Biochemistry

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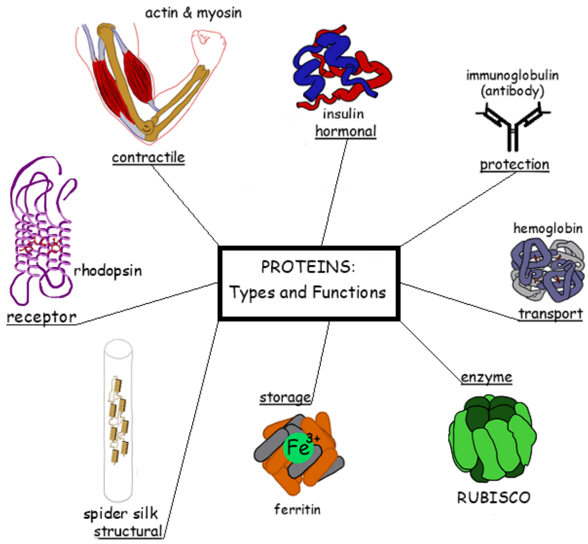
Fibrous Proteins

Structure-function relationship

-<u>structure</u> of biomolecules (carbs, lipids, proteins) correlates with their <u>function</u>.

-different functions of proteins imply that they have different amino acids in their structure. So, amino acids determine the structure of proteins and consequently their function.
In this lecture you need to be able to link structure with function as in why a protein would have a certain amino acid at a particular position, and what would happen if the amino acid changes to a different one.

Biological Functions of Proteins



- **Enzymes** catalysts for reactions; speed up reactions.
- **Transport** molecules.
  Examples:
  A. Hemoglobin; transports molecules like O_2 in and out of the blood.
  B. Channel proteins; transport molecules in and out of the cells, or inside the cells from one region to another.
- **Contractile**/motion.
  Example: myosin, actin that play an important role in muscle contraction.
- **Structural.** (the topic of this sheet)
  Example: actin—that maintain the structure of cells.
  collagen, and keratin—that maintain the structure of a tissue, an organ, or a whole organism.
  -collagen maintains the structure of bones for example.
- **Defense**—antibodies AKA immunoglobulins.
- **Signaling**— transmitting signals from outside of cells to their inside.
  Example: hormones, receptors.
- **Toxins**—diphtheria toxin (الدفتيريا), enterotoxins—toxin that targets the intestines.

Types of Proteins 1.fibrous. 2.globular.

Proteins can be divided into two groups <u>according to structure</u>:

- fibrous (fiber-like elongated proteins with a uniform secondary-structure only, just like collagen has a continuous helical formation).

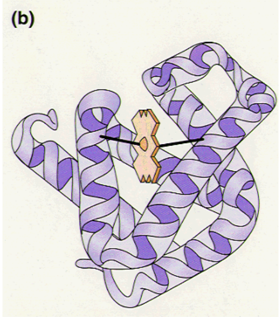
  Function: structural proteins.

  Examples on fibrous proteins: collagens, actin, elastin, and keratins.

Collagen, a fibrous protein

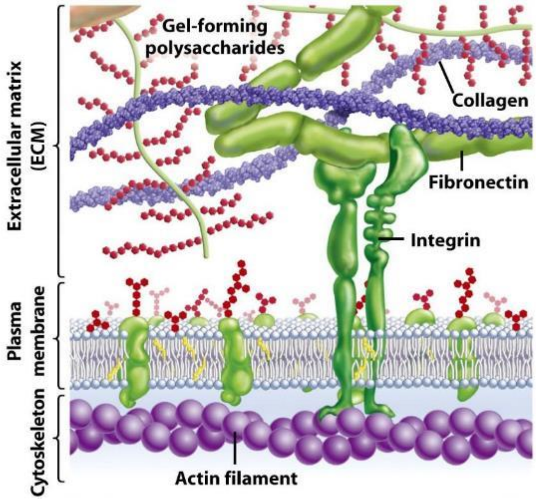
- globular:
  (Globe-like rounded proteins with three-dimensional compact structures, overall composed of several secondary structures—a combination of different alpha helices interrupted by turns or it could be several alpha helices with a number of beta strands).

  Examples: myoglobin, hemoglobin, immunoglobulin, as well as enzymes.



Myoglobin, a globular protein

Fibrous Proteins in the ECM



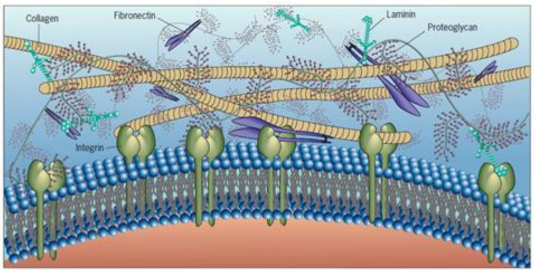
The extracellular space is largely filled by an intricate (complex) network of macromolecules including extracellular proteins and polysaccharides that assemble into an organized meshwork in close association with cell surface.
the matrix functions as a reservoir for nutrients, ions, hormones.

extracellular proteins in the matrix are fibrous proteins (elongated with continuous alpha helices as their secondary structure).
Examples: Collagen, elastin, fibronectin, laminin.

Function: connect cells together, maintain the organization of cells in a tissue or organ and the structure/shape of a tissue, transmit signals inside and outside of cells and allow cells to sense any change in the outer environment because these proteins are connected to the cell surface receptors, for example actin filaments are connected to ECMs' fibronectin via the surface receptor integrin. (see figure above)

collagens

as a matrix protein it exists as a long, thick protein but it has different shapes as well.



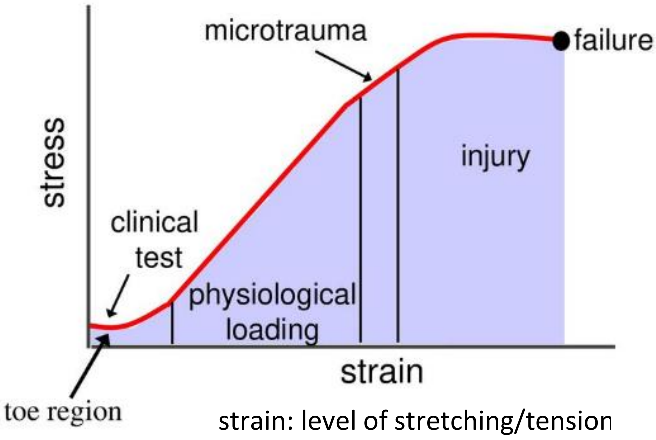
typical structure of collagen: elongated and thick

- Collagens are a family of fibrous proteins of 40 different types found in all multicellular animals.
- Most **abundant** proteins in mammals (constitute 25% of the total protein mass).
- Named as type I collagen, type II collagen, type III collagen, and so on.
- Main function (structural): structural support to tissues.

As a result of its function, collagens' primary feature is that they are stiff, tuff and rigid to provide strength against tension (tensile strength).

increased strain on a tissue subsequently increases stress on it, normally a tissue would go through several stresses, the strain causes physiological loading. if u press on your skin for example, that increases strain, but nothing really happens to that skin then.

BUT if you stretch it too much, that will cause microtrauma, an injury u cannot notice but can be seen under the microscope.
and if the stress continues on the tissue, an observed injury is reached in which the tissue would not be able to withstand the tension or stretching (failure).



strain: level of stretching/tension

Structure

collagen support tissues and it is able to do that because of it's structure.

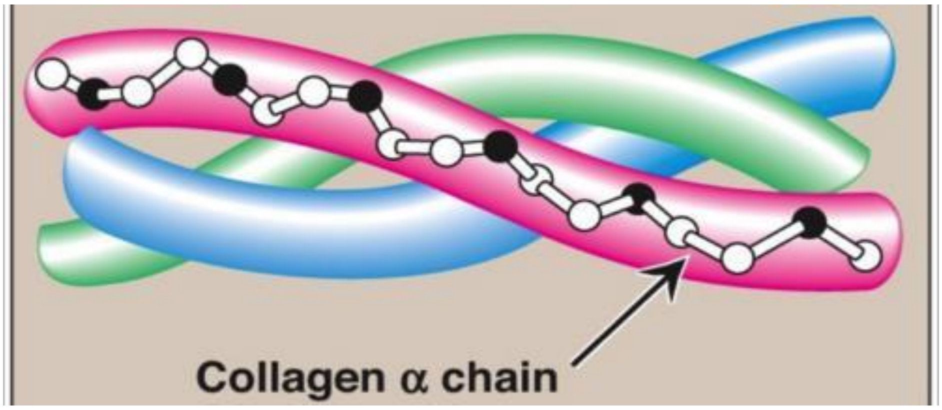
structure of collagen:

- It is a triple-stranded, helical protein—collagen is composed of three strands that intertwine around each other in a helical formation.
- the three collagen polypeptide chains are called α collagen chains, wound around one another in a ropelike superhelix.

there are different types of alpha chains: alpha-1 chains, alpha-2 chains and so on...

a collagen molecule could have different combinations of alpha chains, forming different types of collagen molecules.
For example, a collagen molecule could be made of three alpha-1 chains or two alpha-1 chains along with an alpha-2 chain.

- This basic unit of a collagen molecule is called tropocollagen—a unit composed of 3 alpha collagen chains.
- Compared to the α -helix, the collagen helix is much more extended with 3.3 residues per turn.
  Elaboration: α collagen chain has a different conformation from the typical α -helix, in which the helical structure of collagen chain is more extended so there are less amino acids per full turn in comparison with the α -helix.

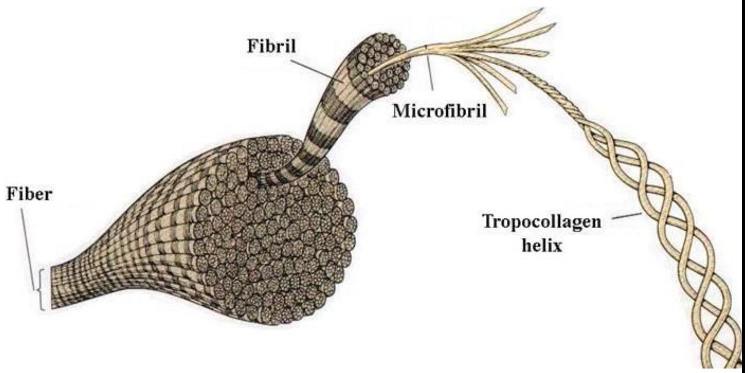


Formation of collagen fibers

- Following cellular release of tropocollagen, 5 of them polymerize into a microfibril, in which they connect via aldehyde links.
- Microfibrils align with each other forming larger collagen fibrils—a structure that can be observed under the microscope.
- Fibrils assemble into collagen fibers.

Tropocollagen, microfibril, fibril, and fibers are covalently crossed linked with each other via lysine residues.

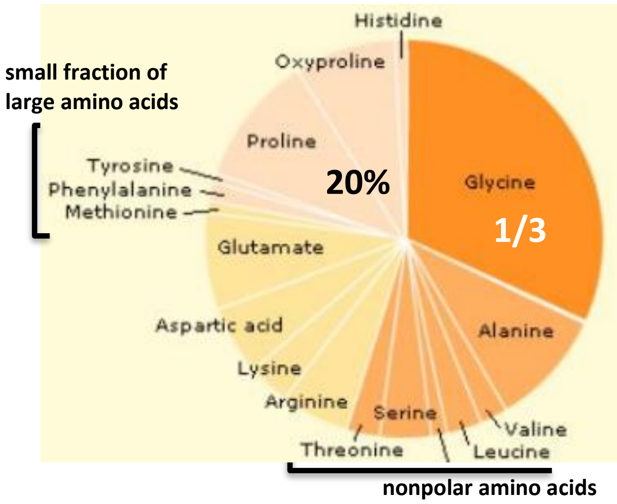
Order of complexity:
Tropocollagen→ microfibril→ fibril→fibers.



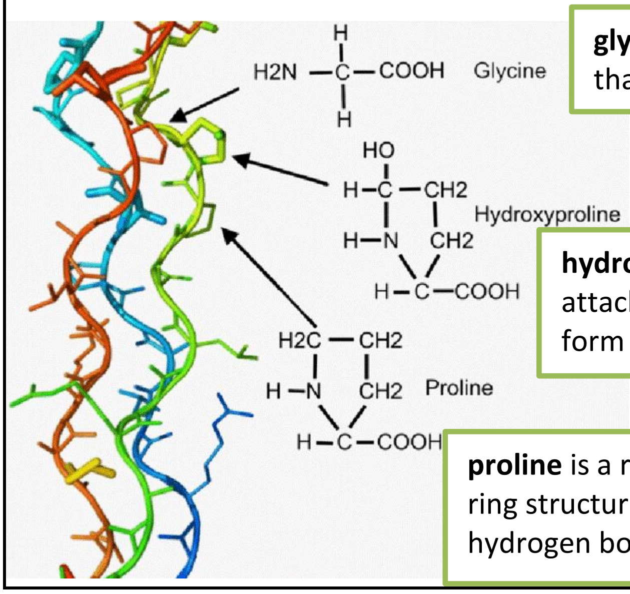
Composition of collagens amino acids that determine the structure of collagen

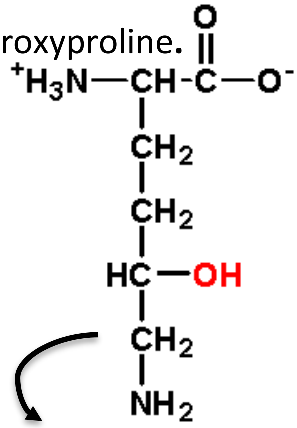
the amino acids that mainly make up the primary structure collagen are hydrophobic and non-polar.

1.**glycine** (33%).
2. **proline** (13%) + (9%) hydroxyproline—modified proline with additional hydroxyl group, so approximately (20%) proline in total.
3.hydroxylysine.
4. non-polar amino acids.
5.large amino acids (e.g. tyrosine, phenylalanine, methionine).
but tryptophane –the largest amino acid isn't present in the structure for a reason.



- A characteristic of Collagens is that they are glycine rich (33%).
  1/3 of all amino acids in collagen is basically glycine.
- Primary structure of collagen: Gly X-Y
  X & Y could be any amino acid, but **X** is often proline and **Y** is often hydroxyproline.





glycine is the smallest amino acid that has H as R-group

hydroxyproline has a hydroxyl group attached to the R-group, which allows it to form hydrogen bonds between strands.

proline is a rigid molecule that forms a ring structure and cannot form hydrogen bonds at the alpha amino.

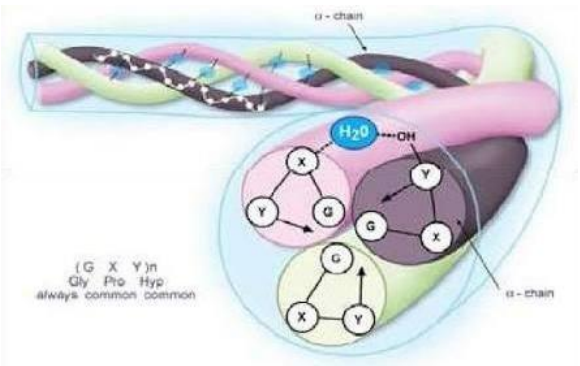
hydroxylysine; lysine + additional hydroxyl group that acts as a reactive group and allow molecules to link to it.

Functional purpose of amino acids why is collagen mainly composed of glycine and proline?

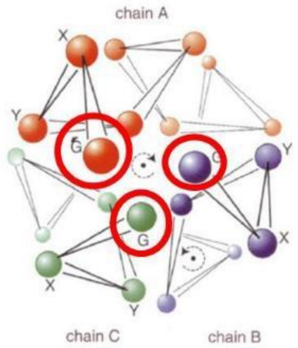
Glycine

- Glycine allows the three helical chains to **pack tightly** together to form the final collagen superhelix and provides **flexibility** to the molecule.

glycine is the smallest amino acid, located at the center of a collagen molecule and plays a role in reducing repulsion between R-groups which allows alpha collagen chains to rotate freely and intertwine tightly forming the helical structure just like threads of a rope would wrap around each other.



notice how the glycine residues are embedded in the center of the collagen molecule



Top view of the collagen molecule: glycine residues are embedded in the center.

Glycine's ability to rotate freely also allows the collagen molecule to be flexible.

Proline

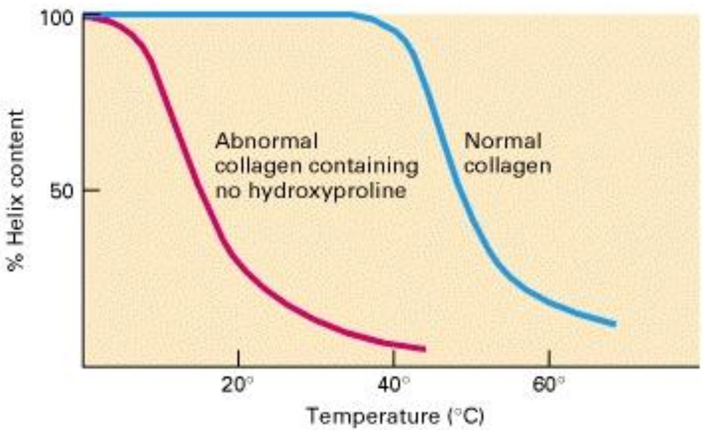
- Proline creates kinks (انحاءات), stabilizes the helical conformation in each chain, and provides rigidity to collagens since it's a cyclic rigid molecule.

proline isn't favorable to be present at α -helices because it tends to break the helical structure, but in a collagen molecule proline creates kinks which allows the three strands to rotate and bend a little, so in a collagen strand there would be a stretch of amino acids interrupted by proline where a kink would be present followed by another stretch then proline followed by a kink and so on which finally forms the helical formation of collagen.

Hydroxyproline

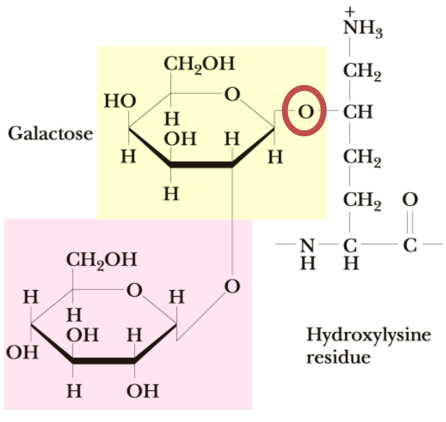
- the presence of OH group in hydroxyproline causes the formation of hydrogen bonds between the 3 collagen strands.
  →this results in getting the strands closer to each other in addition to their tight packing because of glycine.
- Normal collagen is stable even at 40°C.
- Without hydrogen bonds between hydroxyproline residues, the collagen helix is unstable and loses most of its helical content at temperatures above 20°C.

the significance of hydroxyproline is illustrated by the following figure:

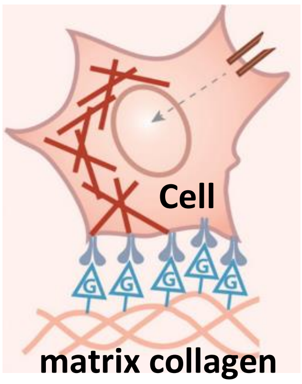


A comparison between a collagen molecule that has normal helical content vs. a collagen molecule that lacks hydroxyproline at different temperatures:
A normal collagen molecule would start to deteriorate where it starts to lose its helical formation at high temperatures.
in contrast a collagen molecule that lacks hydroxyproline would starts to disrupt at normal physiological temperatures as it deteriorates and loses its helical formation.

Hydroxylysine



- Hydroxylysine serves as an attachment site of sugar residues (like galactose and glucose) or polysaccharides at the additional OH group site making collagen a glycoprotein.



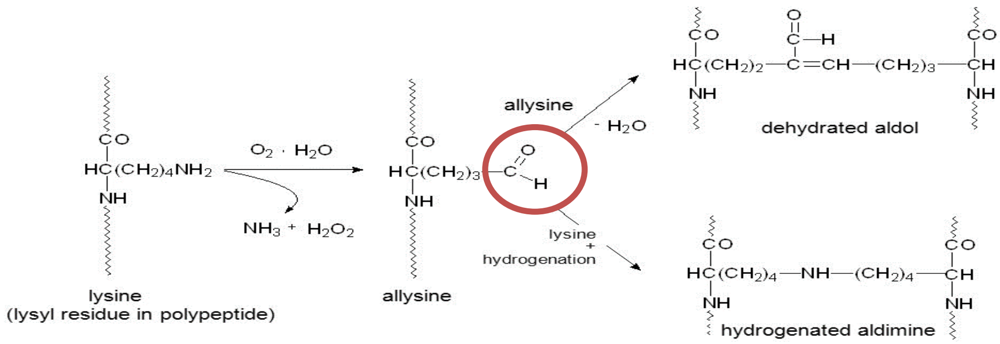
- Sugars allow collagen to recognize and interact with cell surface receptors, as sugars provide linkage points between matrix collagen molecules and cells.

amino acids are also involved in these interactions along with sugars.

Oxidation of lysine

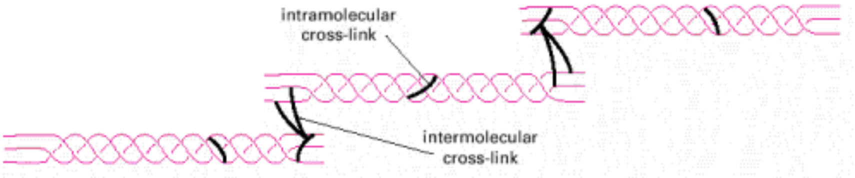
- Some of the lysine side chains are oxidized by certain enzymes to aldehyde derivatives known as allysine (aldolysine).

Allysine has a reactive group that can form covalent linkages and cross-links with another allysine, lysine or hydroxylysine residues within the same or with another tropocollagen.



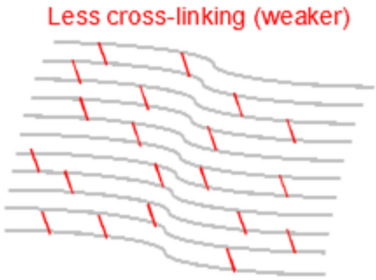
the amino group in lysine residues gets oxidized into aldehyde group

- These cross-links stabilize the side-by-side packing of collagen molecules and generate a strong fibril.

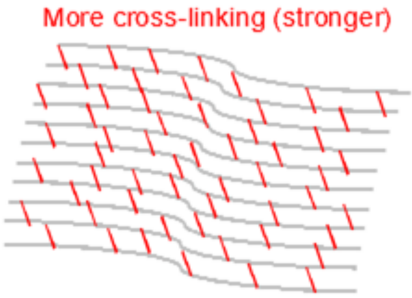


In conclusion:
the 3 alpha collagen strands are packed together because of glycine, the packing increases because of the formation of hydrogen bonds between different hydroxyproline residues, further packing increases due to covalent cross linkages between allysine with different lysine residues within the same or another tropocollagens, microfibrils, or even fibrils, which makes the collagen molecule very rigid and tough.
*see summary at the end of the sheet.

Function of cross-linking

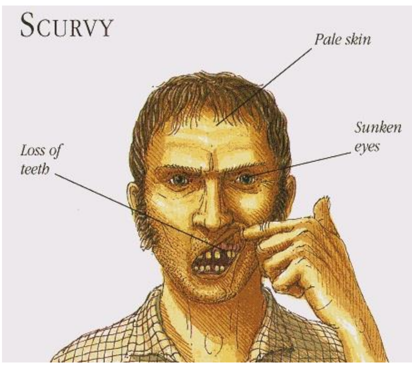


- If cross-linking is inhibited, the tensile strength of the fibrils is drastically reduced; collagenous tissues become fragile and lose their ability to protect the tissue so structures such as skin, tendons, and blood vessels tend to tear easily.
- Deficiency of hydroxylation of proline can cause diseases such as Ehlers-Danlos syndrome (symptoms: severe skin-stretch and easily broken bones).
- The amount of cross-linking in a tissue increases with age. This applies to animals as well that's why meat from older animals is tougher and harder to chew than tender meat from younger animals.



Scurvy

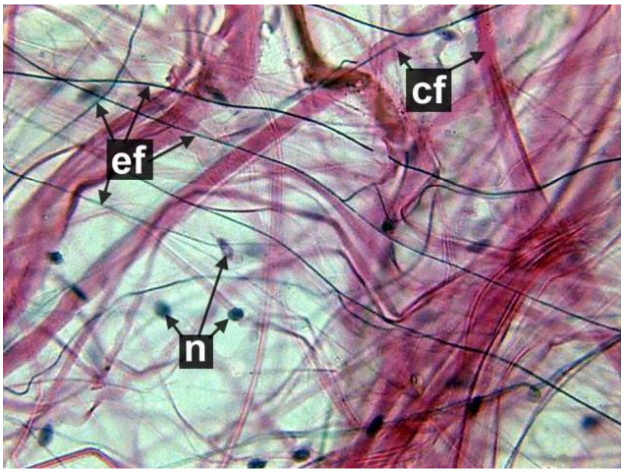
- Scurvy is a disease is caused by a dietary deficiency of ascorbic acid (vitamin C).
  Vitamin C is important for the function of the enzyme proline-hydroxylase.
- Deficiency of vitamin C prevents proline hydroxylation; the collagen molecule then would become fragile as the defective pro-α chains fail to form a stable triple helix and are immediately degraded within the cell.



- Symptoms linked to scurvy include loss of teeth because gum tissues cannot hold teeth in their sockets anymore, blood vessels also become extremely fragile.
- pirates lose most of their teeth due to vitamin C deficiency, stems from the fact that they hardly consume vitamin-C rich fruits like oranges and lemons, these fruits cannot stay fresh for long which means after awhile vitamin C in them would be inactive and compromised.

❖ Now, we will talk about the second type of fibrous proteins which is **Elastin**.

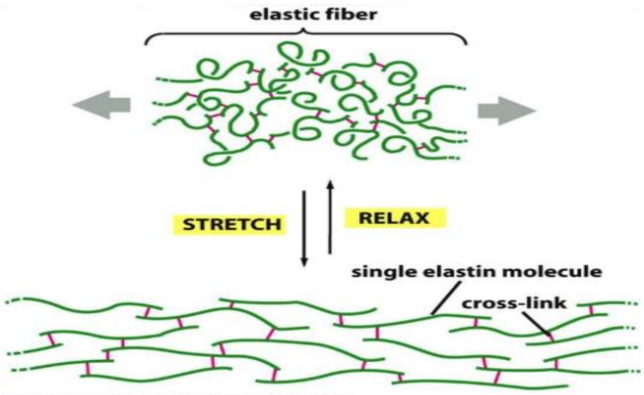
Elastin



Elastin is a long fibrous protein, that exists in tissues such as skin, blood vessels and lungs in addition to many types of cells such as fibroblast, endothelial cells etc. Also, you can see here collagen fibers (thick fibers in pink) and the elastic fibers (thin filaments).

☒ So, what is Elastin?

Elastin is present in tissues that can be **stretched**, a network of elastic fibers in the extracellular matrix of tissues gives them the required resilience (مرونة), so that they can recoil after transient stretch, in addition to long inelastic **collagen fibrils** that are interwoven with the elastic fibers and limit the extent of stretching and prevent the tissue from tearing. That's why you can stretch the skin but you **can't** tear it.



The main component of elastic fibers is **elastin**, which is a highly hydrophobic protein and it is rich in **proline** and **glycine**. It is hydrophobic, because the hydrophobic interactions between these strands are responsible to get the stretched elastic network back to its original shape.

Elastin contains some hydroxyproline, but **no** hydroxylysine. **Recall** that the main purpose of the presence of hydroxylysine in collagen is attaching to sugars and that makes collagen a glycoprotein, so because Elastin lacks hydroxylysine it is **not** considered as a glycoprotein because it is **not** glycosylated.

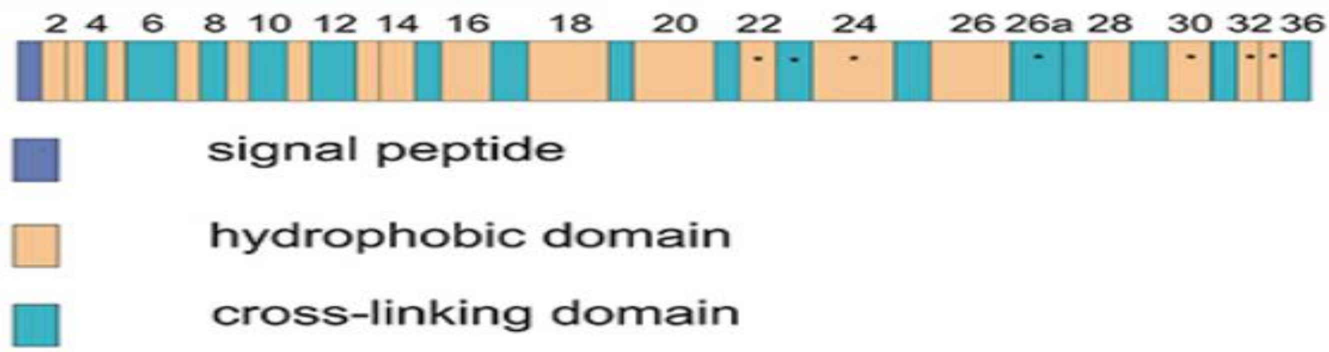
The primary component, tropoelastin molecules, is crosslinked between **lysines** to one another, these lysine residues allow the formation of covalent cross links between elastin strands.

❖ **Now we will get closer to the primary structure of elastin protein.**

The elastin protein is composed largely of **two types** of short segments that alternate along the polypeptide chain:

Hydrophobic domains: which are responsible for the elastic properties such as **stretching.**

Cross-linking domains: which are regions that are rich in **Alanine** and **lysine** that are responsible for the covalent links between strands.



So, we talked about two different fibrous proteins till now, **Collagen** and **Elastin**. Now, we will talk about the third type which is **Keratin**.

Keratin

Keratins belong to family of proteins that can be divided into two types:

1. **α keratins:** α keratin mainly made of **α helices** as secondary structures.

2. **β keratins:** β keratins contain a lot of **Beta strands**.

   ✓ **We will focus on α keratins**

α keratins are important because they made our hair and fingernails as well as animal skin, keratins generally also make a large group of proteins known as intermediate filaments.

Recall that Collagen contains (unusually) hydroxylysine and hydroxyproline, elastin contains unusually hydroxyproline, also α keratin has unusually a high content of **cysteine** residues.

☒ **So, what is the importance of cysteine residues and what is their function?**

✓ The answer is that they form **disulfide bonds** (we will talk about that later on).

α-keratins structure (hair vs. fingernails)

The structure of α-keratin is summarized as the following;

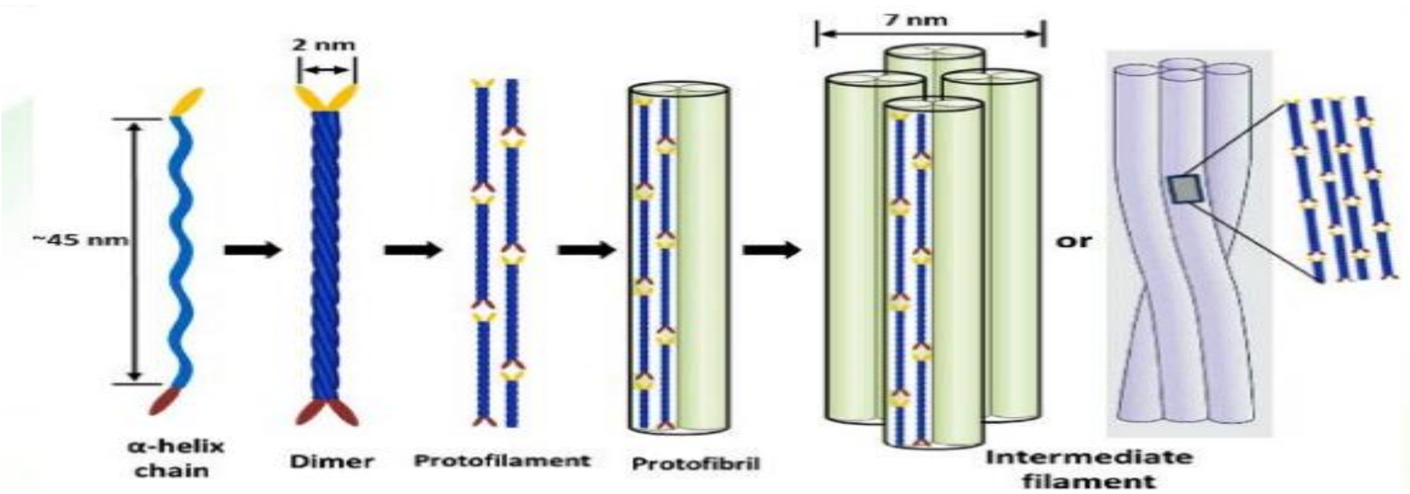
1)Two helical α-keratin molecules form a coiled coil dimer.

2) Two coiled coil dimers associate forming tetramer.

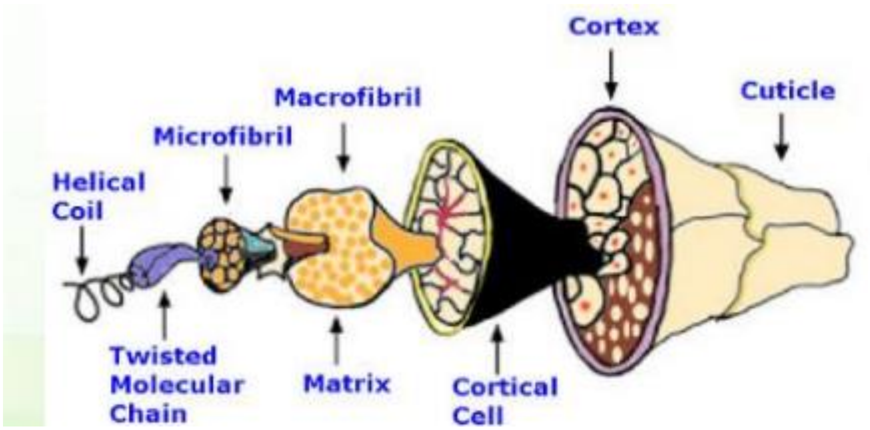
3) tetramers associate head to-tail forming a protofilament.

4) Two protofilaments twist together to form a protofibril.

5) Four protofibrils form an intermediate filament.

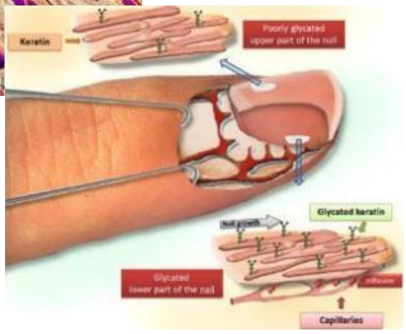


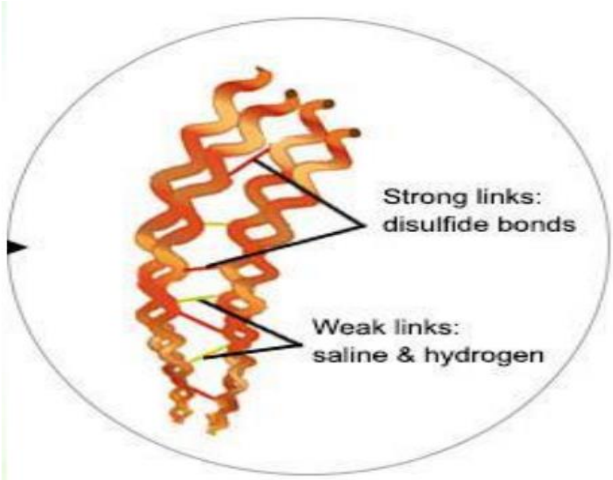
After that, Eight intermediate filaments cluster to make a microfibril, then hundreds of microfibrils are cemented into a macrofibril, many macrofibrils cluster to form a single hair.



Fingernails and hair are both made of α keratin, **so how is it possible that fingernails are hard whereas hair is not?**

The reason is that fingernails have a lot of disulfide cross-links between the α keratin chains, which are not present that much in hair.

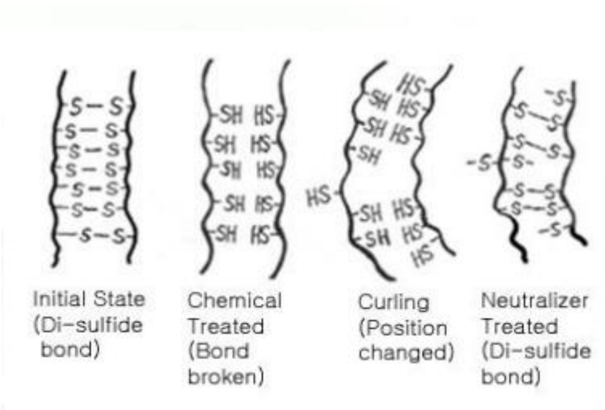




One of the daily habits that is related to α keratins, when we walk up in the morning and our hair is a mess, so we need to wet our hair to be able to comb it, **So why do we do that?**

Biochemically, we do that to break the hydrogen bonds between hair strands. When hair gets wet (by having a shower or by sweating), water molecules disrupt some of the hydrogen bonds which help to keep the alpha-helices aligned. This is known as **temporary wave,** when hair dries up, the hair strands are able to maintain the new curl in the hair for a short time.

In **permanent wave**, there is a reformation of the **disulfide linkages** between the α keratins, by treating the hair with reducing substances like ammonium thioglycolate, that break the disulfide bonds and reduce the Cystines amino acids in the hair, then an oxidizing agent, usually hydrogen peroxide is added to reform the disulfide bonds in the new positions until the hair grows out, so even when the hair gets wet, the hair maintains its design because we are talking about covalent bonds here not hydrogen bonds.



The End

Functional purpose of amino acids (Summary)

Amino Acid in collagen		Characteristics	Function
Glycine		Small in size, reduces repulsion, rotate freely.	Collagen flexibility Tight packing of the alpha collagen chains
Proline		Rigid, create kinks	Collagen rigidity Helical conformation
Hydroxyproline		Additional OH group	Increases tight packing of the alpha collagen chains Stabilizes the collagen molecule and prevents its disruption.
lysine	hydroxylysine	Additional OH group	Glycoprotein(collagen) formation Collagen-cell interaction
	Allysine	Oxidized lysine	side-by-side packing of collagen strands.

Self-assessment Questions

1) Why must glycine be found at regular intervals in the collagen triple helix?

A. Because it is non-polar.
B. Because it is the only amino acid which is small enough to fit at its specific spot
C. Any small amino acid can work not only glycine
D. All the above are incorrect

Answer: B

2) An environmentalist attempted to live in a desolate forest for 6 months but had to cut his experiment short when he began to suffer from bleeding gums, some teeth falling out, and red spots on the thighs and legs. This individual is suffering from an inability to properly synthesize which one of the following proteins?

A. Myoglobin
B. Hb
C. Collagen
D. Insulin
E. Fibrillin

Answer: C

3) The difference between hair and nail keratin is?

A. higher rigidity in hair due to increased amount of hydrogen bonds
B. increased disulfide bridges in nail keratin
C. there is no structural difference just that nails are smaller and therefor stronger
D. none of the above

Answer: B

4) what's shared between elastin and collagen?

A. strength and elasticity
B. hydroxylysine and hydroxyproline
C. their triple helical structure
D. none of the above is correct

Answer: D

5) which of the following disturb disulfide bridges?

A. hot water
B. ammonium thioglycolate
C. ascorbic acid
D. disulfide bonds are covalent and therefor too hard to reduce

Answer: B

6) collagen is different from elastin in all of the following EXCEPT

A. glycosylation
B. presence of proline
C. having obvious secondary structure
D. oxidation of lysine
E. cross linking of monomers

Answer: B

Good luck <3