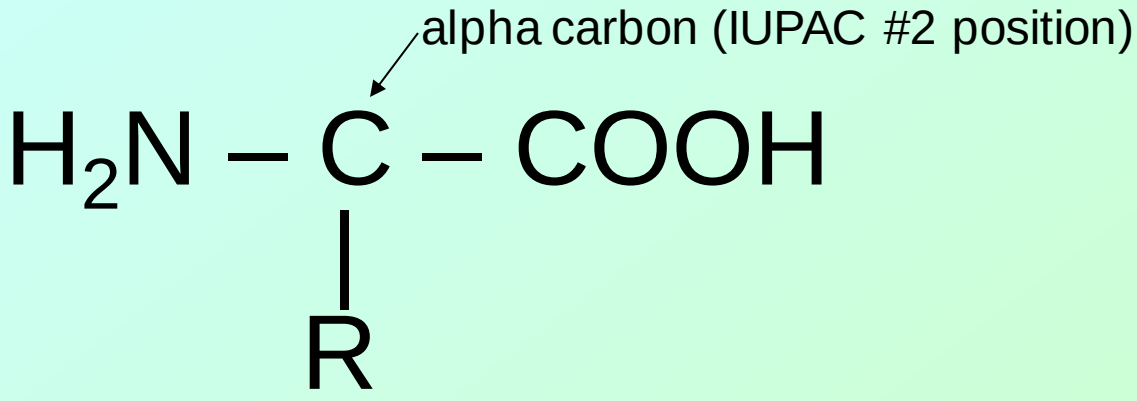
Amino Acids & Proteins

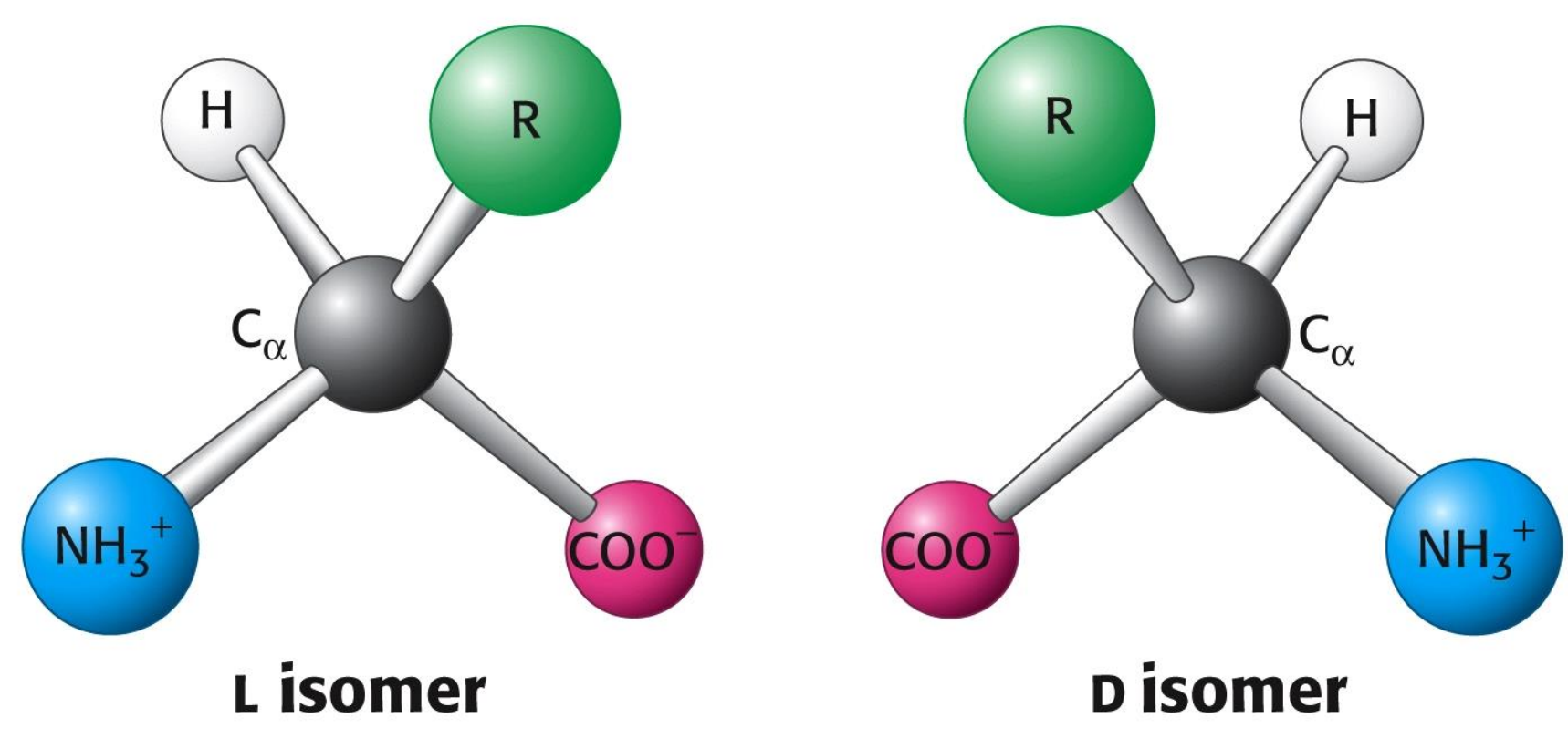
- Proteins are linear copolymers built from monomeric units called amino acids.
- Twenty amino acids are commonly found in proteins.
- These amino acids contain a variety of different functional groups:
  - Alcohols (R-OH)
  - Phenols (Ph-OH)
  - Carboxylic acids (R-COOH)
  - Thiols (R-SH)
  - Amines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



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.

- 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:

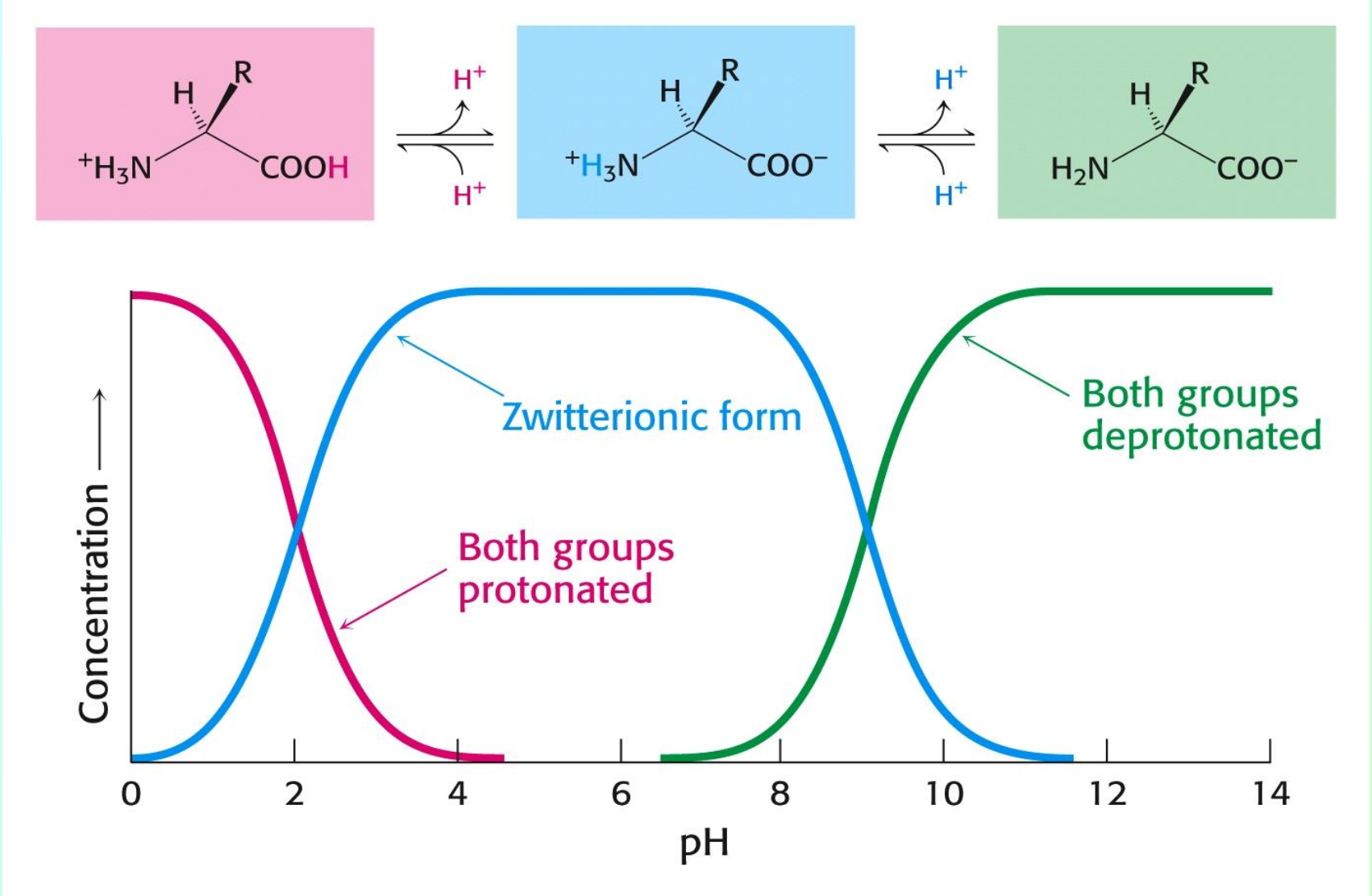


TABLE 3.4 **pK_a values of some amino acids**

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.

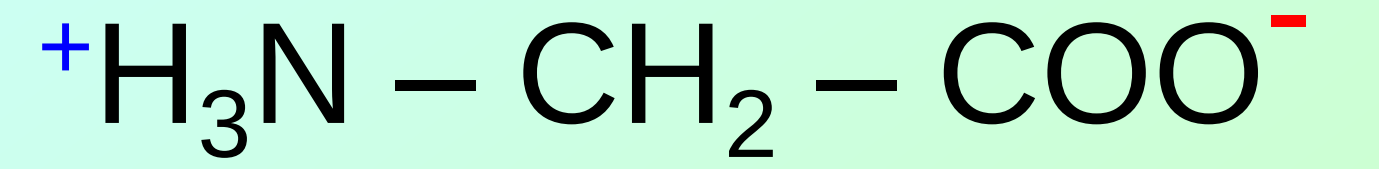
- Summary:

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:



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: $^{+}H_3N - CH_2 - COOH$

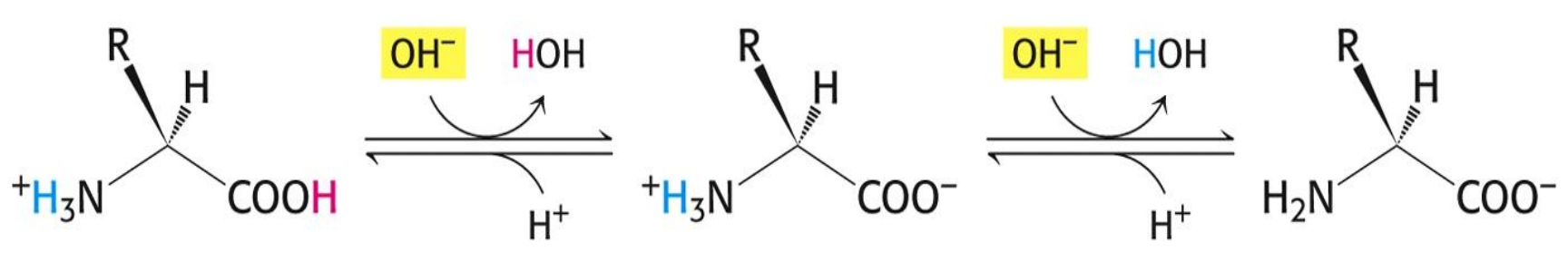
pH=7: $^{+}H_3N - CH_2 - COO^{-}$

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

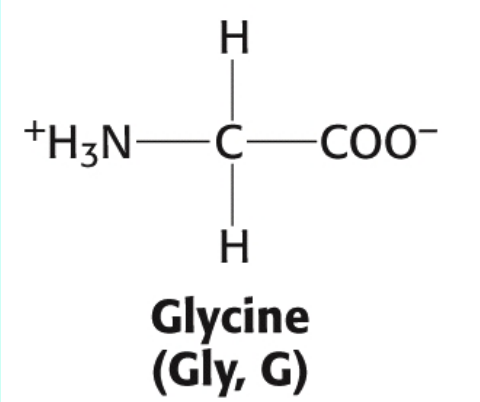
Low pH

Neutral pH

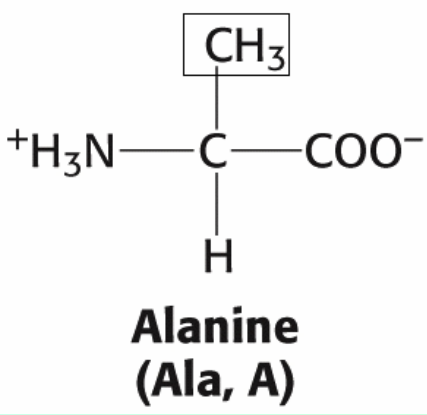
High pH



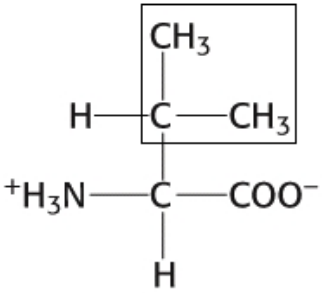
Amino acids: (*Aliphatic*)



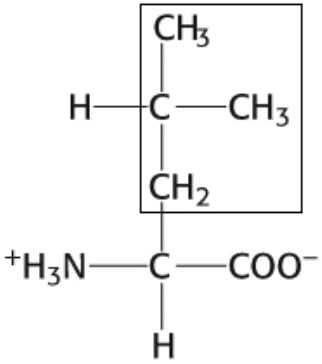
Glycine (Gly, G)



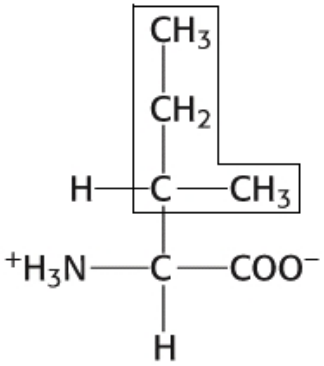
Alanine (Ala, A)



Valine (Val, V)



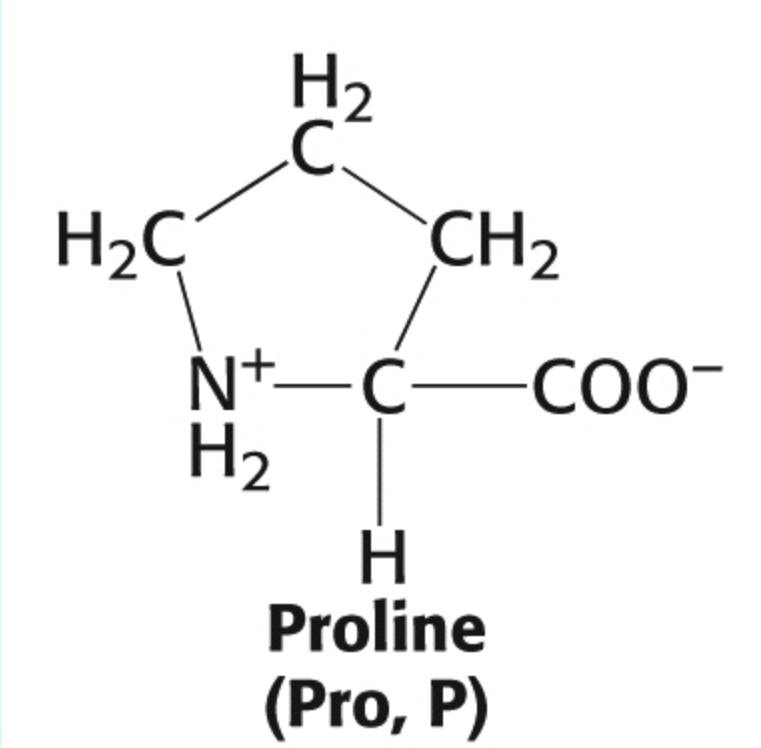
Leucine (Leu, L)



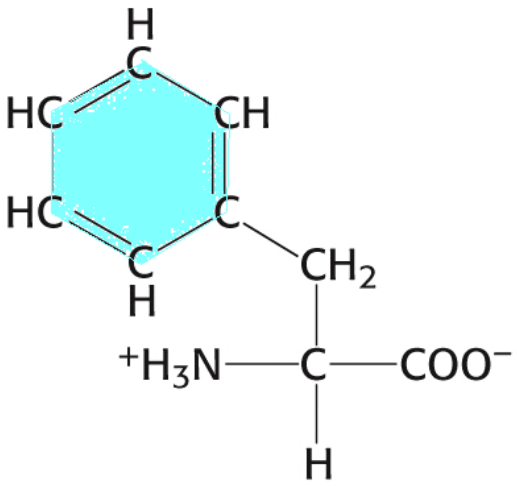
Isoleucine (Ile, I)

- Amino acid Proline

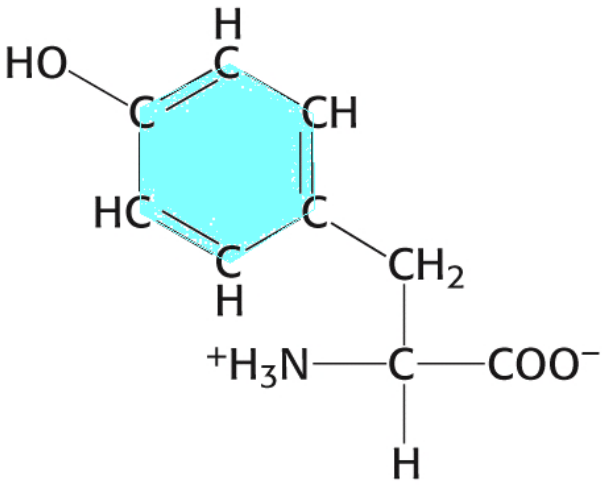
  (The only secondary (2°) amino acid or ("imino" acid.)



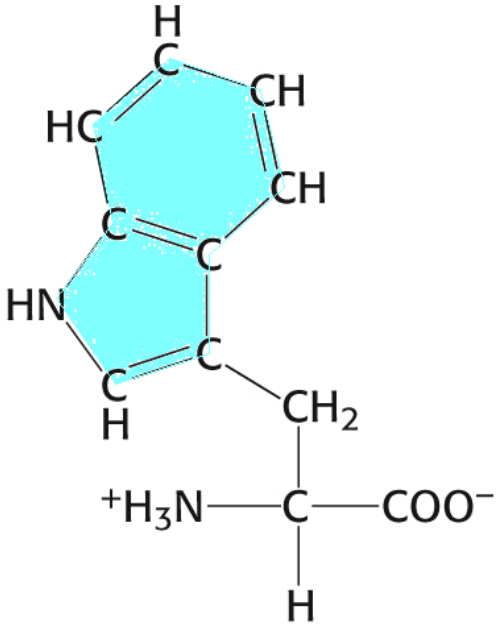
- Amino acids (*Aromatic*)



Phenylalanine (Phe, F)

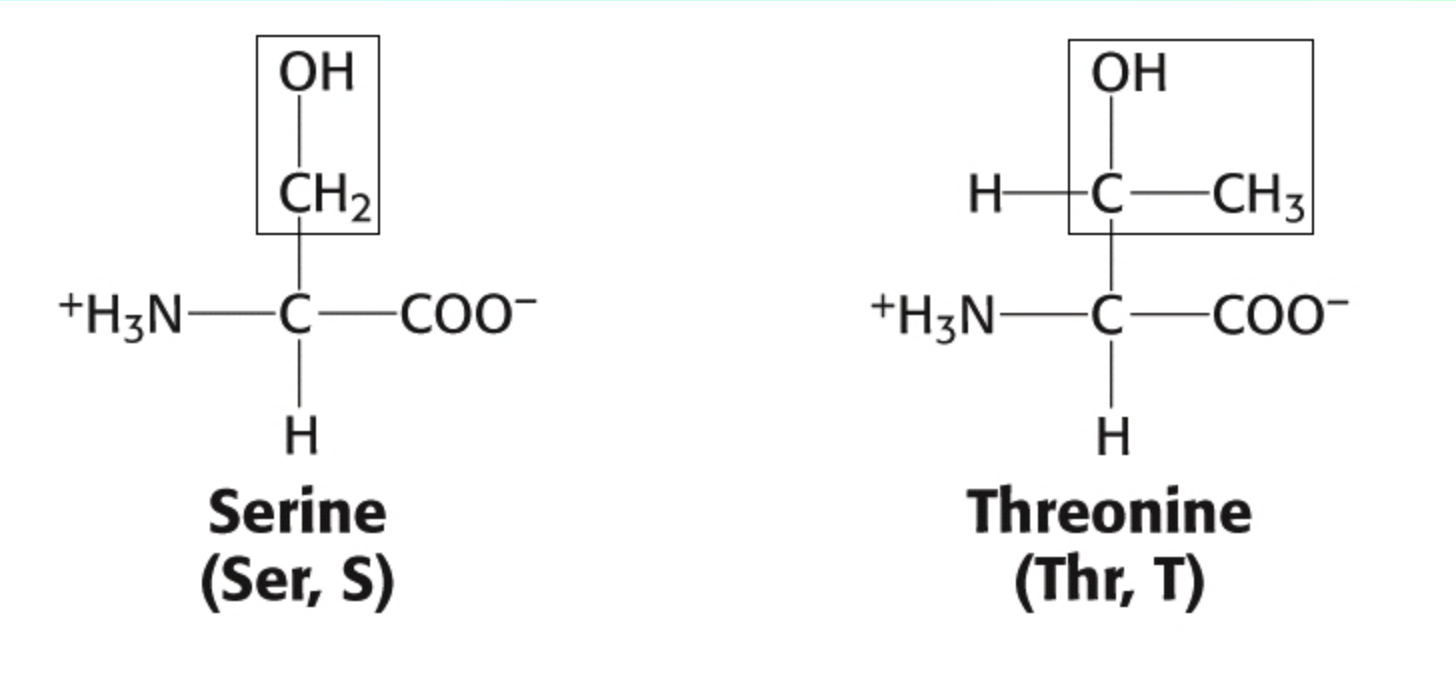


Tyrosine (Tyr, Y)

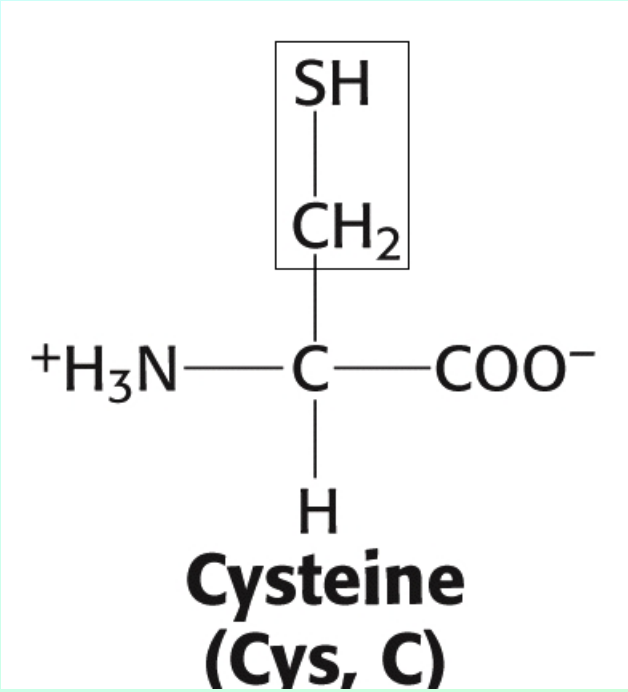


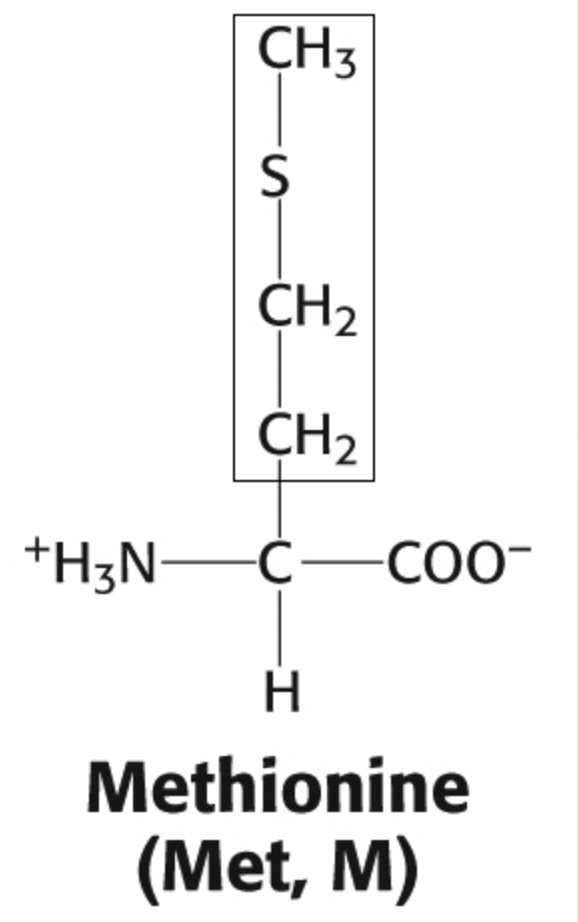
Tryptophan (Trp, W)

- Amino acids (*Alcohols*)

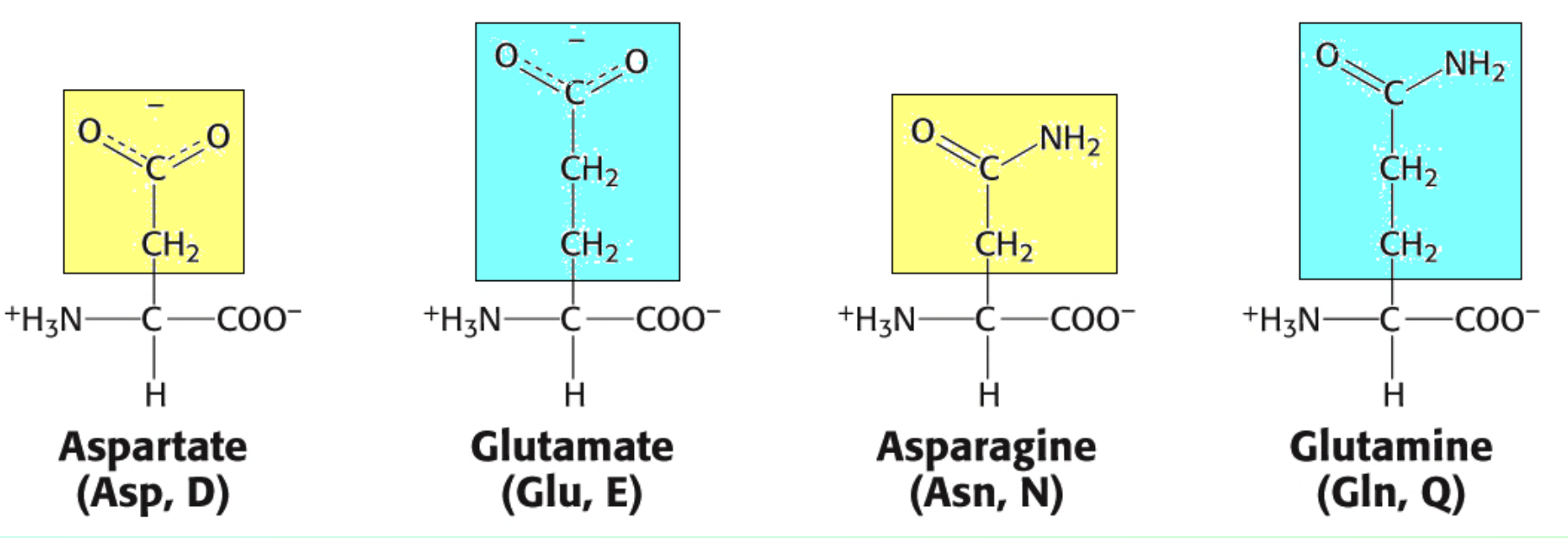


- Amino acids (*Sulfur*)

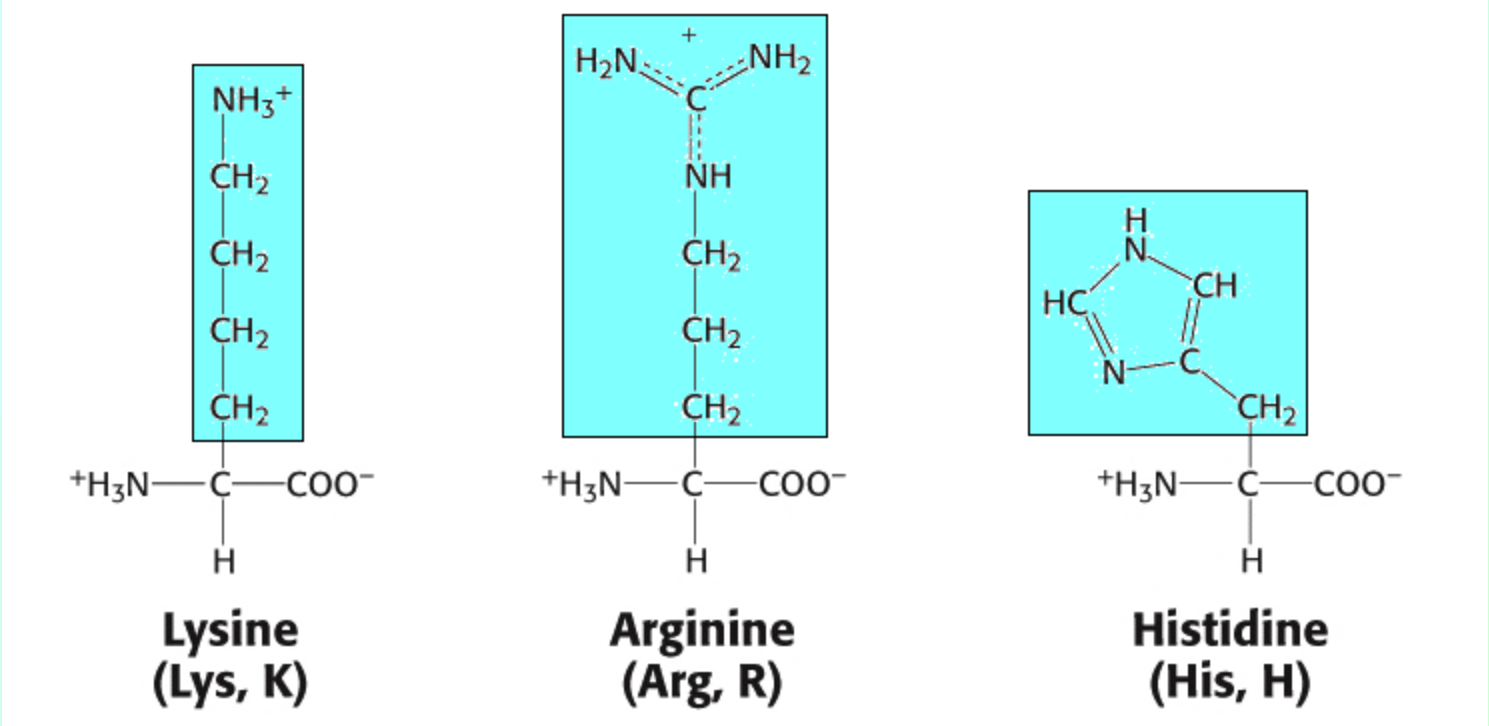




- Amino acids (*Acids and related amides*)



- Amino acids (*Basic*)



- Histidine (*Acid/Base Activity*)

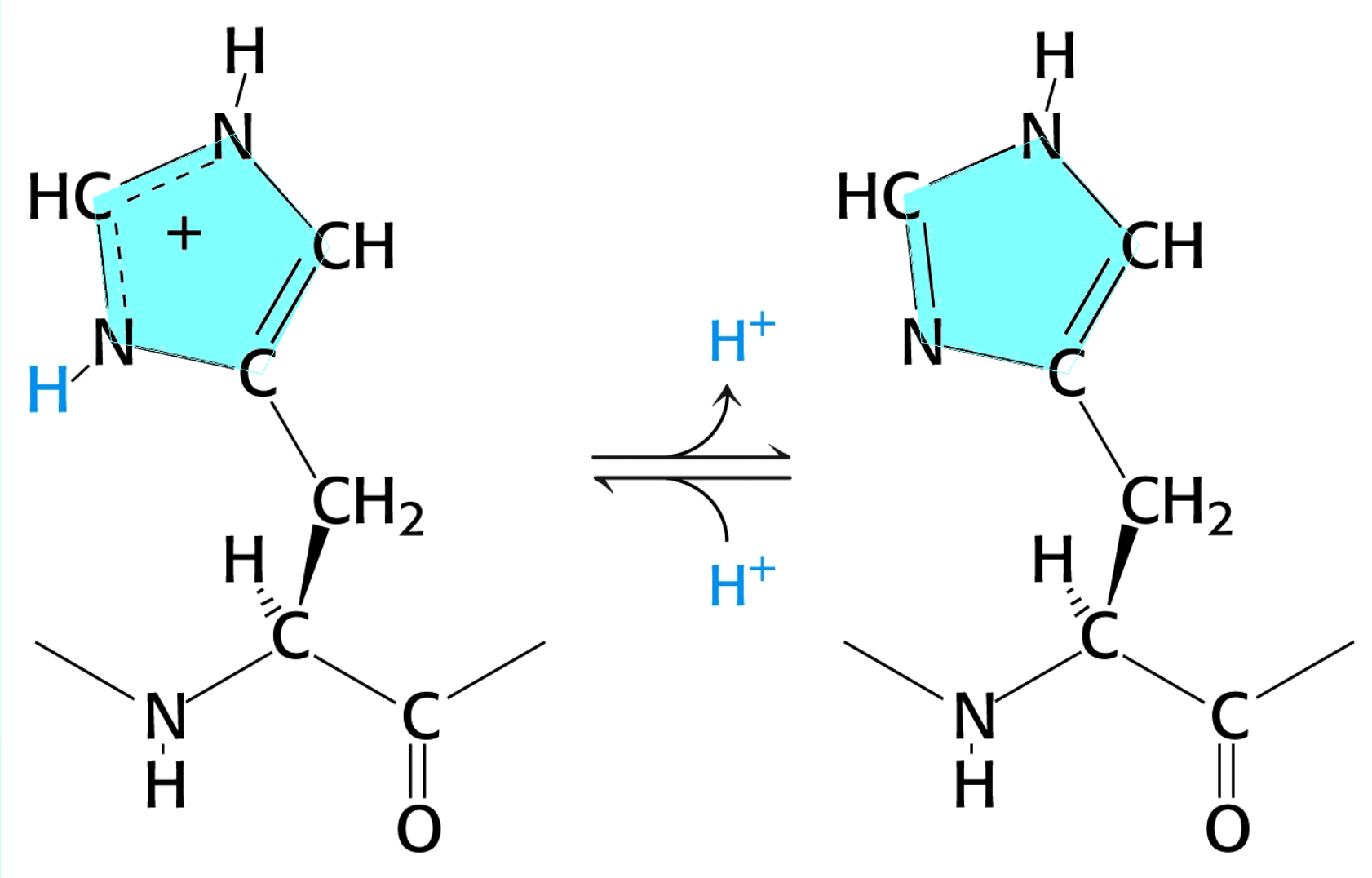
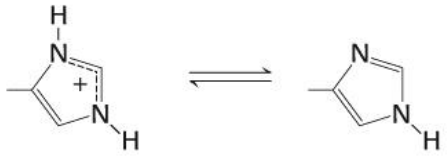
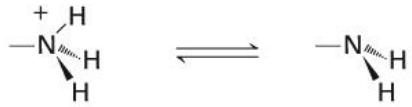
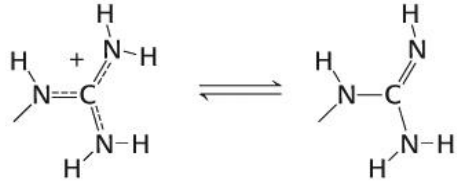


TABLE 3.1 Typical pK_a values of ionizable groups in proteins

Group	Acid ⇌ Base	Typical pK_a*
Terminal α-carboxyl group		3.1
Aspartic acid Glutamic acid		4.1
Histidine		6.0
Terminal α-amino group		8.0
Cysteine		8.3
Tyrosine		10.9
Lysine		10.8
Arginine		12.5

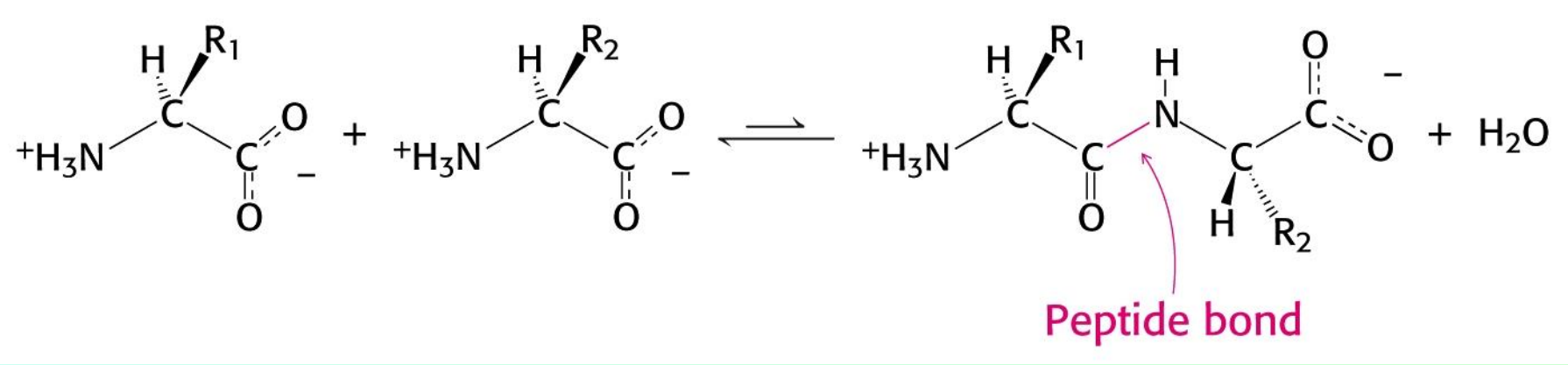
*pK_a values depend on temperature, ionic strength, and the microenvironment of the ionizable group.

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			

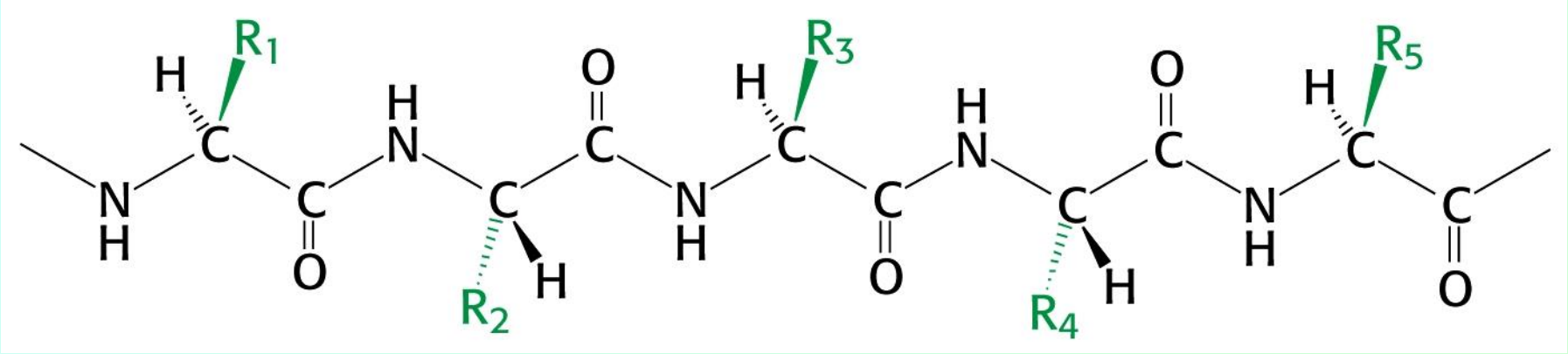
Essential Amino Acids:
Isoleucine
Leucine
Lysine
Methionine
Phenylalanine [a]
Threonine
Tryptophan [a]
Valine
Arginine [b]
Histidine [b]
[a] *Aromatic* [b] *Probably essential*

- Amino acids are polymerized via amide or “peptide” bonds:



- Copolymer of amino acids:

  – a “polypeptide”

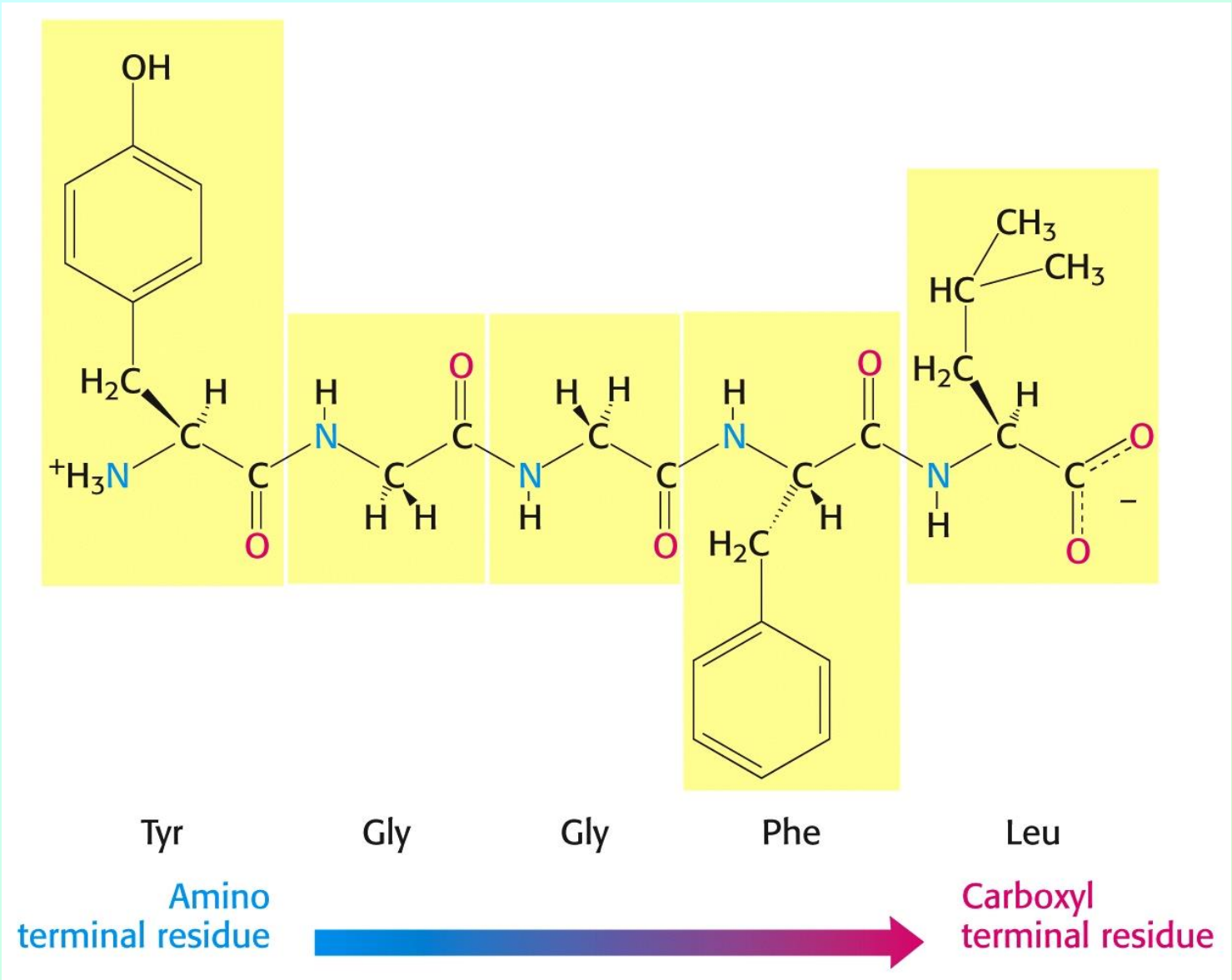


Definition:

Amino acid polymers of ≤50 amino acids are called

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

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


Tyr
Gly
Gly
Phe
Leu
Amino terminal residue
Carboxyl terminal residue

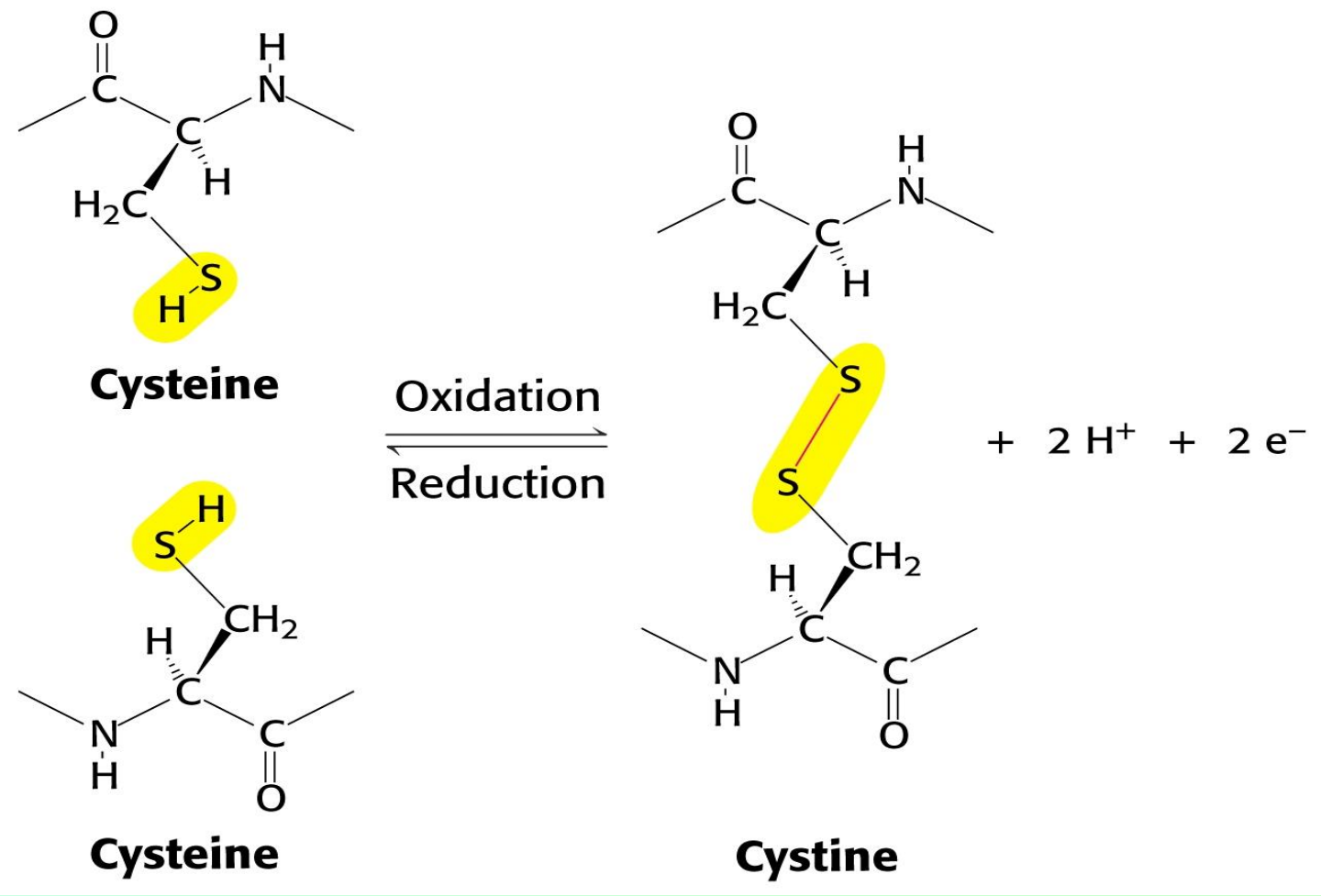
- An example of a “dipeptide” is the sweetener *Aspartame.*
- Other names include:
  - NutraSweet
  - Equal
  - Tri-Sweet
  - Sanecta
- IUPAC Name:

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

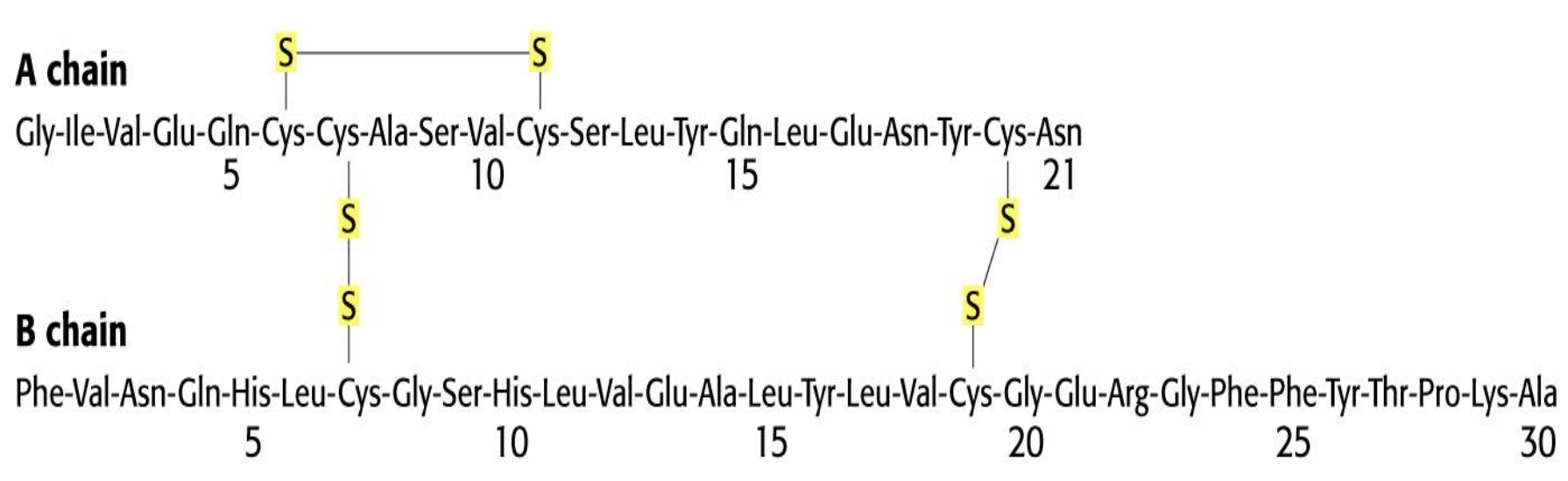
Abbreviated Structure:

Asp – Phe - OCH3

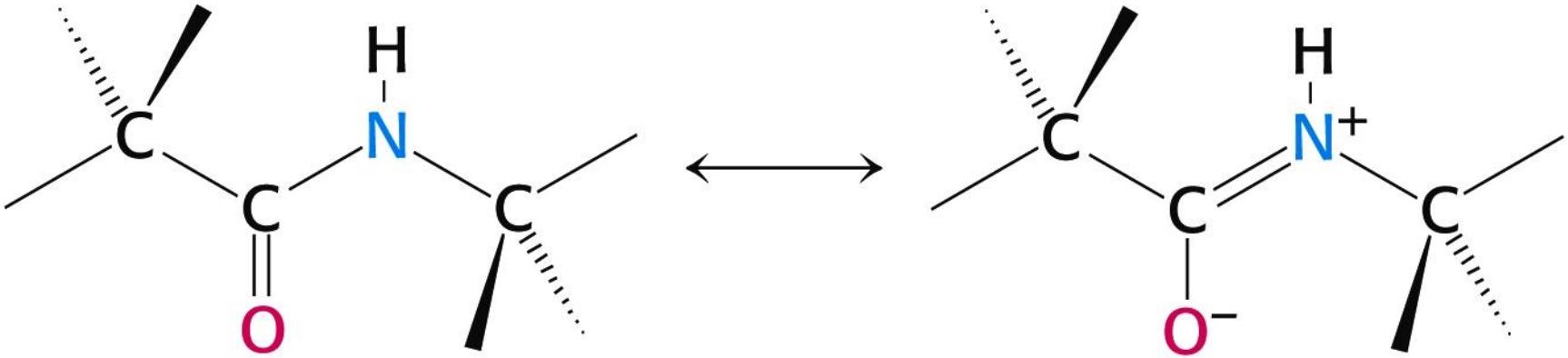
- Cross links between peptide chains:
  - Disulfide linkages between individual "**cysteines**" are called "**cystines:**"



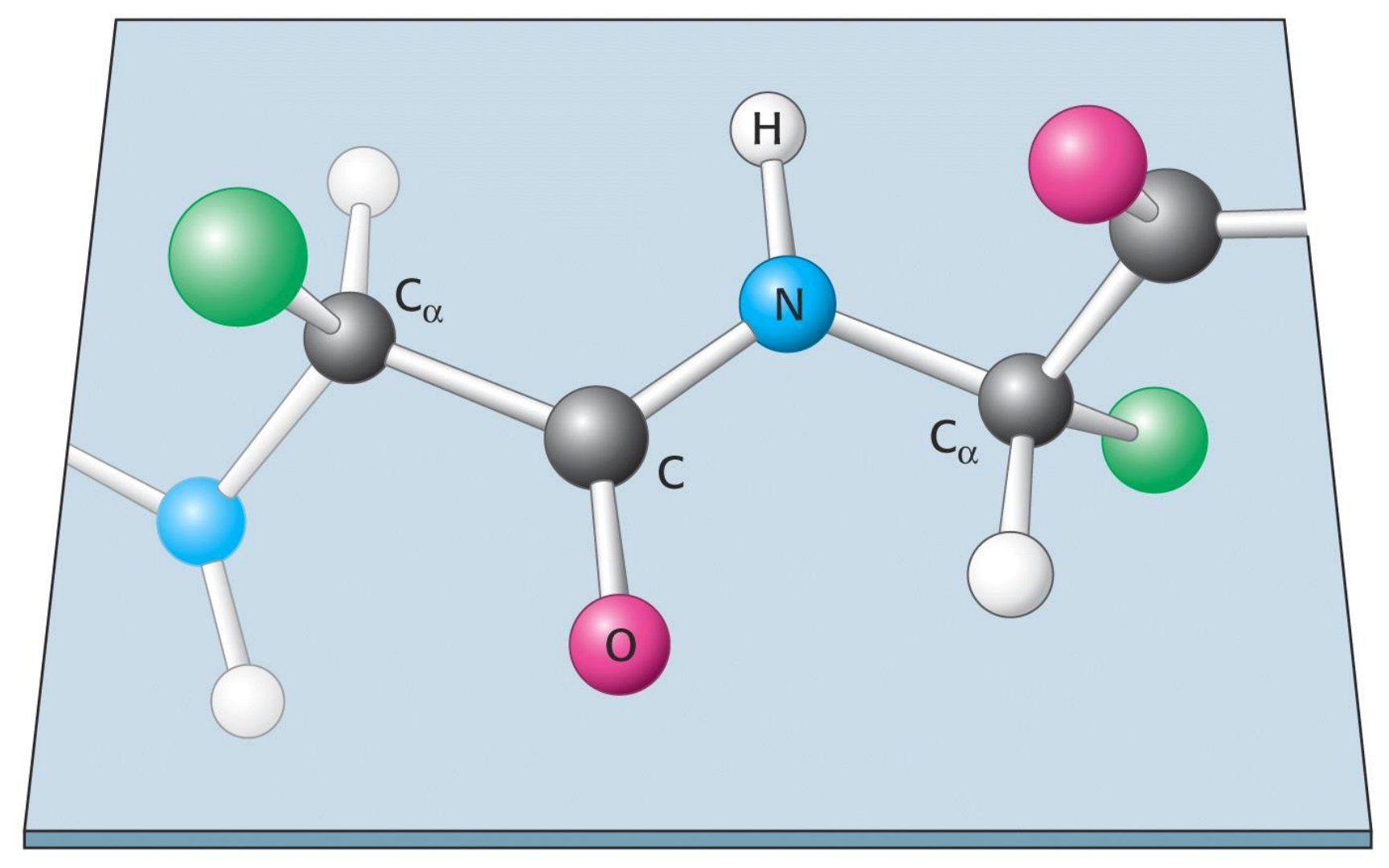
- Insulin is the smallest protein, with 51 amino acids in two chains linked by cystine (disulfide) cross links:



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



Peptide bond resonance structures


H
Cα
N
C
Cα
O

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.

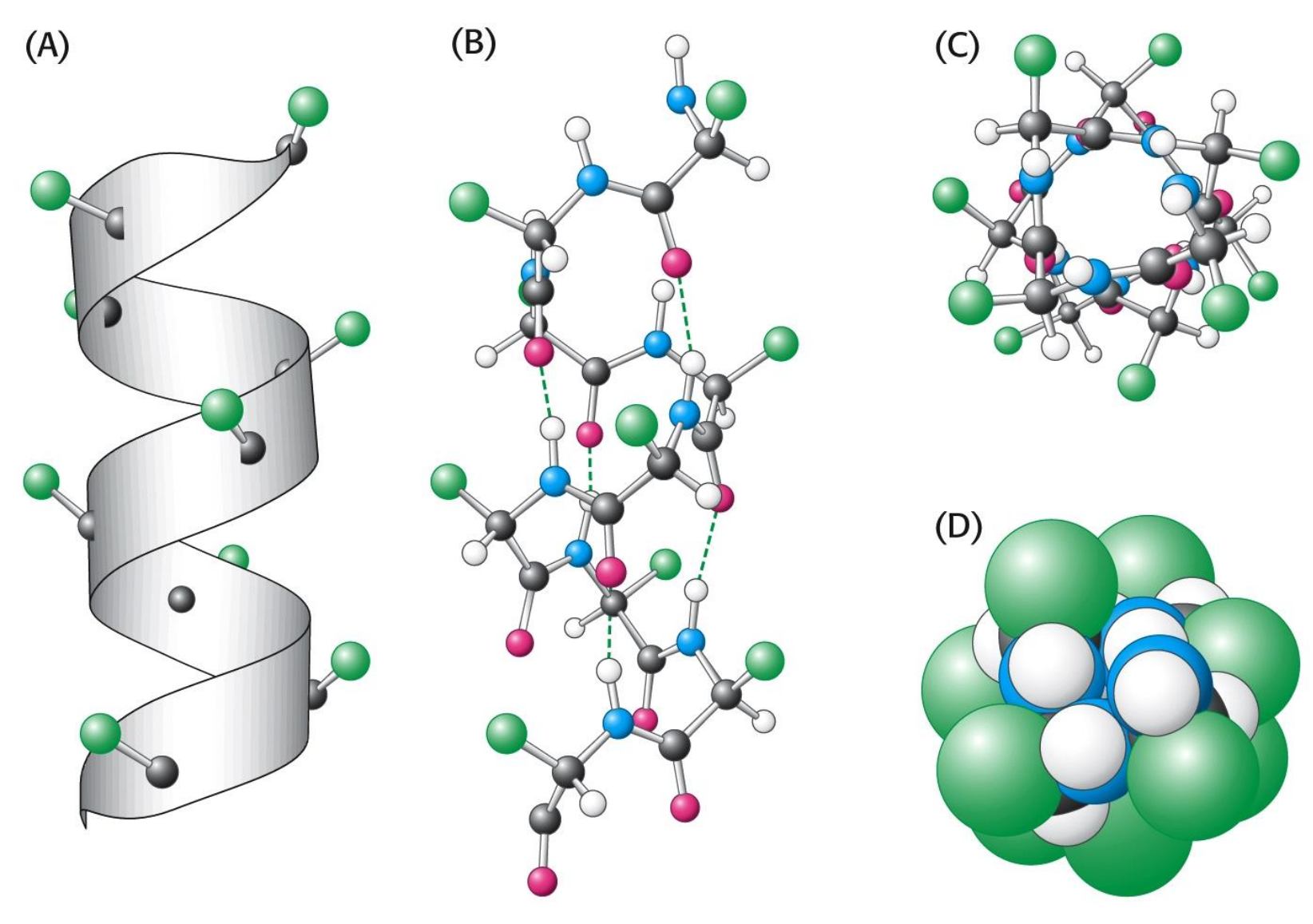
Secondary Structure in Proteins:

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

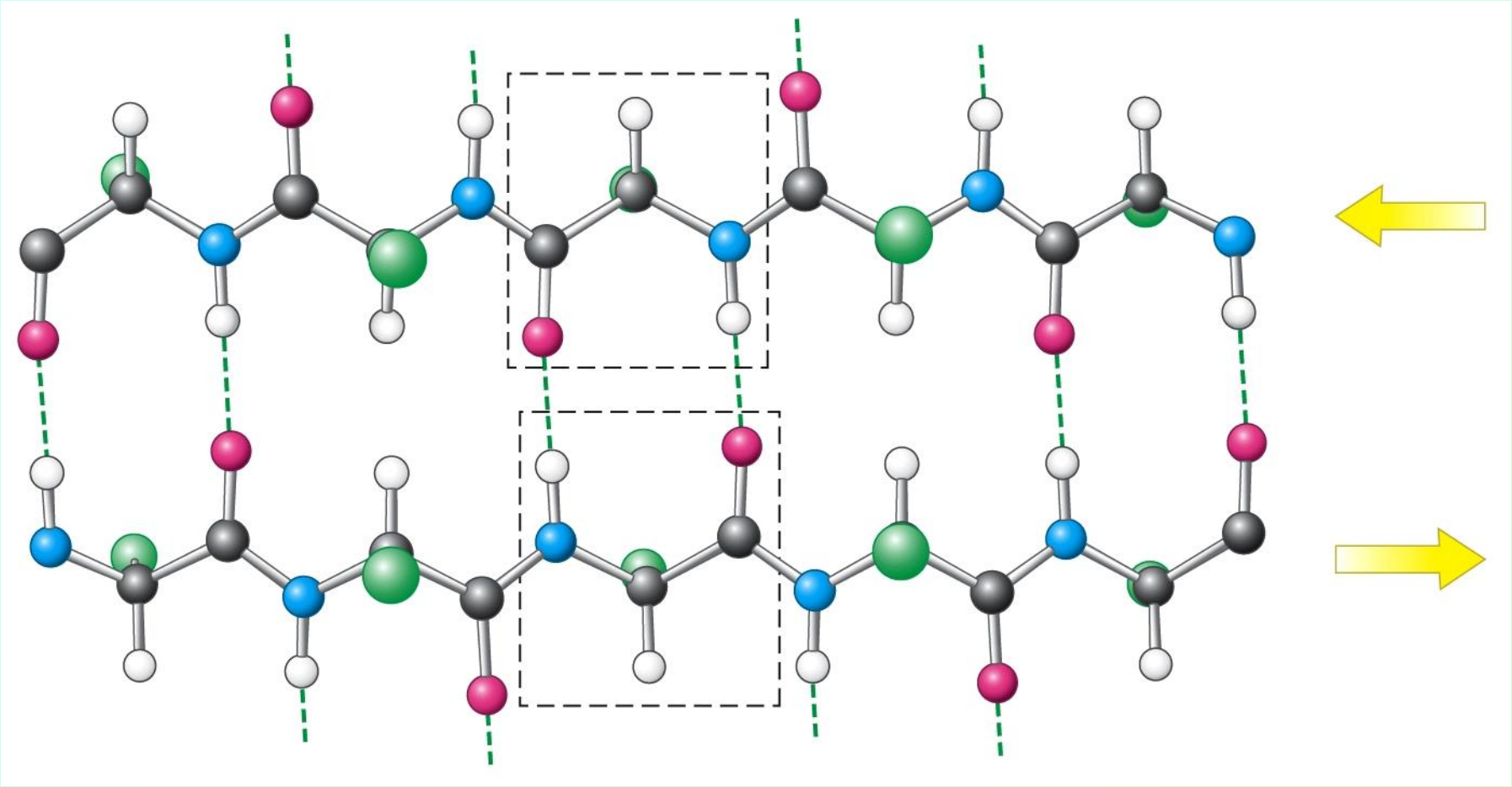
alpha – helix *beta - sheet*

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.

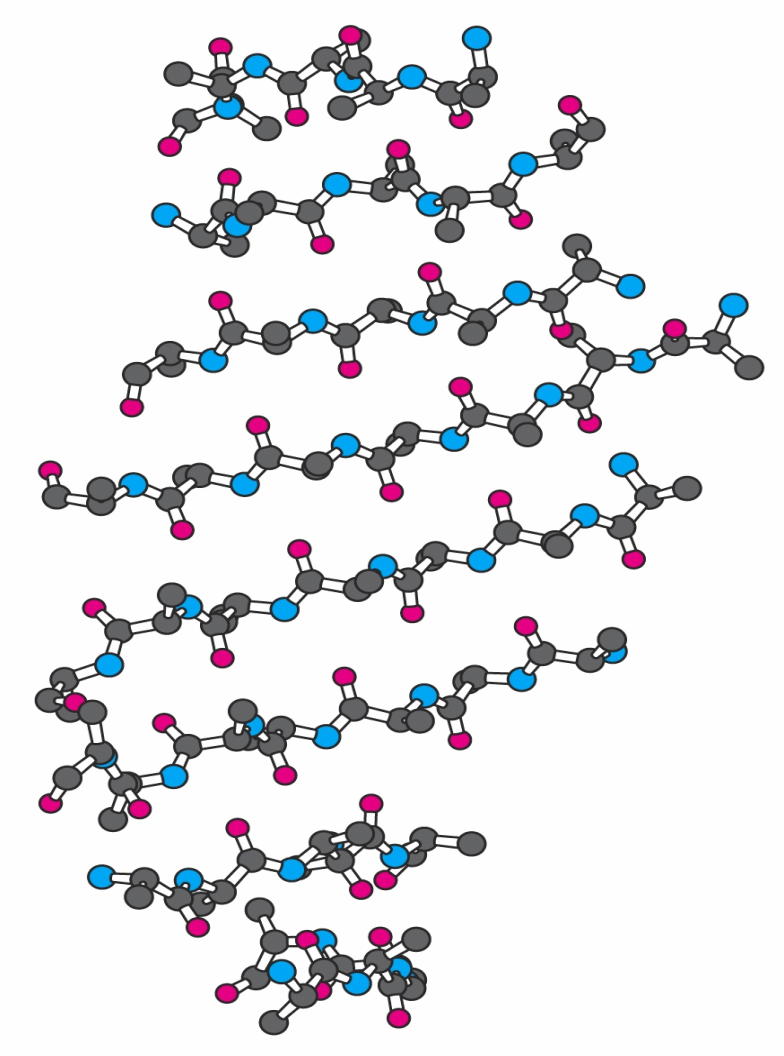
- The *alpha* (*α*) – *helix:*

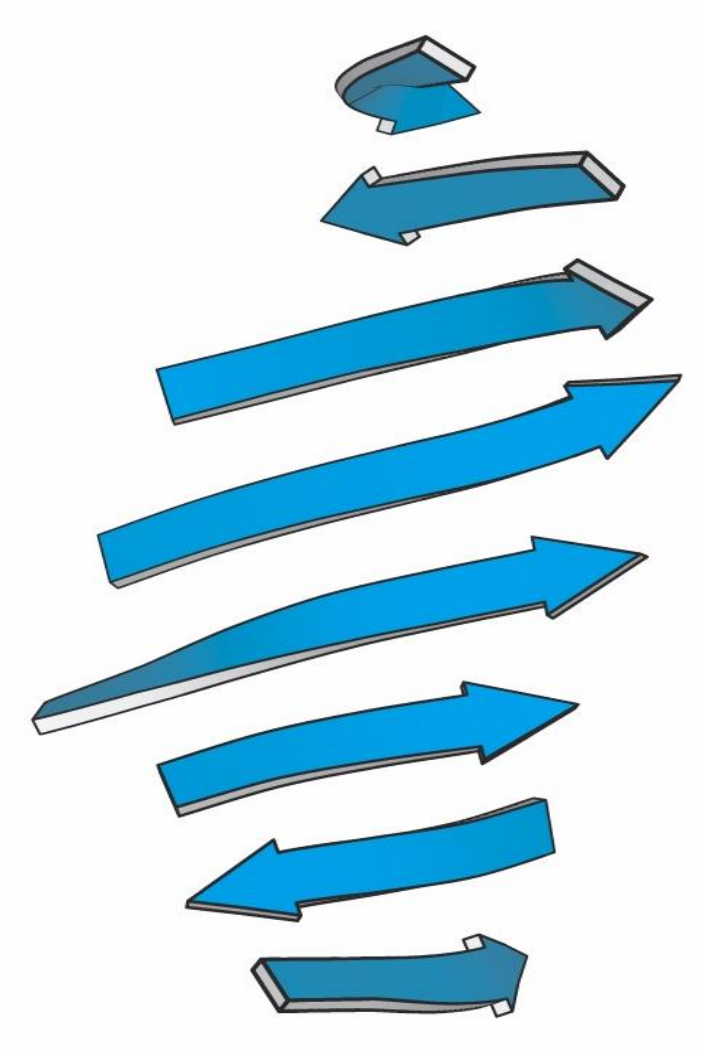


- *beta – sheet (antiparallel):*

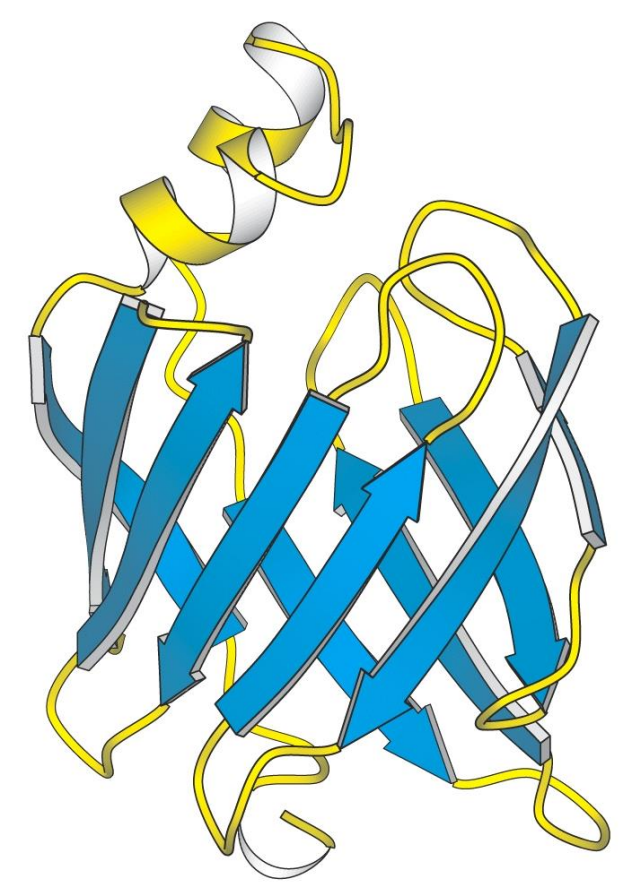


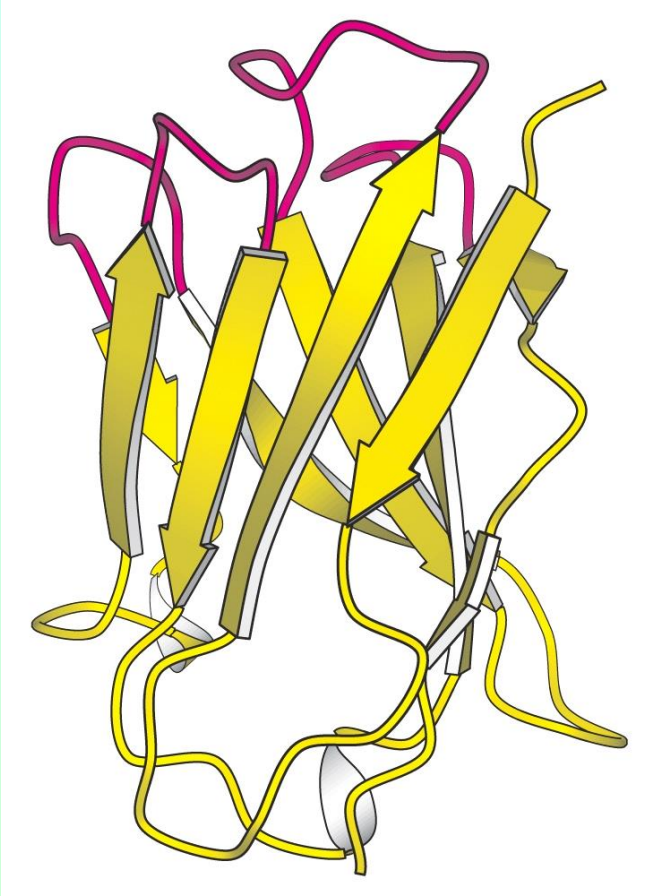
- *beta – sheet (artistic representations):*





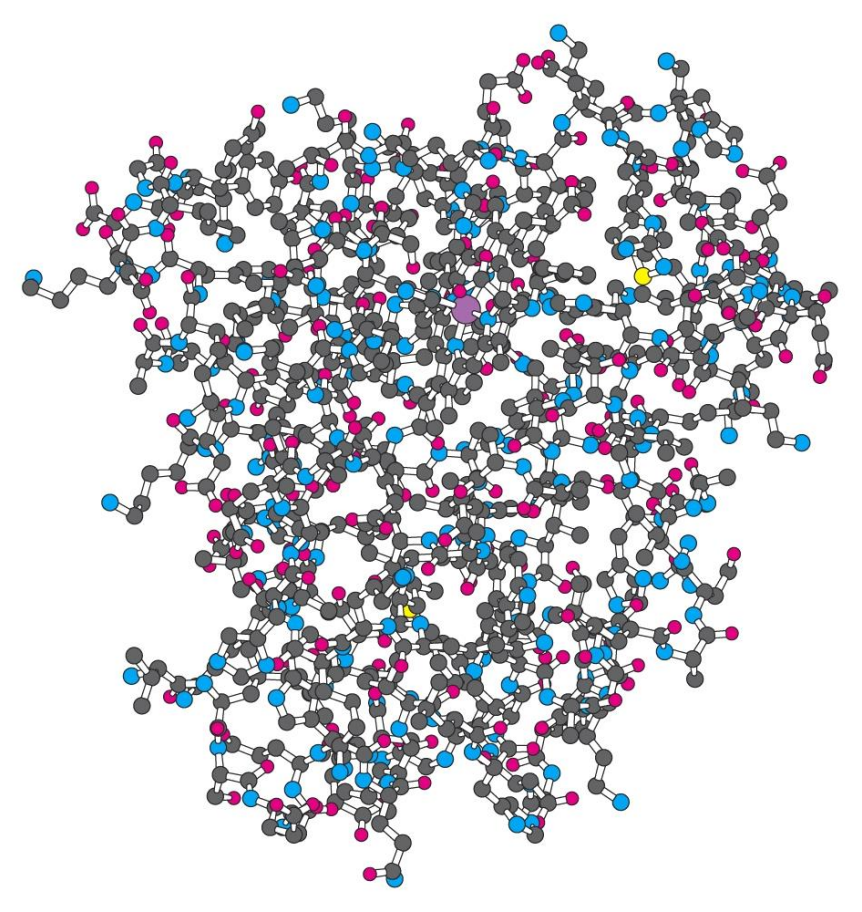
Examples of *beta*-sheet domains in proteins:





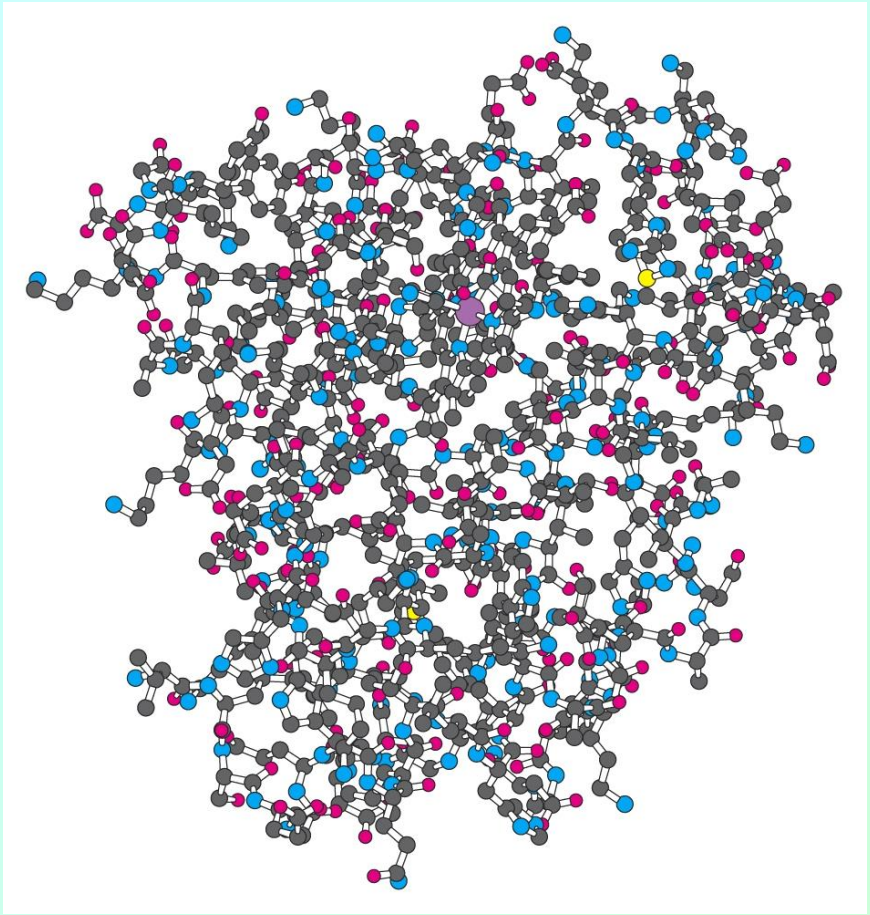
- Tertiary (3°) Structure of Protein

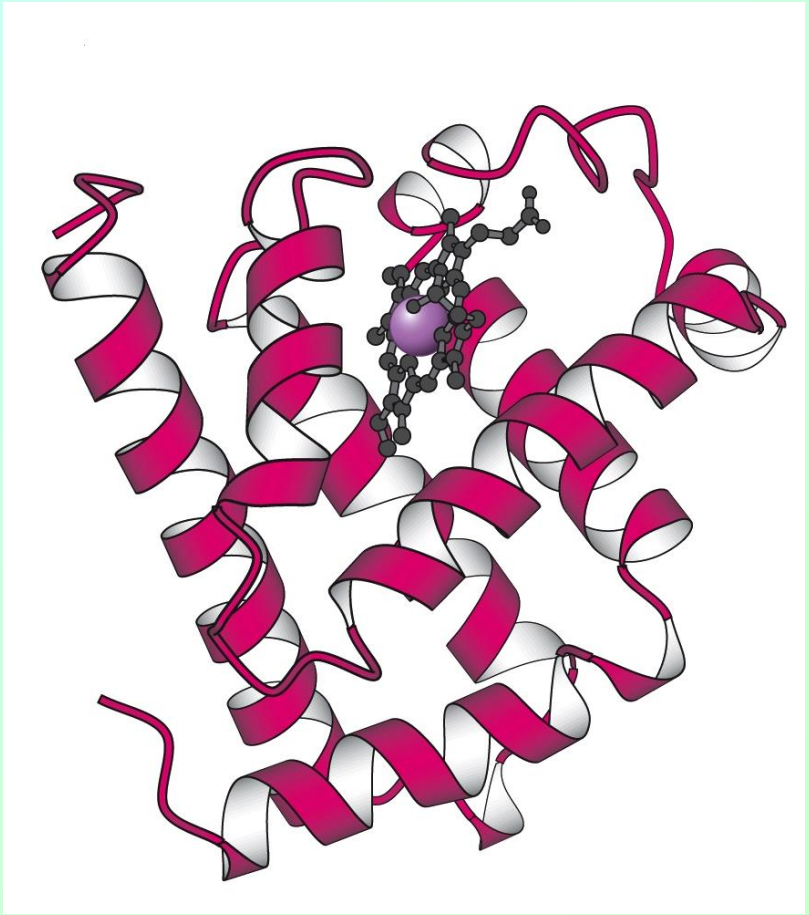
Water-soluble proteins fold into compact structures with nonpolar cores.



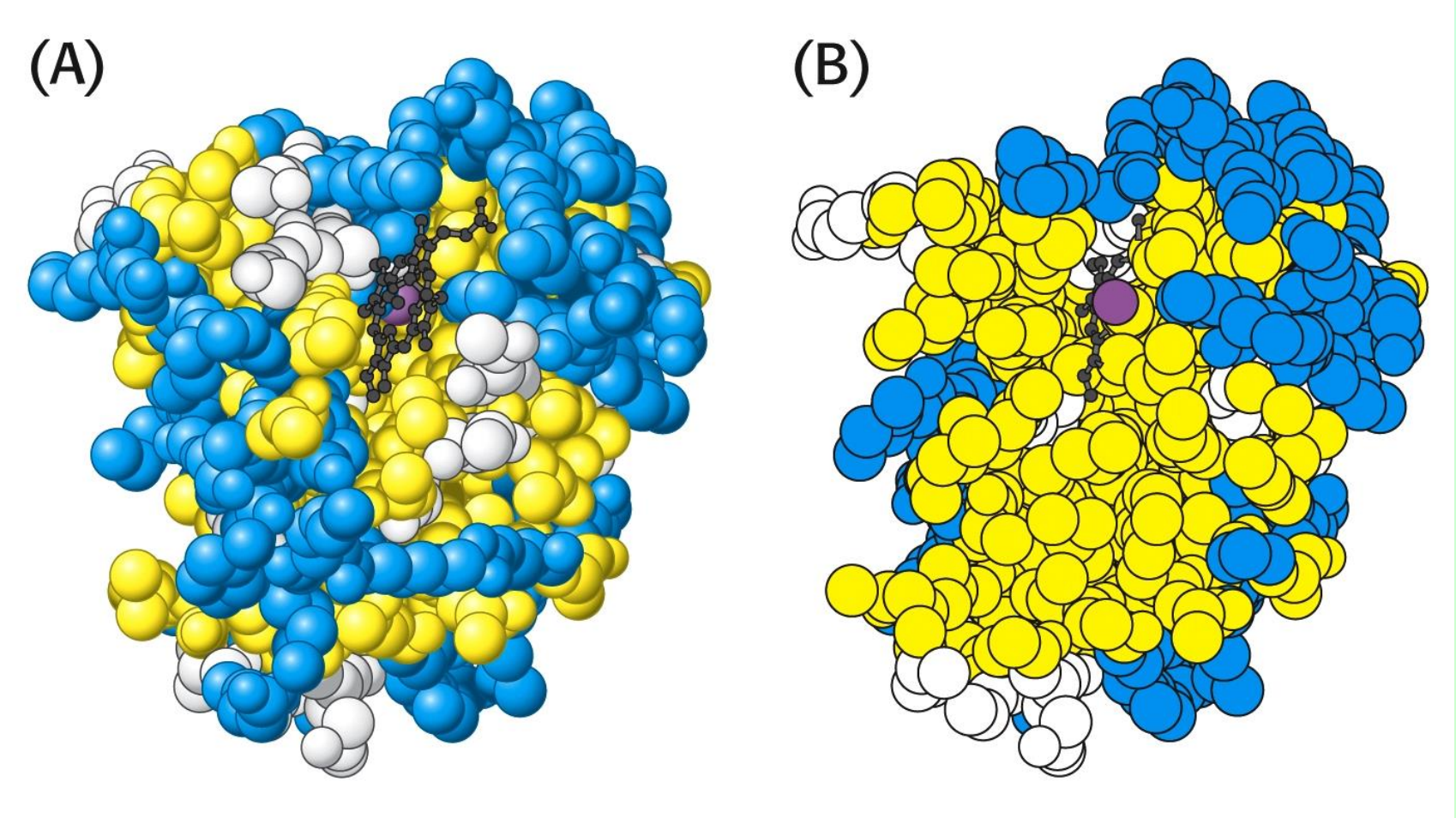
Tertiary (3°) Structure the Protein Myoglobin

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



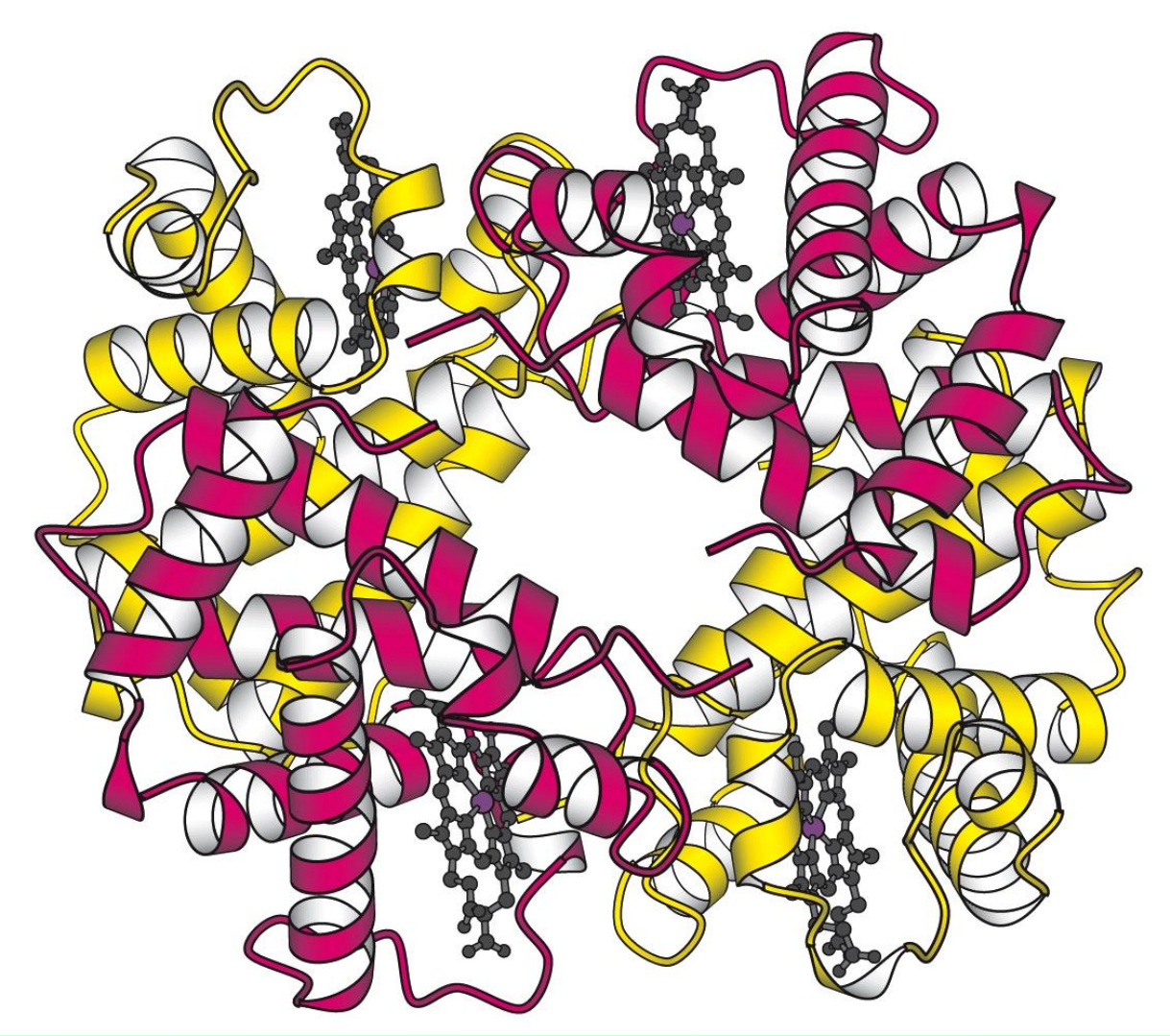


- In the case of myoglobin and many other proteins, the majority of hydrophobic amino acids (yellow) are found inside in structure:

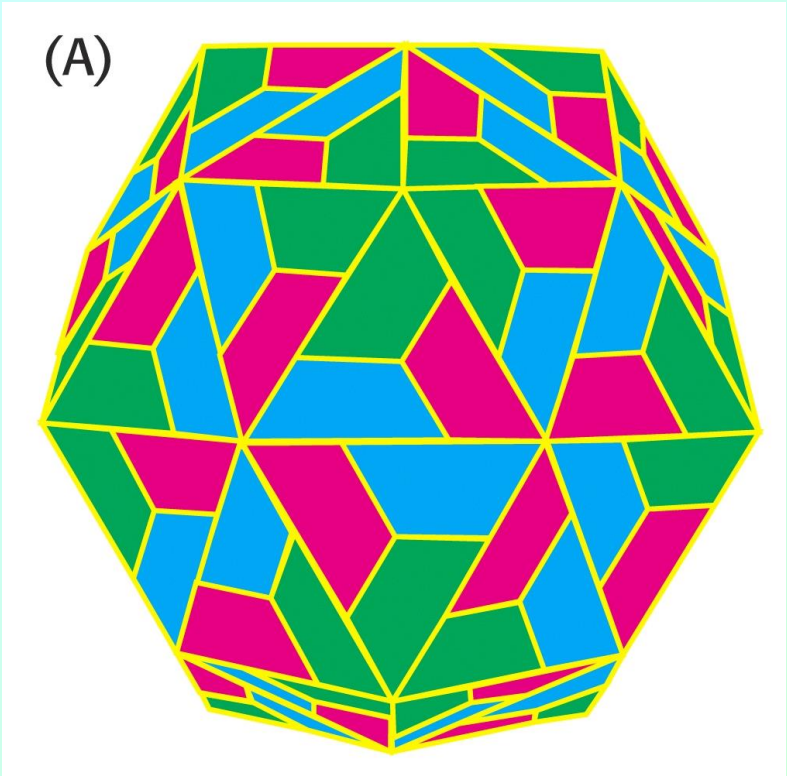


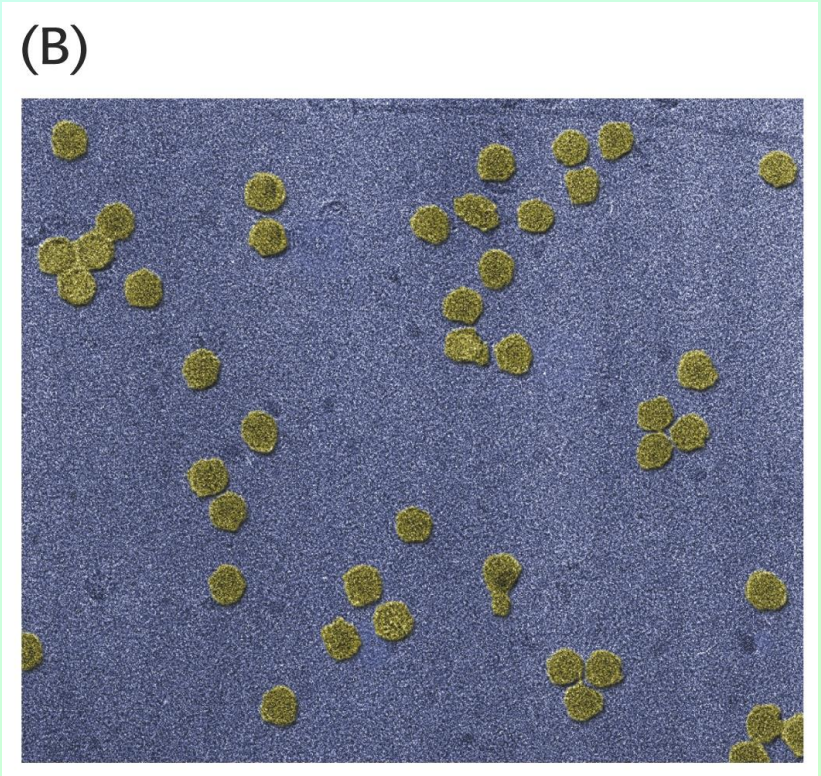
- The Cro protein of Lambda bacteriophage is a dimer of identical subunits:

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



- The coat of rhinovirus contains 60 copies of each of four subunits (*240 total*)!

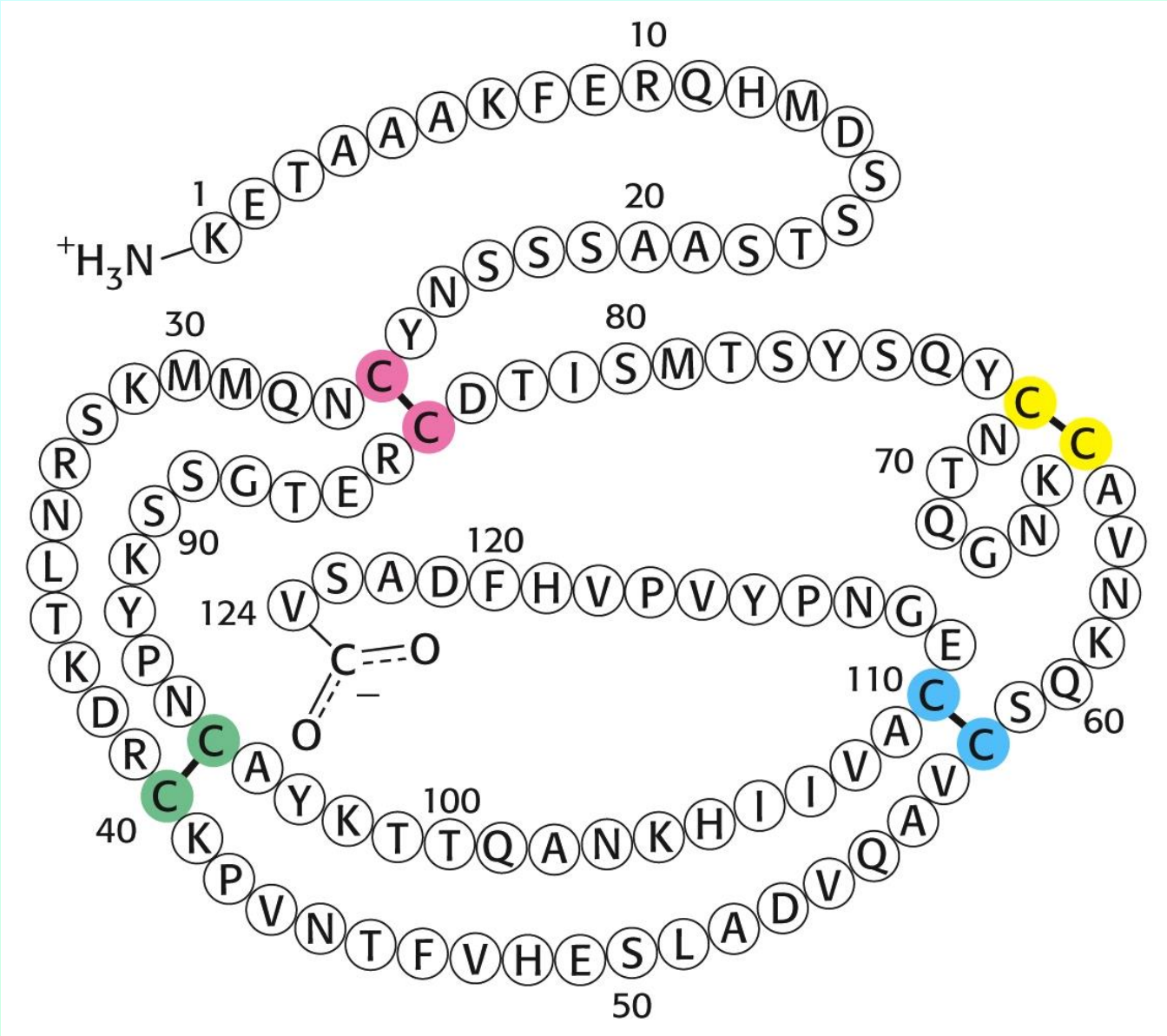




- In 1961 Christian Anfinsen published a classic landmark work that clearly showed tertiary structure was determined by primary structure!
- His experiment was a classic example of well-designed experiments that did not require expensive equipment or years of work.
- It deserves our attention.

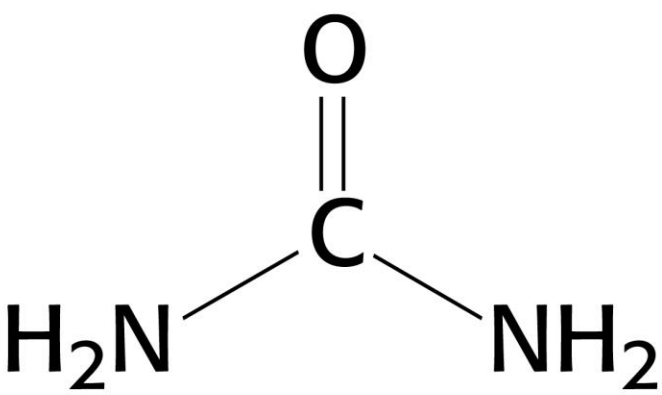
- Anfinson chose the enzyme, *ribonuclease*, for his experiments. This enzyme hydrolyzes RNA and is composed of a single polypeptide chain with 124 amino acids.
- Four disulfide (cystine) linkages are observed in the active enzyme that stabilize the 3-D (3°) shape of the enzyme.
- The enzyme functions only when its 3° structure is properly aligned.

- Amino acid sequence of ribonuclease:

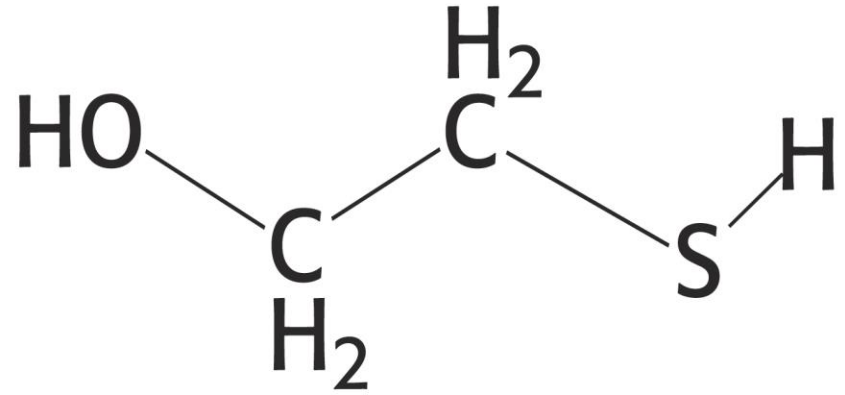


Anfinson used two chemicals to disrupt the enzyme's 3° structure *[DENATURATION]*

1. urea - *disrupts hydrogen bonds*
2. β-mercaptoethanol – *reduces disulfide bonds*

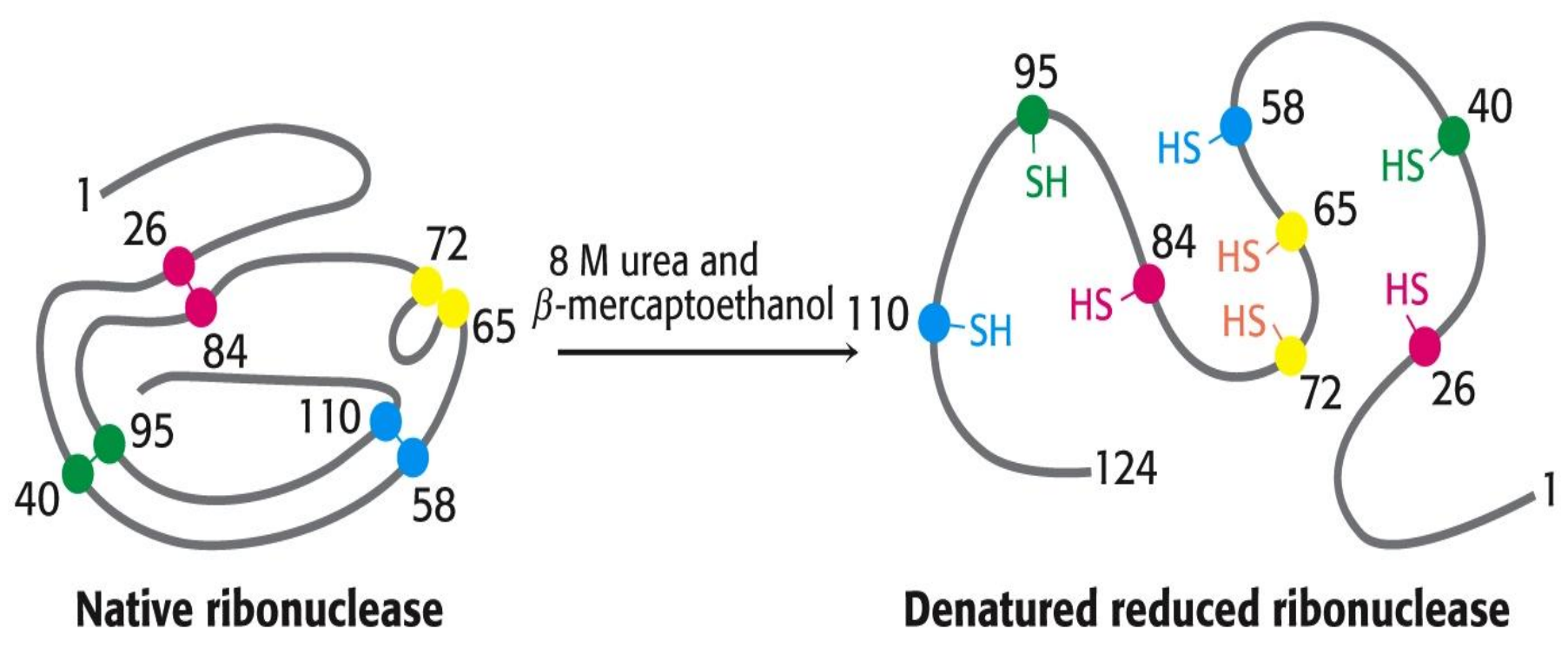


Urea

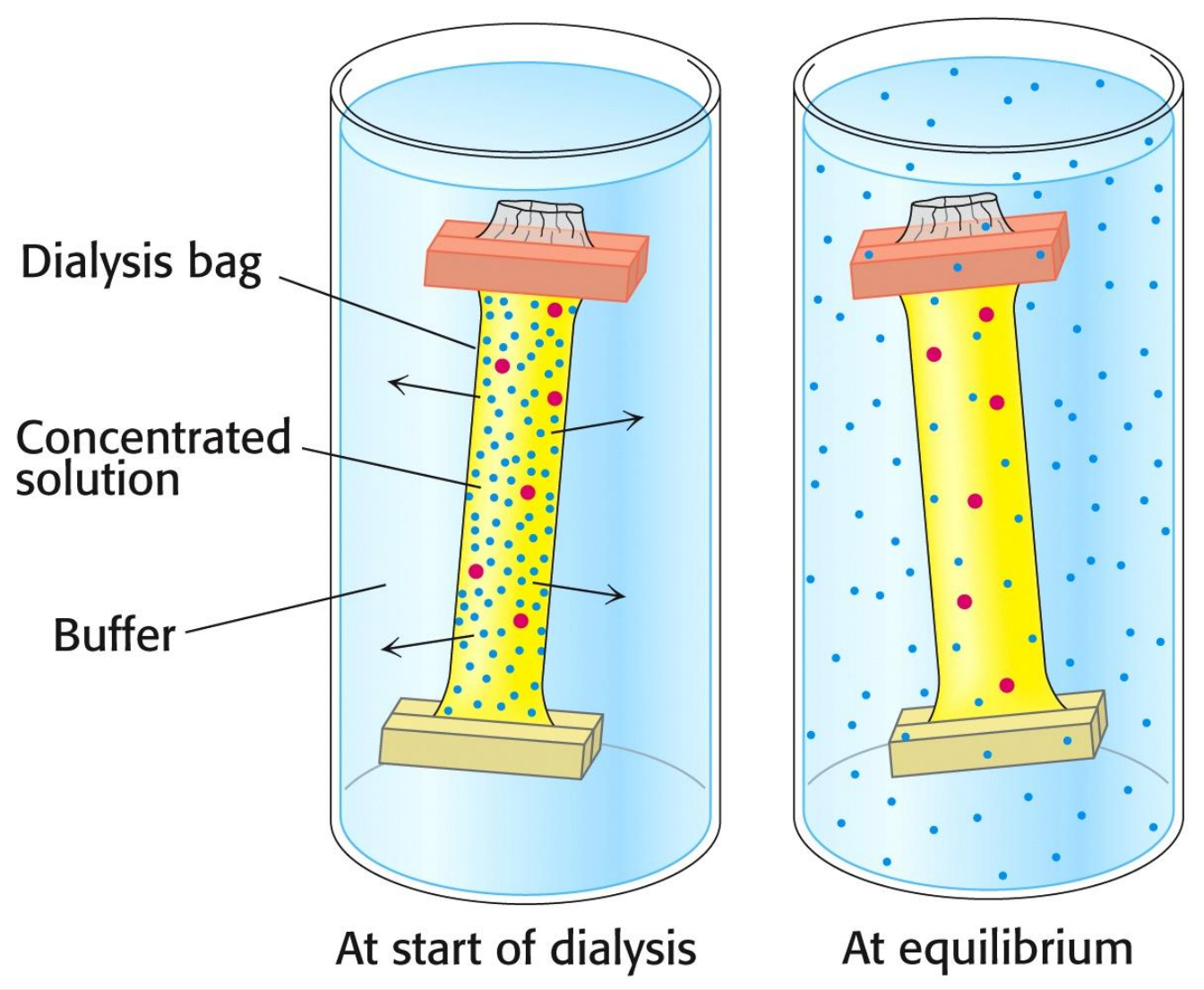


β-Mercaptoethanol

Anfinson's Experiment:



He also used dialysis to separate these chemicals from the enzyme in different orders.



- By adding either one of these two chemicals to the surrounding medium, it is not removed during dialysis.
- In essence, Anfinson could remove either the urea or the β-mercaptoethanol in any order he chose.
- The **order** made a big difference in the enzymes ability to recover from the treatment!

Anfinson's Experiment:

Experiment #1:

1. Add both urea and β-mercaptoethanol to a solution of enzyme.

   Activity is lost.

2. Remove urea by dialysis; then remove β-mercaptoethanol by dialysis.
   Activity is recovered 100%!

Experiment #2:

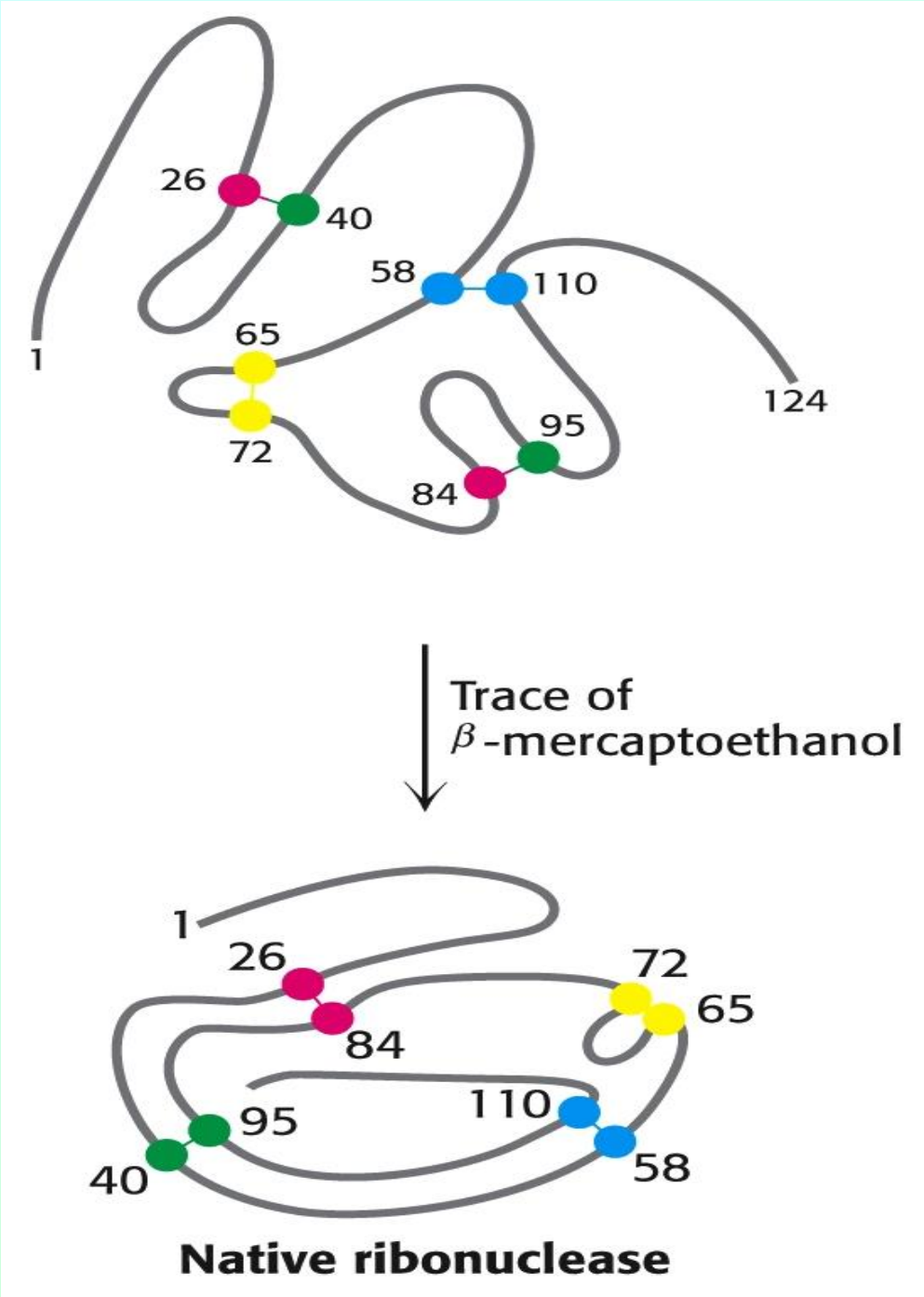
1. Add both urea and β-mercaptoethanol to a solution of enzyme.
   Activity is lost.
2. Remove β-mercaptoethanol by dialysis; then remove urea by dialysis.

   Only ~1% of activity is recovered. $N = 8^2 = 64, 1/64 \sim 1\%$

Experiment #3:

1. Add β-mercaptoethanol to the solution from Exp.#2. Then, remove urea by dialysis;
2. Finally, remove β-mercaptoethanol by dialysis. *Activity is recovered 100%!*

Anfinson's Experiment:



End of Lecture Slides
for
Amino Acids & Proteins

Credits: Most of the diagrams used in these slides were taken from Stryer, et.al, Biochemistry, 5th Ed., Freeman Press, Chapters 3-4 (Our course textbook).