

CLS 281

Basic Biochemistry and Biomolecules

جامعة
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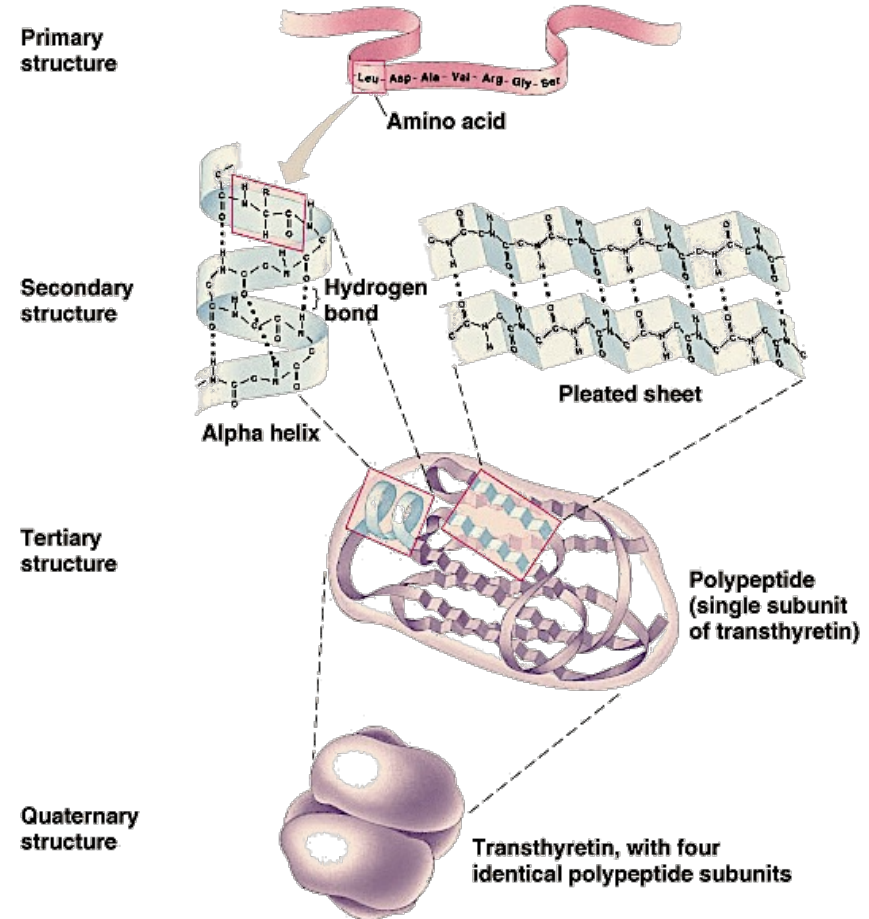
Experiment 3

Precipitation of Protein

Levels of protein structure

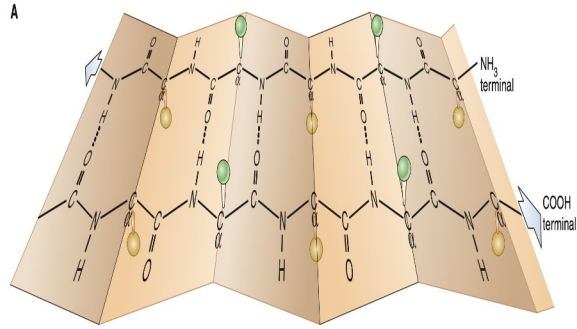
There are four levels of protein structure:

- The **primary structure** (linear sequence of a chain 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)

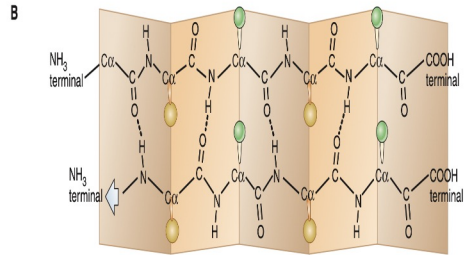


The four hierarchies of protein structure.

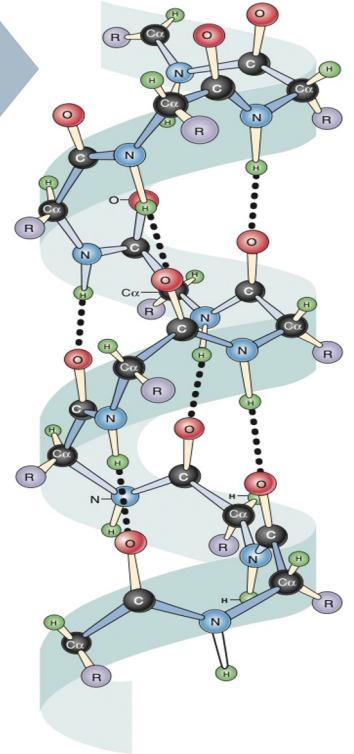
Secondary protein structure



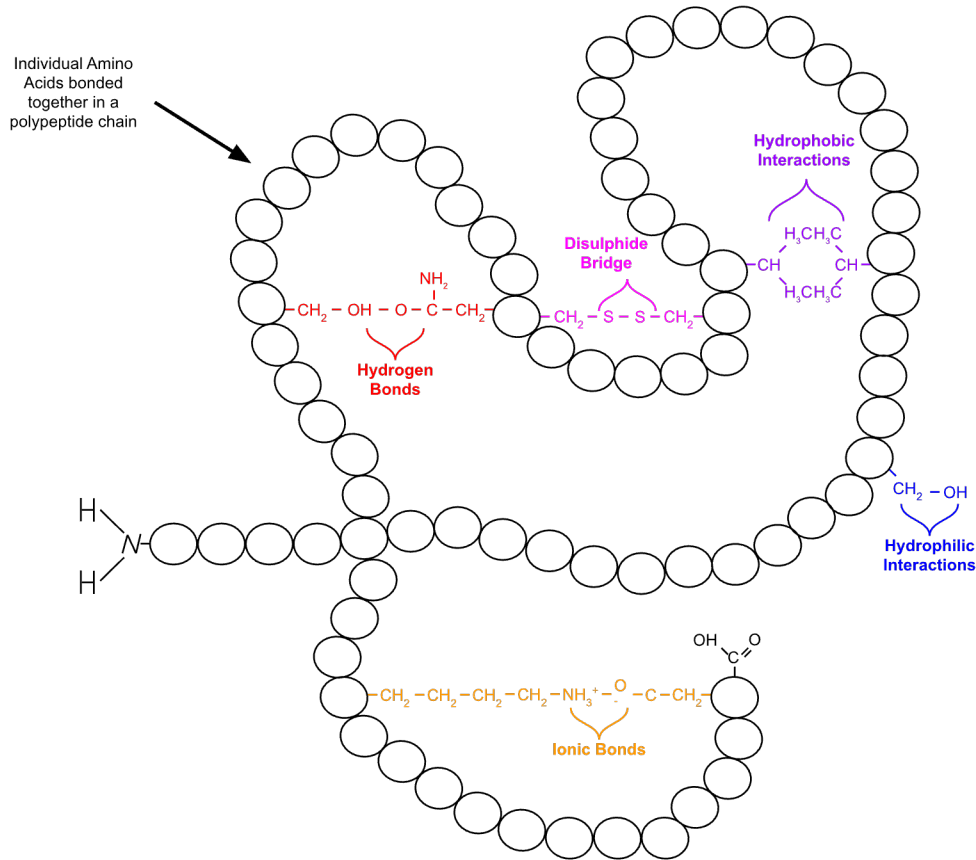
The α -helix.



The β -pleated sheet.



Tertiary protein structure



Bond Types

Hydrophobic Interactions:

These amino acids orient themselves towards the center of the polypeptide to avoid the water

Disulphide Bridge: The amino acid cysteine forms a bond with another cysteine through its R group

Hydrogen Bonds: Polar "R" groups on the amino acids form bonds with other Polar R groups

Hydrophilic Interactions:

These amino acids orient themselves outward to be close to the water

Ionic Bonds: Positively charged R groups bond together

Protein denaturation

- When the structures of native proteins are altered by chemical or physical means, the protein molecules tend to **agglomerate and precipitate**, and the protein becomes **denatured**.
- Denaturation is accompanied by loss of protein biological activity.

Denaturation definition

- It is a process in which proteins or nucleic acids lose the **quaternary structure, tertiary structure, and secondary structure** which is present in their native state, by application of some external stress or compound such as **a strong acid or base, a concentrated inorganic salt, an organic solvent** (e.g., Alcohol or chloroform), **radiation or heat**.

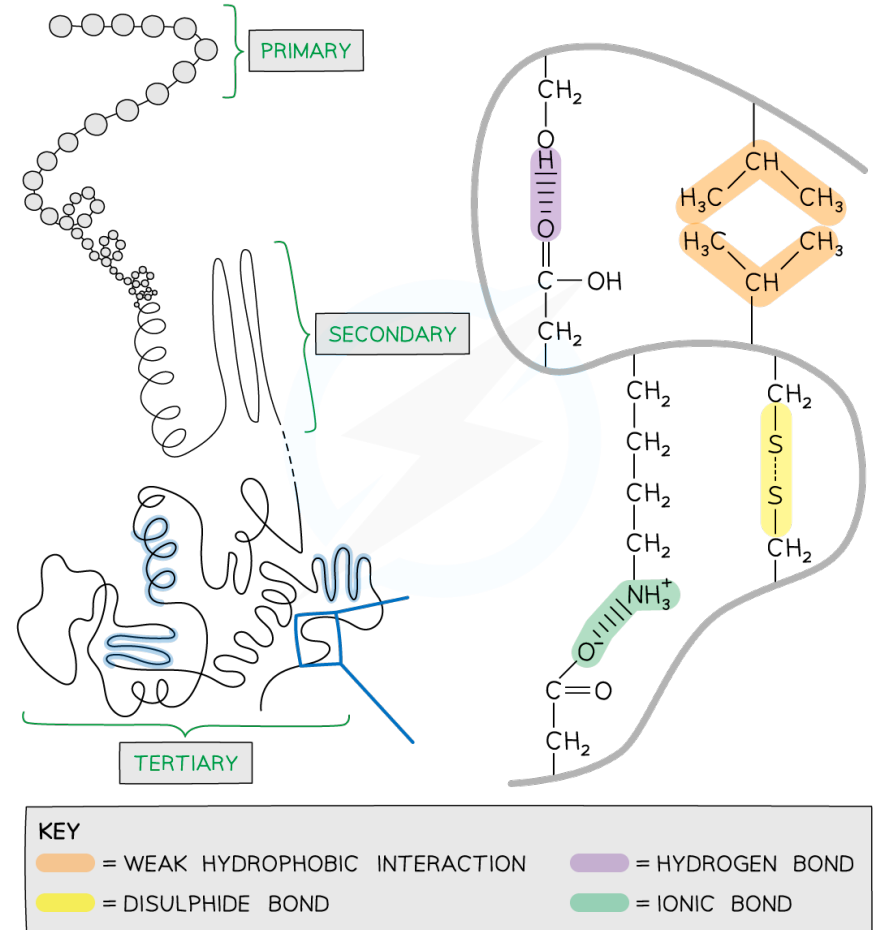
Indication of Denaturation

- Turbidity
- Agglomerate
- Precipitate



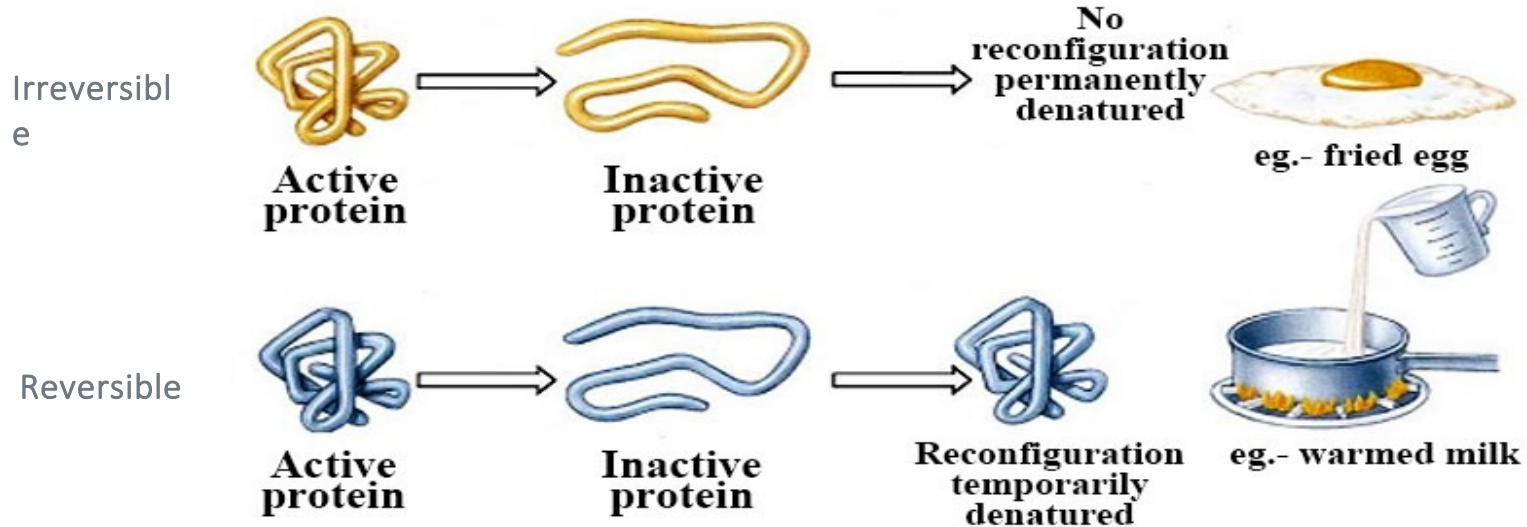
Disruption of bonds causing protein to denature

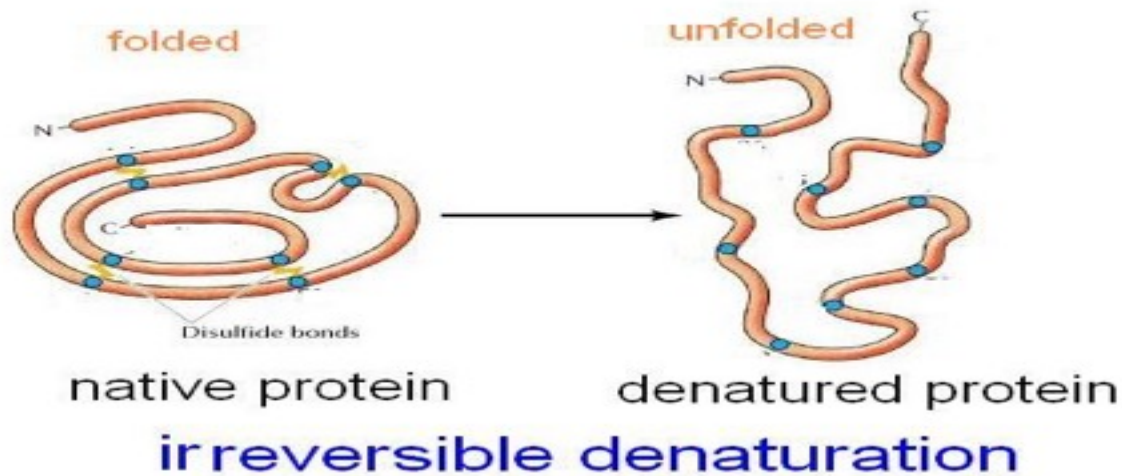
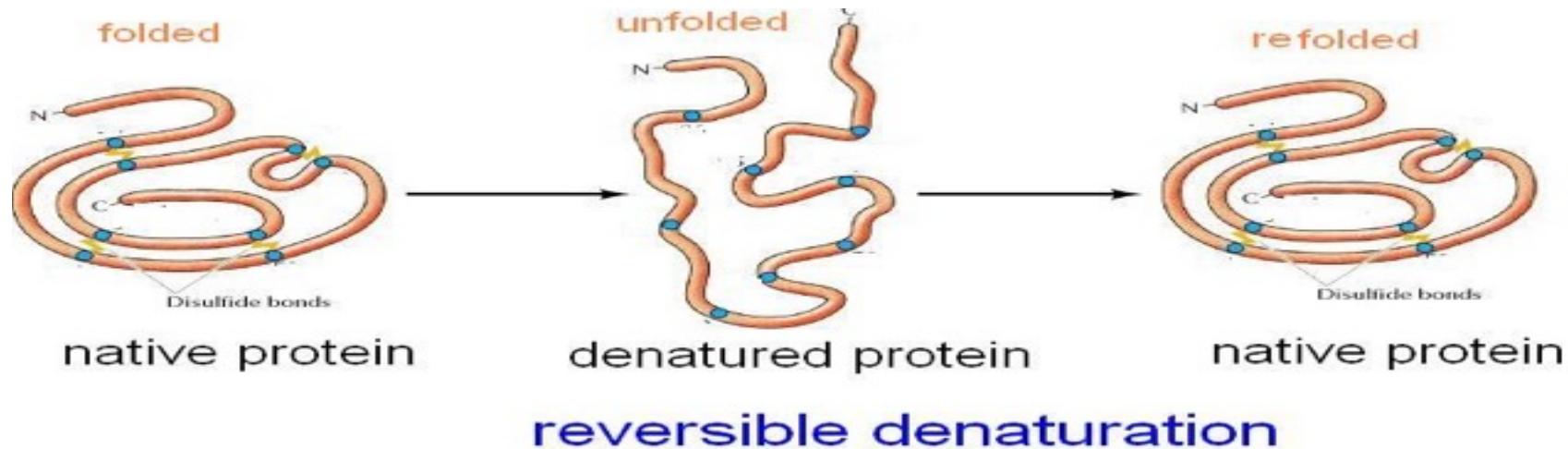
- Protein precipitation is due to a disruption of **hydrogen bonds**, **ionic bonds**, and sometimes the **stronger covalent disulfide bonds**.



Types of Denaturation

1. **Irreversible** (excessive heating or extreme PH changes).
2. **Reversible** (treatment with ammonium sulfate).





Today's experiments

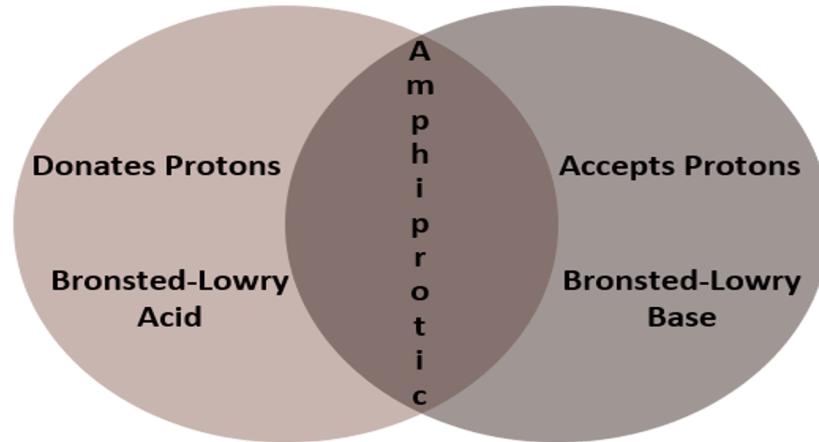
Study protein denaturation by applying different denaturing agents:

1. Strong Mineral Acids
2. Alkaloidal Reagent
3. Metallic Salts

01

Influence of Strong Mineral Acids

- Proteins are **amphiprotic** substances (can accept or give **protons**).
- The protein will precipitate because the ionic bonds and hydrogen bonds are disrupted.

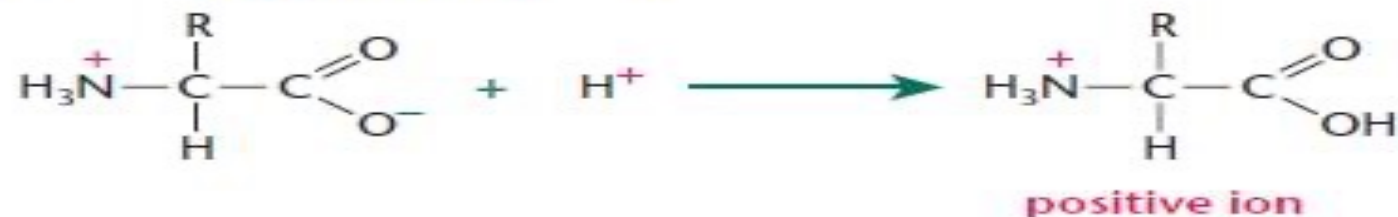


Amphiprotic species
can accept and
donate protons.

Amino acids as bases

In strongly **acidic** conditions a **positive ion** forms:

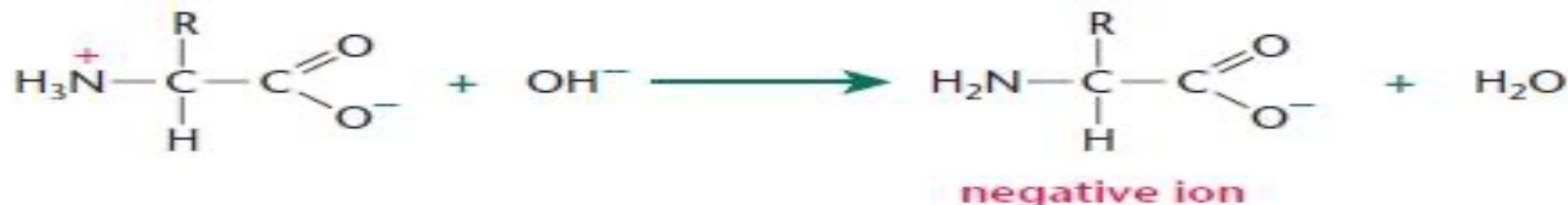
- an amino acid behaves as a **base**
- the COO^- ion gains a proton.



Amino acids as acids

In strongly **alkaline** conditions a **negative ion** forms:

- an amino acid behaves as an **acid**
- the NH_3^+ ion loses a proton.



01

Procedure

Steps		Tube 1	Tube 2	Tube 3
1	Add the protein	3 ml of 1% egg albumin	3 ml of 1% egg albumin	3 ml of 1% egg albumin
2	Add the Acid	2 ml of con. HCL	2 ml of con. HNO ₃	2 ml of con. H ₂ SO ₄

3- Mix, and describe any color change that occurred

02 *Precipitation by Alkaloidal Reagent*

- Alkaloidal reagents are acids that can combine with alkaloids (Alkaloids are organic bases from plants).
- Examples of alkaloidal reagents:
 1. Trichloroacetic acid
 2. Tannic acid
 3. Phosphotungstic acid
 4. Picric acid
 5. Sulfosalicylic acid
- Certain acidic reagents (alkaloidal reagents) combine with protein to form insoluble protein salts (e.g., Protein tannate).

02

Procedure

Steps		Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
1	Sample	3 ml of 1% egg albumin	3 ml of 1% egg albumin	3 ml of 1% egg albumin	3 ml of 1% egg albumin	3 ml of 1% urea	3 ml of 1% alanine
2	Denaturing Reagent	5 drops of 10% Trichloroacetic acid	5 drops of 5% aqueous tannic acid	5 drops of 20% Phosphotungstic acid	5 drops of 20% sulfosalicylic acid	5 drops of 10% Trichloroacetic acid	5 drops of 10% Trichloroacetic acid

3- Mix, and describe any color change that occurred

03

Precipitation by Metallic Salts

- Principle:

When heavy metal cations (positive ions) (e.g. Pb, Cu, Hg, Ag) are added to a protein solution, the metal ions combine with the negatively charged groups to form **insoluble metal ion proteinate**.

03

Procedure

Steps		Tube 1	Tube 2	Tube 3	Tube 4
1	Sample	2 ml of 1% egg albumin	2 ml of 1% egg albumin	2 ml of 1% egg albumin	2 ml of 1% urea albumin
2	Denaturing Reagent	5 drops of 0.2M copper sulfate	5 drops of 0.2M lead acetate	5 drops of 0.2M of mercuric chloride	5 drops of 0.2M copper sulfate

3- Mix well, and describe the result.

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 Comment** (2 mark)

Deadline: Next lab Submission: via email