Biological Physics
Course Code: BIO503

Biophysics is an interdisciplinary science that applies the approaches and methods of physics to study biological systems. Biophysical research shares significant overlap with biochemistry, physical chemistry, nanotechnology, bioengineering, computational biology, biomechanics and systems biology. Biophysics covers all scales of biological organization, from molecular to organismic and populations. Bridge between biology and physics.

Study of biological systems and biological processes using physics-based methods or based on physical principles. Biophysics discovers how atoms are arranged to work in DNA and proteins. Biophysicists are discovering how proteins work, they use these protein structures for discovering how biological machines work, in health and also in diseases. Biophysicists study life at every level, from atoms and molecules to cells, organisms, and environments.

Biophysicists ask questions, such as: How do protein machines work? Even though they are millions of times smaller than everyday machines, molecular machines work on the same principles. They also use energy to do work. How do systems of nerve cells communicate? How do proteins pack DNA into viruses? How do viruses invade cells? How do plants harness sunlight to make food? Understanding of life by studying fundamental physical principles and mechanisms by which living organisms survive, adapt, and grow.

Fundamental experimental work in areas such as molecular structure and dynamics, photosynthesis, or cell membranes.

Study evolution, neural dynamics, electron transfer, and non-linear phenomena such as heart rhythms or organism development.

Today, biophysics seeks to answer diverse biological questions, such as How are tiny molecules in our environment detected by a sense organ and converted into electrical impulses that provide the brain with information about the external world?

Biophysics is a growing enterprise world-wide, driven primarily by the widespread realization of the major contributions made to biological science by a combination of truly state-of-the-art physical measurements with modern molecular biology.

The field occupies a unique and central position at the intersection of the biological, chemical, physical, and computational sciences

Biophysicists are driven primarily by their curiosity about how biological systems work at the molecular level

Development and advancement of new techniques and approaches. Biophysicists as a group most often develop the novel, sophisticated experimental methods that reveal molecular level details with unique clarity. Biophysics is a molecular science that seeks to explain biological function in terms of the molecular structures and properties of specific molecules.

Size of these molecules varies dramatically:

Small fatty acids and sugars (1 nanometer), Macromolecules such as proteins (5 to 10 nm), Starches (greater than 1,000 nm and longer than the thickness of a human hair)

Enormously elongated DNA molecules (more than 1 centimeter long but only 20 nm wide, or the scaled equivalent of a piece of string 45 miles long). These biomolecules, the sole building blocks of living organisms, assemble into cells, tissues and whole organisms by forming complex individual structures that are visible under a light microscope. The most famous biophysicist, Francis Crick, was one of three people who won the Nobel Prize for this accomplishment. The team used data from a technique known as X-ray crystallography, which revealed the physical patterns of DNA.

So exactly what did he do? 50 years before "biophysics" was a trendy college major, he approached DNA as a physics problem. What he and James Watson did was to piece together just exactly what DNA looks like physically, the spiral-staircase shape. Experiments in the 1940's showed that genes are made of a simple chemical–DNA. How such a simple chemical could be the molecule of inheritance remained a mystery until biophysicists discovered the DNA double helix in 1953. During the 2000's, biophysical inventions decoded all the genes in a human being (Human genome project). All the genes of nearly 200 different species, and some genes from more than 100,000 other species have been determined. Biophysicists analyze those genes to learn how organisms are related and how individuals differ.

HISTORY OF BIOPHYSICS

Biophysics is a relatively young discipline (1960) but its origins go back a long way. Basic biophysics concepts can be detected as far back as the mid-nineteenth century, at the Berlin Physiology School, if not earlier still, in the eighteenth-century British Physiology School (Cavendish, Walsh). Biophysics might even be seen as dating back to the times of Volta and Galvani.

The popularity of biophysics rose sharply in 1944 when the book "What is life" by Erwin Schrodinger (1933 nobel prize for physics) was published.

By 1950, as many as 200 institutions throughout the world had adopted the term Biophysics in their name. The American Biophysics Society was founded in 1957.

In Italy, the new discipline was initially promoted by three world-leading scientists: Antonino Borsellino in Genoa, Adriano Gozzini in Pisa and Mario Ageno in Rome.

In 1959 a series of conferences on Bio-Physics and Bio-Chemistry was organized in Genoa and in other cities. Back then, it was considered extremely novel for physics scientists to have common interests with medicine and biology scientists.

The establishment of the Italian Biophysical Society dates back to 1960. The first National Biophysics Congress was held in Italy from 3 to 5 June 1963.

Biophysics Importance

The importance of Biophysics is highlighted by variety of outstanding drug withdrawals in recent years. Each of these withdrawals was preceded by severe or fatal adverse effects that had been unrecognized or underappreciated at the time of approval. exploitation the tools of Biophysics, it's potential to spot adverse effects that will be unnoted in irregular trials as a result of those adverse effects square measure uncommon, represent a rise in risk from Associate in Nursing already high baseline occur primarily in patient teams underrepresented in clinical trials, need several months or years to develop, occur primarily with co-administration of specific alternative medication, and/or occur primarily in patients with a particular comorbidity or genotype. Biophysics importance articles from OMICS Group are an open access articles named in Journal of Physical Chemistry & Biophysics which strives to release issues quarterly and is adamant to publish new findings related to the field of Biophysics importance. Risks and benefits are commonly identified only after a drug is widely used by the general population. Observational study designs are essential for the study of risks and benefits associated with marketed drugs. Regulatory agencies are under pressure to identify and respond to post approval Biophysics issues and work with stakeholders on risk management and risk communication.

While some colleges and universities have dedicated departments of biophysics, usually at the graduate level, many do not have university-level biophysics departments, instead having groups in related departments such as biochemistry, cell biology, chemistry, computer science, engineering, mathematics, medicine, molecular biology, neuroscience, pharmacology, physics, and physiology. Depending on the strengths of a department at a university differing emphasis will be given to fields of biophysics. What follows is a list of examples of how each department applies its efforts toward the study of biophysics. This list is hardly all inclusive. Nor does each subject of study belong exclusively to any particular department. Each academic institution makes its own rules and there is much overlap between departments.

Biology and molecular biology – Almost all forms of biophysics efforts are included in some biology department somewhere. Typical examples include: gene regulation, single protein dynamics, bioenergetics, patch clamping, biomechanics, virophysics.

Structural biology – Ångstrom-resolution structures of proteins, nucleic acids, lipids, carbohydrates, and complexes thereof.

Biochemistry and chemistry – biomolecular structure, siRNA, nucleic acid structure, structure-activity relationships.

Computer science – Neural networks, biomolecular and drug databases.

Computational chemistry – molecular dynamics simulation, molecular docking, quantum chemistry

Bioinformatics – sequence alignment, structural alignment, protein structure prediction

Mathematics – graph/network theory, population modeling, dynamical systems, phylogenetics.

Medicine – biophysical research that emphasizes medicine.

Neuroscience – studying neural networks experimentally (brain slicing) as well as theoretically (computer models), membrane permittivity, gene therapy, understanding tumors.

Pharmacology and physiology – channelomics, biomolecular interactions, cellular membranes, polyketides.

Physics – negentropy, stochastic processes, and the development of new physical techniques and instrumentation as well as their application.

Quantum biology – The field of quantum biology applies quantum mechanics to biological objects and problems. Decohered isomers to yield time-dependent base substitutions. These studies imply applications in quantum computing.

Agronomy and agriculture

Many biophysical techniques are unique to this field. Research efforts in biophysics are often initiated by scientists who were biologists, chemists or physicists by training

Biophysical Techniques

The characterization of molecular structure, the measurement of molecular properties, and the observation of molecular behavior presents an enormous challenge for biological scientists. A wide range of biophysical techniques have been developed to study molecules in crystals, in solution, in cells, and in organisms. These biophysical techniques provide information about the electronic structure, size, shape, dynamics, polarity, and modes of interaction of biological molecules. Some of the most exciting techniques provide images of cells, subcellular structures, and even individual molecules. It is now possible, for example, to directly observe the biological behavior and physical properties of single protein or DNA molecules within a living cell and determine how the behavior of the single molecule influences the biological function of the organism.

Much biophysical research involves either the development of novel techniques to investigate the structure, properties, and biological functions of biomolecules or the application of these techniques to monitor how the structure and dynamics of biomolecules enables specific biological functions

Electrophysiology

- Electrophysiology is the study of the electrical nature of tissues and cells.
- It involves the movement and measurement of voltage and current coursing through the tissues through the ion channel proteins
- It also includes the movement and adverse effects of currents in whole organs such as the heart.
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Hydrodynamics

- The scientific study of the motion of fluids, especially non-compressible liquids, under the influence of internal and external forces.
- The behavior of large biomolecules—proteins, carbohydrates, and nucleic acids—in solution is complex and directly related to molecular size, shape, and flexibility.
- Analysis of hydrodynamic behavior thus provides important information about the structure, dynamics, and interactions of bio macromolecules.

Microscopy & Imaging

- To generate images of cellular and molecular structures with dimensions from microns to nanometers.
- See individual molecules or cellular structures using atomic force, electron, or confocal fluorescence microscopy.

Confocal Fluorescence Microscopy

- An optical imaging technique.
- Optical resolution and contrast of a micrograph.
- Easy
- Extremely high-quality images can be obtained
- Imaging of both fixed and living cells and tissues

Atomic Force Microscopy

- Type of high resolution scanning probe microscope that has a resolution that you can measure in fractions of a nanometer.
- Measure the mechanical properties of living material (such as tissue or cells)

Magnetic Resonance Imaging

- A medical imaging technique.
- Used in radiology to form pictures of the anatomy and the physiological processes of the body in both health and disease.
- MRI scanners use strong magnetic fields, electric field gradients, and radio waves to generate images of the organs in the body

Modeling & Simulation

- Use of computer simulations of the many cellular subsystems
- Such as the networks of metabolites
- Enzymes which comprise metabolism
- Signal transduction pathways

- Gene regulatory networks to both analyze and visualize the complex connections of these cellular processes.
- Protein structure prediction is the prediction of the three-dimensional structure of a protein.

Spectroscopy

- Used for Molecular structure determination.
- Drug metabolism studies & mechanism of drug action.
- Cell research
- Disease detection
- Drug design and pharmaceutical materials
- Characterization of drug-cell interactions
- Microbiology and cell sorting
- Cosmetics and in vivo skin analysis

The interaction of electromagnetic radiation, x-rays, ultraviolet, visible, and infra red light, and radio waves, with molecules provides a wealth of information about the structure, dynamics, and function of biomolecules and biological processes.

Fluorescence

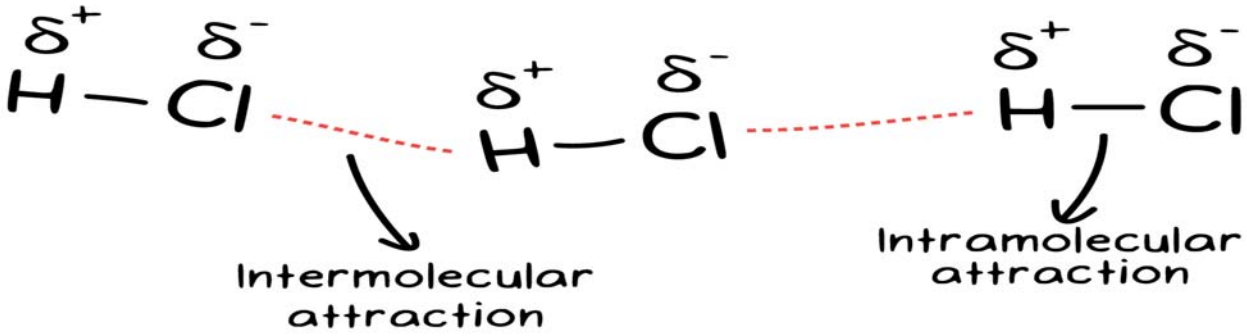
- Non-destructive way of tracking or analyzing biological molecules by means of fluorescence.
- Visualize the dynamics of tissue, cells, individual organelles, and macromolecular assemblies inside the cell.
- Examine and analyze protein–protein, protein–nucleic acid, ligand–receptor, and ligand–lipid interactions.
- Study of protein conformation and orientation.

Forces of Attraction

There are two kinds of forces, or attractions, that operate in a molecule—

1. Intra-molecular.
2. Intermolecular.

- Intramolecular forces are the forces that hold atoms together within a molecule.
- Intermolecular forces are forces that exist between molecules.
- Intermolecular forces are also known as van der Waals forces, named after **Johannes van der Waals** who first postulated them.
- Intermolecular forces are typically weaker than intramolecular forces, and account for the bulk properties of matter (e.g., boiling point, melting point, etc.)



- Forces binding atoms in a molecule are due to chemical bonding.
- The energy required to break a bond is called the bond-energy
- For example the average bond-energy for O-H bonds in water is 463 kJ/mol.
- On average, 463 kJ is required to break 6.023x1023 O-H bonds

Types of intra-molecular forces of attraction

- Ionic bond
- Covalent bond
- Metallic bonding
- Ionic bonding is a type of chemical bond that involves the electrostatic attraction between oppositely charged ions.
- A covalent bond, also called a molecular bond, is a chemical bond that involves the sharing of electron pairs between atoms
- Metallic bonding are the force of attraction between valence electrons and the metal atoms. It is the sharing of many detached electrons between many positive ions, where the electrons act as a "glue" giving the substance a definite structure.

Intermolecular force of attractions

- Mediate interaction between molecules.
- Intermolecular forces are responsible for the physical state of a compound (solid, liquid or gas).
- When we know atoms in molecule are held together?

Intramolecular force

- Then why do molecules in a liquid or solid stick together around each other?
- What makes the molecules attracted to one another?

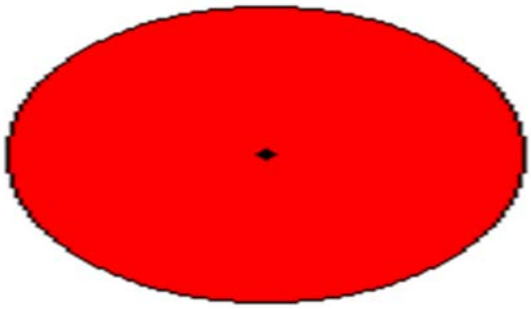
Intermolecular force

Types of intermolecular forces that exist between molecules

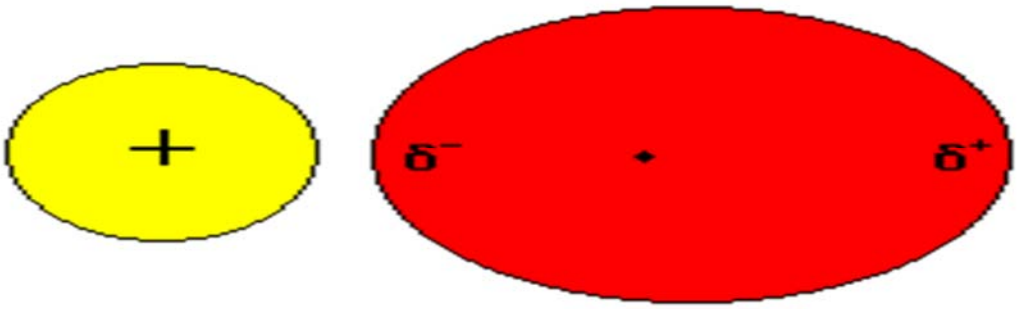
- Dipole-dipole interactions
- Hydrogen bonding:
- London dispersion forces
- Ion dipole forces

Ion-induced dipole forces

An ion-induced dipole attraction is a weak attraction that results when the approach of an ion induces a dipole in an atom or in a nonpolar molecule by disturbing the arrangement of electrons in the nonpolar species.



Spherical atom with no dipole. The dot indicates the location of the nucleus.



Upon approach of a charged ion, electrons in the atom respond and the atom develops a dipole.

- The force of attraction between a non-polar molecule and an ion that may lie in its vicinity is called ion-induced dipole force.

- In this, the ion may attract or repel the electron cloud present on the non-polar molecule and induce the non-polar molecule to become a temporary dipole.

The strength of this induced dipole depends on how easily the electron cloud can be distorted, i.e., the bigger the molecule, the stronger is the dipole induced.

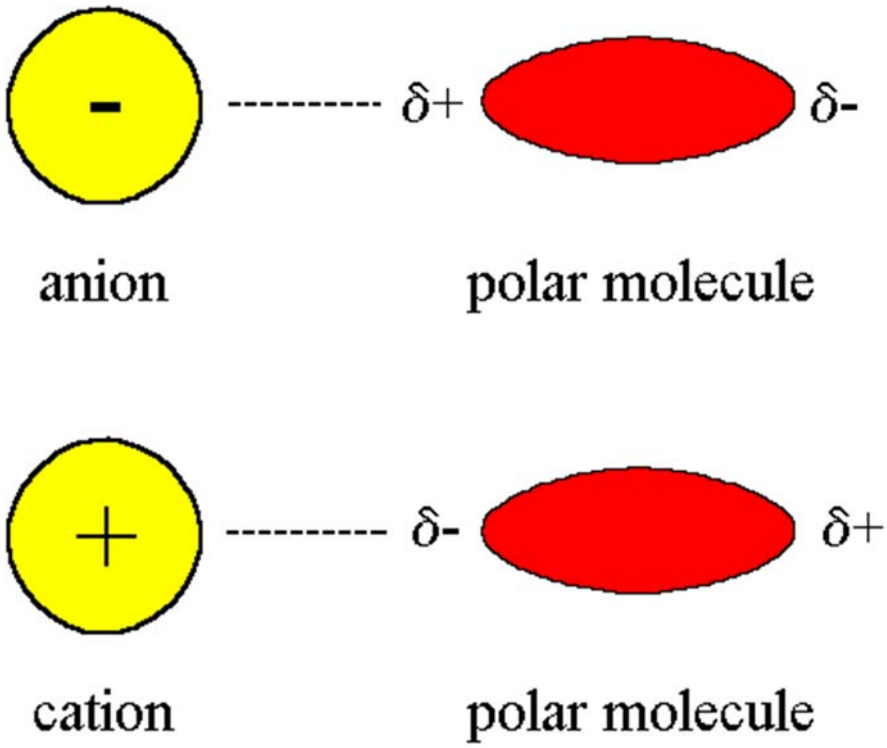
- Hemoglobin is a protein found in the red blood cells, and its function is to carry oxygenated blood to various parts of the body.
- It has an Fe2+ ion in the center of its protein structure.
- This Fe2+ ion attracts the O2 by ion-induced dipole force.

  (Although oxygen is an electronegative atom, in O2, the electron pairs experience an equal pull from both the oxygen atoms, and thus, there is no development of +δ and -δ charge on O2. Hence, the molecule as a whole is non-polar).

Ion-Dipole Forces

An ion-dipole force is an attractive force that results from the electrostatic attraction between an ion and a neutral molecule that has a dipole.

- Most commonly found in solutions. Especially important for solutions of ionic compounds in polar liquids.
- A positive ion (cation) attracts the partially negative end of a neutral polar molecule.
- A negative ion (anion) attracts the partially positive end of a neutral polar molecule.



- Ion-dipole attractions become stronger as either the charge on the ion increases, or as the magnitude of the dipole of the polar molecule increases.

Hydrogen Bonds

Hydrogen bonds are a type of dipole-dipole interactions that occur between hydrogen and either nitrogen, fluorine, or oxygen. Hydrogen bonds are incredibly important in biology, because hydrogen bonds keep the DNA bases paired together, helping DNA maintain its unique structure.

Forming a Hydrogen Bond

A hydrogen bond is the electromagnetic attraction created between a partially positively charged hydrogen atom attached to a highly electronegative atom and another nearby electronegative atom. A hydrogen bond is a type of dipole-dipole interaction; it is not a true chemical bond. These attractions can occur between molecules (*inter*molecularly) or within different parts of a single molecule (*intra*molecularly).

Hydrogen Bond Donor

A hydrogen atom attached to a relatively electronegative atom is a hydrogen bond donor. This electronegative atom is usually fluorine, oxygen, or nitrogen. The electronegative atom attracts the electron cloud from around the hydrogen nucleus and, by decentralizing the cloud, leaves the hydrogen atom with a positive partial charge. Because of the small size of hydrogen relative to other atoms and molecules, the resulting charge, though only partial, is stronger. In the molecule ethanol, there is one hydrogen atom bonded to an oxygen atom, which is very electronegative. This hydrogen atom is a hydrogen bond donor.

Hydrogen Bond Acceptor

A hydrogen bond results when this strong partial positive charge attracts a lone pair of electrons on another atom, which becomes the hydrogen bond acceptor. An electronegative atom such as fluorine, oxygen, or nitrogen is a hydrogen bond acceptor, regardless of whether it is bonded to a hydrogen atom or not. Greater electronegativity of the hydrogen bond acceptor will create a stronger hydrogen bond. The diethyl ether molecule contains an oxygen atom that is not bonded to a hydrogen atom, making it a hydrogen bond acceptor.

Applications for Hydrogen Bonds

Hydrogen bonds occur in inorganic molecules, such as water, and organic molecules, such as DNA and proteins. The two complementary strands of DNA are held together by hydrogen bonds between complementary nucleotides (A&T, C&G). Hydrogen bonding in water contributes to its unique properties, including its high boiling point (100 °C) and surface tension.

Ion-Induced Dipole Force

An ion-induced dipole force occurs when an ion interacts with a non-polar molecule. Like a dipole-induced dipole force, the charge of the ion causes a distortion of the electron cloud in the non-polar molecule, causing a temporary partial charge. The temporary partially charged dipole and the ion are attracted to each other and form a fleeting interaction.

Dispersion Force

Dispersion forces are weak intermolecular forces caused by temporary dipoles.

Temporary Dipoles

Temporary dipoles are created when electrons, which are in constant movement around the nucleus, spontaneously come into close proximity. This uneven distribution of electrons can make one side of the atom more negatively charged than the other, thus creating a temporary dipole, even on a non-polar molecule. The more electrons there are in an atom, the further away the shells are from the nucleus; thus, the electrons can become lopsided more easily, and these forces are stronger and more frequent. These intermolecular forces are also sometimes called "induced dipole-induced dipole" or "momentary dipole" forces.

London Dispersion Forces

Although charges are usually distributed evenly between atoms in non-polar molecules, spontaneous dipoles can still occur. When this occurs, non-polar molecules form weak attractions with other non-polar molecules. These London dispersion forces are often found in the halogens (e.g., F_2 and I_2), the noble gases (e.g., Ne and Ar), and in other non-polar molecules, such as carbon dioxide and methane. London dispersion forces are part of the van der Waals forces, or weak intermolecular attractions.

The Properties of Water

Water is the most abundant compound on Earth's surface. In nature, water exists in the liquid, solid, and gaseous states. It is in dynamic equilibrium between the liquid and gas states at 0 degrees Celsius and 1 atm of pressure. At room temperature (approximately 25 degrees Celsius), it is a tasteless, odorless, and colorless liquid. Many substances dissolve in water, and it is commonly referred to as the universal solvent.

Properties of Water

Molecular formula	H_2O

Properties of Water

Molar mass	18.01528(33) g/mol
Appearance	white solid or almost colorless, transparent, with a hint of blue, crystalline solid or liquid
Density	1000 kg/m^3, liquid (4º C)(62.4 lb/cu. ft)
Melting point	0º C, 32º F, (273.15 K)
Acidity (pK_a)	15.74 ~35–56
Basicity (pK_b)	15.74
Refractive index (n_D)	1.3330
Viscosity	0.001 Pas at 20º C

Structure of Water

Crystal structure	Hexagonal
Molecular shape	Bent
Dipole moment	1.85 D

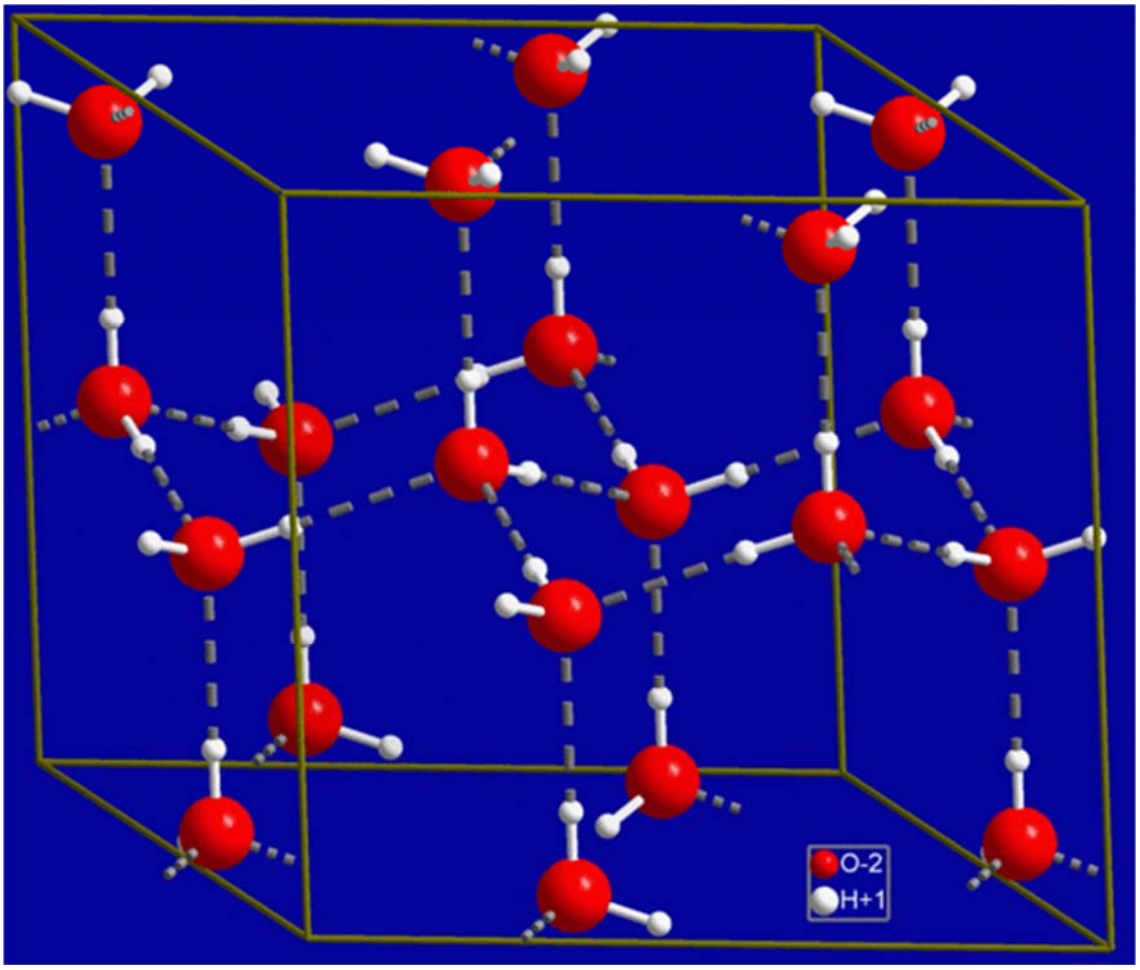
The Phases of Water

Similar to many other substances, water can take numerous forms. Its liquid phase, the most common phase of water on Earth, is the form that is generally meant by the word "water."

Solid Phase (Ice)

The solid phase of water is known as ice and commonly takes the structure of hard, amalgamated crystals, such as ice cubes, or of loosely accumulated granular crystals, such as snow. Unlike most other substances, water's solid form (ice) is *less* dense than its liquid form, as a result of the nature

of its hexagonal packing within its crystalline structure. This lattice contains more space than when the molecules are in the liquid state.



The hexagonal structure of ice: As a naturally occurring crystalline inorganic solid with an ordered structure, ice is considered to be a mineral. It possesses a regular crystalline structure based on the molecular structure of water, which consists of a single oxygen atom covalently bonded to two hydrogen atoms: H-O-H.

Differences Between DNA and RNA

DNA stands for deoxyribonucleic acid, while RNA is ribonucleic acid. Although DNA and RNA both carry genetic information, there are quite a few differences between them. This is a comparison of the differences between DNA versus RNA, including a quick summary and a detailed table of the differences.

Summary of Differences Between DNA and RNA

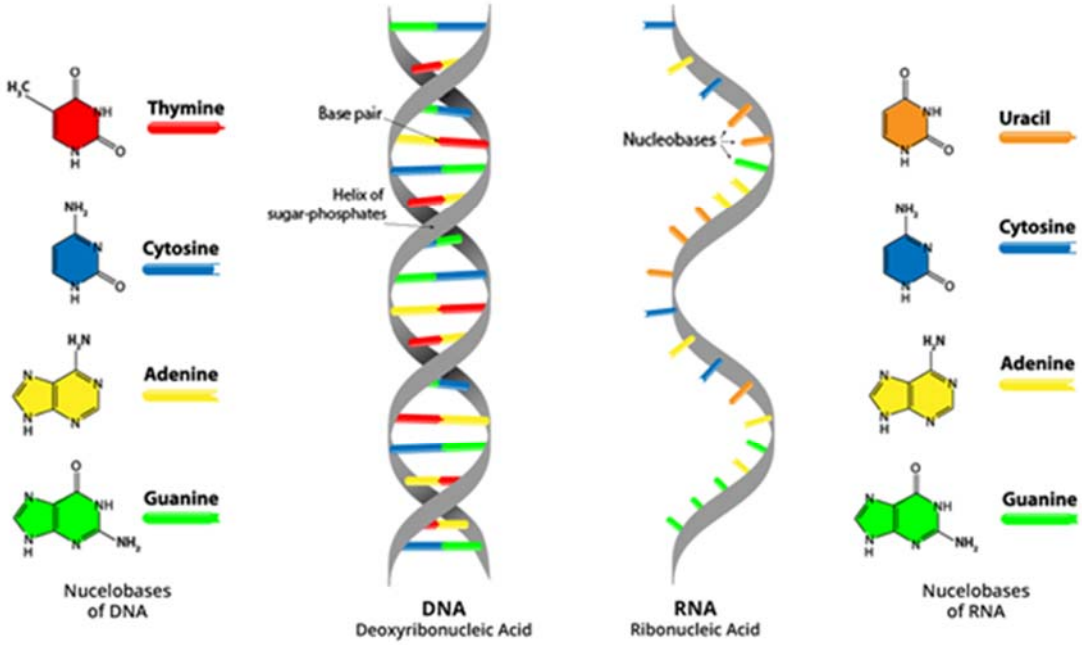
1. DNA contains the sugar deoxyribose, while RNA contains the sugar ribose. The only difference between ribose and deoxyribose is that ribose has one more -OH group than deoxyribose, which has -H attached to the second (2') carbon in the ring.

1. DNA is a double-stranded molecule while RNA is a single stranded molecule.
2. DNA is stable under alkaline conditions while RNA is not stable.
3. DNA and RNA perform different functions in humans. DNA is responsible for storing and transferring genetic information while RNA directly codes for amino acids and as acts as a messenger between DNA and ribosomes to make proteins.
4. DNA and RNA base pairing is slightly different since DNA uses the bases adenine, thymine, cytosine, and guanine; RNA uses adenine, uracil, cytosine, and guanine. Uracil differs from thymine in that it lacks a methyl group on its ring.

Comparison of DNA and RNA

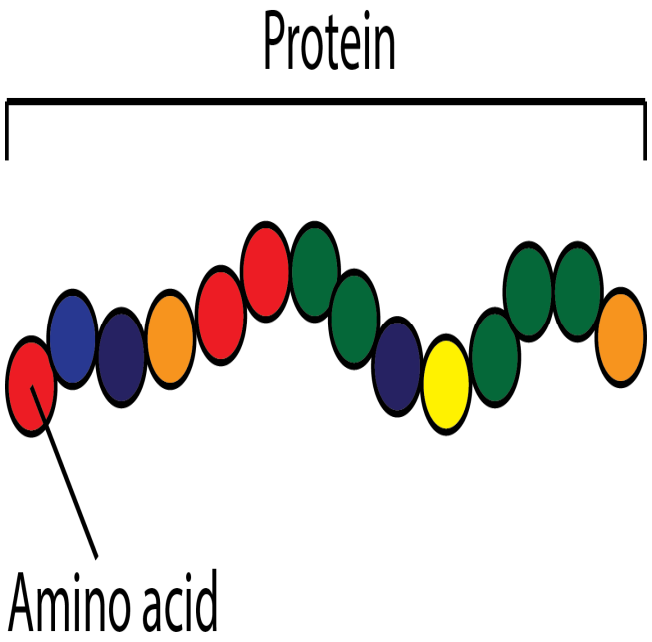
Comparison	**DNA**	**RNA**
Name	DeoxyriboNucleic Acid	RiboNucleic Acid
Function	Long-term storage of genetic information; transmission of genetic information to make other cells and new organisms.	Used to transfer the genetic code from the nucleus to the ribosomes to make proteins. RNA is used to transmit genetic information in some organisms and may have been the molecule used to store genetic blueprints in primitive organisms.
Structural Features	B-form double helix. DNA is a double-stranded molecule consisting of a long chain of nucleotides.	A-form helix. RNA usually is a single-strand helix consisting of shorter chains of nucleotides.
Composition of Bases and Sugars	deoxyribose sugar phosphate backbone adenine, guanine, cytosine, thymine bases	ribose sugar phosphate backbone adenine, guanine, cytosine, uracil bases
Propagation	DNA is self-replicating.	RNA is synthesized from DNA on an as-needed basis.
Base Pairing	AT (adenine-thymine) GC (guanine-cytosine)	AU (adenine-uracil) GC (guanine-cytosine)
Reactivity	The C-H bonds in DNA make it fairly stable, plus the body destroys enzymes that would attack DNA. The small grooves in the helix also serve as	The O-H bond in the ribose of RNA makes the molecule more reactive, compared with DNA. RNA is not stable under alkaline conditions, plus the large grooves in the molecule make it susceptible to

	protection, providing minimal space for enzymes to attach.	enzyme attack. RNA is constantly produced, used, degraded, and recycled.
Ultraviolet Damage	DNA is susceptible to UV damage.	Compared with DNA, RNA is relatively resistant to UV damage.



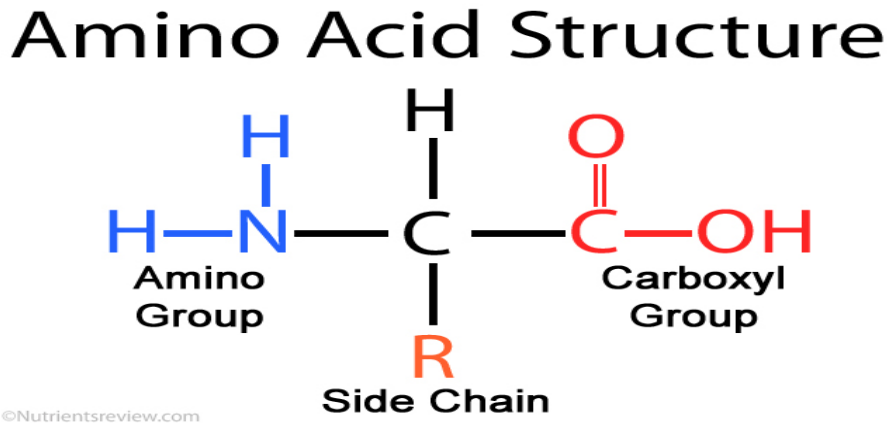
Chemistry of amino acids and protein structure

Proteins are large, complex molecules that are critical for the normal functioning of the human body. They are essential for the structure, function, and regulation of the body's tissues and organs. Proteins are made up of hundreds of smaller units called **amino acids** that are attached to one another by peptide bonds, forming a long chain. You can think of a protein as a string of beads where each bead is an amino acid.



Amino acid structure and its classification

An amino acid contains both a carboxylic group and an amino group. Amino acids that have an amino group bonded directly to the alpha-carbon are referred to as **alpha amino acids**. The simplest representation of an alpha amino acid is shown below.



Isoelectric point (pI) of amino acids

Isoelectric point is the point along the pH scale where the amino acid has a net zero charge. Consider glycine. Look at the equilibrium below; as we add hydroxide ions—in other words, raise the pH—different charged forms of glycine exist. Form B has a net zero charge and is called a **zwitterion**. Form A has a net charge of +1, and form C has a net charge of -1.

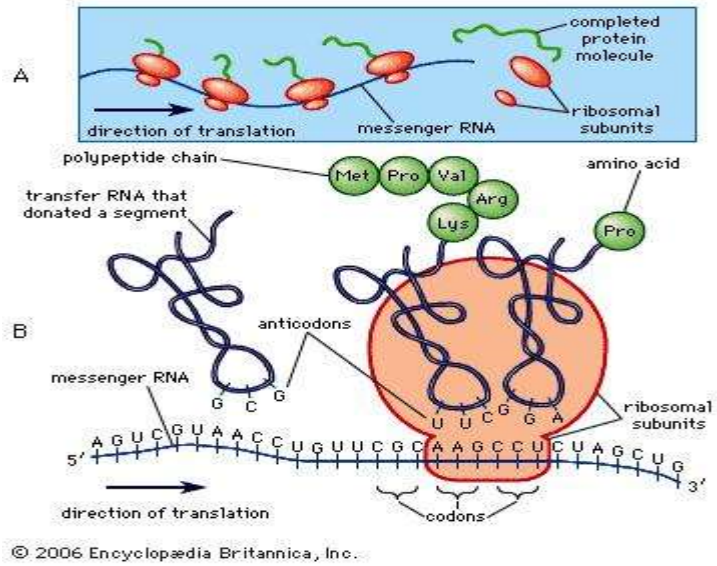
Essential amino acids

Humans can produce 10 of the 20 amino acids. The others must be supplied in the food. Failure to obtain enough of even 1 of the 10 essential amino acids, those that we cannot make, results in degradation of the body's proteins—muscle and so forth—to obtain the one amino acid that is needed. Unlike fat and starch, the human body does not store excess amino acids for later use—the amino acids must be in the food every day.

The 10 amino acids that we can produce are alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine. Tyrosine is produced from phenylalanine, so if the diet is deficient in phenylalanine, tyrosine will be required as well. The essential amino acids are arginine (required for the young, but not for adults), histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. These amino acids are required in the diet. Plants, of course, must be able to make all the amino acids. Humans, on the other hand, do not have all the the enzymes required for the biosynthesis of all of the amino acids.

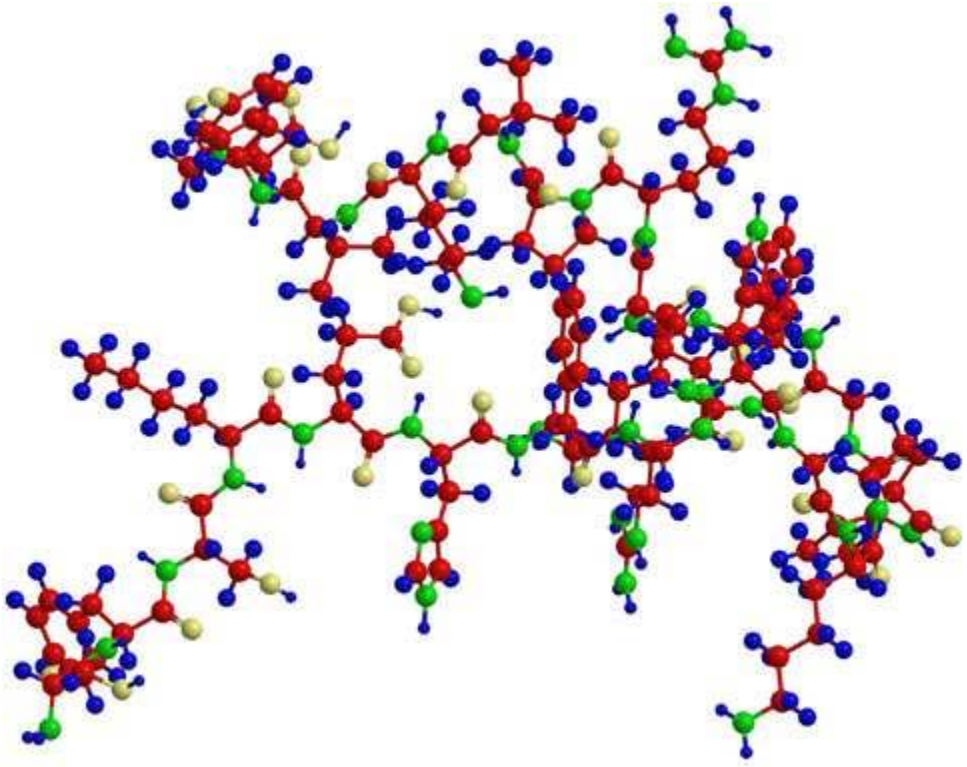
Proteins

Protein, highly complex substance that is present in all living organisms. Proteins are of great nutritional value and are directly involved in the chemical processes essential for life. The importance of proteins was recognized by chemists in the early 19th century, including Swedish chemist Jöns Jacob Berzelius, who in 1838 coined the term *protein*, a word derived from the Greek *proteios*, meaning “holding first place.” Proteins are species-specific; that is, the proteins of one species differ from those of another species. They are also organ-specific; for instance, within a single organism, muscle proteins differ from those of the brain and liver.



Synthesis of protein.

A protein molecule is very large compared with molecules of sugar or salt and consists of many amino acids joined together to form long chains, much as beads are arranged on a string. There are about 20 different amino acids that occur naturally in proteins. Proteins of similar function have similar amino acid composition and sequence. Although it is not yet possible to explain all of the functions of a protein from its amino acid sequence, established correlations between structure and function can be attributed to the properties of the amino acids that compose proteins.

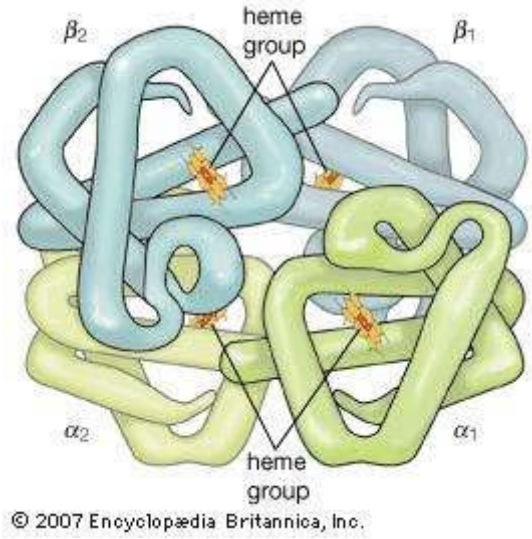


Peptide

Plants can synthesize all of the amino acids; animals cannot, even though all of them are essential for life. Plants can grow in a medium containing inorganic nutrients that provide nitrogen, potassium, and other substances essential for growth. They utilize the carbon dioxide in the air during the process of photosynthesis to form organic compounds such as carbohydrates. Animals, however, must obtain organic nutrients from outside sources. Because the protein content of most plants is low, very large amounts of plant material are required by animals, such as ruminants (e.g., cows), that eat only plant material to meet their amino acid requirements. Nonruminant animals, including humans, obtain proteins principally from animals and their products—e.g., meat, milk, and eggs. The seeds of legumes are increasingly being used to prepare inexpensive protein-rich food(*see* human nutrition).

The protein content of animal organs is usually much higher than that of the blood plasma. Muscles, for example, contain about 30 percent protein, the liver 20 to 30 percent, and red blood cells 30 percent. Higher percentages of protein are found in hair, bones, and other organs and tissues with a low water content. The quantity of free amino acids and peptides in animals is much smaller than the amount of protein; protein molecules are produced in cells by the stepwise alignment of amino acids and are released into the body fluids only after synthesis is complete.

The high protein content of some organs does not mean that the importance of proteins is related to their amount in an organism or tissue; on the contrary, some of the most important proteins, such as enzymes and hormones, occur in extremely small amounts. The importance of proteins is related principally to their function. All enzymes identified thus far are proteins. Enzymes, which are the catalysts of all metabolic reactions, enable an organism to build up the chemical substances necessary for life—proteins, nucleic acids, carbohydrates, and lipids—to convert them into other substances, and to degrade them. Life without enzymes is not possible. There are several protein hormones with important regulatory functions. In all vertebrates, the respiratory protein hemoglobin acts as oxygencarrier in the blood, transporting oxygen from the lung to body organs and tissues. A large group of structural proteins maintains and protects the structure of the animal body.



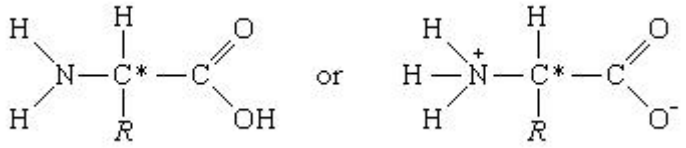
Hemoglobin is a protein made up of four polypeptide chains (α_1, α_2, β_1, and β_2). Each chain is attached to a heme group composed of porphyrin (an organic ringlike compound) attached to an

iron atom. These iron-porphyrin complexes coordinate oxygen molecules reversibly, an ability directly related to the role of hemoglobin in oxygen transport in the blood.*Encyclopædia Britannica, Inc.*

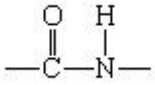
General Structure And Properties Of Proteins

The amino acid composition of proteins

The common property of all proteins is that they consist of long chains of α-amino (alpha amino) acids. The general structure of α-amino acids is shown in . The α-amino acids are so called because the α-carbon atom in the molecule carries an amino group ($-NH_2$); the α-carbon atom also carries

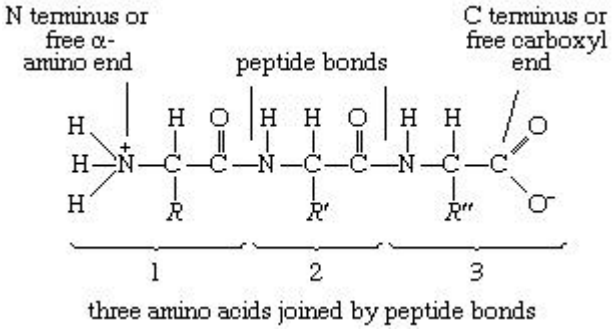


a carboxyl group (−COOH).
In acidic solutions, when the pH is less than 4, the −COO groups combine with hydrogen ions (H^+) and are thus converted into the uncharged form (−COOH). In alkaline solutions, at pH above 9, the ammonium groups ($-NH^+_3$) lose a hydrogen ion and are converted into amino groups ($-NH_2$). In the pH range between 4 and 8, amino acids carry both a positive and a negative charge and therefore do not migrate in an electrical field. Such structures have been designated as dipolar ions, or zwitterions (i.e., hybrid ions).
Although more than 100 amino acids occur in nature, particularly in plants, only 20 types are commonly found in most proteins. In protein molecules the α-amino acids are linked to each other by peptide bonds between the amino group of one amino acid and the carboxyl group of its



neighbour.

The condensation (joining) of three amino acids yields the tripeptide.



three amino acids joined by peptide bonds

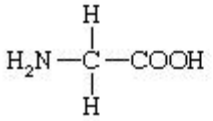
It is customary to write the structure of peptides in such a way that the free α-amino group (also called the N terminus of the peptide) is at the left side and the free carboxyl group (the C terminus) at the right side. Proteins are macromolecular polypeptides—i.e., very large molecules composed of many peptide-bonded amino acids. Most of the common ones contain more than 100 amino acids linked to each other in a long peptide chain. The average molecular weight (based on the weight of a hydrogen atom as 1) of each amino acid is approximately 100 to 125; thus, the molecular weights of proteins are usually in the range of 10,000 to 100,000 daltons (one dalton is

the weight of one hydrogen atom). The species-specificity and organ-specificity of proteins result from differences in the number and sequences of amino acids. Twenty different amino acids in a chain 100 amino acids long can be arranged in far more than 10^{100} ways (10^{100} is the number one followed by 100 zeroes).

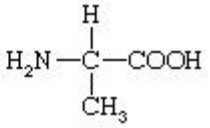
Structures of common amino acids

The amino acids present in proteins differ from each other in the structure of their side (*R*) chains. The simplest amino acid is glycine, in which *R* is a hydrogen atom. In a number of amino acids, *R* represents straight or branched carbon chains. One of these amino acids is alanine, in which *R* is the methyl group($-CH_3$). Valine, leucine, and isoleucine, with longer *R* groups, complete the alkyl side-chain series. The alkyl side chains (*R* groups) of these amino acids are nonpolar; this means that they have no affinity for water but some affinity for each other. Although plants can form all of the alkyl amino acids, animals can synthesize only alanine and glycine; thus valine, leucine, and isoleucine must be supplied in the diet.

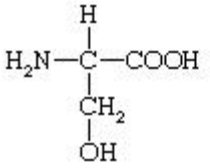
Two amino acids, each containing three carbon atoms, are derived from alanine; they are serine and cysteine. Serine contains an alcohol group ($-CH_2OH$) instead of the methyl group of alanine, and cysteine contains a mercapto group ($-CH_2SH$). Animals can synthesize serine but not cysteine or cystine. Cysteine occurs in proteins predominantly in its oxidized form (oxidation in this sense meaning the removal of hydrogen atoms), called cystine. Cystine consists of two cysteine molecules linked by the disulfide bond ($-S-S-$) that results when a hydrogen atom is removed from the mercapto group of each of the cysteines. Disulfide bonds are important in protein structure because they allow the linkage of two different parts of a protein molecule to—and thus the formation of loops in—the otherwise straight chains. Some proteins contain small amounts of cysteine with free sulfhydryl ($-SH$) groups.



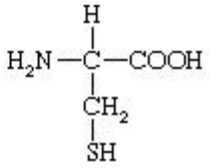
glycine
(Gly, G)



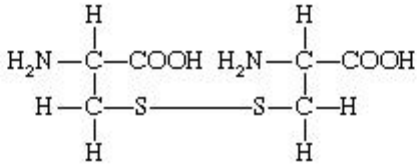
alanine
(Ala, A)



serine
(Ser, S)



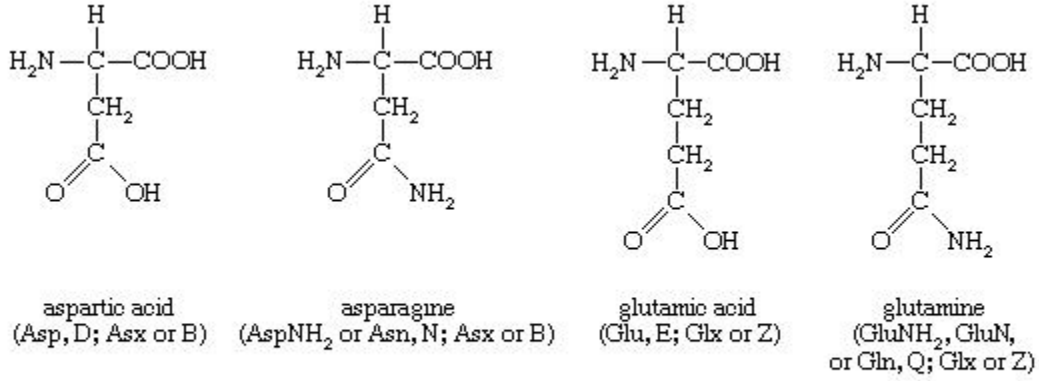
cysteine
(CysH, C)



cystine
(Cys-S-S-Cys, C-C)

Four amino acids, each consisting of four carbon atoms, occur in proteins; they are aspartic acid, asparagine, threonine, and methionine. Aspartic acid and asparagine, which occur in large amounts, can be synthesized by animals. Threonine and methionine cannot be synthesized and thus are essential amino acids; i.e., they must be supplied in the diet. Most proteins contain only small amounts of methionine.

Proteins also contain an amino acid with five carbon atoms (glutamic acid) and a secondary amine (in proline), which is a structure with the amino group ($-NH_2$) bonded to the alkyl side chain, forming a ring. Glutamic acid and aspartic acid are dicarboxylic acids; that is, they have two carboxyl groups ($-COOH$).



aspartic acid (Asp, D; Asx or B)

asparagine ($AspNH_2$ or Asn, N; Asx or B)

glutamic acid (Glu, E; Glx or Z)

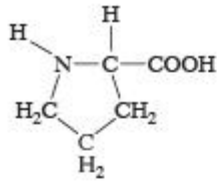
glutamine ($GluNH_2$, GluN, or Gln, Q; Glx or Z)

Glutamine is similar to asparagine in that both are the amides of their corresponding dicarboxylic acid forms; i.e., they have an amide group ($-CONH_2$) in place of the carboxyl ($-COOH$) of the side chain. Glutamic acid and glutamine are abundant in most proteins; e.g., in plant proteins they sometimes comprisemore than one-third of the amino acids present. Both glutamic acid and glutamine can be synthesized by animals.

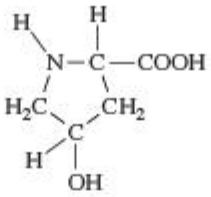
The amino acids proline and hydroxyproline occur in large amounts in collagen, the protein of the connective tissue of animals. Proline and hydroxyproline lack free amino ($-NH_2$) groups because the amino group is enclosed in a ring structure with the side chain; they thus cannot exist in a zwitterion form. Although the nitrogen-containing group (>NH) of these amino acids can form a peptide bond with the carboxyl group of another amino acid, the bond so formed gives rise to a kink in the peptide chain; i.e., the ring structure alters the regular bond angle of normal peptide bonds.

Proteins usually are almost neutral molecules; that is, they have neither acidic nor basic properties. This means that the acidic carboxyl ($-COO^-$) groups of aspartic and glutamic acid are about equal in number to the amino acids with basic side chains. Three such basic amino acids, each containing six carbon atoms, occur in proteins. The one with the simplest structure, lysine, is synthesized by plants but not by animals. Even some plants have a low lysine content. Arginine is found in all proteins; it occurs in particularly high amounts in the strongly basic protamines (simple proteins composed of relatively few amino acids) of fish sperm. The third basic amino acid is histidine. Both arginine and histidine can be synthesized by animals. Histidine is a weaker base than either

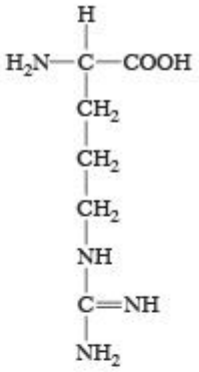
lysine or arginine. ions (H^+) to the nitrogen atoms of the imidazole ring.



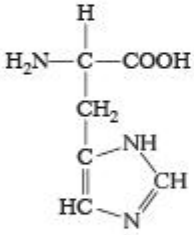
proline
(Pro, P)



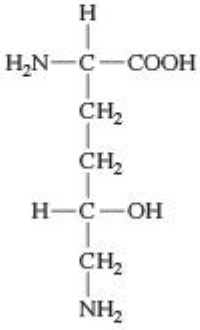
hydroxyproline (Hypro)



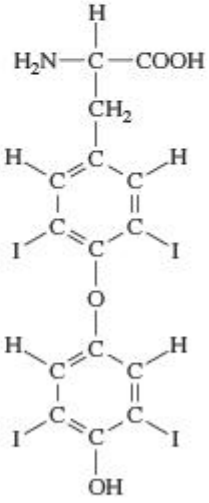
arginine
(Arg, R)



histidine
(His, H)



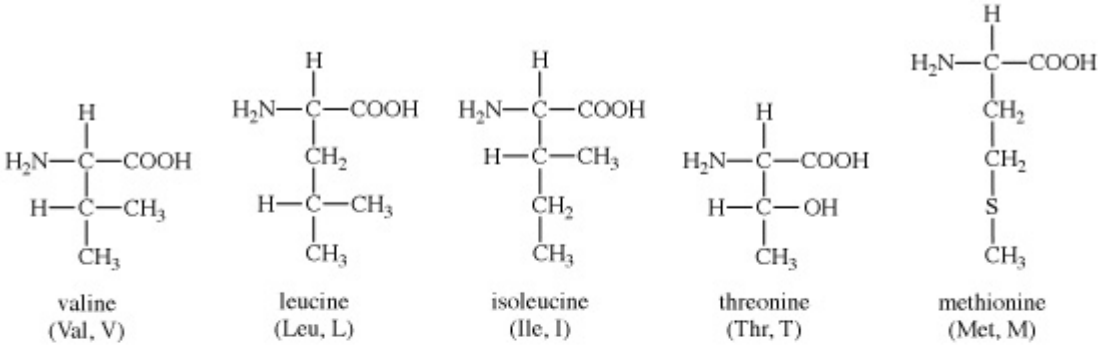
hydroxylysine
(Hylys or Lys−OH)

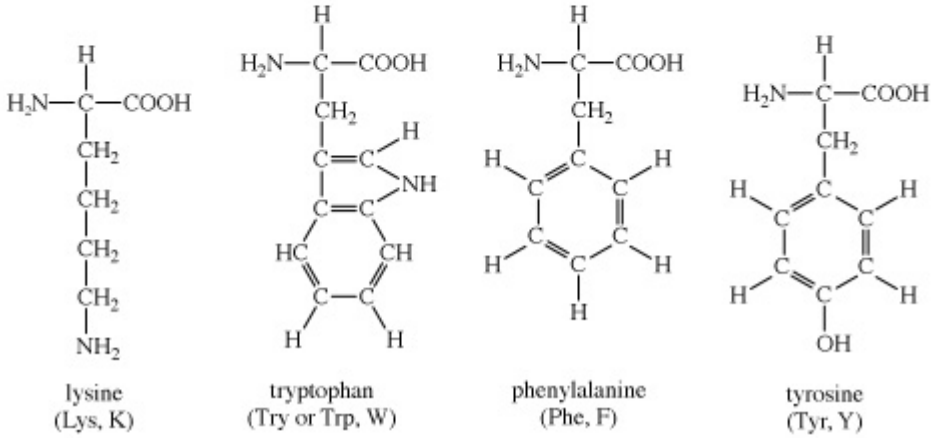


thyroxine (Thy)
occurs only in the
hormone protein
thyroglobulin:
I=iodine

The remaining amino acids—phenylalanine, tyrosine, and tryptophan—have in common an aromatic structure; i.e., a benzene ring is present. These three amino acids are essential, and, while

animals cannot synthesize the benzene ring itself, they can convert phenylalanine to tyrosine.

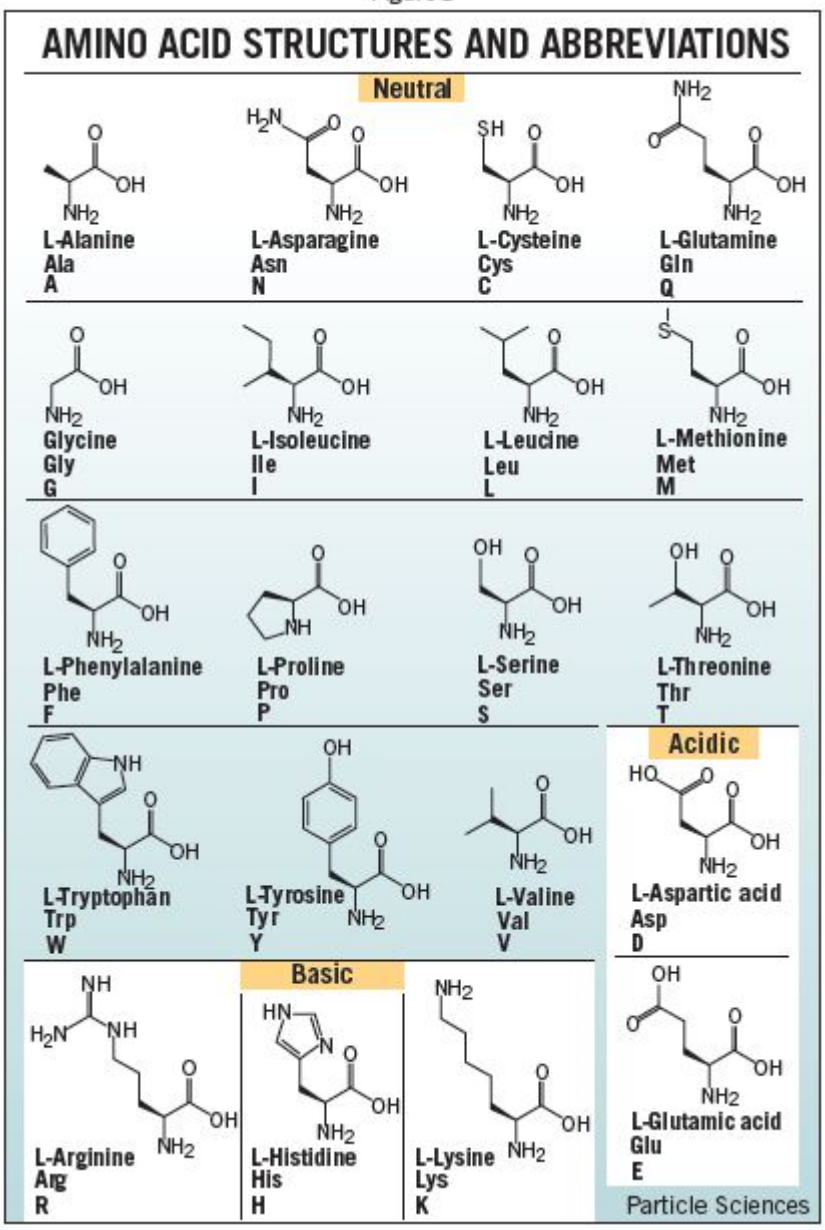




Because these amino acids contain benzene rings, they can absorb ultraviolet light at wavelengths between 270 and 290 nanometres (nm; 1 nanometre = 10^{-9}metre = 10 angstrom units). Phenylalanine absorbs very little ultraviolet light; tyrosine and tryptophan, however, absorb it strongly and are responsible for the absorption band most proteins exhibit at 280–290 nanometres. This absorption is often used to determine the quantity of protein present in protein samples.

Primary Structure

Figure 1



There are 20 different standard L-α-amino acids used by cells for protein construction. Amino acids, as their name indicates, contain both a basic amino group and an acidic carboxyl group. This difunctionality allows the individual amino acids to join together in long chains by forming *peptide bonds:* amide bonds between the -NH2 of one amino acid and the -COOH of another. Sequences with fewer than 50 amino acids are generally referred to as *peptides*, while the terms *protein* or *polypeptide*are used for longer sequences. A protein can be made up of one or more polypeptide molecules. The end of the peptide or protein sequence with a free carboxyl group is called the *carboxy-terminus* or *C-terminus*. The terms *amino-terminus* or *N-terminus* describe the end of the sequence with a free α-amino group.

The amino acids differ in structure by the substituent on their side chains. These side chains confer different chemical, physical and structural properties to the final peptide or protein. The structures of the 20 amino acids commonly found in proteins are shown in Figure 1. Each amino acid has both a one-letter and three-letter abbreviation. These abbreviations are commonly used to simplify the written sequence of a peptide or protein.

Depending on the side-chain substituent, an amino acid can be classified as being acidic, basic or neutral. Although 20 amino acids are required for synthesis of various proteins found in humans, we can synthesize only 10. The remaining 10 are called essential amino acids and must be obtained in the diet.

The amino acid sequence of a protein is encoded in DNA. Proteins are synthesized by a series of steps called transcription (the use of a DNA strand to make a complimentary messenger RNA strand - mRNA) and translation (the mRNA sequence is used as a template to guide the synthesis of the chain of amino acids which make up the protein). Often, post-translational modifications, such as glycosylation or phosphorylation, occur which are necessary for the biological function of the protein. While the amino acid sequence makes up the ***primary structure*** of the protein, the chemical/biological properties of the protein are very much dependent on the three-dimensional or tertiary structure.

Secondary Structure

Stretches or strands of proteins or peptides have distinct characteristic local structural conformations or ***secondary structure***, dependent on hydrogen bonding. The two main types of secondary structure are the α-helix and the ß-sheet.

The *α-helix* is a right-handed coiled strand. The side-chain substituents of the amino acid groups in an α-helix extend to the outside. Hydrogen bonds form between the oxygen of the C=O of each peptide bond in the strand and the hydrogen of the N-H group of the peptide bond four amino acids below it in the helix. The hydrogen bonds make this structure especially stable. The side-chain substituents of the amino acids fit in beside the N-H groups.

The hydrogen bonding in a *ß-sheet* is between strands (inter-strand) rather than within strands (intra-strand). The sheet conformation consists of pairs of strands lying side-by-side. The carbonyl oxygens in one strand hydrogen bond with the amino hydrogens of the adjacent strand. The two strands can be either parallel or anti-parallel depending on whether the strand directions (N-terminus to C-terminus) are the same or opposite. The anti-parallel ß-sheet is more stable due to the more well-aligned hydrogen bonds.

Tertiary Structure

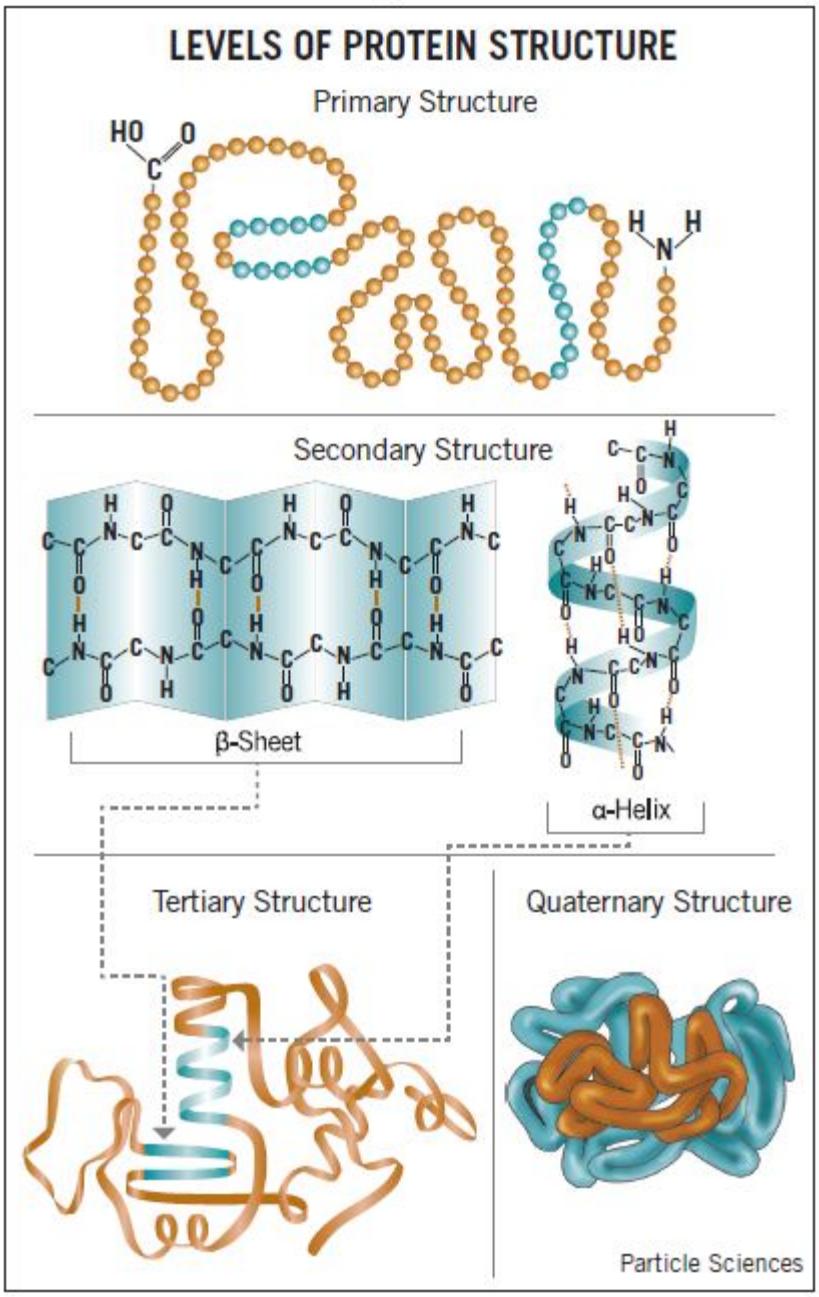
The overall three-dimensional shape of an entire protein molecule is the ***tertiary structure***. The protein molecule will bend and twist in such a way as to achieve maximum stability or lowest energy state. Although the three-dimensional shape of a protein may seem irregular and random,

it is fashioned by many stabilizing forces due to bonding interactions between the side-chain groups of the amino acids.

Under physiologic conditions, the hydrophobic side-chains of neutral, non-polar amino acids such as phenylalanine or isoleucine tend to be buried on the interior of the protein molecule thereby shielding them from the aqueous medium. The alkyl groups of alanine, valine, leucine and isoleucine often form hydrophobic interactions between one-another, while aromatic groups such as those of phenylalanine and tryosine often stack together. Acidic or basic amino acid side-chains will generally be exposed on the surface of the protein as they are hydrophilic.

The formation of disulfide bridges by oxidation of the sulfhydryl groups on cysteine is an important aspect of the stabilization of protein tertiary structure, allowing different parts of the protein chain to be held together covalently. Additionally, hydrogen bonds may form between different side-chain groups. As with *disulfide bridges*, these hydrogen bonds can bring together two parts of a chain that are some distance away in terms of sequence. *Salt bridges*, ionic interactions between positively and negatively charged sites on amino acid side chains, also help to stabilize the tertiary structure of a protein.

Figure 2



Quaternary Structure

Many proteins are made up of multiple polypeptide chains, often referred to as *protein subunits*. These subunits may be the same (as in a homodimer) or different (as in a heterodimer).

The ***quaternary structure*** refers to how these protein subunits interact with each other and arrange themselves to form a larger aggregate protein complex. The final shape of the protein complex is once again stabilized by various interactions, including hydrogen-bonding, disulfide-bridges and salt bridges. The four levels of protein structure are shown in Figure 2.

Protein Stability

Due to the nature of the weak interactions controlling the three-dimensional structure, proteins are very sensitive molecules. The term native state is used to describe the protein in its most stable natural conformation in situ. This native state can be disrupted by a number of external stress factors including temperature, pH, removal of water, presence of hydrophobic surfaces, presence of metal ions and high shear. The loss of secondary, tertiary or quaternary structure due to exposure to a stress factor is called denaturation. Denaturation results in unfolding of the protein into a random or misfolded shape.

A denatured protein can have quite a different activity profile than the protein in its native form, usually losing biological function. In addition to becoming denatured, proteins can also form aggregates under certain stress conditions. Aggregates are often produced during the manufacturing process and are typically undesirable, largely due to the possibility of them causing adverse immune responses when administered.

In addition to these physical forms of protein degradation, it is also important to be aware of the possible pathways of protein chemical degradation. These include oxidation, deamidation, peptide-bond hydrolysis, disulfide-bond reshuffling and cross-linking. The methods used in the processing and the formulation of proteins, including any lyophilization step, must be carefully examined to prevent degradation and to increase the stability of the protein biopharmaceutical both in storage and during drug delivery.

Protein Structure Analysis

The complexities of protein structure make the elucidation of a complete protein structure extremely difficult even with the most advanced analytical equipment. An amino acid analyzer can be used to determine which amino acids are present and the molar ratios of each. The sequence of the protein can then be analyzed by means of peptide mapping and the use of Edman degradation or mass spectroscopy. This process is routine for peptides and small proteins, but becomes more complex for large multimeric proteins.

Peptide mapping generally entails treatment of the protein with different protease enzymes in order to chop up the sequence into smaller peptides at specific cleavage sites. Two commonly used enzymes are trypsin and chymotrypsin. Mass spectroscopy has become an invaluable tool for the analysis of enzyme digested proteins, by means of peptide fingerprinting methods and database searching. Edman degradation involves the cleavage, separation and identification of one amino acid at a time from a short peptide, starting from the N-terminus.

One method used to characterize the secondary structure of a protein is circular dichroism spectroscopy (CD). The different types of secondary structure, α-helix, ß-sheet and random coil, all have characteristic circular dichroism spectra in the far-uv region of the spectrum (190-250 nm). These spectra can be used to approximate the fraction of the entire protein made up of each type of structure.

A more complete, high-resolution analysis of the three-dimensional structure of a protein is carried out using X-ray crystallography or nuclear magnetic resonance (NMR) analysis. To determine the three-dimensional structure of a protein by X-ray diffraction, a large, well-ordered single crystal is required. X-ray diffraction allows measurement of the short distances between atoms and yields a three-dimensional electron density map, which can be used to build a model of the protein structure.

The use of NMR to determine the three-dimensional structure of a protein has some advantages over X-ray diffraction in that it can be carried out in solution and thus the protein is free of the constraints of the crystal lattice. The two-dimensional NMR techniques generally used are NOESY, which measures the distances between atoms through space, and COESY, which measures distances through bonds.

Protein Structure Stability Analysis

Many different techniques can be used to determine the stability of a protein. For the analysis of unfolding of a protein, spectroscopic methods such as fluorescence, UV, infrared and CD can be used. Thermodynamic methods such as differential scanning calorimetry (DSC) can be useful in determining the effect of temperature on protein stability. Comparative peptide-mapping (usually using LC/MS) is an extremely valuable tool in determining chemical changes in a protein such as oxidation or deamidation. HPLC is also an invaluable means of analyzing the purity of a protein. Other analytical methods such as SDS-PAGE, iso-electric focusing and capillary electrophoresis can also be used to determine protein stability, and a suitable bioassay should be used to determine the potency of a protein biopharmaceutical. The state of aggregation can be determined by following “particle” size and arrayed instruments are now available to follow this over time under various conditions.

Thermodynamics

Thermodynamics, science of the relationship between heat, work, temperature, and energy. In broad terms, thermodynamics deals with the transfer of energy from one place to another and from one form to another. The key concept is that heat is a form of energy corresponding to a definite amount of mechanical work.
Heat was not formally recognized as a form of energy until about 1798, when Count Rumford (Sir Benjamin Thompson), a British military engineer, noticed that limitless amounts of heat could be generated in the boring of cannon barrels and that the amount of heat generated is proportional to the work done in turning a blunt boring tool. Rumford’s observation of the proportionality between heat generated and work done lies at the foundation of thermodynamics. Another pioneer was the

French military engineer Sadi Carnot, who introduced the concept of the heat-engine cycle and the principle of reversibility in 1824. Carnot's work concerned the limitations on the maximum amount of work that can be obtained from a steam engine operating with a high-temperature heat transfer as its driving force. Later that century, these ideas were developed by Rudolf Clausius, a German mathematician and physicist, into the first and second laws of thermodynamics, respectively.

The most important laws of thermodynamics are:

- *The zeroth law of thermodynamics.* When two systems are each in thermal equilibrium with a third system, the first two systems are in thermal equilibrium with each other. This property makes it meaningful to use thermometers as the "third system" and to define a temperature scale.
- *The first law of thermodynamics, or the law of conservation of energy.* The change in a system's internal energy is equal to the difference between heat added to the system from its surroundings and work done by the system on its surroundings.
- *The second law of thermodynamics.* Heat does not flow spontaneously from a colder region to a hotter region, or, equivalently, heat at a given temperature cannot be converted entirely into work. Consequently, the entropy of a closed system, or heat energy per unit temperature, increases over time toward some maximum value. Thus, all closed systems tend toward an equilibrium state in which entropy is at a maximum and no energy is available to do useful work. This asymmetry between forward and backward processes gives rise to what is known as the "arrow of time."
- *The third law of thermodynamics.* The entropy of a perfect crystal of an element in its most stable form tends to zero as the temperature approaches absolute zero. This allows an absolute scale for entropy to be established that, from a statistical point of view, determines the degree of randomness or disorder in a system.

Although thermodynamics developed rapidly during the 19th century in response to the need to optimize the performance of steam engines, the sweeping generality of the laws of thermodynamics makes them applicable to all physical and biological systems. In particular, the laws of thermodynamics give a complete description of all changes in the energy state of any system and its ability to perform useful work on its surroundings.

This article covers classical thermodynamics, which does not involve the consideration of individual atoms or molecules. Such concerns are the focus of the branch of thermodynamics known as statistical thermodynamics, or statistical mechanics, which expresses macroscopic thermodynamic properties in terms of the behaviour of individual particles and their interactions. It has its roots in the latter part of the 19th century, when atomic and molecular theories of matter began to be generally accepted.

Thermodynamic states

The application of thermodynamic principles begins by defining a system that is in some sense distinct from its surroundings. For example, the system could be a sample of gas inside a cylinder with a movable piston, an entire steam engine, a marathon runner, the planet Earth, a neutron star, a black hole, or even the entire universe. In general, systems are free to exchange heat, work, and other forms of energy with their surroundings.

A system's condition at any given time is called its thermodynamic state. For a gas in a cylinder with a movable piston, the state of the system is identified by the temperature, pressure, and volume of the gas. These properties are characteristic parameters that have definite values at each state and are independent of the way in which the system arrived at that state. In other words, any change in value of a property depends only on the initial and final states of the system, not on the path followed by the system from one state to another. Such properties are called state functions. In contrast, the work done as the piston moves and the gas expands and the heat the gas absorbs from its surroundings depend on the detailed way in which the expansion occurs.
The behaviour of a complex thermodynamic system, such as Earth's atmosphere, can be understood by first applying the principles of states and properties to its component parts—in this case, water, water vapour, and the various gases making up the atmosphere. By isolating samples of material whose states and properties can be controlled and manipulated, properties and their interrelations can be studied as the system changes from state to state.

Thermodynamic equilibrium

A particularly important concept is thermodynamic equilibrium, in which there is no tendency for the state of a system to change spontaneously. For example, the gas in a cylinder with a movable piston will be at equilibrium if the temperature and pressure inside are uniform and if the restraining force on the piston is just sufficient to keep it from moving. The system can then be made to change to a new state only by an externally imposed change in one of the state functions, such as the temperature by adding heat or the volume by moving the piston. A sequence of one or more such steps connecting different states of the system is called a process. In general, a system is not in equilibrium as it adjusts to an abrupt change in its environment. For example, when a balloon bursts, the compressed gas inside is suddenly far from equilibrium, and it rapidly expands until it reaches a new equilibrium state. However, the same final state could be achieved by placing the same compressed gas in a cylinder with a movable piston and applying a sequence of many small increments in volume (and temperature), with the system being given time to come to equilibrium after each small increment. Such a process is said to be reversible because the system is at (or near) equilibrium at each step along its path, and the direction of change could be reversed at any point. This example illustrates how two different paths can connect the same initial and final states. The first is irreversible (the balloon bursts), and the second is reversible. The concept of reversible processes is something like motion without friction in mechanics. It represents an idealized limiting case that is very useful in discussing the properties of real systems. Many of the results of thermodynamics are derived from the properties of reversible processes.

Temperature

The concept of temperature is fundamental to any discussion of thermodynamics, but its precise definition is not a simple matter. For example, a steel rod feels colder than a wooden rod at room temperature simply because steel is better at conducting heat away from the skin. It is therefore necessary to have an objective way of measuring temperature. In general, when two objects are brought into thermal contact, heat will flow between them until they come into equilibrium with each other. When the flow of heat stops, they are said to be at the same temperature. The zeroth law of thermodynamics formalizes this by asserting that if an object A is in simultaneous thermal equilibrium with two other objects B and C, then B and C will be in thermal equilibrium with each

other if brought into thermal contact. Object *A* can then play the role of a thermometer through some change in its physical properties with temperature, such as its volume or its electrical resistance.

With the definition of equality of temperature in hand, it is possible to establish a temperature scale by assigning numerical values to certain easily reproducible fixed points. For example, in the Celsius (°C) temperature scale, the freezing pointof pure water is arbitrarily assigned a temperature of 0 °C and the boiling point of water the value of 100 °C (in both cases at 1 standard atmosphere; *see*atmospheric pressure). In the Fahrenheit (°F) temperature scale, these same two points are assigned the values 32 °F and 212 °F, respectively. There are absolute temperature scales related to the second law of thermodynamics. The absolute scale related to the Celsius scale is called the Kelvin (K) scale, and that related to the Fahrenheit scale is called the Rankine (°R) scale. These scales are related by the equations K = °C + 273.15, °R = °F + 459.67, and °R = 1.8 K. Zero in both the Kelvin and Rankine scales is at absolute zero.

Work and energy

Energy has a precise meaning in physics that does not always correspond to everyday language, and yet a precise definition is somewhat elusive. The word is derived from the Greek word *ergon*, meaning work, but the term *work* itself acquired a technical meaning with the advent of Newtonian mechanics. For example, a man pushing on a car may feel that he is doing a lot of work, but no work is actually done unless the car moves. The work done is then the product of the force applied by the man multiplied by the distance through which the car moves. If there is no friction and the surface is level, then the car, once set in motion, will continue rolling indefinitely with constant speed. The rolling car has something that a stationary car does not have—it has kinetic energy of motion equal to the work required to achieve that state of motion. The introduction of the concept of energy in this way is of great value in mechanics because, in the absence of friction, energy is never lost from the system, although it can be converted from one form to another. For example, if a coasting car comes to a hill, it will roll some distance up the hill before coming to a temporary stop. At that moment its kinetic energy of motion has been converted into its potential energy of position, which is equal to the work required to lift the car through the same vertical distance. After coming to a stop, the car will then begin rolling back down the hill until it has completely recovered its kinetic energy of motion at the bottom. In the absence of friction, such systems are said to be conservative because at any given moment the total amount of energy (kinetic plus potential) remains equal to the initial work done to set the system in motion.

As the science of physics expanded to cover an ever-wider range of phenomena, it became necessary to include additional forms of energy in order to keep the total amount of energy constant for all closed systems (or to account for changes in total energy for open systems). For example, if work is done to accelerate charged particles, then some of the resultant energy will be stored in the form of electromagnetic fields and carried away from the system as radiation. In turn the electromagnetic energy can be picked up by a remote receiver (antenna) and converted back into an equivalent amount of work. With his theory of special relativity, Albert Einstein realized that energy (*E*) can also be stored as mass (*m*) and converted back into energy, as expressed by his famous equation $E = mc^2$, where *c* is the velocity of light. All of these systems are said to be conservative in the sense that energy can be freely converted from one form to another without limit. Each fundamental advance of physics into new realms has involved a similar extension to the list of the different forms of energy. In addition to preserving the first law of thermodynamics

(*see below*), also called the law of conservation of energy, each form of energy can be related back to an equivalent amount of work required to set the system into motion.
Thermodynamics encompasses all of these forms of energy, with the further addition of heat to the list of different kinds of energy. However, heat is fundamentally different from the others in that the conversion of work (or other forms of energy) into heat is not completely reversible, even in principle. In the example of the rolling car, some of the work done to set the car in motion is inevitably lost as heat due to friction, and the car eventually comes to a stop on a level surface. Even if all the generated heat were collected and stored in some fashion, it could never be converted entirely back into mechanical energy of motion. This fundamental limitation is expressed quantitatively by the second law of thermodynamics (*see below*).
The role of friction in degrading the energy of mechanical systems may seem simple and obvious, but the quantitative connection between heat and work, as first discovered by Count Rumford, played a key role in understanding the operation of steam engines in the 19th century and similarly for all energy-conversion processes today.

Total internal energy

Although classical thermodynamics deals exclusively with the macroscopic properties of materials—such as temperature, pressure, and volume—thermal energy from the addition of heat can be understood at the microscopic level as an increase in the kinetic energy of motion of the molecules making up a substance. For example, gas molecules have translational kinetic energy that is proportional to the temperature of the gas: the molecules can rotate about their centre of mass, and the constituent atoms can vibrate with respect to each other (like masses connected by springs). Additionally, chemical energy is stored in the bonds holding the molecules together, and weaker long-range interactions between the molecules involve yet more energy. The sum total of all these forms of energy constitutes the total internal energy of the substance in a given thermodynamic state. The total energy of a system includes its internal energy plus any other forms of energy, such as kinetic energy due to motion of the system as a whole (e.g., water flowing through a pipe) and gravitational potential energy due to its elevation.

The First Law Of Thermodynamics

The laws of thermodynamics are deceptively simple to state, but they are far-reaching in their consequences. The first law asserts that if heat is recognized as a form of energy, then the total energy of a system plus its surroundings is conserved; in other words, the total energy of the universe remains constant.
The first law is put into action by considering the flow of energy across the boundary separating a system from its surroundings. Consider the classic example of a gas enclosed in a cylinder with a movable piston. The walls of the cylinder act as the boundary separating the gas inside from the world outside, and the movable piston provides a mechanism for the gas to do work by expanding against the force holding the piston (assumed frictionless) in place. If the gas does work W as it expands, and/or absorbs heat Q from its surroundings through the walls of the cylinder, then this corresponds to a net flow of energy $W - Q$ across the boundary to the surroundings. In order to conserve the total energy U, there must be a counterbalancing change$\Delta U = Q - W$ (1)in the internal energy of the gas. The first law provides a kind of strict energy accounting system in

which the change in the energy account (ΔU) equals the difference between deposits (Q) and withdrawals (W).
There is an important distinction between the quantity ΔU and the related energy quantities Q and W. Since the internal energy U is characterized entirely by the quantities (or parameters) that uniquely determine the state of the system at equilibrium, it is said to be a state function such that any change in energy is determined entirely by the initial (i) and final (f) states of the system: $\Delta U = Uf - Ui$. However, Q and W are not state functions. Just as in the example of a bursting balloon, the gas inside may do no work at all in reaching its final expanded state, or it could do maximum work by expanding inside a cylinder with a movable piston to reach the same final state. All that is required is that the change in energy (ΔU) remain the same. By analogy, the same change in one's bank account could be achieved by many different combinations of deposits and withdrawals. Thus, $Q$and W are not state functions, because their values depend on the particular process (or path) connecting the same initial and final states. Just as it is more meaningful to speak of the balance in one's bank account than its deposit or withdrawal content, it is only meaningful to speak of the internal energy of a system and not its heat or work content.
From a formal mathematical point of view, the incremental change dU in the internal energy is an exact differential (*see* differential equation), while the corresponding incremental changes $d'Q$ and $d'W$ in heat and work are not, because the definite integrals of these quantities are path-dependent. These concepts can be used to great advantage in a precise mathematical formulation of thermodynamics

The Second Law Of Thermodynamics

The first law of thermodynamics asserts that energy must be conserved in any process involving the exchange of heat and work between a system and its surroundings. A machine that violated the first law would be called a perpetual motion machine of the first kind because it would manufacture its own energy out of nothing and thereby run forever. Such a machine would be impossible even in theory. However, this impossibility would not prevent the construction of a machine that could extract essentially limitless amounts of heat from its surroundings (earth, air, and sea) and convert it entirely into work. Although such a hypotheticalmachine would not violate conservation of energy, the total failure of inventors to build such a machine, known as a perpetual motion machine of the second kind, led to the discovery of the second law of thermodynamics. The second law of thermodynamics can be precisely stated in the following two forms, as originally formulated in the 19th century by the Scottish physicist William Thomson (Lord Kelvin) and the German physicist Rudolf Clausius, respectively:

A cyclic transformation whose only final result is to transform heat extracted from a source which is at the same temperature throughout into work is impossible.
A cyclic transformation whose only final result is to transfer heat from a body at a given temperature to a body at a higher temperature is impossible.

The two statements are in fact equivalent because, if the first were possible, then the work obtained could be used, for example, to generate electricity that could then be discharged through an electric heater installed in a body at a higher temperature. The net effect would be a flow of heat from a lower temperature to a higher temperature, thereby violating the second (Clausius) form of the

second law. Conversely, if the second form were possible, then the heat transferred to the higher temperature could be used to run a heat engine that would convert part of the heat into work. The final result would be a conversion of heat into work at constant temperature—a violation of the first (Kelvin) form of the second law.

Central to the following discussion of entropy is the concept of a heat reservoir capable of providing essentially limitless amounts of heat at a fixed temperature. This is of course an idealization, but the temperature of a large body of water such as the Atlantic Ocean does not materially change if a small amount of heat is withdrawn to run a heat engine. The essential point is that the heat reservoir is assumed to have a well-defined temperature that does not change as a result of the process being considered.

Entropy

Entropy and efficiency limits

The concept of entropy was first introduced in 1850 by Clausius as a precise mathematical way of testing whether the second law of thermodynamics is violated by a particular process. The test begins with the definition that if an amount of heat Q flows into a heat reservoir at constant temperature T, then its entropy S increases by $\Delta S = Q/T$. (This equation in effect provides a thermodynamic definition of temperature that can be shown to be identical to the conventional thermometric one.) Assume now that there are two heat reservoirs R_1 and R_2 at temperatures T_1 and T_2. If an amount of heat Q flows from R_1 to R_2, then the net entropy change for the two reservoirs is $\Delta S = \frac{Q}{T_2} - \frac{Q}{T_1}$. (3) ΔS is positive, provided that $T_1 > T_2$. Thus, the observation that heat never flows spontaneously from a colder region to a hotter region (the Clausius form of the second law of thermodynamics) is equivalent to requiring the net entropy change to be positive for a spontaneous flow of heat. If $T_1 = T_2$, then the reservoirs are in equilibrium and $\Delta S = 0$.

Entropy and Life

- It is a measure of disorder: cells are NOT disordered and so have low entropy.
- In the process of energy transfer, some energy will dissipate as heat. The flow of energy maintains order and life.
- Entropy wins when organisms cease to take in energy and die.

- All biological organisms require energy to survive.
- In a closed system, such as the universe, this energy is not consumed but transformed from one form to another.
- Cells, for example, perform a number of important processes. These processes require energy.
- In photosynthesis, the energy is supplied by the sun. Light energy is absorbed by cells in plant leaves and converted to chemical energy.
- The chemical energy is stored in the form of glucose, which is used to form complex carbohydrates necessary to build plant mass.
- The energy stored in glucose can also be released through cellular respiration. This process allows plant and animal organisms to access the energy stored in carbohydrates, lipids and other macromolecules through the production of ATP.
- This energy is needed to perform cell functions such as DNA replication, mitosis, meiosis, cell movement, endocytosis, exocytosis, and apoptosis.
- As with other biological processes, the transfer of energy is not 100% efficient.
- In photosynthesis, for example, not all of the light energy is absorbed by the plant. Some energy is reflected and some is lost as heat.

- The loss of energy to the surrounding environment results in an increase of disorder or entropy.
- Unlike plants and other photosynthetic organisms, animals can not generate energy directly from the sunlight.
- They must consume plants or other animal organisms for energy.
- The higher up an organism is on the food chain, the less available energy it receives from its food sources. Much of this energy is lost during metabolic processes performed by the producers and primary consumers that are eaten.
- Therefore, much less energy is available for organisms in higher trophic levels. The lower the available energy, the less number of organisms can be supported. This is why there are more producers than consumers in an ecosystem.
- Living systems require constant energy input to maintain their highly ordered state.
- Cells, for example, are highly ordered and have low entropy. In the process of maintaining this order, some energy is lost to the surroundings or transformed.
- So while cells are ordered, the processes performed to maintain that order result in an increase in entropy in the cell's/organism's surroundings. The transfer of energy causes entropy in the universe to increase.

Gibbs Free Energy (G) - The energy associated with a chemical reaction that can be used to do work. The free energy of a system is the sum of its enthalpy (H) plus the product of the temperature (Kelvin) and the entropy (S) of the system:

$$G = H - TS$$

Free energy of reaction (ΔG)

- The change in the enthalpy (ΔH) of the system minus the product of the temperature (Kelvin) and the change in the entropy (ΔS) of the system:

$$\Delta G = \Delta H - T\Delta S$$

Standard-state free energy of reaction (ΔG°)

- The free energy of reaction at standard state conditions:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

Standard-state conditions

- The partial pressures of any gases involved in the reaction is 0.1 MPa.
- The concentrations of all aqueous solutions are 1 M.

Measurements are also generally taken at a temperature of 25°C (298 K)

Standard-State Free Energy of Formation (ΔG_f°)

- The change in free energy that occurs when a compound is formed form its elements in their most thermodynamically stable states at standard-state conditions. In other words, it is the difference between the free energy of a substance and the free energies of its elements in their most thermodynamically stable states at standard-state conditions.
- The standard-state free energy of reaction can be calculated from the standard-state free energies of formation as well. It is the sum of the free energies of formation of the products minus the sum of the free energies of formation of the reactants:

$$\Delta G^\circ = \sum \Delta G^\circ_{f\,products} - \sum \Delta G^\circ_{f\,reactants}$$

Recall from the enthalpy notes that reactions can be classified according to the change in enthalpy (heat):

- **Endothermic** - absorbs heat, $\Delta H^\circ > 0$
- **Exothermic** - releases heat, $\Delta H^\circ > 0$

Reactions can also be classified according to the change in the free energy of the reaction:

- **Endergonic** - NON-SPONTANEOUS, $\Delta G^\circ > 0$
- **Exergonic** - SPONTANEOUS, $\Delta G^\circ < 0$

Summary

Favorable Conditions	Unfavorable Conditions
$\Delta H^\circ < 0$	$\Delta H^\circ > 0$
$\Delta S^\circ > 0$	$\Delta S^\circ > 0$

SPONTANEOUS: ΔG° is negative ($\Delta G^\circ < 0$)

NON-SPONTANEOUS: ΔG° is positive ($\Delta G^\circ > 0$)

Endergonic vs Exergonic

Endergonic and exergonic are two types of chemical reactions or processes in thermochemistry or physical chemistry. The names describe what happens to energy during the reaction. The classifications are related to endothermic and exothermic reactions, except endergonic and exergonic describe what happens with any form of energy, while endothermic and exothermic relate only to heat or thermal energy.

Endergonic Reactions

- An endergonic reactions may also be called an unfavorable reaction or nonspontaneous reaction. The reaction requires more energy than you get from it.
- Endergonic reactions absorb energy from the surroundings.
- The chemical bonds that are formed from the reaction are weaker than the chemical bonds that were broken.
- The free energy of the system increases. The change in the standard Gibbs Free Energy (G) of an endergonic reaction is positive (greater than 0).
- The change in entropy (S) decreases.
- Endergonic reactions are not spontaneous.
- Examples of endergonic reactions include endothermic reactions, such as photosynthesis and the melting of ice into liquid water.
- If the temperature of the surroundings decreases, the reaction is endothermic.

Exergonic Reactions

- An exergonic reaction may be called a spontaneous reaction or a favorable reaction.
- Exergonic reactions release energy to the surroundings.

- The chemical bonds formed from the reaction are stronger than those that were broken in the reactants.
- The free energy of the system decreases. The change in the standard Gibbs Free Energy (G) of an exergonic reaction is negative (less than 0).
- The change in entropy (S) increases. Another way to look at it is that the disorder or randomness of the system increases.

- Exergonic reactions occur spontaneously (no outside energy is required to start them).
- Examples of exergonic reactions include exothermic reactions, such as mixing sodium and chlorine to make table salt, combustion, and chemiluminescence (light is the energy that is released).
- If the temperature of the surroundings increases, the reaction is exothermic.

Notes About the Reactions

- You cannot tell how quickly a reaction will occur based on whether it is endergonic or exergonic. Catalysts may be needed to cause the reaction to proceed at an observable rate.

For example, rust formation (oxidation of iron) is an exergonic and exothermic reaction, yet it proceeds so slowly it's difficult to notice the release of heat to the environment.

- In biochemical systems, endergonic and exergonic reactions often are coupled, so the energy from one reaction can power another reaction.
- Endergonic reactions always require energy to start. Some exergonic reactions also have an activation energy, but more energy is released by the reaction than is required to initiate it. For example, it takes energy to start a fire, but once combustion starts, the reaction releases more light and heat than it took to get it started.

- Endergonic reactions and exergonic reactions are sometimes called reversible reactions. The quantity of the energy change is the same for both reactions, although the energy is absorbed by the endergonic reaction and released by the exergonic reaction. Whether the reverse reaction actually *can* occur is not a consideration when defining reversibility. For example, while burning wood is a reversible reaction theoretically, it doesn't actually occur in real life.

Perform Simple Endergonic and Exergonic Reactions

In an endergonic reaction, energy is absorbed from the surroundings. Endothermic reactions offer good examples, as they absorb heat. Mix together baking soda (sodium carbonate) and citric acid in water. The liquid will get cold, but not cold enough to cause frostbite.

An exergonic reaction releases energy to the surroundings.

Exothermic reactions are good examples of this type of reaction because they release heat. The next time you do laundry, put some laundry detergent in your hand and add a small amount of water. Do you feel the heat? This is a safe and simple example of an exothermic and thus exergonic reaction.

Structure of the plasma membrane

The biological membrane, which is present in both eukaryotic and prokaryotic cell. It is also called as cell membrane as it is works as a barrier between the inner and outer surface of a cell. In animal cells, the plasma membrane is present in the outer most layer of the cell and in plant cell it is present just beneath the cell wall.

Plasma Membrane Definition

Plasma membrane can be defined as a biological membrane or an outer membrane of a cell, which is composed of two layers of phospholipids and embedded with proteins. It is a thin semi permeable membrane layer, which surrounds the cytoplasm and other constituents of the cell.

Function of Plasma Membrane

1. It separates the contents of the cell from its outside environment and it regulates what enters and exits the cell.
2. Plasma membrane plays a vital role in protecting the integrity of the interior of the cell by allowing only selected substances into the cell and keeping other substances out.
3. It also serves as a base of attachment for the cytoskeleton in some organisms and the cell wall in others. Thus the cell membrane supports the cell and helps in maintaining the shape of the cell.
4. The cell membrane is primarily composed of proteins and lipids. While lipids help to give membranes their flexibility and proteins monitor and maintain the cell's chemical climate and assist in the transfer of molecules across the membrane.
5. The lipid bilayer is semi-permeable, which allows only selected molecules to diffuse across the membrane.

Characteristics of Plasma Membrane
Components of Plasma Membrane

The main components of plasma membrane include:

1. Proteins like glycoprotein, which are used for cell recognition and act as receptors and antigens.
2. Proteins like glycolipids are attached to phospholipids along with the sugar chains.
3. Lipids with short chain of carbohydrates are attached on the extracellular side of the membrane.
4. Phospholipid Bilayer - which are made up of phosphates and lipids. They create a partially permeable membrane, which allows only certain substances to diffuse through the membrane.

5. Cholesterol – it maintains the fluidity of cell surface membrane.

Proteins in Plasma Membrane

In plasma membrane, a protein helps in providing the support and shape to the cell. There are three types of proteins in plasma membrane, which includes:

1. Cell membrane receptor proteins- It helps in communication of a cell with their external environment with the help of hormones, neurotransmitters and other signaling molecules.
2. Transport proteins - It helps in transporting molecules across cell membranes through facilitated diffusion. For example: globular proteins.
3. Glycoprotein - It helps in cell to cell communications and molecule transport across the membrane.

Below you could see characteristics of plasma membrane

1. The plasma membrane (cell membrane) is made of two layers of phospholipids.
2. The plasma membrane has many proteins embedded in it.
3. The plasma membrane regulates the entry and exit of the cell. Many molecules cross the cell membrane by diffusion and osmosis.
4. The fundamental structure of the membrane is phospho lipid bilayer and it forms a stable barrier between two aqueous compartments.
5. The proteins present in the plasma membrane, act as pumps, channels, receptors, enzymes or structural components.

Plasma Membrane Structure

1. It is the boundary, which separates the living cell from their non-living surroundings.
2. It is the phospholipids bilayer.
3. Plasma membrane is an amphipathic, which contains both hydrophilic heads and hydrophobic tails.
4. It is a fluid mosaic of lipids, proteins and carbohydrate.
5. It is lipid bilayer, which contains -two layers of phospholipids, phosphate head is polar (water loving), fatty acid tails non-polar (water fearing) and the proteins embedded in membrane.

Fluid mosaic model

The currently accepted model for the structure of the plasma membrane, called the **fluid mosaic model**, was first proposed in 1972. This model has evolved over time, but it still provides a good basic description of the structure and behavior of membranes in many cells.

According to the fluid mosaic model, the plasma membrane is a mosaic of components—primarily, phospholipids, cholesterol, and proteins—that move freely and fluidly in the plane of the membrane. In other words, a diagram of the membrane (like the one below) is just a snapshot of a dynamic process in which phospholipids and proteins are continually sliding past one another.

Interestingly enough, this fluidity means that if you insert a very fine needle into a cell, the membrane will simply part to flow around the needle; once the needle is removed, the membrane will flow back together seamlessly.

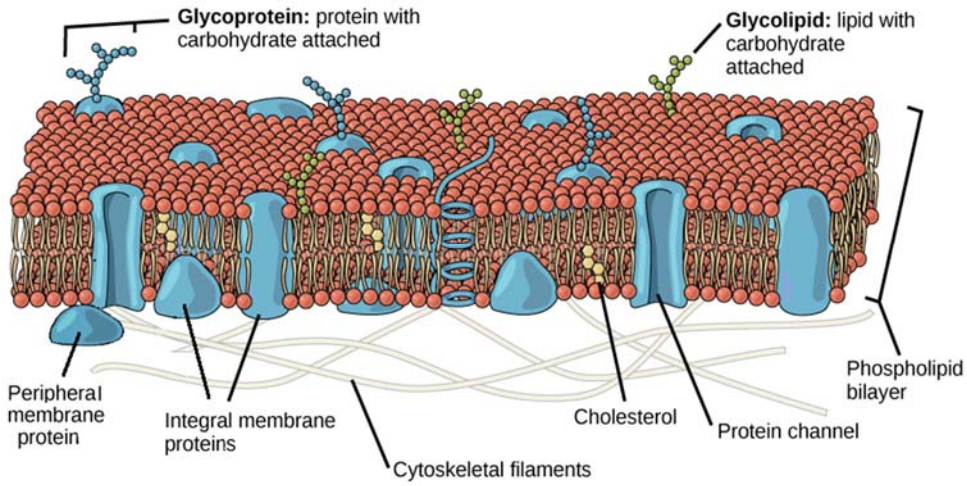


Image of the plasma membrane, showing the phospholipid bilayer with peripheral and integral membrane proteins, glycoproteins (proteins with a carbohydrate attached), glycolipids (lipids with a carbohydrate attached), and cholesterol molecules.

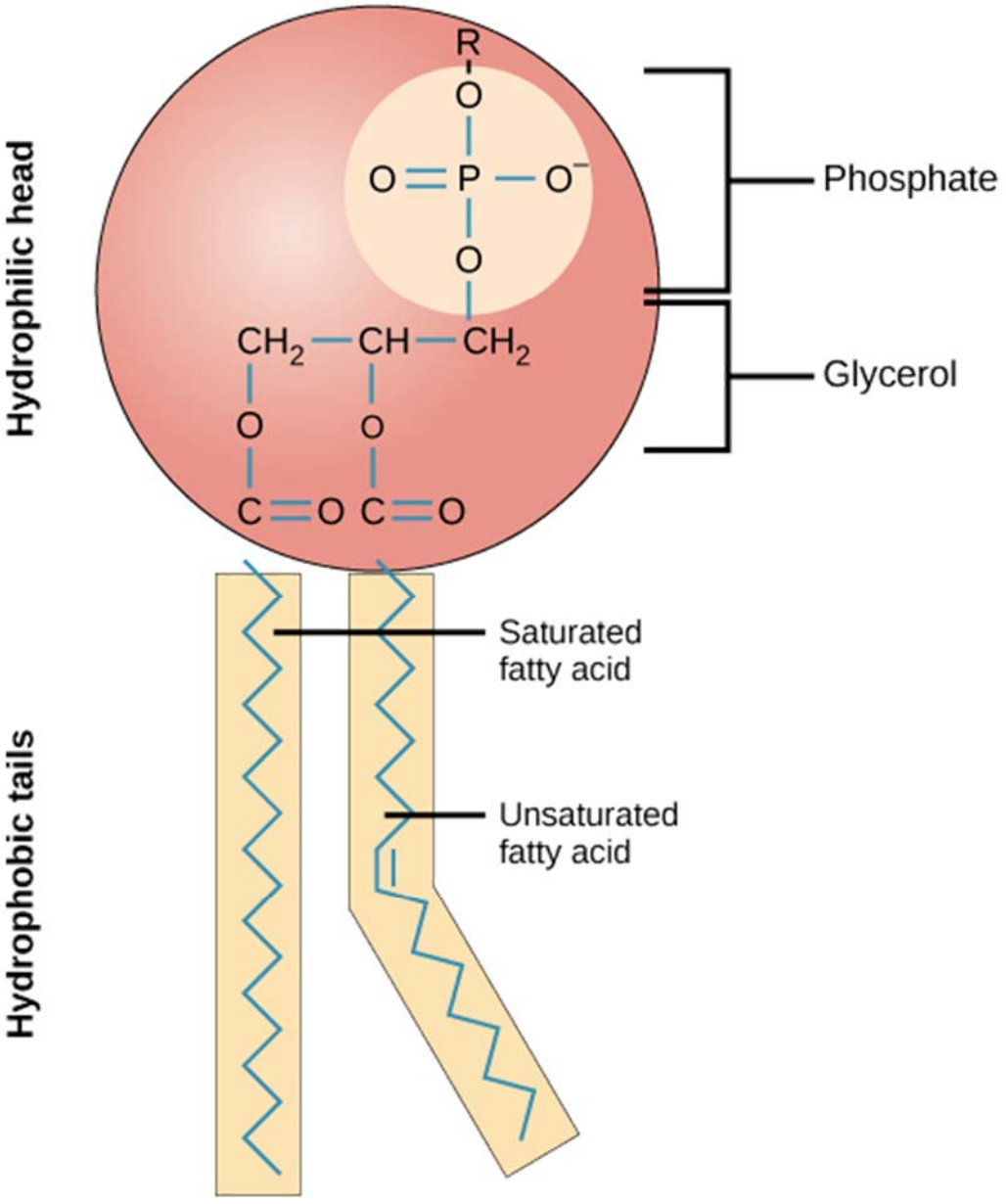
The principal components of the plasma membrane are lipids (phospholipids and cholesterol), proteins, and carbohydrate groups that are attached to some of the lipids and proteins.

- A **phospholipid** is a lipid made of glycerol, two fatty acid tails, and a phosphate-linked head group. Biological membranes usually involve two layers of phospholipids with their tails pointing inward, an arrangement called a **phospholipid bilayer**.
- **Cholesterol**, another lipid composed of four fused carbon rings, is found alongside phospholipids in the core of the membrane.
- Membrane proteins may extend partway into the plasma membrane, cross the membrane entirely, or be loosely attached to its inside or outside face.
- Carbohydrate groups are present only on the outer surface of the plasma membrane and are attached to proteins, forming **glycoproteins**, or lipids, forming **glycolipids**.

  The proportions of proteins, lipids, and carbohydrates in the plasma membrane vary between different types of cells. For a typical human cell, however, proteins account for about 50 percent of the composition by mass, lipids (of all types) account for about 40 percent, and the remaining 10 percent comes from carbohydrates.

Phospholipids

Phospholipids, arranged in a bilayer, make up the basic fabric of the plasma membrane. They are well-suited for this role because they are **amphipathic**, meaning that they have both hydrophilic and hydrophobic regions.



Chemical structure of a phospholipid, showing the hydrophilic head and hydrophobic tails.

Image credit: OpenStax Biology.

The **hydrophilic**, or "water-loving," portion of a phospholipid is its head, which contains a negatively charged phosphate group as well as an additional small group (of varying identity, "R" in the diagram at left), which may also or be charged or polar. The hydrophilic heads of phospholipids in a membrane bilayer face outward, contacting the aqueous (watery) fluid both inside and outside the cell. Since water is a polar molecule, it readily forms electrostatic (charge-based) interactions with the phospholipid heads.

The **hydrophobic**, or "water-fearing," part of a phospholipid consists of its long, nonpolar fatty acid tails. The fatty acid tails can easily interact with other nonpolar molecules, but they interact poorly with water. Because of this, it's more energetically favorable for the phospholipids to tuck their fatty acid tails away in the interior of the membrane, where they are shielded from the surrounding water. The phospholipid bilayer formed by these interactions makes a good barrier between the interior and exterior of the cell, because water and other polar or charged substances cannot easily cross the hydrophobic core of the membrane.

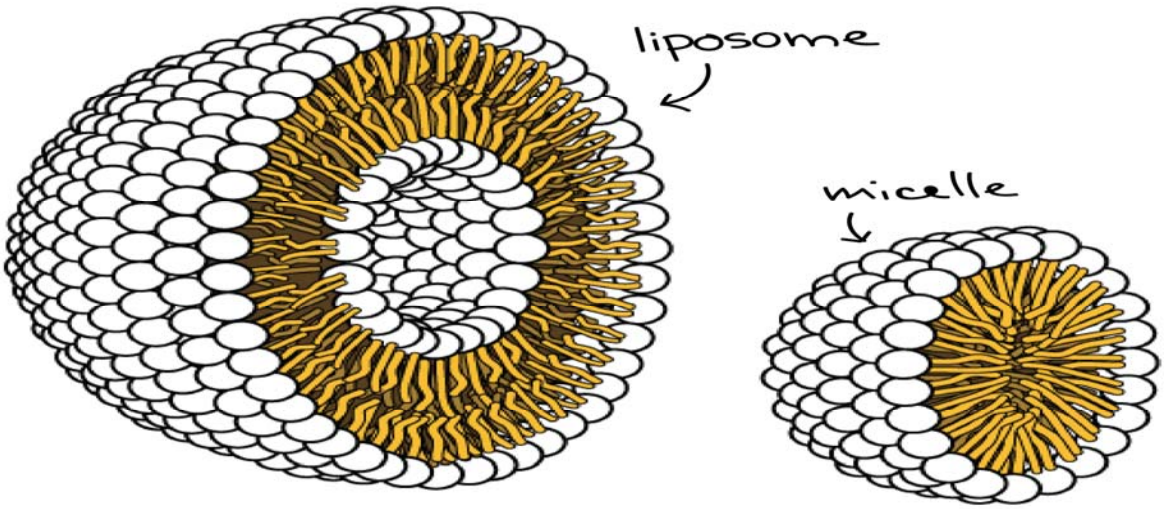


Image of a micelle and a liposome.

Thanks to their amphipathic nature, phospholipids aren't just well-suited to form a membrane bilayer. Instead, this is something they'll do spontaneously under the right conditions! In water or aqueous solution, phospholipids tend to arrange themselves with their hydrophobic tails facing

each other and their hydrophilic heads facing out. If the phospholipids have small tails, they may form a **micelle** (a small, single-layered sphere), while if they have bulkier tails, they may form a **liposome** (a hollow droplet of bilayer membrane)^22start superscript, 2, end superscript.

Proteins

Proteins are the second major component of plasma membranes. There are two main categories of **membra**ne proteins: integral and peripheral.

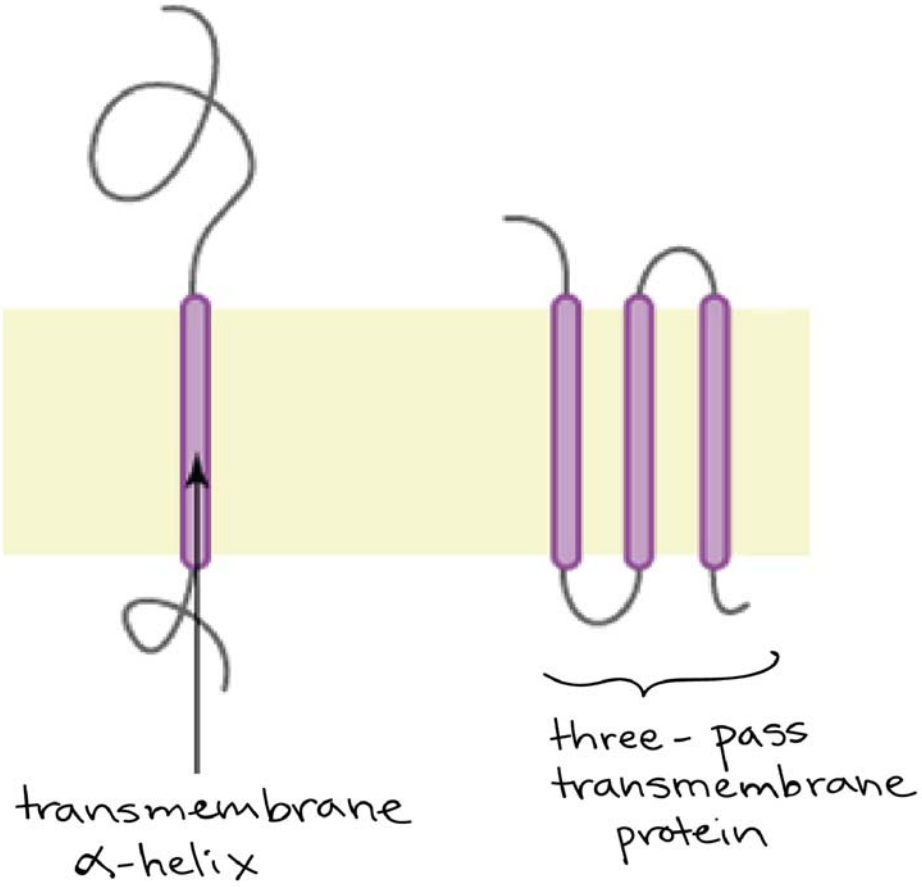
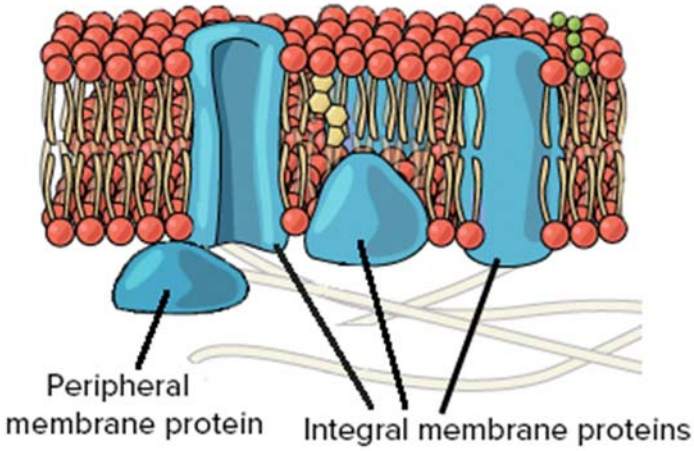


Image of a single-pass transmembrane protein with a single membrane-spanning alpha helix and a three-pass transmembrane protein with three membrane-spanning alpha helices.

Integral membrane proteins are, as their name suggests, integrated into the membrane: they have at least one hydrophobic region that anchors them to the hydrophobic core of the phospholipid bilayer. Some stick only partway into the membrane, while others stretch from one side of the membrane to the other and are exposed on either side^11start superscript, 1, end superscript. Proteins that extend all the way across the membrane are called **transmembrane proteins**.

The portions of an integral membrane protein found inside the membrane are hydrophobic, while those that are exposed to the cytoplasm or extracellular fluid tend to be hydrophilic. Transmembrane proteins may cross the membrane just once, or may have as many as twelve different membrane-spanning sections. A typical membrane-spanning segment consists of 20-25 hydrophobic amino acids arranged in an alpha helix, although not all transmembrane proteins fit this model. Some integral membrane proteins form a channel that allows ions or other small molecules to pass, as shown below.



Peripheral membrane proteins are found on the outside and inside surfaces of membranes, attached either to integral proteins or to phospholipids. Unlike integral membrane proteins, peripheral membrane proteins do not stick into the hydrophobic core of the membrane, and they tend to be more loosely attached.

Carbohydrates

Carbohydrates are the third major component of plasma membranes. In general, they are found on the outside surface of cells and are bound either to proteins (forming **glycoproteins**) or to lipids

(forming **glycolipids**). These carbohydrate chains may consist of 2-60 monosaccharide units and can be either straight or branched.

Along with membrane proteins, these carbohydrates form distinctive cellular markers, sort of like molecular ID badges, that allow cells to recognize each other. These markers are very important in the immune system, allowing immune cells to differentiate between body cells, which they shouldn't attack, and foreign cells or tissues, which they should.

Membrane fluidity

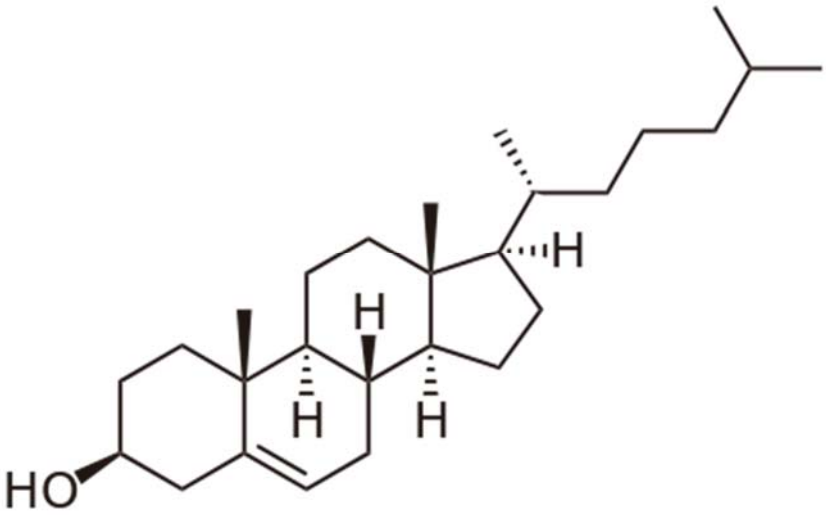
The structure of the fatty acid tails of the phospholipids is important in determining the properties of the membrane, and in particular, how fluid it is.

Saturated fatty acids have no double bonds (are saturated with hydrogens), so they are relatively straight. **Unsaturated** fatty acids, on the other hand, contain one or more double bonds, often resulting in a bend or kink. (You can see an example of a bent, unsaturated tail in the diagram of phospholipid structure that appears earlier in this article.) The saturated and unsaturated fatty acid tails of phospholipids behave differently as temperature drops:

- At cooler temperatures, the straight tails of saturated fatty acids can pack tightly together, making a dense and fairly rigid membrane.
- Phospholipids with unsaturated fatty acid tails cannot pack together as tightly because of the bent structure of the tails. Because of this, a membrane containing unsaturated phospholipids will stay fluid at lower temperatures than a membrane made of saturated ones.

Most cell membranes contain a mixture of phospholipids, some with two saturated (straight) tails and others with one saturated and one unsaturated (bent) tail. Many organisms—fish are one example—can adjust physiologically to cold environments by changing the proportion of unsaturated fatty acids in their membranes. For more information about saturated and unsaturated fatty acids, see the article on lipids.

In addition to phospholipids, animals have an additional membrane component that helps to maintain fluidity. **Cholesterol**, another type of lipid that is embedded among the phospholipids of the membrane, helps to minimize the effects of temperature on fluidity.



At low temperatures, cholesterol increases fluidity by keeping phospholipids from packing tightly together, while at high temperatures, it actually reduces fluidity^{3,4}3,4start superscript, 3, comma, 4, end superscript. In this way, cholesterol expands the range of temperatures at which a membrane maintains a functional, healthy fluidity.

The components of the plasma membrane

Component	Location
Phospholipids	Main fabric of the membrane
Cholesterol	Tucked between the hydrophobic tails of the membrane phospholipids
Integral proteins	Embedded in the phospholipid bilayer; may or may not extend through both layers

Component	Location
Peripheral proteins	On the inner or outer surface of the phospholipid bilayer, but not embedded in its hydrophobic core
Carbohydrates	Attached to proteins or lipids on the extracellular side of the membrane (forming glycoproteins and glycolipids)

Membrane Transport Mechanisms

It is of seminal importance to the cell that it be able to transport molecules in and out of itself. Imagine that a protein having multiple transmembrane domains is structured so that these domains are arrayed in the plane of the membrane in a circle, thereby forming a cylinder, or, better yet, a barrel when viewed from the outside of the cell, with each of the staves of the barrel being one of the transmembrane domains. The center of the barrel could constitute a hole in the plasma membrane that is isolated from the lipid bilayer by an array of transmembrane domains around it. This hole could be used to transport substances into the cell or out from the cell. In fact, this hole can be a relatively hydrophilic environment if hydrophilic side chains from the membrane-spanning chains surrounding the hole protrude into the hole itself.

In practice, given the structure of known membrane proteins, these holes are only large enough to allow the passage of small molecules through the plasma membrane, almost always simple ions like hydrogen, potassium or sodium. The ions may pass through the hole or orifice by passive diffusion, in which case the protein that allows this transport is called an ion channel. Alternatively, the transmembrane protein may invest energy, usually derived from ATP, to actively force ions from one side of the plasma membrane to the other, in which case it will be an ion pump.

Given the importance of membrane transport, cells utilize a wide range of transport mechanisms. The mechanisms fall into one of three categories: simple diffusion, facilitated diffusion, and active transport.

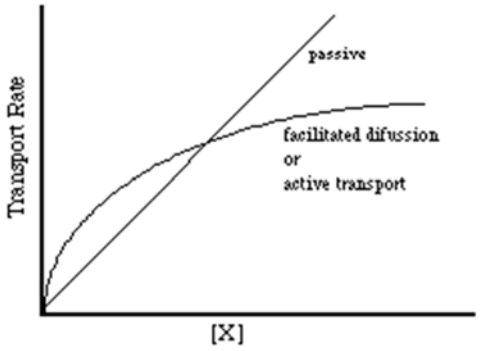
Diffusion

Simple diffusion means that the molecules can pass directly through the membrane. Diffusion is always down a concentration gradient. This limits the maximum possible concentration of the

molecule inside the cell (or outside the cell if it is a waste product). The effectiveness of diffusion is also limited by the diffusion rate of the molecule (see Purves box 5.B). Therefore, though diffusion is an effective enough transport mechanism for some substances (such as H2O), the cell must utilize other mechanisms for many of its transport needs.

Facilitated Diffusion

Facilitated diffusion utilizes membrane protein channels to allow charged molecules (which otherwise could not diffuse across the cell membrane) to freely diffuse in a nd out of the cell. These channels comes into greatest use with small ions like K+, Na+, and Cl-. The speed of facilitated transport is limited by the number of protein channels available, whereas the speed of diffusion is dependent only on the concentration gradient.



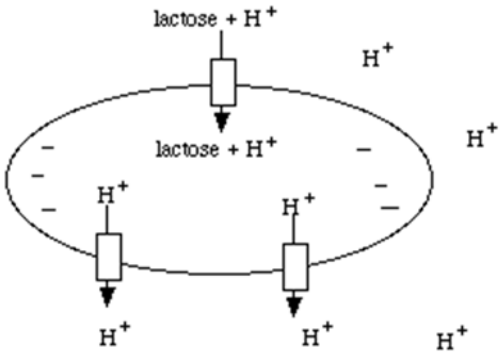
Active Transport

Active transport requires the expenditure of energy to transport the molecule from one side of the membrane to the other, but active transport is the only type of transport that can actually take molecules up their concentration gradient as well as down. Similarly to facilitated transport, active transport is limited by the number of protein transporters present.

We are interested in two general categories of active transport, primary and secondary. Primary active transport involves using energy (usually through ATP hydrolysis) at the membrane protein itself to cause a conformational change that results in the transport of the molecule through the protein. The most well-known example of this is the Na+-K+ pump. The Na+-K+ pump is an antiport, it transports K+ into the cell and Na+ out of the cell at the same time, with the expenditure of ATP.

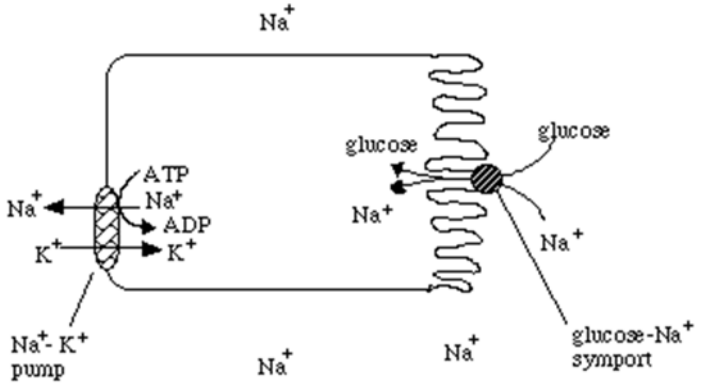
Secondary active transport involves using energy to establish a gradient across the cell membrane, and then utilizing that gradient to transport a molecule of interest up its concentration gradient. An example of this mechanism is as follows: *E. coli* establishes a proton (H+) gradient across the cell membrane by using energy to pump protons out of the cell. Then those protons are coupled to lactose at the lactose permease transmembrane protein. The lactose permease uses the energy of the proton moving down its concentration gradient to transport lactose into the cell. This coupled

transport in the same direction across the cell membrane is known as a symport. *E. coli* uses similar proton driven symports to transport ribose, arabinose, and several amino acids.

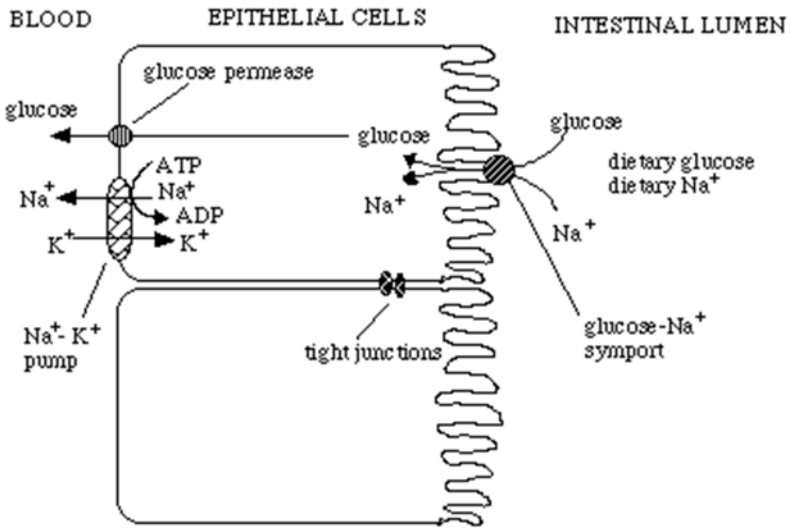


The Na+-glucose secondary transport mechanism

Another secondary active transport system uses the Na+-K+ pump as the first step, generating a strong Na+ gradient across the cell membrane. Then the glucose-Na+ symport protein uses that Na+ gradient to transport glucose into the cell.



This system is used in a novel way in human gut epithelial cells. These cells take in glucose and Na+ from the intestines and transport them through to the blood stream using the concerted actions of Na+-glucose symports, glucose permeases (a glucose facilitated diffusion protein), and Na+-K+ pumps. Note that the epithelial cells are joined together by tight junctions to prevent anything from leaking through from the intestines to the blood stream without first being filtered by the epithelial cells.



Friction

Climbing a vertical rock wall means pitting your strength and stamina against the force of gravity, which pulls you down toward the ground. Another force helps you to climb the vertical rock wall by keeping your hands and feet from slipping. That force is **friction**.

Four Types of Friction

Friction is the force that opposes motion between any surfaces that are in contact. There are four types of friction: static, sliding, rolling, and **fluid** friction. Static, sliding, and rolling friction occur between solid surfaces. Fluid friction occurs in liquids and gases. All four types of friction are described below.

Static Friction

Static friction acts on objects when they are resting on a surface. For example, if you are hiking in the woods, there is static friction between your shoes and the trail each time you put down your foot (see **Figure** below). Without this static friction, your feet would slip out from under you, making it difficult to walk. In fact, that's exactly what happens if you try to walk on ice. That's because ice is very slippery and offers very little friction.

Q: Can you think of other examples of static friction?

A: One example is the friction that helps the people climb the rock wall in the opening picture above. Static friction keeps their hands and feet from slipping.

Sliding Friction

Sliding friction is friction that acts on objects when they are sliding over a surface. Sliding friction is weaker than static friction. That's why it's easier to slide a piece of furniture over the floor after you start it moving than it is to get it moving in the first place. Sliding friction can be useful. For example, you use sliding friction when you write with a pencil. The pencil "lead" slides easily over the paper, but there's just enough friction between the pencil and paper to leave a mark.

Q: How does sliding friction help you ride a bike?

A: There is sliding friction between the brake pads and bike rims each time you use your bike's brakes. This friction slows the rolling wheels so you can stop.

Rolling Friction

Rolling friction is friction that acts on objects when they are rolling over a surface. Rolling friction is much weaker than sliding friction or static friction. This explains why most forms of ground transportation use wheels, including bicycles, cars, 4-wheelers, roller skates, scooters, and skateboards. Ball bearings are another use of rolling friction. You can see what they look like in the **Figure** below. They let parts of a wheel or other machine roll rather than slide over on another.

The ball bearings in this wheel reduce friction between the inner and outer cylinders when they turn.

Fluid Friction

Fluid friction is friction that acts on objects that are moving through a fluid. A **fluid** is a substance that can flow and take the shape of its container. Fluids include liquids and gases. If you've ever tried to push your open hand through the water in a tub or pool, then you've experienced fluid friction. You can feel the resistance of the water against your hand. Look at the skydiver in the

Reducing friction

It is beneficial to reduce the friction between surfaces to make movement easier or reduce the wear and tear on a surface. There are a number of ways to reduce friction:

Make the surfaces smoother. Rough surfaces produce more friction and smooth surfaces reduce friction. Some swimmers wear suits to reduce underwater resistance. These suits mimic the smooth skin of sharks.

Lubrication is another way to make a surface smoother. A **lubricant** is a slippery substance designed to reduce the friction between surfaces. You might use oil to stop a door from squeaking - the oil reduces the friction in the hinge. Water can be used as a lubricant - think of how a floor becomes slippery after it has been mopped.

Make the object more streamlined. A **streamline** shape is one that allows air or water to flow around it easily, offering the least resistance. Compare a boxy old car with a new car that has a rounded shape, allowing it to move with less effort.

Reduce the forces acting on the surfaces. The stronger the forces acting on the surfaces, the higher the friction, so reducing the forces would reduce the friction. If you apply the handbrake when you try to drive a car, the car will have a lot of difficulty moving because of the force immobilising (stopping the movement of) the wheels. If you release the handbrake, the wheels will move more freely because there is no extra force acting on them.

Reduce the contact between the surfaces. Have you ever tried to roll a cube? Spheres are the best shape for reducing friction because very little of a spherical object is in contact with the other surface. Several types of wheels, such as skateboard wheels, contain small spheres called ball bearings to reduce the friction between the moving parts. You can witness the effect of ball bearings by comparing the friction between sliding a book on a table and then doing the same, but using marbles between the book and the surface of the table. Notice how the marbles act as ball bearings, reducing the friction.

- Types of motion in physics

If we observe carefully,we will find that everything in the universe is in motion.However ,different objects move differently.Some objects move along a straight line,some move in a curved path,and

some move in some other way.According to this we can say that there are three types of motion.Which are given as:

- Translatory motion
- Rotatory motion
- Vibratory motion

Translatory motion

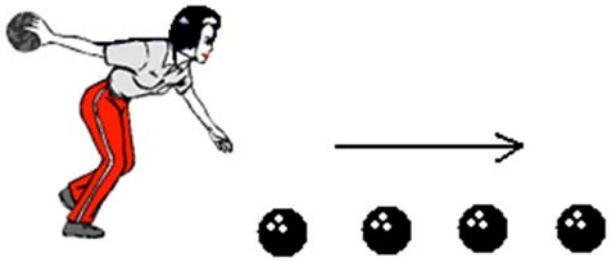
“In transnational motion,a body moves along a line without any rotation.The line may be straight or curved.”Watch how various objects are moving.Do they move along a straight line?Do they move along a circle?A car moving in a straight line has transnational motion.Similarly,an aeroplane moving straight is in transnational motion. Translatory motion is further divided into linear motion,circular motion and random motion.

Examples of translatory motion in daily life

- Motion of train
- motion of earth
- motion of birds
- motion of insects
- motion of aeroplane
- motion of gas molecules

Linear motion

“Straight line motion of a body is known as its linear motion.”



We come across many objects which are moving in a straight line.The motion of objects such as car moving on a straight and level road is linear motion. Aeroplanes flying straight in air and objects failing vertically down are also the examples of linear motion.
In the above diagram a boy is sliding in straight line which is the example of linear motion.

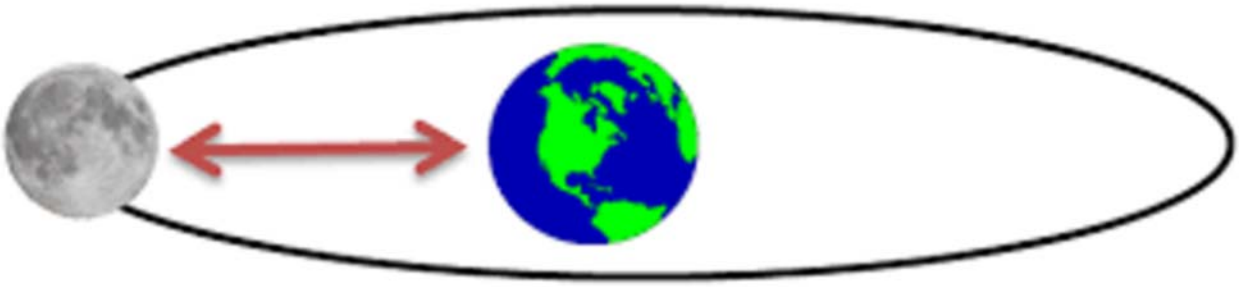
Linear motion examples in daily life

- Motion of car on the road

- Motion of football
- Sliding a boy in straight line is the example of linear motion

Circular motion

“The motion of an object in a circular path is known as circular motion.”A toy train moving on a circular track.Earth revolving around the sun is the example of circular motion.



A bicycle or a car moving along a circular track possesses circular motion.Motion of moon around earth is also example of circular motion.

Examples of circular motion in daily life

- Motion of electron around the nucleus
- Motion of toy car on circular track
- Motion of planets around the sun

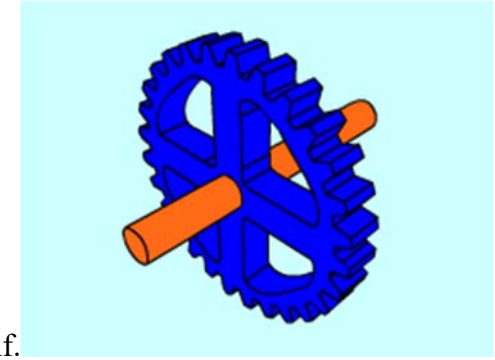
Random motion

“The disordered or irregular motion of a body is called random motion.”Have you noticed the type of motion of insects and birds?Their movements are irregular and disorder.Motion of insects and birds is random motion.The motion of dust or smoke particles in the air is also random motion.The Brownian motion of a gas or liquid molecules along a zig – zag is also an example of random motion.”Random motion of gas molecules is called Brownian motion.”

Rotatory motion

Rotatory motion definition

“The spinning motion of a body about its axis is called its rotatory motion.”
Study the motion of a tap.It is spinning about an axis.Particles of the spinning top move in circles and thus individual particles possess circular motion.Does the top possess circular motion?The top spins about its axis passing through it and thus it possess rotatory motion.An axis is a line around which a body rotates.In circular motion ,the point about which a body goes around ,is

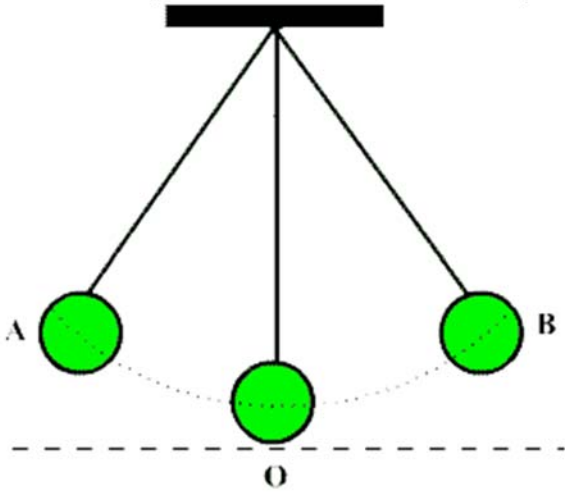
outside the body.In rotatory motion,the lone ,around which a body moves about,is passing through the body itself. 

Examples of rotatory motion in daily life

- The motion of the earth about its geographic axis that causes day and night is rotatory motion.
- The motion of wheel about its axis and that of steering wheel are the examples of rotatory motion.

Vibratory motion

"The motion of a body about its mean position is known as vibratory motion."



Consider a baby in a swing as shown in above figure.As it is pushed,the swing moves back and forth about its mean position.The motion of the baby repeats from one extreme to the other extreme with the swing.Motion of pendulum of a clock about its mean position,is also example of vibratory motion.Children playing in a sea saw is the example of vibratory motion.Besides these examples a baby in a cradle moving to and fro,to and fro motion of the hammer of a ringing electric bell and the motion of the string of a sitar are some of the examples of vibratory motion.Simple harmonic motion is also example of vibratory motion.

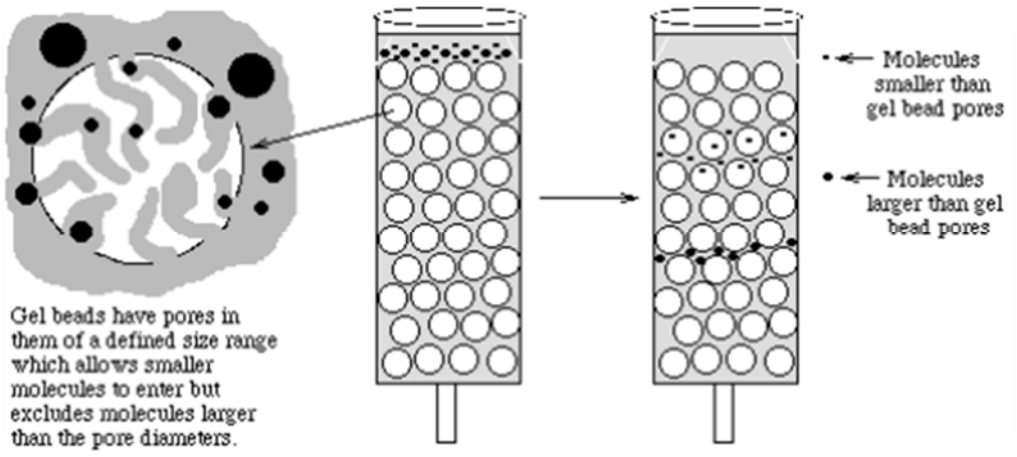
Gel Filtration

This is also known as “Molecular exclusion chromatography” (or) “Molecular sieve chromatography”, “Size exclusion chromatography” and “Permeation chromatography”

Principle:

In exclusion chromatography the separation of molecules is based up on the size and shape. The stationary phase is porous bead material and the mobile phase is the solvent system.

The large molecules cannot enter the pores of the beads so they are excluded out and come down rapidly. Small sized molecules enter the pores of the beads so that their speed is retarded and comedown slowly. The degree of retardation of a molecule is proportional to the time it spends inside the gel pores, which is a function of the molecule’s size and the pore diameter.



Applications:

1) The main application of gel-filtration is the purification of molecules, viruses, nucleic acids, hormones, enzymes, proteins, and antibodies and can be separated and purified by this technique.

2) It is also used for the separation of vitamins, steroids, neuropeptides and drugs.

3) Separations are achieved very quickly by this technique.

4) The molecular weight of the molecule can also be determined by this technique.

5) Protein receptor binding can be understood by this technique.

6) This method is especially useful for the separation of 4S and 5S tRNA.

7) It is also the most satisfactory method for separating DNA (from bacteria, usually Gram positive) from the invariable contaminants, the “Teichoic acid”.

ION-EXCHANGE CHROMATOGRAPHY

Ion exchange protein purification is possible because most proteins bear nonzero net electrostatic charges at all pHs except at pH=pI (isoelectric point). At a pH >pI of a given protein, that protein becomes negatively charged (an anion), at the pH<pI of that same protein, it becomes positively charged (a cation).

Ion exchange chromatography occurs due to electrostatic attraction between buffer-dissolved charged proteins and oppositely charged binding sites on a solid ion exchange adsorbent. An ion exchange adsorbent (also called media, resin, gel, or matrix) usually consists of spherical porous inert beads with charged groups (functional groups) densely grafted onto the beads' surfaces; the charges of functional groups are neutralized by free counter-ions.

General steps for ion exchange chromatographic purification

Protein mixture is transferred into low ionic strength buffer (mobile phase).

Ion exchange adsorbent (stationary phase) is packed into a column, and the column is pre-equilibrated with the buffer of identical pH and similar ionic strength as protein mixture (preferably the same buffer as protein mixture).

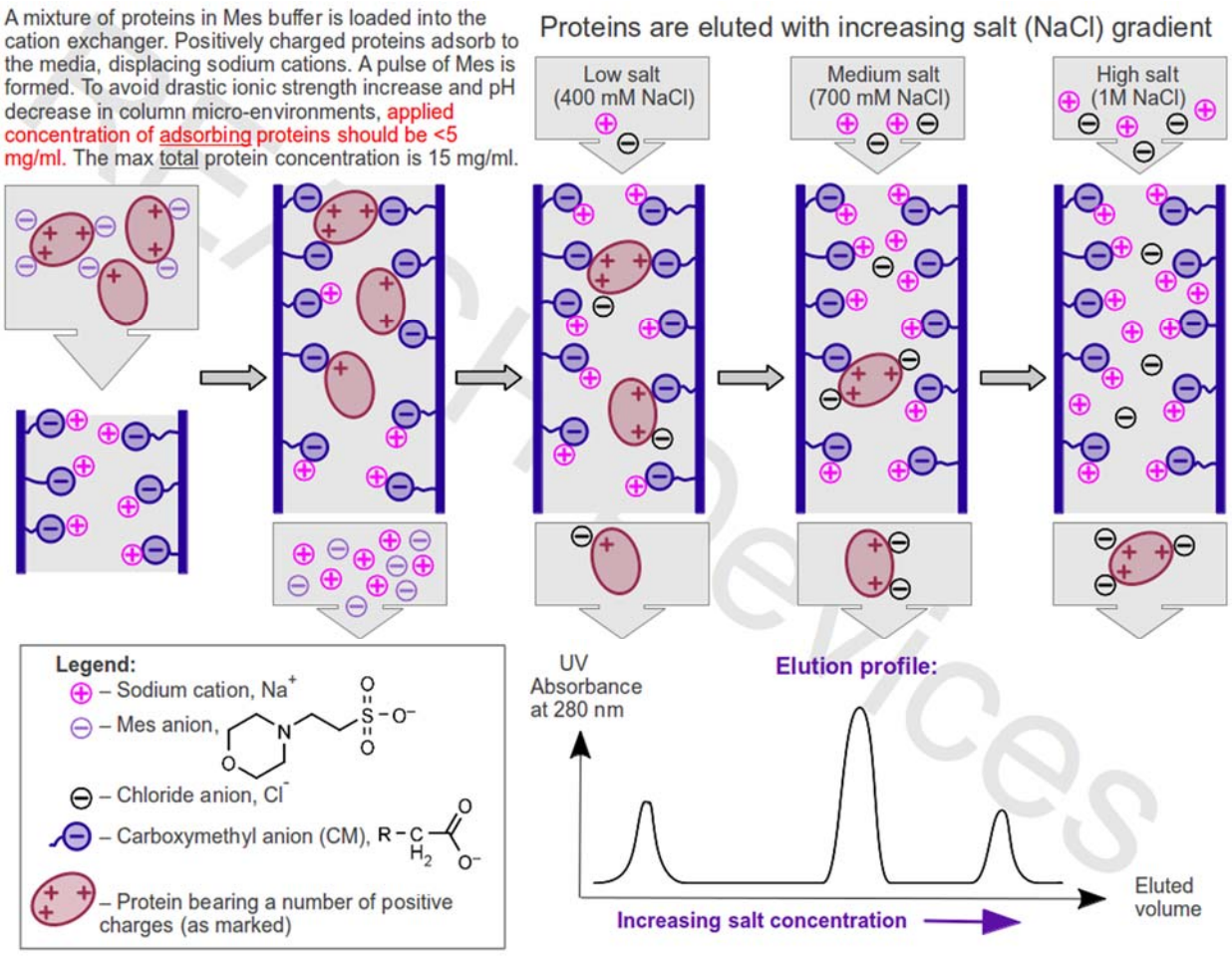
Protein mixture is applied onto the column. Proteins charged oppositely to ion-exchange media are temporarily retained in the column. All other proteins simply pass through the column and are collected during this step.

Retained proteins are eluted from the column by applying a modified buffer. Elution is most commonly achieved by gradually increasing ionic strength of the buffer via salt gradient, and proteins are eluted in order of increasing their net charges. Is specific cases the elution can be accomplished by

(a) pH change

(b) affinity methods.

Ion exchange chromatography can provide high-resolution separation for proteins with the same sign but various total net charge. Due to the high capacity of most ion-exchangers, the technique can also be used for capture of a mixture of same-sign charged proteins from large-volume diluted samples, the proteins are then eluted in considerably decreased sample volume.



There are four basic types of resins which are commonly used in ion exchanging.

1. Strong acidic cation exchange resins
2. Weak acidic cations exchange resins
3. Strong basic anion exchange resins
4. Weak basic anion exchange resins

1. Strong acidic cation exchange resins:

It contains sulphonic acid group. Sulphonated polystyrene resins belong to this class. They are useful in pH range 1-14. They are useful mainly in fractionation of cations, inorganic separation and for separation of vitamins, peptides and amino acids.

2. Weak acidic cations exchange resins:

It contains carboxylic acid group. Carboxylic polymetacrylate (Polymethyl methacrylate) is an example of weak acidic Cation exchange resins. They are useful in pH range 5-14. They are used

in biochemical separation, fractionation of cations, and separation of amino acids, antibiotics and organic bases.

3. Strong basic anion exchange resins:

It contains quaternary ammonium groups. Quaternary ammonium polystyrene belongs to this class. And it is effective between pH 0-12. This type of resins is useful in fractionation of anion and for separation of vitamins and fatty acids.

4. Weak basic anion exchange resins:

It contains phenol, formaldehyde or polyamines group. Phenol formaldehyde and polystyrene resins belongs to this class. They are effective in pH range 0-9. It can be useful in fractionation of anionic complexes of metals and separation of vitamins and amino acids.

Techniques of Ion Exchange Chromatography:

1. Preparation of Column

The ion exchange chromatography is carried out in a chromatographic column which usually consists of a burette provided with a glass wool plug at the lower end. Generally a ratio of 10: 1 or 100:1 between height and diameter is maintained in most of the experiment. Too narrow or too wide column give uneven flow of liquid and sometimes poor separation.

2. Preparation of Ion Exchange

Ion exchange materials are first allowed to swell in buffer or in HCl or NaOH solution for 2-3 hours or sometimes overnight. Almost all ion exchange resin swells when placed in buffer or distilled water and this is due to hydration of their ions. In dry condition, the pore of resins is restricted so in order to swell the pore of resin. Resins are suspended in buffer solution or in distilled water.

3. Washing of Ion Exchangers

The ion exchange material is obtained in required ionic form by washing with appropriate solution. For e.g. the H+ form of cation exchange resins is obtained by washing the material with HCl then with water until the washings are neutral.

Anionic exchangers are generally supplied in the form of salt and amines. Similarly, Na+ form is prepared by washing the resins with NaCl or NaOH solution and then with water.

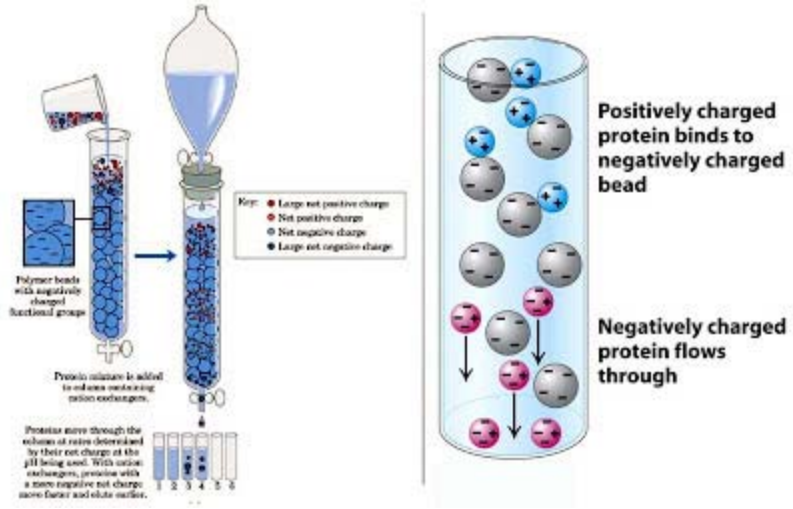


Figure 2: Ion exchange chromatography

4. Packing of Column

This is one of the most critical factors in achieving a successful separation. The column is held in vertical position and the slurry of resins is poured into the column that has its outlet closed. The column is gently tapped to ensure that no air bubbles are trapped and that packing material settles evenly.

5. Sample Application

Sample can be loaded by using pipette or syringe. The amount of sample that can be applied to a column is dependent upon the size of the column and the capacity of resins, If the starting buffer is to be used throughout the development of column, the sample volume be 1 % to 5 % of bed volume.

6. Development an Elution of bound ions

Bound ions can be removed by changing the pH of buffer. E.g. separation of amino acid is usually achieved by using a strong acidic cation exchanger. The sample is introduced onto the column at pH of 1-2, thus ensuring complete binding of all of the amino acids.

Gradient elution used in increasing pH and ionic concentration results in the sequential elution of amino acid. Then acidic amino acid such as aspartic acid and glutamic acid are eluted first. The neutral amino acid such as glycine and valine are eluted. The basic amino acid such as lysine and arginine retain their net positive charge at pH value of 9 to 11 and are eluted at last.

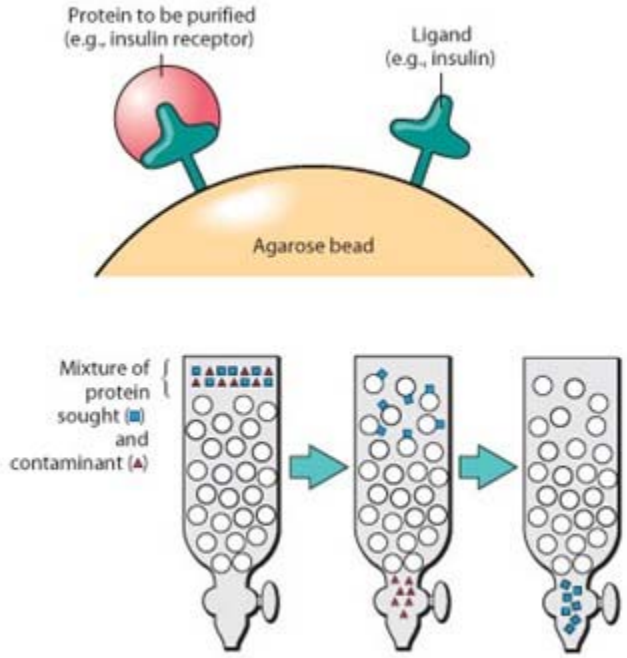
7. Analysis of eluate

Equal fraction of each elute are collected at different test tube keeping the flow rate at 1 ml per minute. The eluate collected in each fraction is mixed with ninhydrin color reagent. The mixture

is then heated to 105°C to develop the color and intensity of color is determined by colorimeter method or spectrophotometer method at 540 to 570 nm.

AFFINITY CHROMATOGRAPHY

It is mainly based on the biological affinity (or) biological specificity. This technique mainly requires previous knowledge of the molecule to be separated a specific ligand will only attach with a specific molecule. The materials to be isolated are capable of binding reversibly to a specific ligand i.e., attached to an insoluble matrix.



The goal of affinity chromatography is to separate all the molecules of a particular specificity from the whole gamut of molecules in a mixture such as a blood serum. For example, the antibodies in a serum sample specific for a particular antigenic determinant can be isolated by the use of affinity chromatography.

Step 1.

An immune-adsorbent is prepared. This consists of a solid matrix to which the antigen (shown in blue) has been coupled (usually covalently). Agarose, sephadex, derivatives of cellulose, or other polymers can be used as the matrix.

Step 2.

The serum is passed over the immune-adsorbent. As long as the capacity of the column is not exceeded, those antibodies in the mixture specific for the antigen (shown in red) will bind (non-covalently) and be retained. Antibodies of other specificities (green) and other serum proteins (yellow) will pass through unimpeded.

Step 3.

Elution. A reagent is passed into the column to release the antibodies from the immune-adsorbent. Buffers containing a high concentration of salts and/or low pH are often used to disrupt the non-covalent interactions between antibodies and antigen. A denaturing agent, such as 8 M urea, will also break the interaction by altering the configuration of the antigen-binding site of the antibody molecule.

Another, gentler, approach is to elute with a soluble form of the antigen. These compete with the immune-adsorbent for the antigen-binding sites of the antibodies and release the antibodies to the fluid phase.

Step 4.

Dialysis. The eluate is then dialyzed against, for example, buffered saline in order to remove the reagent used for elution.

Applications:

- The technique has been used to purify a large variety of macromolecule such as enzymes, Immunoglobulin, membrane receptors, Nucleic acids and even polysaccharides.
- By using affinity chromatography, Whole cells have been purify include fat cells, T and B-lymphocytes, Spleen cells, Lymph node cells, Oocytes and chick embryo neural cells.
- Metal chelate affinity chromatography is the logical extension technique. Same molecular weight protein can be separated by this technique by using the metal ion containing matrix by chelation, because of their difference in their metal binding ability with proteins.
- By using the “Magnetic gel beads affinity chromatography”, immunoglobulin negative thymocytes and neuroblastoma cells have been purified by this method. The magnetic gel beads, usually polyacrylamide (or) agarose have a core made up of Fe3 O4 (Magnetite) and are chemically coupled to a protein ligand.
- Immobilized enzymes (Solid-state enzymes) are also isolated and purified by this method.
- mRNA can be isolated by this technique.
- Native proteins can be separated from denatured proteins by this technique.
- DNA & RNA can be separated from each other
- Papain and Urease can be separated by this technique.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds which are not easily volatalised, thermally unstable and have high molecular weights.

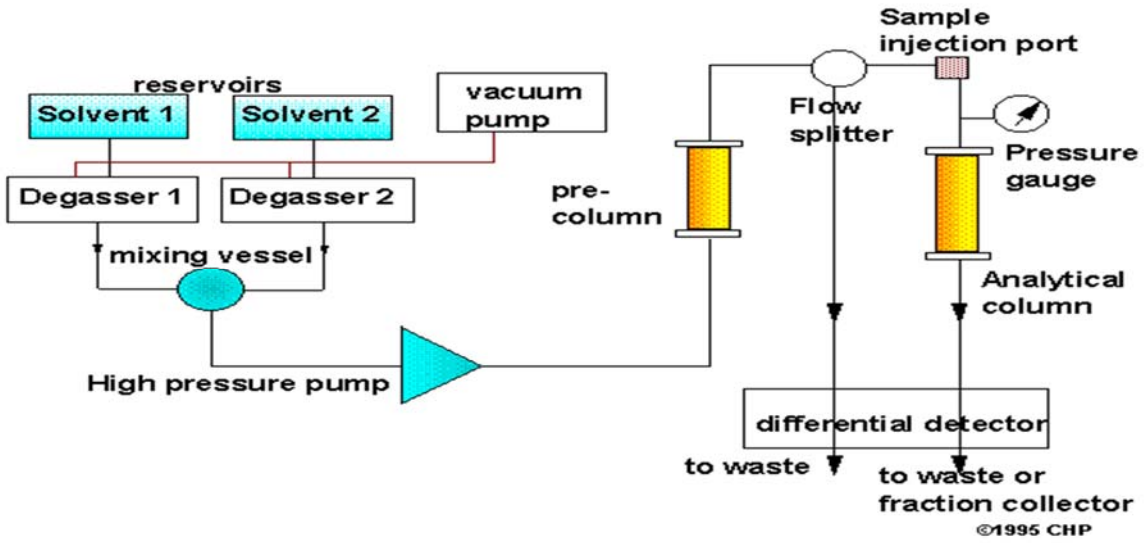
The liquid phase is pumped at a constant rate to the column packed with the stationary phase. Before entering the column the analysis sample is injected into the carrier stream. On reaching the column the sample components are selectively retained on the basis of physico-chemical interactions between the analyte molecules and the stationary phase. The mobile phase moving at a steady rate elutes the components based on the operating conditions. Detection techniques are employed for detection and quantification of the eluted components.

We now introduce you to the significance and role of each component part of the HPLC system.

Principle of High Performance Liquid Chromatography (HPLC)

HPLC is an automated version of column chromatography, which involves use of a stationary phase in the form of a column, a mobile phase, complete with a pump and a detector. The sample is injected within the column and mixed with the mobile phase followed by being pumped under high pressure. The analytes in the sample mixture interact with the stationary phase within the column differently depending on their chemical nature. Some might be retained for a longer time as compared to others and will be hence eluted at a later stage. Finally, all the eluted components are recorded by the detector and expressed in the form of a chromatogram. This chromatogram depicts each analyte within the mixture in the form of a peak plotted against the retention time (RT). The area under the curve (AUC) is generally depictive of the concentration of the analyte.

The main advantage of this technique is the platform is open and can be exploited to develop and standardize any protocol of interest. The sensitivity is very high, and with an appropriate choice of mobile phase, column and detector, multiple analytes can be identified and quantified in a single assay run.



HPLC Mobile Phase

Mobile phase serves to transport the sample to the system. Essential criteria of mobile phase are inertness to the sample components. Pure solvents or buffer combinations are commonly used. The mobile phase should be free of particulate impurities and degassed before use.

HPLC Mobile Phase Reservoirs

These are inert containers for mobile phase storage and transport. Generally transparent glass bottles are used so as to facilitate visual inspection of mobile phase level inside the container. Stainless steel particulate filters are provided inside for removal of particulate impurities in the mobile phase if any.

HPLC Pumps

Variations in flow rates of the mobile phase effect elution time of sample components and result in errors. Pumps provide constant flow of mobile phase to the column under constant pressure.

HPLC Injectors

Injectors are used to provide constant volume injection of sample into the mobile phase stream. Inertness and reproducibility of injection are necessary to maintain high level of accuracy.

HPLC Column

A column is a stainless steel tube packed with stationary phase. It is a vital component and should be maintained properly as per supplier instructions for getting reproducibility separation efficiency run after run.

Column Oven

Variation of temperature during the analytical run can result in changes of retention time of the separated eluting components. A column oven maintains constant column temperature using air circulation. This ensures a constant flow rate of the mobile phase through the column

Detector

A detector gives specific response for the components separated by the column and also provides the required sensitivity. It has to be independent of any changes in mobile phase composition. Majority of the applications require UV-VIS detection though detectors based on other detection technique are also popular these days.

Data Acquisition & Control

Modern HPLC systems are computer based and software controls operational parameters such as mobile phase composition, temperature, flow rate, injection volume and sequence and also acquisition and treatment of output.

These are the main parts of a basic HPLC system more specialized equipment might also have solvent selection valves, vacuum degasser, auto samplers, column switchers, pre or post column derivatization and fraction collectors. These are all covered at length in our upcoming online certificate program on HPLC.

MASS SPECTROMETRY

Mass Spectrometry is widely used to determine and identify the elements present in samples and to determine their concentrations.

Mass Spectrometry is also used to measure the relative atomic mass of an element and to measure the relative molecular mass of a substance.

Mass Spectrometry Process

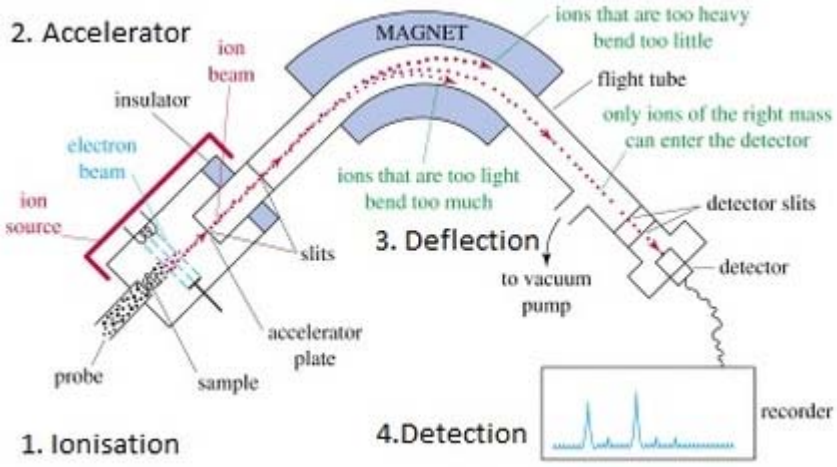
There are four key stages in the process for Mass Spectrometry.

Ionization

Acceleration

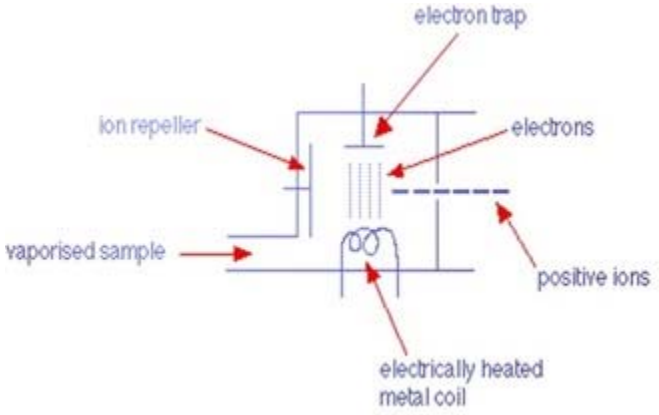
Deflection

Detection



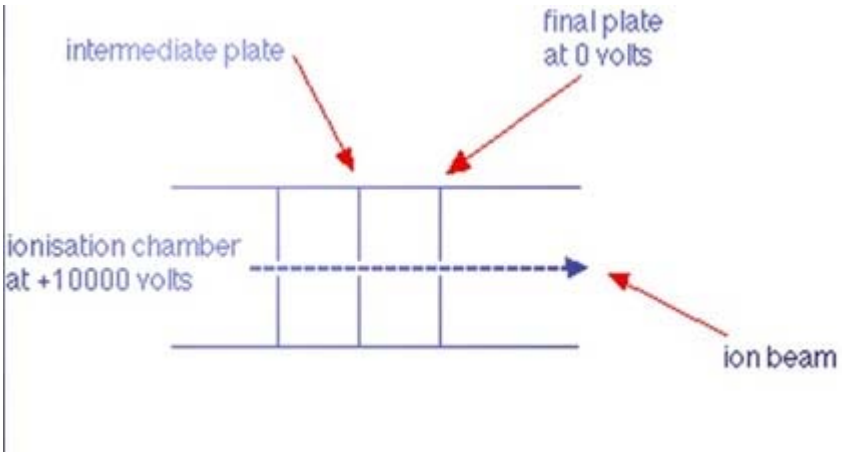
Stage 1 Ionization

1. Electro Ionization is the most common type of ionization.
2. The sample is bombarded by electrons which come from a heated filament.
3. The electrons run in a stream between the cathode and anode.
4. When the sample passes through the electron stream, the high energy electrons in the stream knock electrons out of the sample to form ions.



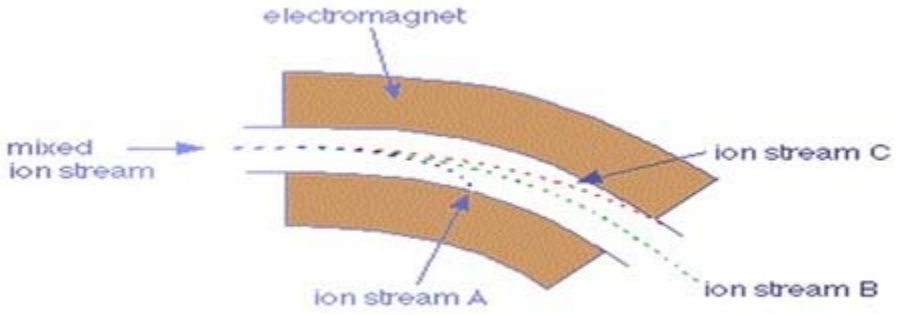
Stage 2 Acceleration

1. Acceleration is a simple step where the ions are placed between a set of charges parallel plates.
2. The ions will then be repelled by one plate and attracted to the other.
3. There is a slit cut in the plate which the ions are attracted to. the force of attraction and repulsion forces the ions through the slit at an accelerated rate.
4. The speed of acceleration can be adjusted by changing the charge on the plates.



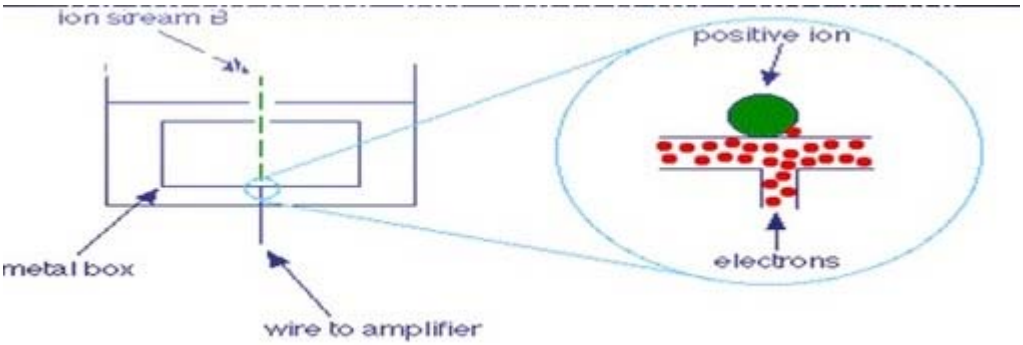
Stage 3 Deflection

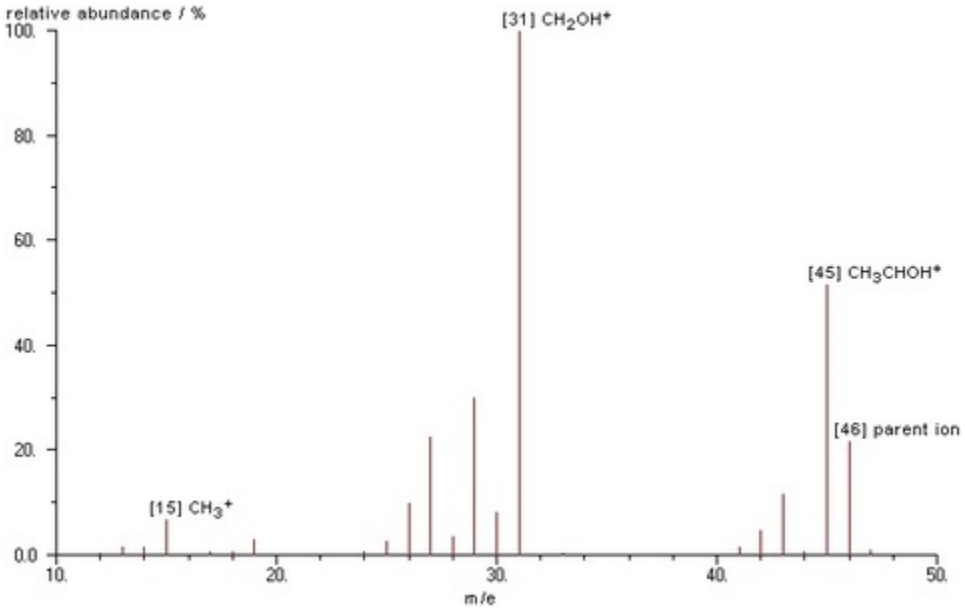
1. Ions are deflected by the magnetic field surrounding the instrument.
2. The amount of deflection depends on the mass and charge of the ions.
3. The heavier ions and ions with a positive charge of 2 or more, are deflected the least (Ion stream C)
4. The lightest ions and ions with 1 positive charge are deflected the most (Ion Stream A)
5. The ions at the correct mass and charge travel to the detector. (Ion Stream B)
6. The mass to charge ratio (m/z) is determined from the ion that hits the detector.



Step 4 Detection

1. When the ion stream reached the detector the hit a wire. On hitting the wire they become neutralised by an electron jumping from the metal wire to the ion.
2. The amplifier picks up on this current being created between the wire and the ion and amplifies the signal being detected.
3. The computer picks up on this and converts it to mass/charge ratio and a spectrum is produced





Applications of Mass Spectrometry

Mass Spectrometry as a technique can be coupled with other techniques such as HPLC and GC.

As it is used in the identification of compounds it is used in all areas of science.

Some of its uses are:

- Trace Gas Analysis
- Pharmaceutical Industry
- Space Exploration
- Forensic Toxicology
- Archaeological Dating.

SPECTROSCOPY

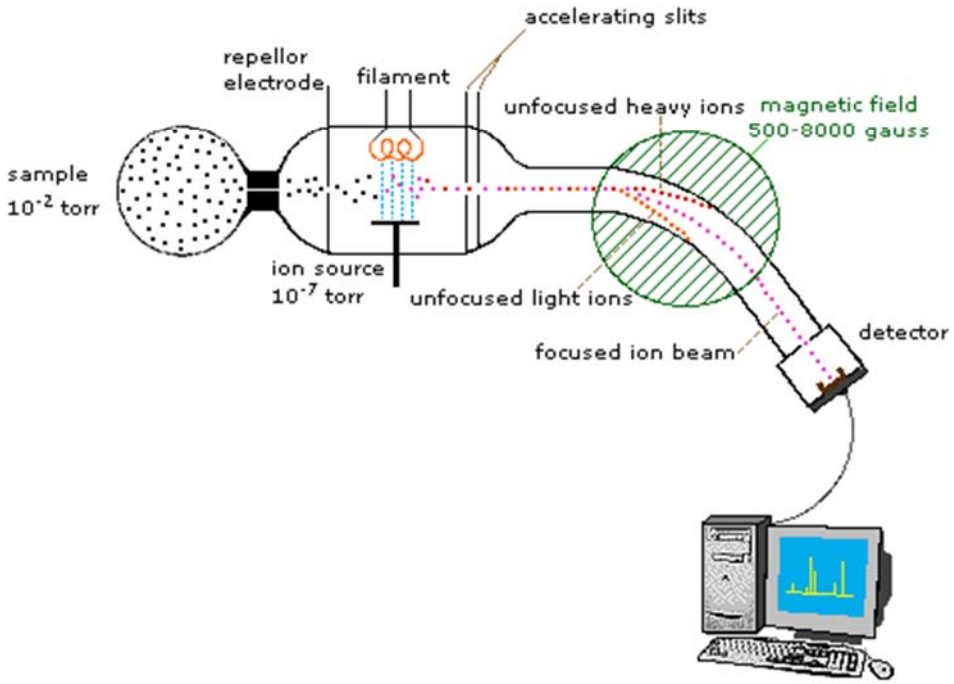
Spectroscopy and spectrography are terms used to refer to the measurement of radiation intensity as a function of wavelength and are often used to describe experimental spectroscopic methods. Spectral measurement devices are referred to as spectrometers, spectrophotometers, spectrographs or spectral analyzers

The Mass Spectrometer

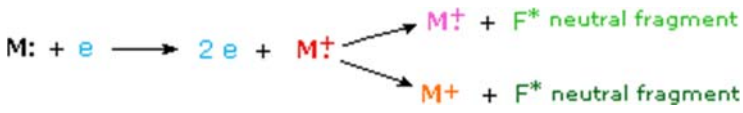
In order to measure the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields. The three essential functions of a mass spectrometer, and the associated components, are:

1. A small sample is ionized, usually to cations by loss of an electron. The Ion Source
2. The ions are sorted and separated according to their mass and charge. The Mass Analyzer
3. The separated ions are then measured, and the results displayed on a chart. The Detector

Because ions are very reactive and short-lived, their formation and manipulation must be conducted in a vacuum. Atmospheric pressure is around 760 torr (mm of mercury). The pressure under which ions may be handled is roughly 10-5 to 10-8 torr (less than a billionth of an atmosphere). Each of the three tasks listed above may be accomplished in different ways. In one common procedure, ionization is effected by a high energy beam of electrons, and ion separation is achieved by accelerating and focusing the ions in a beam, which is then bent by an external magnetic field. The ions are then detected electronically and the resulting information is stored and analyzed in a computer. A mass spectrometer operating in this fashion is outlined in the following diagram. The heart of the spectrometer is the ion source. Here molecules of the sample (black dots) are bombarded by electrons (light blue lines) issuing from a heated filament. This is called an EI (electron-impact) source. Gases and volatile liquid samples are allowed to leak into the ion source from a reservoir (as shown). Non-volatile solids and liquids may be introduced directly. Cations formed by the electron bombardment (red dots) are pushed away by a charged repeller plate (anions are attracted to it), and accelerated toward other electrodes, having slits through which the ions pass as a beam. Some of these ions fragment into smaller cations and neutral fragments. A perpendicular magnetic field deflects the ion beam in an arc whose radius is inversely proportional to the mass of each ion. Lighter ions are deflected more than heavier ions. By varying the strength of the magnetic field, ions of different mass can be focused progressively on a detector fixed at the end of a curved tube (also under a high vacuum).

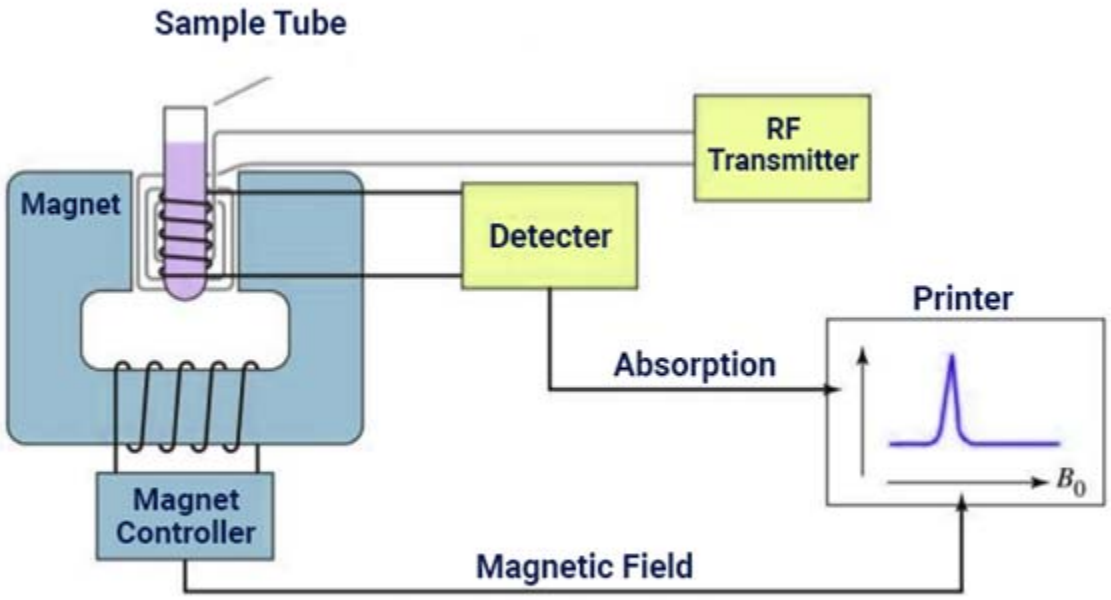


When a high energy electron collides with a molecule it often ionizes it by knocking away one of the molecular electrons (either bonding or non-bonding). This leaves behind a molecular ion (colored red in the following diagram). Residual energy from the collision may cause the molecular ion to fragment into neutral pieces (colored green) and smaller fragment ions (colored pink and orange). The molecular ion is a radical cation, but the fragment ions may either be radical cations (pink) or carbocation (orange), depending on the nature of the neutral fragment. An animated display of this ionization process will appear if you click on the ion source of the mass spectrometer diagram.



NMR SPECTROSCOPY

NMR Spectroscopy is abbreviated as Nuclear Magnetic Resonance spectroscopy. It is a research technique that exploits the magnetic properties of certain atomic nuclei. The NMR spectroscopy determines the physical and chemical properties of atoms or molecules.



It relies on the phenomenon of nuclear magnetic resonance and provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.

NMR Spectroscopy Uses

NMR spectroscopy is used by chemists and biochemists to investigate the properties of organic molecules, although it is applicable to any kind of sample that contains nuclei possessing spin. For example, the NMR can quantitatively analyze mixtures containing known compounds. NMR can either be used to match against spectral libraries or to infer the basic structure directly for unknown compounds.

Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion.

NMR Spectroscopy Techniques

Resonant frequency: It refers to the energy of the absorption, and the intensity of the signal that are proportional to the strength of the magnetic field. NMR active nuclei absorb electromagnetic radiation at a frequency characteristic of the isotope when placed in a magnetic field.

Acquisition of spectra: Upon excitation of the sample with radio frequency pulse, a nuclear magnetic resonance response is obtained. It is a very weak signal, and requires sensitive radio receivers to pick up.

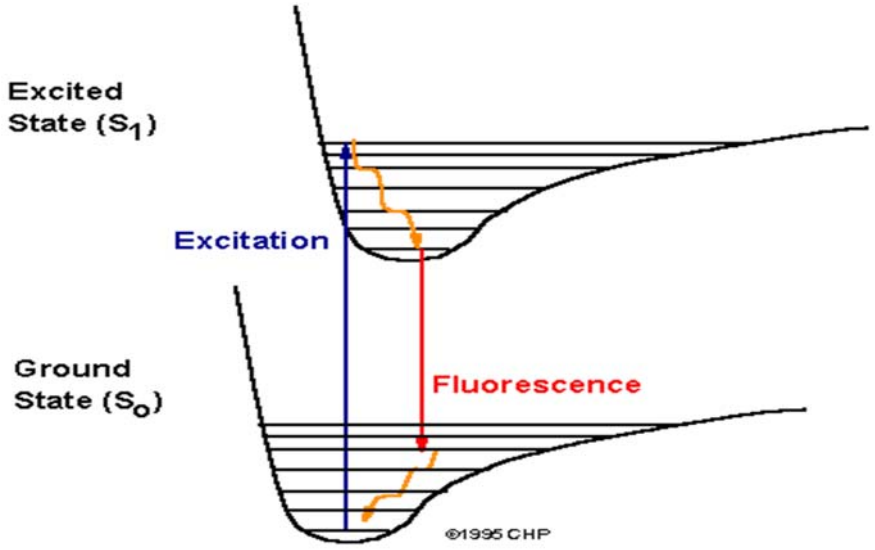
Chemical shift: A spinning charge generates a magnetic field that results in a magnetic moment proportional to the spin. In the presence of an external magnetic field, two spin states exist: one spin up and one spin down, where one aligns with the magnetic field and the other opposes it.

FLUORESCENCE SPECTROSCOPY

Introduction

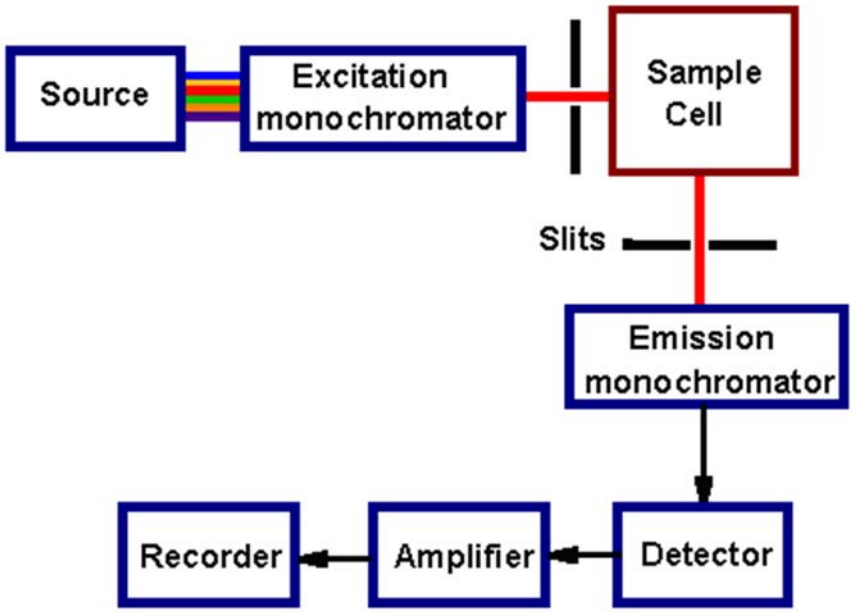
Molecular fluorescence is the optical emission from molecules that have been excited to higher energy levels by absorption of electromagnetic radiation. The main advantage of fluorescence detection compared to absorption measurements is the greater sensitivity achievable because the fluorescence signal has in principle a zero background. Analytical applications include quantitative measurements of molecules in solution and fluorescence detection in liquid chromatography. The theory of quantitative fluorescence measurements is given in a separate document.

Transitions between molecular electronic energy levels:



Instrumentation

A typical fluorometers contains an excitation source, sample cell, fluorescence detector. Molecules in solution are usually excited by UV light and the excitation source is usually a deuterium or xenon lamp. Broad-band excitation light from a lamp passes through a monochromator, which passes only a selected wavelength. The fluorescence is dispersed by another monochromator and detected by a photomultiplier tube. Scanning the excitation monochromator gives the excitation spectrum and scanning the fluorescence monochromator gives the fluorescence spectrum. Simple instruments sometimes use only a band pass filter to select the excitation wavelength.



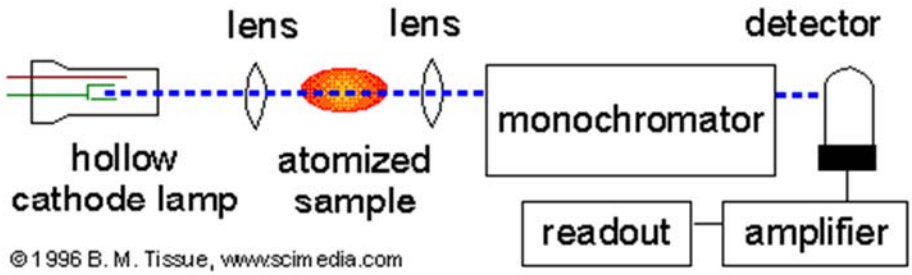
Applications

- Fluorescence spectroscopy is used in, among others, biochemical, medical, and chemical research fields for analyzing organic compounds. There has also been a report of its use in differentiating malignant skin tumors from benign.
- Atomic Fluorescence Spectroscopy (AFS) techniques are useful in other kinds of analysis/measurement of a compound present in air or water, or other media, such as CVAFS which is used for heavy metals detection, such as mercury.
- Fluorescence can also be used to redirect photons, see fluorescent solar collector.
- Additionally, Fluorescence spectroscopy can be adapted to the microscopic level using micro fluorimetry
- In analytical chemistry, fluorescence detectors are used with HPLC.

ATOMIC ABSORPTION SPECTROSCOPY

Introduction

Atomic-absorption (AA) spectroscopy uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption. Applying the Beer-Lambert law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms (in graphite furnace AA). Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.



Light source

The light source is usually a hollow-cathode lamp of the element that is being measured. Lasers are also used in research instruments. Since lasers are intense enough to excite atoms to higher energy levels, they allow AA and atomic fluorescence measurements in a single instrument. The disadvantage of these narrow-band light sources is that only one element is measurable at a time.

Atomizer

AA spectroscopy requires that the analyte atoms be in the gas phase. Ions or atoms in a sample must undergo desolvation and vaporization in a high-temperature source such as a flame or graphite furnace. Flame AA can only analyze solutions, while graphite furnace AA can accept solutions, slurries, or solid samples.

Flame AA uses a slot type burner to increase the path length, and therefore to increase the total absorbance (see Beer-Lambert law). Sample solutions are usually aspirated with the gas flow into a nebulizing/mixing chamber to form small droplets before entering the flame.

The graphite furnace has several advantages over a flame. It is a much more efficient atomizer than a flame and it can directly accept very small absolute quantities of sample. It also provides a reducing environment for easily oxidized elements. Samples are placed directly in the graphite furnace and the furnace is electrically heated in several steps to dry the sample, ash organic matter, and vaporize the analyte atoms.

Light separation and detection

AA spectrometers use monochromators and detectors for uv and visible light. The main purpose of the monochromator is to isolate the absorption line from background light due to interferences. Simple dedicated AA instruments often replace the monochromator with a bandpass interference filter. Photomultiplier tubes are the most common detectors for AA spectroscopy.

ATOMIC EMISSION SPECTROSCOPY

In the twentieth century, Max Planck concluded that energy could be absorbed or emitted discontinuously in the form of packets of energy called quanta. When the atom absorbs energy and jumps from lower stable state the higher state and while coming back to their ground state energy level, they emit the energy equal to the difference between the energies of two levels. This emission radiation is recorded in the atomic emission spectra and is called emission spectroscopy.

When the molecule jumps from the higher energy level to lower energy level, a photon of energy is emitted. The spectrum obtained is called emission spectrum and the spectroscopy is called atomic emission spectroscopy. Atomic emission spectroscopy is a technique to determine the quantities of elements in the sample by using the intensity of light emitted by plasma, spark, flame or arc.

In atomic emission spectrum, the sample is applied with energy in the form of heat or light. The source of energy can be flame, plasma or electrical arc. The excited molecule jumps from lower to higher energy level and comes back from higher energy level to lower energy level emitting radiations of certain wavelength. Each element has different emission level, and this gives the prediction of the different elements in the sample.

Atomic emission spectroscopy is based on the principle that when a molecule is applied energy in the form of light or heat, the molecules get excited and move from lower energy level to higher energy level. At this state the molecules are unstable. Therefore, the excited molecule jumps from higher energy level to lower energy level, emitting radiation. The radiations are emitted in the form of photons. The wavelengths of photons emitted are recorded.

These radiations are recorded in the emission spectrometer. The level of emissions of a substance is the difference between the substance in excited and lower state.

Each element has different and unique level of emission which helps the scientist to detect the elements. The frequencies are recorded as light spectra in the emission spectrometer.

Atomic Emission Spectroscopy Applications

- Atomic emission spectroscopy is used for the regulation of alkali metals in the pharmaceutical processes.
- It is also used for detection of trace metals in different samples.
- Atomic emission spectroscopy is applicable in the smelting process of ores, in the process of extraction of metals.
- Atomic emission spectroscopy helps in the detection of elements in the given sample and hence for structure elucidation.
- It is also used for analyzing motor oils

The instrument used for recording a spectrum is known as spectrometer or spectrophotometer.

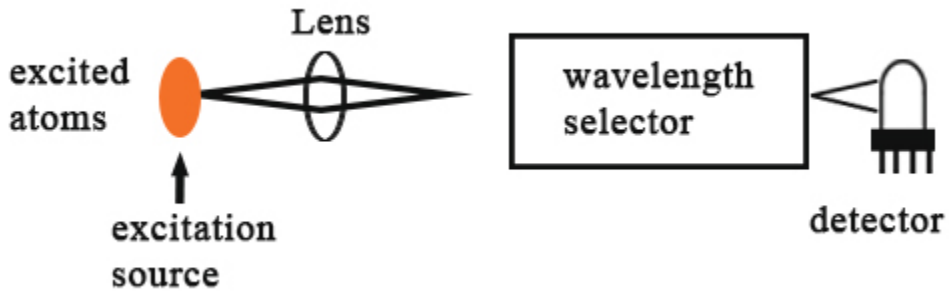
The first step is atomization or excitation. In this solid liquid or solution analyte is converted into gaseous atom. The sample is then made excited and the excitation in emission spectrometer can be thermal or electrical, but often takes the form of electromagnetic radiation. The most common

methods of excitations are flames and plasmas. If the sample is solid, it can be dissolved in solvent for analyzing.

In Atomic emission spectroscopy, in the spectrometer, the sample, after undergoing excitation, is its own source. It is necessary that only the emitted radiation is collected and analyzed and then recorded in the usual way. In case of emission spectrometer, no analyzer is necessary, the source being its own analyzer. A monochromatic is employed which separates radiations into individual wavelengths. The dispersing element is used which is generally a glass prism.

The detector over here is the device which converts spectral radiation into electrical signal that is transmitted to a recording device called a recorder. The recorder produces a print on the chart. It is essential that the detector does not receive radiation directly from the exciting beam and the two are placed at right angles.

A modulator placed between the source of excitation and the sample, ensures that only the emission is recorded which directly arises from the excitation. Any other spontaneous emission in this way is ignored



INDUCTIVELY COUPLED PLASMA EMISSION SPECTROSCOPY (ICPMS)

Inductively Coupled Plasma Mass Spectrometry or ICP-MS is an analytical technique used for elemental determinations. The technique was commercially introduced in 1983 and has gained general acceptance in many types of laboratories. Geochemical analysis labs were early adopters of ICP-MS technology because of its superior detection capabilities, particularly for the rare-earth elements (REEs). ICP-MS has many advantages over other elemental analysis techniques such as atomic absorption and optical emission spectrometry, including ICP Atomic Emission Spectroscopy (ICP-AES), including:

- Detection limits for most elements equal to or better than those obtained by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)
- Higher throughput than GFAAS
- The ability to handle both simple and complex matrices with a minimum of matrix interferences due to the high-temperature of the ICP source

- Superior detection capability to ICP-AES with the same sample throughput
- The ability to obtain isotopic information.

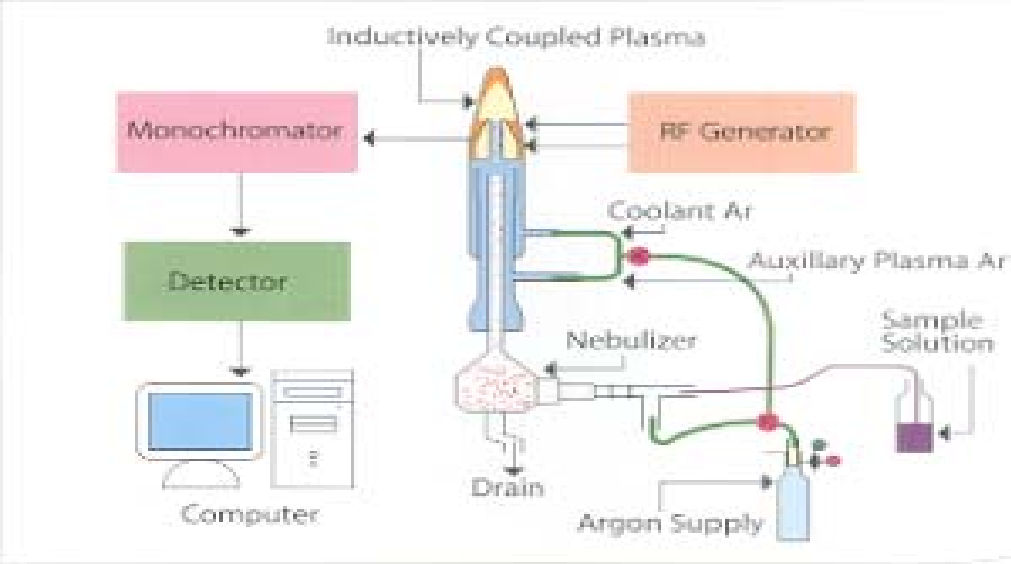
Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP- AES) is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its atomic character. The energy transfer for electrons when they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital. The energy transfer is inversely proportional to the wavelength of electromagnetic radiation,

$$\mathbf{E = hc/ \lambda}$$

(where h is Planck's constant, c the velocity of light and λ is wavelength), and hence the wavelength of light emitted is also unique.

Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is most common to select a single wavelength (or a very few) for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the sample being analyzed. Thus, by determining which wavelengths are emitted by a sample and by determining their intensities, the analyst can qualitatively and quantitatively find the elements from the given sample relative to a reference standard.

The wavelengths used in AES ranges from the upper part of the vacuum ultraviolet (160 nm) to the limit of visible light (800 nm). As borosilicate glass absorbs light below 310 nm and oxygen in air absorbs light below 200 nm, optical lenses and prisms are generally fabricated from quartz glass and optical paths are evacuated or filled by a non-absorbing gas such as Argon.



X-RAY DIFFRACTION

X-ray diffraction (XRD) relies on the dual wave/particle nature of X-rays to obtain information about the structure of crystalline materials. A primary use of the technique is the identification and characterization of compounds based on their diffraction pattern.

The dominant effect that occurs when an incident beam of monochromatic X-rays interacts with a target material is scattering of those X-rays from atoms within the target material. In materials with regular structure (i.e. crystalline), the scattered X-rays undergo constructive and destructive interference. This is the process of diffraction. The diffraction of X-rays by crystals is described by Bragg's Law,

$2d\sin\theta = n\lambda$.

The directions of possible diffractions depend on the size and shape of the unit cell of the material. The intensities of the diffracted waves depend on the kind and arrangement of atoms in the crystal structure. However, most materials are not single crystals, but are composed of many tiny crystallites in all possible orientations called a polycrystalline aggregate or powder. When a powder with randomly oriented crystallites is placed in an X-ray beam, the beam will see all possible interatomic planes. If the experimental angle is systematically changed, all possible diffraction peaks from the powder will be detected.

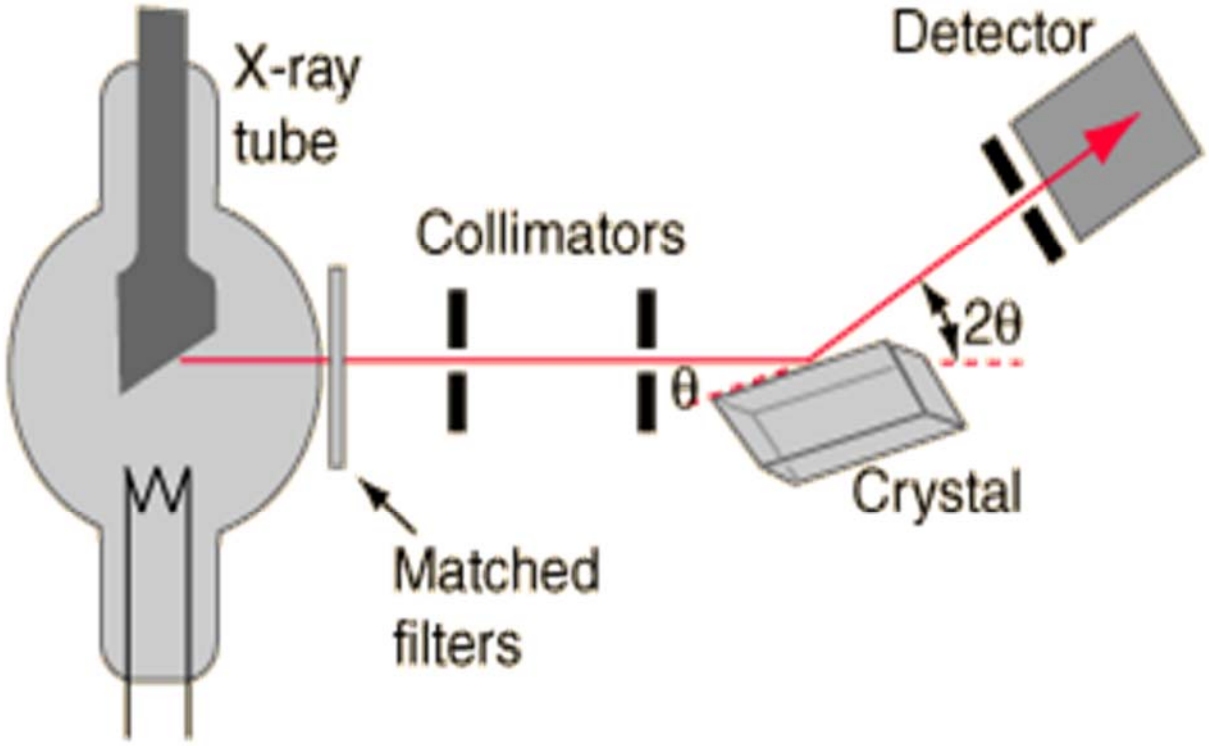
Instrumentation - How Does It Work

X-ray diffractometers consist of three basic elements:

An X-ray tube

A sample holder

An X-ray detector.



X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced.

METHODS OF FRACTIONATION AND CHARACTERIZATION OF PROTEINS AND NUCLEIC ACIDS

PROTEIN ISOLATION AND PURIFICATION

Protein isolation and purification achieved in several steps namely,

Cell disruption

Fractionation

Crude sample preparation

Purification

1. CELL DISRUPTION:

In order to isolate and purify protein, first the location of the protein should be identified. A protein might be present in the cell membrane or in cytosol or in any one of the intracellular organelle. If protein present in the extracellular phase of the membrane i.e. outer leaflet of membrane, then protein might be released from the cell simply using detergents. There is no need for cell disruption in this case. If protein present in other locations then cell disruption has to be carried out.

Cell disruption can be achieved in any one of the following methods.

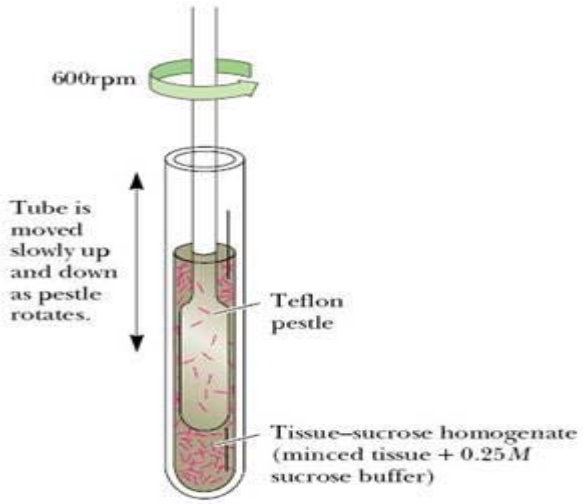
Detergents

Lysozyme

Ultrasonication

Osmotic shock

Mechanical stress (Homogenization)



Detergents generally used to disrupt the cell membrane and release the contents from the cell. Lysozyme mostly used to lyse the cell wall with peptidoglycan layer. So, it was mostly used for lysing gram negative and gram positive bacterial cells. For gram negative bacterial lysis lysozyme require detergents also for its lytic phenomenon due to the presence of outer membrane in gram negative bacteria. Ultrasonic vibrations also used for cell disruption. Osmotic phenomenon used for cell lysis. For example, to lyse RBCs and release hemoglobin, RBCs are placed in water. Due

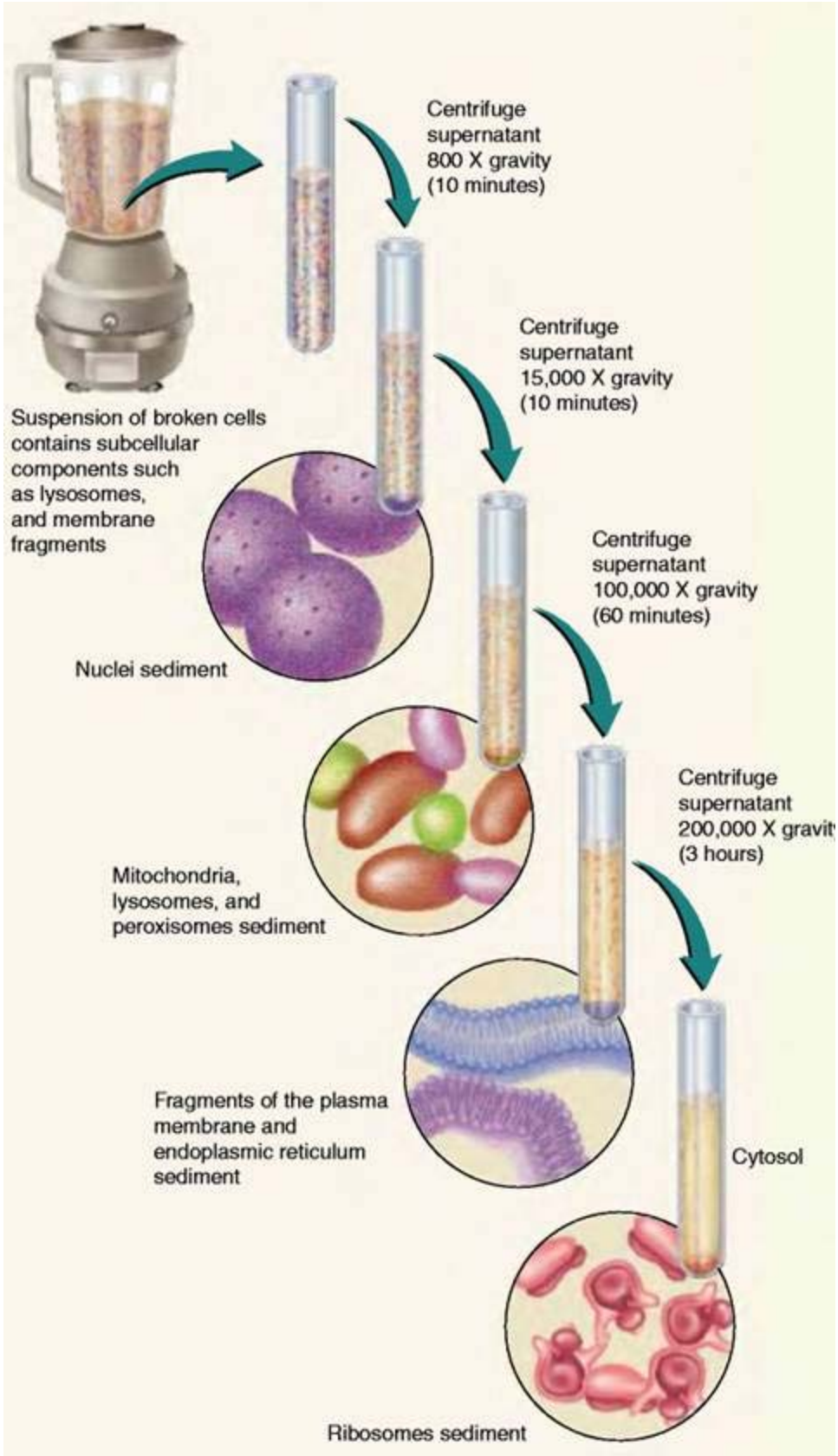
to osmotic pressure water moves into RBCs to neutralize the salt concentration. Because of this endo osmotic action, RBCs lysed and hemoglobin released. Eventhough different methods available for cell disruption, the most common and simple method used was homogenization. This was carried out using Homogenizer and Pestle. For this, cell fraction or mixture mixed with suitable buffer and placed in homogenizer and Teflon pestle rotated at a speed of 600rpm which results in the formation of homogenized mixture by cell disruption.

2. FRACTIONATION:

After homogenized mixture formed, from the mixture particular fraction which contains our protein of interest must be obtained for further analysis. If the protein was present in the intracellular organelle, specific intracellular organelle should be fractionated and it was further disrupted and the extract used for further purification processes. Fractionation was achieved by ultracentrifuge and differential centrifugation.

Initially, the homogenate is spun in the centrifuge at low speed (700 -1,000 g) for 10 to 20 minutes. The heavier particles, such as the nuclei, form sediment, or pellet. Lighter particles, such as mitochondria and lysosomes, remain suspended in the supernatant, the liquid above the pellet. The supernatant is then transferred to another centrifuge tube and spun at a higher speed (15,000 to 20,000 g) for 10-20 minutes. The resulting pellet contains mitochondria, lysosomes, and peroxisomes. The supernatant, which contains microsomes (small closed vesicles formed from ER during homogenization), is transferred to another tube and spun at 100,000 g for 60-120 minutes. Microsomes are deposited in the pellet, and the supernatant contains ribosomes, various cellular membranes, and granules such as glycogen, a carbohydrate polymer. After this latest supernatant is recentrifuged at 200,000 g for 2 to 3 hours, ribosomes and large macromolecules are recovered from the pellet.

The ultracentrifuge is also used to separate cellular components on the basis of their buoyant density, independently of their size and shape. In this case the sample is usually sedimented through a steep density gradient that contains a very high concentration of sucrose or cesium chloride. Each cellular component begins to move down the gradient, but it eventually reaches a position where the density of the solution is equal to its own density. At this point the component floats and can move no farther. A series of distinct bands is thereby produced in the centrifuge tube, with the bands closest to the bottom of the tube containing the components of highest buoyant density.


Centrifuge
supernatant
800 X gravity
(10 minutes)
Centrifuge
supernatant
15,000 X gravity
(10 minutes)
Suspension of broken cells
contains subcellular
components such
as lysosomes,
and membrane
fragments
Nuclei sediment
Centrifuge
supernatant
100,000 X gravity
(60 minutes)
Centrifuge
supernatant
200,000 X gravit
(3 hours)
Mitochondria,
lysosomes, and
peroxisomes sediment
Fragments of the plasma
membrane and
endoplasmic reticulum
sediment
Cytosol
Ribosomes sediment

3. CRUDE SAMPLE PREPARATION:

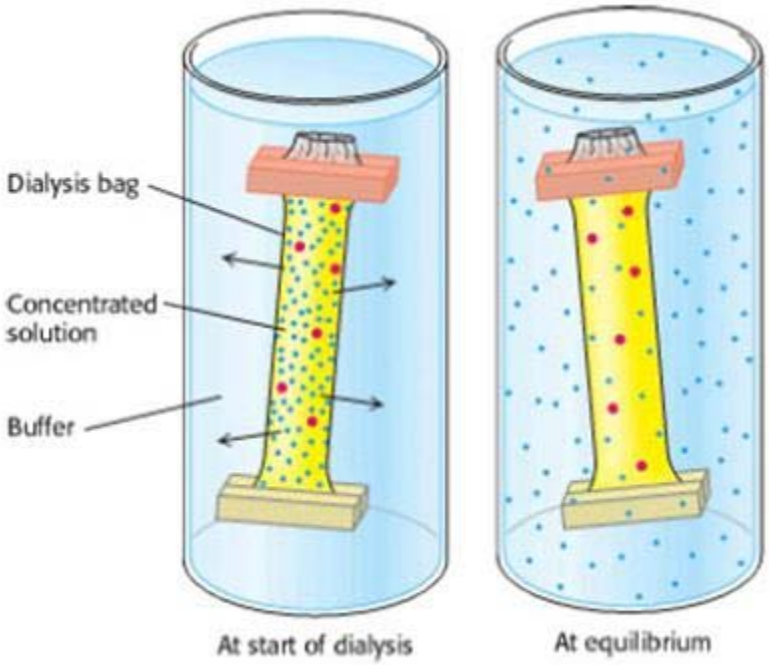
After the protein-containing fraction has been obtained, several relatively crude methods may be used to enhance purification. Processes like Salting out, Dialysis and Gel extrusion chromatography were used for this purpose.

a. Salting out:

It is a technique in which high concentrations of salts such as ammonium sulfate [(NH4JSO4] are used to precipitate proteins. Because each protein has a characteristic salting-out point, this technique removes many impurities. Unwanted proteins that remain in solution are then discarded when the liquid is decanted. When proteins are tightly bound to membrane, organic solvents or detergents often aid in their extraction. For example, 0.8 M ammonium sulfate precipitates fibrinogen, a blood-clotting protein, whereas a concentration of 2.4 M is needed to precipitate serum albumin. Salting out is also useful for concentrating dilute solutions of proteins, including active fractions obtained from other purification steps. Dialysis can be used to remove the salt if necessary.

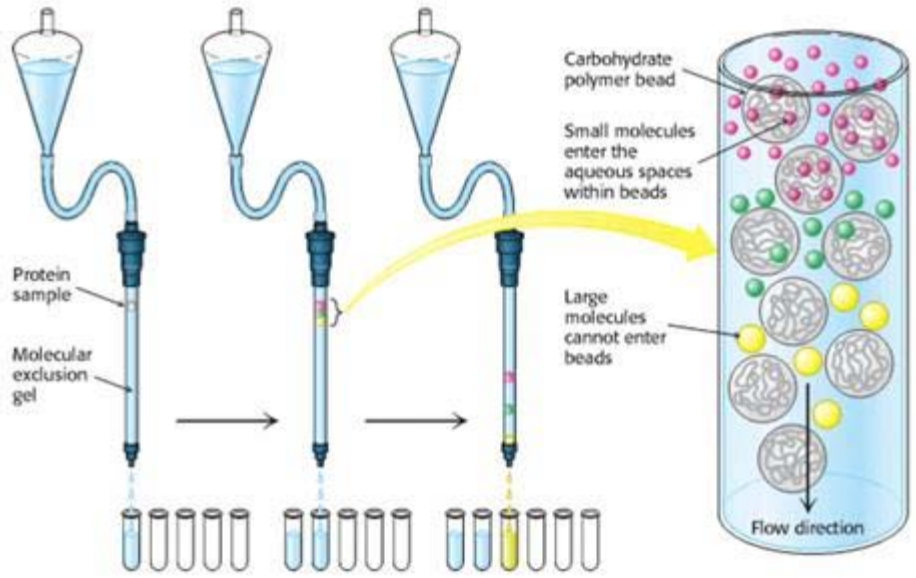
b. Dialysis:

Proteins can be separated from small molecules by dialysis through a semipermeable membrane, such as a cellulose membrane with pores. Molecules having dimensions significantly greater than the pore diameter are retained inside the dialysis bag, whereas smaller molecules and ions traverse the pores of such a membrane and emerge in the dialysate outside the bag. This technique is useful for removing a salt or other small molecule, but it will not distinguish between proteins effectively. The speed of dialysis might be increased by using hot water and electrical field. Ultrafiltration is an improvement on the dialysis principle. Filters having pore sizes over the range of biomolecular dimensions are used to filter solutions to select for molecules in a particular size range. Because the pore sizes in these filters are microscopic, high pressures are often required to force the solution through the filter. This technique is useful for concentrating dilute solutions of macromolecules. The concentrated protein can then be diluted into the solution of choice.



c. Gel Filtration Chromatography:

More discriminating separations on the basis of size can be achieved by the technique of gel-filtration chromatography. The sample is applied to the top of a column consisting of porous beads made of an insoluble but highly hydrated polymer such as dextran or agarose (which are carbohydrates) or polyacrylamide. Sephadex, Sepharose, and Bio-gel are commonly used commercial preparations of these beads, which are typically 100 mm (0.1 mm) in diameter. Small molecules can enter these beads, but large ones cannot. The result is that small molecules are distributed in the aqueous solution both inside the beads and between them, whereas large molecules are located only in the solution between the beads. Large molecules flow more rapidly through this column and emerge first because a smaller volume is accessible to them. Molecules that are of a size to occasionally enter a bead will flow from the column at an intermediate position, and small molecules, which take a longer, tortuous path, will exit last.



4. PURIFICATION:

As a protein sample becomes progressively more pure, more sophisticated methods are used to achieve further purification. The most commonly used techniques include chromatography and electrophoresis. Of these methods mainly two dimensional gel electrophoresis and affinity chromatography mostly used.

PROTEIN ESTIMATION:

To determine the success of a protein purification scheme, monitor the procedure at each step by determining specific activity and by performing an SDS-PAGE analysis. Consider the results for the purification of a fictitious protein. At each step, the following parameters are measured:

Total protein: The quantity of protein present in a fraction is obtained by determining the protein concentration of a part of each fraction and multiplying by the fraction's total volume.

Total activity: The enzyme activity for the fraction is obtained by measuring the enzyme activity in the volume of fraction used in the assay and multiplying by the fraction's total volume.

Specific activity: This parameter is obtained by dividing total activity by total protein.

Yield: This parameter is a measure of the activity retained after each purification step as a percentage of the activity in the crude extract. The amount of activity in the initial extract is taken to be 100%.

Purification level: This parameter is a measure of the increase in purity and is obtained by dividing the specific activity, calculated after each purification step, by the specific activity of the initial extract.

Dissociation constant

The dissociation constant specifies the tendency of a substance A_xB_y to reversibly dissociate (separate) in a solution into smaller components A and B:

AyBy⇌xA+yB(1)(1)AyBy⇌xA+yB

The dissociation constant is denoted K_d and is calculated by

Kd=[A]x[B]y[AxBy](2)(2)Kd=[A]x[B]y[AxBy]

where [A], [B], and $[A_xB_y]$ are the molar concentraions of the entities A, B, and A_xB_y. The dissociation constant is an immediate consequence of the law of mass action which describes equilibria in a more general way. The dissociation constant is also sometimes called ionization constant when applied to salts. The inverse of the dissociation constant is called association constant.

Dissociation constant of water

Formally, the dissociation of water follows the following equation:

H2O⇌H++OH−(3)(3)H2O⇌H++OH−

Thus the dissociation constant is given by

Kd=[H+][OH−][H2O]=2.16×10−16(4)(4)Kd=[H+][OH−][H2O]=2.16×10−16

However, since the concentration of undissociated water is almost unchanged by the dissociation process (due to the low K_d), we can assume that a liter of water at 25°C contains 55.39 mol undissociated water. For the sake of convenience this constant water concentration is combined with the dissociation constant K_d to form the dissociation constant of water K_w:

Kw=[H+][OH−]=2.16×10−16)(55.39)=1.2×10−14(5)(5)Kw=[H+][OH−]=2.16×10−16)(55.39)=1.2×10−14

The value of K_w changes considerably with temperature. Consequently this variation must be taken into account when making precise measurements (i.e. when determining the pH).

Water Temperature [°C]	K_w [10^{-14}]	pK_w
0	0.1	14.92
10	0.3	14.52
18	0.7	14.16
25	1.2	13.92
30	1.8	13.75
50	8.0	13.10
60	12.6	12.90
70	21.2	12.67
80	35	12.46
90	53	12.28
100	73	12.14

Acid base reactions

The dissociation constant can also be applied to the deprotonation of acids. In this case the dissociation constant is denoted as K_a. The greater the dissociation constant of an acid the stronger the acid. Polyprotic acids (e.g. carbonic acid or phosphoric acid) show several dissociation constants, since more than one proton can be separated (one after the other):

$H_3A \rightleftharpoons H^+ + H_2A^-$	$K_{a1} = [H^+][H_2A^-]/[H_3A]$	$pK_{a1} = -\lg(K_{a1})$
$H_2A^- \rightleftharpoons H^+ + HA^{2-}$	$K_{a2} = [H^+][HA^{2-}]/[H_2A^-]$	$pK_{a2} = -\lg(K_{a2})$
$HA^{2-} \rightleftharpoons H^+ + A^{3-}$	$K_{a3} = [H^+][A^{3-}]/[HA^{2-}]$	$pK_{a3} = -\lg(K_{a3})$

A list of acid dissociation constants can be found here.

Other applications

The concept of the dissociation constant is applied in various fields of chemistry and pharmacology. In protein-ligand binding the dissociation constant describes the affinity between a protein and a ligand. A small dissociation constant indicates a more tightly bound the ligand. In the case of antibody-antigen binding the inverted dissociation constant is used and is called affinity constant.

pH, pOH, and the pH scale

let's look at values for **pH and pOH**. pOH is simply the **power of hydroxide ion concentration** and is figured the same way as pH but using the concentration of hydroxide ion instead.) For your notes, expand the table in exercise 24-a by making columns for pH and pOH.)

Let's start by working with the concentrations that are 1.0 x $10^{(\text{raised to some power})}$. These pH and pOH values can be figured very simply. When $[H_3O^+]$ is 10^{-7} M, the pH is 7. Also the $[OH^-]$ is 10^{-7} M and the pOH is 7. Note that the pH and pOH add up to 14.

Now look at the acidic solutions. When the $[H_3O^+]$ is 10^{-6} M, the pH is 6. Also, the $[OH^-]$ is 10^{-8} M and the pOH is 8. Again, the pH and the pOH add up to 14. When the $[H_3O^+]$ is 10^{-5} M,

pH	$[H_3O^+]$	$[OH^-]$	pOH
7	1.0 x 10^{-7} M	1.0 x 10^{-7} M	7
6 5	1.0 x 10^{-6} M 1.0 x 10^{-5} M	1.0 x 10^{-8} M 1.0 x 10^{-9} M	8 9

pH is 5, $[OH^-]$ is 10^{-9} M, and pOH is 9. pH + pOH = 14.

Next, the basic solutions. When $[OH^-]$ is 10^{-6} M, the pOH is 6. Since $[H_3O^+]$ 10^{-8} M, the pH is 8. When $[OH^-]$ is 10^{-5} M, and $[H_3O^+]$ is 10^{-9} M, the pOH is 5 and pH is 9. Again in both cases the sum of pH and pOH is 14.

8 9	1.0×10^{-8} M 1.0×10^{-9} M	1.0×10^{-6} M 1.0×10^{-5} M	6 5

However, the hydrogen ion concentration is not always going to be equal to exactly 1 x 10 raised to a negative number. For example, we skipped over the value of 2.0×10^{-7}. This is more complicated. However, if you use a calculator that will handle logarithms, it is a very simple calculation. First you enter the hydronium ion concentration. You can use decimal format or scientific notation. Next push the log button. Then change the sign by pushing the +/- button. In this case we get 6.70 for the pH. The other values can be obtained in the same way.

6.70 7.30	2.0×10^{-7} M 0.5×10^{-7} M	0.5×10^{-7} M 2.0×10^{-7} M	7.30 6.70

Significant Digits in pH

Now a word about **significant digits** and **log values** like pH's. Let's compare the parts of the pH value to the parts of the value in the scientific notation from which it was derived. The value of 2.0×10^{-7} shows both precision (two significant digits in 2.0) and size or magnitude (shown in the exponent $^{-7}$). In the pH value, the size or magnitude is shown in the number in front of the decimal point. The two significant digits are shown after the decimal point. The size match is a little easier to see if the the concentration is written in **non**standard scientific notation.

$[H_3O^+]$	$= 2.0 \times$		10^{-7}
precision	(two	significant	digits)
size			
pH			= 6.70
precision	(two	significant	digits)
size			
$[H_3O^+]$	=	$0.20 \times$	10^{-6}
precision	(two	significant	digits)
size			

Practice - pH

I'd like you to practice calculating pH values by figuring values for the two empty columns in this table (exercise 26 in your workbook). Write the hydronium ion concentrations in scientific notation and also calculate the pH value for each. Check your answers below, then continue with the lesson.

$[H_3O^+]$	scientific notation	pH
0.1		
0.01		
0.001		
0.0007		
0.0001		
0.000001		
0.0000003		
0.0000001		
0.0000000001		
0.00000000002		

Answers

Your answers should be as follows:

$[H_3O^+]$	scientific notation	pH
0.1	1×10^{-1}	1.0
0.01	1×10^{-2}	2.0
0.001	1×10^{-3}	3.0
0.0007	7×10^{-4}	3.2
0.0001	1×10^{-4}	4.0
0.000001	1×10^{-6}	6.0
0.0000003	3×10^{-7}	6.5

0.0000001	1×10^{-7}	7.0
0.0000000001	1×10^{-10}	10.0
0.00000000002	2×10^{-11}	10.7

Note that each pH value has one digit to the right of the decimal point because each concentration value had one significant digit.

If your answers are not correct, get some help from the instructor.

Hydronium Ion Concentration from pH

It is also possible to run the calculations in the other direction as well. If you know the pH, you can calculate the hydrogen ion concentration. Here is how you do it. Let's say the pH is 4.3. Enter the pH value and make it negative using the **+/- button**. Then press the **10^x button**. It will probably be the **2nd function** or **inverse** of the **log button**. I get 0.00005 or 5×10^{-5} M. If you don't get this value when you try it with your calculator, or if you cannot find the right buttons, check with the instructor in the lab.

Practice - pH and pOH

Once you have that calculation under control, try your hand at filling in the blank spots in this table (exercise 27 in your workbook). Check your answers below then continue with the lesson.

pH	**$[H_3O^+]$**	**pOH**	**$[OH^-]$**
3.2			
8.2			
		6.4	
	0.0050		
			0.0050
			2.3×10^{-5}
		7.8×10^{-4}	

Answers

All concentrations are expressed in molarity.

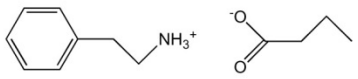
pH	**$[H_3O^+]$**	**pOH**	**$[OH^-]$**
3.2	0.0006 or **6×10^{-4}**	10.8	1.6×10^{-11} or **2×10^{-11}**
8.2	0.000000006 or **6×10^{-9}**	5.8	1.6×10^{-6} or **2×10^{-6}**
7.60	2.5×10^{-8}	6.40	4.0×10^{-7}
2.30	0.0050	11.70	2.0×10^{-12}
11.70	2.0×10^{-12}	2.30	0.0050
9.36	4.3×10^{-10} (from the K_w equation) or 4.4×10^{-10} (if going from pH to conc.)	4.64	2.3×10^{-5}
3.11	7.8×10^{-4}	10.89	1.3×10^{-11}

Molecular forces stabilising ligand binding

The strength of protein-ligand binding is usually determined by a variety of non-covalent interactions (Figures 8.3 and 8.4). These interactions differ from one another in their strength (binding energy) and their range. These interactions are as follows:

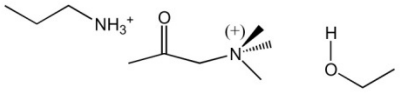
- Covalent bonding. The strongest interaction resulting irreversible binding. Energy: ~ -500 kJ/mol.
- Ionic (Coulomb) interaction: -20 – -40 kJ/mol, distance dependence: 1/*r*.

$$E = \frac{q_1 q_2}{4\pi\varepsilon_o r_{1,2}}$$



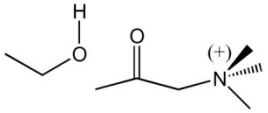
- Charge-dipole interaction: -10 – -30 kJ/mol, $1/r^2$

$$E = \frac{q_1 \mu}{4\pi\varepsilon_o r_{1,2}^2}$$



- Dipole-dipole interaction: -5 – -20 kJ/mol, $1/r^3$

$$E = \frac{\mu_1 \mu_2}{4\pi\varepsilon_o r_{1,2}^3}$$



q, charge; μ, dipole moment; r, distance, ε_0, dielectric constant

- Hydrogen bond: -4 – -30 kJ/mol

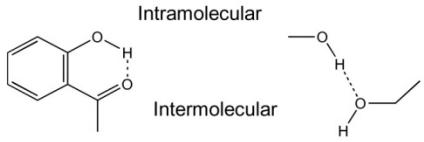
H-bond donor ... acceptor

N-H … O

N-H … N

O-H … N

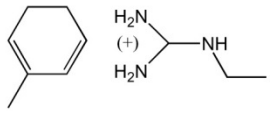
O-H … O



Hydrogen bond energy is largely dependent on the donor-acceptor distance, which is shorter 3,5 Å, and the orientation

- Charge-induced dipole interaction: -20 – -40 kJ/mol, $1/r^4$

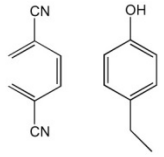
$$E = -\frac{1}{2}\frac{q_1^2 \alpha_2}{r_{1,2}^4}$$



q_1, charge; α_2, polarizability; $r_{1,2}$, distance

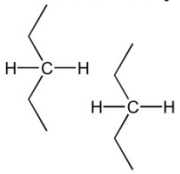
- Dipole-induced dipole interaction: -5 – -30 kJ/mol, $1/r^6$

$$E = -\frac{1}{2}\frac{\mu_1^2 \alpha_2}{r_{1,2}^6}$$



- London-dispersion forces, -2 kJ/mol/methylene group

$$E = -\frac{3 I_1 \alpha_2^2}{4 r_{1,2}^6}$$



μ, dipole moment; α, polarizability; I, ionizability; r, distance

In the case of clashing atoms a strong repulsion is observable, which is explained by the Pauli exclusion principle and is usually taken into account with $1/r^{12}$ distance dependence.

Figure Types of intermolecular interactions

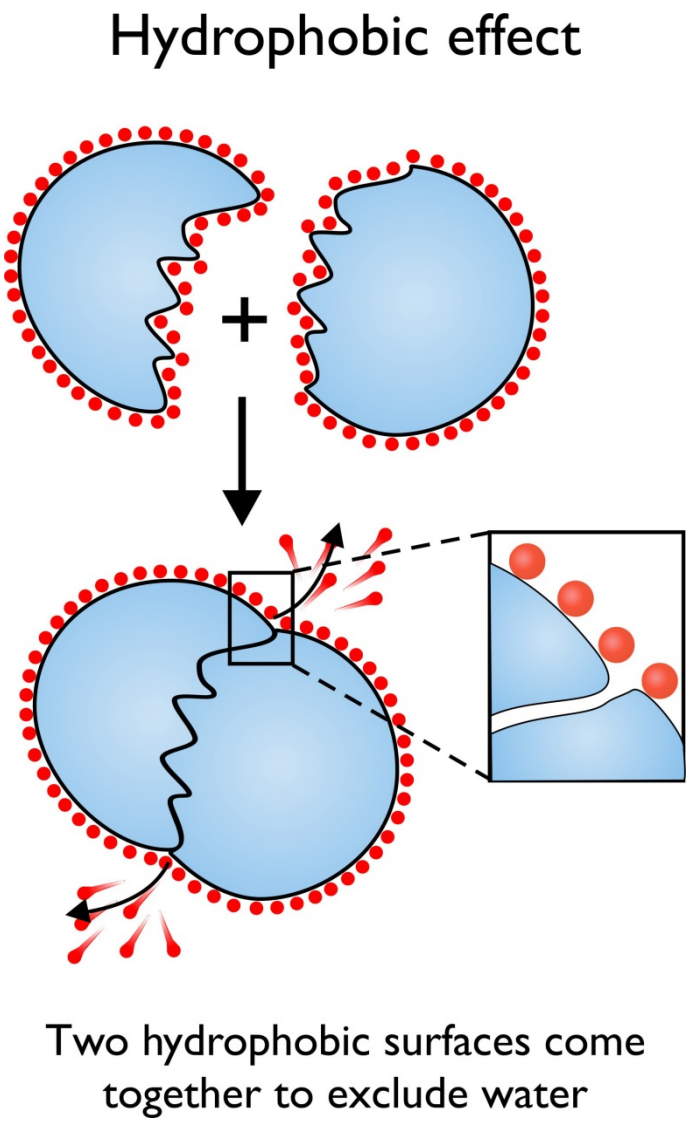


Figure The hydrophobic effect

Beyond the above ones, the hydrophobic effect is a complex, noncovalent interaction in which the hydrophobic part of the molecule becomes buried from water and thus the water-accessible apolar surface area of the macromolecule decreases. The interaction is additive; its energy is roughly proportional to the buried surface area: ~ -80-100 J/mol/Å^2. This represents approximately -3 kJ/mol energy for the burial of a methylene group.

The strong binding affinity and high specificity of protein-ligand interactions result from a high amount of weak noncovalent interactions. Figure 8.5 shows an enzyme-inhibitor complex with a dissociation constant of 10^{-13} M.

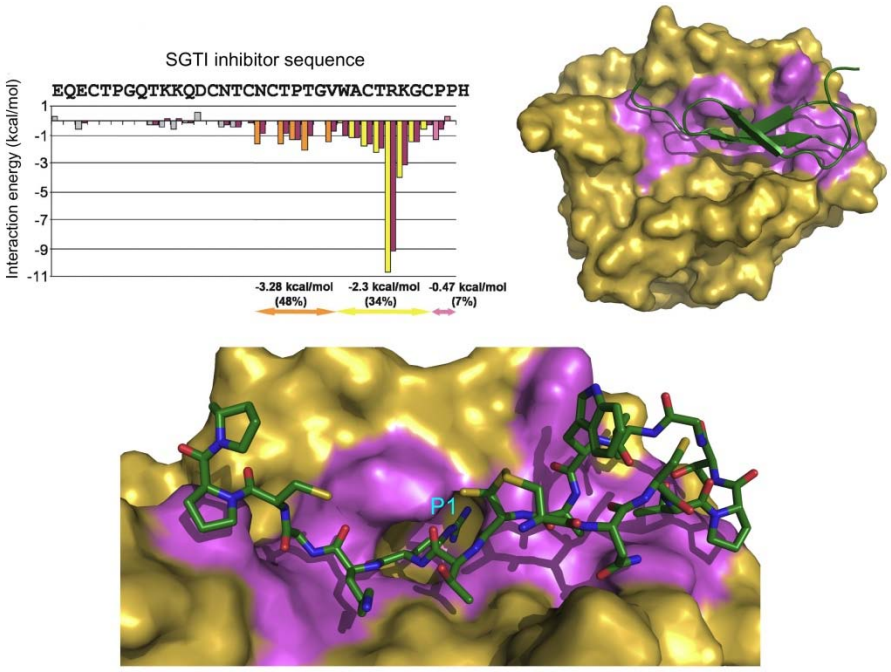


Figure The interaction of a trypsin inhibitor with crayfish trypsin. Upper left panel: distribution of the interaction energies with bovine trypsin among the residues of the inhibitor. Upper right panel: 3D structure of the complex. Bottom panel: close-up view of the binding interface. Note the Arg side chain in the P1 position fitting in the substrate binding site of the protease.

Methods for the experimental determination of the binding constant

Understanding the molecular mechanisms and interactions that govern various processes and reactions in living systems is a central issue in molecular biology, biochemistry, medicine and in the new fields of proteomics and genomics. To reach this goal, it is indispensable to identify the interacting partners and characterise their interactions. A variety of techniques are available for screening and measuring protein-ligand interactions. Methods include affinity chromatography, cross-linking, gel filtration, co-localisation, two-hybrid methods, spectroscopic methods, 3D structure determination, equilibrium dialysis, radioactive labelling, sedimentation velocity measurements, isothermal titration calorimetry, surface plasmon resonance, microarray, immunoblotting and ELISA.

Below we discuss some of the frequently used methods. The radioimmunoassay (RIA) is suitable for the determination of the concentration of various antigens, hormones and drugs in different body fluids such as the human blood. During the experiment, a known amount of a radioactively-labelled antigen is mixed into the solution of its corresponding antibody. Usually, gamma-radiating isotopes such as tyrosine-bound iodine are used. Subsequently, the unlabeled (“cold”) antigen sample is added to the solution and the liberation of the labelled antigen is measured. The unlabelled material competes with the labelled one and may “chase it off” from the antibody, depending on the concentrations. The material bound in complex is separated from the free antigen. This is a technique with high sensitivity; however, the handling of radioactive material needs

special care. Nowadays, the ELISA technique is more popular, which measures the antigen-antibody interactions by applying colour reactions.

In ELISA (enzyme-linked immunosorbent assay), the antigen is bound to a surface and then recognised by a specific antibody. Subsequently, a second antibody is applied that is specific to the first one and usually carries a covalently-bound enzyme molecule. By the addition of the substrate of the enzyme, a colourful and/or fluorescent product will form. This way, the presence of the antigen—or even its concentration—can be determined. A more sophisticated variant of ELISA is the "sandwich" ELISA. In this case, a "capture" antibody is immobilised on the membrane to which the antigen binds in the second step. Then a second antibody is added that also binds the antigen but—similarly to the above procedure—it carries a covalently-bound enzyme. Thus a "sandwich" is being formed, and then detection is carried out by adding substrate. The sandwich ELISA is much more sensitive than the common ELISA technique. Pregnancy tests are usually based on this method.

Molecular motors

Molecular motors are enzyme protein devices that move along cytoskeletal filaments and the proteins are run by ATP hydrolysis.

The main system of Biological motility is driven by the factor of intracellular motility. For example, the microtubules and the actin filaments in cells are *tracks*, and the *molecular motors* have two parts the *tail domain* that are carriers of cargo (organelle or protein) and the *motor domain*, which work along the track causing motion and thus causes movement. Molecular motors convert the chemical energy derived from ATP hydrolysis into mechanical action. Molecular motors are generally of 10^{-8} m sizeand are driven by the Hydrolysis of ATP and have an average speed of $4*10^{-3}$ m/hr

Molecular Motors consist of different varieties

· **Cytoskeletal** – Kinesin, Dynein and Myosin motor groups. These are of large of groups and there are many motors from these groups.

· **DNA Motors** - Helicases and polymerases.

· **Rotatory motors** – Bacterial flagellum, ATPase

for example the aerobic respiration between the mitochondrial matrix and the membrane of the mitochondria.

The Process

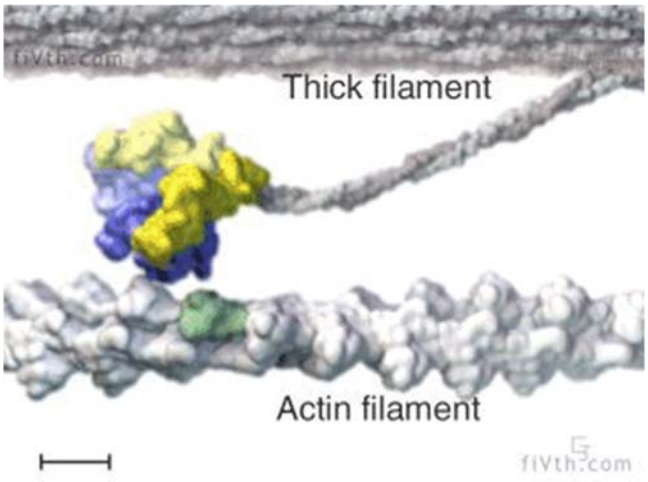
The actual motion as shown in the picture 2 follows a chemical cycle.

The Picture 3 is an example of muscle contraction of a Myosin motor on a actin filament.

This movement is the motor to bind on the actin track and slide the actin filament so that it can contract the sarcomere thus making a contraction.

Firstly in the animation you can see the myosin motor binding to the track and the actin filament causes a phosphate release and that causes a big rotation of 10nm of one part of the motor domain(highlighted red). Then the ATP comes in to the motor which causes the bonded motor to

release from the track. Then the hydrolysis of ATP causes a movement or repositioning of motor domain in the actin filament. Thus, the cycle repeats again: the phosphate release and then a stroke, ATP hydrolysis occurs and reposting of motor.



Sedimentation

- Sedimentation is the tendency for particles in suspension to settle out of the fluid in which they are entrained and come to rest against a barrier.
- This is due to their motion through the fluid in response to the forces acting on them: these forces can be due to gravity, centrifugal acceleration, or electromagnetism.
- In geology, sedimentation is often used as the opposite of erosion, i.e., the terminal end of sediment transport. In that sense, it includes the termination of transport by saltation or true bedload transport.
- Settling is the falling of suspended particles through the liquid, where as sedimentation is the termination of the settling process.
- Sedimentation may pertain to objects of various sizes, ranging from large rocks in flowing water to suspensions of dust and pollen particles to cellular suspensions to solutions of single molecules such as proteins and peptides.
- Even small molecules supply a sufficiently strong force to produce significant sedimentation.

- Whenever a particle is moving through a fluid, a number of forces will be acting on the particle.
- First, a density difference is needed between the particle and the fluid.
- If the densities of the fluid and particle are equal, the buoyant force on the particle will counterbalance the external force and the particle will not move relative to the fluid.
- There are three forces acting on the body:-

1. Gravity Force
2. Buoyant Force

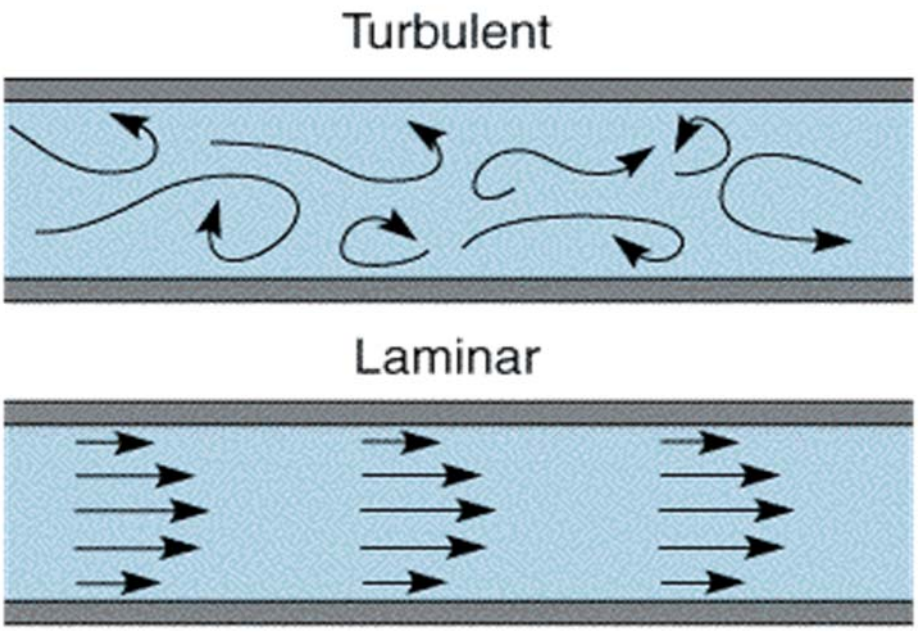
3. Drag Force
4. **Gravity, or gravitation**, is a natural phenomenon by which all things with mass are brought toward (or gravitate toward) one another, including objects ranging from atoms and photons, to planets and stars. Since energy and mass are equivalent, all forms of energy (including light) cause gravitation and are under the influence of it. On Earth, gravity gives weight to physical objects.
5. **Buoyancy** is, basically, the ability of something to float or rise in a fluid. Simple as that. More scientifically, buoyancy refers to a force that arises from the pressure exerted on an object by a fluid (a liquid or a gas). Since it's a force, we call it the **buoyant force**.
6. **Drag** (sometimes called air resistance, a type of friction, or fluid resistance, another type of friction or fluid friction) is a force acting opposite to the relative motion of any object moving with respect to a surrounding fluid. This can exist between two fluid layers (or surfaces) or a fluid and a solid surface.

Reynold's number

- The Reynolds number, referred to as Re, is used to determine whether the fluid flow is laminar or turbulent.
- It is one of the main controlling parameters in all viscous flows where a numerical model is selected according to pre-calculated Reynolds number.
- The dimensionless Reynolds number plays a prominent role in foreseeing the patterns in a fluid's behavior.
- Laminar Flow: the flow of a fluid when each particle of the fluid follows a smooth path, paths which never interfere with one another. One result of laminar flow is that the velocity of the fluid is constant at any point in the fluid.

  Turbulent Flow: irregular flow that is characterized by tiny whirlpool regions. The velocity of this fluid is definitely not constant at every point.

  Typical examples of both Laminar and Turbulent flows can be observed in the cigarette smoke (see image).
- Although the Reynolds number comprises both static and kinematic properties of fluids, it is specified as a flow property since dynamic conditions are investigated.
- Technically speaking, the Reynolds number is the ratio of the inertial forces and the viscous forces.
- In practice, the Reynolds number is used to predict if the flow will be laminar or turbulent.



With respect to laminar and turbulent flow regimes:

- Laminar flow occurs at low Reynolds numbers, where viscous forces are dominant, and is characterized by smooth, constant fluid motion.
- Turbulent flow occurs at high Reynolds numbers and is dominated by inertial forces, which tend to produce chaotic eddies, vortices and other flow instabilities.
- In fluid dynamics, an eddy is the swirling of a fluid and the reverse current created when the fluid is in a turbulent flow regime
- The concept was introduced by Sir George Stokes in 1851, but the Reynolds number was named by Arnold Sommerfeld in 1908 after Osborne Reynolds (1842–1912), who popularized its use in 1883.
- Osborne Reynolds famously studied the conditions in which the flow of fluid in pipes transitioned from laminar flow to turbulent flow.
- In his 1883 paper Reynolds described the transition from laminar to turbulent flow in a classic experiment in which he examined the behavior of water flow under different flow velocities using a small stream of dyed water introduced into the centre of clear water flow in a larger pipe.
- Reynolds number has wide applications, ranging from liquid flow in a pipe to the passage of air over an aircraft wing.
- It is used to predict the transition from laminar to turbulent flow, and used in the scaling of similar but different-sized flow situations, such as between an aircraft model in a wind tunnel and the full size version.
- The predictions of the onset of turbulence and the ability to calculate scaling effects can be used to help predict fluid behavior on a larger scale, such as in local or global air or water movement and thereby the associated meteorological and climatological effects.

Molecular self-assembly

Molecular self-assembly is the process by which molecules adopt a defined arrangement without guidance or management from an outside source.

There are two types of self-assembly. These are

1. Intra-molecular self-assembly
2. Intermolecular self-assembly.

Commonly, the term molecular self-assembly refers to intermolecular self-assembly, while the intra-molecular analog is more commonly called folding.

- Molecular self-assembly is a key concept in supramolecular chemistry.
- This is because assembly of molecules in such systems is directed through non-covalent interactions (e.g., hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions, and/or electrostatic) as well as electromagnetic interactions.
- Common examples include the formation of micelles, vesicles, liquid crystal phases, and Langmuir monolayers by surfactant molecules.
- Further examples of supramolecular assemblies demonstrate that a variety of different shapes and sizes can be obtained using molecular self-assembly
- Molecular self-assembly allows the construction of challenging molecular topologies.
- One example is Borromean rings, interlocking rings wherein removal of one ring unlocks each of the other rings.
- DNA has been used to prepare a molecular analog of Borromean rings.
- More recently, a similar structure has been prepared using non-biological building blocks.
- Molecular self-assembly underlies the construction of biologic macromolecular assemblies in living organisms, and so is crucial to the function of cells.
- It is exhibited in the self-assembly of lipids to form the membrane, the formation of double helical DNA through hydrogen bonding of the individual strands, and the assembly of proteins to form quaternary structures.
- Molecular self-assembly of incorrectly folded proteins into insoluble amyloid fibers is responsible for infectious prion-related neurodegenerative diseases.
- Molecular self-assembly is an important aspect of bottom-up approaches to nanotechnology.
- Using molecular self-assembly the final (desired) structure is programmed in the shape and functional groups of the molecules.
- Self-assembly is referred to as a 'bottom-up' manufacturing technique in contrast to a 'top-down' technique such as lithography where the desired final structure is carved from a larger block of matter.
- In the theoretical vision of molecular nanotechnology, microchips of the future might be made by molecular self-assembly.
- An advantage to constructing nanostructure using molecular self-assembly for biological materials is that they will degrade back into individual molecules that can be broken down by the body.
- Self-assembly is the formation of a well-defined, complex structure under equilibrium conditions spontaneously from non-covalent interactions between two or more molecular components.

- Self-assembly encompasses the self-association of proteins and nucleic acids, such as the formation of different forms of a double-stranded DNA double helix from two complementary poly-deoxynucleotide strands, the association of multiple copies of the same polypeptide chain, the formation of complexes comprised of more than one type of polypeptide chain (ie, heteromultimeric proteins), and the reconstitution of an infectious viral particle, comprised of one or more proteins, plus nucleic acid.

Self-assembly can also be viewed as part of a more general topic of supramolecular assembly or self-organization, which encompasses the formation of a wide variety of structures with masses greater than 1 MDa and sizes greater than 10 nm.

These include pathogens such as:

- Viruses
- large multi-enzyme complexes involved in biosynthetic pathways (eg, pyruvate dehydrogenase) and in protein degradation (eg, the proteasome)
- multiprotein complexes that bind to nucleic acids and are active in DNA replication
- RNA biosynthesis and protein biosynthesis (eg, nucleosomes, polymerases, spliceosomes, ribosomes)
- transmembrane protein complexes (eg, acetylcholine receptor, respiratory enzyme complexes, photosynthetic reaction centers)
- and the protein complexes encaging transport vesicles (clathrin-coated vesicles and circulating lipoproteins).
- The foregoing definition of self-assembly implies that all the necessary information is encoded within the covalent structures of the components and hence requires complete reversibility of the assembly process.
- Reversibility is demonstrated in vitro by the reassembly of the completely dissociated structure upon return to the initial solution conditions.

Dissociation into individual molecules can be effected in many different ways:

- (a) increased hydrostatic pressure
- (b) addition or removal of specific ligands, such as coenzymes, substrates, allosteric effectors or other prosthetic groups
- (c) decrease in concentration
- (d) alteration of pH, temperature, or ionic strength
- (e) the addition of dissociating and denaturing chaotropic agents such as urea, guanidinium salts, and detergents (neutral, cationic, or anionic).

At present, overexpression in Escherichia coli of the individual wild-type or mutagenized subunits makes it possible to elucidate the pathways of self-assembly and the roles in the self-assembly process of various protein domains within each subunit.

- Although the covalent structure of each component encodes all the information necessary for its three-dimensional structure and its interactions with the other components in the self-assembly process, the intermolecular forces involved in both processes are all

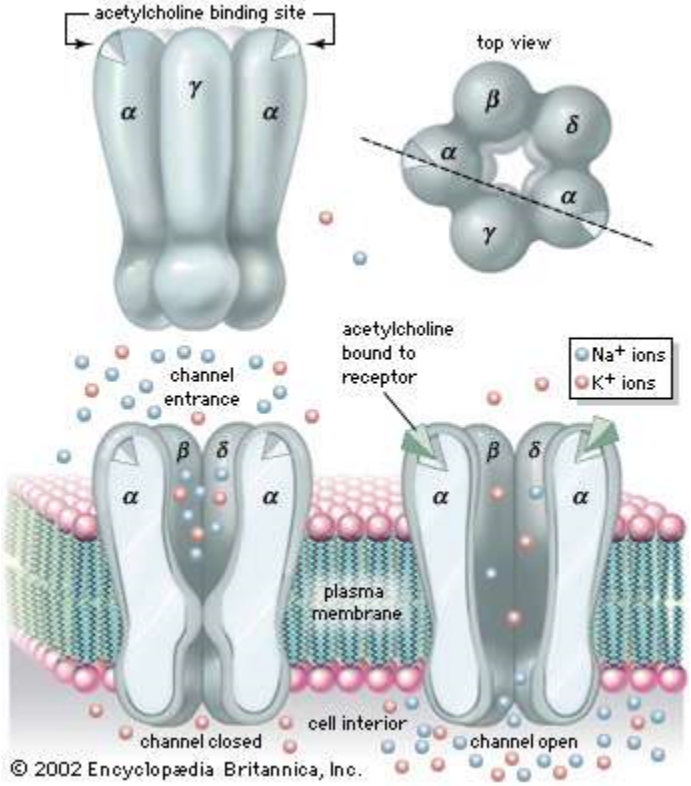
noncovalent, being energetically weaker than covalent bonds by more than an order of magnitude (<10kcal/mol vs ~100kcal/mol).

- These intermolecular forces represent both specific, geometrically directed interactions, like hydrogen bonds and salt bridges (which may be regarded as hydrogen-bonded ion pairs), and relatively nonspecific electrostatic and van der Waals interactions, including the hydrophobic effect.
- The resulting energetic stabilization depends on the amount of accessible surface area buried in the contact between the interacting components and, most critically, on the complementarity in the chemical nature and shapes of the interacting surfaces.
- The term macromolecular assembly (MA) refers to massive chemical structures such as viruses and non-biologic nanoparticles, cellular organelles and membranes and ribosomes, etc. that are complex mixtures of polypeptide, polynucleotide, polysaccharide or other polymeric macromolecules.
- They are generally of more than one of these types, and the mixtures are defined spatially (i.e., with regard to their chemical shape), and with regard to their underlying chemical composition and structure.
- Macromolecules are found in living and nonliving things, and are composed of many hundreds or thousands of atoms held together by covalent bonds; they are often characterized by repeating units (i.e., they are polymers).
- MAs of macromolecules are held in their defined forms by non-covalent intermolecular interactions (rather than covalent bonds), and can be in either non-repeating structures, or in repeating linear, circular, spiral, or other patterns (e.g., as in actin filaments and the flagellar motor, image).
- The process by which MAs are formed has been termed molecular self-assembly, a term especially applied in non-biologic contexts.
- A wide variety of physical/biophysical, chemical/biochemical, and computational methods exist for the study of MA; given the scale (molecular dimensions) of MAs, efforts to elaborate their composition and structure and discern mechanisms underlying their functions are at the forefront of modern structure science.
- The study of MA structure and function is challenging, in particular because of their mega dalton size, but also because of their complex compositions and varying dynamic natures.
- Most have had standard chemical and biochemical methods applied (methods of protein purification and centrifugation, chemical and electrochemical characterization, etc.).
- In addition, their methods of study include modern proteomic approaches, computational and atomic-resolution structural methods (e.g., X-ray crystallography), small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS), force spectroscopy, and transmission electron microscopy and cryo-electron microscopy.

Ion channel

Ion channel, protein expressed by virtually all living cells that creates a pathway for charged ions from dissolved salts, including sodium, potassium, calcium, and chloride ions, to pass through the otherwise impermeant lipid cell membrane. Operation of cells in the nervous system, contraction of the heart and of skeletal muscle, and secretion in the pancreas are examples of physiological processes that require ion channels. In addition, ion channels in the membranes

of intracellular organelles are important for regulating cytoplasmic calcium concentration and acidification of specific subcellular compartments (e.g., lysosomes).



The nicotinic acetylcholine receptor is an example of a ligand-gated ion channel. It is composed of five subunits arranged symmetrically around a central conducting pore. Upon binding acetylcholine, the channel opens and allows diffusion of sodium (Na^+) and potassium (K^+) ions through the conducting pore.*Encyclopædia Britannica, Inc.*

Evolution And Selectivity

Ions flow passively through channels toward equilibrium. This movement may be driven by electrical (voltage) or chemical (concentration) gradients. The ability to alter ion flow as a result of the development of ion channels may have provided an evolutionary advantage by allowing single-celled organisms to regulate their volume in the face of environmental changes. Through subsequent evolution, ion channels have come to play essential roles in cellular secretion and electrical signaling.

Most ion channels are gated—that is, they open and close either spontaneously or in response to a specific stimulus, such as the binding of a small molecule to the channel protein (ligand-gated ion channels) or a change in voltage across the membrane that is sensed by charged segments of the channel protein (voltage-gated ion channels). In addition, most ion channels are selective, allowing only certain ions to pass through. Some channels conduct only one type of ion (e.g., potassium), whereas other channels exhibit relative selectivity—for example, allowing positively charged cations to pass through while excluding negatively charged anions. Cells in higher organisms may express more than 100 different types of ion channel, each with different selectivity and different gating properties.

Function And Structure

The flow of charged ions through open channels represents an electrical current that changes the voltage across the membrane by altering the distribution of charge. In excitable cells, voltage-gated channels that allow transient influx of positive ions (e.g., sodium and calcium ions) underlie brief depolarizations of the membrane known as action potentials. Action potentials can be transmitted rapidly over long distances, allowing for coordination and precise timing of physiological outputs. In nearly all cases, action potentials trigger downstream physiological effects, such as secretion or muscle contraction, by opening voltage-gated calcium-selective ion channels and elevating intracellular calcium concentration.

The amino acid sequences of many different ion channel proteins have been determined, and in a few cases the X-ray crystal structure of the channel is known as well. Based on their structure, the majority of ion channels can be classified into six or seven superfamilies. For potassium-selective channels, which are among the best-characterized ion channels, four homologous transmembrane subunits come together to create a tunnel, known as the conducting pore, that provides a polar pathway through the nonpolar lipid membrane. Other channel types require either three or five homologous subunits to generate the central conducting pore. In solution, ions are stabilized by polarized water molecules in the surrounding environment. Narrow, highly selective ion channels mimic the water environment by lining the conducting pore with polarized carbonyl oxygen atoms. Less-selective channels form pores with a diameter large enough that ions and water molecules may pass through together.

Toxins And Disease

Many natural toxins target ion channels. Examples include the voltage-gated sodium channel blocker tetrodotoxin, which is produced by bacteria resident in puffers (blowfish) and several other organisms; the irreversible nicotinicacetylcholine receptor antagonist alpha-bungarotoxin, from the venom of snakes in the genus *Bungarus* (kraits); and plant-derived alkaloids, such as strychnineand D-tubocurarine, which inhibit the activation of ion channels that are opened by the neurotransmitters glycine and acetylcholine, respectively. In addition, a large number of therapeutic drugs, including local anesthetics, benzodiazepines, and sulfonylurea derivatives, act directly or indirectly to modulate ion channel activity.

Inherited mutations in ion channel genes and in genes encoding proteins that regulate ion channel activity have been implicated in a number of diseases, including ataxia (the inability to coordinate voluntary muscle movements), diabetes mellitus, certain types of epilepsy, and cardiac arrhythmias (irregularities in heartbeat). For example, genetic variations in sodium-selective and potassium-selective channels, or in their associated regulatory subunits, underlie some forms of long-QT syndrome. This syndrome is characterized by a prolongation in the depolarization time-course of cardiac myocyte action potentials, which can lead to fatal arrhythmias. In addition, mutations in adenosine triphosphate (ATP)-sensitive potassium channels that control insulin secretion from cells in the pancreas underlie some forms of diabetes mellitus.

Role In Research

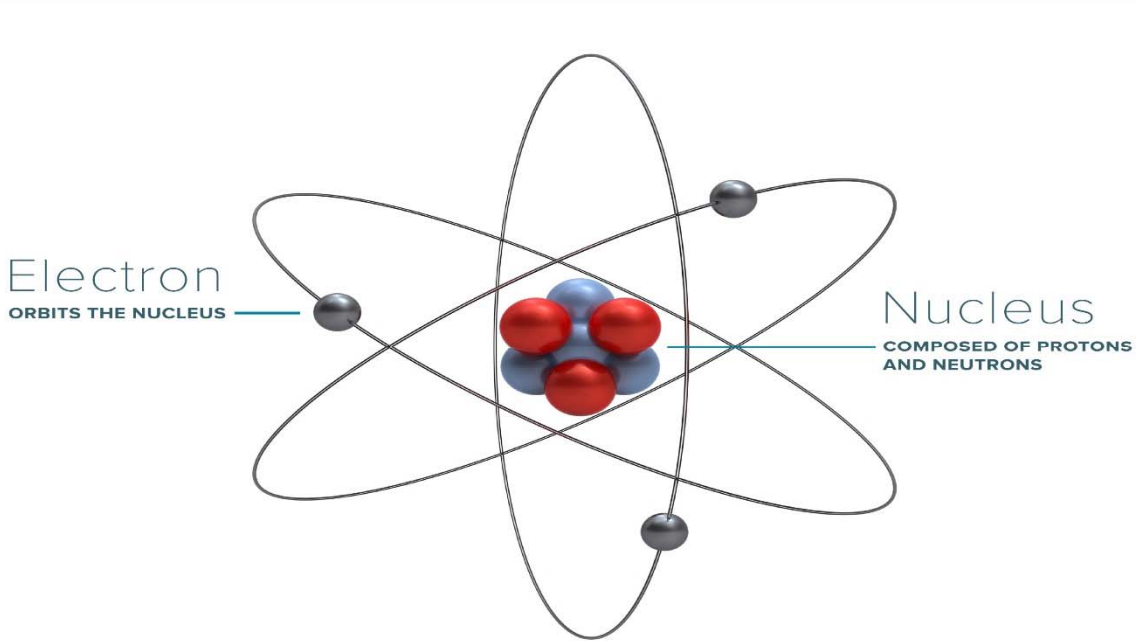
Ongoing basic research on ion channels seeks to understand the structural basis for permeability, ion selectivity, and gating at the molecular level. Research efforts also attempt to answer questions about the cellular regulation of ion channel protein synthesis and about the subcellular distribution and ultimate degradation of channels. In addition, compounds with greater specificity and potency

for channels involved in pain sensation, cardiovascular disease, and other pathological conditions are potential sources for drug development.

WHAT IS RADIATION?

Radiation is the process by which energy is emitted as either particles or waves. Broadly, it can take the form of sound, heat, or light. However, most people generally use it to refer to radiation from electromagnetic waves, ranging from radio waves, though the visible light spectrum, and up through to gamma waves.

ATOMS AND THEIR PARTS



Most of the discussion about radiation, how it works, and what its effects are boil down to the interaction of radiation with atoms (and molecules) that it comes into contact with. Atoms form the basic building blocks of all matter. They consist of a nucleus, made of positively-charged

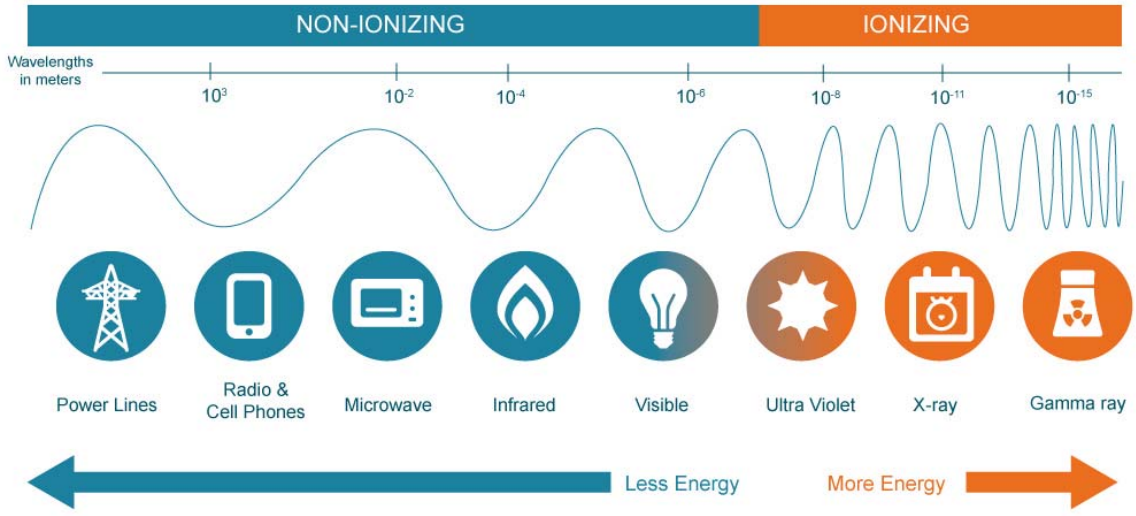
protons (and sometimes neutrally-charged neutrons), and an outer cloud of electrons, which have a negative charge. The positive charge of a single proton is equal to the negative charge of a single electron.

Protons and neutrons have a relatively large size and atomic weight, whereas electrons are extremely small and light by comparison. Due to the nature of opposite charges attracting, atoms tend to have an equal number of protons and electrons, leaving the atom as a whole having a net charge of zero. However, if the atom either loses or gains an electron, it becomes an ion, and carries a charge.

It will seek bonds with other charged particles in order to regain a neutral balance, potentially leading to new molecules being formed.

IONIZING VS NON-IONIZING RADIATION

Radiation is generally classified ionizing or non-ionizing, based on whether it has enough energy to knock electrons off atoms that it interacts with, as well as being able to do lower-energy damage such as breaking chemical bonds in molecules. Ionizing radiation, which is caused by unstable atoms giving off energy to reach a more stable state, is more of a health threat to humans because it involves changing the basic makeup of atoms in cells, and more specifically the DNA molecules inside of cells. It does, of course, take a very strong dose of radiation to substantially damage a cell's structure, as there can be trillions of atoms in a single cell.



The scale of electromagnetic radiation, broken down into categories of ionizing and non-ionizing radiation

Most non-ionizing radiation, such as radio and microwave energy, is considered harmful only to the extent of the amount of heat energy it transfers to whatever it hits. This is, in fact, the way that microwaves cook food. UV light is unique in that while it is non-ionizing, it does have the capacity to cause harmful effects similar to what ionizing radiation can create, such as an increased risk of cancer due to damage to DNA molecules.

HOW IS RADIATION MEASURED?

The radioactivity of a substance, or how "active" it is radioactively, is measured in either curies (Ci) or Becquerel's (Bq). Both are measures of the number of decays per second, or how often an atom in a given sample will undergo radioactive decay and give off a particle or photon of radiation. The curie (1 Ci equals about 37,000,000,000 decays per second) is named after Marie and Pierre Curie, and is equal to roughly the activity of one gram of radium, which they studied. The Becquerel is the SI unit for radioactivity. One Bq equals one decay per second. The Bq is the SI unit, though the curie remains widely used throughout the US in both government and industry.

- RADIATION ACTIVITY IS MEASURED IN AN INTERNATIONAL (SI) UNIT CALLED A BECQUEREL (BQ).
- THE BECQUEREL COUNTS HOW MANY PARTICLES OR PHOTONS (IN THE CASE OF WAVE RADIATION) ARE EMITTED PER SECOND BY A SOURCE.
- THE DEVICE USED FOR MEASUREMENT IS OFTEN THE FAMILIAR GEIGER COUNTER.
- IF YOU PUT A GEIGER COUNTER OVER A GRAM OF SUBSTANCE AND COUNT 3 CLICKS PER SECOND, THE RADIOACTIVITY OF THAT SUBSTANCE WOULD BE 3 BECQUEREL.

UNITS OF RADIOACTIVITY

- Unit of activity is curie (Ci)
- 1 Curie = 3.7×10^{10} disintegration/sec (or Bq)
- SI unit is Becquerel
- 1 Bq = 1 radioactive decay per second
  = 2.703×10^{-11} Ci

Radiation badges

- PHOTOGRAPHIC FILM DARKENS ON EXPOSURE TO RADIATION AND LIGHT.
- LIGHT CANNOT PENETRATE THE BADGE BUT IONIZING RADIATION CAN.
- DARKENING OF THE FILM INDICATES THAT A PERSON HAS BEEN EXPOSED TO TOO MUCH RADIATION.

The Geiger tube

- RADIATION PRODUCES IONS IN A LOW PRESSURE GAS BETWEEN A CENTRAL POSITIVELY CHARGED ELECTRODE AND THE OUTER NEGATIVELY CHARGED TUBE.
- A PULSE OF CURRENT THEN FLOWS THAT IS REGISTERED BY THE COUNTER.
- THE THIN MICA WINDOW ALLOWS THE LEAST PENETRATING RADIATION (ALPHA) TO ENTER THE TUBE.
- GAMMA RADIATION AND MOST BETA CAN ENTER THROUGH THE SIDES OF THE METAL TUBE.

Radiation Exposure

Radiation exposure is expressed in several ways to account for the different levels of harm caused by different forms of radiation and the different sensitivity of body tissues.

RADIATION EXPOSURE IS MEASURED IN AN INTERNATIONAL (SI) UNIT CALLED THE GRAY (GY).

THE RADIATION EXPOSURE IS EQUIVALENT TO THE ENERGY "DEPOSITED" IN A KILOGRAM OF A SUBSTANCE BY THE RADIATION.

EXPOSURE IS ALSO REFERRED TO AS ABSORBED DOSE.

THE IMPORTANT CONCEPT IS THAT EXPOSURE IS MEASURED BY WHAT RADIATION DOES TO SUBSTANCES, NOT ANYTHING PARTICULAR ABOUT THE RADIATION ITSELF.

THIS ALLOWS US TO UNIFY THE MEASUREMENT OF DIFFERENT TYPES OF RADIATION (I.E., PARTICLES AND WAVE) BY MEASURING WHAT THEY DO TO MATERIALS.

    - The gray is a large unit and for normal radiation protection levels a series of prefixes are used:
    - nanogray (nGy) is one thousand millionth of a gray (1/1,000,000,000)
    - microgray (μGy) is one millionth of a gray (1/1,000,000)
    - milligray (mGy) is one thousandth of a gray (1/1,000)
- OFTEN WE ARE INTERESTED IN THE EFFECT OF RADIATION EXPOSURE ON HUMAN TISSUE.
- ENTER A QUANTITY CALLED EQUIVALENT DOSE. THIS RELATES THE ABSORBED DOSE IN HUMAN TISSUE TO THE EFFECTIVE BIOLOGICAL DAMAGE OF THE RADIATION.
- NOT ALL RADIATION HAS THE SAME BIOLOGICAL EFFECT, EVEN FOR THE SAME AMOUNT OF ABSORBED DOSE.

EQUIVALENT DOSE IS MEASURED IN AN INTERNATIONAL (SI) UNIT CALLED THE SIEVERT (SV).

LIKE THE GRAY, THE SIEVERT IS A LARGE UNIT AND FOR NORMAL RADIATION PROTECTION LEVELS A SERIES OF PREFIXES ARE USED:

- NANOSIEVERT (NSV) IS ONE THOUSAND MILLIONTH OF A SIEVERT (1/1,000,000,000)
- MICROSIEVERT (μSV) IS ONE MILLIONTH OF A SIEVERT (1/1,000,000)
- MILLISIEVERT (MSV) IS ONE THOUSANDTH OF A SIEVERT (1/1,000)
- TO DETERMINE EQUIVALENT DOSE (SV), YOU MULTIPLY ABSORBED DOSE (GY) BY A RADIATION WEIGHTING FACTOR THAT IS UNIQUE TO THE TYPE OF RADIATION.
- THE RADIATION WEIGHTING FACTOR (WR) TAKES INTO ACCOUNT THAT SOME KINDS OF RADIATION ARE INHERENTLY MORE DANGEROUS TO BIOLOGICAL TISSUE, EVEN IF THEIR "ENERGY DEPOSITION" LEVELS ARE THE SAME.

- FOR X-RAYS AND GAMMA RAYS AND ELECTRONS ABSORBED BY HUMAN TISSUE, WR IS 1. FOR ALPHA PARTICLES IT IS 20.

    - Radioactive decay (also known as nuclear decay or radioactivity) is the process by which an unstable atomic nucleus loses energy (in terms of mass in its rest frame) by emitting radiation, such as an alpha particle, beta particle with neutrino or only a neutrino in the case of electron capture, gamma ray, or electron in the case of internal conversion.
    - A material containing such unstable nuclei is considered radioactive.
    - Certain highly excited short-lived nuclear states can decay through neutron emission, or more rarely, proton emission.

Radioactivity was discovered in 1896 by the french scientist henri becquerel, while working with phosphorescent materials.

These materials glow in the dark after exposure to light, and he suspected that the glow produced in cathode ray tubes by x-rays might be associated with phosphorescence.

He wrapped a photographic plate in black paper and placed various phosphorescent salts on it. All results were negative until he used uranium salts.

- The uranium salts caused a blackening of the plate in spite of the plate being wrapped in black paper. These radiations were given the name "becquerel rays". An alpha particle is identical to a helium nucleus, being made up of two protons and two neutrons bound together.
- It initially escapes from the nucleus of its parent atom, invariably one of the heaviest elements, by quantum mechanical processes and is repelled further from it by electromagnetism, as both the alpha particle and the nucleus are positively charged.
- The process changes the original atom from which the alpha particle is emitted into a different element.
- Its mass number decreases by four and its atomic number by two. For example, uranium-238 will decay to thorium-234.
- Beta decay itself comes in two kinds: β+ and β-.
- B- emission occurs by the transformation of one of the nucleus's neutrons into a proton, an electron and an antineutrino. Byproducts of fission from nuclear reactors often undergo β- decay as they are likely to have an excess of neutrons.
- B+ decays is a similar process, but involves a proton changing into a neutron, a positron and a neutrino.
- After a nucleus undergoes alpha or beta decay, it is often left in an excited state with excess energy.
- Just as an electron can move to a lower energy state by emitting a photon somewhere in the ultraviolet to infrared range, an atomic nucleus loses energy by emitting a gamma ray.
- Gamma radiation is the most penetrating of the three, and will travel through several centimetres of lead.
- Beta particles will be absorbed by a few millimetres of aluminium, while alpha particles will be stopped in their tracks be a few centimetres of air, or a sheet of paper – although this type of radiation does the most damage to materials it hits.

- Natural radiation
  natural radiation includes radioactivity in the rocks and soil of the earth's crust; radon, a radioactive gas given out by many volcanic rocks and uranium ore; cosmic radiation; and radioactivity in food and drinks.
- Natural radiation accounts for about 80% of the radiation doses to which we are subjected. It may vary from place to place.
-
- Artificial (man-made) radiation
  medical use of radiation is the most significant source of man-made radiation. This includes x-ray radiology, nuclear medicine imaging and radiation therapy.
- Radiation arising from human activities typically accounts for about 20% of public exposure.
- Exposure due to fallout from past testing of nuclear weapons and generation of electricity in nuclear power plants constitutes less than 0.3%.

Dosimeter

A radiation dosimeter is a device that measures exposure to ionizing radiation. It has two main uses: for human radiation protection and for measurement of dose in both medical and industrial processes

Personal dosimeters

The personal ionising radiation dosimeter is of fundamental importance in the disciplines of radiation dosimetry and radiation health physics and is primarily used to estimate the radiation dose deposited in an individual wearing the device.

Ionising radiation damage to the human body is cumulative, and is related to the total dose received, for which the SI unit is the sievert. Workers exposed to radiation, such as radiographers, nuclear power plant workers, doctors using radiotherapy, those in laboratories using radionuclides, and HAZMAT teams are required to wear dosimeters so a record of occupational exposure can be made. Such devices are known as "legal dosimeters" if they have been approved for use in recording personnel dose for regulatory purposes.

Dosimeters can be worn to obtain a whole body dose and there are also specialist types that can be worn on the fingers or clipped to headgear, to measure the localised body irradiation for specific activities.

Types

Common types of personal dosimeters for ionizing radiation include:

The electronic personal dosimeter (EPD) is an electronic device that has a number of sophisticated functions, such as continual monitoring which allows alarm warnings at preset levels and live readout of dose accumulated. These are especially useful in high dose areas where residence time of the wearer is limited due to dose constraints. The dosimeter can be reset, usually after taking a reading for record purposes, and thereby re-used multiple times.

MOSFET dosimete

MOSFET dosimeters [4] are now used as clinical dosimeters for radiotherapy radiation beams. The main advantages of MOSFET devices are:

1. The MOSFET dosimeter is direct reading with a very thin active area (less than 2 μm).

2. The physical size of the MOSFET when packaged is less than 4 mm.

3. The post radiation signal is permanently stored and is dose rate independent.

Film badge dosimeters are for one-time use only. The level of radiation absorption is indicated by a change to the film emulsion, which is shown when the film is developed.

Quartz fiber dosimeters are charged to a high voltage, and are usually used for one work period only. As the gas in the dosimeter chamber becomes ionized by radiation the charge leaks away, causing the fiber indicator to change against a graduated scale.These are now being superseded by more modern types, such as electronic personal dosimeters.

A thermoluminescent dosimeter measures ionizing radiation exposure by measuring the intensity of visible light emitted from a crystal in the detector when heated. The intensity of light emitted is dependent upon the radiation exposure.

Both the quartz fiber and film badge types are being superseded by the TLD and the EPD.

Radiation protection instruments

Practical radiation measurement using calibrated radiation protection instruments is essential in evaluating the effectiveness of protection measures, and in assessing the radiation dose likely to be received by individuals.

The measuring instruments for radiation protection are both:

1. “Installed" (in a fixed position)

2. Portable (hand-held or transportable).

- Installed instruments are fixed in positions which are known to be important in assessing the general radiation hazard in an area.
- Examples are installed "area" radiation monitors, Gamma interlock monitors, personnel exit monitors, and airborne particulate monitors.
- The area radiation monitor will measure the ambient radiation, usually X-Ray, Gamma or neutrons; these are radiations which can have significant radiation levels over a range in excess of tens of metres from their source, and thereby cover a wide area.
- Gamma radiation "interlock monitors" are used in applications to prevent inadvertent exposure of workers to an excess dose by preventing personnel access to an area when a high radiation level is present. These interlock the process access directly.
- Airborne contamination monitors measure the concentration of radioactive particles in the ambient air to guard against radioactive particles being ingested, or deposited in the lungs of personnel.

- These instruments will normally give a local alarm, but are often connected to an integrated safety system so that areas of plant can be evacuated and personnel are prevented from entering an air of high airborne contamination.
- Personnel exit monitors (PEM) are used to monitor workers who are exiting a "contamination controlled" or potentially contaminated area. These can be in the form of hand monitors, clothing frisk probes, or whole body monitors.
- These monitor the surface of the workers body and clothing to check if any radioactive contamination has been deposited. These generally measure alpha or beta or gamma, or combinations of these.
- Portable instruments are hand-held or transportable. The hand-held instrument is generally used as a survey meter to check an object or person in detail, or assess an area where no installed instrumentation exists.
- They can also be used for personnel exit monitoring or personnel contamination checks in the field. These generally measure alpha, beta or gamma, or combinations of these.
- Transportable instruments are generally instruments that would have been permanently installed, but are temporarily placed in an area to provide continuous monitoring where it is likely there will be a hazard.
- Such instruments are often installed on trolleys to allow easy deployment, and are associated with temporary operational situations.

Bio-electromagnetics

- Bio-electromagnetics, also known as bio-electromagnetism, is the study of the interaction between electromagnetic fields and biological entities.
- Areas of study include electrical or electromagnetic fields produced by living cells, tissues or organisms, including bioluminescent bacteria; for example, the cell membrane potential and the electric currents that flow in nerves and muscles, as a result of action potentials.
- Others include animal navigation utilizing the geomagnetic field; potential effects of man-made sources of electromagnetic fields like mobile phones; and developing new therapies to treat various conditions.
- The term can also refer to the ability of living cells, tissues, and organisms to produce electrical fields and the response of cells to electromagnetic fields
- Electromagnetic energy is used extensively for many biomedical applications including:
- Imaging
- Electrical stimulation of the heart, brain, and other tissues
- Hyperthermia
- Sensing

Researchers are currently:

- Studying the microwave aspects of high field magnetic resonance imaging (MRI) and electron paramagnetic resonance (EPR) imaging.
- Developing game-changing:
- (a) wireless and fully-passive implants for continuous and unobtrusive monitoring of brain signals with minimum impact to the individual’s activity, and

- (b) non-invasive and inconspicuous epidermal textile sensors for real-time monitoring of internal organs deep into the body in a carefree manner.
- Short-lived electrical events called action potentials occur in several types of animal cells which are called excitable cells, a category of cell include neurons, muscle cells, and endocrine cells, as well as in some plant cells.
- These action potentials are used to facilitate inter-cellular communication and activate intracellular processes.
- The physiological phenomena of action potentials are possible because voltage-gated ion channels allow the resting potential caused by electrochemical gradient on either side of a cell membrane to resolve.
- Bio-electromagnetism is studied primarily through the techniques of electrophysiology. In the late eighteenth century, the Italian physician and physicist **Luigi Galvani** first recorded the phenomenon while dissecting a frog at a table where he had been conducting experiments with static electricity.
- Galvani coined the term animal electricity to describe the phenomenon, while contemporaries labeled it galvanism.
- Galvani and contemporaries regarded muscle activation as resulting from an electrical fluid or substance in the nerves.
- Some usually aquatic animals, such as sharks, have acute bioelectric sensors providing a sense known as electroreception, while migratory birds navigate in part by orienteering with respect to the Earth's magnetic field.
- In an extreme application of electromagnetism the electric eel is able to generate a large electric field outside its body used for hunting and self-defense through a dedicated electric organ.
- Electroreception or electroception is the biological ability to perceive natural electrical stimuli.
- It has been observed almost exclusively in aquatic or amphibious animals, since salt-water is a much better conductor than air; the currently known exceptions being the monotremes (echidnas and platypuses), cockroaches and bees.
- Electroreception is used in electrolocation (detecting objects) and for electrocommunication.

Electrolocation

- Electroreceptive animals use this sense to locate objects around them.
- This is important in ecological niches where the animal cannot depend on vision: for example in caves, in murky water and at night.
- Many fish use electric fields to detect buried prey.
- Some shark embryos and pups "freeze" when they detect the characteristic electric signal of their predators.
- It has been proposed that sharks can use their acute electric sense to detect the earth's magnetic field by detecting the weak electric currents induced by their swimming or by the flow of ocean currents.
- The walking behavior of cockroaches can be affected by the presence of a static electric field: they like to avoid the electric field.
- Cabbage loopers are also known to avoid electric fields

- In active electrolocation, the animal senses its surrounding environment by generating electric fields and detecting distortions in these fields using electroreceptor organs.
- This electric field is generated by means of a specialized electric organ consisting of modified muscle or nerves.
- This field may be modulated so that its frequency and wave form are unique to the species and sometimes, the individual.
- Animals that use active electroreception include the weakly electric fish, which either generate small electrical pulses ("pulse-type") or produce a quasi-sinusoidal discharge from the electric organ (termed "wave-type").
- These fish create a potential which is usually smaller than one volt.
- Weakly electric fish can discriminate between objects with different resistance and capacitance values, which may help in identifying the object.
- Active electroreception typically has a range of about one body length, though objects with an electrical impedance similar to that of the surrounding water are nearly undetectable.
- In passive electrolocation, the animal senses the weak bioelectric fields generated by other animals and uses it to locate them.
- These electric fields are generated by all animals due to the activity of their nerves and muscles. A second source of electric fields in fish is the ion pumps associated with osmoregulation at the gill membrane. This field is modulated by the opening and closing of the mouth and gill slits.
- Many fish that prey on electrogenic fish use the discharges of their prey to detect them. This has driven the prey to evolve more complex or higher frequency signals that are harder to detect.
- Passive electroreception is carried out solely by ampullary electroreceptors in fish. It is tuned to low frequency signals (below one up to tens of Hertz)
- Fish use passive electroreception to supplement or replace their other senses when detecting prey and predators. In sharks, sensing an electric dipole alone is sufficient to cause them to try to eat it
- Weakly electric fish can also communicate by modulating the electrical waveform they generate, an ability known as electro-communication.
- They may use this for mate attraction and territorial displays. Some species of catfish use their electric discharges only in agonistic displays.
- **Sharks and rays**
- Sharks and rays (members of the subclass Elasmobranchii), such as the lemon shark, rely heavily on electrolocation in the final stages of their attacks, as can be demonstrated by the robust feeding response elicited by electric fields similar to those of their prey.
- Sharks are the most electrically sensitive animals known, responding to DC fields as low as 5 nV/cm.
- The electric eel (actually a knifefish, not an eel), besides its ability to generate high voltage electric shocks, uses lower voltage pulses for navigation and prey detection in its turbid habitat.
- Dolphins have evolved electroreception in structures different from those of fish, amphibians and monotremes.
- Bees collect a positive static charge while flying through the air.
- When a bee visits a flower, the charge deposited on the flower takes a while to leak away into the ground. Bees can detect both the presence and the pattern of electric fields on

flowers, and use this information to know if a flower has been recently visited by another bee and is therefore likely to have a reduced concentration of nectar.

- Animals detect electric fields through insulating air by mechano-reception, not electroreception. Bees sense the electric field changes via the Johnston's organs in their antennae and possibly other mechano-receptors.
- They distinguish different temporal patterns and learn them. During the waggle dance, honeybees appear to use the electric field emanating from the dancing bee for distance communication.

Echolocation in bats

- Microbats use echolocation to navigate and forage, often in total darkness.
- They generally emerge from their roosts in caves, attics, or trees at dusk and hunt for insects into the night.
- Their use of echolocation allows them to occupy a niche where there are often many insects (that come out at night since there are fewer predators then), less competition for food, and fewer species that may prey on the bats themselves.
- Microbats generate ultrasound via the larynx and emit the sound through the open mouth or, much more rarely, the nose.
- About this sound calls range in frequency from 14,000 to well over 100,000 Hz, mostly beyond the range of the human ear (typical human hearing range is considered to be from 20 Hz to 20,000 Hz).
- Bats may estimate the elevation of targets by interpreting the interference patterns caused by the echoes reflecting from the tragus, a flap of skin in the external ear.
- Using echolocation, bats can detect objects as thin as a human hair in complete darkness.
- Echolocation allows bats to find insects the size of mosquitoes, which many bats like to eat.
- Bats aren't blind, but they can use echolocation to find their way around very quickly in total darkness.
- Bats belonging to the suborder Microchiroptera (microbats) occupy a diverse set of ecological conditions – they can be found living in environments as different as Europe and Madagascar, and hunting for food sources as different as insects, frogs, nectar, fruit, and blood.
- Additionally, the characteristics of an echolocation call are adapted to the particular environment, hunting behavior, and food source of the particular bat

Human echolocation

- Human echolocation is the ability of humans to detect objects in their environment by sensing echoes from those objects, by actively creating sounds – for example, by tapping their canes, lightly stomping their foot, snapping their fingers, or making clicking noises with their mouths – people trained to orient by echolocation can interpret the sound waves reflected by nearby objects, accurately identifying their location and size.
- Human Echolocation is the ability of humans to use sound echoes to help determine their immediate and nearby surroundings.

- Using methods such as tapping a cane on the ground, stamping their feet, or making clicking sounds with their mouths they are able to interpret the resulting echoes/reflections to provide them with an internal “map” of their surroundings.
- Similar in principle to sonar used by bats, toothed whales and dolphins, reflecting sound waves returning to the ears provide important clues such as proximity, density, shape and size of nearby objects.
- Vision and hearing are closely related in that they can process reflected waves of energy.
- Vision processes light waves as they travel from their source, bounce off surfaces throughout the environment and enter the eyes.
- Similarly, the auditory system processes sound waves as they travel from their source, bounce off surfaces and enter the ears.
- Both systems can extract a great deal of information about the environment by interpreting the complex patterns of reflected energy that they receive. In the case of sound, these waves of reflected energy are called "echoes".
- Echoes and other sounds can convey spatial information that is comparable in many respects to that conveyed by light.
- With echoes, a blind traveler can perceive very complex, detailed, and specific information from distances far beyond the reach of the longest cane or arm.
- Echoes make information available about the nature and arrangement of objects and environmental features such as overhangs, walls, doorways and recesses, poles, ascending curbs and steps, planter boxes, pedestrians, fire hydrants, parked or moving vehicles, trees and other foliage, and much more.

Molecular Imaging

- Molecular imaging originated from the field of radio-pharmacology due to the need to better understand fundamental molecular pathways inside organisms in a noninvasive manner.
- It enables the visualization of the cellular function and the follow-up of the molecular process in living organisms without perturbing them.
- The multiple and numerous potentialities of this field are applicable to the diagnosis of diseases such as cancer, and neurological and cardiovascular diseases.
- This technique also contributes to improving the treatment of these disorders by optimizing the pre-clinical and clinical tests of new medication.
- Molecular imaging differs from traditional imaging in that probes known as biomarkers are used to help image particular targets or pathways.
- Biomarkers interact chemically with their surroundings and in turn alter the image according to molecular changes occurring within the area of interest.
- This process is markedly different from previous methods of imaging which primarily imaged differences in qualities such as density or water content.
- This ability to image fine molecular changes opens up an incredible number of exciting possibilities for medical application, including early detection and treatment of disease and basic pharmaceutical development.
- Furthermore, molecular imaging allows for quantitative tests, imparting a greater degree of objectivity to the study of these areas

- Molecular imaging, "the visualization, characterization and measurement of biological processes at the molecular and cellular levels in humans and other living systems", can play pivotal roles in disease diagnosis, treatment efficacy assessment, drug discovery, and the understanding of fundamental biology.
- In addition, molecular imaging is also an extremely powerful tool in multiple disciplines of biomedical research (e.g. stem cell research, RNA interference, nanomedicine, gene/cancer therapy, etc.).

There are many different modalities that can be used for noninvasive molecular imaging. Each have their different strengths and weaknesses and some are more adept at imaging multiple targets than others.

1. **Magnetic resonance imaging**
2. **Optical imaging**
3. **Near Infrared imaging**
4. **Single photon emission computed tomography**
5. **Positron emission tomography**
6. **Probes and the imaging of molecular interactions**
7. MRI has the advantages of having very high spatial resolution and is very adept at morphological imaging and functional imaging.
8. MRI does have several disadvantages though. First, MRI has a sensitivity of around 10−3 mol/L to 10−5 mol/L which, compared to other types of imaging, can be very limiting.
9. Improvements to increase MR sensitivity include increasing magnetic field strength, and hyperpolarization via optical pumping, dynamic nuclear polarization or para hydrogen induced polarization.

Bio-mineralization

- Bio-mineralization is the process by which living organisms produce minerals, often to harden or stiffen existing tissues. Such tissues are called mineralized tissues.
- It is an extremely widespread phenomenon; all six taxonomic kingdoms contain members that are able to form minerals, and over 60 different minerals have been identified in organisms.
- Examples include silicates in algae and diatoms, carbonates in invertebrates, and calcium phosphates and carbonates in vertebrates.
- Organisms have been producing mineralized skeletons for the past 550 million years.
- Other examples include copper, iron and gold deposits involving bacteria.
- Biologically-formed minerals often have special uses such as magnetic sensors in magneto tactic bacteria (Fe_3O_4), gravity sensing devices ($CaCO_3$, $CaSO_4$, $BaSO_4$) and iron storage and mobilization ($Fe_2O_3 \cdot H_2O$ in the protein ferritin).

- In terms of taxonomic distribution, the most common bio minerals are the phosphate and carbonate salts of calcium that are used in conjunction with organic polymers such as collagen and chitin to give structural support to bones and shells.
- The structures of these bio composite materials are highly controlled from the nanometer to the macroscopic level, resulting in complex architectures that provide multifunctional properties.
- Because this range of control over mineral growth is desirable for materials engineering applications, there is significant interest in understanding and elucidating the mechanisms of biologically controlled bio mineralization
- Among the most spectacular examples of bio mineralization are the intricately structured cell walls of diatoms, a large group of single-celled eukaryotic algae that are present in almost all water habitats.
- Diatom cell walls are made of amorphous, hydrated SiO_2 (silica) and exhibit highly regular porous patterns which are hierarchically arranged from the nano- to micrometer scale.
- The silica structures are produced by poly-condensation of $Si(OH)_4$ (silicic acid) molecules which occurs in a specific intracellular compartment, termed the silica deposition vesicle (SDV).
- The first evidence of bio mineralization dates to some 750 million years ago, and sponge-grade organisms may have formed calcite skeletons 630 million years ago.
- But in most lineages, bio mineralization first occurred in the Cambrian or Ordovician periods.
- Organisms used whichever form of calcium carbonate was more stable in the water column at the point in time when they became bio mineralized, and stuck with that form for the remainder of their biological history.
- The stability is dependent on the Ca/Mg ratio of seawater, which is thought to be controlled primarily by the rate of sea floor spreading, although atmospheric CO_2 levels may also play a role.

Bioacoustics

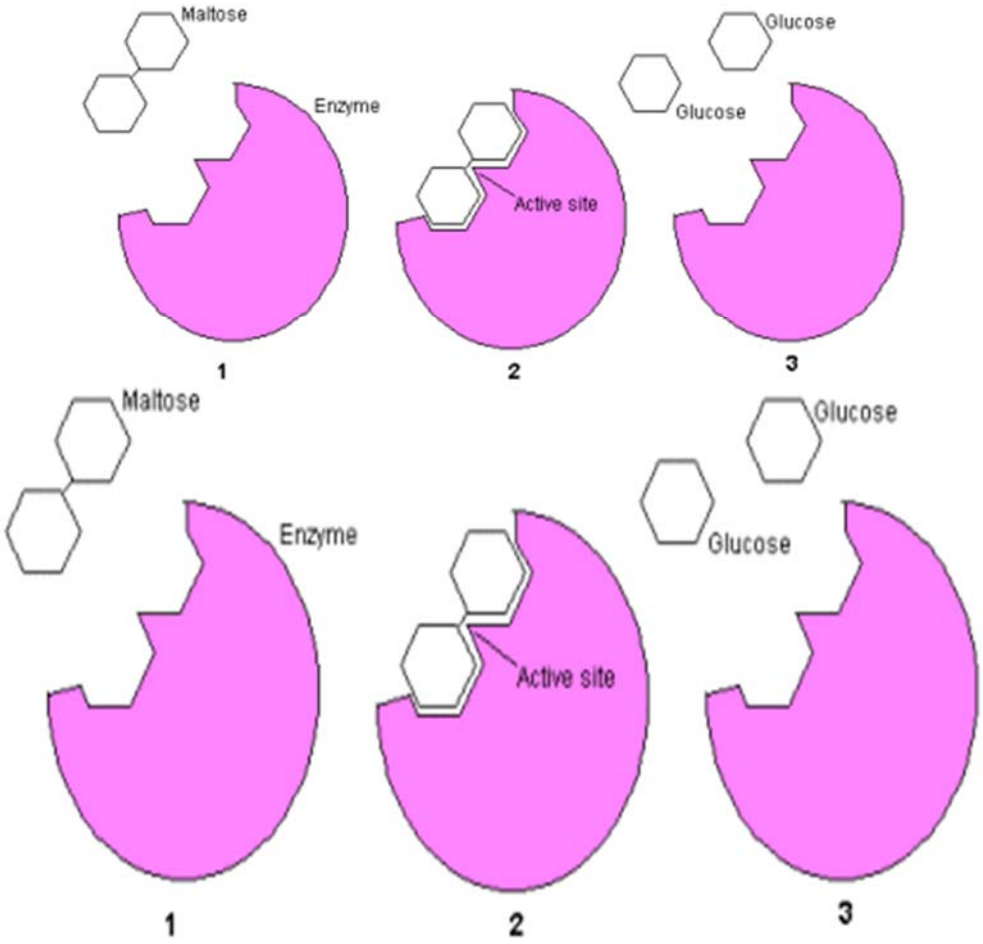
- Bioacoustics is a cross-disciplinary science that combines biology and acoustics. Usually it refers to the investigation of sound production, dispersion and reception in animals (including humans).
- Acoustics is the branch of physics that deals with the study of all mechanical waves in gases, liquids, and solids including topics such as vibration, sound, ultrasound and infrasound.

- A scientist who works in the field of acoustics is an acoustician while someone working in the field of acoustics technology may be called an acoustical engineer.
- The application of acoustics is present in almost all aspects of modern society with the most obvious being the audio and noise control industries.
- This involves neurophysiological and anatomical basis of sound production and detection, and relation of acoustic signals to the medium they disperse through.
- The findings provide clues about the evolution of acoustic mechanisms, and from that, the evolution of animals that employ them.
- Bioacoustic techniques have recently been proposed as a non-destructive method for estimating biodiversity of an area
- In underwater acoustics and fisheries acoustics the term is also used to mean the effect of plants and animals on sound propagated underwater, usually in reference to the use of sonar technology for biomass estimation.
- The study of substrate-borne vibrations used by animals is considered by some a distinct field called biotremology.
- Biotremology is the study of production, dispersion and reception of mechanical vibrations by animals, and their effect on behaviour
- Bioacoustics as a scientific discipline was established by the Ivan Regen who began systematically to study insect sounds. In 1925 he used a special stridulatory device to play in a duet with an insect.
- Stridulation is the act of producing sound by rubbing together certain body parts. This behavior is mostly associated with insects, but other animals are known to do this as well, such as a number of species of fish, snakes and spiders.
- Later, he put a male cricket behind a microphone and female crickets in front of a loudspeaker. The females were not moving towards the male but towards the loudspeaker.
- Regen's most important contribution to the field apart from realization that insects also detect airborne sounds was the discovery of tympanal organ's function

Enzymes

Enzymes are made from **amino acids**, and they are proteins. When an enzyme is formed, it is made by stringing together between 100 and 1,000 amino acids in a very specific and unique order. The chain of amino acids then folds into a unique shape. That shape allows the enzyme to carry out specific chemical reactions -- an enzyme acts as a very efficient catalyst for a specific chemical reaction. The enzyme speeds that reaction up tremendously.

For example, the sugar maltose is made from two glucose molecules bonded together. The enzyme **maltase**is shaped in such a way that it can break the bond and free the two glucose pieces. The only thing maltase can do is break maltose molecules, but it can do that very rapidly and efficiently. Other types of enzymes can put atoms and molecules together. Breaking molecules apart and putting molecules together is what enzymes do, and there is a specific enzyme for each chemical reaction needed to make the cell work properly.



Maltose is made of two glucose molecules bonded together (1). The maltase enzyme is a protein that is perfectly shaped to accept a maltose molecule and break the bond (2). The two glucose molecules are released (3). A single maltase enzyme can break in excess of 1,000 maltose bonds per second, and will only accept maltose molecules.

You can see in the diagram above the basic action of an enzyme. A maltose molecule floats near and is captured at a specific site on the maltase enzyme. The **active site** on the enzyme breaks the bond, and then the two glucose molecules float away.

You may have heard of people who are **lactose intolerant**, or you may suffer from this problem yourself. The problem arises because the sugar in milk -- lactose -- does not get broken into its glucose components. Therefore, it cannot be digested. The intestinal cells of lactose-intolerant people do not produce **lactase**, the enzyme needed to break down lactose. This problem shows how the lack of just one enzyme in the human body can lead to problems. A person who is lactose intolerant can swallow a drop of lactase prior to drinking milk and the problem is solved. Many enzyme deficiencies are not nearly so easy to fix.

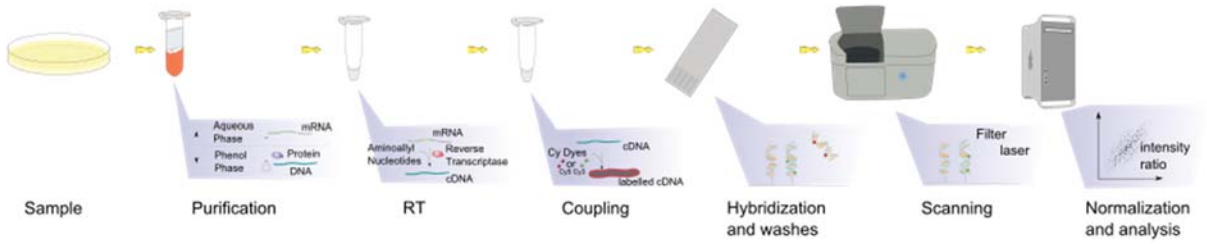
Inside a bacterium there are about 1,000 types of enzymes (lactase being one of them). All of the enzymes float freely in the cytoplasm waiting for the chemical they recognize to float by. There are hundreds or millions of copies of each different type of enzyme, depending on how important a reaction is to a cell and how often the reaction is needed. These enzymes do everything from breaking glucose down for energy to building cell walls, constructing new enzymes and allowing the cell to reproduce. Enzymes do all of the work inside cells.

DNA microarray

A DNA microarray (also commonly known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. Each DNA spot contains picomoles (10−12 moles) of a specific DNA sequence, known as probes (or reporters or oligos). These can be a short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA (also called anti-sense RNA) sample (called target) under high-stringency conditions. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. The original nucleic acid arrays were macro arrays approximately 9 cm × 12 cm and the first computerized image based analysis was published in 1981.

The core principle behind microarrays is hybridization between two DNA strands, the property of complementarynucleic acid sequences to specifically pair with each other by forming hydrogen bonds between complementary nucleotide base pairs. A high number of complementary base pairs in a nucleotide sequence means tighter non-covalent bonding between the two strands. After washing off non-specific bonding sequences, only strongly paired strands will remain hybridized. Fluorescently labeled target sequences that bind to a probe sequence generate a signal that depends on the hybridization conditions (such as temperature), and washing after hybridization. Total strength of the signal, from a spot (feature), depends upon the amount of target sample binding to the probes present on that spot. Microarrays use relative quantitation in which the intensity of a

feature is compared to the intensity of the same feature under a different condition, and the identity of the feature is known by its position.



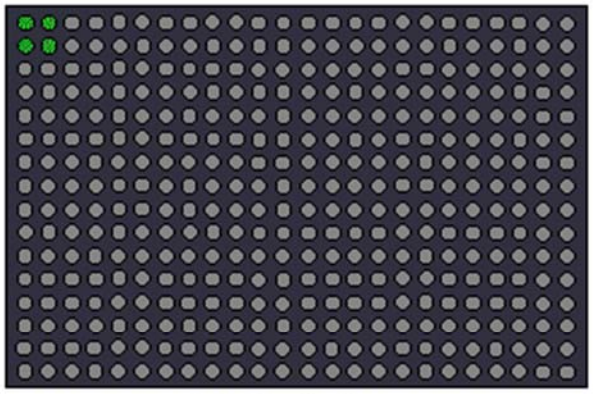
Microarray Technique

An array is an orderly arrangement of samples where matching of known and unknown DNA samples is done based on base pairing rules. An array experiment makes use of common assay systems such as microplates or standard blotting membranes. The sample spot sizes are typically less than 200 microns in diameter usually contain thousands of spots.

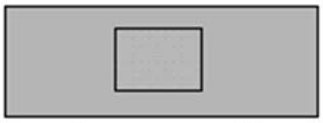
Thousands of spotted samples known as probes (with known identity) are immobilized on a solid support (a microscope glass slides or silicon chips or nylon membrane). The spots can be DNA, cDNA, or oligonucleotides. These are used to determine complementary binding of the unknown sequences thus allowing parallel analysis for gene expression and gene discovery. An experiment with a single DNA chip can provide information on thousands of genes simultaneously. An orderly arrangement of the probes on the support is important as the location of each spot on the array is used for the identification of a gene.

Types of Microarrays

Depending upon the kind of immobilized sample used construct arrays and the information fetched, the Microarray experiments can be categorized in three ways:



384 Well Plate



Microarry Chip

1. **Microarray Expression Analysis:** In this experimental setup, the cDNA derived from the mRNA of known genes is immobilized. The sample has genes from both the normal as well as the diseased tissues. Spots with more intensity are obtained for diseased tissue gene if the gene is over expressed in the diseased condition. This expression pattern is then compared to the expression pattern of a gene responsible for a disease.

2. **Microarray for Mutation Analysis:** For this analysis, the researchers use gDNA. The genes might differ from each other by as less as a single nucleotide base.

A single base difference between two sequences is known as Single Nucleotide Polymorphism (SNP) and detecting them is known as SNP detection.

3. **Comparative Genomic Hybridization:** It is used for the identification in the increase or decrease of the important chromosomal fragments harboring genes involved in a disease.

Applications of Microarrays

Gene Discovery: DNA Microarray technology helps in the identification of new genes, know about their functioning and expression levels under different conditions.

Disease Diagnosis: DNA Microarray technology helps researchers learn more about different diseases such as heart diseases, mental illness, infectious disease and especially the study of cancer. Until recently, different types of cancer have been classified on the basis of the organs in which the tumors develop. Now, with the evolution of microarray technology, it will be possible for the researchers to further classify the types of cancer on the basis of the patterns of gene activity in the tumor cells. This will tremendously help the pharmaceutical community to develop more effective drugs as the treatment strategies will be targeted directly to the specific type of cancer.

Drug Discovery: Microarray technology has extensive application in *Pharmacogenomics.*Pharmacogenomics is the study of correlations between therapeutic responses to drugs and the genetic profiles of the patients. Comparative

analysis of the genes from a diseased and a normal cell will help the identification of the biochemical constitution of the proteins synthesized by the diseased genes. The researchers can use this information to synthesize drugs which combat with these proteins and reduce their effect.

Toxicological Research: Microarray technology provides a robust platform for the research of the impact of toxins on the cells and their passing on to the progeny. Toxicogenomics establishes correlation between responses to toxicants and the changes in the genetic profiles of the cells exposed to such toxicants.