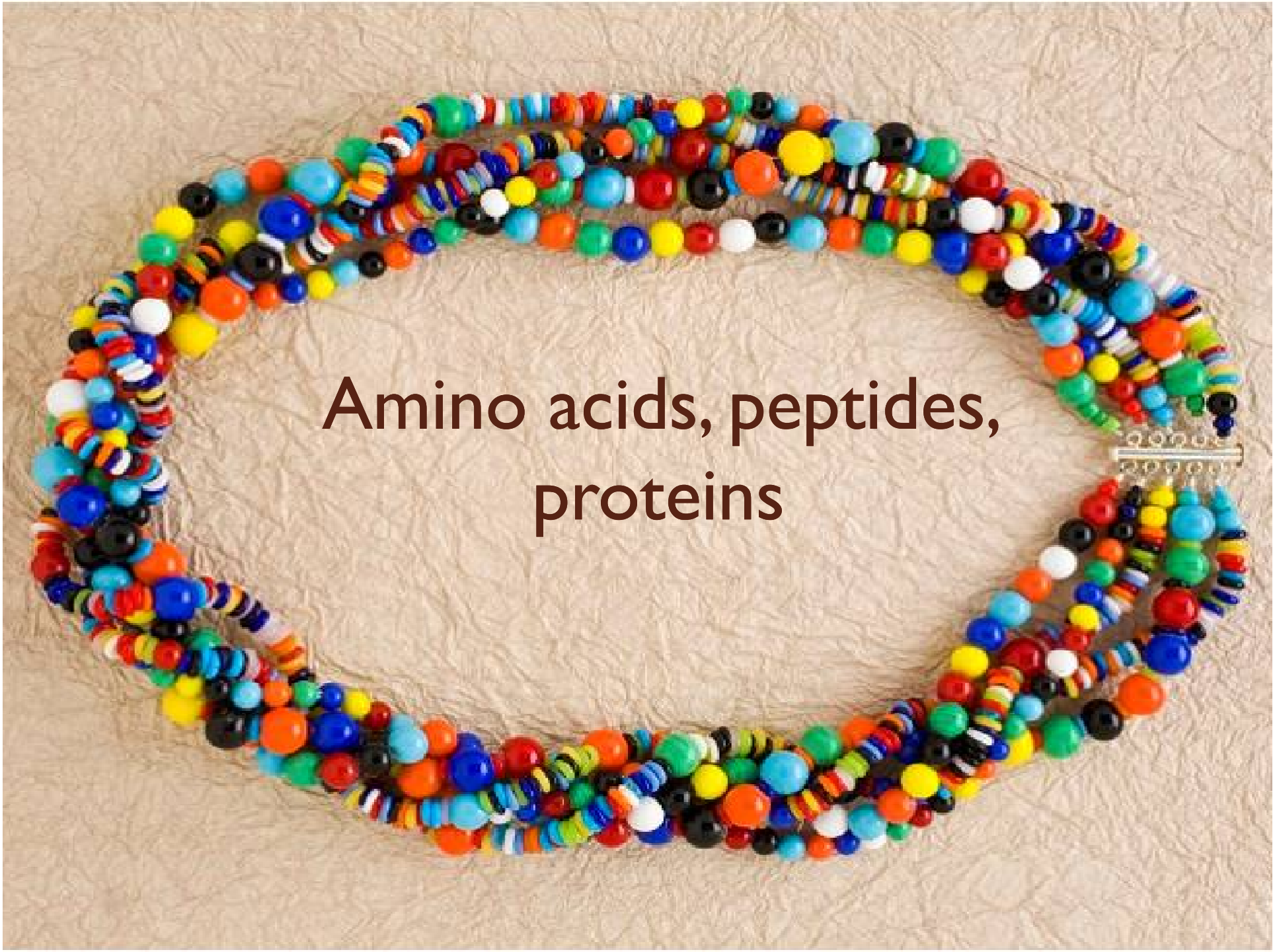
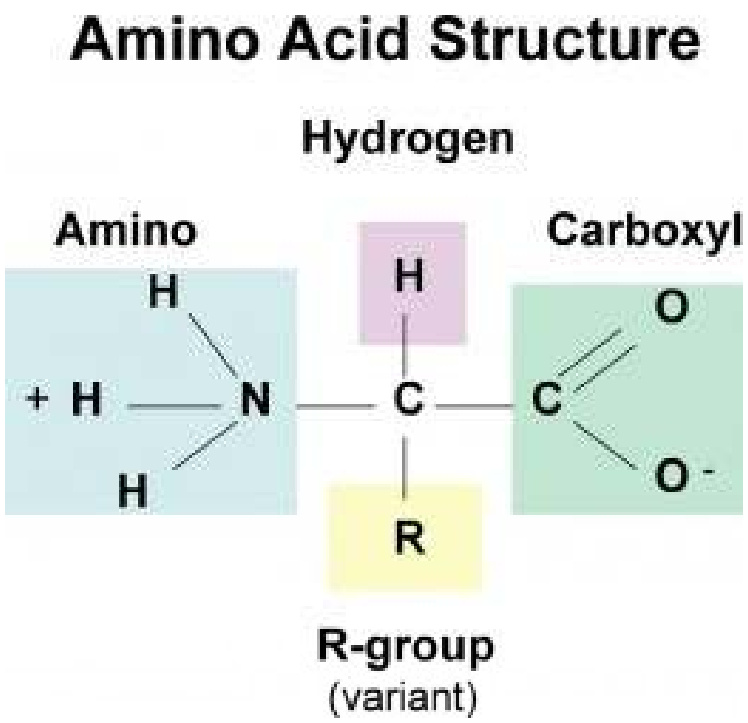

Amino acids, peptides,
proteins

Biomedical Importance of Amino Acids

- protein structural compounds
- precursors for the synthesis of:
  - glucose, fatty acids and ketone bodies
  - biologically important nitrogen compounds: hem, amines, porphyrins, purines, pyrimidines, and urea, hormones, hormone-releasing factors, neuromodulators, or neurotransmitters
- source of metabolic fuel

L-Amino Acids Present in Proteins

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



Two functional groups:

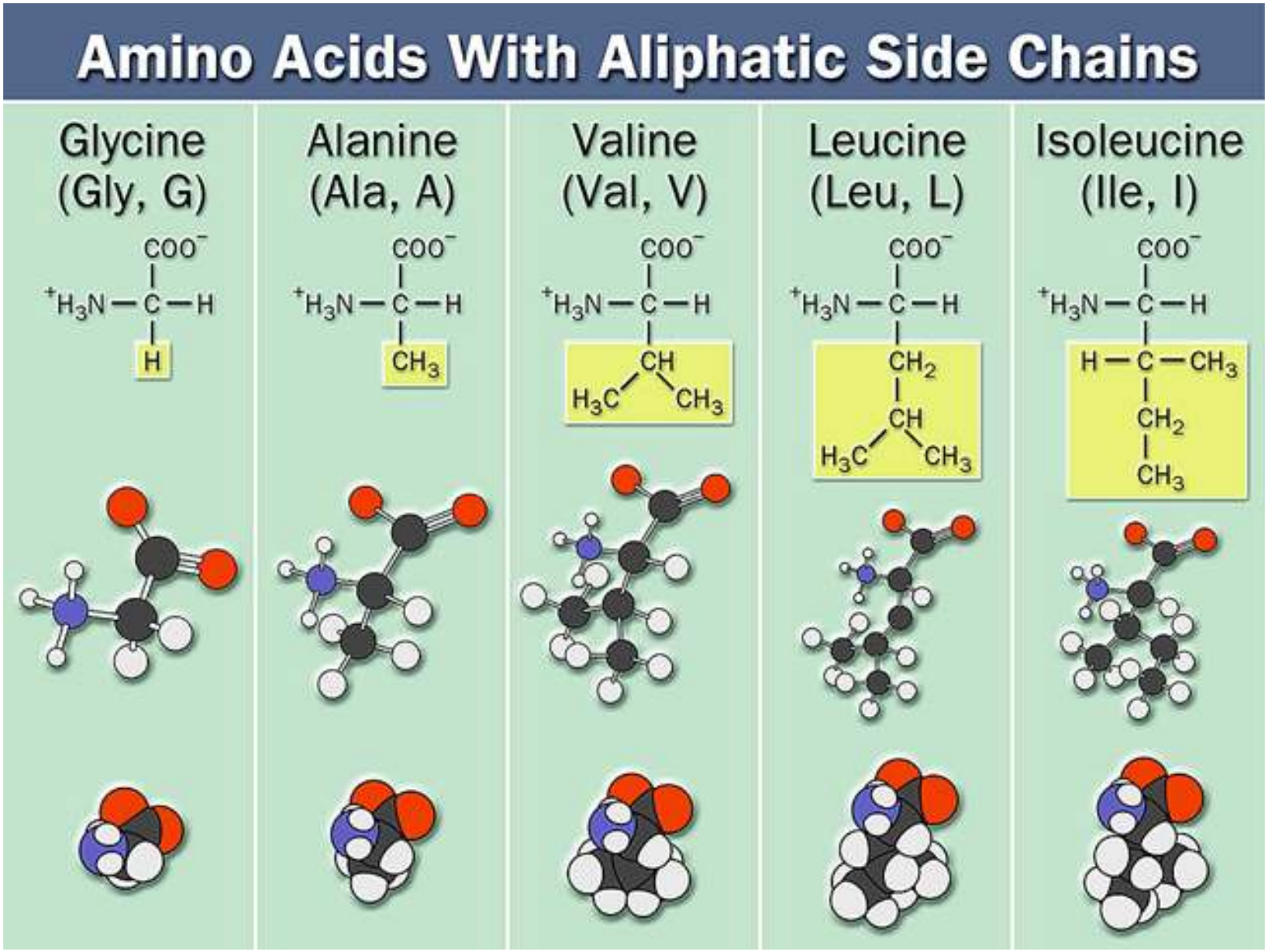
- carboxyl group
- amino group

R-amino acid side chain distinguishes one amino acid from another

Three main R-groups of amino-acids:

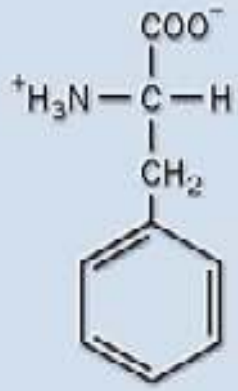
- Hydrophobic side chain (UNPOLAR)
- Uncharged hydrophilic side chain (POLAR)
- Charged hydrophilic side chain (POLAR)

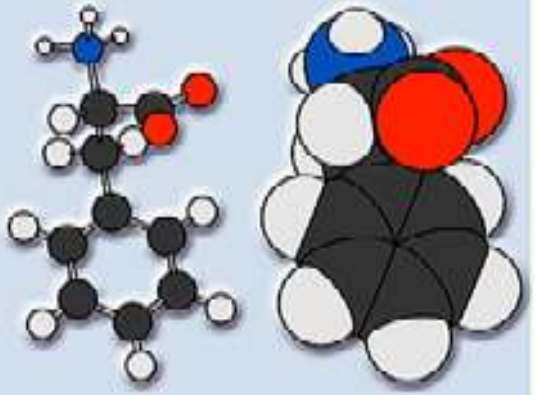
Amino Acids With Aliphatic Side Chains



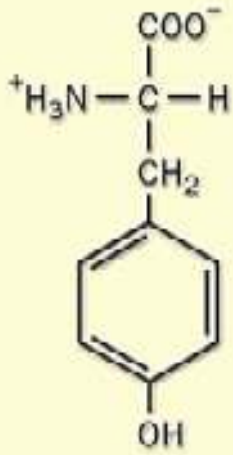
Aromatic Amino Acids

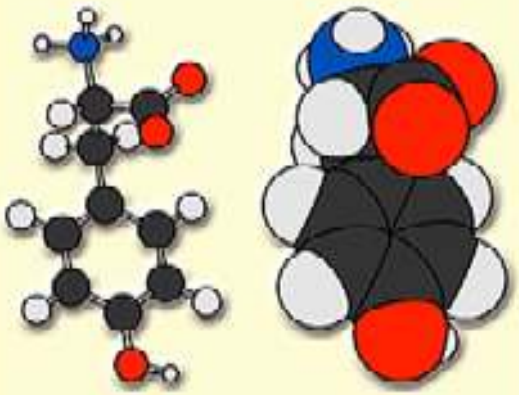
Phenylalanine
(Phe, F)



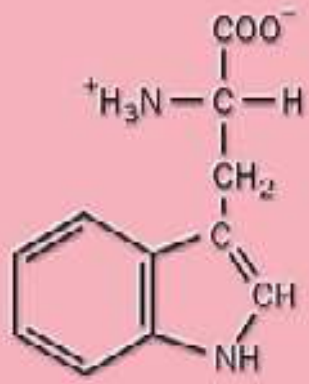


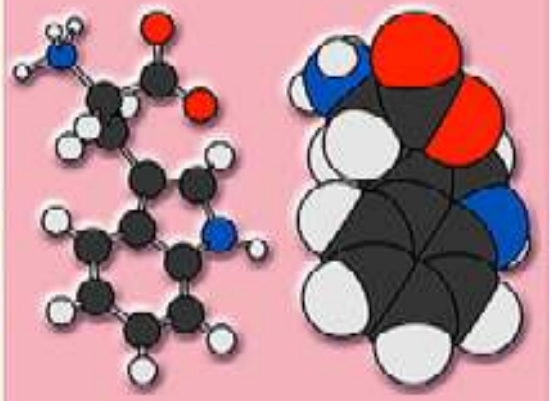
Tyrosine
(Tyr, Y)





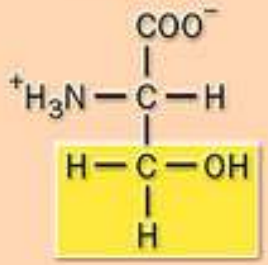
Tryptophan
(Trp, W)

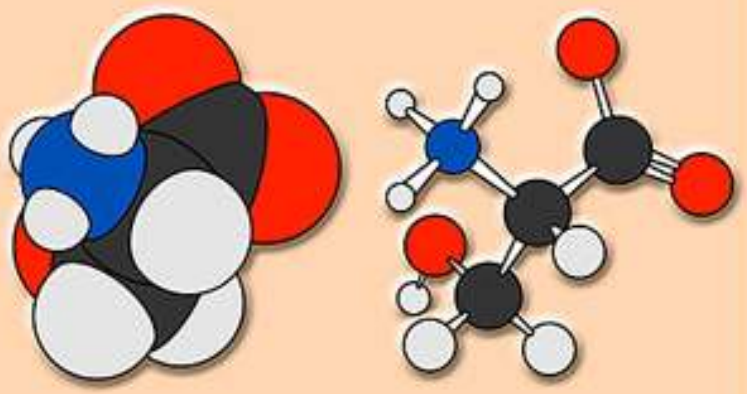




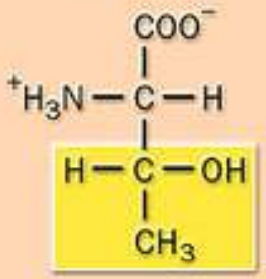
Amino Acids with Aliphatic Hydroxyl-containing Side Chains

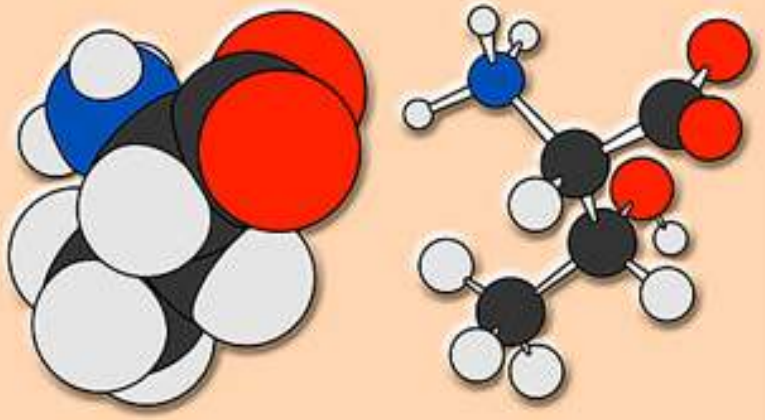
Serine
(Ser, S)



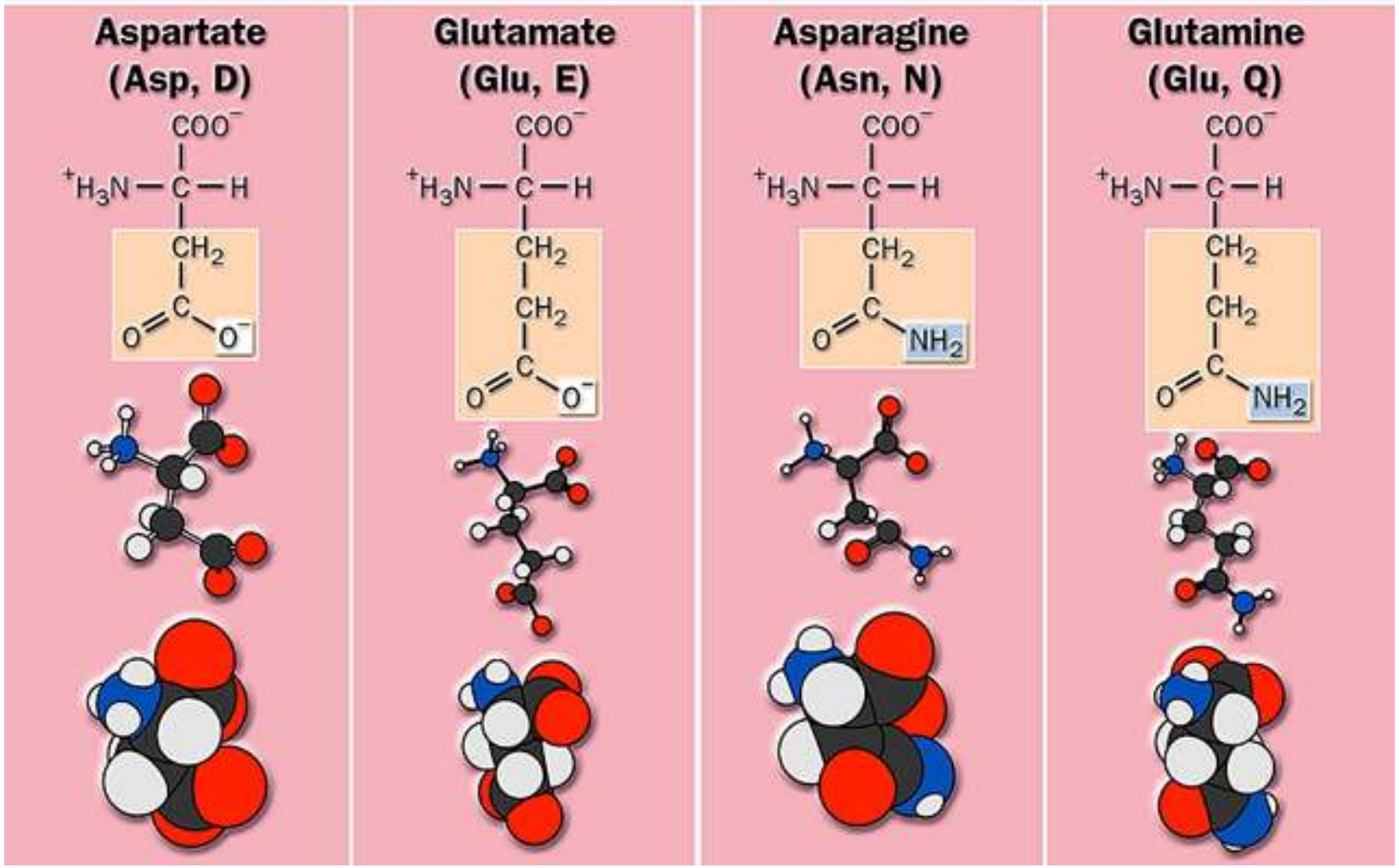


Threonine
(Thr, T)

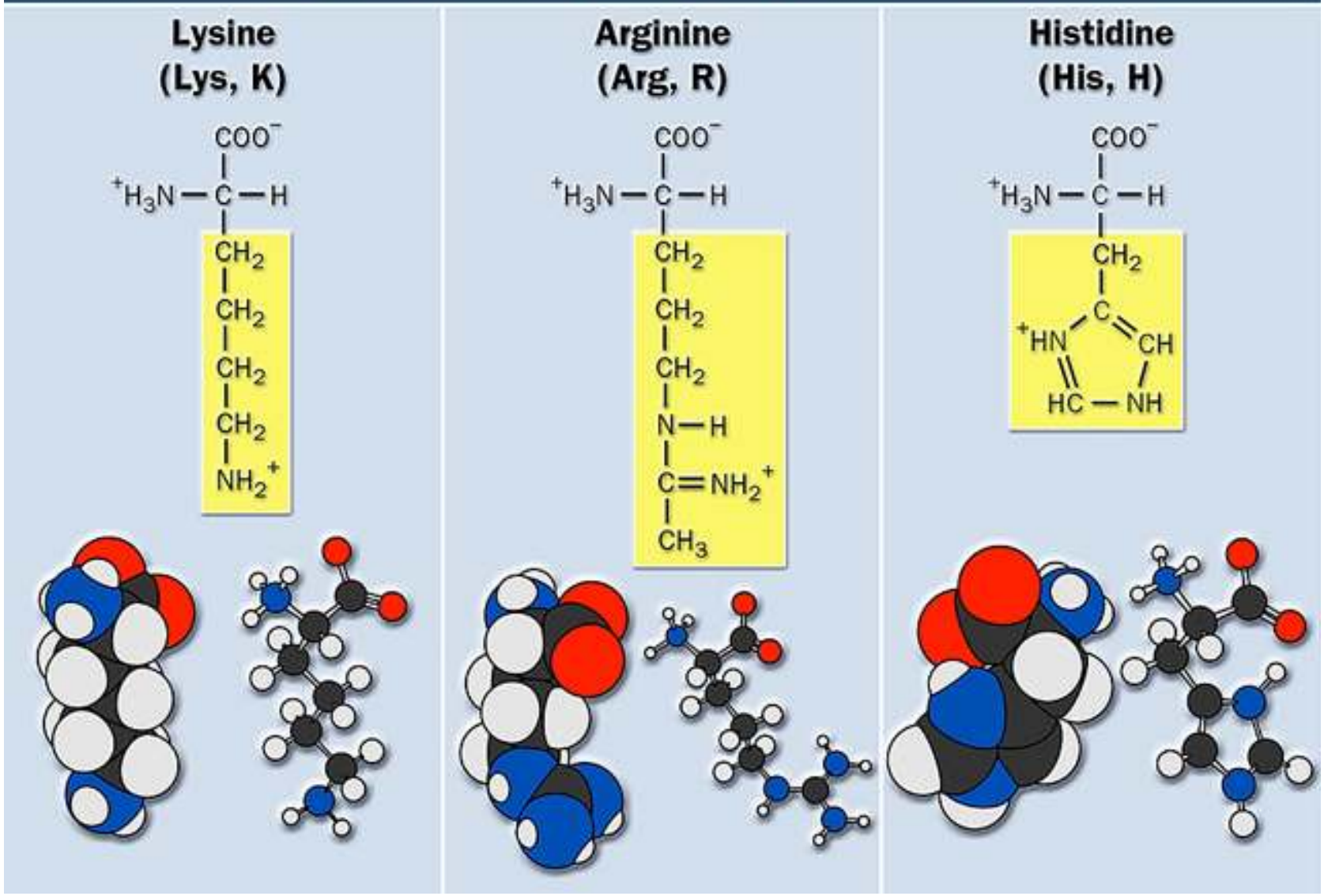




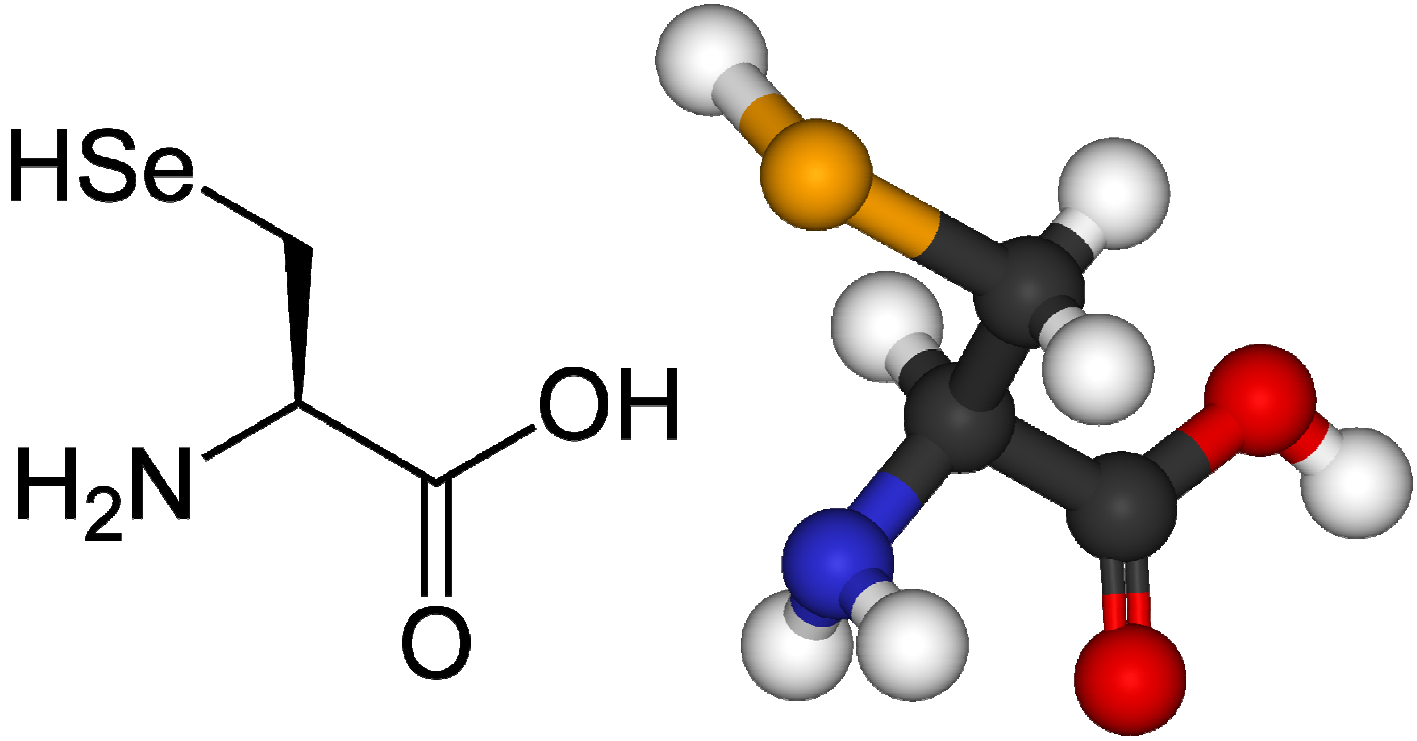
Amino Acids with Acidic Side Chains and Their Derivatives



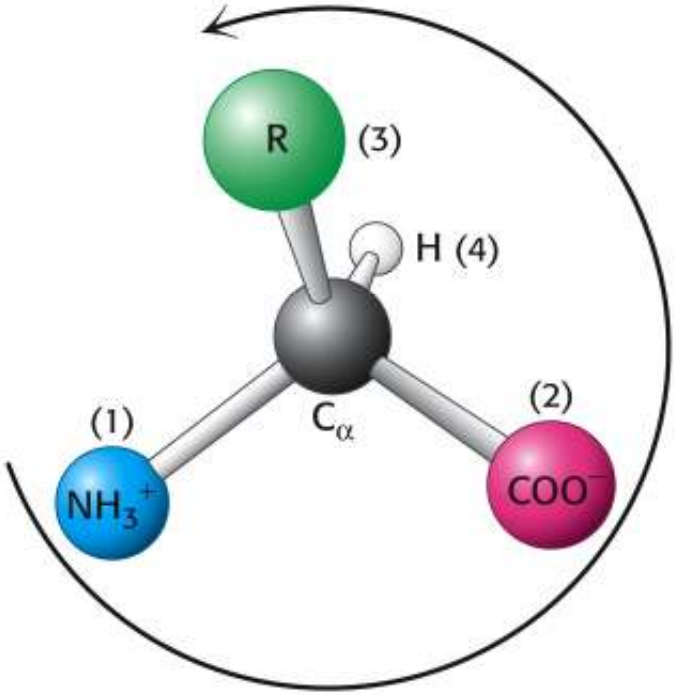
Amino Acids with Basic Side Chains

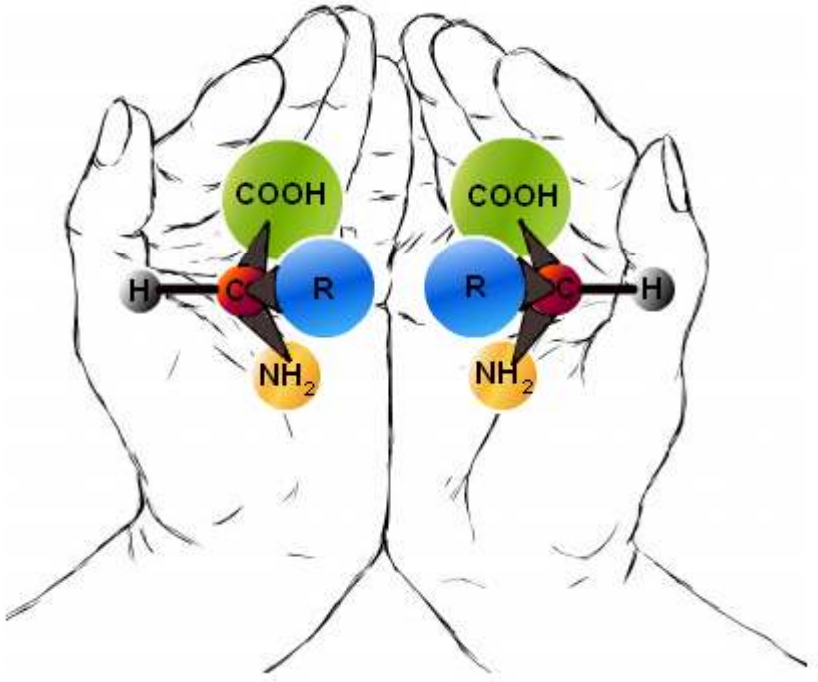


Selenocysteine, the 21st L-Amino Acid

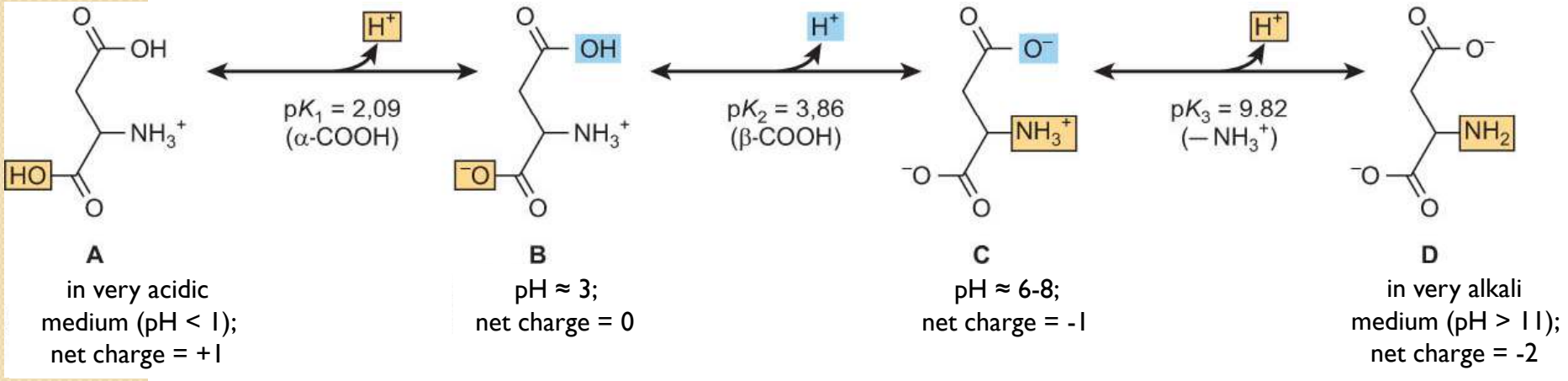


Only L-α-amino acids occur in proteins

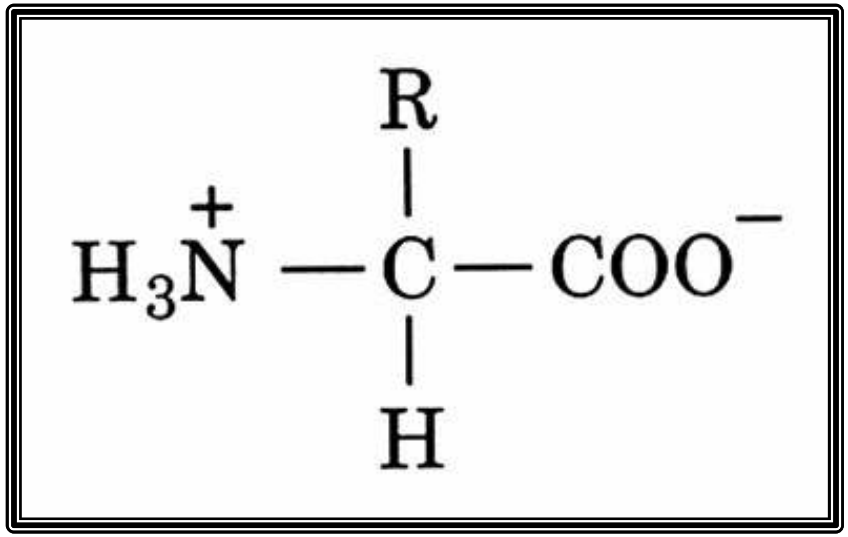


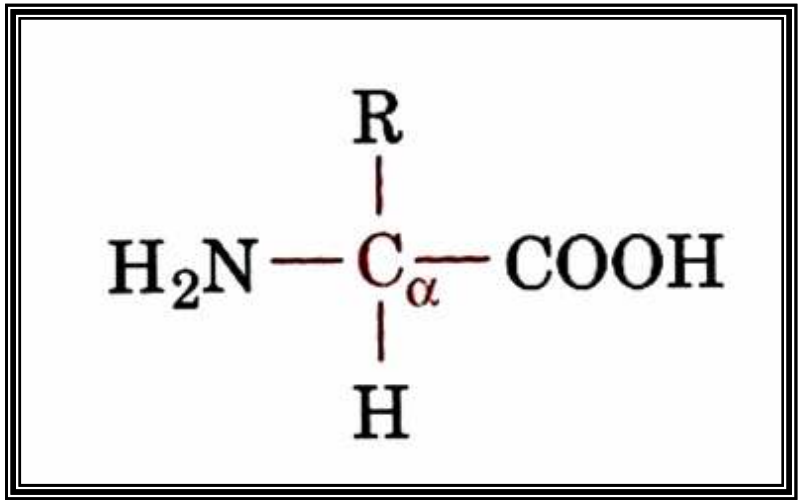


Amino Acids May Have Positive, Negative, or Zero Net Charge



Protonic equilibria of aspartic acid



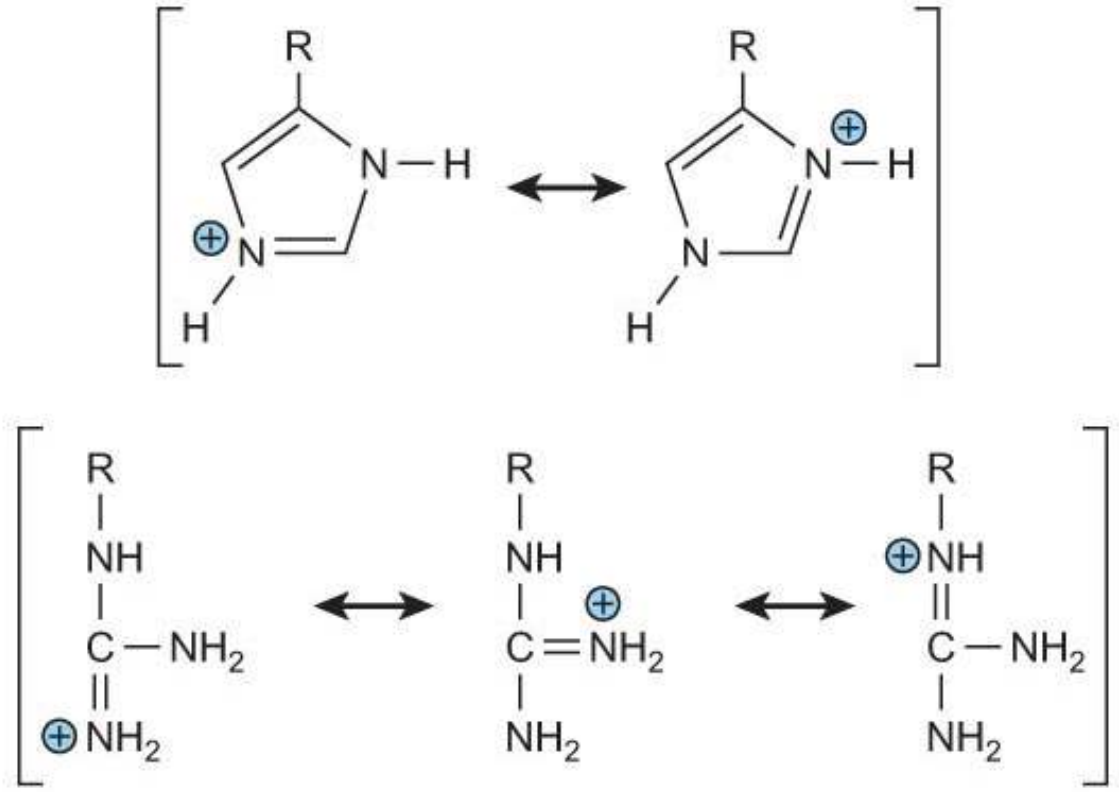


dual "ZWITTER" ION
(physiological pH)

Amino Acid	Abbreviation		pK_1	pK_2	pK_R	pI
	3-Letters	1-Letter	-COOH	$-NH_3^+$	R group	
Alanine	Ala	A	2.34	9.69	-	6.00
Arginine	Arg	R	2.17	9.04	12.48	10.76
Asparagine	Asn	N	2.02	8.80	-	5.41
Aspartic Acid	Asp	D	1.88	9.60	3.65	2.77
Cysteine	Cys	C	1.96	10.128	8.18	5.07
Glutamic Acid	Glu	E	2.19	9.67	4.25	3.22
Glutamine	Gln	Q	2.17	9.13	-	5.65
Glycine	Gly	G	2.34	9.60	-	5.97
Histidine	His	H	1.82	9.17	6.00	7.59
Isoleucine	Ile	I	2.36	9.60	-	6.02
Leucine	Leu	L	2.36	9.60	-	5.98
Lysine	Lys	K	2.18	8.95	10.53	9.74
Methionine	Met	M	2.28	9.21	-	5.74
Phenylalanine	Phe	F	1.83	9.13	-	5.48
Proline	Pro	P	1.99	10.60	-	6.30
Serine	Ser	S	2.21	9.15	-	5.58
Threonine	Thr	T	2.09	9.10	-	5.60
Tryptophan	Trp	W	2.83	9.39	-	5.89
Tyrosine	Tyr	Y	2.20	9.11	10.07	5.66
Valine	Val	V	2.32	9.62	-	5.96

From Lehninger Principle of Biochemistry.

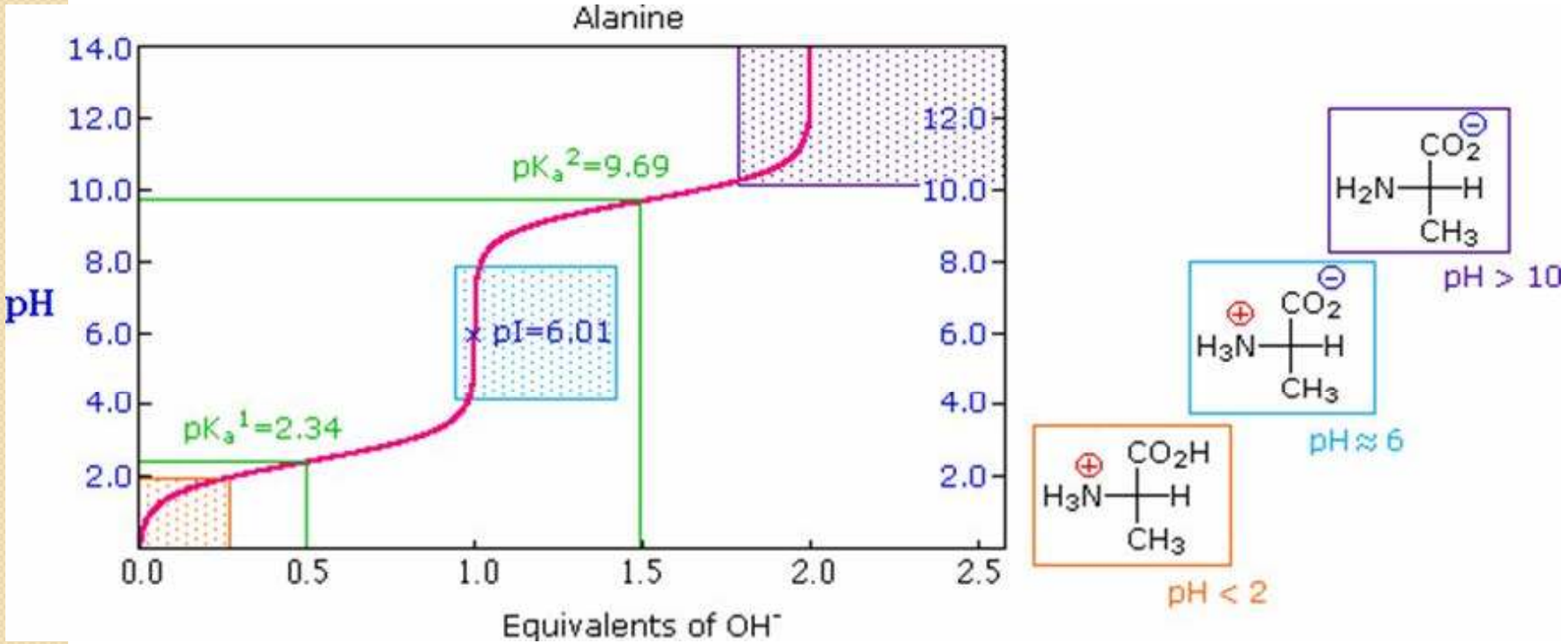
Resonance hybrids of the protonated forms of the R groups of histidine and arginine



Isoelectric pH

- The isoelectric pH, also called the pI, is the pH midway between pK_a values for the ionizations on either side of the isoelectric species

$$pI = \frac{pK1 + pK2}{2}$$


Alanine
pH
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.5
1.0
1.5
2.0
2.5
Equivalents of OH⁻
pK$_a$^{2}=9.69
pI=6.01
pK$_a$^{1}=2.34
pH > 10
pH ≈ 6
pH < 2

Typical Range of pK_a Values for Ionizable Groups in Proteins

Dissociating Group	pK_a Range
α-Carboxyl	3.5–4.0
Non α- COOH of Asp or Glu	4.0–4.8
Imidazole of His	6.5–7.4
SH of Cys	8.5–9.0
OH of Tyr	9.5–10.5
α-Amino	8.0–9.0
ε-Amino of Lys	9.8–10.4
Guanidinium of Arg	~12.0

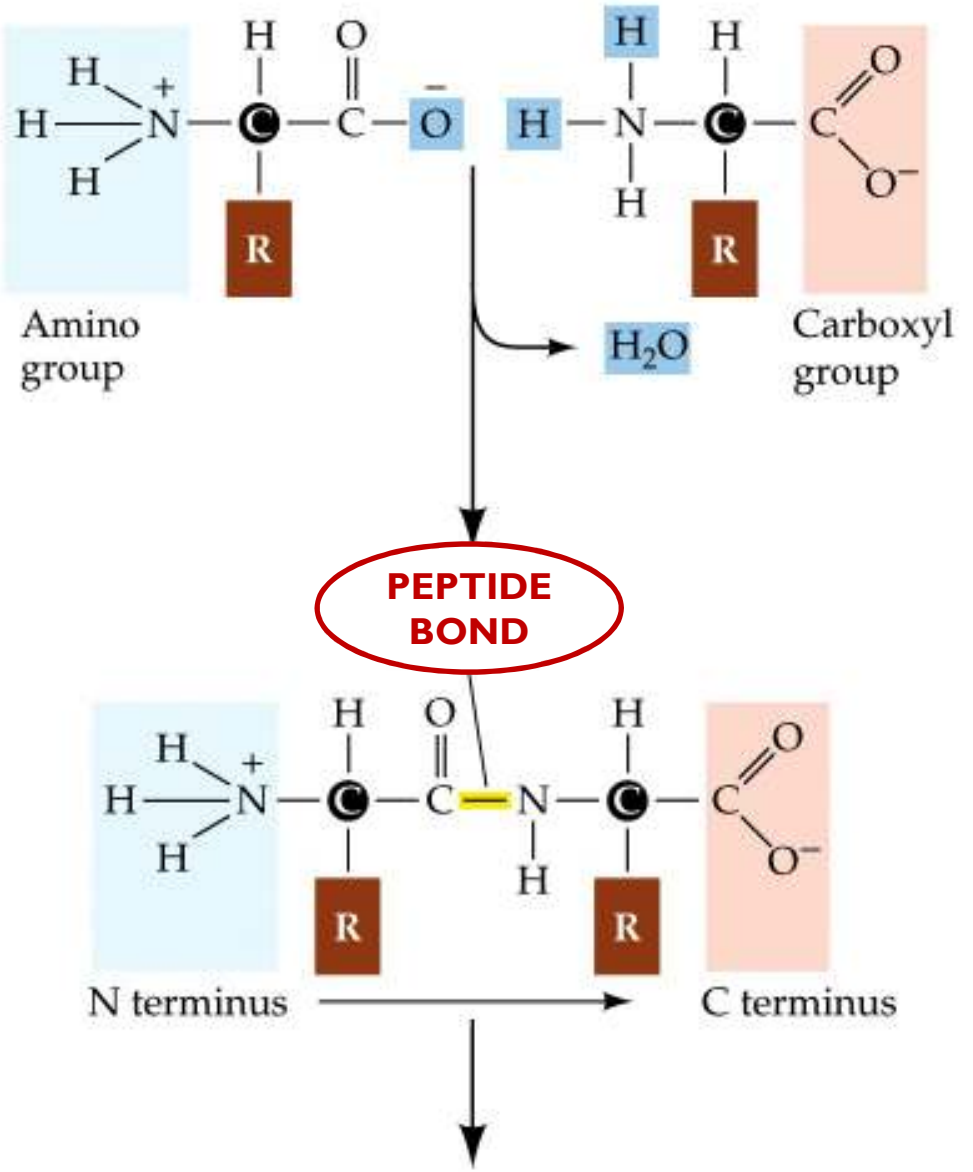
The Solubility of Amino Acids Reflects Their Ionic Character

- soluble in polar solvents, insoluble in nonpolar solvents
- amino acids do not absorb visible light
- Tyr, Phe and Trp absorb UV light

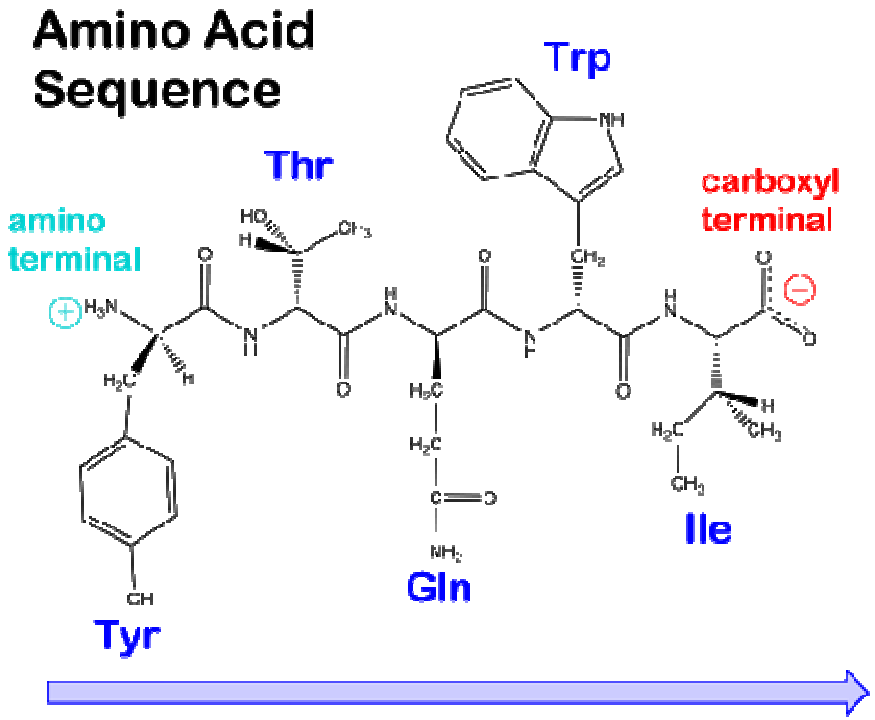
- α-R-GROUPS determine the properties of amino acids
- FUNCTIONAL GROUPS dictate the chemical reactions of amino acids

Reactions of amino acids

- R-groups is changing after incorporation of amino acid in protein
- OXIDATION -SH group in Cys builds DISULPHID BONDS (stabilize structure of secretory proteins)
- HYDROXYLATION Prol, Lys (stabilize collagen, crucial in vitamin C)
- GLYCOSYLATION (on Ser, Thr, Asn; for synthesis of secretory and membrane proteins; glycosylation of Hb in HbA_{1c}, in weakly controlled diabetes)
- PHOSPHORILATION (Ser, Thr, Tyr; modifies activity of many enzymes (i.e. glycogen synthetase)
- The most important: forming of PEPTIDE BOND



Amino Acid Sequence Determines PRIMARY STRUCTURE



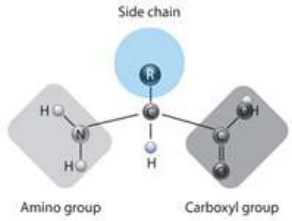
tyrosyl-threonyl-glutaminyl-tryptophanyl-isoleucine

Tyr-Thr-Gln-Trp-Ile

YTQWI

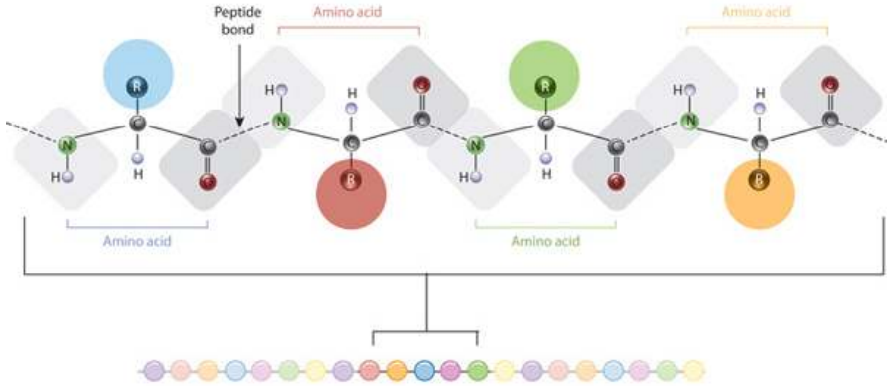
DIPEPTIDE, TRIPEPTIDE:

2, or 3 amino acid residues



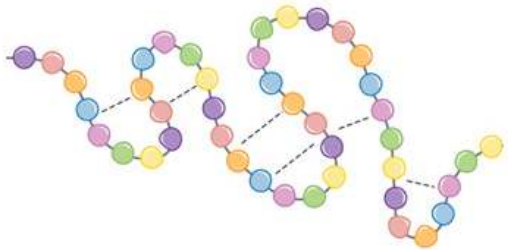
OLIGOPEPTIDE:

from 3 to 10 amino acid residues



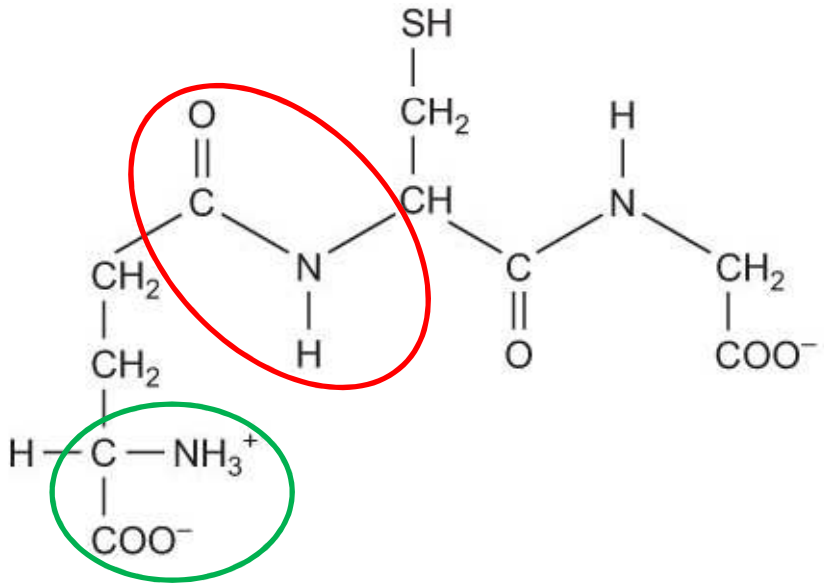
POLYPEPTIDE:

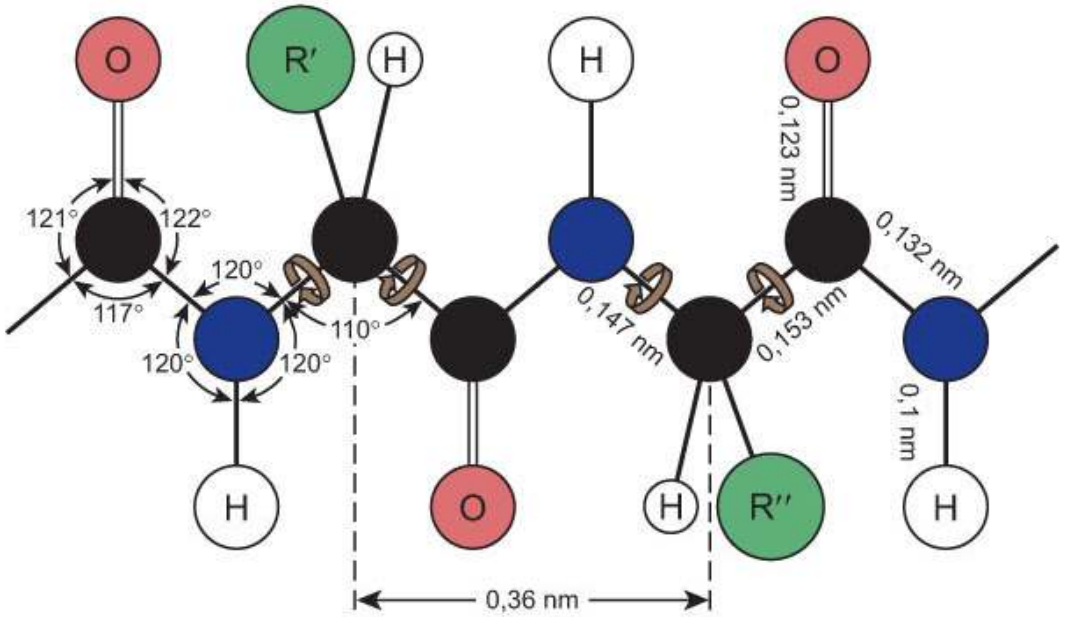
more than 10 amino acid residues



Glutathione (-glutamyl-cysteinyl-glycine).

Note the non- peptide bond that links Glu to Cys.

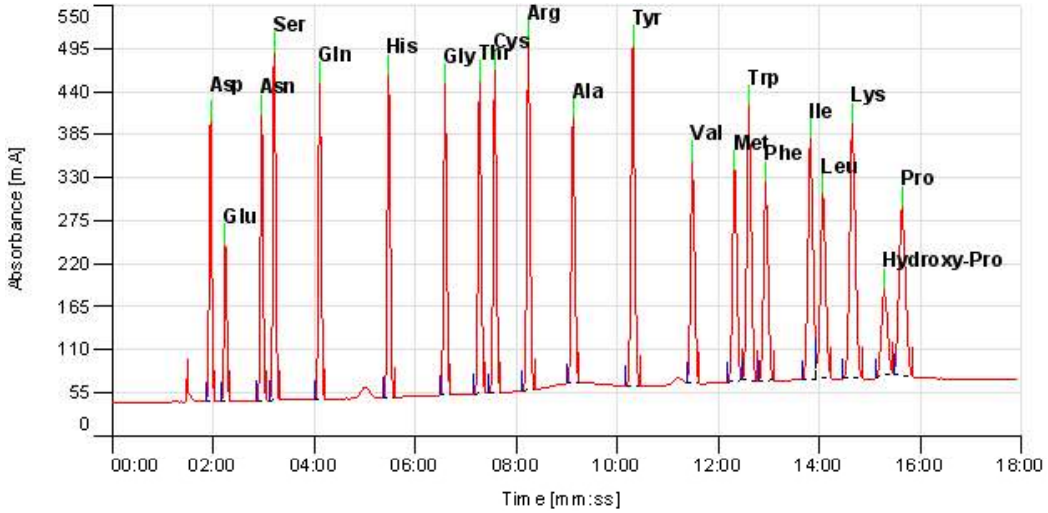




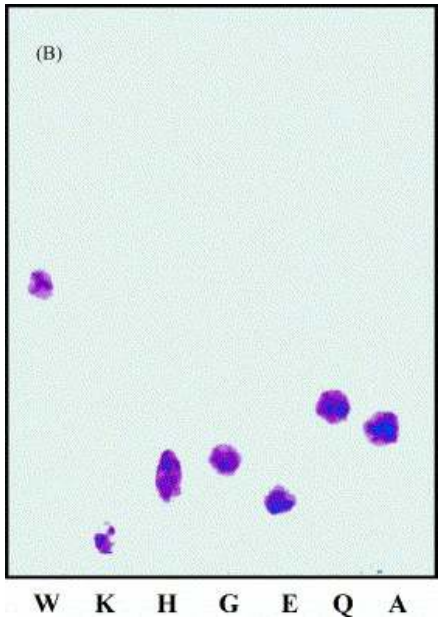
Dimensions of a fully extended polypeptide chain. The four atoms of the peptide bond are coplanar. Free rotation can occur about the bonds that connect the -carbon with the -nitrogen and with the -carbonyl carbon (brown arrows). The extended polypeptide chain is thus a semirigid structure with two-thirds of the atoms of the backbone held in a fixed planar relationship one to another. The distance between adjacent -carbon atoms is 0.36 nm (3.6 Å). The interatomic distances and bond angles, which are not equivalent, are also shown.

Analysis of the Amino Acid Content

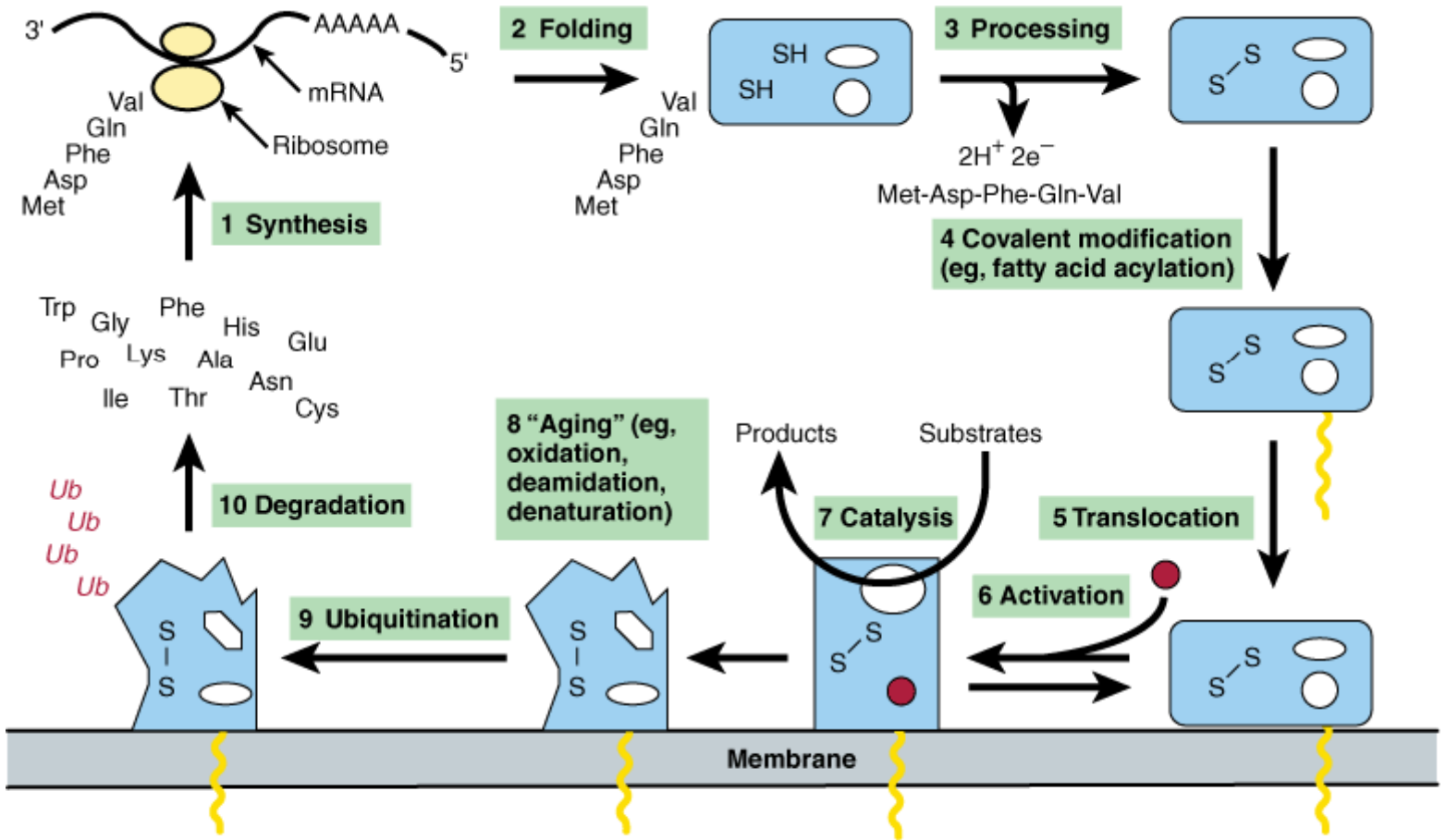
HPLC



TLC



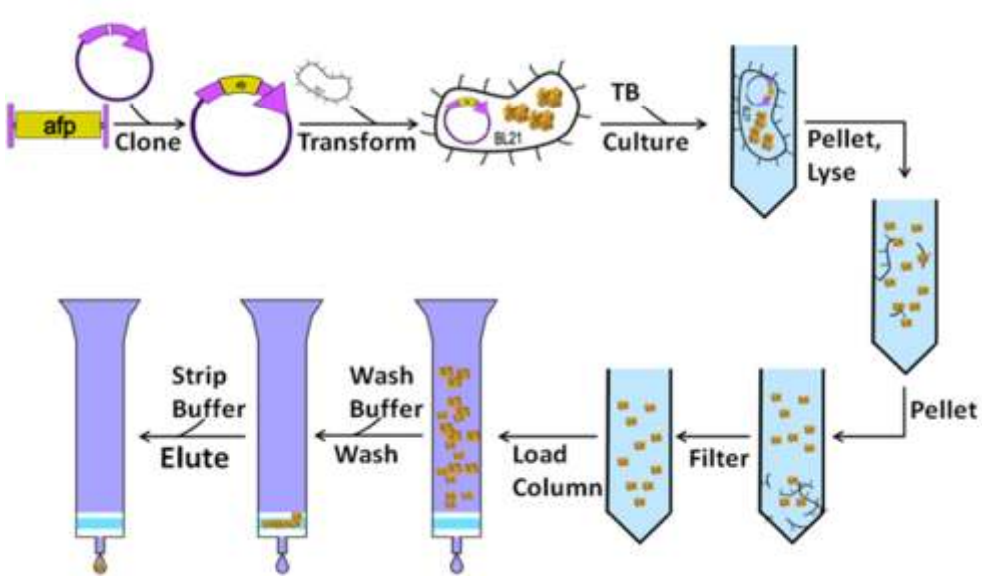
"Life cycle" of a protein



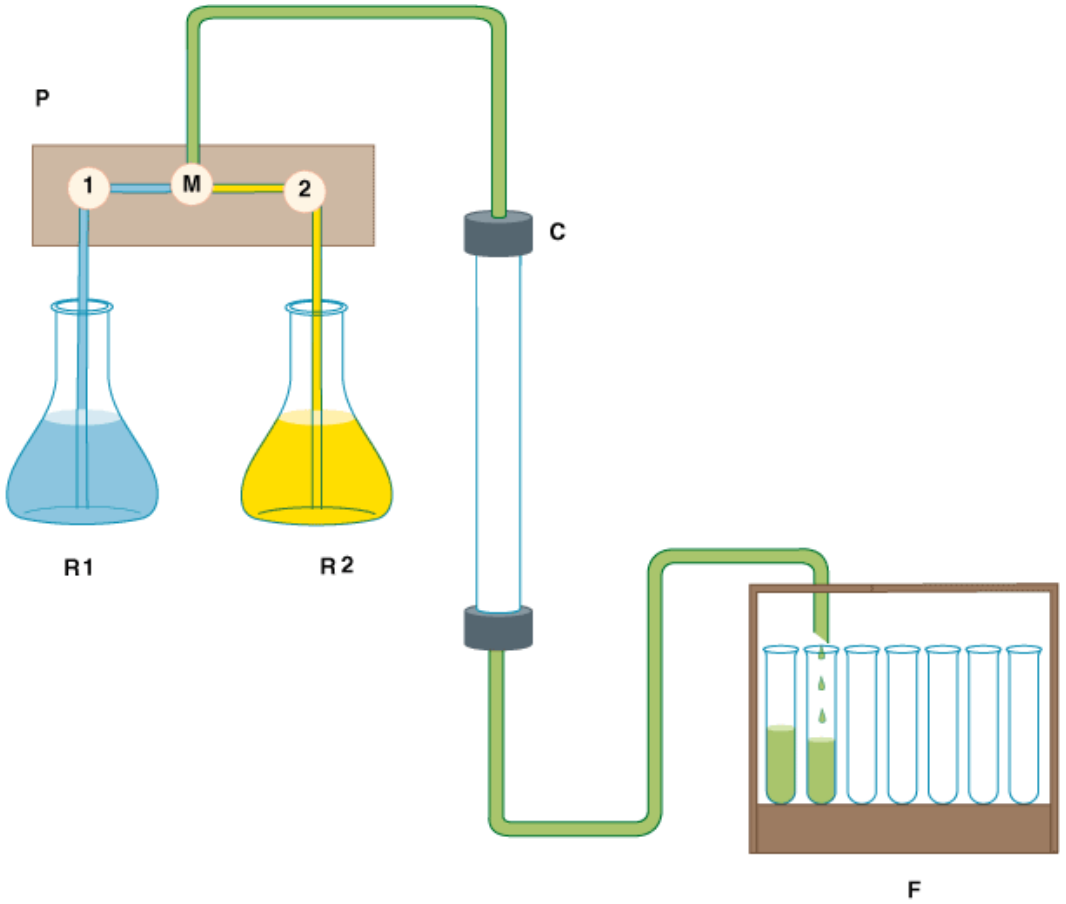
Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 29th Edition*: www.accessmedicine.com

Determination of Primary Structure

- Proteins & Peptides Must Be Purified Prior to Analysis

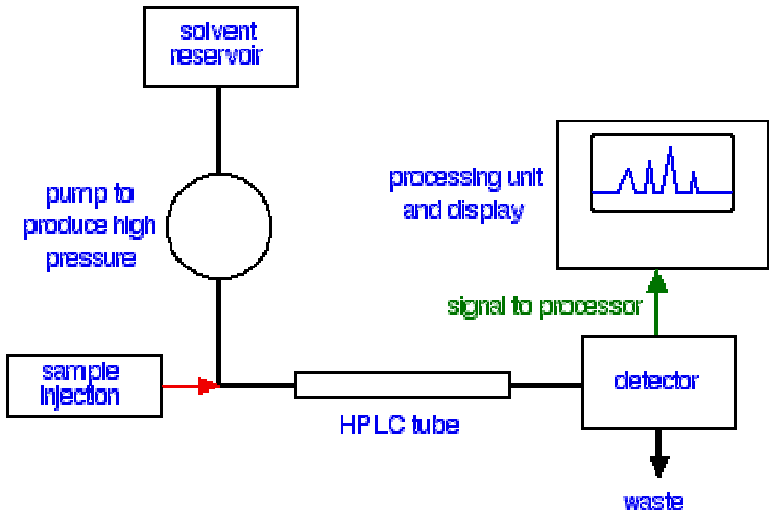


Column Chromatography

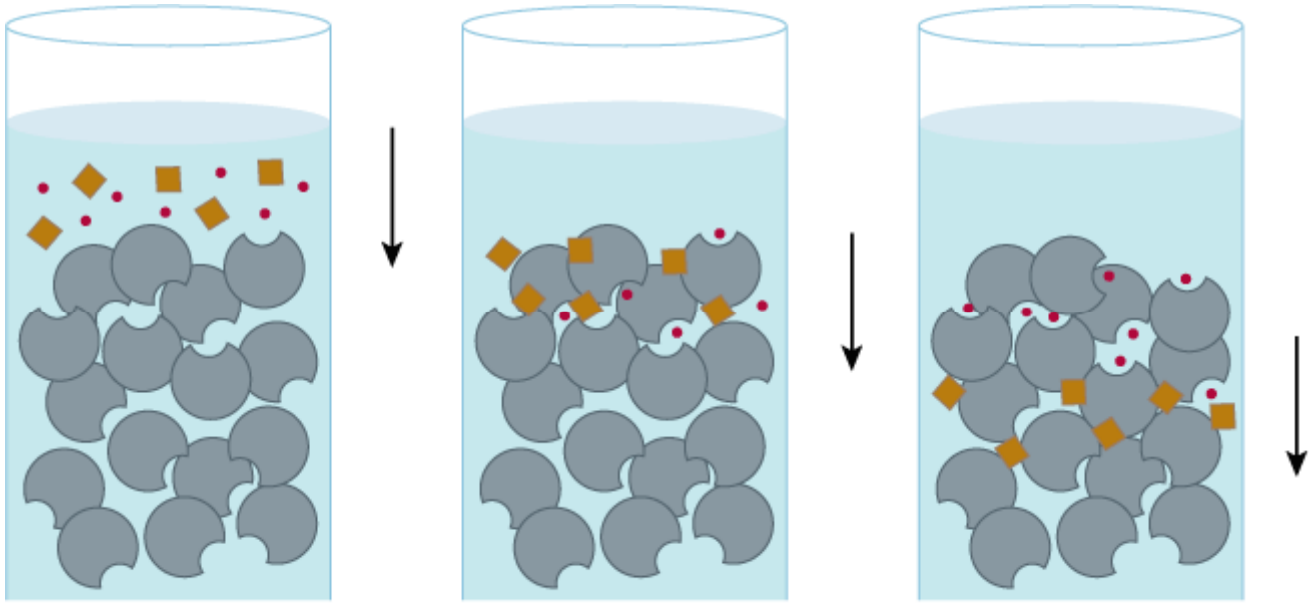


Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 29th Edition*: www.accessmedicine.com

HPLC—High-Pressure Liquid Chromatography

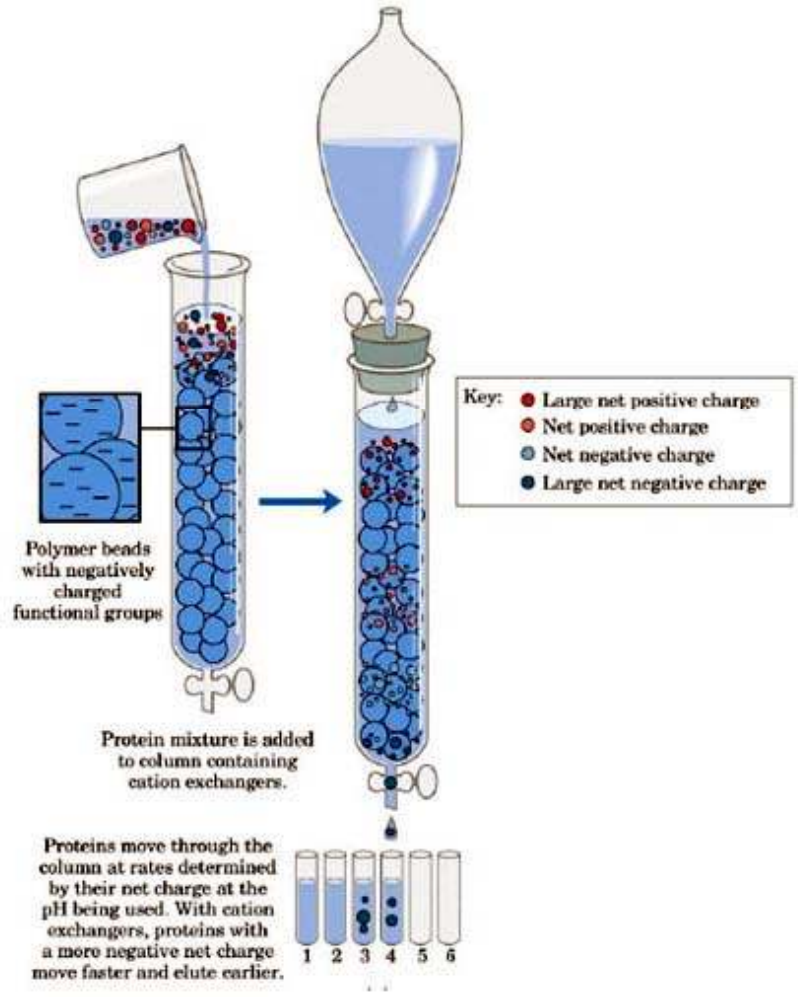


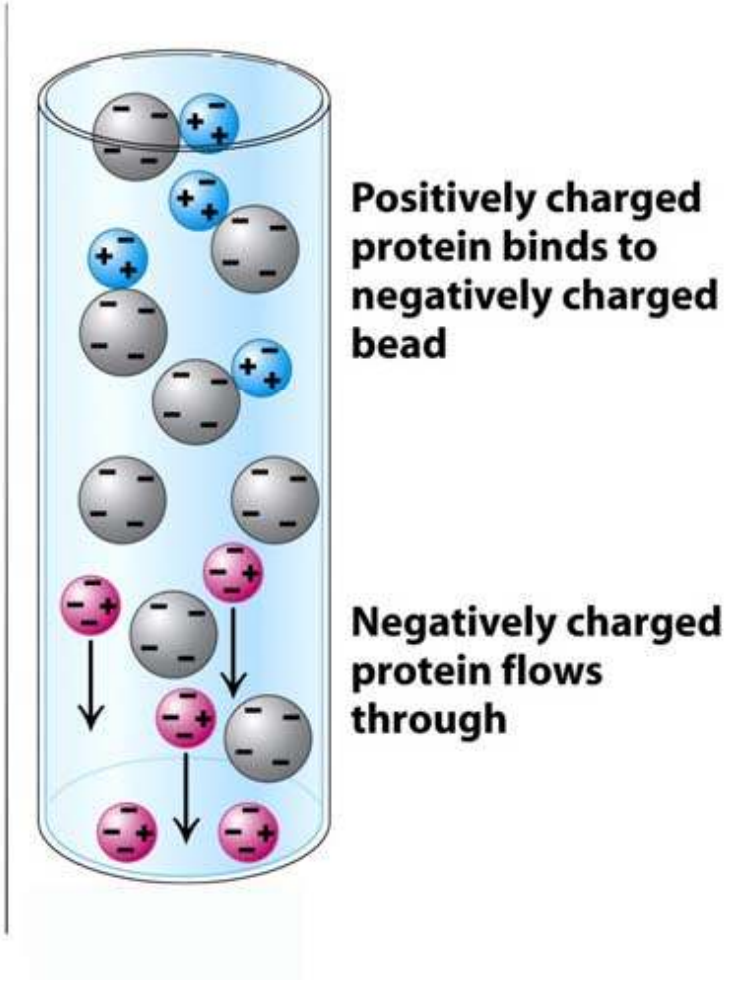
Size-Exclusion Chromatography



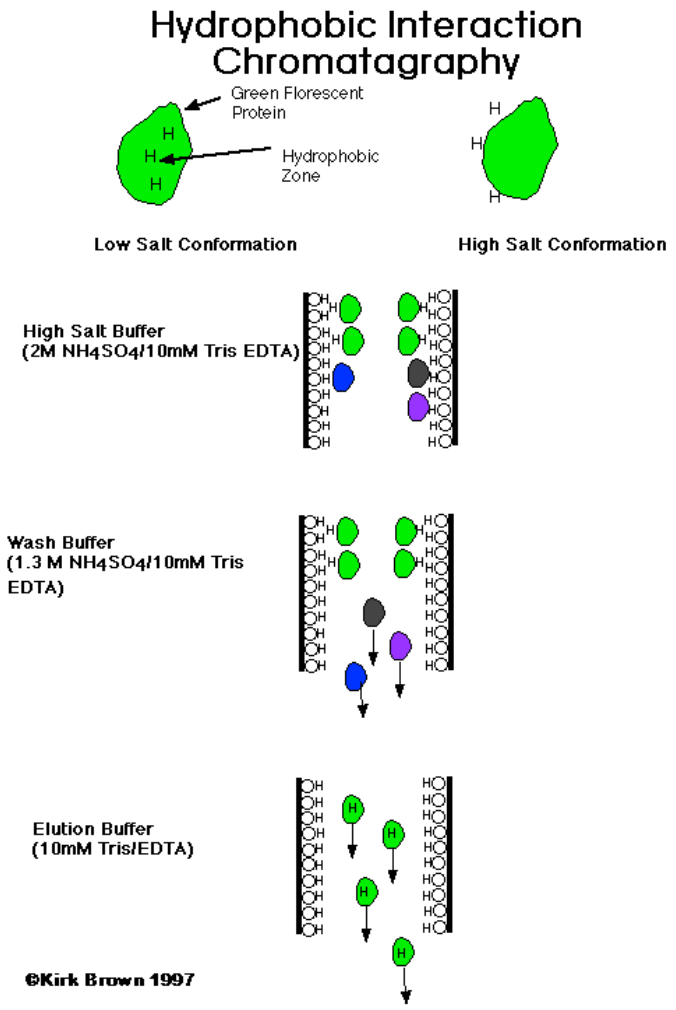
Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 29th Edition*: www.accessmedicine.com

Ion-Exchange Chromatography

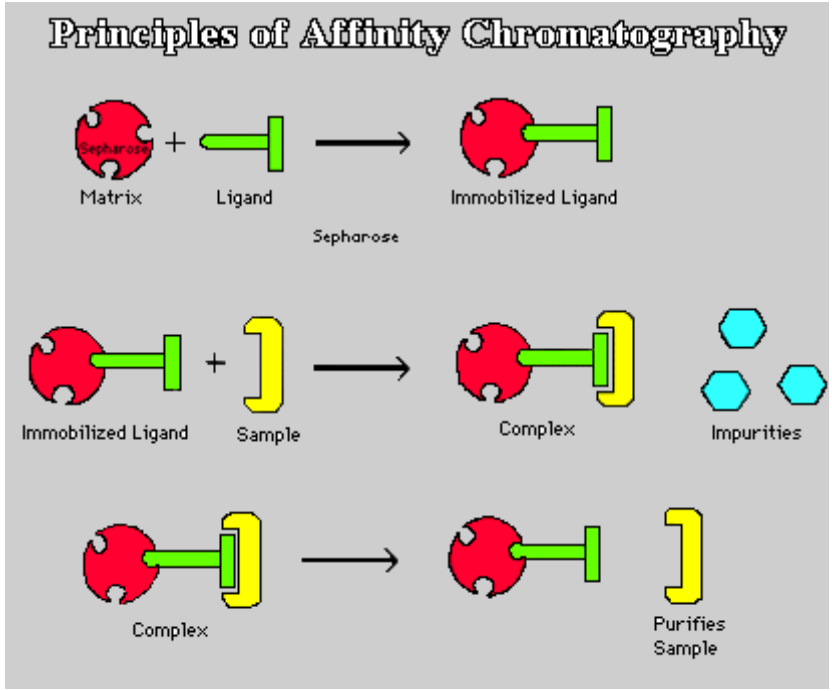




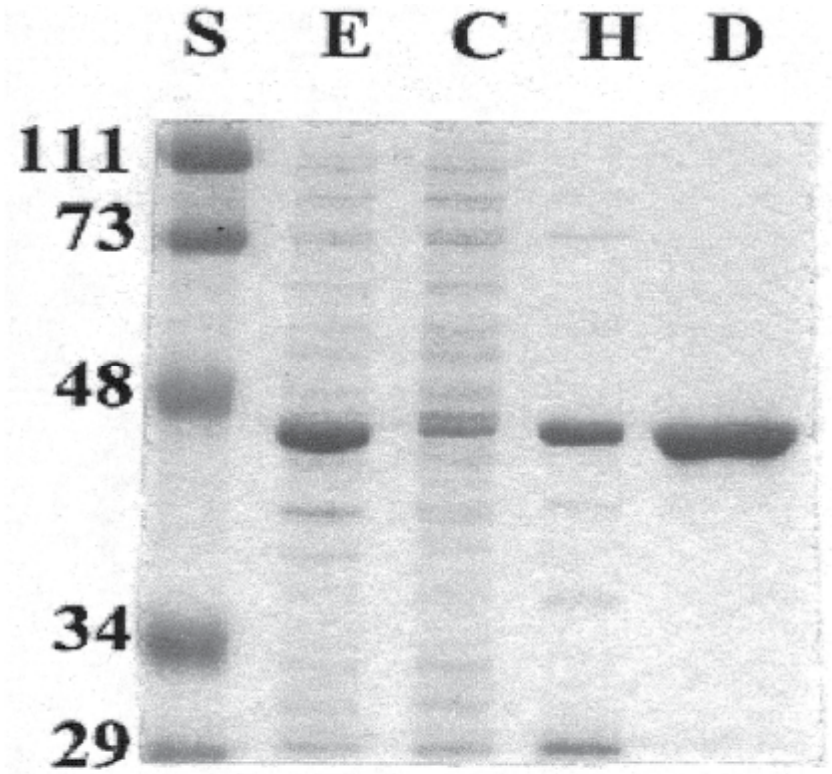
Hydrophobic Interaction Chromatography



Affinity Chromatography

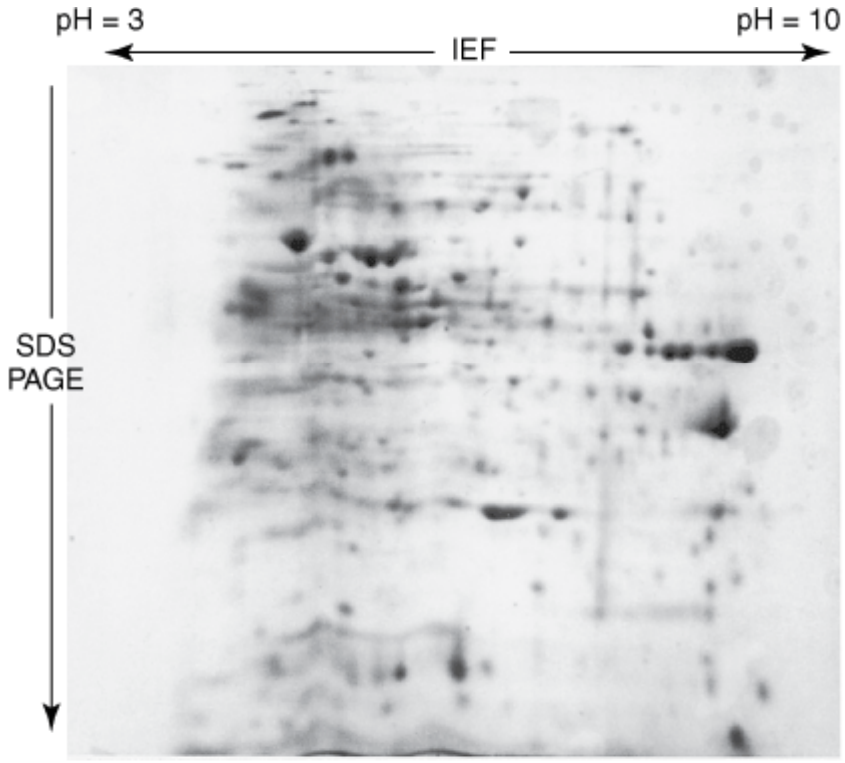


Protein Purity Is Assessed by Polyacrylamide Gel Electrophoresis (PAGE)



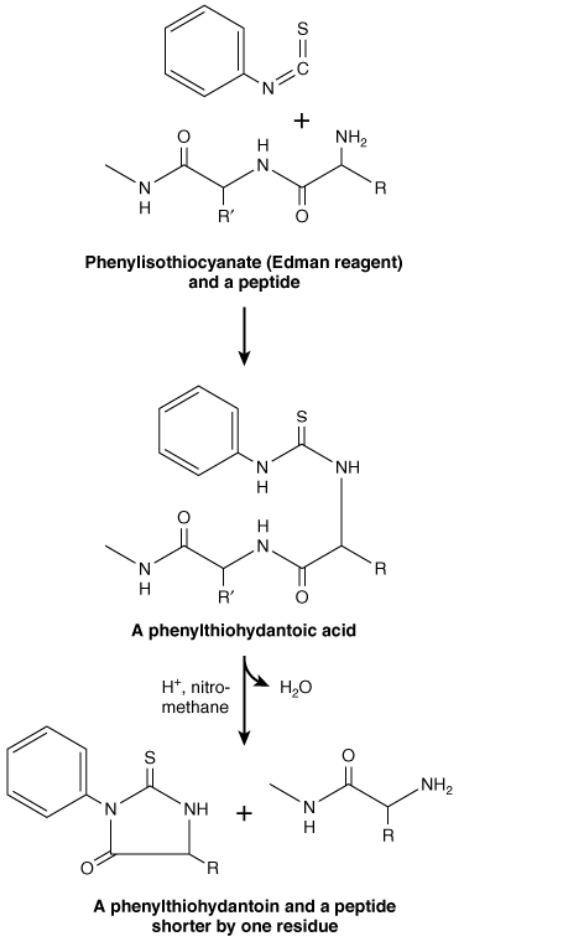
Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 29th Edition*: www.accessmedicine.com

Isoelectric Focusing (IEF)

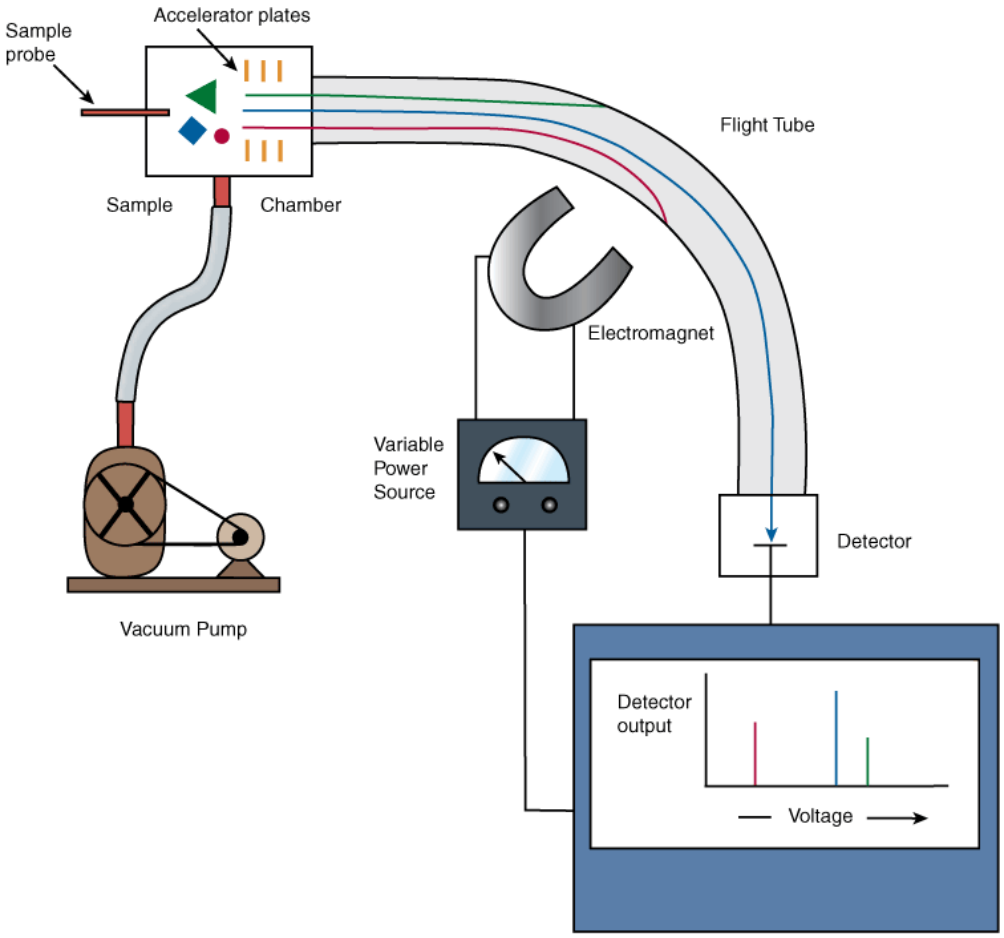


Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 29th Edition*: www.accessmedicine.com

Edman's reaction



Mass Spectrometry



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 29th Edition*: www.accessmedicine.com

Summary

- Only L-amino acids are present in proteins
- Two functional groups, R—NH_3^+ and R—COOH
- pI is the pH at which an amino acid bears no net charge
- The most important biochemical reaction of amino acids is the formation of peptide bonds
- Amino acids are classified as basic, acidic, aromatic, aliphatic, or sulfur containing based on the properties of their R groups

Summary

- Peptides are named for the number of amino acid residues present, and as derivatives of the carboxyl terminal residue
- The primary structure of a peptide is its amino acid sequence, starting from the amino-terminal residue
- Proteins undergo post-transitional alterations during their lifetime
- All proteins expressed in a living cell = proteome