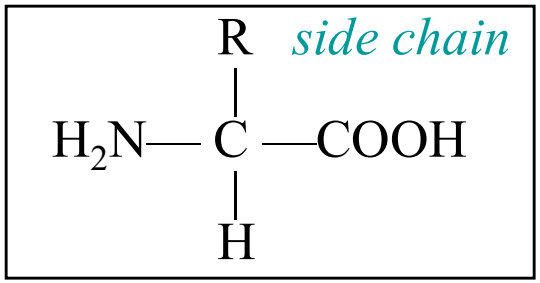
Amino Acids, Peptides, and Proteins

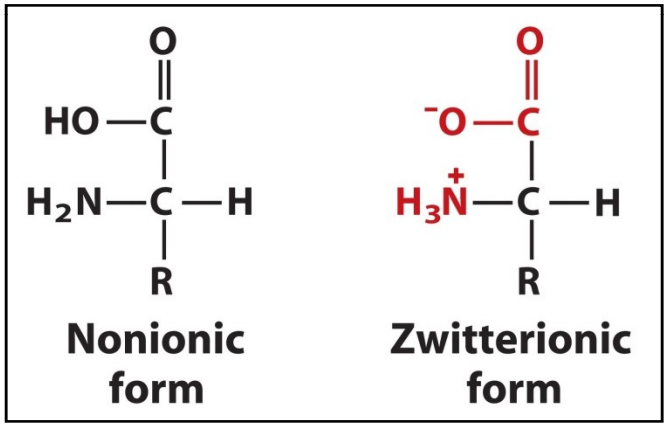
Amino Acids

- The building blocks of proteins
- Also used as single molecules in biochemical pathways
- 20 standard amino acids (a-amino acids)
- Two functional groups:
  - carboxylic acid group
  - amino group on the alpha (α) carbon
- Have different side groups (R)
  - Properties dictate behavior of AAs



Zwitterions

- Both the $-NH_2$ and the –COOH groups in an amino acid undergo ionization in water.
- At physiological pH (7.4), a zwitterion forms
  - Both + and – charges
  - Overall neutral
  - Amphoteric
    - Amino group is protonated
    - Carboxyl group is deprotonated



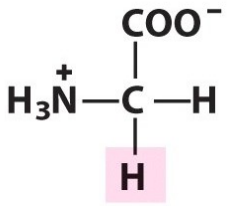
- Soluble in polar solvents due to ionic character
- Structure of R also influence solubility

Classification of Amino Acids

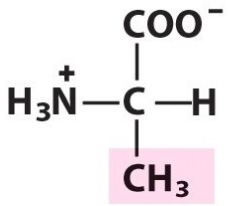
- Classify by structure of R
  - Nonpolar
  - Polar
  - Aromatic
  - Acidic
  - Basic

Nonpolar Amino Acids

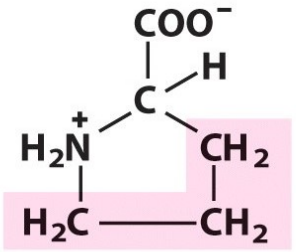
- Hydrophobic, neutral, aliphatic



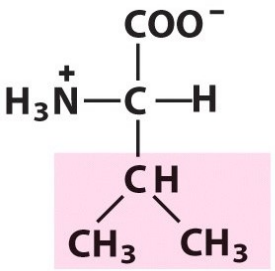
Glycine



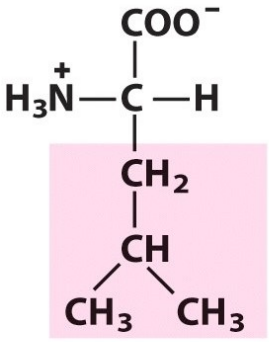
Alanine



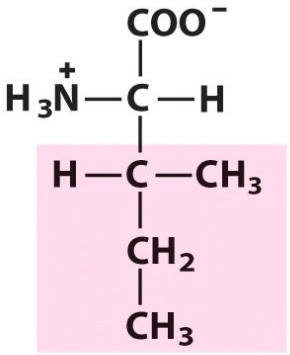
Proline



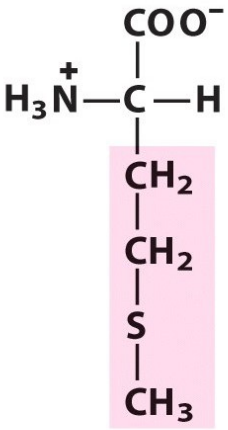
Valine



Leucine



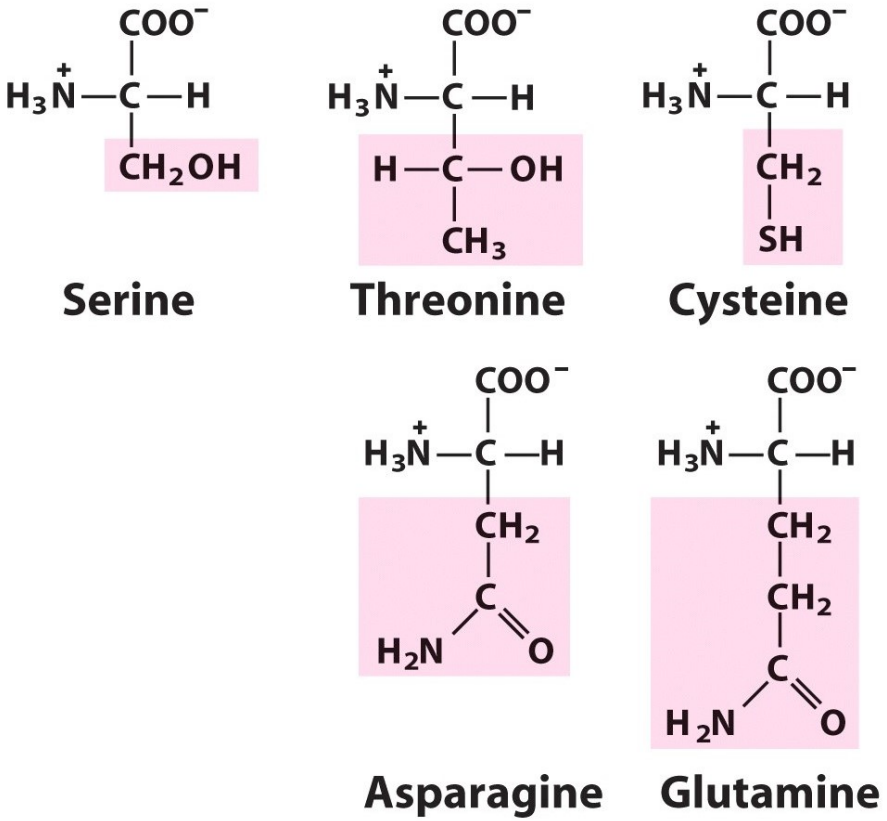
Isoleucine



Methionine

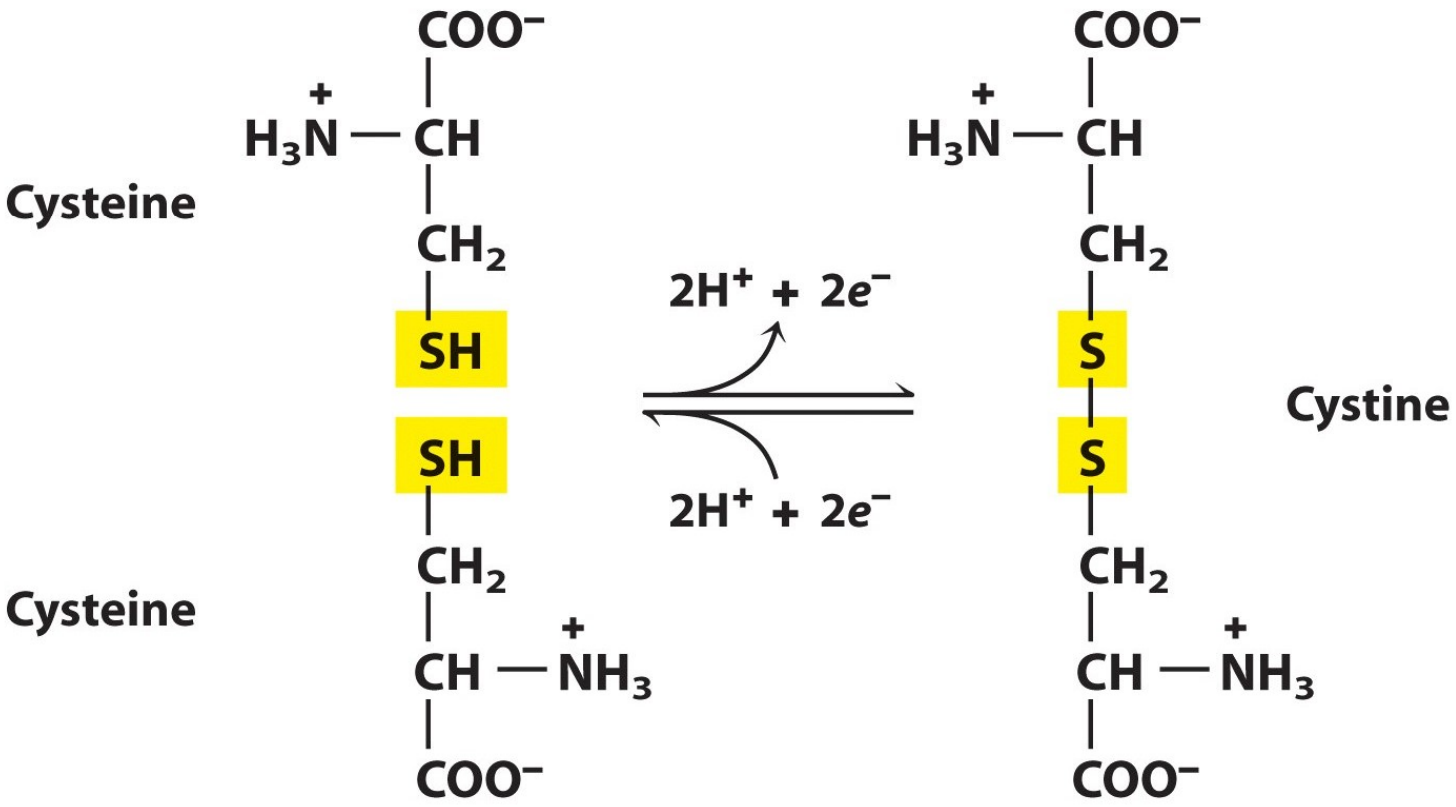
Polar Amino Acids

- Hydrophilic, neutral, typically H-bond



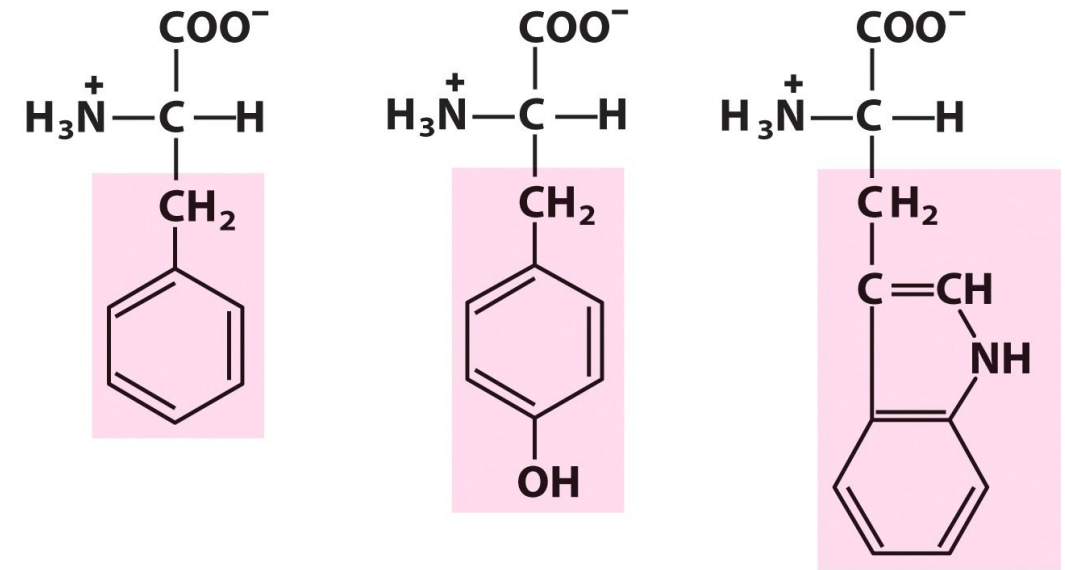
Disulfide Bonds

- Formed from oxidation of cysteine residues



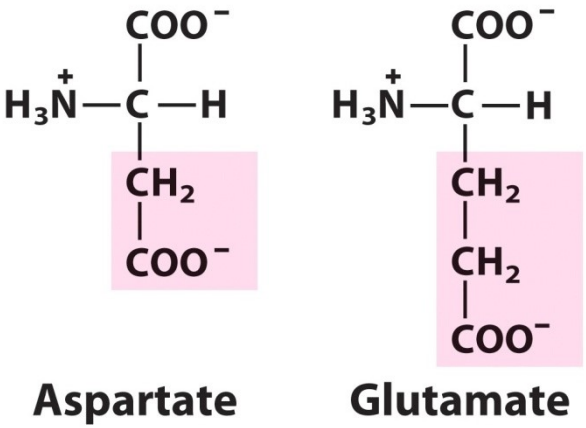
Aromatic Amino Acids

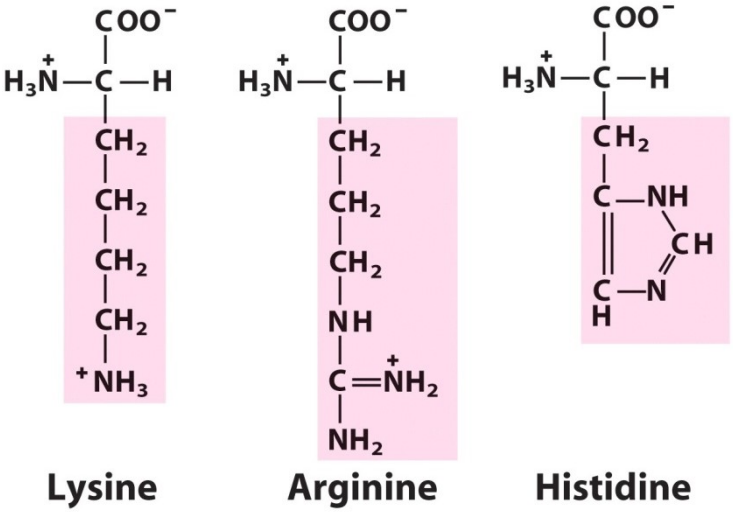
- Bulky, neutral, polarity depend on R



Acidic and Basic Amino Acids

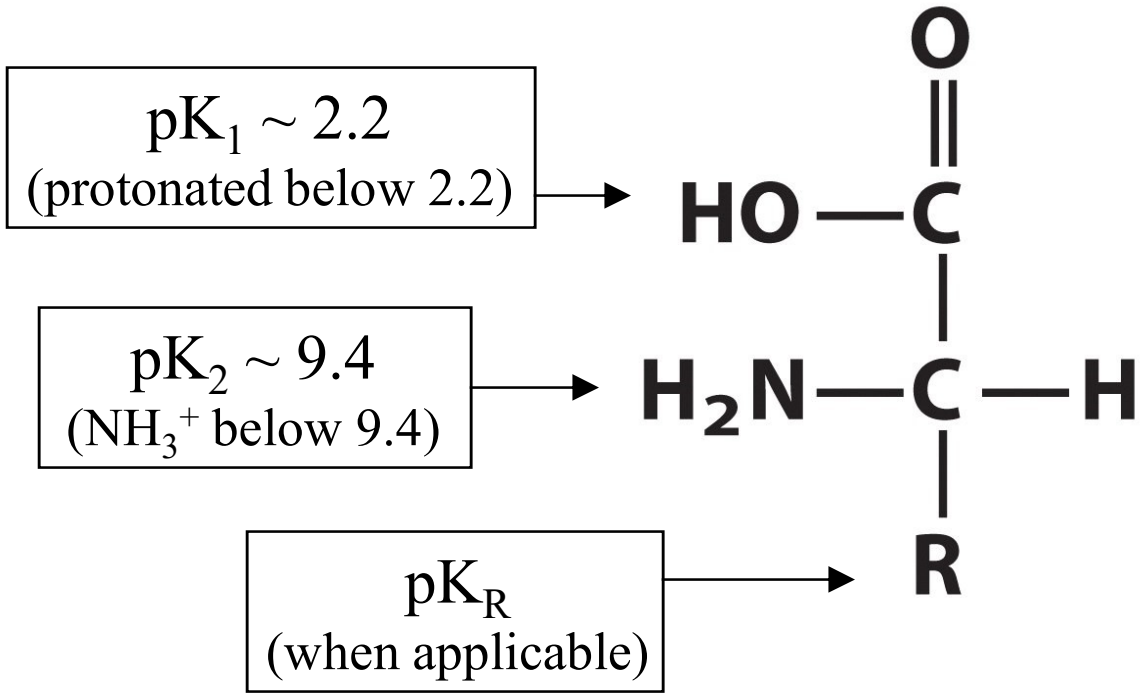
- Acidic
  - R group = carboxylic acid
  - Donates H^+
  - Negatively charged
- Basic
  - R group = amine
  - Accepts H^+
  - Positively charged
  - His ionizes at pH 6.0





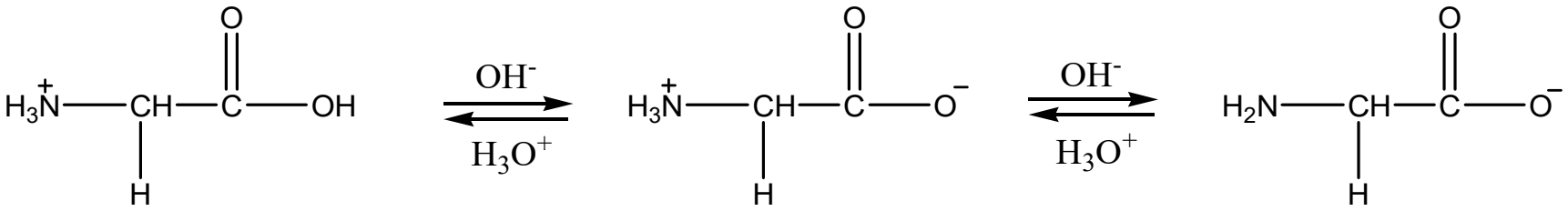
Acid-base Properties

- Remember H_3PO_4 (multiple pK_a's)
- AAs also have multiple pK_a's due to multiple ionizable groups



pH and Ionization

- Consider glycine:



Glycine ion at acidic pH (charge = 1+)

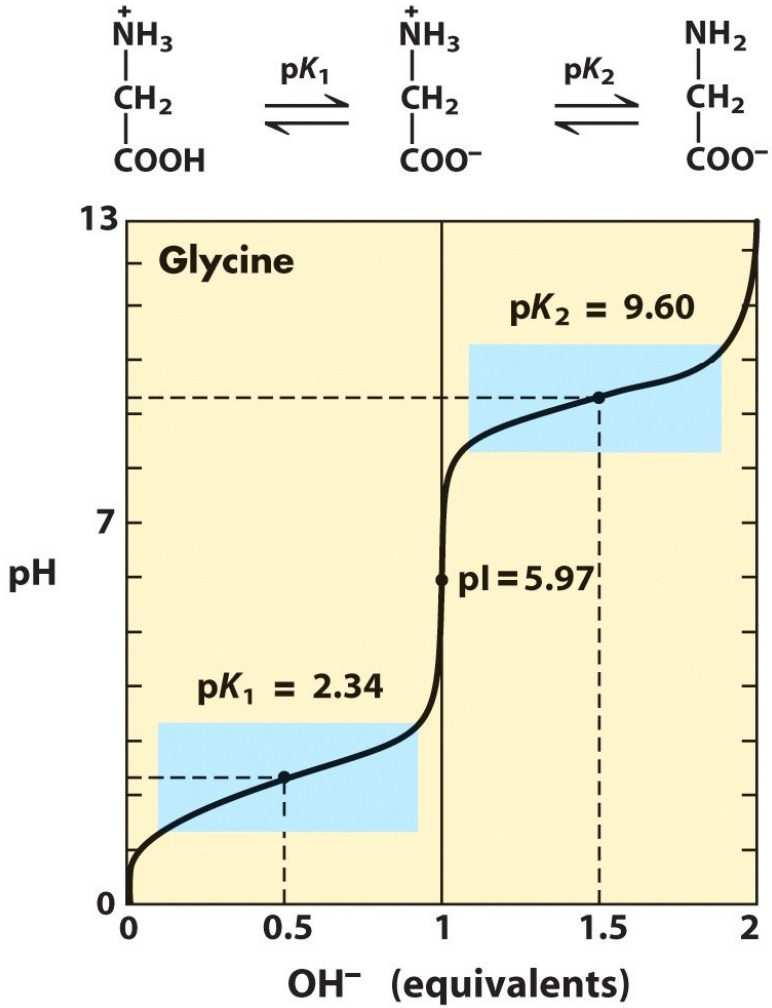
Zwitterion of glycine (charge = 0)

Glycine ion at basic pH (charge = 1-)

- Note that the uncharged species never forms

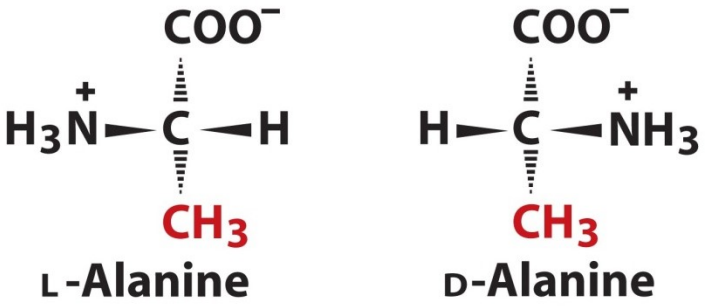
Titration of Glycine

- pK_1
  - [cation] = [zwitterion]
- pK_2
  - [zwitterion] = [anion]
- First equivalence point
  - Zwitterion
  - Molecule has no net charge
  - pH = pI (Isoelectric point)
  - pI = average of pK_a's = ½ (pK_1 + pK_2)
  - $pI_{glycine}$ = ½ (2.34 + 9.60) = 5.97

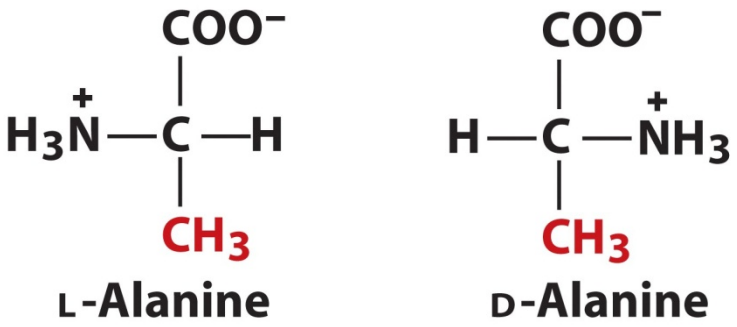


Stereochemistry of AAs

- All amino acids (except glycine) are optically active

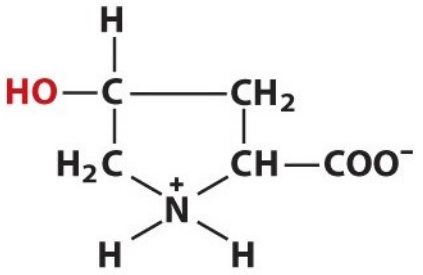


- Fischer projections:

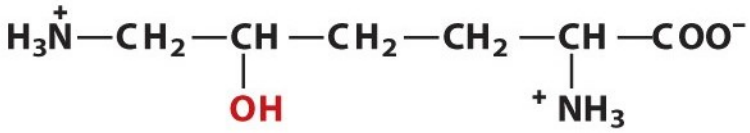


Non-standard Amino Acids

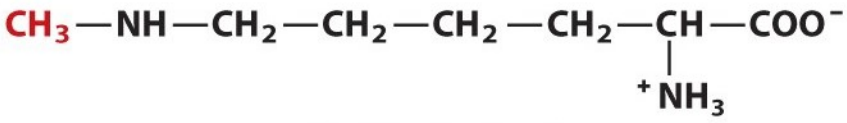
- AA derivatives
  - Modification of AA after protein synthesized
  - Terminal residues or R groups
  - Addition of small alkyl group, hydroxyl, etc.
- D-AAs
  - Bacteria



4-Hydroxyproline



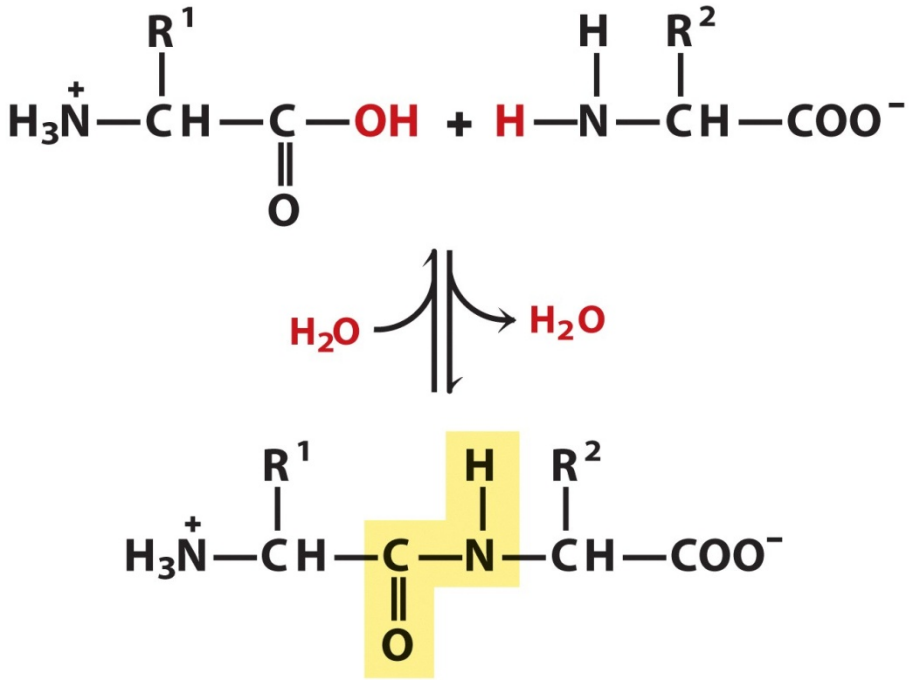
5-Hydroxylysine



6-*N*-Methyllysine

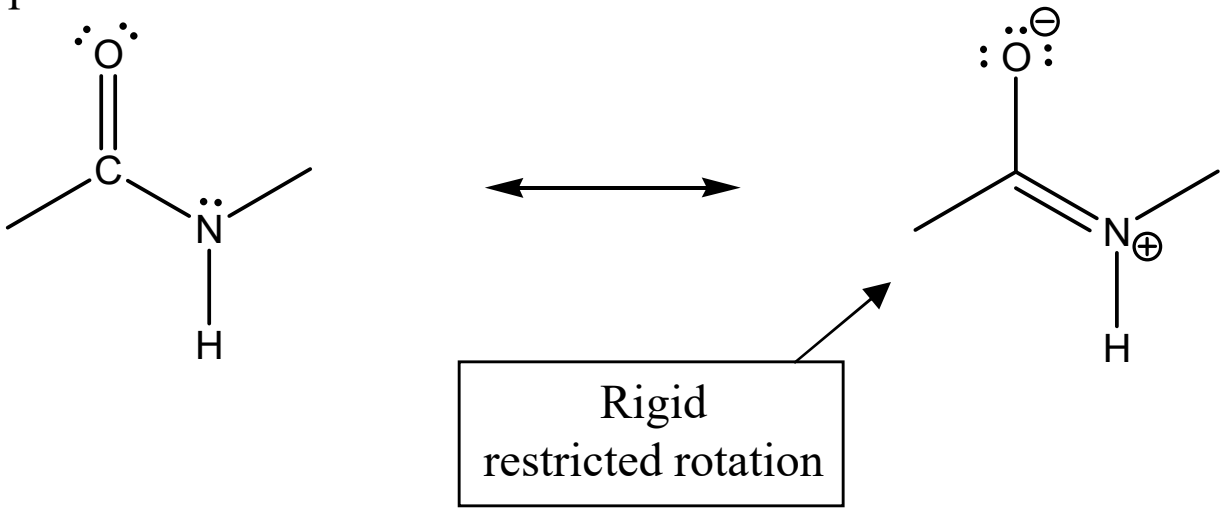
The Peptide Bond

- Chain of amino acids = peptide or protein
- Amino acid residues connected by peptide bonds
- Residue = AA – H_2O



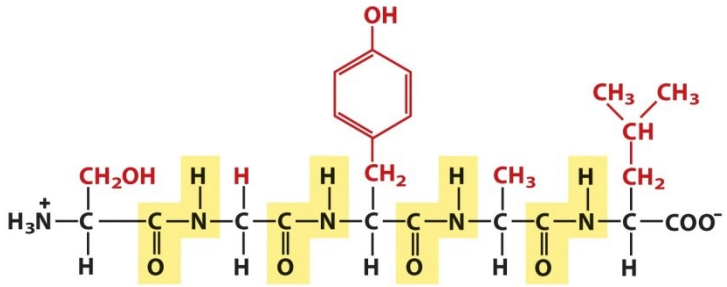
The Peptide Bond

- A **peptide bond (amide bond)** is a covalent chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amine group of the other molecule, thereby releasing a molecule of water (H_2O). This is a dehydration synthesis reaction (also known as a condensation reaction), and usually occurs between amino acids. The resulting C(O)NH bond is called a peptide bond, and the resulting molecule is an amide. The four-atom functional group -C(=O)NH- is called a **peptide link**. Polypeptides and proteins are chains of amino acids held together by peptide bonds



- Amide linkage is planar, NH and CO are anti

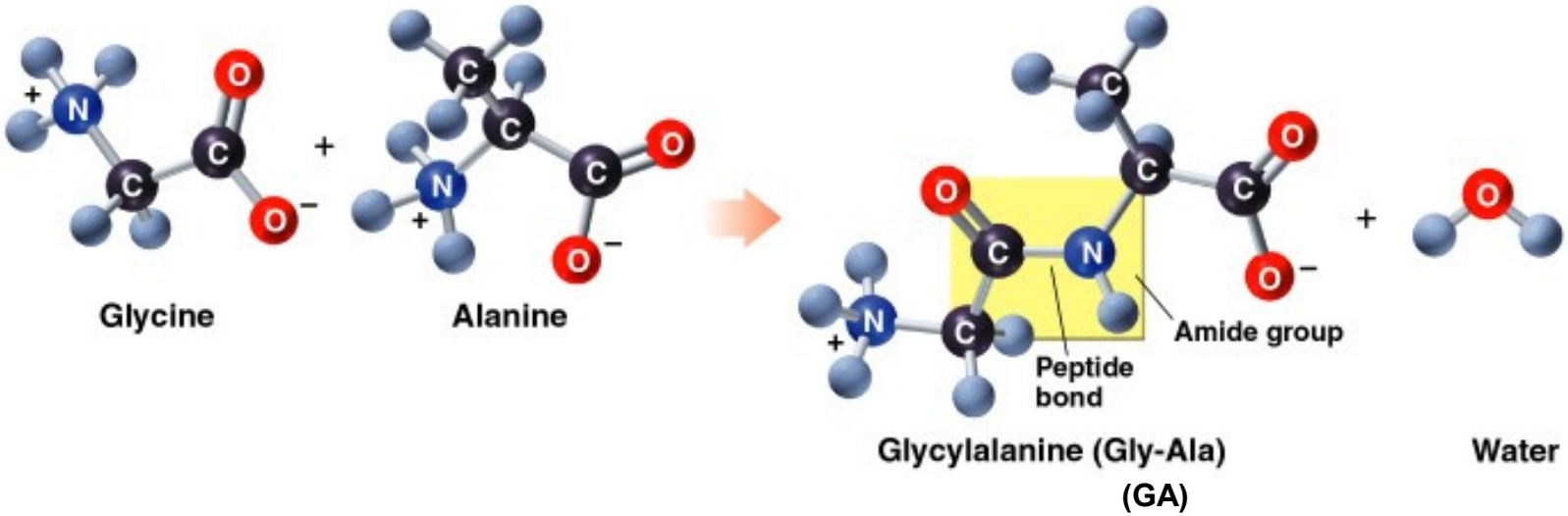
Polypeptides



- Linear polymers (no branches)
- AA monomers linked head to tail
- Terminal residues:
  - Free amino group (N-terminus)
    - Draw on left
  - Free carboxylate group (C-terminus)
    - Draw on right
- pK_a values of AAs in polypeptides differ slightly from pK_a values of free AAs

Naming Peptides

- Name from the free amine (NH_3^+)
- Use *-yl* endings for the names of the amino acids
- The last amino acid with the free carboxyl group (COO^-) uses its amino acid name



Protein size

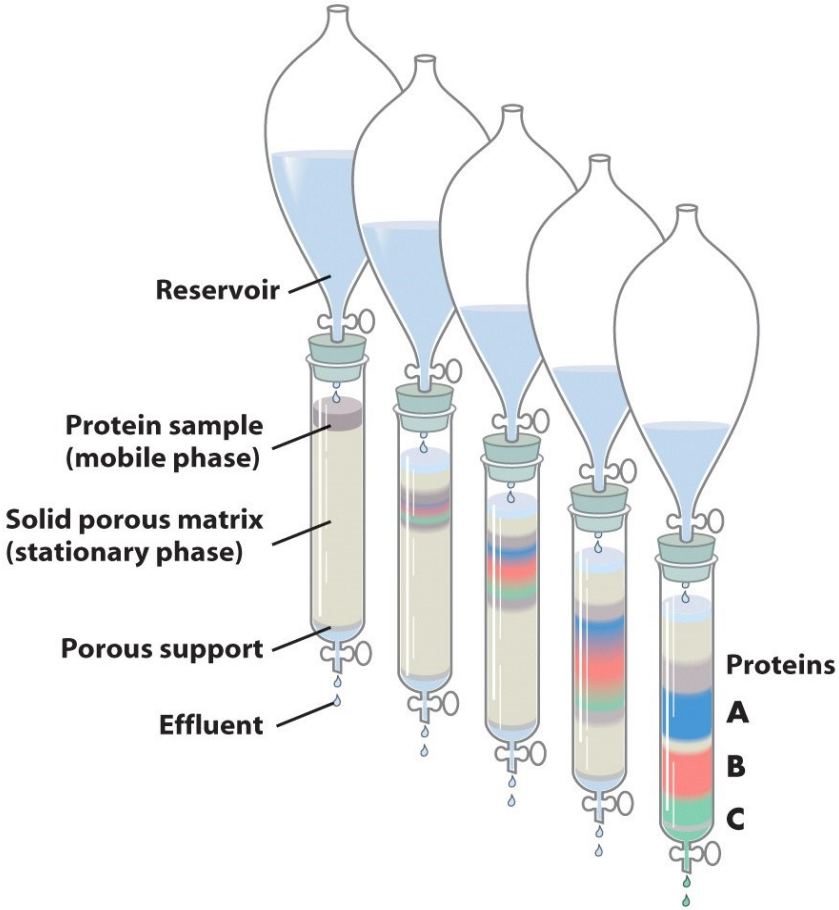
- In general, proteins contain > 40 residues
  - Minimum needed to fold into tertiary structure
- Usually 100-1000 residues
- Percent of each AA varies
- Proteins separated based on differences in size and composition
- Proteins must be pure to analyze, determine structure/function

General Separation Procedure

- Detect/quantitate protein (assay)
- Determine a source (tissue)
- Extract protein
  - Suspend cell source in buffer
  - Homogenize
    - Break into fine pieces
    - Cells disrupted
    - Soluble contents mix with buffer
    - Centrifuge to separate soluble and insoluble
- Separate protein of interest
  - Based on solubility, size, charge, or binding ability

Chromatography

- Mobile phase
  - Mixture dissolved in liquid or solid
- Stationary phase
  - Porous solid matrix
- Components of mixture pass through the column at different rates based on properties

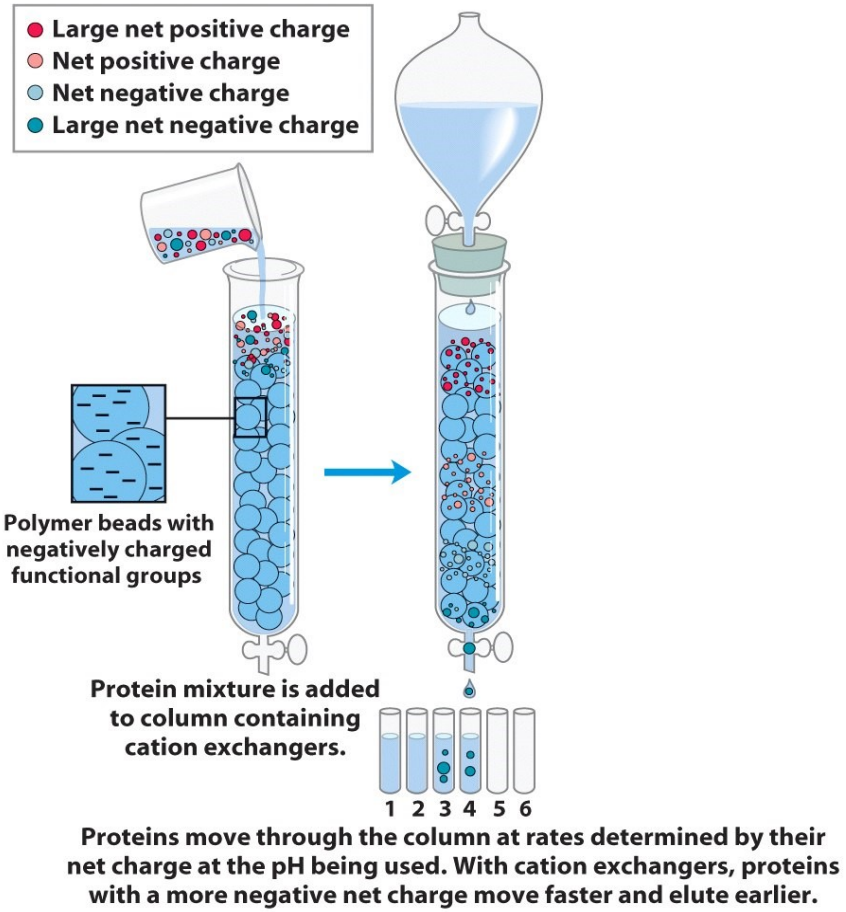


Types of Chromatography

- Paper
  - Stationary phase = filter paper
  - Same theory as thin layer chromatography (TLC)
  - Components separate based on polarity
- High-performance liquid (HPLC)
  - Stationary phase = small uniform particles, large surface area
  - Adapt to separate based on polarity, size, etc.
- Hydrophobic Interaction
  - Hydrophobic groups on matrix
  - Attract hydrophobic portions of protein

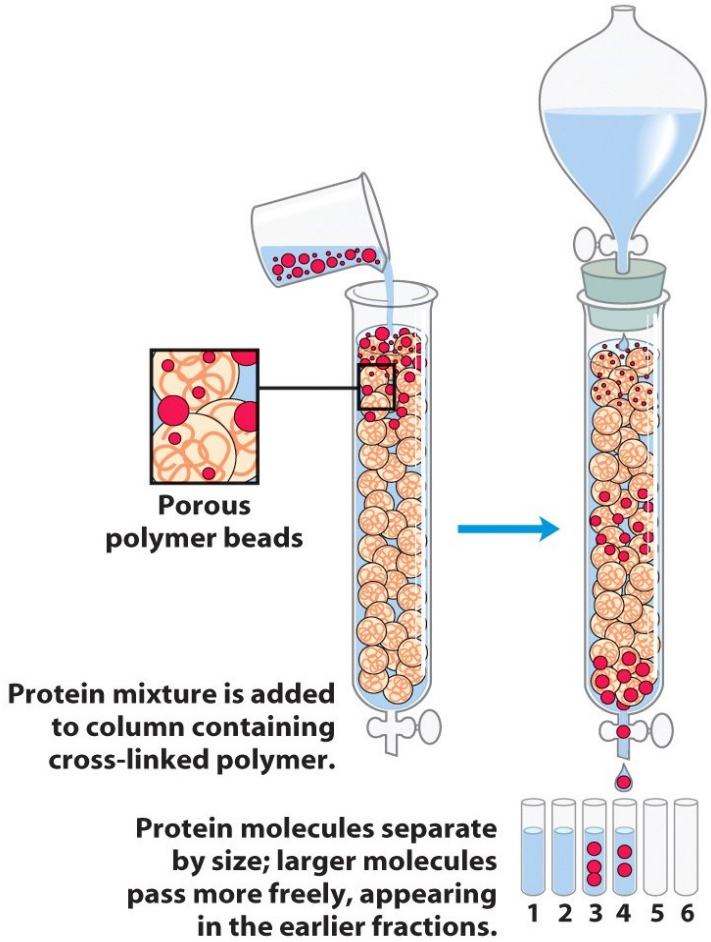
Types of Chromatography

- Ion-exchange
  - Stationary phase = chemically modified to include charged groups
  - Separate based on net charge of proteins
  - Anion exchangers
    - Cation groups (protonated amines) bind anions
  - Cation exchangers
    - Anion groups (carboxylates) bind cations



Types of Chromatography

- Gel-filtration
  - Size/molecular exclusion chromatography
  - Stationary phase = gels with pores of particular size
  - Molecules separate based on size
    - Small molecules caught in pores
    - Large molecules pass through



Types of Chromatography

- Affinity
  - Matrix chemically altered to include a molecule designed to bind a particular protein
  - Other proteins pass through