



Assiut University



# Protein structure, function and Isolation

By

**Dr. Alaa El-Din Hamid Sayed**

Zoology Department, Faculty of Science, Assiut University

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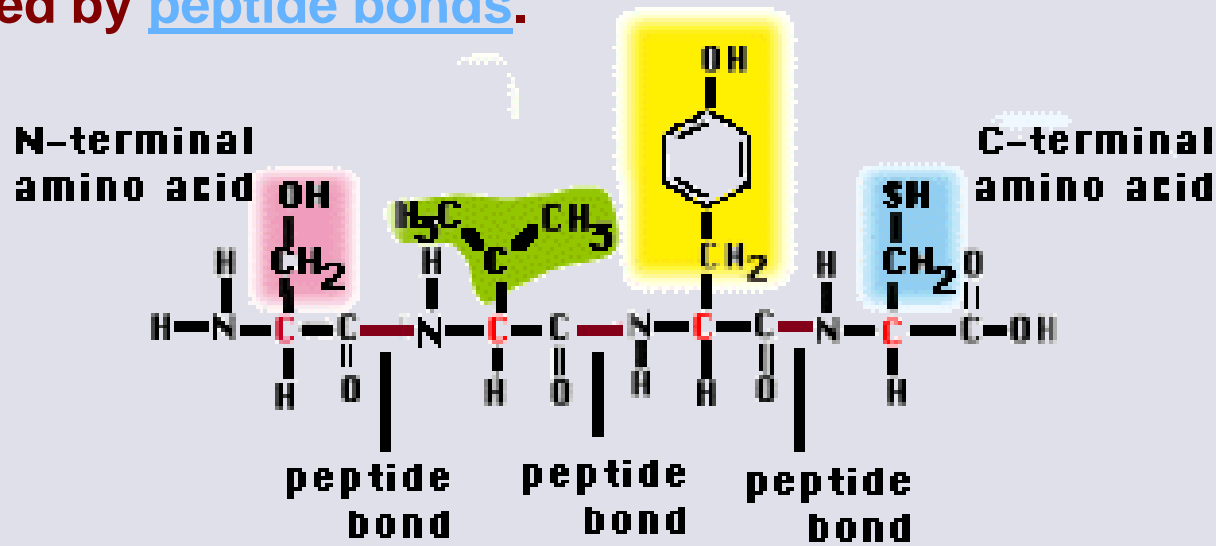
# **Contents**

- 1. What are proteins?**
- 2. Types of protein structure**
- 3. Protein Function**
- 4. Isolation of protein**

**What are proteins?**

# What are proteins

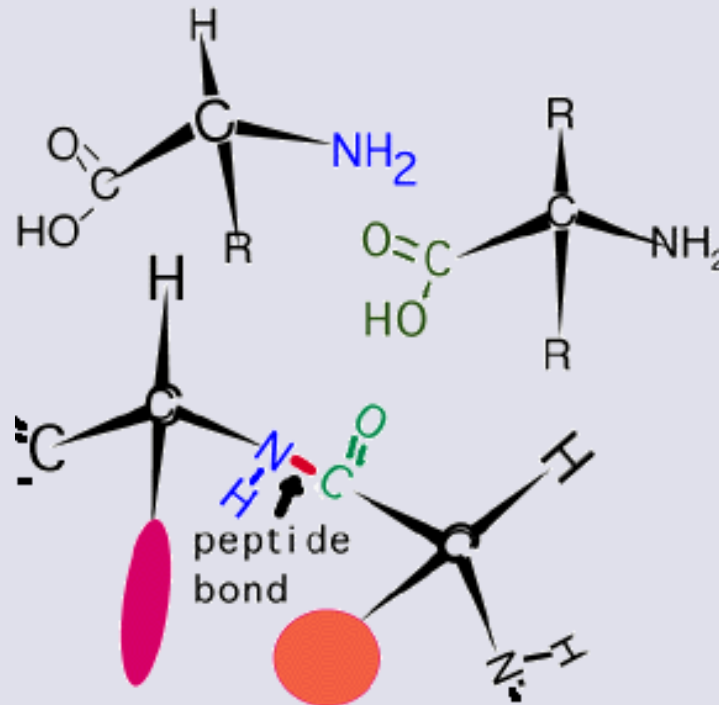
- ❑ Proteins are polymers of amino acids in which the adjacent amino acids are connected by peptide bonds.



- ❑ This polypeptide is short but has several features that are common to all proteins.
  - All proteins have an N-terminal end.
  - All proteins have a C-terminal end.

# Peptide bonds

## What is a peptide bond?



A C-N covalent bond between the Nitrogen of one amino acid and the carboxyl carbon of an adjacent amino acid.

# Amino acids

## What are amino acids?

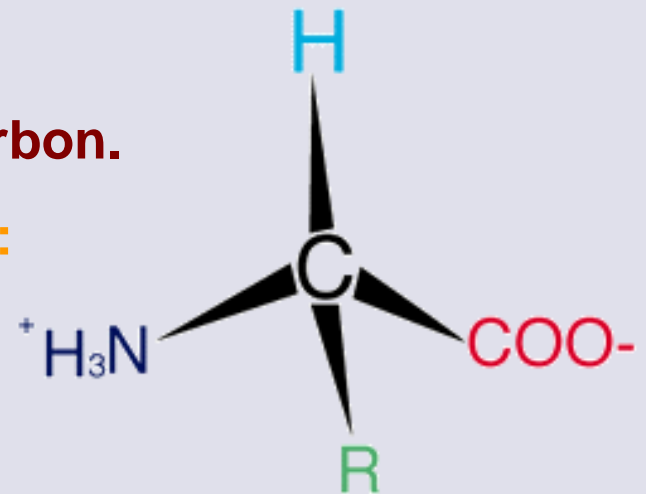
- The term amino acids refers to a groups of molecules which comprise the primary structure of a protein

In general, amino acids have several features in common.

- The figure shows that amino acids have a central carbon, referred to as the alpha carbon.

The alpha carbon is covalently bounded to:

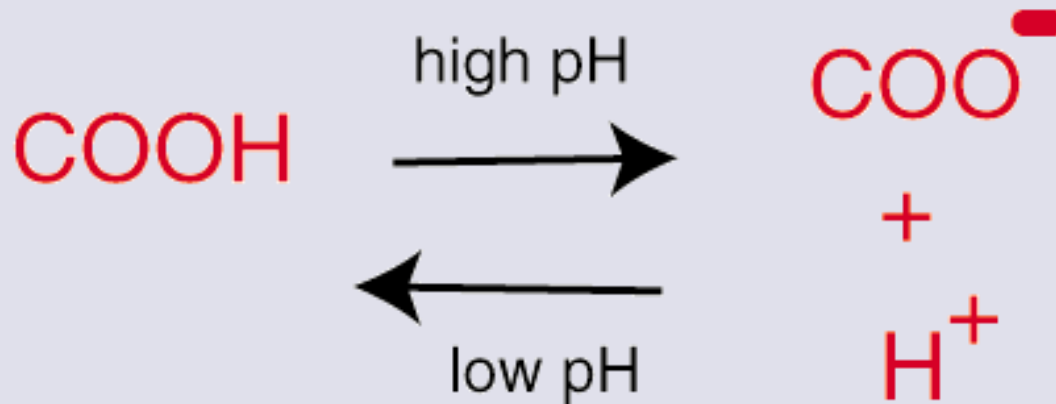
- A hydrogen (H)
- A carboxyl functional group (COO<sup>-</sup>).
- An amine functional group (NH<sub>3</sub><sup>+</sup>).
- A side chain that distinguishes one kind of amino acid from another kind.



# Carboxyl Functional Group

- It is an acidic functional group frequently found in biological molecules.
- It is found in amino acids, proteins, fatty acids, acetic acids and other organic acid.

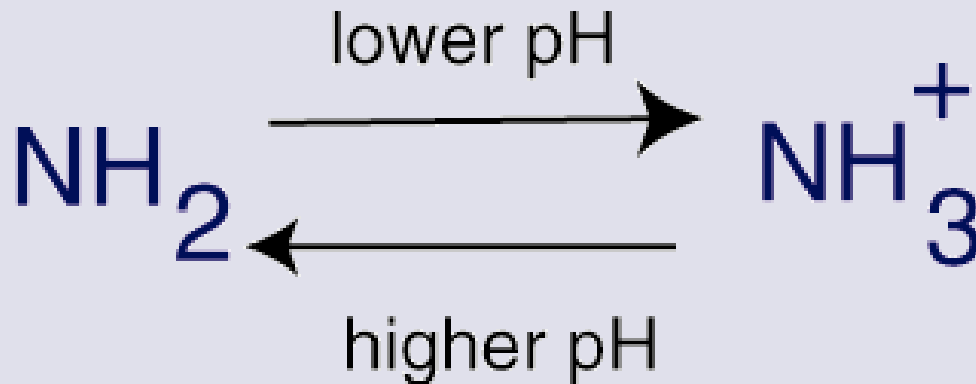
Because the carboxyl functional group is a weak acid it will dissociate.



- At a pH of 7 the carboxyl group is in the dissociated form (COO-).
- The carboxyl group of an amino acid will contribute a negative charge at neutral pH.

# Amino Functional Group

- The amino functional group is basic and found in proteins, amino acids and the nitrogenous bases of DNA and RNA .
- Under certain pH conditions the amino group can accept a proton and gain a positive charge of +1.



- At a pH of 7 the amino group of an amino acid has a positive charge. The amino group then contributes a positive charge to the amino acid at pH 7 .

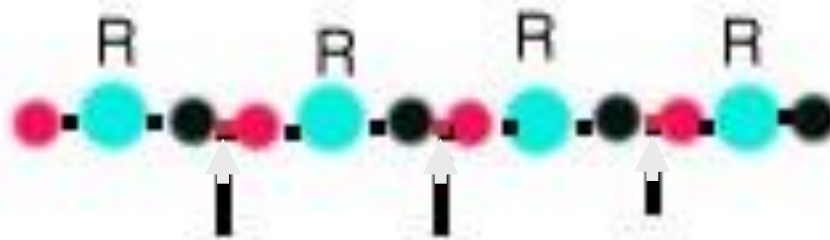
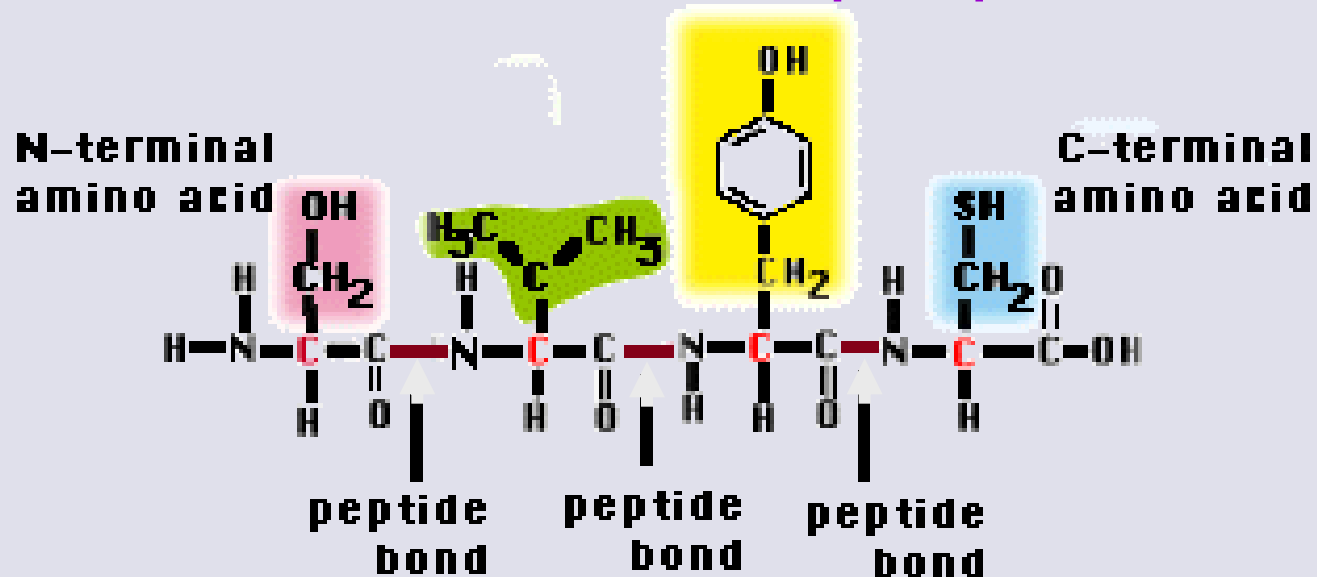


# **Types of protein structure**

# I- Primary structure of proteins

One component of the primary structure of a protein is:

- The sequence of amino acids that make up the protein

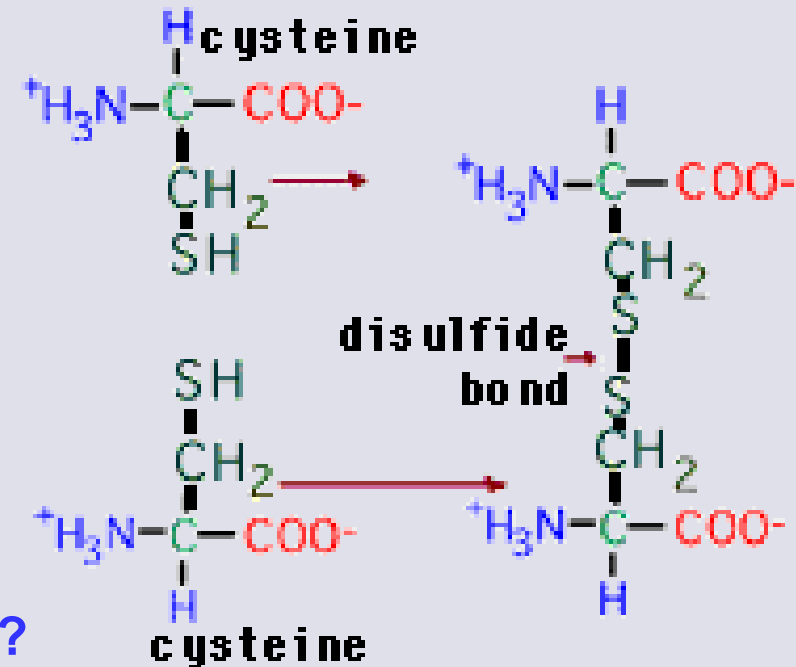


The sequence of the amino acids is:  
N-serine, valine, tyrosine, cysteine-C.

Another component of the primary structure is:

➤ The covalent bonds in the protein.

A single covalent bond between the sulfur atoms to two amino acids called cysteine .



What is the significance of disulfide bonds?

- Because it is a covalent bond, the disulfide bond can be considered as part of the primary structure of a protein.
- They are very important in determining the tertiary structure of proteins.
- they are very important in determining the quaternary structure of some proteins.

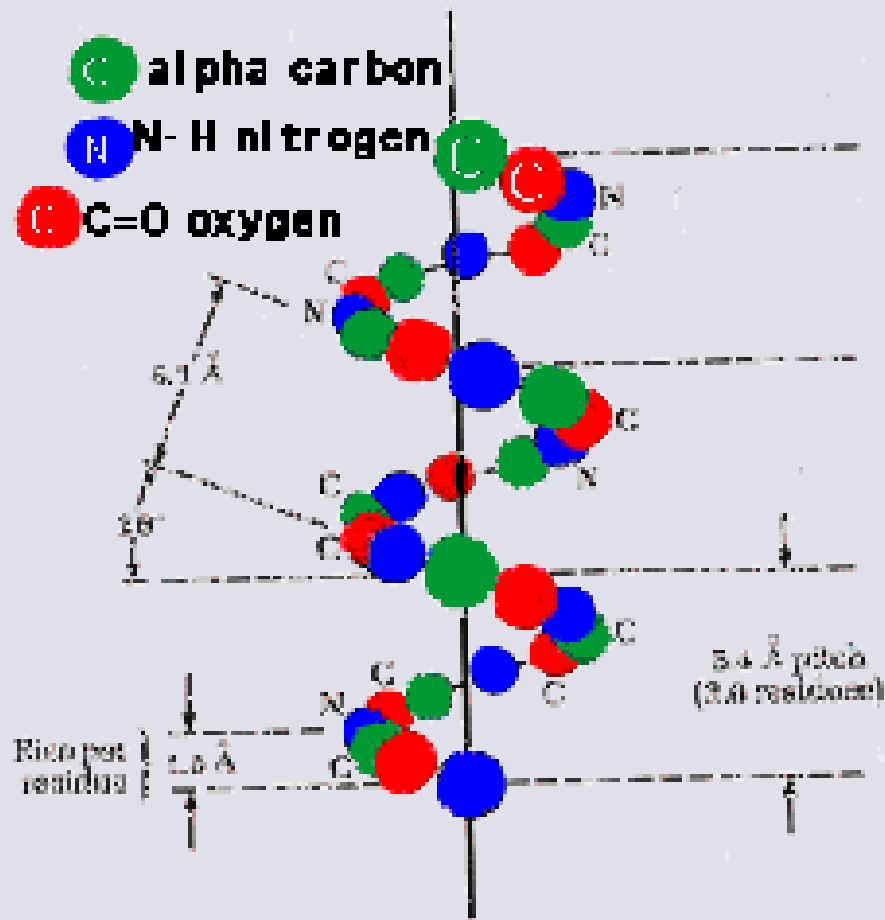
The structure of antibody molecules is a very prominent example showing the role of disulfide bonds.

## II- The Secondary Structure of Protein

- **Secondary protein structure:** It occurs when the sequence of amino acids are linked by hydrogen bonds.
- The secondary structure of protein is formed of  $\alpha$ -helical regions and  $\beta$ -pleated sheets.

### What is an alpha helix?

- An alpha helix can be formed by making a rope coil in a left handed direction.
- The rope is represented by the N-C-C-N-C-C-N .... backbone of the polypeptide chain.



- The alpha helix structure of protein depends on; **bond angles, lengths and rotations.**
- The alpha helix could be a very stable structure because intra-chain hydrogen bonds could be formed that stabilized the helix.

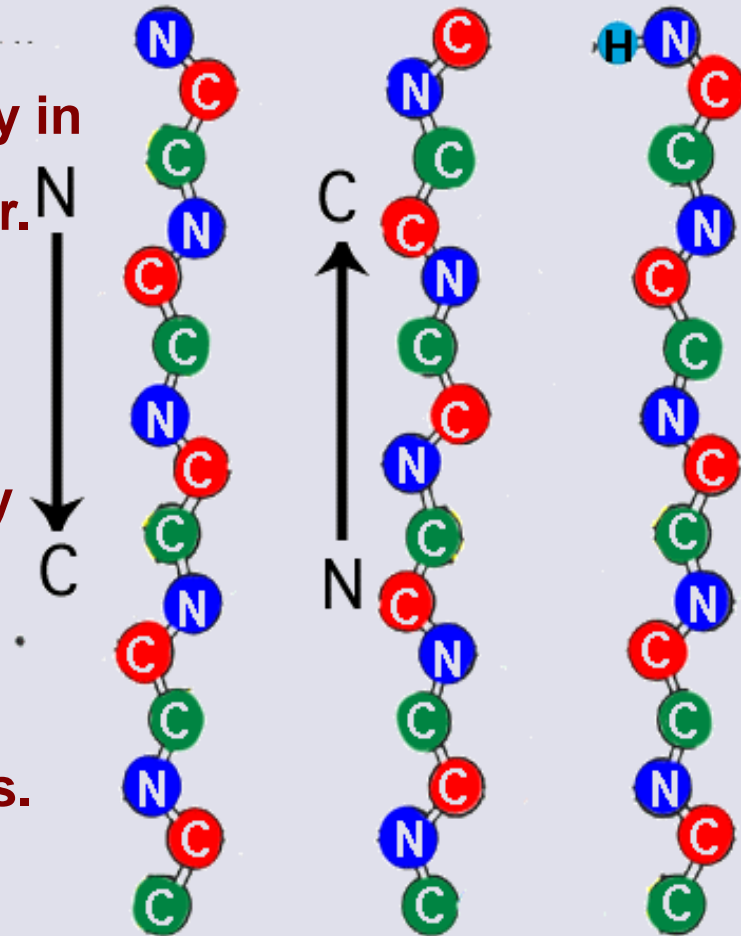
# Significance of alpha helix

- The alpha helix is one of the structures that together with the  $\beta$ -pleated sheets are called the secondary structures of proteins.
- Some proteins like **keratin** and **collagen** are almost entirely  $\alpha$ -helical in structure.
- Most globular proteins have  $\alpha$ -helical and  $\beta$ -pleated sheet regions.
- Charge amino acid side chains have a tendency to **destabilize** the  $\alpha$ -helical or  $\beta$ -pleated sheet structures.
- Amino acids with hydrophobic side chains are compatible with the formation of  $\alpha$ -helices and  $\beta$ -pleated sheets.

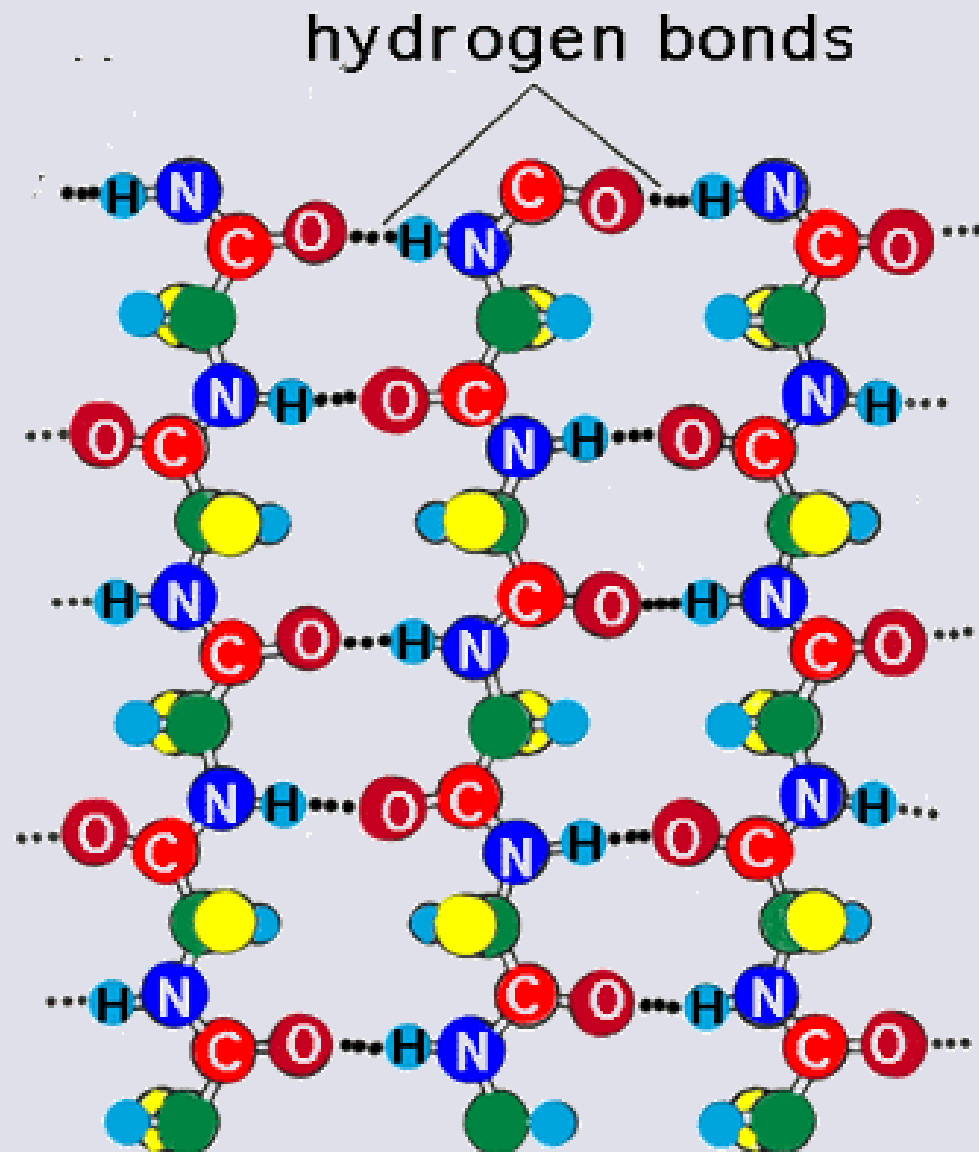
# What is a beta pleated sheet?

A beta pleated sheet is a crease ed structure that is composed of the C-C-N-C-C backbone of a polypeptide .

- Each chain of CCNCC... has a N to C polarity in the direction opposite to that of its neighbor.
- The line on the left and far right have the N-to-C polarity from top to bottom .
- The line in the middle has the N-to-C polarity from bottom to top.
- ❖ These chains are said to be **antiparallel** because they run in the opposite directions.
- ❖ If the chains run in the same N-C direction they are said to be **parallel**.



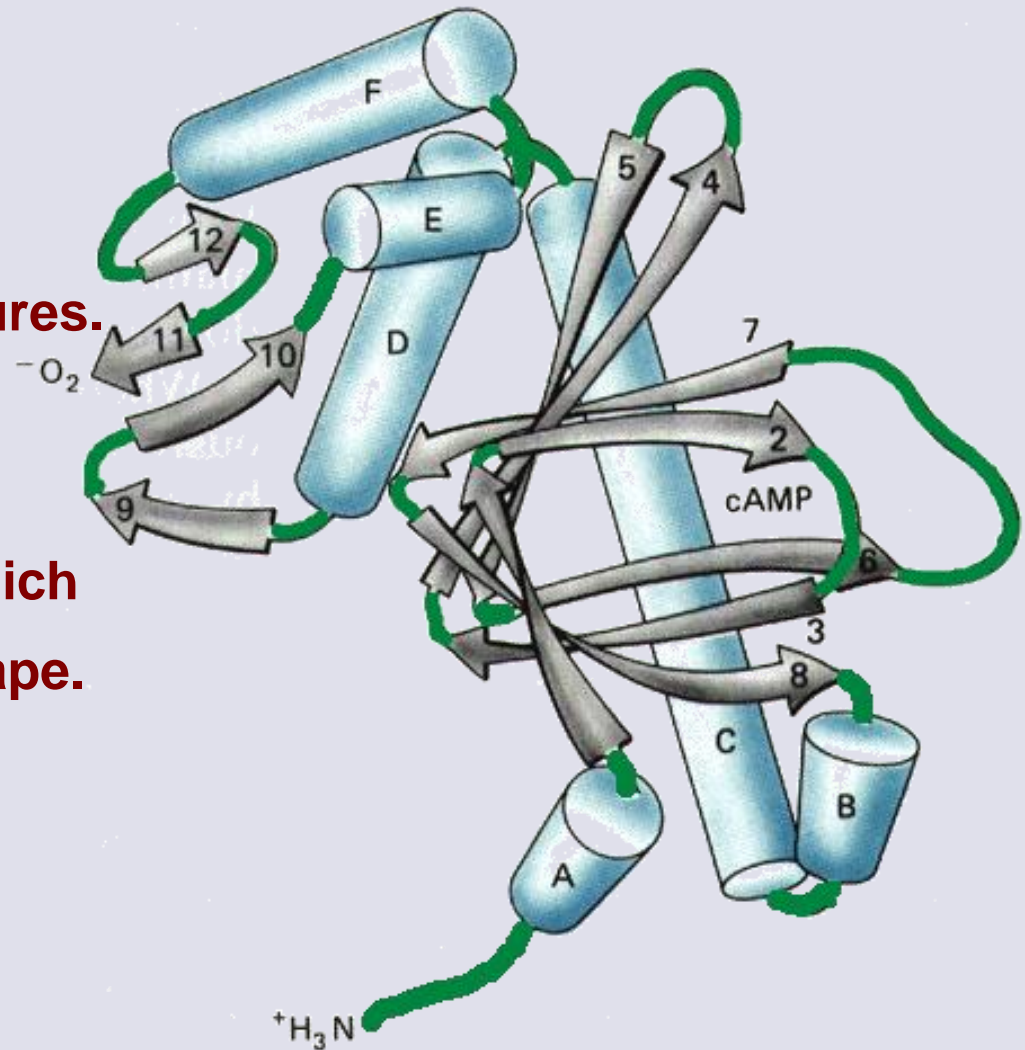
- The beta pleated sheet structure is stabilized by hydrogen bonds between the different chains.





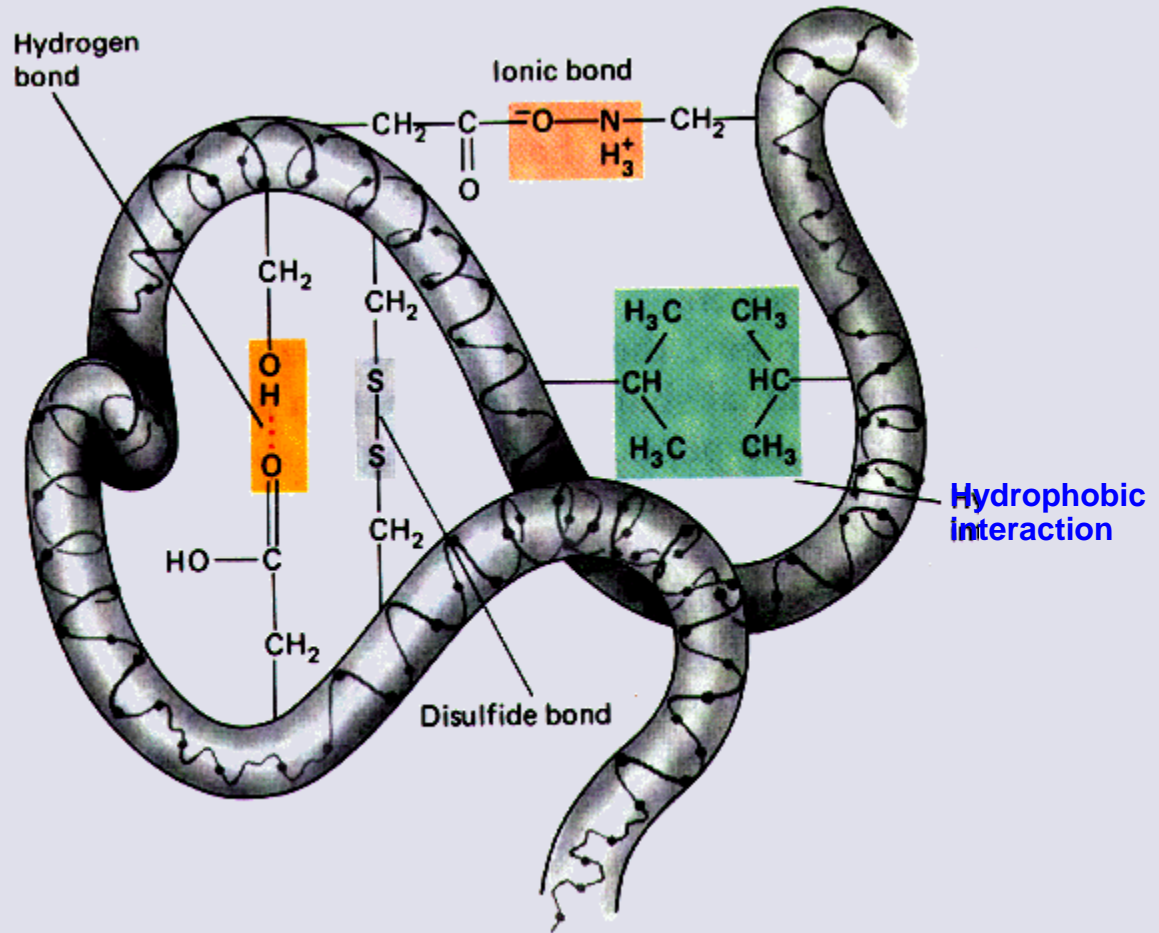
# III- Tertiary Structure of Proteins

- The  $\beta$ -pleated sheets (ribbons with arrows) and the  $\alpha$ -helical regions (barrel shaped structures).
- In addition to these secondary structures, the protein has additional twists and turns which give the protein its unique shape.



# Forces that give rise to tertiary structure

- Ionic bonding.
- **Hydrogen bonding.**
- Hydrophobic interaction.
- **Disulfide bonds.**

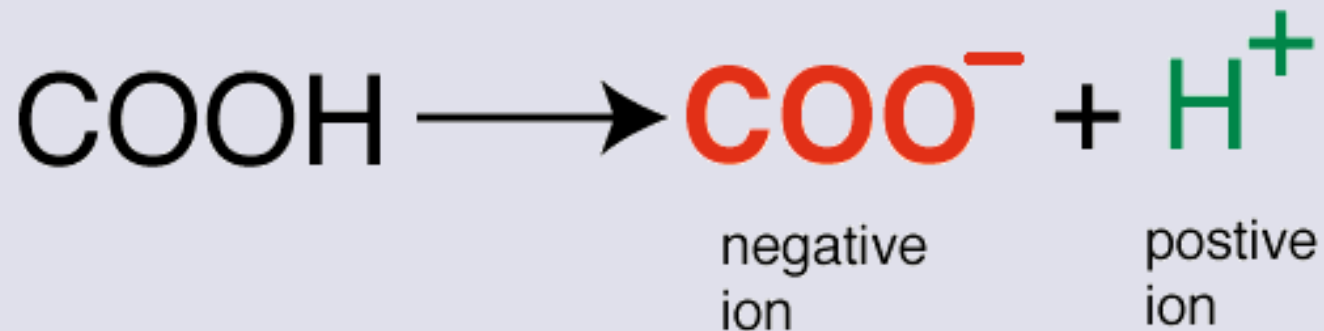


# 1- Ionic bonding.

They are forces of attraction between ions of opposite charge ( + and - )

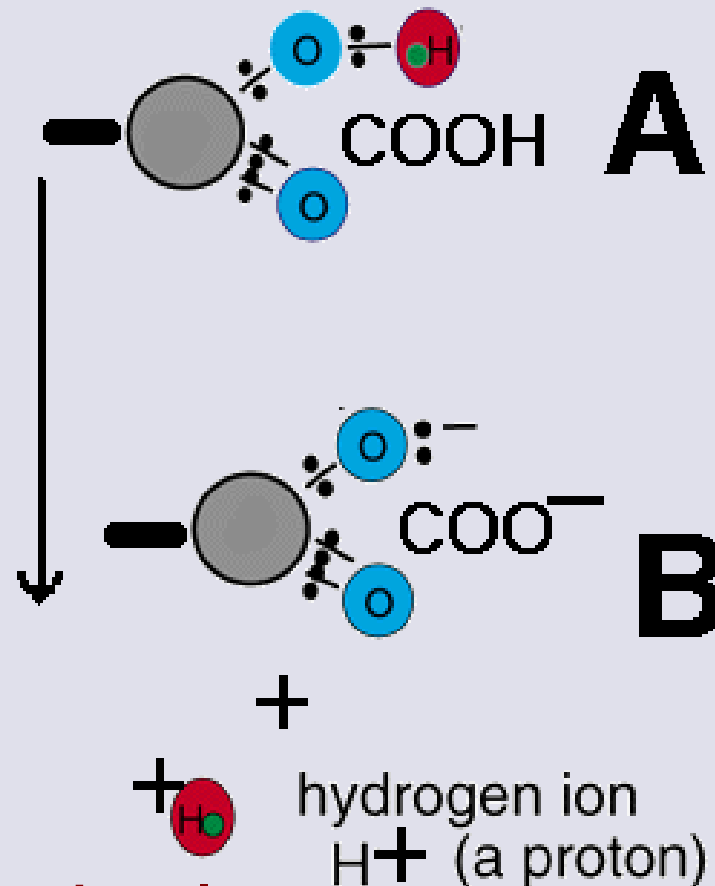
What kinds of biological molecules form ionic bonds?

Any kind of biological molecule that can form ions



- An example of a functional group that can enter into ionic bonds is shown. The carboxyl group is shown.
- Under the right conditions of pH the carboxyl group will ionize and form the negatively charged COO ion and a positively charged H ion (or proton)

Here is another representation of the carboxyl group. In this case the covalent bonds are shown by the lines and the shared electrons are shown by the black dots .



When ionization of the carboxyl group occurs a proton dissociates from the OH group, leaving the shared electrons behind with the oxygen. Thus the COO ion has an excess of electrons over protons and is an anion. The proton that is released has no associated electron and is therefore a cation .

## Functions of ionic bonds in biology?

- They play an important role in determining the shapes (tertiary and quaternary structures) of proteins.
- They are involved in the process of enzymatic catalysis.
- They are important in determining the shapes of chromosomes.
- They play a role in muscle contraction and.
- They are important in cell shape, establishing polarized membranes for neuron function and muscle contraction.

## 2- Hydrogen Bonds

### Properties of hydrogen bonds.

- Is formed when a charged part of a molecule having polar covalent bonds forms an electrostatic interaction with a substance of opposite charge.

- **Strength**

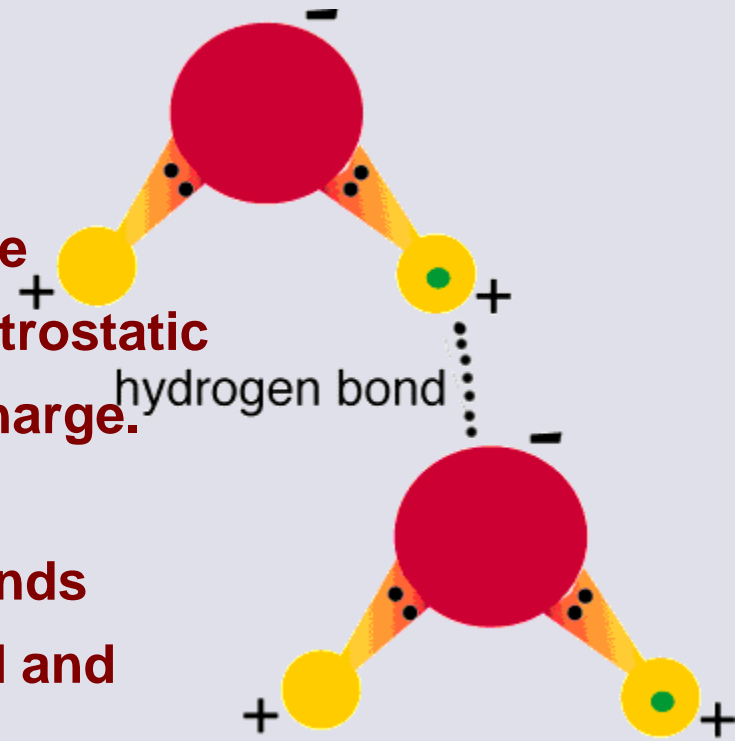
Hydrogen bonds are classified as weak bonds because they are easily and rapidly formed and broken under normal biological conditions.

- **What classes of compounds can form hydrogen bonds?**

Under the right environmental conditions, any compound that has polar covalent bonds can form hydrogen bonds.

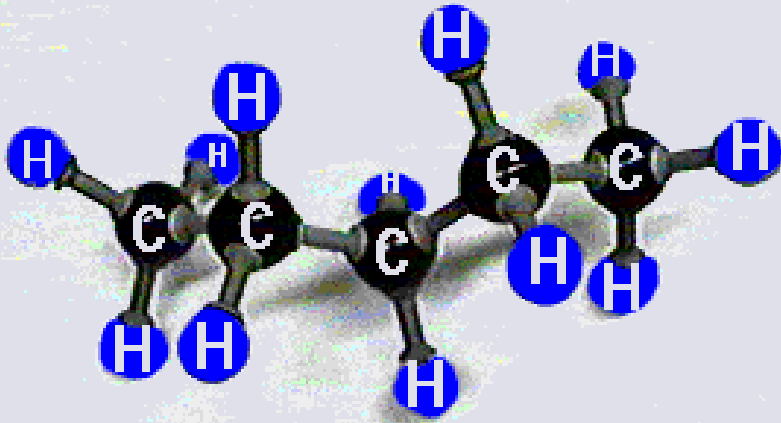
- **Importance in biological systems.**

- \* Stabilizing and determining the structure of large macromolecules like **proteins** and **nucleic acids**.
- \* They are involved in the mechanism of enzyme catalysis



# The Covalent Bond

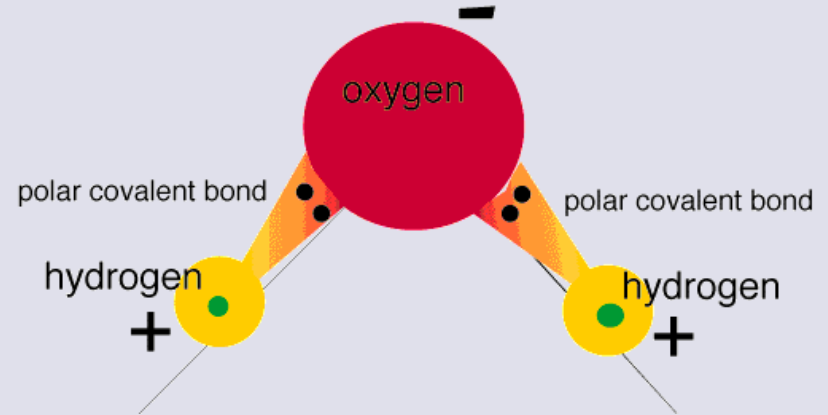
## Non-polar Covalent Bond



### Hydrocarbons

In Biological systems:  
The molecule that predominance  
in non-polar covalent bonds is  
called **hydrophobic**

## Polar Covalent Bond

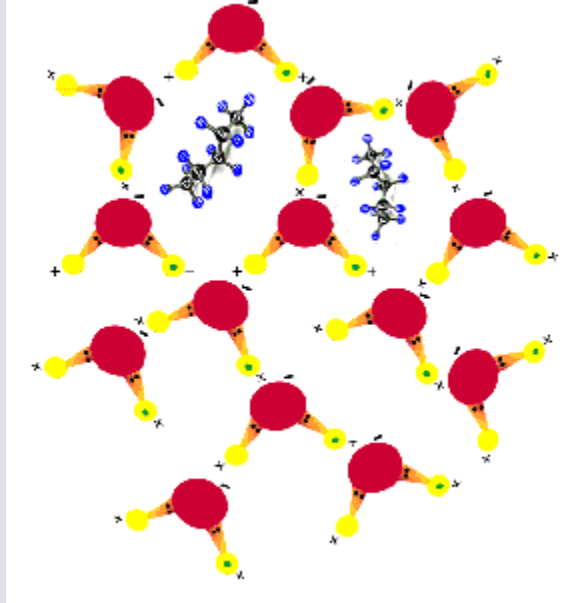


### Water

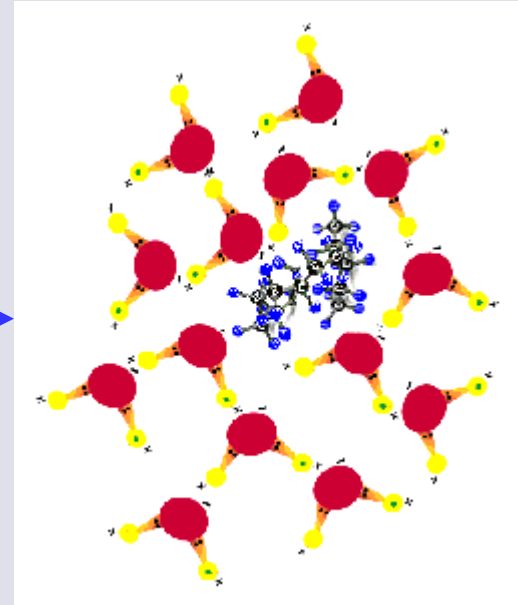
In Biological systems:  
It allow the formation of the weak  
**Hydrogen bond**.

# 3- Hydrophobic Interactions

- ❑ Hydrophobic interactions are more correctly called **hydrophobic exclusions**.



Over period of time



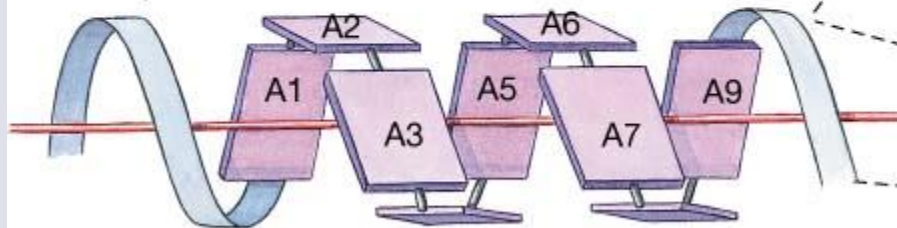
- There are two regions containing hydrophobic substances.
- Each of the substances is excluded from the water matrix.

- The two areas of hydrophobic substances will encounter one another, combine and form one larger hydrophobic region that is excluded from the water matrix.
- This combined state is more energetically favorable than the one in which the hydrophobic substances were separate. Thus this combined state will persist.

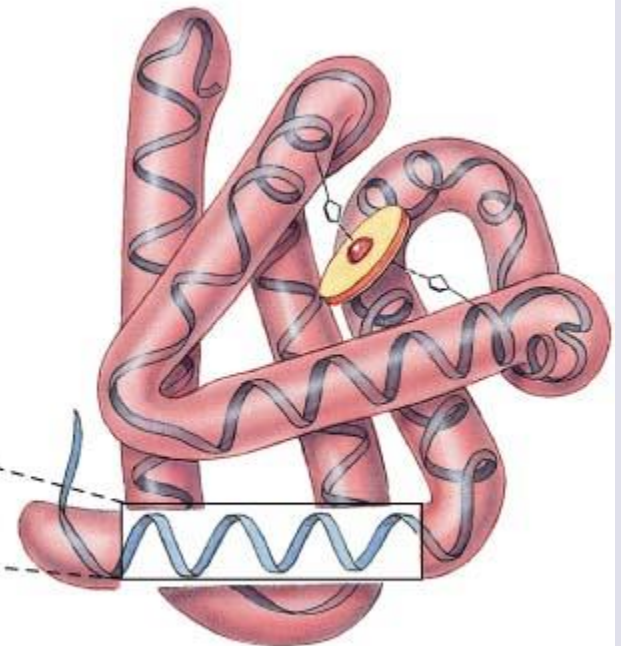




**(a) Primary structure**



**(b) Secondary structure**



**(c) Tertiary structure**

# VI- Quaternary Structure of Proteins

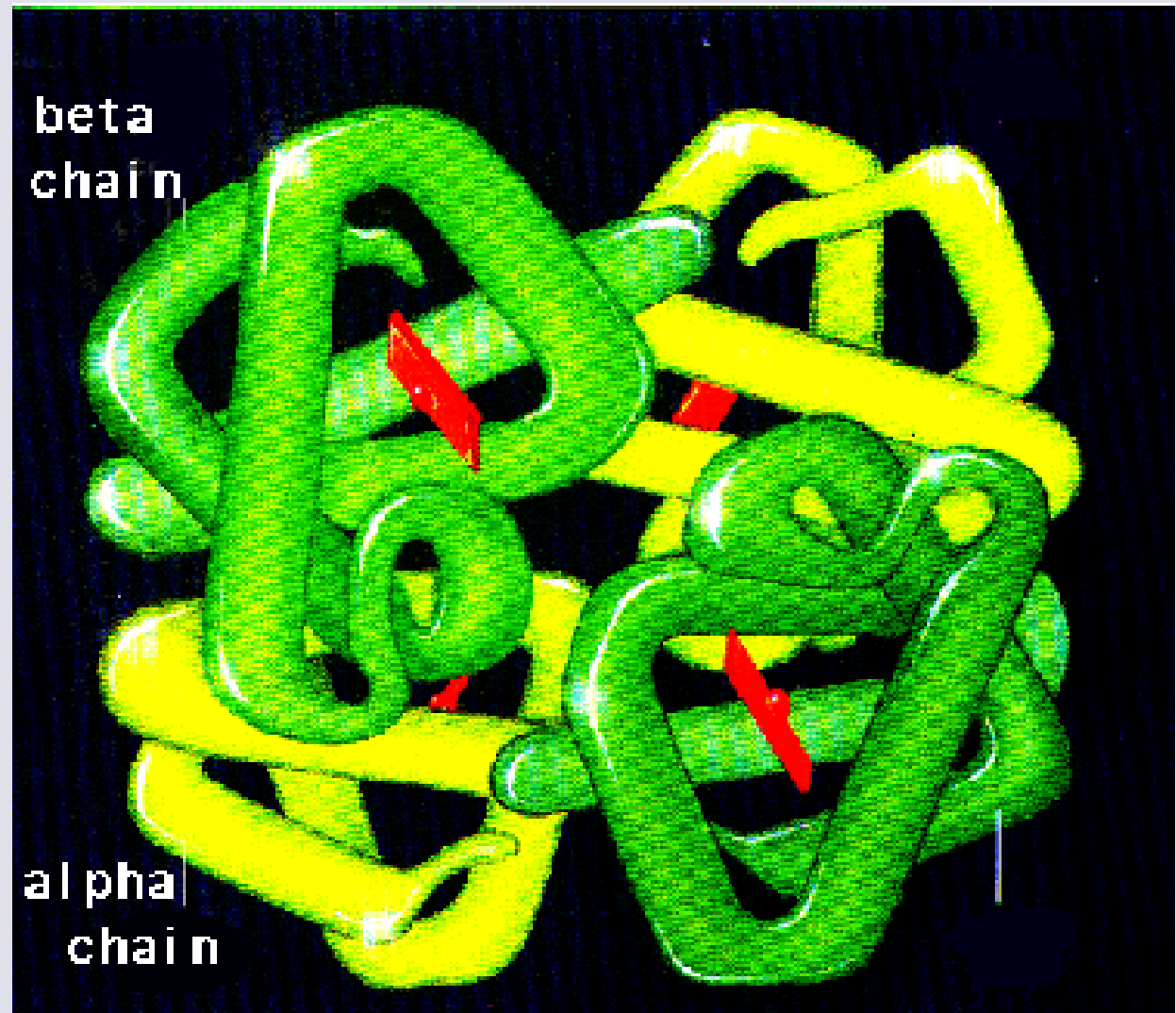
**The quaternary structure of proteins is the shape that results from the orderly interaction of the polypeptides of a multisubunit protein .**

## **Multisubunit protein**

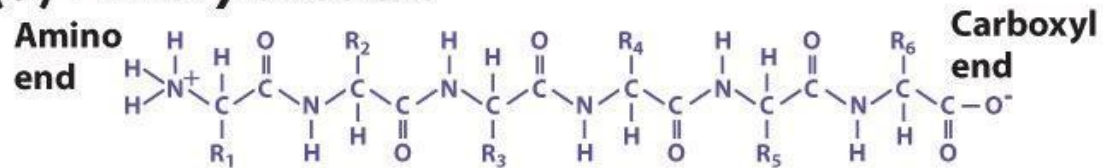
- **Some proteins are composed of more than one polypeptide chain.**
- **Each polypeptide chain is called a subunit. For example, if a protein is composed of two polypeptides, then it has two subunits.**
- **The polypeptides may or may not be different in primary structure.**  
**This is dependent upon the nature of the protein.**

# Forces that give rise to the quaternary structure

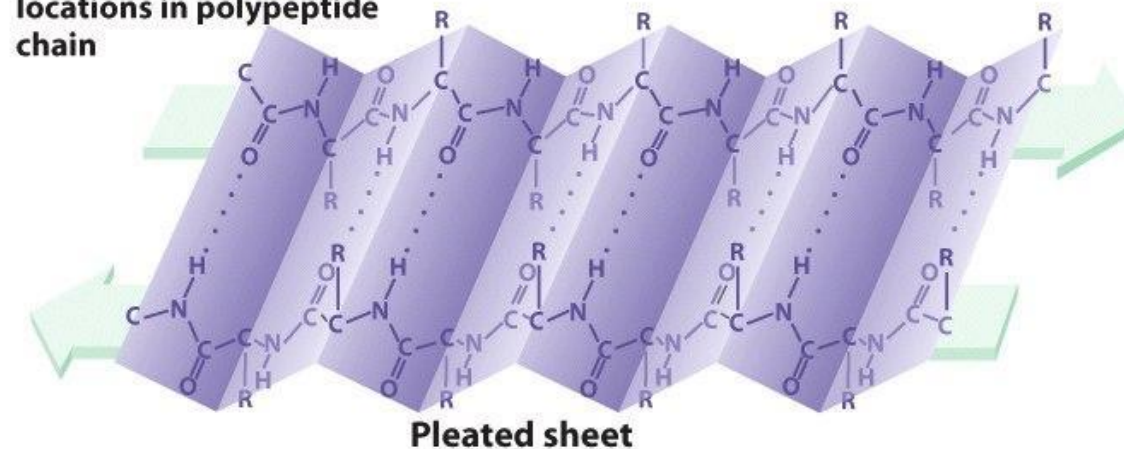
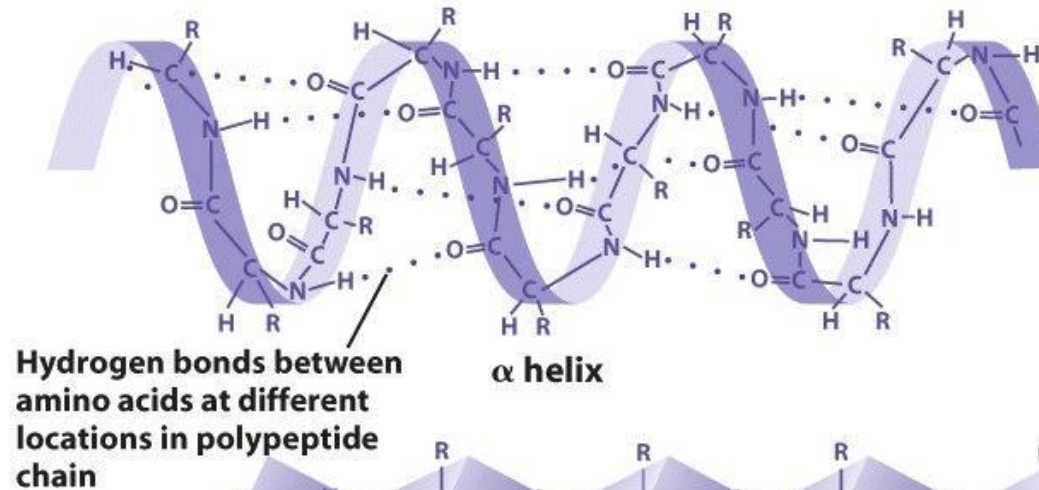
- ❖ Ionic bonding
- ❖ Hydrogen bonding
- ❖ Hydrophobic interaction
- ❖ Disulfide bonds
- ❖ Covalent bonds



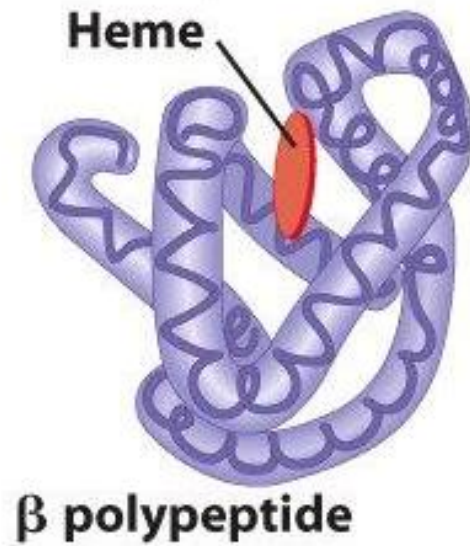
### (a) Primary structure



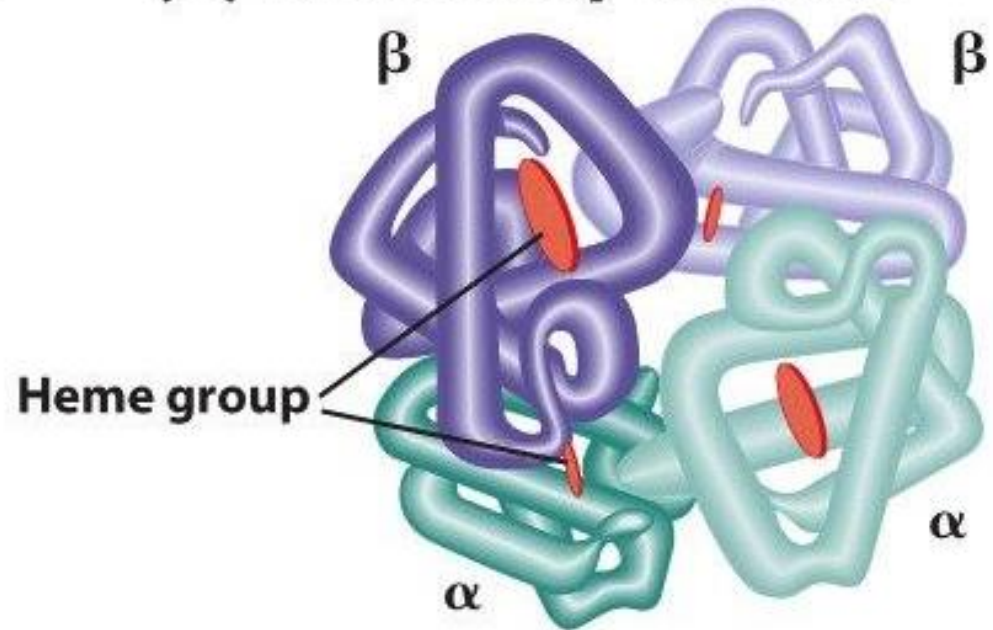
### (b) Secondary structure



**(c) Tertiary structure**



**(d) Quaternary structure**





**Primary protein structure**  
is sequence of a chain of  
amino acids.

Amino Acids



Pleated sheet



Alpha helix

**Secondary protein structure**  
occurs when the sequence of  
aminoacids are linked by hydrogen  
bonds.



Pleated sheet

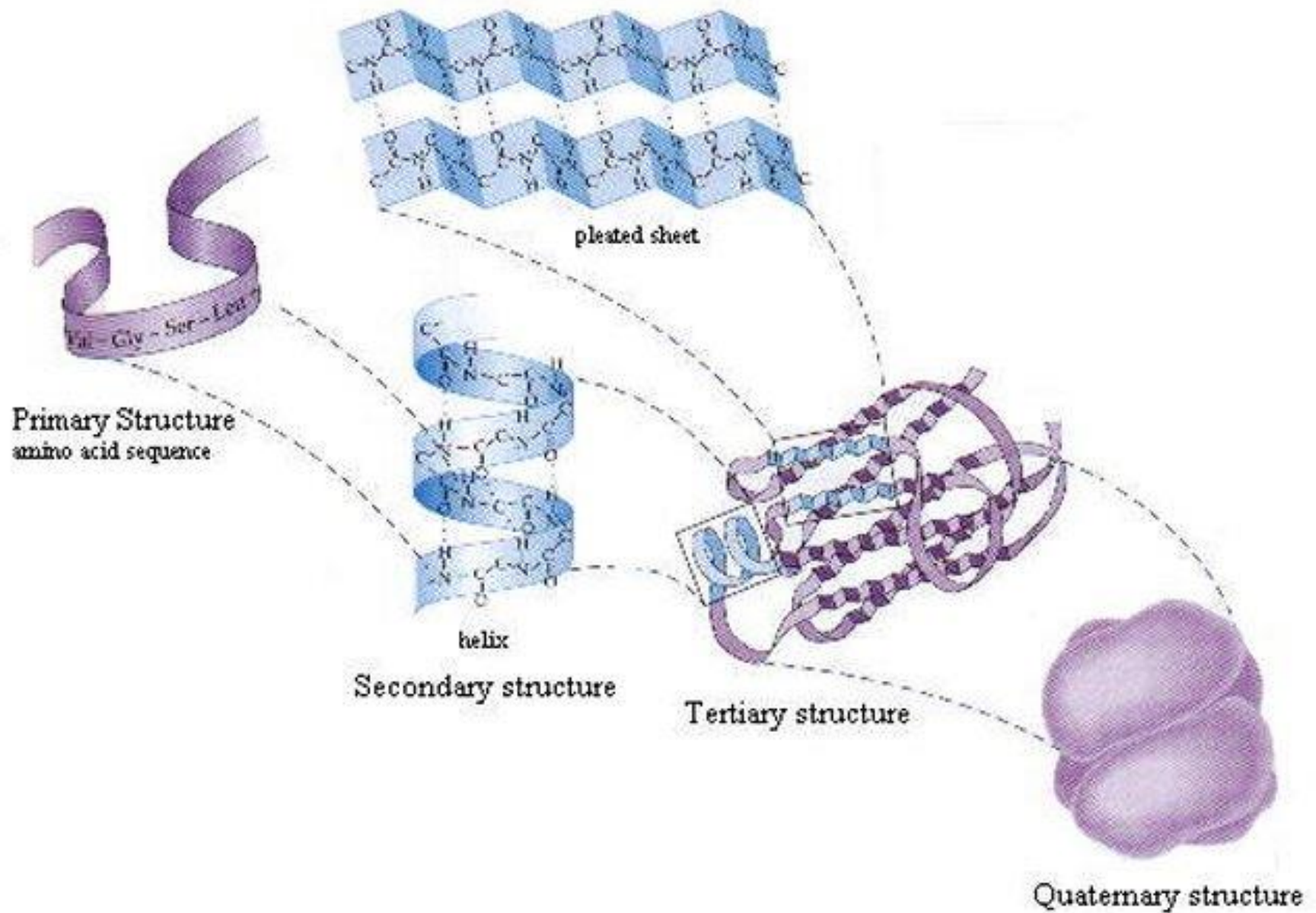
Alpha helix

**Tertiary protein structure**  
occurs when certain attractions  
are present between alpha helices  
and pleated sheets.



**Quaternary protein structure**  
is a protein consisting of more  
than one amino acid chain.





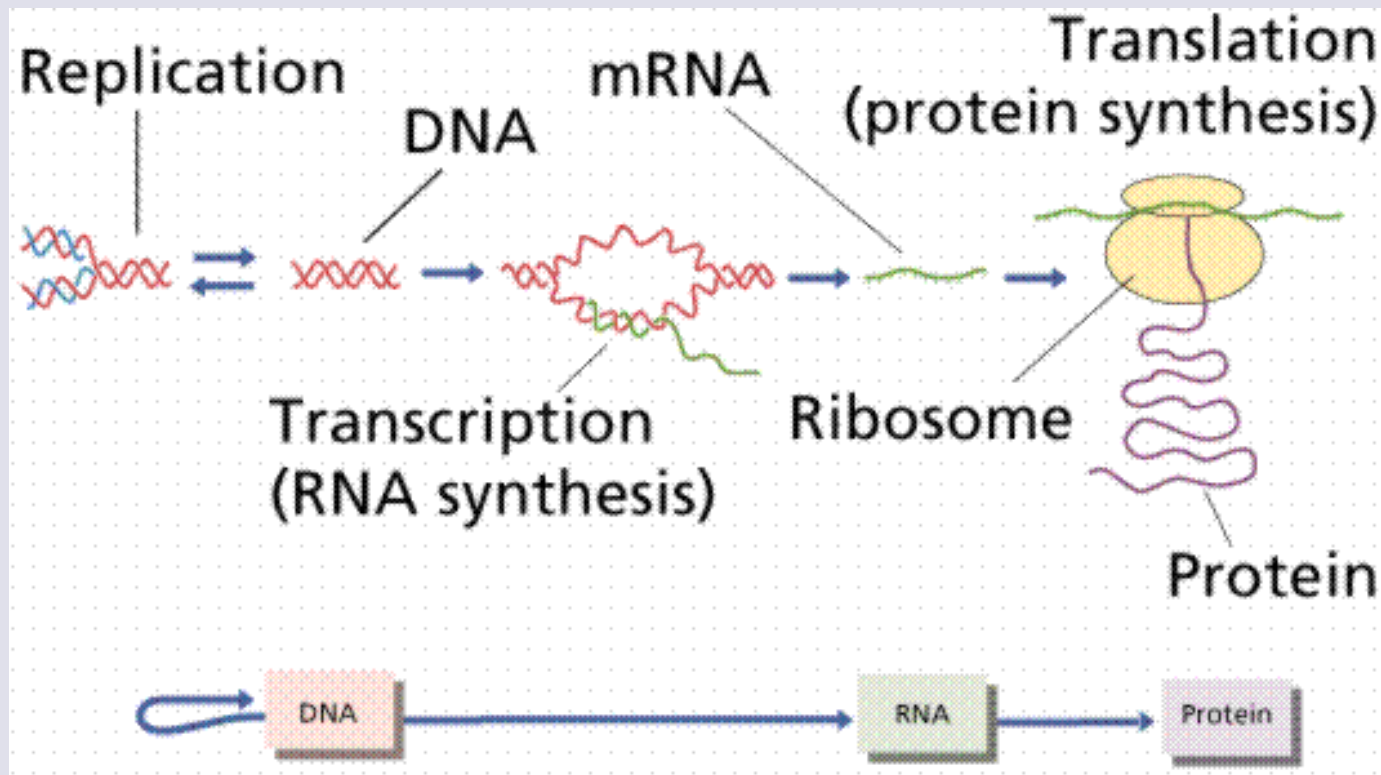
# Protein Function



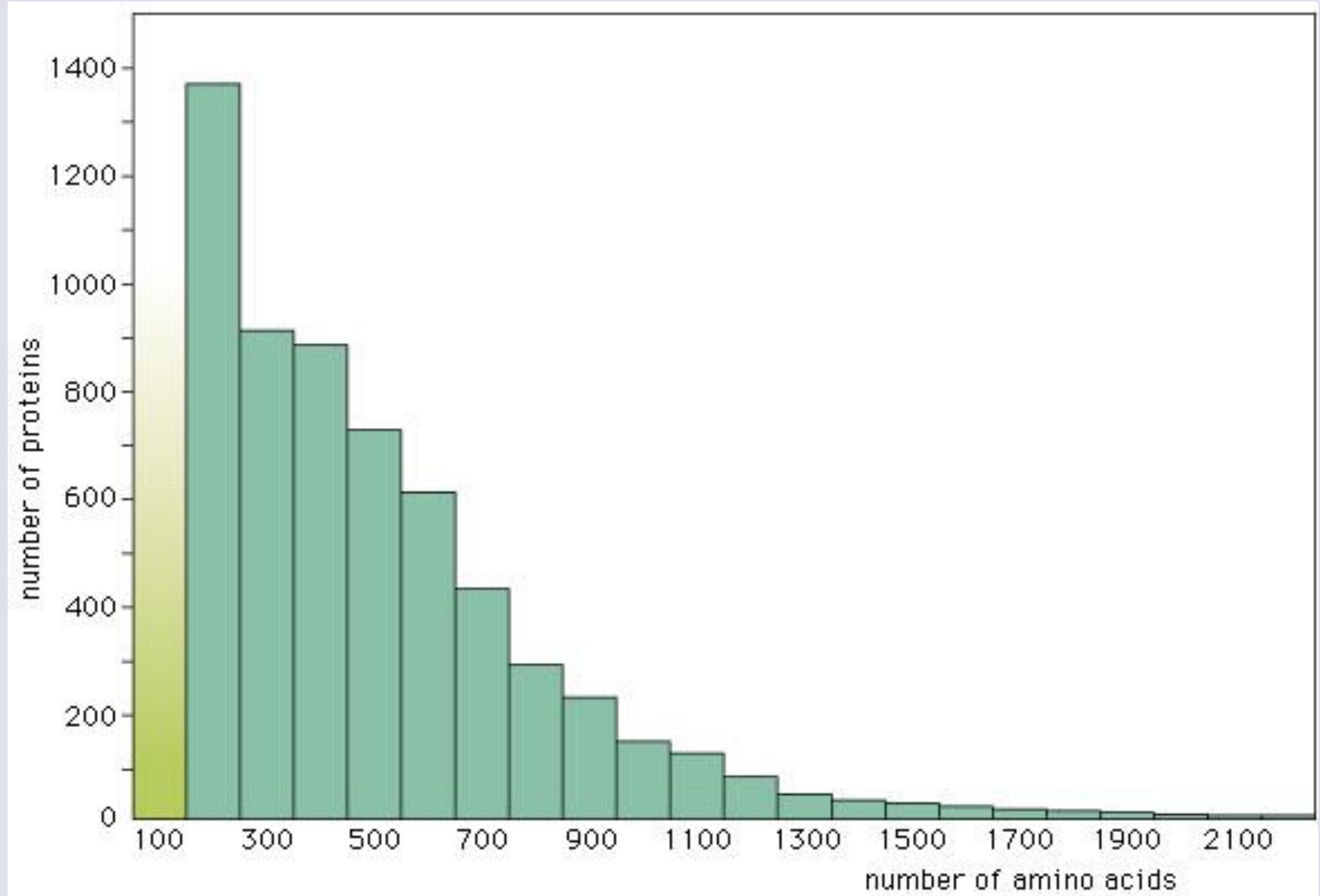
# Protein Function

- **Enzymes** - proteases, synthetases, polymerases, kinases
- **Structural** tubulin collagen, elastin a-keratin
- **Transport** - serum albumin, hemoglobin, transferrin
- **Motor** - myosin, kinesin, dynein
- **Storage** - ferritin, ovalbumin, calmodulin
- **Signaling** - insulin, nerve growth factor, integrins
- **Receptor** - acetylcholine receptor, insulin receptor, EG recept
- **Gene regulatory** - lactose repressor, homeodomain proteins
- **Special purpose** - green fluorescent protein, glue proteins

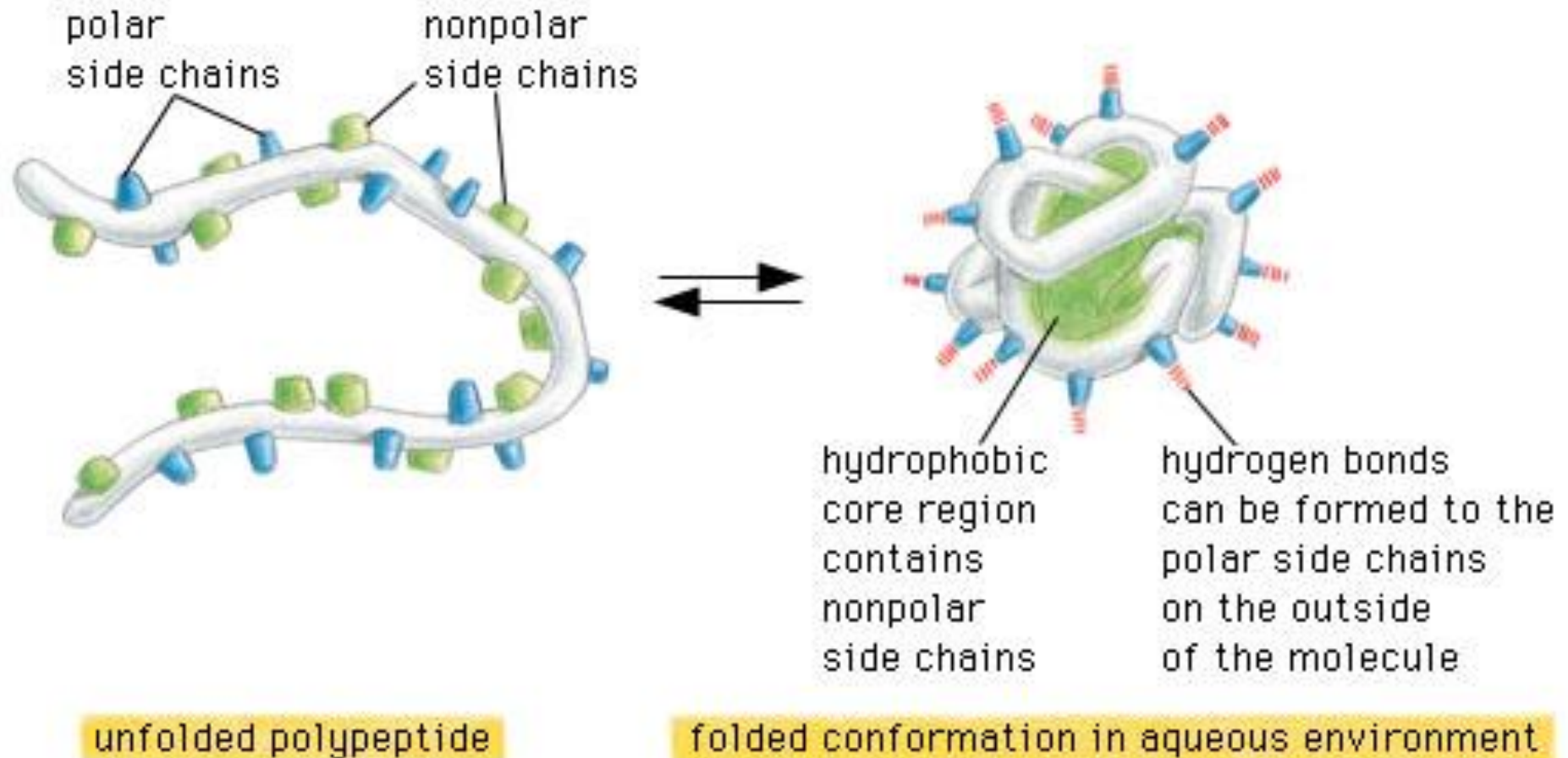
**Protein synthesized by ribosomal machinery which translate the nucleotide sequence in mRNA into amino acid sequence of protein**



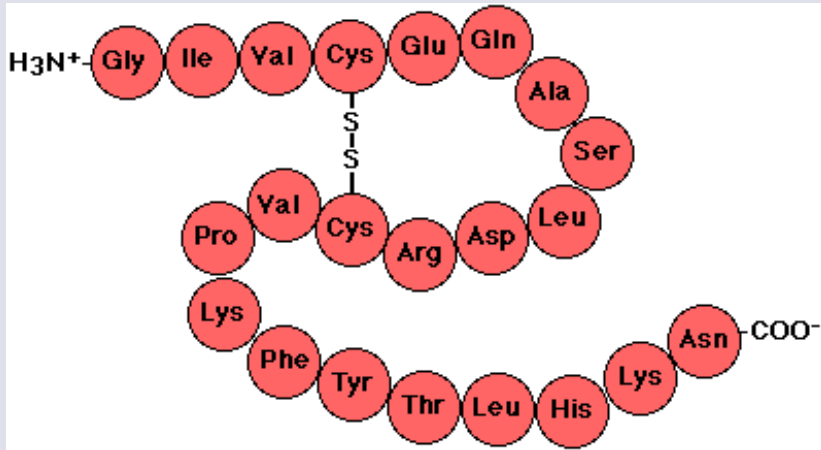
# Number & Size Distribution of Cellular Proteins



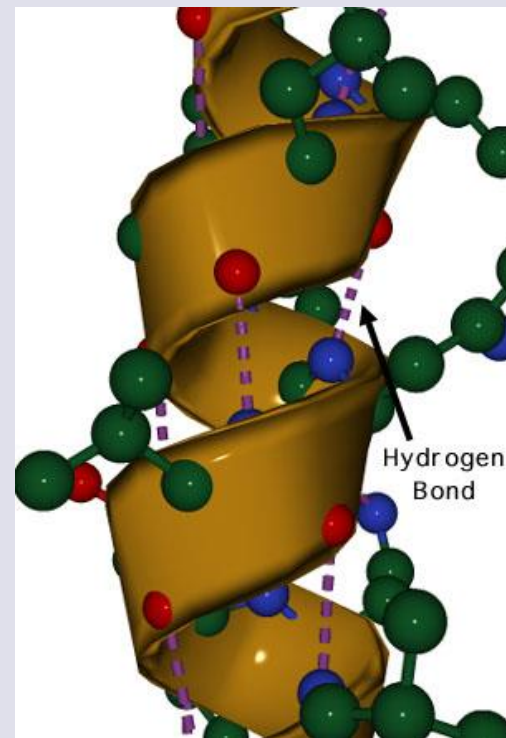
# Protein Folding



# Disulfide Bridge – Linking Distant Amino Acids



## Hydrogen Bonding And Secondary Structure



# **Isolation of protein**

# Why purify a protein?

- Characterize function, activity, structure
- Use in assays
- Raise antibodies
- many other reasons ...

# How pure should my protein be?

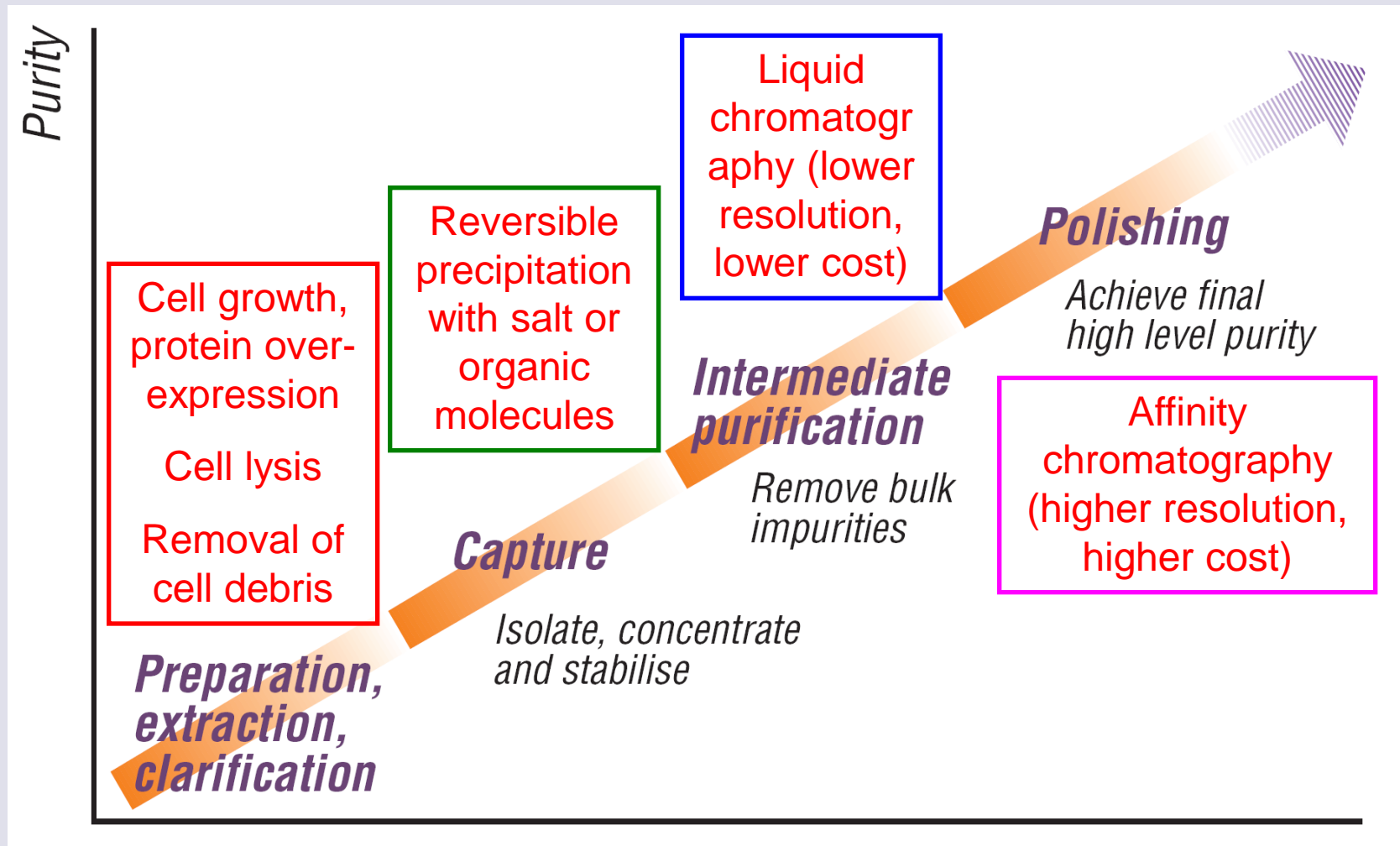
Application	Required Purity
Therapeutic use, <i>in vivo</i> studies	Extremely high > 99%
Biochemical assays, X-ray crystallography	High 95-99%
N-terminal sequencing, antigen for antibody production, NMR	Moderately high < 95%



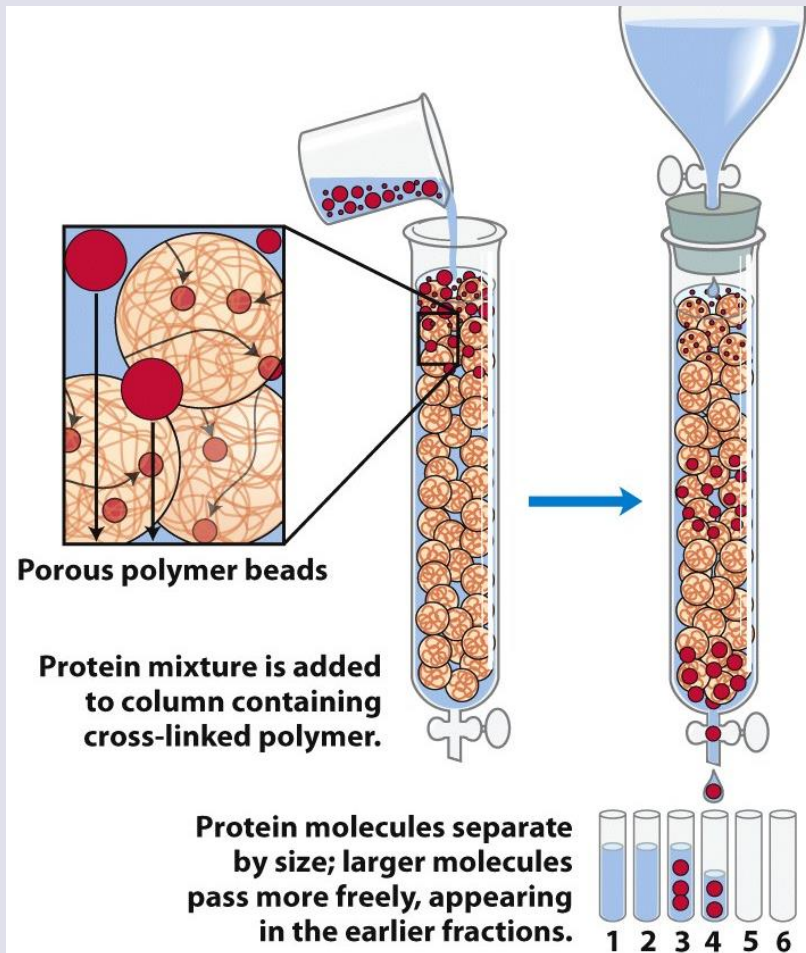
# **Separation of proteins based on physical and chemical properties**

- **Solubility**
- **Binding interactions**
- **Surface-exposed hydrophobic residues**
- **Charged surface residues**
- **Isoelectric Point**

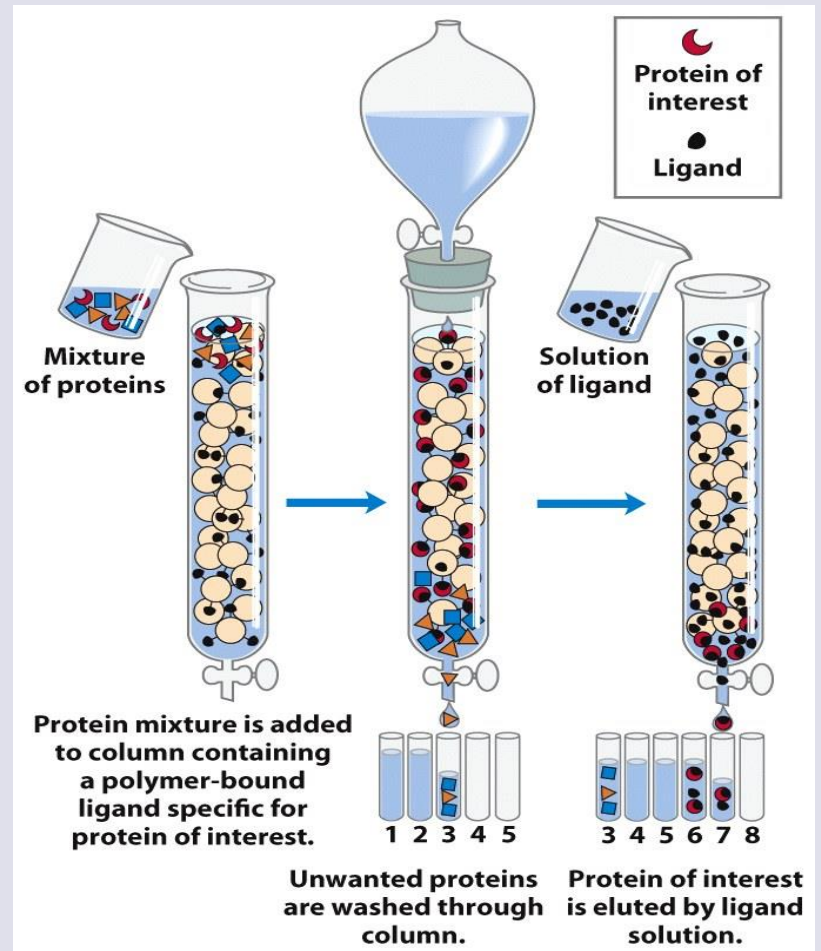
# Protein isolation, concentration, and stabilization



# Size exclusion chromatography



# Affinity Chromatography



# Separation of Protein

- Once the cell is broken open, lysate is collected for further purification based on properties of the protein.

Proteins are separated on the basis of



Molecular approaches of separation

**Chromatography**

- Stationary phase: Gel
- Mobile phase: Solvent-containing molecules.
- Differential interaction of molecules With Stationary phase and solvent.

**Electrophoresis**

- It doesn't use mobile phase.
- It separates charged molecules according to size or charge.
- Molecules move in an electric field through a fluid phase.

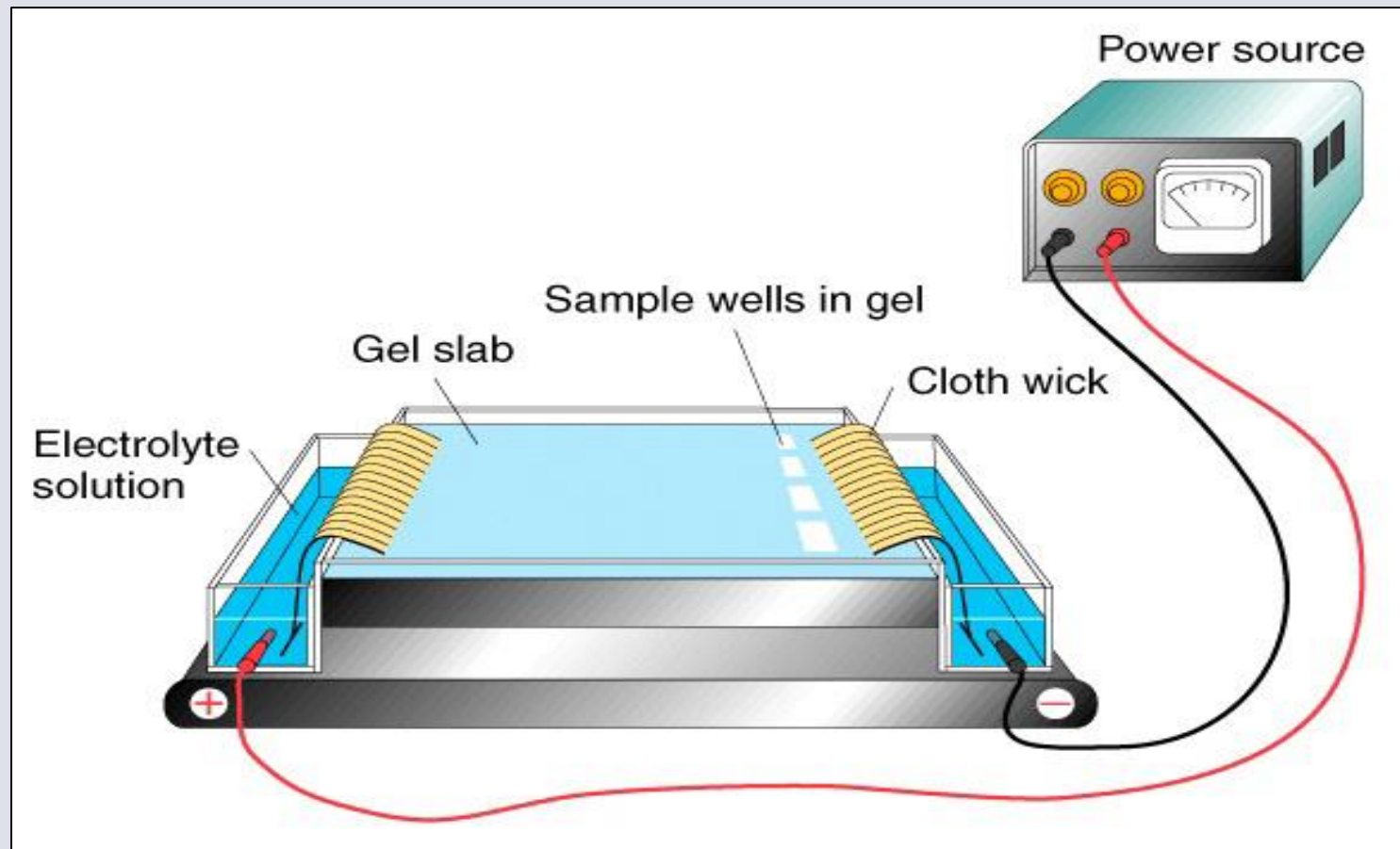
# Protein detection methods

- SDS-PAGE
  - Visual confirmation
- UV Spectrophotometry
  - Absorbance @ 280 nm
- Colorimetric Techniques
  - Color change proportional to [protein]
  - Bradford, Lowry, BCA

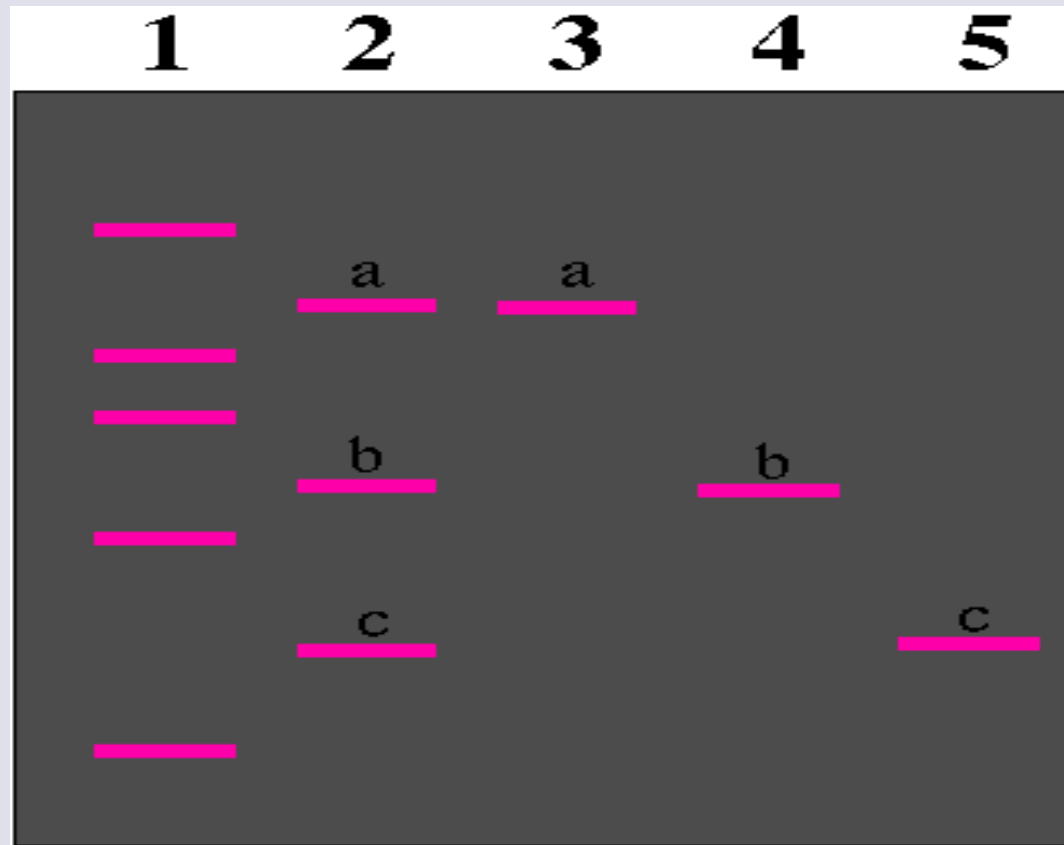
# Electrophoresis

# Electrophoresis

- **Cation** = positively charged ion, it moves toward the cathode (-)
- **Anion** = negatively charged ion, it moves toward the anode (+)
- **Amphoteric substance** = can have a positive/negative/zero charge, it depends on conditions
- **Principle:**
  - Some substances have different net charges and can be separated into several fractions in external electric field.
  - But velocity of a particle also depends on the:
    - **size, shape of the particle and given applied voltage**



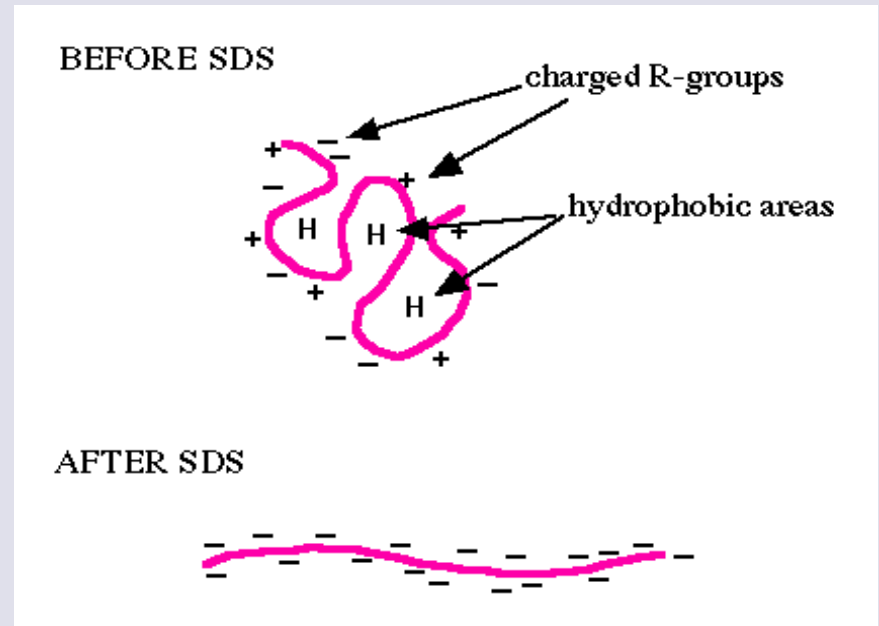




**The actual bands are equal in size, but the proteins within each band are of different sizes.**

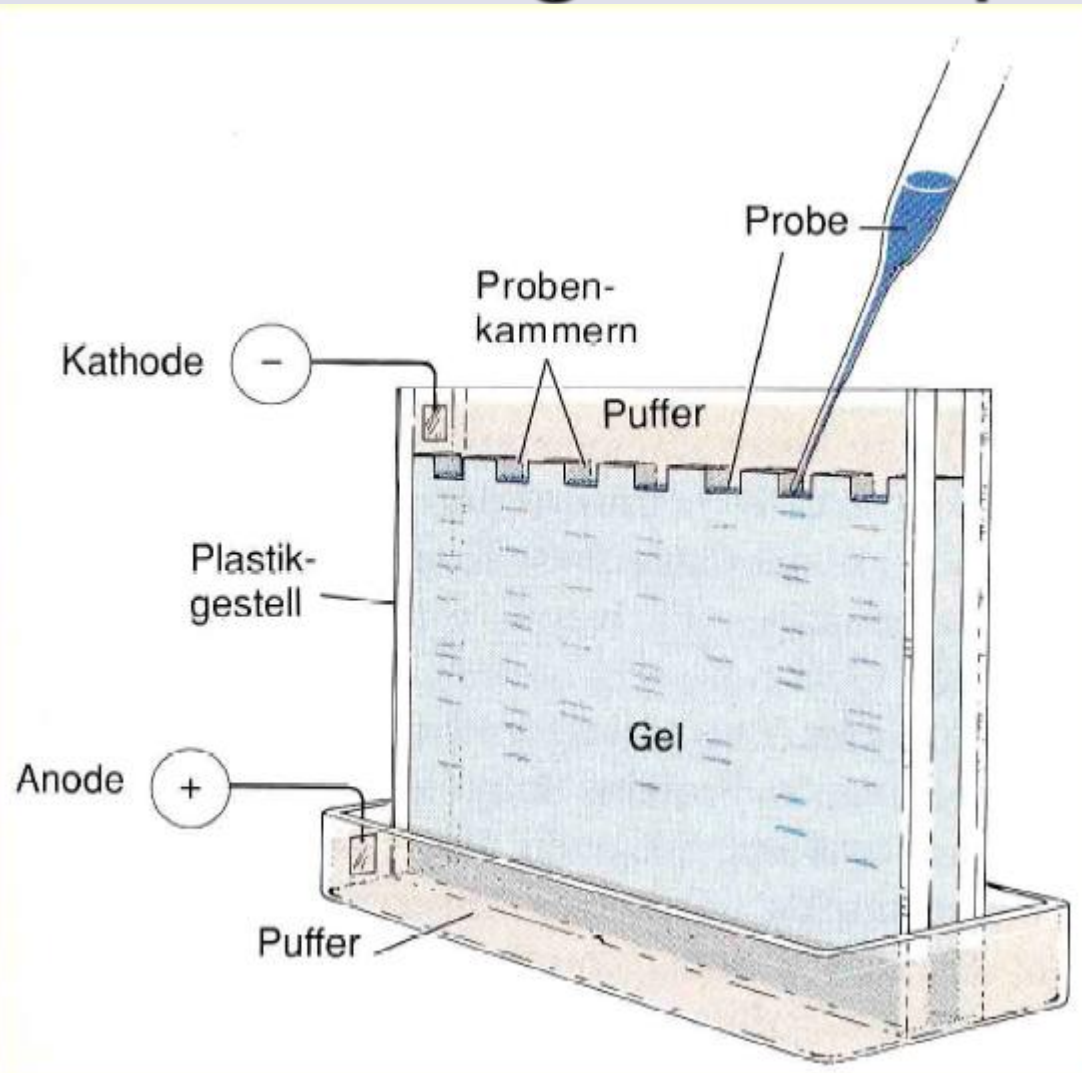
# SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis (Laemmli 1970)

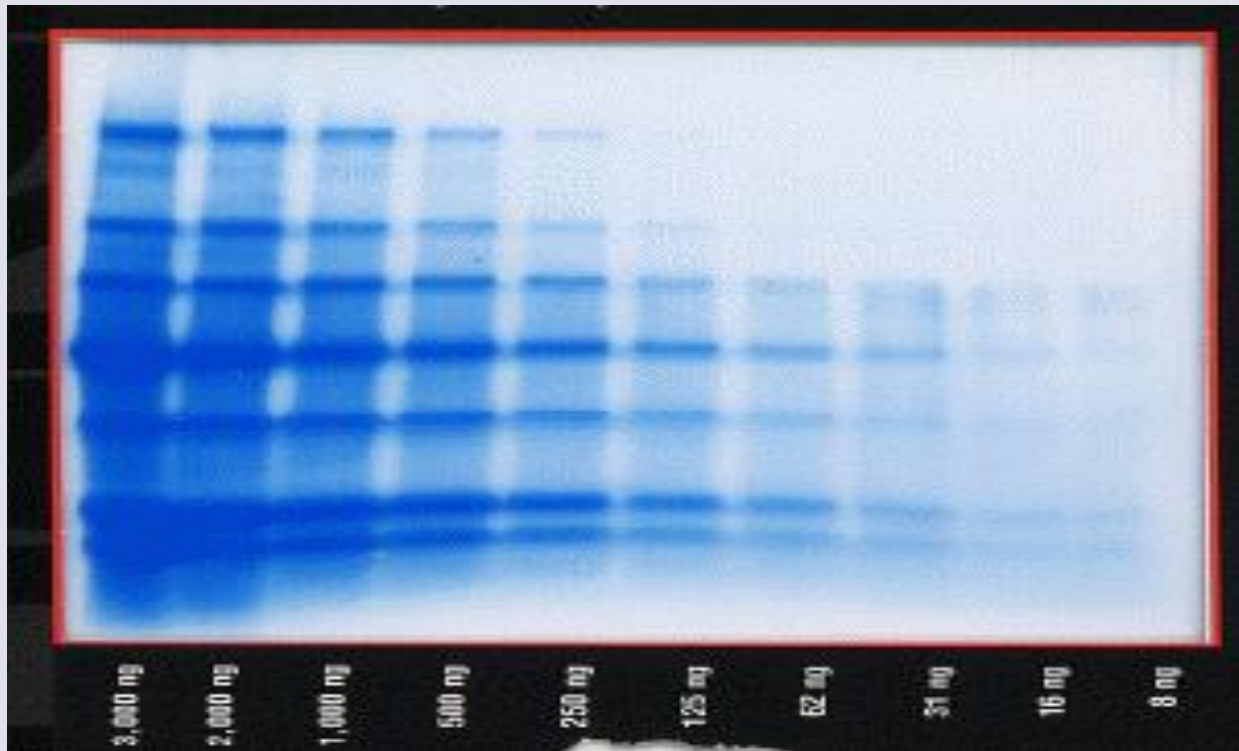
**SDS (sodium dodecyl sulfate) is a detergent (soap) that can dissolve hydrophobic molecules but also has a negative charge**



**Therefore, if a cell is incubated with SDS, the • membranes will be dissolved, all the proteins will be solubilized by the detergent and all the proteins will be covered with many negative charges.**

# Vertical gel set up





**Protein gel (SDS-PAGE) that has  
been stained with Coomassie  
Blue.**



- Serum proteins are separated into 6 groups:
- Albumin
- $\alpha_1$  - globulins
- $\alpha_2$  - globulins
- $\beta_1$  - globulins
- $\beta_2$  - globulins
- $\gamma$  - globulins

# Terminologies..

- The **Western blot** (alternatively, **protein immunoblot**) is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract.
- A **Southern blot** is a method routinely used in molecular biology for detection of a specific DNA sequence in DNA samples.
- The **northern blot** is a technique used in molecular biology research to study gene expression by detection of RNA.
- **Southwestern blotting**, based along the lines of Southern blotting (which was created by Edwin Southern) and first described by B. Bowen and colleagues in 1980, is a lab technique which involves identifying and characterizing DNA-binding proteins (proteins that bind to DNA).

# **Western Blotting (WB)**

**WB is a protein detection technique that combines the separation power of SDS PAGE together with high recognition specificity of antibodies**

**An antibody against the target protein could be purified from serum of animals (mice, rabbits, goats) immunized with this protein**

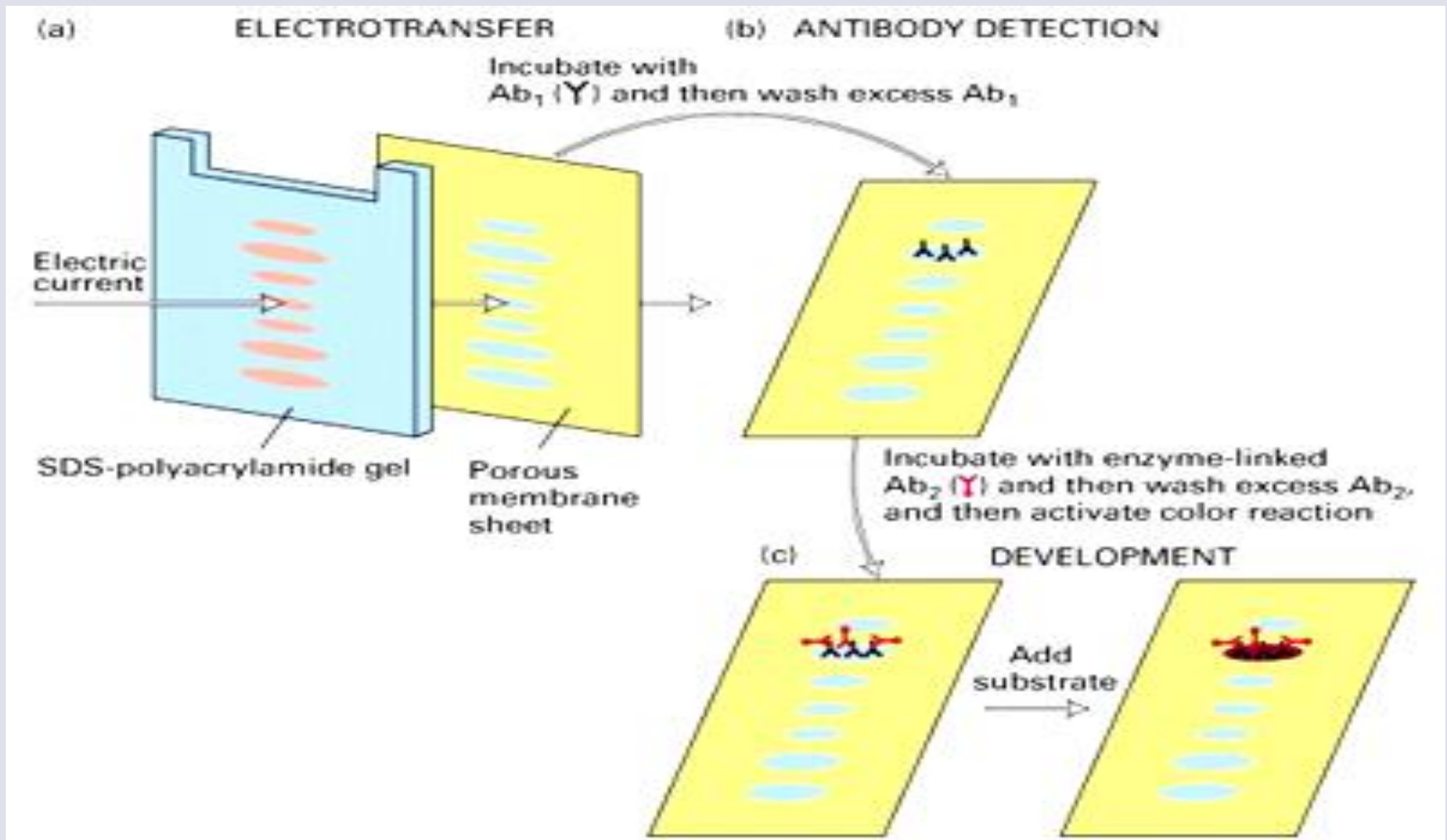
**Alternatively, if protein contains a commonly used tag or epitope, an antibody against the tag/epitope could be purchase from a commercial source (e.g. anti-6 His antibody)**

# **WB: 4 steps**

- 1. Separation of proteins using SDS PAGE**
- 2. Transfer of the proteins onto e.g. a nitrocellulose membrane (blotting)**
- 3. Immune reactions**
- 4. Visualization**



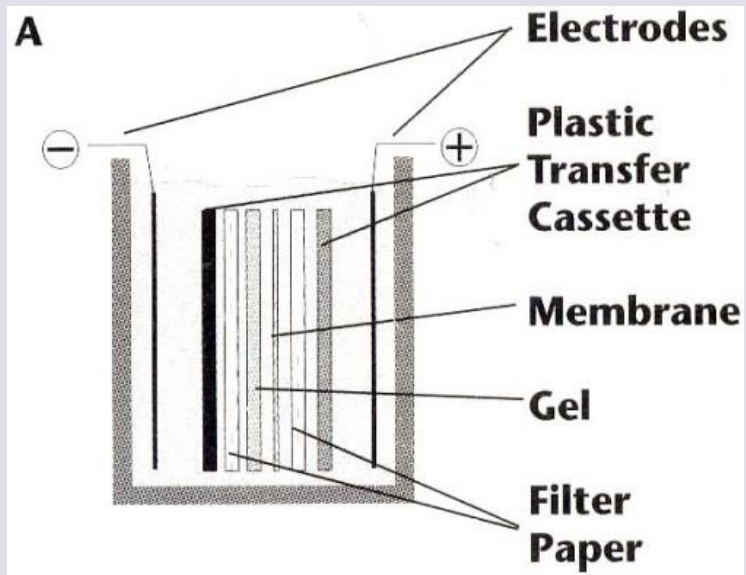
# The essence of Western-blot



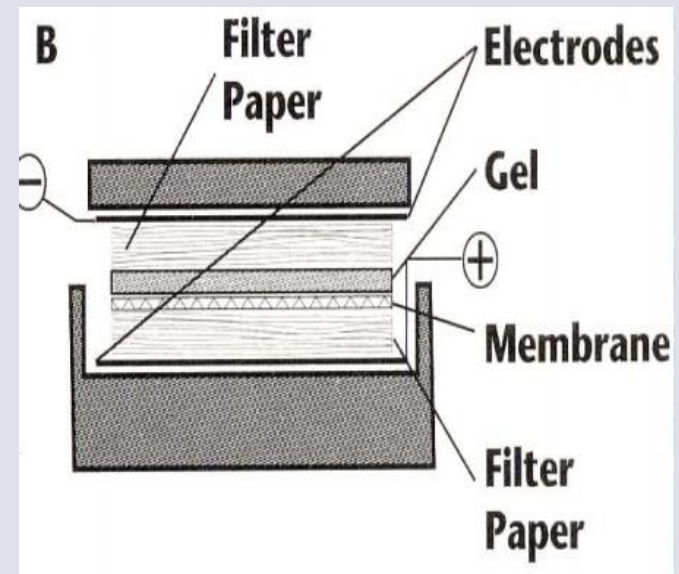
# Transfer



## Wet



## Semi-dry



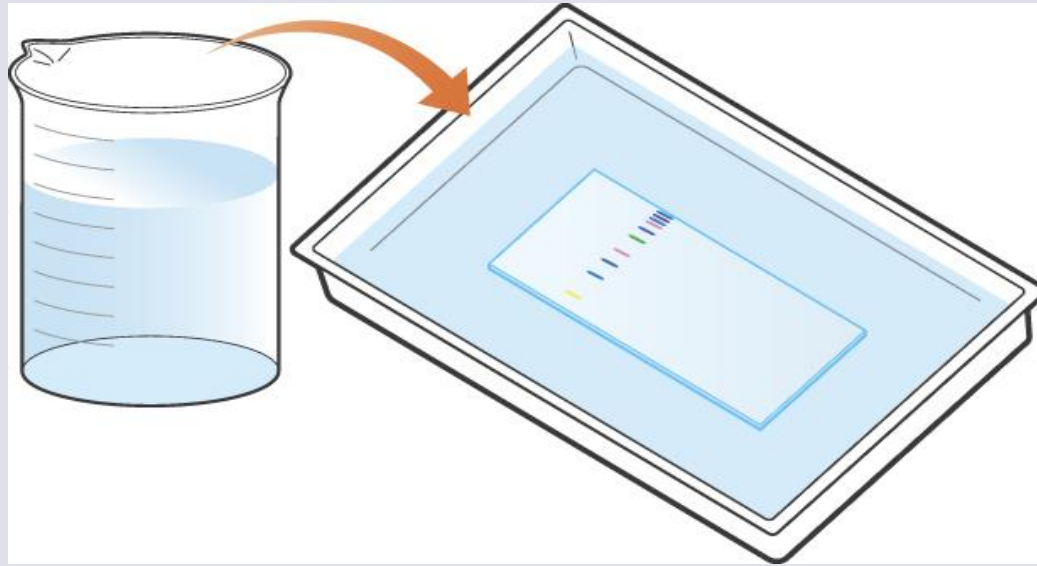
# Types of membranes

**Nitrocellulose (NC) □ high binding capacity, works well with both protein and DNA not need methanol to preparation.**

**Polyvinylidene difluoride (PVDF) □ high capacity and stable, need methanol for preparation.**

**These both membranes bind proteins non-covalently.**

# Blocking

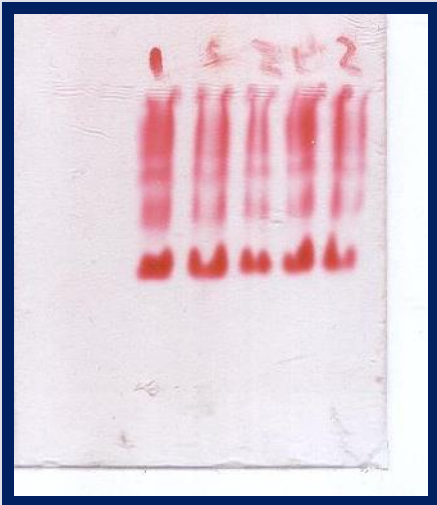


**5% non-fat milk or BSA with Tween 20: Prevents the primary antibody from binding randomly to the membrane**

**After blocking apply your first Ab at the specific concentration, learn how...? Wash carefully, apply secondary Ab HRP conjugated, wash carefully, detect your specific protein by detection reagent.**

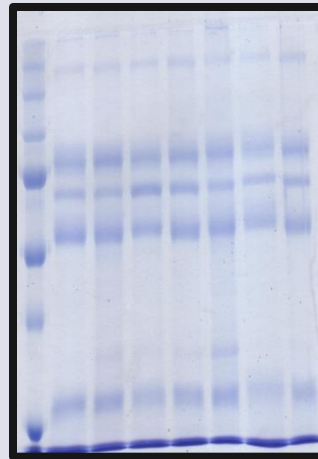
# Methods of protein Detection

**Cellulose acetate plates**

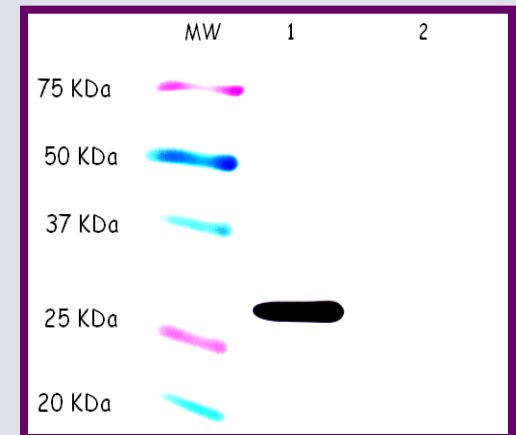


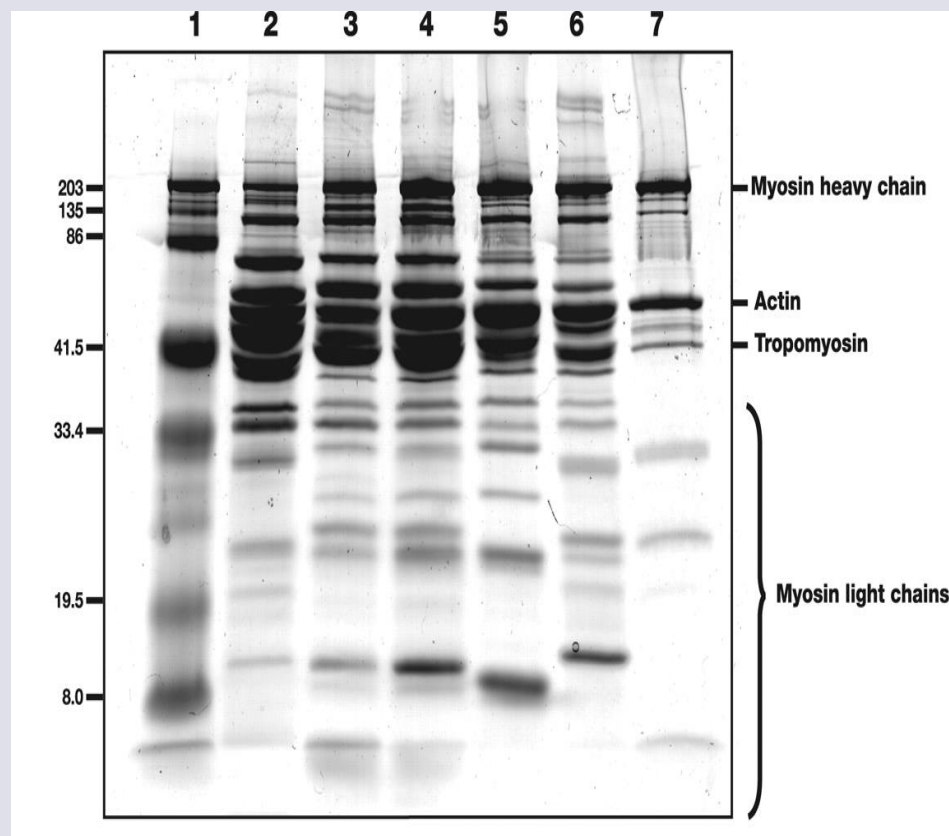
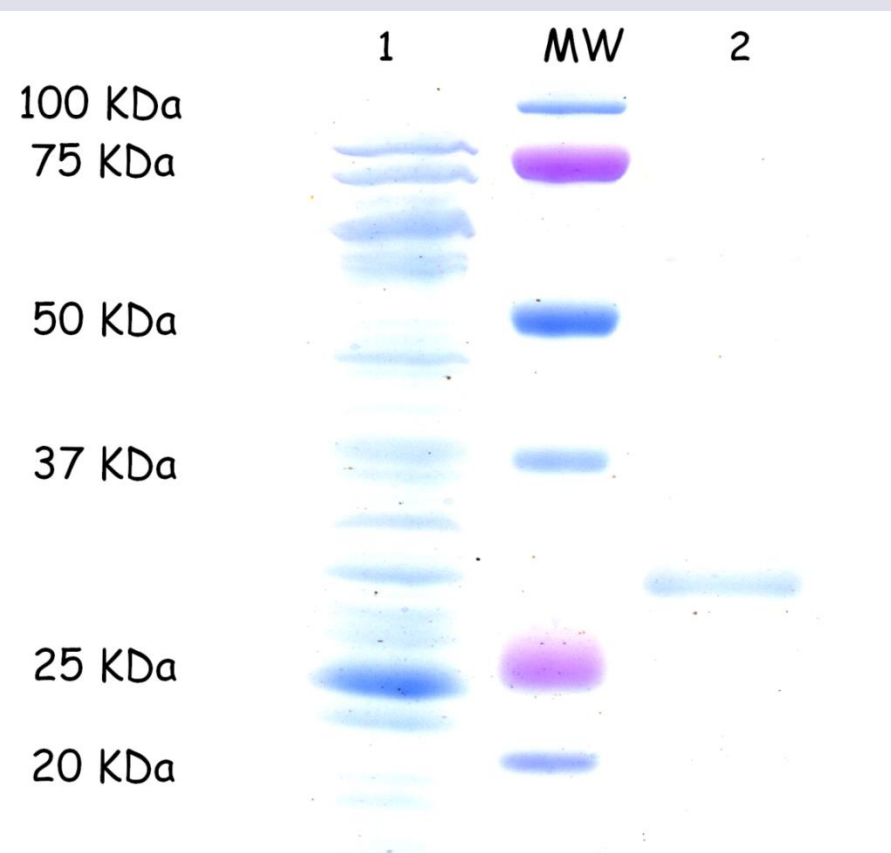
**Sodium Dodecyl Sulfate (SDS)-  
Polyacrylamide Gel Electrophoresis  
(SDS-PAGE)**

**\* Visualize many proteins at once**



**SDS-PAGE combined  
with immunoblotting  
(Western blot)**  
**\* Visualize one  
protein out of complex**





# **Gel Analysis**

**you can analyze your profile  
using suitable software  
like such as**

**1- NIH Image Software**

**2- Lab Image**

**3- Gel Pro Analyzer**

# References and additional Reading

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A composite image featuring a baby in the lower-left foreground, looking up and reaching towards a large, bright full moon in a dark blue night sky. The sky is filled with wispy white clouds and small white stars. A bright green speech bubble with a white outline points from the baby's mouth towards the moon. Inside the bubble, the word "Questions" is written in white, bold, sans-serif font.

Questions