CLS 281

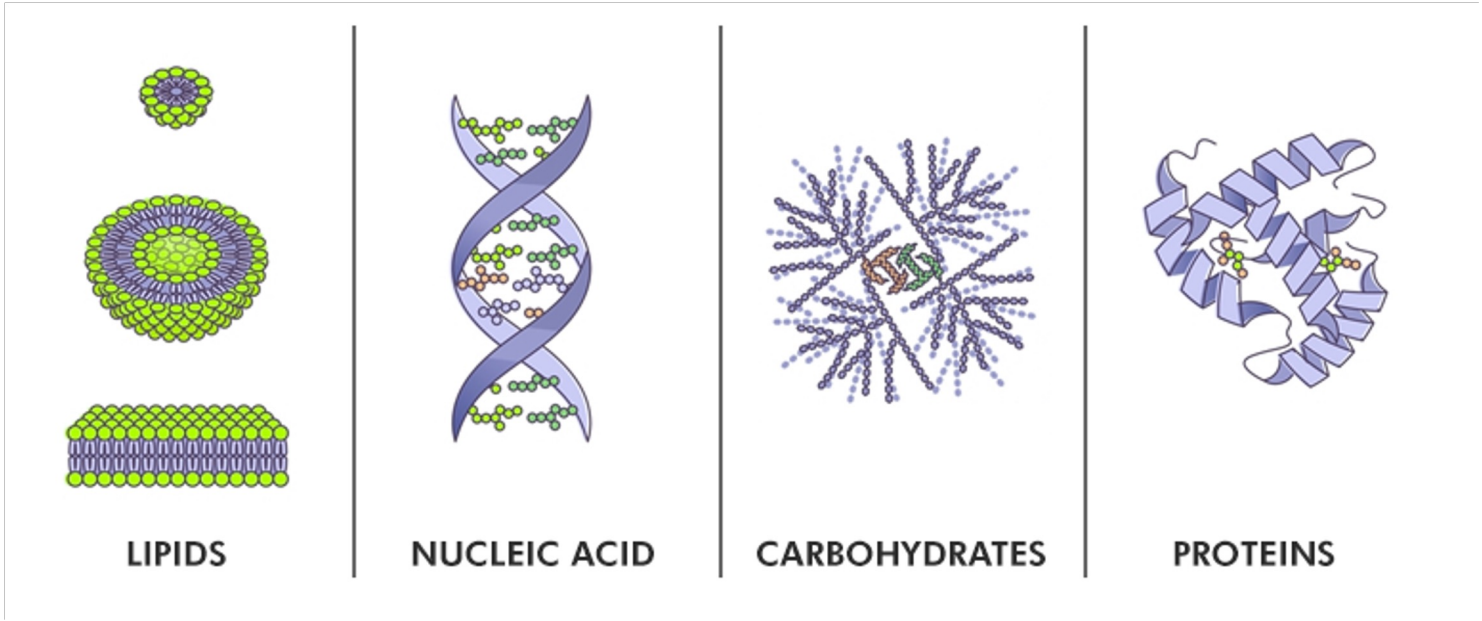
Basic Biochemistry and Biomolecules

جامعة الملك سعود
King Saud University
1957

Experiment 1

General Color Tests for Proteins

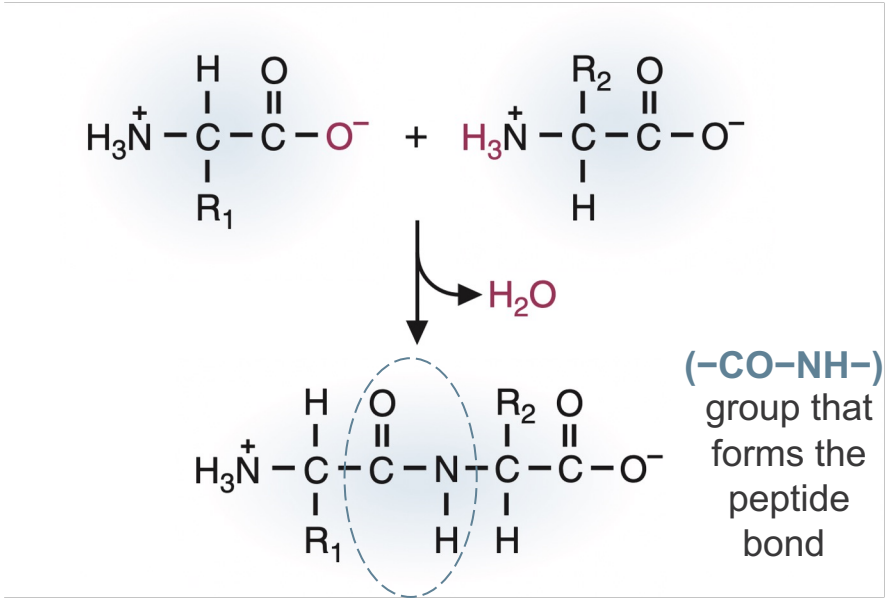
Biomolecules



The four significant classes of Biomolecules: Lipids, Nucleic acids, Carbohydrates, and Proteins.

Background

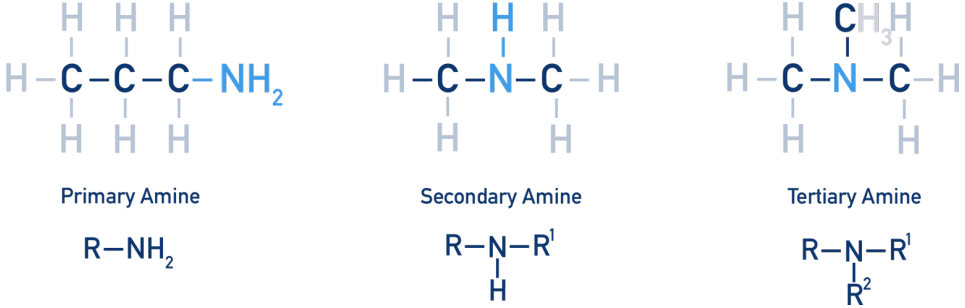
- **Proteins** are the most abundant and functionally diverse molecules in living systems.
- **Proteins** display an incredible diversity of functions, yet all share the common structural feature of being linear **polymers of amino acids.**
- **Amino acids** are covalently linked through (–CO–NH–) peptide bonds.

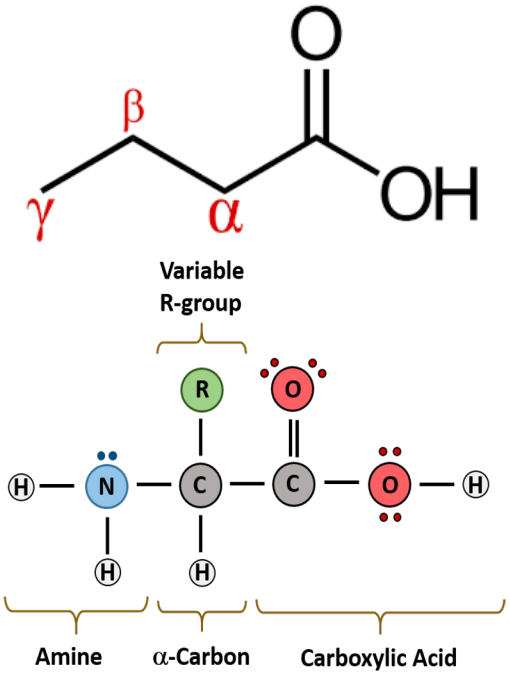


Peptide bonds. Amino acids in a polypeptide chain are joined through peptide bonds between the carboxyl group of one amino acid and the amino group of the next amino acid in the sequence.

Background

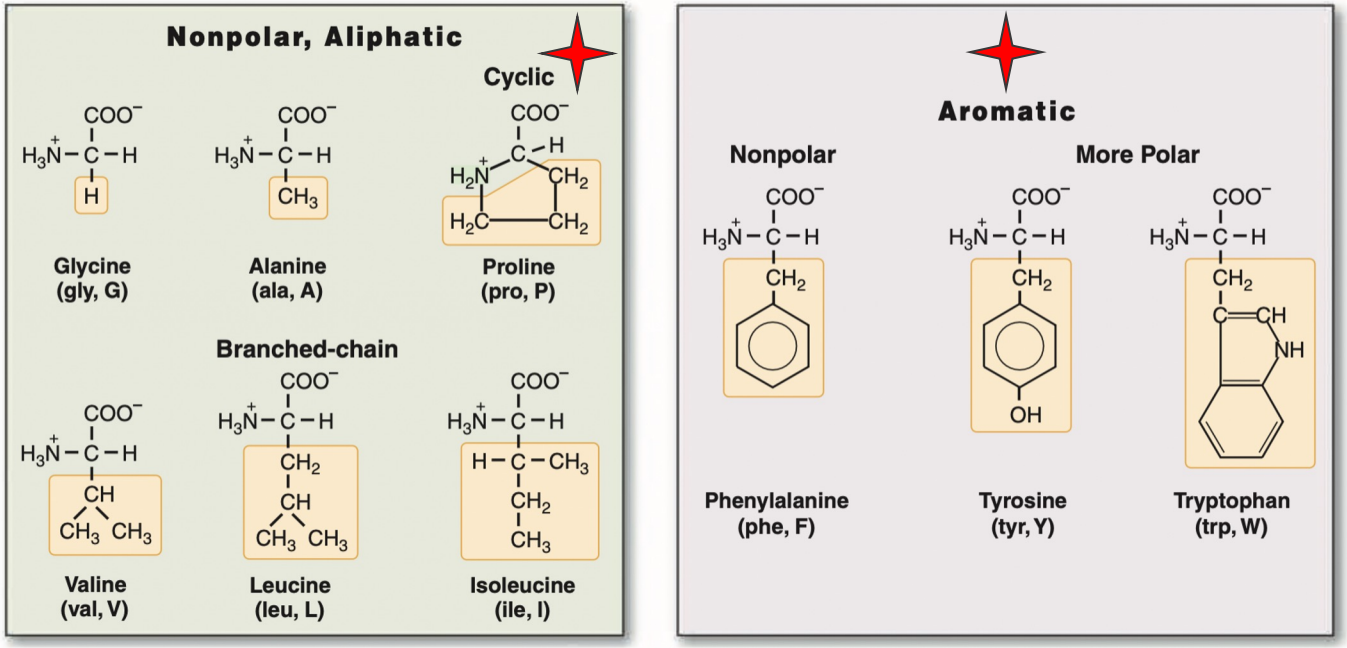
- The first carbon following the carboxyl carbon is the **alpha carbon**. The second carbon following the carboxyl carbon is the **beta carbon.**
- There are 20 amino acids commonly found as constituents of mammalian proteins.
- Each amino acid has a **primary amino group** bonded to the α-carbon atom, except for **proline**, which has a **secondary amino group.**



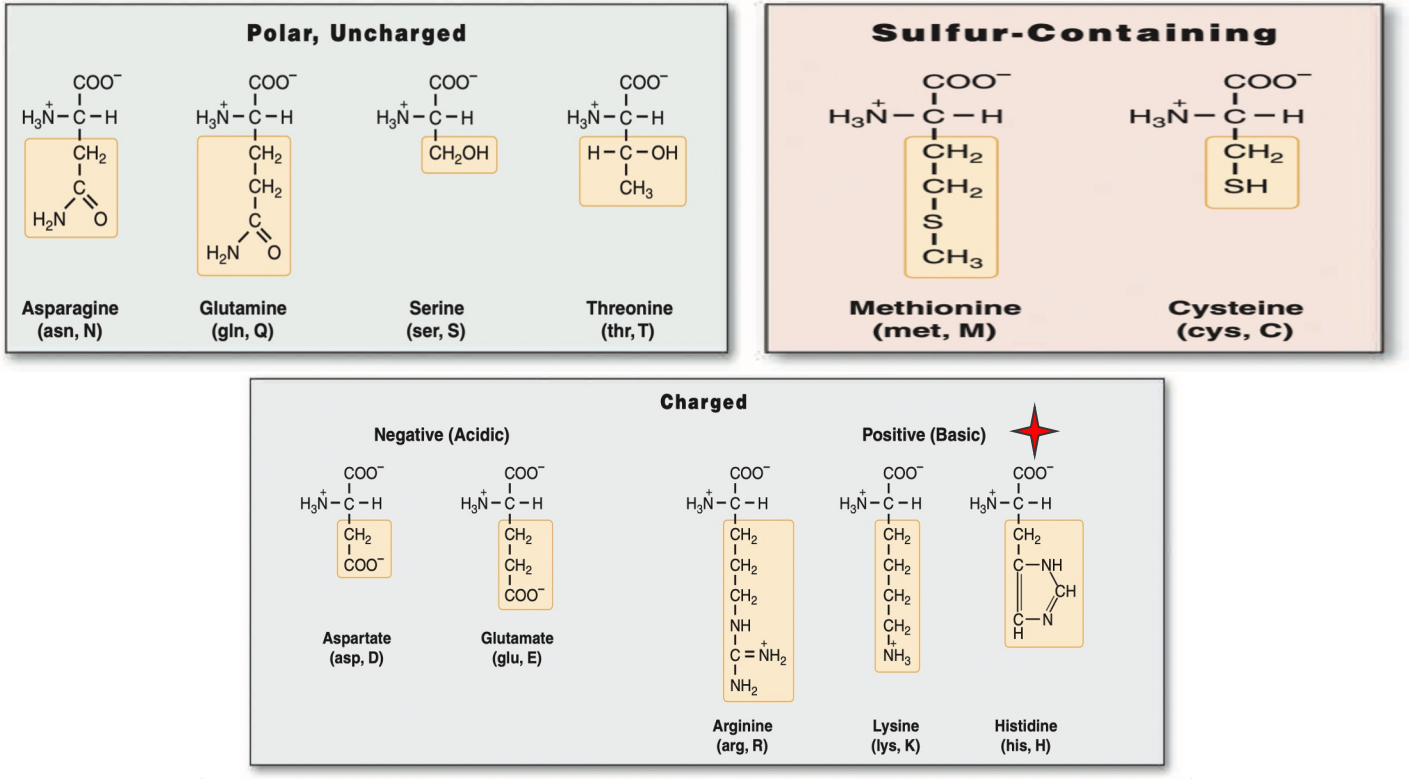


Structural features of free amino acid.

Classification of Amino Acid



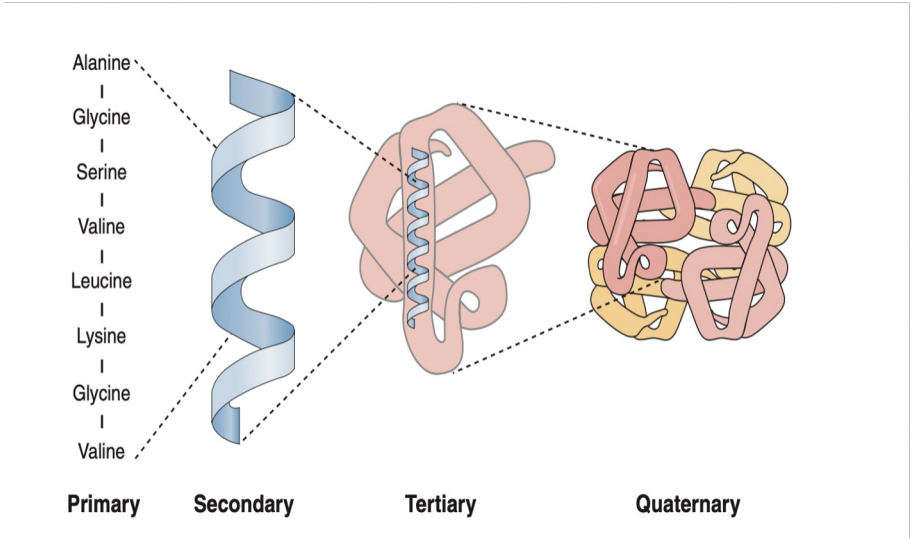
Classification of Amino Acid



Levels of protein structure

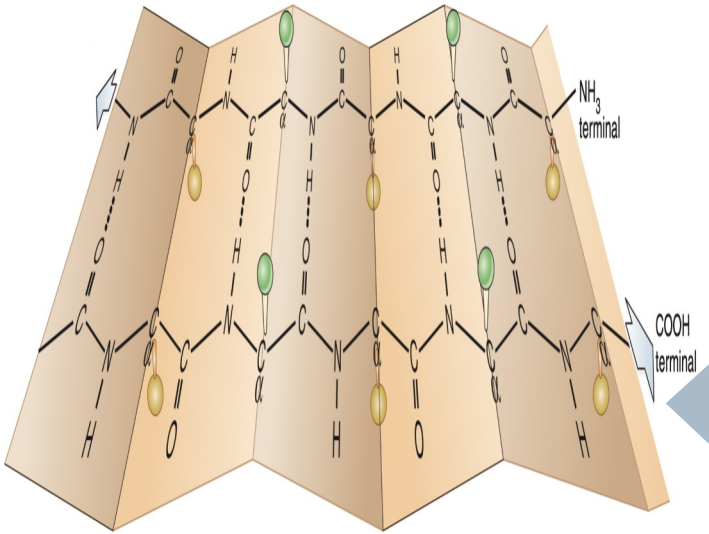
There are four levels of protein structure:

- The **primary structure** (linear sequence of amino acids within the protein)
- The **secondary structure** (a regular, repeating pattern of hydrogen bonds stabilizing a particular structure)
- The **tertiary structure** (the folding of the secondary structure elements into a three-dimensional conformation)
- The **quaternary structure** (the association of subunits within a protein)



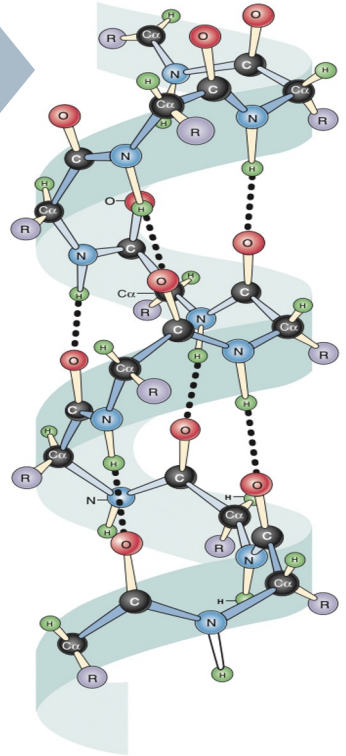
The four hierarchies of protein structure.

Secondary protein structure



The β-pleated sheet.

The α-helix.



Methods of protein detection

- Several techniques have been developed for detecting or measuring protein in a sample.
- Each technique has its own advantages, limitations, and uses.

General Proteins Color Tests:

1. Biuret Test
2. Ninhydrin Test
3. Xanthoproteic Test
4. Lowry
5. Bradford
6. BCA
7. Direct spectrophotometry at 280 nm.

Table.1 Methods of protein measurements.

Method	Sensitivity	Time	Principle	Interferences	Comments
Biuret	Low 1–20 mg	Moderate 20–30 min	Peptide bonds + alkaline $Cu^{2+} \rightarrow$ purple complex	Zwitterionic buffers, some amino acids	Similar color with all proteins. Destructive to protein samples.
Lowry	High ~5 μg	Slow 40–60 min	(1) Biuret reaction (2) Reduction of phosphomolybdate-phosphotungstate by Tyr and Trp	Ammonium sulfate, glycine, zwitterionic buffers, mercaptans	Time-consuming. Intensity of color varies with proteins. Critical timing of procedure. Destructive to protein samples.
Bradford	High ~1 μg	Rapid 15 min	λ_{max} of Coomassie dye shifts from 465 nm to 595 nm when protein-bound	Strongly basic buffers; detergents Triton X-100, SDS	Stable color that varies with proteins. Reagents commercially available. Destructive to protein samples. Discoloration of glassware.
BCA	High 1 μg	Slow 60 min	(1) Biuret reaction (2) Copper complex with BCA; λ_{max} = 562 nm	EDTA, DTT, ammonium sulfate	Compatible with detergents. Reagents commercially available. Destructive to protein samples.
Spectro-photometric (A_{280})	Moderate 50 – 1000 μg	Rapid 5–10 min	Absorption of 280-nm light by aromatic residues	Purines, pyrimidines, nucleic acids	Useful for monitoring column eluents. Nucleic acid absorption can be corrected. Nondestructive to protein samples. Varies with proteins.

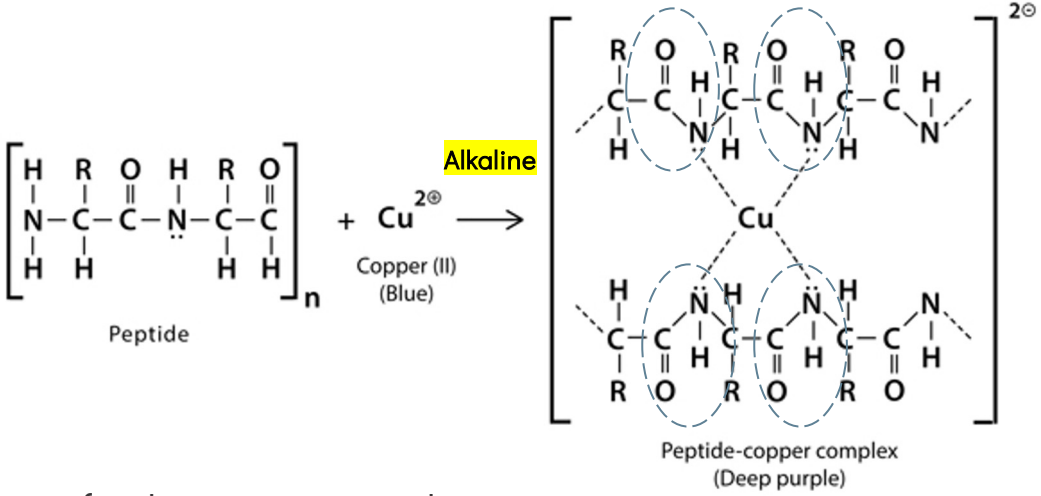
Type of Testing

- **Qualitative** examinations measure the presence or absence of a substance.

- **Quantitative** examinations are used for determining the amount of an analyte in a sample. The amount is always expressed as a number with appropriate units.

Biuret Test

- The biuret procedure is the **most widely** used method for the determination of total protein.
- In this reaction, **cupric ions (Cu2+)** are complex with the groups involved in the peptide bond.
- Cupric ions react with the NHCO group that occurs in the peptide bond.
- In an alkaline medium and the presence of at least two peptide bonds, a violet-colored is formed.



copper co-ordinated complex.

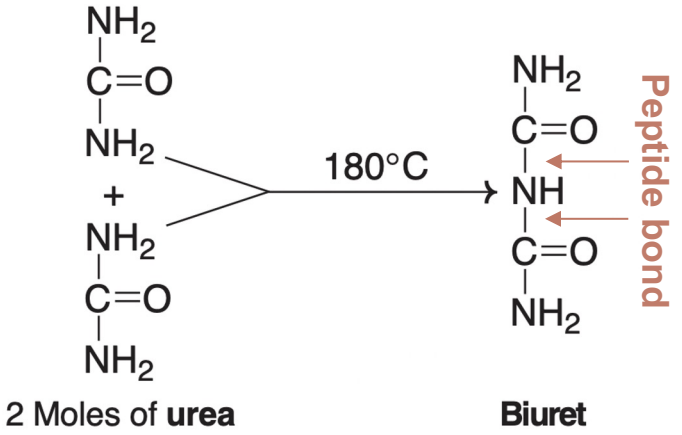
Biuret Test

Why its called the Biuret test?

- The method was named because a substance called biuret (NH2CONHCONH2) reacted with cupric ions in the same manner as protein.

- **Biuret** is a compound formed by heating 2 molecules of urea to 180°C which results in the **condensation** of 2 molecules of urea.

- When biuret is treated with dilute copper sulfate in an alkaline medium, a purple color is obtained due to the presence of 2 peptide bonds.



Bi = 2
>> 2 urea compounds

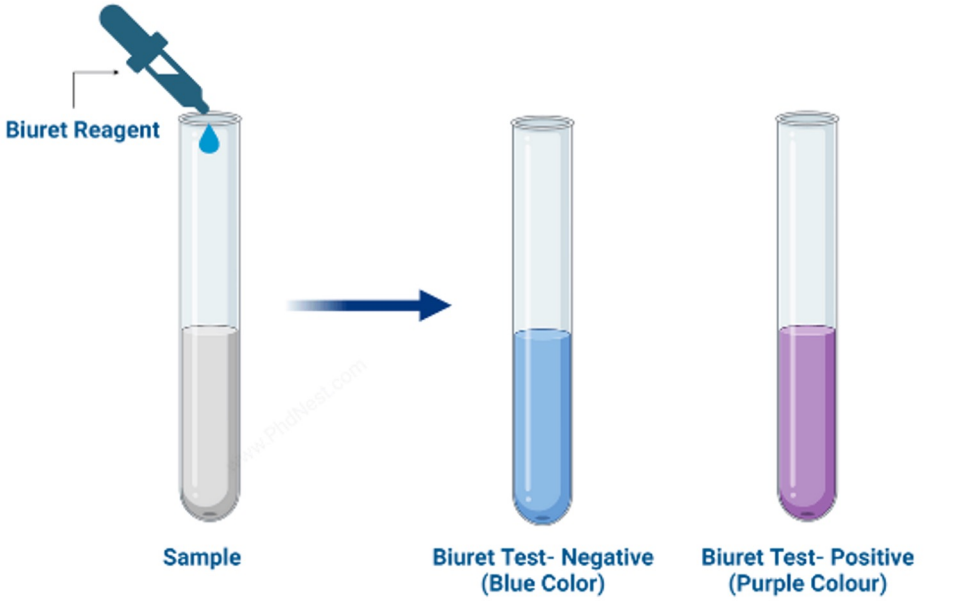
Biuret Test

Does Not Detect:

- Amino Acid
- **Dipeptides**: a dipeptide is an organic compound derived from two amino acids

Detect:

- **Tripeptides:** a tripeptide is a peptide derived from three amino acids joined by two or sometimes three peptide bonds.
- Proteins
- Histidine is the only amino acid that gives a positive result in the Biuret test.



Biuret Test Procedure

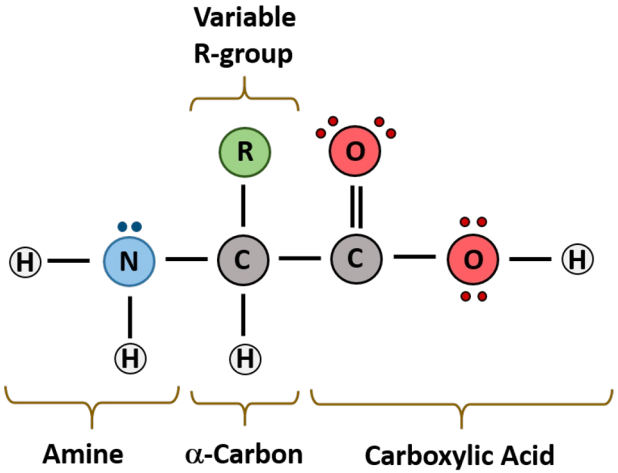
Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
1	Sample	1 %casein	1%glucose	1%sucrose	1%alanine	1%egg albumin	H2O
	Volume	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
2	Alkaline Reagent	10% NaOH					
	Volume	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
3	Reagent	0.1% CUSO4					
	Volume	5 drops	5 drops	5 drops	5 drops	5 drops	5 drops

Mix, and describe any color change that occurred

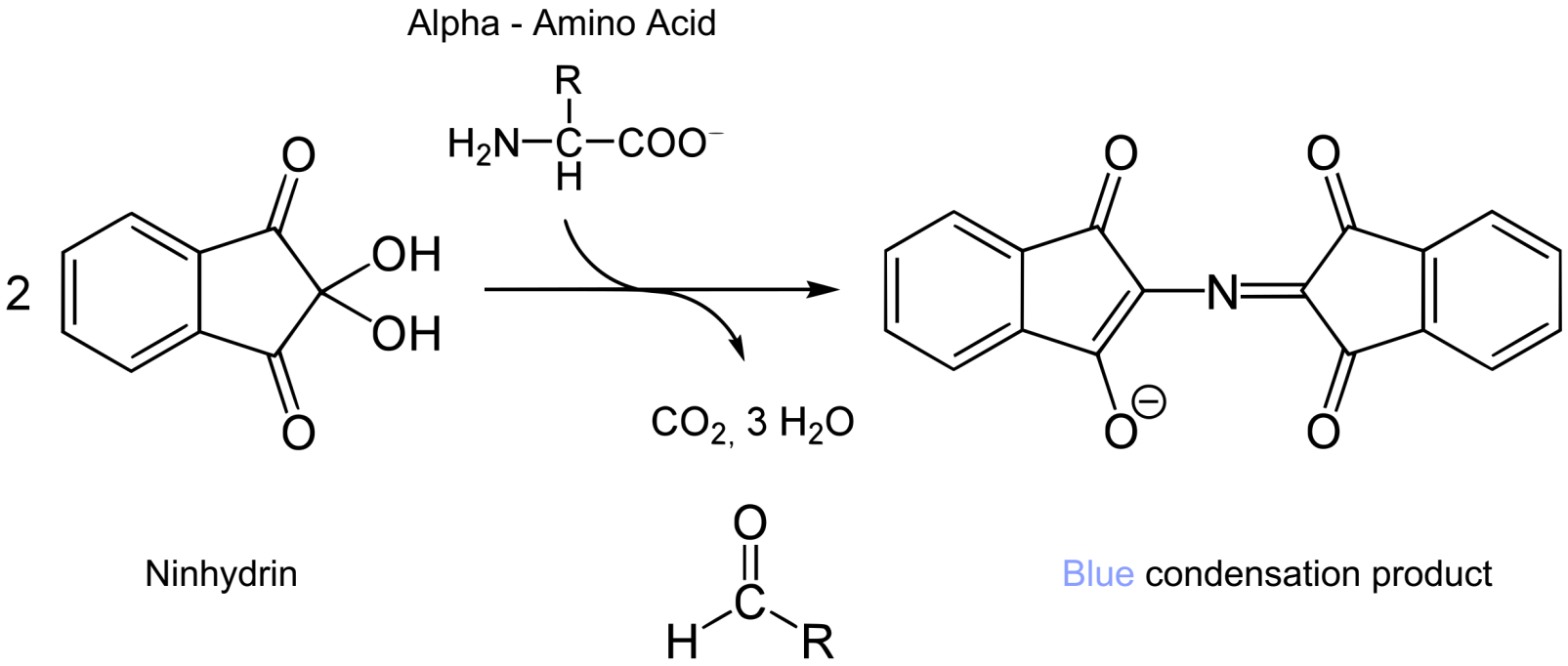
Ninhydrin Test

- It is a general test used for detecting the presence of **proteins** and **peptides,** and **amino acids.**
- Amino acid (that has an **α-amino group**) reacts with **ninhydrin** to form a blue colored complex.
- This color is due to liberates NH3 with ninhydrin.
- What is Alpha-amino acid?

  α-Amino acids are simple molecules that are made of a central C-atom, labeled Ca (Alpha carbon), that is bound to an **alpha amine group NH2 called (the free amino group, if it is attached to a single carbon atom).**

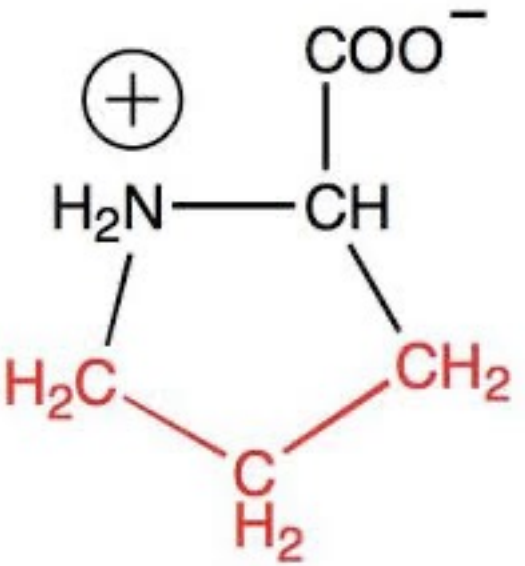


Ninhydrin Test Chemical Reaction



Proline gives yellow color due to the lack of

a free (primary) amino group.

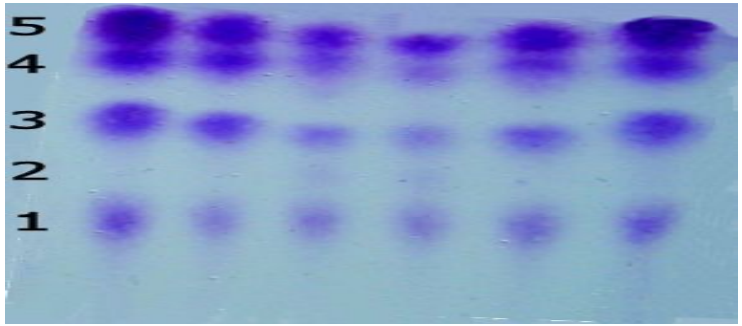


Proline

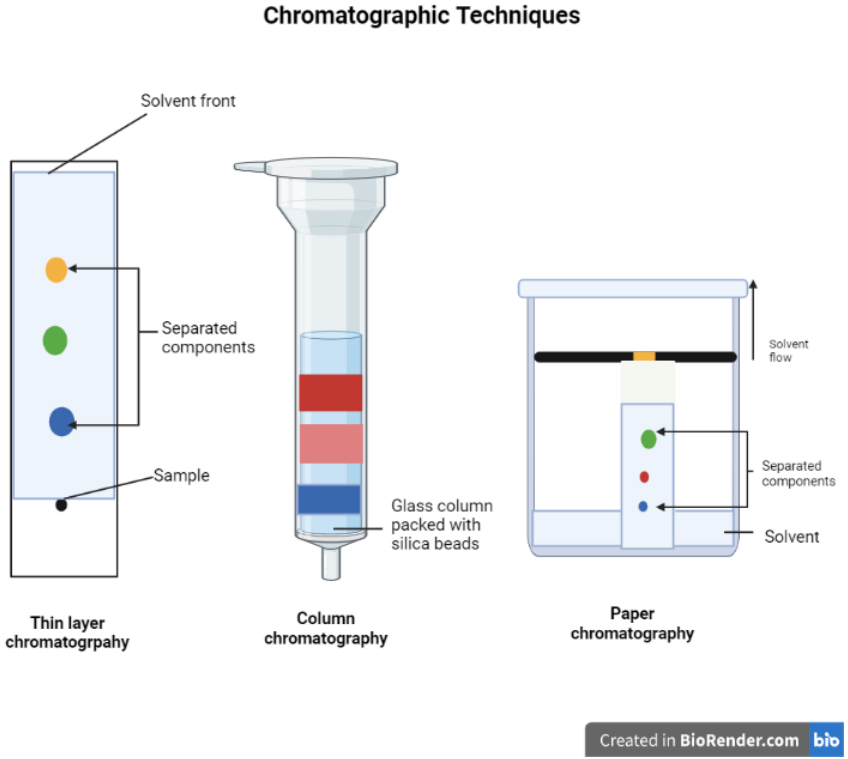
Ninhydrin Test

Application:

- Ninhydrin is used to locate the α-amino acid in **paper chromatography** as blue to purple spots.
- Also, it permits the quantitative estimation of α-amino acid and peptides in **column chromatography.**



A chromatogram of a protein sample stained purple with ninhydrin showing 4 amino acid pieces present at different positions



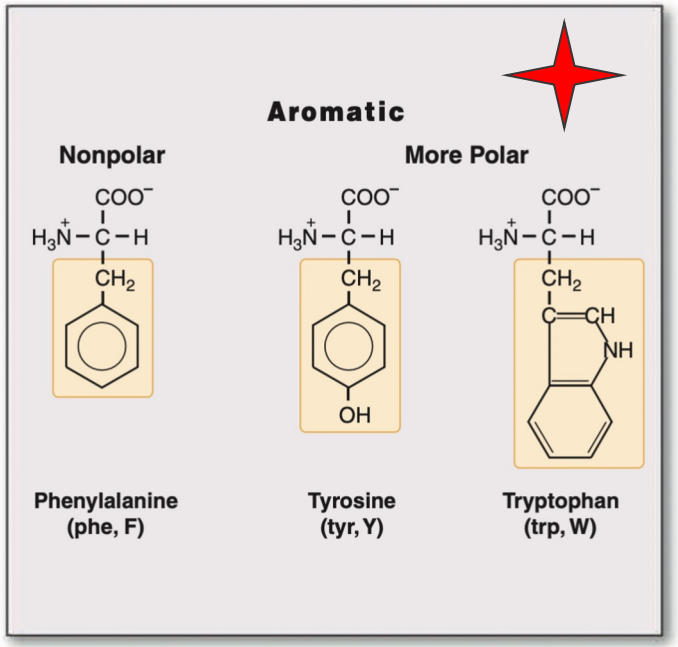
Ninhydrin Test Procedure

Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
1	Sample	Dilute ammonia	1%casein	1%sucrose	1%proline	1%alanine	1%egg albumin	H2O
	Volume	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
2	Reagent	0.1% aqueous ninhydrin						
	Volume	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

3- Mix, incubate in a **boiling water bath for 4 minutes**, then cool.
4- Describe the color changes that occur in each test tube.

Xanthoproteic Test

- The xanthoproteic test is a method that can be used to determine the amount of protein soluble in a solution using concentrated **nitric acid.**
- The test gives a positive result in those **proteins with amino acids carrying aromatic groups.**
- **Nitration** of the aromatic rings in **Tyrosine** and **Tryptophan**, with concentrated HNO3 nitric acid, produces a yellow color.



Xanthoproteic Test Principle

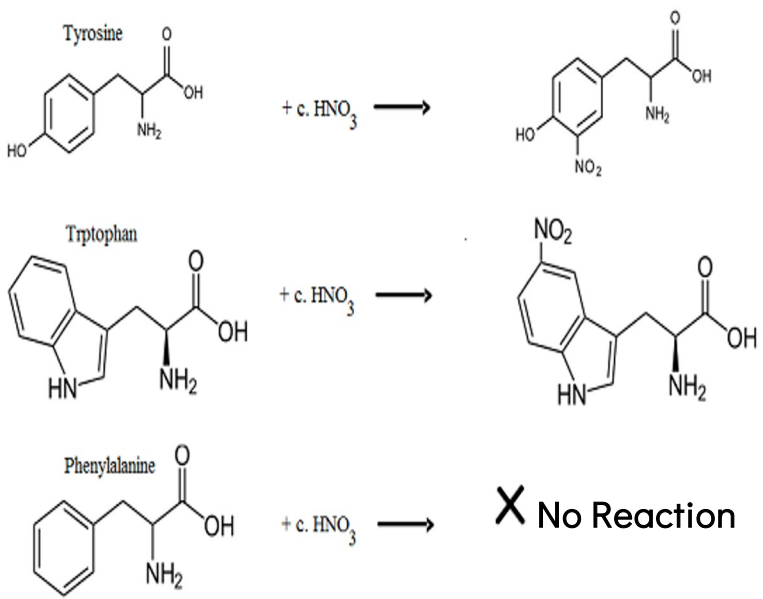
- Tyrosine or Tryptophan + con.HNO3 —--(heat)-------> yellow color

- The yellow color is due to **Xanthoproteic acid**, which is formed due to the nitration of certain amino acids.

- Xanthoproteic acid is a non-crystallizable yellow substance derived from proteins upon treatment with nitric acid.

Note:

- **Phenylalanine** does not produce the color because the benzene ring is not activated for **nitration**. However, at alkaline pH, the color changes to orange due to the ionization of the phenolic group.
- The salts of these derivatives are orange in color.

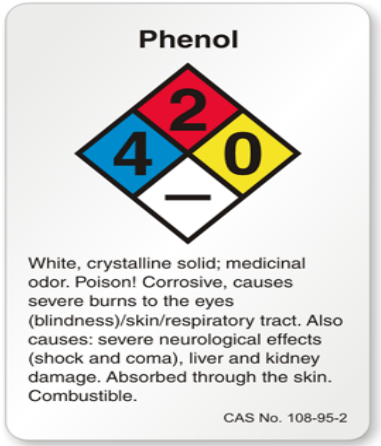


Xanthoproteic Test Procedure

Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
1	Sample	0.02% tryptophan	1%phenol	1%sucrose	1% phenylalanine	1%alanine	1%egg albumin	H2O
	Volume	2 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
2	Reagent	Con. HNO3						
	Volume	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

3- Incubate in **boiling water bath for 2 mins**, then Cool.
4- Describe the change in color in each test tube.

Safety Tips



HMIS Classification

NFPA Classification

Chemical	Hazards
NaOH	Corrosive
CUSO4	Toxic
HNO3	Corrosive
phenol	Irritation

Summary

Test	Detect	Principle	Positive Result	Negative Result
Biuret Test	Proteins, peptide of at least 2 peptide bond	(Cu2+) binding to NHCO that forms the peptide bond.	Violet	Colorless-light Blue
Ninhydrin Test	Alpha amino acids	Ninhydrin bounding to alpha-amino group	Blue	Colorless
Xanthoproteic Test	Aromatic amino acid (tyrosine and tryptophan)	HNO3 nitration to an aromatic amino acid group.	Yellow	Colorless

Guideline for writing the lab report

Total: 5 marks

All the following information should be included in your report:

a) Course # (CLS 281)
b) Experiment title
c) Date of the experiment
d) Student's names and university ID#
e) Section #

The lab report is broken down into 6 sections:

1. Experiment **title**
2. The **aim** of the experiment (objective, or what the test detects specifically) (1 mark)
3. **Principle** (chemical reaction) (1 mark)
4. **Methodology** (written in **steps**, not in tables)
5. **Result** (1 mark)
6. **Interpretation or Comme**nt (2 mark)

Deadline: Next lab Submission: Handout next lab