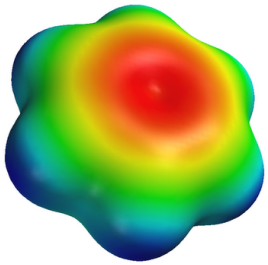

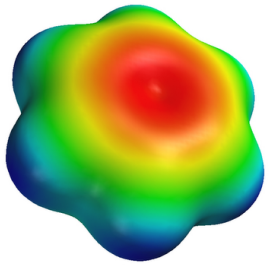

Chem 109 C

Fall 2014

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Proteins: Structure

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

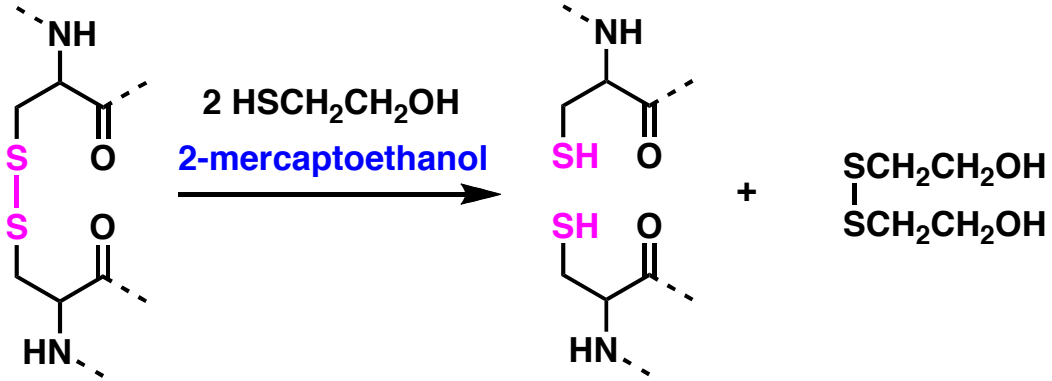
strategy for determining the primary structure:

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: $HSCH_2CH_2OH$



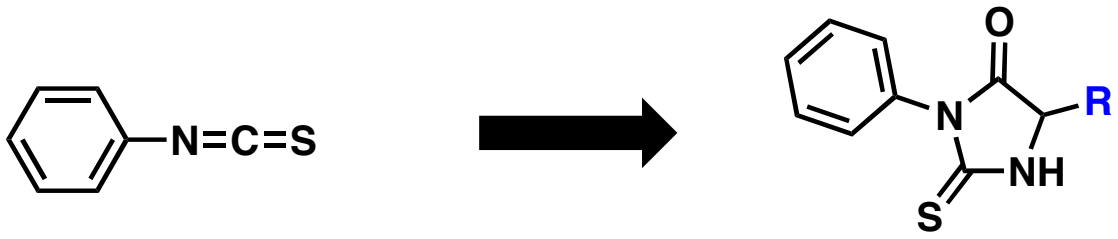
complete hydrolysis: 6 M HCl, H_2O, 100 ºC, 24 h

then use amino acid analyser…

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 sequencator

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…

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…

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

Gly-Arg-Trp-Ala-Glu-Leu-Met-Pro-Val-Asp

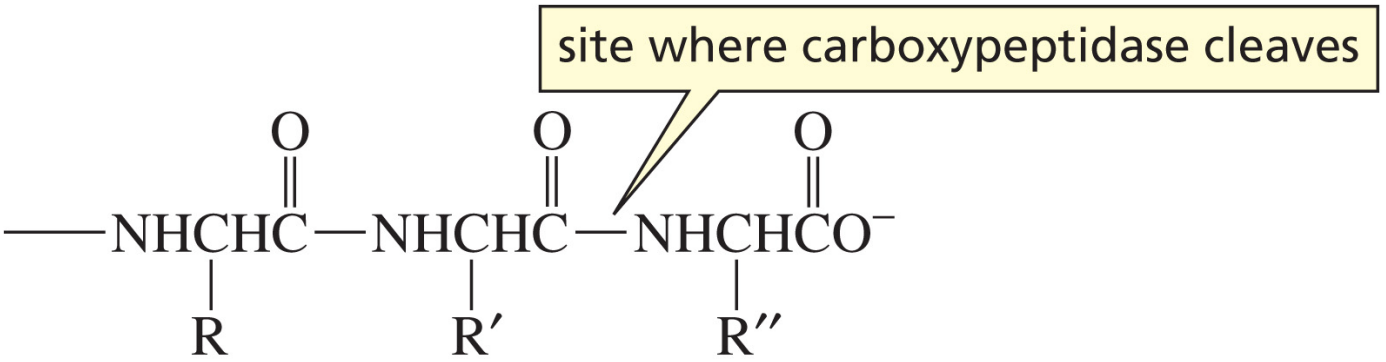
selective reagents to break amide bonds:

for C-terminal amino acid:

exopeptidases:

carboxypeptidase A: all but Arg and Lys

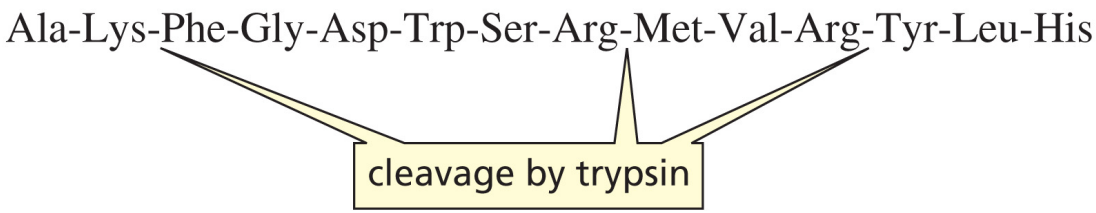
carboxypeptidase B: only Arg and Lys



selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

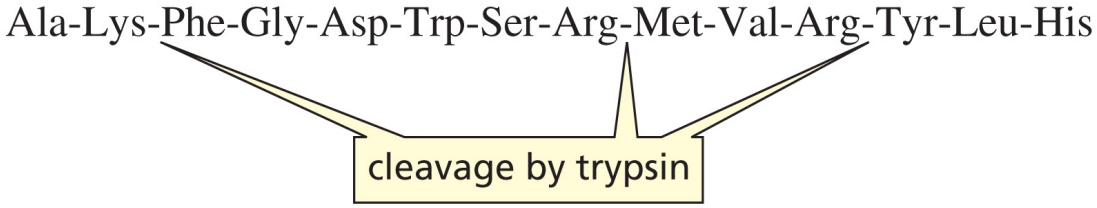
Trypsin: C-side of Arg and Lys



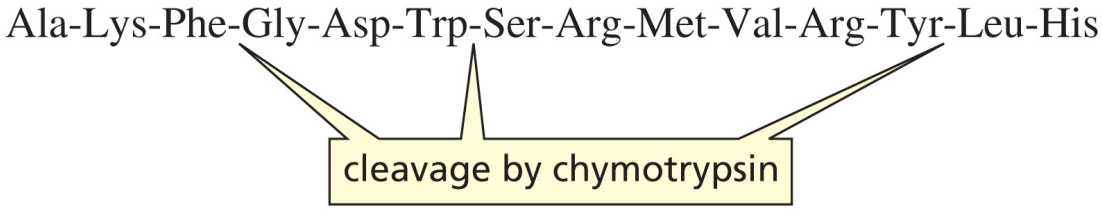
selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

Trypsin: C-side of Arg and Lys



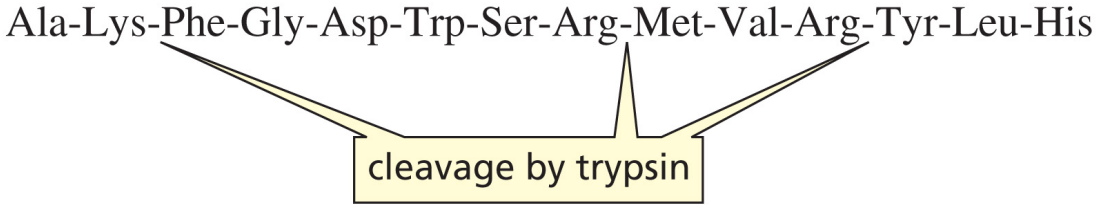
Chymotrypsin: C-side of Phe, Tyr and Trp



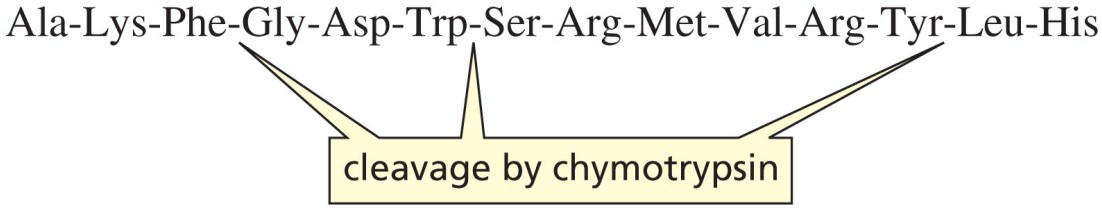
selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

Trypsin: C-side of Arg and Lys



Chymotrypsin: C-side of Phe, Tyr and Trp

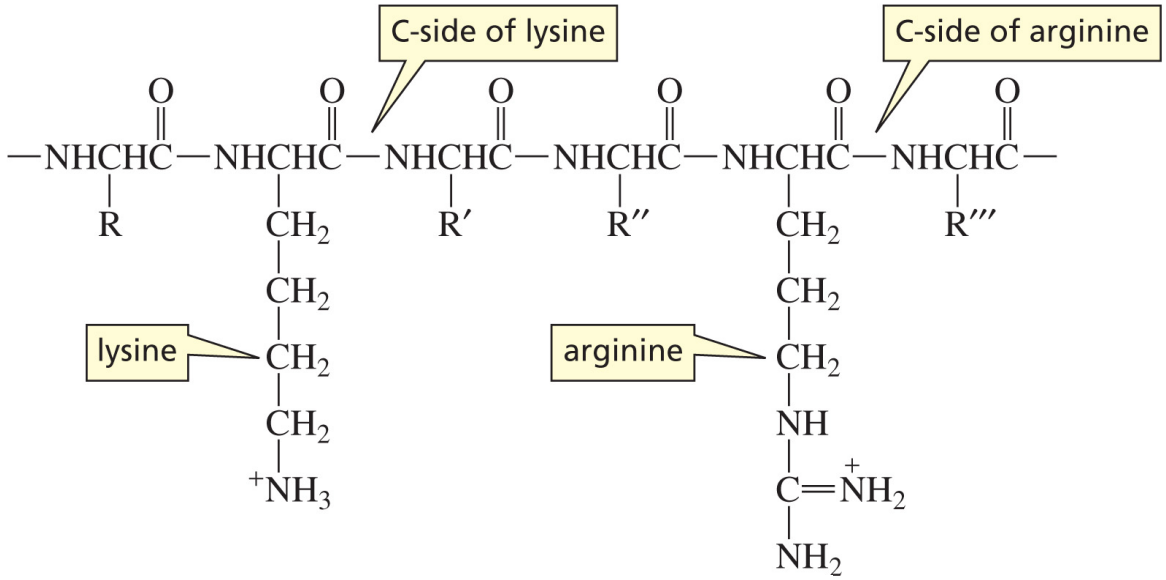


Elastase: C-side of Gly and Ala

selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

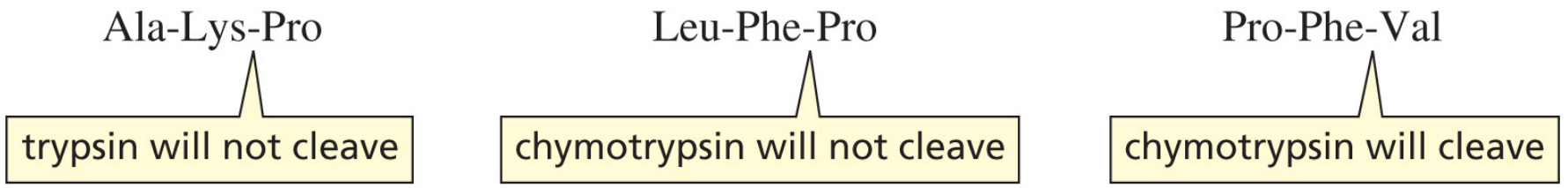
Trypsin: C-side of Arg and Lys



selective reagents to break amide bonds:

for endo- and exopeptidases,

no cleavage next to 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 22.4 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*

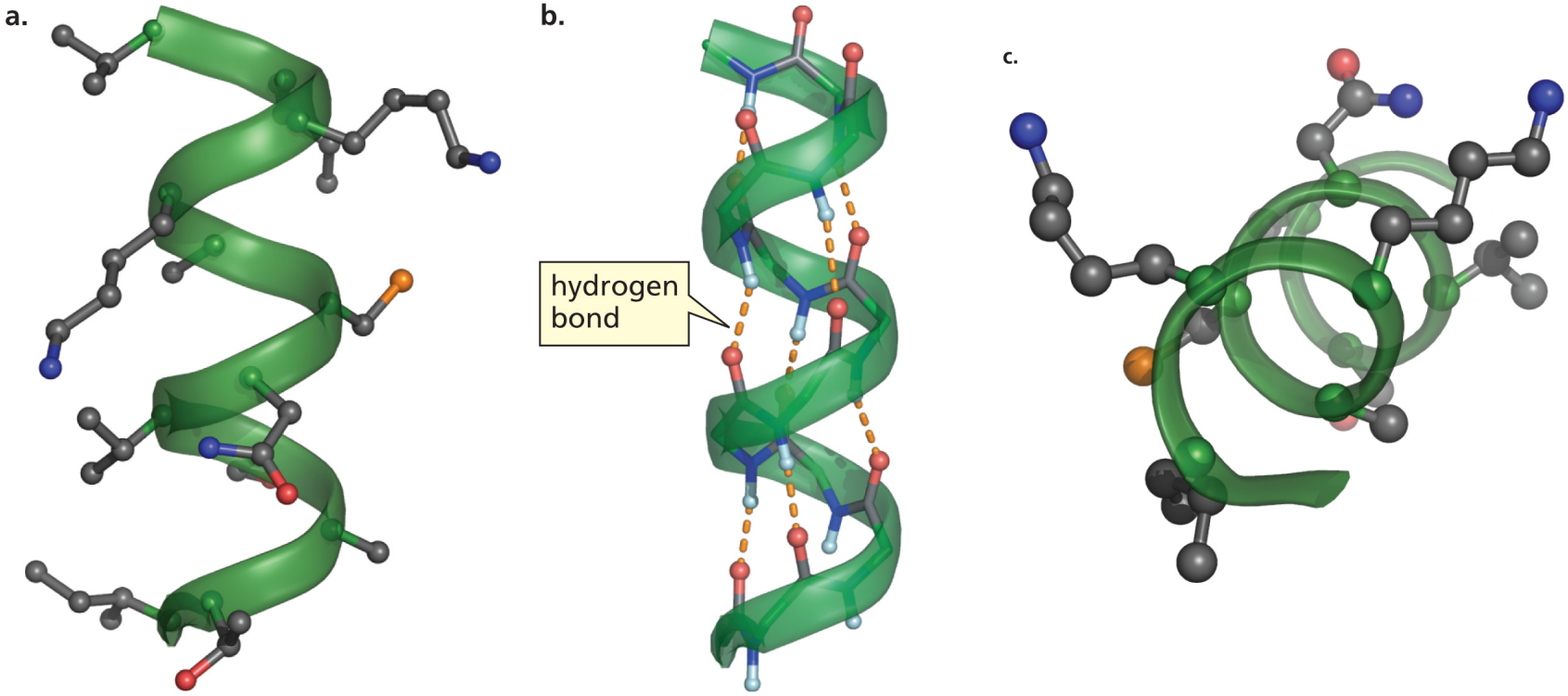
- **obtained from sequencing a protein**

***Secondary structure** describes the repetitive conformation of segments of a protein*

three types:

Proteins: Secondary Structure

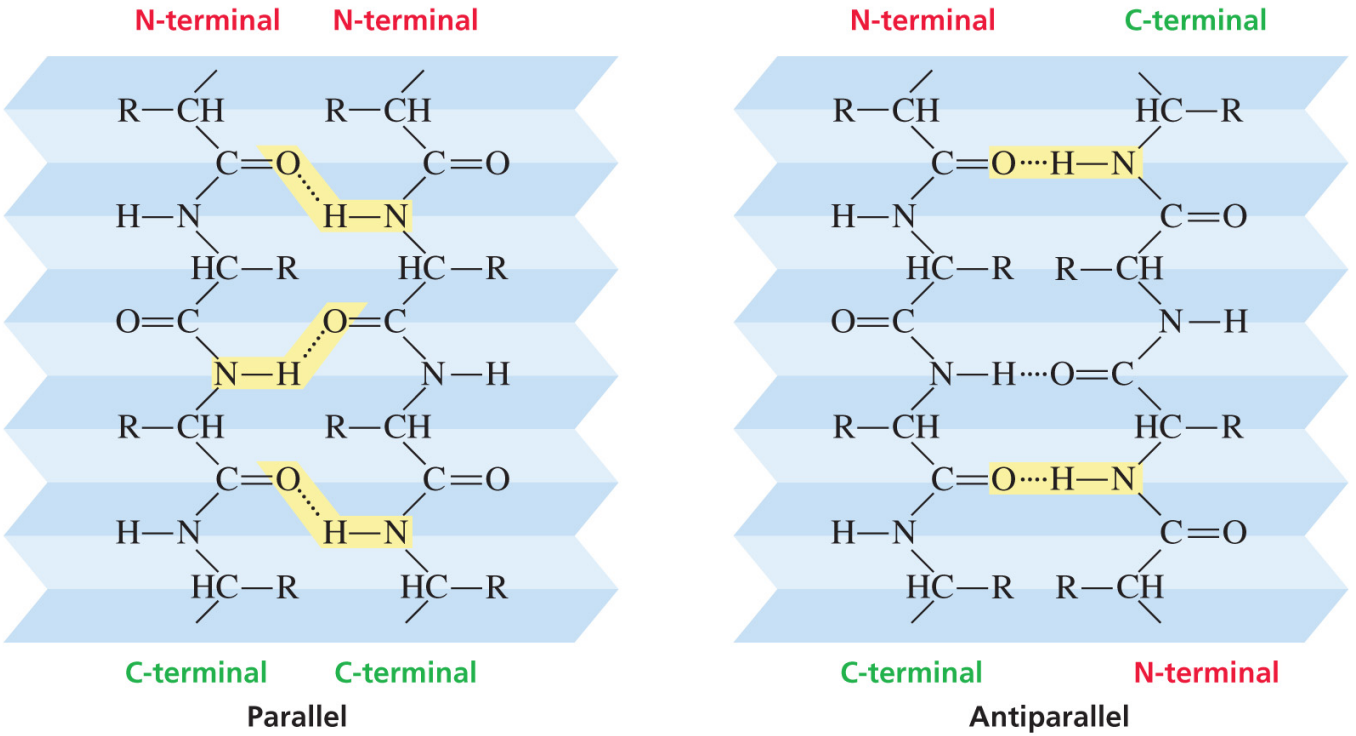
α-Helix



one turn: 3.6 aa, 5.4 Å repeat distance

Proteins: Secondary Structure

β-Pleated Sheet



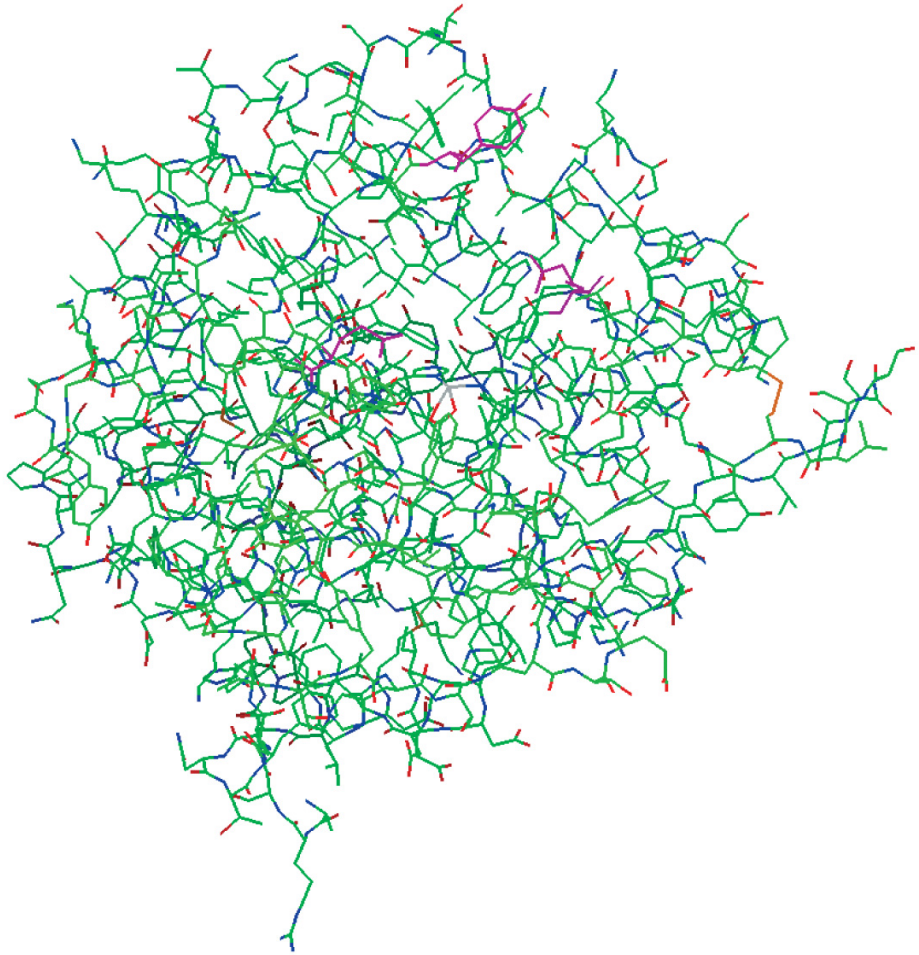
average two residue repeat distance is 7.0 Å

Proteins: Secondary Structure

Coils or Loops

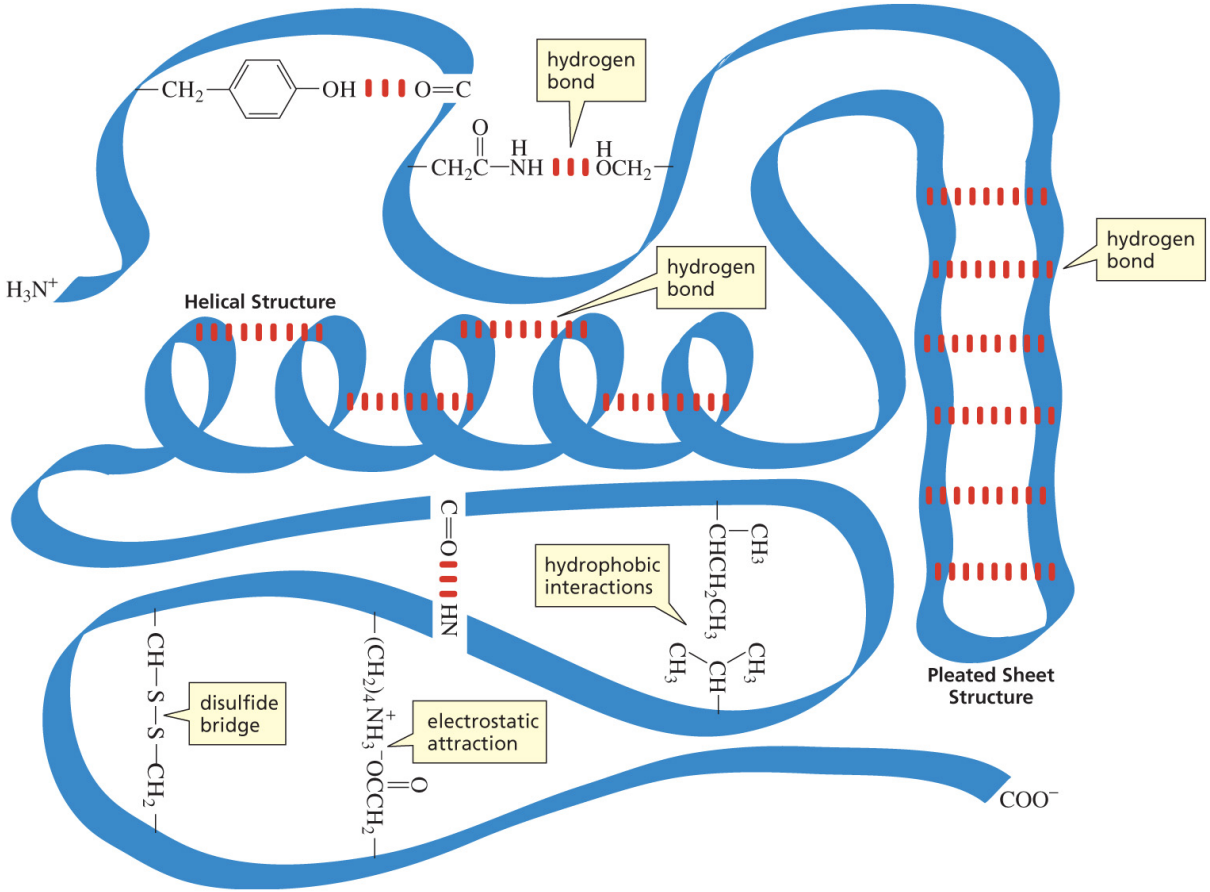
Proteins: Tertiary Structure

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



Proteins: Tertiary Structure

Tertiary structure ***describes the 3D arrangement of all the atoms in the protein (subunit)***

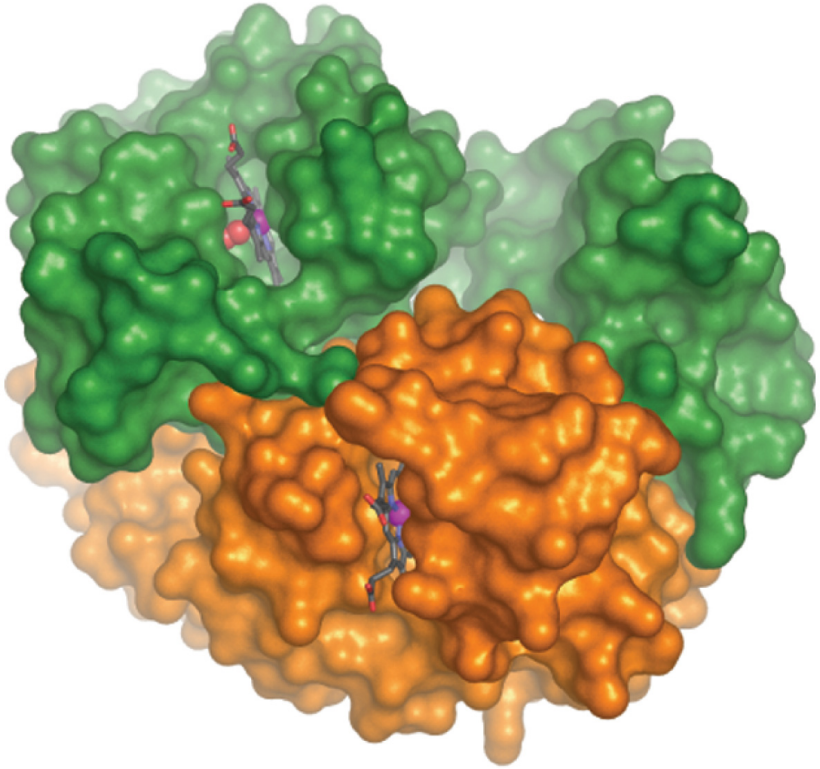


Proteins: Quaternary Structure

Quaternary structure *aggregates of proteins: each is called a subunit*

Proteins: Quaternary Structure

Quaternary structure *aggregates of proteins: each is called a subunit*

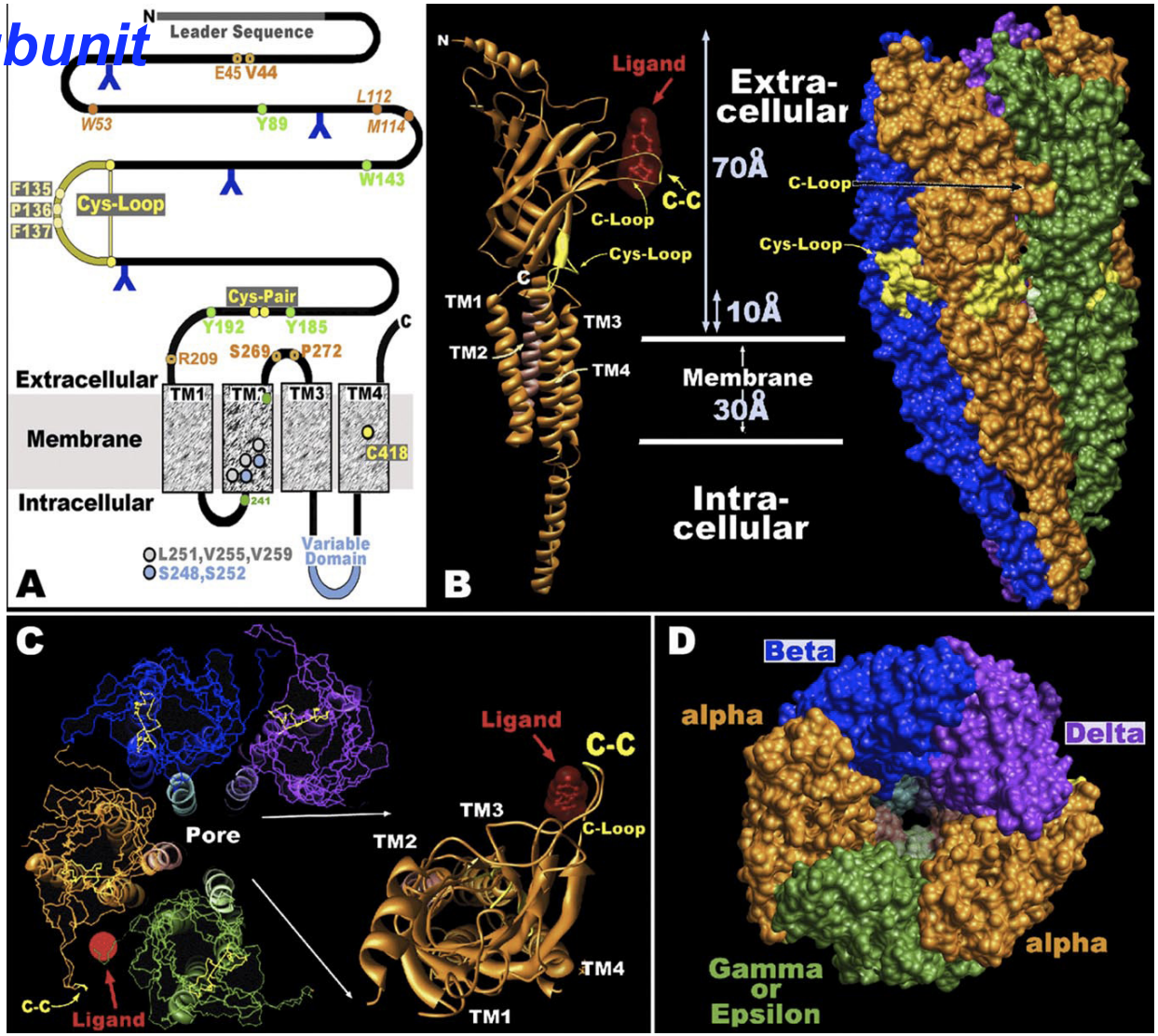


hemoglobin

at position 6: glutamate → valine: sickle cell anemia

Proteins: Quaternary Structure

Quaternary structure *aggregates of proteins: each is called a subunit*



nicotinic acetylcholine receptor (nAChR)

Proteins: Denaturation

Protein denaturation: **is destruction of the highly organized tertiary structure**

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