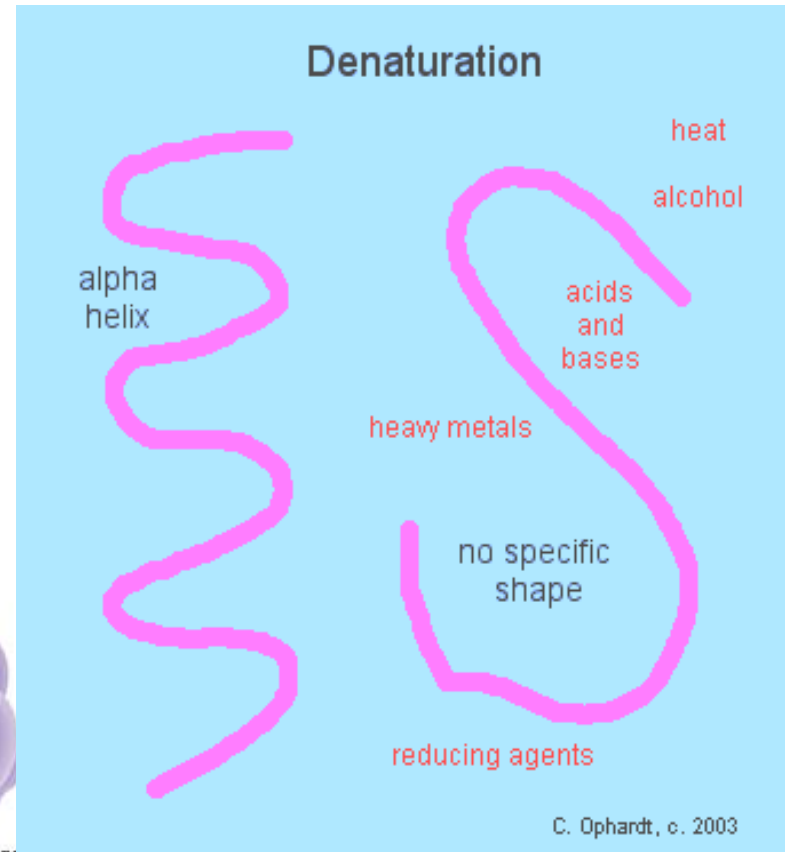
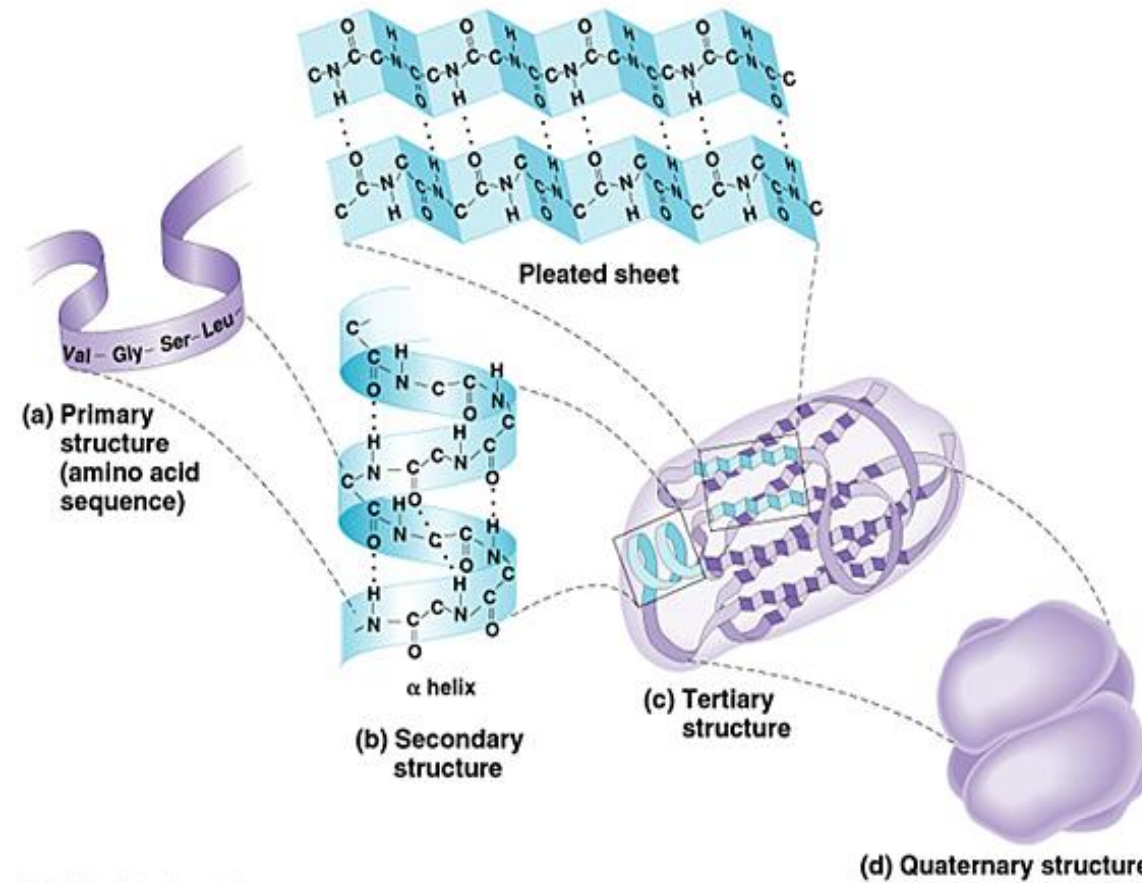


Qualitative chemical reaction of functional group in protein



C. Ophardt, c. 2003

- Certain functional **groups** in proteins can react to **produce characteristically colored products**.
- The **color intensity** of the product formed by a particular group varies among proteins in proportion to the number of reacting functional or free groups present and their accessibility to the reagent.

Egg Proteins:

Albumin and globulin

Separated by centrifugation at 3000 rpm for 20 min.

The **albumin** is the **supernatant** because it is has **low Mwt.** and globulin is the precipitate which is higher Mwt. Than albumin.



Today Experiments

1- Biuret test

Protein Precipitation:

2- Effect of salt concentration on the protein solubility

3- Acid precipitation of proteins

4- Precipitation of protein by salts of heavy metals

5- Protein denaturation by Heat

Experiment(1): Biuret test :

Objective:

-To detect the presence of a protein or peptides.

This test is specific for the peptide bond.

Substances(protein),containing

not less than two

peptide linkages give positive

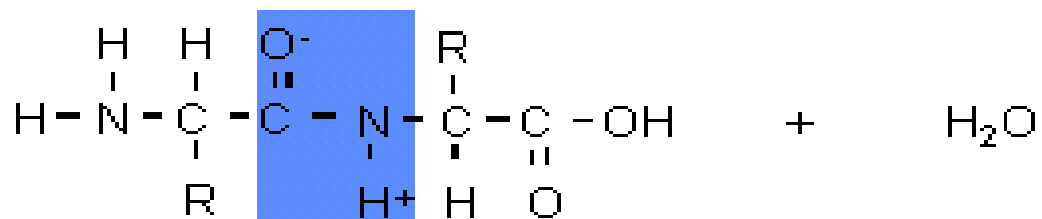
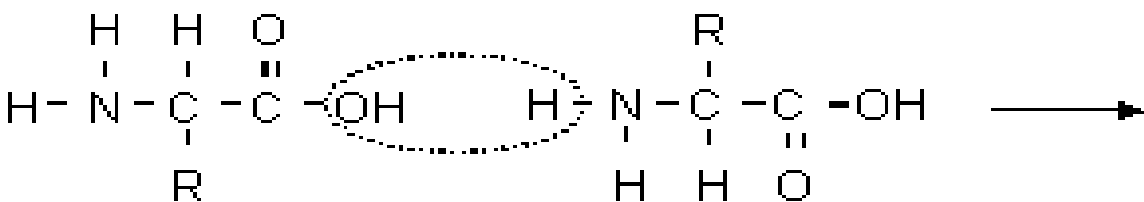
result with this test



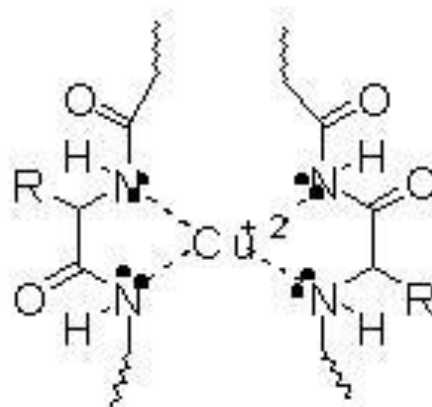
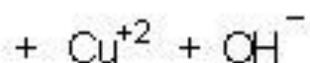
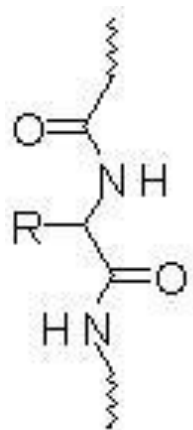
Principle:

This test is specific for the peptide bond. Substances containing not less than two peptide linkages give this test. In this reaction, proteins form a purple colored complex with CuSO_4 in a strongly alkaline solution.

When *proteins and peptides* (i.e peptide bonds) treated with an **alkaline solution of dilute copper sulfate a violet color** is formed . A positive test is indicated by the formation of a **violet color**. The name of the test is derived from a specific compound, **biuret** which give a positive test with this reagent.



Peptide bond



Biuret complex
pink or violet blue



Method:

- 1- add 3ml of protein Albumin
- 2- Add 1 ml of 10% NaOH
- 3- Add 1 ml of CuSO_4 (Biuret reagent) and **mix well**.

protein	Observation	Comment
Albumin		

Precipitation of the protein:

A-By salt "Salting out"(NH₄SO₄)

B- By strong acids(HNO₃,TCA)

C-by salts of heavy metals(Hg⁺², Pb⁺², Ag⁺¹
Tl⁺¹, Cd⁺²)

Experiment (2): Effect of salt concentration on the protein solubility :

Objective:

This experiment is used to **separate different proteins using salting-out theory.**

Each protein can be precipitated at **specific salt concentration.**

Salting out: separate different protein by using different salt concentration.

In salting out you must take into account the following:

- 1-The **type of salt** (ammonium sulfate , $(\text{NH}_4)_2\text{SO}_4$ or NaCl)
- 2-The **molecular weight of protein** , the high Mwt. will precipitate first .
- 3-there is **inverse relationship** between the **molecular weight of protein** and the concentration of precipitation salt.
High molecular weight need low concentration of salt (low percentage of saturation)
Low molecular weight need high concentration of salt (High percentage of saturation)
- 4-It is Reverse process, the protein can again become soluble when we add water
- 5- Application , in separate mixture protein

Principle:

The **low salt concentration** solutions make protein **solubility easier** using the **attraction of salt ions to the functional groups of the protein**. e.g NaCl

On contrast, **high salt concentration** or solids dissolved in the reaction medium up till saturation solutions **causes the protein to precipitate** since salt ions, in this case, **compete with the protein molecules in binding water** molecules. So the salt it just cause *protein dehydration*. e.g (NH₄)SO₄

Method:

T1	T2
Take 2 ml of your albumin sample	Take 2 ml of your albumin sample
Slightly add of 50% saturated (NH₄)₂SO₄ solution	Add a few amount of 100% solid (NH₄)₂SO₄
Shake it well and write your observation	Shake it well and write your observation
record your observation .	Compare between T1 and T2

Results:

Tube	Observation	Comment
Albumin+50% saturated (NH ₄) ₂ SO ₄		
(Albumin+ 100% saturated (NH ₄) ₂ SO ₄		

**Discusses each result and Compare between them
what and why you obtain it ...**

Experiment(3): Acid precipitation of proteins

Objective:

To investigate the ***effects of strong acids*** on the protein solubility.

Applications:

- Separation and purification
- Detection of small amount of protein in urea sample
- Stop the enzyme reaction

Principle:

By changing the PH value of protein the addition of acid will **reducing the Optimum PH** of protein until to be equal to PI (isoelectric point) (i.e. when positive charged are equal to negative in protein).

The **weak bond will affect** , and this cause protein precipitate .

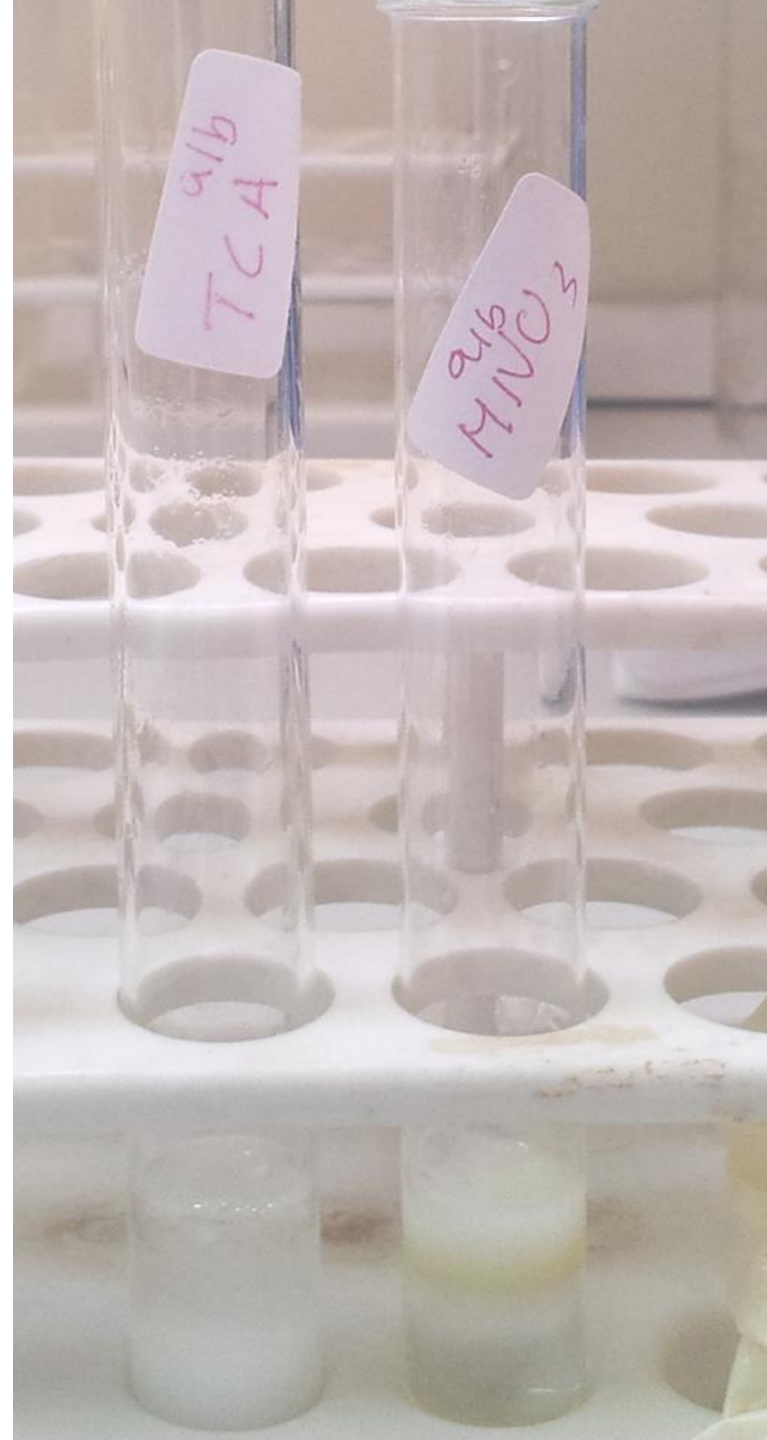
Method

HNO ₃	TCA
In a test tube, put 3ml of conc. nitric acid carefully	Put 3 ml of the albumin solution
Using a dropper add to (albumin) on the inner wall of the tube to form a layer up the acid	add 5-7 drops of T.C.A solution carefully
Record your observation	Record your observation

Results:

Tube	Observation	Comment
Conc. HNO ₃ + Albumin		
Albumin + TCA		

**Discusses each result
what and why you
obtain it ...**



Experiment(4):precipitation of proteins by salts of heavy metals:

Heavy metal salts usually contain Hg^{+2} , Pb^{+2} , Ag^{+1} , Tl^{+1} , Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.

Objective:

to identify the effect of heavy metal salt on protein

Principle

Heavy metal salt will neutralize the protein .

By the **negative** charge of **protein** will bind with positive charge of metal ion . Then the protein will precipitate as **insoluble metal protein salt** .

Application:

To eliminate the poisoning by palladium Pb^{++} , mercury salts Hg^{++}How???

Method

A	B
In a test tube, put 1 ml of Albumin sample	In a test tube, put 1 ml of Albumin sample
Using a dropper add to (albumin) few drops of AgNO_3	Using a dropper add to (albumin) few drops of HgCl_2
Record your observation	Record your observation

Results:

Tube	Observation	Comment
Albumin + AgNO ₃		
Albumin + HgCl ₂		

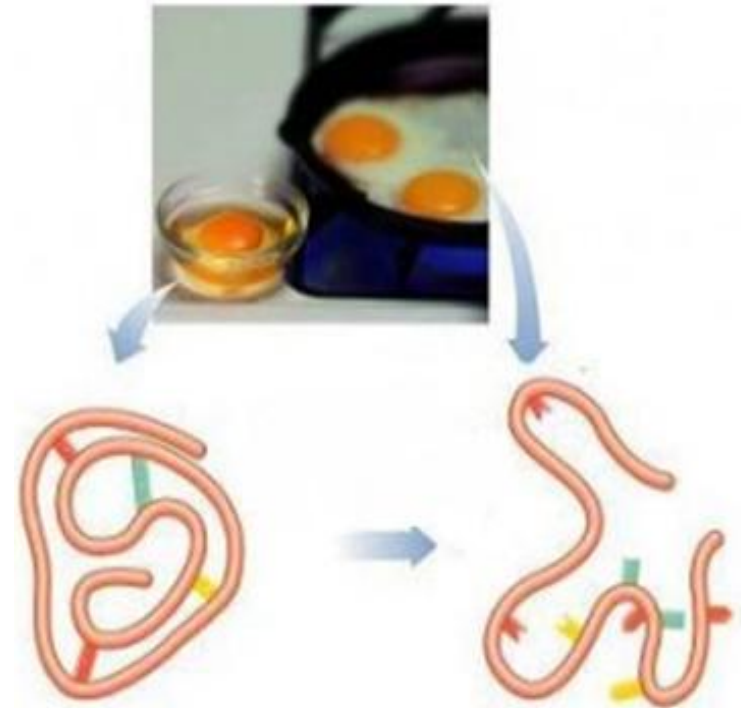
**Discusses each result
what and why you
obtain it ...**



Denaturation of Proteins

Denaturation is a process in which the proteins losing it tertiary structure and secondary structure, by application of some external factor or compound such as a **strong acid** or **base**, a conc. **inorganic salt**, an **organic solvent** (e.g., **alcohol** or **chloroform**), or **heat**.

If proteins in a living cell are denatured, this results in disruption of cell activity and possibly cell death. Denatured proteins can exhibit a wide range of characteristics, from **loss of solubility** to **communal aggregation**.

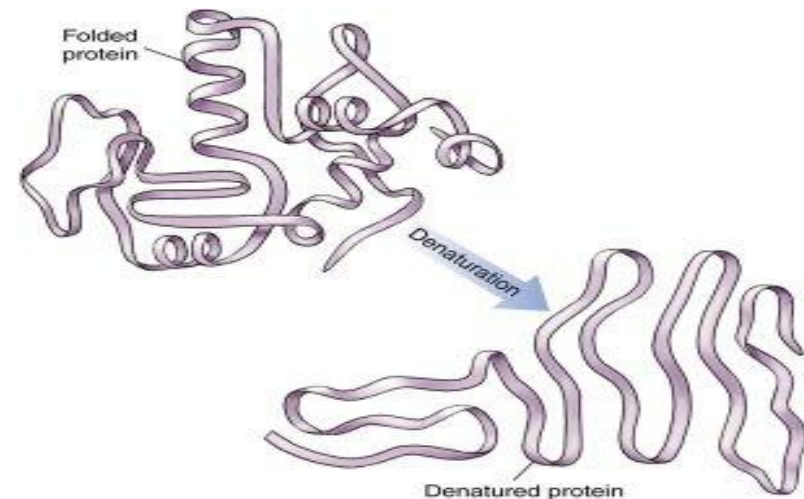


Experiment(5):proteins denaturation by heating

Denaturation is a major change from the original native state without alteration of the molecule's primary structure, i.e., without cleavage of any of the primary chemical bonds that link one amino acid to another.

Denaturation Factors:

Heat , inorganic salt ,
organic solvent ,
irradiation ,strong acid ,
strong base



Method:

- 1- Take 1 ml of protein Albumin and drops of acetic acid
- 2- add 0.5 ml of acetic acid
- 3- Place it in a boiling water bath for 5-10 minutes
- 4- Remove aside to cool to room temperature.

Result:

protein	Observation	Comment
Albumin		

Discusses the result what and why you obtain it ...

HgCl₂

AgNO₃

Heat

91b
TCA

91b
MNO₃

Bimnet



Thank You

