Working with proteins

- Techniques in protein purification
- Determine the primary structure of protein

學習目標:(p. 85-100)

a. 熟悉純化蛋白質常用的技術及原理；

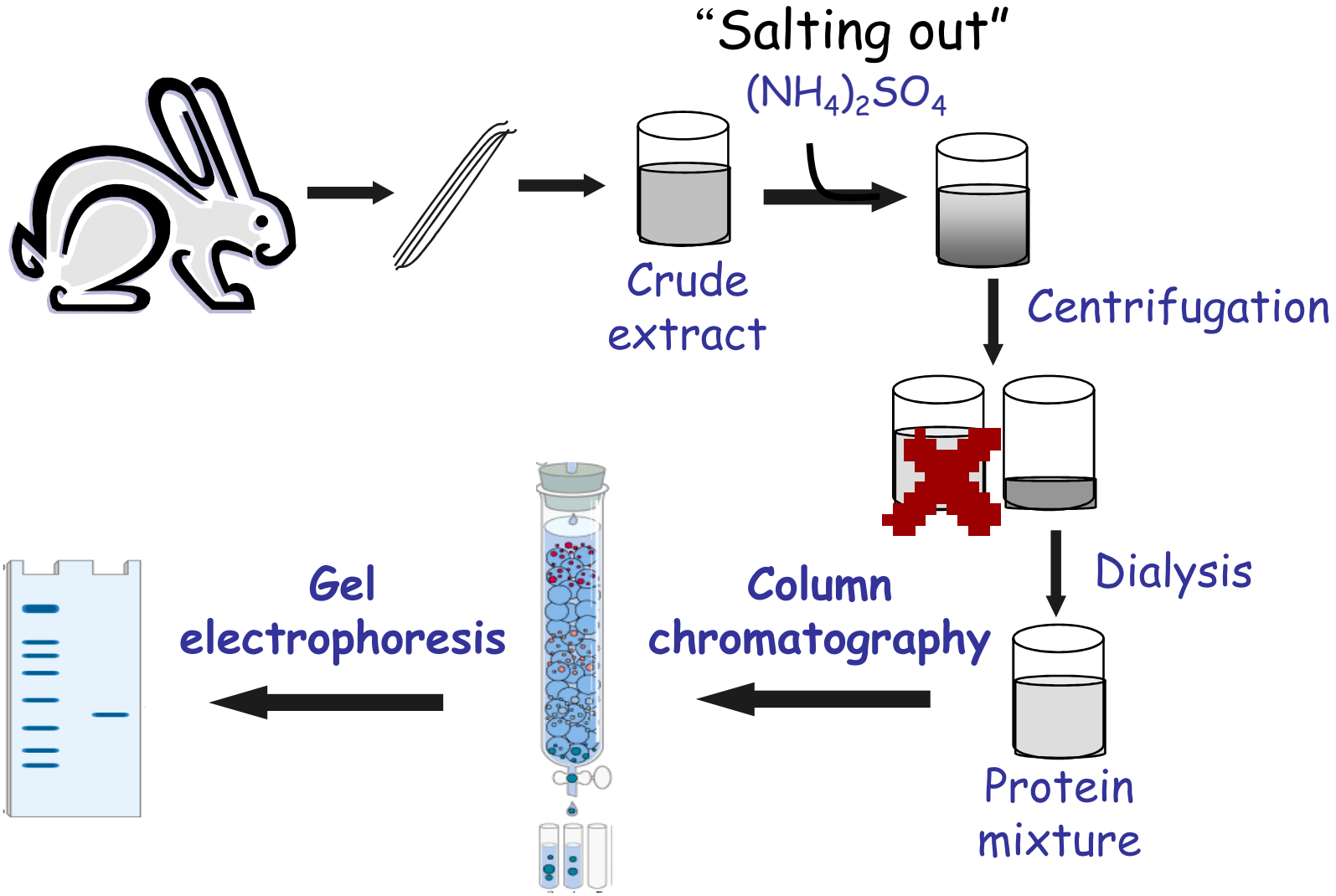
b. 瞭解各種技術使用的時機及限制。

Protein/peptide preparation

1. From biological tissue (*in vivo*)
2. Genetic engineering (Ch 9 of 5th ed.)
3. Chemical synthesis (*in vitro*)
   - In the reverse order (Fig 3-29)
   - Not very efficient
     - A protein of 100 a.a.:
       days by machine vs. 5 sec in bacteria

Protein purification

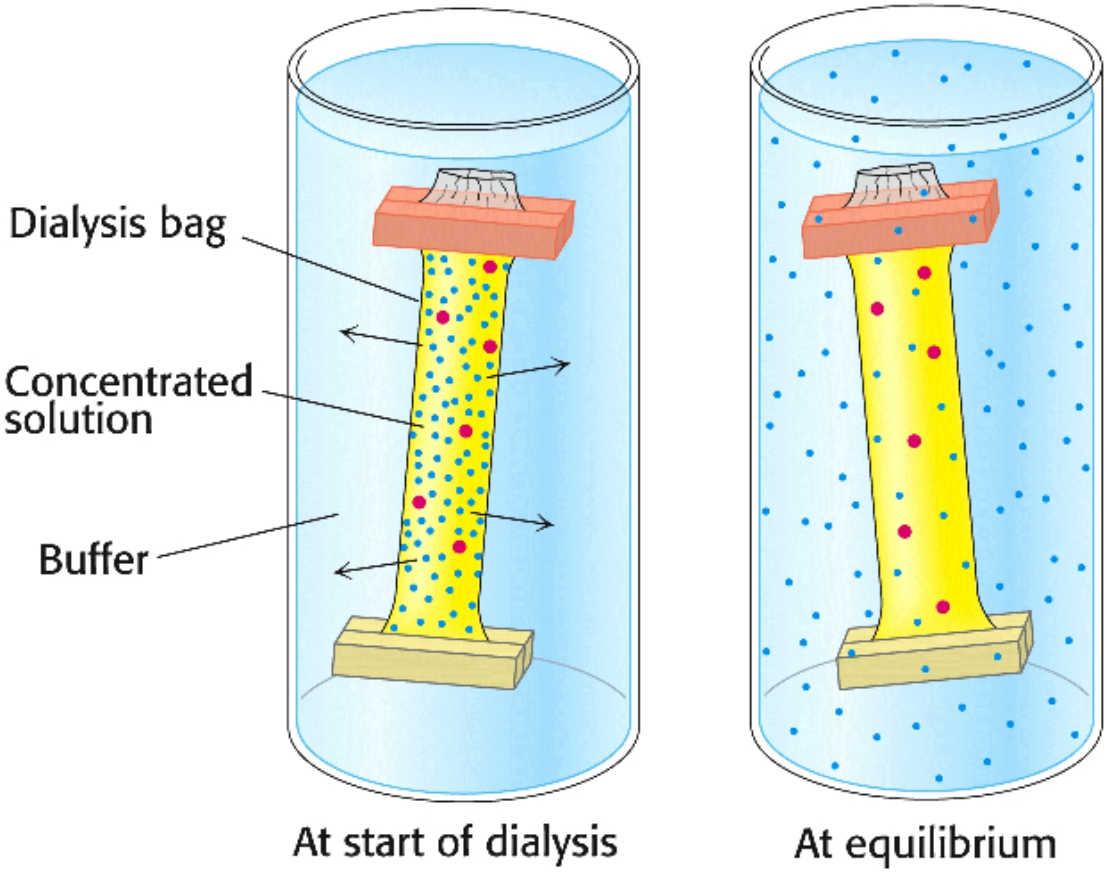
p. 85



Dialysis

- Semi-permeable membrane
- Concentration gradient

From Stryer 5th ed.



Column Chromatography

- Stationary phase + mobile phase
- Fractionation
- By difference among molecules
  - Size
  - Charge
  - Binding affinity

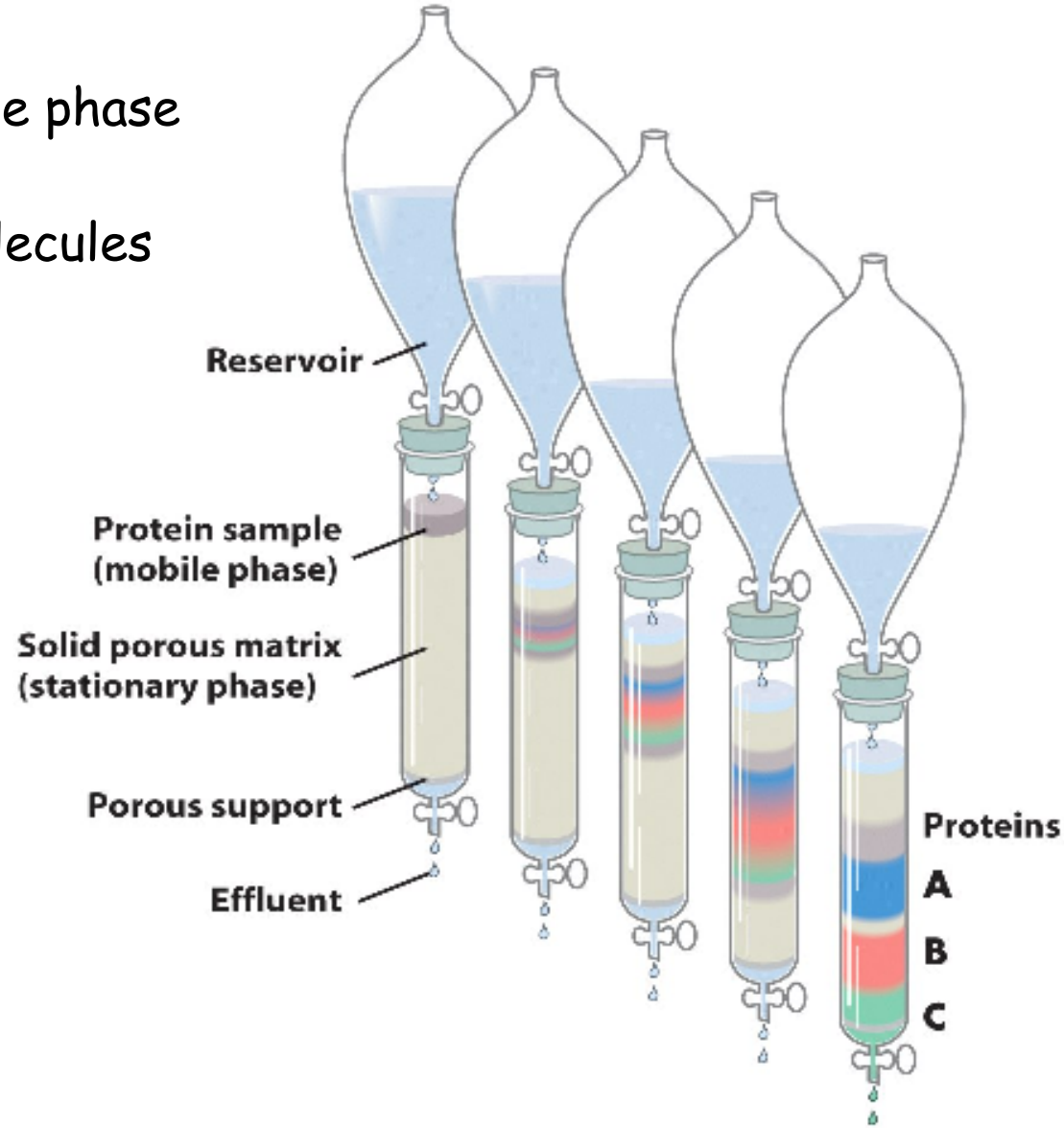


Fig 3-16, p.86

Ion Exchange

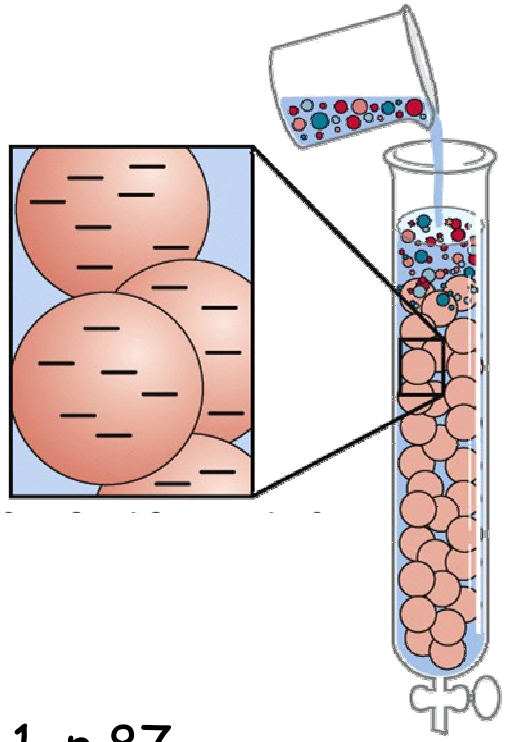
Fig 3-17a, p.87

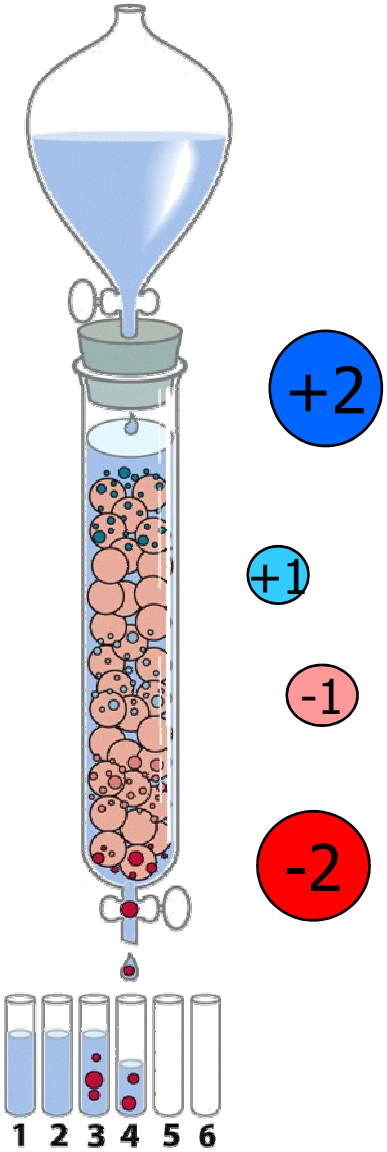
- By charge difference
- Cation and anion exchanger
  - Refers to the target interested
- Changing pH or salt conc.
  - Often increase salt conc. (salt gradient)

Negatively charged beads

||

Cation exchangers

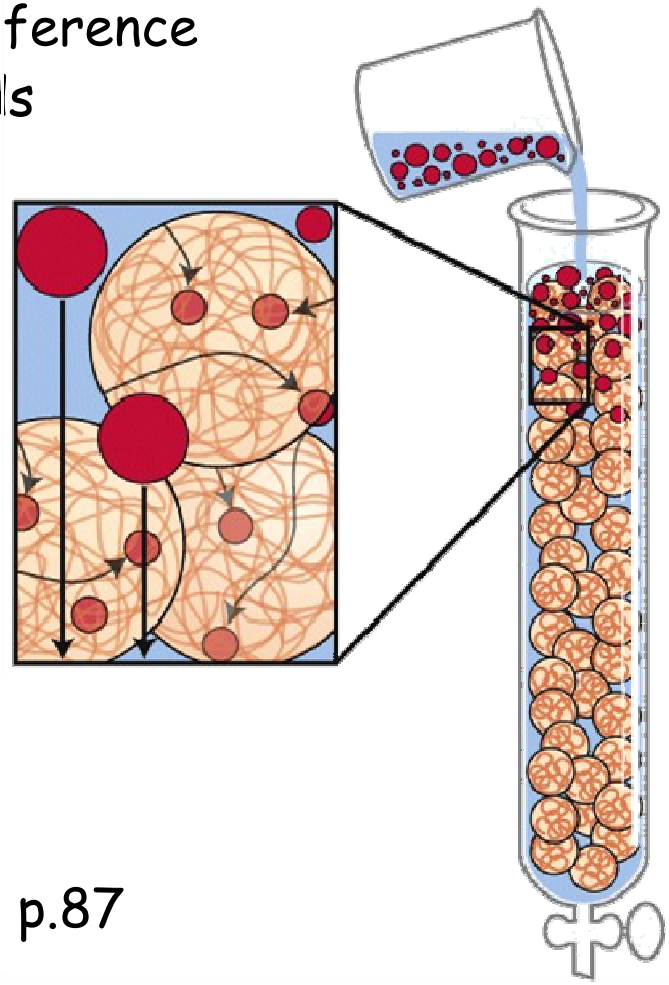




See Example 3-1, p.87

Size-exclusion

- Also called Gel filtration
- By size difference
- Pores beads



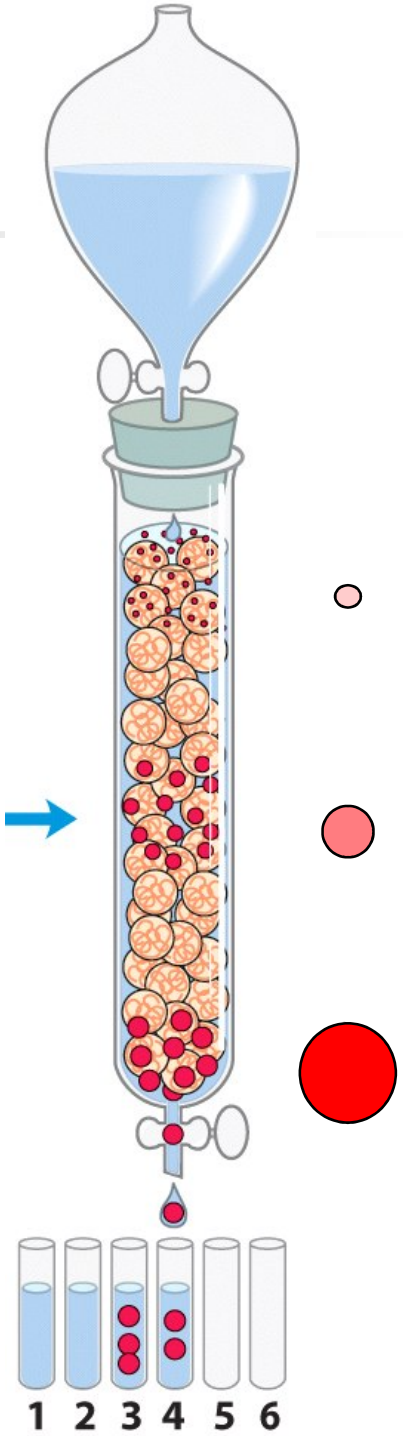
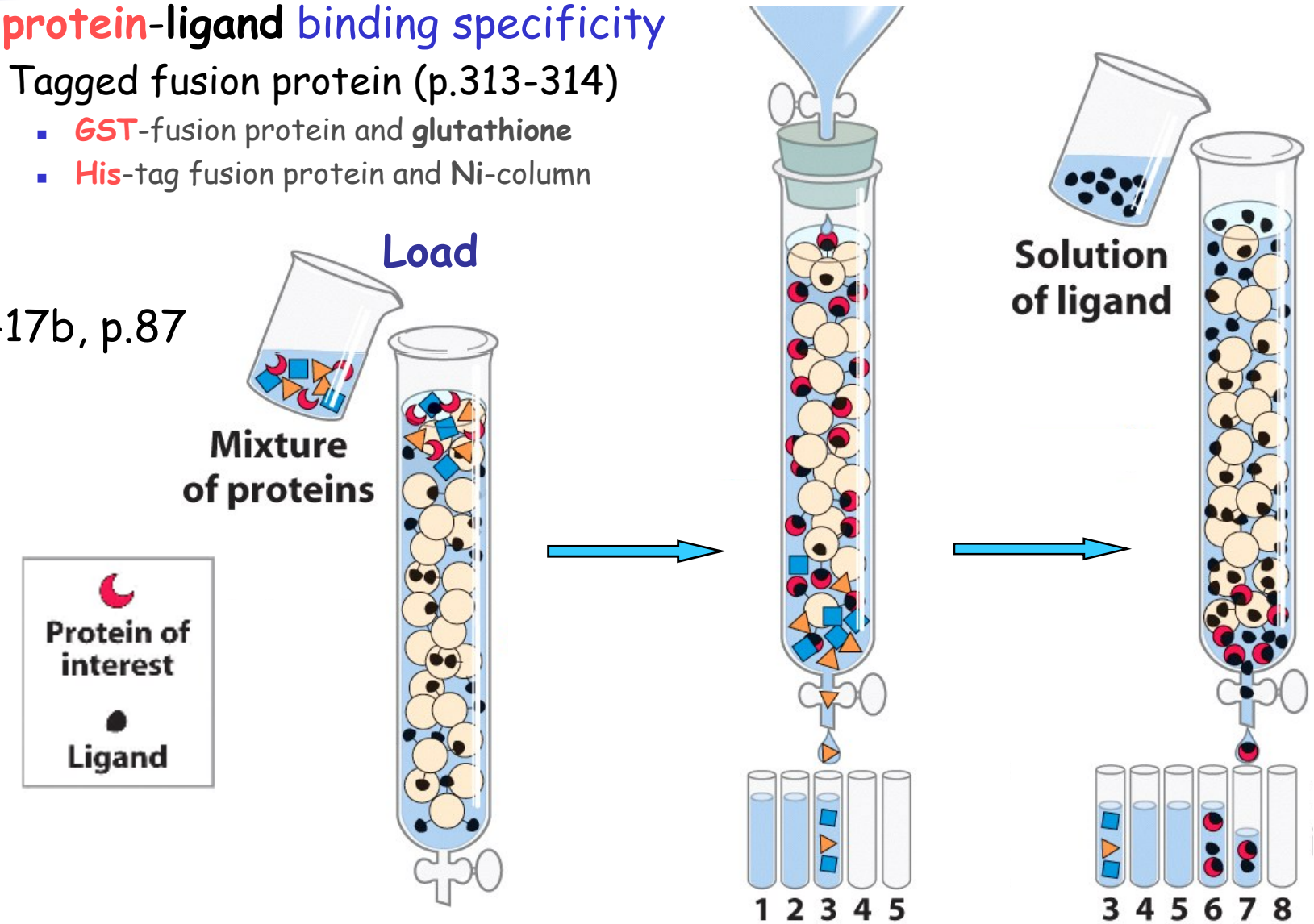


Fig 3-17b, p.87

Affinity

- By **protein-ligand** binding specificity
  - Tagged fusion protein (p.313-314)
    - **GST**-fusion protein and **glutathione**
    - **His**-tag fusion protein and **Ni**-column

Fig 3-17b, p.87



General approach (p.88)

- Inexpensive procedures is used first.
  - Large sample volume, more contaminants
- Sophisticated (and expensive) procedures at later stages.
  - Example: HPLC

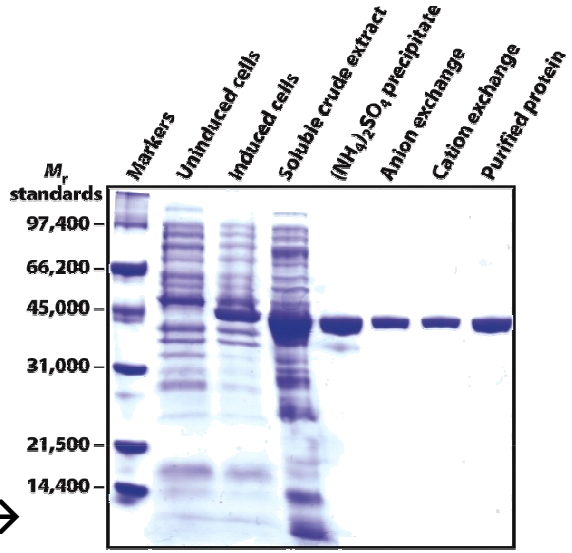


Fig 3-18b, p.89→

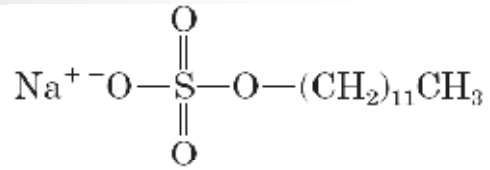
TABLE 3–5 A Purification Table for a Hypothetical Enzyme

Procedure or step	Fraction volume (mL)	Total protein (mg)	Activity (units)	Specific activity (units/mg)
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	60,000	600
5. Affinity chromatography	6	3	45,000	15,000

Note: All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 91.

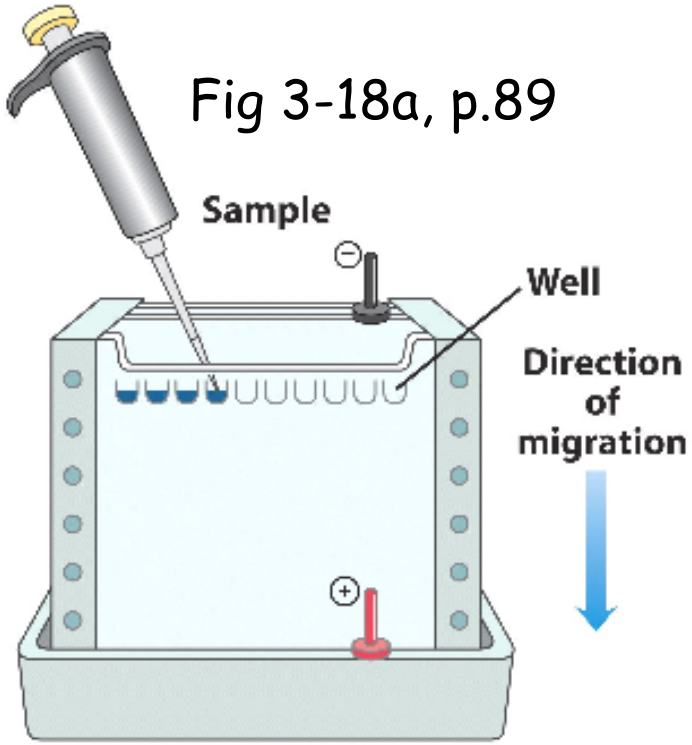
Electrophoresis

- SDS-PAGE
  - Sodium dodecyl sulfate (p. 89)
    - Same shape (denatured) and e/m ratio
  - Polyacrylamide gel electrophoresis
    - Molecular sieve that provides friction



Sodium dodecyl sulfate (SDS)

Fig 3-18a, p.89



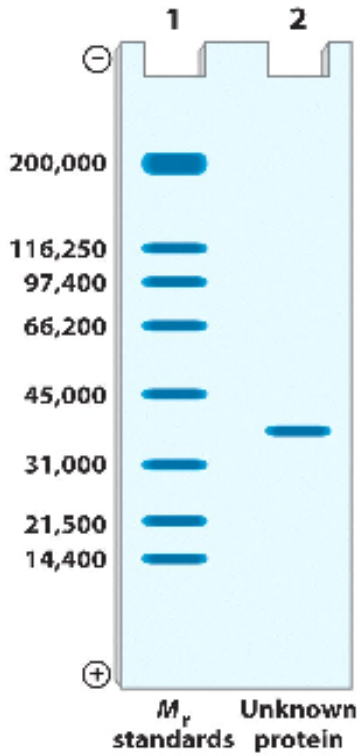
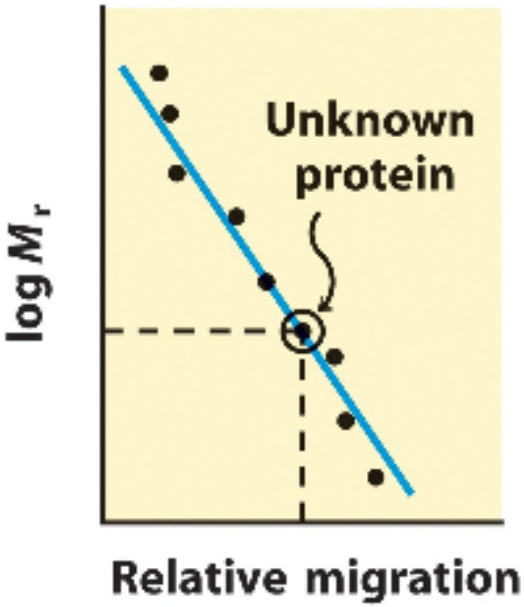


Fig 3-19, p.90



Isoelectric focusing (IEF)

- Separate proteins according to pI

Fig 3-20, p.90

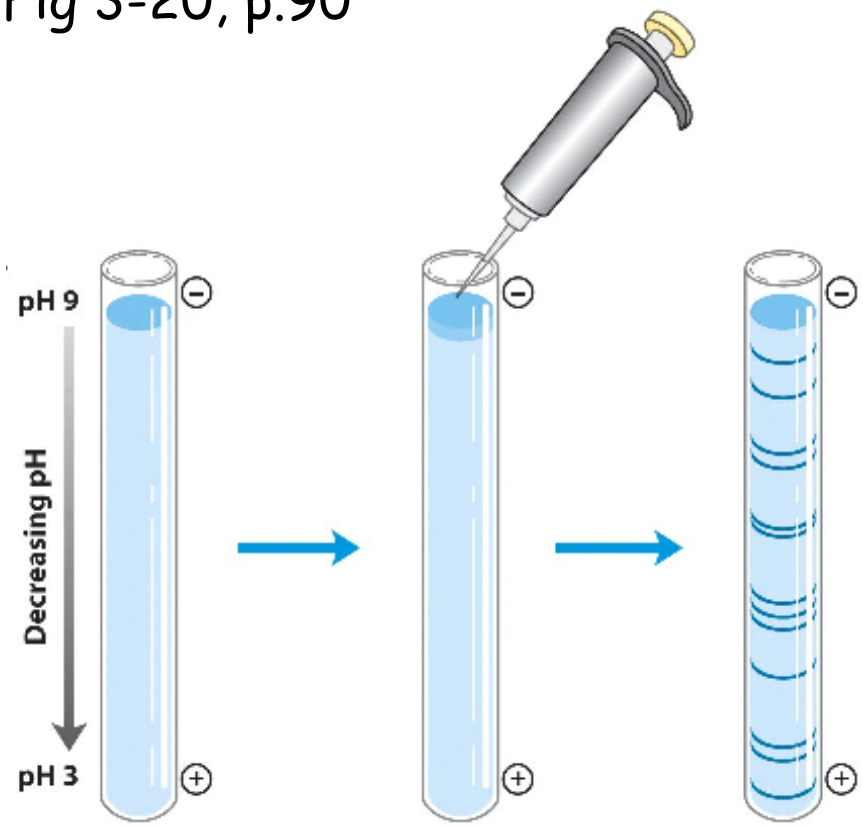
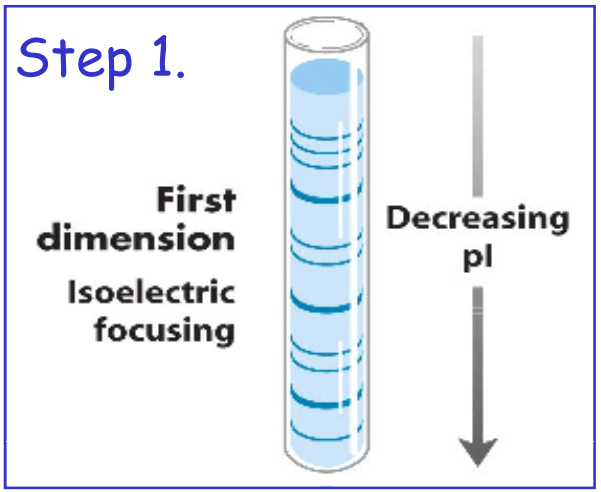
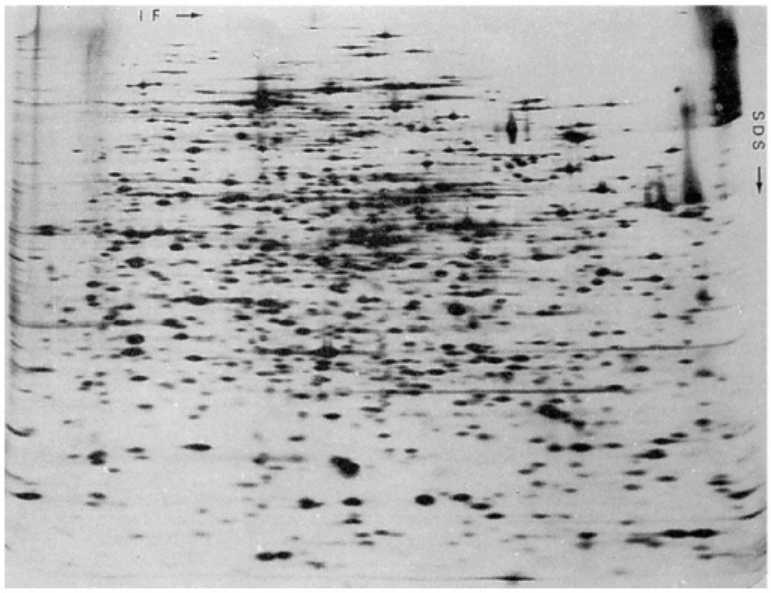


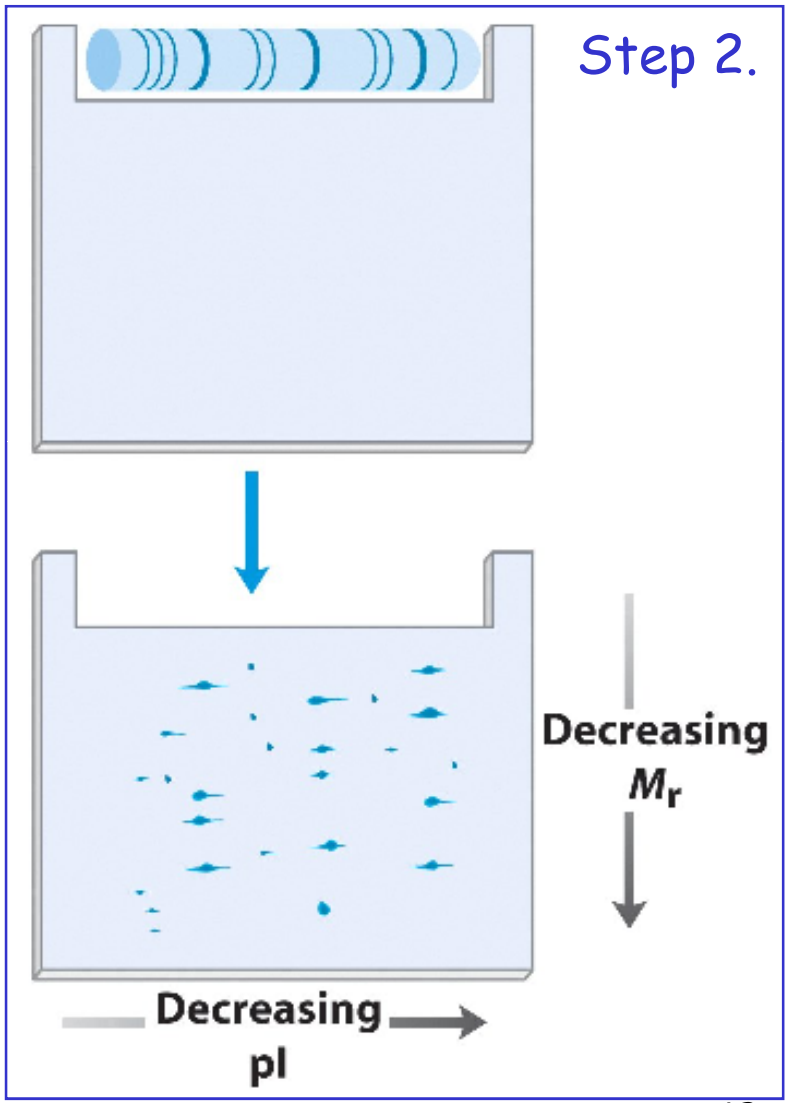
TABLE 3–6 The Isoelectric Points of Some Proteins	
Protein	**pI**
Pepsin	<1.0
Egg albumin	4.6
Serum albumin	4.9
Urease	5.0
β-Lactoglobulin	5.2
Hemoglobin	6.8
Myoglobin	7.0
Chymotrypsinogen	9.5
Cytochrome *c*	10.7
Lysozyme	11.0

2D electrophoresis

Fig 3-21, p.91







Activity vs. Specific Activity

- Activity = "red marble"
  - Total units of enzyme
- Specific activity
  - The units/mg enzyme
  - A measure of enzyme purity

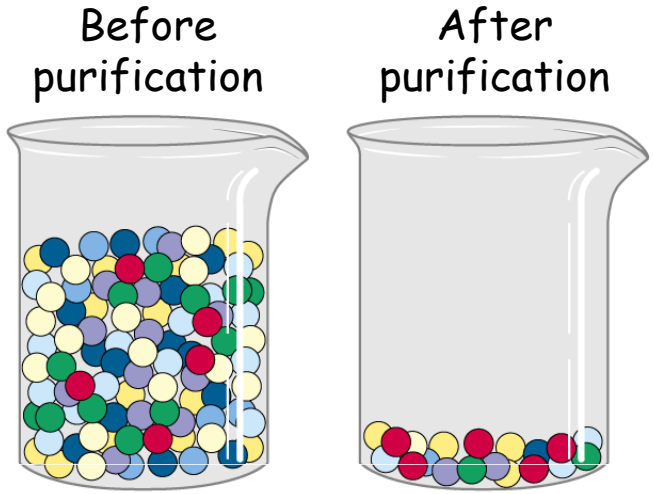


Fig 3-22, p. 91

p. 88

TABLE 3–5 A Purification Table for a Hypothetical Enzyme

Procedure or step	*Fraction volume (ml)*	*Total protein (mg)*	*Activity (units)*	*Specific activity (units/mg)*
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	60,000	600
5. Affinity chromatography	6	3	45,000	15,000

Note: All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 91.

Structural hierarchy

1. Primary structure
   - Amino acid sequence
2. Secondary structure
   - Recurring structural pattern
3. Tertiary structure
   - 3D folding of a polypeptide chain
4. Quaternary structure
   - Subunits arrangement within a protein

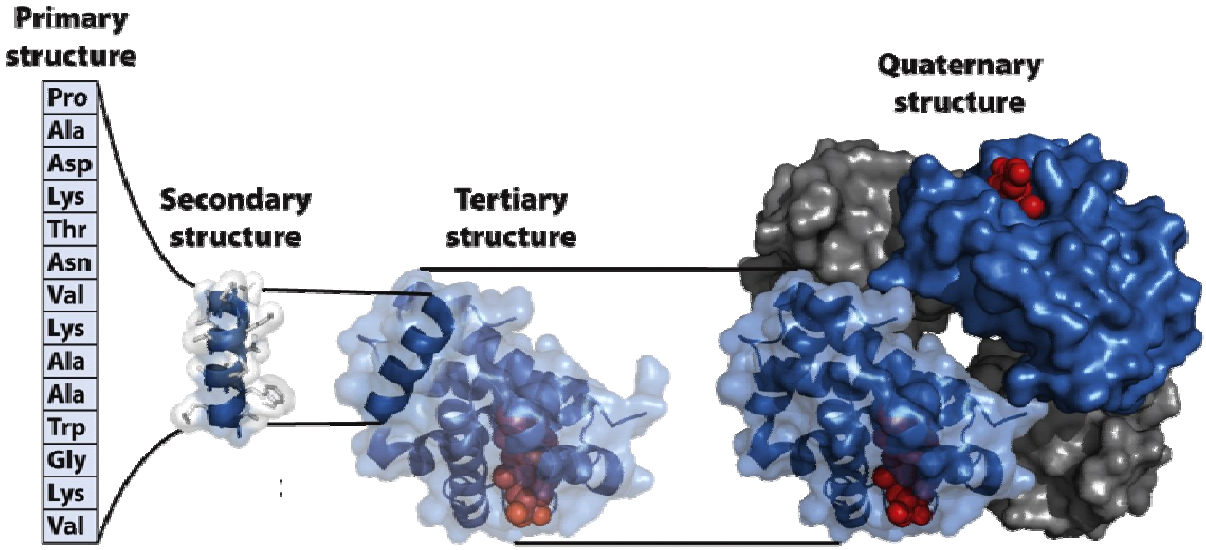
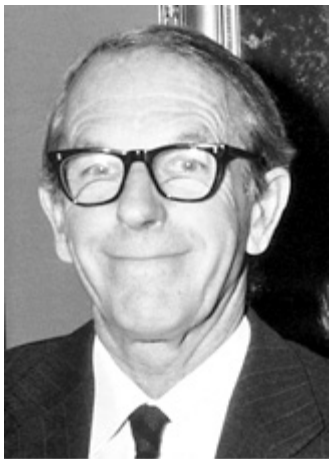


Fig 3-23, p.92

1º structure

50 years ago in 1953

- Double-helical structural of DNA by **Watson and Crick**
- A.A. sequence of insulin by **Frederic Sanger**
  - Covalent structure of protein (N.P. 1958)
  - DNA sequence (N.P. 1980) vs. Protein sequence
    - Protein function



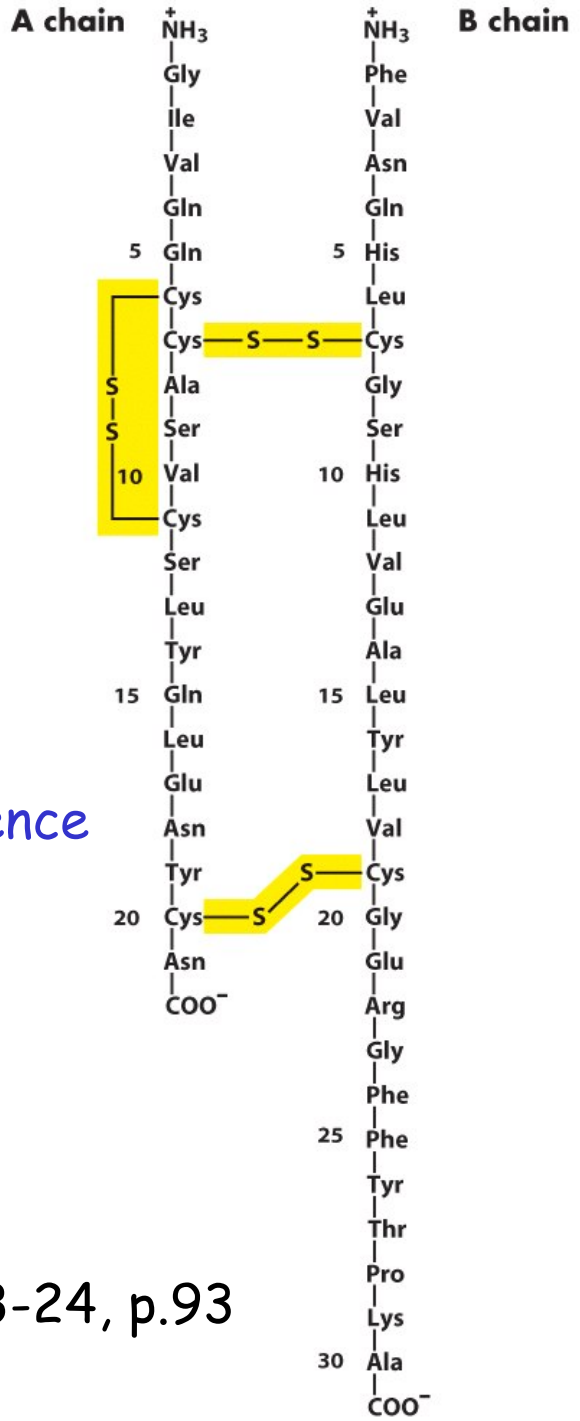


Fig 3-24, p.93

Peptide sequencing (I)

- Acid hydrolysis
  - Determine types and amounts of amino acids in the polypeptide.

Polypeptide

6M HCl
110°C 12 hr

→

Free amino acids mixture

Chromatography separation

→

Amino acids composition

TABLE 3–3 Amino Acid Composition of Two Proteins

Amino acid	Number of residues per molecule of protein* Bovine cytochrome c	Bovine chymotrypsinogen
Ala	6	22
Arg	2	4
Asn	5	15
Asp	3	8
Cys	2	10
Gln	3	10
Glu	9	5
Gly	14	23
His	3	2
Ile	6	10
Leu	6	19
Lys	18	14
Met	2	2
Phe	4	6
Pro	4	9
Ser	1	28
Thr	8	23
Trp	1	8
Tyr	4	4
Val	3	23
Total	104	245

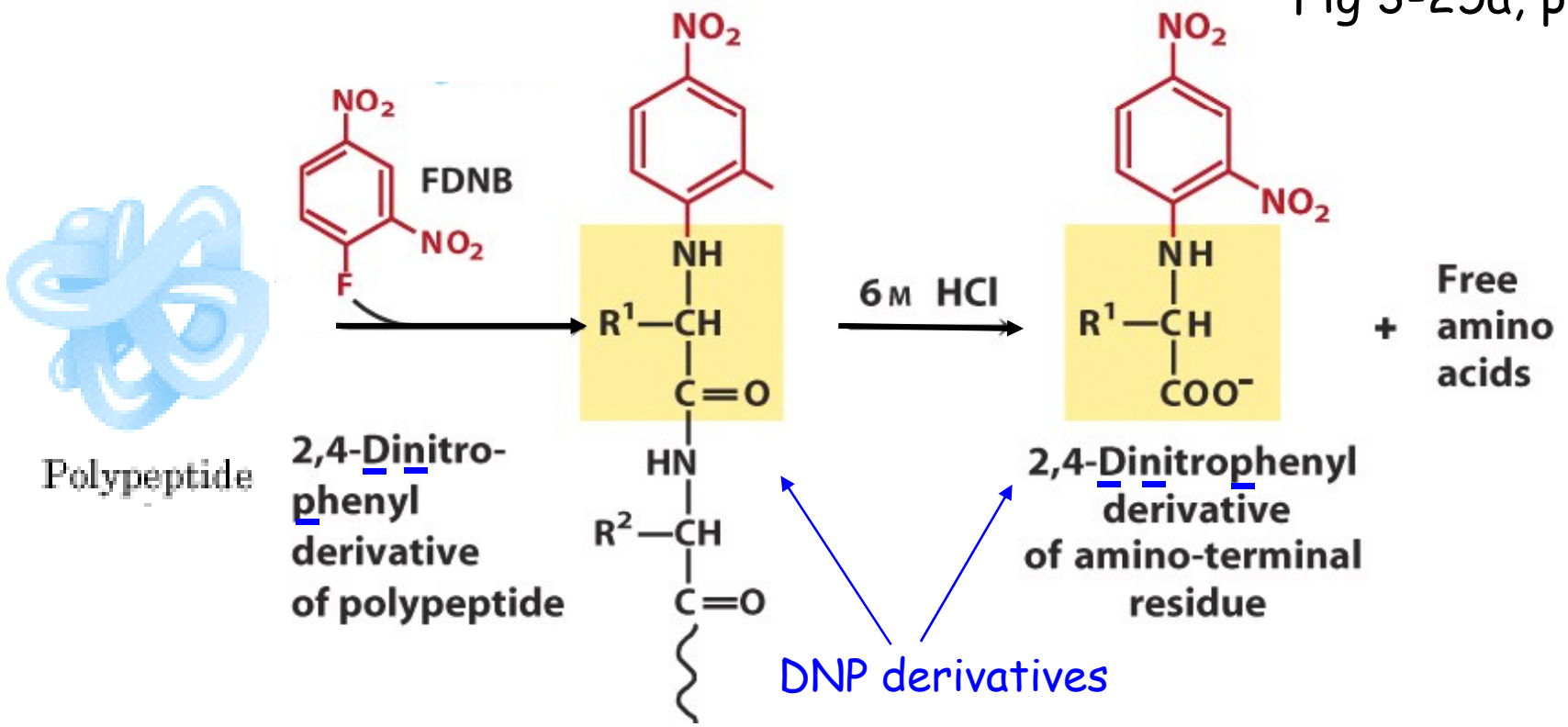
p. 94

p. 84 →

Peptide sequencing (II)

- N-terminal labeling + acid hydrolysis
  - Identify N-terminal residue.
  - Determine # of polypeptides in a protein

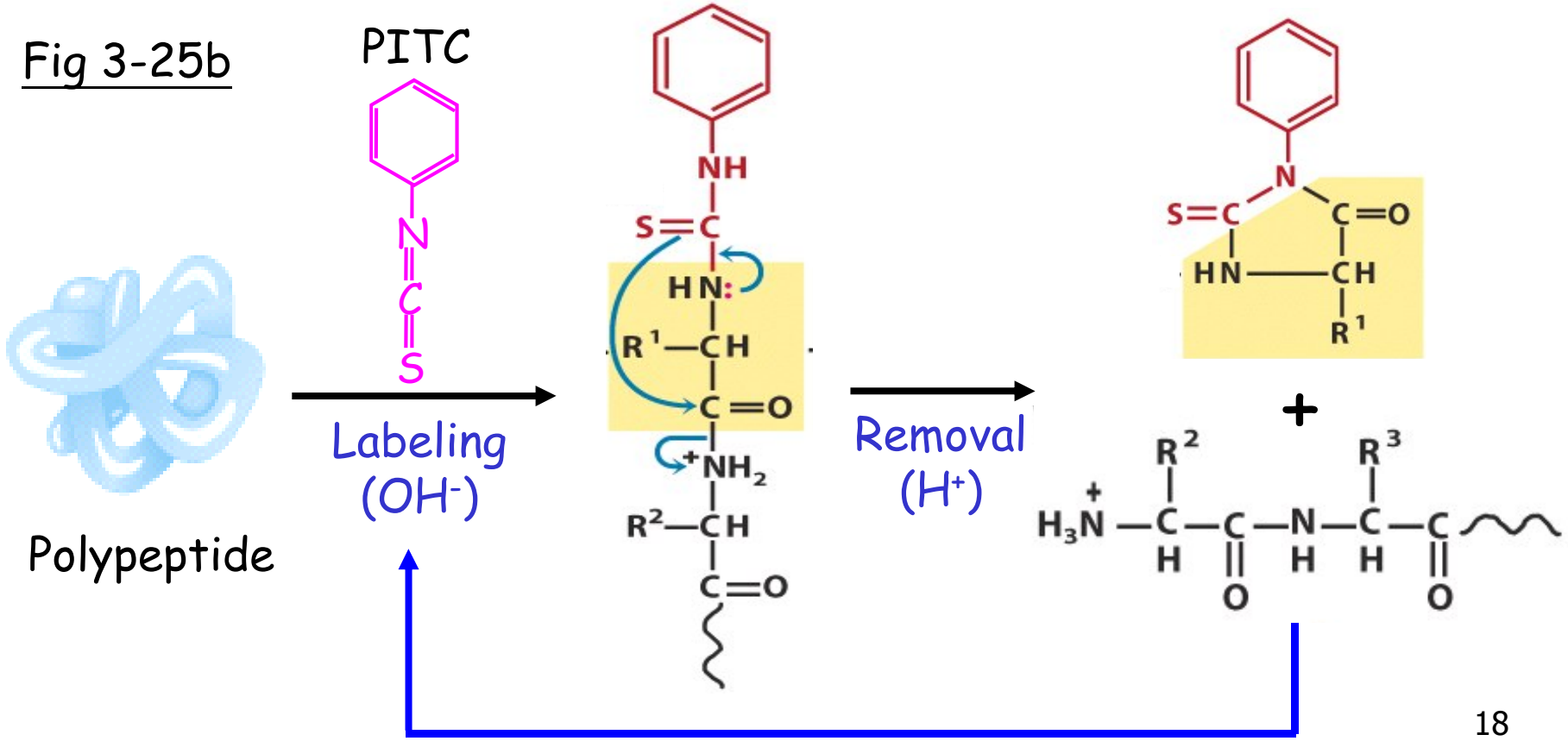
Fig 3-25a, p.94



Peptide sequencing (III)

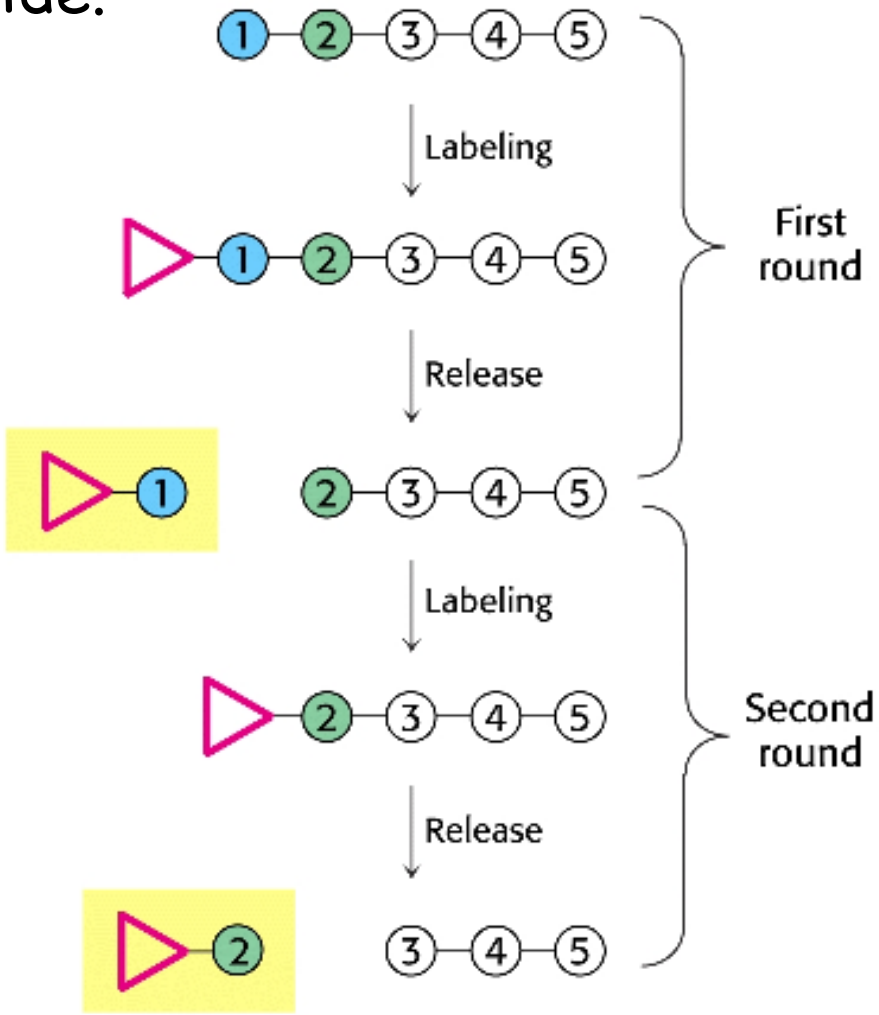
- N-terminal labeling and removal (Edman degradation)
  - Automated sequencer (10 years vs. 2 days)
  - Efficiency vs. polypeptide length

Fig 3-25b



Edman degradation

- Sequentially remove one residue at a time from the N-terminal of a peptide.

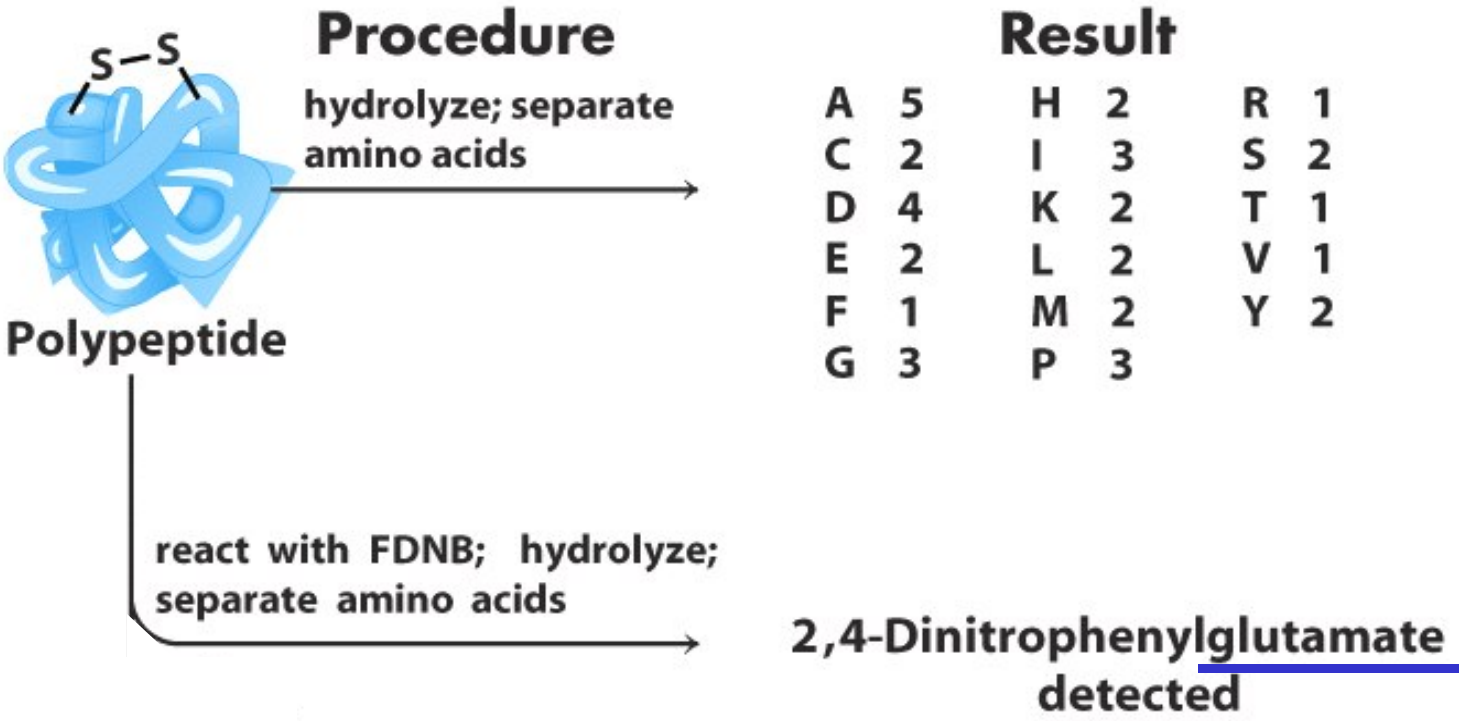


From Stryer
5th ed. Fig 4.21

Protein sequencing (I)

- Determine a.a. composition and N-terminal residue of intact sample (~ 50 a.a.)

Fig 3-27, p. 97



- 38 a.a. total
- N-terminus: Glu (E)

Protein sequencing (IIa)

- Remove S-S bond (by DTT)
- Protease treatment
- Sequencing of each peptide fragment

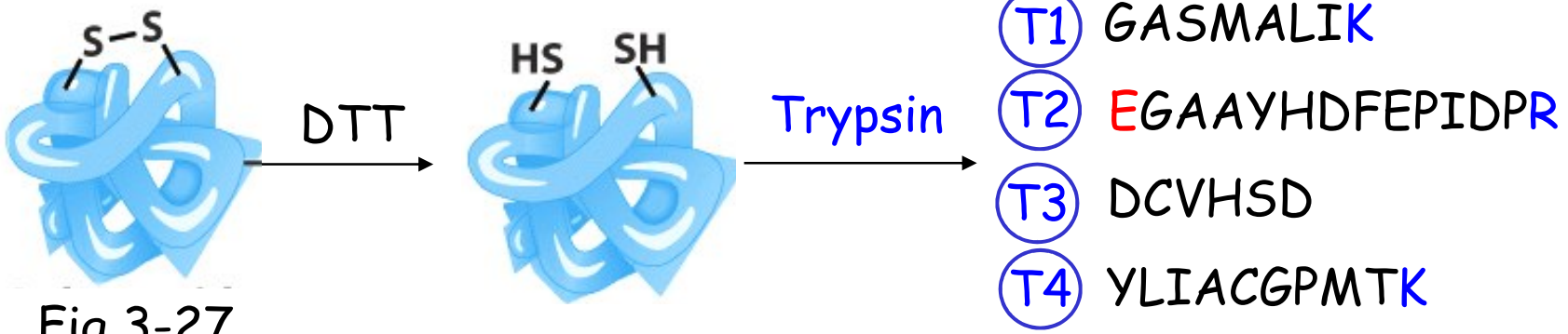


Fig 3-27

Table 3-7, p. 96

The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

Treatment*	Cleavage points†
Trypsin	Lys, Arg (C)
Cyanogen bromide	Met (C)

- N-terminus: Glu (E)
- Trypsin: cleaves 3 times

Protein sequencing (IIb)

- Remove S-S bond (by DTT)
- Chemical treatment
- Sequencing of each peptide fragment

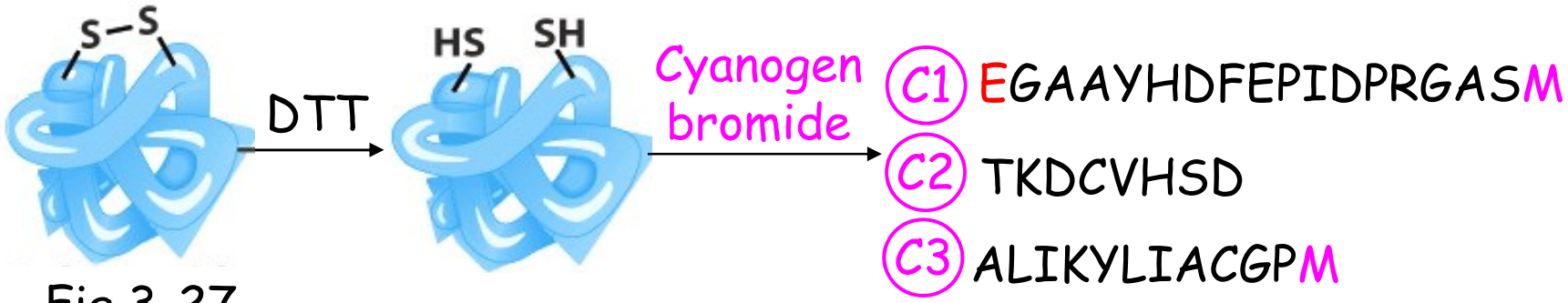


Fig 3-27

Table 3-7, p. 96

The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

Treatment*	Cleavage points†
Trypsin	Lys, Arg (C)
Cyanogen bromide	Met (C)

- N-terminus: Glu (E)
- CNBr: cleaves twice

Protein sequencing (III)

- Ordering peptide fragments
- Locating disulfide bonds

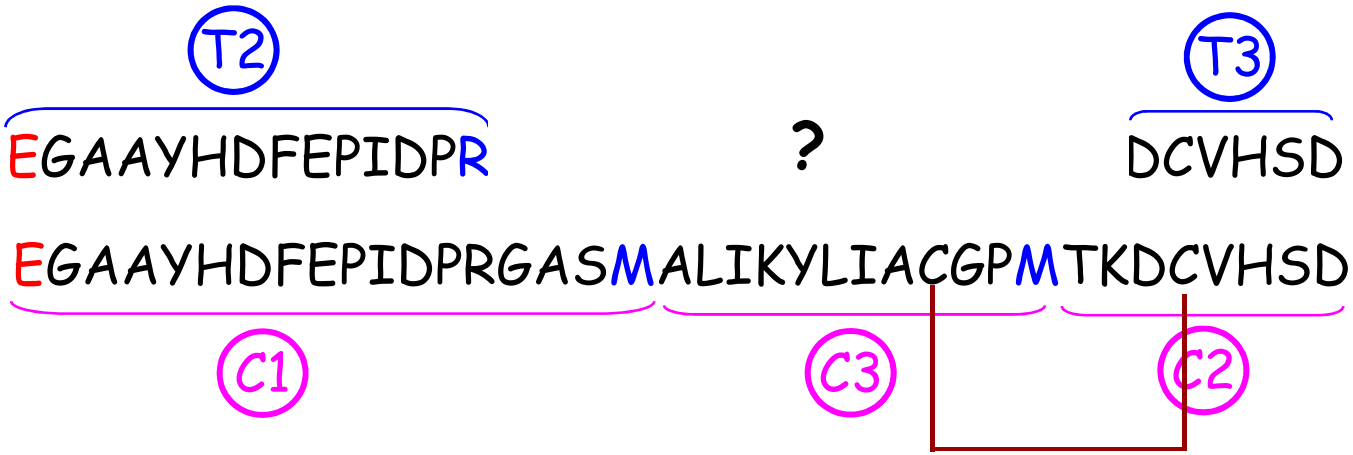


Fig 3-27

Steps in protein sequencing

1. Breaking disulfide bond
2. Cleaving the polypeptide chain
3. Purifying each fragment
4. Sequencing of peptides
5. Ordering peptide fragments
6. Locating disulfide bonds

Other sequencing methods

- By mass spectrometry (Box 3-2)
- Deduced from DNA sequence

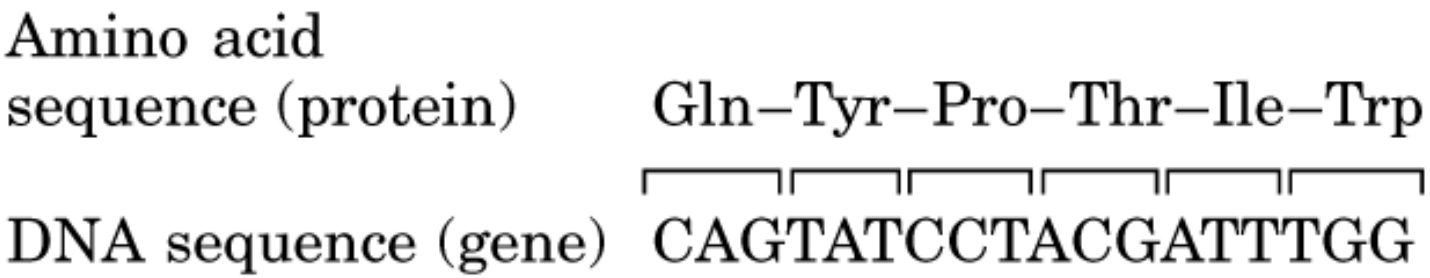


Fig 3-28

Proteome 蛋白質體

p. 100 and p. 324

- The entire protein complement encoded by an organism's DNA
  - Gene : Genome
  - Protein : Proteome
- Proteomics can provide clues to the role of proteins whose functions are as yet unknown.

Proteomics

- Protein mixtures from cells
- 2-D gel electrophoresis
- Extract individual protein spot from gel
- Sequenced by mass spectrometry
- Compare with genomic sequence to identify the protein
- Identify new protein and changes in protein due to modification.

Ch 3 Review

- 20 amino acids
  - Full name, 3 letter code
  - Structure (functional group)
  - Physical and chemical properties
  - Titration curve and pI
- Peptide and protein
  - Peptide bond
  - Properties
  - Structural hierarchy
- Working with proteins
  - Sequencing methods
  - Protein chemistry techniques
  - Proteomics
- Problems: 2, 5, 10, 15