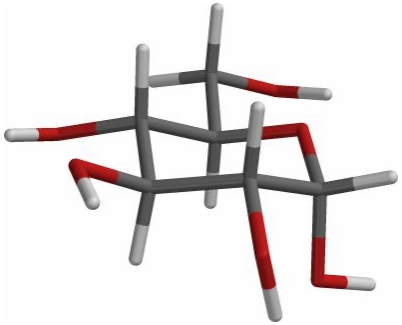
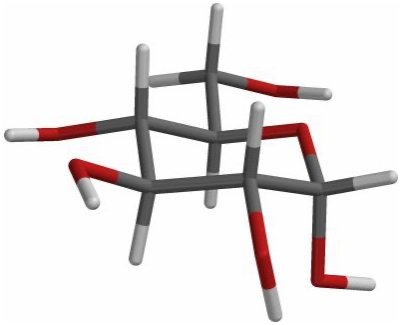




Chem 109 C
Bioorganic Compounds

Armen Zakarian

Office: Chemistry Bldn 2217

http://labs.chem.ucsb.edu/~zakariangroup/courses.html

Proteins: Structure

Primary structure is the sequence of amino acids in a protein and the location of disulfide bridges

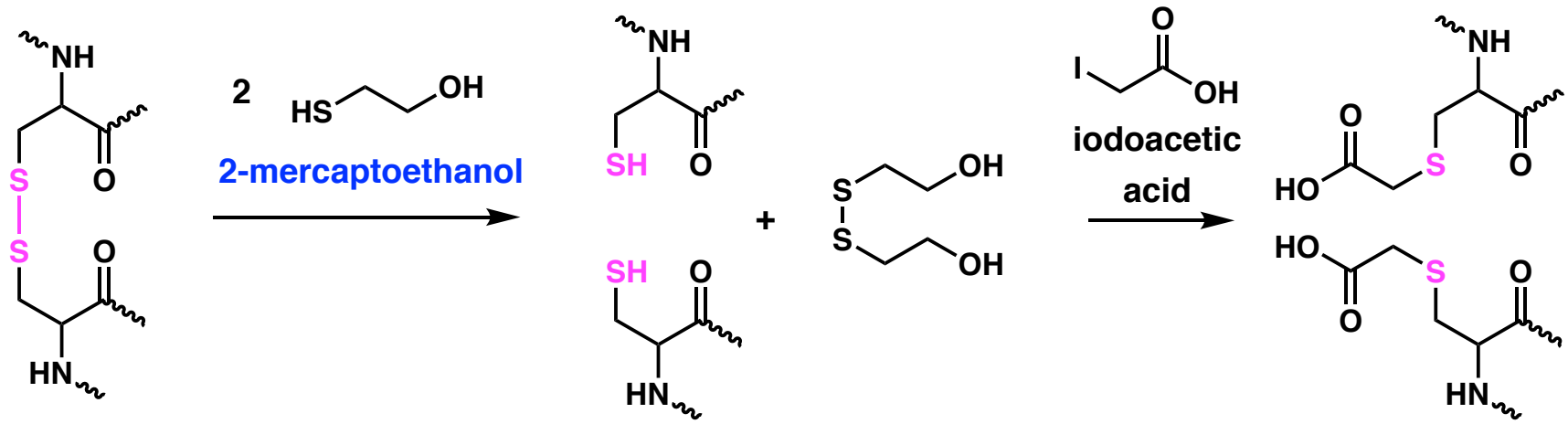
strategy for determining the primary structure:

1. **break down peptide into individual amino acids to determine composition**

2. **use selective reagents to determine sequence**

first steps - overall composition:

cleaving disulfide bridges: $HSCH_2CH_2OH$



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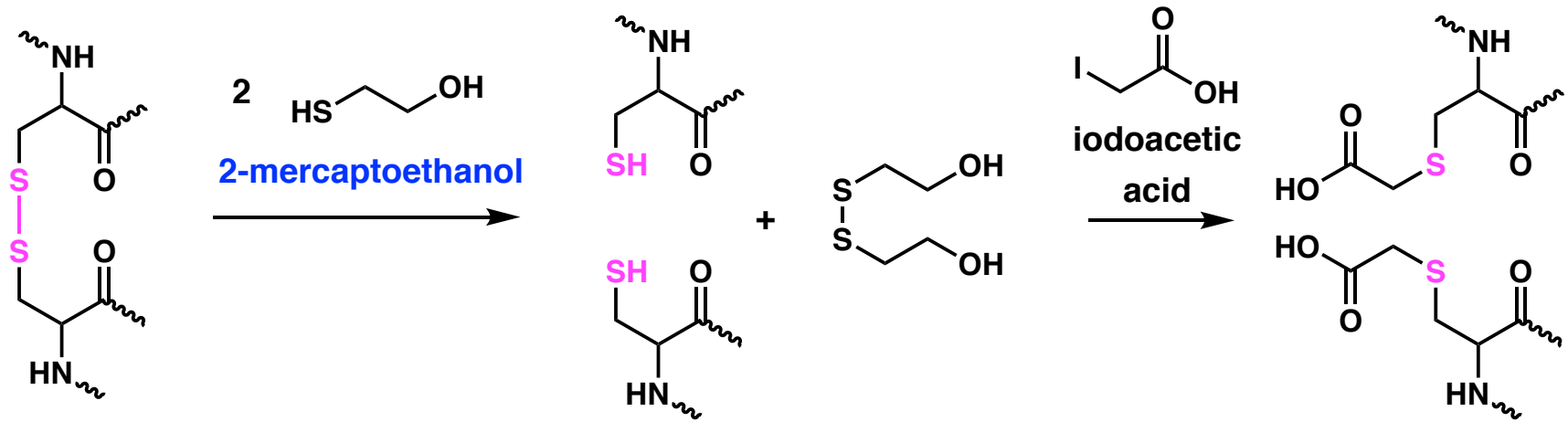


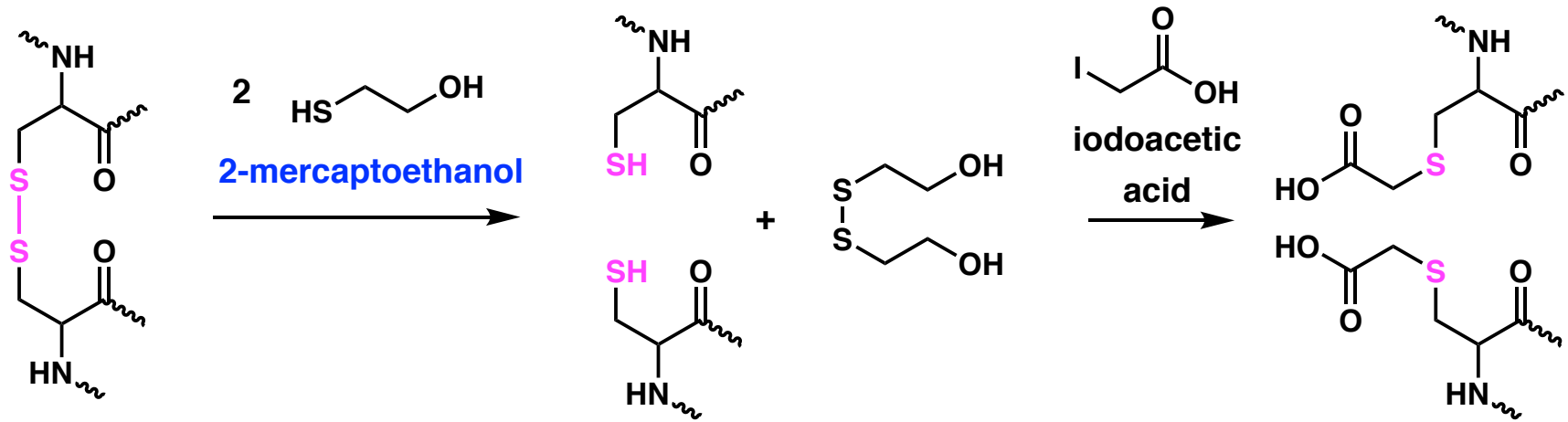
complete hydrolysis: 6 M HCl, H_20, 100 °C, 24 h

protein $\xrightarrow[100\ °C]{6\ M\ HCl}$ amino acids

Proteins: Structure

first steps - overall composition:

cleaving disulfide bridges: $HSCH_2CH_2OH$

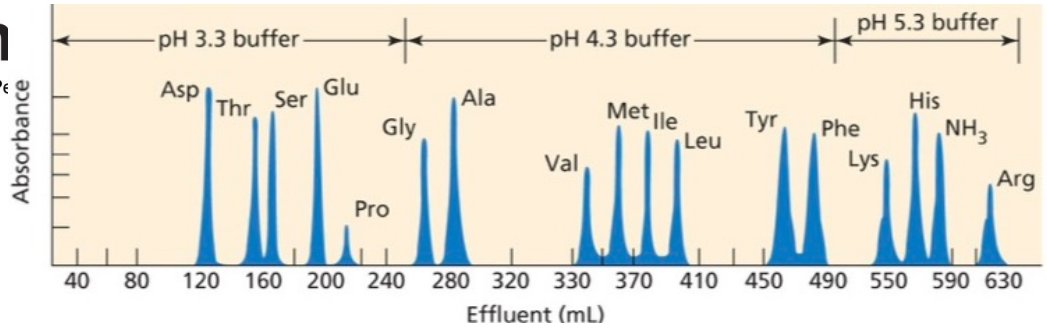


complete hydrolysis: 6 M HCl, H_20, 100 °C, 24 h

protein $\xrightarrow[100\ °C]{6\ M\ HCl}$ amino acids

24 h

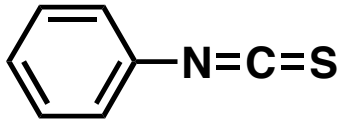
then use amino acid analyzer…



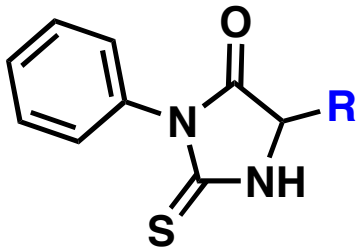
selective reagents to break amide bonds:

for *N*-terminal amino acid

Edman's reagent:



phenyl isothiocyanate



PTH-amino acid

from *N*-terminal amino acid

can be repeated up to 50 times in sequencer

selective reagents to break amide bonds:

partial hydrolysis with dilute acid into smaller pieces

Ala-Lys-Phe-Gly-Asp-Trp-Ser-Arg-Met-Val-Arg-Tyr-Leu-His

Sequencing an oligopeptide...

PRACTICE PROBLEM

A decapeptide undergoes partial hydrolysis to give peptides whose amino acid compositions are shown. Reaction of the intact decapeptide with Edman's reagent releases PTH-Gly. What is the sequence of the decapeptide?

1. Ala, Trp
2. Val, Pro, Asp
3. Pro, Val
4. Ala, Glu
5. Trp, Ala, Arg
6. Arg, Gly
7. Glu, Ala, Leu
8. Met, Pro, Leu, Glu

Sequencing an oligopeptide...

PRACTICE PROBLEM

A decapeptide undergoes partial hydrolysis to give peptides whose amino acid compositions are shown. Reaction of the intact decapeptide with Edman's reagent releases PTH-Gly. What is the sequence of the decapeptide?

1. Ala, Trp
2. Val, Pro, Asp
3. Pro, Val
4. Ala, Glu
5. Trp, Ala, Arg
6. Arg, Gly
7. Glu, Ala, Leu
8. Met, Pro, Leu, Glu

Midterm 1

Wednesday, October 16, HFH 1104, 8 – 8:50 am

- **Chapter 20. Carbohydrates.**

  All Sections except 20.13, 20.17, 20.19

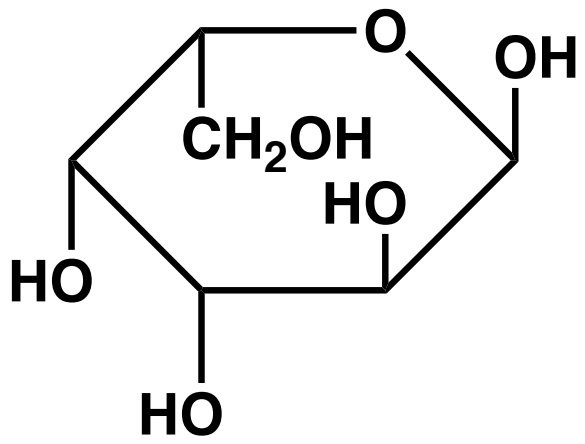
- **Chapter 21. Amino acids, Proteins.**

  All sections Up to 21.11, including 21.11

structures of carbohydrates (except glucose, mannose, and galactose) and amino acids will be provided

PRACTICE PROBLEM

Name the following monosaccharide and draw a Fischer projection for the open form



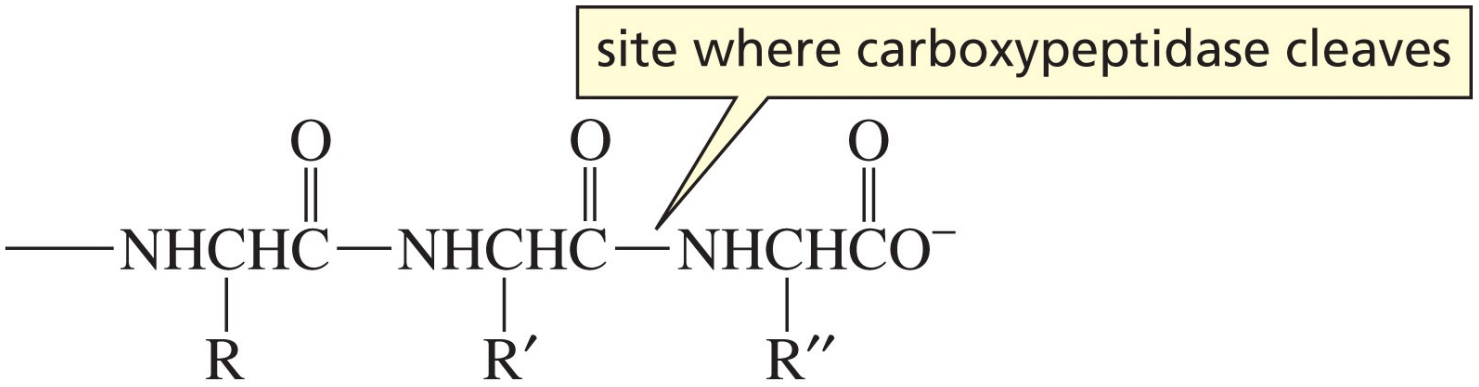
selective reagents to break amide bonds:

for C-terminal amino acid:

exopeptidases:

carboxypeptidase A: all but Arg and Lys

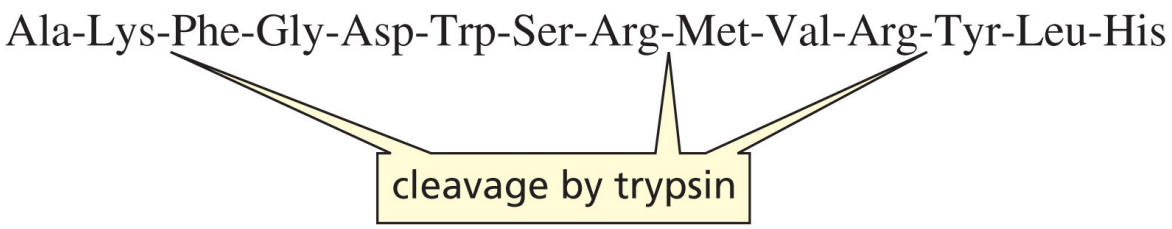
carboxypeptidase B: only Arg and Lys



selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

Trypsin: C-side of Arg and Lys



selective reagents to break amide bonds:

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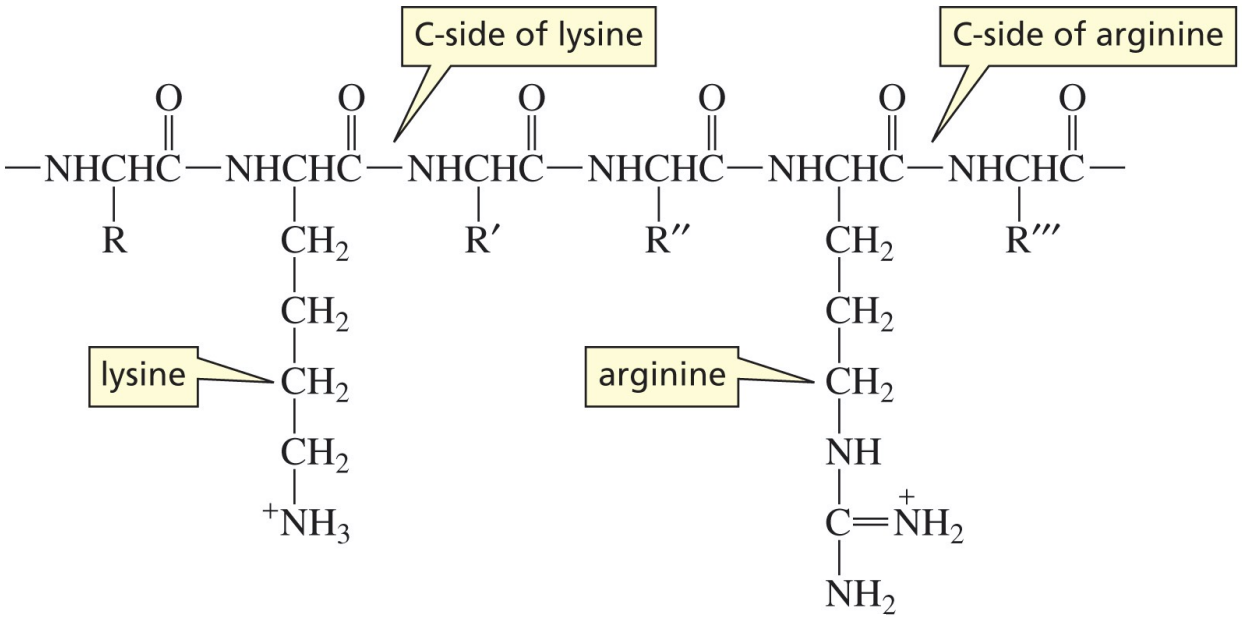
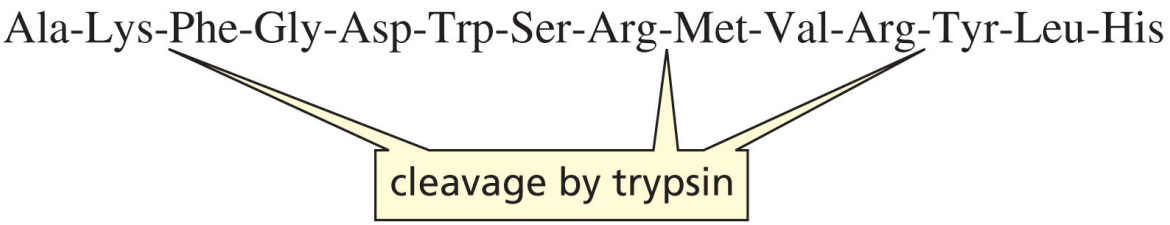
Trypsin: C-side of Arg and Lys



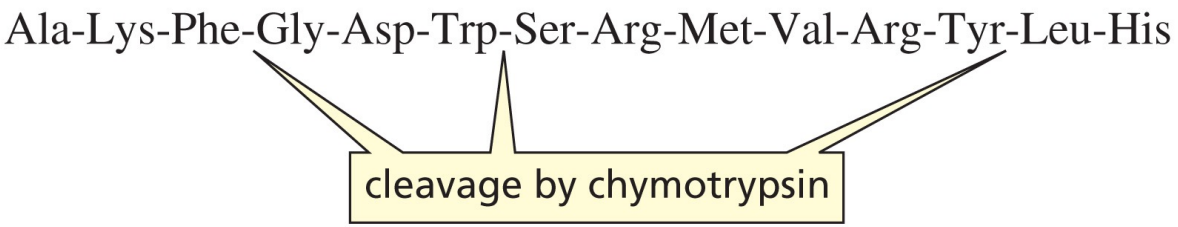
selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

Trypsin: C-side of Arg and Lys



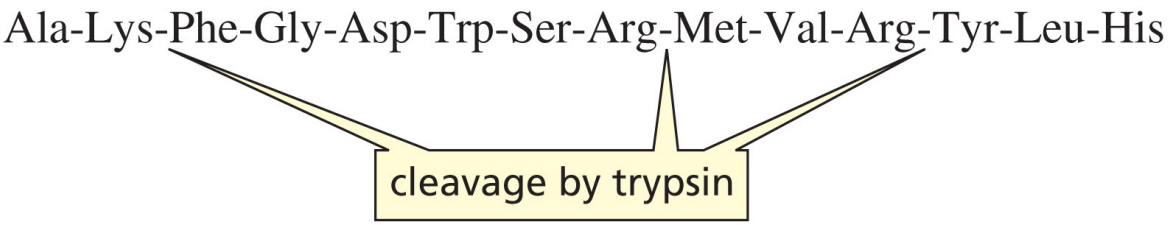
Chymotrypsin: C-side of Phe, Tyr and Trp



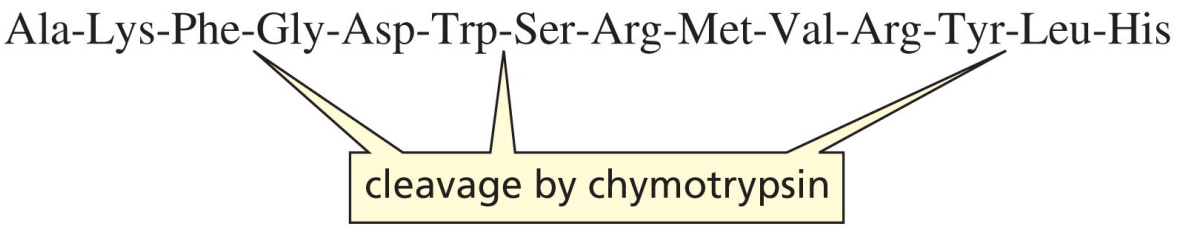
selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

Trypsin: C-side of Arg and Lys



Chymotrypsin: C-side of Phe, Tyr and Trp

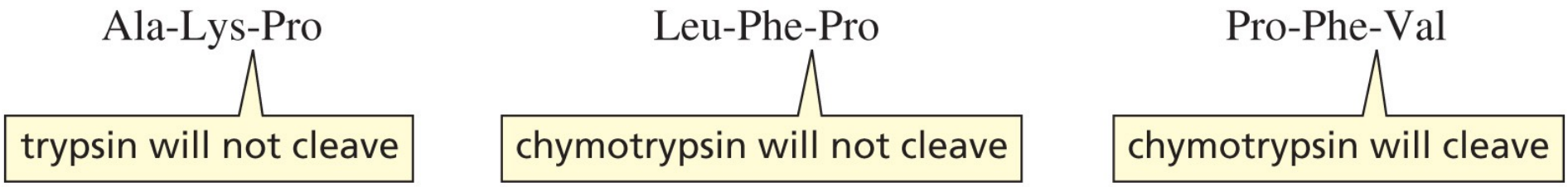


Elastase: C-side of Gly and Ala

selective reagents to break amide bonds:

for endo- and exopeptidases,

no reaction at Pro:



selective reagents to break amide bonds:

cyanogen bromide: BrCN,

C-side of **Met**

Ala-Lys-Phe-Gly-Asp-Trp-Ser-Arg-Met-Val-Arg-Tyr-Leu-His

BrCN will cleave next to proline (Pro)

Table 21.4 Specificity of Peptide or Protein Cleavage

Reagent	Specificity
Chemical reagents	
Edman's reagent	removes the N-terminal amino acid
Cyanogen bromide	hydrolyzes on the C-side of Met
Exopeptidases*	
Carboxypeptidase A	removes the C-terminal amino acid (not Arg or Lys)
Carboxypeptidase B	removes the C-terminal amino acid (only Arg or Lys)
Endopeptidases*	
Trypsin	hydrolyzes on the C-side of Arg and Lys
Chymotrypsin	hydrolyzes on the C-side of amino acids that contain aromatic six-membered rings (Phe, Tyr, Trp)
Elastase	hydrolyzes on the C-side of small amino acids (Gly and Ala)

* Cleavage will not occur if Pro is on either side of the bond to be hydrolyzed.

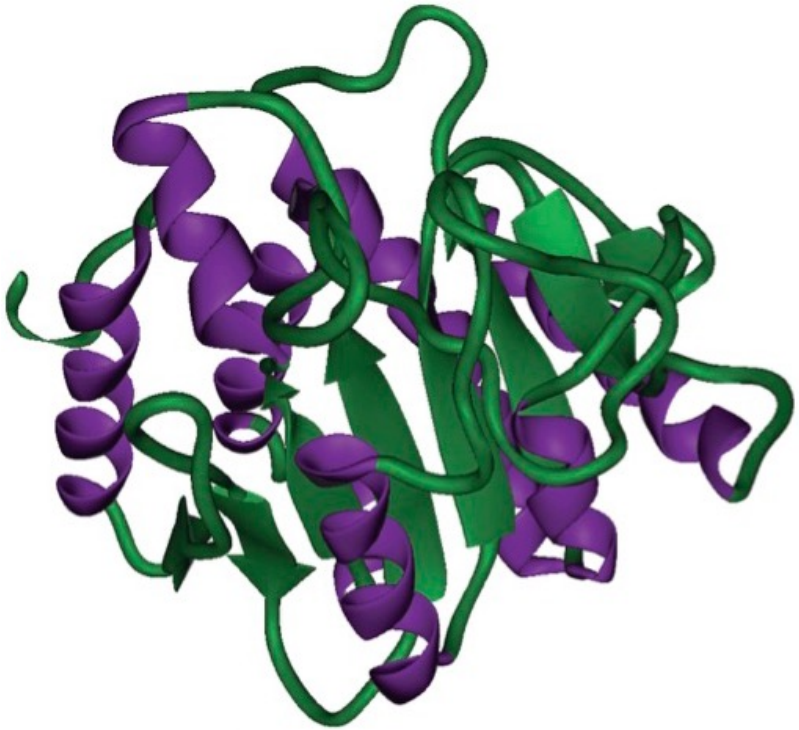
Proteins: Structure

***Primary structure** is the sequence of amino acids in a protein and the location of disulfide bridges*

- **obtained from sequencing a protein**

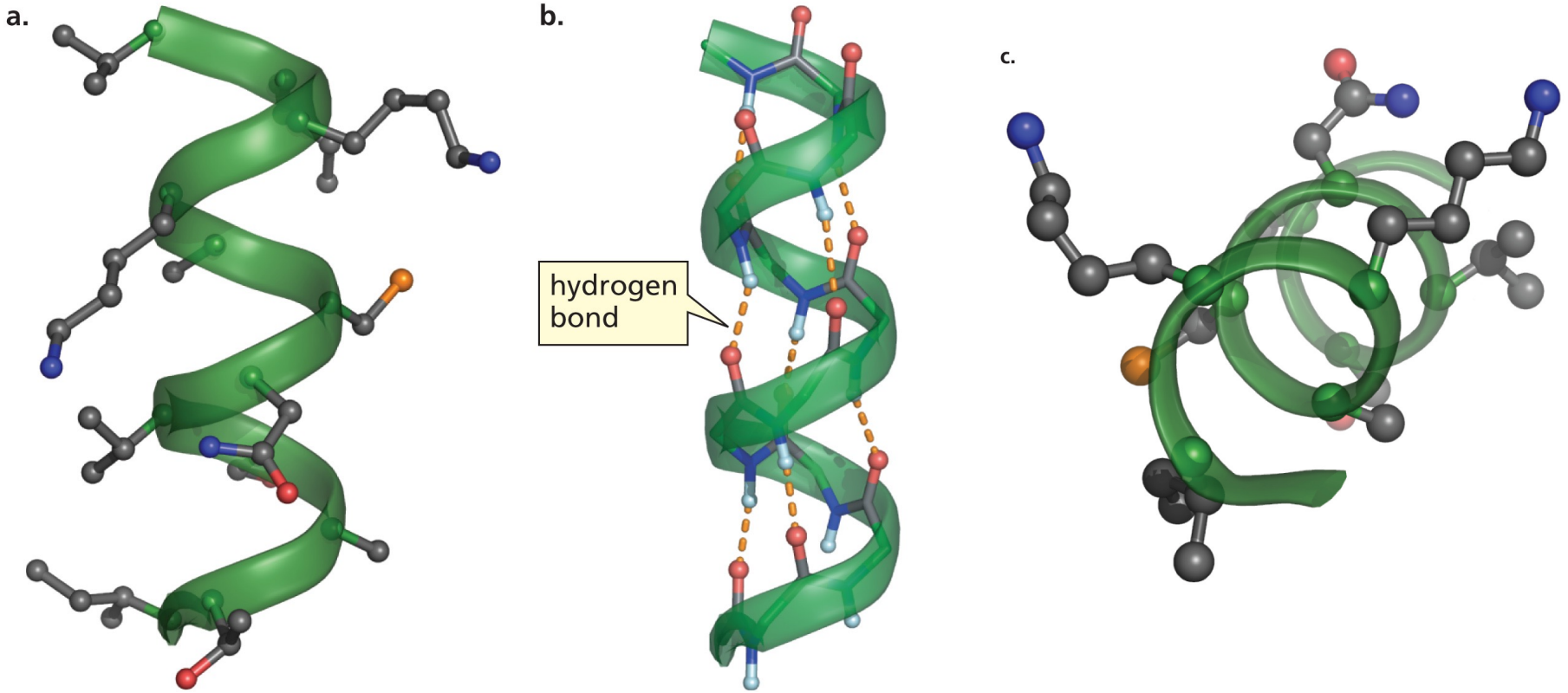
***Secondary structure** describes the common conformations of segments of a protein*

three types:



Proteins: Secondary Structure

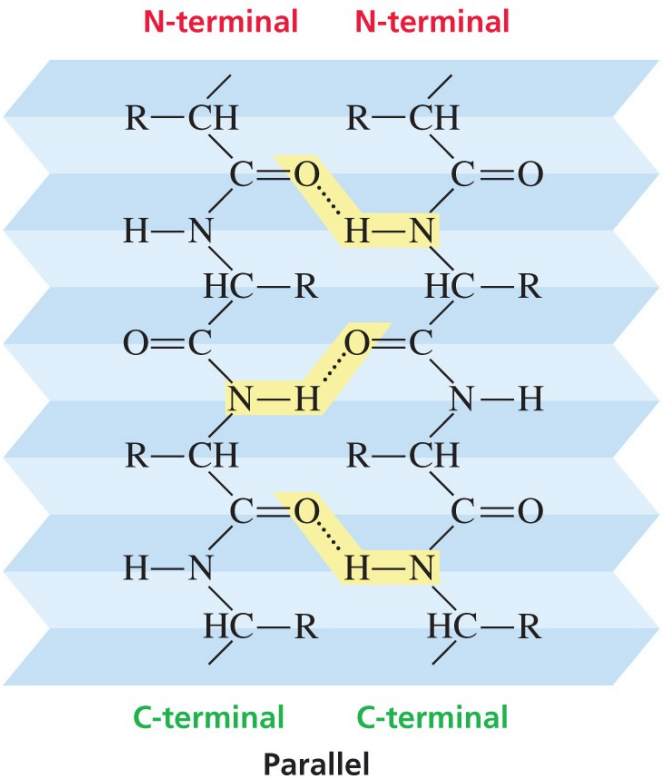
α-Helix



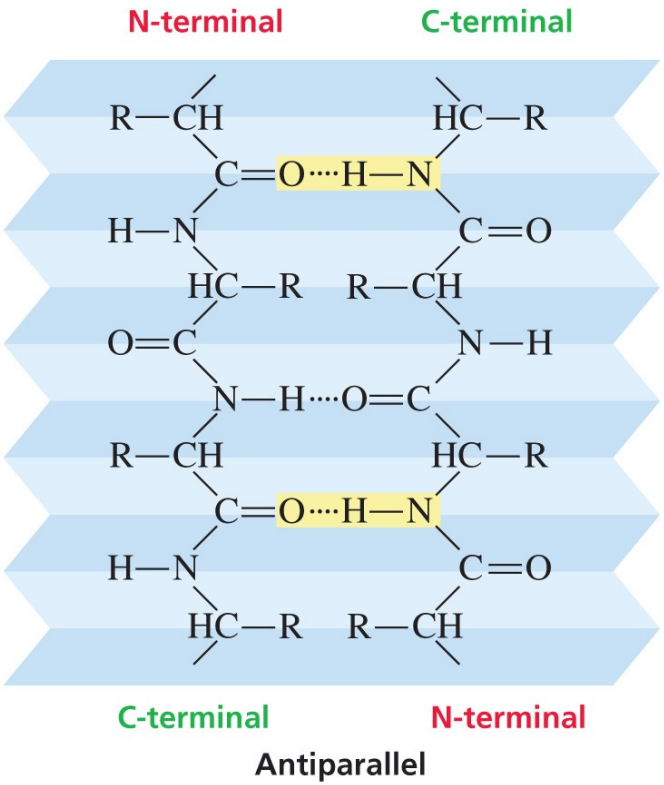
one turn: 3.6 aa, 5.4 Å repeat distance

Proteins: Secondary Structure

β-Pleated Sheet



Parallel

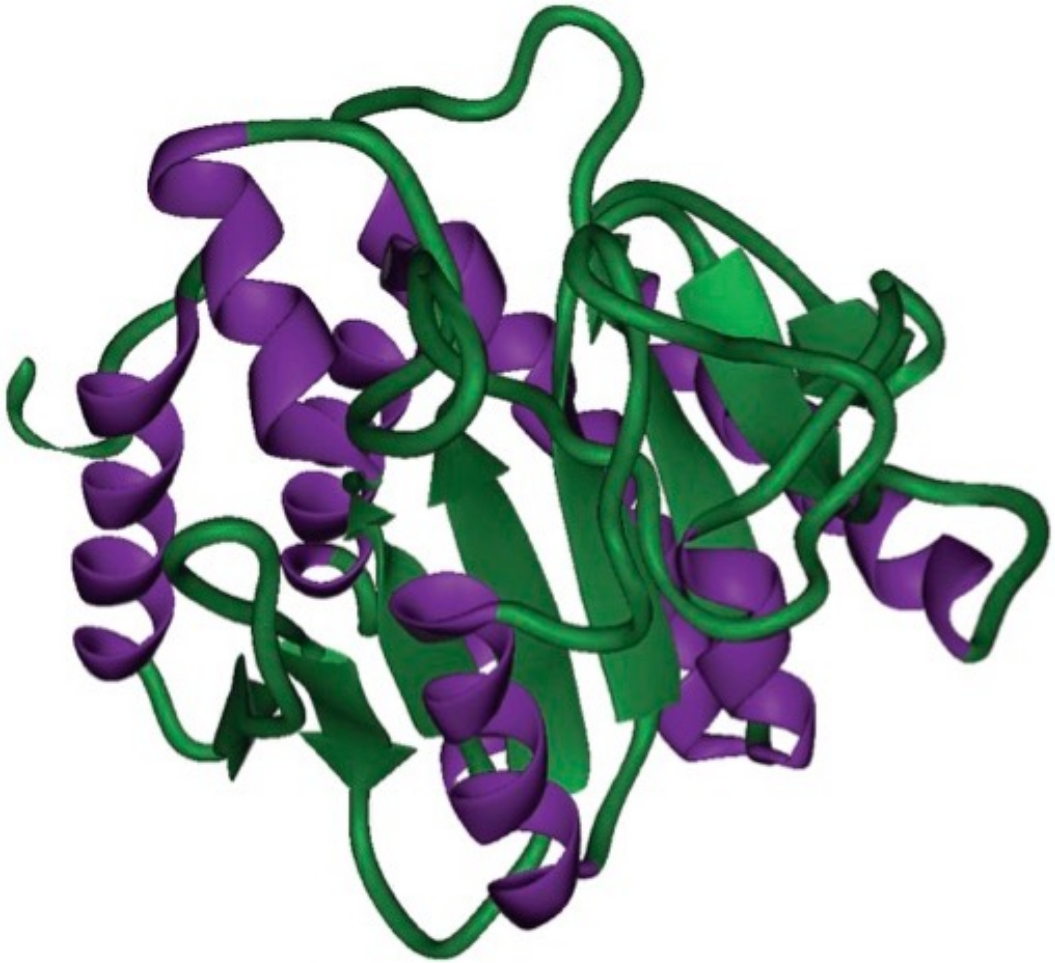


Antiparallel

average two residue repeat distance is 7.0 Å

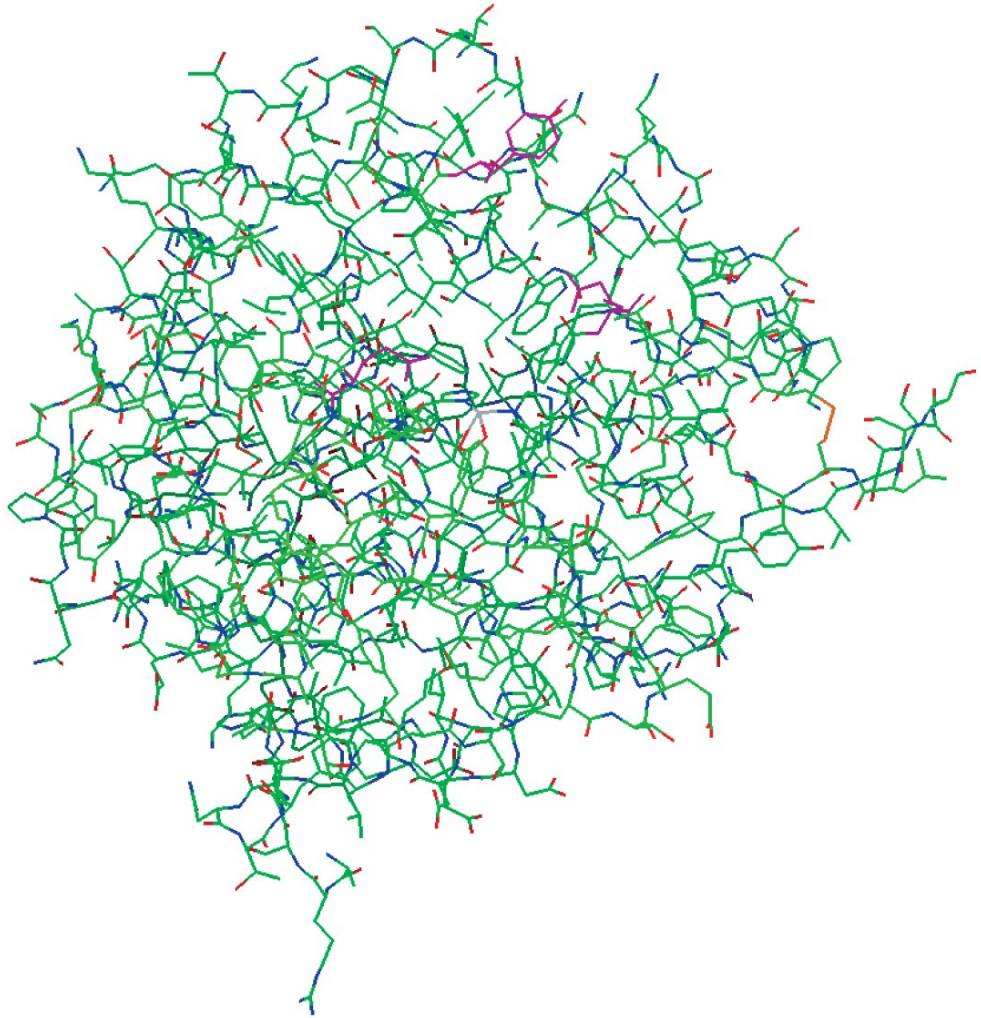
Proteins: Secondary Structure

Coils or Loops



Proteins: Tertiary Structure

Tertiary structure describes the 3D arrangement of all the atoms in the protein



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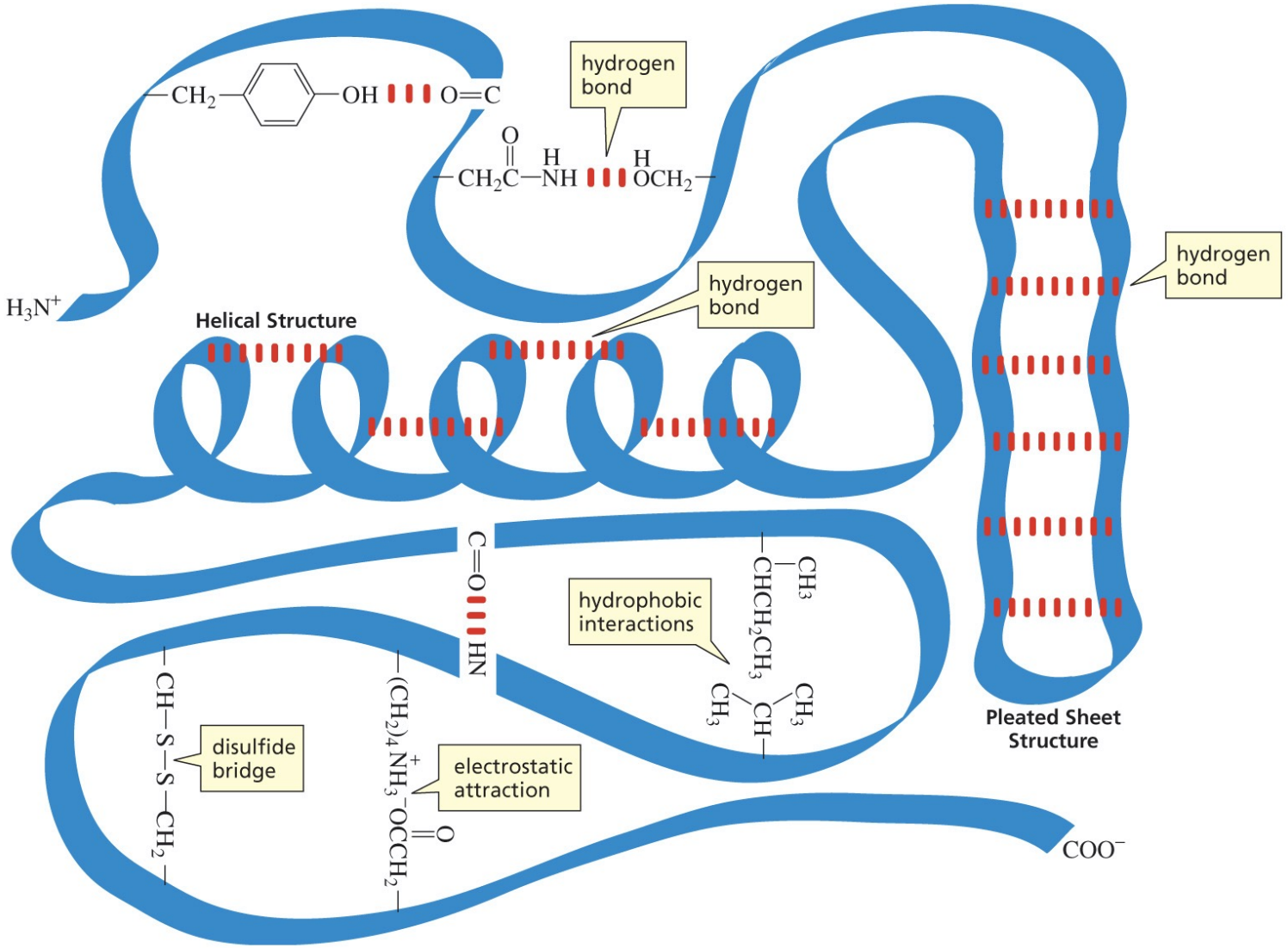
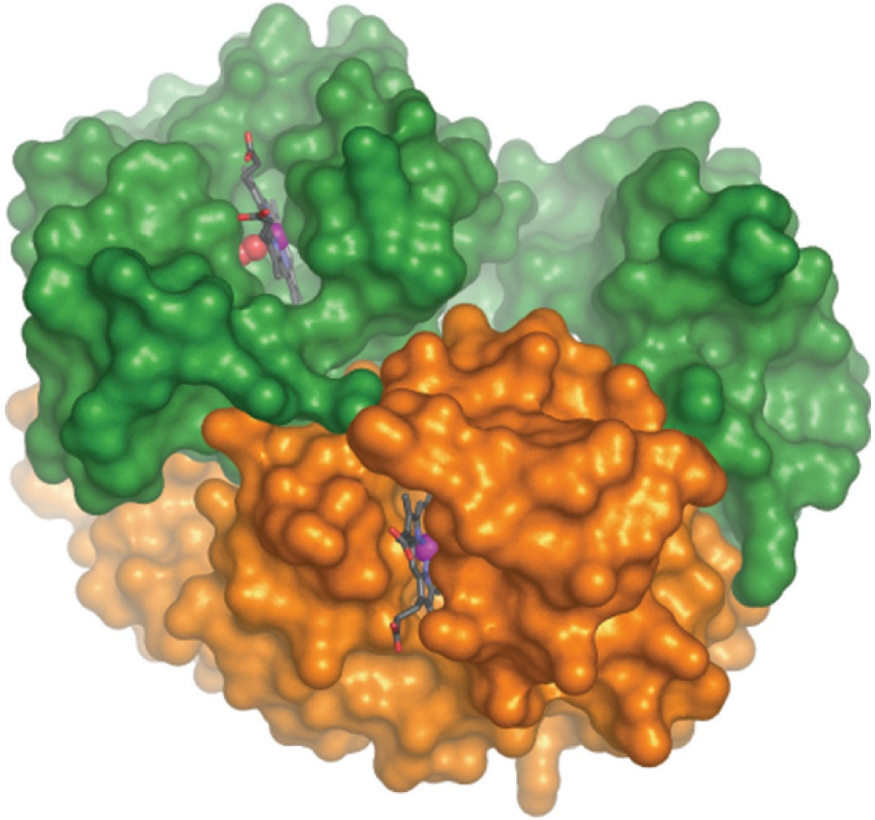


Proteins: Quaternary Structure

Quaternary structure aggregates of proteins: each is called a subunit

Proteins: Quaternary Structure

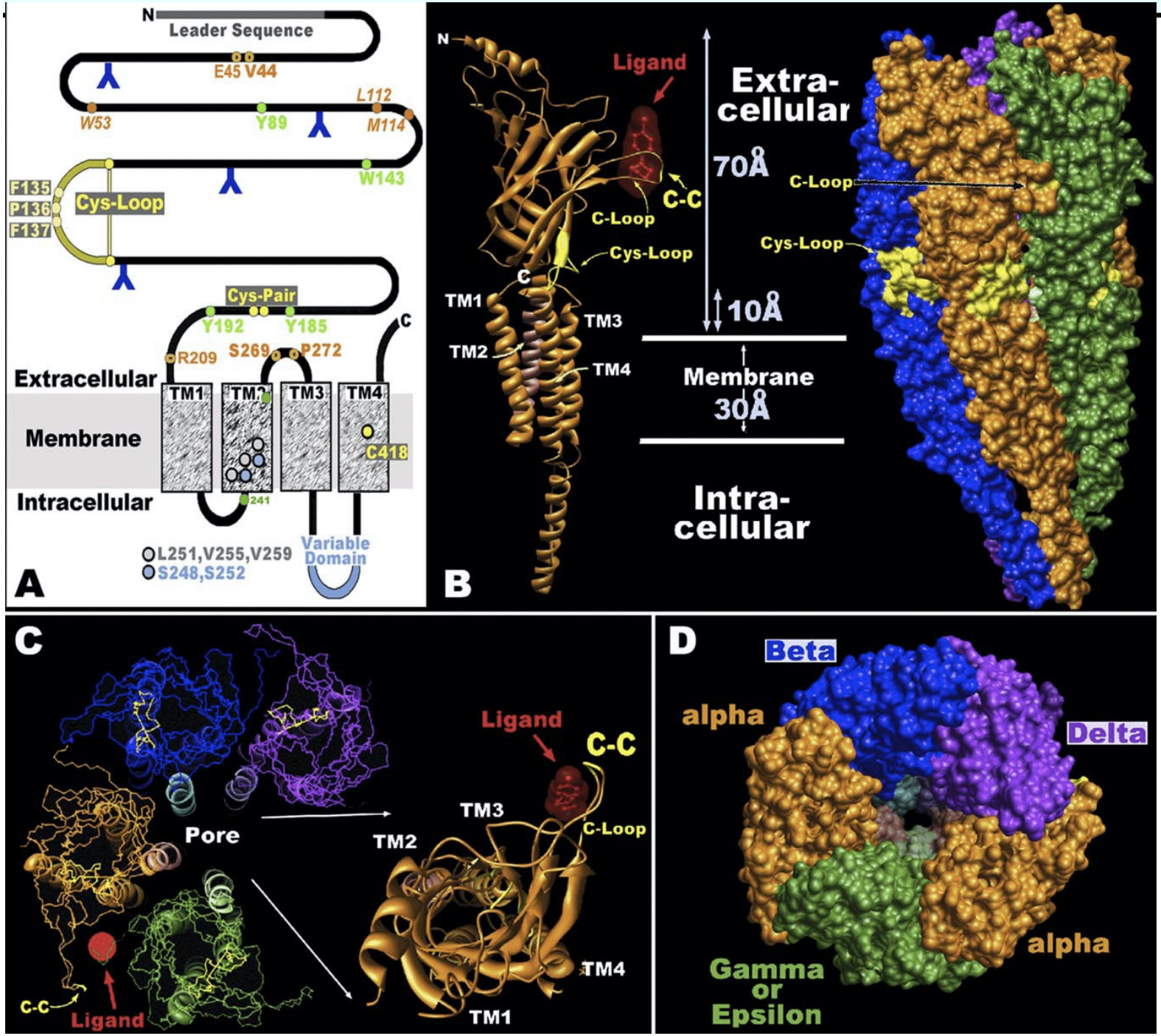
Quaternary structure aggregates of proteins: each is called a subunit



hemoglobin

mutation at position 6: glutamate → valine: sickle cell anemia

Proteins: Quaternary Structure



nicotinic acetylcholine receptor (nAChR)

Proteins: Denaturation

Protein denaturation: **is destruction of the highly organized tertiary structure, results in [irreversible] loss of function**

factors that can cause denaturation:

- **changing acidity (pH)**
- **temperature**
- **some reagents (urea, guanidine)**
- **detergents**
- **organic solvents**

Protein Structure and Analysis

structure: primary, secondary, tertiary, quaternary
parameters of alpha-helix, beta sheet, loops/coils
and tertiary structures

structure determination, reagents:
$HSCH_2CH_2OH$
6M HCl

Edman's reagent
cyanogen bromide BrCN

exopeptidases: carboxypeptidase A
carboxypeptidase B

endopeptidases:
trypsin
chymotripsin
elastase

PROBLEM 48

Determine the primary structure of an octapeptide from the following data:

-acid-catalyzed hydrolysis gives 2 Arg, Leu, Lys, Met, Phe, Ser, and Tyr

-carboxypeptidase A releases Ser

-Edman's reagent releases Leu

-BrCN forms two peptides with the following composition:

1. Arg, Phe, Ser and 2. Arg, Leu, Lys, Met, Tyr

-trypsin-catalyzed hydrolysis forms the following two amino acids and two peptides:

1. Arg 2. Ser 3. Arg, Met, Phe 4. Leu, Lys, Tyr