Proteins





Learning Outcomes

1(e)iii. Describe the **structure** and **properties** of **amino acids** (in proteins)

1(f)iii. Describe the **formation** and **breakage of a peptide bond**.

1(k) Explain **primary** structure, **secondary** structure, **tertiary** structure and **quaternary structure of proteins**, and describe the **types of bonds** that hold the molecule in shape (hydrogen, ionic, disulfide bonds and hydrophobic interactions).



Learning Outcomes

1(I) Explain the **effects of temperature** and **pH** on protein structure.

1(m) Describe the molecular **structure** of the **haemoglobin** protein and explain how its **structure** relates to its **function** in transport

- 1. Amino acids
- **2.** Formation of peptide bonds
- **3.** Levels of organisation in proteins
 - A. Primary Structure
 - B. Secondary Structure
- C. Tertiary Structure
- D. Quaternary Structure
- 4. Case Studies: Haemoglobin

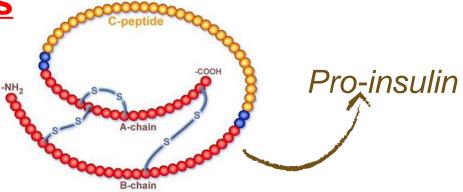
WHAT YOU

NEED TO KNOW

Introduction

Hade up of the elements <u>carbon</u>, <u>hydrogen</u>
& <u>oxygen</u>, <u>nitrogen</u> and sometimes <u>sulphur</u>

+Building blocks of proteins are monomers called <u>amino acids</u>

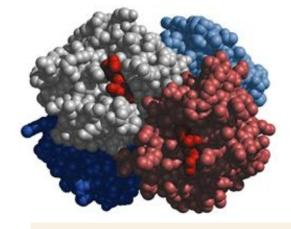


Functions of Proteins

vely. Colla- Keratin is
ne develop- s for baby
oxygen ules across
ion of
oteins are

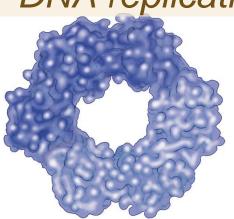


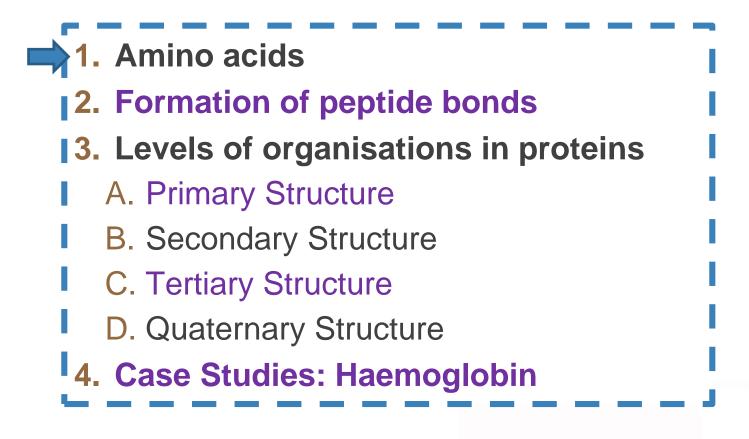
Collagen – cartilage & tendons



Haemoglobin

Antibod **DNA polymerase III** - DNA replication





WHAT YOU NEED TO KNOW



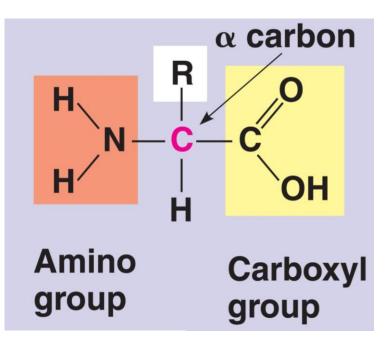
Learning Outcome

1(e)iii. **Describe** the <u>structure</u> & <u>properties</u> of amino acids



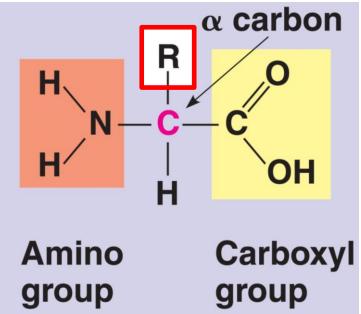


Structure of an amino acid





Types of amino acids

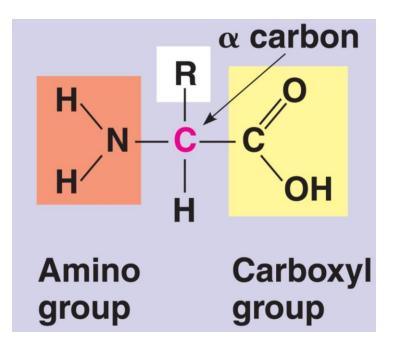


20 different amino acids found in proteins

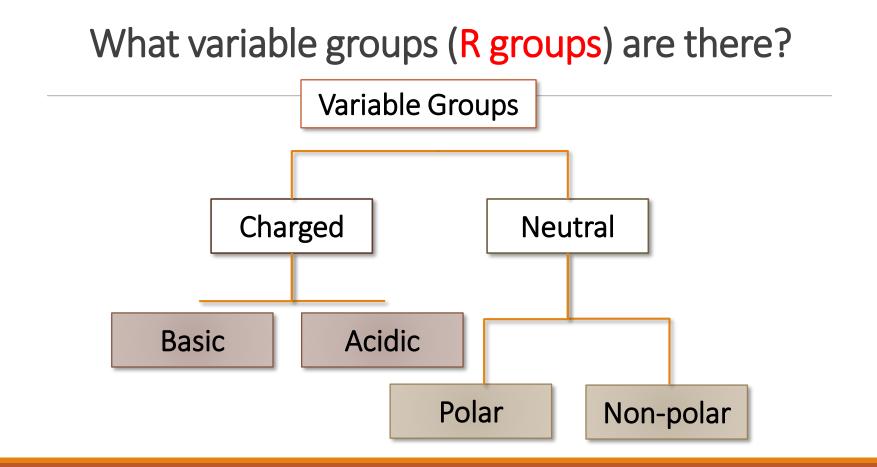
Differs only in R groups



Types of amino acids



Classified based on the different chemical properties of the <u>R groups</u>

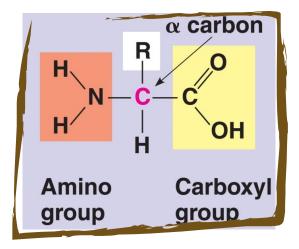




Classification of amino acids

Amino acids may be:

- 1. Non-polar
- 2. Polar
- 3. Charged
 - Acidic
 - Basic

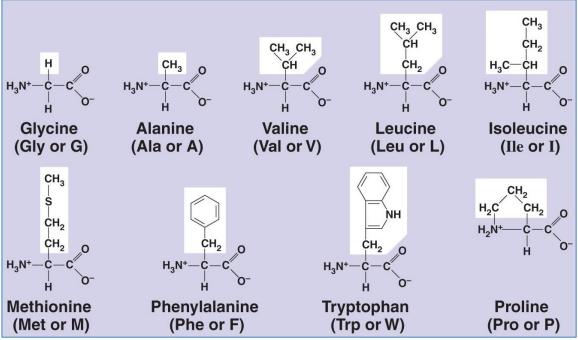




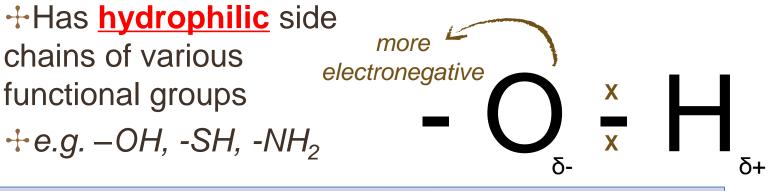
Non-polar amino acids

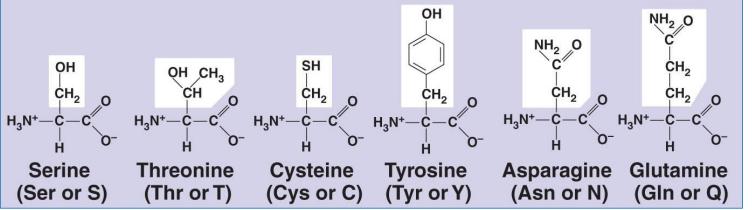
Side chains with hydrophobic properties:

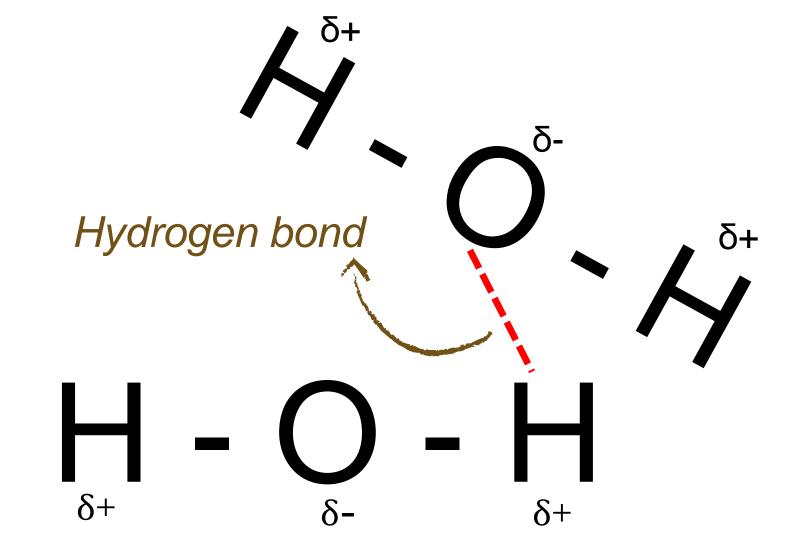
- Pure hydrocarbon alkyl groups or
- Aromatic (benzene) rings



Polar amino acids







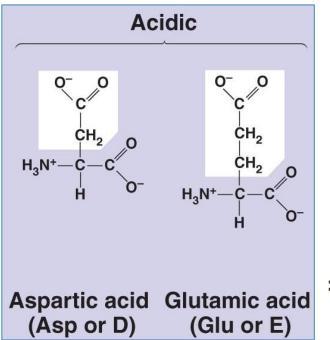


Charged amino acids

+ Determined by Acidic **Basic** the acidic or NH3+ NH₂ ·NH⁴ 0-0-0 ĊH, C=NH_o+ basic ĊH2 ŃΗ ĊH ÇH, ĊH, functional CH_ H₂N CH2 ÇH2 H₃N+groups of the 0 н H₃N+side chain Lysine **Histidine** Arginine Aspartic acid **Glutamic acid** (Lys or K) (Arg or R) (His or H) (Asp or D) (Glu or E)



Acidic amino acids

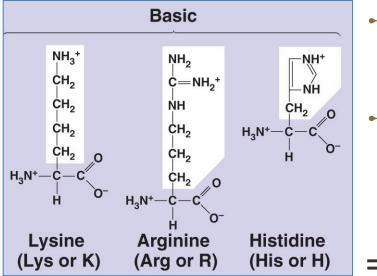


- + Have additional –COOH groups
- which ionize in aqueous environment to produce
 COO⁻ groups

=> Forming an **acidic** solution



Basic amino acids

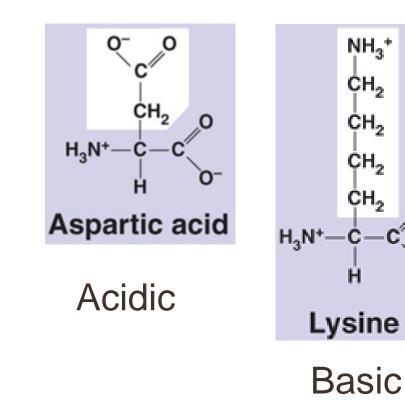


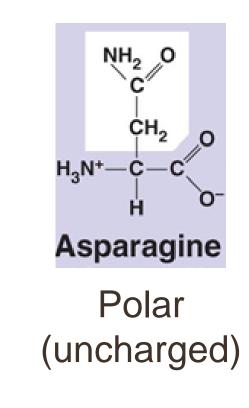
- + Have additional -NH₂ groups
- which ionize in aqueous environment to produce NH₃⁺ groups,

=> forming a **basic** solution

Spot the difference

O

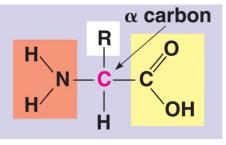








+ Describe the structure of the amino acid.



- + What are the 4 types of amino acids?
 - 1. Non-polar
 - 2. Polar
 - 3. Charged Acidic
 - 4. Charged Basic

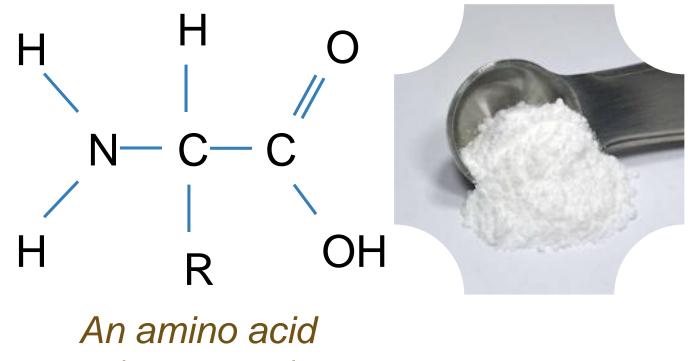
VIDEO: AMINO ACIDS https://youtu.be/652GrZpLkPs



Soluble in water but insoluble in organic solvents

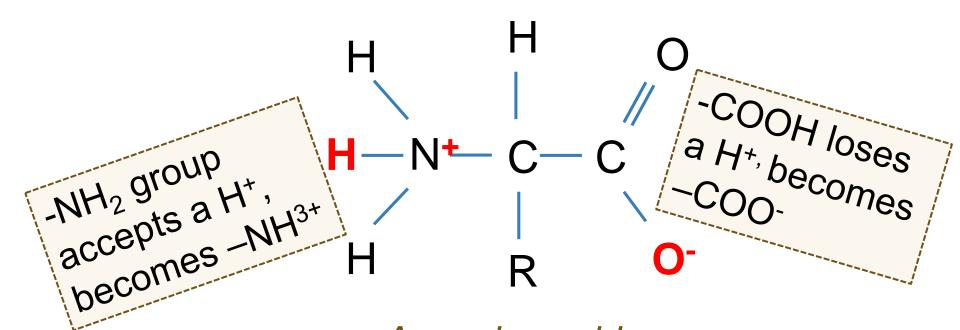
Dissolve in water to form ions

Pg 104



(powdered)

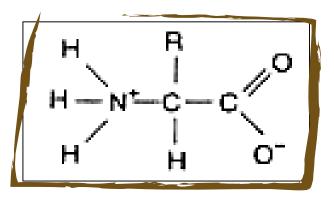
Pg 104



An amino acid (dissolved)

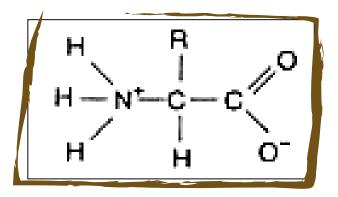


Amino acid that has both positive & negative charges \rightarrow known as a <u>zwitterion</u>





The **pH** at which an amino acid is a zwitterion is its **isoelectric point**



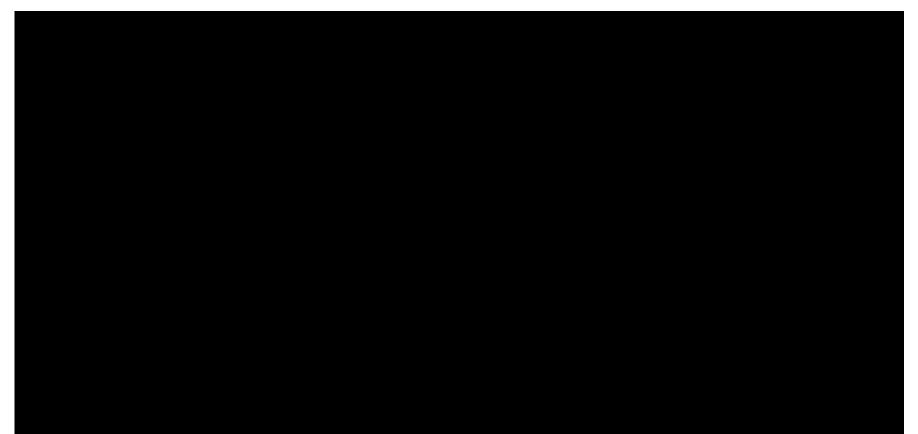


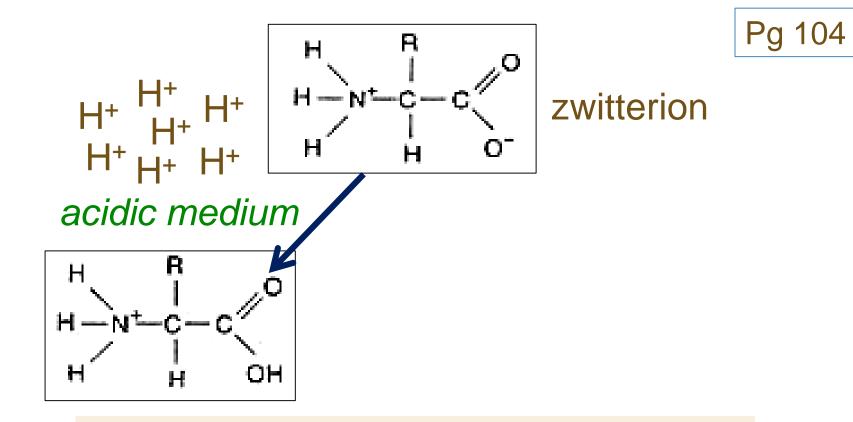
All amino acids can act as either acids or bases (i.e. <u>amphoteric</u>)

+ Can act as **<u>pH buffers</u>** in solutions

Able to resist changes in pH when a small amount of acid/base is added

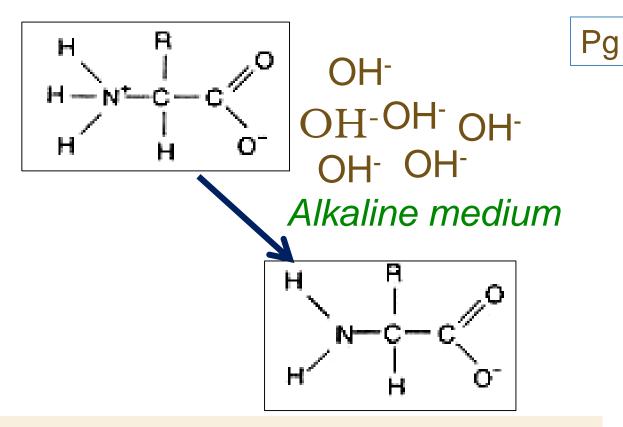
VIDEO: AMINO ACIDS AS PH BUFFERS <u>https://youtu.be/fhosQQeCjN4</u>





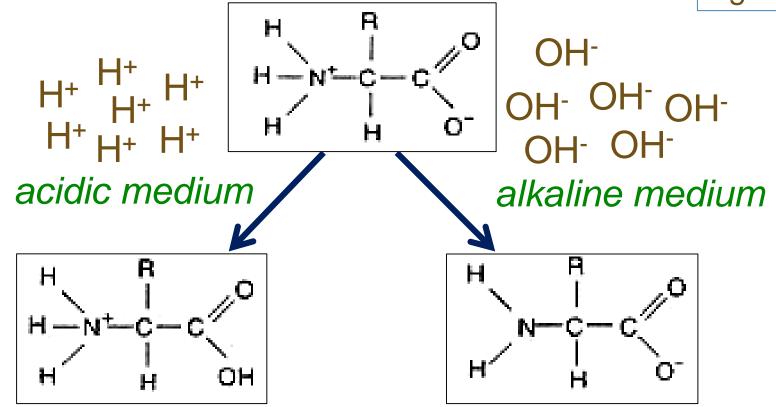
- Amino acid behaves as a base
- -COO⁻ gains a H⁺ & becomes -COOH





- Amino acid behaves as an acid
- -NH³⁺ loses a proton and becomes –NH₂







- ✤ What are some properties of an amino acid?
 - Soluble in water
 - Exist as Zwitterions
 - Amphoteric → Can act as buffers



In aqueous medium, an amino acid can become a zwitterion. What is a zwitterion?

An Amino acid with **both** positive and negative charges

+ What is isoelectric point?

pH at which an amino acid is a zwitterion is its **isoelectric point**

1. Amino acids **2**. Formation of peptide bonds **3**. Levels of organisations in proteins A. Primary Structure **B.** Secondary Structure C. Tertiary Structure D. Quaternary Structure 4. Case Studies: Haemoglobin

WHAT YOU NEED TO KNOW



Learning Outcome

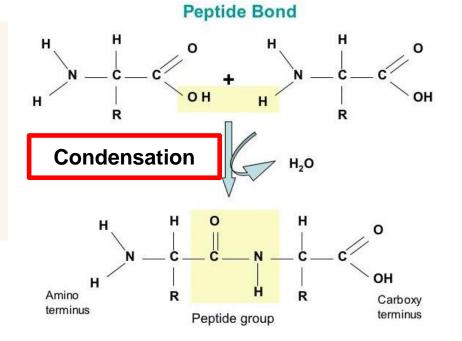
1(f)iii. Describe the formation & breakage of a peptide bond



Amino acids are linked by peptide bonds

A <u>covalent</u> bond formed between -<u>COOH</u> group of one a.a. & the -<u>NH₂</u> group of the next a.a.

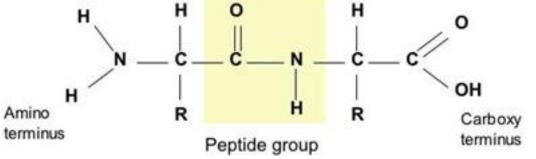
A water molecule is released



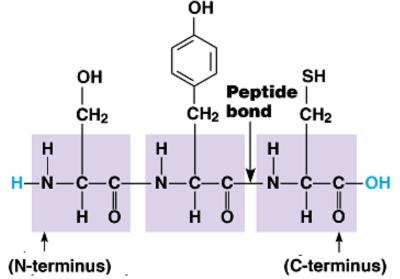


Once 2 a.a. are joined via a peptide bond, a dipeptide is formed

+ Has a free NH₂ at one end & a free COOH at the other

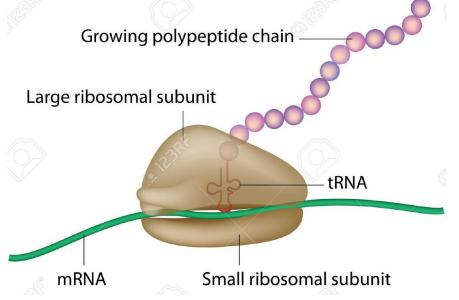


Continued condensation leads to formation of a long chain of a.a. called a polypeptide.





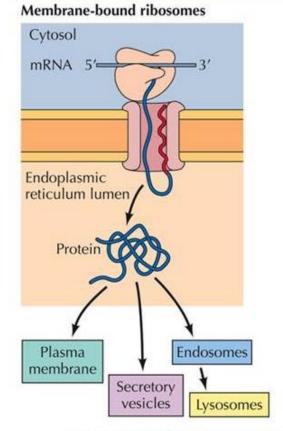
Occurs at ribosomes during translation, catalyzed by peptidyl transferase



Linear polypeptide will then fold, coil, or attach to other polypeptide chains to form a functional protein.

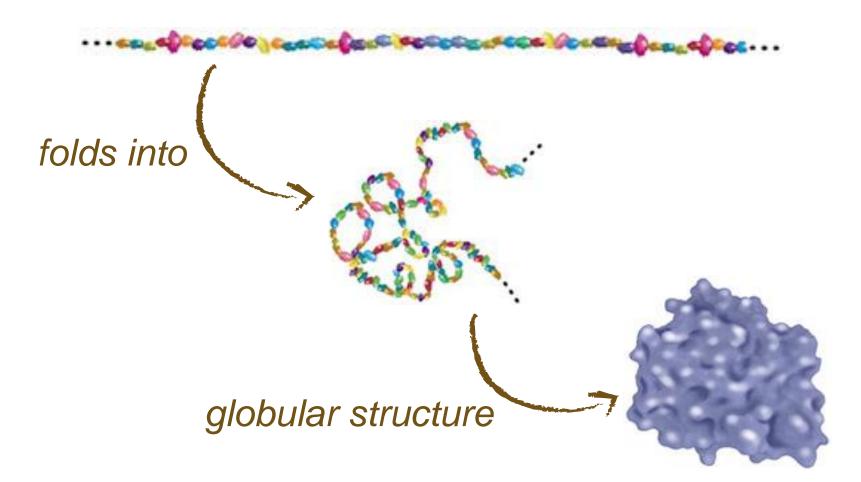
> Occurs in 🖉 RER & Golgi body

Pg106



THE CELL, Fourth Edition, Figure 10.3 G 2008 ASM Prets and Sinauer Associates, Inc.

Pg 106





Are these two tripeptides the same?

- N alanine lysine serine C N - serine – lysine – alanine - C
- A polypeptide has direction
- N-terminus is the beginning of the polypeptide
- C-terminus is the end



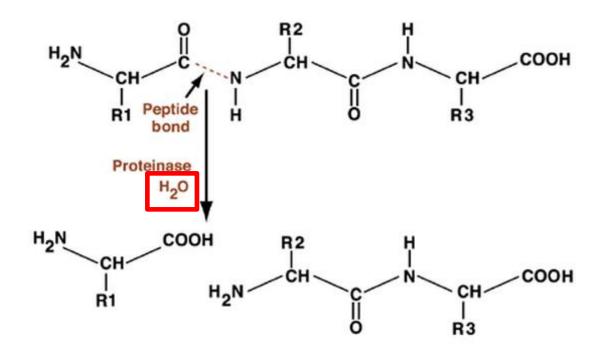
Hydrolysis of peptide bonds

+Add one H_2O across each peptide bond

Can be catalysed At high by enzymes temperature, (e.g. protease) with acid



Hydrolysis of peptide bonds



Learning Outcomes

1(Finil Describe the structure and properties of amino acids (in proteins)

1(finition) 1(finition) 1(finition) 1(finition)
 1(k) Explain primary structure, secondary structure, tertiary structure and quaternary structure of proteins, and describe the types of bonds that hold the molecule in shape (hydrogen, ionic, disulfide bonds and hydrophobic interactions).

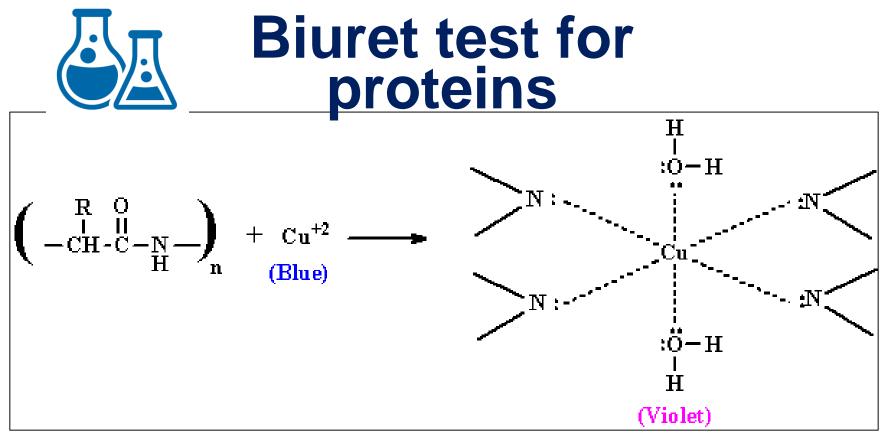




- Add an <u>equal volume</u> of Biuret's solution to sample solution.
- + Shake to mix contents thoroughly.
- Add dilute CuSO₄ drop by drop, shaking after every drop







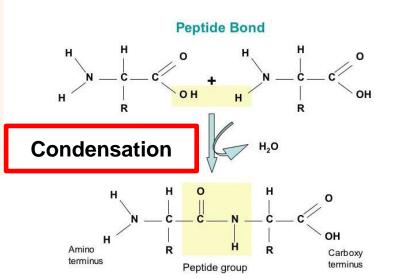




+ Describe how two amino acids are joined together.

Amino acids are linked by peptide bonds

A <u>covalent</u> bond formed between -<u>COOH</u> group of one a.a. & the -<u>NH₂</u> group of the next a.a. and a water **molecule** is being released

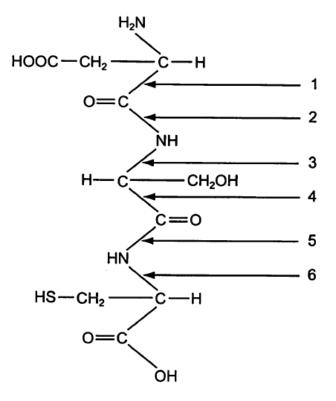


Tutorial 2: Proteins MCQ 3

+The diagram represents a tripeptide.

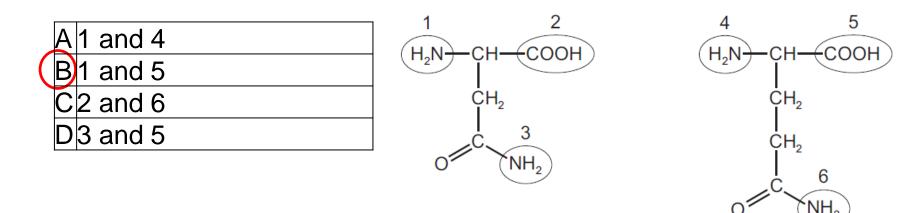
+At which bonds does hydrolysis occur to release the amino acids from the tripeptide?

\langle	A	1 and 4
	B)	2 and 5
	С	2 and 6
	D	3 and 6



Tutorial 2: Proteins MCQ 4

The diagrams show the structures of two amino acids, each of which has two amino (–NH2) groups. A peptide bond is formed between the two amino acids. Which groups form the peptide bond?



1. Amino acids 2. Formation of peptide bonds → 3. Levels of organisations in proteins A. Primary Structure **B.** Secondary Structure C. Tertiary Structure D. Quaternary Structure **4.** Case Studies: Haemoglobin

WHAT YOU NEED TO KNOW

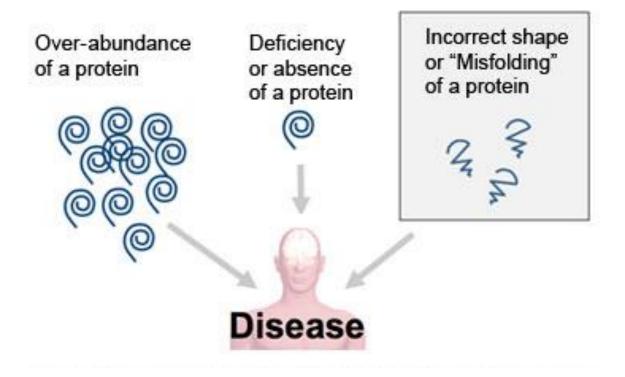


Learning Outcome

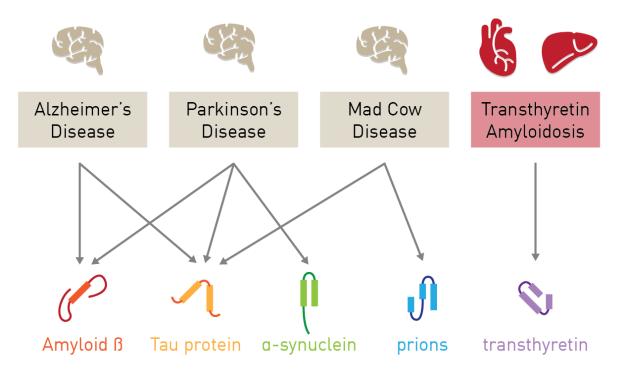


1(*m*) **Explain** the meaning of the terms <u>primary</u>, <u>secondary</u>, <u>tertiary</u> & <u>quaternary structure</u> of proteins & describe the <u>types of bonds</u> which hold the molecule in shape.

Why is protein folding important?



Some diseases caused by protein misfolding



Some Diseases CAUSED BY ABNORMAL proteins

What causes fatal mad cow disease?

A cow eats feed supplemented with sheep bone meal containing infectious proteins called prions. There has been a ban on such rendered feed since 1997.

O The prions are suspected of corrupting normal protein production.

O Prions are absorbed by the stomach and are thought to travel along nerve fibers to the brain stem, destroying brain tissue.

Empty area

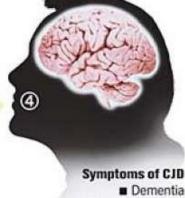
Cow brain tissue showing bovine spongiform encephalopathy (BSE)

> When humans eat processed meat products that might contain prioninfected tissues, they too can come down with a similar fatal

syndrome, dubbed variant Creutzfeldt-Jakob disease. All the various bans on cattle and beef products are designed to eliminate human consumption

of the tainted meat.

Human brain tissue showing Creutzfeldt-Jakob disease (CJD)



Sleep disorders
 Schizophrenia-like symptoms

Symptoms of BSE

- Disorientation
- Fearfulness
- Wobbly movement

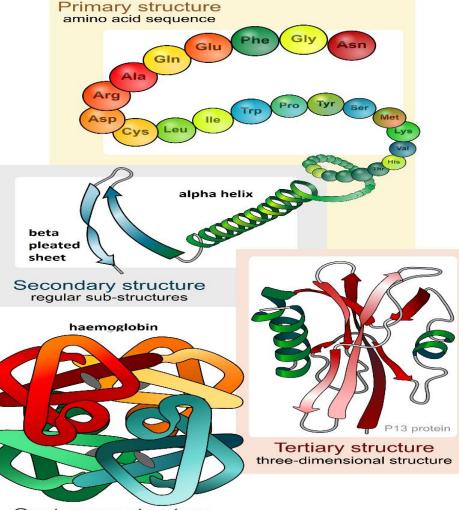
Sources- UCSF, World Health Organ

Cumulatively, through the end of 2015, more than **184,500 cases of BSE** had been confirmed in the United Kingdom alone in more than 35,000 herds

JOHN BLANCHARD / The Chronicle

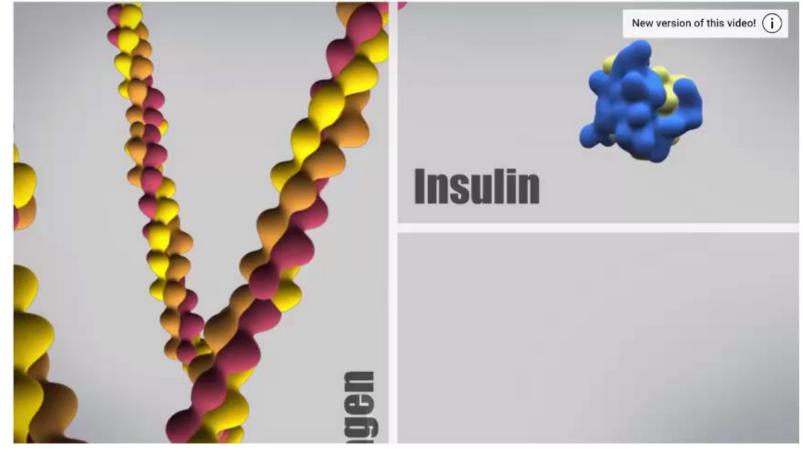
VIDEO: WHY STUDY PROTEIN FOLDING? <u>https://youtu.be/KpedmJdrTpy</u>

4 levels of protein folding



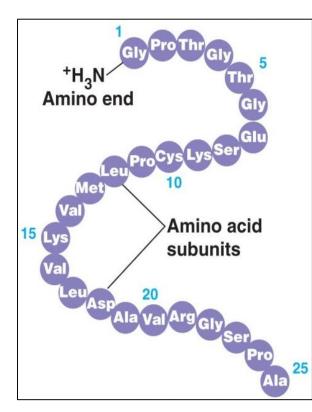
Quaternary structure

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





Primary structure

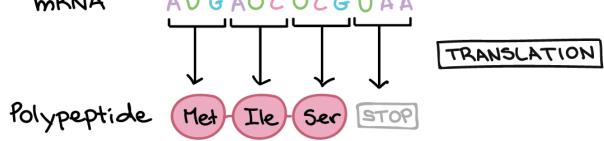


Refers to the <u>number</u>, <u>sequence</u> & <u>type</u> of a.a. in a linear polypeptide chain.

Peptide bonds are involved



Primary structure Seq. of a.a. is determined by seq. of nucleotide bases in the gene encoding the protein. DNA TRANSCRIPTION mRNA AVGAUCUCGUAA

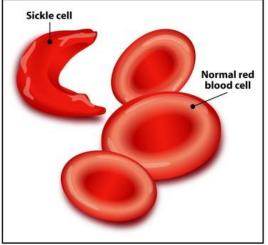




Primary structure

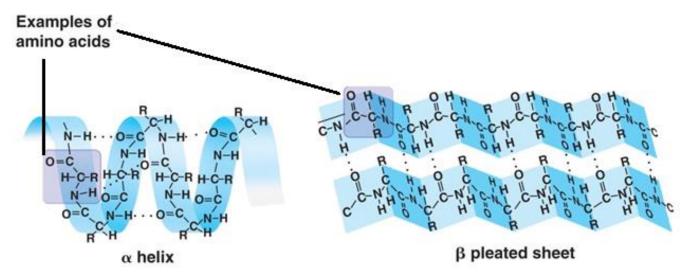
A change in the DNA seq. may change the polypeptide sequence & can affect a protein's
 <u>3D conformation</u> & <u>function</u>

+e.g. sickle cell anaemia



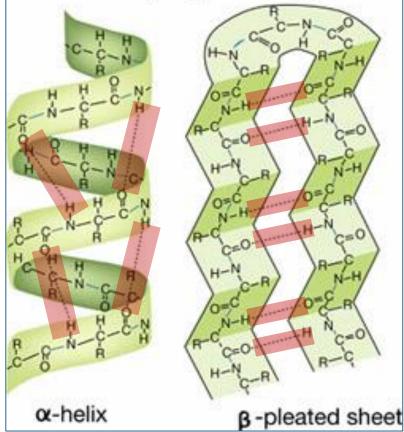


Secondary structure Refers to the folding of polypeptide chain into <u>regular structures</u> such as <u>α-helices</u> & <u>β-pleated sheets</u>



Pg 110

Secondary structure is the result of hydrogen bonding



Bonds involved:

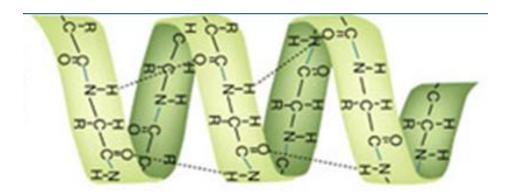
Hydrogen bonds form between -CO group of a peptide bond on one a.a. & the -NH group on a peptide bond of another a.a. in the <u>same</u> polypeptide chain.

hydrogen bonds



α-helix

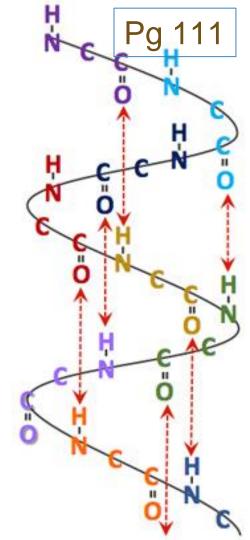
Coiling of polypeptide chain into a **helical** conformation



α-helix

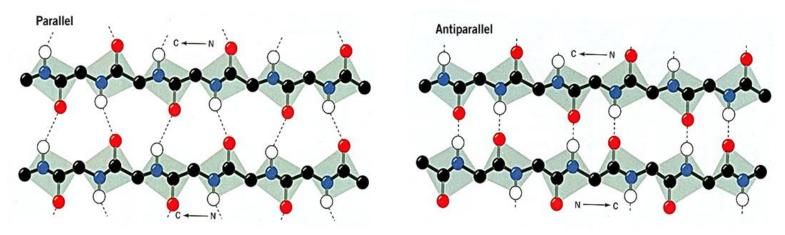
Carbonyl oxygen (**C=O**) of each a.a. in the peptide bond is **hydrogen bonded** to the hydrogen on the amino group (**N-H**) of the a.a. that is **four a.a. away** in the linear sequence

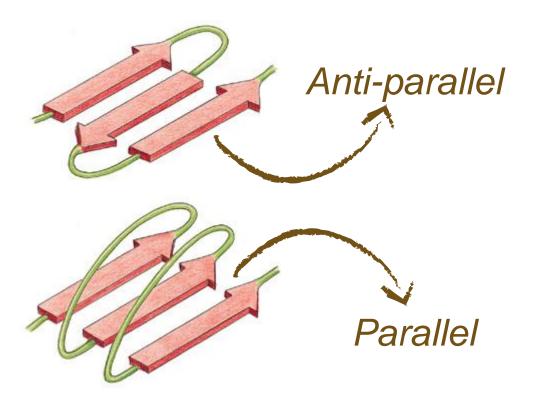
e.g. keratin





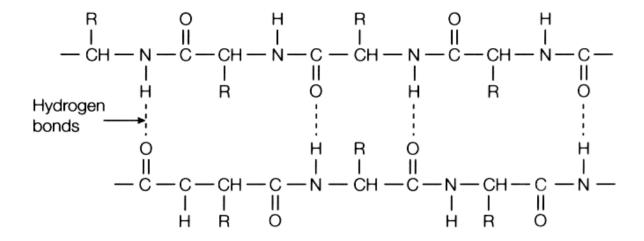
Made up of different sections of a single polypeptide running parallel or anti-parallel to each other







Stabilised by <u>hydrogen bonds</u> formed between the peptide bonds in different sections of the <u>same</u> polypeptide chain.

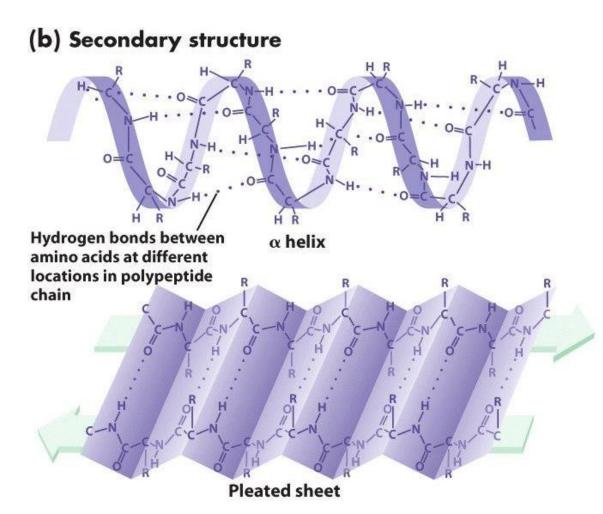




 Multiple β –pleated sheets provide strength & resilience in many structural proteins
 e.g. silk fibroin consists of stacks of antiparallel β – pleated sheets





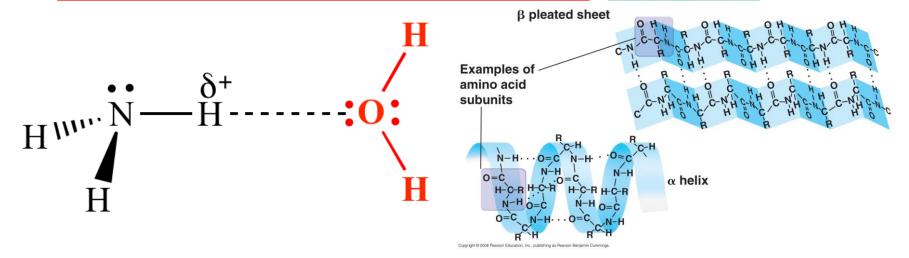




Are Hydrogen Bonds Strong Bonds??

Ans: While each hydrogen bond is very <u>weak</u>, the <u>large</u> number of such bonds means that they play an important role in maintaining the shape and stability of a polypeptide molecule.

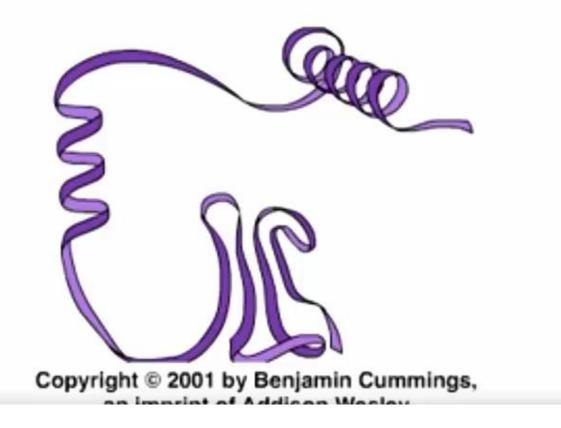




- **1.** Amino acids
- **2.** Formation of peptide bonds
- **3.** Levels of organisations in proteins
 - A. Primary Structure
 - B. Secondary Structure
 - C. Tertiary Structure
 - D. Quaternary Structure
 - 4. Case Studies: Haemoglobin

WHAT YOU NEED TO KNOW

Video: Tertiary structure



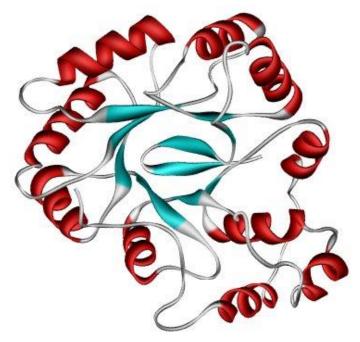
114

Pq



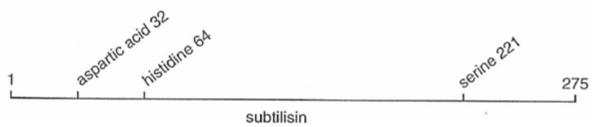
Refers to the folding of the polypeptide chain into its unique 3D globular conformation

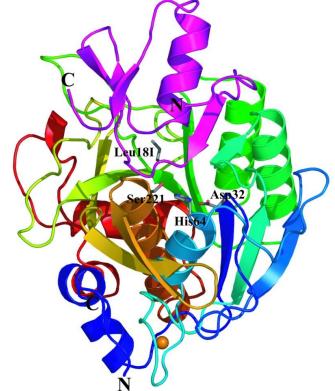
Folding from primary to secondary to tertiary structure





Refers to the relationship of a.a. residues that are far apart in the linear sequence



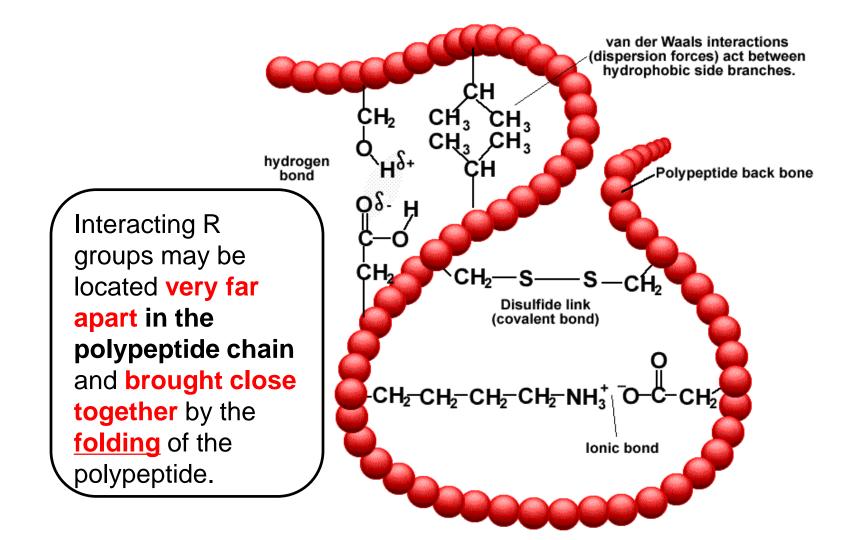




Bonds in 3° structure

Hydrogen bonds b/w polar R groups Ionic bonds b/w acidic & basic R groups

Disulfide bonds b/w -SH groups of cysteine Hydrophobic interactions b/w non-polar R groups





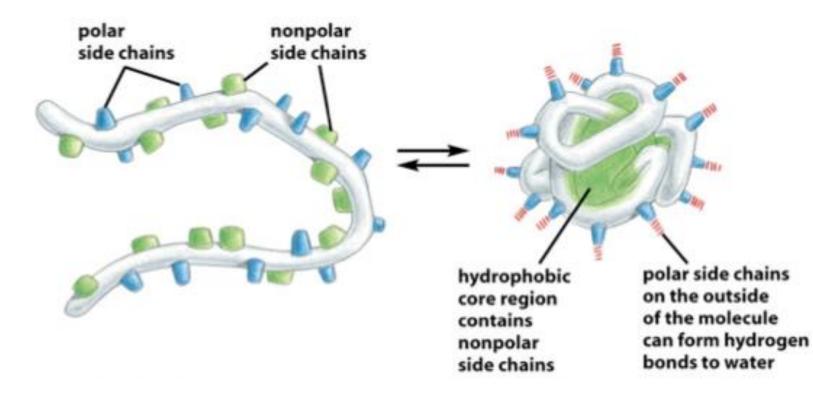
Water-soluble globular proteins fold spontaneously

so that

majority of its hydrophobic side chains of non-polar a.a are buried in the interior

majority of polar, charged side chains of polar and charged a.a. are on the surface



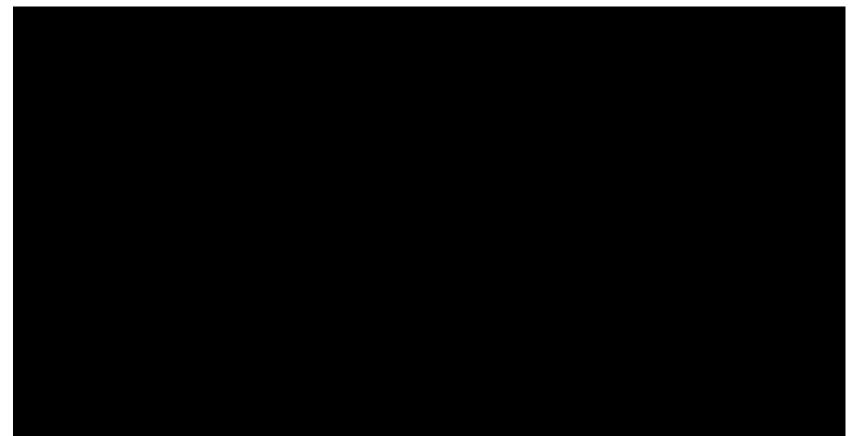




Once folded, the 3-dimensional biologically active conformation of the protein is maintained by the four types of bonds



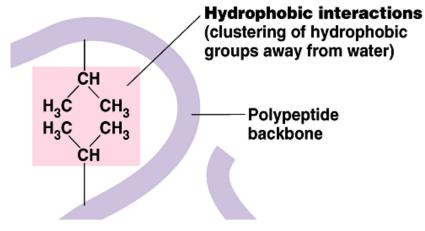
VIDEO: PROTEIN FOLDING <u>https://youtu.be/hok2hyED9go</u>





Hydrophobic interactions

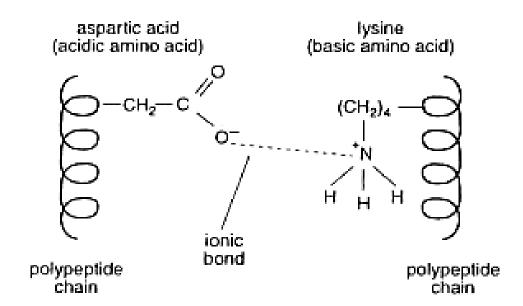
During polypeptide folding, hydrophobic R groups will cluster at interior of protein, out of contact with water





lonic bonds

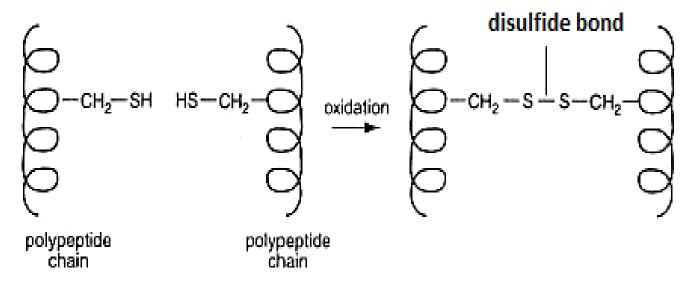
Formed between acidic (-ve) & basic (+ve) R groups





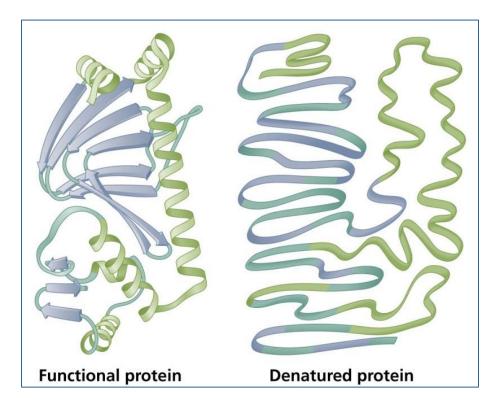
Disulfide bonds

Formed between two –SH groups of cysteine residues





Denaturation





Denaturation

Loss of / alteration of the specific 3D conformation of a protein molecule

- + May be permanent or temporary
- +1° structure remains unchanged
- Once denaturation occurs, the protein molecule unfolds & loses normal biological function.



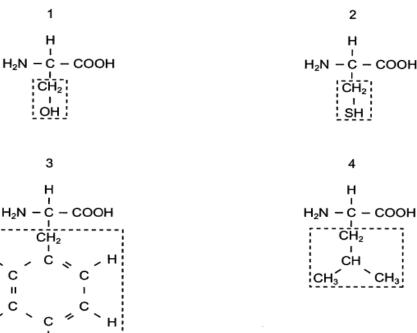
Denaturation

Caused by the following factors :

- * High temperature
- * pH alteration
- W UV irradiation
- * High ionic concentration
- * Heavy metals
- * Organic solvents

The diagram shows four different amino acids, each with a different R group (side chain). ¹

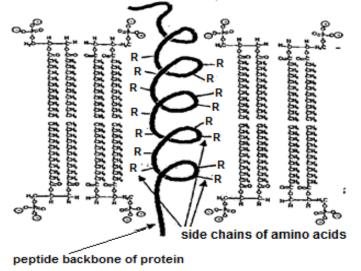
Which amino acids could form a hydrophobic interaction between their R groups?

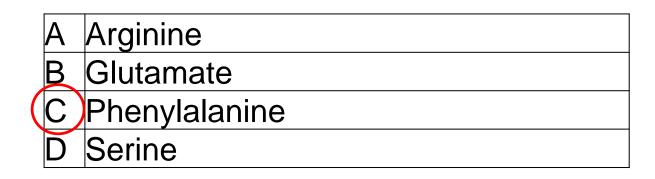


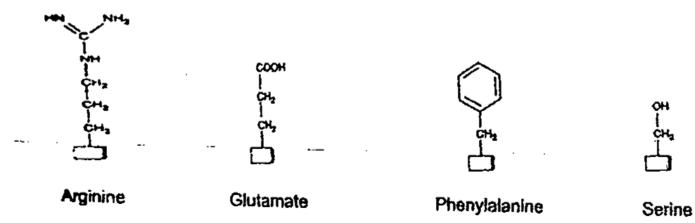
Which amino acids could form a hydrophobic interaction between their R groups?

Α	1 and 2	
В	1 and 3	
С	2 and 4	
) 3 and 4	

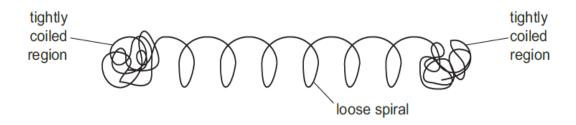
+With reference to the figure, which one of the following amino acids, with only the side chains indicated, is likely to be found at any of the positions protruding from the peptide backbone of the protein?







+The protein glutenin gives bread dough its elasticity. The diagram represents a polypeptide of glutenin.



+What describes the structure of glutenin?

- secondary structure because the loose spiral is an α -helix
- B) tertiary structure because the different regions form a 3D shape
 - quaternary structure because there are both globular and fibrous regions
 - O quaternary structure because there are both spiral and tightly coiled regions



Learning Outcome



1(*I*) <u>Explain</u> the effects of temperature and pH on protein structure.

Denaturation by high temperature

At high temperature, protein gains kinetic energy Thermal agitation disrupts hydrogen, ionic bonds & hydrophobic interactions Loses specific 3D DENATURATION conformation & biological function

Folded Protein

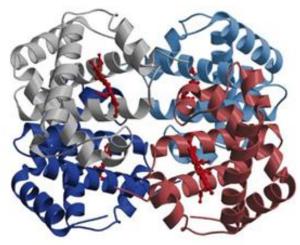
Unfolded Protein

Denaturation by alteration of pH

Δ in pH alters [H⁺] & [OH⁻] in environment Alters ionic charges of acidic & basic R groups → disrupts ionic & hydrogen bonds Loses specific 3D conformation & biological function

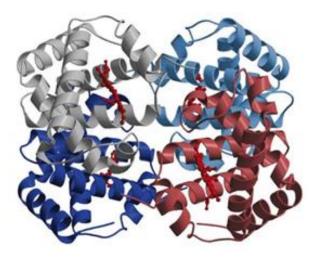


Refers to arrangement of polypeptide subunits within a protein that is made up of **more than one polypeptide chains**





i.e. spatial arrangement of more than one polypeptide chain





Bonds involved :

- * Same as those in tertiary structure
- * But they form between R groups of different polypeptide chains in the same protein



Examples:

, Haemoglobin

i globular

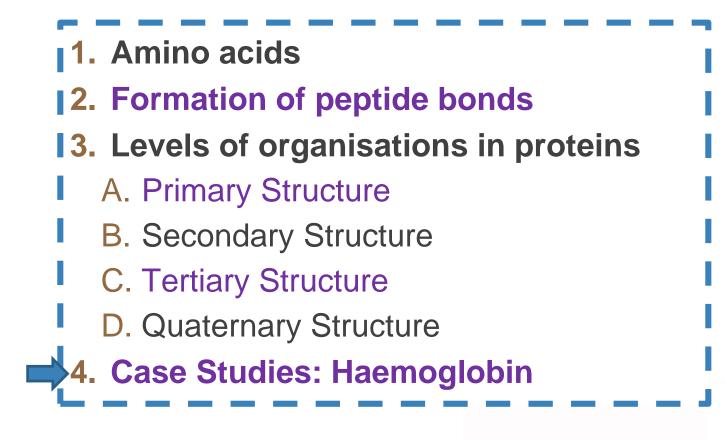
Collagen.

(Not in H1 Syllabus)

fibrous



Level of organisation	Bonds holding molecule in shape	Formed between
Primary	Peptide bonds	NH ₂ & COOH groups of adjacent amino acids
Secondary	Hydrogen bonds	-CO & -NH of peptide bonds
Tertiary	Ionic bonds Hydrogen bonds Disulfide bonds Hydrophobic interactions	R groups of amino acids within same polypeptide
Quaternary	Ionic bonds Hydrogen bonds Disulfide bonds Hydrophobic interactions	R groups of amino acids of different polypeptides



WHAT YOU NEED TO KNOW

Learning Outcome

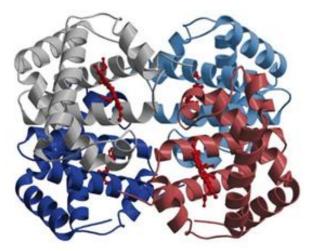


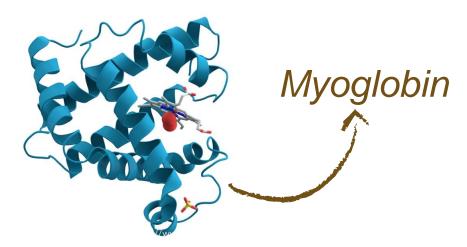
1(*m*) <u>Describe</u> the <u>molecular</u> <u>structure</u> of the <u>haemoglobin</u> protein and explain how its structure relates to <u>its function</u> in transport

Haemoglobin

Haemoglobin is one of two oxygen-binding proteins found in vertebrates.

+ It is found exclusively in red blood cells.

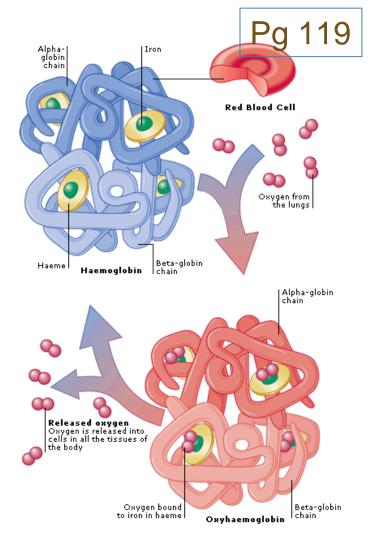




Haemoglobin

Function:

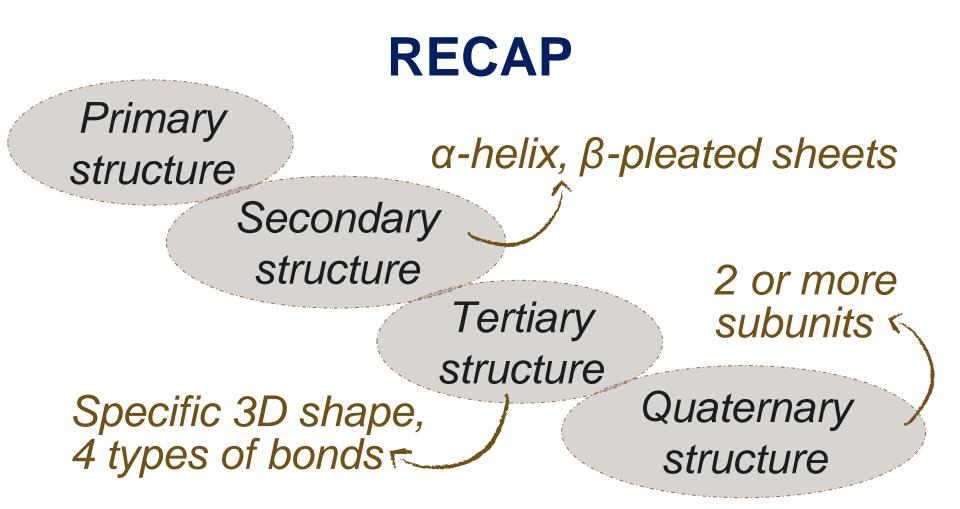
A transport protein, it <u>carries</u> O_2 in the blood from the lungs to the capillaries of tissues, in order to supply cells with O_2 .





Haemoglobin Structure

Quaternary – 4 subunits (each subunit has tertiary structure) > 2α -chains & 2β -chains subunits Each chain is primarily α-helical



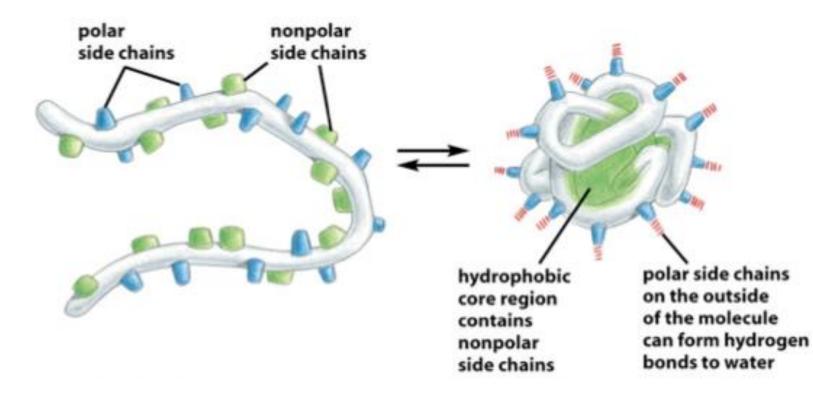


Each polypeptide has hydrophobic & hydrophilic a.a. residues

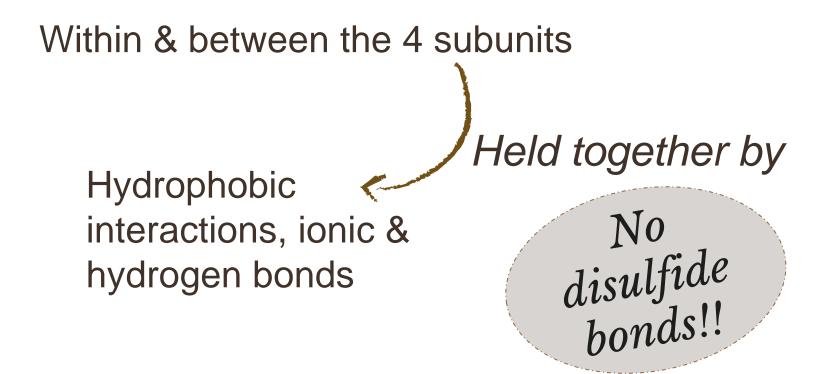
3° structure folded so that non-polar R groups are buried in the interior Polar & charged R groups are on the surface



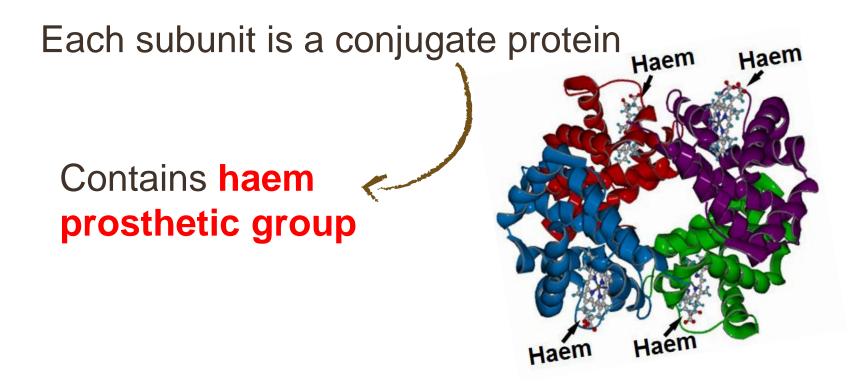
Tertiary structure





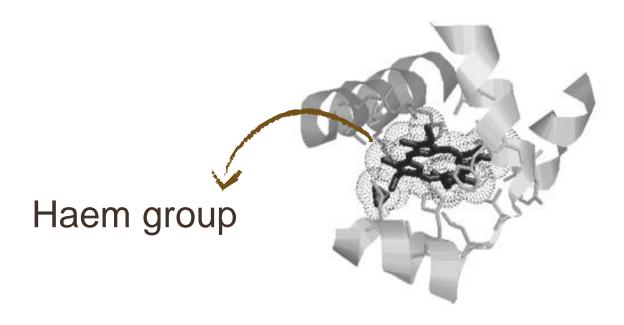








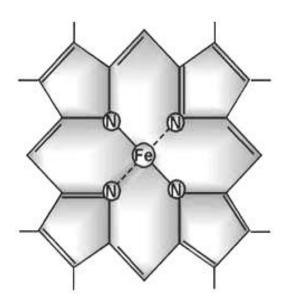
Haem-binding site





Porphyrin ring & Fe²⁺

Haem group = a porphyrin ring + Fe^{2+}

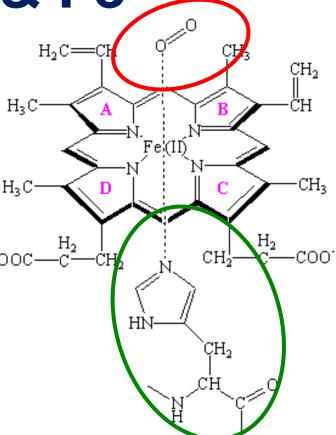


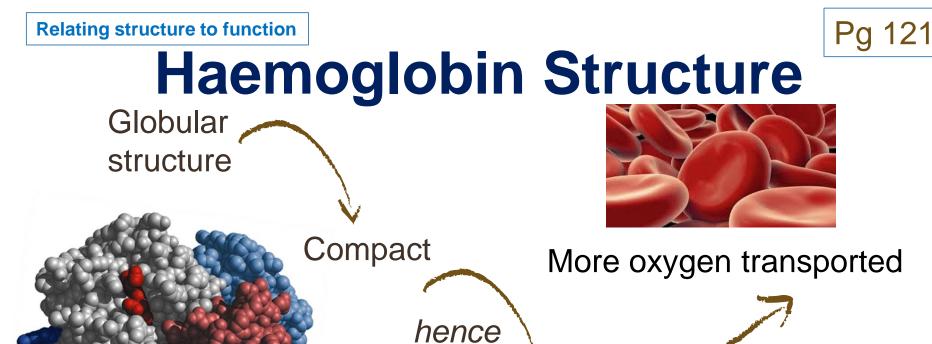


Porphyrin ring & Fe²⁺

Fe²⁺ is at the center of the planar porphyrin ring.

The haem is orientated so that its Fe²⁺ on **one face is complexed to an a.a. residue**, while the **other face is accessible for O**₂ **binding.**





Many Hb molecules can be packed into a RBC



Haemoglobin Structure 4 subunits Each bind 1 oxygen molecule Increases capacity for transport of oxygen. More oxygen transported



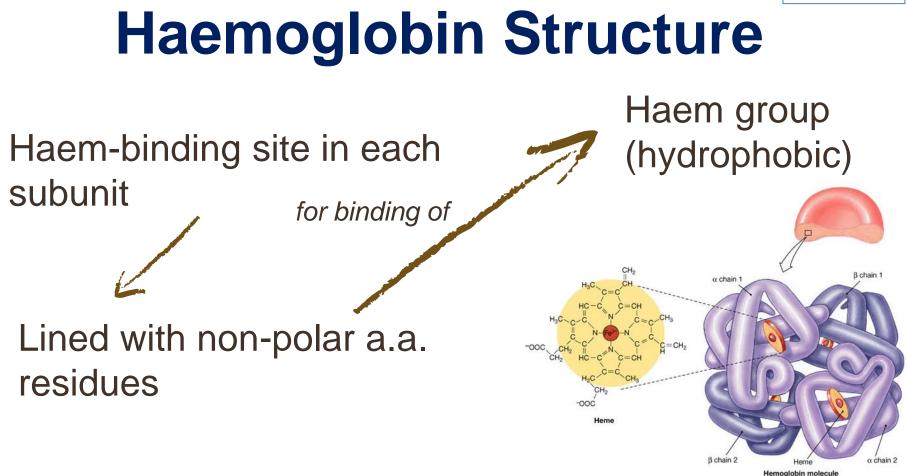
Globular structure with a hydrophobic core and a hydrophilic exterior.

Hb soluble in RBC cytoplasm ⁴

A good transport protein for O_2 in blood





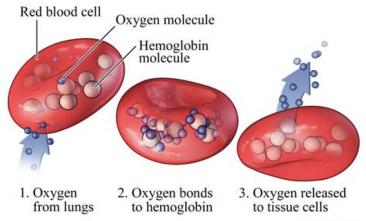




Haem group (hydrophobic) Allows <u>reversible</u> binding of oxygen, enhancing release in metabolically active tissues

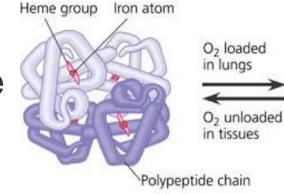


porphyrin ring bound to an iron ion





+ Each Hb molecule consists of four subunits, each capable of binding one O_2 + This greatly facilitates transport of O₂ by Hb.

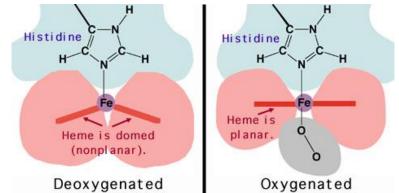






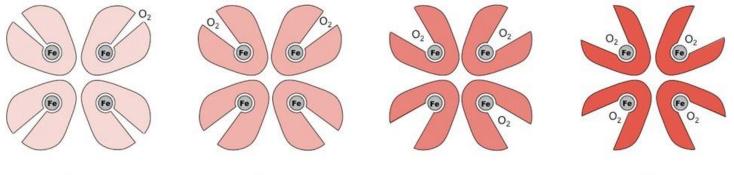
Binding of one O_2 molecule to one subunit induces the remaining unfilled subunits to **change their shape slightly** so that their **affinity for O₂ increases**.

Loading of the first O_2 molecule results in rapid loading of 3 more O_2



+ Binding of O_2 to haemoglobin is said to be <u>cooperative</u>

Conversely, when one subunit unloads O₂, the other three more readily unload as a conformation change lowers their affinity of O₂.



HbO₂

HbO₄

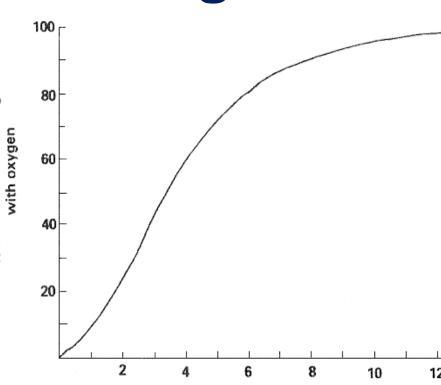
HbO₆

HbO₈

121

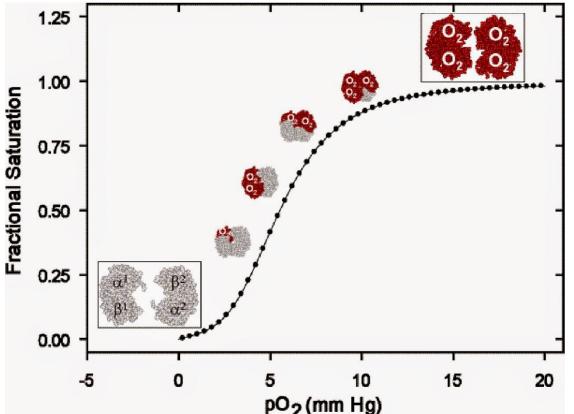


The oxygen dissociation nidolgor curve of haemoglobin has a % saturation of haem steep slope (S-shaped or sigmoid), as even a slight change in the $[O_2]$ causes haemoglobin to load/unload a substantial amount of O_2



Partial Pressure of Oxygen / kPa

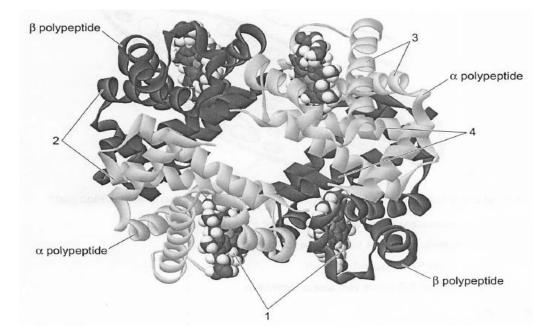




Tutorial 2: Proteins MCQ 6

+The diagram shows a haemoglobin molecule.

+Which identifies the different parts of the molecule?



Tutorial 2: Proteins MCQ 6

Which identifies the different parts of the molecule?

	1	2	3	4
Α	alpha helix	beta pleated sheet	binding site	hydrophobic amino acids
В	hydrophobic amino acids	beta pleated sheet	prosthetic group	binding site
С	prosthetic group	hydrophobic amino acids	alpha helix	hydrophilic amino acids
D	prosthetic group	hydrophilic amino acids	alpha helix	hydrophobic amino acids

Video

