PROTEIN STRUCTURE:

INTRODUCTION:

-Proteins have different structures, and some have repeating inner structures, other do not.

-A protein may have gazillion possibilities of structures, but a few would be active.

-These active structures are known as **native conformations;** the 3- dimensional structure of a properly folded and functional protein.

LEVELS OF PROTEIN STRUCTURE:

1) Primary Structure:

The order in which the amino acids are covalently linked together, **i.e.**

Leu—Gly—Thr—Val—Arg—Asp—His

-The primary structure of a protein determines the other levels of structure

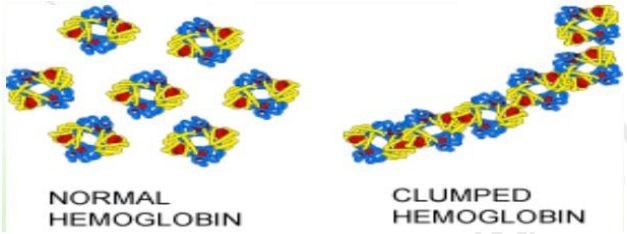
-A single amino acid substitution can give rise to a malfunctioning protein, **as is the case with** sickle-cell anemia.

◊ Sickle-Cell Anemia (Hbs):

-It is a hereditary disorder

-It is caused by a change of amino acids in the 6th position of β globin (Glu to Val)

-What happens is that Glu is polar and charged, while Val is nonpolar, thus; what was able to face the aqueous environment (Glu) is now trying to hide/escape from it (Val)



-The mutation results in:

- Arrays of aggregates of hemoglobin molecules
- Deformation of the red blood cell
- Clotting in blood vessels and tissues

2) Secondary Structure:

The content of α-helices, β-sheets and other forms, but not their orientation relative to each other

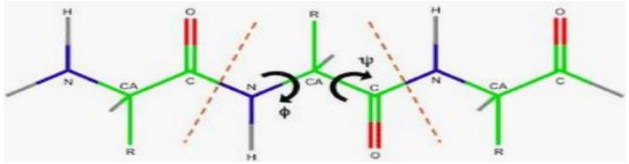
[No 3D structure is known]

***Why Does it Form?**

Due to the noncovalent interactions between the backbone atoms

RECALL:

Rotation of bonds and un-rotation of peptide bonds affect the **secondary structure**



***Common Secondary Structures:**

-A hydrogen-bonded, local arrangement of the backbone of a polypeptide chain

-Polypeptide chains can fold into regular structures such as:

- Alpha helix
- Beta-pleated sheet
- Turns
- Loops

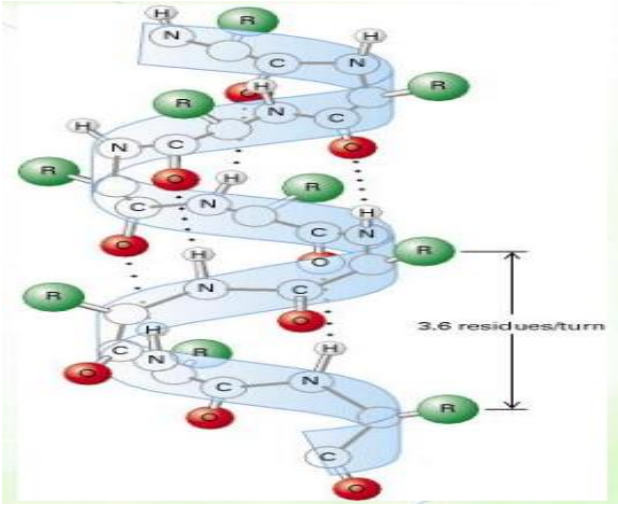
◊ The α Helix:

-It looks like a helical rod

-The helix has an average of 3.6 amino acids **per** turn.

-The pitch of the helix; the linear distance between corresponding points on successive turns **is** 5.4 Å [1 Å = 10^{-10} m]

-It is very stable **because of** the linear hydrogen bonding



-Amino Acids NOT Found in an α-Helix *Next to Each Other*:

[This means that we can find them but not next to each other**]**

- Too small amino acids; Glycine
- Too large amino acids; Tryptophan, Tyrosine and Phenylalanine
- β-C-branched amino acids; Isoleucine, Valine and Threonine
- Close proximity of a pair of charged amino acids with similar charges

-Amino Acids NOT Found in an α-Helix at All:

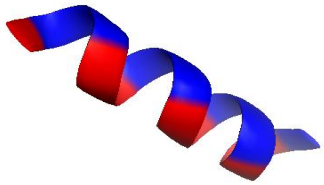
- Proline:

No rotation around N-Cα bond

No hydrogen bonding of α-amino group

-Amphipathic α-Helices:

i.e. ions channels within the plasma membrane

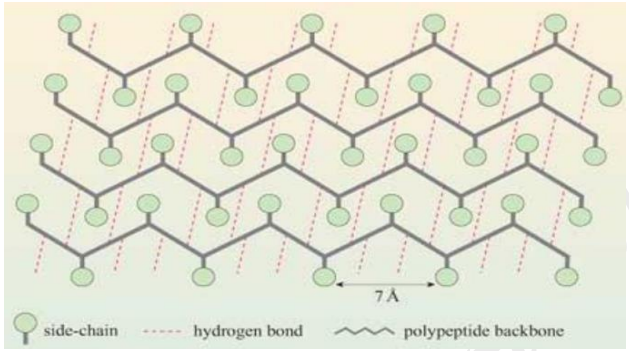


Blue (for example) = Polar amino acids will face the interior of the channel

Red (for example) = Non-polar amino acids will face the exterior of the channel (interacting with the hydrophobic tails)

◊ The β Pleated Sheet (β-Sheet):

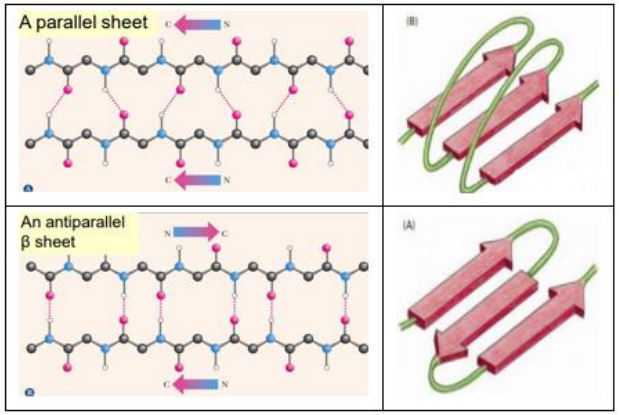
-It is a zigzag-like chains (strands), that are held together via hydrogen bonding



NOTE:

H-bonding is cross-linking the strands, thus, adds to the mechanical properties of this structure

-Orientation of β-Sheets Relative to Each Other:



NOTE:

β sheets can be purely antiparallel, purely parallel, or mixed

NOTE:

β-sheets can be found along with **α-helices** in the same protein

-How many β strands can a β sheet have?

β sheets can form between many strands, typically 4 or 5 but as many as 10 or more

NOTE:

-Amino acids that cannot be found next to each other in an α-helix can be found next to each other in a β-sheet

-Amino Acids NOT Found in an α-Helix at All:

- Proline:

No rotation around N-Cα bond

No hydrogen bonding of α-amino group

◊ β-Turns:

-They are compact, U-shaped secondary structures

-They are flexible; thus, no fixed structure is known. Furthermore, we must have small and appropriate-shaped amino acids, like **Glycine** and **Proline**, respectively

-Used to link α-helices and β-sheets

◊ Loops:

-They are larger turns

2⁺) Super-Secondary Structures:

They are regions in proteins that contain an ordered organization of secondary structures.

Examples:

- Motifs
- Domains

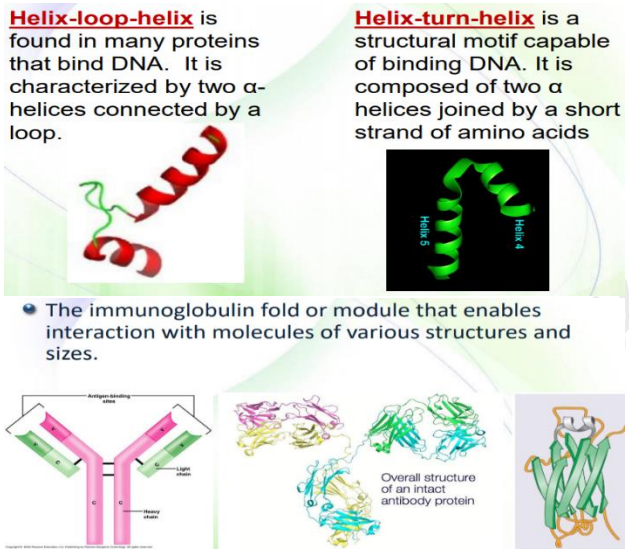
◊ Motifs (Modules):

-A repetitive super-secondary structure (repetitive secondary structures), which can often be repeated and organized into larger motifs

-It usually constitutes a small portion of a protein (typically less than 20 amino acids)

-It tells us about the shape (folding), but not the function

-Examples:



◊ Domains:

-A compactly folded region of polypeptide found in proteins with similar function and/or structure.

-Domains with similar conformations are associated with the particular function.

-A structural domain may consist of 100–200 residues in various combinations of α helices, β sheets, turns, and random coils.

-They fold independently of the rest of the protein.

-Domains may also be defined in functional terms;

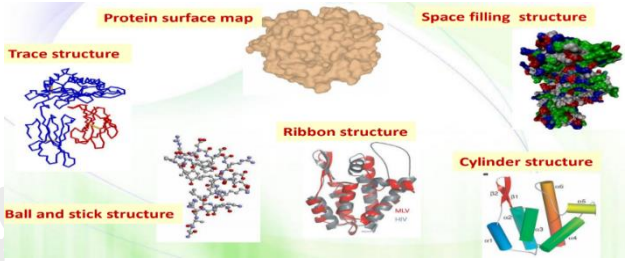
- Enzymatic activity
- Binding ability (e.g., a DNA-binding domain)

3) Tertiary Structure:

***Definitions:**

- The overall conformation of only one polypeptide chain
- The three-dimensional arrangement of all the amino acids residues
- The spatial arrangement of amino acid residues that are far apart in the sequence

***Common Representations of Proteins:**



***Shape Determining Forces:**

They are <u>Noncovalent Interactions</u>:

◊ Hydrogen Bonding:

between;

two side chains

two peptide bonds

one side chain and one peptide group

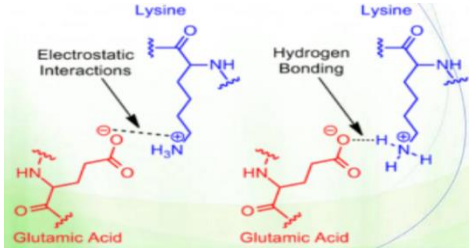
with the surrounding aqueous medium

◊ Charge-Charge Interactions (Salt Bridges):

between oppositely charged R-groups of amino acids

NOTE:

The same charged group can form either hydrogen bonding or electrostatic interactions



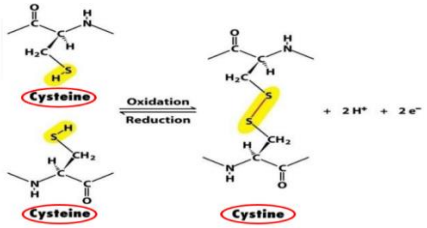
◊ **Charge-Dipole Interactions:**
between charged R groups with the partial charges of water

◊ **Hydrophobic Interactions:**
- between non-polar side chains
- A system is more thermodynamically (energetically) stable when hydrophobic groups are clustered together rather than extended into the aqueous surroundings

Q: Can polar amino acids be found in the interior?
Yes, in this case, they form hydrogen bonds to other amino acids or to the polypeptide backbone. **i.e.** membrane ionic channels

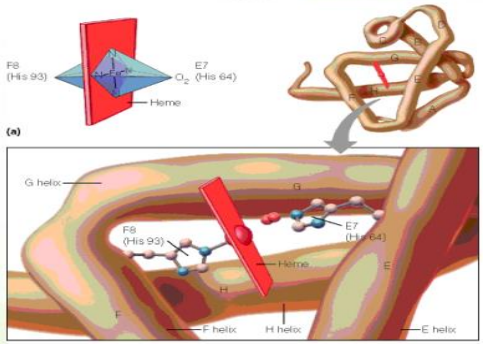
◊ **Van Der Waals Interactions:**
- Attractive and repulsive forces
- Although van der Waals forces are extremely weak; they are significant because there are so many of them in large protein molecules

***Stabilizing Forces:**
They can be:
-Covalent Interactions:
◊ **Disulfide Bridges:**
between side chains of two cysteine molecules, **i.e.** in insulin



◊ **Covalent Interaction, with the involvement of metal ions [a non-protein component]:**
Non-Protein Components:
Metal ions/ Sugar molecules/ Lipid molecules/ Heme groups

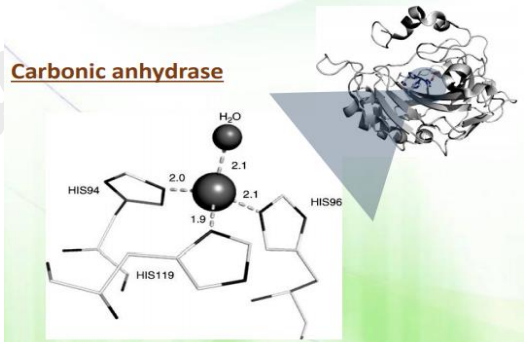
- Iron (Ferrous, Fe^{2+}) in myoglobin:



-as well as hemoglobin-

- Zinc (Zn) in some transcriptional factors

-Noncovalent Interactions:
◊ **Salt Bridges, with the involvement of metal ions:**
- Zinc, Zn in Carbonic Anhydrase:



RECALL:

$$CO_2 + H_2O \xrightarrow[\text{In RBCs}]{\text{carbonic anhydrase}} H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

How Does it Work?
By interacting with H_2O; thus, bringing it closer to the enzyme to react with CO_2 forming H_2CO_3

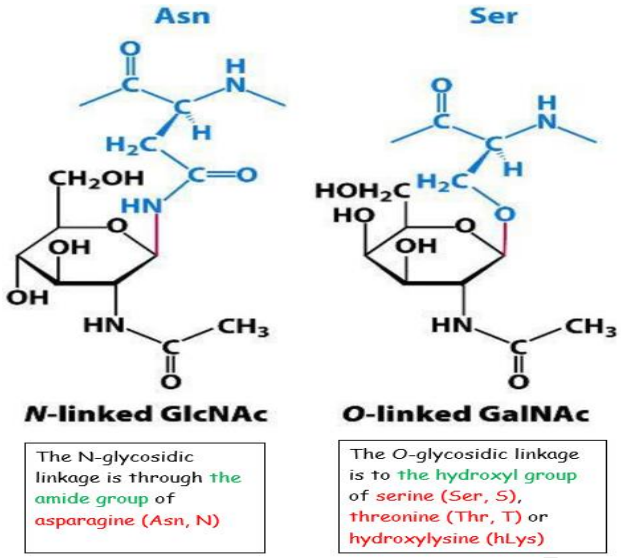
COMPLEX PROTEIN STRUCTURES:
They are proteins linked to a non-protein component (prosthetic group) in order to be active or inactive
Holo- protein is the protein that is connected/linked/conjugated with a prosthetic group
Apo- protein is the protein that is NOT connected/linked/conjugated with a prosthetic group

***Non-Protein Components (Prosthetic Groups):**
Metal ions/ Sugar molecules/ Lipid molecules/ Heme groups

→ Proteins can be either holo- or apo-, but each is functional in only one form

***Proteins that are Functional (Active) in the Holo- Protein Form:**
◊ Glycoproteins:
the sugar component is *covalently linked* to the protein (N-linked glycosylation and O-linked glycosylation for proteins).

RECALL:



i.e. Antibodies
with a sugar component added to the heavy chains

◊ Lipoproteins

◊ Phosphoprotein

◊ Proteins activated via phosphorylation

***Proteins that are Functional (Active) in the Apo- Protein Form:**
◊ Proteins inactivated via phosphorylation

4) Quaternary Structure:
The association of several protein chains or subunits into a closely packed arrangement, where each subunit has its own primary, secondary and tertiary structure.

[This level of protein structure is specific for proteins with more than one polypeptide chain**]**
Proteins that are composed of one subunit are *monomers* and they don't actually have a **quaternary structure**.
→ Proteins made of
Two subunits = dimers
Three subunits = trimers
Four subunits = tetramers
etc.
→ The subunits making a protein may be the same polypeptides (homo-) or different ones (hetero-)

i.e.
A protein with two identical subunits
= homodimer **[The Simplest Protein with Quaternary Structure]**
A protein with four different subunits
= heterotetramer

***How are these Subunits Connected?**
Via *the stabilizing forces* mentioned earlier

NOTE:
-There is no peptide bonds between subunits
-The most common covalent bond between subunits is the <u>disulfide bond</u>

***Examples:**
-Hemoglobin:
No. of subunits = 4
Type of subunits = 2α + 2β
Type of Protein = heterotetramer
Connections between subunits = Noncovalent interactions and disulfide bonds

-Antibodies (Immunoglobulins):
No. of subunits = 4
Type of subunits = 2 light + 2 heavy
Type of Protein = heterotetramer
Connections between subunits = Disulfide bonds

DENATURATION & RENATURATION OF PROTEINS:

◊ Denaturation:

-The process in which the native conformation (3D shape) is disrupted

-How Does it Occur?

Denaturation involves the breaking of the *non-covalent bonds* as well as disruption of *disulfide bridges*; which results in a complete disruption of the tertiary structure

-Why Does it Occur (How Can We Denature Proteins)?

By using denaturing agents such as:

- **Heat**; which can disrupt low-energy *van der Waals forces* in proteins

i.e. the change between the jelly like material that made up the proteins inside an egg to become in the solid state after **heating** the egg

- **Using some chemical detergents like <u>triton x-100</u> (nonionic, uncharged) and <u>sodium dodecyl sulfate</u> (SDS) (anionic, charged):**

Disruption of *hydrophobic interactions*.

NOTE:

If a detergent is charged (like SDS), it can also disrupt *electrostatic interactions* -along with *hydrophobic interactions*-

- **Extreme Changes of pH (Either High or Low):**

Disruption of different types of *noncovalent interactions* specifically the *electrostatic interactions* and *hydrogen bonding*.

WHY?

Because the changes in the protonation and deprotonation particularly for acidic and basic amino acids, so they would accept or donate their protons and become with a different charge than the original one at physiological conditions.

- **Other reagents, such as <u>urea</u> and <u>guanidine hydrochloride</u>:**

Disruption *hydrogen bonding* and *hydrophobic interactions*.

- **Reducing agents such as <u>β-Mercaptoethanol</u> (βME) and <u>dithiothreitol</u> (DTT):**

Reduction of *disulfide bridges* to two sulfhydryl groups.

◊ Renaturation:

-The process in which the native conformation of a protein is re-acquired

-When denaturing conditions are removed like **reducing agents** or exposing proteins to **oxidizing agent,** some of them, specifically those dependent on *disulfide bonding*, can renaturate and their activity is recovered. This process occurs <u>spontaneously</u>

NOTE:

-NOT all proteins are able to be renatured

-For denaturation that happened due to disruption of *non-covalent interaction*, it will be harder to go back , so it is mostly <u>irreversible</u>

***Factors Determining Protein Structure:**

<u>◊ The least amount of energy needed to stabilize the protein</u>. **This is determined by:**

- The amino acid sequence (the primary structure) mainly the internal residues. [The most important factor]
- The proper angles between the amino acids, specifically between R-groups

<u>◊ The different sets of weak non-covalent bonds that form between mainly the R-groups</u>

◊ Non-protein molecules like prosthetic groups of different types, metal ions, heme groups, sugar and lipid components

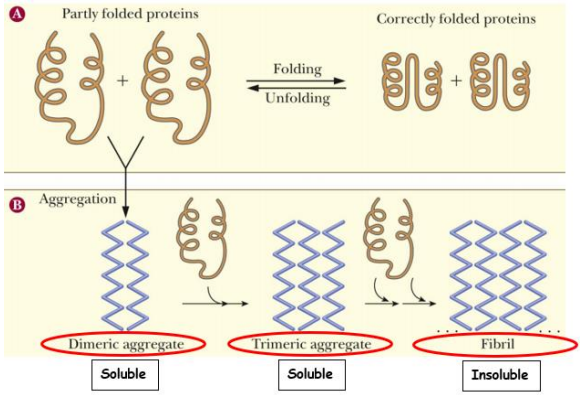
PROTEIN MISFOLDING:

***How Does it Occur?**

The problem starts with a partly folded proteins (= partly unfolded proteins);

If these were exposed to certain conditions, they will be correctly folded; thus, the **hydrophobic regions** are faced inwards from the aqueous environment

If these were NOT exposed to those certain conditions, they will completely misfolded; thus, the **hydrophobic regions** are faced outwards to the aqueous environment, interacting with other hydrophobic regions of other misfolded proteins, forming **aggregates** [a stabilizing pathway not more]



***The Effect of Aggregates:**

- Accumulation; thus, changing the shape of cell, reflecting on its function

[May reach to the point of forming fibril structure or amyloid like what we can see in Alzheimer disease]

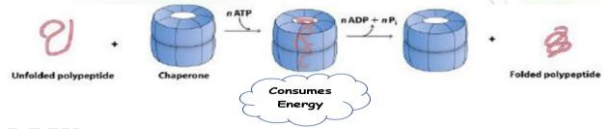
- Affecting the rigidity and bendability of the cell
- They (soluble/insoluble) can be toxic

***How to Solve the Problem of Misfolding and Aggregates?**

-By using chaperones that;

- fold proteins

[while they are being synthesized or after synthesis is done to fold into the proper 3D shape. Even if the proteins failed to fold properly, chaperons may unwind the proteins and refold]



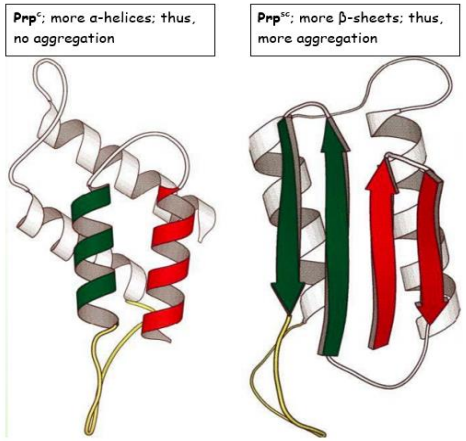
- prevent the hydrophobic regions in newly synthesized protein chains from associating with each other to form protein aggregates
- target misfolded proteins for degradation

***Related Diseases:**

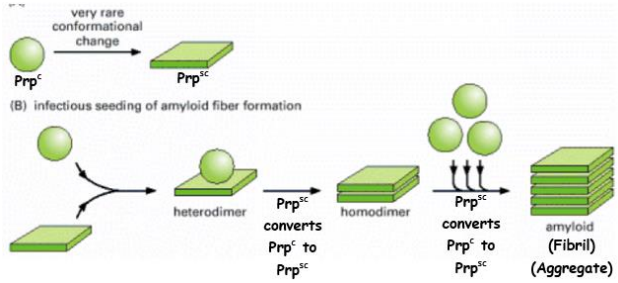
◊ Prion Diseases:

Prion protein (Prp^c) is a protein that is found in the neurons.

As other proteins, **Prp^c** can face misfolding and then called **prion (Prp^{sc})**.



The problem is that: **misfolded Prp (prion, Prp^{sc})** can interact with normal, correctly folded **Prp^c** proteins; converting them to **prions (Prp^{sc})**.



Prion diseases are caused by a transmissible agent. So, there has to be some sort of transfer of the abnormal misfolded prion from a cow to a human, for example.

Then the "infection" between cells starts.

NOTE:
Abnormal protein can be acquired by:
- Infection
- Inheritance
- Spontaneously

The accumulation of **prions (Prp^{sc})** results in a number of diseases referred to as **prion diseases**, such as:
- Mad cow disease (in cow).
- Creutzfeldt-Jakob Disease (CJD) (in humans); it is a form of encephalopathy (problem in the neural system)
- Scrapie (in sheep)

◊ Alzheimer Disease:
-This disease is not transmissible between individuals (It's not an infectious disease)
-Family history may increase the probability of getting this disease
-The age of the onset; the age when we start to notice the symptoms of the disease, might be earlier in some families in which this disease runs

In this disease, there would be accumulation of certain types of aggregates or plaques which are called **(amyloid plaques),** it's not normal for these plaques to be present in the interstitial fluid between neurons.

These plaques are actually made of **amyloid peptides** and also **a protein** called **(tau),** so they start damaging the neurons and this results in **Alzheimer's disease.**

To understand how does this occur, check this video from 1:00 to 2:00
https://www.youtube.com/watch?v=v5gdH_Hydes

STRUCTURE-FUNCTION RELATIONSHIP:

INTRODUCTION:
***Biological Functions of Proteins:**

Protein(s)	Function(s)
Enzymes	Catalysts for reactions; most of the enzymes are proteins depending on their molecular classification
Hemoglobin, Lipoproteins, Channel proteins	Transport different materials
Myosin, Actin, …	Contractile/motion
Collagen, Keratin, Actin, Elastin, …	Structural function
Antibodies	Defense
Hormones -proteins can be hormones-, Receptors, Transducers, Transcription factors, …	Signaling
Diphtheria, Enterotoxins	Toxins

***Classification of Protein *According to* Structure:**
◊ Fibrous; fiber-like with a uniform secondary-structure only), having an elongated structure to perform mechanical and structural functions
i.e. Collagen, Keratin, Actin, Elastin

◊ Globular; globe-like with three-dimensional compact structures, more compact in comparison with fibrous proteins
i.e. Myoglobin, Hemoglobin, Immunoglobulin

FIBROUS PROTEINS:
-Abundant in the ECM
-Examples: Collagen, Fibronectins, Integrins

***Collagen:**
◊ Introduction:
-A family of fibrous proteins with 25 different types found in all multicellular animals.

They are named as type I collagen, type II collagen, type III collagen, and so on **-They are** the most abundant proteins in mammals, **constituting** 25% of the total protein mass in these animals

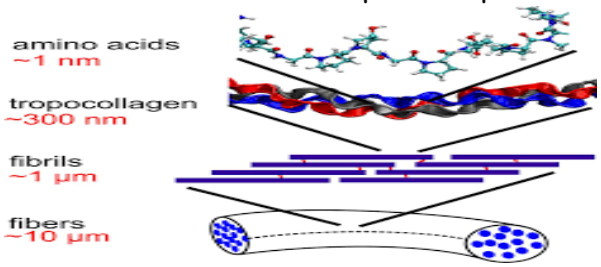
-Functions to provide structural support to tissues

[The primary feature of a typical collagen molecule is its stiffness**]**

◊ Structure of Collagen:

-This basic unit of collagen is called tropocollagen, **and it is made of** 3 helices; thus, **collagen has** a quaternary structure

- It is a left-handed, triple-stranded, helical protein, in which three collagen polypeptide chains, **called** a-chains; wound around one another in a ropelike superhelix



- Compared to the a-helix, the collagen helix is much more extended with 3.3 residues (amino acids) per turn, and that is why it's more extended and needs more space to complete each turn

◊ Composition of Collagen:

-Glycine (33%) + Proline (13%) + Hydroxyproline (9%) + Hydroxylysine

NOTE:

Hydroxy- proline/lysine is a *post-transitional modification* by hydroxylation of proline (on C4) /lysine (on C5)

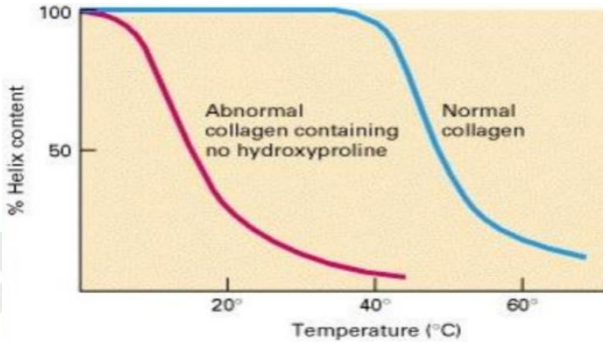
-Every third residue is **glycine**, with the preceding residue being **proline** or **hydroxyproline** in a repetitive fashion as follows:

◊ Functional Purposes of Amino Acids:

-The presence of large percentage of amino acid glycine allows the three helical a-chains to pack tightly together and increase its ability to compact because this amino acid is small in size

-Proline with the ring structure inside creates the kinks and bends the structure, so it stabilizes the helical conformation in each a-chain

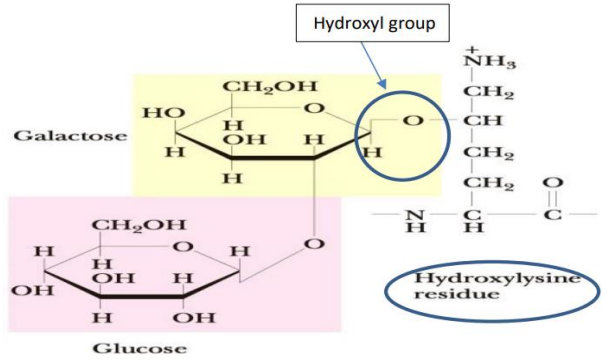
-Hydroxyproline increases the ability of these **prolines** to form *hydrogen bonding*



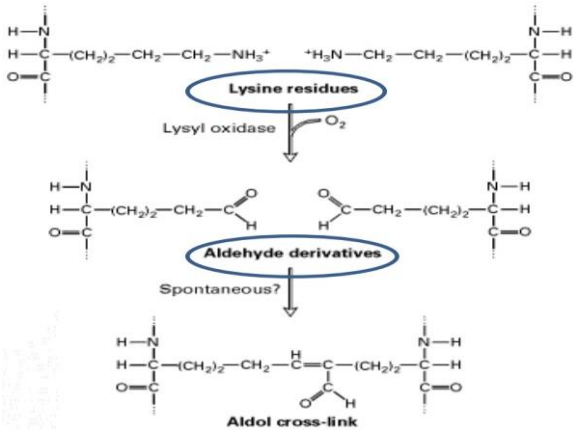
-The normal collagen is stable even at 40°C

-The abnormal collagen helix is unstable and loses most of its helical content at temperature above 20°C

-Hydroxylysine serves as attachment sites of polysaccharides making collagen a glycoprotein



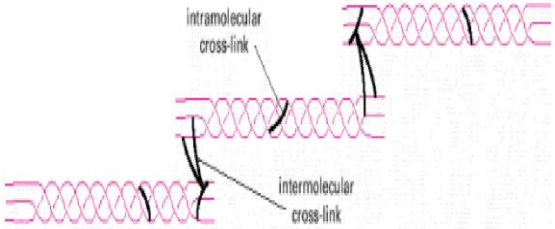
-The presence of allysine (oxidized lysine) along with other allysine, hydroxylysine or lysine molecules stabilize and strengthen collagen due to *covalent aldol cross-links*



•**Cross-links can be:**

Intramolecular; within the same tropocollagen unit

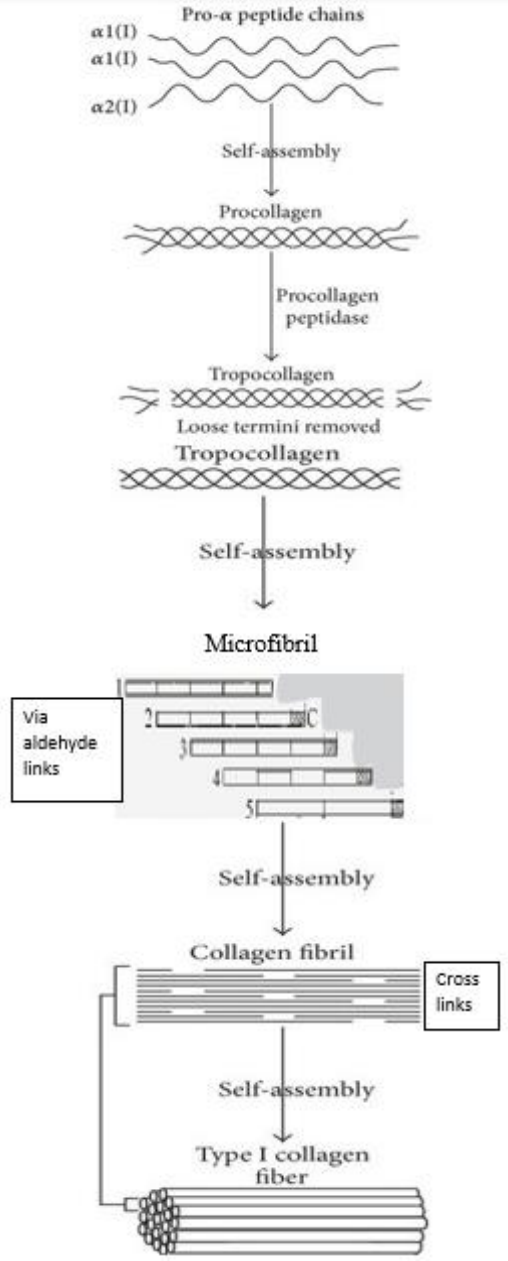
Intermolecular; between one tropocollagen and another



These cross-links:

- stabilize the side-by-side packing of collagen molecules
- generate a strong fibril
- increases with aging
- **If they are inhibited**, the tensile strength of the fibrils is drastically reduced; collagenous tissues become fragile, and structures such as skin, tendons, and blood vessels tend to tear

◊ Formation of Collagen Fibers:



◊ Related Medical Conditions; Scurvy:

a disease that occurs most often in sailors **caused by** a dietary deficiency of ascorbic acid (vitamin C)

Why?

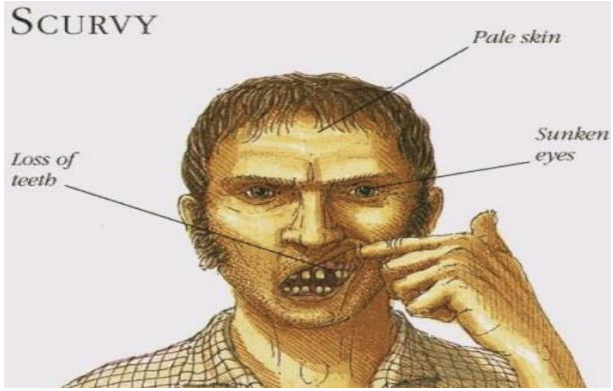
Because they spend a long time on ships, so they don't have the chance to eat fresh fruits rich in vitamin C

RECALL:

The Importance of Vitamin C:

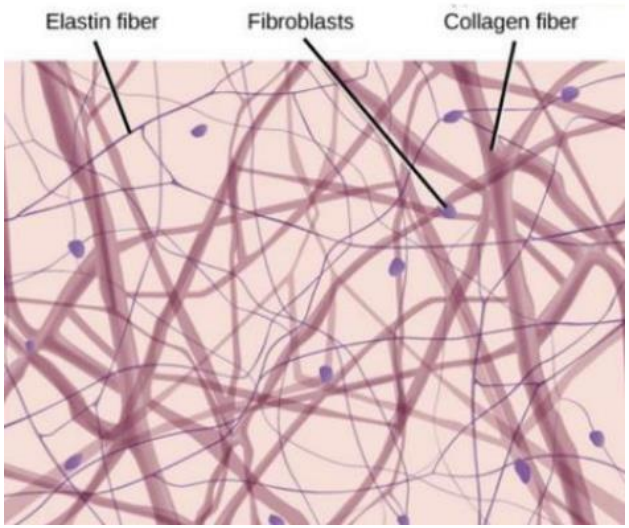
- in the immune system
- acts as an antioxidant
- involved in the hydroxylation of proline in collagen

Thus; less hydrogen → low/reduced mechanical properties of collagen as a protein, which then gets degraded easily, so the patient will suffer from tears in their blood vessels and gums; therefore, patients start to lose their teeth



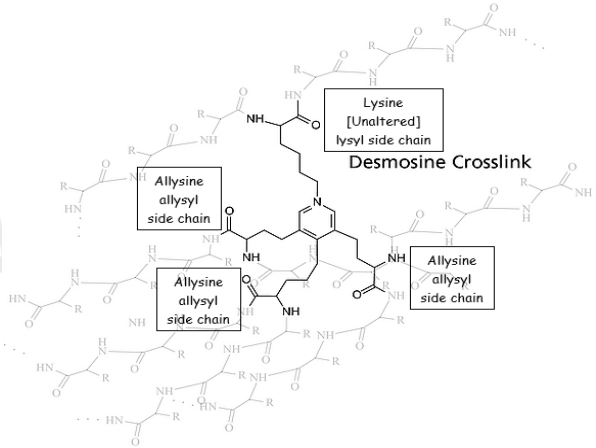
NOTE:
The defective pro-α chains fail to form a stable triple helix and are immediately degraded within the cell

***Elastin:**
Elastin fibers have *elasticity* and *resilience*, so they can come back to their normal shape and size after a transient stretching.
These fibers are important for many tissues, **i.e.** skin, blood vessels and lungs



NOTE:
Long, inelastic collagen fibrils are interwoven with the elastic fibers. Why?
to limit the extent of stretching and prevent the tissue from tearing

◊ Structure of Elastin:
-Hydrophobic segments; which are rich mainly with **proline** and **glycine.**
These are responsible for the *elastic properties.* **HOW?**
Glycine [small in size] → can be compacted easily
Proline [has the ring structure inside it] → that bends or introduces kinks into the structure of **proline**, and this makes the curving of this molecule easier and makes the molecule more elastic
-Alanine and Lysine rich α-helical segments; which form cross-links between adjacent molecules of **elastin** which contributes to the *mechanical properties.*



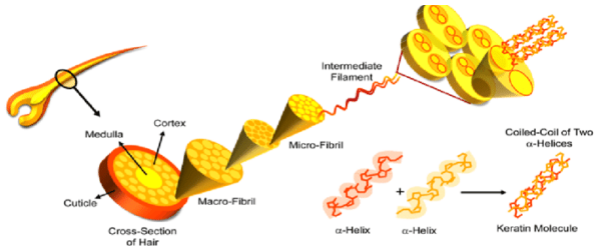
-Some **hydroxyproline** but less than that present in **collagen,** and NO **hydroxylysine**; thus, no glycosylation

NOTE:
The basic unit (primary component) = Tropoelastin

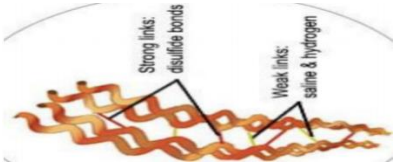
***Keratin:**
-It is known to be involved in making some types of *intermediate filaments*
-There are two types of Keratins:
α keratins and β keratins [with similar amino acid sequences and biological function]

NOTE:
α-Keratin is the major protein of hair and fingernails as well as animal skin.
It has a high content of **cysteine** amino acids that contain **thiol groups**, which means that there are *disulfide bridges.*

◊ α-Keratin Structure in Hair:
Helical α-keratin molecule (x)
2x [dimer] = protofilament
4x [tetramer] = protofibril
32x [8 tetramer] = microfibril
hundreds of microfibrils = microfibril



◊ α-Keratin Structure in Hair:
Similar to that in hair, but with *disulfide bridges*.



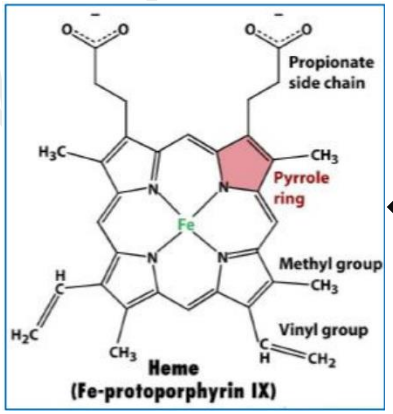
◊ An Application Upon Keratins:
Temporary wave (related to having a shower):
When hair gets wet, water molecules disrupt some of the *hydrogen bonds* between **keratin** molecules, so new *hydrogen bonds* between **water** and **keratin** form, which help to keep the alpha helices aligned, that is why the hair is a bit straight when its wet. Then when hair dries up, the hair strands are able to maintain the new curl in the hair for a short time.

Permanent wave:
In this type the hairdresser:
1) uses a reducing substance (usually **ammonium thioglycolate**) to reduce some of the disulfide cross-links. So now the cystine amino acids have their SH- group reduced
2) Then the hair is put on rollers or curlers to shift positions of alpha-helices, during this process the hair is shifting from straight to curly
3) After we reach the final look of curls, an oxidizing agent (usually **hydrogen peroxide**) is added to reform and stabilize the disulfide bonds in the new positions of the alpha-helices until the hair grows out

NOTE:
Those curls don't stay forever, only for 6 months to a year

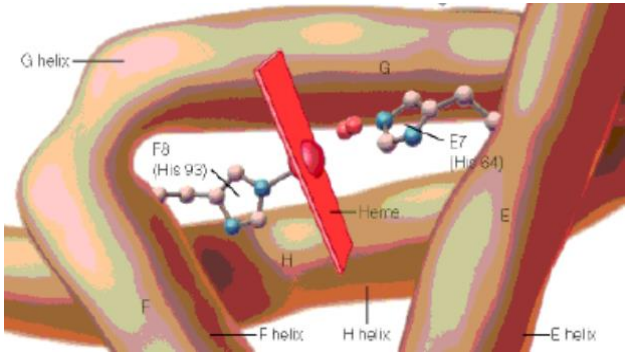
GLOBULAR PROTEINS:
i.e. Myoglobin, Hemoglobin, Cytochrome P450, …

***Introduction:**
We're going to discuss some *heme proteins*, and **heme is** a prosthetic group that belongs to **perfurins family** [made of 4 rings, each is called a **pyrrole ring**], so it is a tetrapyral ring, on each ring; there're two side chains, in addition to the presence of **ferrous ion** in the middle



-Ring
-Mostly hydro-phobic
-Planar
-Rigid

NOTE:
◊ For Iron (Fe) within the heme group;
-It has to be *ferrous* -with +2 charge-, it can't be ferric -with +3 charge-. Once it becomes oxidized to Fe^{3+}, that's going to make the same group incapable to bind to oxygen, and the heme group becomes **hemen**
-It can form 6 bonds; 4 with the N atoms within the pyrrole rings + 1 with Oxygen + 1 with the **proximal histidine** (F8, His 93)



◊ The distal histidine (E7, His 64):
-A gate that allows the entry of oxygen molecules and regulates the binding to the heme group
-When O_2 binds to Fe, His-E7 stabilizes this binding with an angle of 120° instead of 90° (perpendicular) to the heme molecule

A Quick Clarification:
F8 = α-helix number 6 [F = 6] / on the 8th histidine
E7 = α-helix number 5 [E = 5] / on the 7th histidine

◊ **Surrounding the heme group itself,** there're only hydrophobic amino acids with the exception for the two histidine molecules (basic)

◊ **The presence or location of the heme group inside the protein** to maintain the hydrophobic environment and a non-oxidizing environment so we would keep the ferrous ion in its state

***Myoglobulin:**

◊ **Structure of Myoglobin:**

-*A* monomeric protein that is made of one polypeptide chain

-Mainly found in muscle tissue

-It includes a prosthetic group, the heme group [1 O_2 per time]

-It can be present in two forms: Oxymyoglobin (oxygen-bound) & Deoxy myoglobin (oxygen-free)

-No **quaternary structure for myoglobin**

-The tertiary structure of myoglobin is 8 a-helices, designated A to H, that are connected by short non-helical regions; turns and loops

-The chain in myoglobin has hydrophobic (nonpolar) amino acids on the inside -with the exception for the two histidine molecules (basic)- and hydrophilic (polar) amino acids on the outside

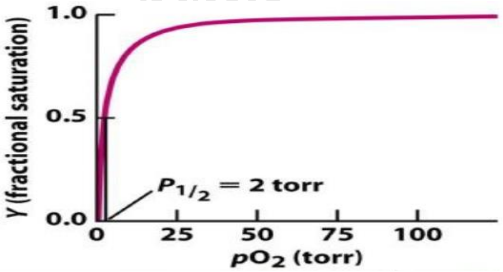
◊ **Functions of Myoglobin:**

• Storing oxygen in muscles. Once the cells need them, they can be released and used in the combustion reaction that generate energy [An Oxygen Sensor]

◊ **Oxygen Binding to Myoglobin:**

The saturation curve is considered as a *hyperbolic* saturation curve, just a very low concentration of O_2 is going to be sensed by myoglobin and the binding of this oxygen is going to be accelerated or it's going to be very steep so a very high percentage or fractional saturation of myoglobin is achieved with a very low pressure of O_2

This indicates the high affinity of myoglobin to O_2



Depending on this curve;

2 mmHg → 50% saturated myoglobin

In our tissues, 20 - 30 mmHg (What do you think 😐?) → almost 100% saturated myoglobin

***Hemoglobin:**

◊ **Structure of Myoglobin:**

-*A* tetrameric protein that is made of four polypeptide chains

-Mainly found in blood stream

-It includes a prosthetic group, the heme group in each subunit; thus, 4 heme groups [4 O_2 per time]

-The quaternary structure of hemoglobin is 4 subunits, 2α + 2β

-The tertiary structure of the α subunit is 7 α-helices, **while for the β subunit is** 8 α-helices [just similar to myoglobin chain]

-Each chain in hemoglobin has hydrophobic (nonpolar) amino acids on the inside -with the exception for the two histidine molecules (basic)- and hydrophobic (nonpolar) amino acids on the outside

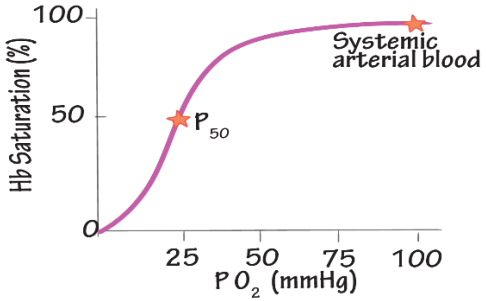
Thus, *hydrophobic interactions* between the chains occur. In addition, there're *electrostatic interactions (salt bridges)* and *hydrogen bonding* between them

◊ **Functions of Hemoglobin:**

• Transport of O_2 and CO_2; transporting oxygens in lungs to these cells and taking back CO_2 from cells back to lungs)

• Blood buffering; it's also responsible for buffering blood through the histidine amino acid present in the structure of this protein

◊ **Oxygen Binding to Myoglobin:**



NOTE: *A sigmoidal curve*

Depending on this curve;
26 mmHg → 50% saturated hemoglobin
In our lungs, 100 mmHg → 95-98% saturated hemoglobin

NOTE: 1 mmHg = 1 torr
→ **Saturation Curve; Myoglobin vs. Hemoglobin:**

	Myoglobin	**Hemoglobin**
Description	Hyperbolic	Sigmoidal
P50 ($P_{\frac{1}{2}}$)	~2 torr	~26 torr
~P100 [~Complete Saturation]	20 – 30 torr	100 torr
Affinity to O_2	High; either at low or high pressure	Low; at low pressure High; at high pressure

NOTE:
A sudden decrease or reduction in the pulmonary capillary oxygen tension so that would not be reflected as a big change in the saturation [important at high altitudes]
But this would work until certain limit but whenever we have a very low O_2 pressure; which is important to be able to release this O_2 at the peripheral tissues

◊ **Hemoglobin is an Allosteric Protein:**
Allosteric means that this protein once bound to one molecule or one ligand, O_2 molecule to one of the chains in this case, this is going to encourage and affect the binding of the second, third and fourth molecules of O_2. **This is called** positive cooperativity

◊ **R – T Conformations:**
α1 + β1 = αβ dimer 1
α2 + β2 = αβ dimer 2
The type of interactions between α subunit and β subunit in the same dimer = Hydrophobic interactions
The type of interactions between dimer 1 and dimer 2 = Hydrophobic interactions + Ionic (Electrostatic) interactions + Hydrogen bonds

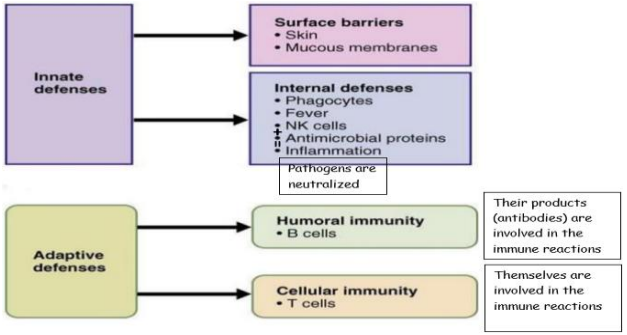
More Ionic interactions + H-bonds = شدّ
[T state (conformation) Taut]
Less Ionic interactions + H-bonds = ارتخاء
[R state (conformation) Relaxed]

NOTE:
The tertiary structure of the subunits change

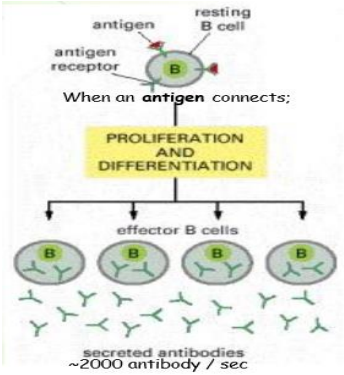
T = Deoxy-hemoglobin (O_2 unbound)
R = Oxy-hemoglobin (O_2 bound)

WHY?
Because the presence of O_2 bound to the Fe in the heme group reduces *ionic interactions & H-bonds*; since a dome-like structure (Fe is upper from the planar heme level) appears in the deoxy- form, and disappear [completely planar heme] in the oxy- form
https://www.youtube.com/watch?v=jVUwn4wWTXI

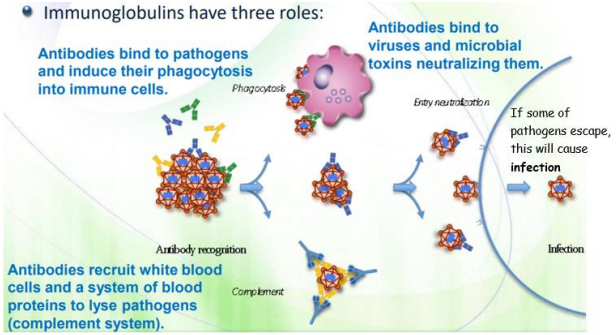
IMMUNOGLOBULINS:
RECALL: Types of Immunity:



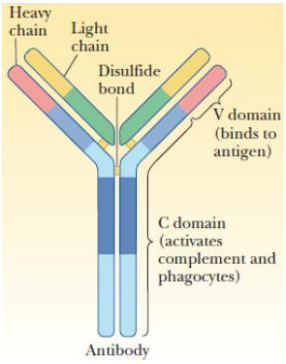
*How Do B-Cells Work?



*How Do Antibodies (Immunoglobulins) Produced from B-Cells Work?



*General Structure of an Antibody:



-Y-shaped molecules consisting of two identical heavy chains and two identical light chains

-*Disulfide bonds*, which are covalent bonds, hold the chains together (inter-chain). Furthermore, they're present within the same chain (intra-chain). Thus, they maintain the quaternary structure of the protein

-There's an attachment site here for sugars, so antibodies are **glycoproteins**

-They are soluble and aren't a part of the membrane

*Regions of an Antibody:

Keywords:

L: Light chain/ **H**: Heavy chain

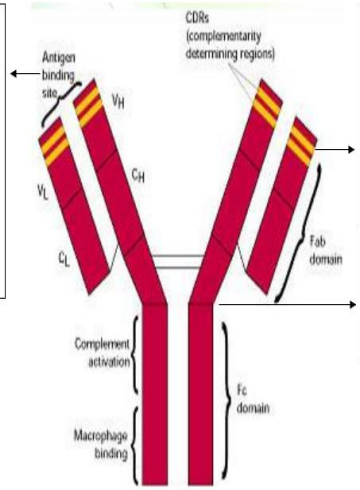
V: Variable/ **C**: Constant

Each Light chain = 1 VL + 1 CL

Each Heavy chain = 1 VH + 3 CH

-Within the variable region
-Epitope binding site
-About 7-12 amino acids contribute to this site

NOTE:
Each type of B cell produces only **one type of antibodies,** and each **antibody** can bind **two types of antigens**



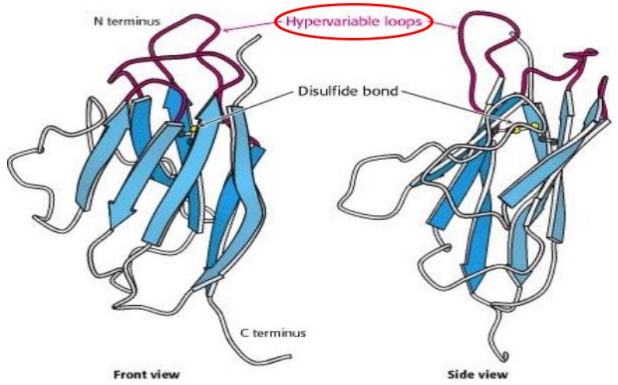
-Within the variable region
-Called the **hypervariable region**
-They bind specifically to antigen that is specific and have high affinity; $K_d = 10^{-12} - 10^{-7}$, which is relatively low, so the binding is strong

-Called the **hypervariable region**
-It's made of flexible molecules, so that allows the bend of that molecule easily

NOTE:

Regarding the structure of the **hypervariable regions;** these are actually represent **loops** exist as a **specialized domain** (*super secondary structure*) called **an Immunoglobulin fold,** which is a motif that is present in every immunoglobulin. They are presented in every Immunoglobulin.

The hypervariable regions are specifically in **three loops** connecting the **β sheets** to each other



***Diversity of Antibodies:**

-Antigen-antibody binding is mediated by *noncovalent interactions*, that is relatively weak, so there must be a sort of specificity

-The enormous diversity of antigen-binding sites can be generated by changing only the lengths and amino acid sequences of the hypervariable loops

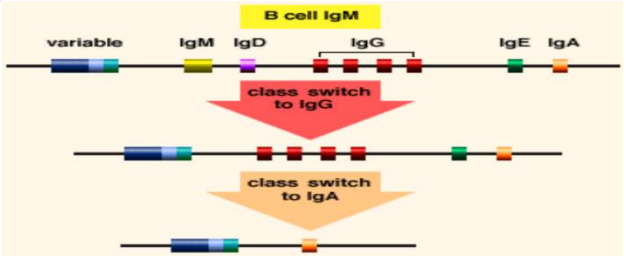
-Each individual is capable of producing more than 1011 different antibody molecules. **This is done via:**

- DNA rearrangement of the different genes
- Imprecise joining of different regions within the genes
- Addition/deletion of nucleotides during rearrangement and that also change the whole product of that gene
- Somatic hypermutation in which some nucleotides even if it is just one nucleotide is change within the DNA and this would result in kind of changes in the messenger RNA and then changing in the resulting products

***Class Switching:**

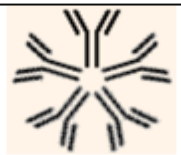
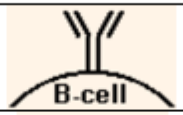
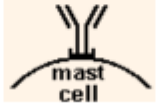
B cells that is in a resting state, before binding to any antigen, contain IgM type of antibodies only.

Then, once an antigen binds, it gets activated and **class switching** occurs [DNA rearrangement as a mechanism is involved and it's going to change the heavy chain constant region and this would result a production of other types of antibodies like IgG, IgA and IgE]



https://www.youtube.com/watch?v=gyTHXjVUPWw

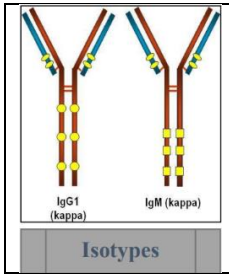
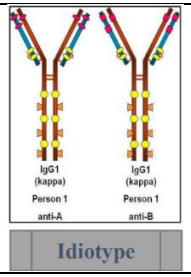
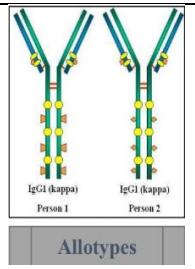
***Immunoglobulins Isotypes:**

Isotype	Structure	H-Chains	What Does it Do?	Notes
IgM	 Pentamer	μ (mu)	Promotes phagocytosis and activate the complement system that leads to cell killing	-The most important one **-Expressed** on the surface of inactive B-cells **-Found primarily in** plasma cells -The first antibodies produced in significant quantities against an antigen
IgG	 Monomer	γ (gamma)	Promotes phagocytosis and activate the complement system	-Most abundant immunoglobulins in sera (600-1800 mg/dL) -Only type of antibodies that can cross the placenta -Not associated with the membrane
IgD		Δ (delta)		**- Present on** surface of B-cell that have not been exposed to antigens
IgE	 Monomer	ϵ (epsilon)	Plays an important role in allergic reactions	
IgA	 Dimer	α (alpha)		**-Found mainly in** mucosal secretion -The initial defense in mucosa against pathogen agents

NOTE:

There are two "light" chains (lambda and kappa). On the other hand, there are five "heavy" chains (alpha, delta, gamma, epsilon and mu) that make five types (isotopes) of immunoglobulins known as immunoglobulins isotype (IgA, IgD, IgG, IgE, IgM)

***Isotypes vs. Idiotypes vs. Allotypes:**

	Immunoglobulins with changes, modifications or class switching in the constant region of the heavy chains		Immunoglobulins with different variable domains of both their light (VL) chains and heavy (VH) chains [or one of them]		Immunoglobulins belong to the same class but different among individuals of the same species due to different genetics

***Hybridoma and Monoclonal Antibodies:**

mono- أحادي

clonal مجموعة

antibodies أجسام مضادّة

إذن؛ مجموعة من الأجسام المضادّة أحادية الهدف. بالتالي، تمتاز بتمييز عالٍ جدًّا لنوع محدّد من الأجسام الغريبة

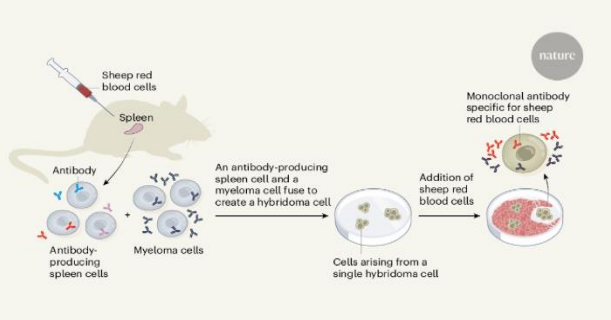
◊ How Can We Produce Monoclonal Antibodies?

1) Isolating B cells that produces our aimed antibody

By injecting the specific antibody's antigen in a mouse for example, and collect those B cells that produce the wanted antibody

2) Amplifying these B cells

First, by creating **immortal B cells**, which we can get from **cancer cells** [cancer cells are immortal]. Then, hybridizing of a **B cell** with **myeloma cell;** which is a blood cancer type, so once they hybrid with each other, the produce cells with the hybrid cell called **hybridoma cells** which are immortal ones. **This hybridoma (immortal) cells** can produce single/specific (monoclonal) antibodies in large numbers



NOTE:

To be able to use these antibodies in human beings, we need them to be humanized for different reasons. then, they can be humanized by attaching the CDRs (because they are the important parts that bind to the antigen) into appropriate sites in a human immunoglobulin molecule

◊ Benefits of Monoclonal Antibodies:

- Uses can be seen in the medical labs, to measure the amounts (concentrations) of many individual proteins and molecules (e.g. plasma proteins, steroid hormones), present in different ceps and they are going to bind to these molecules and induce certain changes (such as color change), indicating the concentration of these molecules in the patient's sample
- They determine the nature of infectious agents (e.g. types of bacteria) so we can determine the type of bacteria that infects that person by identifying certain antigen by these monoclonal antibodies in the lab
- They are used to direct therapeutic agents to tumor cells [cancer treatment, in immunotherapy instead of chemotherapy or radiation, by monoclonal antibodies]
- They are used to accelerate removal of drugs from the circulation when they reach toxic levels; these monoclonal antibodies can bind to these toxic drugs in the blood and damage them and accelerate their removal out of the patient's blood

Done by: Abdullah Al-Jaouni