- Reading: Ch3; Fig 3-23
  Ch4; 106-110
- Homework: #7

Lecture 7 (9/24/25)

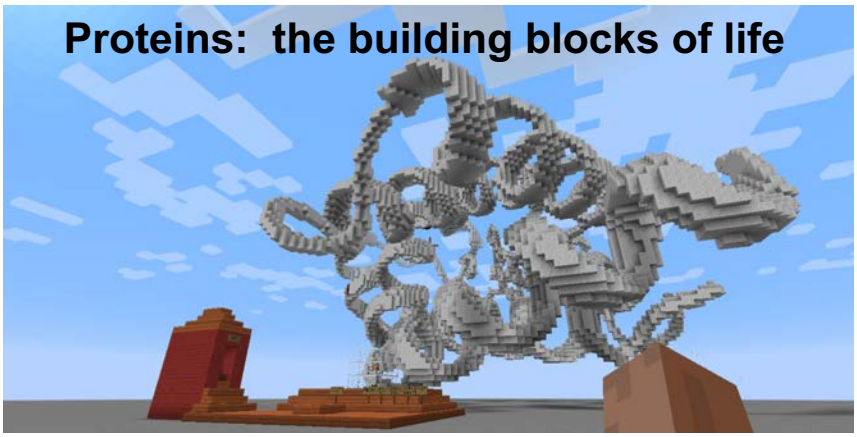
NEXT

- Reading: Ch3; 90-93, Box 3-2
- Homework: #8

OUTLINE

I. Protein Structure
   A. Hierarchy; 1°, 2°, 3°, 4°
   B. Primary
      1. The peptide bond
      2. 4 S's
      3. Determination of 1° structure
         a. Chemical
         b. Physical
         c. Biological

Protein Structure



4 Levels of Protein Structure

The **STRUCTURE** of proteins has been divided into **four** categories:

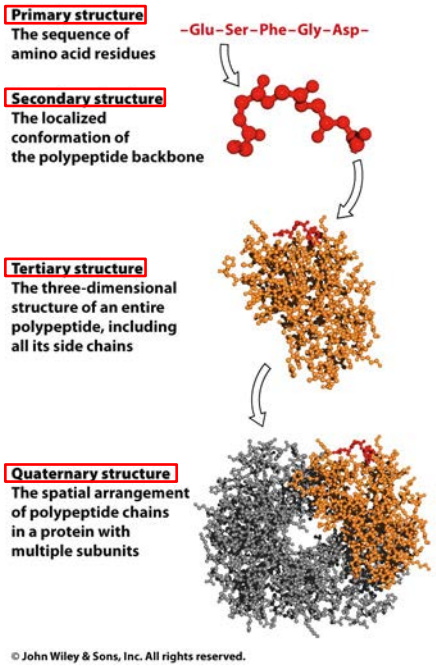
1) **primary structure** – sequence of amino acids
2) **secondary structure** – small units of repetitive structure
3) **tertiary structure** – overall 3D shape
4) **quaternary structure** – shape of ≥2 chains

15

4 levels of protein structure

In order to understand these levels of structure, you need to understand the nature of the polymer first.

In other words, the linkage or PEPTIDE BOND



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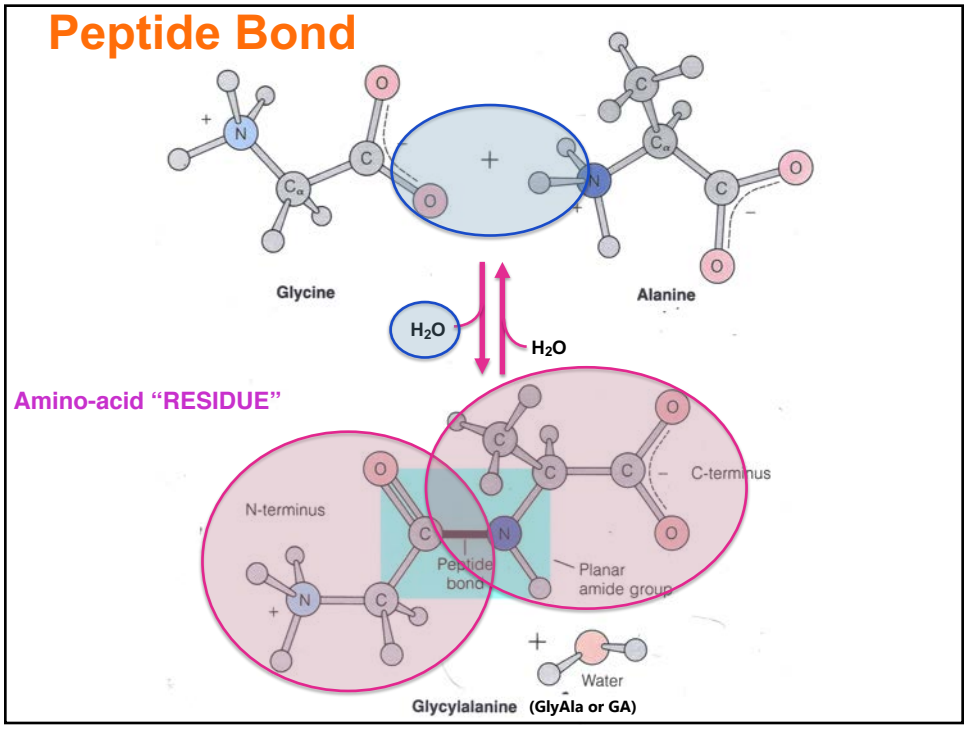
The Peptide Bond

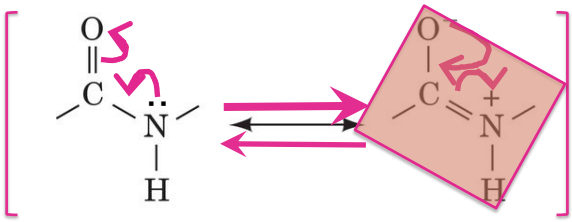


Peptide Bond

The 4 S's for the Peptide Bond:

Shape
Size
Solubility
Stability

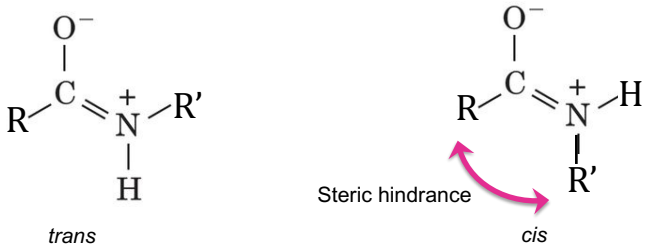
Peptide Bond - Shape



Resonance of Peptide Bond

Consequences:
1) Double-bond Character
   a) Length
   b) Strength
   c) Planarity

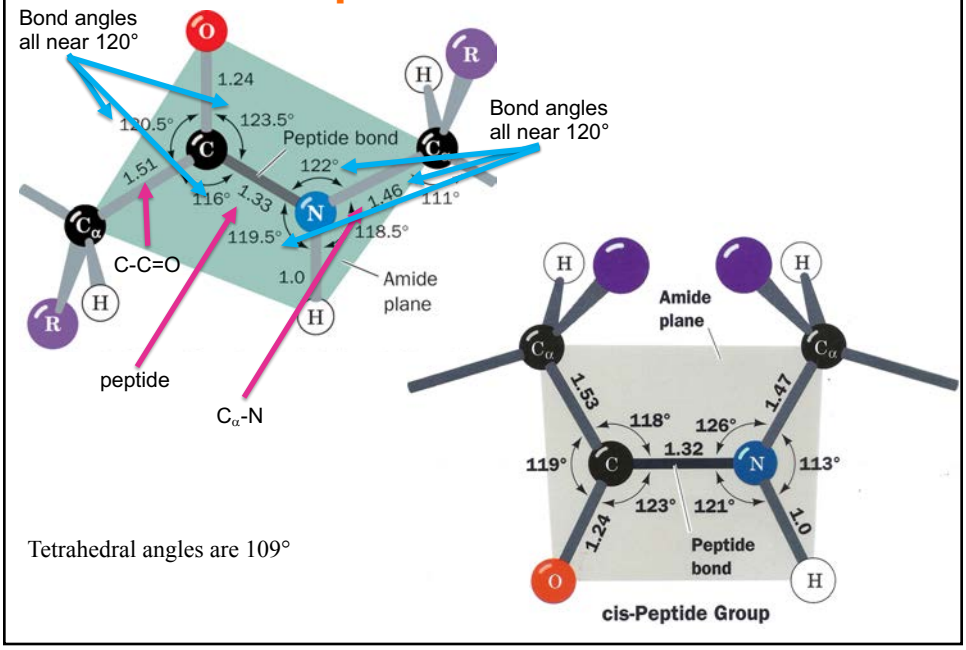
Peptide Bond - Shape



Trans vs. Cis of Peptide Bond

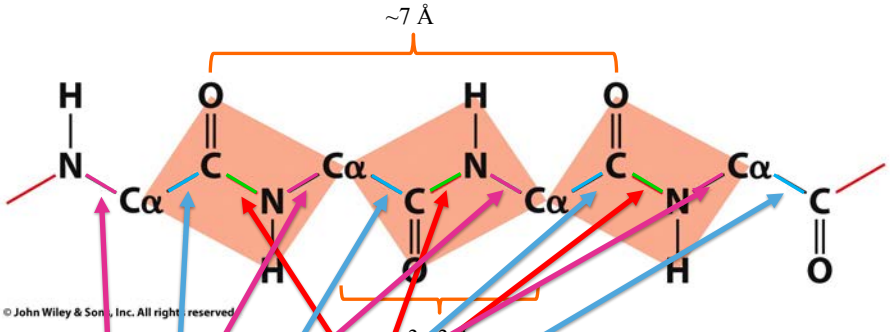
Most Peptide Bonds in Proteins Assume Trans Configuration

Peptide Bond - Size



Tetrahedral angles are 109°

Peptide Bond - Size



No free rotation of peptide bonds

Therefore, there are only TWO types of bonds in the whole polymer that are flexible:

$N–C_\alpha$

$C_\alpha–C=O$

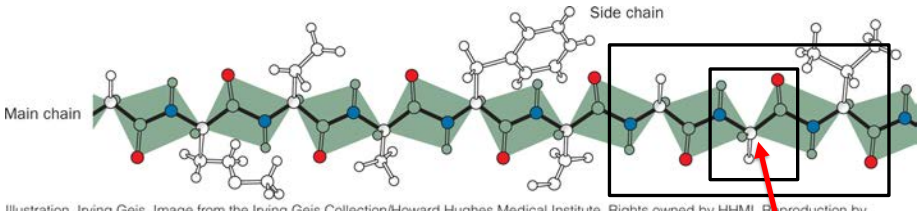
$N–C_\alpha$ is called the Phi (ϕ)

$C_\alpha–C=O$ is called the Psi (ψ)

Peptide Bond - Size

One Amino-acid "residue"

Three Amino-acid "residues" in sequence



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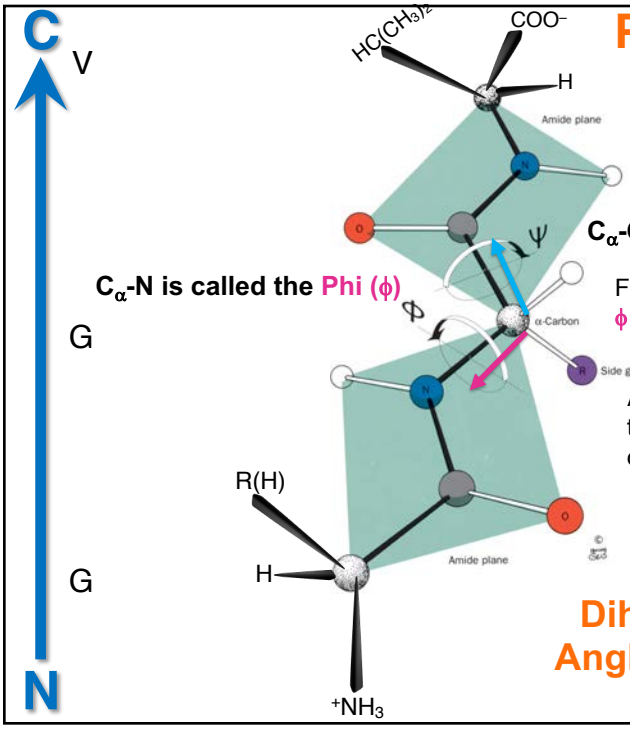
G M D A F S G G V

Extended Conformation of Polypeptide

Let's take this tripeptide with two peptide bonds, get small, and sit on this alpha carbon

Peptide Bond - Size



C_α-N is called the Phi (ϕ)

C_α-C=O is called the Psi (ψ)

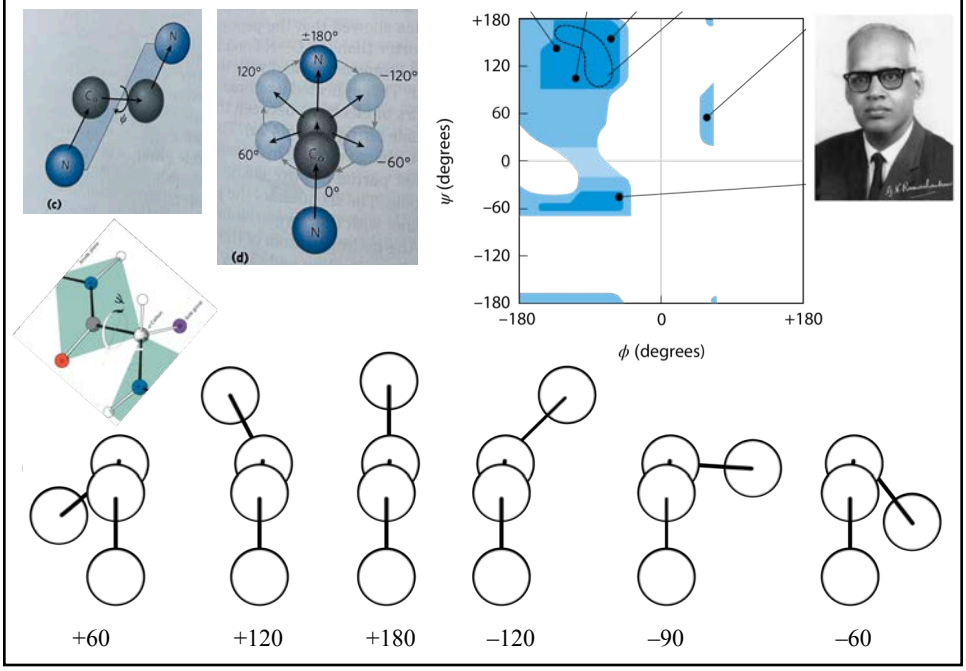
Fully extended all trans peptide: ϕ = -180° and ψ = +180°

Angles increase as you turn the peptide-bond plane clockwise

Angles decrease as you turn the peptide-bond plane counter-clockwise

Dihedral or Torsion Angles of Polypeptide Backbone

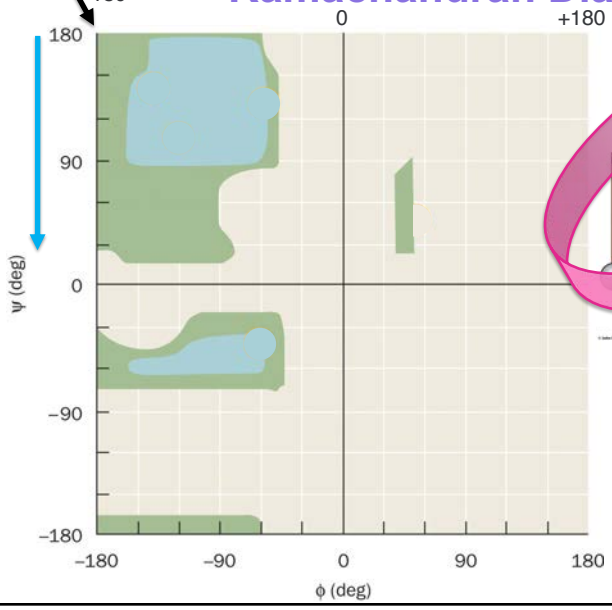
Peptide Bond - Size

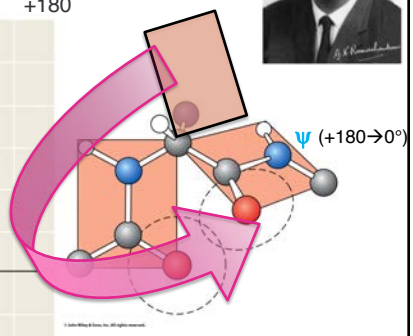


Peptide Bond - Size

Fully extended
-180

Ramachandran Diagram





ψ went from +180° to smaller angles on Ramachandran Plot

Recall: Angles decrease as you turn the peptide-bond plane counter-clockwise

Peptide Bond - Size

Only 25% of allowable angles used

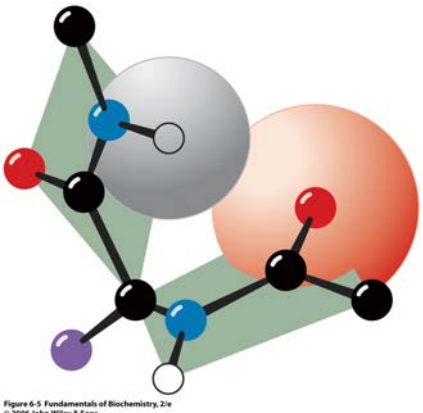


Figure 6-5 Fundamentals of Biochemistry, 2/e
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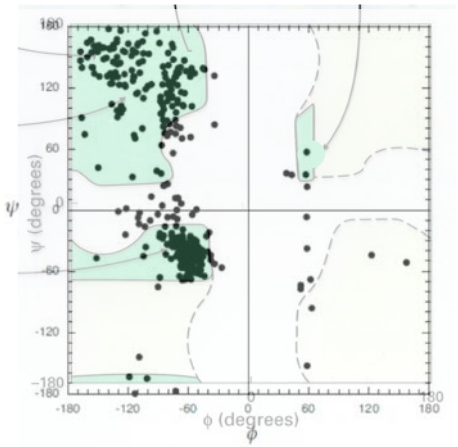
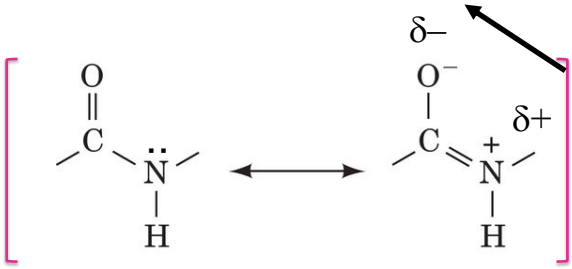
Ramachandran Plot of Yeast Hexokinase



- Glycine has more allowable angles (in yellow) Why?

Peptide Bond - Solubility



Resonance of Peptide Bond

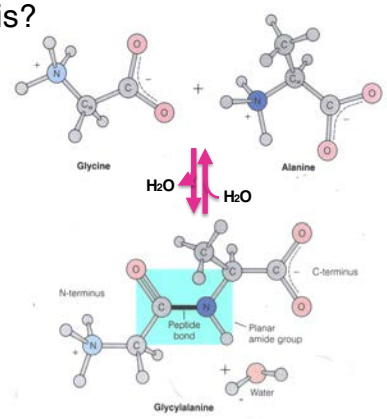
Consequences:

1) Double-bond Character
   a) Length
   b) Strength
   c) Planarity
2) Partial charges on O & N – Polarity of peptide bond
   a) O is excellent H-bond acceptor
   b) N is excellent H-bond donor

This makes the polymer (called "alpha-carbon backbone") very water soluble

Peptide Bond - Stability

What is the energetics of hydrolysis?



The ΔG of hydrolysis is –2.4 kcal/mole (–10 kJ/mole)

Glycylalanine + H_2O ⇌ Glycine + Alanine

Why doesn't your hair fall apart into AA & water, or all other proteins?

Kinetically stable, thermodynamically unstable

What does it take to hydrolyze proteins?

Peptide Bond

The catalysis of the hydrolysis reaction to cleave the Peptide Bond:

Acid	– 6 N HCl, 16 hr at 110 C° All peptide (and amide) bonds cleaved
Base	–3 N NaOH, 6 hr at 100 C° All peptide (and amide) bonds cleaved, but hydroxyl groups and guanidino groups are destroyed
Enzymes	–proteolysis; incomplete due to specificity of enzymes

Use hydrolysis to answer 2 questions:

1) determine amino acid **composition**
2) Help determine entire amino-acid **sequence** by fragmenting into small peptides

Peptide Bond

The 4 S's for the Peptide Bond (recap):

Shape	–trans & double-bond, planar character due to resonance
Size	–7 Å repeat length & two rotatable bonds at Cα (ϕ & ψ)
Solubility	–highly polar bond due to resonance
Stability	–high bond energy due to resonance

Determination of the Primary Structure

Fred Sanger
1918-2013
1958 Nobel Prize

[TABLE 4-2] Composition of Some Proteins

Protein	Number of Amino Acid Residues	Number of Polypeptide Chains	Molecular Mass (D)*	
Insulin (bovine)	51	2	5733	
Rubredoxin (*Pyrococcus*)	53	1	5878	110.9
Myoglobin (human)	153	1	17,053	
Phosphorylase kinase (yeast)	416	1	44,552	
Hemoglobin (human)	574	4	61,972	
Reverse transcriptase (HIV)	986	2	114,097	
Nitrite reductase (*Alcaligenes*)	1029	3	111,027	
C-reactive protein (human)	1030	5	115,160	107.8
Pyruvate decarboxylase (yeast)	1112	2	121,600	
Immunoglobulin (mouse)	1316	4	145,228	
Ribulose bisphosphate carboxylase (spinach)	5048	16	567,960	
Glutamine synthetase (*Salmonella*)	5628	12	621,600	110.5
Carbamoyl phosphate synthetase (*E. coli*)	5820	8	637,020	

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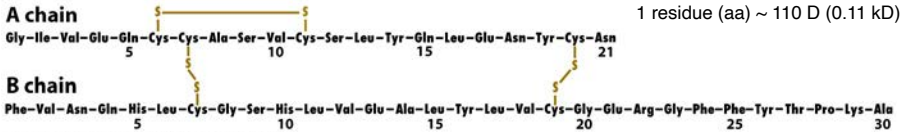
* Dalton (D) = amu = g/mol
kD = kilodalton = 1000 D

1 residue (aa) ~ 110 D (0.11 kD)



Determination of primary structure

1) Purify protein
2) Determine the amino-acid composition, including stoichiometry
3) Disrupt structure (2°, 3°, 4°, and disulfides)
4) Determine the number of peptide chains by counting number of amino terminal ends
5) Divide into fragments and determine sequence
6) Divide into different set of fragments and determine sequence
7) Determine overlaps and piece original sequence back together

28

Determination of primary structure

1) Purify protein
2) Determine the amino-acid composition, including stoichiometry
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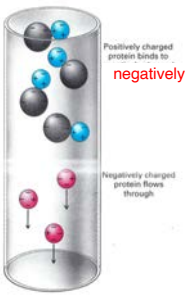
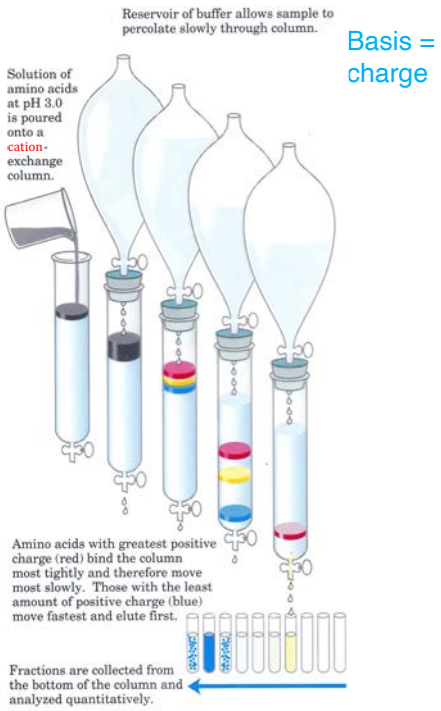
Ion Exchange Chromatography: Example of amino-acid mixture



If this were a mixture of G, K, and D, what would the order of elution be?

1st= D, 2nd = G, 3rd = K



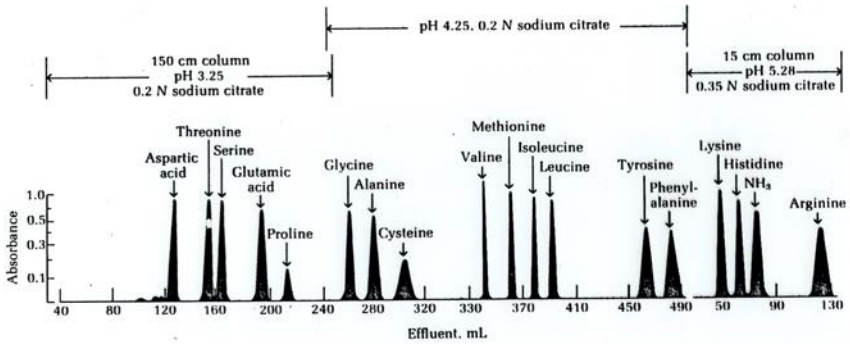
Basis = charge

Determination of primary structure

Wm H. Stein
Stanford Moore

Amino Acid Composition

Figure 5-15
Automatically recorded chromatographic analysis of amino acids on a cation-exchange resin. The elution is carried out with different buffers of successively higher pH. The effluent is caught in small volumes, and the amino acid content of each tube is automatically analyzed. The area under each peak is proportional to the amount of each amino acid in the mixture.



Absorbance is at 570 nm (purple). How did they make the amino acid residues colored? .....ninhydrin

How many AA are "resolved?" 17

Where are the other 3? .....Asn & Gln have already been converted to Asp & Glu, respectively
.....enough Trp was not in this protein to "see"

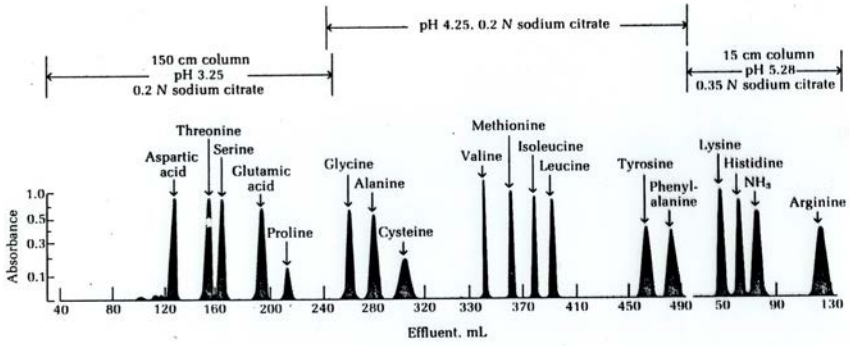
Determination of primary structure

Wm H. Stein
Stanford Moore

Amino Acid Composition

Figure 5-15
Automatically recorded chromatographic analysis of amino acids on a cation-exchange resin. The elution is carried out with different buffers of successively higher pH. The effluent is caught in small volumes, and the amino acid content of each tube is automatically analyzed. The area under each peak is proportional to the amount of each amino acid in the mixture.

Cation exchanger=Dowex-50 (sulfonic acid on polystyrene)



Calculate the "mole%" or "mole fraction"; in other words the stoichiometry:

Example; protein MW = 11,000 Da

Approximate #AA = 11,000/110 (Ave MW for AA) = 100 AA

If 10% of A_{570} is 10% for Arg: 100 x 0.10 = 10 Arg in protein