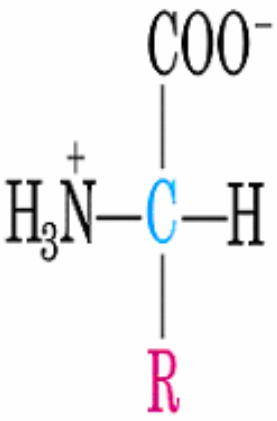
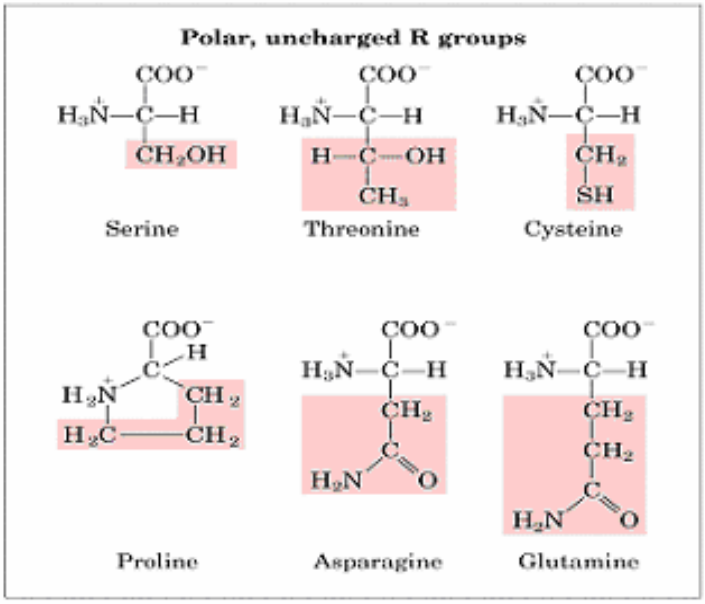
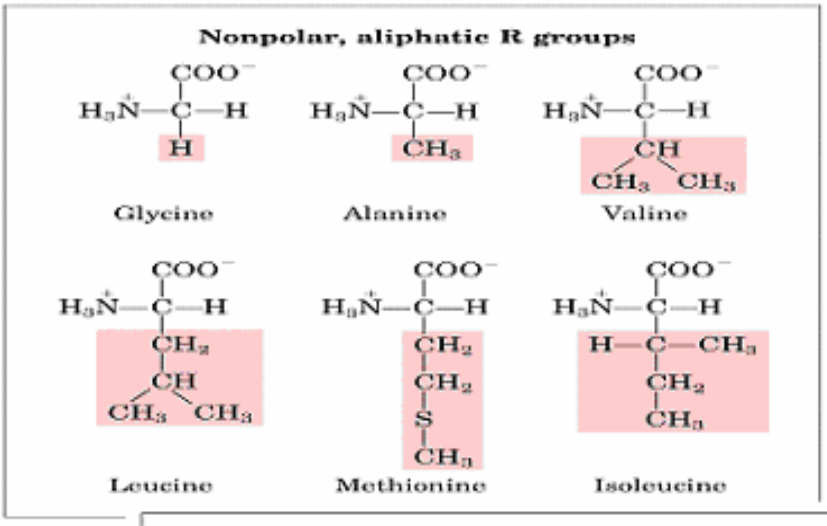
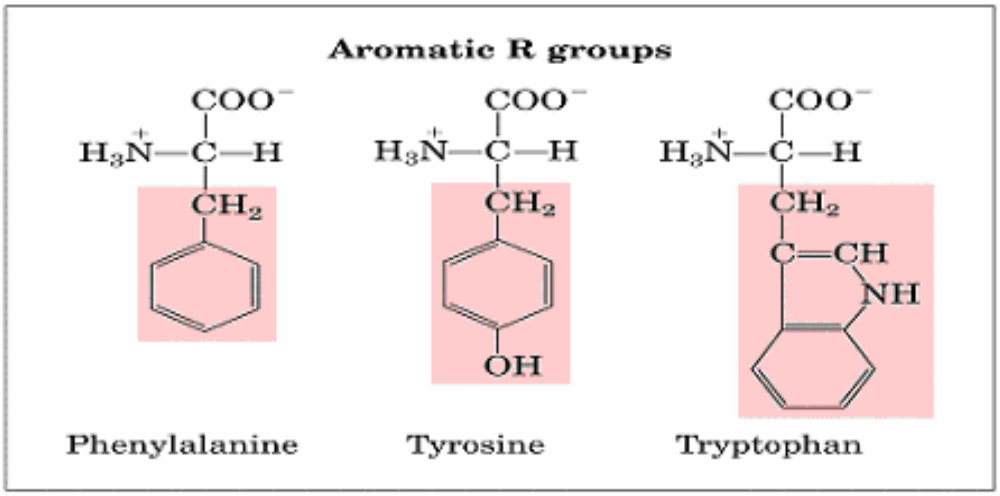
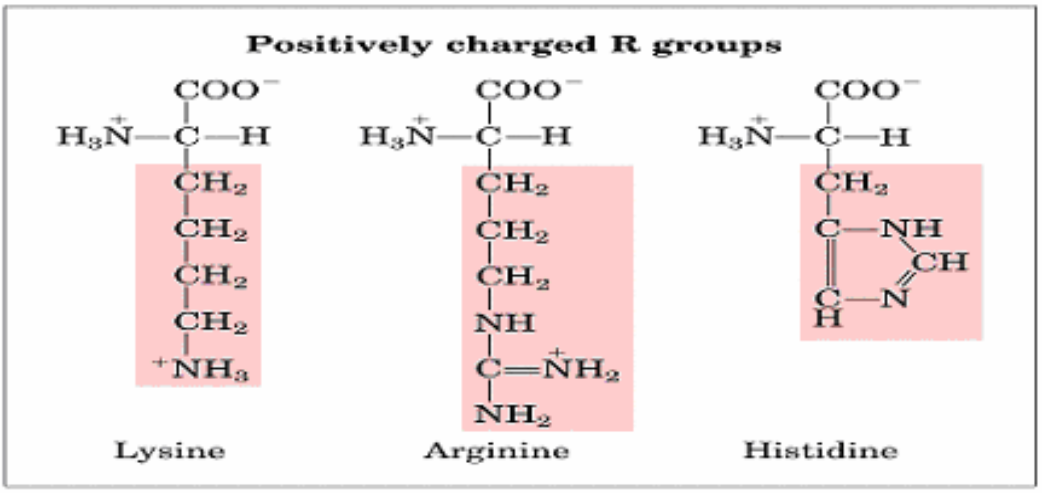
Amino Acids, Peptides and Proteins

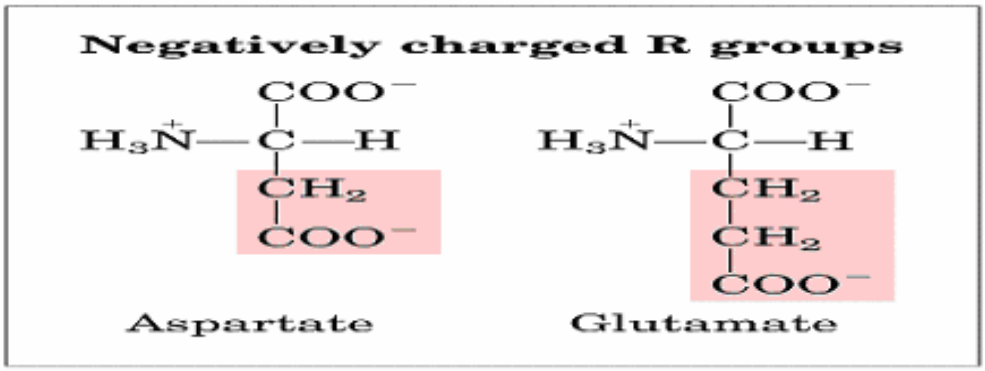




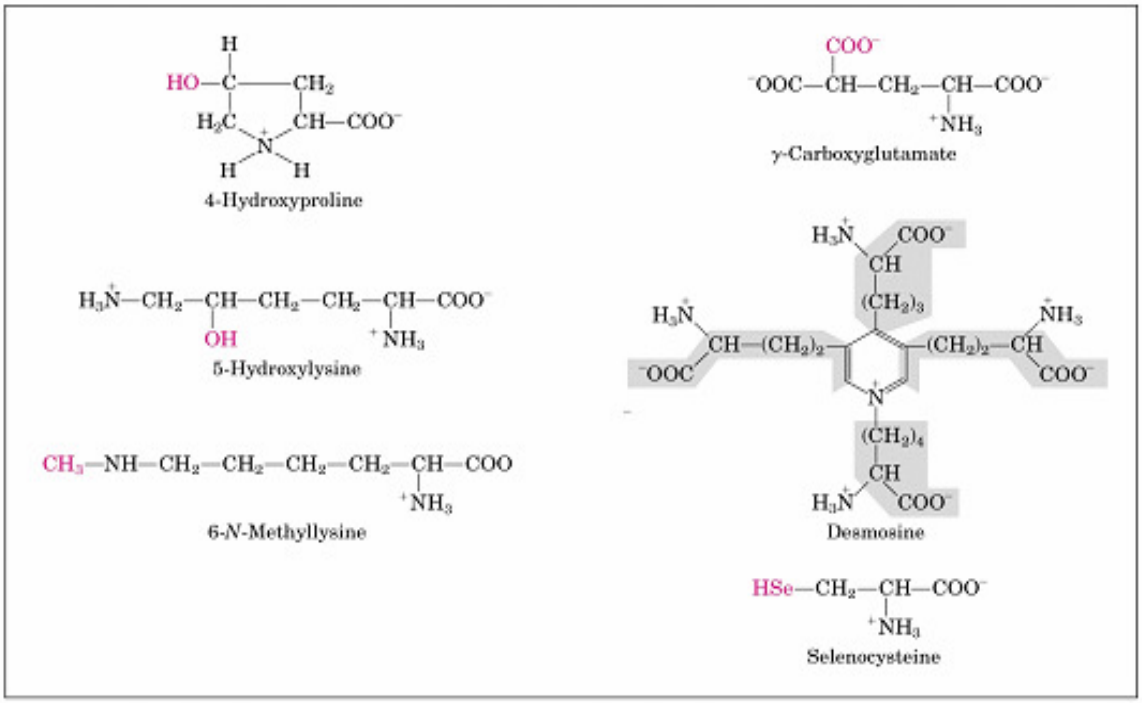




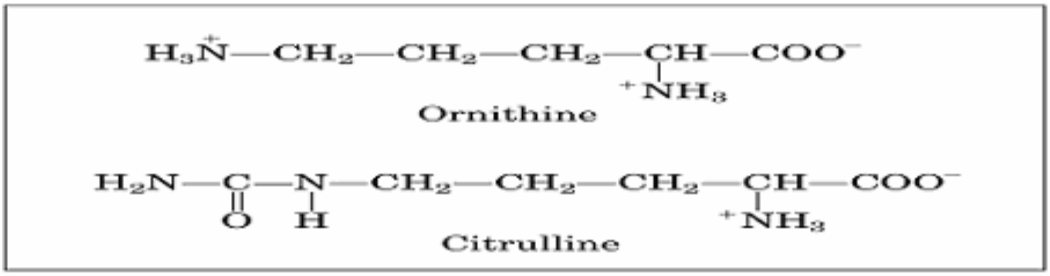




Non-standard amino acids

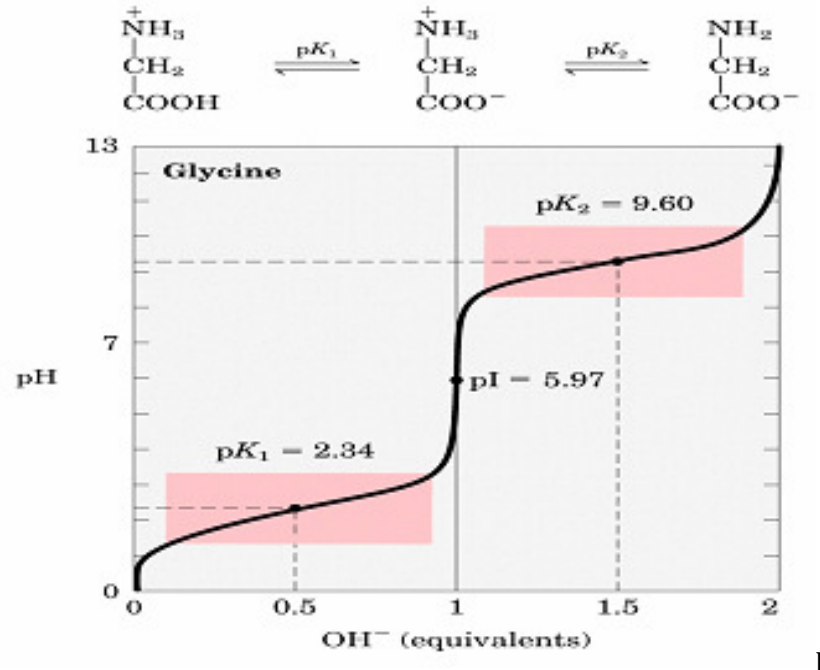


(a)



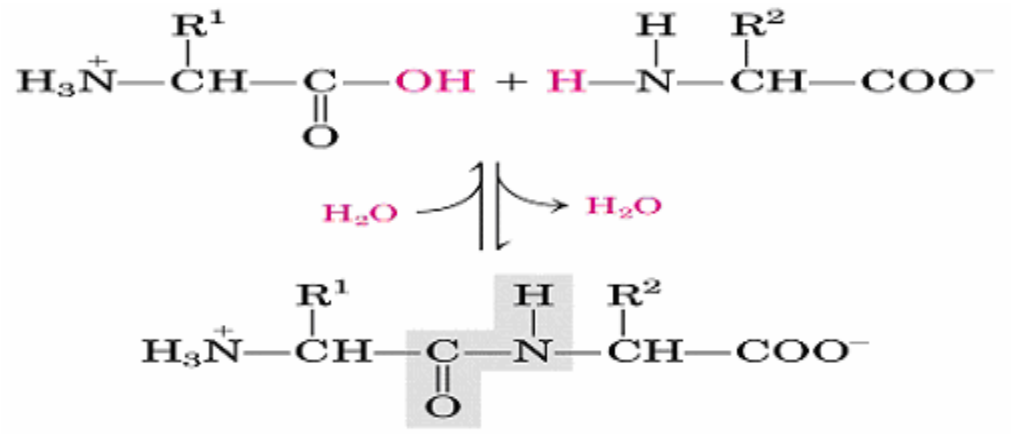
(b)

Titration of Amino acids

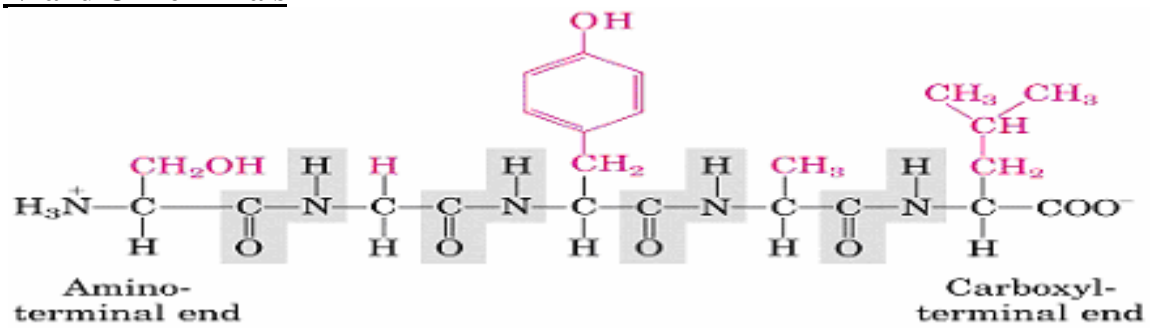


pI = Isolelectric point

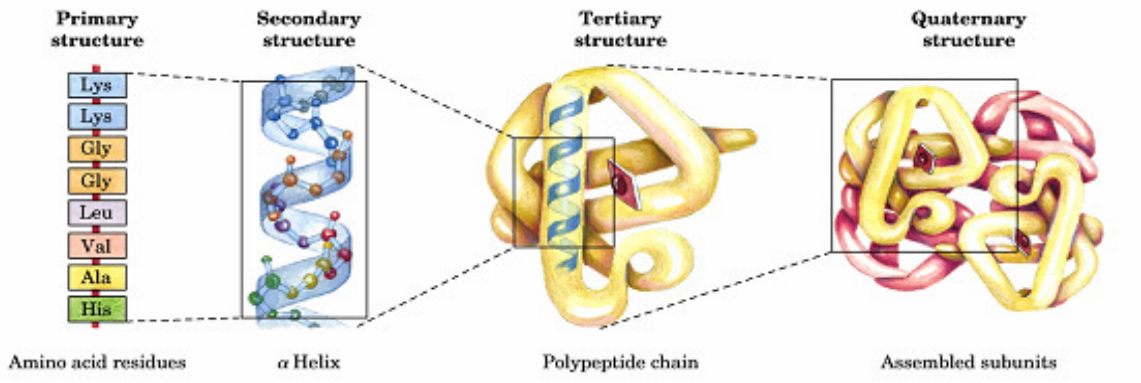
Formation of a peptide bond by condensation



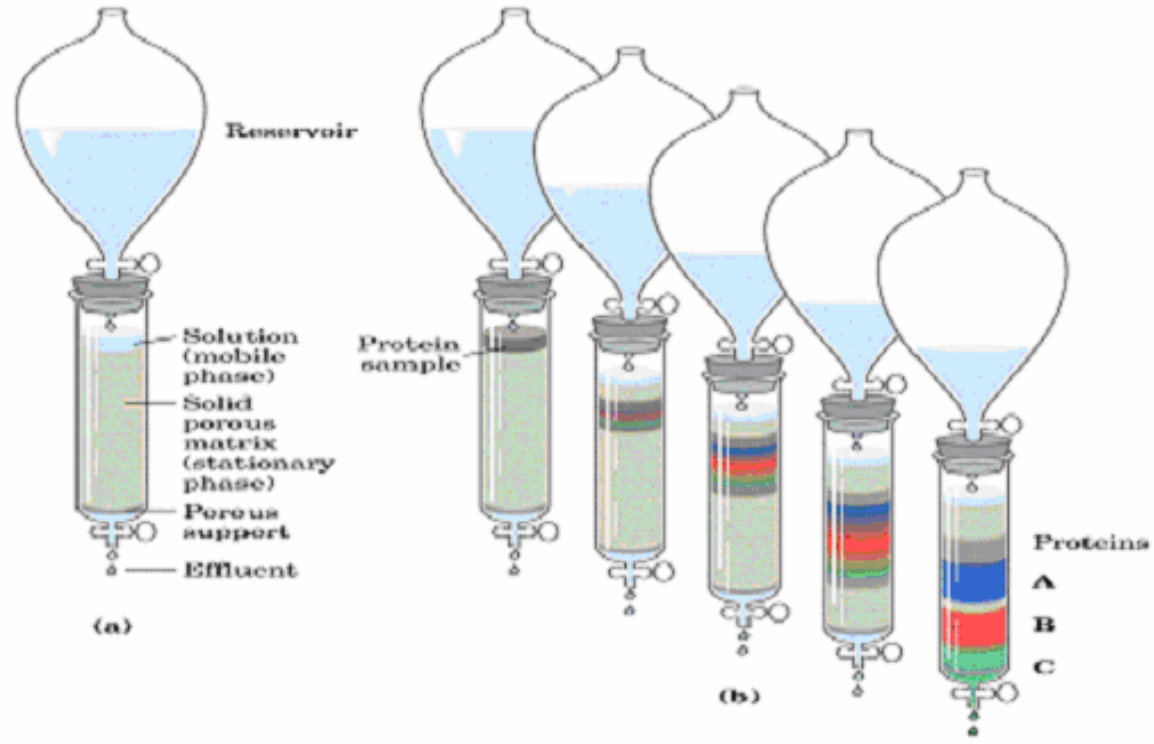
N- and C- Terminals



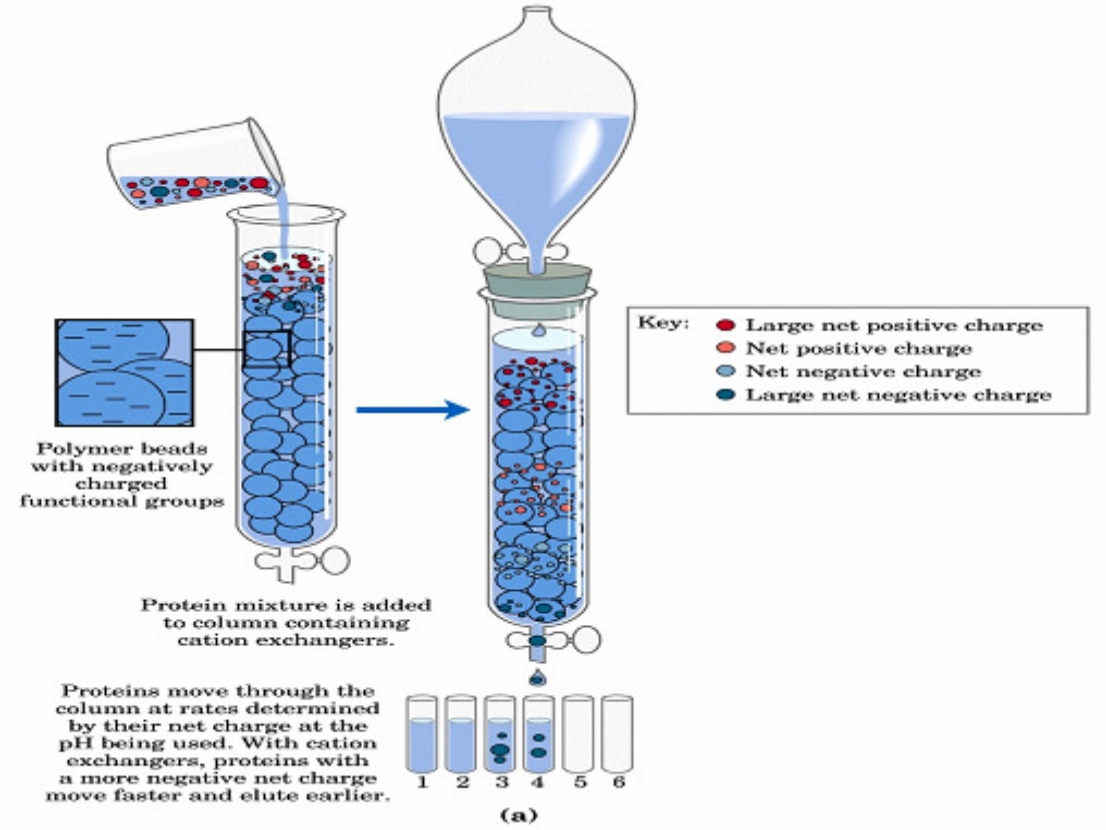
Levels of structure in proteins



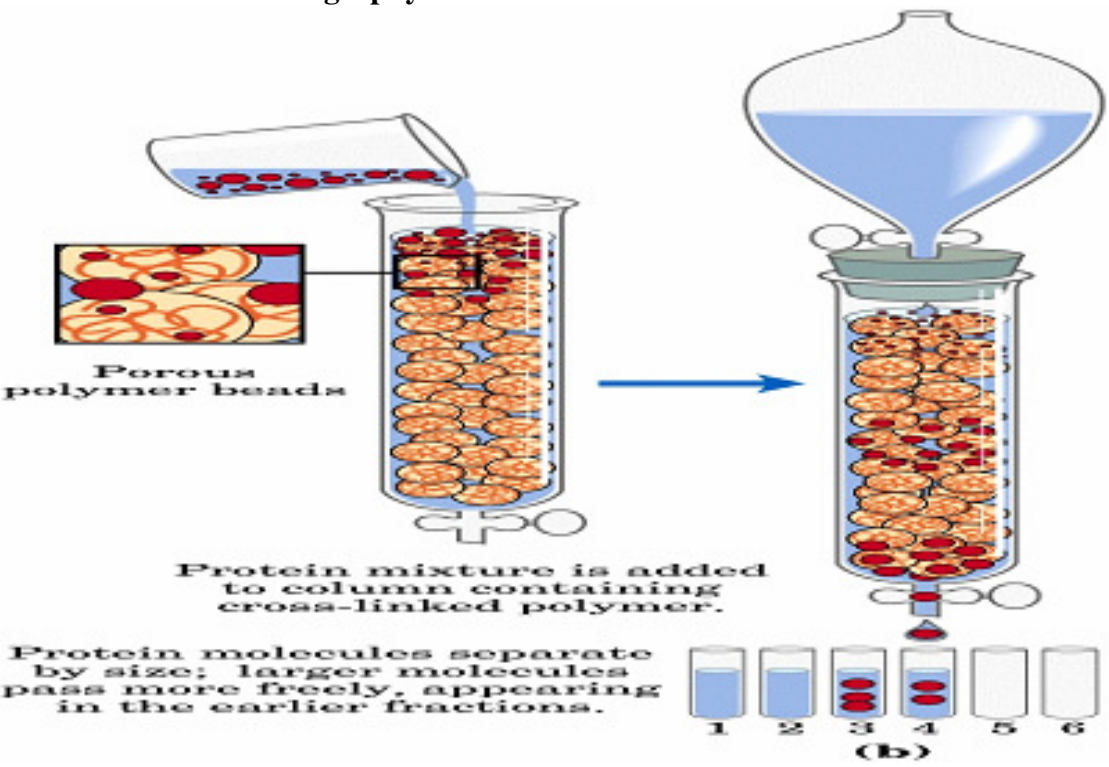
Column chromatography



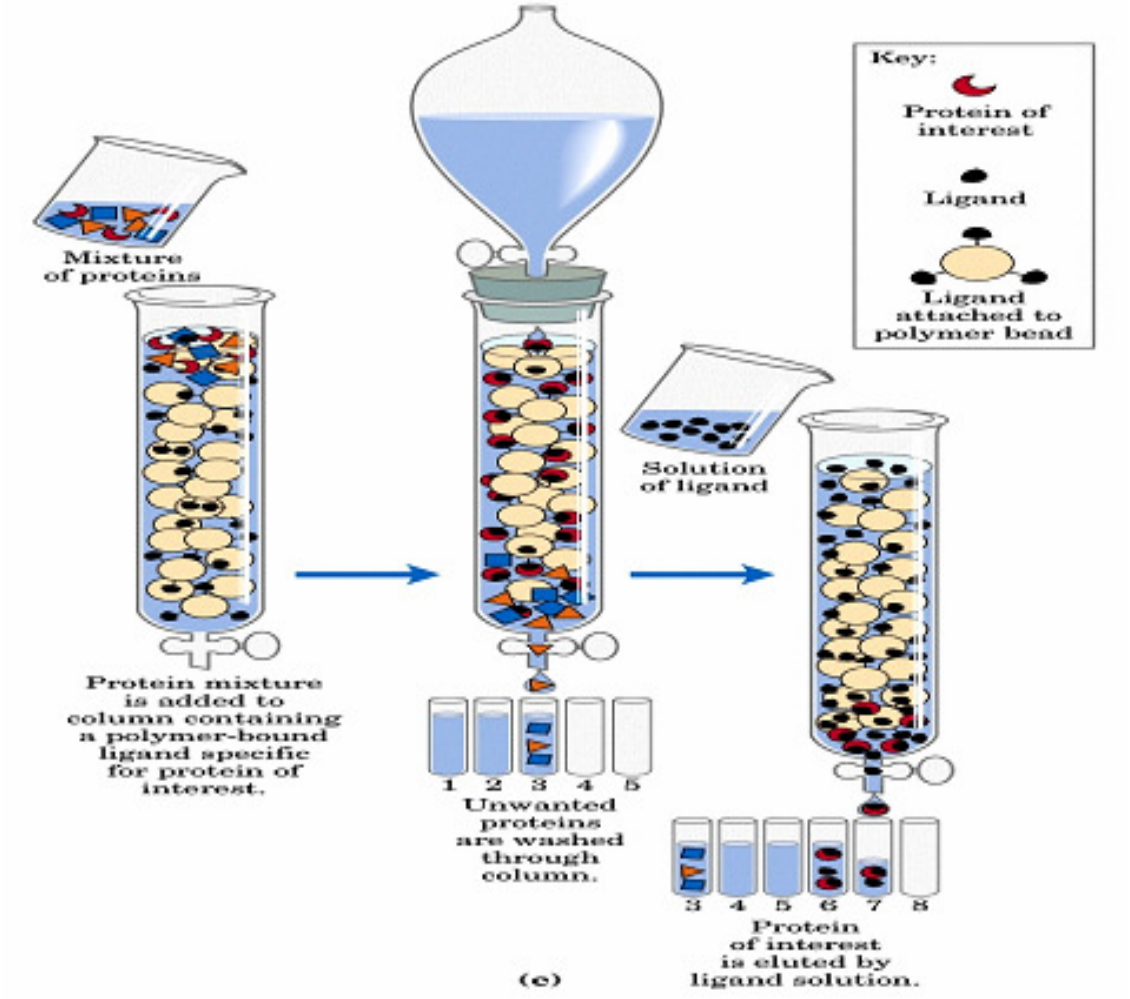
Ion-Exchange chromatography



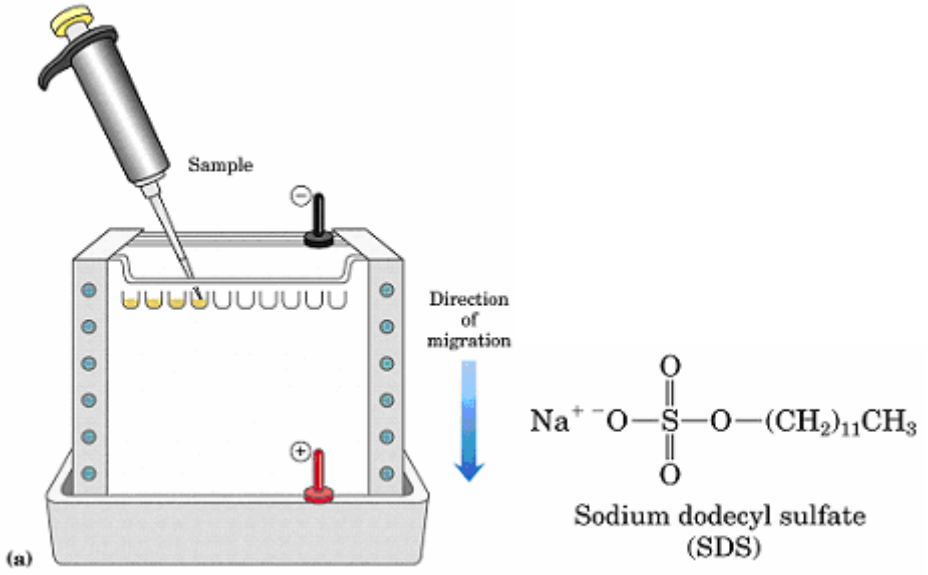
Size Exclusion Chromatography

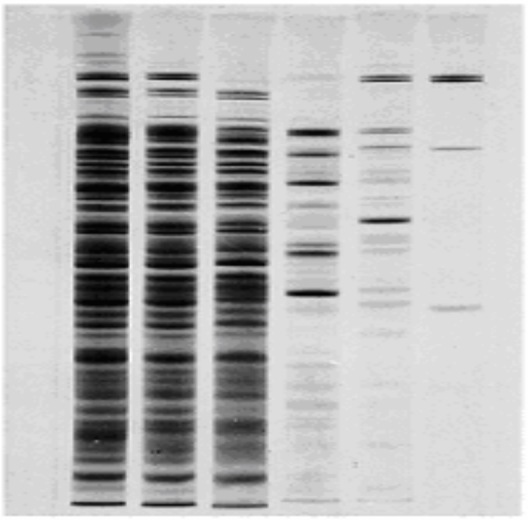


Affinity Chromatography

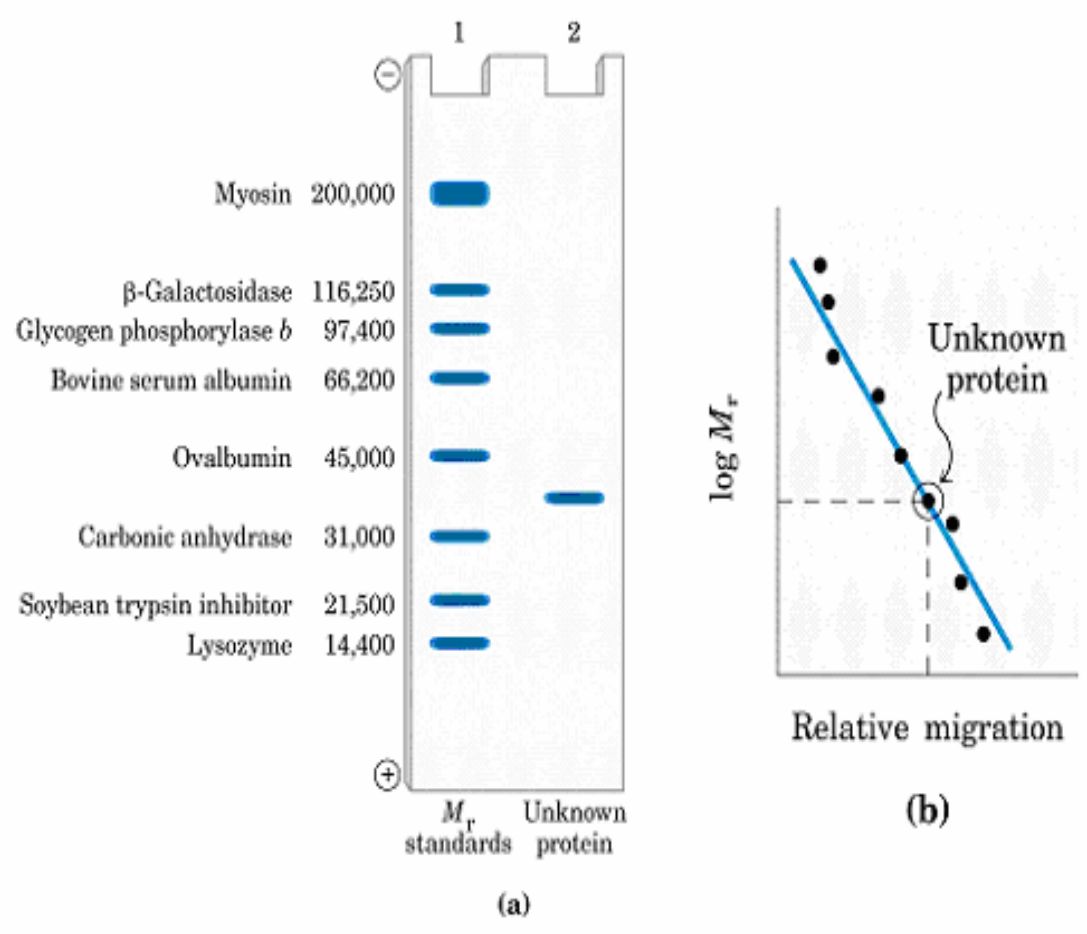


Electrophoresis

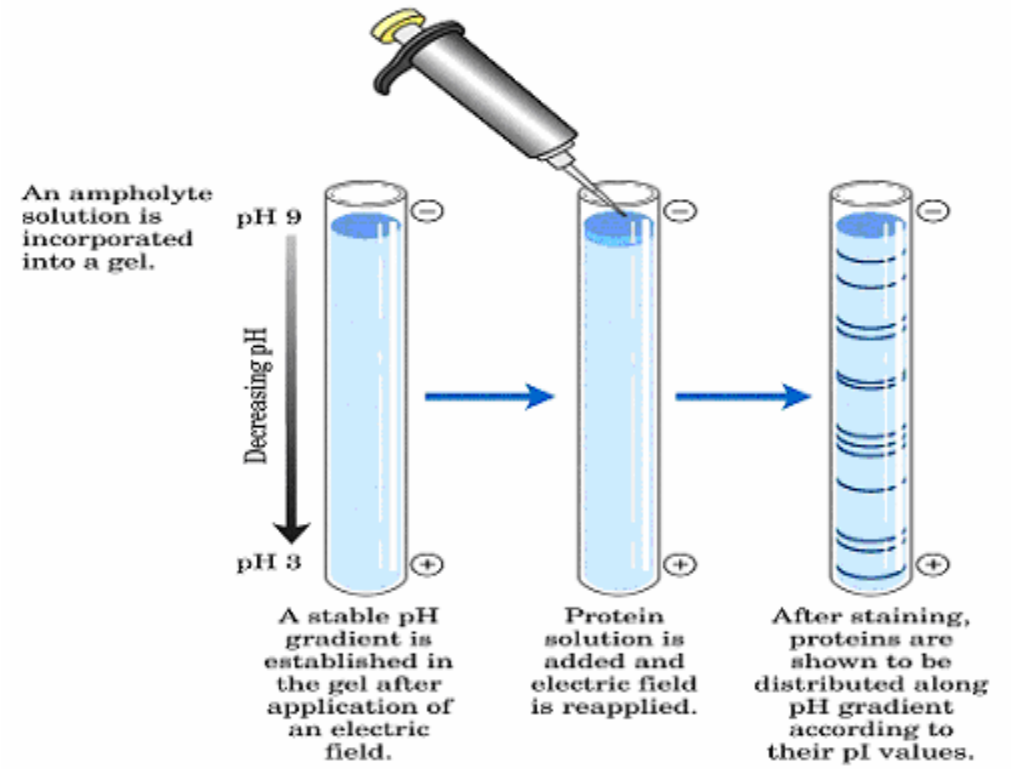




Estimating the molecular weight of a protein



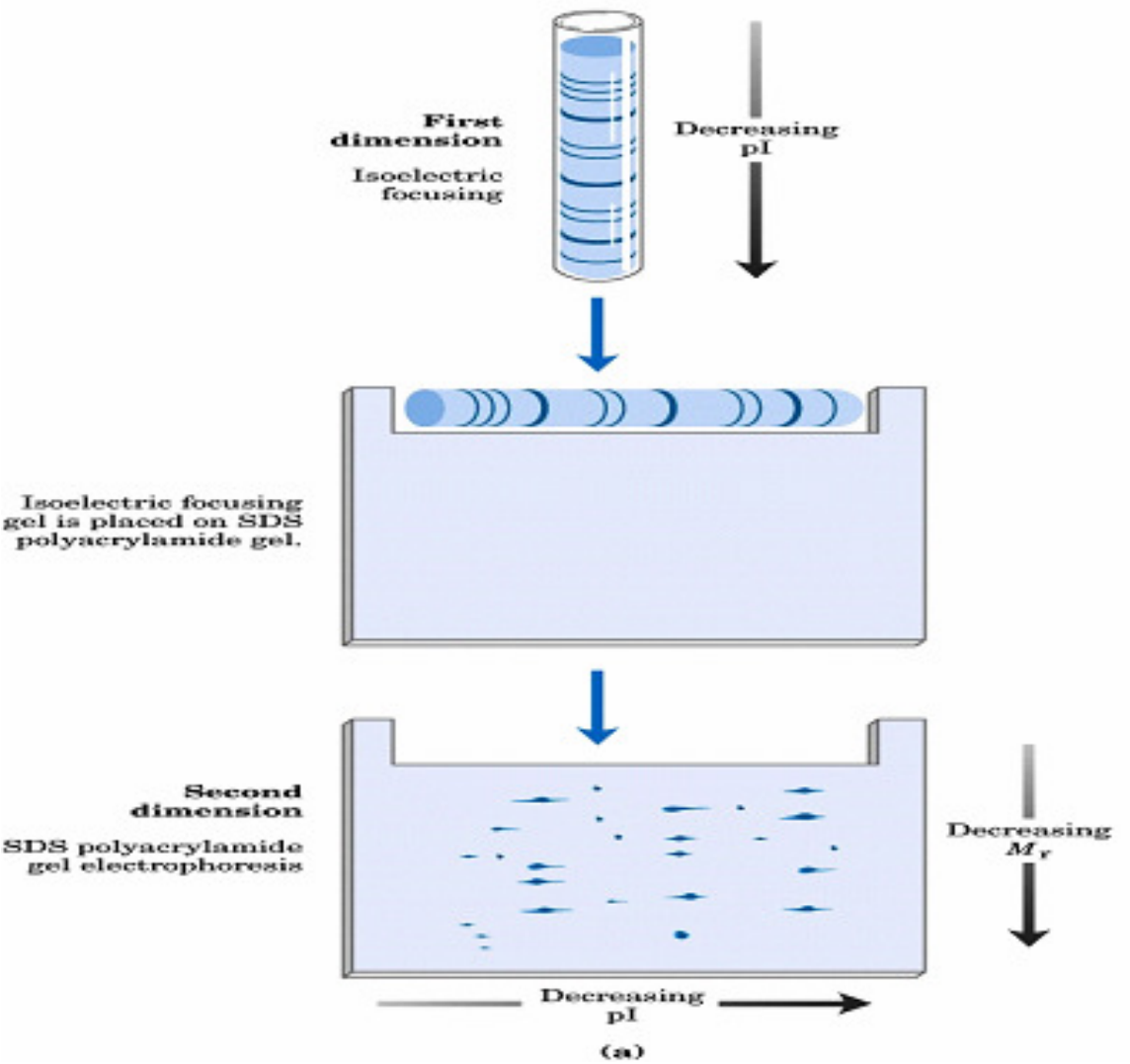
Isoelectric Focusing



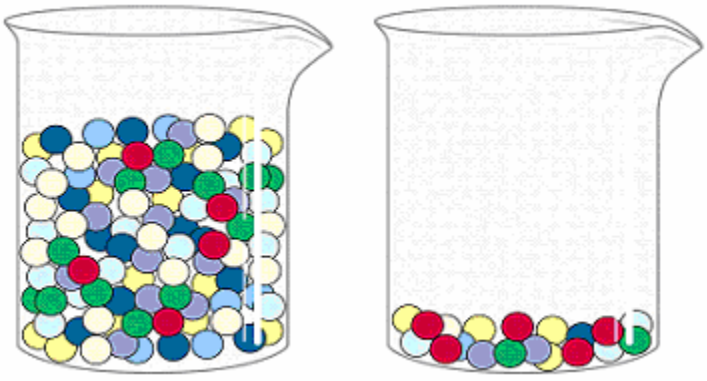
The Isoelectric Points of Some Proteins

Protein	pI
Pepsin	~1.0
Egg albumin	4.6
Serum albumin	4.9
Urease	5.0
β-Lactoglobulin	5.2
Hemoglobin	6.8
Myoglobin	7.0
Chymotrypsinogen	9.5
Cytochrome *c*	10.7
Lysozyme	11.0

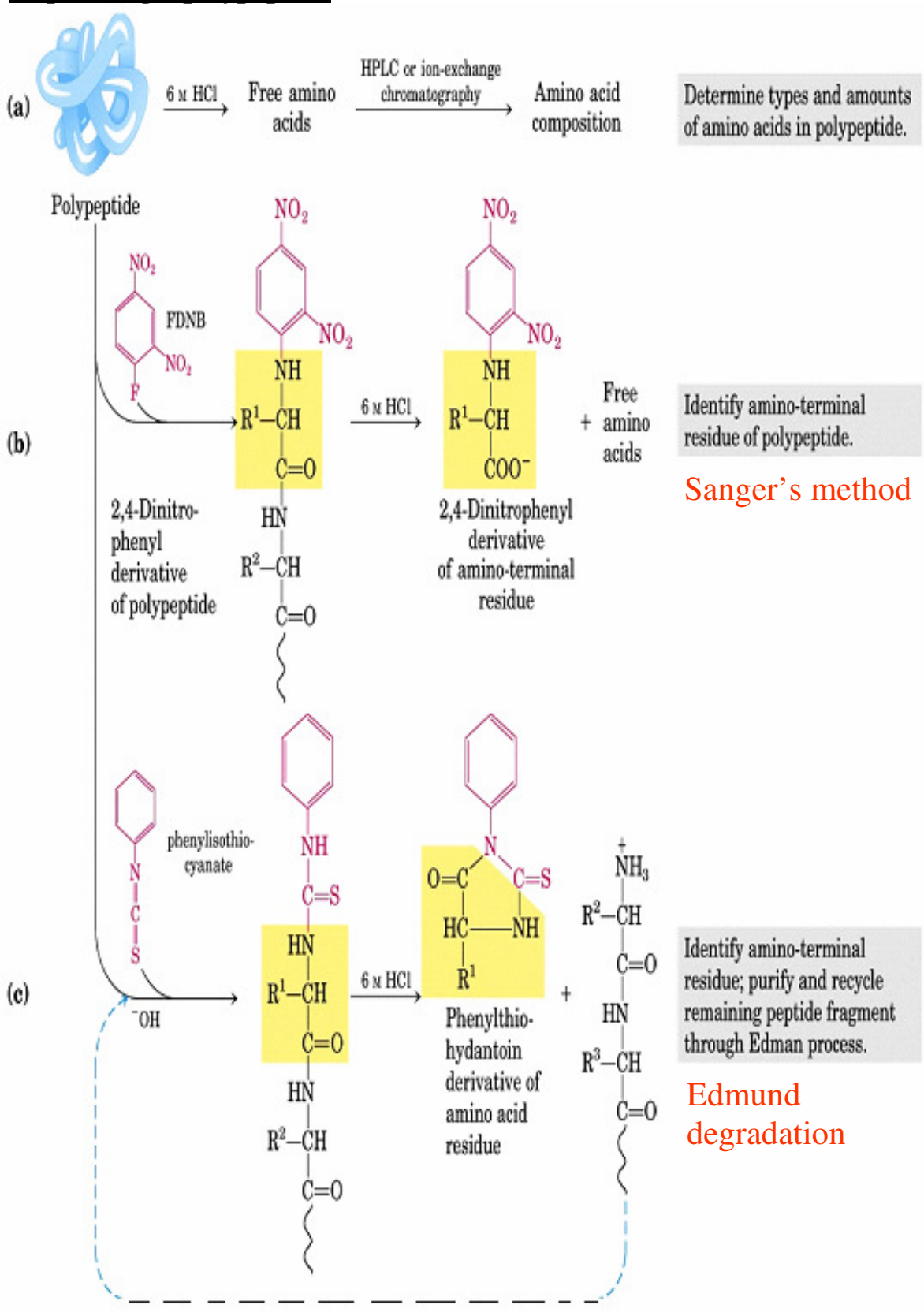
Two-dimensional electrophoresis



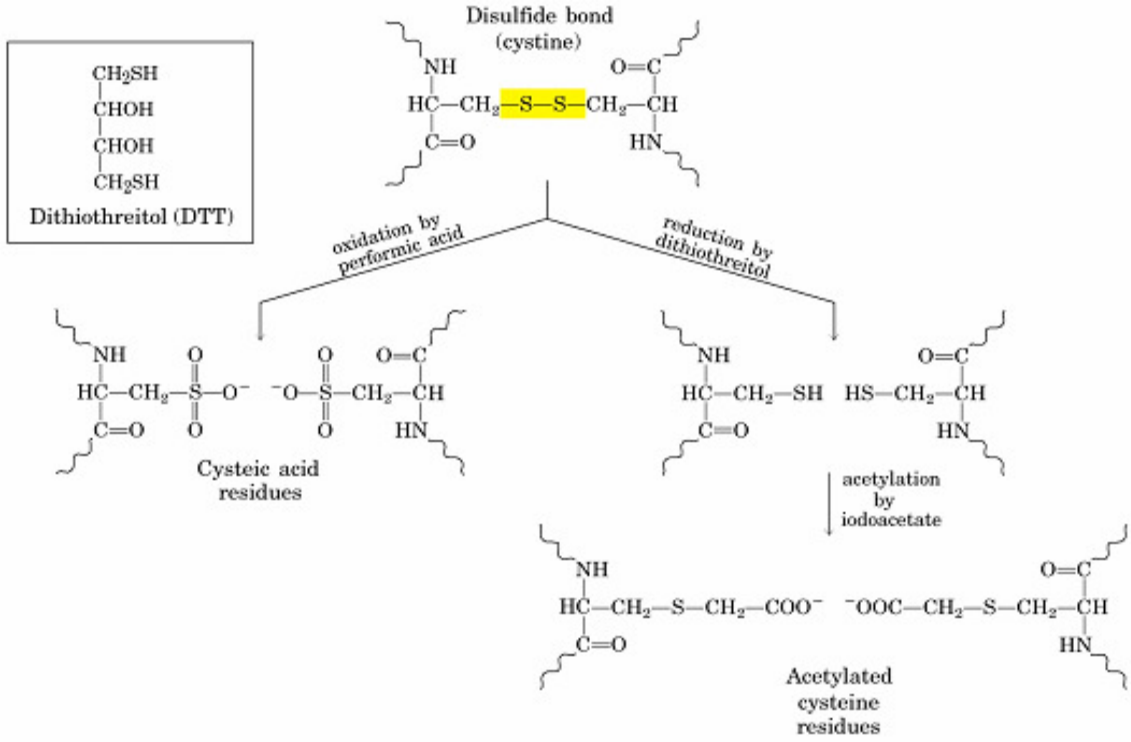
Activity versus specific activity



Sequencing a polypeptide



Breaking disulfide bonds in proteins.

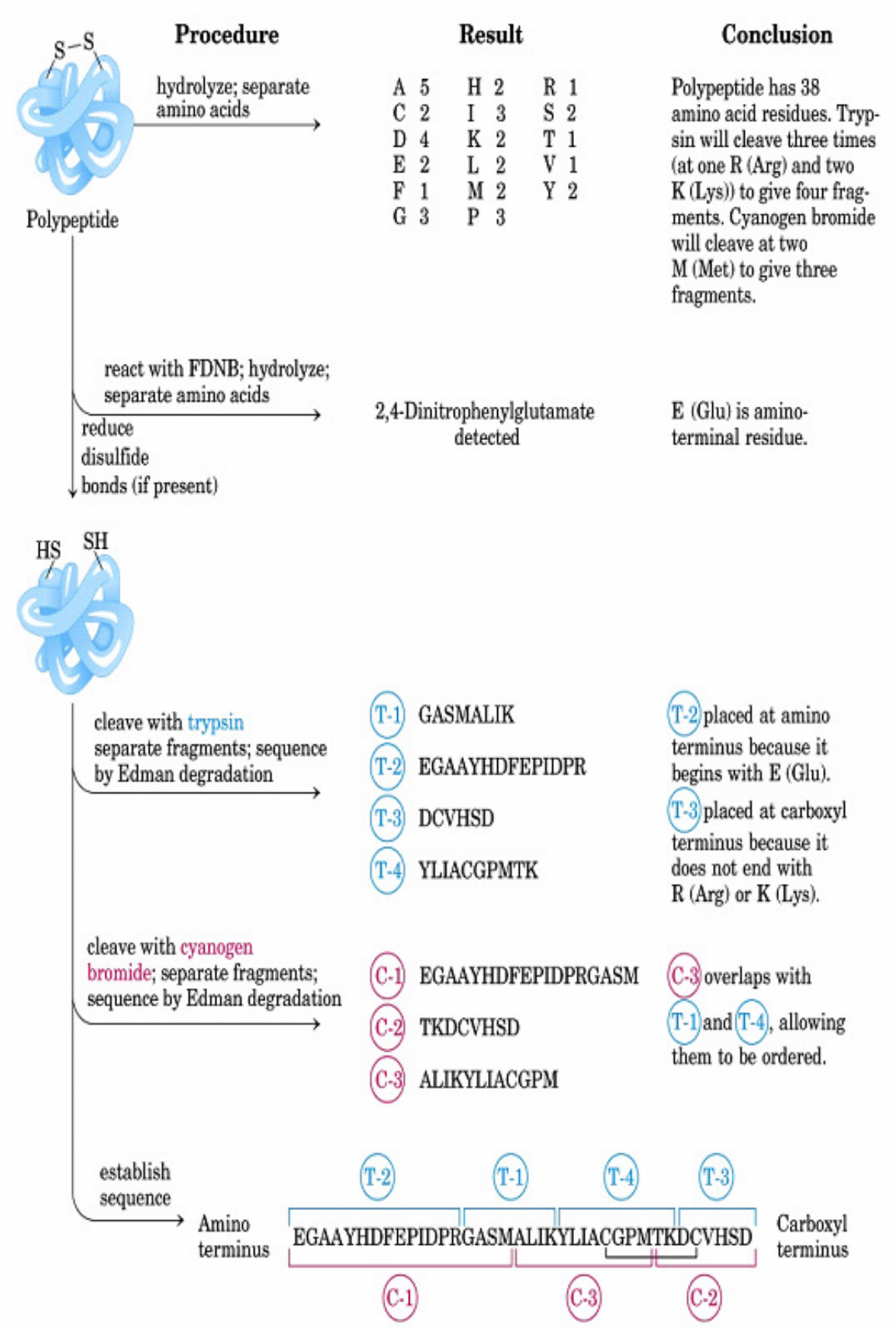


The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

Treatment*	Cleavage points†
Trypsin	Lys, Arg (C)
Submaxillarus protease	Arg (C)
Chymotrypsin	Phe, Trp, Tyr (C)
Staphylococcus aureus V8 protease	Asp, Glu (C)
Asp-*N*-protease	Asp, Glu (N)
Pepsin	Phe, Trp, Tyr (N)
Endoproteinase Lys C	Lys (C)
Cyanogen bromide	Met (C)

*All except cyanogen bromide are proteases. All are available from commercial sources.

†Residues furnishing the primary recognition point for the protease or reagent; peptide bond cleavage occurs on either the carbonyl (C) or the amino (N) side of the indicated amino acid residues.


Procedure
Result
Conclusion
S–S
hydrolyze; separate amino acids
A 5 H 2 R 1
C 2 I 3 S 2
D 4 K 2 T 1
E 2 L 2 V 1
F 1 M 2 Y 2
G 3 P 3
Polypeptide has 38 amino acid residues. Trypsin will cleave three times (at one R (Arg) and two K (Lys)) to give four fragments. Cyanogen bromide will cleave at two M (Met) to give three fragments.
Polypeptide
react with FDNB; hydrolyze; separate amino acids
2,4-Dinitrophenylglutamate detected
E (Glu) is amino-terminal residue.
reduce disulfide bonds (if present)
HS
SH
cleave with trypsin separate fragments; sequence by Edman degradation
T-1 GASMALIK
T-2 EGAAYHDFEPIDPR
T-3 DCVHSD
T-4 YLIACGPMTK
T-2 placed at amino terminus because it begins with E (Glu).
T-3 placed at carboxyl terminus because it does not end with R (Arg) or K (Lys).
cleave with cyanogen bromide; separate fragments; sequence by Edman degradation
C-1 EGAAYHDFEPIDPRGASM
C-2 TKDCVHSD
C-3 ALIKYLIACGPM
C-3 overlaps with T-1 and T-4, allowing them to be ordered.
establish sequence
Amino terminus
T-2
T-1
T-4
T-3
EGAAYHDFEPIDPRGASMALIKYLIACGPMTKDCVHSD
Carboxyl terminus
C-1
C-3
C-2

Synthesis of a peptide