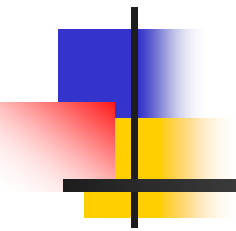
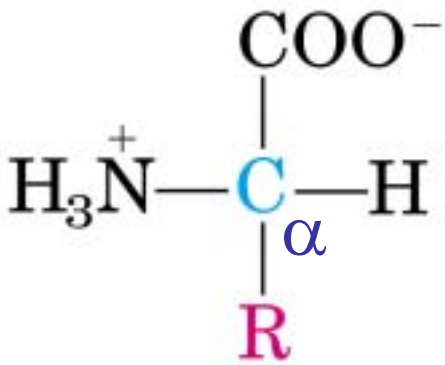
1. 氨基酸、蛋白質與酵素、維生素及輔脢之結構與功能.
2. 核甘酸與核酸結構、DNA複製與修補、基因表現與調控及蛋白質合成
3. 中間代謝及調控（包括醣類代謝、脂肪酸氧化及含氮化合物代謝、呼吸鏈及氧化磷酸化作用、賀爾蒙作用及訊號傳遞等

1. Biochemistry (5th ed.) Geoffery L. Zubay. Mc Graw-Hill. 1998.
2. Harper's Biochemistry (24th ed.) Robert K. Murray, Daryl K. Granner. Mayers & Rodwell Prentice-Hall. 1996.
3. Biochemistry (4th ed.) Lubert Stryer. W. H. Freeman and Co. 1995.
4. Biochemistry (2nd ed.) C. K. Mathews and K. E. van Holde. Benjamin/Cummings 1996.
5. Principles of Biochemistry (2nd ed.) Albert, L. Lehninger, David, L. Nelson Michael, M. Cox. Worth Publishers. 1993.



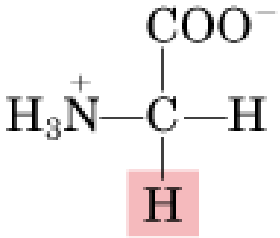
Amino acids, peptides, and proteins

α-Amino acid (residue)

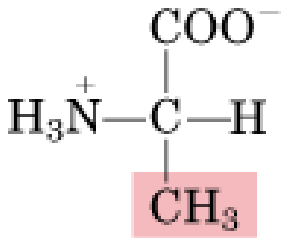


- Common structure
  - α-carbon (chiral center)
    - L-amino acid and D-amino acid
    - Proteins are made exclusively from L-form a.a.
    - Free D-serine, D-glutamate in brain tissue
    - D-alanine and D-glutamate in cell walls of gram+ bacteria
  - Acid group (carboxyl group)
  - Amino group
  - Functional group, side chain, R group (chemical property)
    - Nonpolar aliphatic (hydrophobic)
    - Aromatic
    - Polar, uncharged
    - Negatively charged
    - Positively charged

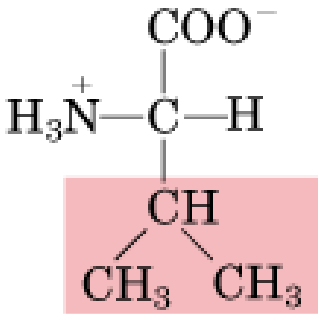
Hydrophobic R groups



Glycine

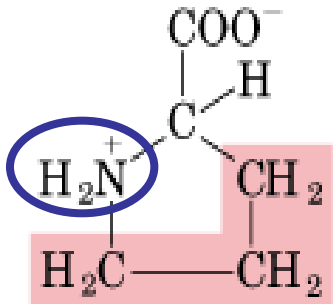


Alanine

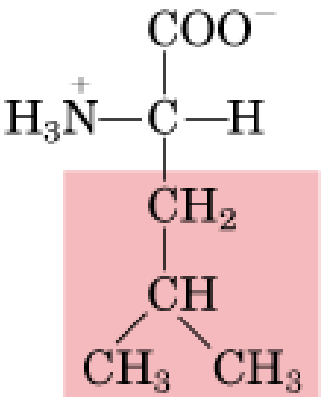


Valine

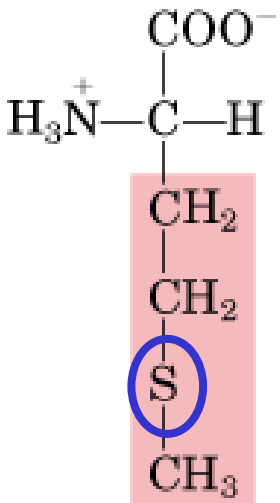
Secondary amino (imino) group



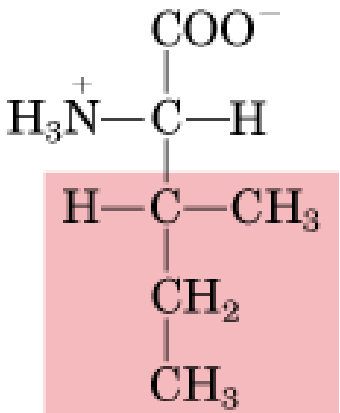
Proline



Leucine



Methionine

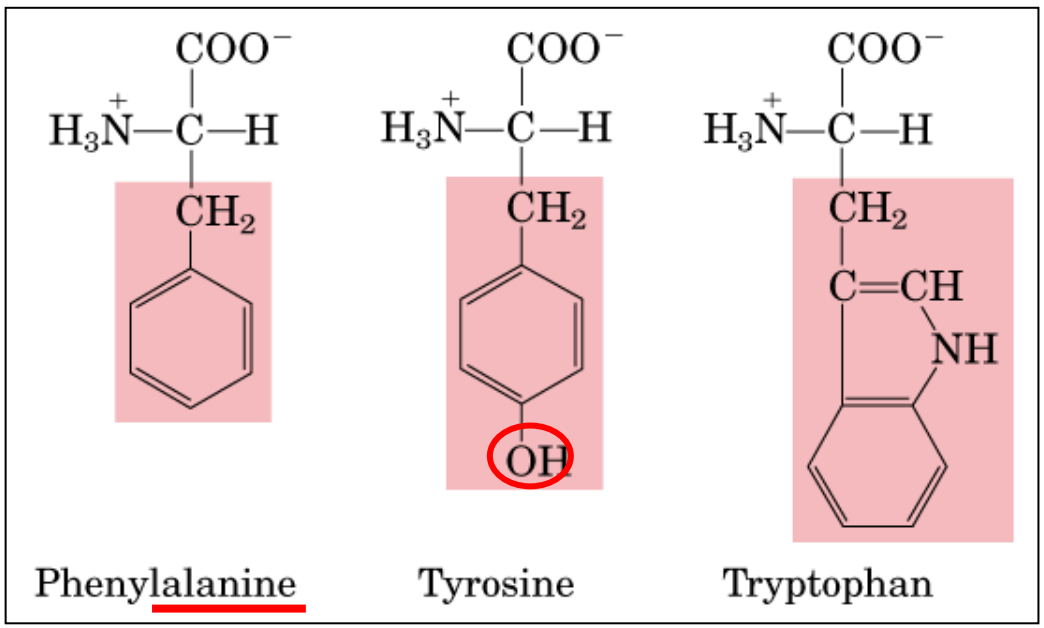


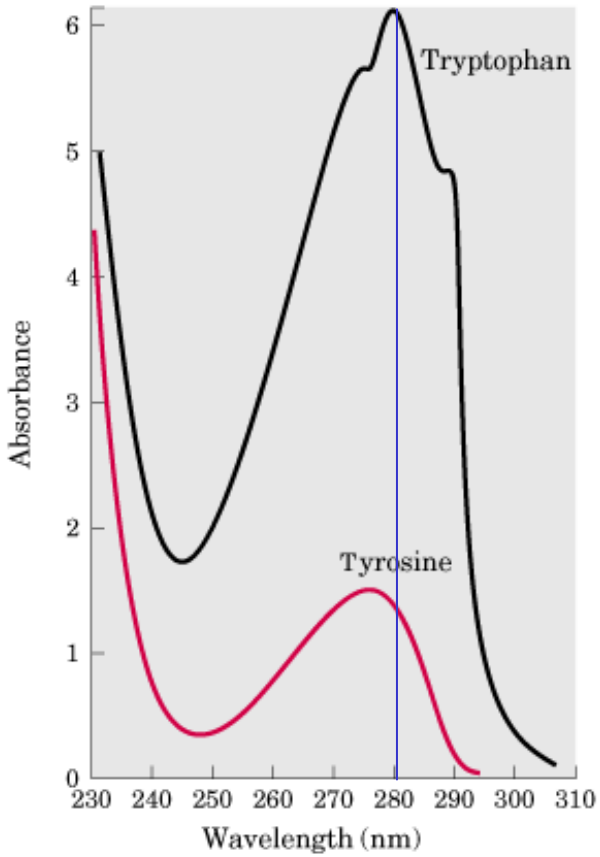
Isoleucine

Branched-chain a.a.

- Val, Leu, Ile

Aromatic R groups





Lambert-Beer Law

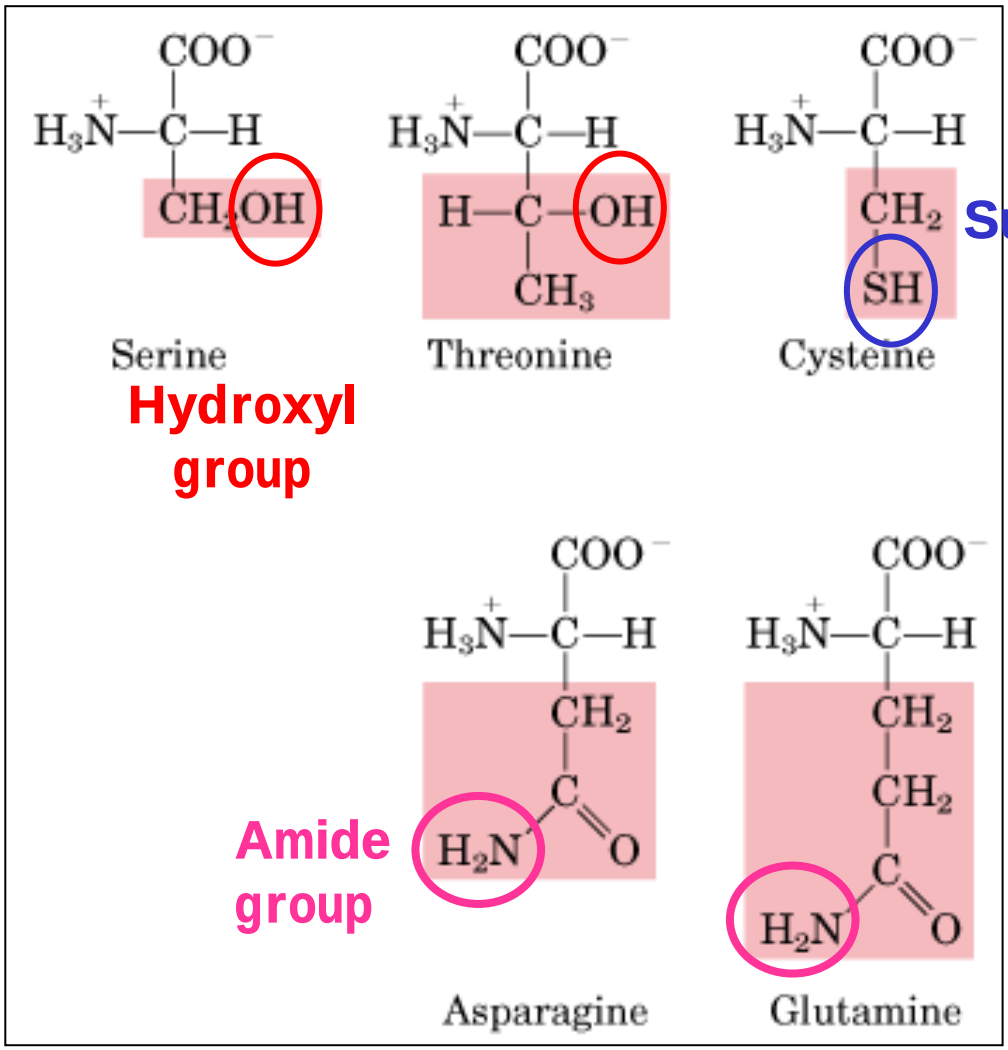
Absorbance (A) = $\log(I_0 / I) = \varepsilon c l$

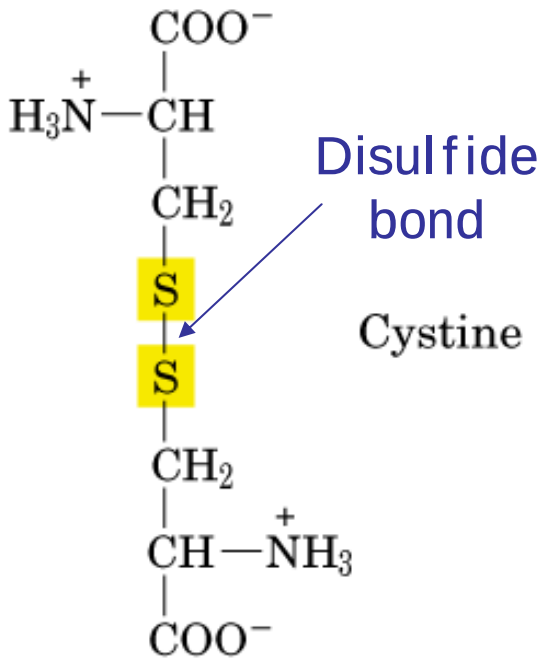
ε : molar extinction coefficient

→ A ∝ concentration (c)

$\varepsilon_{280\ nm}$: Trp > Tyr >> Phe

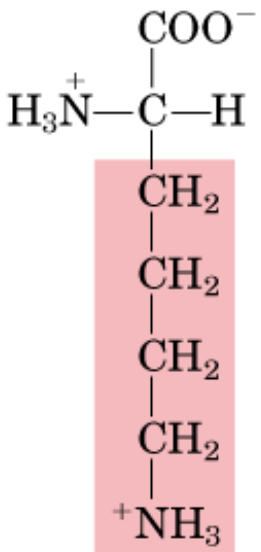
Polar, hydrophilic R groups



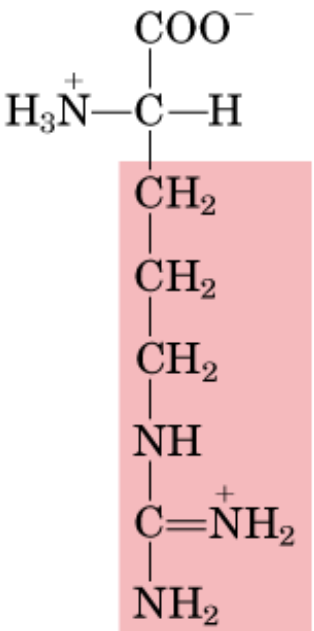


Charged R groups

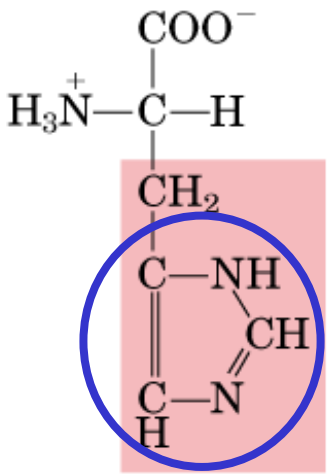
At pH = 7.0



Lysine



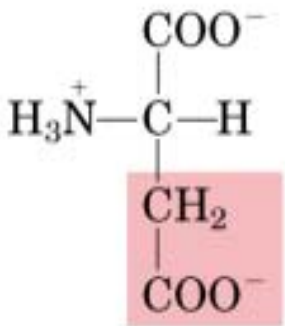
Arginine



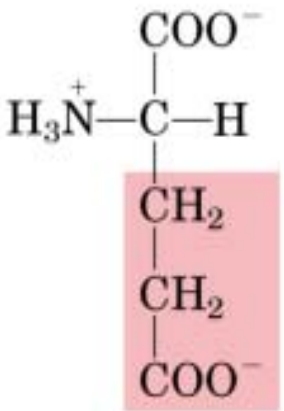
Histidine

Imidazole group

pK_R ~ 7.0



Aspartate

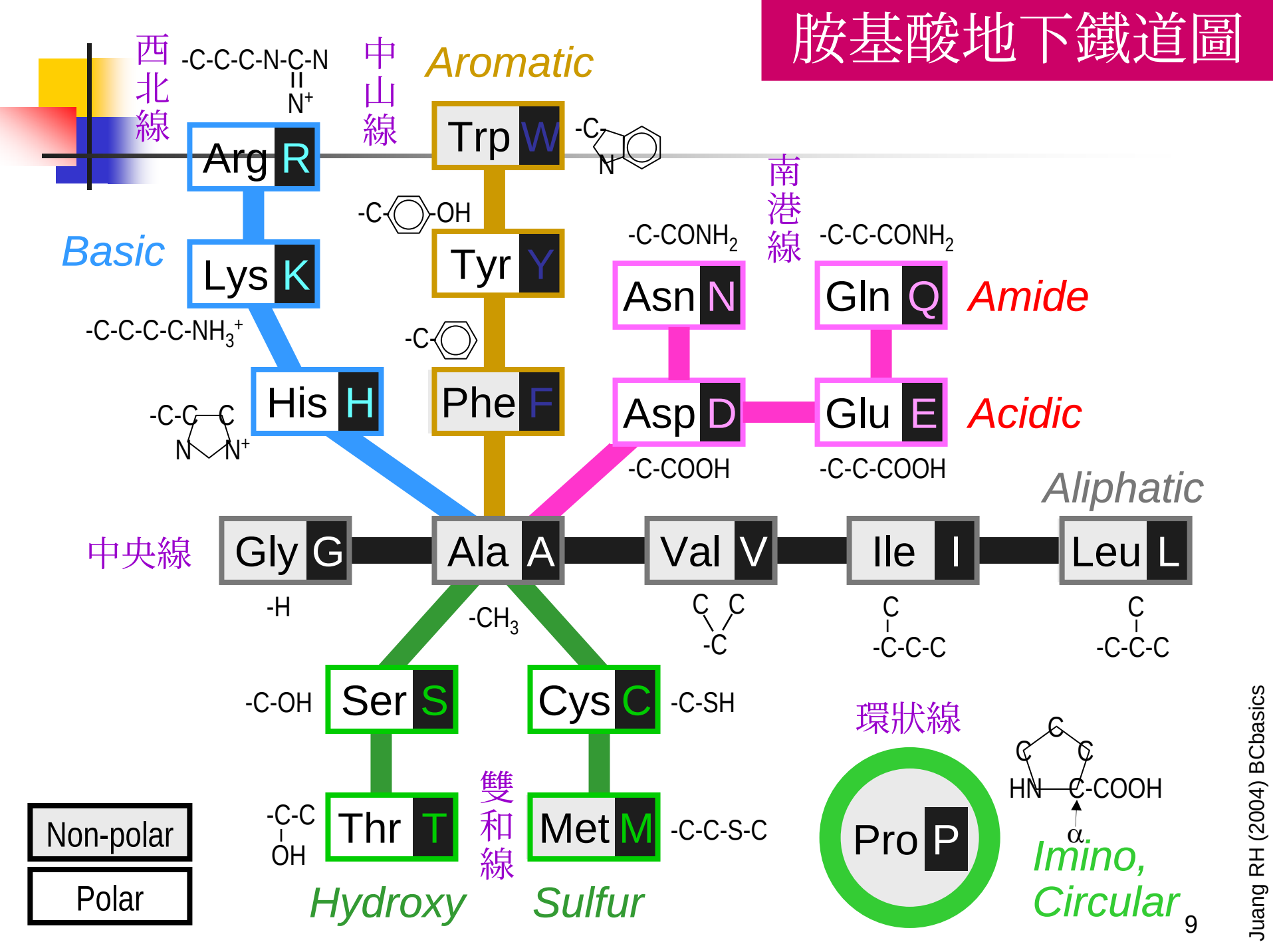


Glutamate

Amino acids

- Asparagine (天門冬醯胺)
- Cysteine (半胱胺酸)
- Leucine (白胺酸)
- Glycine (甘胺酸)
- Tyrosine (酪胺酸)
- Aspartate (天門冬酸)
- Alanine (丙胺酸)
- Valine (纈胺酸)
- Serine (絲胺酸)
- Glutamate (麩胺酸)
- Phenylalanine (苯丙胺酸)
- Arginine (精胺酸)
- Lysine (離胺酸)
- Histidine (組織胺酸)
- Proline (脯胺酸)
- Tryptophan (色胺酸)
- Isoleucine (異白胺酸)
- Methionine (甲硫胺酸)
- Threonine (酥胺酸)
- Glutamine (麩胺醯胺)

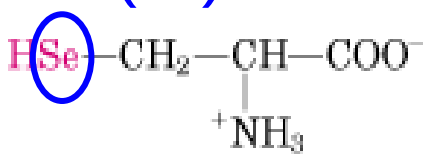
胺基酸地下鐵道圖



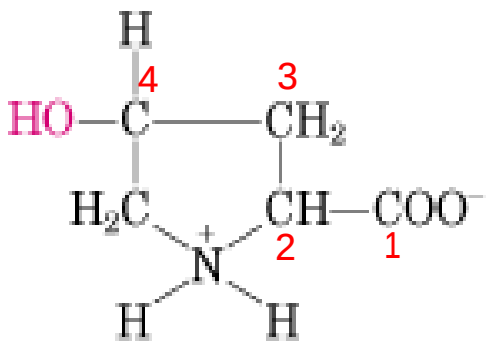
Nonstandard amino acids

- Derived from standard amino acid
  - 4-hydroxyproline, 5-hydroxylysine
    - Collagen
  - Selenocysteine
    - Glutathione peroxidase (GSH oxidation)
  - Ornithine and citrulline
    - Urea cycle

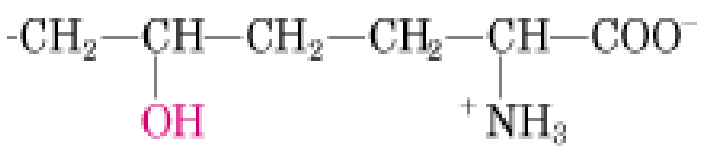
selenium (硒)



Selenocysteine



4-Hydroxyproline



5-Hydroxylysine

If only one amino acid (a.a.) begins with a certain letter, that letter is used

Cysteine = Cys = C

Histidine = His = H

Isoleucine = Ile = I

Methionine = Met = M

Serine = Ser = S

Valine = Val = V

If more than one a.a. begins with a certain letter, that letter is assigned to the most commonly occurring one

Alanine = Ala = A

Glycine = Gly = G

Leucine = Leu = L

Proline = Pro = P

Threonine = Thr = T

Phonetically suggestive

Phenylalanine ("Fenylalanine") = Phe = F

Arginine ("aRginine") = Arg = R

Tyrosine ("tYrosine") = Tyr = Y

Tryptophan (double ring in the molecule) = Trp = W

A letter close to the initial is used

Aspartic acid (near A) = Asp = D

Asparagine (contains N) = Asn = N

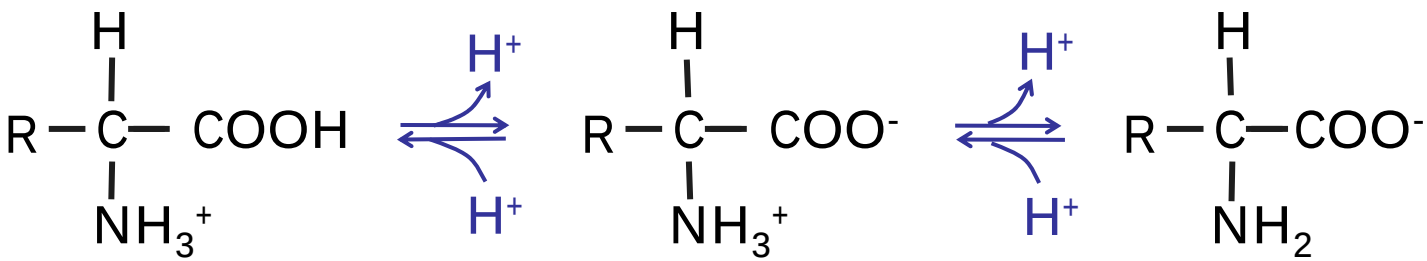
Glutamic acid (near G) = Glu = E

Glutamine ("Q-tamine") = Gln = Q

Lysine (near L) = Lys = K

Chemical properties of A.A.

- Can act as an acid or a base
  - Zwitterion (zwitterionic)
  - Amphorlyte (amphoteric)
  - Dipolar ion
- Min. 2 proton yielding groups per a.a.



Net charge:

+ 1 0 - 1

K_a: dissociation constant

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$$

$$HA \rightleftharpoons H^+ + A^-$$

$$K_{eq} = \frac{[H^+]\,[A^-]}{[HA]} = K_a$$

兩邊倒數，取 log

$$\log \frac{[HA]}{[H^+]\,[A^-]} = \log \frac{1}{K_a}$$

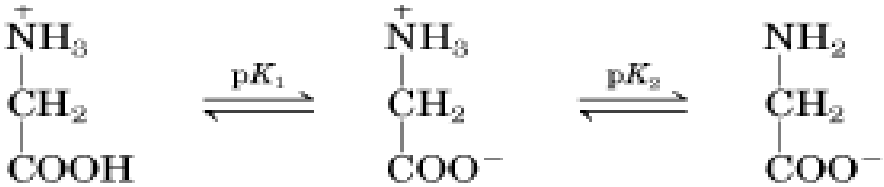
At $K_{eq,}$ $[HA] = [A^-]$

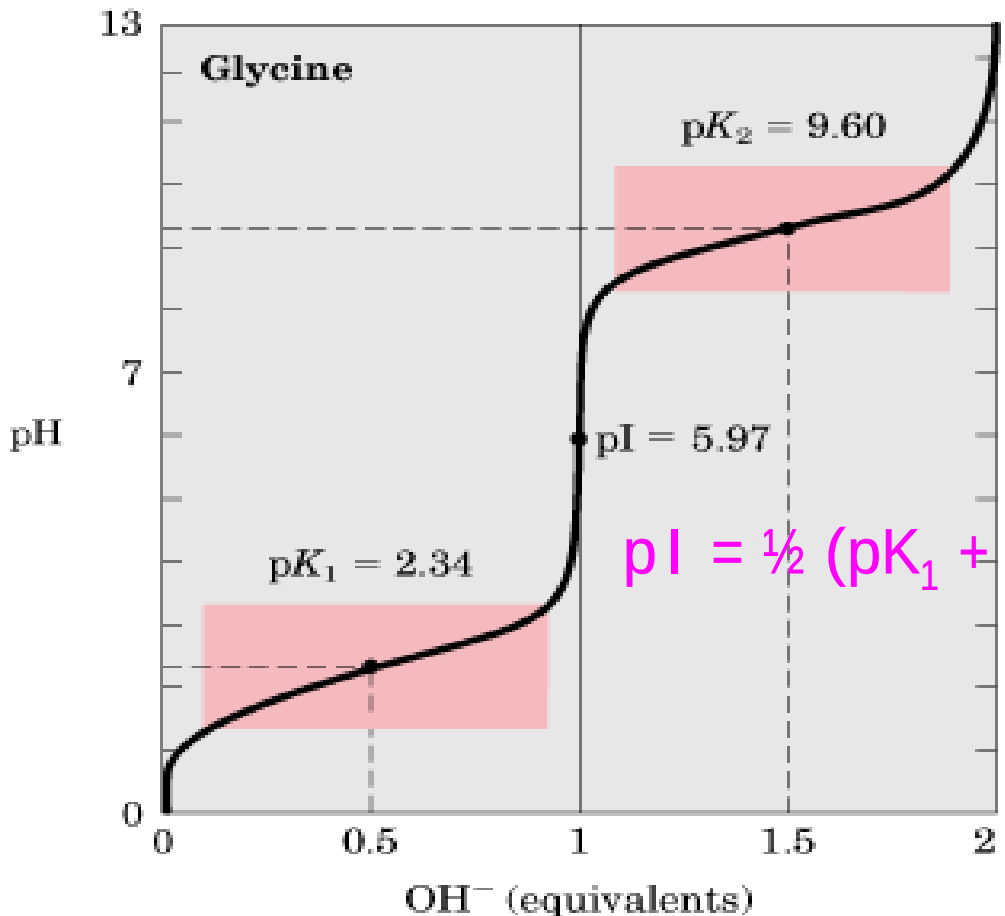
$pH = pK_a$

(p□ = - log □)

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

Titration curve of Gly





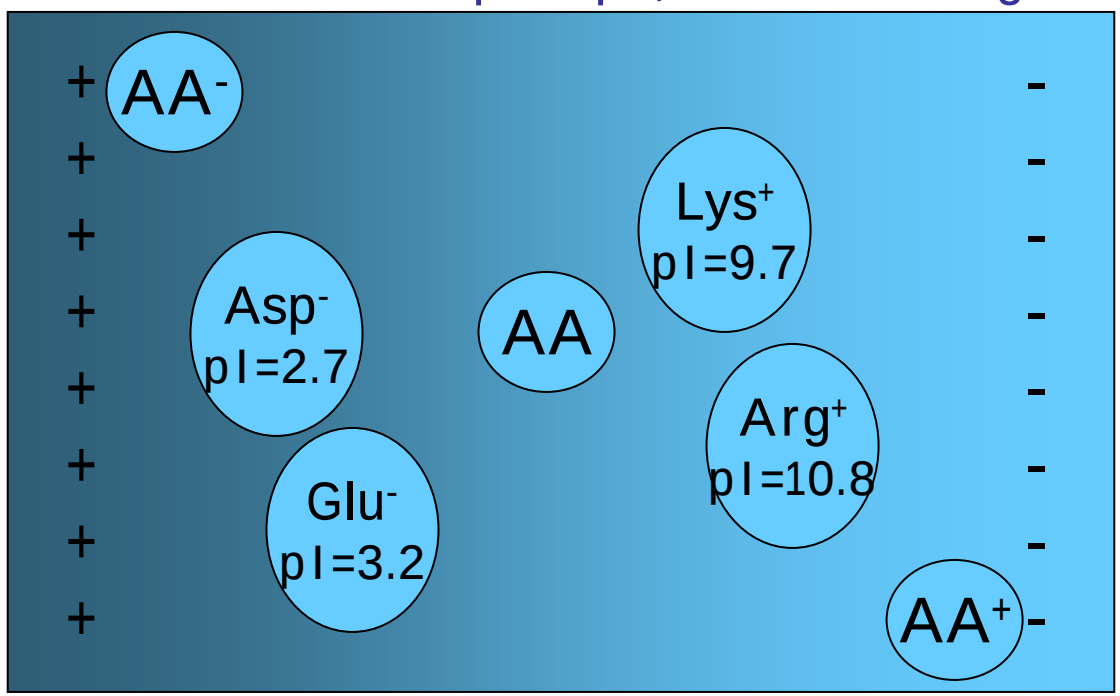
- Two buffer zones
- No ionizing R group.
- Net charge and pH relationship
  - Isoelectric point
  - Isoelectric pH (pI)

$pI = \frac{1}{2} (pK_1 + pK_2)$

What is isoelectric point (pI)?

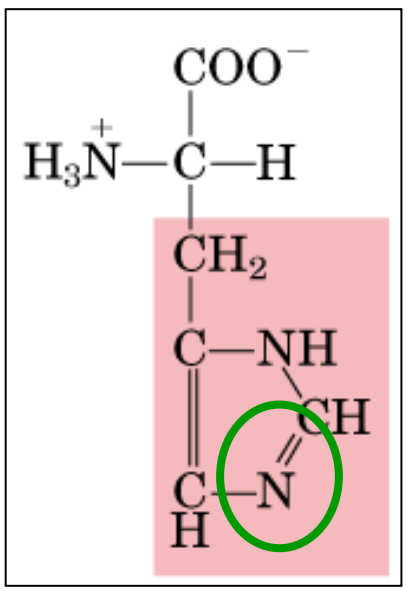
- pI = The pH at which the net charges equal zero
- At its pI, the amino acid will no longer move in an electrical field

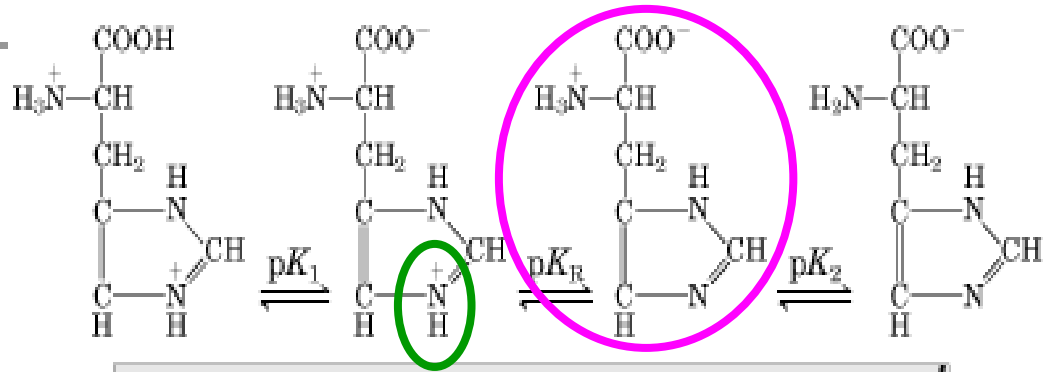
When pH < pI, the a.a. is positively charged
When pH > pI, the a.a. is negatively charged

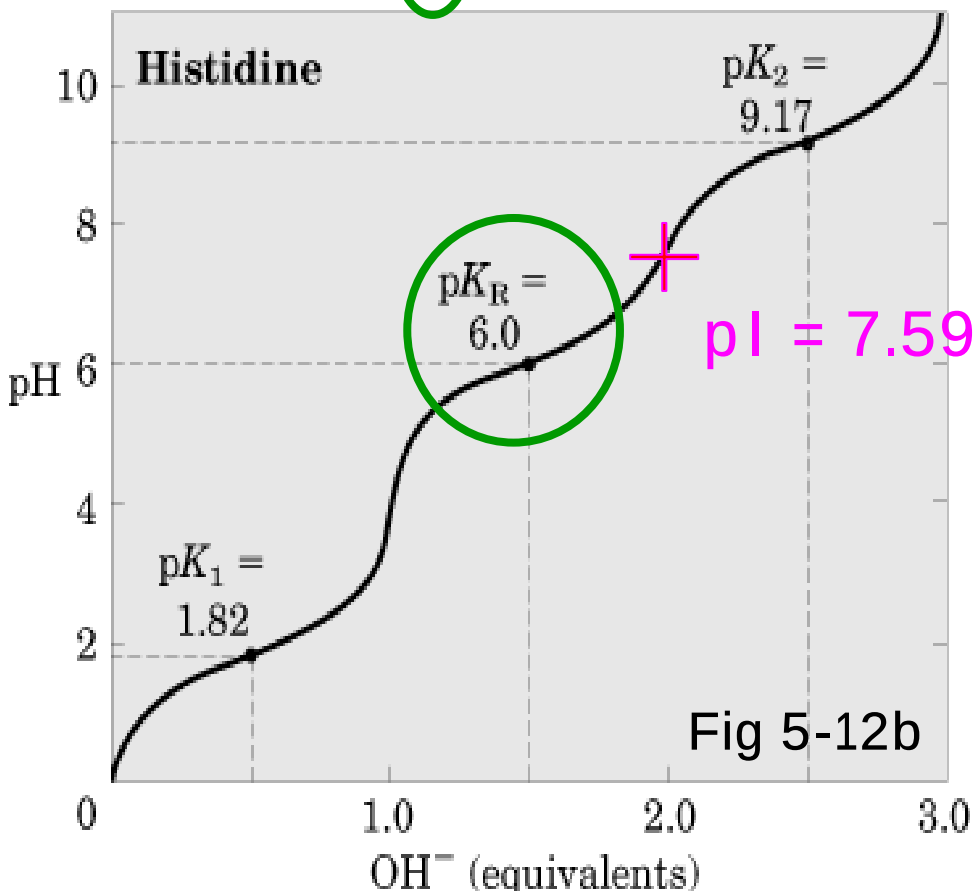


Titration curve of His

- An ionizable R group (imidizole)
- pK_R near 7







Monomer → Polymer

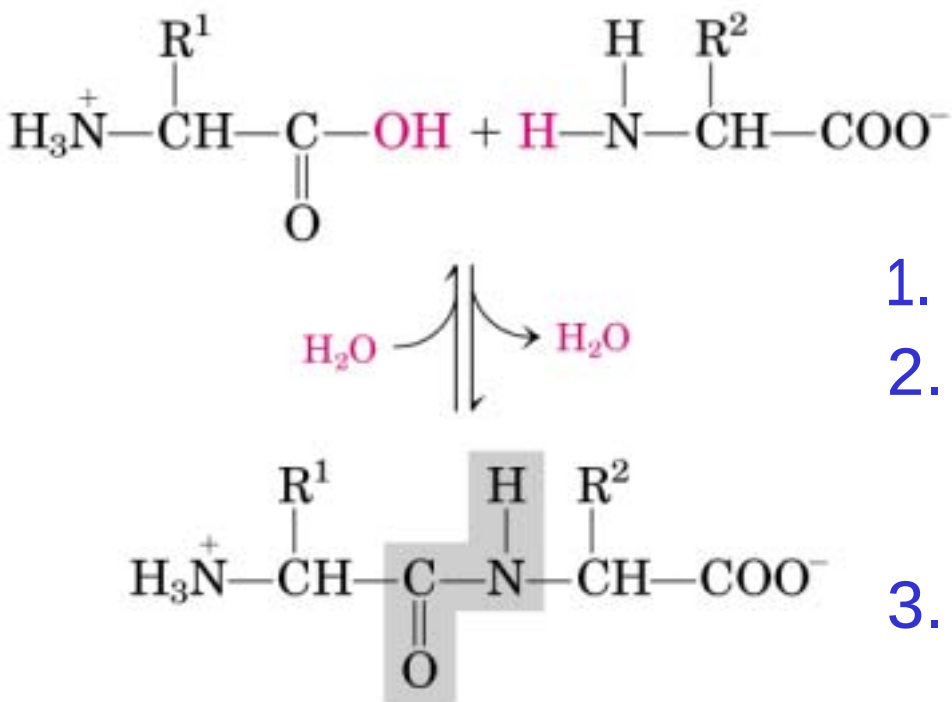
Amino Acid

Peptide

Protein

The Peptide Bond

Fig 5-13

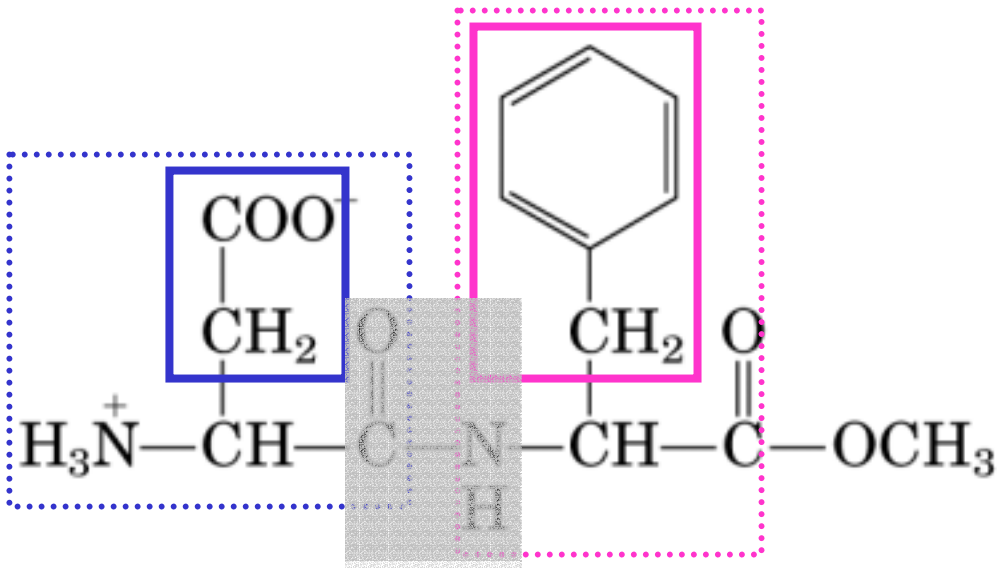


Peptide bond (covalent)

1. Two amino acids
2. Removal of one water molecule (condensation)
3. Formation of the **CO-NH**

NutraSweet (aspartame)

p. 127



- Artificial sweetener
- Dipeptide (made of 2 amino acids)
- A.A. sequence: Aspartate + phenylalanine

Chemical properties of peptides

Determined by

1. Free α-amino
2. Free α-carboxyl
3. Nature and number of ionizable R groups

At pH 7, R-group only…

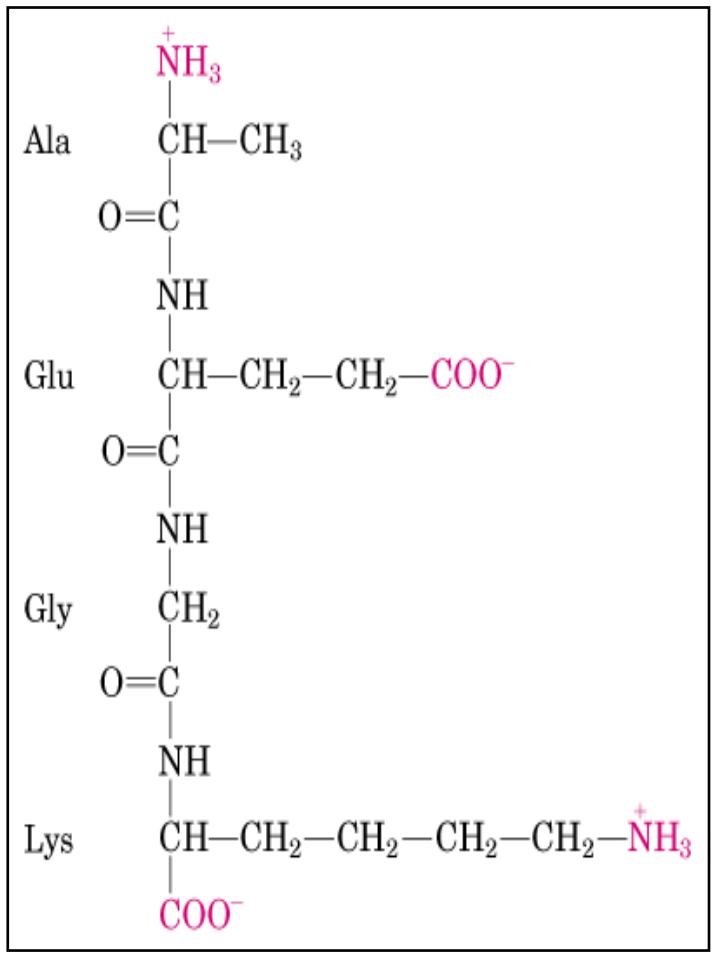


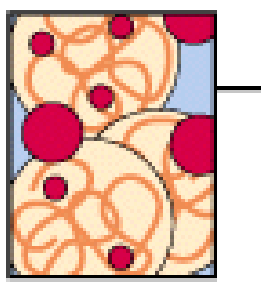
Fig 5-15

Working with proteins

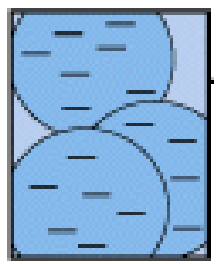
- From biological tissue (*in vivo*)
- Genetic engineering (cloning)
- Chemical synthesis (*in vitro*)
  - In the reverse order (from C to N)
  - Not very efficient
    - A protein of 100 a.a. = days by machine vs. 5 sec in bacteria

Column Chromatography

- Stationary phase + mobile phase
- By charge, size, binding affinity difference
  - Size exclusion or gel filtration
    - Stokes radius (function of mass and shape)
  - Ion exchange
  - Affinity (binding specificity)
    - His-tag fusion protein and Ni^{2+}-column (Co^{2+})
    - GST-fusion protein and GST column
    - Ag-Ab



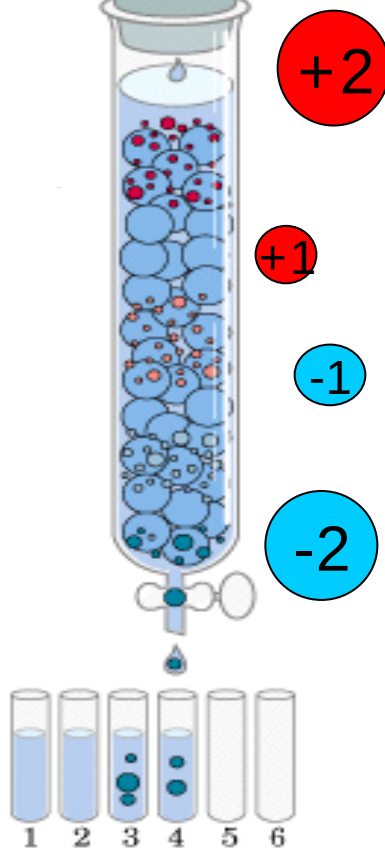
Porous polymer beads



Negatively charged beads

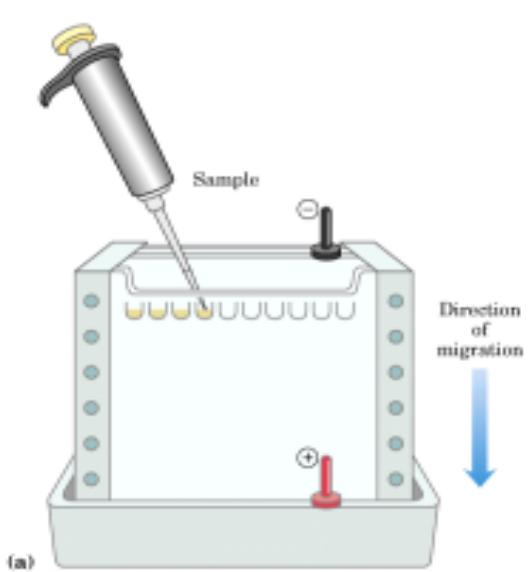
‖

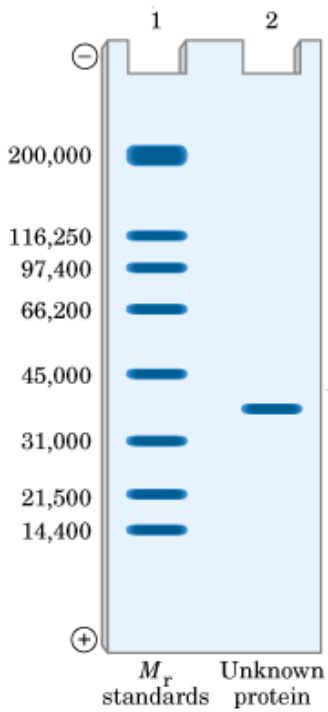
Cation exchanger



Electrophoresis

- SDS-PAGE
  - Sodium dodecyl sulfate (detergent)
    - Denature protein (rod-like structure)
    - Confer negative charge to protein
  - Polyacrylamide gel = pores matrix (provide friction)
  - Separate protein according to molecular weight





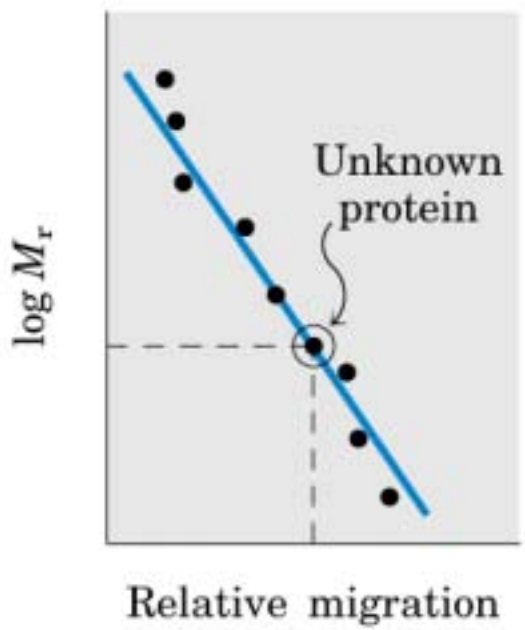
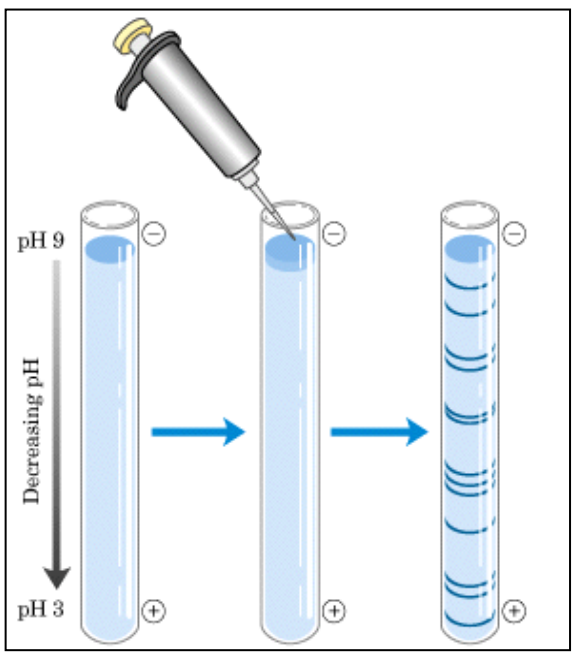
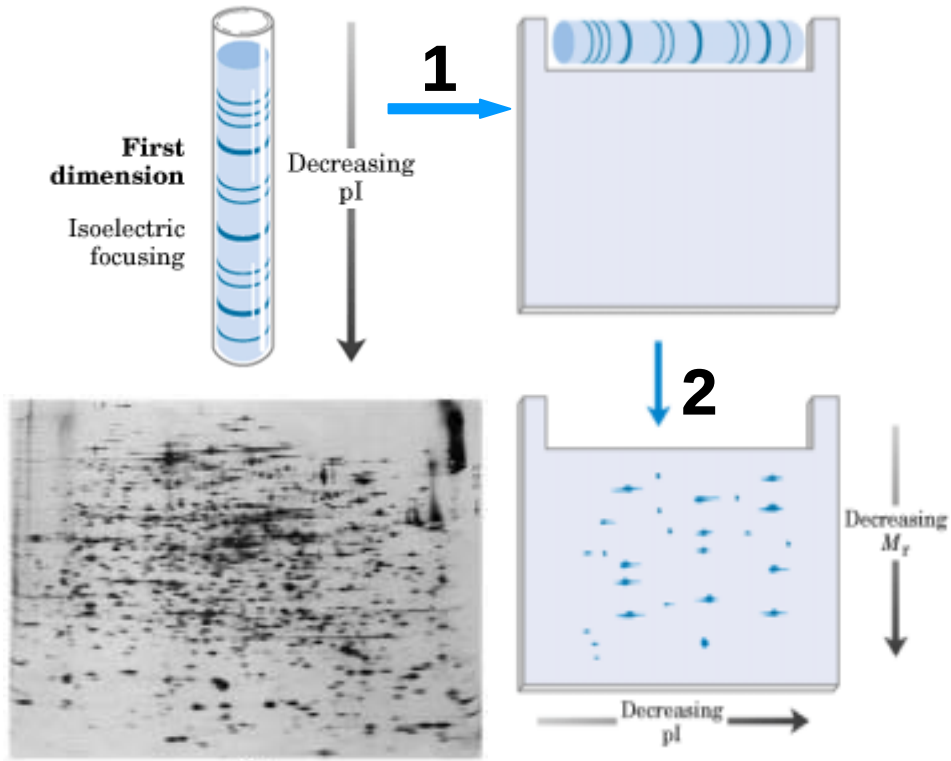


Fig 5-19

Others

- Isoelectric focusing (IEF)
  - Separate proteins according to pI
- 2D electrophoresis
  - pI first, molecular weight second
  - Often used in proteomics





Activity vs. Specific Activity

- Activity = "red marble"

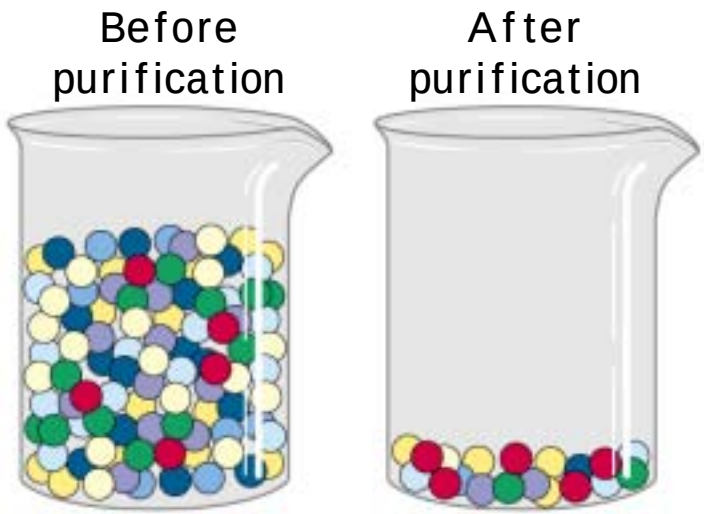


Fig 5-23, p. 137

p. 133

table 5–5

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

Peptide sequencing (I)

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

p. 141

Fig 5-25a

Polypeptide

6M HCl
110°C 12 hr

Free amino acids mixture

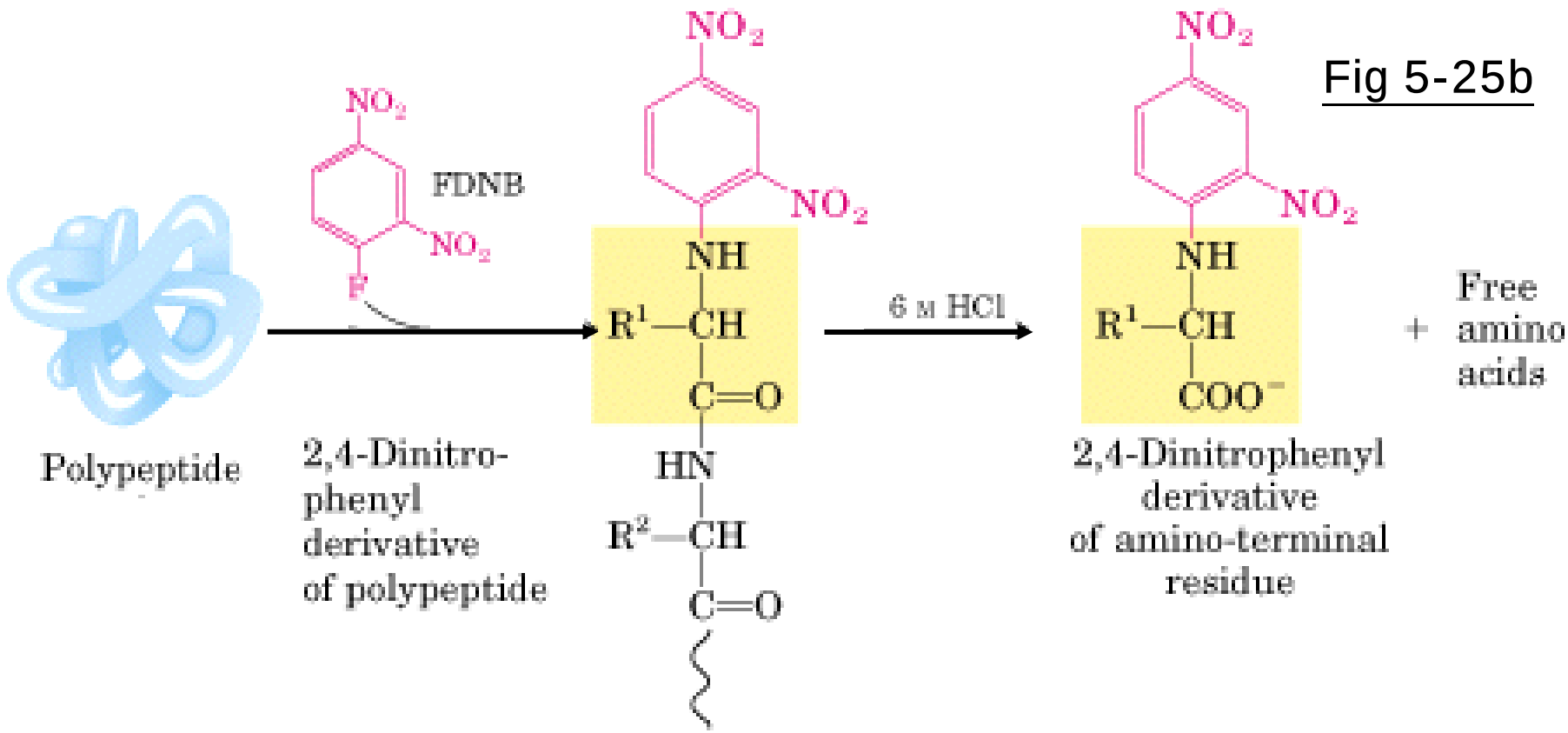
Chromatography separation

Amino acids composition

e.g. Table 5-3

Peptide sequencing (II)

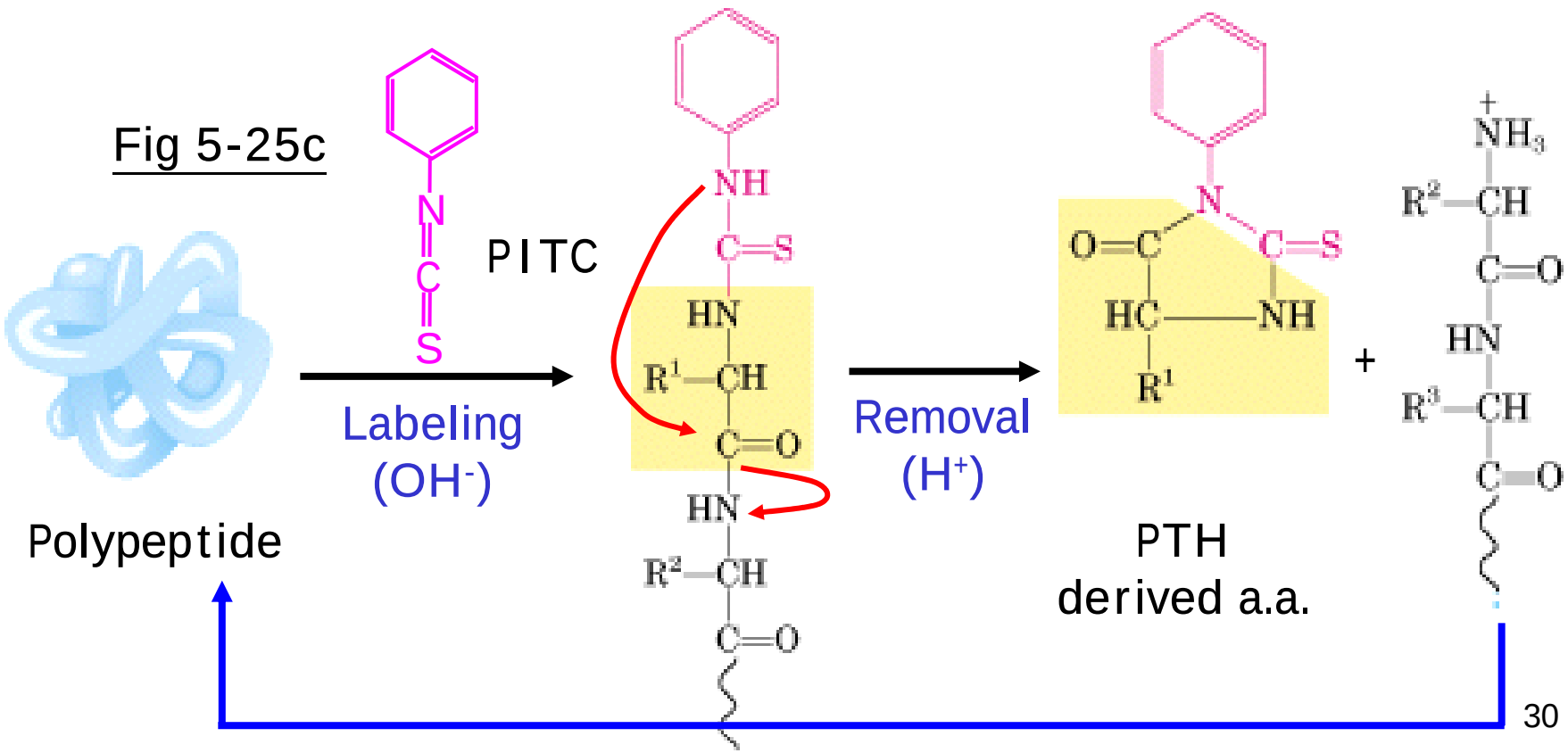
- N-terminal labeling + acid hydrolysis
  - Identify N-terminal residue (DNB-a.a.).
  - Determine # of polypeptides in a protein



Peptide sequencing (III)

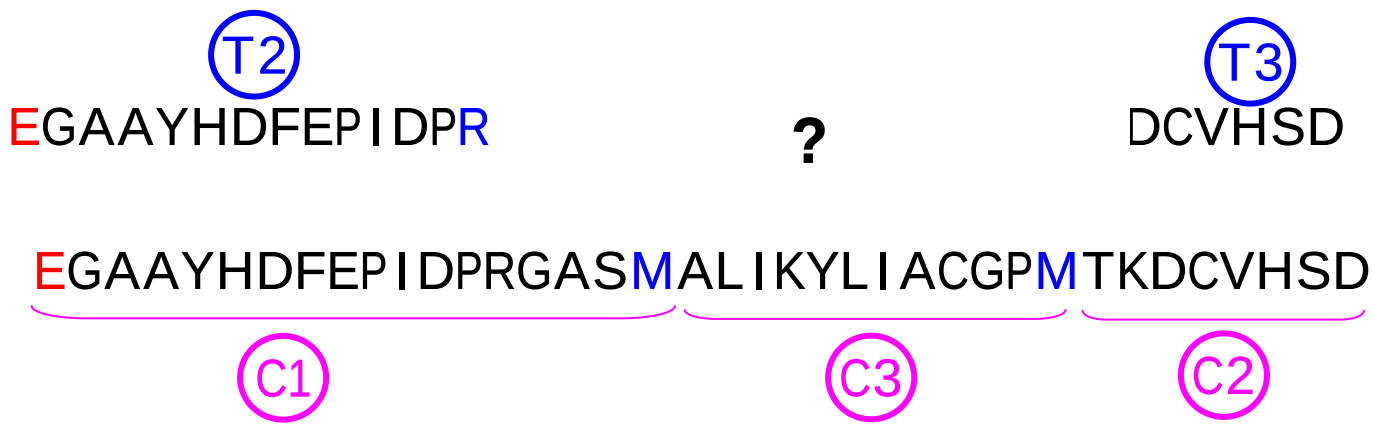
- N-terminal labeling and removal (Edman degradation)
  - Automated sequencer (10 years vs. 2 days)
  - Efficiency vs. polypeptide length

Fig 5-25c

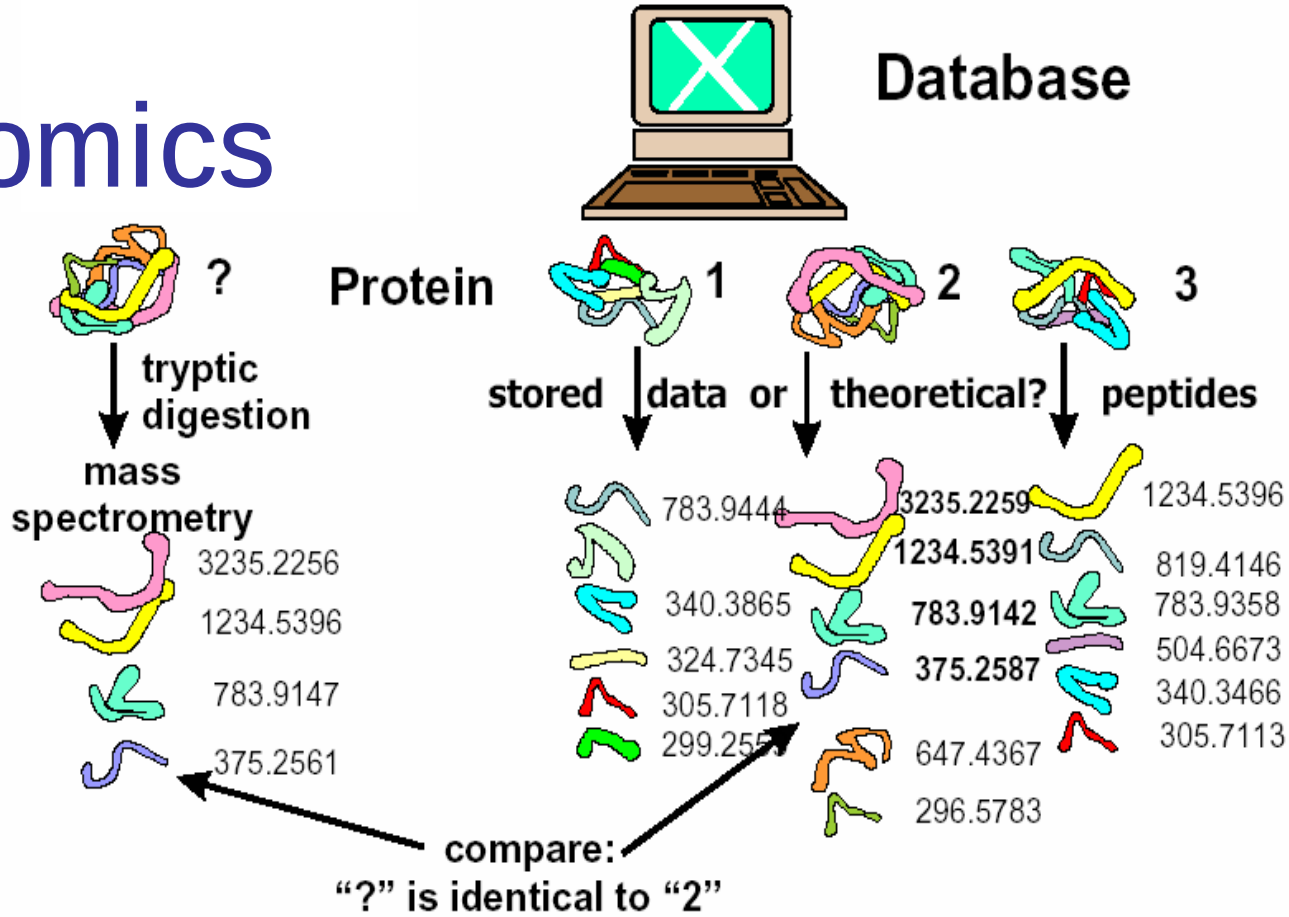


Steps in protein sequencing

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



Proteomics



- The entire protein complement encoded by an organism's DNA
  - 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.

Molecular evolution

Lehninger, 4th ed. p. 107

- Molecular evolution
  - Premise (Emile Zuckerkandl and Linus Pauling, mid 1960's)
    - If 2 organisms are close related, the sequences of their genes and proteins should be similar
- Residue variation of a given protein
  - Residues essential for function
    - Conserved over time
  - Residues non-essential for function
    - Tend to vary over time
  - Variation
    - Random
    - Non-random (conservative), p. 108
      - Substitute with a.a. of similar chemical properties
- Homologous proteins (homologs)
  - Members of a family of proteins that share a common ancestor (see also p. 37)
  - Paralogs
    - 2 proteins within a family (homologs) are present in the same species
  - Orthologs
    - From different species