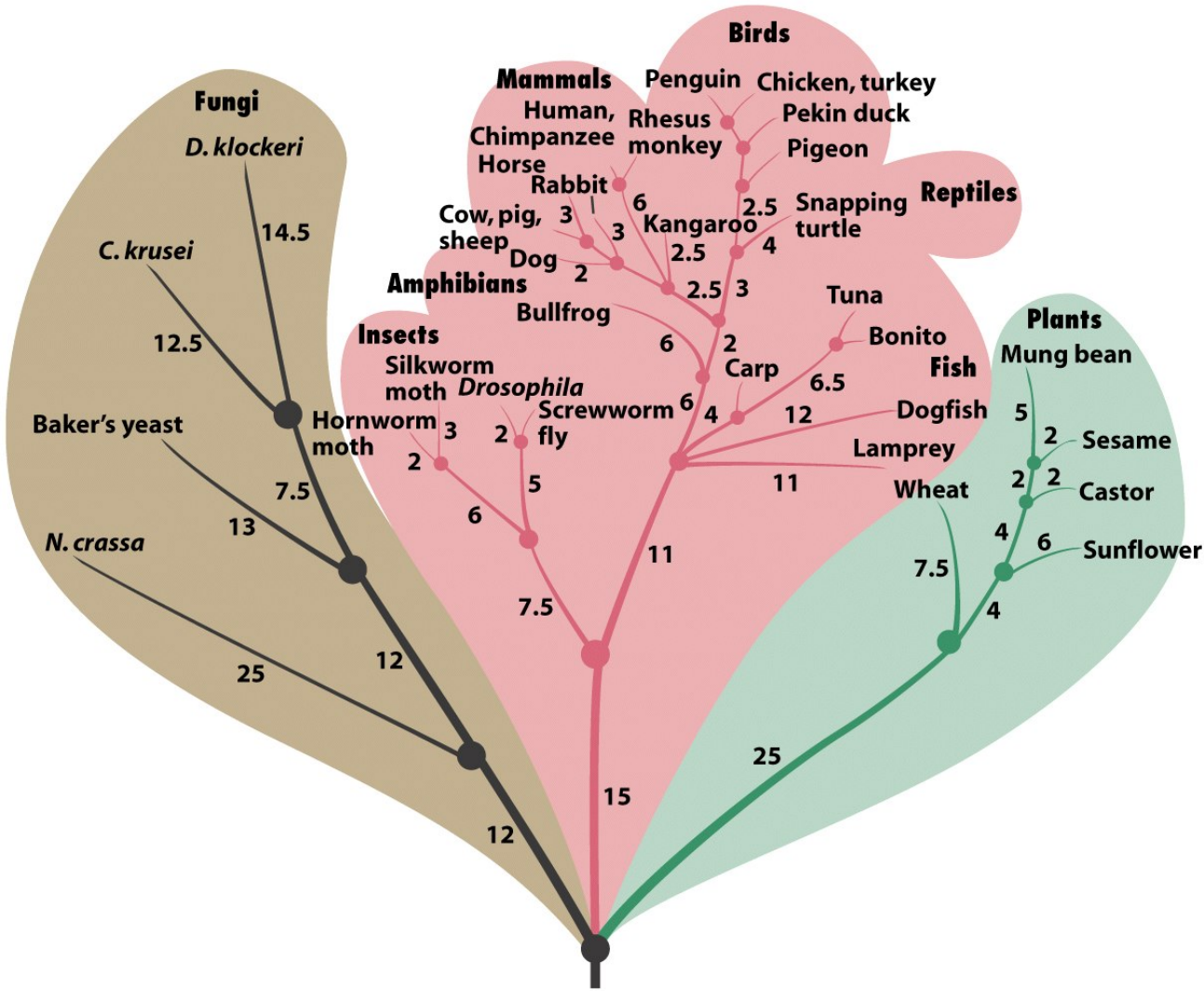
Hydrophilic, basic: H His, K Lys, R Arg, X TrimethylLys

Polar, uncharged: B Asn or Asp, G Gly, N Asn, Q Gln, S Ser, T Thr, W Trp, Y Tyr, Z Gln or Glu

Hydrophobic: A Ala, C Cys, F Phe, I Ile, L Leu, M Met, P Pro, V Val

Table 5-5 part 2 Fundamentals of Biochemistry, 2/e

Phylogenetic trees



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Protein evolve at characteristic rates

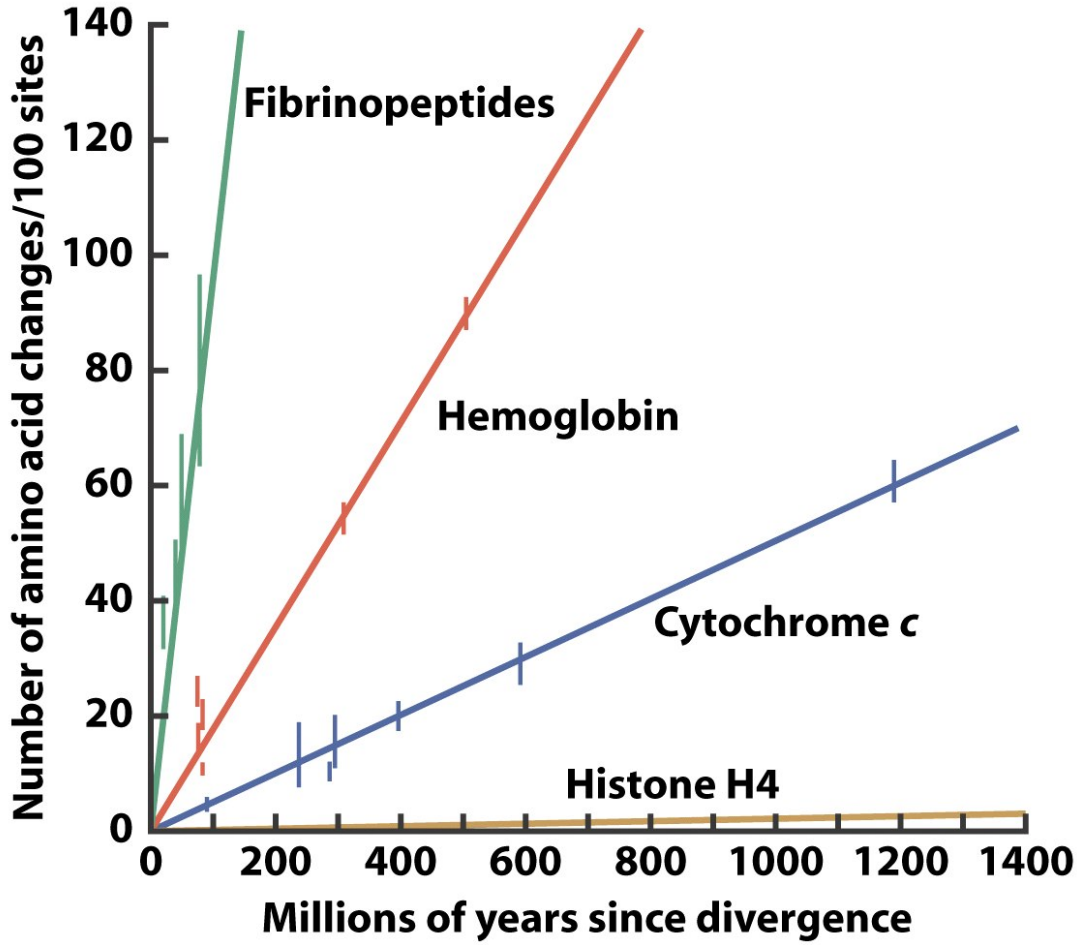

Figure 5-24 Fundamentals of Biochemistry, 2/e
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Gene duplication and protein families

Homologous
Orthologous
Paralogous
Pseudogenes

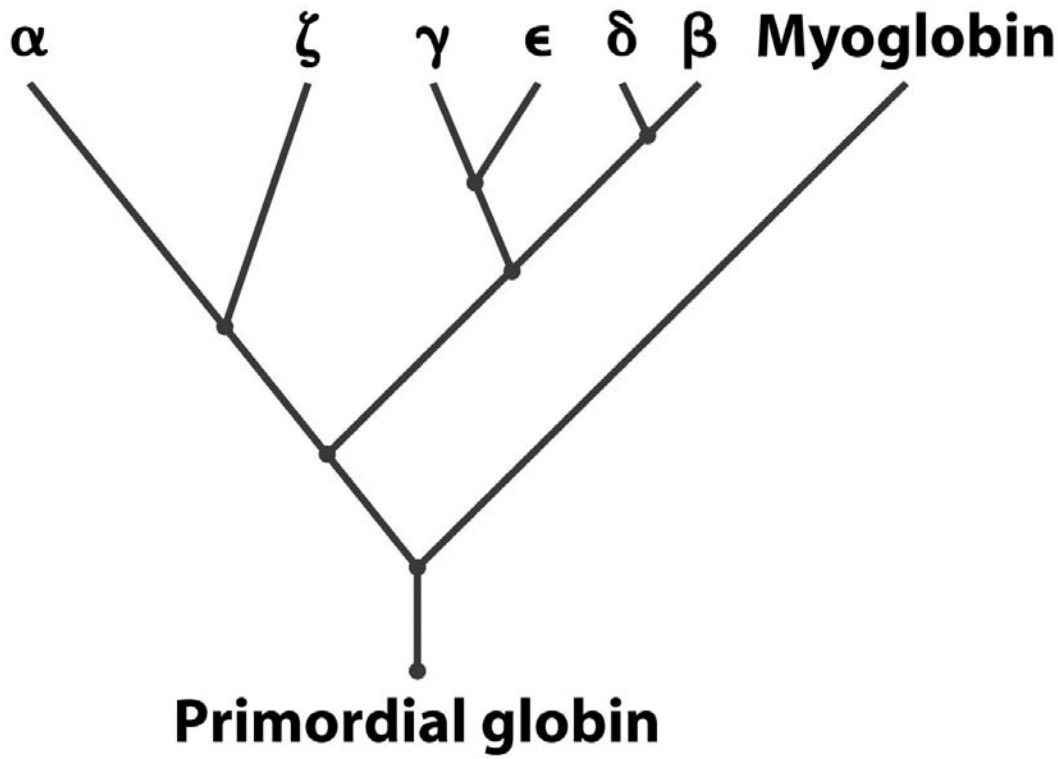

Figure 5-25 Fundamentals of Biochemistry, 2/e
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Protein modules

Module

Understanding of modular structure

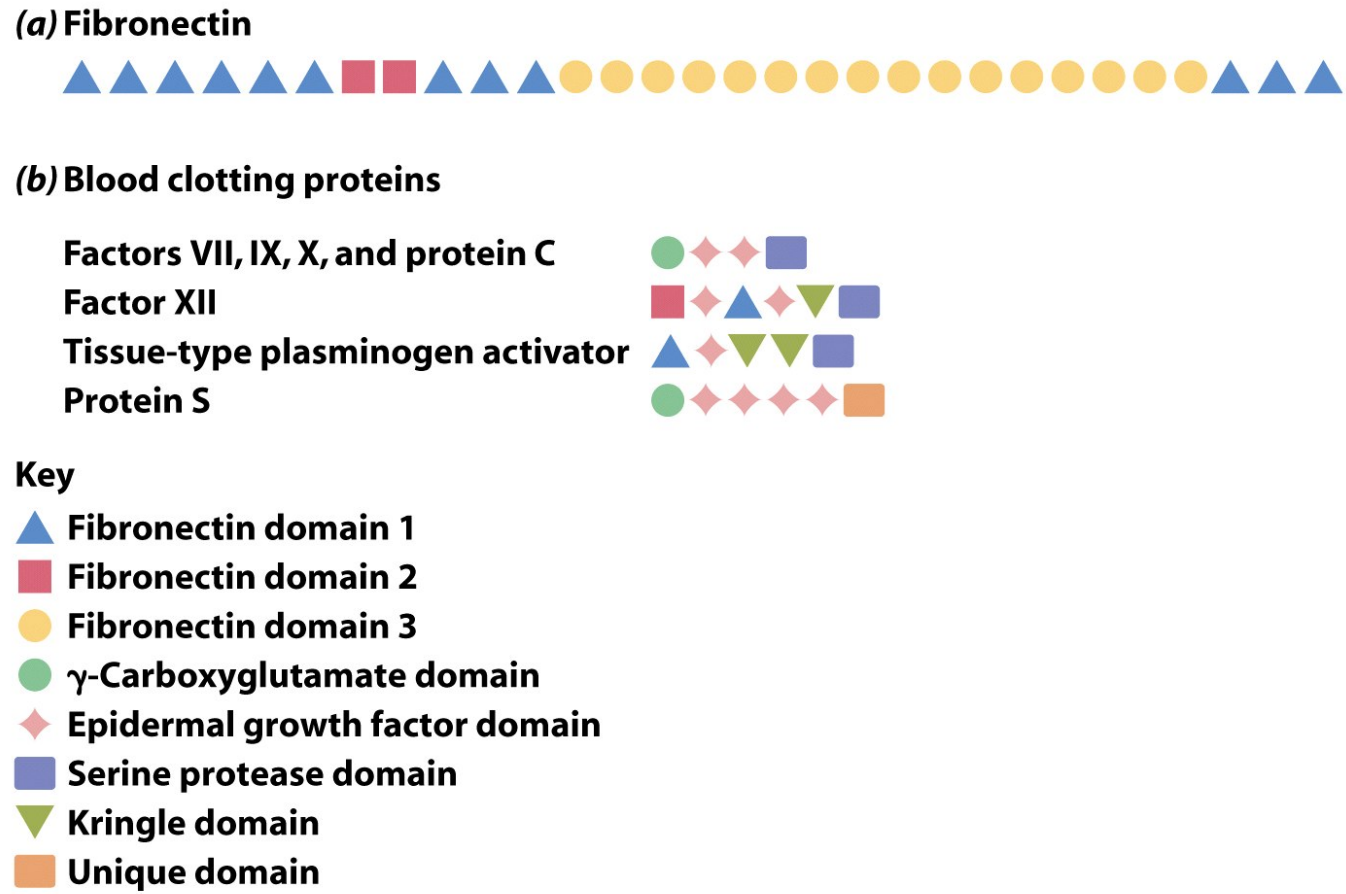

Figure 5-26 Fundamentals of Biochemistry, 2/e

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