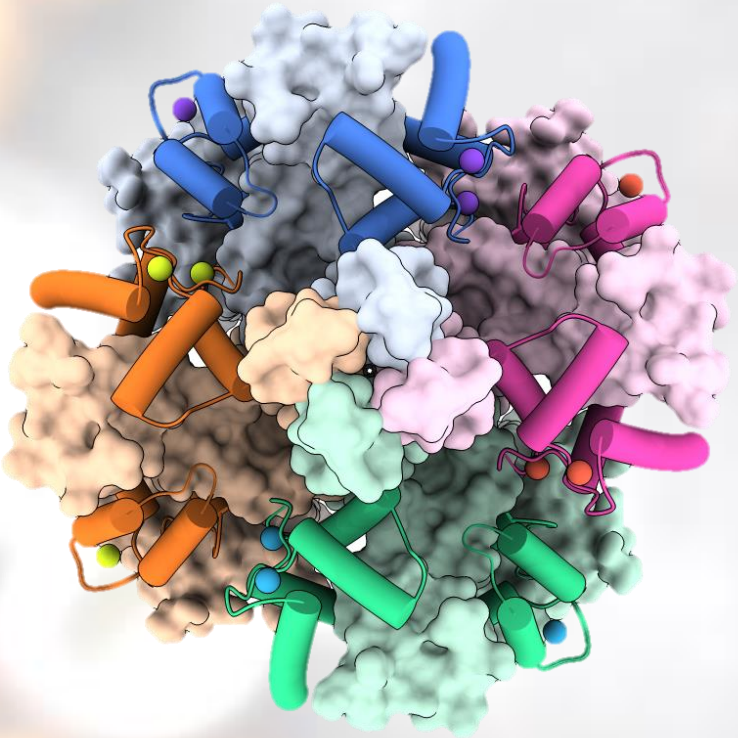
Chemistry of amino acids and proteins



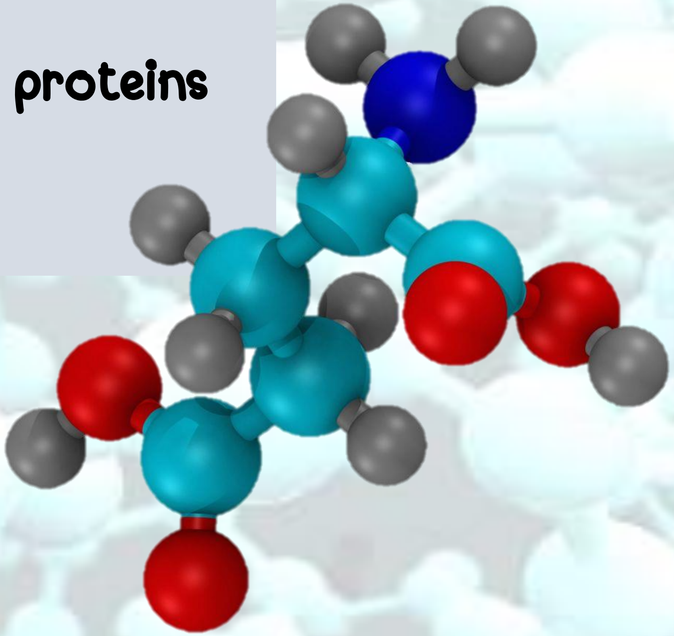
Asst. Prof. Panupong Mahalapbutr, Ph.D.

Department of Biochemistry, Faculty of Medicine, Khon Kaen University

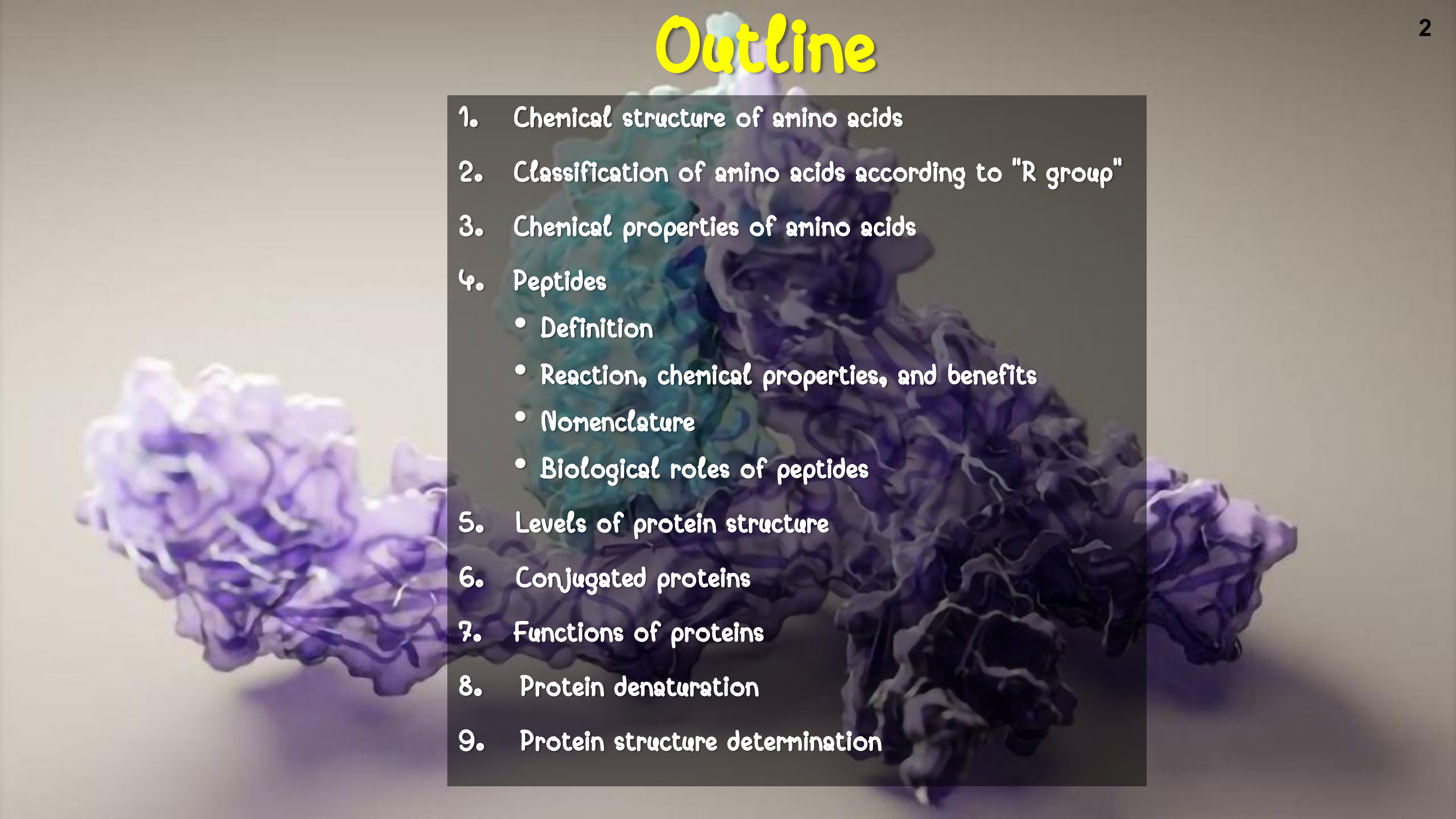
E-mail: panupma@kku.ac.th

Objectives……

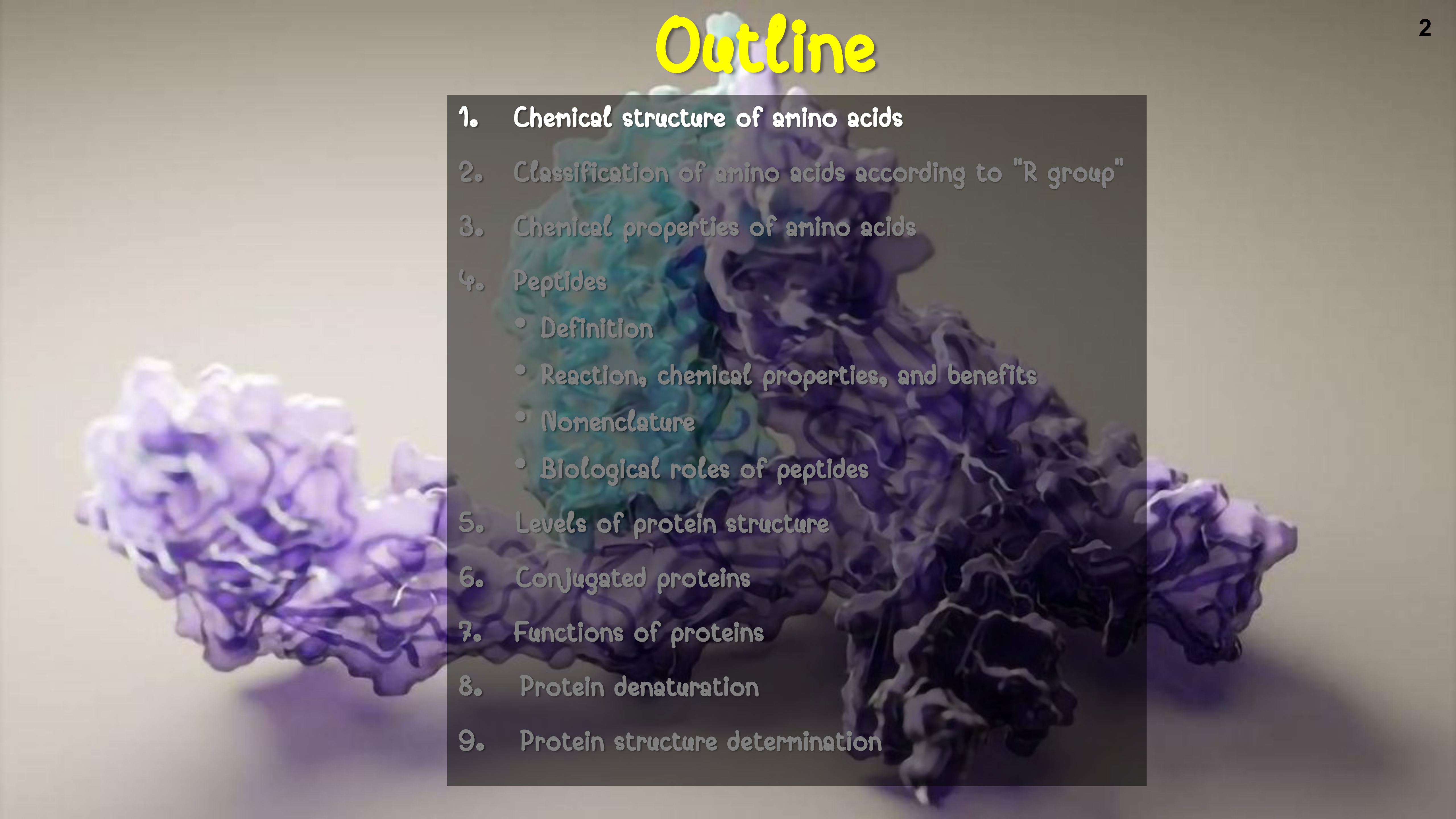
1. Explain the structure and chemical properties of amino acids, peptides, and proteins
2. Classify amino acids according to their R group properties
3. Describe the 4 levels of protein structure, including the bonds and interactions involved in each level.
4. Describe the biological functions of amino acids, peptides, and proteins
5. State the factors that affect the denaturation of proteins



Outline



1. Chemical structure of amino acids
2. Classification of amino acids according to "R group"
3. Chemical properties of amino acids
4. Peptides
    - Definition
    - Reaction, chemical properties, and benefits
    - Nomenclature
    - Biological roles of peptides
5. Levels of protein structure
6. Conjugated proteins
7. Functions of proteins
8. Protein denaturation
9. Protein structure determination



Outline

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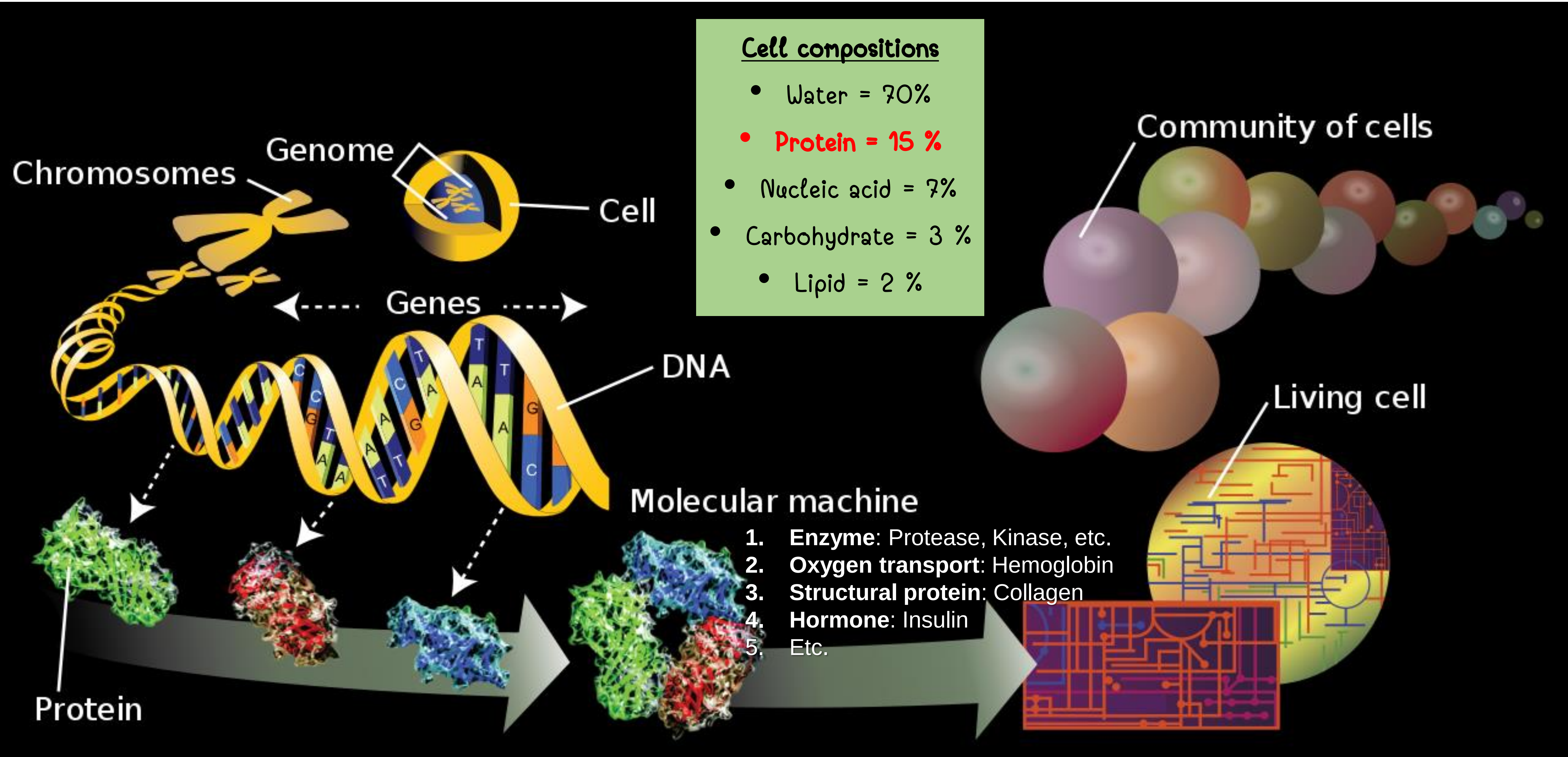
Proteins are one of the most abundant organic molecules in living systems



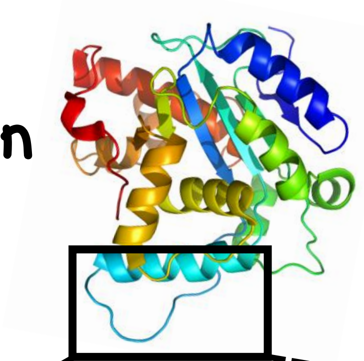
Cell compositions

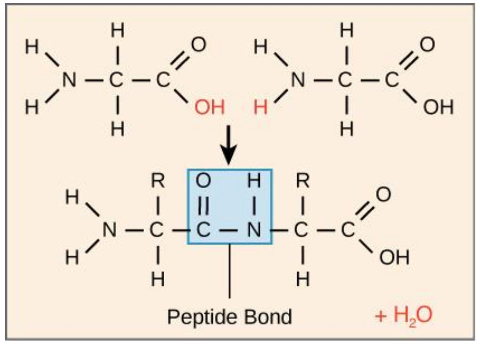
- Water = 70%
- Protein = 15 %
- Nucleic acid = 7%
- Carbohydrate = 3 %
- Lipid = 2 %

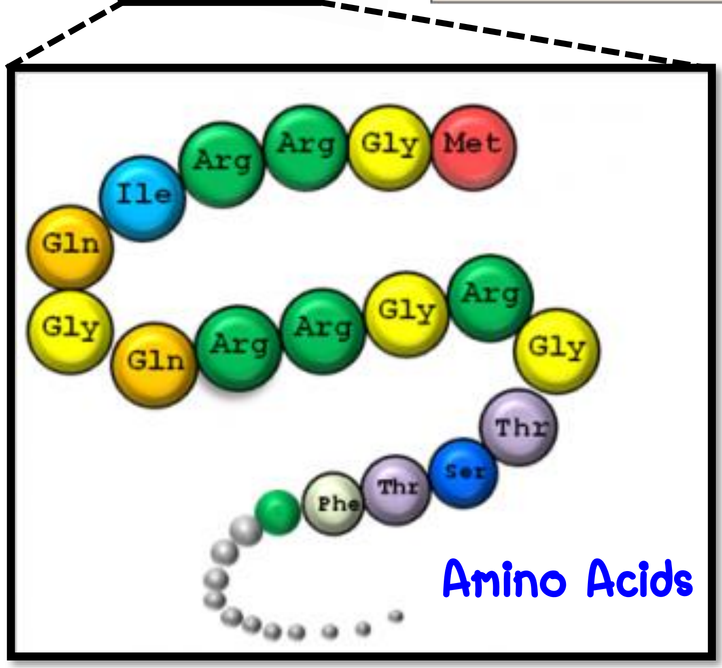
1. **Enzyme**: Protease, Kinase, etc.
2. **Oxygen transport**: Hemoglobin
3. **Structural protein**: Collagen
4. **Hormone**: Insulin
5. Etc.

The smallest units of protein are amino acids

Protein = Polymer of amino acids linked together by peptide bonds

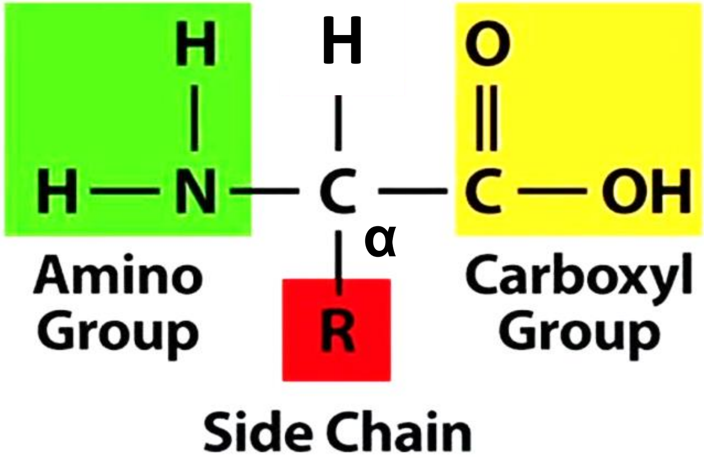






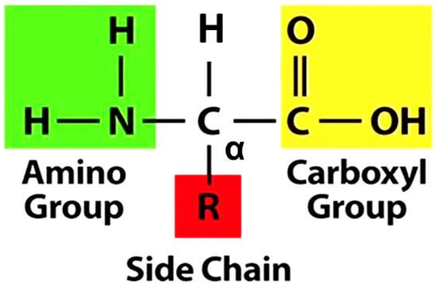
Structure of amino acid

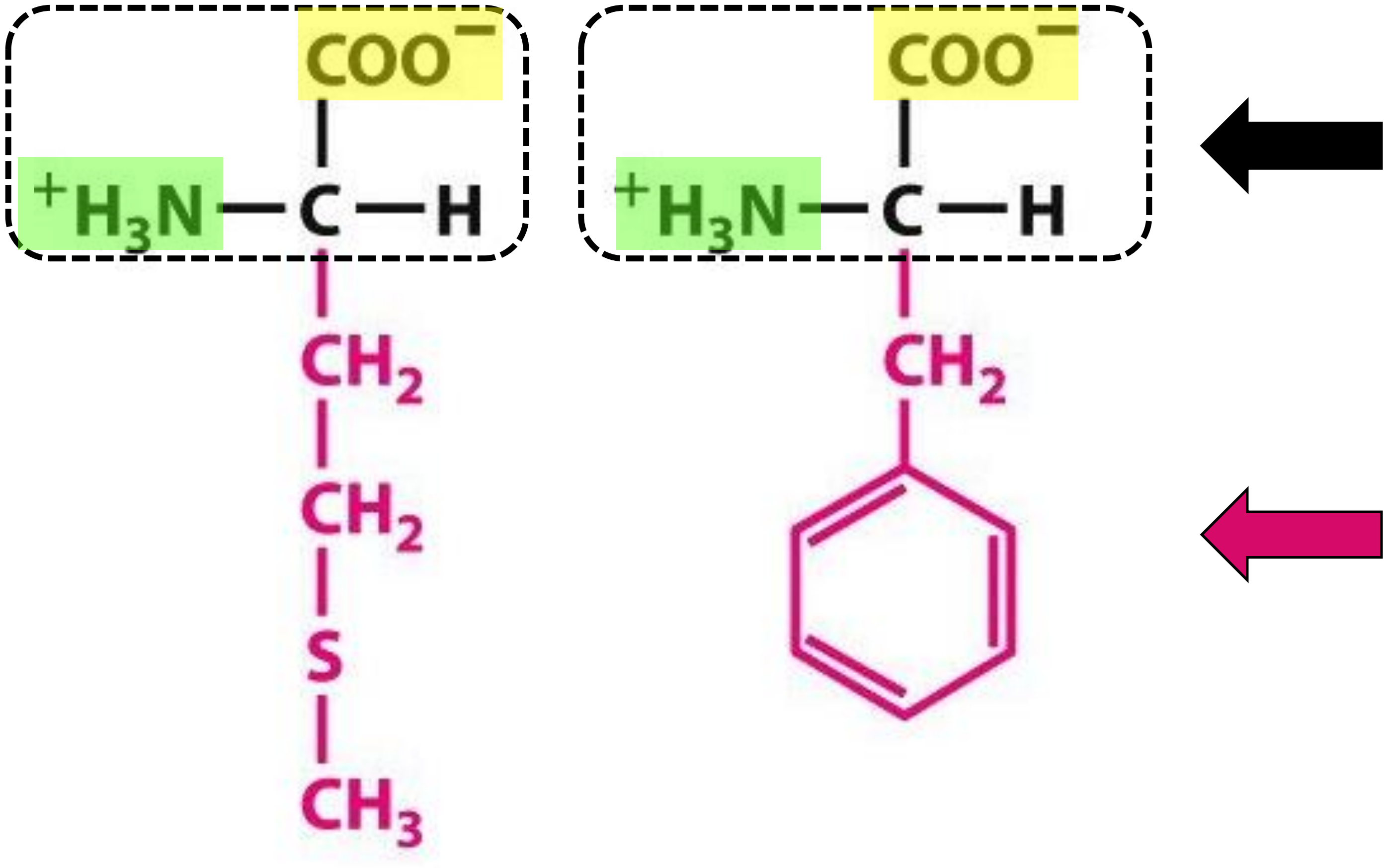
1. Alpha carbon (C_α)
2. Amino group
3. Carboxyl (Acid) group
4. R group (side chain)



***R group determines the differences in each amino acid

The smallest units of protein are amino acids





Same

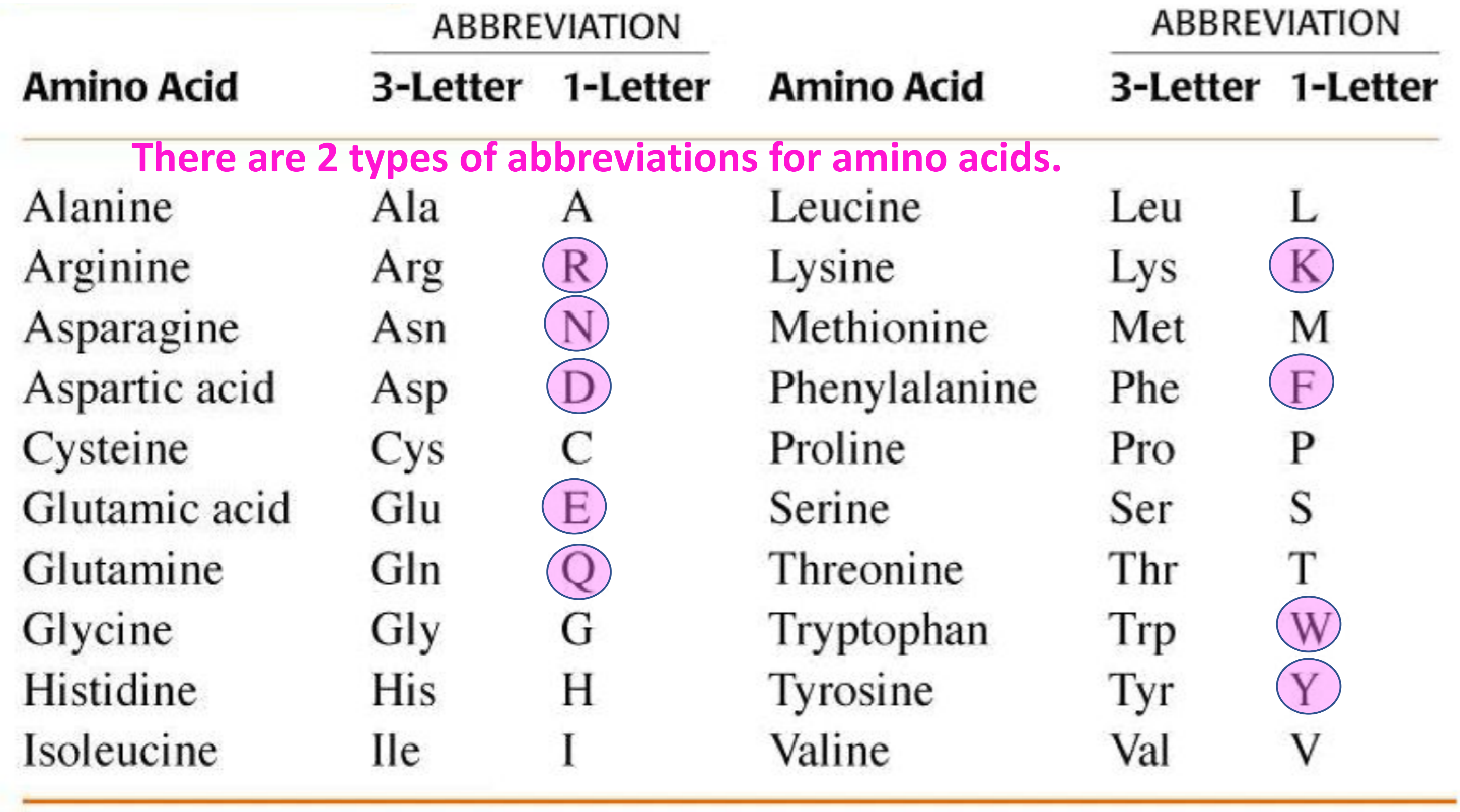
R groups are different

Methionine
(Met or M)

Phenylalanine
(Phe or F)

There are more than 300 amino acids in nature, but only 20 are found as building blocks of proteins.

They are called common amino acids or standard amino acids.

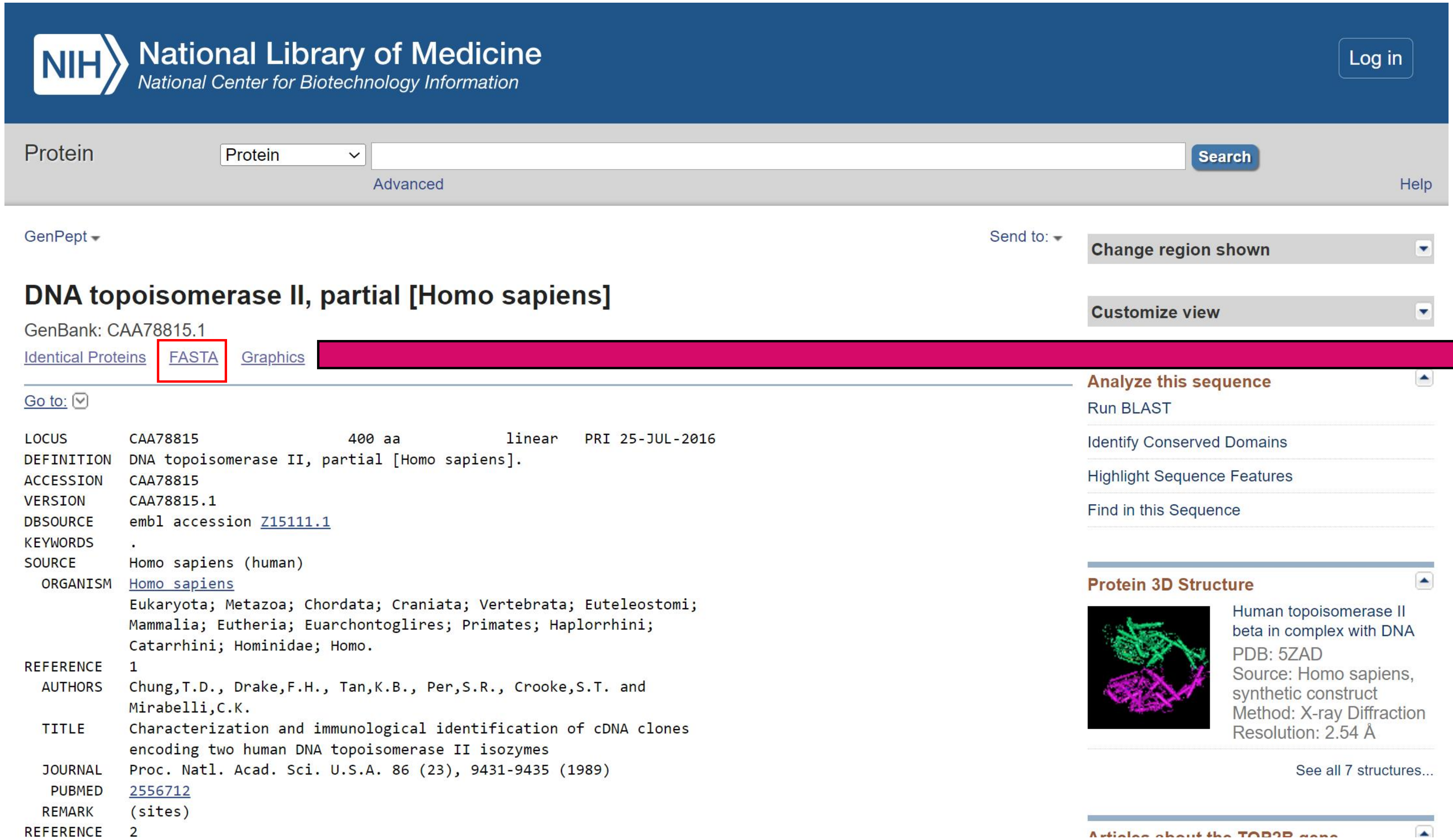


There are 2 types of abbreviations for amino acids.

Amino Acid	ABBREVIATION 3-Letter	 1-Letter	Amino Acid	ABBREVIATION 3-Letter	 1-Letter
Alanine	Ala	A	Leucine	Leu	L
Arginine	Arg	R	Lysine	Lys	K
Asparagine	Asn	N	Methionine	Met	M
Aspartic acid	Asp	D	Phenylalanine	Phe	F
Cysteine	Cys	C	Proline	Pro	P
Glutamic acid	Glu	E	Serine	Ser	S
Glutamine	Gln	Q	Threonine	Thr	T
Glycine	Gly	G	Tryptophan	Trp	W
Histidine	His	H	Tyrosine	Tyr	Y
Isoleucine	Ile	I	Valine	Val	V

FASTA format (1-letter symbol)

https://www.ncbi.nlm.nih.gov/protein/CAA78815.1



DNA topoisomerase II, partial [Homo sapiens]

GenBank: CAA78815.1

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DEFINITION  DNA topoisomerase II, partial [Homo sapiens].
ACCESSION   CAA78815
VERSION     CAA78815.1
DBSOURCE    embl accession Z15111.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Chung,T.D., Drake,F.H., Tan,K.B., Per,S.R., Crooke,S.T. and
            Mirabelli,C.K.
  TITLE     Characterization and immunological identification of cDNA clones
            encoding two human DNA topoisomerase II isozymes
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 86 (23), 9431-9435 (1989)
   PUBMED   2556712
  REMARK    (sites)
REFERENCE   2
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DNA topoisomerase II, partial [Homo sapiens]

GenBank: CAA78815.1

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>CAA78815.1 DNA topoisomerase II, partial [Homo sapiens]
MAKSGGCGAGAGVGGGNGALTWVNNAAKKEESETANKNDSSKKLSVERVYQKKTQLEHILLRPDTYIGSV
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MNNMMKTSEAKIKHFDGEDYTCITFQPDLSKFKMEKLDKDIVALMTRRAYDLAGSCRGVKVMFNGKKLPV
NGFRSYVDLYVKDKLDETGVALKVIHELANERWDVCLTLSEKGFQQISFVNSIATTKGGRHVDYVVDQVV
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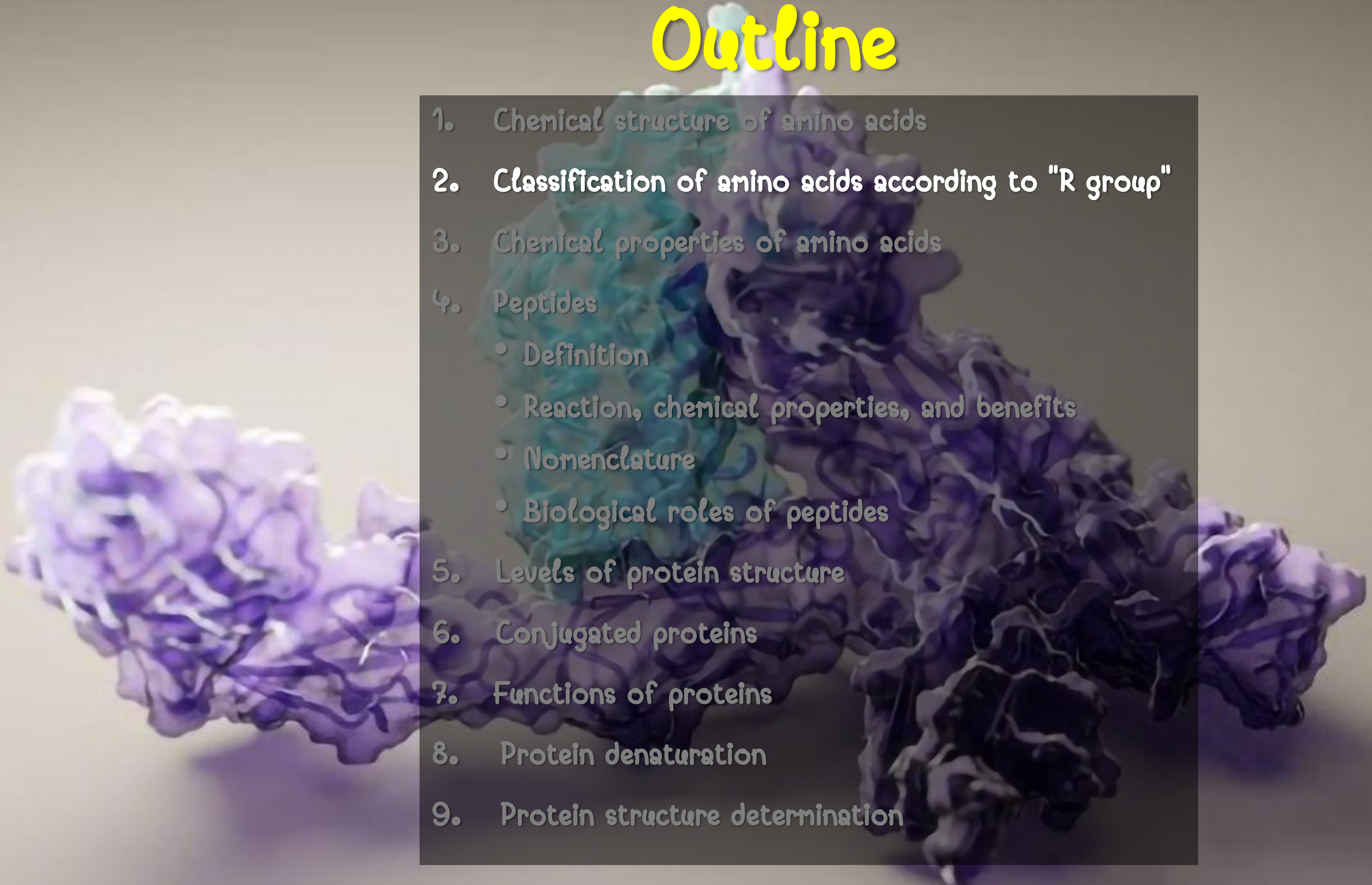
Outline



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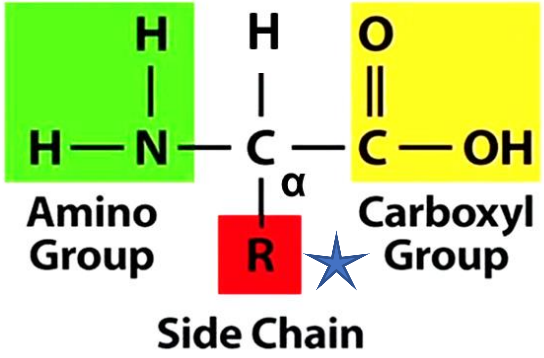
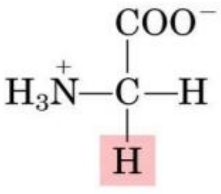
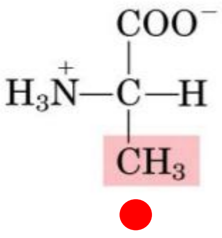


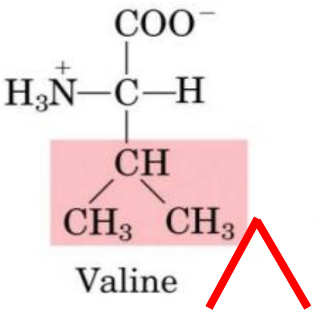
1. Nonpolar, aliphatic R groups
2. Polar, uncharged R groups
3. Positively charged R groups
4. Negatively charged R groups
5. Aromatic R groups

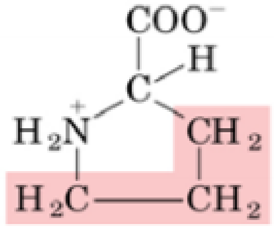
Classification of amino acids according to "R group"

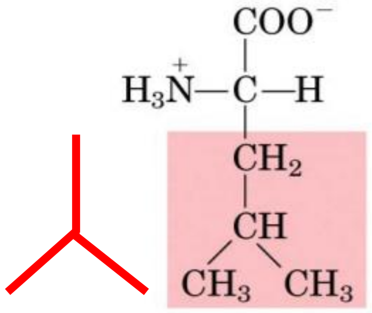
1. Non-polar, aliphatic R groups

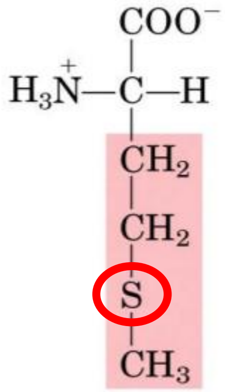

Glycine

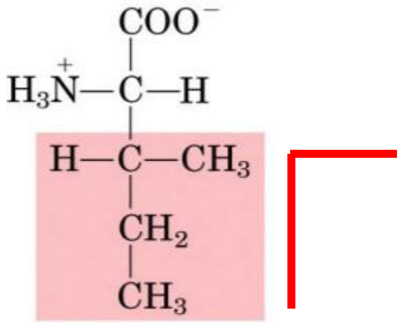

Alanine


Valine

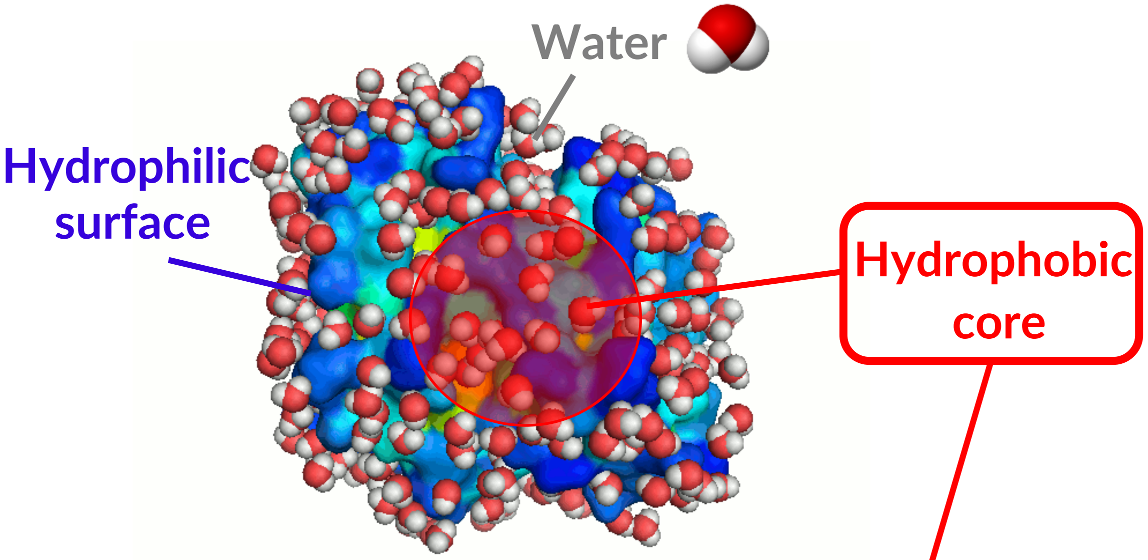

Proline


Leucine


Methionine


Isoleucine

- This group consists of 7 amino acids
- The hydrophobic -R group characteristic leads them to cluster within the protein structure through hydrophobic interactions, evading contact with water.



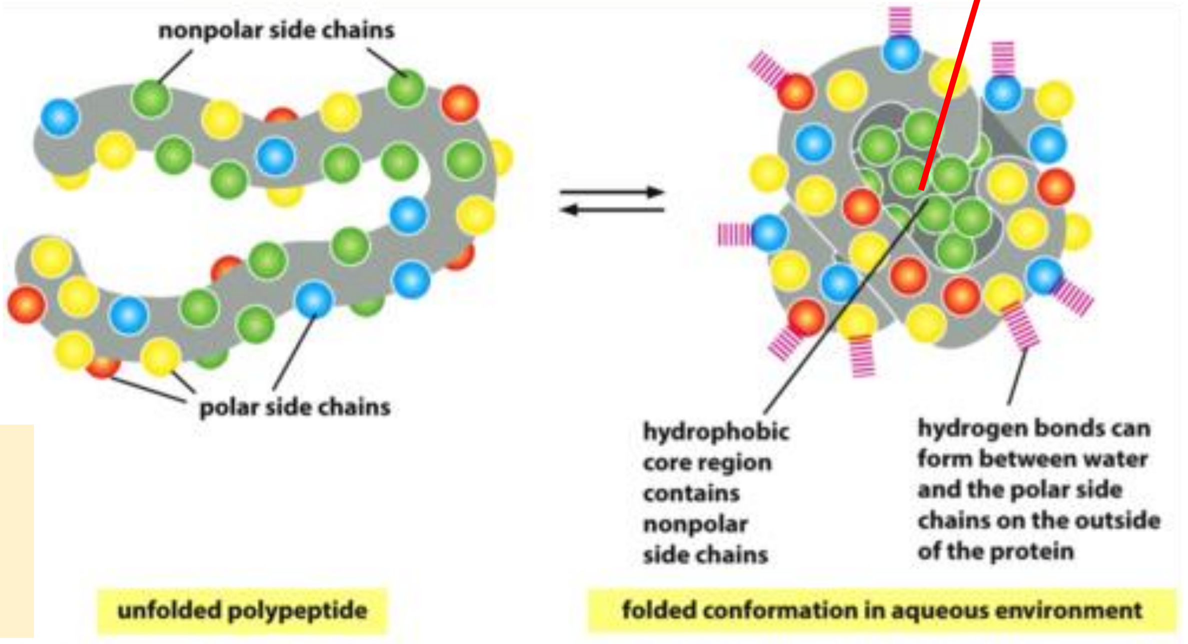
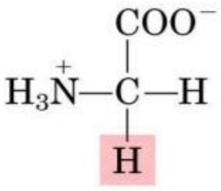


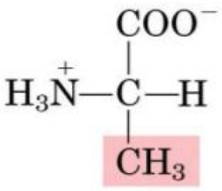
Figure 4-5 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Classification of amino acids according to "R group"

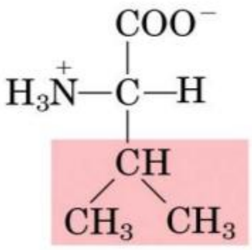
1. Non-polar, aliphatic R groups



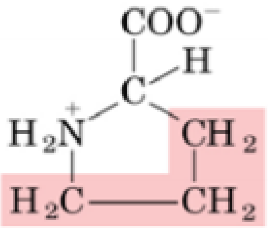
Glycine



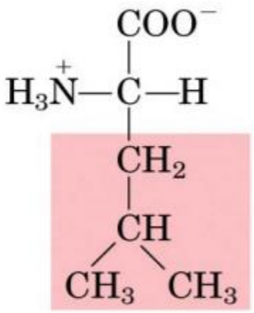
Alanine



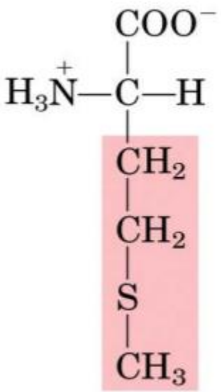
Valine



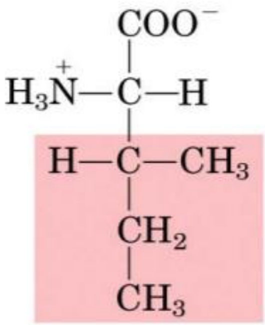
Proline



Leucine



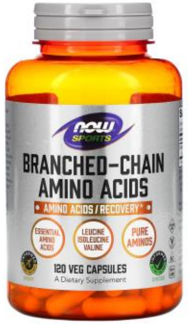
Methionine



Isoleucine

Branched-Chain Amino acids (BCAAs)

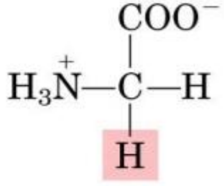
- Amino acids that have a branched structure
- Essential amino acids
- Consisting of Leucine, Isoleucine, and Valine
- BCAAs play a crucial role in the body as they constitute 35% of muscle protein and represent 40% of the essential amino acids required by the body
- Found in foods such as meat, dairy products, eggs, and soy products



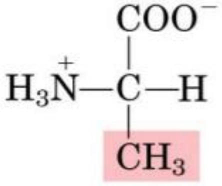
https://fitbod.me/blog/natural-food-sources-of-bcaa/

Classification of amino acids according to "R group"

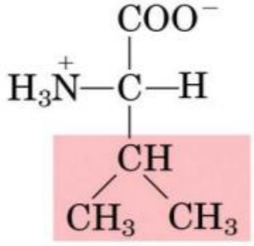
1. Non-polar, aliphatic R groups



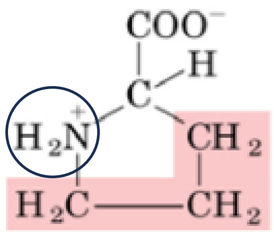
Glycine



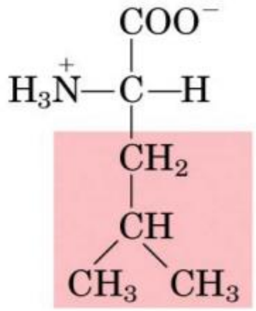
Alanine



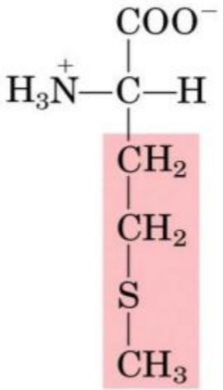
Valine



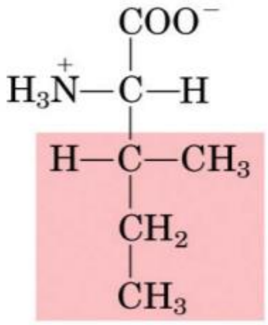
Proline



Leucine



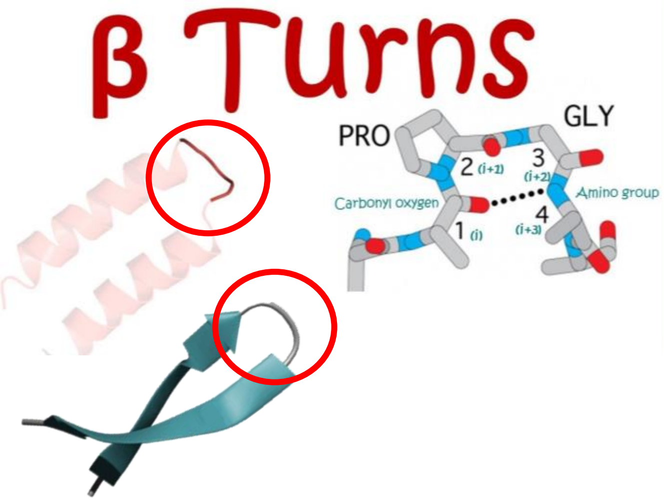
Methionine

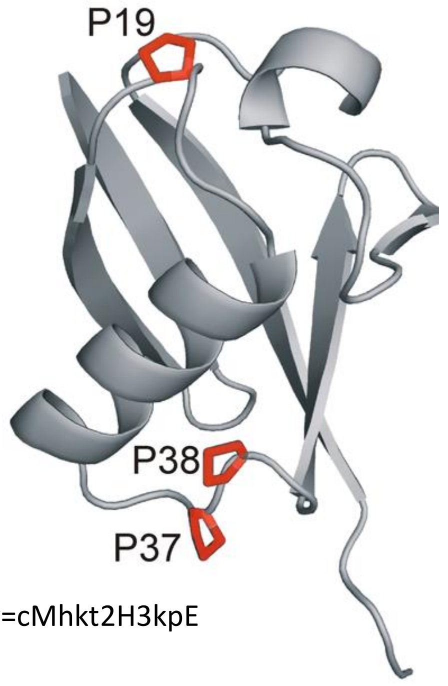


Isoleucine

Proline has special characteristics:

- No free amino group >> Imino group >> Imino acid
- R group is cyclic → When forming a peptide bond, it causes a bend in the amino acid chain
- Commonly positioned in the turns/loops region, since it causes a change in the direction of the polypeptide chain, leading to a more compact folding of the polypeptide chain.

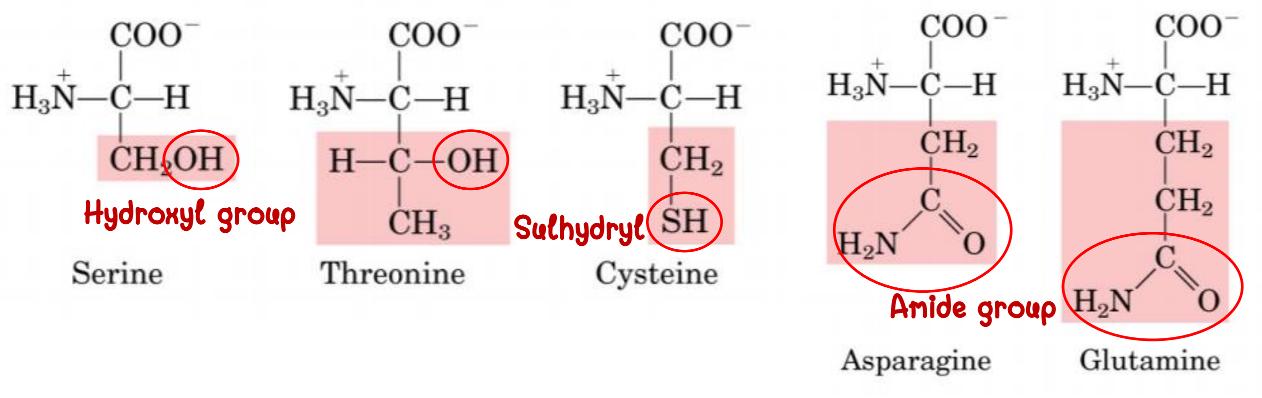




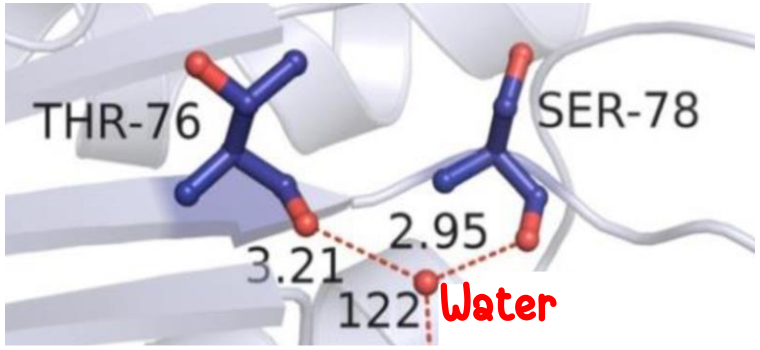
https://www.youtube.com/watch?app=desktop&v=cMhkt2H3kpE

Classification of amino acids according to "R group"

2. Polar, uncharged R groups

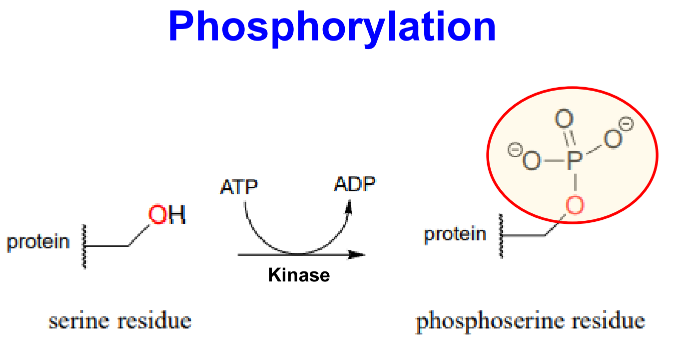


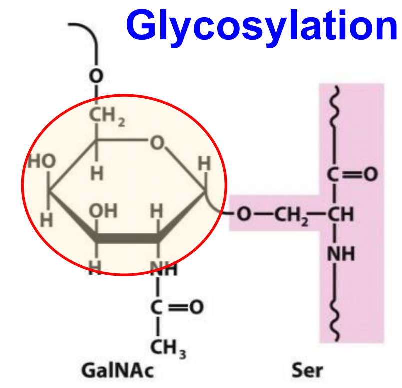
- This group consists of 5 amino acids
- The polar nature of R-groups allows them to engage in hydrogen bonding with other polar molecules.



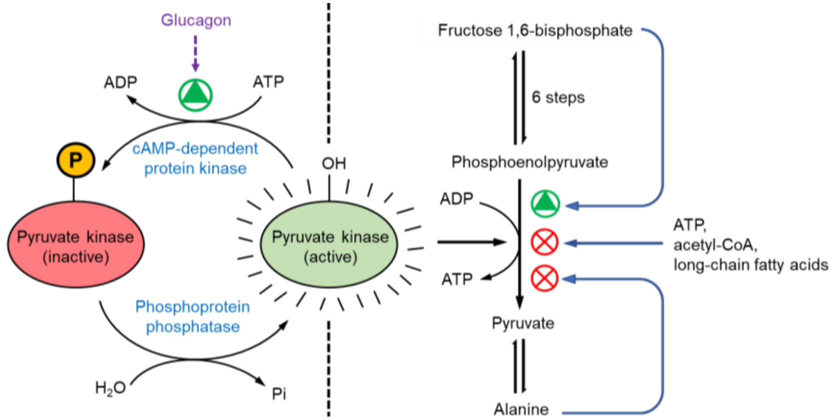
Molecules **2018**, *23*(9), 2321

- The -OH group of Serine and Threonine can be phosphorylated and glycosylated



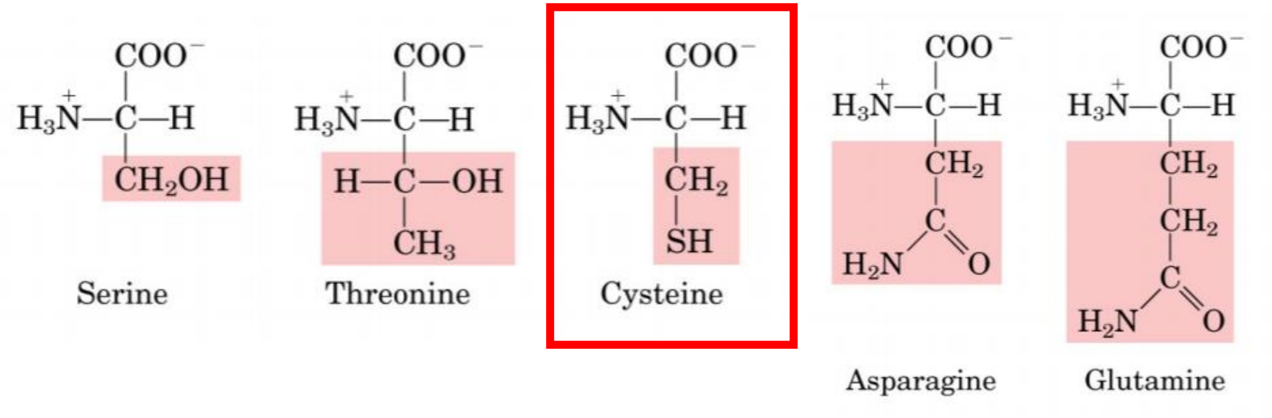


"Post-translational modifications" → Control the function of proteins



Classification of amino acids according to "R group"

2. Polar, uncharged R groups



- The sulfhydryl (-SH) group in two cysteine molecules can form covalent bond, known as **a disulfide bond or S-S bond**, enhancing the strength of the protein structure.

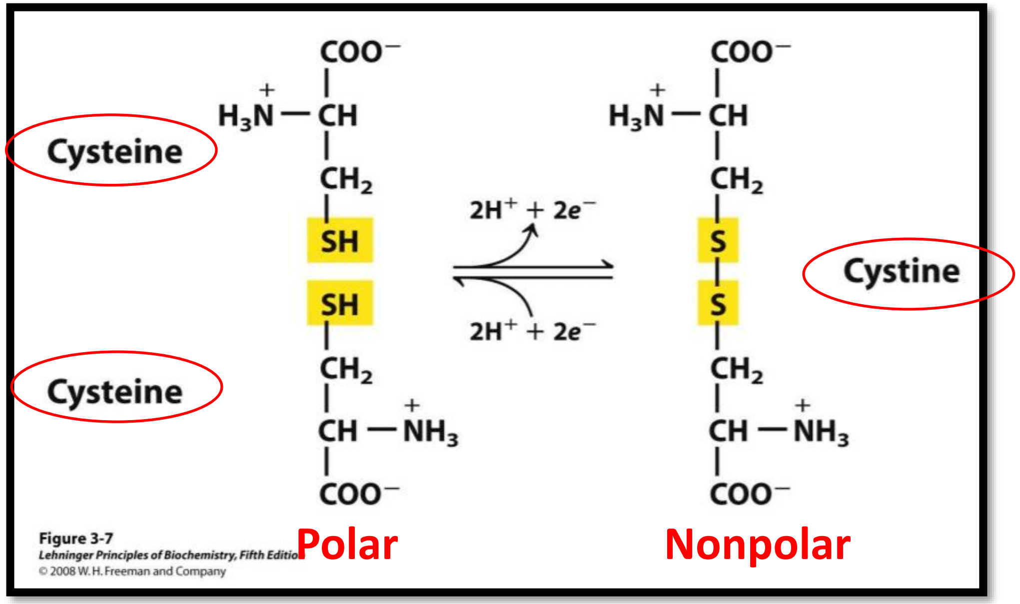


Figure 3-7
Lehninger Principles of Biochemistry, Fifth Edition
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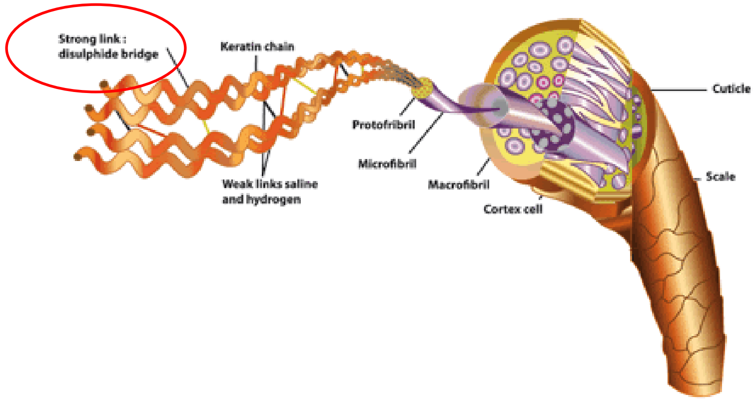
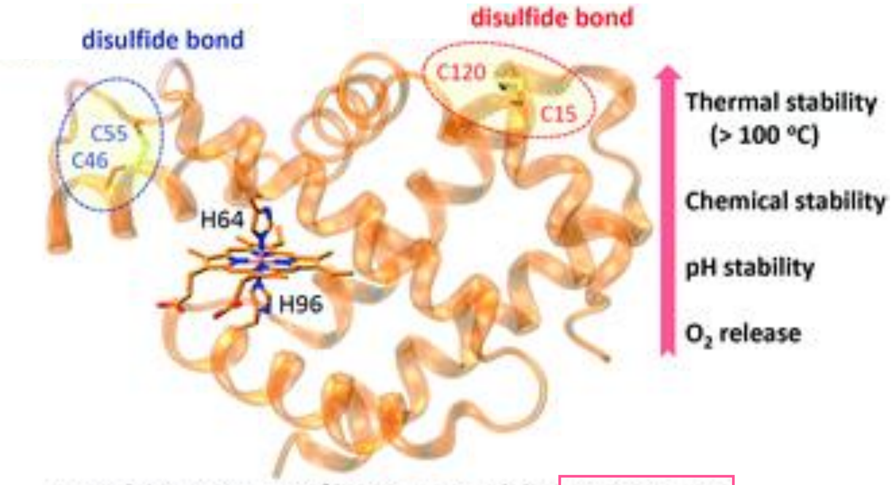


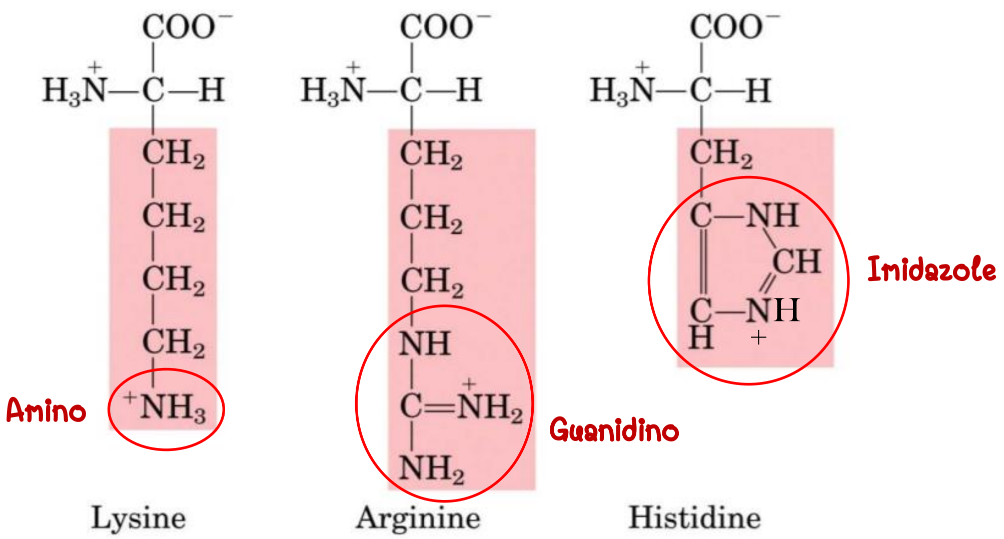


A modeling structure of human neuroglobin A15C mutant

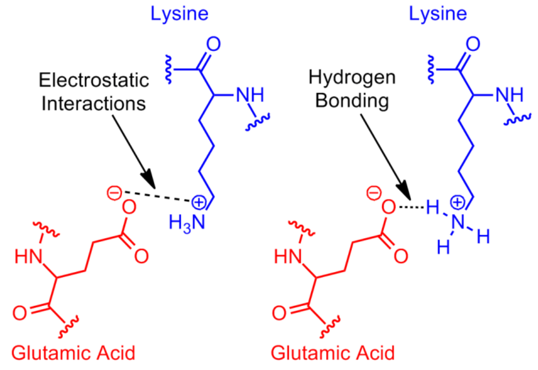
RSC Adv., 2019, **9**, 4172-4179

Classification of amino acids according to "R group"

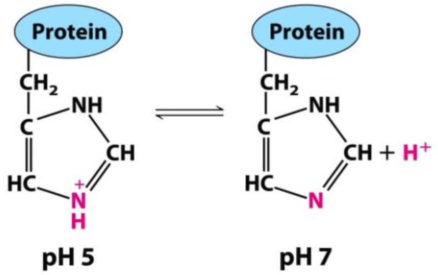
3. Positively charged R groups



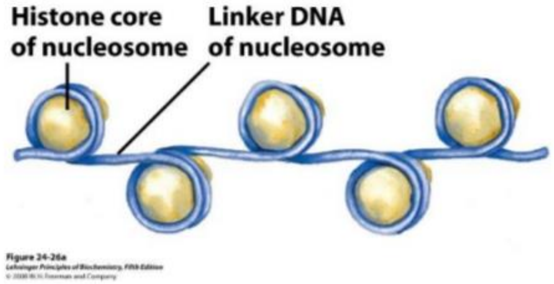
- The positively charged nature of R-groups allows them to engage in salt bridge/electrostatic interaction and Hydrogen bonding with other charged molecules.

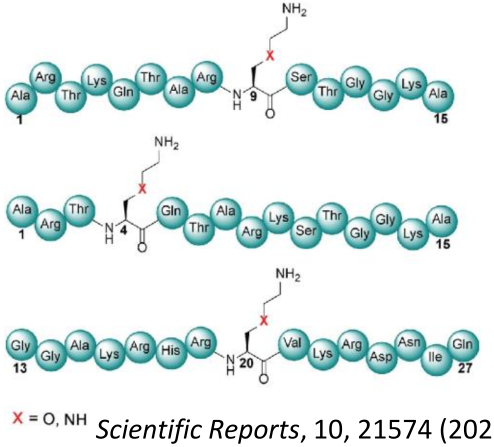


- This group consists of 3 amino acids
- At physiological pH (7.4) Lys and Arg have a positive charge
- At pH < 6 His has a positive charge



- This group is an important component of histone proteins.

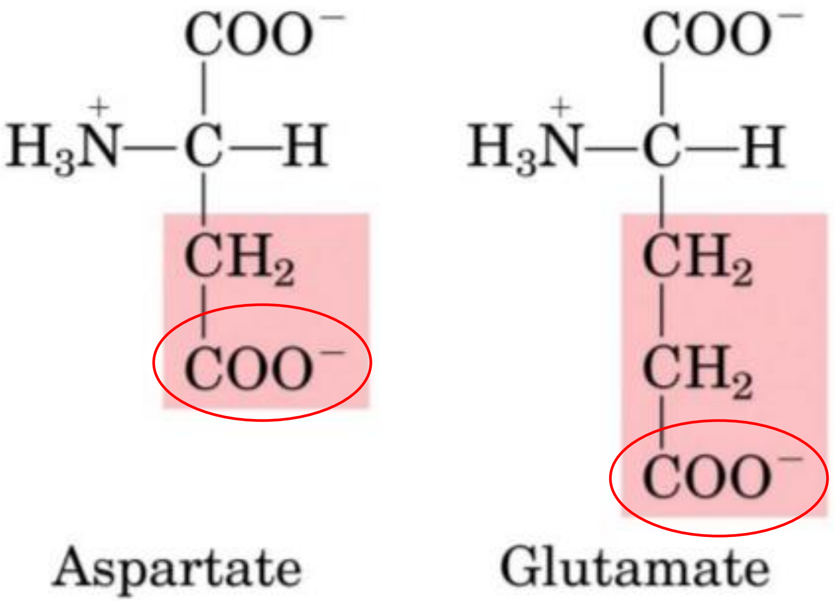




Scientific Reports, 10, 21574 (2020)

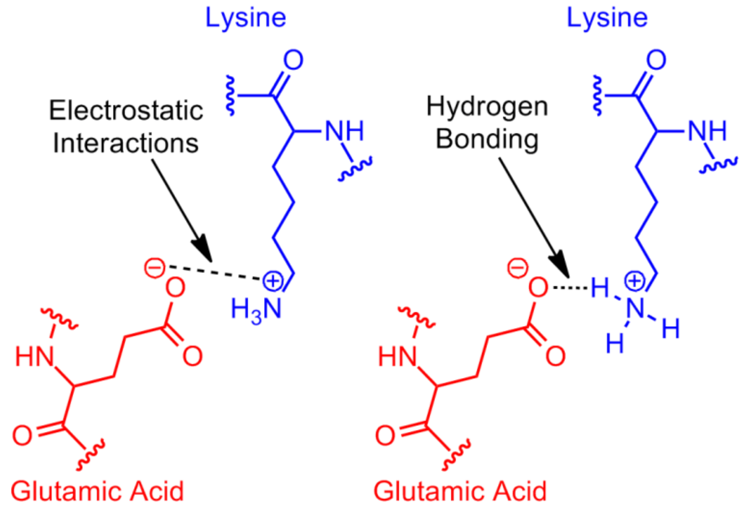
Classification of amino acids according to "R group"

4. Negatively charged R groups



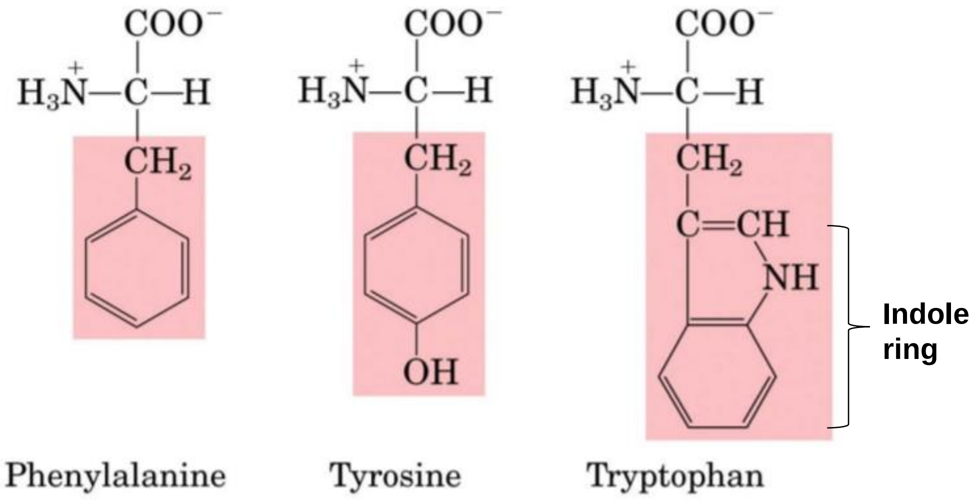
- This group consists of 2 amino acids
- At physiological pH (7.4), both amino acids have a negative charge

- The nagatively charged nature of R-groups allows them to engage in salt bridge/electrostatic interaction and Hydrogen bonding with other charged molecules.



Classification of amino acids according to "R group"

5. Aromatic R groups



- The hydrophobic -R group in phenylalanine can form hydrophobic interactions with other non-polar molecules

- Tyrosine and tryptophan contain polar groups (-OH, -NH), allowing them to engage in hydrogen bonding along with hydrophobic interactions

- The aromatic ring can absorb UV light at a wavelength of 280 nm, enabling a rough quantification of the protein

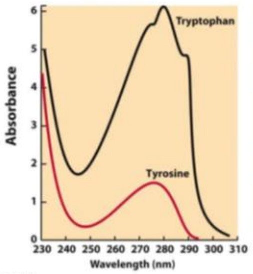
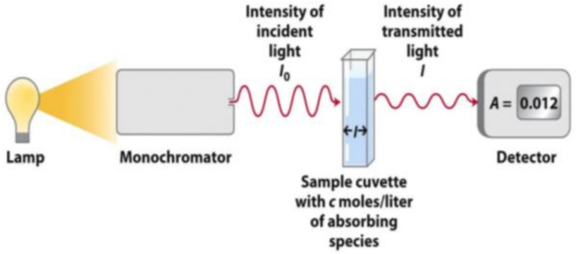


Figure 3-6
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W.H. Freeman and Company



Box 3-1 figure 1
Lehninger Principles of Biochemistry, Fifth Edition
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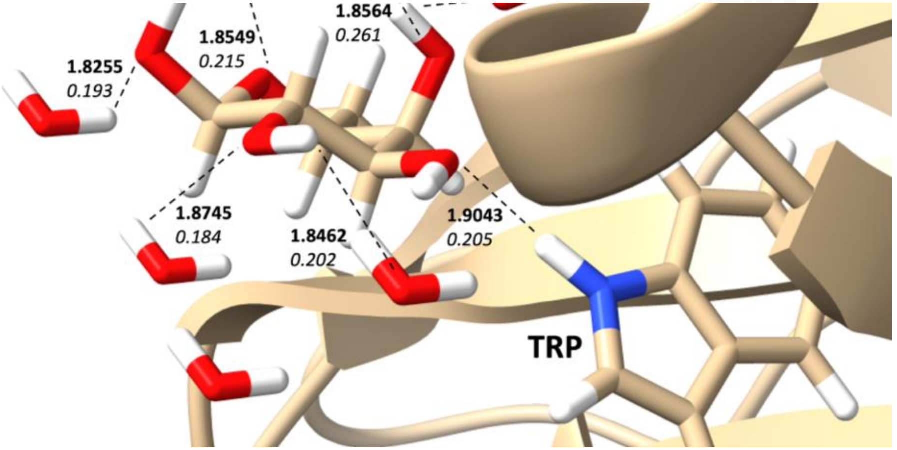


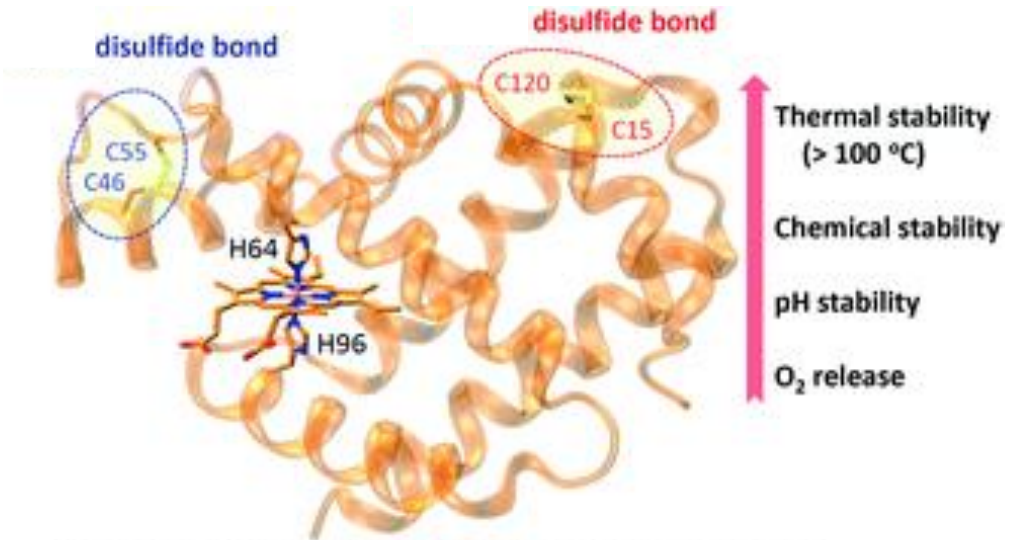
Int. J. Mol. Sci. **2023**, *24*(7), 6311

Summary

- The smallest amino acid and does not contain carbon =
- Amino acids that contain sulfur =
- Amino acids that can form disulfide bonds =
- Amino acids with aromatic rings =
- Amino acids without a free amino group =

Applications

Protein design



A modeling structure of human neuroglobin A15C mutant

RSC Adv., 2019,**9**, 4172-4179

Drug design

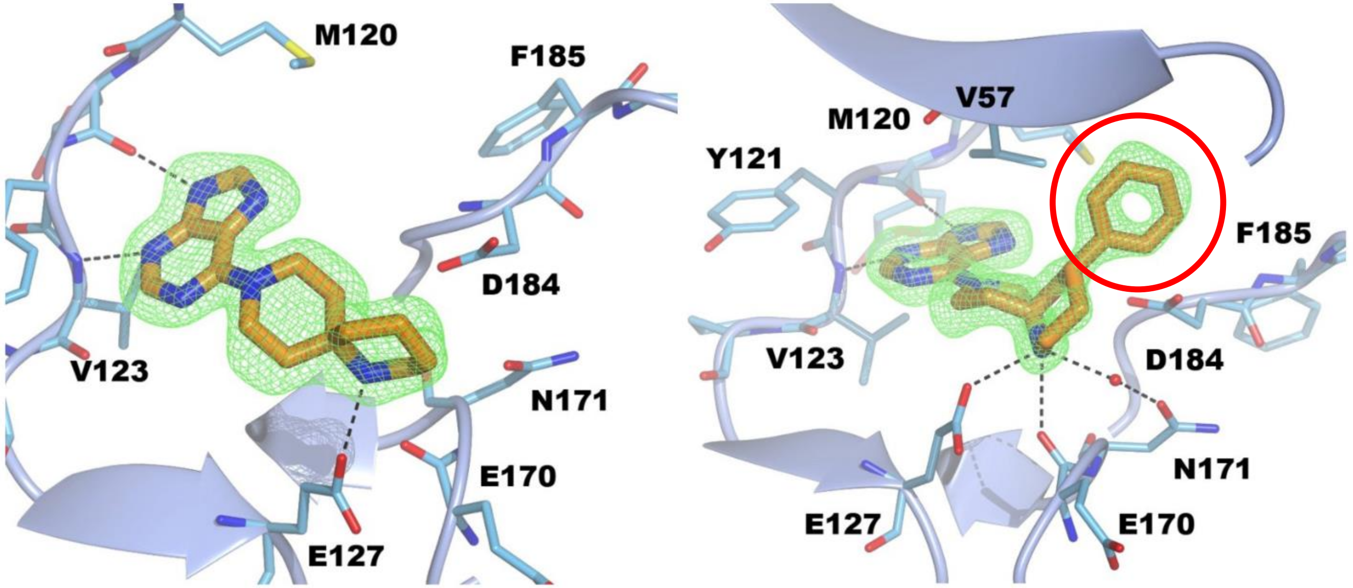
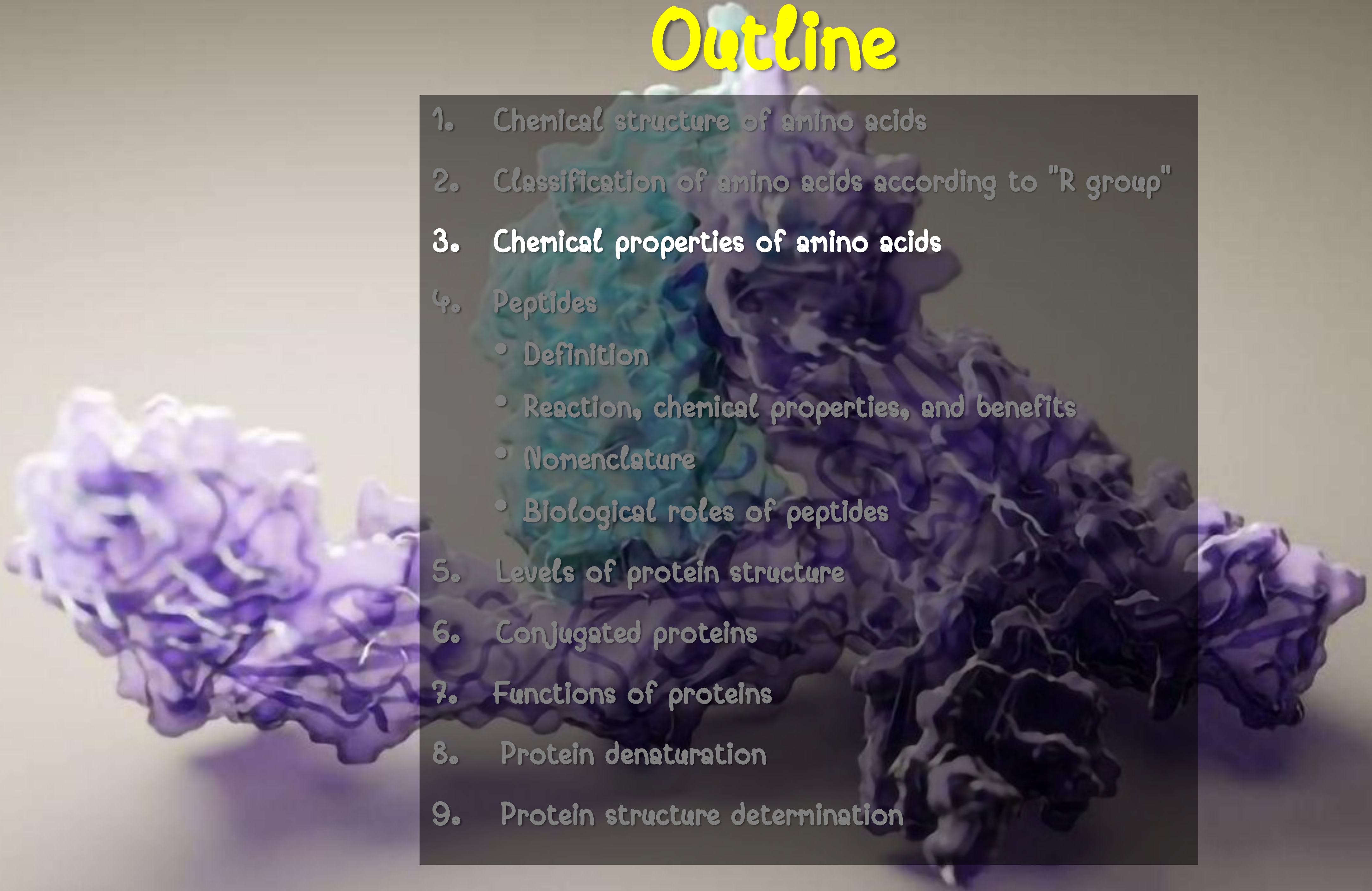


Figure 3: Crystal structures of PKA in complex with two different spirocycle-based inhibitors.

cAMP-dependent protein kinase A (PKA)

Just introducing phenyl ring > #H-bonds ↑

Outline



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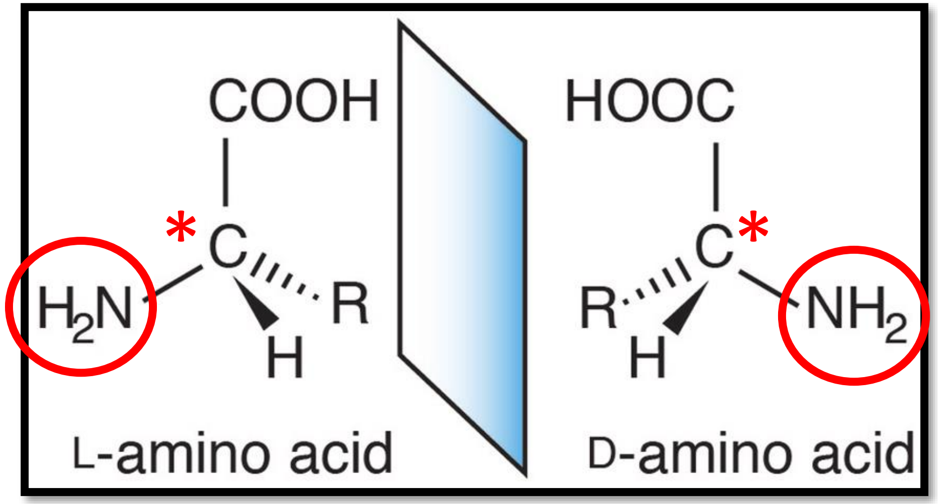
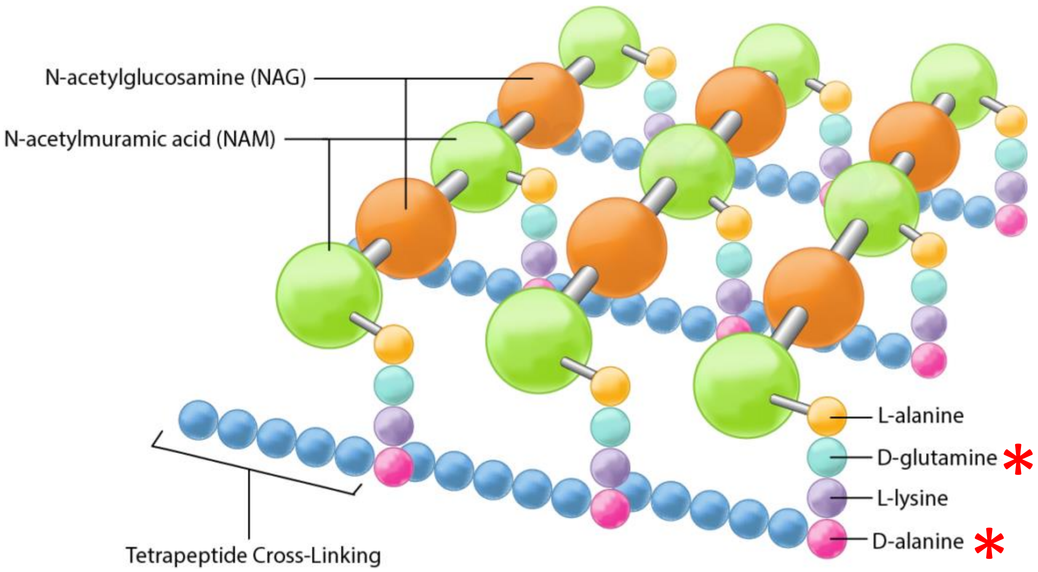
Chemical properties of Amino Acids

1. Enantiomer (mirror images of one another but cannot be superimposed)



Bacterial cell walls



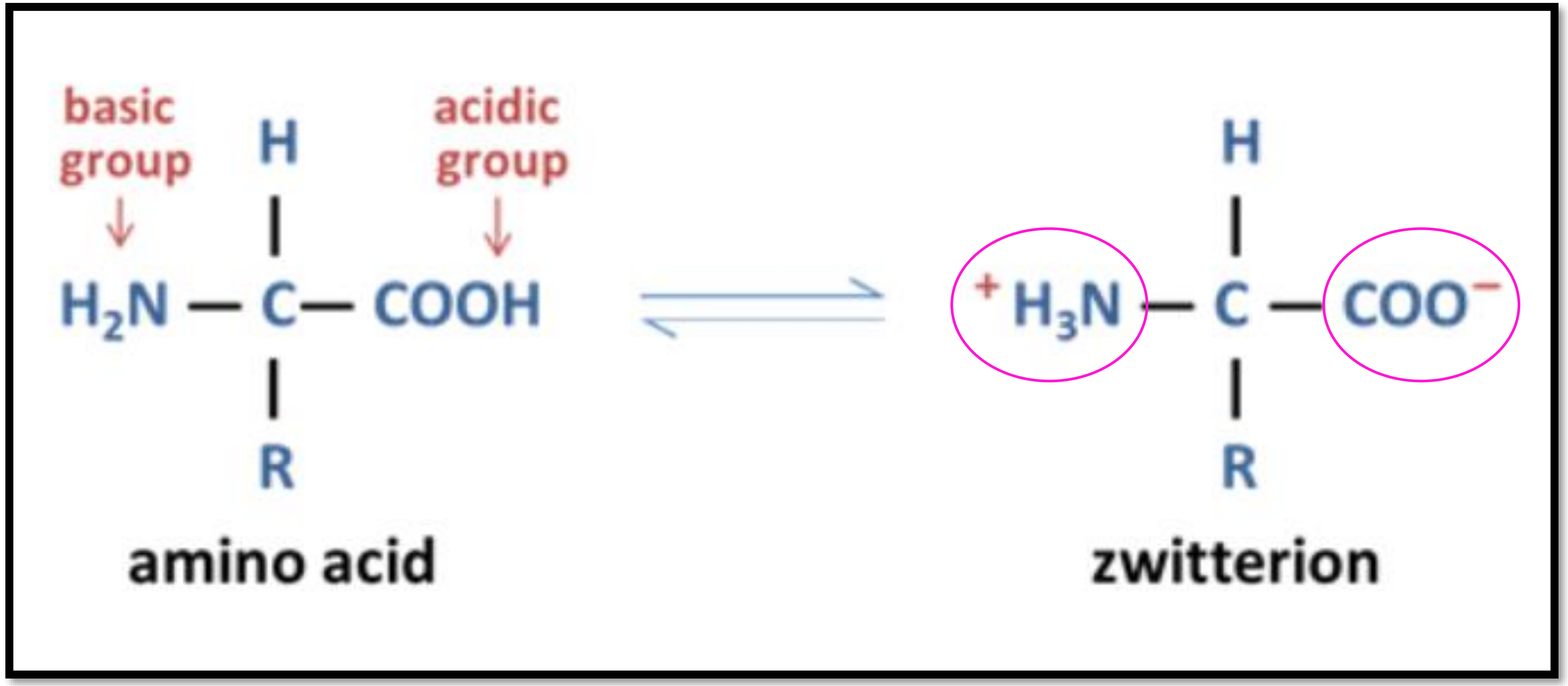
- Since the alpha carbon (C_α) is asymmetric, there are two possible, non-superimposable, mirror images of the amino acids: D-amino acids & L-amino acids
- L-amino acid are abundant in nature, serving as components of protein structures. In contrast, D-amino acids are less common and are frequently found in bacterial cell walls

NH_2 is on the "right" = D-form

NH_2 is on the "left" = L-form

Chemical properties of Amino Acids

2. Zwitterionic property (Dipolar)

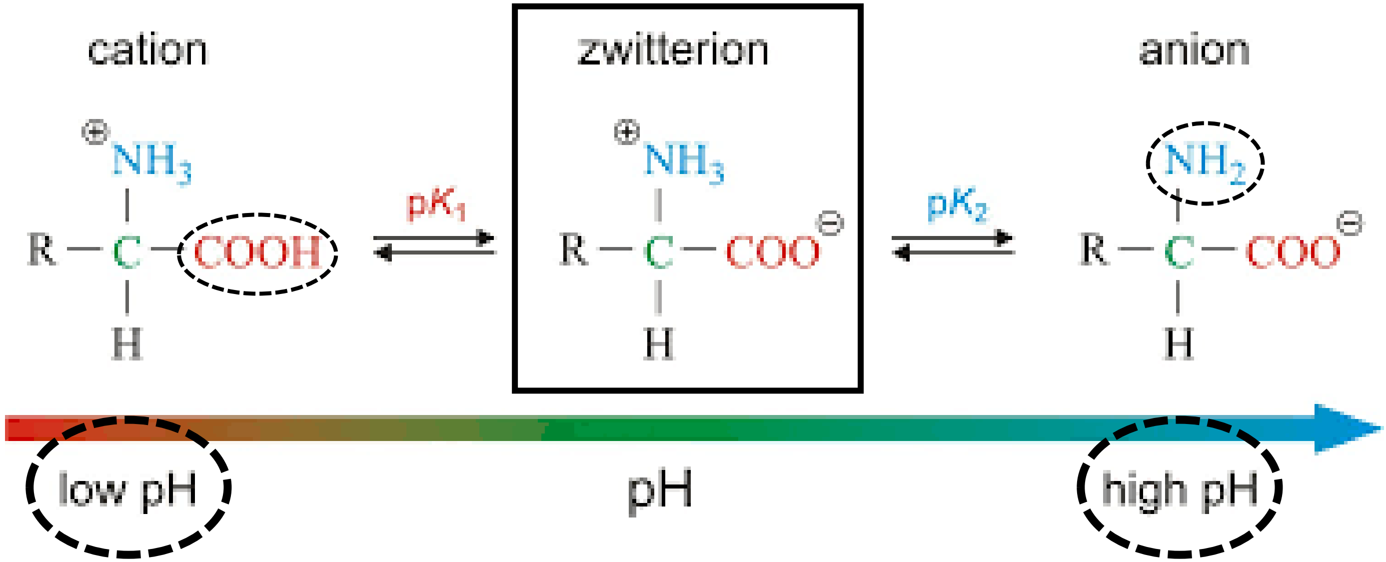


When amino acids with a non-ionizable R group dissolves in water at neutral pH, they undergo ionization, forming a dipolar molecule containing both positive and negative charges within the same molecule

Chemical properties of Amino Acids

3. Amphoteric property

They act as <u>both acid and base</u> due to the presence of amino and carboxylic groups.



Amino acid acts as
a base (absorb H^+).

Amino acid acts as
an acid (release H^+).

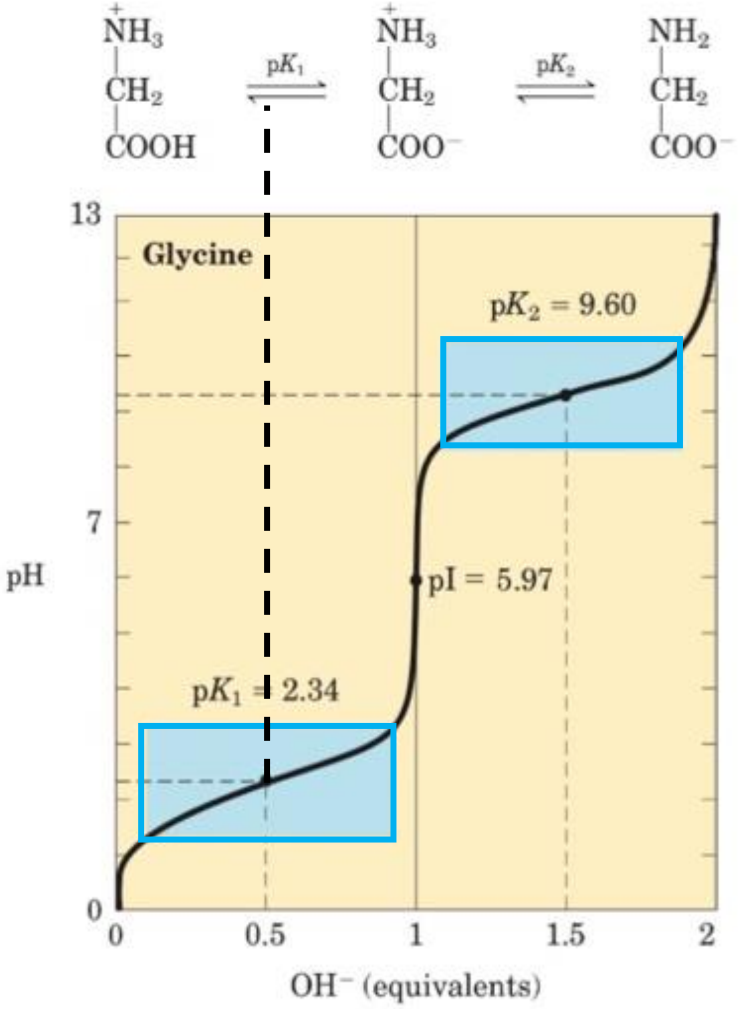
Fully protonated

Fully deprotonated

Chemical properties of Amino Acids

4. Buffer

Amino acids are able to maintain pH levels because they can act as both acid and base



- Glycine functions as a buffer at 2 Buffering regions

1. pKa1 ± 1
2. pKa2 ± 1

Henderson-Hasselbalch Equation

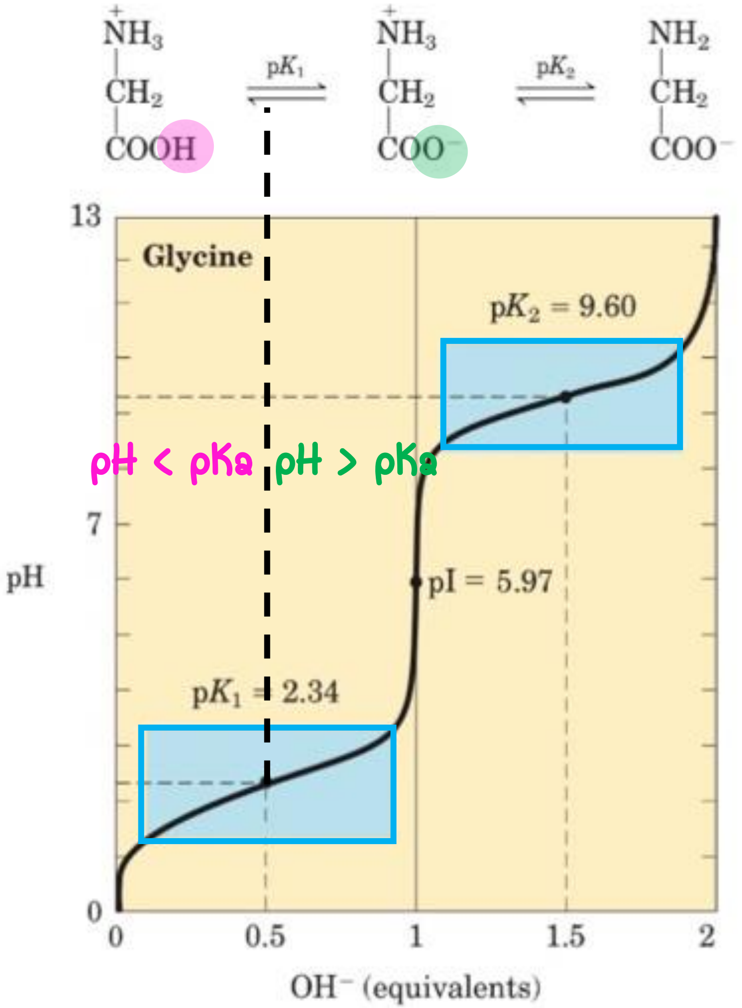
$$pH = pK_a - \cancel{\log \frac{[HA]}{[A^-]}}$$

Because there are 2 groups that can give H^+: COOH & NH_3^+

Chemical properties of Amino Acids

4. Buffer

Amino acids are able to maintain pH levels because they can act as both acid and base



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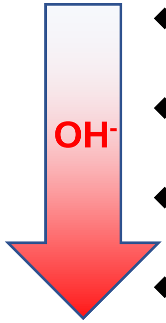
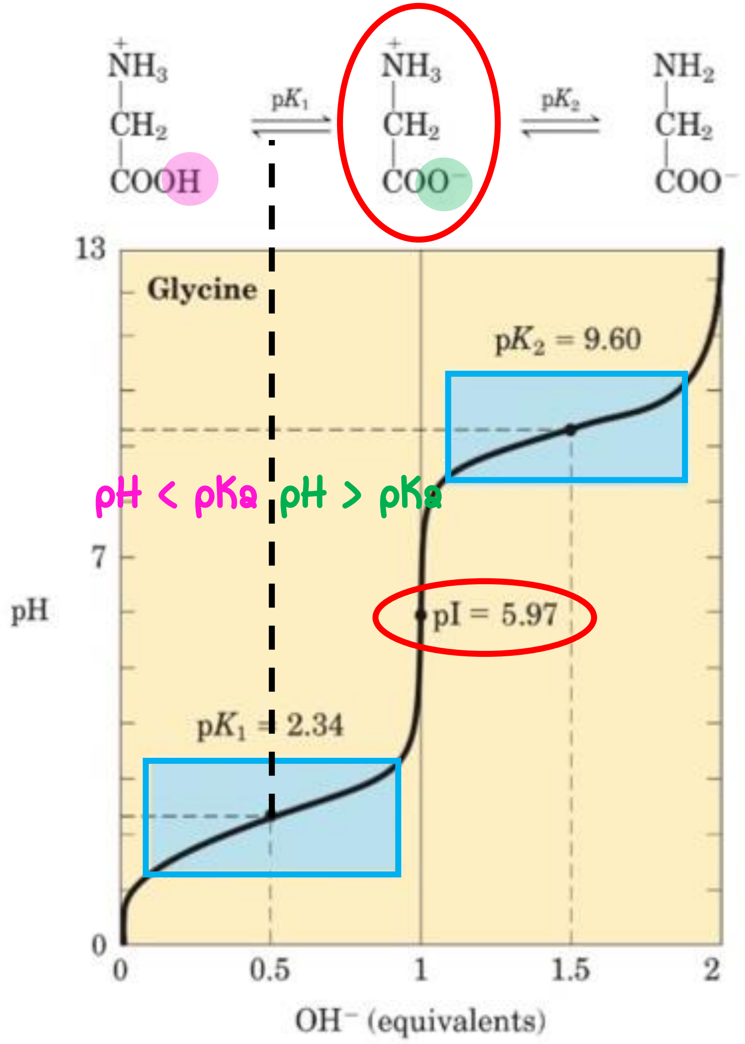


- at low pH Protonated form (H)
- at pH < pKa_1 Protonated form (H) > Deprotonated form (-)
- at pH = pKa_1 Protonated form (H) = Deprotonated form (-) ***
- at pH > pKa_1 Protonated form (H) < Deprotonated form (-)
- at pH = 5.97 Deprotonated form (-)

Chemical properties of Amino Acids

4. Buffer

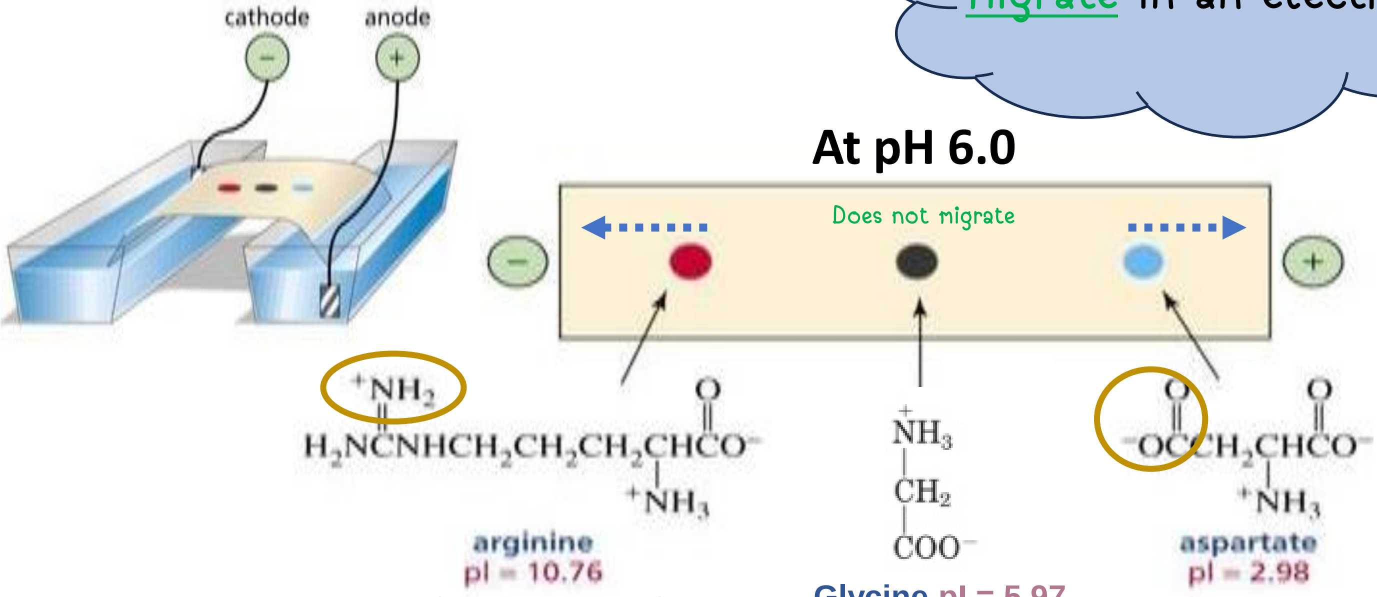
Amino acids are able to maintain pH levels because they can act as both acid and base



$$pI = \frac{(pK_1 + pK_2)}{2}$$

❖ **Isoelectric point (pI)** the pH at which the net charge of a protein molecule is zero

Amino acid does not migrate in an electric field.



(pH < pI, +)

Glycine pI = 5.97

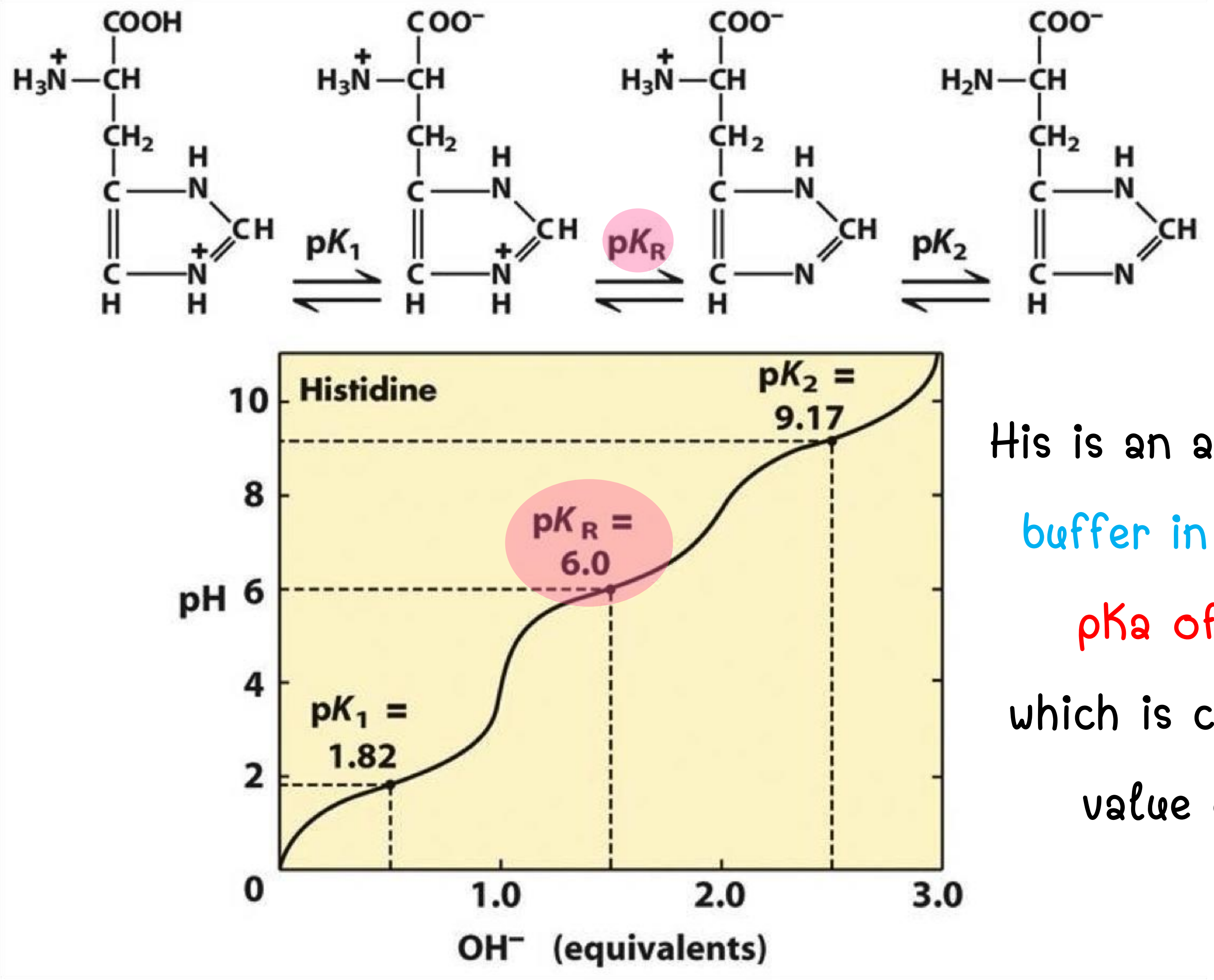
(pH = pI, 0)

(pH > pI, -)

https://slideplayer.com/slide/12720501/

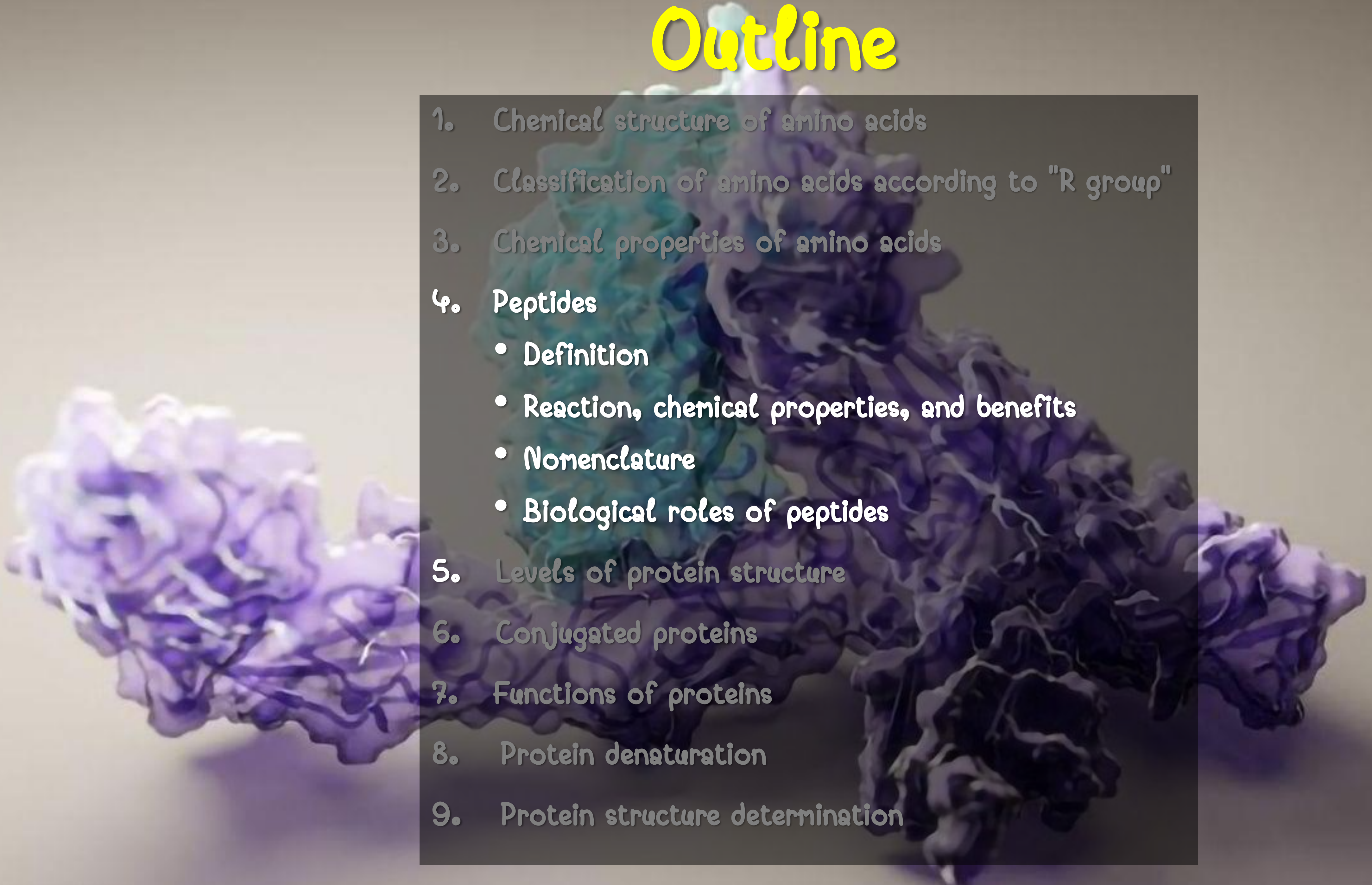
Chemical properties of Amino Acids

4. Buffer



His is an amino acid that acts as a good buffer in the human body because the pKa of its imidazole ring is = 6, which is close to the physiological pH value of 7.4 in the human body

Outline



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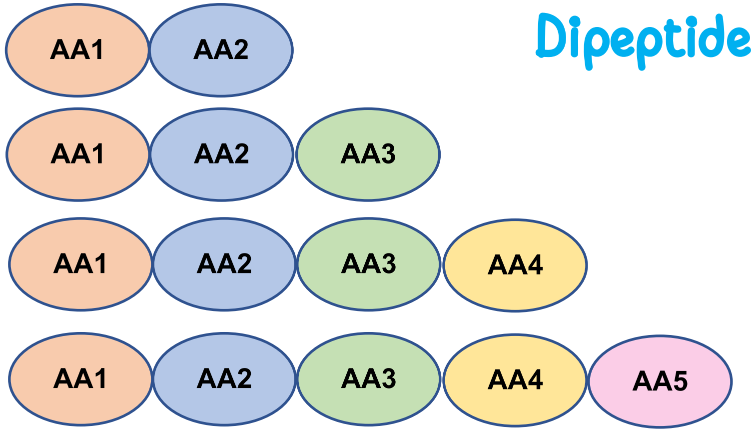
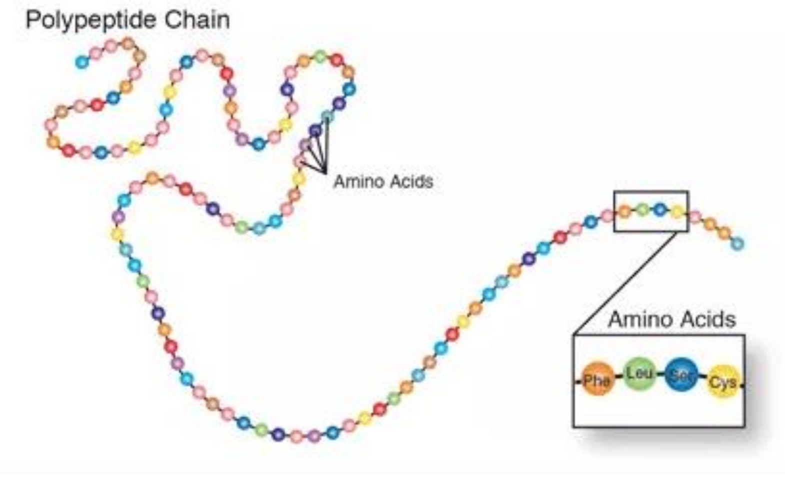
Peptides are chains of amino acids)

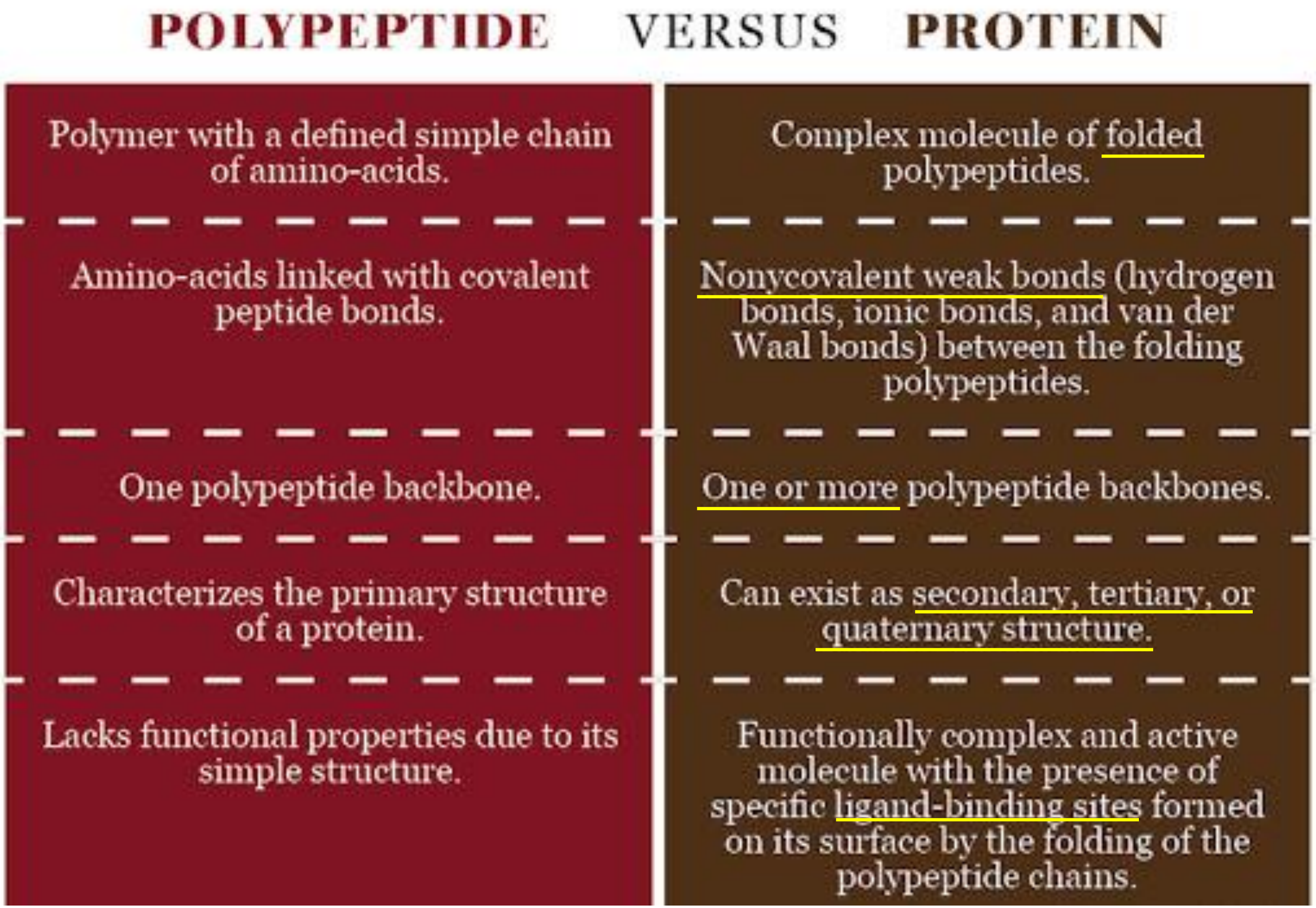
- When **2-20 amino acids** are joined by peptide bonds, the structure is called "**Oligopeptide**".
  - **Dipeptide**
  - **Tripeptide**
  - **Tetrapeptide**
  - **Pentapeptide**



Dipeptide $\neq$ 2 peptide bonds

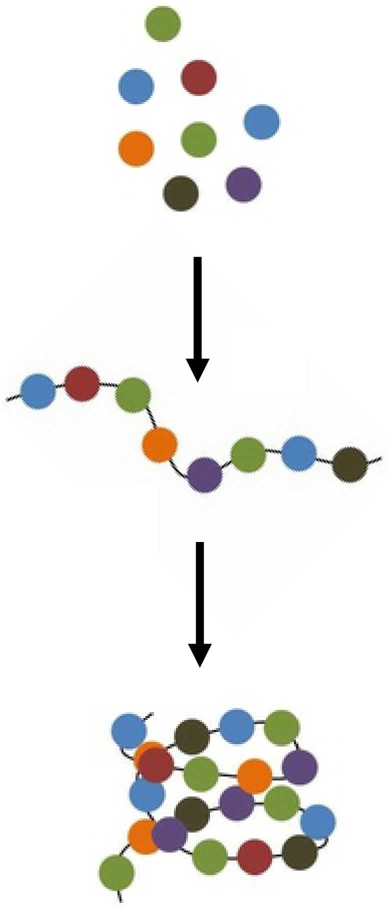
- When **>20 amino acids** are joined by peptide bonds,
  - MW <10 kDa → "**Polypeptide**"
  - MW >10 kDa → "**Protein**"





POLYPEPTIDE VERSUS PROTEIN

POLYPEPTIDE	PROTEIN
Polymer with a defined simple chain of amino-acids.	Complex molecule of folded polypeptides.
Amino-acids linked with covalent peptide bonds.	Noncovalent weak bonds (hydrogen bonds, ionic bonds, and van der Waal bonds) between the folding polypeptides.
One polypeptide backbone.	One or more polypeptide backbones.
Characterizes the primary structure of a protein.	Can exist as secondary, tertiary, or quaternary structure.
Lacks functional properties due to its simple structure.	Functionally complex and active molecule with the presence of specific ligand-binding sites formed on its surface by the folding of the polypeptide chains.



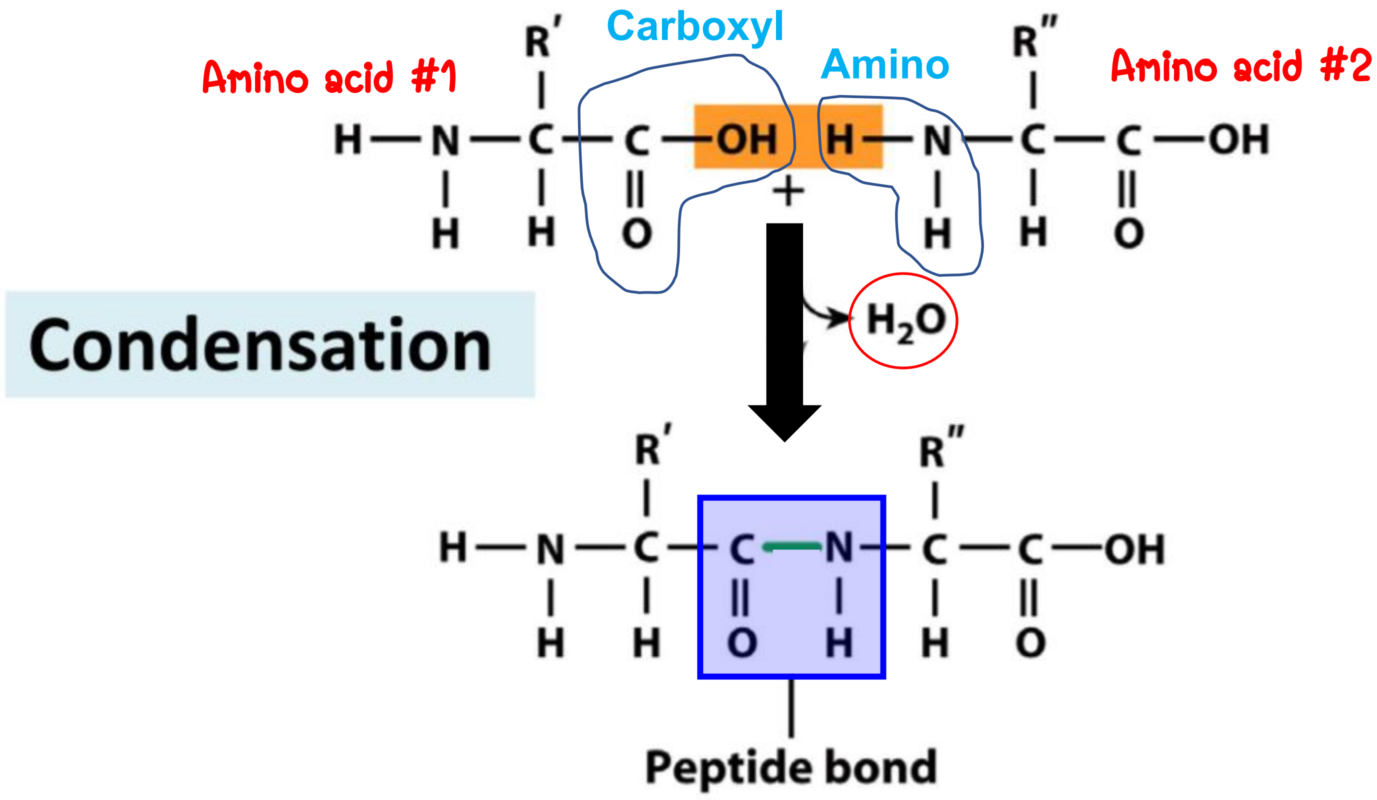
Amino acids

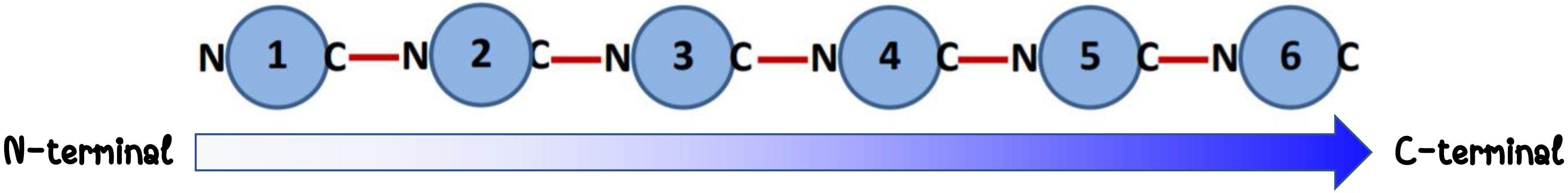
Polypeptide

Protein

http://www.differencebetween.net/science/difference-between-polypeptide-and-protein/

Peptide bonds are formed by condensation reactions between two amino acids



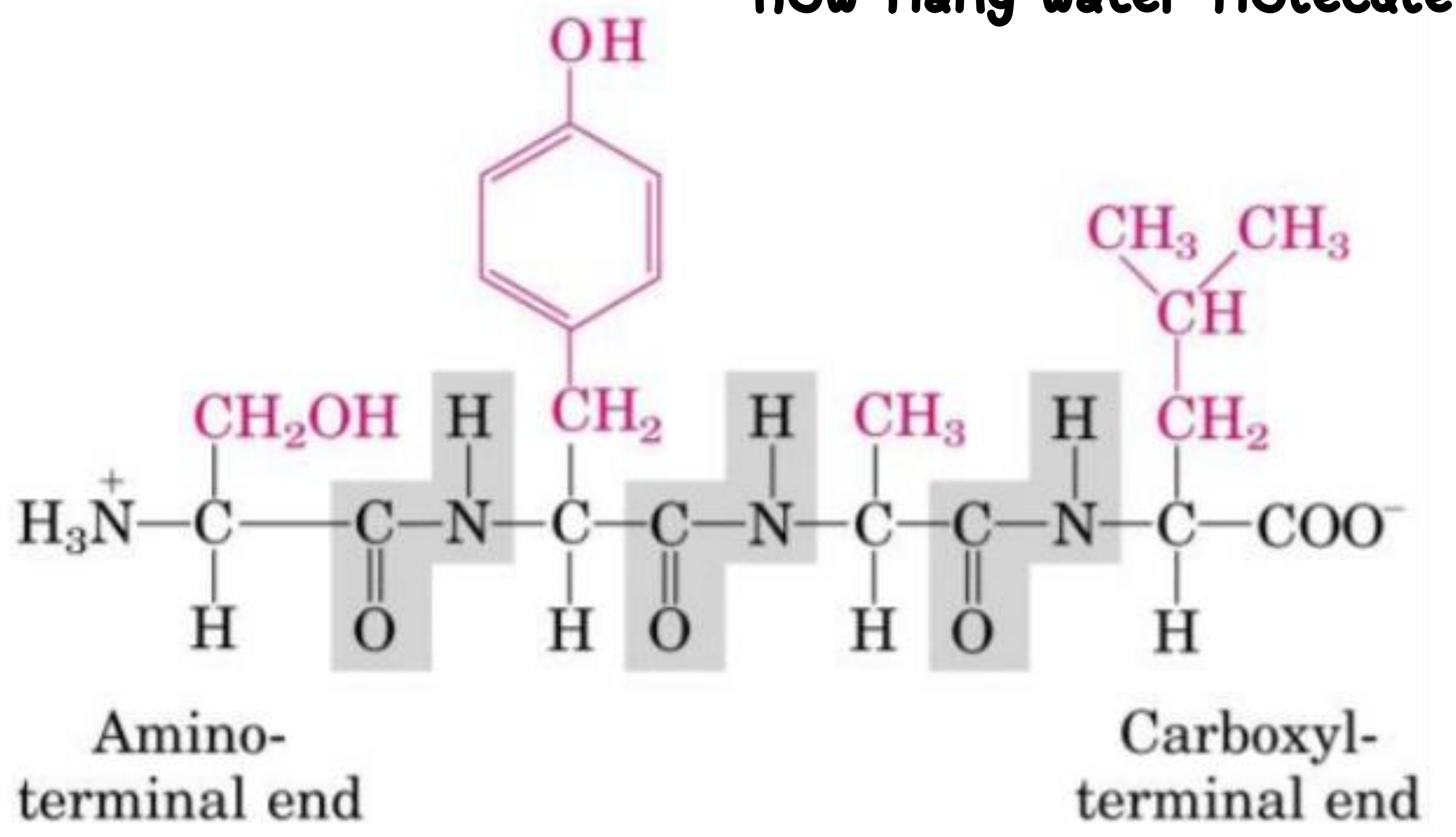


Synthesis direction

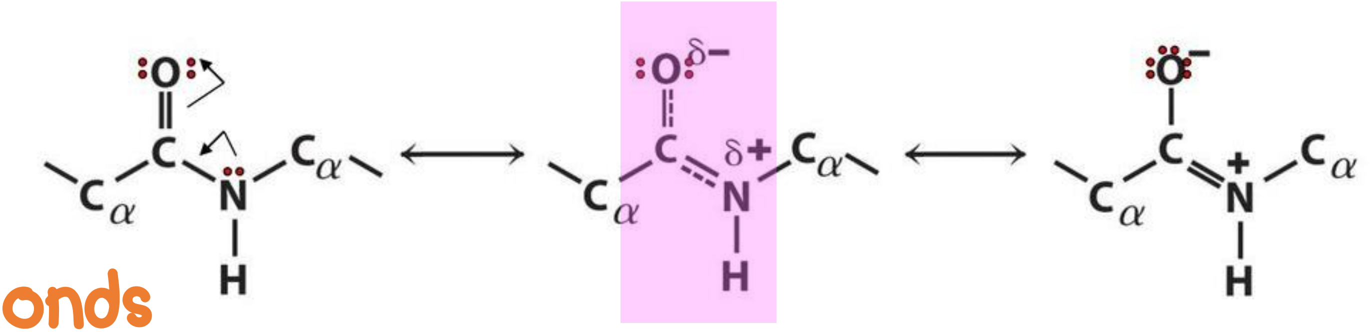
Peptide bonds are formed by condensation reactions between two amino acids

When **4 amino acids** are joined together,

how many water molecules will be lost?

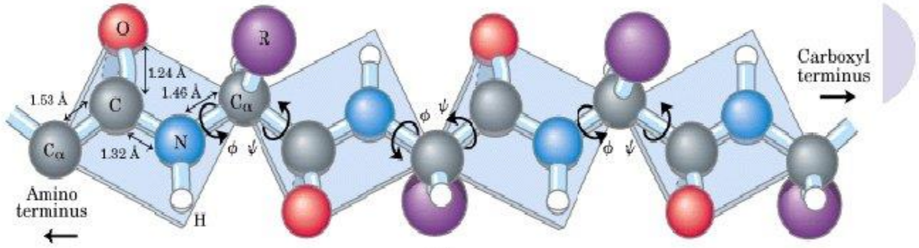


Peptide bonds are formed by condensation reactions between two amino acids



Chemical properties of peptide bonds

1. Partial double bond ($\mathrm{C}\overset{\cdots\cdots}{—}\mathrm{N}$) due to resonance in a structure

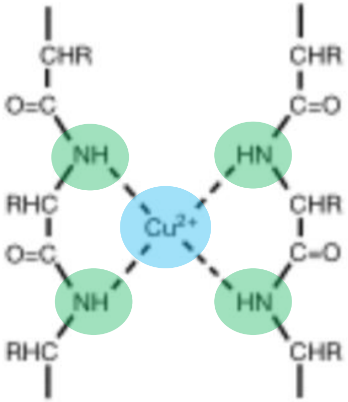


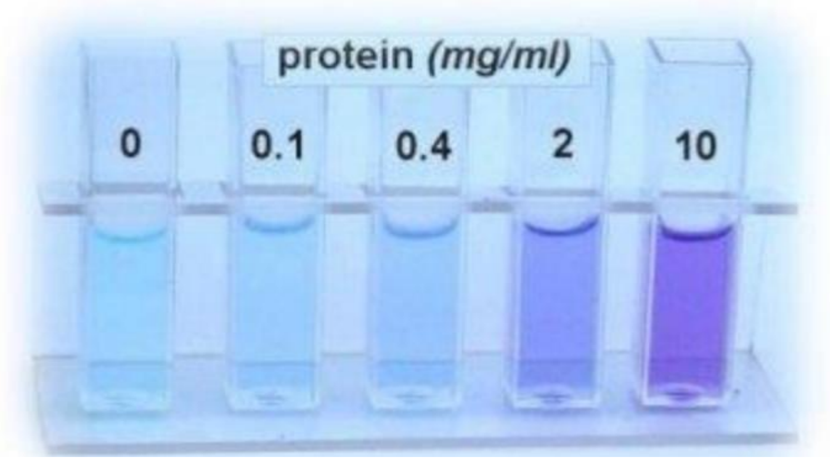
2. Planar
3. Rigid (unable to rotate freely)
4. Bond length: $\mathrm{C}—\mathrm{N} > \mathrm{C}\overset{\cdots\cdots}{—}\mathrm{N} > \mathrm{C}\equiv\mathrm{N}$
5. Bond strength: $\mathrm{C}\equiv\mathrm{N} > \mathrm{C}\overset{\cdots\cdots}{—}\mathrm{N} > \mathrm{C}—\mathrm{N}$

Benefits of peptide bonds

Used to find "protein concentration"

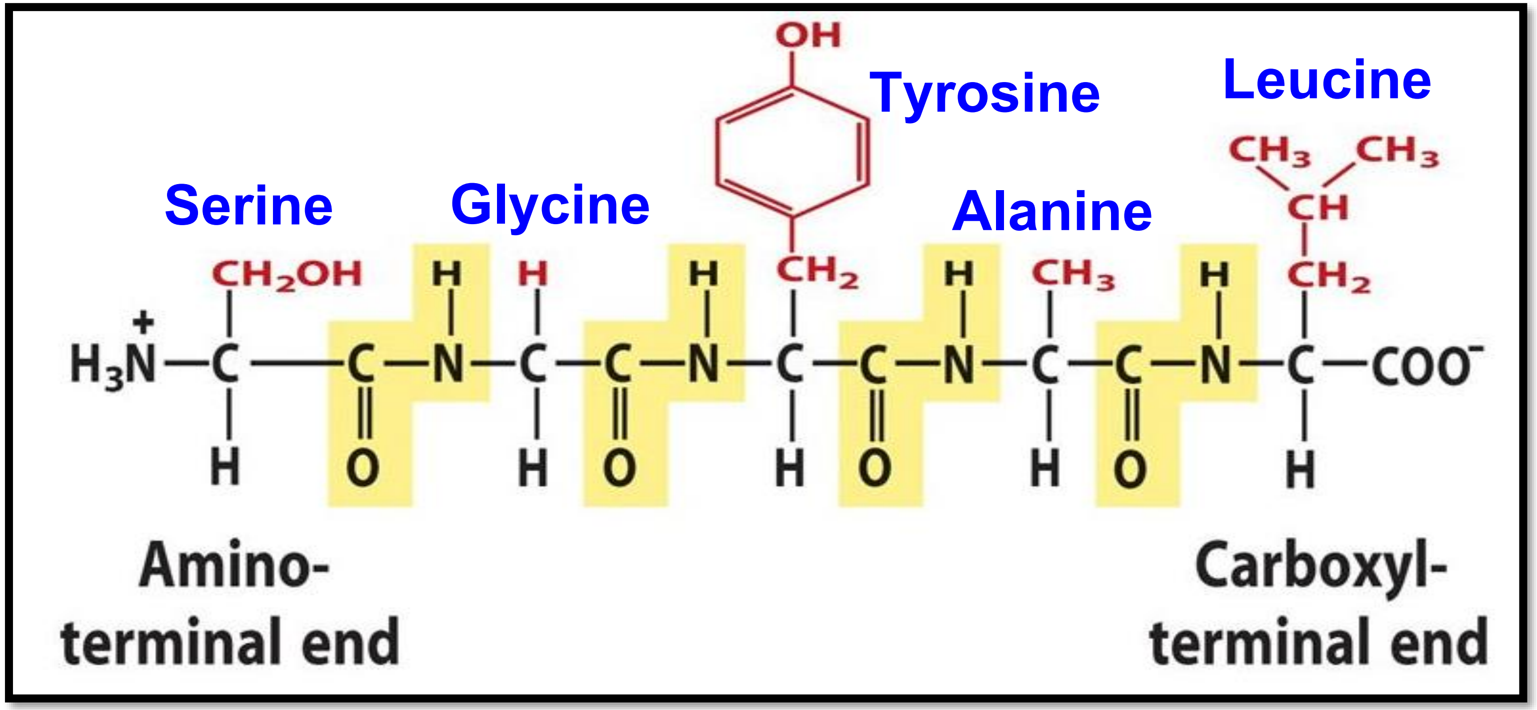
- **"Biuret test"** is the test used to detect the presence of peptide bonds in the sample
- Biuret reagent contains Cu^{2+} ions that can react with the peptide bonds in the protein to form a purple-colored complex
- The intensity of the purple color is directly proportional to the protein concentration in the sample

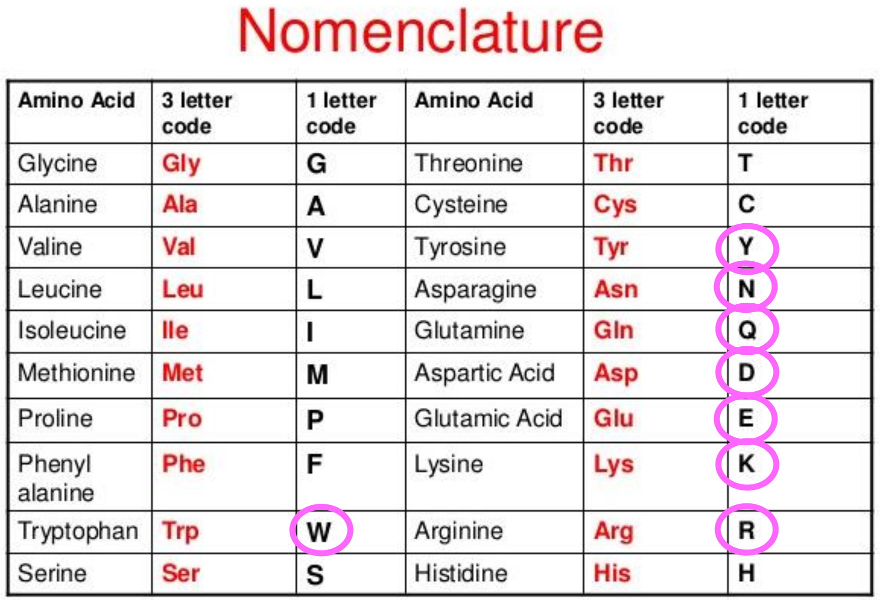




https://biology-igcse.weebly.com/food-test-4---biuret-test-for-proteins.html

Peptide nomenclature





Nomenclature

Amino Acid	3 letter code	1 letter code	Amino Acid	3 letter code	1 letter code
Glycine	Gly	G	Threonine	Thr	T
Alanine	Ala	A	Cysteine	Cys	C
Valine	Val	V	Tyrosine	Tyr	Y
Leucine	Leu	L	Asparagine	Asn	N
Isoleucine	Ile	I	Glutamine	Gln	Q
Methionine	Met	M	Aspartic Acid	Asp	D
Proline	Pro	P	Glutamic Acid	Glu	E
Phenyl alanine	Phe	F	Lysine	Lys	K
Tryptophan	Trp	W	Arginine	Arg	R
Serine	Ser	S	Histidine	His	H

Nomenclature#1 Serylglycyltyrosylalanylleucine

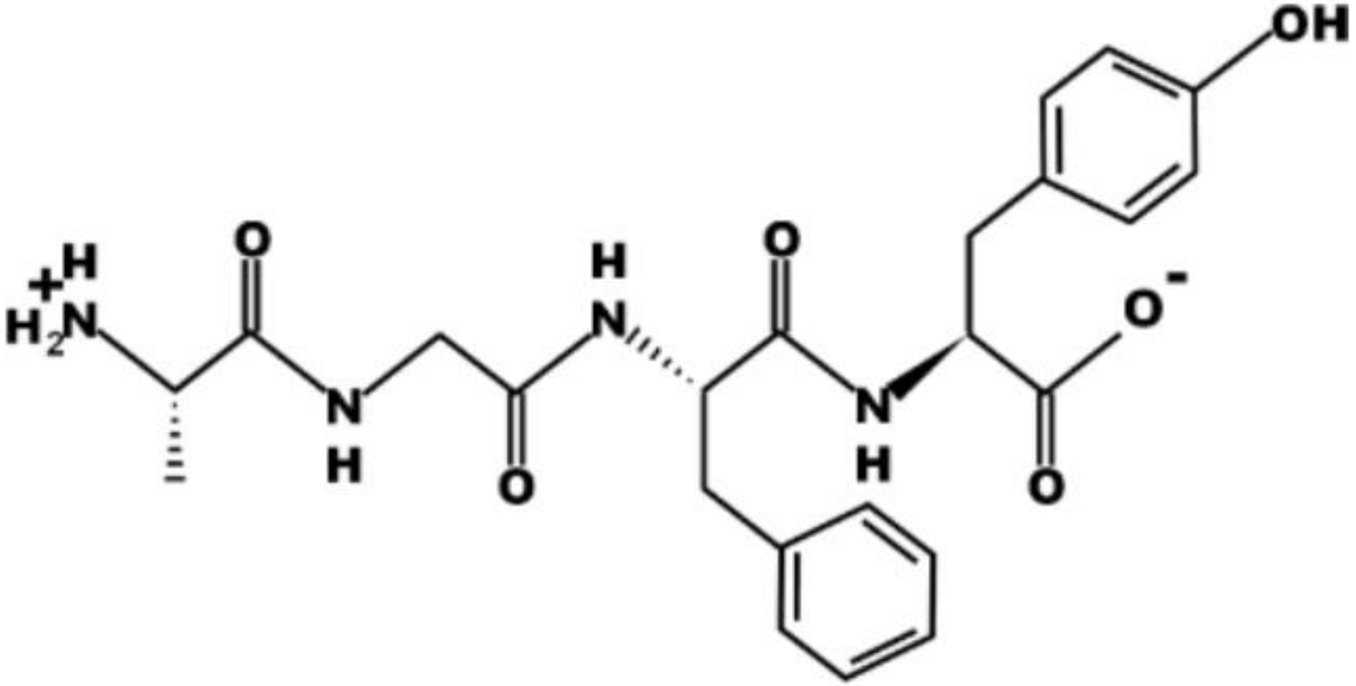
Nomenclature#2 Ser–Gly–Tyr–Ala–Leu

Nomenclature#3 SGYAL

Change the suffix from '-ine' to '-yl' without changing the name of the last amino acid

Peptide nomenclature

Exercise 1.



How many amino acids? ……………………………

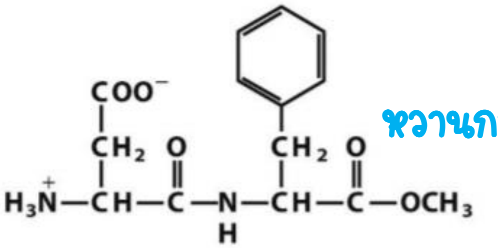
Nomenclature#1 ……………………………

Nomenclature#2……………………………

Nomenclature#3……………………………

Biological functions of peptides

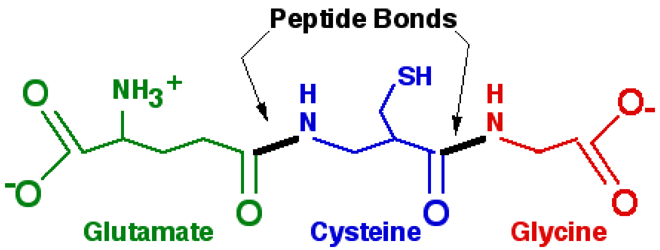
- **As a sweetener**
  - Aspartame

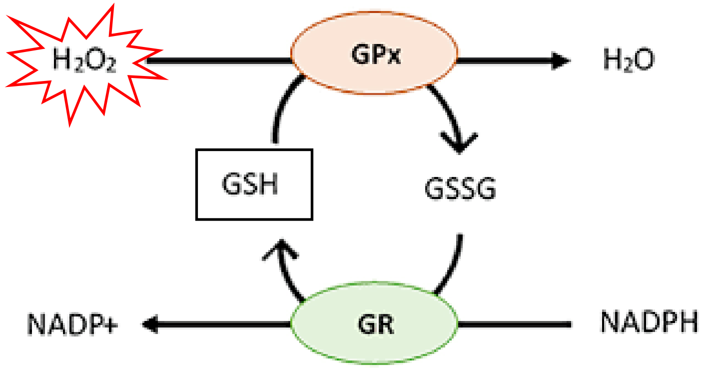


L-Aspartyl-L-phenylalanine methyl ester (aspartame)

หวานกว่า sucrose 300 เท่า

- **As an antioxidant**
  - GSH (glutathione)

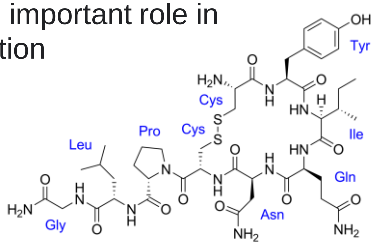




- **As a hormone**
  - Oxytocin
  - Vasopressin
  - Glucagon

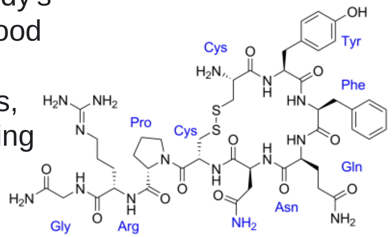
Oxytocin

-neurotransmitter in the brain
-plays an important role in reproduction

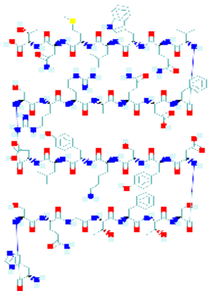


Vasopressin

play essential roles in the control of the body's osmotic balance, blood pressure regulation, sodium homeostasis, and kidney functioning

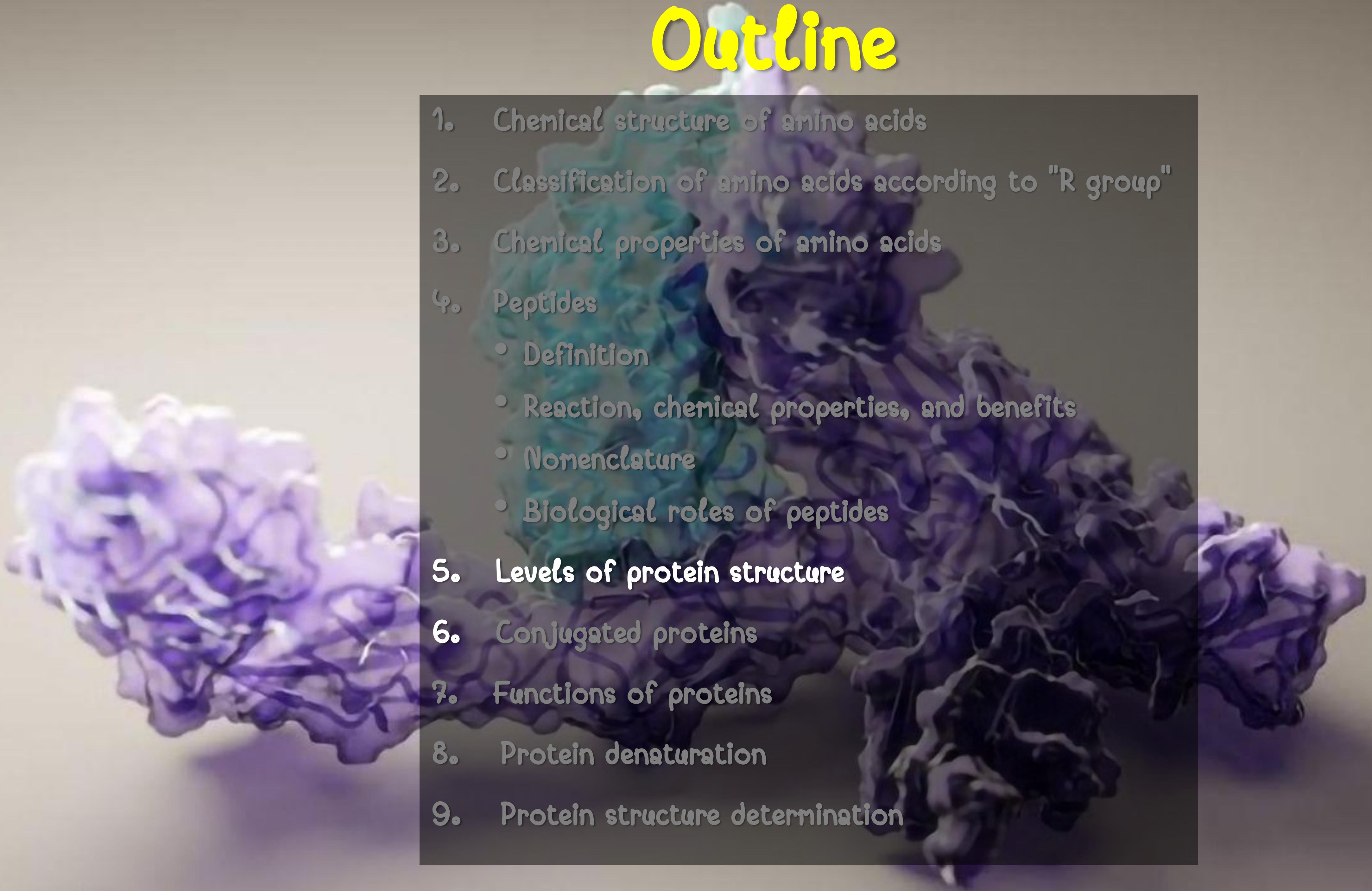


Glucagon



regulate the blood sugar (glucose) levels in the body

Outline



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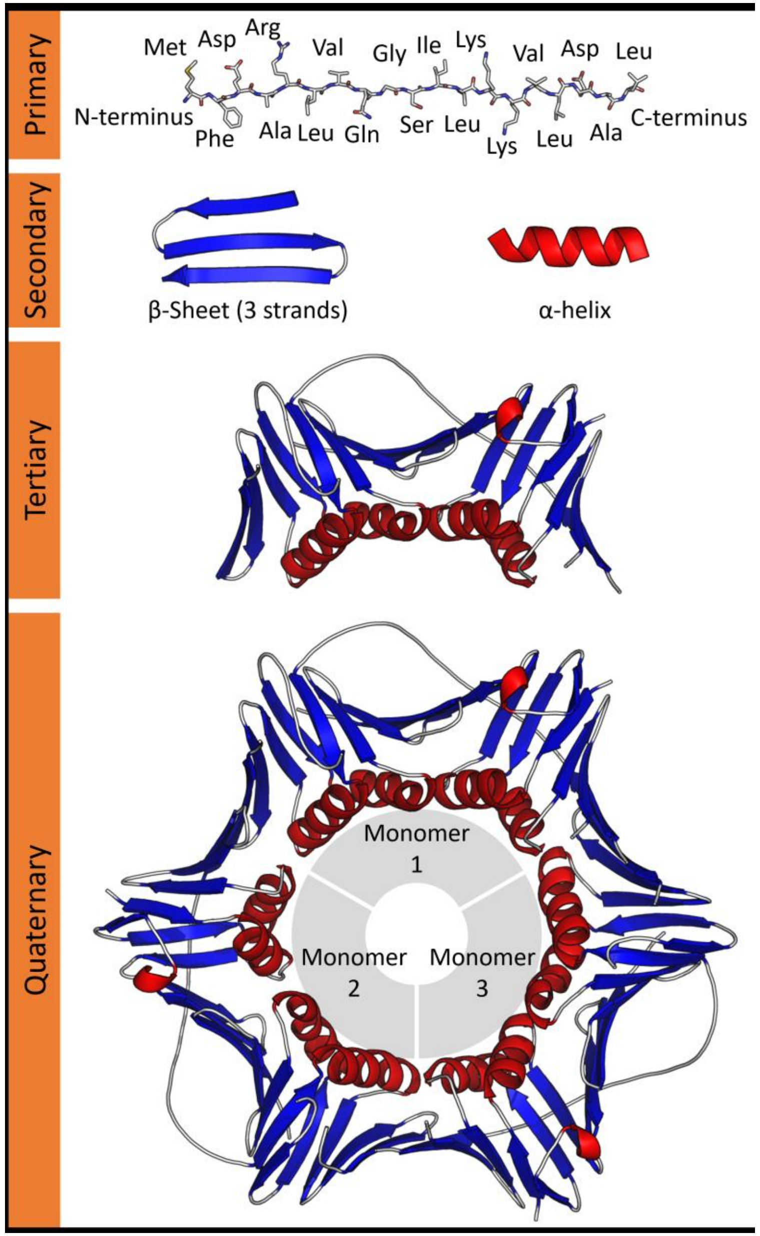
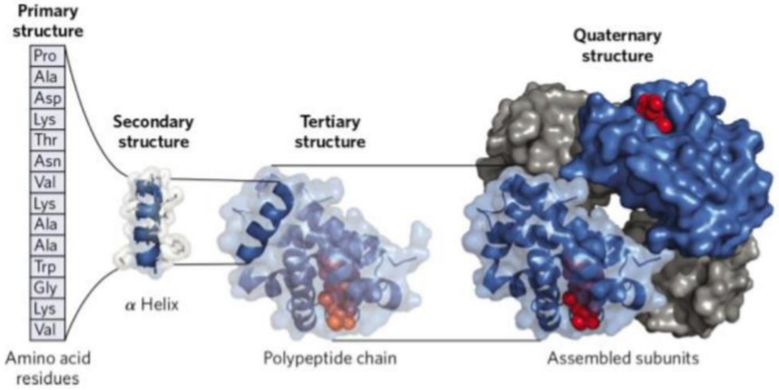
Levels of protein structure

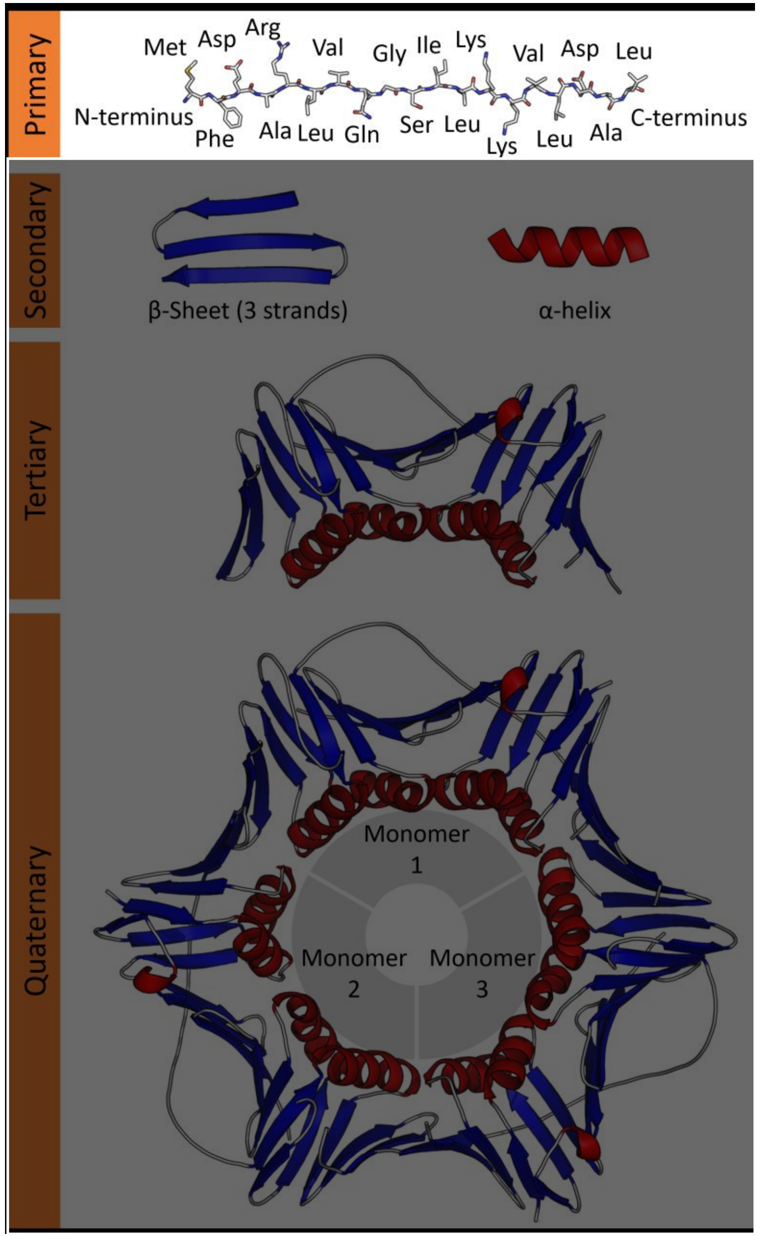


4 Levels of Protein Structure

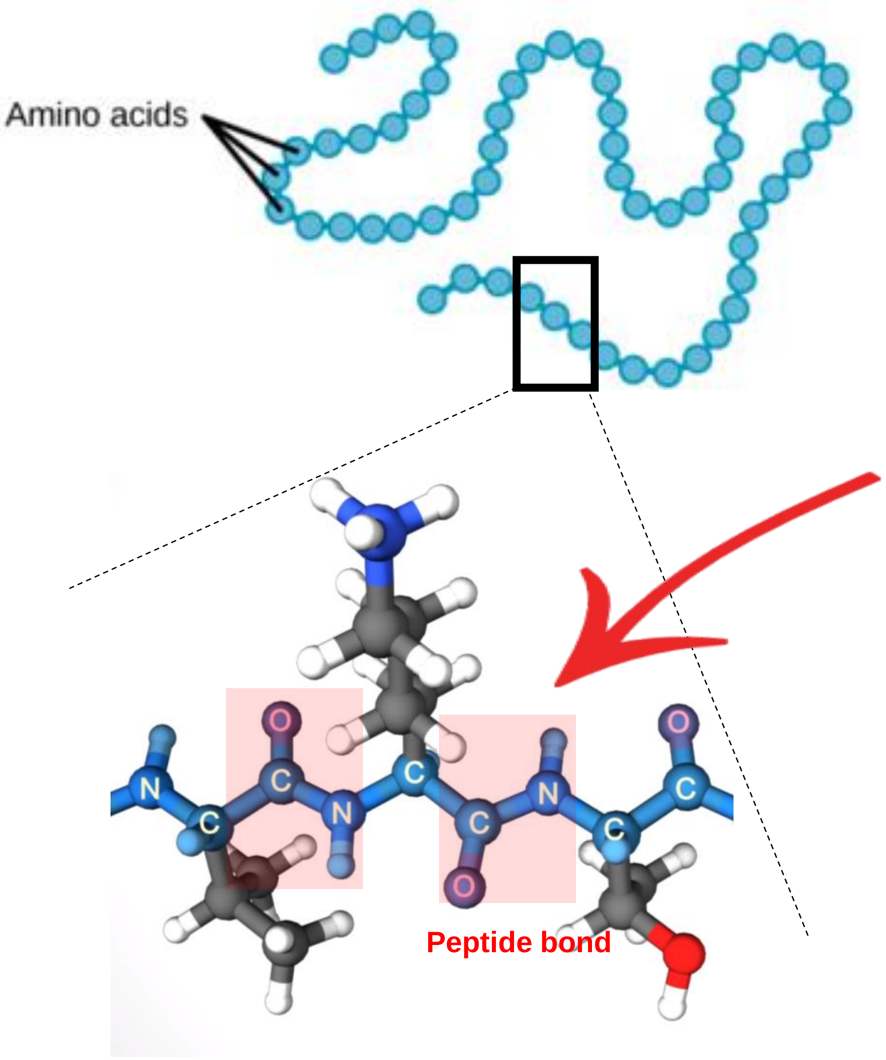
1. **Primary structure:** the sequence of amino acids in a polypeptide chain
2. **Secondary structure:** the local folded structures that form within a polypeptide due to hydrogen bonding between **atoms of the backbone**
3. **Tertiary structure:** the overall folding of the polypeptide chains due to interactions between **the R groups of the amino acid**
4. **Quaternary structure:** some proteins are made up of multiple polypeptide chains (subunit)



Levels of protein structure: 1° structure

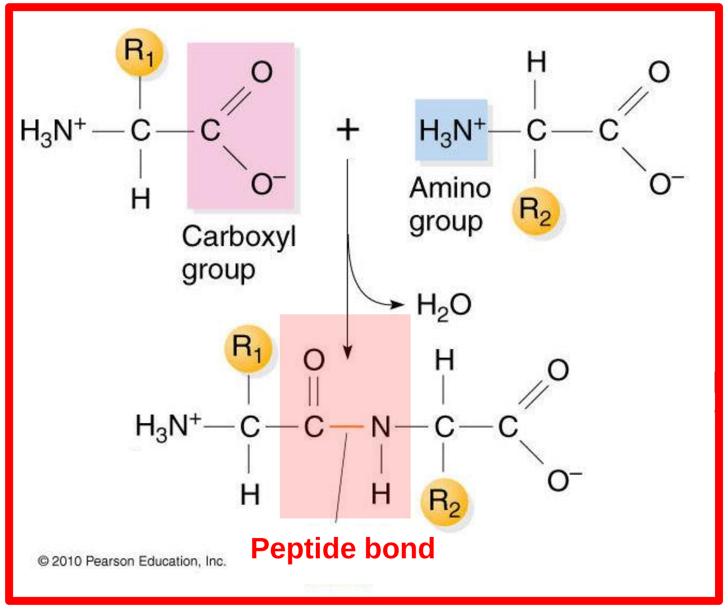


1. **Primary structure:** the sequence of amino acids in a polypeptide chain



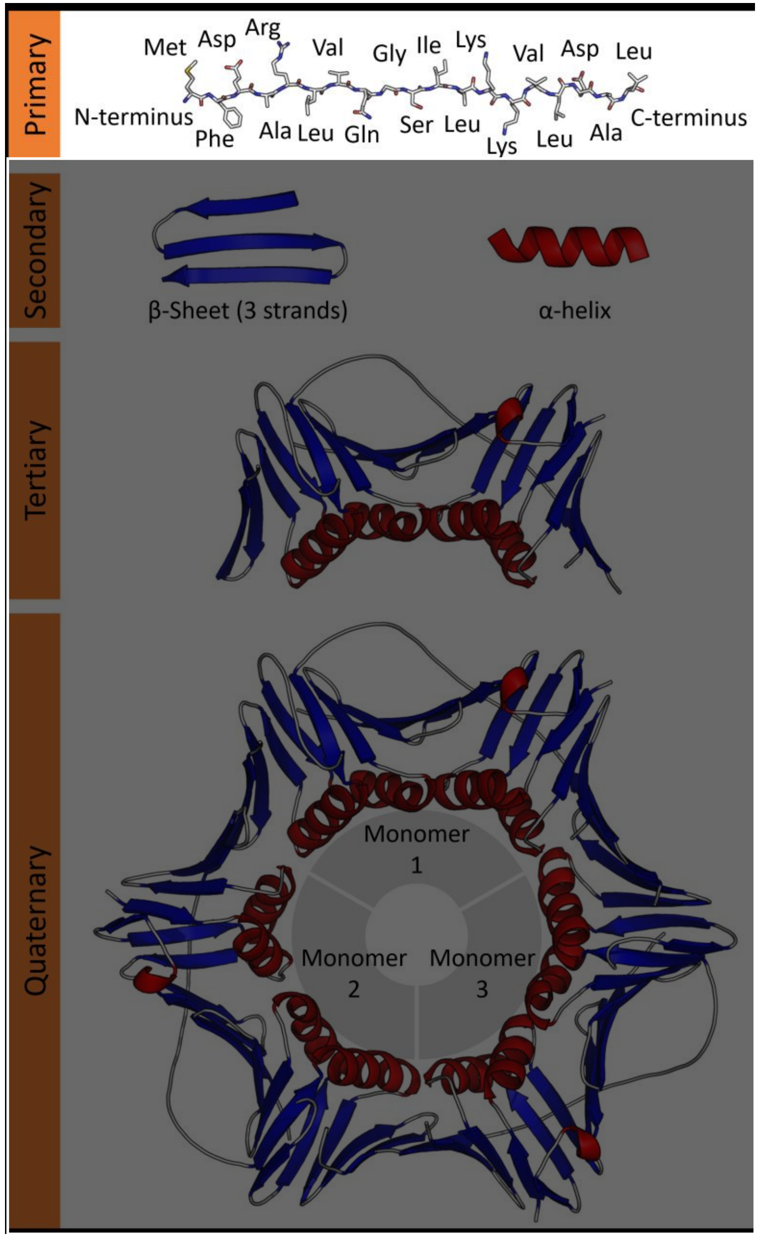
Peptide bond

Peptide bond formation

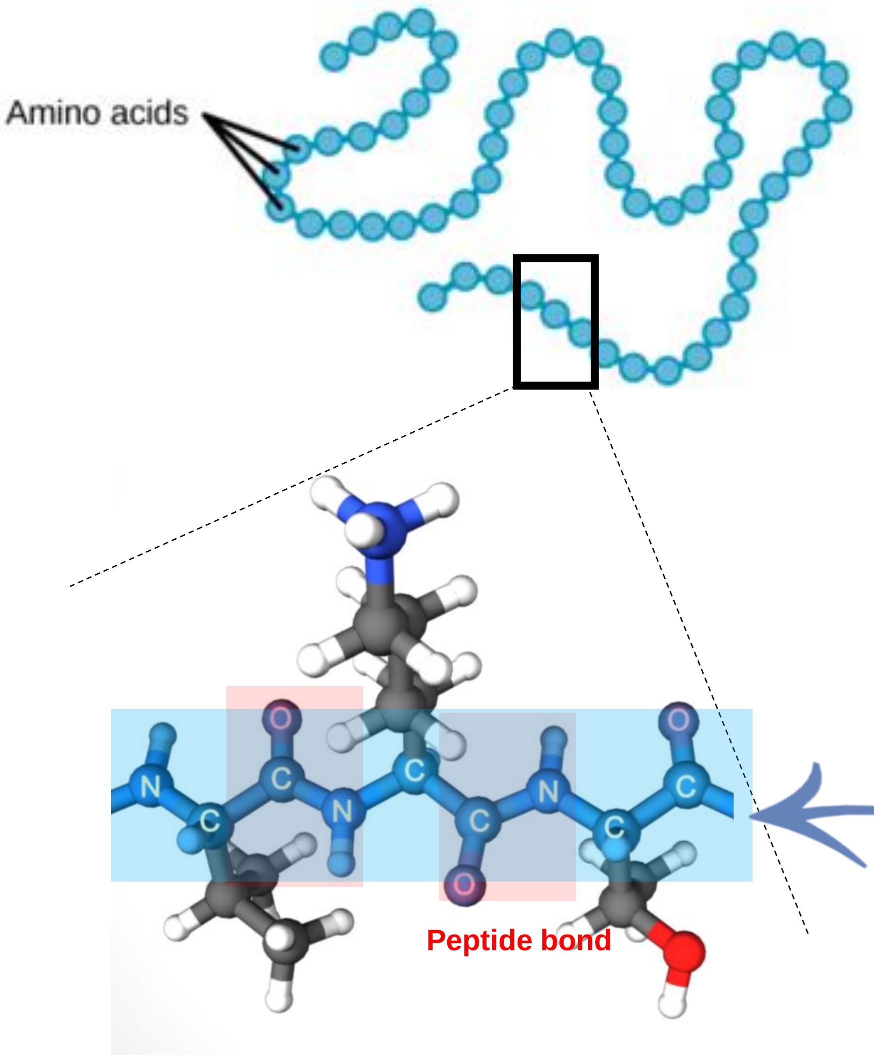


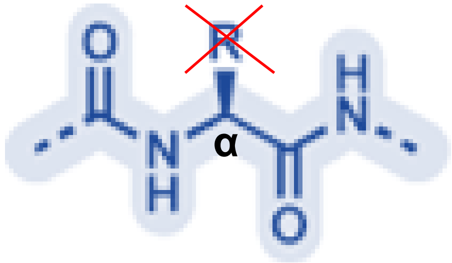
Peptide bond

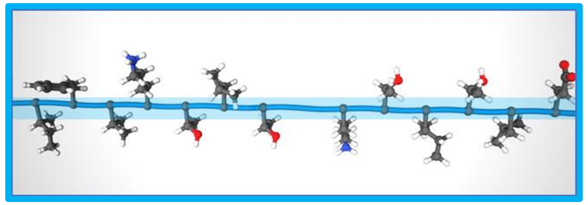
Levels of protein structure: 1° structure



1. **Primary structure:** the sequence of amino acids in a polypeptide chain



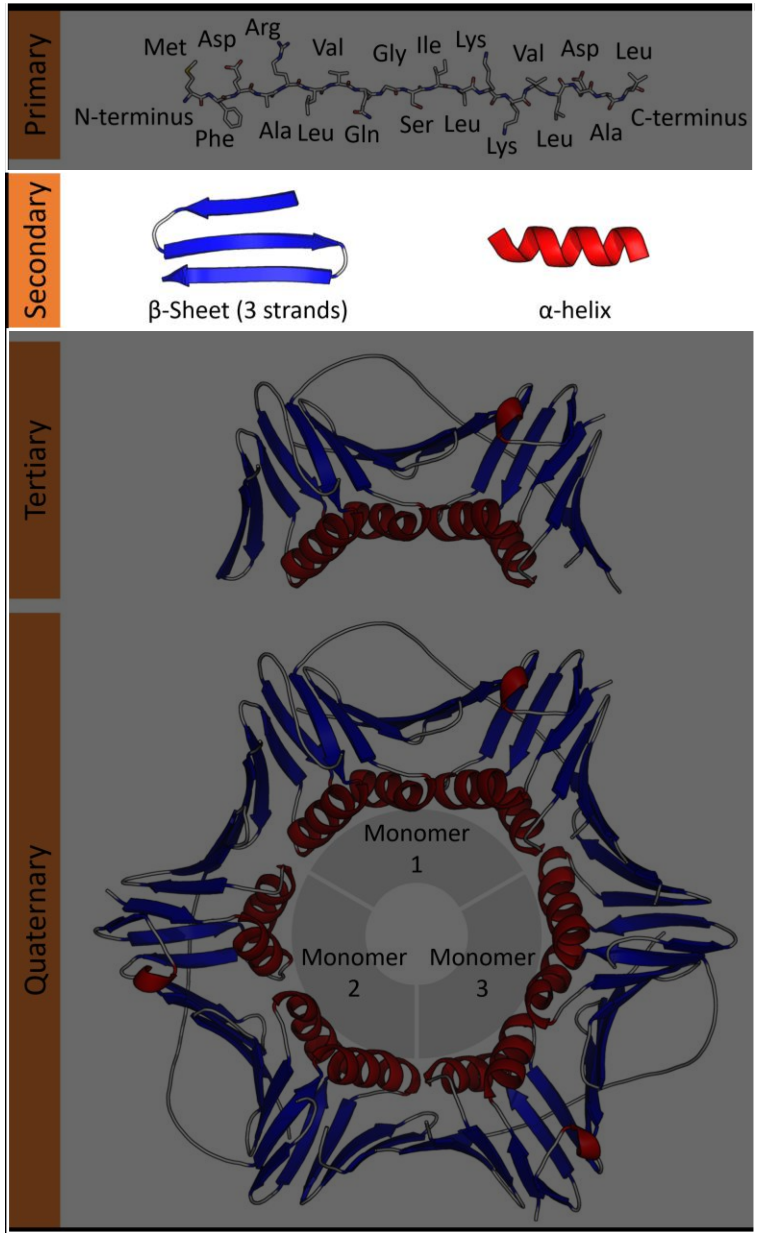




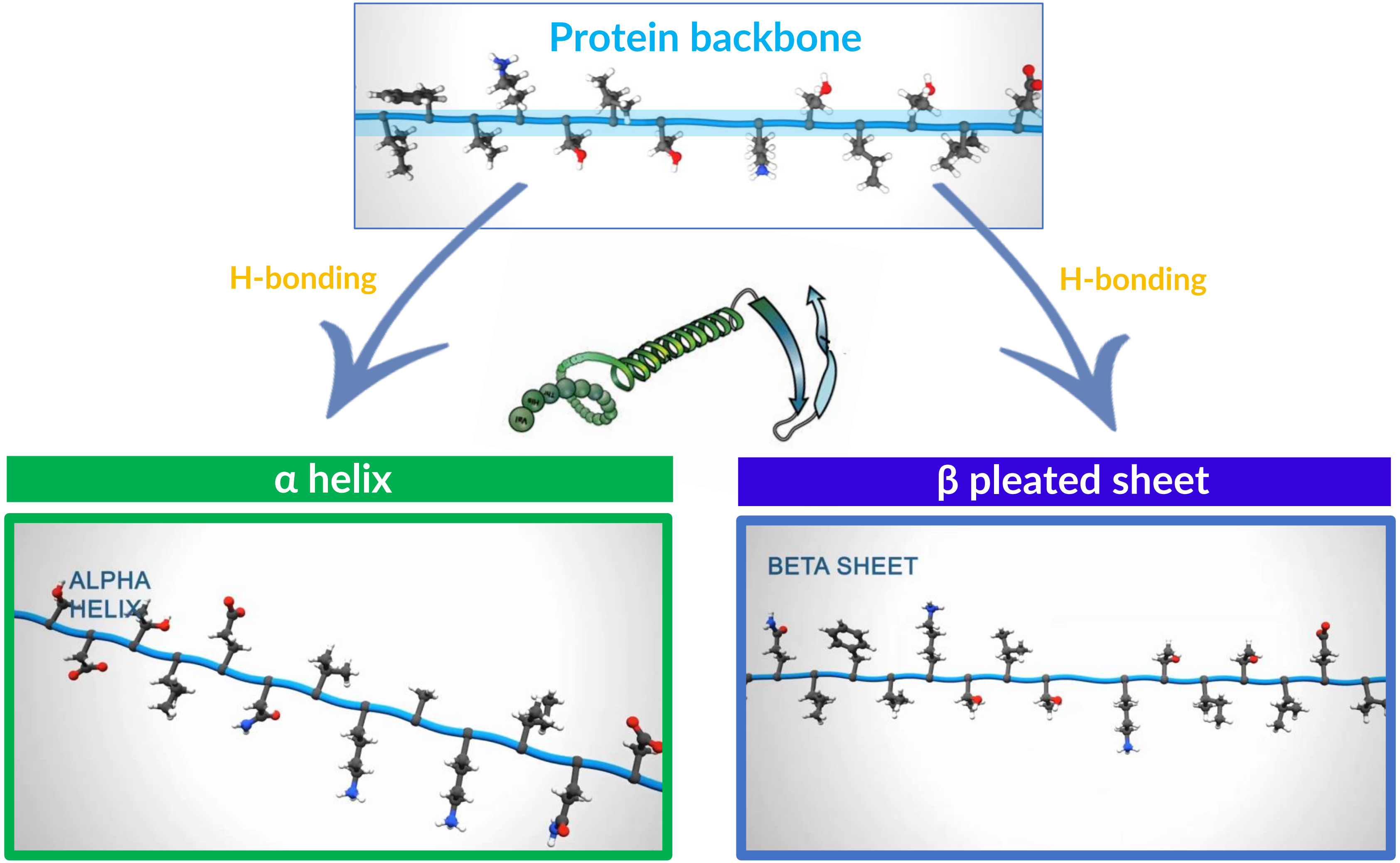
Protein backbone

Count every atom except R group

Levels of protein structure: 2° structure



2. Secondary structure: the local folded structures that form within a polypeptide due to hydrogen bonding between atoms of the backbone



Protein backbone

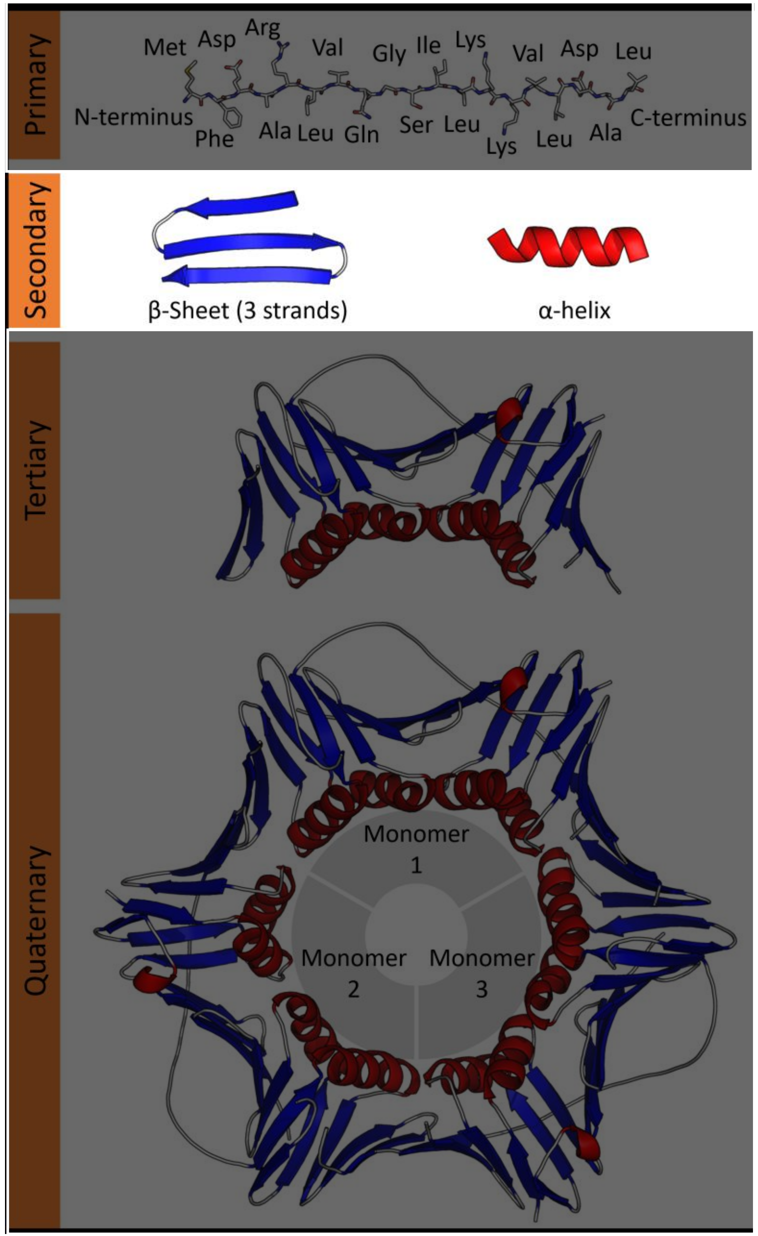
H-bonding

H-bonding

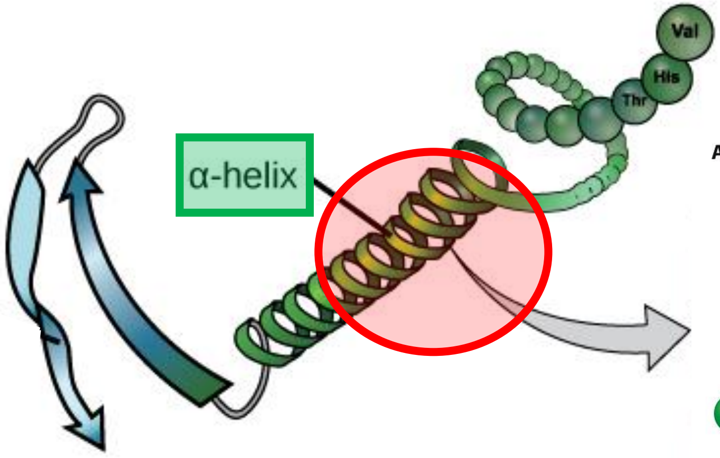
α helix

β pleated sheet

Levels of protein structure: 2° structure



2. **Secondary structure:** the local folded structures that form within a polypeptide due to hydrogen bonding between **atoms of the backbone**



α-helix

- a right-handed helix
- has 3.6 residues per turn
- are stabilized by hydrogen bond formation

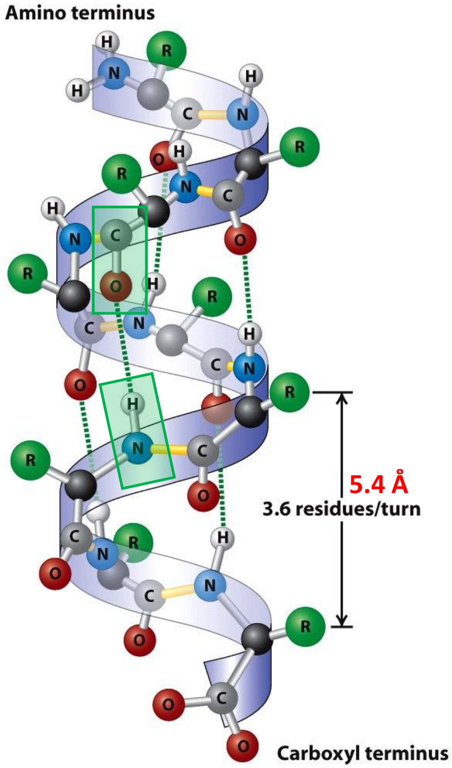


Figure 3-4
Molecular Cell Biology, Sixth Edition
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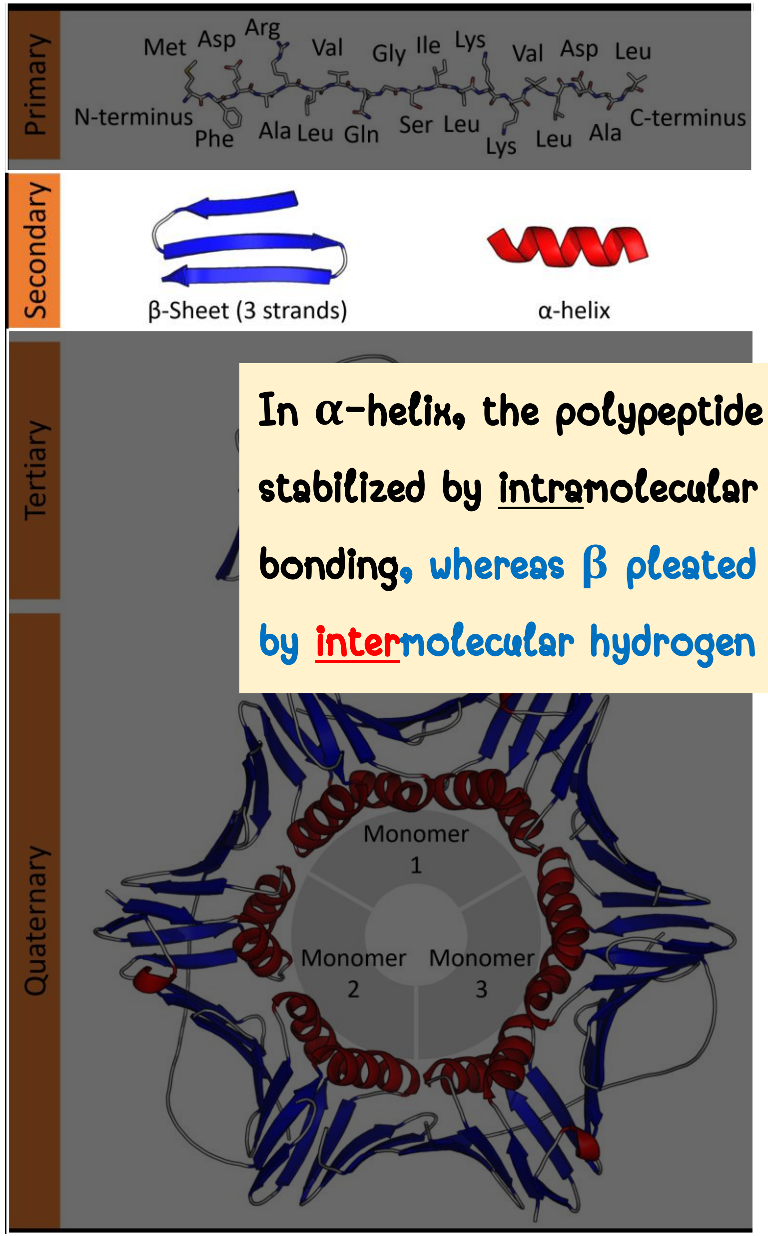
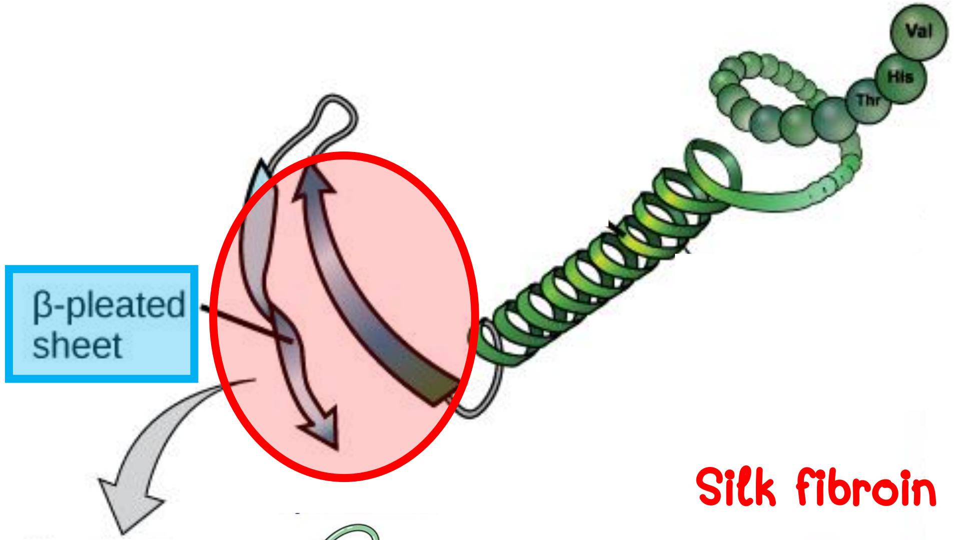
Levels of protein structure: 2° structure

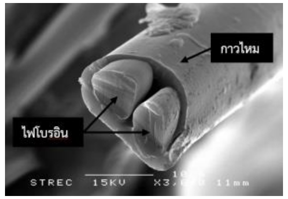
2. **Secondary structure:** the local folded structures that form within a polypeptide due to hydrogen bonding between **atoms of the backbone**

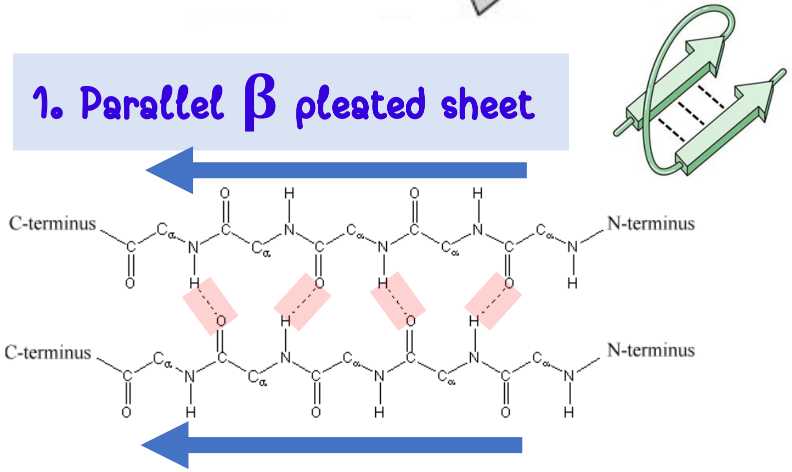


In α-helix, the polypeptide chains are stabilized by intramolecular hydrogen bonding, whereas β pleated sheet is stabilized by intermolecular hydrogen bonding

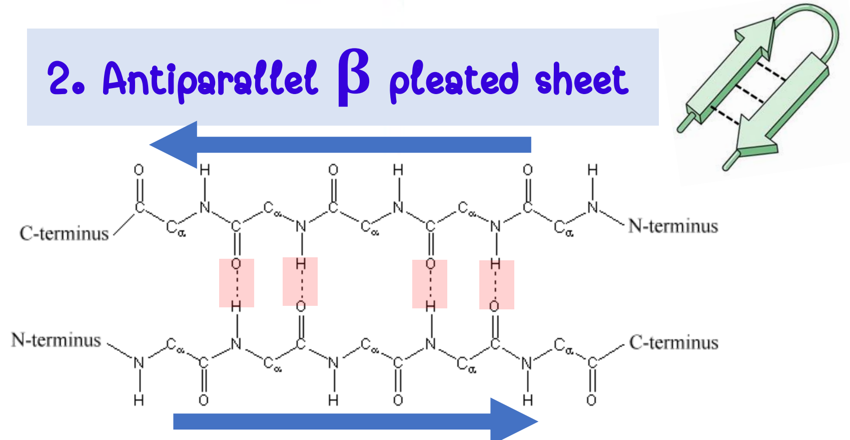


Silk fibroin





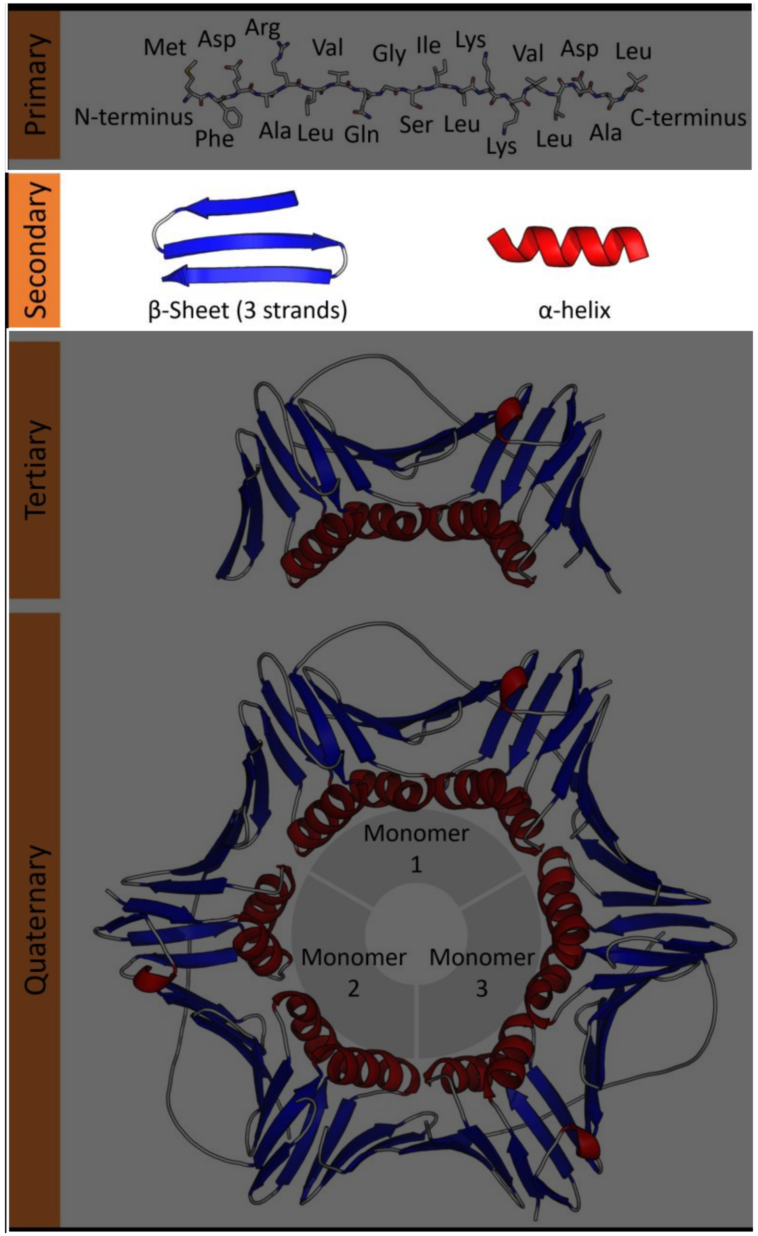
1. Parallel β pleated sheet



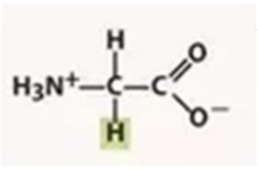
2. Antiparallel β pleated sheet

Stronger!!

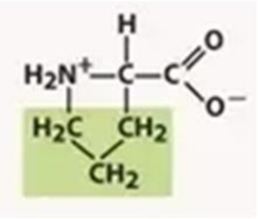
Levels of protein structure: 2° structure



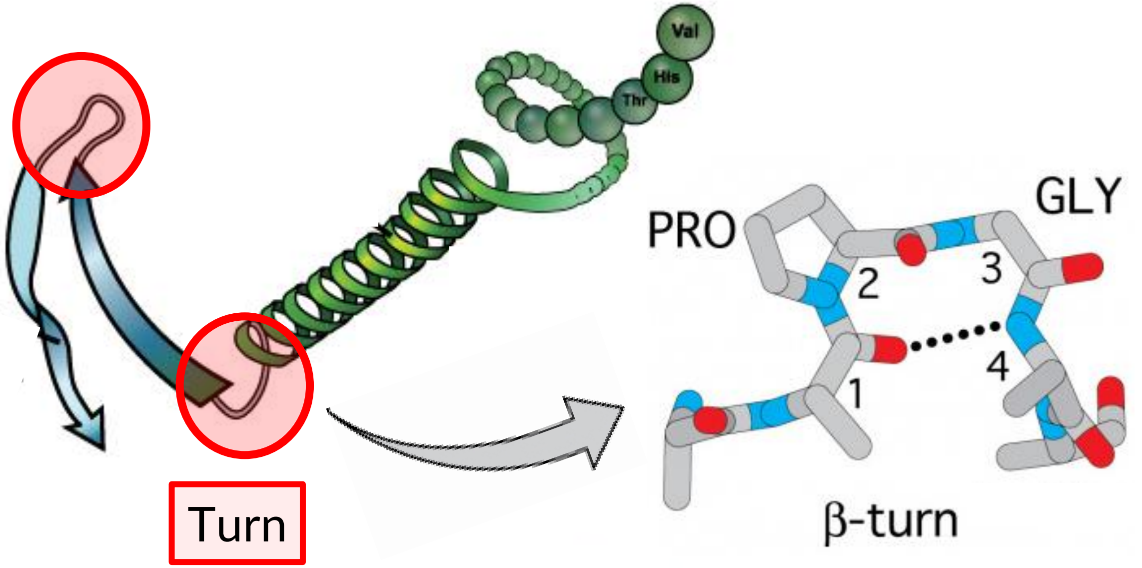
2. **Secondary structure:** the local folded structures that form within a polypeptide due to hydrogen bonding between **atoms of the backbone**



Glycine (Gly)

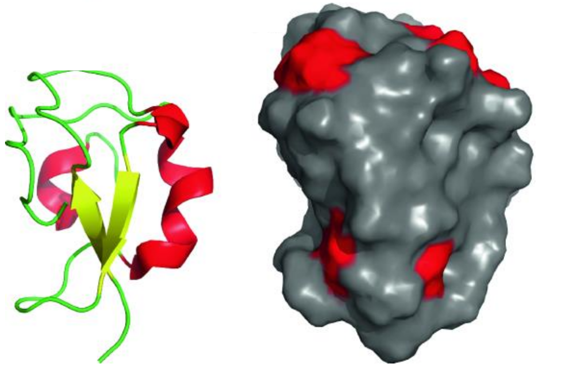


Proline (Pro)

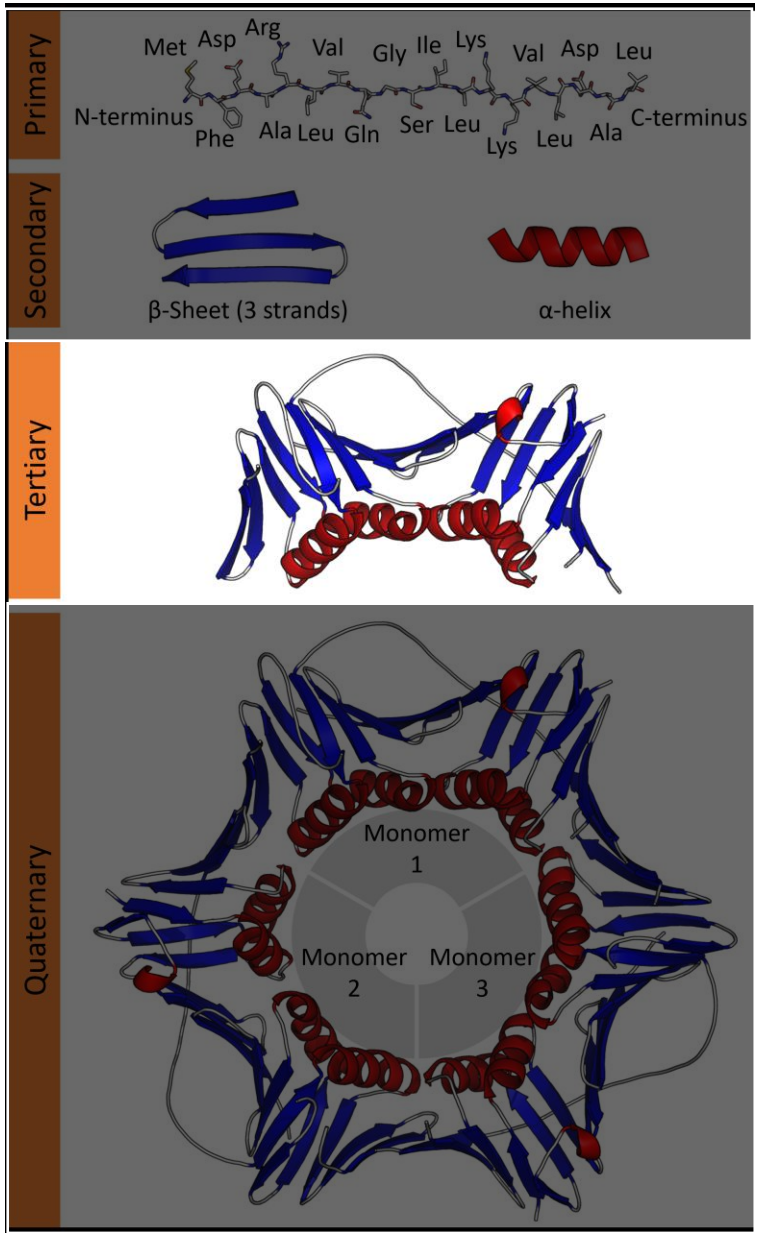


Turn

- Its role involves altering the direction of the polypeptide structure or linking the alpha helix and beta pleat, leading to a more compact folding of the polypeptide chain
- Pro and Gly residues are favored in β-turns due to the cyclic structure of Pro and the flexibility of Gly
- very abundant in globular protein

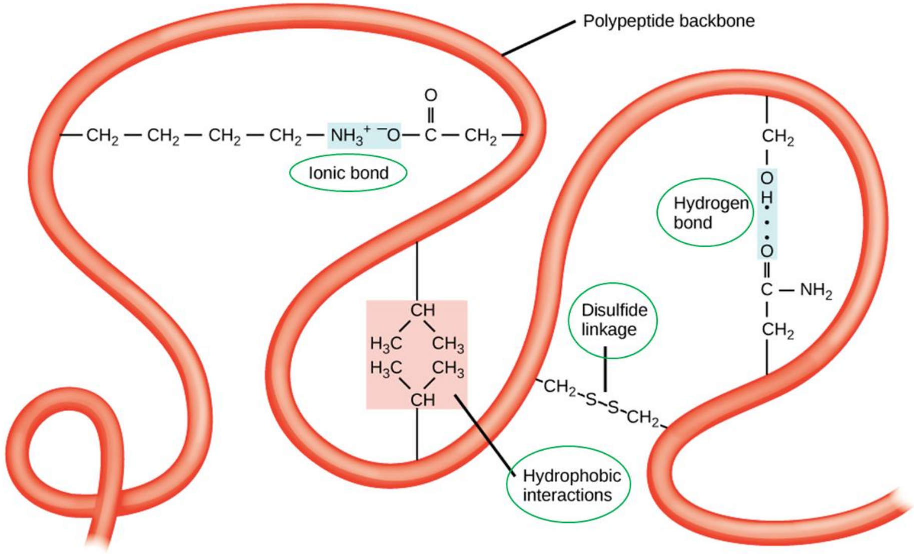


Levels of protein structure: 3° structure

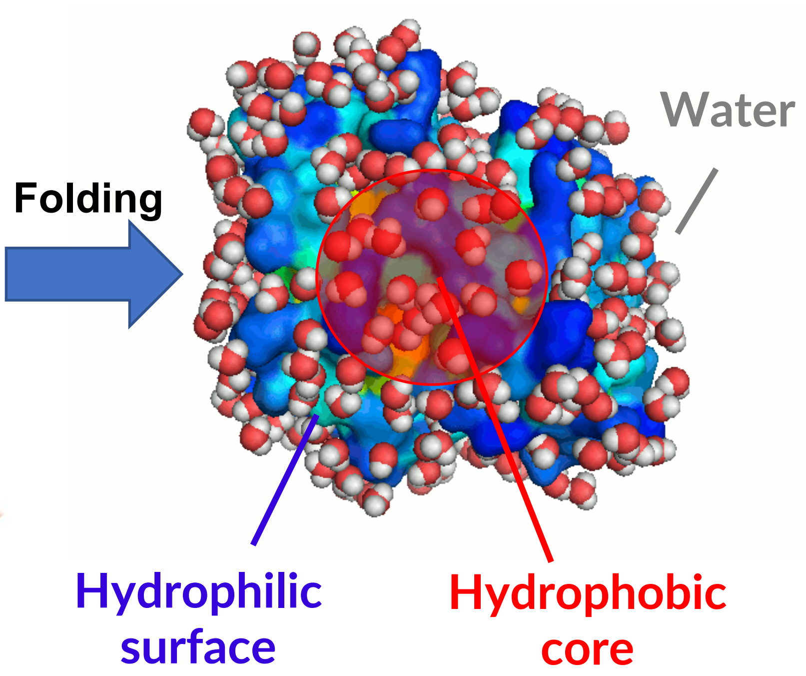


3. **Tertiary structure:** the overall folding of the polypeptide chains due to interactions between **the R groups of the amino acid**

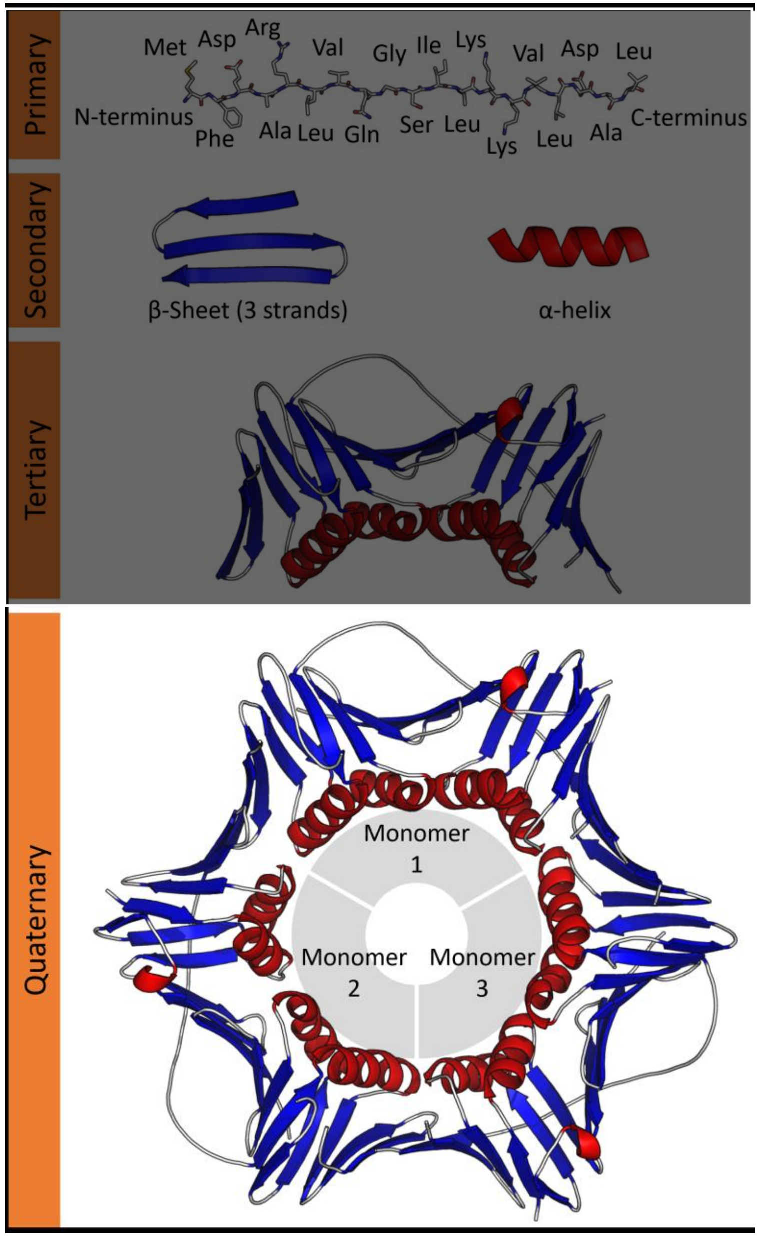
- Ionic bond (electrostatic interaction)
- Hydrophobic interaction
- H-bond
- Disulfide linkage



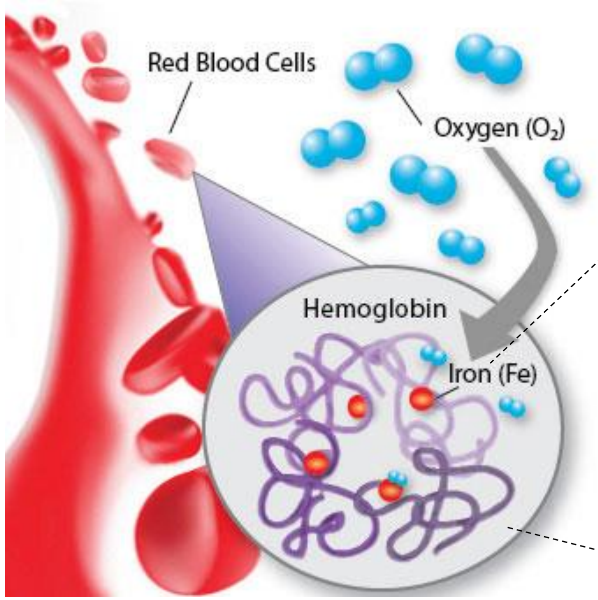
3D structure



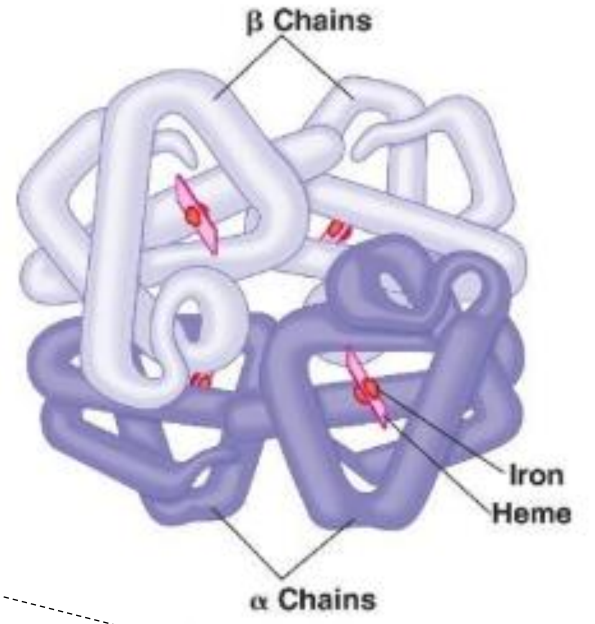
Levels of protein structure: 4° structure



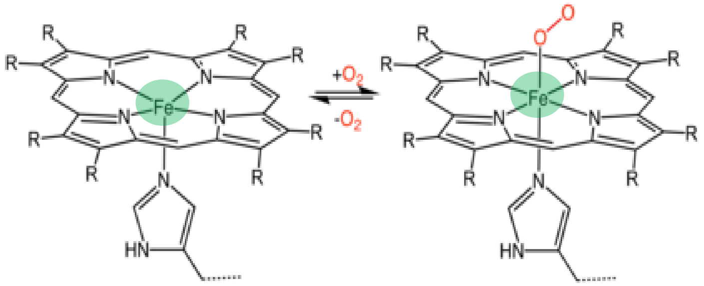
4. Quaternary structure: some proteins are made up of multiple polypeptide chains (subunit)



Hemoglobin

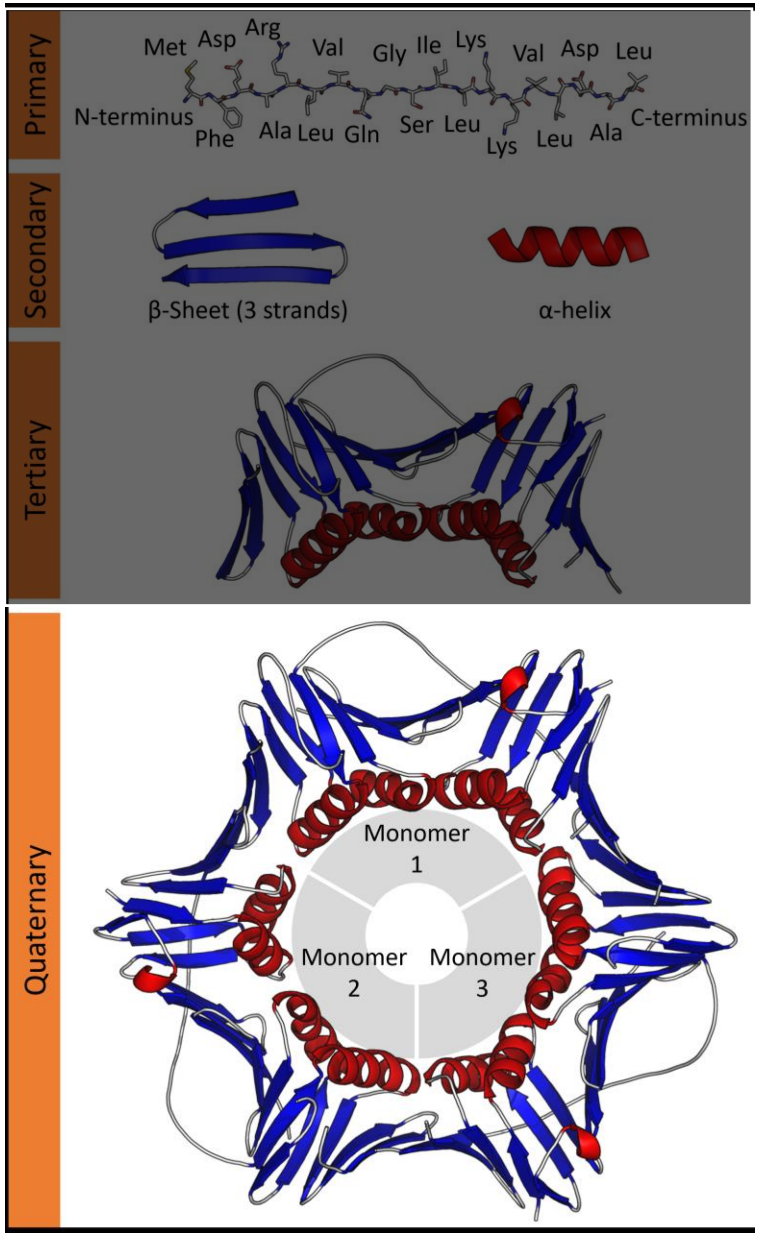


- A hemoglobin molecule is made up of four polypeptide chains:
  - 2 Alpha-globin chains
  - 2 Beta-globin chains
- Each chain contains a heme molecule which binds to oxygen



Levels of protein structure: 4° structure

4. Quaternary structure: some proteins are made up of multiple polypeptide chains (subunit)

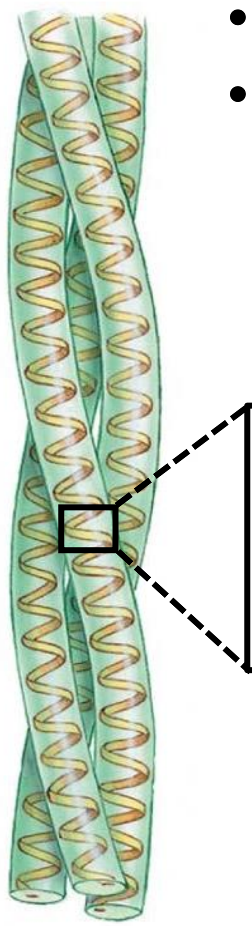


- Collagen is made up of three polypeptide chains called **a triple helix**
- Each chain is made from **$(G\text{-}X\text{-}Y)_n$**

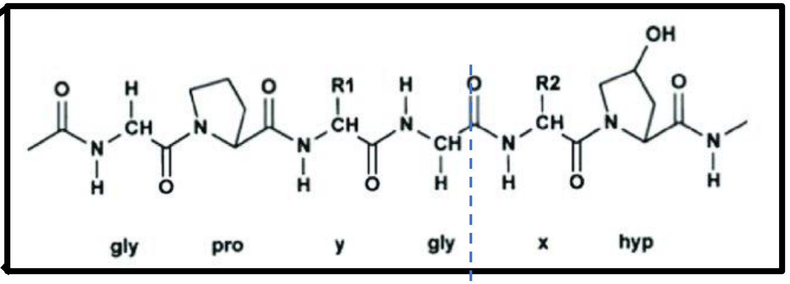
G = glycine, X = Proline, Y = hydroxyproline (Hyp)

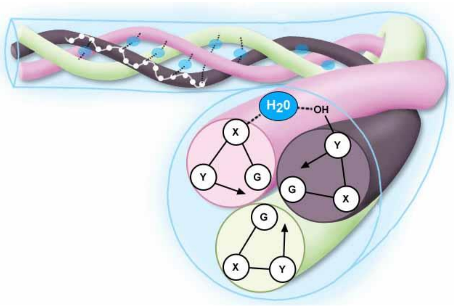
*Hyp is produced by hydroxylation of proline by prolyl hydroxylase

*L-ascorbic acid is an essential coenzyme of prolyl hydroxylase



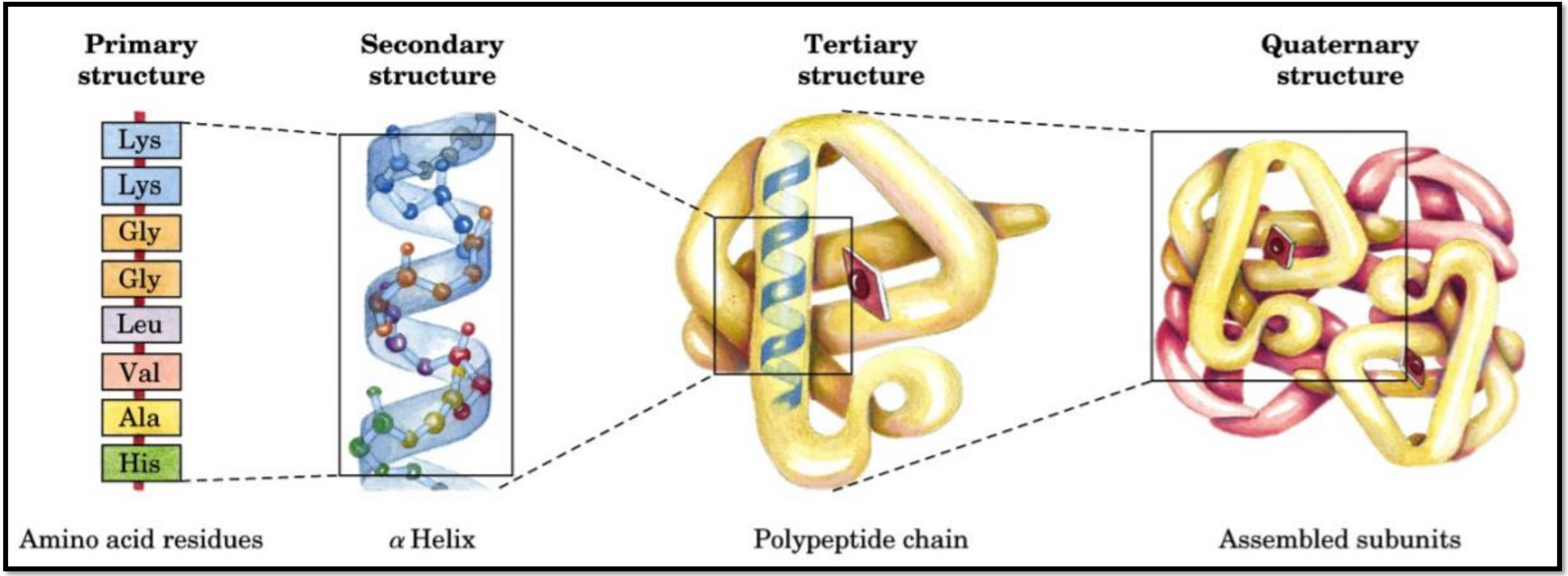
Collagen
(fibrous protein)





Collagen is a protein found in connective tissue, skin, tendon, bone, etc.

SUMMARY



Amino acids are linked by Peptide bond

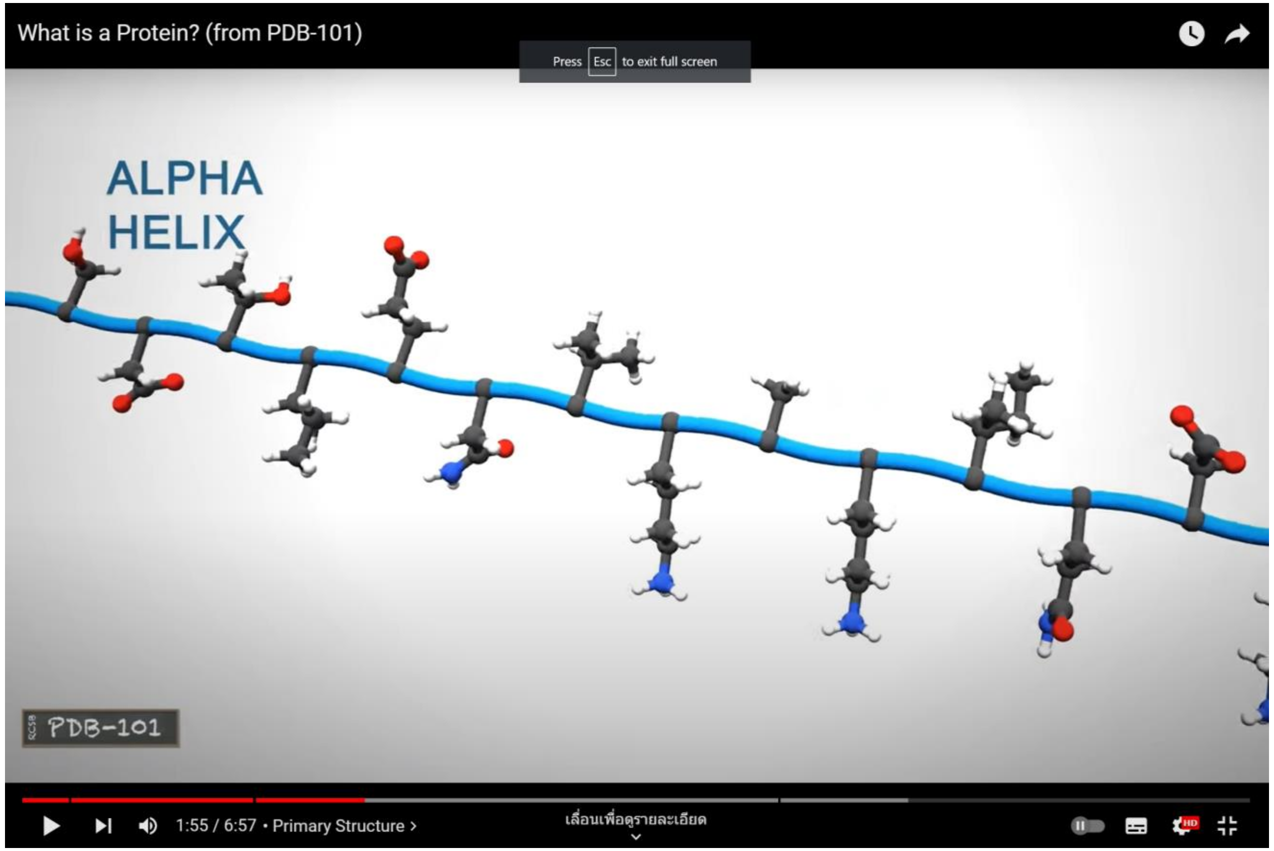
Hydrogen bond between Protein backbone

Interactions (hydrophobic, Ionic bond, H-bond, disulfide bond) between R-group of amino acids in <u>the same polypeptide chain</u>

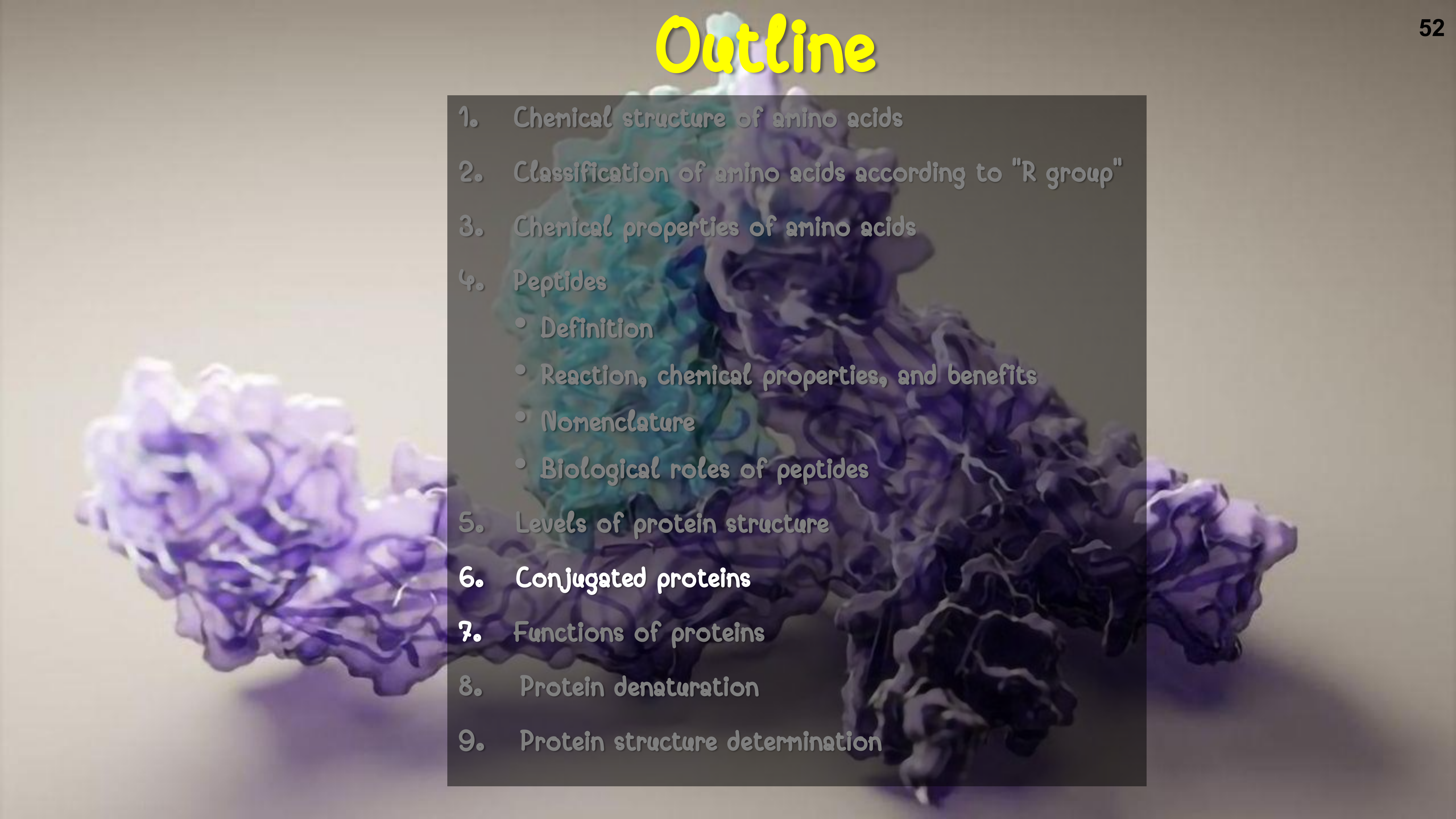
Interactions (hydrophobic, Ionic bond, H-bond, disulfide bond) between R-group of amino acids in <u>another polypeptide chain</u>

4 levels of Protein Structure

https://www.youtube.com/watch?v=wvTv8TqWC48&t=124s



Outline



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3. Chemical properties of amino acids
4. Peptides
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   - Reaction, chemical properties, and benefits
   - Nomenclature
   - Biological roles of peptides
5. Levels of protein structure
6. **Conjugated proteins**
7. Functions of proteins
8. Protein denaturation
9. Protein structure determination

Conjugated proteins

A protein to which another chemical group (e.g., lipids) is attached by either covalent bonding or other interactions

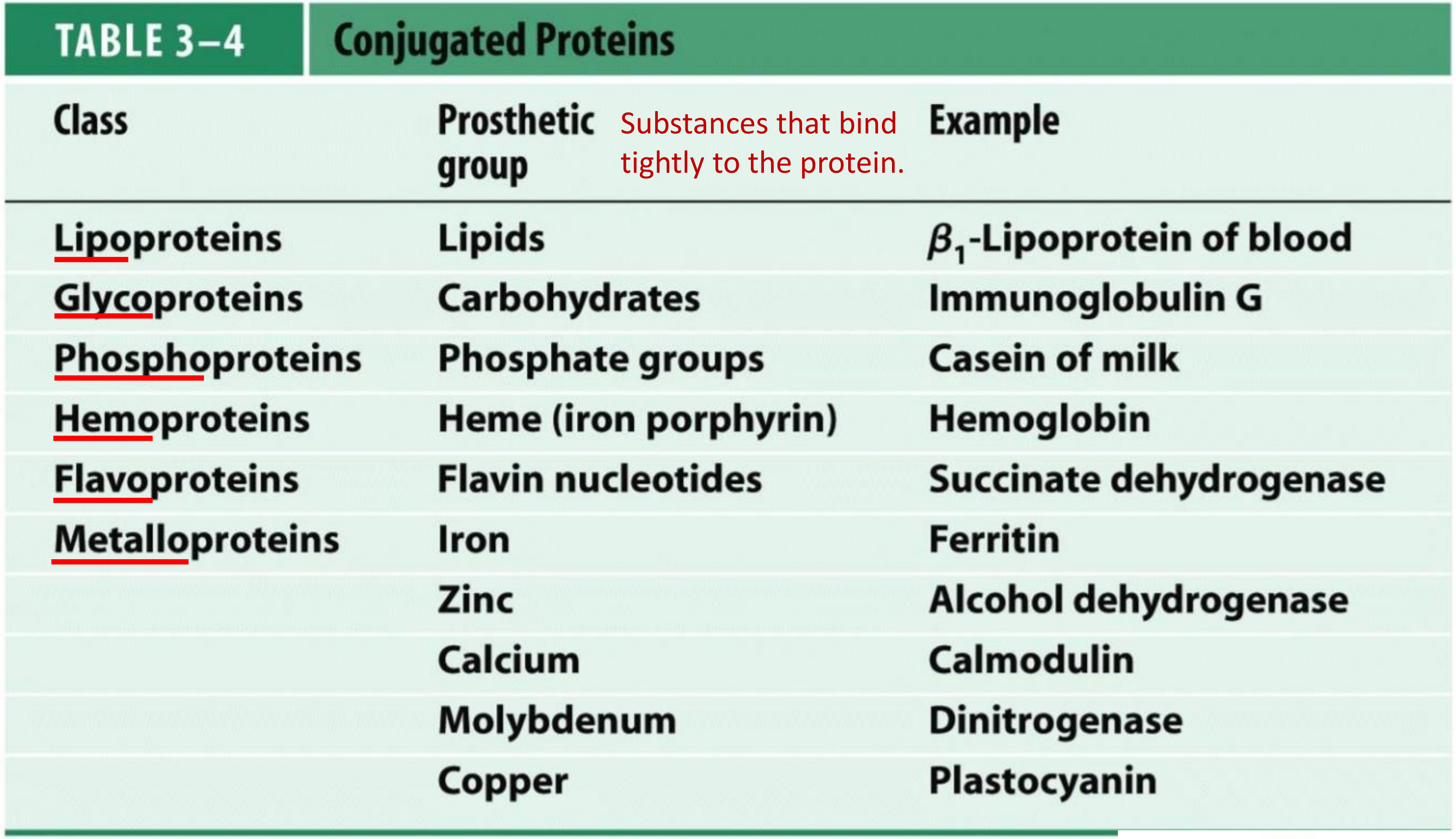
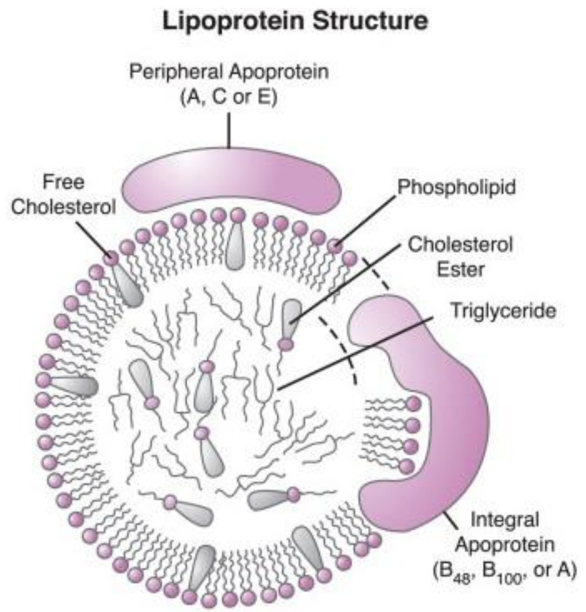


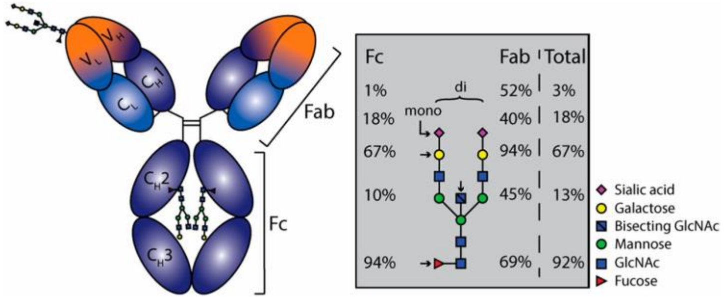
TABLE 3–4 Conjugated Proteins

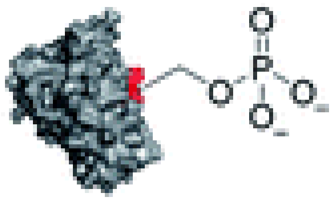
Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

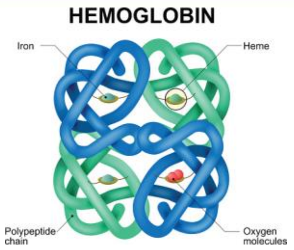
Substances that bind tightly to the protein.

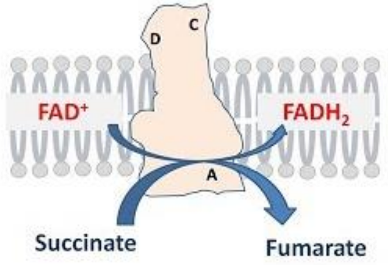
Table 3-4
Lehninger Principles of Biochemistry, Fifth Edition

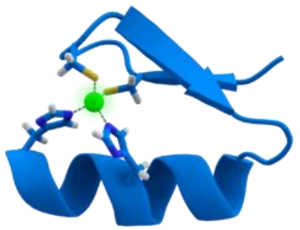












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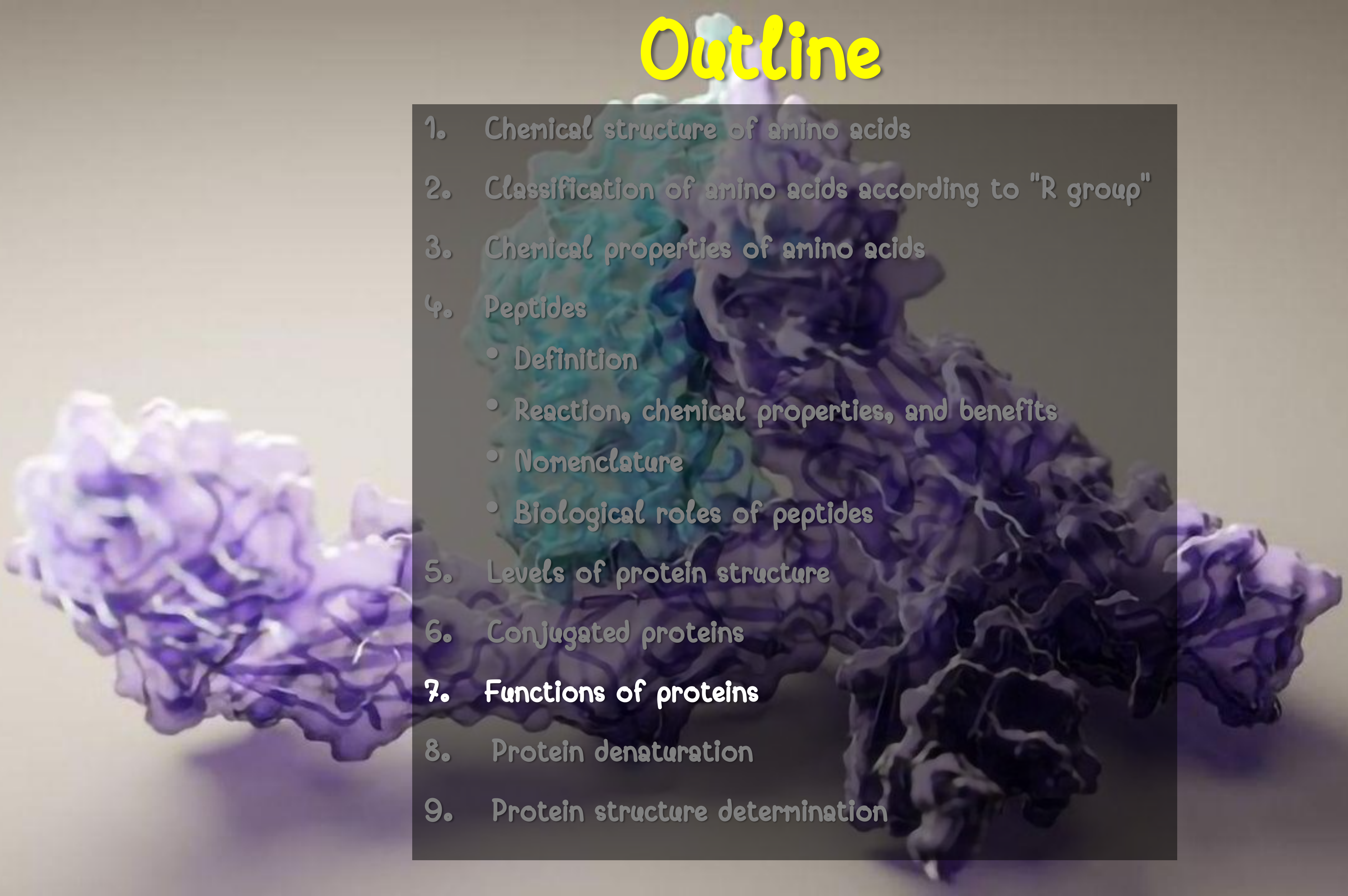

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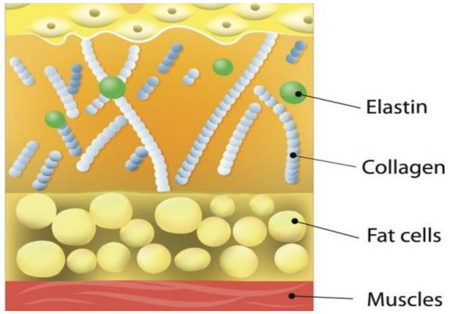
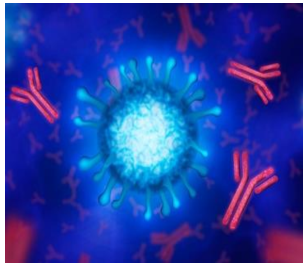
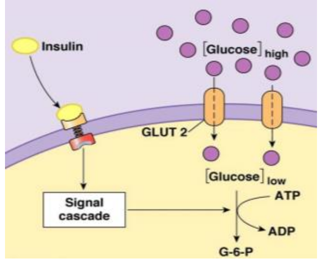
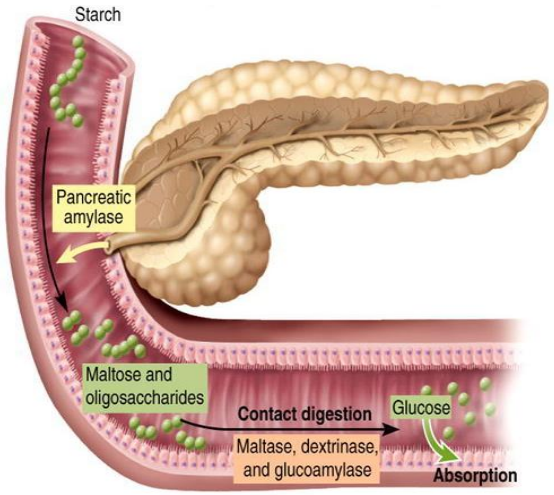
Functions of proteins

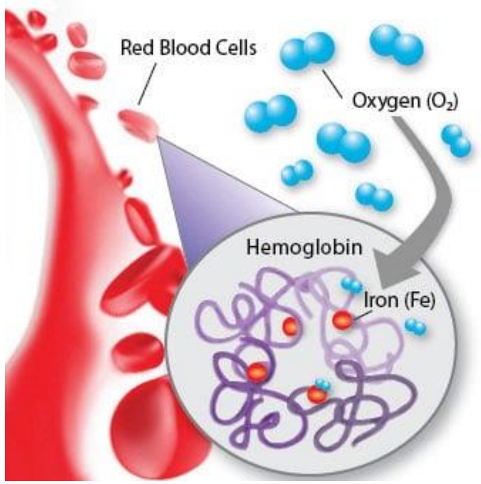
1. **Enzyme:** Amylase, Lipase, Protease
2. **Oxygen transport:** Hemoglobin
3. **Structural protein:** Collagen, Keratin
4. **Immunity:** Immunoglobulin
5. **Hormone:** Insulin, Glucagon
6. **Sensation:** T1R2/T1R3 sweet receptor
7. **Cell proliferation:** EGFR

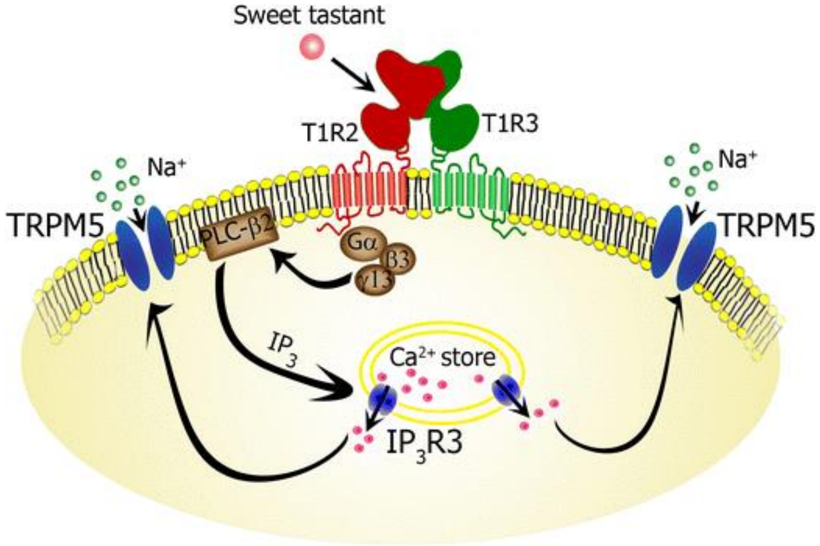


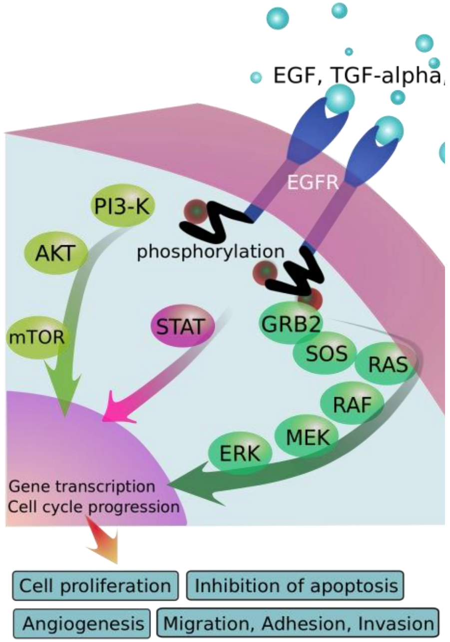












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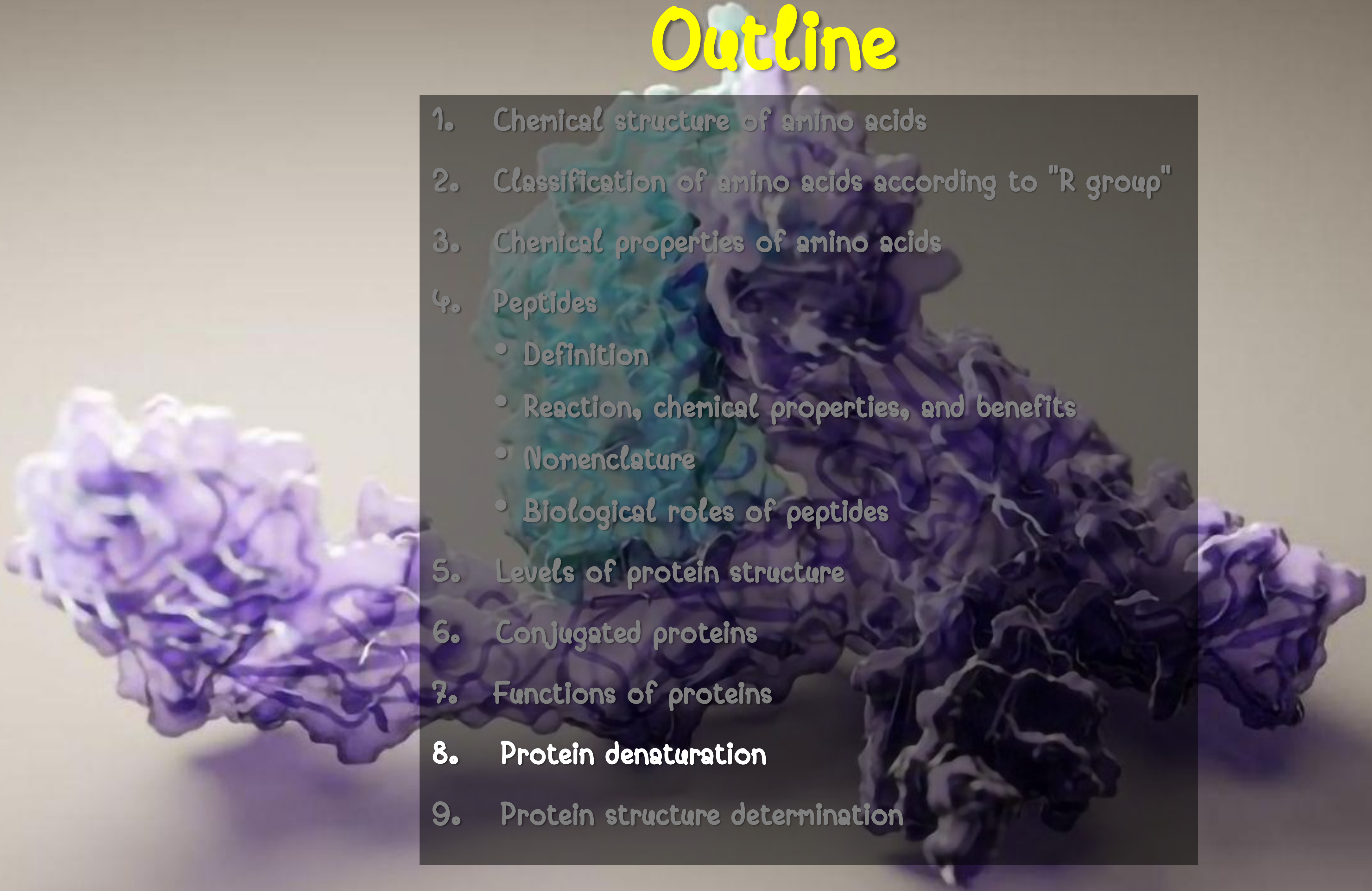

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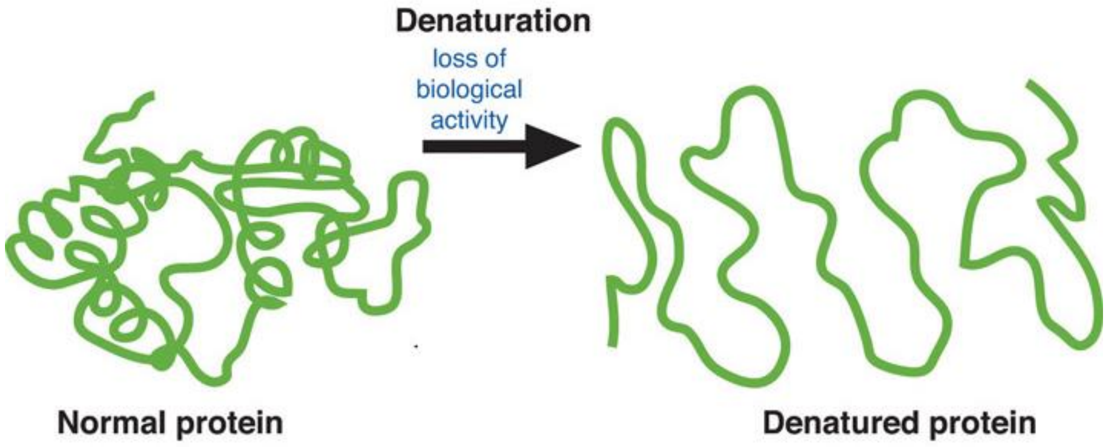
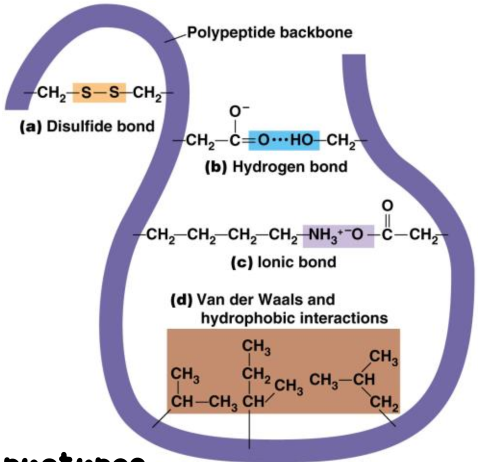
9. Protein structure determination

Protein denaturation

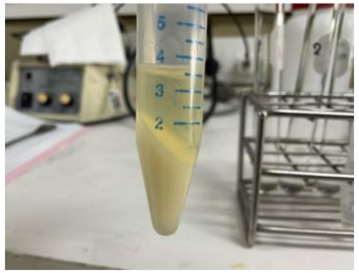


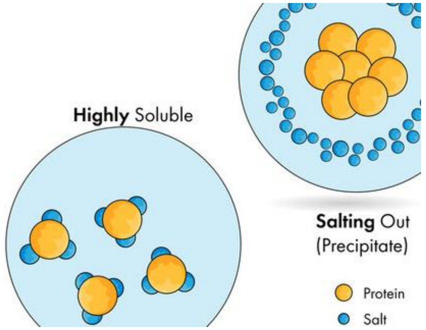


- **The process by which a protein undergoes the loss of its quaternary, tertiary, and secondary structures, becoming a primary structure (a long chain of amino acids) <u>without breaking the peptide bond</u>**

Reversible denaturation

- Proteins can return to work
- Such as salting out (using high concentrations of salt to precipitate proteins in the solution)





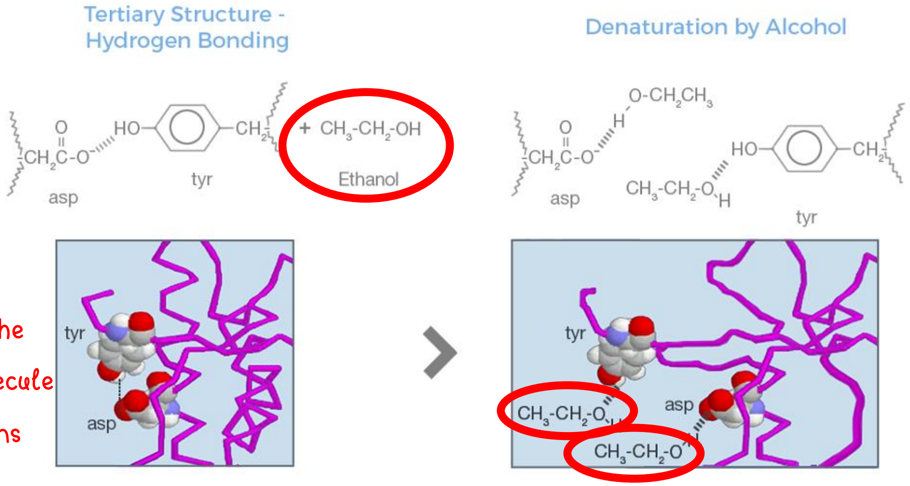
https://stock.adobe.com/th/search/images?k=solubility

Irreversible denaturation

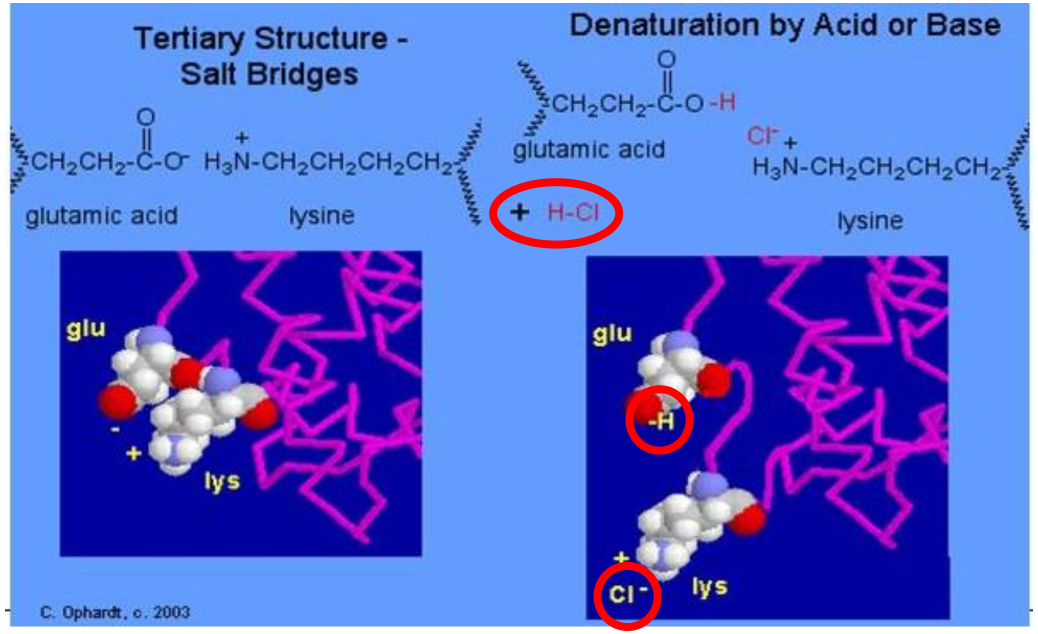
- Proteins <u>cannot</u> return to work
- Such as exposing protein solutions to heat, potent acids, and strong bases

Factors causing protein denaturation

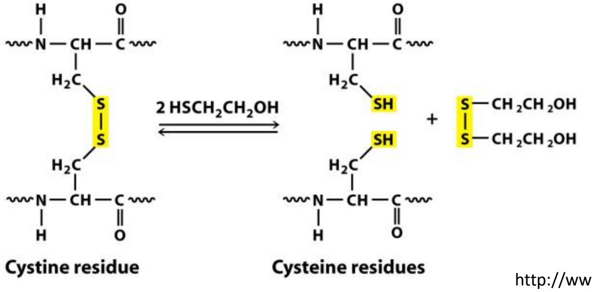
- **Heat** destroys H-bonds and hydrophobic interactions
- **Organic solvents** destroy H-bonds and/or hydrophobic interaction (in case of solvent with very low polarity)
- **Acids/Bases (extreme pH)** destroy H-bond and ionic bond between R- of polar amino acids
- **Reducing agent** e.g., β-mercaptoethanol destroys disulfide bond between two cysteine residues
- **Heavy metals** e.g., Hb^{2+}, Ag^{+}, Pb^{2+} bind to carboxylate group (-COO^{-}), leading to an insoluble metal protein salt



New hydrogen bonds are formed instead between the new organic solvent molecule and the protein side chains

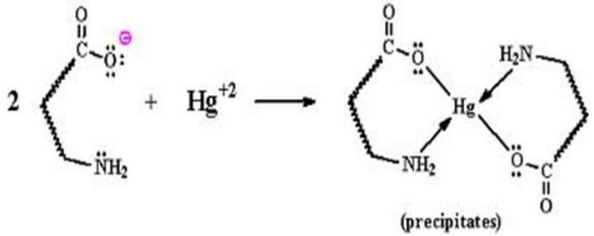


C. Ophardt, c. 2003



Cystine residue

Cysteine residues



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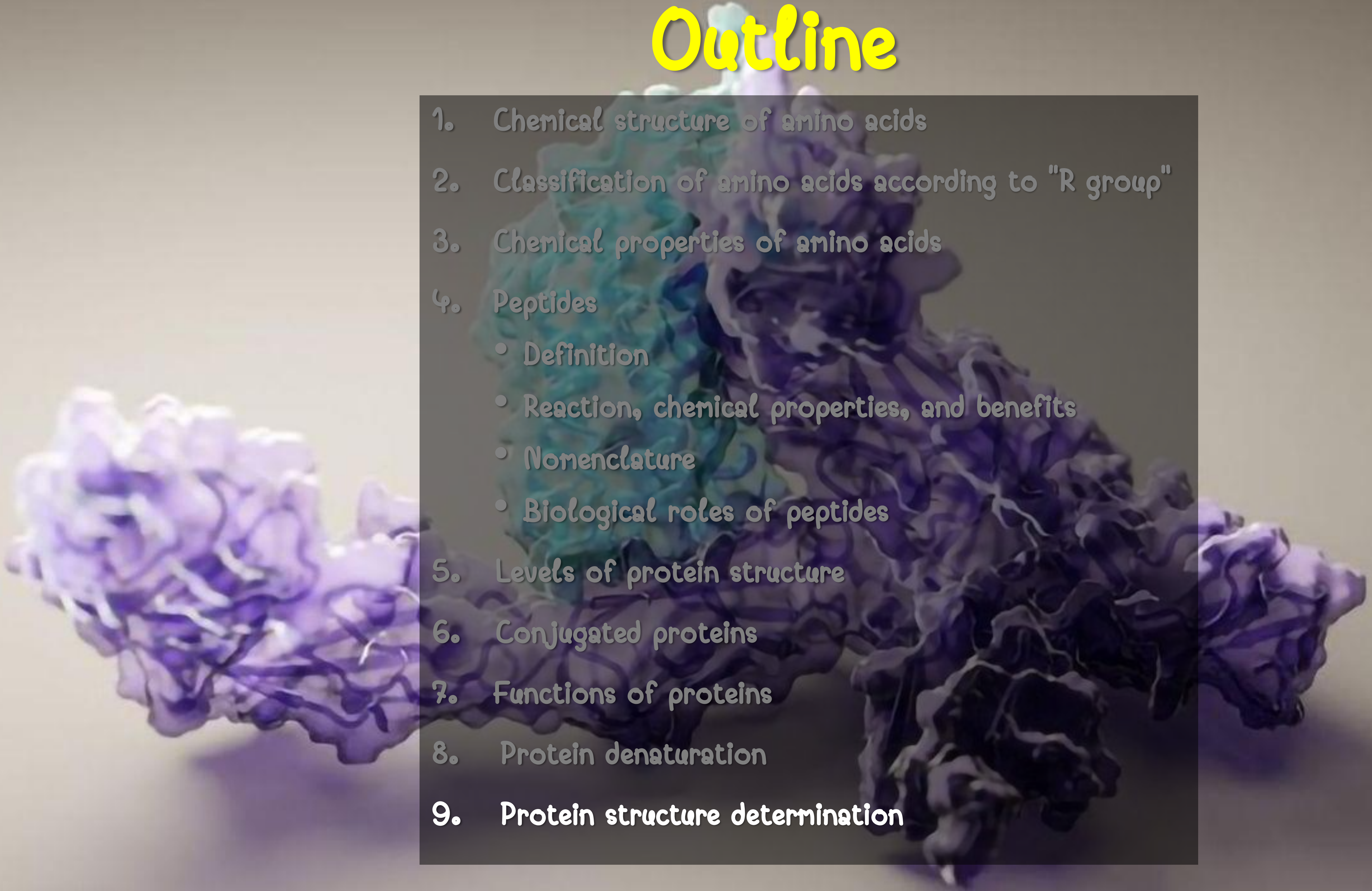
Outline



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9. **Protein structure determination**

Protein structure determination

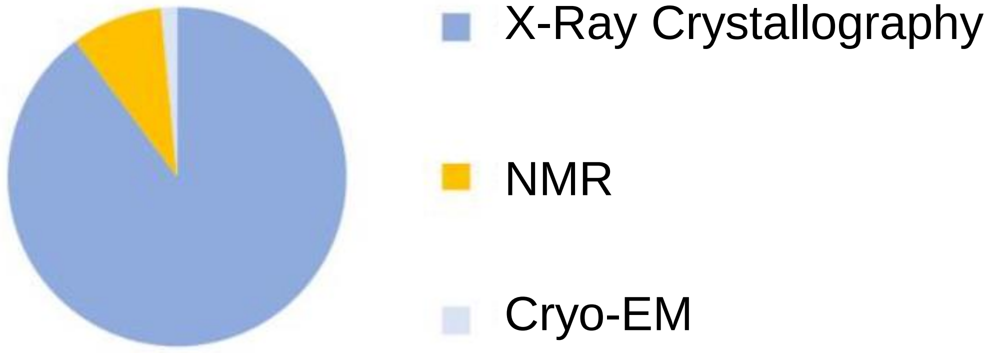
To understand the functions of proteins at a molecular level, it is often necessary to determine their three-dimensional structure!

- How proteins interacts with other molecules
- How they perform catalysis (in the case of enzymes)
- Miscoding and/or misfolding of proteins associated with diseases

https://www.slideshare.net/AmjadIbrahim/protein-structure-determination

Techniques for protein structure determination

- **X-Ray Crystallography**
- **Nuclear magnetic resonance (NMR)**
- **Cryo-Electron microscopy (Cryo-EM)**

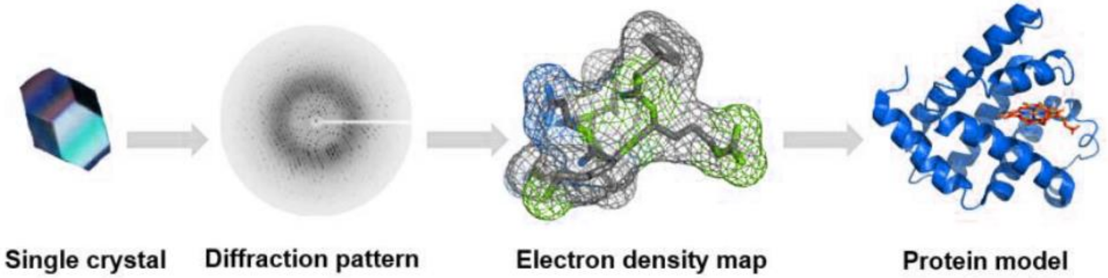


According to the statistics of PDB (https://www.rcsb.org/), more than 120,000 protein structures resolved by X-Ray crystallography, accounting for nearly 90% of the total.

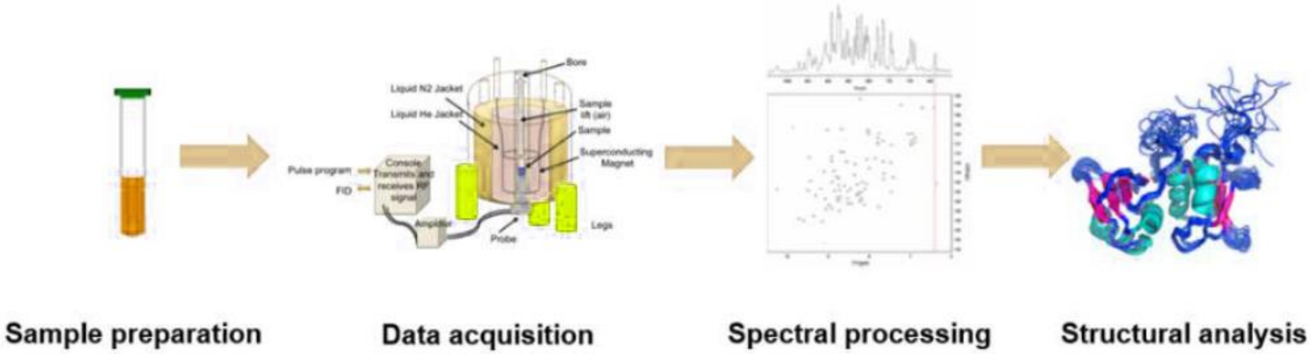
https://www.creative-biostructure.com/comparison-of-crystallography-nmr-and-em_6.htm

Techniques for protein structure determination

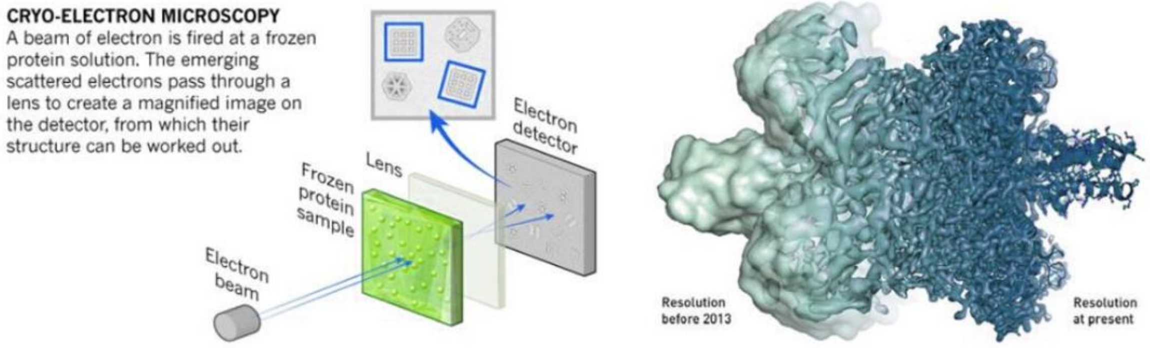
- **X-ray crystallography** uses X-ray to determine the position and arrangement of atoms in a crystal
- Based on this electron density map, the average position of atoms in the crystal, chemical bonds, and various information can be determined



- **NMR analysis** is performed on aqueous samples of protein with high purity, high stability, and high concentration
- NMR structure is calculated from magnetic properties of several nuclei



- The essential mechanism of **Cryo-EM** is electron scattering
- The coherent electrons are used as a light source to measure the sample
- After the electron beam passes through the sample, the lens system converts the scattered signal into a magnified image recorded on the detector
- Signal processing is performed to obtain the three-dimensional structure of the sample



CRYO-ELECTRON MICROSCOPY
A beam of electron is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, from which their structure can be worked out.

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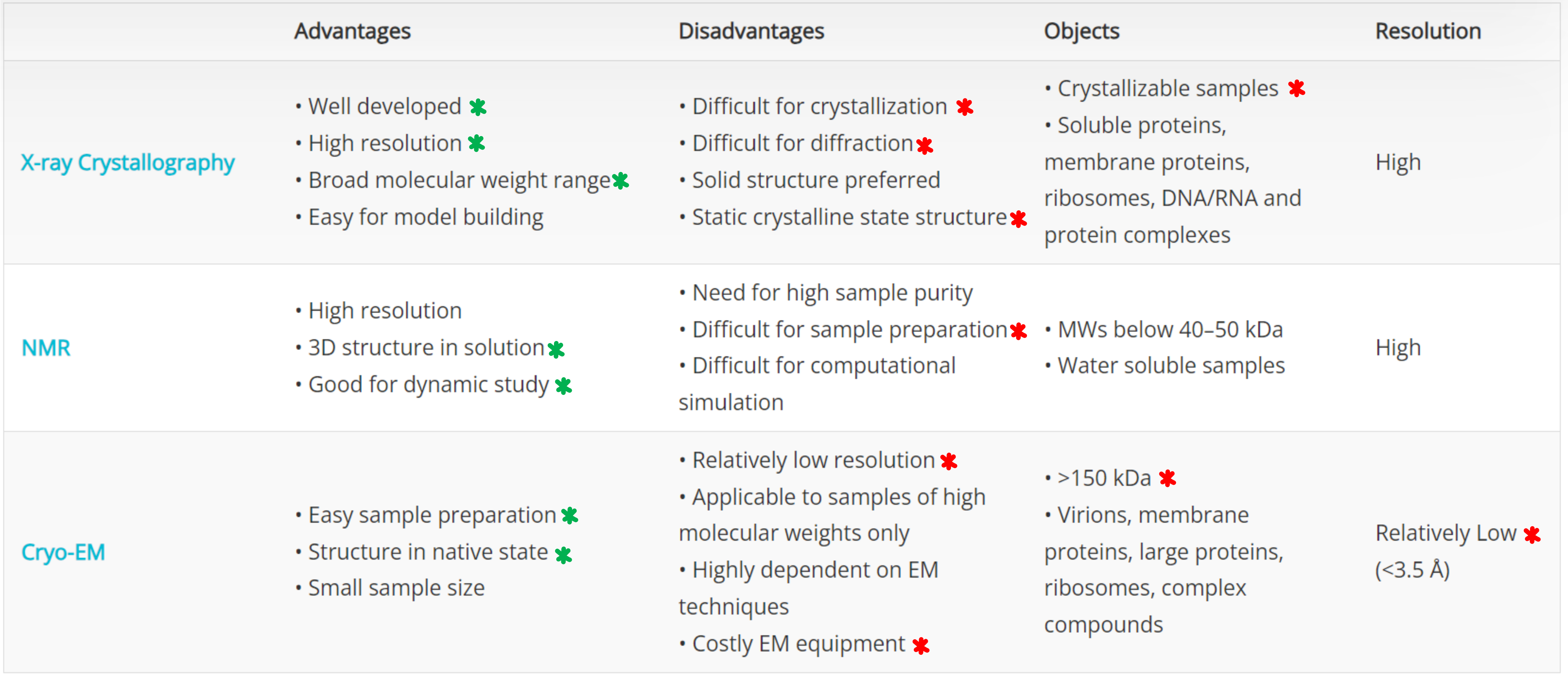
Techniques for protein structure determination



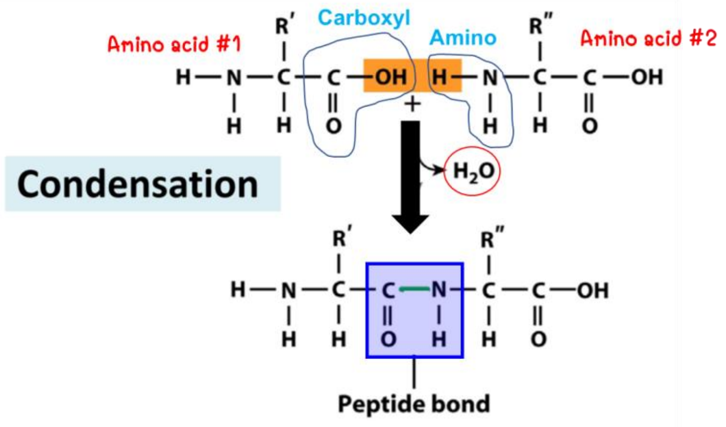
	Advantages	Disadvantages	Objects	Resolution
X-ray Crystallography	• Well developed ❇ • High resolution ❇ • Broad molecular weight range❇ • Easy for model building	• Difficult for crystallization ✱ • Difficult for diffraction✱ • Solid structure preferred • Static crystalline state structure✱	• Crystallizable samples ✱ • Soluble proteins, membrane proteins, ribosomes, DNA/RNA and protein complexes	High
NMR	• High resolution • 3D structure in solution❇ • Good for dynamic study ❇	• Need for high sample purity • Difficult for sample preparation✱ • Difficult for computational simulation	• MWs below 40–50 kDa • Water soluble samples	High
Cryo-EM	• Easy sample preparation❇ • Structure in native state ❇ • Small sample size	• Relatively low resolution✱ • Applicable to samples of high molecular weights only • Highly dependent on EM techniques • Costly EM equipment ✱	• >150 kDa ✱ • Virions, membrane proteins, large proteins, ribosomes, complex compounds	Relatively Low ✱ (<3.5 Å)

Table 1 The comparison of X-ray crystallography, NMR and Cryo-EM

https://www.creative-biostructure.com/comparison-of-crystallography-nmr-and-em_6.htm

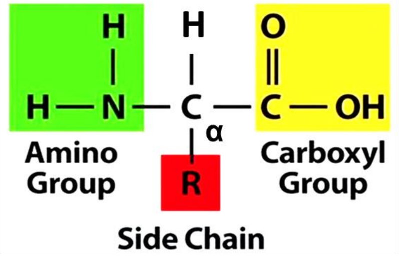
Summary

- When **2-20 amino acids** are joined by peptide bonds, the structure is called "**Oligopeptide**".
- When **>20 amino acids** are joined by peptide bonds,
  - MW <10 kDa → "**Polypeptide**"
  - MW >10 kDa → "**Protein**"



Structure of amino acid

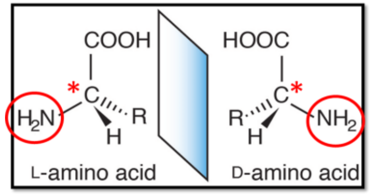
1. Alpha carbon (C_α)
2. Amino group
3. Carboxyl (Acid) group
4. R group (side chain)

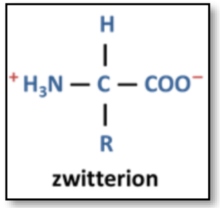


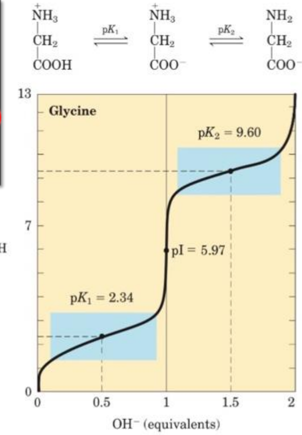
***R group determines the differences in each amino acid

Chemical properties of Amino Acids

1. Enantiomer (Mirror image)
2. Zwitterionic property (Dipolar +,-)
3. Amphoteric property (Acid+Base)
4. Buffer (maintain pH)





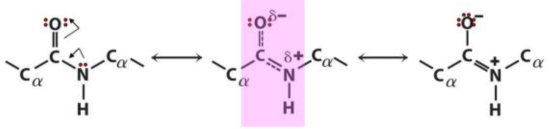


Classification of amino acids according to "R group"

1. **Non-polar, aliphatic R groups:** Ala, Val
2. **Polar, uncharged R groups:** Ser, Asn
3. **Positively charged R groups:** Lys, Arg
4. **Negatively charged R groups:** Asp, Glu
5. **Aromatic R groups:** Phe, Tyr, Trp

Chemical properties of peptide bonds

1. Partial double bond ($C \overset{\cdots}{-} N$) due to resonance in a structure
2. Planar
3. Rigid (unable to rotate freely)
4. Bond length: $C - N > C \overset{\cdots}{-} N > C \equiv N$
5. Bond strength: $C \equiv N > C \overset{\cdots}{-} N > C - N$



4 Levels of Protein Structure

1. **Primary structure**: the sequence of amino acids in a polypeptide chain

2. **Secondary structure**: the local folded structures that form within a polypeptide due to hydrogen bonding between **atoms of the backbone**

    2.1 Alpha helix

    2.2 Beta pleated sheet (Parallel, Antiparallel)

    2.3 Turn

3. **Tertiary structure**: the overall folding of the polypeptide chains due to interactions between **the R groups of the amino acid**

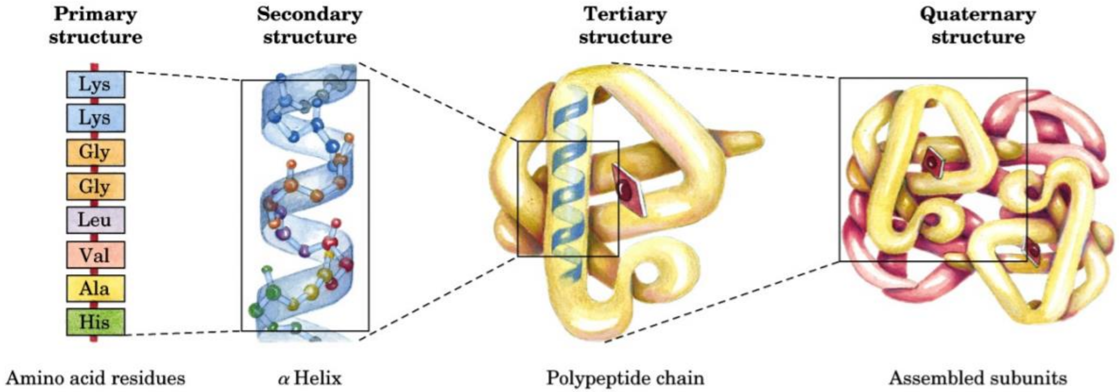
    3.1 Hydrophobic interaction

    3.2 Ionic bond

    3.3 Disulfide bond

    3.4 Hydrogen bond

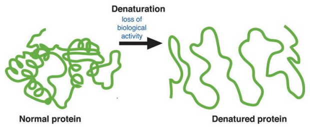
4. **Quaternary structure**: some proteins are made up of multiple polypeptide chains (subunit)



Functions of proteins

1. **Enzyme**: Amylase, Lipase, Protease
2. **Oxygen transport**: Hemoglobin
3. **Structural protein**: Collagen, Keratin
4. **Immunity**: Immunoglobulin
5. **Hormone**: Insulin, Glucagon
6. **Sensation**: T1R2/T1R3 sweet receptor
7. **Cell proliferation**: EGFR

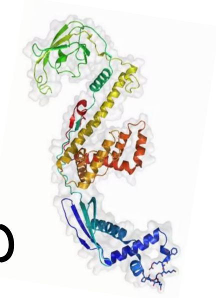
Protein denaturation



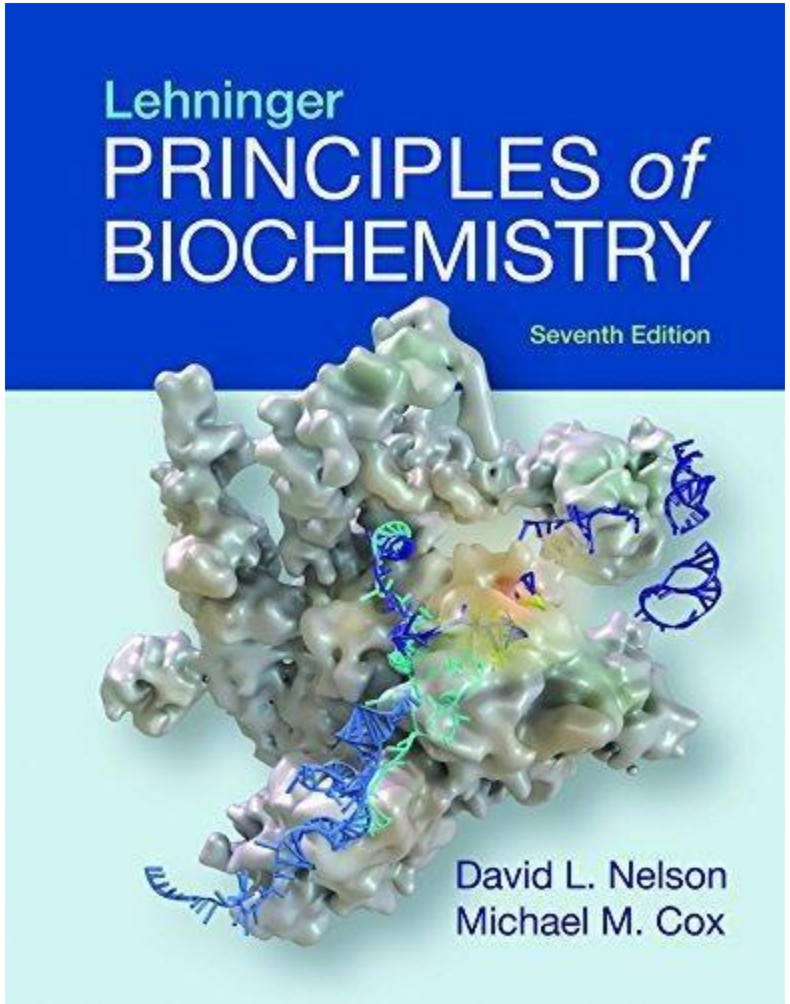
The process by which a protein undergoes the loss of its quaternary, tertiary, and secondary structures, becoming a primary structure (a long chain of amino acids) **without breaking the peptide bond**

Protein structure determination

- X-Ray Crystallography
- Nuclear magnetic resonance (NMR)
- Cryo-Electron microscopy (Cryo-EM)

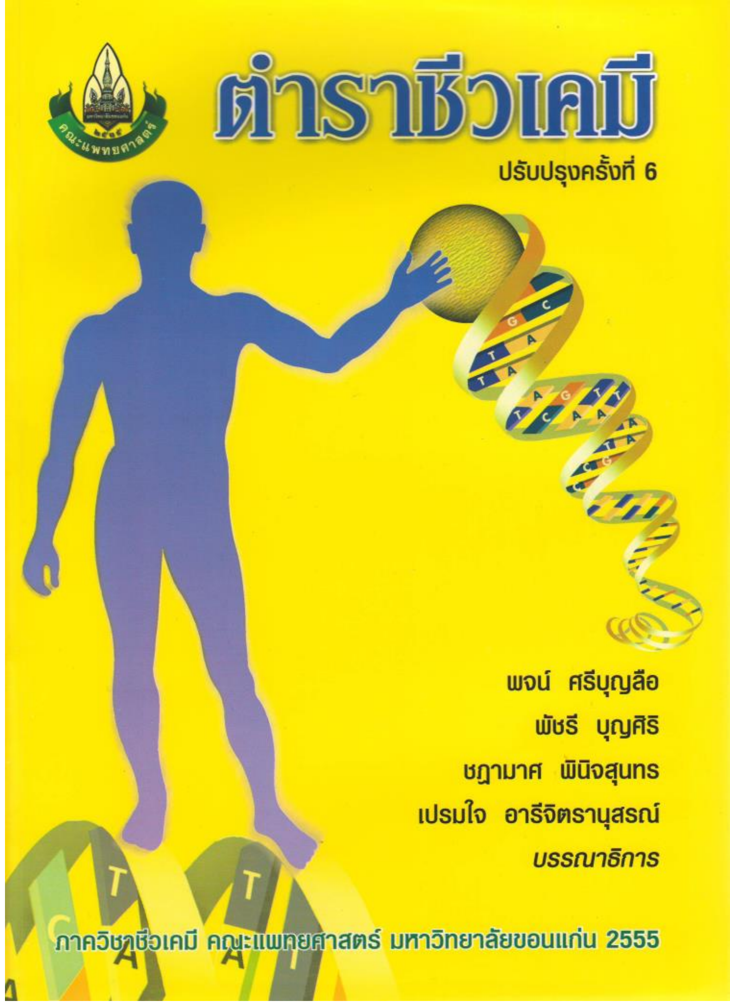


Suggested books



Lehninger
PRINCIPLES of BIOCHEMISTRY
Seventh Edition

David L. Nelson
Michael M. Cox



ตำราชีวเคมี
ปรับปรุงครั้งที่ 6

พจน์ ศรีบุญลือ
พัชรี บุญศิริ
ชฎามาศ พินิจสุนทร
เปรมใจ อารีจิตรานุสรณ์
บรรณาธิการ

ภาควิชาชีวเคมี คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น 2555

E-mail: panupma@kku.ac.th