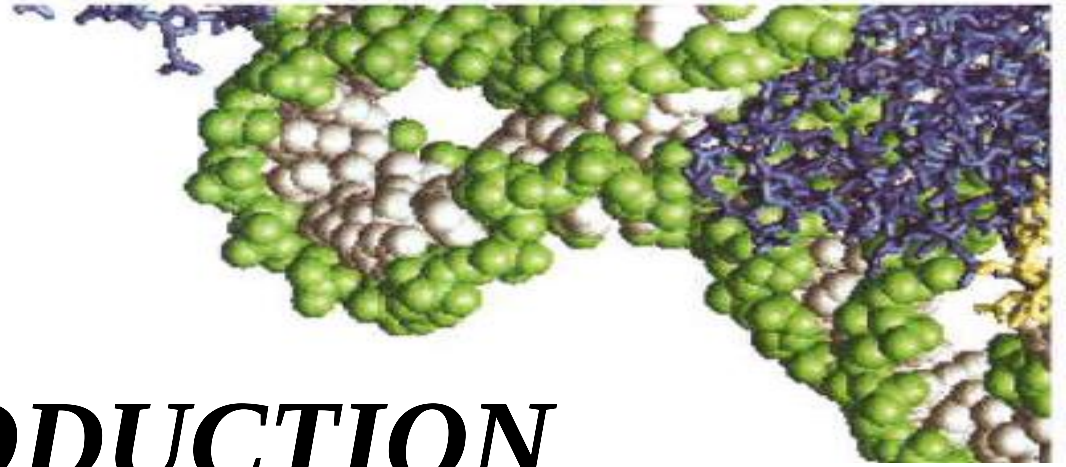


INTRODUCTION
into
BIOCHEMISTRY

Biochemistry

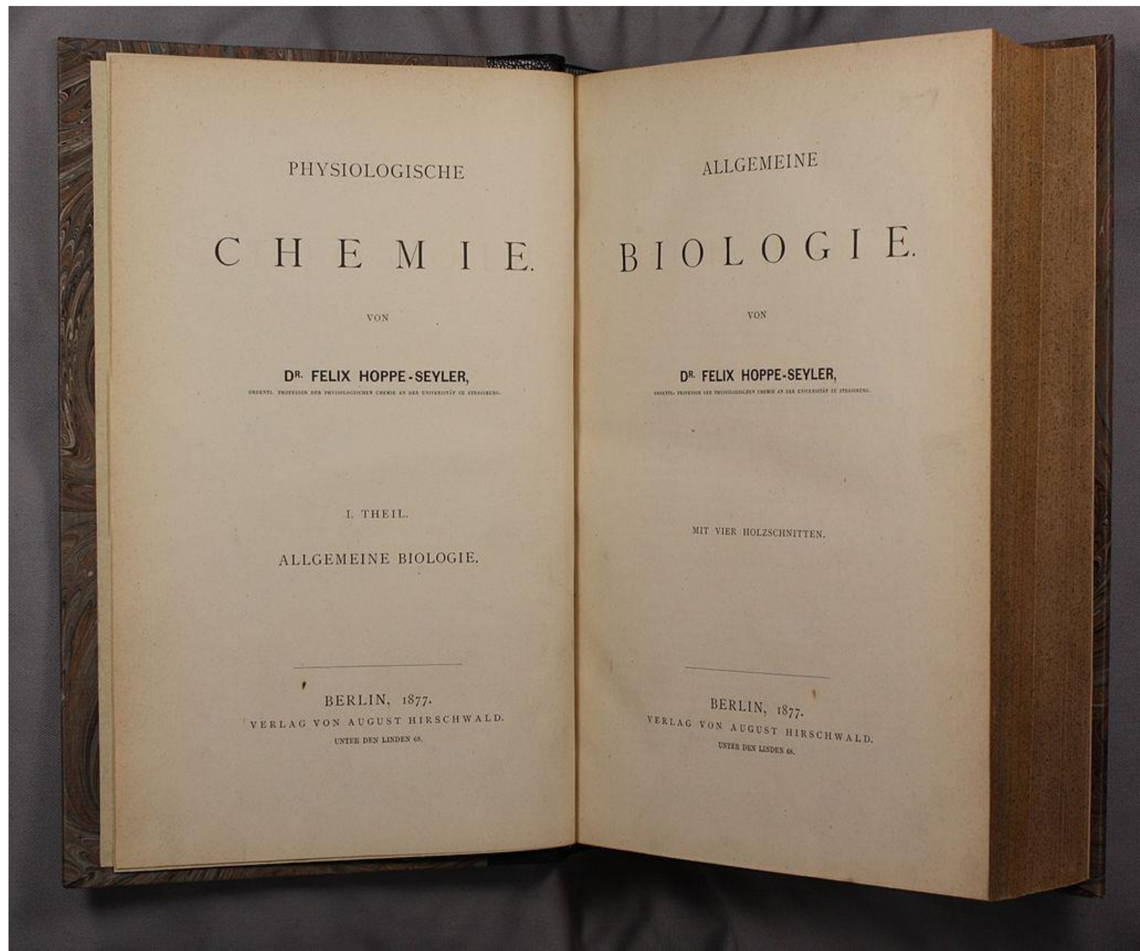
the science of the chemical constituents of living cells

and of the reactions and processes they undergo

The term "biochemistry" itself is derived from a combination of *biology* and *chemistry*

In 1877, Felix Hoppe-Seyler used the term as a synonym for *physiological chemistry* in the first issue of Journal of Physiological Chemistry

Felix Hoppe-Seyler, a physiologist and chemist, became the principal founder of biochemistry. His text **Physiological Chemistry** became the standard text for this new branch of applied chemistry



Although the term “biochemistry” seems to have been first used earlier, it is generally accepted that the word "biochemistry" was first proposed in 1903 by Carl Neuberg, a German chemist.

Because life depends on biochemical reactions,

Biochemistry

has become the basic language of all biological sciences

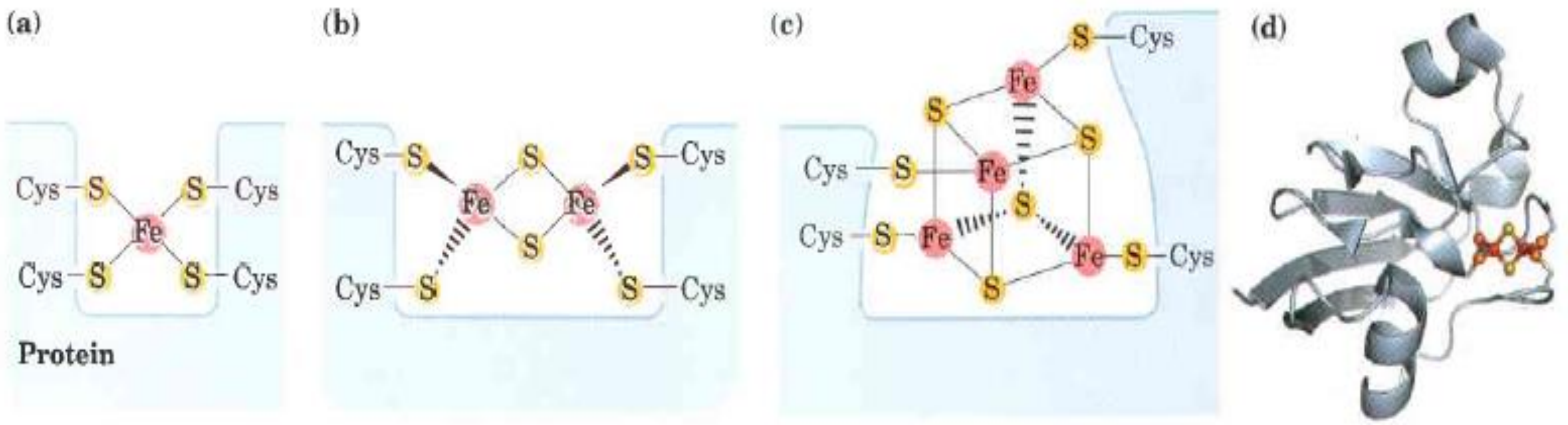
Biochemistry

is a field of science concerned with the study of:

- **chemical properties of the compounds constitutive of the living organism**
- **their conversions**
- **relation of these conversions to the activity of organs and tissues**

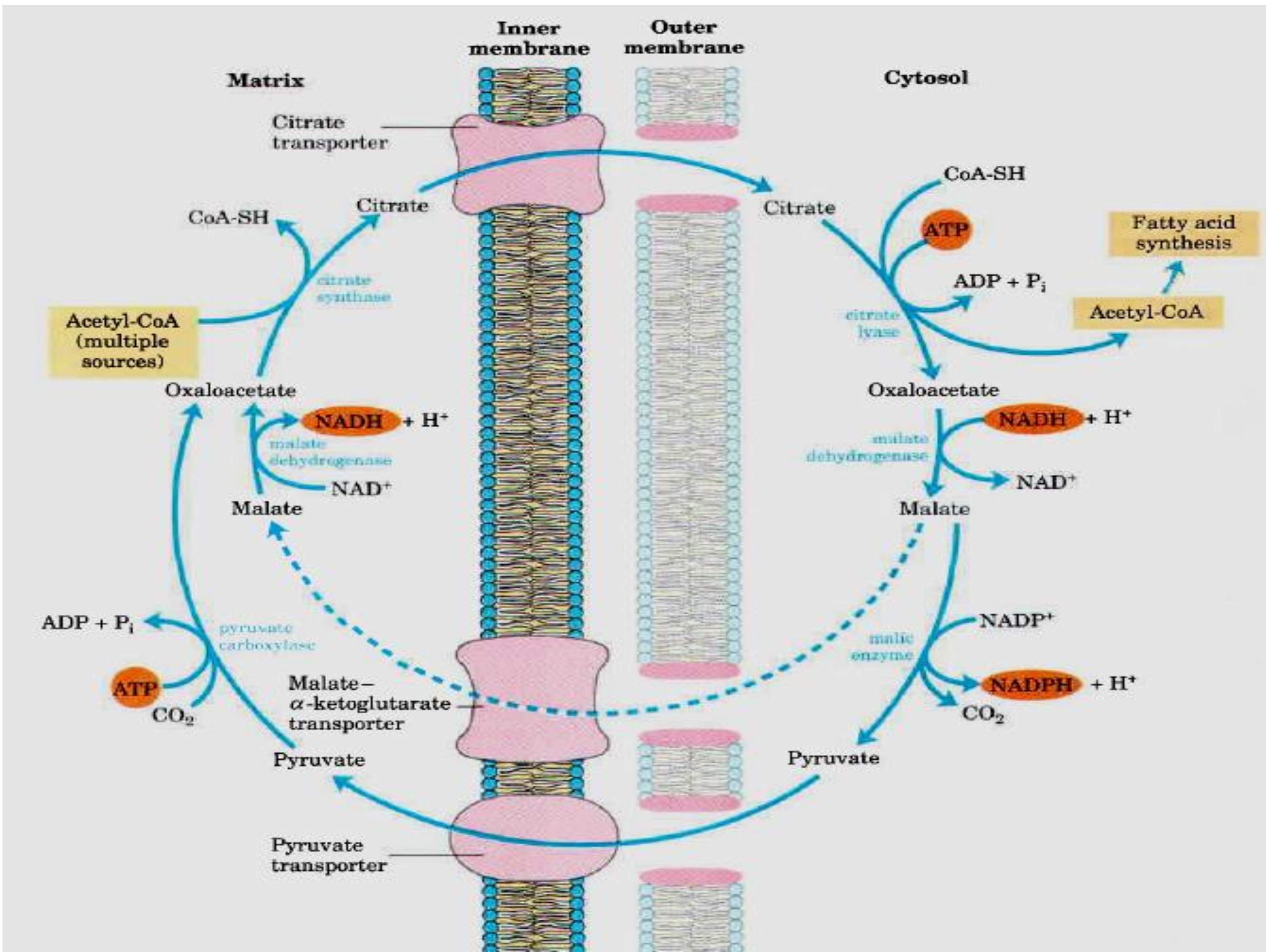
Static biochemistry

analysis of the chemical composition of the living organism



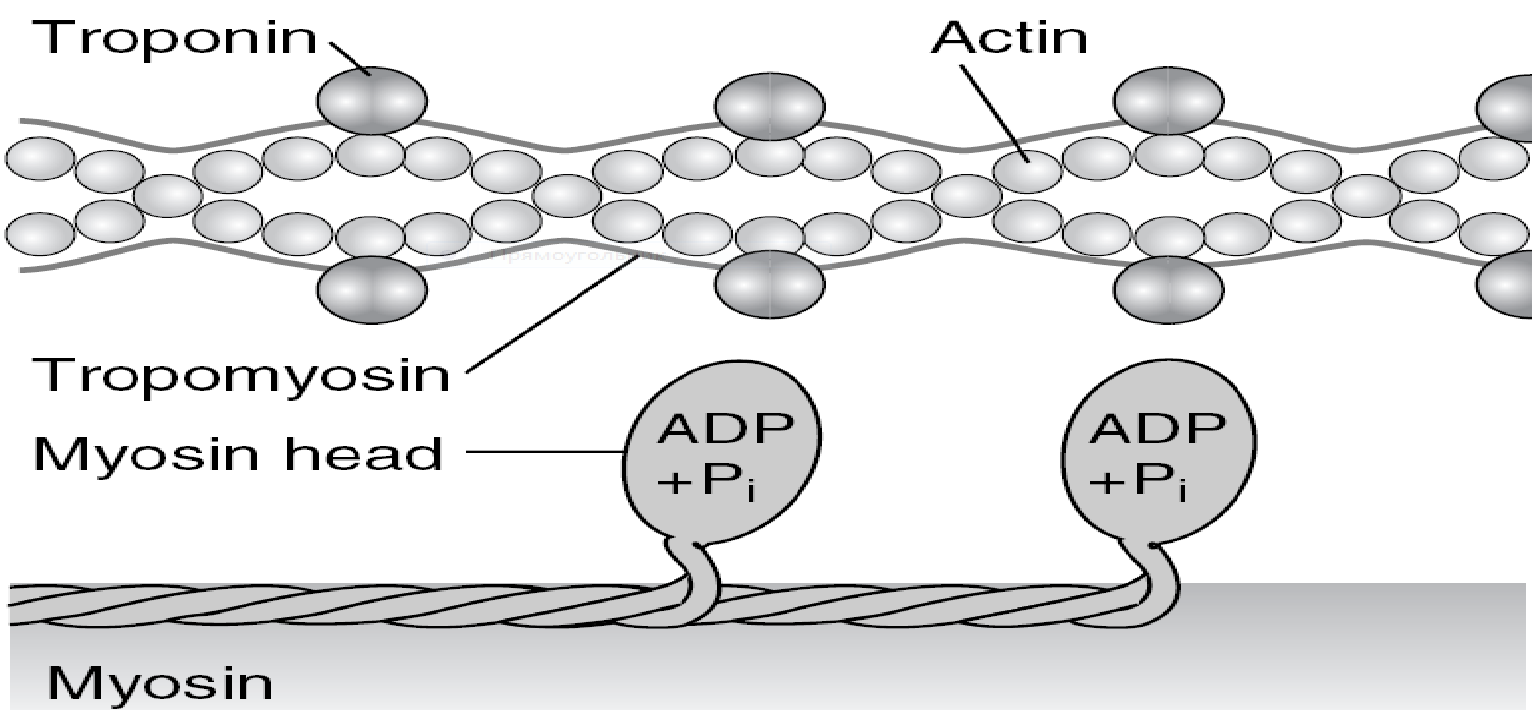
Dynamic biochemistry

study of the whole variety of metabolic conversions in organism



Functional biochemistry

concerned with the chemical processes that constitute a basis of various manifestations of vital activity



Biochemistry is concerned with the entire spectrum of life forms,

from relatively simple viruses and bacteria

to complex human beings

Depending on the object of study biochemistry divided into:

- **biochemistry of humans and animals**
- **biochemistry of plants**
- **biochemistry of microorganisms**

Major objective of biochemistry:

complete understanding,

at the molecular level,

of all the chemical processes associated with living cells

Methods used in biochemistry

Researchers in biochemistry use specific techniques native to biochemistry, but increasingly combine these with techniques and ideas developed in the fields of genetics, molecular biology and biophysics.

Methods used in biochemistry

- **for separating and purifying biomolecules**

  - **salt fractionation**
  - **chromatography**
  - **electrophoresis**
  - **ultracentrifugation**

Methods used in biochemistry

- **for determining biomolecular structure**

- **elemental analysis**
- **spectroscopy**
- **mass spectrometry**
- **X-ray crystallography**
- **use hydrolysis and enzymes to degrade the biomolecules**

Methods used in biochemistry

- ***for determining substances concentrations***

- spectrophotometry

- colourimetry

Preparations for biochemical studies

- **whole organism**
- **isolated perfused organ**
- **tissue slice**
- **whole cells**
- **homogenates**
- **isolated cell organelles**
- **purified metabolites and enzymes**
- **isolated genes**

History of biochemistry

The modern biochemistry as an independent discipline has emerged at the turn of the 20th century. In prior periods the problems of biochemistry were studied by classical chemistry and physiology.

History of biochemistry

<u>I period</u>

ancient time – 15th century

In this period people used biochemical processes to make bread, cheese, wine, through the essence of these processes was unknown to them

History of biochemistry

II period: 15th century– first half of the 19th century

German physician Paracelsus (1493-1541) put forward the concept of a close relationship between chemistry and medicine: chemical reactions formed the basis of vital activity and the cause of any disease is a disturbance of the natural course of chemical processes within the organism.

II period: 15th century– first half of the 19th century

Russian scientist
(1711-1765) –

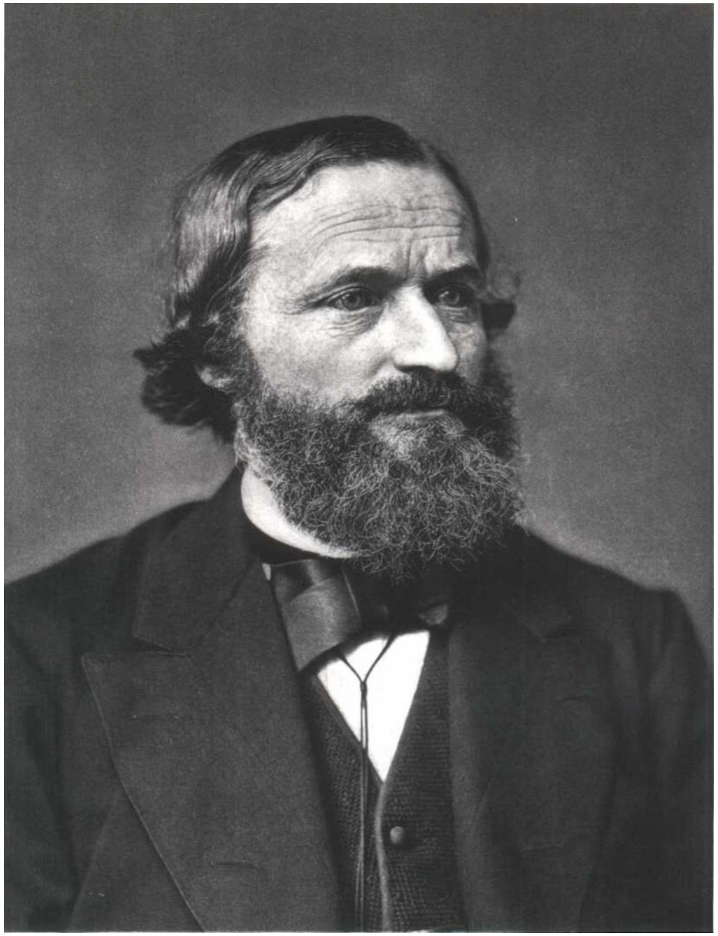
formulation of the law of conservation of mass

<u>II period</u>: 15th century– first half of the 19th century

French chemist
Lavoisier (1743-1794) –

in respiration of living organisms oxygen consumed and carbon dioxide evolved

II period: 15th century– first half of the 19th century



Russian chemist
Kirchhoff (1764-1833)

described in 1814 an enzymatic process of starch saccharization by the action of an extract from the germinated barley corn

<u>II period</u>: 15th century– first half of the 19th century

By the 1850s, other enzymes were discovered:

salivary amylase, pepsin in gastric juice, trypsin of pancreatic juice

II period: 15th century– first half of the 19th century

Justus von Liebig

1842

influential work

Animal chemistry, or, Organic chemistry in its applications to physiology and pathology,

which presented a chemical theory of metabolism

History of biochemistry

<u>III period</u>:

second half of the 19th century – first half of the 20th century

French scientist Pasteur (1821-1895) – studies of fermentation with participation of living yeast cells

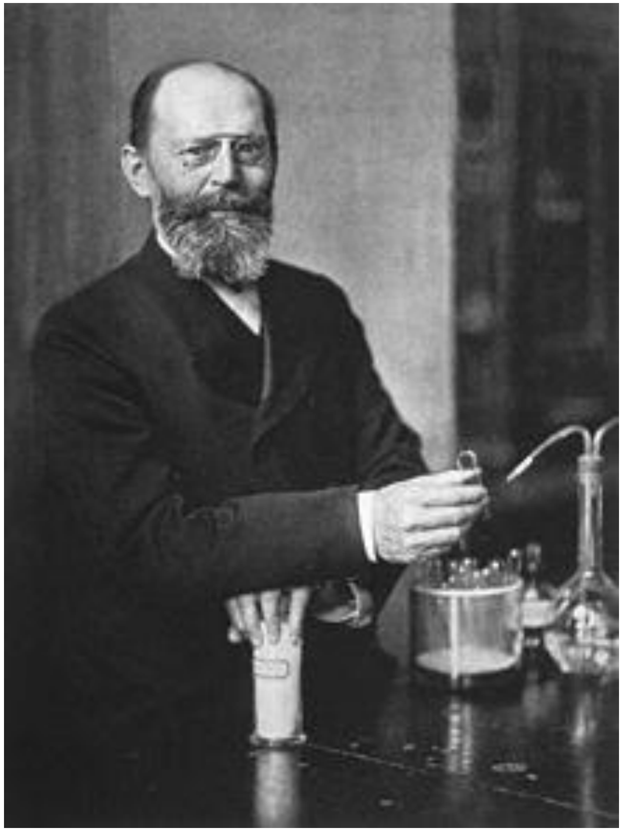
<u>III period</u>: second half of the 19th century – first half of the 20th century

German chemist Buchner in 1897 provide evidence for ability of a cell-free yeast to produce alcoholic fermentation. For his works cell-free fermentation was awarded a Nobel Prize chemistry in 1907)

***III period*: second half of the 19th century – first half of the 20th century**

In the second half of the 19th century special chairs of medical, or physiological, chemistry were instituted at the medical departments of many European universities

***III period*: second half of the 19th century – first half of the 20th century**

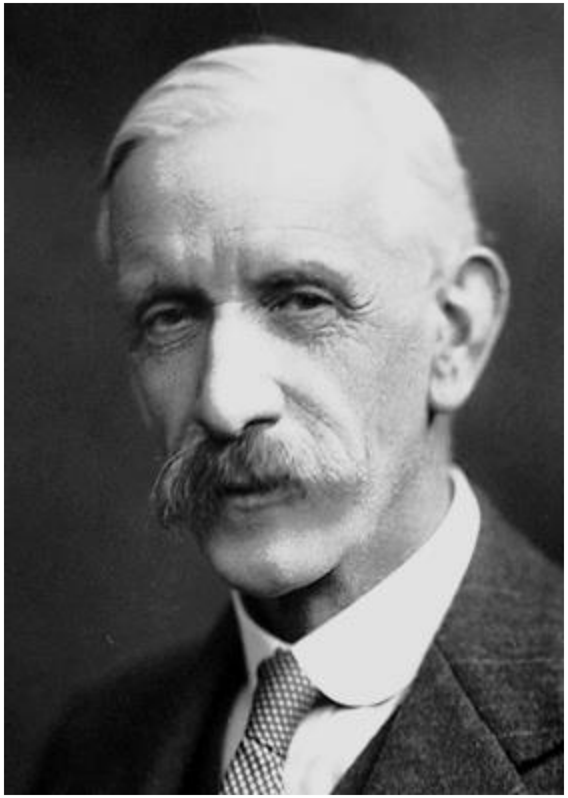


Emil Fischer

chemistry of proteins

***III period*: second half of the 19th century – first half of the 20th century**

Frederick Gowland Hopkins
English biochemist who was awarded the Nobel Prize in Physiology or Medicine in 1929, with Christiaan Eijkman for the discovery of vitamins



History of biochemistry

IV period:

1950s – present time

advent of biochemistry, development of new methodologic principles and techniques

such as chromatography, X-ray diffraction,

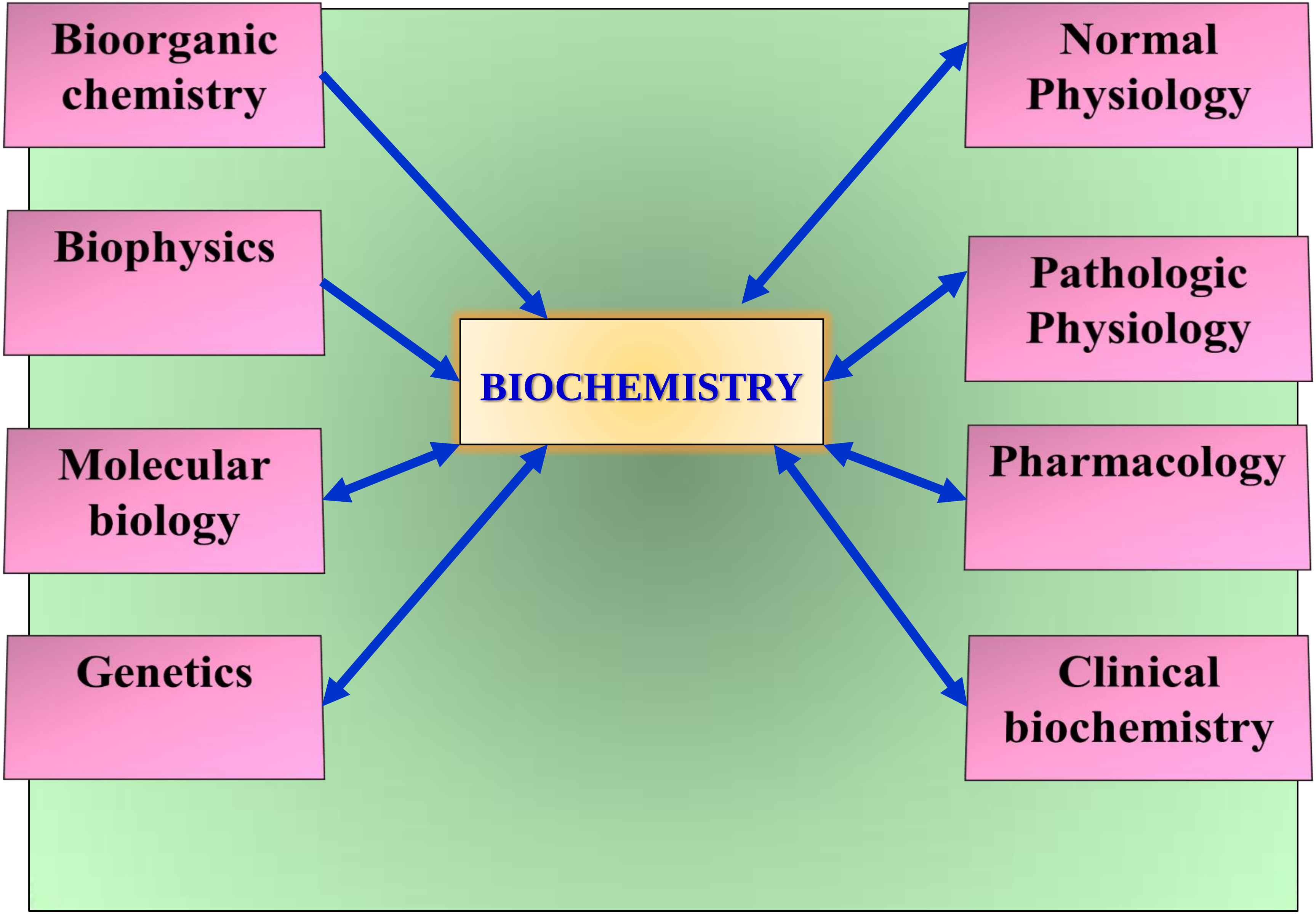
NMR spectroscopy, radioisotopic labelling, electron microscopy and molecular dynamics simulations.

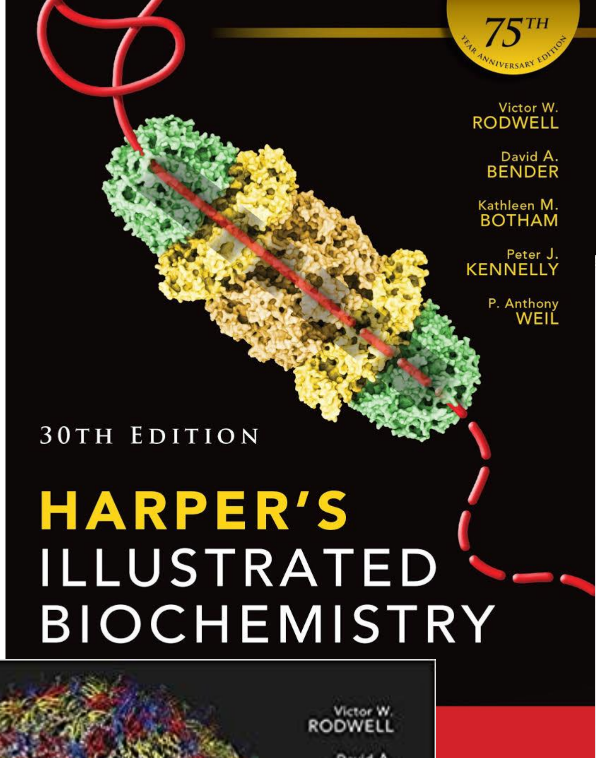
These techniques allowed for the discovery and detailed analysis of many molecules and metabolic pathways of the cell, such as glycolysis and the Krebs cycle (citric acid cycle)

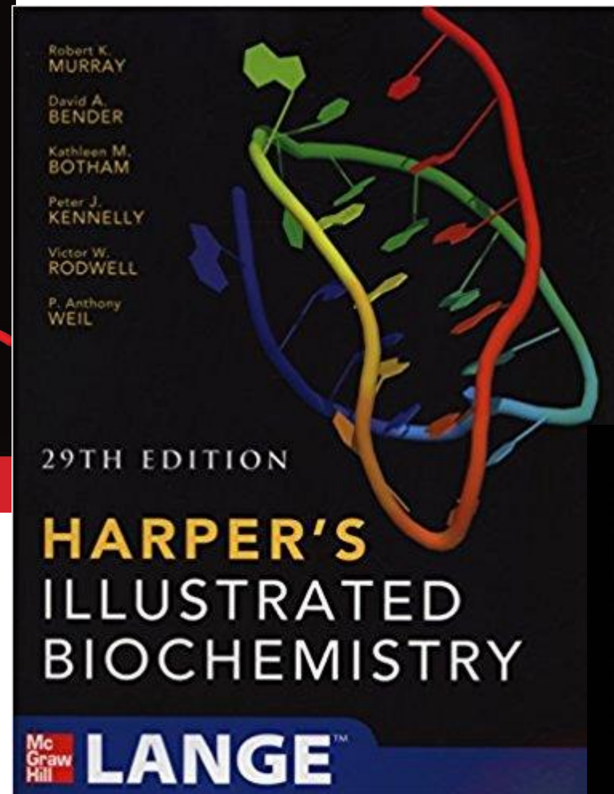
Another significant historic event in biochemistry is the discovery of the gene and its role in the transfer of information in the cell. This part of biochemistry is often called molecular biology. In the 1950s, James D. Watson, Francis Crick, Rosalind Franklin, and Maurice Wilkins were instrumental in solving DNA structure and suggesting its relationship with genetic transfer of information.

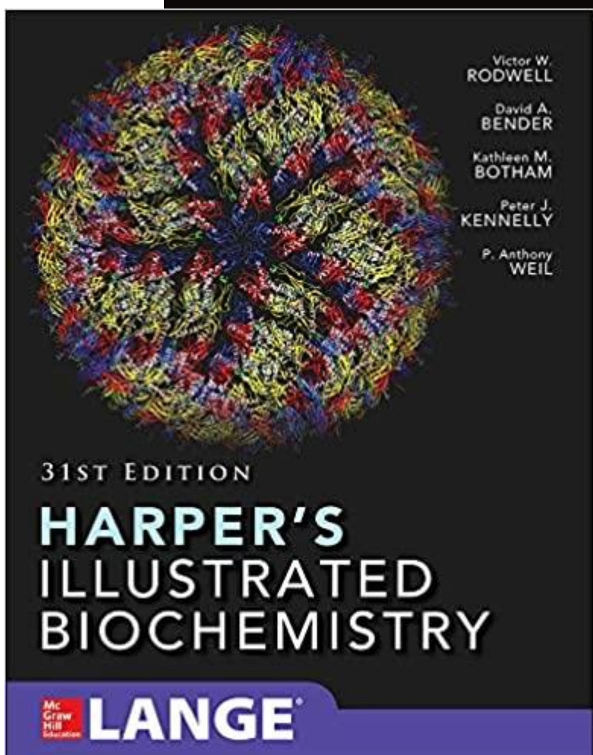
- **In 1958, George Beadle and Edward Tatum received the Nobel Prize for work in fungi showing that one gene produces one enzyme.**

- **In 1988, Colin Pitchfork was the first person convicted of murder with DNA evidence, which led to growth of forensic science.**


Bioorganic chemistry
Biophysics
Molecular biology
Genetics
BIOCHEMISTRY
Normal Physiology
Pathologic Physiology
Pharmacology
Clinical biochemistry


75TH
YEAR ANNIVERSARY EDITION
Victor W. RODWELL
David A. BENDER
Kathleen M. BOTHAM
Peter J. KENNELLY
P. Anthony WEIL
30TH EDITION
HARPER'S
ILLUSTRATED
BIOCHEMISTRY


Robert K. MURRAY
David A. BENDER
Kathleen M. BOTHAM
Peter J. KENNELLY
Victor W. RODWELL
P. Anthony WEIL
29TH EDITION
HARPER'S
ILLUSTRATED
BIOCHEMISTRY
Mc Graw Hill
LANGE


Victor W. RODWELL
David A. BENDER
Kathleen M. BOTHAM
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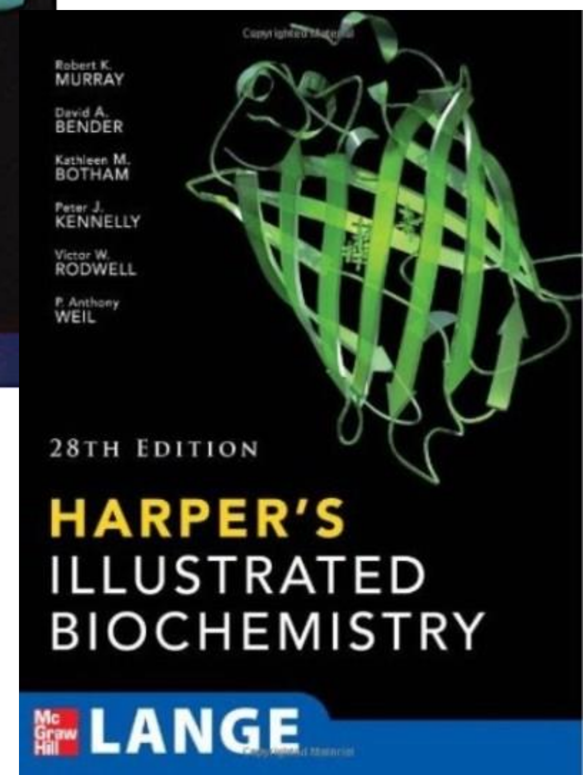
31ST EDITION
HARPER'S
ILLUSTRATED
BIOCHEMISTRY
LANGE


Robert K. MURRAY
David A. BENDER
Kathleen M. BOTHAM
Peter J. KENNELLY
Victor W. RODWELL
P. Anthony WEIL
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BIOCHEMISTRY
Mc Graw Hill
LANGE

STRUCTURE and FUNCTIONS of PROTEINS

Lecture I

PROTEINS

high-molecular nitrogen-containing organic compounds whose molecules are built up of amino acid residues

Short polymers of amino acids called

PEPTIDES

1838 - G.Mulder **proteins were first described**

1838 – J.Berzelius **term "protein"**

1888 - A.Danilevsky **peptide bond**

1925-1930 –T.Svedberg **sedimentation analysis**

1951 – L.Pauling **prediction of regular protein secondary structure**

1952 – K.Linderstrom-Lang **protein folding and structure mediated by hydrophobic interactions**

1953 – F.Sanger **determined the amino acid sequence of insulin**

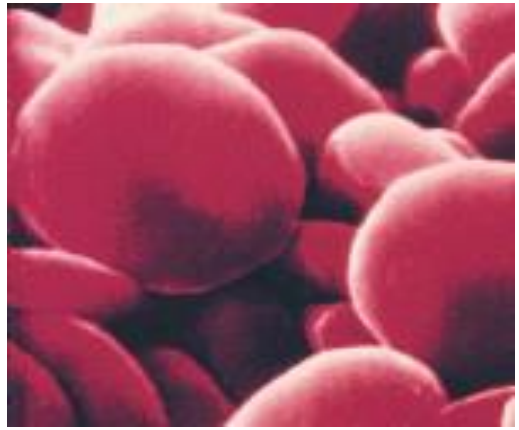
1958 - J. Kendrew **structure of myoglobin**

1959 – M.Perutz **structure of hemoglobin**

- **Proteins are the most abundant biological macromolecules, occurring in all cells and all parts of cells.**
- **Proteins also occur in great variety; thousands of different kinds, ranging in size from relatively small peptides to huge polymers with molecular weights in the millions.**
- **Proteins exhibit enormous diversity of biological function and are the most important final products of the information pathways.**

Functions of proteins

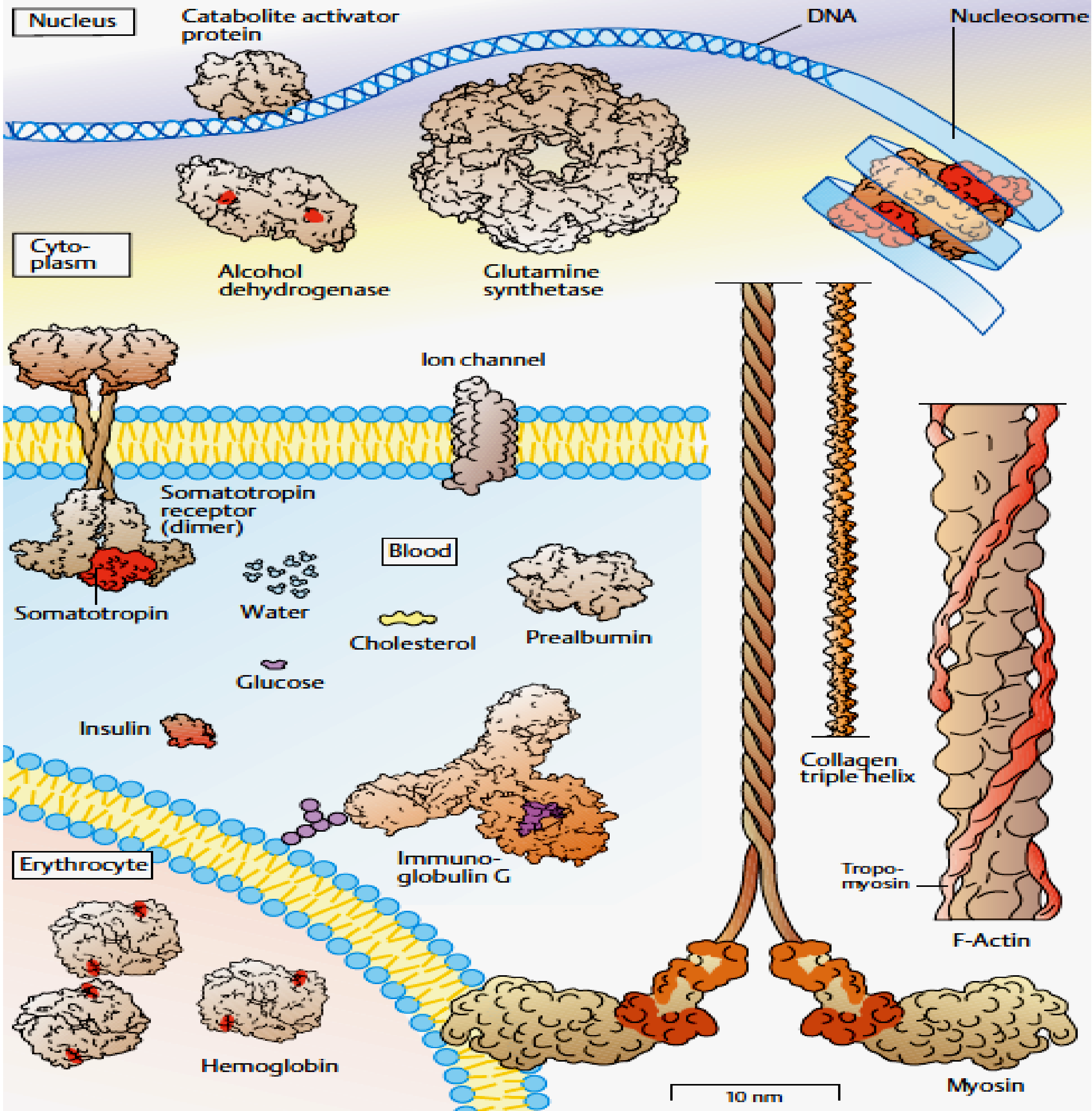
- Catalytic
- Nutritive (reserve)
- Transport
- Protective
- Contractile
- Structural
- Hormonal
- Receptors
- Hemostatic
- Toxigenic
- Antitoxic

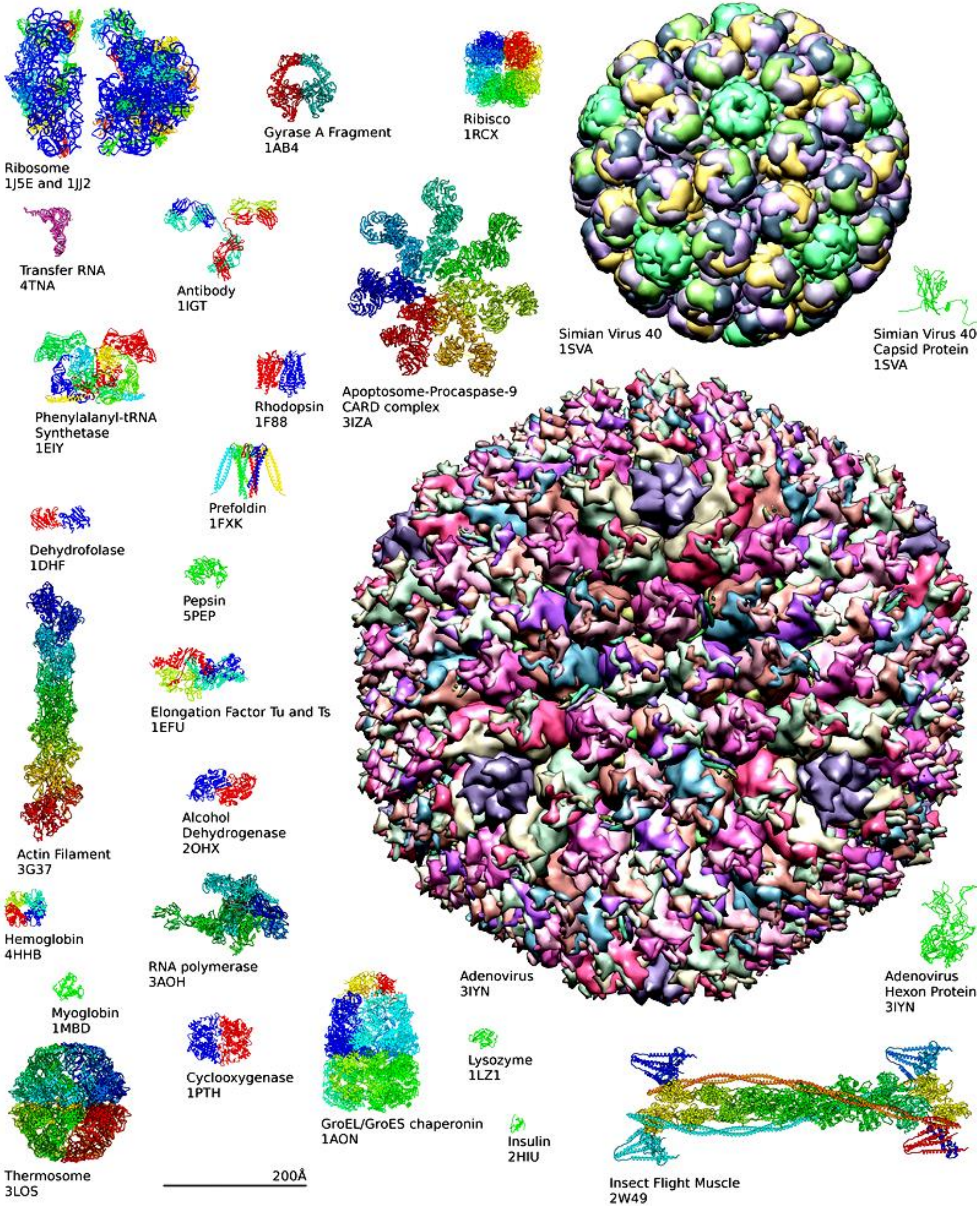
The light produced by fireflies is the result of a reaction involving the protein luciferin and ATP, catalyzed by the enzyme luciferase.

Erythrocytes contain large amounts of the oxygen-transporting protein hemoglobin.

The protein keratin, formed by all vertebrates, is the chief structural component of hair, scales, horn, wool, nails, and feathers.



Nucleus
Catabolite activator protein
DNA
Nucleosome
Cyto-plasm
Alcohol dehydrogenase
Glutamine synthetase
Ion channel
Somatotropin receptor (dimer)
Blood
Somatotropin
Water
Cholesterol
Prealbumin
Glucose
Insulin
Collagen triple helix
Tropo-myosin
F-Actin
Immuno-globulin G
Erythrocyte
Hemoglobin
10 nm
Myosin


Ribosome
1J5E and 1JJ2
Gyrase A Fragment
1AB4
Ribisco
1RCX
Simian Virus 40
1SVA
Simian Virus 40
Capsid Protein
1SVA
Transfer RNA
4TNA
Antibody
1IGT
Apoptosome-Procaspase-9
CARD complex
3IZA
Phenylalanyl-tRNA
Synthetase
1EIY
Rhodopsin
1F88
Prefoldin
1FXK
Dehydrofolase
1DHF
Pepsin
5PEP
Elongation Factor Tu and Ts
1EFU
Actin Filament
3G37
Alcohol
Dehydrogenase
2OHX
Hemoglobin
4HHB
RNA polymerase
3AOH
Myoglobin
1MBD
Adenovirus
3IYN
Adenovirus
Hexon Protein
3IYN
Cyclooxygenase
1PTH
Lysozyme
1LZ1
GroEL/GroES chaperonin
1AON
Insulin
2HIU
Thermosome
3LOS
200Å
Insect Flight Muscle
2W49

Amino acid composition of proteins

- **Structure of amino acids**
- **Classification**
- **Peptide bond formation**

Harper's Illustrated Biochemistry

In addition to the 20 common amino acids, proteins may contain residues created by modification of common residues already incorporated into a polypeptide.

Among these uncommon amino acids are

4-hydroxyproline, a derivative of proline,

and 5-hydroxylysine, derived from lysine.

The former is found in plant cell wall proteins, and both are found in collagen, a fibrous protein of connective tissues.

New Amino Acids

In addition to 20 L-amino acids that take part in protein synthesis, recently two more new amino acids described.

They are:

- **Selenocysteine** - 21st amino acids

Selenocysteine occurs at the active site of several enzymes

Some 300 additional amino acids have been found in cells. They have a variety of functions but are not constituents of proteins.

Ornithine and citrulline deserve special note because they are key metabolites in the biosynthesis of arginine and in the urea cycle.

Protein content of the organs and tissues

In total human body the proteins account for 45% of dry mass

	(dry tissue mass)	(fresh tissue mass)
Muscle	80%	18-23%
Lung	82%	14-15%
Spleen	84%	17-18%
Kidney	72%	16-17%
Liver	57%	18-19%

Physico-chemical properties of proteins

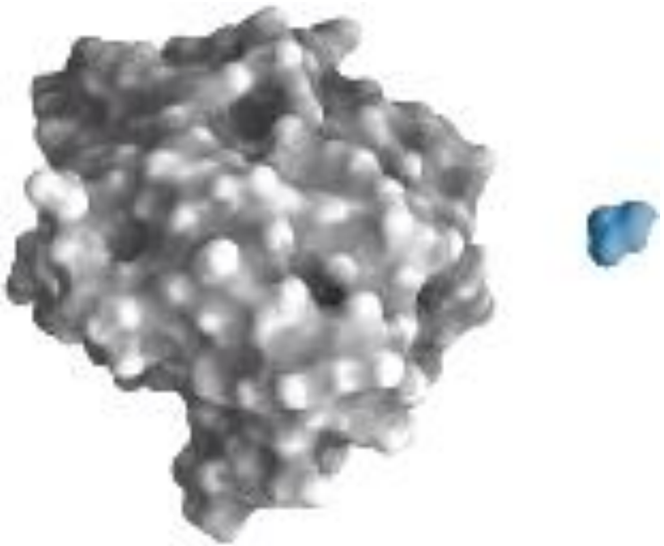
- **High molecular weight**
- **Shape and size of molecules**
- **High viscosity in solution**
- **Low diffusion**
- **Pronounced swelling ability**
- **Optical activity**

Physico-chemical properties of proteins

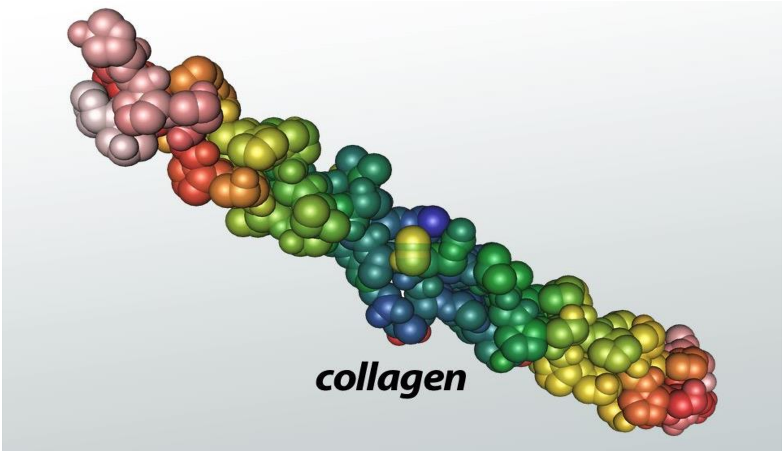
- **Carge**
- **Mobility in electric field**
- **Low osmotic and high oncotic pressures**
- **Solubility**
- **Ability to absorhb UV light at 280 nm**
- **Amphoteric**
- **Denaturation**

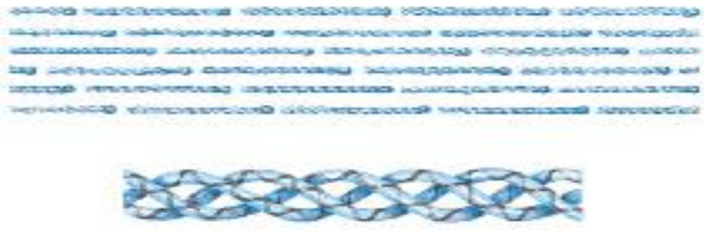
Shape of protein molecules

- **Globular**



- **Fibrous**





	Fibrous	Globular
Shape	Long and narrow	Round / spherical
Purpose	Structural	Functional
Acid Sequence	Repetitive amino acid sequence	Irregular amino acid sequence
Durability	Less sensitive to changes in pH, temperature, etc.	More sensitive to changes in pH, temperature, etc.
Examples	Collagen, myosin, fibrin, actin, keratin, elastin	Enzymes, haemoglobin, insulin, immunoglobulin
Solubility	(Generally) insoluble in water	(Generally) soluble in water

Denaturation of proteins

destruction of three-dimensional structure of protein molecule

with loss of specific properties

(solubility, electrophoretic mobility, biological activity and other)

Denaturation of proteins

Most proteins can be denatured:

- **by heat, which affects the weak interactions in a protein,**
- **by extremes of pH,**
- **by certain organic solvents such as alcohol or acetone,**
- **by certain solutes such as urea and guanidine hydrochloride,**
- **by detergents**
- **by heavy metal salts**

.

Molecular weight of protein

Varies from 6,000 Da

to over 1,000,000 Da

Molecular Weight of Some Proteins

Cytochrome *c* (human) 13,000
Ribonuclease A (bovine pancreas) 13,700
Lysozyme (chicken egg white) 13,930
Myoglobin (equine heart) 16,890
Chymotrypsin (bovine pancreas) 21,600
Chymotrypsinogen (bovine) 22,000
Hemoglobin (human) 64,500
Serum albumin (human) 68,500
Hexokinase (yeast) 102,000
RNA polymerase (*E. coli*) 450,000
Apolipoprotein B (human) 513,000
Glutamine synthetase (*E. coli*) 619,000
Titin (human) 2,993,000

Determination of molecular weight

- **Sedimentation analysis**
- **Gel chromatography**
- **Gel electrophoresis**

Sedimentation analysis

carried out by means of ultracentrifuges

(special centrifuge with great speed)

Theodor Svedberg


BECKMAN
COULTER

Sedimentation analysis

$$M = \frac{R\,T\,s}{D\,(1 - \nu\,\rho)}$$

R – gas constant

T – absolute temperature

s – sedimentation constant

ν **– the partial specific volume of the protein molecule**

D – diffusion coefficient

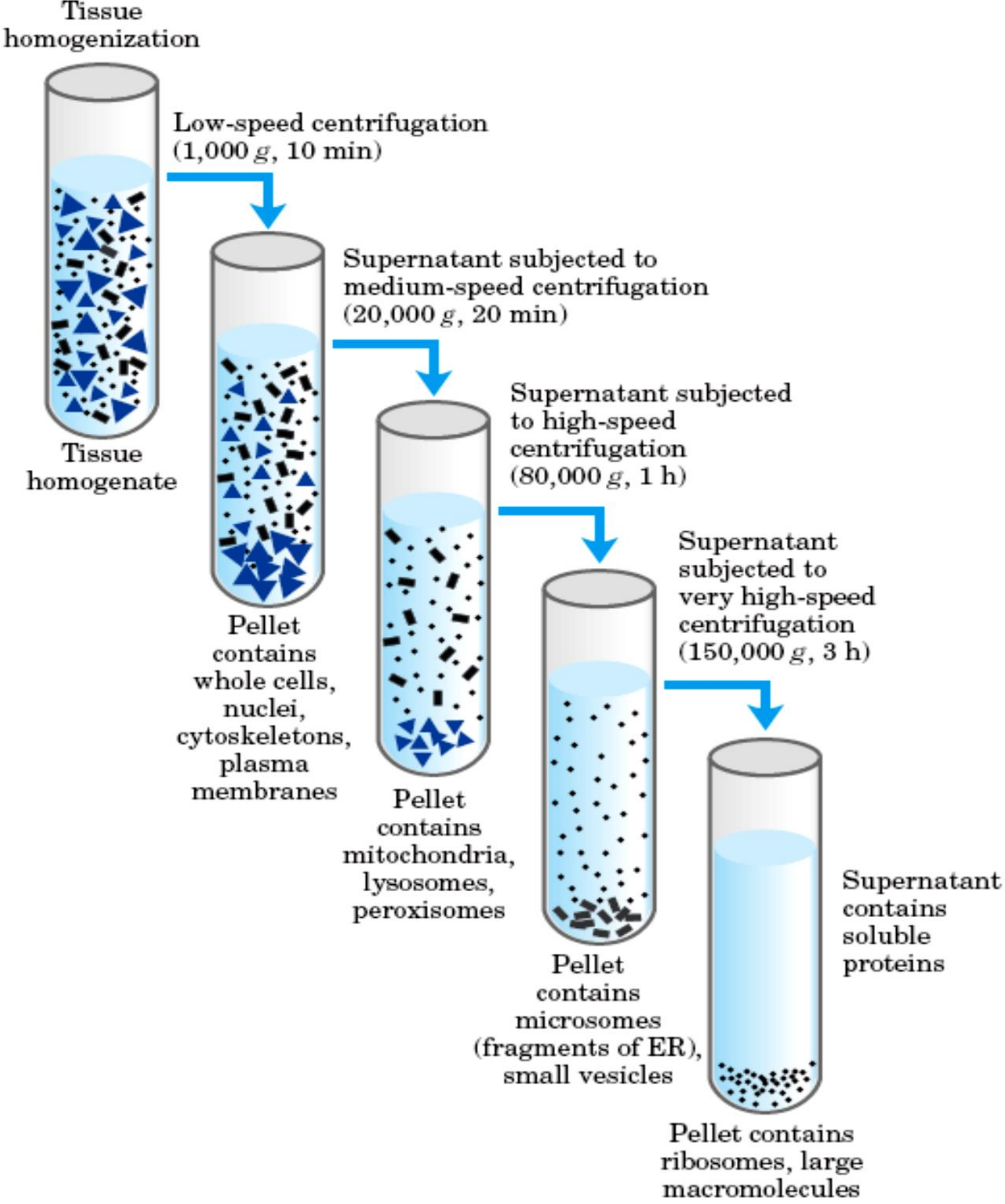
ρ **– solvent density**

Protein purification

The aim of protein purification is to isolate one particular protein from all the others in the starting material

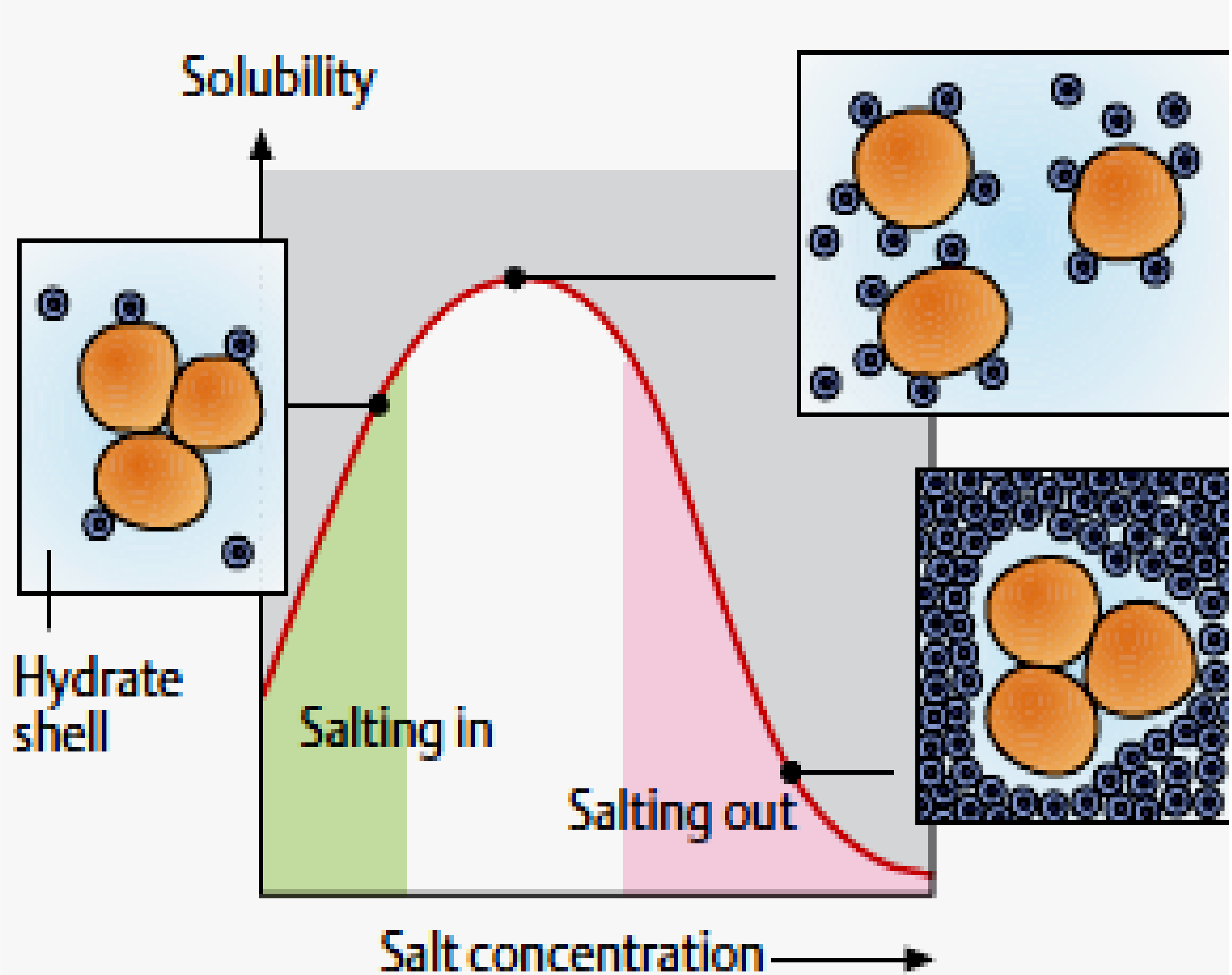
Protein purification stages

- **Selecton of a protein source**
- **Homogenization and solubilization**
- **Fractionation**
  - **salting-out**
  - **chromatography**
  - **electrophoresis**
- **Dialysis**



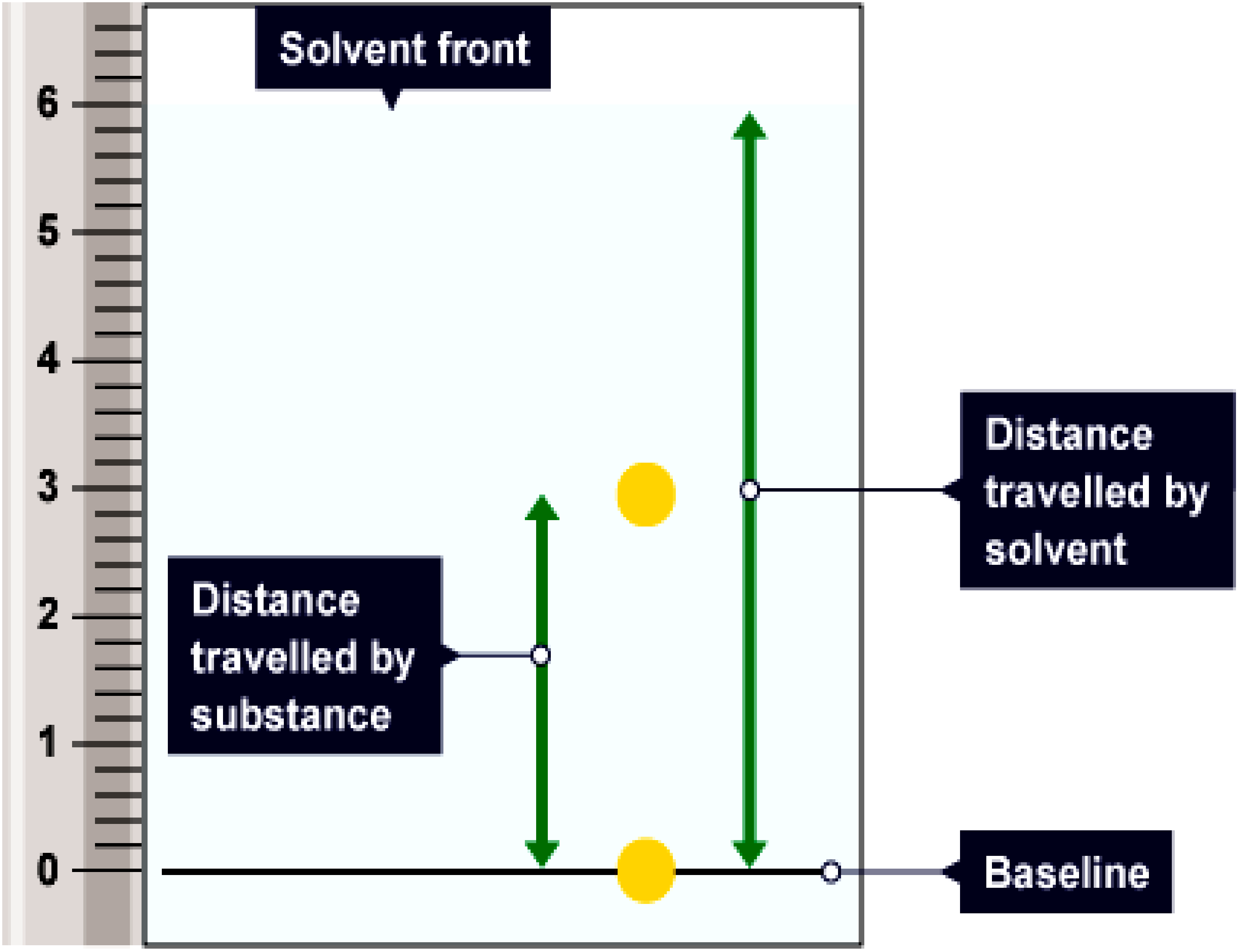
Tissue homogenization
Low-speed centrifugation (1,000 g, 10 min)
Tissue homogenate
Supernatant subjected to medium-speed centrifugation (20,000 g, 20 min)
Pellet contains whole cells, nuclei, cytoskeletons, plasma membranes
Supernatant subjected to high-speed centrifugation (80,000 g, 1 h)
Pellet contains mitochondria, lysosomes, peroxisomes
Supernatant subjected to very high-speed centrifugation (150,000 g, 3 h)
Pellet contains microsomes (fragments of ER), small vesicles
Supernatant contains soluble proteins
Pellet contains ribosomes, large macromolecules

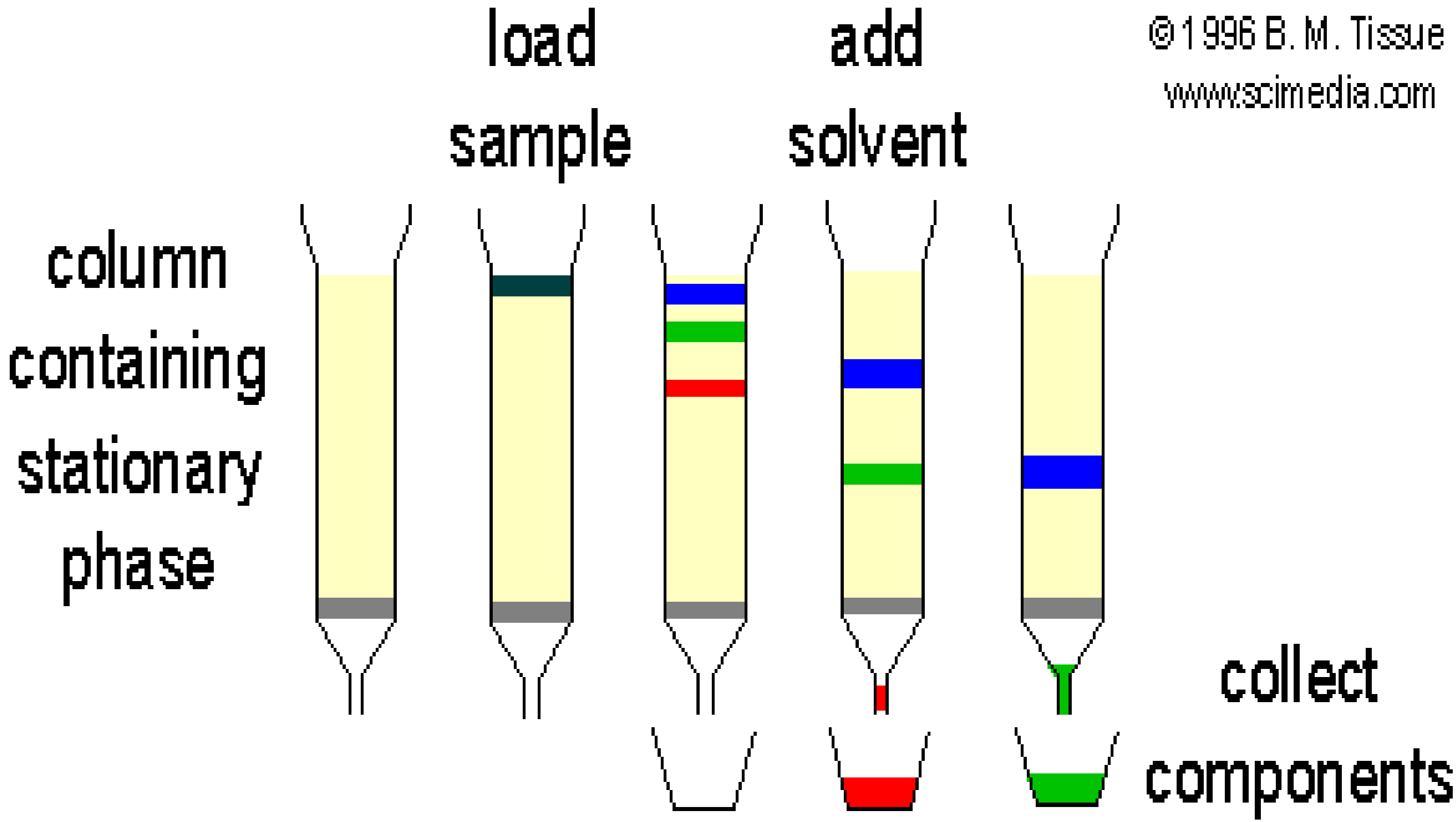
Salting-out

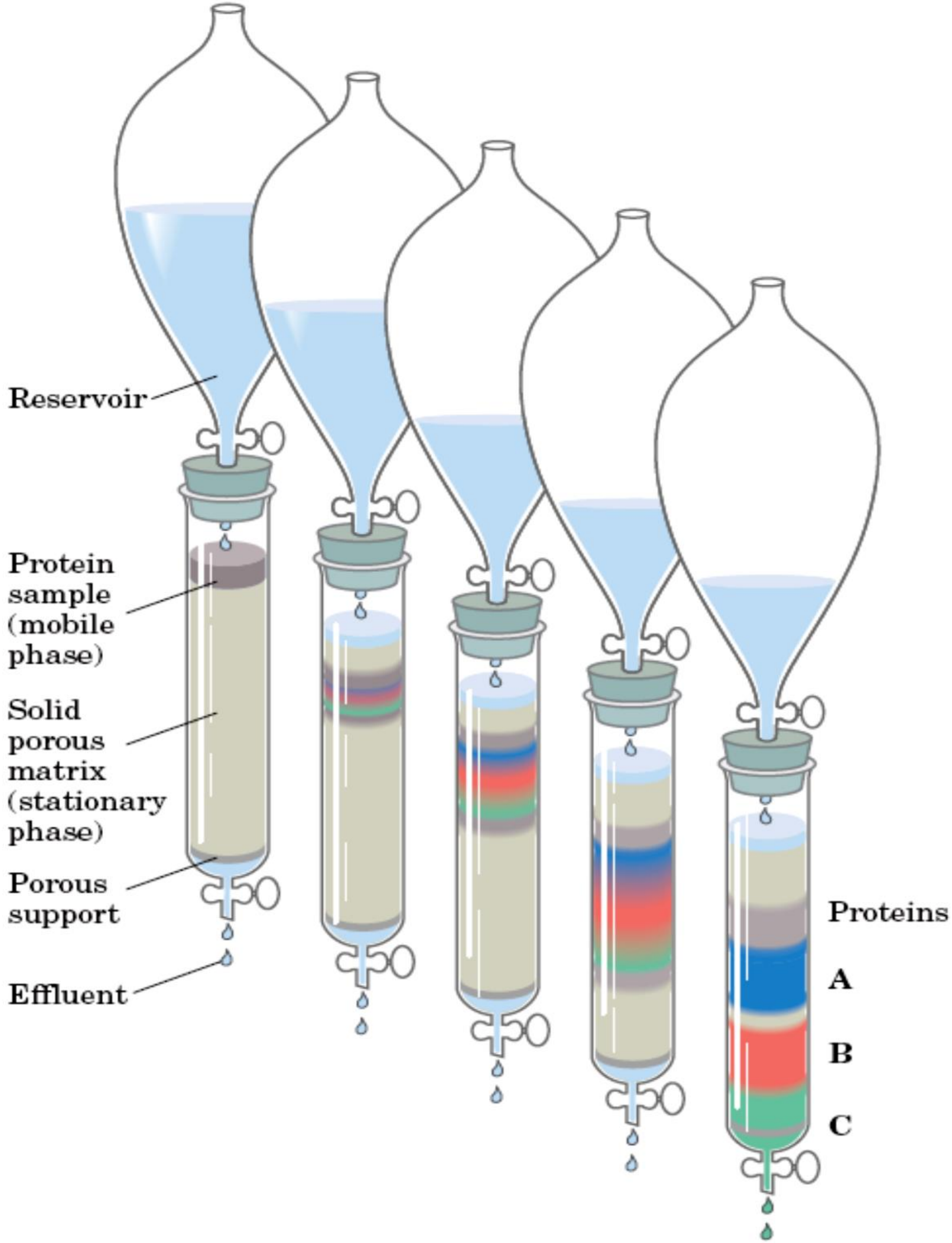

Solubility
Hydrate shell
Salting in
Salting out
Salt concentration

Chromatography

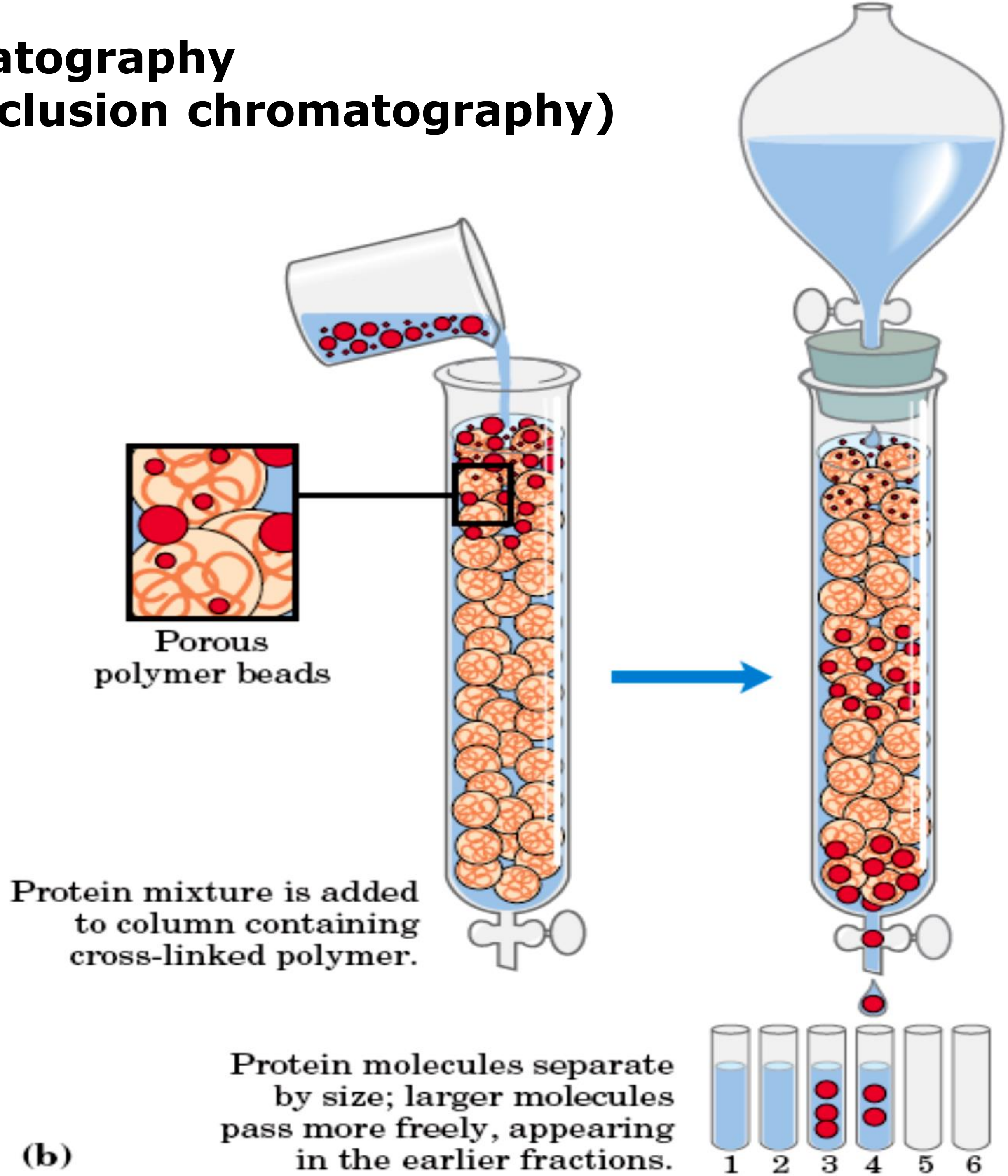
is a physical method of separation that distributes components to separate between two phases, one stationary (stationary phase), the other (the mobile phase) moving in a definite direction

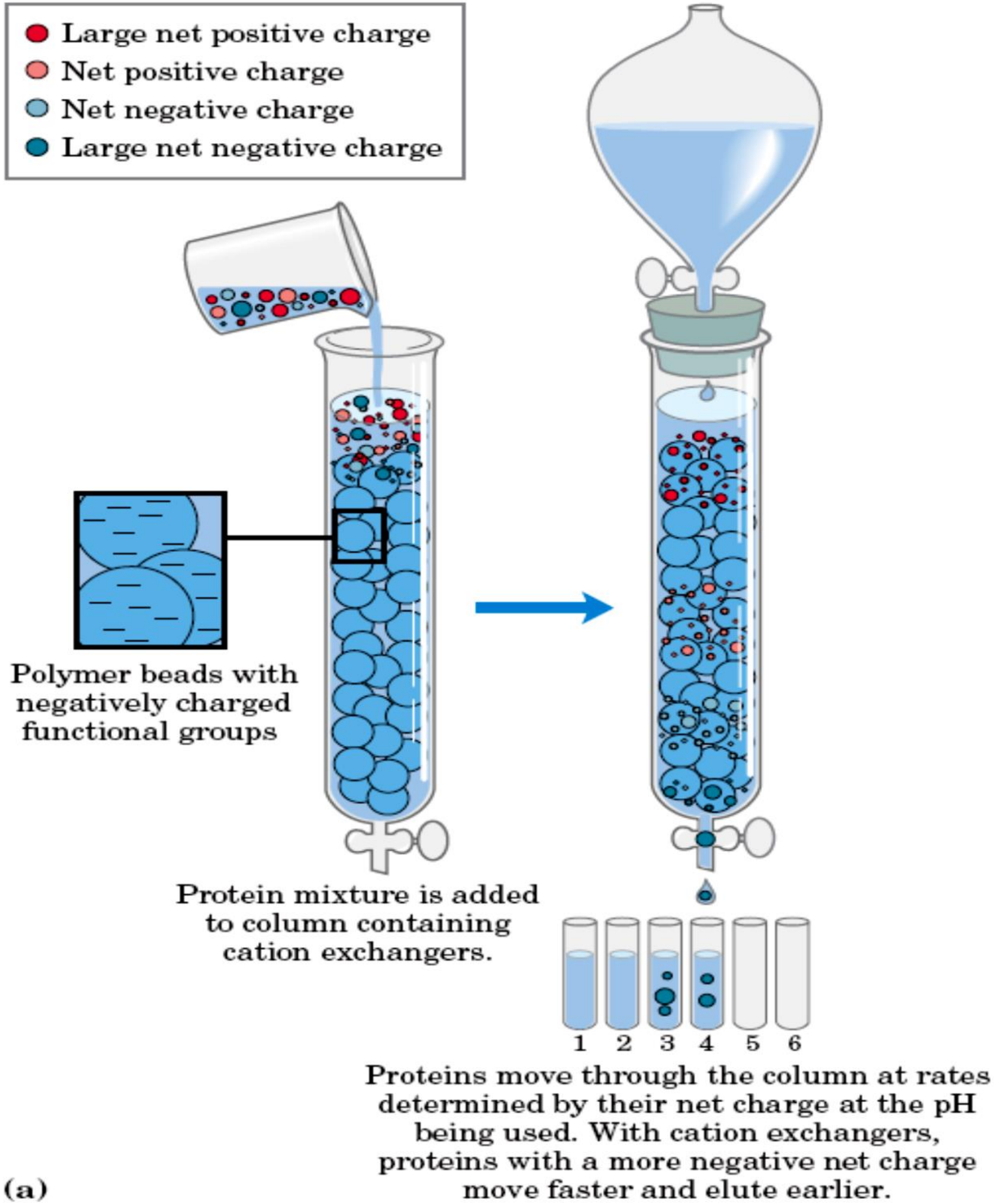

6
5
4
3
2
1
0
Solvent front
Distance travelled by solvent
Distance travelled by substance
Baseline

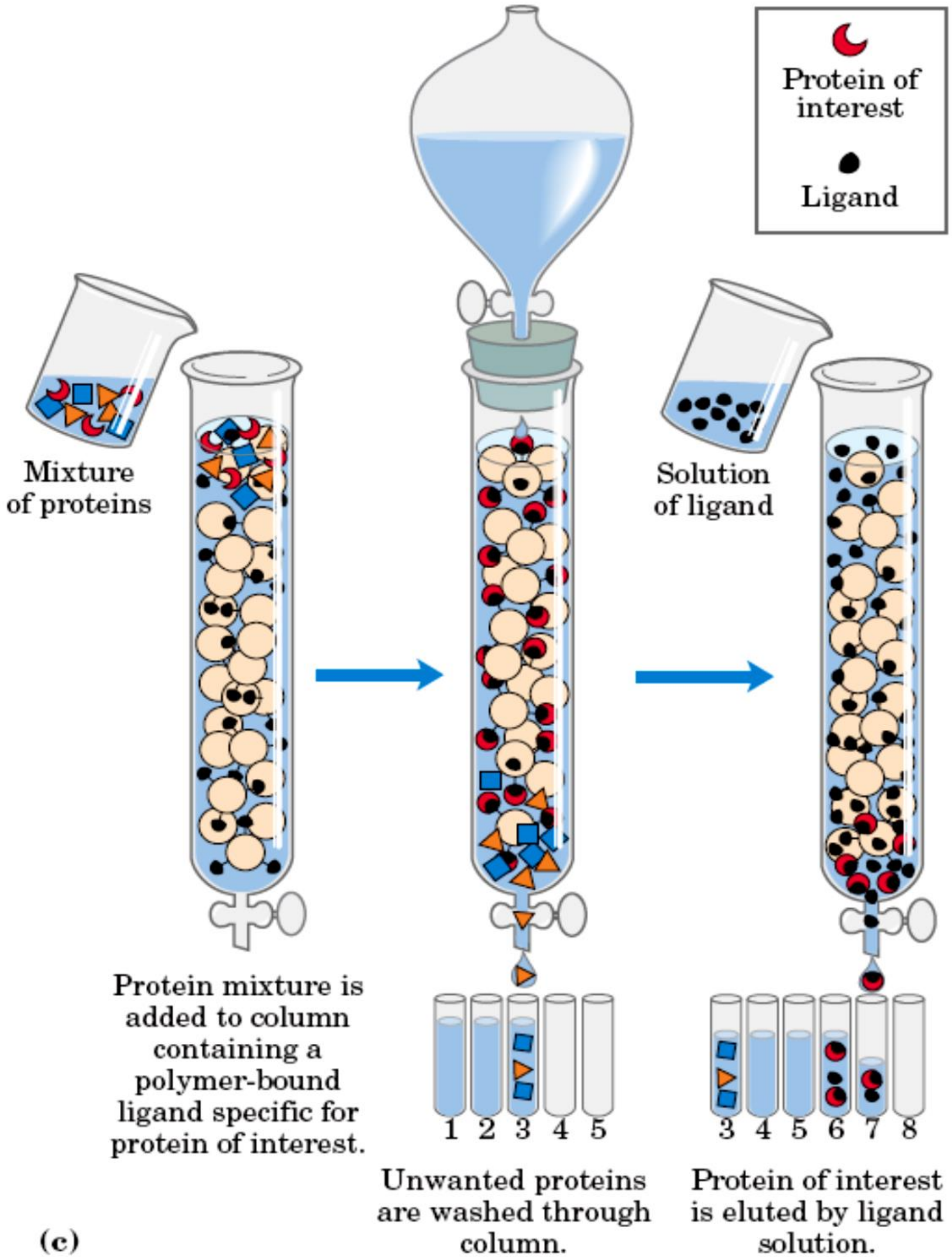

load sample
add solvent
© 1996 B. M. Tissue
www.scimedia.com
column containing stationary phase
collect components


Reservoir
Protein sample (mobile phase)
Solid porous matrix (stationary phase)
Porous support
Effluent
Proteins
A
B
C

Gel chromatography
(or size exclusion chromatography)




Large net positive charge
Net positive charge
Net negative charge
Large net negative charge
Polymer beads with negatively charged functional groups
Protein mixture is added to column containing cation exchangers.
1 2 3 4 5 6
Proteins move through the column at rates determined by their net charge at the pH being used. With cation exchangers, proteins with a more negative net charge move faster and elute earlier.
(a)

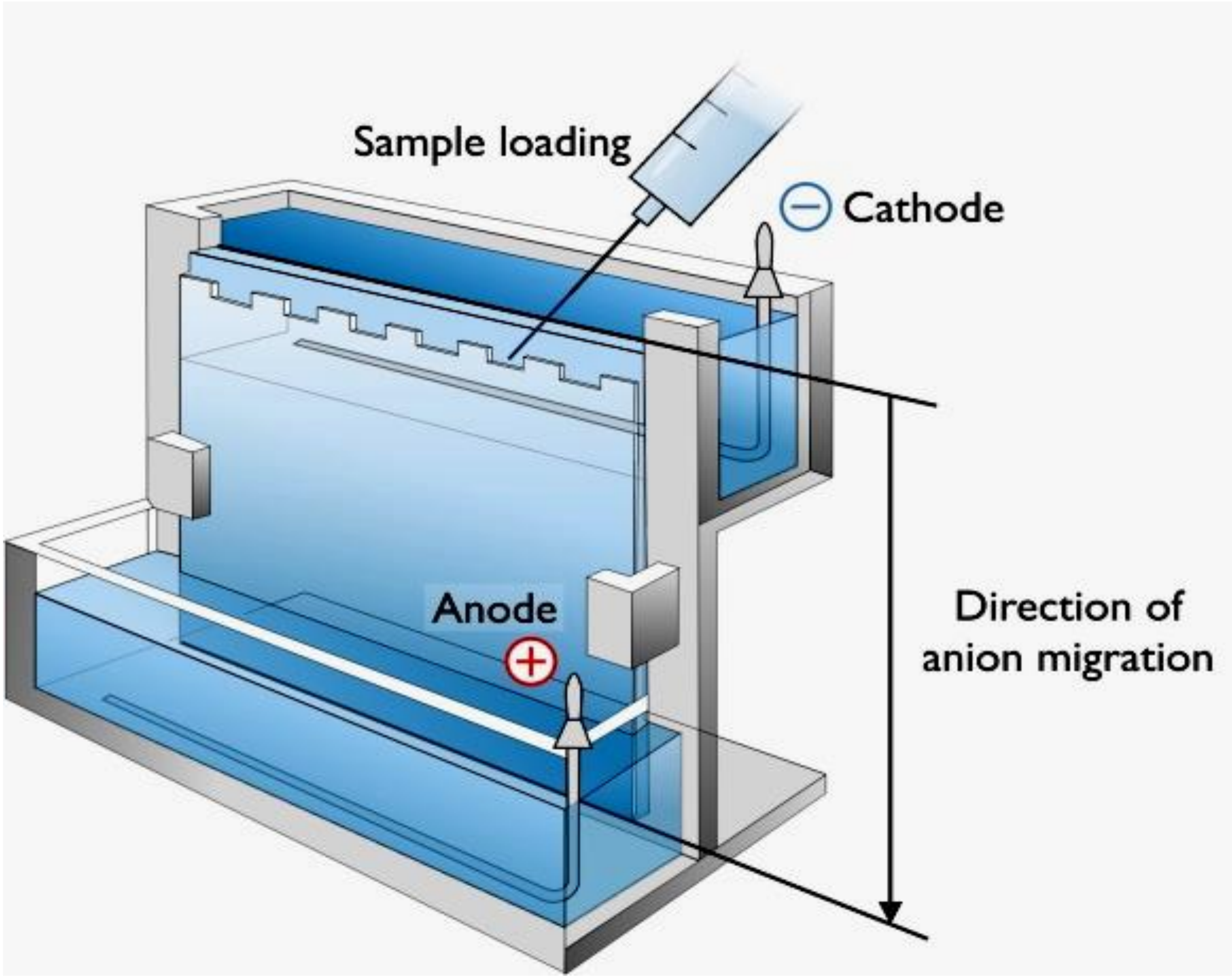


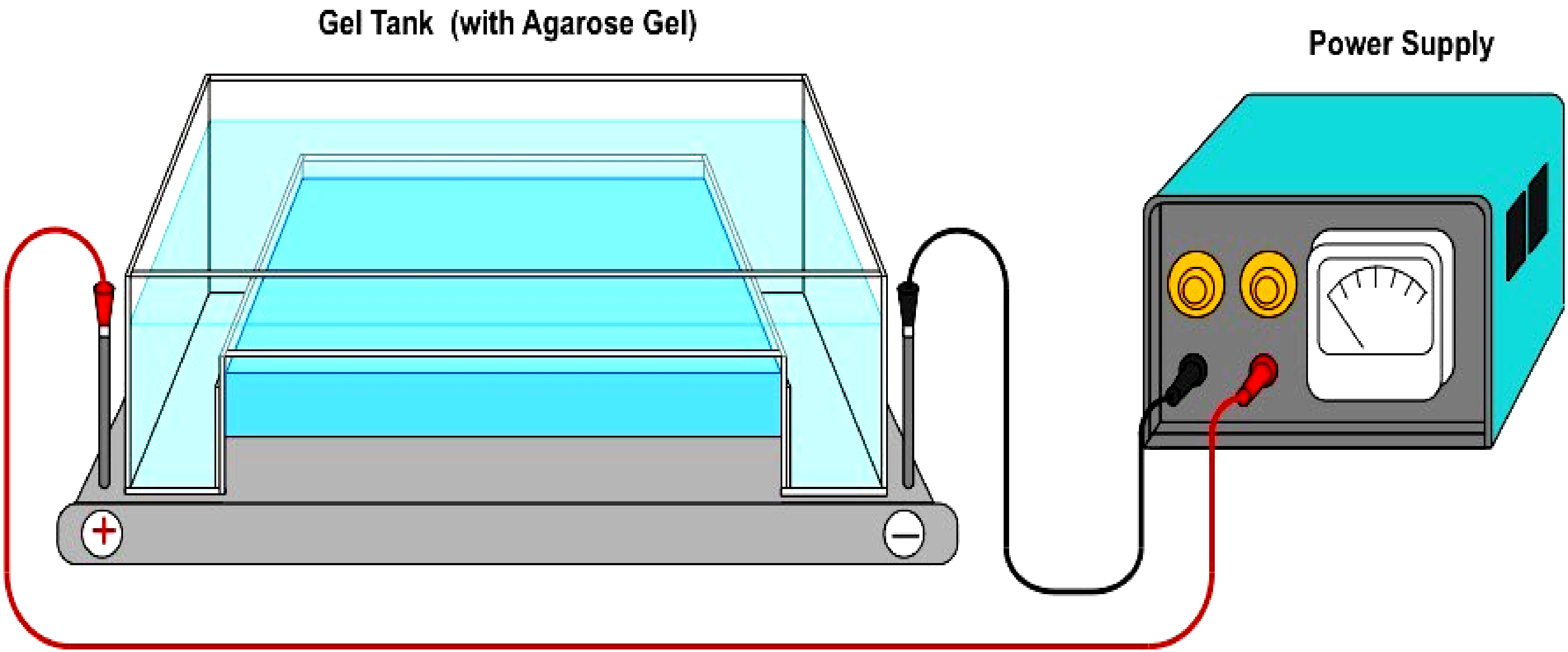
Protein of interest
Ligand
Mixture of proteins
Solution of ligand
Protein mixture is added to column containing a polymer-bound ligand specific for protein of interest.
1 2 3 4 5
Unwanted proteins are washed through column.
3 4 5 6 7 8
Protein of interest is eluted by ligand solution.
(c)

Electrophoresis

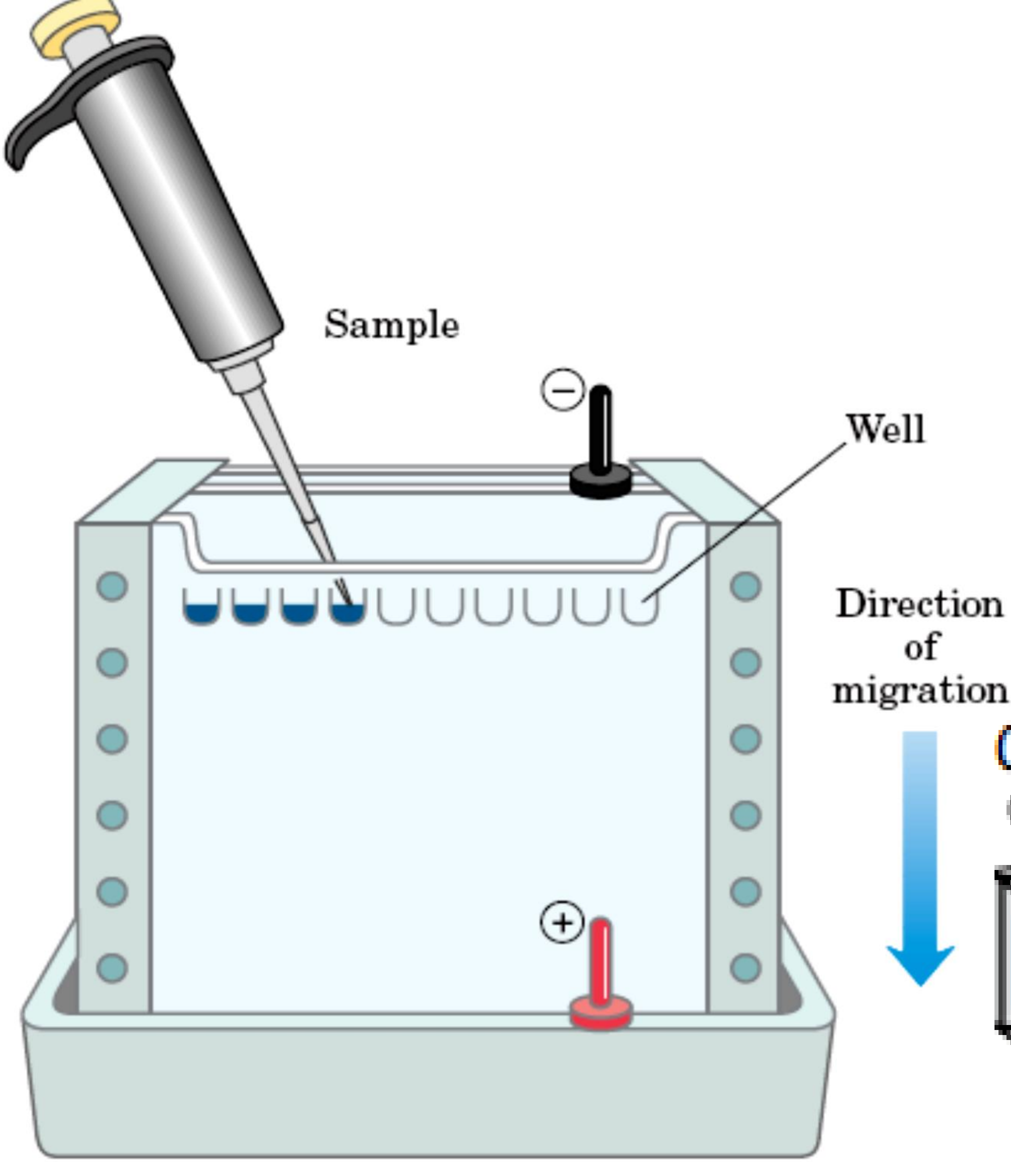
is the motion of dispersed particles under the influence of electric field.

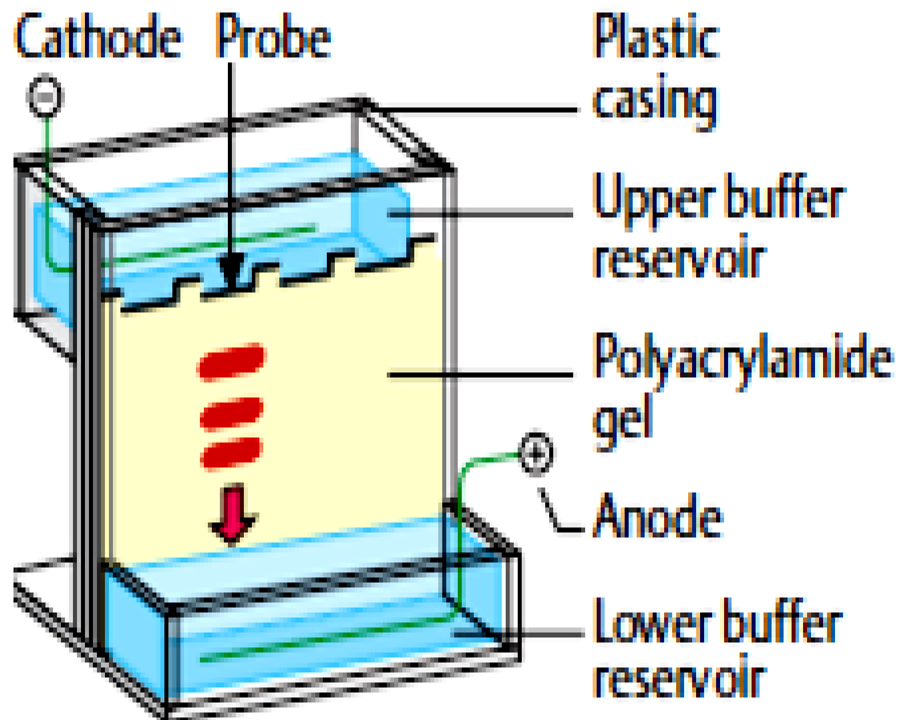
It can be used to separate proteins by size, density and purity.

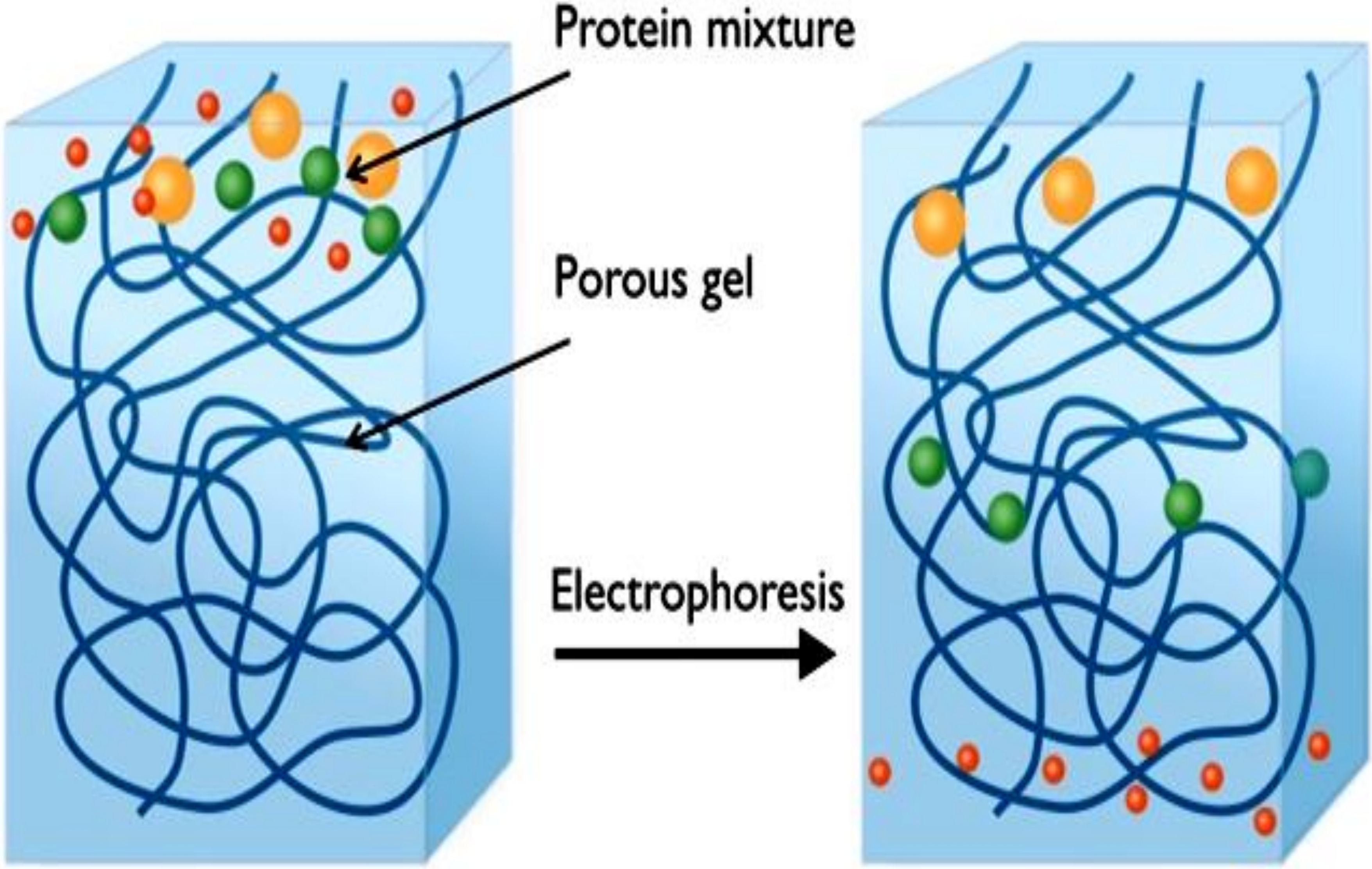

Sample loading
Cathode
Anode
Direction of anion migration

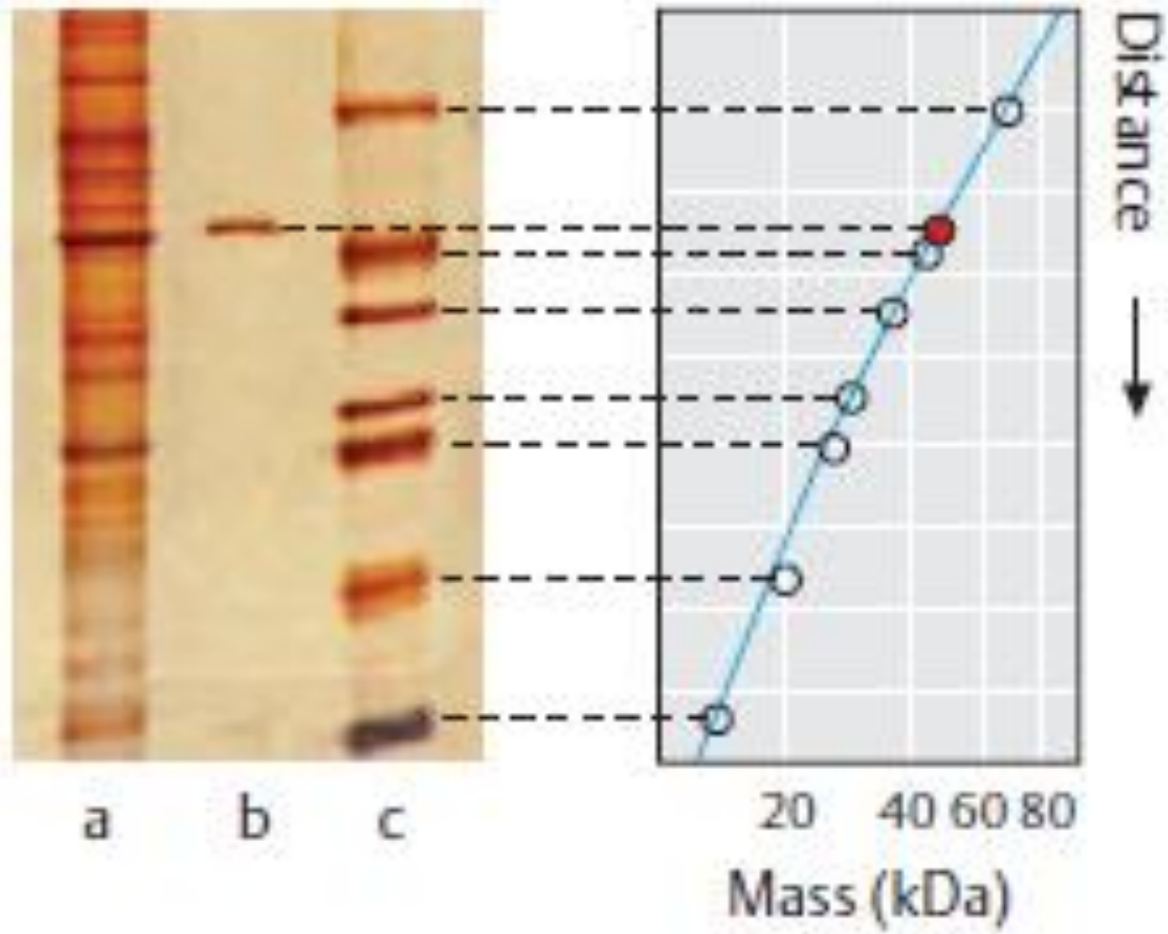


Gel Tank (with Agarose Gel)
Power Supply
+
−

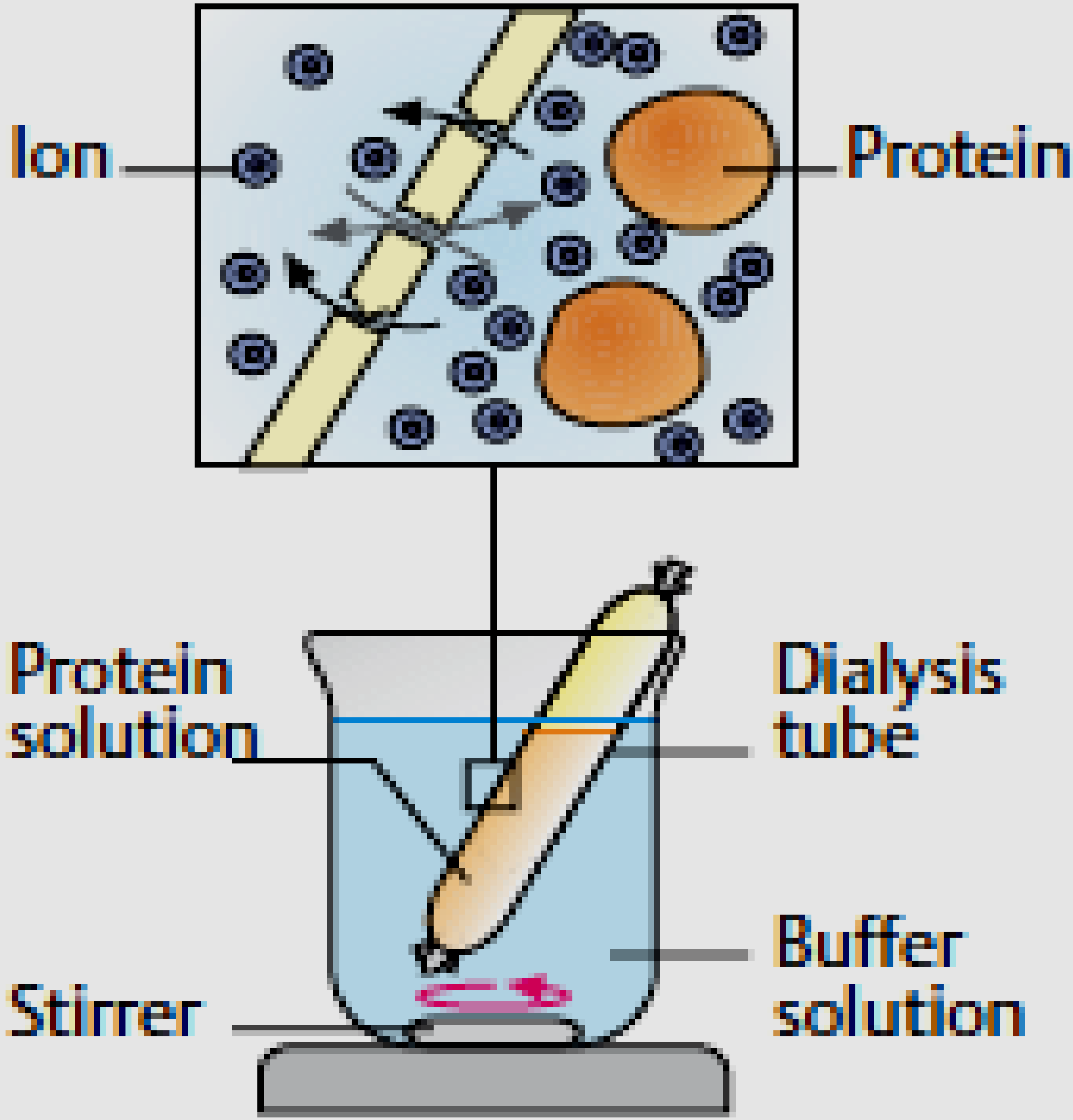

Sample
Well
Direction of migration


Cathode
Probe
Plastic casing
Upper buffer reservoir
Polyacrylamide gel
Anode
Lower buffer reservoir


Protein mixture
Porous gel
Electrophoresis


Distance
a
b
c
20
40
60
80
Mass (kDa)

Dialysis



Ion
Protein
Protein solution
Dialysis tube
Buffer solution
Stirrer