CHEM 161B EXAM 1

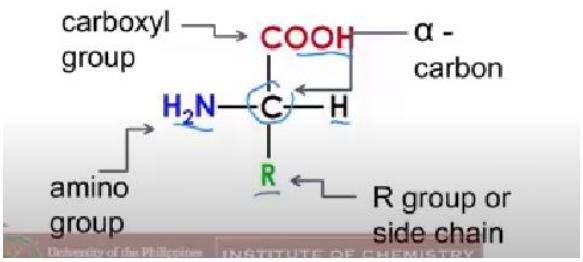
AMINO ACIDS

Amino Acids are the building blocks of proteins.

General Structure of Amino Acids

An alpha amino acid consists of:

- Carboxyl group
- Amino group
- R group (side chain)



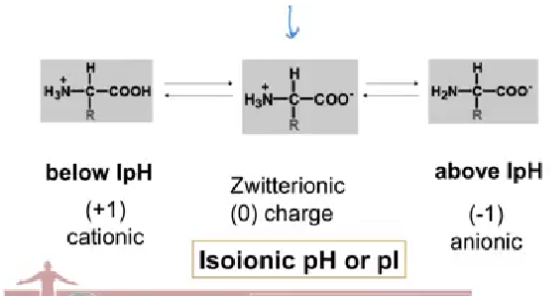
- Amino acids are **amphoteric.**
  - Acts both as an acid and as a base
  - As an **acid** due to the **COOH group**
  - As a **base** due to the **NH2 group**
- Structure at neutral pH
  - Predominantly dipolar ions or **zwitterions**
  - Contains both the negative and the positive charges of the same molecule.
  - Formation of zwitterions are due to the **intramolecular acid base reaction** between the carboxylic acid (-) and the amino group (+).
  - **Zwitterionic structure** explains the physical properties of amino acids.
    - Crystalline solid
    - High melting point
- Amino acids have an **asymmetric carbon** (except glycine)
  - Chiral molecule
  - Optically active
- Stereochemistry
  - L- configuration rotates the polarized light counterclockwise.
  - D- configuration rotates the polarized light clockwise.

Classification of Amino Acids

- Based on **nutritional requirement**
  - Essential AA (PVT TIM HALL)
    - Arg
    - His
    - Ile
    - Leu
    - Lys
    - Met
    - Phe
    - Thr
    - Trp
    - Val
  - Non-essential AA (Almost All Girls Go Crazy After Getting Taken Prom Shopping)
    - Ala
    - Asn
    - Asp
    - Cys
    - Glu
    - Gln
    - Gly
    - Pro
    - Ser
    - Tyr
- Based on **structure**
  - **Nonpolar aliphatic R groups** - hydrocarbons in the sidechains
    - Gly - achiral
    - Ala ï methyl group
    - Val
    - Ile ï 2 chiral Carbons
    - Leu
    - Pro ï cyclic imino acid
    - Met ï thioether group
  - **Aromatic R groups**
    - Tyr ï hydroxyl group

        - Phe ï phenyl ring
        - Trp ï bicyclic indole ring
    - **Polar but uncharged R groups** ï hetero atoms in the side chain but no charges
        - Ser ï OH
        - Thr ï 2 chiral carbons (OH)
        - Cys ï thiol/sulfhydryl group (OH)
        - Asn ï amide
        - Gln ï amide
    - **Positively charged R groups** ï have basic -NH2 side chains.
        - Arg
        - His
        - Lys
    - **Negatively charged R groups** ï have acidic -COOH side chains, negatively-charged at pH 7.
        - Asp
        - Glu

Protonic Equilibria of Amino Acids



- Isoionic pH or pI ï AA has 0 charge
- Lower IpH ï higher concentration of H+, cationic
- Higher IpH ï higher concentration of OH-, anionic

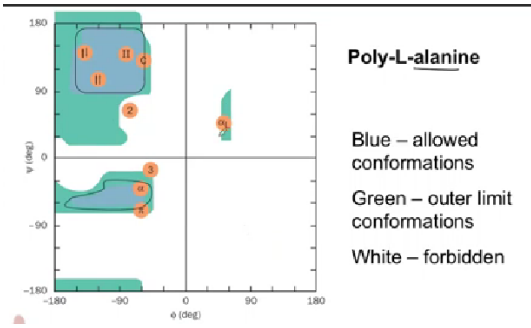
$$IpH = \frac{pK_a(x) + pK_a(y)}{2}$$

Analysis of Amino Acids

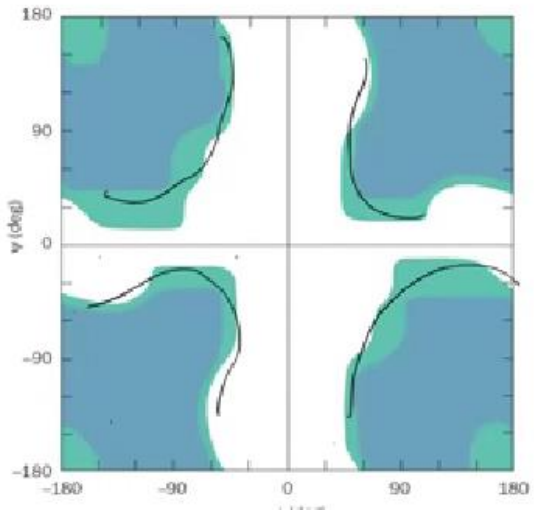
- **Paper Electrophoresis** ï separation of AA based on electric charge
- **Reaction with ninhydrin** ï addition of ninhydrin would render the reaction with the AA to turn the product into blue or purple-colored
- **Spectrophotometry** ï amino acids capable of absorbing light are those that contain aromatic groups (Tyr, Phe, Trp).
    - Trp has a higher absorption of light than that of Tyr because of the bicyclic structure of Trp (resonance-stabilized and conjugated).

PEPTIDES

- Proteins are linear polymers of amino acids that are formed by linking together the **carboxyl group** of one amino acid and the **amino group** of another amino acid through **peptide bond**.
- Peptide-bond formation is accompanied by the **loss of a molecule of water**.
    - The reaction is reversible.
    - The equilibrium favors the side of the hydrolysis rather than the synthesis.
    - The synthesis of peptide bonds would require input of free energy.
- Peptide bonds are **planar**.
- The **trans peptide bonds** is strongly favored because of steric clashes in the cis form.
- N-CŬ ï phi torsion angle
- CŬ-$C_{(C=O)}$ ï psi torsion angle
- Allowed configurations of polypeptides are indicated by the **Ramachandran diagram**.



- Factors involved in allowed conformations in polypeptides:
  - Psi and phi values
  - Van der Waals distances for interatomic contacts (greater distances between groups are allowed)
- Glycineôs conformation has larger regions for it to be allowed to exist.
  - Glycine is less sterically hindered.
  - Has greater conformational freedom
  - Can occupy positions where a polypeptide makes a sharp turn.



Biological Activities of Some Peptides

A. HORMONES

- **Insulin** ï contain 2 polypeptide chains (30 and 21 residues)
  - Secreted by the beta-cells of the pancreas
  - Involved in glucose metabolism
  - Joined together by disulfide linkages in cysteine
- **Glucagon** ï 29 residues; secreted by the alpha-cells of the pancreas.
  - Suppresses insulin
  - Released during fasting or starvation state
- **Oxytocin** ï 9 residues; stimulates uterine contractions.
- **Vasopressin** ï 9 residues; causes rise in blood pressure; prevents urination at night.

B. SYNTHETIC PEPTIDES

- **Aspartame** ï artificial sweetener

PROTEINS

- ***ñProteiosò*** ï meaning ñfirst of rankò
- Most abundant macromolecules in living cells.
- **Polypeptide chain** ï linear polymer of amino acids.
- **Proteins** ï generally have very high molecular weight.
- **Oligomers** ï more than one polypeptide chain.

CLASSIFICATION OF PROTEINS

a. COMPOSITION

- Simple Proteins ï yield only AA upon hydrolysis
- Conjugated proteins ï simple proteins + non-protein substances

b. SOLUBILITY

- Albumins ï soluble in water and dilute aq solutions
- Globulins ï soluble in dilute salt solutions but are insoluble or sparingly soluble in water
- Glutelins ï soluble in dilute solutions of acids and bases; insoluble in neutral solvents

- Albuminoids/Scleroproteins ï insoluble in most ordinary solvents
- Prolamins ï soluble in 50=90% alcohol
- Insoluble in water, neutral solvents or absolute alcohol.

c. SHAPE

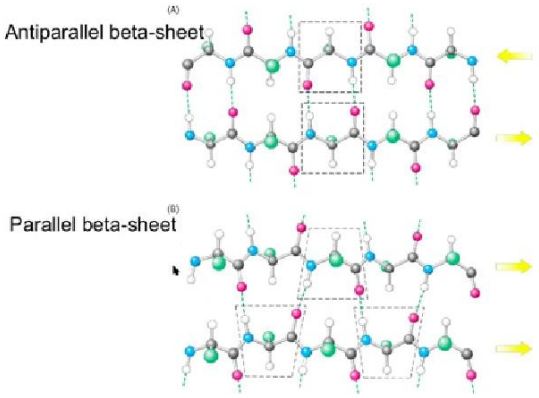
- Globular proteins ï tightly folded into compact spherical shape; soluble in aqueous system.
- Fibrous proteins ï long, stringy molecules; water-insoluble; structural and protective functions.
    - E.g., collagen, keratin

d. FUNCTION

- Enzymes
- Transport
- Nutrient and storage
- Contractile or motile function
- Structural roles
- Defense
- Regulatory roles
- Information decoding roles

FOUR LEVELS OF PROTEIN ORGANIZATION

- **Primary Structure** ï amino acid sequence
- **Secondary Structure**
    - Ŭ-helix ʁ-pleated sheet
    - formed by regular patterns of **hydrogen bonds** between N-H and C=O groups of amino acids that are near each other in the linear sequence.
    - 3.6 residues per turn
    - Essentially all Ŭ-helices found in proteins are **right-handed**.
    - Antiparallel beta-sheet is **more stable** than parallel beta-sheet because of its geometry.



- **Tertiary Structure** ï refers to the spatial arrangement of AA that are far apart in the sequence.
    - e.g. **myoglobin** ï has 1 sub-unit; oxygen-carrier in **muscle**; has a heme group
    - Water-soluble proteins fold into compact structure with nonpolar cores.
    - Interactions between R groups: hydrophobic interactions (nonpolar portions, hydrocarbons), H-bonding (hetero atoms, N, O, S (very weakly)), ionic interaction (+/- charges), disulfide linkage (cysteine)
- **Quaternary Structure** ï spatial arrangement of protein subunits. **Noncovalent** interactions between tertiary structures.
    - e.g. **hemoglobin ï** oligomeric protein made up of 4 polypeptide chains and a heme group
- A **protein domain** is a conserved part of a given protein sequence and tertiary structure that can **evolve, function**, and **exist** independently of the rest of the protein chain.

PROTEIN PURIFICATION

- **Why** purify proteins? To study structure, function, information about genes, for use in other applications (e.g., research, medical, industrial, etc.)

TYPICAL SCHEME TO PURIFY PROTEINS

- **Develop assays to identify and quantify the protein**
  - Monitor purification progress
  - Perform in every step
  - Assays to **identify** proteins:
    - Characteristic properties (size, molar mass, spectroscopic profile)
    - Enzymatic activities (measure extent of product conversion)
  - Assays to **quantify** proteins:
    - Biuret method: strong alkaline **copper** agent, **purple** complex with peptide bonds
    - Lowry method: **copper** reagent + **Folin-Ciocalteau** phenol reagent; **dark blue** color
    - Bradford method: Coomassie brilliant blue G-250 (**red dye becomes blue** in the presence of protein)
- **Select source and prepare homogenate**
  - Possible sources: animal, plant tissues, microorganisms, genetically modified bacteria, yeast
  - Prepare homogenate ï break the cells
- **Separate cell homogenate into fractions** ï use properties unique to the protein
  - Separation of proteins:
    - Based on **size or molecular weight**: centrifugation, dialysis, size exclusion chromatography
    - Based on **solubility differences**: salting-out
    - Based on **difference in electric charge**: ion-exchange chromatography, isoelectric focusing
    - Based on **size and electric charge**: electrophoresis
    - Based on **affinity to other molecules**: affinity chromatography
- **Perform Chromatography**
  - Size exclusion/gel filtration chromatography
  - Ion-exchange chromatography
  - Affinity chromatography
- **Determine protein purity and size by gel electrophoresis**
  - **SDS ï** denatures protein giving them a NET negative charge
  - Smaller molecules migrate faster to the positive charge whereas the large ones are retarded to the pores of the gel.

DETERMINING PRIMARY STRUCTURE OF PROTEIN

- **Determine Amino Acid Composition**
  - Acid or base hydrolysis

- Separate free amino acids (ion exchange chromatography)
- Measure the amount of each AA

COMMON STRATEGY FOR SEQUENCE DETERMINATION

- **Purification of the polypeptide**
- **Cleavage of S-S (**using в-mercaptoethanol, SH SH, or performic acid, SO3- SO3-)
- **Determine N and C terminal residues**
  - N ï terminal
    - Sangerôs method -> DNP derivative
    - Dansyl chloride -> sulfonamide derivative
    - Dabsyl chloride -> sulfonamide derivative; cannot be repeatedly used for the same peptide due to the complete hydrolysis step
    - **Edman degradation -> phenylthiolhydantoin-derivative**
      - Releases N-terminal residue, leaves the rest of the polypeptide intact
      - Limited to first ~50 AA
  - C-terminal
    - Carboxypeptidase ï cleaves at amino side of C-terminal (not for Arg, Lys, Pro); not as reliable.
- **Internal cleavages**
  - **Cyanogen bromide (CNBr-Met)**
    - Cleaves **carboxyl side** of **methionine residues** (result: methioninehomoserine lactone)
  - **Trypsin (T-ALC)**
    - Cleaves **carboxyl side** of **arginine** and **lysine residues**
  - **Chymotrypsin (C-PTrpTyrLC)**
    - Cleaves **carboxyl side** of **phenylalanine, tryptophan, tyrosine,** and **leucine residues**
  - **Thermolysin (T-PTrpTyrIleLeuValA)**
    - Cleaves **amino side** of **phenylalanine, tryptophan, tyrosine, leucine, isoleucine,** and **valine residues**
  - For 2 or more polypeptide chains:
    - Denature using **urea or guanidine HCl**
    - Treatment with **dithiothreitol**, then **iodoacetate** to keep the chains separated