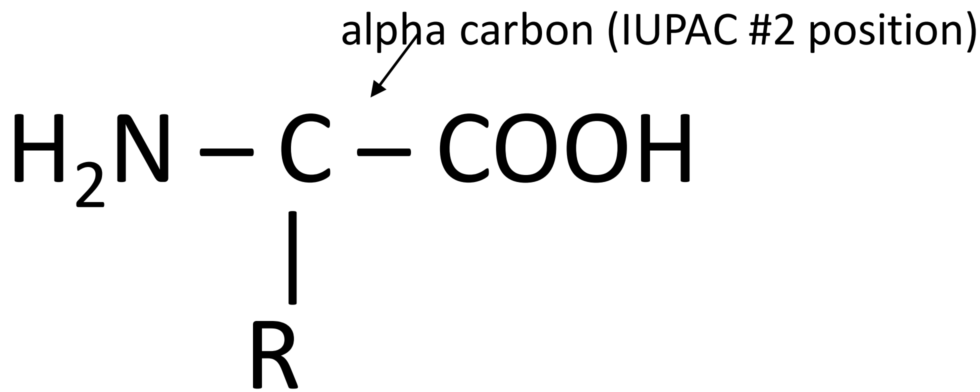
Amino Acids
&
Proteins

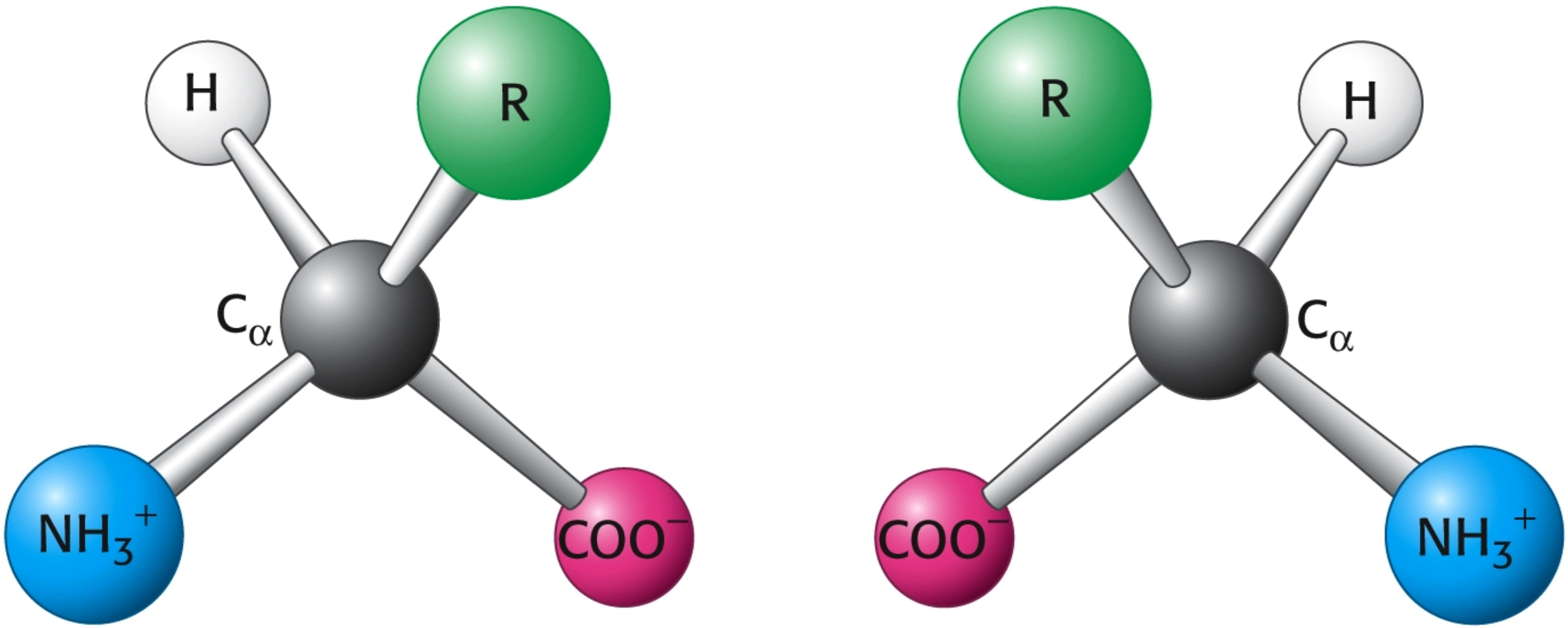
- Proteins are linear copolymers built from monomeric units called amino acids.
- Twenty amino acids are commonly found in proteins.
- These amino acids contain a variety of different functional groups:
  - Alcohols (R-OH)
  - Phenols (Ph-OH)
  - Carboxylic acids (R-COOH)
  - Thiols (R-SH)
  - Amines ($R\text{-}NH_2$)
  - *and others…*

- Protein function depends on both
  - amino acid content, and
  - amino acid sequence.
- Protein fold into diverse shapes such as
  - spherical
  - elipsoidal
  - long strands, etc.
- All information for 3-D structure is contained in the <u>linear sequence</u> of amino acids.

- To understand protein function, we must first understand the nature of amino acids.
- Amino acids are essentially α-amino acids:



- When R is not H, the alpha carbon is asymetric, giving rise to isomers.



L isomer **D isomer**

Only L-amino acids are constituents of proteins.

- Carboxylic acids are traditional Bronsted-Lowery acids, donating a proton in aqueous solution.
- The pKa for caroboxylic acids is normally around 2 to 5. That is, the pH at which these acids are 50% ionized:

$$R\text{-}COOH \rightarrow R\text{-}COO^{-} + H^{+}$$

pH= [less than 2] → [above 5]

- Amino groups function as bases, accepting a proton.
- The pKa for amino groups is usually around 9 – 10. Again, at the pKa these groups are 50% ionized:

$$R\text{-}NH_3^+ \rightarrow R\text{-}NH_2 + H^+$$

pH= [below 8] → [above 9]

- Even though both acids and amines are present in the same molecule, they mostly behave as though they were separate entities:

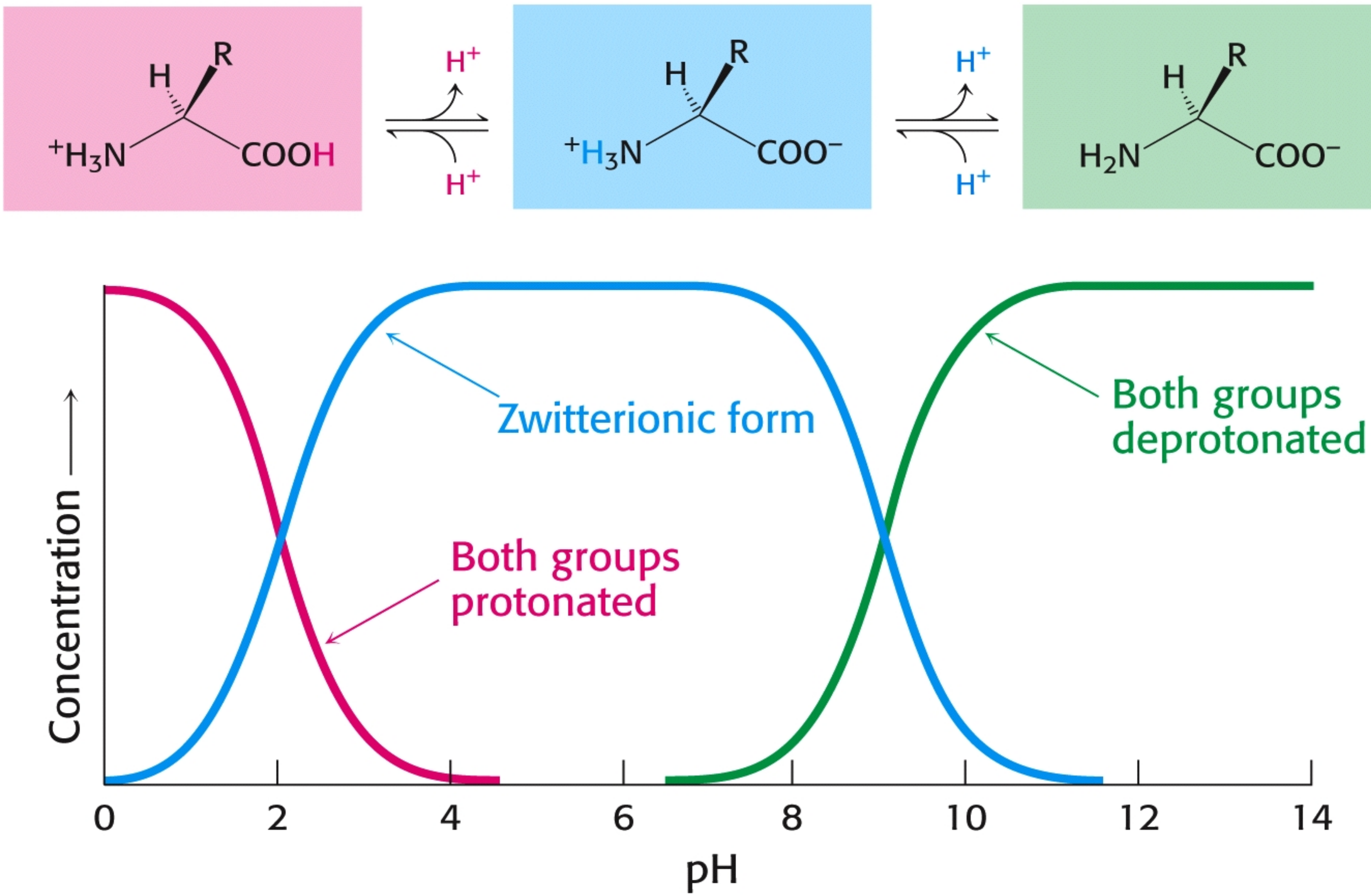


TABLE 3.4 **pK_a values of some amino acids**

Amino acid	pK_a values (25°C) α-COOH group	α-NH_3^+ group	Side chain
Alanine	2.3	9.9	
Glycine	2.4	9.8	
Phenylalanine	1.8	9.1	
Serine	2.1	9.2	
Valine	2.3	9.6	
Aspartic acid	2.0	10.0	3.9
Glutamic acid	2.2	9.7	4.3
Histidine	1.8	9.2	6.0
Cysteine	1.8	10.8	8.3
Tyrosine	2.2	9.1	10.9
Lysine	2.2	9.2	10.8
Arginine	1.8	9.0	12.5

After J. T. Edsall and J. Wyman, *Biophysical Chemistry* (Academic Press, 1958), Chapter 8.

- Summary:

At low pH, proton concentration [H+]is high. Therefore, both amines and carboxylic acids are protonated. ($-NH_3^+$ & $-COOH$)

At high pH, proton concentration is low. Therefore, both amines and carboxylic acids are deprotonated. ($-NH_2$ & $-COO^-$)

At neutral pH, amines are protonated($-NH_3^+$) and carboxylates are deprotonated($-COO^-$)

- “Zwitter” Ions:
- Ions bearing two charges were named zwitter ions by German scientists; the name still applies today, especially for amino acids at neutral pH:

$$^{+}H_3N - CH_2 - COO^{-}$$

Acid-Base Properties of Amino Acids

Draw the following chemical structures for glycine:

(Non-existent form:) **H_2N – CH_2 - COOH**

pH=1:

pH=7:

pH=12:

Acid-Base Properties of Amino Acids

Draw the following chemical structures for glycine:

(Non-existent form:) $H_2N - CH_2 - COOH$

pH=1: $^{+}H_3N - CH_2 - COOH$

pH=7:

pH=12:

Acid-Base Properties of Amino Acids

Draw the following chemical structures for glycine:

(Non-existent form:) $H_2N - CH_2 - COOH$

pH=1: $^{+}H_3N - CH_2 - COOH$

pH=7: $^{+}H_3N - CH_2 - COO^{-}$

pH=12:

Acid-Base Properties of Amino Acids

Draw the following chemical structures for glycine:

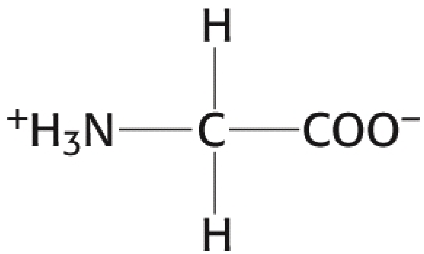
(Non-existent form:) $H_2N – CH_2 - COOH$

pH=1: $^{+}H_3N – CH_2 - COOH$

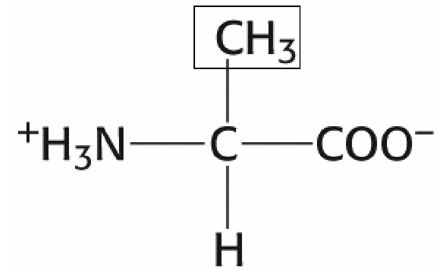
pH=7: $^{+}H_3N – CH_2 – COO^{-}$

pH=12: $H_2N – CH_2 – COO^{-}$

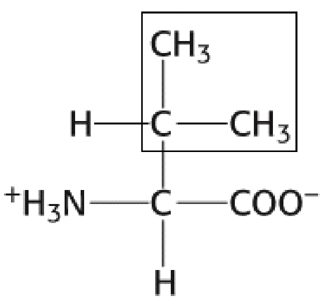
Amino *tic*)



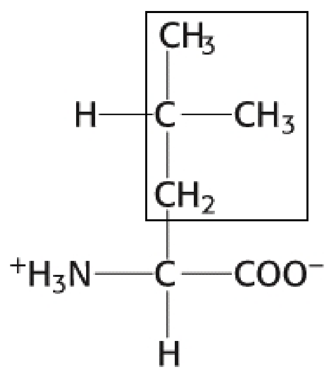
Glycine
(Gly, G)



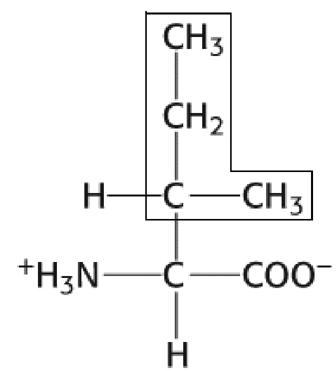
Alanine
(Ala, A)



Valine
(Val, V)



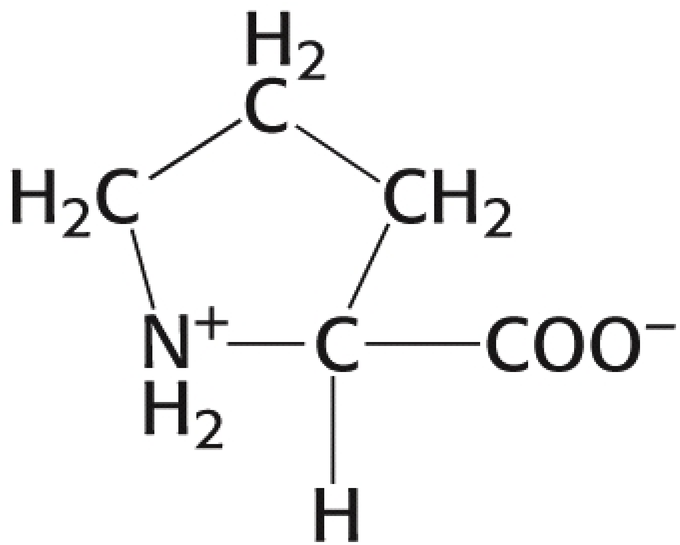
Leucine
(Leu, L)



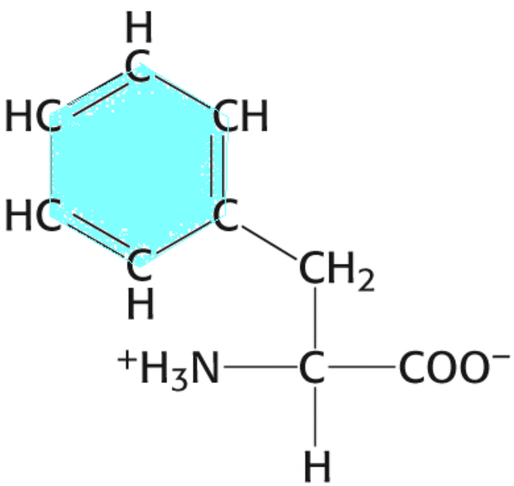
Isoleucine
(Ile, I)

- Amino acid Proline

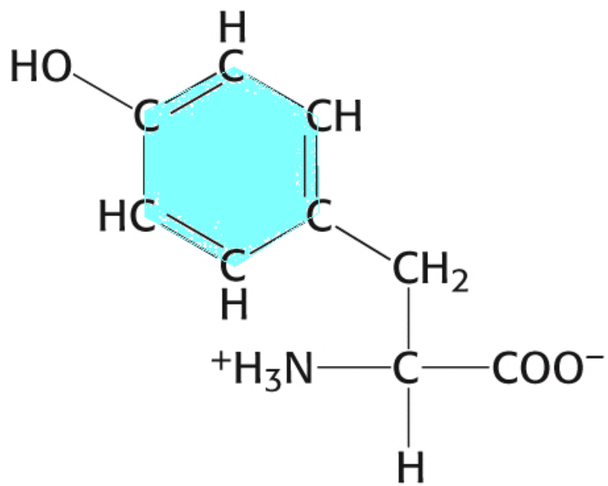
(The only se … *imino” acid.)*



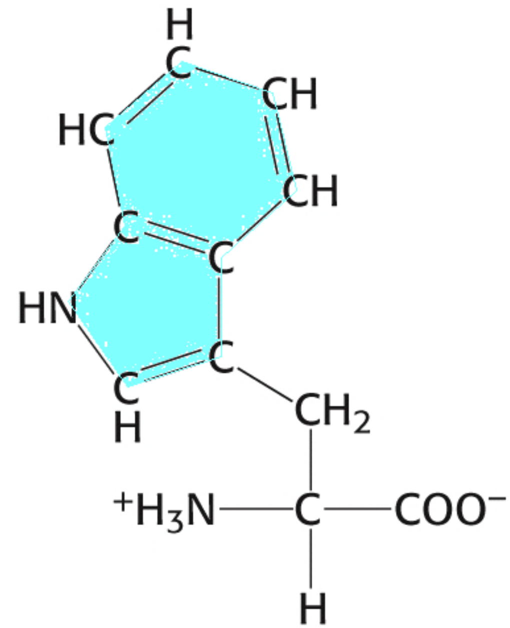
Proline
(Pro, P)



Phenylalanine
(Phe, F)

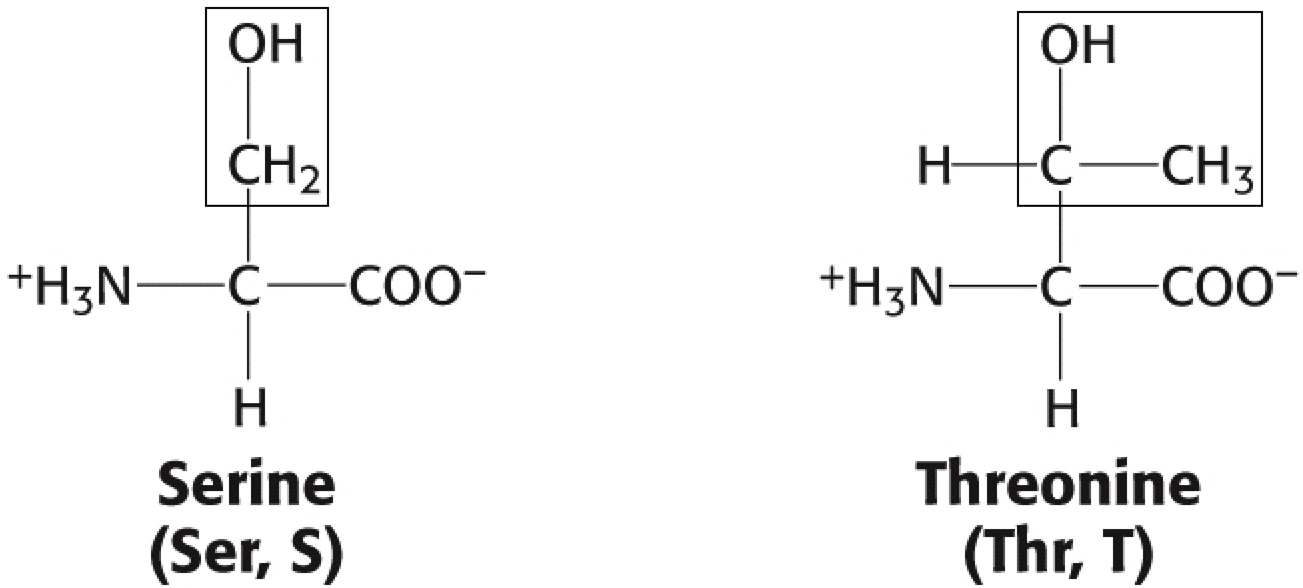


Tyrosine
(Tyr, Y)



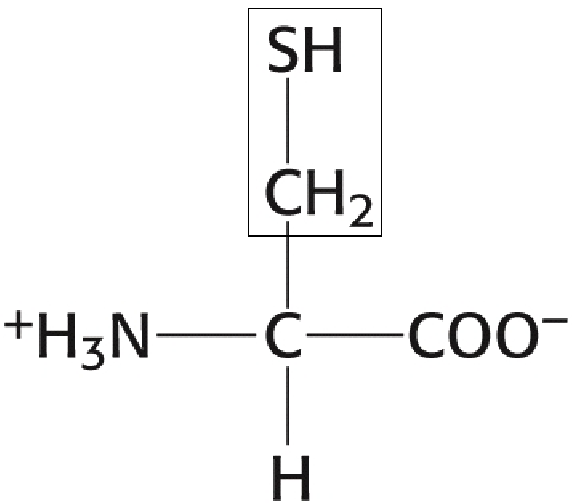
Tryptophan
(Trp, W)

- A

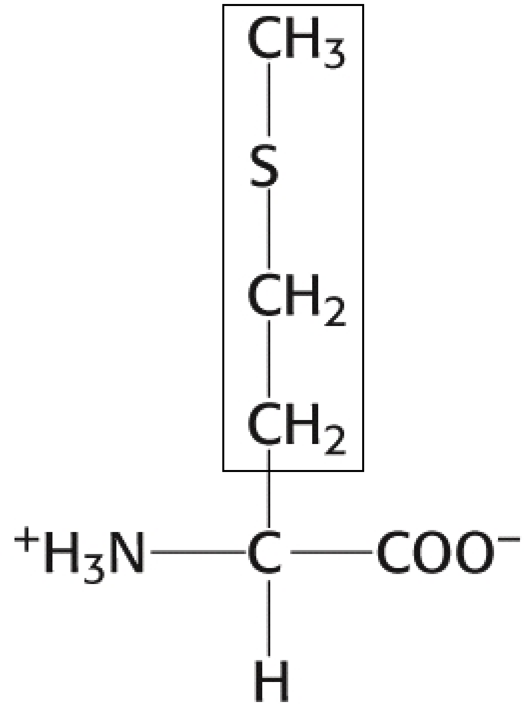


OH
CH2
+H3N
C
COO−
H
Serine
(Ser, S)
OH
H
C
CH3
+H3N
C
COO−
H
Threonine
(Thr, T)

- Amino acids (*Sulfur*)

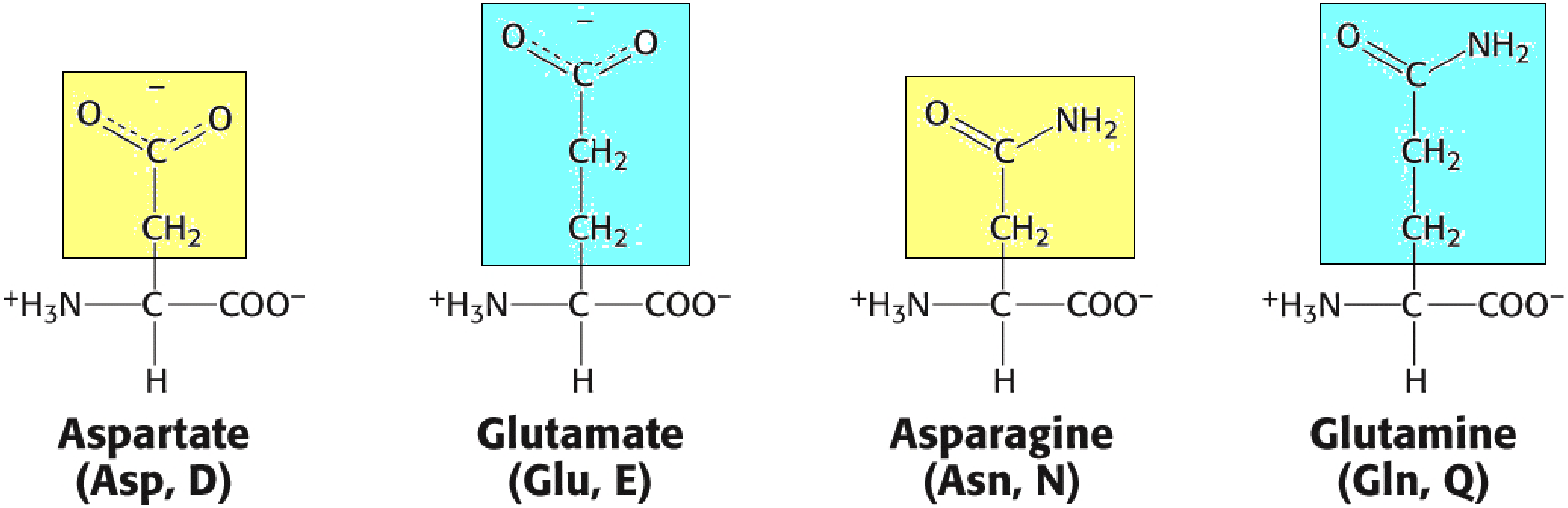


Cysteine (Cys, C)

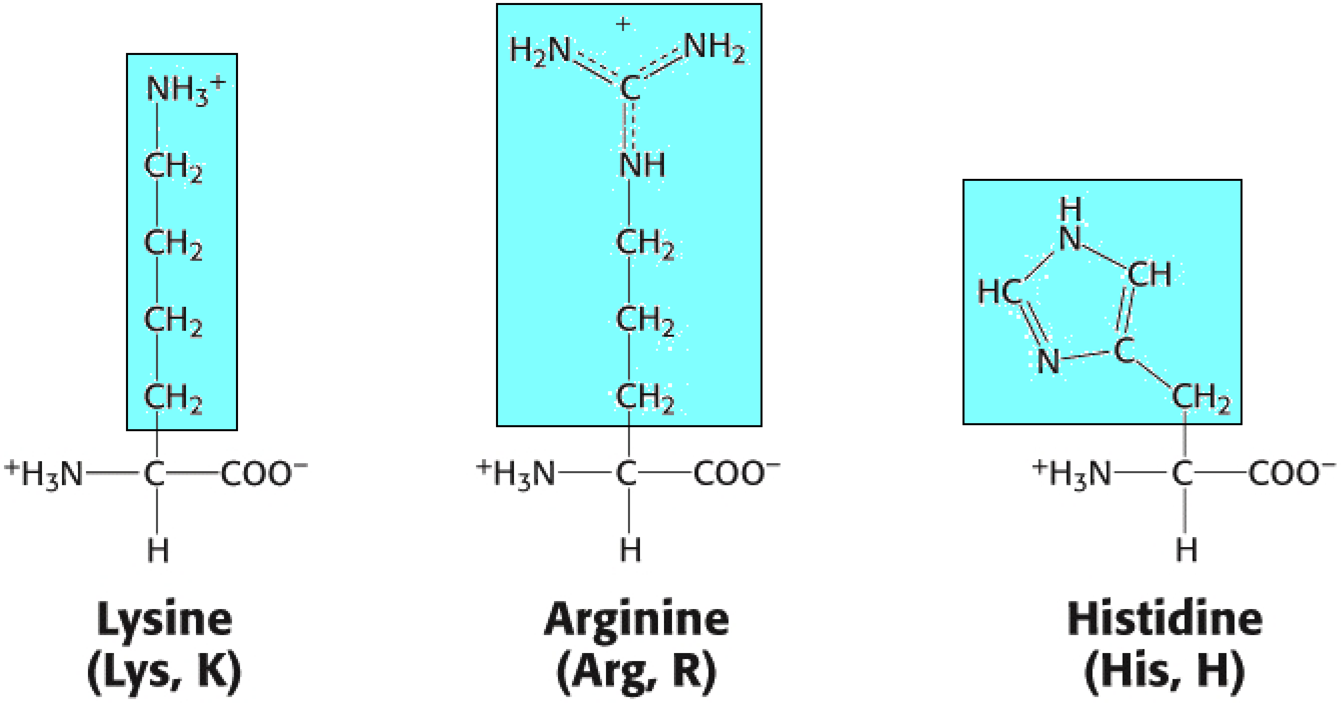


Methionine (Met, M)

- Amino acids (*Acids and related amides*)



- A



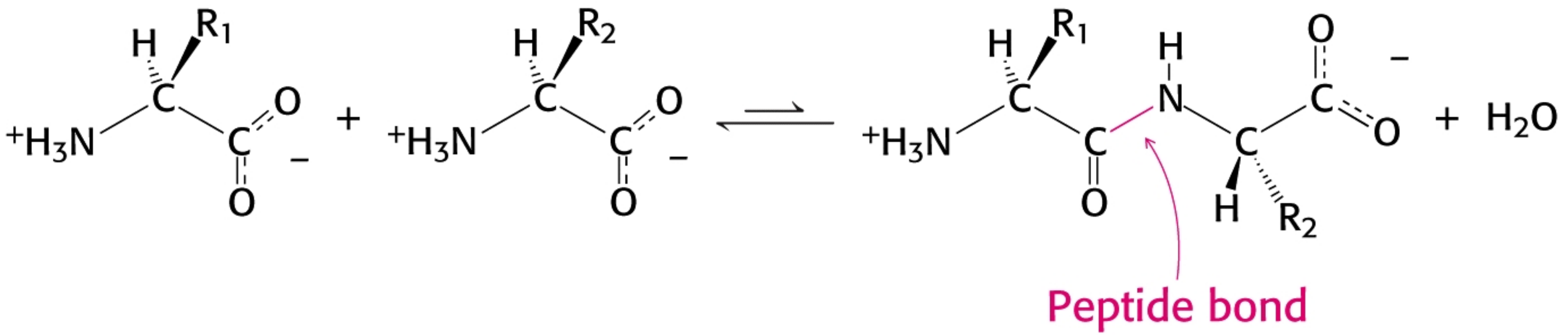
NH_3^+
CH_2
H_2N
NH_2
C
NH
H
N
CH
HC
N
C
CH_2
^+H_3N
C
COO^-
H
Lysine
(Lys, K)
Arginine
(Arg, R)
Histidine
(His, H)

TABLE 3.2 Abbreviations for amino acids

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

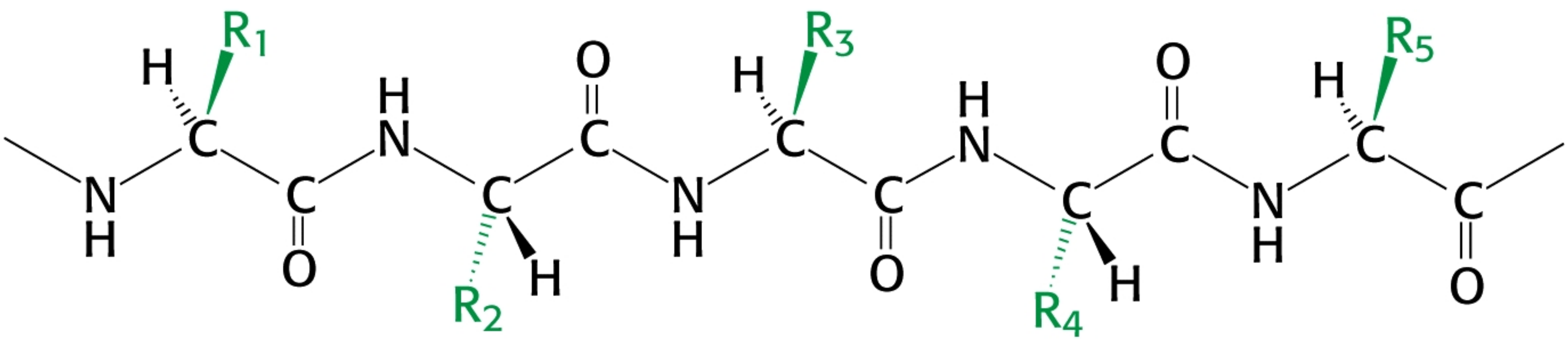
Essential Amino Acids:
Isoleucine
Leucine
Lysine
Methionine
Phenylalanine [a]
Threonine
Tryptophan [a]
Valine
Arginine [b]
Histidine [b]
[a] *Aromatic* [b] *Probably essential*

- Amino acids are polymerized via amide or "peptide" bonds:



- Copolymer of amino acids:

a “polypeptide”

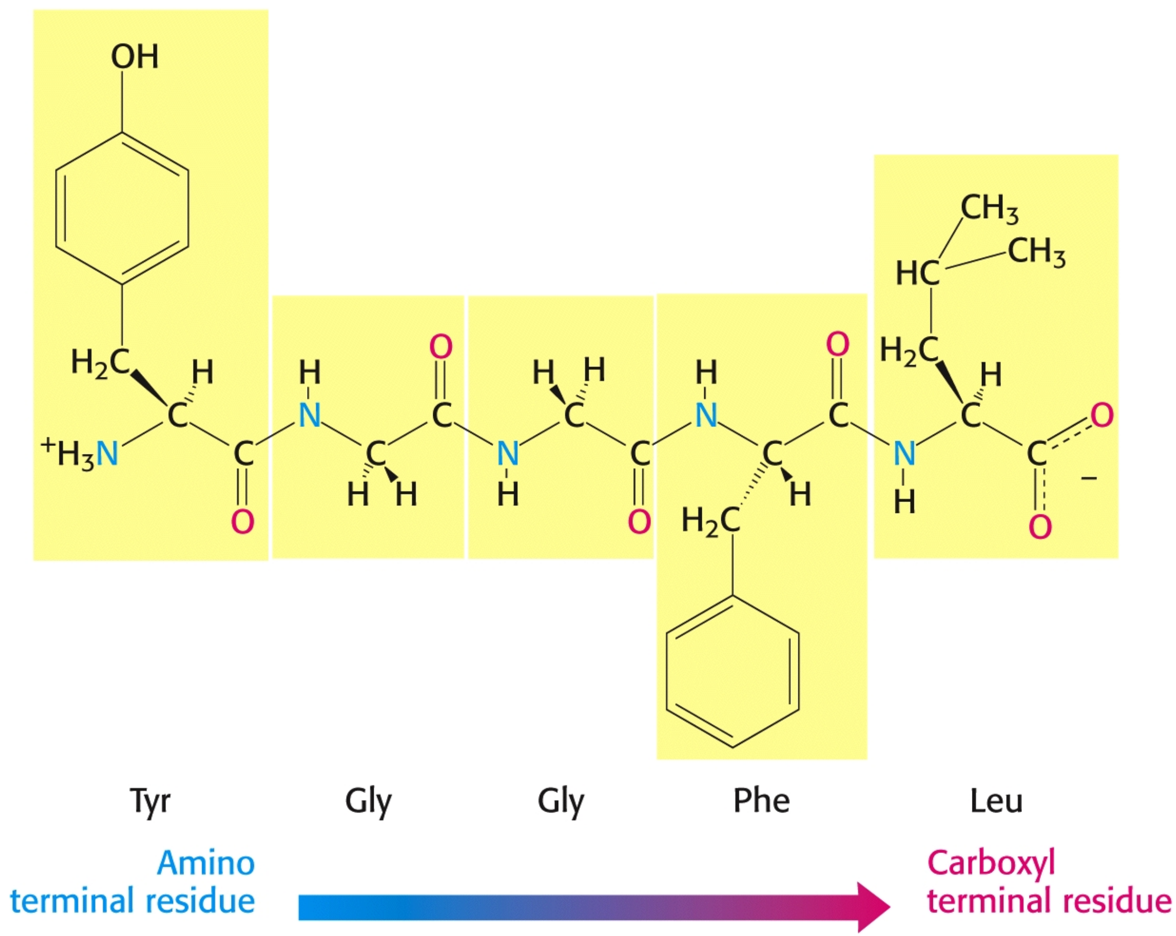


Definition:

Amino acid polymers of ≤50 amino acids are called

*“**polypeptides, peptides, oligopeptides, etc**.”*

*Amino acids polymer of >50 amino acids are called “**proteins**.”*



OH
H_2C
H
^+H_3N
C
C
O
H
N
C
H H
O
C
H H
C
N
H
C
O
H
N
C
H
H_2C
O
C
CH_3
CH_3
HC
H_2C
H
C
N
H
C
O
O
–
Tyr
Gly
Gly
Phe
Leu
Amino
terminal residue
Carboxyl
terminal residue

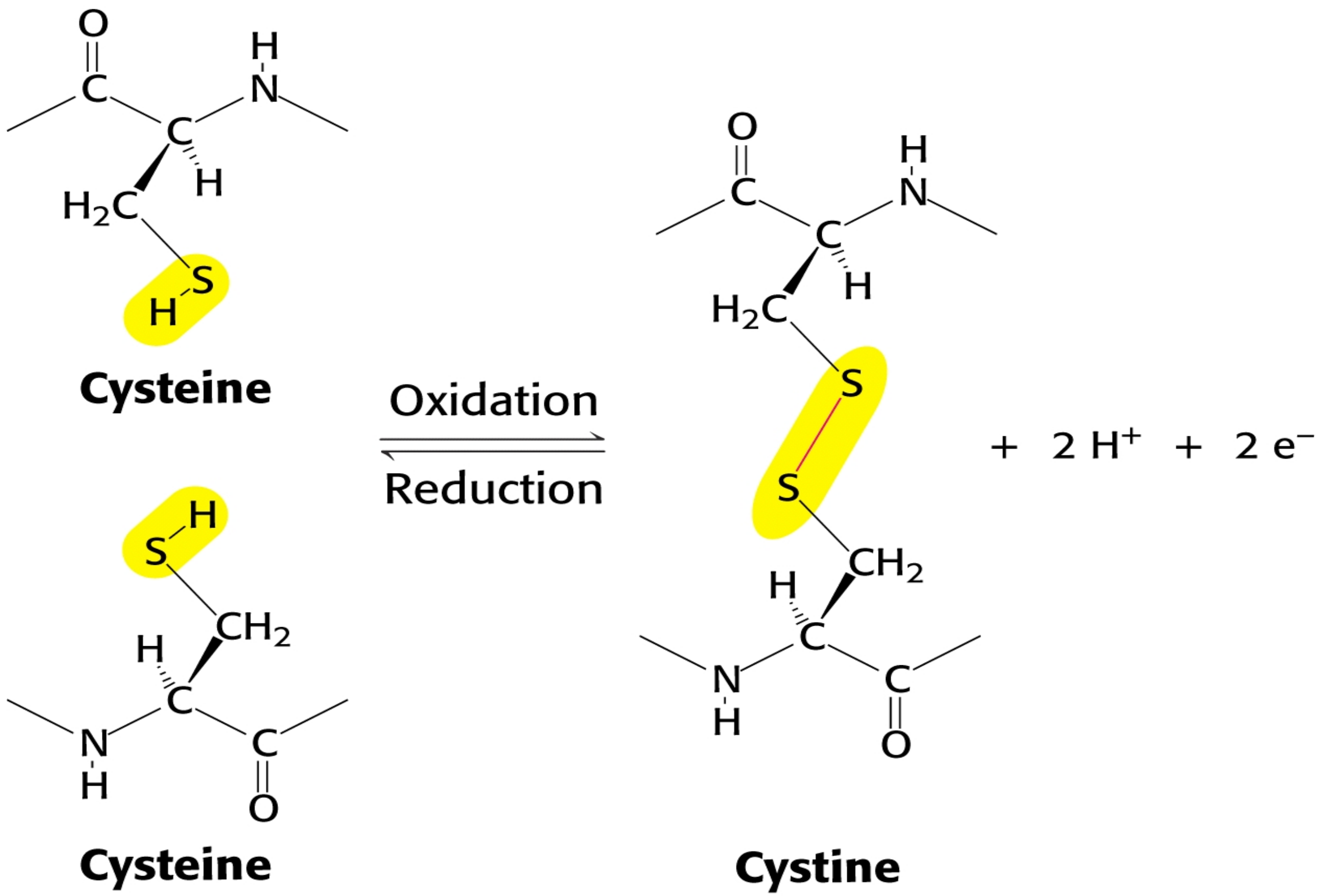
- An example of a “dipeptide” is the sweetener *Aspartame.*
- Other names include:
  - NutraSweet
  - Equal
  - Tri-Sweet
  - Sanecta
- IUPAC Name:

“N-*L- α – Aspartyl-L-phenylalanine 1-methyl ester”*

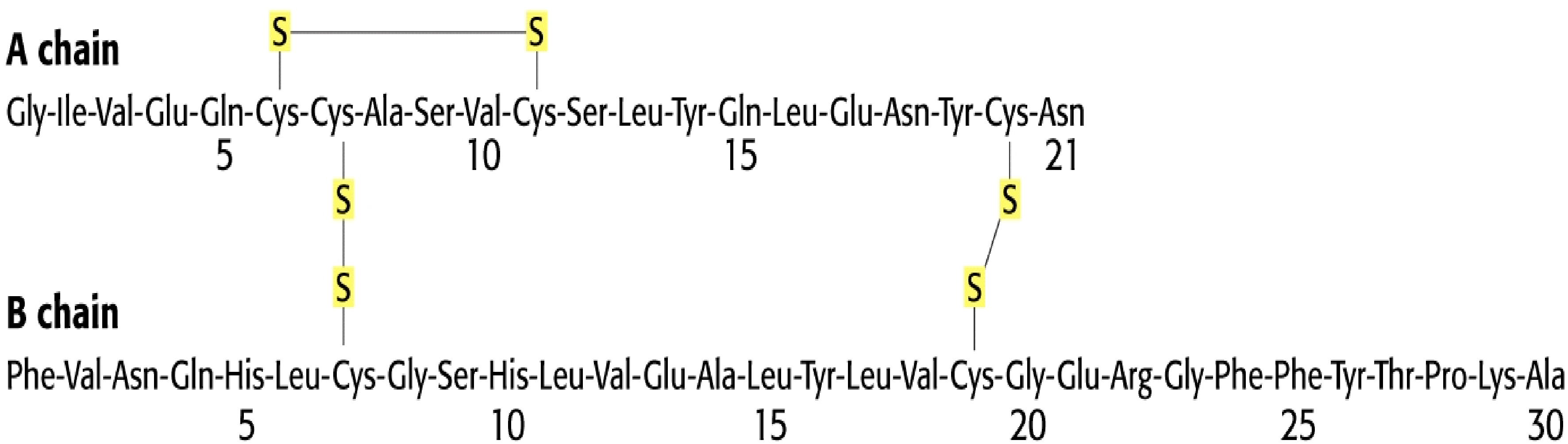
Asp – Phe – OCH3

Abbreviated Structure:

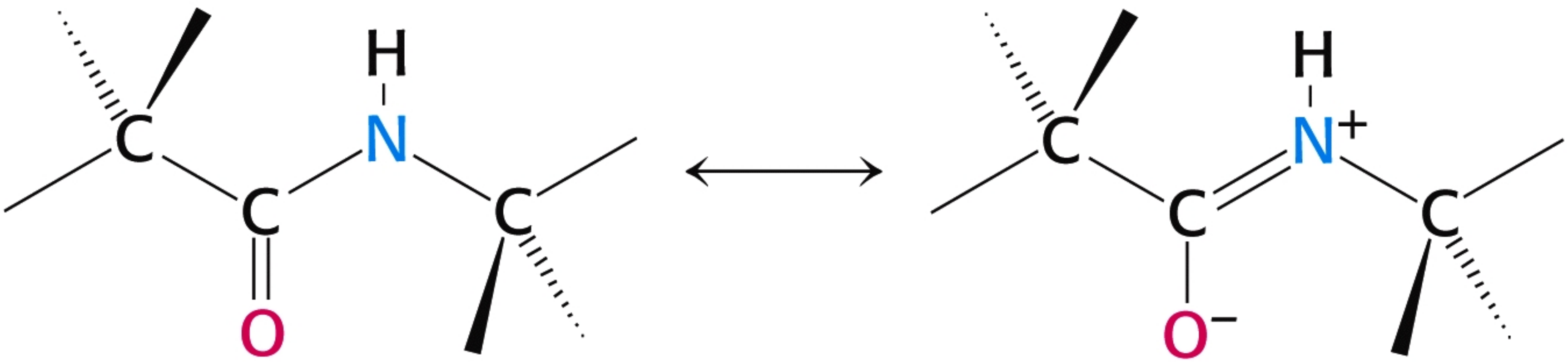
- Cross links between peptide chains:
  - Disulfide linkages between individual "**cysteines**" are called "**cystines:**"



- Insulin is the smallest protein, with 51 amino acids in two chains linked by cystine (disulfide)



- Peptide bonds have *partial* double bond character due to resonance that limits

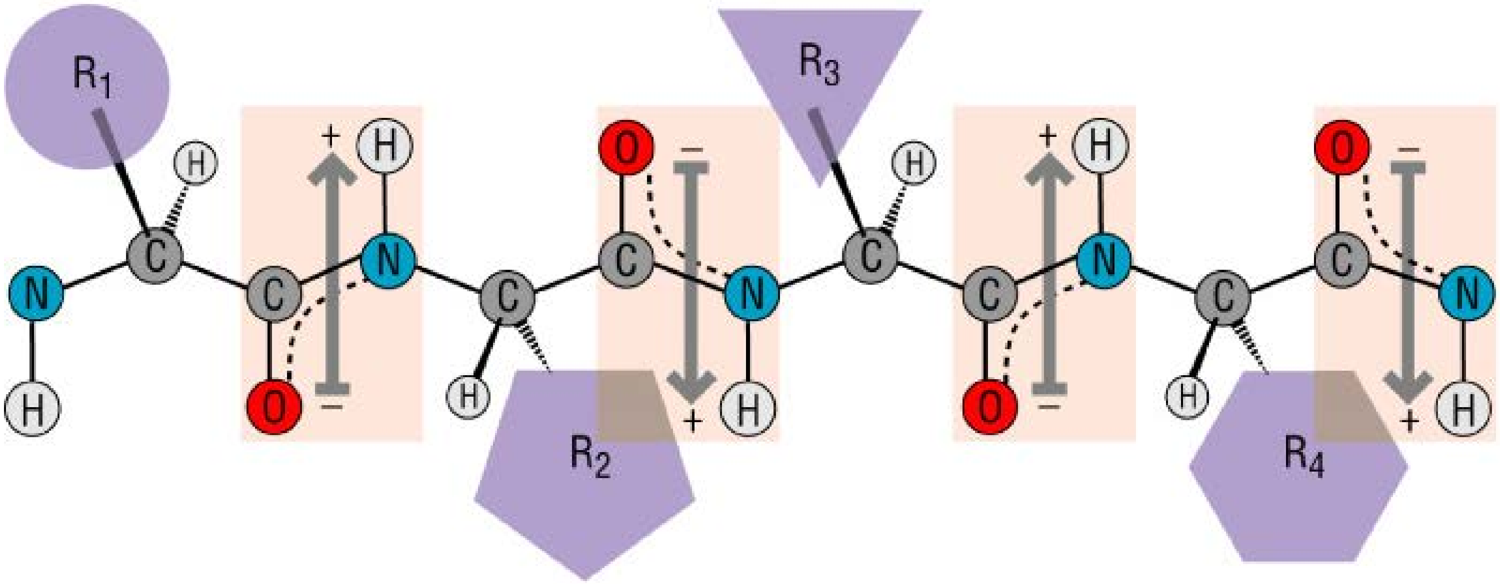


Peptide bond resonance structures

Levels of Protein Structure

- **Primary (1°) Protein Structure**
  - linear sequence of amino acids.
- **Secondary (2°) Protein Structure**
  - localized regional structures
- **Teritary (3°) Protein Structure**
  - overal shape of proteins
- **Quaternary (4°) Protein Structure**
  - interactions between proteins

Primary Structure

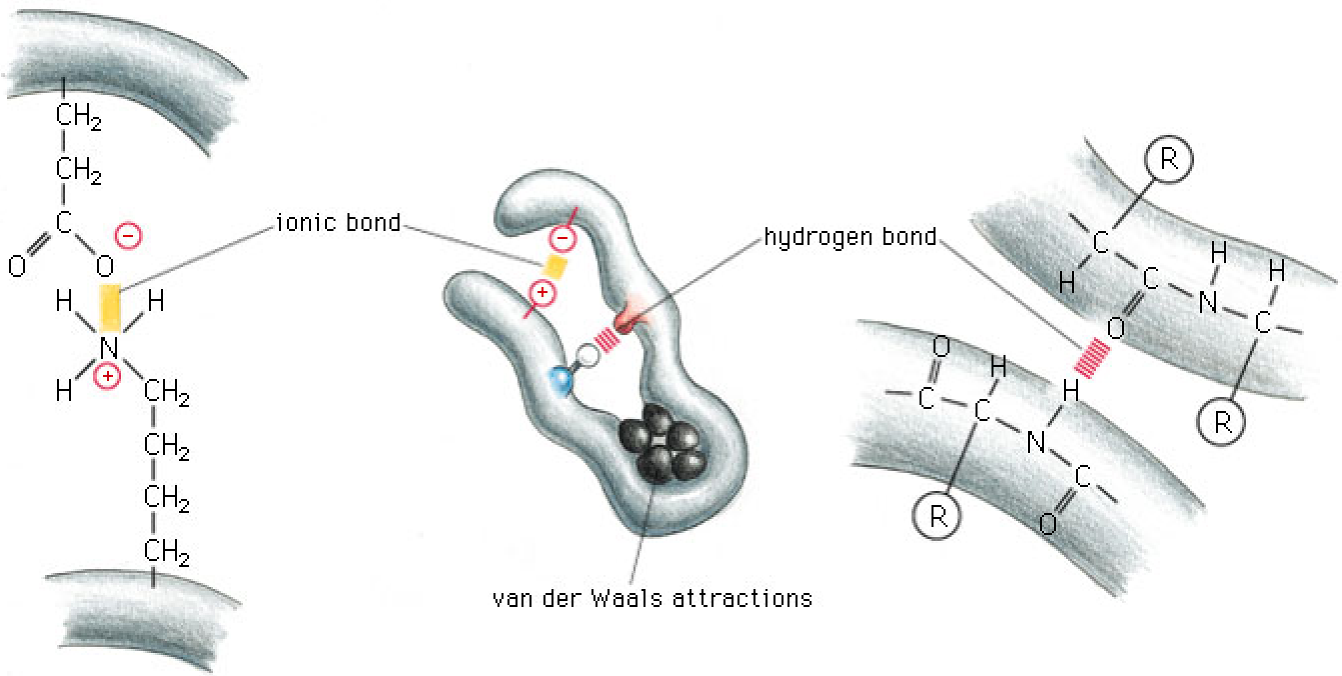


Linear polypeptide where amino acid residues linked via peptide bond

Protein Structure:

- Twisting about various bonds in the polypeptide backbone gives proteins a variety of shapes.
- **The peptide bond allows for rotation around it and therefore the protein can fold and orient the R groups in favorable positions**
- **Weak non-covalent interactions** will hold the protein in its functional shape – these are weak and will take many to hold the shape

Non-covalent Bonds in Proteins

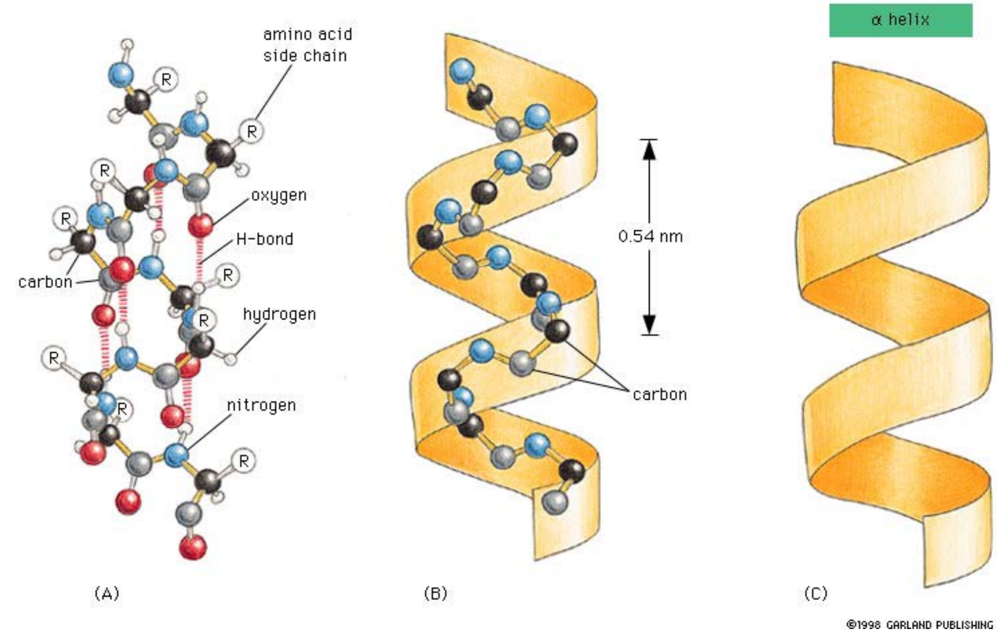


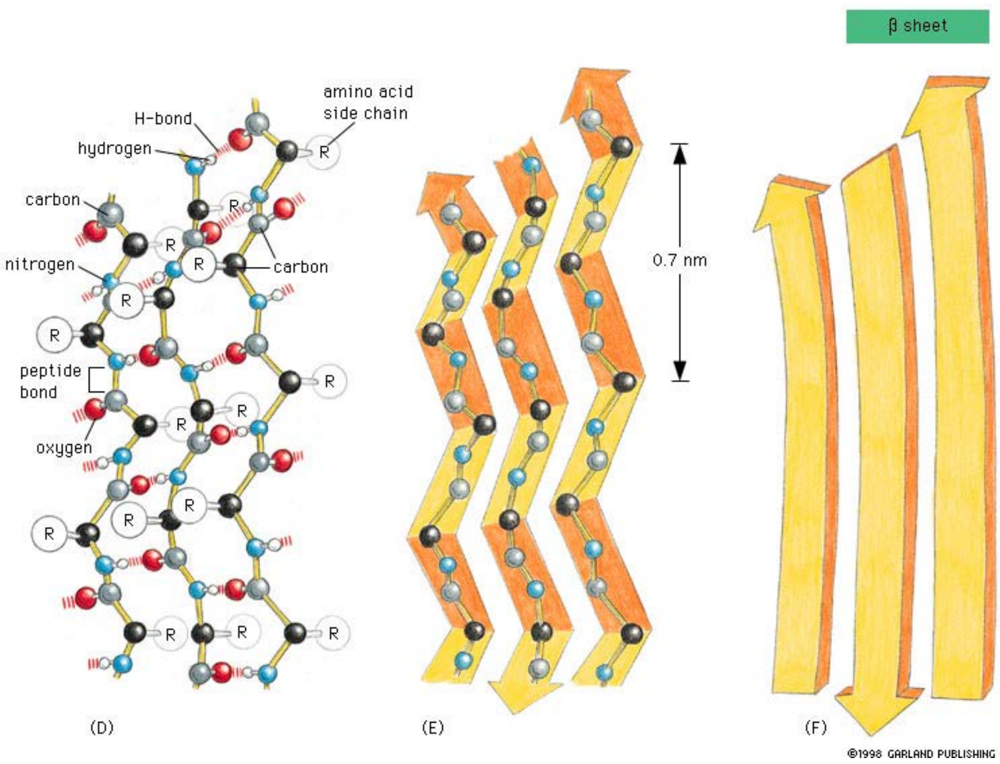
Secondary Structure in Proteins

- Pauling and Corey proposed two secondary structures in proteins many years before they were actually proven:

alpha – helix　　　　*beta - sheet*

Both of these secondary protein structures are stabilized by hydrogen bonding between the carbonyl oxygen atoms and the nitrogen atoms of amino acids in the protein chain.





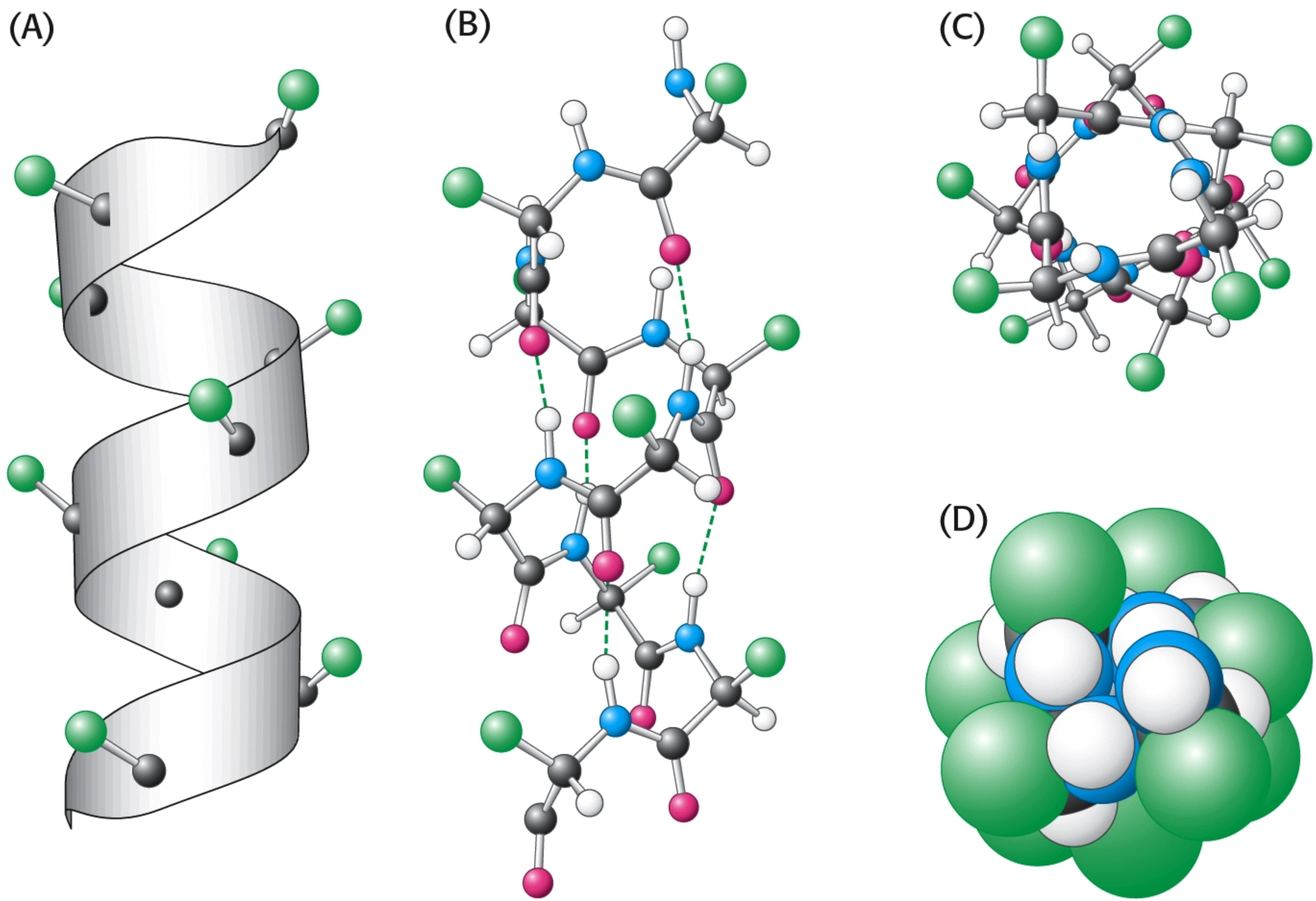
Protein Folding

- 2 regular folding patterns have been identified – formed between the bonds of the peptide backbone

- **α-helix** – protein turns like a spiral – fibrous proteins (hair, nails, horns)

- **β-sheet** – protein folds back on itself as in a ribbon – globular protein

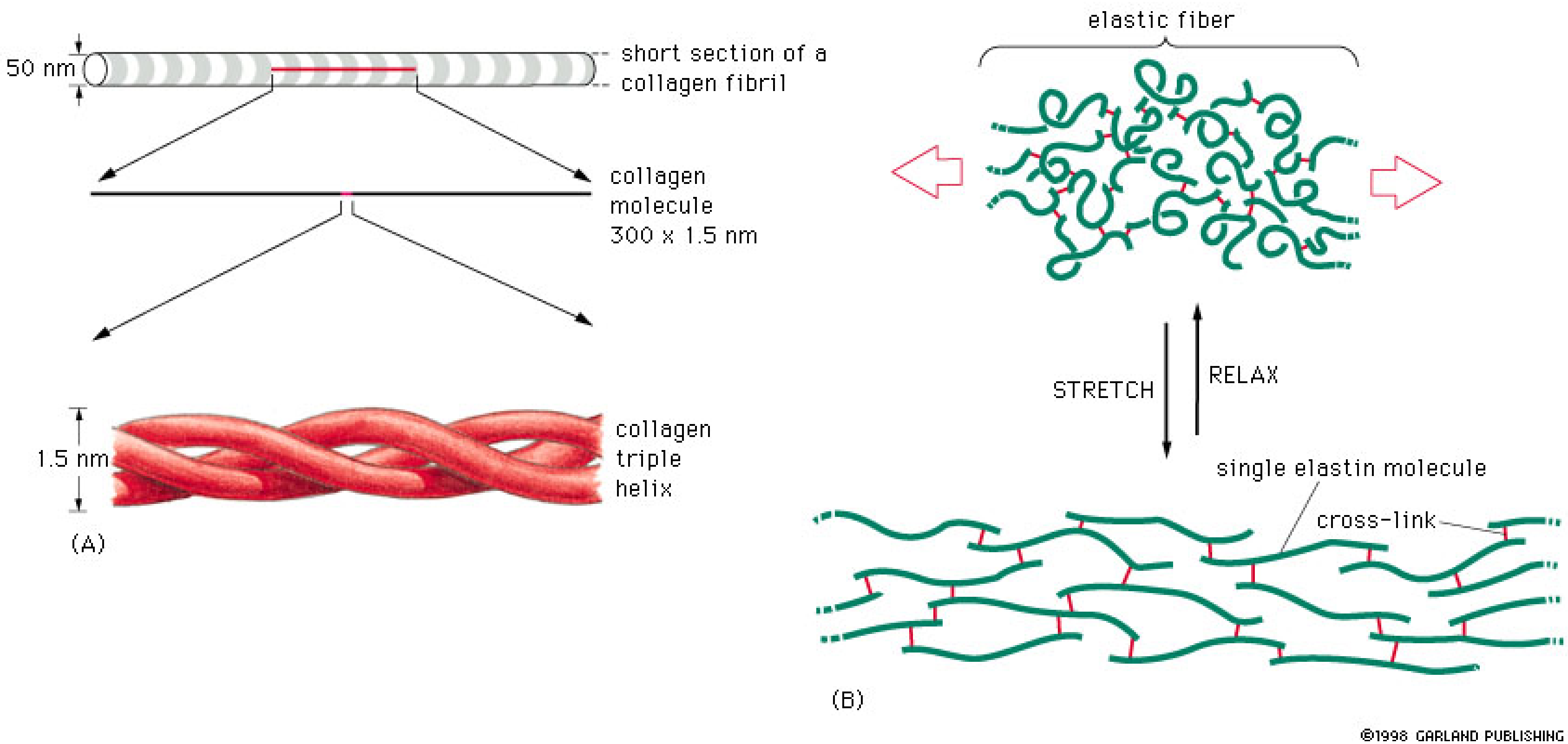
The *alpha* (α) – *helix*



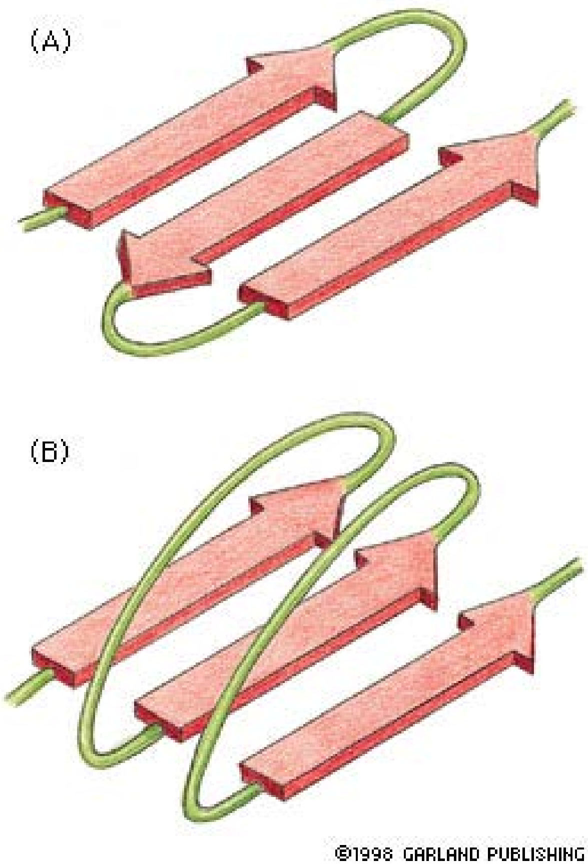
Important Fibrous Proteins

- Intermediate filaments of the cytoskeleton
  - Structural scaffold inside the cell
    - Keratin in hair, horns and nails
- Extracellular matrix
  - Bind cells together to make tissues
  - Secreted from cells and assemble in long fibers
    - Collagen – fiber with a glycine every third amino acid in the protein
    - Elastin – unstructured fibers that gives tissue an elastic characteristic

Collagen and Elastin



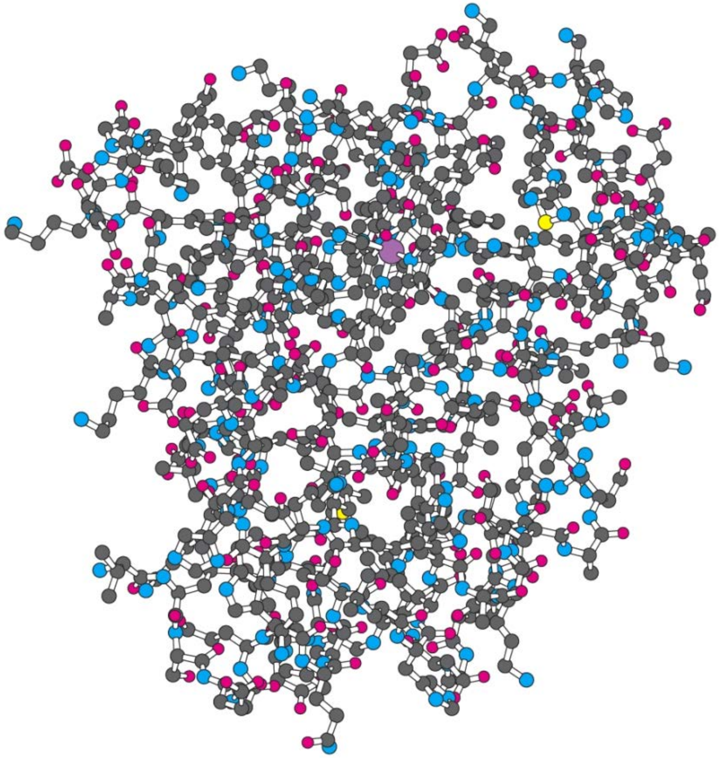
β Sheets



- Core of many proteins is the β sheet
- Form rigid structures with the H-bond
- Can be of 2 types
  - Anti-parallel – run in an opposite direction of its neighbor (A)
  - Parallel – run in the same direction with longer looping sections between them (B)

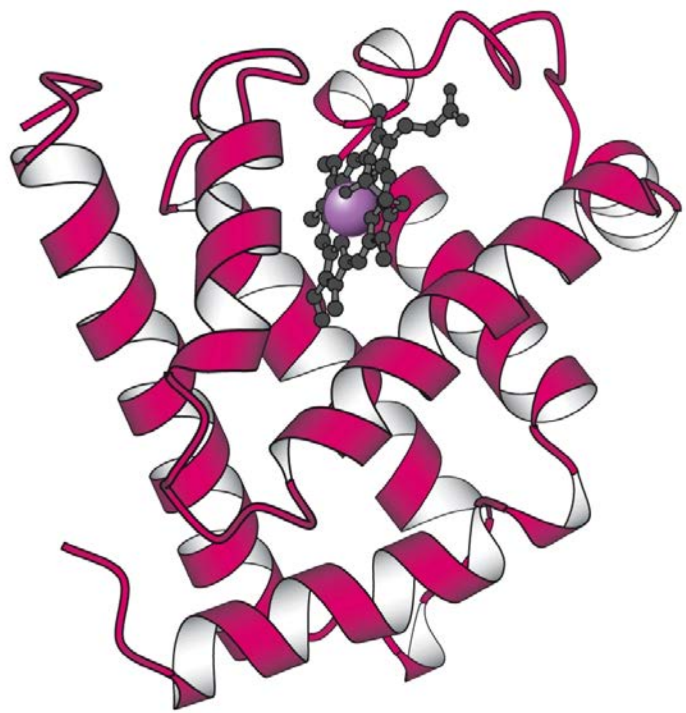
Tertiary (3°) Structure of Protein

- Describes all aspects of the three-dimensional folding of a polypeptide

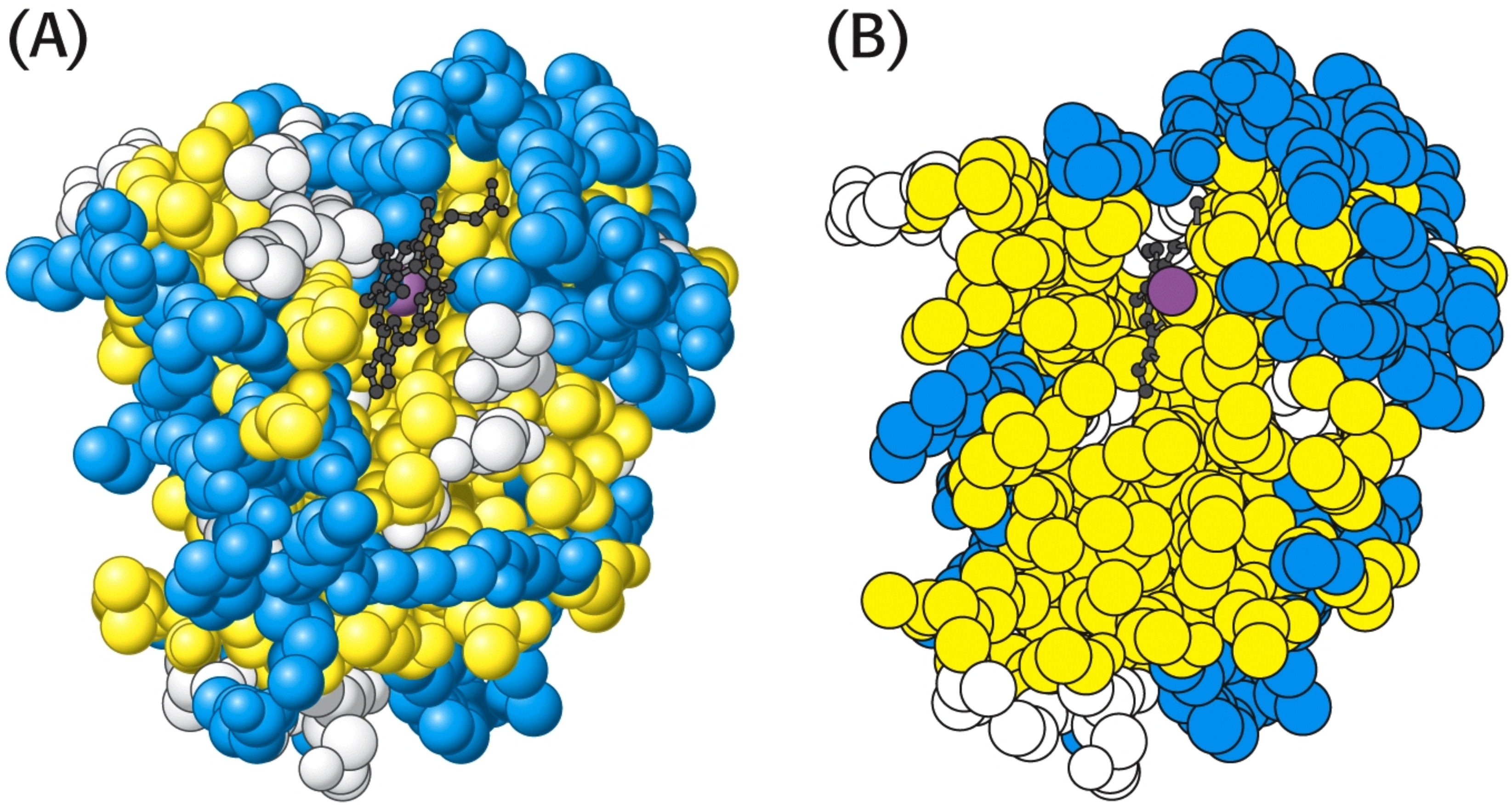


Tertiary (3°) Structure the Protein Myoglobin

Water-soluble proteins fold into compact structures with non-polar cores.

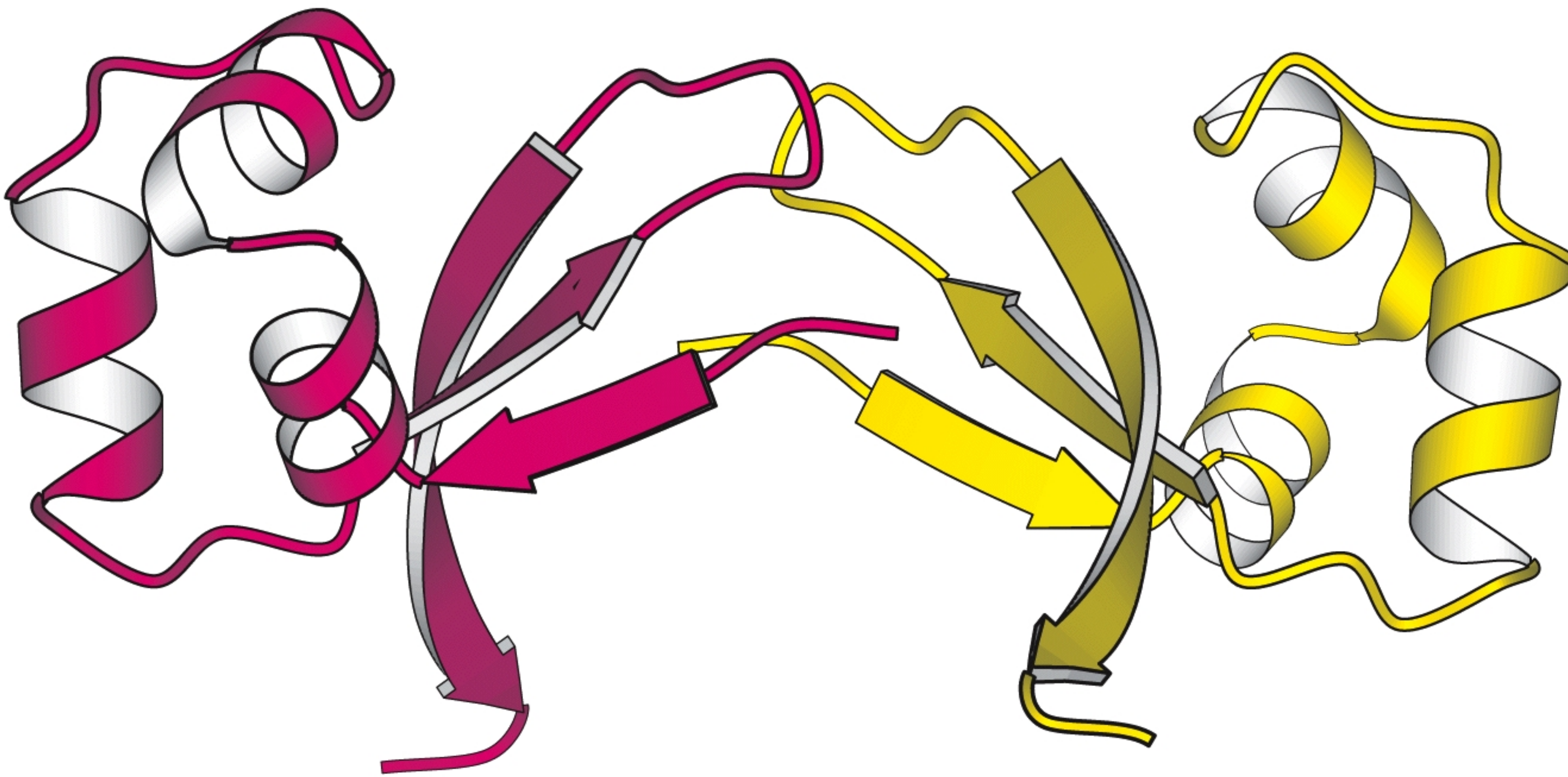


- In the case of myoglobin and many other proteins, the majority of hydrophobic amino acids (yellow) are found inside in structure:

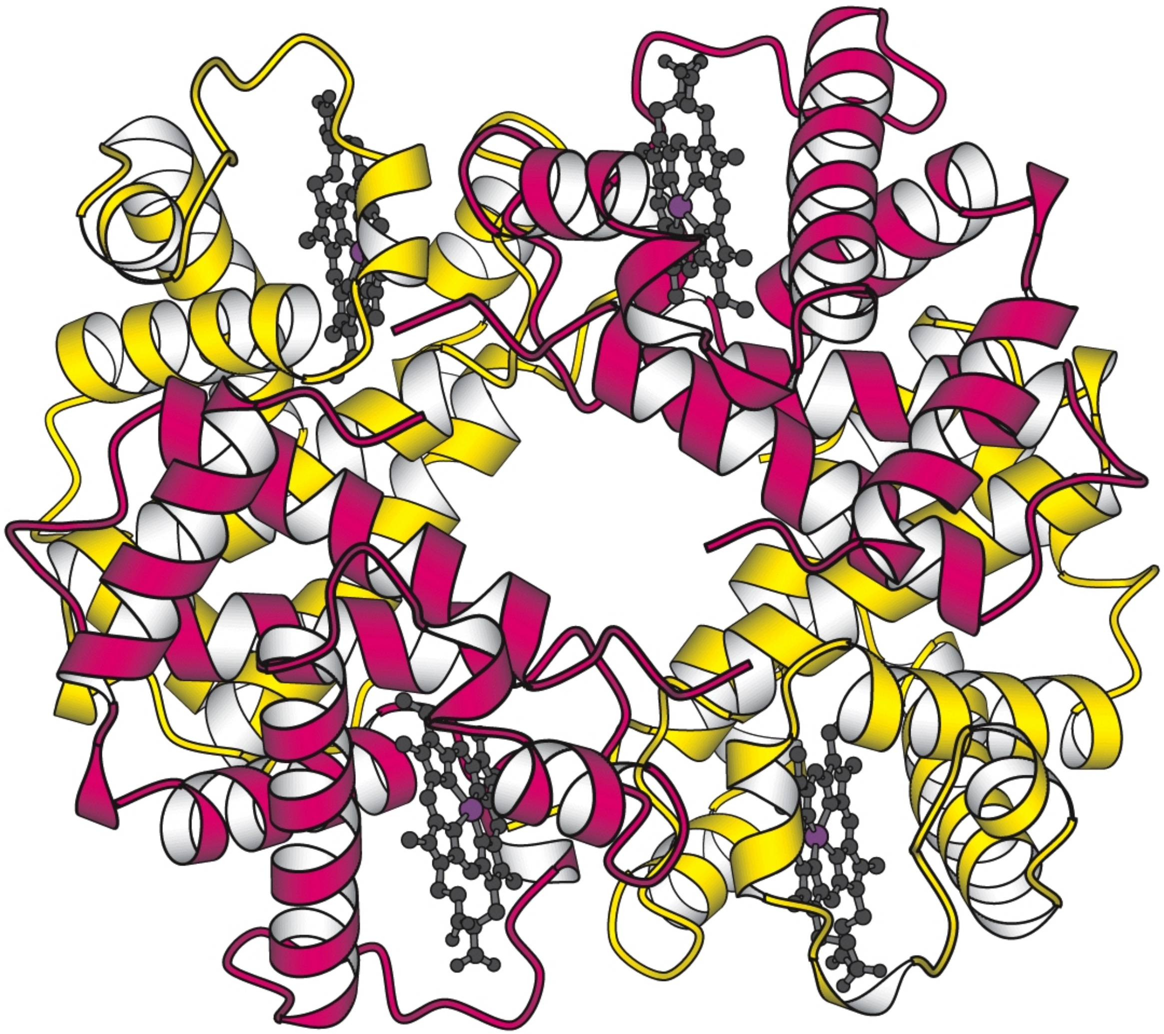


Quaternary structure of Protein

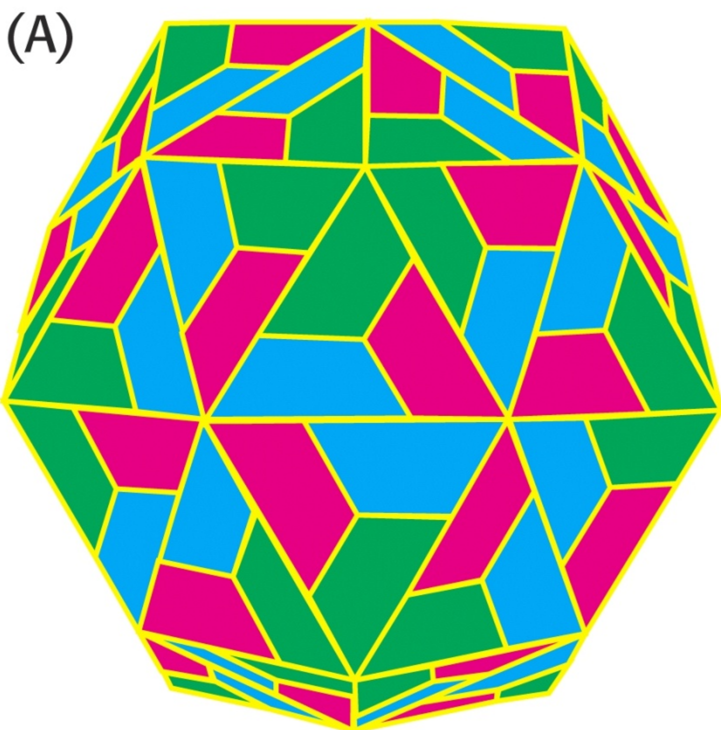
When a protein has two or more polypeptide subunits, their arrangement in space is referred to as quaternary structure

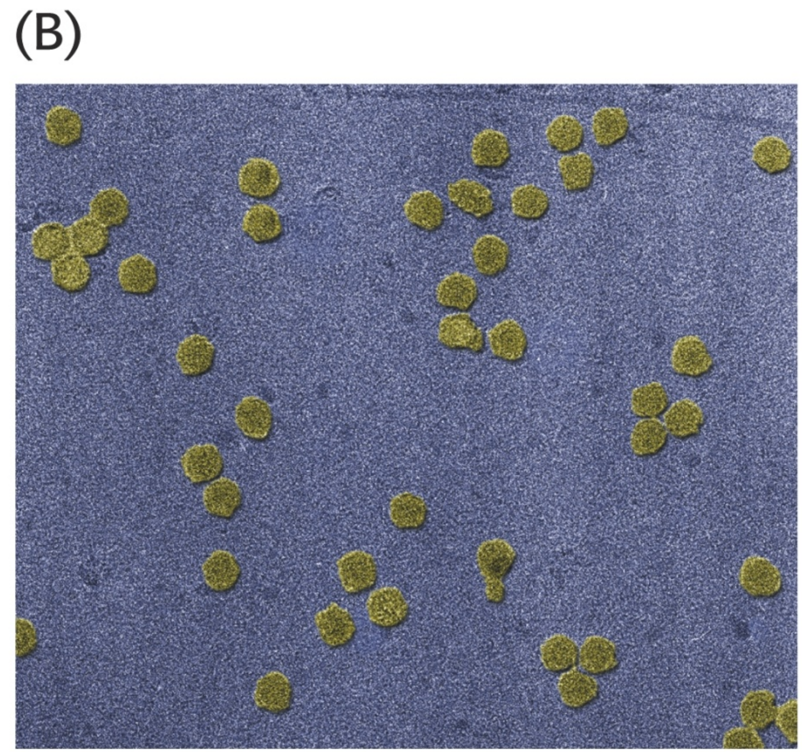


- Hemoglobin is a protein tetramer, containing two identical pairs of subunits:



- The coat of rhinovirus contains 60 copies of (2





Biuret test

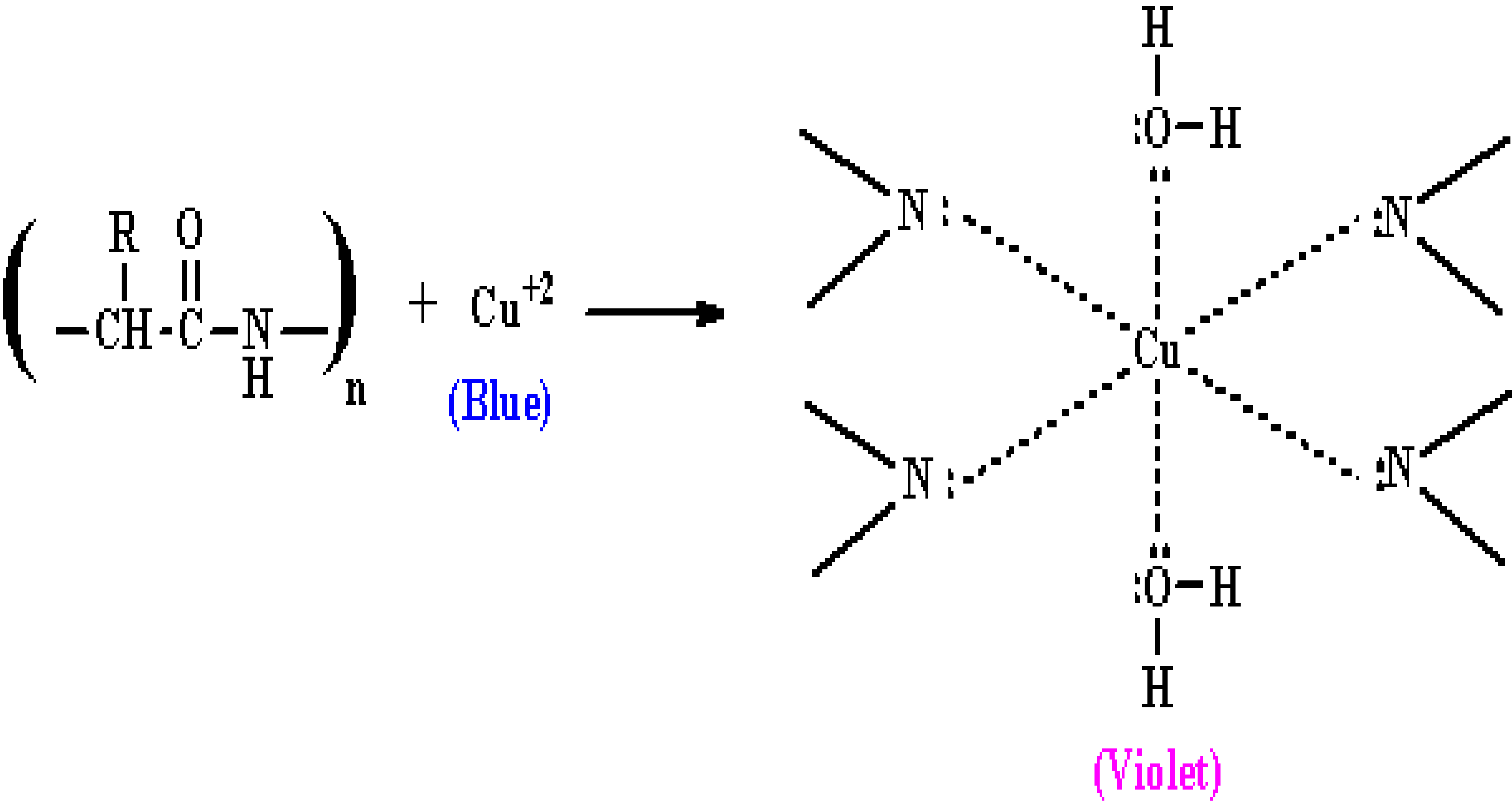
- Biuret structure:

it is result of condensation of two molecule of urea

- **Principle**:
- The biuret reagent (copper sulfate in a strong base) reacts with peptide bonds in proteins to form a blue to violet complex known as the “**biuret complex**”.
- This color change is dependent on the **number** of **peptide bonds** in the solution, so the more protein, the more intense the change.

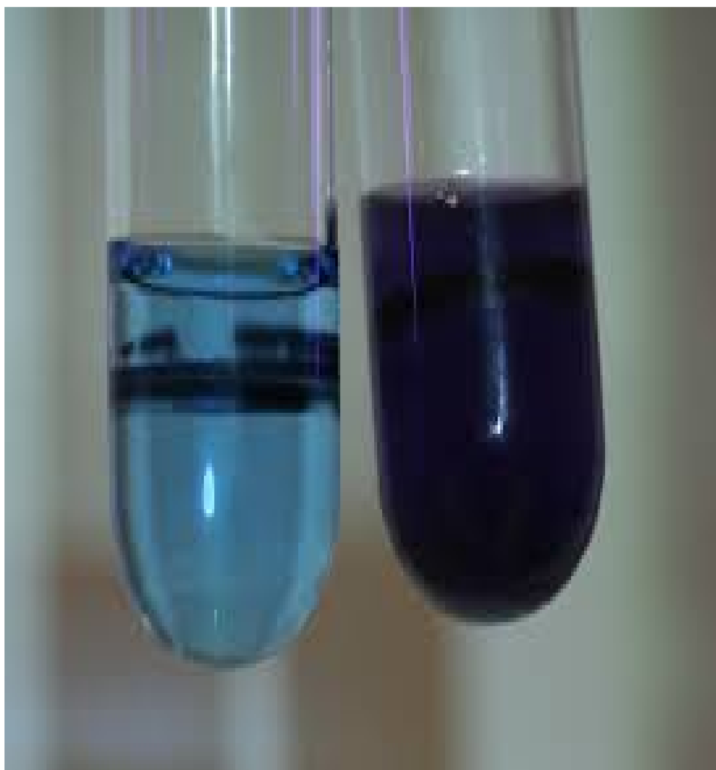
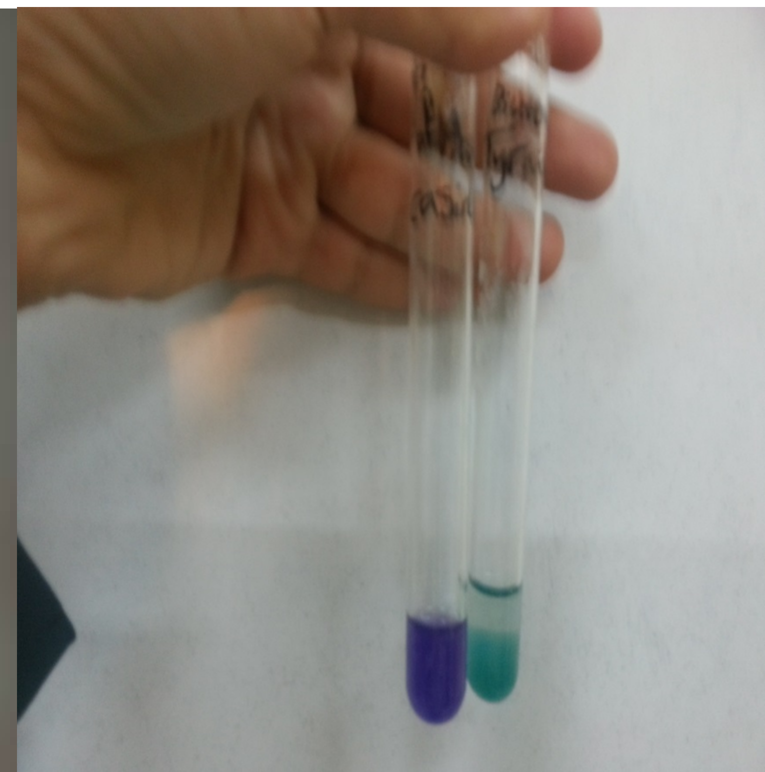
- The NaOH is there to raise the **pH** of the solution to **alkaline** levels; the crucial component is the **copper II** ion (**Cu^{2+}**) from the $CuSO_4$.
- When **peptide bonds** are present in this **alkaline** solution, the **Cu^{2+}**ions will form a coordination **complex** with 4 **nitrogen** atoms from peptide bonds.
- **N.B.** Two peptide bonds at least are required for the formation of this complex.

A chelate is a chemical compound composed of a metal ion and a chelating agent. A chelating agent is a substance whose molecules can form several bonds to a single metal ion. In other words, a chelating agent is a multidentate ligand.



Procedure

- To 2 ml of protein solution in a test tube, add 4ml of reagent incubation 30 min
- Result :

	Observations	Interpretation
	No change (solution remains blue)	Proteins are not present
	The solution turns from blue to violet(purple)	Proteins are present
	The solution turns from blue to pink	Peptides are present (Peptides or peptones are short chains of amino acid residues)

Xanthoproteic test :

Objective:

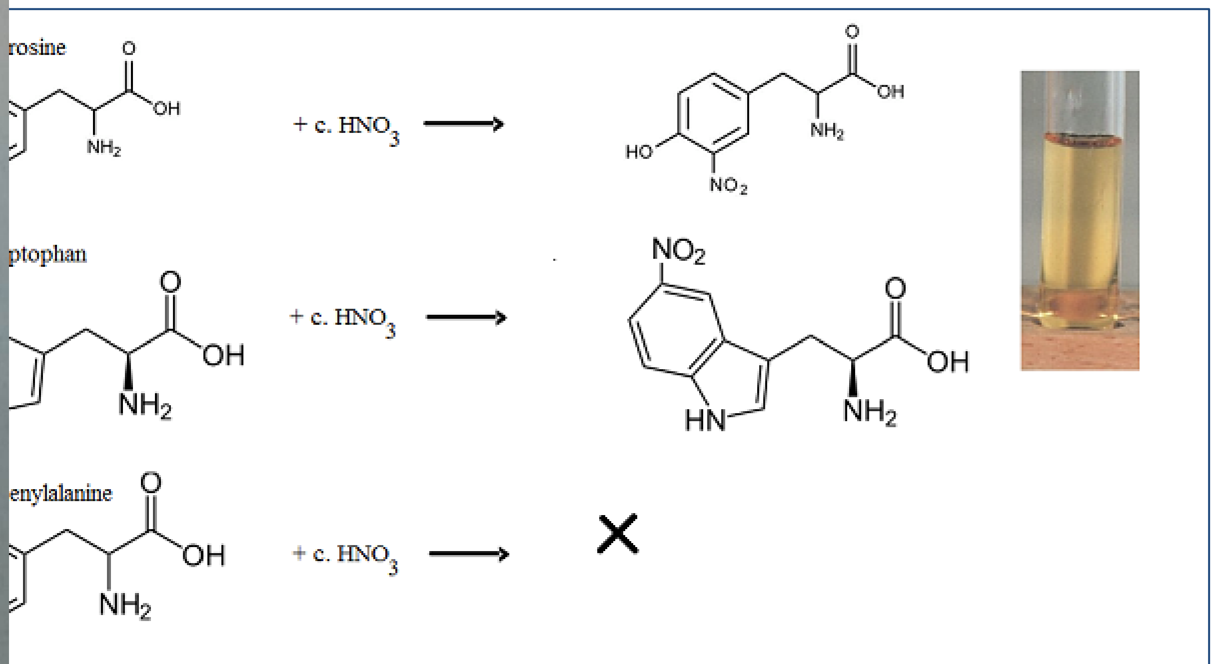
- To differentiate between aromatic amino acids which give positive results [yellow color] and other amino acids.

Principle:

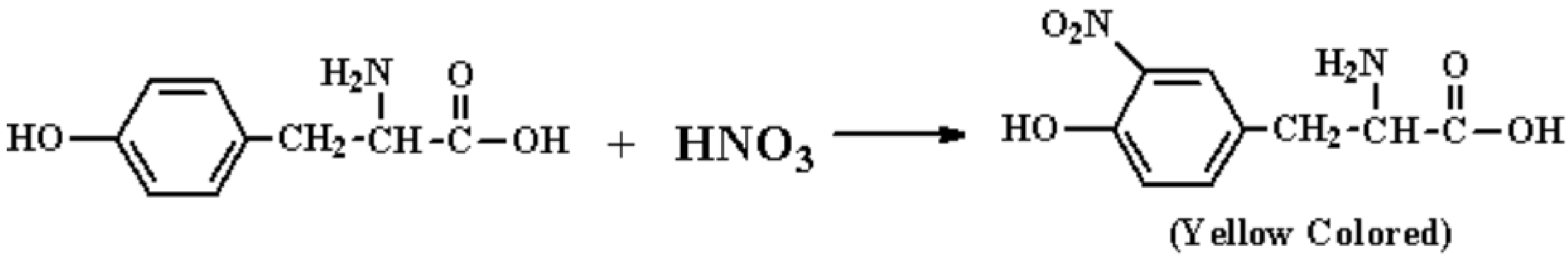
- Concentrated nitric acid react with aromatic nucleus present in the amino acid side chain [nitration reaction] →giving the solution yellow color.

Note:

- Amino acids tyrosine and tryptophan →contain activated benzene rings [aromatic nucleus] which are easily nitrated to yellow colored compounds.
- The aromatic ring of phenyl alanine does not react readily with nitric acid despite it contains a benzene ring, because it is not activated, therefore it will not react

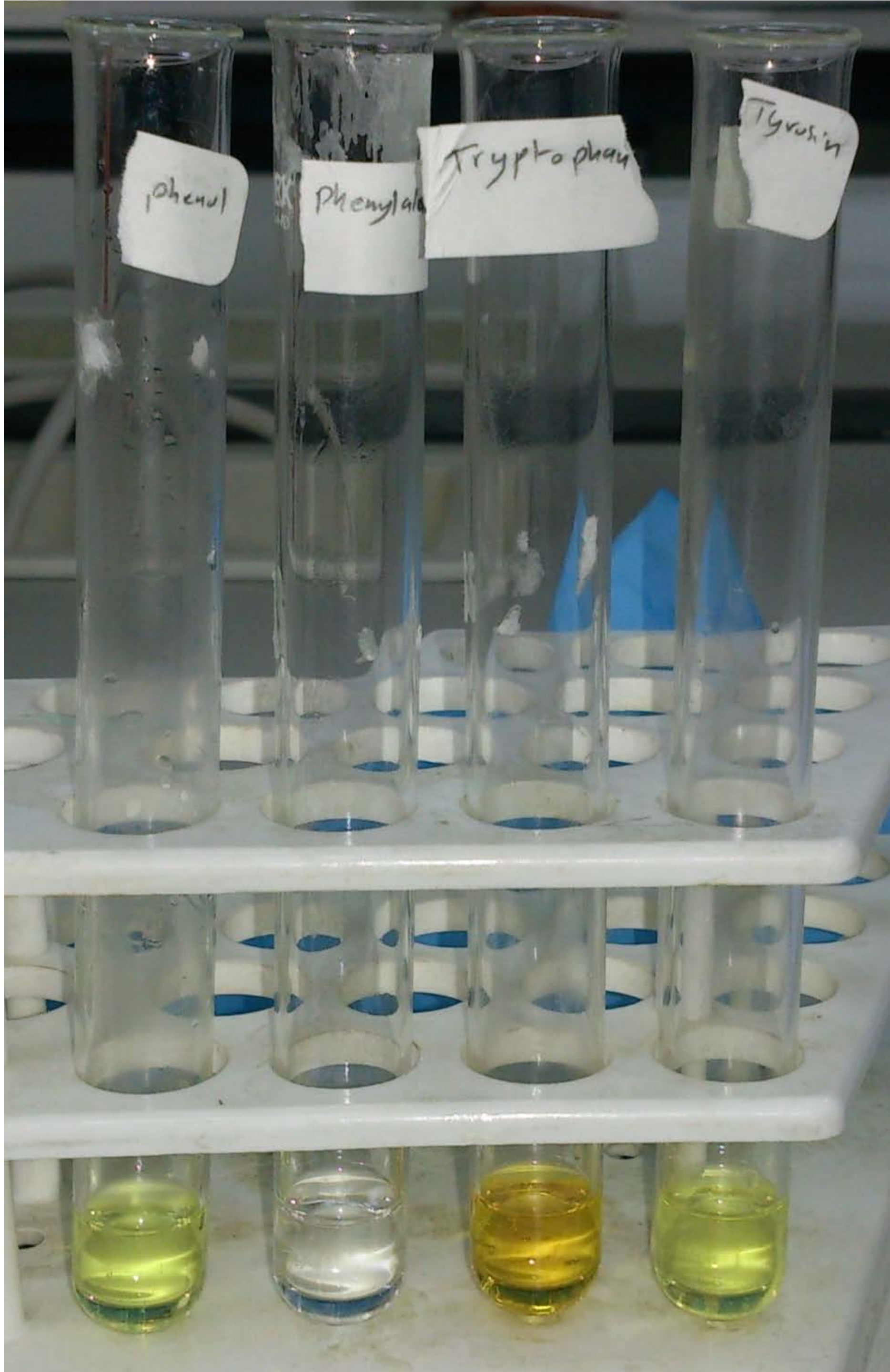


rosine
+ c. HNO3
ptophan
+ c. HNO3
enylalanine
+ c. HNO3


HO
H2N
O
CH2-CH-C-OH
+
HNO3
O2N
HO
H2N
O
CH2-CH-C-OH
(Yellow Colored)

Procedure

- To 2 mL amino acid solution in a boiling test tube, add equal volume of concentrated HNO_3.
- Heat over a flame for 2 min and observe the color.
- Now cool thoroughly and add 3ml of NaOH to neutralize the excess acid
- Observe the color of the nitro derivative of aromatic nucleus.



Digestion & Absorption

- Total protein load received by the gut is derived from 2 sources:

- 70-100 g dietary protein per day

- 35-200 g of endogenous protein secreted into the gut (enzymes) or shed from the epithelium as a result of cell turnover.

Digestion and absorption of protein is extremely efficient

- Only 1-2 g of nitrogen=equivalent to 6-12 of protein is lost into the feces daily.

- Proteins are hydrolyzed by peptidases

- **Endopeptidases (cleave internal peptide bonds)**
- **Exopeptidases**
- **Carboxypeptidases**
- **Aminopeptidases**

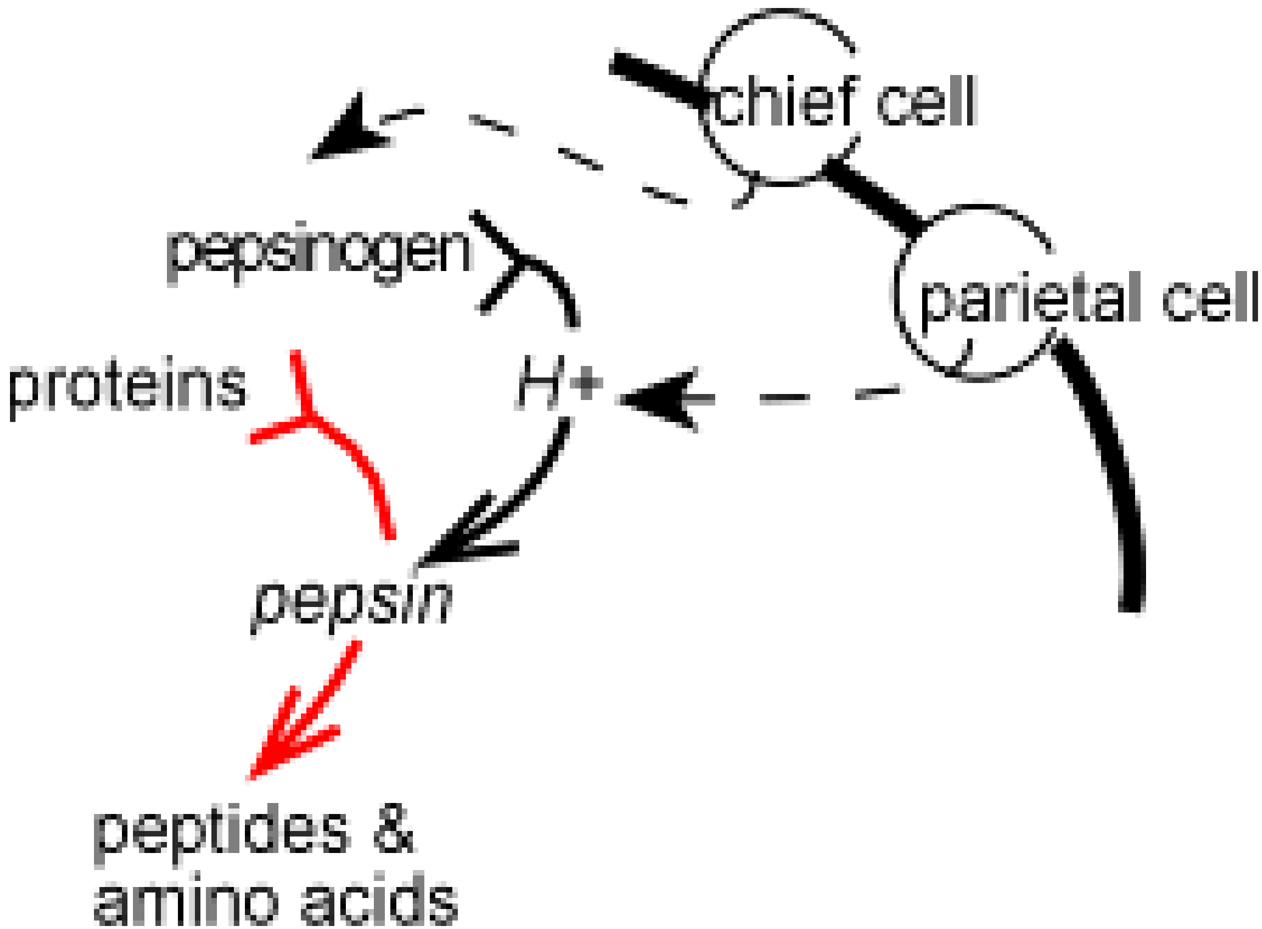
Protein Digestion

Ingested proteins are first split into smaller fragments by pepsin in the stomach.

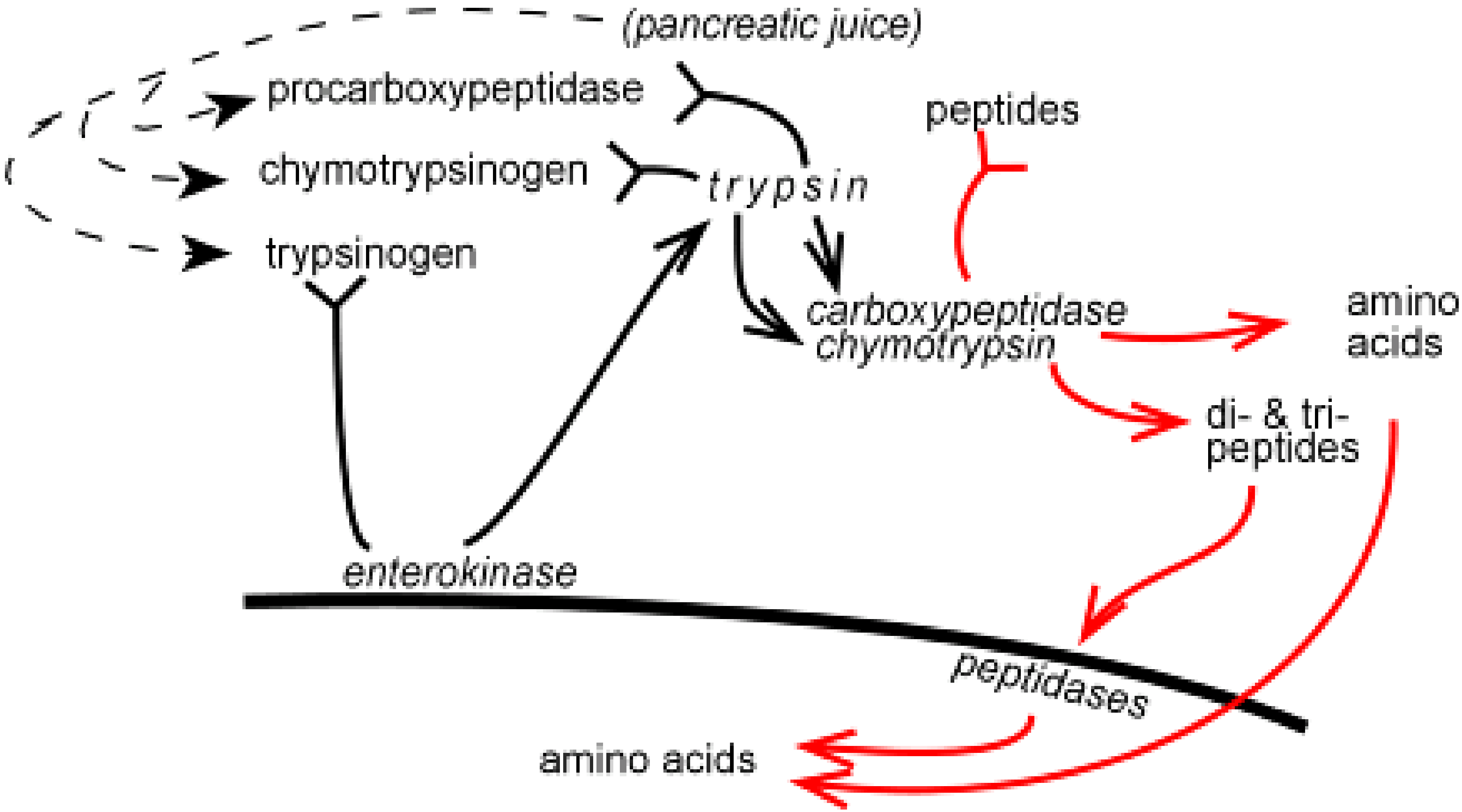
Trypsin (TRYP) and chymotrypsin (CHY) are proteolytic enzymes secreted by the pancreas and liberated in the digestive tract as inactive precursors, respectively. Trypsinogen and chymotrypsinogen, activated once in the digestive tract. They are both endopeptidases that break peptide bonds of specific non-terminal amino acids within the protein.

These peptides are then further reduced by the action of carboxypeptidase which hydrolyzes off one amino acid at a time beginning at the free carboxyl end of the molecule or by aminopeptidase which splits off one amino acid at a time beginning at the free amino end of the polypeptide chain

Proteolysis in Stomach



Proteolysis in Intestine



Amino acid, di & tripeptide absorption

- Amino acids, dipeptides, and tripeptides are imported against its concentration gradient from the intestinal lumen across the apical surface of the epithelial cells by a two-Na^+/one-peptide symporter located in the microvillar membranes.

- Transported dipeptides, and tripeptides molecule are broken into amino acid by the activity of cytosolic peptidase

- Amino acids enters the blood steam by the help of amino acid transporters present on basolateral side.

- All the Na^+ ions transported from the intestinal lumen into the cell during Na+/peptide symport, are pumped out across the basolateral membrane by Na+/K+ ATPase channel. Thus the low intracellular Na^+ concentration is maintained.

Amino acid Absorption