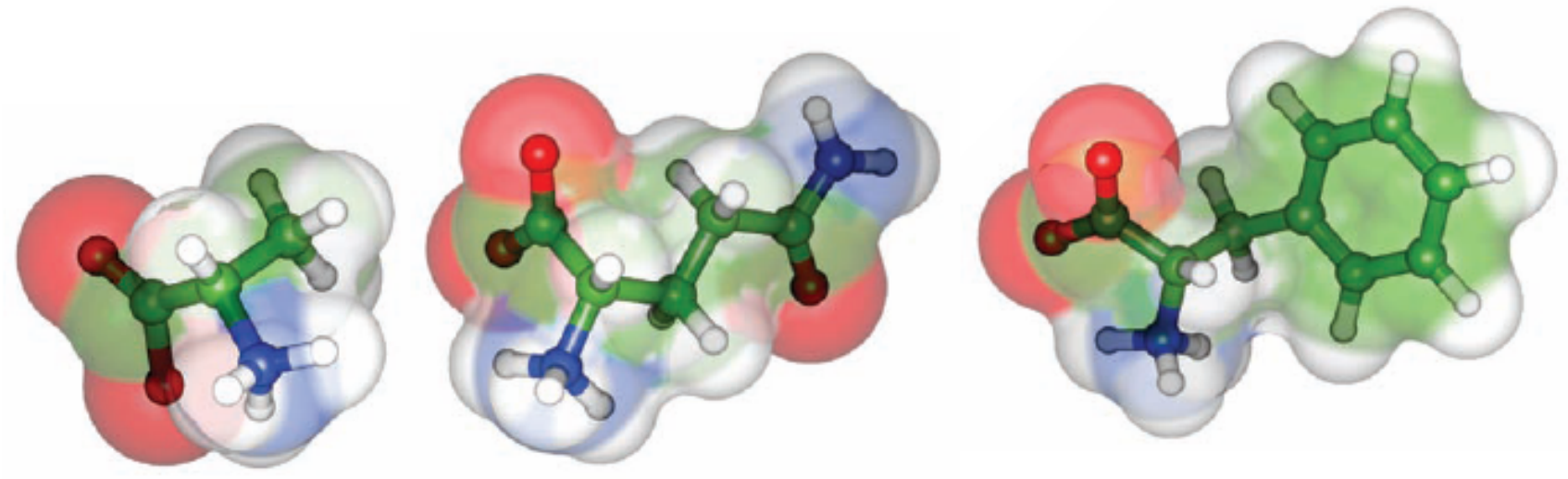
SAPIENZA
UNIVERSITÀ DI ROMA

Amino acids



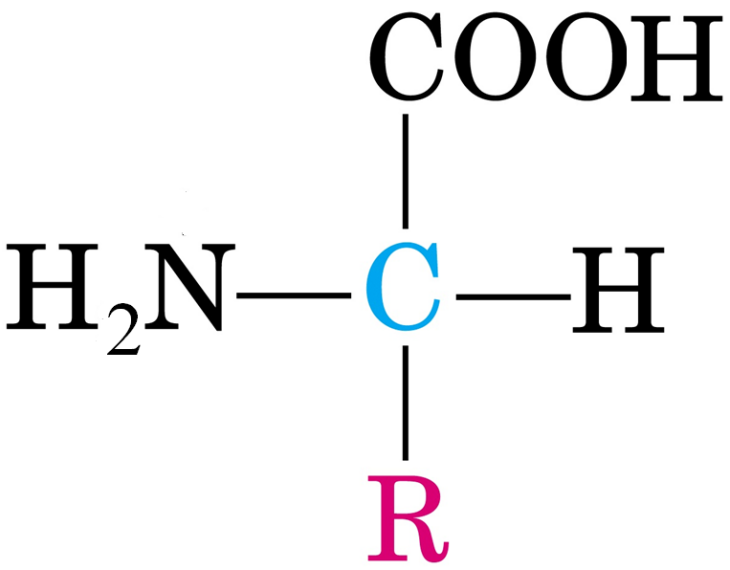
Alanine Glutamine Phenylalanine

Prof. Francesco Malatesta

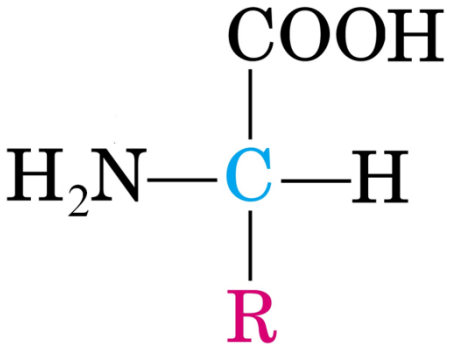
Physical-chemical properties of amino acids

- Amino acids are found in proteins but also in hormones and neurotransmitters

- Amino acids contain both an **amino** group and a **carboxylic** group

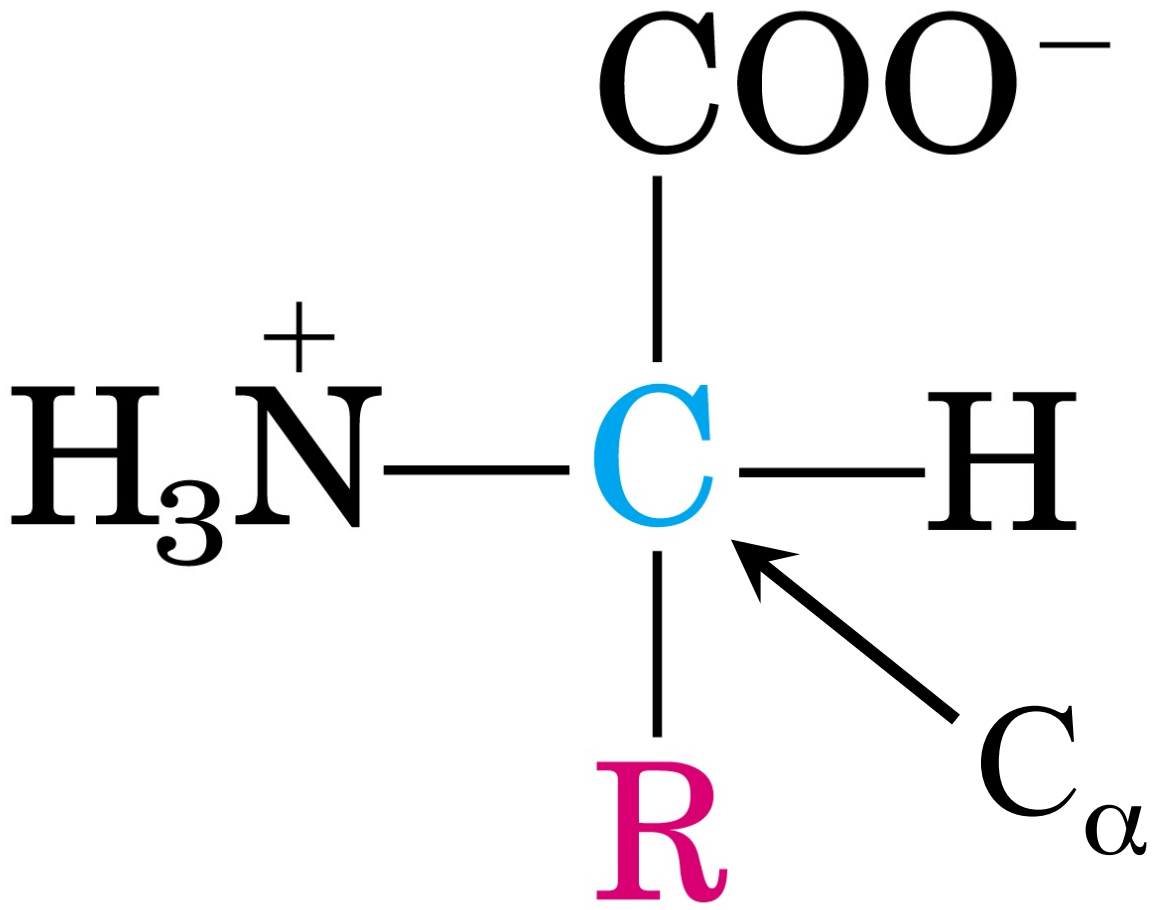


Some properties, both chemical and physical are not in agreement with this structure:



- contrary to alifatic amines (R-NH_2) and carboxylic acids (R-COOH), amino acids are non-volatile crystalline solids that melt at high temperatures (200-300 °C)
- amino acids are insoluble in apolar solvents (benzene, ether, etc) and are instead soluble in H_2O
- their aqueous solutions behave as solutions of compounds with a high dipole moment
- the **acidity constants** (R-COOH) are **higher** than the corresponding carboxylic acids ($K_A \approx 10^{-2}$ M) and the **basicity constants** are lower than the corresponding aliphatic amines ($K_B \approx 10^{-4}$ M)

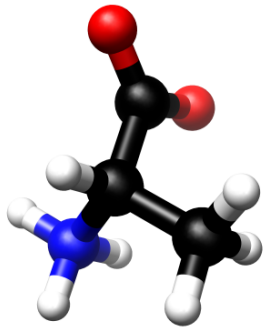
All these properties agree with the presence of a dipolar species:

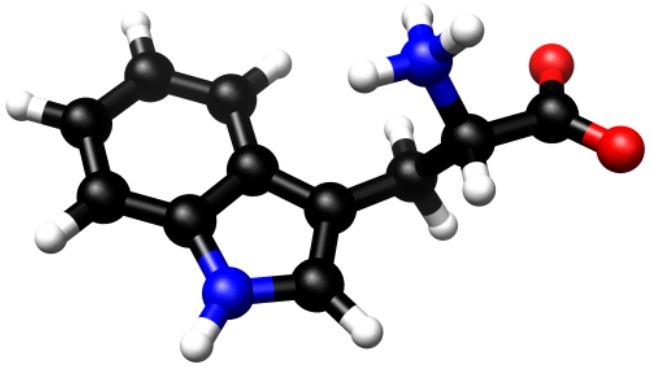


Amino acids are dipolar ions or zwitterions

NOMENCLATURE

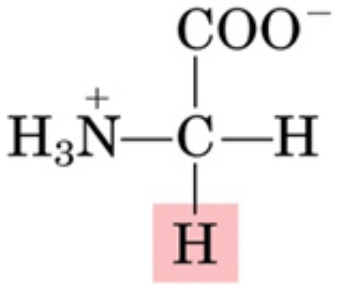
ALANINE	ALA	A
CYSTEINE *	CYS	C
ASPARTIC ACID	ASP	D
GLUTAMIC ACID	GLU	E
PHENYLALANINE *	PHE	F
GLYCINE	GLY	G
HISTIDINE *	HIS	H
ISOLEUCINE *	ILE	I
LYSINE *	LYS	K
LEUCINE *	LEU	L
METHIONINE *	MET	M
ASPARAGINE	ASN	N
PROLINE	PRO	P
GLUTAMINE	GLN	Q
ARGININE	ARG	R
SERINE	SER	S
THREONINE *	THR	T
VALINE *	VAL	V
TRYPTOPHAN *	TRP	W
TYROSINE	TYR	Y



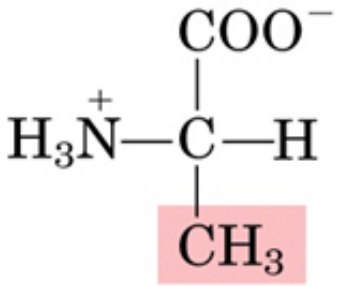


* essential amino acid

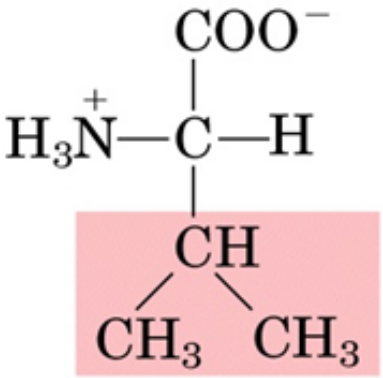
Nonpolar, aliphatic R groups



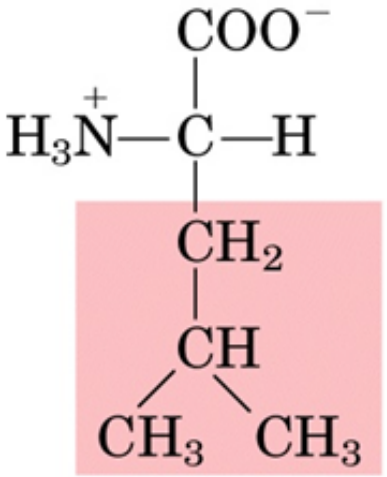
Glycine



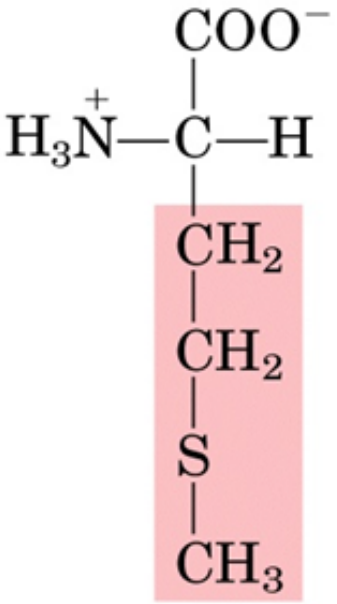
Alanine



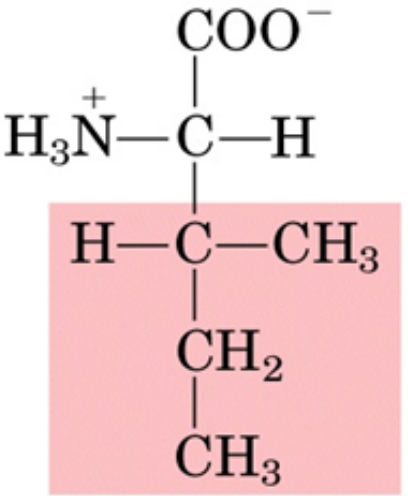
Valine



Leucine

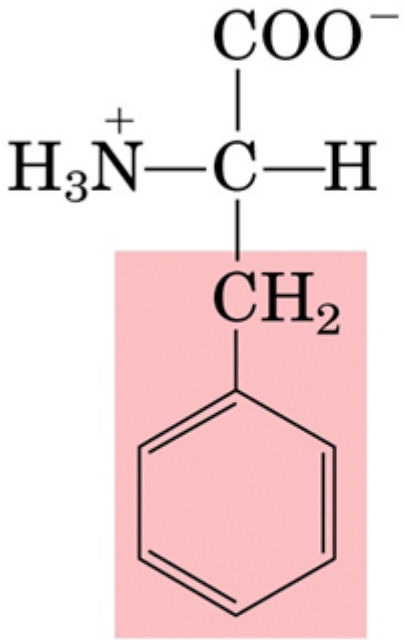


Methionine

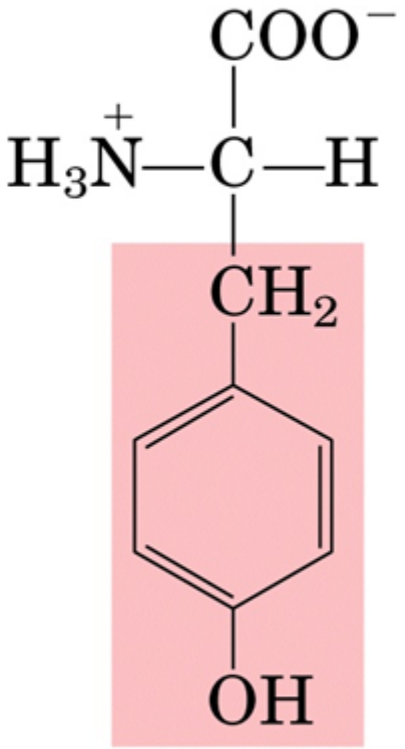


Isoleucine

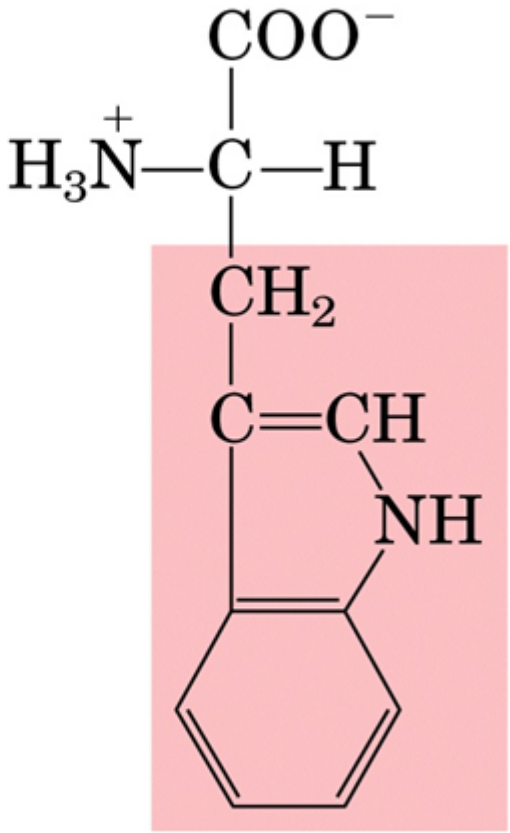
Aromatic R groups



Phenylalanine

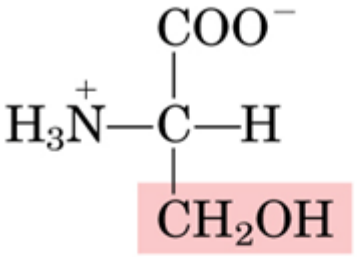


Tyrosine

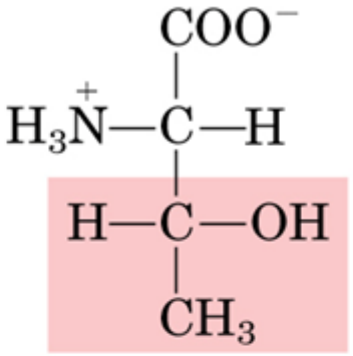


Tryptophan

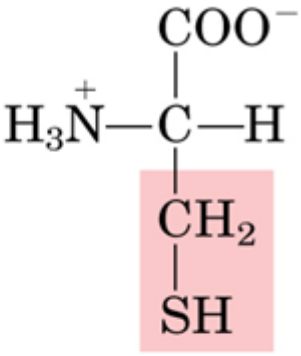
Polar, uncharged R groups



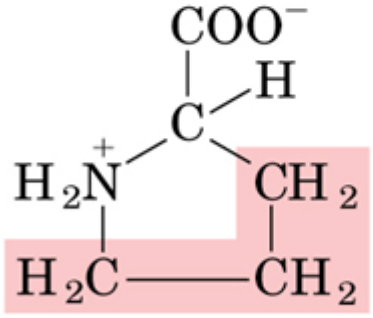
Serine



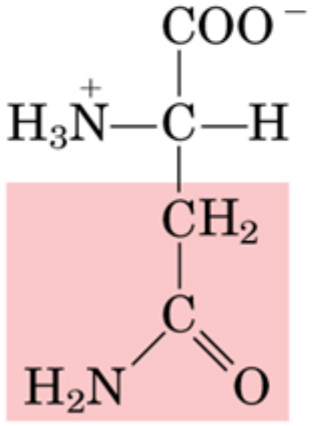
Threonine



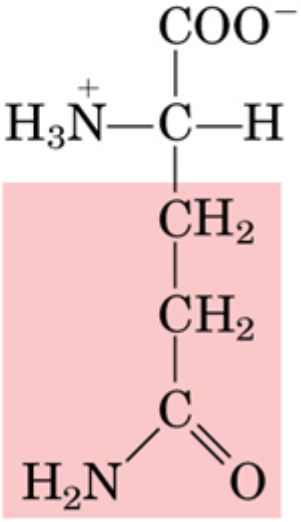
Cysteine



Proline

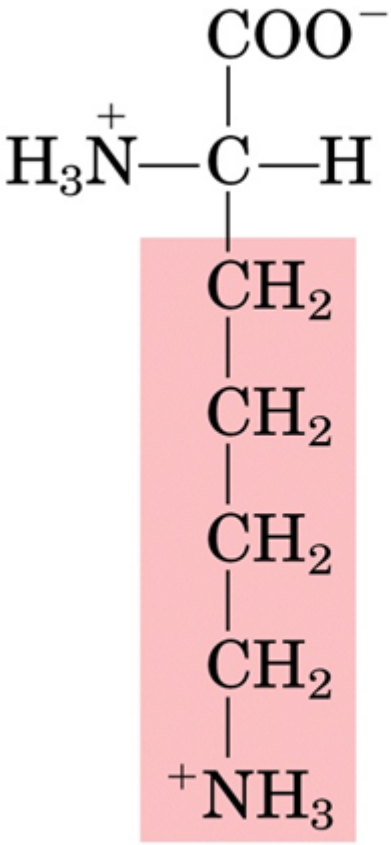


Asparagine

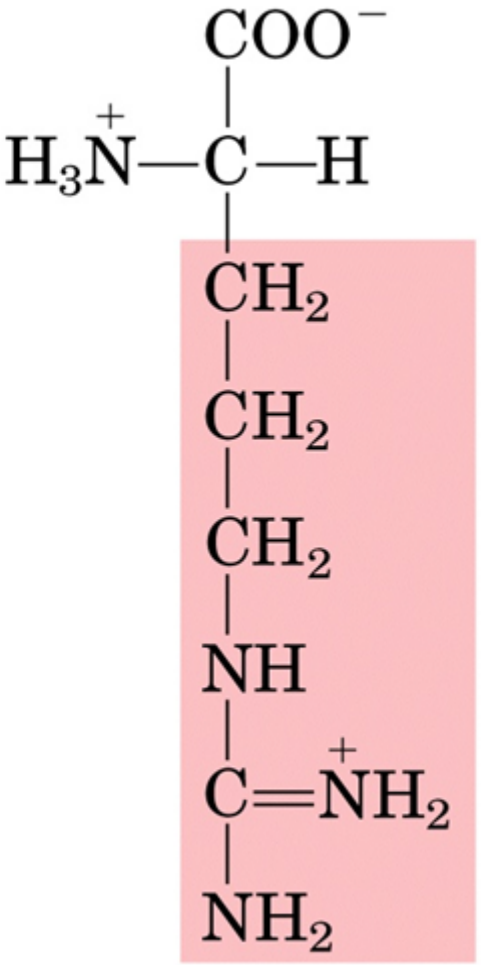


Glutamine

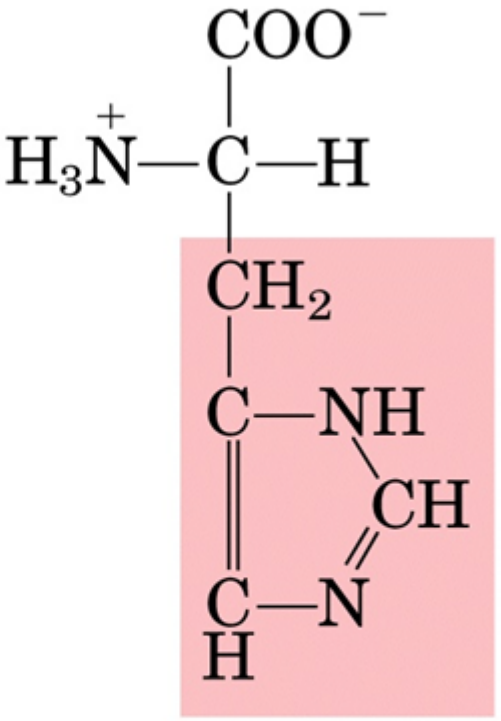
Positively charged R groups



Lysine

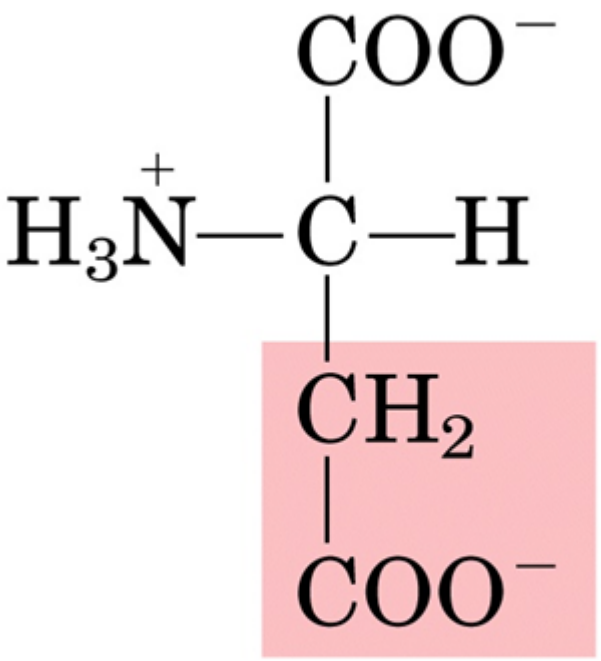


Arginine

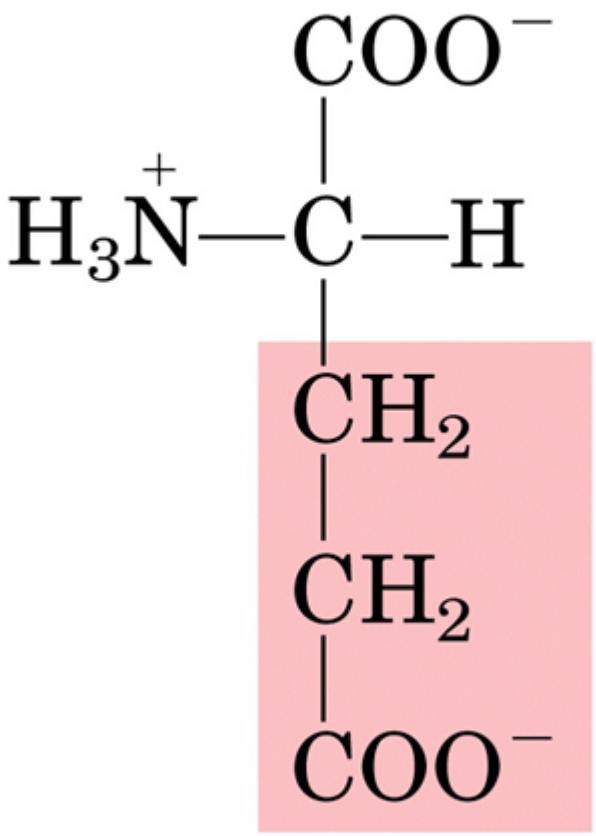


Histidine

Negatively charged R groups

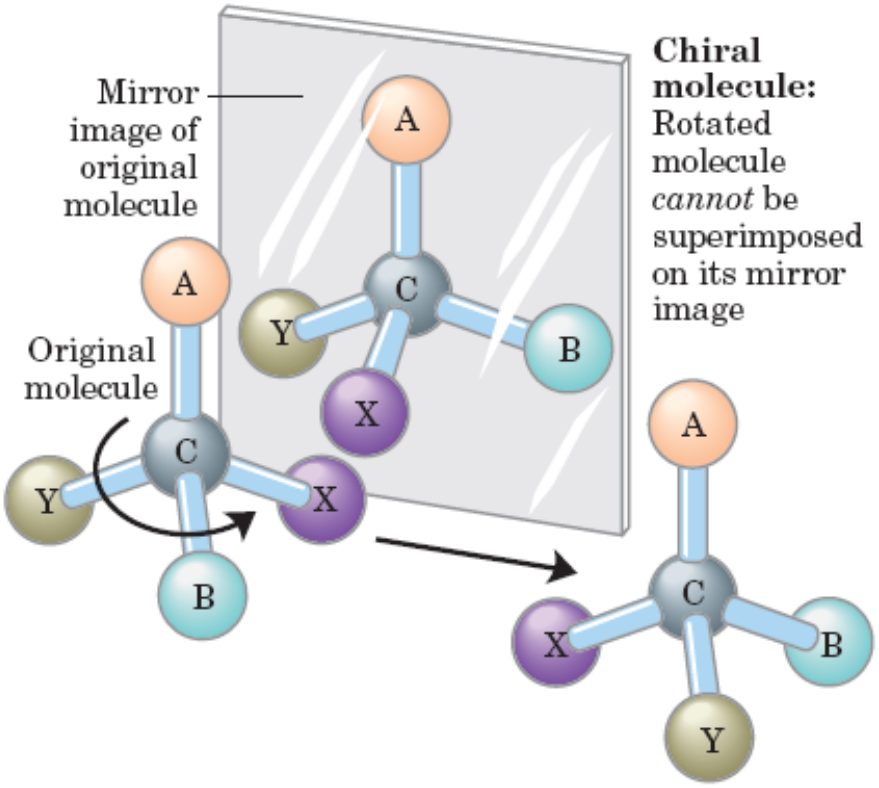


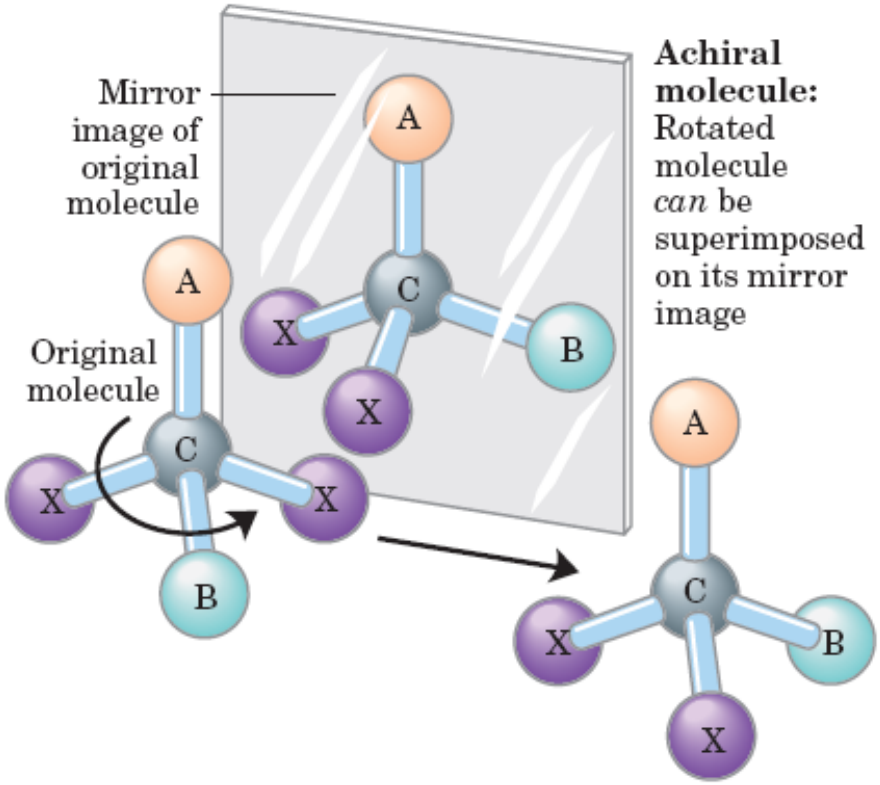
Aspartate



Glutamate

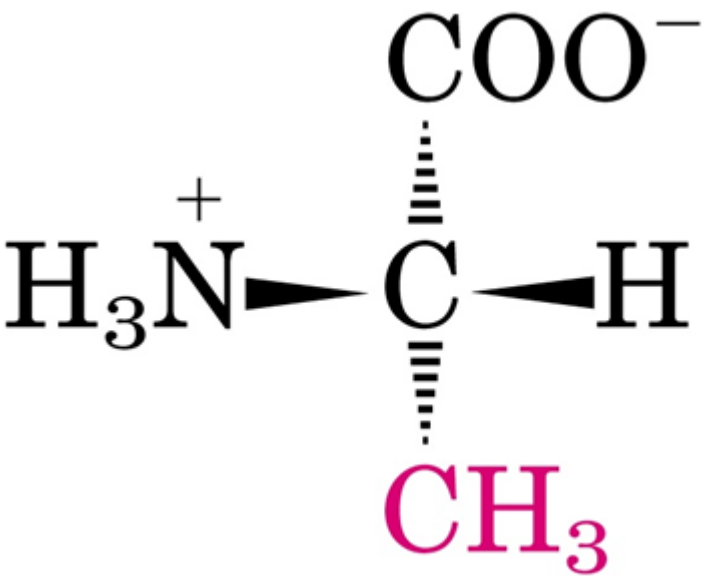
Stereoisomerism of amino acids



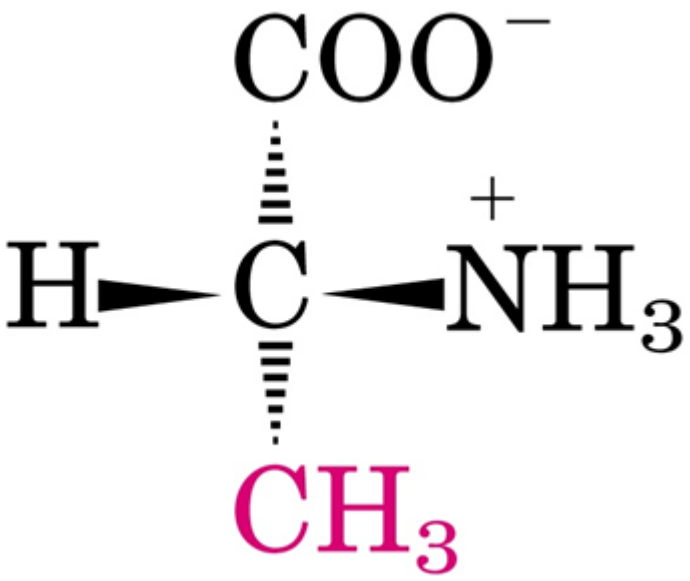


Stereoisomerism of amino acids

Amino acids are chiral (except Gly) and only the L series are present in natural proteins:

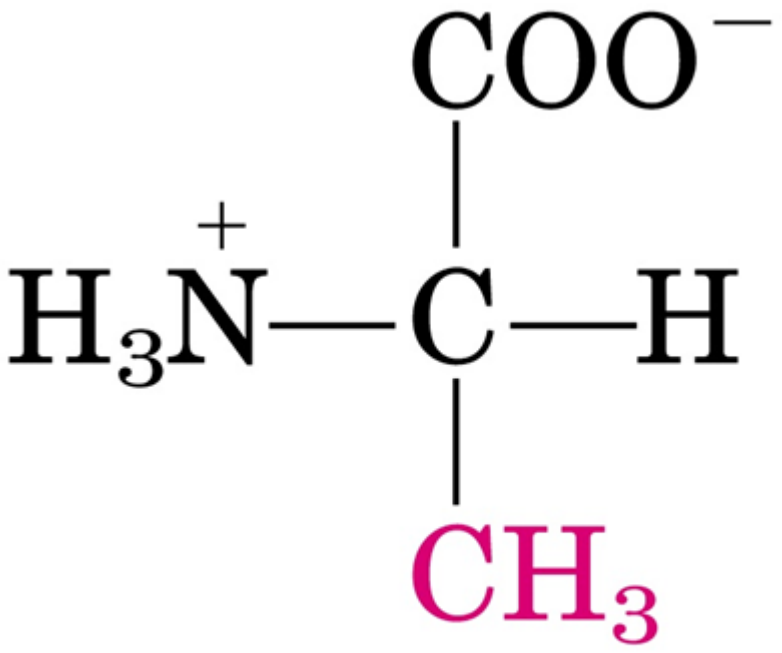


L-alanine

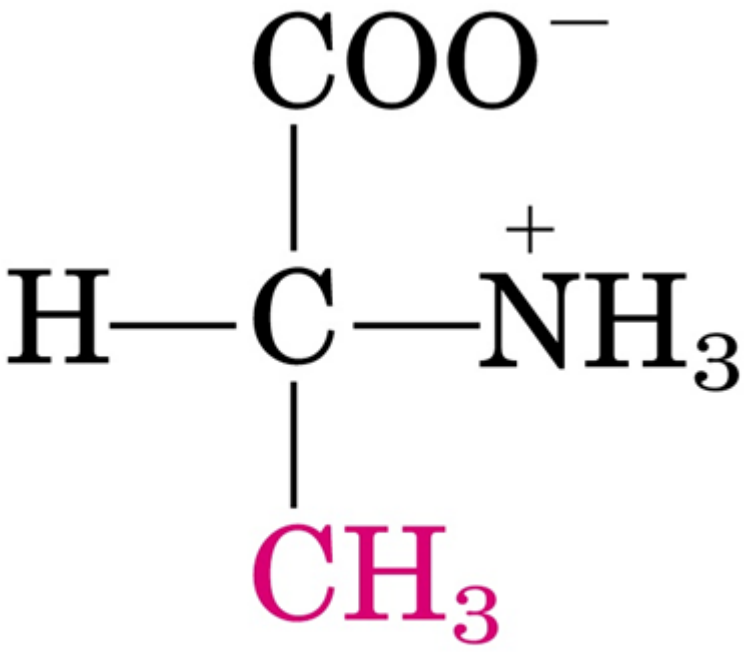


D-alanine

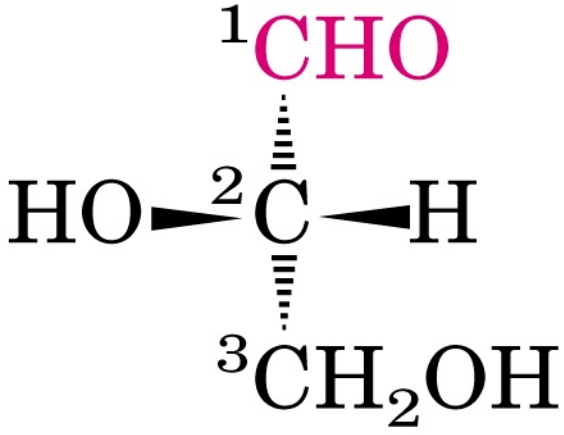
Fischer notation



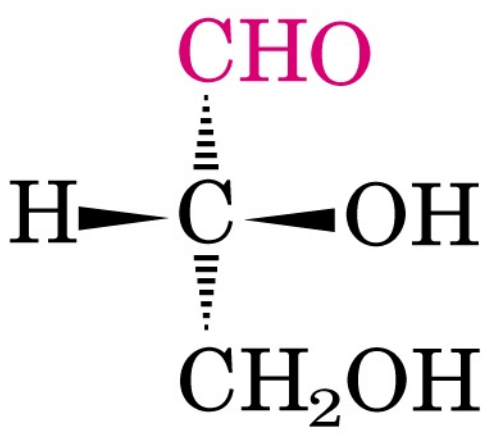
L-Alanine



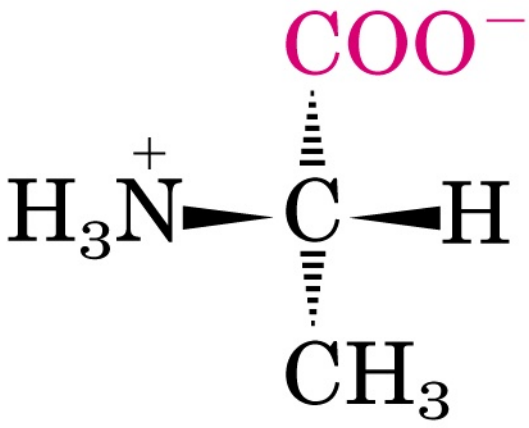
D-Alanine



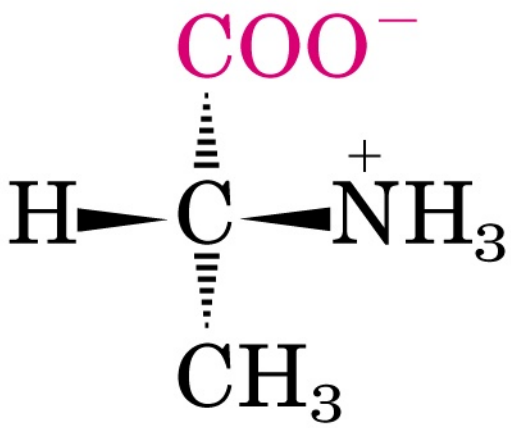
L-Glyceraldehyde



D-Glyceraldehyde

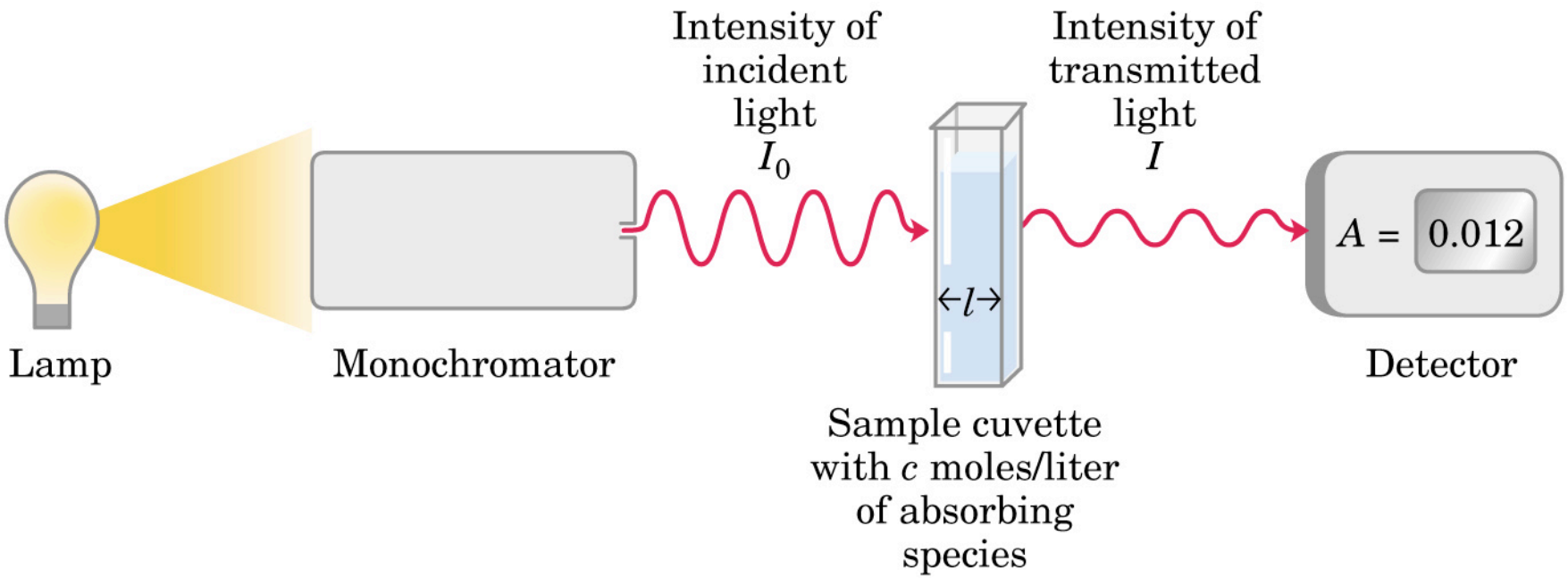


L-Alanine

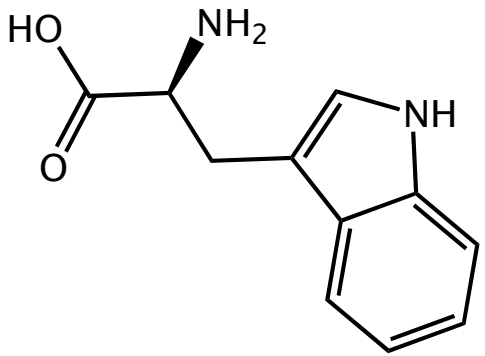


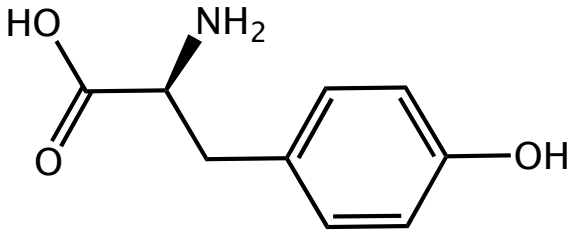
D-Alanine

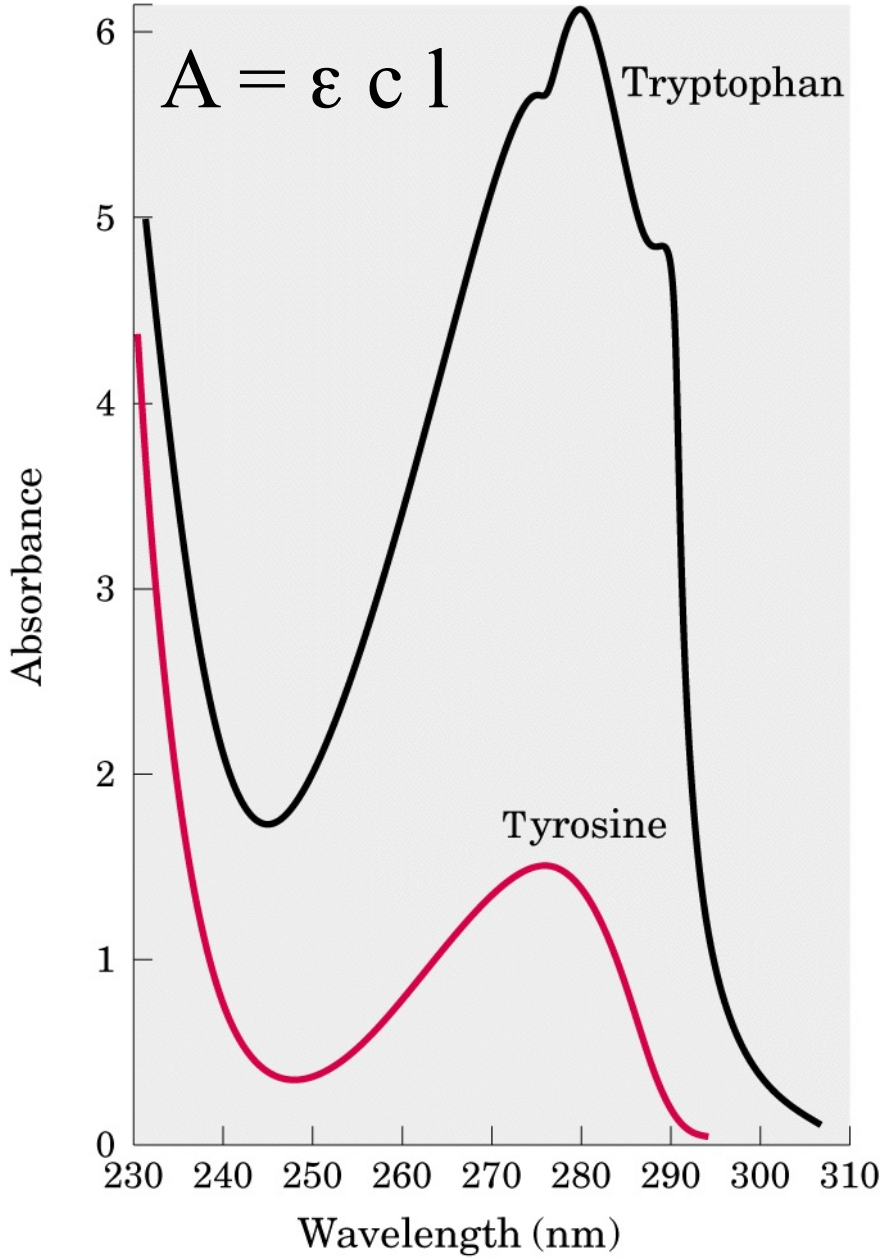
Lambert-Beer law



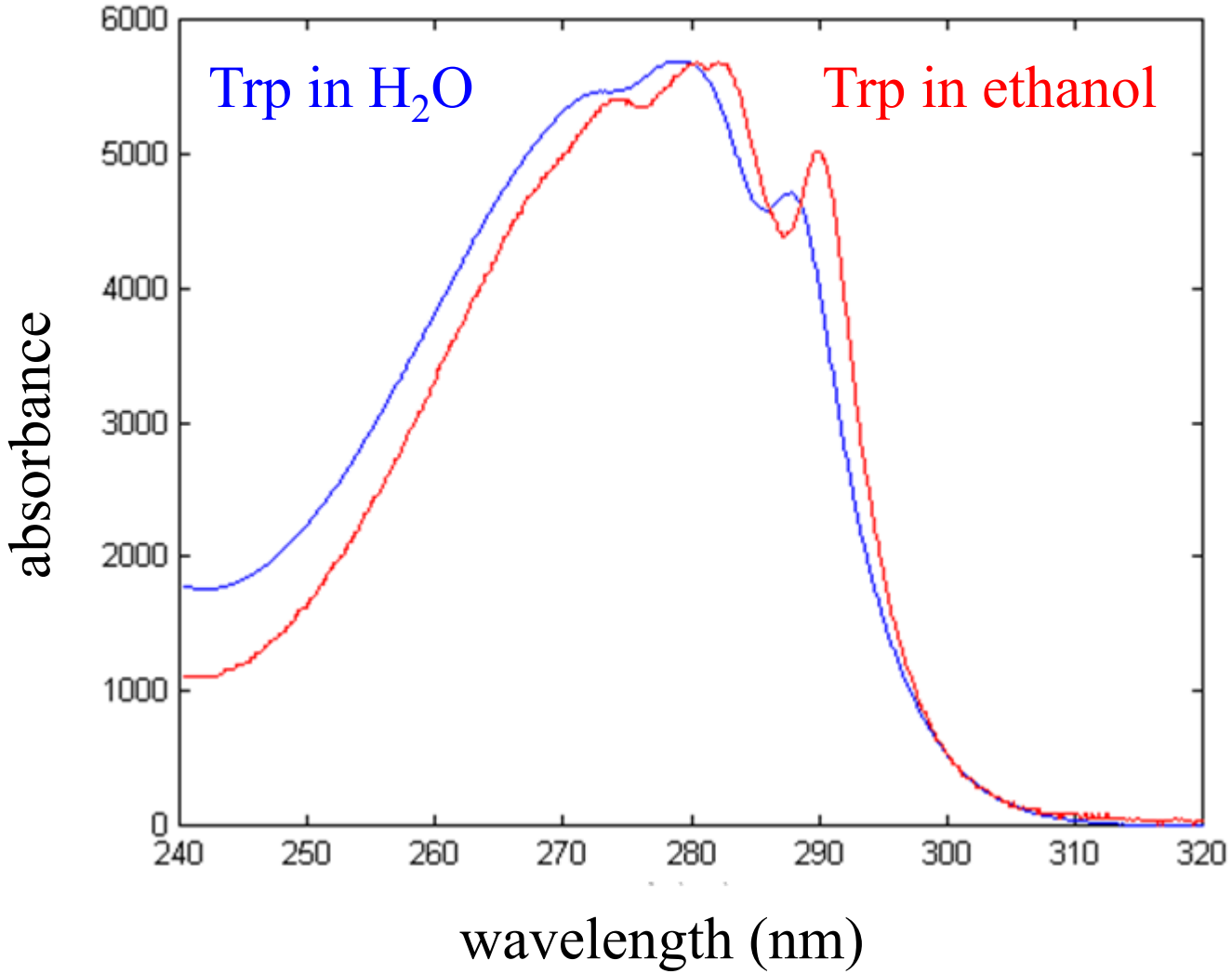
$$A = \log (I_0/I) = \varepsilon\ c\ l$$


HO
NH2
O
NH

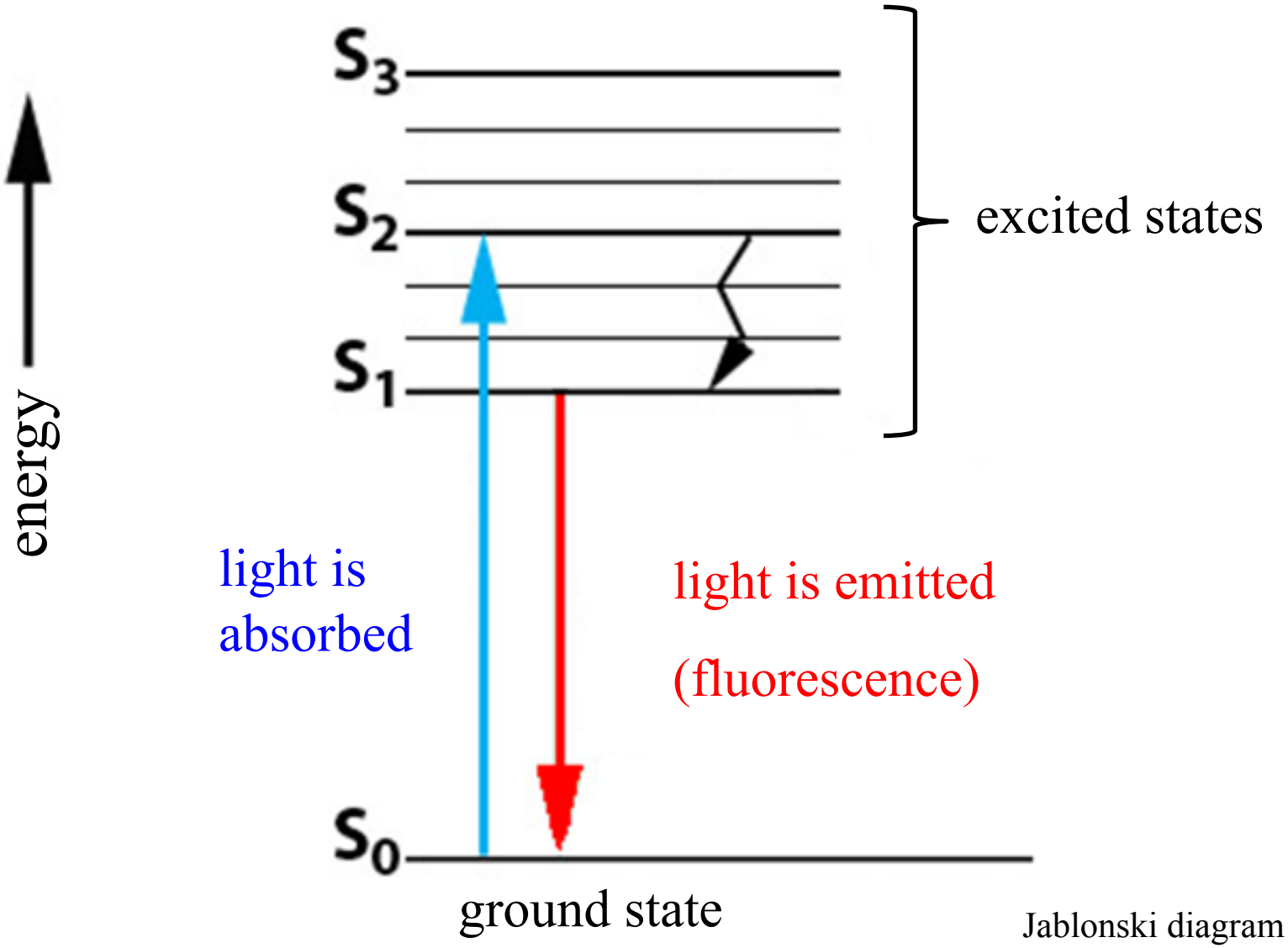

HO
NH2
O
OH


A = ε c l
Tryptophan
Tyrosine
Absorbance
Wavelength (nm)
0
1
2
3
4
5
6
230
240
250
260
270
280
290
300
310

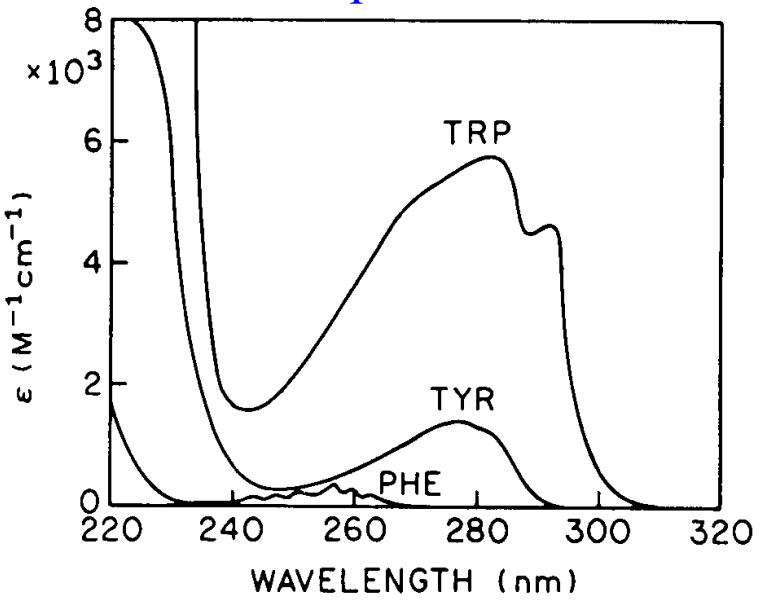
The absorption spectrum depends on the environment of the chromophore



Some amino acids are fluorescent



Absorption



Fluorescence

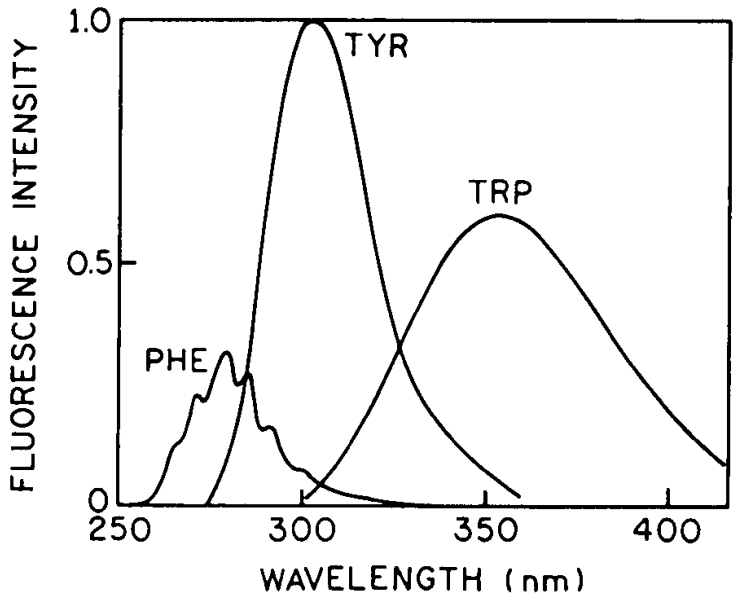


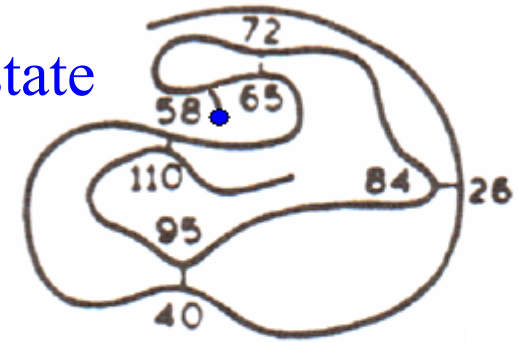
Table 3.1. Fluorescence Parameters of Aromatic Amino Acids in Water at Neutral pH[a]

Species	λ_{ex} (nm)	λ_{em} (nm)	Bandwidth (nm)	Quantum yield	Lifetime (ns)
Phenylalanine	260	282	—	0.02	6.8
Tyrosine	275	304	34	0.14	3.6
Tryptophan	295	353	60	0.13	3.1[b]

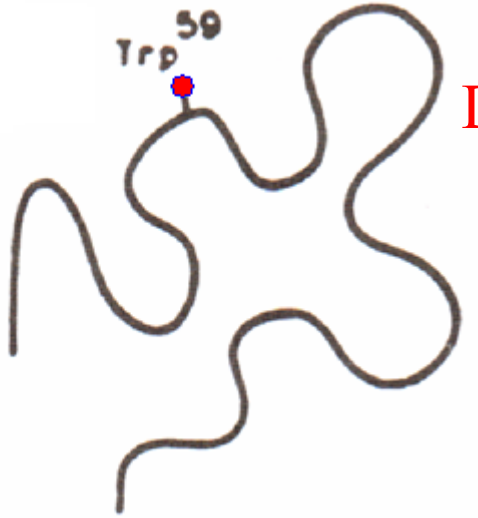
buried Trp

solvent exposed Trp

N state

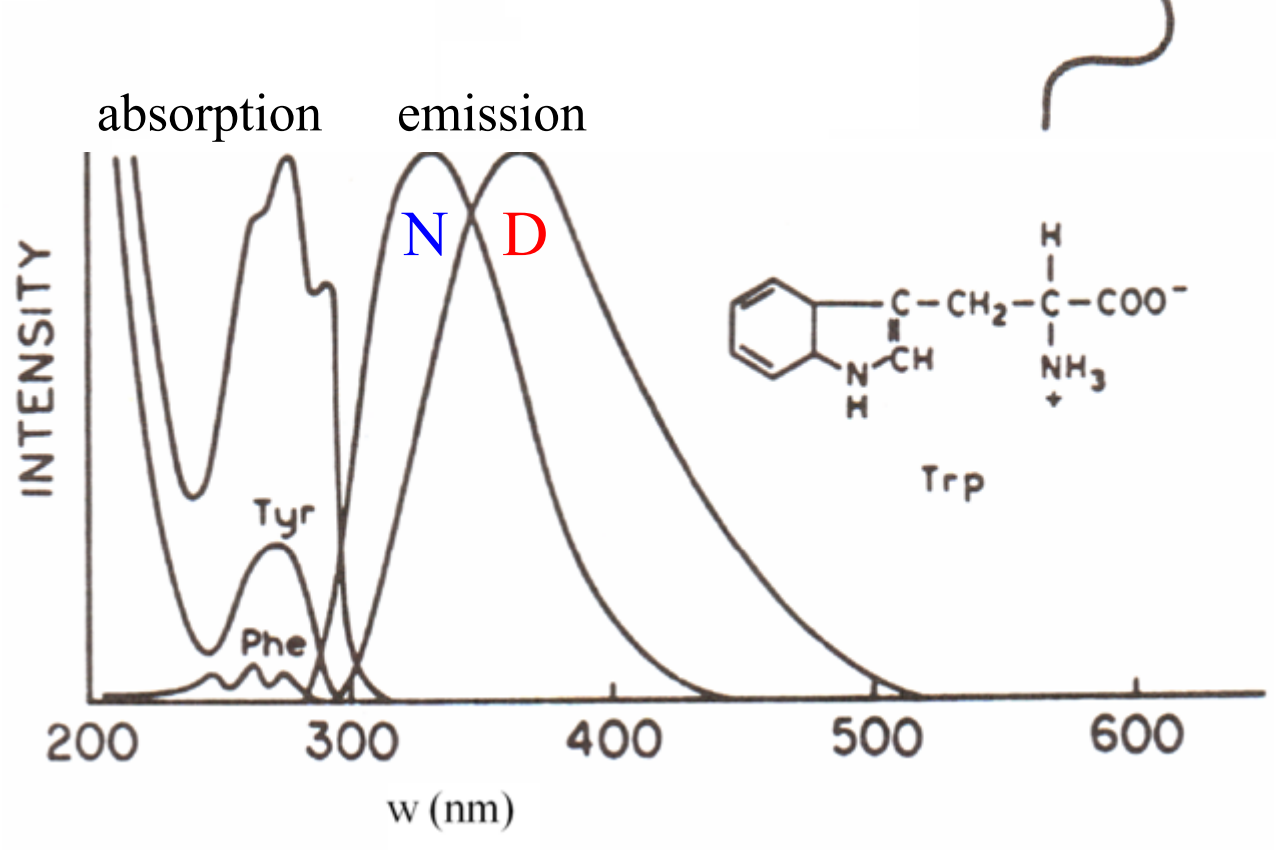


denaturant



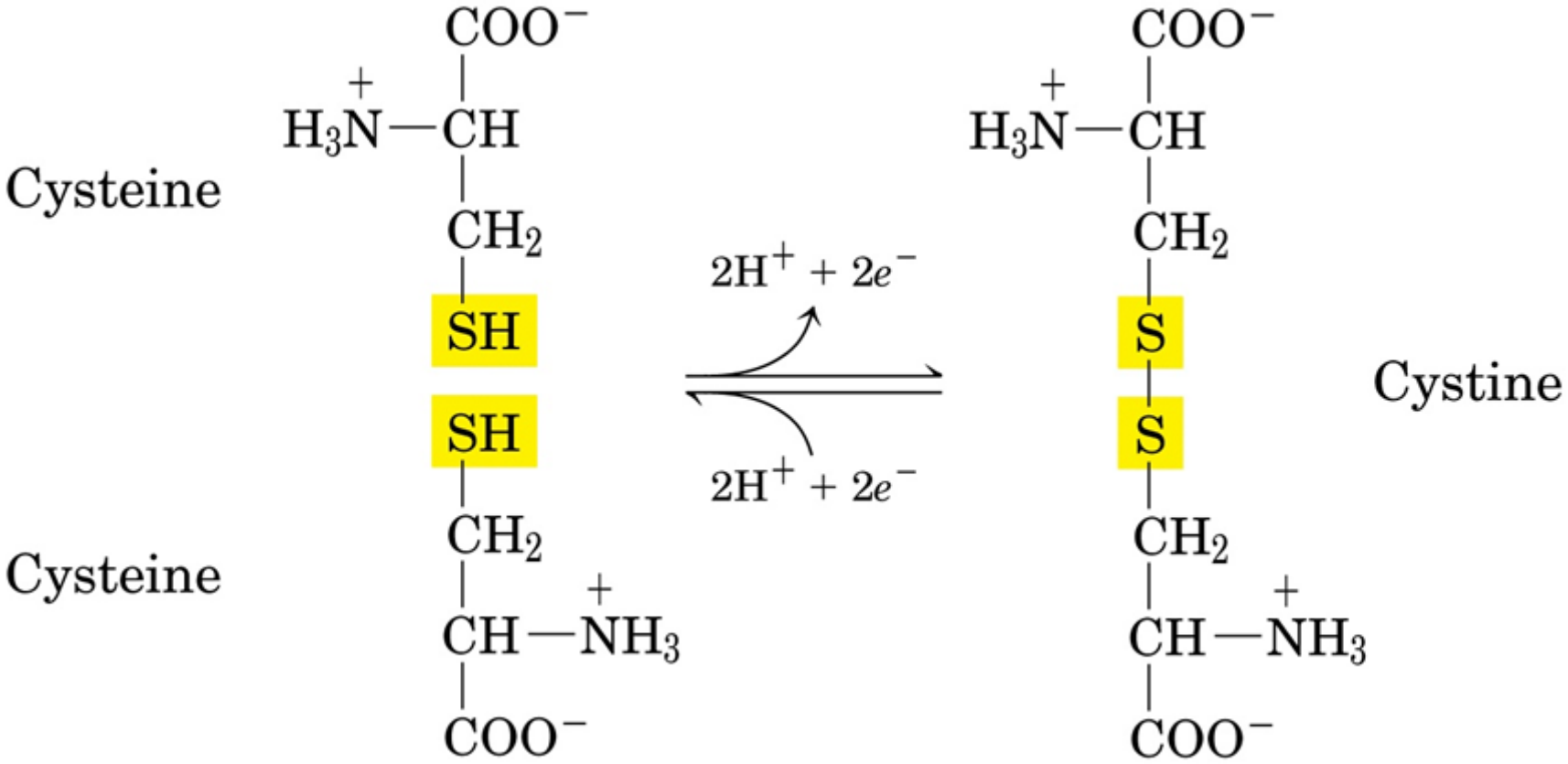
D state

absorption emission

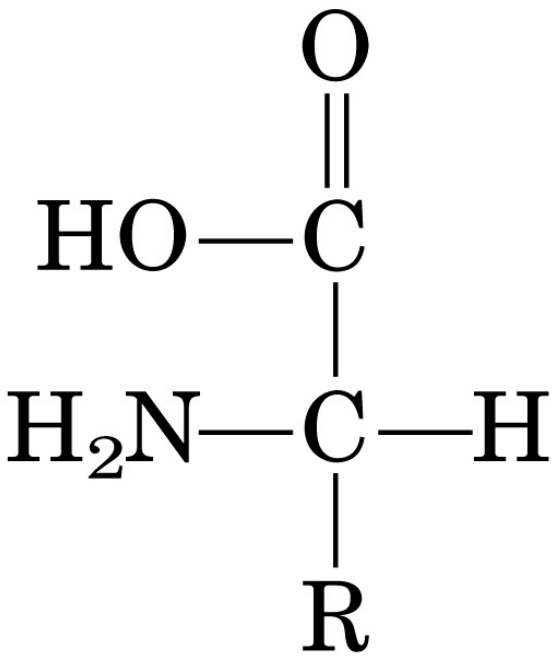


Trp

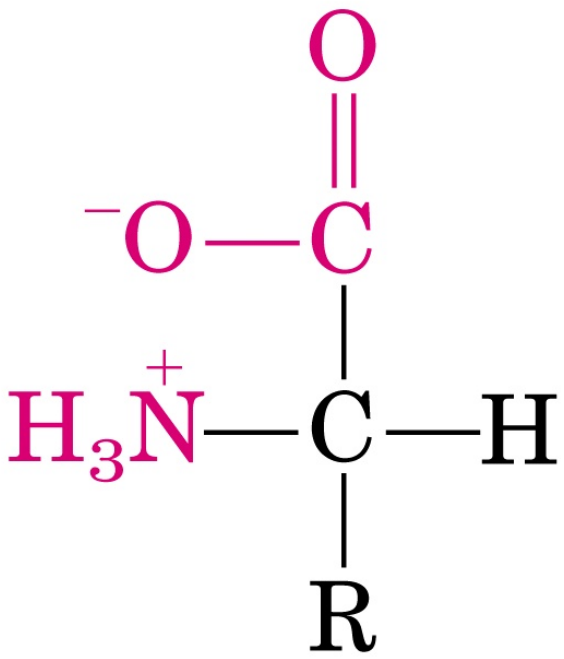
Disulphide bridges



Acid-base properties of amino acids



Nonionic form

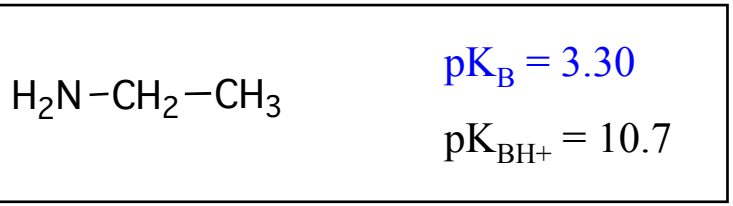


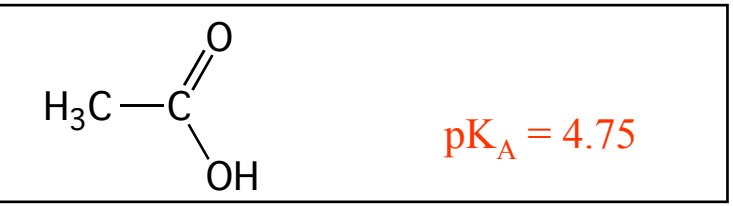
Zwitterionic form

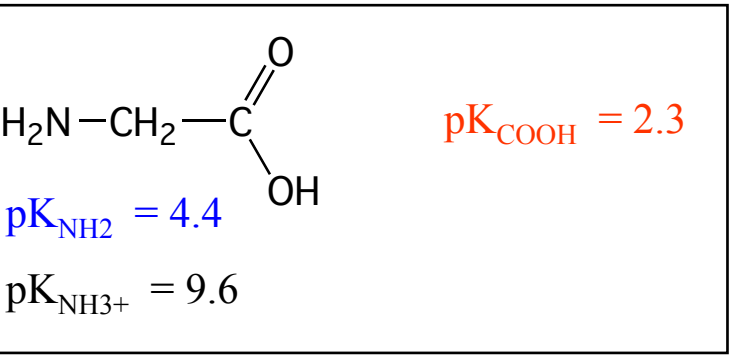
Acid-base properties

	pK_{COOH}	pK_{NH3+}	pK_R	pI
GLY	2.3	9.6	-	6.0
ALA	2.3	9.7	-	6.0
VAL	2.3	9.6	-	6.0
PRO	2.0	10.6	-	6.3
LEU	2.4	9.7	-	6.0
MET	2.3	9.2	-	5.8
ILE	2.4	9.7	-	6.1
PHE	1.8	9.1	-	5.5
TYR	2.2	9.1	10.1	5.7
TRP	2.4	9.4	-	5.9
SER	2.2	9.2	-	5.7
THR	2.6	10.4	-	6.5
CYS	1.8	10.8	8.3	5.0
ASN	2.0	8.8	-	5.4
GLN	2.2	9.1	-	5.7
LYS	2.2	9.0	10.5	9.8
ARG	2.2	9.0	12.5	10.8
HIS	1.8	9.2	6.0	7.6
ASP	2.1	9.8	3.9	3.0
GLU	2.2	9.7	4.3	3.2

The acidity constants (R-COOH) are higher than the corresponding carboxylic acids ($pK_A \approx 2.2$) and the basicity constants are lower than the corresponding aliphatic amines ($pK_B \approx 4.4$)





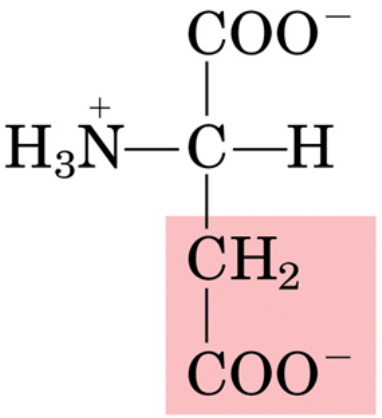


Effect of substituents on the dissociation constants of some acids

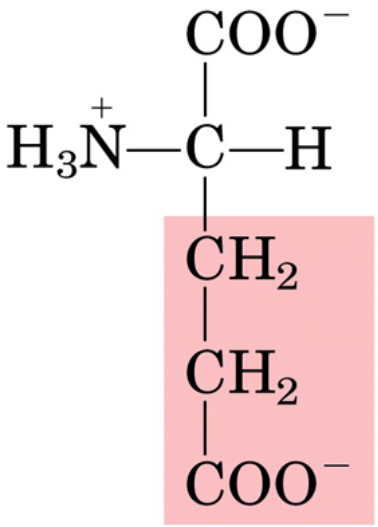
ACID	FORMULA	pK
acetic	CH_3-COOH	
chloroacetic	ClCH_2-COOH	2.87
dichloroacetic	Cl_2CH-COOH	1.48
aminoacetic	$^{+}H_3N$-CH_2-COOH	2.35
malonic	$^{-}$OOC-CH_2-COOH	5.70

from H. Gutfreund, Enzyme physical properties, Wiley Interscience, 1972, London

The R group of Asp is more acidic than the R group of Glu: the position effect



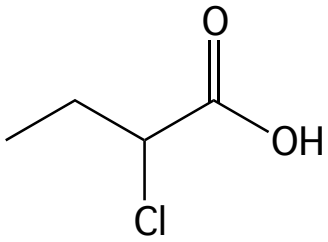
Aspartate



Glutamate

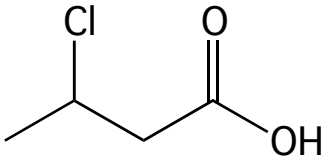
	pK_{COOH}	pK_{NH3+}	pK_R
ASP	2.1	9.8	3.9
GLU	2.2	9.7	4.3

Acid strength



2-chloro-butanoic acid

>



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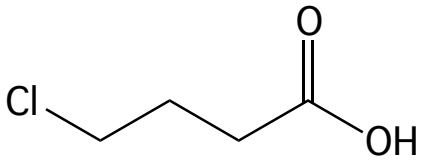
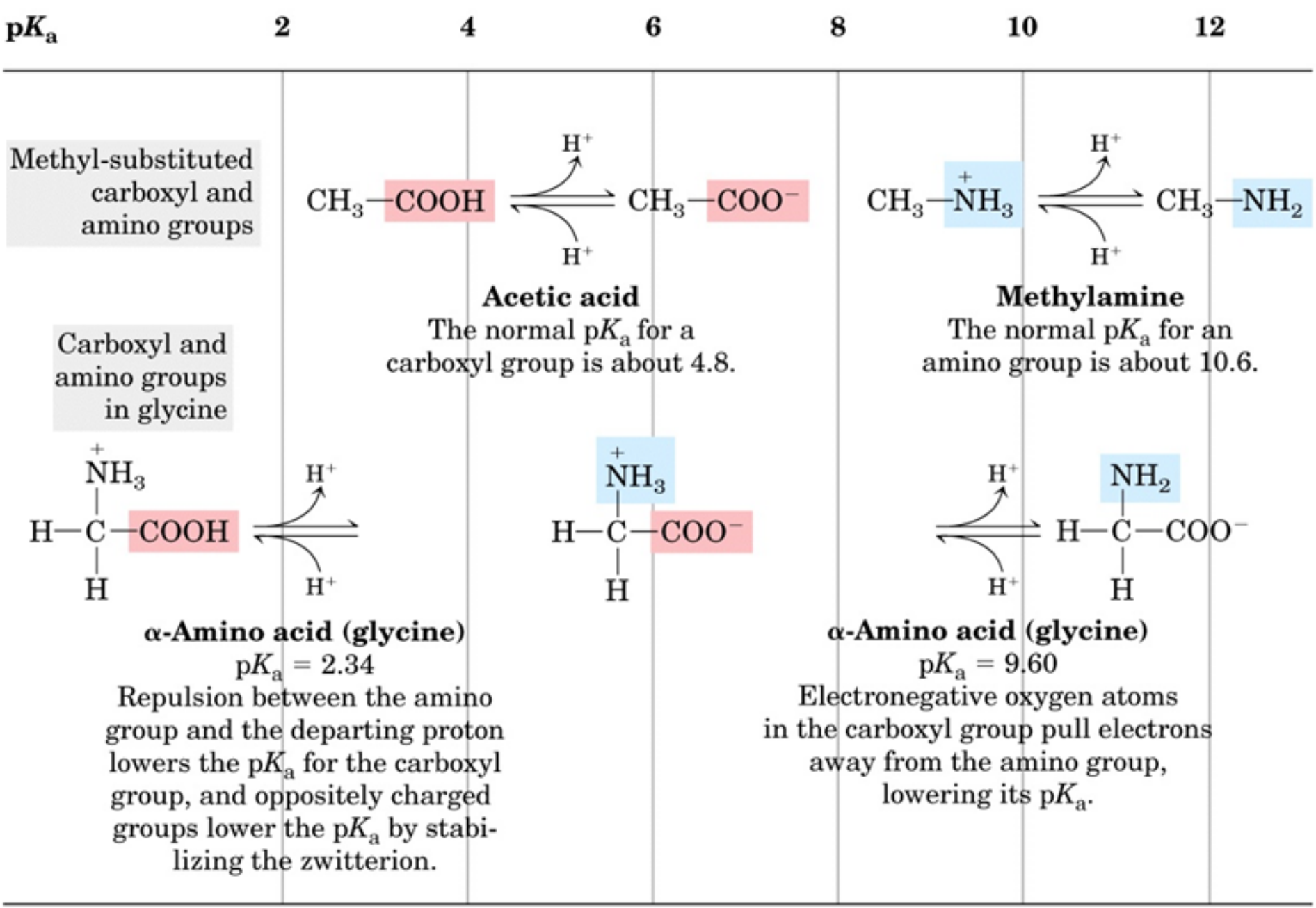
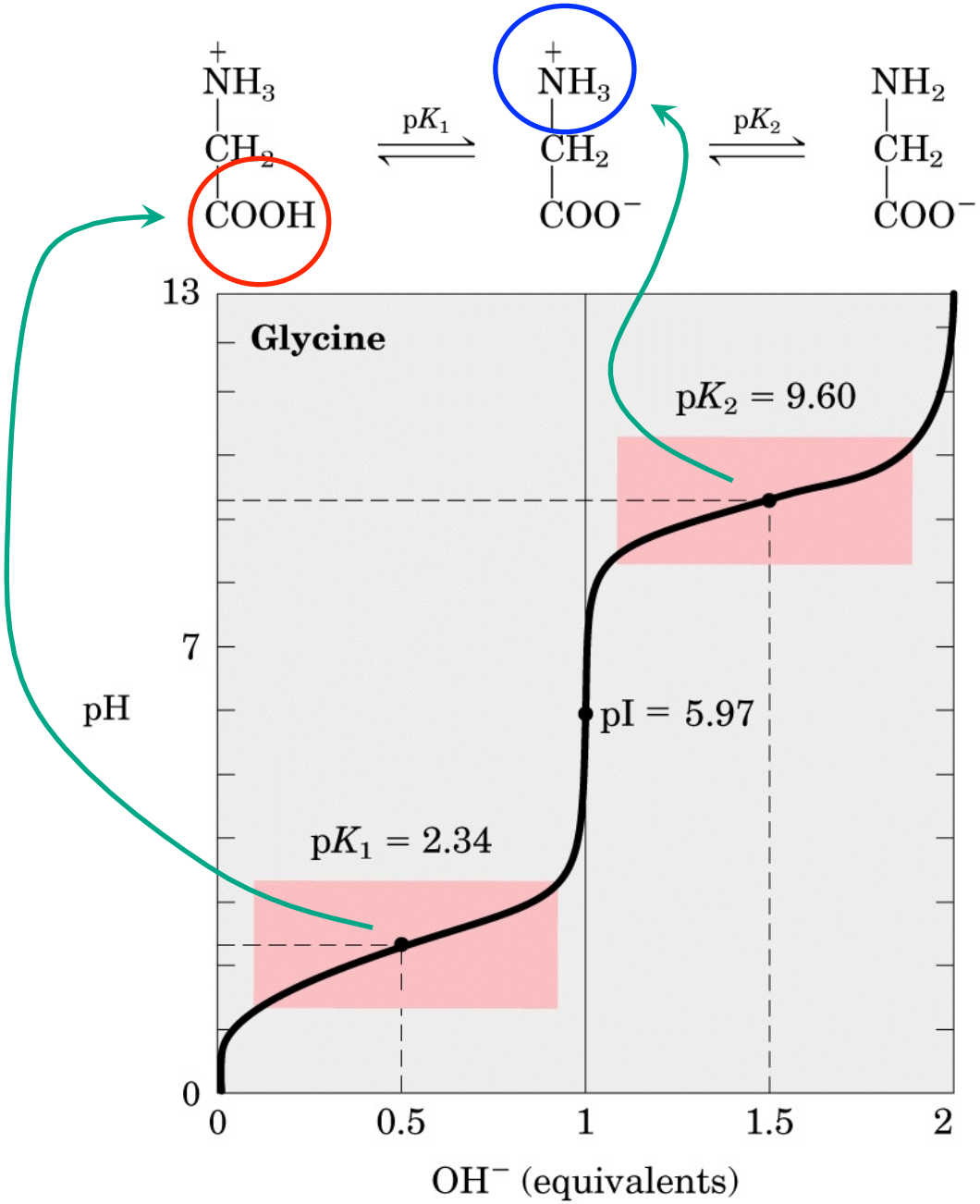
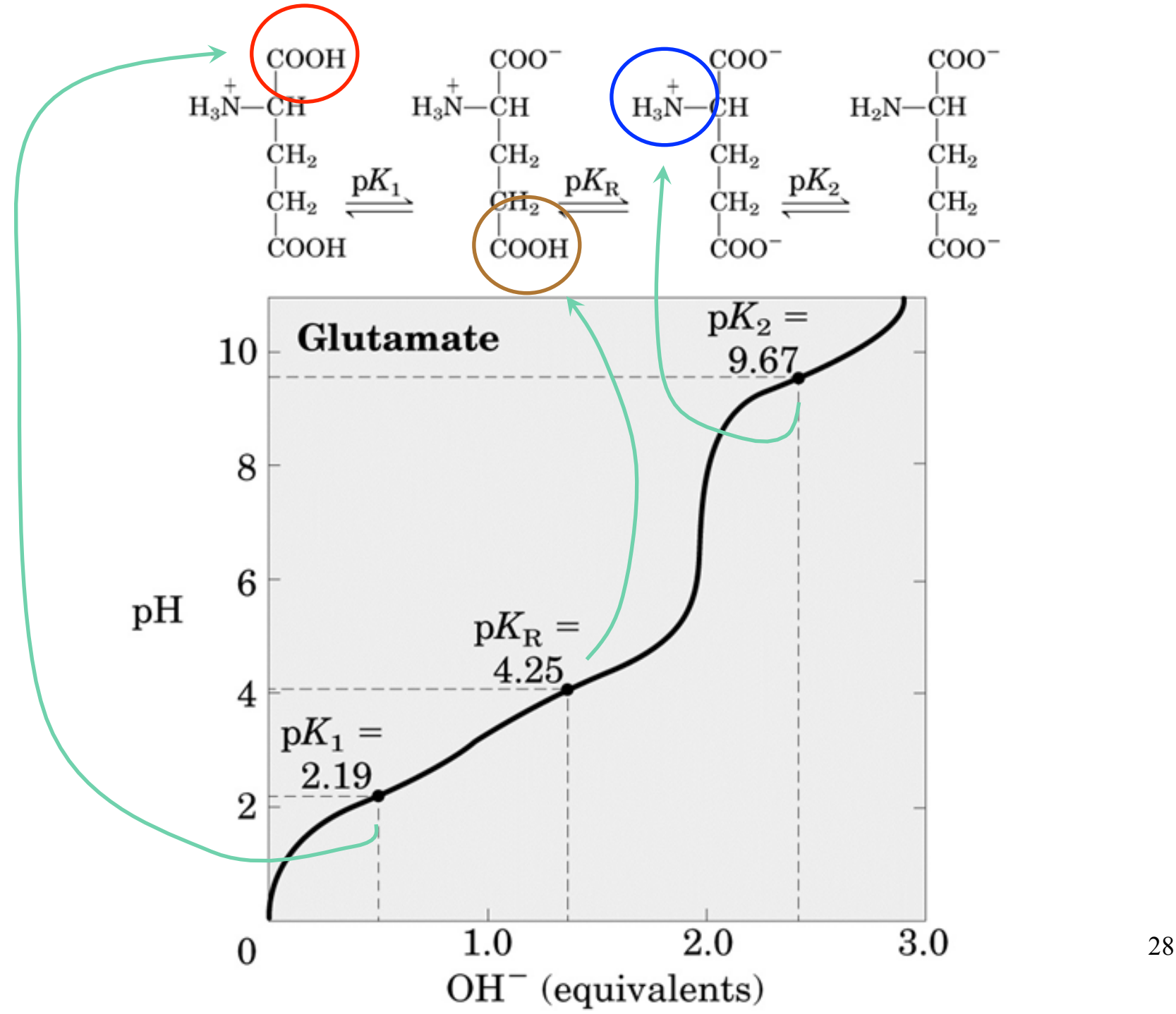
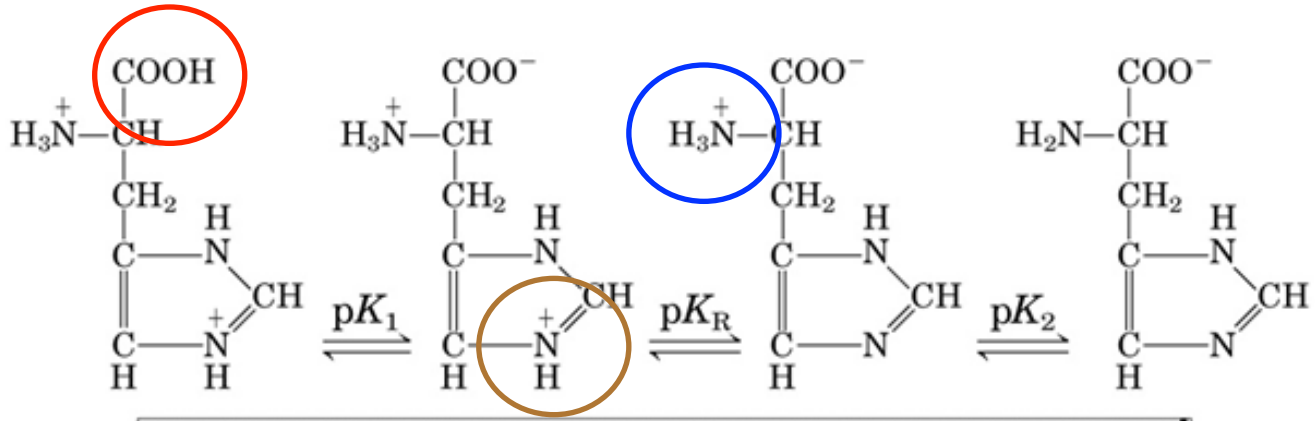


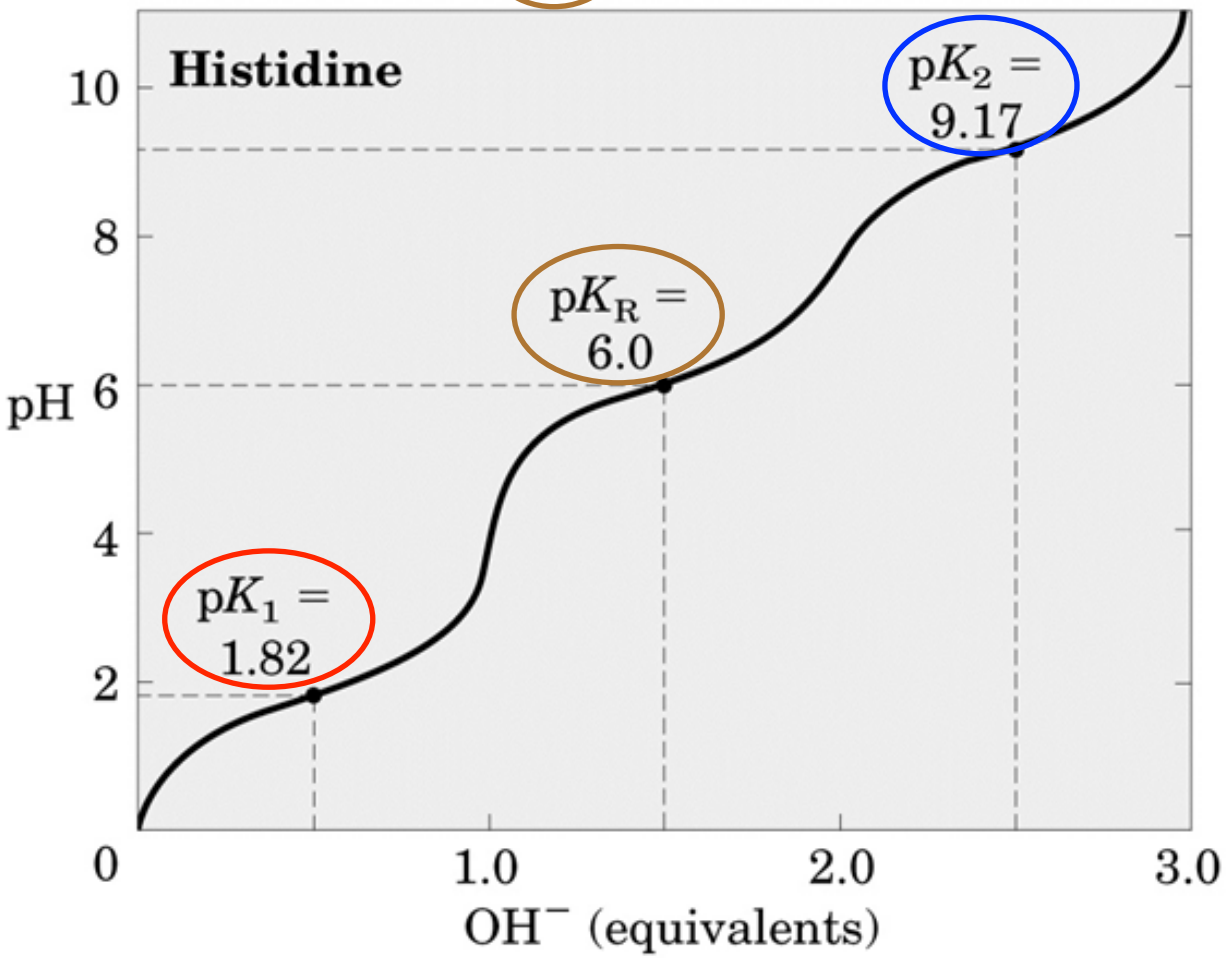

pKa
2
4
6
8
10
12
Methyl-substituted carboxyl and amino groups
CH3—COOH
H+
H+
CH3—COO−
CH3—NH3+
H+
H+
CH3—NH2
Acetic acid
The normal pKa for a carboxyl group is about 4.8.
Methylamine
The normal pKa for an amino group is about 10.6.
Carboxyl and amino groups in glycine
NH3+
H—C—COOH
H
H+
H+
NH3+
H—C—COO−
H
H+
H+
NH2
H—C—COO−
H
α-Amino acid (glycine)
pKa = 2.34
Repulsion between the amino group and the departing proton lowers the pKa for the carboxyl group, and oppositely charged groups lower the pKa by stabilizing the zwitterion.
α-Amino acid (glycine)
pKa = 9.60
Electronegative oxygen atoms in the carboxyl group pull electrons away from the amino group, lowering its pKa.

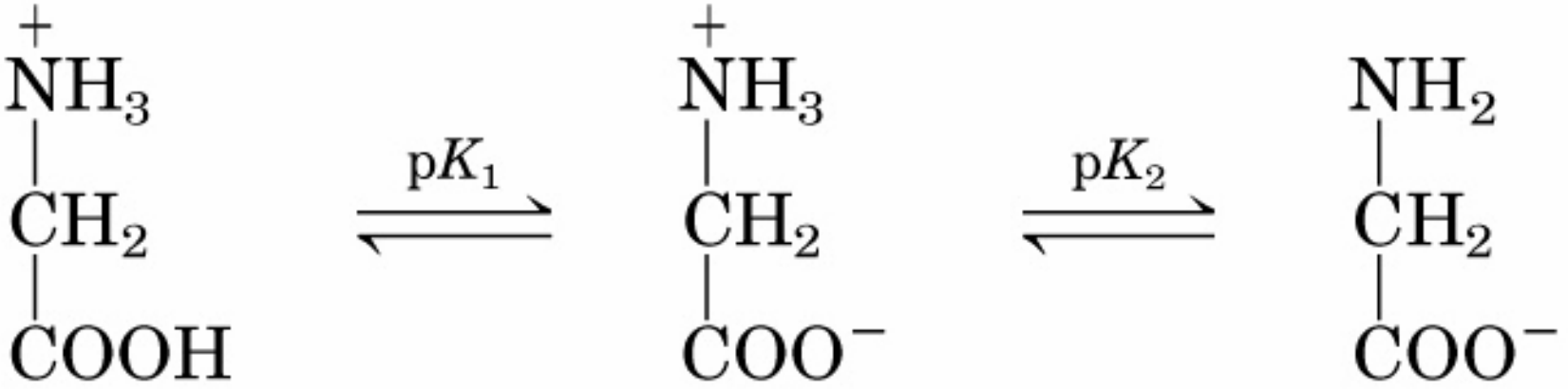
Amino acids have characteristic titration curves




COOH
H3N—CH
CH2
CH2
COOH
pK1
COO⁻
H3N—CH
CH2
CH2
COOH
pKR
COO⁻
H3N—CH
CH2
CH2
COO⁻
pK2
COO⁻
H2N—CH
CH2
CH2
COO⁻
Glutamate
pK2 = 9.67
pKR = 4.25
pK1 = 2.19
pH
10
8
6
4
2
0
1.0
2.0
3.0
OH⁻ (equivalents)


COOH
COO⁻
COO⁻
COO⁻
pK1
pKR
pK2


Histidine
pK2 = 9.17
pKR = 6.0
pK1 = 1.82
pH
10
8
6
4
2
0
1.0
2.0
3.0
OH⁻ (equivalents)



cationic species

charge = +1

zwitterionic species

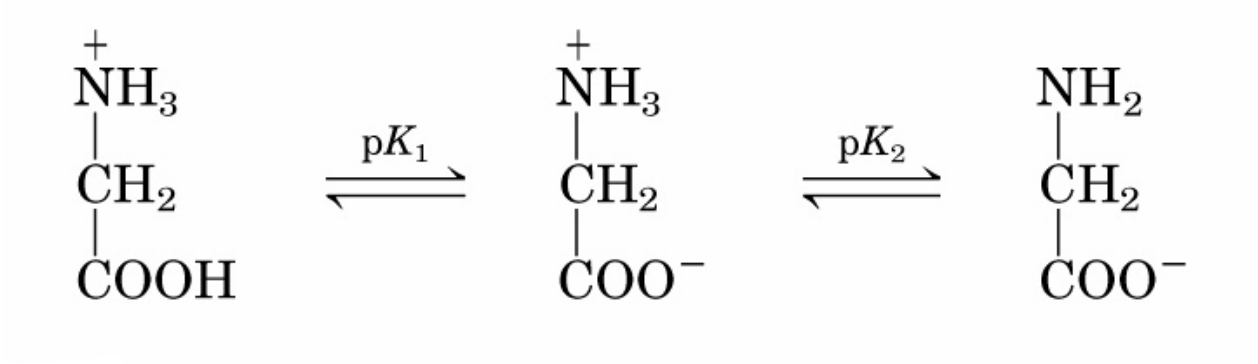
charge = 0

anionic species

charge = −1

The concentrations of these species are pH dependent

The degree of ionization of an amino acid is pH dependent



H_2A^+ $\quad$ HA $\quad$ A^-

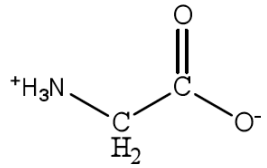
$$\begin{cases} K_1 = \dfrac{[HA][H^+]}{[H_2A^+]} \\ K_2 = \dfrac{[A^-][H^+]}{[HA]} \\ c = [H_2A^+] + [HA] + [A^-] \end{cases}$$

We wish to determine the concentration dependence of each species on pH

$$\begin{cases} [H_2A^+] = \dfrac{[H^+]}{K_1}[HA\ ] \\ [A^-] = \dfrac{K_2}{[H^+]}[HA\ ] \\ c = [H_2A^+] + [HA\ ] + [A^-] = \left(\dfrac{[H^+]}{K_1} + 1 + \dfrac{K_2}{[H^+]}\right)[HA\ ] = \left(\dfrac{[H^+]^2 + K_1[H^+] + K_1K_2}{K_1[H^+]}\right)[HA\ ] \end{cases}$$

$$\begin{cases} [HA\ ] = \dfrac{K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2}c & \text{zwitterionic species} \\ [H_2A^+] = \dfrac{[H^+]^2}{[H^+]^2 + K_1[H^+] + K_1K_2}c & \text{cationic species} \\ [A^-] = \dfrac{K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2}c & \text{anionic species} \end{cases}$$

The degree of ionization of an amino acid is pH dependent

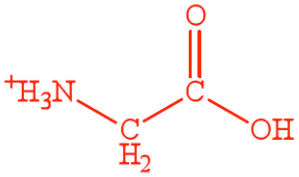


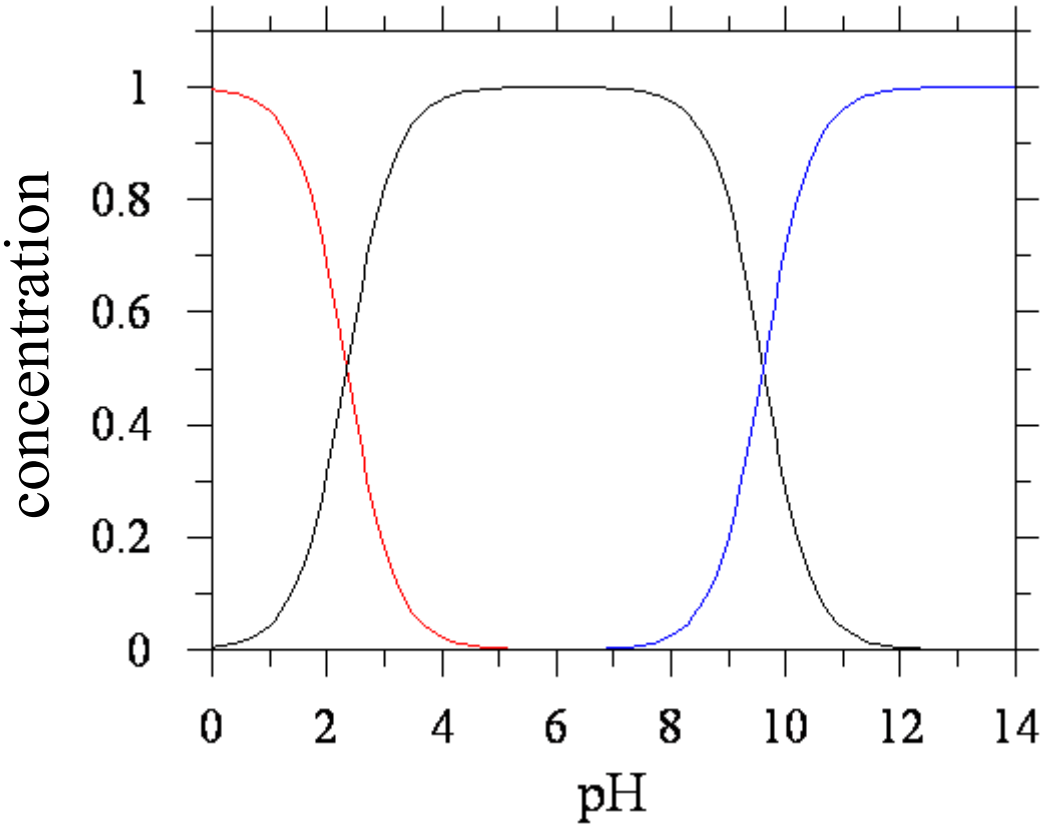
$$[HA] = \frac{K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2}c$$

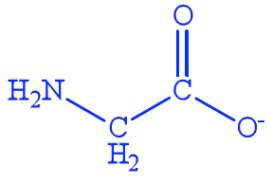
zwitterion

$$[H_2A^+] = \frac{[H^+]^2}{[H^+]^2 + K_1[H^+] + K_1K_2}c$$

$$[A^-] = \frac{K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2}c$$



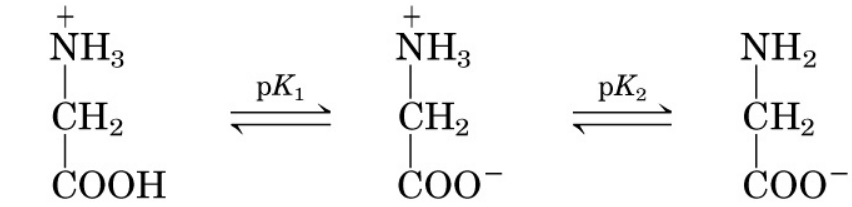


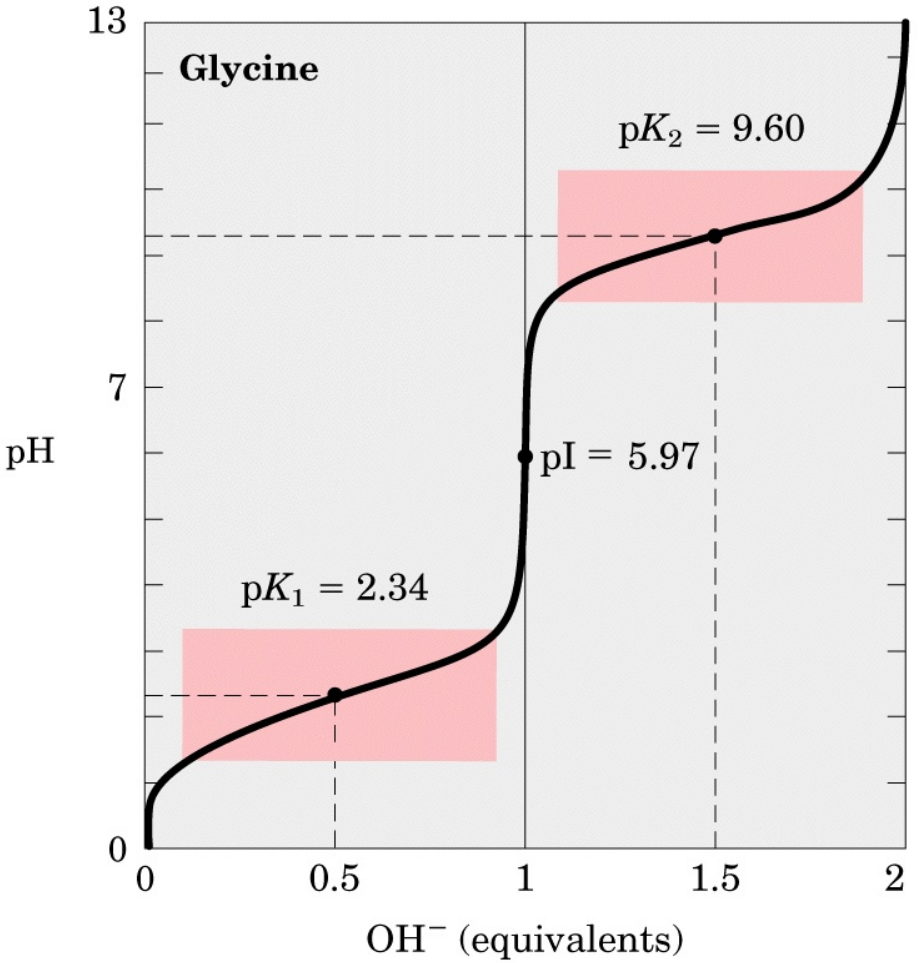


pK_1=2.34 pK_2=9.60

	pK_{COOH}	pK_{NH3+}	pK_R	pI
GLY	2.3	9.6	-	6.0
ALA	2.3	9.7	-	6.0
VAL	2.3	9.6	-	6.0
PRO	2.0	10.6	-	6.3
LEU	2.4	9.7	-	6.0
MET	2.3	9.2	-	5.8
ILE	2.4	9.7	-	6.1
PHE	1.8	9.1	-	5.5
TYR	2.2	9.1	10.1	5.7
TRP	2.4	9.4	-	5.9
SER	2.2	9.2	-	5.7
THR	2.6	10.4	-	6.5
CYS	1.8	10.8	8.3	5.0
ASN	2.0	8.8	-	5.4
GLN	2.2	9.1	-	5.7
LYS	2.2	9.0	10.5	9.8
ARG	2.2	9.0	12.5	10.8
HIS	1.8	9.2	6.0	7.6
ASP	2.1	9.8	3.9	3.0
GLU	2.2	9.7	4.3	3.2

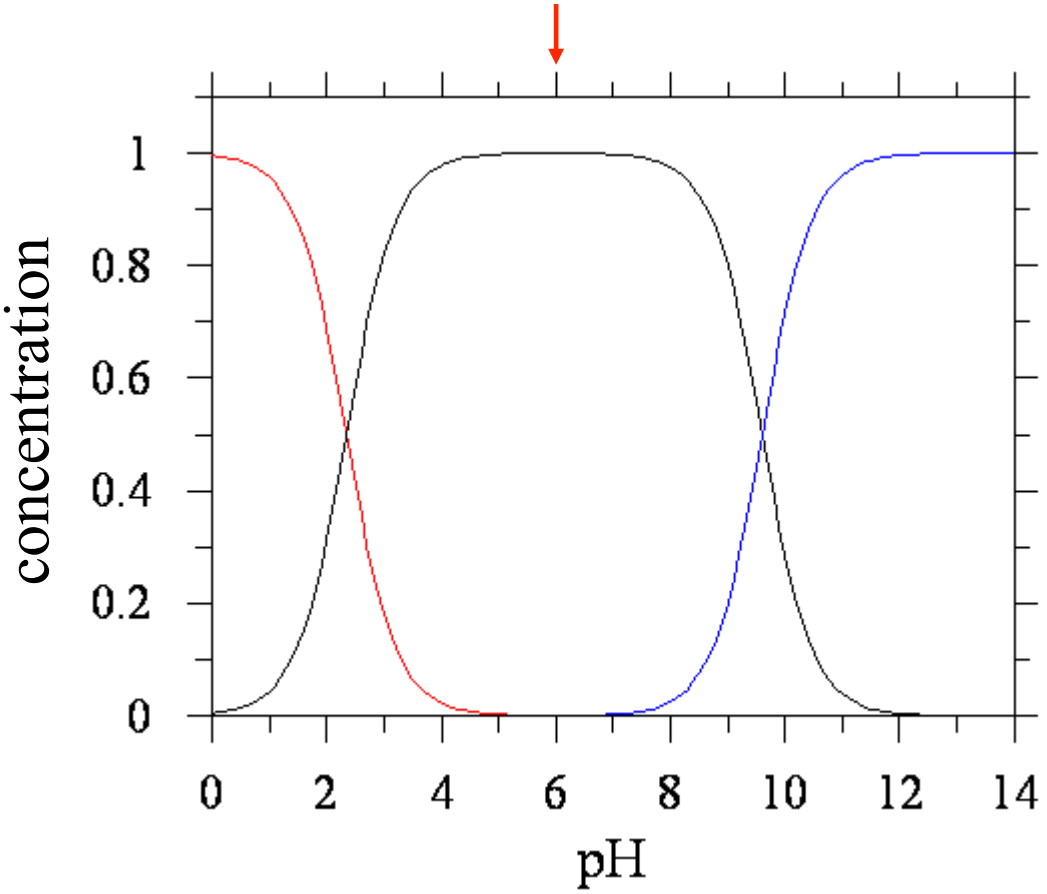
pI: the isoelectric pH (or point)





The isoelectric pH (or point)

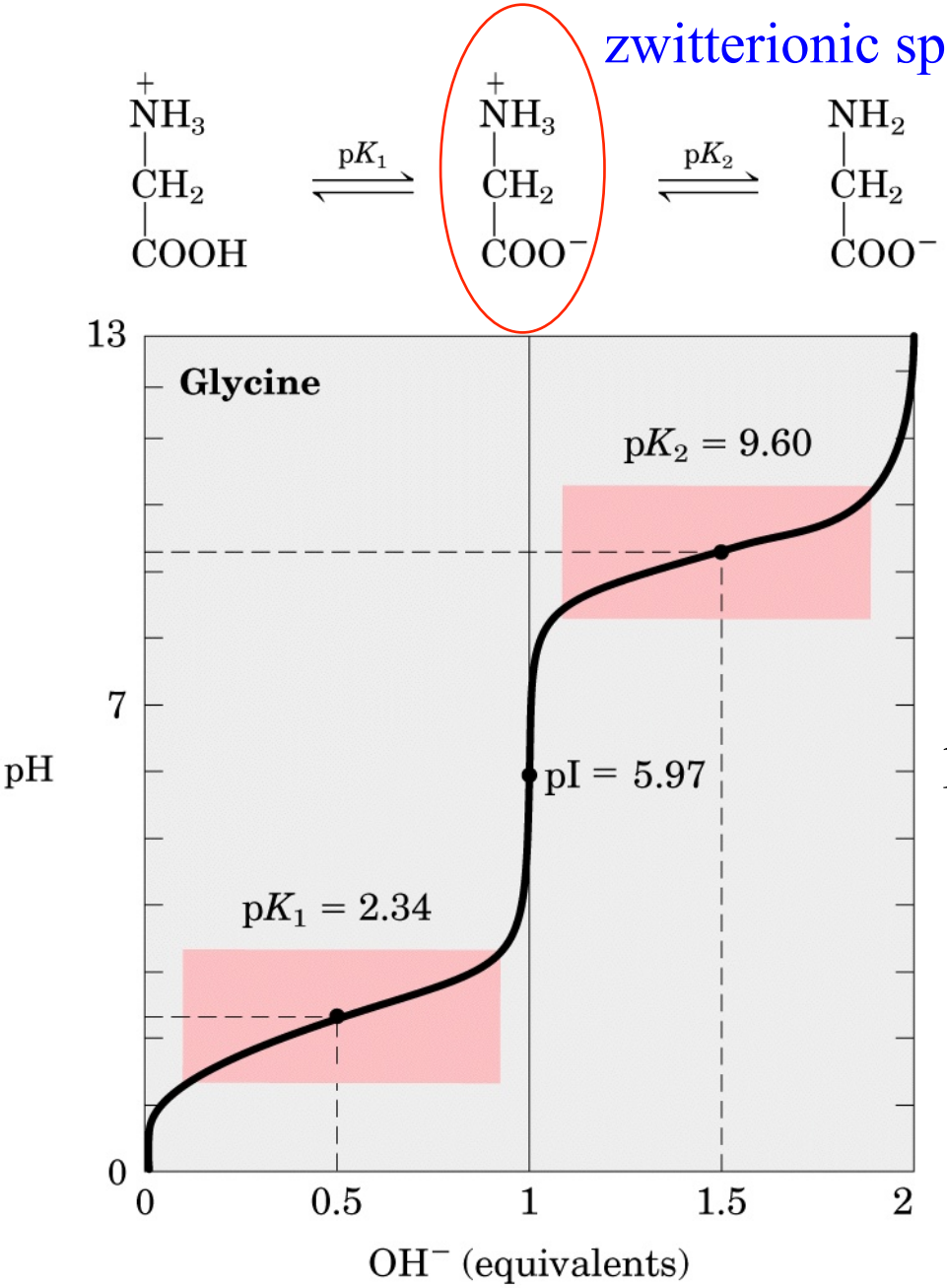
The isoelectric pH is that pH value at which the concentration of the zwitterionic species is maximal



pH < pI → cation
pH = pI → zwitterion
pH > pI → anion

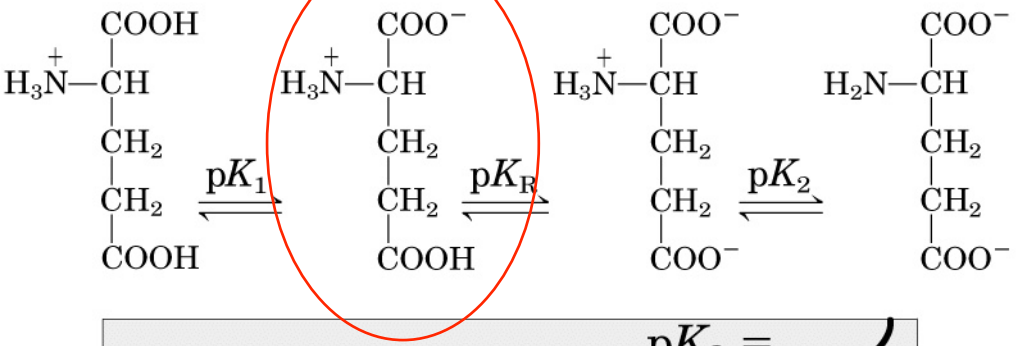
$$pI = \frac{pK_1 + pK_2}{2}$$

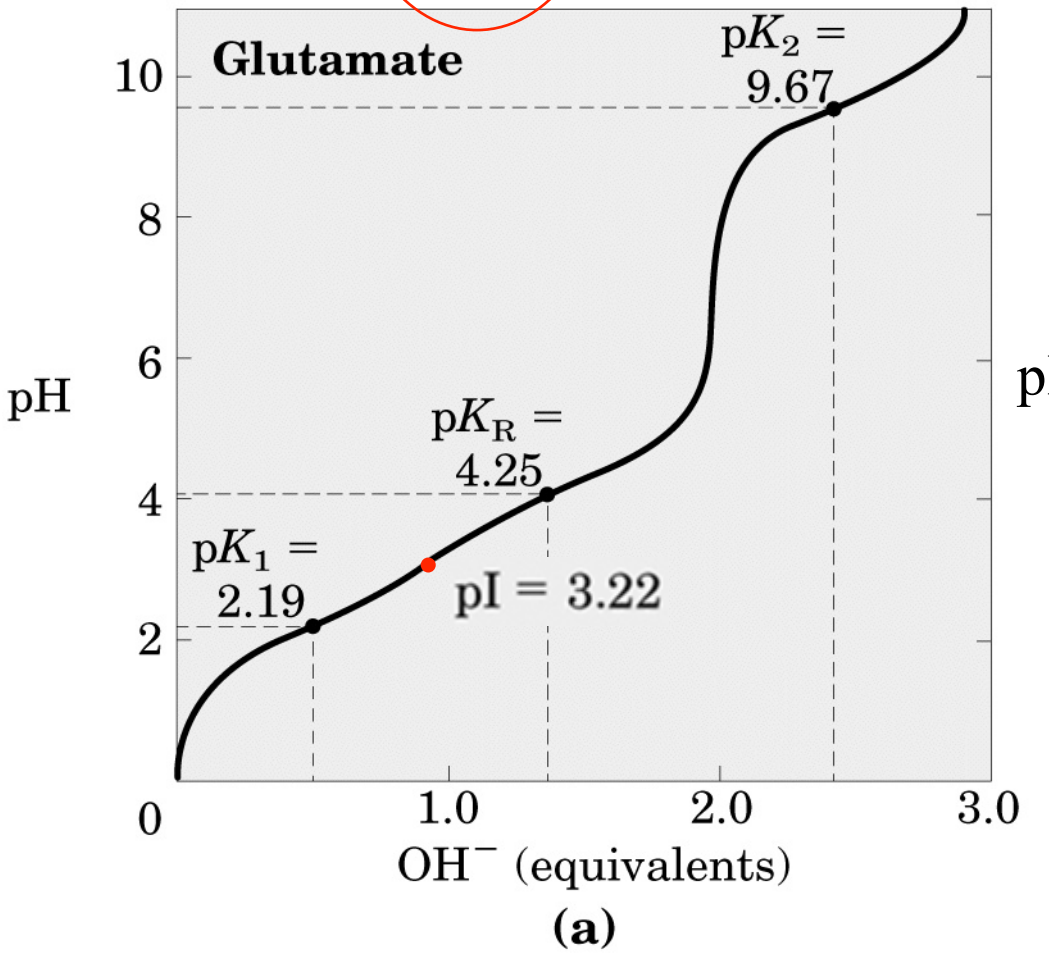
zwitterionic species



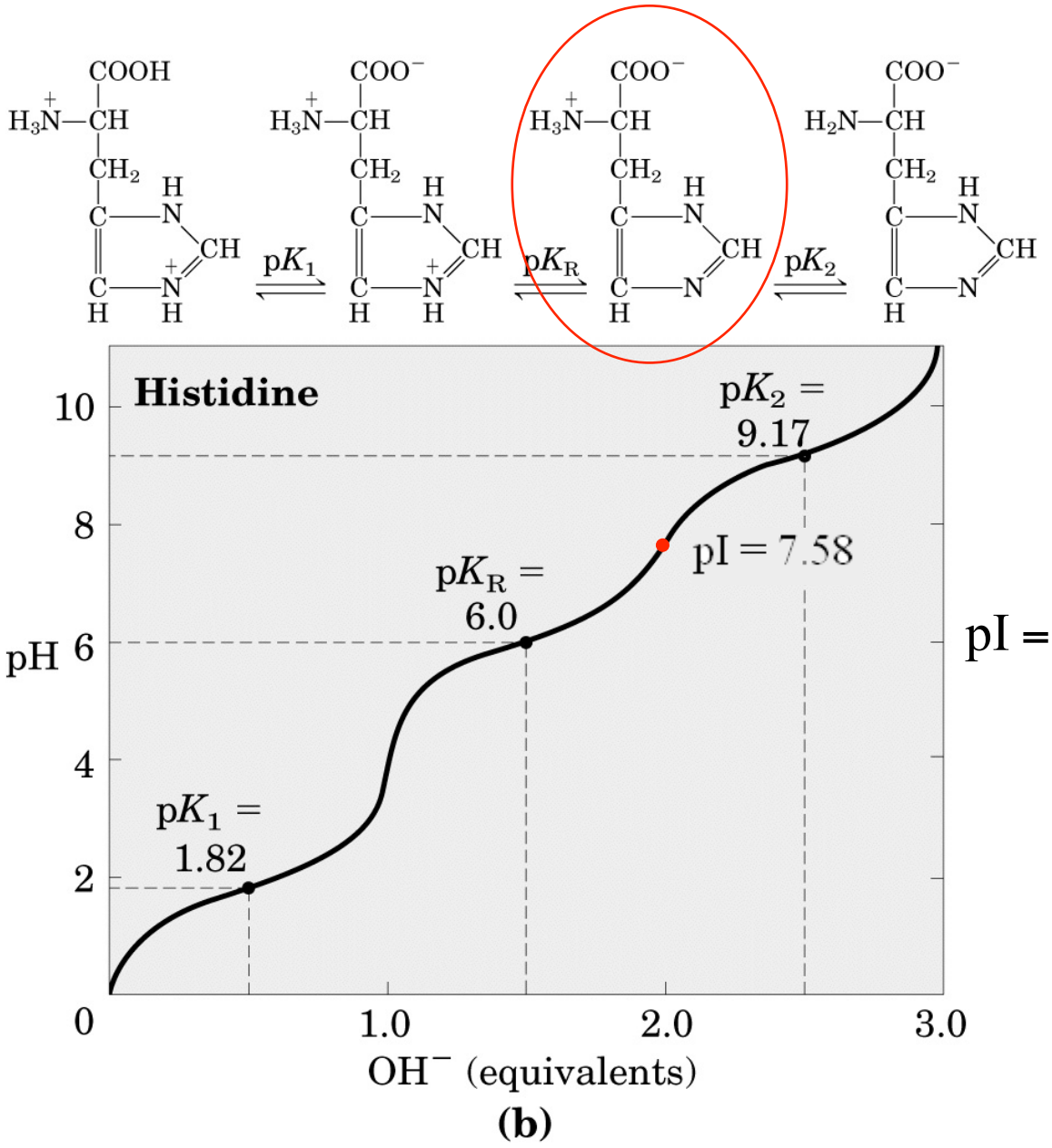
$$pI = \frac{pK_1 + pK_2}{2} = \frac{2.34 + 9.60}{2} = 5.97$$

zwitterionic species





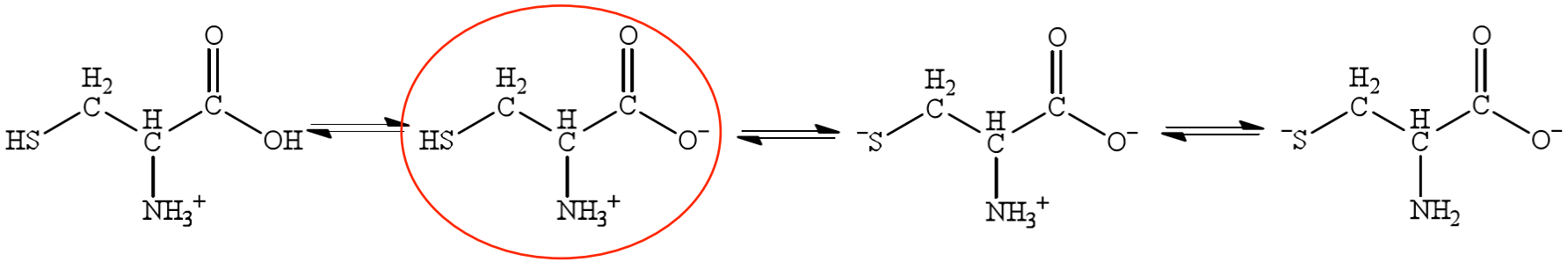
$$pI = \frac{pK_1 + pK_R}{2} = \frac{2.19 + 4.25}{2} = 3.22$$



$$pI = \frac{pK_R + pK_2}{2} = \frac{6.0 + 9.17}{2} = 7.58$$

Calculate the pI of cysteine

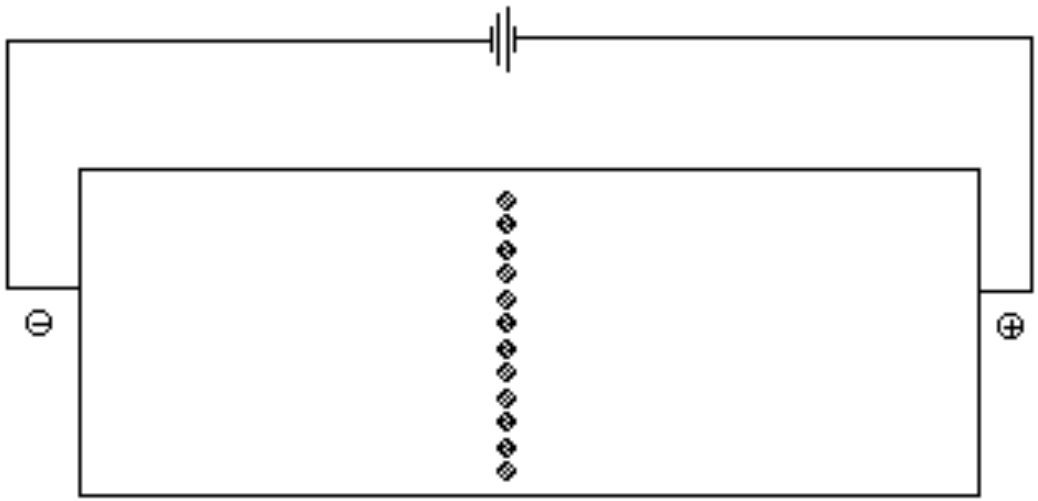
$pK_{COOH} = 1.96$ $pK_{SH} = 8.18$ $pK_{NH3+} = 10.28$



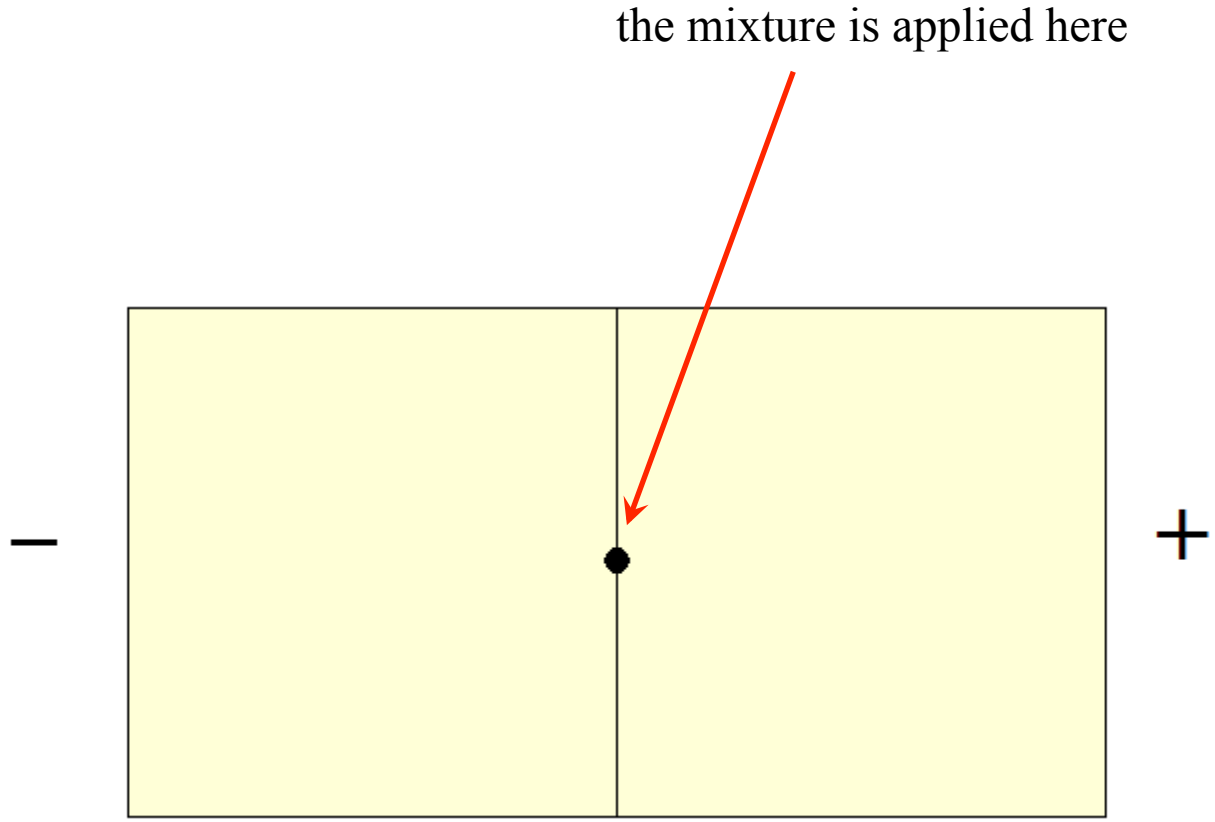
1+ 0 1- 2-

$$pI = \frac{pK_{COOH} + pK_{SH}}{2} = \frac{1.96 + 8.18}{2} = 5.07$$

Electrophoresis



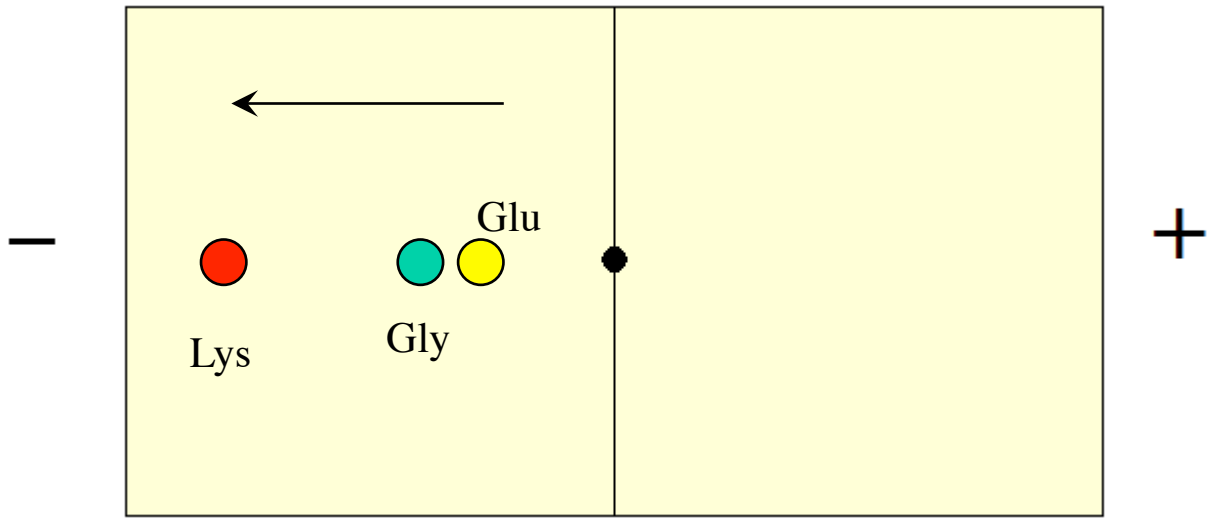
We have a mixture of Gly (pI=5.97), Lys (pI=9.74) and Glu (pI=3.22). If the electrophoresis is carried out at pH 1, 6 and 11, is it possible to separate the mixture?



We have a mixture of Gly (pI=5.97), Lys (pI=9.74) and Glu (pI=3.22). If the electrophoresis is carried out at pH 1, 6 and 11, is it possible to separate the mixture?

pH=1

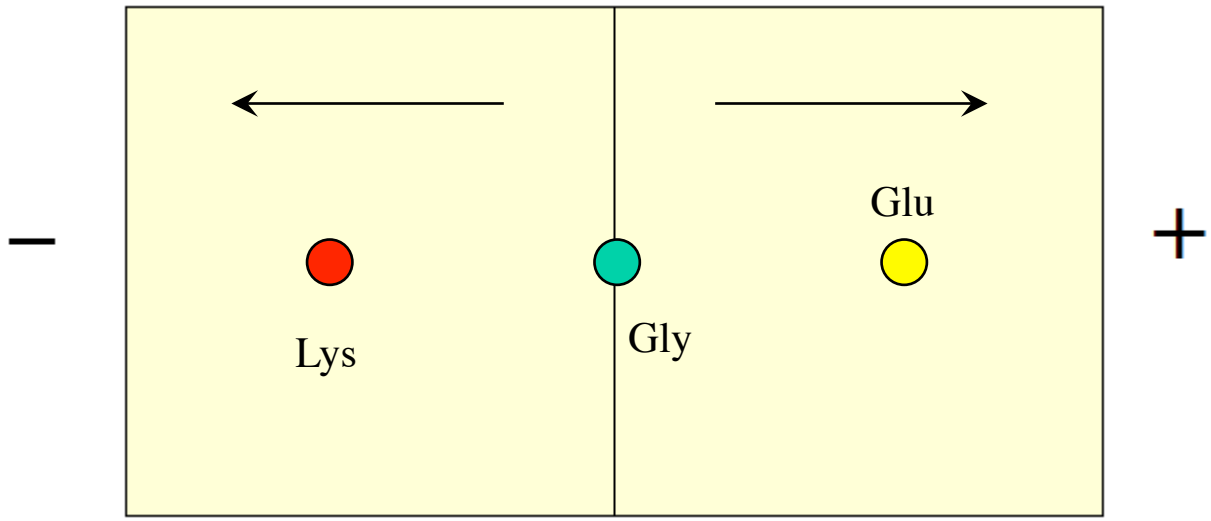
In each case pH < pI and the amino acids will be in cationic form



We have a mixture of Gly (pI=5.97), Lys (pI=9.74) and Glu (pI=3.22). If the electrophoresis is carried out at pH 1, 6 and 11, is it possible to separate the mixture?

pH=6

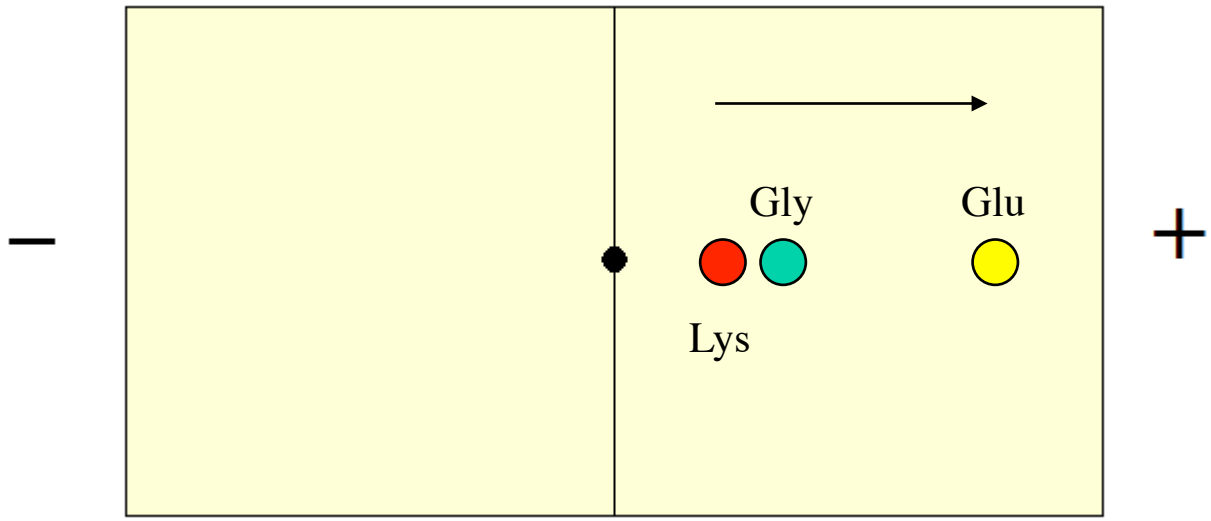
Gly doesn't move (pH = pI), Glu is anionic (pH > pI) and Lys is cationic (pH < pI)



We have a mixture of Gly (pI=5.97), Lys (pI=9.74) and Glu (pI=3.22). If the electrophoresis is carried out at pH 1, 6 and 11, is it possible to separate the mixture?

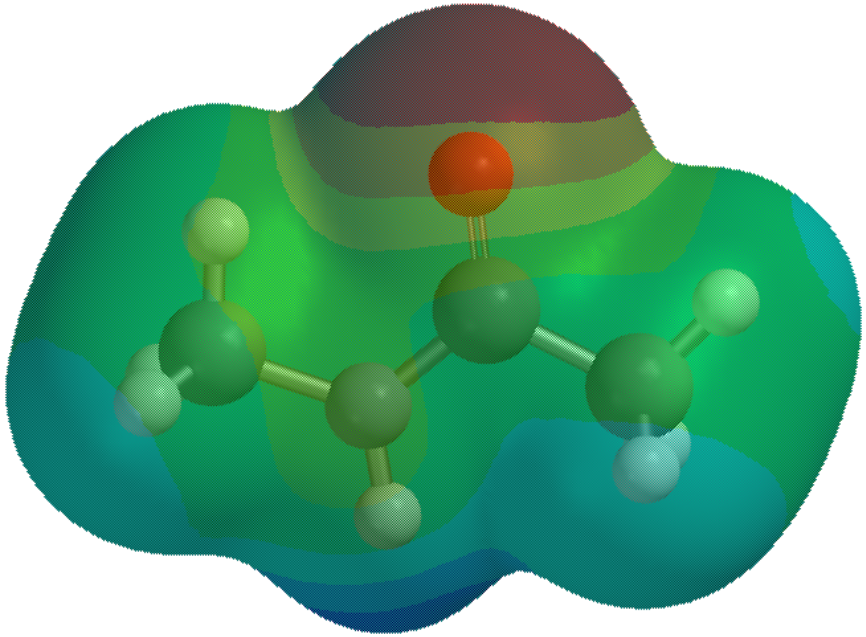
pH=11

In each case pH > pI and the amino acid will be in anionic form



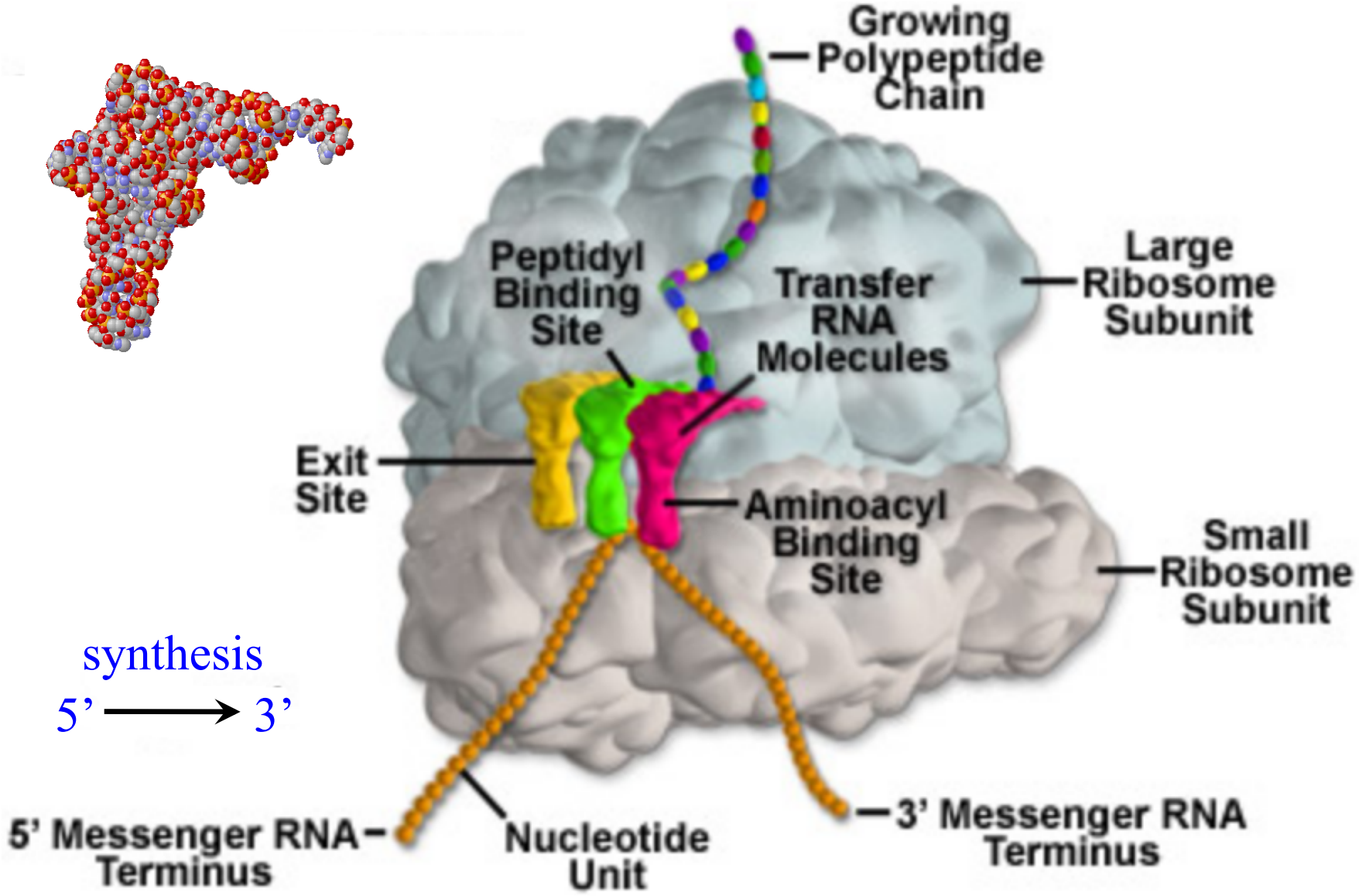
SAPIENZA
UNIVERSITÀ DI ROMA

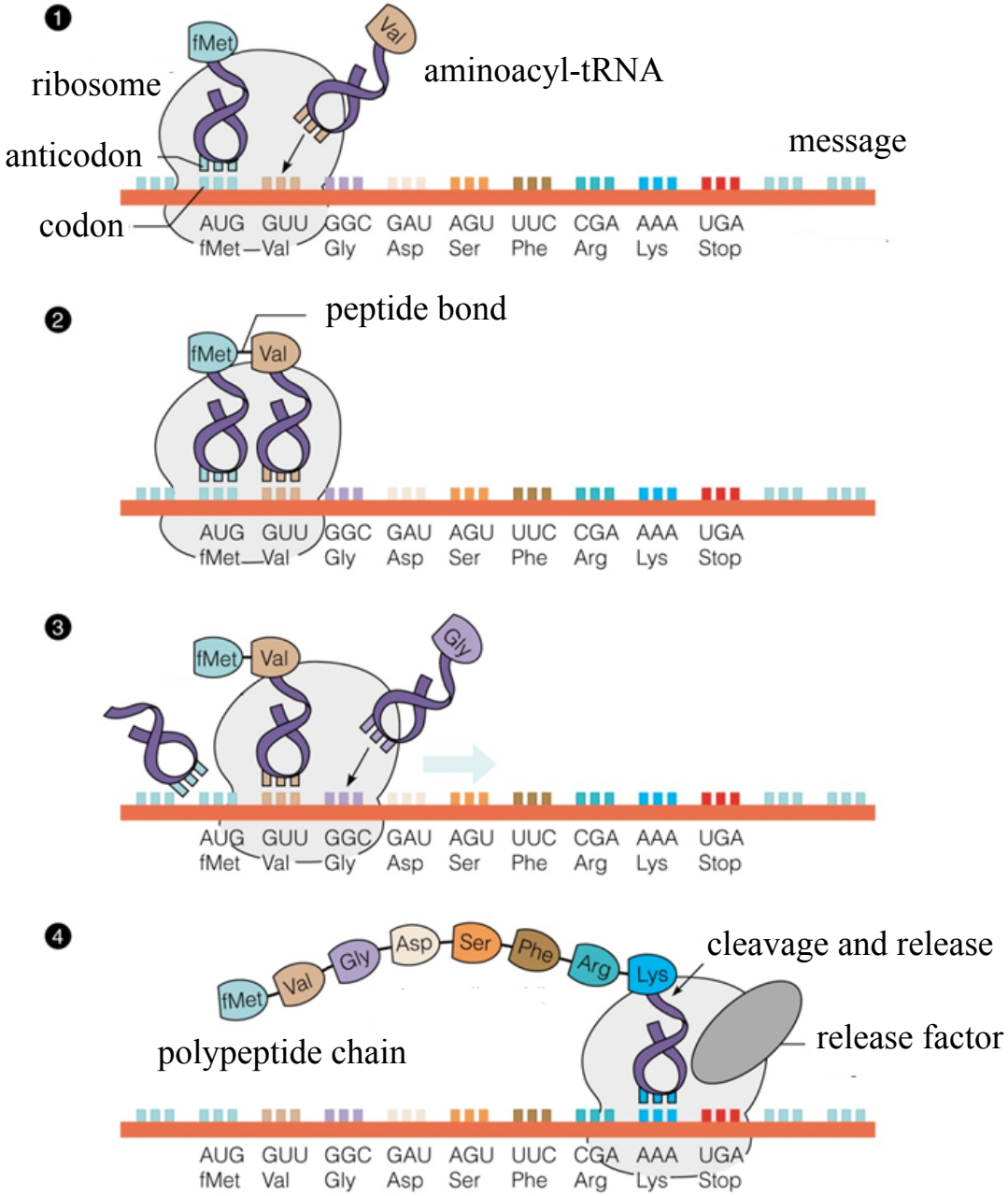
The peptide bond, peptides & proteins

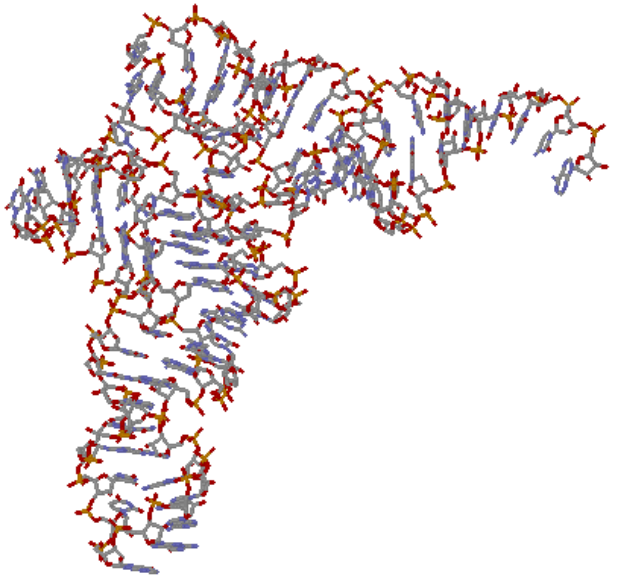


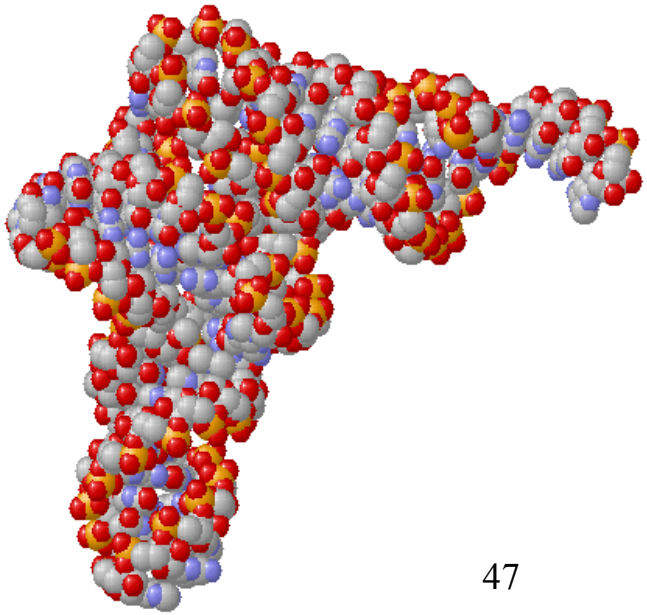
Prof. Francesco Malatesta

Protein synthesis takes place on the ribosome

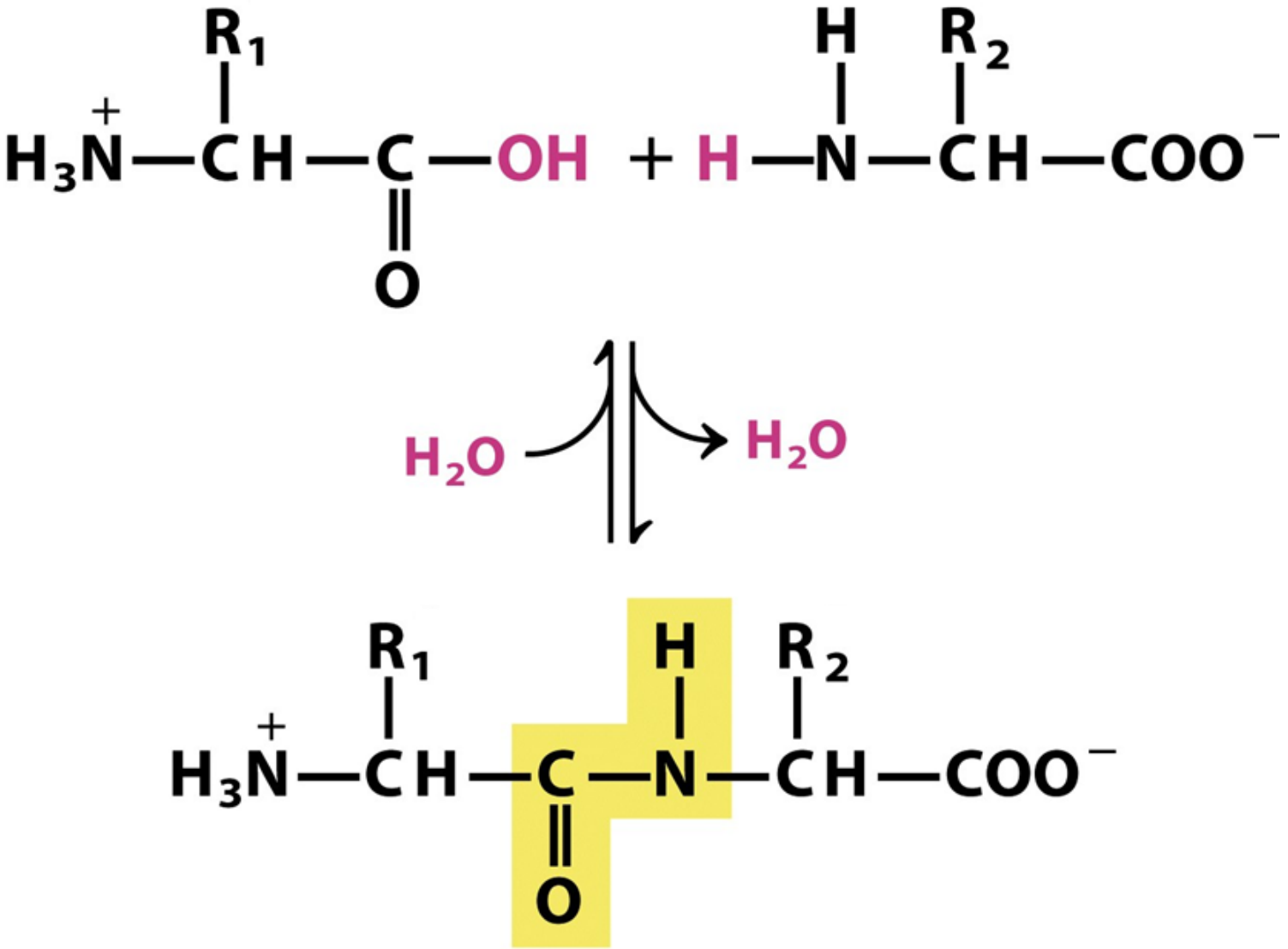



1
ribosome
aminoacyl-tRNA
anticodon
message
codon
fMet
Val
AUG GUU GGC GAU AGU UUC CGA AAA UGA
fMet Val Gly Asp Ser Phe Arg Lys Stop
2
peptide bond
3
Gly
4
Asp Ser Phe Arg Lys
cleavage and release
release factor
polypeptide chain





The peptide bond



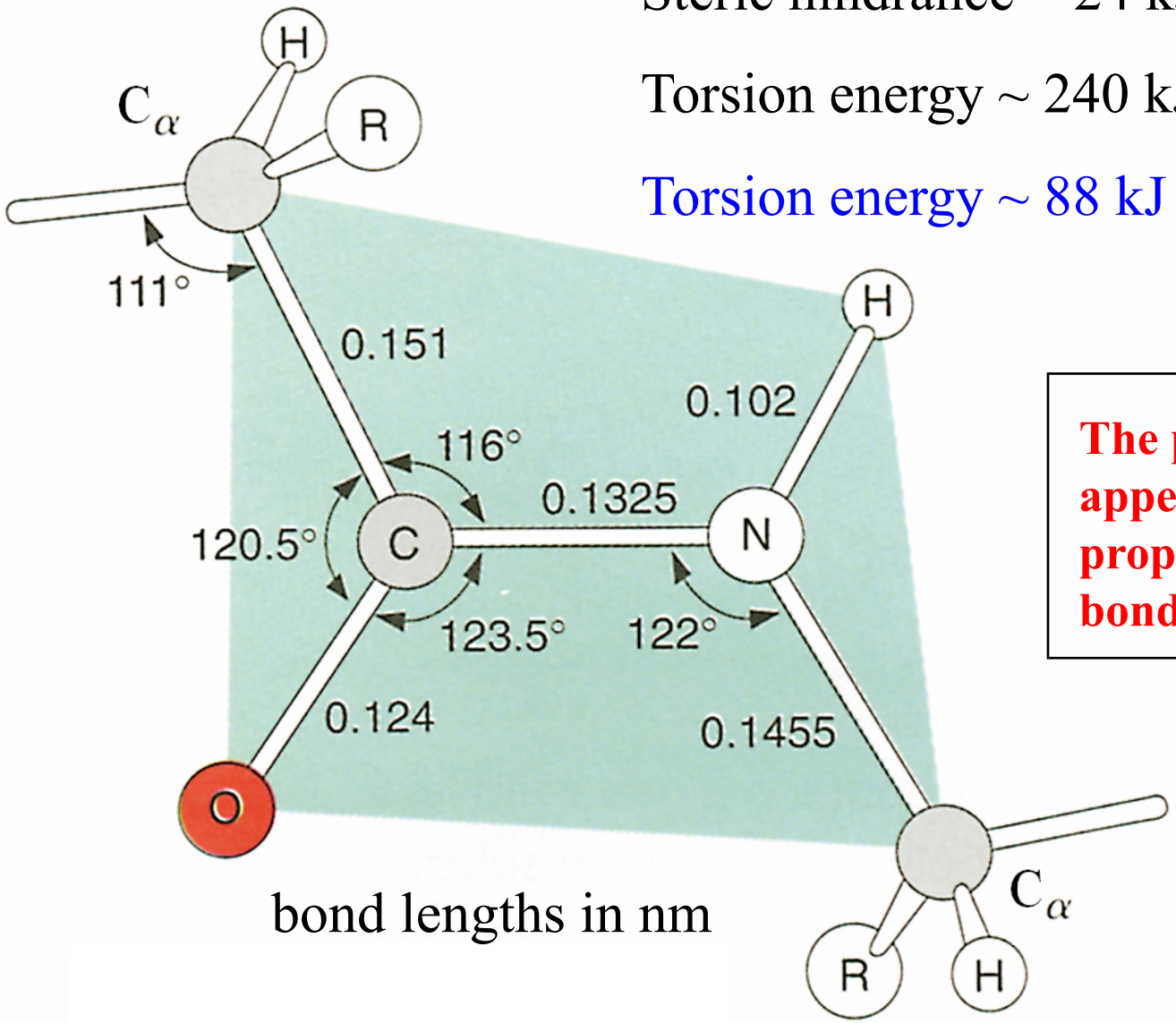
The peptide bond

Torsion energy ~ 12 kJ mol^{-1} (ethane)

Steric hindrance ~ 24 kJ mol^{-1} (butane)

Torsion energy ~ 240 kJ mol^{-1} (2-butene)

Torsion energy ~ 88 kJ mol^{-1} (peptide bond)

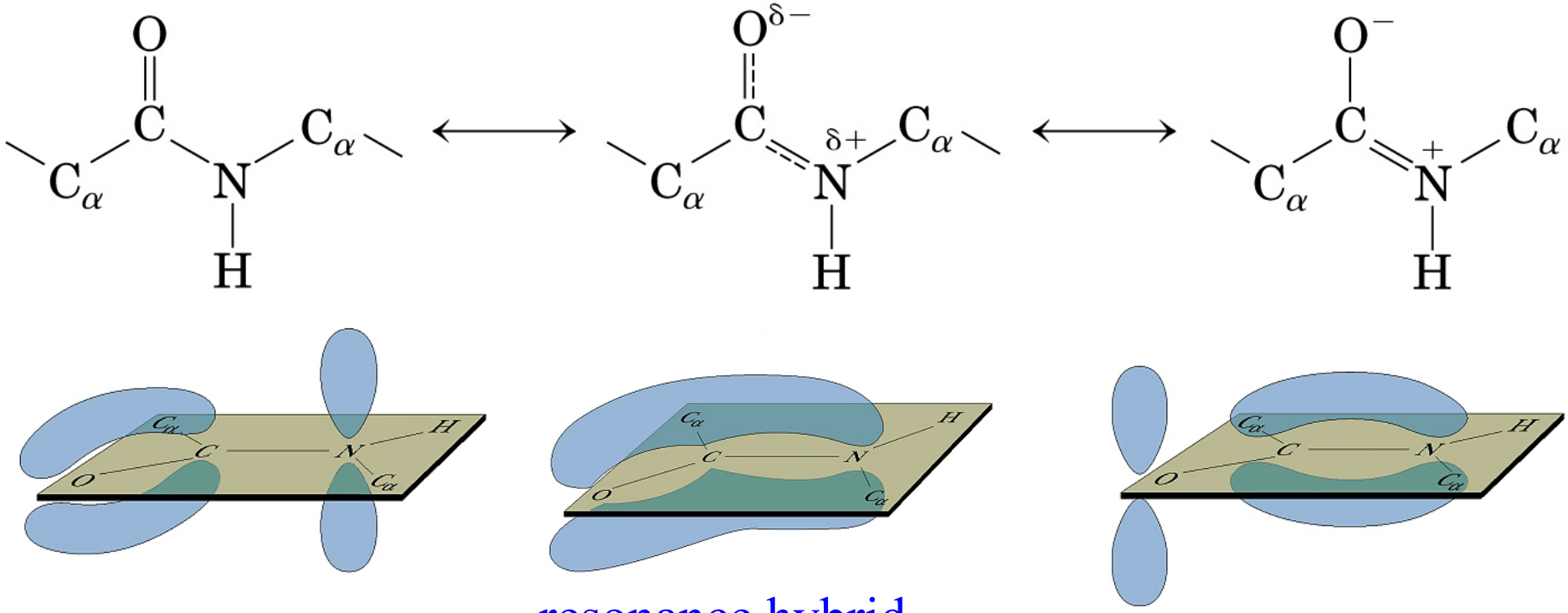


bond lengths in nm

The peptide bond appears not to have the properties of a single bond

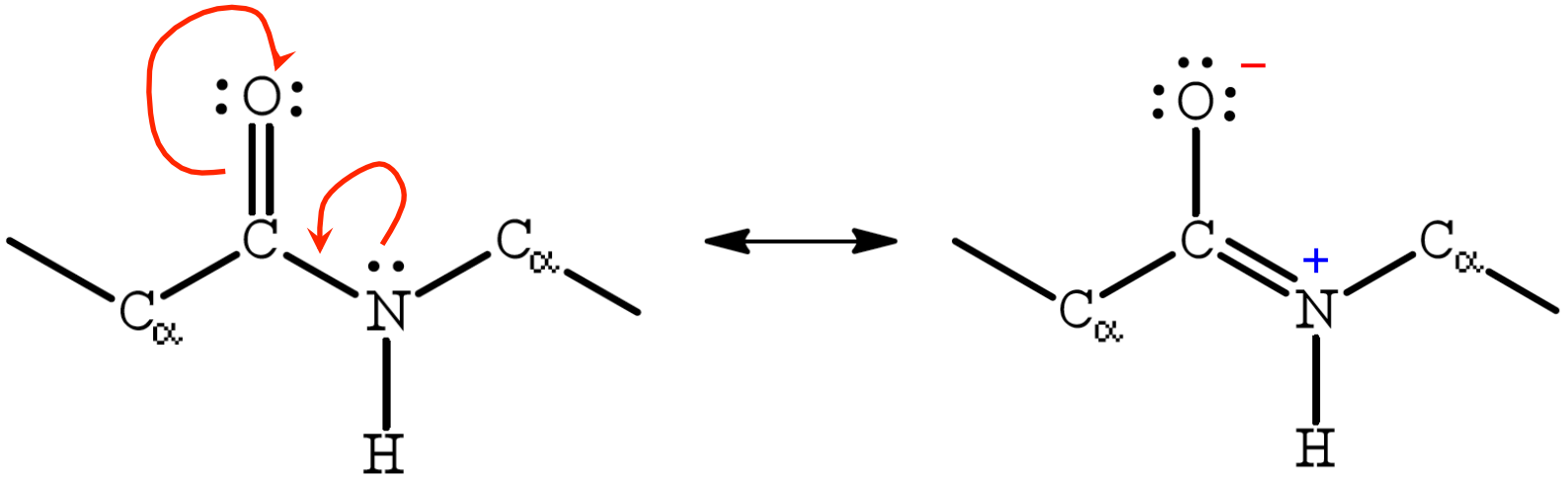
The peptide bond has partial double bond character.

The **carbonyl oxygen** bears a partial negative charge and the **amide nitrogen** a partial positive charge, generating an electric dipole in the **resonance hybrid**.

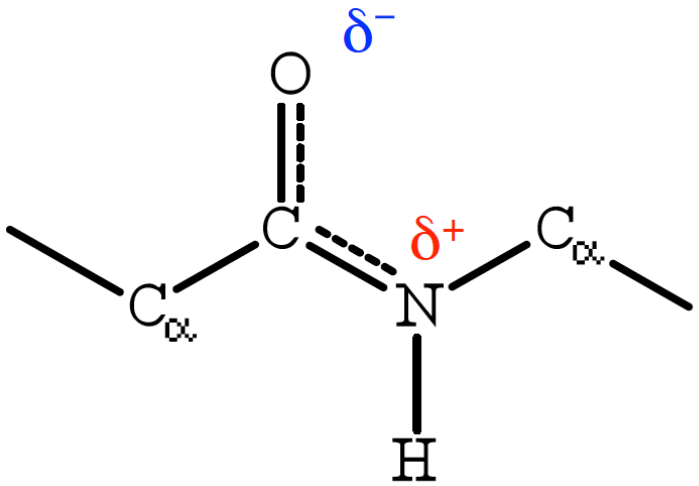


resonance hybrid

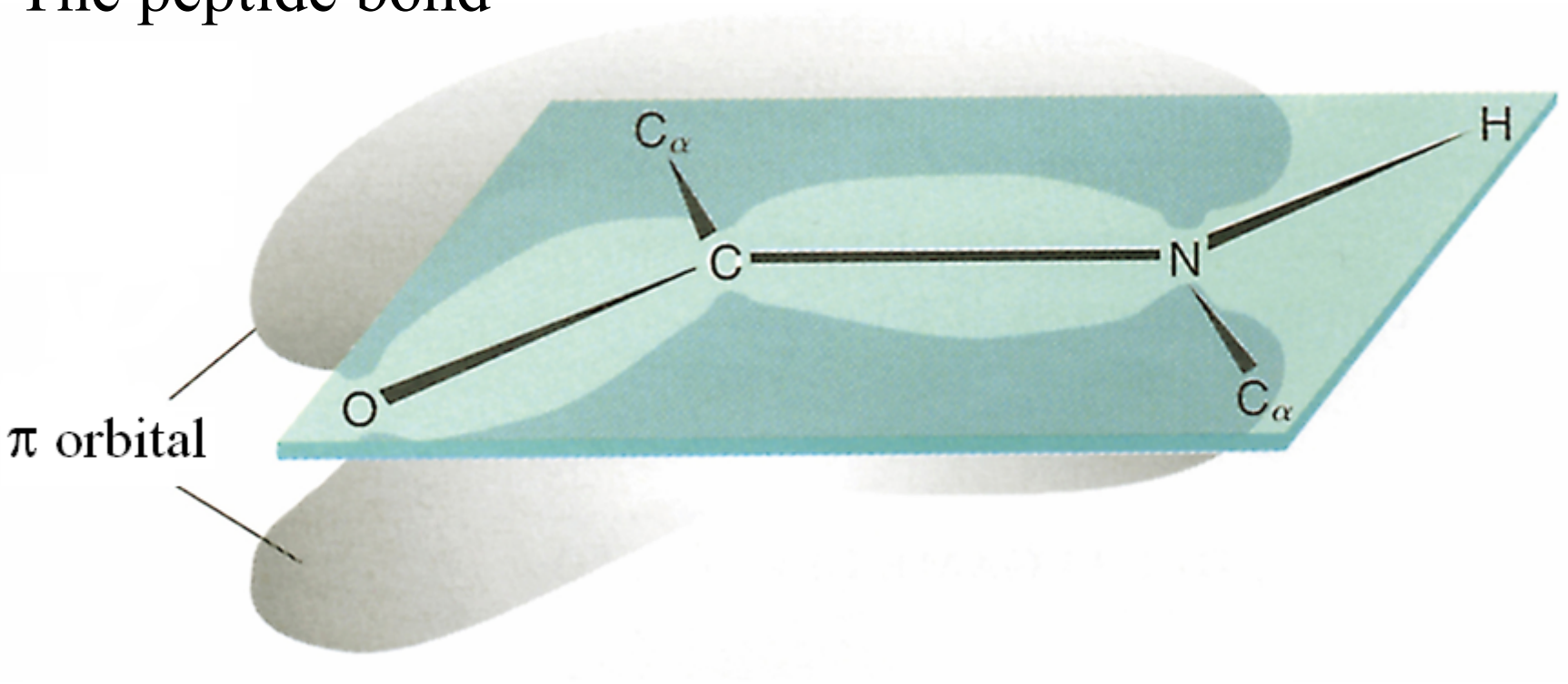
Resonance limit formulas in the peptide bond



resonance hybrid

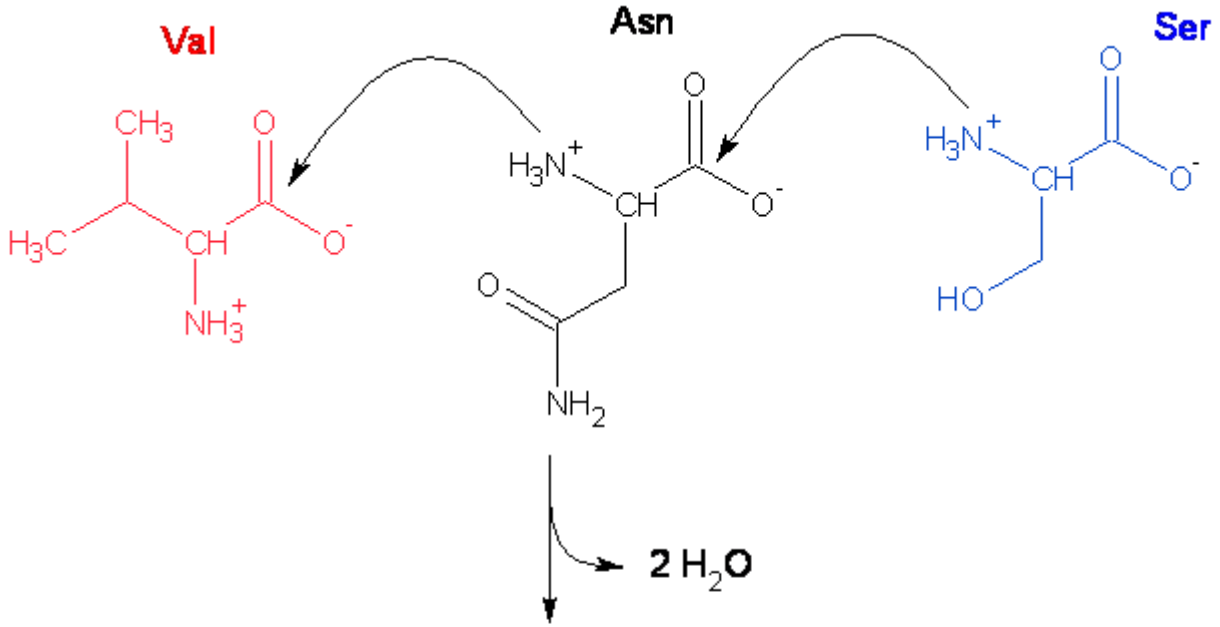


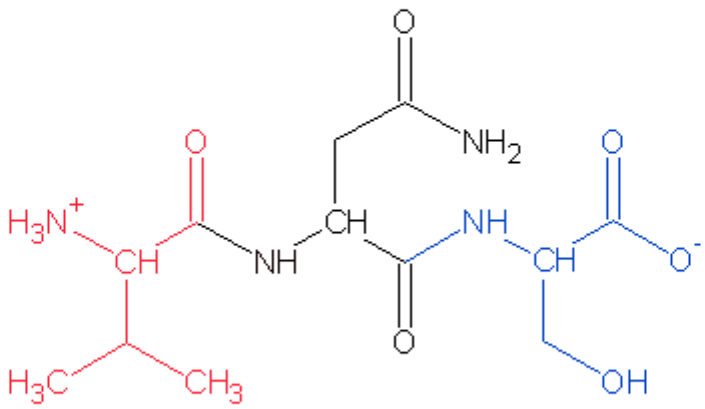
The peptide bond



- partial double bond character : ~ 40%
- 6 atoms are co-planar (nitrogen retains some piramidality)
- virtually all peptide bonds in proteins are present in the *trans* configuration.

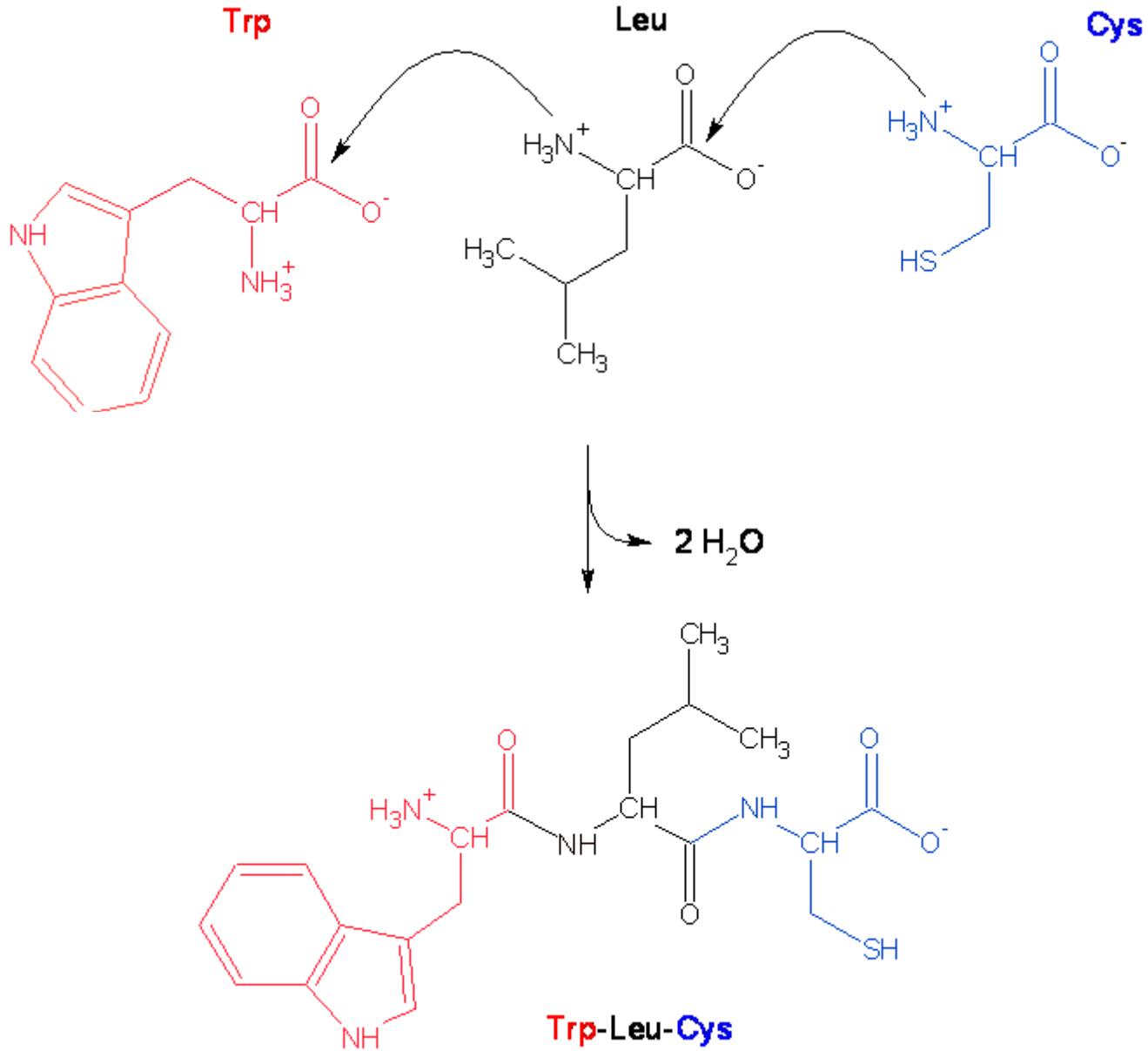
Write the structure of the following tripeptide: Val-Asn-Ser



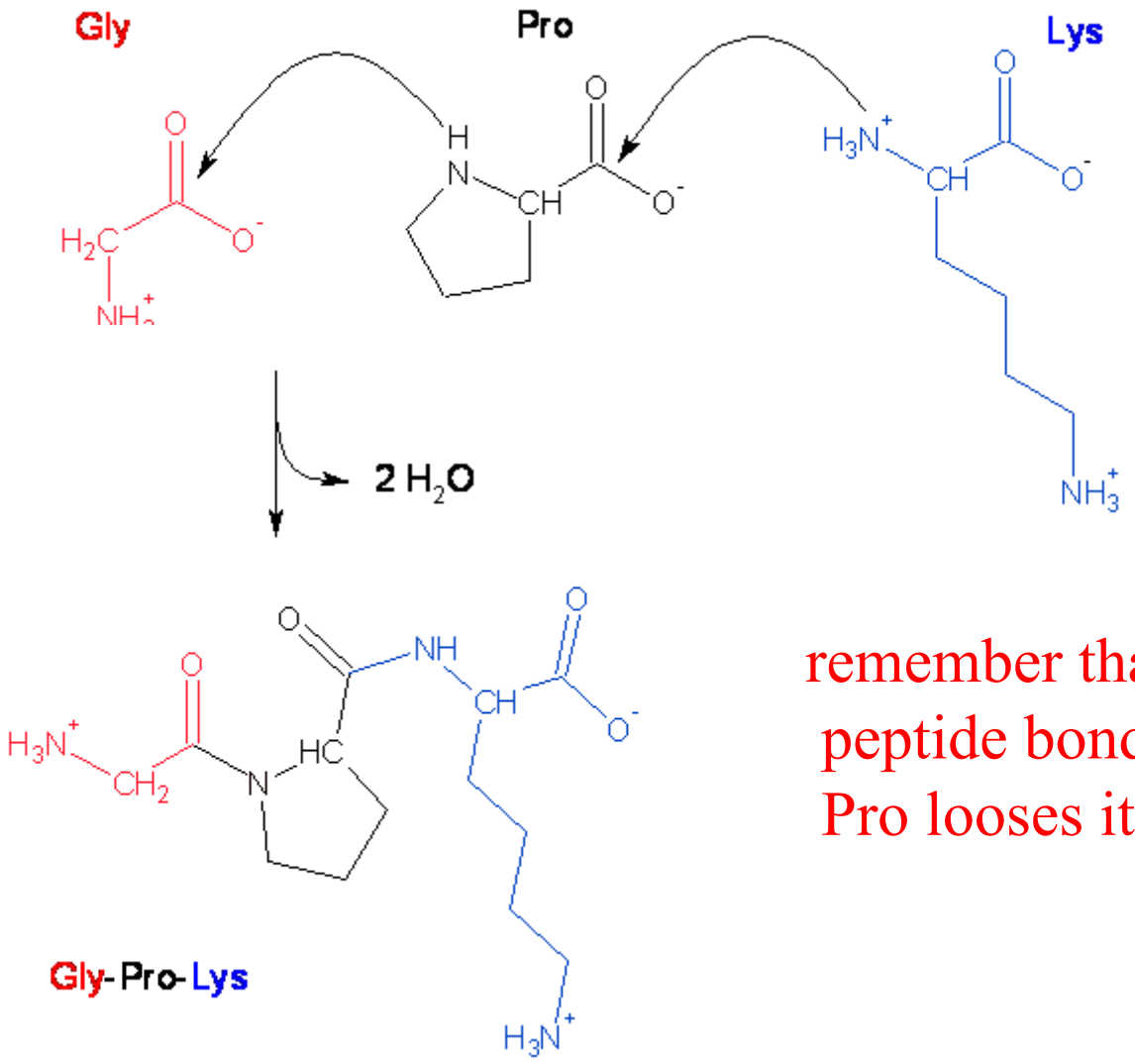


Val-Asn-Ser

Write the structure of the following tripeptide: Trp-Leu-Cys



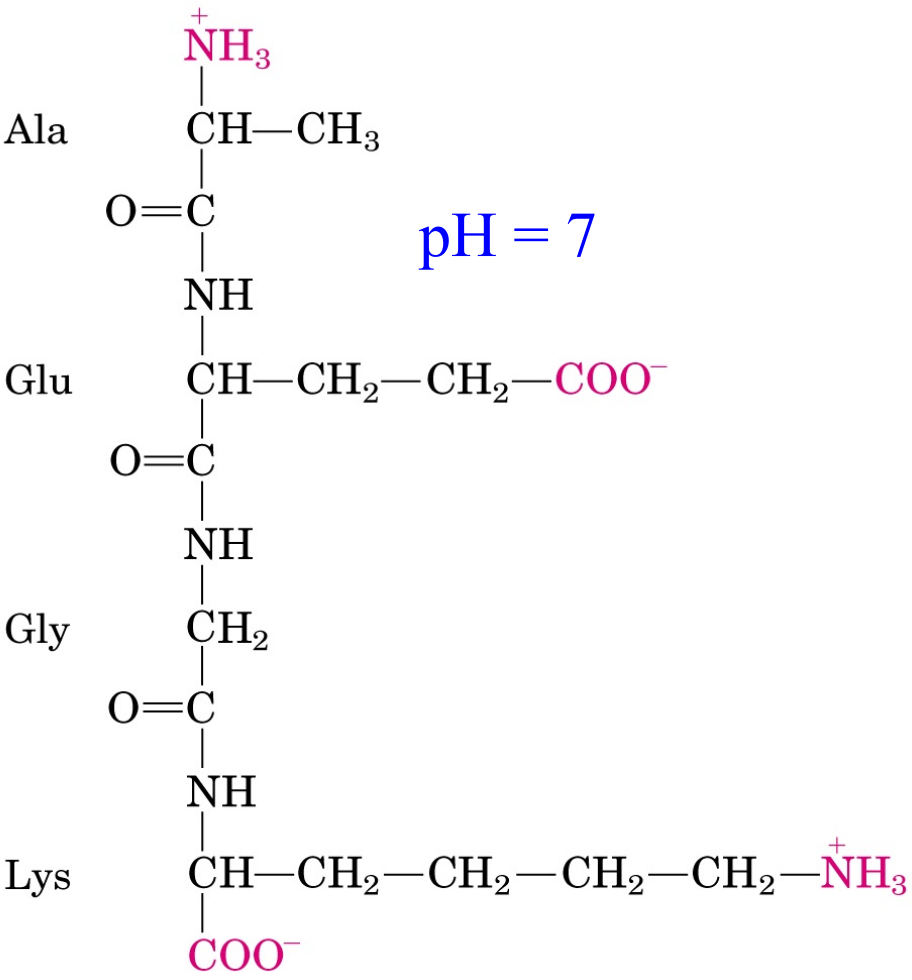
Write the structure of the following tripeptide: Gly-Pro-Lys

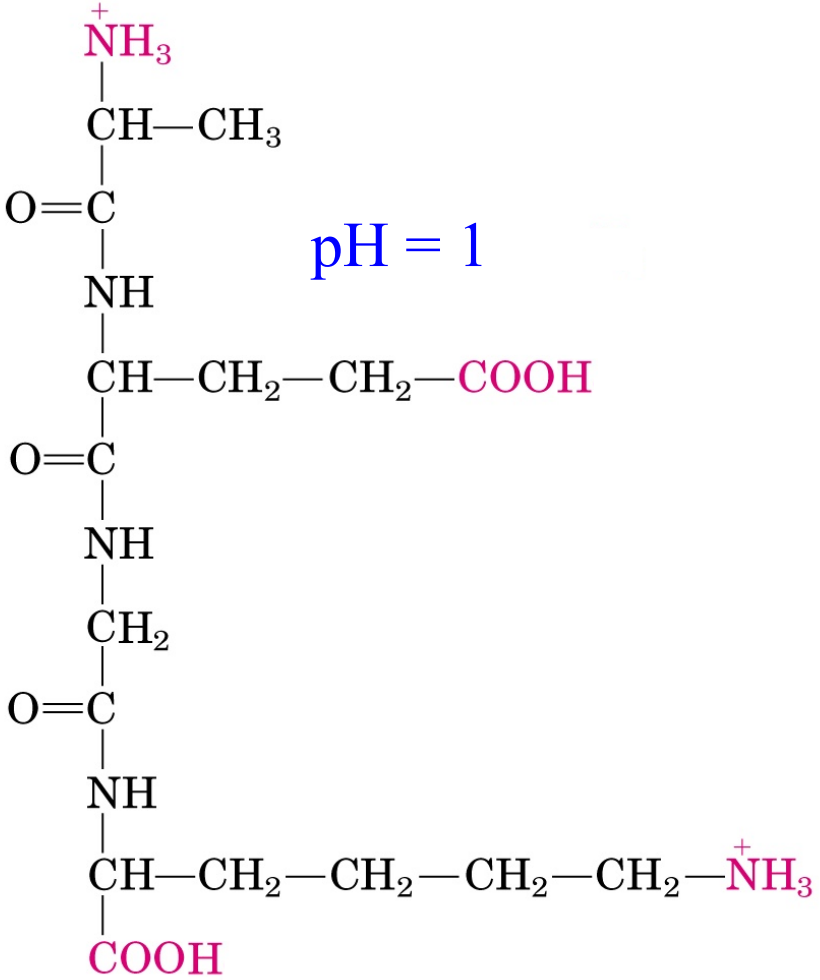


remember that upon
peptide bond formation
Pro looses it NH proton

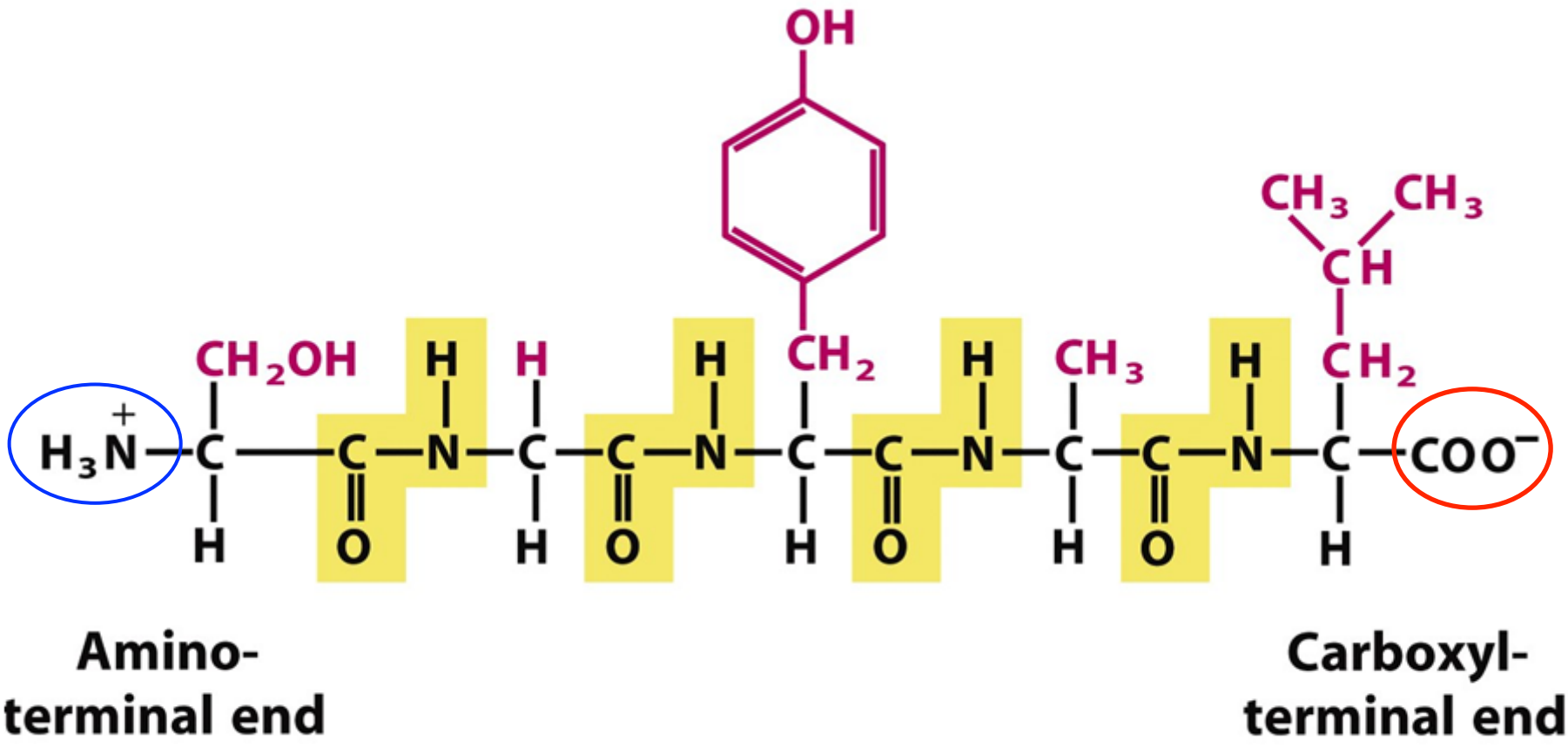
Write the structure of the following tetrapeptide at pH 1 & 7:

Ala-Glu-Gly-Lys (A-E-G-K)

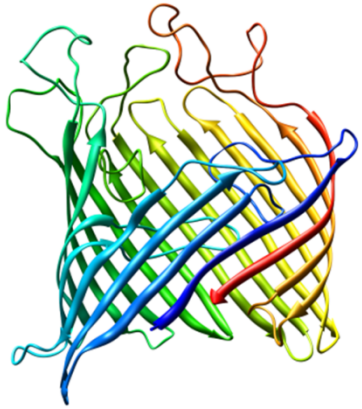




The polypeptide chain has a directionality



Maltoporin



N-terminal residue

```
VAL ASP PHE HIS GLY TYR ALA ARG SER GLY ILE GLY TRP THR GLY SER GLY GLY GLU GLN   20
GLN CYS PHE GLN THR THR GLY ALA GLN SER LYS TYR ARG LEU GLY ASN GLU CYS GLU THR   40
TYR ALA GLU LEU LYS LEU GLY GLN GLU VAL TRP LYS GLU GLY ASP LYS SER PHE TYR PHE   60
ASP THR ASN VAL ALA TYR SER VAL ALA GLN GLN ASN ASP TRP GLU ALA THR ASP PRO ALA   80
PHE ARG GLU ALA ASN VAL GLN GLY LYS ASN LEU ILE GLU TRP LEU PRO GLY SER THR ILE  100
TRP ALA GLY LYS ARG PHE TYR GLN ARG HIS ASP VAL HIS MET ILE ASP PHE TYR TYR TRP  120
ASP ILE SER GLY PRO GLY ALA GLY LEU GLU ASN ILE ASP VAL GLY PHE GLY LYS LEU SER  140
LEU ALA ALA THR ARG SER SER GLU ALA GLY GLY SER SER SER PHE ALA SER ASN ASN ILE  160
TYR ASP TYR THR ASN GLU THR ALA ASN ASP VAL PHE ASP VAL ARG LEU ALA GLN MET GLU  180
ILE ASN PRO GLY GLY THR LEU GLU LEU GLY VAL ASP TYR GLY ARG ALA ASN LEU ARG ASP  200
ASN TYR ARG LEU VAL ASP GLY ALA SER LYS ASP GLY TRP LEU PHE THR ALA GLU HIS THR  220
GLN SER VAL LEU LYS GLY PHE ASN LYS PHE VAL VAL GLN TYR ALA THR ASP SER MET THR  240
SER GLN GLY LYS GLY LEU SER GLN GLY SER GLY VAL ALA PHE ASP ASN GLU LYS PHE ALA  260
TYR ASN ILE ASN ASN ASN GLY HIS MET LEU ARG ILE LEU ASP HIS GLY ALA ILE SER MET  280
GLY ASP ASN TRP ASP MET MET TYR VAL GLY MET TYR GLN ASP ILE ASN TRP ASP ASN ASP  300
ASN GLY THR LYS TRP TRP THR VAL GLY ILE ARG PRO MET TYR LYS TRP THR PRO ILE MET  320
SER THR VAL MET GLU ILE GLY TYR ASP ASN VAL GLU SER GLN ARG THR GLY ASP LYS ASN  340
ASN GLN TYR LYS ILE THR LEU ALA GLN GLN TRP GLN ALA GLY ASP SER ILE TRP SER ARG  360
PRO ALA ILE ARG VAL PHE ALA THR TYR ALA LYS TRP ASP GLU LYS TRP GLY TYR ASP TYR  380
THR GLY ASN ALA ASP ASN ASN ALA ASN PHE GLY LYS ALA VAL PRO ALA ASP PHE ASN GLY  400
GLY SER PHE GLY ARG GLY ASP SER ASP GLU TRP THR PHE GLY ALA GLN MET GLU ILE TRP  420
TRP                                                                              421
```

C-terminal residue

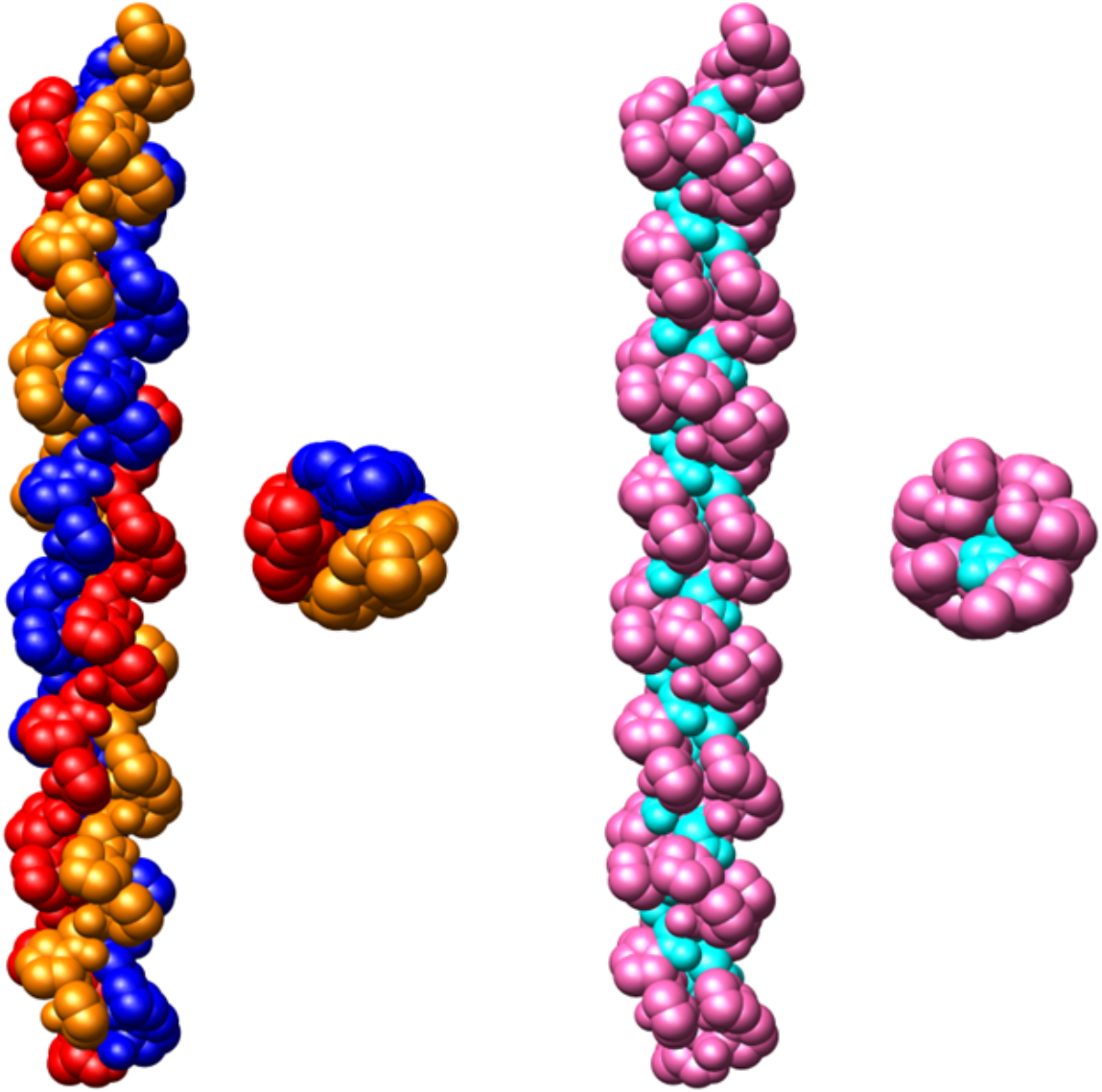
Molecular Data on Some Proteins

	Molecular weight	Number of residues	Number of polypeptide chains
Cytochrome *c* (human)	13,000	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (chicken egg white)	13,930	129	1
Myoglobin (equine heart)	16,890	153	1
Chymotrypsin (bovine pancreas)	21,600	241	3
Chymotrypsinogen (bovine)	22,000	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	68,500	609	1
Hexokinase (yeast)	102,000	972	2
RNA polymerase (*E. coli*)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (*E. coli*)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

Conjugated Proteins

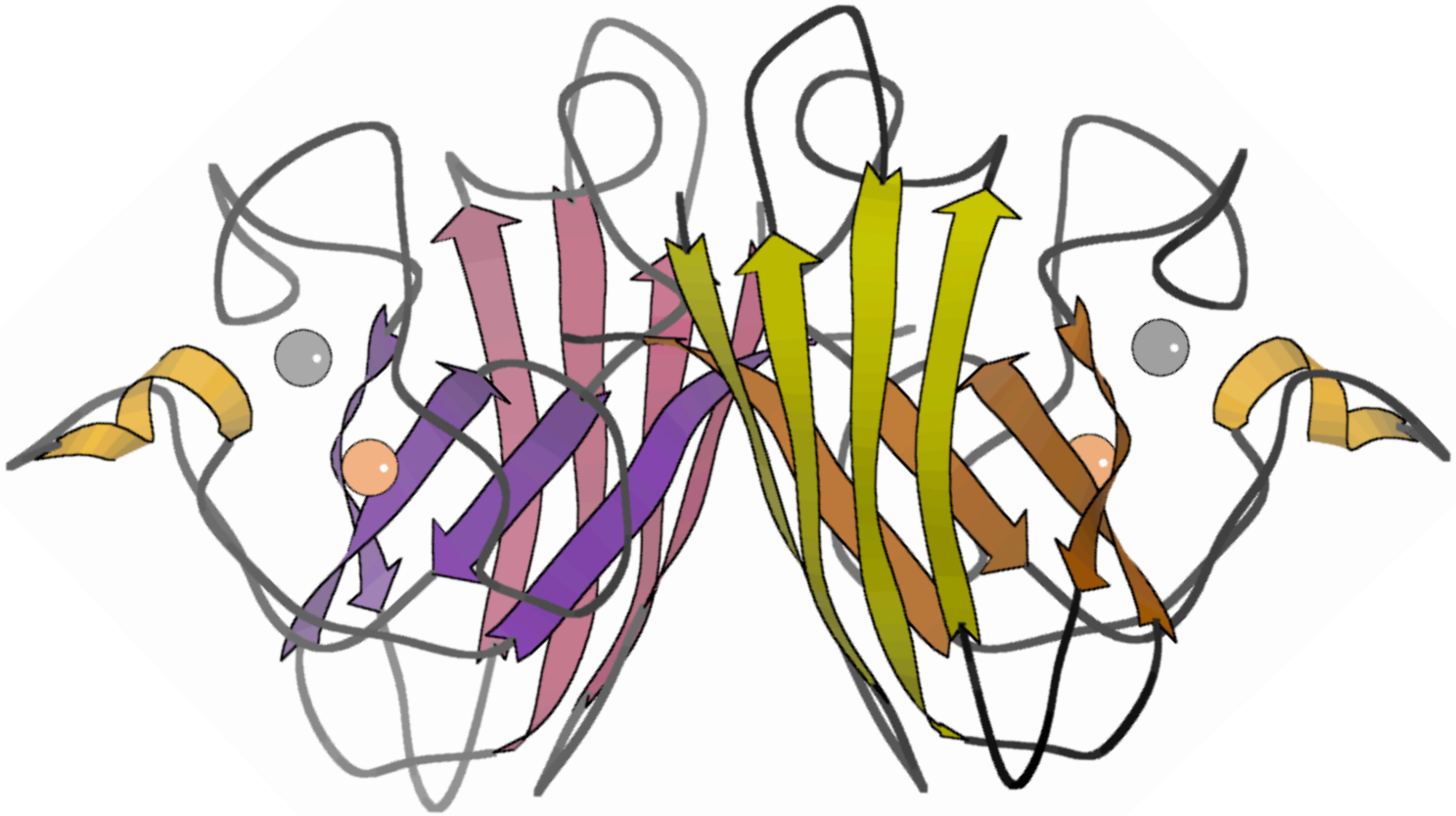
Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

Collagen: a fibrous protein

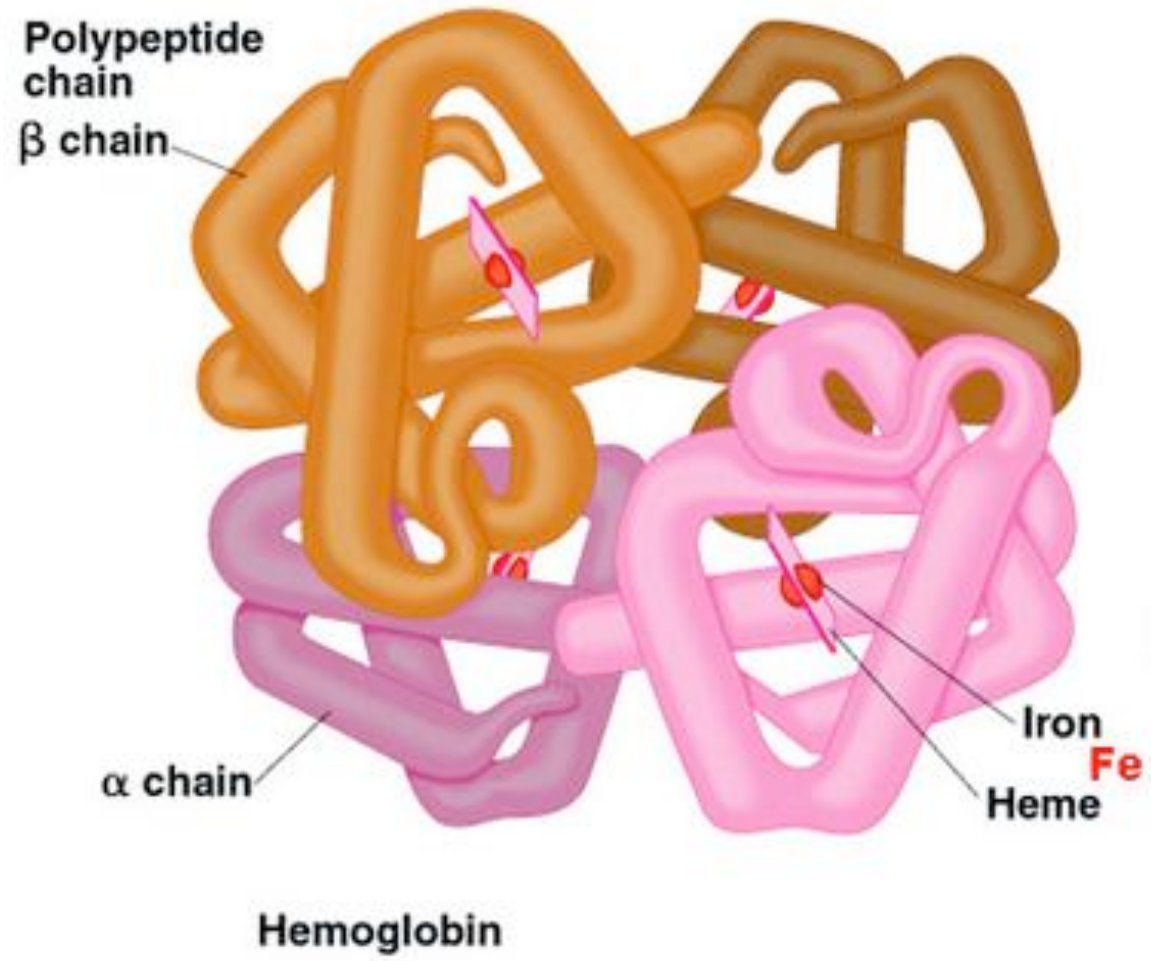


Superoxide dismutase: an enzyme

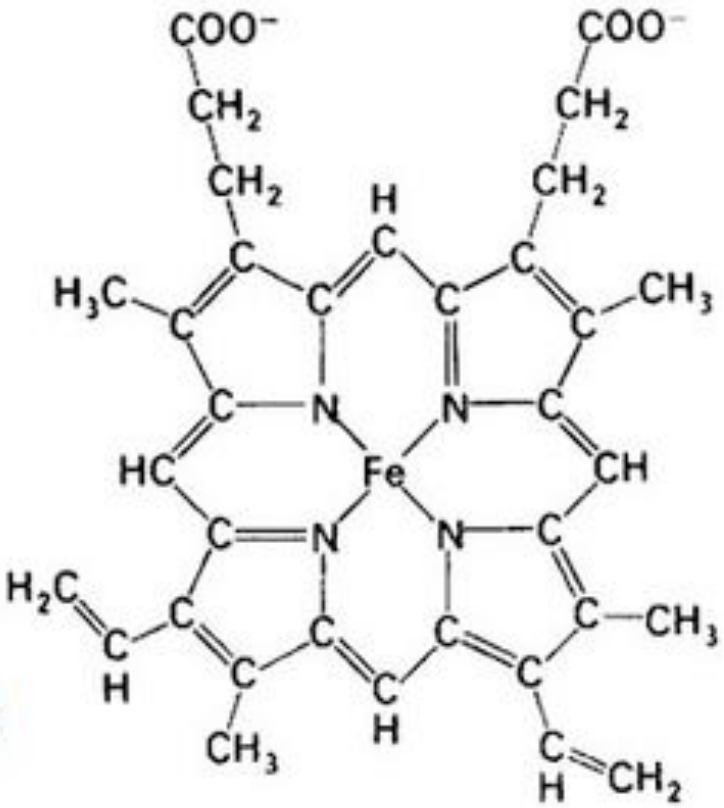
$$2\ O_2^- + 2\ H^+ \rightarrow\ O_2 + H_2O_2$$



Hemoglobin: an O_2 transport protein

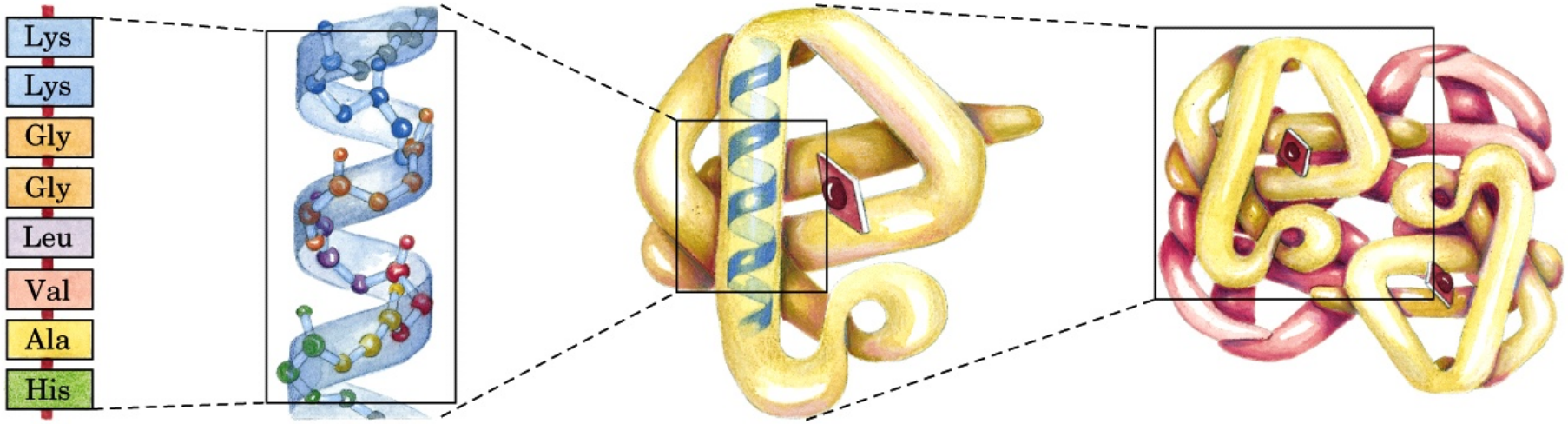


Hemoglobin



Heme
(Fe-protoporphyrin IX)

Structural organization levels of proteins



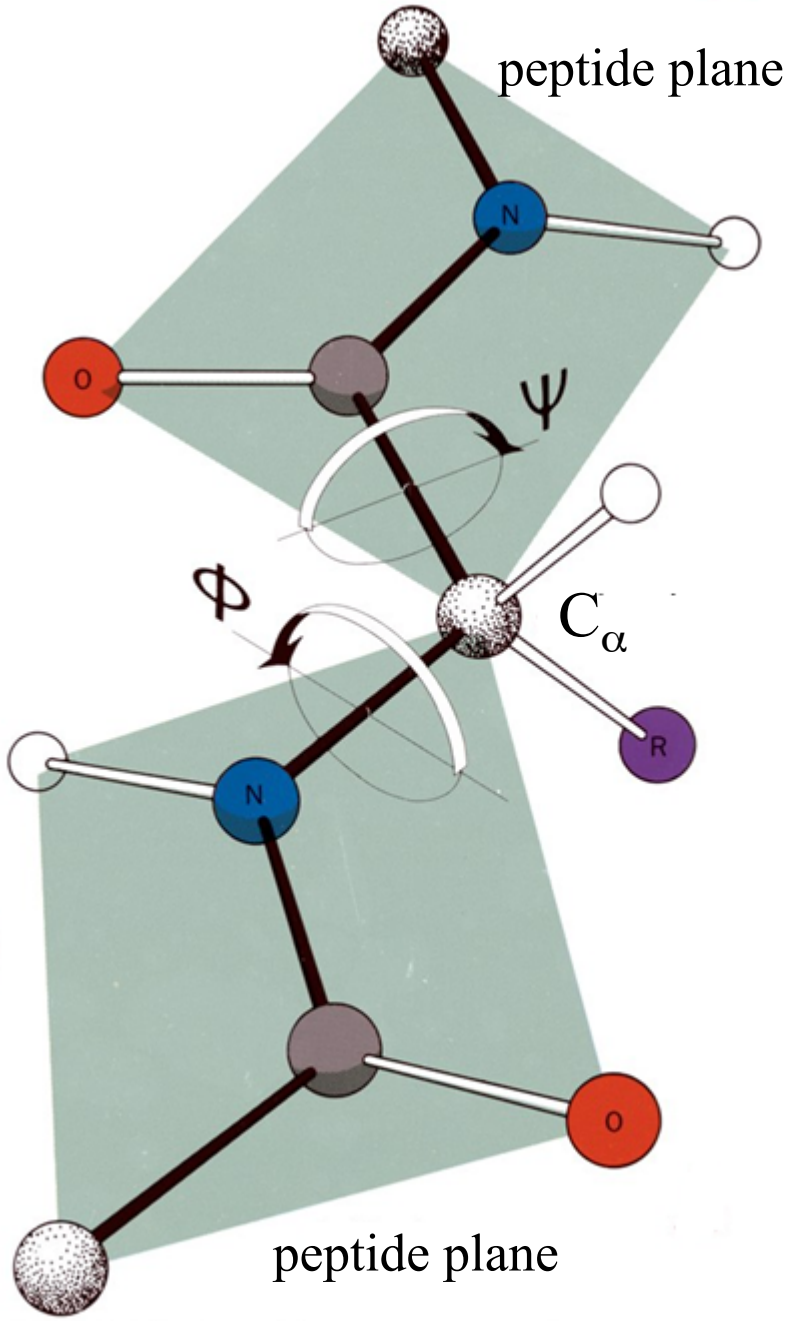
Primary structure: amino acid sequence

Secondary structure: regular and recurrent spatial organization of the polypeptide chain, or the local polypeptide conformation

Tertiary structure: threedimensional structure of the polypeptide chain

Quaternary structure: structural assemby of several (≥ 2) polypeptide chains

Every level is studied with different experimental techniques

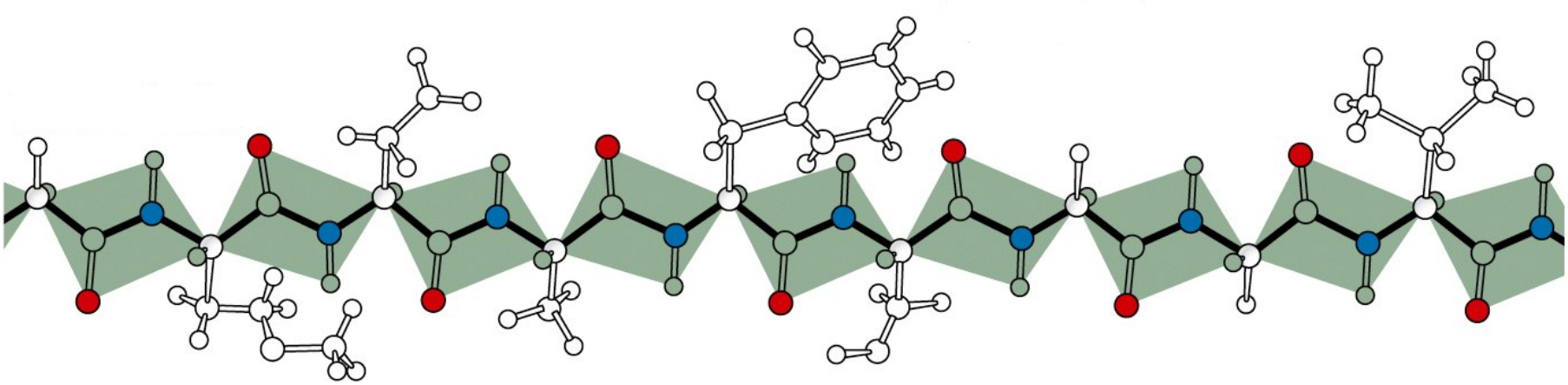


Solid angles ϕ e ψ

ϕ: rotation around the C_α-N bond

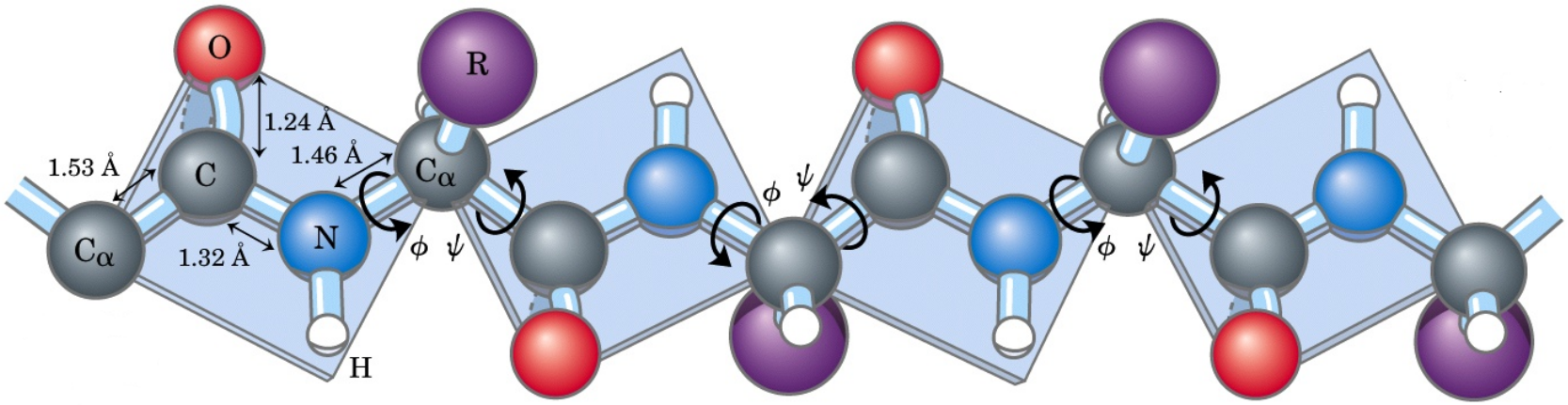
ψ: rotation around the C_α-C bond

The numerical values of the solid angles determines the conformation of the polypepide chain

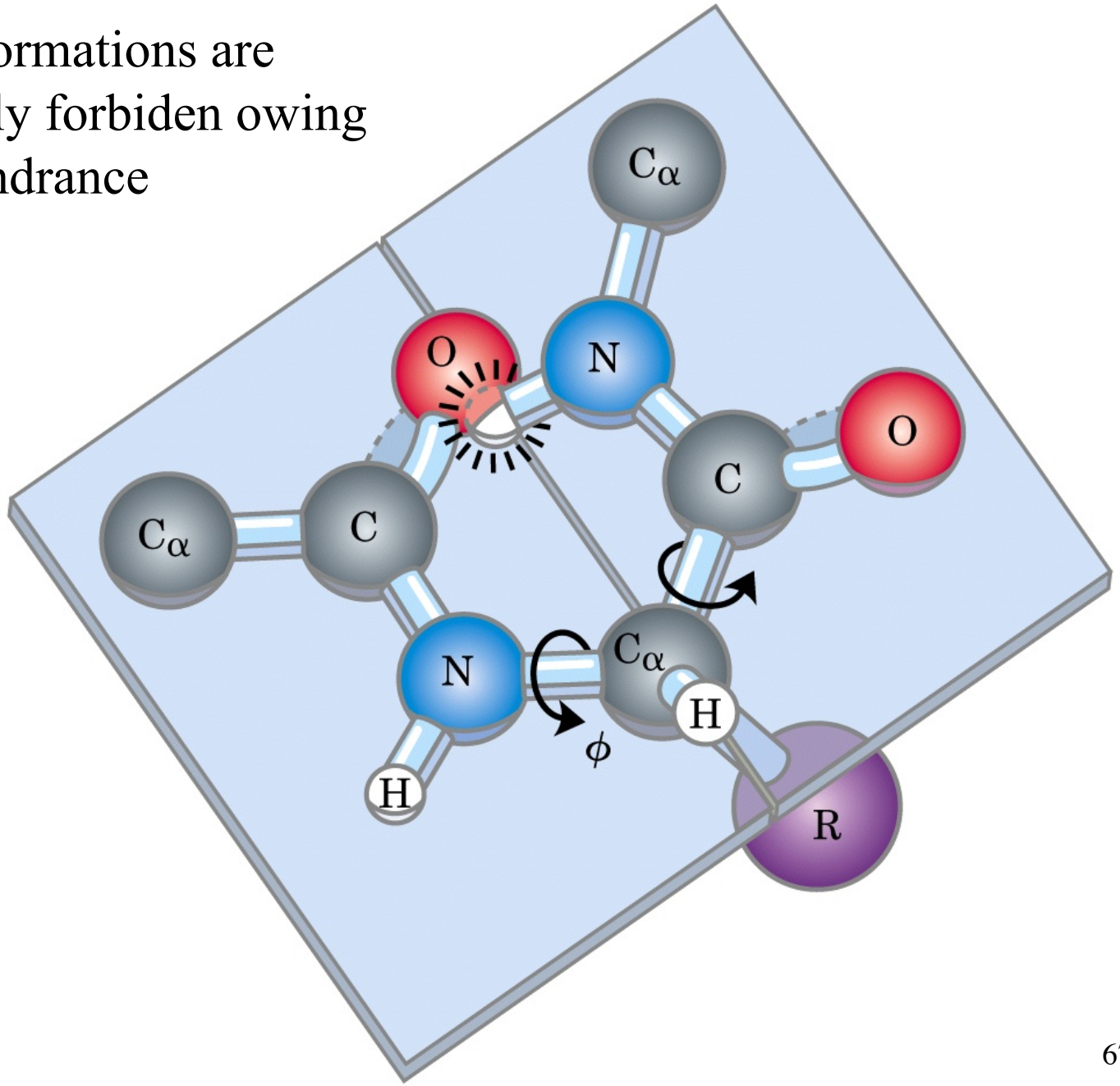


← N-terminal

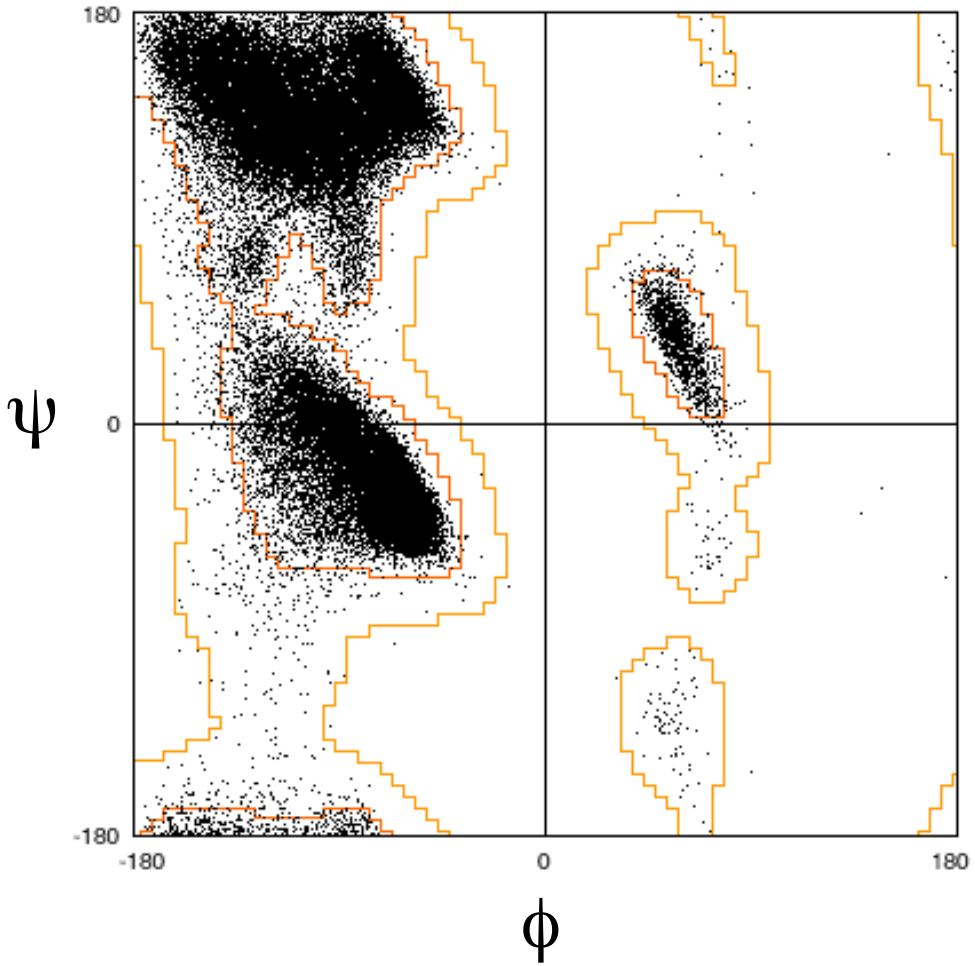
C-terminal →



Some conformations are energetically forbiden owing to steric hindrance

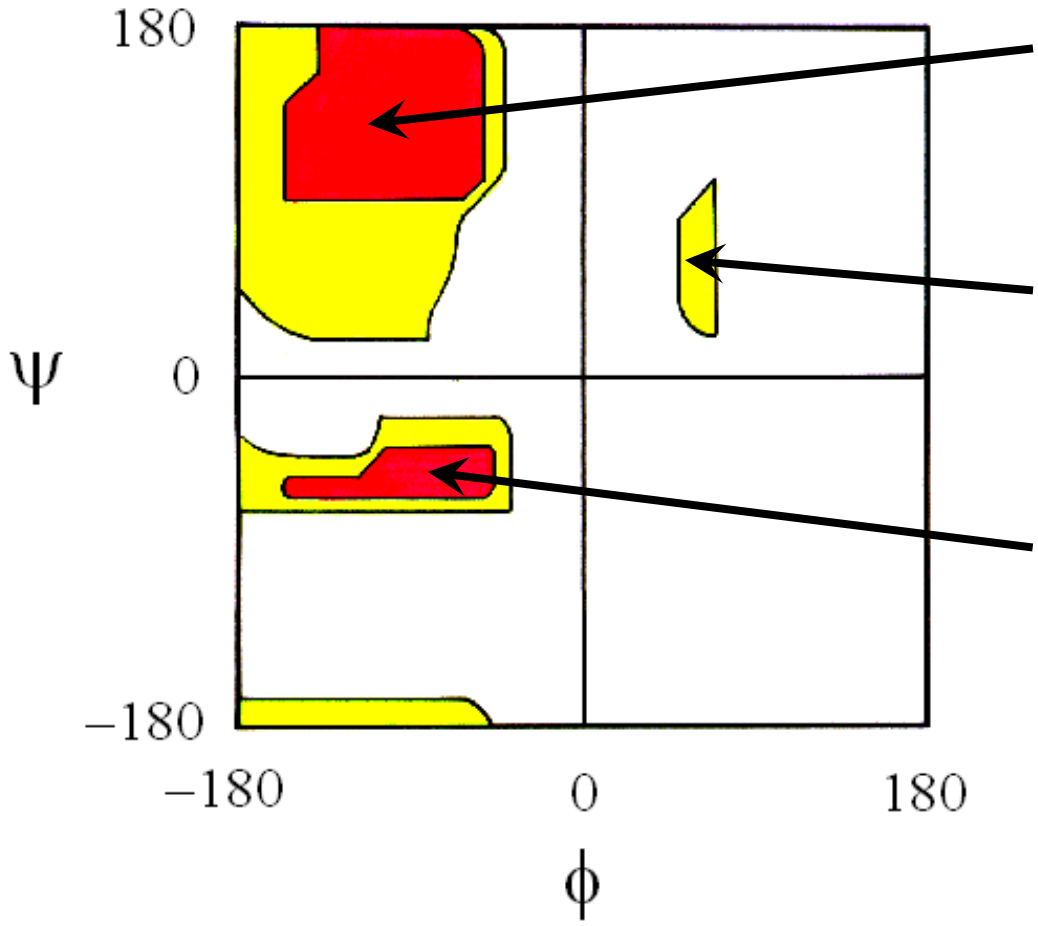


The Ramachandran plot: a diagram in which the ψ angle is plotted as a function of the ϕ angle



500 high resolution (≤ 1.8 Å) proteins (100000 data points) Distribution of non-Gly e non-Pro residues.

The Ramachandran plot



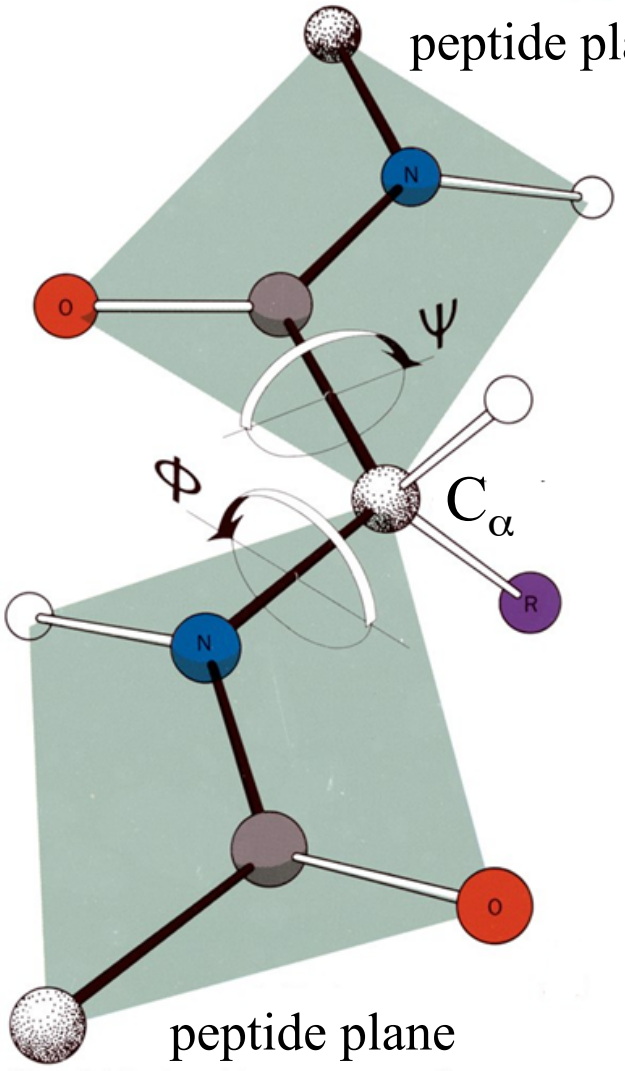
β sheets

left-handed α-helix

right-handed α-helix

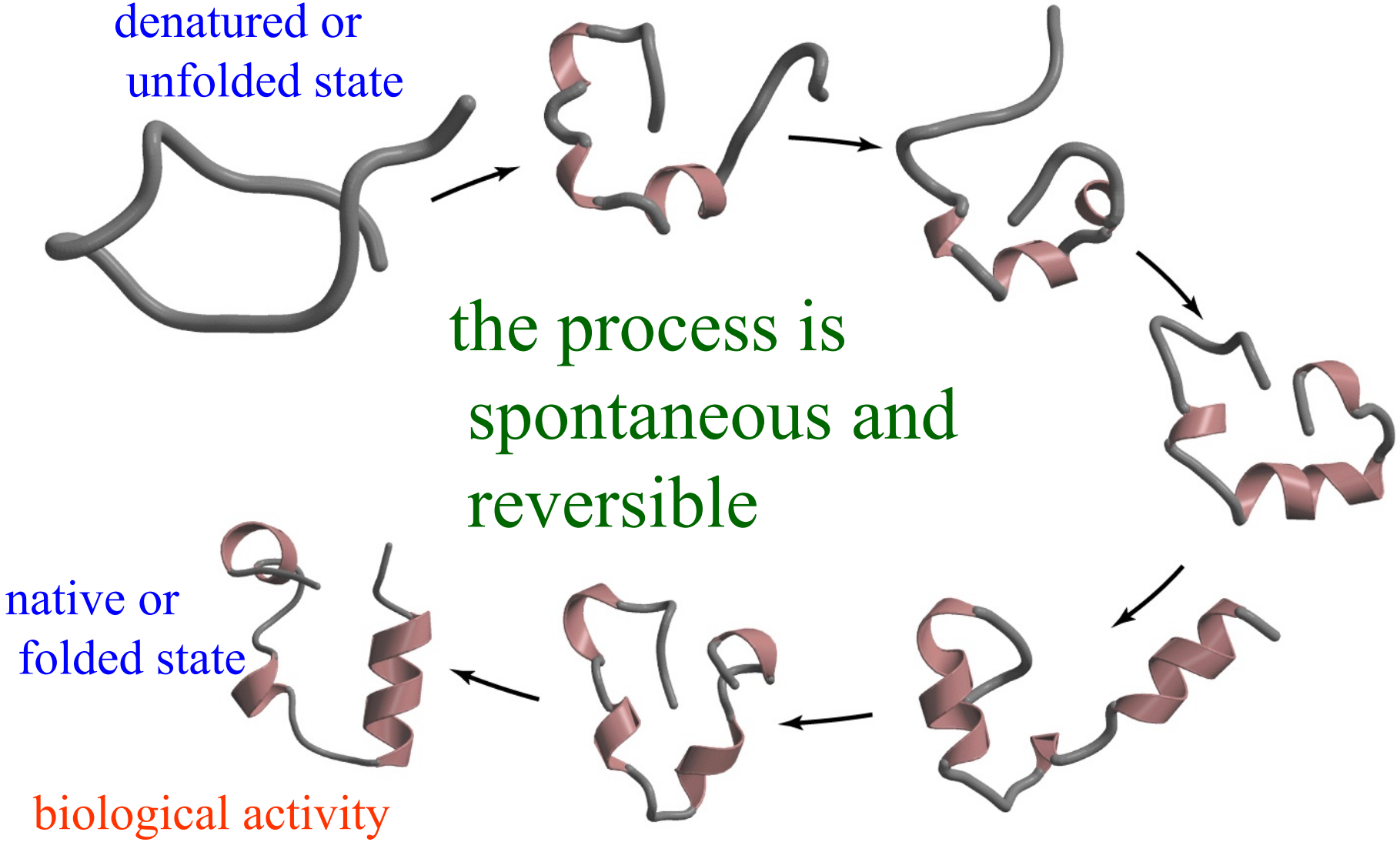
Red: allowed regions (normal contact radii). Yellow: allowed regions (shorter contact radii, as found in protein crystals)

Torsion angles of the protein backbone



Structure	φ	ψ
right-handed α helix	-57°	-47°
left-handed α helix	+57°	+47°
parallel β sheet	-119°	+113°
anti-parallel β sheet	-139°	+135°
polyglicine II	-80°	+150°
poly-L-proline I (*cis*)	-83°	+158°
poly-L-proline II (*trans*)	-78°	+149°

Protein folding



Key concepts

Proteins fold spontaneously under physiological conditions

• in the equilibrium between the denatured state (unfolded or partially unfolded) and the native state (folded, biologically functional), under physiological conditions the vast majority of molecules are in the native state.

Primary structure determines secondary, tertiary (and quaternary) structures

• many proteins can refold from a random coil set of conformations without "instructions" from any other cellular components

• All the information for 3-D structure is provided by the amino acid sequence.

Proteins can be unfolded (denatured) *in vitro* by chemical or physical (urea, pH, heat) **and then refolded** (renatured) by diluting out the chemical denaturant

Proteins fold on a defined pathway (or alternative pathways)

• all possible conformations are not searched randomly to reach the most stable (lowest free energy) structure.

Key concepts continued

Some proteins don't fold on their own

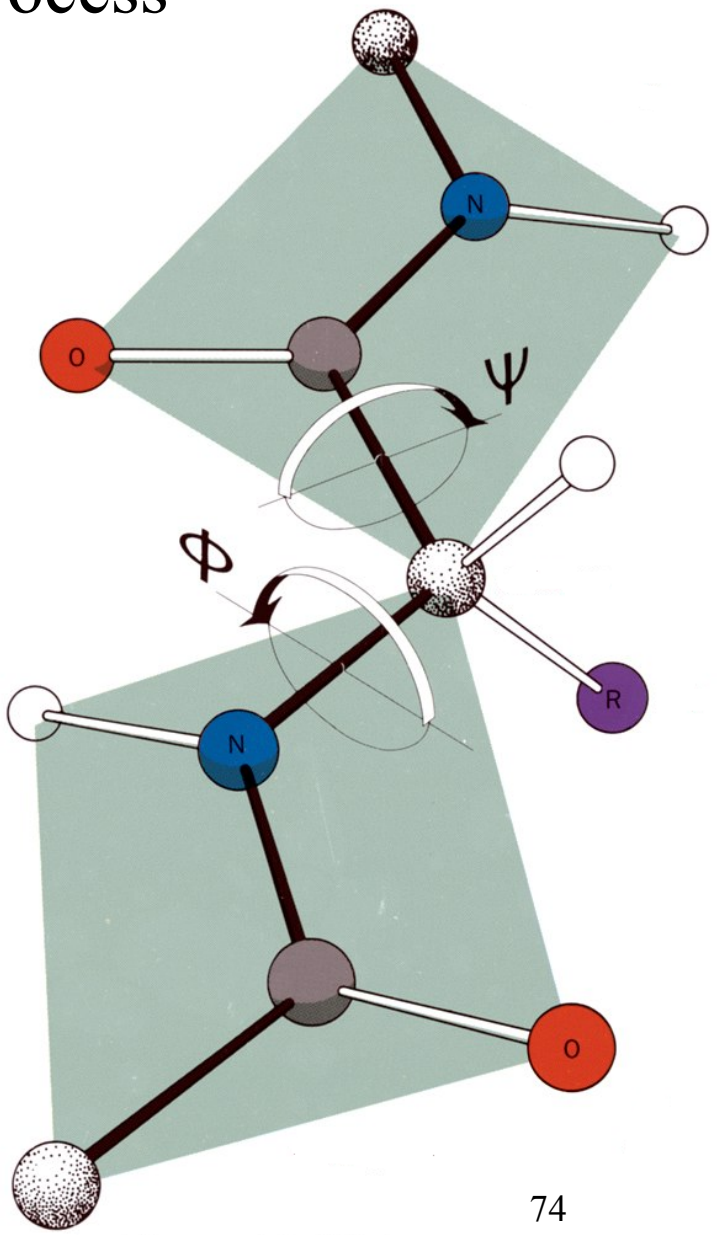
- These proteins need molecular chaperones (also proteins) to keep them from slipping off the folding pathway or to help them to get back on it
- Some chaperones require energy (ATP hydrolysis) to carry out their function.

Many diseases are the result of defects in protein folding

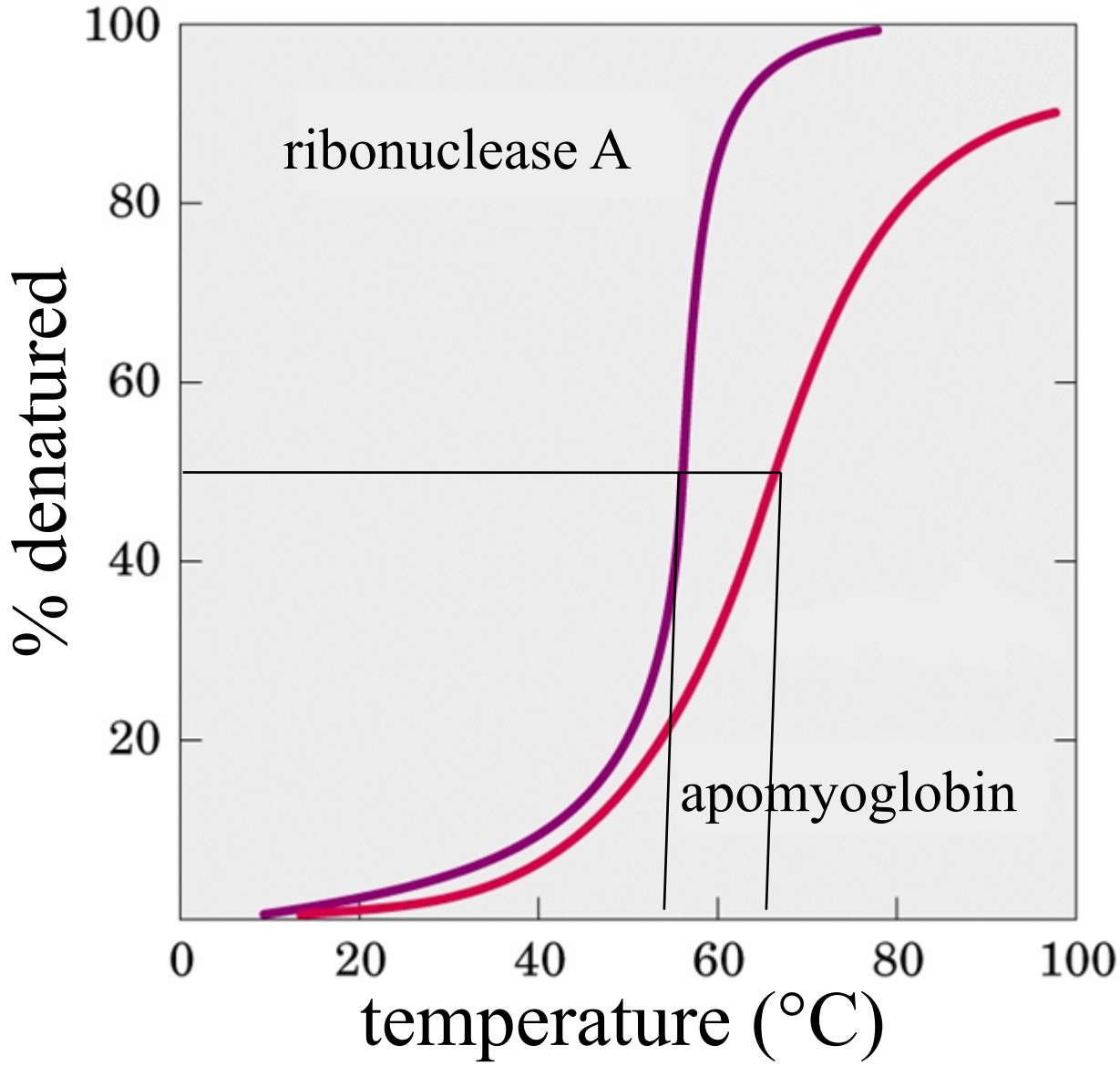
- the spongiform encephalopathies (transmissible spongiform encephalopathies, human CJD, bovine "mad cow" disease), Alzheimer's , Parkinson's and Huntington's diseases
- diseases involving deposits of misfolded proteins (amyloid deposits) result from aggregation of a specific protein, different for different diseases, that has misfolded and formed cross-β structures that form higher order structures (protofibrils, fibrils &fibers) that are very stable.
- one hypothesis is that cellular degradation apparatus can't keep up with disposal of the abnormally folded protein (proteasome).

Protein folding is a conformational process

- the folding of a protein is a series of consecutive spontaneous reactions in the conformation of a protein changes from a denatured state to a folded state
- the dihedral angles ϕ e ψ change during the folding process
- the process is reversible
- all the information necessary to generate the native conformation is coded by the primary structure

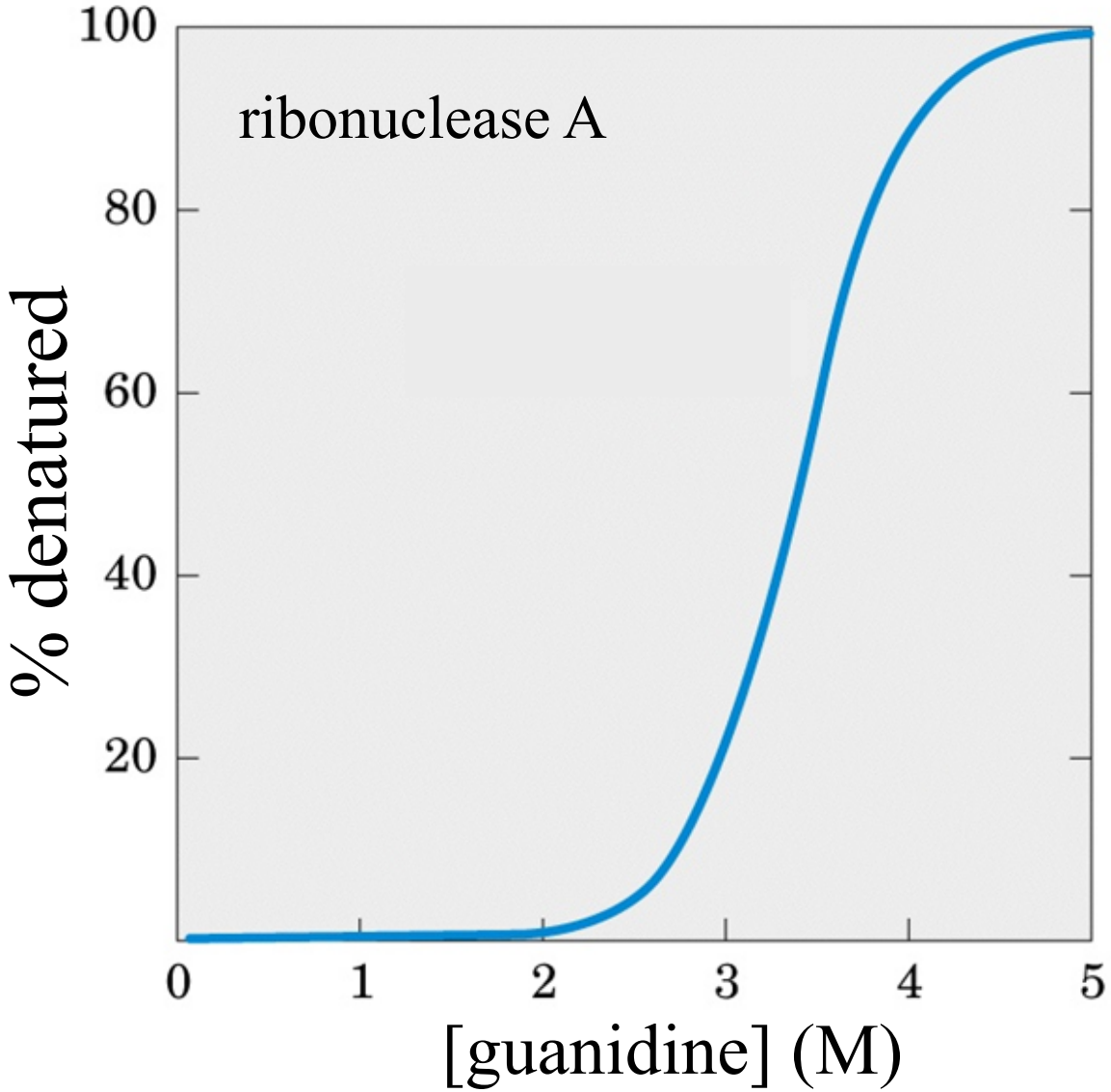


Protein folding may be studied by using either physical or chemical methods



Several types of spectroscopic signals are followed: absorption, fluorescence, circular dichroism, NMR, etc., which take advantage of the different spectroscopic properties of the native and denatured states.

Compounds known as denaturants (urea, guanidine) cause protein denaturation (reversibile destruction of the secondary and tertiary (quaternary) structures.

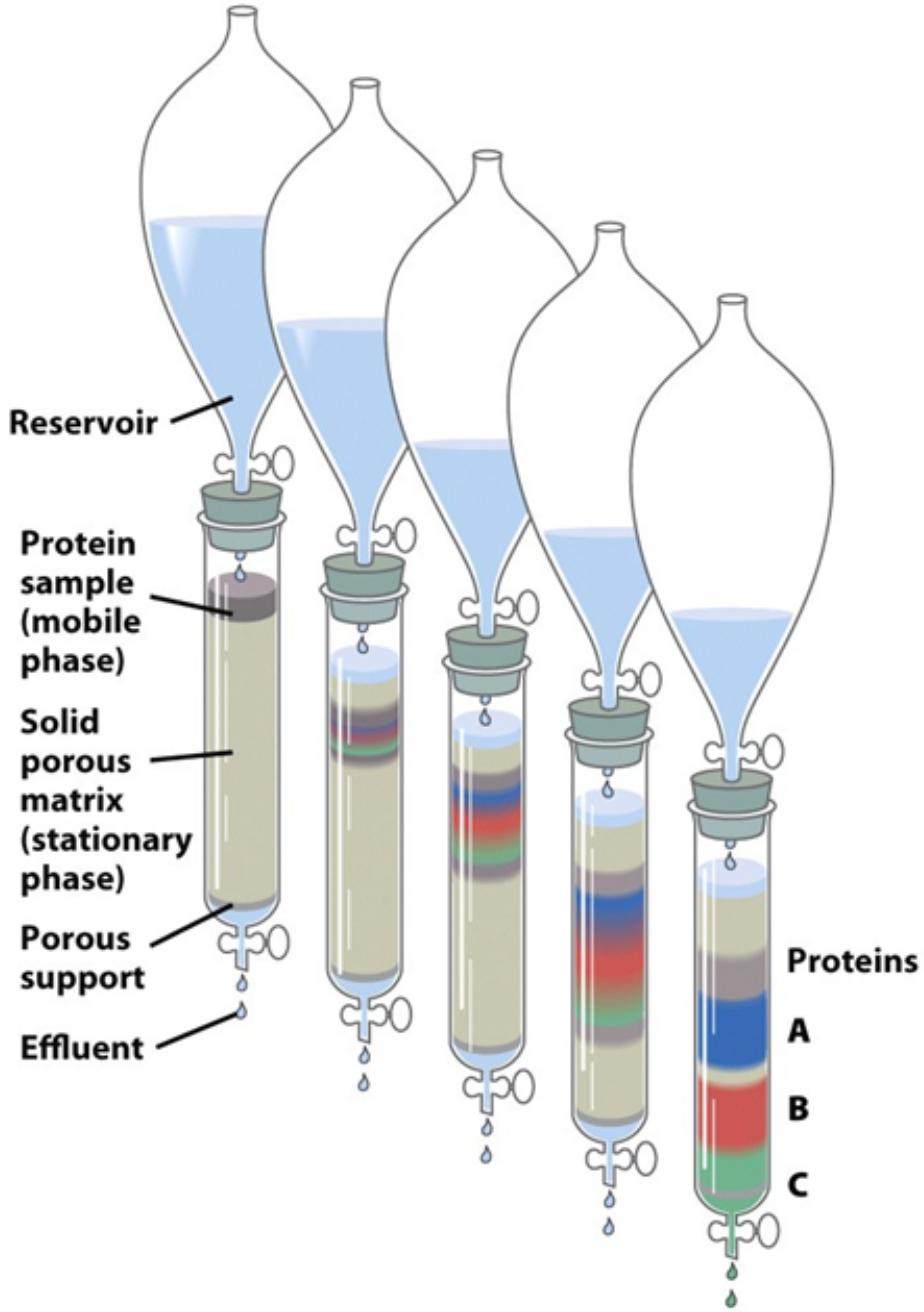


guanidine

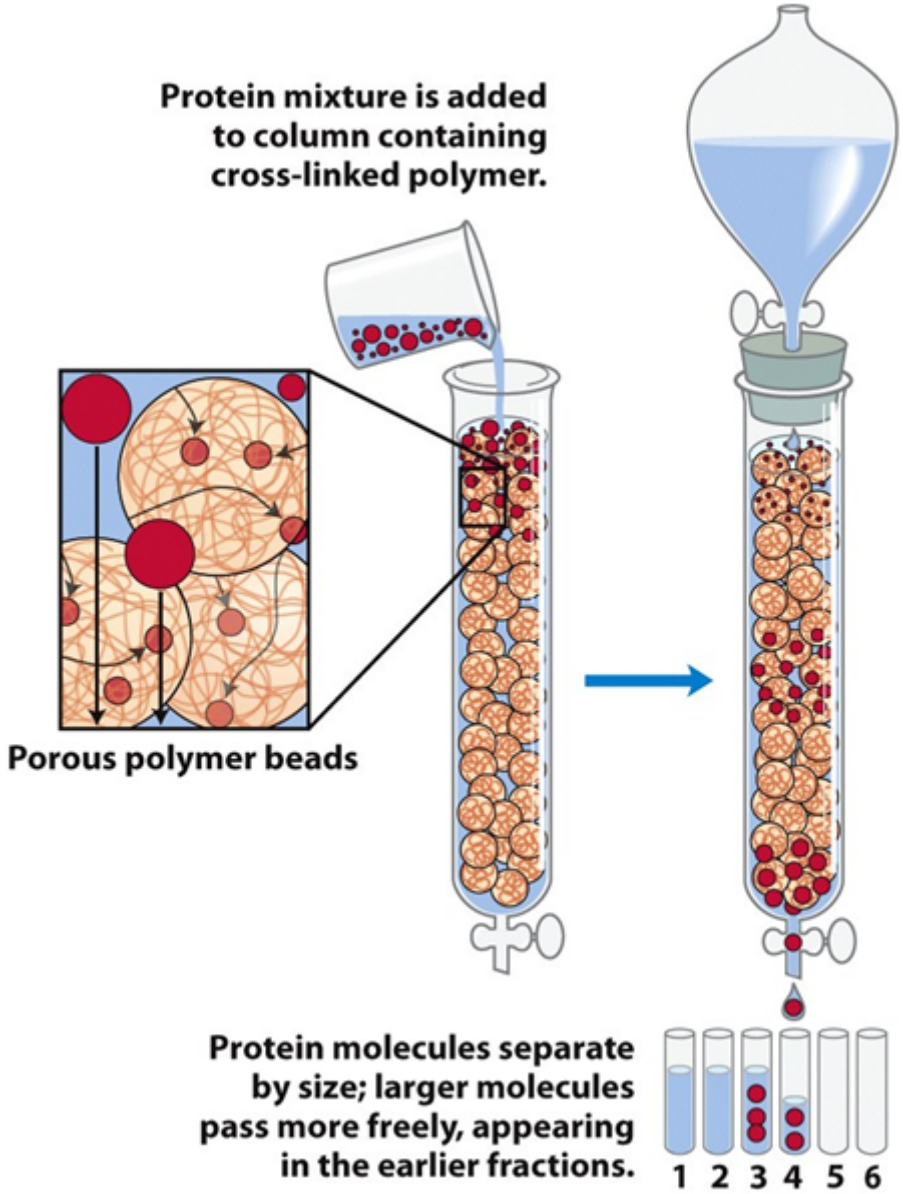
urea

pH

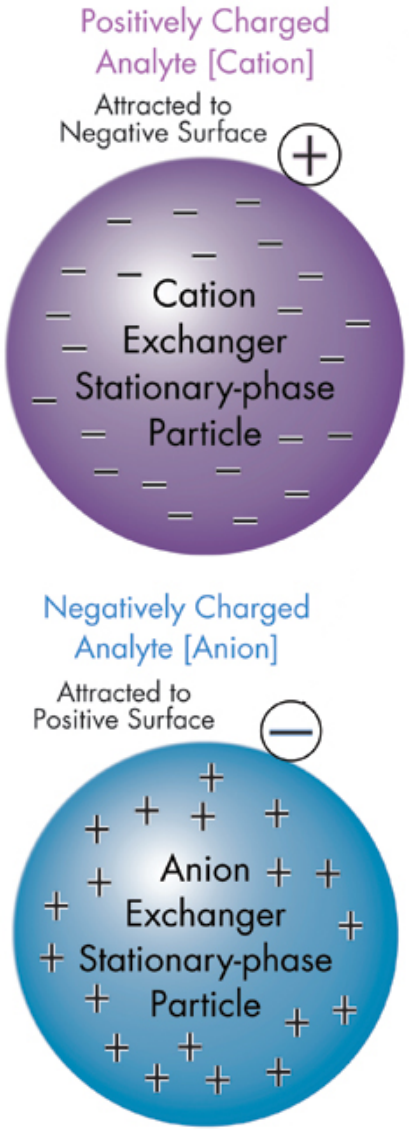
Chromatography: how are proteins purified

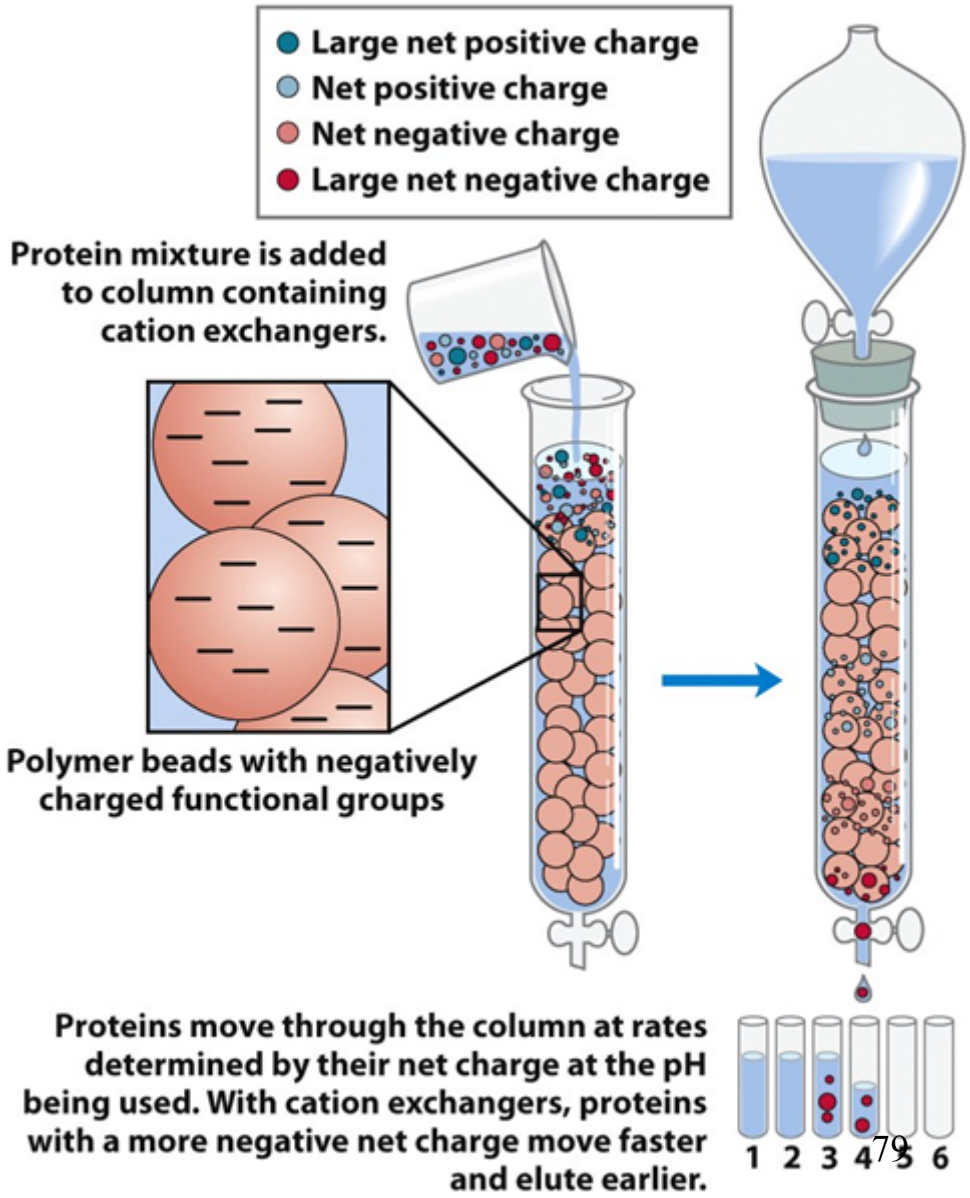


Size exclusion chromatography

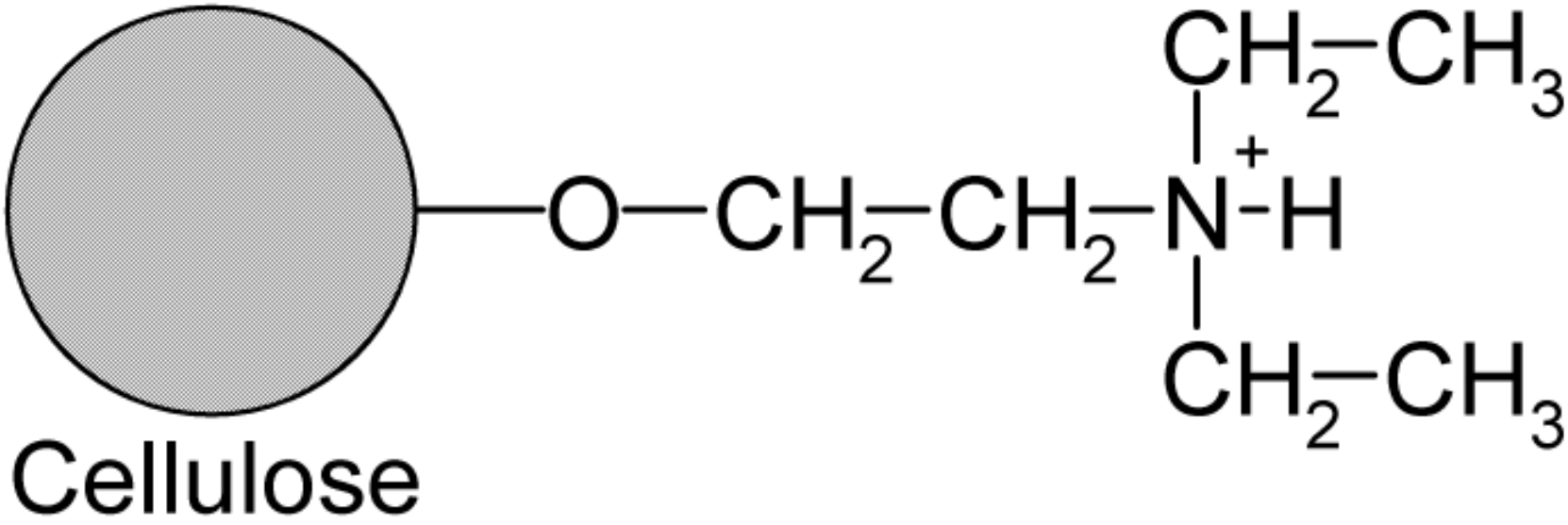


Ion-exchange chromatography

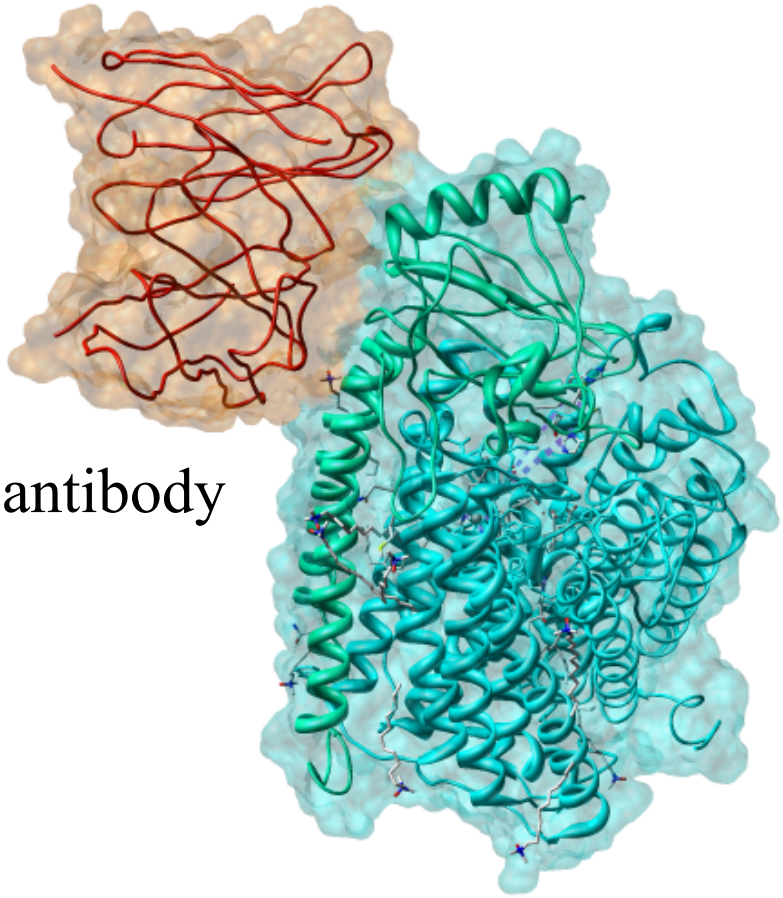




Anion-exchange chromatography

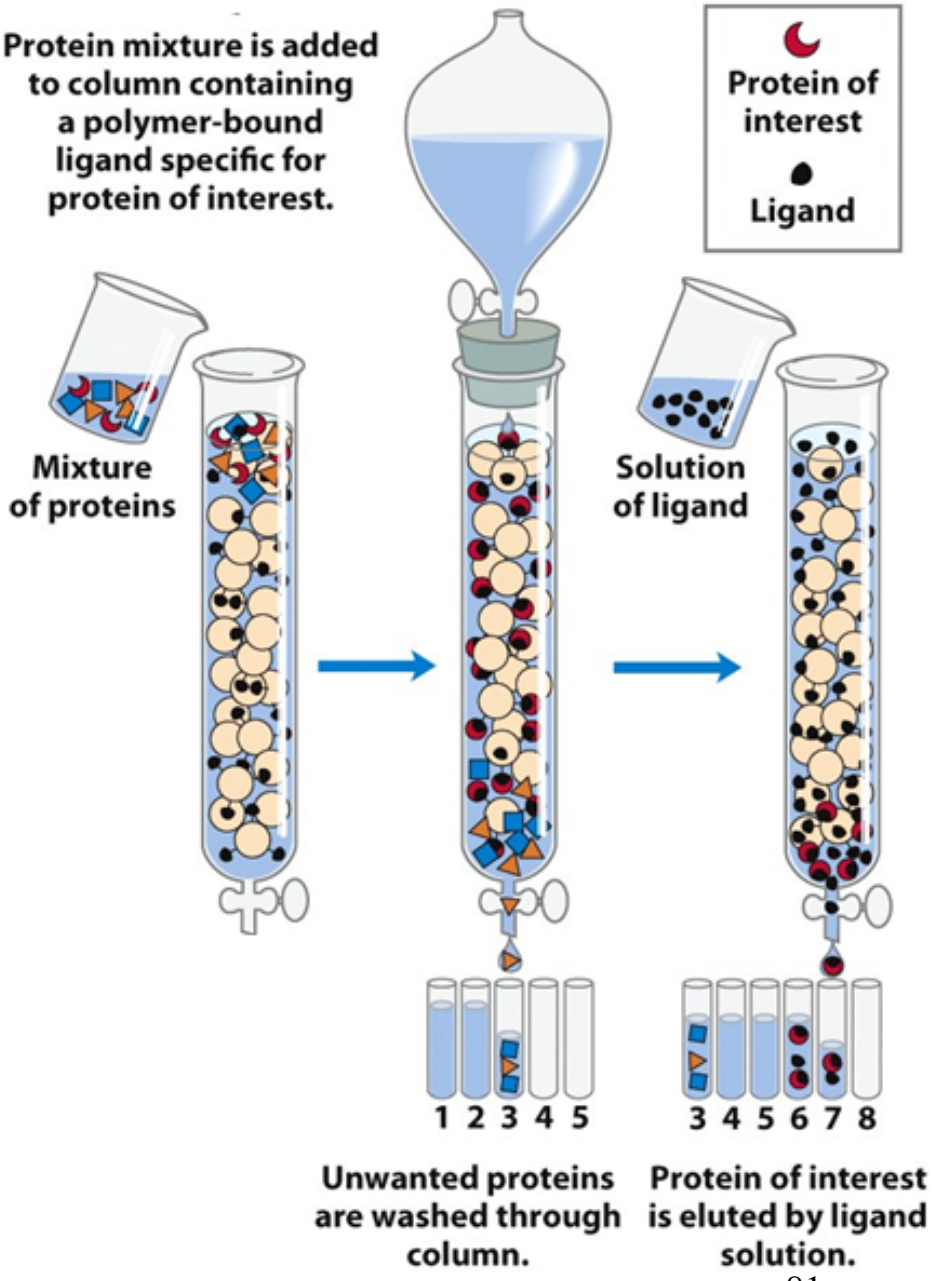


Affinity chromatography



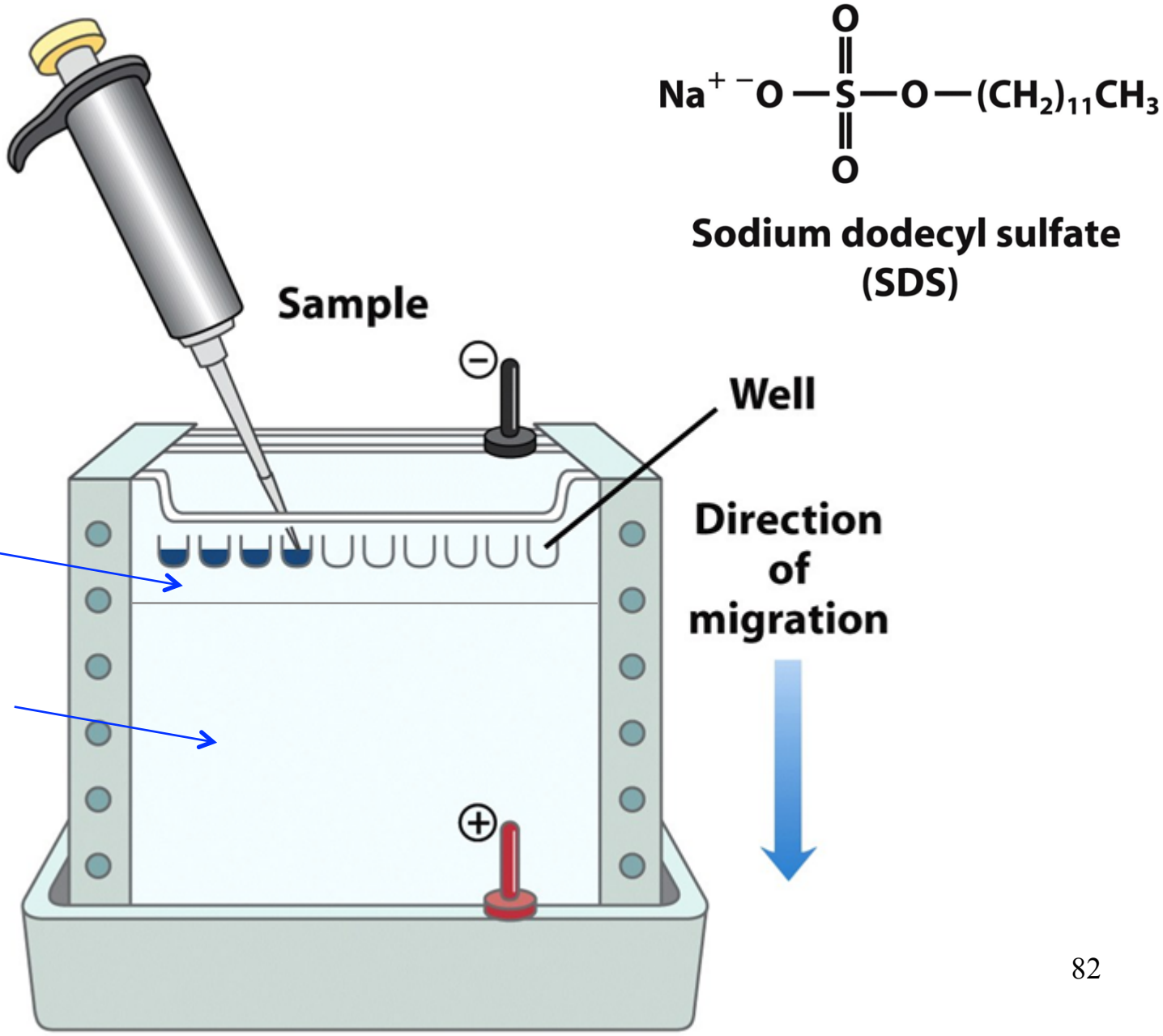
antibody

antigen

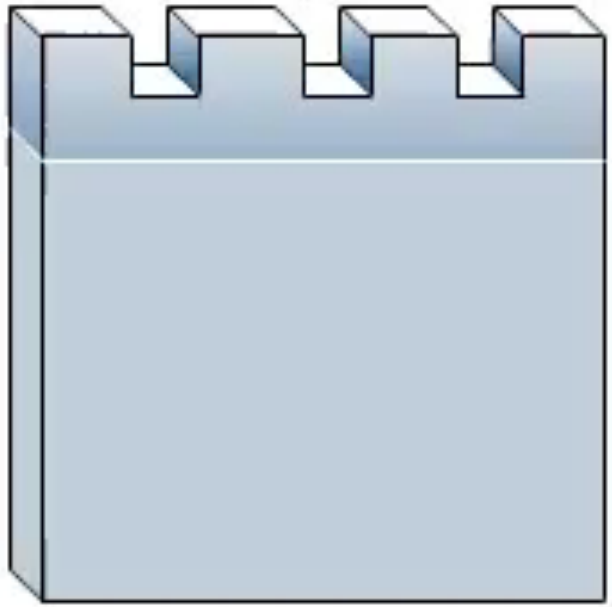


Sodium dodecyl sulfate polyacryamide gel electrophoresis

SDS-PAGE



SDS-PAGE



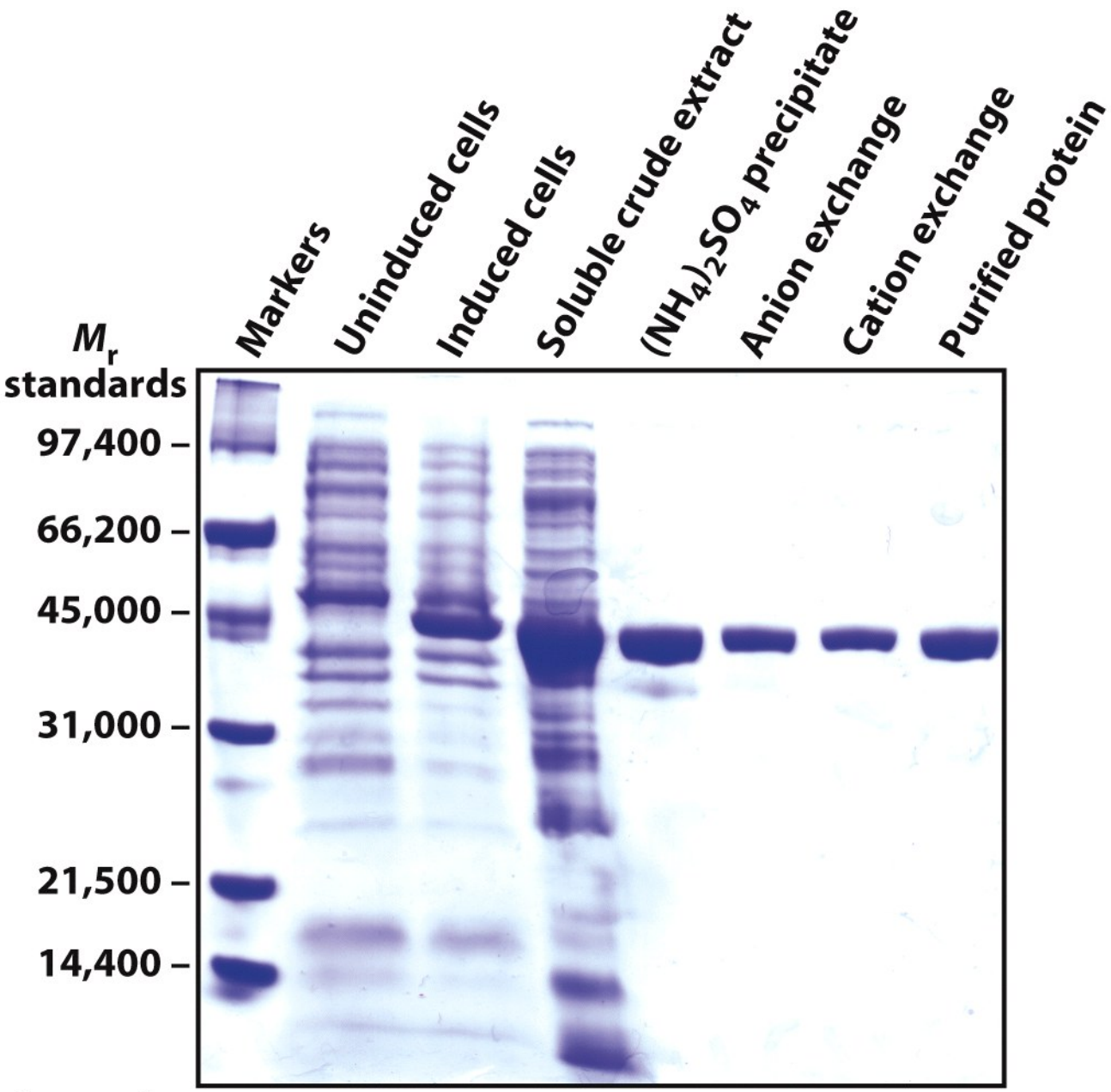


Figure 3-18b
Lehninger Principles of Biochemistry, Fifth Edition

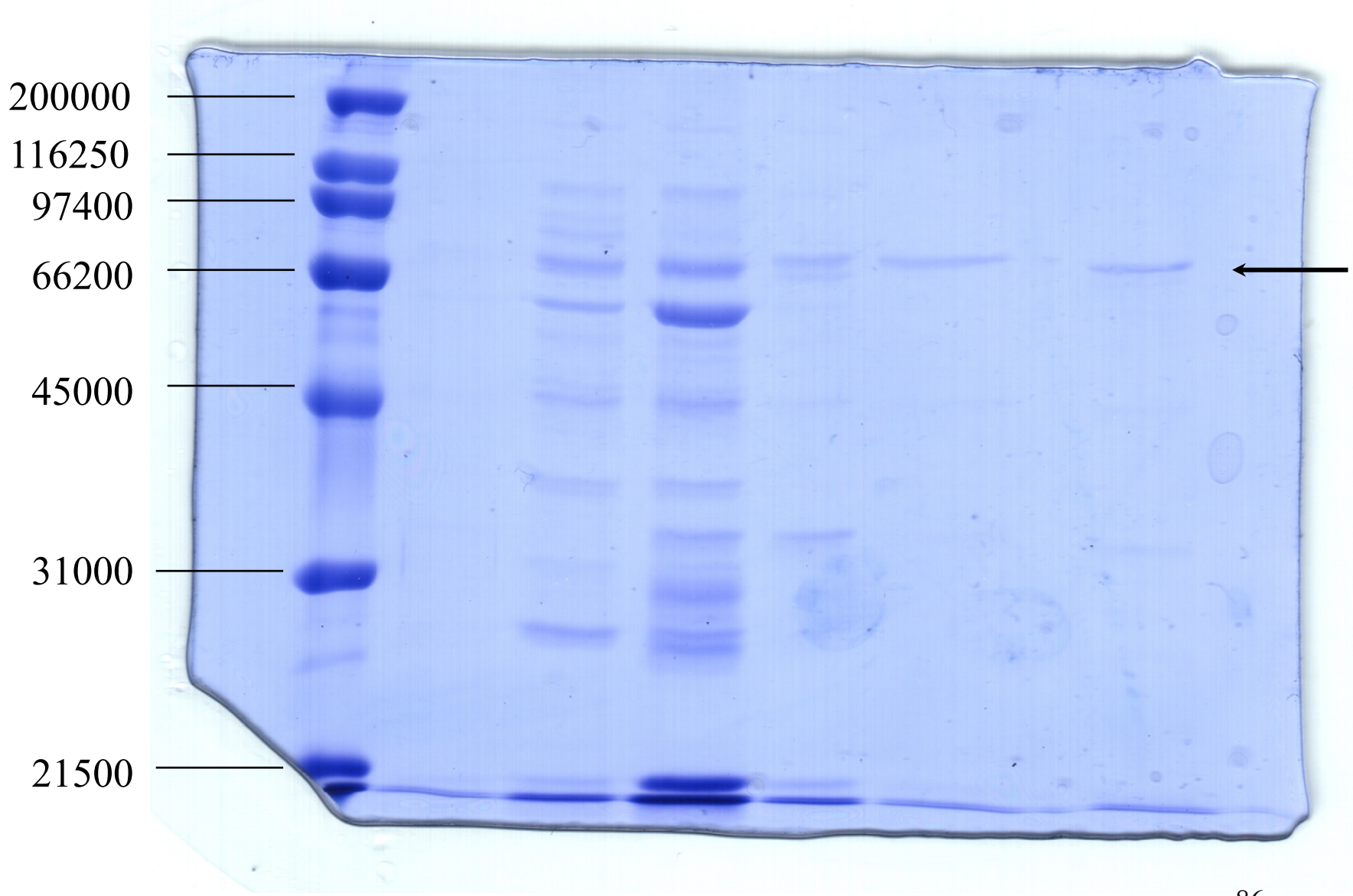
Purification of proteins

A Purification Table for a Hypothetical Enzyme

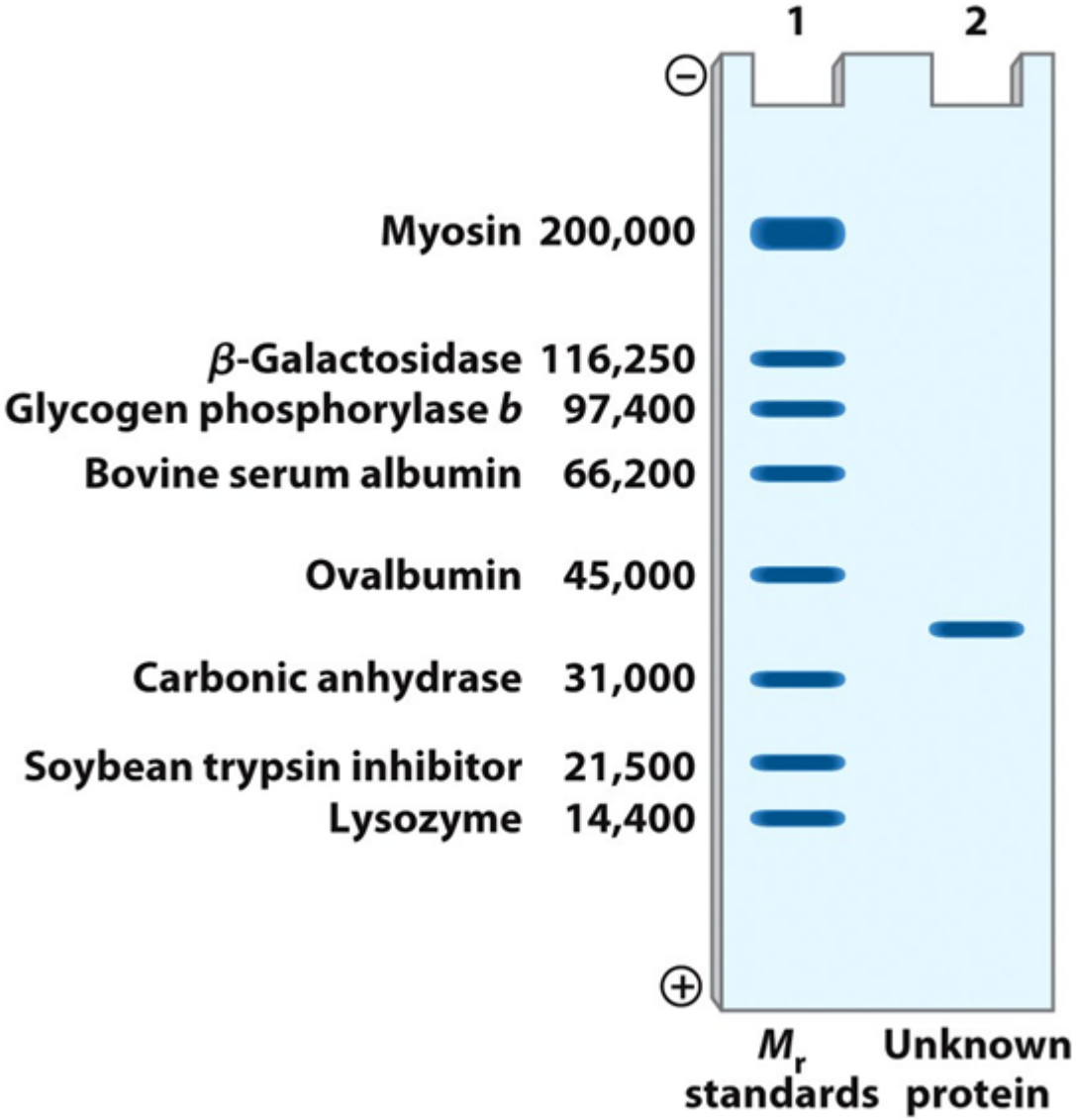
Procedure or step	Fraction volume (mL)	Total protein (mg)	Activity (units)	Specific activity (units/mg)
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100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

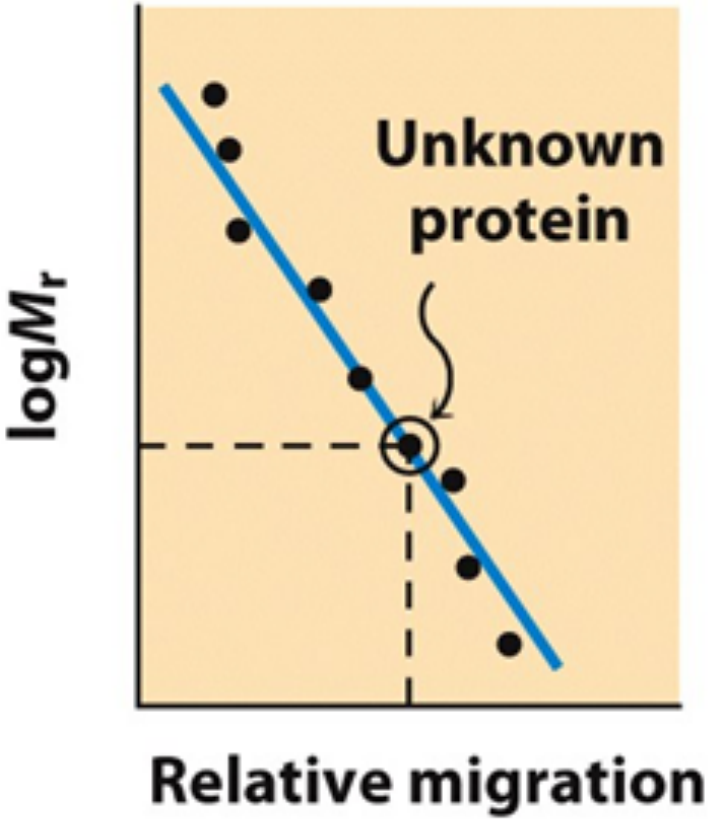
Note: All data represent the status of the sample *after* the designated procedure has been carried out.

Polyphosphate kinase from *Marinobacter hydrocarbonoclasticus*

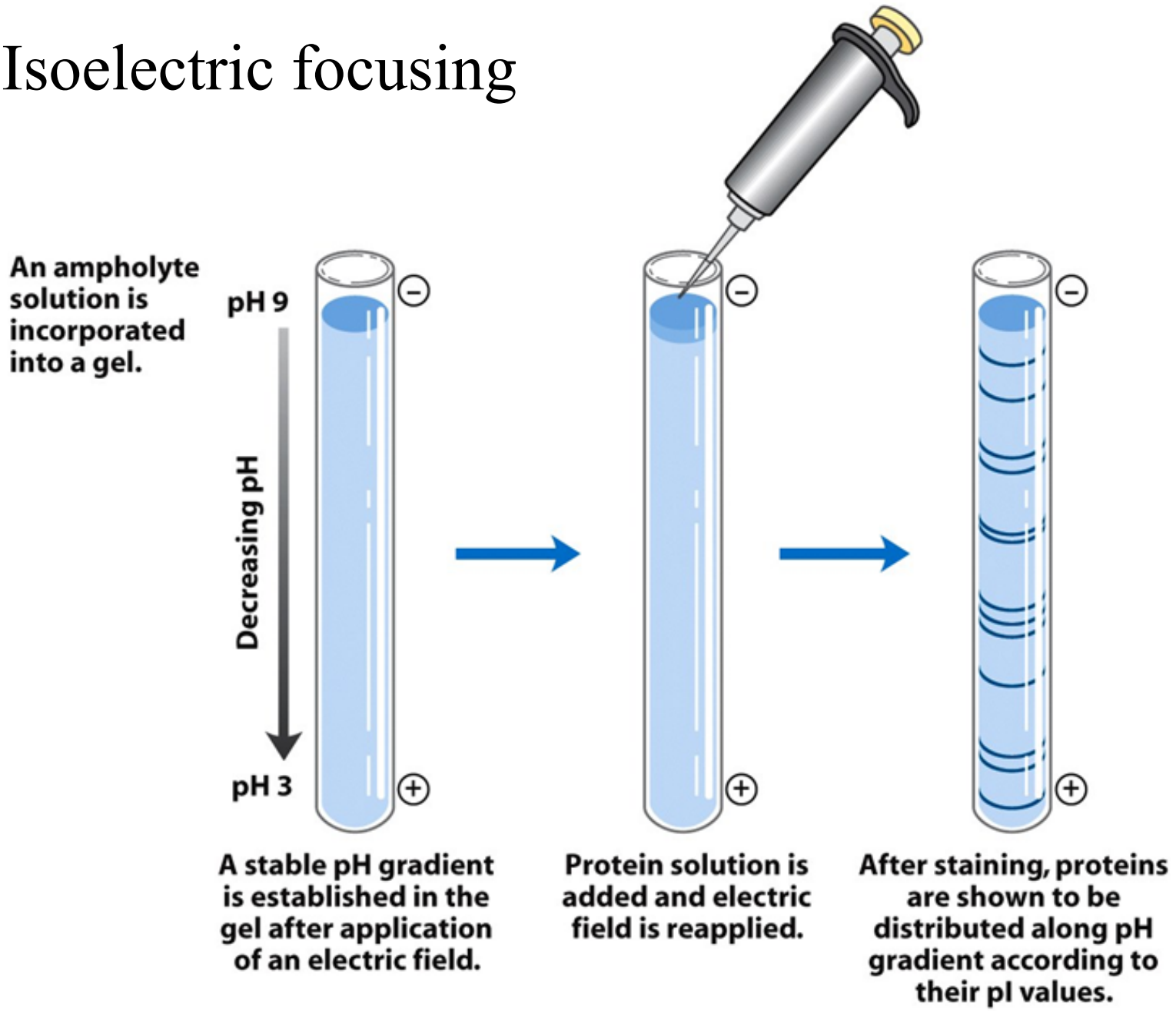


Molecular weight determination

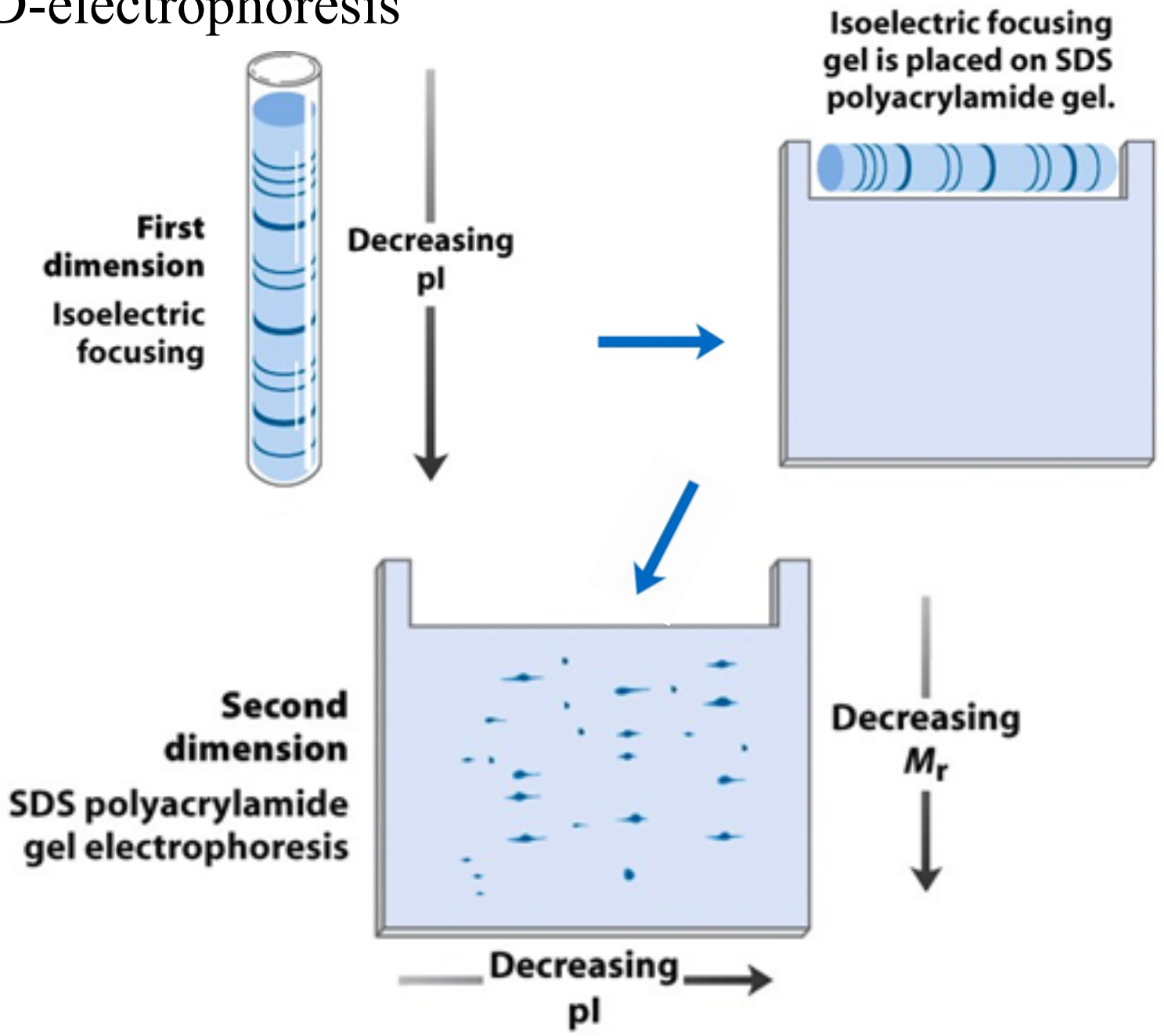




Isoelectric focusing



2D-electrophoresis



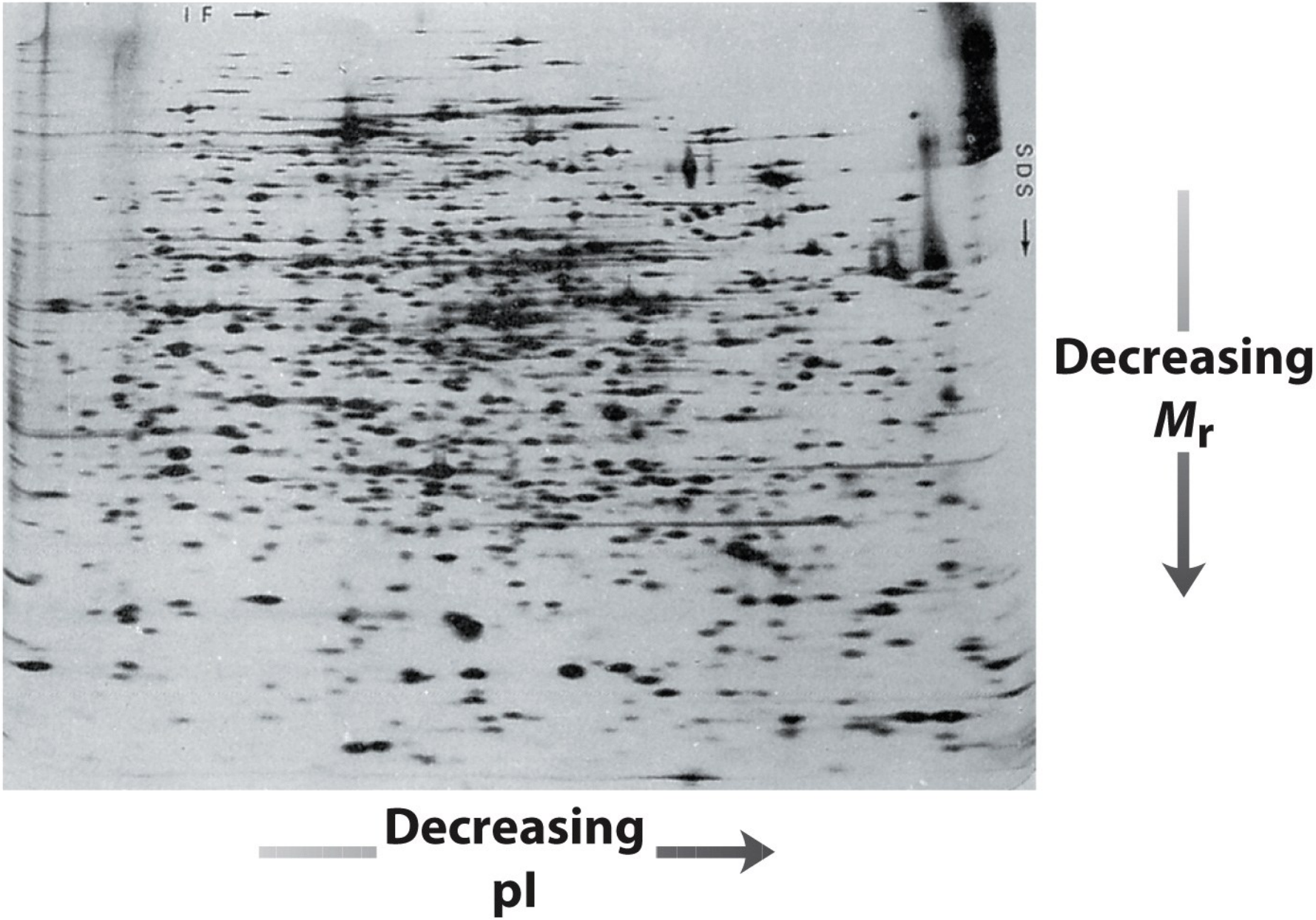
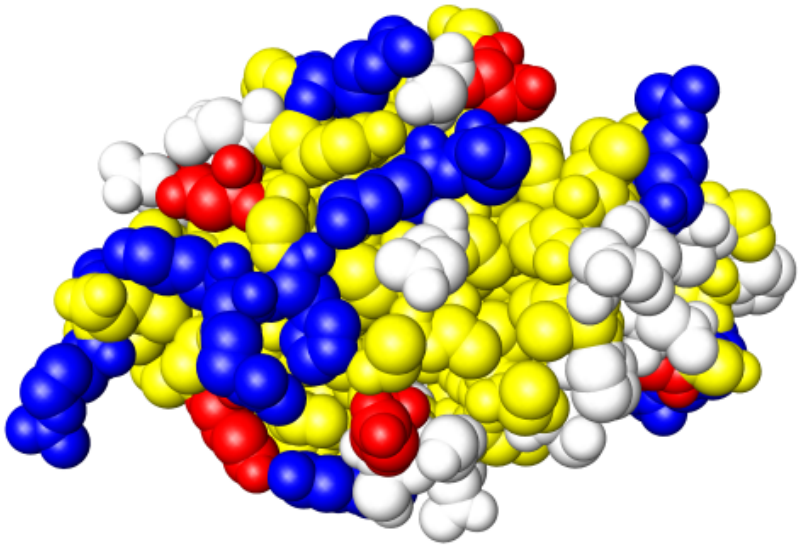


Figure 3-21b
Lehninger Principles of Biochemistry, Fifth Edition

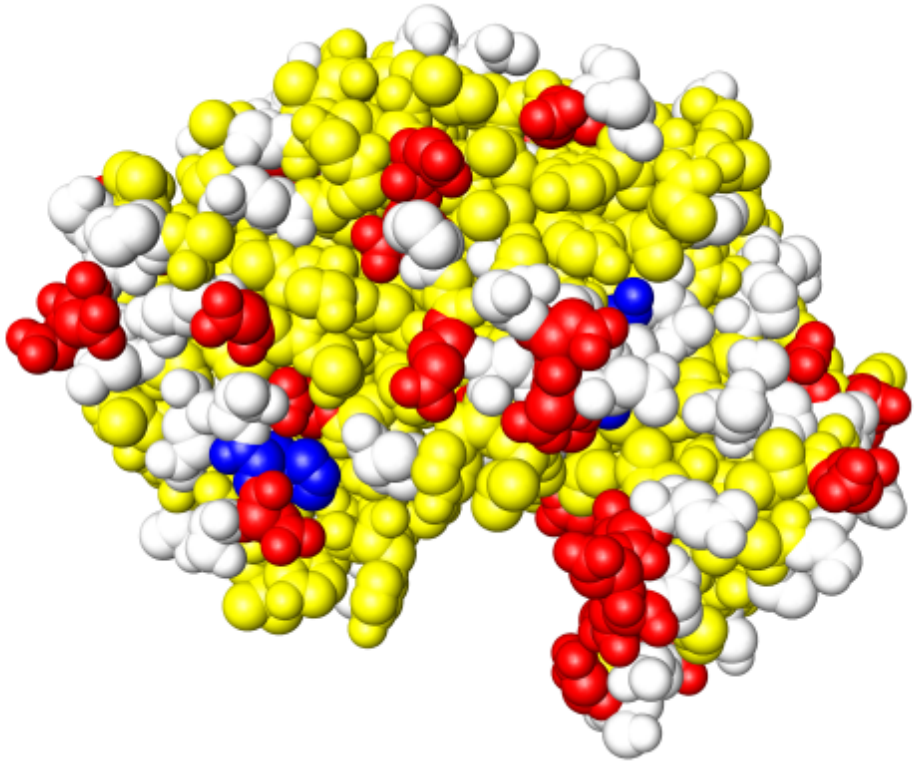
Isoelectric Points of Several Common Proteins

Protein	p*I*
Pepsin	<1.0
Ovalbumin (hen)	4.6
Serum albumin (human)	4.9
Tropomyosin	5.1
Insulin (bovine)	5.4
Fibrinogen (human)	5.8
γ-Globulin (human)	6.6
Collagen	6.6
Myoglobin (horse)	7.0
Hemoglobin (human)	7.1
Ribonuclease A (bovine)	9.4
Cytochrome *c* (horse)	10.6
Histone (bovine)	10.8
Lysozyme (hen)	11.0
Salmine (salmon)	12.1

Lisozyme (*Gallus gallus*) pI = 11



Pepsin (*Sus scropha*) pI = 1



Color code: acidic = red; basic = blue; polar = white; hydrofobic = yellow