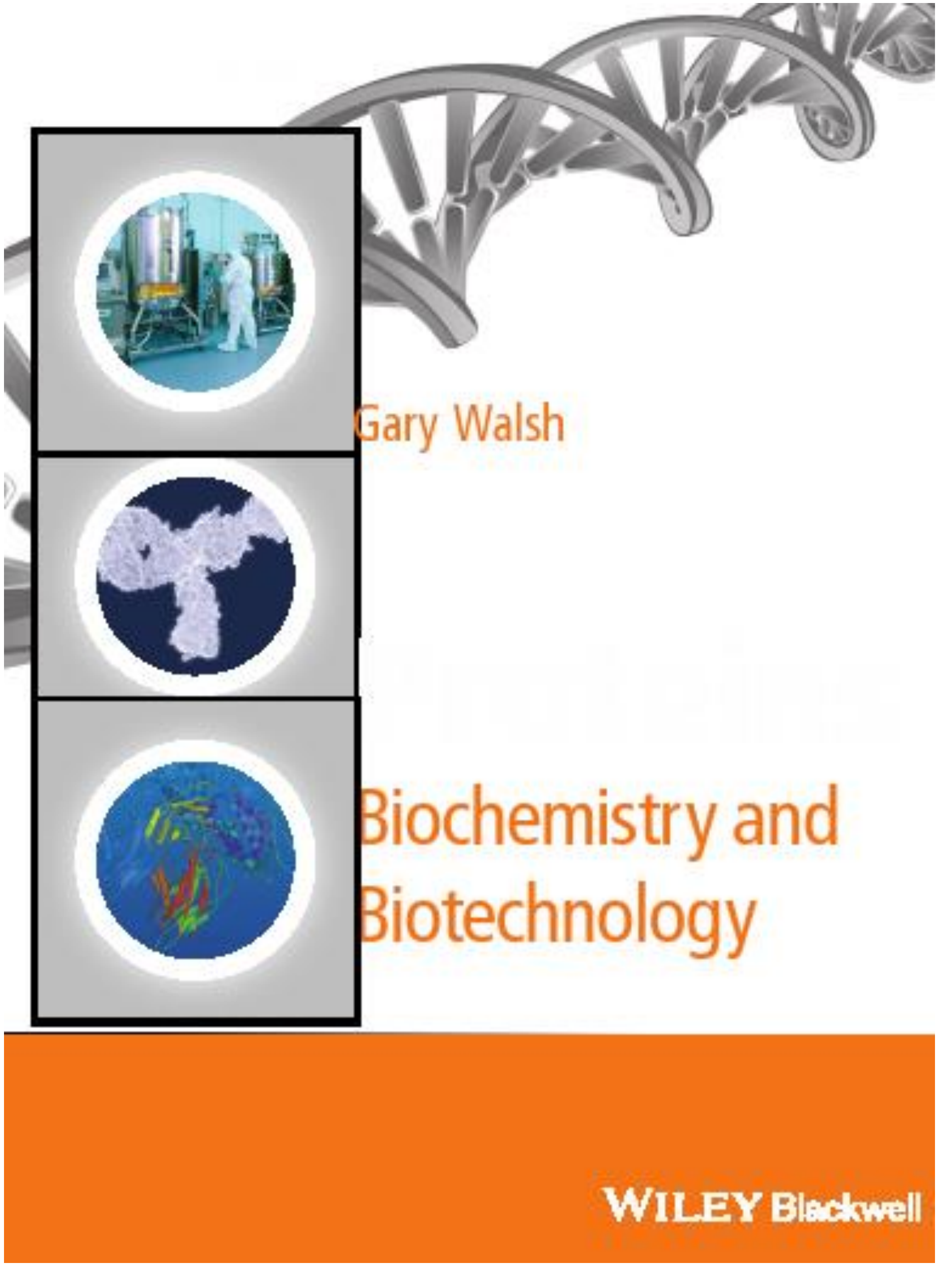

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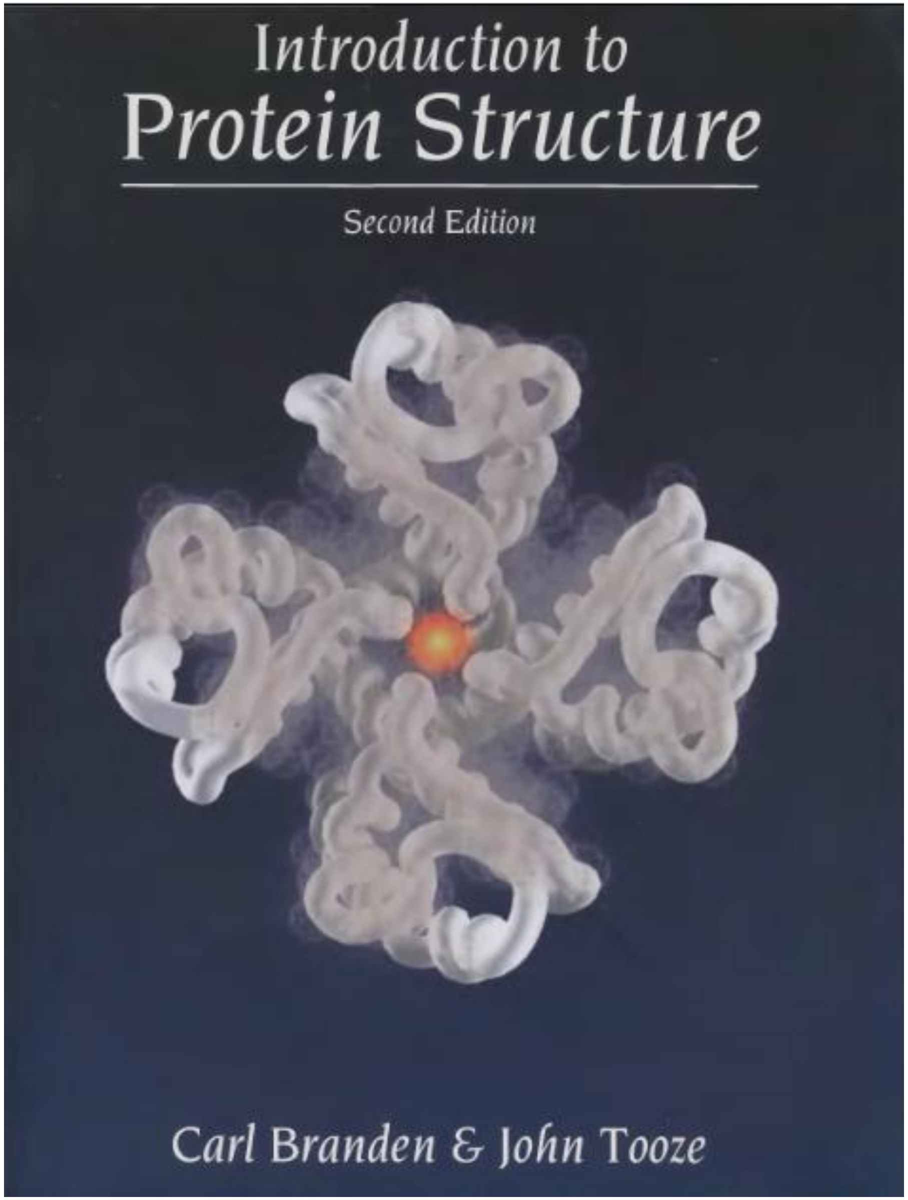

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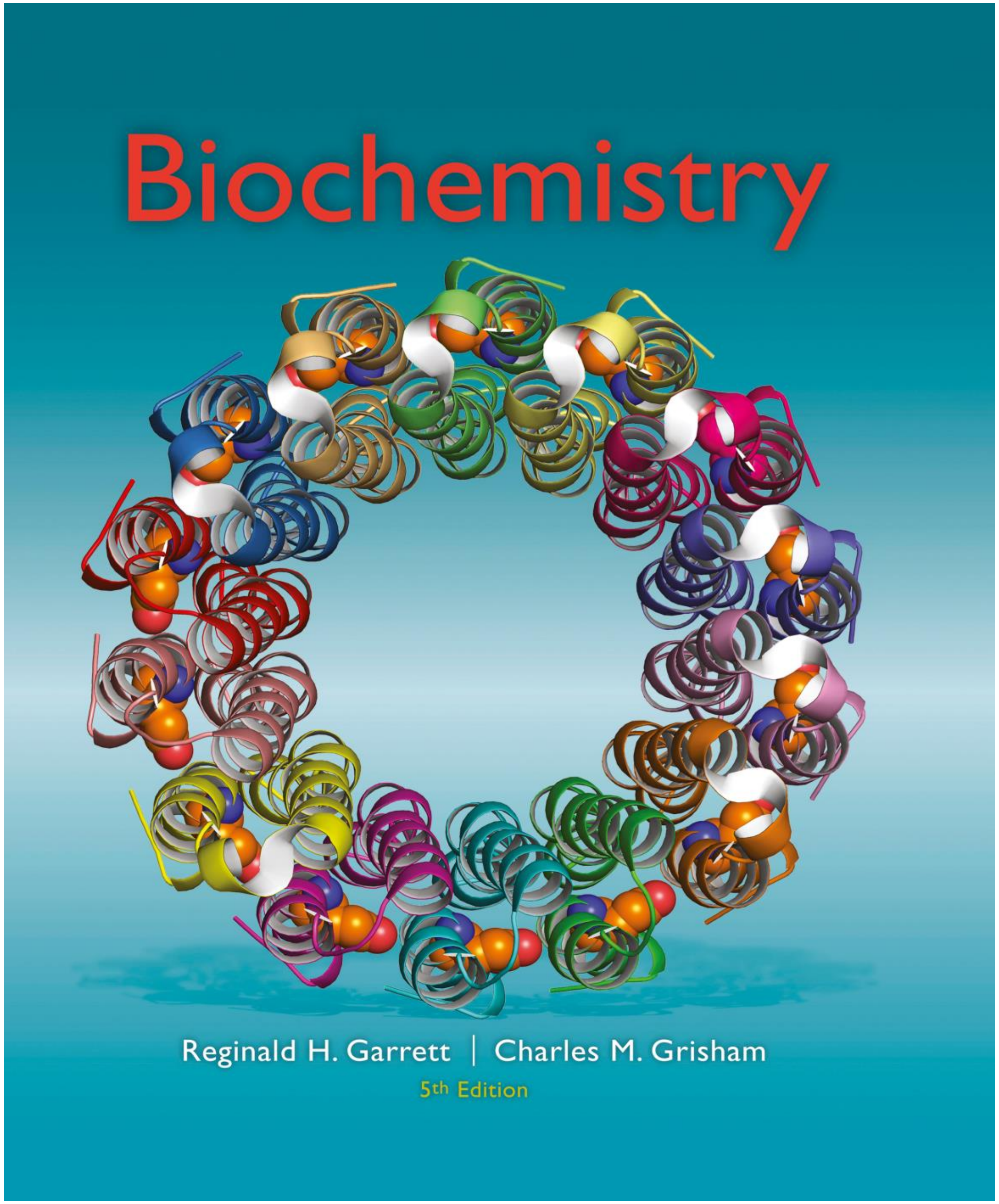

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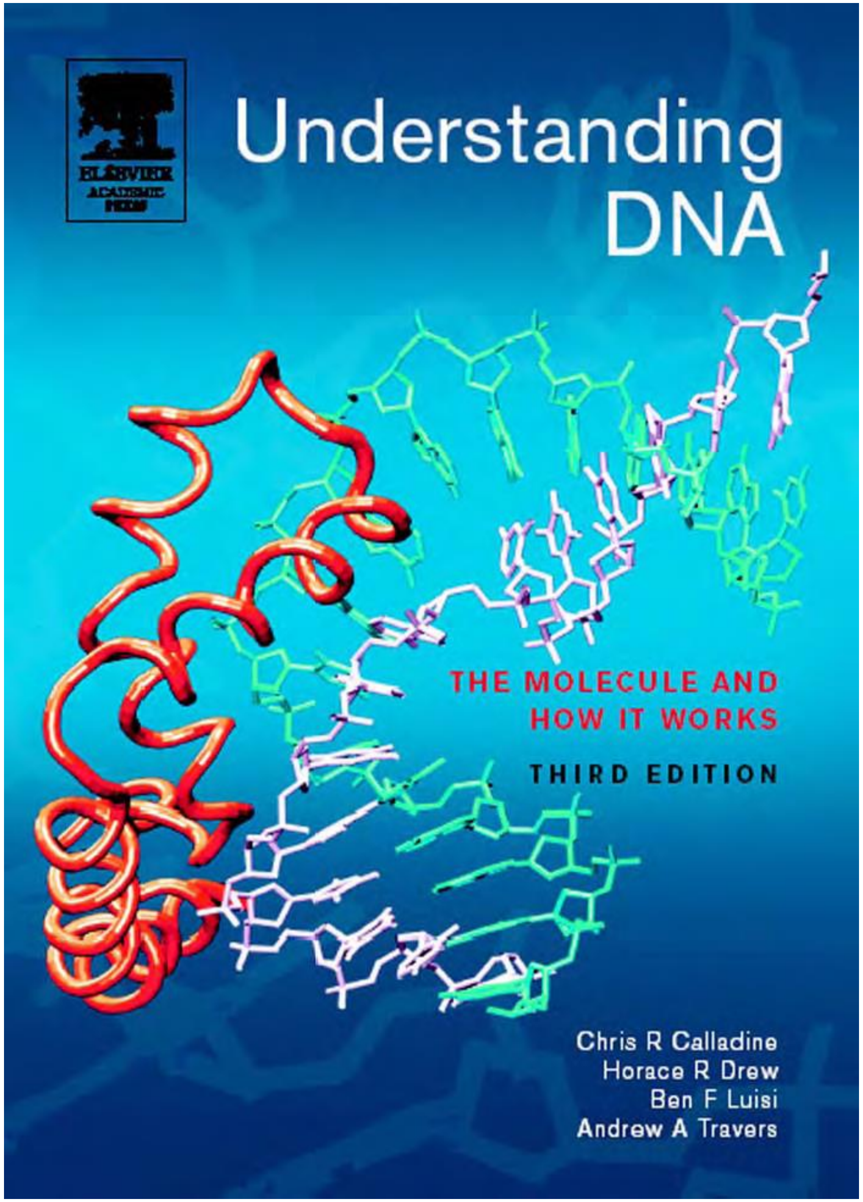

Understanding DNA

THE MOLECULE AND HOW IT WORKS

THIRD EDITION

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Table 2.1 The 20 commonly occurring amino acids. They may be subdivided into five groups on the basis of side-chain structure. Their three- and one-letter abbreviations are also listed (one-letter abbreviations are generally used only when compiling extended sequence data, mainly to minimize writing space and effort). In addition to their individual molecular masses, the per cent occurrence of each amino acid in an 'average' protein is also presented. This data was generated from sequence analysis of over 1000 different proteins.

R group classification	Amino acid	Abbreviated name (3 letter)	Abbreviated name (1 letter)	Molecular mass (Da)	Per cent occurrence in 'average' protein
Non-polar, aliphatic	Glycine	Gly	G	75	7.2
	Alanine	Ala	A	89	8.3
	Valine	Val	V	117	6.6
	Leucine	Leu	L	131	9.0
	Isoleucine	Ile	I	131	5.2
	Proline	Pro	P	115	5.1
Aromatic	Tyrosine	Tyr	Y	181	3.2
	Phenylalanine	Phe	F	165	3.9
	Tryptophan	Trp	W	204	1.3
Polar but uncharged	Cysteine	Cys	C	121	1.7
	Serine	Ser	S	105	6.0
	Methionine	Met	M	149	2.4
	Threonine	Thr	T	119	5.8
	Asparagine	Asn	N	132	4.4
	Glutamine	Gln	Q	146	4.0
Positively charged	Arginine	Arg	R	174	5.7
	Lysine	Lys	K	146	5.7
	Histidine	His	H	155	2.2
Negatively charged	Aspartic acid	Asp	D	133	5.3
	Glutamic acid	Glu	E	147	6.2

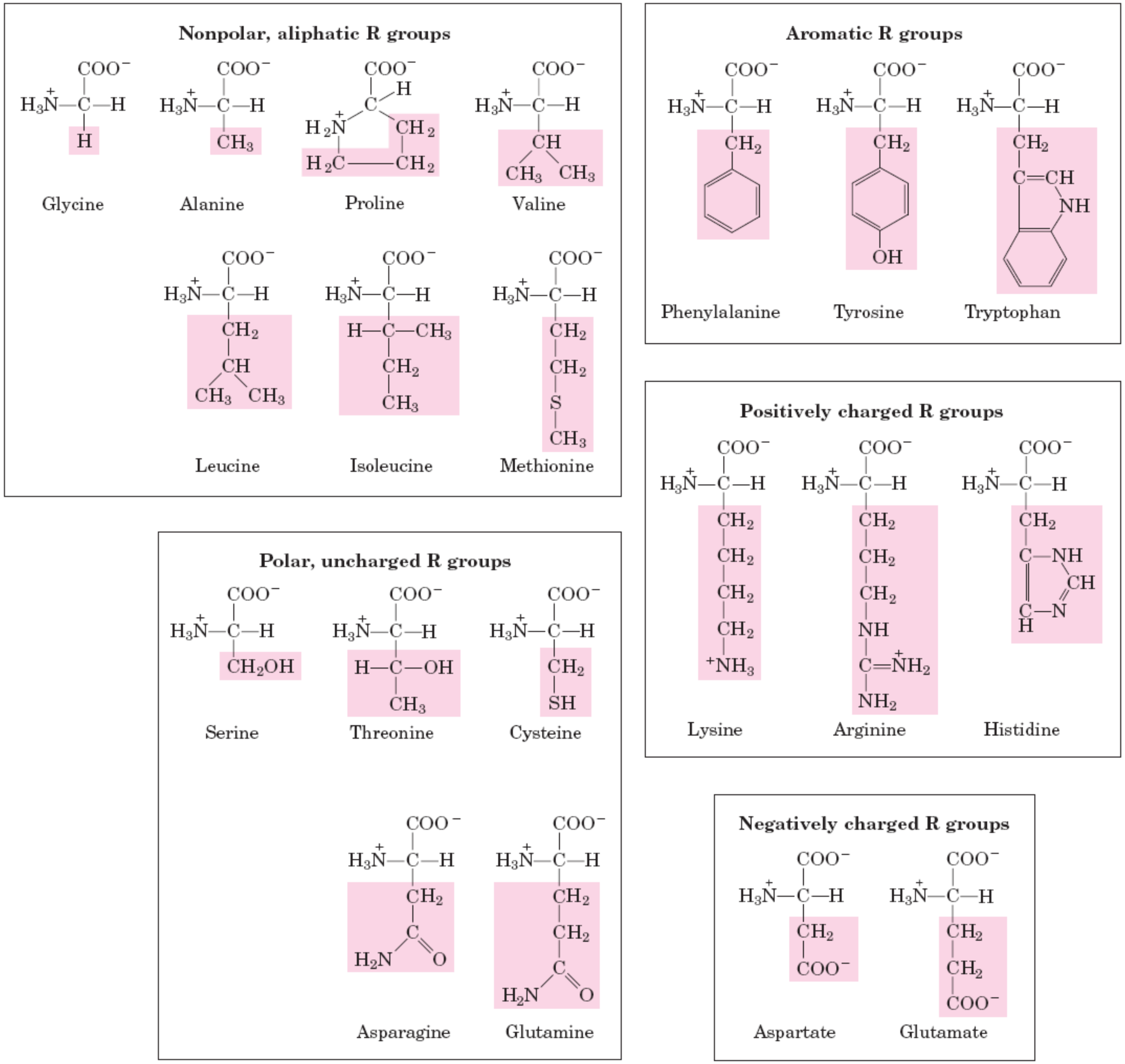
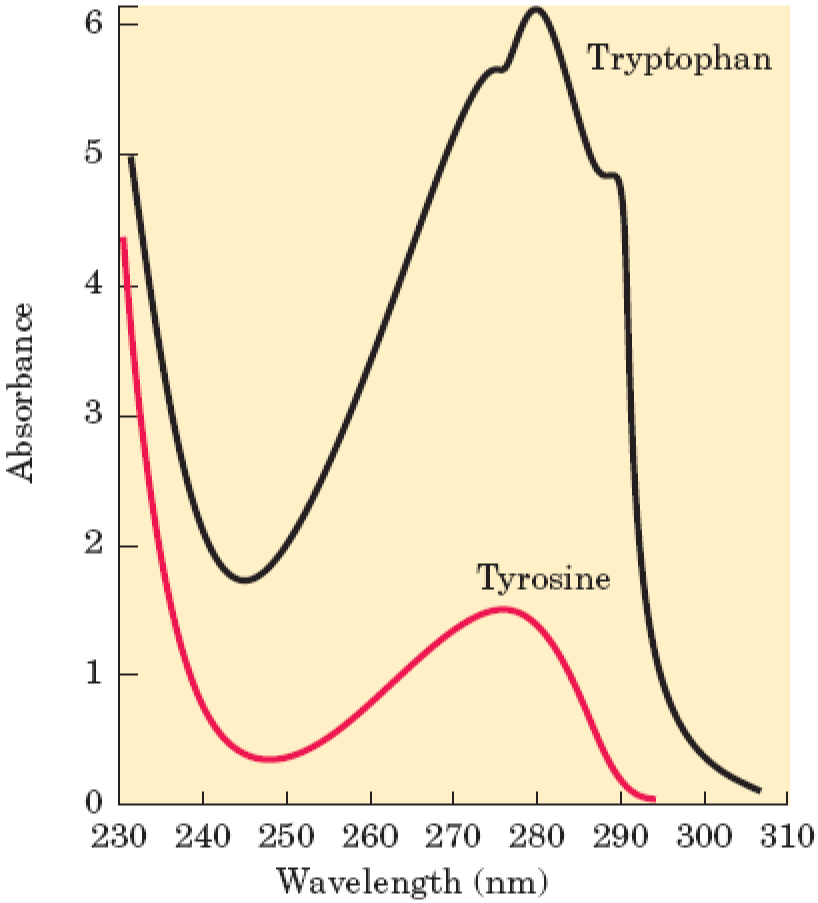


FIGURE 3–5 The 20 common amino acids of proteins. The structural formulas show the state of ionization that would predominate at pH 7.0. The unshaded portions are those common to all the amino acids; the portions shaded in red are the R groups. Although the R group of histidine is shown uncharged, its pK_a (see Table 3–1) is such that a small but significant fraction of these groups are positively charged at pH 7.0.



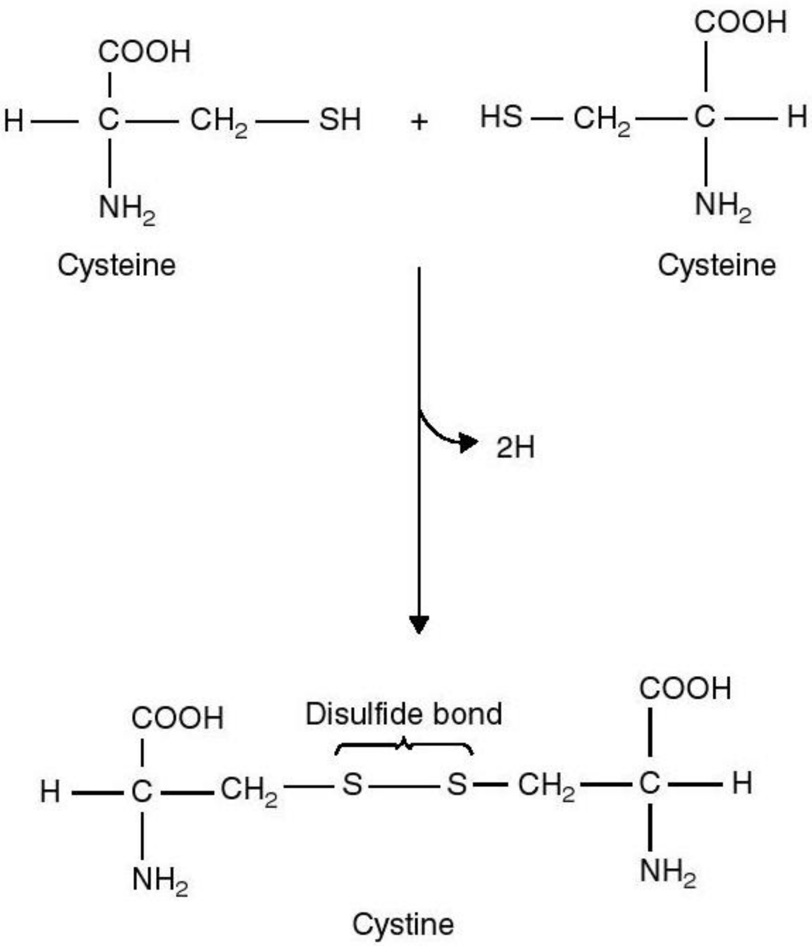
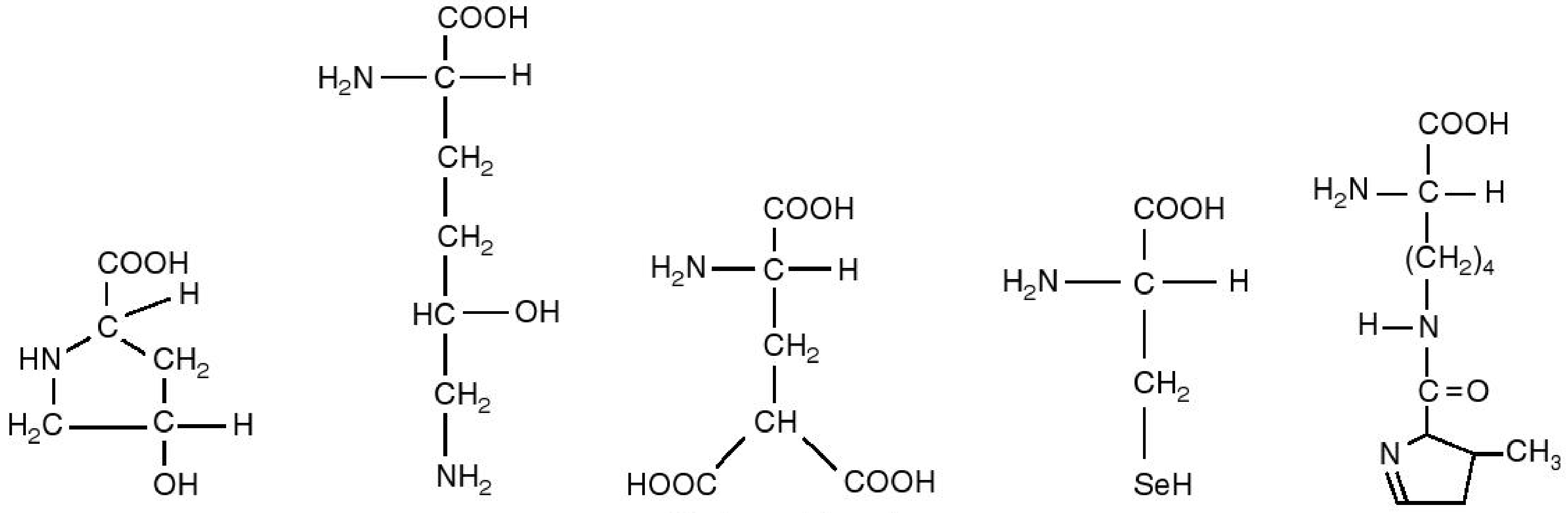


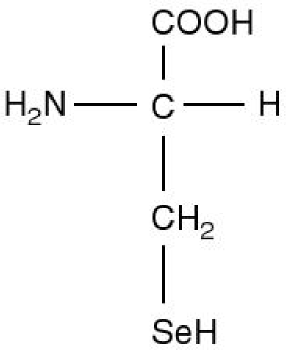
Figure 2.2 The formation of cystine via disulfide bond formation between two cysteines.



4-Hydroxyproline 5-Hydroxylysine γ-Carboxyglutamate Selenocysteine Pyrrolysine

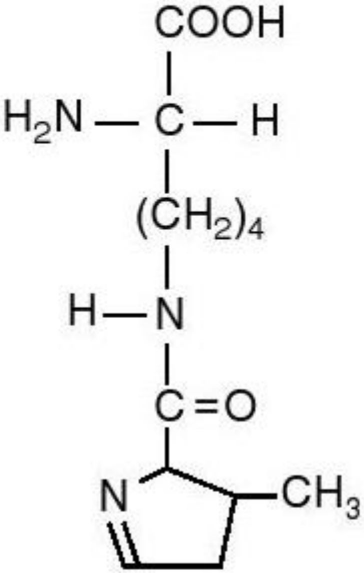
Figure 2.3 Structure of some modified amino acids.

In addition to the 20 common amino acids, some modified amino acids are also found in several proteins. In most instances these modified amino acids are formed by PTM reactions, as discussed later in this chapter. However, two amino acids (selenocysteine and pyrrolysine; Figure 2.3) exist as a preformed amino acid in their own right and are hence sometimes called the 21st and 22nd proteinogenic amino acids.



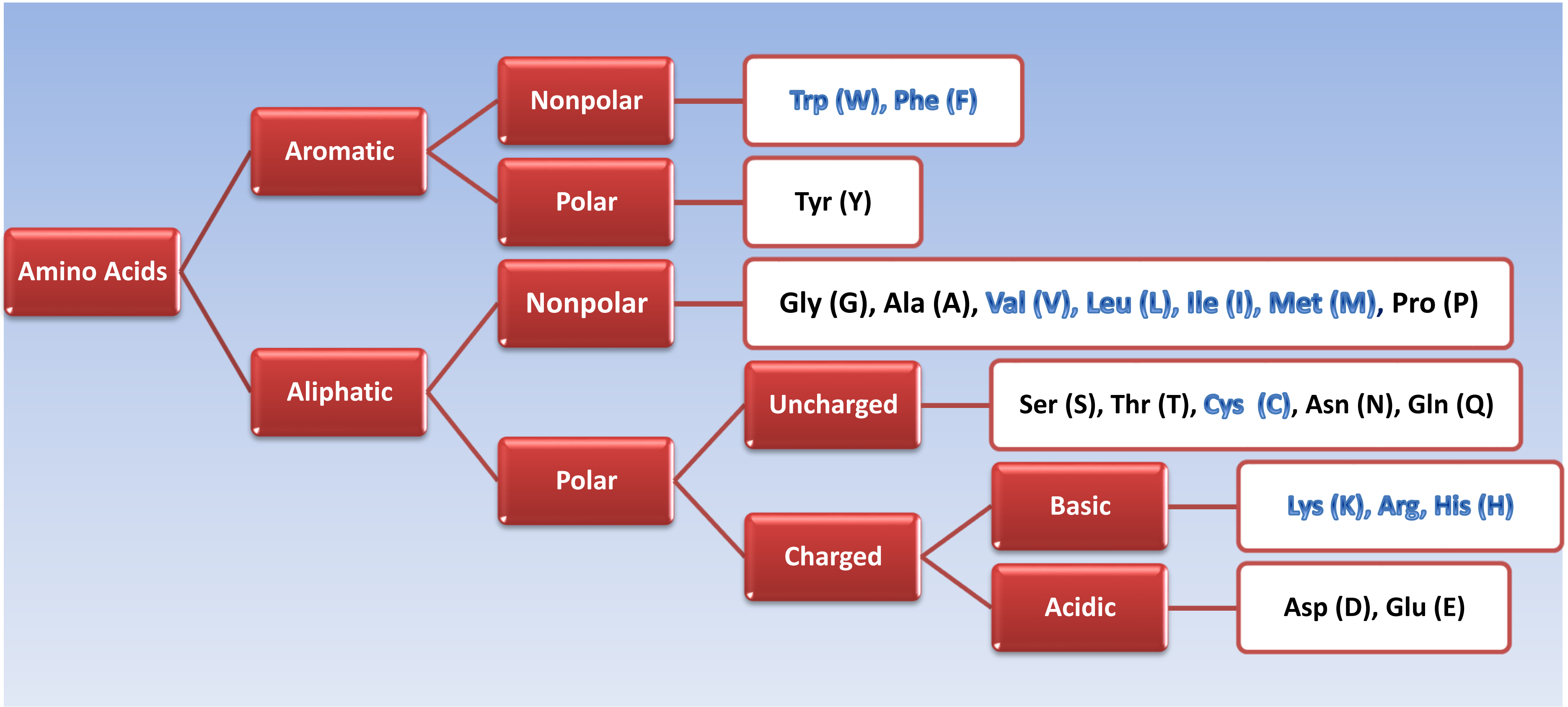
Selenocysteine

Selenium in the form of selenocysteine (Sec or U) is an essential component of a small number of enzymes in some species (including glutathione peroxidase, thioredoxin reductases and some hydrogenases). The nucleotide sequence of the genes coding for such enzymes contains a UGA codon, which codes for selenocysteine. In non-selenocysteine proteins, UGA normally functions as a termination codon. The reading of UGA as selenocysteine rather than the more usual stop codon is apparently dependent on the presence of a so-called *cis*-acting selenocysteine insertion sequence element.

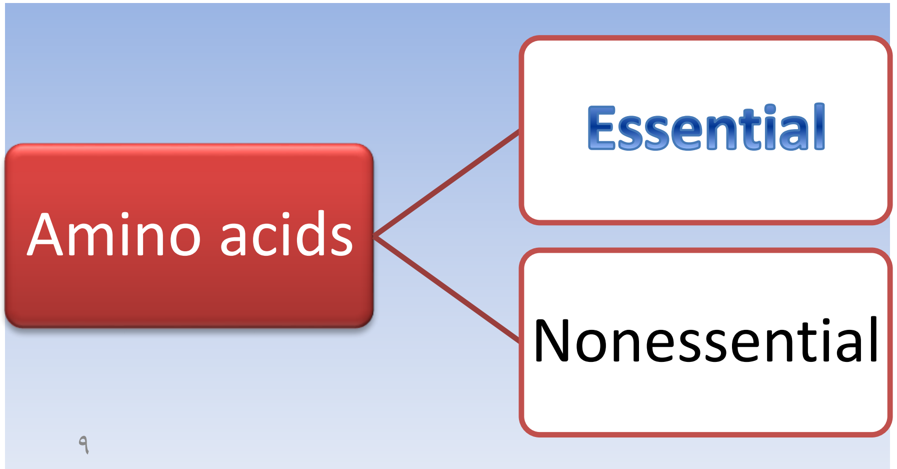


Pyrrolysine

Pyrrolysine (Pyl or O) displays a side chain similar to lysine, with the presence of an added pyrroline ring at the end of the lysine side chain. Similarly to Sec, Pyl is encoded by a codon which normally functions as a stop signal (UAG), with Pyl insertion likely requiring a pyrrolysine insertion sequence element. Its presence appears to be restricted to a small number of methanogenic, mainly archael, microorganisms, where it appears to reside within the active site of several methyltransferase enzymes, playing a direct catalytic role therein.

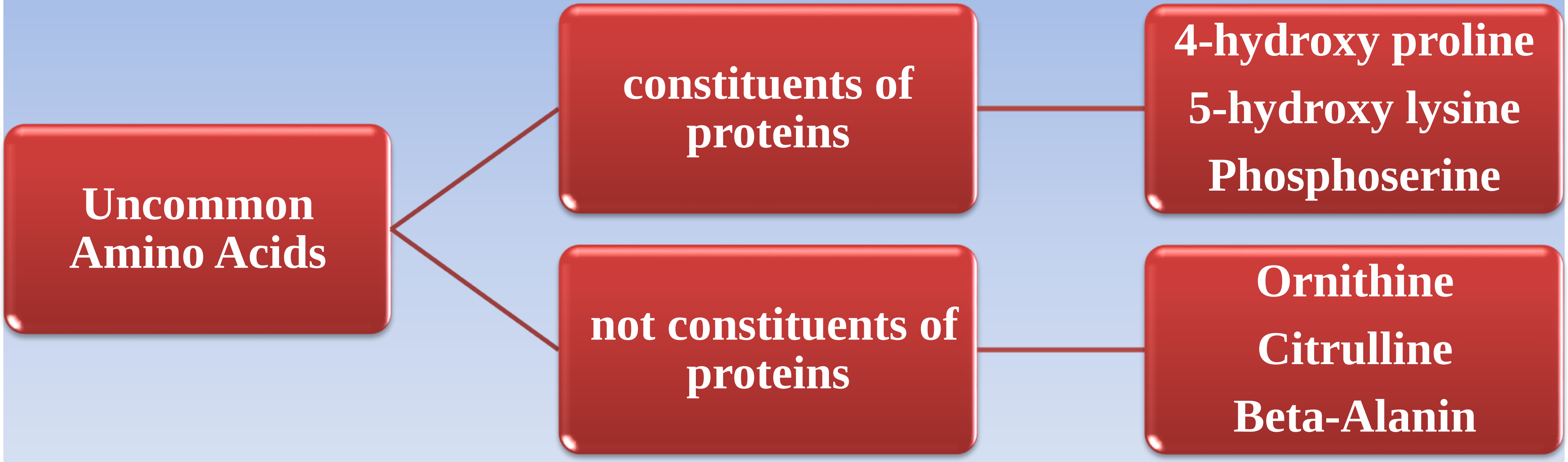


- Amino Acids
  - Aromatic
    - Nonpolar
      - Trp (W), Phe (F)
    - Polar
      - Tyr (Y)
  - Aliphatic
    - Nonpolar
      - Gly (G), Ala (A), Val (V), Leu (L), Ile (I), Met (M), Pro (P)
    - Polar
      - Uncharged
        - Ser (S), Thr (T), Cys (C), Asn (N), Gln (Q)
      - Charged
        - Basic
          - Lys (K), Arg, His (H)
        - Acidic
          - Asp (D), Glu (E)



- Amino acids
  - Essential
  - Nonessential

- ❖ Standard amino acids (20)
- ❖ Prolin (P) → imino acid
- ❖ Ile and Thr → Two chiral center
- ❖ Gly → without chiral center



Uncommon Amino Acids

constituents of proteins

4-hydroxy proline

5-hydroxy lysine

Phosphoserine

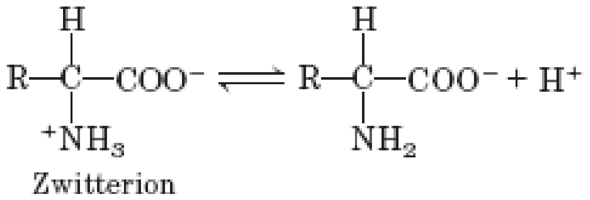
not constituents of proteins

Ornithine

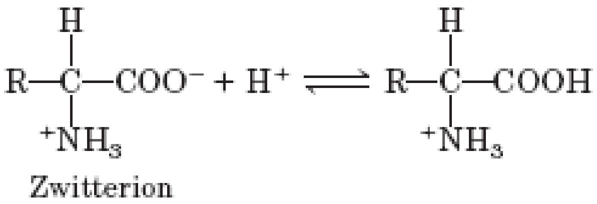
Citrulline

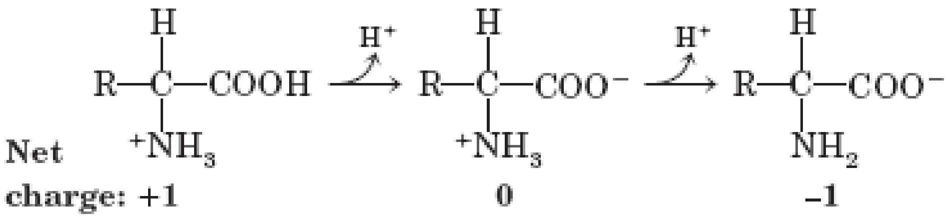
Beta-Alanin

Uncommon amino acids created by modification of common residues already incorporated into a polypeptide.



or a base (proton acceptor):





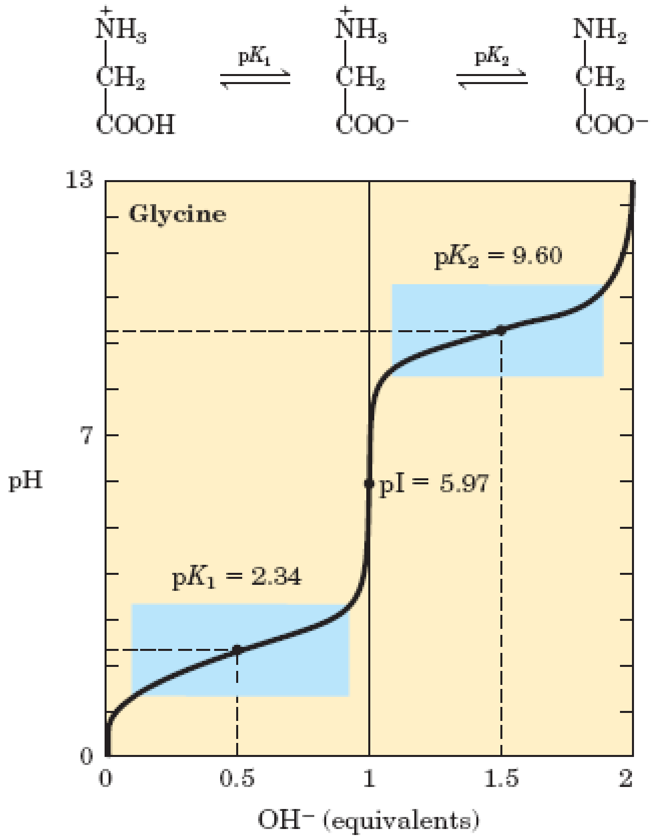
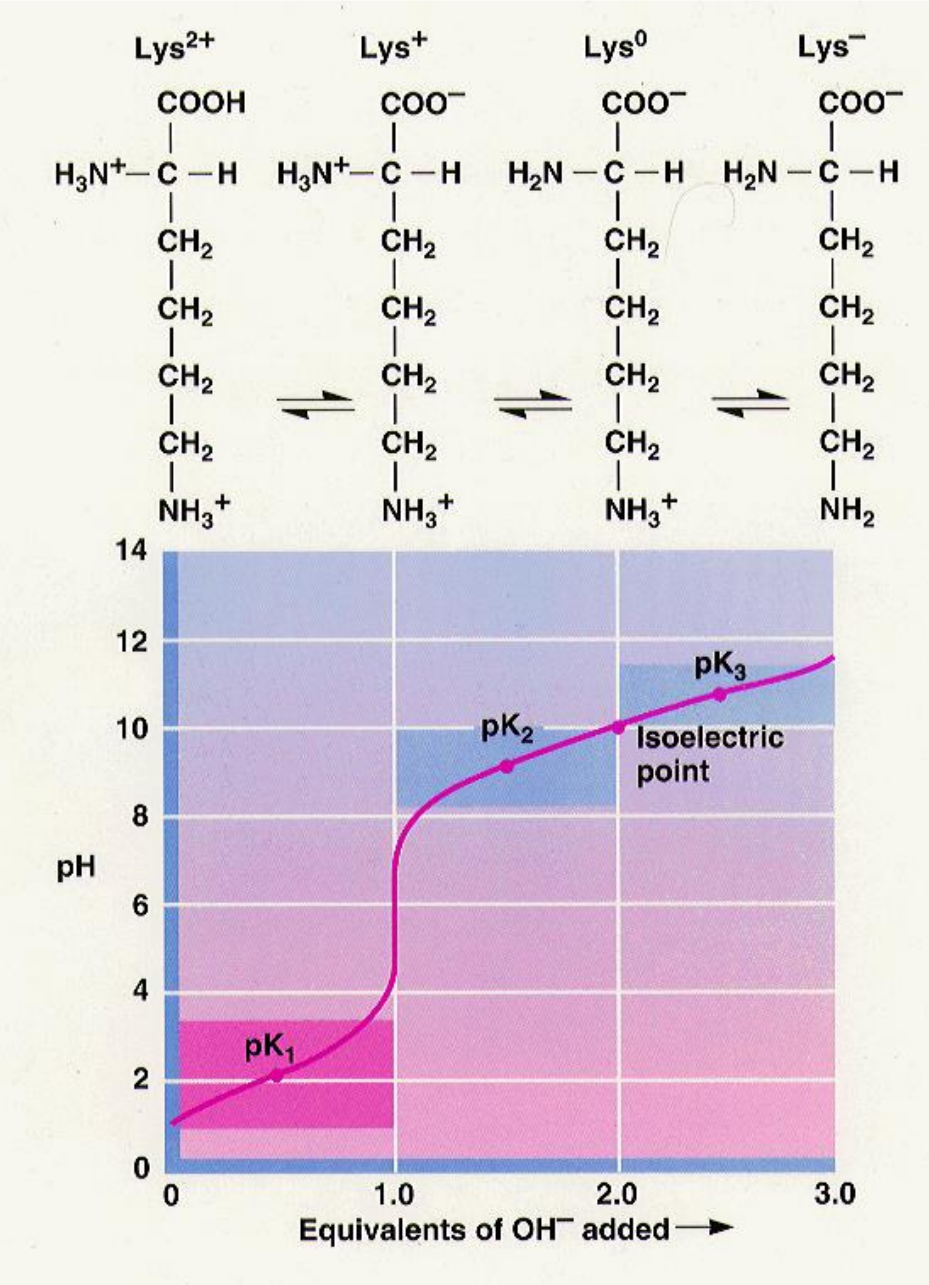
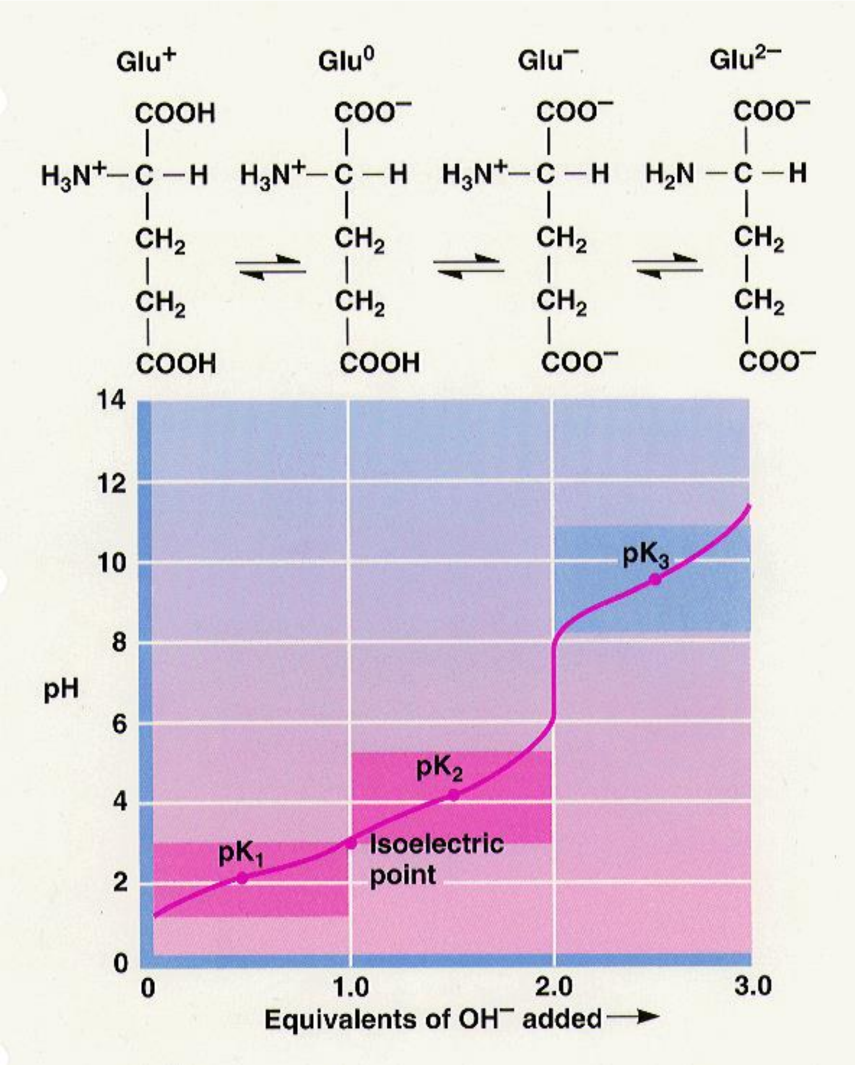


FIGURE 3–10 Titration of an amino acid. Shown here is the titration curve of 0.1 M glycine at 25 °C. The ionic species predominating at key points in the titration are shown above the graph. The shaded boxes, centered at about $pK_1 = 2.34$ and $pK_2 = 9.60$, indicate the regions of greatest buffering power.


Lys2+
Lys+
Lys0
Lys−
COOH
COO−
H3N+ — C — H
H2N — C — H
CH2
NH3+
NH2
14
12
10
8
6
4
2
0
pH
pK1
pK2
pK3
Isoelectric point
0
1.0
2.0
3.0
Equivalents of OH− added →


Glu+
Glu0
Glu−
Glu2−
COOH
COO−
H3N+ — C — H
H2N — C — H
CH2
14
12
10
8
6
4
2
0
pH
pK1
pK2
pK3
Isoelectric point
0
1.0
2.0
3.0
Equivalents of OH− added →

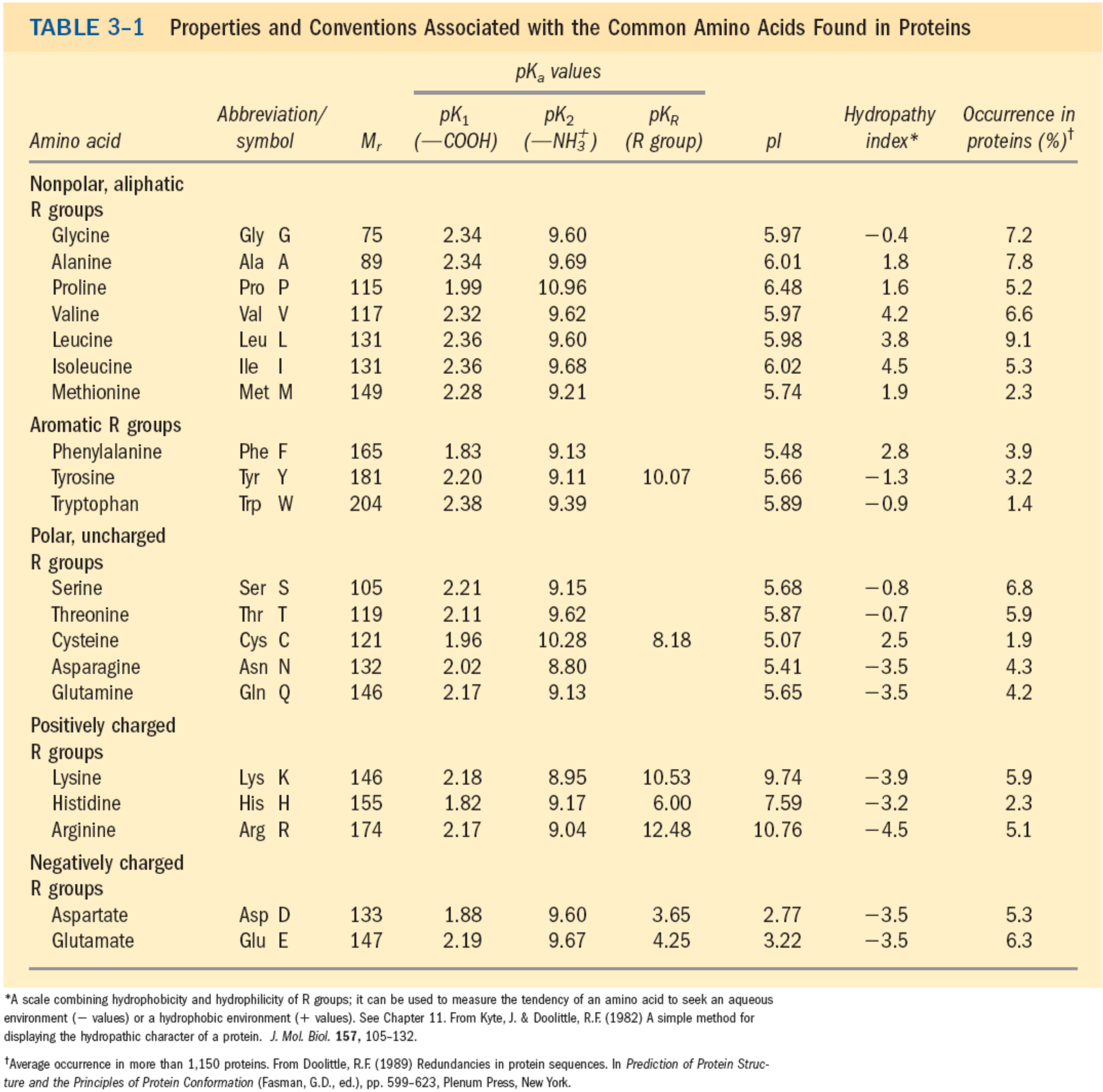


TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

			pK_a values					
Amino acid	Abbreviation/ symbol	M_r	pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups								
Glycine	Gly G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups								
Serine	Ser S	105	2.21	9.15		5.68	−0.8	6.8
Threonine	Thr T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5	6.3

*A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (− values) or a hydrophobic environment (+ values). See Chapter 11. From Kyte, J. & Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105–132.

†Average occurrence in more than 1,150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed.), pp. 599–623, Plenum Press, New York.

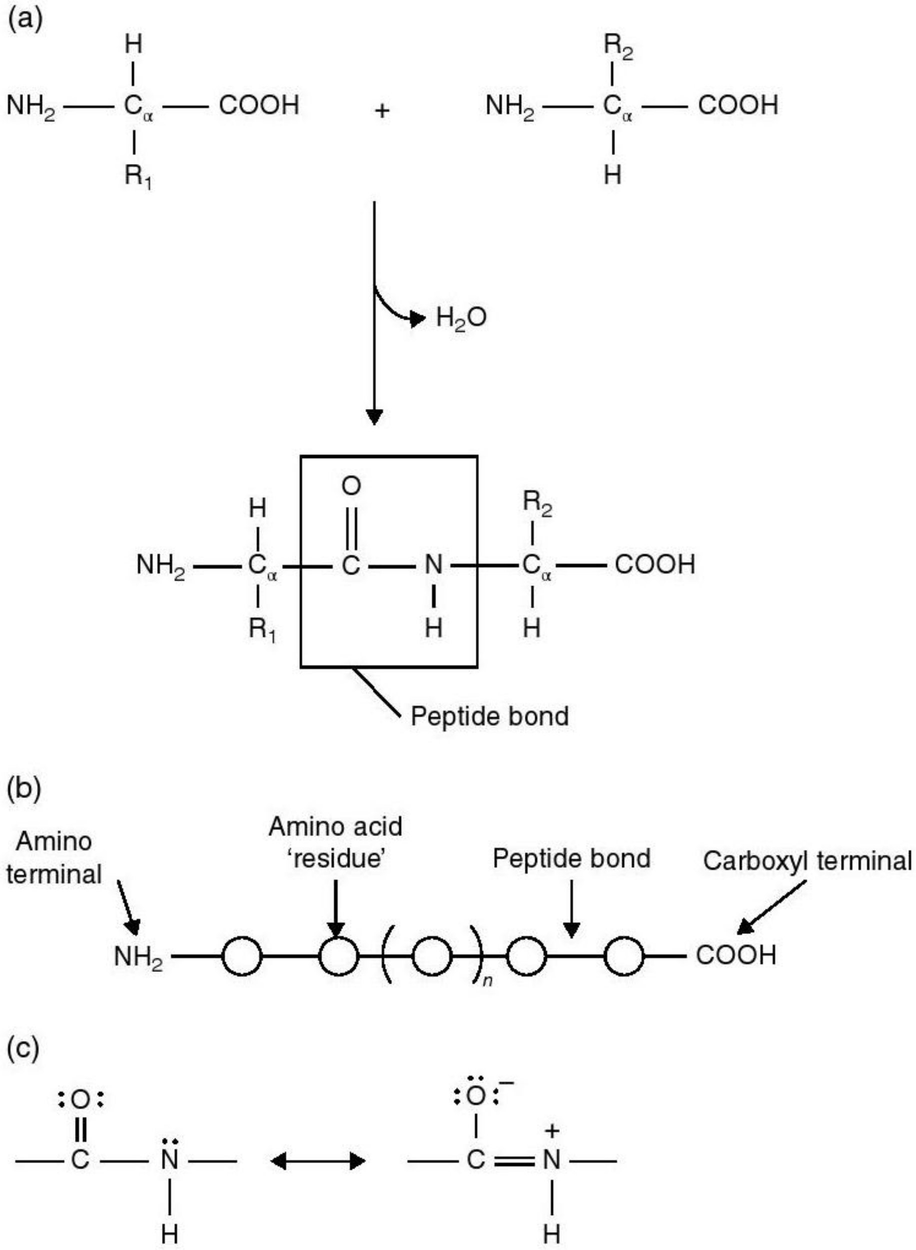


Figure 2.4 (a) Peptide bond formation. (b) Polypeptides consist of a linear chain of amino acids successively linked via peptide bonds. (c) The peptide bond displays partial double-bonded character.

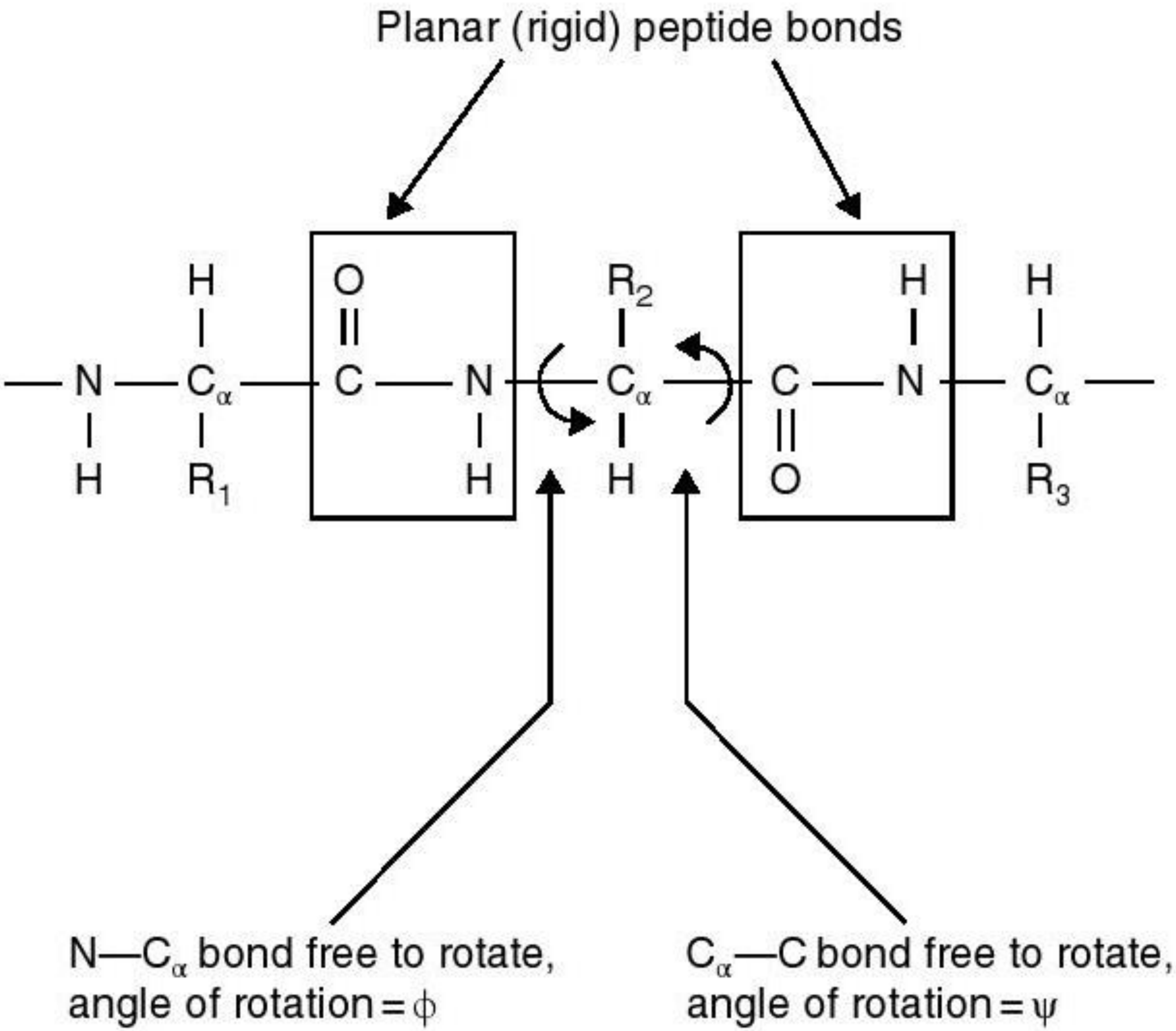

Planar (rigid) peptide bonds
H
N
H
C_α
R_1
O
C
N
H
R_2
C_α
H
C
O
H
N
H
C_α
R_3
N—C_α bond free to rotate, angle of rotation = φ
C_α—C bond free to rotate, angle of rotation = ψ

Figure 2.5 Fragment of polypeptide chain backbone illustrating rigid peptide bonds and the intervening N—Cα and Cα—C backbone linkages, which are free to rotate.

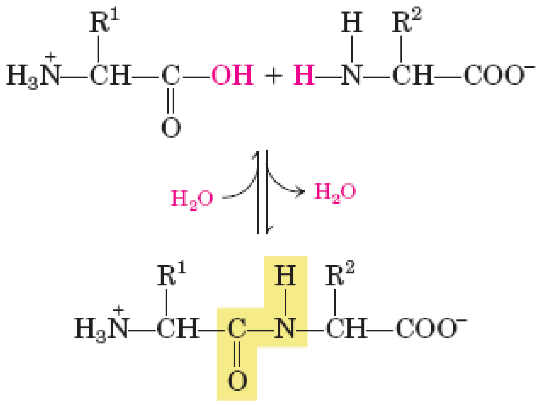


FIGURE 3–13 Formation of a peptide bond by condensation. The α-amino group of one amino acid (with R² group) acts as a nucleophile to displace the hydroxyl group of another amino acid (with R¹ group), forming a peptide bond (shaded in yellow). Amino groups are good nucleophiles, but the hydroxyl group is a poor leaving group and is not readily displaced. At physiological pH, the reaction shown does not occur to any appreciable extent.

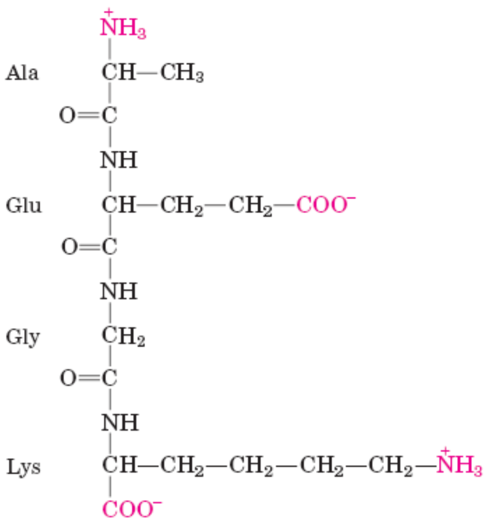


FIGURE 3–15 Alanylglutamylglycyllysine. This tetrapeptide has one free α-amino group, one free α-carboxyl group, and two ionizable R groups. The groups ionized at pH 7.0 are in red.

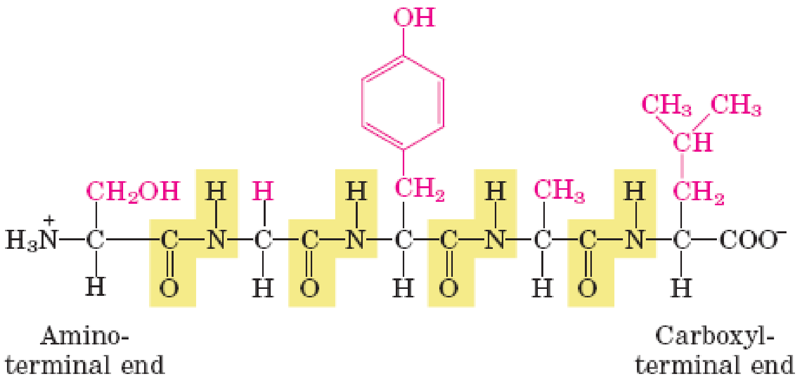


FIGURE 3–14 The pentapeptide serylglycyltyrosylalanylleucine, or Ser–Gly–Tyr–Ala–Leu. Peptides are named beginning with the amino-terminal residue, which by convention is placed at the left. The peptide bonds are shaded in yellow; the R groups are in red.

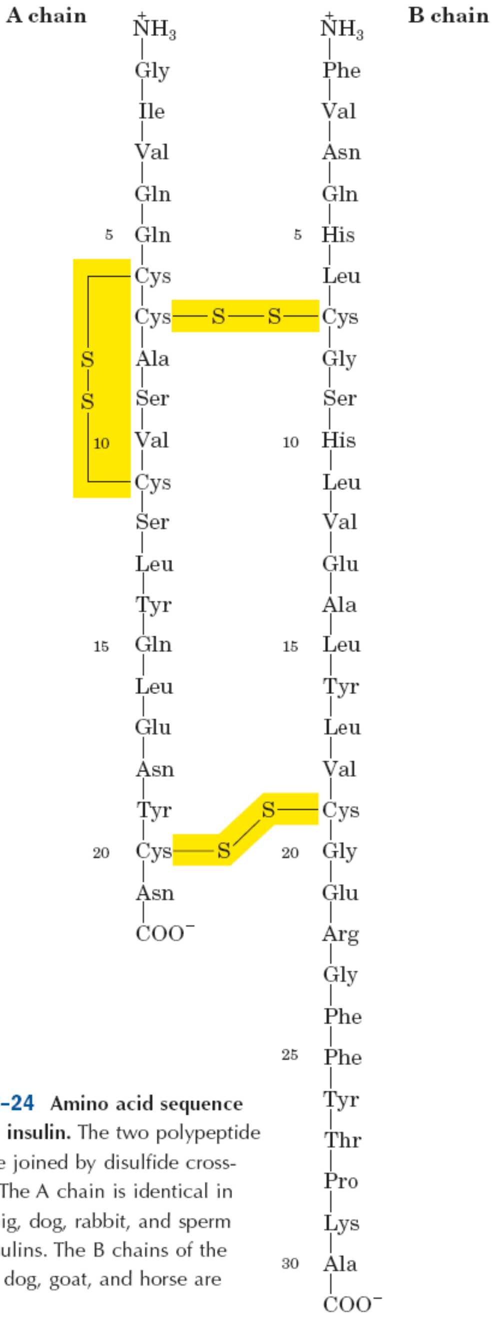


FIGURE 3–24 Amino acid sequence of bovine insulin. The two polypeptide chains are joined by disulfide cross-linkages. The A chain is identical in human, pig, dog, rabbit, and sperm whale insulins. The B chains of the cow, pig, dog, goat, and horse are identical.

Biologically Active Peptides and Polypeptides Occur in a Vast Range of Sizes

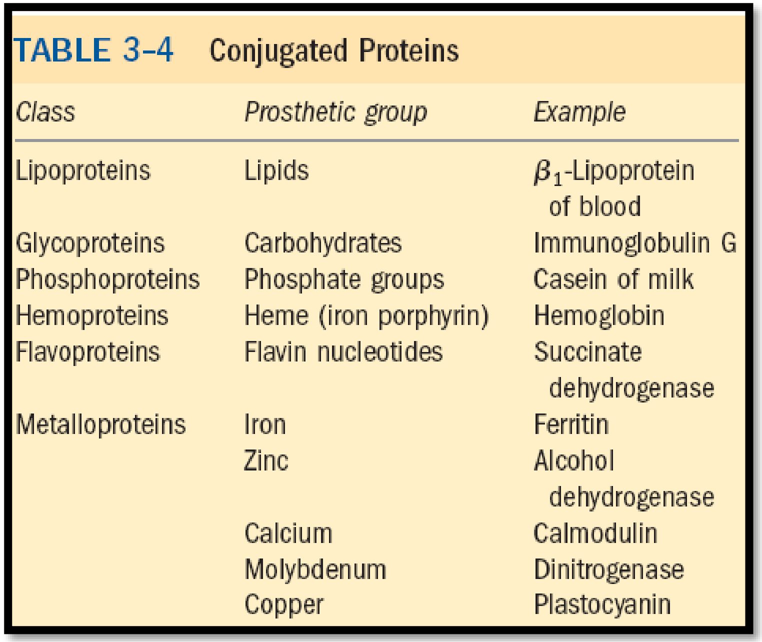
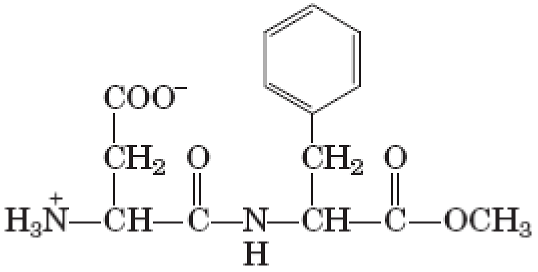


TABLE 3–4 Conjugated Proteins

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



L-Aspartyl-L-phenylalanine methyl ester (aspartame)

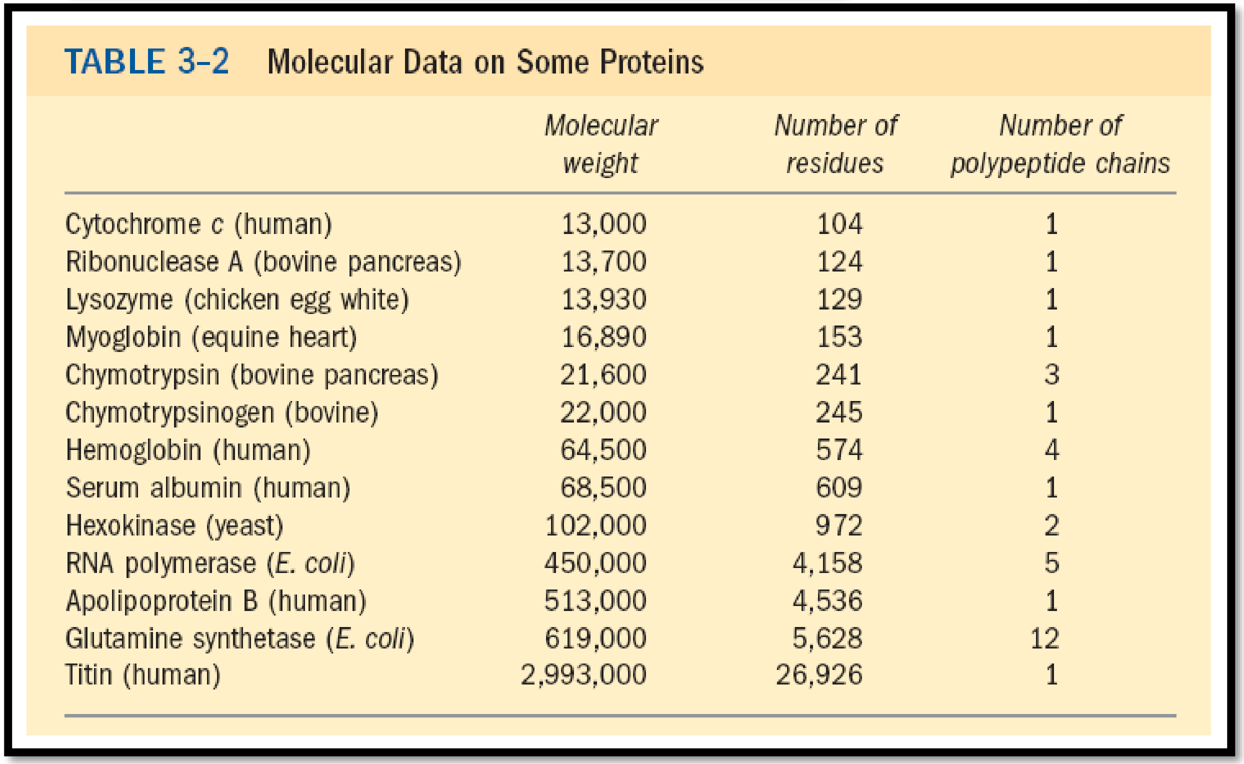


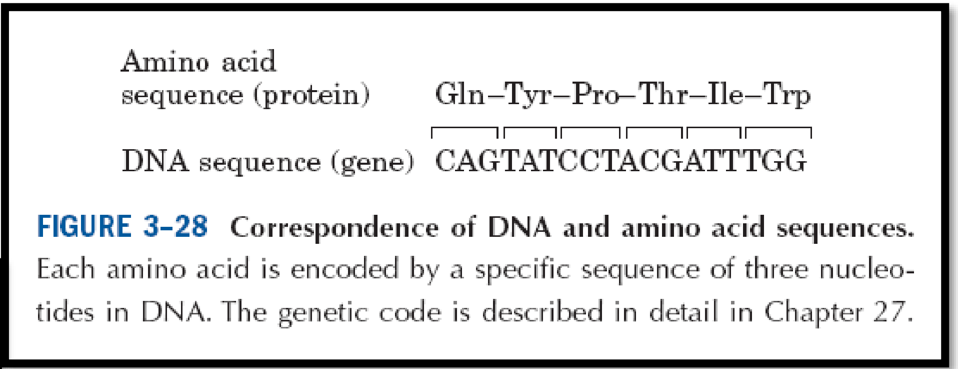
TABLE 3–2 Molecular Data on Some Proteins

	Molecular weight	*Number of residues*	*Number of polypeptide chains*
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 (*E. coli*)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (*E. coli*)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

THE THREE-DIMENSIONAL STRUCTURE OF PROTEINS

The Function of a Protein Depends on Its Amino Acid Sequence

A Protein's Conformation Is Stabilized Largely by Weak Interactions



Amino acid sequence (protein) Gln–Tyr–Pro–Thr–Ile–Trp

DNA sequence (gene) CAGTATCCTACGATTTGG

FIGURE 3–28 Correspondence of DNA and amino acid sequences. Each amino acid is encoded by a specific sequence of three nucleotides in DNA. The genetic code is described in detail in Chapter 27.

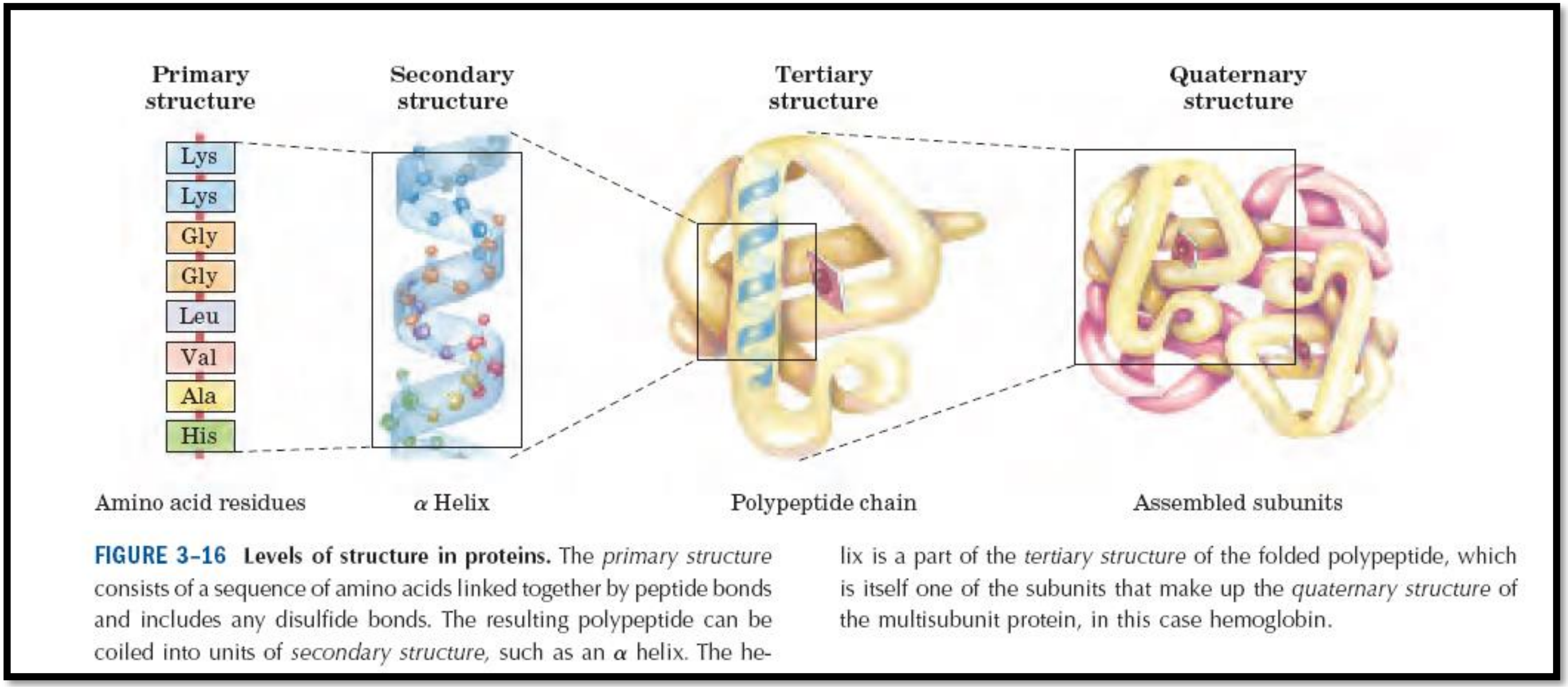
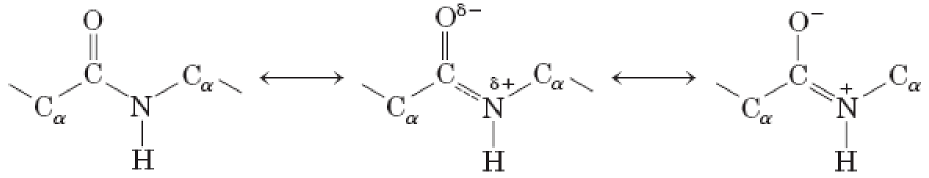
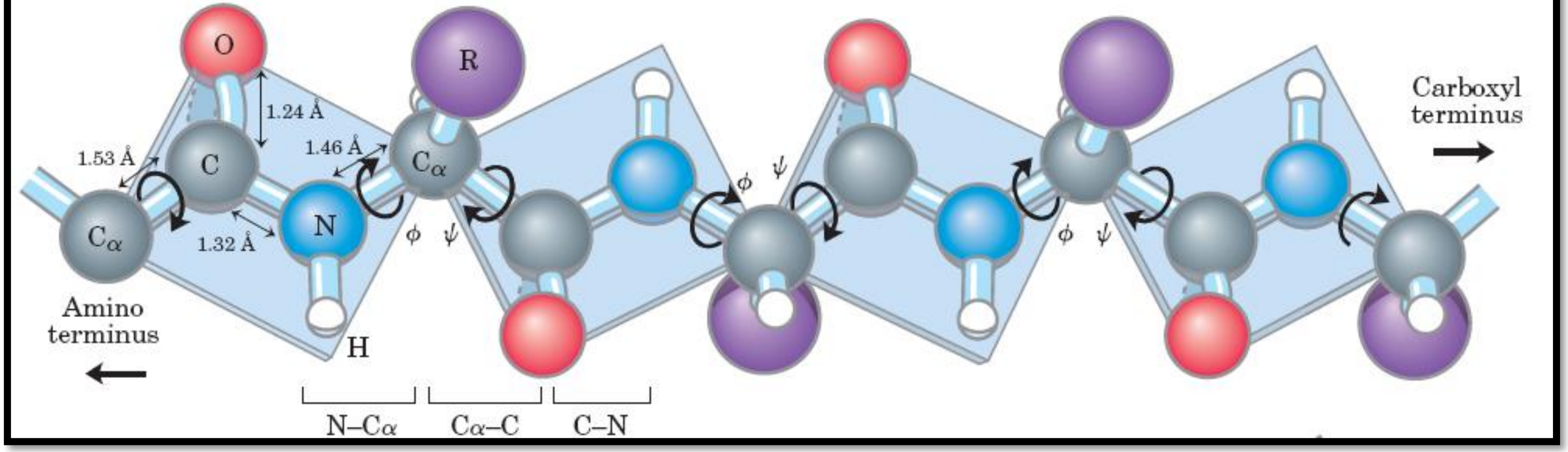


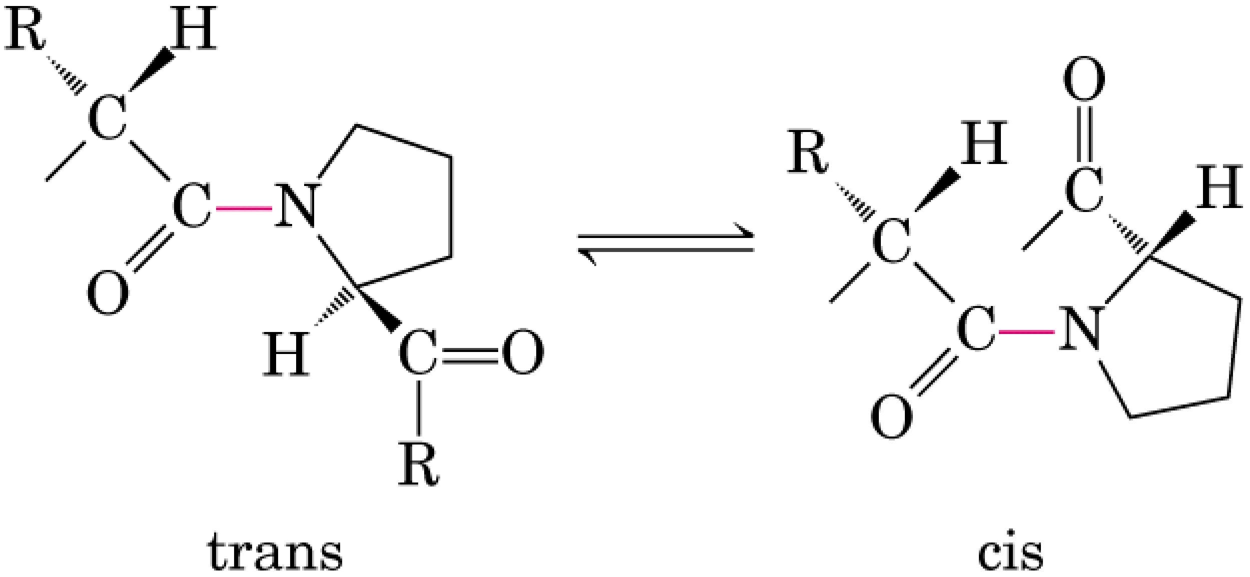
FIGURE 3–16 Levels of structure in proteins. The *primary structure* consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. The resulting polypeptide can be coiled into units of *secondary structure*, such as an α helix. The helix is a part of the *tertiary structure* of the folded polypeptide, which is itself one of the subunits that make up the *quaternary structure* of the multisubunit protein, in this case hemoglobin.

The Peptide Bond Is Rigid



The carbonyl oxygen has a partial negative charge and the amide nitrogen a partial positive charge, setting up a small electric dipole. Virtually all peptide bonds in proteins occur in this trans configuration; an exception is noted in Figure 4–8b.

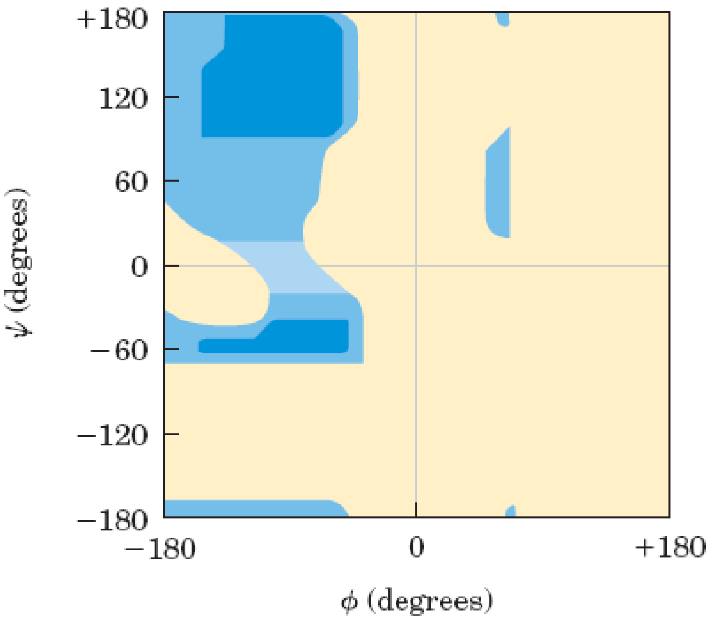


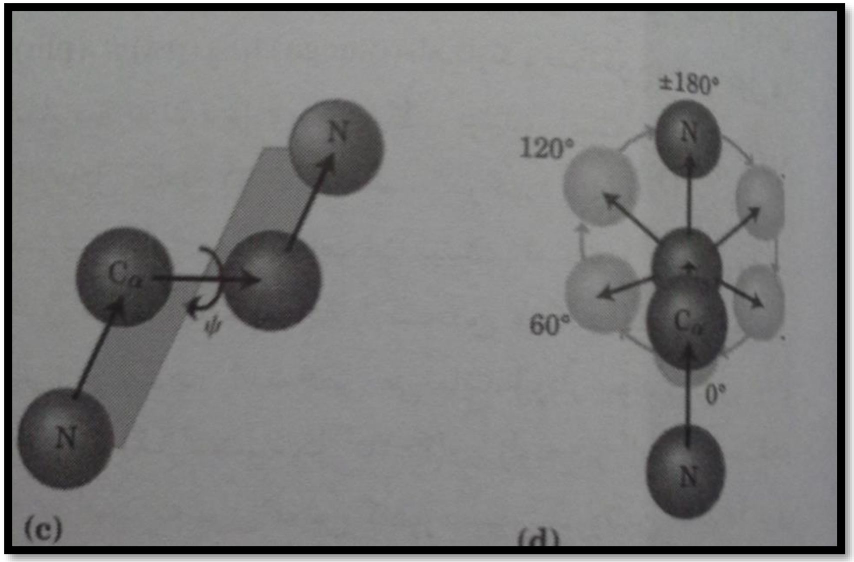


Proline isomers

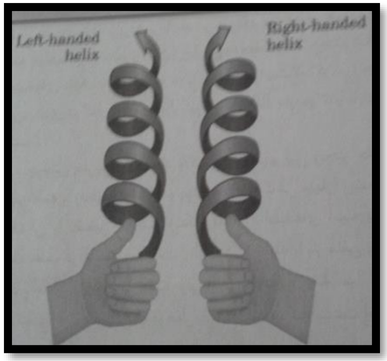
(b)

FIGURE 4–3 Ramachandran plot for L-Ala residues. The conformations of peptides are defined by the values of ϕ and ψ. Conformations deemed possible are those that involve little or no steric interference, based on calculations using known van der Waals radii and bond angles. The areas shaded dark blue reflect conformations that involve no steric overlap and thus are fully allowed; medium blue indicates conformations allowed at the extreme limits for unfavorable atomic contacts; the lightest blue area reflects conformations that are permissible if a little flexibility is allowed in the bond angles. The asymmetry of the plot results from the L stereochemistry of the amino acid residues. The plots for other L-amino acid residues with unbranched side chains are nearly identical. The allowed ranges for branched amino acid residues such as Val, Ile, and Thr are somewhat smaller than for Ala. The Gly residue, which is less sterically hindered, exhibits a much broader range of allowed conformations. The range for Pro residues is greatly restricted because ϕ is limited by the cyclic side chain to the range of −35° to −85°.





The α Helix Is a Common Protein Secondary Structure



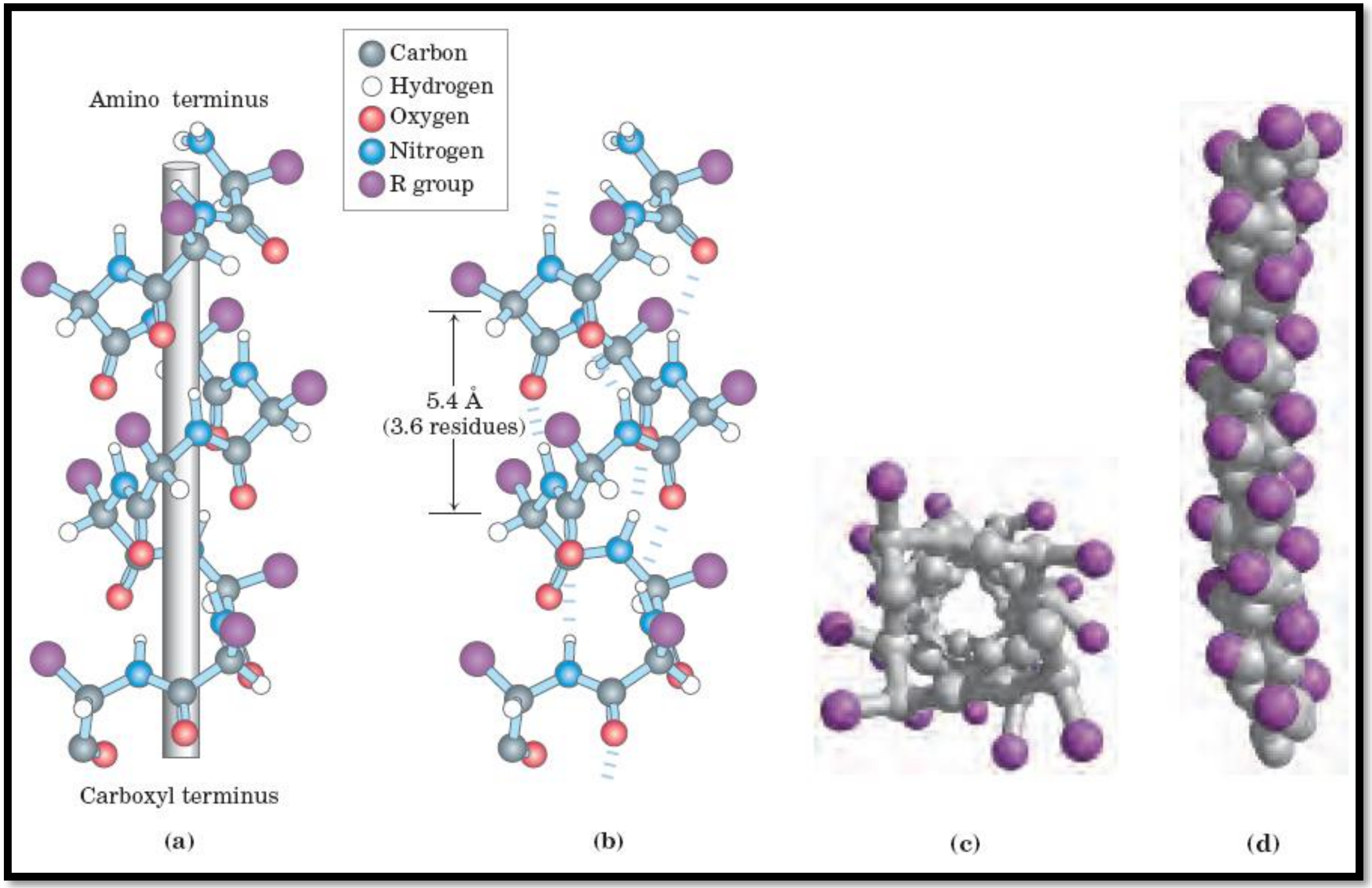
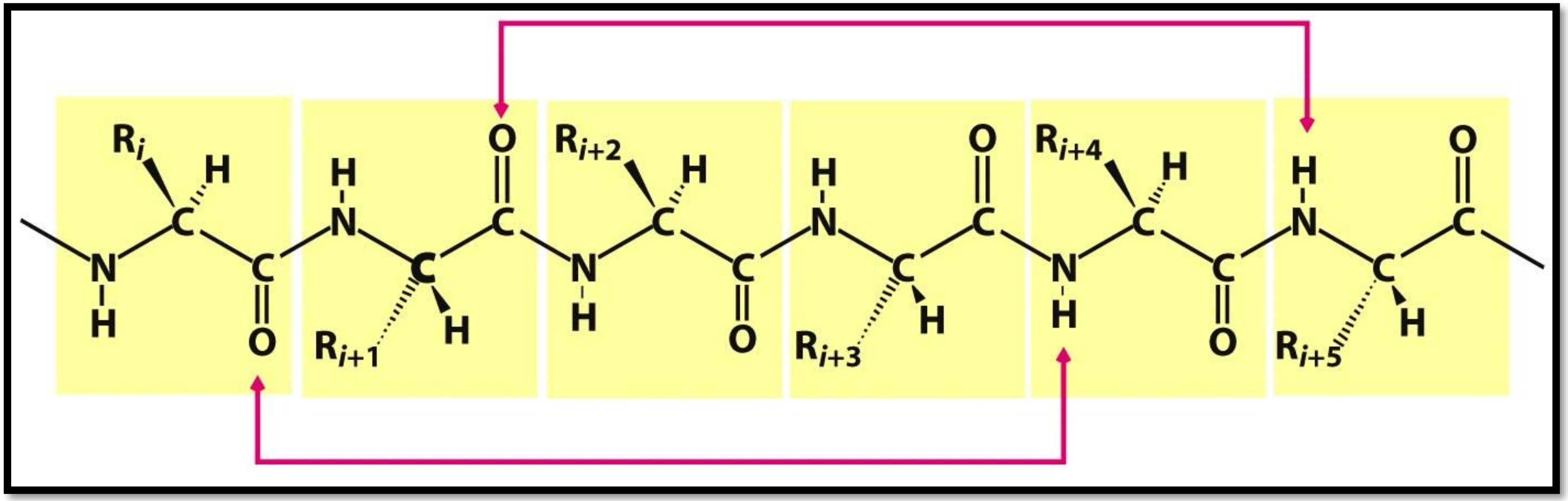
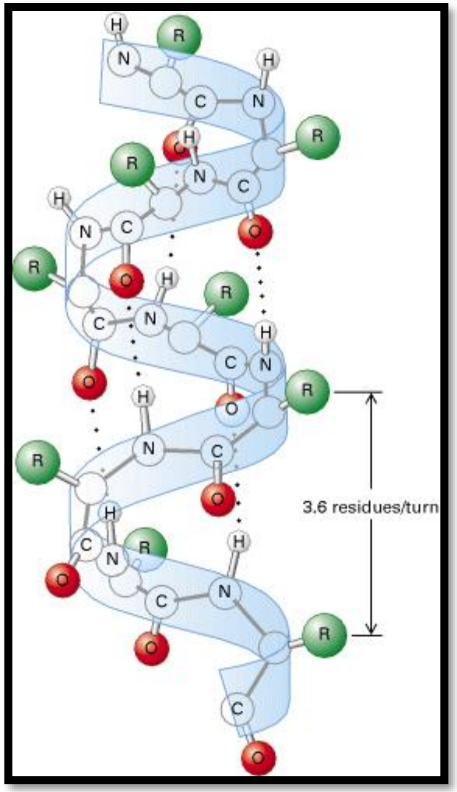
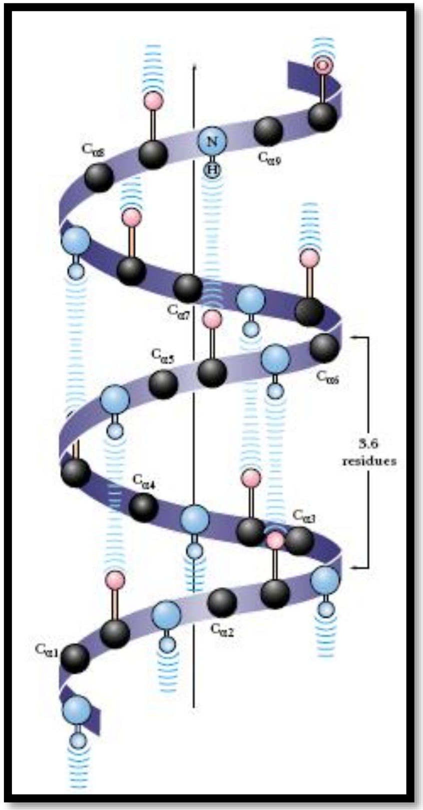


FIGURE 4–4 Four models of the α helix, showing different aspects of its structure. **(a)** Formation of a right-handed α helix. The planes of the rigid peptide bonds are parallel to the long axis of the helix, depicted here as a vertical rod. **(b)** Ball-and-stick model of a right-handed α helix, showing the intrachain hydrogen bonds. The repeat unit is a single turn of the helix, 3.6 residues. **(c)** The α helix as viewed from one end, looking down the longitudinal axis (derived from PDB ID 4TNC). Note the positions of the R groups, represented by purple spheres. This ball-and-stick model, used to emphasize the helical arrangement, gives the false impression that the helix is hollow, because the balls do not represent the van der Waals radii of the individual atoms. As the space-filling model **(d)** shows, the atoms in the center of the α helix are in very close contact.


R_i
H
N
C
O
R_{i+1}
R_{i+2}
R_{i+3}
R_{i+4}
R_{i+5}


H
N
C
O
R
3.6 residues/turn


Cα1
Cα2
Cα3
Cα4
Cα5
Cα6
Cα7
Cα8
Cα9
N
H
3.6
residues

The β Conformation Organizes Polypeptide Chains into Sheets

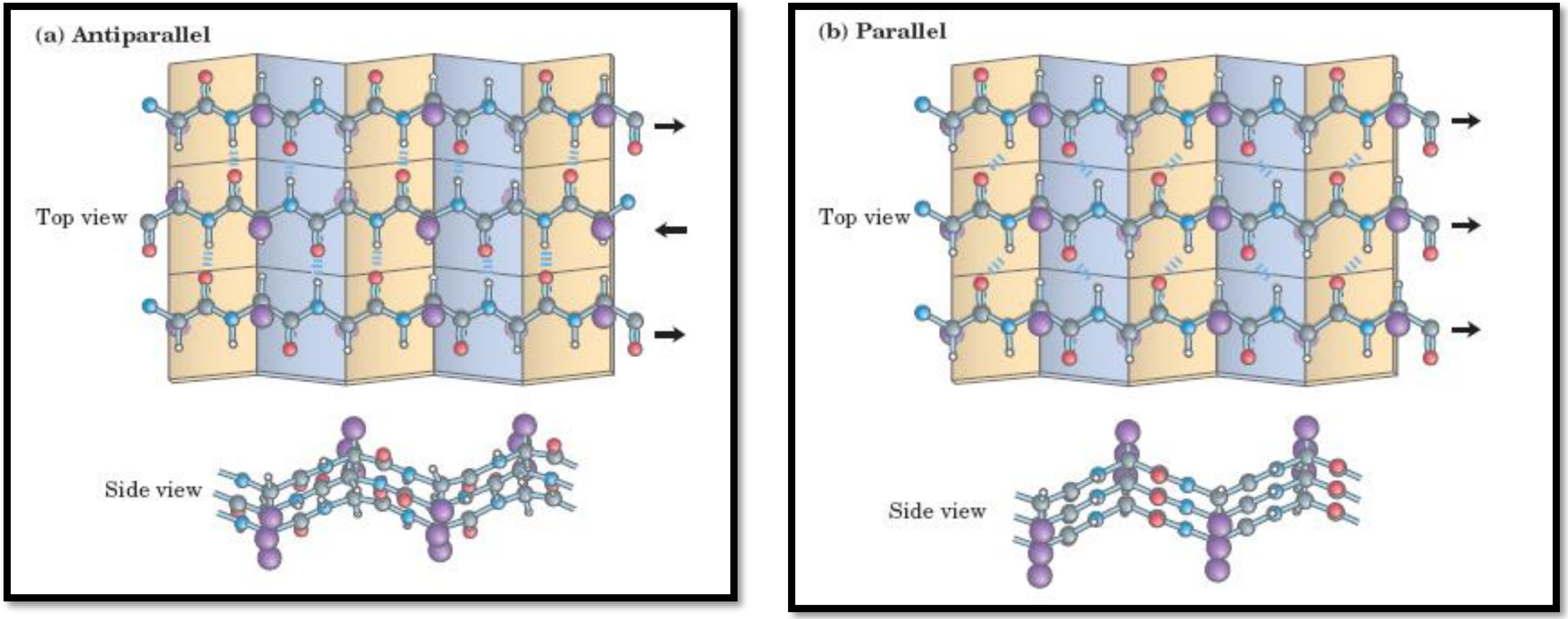
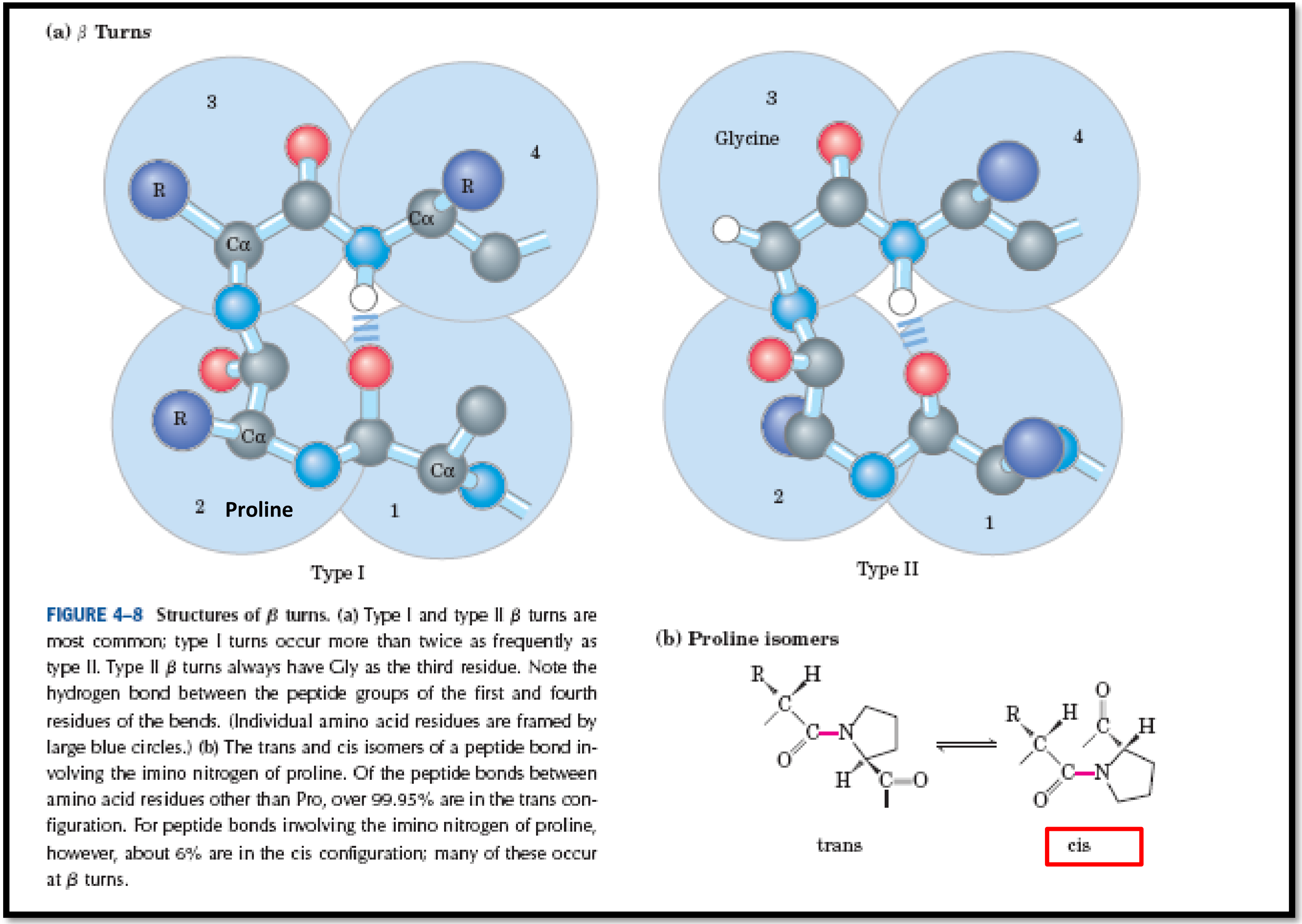


FIGURE 4–7 **The β conformation of polypeptide chains.** These top and side views reveal the R groups extending out from the β sheet and emphasize the pleated shape described by the planes of the peptide bonds. (An alternative name for this structure is β-pleated sheet.) Hydrogen-bond cross-links between adjacent chains are also shown. (a) Antiparallel β sheet, in which the amino-terminal to carboxyl-terminal orientation of adjacent chains (arrows) is inverse. (b) Parallel β sheet.

β Turns Are Common in Proteins



(a) β Turns

FIGURE 4–8 Structures of β turns. (a) Type I and type II β turns are most common; type I turns occur more than twice as frequently as type II. Type II β turns always have Gly as the third residue. Note the hydrogen bond between the peptide groups of the first and fourth residues of the bends. (Individual amino acid residues are framed by large blue circles.) (b) The trans and cis isomers of a peptide bond involving the imino nitrogen of proline. Of the peptide bonds between amino acid residues other than Pro, over 99.95% are in the trans configuration. For peptide bonds involving the imino nitrogen of proline, however, about 6% are in the cis configuration; many of these occur at β turns.

(b) Proline isomers

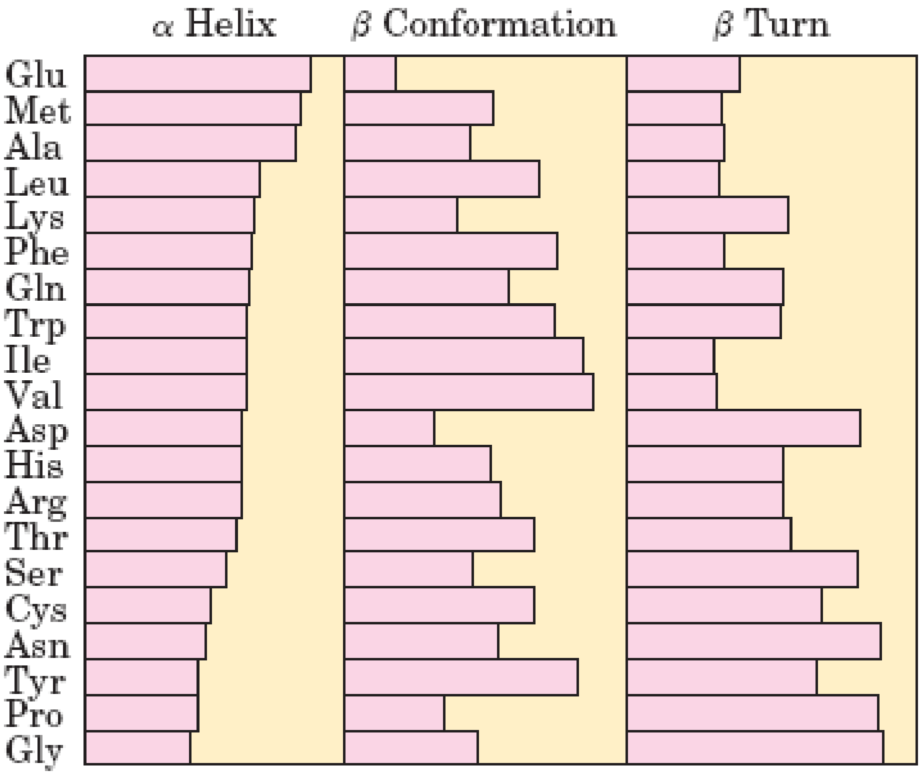


FIGURE 4–10 Relative probabilities that a given amino acid will occur in the three common types of secondary structure.

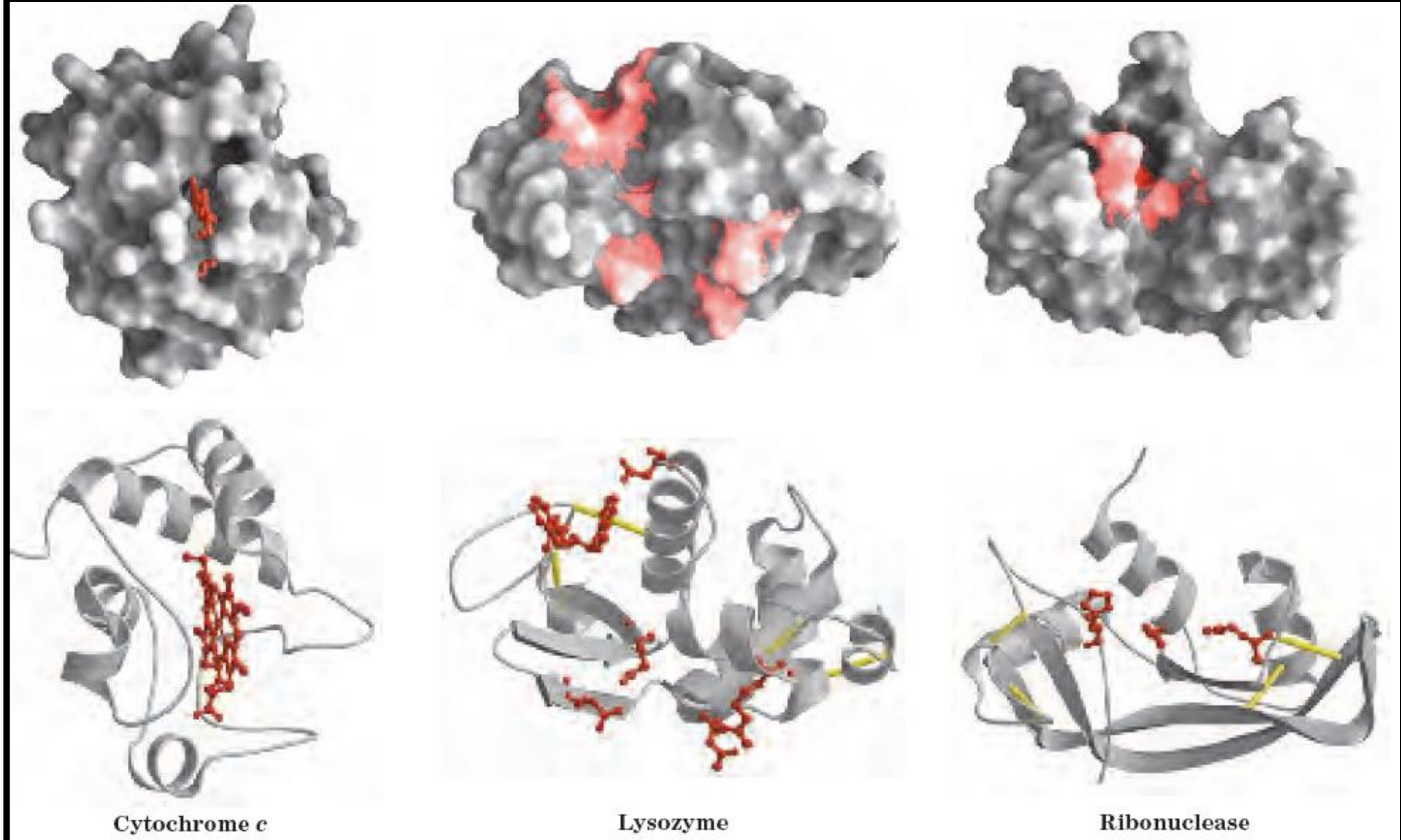
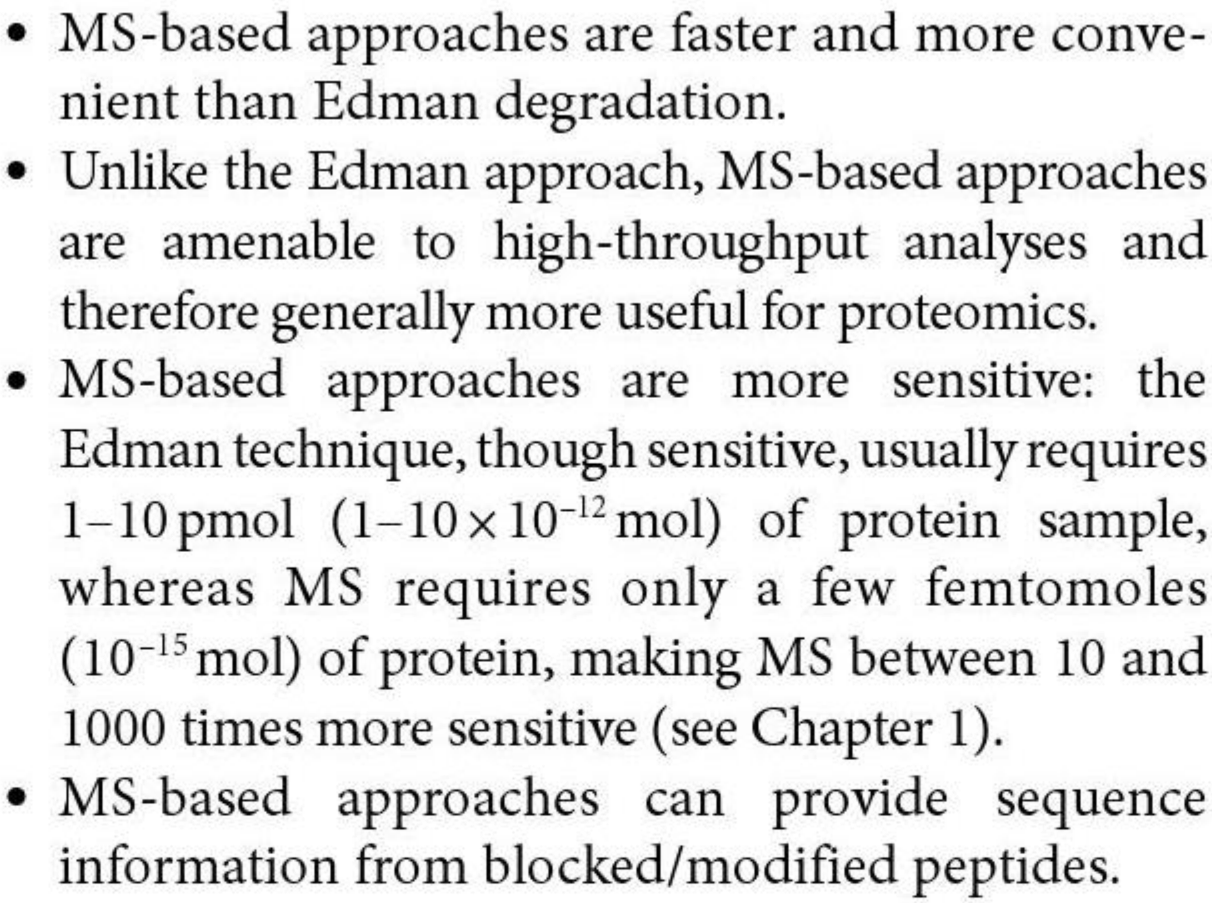


FIGURE 4–18 Three-dimensional structures of some small proteins. Shown here are cytochrome *c* (PDB ID 1CCR), lysozyme (PDB ID 3LYM), and ribonuclease (PDB ID 3RN3). Each protein is shown in surface contour and in a ribbon representation, in the same orientation. In the ribbon depictions, regions in the β conformation are represented by flat arrows and the α helices are represented by spiral ribbons. Key functional groups (the heme in cytochrome *c;* amino acid side chains in the active site of lysozyme and ribonuclease) are shown in red. Disulfide bonds are shown (in the ribbon representations) in yellow.

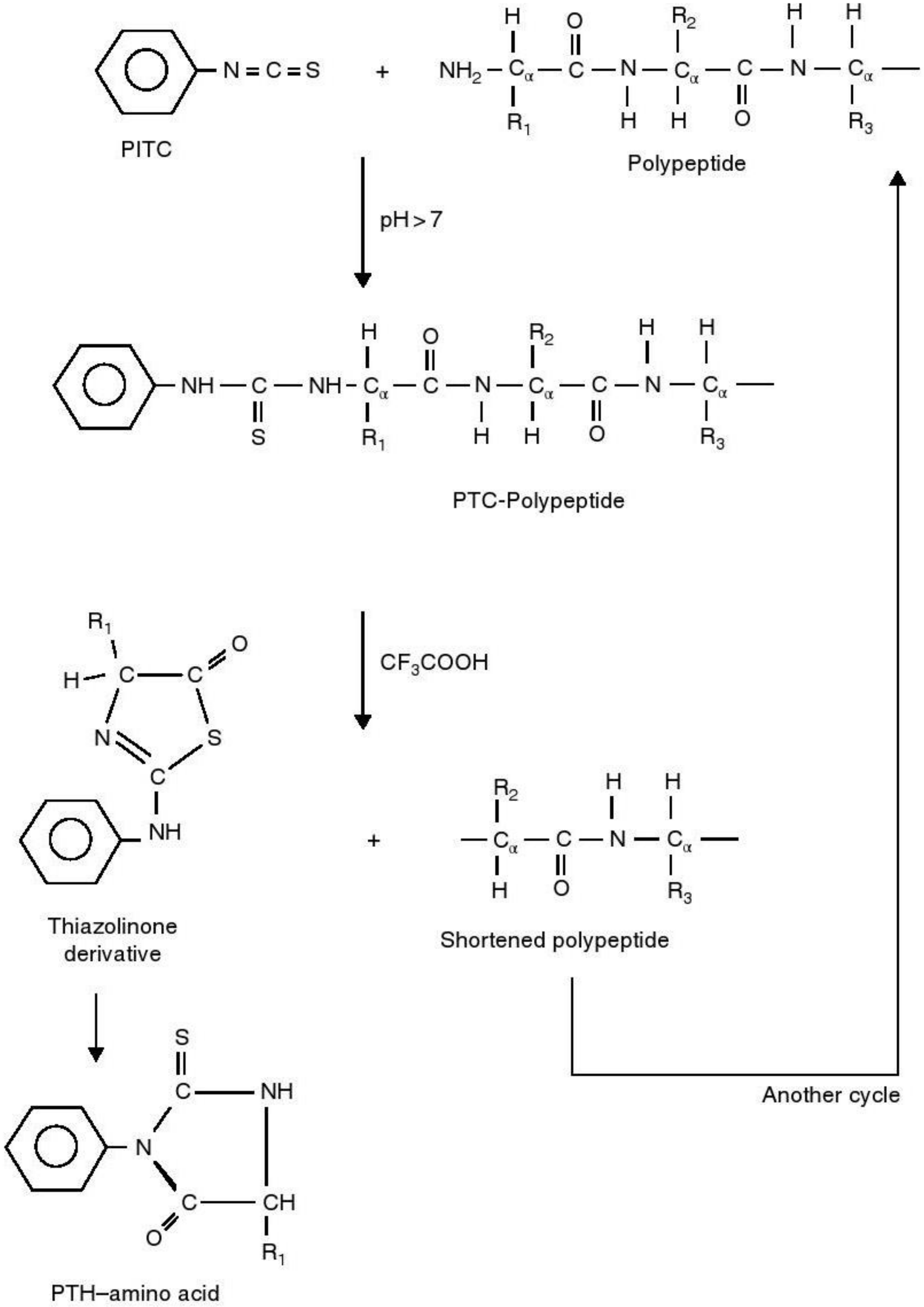
Amino Acid Sequence Determination

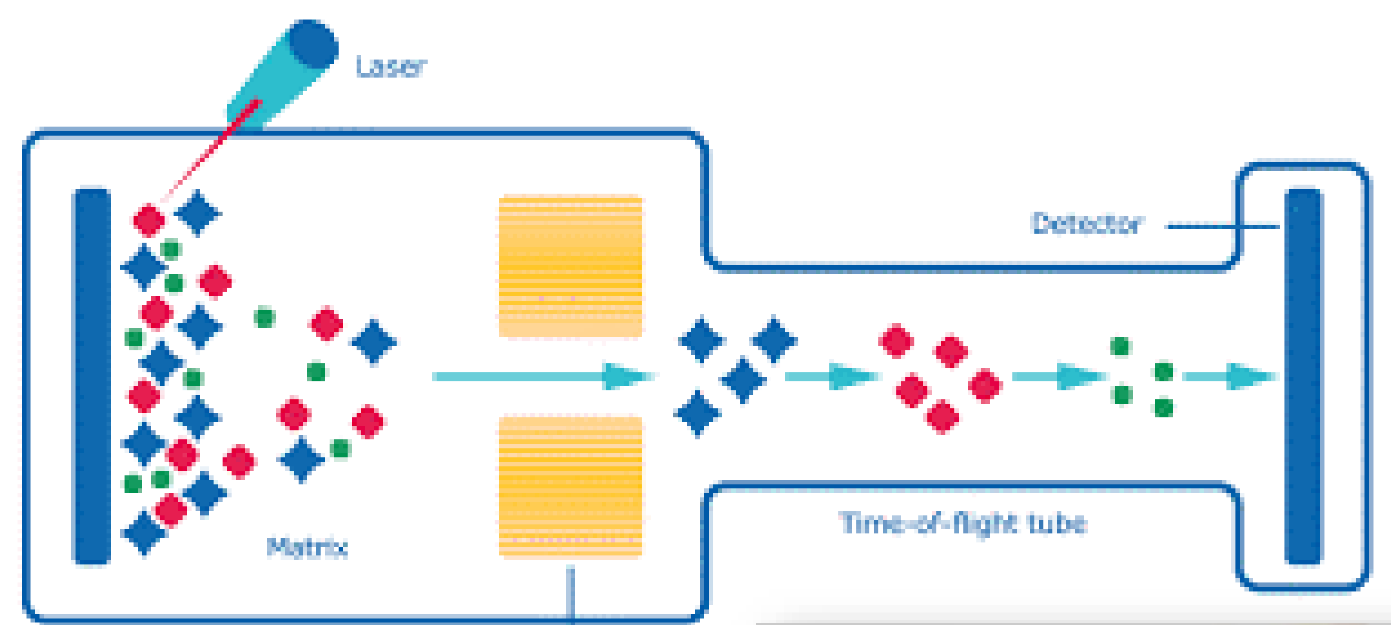
❑ **Edman degradation**

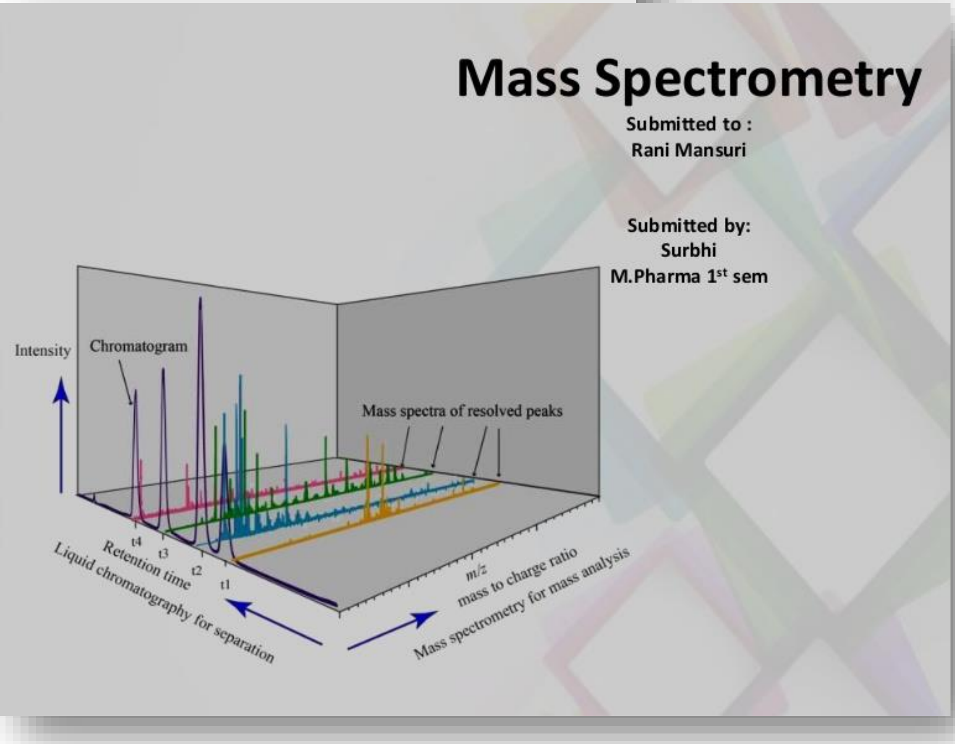
❑ **MS (Mass Spectrometry)**



- MS-based approaches are faster and more convenient than Edman degradation.
- Unlike the Edman approach, MS-based approaches are amenable to high-throughput analyses and therefore generally more useful for proteomics.
- MS-based approaches are more sensitive: the Edman technique, though sensitive, usually requires 1–10 pmol ($1–10 \times 10^{-12}$ mol) of protein sample, whereas MS requires only a few femtomoles (10^{-15} mol) of protein, making MS between 10 and 1000 times more sensitive (see Chapter 1).
- MS-based approaches can provide sequence information from blocked/modified peptides.


PITC
Polypeptide
pH > 7
PTC-Polypeptide
CF₃COOH
Thiazolinone derivative
Shortened polypeptide
Another cycle
PTH–amino acid





Mass Spectrometry

Submitted to :
Rani Mansuri

Submitted by:
Surbhi
M.Pharma 1st sem

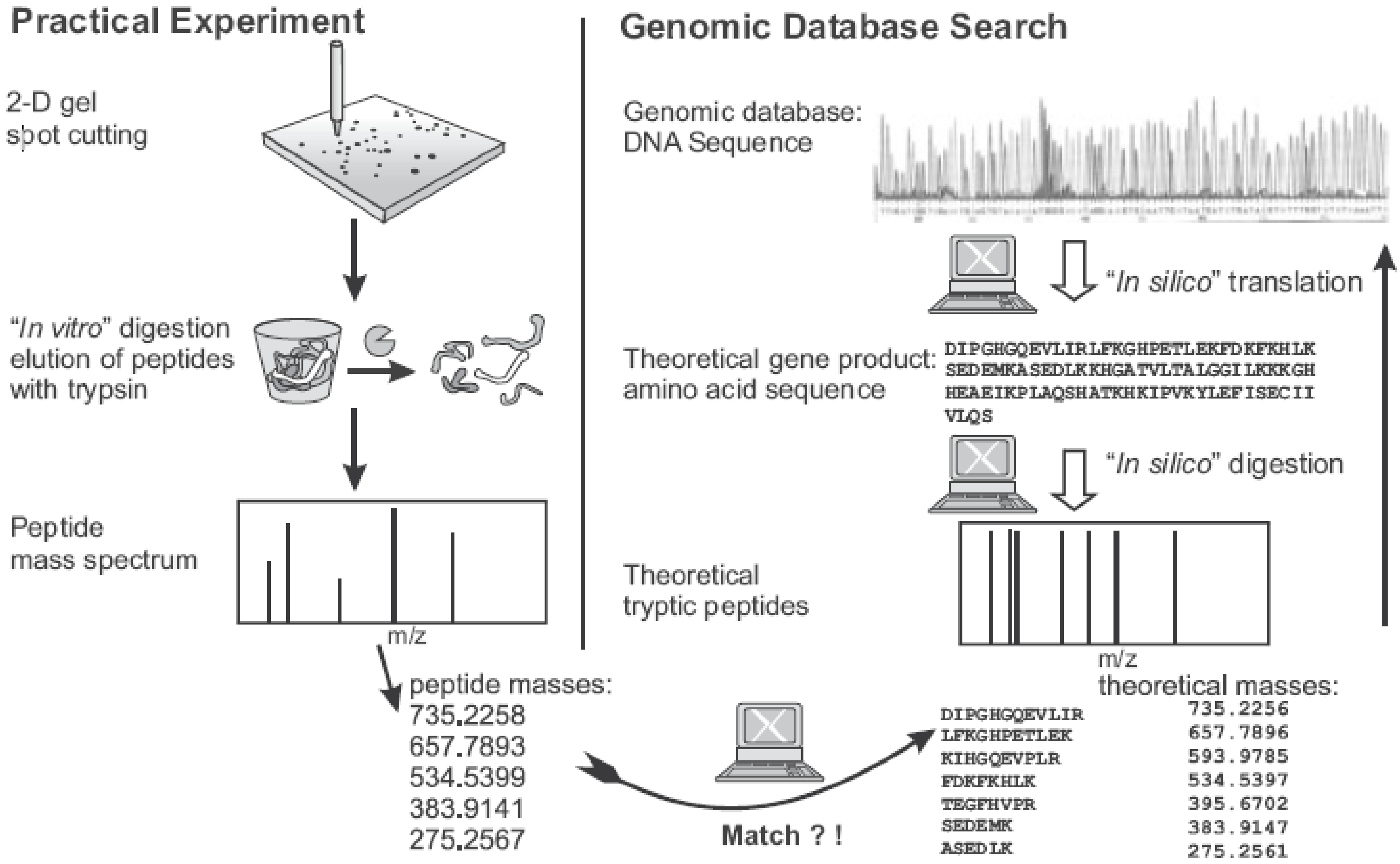
Intensity
Chromatogram
Mass spectra of resolved peaks
t4
t3
t2
t1
Retention time
Liquid chromatography for separation
m/z
mass to charge ratio
Mass spectrometry for mass analysis

Pappin DJ, Hojrup P, Bleasby JA. Curr biol 3 (1993) 327–332.
The techniques and its background will be described in detail in the method chapters.

Peptide mass fingerprinting The easiest and fastest way to identify proteins is shown in figure 2: peptide mass fingerprinting (PMF), which was introduced by four independent groups, including Pappin *et al*. (1993). The gel plug containing the protein of interest is cut out of the gel slab, the protein is digested inside the gel plug with a proteolytic enzyme, mostly trypsin. The cleavage products, the peptides, are eluted from the plug and submitted to mass spectrometry analysis. Mostly MALDI ToF instruments are employed, because they are easier to handle than electrospray systems. The mass spectrum with the accurately measured peptide masses is matched with theoretical peptide spectra in various databases using adequate bioinformatics tools. When no match is found in peptide and protein databases, genomic databases can be searched. The DNA sequence in the open reading frames can be theoretically translated into the amino acid sequence we have to remove this because it is not very practical to search DNA with MALDI data, it is not specific enough. You can do it easily with MS/MS though. Since the cleavage sites of trypsin are known, theoretical tryptic peptide masses can be generated and compared with the experimentally determined masses. If a sufficient number of experimental peptide masses match with the theoretical peptides within a protein, then protein identification with high confidence can be achieved.

This procedure works very well for protein identification. However, the method can be compromised for a number of reasons. In these circumstances, more specific information is needed for unambiguous protein identification, specifically peptide sequence information.

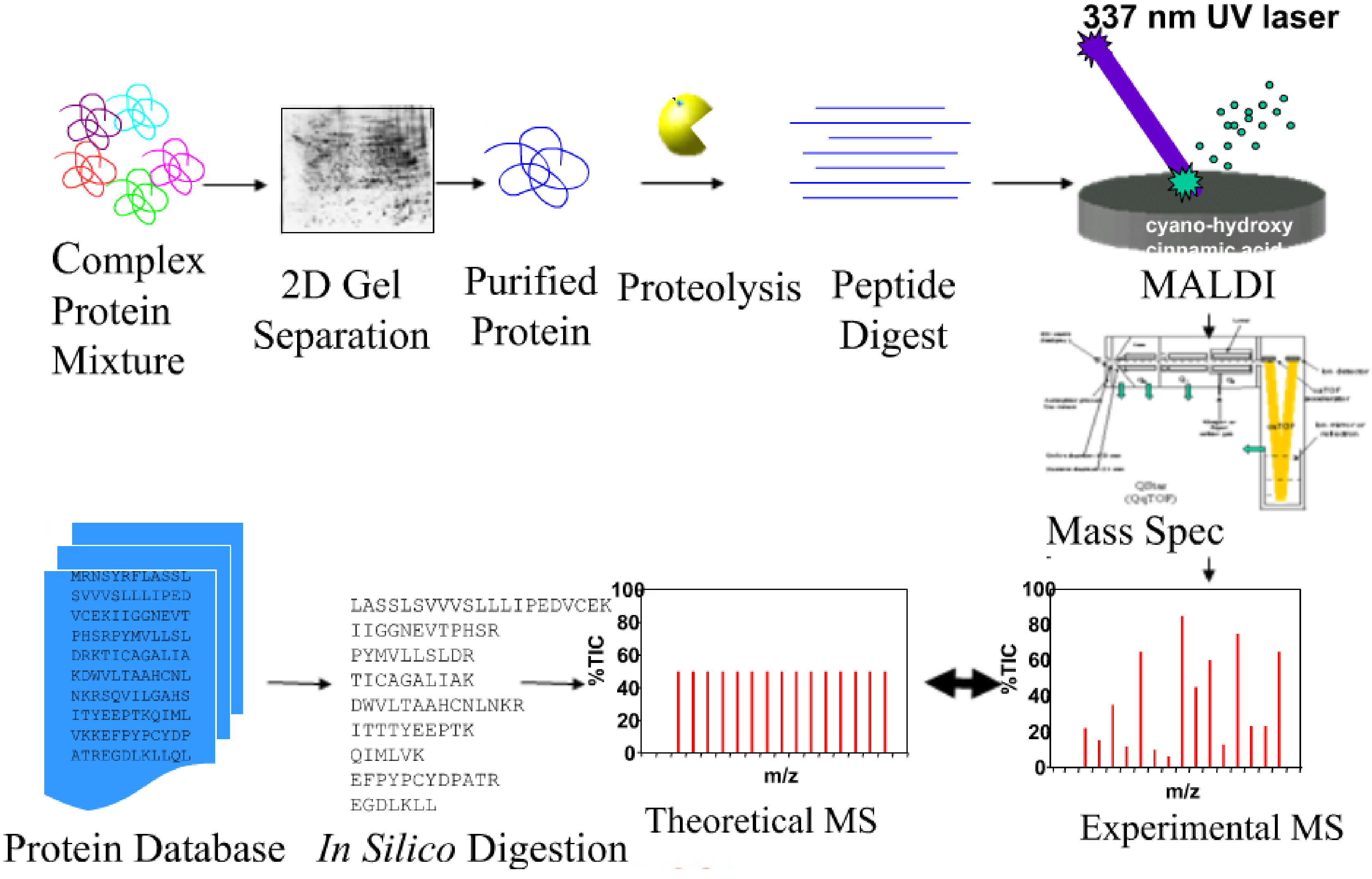
Peptide Mass Fingerprinting (PMF)



DIPGHGQEVLIR	735.2256
LFKGHPETLEK	657.7896
KIHGQEVPLR	593.9785
FDKFKHLK	534.5397
TEGFHVPR	395.6702
SEDEMK	383.9147
ASEDLK	275.2561

Fig. 2: Protein identification with peptide mass fingerprinting. The peptide masses of the digested protein are matched with a list of theoretical masses of peptides, which are mathematically derived from the open reading frames of the genome database of a certain organism.

Review: Peptide Mass Fingerprinting



337 nm UV laser

cyano-hydroxy cinnamic acid

Complex Protein Mixture → 2D Gel Separation → Purified Protein → Proteolysis → Peptide Digest → MALDI

Mass Spec

Protein Database

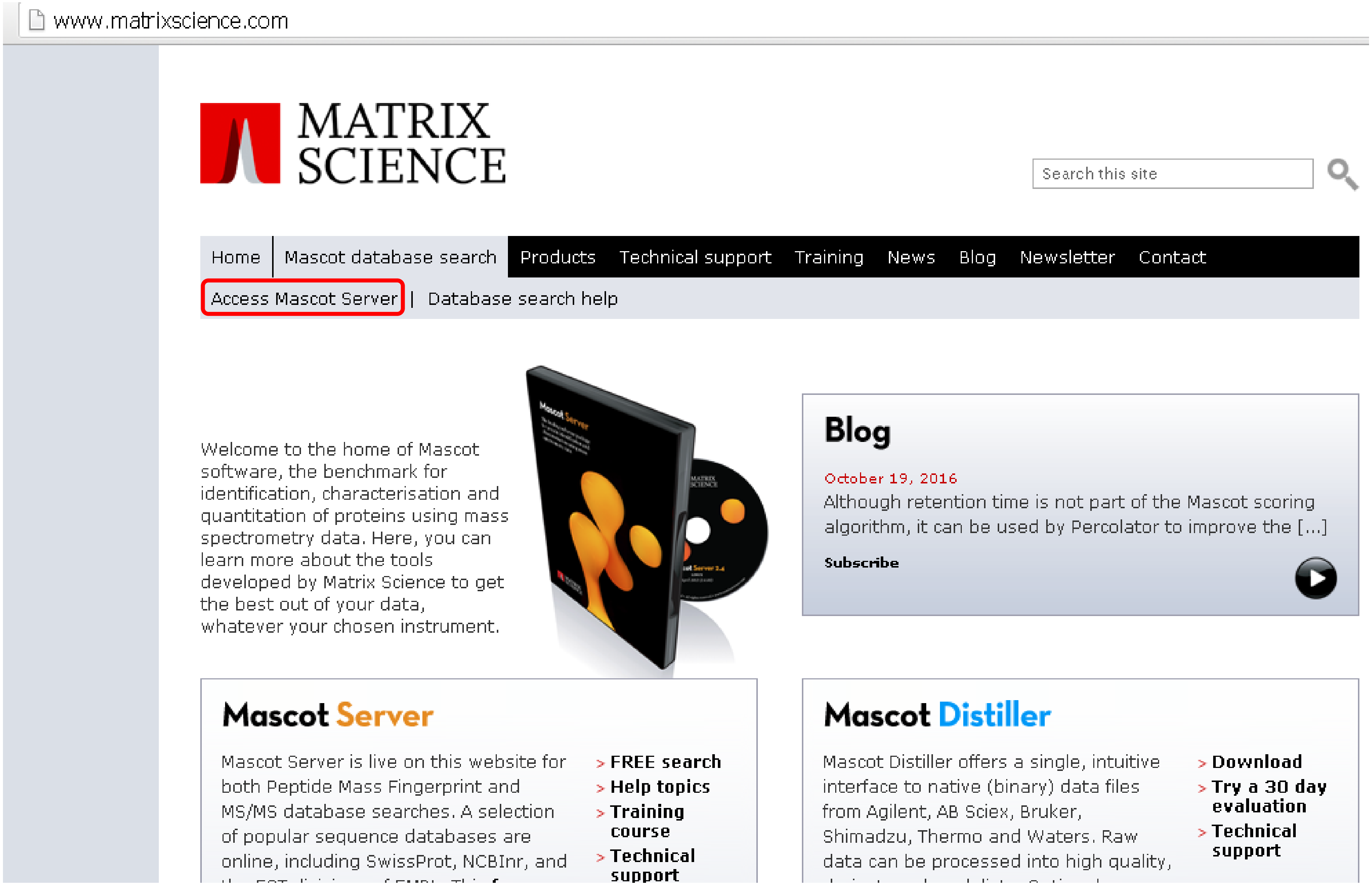
```
MRNSYRFLASSL
SVVVSLLLIPED
VCEKIIGGNEVT
PHSRPYMVLLSL
DRKTICAGALIA
KDWVLTAAHCNL
NKRSQVILGAHS
ITYEEPTKQIML
VKKEFPYPCYDP
ATREGDLKLLQL
```

In Silico Digestion

```
LASSLSVVVSLLLIPEDVCEK
IIGGNEVTPHSR
PYMVLLSLDR
TICAGALIAK
DWVLTAAHCNLNKR
ITTTYEEPTK
QIMLVK
EFPYPCYDPATR
EGDLKLL
```

Theoretical MS

Experimental MS



Welcome to the home of Mascot software, the benchmark for identification, characterisation and quantitation of proteins using mass spectrometry data. Here, you can learn more about the tools developed by Matrix Science to get the best out of your data, whatever your chosen instrument.

Blog

October 19, 2016

Although retention time is not part of the Mascot scoring algorithm, it can be used by Percolator to improve the [...]

Subscribe

Mascot Server

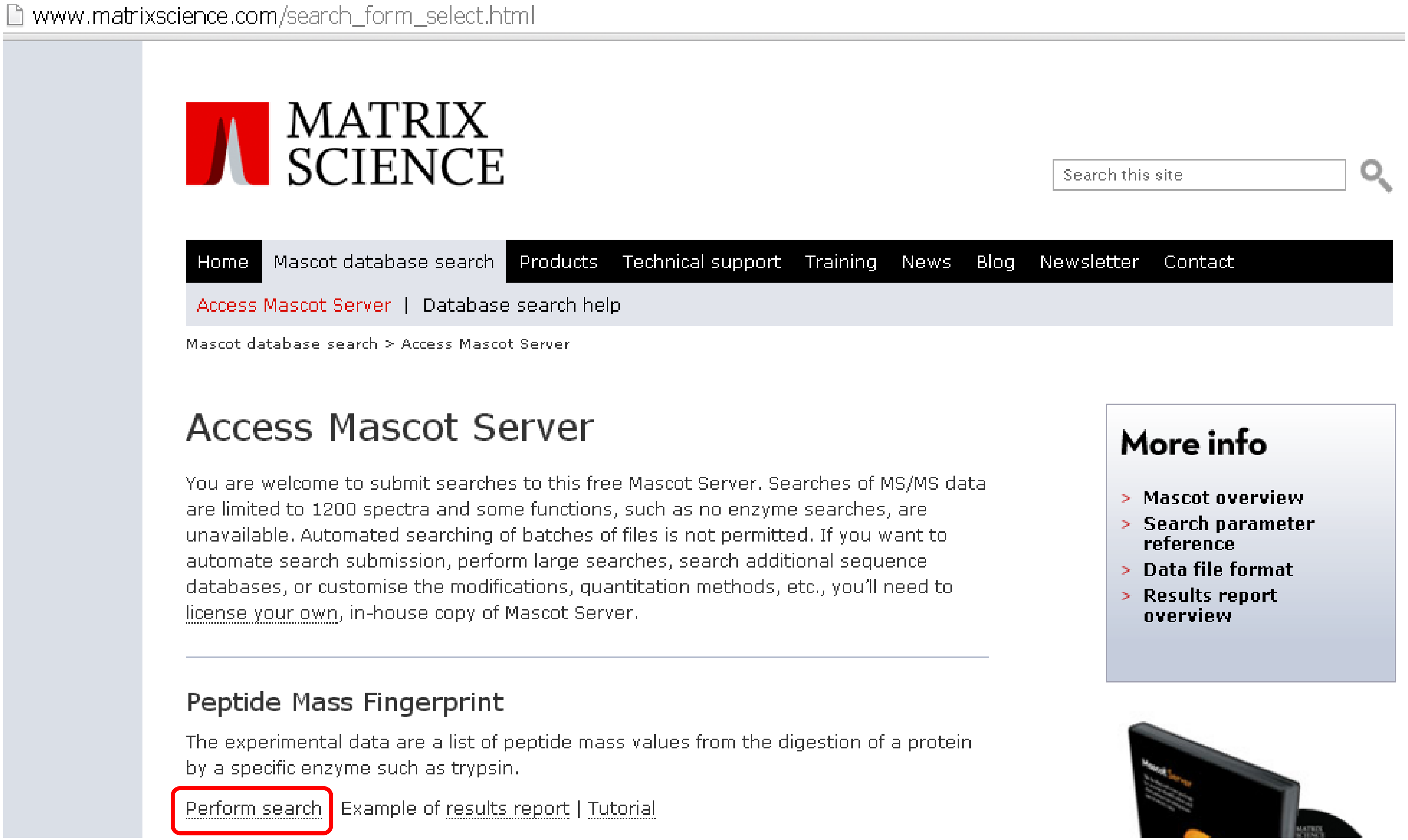
Mascot Server is live on this website for both Peptide Mass Fingerprint and MS/MS database searches. A selection of popular sequence databases are online, including SwissProt, NCBInr, and

- > **FREE search**
- > **Help topics**
- > **Training course**
- > **Technical support**

Mascot Distiller

Mascot Distiller offers a single, intuitive interface to native (binary) data files from Agilent, AB Sciex, Bruker, Shimadzu, Thermo and Waters. Raw data can be processed into high quality,

- > **Download**
- > **Try a 30 day evaluation**
- > **Technical support**


www.matrixscience.com/search_form_select.html
MATRIX SCIENCE
Search this site
Home
Mascot database search
Products
Technical support
Training
News
Blog
Newsletter
Contact
Access Mascot Server | Database search help
Mascot database search > Access Mascot Server
More info
> Mascot overview
> Search parameter reference
> Data file format
> Results report overview

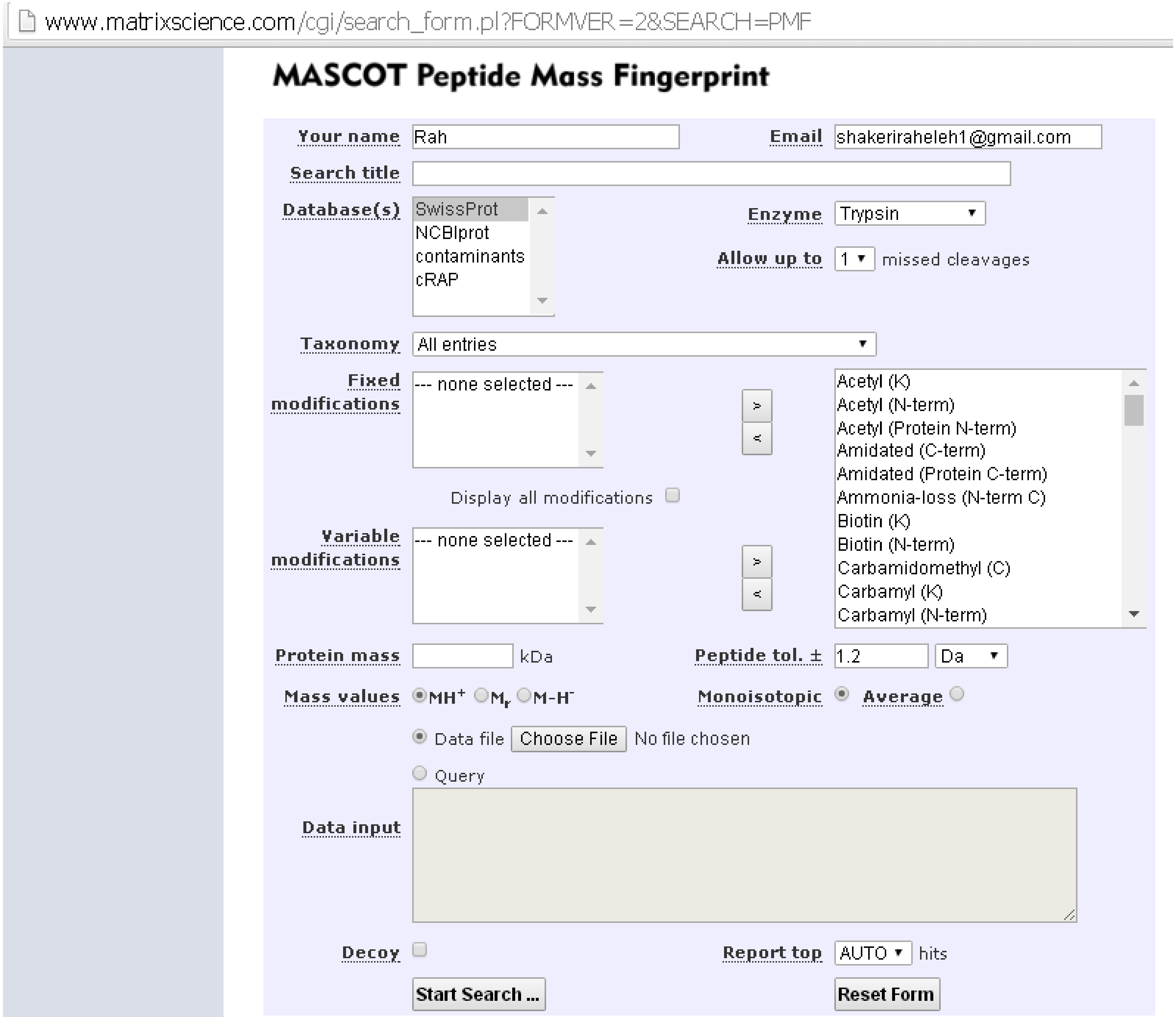
Access Mascot Server

You are welcome to submit searches to this free Mascot Server. Searches of MS/MS data are limited to 1200 spectra and some functions, such as no enzyme searches, are unavailable. Automated searching of batches of files is not permitted. If you want to automate search submission, perform large searches, search additional sequence databases, or customise the modifications, quantitation methods, etc., you'll need to license your own, in-house copy of Mascot Server.

Peptide Mass Fingerprint

The experimental data are a list of peptide mass values from the digestion of a protein by a specific enzyme such as trypsin.

Perform search Example of results report | Tutorial



www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=PMF

MASCOT Peptide Mass Fingerprint

Your name: Rah

Email: shakeriraheleh1@gmail.com

Search title:

Database(s): SwissProt (selected), NCBIprot, contaminants, cRAP

Enzyme: Trypsin

Allow up to 1 missed cleavages

Taxonomy: All entries

Fixed modifications: --- none selected ---

Display all modifications □

Variable modifications: --- none selected ---

Acetyl (K)
Acetyl (N-term)
Acetyl (Protein N-term)
Amidated (C-term)
Amidated (Protein C-term)
Ammonia-loss (N-term C)
Biotin (K)
Biotin (N-term)
Carbamidomethyl (C)
Carbamyl (K)
Carbamyl (N-term)

Protein mass: kDa

Peptide tol. ± 1.2 Da

Mass values: ● MH^+ ○ M_r ○ $M-H^-$

Monoisotopic ● Average ○

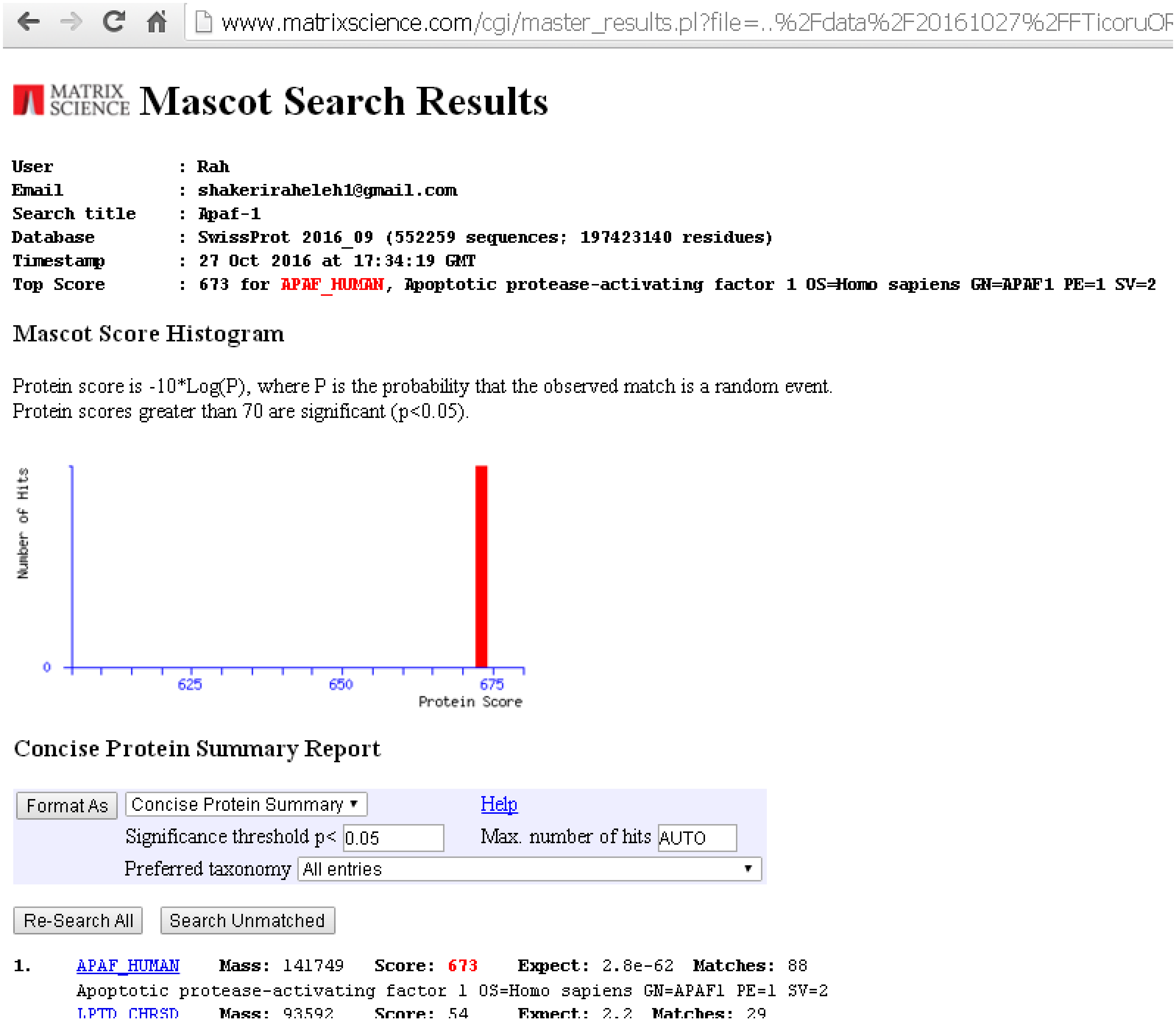
● Data file Choose File No file chosen

○ Query

Data input:

Decoy □

Report top AUTO hits


www.matrixscience.com/cgi/master_results.pl?file=..%2Fdata%2F20161027%2FFTicoruOF
MATRIX SCIENCE

Mascot Search Results

```
User          : Rah
Email         : shakeriraheleh1@gmail.com
Search title  : Apaf-1
Database      : SwissProt 2016_09 (552259 sequences; 197423140 residues)
Timestamp     : 27 Oct 2016 at 17:34:19 GMT
Top Score     : 673 for APAF_HUMAN, Apoptotic protease-activating factor 1 OS=Homo sapiens GN=APAF1 PE=1 SV=2
```

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 70 are significant (p<0.05).

Concise Protein Summary Report

Significance threshold p< 0.05

Max. number of hits AUTO

Preferred taxonomy All entries ▾

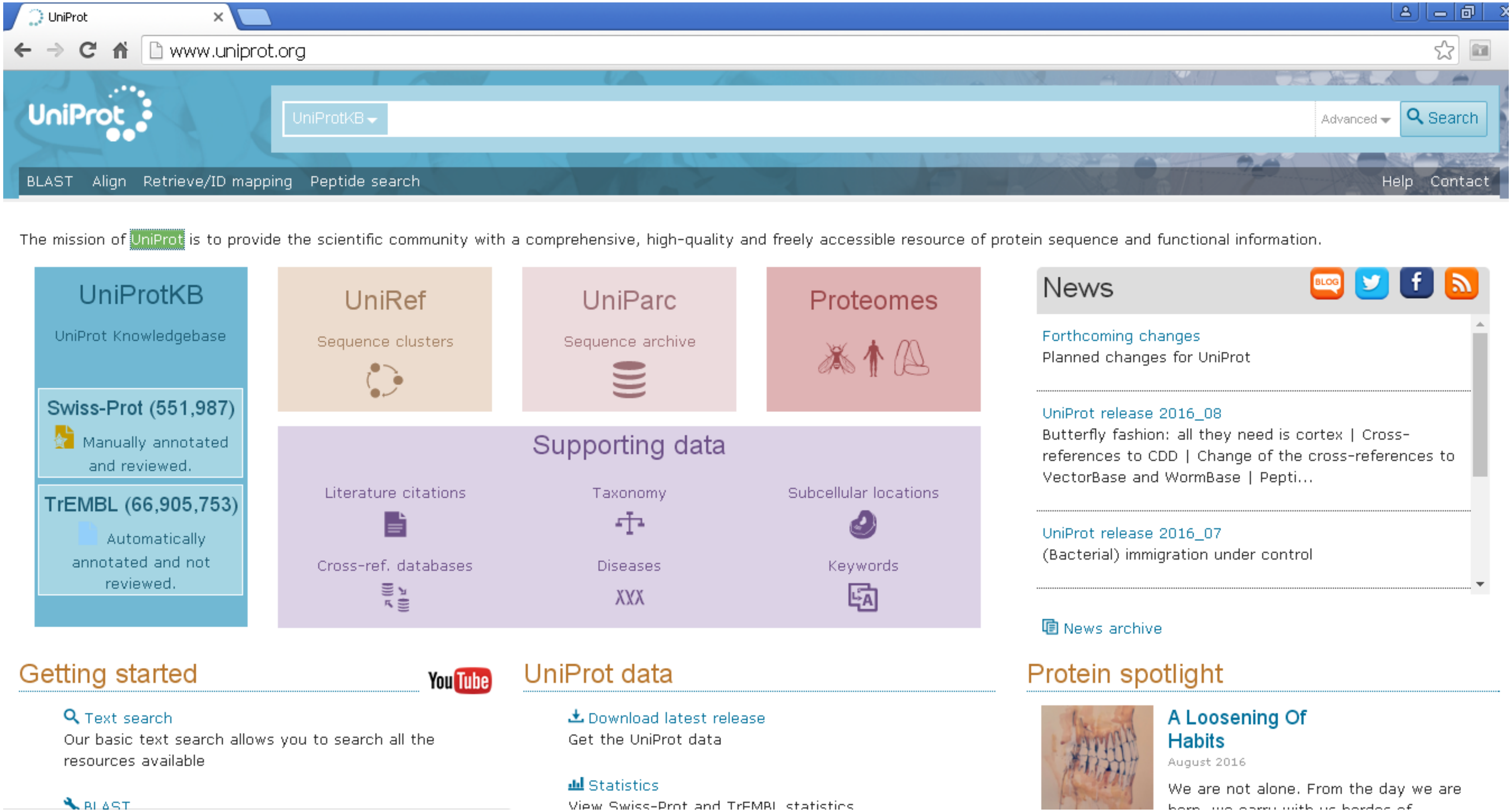
```
1.   APAF_HUMAN   Mass: 141749   Score: 673   Expect: 2.8e-62  Matches: 88
     Apoptotic protease-activating factor 1 OS=Homo sapiens GN=APAF1 PE=1 SV=2
     LPTD_CHRSD   Mass: 93592    Score: 54    Expect: 2.2   Matches: 29
```

The last point in particular has always been a complicating factor when applying the Edman approach to eukaryotic-derived proteins. Up to 80% of such proteins display chemically altered N-terminal amino acid residues, which do not react with the Edman PITC reagent (Box 2.1). The most common N-terminal chemical alteration observed is acetylation (see section 2.9.4), but blocking may also be the result of glycosylation and formylation for example.

Today, however, the vast majority of protein sequences are obtained/predicted indirectly via nucleotide sequence data generated from genome sequencing projects (Chapter 1), which now means that amino acid sequence data for several tens of millions of different proteins are available and may be accessed and interrogated through databases such as the Uniprot database (www.uniprot.org; Box 2.2).

Despite the central importance of the genomic approach, direct sequencing methods remain important/essential for a number of applications. For example, direct sequencing (full-length or at least partial sequencing of the first 10–20 amino acids at the N-terminus of a protein) can be used to:

- design polymerase chain reaction (PCR) primers to assist in the ultimate cloning of the gene coding for the protein if the protein has been purified directly from, for example, a source for which no genome sequence data is available;
- serve as a quality control tool to directly verify the identity/sequence of protein products such as biopharmaceuticals.



The mission of UniProt is to provide the scientific community with a comprehensive, high-quality and freely accessible resource of protein sequence and functional information.

UniProtKB

UniProt Knowledgebase

Swiss-Prot (551,987)

Manually annotated and reviewed.

TrEMBL (66,905,753)

Automatically annotated and not reviewed.

UniRef

Sequence clusters

UniParc

Sequence archive

Proteomes

Supporting data

Literature citations

Taxonomy

Subcellular locations

Cross-ref. databases

Diseases

Keywords

News

Forthcoming changes

Planned changes for UniProt

UniProt release 2016_08

Butterfly fashion: all they need is cortex | Cross-references to CDD | Change of the cross-references to VectorBase and WormBase | Pepti...

UniProt release 2016_07

(Bacterial) immigration under control

News archive

Getting started

Text search

Our basic text search allows you to search all the resources available

BLAST

UniProt data

Download latest release

Get the UniProt data

Statistics

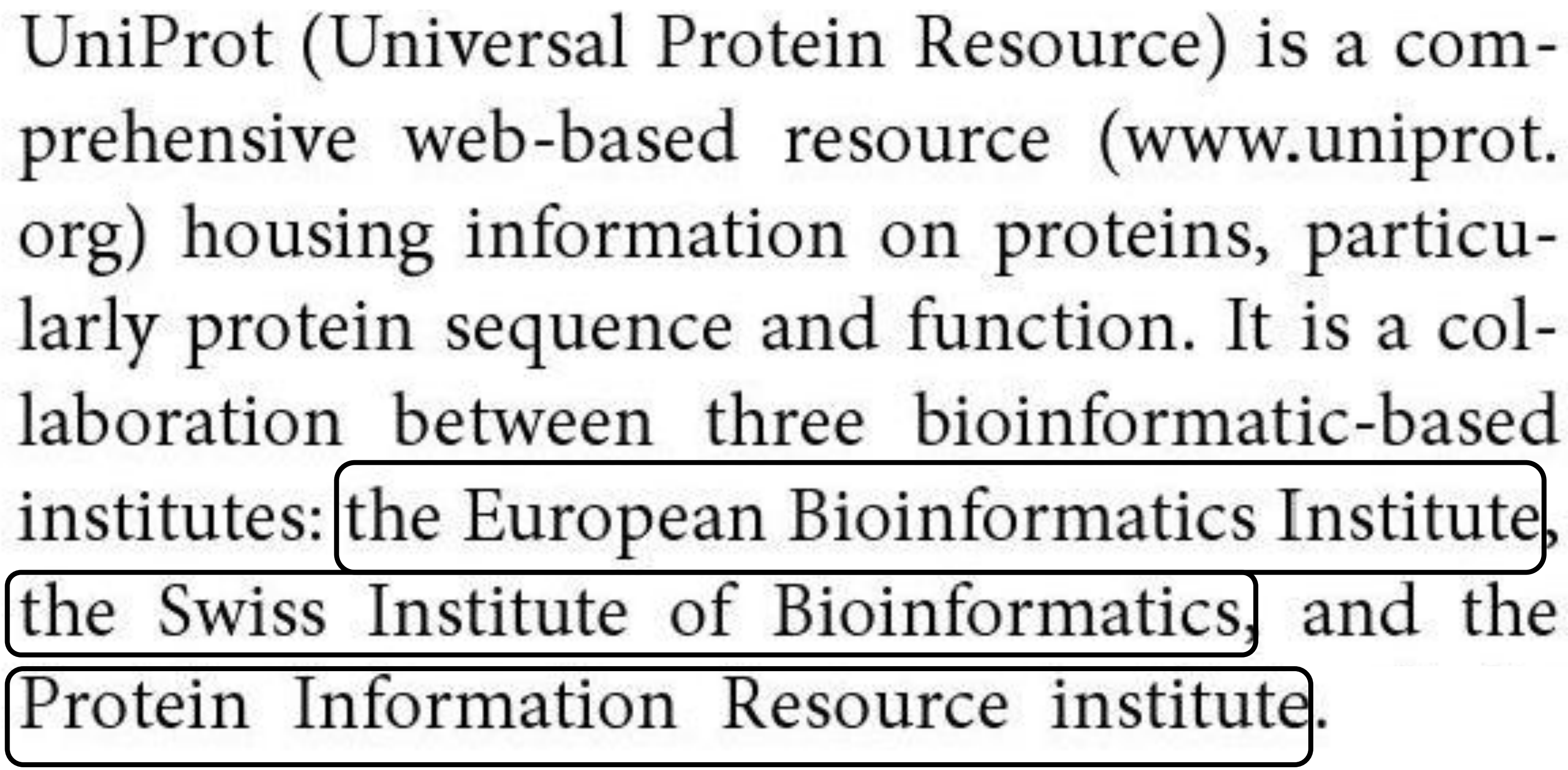
View Swiss-Prot and TrEMBL statistics

Protein spotlight

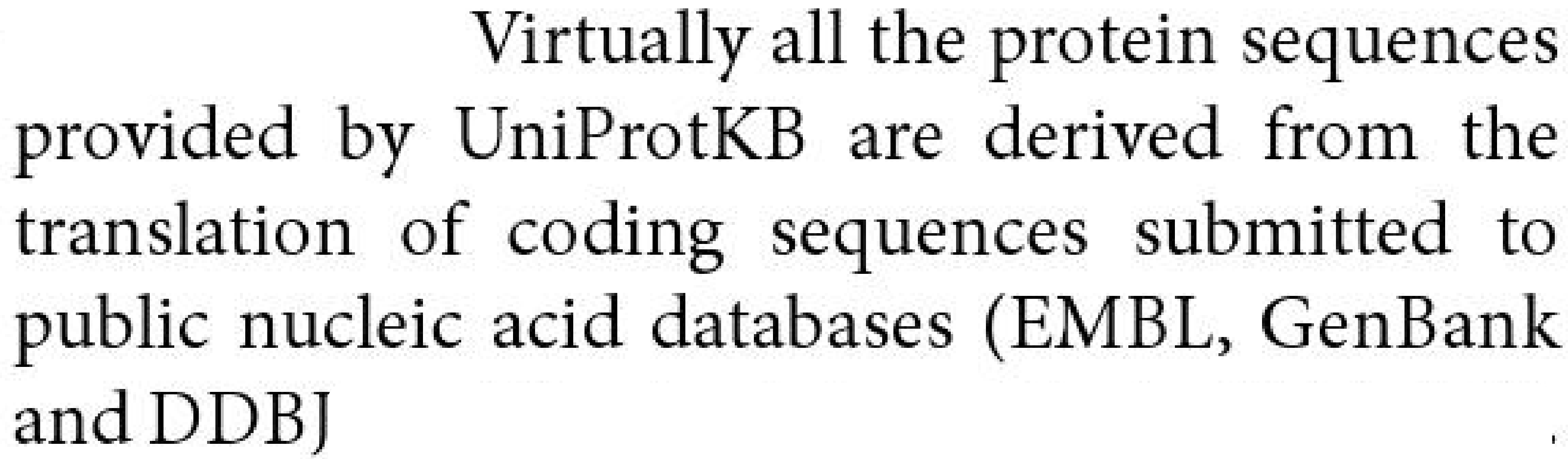
A Loosening Of Habits

August 2016

We are not alone. From the day we are born, we carry with us hordes of

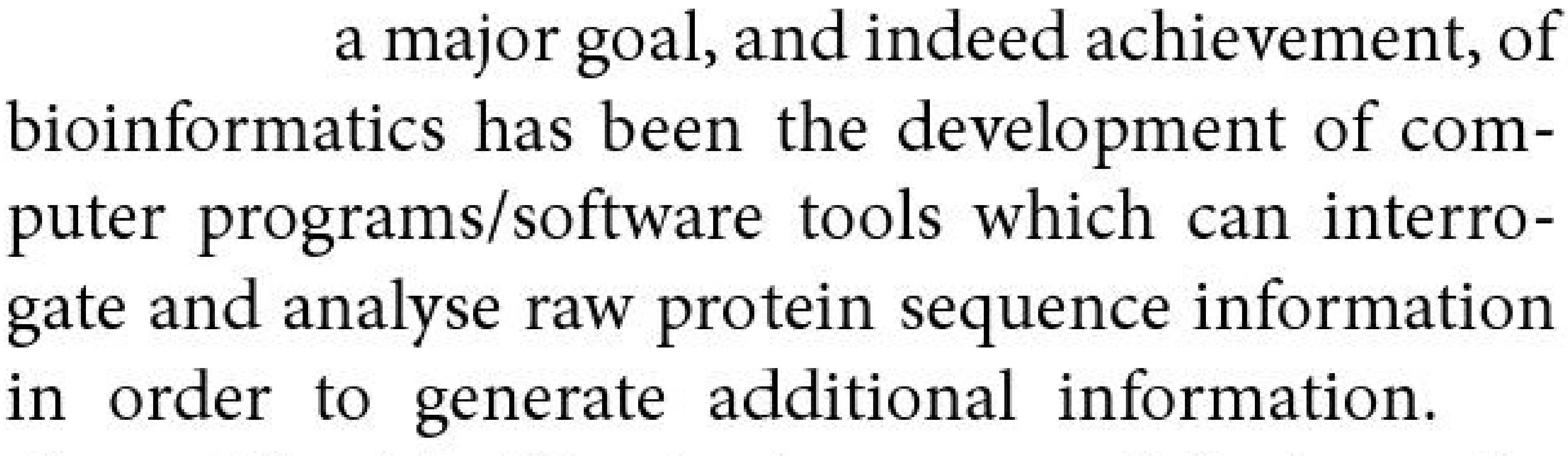


UniProt (Universal Protein Resource) is a comprehensive web-based resource (www.uniprot.org) housing information on proteins, particularly protein sequence and function. It is a collaboration between three bioinformatic-based institutes: the European Bioinformatics Institute, the Swiss Institute of Bioinformatics, and the Protein Information Resource institute.



Virtually all the protein sequences provided by UniProtKB are derived from the translation of coding sequences submitted to public nucleic acid databases (EMBL, GenBank and DDBJ

Bioinformatic analysis of sequence data



a major goal, and indeed achievement, of bioinformatics has been the development of computer programs/software tools which can interrogate and analyse raw protein sequence information in order to generate additional information.

Various and often multiple different bioinformatic programs/tools are available that interrogate protein sequence information/databases in order to:

- identify proteins containing similar amino acid sequences (i.e. run similarity searches) and assess how closely related two (or more) proteins are, or if there is a high probability that they undertake similar functions (see next section);
- calculate a theoretical molecular mass, isoelectric point (see Chapter 4) or other physicochemical property of a protein;
- predict elements of a protein's higher-order structure (secondary and tertiary structure, or for example protein domains, as discussed in section 2.2.2);
- predict if a protein is likely to undergo PTMs (see section 2.9), and at what point(s) along the protein backbone this is likely to occur;
- predict where in the cell the protein is likely to function (or if it is likely exported from the cell).

Sequence similarity and sequence alignment analysis

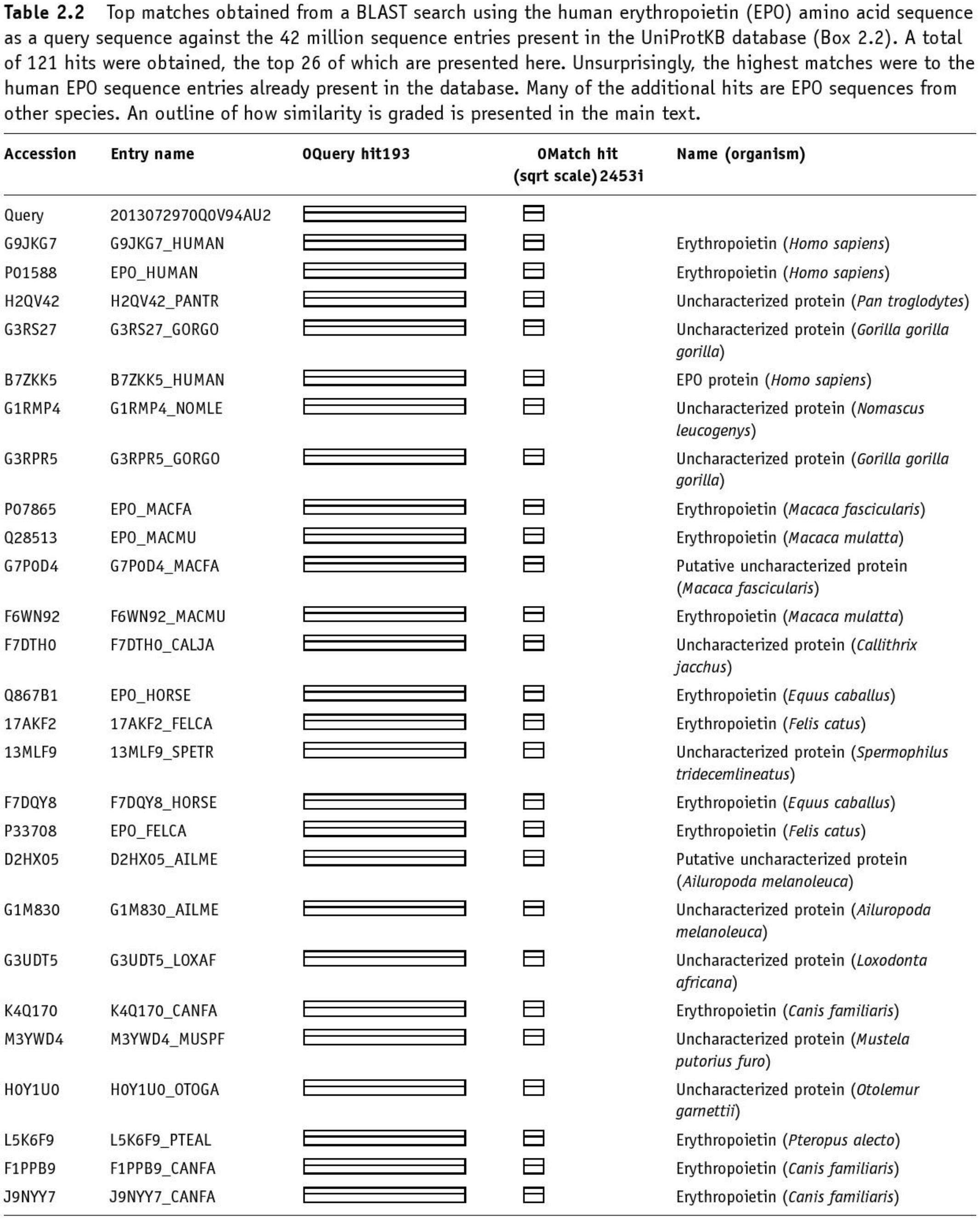


Table 2.2 Top matches obtained from a BLAST search using the human erythropoietin (EPO) amino acid sequence as a query sequence against the 42 million sequence entries present in the UniProtKB database (Box 2.2). A total of 121 hits were obtained, the top 26 of which are presented here. Unsurprisingly, the highest matches were to the human EPO sequence entries already present in the database. Many of the additional hits are EPO sequences from other species. An outline of how similarity is graded is presented in the main text.

Accession	Entry name	0Query hit193	0Match hit (sqrt scale) 2453i	Name (organism)
Query	2013072970Q0V94AU2	▭	▭	
G9JKG7	G9JKG7_HUMAN	▭	▭	Erythropoietin (*Homo sapiens*)
P01588	EPO_HUMAN	▭	▭	Erythropoietin (*Homo sapiens*)
H2QV42	H2QV42_PANTR	▭	▭	Uncharacterized protein (*Pan troglodytes*)
G3RS27	G3RS27_GORGO	▭	▭	Uncharacterized protein (*Gorilla gorilla gorilla*)
B7ZKK5	B7ZKK5_HUMAN	▭	▭	EPO protein (*Homo sapiens*)
G1RMP4	G1RMP4_NOMLE	▭	▭	Uncharacterized protein (*Nomascus leucogenys*)
G3RPR5	G3RPR5_GORGO	▭	▭	Uncharacterized protein (*Gorilla gorilla gorilla*)
P07865	EPO_MACFA	▭	▭	Erythropoietin (*Macaca fascicularis*)
Q28513	EPO_MACMU	▭	▭	Erythropoietin (*Macaca mulatta*)
G7P0D4	G7P0D4_MACFA	▭	▭	Putative uncharacterized protein (*Macaca fascicularis*)
F6WN92	F6WN92_MACMU	▭	▭	Erythropoietin (*Macaca mulatta*)
F7DTH0	F7DTH0_CALJA	▭	▭	Uncharacterized protein (*Callithrix jacchus*)
Q867B1	EPO_HORSE	▭	▭	Erythropoietin (*Equus caballus*)
17AKF2	17AKF2_FELCA	▭	▭	Erythropoietin (*Felis catus*)
13MLF9	13MLF9_SPETR	▭	▭	Uncharacterized protein (*Spermophilus tridecemlineatus*)
F7DQY8	F7DQY8_HORSE	▭	▭	Erythropoietin (*Equus caballus*)
P33708	EPO_FELCA	▭	▭	Erythropoietin (*Felis catus*)
D2HX05	D2HX05_AILME	▭	▭	Putative uncharacterized protein (*Ailuropoda melanoleuca*)
G1M830	G1M830_AILME	▭	▭	Uncharacterized protein (*Ailuropoda melanoleuca*)
G3UDT5	G3UDT5_LOXAF	▭	▭	Uncharacterized protein (*Loxodonta africana*)
K4Q170	K4Q170_CANFA	▭	▭	Erythropoietin (*Canis familiaris*)
M3YWD4	M3YWD4_MUSPF	▭	▭	Uncharacterized protein (*Mustela putorius furo*)
H0Y1U0	H0Y1U0_OTOGA	▭	▭	Uncharacterized protein (*Otolemur garnettii*)
L5K6F9	L5K6F9_PTEAL	▭	▭	Erythropoietin (*Pteropus alecto*)
F1PPB9	F1PPB9_CANFA	▭	▭	Erythropoietin (*Canis familiaris*)
J9NYY7	J9NYY7_CANFA	▭	▭	Erythropoietin (*Canis familiaris*)

BLAST
(Basic Local Alignment Search Tool): UniProt or NCBI

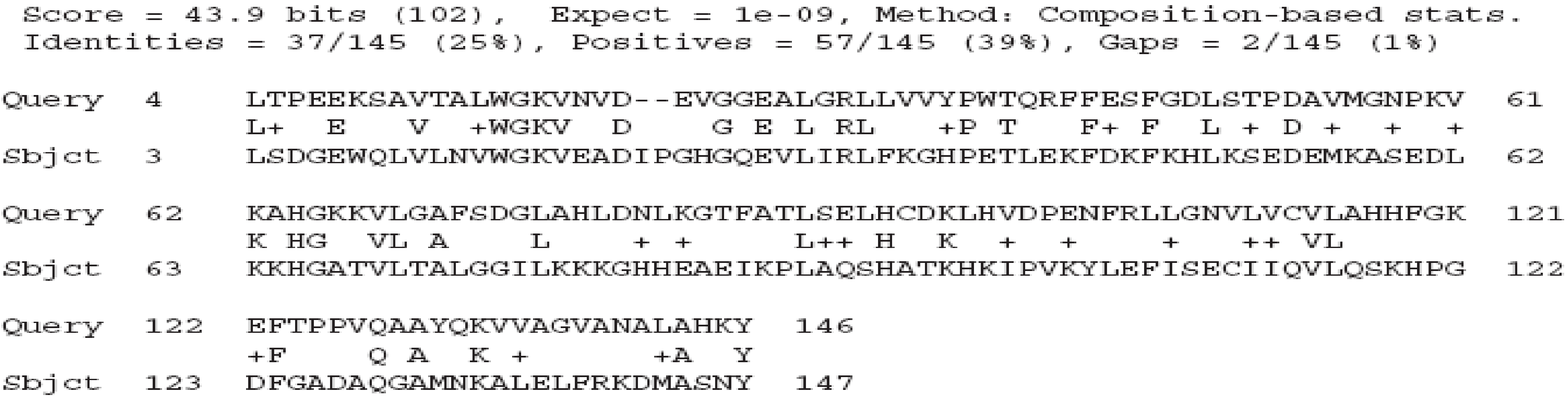
Alignment
Pairwaise alignment
Multiple alignment

Homology
Similarity
Identity

```
1    -------------MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAK   47
1    MCEPAPPPTQSAWHSFPECPA-LFLLLSLLLLPLGLPVLGAPPRLICDSRVLERYILEAR   59
                   .. **** *:****** ************************:***:

48   EAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQAL  107
60   EAENVTMGCAQGCSFSENITVPDTKVNFYTWKRMDVGQQALEVWQGLALLSEAILRGQAL  119
     ****:* ***: **:.*************:****:*****:************:******

108  EVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTTTADTFRK  167
120  LANASQPSETPQLHVDKAVSSLRSLTSLLRALGAQKEAMSLPEEASPAPLRTFTVDTLCK  179
     *.*:*** *  *********.*****:***********:* *:*** *****:*.**: *

168  LFRVYSNFLRGKLKLYTGEACRTGDR  193  P01588   EPO_HUMAN
180  LFRIYSNFLRGKLTLYTGEACRRGDR  205  J9NYY7   J9NYY7_CANFA
     ***:*********.******** ***
```

Figure 2.6 A pairwise sequence alignment between the amino acid sequence of human erythropoietin (EPO, top line of each twin sequence) and canine EPO (bottom line of each twin sequence) (a). The sequence alignment was undertaken via the UniProt website. Asterisks are automatically placed underneath sequence positions housing identical amino acid residues while double or single dots (i.e. a colon or a period) appear underneath residue positions which display strongly or weakly similar properties, respectively. Thus, human and canine EPOs contain identical residues at 155 positions (i.e. they display approximately 75% identity) and similar residues at a further 24 positions. The software also facilitates the generation of additional information such as the positioning of amino acid residues with particular properties.

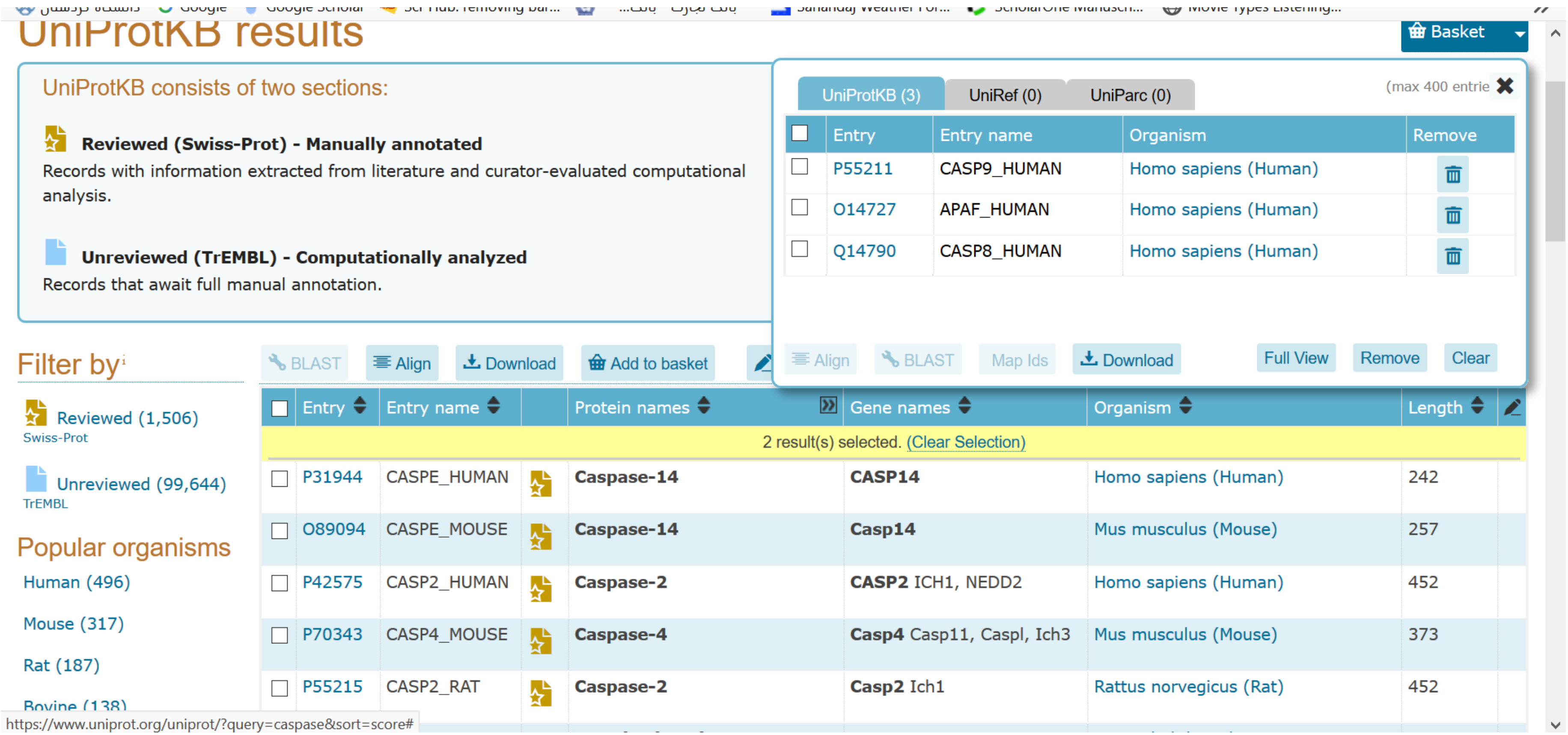
57 similar amino acides
37 full match
20 +



```
 Score = 43.9 bits (102),  Expect = 1e-09, Method: Composition-based stats.
 Identities = 37/145 (25%), Positives = 57/145 (39%), Gaps = 2/145 (1%)

Query  4     LTPEEKSAVTALWGKVNVD--EVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKV  61
             L+  E   V  +WGKV  D    G E L RL   +P T   F+ F  L + D +  +  +
Sbjct  3     LSDGEWQLVLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKASEDL  62

Query  62    KAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGK  121
             K HG  VL A    L    + +     L++ H  K  +  +    +   ++ VL
Sbjct  63    KKHGATVLTALGGILKKKGHHEAEIKPLAQSHATKHKIPVKYLEFISECIIQVLQSKHPG  122

Query  122   EFTPPVQAAYQKVVAGVANALAHKY  146
             +F    Q A  K +      +A  Y
Sbjct  123   DFGADAQGAMNKALELFRKDMASNY  147
```

% identity = ۱۰۰×(طول ناحیه انطباق /تعداد ریشه های یکسان)

% Similarity = ۱۰۰×(طول ناحیه انطباق /تعداد ریشه های مشابه + تعداد ریشه های یکسان)



UniProtKB results

UniProtKB consists of two sections:

Reviewed (Swiss-Prot) - Manually annotated
Records with information extracted from literature and curator-evaluated computational analysis.

Unreviewed (TrEMBL) - Computationally analyzed
Records that await full manual annotation.

UniProtKB (3) | UniRef (0) | UniParc (0) | (max 400 entrie ✖

□	Entry	Entry name	Organism	Remove
□	P55211	CASP9_HUMAN	Homo sapiens (Human)	🗑
□	O14727	APAF_HUMAN	Homo sapiens (Human)	🗑
□	Q14790	CASP8_HUMAN	Homo sapiens (Human)	🗑

Filter by

Reviewed (1,506)
Swiss-Prot

Unreviewed (99,644)
TrEMBL

Popular organisms

Human (496)

Mouse (317)

Rat (187)

Bovine (138)

□	Entry	Entry name		Protein names	Gene names	Organism	Length
2 result(s) selected. (Clear Selection)							
□	P31944	CASPE_HUMAN	★	**Caspase-14**	**CASP14**	Homo sapiens (Human)	242
□	O89094	CASPE_MOUSE	★	**Caspase-14**	**Casp14**	Mus musculus (Mouse)	257
□	P42575	CASP2_HUMAN	★	**Caspase-2**	**CASP2** ICH1, NEDD2	Homo sapiens (Human)	452
□	P70343	CASP4_MOUSE	★	**Caspase-4**	**Casp4** Casp11, Caspl, Ich3	Mus musculus (Mouse)	373
□	P55215	CASP2_RAT	★	**Caspase-2**	**Casp2** Ich1	Rattus norvegicus (Rat)	452

https://www.uniprot.org/uniprot/?query=caspase&sort=score#

Higher-level structure

Secondary structure: α-helix and β-strand
Tertiary structure
Quaternary structure

❑Fibrous proteins versus Globular proteins
❑Why Secondary structures are formed?
❑α-helix and β-strand properties
❑Loops such as β-turn
❑Types of β-sheets: Parallel, Antiparallel and Mixed
❑Detection of secondary structures by???

Domain and Motif

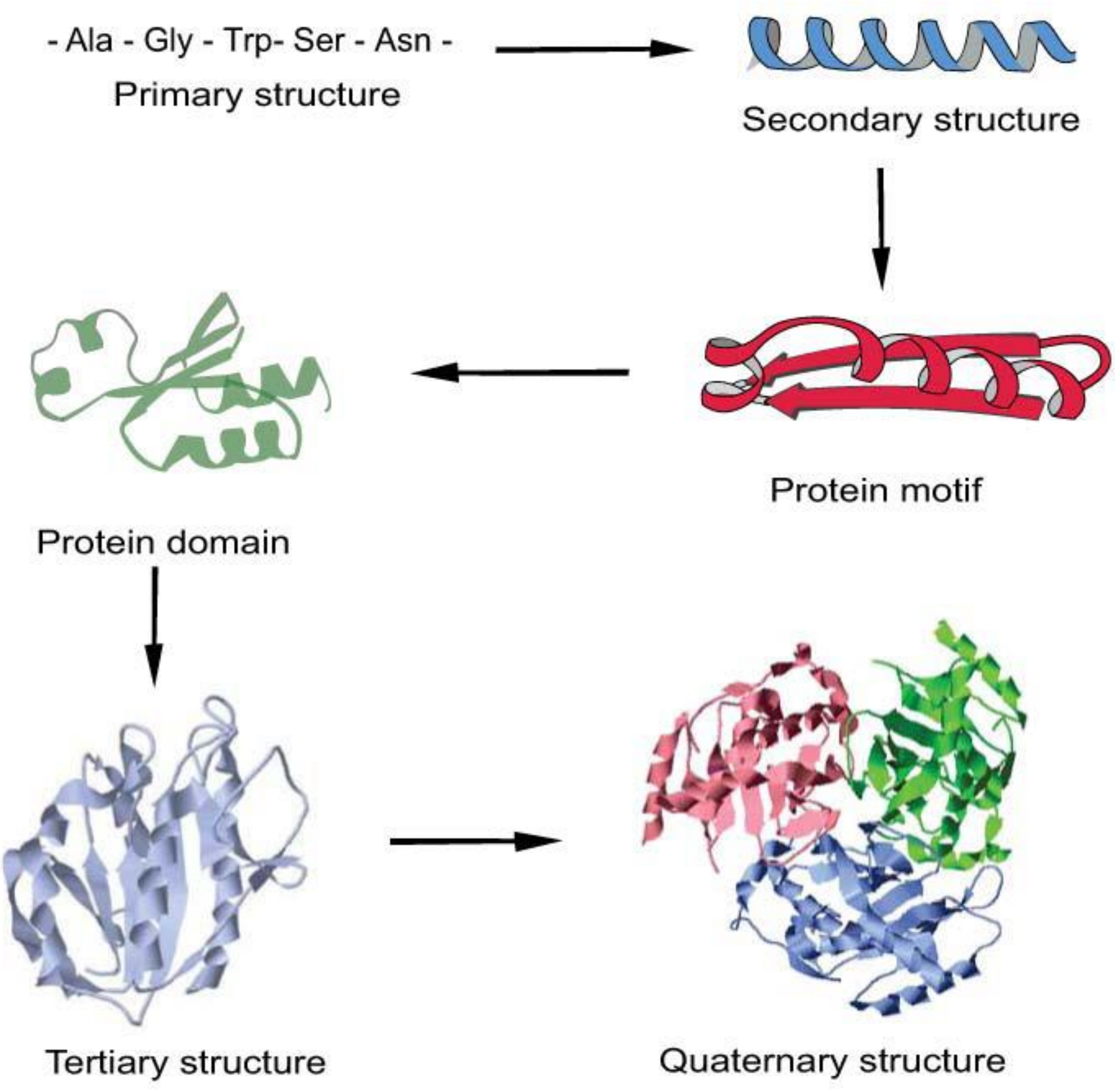
- Several motifs usually combine to form compact globular structures, which are called **domains** (fundamental functional and structural units).

- **Tertiary structure**: the way motifs are arranged into domain structures and for the way a single polypeptide chain folds into one or several domains.
- Large polypeptide chains fold into several domains.

- There are many known examples where several biological functions that are carried out by separate polypeptide chains in one species are performed by domains of a single protein in another species.

- Sequences → Structural Motifs → Domain → Tertiary structure

  The number of such combinations is limited.



- Ala - Gly - Trp- Ser - Asn -

Primary structure

Secondary structure

Protein motif

Protein domain

Tertiary structure

Quaternary structure

Tertiary structure

❑Domain, Motif (structural motif, sequence motif, functional motif) and Fold

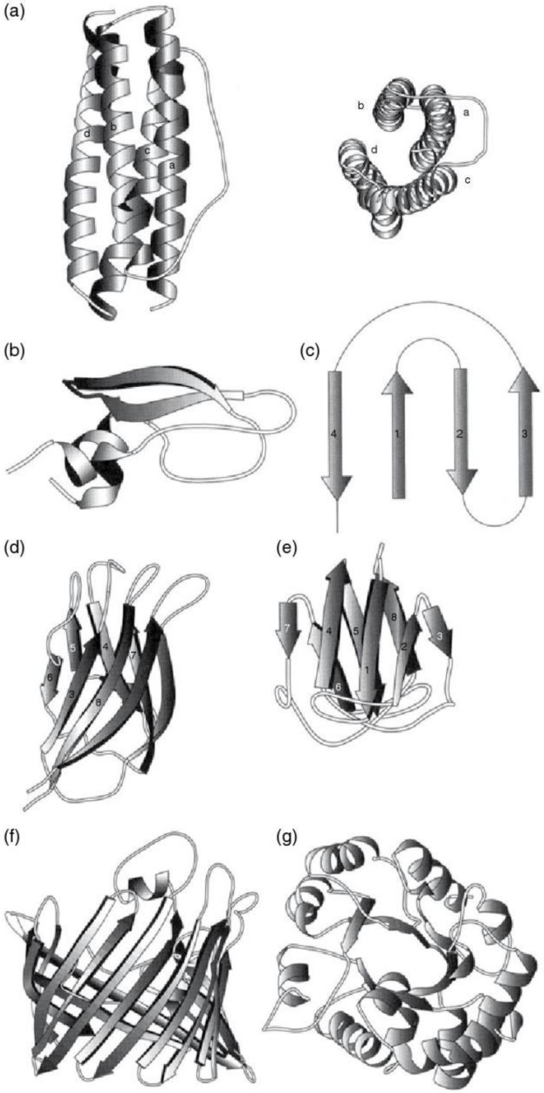
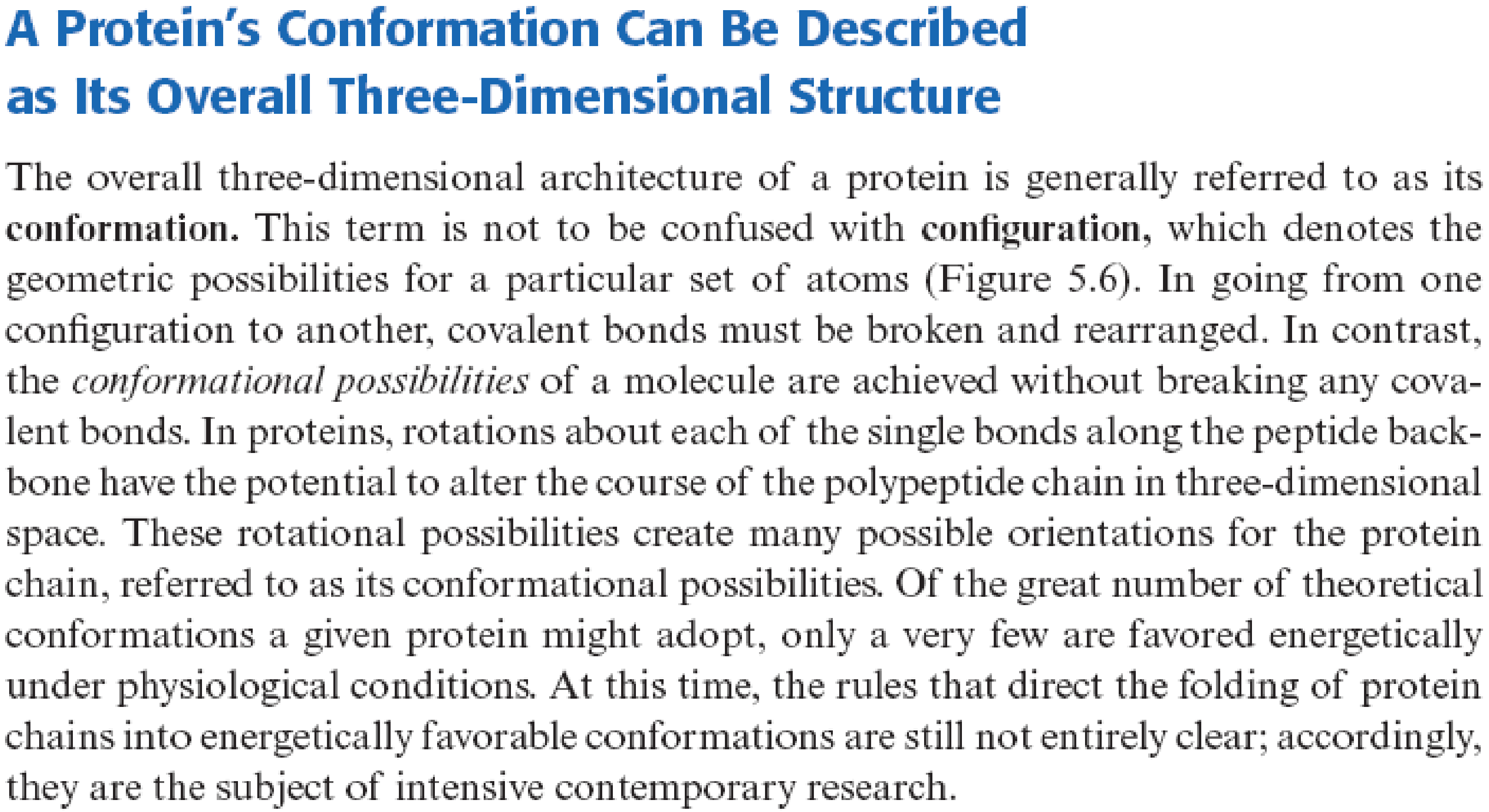


Figure 2.11 Some structural motifs commonly associated with (globular) polypeptides: (a) a four-helix bundle (b) a hairpin structure (c) a β sheet with a Greek key topology (d) a jelly roll motif (e) a β sandwich (f) a β barrel (g) an α/β barrel. Refer to text for further details. Reproduced from *Current Protocols in Protein Science* by kind permission of the publisher, John Wiley & Sons, Ltd.



A Protein's Conformation Can Be Described as Its Overall Three-Dimensional Structure

The overall three-dimensional architecture of a protein is generally referred to as its **conformation.** This term is not to be confused with **configuration,** which denotes the geometric possibilities for a particular set of atoms (Figure 5.6). In going from one configuration to another, covalent bonds must be broken and rearranged. In contrast, the *conformational possibilities* of a molecule are achieved without breaking any covalent bonds. In proteins, rotations about each of the single bonds along the peptide backbone have the potential to alter the course of the polypeptide chain in three-dimensional space. These rotational possibilities create many possible orientations for the protein chain, referred to as its conformational possibilities. Of the great number of theoretical conformations a given protein might adopt, only a very few are favored energetically under physiological conditions. At this time, the rules that direct the folding of protein chains into energetically favorable conformations are still not entirely clear; accordingly, they are the subject of intensive contemporary research.

Difference between Conformation and Configuration

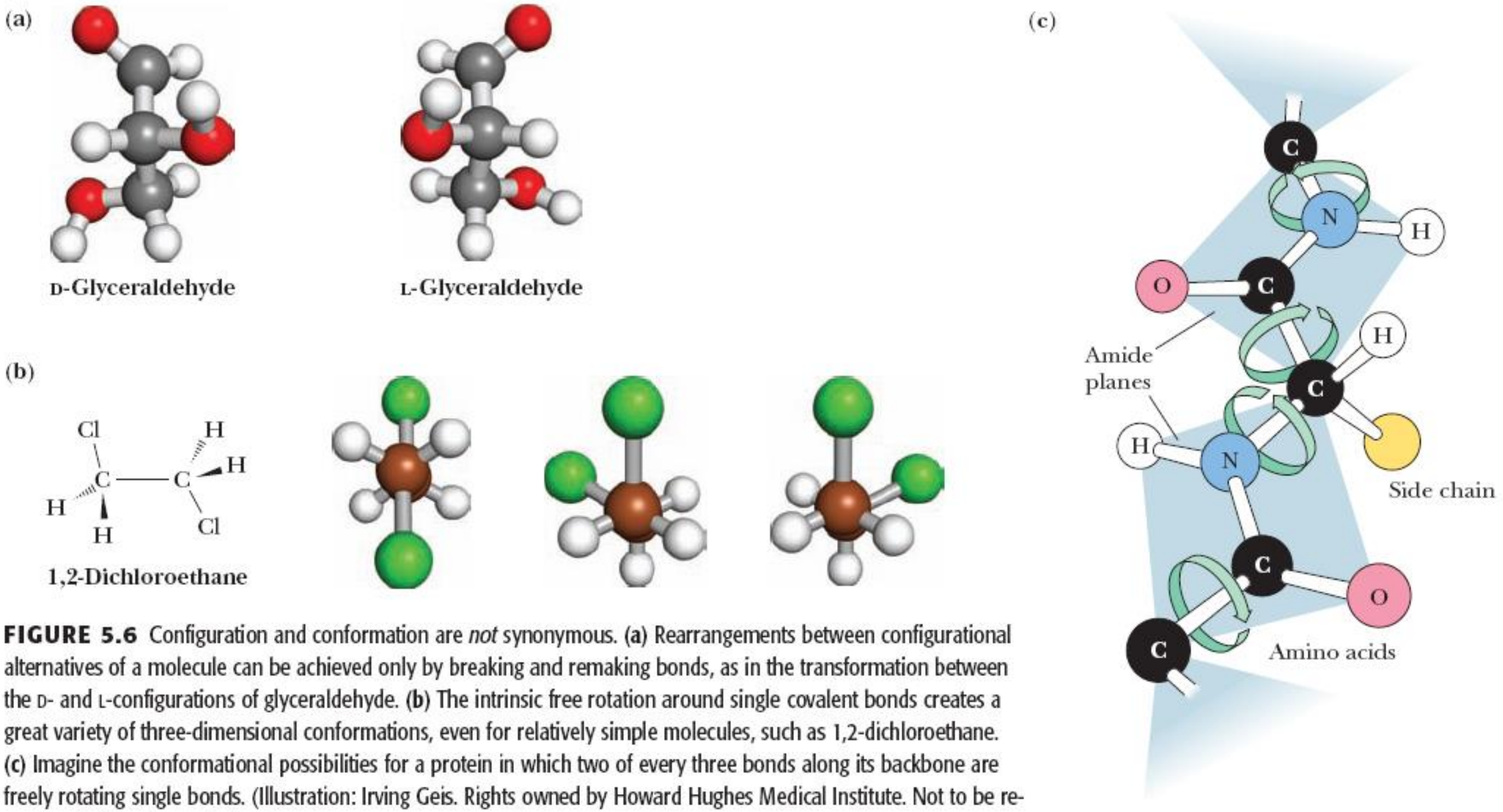


FIGURE 5.6 Configuration and conformation are *not* synonymous. **(a)** Rearrangements between configurational alternatives of a molecule can be achieved only by breaking and remaking bonds, as in the transformation between the D- and L-configurations of glyceraldehyde. **(b)** The intrinsic free rotation around single covalent bonds creates a great variety of three-dimensional conformations, even for relatively simple molecules, such as 1,2-dichloroethane. **(c)** Imagine the conformational possibilities for a protein in which two of every three bonds along its backbone are freely rotating single bonds. (Illustration: Irving Geis. Rights owned by Howard Hughes Medical Institute. Not to be reproduced without permission.)

تفاوت کانفورماسیون و کانفیگوراسیون