Peptides:

polypeptide vs protein:

polypeptide: a long peptide with NO particular structure

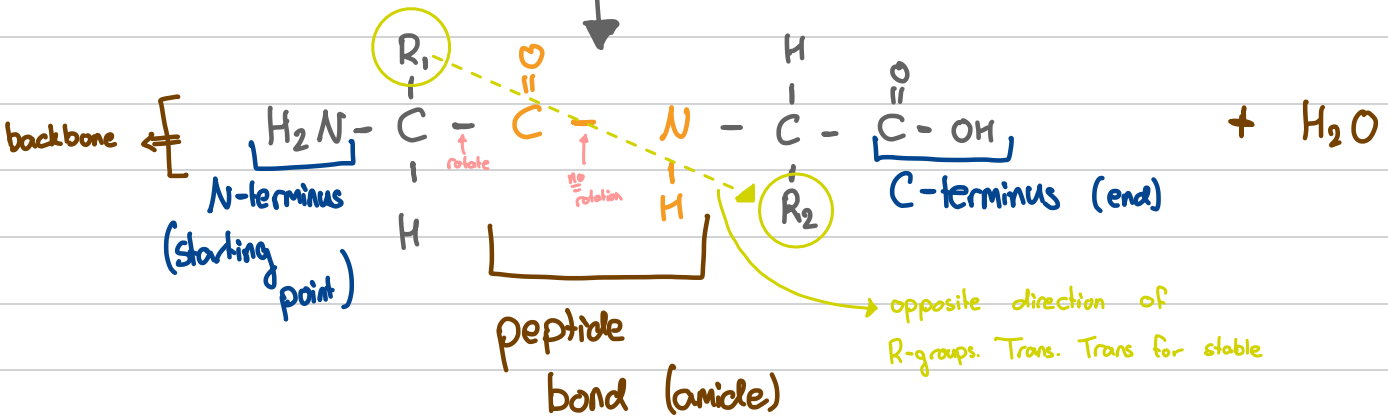
protein: polypeptide chains with an organized 3D structure.

oligopeptide: 20-30 amino acids

molecular weight measured in Daltons.

Peptide bond:

Condensation / Dehydration reaction between two amino acids.

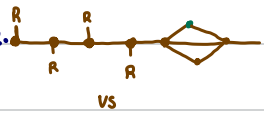


Properties of peptide bonds:

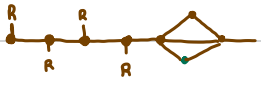
1 → Trans R-groups. Trans more stable due to less steric hindrance (less repulsion). ← due to van der waals interactions

↳ In all amino acids in polypeptide they exist as trans... except for proline.

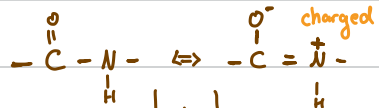
↳ Proline has equal cis & trans. Because the cyclic causes equal steric hindrance.

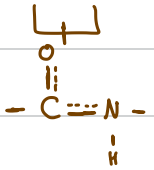


VS



2 → Double bond + Resonance in the peptide bond.

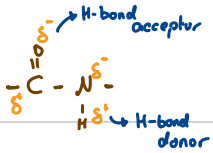




↳ Rigid. No rotation in peptide bond. Other bonds rotate

↳ Planar (flat / sp2)

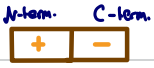
↳ Charged



3→ Hydrogen Bonding (Secondary structure)

↳ Proline doesn't form hydrogen bonding due to N being tertiary N & lacking hydrogens. So in proline no H-bond donor.

4→ Peptide is polar. It has two opposite charged ends.

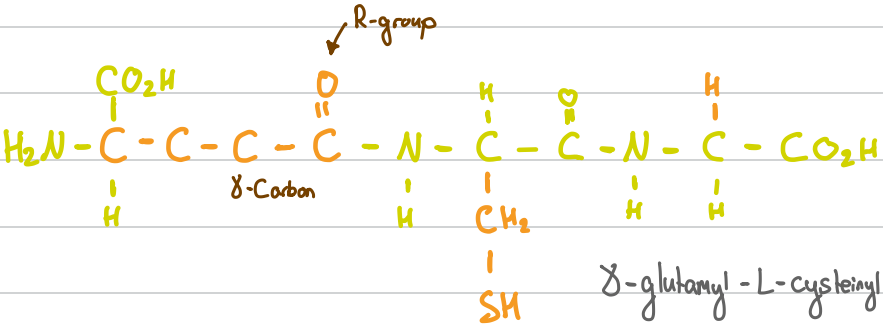


5→ Zig-Zag arrangment of amino acids.

Functional Peptides Examples:

1→

Name: Glutathione



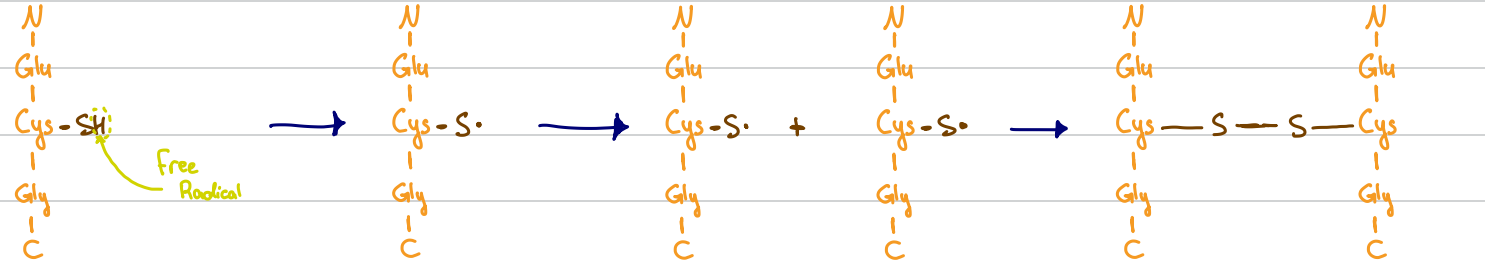
Structure:

* Tripeptide
* H_2N - γGlu- Cys - Gly - CO_2H

SH (thio)

Functions:

* Anti-oxidant
* Sacrifices its self by reacting with the harmful free radicals (oxidizing agents)
* Glutathione gets oxidized & forming disulfide between each other.



Reduced Glutathione

Oxidized Glutathione (lost e⁻ to free radical)

Memorization Tip:

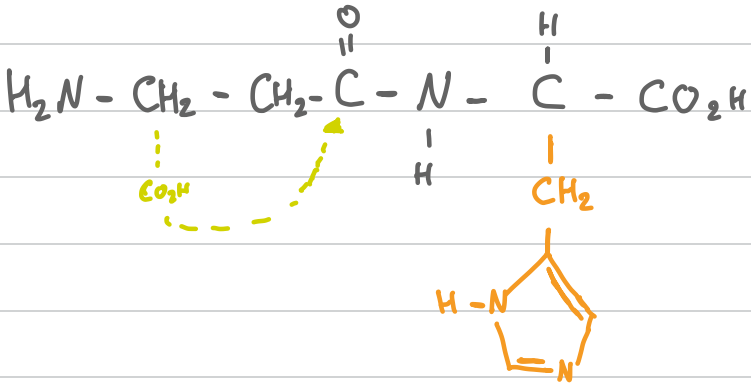
Glutathione ⇒ Glutamic Acid + thio... cydeine + glycine... simplest a.a at end to form tripeptide

2 →

Name: Carnosine:

Structure:

* Dipeptide
* H_2N - β Ala - His - CO_2H



β-alanyl-L-histidine

β-alanine : $H_2N - CH_2 - CH_2 - CO_2H$

Functions:

* Anti-oxidant. Like Glutathione. Protects against free radicals like oxygen & peroxides
* Plays a role in muscle contraction

Memorization Tip:

Carnosine ⟹ Ca ⇒ β alanine + sine ⇒ histidine

3 →

Name: Enkephalins → Met-enkephalins
→ Leu-enkephalins

Structure:

* Pentapeptide (5 amino acids)
* Met-enkephalins: Tyr - Gly - Gly - Phe - Met
* Leu-enkephalins: Tyr - Gly - Gly - Phe - Leu

} differ at the terminal carbonyl end

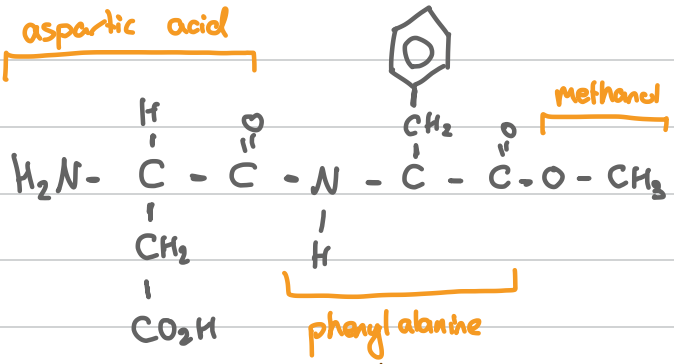
DONT memorize sequence

Function:

* Analgesics (pain blockers)
* There structure very similar to morphine (opioid)
* The aromatic R-groups of Tyrosine & phenylalanine play a role in its analgesic properties

4 →

Name: Aspartame



L-aspartyl-L-phenylalanine methyl ester

Structure:

* Dipeptide + methanol => ester bond
* H_2N - Asp - Phe - C(=O) - O - CH_3
* L-amino acid => sweet. D-amino acid => bitter] all peptides we studied are L-amino acids

Functions:

* Zero-calorie sweetner sugar substitute
* Some research shows it may cause cancer.
* Phenylketouria. Due to phenylalanine. Use alitame instead. Alitame => aspartic acid + alanine

5→

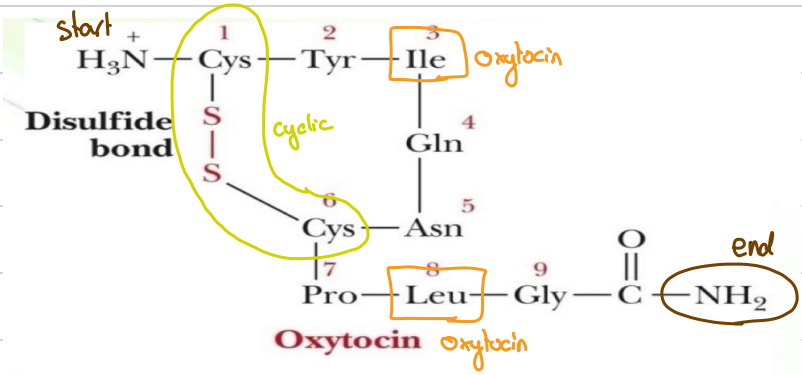
Name: Oxytocin and Vasopressin (ADH)

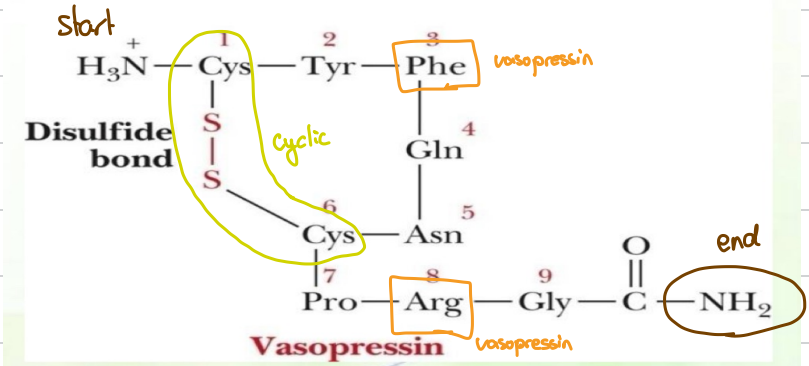
Structure:

* Cyclic structure due to disulfide bonds. -S-S- between Cys.

* Amide group at C-terminal

* Oxytocin → Has Leu & Ile (tip: oxytocin female hormone → lucy → Leu & Ile)
* Vasopressin → Has Phe & Arg





Functions:

* Oxytocin (Love hormone)

↳ uterus contraction during child birth

↳ lactation (milk ejection)

* Vasopressin (ADH)

* Increase water retention (more water reabsorption into blood from urine)
* Smooth muscle contraction
* Increase blood pressure

} ⇒ these two increase blood pressure

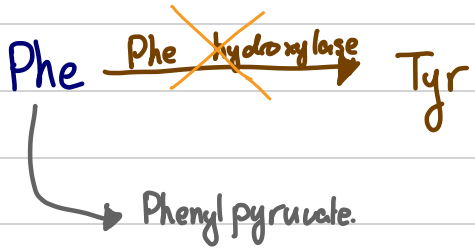
Phenylketouria:

Phenyl + keto + uria => phenylalanine not metabolized well and released in urine.

Mechanism:

* Lacking phenylalanine hydroxylase enzyme. Phy to Tyr conversion blocked

* Phe converted to phenyl pyruvate



* Accumilation of phenylpyruvate causes mental retardation.

* Decrease Phe consumption... like aspartame. Use alitame.

Protein Structure

When a protein is formed it will always have the same 3D structure according to its amino acid sequence. This 3D structure is the most energetically stable. It is called the native conformation.

Protein Structure
- Primary 1° structure => Sequence of amino acids. Ex: Gly - Glu - Val - Asp - Arg
- Secondary 2° structure => Localized 3D organization of polypeptide chain
  - regular: α-helix, β-pleated sheat
  - non-regular: turns, loops, coils, bends
- Tertiary 3° structure => Overall 3D shape of entire protein (Many 2°)
- Quaternary 4° structure => Two or more polypeptide chains (ex: hemoglobin)

① Primary Structure:

Sequence of amino acids. This sequence determines the 2° and 3° structures.

N terminus ⟶ C terminus
start end

→ Sickle Cell anemia:

* Substitution of a single amino acid. Changes 1° & 2° & 3° structures.

* Substitution of amino acid no 6 Glu ⟶ Val of β-globin

normal: 6: Glu

sickle cell anemia: 6: Val

→ Hemoglobin aggregates inside RBC. Causing sickle-shape RBCs.

→ Sickle RBC increase blood clotting chance.

② Secondary Structure:

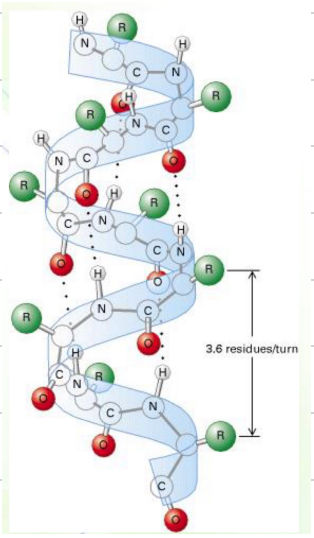
Local shape ⇒ Due to Hydrogen bonding BETWEEN back bone.

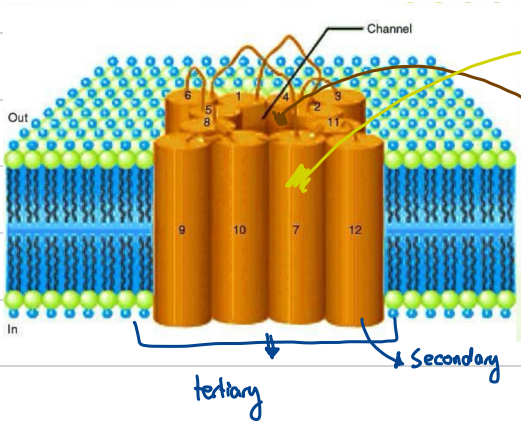
2° ⇒ H-bonding between peptide bonds... so not R-groups.

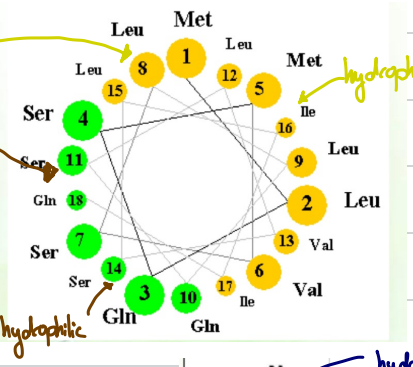
3° ⇒ interactions between R-groups

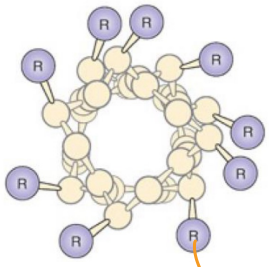
→ α-helix : Regular

* helical rod structure
* 3.6 amino acid per turn
* Very Stable due to linear H-bonding
* Form part of the membrane acting as ion channels
* Are amphipathic α-helices in ion channels

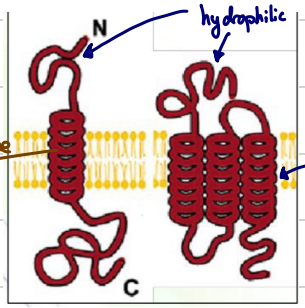








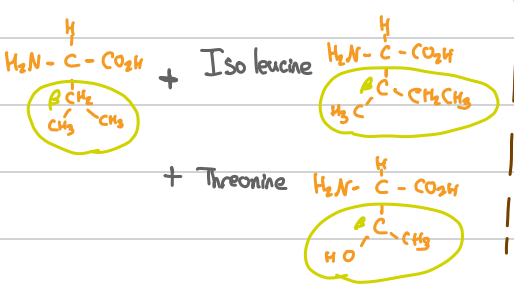
side chains projecting outside.
↓
to avoid steric hindrance.



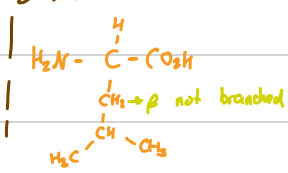
α-helices in trans-membrane proteins

Amino Acids NOT in α-helix:

* Glycine - too small
* Proline
  - Rigid peptide bond. No rotation
  - Bent amino acid
  - CAN'T form hydrogen bonding
* Branched R-group AT β-carbon
  - Valine
  - Isoleucine
  - Threonine



Leucine not branched at β-carbon. Leu Forms α-helix.



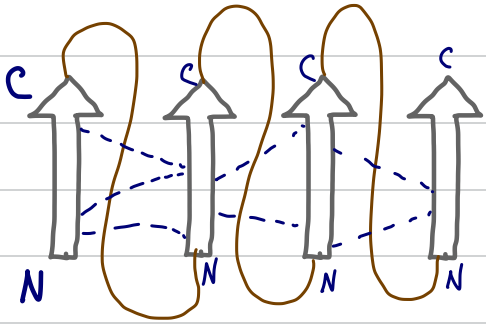
* Similar charge R-group near each other

→ β-pleated sheet. Regular

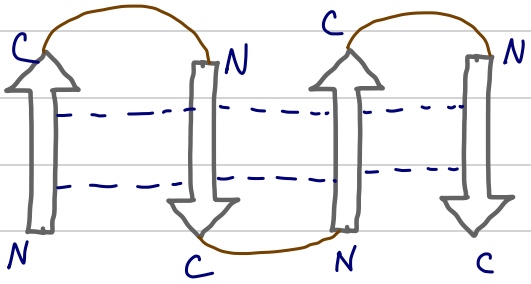
Two parallel β-strands forming H-bonds with each other.

Can be parallel or anti-parallel or mixed.

Parallel:

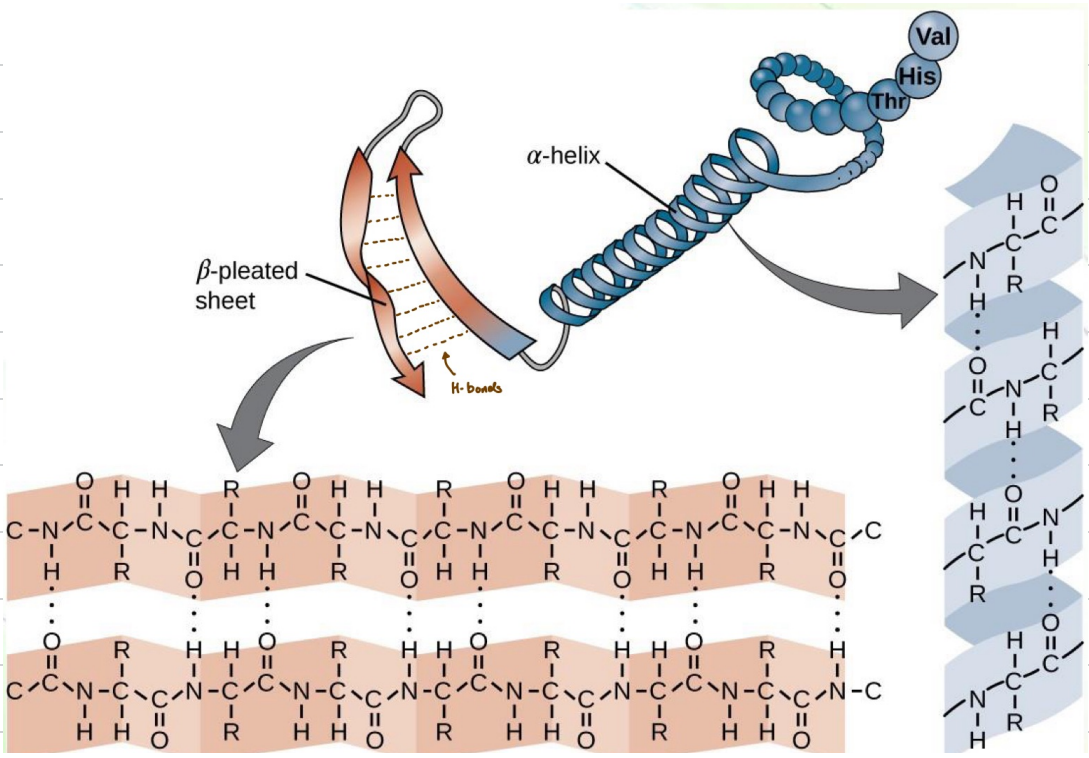


Anti-parallel:



Anti-parallel is more stable! (linear H-bond)

* We can't have Proline in β-sheat because proline no H-bonding.

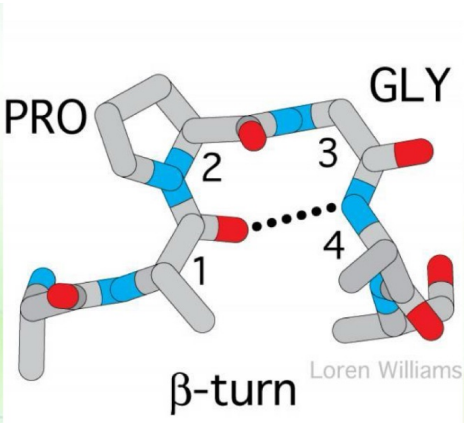


→ β- turns / hairpin bends: non-regular

* U-shaped. Turns the polypeptide chain

* 2nd is proline → cyclic so bends

* 3rd is glycine → small

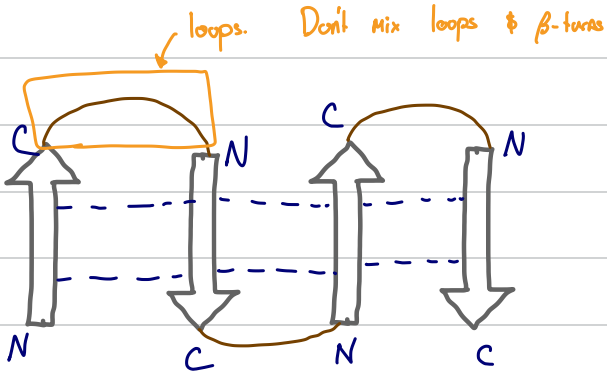


→ Loops & coils:

* Connect α-helix / β-sheets together

* No regular sequence of amino acid.

* Are flexible



* When we have α-helix / β-pleated directly connected by loops. Called motifs. A super-secondary structure

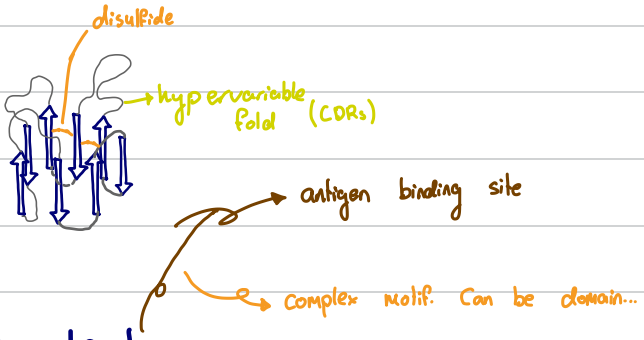
→ Super- Secondary structures: → motifs (DNA-binding); → Domains (tertiary)

motifs: Repeated secondary structures Connected by loops

* Small portion of protein. They DONT provide us with the biological function of proteins.

1→ Immunoglobin folds. (antibodies) (Motif/Domain/Fold)

* Anti-parallel β-sheets connected by loops

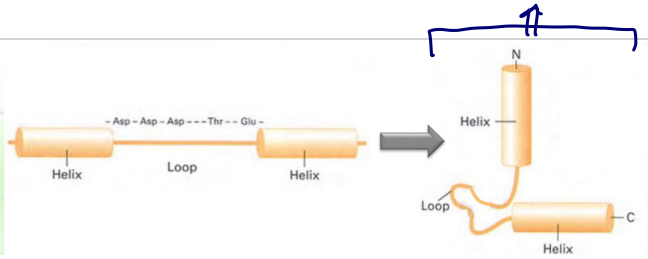


* Allows immunoglobin to interact with various structures

2→ Helix - loop - helix:

* α-helix → loop → α-helix
* DNA-binding protein

A motif



3→ Helix - turn - helix:

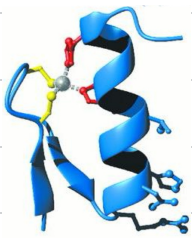
* α-helix → turn → α-helix
* DNA-binding proteins

4→ Beta hairpin: (β-turn... proline + glycine)

* Two anti-parallel β-sheets connected by turn / bend:

5→ Zinc Fingers:

* β-sheets antiparallel connected by loops. Then connected to α-helix. Folded and bound by a zinc ion.
* DNA-binding proteins.

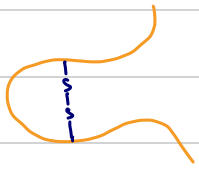


③ Tertiary Structure:

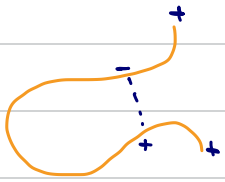
* Overall 3D shape of protein due to interactions between R-groups
* Protein folding happens gradually. Not suddenly.
* Proteins are not static. They are in constant movement. Their structures are flexible.

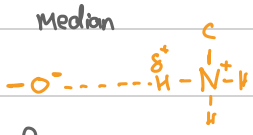
Interactions b/w R-groups:

* **Covalent** (disulfide bridge) - strongest
  - → disulfide bridge → stabilizing & not shape determining
  - → strongest
  - → Cysteine gets oxidized. Cross-linking of two cysteines is called **Cystine**



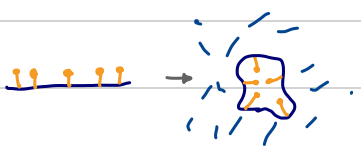
* **Ionic** (salt bridge)
  - → Between charged groups
  - → Ex: Lysine & glutamic acid



* **H-bonding**
  - → Between R-groups
  - → Between R-group & aqueous medium
  - → Charge Dipole interactions 
  - → Polar amino acids can be found in either interior or exterior → these amino acids have a special role.
  - → Many of them... so they can be strong

* **Van der waals**
  - → Due to uneven distribution of electrons Temperary induced di-di interactions
  - → Can be repulsive or attractive
  - → One is weak. But many of them strong

* **Hydrophobic interactions**
  - → Non-polar R-groups cluster together inside... to form a hydrophobic core.



⇒ Forces that stabilize but DON'T determine shape:

* Disulfide bridge

* Metal ions

↳ Salt-bridge (ionic) metallic → carbonic anhydrase (iron)

↳ Covalent metallic → myoglobin (zinc)

What affects the 3D protein structure:

* Primary Structure, the amino acid sequence ☆ this causes the rest

* Angles between amino acids...

Due to:

* Non-covalent interactions

* non-protein molecules such as metal ions.

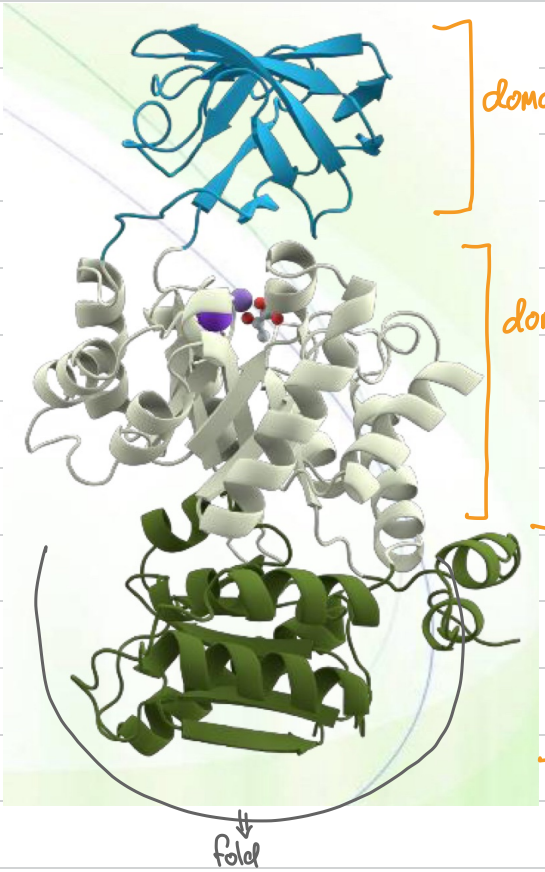
Domains: combination of many α-helices / β-sheets connected via loops/coils/turns. It is a super secondary structure. Not tertiary.

Domain larger than motifs, having 100 → 200 amino acids.

Domain associated with a specific function. Motif not necessarily to have a specific function.

Similar domains associated with proteins with similar function.

Domains fold independantly of each other... maintaining its structure if seperated



all these 3 domains are independent of one another.

⇒ combination of domains to perform a specific task is called a **Fold**

Types of folds: combination of multiple domains

* Actin fold
* nucleotide-binding fold

Order of protein folding complexity:

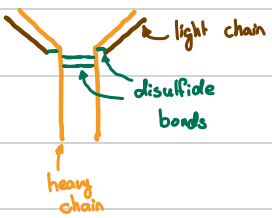
1. primary structure
2. secondary structure
3. motifs } => super secondary
4. Domains
5. Fold => combination of domains
6. Tertiary Structure
7. Quaternary Structure

Quaternary Structure:

* Having two or more polypeptide subunits.
* Oligomeric proteins
* monomeric / dimeric / trimeric / tetrameric .... etc.
* homooligomeric protein → identical polypeptide subunits
* heterooligomeric protein → different polypeptide subunits

Bonds between polypeptide subunits:

* Covalent disulfide bridge
  ↳ Present in immunoglobins



* Non-covalent electrostatic attractions due to metal ions
  ↳ Present in hemoglobin

Denaturation:

* Can be irreverable or reversable (renaturation)
* Breaking non-covalent interactions
* When disulfide bridge breaks... the 3D shape is completely disrupted

Renaturation:

* Can be quick & spontaneous
* More common in small proteins
* Placing —S-S— bonds in correct orientation.

Denaturating Agents:

* High temperatures
  ↳ Distrupt low energy van der waals

* Extremes of pH
  ↳ Distrupt electrostatic attractions & hydrogen bonding

* Detergents
  ↳ Triton X-100 ~ not charged ⇒ disrupt hydrophobic interactions
  ↳ SDS ~ anionic, charged ⇒ disrupt hydrophobic interactions + electrostatic attractions

* Hydrogen bonding + hydrophobic interactions disruption
  ↳ Urea
  ↳ Guanidine Hydrochloride

* Reducing Agents. ~ Reduce disulfide bridge —S—S— into $\overset{-SH}{-SH}$. Breaking it.
  ↳ β-ME
  ↳ DTT

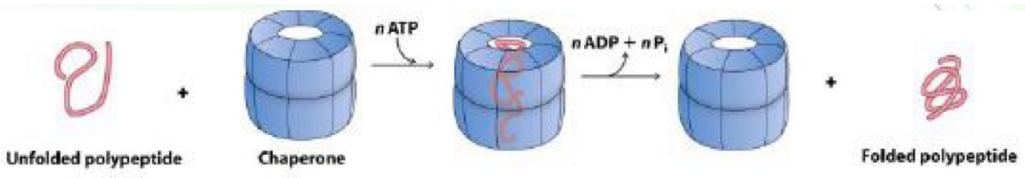
Protein misfolding:

What fixes protein misfolding:

1 → Chaperones:

* Use ATP to properly fold proteins

* Prevent hydrophobic regions of polypeptides from associating with each other and forming aggregates. Aggregates are the causes of diseases.

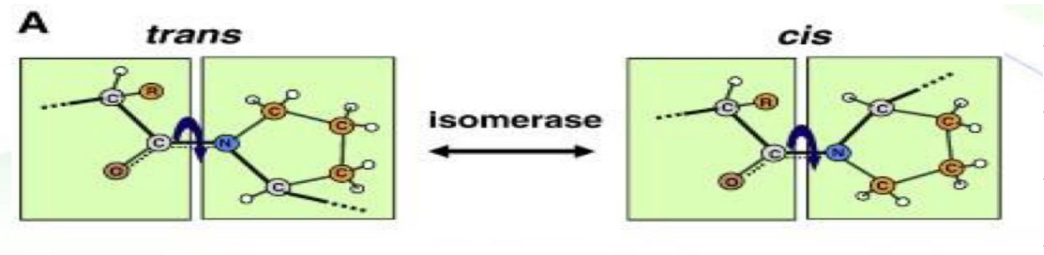


2 → Cis - Trans isomerase:

* Reversible reaction between cis peptide bond & trans peptide bond after proline.

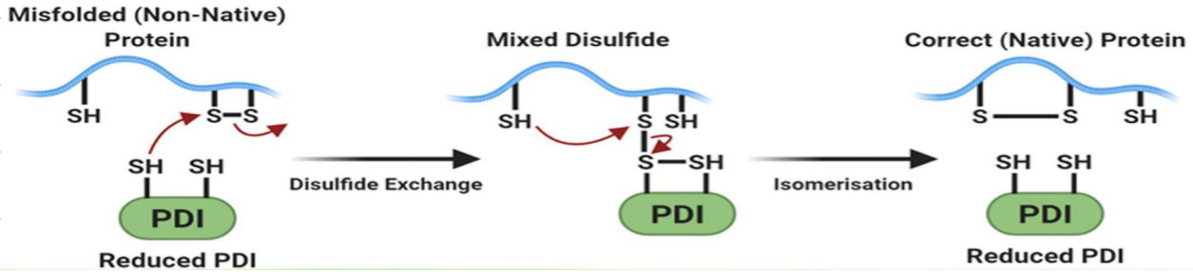
* Changes the configuration depending on most suitable for protein.

* Cis is suitable for hairpin turns (β-turns)



3 → Protein disulfide isomerase:

* Breaks and reforms disulfide bonds to correct them to fix protein folding.



Forming Toxic Aggregates:

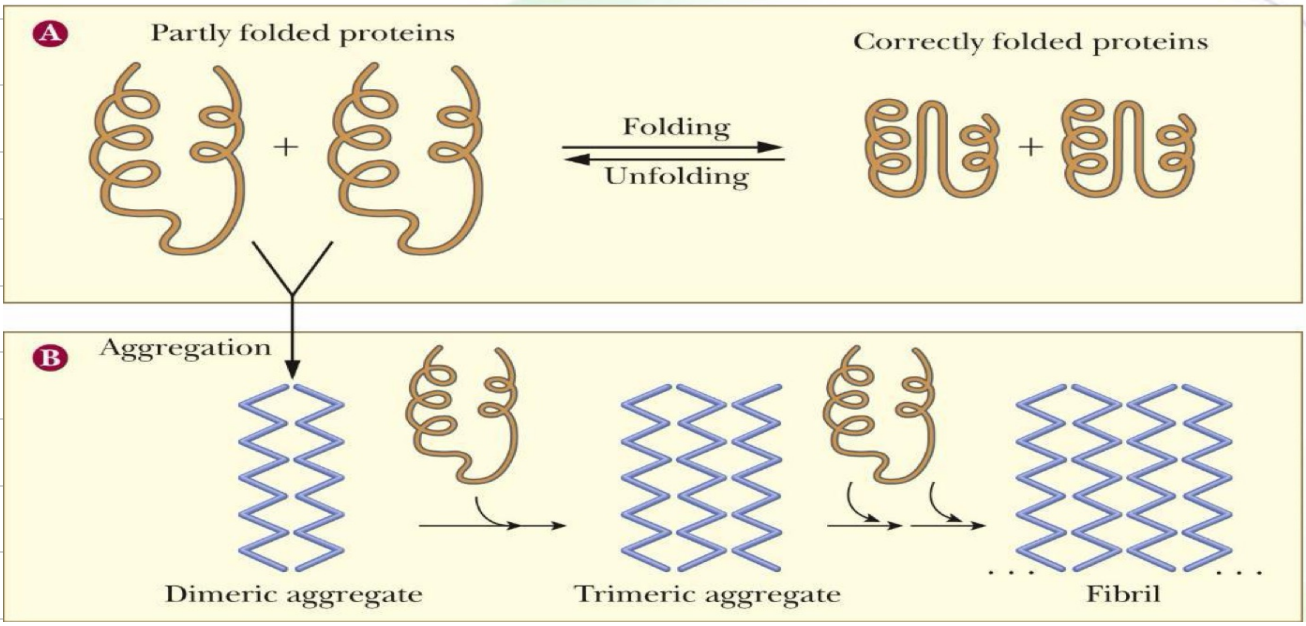
* Exposed hydrophobic regions of misfolded proteins interact with one another and form aggregates.

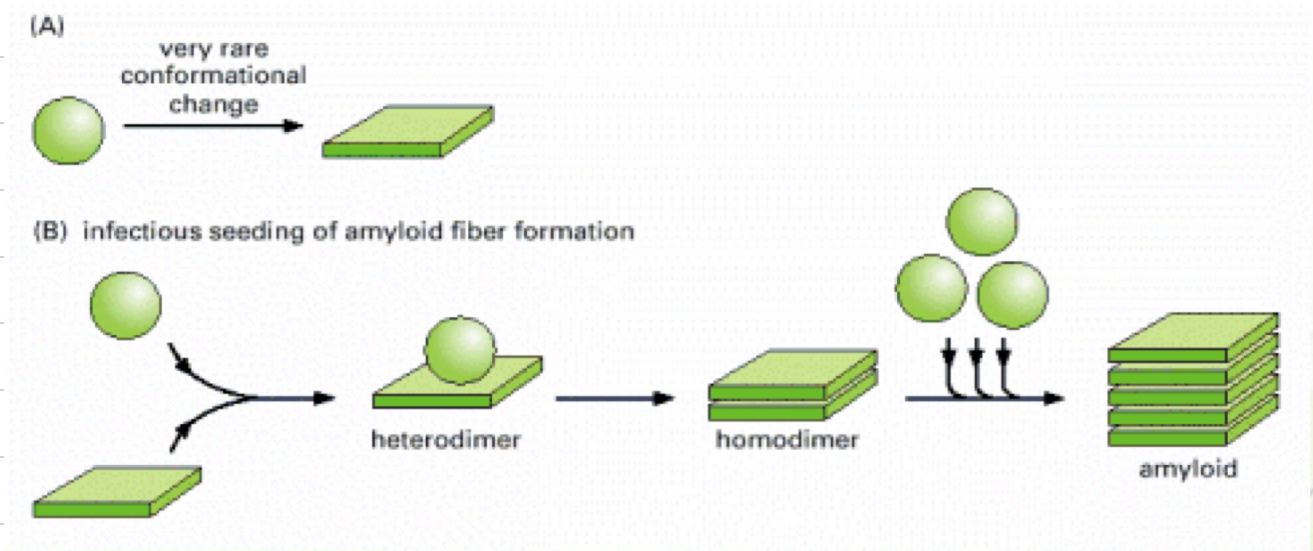
* Vary in size

* Can be polar or non-polar

* Cluster of aggregates form insoluble fibrillar structure called **amyloid**

* Very toxic





Diseases due to protein misfolding:

① Alzheimer's Disease:

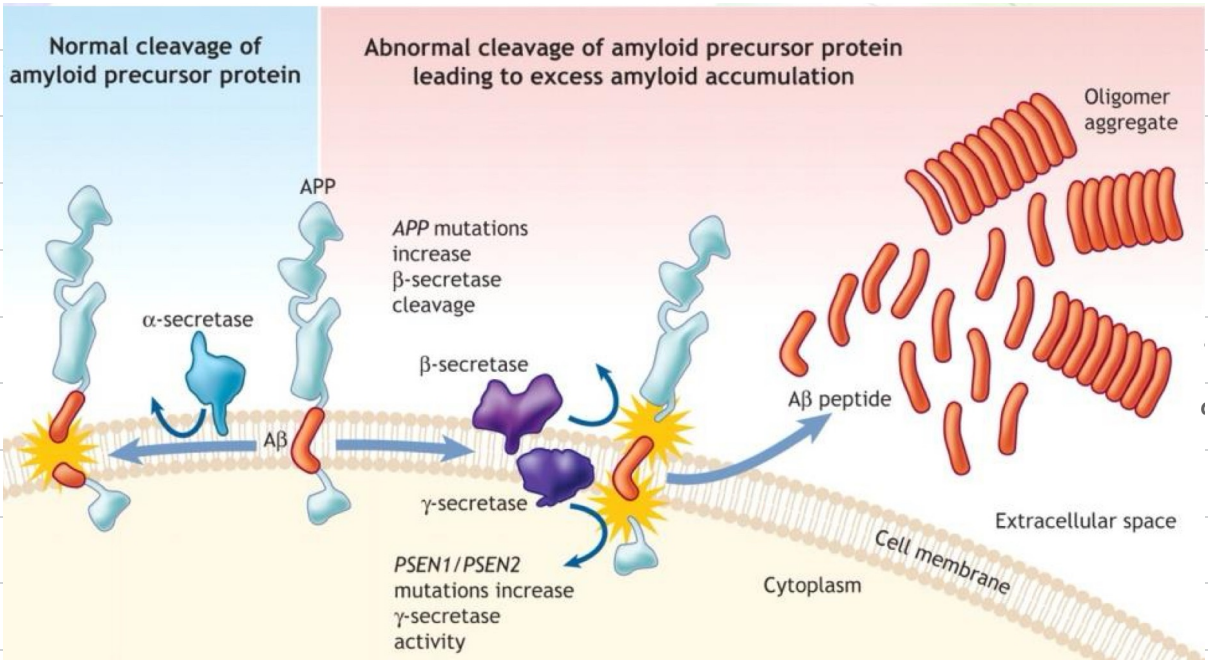
* Not transmissible... not infectious

* Due to misfolding of 2 proteins:

1→ Tau protein

2→ amyloid (Aβ) protein → forms amyloid plaque aggregates

* The amyloid plaque deposits in the brain... killing neurons.



Details not required. Just know that:

* Abnormal shedding of amyloid peptide (Aβ). causes hydrophobic Aβ to be shed in large amounts and the form aggregates.

↓

amyloid plaque

② Prion Diseases:

* Infectious protein diseases → Can be transmitted
  → Can be inherited (genetic)
  → Can occur spontaneously

* In cows: Mad cow disease

* In humans: Creutzfeldt-Jacob disease (CJ-disease)

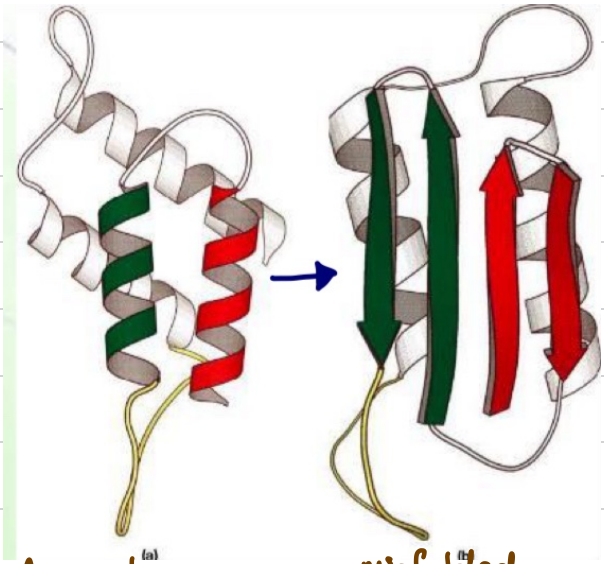
* In sheep: Scrapie

* Misfolded prion protein (Pr^{Psc}) ruins and misfolds the normal folded prion protein (Pr^{Pc})

* Since Pr^{Psc} can misfold the normal Pr^{Pc}. This makes prion disease infectious.

* Pr^{Pc} is a brain protein. So prion's disease affects the brain

* Normal Pr^{Pc} has many α-helices. When it misfolds into Pr^{Psc} it gets alot of β-pleated sheets. This excess in β-pleated causes the protein to form aggregates.



Normal Pr^{Pc}

Misfolded Pr^{Psc} which forms aggregates

Pr^{Pc} → normal. many α-helices

Pr^{Psc} → misfolded. many β-pleated

Complex Proteins:

Protein + non-protein component

* Lipo protein
* Phosphoprotein
* Hemo protein
* Nucleoprotein
* Glycoprotein
  - N-glycosidic → Asn
  - O-glycosidic → Thr / Ser / hydroxy Lys

Holoprotein: protein + non-protein part Ex: Lipoprotein

Apoprotein: protein portion of holo protein Ex: Apolipoprotein

Ex:

Lipoprotein => the protein part only is called Apolipoprotein

Glycoprotein => " " " " Apoglycoprotein

Co-enzyme: molecule that assists enzymes

Prosthetic group: Molecules tightly bound to proteins such as co-enzymes & metals

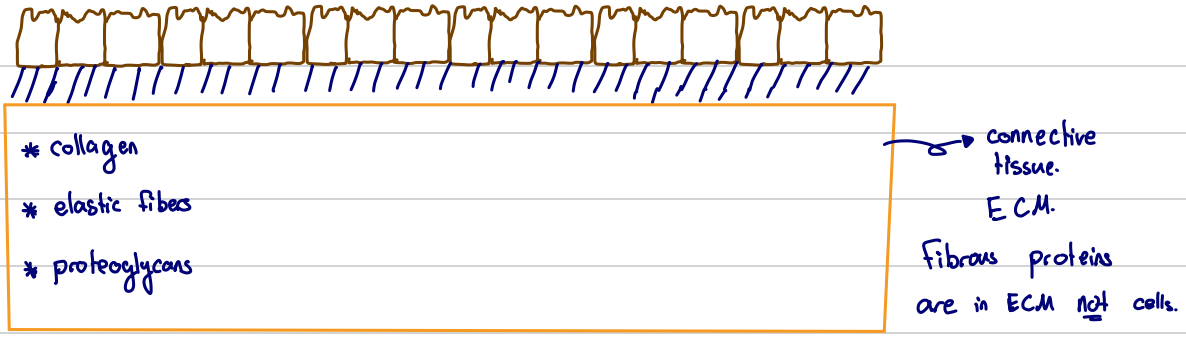
Fibrous Proteins:

Types of proteins:

* **Contraction** - actin / myosin
* **Structural** - collagen / keratin (intermediate filaments) / actin (microfilaments)
* **Defense** - Antibodies (immunoglobins)
* **Transport** - Hemoglobin / channel proteins
* **Signalling** - Hormones / Receptors
* **Toxins** - Enterotoxins / Diphtheria

Proteins
- Fibrous (fiber-like) ⇒ collagen / elastin / keratin — Secondary structure only. NOT 3D
- Globular (3D globe-like) ⇒ hemoglobin / myoglobin / immunoglobins

Connective Tissue (ECM):



Collagen ! :

* Most abundant protein in body (25%)

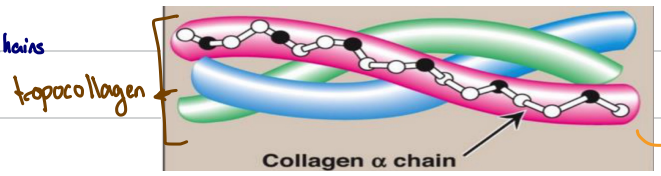
* Many Many types.. type I .. type II .. type III ... etc. Fibril forming / network forming / transmembrane ... etc.

* Provide structure support. Very stiff and high tensile strength
  ↳ like rope. NO compressive force.

Collagen's Structure:

* Made of 3 α-chains (not α-helix). The 3 α-chains cross linked with each other to form tropocollagen. They are tightly bound by cross-links (covalent) and Hydrogen bonds.
  ↳ aldehyde covalent links via oxidized lysine

→ tropocollagen.. made of 3 α-chains



↳ looks like rope

* Collagen Organization: (4 levels)

1→ 5 tropomyosin make 1 collagen microfibril. Covalent cross-links

2→ many microfibrils make one fibril. Covalent cross-links

3→ many fibrils make one fiber

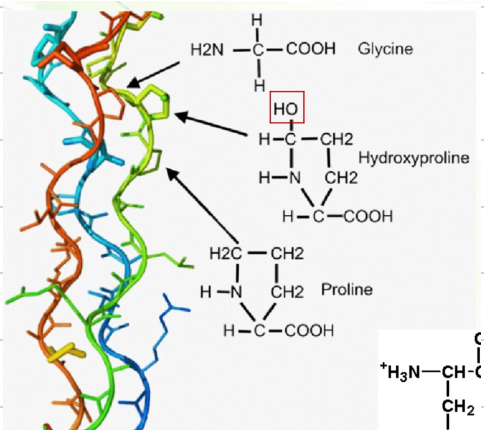
* 3.3 amino acids per turn of α-chain

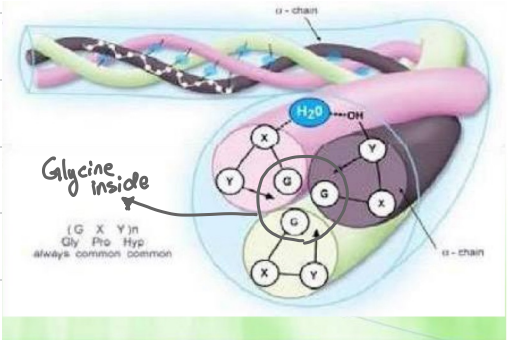
* α-chain is more extended than α-helix.

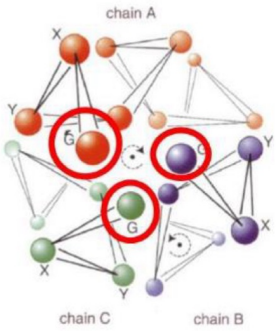
* α-helix more compact thus more amino acids per turn (3.6)

* Amino-Acid organization:

→ 33% Glycine → 13% Proline → 9% hydroxyproline → lysine → hydroxylysine







1→ Glycine:

* Most common. 33%. Every third amino acid is glycine. -Gly-X-Y-Gly-X-Y-Gly-

* It is small... it allows the 3 α-chains to pack closely together

* It is situated towards the center of the triple helix

* Can rotate freely.. it provides flexibility

2→ Proline:

* Usually.. Gly-X-Y where X & Y are proline & hydroxyproline

* Proline's cyclic structure creates kinks in α-chain structure.

* It stabilizes the helical structure

* It is rigid. Provides rigidity

3→ Hydroxyproline:

* Allows the formation of H-bonds b/w α-chains. Increases strength & stability

4→ Hydroxylysine:

* Glycosylation.. adding polysaccharides to form glycoproteins.

* Function of glycosylated collagen: recognize & interact with cell surface receptors. Cell signaling.

Hydroxylation of proline & lysine:

→ prolyl hydroxylase & lysyl hydroxylase... using ascorbate/ascorbic acid. (Vitamin C).

→ hydroxylation occurs after α-chain is translated

Scurvy:

* Lack of vitamin C
* No hydroxylation of proline & lysine
* Less H-bonding. Unstable triple helix. Weak collagen structure
* Weakened tissue. Fragile blood vessels. Weak gums.. teeth become loose.

5→ Lysine

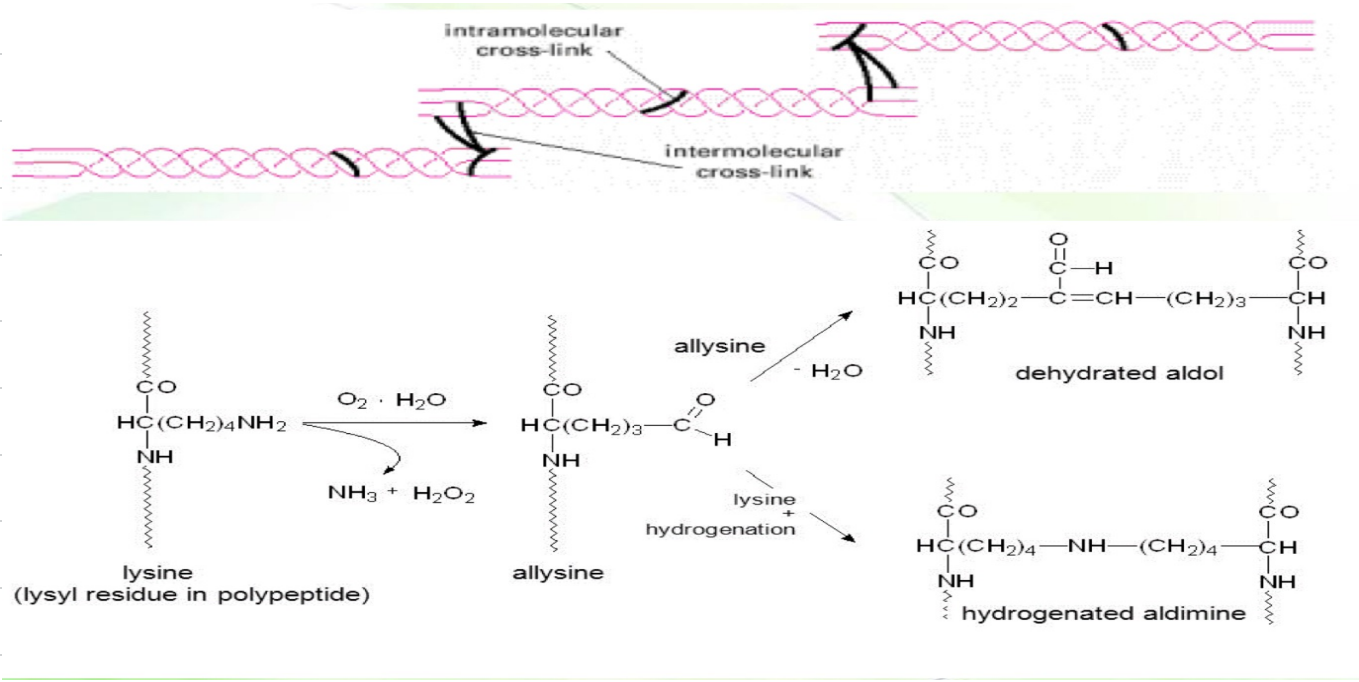
* Responsible for covalent cross-links. How?

* Lysine (or hydroxylysine) gets oxidized to allysine (aldehyde lysine)

* Allysine + Allysine OR Allysine + Lysine react together & form covalent cross-links

* Between same tropocollagen OR b/w different tropocollagen. This strengthens the collagen's tensile strength

* The older we get. The more cross-links between collagen molecules



Ehlers-Danlos Syndrome:

* Weakened collagen due to deficiency in hydroxylation.

Collagen Synthesis:

① Transcription of DNA to produce mRNA. mRNA translated into α-chain in the RER

② Collagen gets hydroxylated & glycosylated (hydroxylysine)

③ Assembly of tropocollagen from three α-chains

④ Modification by cleavage ... then assembly.

5 tropocollagen → collagen microfibril → collagen fibril → collagen fibers

Advanced Glycosylated End products (AGE's):

* Glucose deposited on proteins such as collagen non-enzymatically. Hard to remove this glucose.

* More glucose in blood (hyperglycemia) the more the AGEs.

* Glycosylated collagen gets oxidized (non-enzymatically) & produces more cross links.

↳ this causes the production of protein aggregates called Advanced Glycosylated End products (AGE's). We measure this for diabetes patients.

Side affects of AGE's:

* Inflammation
* Apoptosis
* Nephropathy - kidney function problems
* Retinopathy - eye problems
* Cardiomyopathy - heart problems
* Atherosclerosis - hypertension

⇒ all common problems with diabetes patients

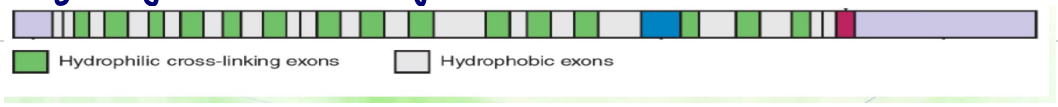
Elastins:

* Provide elasticity, can stretch & then relax and go back to original shape.

* Collagen fibers are interwoven with elastic fibes to limit elasticity. Prevents tissues from tearing.

* Many Cross-links between tropoelastin molecules. When stretched, the cross-links hold tropoelastin together.
  - ↳ Tropoelastin - Soluble
  - ↳ Elastin - insoluble... due to many cross-links

* Repeating hydrophilic & hydrophobic amino acid domains



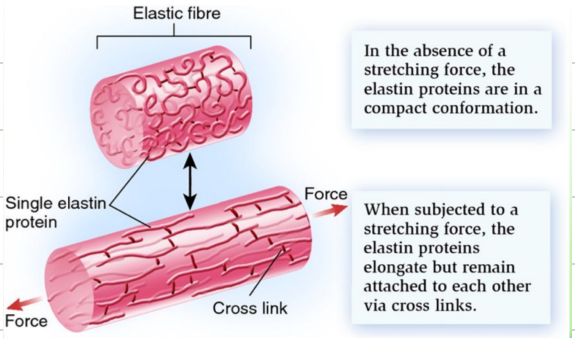
- ↳ Hydrophilic - Lysine + Alanine ⇒ Responsible for forming covalent cross-links
- ↳ Hydrophobic - Glycine + Proline + Valine ⇒ Responsible for hydrophobic interactions... Reversible. Stretch & relax.

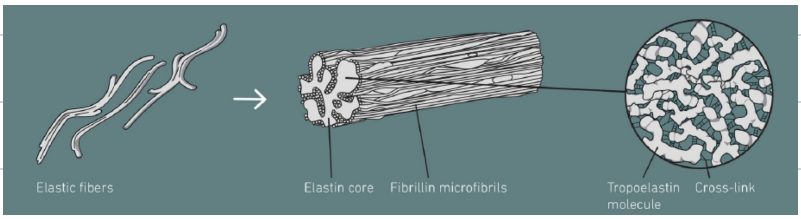
* Has hydroxyproline ✓

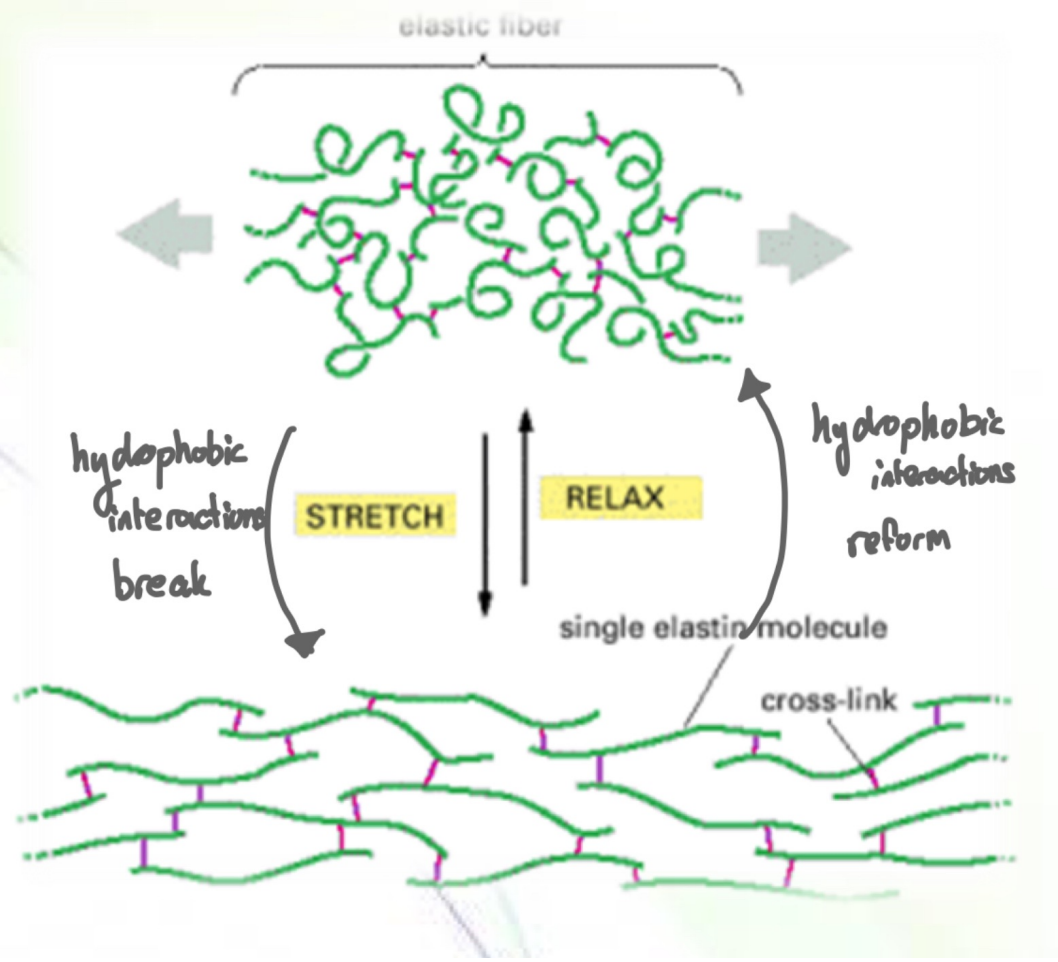
* NO hydroxylysine ✗ ⇒ no glycosylation of elastin
  - ↳ it still has lysine which forms cross-links.

* Lysyl oxidase oxidizes lysine to allysine & forms covalent cross-links

* Cross-links prevent elastin molecules from seperating when stretched.
* Hydrophobic interactions bring back the elastin molecules together when relaxed.

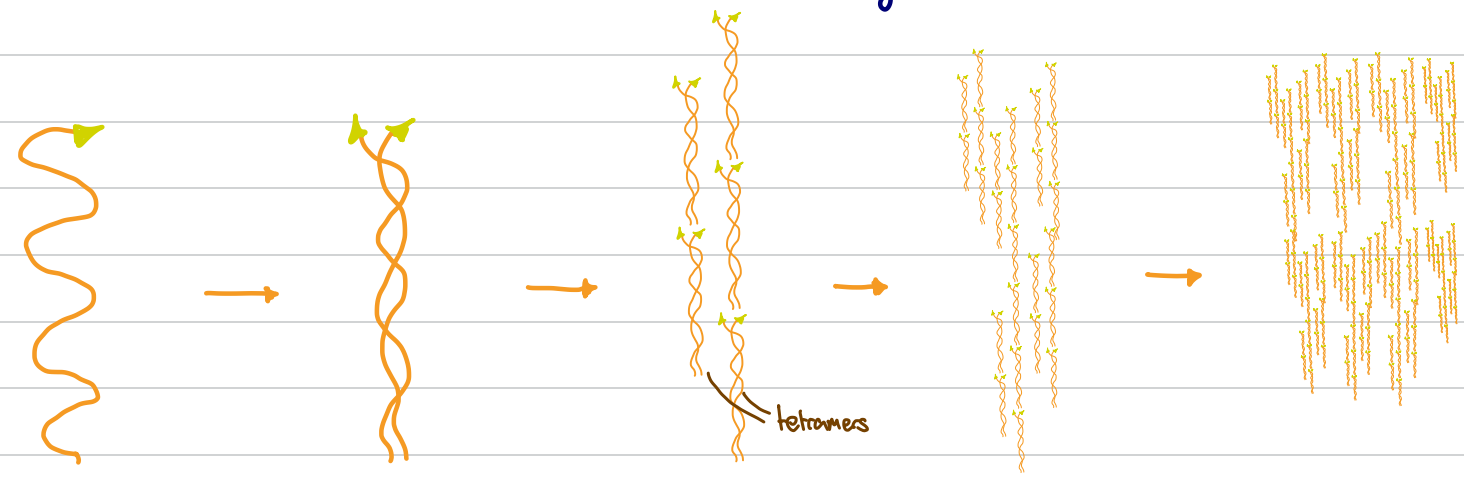





elastic fiber
hydrophobic interactions break
STRETCH
RELAX
hydrophobic interactions reform
single elastin molecule
cross-link

α - keratins:

* Found in skin, nails & hair. Provides hardness + dryness



α-helix → Dimer → Protofilament → Protofibril → Intermediate Filament

Disulfide Cross-links

* Two α-helix twist & form dimers
* Two dimers cross-link & form tetramers. Tetramers stack to form protofilaments
* 2 protofilaments form protofibril
* 4 protofibrils form intermediate filament ×2
* 8 intermediate filaments form microfibril ×2
* Many microfibrils make macrofibril
* Many macrofibrils make a single hair strand.

* Has many cysteines that form disulfide bridges.
  - ↳ Collagen / Elastin ⇒ lysine cross-links
  - ↳ Keratin ⇒ Cysteine cross-links (disulfide)

* Cysteine:
  - → Many in nails to harden them. Disulfide cross-links
  - → Curly hair has more cysteine than smooth hair... more disulfide cross-links

Temporary Waves:

* Starting straight hair...

* A bit curly due to H-bonding b/w keratin.

* Add water or heat which breaks H-bonding & makes hair more straight.

Permanent Waves:

* Using chemicals to break & reform disulfide bonds.

* Reducing agents to break disulfide bonds. Ammonium thioglycate

* Fix hair / make it curly. Use oxidizing agent H_2O_2 to reform disulfide bonds.

* The hair will be stuck in this new style due to disulfide bonds.

Globular Proteins:

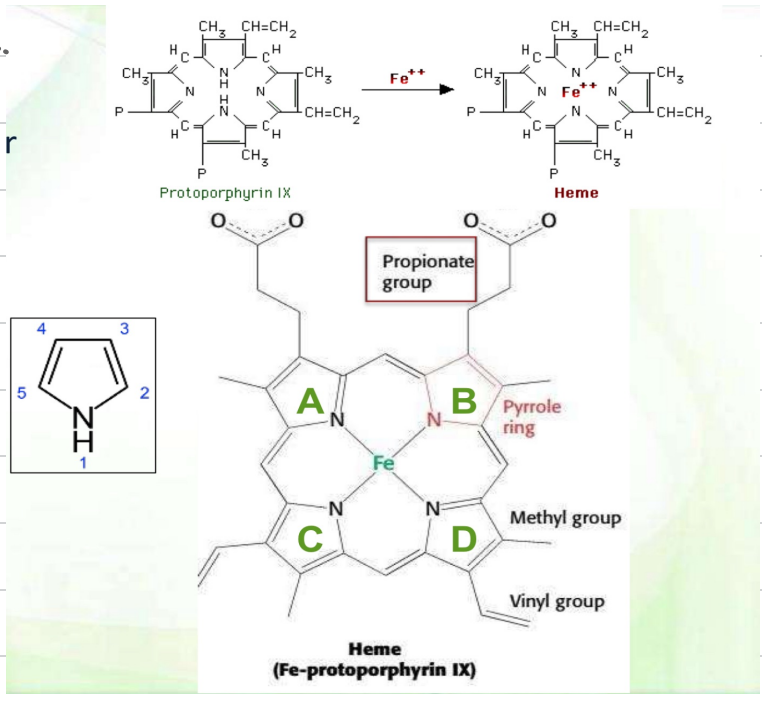
Hemoglobin: Present in blood. Transport CO_2 & O_2. Blood buffering (HCO_3^-/H_2CO_4)

Myoglobin: Present in muscles. Oxygen storage. Releases oxygen during hypoxia
↳ low O_2 in tissue

Heme = protoporphyrin IX + heme

Protoporphyrin & heme:

* Planar (flat)
* 4 rings called pyrrole rings (A → D)
* Very Hydrophobic
* Has two branched hydrophilic propionate groups
* Fe^{2+} + protoporphyrin IX = heme
* Since heme is hydrophobic... it is located in the inside of hemoglobin.
* Fe^{2+} forms 6 bonds. 4 with the 4 pyrrole rings of protoporphyrin. 1 with histidine of the globin. One with O_2.
* Heme is a prosthetic group. Non-protein part.
* Has methyl & vinyl groups
* Heme gives a red color



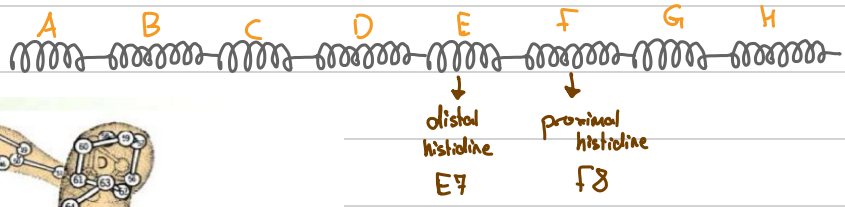
Other heme proteins: (heme + protein)

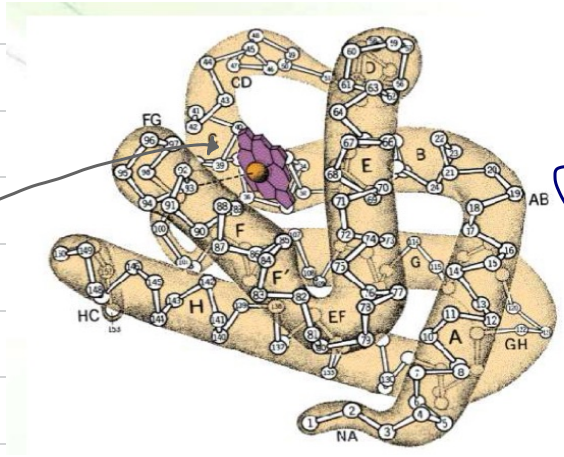
→ cytochromes - e^- transfer
→ Sensor proteins - senses heme & gases $CO/O_2/NO$
→ NOS, P450 - oxygenation

Myoglobin Structure:

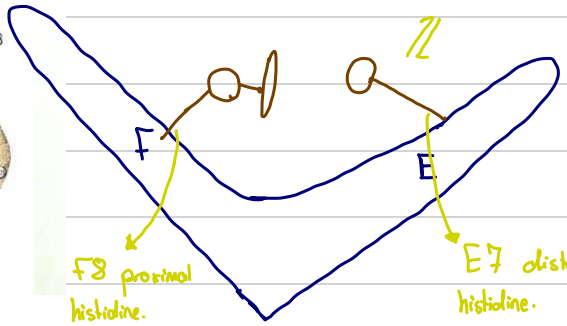
* One subunit.. not 4. Tertiary Structure NOT quaternary

* Made of 8 α-helices (A → H)





globin fold.
Inside.. so hydrophobic
heme hydrophobic.



F8 proximal histidine. Directly binds to heme. Fe^{2+} has 1 free bond with oxygen.

E7 distal histidine. Acts like a gate. Stabalization. Lowers CO affinity.

* Proximal histidine (F8) & distal histidine (E7) are present internally even tho they are hydrophilic.

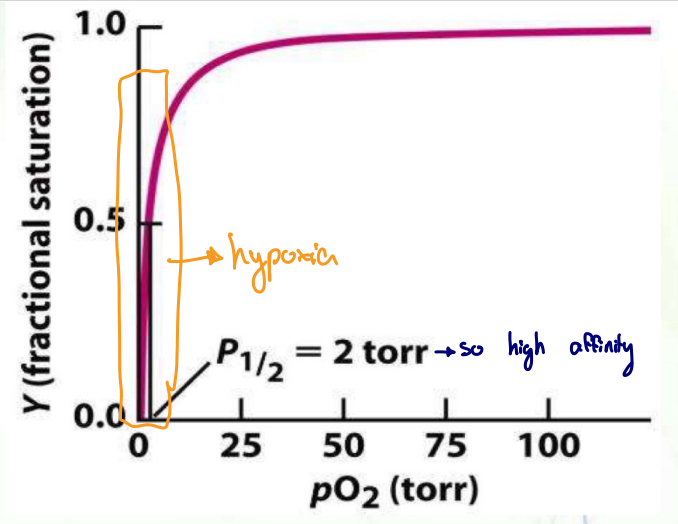
* Heme stabalized by hydrophobic interactions

* Heme stabalizes tertiary interaction but not shape-determining

* Hydrophobic region surrounding heme ensures it remains ferrous (Fe^{2+}) & not ferric (Fe^{3+}) iron.

* Distal histidine (E7) stabalizes by forming hydrogen bonding

Myoglobin Saturation curve:



* Hyperbolic curve

* Lower P50 means higher affinity..

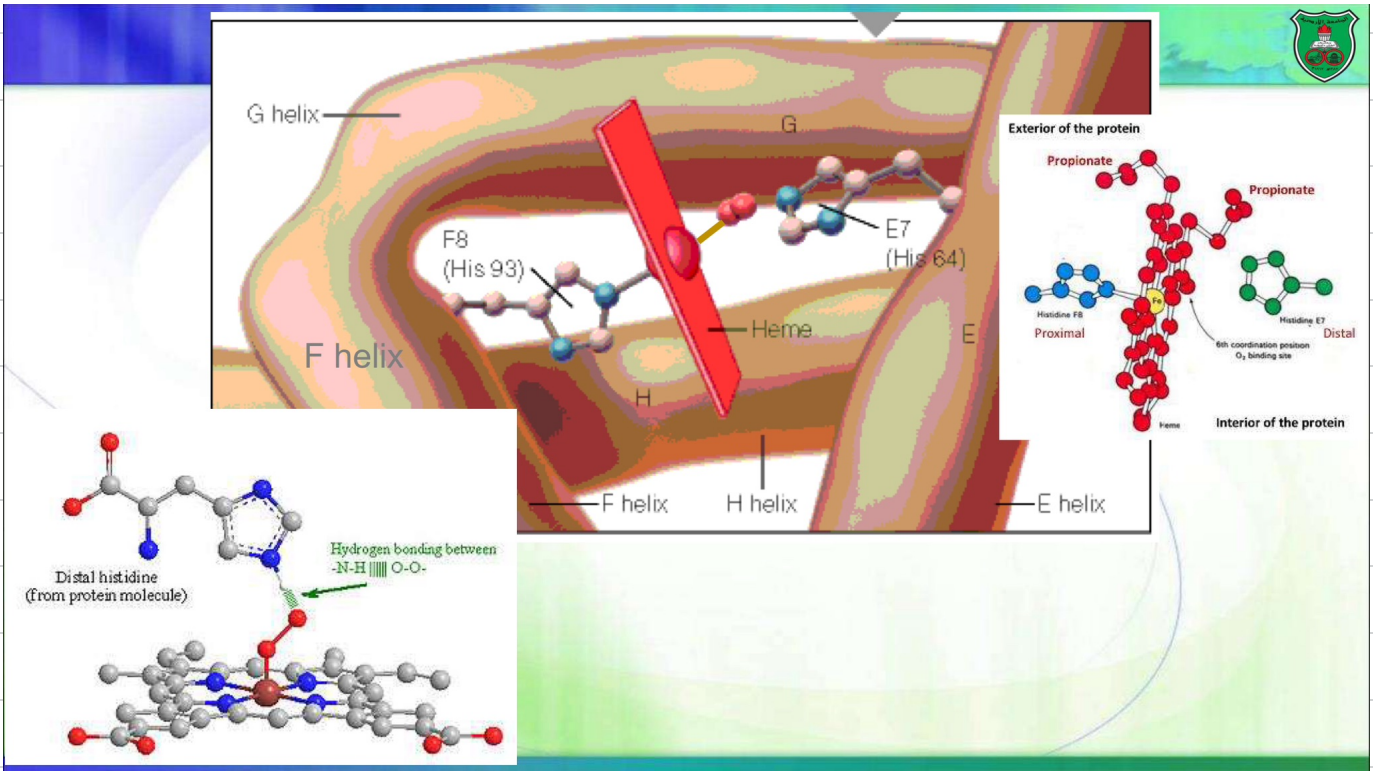
* P50 = pO_2 at which half of myoglobin/hemoglobin is saturated.

* Myoglobin very low P50 (~2.8 torr/mmHg) so very very high affinity for O_2.

* As pO_2 increases.. affinity for oxygen increases

* At low pO_2 (hypoxia) the oxymyoglobin affinity decreases & releases O_2 to the muscles forming deoxymyoglobin

* In normal conditions.. high oxygen affinity so it stores it.



Hemoglobin Structure:

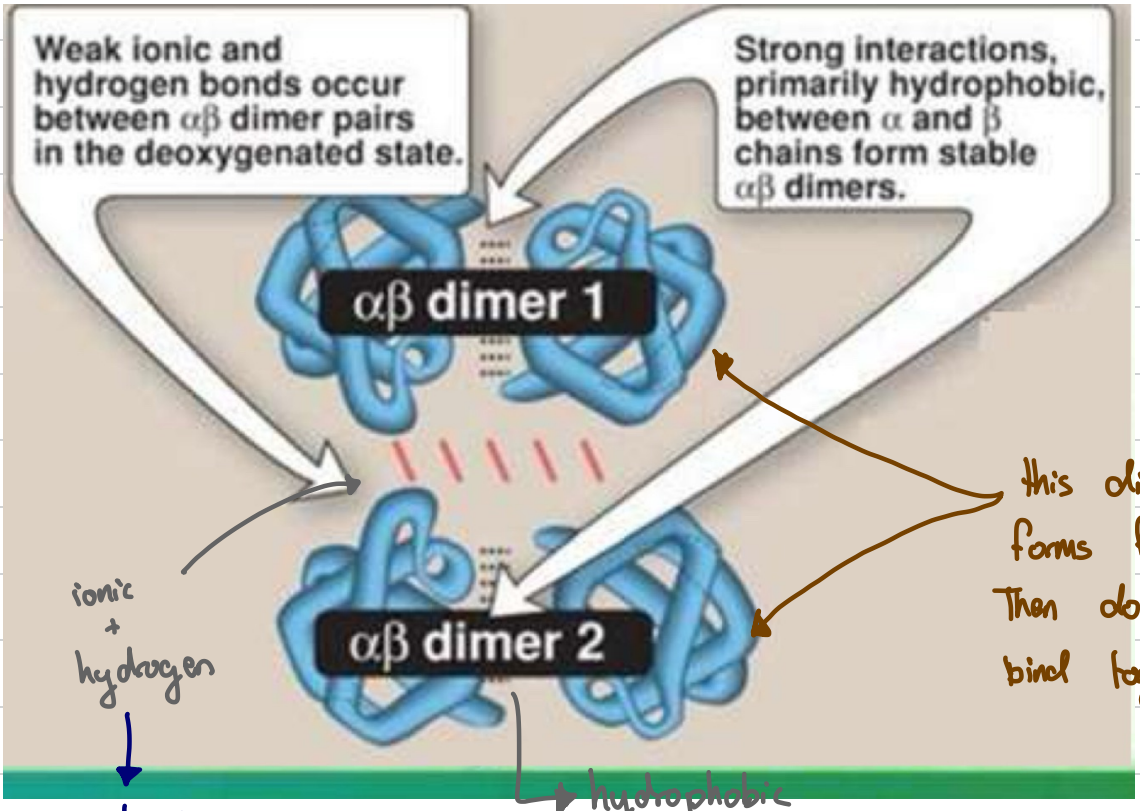
- Hemoglobin
  - heme prosthetic group
    - Ferrous Iron. Fe^{2+}
    - Protoporphyrin IX
  - 2 α-globin + 2 β-globin ⇒ α2 β2 tetrameric hemeprotein (quaternary)
    - 141 a.a
    - 146 a.a

* 4 subunits. Quaternary

* 2 α and 2 β ⇒ two αβ-protomers. So $\alpha_1 + \beta_1 = \alpha_1\beta_1$.
$\alpha_1\beta_1 + \alpha_2\beta_2 = (\alpha\text{-}\beta)_2$

* The αβ dimer is held together by many hydrophobic interactions (strong).

* One αβ dimer with other αβ dimer have electrostatic attractions and hydrogen-bonding.



When O_2 binds & we go from T state to R state.. these interactions are broken

Continued...

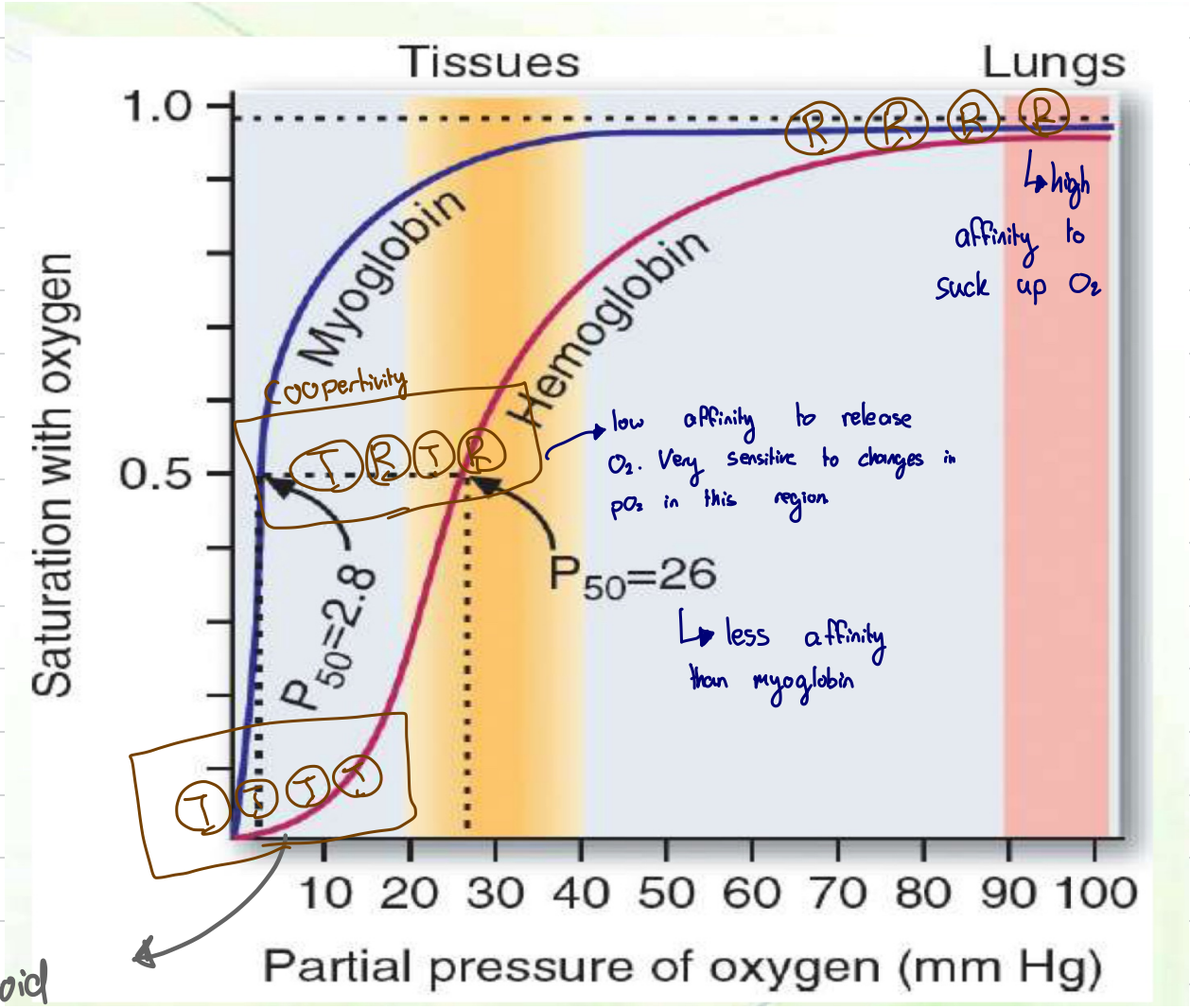
* Each globin has a heme group (protoporphyrin IX + Fe^{2+}). So one hemoglobin binds 4 O_2 molecules. 8 O atoms.

* α-globin less a.a than β-globin

* α-globin made of 7 α-helices (141 a.a)

* β-globin made of 8 α-helices (just like myoglobin) (146 a.a)

Hemoglobin Saturation Curve:



sigmoid
S-shape

Continued...

* Transport function.. so lower affinity than myoglobin

* Lungs high affinity. Tissue low affinity. More pO_2 the more affinity.

* Less affinity than myoglobin (p50 ~ 25 torr)

* Curve is sigmoid (s-shape). 3 phases.

Why is hemoglobin saturation curve sigmoidal ?

→ Hemoglobin is allosteric protein.. when first O_2 binds it changes shape and increases affinity for second O_2 to bind.

→ T-state. Tense / Taught state. Low affinity of oxygen

→ R-state. Relaxed state. High affinity for oxygen. 500x more affinity.

→ When first O_2 binds, hemoglobin changes from T-state to R-state. Increasing affinity for second O_2 to bind and so on.

→ This is called coopertivity.

→ Oxygen is a positive homotropic effector.

Positive => increase affinity

Homotropic => homo → oxygen changes affinity for other oxygens... so homo since same.

H^+ is a negative heterotrophic effector
↳ ↓ affinity ↳ H^+ & O_2 => different

How Does Hemoglobin structure change shape?

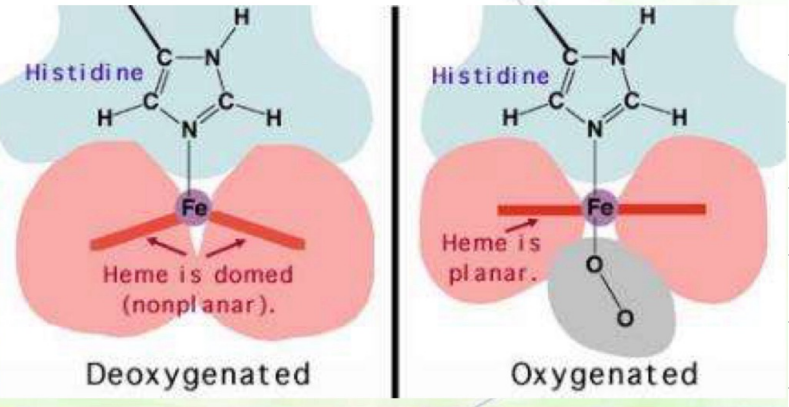
* Oxygen binds to Fe^{2+}.. pulls heme a tiny bit. Heme goes from dome (non-planar) to planar. Fe → — Fe — → F8 pulled a bit & pulls all other & changes shape
  ↳ domed due to hydrophobic heme repelled by proximal His F8

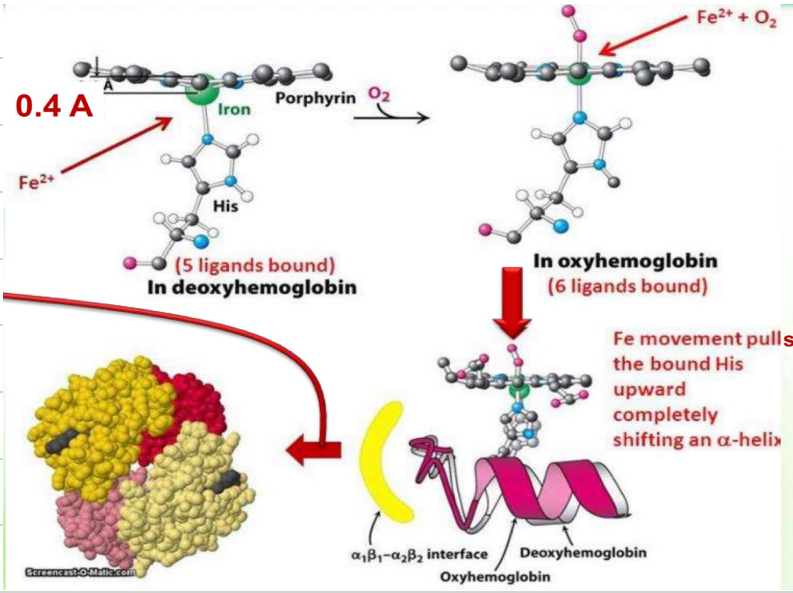
* The pulling of heme pulls amino acids a bit. This changes shape. The ionic & H-bonding between $\alpha_1\beta_1$ dimer & $\alpha_2\beta_2$ dimer are broken a bit.

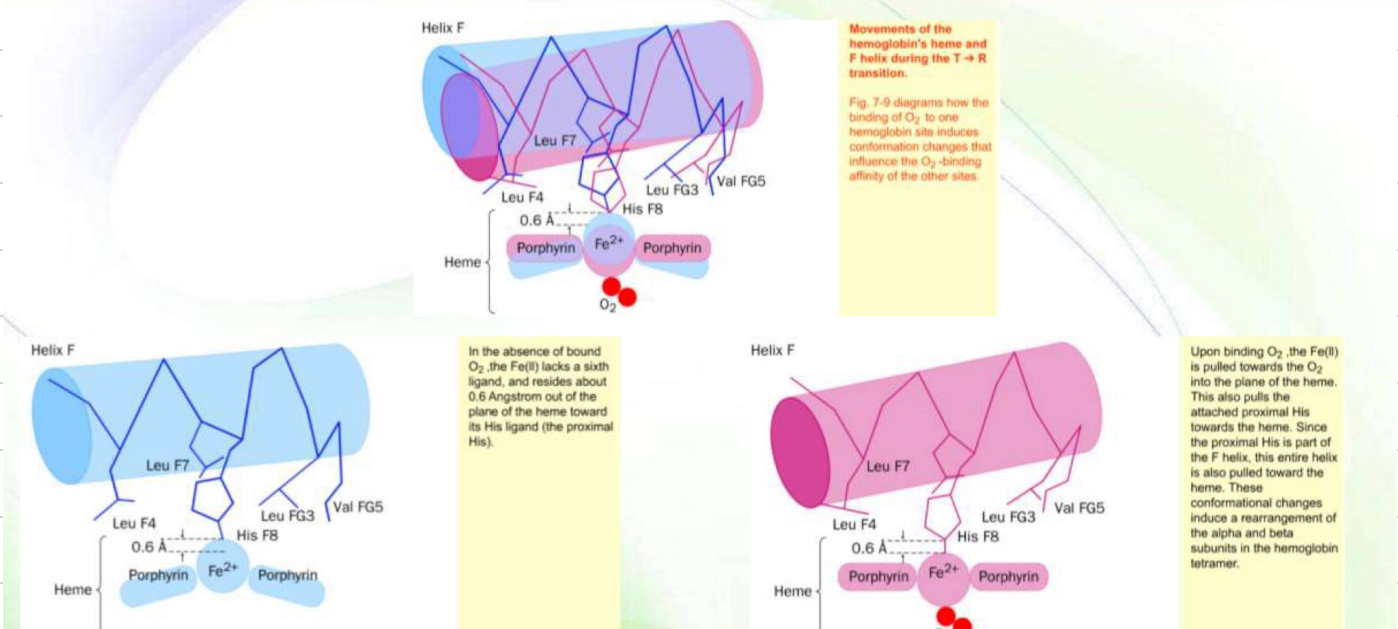
* All other heme change shape & go from T to R.

* Change in tertiary structure of all 4 globins

* In myoglobin... this change in heme structure does not affect function.







Summary:

T state... O_2 added → Distal His (E7) forms H-bonding with O_2 → Pulls proximal His (F8) → Heme goes from dome to flat → entire structure of all polypeptides changes → ionic & H-bonding break between $\alpha_1\beta_1$ & $\alpha_2\beta_2$ dimers. → change from low affinity T state to high affinity R-state → sigmoid graph.

Cooperativity Theories:

① Concerted Theory:

T T T T → R R R R. Either T or R... no intermediates

* When O_2 binds.. the probability that hemoglobin exists as R increases.

② Sequential Theory:

T T T T → R T T T → R R T T → R R R T → R R R R

Both theories are valid

Distal Histidine (E7) & CO affinity:

* When bond to Fe^{2+} in heme is linear... will be sooo stable & so high affinity.

* CO forms linear bond while O_2 forms bent bonds. So CO has thousands of times higher affinity than O_2.

* Distal His (E7) comes & bends the F8 – Fe – C ≡ O bond and thus decreasing the affinity to CO.

* After bending CO is 250 times more affinity than O_2.

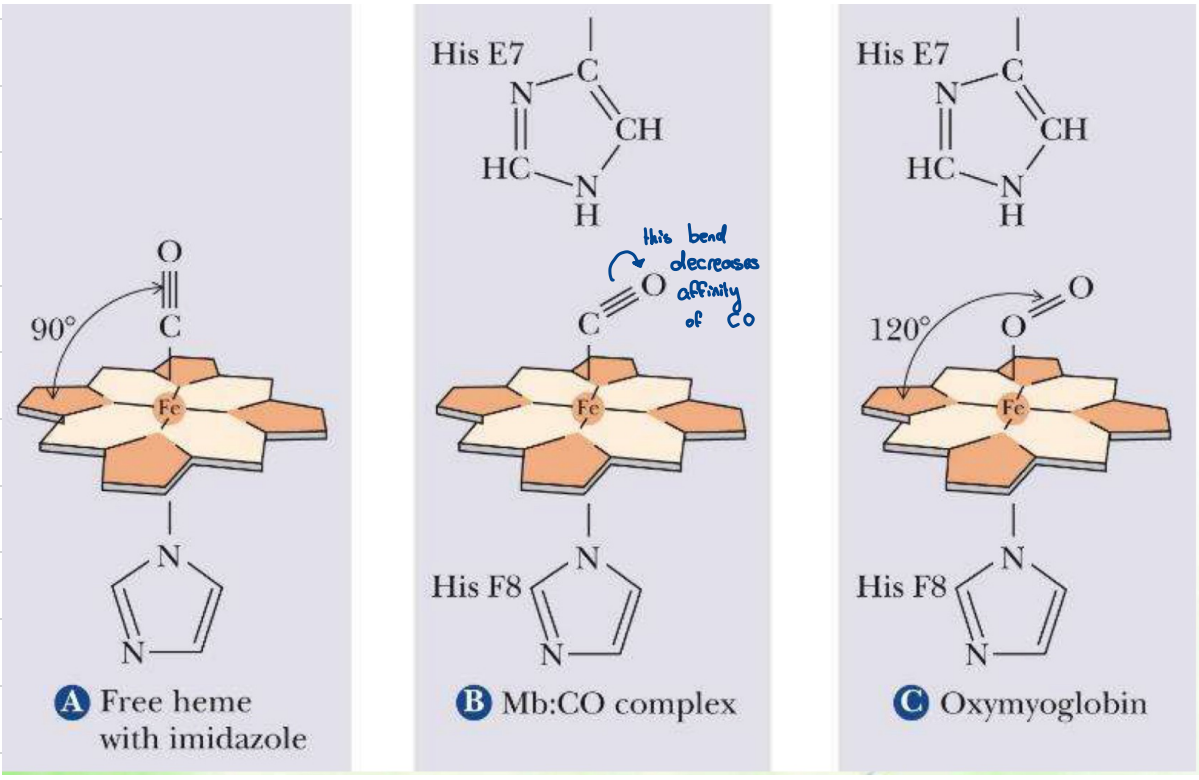
* So distal histidine has 3 function.

1→ Acts as gate.

2→ Stabilizes hemoglobin by H. bonding with O_2

3→ Reduces CO affinity by bending Fe - CO bond. Helps combat CO poisoning.

CO poisoning is FATAL !!



Types of Hemoglobin:

Adult hemoglobin:

* HbA1 ⇒ $\alpha_2\beta_2$ ($\alpha\beta + \alpha\beta$) major
* Hb A2 ⇒ $\alpha_2\delta_2$ ($\alpha\delta + \alpha\delta$) Minor
  - ↳ delta

⇒ synthesis of hemoglobin occurs in bone marrow in adults

* HbA1c ⇒ Glycosylated hemoglobin

→ non-enzymatic (like collagen)

→ Usually.. addition of Glucose onto Valine in the β-chain

→ Present in high amount in people with diabetes

→ HbA1c provides a long term test of blood sugar as opposed to blood fasting sugar.

Diabetes Sugar Levels:

	Fasting Blood Glucose	HbA1c	⇒ Status
①	< 100	< 5.7	normal
②	100 - 126	5.7 - 6.5	Pre DM
③	126 >	> 6.5	DM

↓

long-term (2-3) months

Glucose → Valine → β-chain

Fetal Hemoglobin:

* HbF ⇒ $\alpha_2\gamma_2$ (γ → gamma)

→ no β-chain.. doesn't bind with 2-3 DPG

→ High affinity. We need high affinity in fetus + embryo to steal oxygen from mother.

Embryonic Hemoglobin:

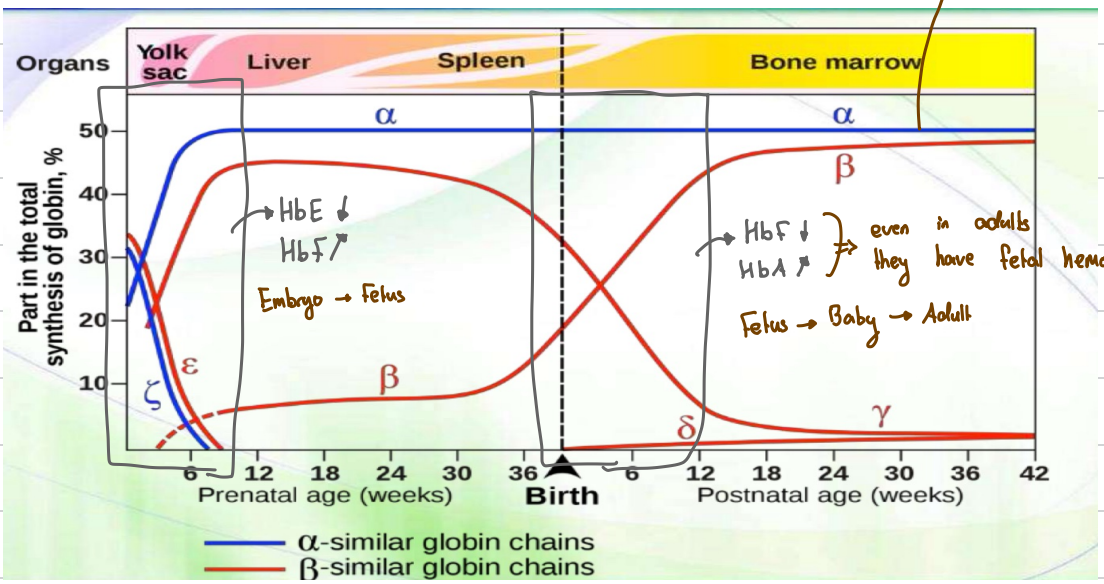
* HbE Gower 1 ⇒ $\zeta_2\varepsilon_2$ (ζ → zeta, ε → epsilon)

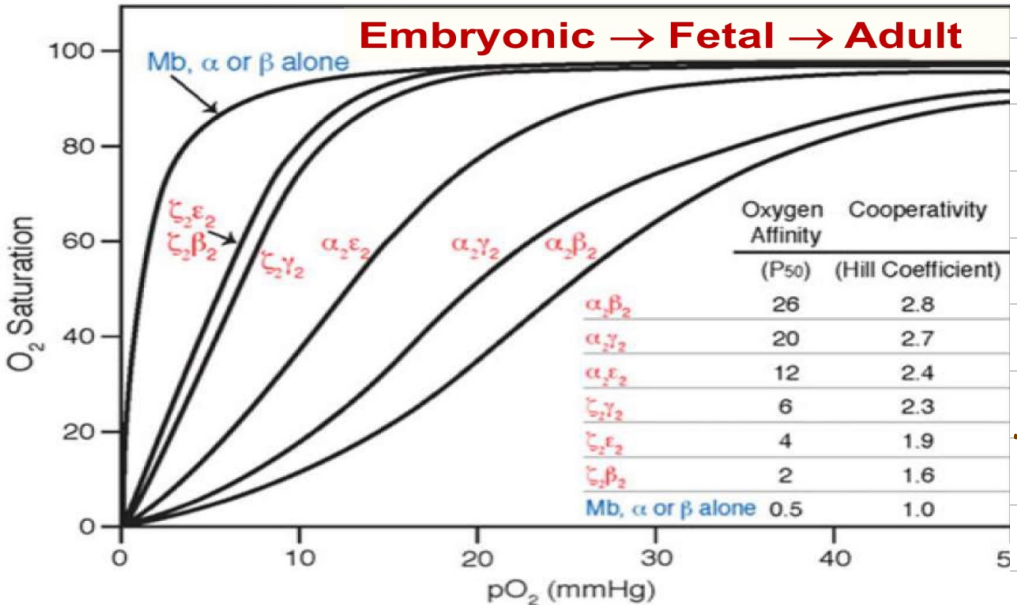
* Other forms

α is in both HbA & HbF

HbA: $\alpha_2\beta_2$ / $\alpha_2\delta_2$

HbF: $\alpha_2\gamma_2$





	Oxygen Affinity (P50)	Cooperativity (Hill Coefficient)
$\alpha_2\beta_2$	26	2.8
$\alpha_2\gamma_2$	20	2.7
$\alpha_2\varepsilon_2$	12	2.4
$\zeta_2\gamma_2$	6	2.3
$\zeta_2\varepsilon_2$	4	1.9
$\zeta_2\beta_2$	2	1.6
Mb, α or β alone	0.5	1.0

E → F → A

P_{50} increasing

Affinity decreasing

Affinity:

Emb. > Fetus > Adult

Regulation of hemoglobin function:

Allosteric effector => binds to allosteric protein to either increase or decrease affinity to ligand

Homotropic = same as ligand (O_2 in hemoglobin) (positive) → allosteric regulators

Heterotropic = different than ligand

Hemoglobin Negative Heterotropic effectors:

① $H^+ \nearrow$ / pH $\searrow$

② $CO_2 \nearrow$

③ 2-3 BPG $\nearrow$

④ $Cl^- \nearrow$

⑤ Heat $\nearrow$

all these have shift to right

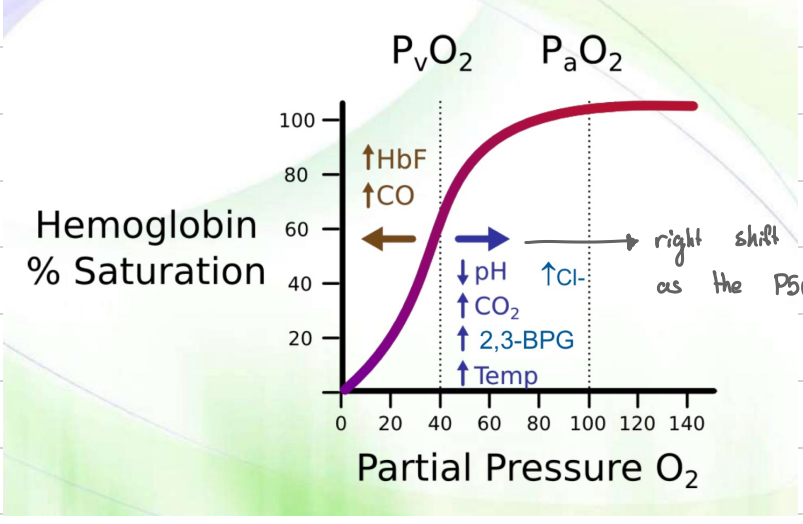
=> all these decrease HbA1 affinity to oxygen.

Think of a working tissue. Leg muscle while running. For the muscle the Hb needs to have low affinity so it releases its O_2.

$CO_2 \nearrow$ $H \nearrow$ pH $\searrow$ Heat $\nearrow$ 2-3 BPG $\nearrow$ $Cl \nearrow$

All these are a result of respiring tissue.. so with these the affinity for O_2 decreases.

↳ in lungs... high O_2 affinity and releases CO_2 also.



right shift decreases O_2 affinity as the P50 increases

H^+ & CO_2 directly propotional...

$$H_2O + CO_2 \underset{\text{carbonic anhydrase}}{\rightleftharpoons} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

HCO_3^- ↓ exits the RBC & transported in plasma

more CO_2 ... less pH ... lower O_2 affinity

How does H^+ (low pH) reduces O_2 affinity (bohr affect):

* High H^+ lowers O_2 affinity. Right shift. Bohr affect

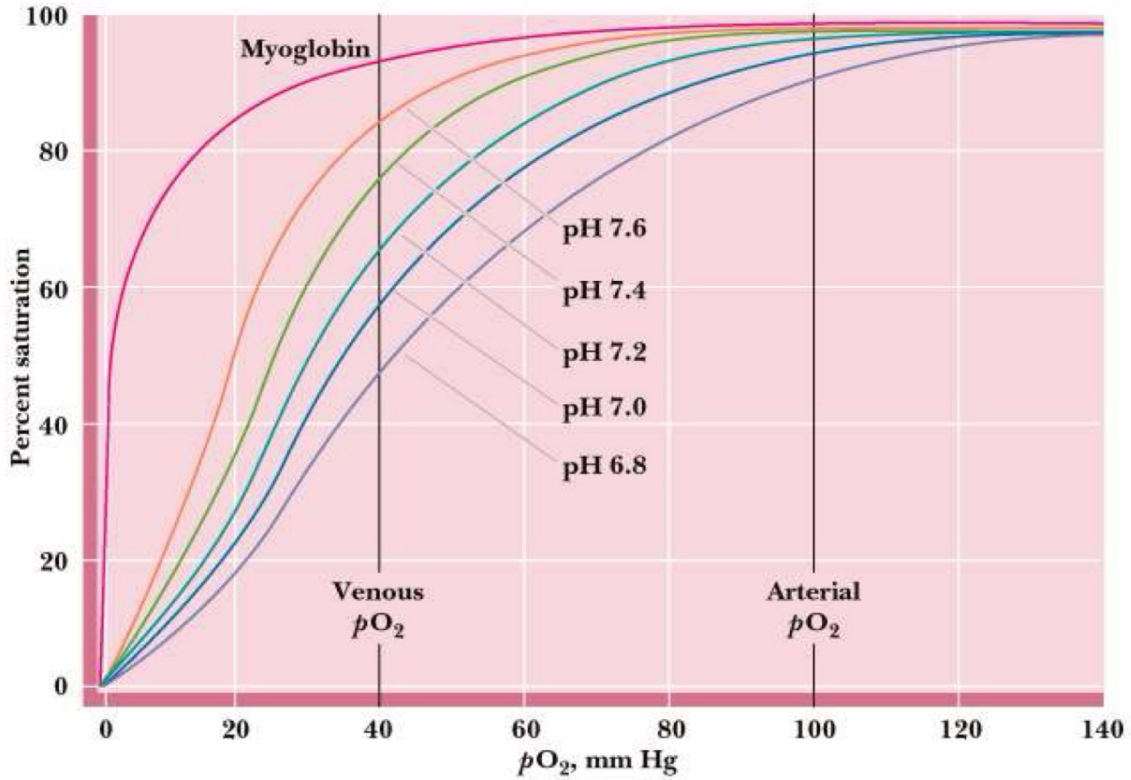
* Affects protonation. H^+ changes will change electrostatic attractions (His146 β + Lys α ; His146 β + Asp 94 β) which favor T-state

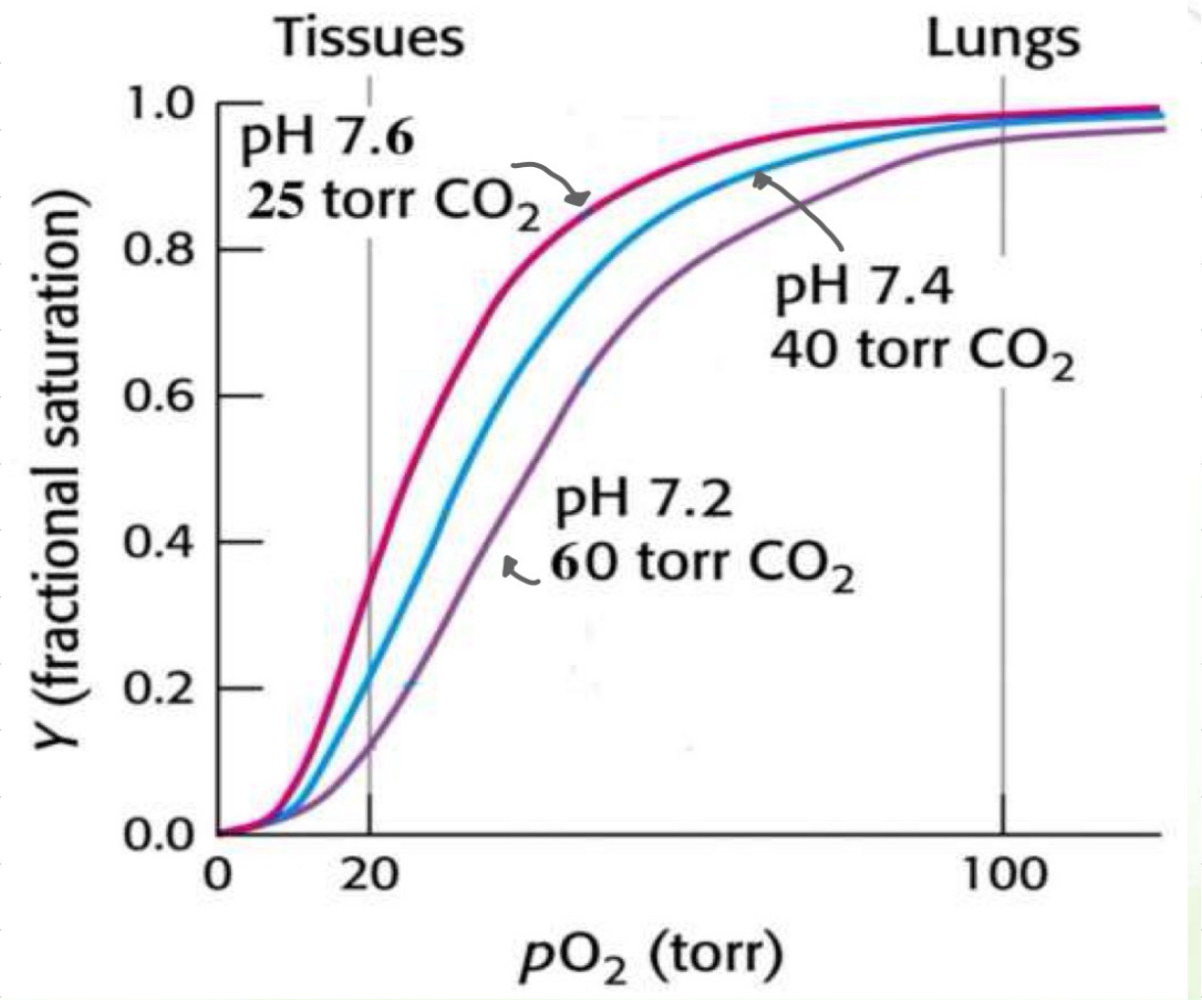
* High H^+ will protonate His 146 of β-chain

* pKa of His in T-state: 7.7 protonated
* pka of His in R-state: 7.3 deprotonated

* The low pH (extra H^+) will protonate His 146. This allows for formation of electrostatic attractions which stabilize the T-state.

* T-state favored = more H^+ means lower O_2 affinity


Myoglobin
pH 7.6
pH 7.4
pH 7.2
pH 7.0
pH 6.8
Venous
pO_2
Arterial
pO_2
Percent saturation
100
80
60
40
20
0
0
20
40
60
80
100
120
140
pO_2, mm Hg


Tissues
Lungs
pH 7.6
25 torr CO_2
pH 7.4
40 torr CO_2
pH 7.2
60 torr CO_2
Y (fractional saturation)
1.0
0.8
0.6
0.4
0.2
0.0
0
20
100
pO_2 (torr)

How does $Hb\text{-}CO_2$ lower O_2 affinity:

* CO_2 directly binds with Hb at α-amino N-terminals of globin chains.

* This binding forms carbaminoglobin ($Hb\text{-}CO_2$). The functional group is called carbamate & is negatively charged.

* $R\text{-}\underset{\underset{H}{|}}{N}\text{-}H + CO_2 \rightarrow R\text{-}\underset{\underset{H}{|}}{N}\text{-}\overset{\overset{O}{\|}}{C}\text{-}O^- + H^+$ (carbamate)

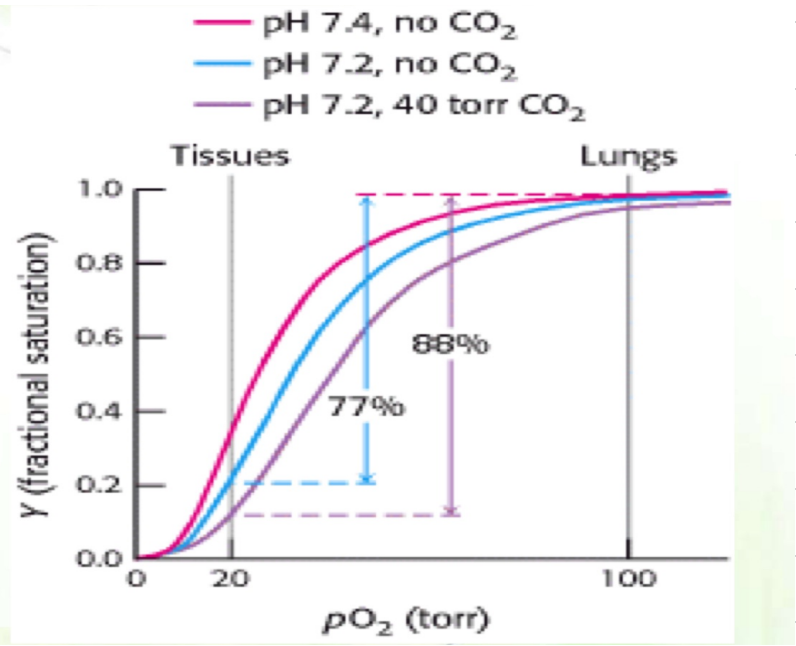
* The negative carbamate stabilize electrostatic attractions that stabilize the T-state.

① CO_2 → production of H^+ / HCO_3^- → H^+ stabalize T-state → Less O_2 affinity

② CO_2 → bind to amino N-terminals → -ve carbamate stabilize T-state → Less O_2 affinity

③ Cl^- (chloride shift) → electrostatic attractions in α-chain favour the T-state
↳ H^+ result in 75% in right shift
↳ $Hb\text{-}CO_2$ result in 25% in right shift

⇒ because majority of CO_2 transported in form of HCO_3^-/H^+

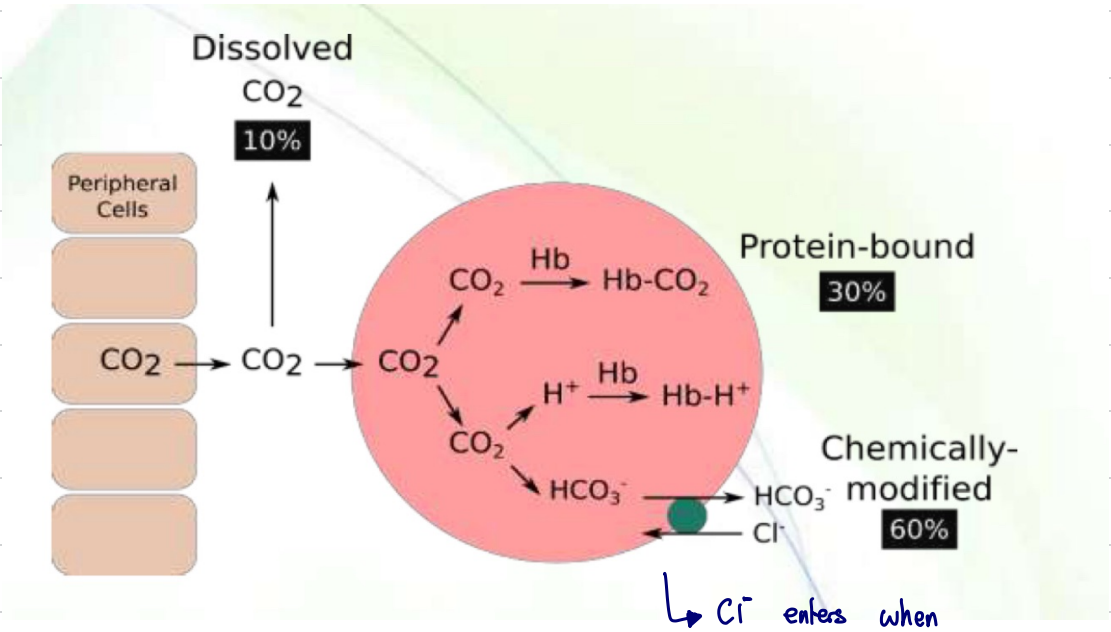


CO_2 transport & the isohydric shift:

1 → 60% - HCO_3^- in plasma & Hb-H^+ in RBC

2 → 30% - Hb-CO_2, carbamates. CO_2 directly bound to Hb

3 → 10% - dissolved in plasma



↳ Cl^- enters when HCO_3^- leaves. Chloride shift.

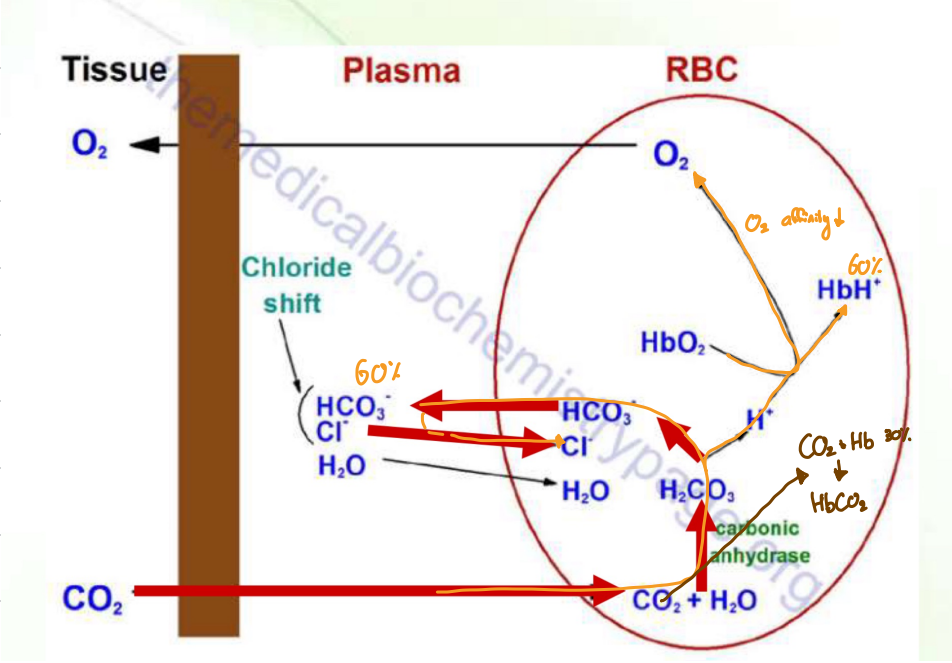
The Cl^- lowers O_2 affinity

Isohydric shift ⇒ constant pH maintained even with release of H^+, due to Hb acting as a buffer.

Chloride shift & how chloride decreases O_2 affinity:

* HCO_3^- leaves RBC & Cl^- enters RBC. (in venous blood where pCO_2 is high... in arteries like in lungs where pCO_2 is low & pO_2 is high the opposite happens.)

* Cl^- forms electrostatic attractions between N-terminus & Arg 141 in the α-chain. These interactions stabilize the T-state & lower O_2 affinity.



How does 2,3-Bisphosphoglycerate (2,3-BPG) reduce O_2 affinity:

Lack of O_2:

Glucose → 1,3 BPG → 3PG → Pyruvate → Lactate

- 1,3 BPG ⇄ (isomer) 2,3 BPG
- 2,3 BPG → (loses P_i) 3PG
- Pyruvate → Lactate: NADH → NAD+
  - gets reduced.
  - NADH oxidized to NAD+.
  - NAD+ recycled into glycolysis
- 2,3 BPG ↓ O_2 affinity.
- More O_2 unloading

* One 2,3 BPG binds to the central cavity of one Hb tetrode

* Forms electrostatic attractions with β-chain only!!! HbA2 & Hbf not affected by 2,3 BPG.

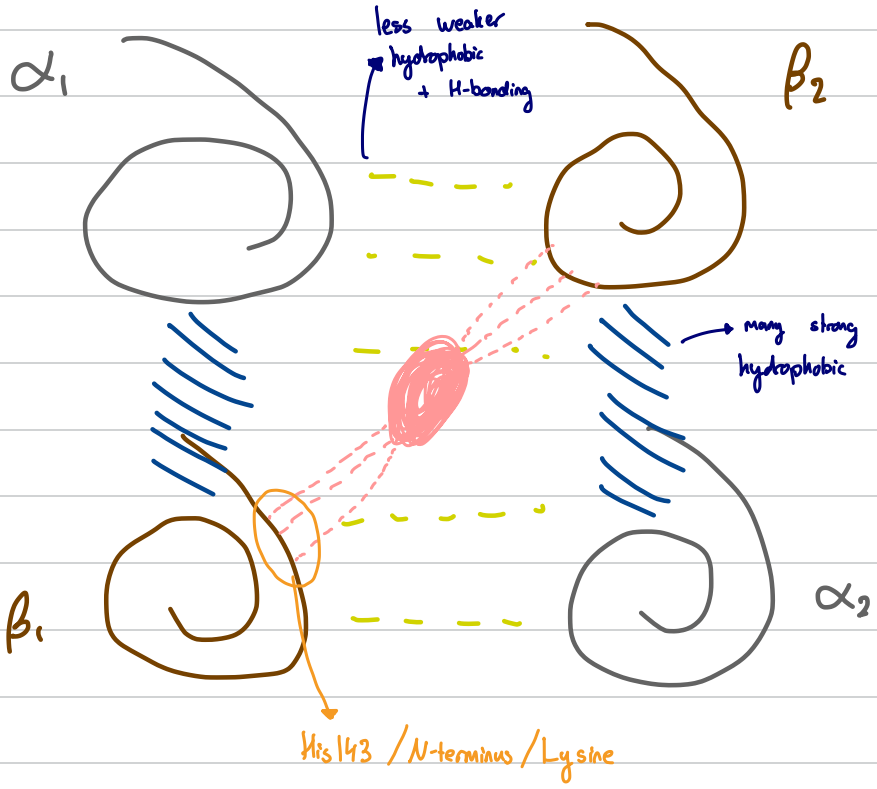
Attractions b/w 2,3 BPG:

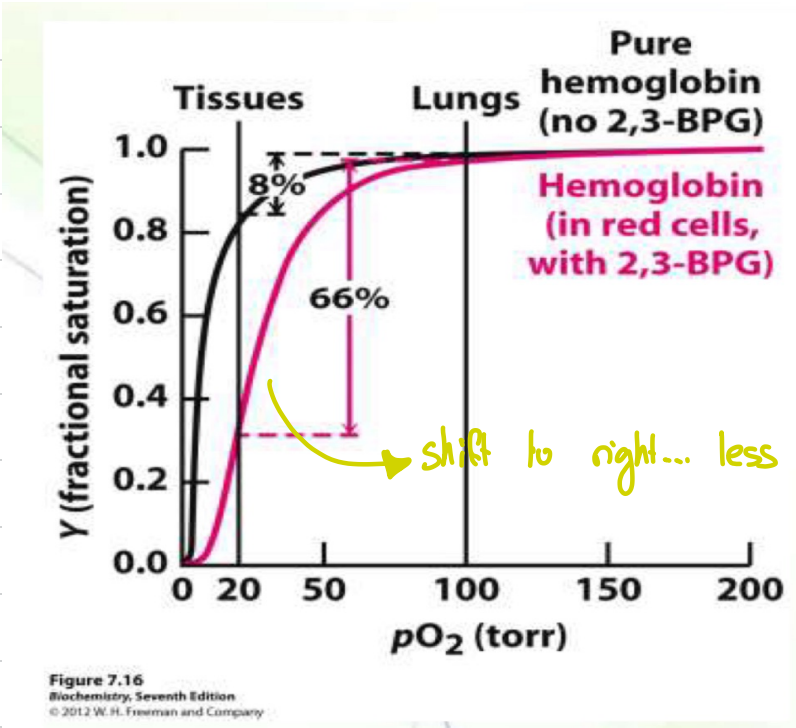
- Terminal N-terminus of both β-chains
- His 143 of β-chain
- Lysine of β-chain

* These attractions stabilize the T-state. Lowering O_2 affinity.

* Without 2,3 BPG the affinity is so high (P50 = 1). With 2,3 BPG P50 is 26 Torr (normal).

* 2,3 BPG is sooo important in lowering affinity of O_2 so that more O_2 is unloaded & that O_2 isn't just stuck onto Hb.



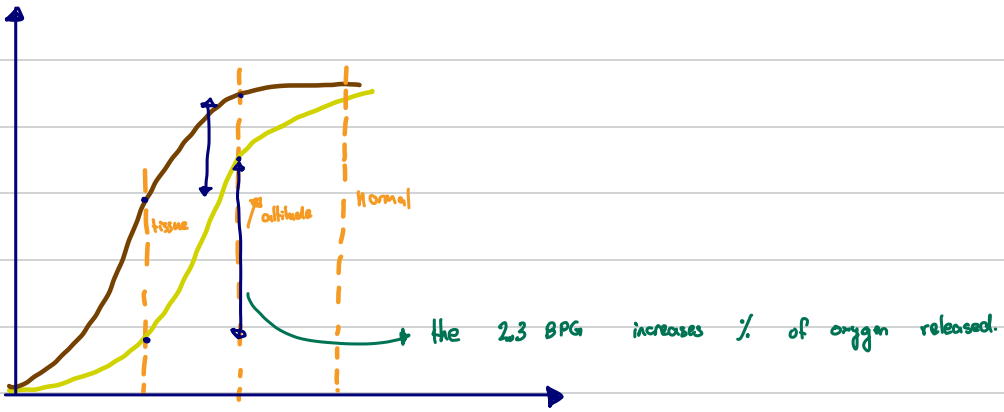


High altitudes & 2,3 -BPG:

* Low pO_2 high altitudes

* 2,3 BPG lowers O_2 affinity, more O_2 unloading

* Balance between less binding... more release & more binding ... less release.



2,3 BPG notes:

* When blood is stored... 2,3 BPG & ATP are broken down. So when blood given to patients we add the 2,3 BPG & ATP to the blood. Else the blood can trap O_2 due to high affinity for O_2.

* Both CO_2 & 2,3 BPG are important for lowering O_2 affinity to allow for unloading.

* Heat causes right shift. Also heat increases metabolism & increases 2,3 BPG levels.

fetal Hemoglobin (HbF) & 2,3 BPG:

* HbF => $\alpha_2\gamma_2$. No β-globin.

* γ-globin has **serine** in place of **His 143** of β-globin

* 2,3 BPG cant bind to γ... so no affect on HbF. HbF has high O_2 affinity. Needed to steal O_2 from mother.

CO & Hb:

* CO INCREASES Hb affinity to O_2. Holding onto O_2 more & not releasing it. Less O_2 unloading.

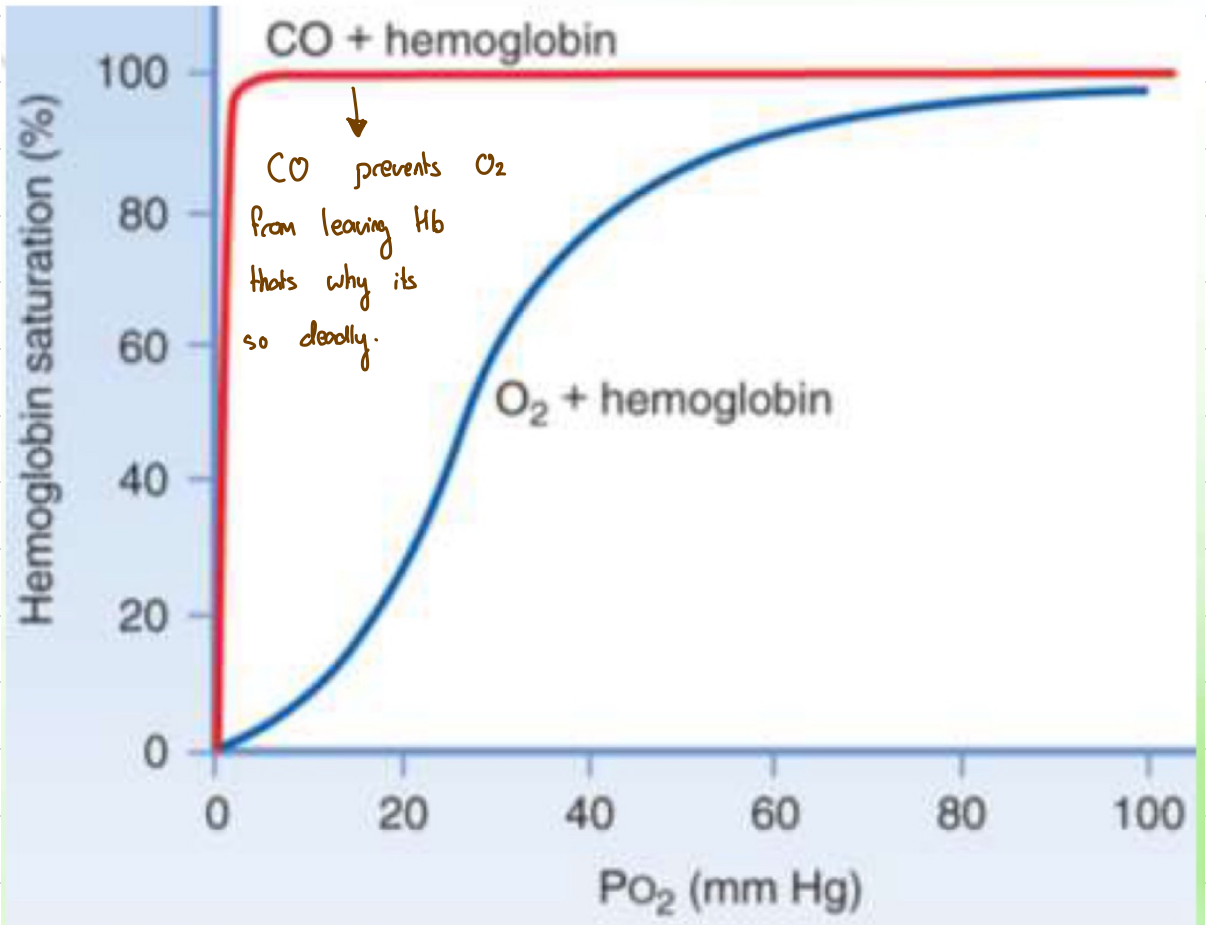
* CO 200 times more affinity than O_2. CO directly competes with O_2, it has same binding site. The proximal histidine, F8, directly binds with CO.

* CO causes shift to left. (CO stabilizes R-state.) Very high O_2 affinity... no release of O_2.

* Normal people: 1% Hb-CO

* Smokers: 10% Hb-CO

* Fatal levels: 40% Hb-CO



Immunoglobins:

Immunity Types:

① Innate defense . If Innate fails then ② adaptive defense takes over

innate defense

① Surface Barrier skin /mucus

② Internal Defense macrophages /neutrophils

Adaptive Defense

① Humoral Immunity B-cells → the B-cells release antibodies / immunoglobins

② Cellular Immunity T-cells

B-cell detects antigen

Divides & differentiates into antibody secreting cell... very large number of antibodies produced (2000/s)

How do immunoglobins work:

* very specific. They bind to specific antigens.

① ⇒ Inactivation & neutralization of viruses / toxins

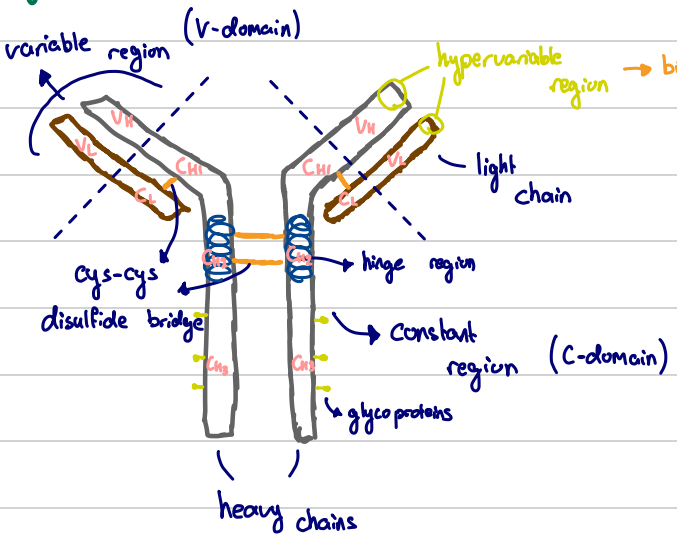
② ⇒ Induce phagocytosis.. they bind to pathogen & make its location clear to phagocytes

③ ⇒ Complement system ~ recruits WBC's & proteins which puncture pathogen and cause it to lyse.

* Antibodies don't directly kill pathogens

* We have more than 10^{11} different antibodies !

Antibody Structure:



* Each chain has more than one domain

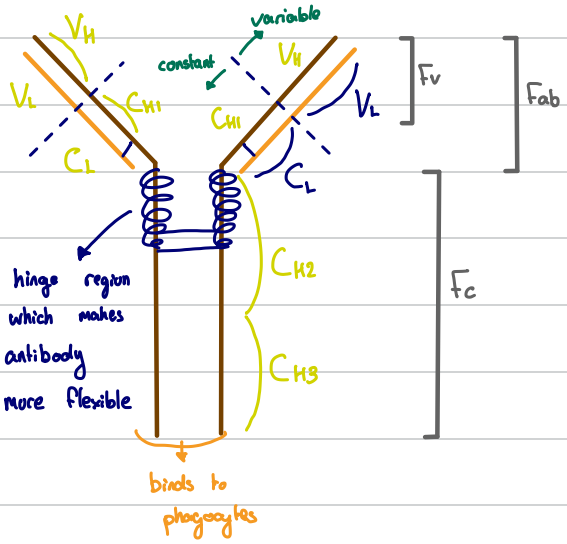
* Disulfide bonds between different chains.

* Disulfide within same chain (intra-chain disulfide bond)

* Contains glycoproteins. protein alot + oligosaccharide chain.

* Light chain => one variable region V_L + one constant region C_L

* Heavy chain => one variable region V_H + three constant regions $C_{H1} + C_{H2} + C_{H3}$



* Right side chains = Left side chains

* V_L pairs V_H. C_L pairs C_{H1}

* For same isotype all antibodies have the same constant regions.. C_L / C_{H1} / C_{H2} / C_{H3}

* Fc regions binds to phagocyte immune cells

↳ Fc = $C_{H2} + C_{H3}$

The variable region Fv (V_L + V_H):

* Has 7 → 12 amino acids that contribute to antigen-binding site. This amino acid sequence is <u>different</u> in different anti-bodies

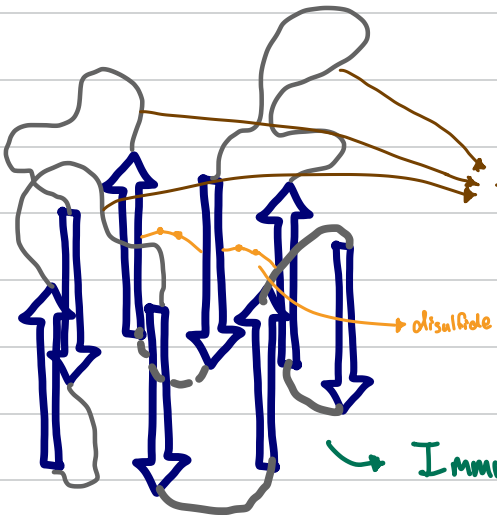
* Each B-cell produces one type of unique antibody. The variable region is different in every single antibodies. The constant region remains the same for the same isotype.

* Each antibody has its own unique very specific region called: Hypervariable Region OR Complemetarity Determing Regions. (CDRs)

* The CDRs are present inside the immunoglobin fold. They are <u>three</u> loops connecting the β-sheets

* Immunoglobin fold made up of β-sheets in a circular barrel shape which are held together by disulfide bridges. Immunoglobin fold provide specificity and allow antibody to bind to antigens.

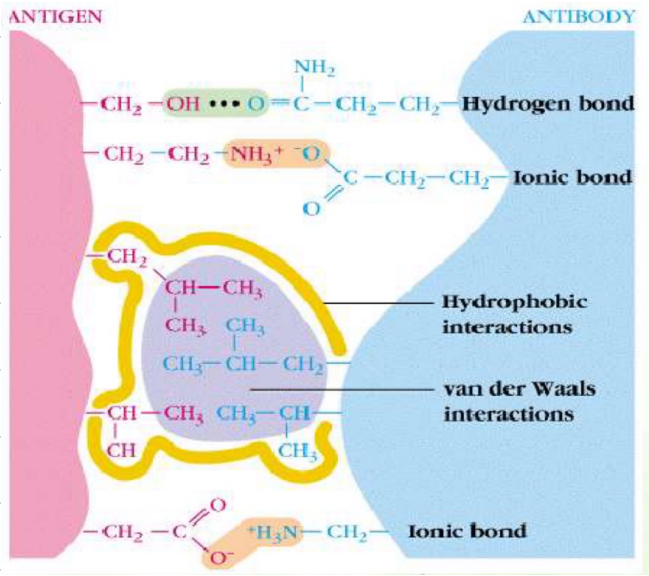
* Purpose of CDRs?
↳ Recognize & bind with antigens with high affinity / low K_D
↳ K_D is rate at which antibody dissociates from antigen. High K_D means low affinity. Inversely proportional



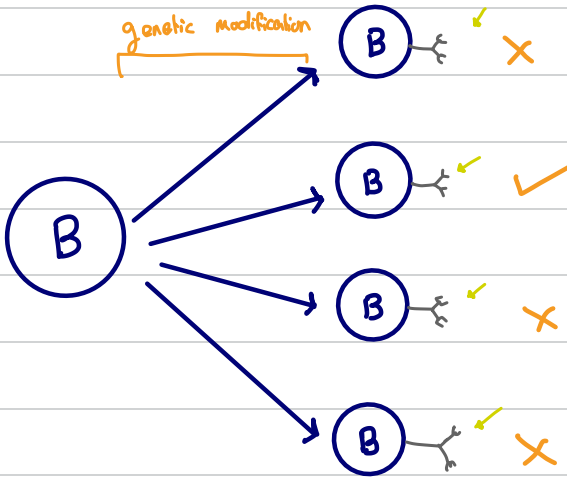
↳ Immunoglobin fold → Binds to low K_D antigens

Antibody - Antigen interactions:

* Complementary interactions between variable region. Non-covalent interactions. Like Lego pieces



Diversity in Antibodies Variable Regions:



appropriate antibody for pathogen antigen.

B-cell keeps undergoing genetic mutations & producing many different antibodies until we find the correct one. This can take days to weeks.

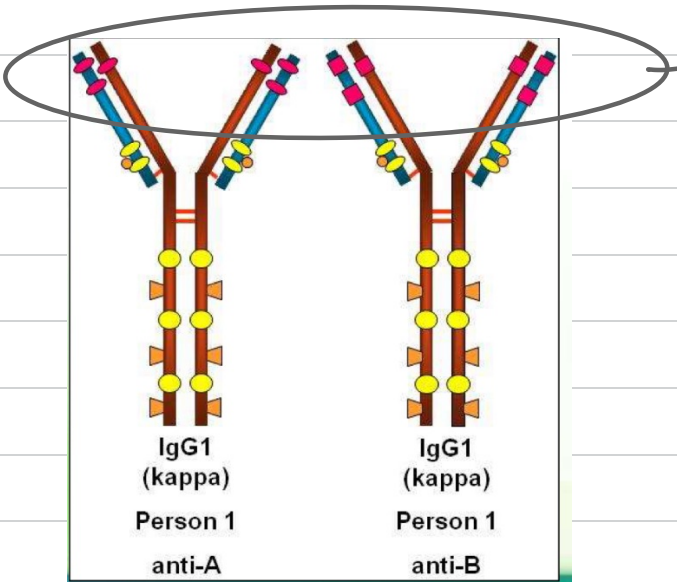
* Genetic variation by taking combination of genes. Genetic Recombination

* Genetic variation by undergoing genetic mutations. Changing sequence & length of hypervariable loops.

* For same antigen we can change the constant regions to give different functions.

Antibody Types:

① Idiotype => same constant region. Different variable regions.



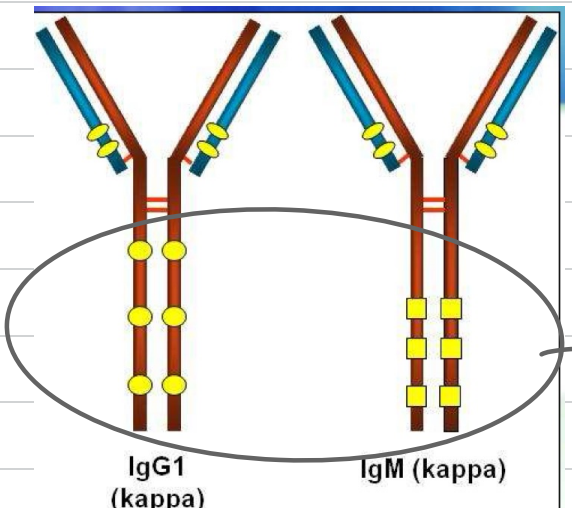
→ idiotypes:
only variable different.

V_L & V_H different

C_L & C_{H1} / C_{H2} / C_{H3} different

* Same function but different antigens

② Isotypes => same variable region. Different constant heavy regions.



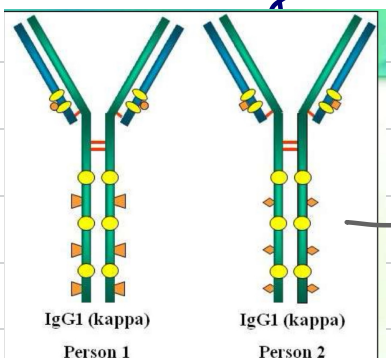
* Same antigen but different types

→ isotypes.

V_L & V_H same. C_{H1} / C_{H2} / C_{H3} different

C_L same.

③ Allotype => very slight difference in constant regions of same isotype due to different genetics in different people.



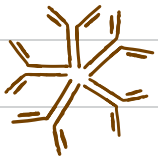
→ slight differences in the heavy regions. Same heavy chain type.

Isotypes: Important!

Different light chains: Lambda / Kappa

Different heavy chains: → same variable region but the constant changes. So same antigen-binding but different propeties of the antibodies

① Mu - IgM

* exists as a pentamer... binds to 10 antigens. 

* exists as a monomer on surface of plasma cells. Antigen receptor.

* First antibody produced & secreted

* Activates phagocytes & the complement protein system which kills the pathogen. It is the first defense.

② Gamma - Ig G

* exists as a monomer 

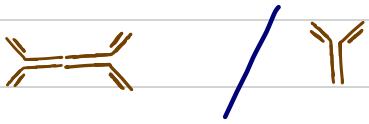
* Constantly being produced in blood. Due to past infections... it prevents future infections

* Most abundant anti-body in blood (600 - 1800 mg/dl)

* Only antibody which crosses the placenta... gives fetus immunity. When baby is born it still has immunity from mother for a period of time.

* Activates phagocytes & the complement protein system which kills the pathogen.

③ Alpha - Ig A

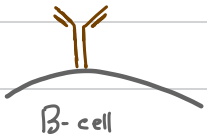
* Usually exists as dimers 

* Present on mucousal secretions (barriers)

* NO recruitment of protein complement system

④ Delta - Ig D

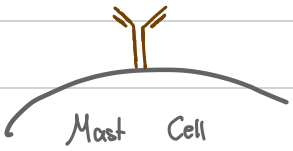
* On surface of B-cells NOT exposed to antigens. Ig M & Ig D are before antigen.. they are removed in class switch.



⑤ Epsilon - Ig E

* Present on mast cells

* Responsible for allergic reactions



* Released as a monomer

Class - Switch:

→ ① M ② G ③ A

① B-cell has antigen receptor.. no antigen.

→ IgD + IgM

② Antigen detected & IgM released. B-cell now has IgG.

→ IgD & IgM switched to IgG

→ IgG released constantly as long-term immunity. Most abundant.

③ Sometimes IgG switched to IgA

→ IgA dimers released which act in mucousal secretions.

Class switching due to DNA rearrangment.

Monoclonal Antibodies:

* Very specific

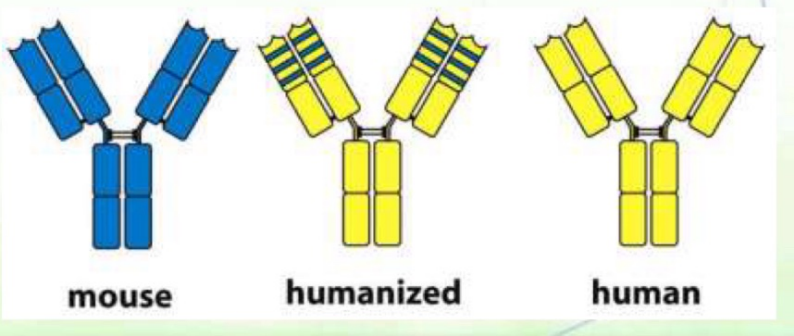
* Made by fusing a myeloma cancer cell with a plasma cell.
→ This forms a hybridoma cell
→ Hybridoma immortal
→ Hybridoma keeps dividing & producing many antibodies
→ Different plasma cells result in polyclonal antibodies. The hybridoma produces monoclonal antibodies.

* Inject antigen in mouse

* Extract & isolate polyclonal plasma cell from mouse

* Fuse myeloma (↳ B-cancer cell) with plasma cell to produce the hybridoma.

* We then humanize the antibodies by taking the mouse CDRs and attaching them to human antibodies



Benefits:

→ measures the protein levels in the blood
→ Directs medicine to tumor cells
→ Determines the type of pathogen
→ Removes drugs from circulation if they reach toxic levels.

Plasma Proteins:

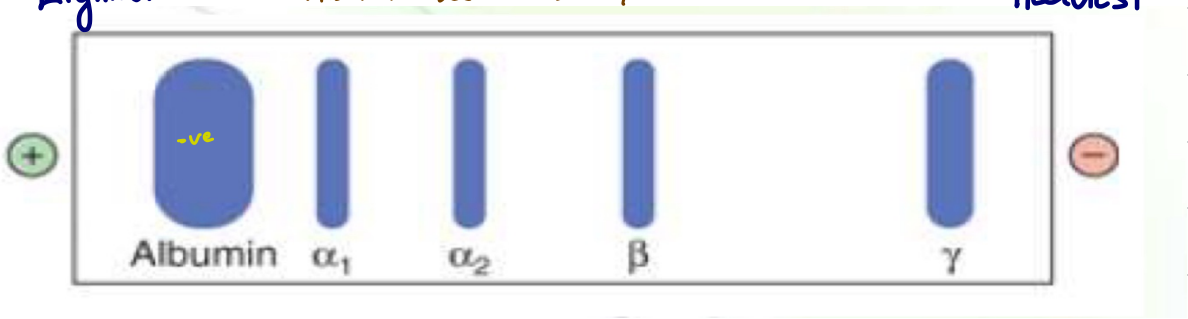
Blood
- Plasma 55% → contains many stuff like waste, proteins, amino acids sugars, lipids, inorganic ions etc.
- Cells 45%

Electrophoresis & Bands:

+5% + gets heavier

60 5 10 18 20

Lightest — Albumin > α1 > α2 > β1 > γ — Heaviest



Thicker Band ⇒ more %

Albumin Band:
* ~ 60 %

α1- Globulins Band
* ~ 5%
* α1- antitrypsin → an anti-protease. Neutralizes Trypsin / Elastase ... Protects tissues
* α1- fetoprotein → No info in slides.

α1 ⇒ AF

α2- Globulins Band:

* ~ 10 %
* Ceruloplasmin → Copper transport & iron oxidation
* Haptoglobin (Hp) → Hemoglobin binding and Iron protection
* α2-macroglobulin → Large. Zinc & cytokines transport. Protease inactivation.
  ↳ like antitrypsin

β- Globulins Band:

* ~ 15%
* C-reactive Protein (CRP) → Activates protein compliment system. Immune response.
* Transferrin → Iron transport (Fe^{3+})
* LDL

β ⇒ CT

γ- Globulins Band:

* ~ 20%
* Ig M → Pentamer. First Ig released. No inflammation
* Ig G → Monomer. Most abundant. Can cross placenta. Inflammation
* Ig A → Dimer. Mucousal secretions. No inflammation
* Ig D → Present on surface of B-cells not exposed to antigens.
* Ig E → On mast cells. Allergy.

Lightest → Heaviest:

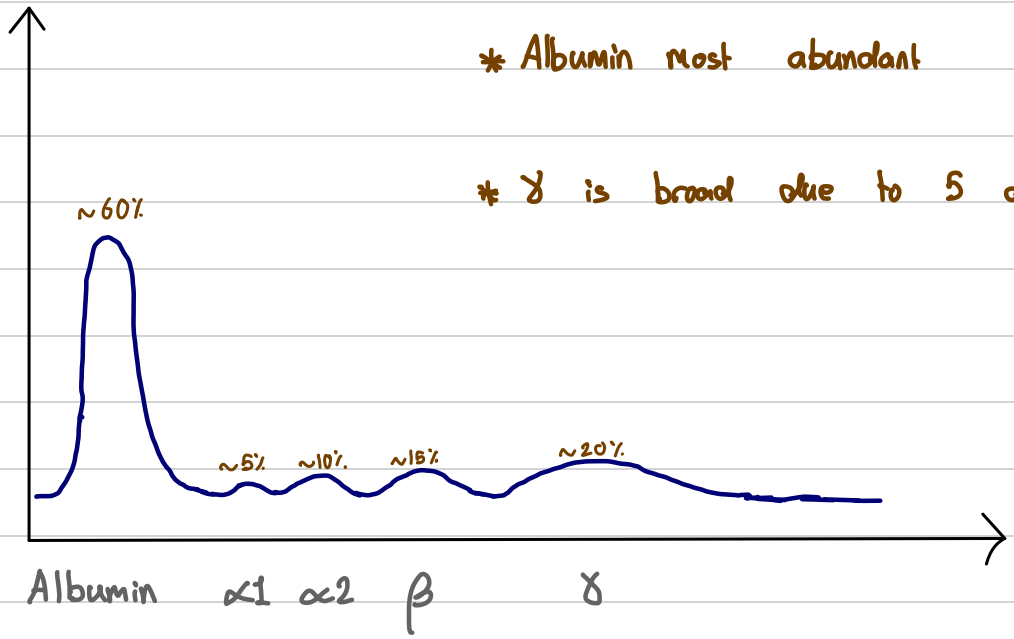
60% Albumin > 5% α-1 > 10% α-2 > 15% β > 20% γ

* α-1: antitrypsin, fetoprotein
* α-2: ceruloplasmin, Hepatoglobin, Macroglobulin
* β: Transferrin, CRP
* γ: Imunnoglobulins

α1 / α2 / β ⇒ acute phase proteins

Reading Electrophoresis Chromatographs:

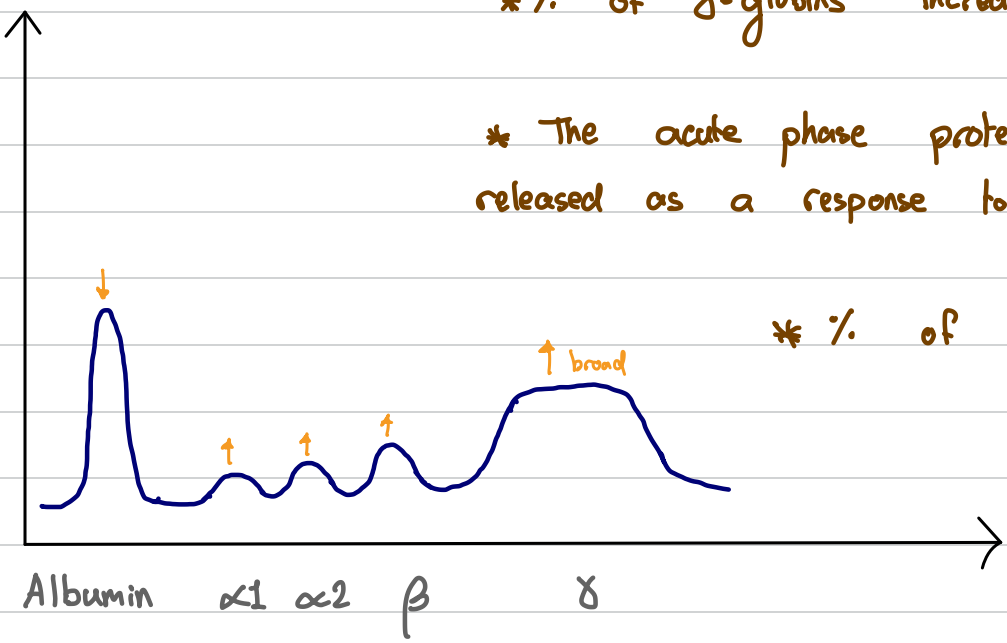
① Normal Levels:



* Albumin most abundant

* γ is broad due to 5 different immunoglobins

② Long standing inflammation / Delayed Response Pattern / Immune Response:



* % of γ-globins increase

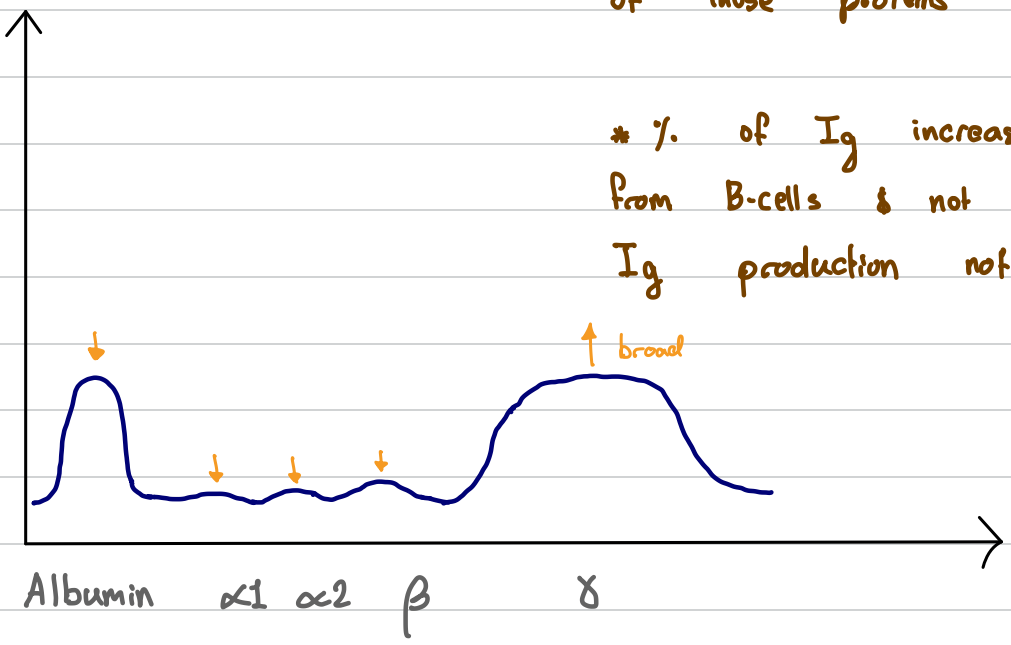
* The acute phase proteins increase, they are released as a response to inflammation.

* % of Albumin decreases

③ Chronic Liver Failure / Hepatic Cirrhosis / Polyclonal Gammopathy:

* Albumin & acute phase proteins decrease due to liver damage. Less production of those proteins

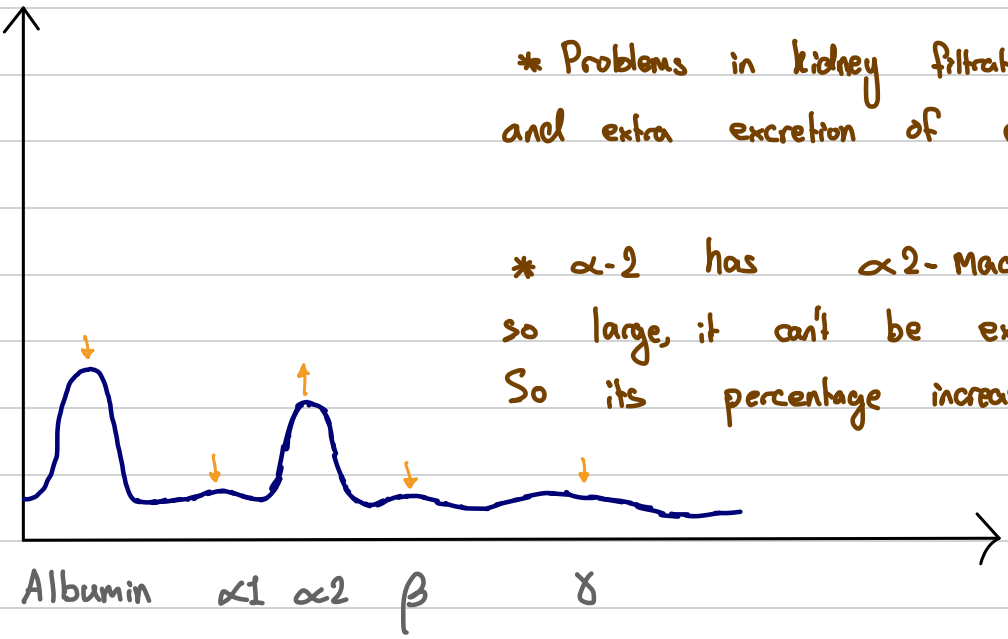
* % of Ig increases cuz Ig produced from B-cells & not from liver cells. So Ig production not affected.



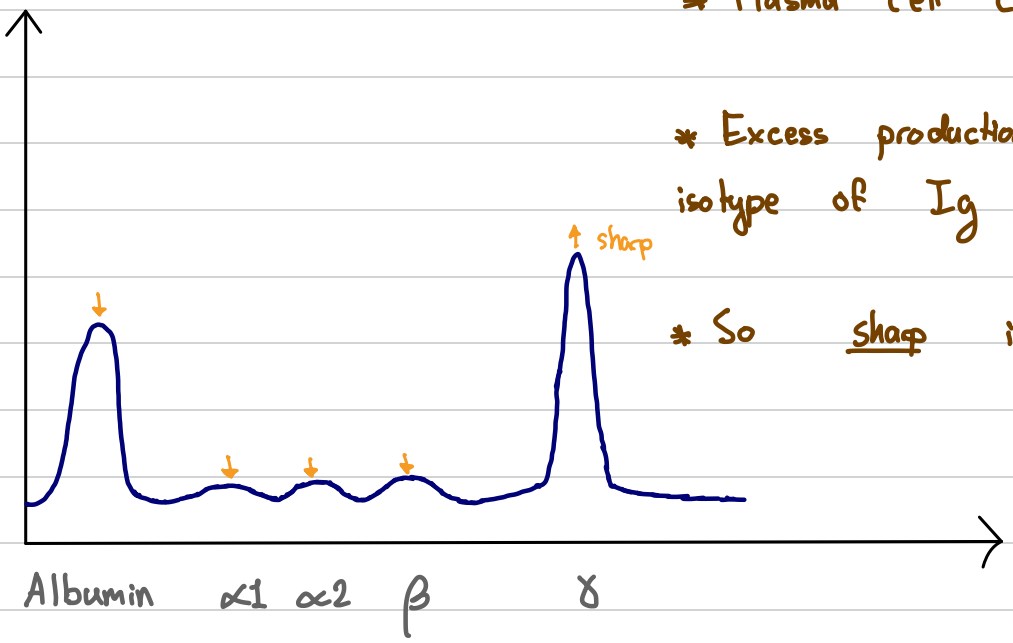
④ Nephrotic Syndrome / Protein losing enteropathy:

* Problems in kidney filtration. Weak filtering and extra excretion of proteins. Protein loss.

* α-2 has α2-Macroglobulin which is so large, it can't be excreted in nephrons. So its percentage increases as it stays in blood.

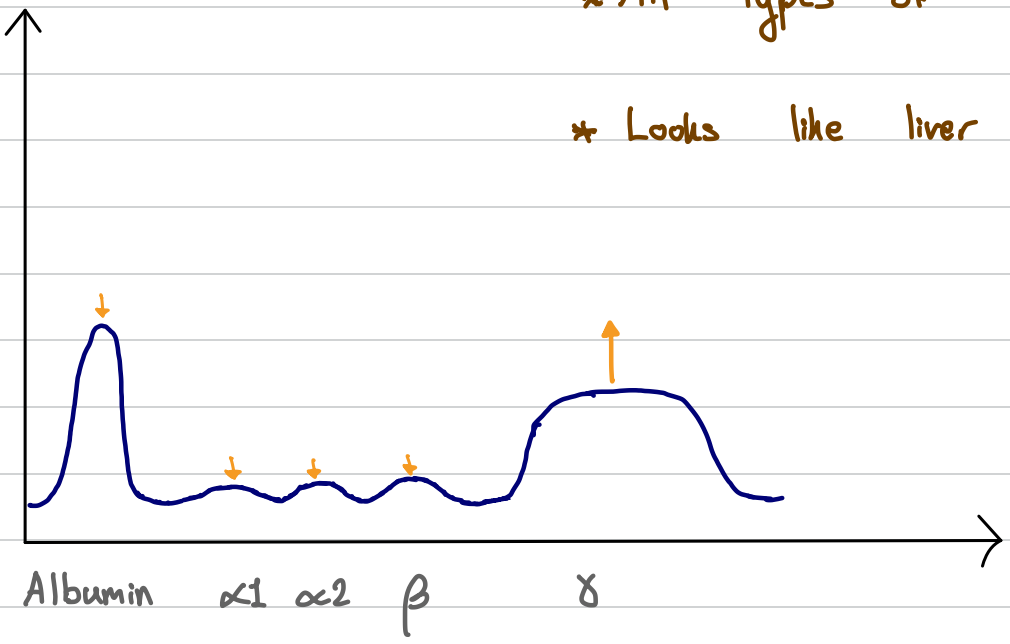


⑤ Plasma cell myeloma / Monoclonal gamopathy



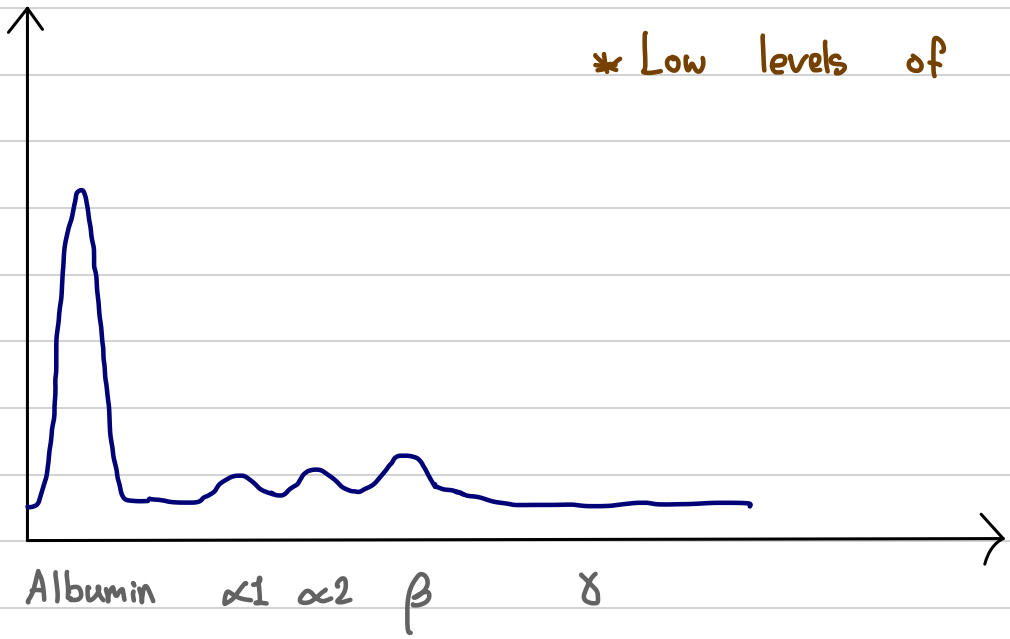
* Plasma cell cancer
* Excess production of only one isotype of Ig
* So sharp increase

⑥ Polyclonal Gammopathy:



* All types of Ig are released
* Looks like liver failure.

⑦ Hypogammaglobulinemia:



Plasma Protein Synthesis:

→ Most in Liver: Albumin + Acute phase proteins (α1-antitrypsin / α1-fetoprotein / α2-ceruloplasmins / α2-haptoglobins / α2-macroglobulins / β-transferrins / CRPs

→ Immunoglobins from plasma cells from the spleen, lymph nodes, bone marrow

→ Most are synthesized from pre-proteins (protein + signal peptide) except Albumin.

inactive preprotein $\xrightarrow[\text{cutting}]{\text{modification}}$ active protein + signal peptide

→ Albumin is preproprotein.

prepro albumin ⟶ pro albumin ⟶ albumin

→ Post-translation modification: Glycosylated (→ makes viscous glycoproteins) / Proteolysis / phosphorylation

→ Albumin IS NOT glycosylated. We don't want Albumin to be viscous. Most are glycoproteins except albumin.

→ Time to degrade (transit time) is 0.5 hr → few hours

Polymorphism:

→ The proteins have different shapes & traits according to different people due to genetics. Proteins have multiple forms.

→ Normal function

→ Monogenic / Mendelian trait ~ coded for by one gene.

→ Exists in >2 phenotypes

→ We can use electrophoresis OR isoelectric focusing to analyse the proteins

→ Examples: A/B/AB/O blood groups | eye color | hair color

Plasma Proteins half-lives:

- Albumin: 20 days
- Crohn's disease, Albumin: 1 day

reduction

Crohn's disease: Digestive tract inflammation. Protein-losing gastroenteropathy.

- Haptoglobin (α_2-globulin): 5 days
- Hemoglobin - Haptoglobin complex (Hb-Hp): 90 minutes

reduction

- Prealbumin / Transthyretin: 2 days

Albumin:

→ size: 69,000 Da . very light

→ half-life: 20 days

→ Crohn's disease half-life: 1 day

→ Produced by liver at 12g /day. Used as a Liver function test. ~25% of total protein synthesized by liver.

→ Most abundant in blood. ~ 60 % . ~ 4 g /dL

→ Monomeric. One polypeptide only.

→ Ellipsoid shape not to increase viscosity. fibrinogen is Flat & increases viscosity. Not perfectly circular

→ At pH 7 it has 20 negative charges.

→ Not glycolipid not to increase viscosity

→ Preproalbumin. Not prealbumin

Albumin Functions:

1→ Main factor in increasing blood osmotic pressure (oncotic pressure). Preventing edema. More oncotic p. = less edema.
Less oncotic p. = more edema

2→ Acts as a buffer... like hemoglobin

3→ Transport!!

* Free fatty acids → cuz non-polar

* Steroids → cuz non-polar

* Tryptophan → cuz non-polar

* Bilirubin → Results from breakdown of heme. Non-polar. VERY toxic.
↳ Bilirubin is yellowish color

* Metals & heavy metals → metals are toxic in blood
⇒ Major Copper transport. ceruloplasmins are for storage of Cu.
⇒ Calcium transport. imp. too

* Most drugs.
↳ Drug - Drug interactions, two drugs have same binding site on albumin.
↳ Drug - Drug interactions result in high toxic levels of drugs as more free drugs in blood because binding site are occupied.
↳ Aspirin, competes with bilirubin
↳ Phenytoin - epilepsy - competes with Dicoumarol (like heparin) -anticoagulant-
↳ Also transports sulfonamides & penicillin G.

Clinical Disorders Regarding Albumin.

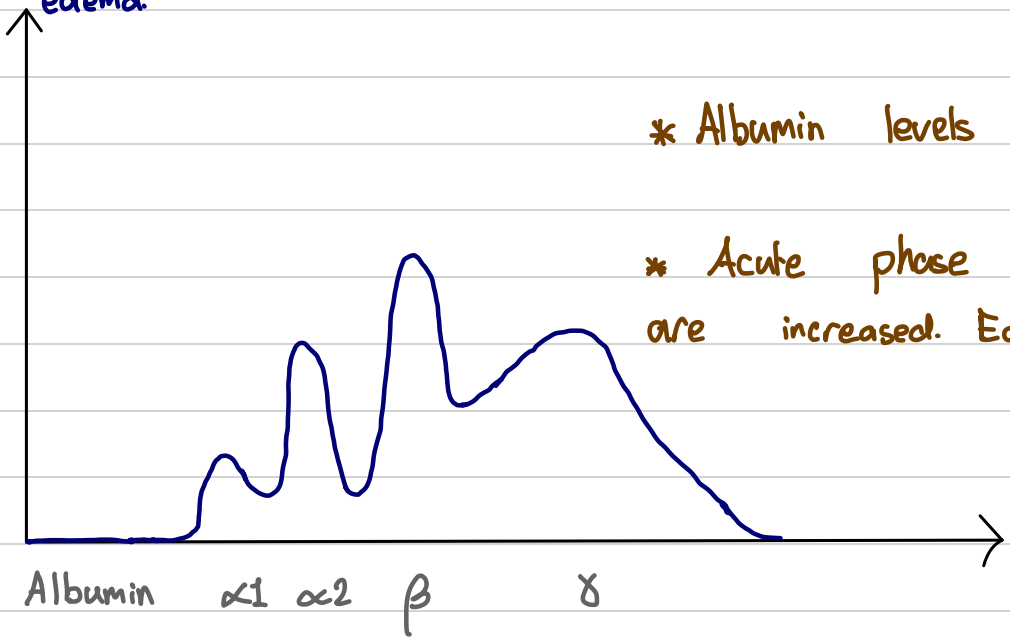
1→ Analbuminemia:

* Total lack of albumin. Zero albumin in blood. Fatal !

* Rare.. it is recessive

* MODERATE edema... not severe.

* Body compensates by increasing other plasma proteins which helps lower edema.



* Albumin levels zero

* Acute phase proteins & Ig are increased. Edema is moderate.

2→ Hypoalbuminemia:

* Low albumin concentration in blood. <2 g/dL

* Causes severe generalized Edema

* Severe edema cuz no protein compensation

* Due to malnutrition or loss of protein (gastrointestinal loss / nephrotic syndrome). Or due to decreased production from Liver failure.

3 → Hyper albuminemia:

* High concentration of Albumin in blood

* Hyper albuminemia is due to dehydration. Less water so higher concentration of Albumin.

4 → Drug - Drug interactions:

① Bilirubin & Aspirin

⇒ If little kids take aspirin... it competes with bilirubin for same binding spot in Albumin.

⇒ Extra levels of Bilirubin in blood.

Bilirubin Toxicity:

→ Jaundice : yellowing of eyes & skins. Not dangerous

→ Kernicterus: Bilirubin goes to brain. Mental retardation. Dangerous.

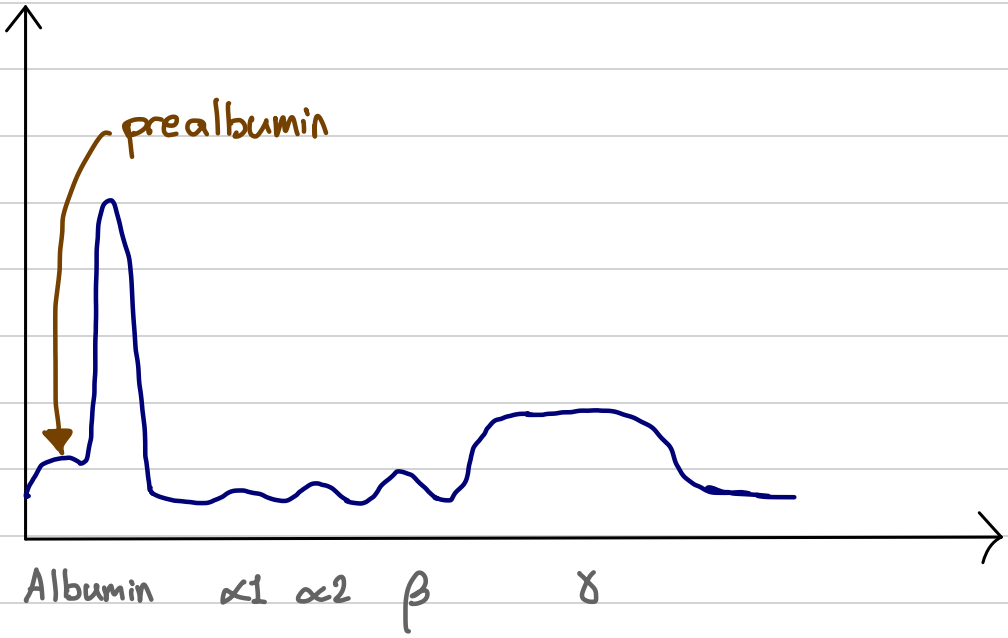
② Phenytoin & Dicoumarol

Phenytoin => treats epilepsy
Dicoumarol => anti-coagulant

These drugs taken together cause problems.

Pre albumin / transthyritin:

* NOT albumin precursor. Different protein. (proalbumin)
(prepro → pro . No pre alone)



* Lighter than albumin.

Prealbumin / Transthyretin => 62,000 Da

Albumin => 69,000 Da

* Short half-life:

Prealbumin / Transthyretin: 2 days

Albumin: 20 days

Albumin + Crohns: 1 day

Haptoglobin: 5 days

Hp + Hb: 90 mins

Pre albumin / Transthyretin function:

1→ Transports & carries thyroid hormones. T3 & T4

2→ Used as a measure for protein nutrition when (due to short half-life) analyzing chromatograph. Same idea how we use albumin to study liver function.

Acute Phase Proteins:

→ These proteins respond to: (increase levels by x1000)

* Acute inflammation
* Chronic inflammation
* Tissue damage
* Cancer

⇒ basically inflammation proteins

→ Acute-phase proteins are stimulated by Interleukin-1 (IL-1)

→ Types:

* Fibrinogen - Blood clotting

* α1-globulins:
→ Antitrypsin - Neutralizes proteases
→ feto protein

* α2-globulins:
→ ceruloplasmins - Copper storage & transport. Iron oxidation to Fe^{3+}
→ Haptoglobins - Iron preservation & Hb binding
→ macroglobulins - Zinc & cytokines transport

* β-globulins
→ CRP - Complement protein system recruitment
→ transferrins - Fe^{3+} transport

* γ-globulins
→ IgM, IgG, IgA, IgD, IgE

α1-Antitrypsin: (α1-antiprotease)

→ Neutralizes and inactivates trypsin and elastase

→ Most abundant α1-globulin. 90%.

→ Polymorphic! Has 75 different forms.

→ Coded for by 4 alleles. P_i^M / P_i^S / P_i^Z / P_i^F

↳ MM most common & safe

↳ MS & MZ are safe. Basically M is safe

↳ ZZ & SZ have α1-Antitrypsin deficiency... causing emphysema.

→ Work mostly in Lungs. Elastase & Trypsin cause proteolysis of lung tissue, damaging the lung tissue.

→ Anti-trypsin neutralize the elastase & trypsin so they no longer break down lung tissue.

α1-Antitrypsin and Lung problems (emphysema)

① Due to genetic... ZZ & SZ cause deficiency in antitrypsin. Activated trypsin & elastase break down lung tissue & cause emphysema.

② Due to smoking...

Smoking causes oxidation of the thioether sulfur.

Methionine → Methionine-Sulfoxide

Methionine-Sulfoxide causes emphysema

Emphysema is chronic.

α1- Antitrypsin and Liver problems:

→ ZZ causes polymerization of loop within β-sheat structure.

→ This results in protein aggrigation in Liver which causes Liver cirrhosis

→ ZZ causes Liver cirrhosis and emphysema.

α-2-Haptoglobins (Hp):

* α2- globulin

* A glycoprotein

* A tetramer... 2 α chains & 2 β chains. Like hemoglobin

* Haptoglobin is larger than Hb.

* Half-Live:
Hp: 5 days
Hp + Hb: 90 minutes. Quickly degrades

* Polymorphic. Three phenotypes.
Alleles: Hp1 & Hp23
Phenotypes differ in α-chain.
→ 2β + α1 + α1
→ 2β + α1 + α2
→ 2β + α2 + α2

* Binds with Hb... preventing loss of Hb & IRON

* Main function is conserving iron by binding to Hb.

α-2-Haptoglobins & clinical applications.

→ Hemolytic Anemia: RBC's burst (lyse) & release hemoglobin in the blood.

→ Extra Hb in blood. Hp binds to Hb to conserve Fe. This causes a decreased level of Haptoglobins in the blood.

→ Decreased level of Haptoglobin is a test for hemolysis.

α-2- ceruloplasmins:

* Contains 6 atoms of copper

* Glycoprotein

Functions:

1→ Storage of Copper (90% of blood Cu in ceruloplasmins.)

2→ Regulates copper levels by releasing and binding.
↳ By using the cysteine rich proteins called metallothioneins which binds to Cu.

3→ Albumin used for Cu transport (10% of total Cu)
Albumin: Cu transport
Ceruloplasmins: Cu storage & regulation.

4→ Acts as ferroxidase enzyme.
↳ Oxidizes Fe^{2+} to Fe^{3+} which is transported by transferrins.

5→ Ceruloplasmin can act as different enzymes.

α-2- ceruloplasmins and clinical Disorders:

→ Can be genetic or lack of copper in diet... so no copper to build the ceruloplasmins.

Continued... ↓

① Hyperceruloplasminemia:

→ Low levels of ceruloplasmins (50%)

→ No serious health complications.

② Aceruloplasminemia:

→ Lack of ferrous oxidase activity.

→ No oxidation of Fe^{2+} to Fe^{3+}.

→ No Fe^{3+} so no transport by transferrins.

→ Accumulation of iron in tissue resulting in organ failure.

④ Wilson's Disease:

→ Defective ceruloplasmins

→ Copper toxicity... bronze skin color.

→ Precipitates in liver. Liver failure.

→ Production of apoceruloplasmin. Removing Cu from holoprotein We remain with protein-containing part which is the apoprotein.

α-2-macroglobulin:

* Macro... Large plasma protein !

* Since large. Not excreted in nephrotic syndrome. The chromatograph will show large α2 peak due to α-2-macroglobulin.

Functions:

→ Transports 10% of Zinc & cytokines

→ Inactivates / inhibits proteases like inactivating blood clotting factors.

→ Inhibits blood coagulation factors. Anti-coagulant

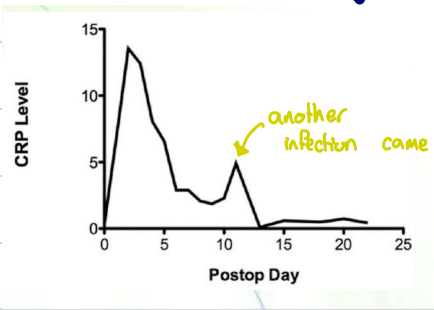
β - C-reaction protein (CRP):

* Part of inflammation. Used by IgM & IgG. Pathogen defence.

* Binds to polysaccharide called fraction C present on pneumococci bacteria.

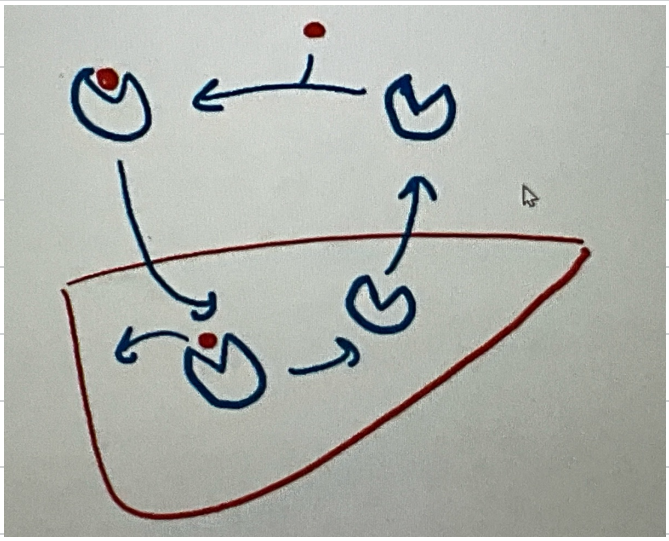
* Normal conditions its not detected. During inflammation & tissue damage its detectible.

* Peak after 48 hours / two days of infection.



β- Transferrins:

* part β1- globulins
* Glyprotein. Made in liver
* Transports Fe^{3+} in blood.
* Apotransferrin + Fe^{3+} → Holotransferrin → enters liver and fe is deposited & apotransferrin released again and ready to bind fe^{3+} again



→ Fe^{2+} conservation: α-2- haptoglobin

→ Fe^{2+} to Fe^{3+}: α- 2- ceruloplasmins

→ Fe^{3+} transport: β1- transferrins

Due to no time...

VERY SUMMARIZED ENZYME NOTES.

Includes ONLY: new memorization info.

Enzymes - Introduction:

5 Features of Active sites:

1 → Is an internal canal, made of non-polar & polar a.a. Water is excluded from the canal.

2 → 3D shape. The 3D shape is determined by the big non-active site. Active site from distance parts of a.a, Ex: 1, 2, 7, 8, 11, 13, 14. Not 1,2,3,4,5,6...

3 → Substrate binding is reversible... due to weak non-covalent interactions.

4 → ≥ 3 point of contact for substrate. Makes it more specific. Chirality is important

5 → Active site is small relative to entire enzyme

Glucokinase:

kinase ⇒ adding Pi

Glucokinase adds Pi to glucose ① using ATP ②

1 → Glucose binds lock & key

2 → This binding changes 3D shape... ATP binds induced fit.

ΔG :

* $\Delta G = G_{products} - G_{reactants}$

* ΔG = -ve ⇒ exergenic (spontaneous) ⇒ releases energy ⇒ catabolism ⇒ exothermic

* ΔG = +ve ⇒ endergenic (non-spontaneous) ⇒ requires energy ⇒ anabolism ⇒ endothermic

* Reversible reaction, same ΔG value but different sign.

Apoprotein ⇒ Inactive enzyme. Protein portion only.

Holoprotein ⇒ Active enzyme. Protein portion + non-protein activating co-enzyme (co-factor)

Classification:

① Over ② The ③ ④ ⑤ ⑥ HILL

1→ O => oxidoreductases

2→ T => Transferases

3→ H => Hydrolases

4→ L => Lyases

5→ I => Isomerases

6→ L => Ligases

1→ Oxidoreductases:

→ Catalyze oxidation - reduction (reversible so can do BOTH) reactions. Transfer of e^- / H^+ / O

1.a→ Dehydrogenase

* Removes (or adds.. all enzymes reversible) H / H^- using NAD^+ / NADH or FAD / $FADH_2$

Removing H => Oxidation

Adding H => Reduction

=> memo: $R\text{-}\overset{H}{\underset{H}{C}}\text{-}OH + NAD^+ \rightarrow R\text{-}\overset{O}{\overset{\|}{C}}\text{-}H + NADH$

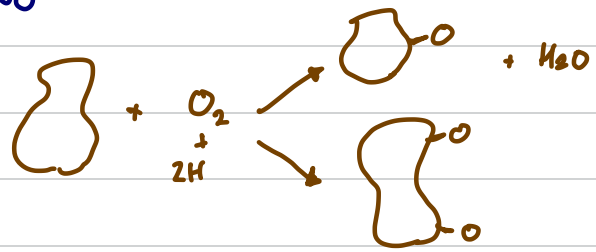
1.b → Oxidase:

* Removes H and adds it to O_2 forming H_2O_2.
* Oxidation by removing H.

memo: 

2. c → Oxygenase:

* Oxidation by adding O_2. Produce H_2O NOT H_2O_2
* Monooxygenase → One O added. H_2O produced
* Dioxygenase → Two O added. No H_2O produced

memo: 

2. d → Peroxidase:

* Oxidizes substrate using H_2O_2.
* Glutathione (→ GSH) oxidation... protects against H_2O_2.
* Produce H_2O & $\frac{1}{2} O_2$
* $2GSH + H_2O_2 \longrightarrow GSSG + H_2O + \frac{1}{2} O_2$

1- Oxidoreductases:

→ Dehydrogenase : H / H^- removal using $NAD^+ / FAD \rightarrow NADH / FADH_2$ formed

→ Oxidases : H / H^- removal using O_2. H_2O_2 produced

→ Oxygenase: Adding O_2. Producing H_2O

→ Peroxidases: Using H_2O_2 to oxidize substrate. (H loss). $H_2O + \frac{1}{2}O_2$ produced

2 → Transferase:

* Transferring group from one molecule to another

2. a → Kinases

* Adds Phosphate

2. b → Transaminase

* Transfer amino group from amino acid to keto acid... Forming another amino acid and turning old amino acid into keto acid

* Interconversion of amino acids.

* Name of enzyme after amino acid formed.

* $-NH_2$ exchanged with $=O$

Continued...

amino acid A + keto acid B → Keto Acid A + Amino Acid B.

Amino Acid → Keto Acid Examples ($-NH_2 \Leftrightarrow =O$)

* Alanine → Pyruvate (3 C)
* Glutamate → α-keto glutarate
* Aspartate → Oxalo acetate (4 C)

2. C → Synthases

* Transfering residue to larger important molecule. Like adding Glucose to glycogen.

→ Glycogen Synthase.

Glu - UDP + Glu - Glu - Glu - Glu - Glu - Glu

↓ Glycogen Synthase ~ UDP-glucose – glycogen glycosyl transferase

UDP + Glu - Glu - Glu - Glu - Glu - Glu - Glu

3 → Hydrolases:

* Catalyze hydrolysis of reactions. Using H_2O to break large molecules into smaller one.

* Proteases are a type of hydrolases. Like Trypsin

Types of proteases (hydrolase):

① Trypsin => Breaks peptide bond between Lysine & Arginine.
Tip: LA => Lysine & Arginine

② Chymotrypsin => Breaks peptide bond between Phenylalanine / Tyrosine / Tryptophan
Tip: Bulky amino acids

③ Elastase => Breaks peptide bond between Glycine / Alanine / Valine
Tip: Small uncharged groups

4→ Lyases:

Breaks / Forms double bonds & ring structures. WITHOUT using oxygen or water.

4.a → Dehydrases:

Removes -OH & -H to form H_2O & double bond. $-\overset{OH}{\overset{|}{C}}-\overset{H}{\overset{|}{C}}- \rightarrow -C=C-$
Ex: enolase (en + ol → C=C + -OH)

4.b → Decarboxylase:

Removing $-CO_2^-$ to form $-CO_2 \ldots$ H is added instead

4.c → Synthase:

Transferase? NO... in Lyases it is adding residue to a bigger compound. BUT addition is to a double bond OR addition forms C-C bonds.

4.d → Aldolase:

Breaks / forms C-C bond... 1,6 bisphosphate into DHAP & GAP.

5 → Isomerase

Isomerase: Catalyses isomerization by rearranging bond structure. Ex: Glu ⇌ Fru

Mutase: Changes Phospate position. Ex: 1,3 BPG ⇌ 2,3 BPG.

6 → Ligases:

Joining molecules together via carbon containing bonds... using ATP as energy source.

* Synthetases ⇒ ligases that use ATP. Synthases are transferases or Lyases, not Ligases

* Carboxylases ⇒ Add CO_2 using energy from ATP.

Specific Catalysts:

① Abzymes:

* Antibodies acting as enzymes.

* Very specific

* Rat injected with transition state.

* Clinical application: Abzyme mimicking cocaine esterase.. used to help addicts.

② Ribozymes:

* RNA acting as a catalyst

Ex: Ribonucleoproteins:

→ made of both protein & RNA... RNA part acts as catalyst

→ catalyzes RNA splicing.

→ catalyzes protein synthesis in proteins.

Enzyme Type Summary:

O T H L IL

1→ Oxidoreductases:

a→ Dehydrogenases
↳ Remove H. Using NAD^+ or FAD

b→ Oxidases
↳ Remove H. Using O_2. Forming H_2O_2

c → Oxygenases
↳ Add O_2 + H... Forming H_2O. (-OH can either form or not)

d→ Peroxidases
↳ Oxidize molecule using H_2O_2. $\frac{1}{2}O_2$ + H_2O formed. $2GSH + H_2O_2 \rightarrow GSSG + H_2O + \frac{1}{2}O_2$

2→ Transferases:

a→ Kinases
↳ Adds Pi to molecules using ATP. Phosphorylation

b→ Transaminase
↳ Transfers between $-NH_2$ and $=O$ from different molecules.
Interconversion between amino acids and keto acids.

C → Synthases
↳ Adding residue to big structure. Ex: Transferring Glucose from UDP-glucose onto glycogen.
UDP left behind

3 → Hydrolases:

↳ Hydrolysis of bonds using H_2O. Breaking bonds.

↳ Proteases are hydrolases:

① Trypsin ⇒ LA ⇒ Between Lys & Arg

② Chymotrypsin ⇒ Big aromatic rings: Phe & Tyr & Trp

③ Elastase ⇒ Between small a.a: Gly & Ala & Val

4 → Lyases:

↳ Breaking / Forming C=C or rings or C-C.

a → Dehydrase

↳ Dehydration. Removing -H & -OH forming H_2O & C=C

↳ Enolase (ene + ol) (C=C + -OH)

note: not all dehydration reactions are dehydrases.

dehydrase: Produces double bond. H_2O produced

Ligase: Uses ATP as energy source. H_2O produced sometimes

b → Decarboxylation

↳ Removing $-\overset{O}{\overset{\|}{C}}-O^-$ replacing it with H. Forming CO_2

c → Synthases

↳ adding residue to big polymer involving C=C / C-C bonds

d → Aldolase

↳ Breaks C-C bond in fructose-1,6-Bisphosphate forming DHAP & GAP

5 → Isomerase

a → Isomerase: isomers by changing atom arrangment

b → Mutase: isomers by changing Pi position

6 → Ligases:

↳ Joins together residues using energy from ATP.

↳ Synthetases. NOT synthase

↳ Carboxylases ⇒ addition of CO_2.

Extra:

Ribozyme:

↳ ribonucleoprotein (catalysing region)

↳ RNA splicing catalyst

↳ Protein synthesis catalyst

Abzyme:

↳ Can mimick other enzymes. Very specific.

Enzymes - Kinetics

Rate Law:

rate: $k[S]$

$k = \frac{1}{\text{time}} = sec^{-1}$

Michaelis - Menten Equation:

①

V. Imp.

$$V_o = V_{max} \times \frac{[S]}{[S] + K_m}$$

* V_o = rate at a specific $[S]$

$\Rightarrow V_o = k_2[ES]$

* at V_{max} : $[ES] = [E]_t$

all enzymes exist as ES complex.

②

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$K_m = \frac{k \text{ of dissociation ES}}{k \text{ of formation ES}}$$

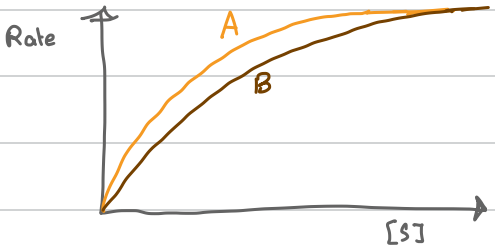
$\therefore$ $$K_m = \frac{k_{-1} + k_2}{k_1}$$

k_2 is relatively low... $\Rightarrow$ $$K_m = \frac{k_{-1}}{k_1}$$

Cases:

① Same enzyme + same reaction but two substrates.

↳ same Vmax. Different Km.



$A + B \xrightarrow{E} C$

↳ same Vmax because it will be totally saturated, cuz in end both will be same conc.

Fixed large [A] + increasing [B] = Fixed large [B] + increasing [A]

Both large = Both large

↳ enzymes fully saturated

↳ same Vmax

② Same enzyme but different reactions with different substrates.

↳ Different Vmax and Different Km

③ Different enzymes + same reaction. Isozymes

↳ Different Vmax and Different Km

④ Doubling / Tripling ... concentration of enzyme. Same reaction.

↳ Doubles / Triples ... the Vmax. Km stays the same.

K_{cat}:

$$K_{cat} = \frac{V_{max}}{[E]_t}$$

at V_{max}

$K_{cat} = k_2$

$K_{cat} : \frac{1}{s} = s^{-1}$

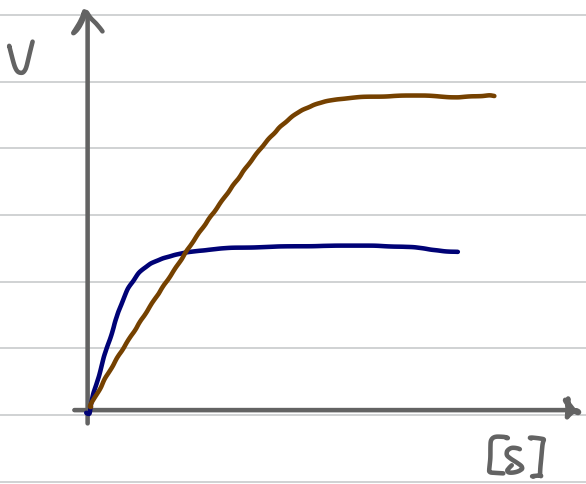
* Higher K_{cat} => more efficiency

* Look at units.. V_{max} = M/t or mol/t .
Make sure that [E] corresponds to mol or M.

enzyme efficiency $\propto \frac{K_{cat}}{K_m}$

Usually (not always) K_{cat} & K_m are directly prop.

Less K_{cat} = Less V_{max} = Less K_m = more affinity



Lineweaver - Burk / Double-reciprocal plot:

* Going from hyperbolic to linear graph.

$\frac{V_0}{[S]}$ = hyperbolic $\qquad \frac{\frac{1}{V_0}}{\frac{1}{[S]}}$ = Linear

$$V_0 = V_{max} \times \frac{[S]}{[S]+K_m} \Rightarrow \frac{1}{V_0} = \frac{[S]+K_m}{[S] \times V_{max}}$$

$$\Rightarrow \frac{1}{V_0} = \frac{[S]}{[S] \times V_{max}} + \frac{K_m}{[S] \times V_{max}} \Rightarrow \frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$

($\frac{K_m}{V_{max}}$: constant)

$$\therefore \quad y = mx + c \quad \Leftrightarrow \quad \underbrace{\frac{1}{V_0}}_{y} = \underbrace{\frac{K_m}{V_{max}}}_{m} \times \underbrace{\frac{1}{[S]}}_{x} + \underbrace{\frac{1}{V_{max}}}_{c}$$

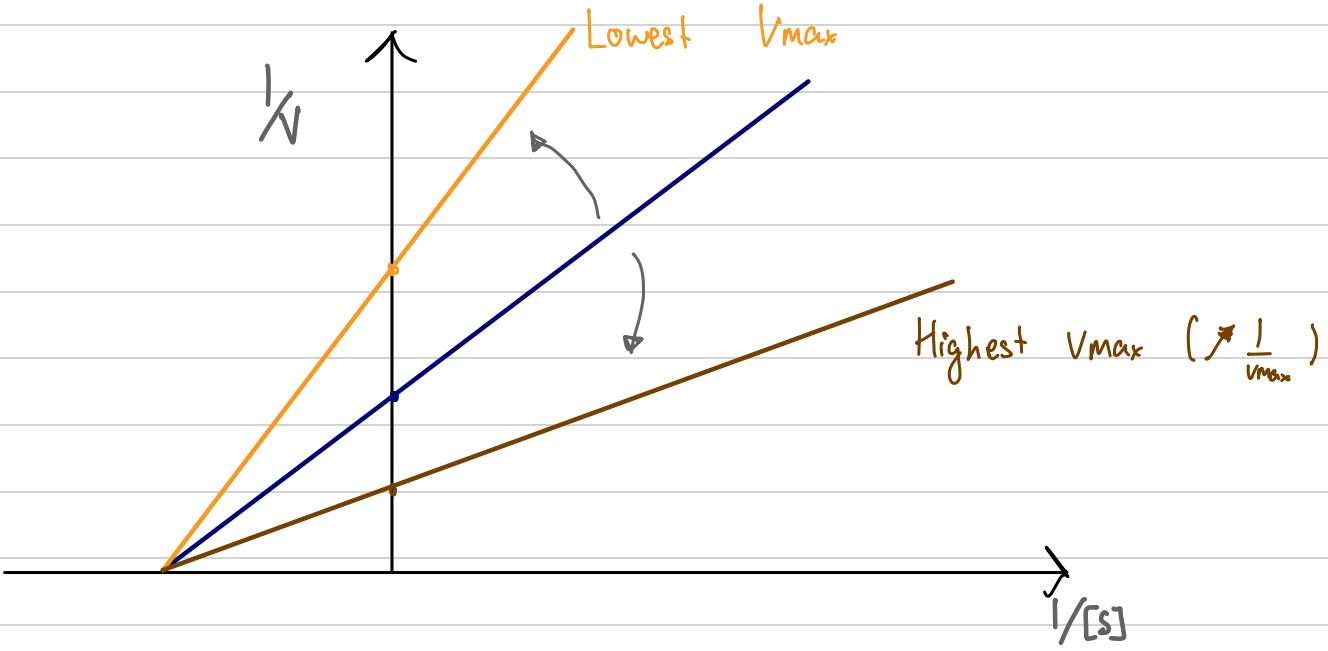
y-int $= \frac{1}{V_{max}}$

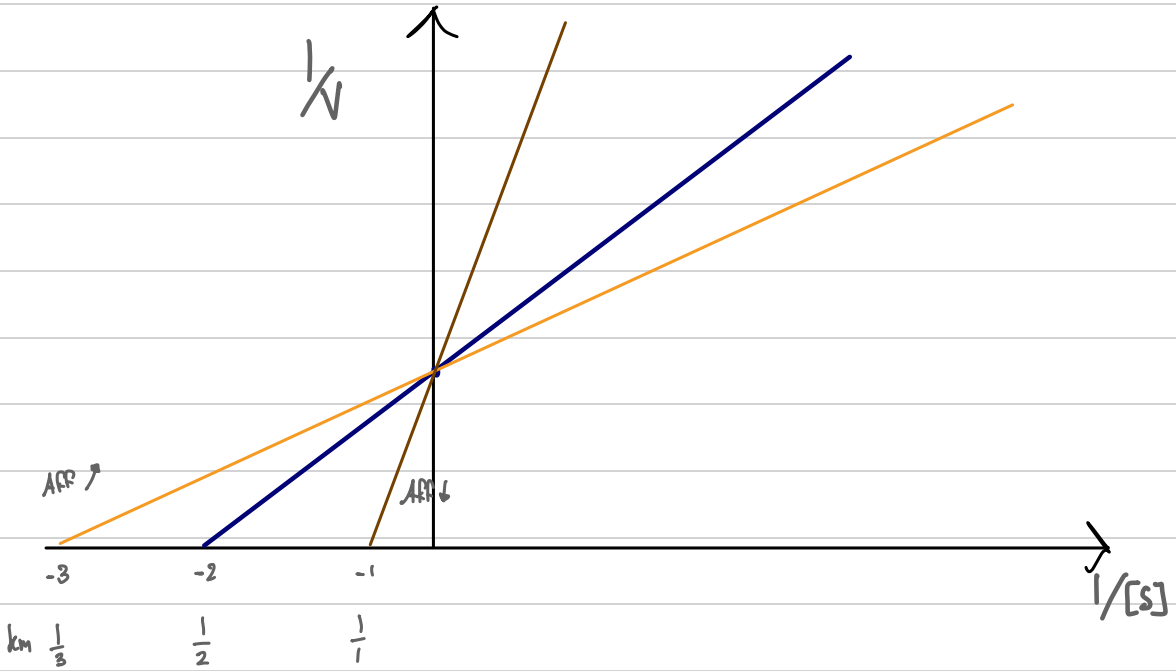
gradient $= m$

x-int $= \frac{-1}{K_m}$

$$0 = \frac{K_m}{V_{max}} \times X + \frac{1}{V_{max}} \Rightarrow \frac{K_m \cdot X}{V_{max}} = -\frac{1}{V_{max}} \Rightarrow X = -\frac{1}{K_m}$$

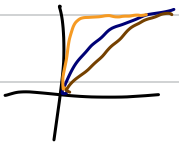
$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$





Equation Summary:

① Michaelis - Menten Equation: Hyperbolic. V on [S]



$$V_o = V_{max} \times \frac{[S]}{[S] + K_m}$$

* Left shift ... K_m ↓ & Aff. ↑

* Right shift ... K_m ↑ & Aff ↓

② Lineweaver - Burk: Linear. 1/v on 1/s

$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \qquad y = mx + c$$

* y-int = $\frac{1}{V_{max}}$ x-int = $\frac{-1}{K_m}$ m = $\frac{K_m}{V_{max}}$

* Shift up = $\frac{1}{V_{max}}$ ↑ = V_{max} ↓

* Shift down = $\frac{1}{V_{max}}$ ↓ = V_{max} ↑

* Shift right = $-\frac{1}{K_m}$ ↓ = K_m ↑ = Aff. ↓

* Shift Left = $-\frac{1}{K_m}$ ↑ = K_m ↓ = Aff. ↑

③ K_M constant:

$$K_M = \frac{k_{-1} + k_2}{k_1} \qquad \frac{\text{Dissociation}}{\text{Formation}}$$

* k_2 can be neglected

* $k_2 = k_{cat}$

④ k_{cat}:

$$k_{cat} = \frac{V_{max}}{[E]_t}$$

* Units $\Rightarrow$ s^{-1}

* $k_{cat} = k_2$ at V_{max}

* $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$

if $[ES] = [E]_t$ at V_{max}.

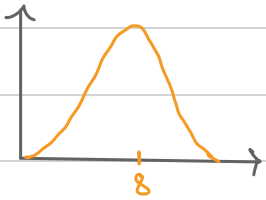
so.. $V_{max} = [ES] \times k_2$

$\Rightarrow V_{max} = [E]_t \times k_{cat}$

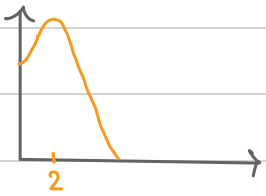
Enzymes - Regulation:

Just weird pure memo info.

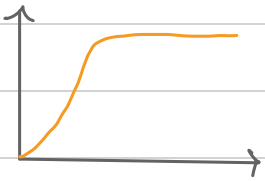
Optimum pH:

* Chymotrypsin: 

intestine, basic

* Pepsin. 

stomach, acidic

* Cholinesterase 

* Papain 

Rate of diffusion:

1→ Compartmentalization

2→ Enzyme complexing

Enzyme Complexing:

Pyruvate Dehydrogenase complex... three reactions.

Pyruvate Dehydrogenase:

* Pyruvate (3 C) + CoA $\xrightarrow{NAD^+ \rightarrow NADH}$ Acetyl CoA (2 C) + CO_2

Link Reaction

1→ Dehydrogenation - Oxidoreductase

2→ Decarboxylation - Lyase

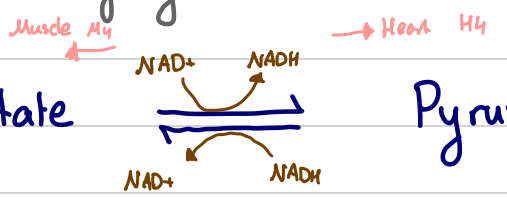
3→ Transfer of CoA - Transferase

Lactate Dehydrogenase (LDH):

H = Heart Subunit M = Muscle subunit. LDH = tetramer (4 subunits)

① LDH_1 (H_4) → Present in heart. Favors Pyruvate production.
② LDH_2 (H_3M) → RBCs
③ LDH_3 (H_2M_2) → Lungs
④ LDH_4 (H_1M_3) → kidneys
⑤ LDH_5 (M_4) → Present in skeletal muscles & Liver. Favours Lactate production

Lactate Dehydrogenase:



* Lactate ⇌ Pyruvate

Irreversible inhibitors:

① Covalent Inhibitors:

→ Organophosphates

↳ DFP (di-isopropyl flouro phosphate)

↳ Sarin gas

↳ Malathion & Parathione

& serine a.a

=> Organophosphates bind to Acetyl Cholinesterase. (Deadly)

=> // // // Serine enzymes (Not deadly)

→ Aspirin

=> Binds to COX. (mimicks arachidonic acid structure)

=> Adds acetyl group to serine.

② Transition state analogues (suicide inhibitors):

→ Penicillin

=> Binds to glycopeptidyl transpeptidase. Bacteria cell wall.

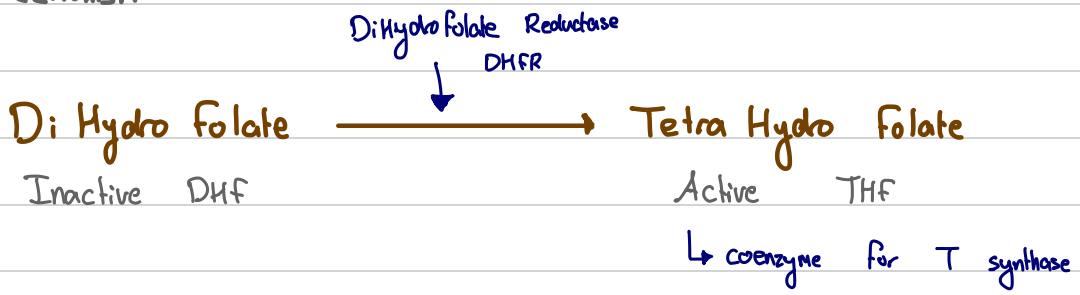
=> β-Lactam ring mimic the transition state analogue.

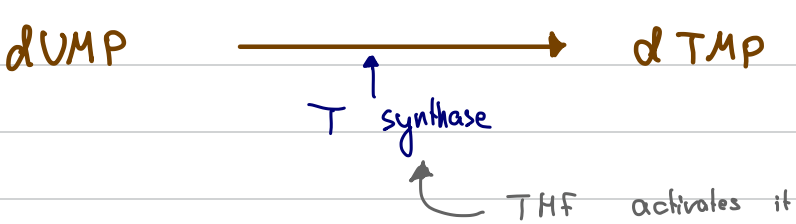
=> Serine

→ Methotrexate:

* Mimics folate. Folate used in nucleotide synthesis
* Mimics substrate analogue

Mechanism:





* Methotrexate binds to DHFR with High affinity. No THF... no T synthase so no nucleotides produced... slows cancer growth

③ Heavy Metals:

① Hg / Pb / Al / Fe ⇒ non-specific inhibition in high doses

② Hg poisoning:
* Binds to -SH groups in non-active site region
* Changes active site & inactivates enzyme

③ Pb poisoning:
* Replaces Ca^{2+} / Fe / Zn
* Affects CNS due to Ca^{2+} replacement
* Affects RBCs due to Fe^{2+} replacement. Anemia.

Allosteric Regulation:

Aspartate transcarbamoylase (ATCase):

* 12 subunits. 6 catalytic. 6 Regulatory. C_6r_6. Allosteric enzyme
* ATCase produces CTP, TTP, UTP. Aspartate is substrate
* CTP inhibits ATCase (-ve feedback) on regulatory site
* ATP activates ATCase on regulatory site
* CTP → shift to right → T-state → k_{so} ↑ & Aff. ↓
* ATP → shift to left → R-state → k_{so} ↓ & Aff. ↑

Covalent Regulation:

* Addition of Pi on aa to either activate or inhibit this enzyme.
* Addition of Pi on aa containing -OH. The /Ser/Tyr

Reversible Covelent Modifications:

Phosphorylation on Glucagon-Related (Glycogen phosphorylase) enzymes ⇒ Activation

Phosphorylation on Insulin-Related (Glycogen synthase) enzymes ⇒ Inactivation

Glucagon Pathway Activation ⇒ Phosphorylation by kinases

Insulin Pathway Activation ⇒ Dephosphorylation by phosphatases

Glycogen phosphorylase exists in R & T form. Phosphorylation stabilizes the R-form... stabilizing the enzyme.

Glycogen phosphorylase

⇒ Phosphorylase a = R form, phosphorylated (a = active ... +Pi)

⇒ Phosphorylase b = T form, dephosphorylated (b = inactive ... -Pi)

⇒ ↑AMP : R form

⇒ ↑ATP : T form

⇒ ↑ Glucose : T form

⇒ -ve feedback.
-ve allosteric effector

Zymogens:

inactive proprotein → active protein

('pro' strand)

Ex

inactive Angiotensinogen → active Angiotensin

('ogen' strand)

* trypsinogen → Trypsin

first 6 amino acids at N-terminus removed & trypsinogen converted to active trypsin

Quick Summary on different examples of regulations:

① Irreversible covalent inhibition
- Organophosphates ~ acetyl cholinesterase
- Aspirin ~ COX

② Transition state analogs inhibitors
- Methotrexate ~ DHFR
- Penicillin ~ glycopeptidyl transpeptidase

③ Metal inhibitors
- Hg^{2+} ~ disulfhydryl bonds on enzymes
- Pb^{2+} ~ Replaces Ca^{2+} / Zn^{2+} / Fe^{2+}

④ Reversible covalent modification regulation:
- Phosphorylation ~ Glycogen phosphorylase
  - → activated: P_i / AMP
  - → inactivated: ATP / Glucose-6-phosphate

⑤ Irreversible covalent modification regulation:
- Trypsinogen ⟶ Trypsin
  - ↓ - 6 amino acids N-terminus

⑥ Allosteric binding regulation:
- ATCase ~ Activated: ATP / Inhibited: CTP
  - ATP → -ve heterotrophic
  - CTP → +ve heterotrophic

Metabolic Regulation Examples:

① Feed back inhibition / Product inhibition:

- Hexokinase & Glucose-6-phosphate

- Glycogen phosphorylase & Glucose-6-phosphate /ATP

- ATCase & CTP

② Feedback activation: (amplification)

- Blood coagulation

③ Feed forward activation: (faster)

- Increase products fast!

↳ Glycolysis: After rate determining step... fructose-1,6-bisphosphate activates pyruvate kinase... phosphoenol pyruvate to pyruvate... faster!

- Decrease substrate fast!

↳ Removing toxic /poisonous substrates by doing feedforward activation to be faster

④ Committed Step in Glycolysis: (irreversible + unique + exergenic)

- Glucokinase / Hexokinase NOT commited step
- Phosphofructokinase (PFK) IS commited step

Why?

Because glucose-6-phosphate can undergo many different reactions... glycogen (not unique) formation... nucleic acid synthesis... etc.

BUT... the Fructose-1,6-BP WILL 100% form pyruvate only. So:

↳ GK / HK not committed step.. PFK is committed step.

⑤ Rate-Limiting in Glycolysis:

- Slowest
- Highest Ea... requires ATP usually
- High Km value (low affinity)
- Committed step ≠ Rate-limiting step

Glycolysis: Phosphofructokinase... Fructose-6-P → Fructose-1,6-BP

=> Rate-Limiting step: Glucokinase.. Glucose → Glucose-6-phosphate slowest

=> Committed step: Phosphofructokinase... Fructose-6-P → Fructose-1,6-BP

Disease Diagnosis:

- Creatine Phosphokinase (CPK):

- CPK1 : CPK-BB ~ Brain
- CPK2 : CPK-MB ~ heart / cardiac muscles (MM 80% : MB 20%)
- CPK3 : CPK-MM ~ Skeletal muscle

- Elevated level in one of those isozymes indicate damage to its corresponding tissue

  ↳ By:
  - necrosis
  - blebbing

- High levels of CPK-MB... Heart Attack!

Enzymes - Cofactors & Vitamins :

Summary:

Water soluble + Activation-Transfer coenzymes

① Vitamin B1 ~ Thiamin Pyrophosphate:

Decarboxylation.

Binds to enzyme via Mg^{2+}... chelation.

Pyruvate Dehydrogenase (3C pyruvate to 2C Acetyl CoA)

α-ketoglutarate dehydrogenase (5C α-ketoglutarate to 4C succinyl CoA)

② Vitamin B5 ~ Pantothenic Acid:

Forms Coenzyme A... Pantothenic acid = pantoic Acid + β-alanine

Catalyses carbs/proteins/Lipids metabolism at C=O. Forms $R-\overset{\overset{O}{\|}}{C}-S-R'$

Pyruvate Dehydrogenase (3C pyruvate to 2C Acetyl CoA)

Citrate synthase (4C oxaloacetate + 2C acetyl CoA → Citrate)

③ Vitamin B6 ~ Pyridoxal Phosphate:

Amino Acid metabolism by using transaminases

A.As ⟷ keto acid conversion

Alanine + α-ketoglutarate ⇌ Pyruvate + Glutamate

Aspartate + α-ketoglutarate ⇌ oxaloacetate + Glutamate

④ Vitamin B7 ~ Biotin

Carboxylation

Covalent bond via Lysine in active site

Comes from food & bacteria

Avidin from raw eggs binds to biotin. Biotin deficency.

Pyruvate Carboxylase (^{3C}pyruvate → ^{4C}oxaloacetate)

Acetyl CoA Carboxylase (^{2C}Acetyl CoA → ^{3C}Malonyl CoA) → fatty Acid synthesis

Water-Soluble + oxidation-reduction coenzymes:

① Vitamin A (lipid-soluble) & C:

Use metals for oxygen transfer

② Vitamin B2 ~ Riboflavin:

Produces Flavin Adenine Dinucleotide (FAD)

Flavin + Pi → FMN

FMN + adenylate → FAD

FAD binds covalenty to active site. Prosthetic group.

Takes H (e^-) one by one

Forms double bonds & disulfide bonds

Succinate dehydrogenase (succinate → fumarate)

③ Vitamin B3 ~ Niacin

Produces NAD^+ & $NADP^+$

cosubstrate.. non-covalent binding

Many dehydrogenases use it.

His in Lactate dehydrogenase active site binds to H on -OH & weakens the bond.

Lactate dehydrogenase (3C Lactate → 3C pyruvate)

Pyruvate dehydrogenase (3C pyruvate → 2C Acetyl CoA)

α-ketoglutarate dehydrogenase (5C α-ketoglutarate → 4C succinyl CoA)

Vitamin B1 + B3 are used together in some dehydrogenase

Thiamin P.P Niacin

④ Vitamin C ~ Ascorbic Acid

Antioxidant against radicals

Hydroxylation of Pro & Lys. Prolyl hydroxylase

Proline + α-ketoglutarate (5C) + Vit-C ⟶ Hyd-Pro + succinate (4C) + Dehyd-Vit.C + CO_2

Proline acts as a oxidizing agent, turning α-ketoglutarate into succinate.

Vit-C + R· → Vit-C· + RH

Radical Vit-C is stable due to ring structure. Electron moves in the ring.

Metals in enzymes:

① Fe^{2+}: Hemoglobin / Myoglobin

② Zn^{2+}: Carbonic Anhydrase, Alcohol dehydrogenase

③ Mg^{2+}: Hexokinase, thiamine pyrophosphate

↳ Chelation

Alcohol Dehydrogenase & Zinc:

Serine & Histidine pull H^+ from alcohol. $R-O^-$ formed.
The -ve ethoxide is stabalized by Zn^{2+} ... aldehyde formed.

Alcohol dehydrase → Zn^{2+} not part of rxn... only stabilizes.
Carbonic Anhydrase → Zn^{2+} part of rxn.. Zn^{2+} pulls H^+ and binds -OH with CO_2

Lipid-soluble Vitamins:

Summary:

A - vision + growth + reproduction
K - blood coagulation
E - antioxidant
D - Regulating Ca^{2+} & phosphate metabolism

They are transported in blood via the lipoprotein Chylomicrons

Vitamin A:

* Called Retinoids (Retinol / Retinal / Retinoic Acid) — isomers

* Derived from β-carotene ... cleavage in small intestines to give two molecules of Retinal

1 β-carotene ⟶ 2 Retina

Transport:

① β-carotene absorbed
② Cleavage int 2 Retinal. Retinal → Retinol
③ Retinol + fatty acid = Retinyl Ester
④ Transported via chylomicrons lipoprotein in blood
⑤ Goes to liver & stored
⑥ When needed it releases retinol into blood. — Prealbumin / transthyretin ... T3/T4 transport into target cells
⑦ Retinol + Retinol Bind Protein (RBP) + Transthyretin Complex
⑧ Retinol complex goes to target cells
⑨ Retinol to Retinal OR Retinoic Acid depending on cell

Vitamin A Functions:

① Spermatogenisis:

* Retinol * Retinal * NOT Retinoic Acid

② Vision:

* Retinal * NO Retinol * NO Retinoic Acid
* Retinol oxidized to Retinal in Retina cells in eye.

③ Growth:

* Retinoic Acid * No Retinol * No Retinal
* Retinol oxidized to Retinoic Acid in target cells ... used for DNA transcription Regulation.

Memo!

Retinoic Acid: Growth only

Retinal: Vision + Reproduction

Retinol: Reproduction only.

Vitamin D ~ Calcitriol:

* Comes from cholesterol

* Synthesized in body OR from diet

Vitamin D synthesis:

① 7-dehydrocholesterol —(UV-light)→ Vitamin D3 => Skin
↳ Transport: Vitamin D-binding protein

② Ergocalciferol D2 + Cholecalciferol D3 => Vitamin D2 & D3 => Diet
↳ Transport: Chylomicrons

③ D2 & D3 goes to liver.

④ D2 / D3 —(25-hydroxylase, Liver)→ Calcidiol

⑤ Calcidiol goes to kidneys

⑥ Calcidiol —(1-hydroxylase, kidneys)→ Calcitriol
↓
Active form

Vitamin D function:

→ Active Vit. D... calcitriol enters cell... binds to intracellular Vit. D receptor

→ Vitamin D interacts & Regulates DNA transcription (like retinoic Acid!)

Outcome!

* Regulating Ca^{2+} & Phosphate serum levels

outside idea:

rickets is Vit. D deefficiency.

No Vit.D => no absorption of Ca^{2+} & Phosphates from food => weak bones

Vitamin K:

* Found in three forms ... K1 /K2 /K3

* Found in plants & intestinal bacteria

* A quinone ... made from quinone

* Synthesized from gut bacteria!

Vitamin K function:

Blood coagulation.

Takes part in carboxylation of Glutamic Acid into carboxyglutamate.

Carboxyglutamate helps in coagulation. SO! Vit K = Blood coagulation.

Vitamin E:

Function: Antioxidant (it has ring like Vit. C)

Active Form: α-Tocopherol

↓

Memo: E = antioxidant = phenol = ring

↓

looks like phenol

Protein Purification:

Fixed pI:

Low pH => +ve charge

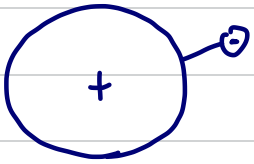
High pH => -ve charge

Fixed pH:

Low pI => more -ve

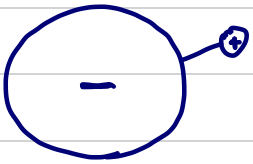
High pI => more +ve

Anion-Exchange Chromatography:



* All +ve goes out first
* Less -ve goes first
* more pI to Less pI goes out first

Cation-Exchange Chromatography:



* All -ve goes out first
* Less +ve goes first
* less pI to more pI goes out first

Affinity Chromatography:

- concanavalin A high affinity to glucose.

- Concanavalin A binds to beads with glucose... add alot extra free glucose to detach the concanavalin A.

Protein Analysis:

① SDS:

- Detergent

- Makes all proteins in SDS-PAGE same shape & same CHARGE (negative)

② Reducing Agents, β-ME / DTT:

- Reduces & Breaks the disulfide bonds

③ 2D-PAGE

- Isoelectric focusing + SDS-page

Low pI = -ve = goes to +ve

High pI: +ve = goes to -ve

④ Immunoblotting / Western Blotting:

1- Carry out SDS-PAGE

2- Transfer from Gel onto paper

3- Use antibodies to turn on a signal by activating enzyme.

4- Very specific to individual proteins.

⑤ ELISA:

- Enzyme Linked Immuno Sorbet Assay

- More sensitive & rapid than immunoblotting.

- Antibodies on plate detect antigen & bind

- Antibody linked to enzyme is added which binds to antigen-antibody complex.

- Washing

- If protein needed to detect is present... antibody-enzyme is bound & gives a color

- If protein not present... antibody goes away with washing & no color.

⑥ Edman Degregation:

- PITC is used to cleavage one A.A at (N-terminus)
- We take PITC - A.A and analyze type of A.A... using chromatography.
- Works for ONLY 50 amino acids

⑦ Peptide cleavage:

- Cyanogen Bromide: After Methionine
- Trypsin: After Lysine or Arginine (LA). NOT if after is Proline
- Chymotrypsin: After Phe ... Tyr ... Trp. NOT if after is Proline
- Elastase: After Gly ... Ala ... Val ... Ser. NOT if after is Proline
- Pepsin: Before... Like chymotrypsin, add Leu Not if AFTER is Proline

⑧ 3D - shape determination:

X-ray Crystallography	VS	NMR - spectroscopy
- Protein in crystal form		- Protein in aqueous form
- Doesn't give shape when protein is functioning		- Gives shape when protein is functioning Like T-state Hb to R-state Hb.
- Detect electrons		- Magnetic fields.