Heterofunctional compounds. Aminoacids and proteins

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Structural Differences Between Carbohydrates, Lipids, and Proteins

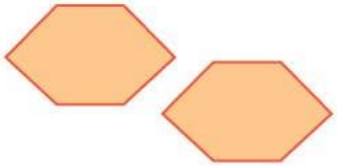
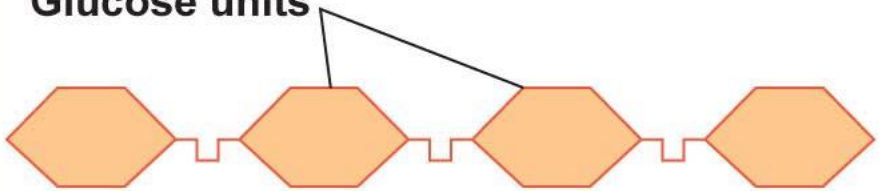
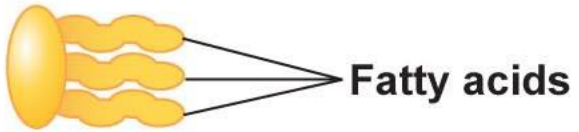
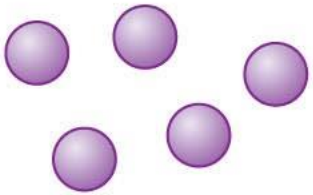
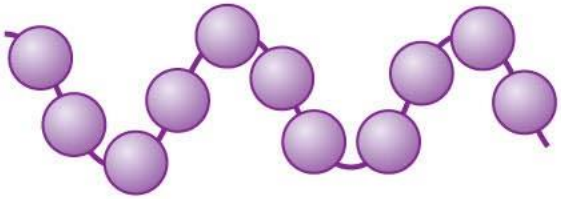
Macronutrients	Chains of	Example
Carbohydrates	Glucose 	Glucose units 
Lipids	Fatty acids 	Triglyceride 
Proteins	Amino acids 	Amino acids 

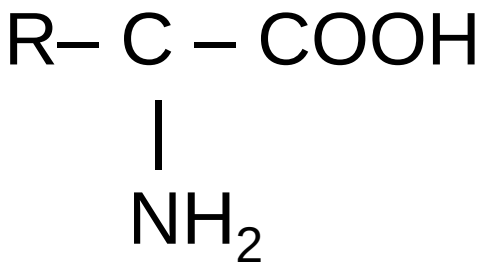
Figure 6.1

General principles

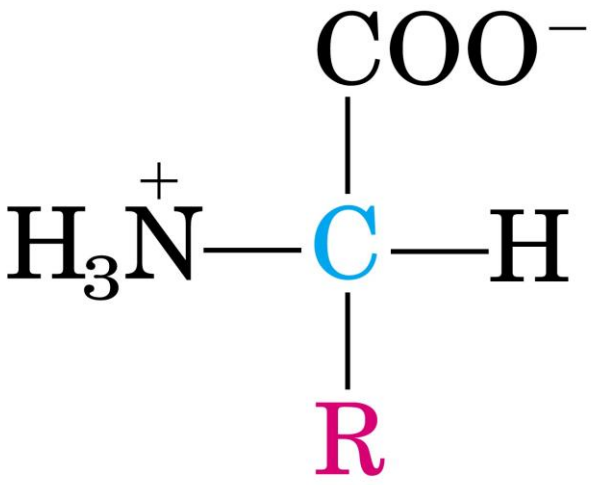
- Amino acids are the building blocks of proteins

•While their name implies that amino acids are compounds that contain an —NH_2 group and a —COOH group, these groups are actually present as —NH_3^+ and —COO^- respectively.

•They are classified as α, β, γ, *etc*. amino acids according the carbon that bears the nitrogen.

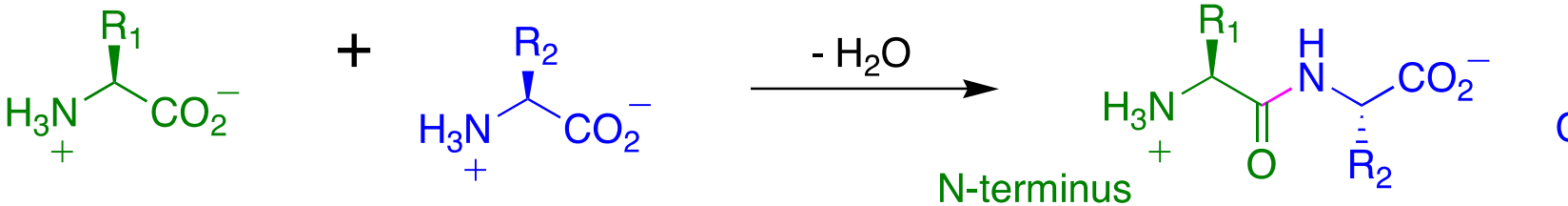


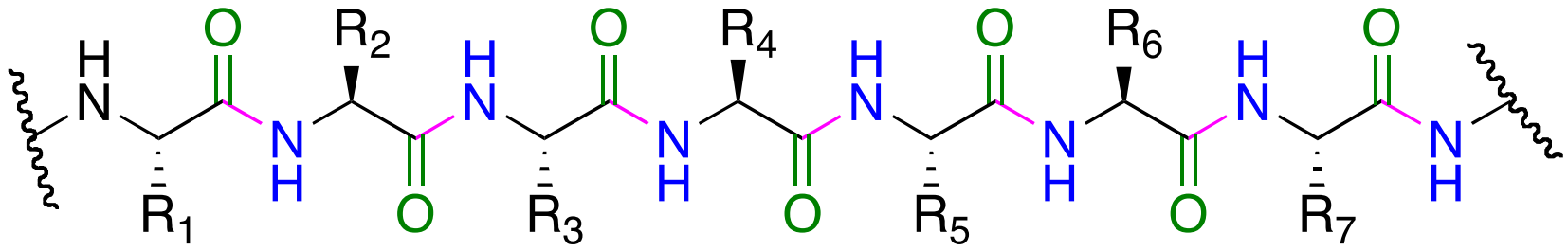
α- amino acid



- 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…*

Biopolymer: the monomeric amino acids are linked through an amide bond (the carboxylic acids of one AA with the α-amino group of a second)

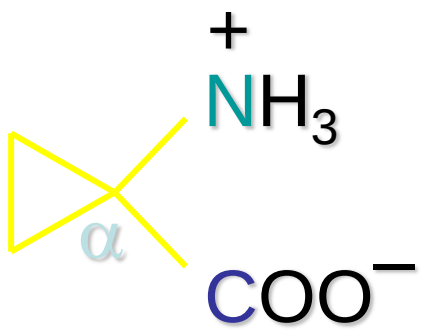




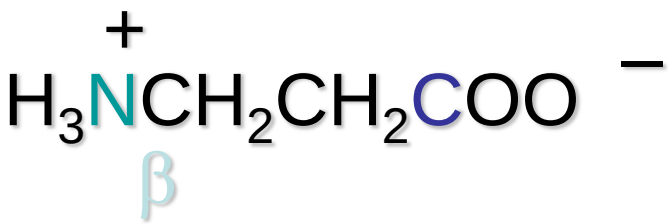
Peptide or protein (polypeptide)

peptide (< 50 amino acids) protein (> 50 amino acids)

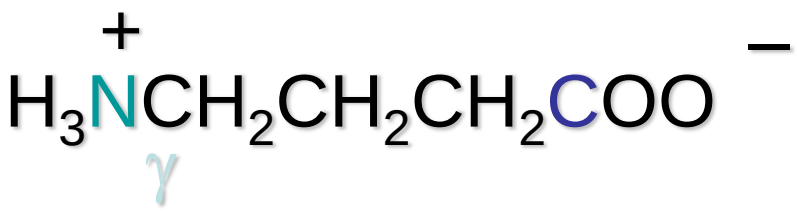
Amino Acids



an α-amino acid that is an intermediate in the biosynthesis of ethylene



a β-amino acid that is one of the structural units present in coenzyme A

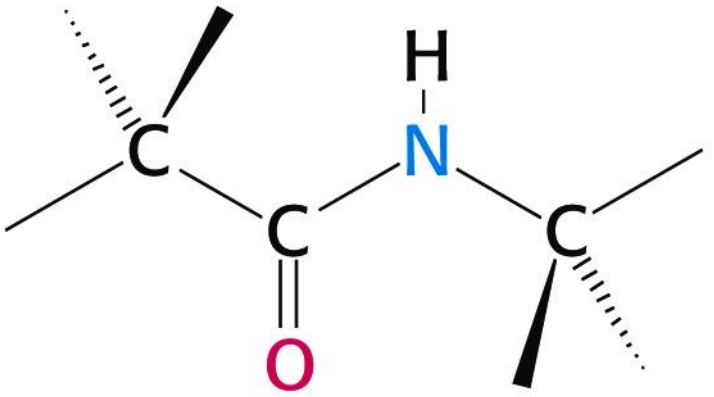


a γ-amino acid involved in the transmission of nerve impulses

Structural features of Amino acids

- All 20 amino acids have common structural features
- All amino acids have an amino group ($-NH_3^+$), a carboxylate ($-COO^-$) group and a hydrogen bonded to the same carbon atom (the α-carbon)
- They differ from each other in their side chain called R group.
- R groups vary in structure, size and electric charges and influence the solubility of amino acids in water.

Peptide bonds have *partial* double bond character due to resonance that limits rotation about this bond:



Nomenclature of aminoacids

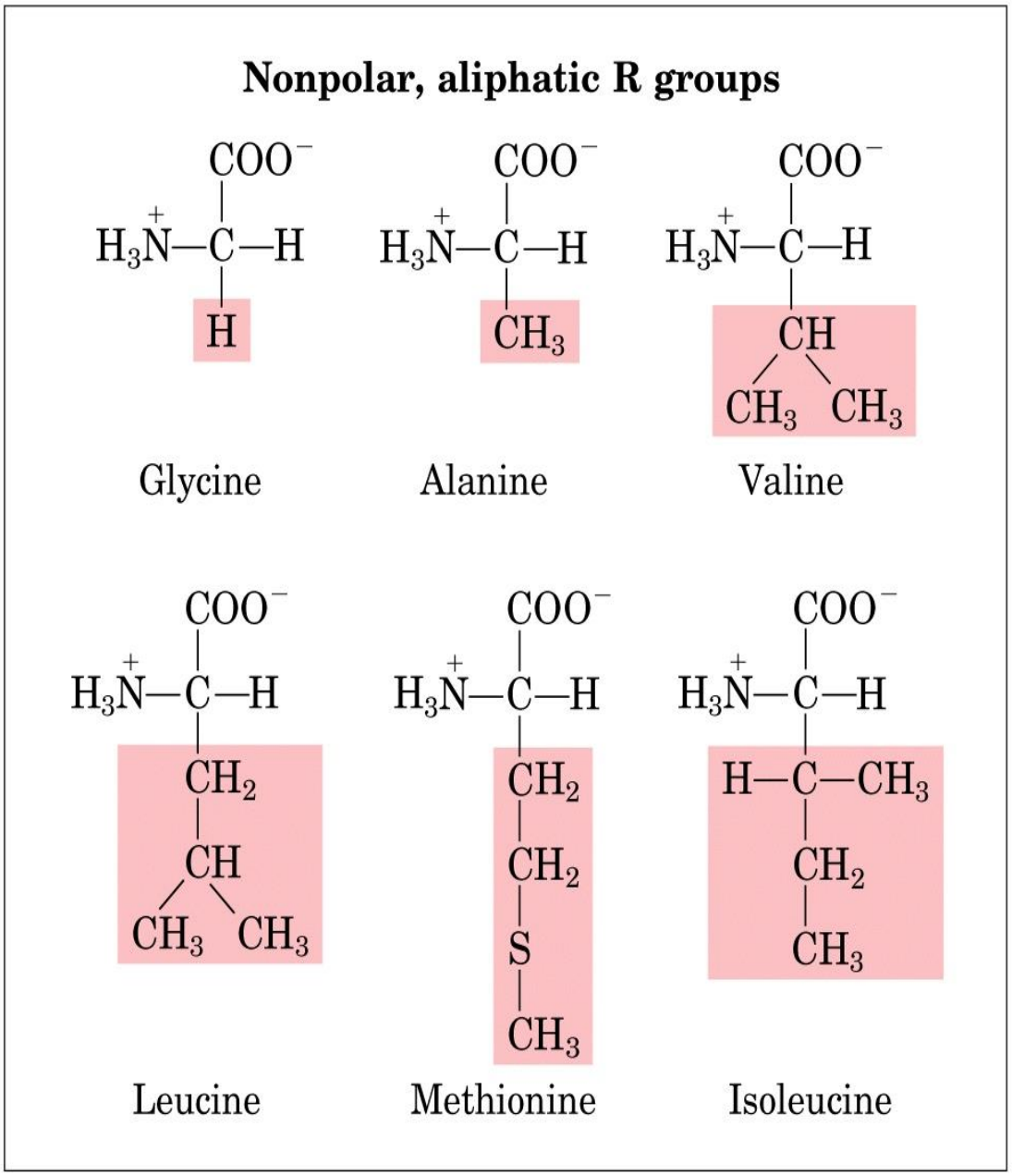
Amino acid	Three-letter abbreviation	One-letter abbreviation	Amino acid	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A	Methionine	Met	M
Arginine	Arg	R	Phenylalanine	Phe	F
Asparagine	Asn	N	Proline	Pro	P
Aspartic Acid	Asp	D	Serine	Ser	S
Cysteine	Cys	C	Threonine	Thr	T
Glutamine	Gln	Q	Tryptophan	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			

Classification of Amino Acids

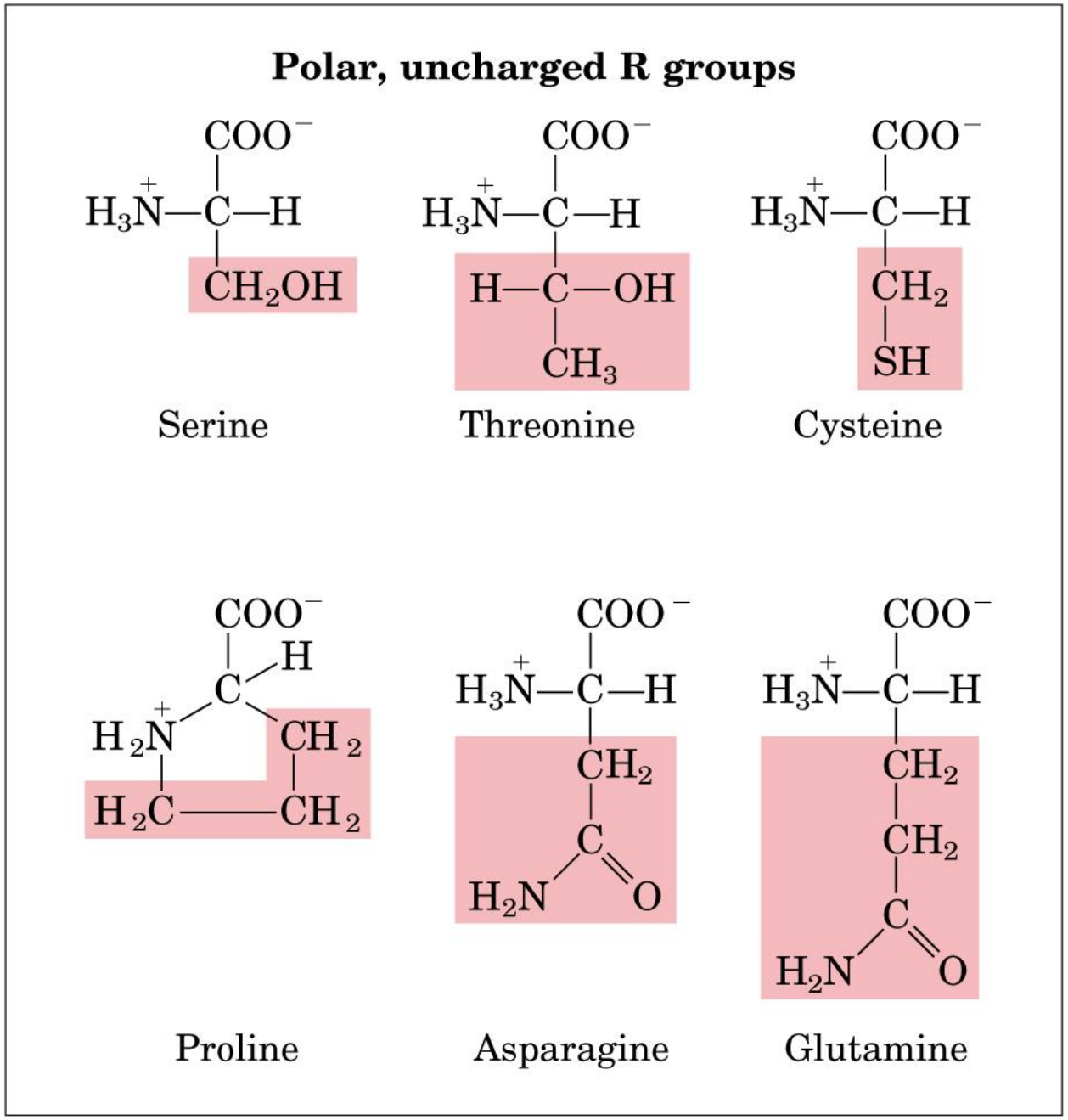
- Nutritional
  - Essential
  - Non-essential

Based on R group

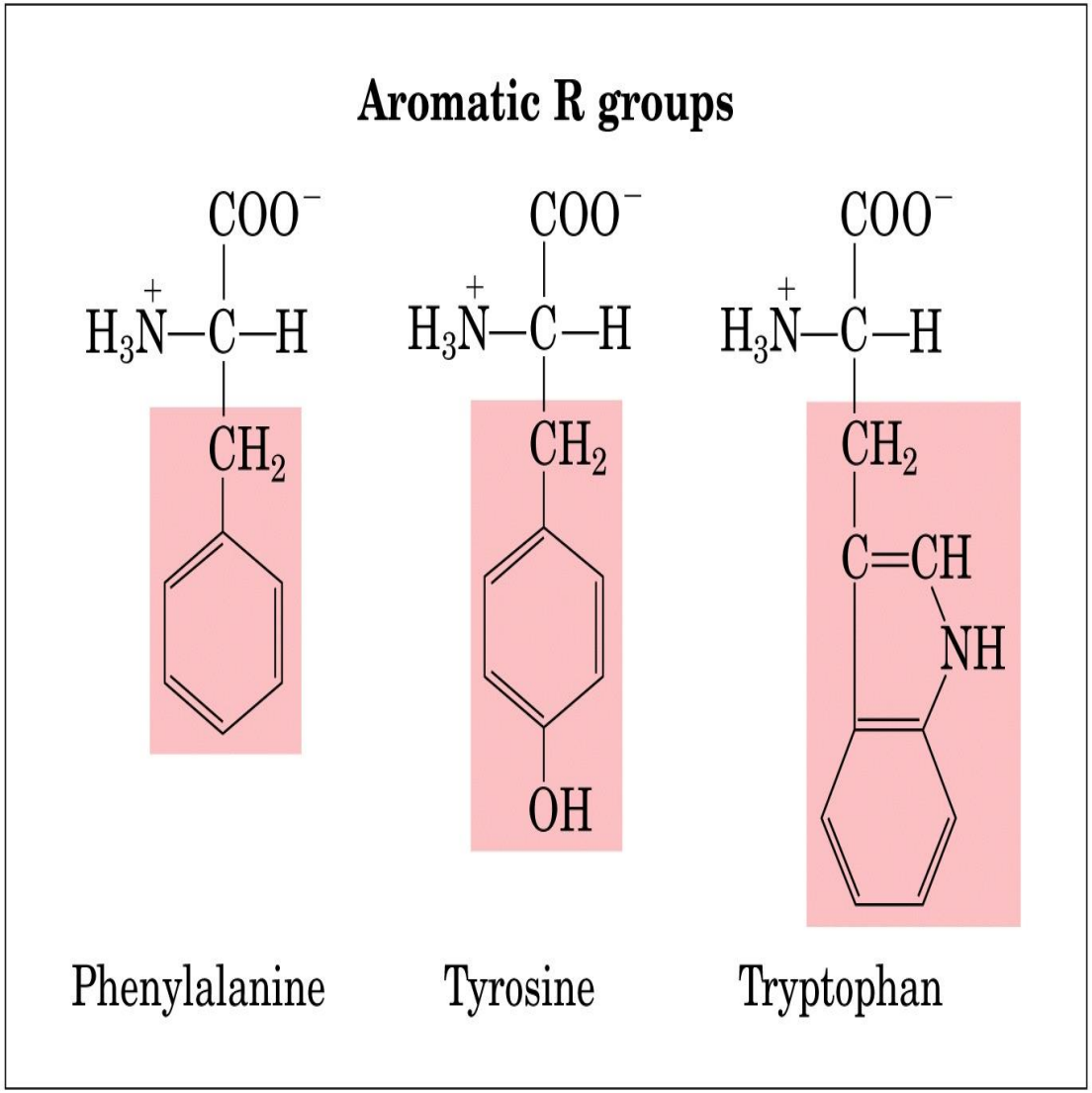
- Non polar aliphatic R group
- Polar uncharged R group
- Aromatic R group
- Positively charged R group
- Negatively charged R group



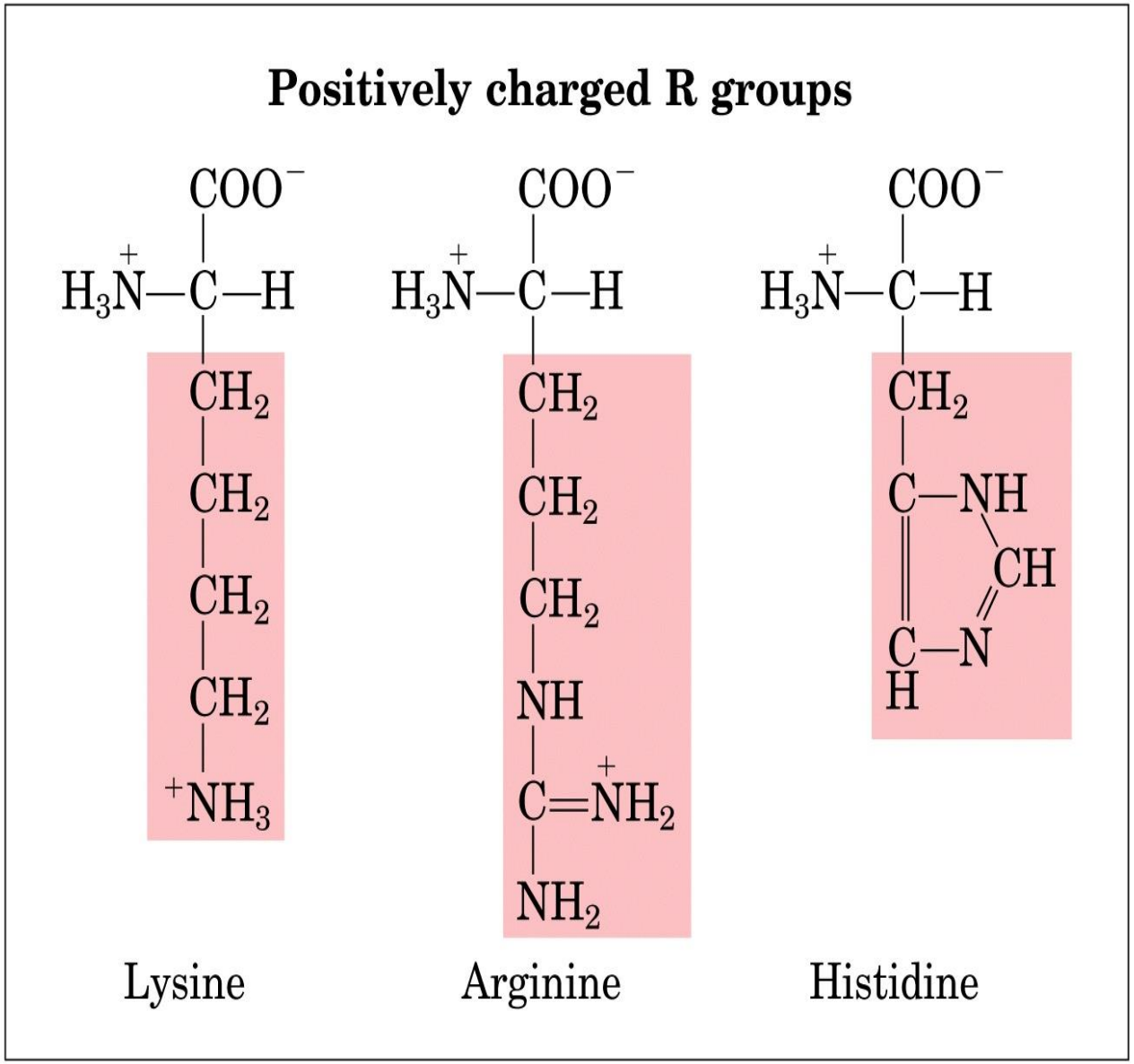
The hydrocarbon R group in this class of amino acids is nonpolar and hydrophobic. Glycine has the simplest amino acid structure. The bulky side chain of valine, isoleucine and leucine are important in promoting hydrophobic interactions within protein structures.



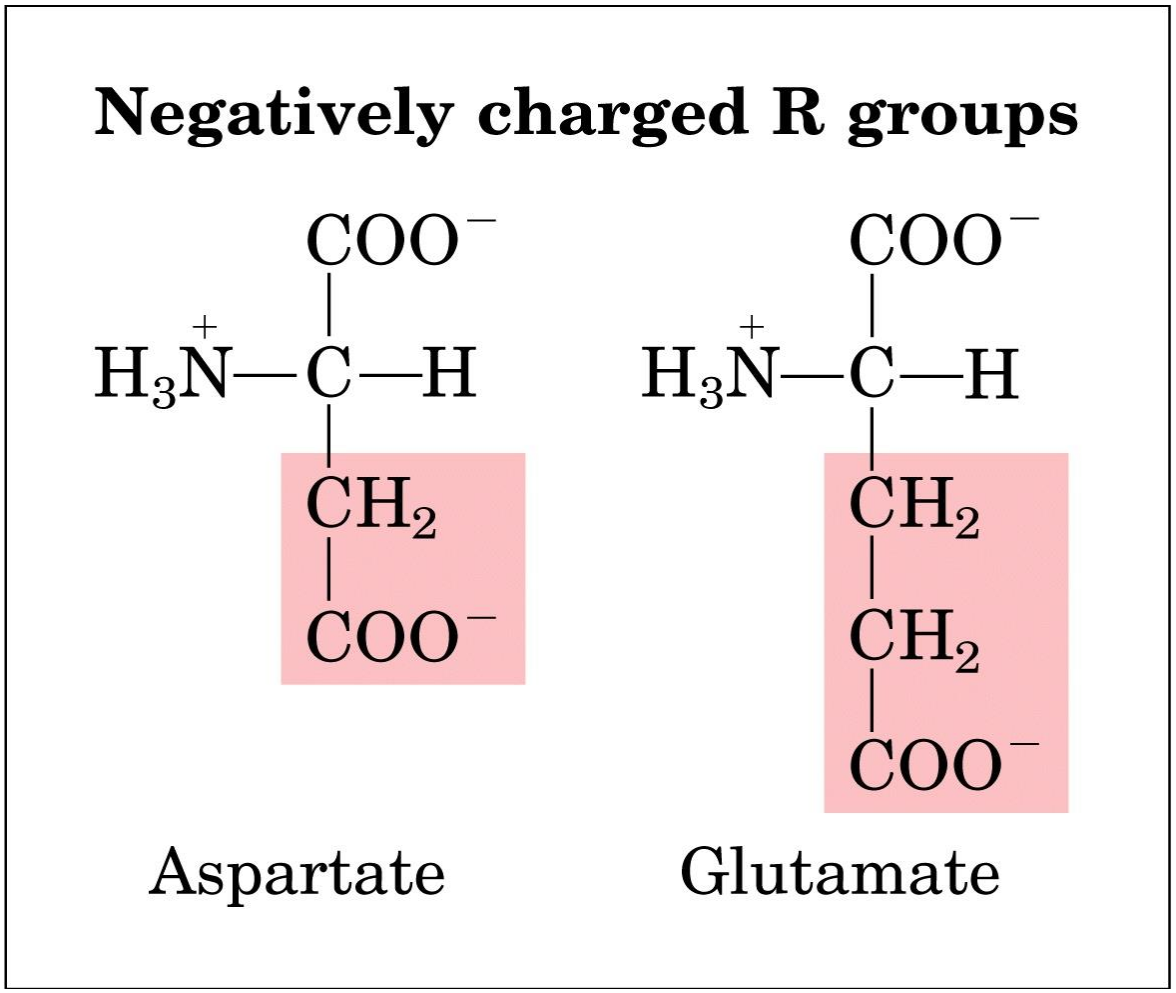
The R group of these amino acids is more soluble in water, or hydrophilic than those of non polar amino acids, because they contain functional groups that form hydrogen bond with water



Their aromatic side chains are relatively nonpolar. All can participate in hydrophobic interactions. The OH group of tyrosine can form hydrogen bond and can act as an important functional group in the activity of some enzymes.



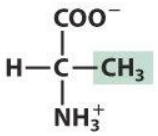
The amino acids in which the R group have a net positive charge at pH 7.0

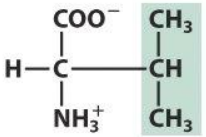


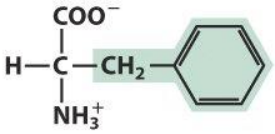
Amino acids having R group with a net negative charge at pH 7.0, with a second carboxyl group

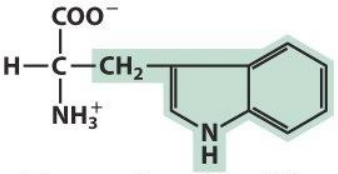
Structures and abbreviations of the standard amino acids

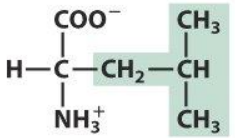
Hydrophobic amino acids

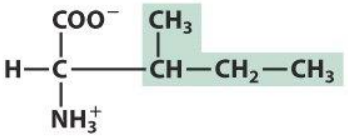

Alanine (Ala, A)

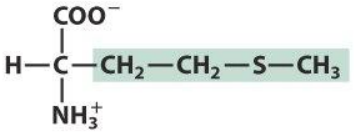

Valine (Val, V)

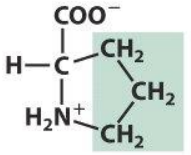

Phenylalanine (Phe, F)


Tryptophan (Trp, W)

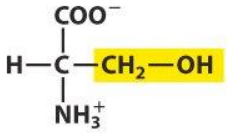

Leucine (Leu, L)

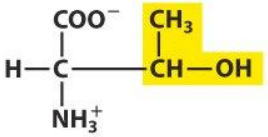

Isoleucine (Ile, I)

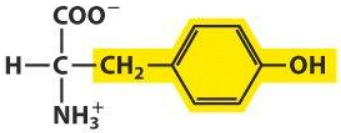

Methionine (Met, M)

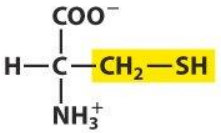

Proline (Pro, P)

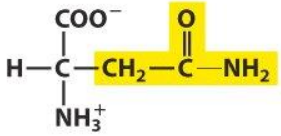
Polar amino acids

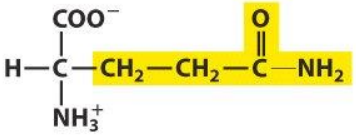

Serine (Ser, S)

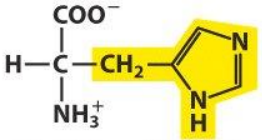

Threonine (Thr, T)

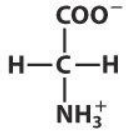

Tyrosine (Tyr, Y)


Cysteine (Cys, C)

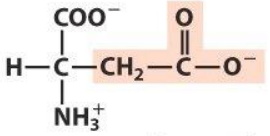

Asparagine (Asn, N)

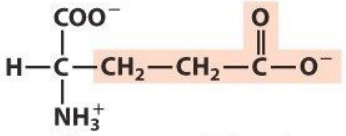

Glutamine (Gln, Q)

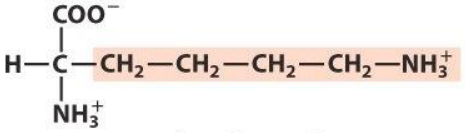

Histidine (His, H)

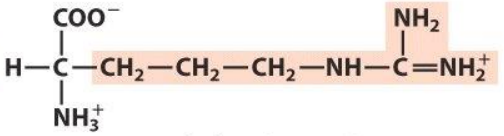

Glycine (Gly, G)

Charged amino acids


Aspartate (Asp, D)


Glutamate (Glu, E)


Lysine (Lys, K)


Arginine (Arg, R)

Nutritional Classification of Amino acids

Essential Amino Acids:

Need to supplied in daily diet

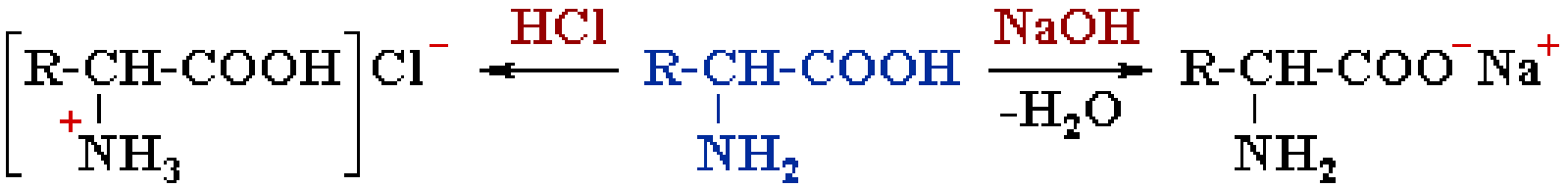
1. Lysine
2. Leucine
3. Isoleucine
4. Metionine
5. Tryptophan
6. Phenylalanine
7. Threonine
8. Valine

Nonessential Amino Acids:

Need not be supplied in daily diet

- Alanine
- Asparagine
- Glycine
- Tyrosine
- Serine
- Proline
- Cysteine
- Cystine
- Histidine (essential for children)
- Glutamine (conditionally essential)
- Arginine (conditionally essential)
- Glutamate

Chemical properties of aminoacids

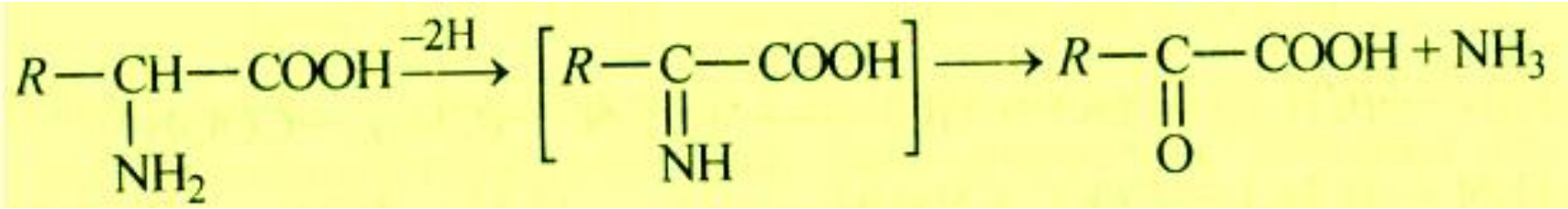


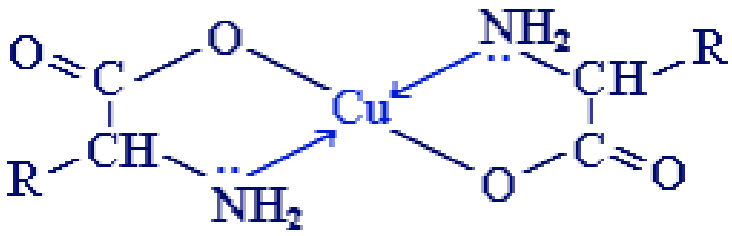
$$H_2N–CH_2–COOH + NaOH \longrightarrow H_2N–CH_2–COO^- Na^+ + H_2O$$

$$H_2N\text{-}CH_2\text{-}COOH + C_2H_5OH \xrightarrow{H^+} H_2N\text{-}CH_2\text{-}COOC_2H_5 + H_2O$$

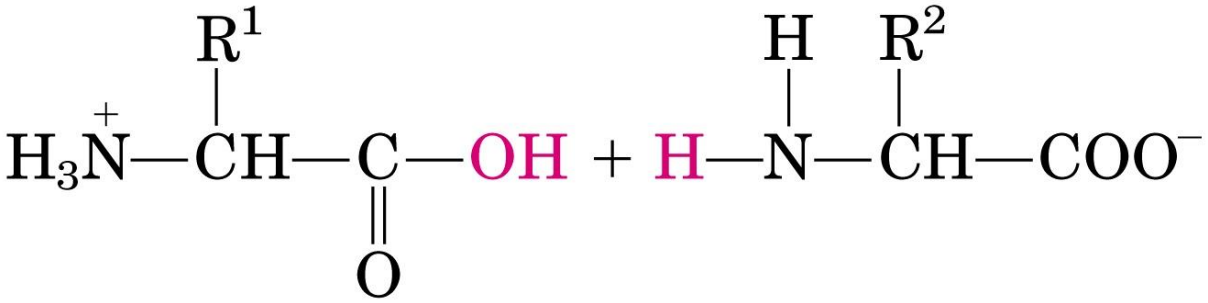
$$H_2N–CH_2–COOH + HNO_2 \longrightarrow HO–CH_2–COOH + N_2\uparrow + H_2O$$

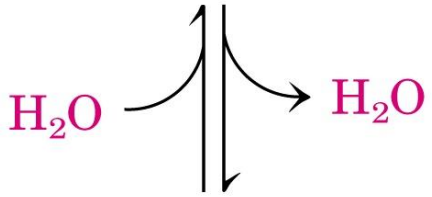
$$H_2N–CH_2–COOH \xrightarrow{Ba(OH)_2,\,t} H_2N–CH_3 + BaCO_3\downarrow + H_2O$$

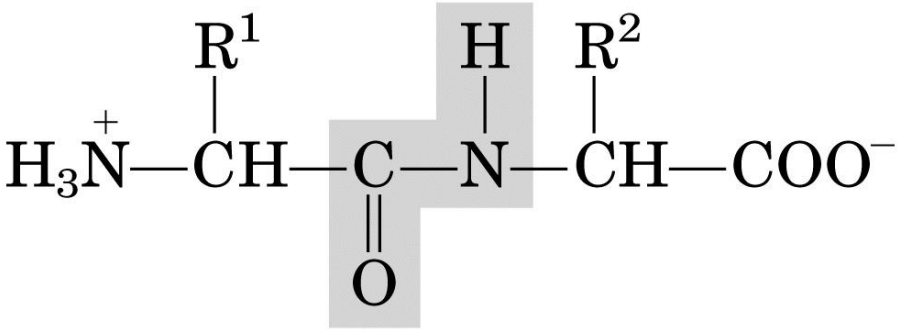




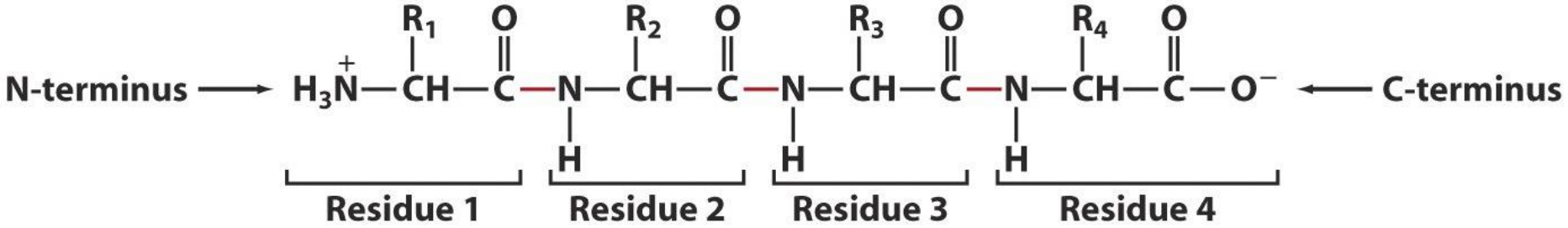
Formation of a peptide bond

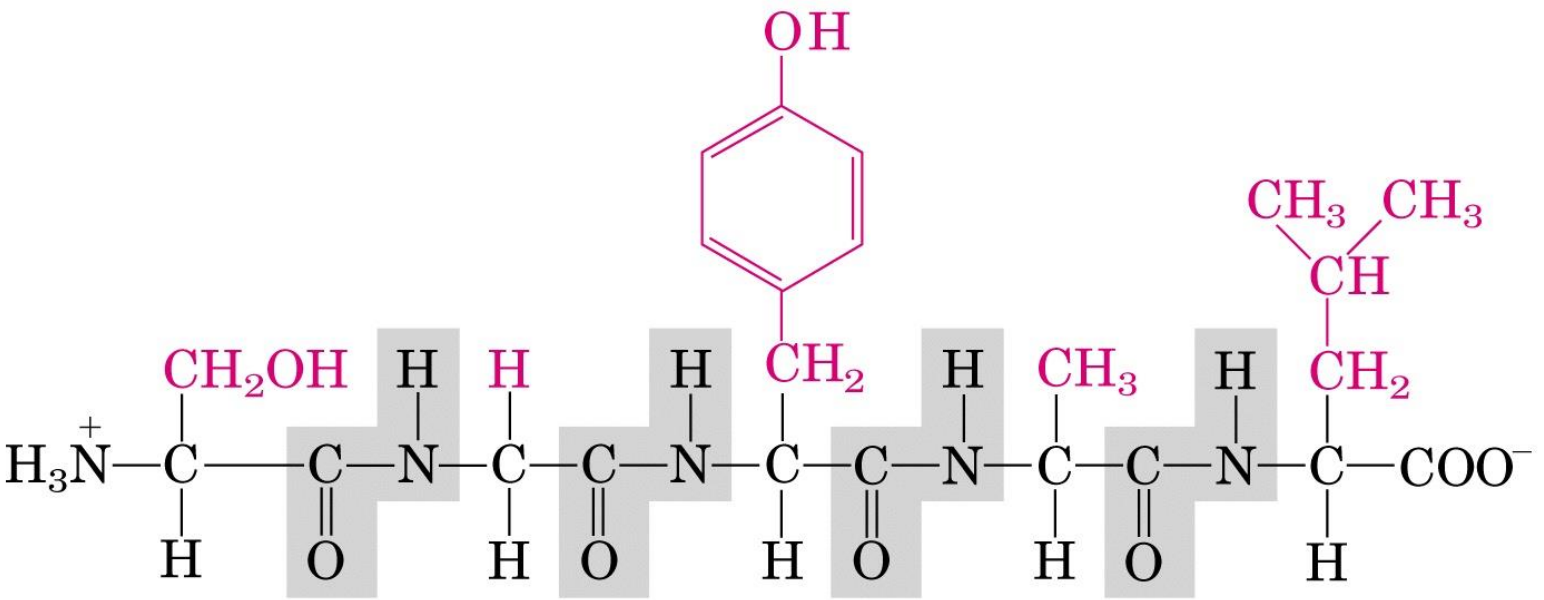




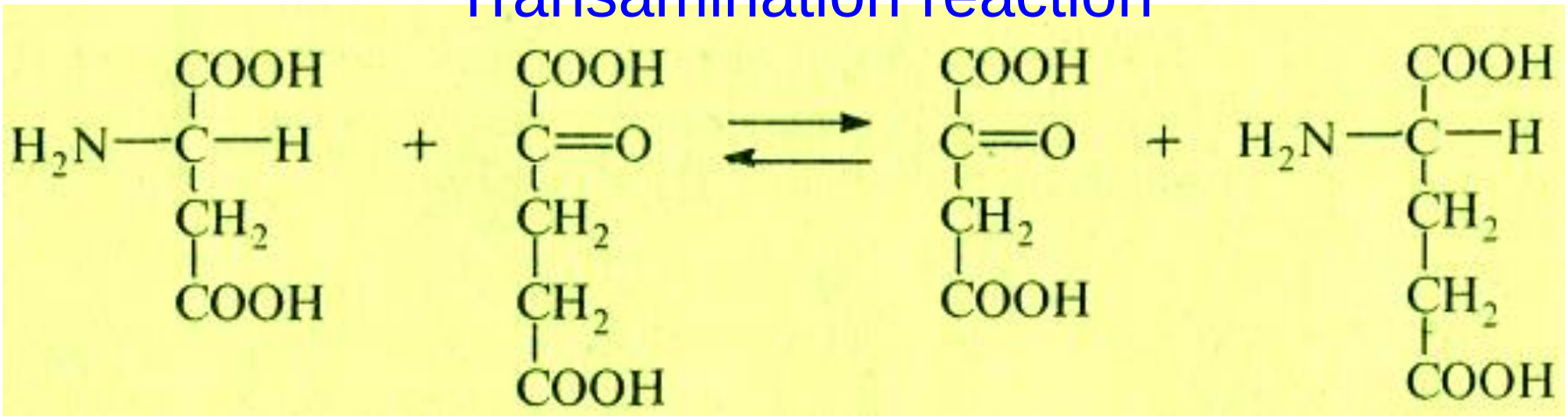


Amino acid residues



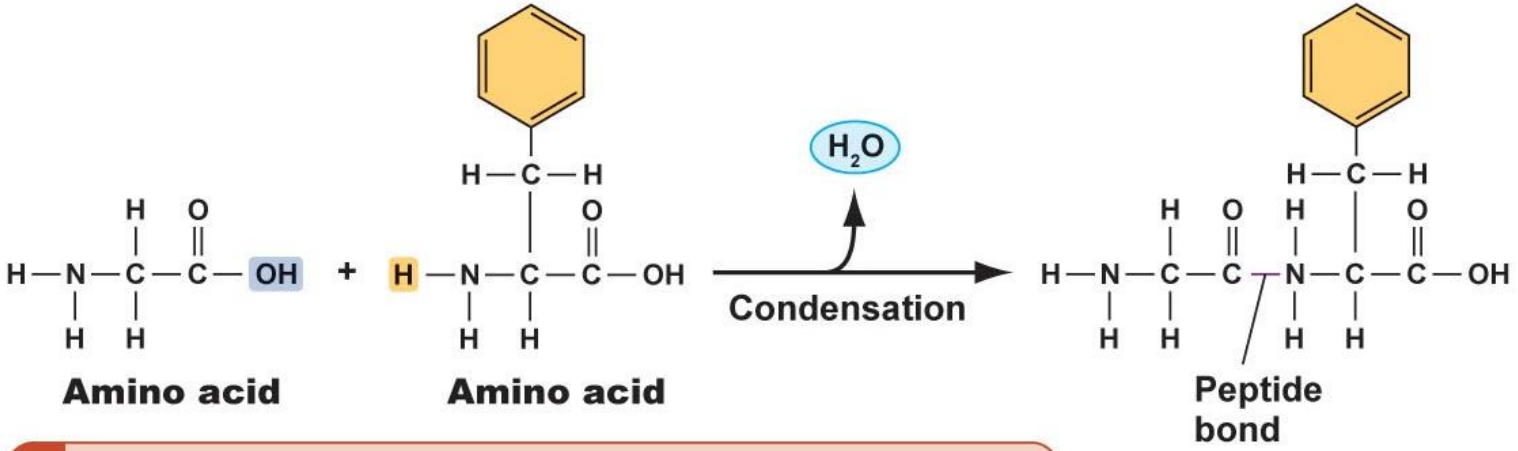


Transamination reaction

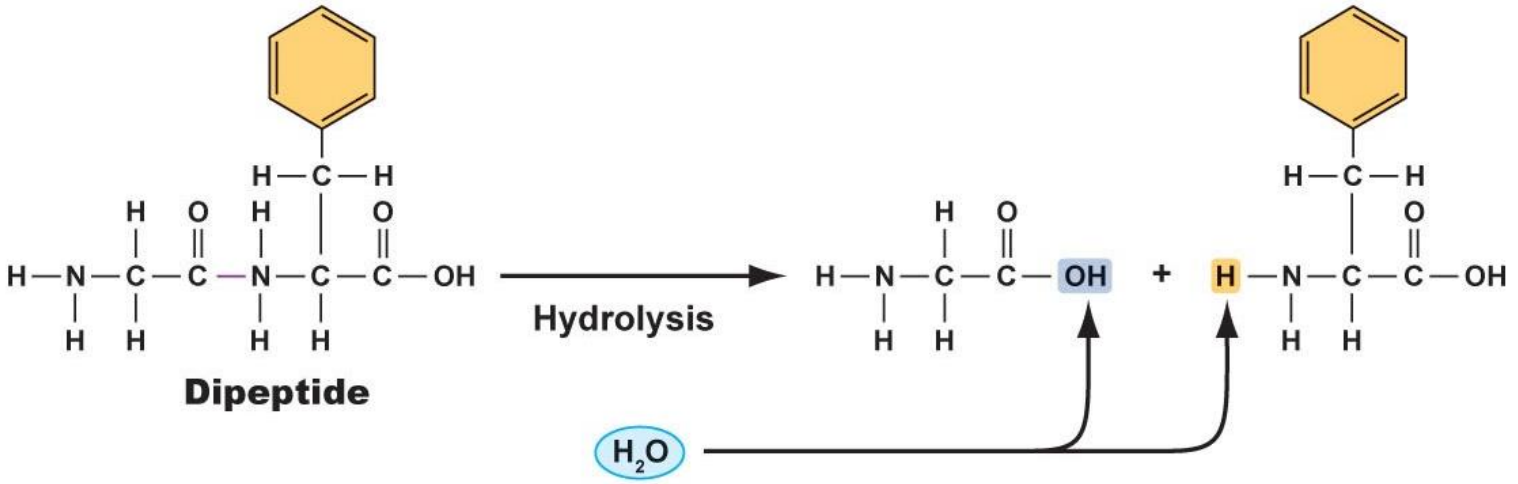


Asparagine acid α-ketoglutaric acid oxaloacetic acid glutamine acid

Condensation and Hydrolytic Reactions

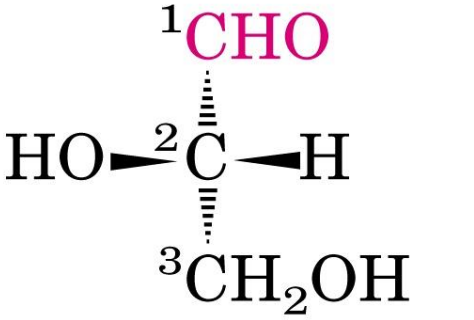


a A peptide bond forms by condensation when the acid group (COOH) and amine group of two different amino acids join and release a molecule of water.

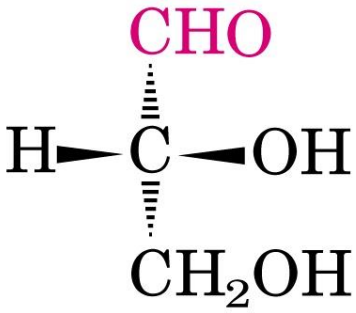


b When peptide bonds are broken by hydrolysis, the hydroxyl group (OH) and hydrogen (H) from water are added.

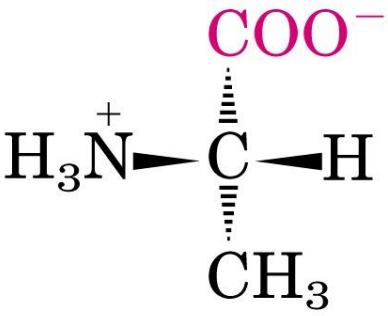
Handedness of Amino Acids



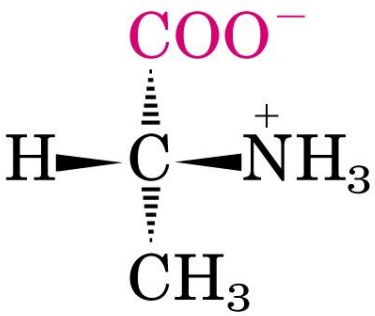
L-Glyceraldehyde



D-Glyceraldehyde



L-Alanine

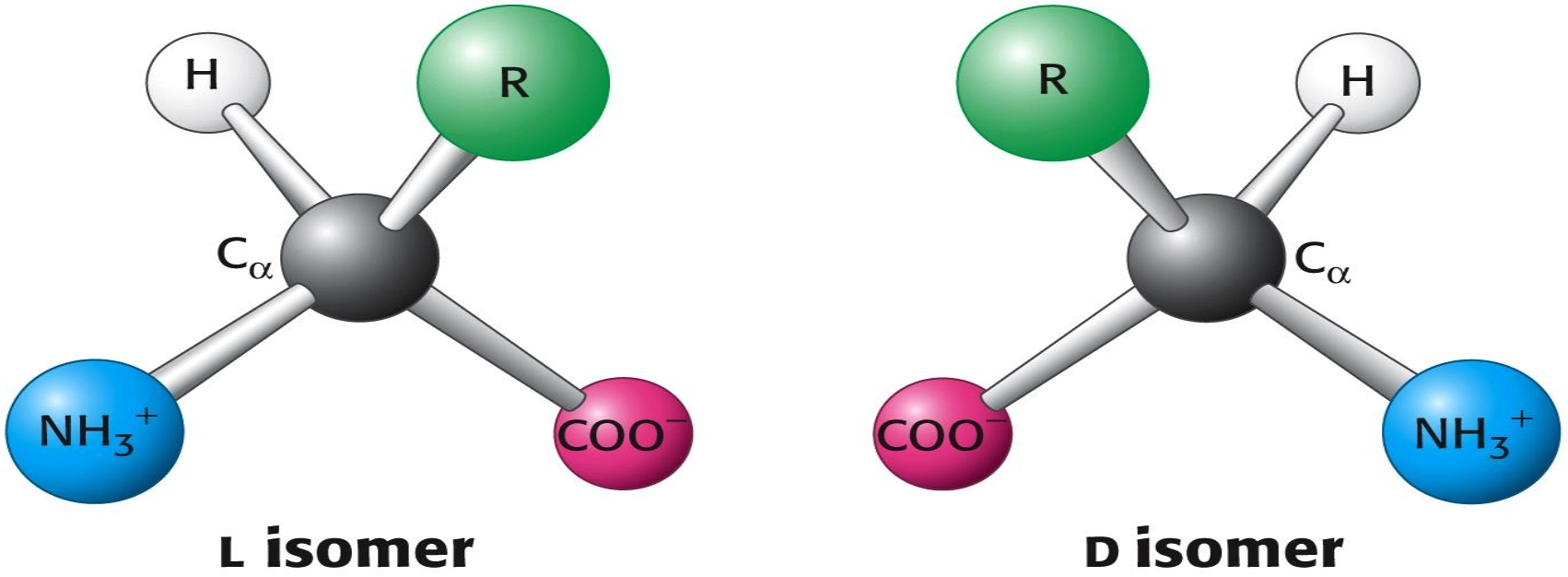


D-Alanine

Perspective formula: the wedge-shaped bonds project out of the plane of the paper and the dashed bonds behind it.

Isomerism

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

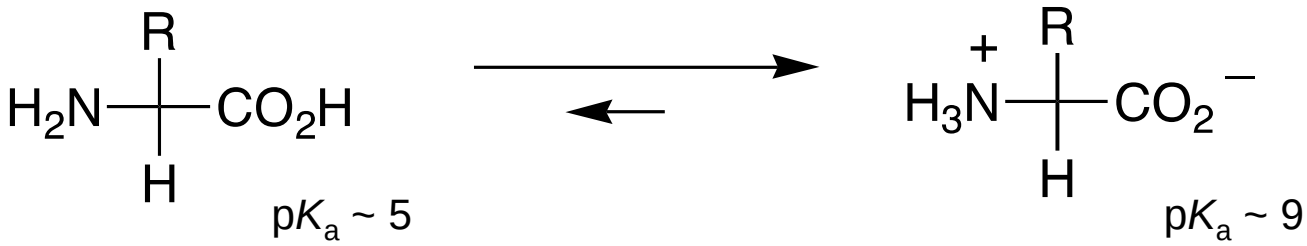


L isomer **D isomer**

Only L-amino acids are constituents of proteins.

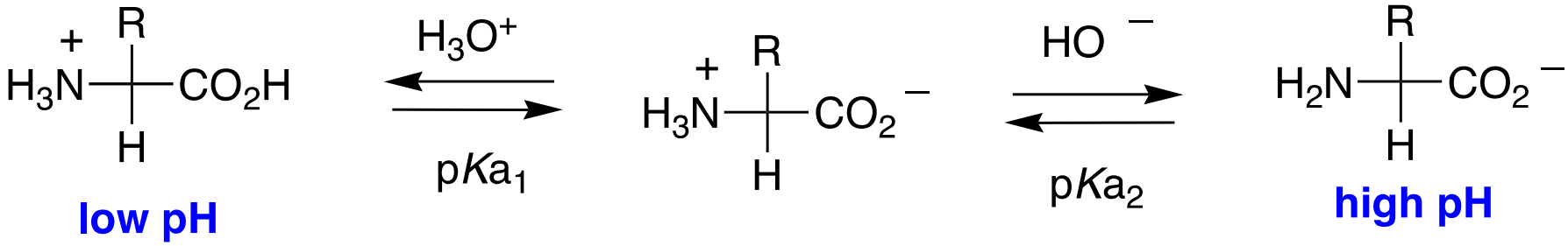
"L" and "D" isomeric nomenclature is similar to the "R" and "S" utilized in modern organic chemistry.

Acid-Base Behavior of Amino Acids. Amino acids exist as a zwitterion: a dipolar ion having both a formal positive and formal negative charge (overall charge neutral).



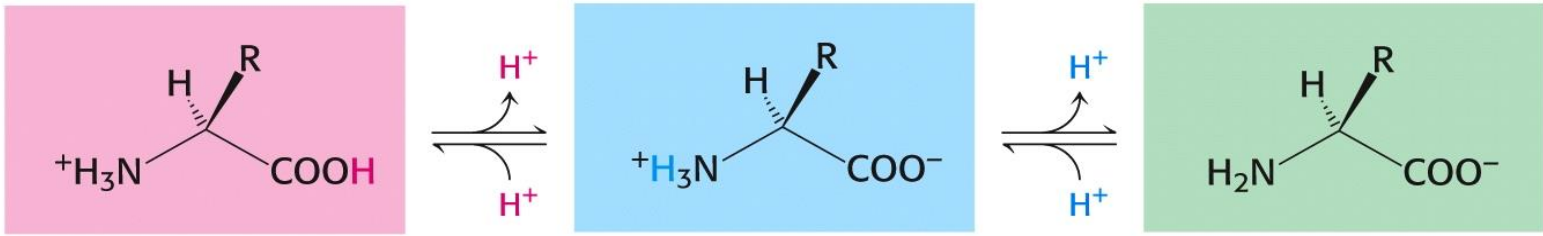
Amino acids are ***amphoteric***: they can react as either an acid or a base. Ammonium ion acts as an acid, the carboxylate as a base.

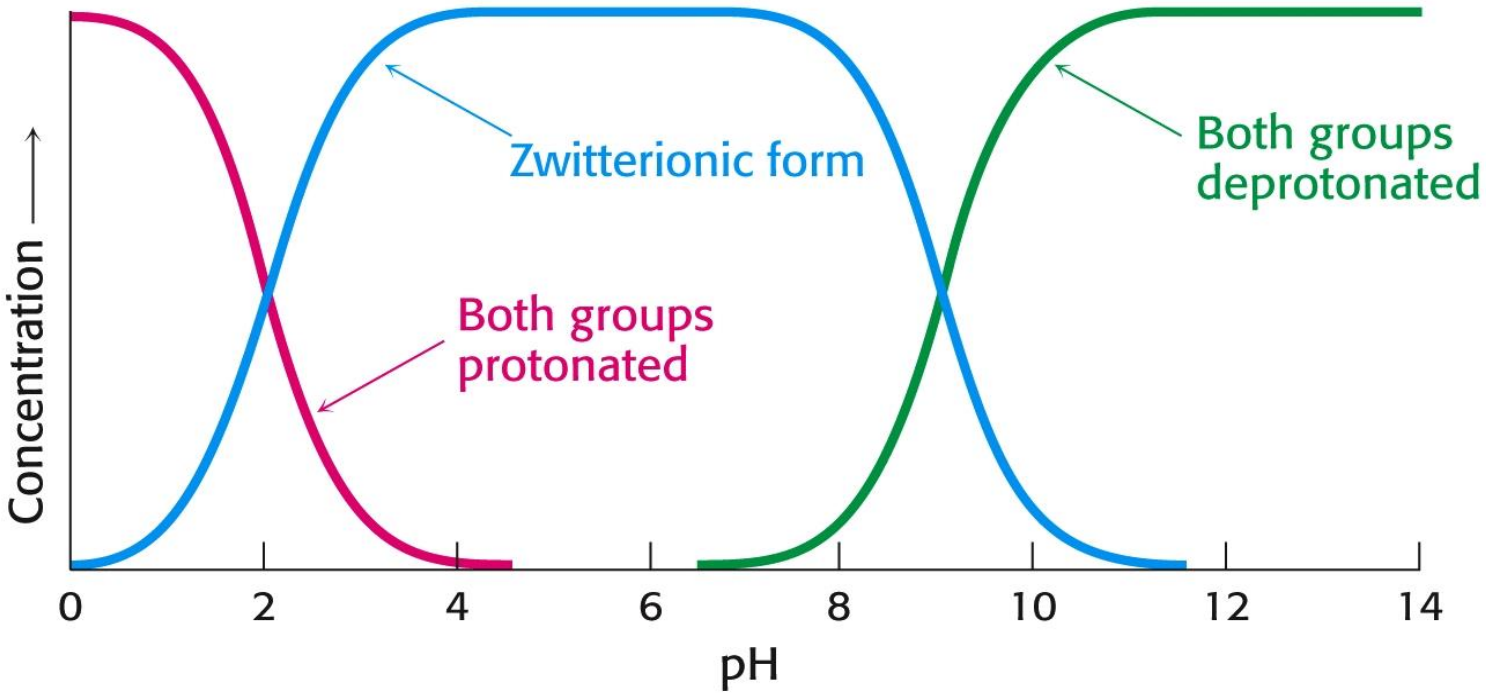
Isoelectric point (pI): The pH at which the amino acid exists largely in a neutral, zwitterionic form (influenced by the nature of the sidechain)



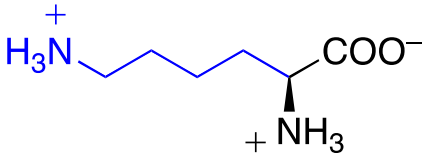
Acid-Base Behavior of Amino Acids

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

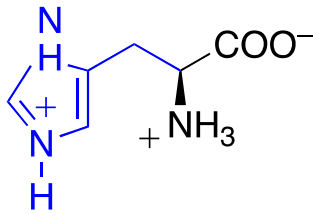




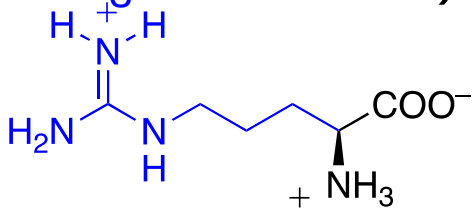
At low pH, proton concentration [H^+] is high. Therefore, both amines and carboxylic acids are protonated. (-NH_3^+ & -COOH)



Basic



Basic



(S)-(+)-Lysine *(Lys, K)*

pKa ~ 10.5

(S)-(–)-Histidine *(His, H)*

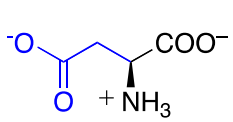
pKa ~ 6.0

(S)-(+)-Arginine *(Arg, R)*

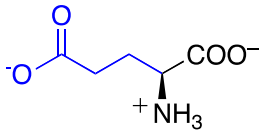
pKa ~ 12.5

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.



Acidic



(S)-(+)-Aspartic Acid *(Asp, D)*

pKa ~ 3.6

(S)-(+)-Glutamic Acid *(Glu, E)*

pKa ~ 4.2

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^-)

Acid-Base Properties of Amino Acids

Draw the following chemical structures for glycine:

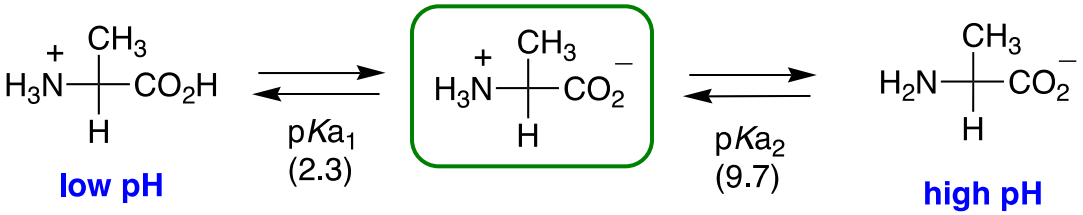
(Non-existent form:) $H_2N - CH_2 - COOH$

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

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

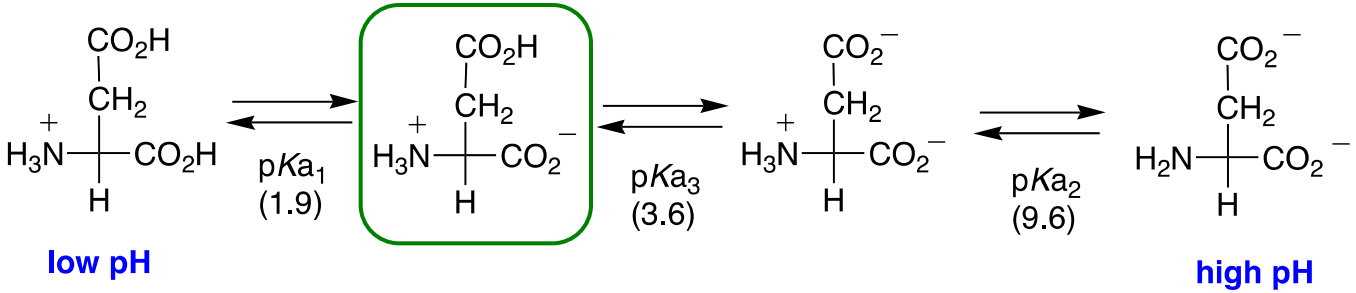
pH=12: $H_2N - CH_2 - COO^{-}$

$$pI = \frac{pKa_x + pKa_y}{2}$$



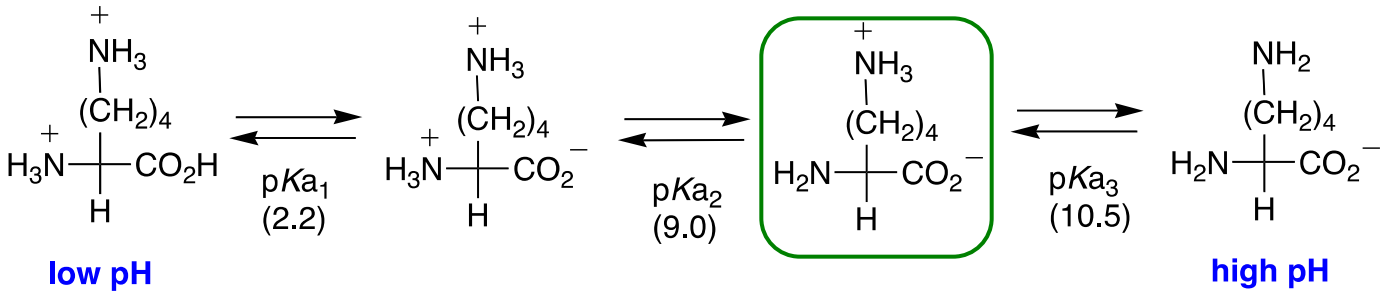
$$pI = \frac{pKa_1 + pKa_2}{2}$$

$$pI = 6.0$$



$$pI = \frac{pKa_1 + pKa_3}{2}$$

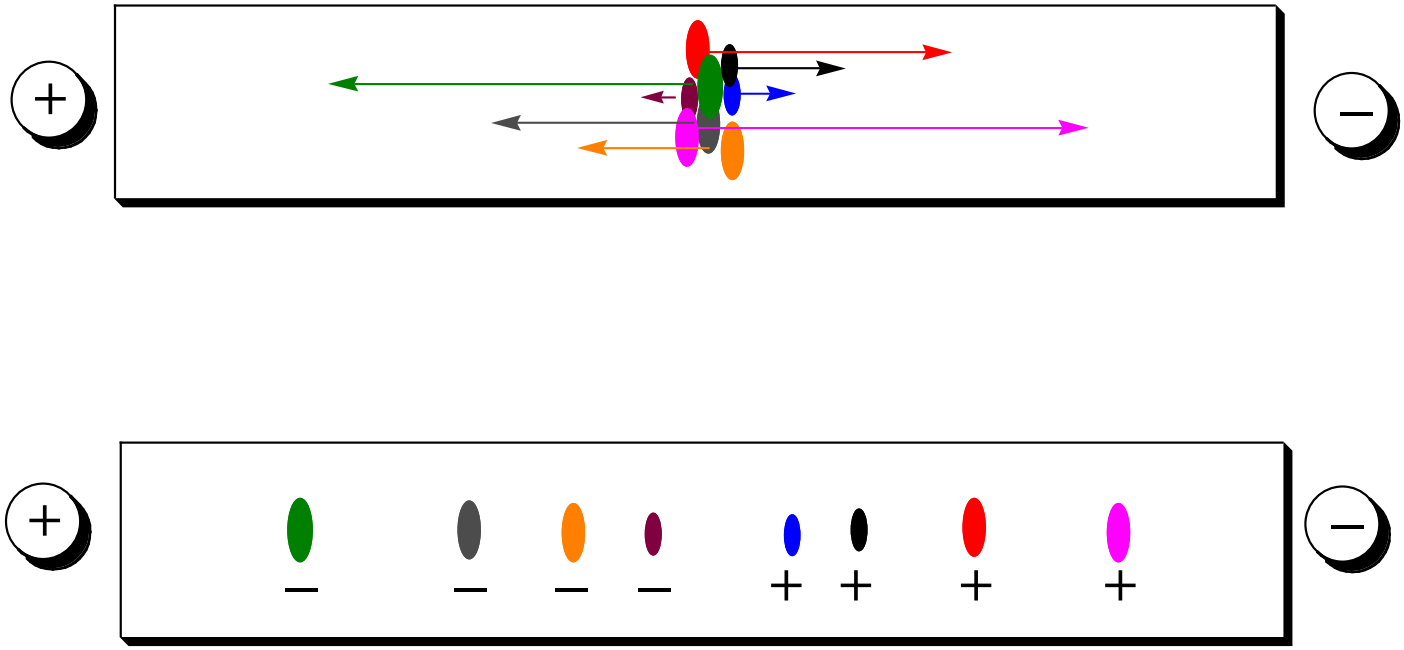
$$pI = 2.7$$



$$pI = \frac{pKa_2 + pKa_3}{2}$$

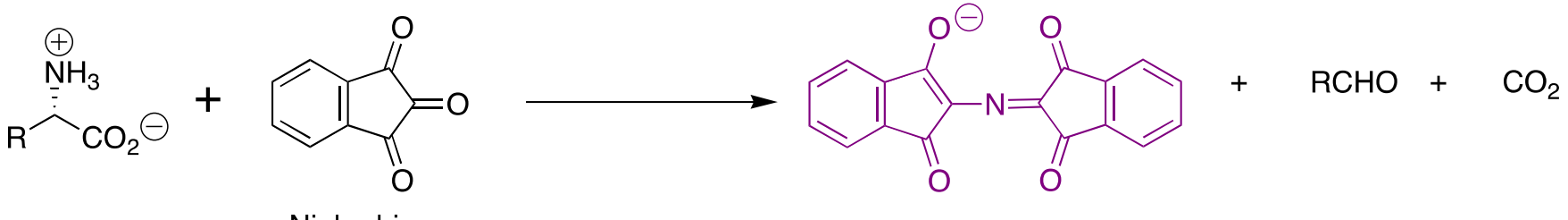
$$pI = 9.7$$

Electrophoresis: separation of polar compounds based on their mobility through a solid support. The separation is based on charge (pI) or molecular mass.



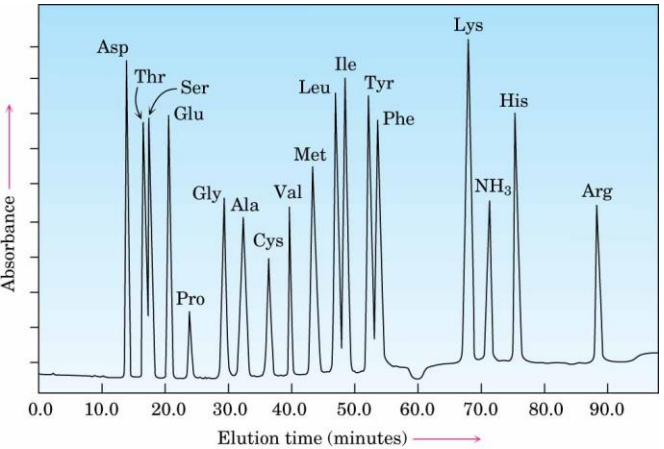
Amino Acid Analysis automated method to determine the amino acid content of a peptide or protein

Reaction of primary amines with ninhydrin



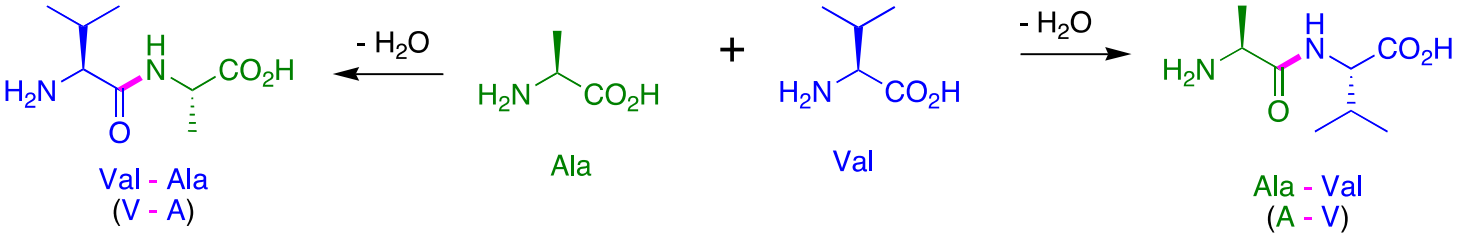
peptide -or- protein → [H] → reduce any disulfide bonds → Enzymatic digestion -or- H_3O^+, Δ → **individual amino acids**

→ liquid chromatography → derivatize w/ ninhydrin → Detected w/ UV-vis

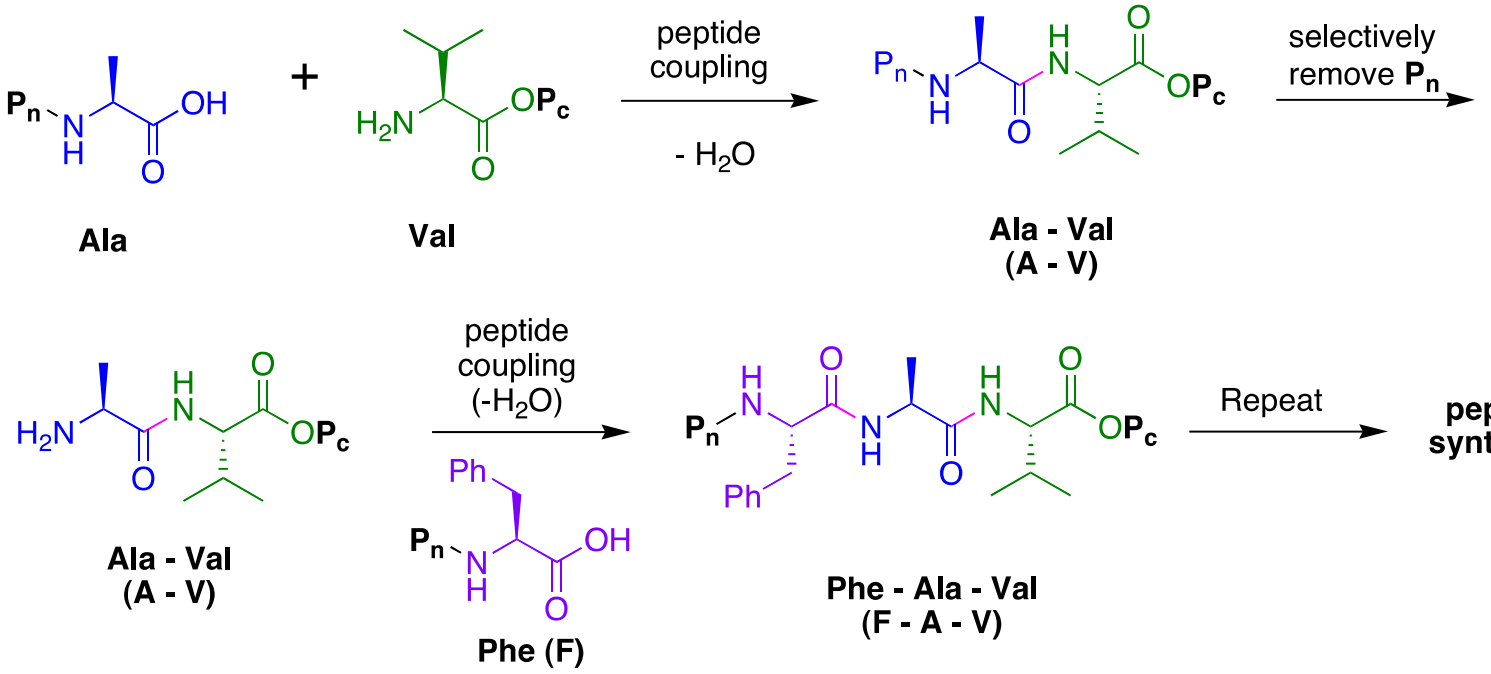


Different amino acids have different chromatographic mobilities (retention times)

1972 Nobel Prize in Chemistry
William Stein
Stanford Moore

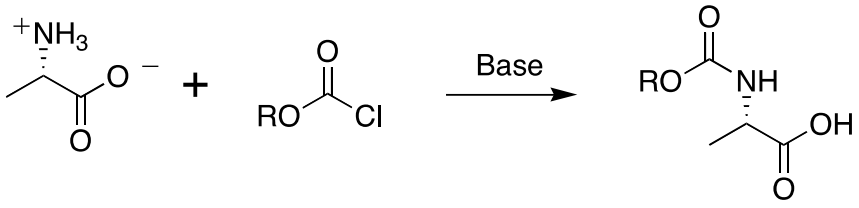


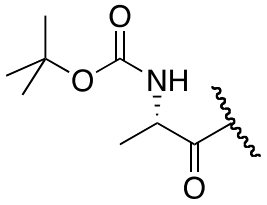
The need for protecting groups



Orthogonal protecting group strategy: the carboxylate protecting group must be stable to the reaction conditions for the removal of the α-amino protecting group and (*vice versa*)

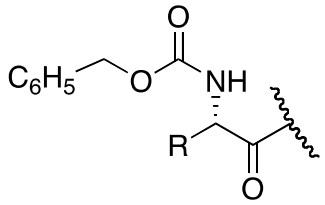
Amino Group Protection. The α-amino group is protected as a carbamate





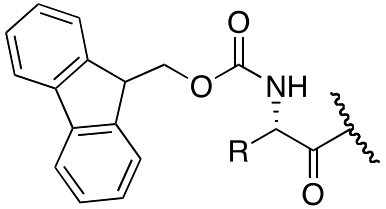
tert-butoxycarbonyl
(t-BOC)

removed with
mild acid



benzyloxycarbonyl
(cBz)

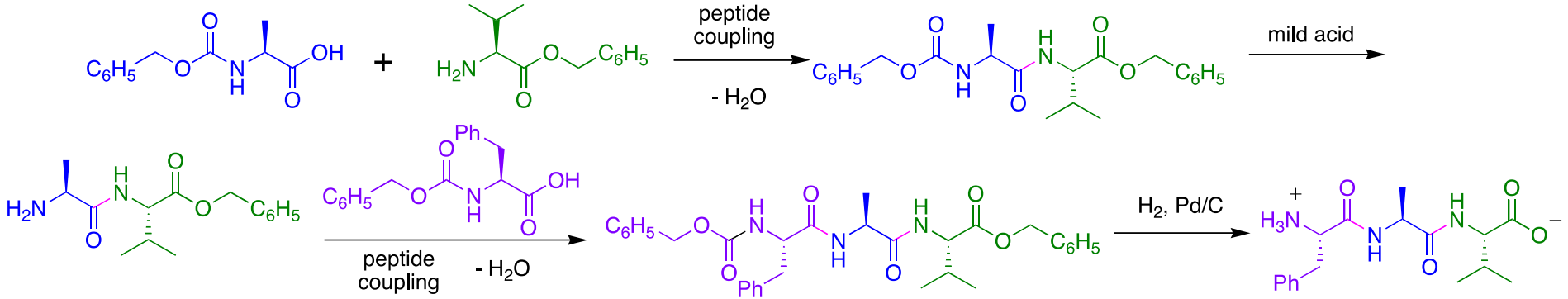
removed with mild acid
or by hydrogenolysis



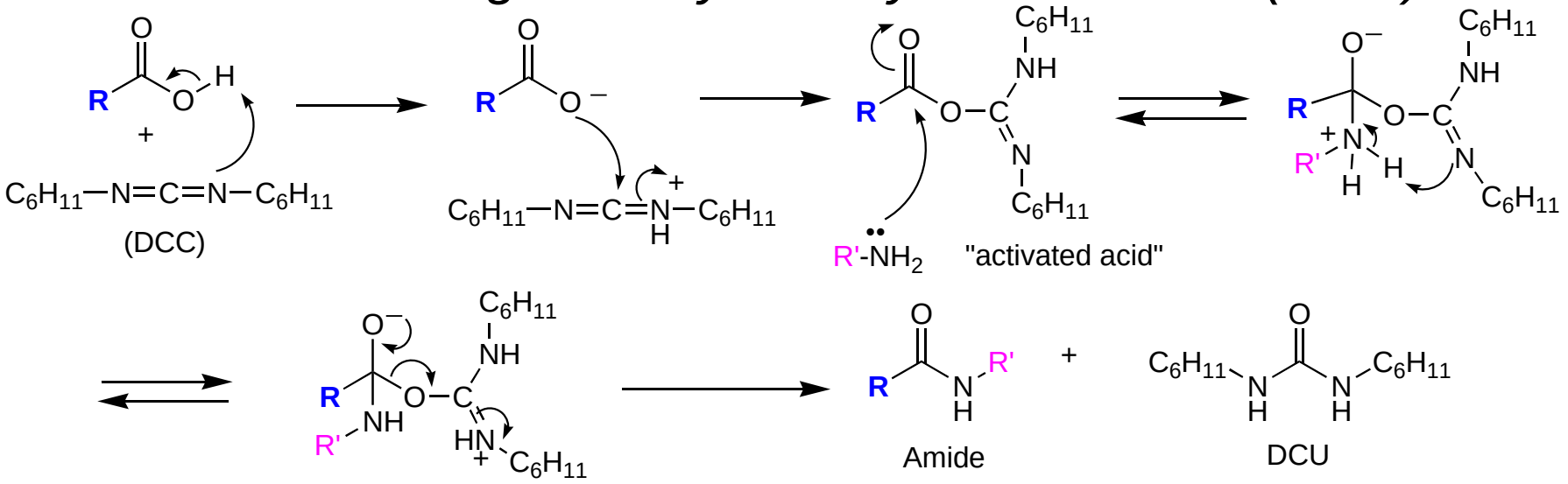
fluorenylmethylcarbonyl
(FMOC)

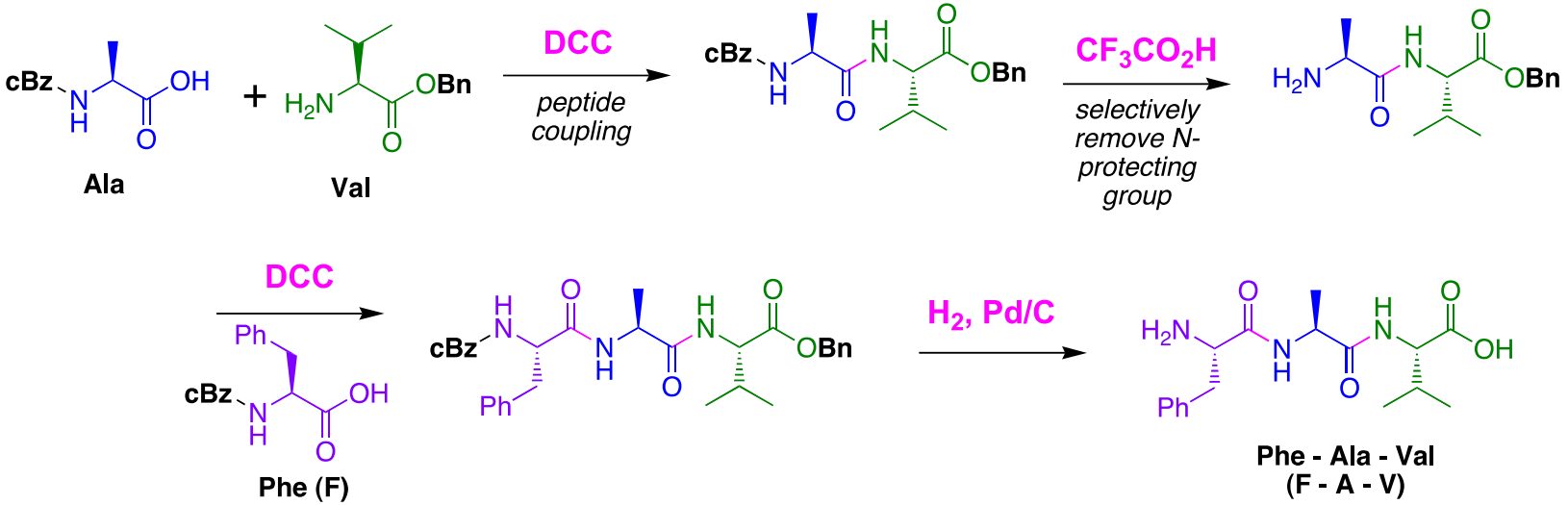
removed with mild base
(piperidine)

Carboxyl Group Protection. Protected as a benzyl ester; removed by hydrogenolysis



Peptide Bond Formation. Amide formation from the reaction of an amine with a carboxylic acid is slow. Amide bond formation (peptide coupling) can be accelerated if the carboxylic acid is activated. *Reagent: dicyclohexylcarbodiimide (**DCC**)*





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

Protein Structure:

- Twisting about various bonds in the polypeptide backbone gives proteins a variety of shapes.

- Bond angles give rise to secondary structures. Then, localized secondary structures help drive the peptide folding that gives rise to tertiary structure.

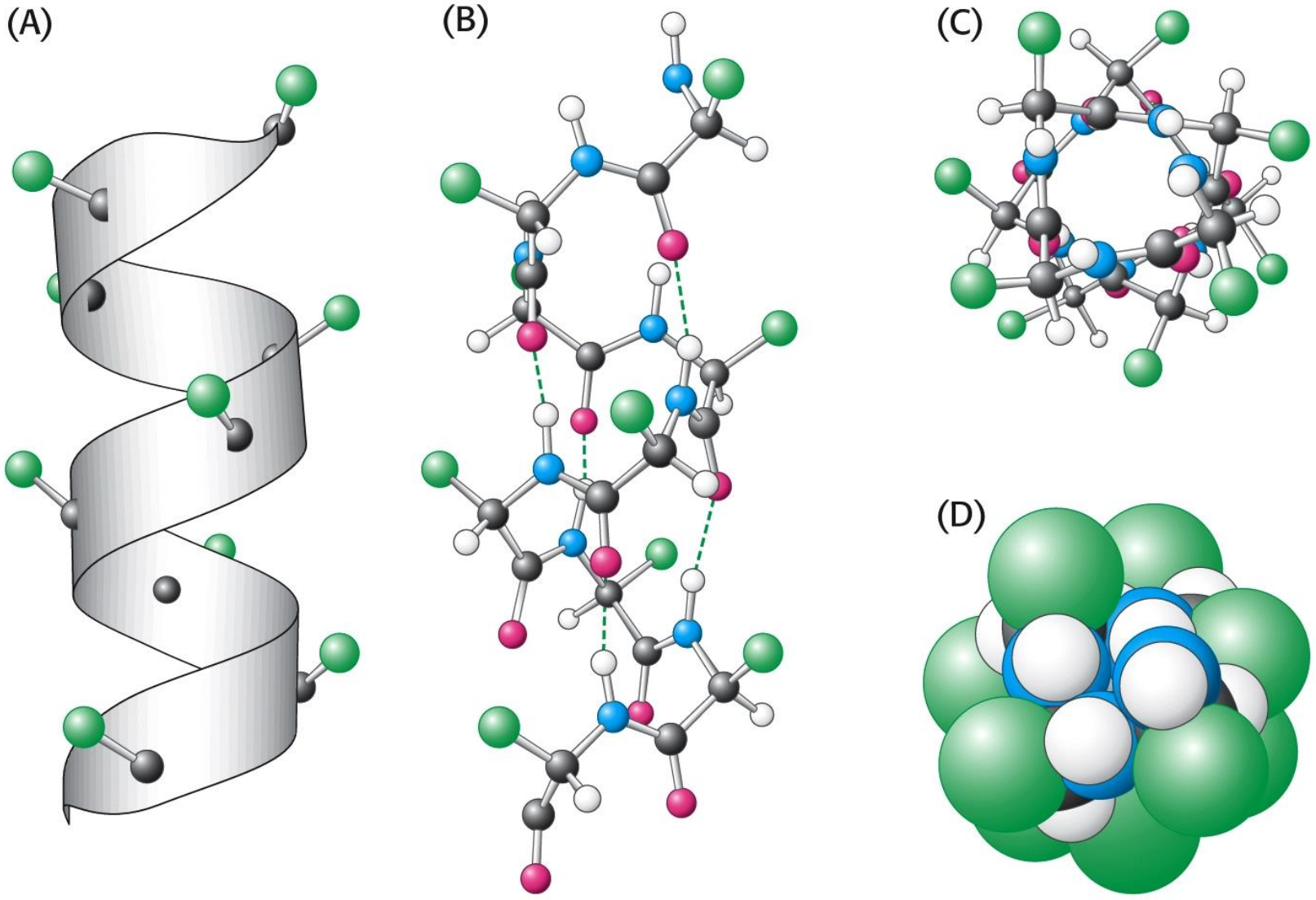
Secondary Structure in Proteins:

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

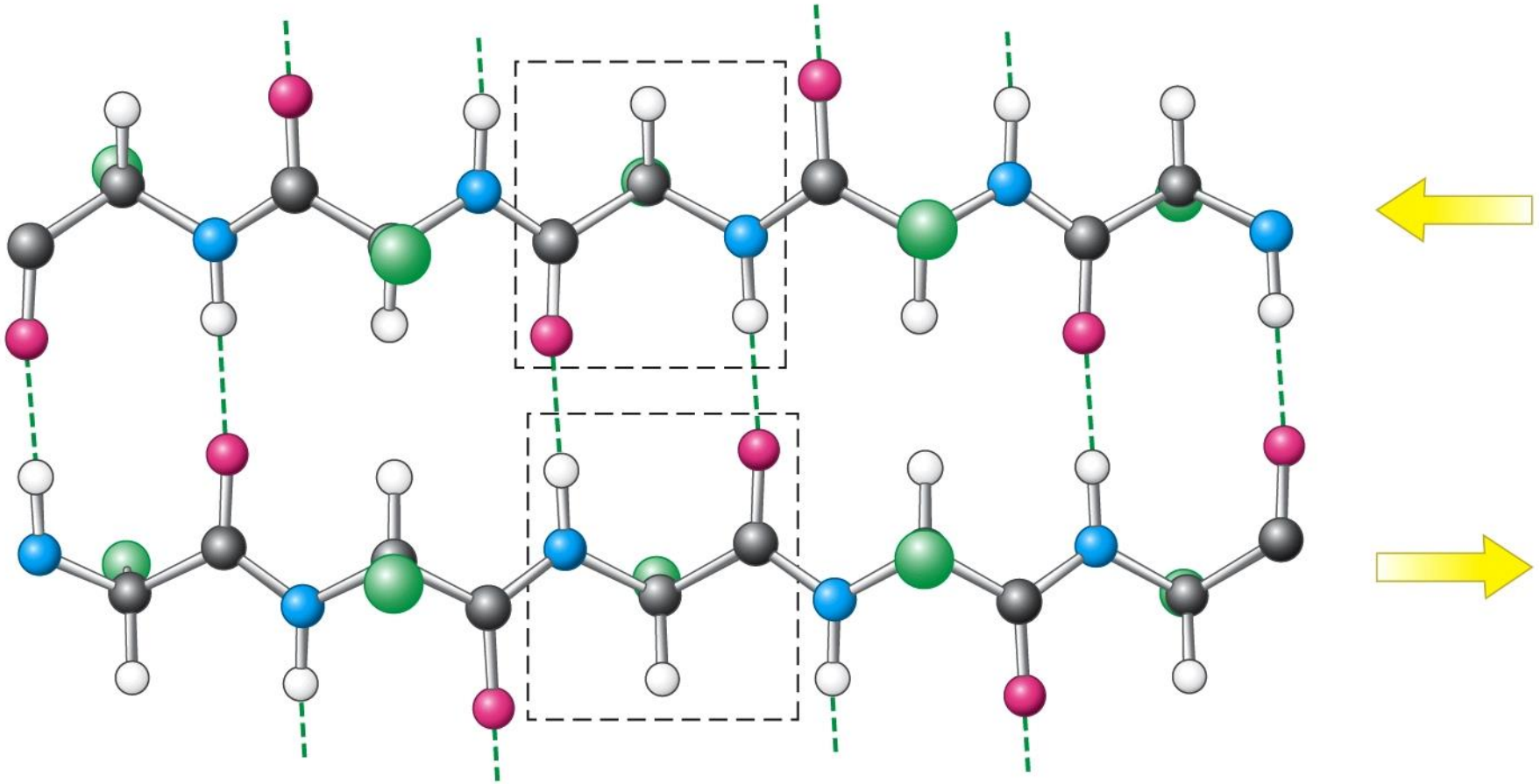
alpha – helix *beta - sheet*

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.

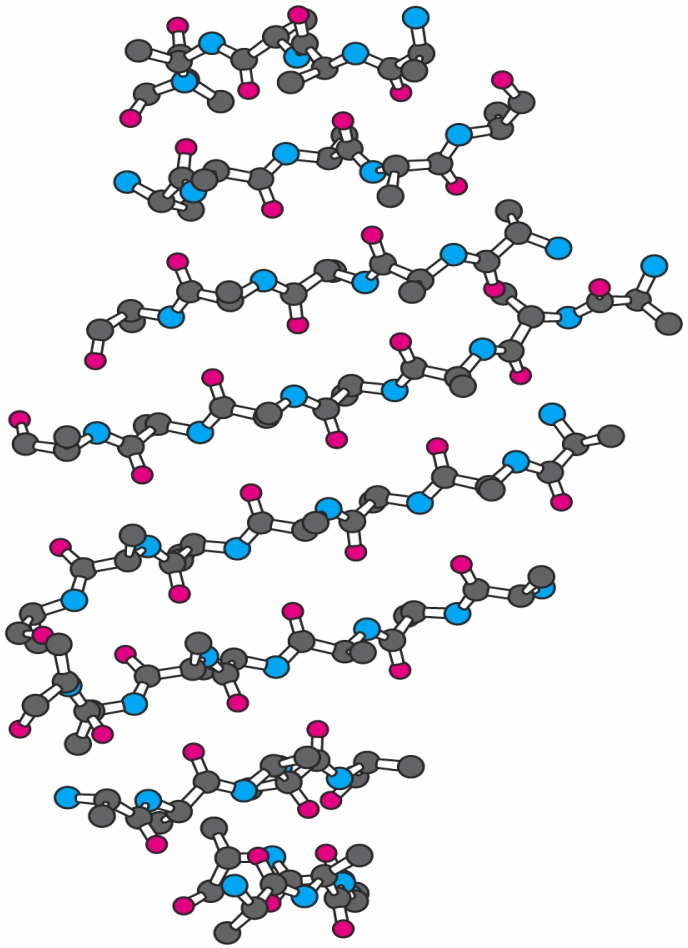
- The *alpha* (*α*) – *helix:*

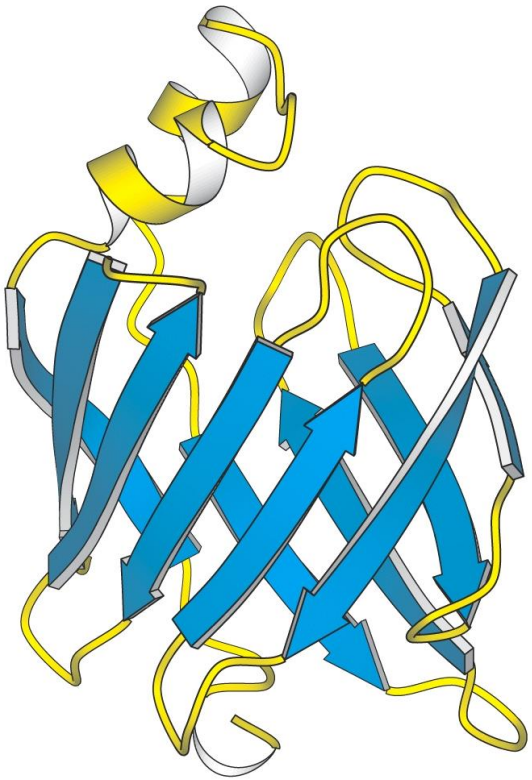


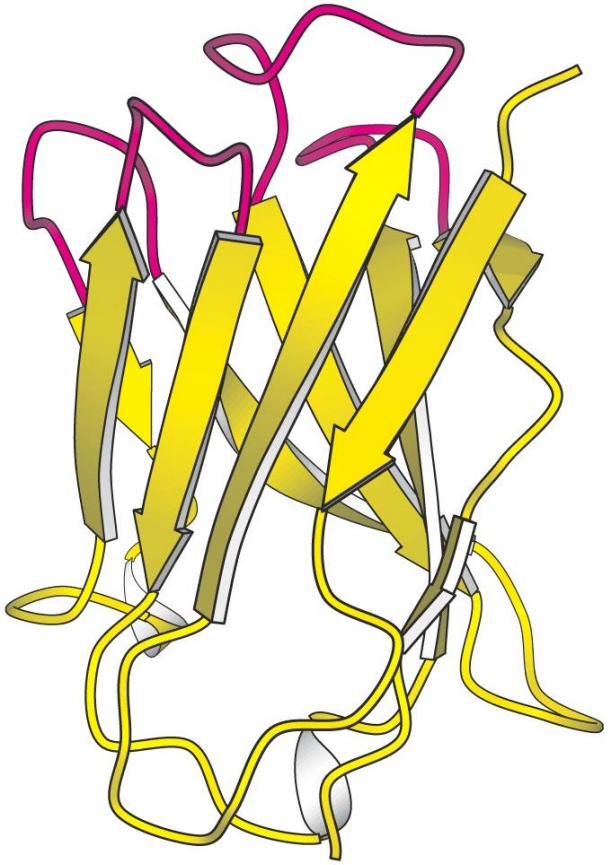
beta – sheet (antiparallel):



Examples of *beta*-sheet domains in proteins:

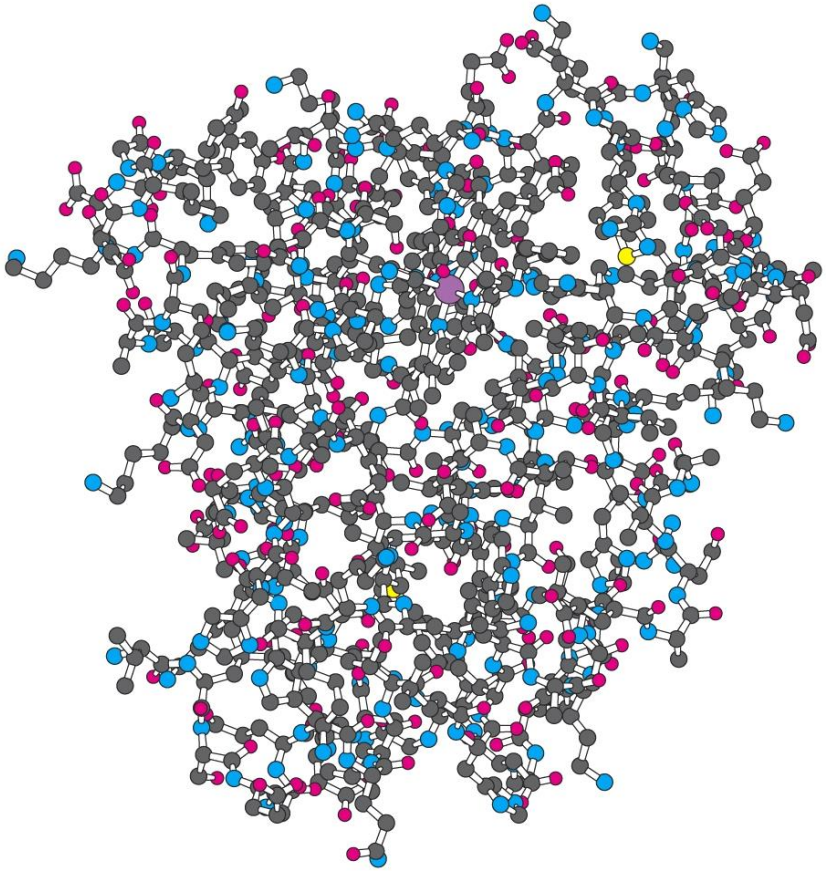


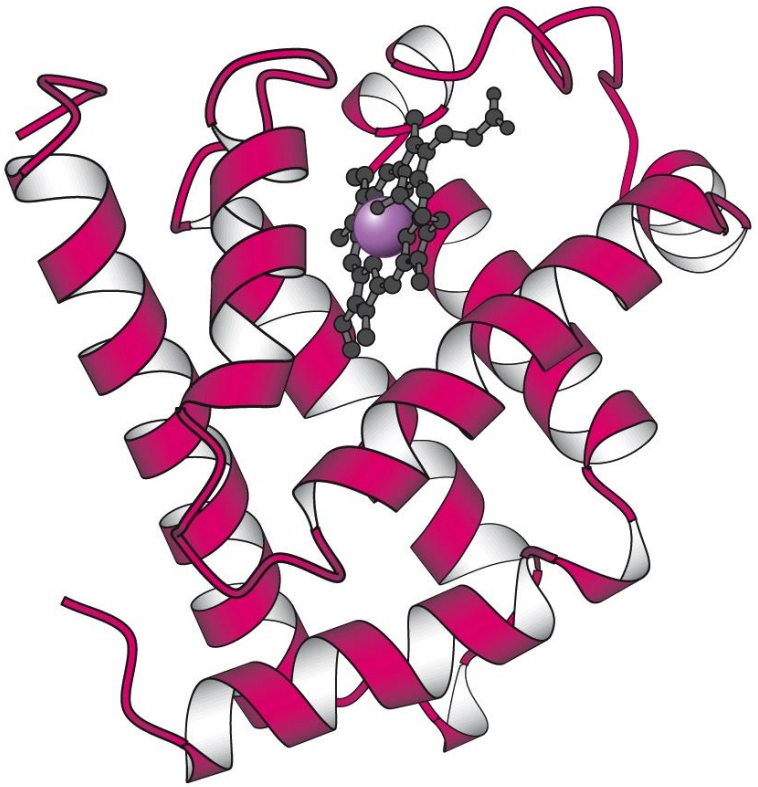




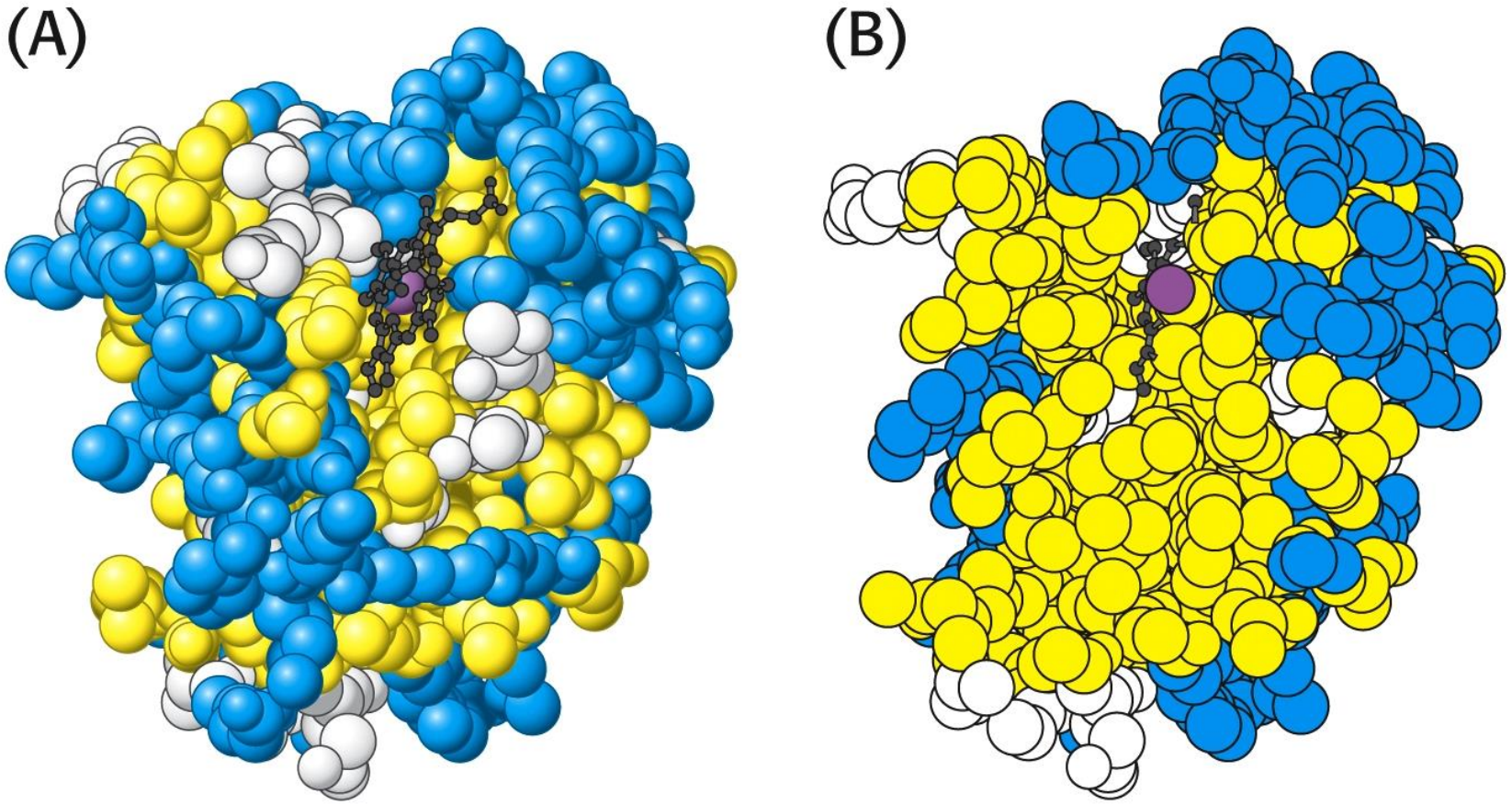
Tertiary (3°) Structure the Protein Myoglobin

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

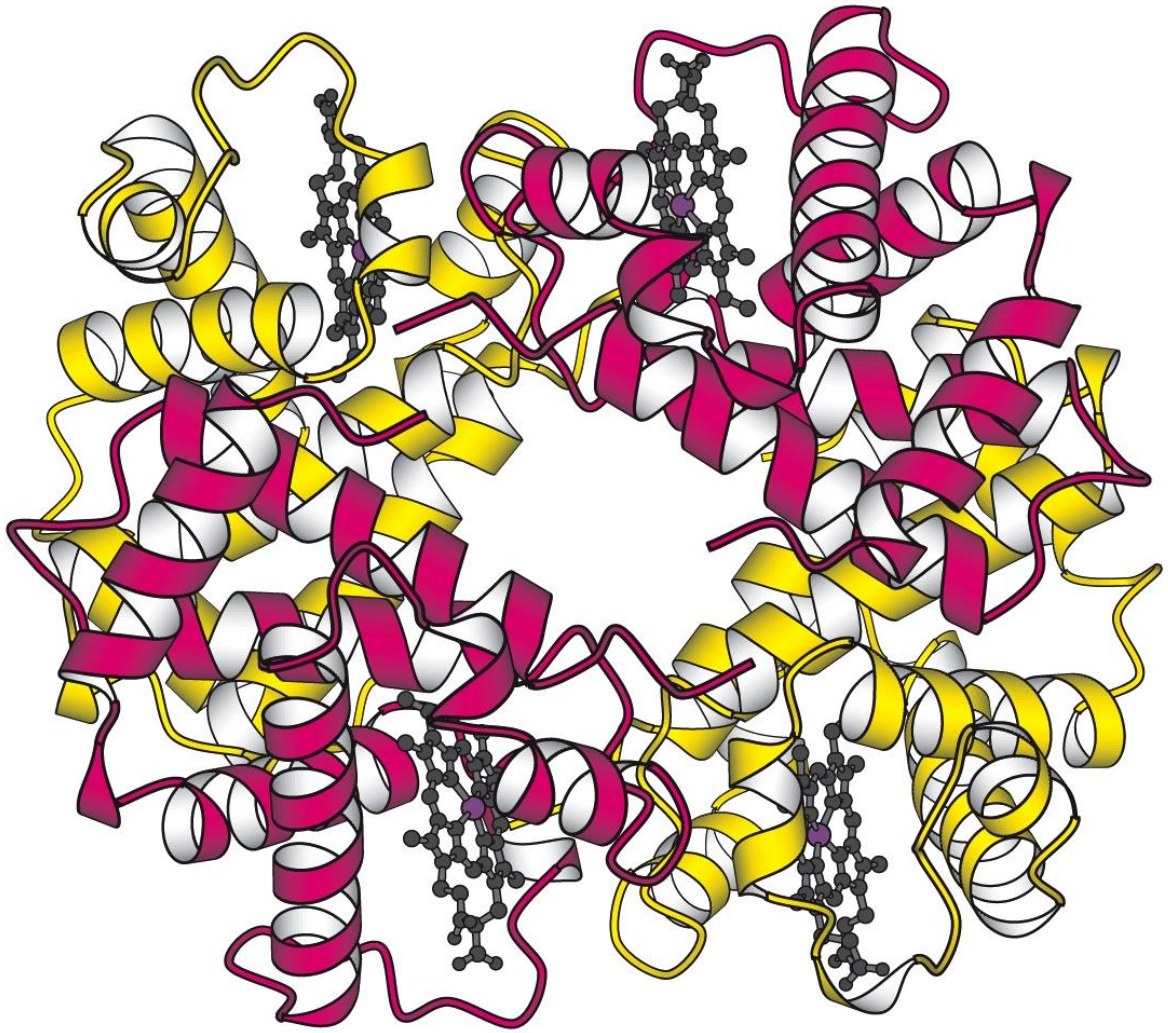




- In the case of myoglobin and many other proteins, the majority of hydrophobic amino acids (yellow) are found inside in structure:



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

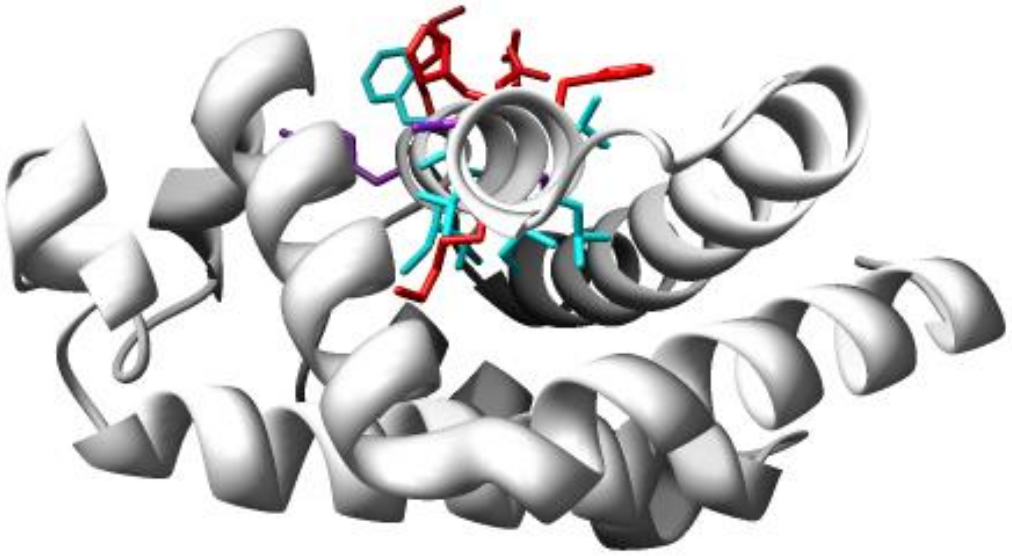


Tertiary Structure of polypeptides and Proteins

Fibrous. Polypeptides strands that "bundle" to form elongated fibrous assemblies; insoluble.

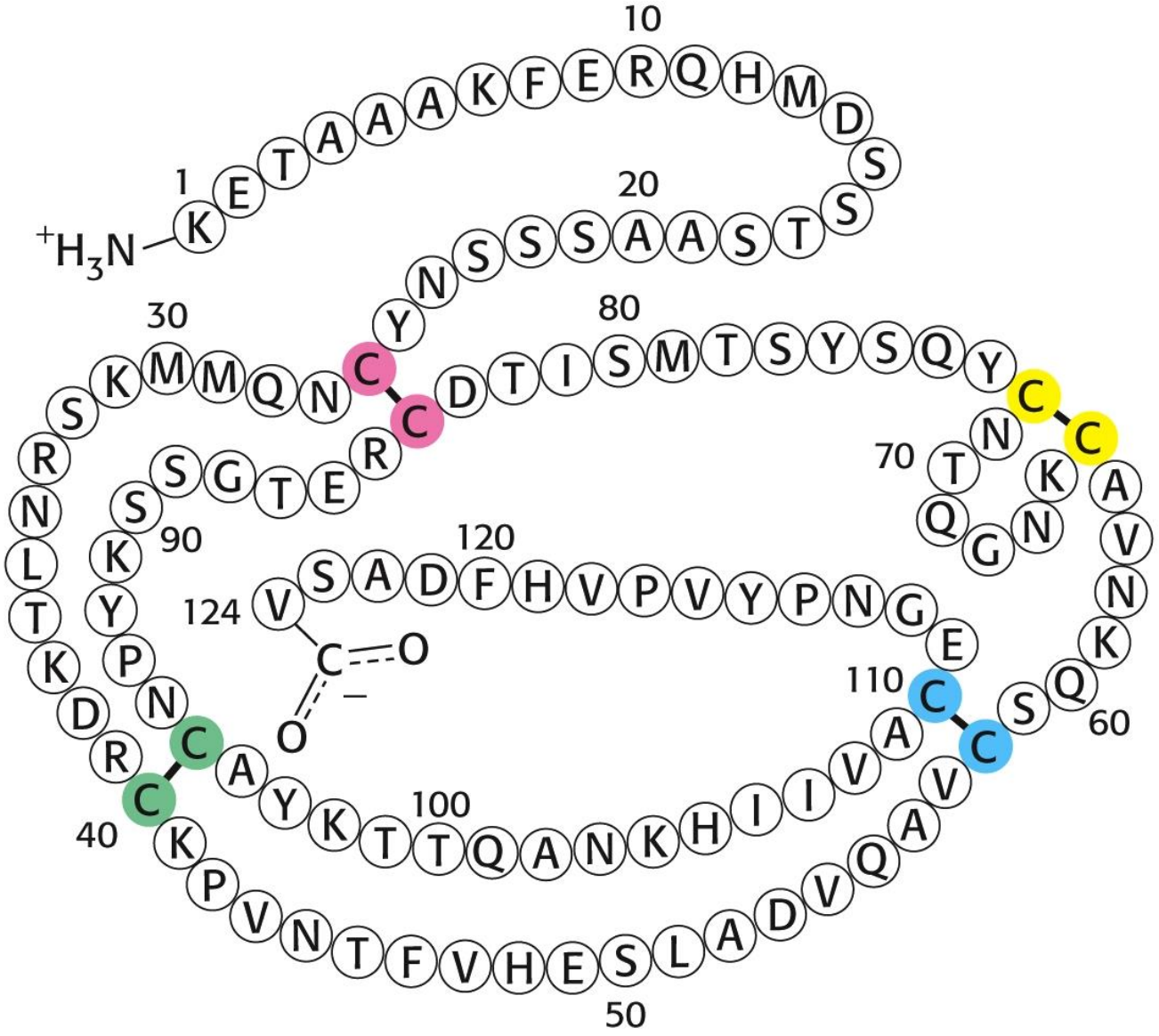
Globular. Proteins that fold into a "spherical" conformation.

Hydrophobic effect. Proteins will fold so that *hydrophobic* amino acids are on the inside (shielded from water) and *hydrophilic* amino acids are on the outside (exposed to water)

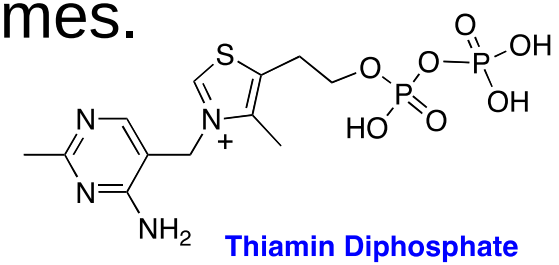


Pro • Ile • Lys • Tyr • Leu • Glu • Phe • Ile • Ser • Asp • Ala • Ile • Ile • His •Val • His • Ser • Lys

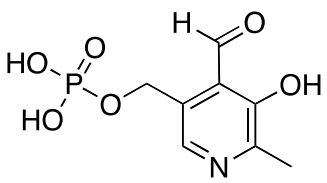
- Amino acid sequence of ribonuclease:



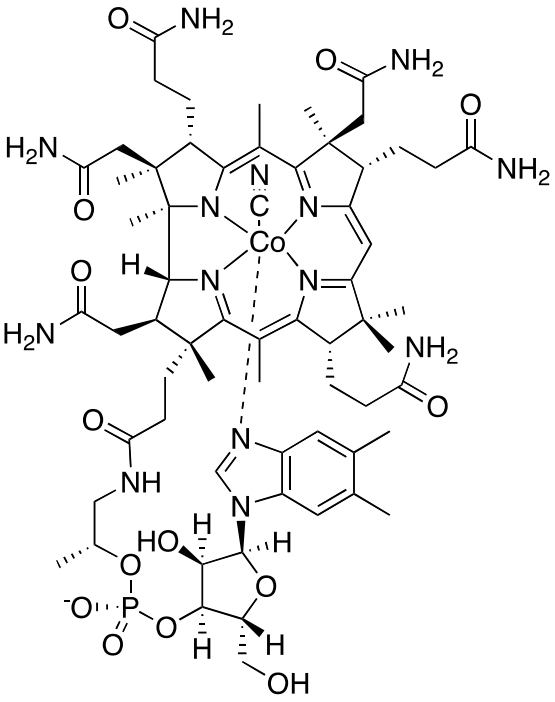
Coenzymes. Some reactions require additional organic molecules or metal ions. These are referred to as cofactors or coenzymes.



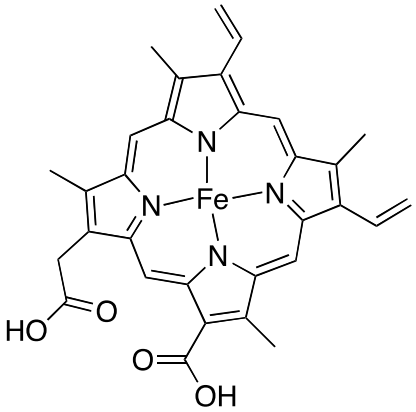
Thiamin Diphosphate
(vitamin B_1)



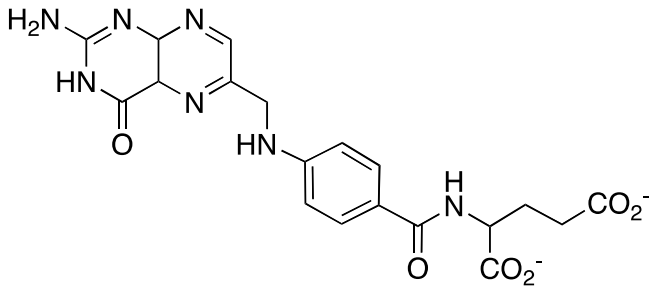
Pyridoxal Phophates
(vitamin B_6)



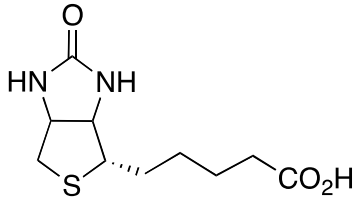
Vitamin B_{12}
(cyanocobalamin)



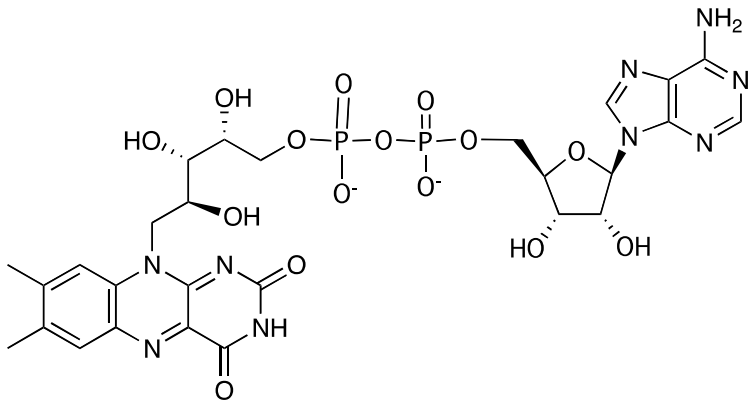
Heme



Folic Acid
(vitamin B_9)



Biotin
(vitamin B_7)



Flavin Adenine Diphosphate (FAD)
(Vitamin B_2)

Denaturing

- Alteration of the protein's shape and thus functions through the use of
  - Heat
  - Acids
  - Bases
  - Salts
  - Mechanical agitation
- Primary structure is unchanged by denaturing