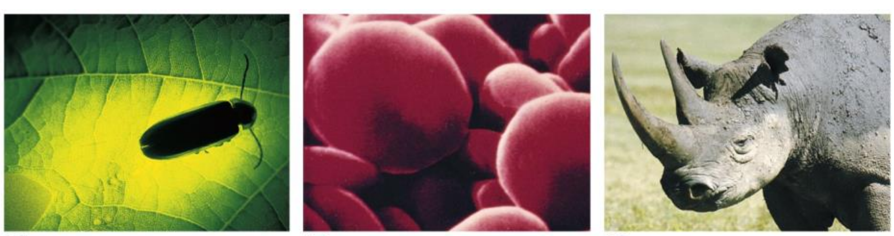
AMINO ACIDS AND PROTEINS

Course: Biochemistry I (BIOC 230)

Textbook:
Principles of Biochemistry, 5th Ed., by L. A. Moran and others. 2014, Pearson. . Chapter 3

Some functions of proteins



(a) (b) (c)

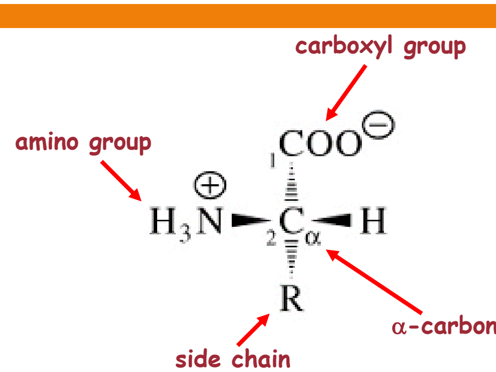
Figure 3-1
Lehninger Principles of Biochemistry, Fifth Edition

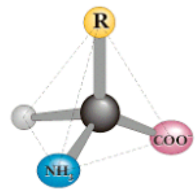
Properties of Amino Acids

- Capacity to polymerize
- Novel acid-base properties
- Varied structure and chemical functionality
- Chirality

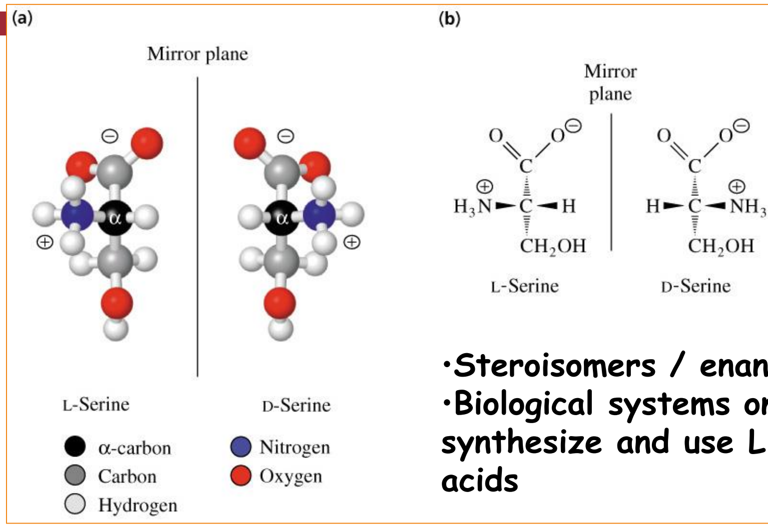
Basic Amino Acid Structure

- a-carbon is chiral (except for glycine)
- at pH 7.0 uncharged amino acids are zwitterions
- amino acids have a tetrahedral structure





Amino Acid Enantiomers



- **Steroisomers / enantiomers**
- **Biological systems only synthesize and use L-amino-acids**

AA can be classified by R group

- Aa are classified into 5 groups based on the properties of their R group , in particular their polarity or tendency to interact with water at biological pH
- The polarity of R group varies from nonpolar and hydrophobic (water insoluble) to highly polar and hydrophilic (water soluble)

Amino Acid Classification

- Aliphatic
- Aromatic

Hydrophobic

- Sulfur containing
- Polar/uncharged
- basic/acidic

Hydrophillic

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

Amino acid	Abbreviation/ symbol	M_r*	pK_a values: 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

Abbreviations and symbols of AAs

Unique first letter		
Cysteine	Cys	C
Histidine	His	H
Isoleucine	Ile	I
Methionine	Met	M
Serine	Ser	S
Valine	val	V

Common AAs have priority		
Alanine	Ala	A
Glycine	Gly	G
Leucine	Leu	L
Proline	Pro	P
Threonine	Thr	T

Abbreviations and symbols of AAs

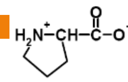
Similar sounding names		
Arginine	Arg	R
Aspargine	Asn	N
Aspartate	Asp	D
Glutamate	Glu	E
Glutamine	Gln	Q
Phenylalanine	Phe	F
Tyrosine	Tyr	Y
Tryptophan	trp	W

Letter close to initial letter		
Aspartate or aspargine	Asx	B
Glutamate or glutamine	Glx	Z
lysine	Lys	K (near L)

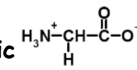
Aliphatic (alkane) Amino Acids

Hydrophobicity

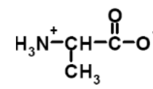
- Proline (pro, P)- cyclic “imino acid”



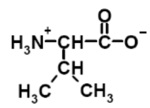
- Glycine(gly, G)-only non-chiral amino acid, not hydrophobic



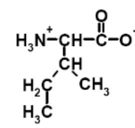
- Alanine (ala, A) – R-group = methyl-group

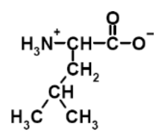


- Valine (Val, V) –Think V!



- Leucine (Leu, L) –
- Isoleucine (Ile, I) –2 chiral carbons





Nonpolar, aliphatic R groups

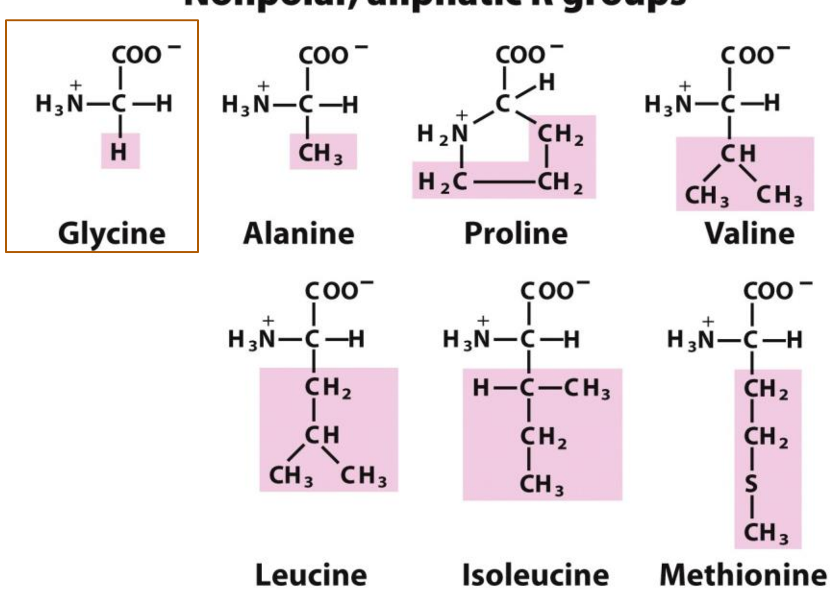
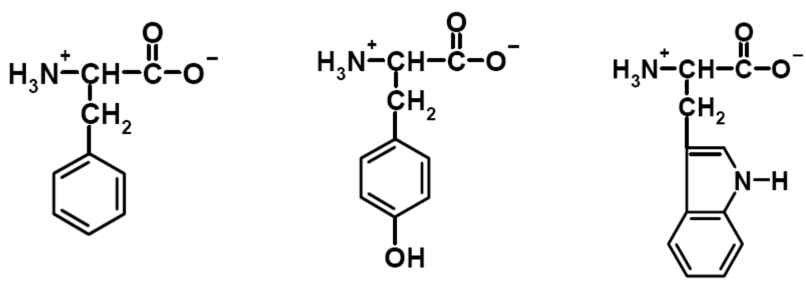


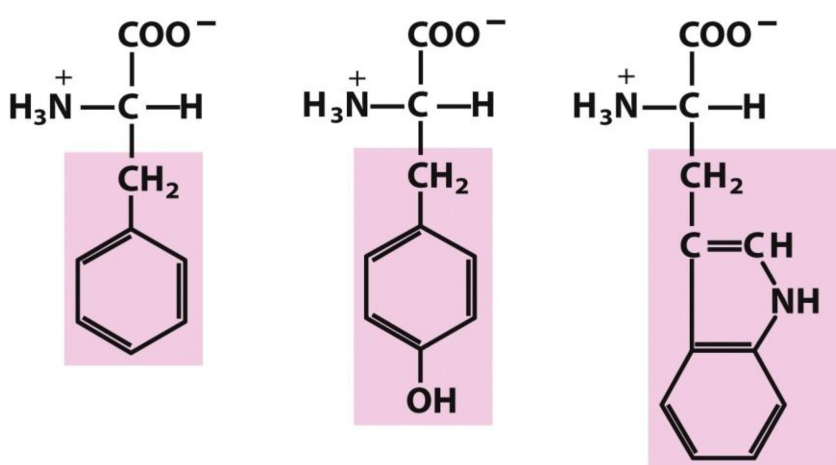
Figure 3-5 part 1
Lehninger Principles of Biochemistry, Fifth Edition

Aromatic Amino Acids

- All very hydrophobic
- All contain aromatic group
- Absorb UV at 280 nm
- Phenylalanine (Phe, F)
- Tyrosine (Tyr, Y) – -OH ionizable (pKa = 10.5), H-Bonding
- Tryptophan (Trp, W) – bicyclic indole ring, H-Bonding



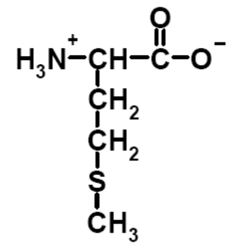
Aromatic R groups



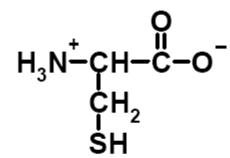
Phenylalanine Tyrosine Tryptophan

Figure 3-5 part 2
Lehninger Principles of Biochemistry, Fifth Edition

Sulfur Containing Amino Acids

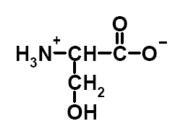


- Methionine (Met, M) – "start" amino acid, very hydrophobic
- Cysteine (Cys, C) – sulfur in form of sulfhydroyl, important in disulfide linkages, weak acid, can form hydrogen bonds.

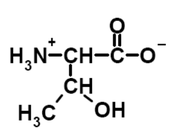


Polar Uncharged Amino Acids

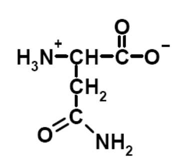
- Polar side groups, hydrophillic in nature, can form hydrogen bonds



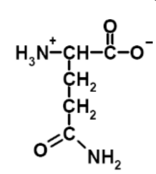
- Hydroxyls of Ser and Thr weakly ionizable
- Serine (Ser, S) – looks like Ala w/ -OH



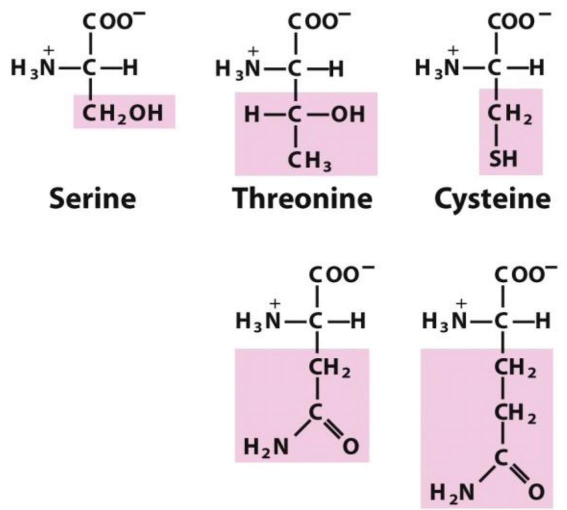
- Threonine (Thr, T) – 2 chiral carbons



- Asparagine (Asn, N) – amide of aspartic acid
- Glutamine (Gln, Q) – amide of glutamic acid



Polar, uncharged R groups



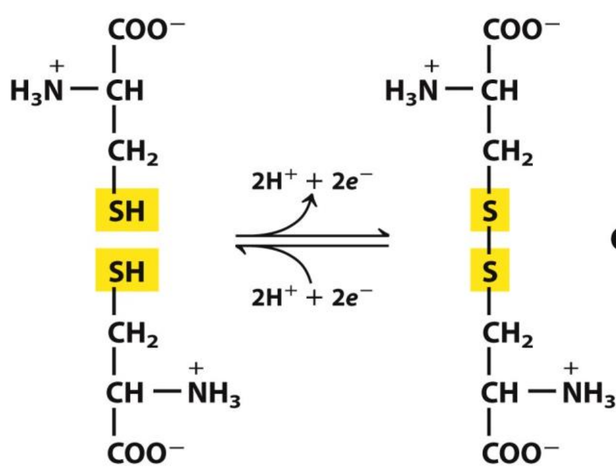
Serine Threonine Cysteine

Asparagine Glutamine

Figure 3-5 part 3
Lehninger Principles of Biochemistry, Fifth Edition

Cysteine

Cysteine



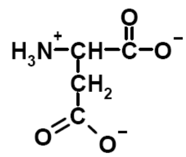
Cystine

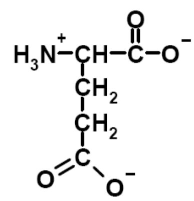
Figure 3-7
Lehninger Principles of Biochemistry, Fifth Edition

Reversible formation of a disulfide bond by the oxidation of two molecules of cysteine

Acidic Amino Acids

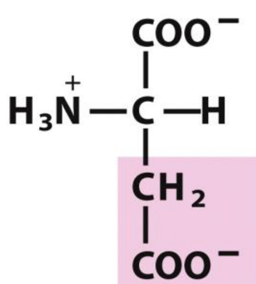
- Contain carboxyl groups (weaker acids than a-carboxyl-group)
- Negatively charged at physiological pH, present as conjugate bases (therefore –ate not –ic acids)
- Carboxyl groups function as nucleophiles in some enzymatic reactions
- Aspartate –

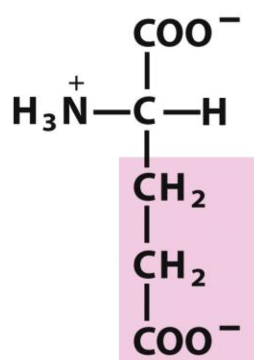




- Glutamate –

Negatively charged R groups



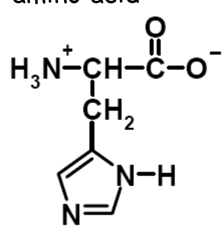


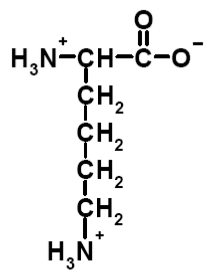
Aspartate Glutamate

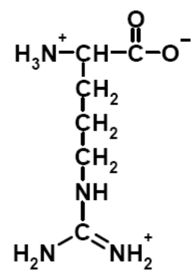
Figure 3-5 part 5
Lehninger Principles of Biochemistry, Fifth Edition

Basic Amino Acids

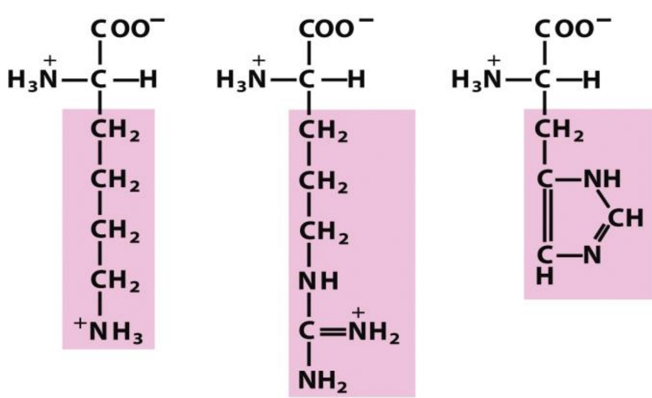
- Hydrophillic nitrogenous bases
- Positively charged at physiological pH
- Histidine – imidazole ring protonated/ionized, only amino acid that functions as buffer in physiol range.
- Lysine - diamino acid, protonated at pH 7.0
- Arginine - guianidinium ion always protonated, most basic amino acid







Positively charged R groups



Lysine Arginine Histidine

Figure 3-5 part 4
Lehninger Principles of Biochemistry, Fifth Edition

Arg has a guanidinium group and His has an imidazole.
His is the only common aa with an ionizable R group with pk_a near neutrality, thus can be charged or uncharged at pH 7.0

Essential/Non-Essential Amino Acids

- **Essential amino acids:** can't be synthesized by the body. Nine AAs are essential.
- Essential AAs (9): isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, histidine
- Non-essential: alanine^, aspartate^, serine^, asparagine^, glutamate^, glutamine*, glycine*, proline*, tyrosine*, arginine*, cysteine*,
- Conditionally essential * (6): synthesis can be limited under special pathophysiological conditions, such as prematurity in the infant or individuals in severe catabolic distress
- Dispensable^ (5): can be synthesized in the body

Essential amino acids (cont'd)

- Most animal proteins contain all essential aa in about the quantities needed by human body.
- Vegetable proteins often lack one or more essential aa and may in some cases be difficult to digest. Thus mixed vegetables are needed to complement each other.
- Example: Corn is deficient in Lysine; legumes are deficient in methionine

Amino acids can act as acids and bases

- Amino and carboxyl groups of aa along with ionizable R groups of some aa function as weak acids or bases
- In water at neutral pH, an aa that lacks ionizable R group, exists as dipolar ion or **zwitterion** (hybrid ion) which can act as either acid or base
- Substances having this dual (acid-base) nature are amphoteric and are often called ampholytes

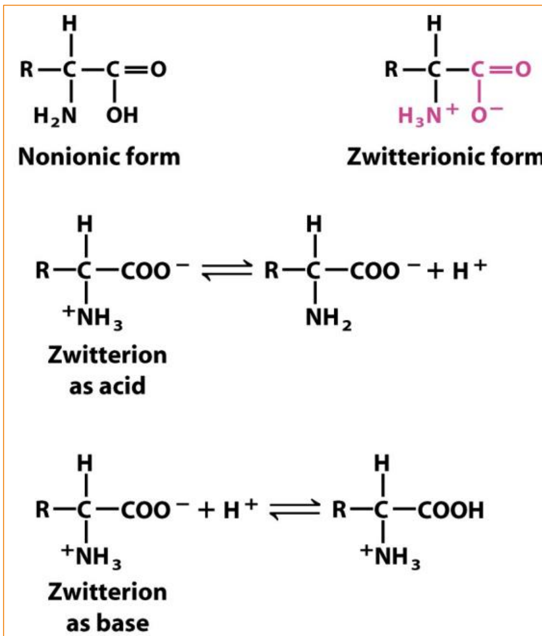


Figure 3-9
Lehninger Principles of Biochemistry, Fifth Edition

Class activity!

1. Amino acids exist in human cells mostly in the form:
   a. L-isomer
   b. D-isomer
2. Amino acids have one or more chiral centers, except:
   a. Ala
   b. Gly
   c. Ile
   d. Pro

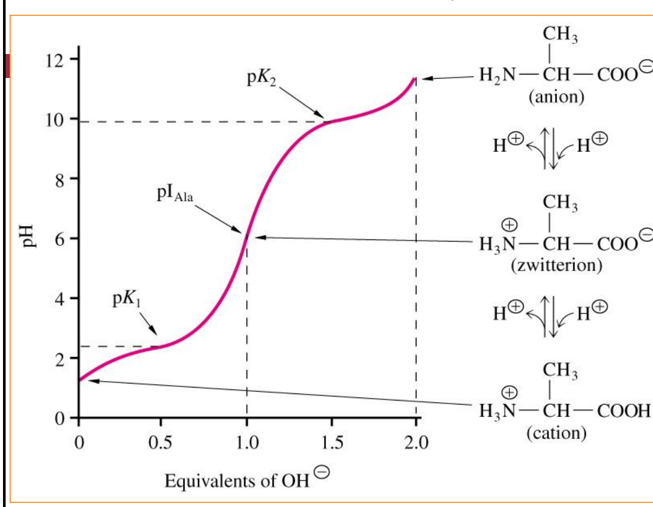
Class activity!

1. Which of the following amino acids is negatively charged at physiological pH?
   a. Ala
   b. Glu
   c. Gln
   d. Lys
2. The amino acid that can for disulfide bridges is/are:
   a. Val
   b. Met
   c. Cys
   d. Tyr

Amino acids have characteristic titration curves

- Acid-base titration involves the gradual addition or removal of protons
- *pK_a*: a measure of the tendency of a group to give up a proton, with that tendency decreasing tenfold as the *pK_a* increases by one unit
- What type of information can be deduced from the titration curve?

Titration Curve for **Alanine**



pK_1 carboxylic acid = 2
pK_2 amino group = 10

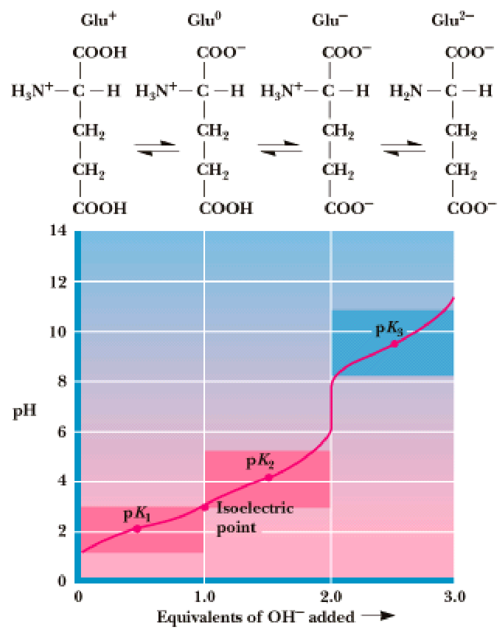
$pI = (pK_1 + pK_2)/2$

pI (isoelectric point) = the pH at which the number of positive and negative charges on a population of molecules is equal (i.e. no net charge).

Titration Curve for **Glutamic Acid**

pK_1 carboxylic acid = 2.2
pK_2 R group = 4.3
pK_3 amino group = 9.7

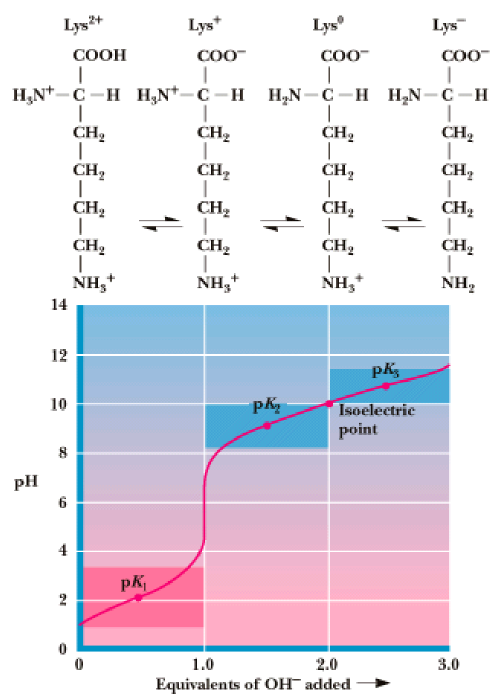
$pI = (pK_1 + pK_2)/2$
$pI = (2.2+4.3)/2$
$pI = 3.25$



Titration Curve for **Lysine**

pK_1 carboxylic acid = 2.2
pK_2 amino group = 9.0
pK_3 R group = 10.5

$pI = (pK_2 + pK_3)/2$
$pI = (9+10.5)/2$
$pI = 9.75$



pKa's of charged amino acids R-groups

- Aspartate/Glutamate = 4.0
- Histidine = 6.0
- Cysteine = 8.4
- Tyrosine = 10.5
- Lysine = 10.5
- Arginine = 12.5

Uncommon amino acids

- In addition to the 20 common aa, proteins can have residues created by modification of common residues already incorporated into a polypeptide
- Examples:
  - 4-hydroxyproline: found in plant cell wall, collagen
  - 5-hydroxylysine: found in collagen
  - 6-N-methyllysine: found n myosin
  - γ-caboxyglutamate: found in prothrombin and other proteins that bind Ca++
  - Desmosine: a derivative of 4 lysines found in elastin
  - **Selenocysteine**: a special and rare aa, incorporated into proteins during synthesis. It contains selenium rather than sulfur. It is actually derived from serine.

Uncommon amino acids.

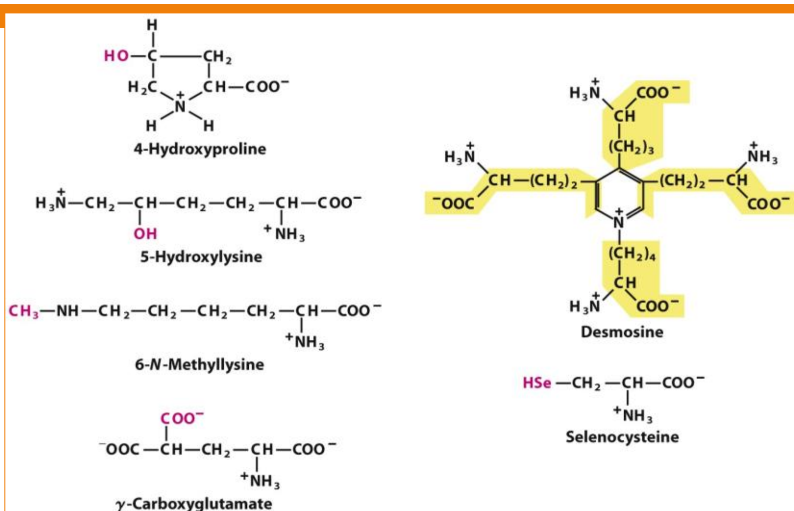


Figure 3-8a
Lehninger Principles of Biochemistry, Fifth Edition

Review of Ser & Cys structure!

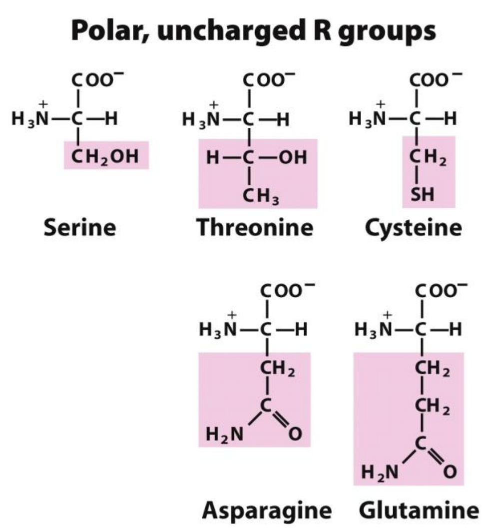


Figure 3-5 part 3
Lehninger Principles of Biochemistry, Fifth Edition

Uncommon aa

- Some aa residues may be modified transiently in proteins to alter protein's function
- Examples include addition of phosphoryl, methyl, acetyl, adenyly, ADP-ribosyl, or other groups to particular aa to increase or decrease a protein's activity
- Some 300 additional aa have been found in cells. They have a variety of functions but are not all constituents of proteins.

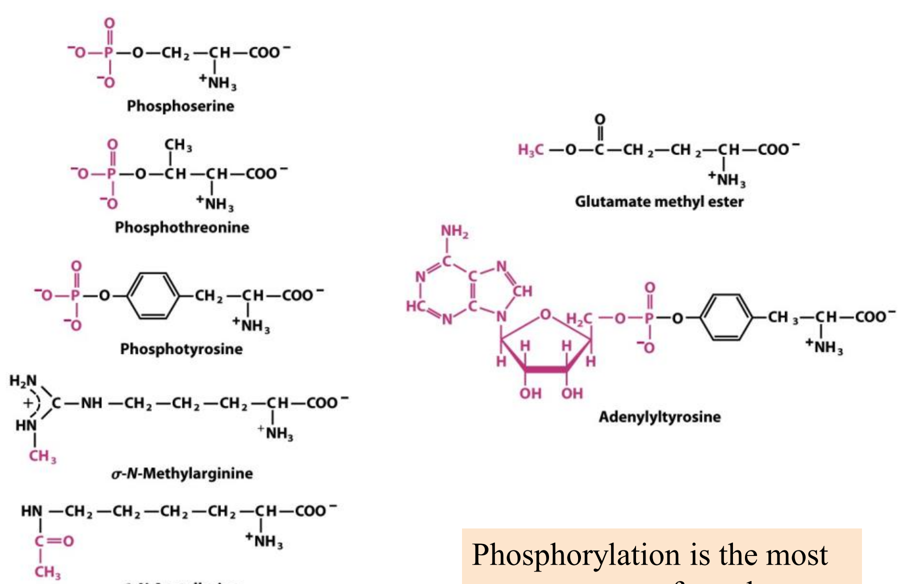
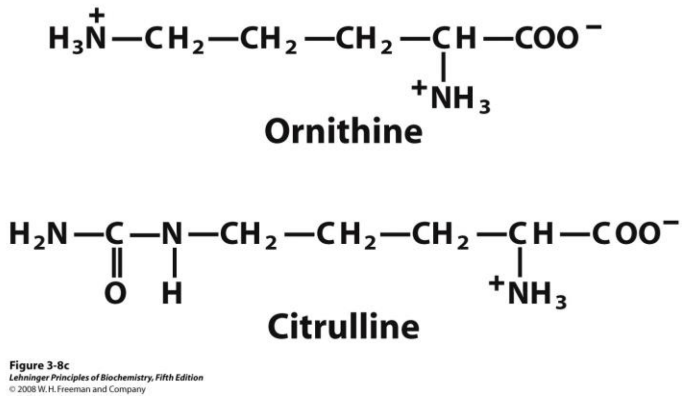


Figure 3-8b
Lehninger Principles of Biochemistry, Fifth Edition

Phosphorylation is the most common type of regulatory modification



Ornithine and citrulline, are not found in proteins, but occur as intermediates in the biosynthesis of arginine and in the urea cycle.

Use of amino acids in diagnosis of disease

- Elevated conc of amino acids are found in plasma or urine in a number of clinical disorders
- Abnormally high conc in urine is called an aminoaciduria

- **Phenylketonuria (PKU)**
- **Cystinuria**
- **Hartnup disease**

Phenylketonuria (PKU)

- PKU: a metabolic defect in which patients lack sufficient amounts of phenylalanine hydroxylase which converts Phe to Tyr.
- Phe, phenylpyruvate and phenyllactate accumulate in plasma and urine.
- If not put on special diet low in Phe, it leads to mental retardation. Incidence 1/10,000-25,000.
- Included in newborn screening.

Cystinuria

- **Cystinuria**: a genetic defect in membrane transport system of Cystine and the basic amino acids (Lys, Arg & derive ornithine) in epithelial cells.
- Large amounts of these aa are excreted in urine.
- Symptoms arise from formation of cystine stones in kidneys, ureter and bladder.

Hartnup disease

- **Hartnup disease:** a genetic defect in epithelial cell transport of neutral type amino acids (particularly Trp) and high conc of these are found in urine.
- Symptoms are primarily caused by a deficiency of Trp.
- Symptoms include a pellagra-like rash, cerebellar ataxia (irregular jerky muscle movements due to toxic effects of indole derived from bacterial degradation of unabsorbed trp in gut

Proteins

Protein Nomenclature

- Peptides 2 – 50 amino acids
- Proteins >50 amino acids
- Amino acid with free α-amino group is the amino-terminal or N-terminal **residue**
- Amino acid with free α -carboxyl group is the carboxyl-terminal or C-terminal **residue**
- Three letter code – Met-Gly-Glu-Thr-Arg-His
- Single letter code – M-G-E-T-R-H
- Linked amino acid moieties in a polypeptide chain are called amino acid residues

Formation of a peptide bond by condensation

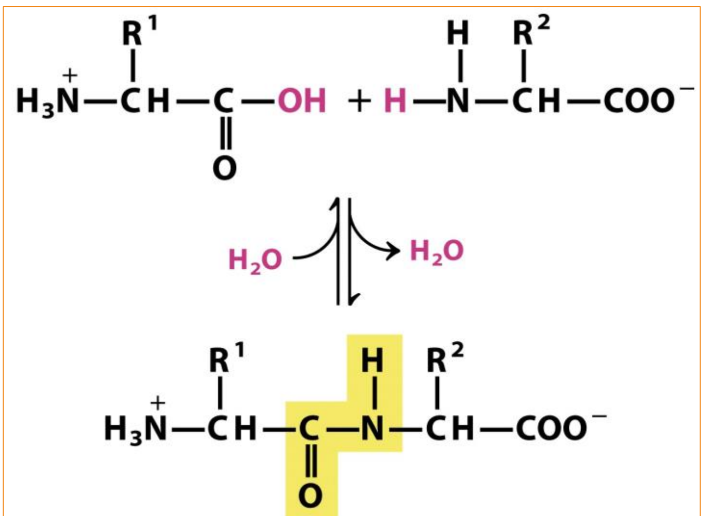


Figure 3-13
Lehninger Principles of Biochemistry, Fifth Edition

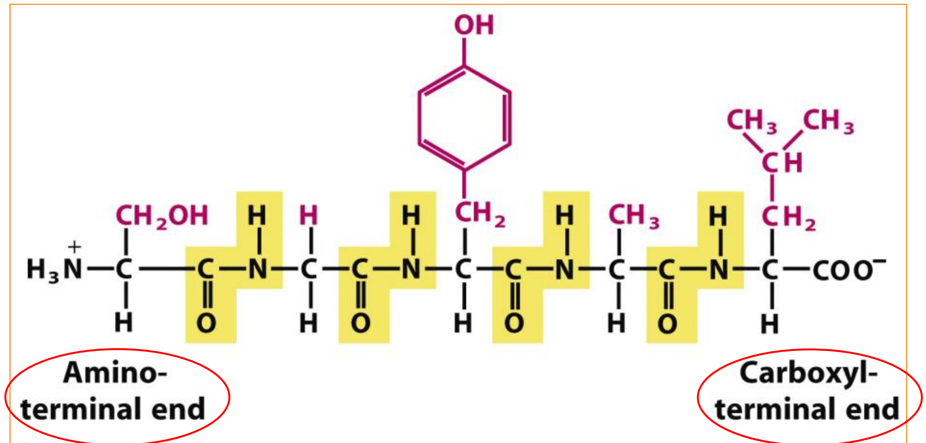


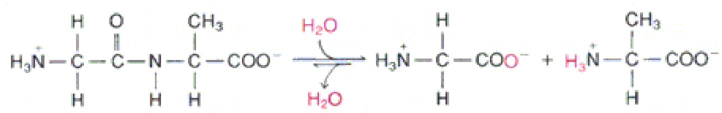
Figure 3-14
Lehninger Principles of Biochemistry, Fifth Edition

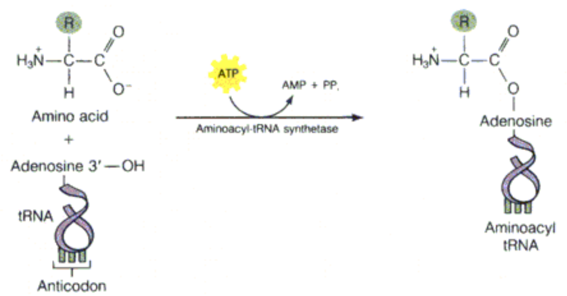
The pentapeptide serylglycyltyrosylalanylleucine, Ser–Gly–Tyr–Ala–Leu, or SGYAL

Stability and Formation of the Peptide Bond

- Hydrolysis of peptide bond favored energetically, but uncatalyzed reaction very slow.
- Strong mineral acid, such as 6 M HCl, good catalyst for hydrolysis
- Amino acids must be "activated" by ATP-driven reaction to be incorporated into proteins

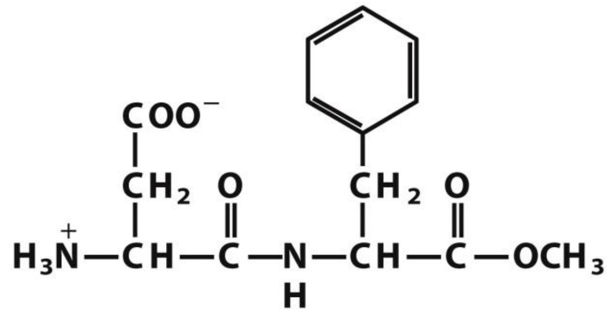
Stability and Formation of the Peptide Bond





Biologically active peptides and polypeptides occur in a variety of forms

- Naturally occurring peptides range in size from 2 to thousands of aa residues
- Many small peptides like some hormones exert their effects at very low concentration; for example Oxytocin (9 aa residues); Thyrotropin-releasing factor (3 aa residues)

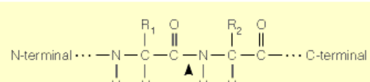


L-Aspartyl-L-phenylalanine methyl ester (aspartame)

Unnumbered 3 p83
Lehninger Principles of Biochemistry, Fifth Edition

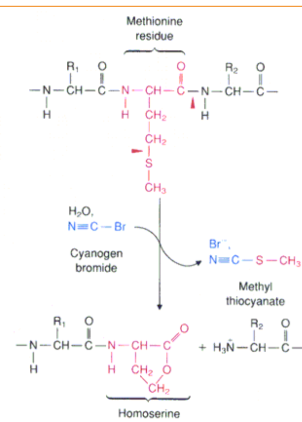
Aspartame: artificial sweetener. It is 200 times sweeter than table sugar and is widely used in diet drinks.

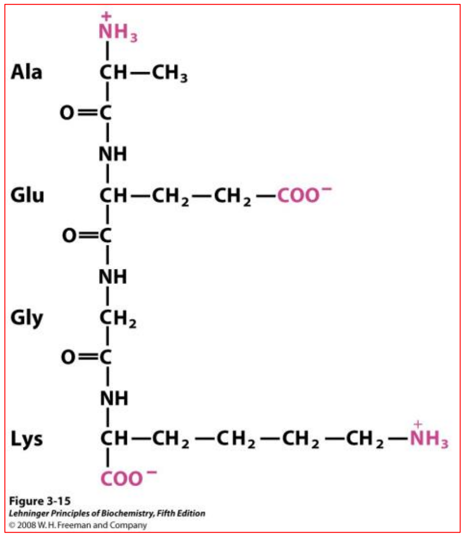
Enzymatic and Chemical Cleavage of Peptide Linkage



Enzyme	Preferred Site[a]	Source
Trypsin	R_1 = Lys, Arg	From digestive systems of animals, many other sources
Chymotrypsin	R_1 = Tyr, Trp, Phe, Leu	Same as trypsin
Thrombin	R_1 = Arg	From blood; involved in coagulation
V-8 protease	R_1 = Asp, Glu	From *Staphylococcus aureus*
Prolyl endopeptidase	R_1 = Pro	Lamb kidney, other tissues
Subtilisin	Very little specificity	From various bacilli
Carboxypeptidase A	R_2 = C-terminal amino acid	From digestive systems of animals
Thermolysin	R_2 = Leu, Val, Ile, Met	From *Bacillus thermoproteolyticus*

[a]The residues indicated are those next to which cleavage is most likely. Note that in some cases preference is determined by the residue on the N-terminal side of the cleaved bond (R_1) and sometimes by the residue to the C-terminal side (R_2). Generally, proteases do not cleave where proline is on the other side of the bond. Even prolyl endopeptidase will not cleave if R_2 = Pro.





Peptides have characteristic titration curves and isoelectric pH (pI)

Assignment!!!

Ala-Ser-Glu-Tyr-Trp-Lys-Arg-His-Pro-Gly

- Draw the decapeptide at pH 1, 7, and 12. (pay attention to the form the N- and C- terminal and each R-group takes on at each pH)
- Calculate the overall charge at each pH.
- Write out the one letter code for the decapeptide

Assignment!!!

- Refer to the "Problems at end of chapter 2" and answer questions: 2, 4, 5, 15, 16.
- Refer to the "Problems at end of chapter 3" and answer questions: 8, 9, 10,

Protein purification techniques

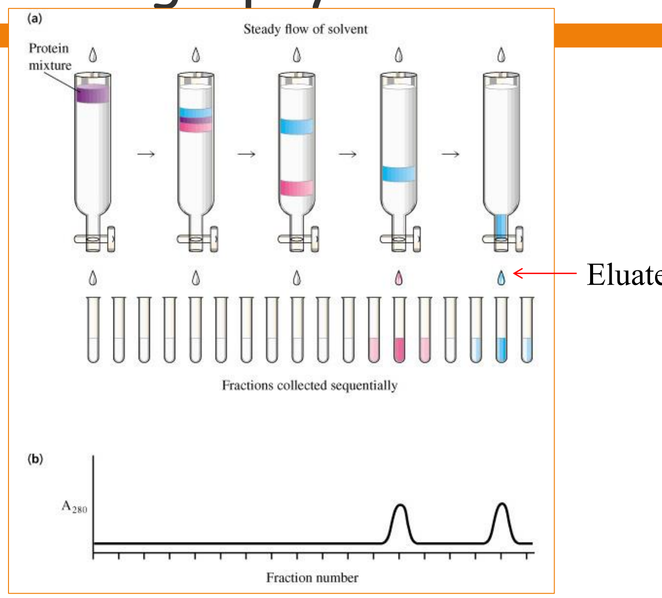
Why purify proteins?

- Pure proteins are required to study enzyme function
- Pure proteins are required for structural analysis (x-ray crystallography, NMR spectroscopy)
- Pure proteins are required to obtain amino acid sequence

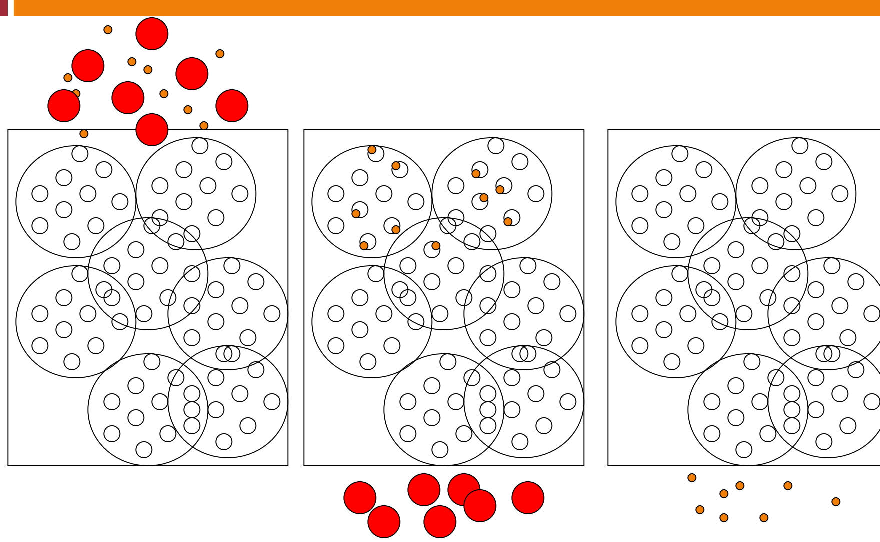
Steps in protein purification

- Develop assay
- Choose source of protein
- Prepare tissue extract
  - cell disruption
  - subcellular fractionation
- Protein fractionation (several steps)
- Determination of purity

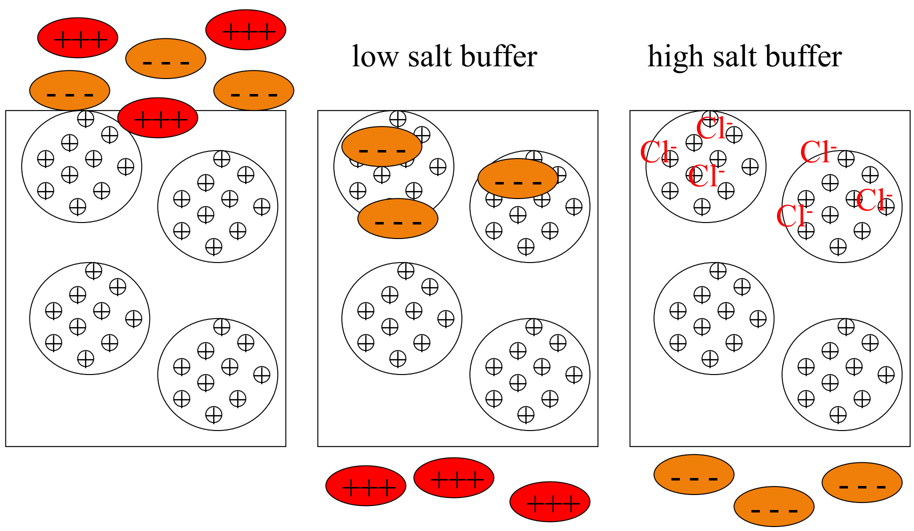
Chromatography



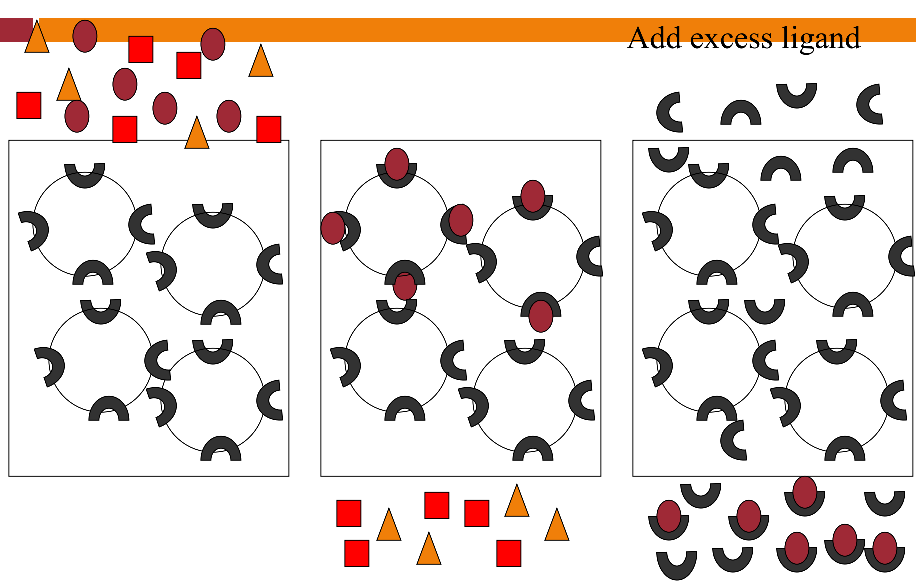
Gel filtration Chromatography or size-exclusion



Ion-exchange Chromatography



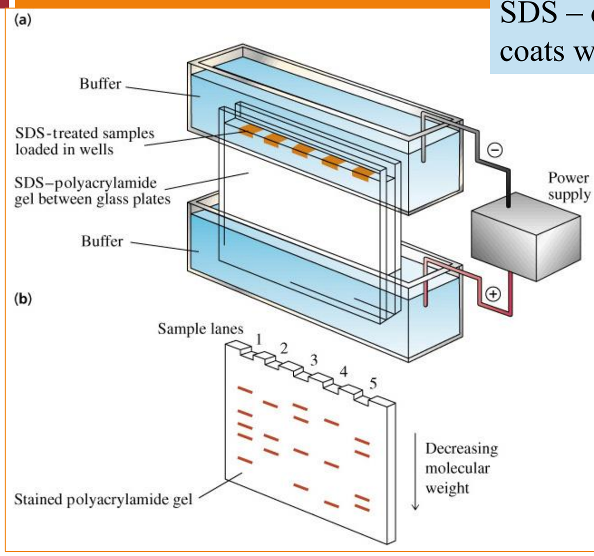
Affinity Chromatography

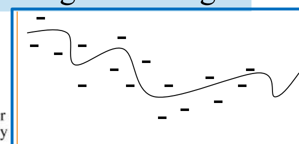


SDS poly acrylamide electrophoresis (PAGE)

SDS = $H_3C\text{-}(CH_2)_{10}\text{-}CH_2\text{-}OSO_3^-$

SDS – denatures protein coats w/ negative charge



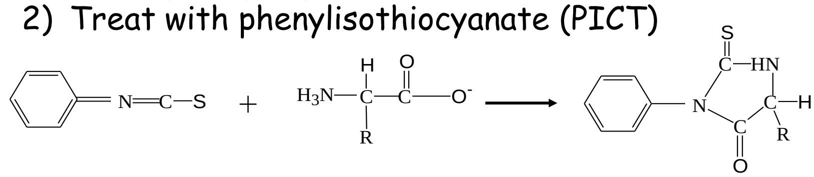


Applications of SDS-PAGE

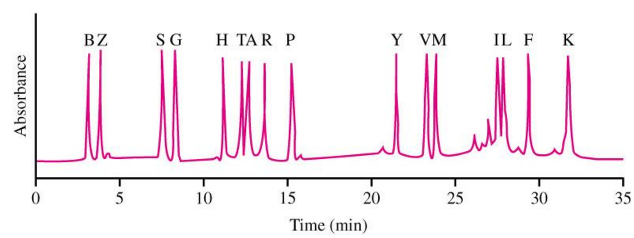
- Used to determine protein MW
- And purity of protein prep

Amino Acid Analysis

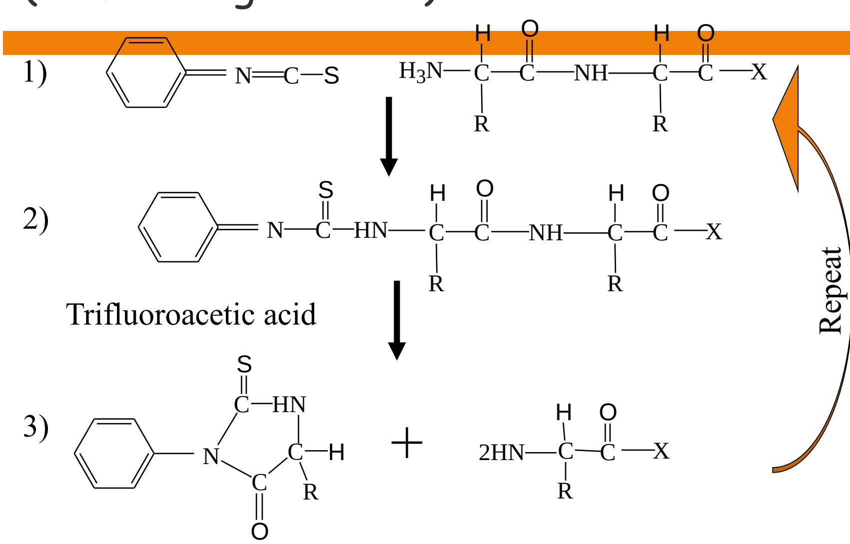
1) Acid hydrolyze protein
2) Treat with phenylisothiocyanate (PICT)



3) Separate derivatized AA's by HPLC



Protein Sequencing (Edman Degradation)



Can sequence 30 to 60 AA's from N-terminus

Generate Proteolytic Fragments

Endopeptidases

•Typsin cleaves at COOH end of Lys and Arg
•Chymotrypsin cleaves at COOH end of Phe, Tyr, Trp

Chemical Cleavages

•Cyanogen Bromide cleaves at COOH end of Met

Generate overlapping fragments
Sequence individual fragments and piece together sequence

Peptide mapping exercise

Met-Ala-Arg- Gly-Glu-Tyr-Met-Cys-Lys-Phe-Ala-Glu-Gln-Asp

Trypsin
Met-Ala-Arg
Phe-Ala-Glu-Gln-Asp
Gly-Glu-Tyr-Met-Cys-Lys

Chymotrysin
Met-Ala-Arg- Gly-Glu-Tyr
Met-Cys-Lys –Phe
Ala-Glu-Gln-Asp

CNBr
Met
Ala-Arg-Gly-Glu-Tyr-Met
Cys-Lys-Phe-Ala-Glu-Gln-Asp