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SCHOOL OF SCIENCE AND HUMANITIES

DEPARTMENT OF PHYSICS

UNIT – I - BIOPHYSICS – SPHA7410

UNIT I

The reductionist program in biology - the use of physics and chemistry in biology - introduction to modern molecular biology - elementary description of the cell - DNA, proteins and the molecules of Life - the central dogma of information transfer within a cell - introduction to Darwinian evolution and prebiotic evolution.

MODERN BIOLOGY

The reductionist program in biology

The reductionist method of dissecting biological systems into their constituent parts has been effective in explaining the chemical basis of numerous living processes. However, many biologists now realize that this approach has reached its limit. Biological systems are extremely complex and have emergent properties that cannot be explained, or even predicted, by studying their individual parts. The reductionist approach—although successful in the early days of molecular biology—underestimates this complexity and therefore has an increasingly detrimental influence on many areas of biomedical research, including drug discovery and vaccine development.

The claim made by Francis Crick (1966) that "The ultimate aim of the modern movement in biology is to explain all biology in terms of physics and chemistry" epitomizes the reductionist mindset that has pervaded molecular biology for half a century. The theory is that because biological systems are composed solely of atoms and molecules, without the influence of 'alien' or 'spiritual' forces, it should be possible to explain them using the physicochemical properties of their individual components, down to the atomic level. The most extreme manifestation of the reductionist view is the belief that is held by some neuroscientists that consciousness and mental states can be reduced to chemical reactions that occur in the brain (Bickle, 2003; Van Regenmortel, 2004).

Reductionists analyse a larger system by breaking it down into pieces and determining the connections between the parts. They assume that the isolated molecules and their structure have sufficient explanatory power to provide an understanding of the whole system. As the value of methodo-logical reductionism has been particularly evident in molecular biology, it might seem odd that, in recent years, biologists have become increasingly critical of the idea that biological systems can be fully explained using physics and chemistry. Their situation is similar to that of an art student asking about the significance of Michelangelo's *David* and being told that it is just a piece of marble hewn into a statue in 1504. This is certainly true, but it evades pertinent questions about the anatomy of the statue, its creation at the beginning of the Florentine Renaissance, its significance in European art history, or even the scars on its left arm that were plastered after it was broken in three places during the anti-Medici revolt of 1527. In an analogous way, the biology, development, physiology, behaviour or fate of a human being cannot be adequately explained along reductionist lines that consider only chemical composition. Anti-reductionists therefore regard biology as an autonomous discipline

that requires its own vocabulary and concepts that are not found in chemistry and physics. Both sides have discussed their standpoints at several recent international meetings (Bock & Goode, 1998; Van Regenmortel & Hull, 2002; Van Regenmortel, 2004) and the main disagreement between the protagonists is about what constitutes a good scientific explanation.

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Today, it is clear that the specificity of a complex biological activity does not arise from the specificity of the individual molecules that are involved, as these components frequently function in many different processes.

Biological specificity results from the way in which these components assemble and function together (Morange, 2001a). Interactions between the parts, as well as influences from the environment, give rise to new features, such as network behaviour (Alm & Arkin, 2003), which are absent in the isolated components.
Consequently, 'emergence' has appeared as a new concept that complements 'reduction' when reduction fails (Van Regenmortel, 2004). Emergent properties resist any attempt at being predicted or deduced by explicit calculation or any other means. In this regard, emergent properties differ from resultant properties, which can be predicted from lower-level information. For instance, the resultant mass of a multi-component protein assembly is simply equal to the sum of the masses of each individual component. However, the way in which we taste the saltiness of sodium chloride is not reducible to the properties of sodium and chlorine gas. An important aspect of emergent properties is that they have their own causal powers, which are not reducible to the powers of their constituents. For instance, the experience of pain can alter human behaviour, but the lower-level chemical reactions in the neurons that are involved in the perception of pain are not the cause of the altered behaviour, as the pain itself has causal efficacy. According to the principles of emergence, the natural world is divided into hierarchies that have evolved over evolutionary time (Kim, 1999; Morowitz, 2002). Reductionists advocate the idea of 'upward causation' by which molecular states bring about higher-level phenomena, whereas proponents of emergence accept 'downward causation' by which higher-level systems influence lower-level configurations (Kim, 1999).

Anti-reductionists therefore regard biology as an autonomous discipline that requires its own vocabulary and concepts that are not found in chemistry and physics

Reference:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1299179/

Physics in Biology

The aim of most biomedical research is to uncover new knowledge that will lead to better health. At the National Institutes of Health (NIH) in the US we do this by supporting research

on the prevention, detection, diagnosis and treatment of disease and disability, from the rarest genetic disorder to the common cold, as well as research on the basic principles of biology.

Physicists, heredity and the rise of molecular biology

Exactly 50 years ago, in a speech entitled "A physicist looks at biology", Max Delbruck, a leading physicist who had made a conversion to biology some years earlier, attempted to describe the transition. In the speech, delivered to the 1000th meeting of the Connecticut Academy of Arts and Sciences, Delbruck said: "A mature physicist, acquainting himself for the first time with the problems of biology, is puzzled by the circumstance that there are no 'absolute phenomena'….The animal or plant or micro-organism he is working with is but a link in an evolutionary chain of changing forms, none of which has any permanent validity. Even the molecular species and the chemical reactions which he encounters are the fashions of today to be replaced by others as evolution goes on. The organism he is working with is not a particular expression of an ideal organism, but one thread in the infinite web of all living forms, all interrelated and all interdependent. The physicist has been reared in a different atmosphere. The materials and phenomena he works with are the same here and now as they were at all times and as they are on the most distant star."

Delbruck had been a student of Niels Bohr and then a powerful proselytizer for biology. With the assistance of Bohr's book *Light and Life* and, more importantly, Schrödinger's book *What is Life?*, he attracted many other physicists to biology. The effects of his missionary zeal were powerful – not just because some very smart people started to do biology, but because they brought to biological problems a quantitative, analytic approach – an approach that created the atmosphere in which principles of molecular biology were discovered by seeking the physical basis of heredity.

The leading physicist Leo Szilard was among the converts, and claimed that what physicists brought to biology was "not any skills acquired in physics, but rather an attitude: the conviction which few biologists had at that time, that mysteries can be solved" (see Fleming in further reading).

Delbruck and his friends were gripped by some fundamental questions: what is the physical form in which hereditary information is stored? How is it reproduced when a cell divides, or when a single virus particle invades a cell and makes hundreds or thousands of copies of itself? How is the information reassorted during sexual reproduction? How does the information change when mutations occur?

Answers to many of these questions came from the "phage school" that Delbruck founded. The phage school was a group of former physicists and some biologists who shared his passion for reducing the problem of heredity to simple rules, physical entities and conserved energy by studying the replication and genetic behaviour of bacterial viruses (also called bacteriophage or "phage") in their bacterial hosts. The studies culminated in findings that form the pillars of modern molecular biology: the identification of deoxyribonucleic acid (DNA) as genetic material, a description of the physical organization of DNA through X-ray crystallography, the deduction of the principles of base pairing and the strategy of replication from the organization of the double helix, and the deciphering of the genetic code as triplets chosen from a set of four nucleotides.

Delbruck and his phage school were important, but there were, in fact, multiple intellectual lineages connected with physics that helped to create the modern world of molecular biology

(see Keller in further reading). For instance, Warren Weaver was a mathematical physicist turned science administrator who, in 1932, first used the term "molecular biology". He chose this phrase because he foresaw "that the moment would arrive when the distinction between chemistry and physics and even mathematics on the one hand and biology on the other would be so illusory and in fact so unfortunate" that he did not want to use the word "biology" to describe the programmes he was supporting at the Rockefeller Foundation.

British scientists with a strong physical bent, such as Astbury, Bragg and others, used X-ray diffraction to study the organization of fibres of many kinds, mainly proteins found in textiles, in an intellectual lineage that led to Wilkins and Franklin and, of course, DNA. The American geneticists T H Morgan and H J Muller used physical agents – namely X-rays – to induce mutations in fruit flies. Muller's affinity for the principles of physics was especially strong. He was fond of noting the potential similarities of mutation of genes to transmutation of elements, calling the prospect of understanding these events in physical terms "the two keystones of our rainbow bridges to power" (see Carlson in further reading)

Bringing physics, not just physicists, to biology

To the birth of modern molecular genetics, physicists contributed their analytic skills but they were not really doing physics, and many were not even using the computational or imaging tools of physics as many biologists do. Delbruck and his colleague Salvador Luria laboriously counted virus infections by hand and eye, just like any other biologist. But contemporary biology, especially the deciphering of genomes by nucleotide sequencing, is about to change that. Biology is rapidly becoming a science that demands more intense mathematical and physical analysis than biologists have been accustomed to, and such analysis will be required to understand the workings of cells.

This change was clearly foreshadowed in Delbruck's 1949 lecture in Connecticut. He first described his awe at the complexity of biology: "The closer one looks at [the] performances of matter in living organisms the more impressive the show becomes. The meanest living cell becomes a magic puzzle box full of elaborate and changing molecules, and far outstrips all chemical laboratories of man in the skill of organic synthesis…."

But Delbruck also sounded a warning: "Biology is a very interesting field…[because of] the vastness of its structure and the extraordinary variety of strange facts…but to the physicist it is also a depressing subject, because…the analysis seems to have stalled around in a semi-descriptive manner without noticeably progressing towards a radical physical explanation…we are not yet at the point where we are presented with clear paradoxes and this will not happen until the analysis of the behaviour of living cells has been carried into far greater detail."

In the past 50 years, and especially in the past 20, molecular and cell biologists have moved much closer to the "radical physical explanation" of cell behaviour that Delbruck sought. Certainly the chemical elements – especially the genes, the ribonucleic acid (RNA), and the proteins – and some of their basic functions are coming into view. What is lacking is a sense of how these functions are integrated to allow cells to manifest their physiological traits.

Reference:

https://physicsworld.com/a/the-impact-of-physics-on-biology-and-medicine/

Chemistry and Biology

The intrinsic relationship between chemistry and biology transcends the formal division of scientific disciplines as we know them today. Why then has the interface between these fields remained an interface and not emerged as a distinct discipline? If we consider Biology to be the study of life and life processes, and Chemistry as the study of molecular behavior, the importance of chemistry in biological processes is not always immediately evident. For example, the study of synthetic chemistry was considered orthogonal to Biology until the chemical synthesis of urea proved that a component of "life" could be made in the laboratory. Since this time, the challenge of synthesizing molecules of nature has become so complex that the synthesis of one molecule is often the subject of many doctoral dissertations. This complexity has resulted in the development of Natural Product Synthesis as a distinct scientific discipline. Likewise, techniques for the isolation and biological evaluation of natural products has become a fundamental subdiscipline in Biology, often referred to as Chemical Ecology.

The difference between these fields does not end with experimental technique, however, and these differences can perhaps best be exemplified by the differences in each scientist's approach to a scientific problem. To me it seems that biologically trained scientists often ask the question "why or how did nature evolve to its present state?" while chemically trained scientists ask "what physical principle can we use to describe our observation?" This is not to say that the biologist does not deal with basic physical principles nor that the chemist does not believe in evolution. But biological systems do evolve and chemical systems do follow the laws of physical principles, and these questions are a fundamental product of classical training in each respective field. Therefore, it is appropriate that each scientist asks the first question that is likely to provide a rationale for observed behavior.

In nature, however, Chemistry and Biology are not distinct, and historically there have been many instances where the two fields have had an extensive amount of interplay. Biochemistry is perhaps the most recognized field at the interface of Chemistry and Biology, but other fields have emerged out of the combination of these two disciplines, namely Biological Chemistry, Chemical Ecology, Bio-organic Chemistry and most recently Chemical Biology. Biochemistry departments are prolific and can be found in medical schools, as basic science departments, and even as divisions within Chemistry departments. Biological Chemistry departments are most often found in medical schools and appear to include almost any area of biological research that involves a molecular component, be that protein, DNA, RNA or small molecule. Bio-organic Chemistry is most often found in Chemistry departments and emerged as an interface field at a time when it was believed that enzyme mimics—molecules that could potentially function as catalysts—could be designed to replace natural enzymes. In the precloning era, enzymes were often difficult to isolate and mutagenesis was not a plausible option. The development of PCR and molecular cloning techniques, however, has limited the need for enzyme mimics, since protein evolution techniques have made the development of proteins with a specific or altered function more easily than the chemical synthesis of the enzyme mimics themselves. This field still thrives, however, and has evolved into the study of molecular recognition and supramolecular chemistry.

Chemical Biology is the youngest of the fields that has emerged from the Chemistry and Biology interface and includes research that involves the synthetic manipulation of chemical species, in order to study biological systems. The emphasis of this definition is on both "chemical synthesis" and "biological systems," since each represent a very distinct and mature science in their own right. The beginnings can be traced to the initial investigations of small-molecule mediated DNA cleavage (Hertzberg and Dervan, 1982). This was followed by other breakthrough studies including the use of antibodies, which rely on the transition-state analog model of enzyme catalysis to catalyze chemical reactions (Lerner et al., 1991), the incorporation of unnatural amino acids into recombinant proteins (Noren et al., 1989; van Hest and Tirrell, 1998) and the elucidation of signal transduction using natural product probes (Rosen and Schreiber, 1992). More recent advances in Chemical Biology include the synthesis of molecules to explore cell recognition (Gordon et al., 1998), chemical modifications of cellular surfaces (Mahal et al., 1997), the functional cloning of natural product receptors (Sche et al., 1999), the modification of ATP to explore kinase specificity (Bishop et al., 1998), the synthesis of novel hormone-binding compounds to explore nuclear hormone receptor specificity and function (Chiellini et al., 1998), synthetic modifications of nucleotides to study RNA structure and catalysis (Strobel, 1999), chemical inducers of signaling events (Crabtree and Schreiber, 1996), and the development of sequence specific DNA-binding small molecules (Dervan and Burli, 1999). These are only a few examples that are meant to be representative, not inclusive, of this emerging field. A common element in these studies, however, is the systematic manipulation of small molecules in order to address fundamental biological questions. Another interesting feature of these studies is that they are largely the product of a single research laboratory, which implies the emergence of a new type of scientist, capable of incorporating nontrivial techniques from both disciplines. This unique bridging of fields will ultimately lead to a new understanding and a novel approach to experimental design that might not otherwise be achieved through collaboration.

For a young scientist enamored with the emerging field of Chemical Biology, the book *Proteins, Enzymes, Genes: The Interplay of Chemistry and Biology* by Joseph S. Fruton is an eye-opening and learning experience. Fruton presents an incredible compilation of the history of the study of biological processes and the related fields from which that study has drawn. The book begins with an initial chapter that describes, and in part rationalizes, the need for an historical account of science by a nonhistorian. The book goes on to describe the history of this science as no historian without a science background ever could. This is entirely understandable since an historian is trained in the art of history, specifically to catalogue and present history within the context of the past. Since past discoveries have always impacted our current scientific thought, as Fruton proves, a scientific historical account is best accomplished by a scientist. Of course, once this is achieved, the scientist becomes the historian.

Although daunting in size, this text is surprisingly easy to peruse and does not require reading in any particular order. The index is large and descriptive and allows the reader to jump from topic to topic without penalty. The sections are clearly marked and the references provide a source of clarification and further reading. Having said this, it must be pointed out that the book reads very well and can be understood by the lay person. If you want proof of this, just read the section on Gibbs free energy (p. 249). The organization of the book is by area and the material is presented essentially in chronological order within each section. The major sections include: the impact of institutional settings, the philosophy of Chemistry and Biology, fermentation as an enzyme source, the function of proteins, chemical energy in biology, biosynthesis, heredity and signal transduction. Each area is discussed with an emphasis on the scientists, their experiments, what they taught us, and how their research was interpreted at the time of discovery. This provides an invaluable insight into how we came to learn what we now consider common knowledge.

Perhaps it is best that the interface of Chemistry and Biology continuously redefines itself and spawns new subdisciplines. Both fields change as new techniques are developed and new breakthroughs discovered. The future of this interface, whether or not it defines itself as a distinct discipline, will likely reflect techniques available from each field. The sequence of the human genome and the function of its gene products is perhaps one of the most important and awe-inspiring projects of modern science, and the Chemistry and Biology interface is likely to play an important role in its elucidation. While we look forward to the future interplay of Chemistry and Biology, Fruton has provided us with a valuable glimpse of its past.

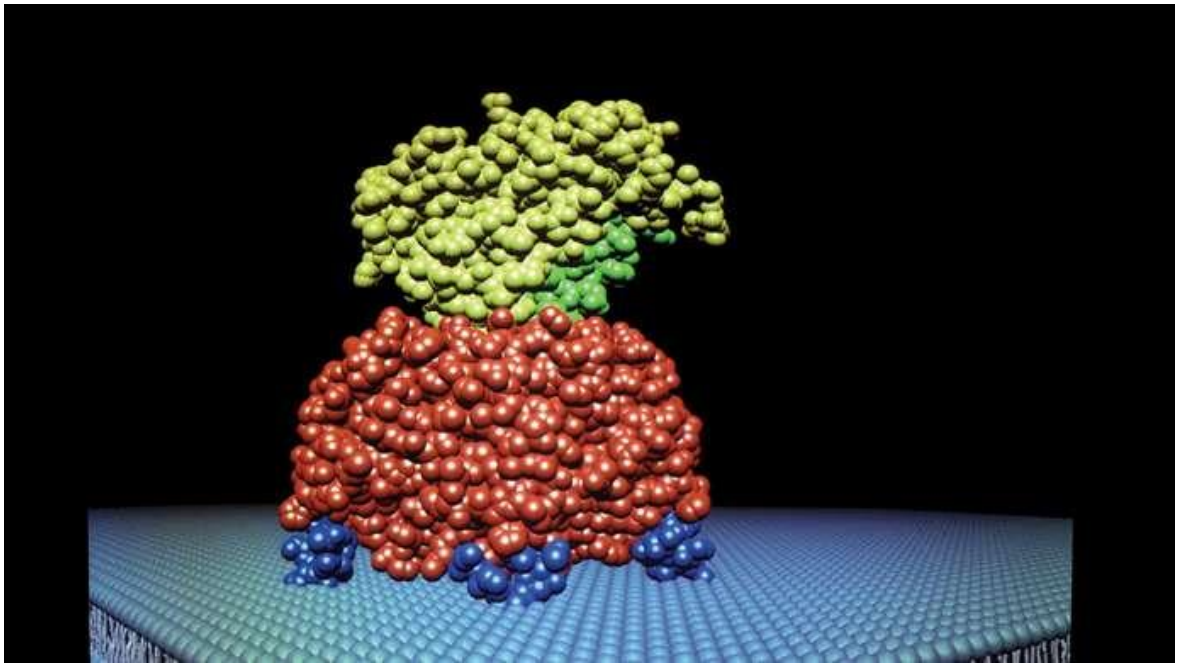
References:

https://www.cell.com/cell/pdf/S0092-8674(00)80808-9.pdf

https://www.infoplease.com/math-science/chemistry/the-chemistry-of-biology-introduction

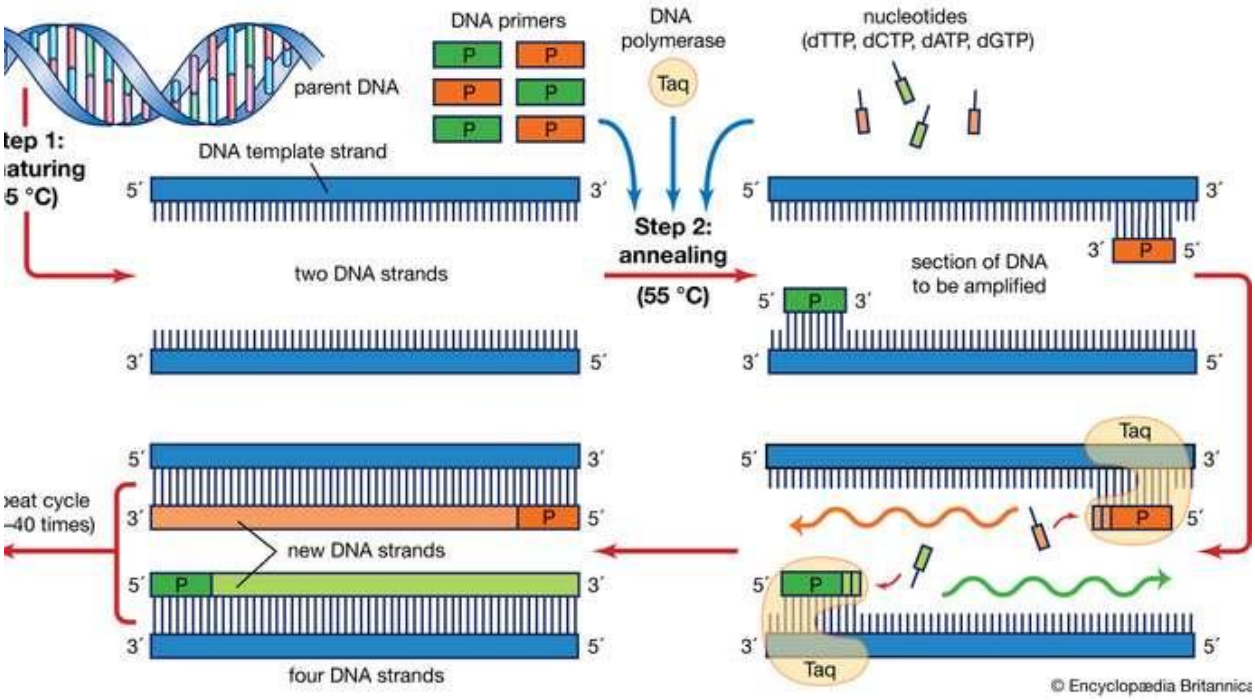
Modern Molecular Biology

Molecular biology, field of science concerned with studying the chemical structures and processes of biological phenomena that involve the basic units of life, molecules. The field of molecular biology is focused especially on nucleic acids (e.g., DNA and RNA) and proteins—macromolecules that are essential to life processes—and how these molecules interact and behave within cells. Molecular biology emerged in the 1930s, having developed out of the related fields of biochemistry, genetics, and biophysics; today it remains closely associated with those fields.



X-ray protein crystallography in molecular biology
The structure of the cholera enterotoxin, shown in a false-colour image obtained by X-ray protein crystallography. *Argonne National Laboratory*

Various techniques have been developed for molecular biology, though researchers in the field may also employ methods and techniques native to genetics and other closely associated fields. In particular, molecular biology seeks to understand the three-dimensional structure of biological macromolecules through techniques such as X-ray diffraction and electron microscopy. The discipline particularly seeks to understand the molecular basis of genetic processes; molecular biologists map the location of genes on specific chromosomes, associate these genes with particular characters of an organism, and use genetic engineering (recombinant DNA technology) to isolate, sequence, and modify specific genes. These approaches can also include techniques such as polymerase chain reaction, western blotting, and microarray analysis.



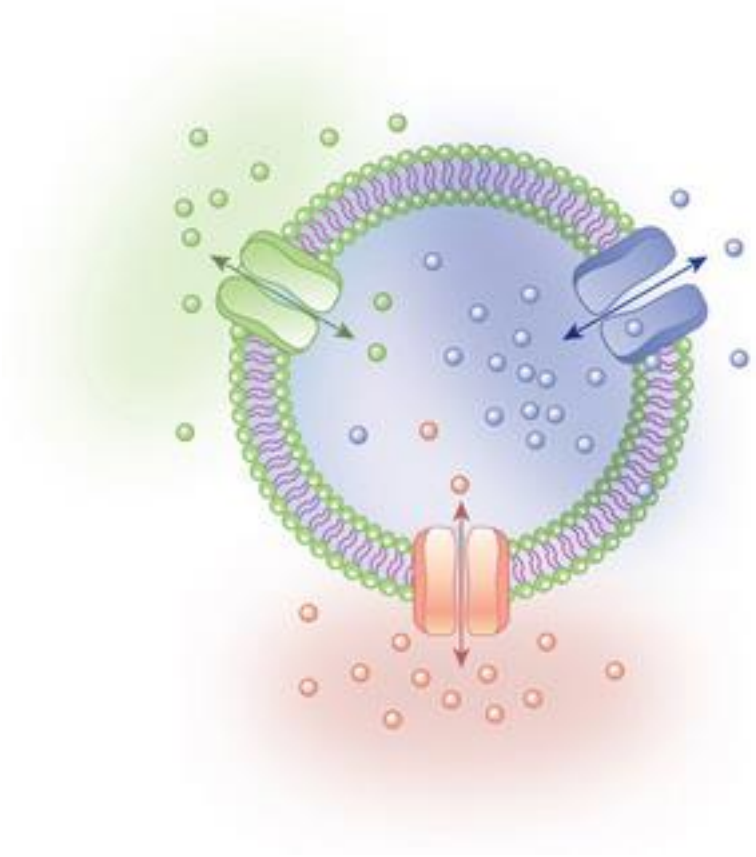
polymerase chain reaction
The three-step process of the polymerase chain reaction.
Encyclopædia Britannica, Inc.

In its early period during the 1940s, the field of molecular biology was concerned with elucidating the basic three-dimensional structure of proteins. Growing knowledge of the structure of proteins in the early 1950s enabled the structure of deoxyribonucleic acid (DNA)—the genetic blueprint found in all living things—to be described in 1953. Further research enabled scientists to gain an increasingly detailed knowledge not only of DNA and ribonucleic acid (RNA) but also of the chemical sequences within these substances that instruct the cells and viruses to make proteins.

Molecular biology remained a pure science with few practical applications until the 1970s, when certain types of enzymes were discovered that could cut and recombine segments of DNA in the chromosomes of certain bacteria. The resulting recombinant DNA technology became one of the most active branches of molecular biology because it allows the manipulation of the genetic sequences that determine the basic characters of organisms.

Reference:
https://www.britannica.com/science/molecular-biology

Description of the Cell



Transport proteins in the cell membrane
A plasma membrane is permeable to specific molecules that a cell needs. Transport proteins in the cell membrane allow for selective passage of specific molecules from the external environment. Each transport protein is specific to a certian molecule (indicated by matching colors).

Cells are considered the basic units of life in part because they come in discrete and easily recognizable packages. That's because all cells are surrounded by a structure called the cell membrane — which, much like the walls of a house, serves as a clear boundary between the cell's internal and external environments. The cell membrane is sometimes also referred to as the plasma membrane.

Cell membranes are based on a framework of fat-based molecules called phospholipids, which physically prevent water-loving, or hydrophilic, substances from entering or escaping the cell. These membranes are also studded with proteins that serve various functions. Some of these proteins act as gatekeepers, determining what substances can and cannot cross the membrane. Others function as markers, identifying the cell as part of the same organism or as foreign. Still others work like fasteners, binding cells together so they can function as a unit. Yet other membrane proteins serve as communicators, sending and receiving signals from neighboring cells and the environment — whether friendly or alarming.

Within this membrane, a cell's interior environment is water based. Called cytoplasm, this liquid environment is packed full of cellular machinery and structural elements. In fact, the concentrations of proteins inside a cell far outnumber those on the outside — whether the outside is ocean water (as in the case of a single-celled alga) or blood serum (as in the case of

a red blood cell). Although cell membranes form natural barriers in watery environments, a cell must nonetheless expend quite a bit of energy to maintain the high concentrations of intracellular constituents necessary for its survival. Indeed, cells may use as much as 30 percent of their energy just to maintain the composition of their cytoplasm.

What Other Components Do Cells Have?

As previously mentioned, a cell's cytoplasm is home to numerous functional and structural elements. These elements exist in the form of molecules and organelles — picture them as the tools, appliances, and inner rooms of the cell. Major classes of intracellular organic molecules include nucleic acids, proteins, carbohydrates, and lipids, all of which are essential to the cell's functions.

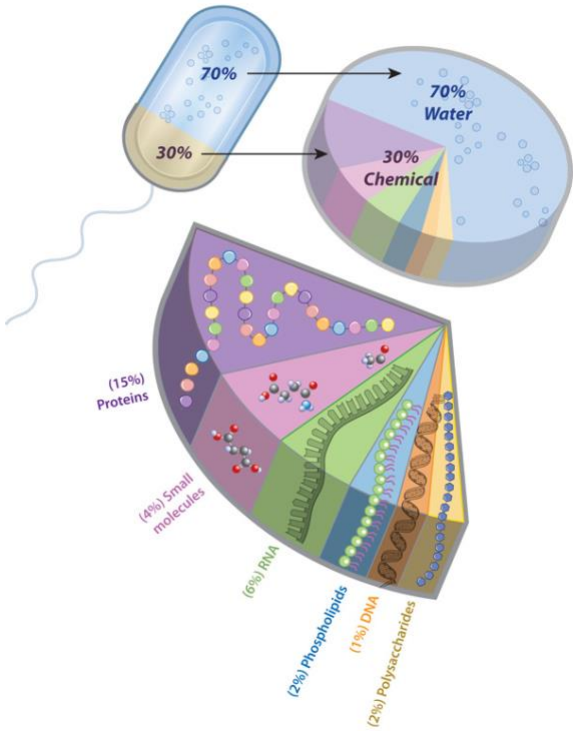
Nucleic acids are the molecules that contain and help express a cell's genetic code. There are two major classes of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the molecule that contains all of the information required to build and maintain the cell; RNA has several roles associated with expression of the information stored in DNA. Of course, nucleic acids alone aren't responsible for the preservation and expression of genetic material: Cells also use proteins to help replicate the genome and accomplish the profound structural changes that underlie cell division.

Proteins are a second type of intracellular organic molecule. These substances are made from chains of smaller molecules called amino acids, and they serve a variety of functions in the cell, both catalytic and structural. For example, proteins called enzymes convert cellular molecules (whether proteins, carbohydrates, lipids, or nucleic acids) into other forms that might help a cell meet its energy needs, build support structures, or pump out wastes.

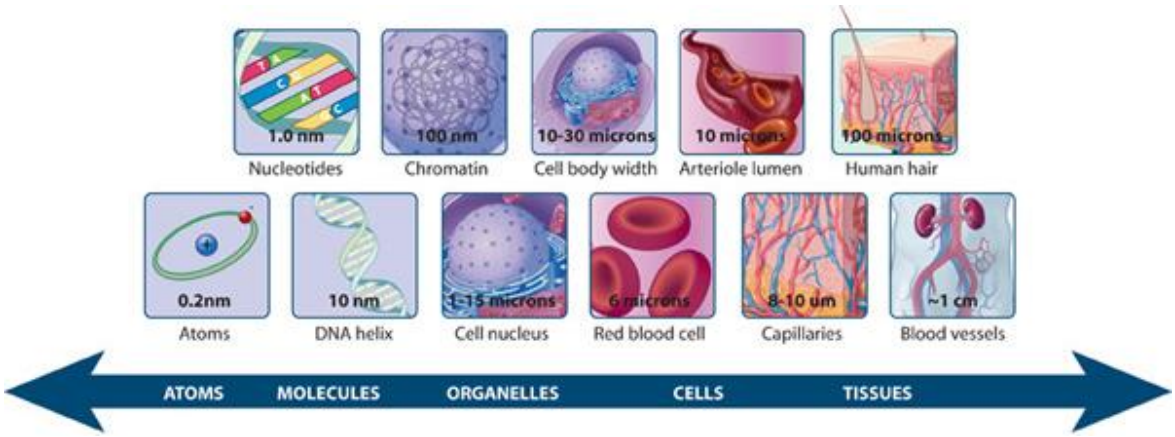
Carbohydrates, the starches and sugars in cells, are another important type of organic molecule. Simple carbohydrates are used for the cell's immediate energy demands, whereas complex carbohydrates serve as intracellular energy stores. Complex carbohydrates are also found on a cell's surface, where they play a crucial role in cell recognition.

Finally, lipids or fat molecules are components of cell membranes — both the plasma membrane and various intracellular membranes. They are also involved in energy storage, as well as relaying signals within cells and from the bloodstream to a cell's interior.

Some cells also feature orderly arrangements of molecules called organelles. Similar to the rooms in a house, these structures are partitioned off from the rest of a cell's interior by their own intracellular membrane. Organelles contain highly technical equipment required for specific jobs within the cell. One example is the mitochondrion — commonly known as the cell's "power plant" — which is the organelle that holds and maintains the machinery involved in energy-producing chemical reactions.



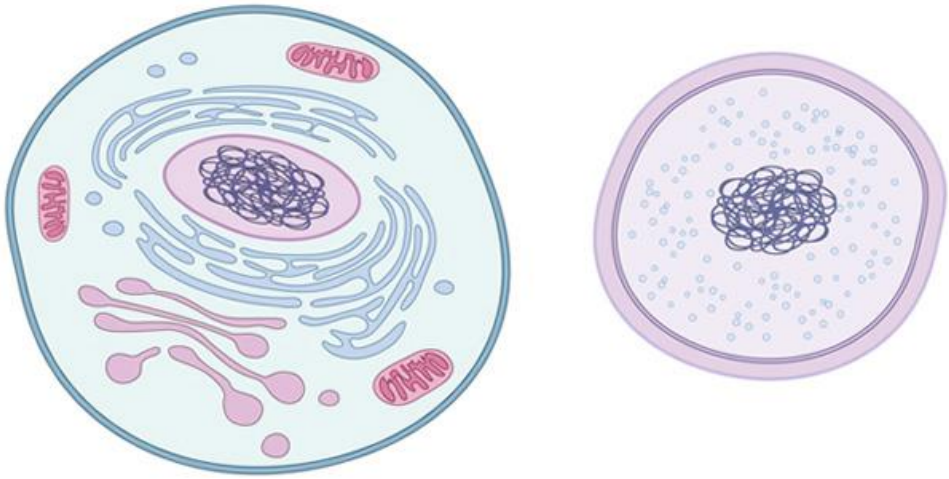
The composition of a bacterial cell
Most of a cell is water (70%). The remaining 30% contains varying proportions of structural and functional molecules.



The relative scale of biological molecules and structures
Cells can vary between 1 micrometer (μm) and hundreds of micrometers in diameter. Within a cell, a DNA double helix is approximately 10 nanometers (nm) wide, whereas the cellular organelle called a nucleus that encloses this DNA can be approximately 1000 times bigger (about 10 μm). See how cells compare along a relative scale axis with other molecules, tissues, and biological structures (blue arrow at bottom). Note that a micrometer (μm) is also known as a micron.

What Are the Different Categories of Cells?

Rather than grouping cells by their size or shape, scientists typically categorize them by how their genetic material is packaged. If the DNA within a cell is not separated from the cytoplasm, then that cell is a prokaryote. All known prokaryotes, such as bacteria and archaea, are single cells. In contrast, if the DNA is partitioned off in its own membrane-bound room called the nucleus, then that cell is a eukaryote. Some eukaryotes, like amoebae, are free-living, single-celled entities. Other eukaryotic cells are part of multicellular organisms. For instance, all plants and animals are made of eukaryotic cells — sometimes even trillions of them.



Comparing basic eukaryotic and prokaryotic differences

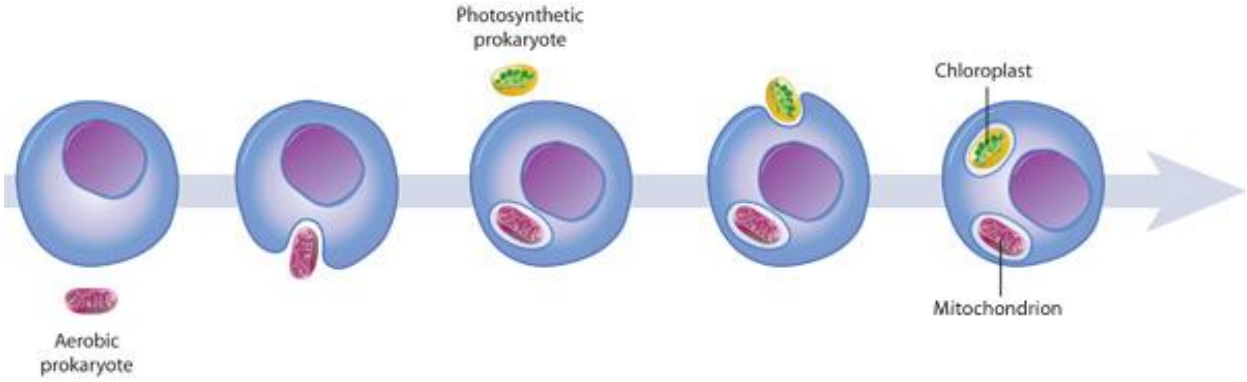
A eukaryotic cell (left) has membrane-enclosed DNA, which forms a structure called the nucleus (located at center of the eukaryotic cell; note the purple DNA enclosed in the pink nucleus). A typical eukaryotic cell also has additional membrane-bound organelles of varying shapes and sizes. In contrast, a prokaryotic cell (right) does not have membrane-bound DNA and also lacks other membrane-bound organelles as well.

How Did Cells Originate?

Researchers hypothesize that all organisms on Earth today originated from a single cell that existed some 3.5 to 3.8 billion years ago. This original cell was likely little more than a sac of small organic molecules and RNA-like material that had both informational and catalytic functions. Over time, the more stable DNA molecule evolved to take over the information storage function, whereas proteins, with a greater variety of structures than nucleic acids, took over the catalytic functions.

As described in the previous section, the absence or presence of a nucleus — and indeed, of all membrane-bound organelles — is important enough to be a defining feature by which cells are categorized as either prokaryotes or eukaryotes. Scientists believe that the appearance of self-contained nuclei and other organelles represents a major advance in the evolution of cells. But where did these structures come from? More than one billion years ago, some cells "ate" by engulfing objects that floated in the liquid environment in which they existed. Then, according to some theories of cellular evolution, one of the early eukaryotic cells engulfed a prokaryote,

and together the two cells formed a symbiotic relationship. In particular, the engulfed cell began to function as an organelle within the larger eukaryotic cell that consumed it. Both chloroplasts and mitochondria, which exist in modern eukaryotic cells and still retain their own genomes, are thought to have arisen in this manner.



Of course, prokaryotic cells have continued to evolve as well. Different species of bacteria and archaea have adapted to specific environments, and these prokaryotes not only survive but thrive without having their genetic material in its own compartment. For example, certain bacterial species that live in thermal vents along the ocean floor can withstand higher temperatures than any other organisms on Earth.

Conclusion

Cells are the smallest common denominator of life. Some cells are organisms unto themselves; others are part of multicellular organisms. All cells are made from the same major classes of organic molecules: nucleic acids, proteins, carbohydrates, and lipids. In addition, cells can be placed in two major categories as a result of ancient evolutionary events: prokaryotes, with their cytoplasmic genomes, and eukaryotes, with their nuclear-encased genomes and other membrane-bound organelles. Though they are small, cells have evolved into a vast variety of shapes and sizes. Together they form tissues that themselves form organs, and eventually entire organisms.

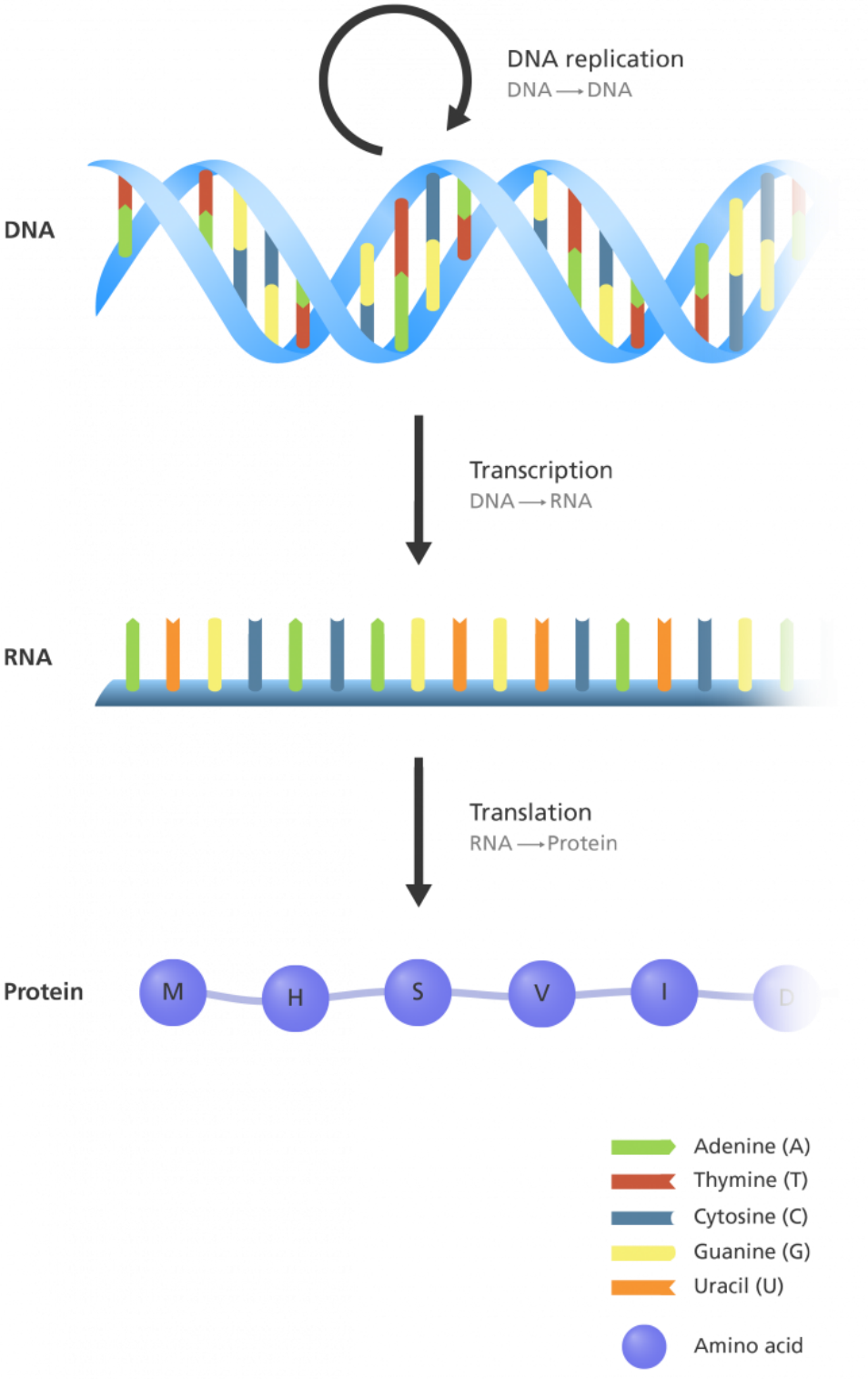
https://www.nature.com/scitable/topicpage/what-is-a-cell-14023083/

The Central Dogma

The 'Central Dogma' is the process by which the instructions in DNA are converted into a functional product. It was first proposed in 1958 by Francis Crick, discoverer of the structure of DNA.

- The central dogma of molecular biology explains the flow of genetic information, from DNA to RNA, to make a functional product, a protein.
- The central dogma suggests that DNA contains the information needed to make all of our proteins, and that RNA is a messenger that carries this information to the ribosomes.
- The ribosomes serve as factories in the cell where the information is 'translated' from a code into the functional product.
- The process by which the DNA instructions are converted into the functional product is called gene expression.

- Gene expression has two key stages - transcription and translation.
- In transcription, the information in the DNA of every cell is converted into small, portable RNA messages.
- During translation, these messages travel from where the DNA is in the cell nucleus to the ribosomes where they are ‘read’ to make specific proteins.
- The central dogma states that the pattern of information that occurs most frequently in our cells is:
    - From existing DNA to make new DNA (DNA replication)
    - From DNA to make new RNA (transcription)
    - From RNA to make new proteins (translation).



An illustration showing the flow of information between DNA, RNA and protein.
Image credit: Genome Research Limited

- Reverse transcription is the transfer of information from RNA to make new DNA, this occurs in the case of retroviruses, such as HIV. It is the process by which the genetic information from RNA is assembled into new DNA.

Does the 'Central Dogma' always apply?

- With modern research it is becoming clear that some aspects of the central dogma are not entirely accurate.
- Current research is focusing on investigating the function of non-coding RNA.
- Although this does not follow the central dogma it still has a functional role in the cell.

Darwinian Evolution

In biology, evolution is the change in the characteristics of a species over several generations and relies on the process of natural selection.

- The theory of evolution is based on the idea that all species[?] are related and gradually change over time.
- Evolution relies on there being genetic variation[?] in a population which affects the physical characteristics (phenotype) of an organism.
- Some of these characteristics may give the individual an advantage over other individuals which they can then pass on to their offspring.

What is natural selection?

- Charles Darwin's theory of evolution states that evolution happens by natural selection.
- Individuals in a species show variation in physical characteristics. This variation is because of differences in their genes[?].
- Individuals with characteristics best suited to their environment are more likely to survive, finding food, avoiding predators and resisting disease. These individuals are more likely to reproduce and pass their genes on to their children.
- Individuals that are poorly adapted to their environment are less likely to survive and reproduce. Therefore their genes are less likely to be passed on to the next generation.
- As a consequence those individuals most suited to their environment survive and, given enough time, the species will gradually evolve.

Natural selection in action: the Peppered moth

- Before the industrial revolution in the mid-1700s, the peppered moth was most commonly a pale whitish colour with black spots.
- This colouring enabled them to hide from potential predators on trees with pale-coloured bark, such as birch trees.
- The rarer dark-coloured peppered moths were easily seen against the pale bark of trees and therefore more easily seen by predators.

A pale peppered moth on an oak tree.
Image credit: Shutterstock

- As the Industrial Revolution reached its peak, the air in industrial areas became full of soot. This stained trees and buildings black.
- As a result, the lighter moths became much easier to spot than the darker ones, making them vulnerable to being eaten by birds.
- The darker moths were now camouflaged against the soot-stained trees and therefore less likely to be eaten.
- Over time this change in the environment led to the darker moths becoming more common and the pale moths rarer.

What have genes got to do with it?

- The mechanisms of evolution operate at the genomic level. Changes in DNA? sequences affect the composition and expression? of our genes, the basic units of inheritance?.
- To understand how different species have evolved we have to look at the DNA sequences in their genomes.
- Our evolutionary history is written into our genome. The human genome looks the way it does because of all the genetic changes that affected our ancestors.
- When DNA and genes in different species look very similar, this is usually taken as evidence of them sharing ancestors.
- For example, humans and the fruit fly, *Drosophila melanogaster*, share much of their DNA. 75 per cent of genes that cause diseases in humans are also found in the fruit fly.
- DNA accumulates changes over time. Some of these changes can be beneficial, and provide a selective advantage for an organism.

- Other changes may be harmful if they affect an important, everyday function. As a result some genes do not change much. They are said to be conserved.

Different types of evolution

Convergent evolution

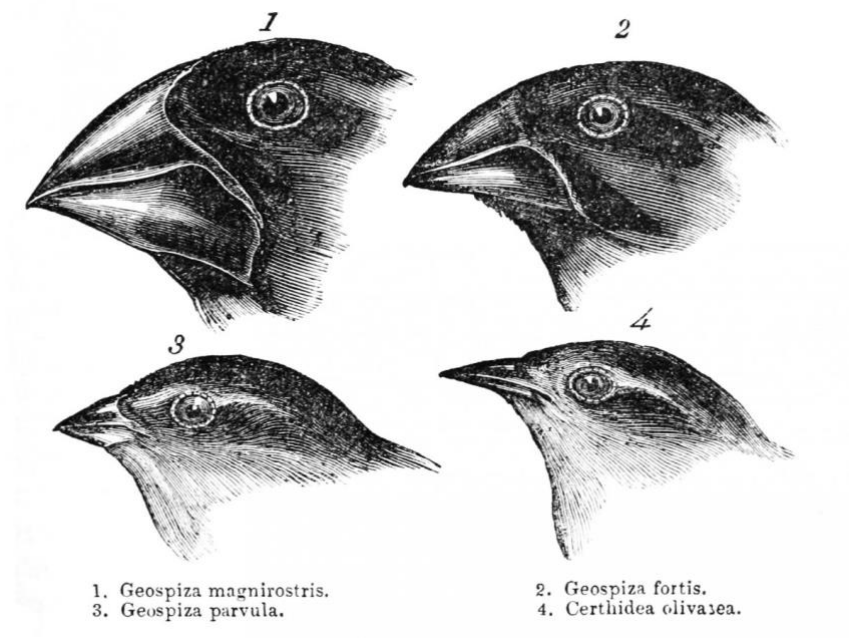
- When the same adaptations evolve independently, under similar selection pressures.
- For example, flying insects, birds and bats have all evolved the ability to fly, but independently of each other.

Co-evolution

- When two species or groups of species have evolved alongside each other where one adapts to changes in the other.
- For example, flowering plants and pollinating insects such as bees.

Adaptive radiation

- When a species splits into a number of new forms when a change in the environment makes new resources available or creates new environmental challenges.
- For example, finches on the Galapagos Islands have developed different shaped beaks to take advantage of the different kinds of food available on different islands.



Sketches of the heads of finches from the Galapagos Islands showing the differences in their beak shapes due to evolution.

References:
https://www.yourgenome.org/facts/what-is-evolution

Prebiotic Evolution

The origin of life on Earth comprised a long series of steps: from the synthesis of small molecules within the primordial atmosphere or near hydrothermal vents, through the formation of biomonomers and biopolymers, culminating in the emergence of a self-replicating, autonomous organism. This philosophical outlook, if not the intimate details, began with the Russian biochemist Alexander Oparin and the British biologist J. B. S. Haldane, who, in the 1920s independently proposed a sequential model for the origin of life (1). Although the process of Darwinian selection may have modulated the populations of genetic macromolecules once the stage of an RNA (or "pre-RNA") world developed, the term "prebiotic evolution" is used here to describe the presumed earlier era of synthesis and degradation that preceded self-replication. A common theme is that the ingredients for life were generated by the flow of energy (sunlight, lightning, or thermal radiation) through the primordial hydrosphere so that the putative mechanisms for the origin of life should be compatible with the conditions that would have prevailed in the early atmosphere and oceans.

The time frame for the emergence of life must be constrained by the physical and biological history of the Earth, but firm dates are difficult to establish. The age of our planet is approximately 4.5 billion years, and life cannot be more ancient unless it came from an extraterrestrial environment. The latter possibility should not be dismissed and may gain credence if the existence of past (or current) life on other planets is confirmed. Regardless of the source, organisms would not have survived until the Earth cooled and meteoritic bombardment subsided sufficiently such that the oceans remained in a liquid state. The

conditions necessary for sustainable life may not have persisted until about four billion years ago. However, evidence of extant organisms appears in fossilized stromatolites in Western Australia from 3.5 billion years ago, and possibly in apatite inclusions from rocks in Greenland dated at almost 3.9 billion years, suggesting that the appearance of life occurred quite rapidly on a geologic time scale once the conditions were favorable.

A historic demonstration of the feasibility of prebiotic simulations was performed by Stanley Miller during the fall of 1952 in the laboratory of Harold Urey at the University of Chicago. Based on Urey's cold-accretion theory for the origin of the planets, Miller subjected a gaseous mixture of methane, ammonia, hydrogen, and water to an electrical discharge, analogous to the effect of lightning in the atmosphere of the young Earth. Chromatographic analysis revealed the presence of three biological amino acids (glycine, alanine, and aspartic acid) along with other products. Further work by Miller and by many other research groups extended the suite of presumed prebiotic amino acids to include glutamic acid, leucine, isoleucine, serine, and threonine. All of the chiral amino acids were obtained as a racemic mixture of left- and right-handed forms, as expected from the achiral starting materials.

The apparent success of these early experiments, which depended on the accessibility and sensitivity of assays specific for amino acids, heightened interest in the nascent discipline of origins-of-life studies. However, prescient objections to the reducing atmosphere of the Miller-Urey simulations were raised first by Philip Abelson, a geochemist at the Carnegie Institution, who argued that the hydrogen-rich gases would have been rapidly replaced by a secondary atmosphere in which carbon was present as carbon dioxide or carbon monoxide, while nitrogen was probably present as molecular dinitrogen. By the early 1980s, a growing body of computational and experimental evidence slowly led to a revised view of the dominant atmosphere during the era before life began. Unfortunately, as shown by Miller and others, these non-reducing gas mixtures (CO2/N2/H2O or CO/N2/H2O) give dramatically lower yields and less variety in amino acids produced by electric discharge (6). If gas-phase syntheses of the Miller-Urey design were important in the origin of life, they must either have proceeded during a brief period when the Earth was very rich in hydrogen, or there may have been another unidentified source of reducing equivalents that maintained a source of hydrogen over a longer stretch of geologic time.

Alternatively, the formation of amino acids and other organic molecules may have been favored near submarine hydrothermal vents, where reducing equivalents would have been present in the extruded gases; the technical challenges inherent in high pressures and temperatures have necessarily restricted the number of such simulations, but compounds as complex as pyruvic acid have been detected using formic acid as the carbon source. Nevertheless, the existence of both thermodynamic and kinetic barriers to the reduction of CO2 (the preferred starting point for any prebiotic synthesis) raises many questions about the availability of the organic precursors in hydrothermal models.

A very different source of the vital ingredients may exist beyond the Earth, in the form of comets, interplanetary dust, and asteroidal debris. These materials are believed to contain carbon compounds, some of which can survive passage through the atmosphere when such objects approach our planet. An intriguing possibility is that their cargo could already be enriched in the left-handed form of amino acids that are required to make modern proteins. While much remains to be understood about the chemical processing of these extraterrestrial bodies, there is great interest in their possible contribution to the primordial soup.

Reference:
http://what-when-how.com/molecular-biology/prebiotic-evolution-molecular-biology/

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DEPARTMENT OF PHYSICS

UNIT – II - BIOPHYSICS – SPHA7410

UNIT II

SEPARATION TECHNIQUES AND PHYSICO-CHEMICALTECHNIQUES - Chromatography - column chromatography - thin layer chromatography - Ion exchange, molecular exclusion and partition chromatography - electrophoresis - gel electrophoresis - SDS - PAGE - hydration of molecules - role of friction - diffusion - sedimentation - ultracentrifuge - viscosity - rotational diffusion - light scattering.

Chromatography is an analytical technique that separates components in a mixture. Chromatographic columns are part of the instrumentation that is used in chromatography. Five chromatographic methods that use columns are gas chromatography (GC), liquid chromatography (LC), Ion exchange chromatography (IEC), size exclusion chromatography (SEC), and chiral chromatography. The basic principles of chromatography can be applied to all five methods.

Gas Chromatographic Columns

In gas chromatography the mobile phase is a gas. Gas chromatographic columns are usually between 1 and 100 meters long. *Gas liquid chromatography(GLC):* The liquid stationary phase is bonded or adsorbed onto the surface of an open tubular (capillary) column, or onto a packed solid support inside the column. Matching the polarities of the analyte and stationary phase is not an exact science. The two should have similar polarities. The thickness of the stationary phase ranges between 0.1 and 8 µm. The thicker the layer the more volatile the analyte can be.

High Performance Liquid Chromatographic Columns

High performance liquid chromatography (HPLC) is a type of liquid chromatography that uses a liquid moblie phase. The same basic principals from gas chromatography are applied to liquid chromatography. There are three basic types of liquid chromatographic columns: liquid-liquid, liquid-solid, and ion-exchange. Liquid-liquid chromatographic columns have the liquid stationary phase bonded or absorbed to the surface of the column, or packed material. liquid-liquid chromatographic columns are not as popular because they have limited stability and they are inconvenient. Partitioning occurs between the two different liquids of the mobile and stationary phases. In liquid-solid chromatographic columns the stationary phase is a solid and the analyte absorbs onto the stationary phase which separates the components of the mixture. In ion-exchange chromatographic columns the stationary phase is an ion-exchange resin and partitioning occurs with ion exchanges that occur between the analyte and stationary phase.
Usually HPLC has a guard column ahead of the analytical column to protect and extend the life of the analytical column. The guard column removes particulate matter, contaminants, and molecules that bind irreversibly to the column. The guard column has a stationary phase similar to the analytical column.

The most common HPLC columns are made from stainless steel, but they can be also made out of thick glass, polymers such as polyetherethelketone, a combination of stainless steel and glass, or a combination of stainless steel and polymers. Typical HPLC analytical columns are between 3 and 25 cm long and have a diameter of 1 to 5 mm. The columns are usually straight unlike GC columns. Particles that pack the columns have a typical diameter between 3 to 5 µm. Liquid chromatographic columns will increase in efficiency when the diameter of the packed particles inside the column decreases.

Packing Material

HPLC columns are usually packed with pellicular, or porous particles. Pellicular particles are made from polymer, or glass beads. Pellicular particles are surrounded by a thin uniform layer of silica, polystyrene-divinyl-benzene synthetic resin, alumina, or other type of ion-exchange resin. The diameter of the pellicular beads is between 30 and 40 µm. Porous particles are more commonly used and have diameters between 3 to 10 µm. Porous particles are made up silica, polystyrene-divinyl-benzene synthetic resin, alumina, or other type of ion-exchange resin. Silica is the most common type of porous particle packing material.

Partition HPLC uses liquid bonded phase columns, where the liquid stationary phase is chemically bonded to the packing material. The packing material is usually hydrolyzed silica which reacts with the bond-phase coating. Common bond phase coatings are siloxanes. The relative structure of the siloxane is shown in Figure 11.

Table 11: This table shows the R groups that can be attached to the siloxane and what chromatographic method it is commonly applied to.

R group attached to siloxane	Chromatography method application
Alkyl	Reverse phase
Fluoroalkyl	Reverse phase
Cyano	Normal and reverse phase
Amide	Reverse phase
Amino	Normal and reverse phase
dimethylamine	Weak anion exchanger
Quaternary Amine	Strong anion exchanger
Sulfonic Acid	Strong cation exchanger
Carboxylic Acid	Weak cation exchanger
Diol	reverse phase
Phenyl	Reverse phase
Carbamate	Reverse Phase

Reverse and Normal Phase HPLC

A polar stationary phase and a non-polar mobile phase are used for normal phase HPLC. In normal phase, the most common R groups attached to the siloxane are: diol, amino, cyano, inorganic oxides, and dimethylamino. Normal phase is also a form of liquid-solid chromatography. The most non-polar compounds will elute first when doing normal phase HPLC.

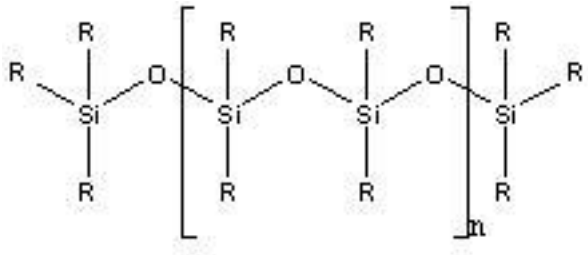

Figure 11: Basic structure of a siloxane. The R groups can be varied depending on the type of column and analyte being analyzed. This figure was created with ChemBioDraw Ultra 12.0.

Reverse phase HPLC uses a polar mobile phase and a non-polar stationary phase. Reverse phase HPLC is the most common liquid chromatography method used. The R groups usually attached to the siloxane for reverse phase HPLC are: C_8, C_{18},or any hydrocarbon. Reverse phase can also use water as the mobile phase, which is advantageous because water is cheap, nontoxic, and invisible in the UV region. The most polar compounds will elute first when performing reverse phase HPLC. Check the animation on the principle of reversed-phase chromatography to understand its principle.

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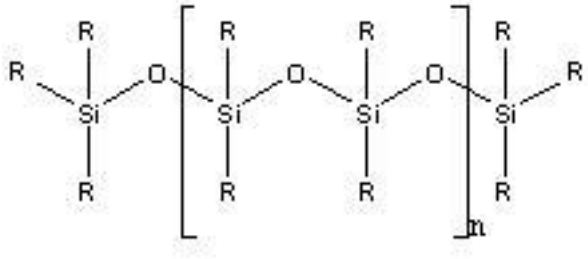

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Ion Exchange Chromatographic Columns

Ion exchange columns are used to separate ions and molecules that can be easily ionized. Separation of the ions depends on the ion's affinity for the stationary phase, which creates an ion exchange system. The electrostatic interactions between the analytes, moble phase, and the stationary phase, contribute to the separation of ions in the sample. Only positively or negatively charged complexes can interact with their respective cation or anion exchangers. Common packing materials for ion exchange columns are amines, sulfonic acid, diatomaceous earth, styrene-divinylbenzene, and cross-linked polystyrene resins. Some of the first ion exchangers used were inorganic and made from aluminosilicates (zeolites). Although aluminosilicates are not widely used as ion exchange resins used.

Size Exclusion Chromatographic Columns

Size Exclusion Chromatographic columns separate molecules based upon their size, not molecular weight. A common packing material for these columns is molecular sieves. Zeolites are a common molecular sieve that is used. The molecular sieves have pores that small molecules can go into, but large molecules cannot. This allows the larger molecules to pass through the column faster than the smaller ones. Other packing materials for size exclusion chromatographic columns are polysaccharides and other polymers, and silica. The pore size for size exclusion separations varies between 4 and 200 nm.

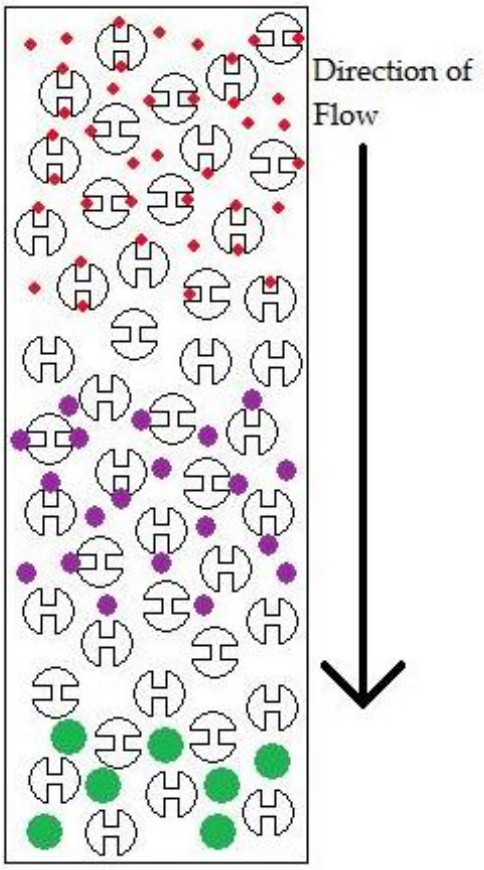


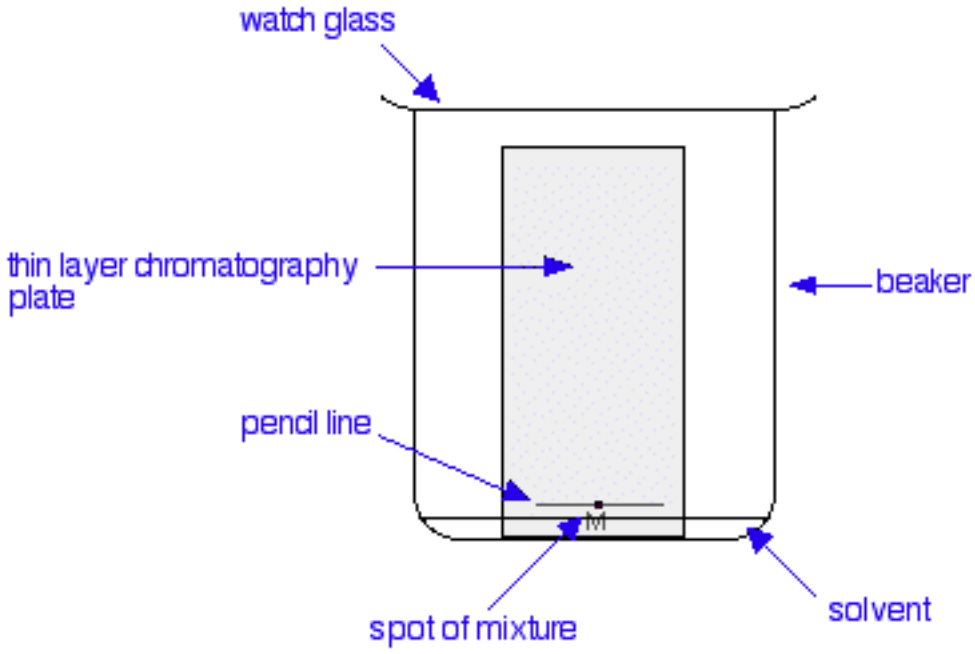
Figure 22: Schematic of a size exclusion column. The larger particles will elute first because they are too big to fit inside the pores. The smallest particles will elute last because they fit very well inside the pores.

Thin Layer Chromatography

Chromatography is used to separate mixtures of substances into their components. All forms of chromatography work on the same principle. They all have a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates.

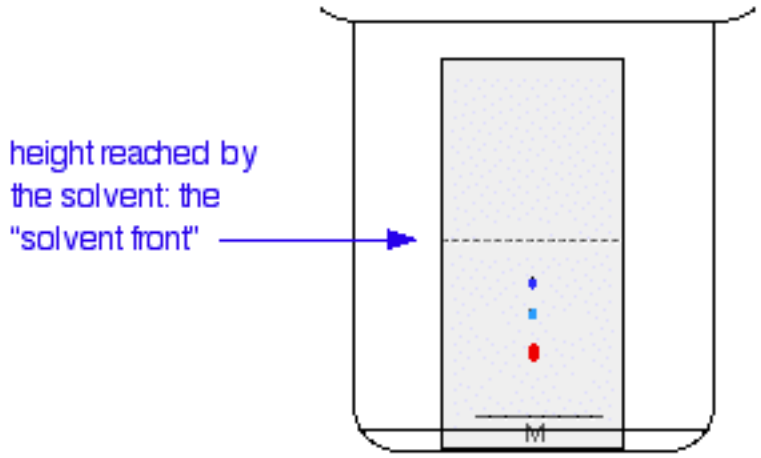
Thin layer chromatography is done exactly as it says - using a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic. The silica gel (or the alumina) is the stationary phase. The stationary phase for thin layer chromatography also often contains a

substance which fluoresces in UV light - for reasons you will see later. The mobile phase is a suitable liquid solvent or mixture of solvents.



A pencil line is drawn near the bottom of the plate and a small drop of a solution of the dye mixture is placed on it. Any labelling on the plate to show the original position of the drop must also be in pencil. If any of this was done in ink, dyes from the ink would also move as the chromatogram developed. When the spot of mixture is dry, the plate is stood in a shallow layer of solvent in a covered beaker. It is important that the solvent level is below the line with the spot on it.

The reason for covering the beaker is to make sure that the atmosphere in the beaker is saturated with solvent vapor. To help this, the beaker is often lined with some filter paper soaked in solvent. Saturating the atmosphere in the beaker with vapor stops the solvent from evaporating as it rises up the plate. As the solvent slowly travels up the plate, the different components of the dye mixture travel at different rates and the mixture is separated into different coloured spots.

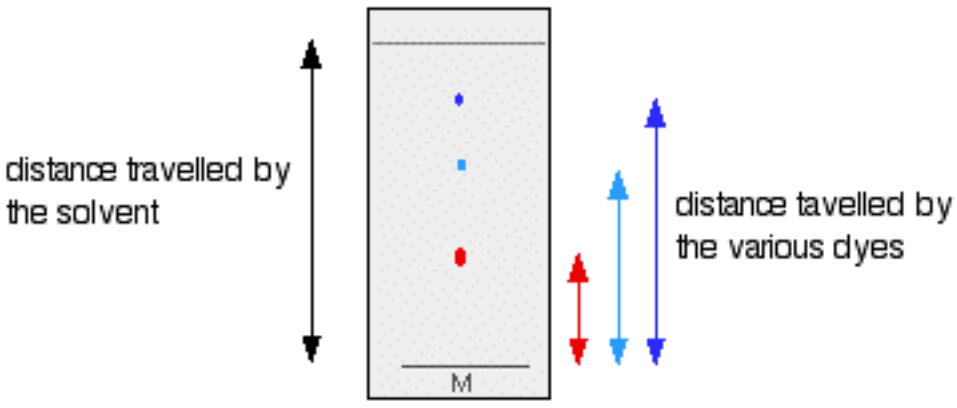


The diagram shows the plate after the solvent has moved about half way up it. The solvent is allowed to rise until it almost reaches the top of the plate. That will give the maximum separation of the dye components for this particular combination of solvent and stationary phase.

Measuring R_f values

If all you wanted to know is how many different dyes made up the mixture, you could just stop there. However, measurements are often taken from the plate in order to help identify the compounds present. These measurements are the distance traveled by the solvent, and the distance traveled by individual spots. When the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.

These measurements are then taken:



The R_f value for each dye is then worked out using the formula:

$$R_f = \frac{\text{distance traveled by sample}}{\text{distance traveled by solvent}}$$

For example, if the red component traveled 1.7 cm from the base line while the solvent had traveled 5.0 cm, then the Rf value for the red dye is:

$$R_f = \frac{1.7}{5.0}$$
$$= 0.34$$

If you could repeat this experiment under exactly the same conditions, then the R_f values for each dye would always be the same. For example, the R_f value for the red dye would always be 0.34. However, if anything changes (the temperature, the exact composition of the solvent, and so on), that is no longer true. You have to bear this in mind if you want to use this technique to identify a particular dye.

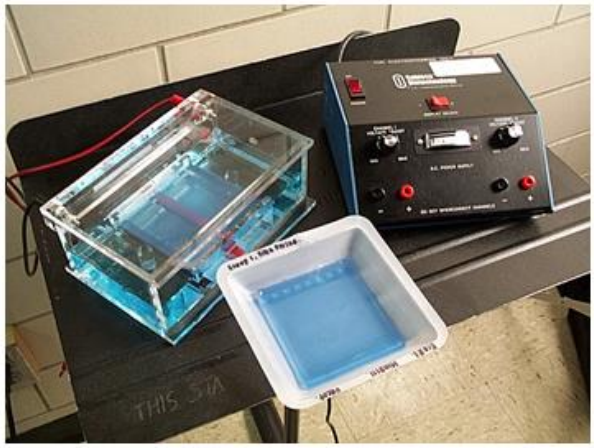
Electrophoresis is a class of separation techniques in which we separate analytes by their ability to move through a conductive medium—usually an aqueous buffer—in response to an applied electric field. In the absence of other effects, cations migrate toward the electric field's negatively charged cathode. Cations with larger charge-to-size ratios—which favors ions of larger charge and of smaller size—migrate at a faster rate than larger cations with smaller charges. Anions migrate toward the positively charged anode and neutral species do not experience the electrical field and remain stationary.

There are several forms of electrophoresis. In slab gel electrophoresis the conducting buffer is retained within a porous gel of agarose or polyacrylamide. Slabs are formed by pouring the gel between two glass plates separated by spacers. Typical thicknesses are 0.25–1 mm. Gel electrophoresis is an important technique in biochemistry where it is frequently used for separating DNA fragments and proteins. Although it is a powerful tool for the qualitative analysis of complex mixtures, it is less useful for quantitative work.

In capillary electrophoresis, the conducting buffer is retained within a capillary tube whose inner diameter is typically 25–75 μm. Samples are injected into one end of the capillary tube. As the sample migrates through the capillary its components separate and elute from the column at different times. The resulting **electropherogram** looks similar to a GC or an HPLC chromatogram, providing both qualitative and quantitative information. Only capillary electrophoretic methods receive further consideration in this section.

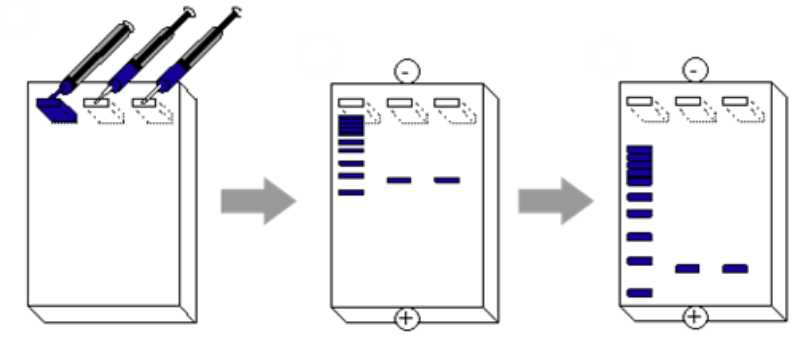
DNA Analysis - Gel Electrophoresis

A solution of DNA is colorless, and except for being viscous at high concentrations, is visually indistinguishable from water. Therefore, techniques such as **gel electrophoresis** have been developed to detect and analyze DNA.



Apparatus for agarose gel electrophoresis. A waterproof tank is used to pass current through a slab gel, which is submerged in a buffer in the tank. The current is supplied by an adjustable power supply. A gel (stained blue by a dye sometimes used when loading DNA on the gel) sits in a tray, awaiting further analysis, such as photography under a UV light source.

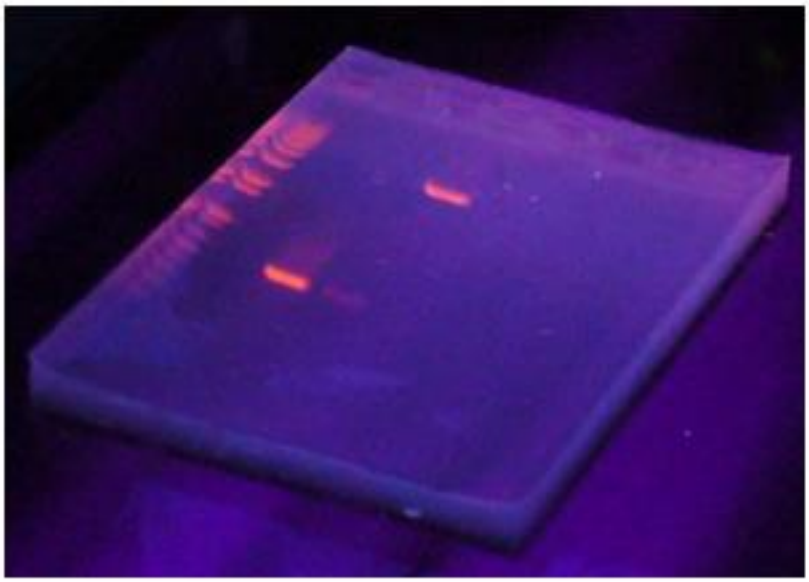
This analysis starts when a solution of DNA is deposited at one end of a gel slab. This gel is made from polymers such as **agarose**, which is a polysaccharide isolated from seaweed. The DNA is then forced through the gel by an electrical current, with DNA molecules moving toward the positive electrode.



Agarose gel electrophoresis. DNA is loaded into wells at the top of a gel. A current is passed through the gel, pulling DNA towards the positively charged electrode. The DNA fragments are separated by size, with smaller fragments moving fastest towards the electrode.

As it migrates, each piece of DNA threads its way through the pores, which form between the polymers in the gel. Because shorter pieces can move through these pores faster than longer pieces, gel electrophoresis separates molecules based on their size (length), with smaller DNA pieces moving faster than long ones. DNA molecules of a similar size migrate to a similar

location in each gel, called a **band**. This feature makes it easy to see DNA after staining the DNA with a fluorescent dye such as **ethidium bromide**. By separating a mixture of DNA molecules of known size (**size markers**) in adjacent lanes on the same gel, the length of an uncharacterized DNA fragment can be estimated. Gel segments containing the DNA bands can also be cut out of the gel, and the size-selected DNA extracted and used in other types of reactions, such as sequencing and cloning.



An agarose gel stained with ethidium bromide and illuminated by UV light. The stain associated with DNA is fluorescent.

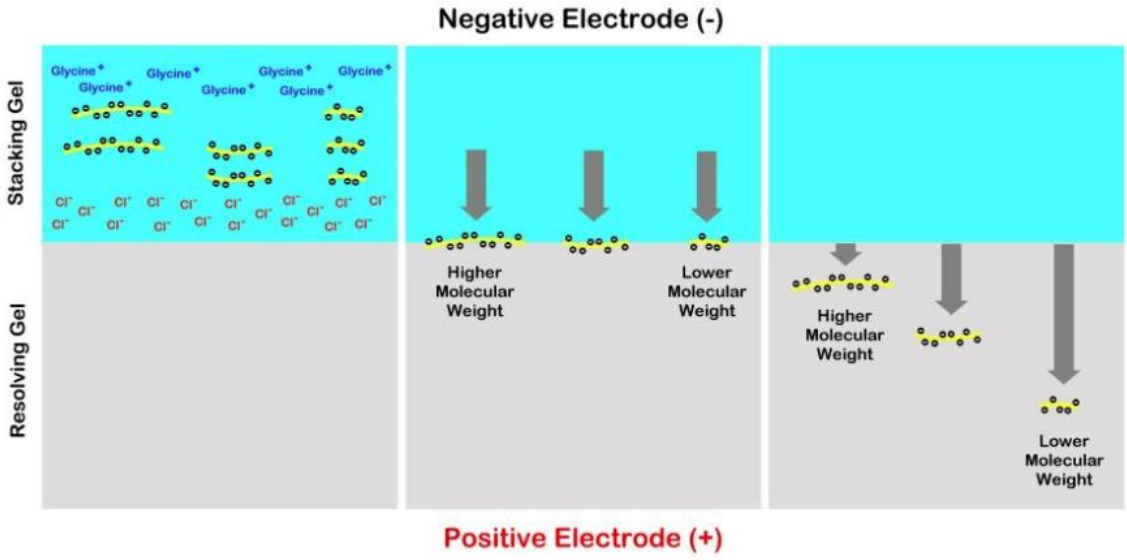
SDS-PAGE

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) is probably the most common analytical technique used to separate and characterize proteins. A solution of **acrylamide** and bisacrylamide is polymerized. Acrylamide alone forms linear polymers. The bisacrylamide introduces crosslinks between polyacrylamide chains. The 'pore size' is determined by the ratio of acrylamide to bisacrylamide, and by the concentration of acrylamide. A high ratio of bisacrylamide to acrylamide and a high acrylamide concentration cause low electrophoretic mobility. Polymerization of acrylamide and bisacrylamide **monomers** is induced by ammonium persulfate (APS), which spontaneously decomposes to form free radicals. TEMED, a free radical stabilizer, is generally included to promote polymerization.

The gels are usually prepared with the top portion of the gel under the sample wells made less dense than the remainder of the gel below that is intentionally made denser. The top portion is referred to as the “stacking gel” and the lower portion is termed the “running gel” or “separating gel”. The purpose of the stacking gel is to concentrate all of the different sized proteins into a compact horizontal zone by sandwiching them between a gradient of glycine molecules above and chloride ions below. This way most of the proteins will enter the denser resolving gel simultaneously before they begin to migrate downwards at different rates based on their size. This way, the bands are much clearer and better separated for visualization and analysis.

Without the stacking gel, the proteins will produce a long smear through the resolving gel instead of tight distinct bands for us to analyze.



PAGE gel. A protein first runs through the stacking gel, where the samples spread out. Once a protein reaches the separating gel, the proteins pack together in tight bands. As they move through the resolving gel they separate by size.

SDS-PAGE

Sodium dodecyl sulfate (SDS) is an **amphipathic** detergent. It has an anionic head group and a **lipophilic** tail. It binds non-covalently to proteins, where roughly one SDS molecule is attracted to every two amino acids. SDS causes proteins to denature and disassociate from each other (excluding covalent cross-linking) and essentially unravel into linear molecules. It also confers negative charge. In the presence of SDS, the intrinsic charge of a protein is masked. During SDS-PAGE, all proteins migrate towards the anode (the positively charged electrode). SDS-treated proteins have very similar charge-to-mass ratios, and similar shapes. During PAGE, the rate of migration of SDS-treated proteins is effectively determined by their unfolded length, which is related to their molecular weight.

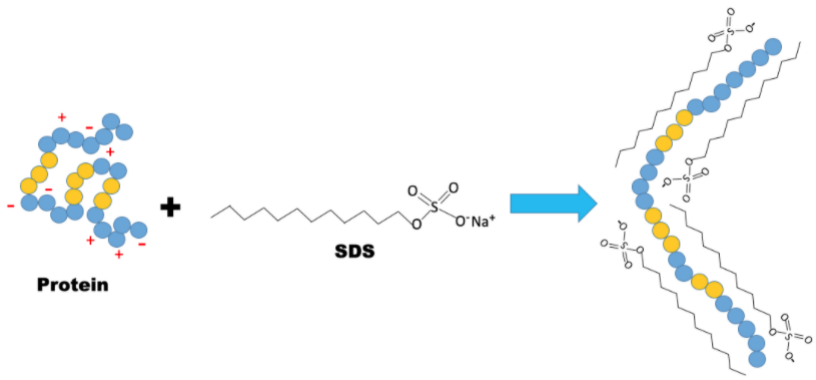
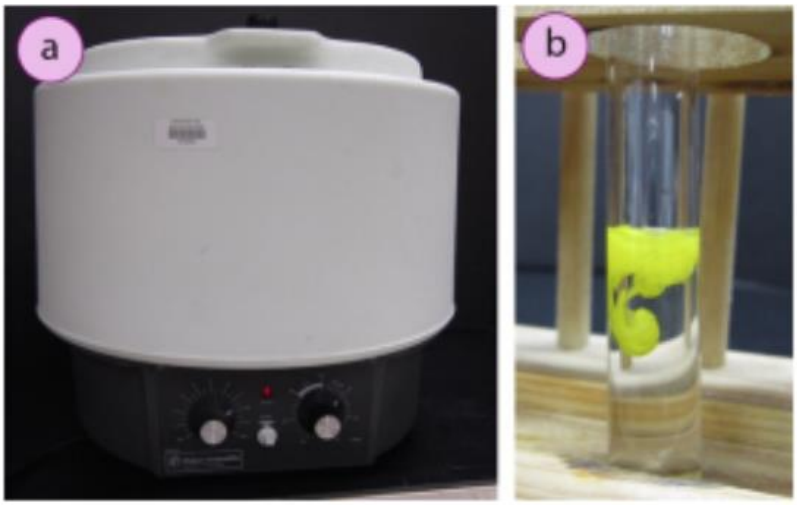


Figure 2: A protein surrounded by the SDS molecules.

Centrifugation

Centrifugation is used for the separation of solid-liquid mixtures that are stubborn to settle or difficult to otherwise filter. It uses centrifugal force by rapidly spinning samples so that the solid is forced to the bottom of the tube. In this section is shown centrifugation of a suspension of yellow lead(II) iodide in water.

As a centrifuge can spin up to 10,000 rotations per minute, an unbalanced load will cause the centrifuge to knock and wobble. If severely unbalanced, the centrifuge can even wobble off the benchtop, causing harm to anything in its way (they are heavy!). To prevent wobbling, every sample in the centrifuge needs to be balanced by an equal volume of liquid in the opposite chamber in the centrifuge.



a) Centrifuge, b) Formation of solid lead(II) iodide.

Special test tubes or centrifuge tubes must be used that *exactly* fit the width of the chambers in the centrifuge. Each tube should be filled to no greater than three-quarters full as the samples will be tilted in the centrifuge and could spill out (Figure 1.91b). If only one sample is to be centrifuged, a tube of water that contains an equal height of liquid should be placed into an opposing slot in the centrifuge (Figure 1.91a-c). More than one sample can be centrifuged at a time, with the only requirement being that each opposing tube must have nearly the same volume. It is acceptable for one pair of tubes to have different volumes as another pair, as long as the entire centrifuge is symmetrically balanced.

To operate the centrifuge, close the lid and turn on the centrifuge. Set the rotation speed if your instrument allows for it (a good general speed is 8,000 rotations per minute) and turn the dial to the recommended amount of time (set by your instructor or experimentation). Allow the system to spin for the designated time and turn it off. Although a centrifuge has a braking mechanism, it is not recommended to use it as the jostling can stir up sediments. After the set amount of time has expired, simply let a centrifuge slow and come to a stop on its own. The solid can then be separated from the liquid by decantation or by pipette.

Ultracentrifuge is a sophisticated and advanced centrifuge that operates at an extremely high speed and separates smaller molecules that cannot be separated from the traditional centrifuges.

- The speed of the rotors in ultracentrifuge can range from 60,000 rpm to 150,000 rpm.
- Ultracentrifuges are mostly operated in more facilitated laboratories to perform more advanced operations.
- These are larger in size and can operate samples either in batches or as a continuous flow system.
- Most ultracentrifuges are refrigerated in order to control the heat that might be generated due to the excessive speed.

Principle of Ultracentrifuge

- The ultracentrifuge works on the same principle as all other centrifuges.
- The working of an ultracentrifuge is based on the sedimentation principle, which states that the denser particles settle down faster when compared to less dense particles under gravity.
- However, the sedimentation of particles under gravity would take a larger amount of time, and that is why an additional force is applied to aid the sedimentation process.
- In an ultracentrifuge, the sample is rotated about an axis, resulting in a perpendicular force, called centrifugal force, that acts on different particles on the sample.
- The larger molecules move faster, whereas the smaller molecules move slower.
- At the same time, denser molecules are moved outwards to the periphery of the tubes whereas the less dense molecules are rotated towards the center of the tube.
- Once the process is completed, the larger and more dense particles settle down, forming pellets at the bottom of the tube. In comparison, the smaller and less dense particles remain either in the suspended in the supernatant or float on the surface.

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DEPARTMENT OF PHYSICS

UNIT – III - BIOPHYSICS – SPHA7410

UNIT – III - EXPERIMENTAL TECHNIQUES

General Introduction to Spectroscopic Techniques in Biology - UV - Visible Spectroscopy - Applications and Results in Biology - IR And Raman Spectroscopy - Applications And Results - Circular Dichroism And Optical Rotatory Dispersion - Fluorescence Spectroscopy - NMR - Use Of NMR In Biological Structure Determination And Medical Imaging - X-Ray Crystallography, Its Use To Determine The Structures Of Biological Molecules.

Circular Dichroism

Circular Dichroism, an absorption spectroscopy, uses circularly polarized light to investigate structural aspects of optically active chiral media. It is mostly used to study biological molecules, their structure, and interactions with metals and other molecules.

Introduction

Circular Dichroism (CD) is an absorption spectroscopy method based on the differential absorption of left and right circularly polarized light. Optically active chiral molecules will preferentially absorb one direction of the circularly polarized light. The difference in absorption of the left and right circularly polarized light can be measured and quantified. UV CD is used to determine aspects of protein secondary structure. Vibrational CD, IR CD, is used to study the structure of small organic molecules, proteins and DNA. UV/Vis CD investigates charge transfer transitions in metal-protein complexes.

Circular Polarization of Light

Electromagnetic radiation consists of oscillating electric and magnetic fields perpendicular to each other and the direction of propagation. Most light sources emit waves where these fields oscillate in all directions perpendicular to the propagation vector. Linear polarized light occurs when the electric field vector oscillates in only one plane. In circularly polarized light, the electric field vector rotates around the propagation axis maintaining a constant magnitude. When looked at down the axis of propagation the vector appears to trace a circle over the period of one wave frequency (one full rotation occurs in the distance equal to the wavelength). In linear polarized light the direction of the vector stays constant and the magnitude oscillates. In circularly polarized light the magnitude stays constant while the direction oscillates.

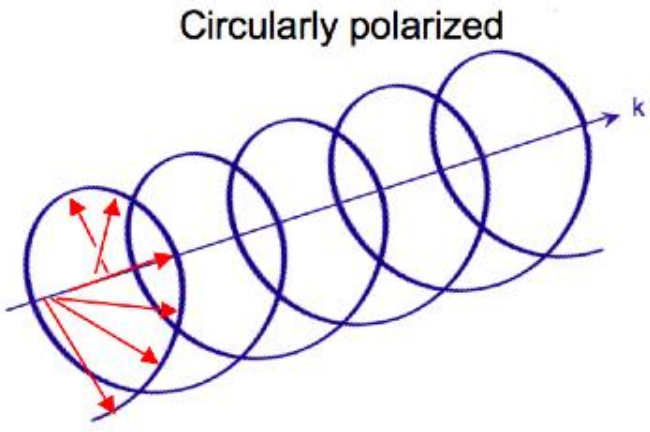

Figure 1: Diagram of linearly polarized and circularly polarized light

As the radiation propagates the electric field vector traces out a helix. The magnetic field vector is out of phase with the electric field vector by a quarter turn. When traced together the vectors form a double helix.

Light can be circularly polarized in two directions: left and right. If the vector rotates counterclockwise when the observer looks down the axis of propagation, the light is left circularly polarized (LCP). If it rotates clockwise, it is right circularly polarized (RCP). If LCP and RCP of the same amplitude, they are superimposed on one another and the resulting wave will be linearly polarized.

Applications

Instrumentation

Most commercial CD instruments are based on the modulation techniques introduced by Grosjean and Legrand. Light is linearly polarized and passed through a monochromator. The single wavelength light is then passed through a modulating device, usually a photoelastic modulator (PEM), which transforms the linear light to circular polarized light. The incident light on the sample switches between LCP and RCP light. As the incident light swtches direction of polarization the absorption changes and the differention molar absorptivity can be calculated.

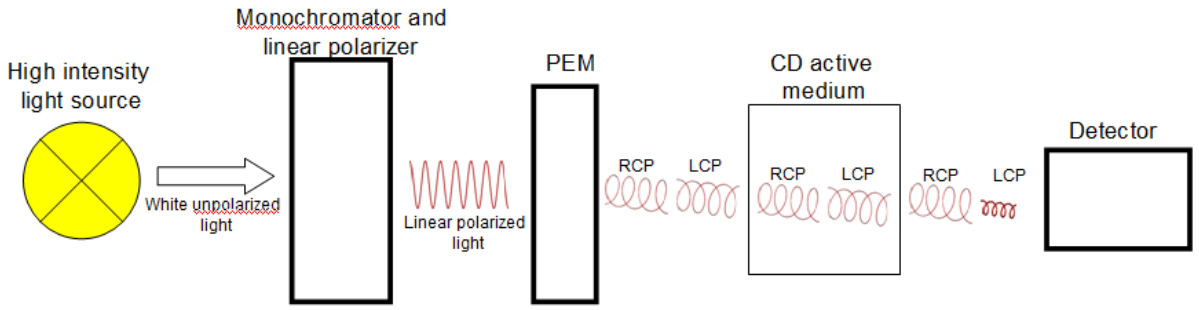

Figure 5: The instrumentation for a common CD spectrometer showing the polarization of light and the differential absorption of LCP and RCP light.

Biological molecules

The most widely used application of CD spectroscopy is identifying structural aspects of proteins and DNA. The peptide bonds in proteins are optically active and the ellipticity they exhibit changes based on the local conformation of the molecule. Secondary structures of proteins can be analyzed using the far-UV (190-250 nm) region of light. The ordered αα-helices, ββ-sheets, ββ-turn, and random coil conformations all have characteristic spectra. These unique spectra form the basis for protein secondary structure analysis. It should be noted that in CD only the relative fractions of residues in each conformation can be determined but not specifically where each structural feature lies in the molecule. In reporting CD data for

large biomolecules it is necessary to convert the data into a normalized value that is independent of molecular length. To do this the molar ellipticity is divided by the number of residues or monomer units in the molecule.

The real value in CD comes from the ability to show conformational changes in molecules. It can be used to determine how similar a wild type protein is to mutant or show the extent of denaturation with a change in temperature or chemical environment. It can also provide information about structural changes upon ligand binding. In order to interpret any of this information the spectrum of the native conformation must be determined.

Some information about the tertiary structure of proteins can be determined using near-UV spectroscopy. Absorptions between 250-300 nm are due to the dipole orientation and surrounding environment of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, and cysteine residues which can form disulfide bonds. Near-UV techniques can also be used to provide structural information about the binding of prosthetic groups in proteins.

Metal containing proteins can be studied by visible CD spectroscopy. Visible CD light excites the d-d transitions of metals in chiral environments. Free ions in solution will not absorb CD light so the pH dependence of the metal binding and the stoichiometry can be determined.

Vibrational CD (VCD) spectroscopy uses IR light to determine 3D structures of short peptides, nucleic acids, and carbohydrates. VCD has been used to show the shape and number of helices in A-, B-, and Z-DNA. VCD is still a relatively new technique and has the potential to be a very powerful tool. Resolving the spectra requires extensive *ab initio* calculations, as well as, high concentrations and must be performed in water, which may force the molecule into a nonnative conformation.

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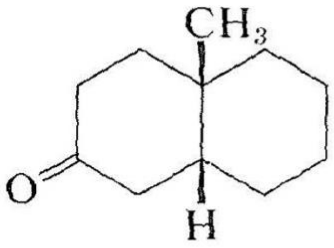
Optical Rotatory Dispersion

Optical rotations usually are measured at just one wavelength, namely 589.3nm , simply because sodium-vapor lamps provide an especially convenient source of monochromatic light. Measurements at other wavelengths are less easily made without specialized instruments, with which relatively few laboratories are currently equipped. Nevertheless, much information has been obtained about structure, conformation, and configuration of organic compounds from measurements of optical rotation as a function of wavelength (i.e., optical rotatory dispersion).

Like other phenomena involving interactions between electromagnetic radiation and organic molecules, as in infrared, ultraviolet, and nmr spectroscopy, optical rotatory dispersion curves often are quite sensitive to small changes in structure. As an example, the rotatory dispersion curves for enantiomers of cis- and trans-10-methyl-2-decalones, 16 and 17 , are reproduced in Figure 19-7:



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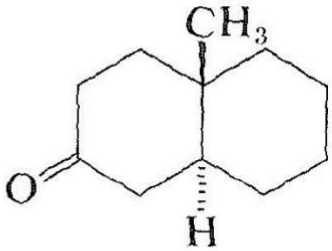

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Figure 19-7: Rotatory dispersion curves for cis-10-methyl-2-decalone, 1616, and trans-10-methyl-2-decalone 1717. *(By permission from C. Djerassi, O*ptical Rotatory Dispersion, *McGraw-Hill Book Co., New York, 1960.)*

Only a small positive rotation is observed for the particular enantiomers at the wavelength of the sodium line (589.3nm)(589.3nm) compared to the large, both positive and negative, rotations found at wavelengths between 270nm270nm and 400nm400nm. If we measure the rotations as a function of wavelength and if, as we approach shorter wavelengths, the rotation rises to a *maximum* before changing sign, as it does with the trans isomer, 1717, then the compound is said to exhibit a **positive Cotton effect**. The opposite behavior, as with the cis isomer, 1616, is called a **negative Cotton effect**. The wavelength at the center point for the very rapid change in rotation for 1717 is 300nm300nm and corresponds to the $n \rightarrow \pi^* n \rightarrow \pi^*$ absorption maximum of the carbonyl group in the ultraviolet absorption curve of the same compound. Thus excitation of the carbonyl group by absorption of ultraviolet light and strong rotatory dispersion of polarized light are associated phenomena. In fact, when a substance exhibits a Cotton effect, not only does it transmit clockwise and counterclockwise circularly polarized light with unequal velocities (Section 19-1), it also absorbs the two forms of light unequally.

Reference

https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Book%3A_Basic_Principles_of_Organic_Chemistry_(Roberts_and_Caserio)/19%3A_More_on_Stereochemistry/19.09%3A_Optical_Rotatory_Dispersion_and_Circular_Dichroism

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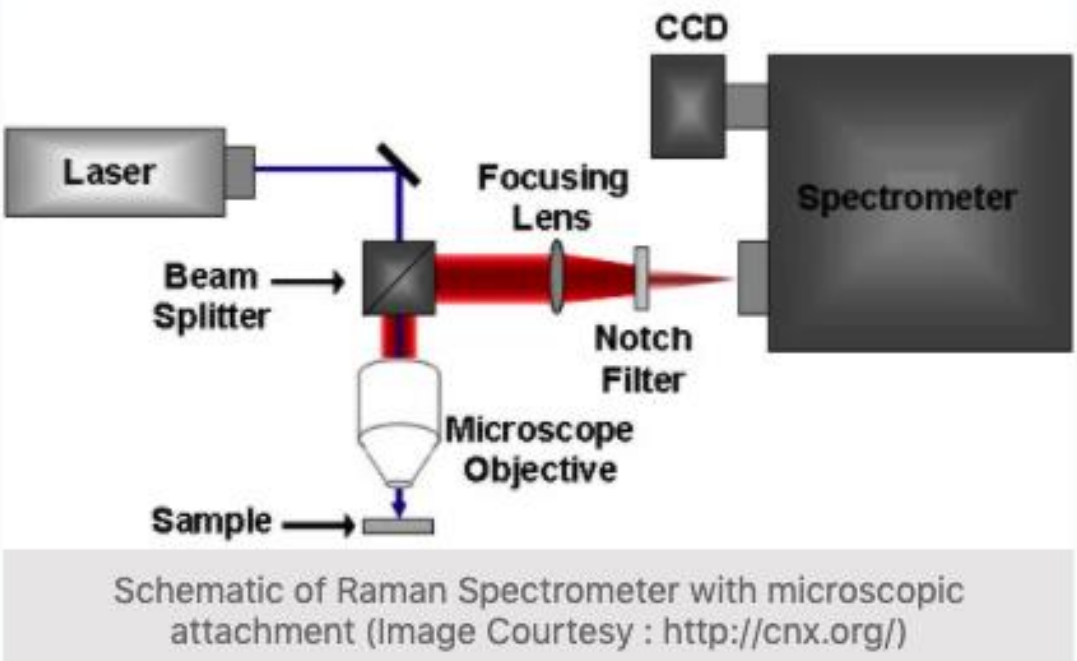
Raman and IR Spectroscopy

Raman and IR spectroscopy are complementary techniques used for fingerprinting of molecules. Raman spectroscopy offers significant advantages over IR and other spectroscopy techniques.



Schematic of Raman Spectrometer with microscopic attachment (Image Courtesy : http://cnx.org/)

Both Raman and IR spectra result due to changes in vibration modes of molecules. However, only those vibration modes which result in changes in the dipole moment of a module are IR active and those that result in change in polarizability are Raman active. IR and Raman activities are related to symmetry of molecules and can be expressed in terms of Rule of mutual exclusion which states that for a molecule having a centre of symmetry the Raman active vibrations are IR inactive and vice versa. However, this rule is not applicable to molecules having no centre of symmetry. In such case vibrations may be both Raman as well as IR active.

The rule is valuable in establishing the structure of a molecule such N2O. The spectra of the molecule suggests that the molecule does not have a center of symmetry. It can be concluded that the molecule has a structure N-N-O and not N-O-N.

The dissimilarities between the two spectroscopic techniques are summarized below:

- Raman spectra result from scattering of light by vibrating molecules whereas IR spectra result from light absorption by vibrating molecules
- Raman activity results from change of polarizability of a molecule whereas IR activity results from changing dipole moment
- A monochromatic light beam of high intensity laser can be used in UV, visible or IR regions in Raman measurements whereas in IR spectroscopy the range is limited to IR frequencies
- In case of Raman scattered light is observed at right angles to the direction of the incident beam whereas in case of IR the absorption signal is measured in the same direction as the incident beam.
- Raman technique is non-destructive. The sample can be measured directly in glass container or in case of pharmaceuticals samples can be measured in original sachets.. IR technique requires solid sample preparation using KBr or CSi powder though accessories such as HATR permit direct observation of liquids, films and gels.
- Laser sources in Raman technique are highly intense and these facilitate focusing the coherent beam on small sample area or on exceedingly small sample volumes. This is a key advantage when only limited sample quantities are available
- A high degree of amplification of weak Stoke signals is necessary in presence of intense Rayleigh light scattering component. This results in higher cost of the Raman spectrometer. Higher cost can be easily justified against the benefits offered by the technique.

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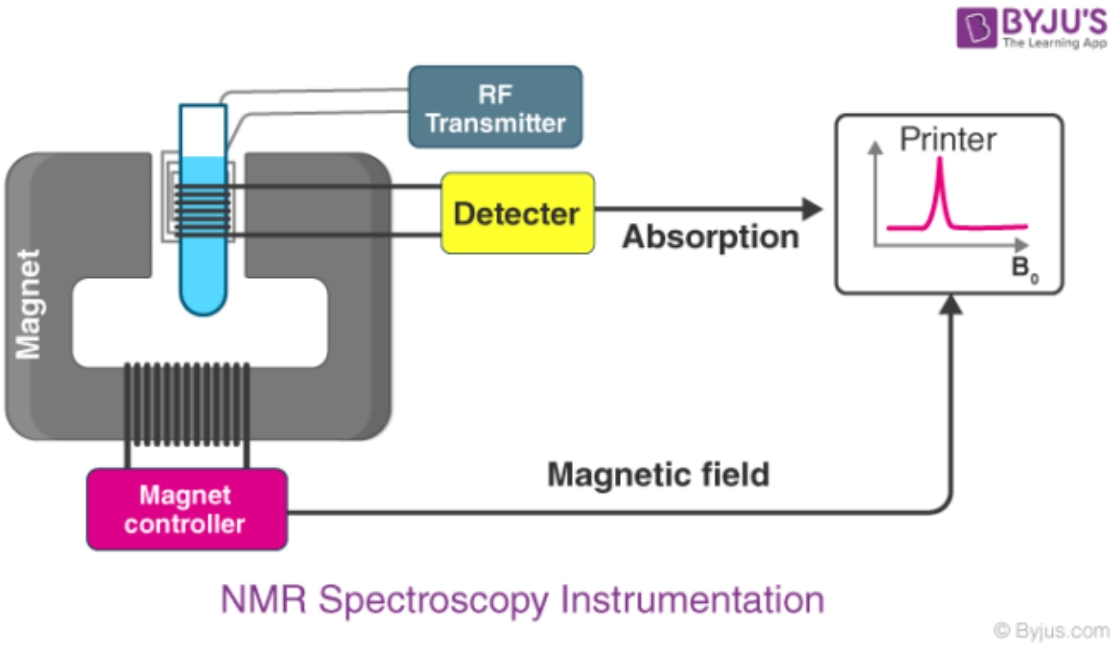
Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy is the study of molecules by recording the interaction of radiofrequency (Rf) electromagnetic radiations with the nuclei of molecules placed in a strong magnetic field.

Zeeman first observed the strange behaviour of certain nuclei when subjected to a strong magnetic field at the end of the nineteenth century, but the practical use of the so-called ***"Zeeman effect"*** was only made in the 1950s when NMR spectrometers became commercially available.

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.

Basis of NMR Spectroscopy

Nuclear Magnetic Resonance (NMR) was first detected experimentally at the end of 1945, nearly concurrently with the work groups Felix Bloch, Stanford University and Edward Purcell, Harvard University. The first NMR spectra was first published in the same issue of the Physical Review in January 1946. Bloch and Purcell were jointly awarded the 1952 Nobel Prize in Physics for their research of Nuclear Magnetic Resonance Spectroscopy.

Nuclear magnetic resonance (NMR) spectroscopy is a crucial analytical tool for organic chemists. The research in the organic lab has been significantly improved with the aid of the NMR. Not only can it provide information on the structure of the molecule, it can also determine the content and purity of the sample. Proton (1H) NMR is one of the most widely used NMR methods by organic chemists. The protons present in the molecule will behave differently depending on the surrounding chemical environment, making it possible to elucidate their structure.

NMR Spectroscopy Principle

- All nuclei are electrically charged and many have spin.
- Transfer of energy is possible from base energy to higher energy levels when an external magnetic field is applied.
- The transfer of energy occurs at a wavelength that coincides with the radio frequency.
- Also, energy is emitted at the same frequency when the spin comes back to its base level.
- Therefore, by measuring the signal which matches this transfer the processing of the NMR spectrum for the concerned nucleus is yield.

NMR Spectroscopy Working

- Place the sample in a magnetic field.
- Excite the nuclei sample into nuclear magnetic resonance with the help of radio waves to produce NMR signals.
- These NMR signals are detected with sensitive radio receivers.
- The resonance frequency of an atom in a molecule is changed by the intramolecular magnetic field surrounding it.
- This gives details of a molecule's individual functional groups and its electronic structure.
- Nuclear magnetic resonance spectroscopy is a conclusive method of identifying monomolecular organic compounds.
- This method provides details of the reaction state, structure, chemical environment and dynamics of a molecule.

Chemical Shift in NMR Spectroscopy

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.

Chemical shift is characterized as the difference between the resonant frequency of the spinning protons and the signal of the reference molecule. Nuclear magnetic resonance chemical change is one of the most important properties usable for molecular structure determination. There are also different nuclei that can be detected by NMR spectroscopy, 1H (proton), 13C (carbon 13), 15N (nitrogen 15), 19F (fluorine 19), among many more. 1H and 13C are the most widely used. The definition of 1H as it is very descriptive of the spectroscopy of the NMR. Both the nuts have a good charge and are constantly revolving like a cloud. Through mechanics, we learn that a charge in motion produces a magnetic field. In NMR, when we reach the radio frequency (Rf) radiation nucleus, it causes the nucleus and its magnetic field to turn (or it causes the nuclear magnet to pulse, thus the term NMR).

NMR Spectroscopy Instrumentation

This instrument consists of nine major parts. They are discussed below:

- **Sample holder –** It is a glass tube which is 8.5 cm long and 0.3 cm in diameter.
- **Magnetic coils –** Magnetic coil generates magnetic field whenever current flows through it
- **Permanent magnet –** It helps in providing a homogenous magnetic field at 60 – 100 MHZ
- **Sweep generator –** Modifies the strength of the magnetic field which is already applied.
- **Radiofrequency transmitter –** It produces a powerful but short pulse of the radio waves.
- **Radiofrequency –** It helps in detecting receiver radio frequencies.
- **RF detector –** It helps in determining unabsorbed radio frequencies**.**
- **Recorder –** It records the NMR signals which are received by the RF detector.
- **Readout system –** A computer that records the data.

NMR Spectroscopy Techniques

1. Resonant Frequency

It refers to the energy of the absorption, and the intensity of the signal that is 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.

2. Acquisition of Spectra

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

NMR Spectroscopy Applications

1. NMR spectroscopy is a Spectroscopy technique 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.
2. 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.
3. Once the basic structure is known, NMR can be used to determine molecular conformation in solutions as well as in studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion.

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X-ray crystallography

X-ray crystallography is a powerful non-destructive technique for determining the molecular structure of a crystal. X-ray crystallography uses the principles of X-ray diffraction to analyze the sample, but it is done in many different directions so that the 3D structure can be built up. It is a technique that has helped to deduce the 3D crystal structure of many materials, especially biological materials.

When you think of X-ray diffraction (XRD), a 2D diffraction pattern comes to mind for most. The basic patterns generated in X-ray crystallography are still 2D diffraction patterns, but the

key difference is that the sample is scanned in multiple directions. These diffraction patterns are then pieced together and refined multiple times so that they analyze and determine the molecular structure of the sample. It is even possible to do this for very large and complex molecules, with proteins being a key example.

Key Principles and Workings

In the instrument, the sample is mounted on to a goniometer, which is used to position the crystal into specific orientations so that it can be analyzed from multiple angles. In some cases where the sample is impure and the crystal structure is not clear, the crystalline sample will need to be purified before analysis.

X-rays are generated from an X-ray tube, and they are then filtered so that they are monochromatic, i.e. of a single wavelength frequency. The atoms in the crystal refract the X-rays and the X-rays are elastically scattered on to a detector. Because they are elastically scattered, they have the same energy as the incident X-rays that are fired at the sample. This generates a 2D diffraction pattern of the crystal in a single orientation.

If the diffraction pattern is not clear, then the sample may not be pure and will be purified at this point. But other factors can prevent a diffraction pattern from being generated including a too-small sample (needs to be at 0.1 nm in each dimension), an irregular crystal structure, and the presence of any internal imperfections—such as cracks—in the crystal.

If the crystal is deemed to be ok, then the analysis and X-ray bombardment towards the sample continues. The sample rotates on the goniometer so that a series of 2D diffraction patterns are generated from various sides of the sample. The intensity is recorded at every orientation and the result is thousands of 2D diffraction patterns that correspond to different parts of the 3D structure. From here, a computational approach analyses the different diffraction phases, angles and intensities to generate an electron density map of the sample. The electron density map is used to generate an atomic model of the sample. The model is constantly refined to ensure that it is accurate, and once the final atomic model has been established, the data goes into a central database to act as a known reference.

Applications

In terms of applications, X-ray crystallography is used in many scientific fields. When it was first established as a useful technique, it was primarily used in fundamental science applications for determining the size of atoms, the lengths and different types of chemical bonds, the atomic arrangement of materials, the difference between materials at the atomic level, and for determining the crystalline integrity, grain orientation, grain size, film thickness and interface roughness of alloys and minerals.

Science has come a long way since then and while these areas are still important for analyzing new materials, it is now often used to identify the structure of various biological materials, vitamins, pharmaceutical drugs, thin-film materials and multi-layered materials. It has become one of the standard ways of analyzing a material if the structure is unknown across the geological, environmental, chemical, material science and pharmaceutical sectors (plus many others) due to its non-destructive nature and its high accuracy and precision.

Nowadays, it is often used to probe specific ways in how the structure of a material, drug, or substance will interact in certain environments. This is has become particularly useful across the proteomics and pharmaceutical sectors. Some of the specific areas that can now be probed with X-ray crystallography include measuring the thickness of films, identifying specific crystal phases and orientations that can help to determine the catalytic activity of materials, determining the purity of a sample, determining how a drug might interact with specific proteins and how the drug can be improved, analyzing how proteins interact with other proteins, for investigating microstructures, and for analyzing what amino acids are present in a protein which can help with determining how catalytically active an enzyme is. These are just a few specific examples as the use of X-ray crystallography is widespread.

Sources and Further Reading

- Creative Biomart: https://www.creativebiomart.net/resource/principle-protocol-x-ray-crystallography-393.htm

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DEPARTMENT OF PHYSICS

UNIT – IV - BIOPHYSICS – SPHA7410

Unit IV

STRUCTURES OF BIOLOGICAL MOLECULES

Level of protein structure - amino acids and the primary structure of proteins - the peptide bond and the secondary structure of proteins - the Ramachandran Plot - tertiary and quaternary structure of proteins the double helical structure of DNA - how it explains DNA function - the structure of viruses.

Protein Structure

Proteins perform multiple functions in a cell and they are the factors to control several events. They are the building blocks and work as enzyme to participate in metabolic reactions of the organism.

Peptide Bonds: Proteins are polymers of amino acids, joined by the covalent bonds, known as peptide bond. A peptide bond is formed between carboxyl group of first and amino group of second amino acid with release of water (Figure 32.1, A,B). it is a dehydration synthesis or condensation reaction. The peptide bond has partial double bond character due to resonance and C-N bond is not free to rotate. But the bond between NCα and C-Cα can be able to rotate through dihedral angles designated by φ (phi) and ψ (psi). These angles can be able to rotate from -180 to +180 with few restriction. The Indian scientist G.N. Ramachandran has determined the possible φ (phi) and ψ (psi) for a particular amino acid by synthesizing tripeptide with the amino of interest in the middle. Based on these calculations, he has constructed Ramchandran plot to define the region of allowed rotation for amino acids present in a protein structure and proposed to use this to validate the 3-D structure of a protein model (Figure 32.1, C).

Amino Acids: As discussed earlier, proteins are madeup of amino acids joined by peptide bonds. Each protein can be broken into the constituents amino acids by a variety of methods to study the free amino acids. Twenty different amino acids are found in protein. The first amino acid discovered was asparagines in 1806. The name of amino acids were trivial or classical or in few cases derived from the food source from which they were isolated first. For examples; Asparagine was isolated from asparagus, glutamate from wheat gluten, tyrosine from cheese (greek tyros, cheese) and glycine has derived its name due to sweet taste (greek Glycos; sweet).

Amino acids share common structure: All 20 amino acids are α-amino acids with a common structure. Each amino acid has a carboxyl group and amine group attached to the primary carbon (the α-carbon). They differ from each other in terms of side chain or R group (Figure 32.2, A). The side chain varies in structure, chemical nature and that has influence on the over all property of amino acid. Except Glycine, each carbon is attached to the four different groups; making it a chiral centre to give streoisomers. There are two common forms of streoisomers called as enantiomers found in the amino acids. These are non-superimposable mirror images to each other, for example, L and D-alanine as given in Figure 32.2, B.

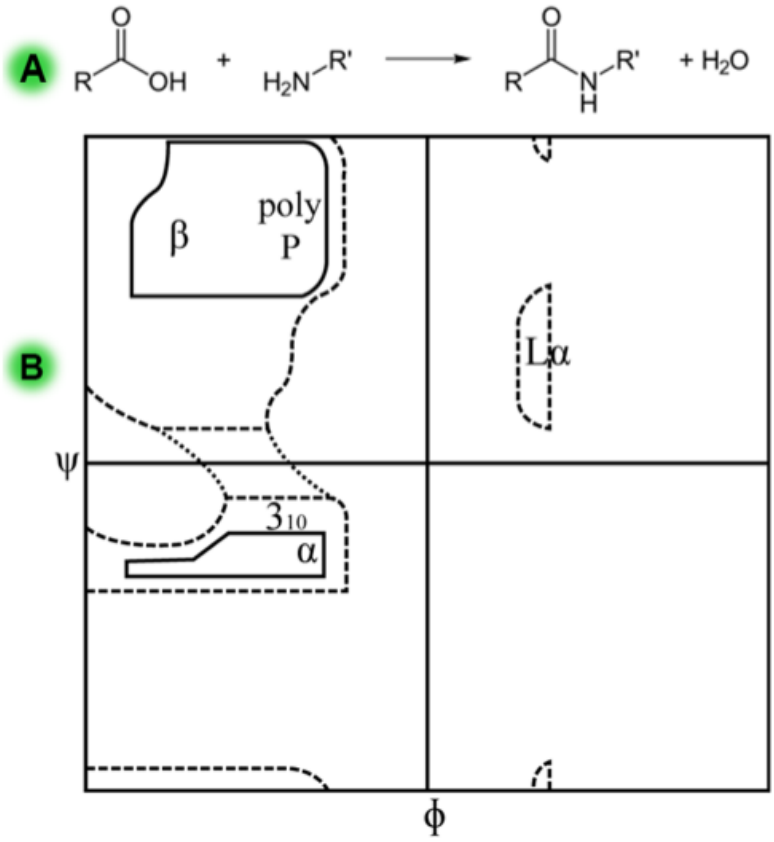


Figure 32.1: Peptide bond and Ramchandran Plot.

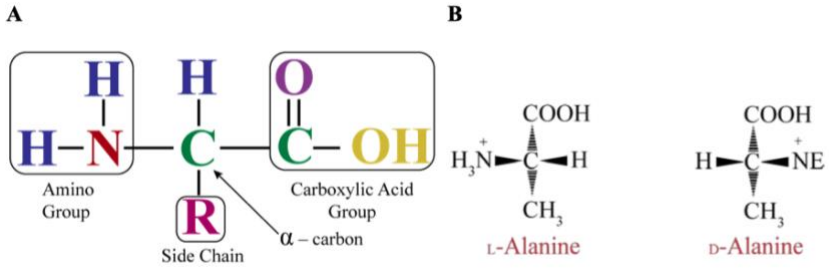


Figure 32.2: (A) A generalized structure of amino acid. (B) L and D-alinine [IMAGE REDRAW]

Amino acids are classified by R groups: As discussed, different amino acids are

classified basd on the side chain or R group. All these 20 amino acids are denotaed by first letter (3 or single) or other letter (3 or single). The structure of these amino acids are given in Figure 32.3 and their different properties are given in Table 32.1. The Different amino acids are as follows:

NONPOLAR, Aliphatic R Group: The R group in this amino acids are non-polar and hydrophobic. Examples include are alanine, valine, leucine, isoleucine and glycine, methionine, proline.

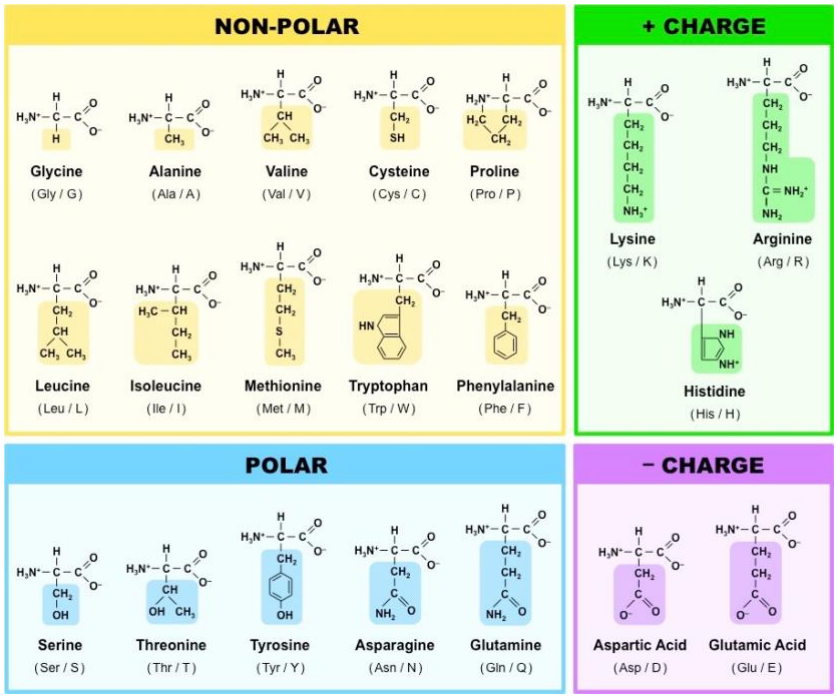


Table 32.1: Properties of common amino acids founds in protein.

Name	3-Letter Symbol	1-Letter Symbol	Molecular weight	Molecular Formula	Residue Formula	Residue Weight ($-H_2O$)	pK_a [1]	pK_b [2]	pK_x [3]	pI [4]
Alanine	Ala	A	89.10	$C_3H_7NO_2$	C_3H_5NO	71.08	2.34	9.69	—	6.00
Arginine	Arg	R	174.20	$C_6H_{14}N_4O_2$	$C_6H_{12}N_4O$	156.19	2.17	9.04	12.48	10.76
Asparagine	Asn	N	132.12	$C_4H_8N_2O_3$	$C_4H_6N_2O_2$	114.11	2.02	8.80	—	5.41
Aspartic acid	Asp	D	133.11	$C_4H_7NO_4$	$C_4H_5NO_3$	115.09	1.88	9.60	3.65	2.77
Cysteine	Cys	C	121.16	$C_3H_7NO_2S$	C_3H_5NOS	103.15	1.96	10.28	8.18	5.07
Glutamic acid	Glu	E	147.13	$C_5H_9NO_4$	$C_5H_7NO_3$	129.12	2.19	9.67	4.25	3.22
Glutamine	Gln	Q	146.15	$C_5H_{10}N_2O_3$	$C_5H_8N_2O_2$	128.13	2.17	9.13	—	5.65
Glycine	Gly	G	75.07	$C_2H_5NO_2$	C_2H_3NO	57.05	2.34	9.60	—	5.97
Histidine	His	H	155.16	$C_6H_9N_3O_2$	$C_6H_7N_3O$	137.14	1.82	9.17	6.00	7.59
Hydroxyproline	Hyp	O	131.13	$C_5H_9NO_3$	$C_5H_7NO_2$	113.11	1.82	9.65	—	—
Isoleucine	Ile	I	131.18	$C_6H_{13}NO_2$	$C_6H_{11}NO$	113.16	2.36	9.60	—	6.02
Leucine	Leu	L	131.18	$C_6H_{13}NO_2$	$C_6H_{11}NO$	113.16	2.36	9.60	—	5.98
Lysine	Lys	K	146.19	$C_6H_{14}N_2O_2$	$C_6H_{12}N_2O$	128.18	2.18	8.95	10.53	9.74
Methionine	Met	M	149.21	$C_5H_{11}NO_2S$	C_5H_9NOS	131.20	2.28	9.21	—	5.74
Phenylalanine	Phe	F	165.19	$C_9H_{11}NO_2$	C_9H_9NO	147.18	1.83	9.13	—	5.48
Proline	Pro	P	115.13	$C_5H_9NO_2$	C_5H_7NO	97.12	1.99	10.60	—	6.30
Pyroglutamatic	Glp	U	139.11	$C_5H_7NO_3$	$C_5H_5NO_2$	121.09	—	—	—	5.68
Serine	Ser	S	105.09	$C_3H_7NO_3$	$C_3H_5NO_2$	87.08	2.21	9.15	—	5.68
Threonine	Thr	T	119.12	$C_4H_9NO_3$	$C_4H_7NO_2$	101.11	2.09	9.10	—	5.60
Tryptophan	Trp	W	204.23	$C_{11}H_{12}N_2O_2$	$C_{11}H_{10}N_2O$	186.22	2.83	9.39	—	5.89
Tyrosine	Tyr	Y	181.19	$C_9H_{11}NO_3$	$C_9H_9NO_2$	163.18	2.20	9.11	10.07	5.66
Valine	Val	V	117.15	$C_5H_{11}NO_2$	C_5H_9NO	99.13	2.32	9.62	—	5.96

[1] pK_a is the negative of the logarithm of the dissociation constant for the -COOH group
[2] pK_b is the negative of the logarithm of the dissociation constant for the $-NH_3^+$ group
[3] pK_x is the negative of the logarithm of the dissociation constant for any other group in the molecule
[4] pI is the pH at the isoelectric point

References: D. R. Lide, *Handbook of Chemistry and Physics, 72nd Edition*, CRC Press, Boca Raton, FL, 1991.

AROMATIC R Groups: The R group in this amino acids are hydrophobic side chain. Examples include are Phenylalanine, tyrosine and tryptophan.

POLAR, Uncharged R Groups: The R group in this amino acids are uncharged and they are more polar than hydrophoc amino acids. Examples include are serine, threonine, cysteine, asparagines and glutamine.

POSITVELY Charged R Groups: The R group in this amino acids are acidic with net negatie charge. Examples include are aspartate and glutamate.

NEGATIVELY Charged, R Groups: The R group in this amino acids are basic with net positive charge. Examples include are Arginine and Lysine.

Structure of protein

As disussed in previous lecture, Protein is made up of 20 naturally occurring amino acids. A typical amino acid contains a amino and a carboxyl group attached to the central α-carbon atom (Figure 33.1). The side chain attached to the αcentral carbon atom determines the chemical nature of different amino acids. Peptide bonds connect individual amino acids in a polypeptide chain. Each amino acid is linked to the neighboring amino acid through a acid amide bond between carboxyl group and amino group of the next amino acid. Every polypeptide chain has a free N- and Cterminals (Figure 33.1). Primary structure of a protein is defined as the amino acid sequence from N- to the C-terminus with a length of several hundred amino acids.

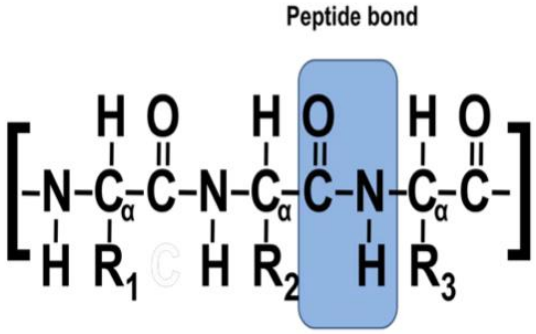


Figure 38.3: The connection between two adjacent amino acids in a polypeptide.

The ordered folding of polypeptide chain give rise to the 3-D conformation known as secondary structure of the protein such as helices, sheet and loops. Arrangement of the secondary structure gives rise to the tertiary structure. α-helix and β-sheet are connected via unstructured loops to arrange themselves in the protein structure and it allows the

secondary structure to change their direction. Tertiary structure defines the function of a protein, enzymatic activity or a nature of structural protein. Different polypeptide chains are arranged to give quaternary structure (Figure 33.2).

Primary Struture: The amino acid sequence of the protein is known as primary structure (Figure 33.2). The order of amino acid determine the folding of the protein to achieve net minimum free energy. This is achived in multiple steps collectively known as folding.

Secondary Structure: The amino acid interact with each and as a result peptide chain folds into the secondary structures. These secondary structures are the building blocks for the tertiary structures. These secondary structures are as follows:

α-Helix: it is a helical structure termed as α-helix by Linus pauling. In this structure, the polypeptide backbone is wound around the central axis with R group of the amino acid protrude outward from the helix backbone (Figure 32.2). In most of the protein, α-helix is right handed.

β-sheet: This is more extended conformation of polypeptide chain where R groups protrude from zigzag strture in opposite directions, giving a alternating structure (Figure 32.2).

Turns: These secondary structure has no definite structure and these are present in protein structure to change the direction of running polypeptide (Figure 32.2). These are also found to the places to connect the successive α-helix and β-sheet. The number of amino acids and their preference in turn is not consistent. The two protein can adopt similar 3-D conformation by changing the length and keeping amino acids in the tuen region of the structure.

Tertiary structure: Secondary structure folds to give rise higher order organization, commonly known as tertiary structure.

Quaternary Struture: If multiple polypeptides are involved in the constitution of protein, the tertiary structure of these different polypeptide chains come together to form quarternary structure.

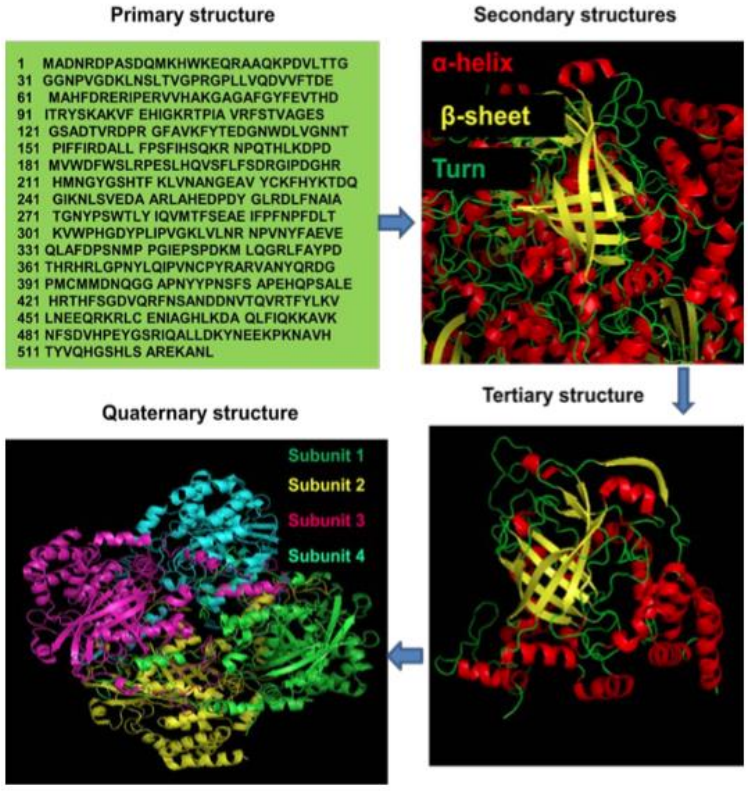
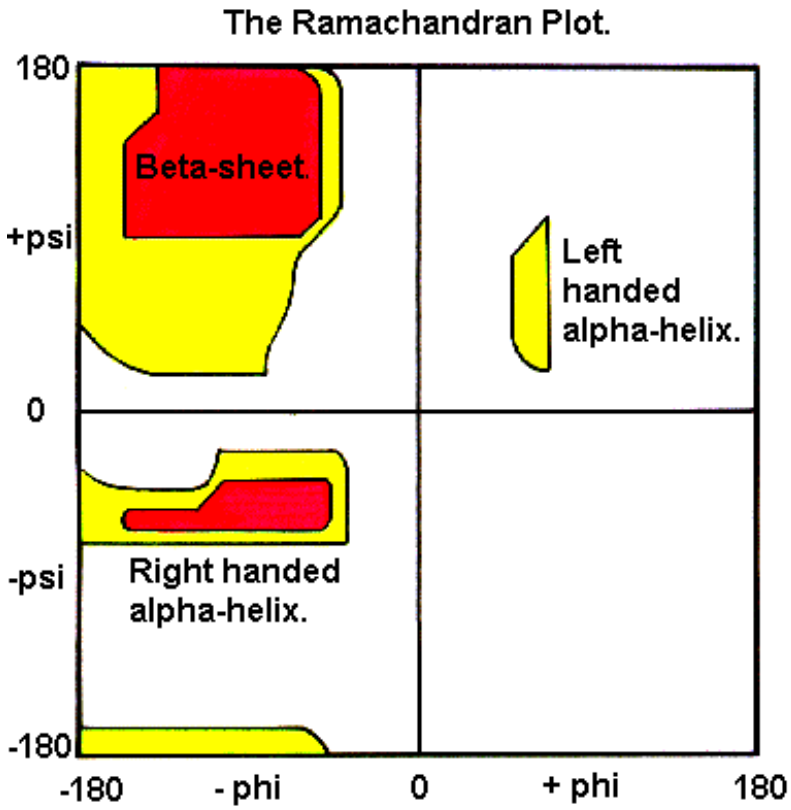


Figure 33.2: The different levels of organization in a protein structure.

The Ramachandran Plot

In a polypeptide the main chain N-Calpha and Calpha-C bonds relatively are free to rotate. These rotations are represented by the torsion angles phi and psi, respectively.

G N Ramachandran used computer models of small polypeptides to systematically vary phi and psi with the objective of finding stable conformations. For each conformation, the structure was examined for close contacts between atoms. Atoms were treated as hard spheres with dimensions corresponding to their van der Waals radii. Therefore, phi and psi angles which cause spheres to collide correspond to sterically disallowed conformations of the polypeptide backbone.



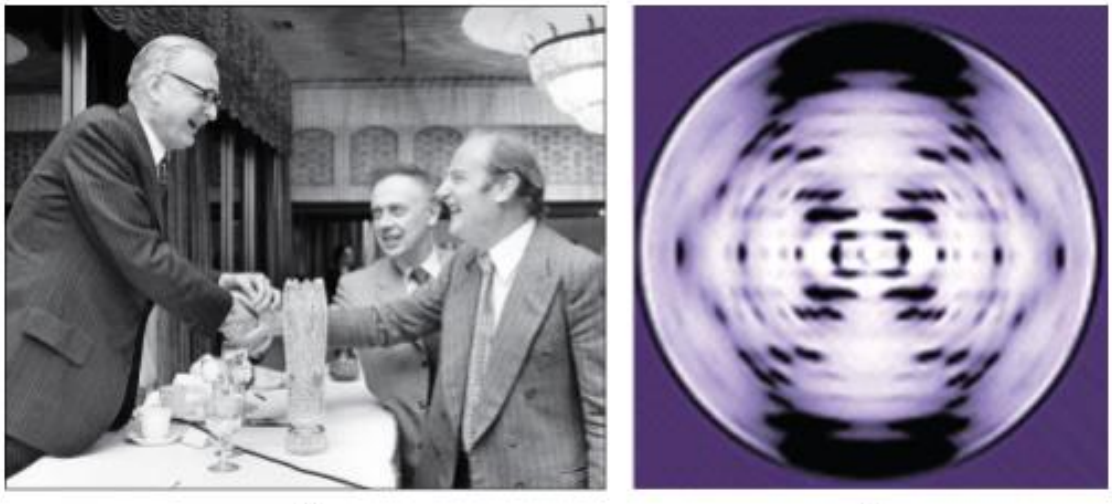
In the diagram above the white areas correspond to conformations where atoms in the polypeptide come closer than the sum of their van der Waals radi. These regions are sterically disallowed for all amino acids except glycine which is unique in that it lacks a side chain. The red regions correspond to conformations where there are no steric clashes, ie these are the allowed regions namely the alpha-helical and beta-sheet conformations. The yellow areas show the allowed regions if slightly shorter van der Waals radi are used in the calculation, ie the atoms are allowed to come a little closer together. This brings out an additional region which corresponds to the left-handed alpha-helix.

L-amino acids cannot form extended regions of left-handed helix but occassionally individual residues adopt this conformation. These residues are usually glycine but can also be asparagine or aspartate where the side chain forms a hydrogen bond with the main chain and therefore stabilises this otherwise unfavourable conformation. The 3(10) helix occurs close to the upper right of the alpha-helical region and is on the edge of allowed region indicating lower stability.

Disallowed regions generally involve steric hindrance between the side chain C-beta methylene group and main chain atoms. Glycine has no side chain and therefore can adopt phi and psi angles in all four quadrants of the Ramachandran plot. Hence it frequently occurs in turn regions of proteins where any other residue would be sterically hindered.

The Structure of DNA

In the 1950s, Francis Crick and James Watson worked together at the University of Cambridge, England, to determine the structure of DNA. Other scientists, such as Linus Pauling and Maurice Wilkins, were also actively exploring this field. Pauling had discovered the secondary structure of proteins using X-ray crystallography. X-ray crystallography is a method for investigating molecular structure by observing the patterns formed by X-rays shot through a crystal of the substance. The patterns give important information about the structure of the molecule of interest. In Wilkins' lab, researcher Rosalind Franklin was using X-ray crystallography to understand the structure of DNA. Watson and Crick were able to piece together the puzzle of the DNA molecule using Franklin's data ([Figure 1]). Watson and Crick also had key pieces of information available from other researchers such as Chargaff's rules. Chargaff had shown that of the four kinds of monomers (nucleotides) present in a DNA molecule, two types were always present in equal amounts and the remaining two types were also always present in equal amounts. This meant they were always paired in some way. In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Medicine for their work in determining the structure of DNA.



(a) (b)

Figure 1: Pioneering scientists (a) James Watson and Francis Crick are pictured here with American geneticist Maclyn McCarty. Scientist Rosalind Franklin discovered (b) the X-ray diffraction pattern of DNA, which helped to elucidate its double helix structure. (credit a: modification of work by Marjorie McCarty; b: modification of work by NIH)

Now let's consider the structure of the two types of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The building blocks of DNA are nucleotides, which are made up of three parts: a deoxyribose (5-carbon sugar), a phosphate group, and a nitrogenous

base ([Figure 2]). There are four types of nitrogenous bases in DNA. Adenine (A) and guanine (G) are double-ringed purines, and cytosine (C) and thymine (T) are smaller, single-ringed pyrimidines. The nucleotide is named according to the nitrogenous base it contains.

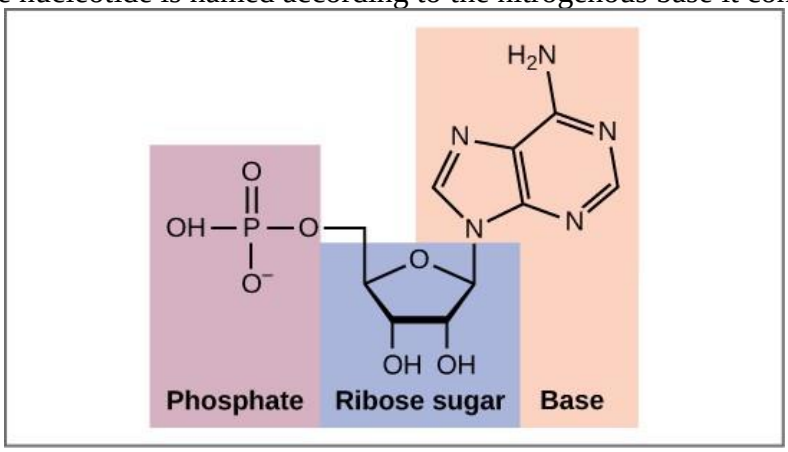


Figure 2: (a) Each DNA nucleotide is made up of a sugar, a phosphate group, and a base. (b) Cytosine and thymine are pyrimidines. Guanine and adenine are purines.

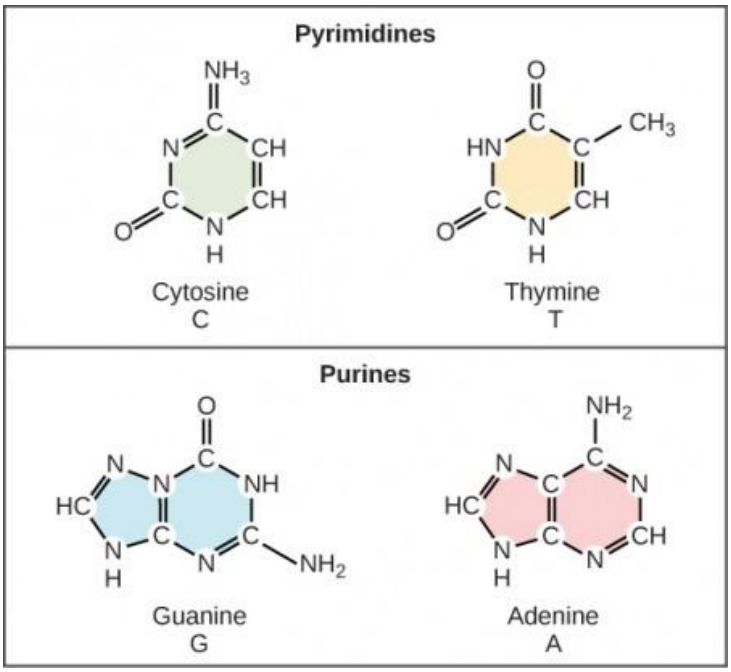


Figure 2: (a) Each DNA nucleotide is made up of a sugar, a phosphate group, and a base. (b) Cytosine and thymine are pyrimidines. Guanine and adenine are purines.

The phosphate group of one nucleotide bonds covalently with the sugar molecule of the next nucleotide, and so on, forming a long polymer of nucleotide monomers. The sugar–phosphate groups line up in a "backbone" for each single strand of DNA, and the nucleotide bases stick out from this backbone. The carbon atoms of the five-carbon sugar are numbered clockwise from the oxygen as 1′, 2′, 3′, 4′, and 5′ (1′ is read as "one prime"). The phosphate group is attached to the 5′ carbon of one nucleotide and the 3′ carbon of the next nucleotide. In its natural state, each DNA molecule is actually composed of two single strands held together along their length with hydrogen bonds between the bases.

Watson and Crick proposed that the DNA is made up of two strands that are twisted around each other to form a right-handed helix, called a double helix. Base-pairing takes place between a purine and pyrimidine: namely, A pairs with T, and G pairs with C. In other words, adenine and thymine are complementary base pairs, and cytosine and guanine are also complementary base pairs. This is the basis for Chargaff's rule; because of their complementarity, there is as much adenine as thymine in a DNA molecule and as much guanine as cytosine. Adenine and thymine are connected by two hydrogen bonds, and cytosine and guanine are connected by three hydrogen bonds. The two strands are anti-parallel in nature; that is, one strand will have the 3′ carbon of the sugar in the "upward" position, whereas the other strand will have the 5′ carbon in the upward position. The diameter of the DNA double helix is uniform throughout because a purine (two rings) always pairs with a pyrimidine (one ring) and their combined lengths are always equal. ([Figure 3]).

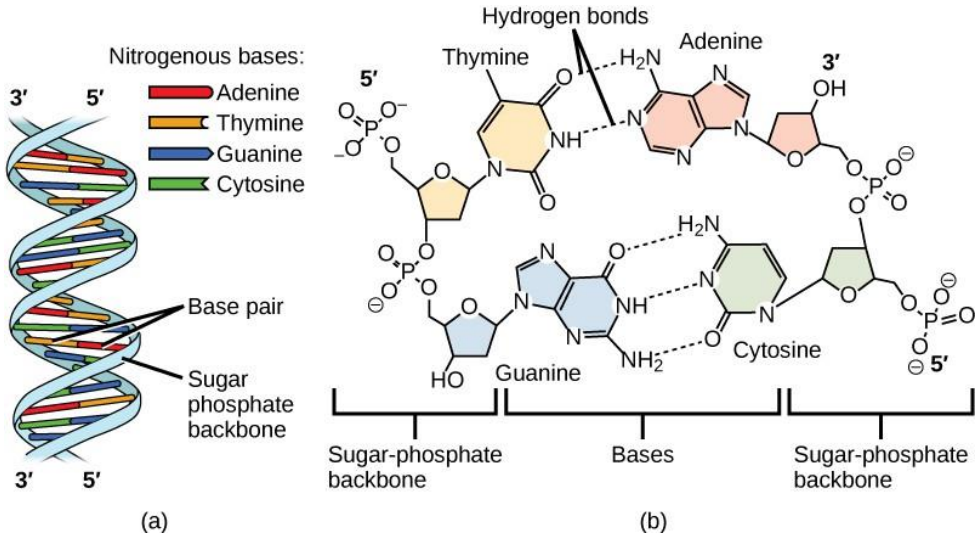


Figure 3: DNA (a) forms a double stranded helix, and (b) adenine pairs with thymine and cytosine pairs with guanine. (credit a: modification of work by Jerome Walker, Dennis Myts)

DNA Replication

When a cell divides, it is important that each daughter cell receives an identical copy of the DNA. This is accomplished by the process of DNA replication. The replication of DNA occurs during the synthesis phase, or S phase, of the cell cycle, before the cell enters mitosis or meiosis.

The elucidation of the structure of the double helix provided a hint as to how DNA is copied. Recall that adenine nucleotides pair with thymine nucleotides, and cytosine with guanine. This means that the two strands are complementary to each other. For example, a strand of DNA with a nucleotide sequence of AGTCATGA will have a complementary strand with the sequence TCAGTACT ([Figure 1]).

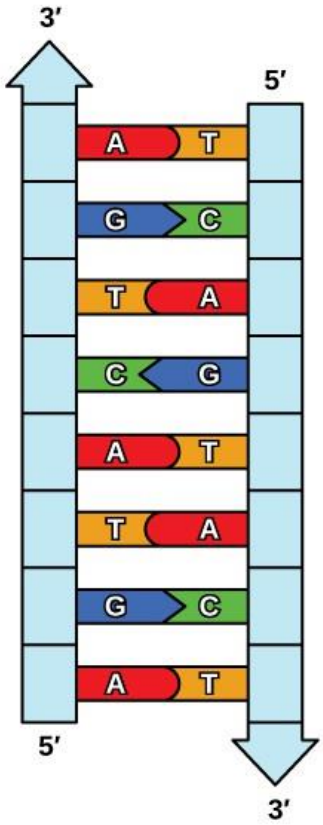


Figure 1: The two strands of DNA are complementary, meaning the sequence of bases in one strand can be used to create the correct sequence of bases in the other strand.

Because of the complementarity of the two strands, having one strand means that it is possible to recreate the other strand. This model for replication suggests that the two strands of the double helix separate during replication, and each strand serves as a template from which the new complementary strand is copied ([Figure 2]).

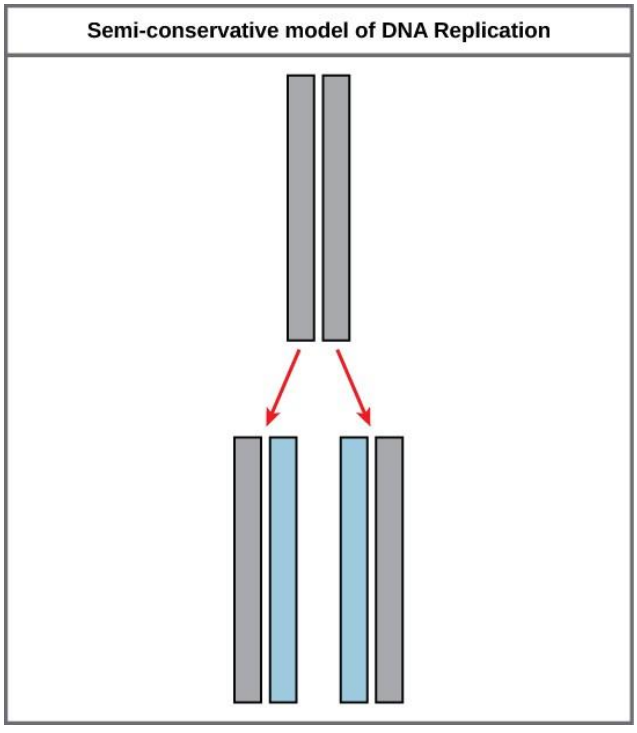


Figure 2: The semiconservative model of DNA replication is shown. Gray indicates the original DNA strands, and blue indicates newly synthesized DNA.

During DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are copied. The new strand will be complementary to the parental or "old" strand. Each new double strand consists of one parental strand and one new daughter strand. This is known as semiconservative replication. When two DNA copies are formed, they have an identical sequence of nucleotide bases and are divided equally into two daughter cells.

Eukaryotic DNA replication is a highly dynamic process that requires the coordinated and tightly regulated action of many molecular machines. Eukaryotic cells must assemble many protein complexes (referred to here as DNA synthesomes) at hundreds of origins of replication, and must activate them according to a strict temporal programme. The combination of genetic and biochemical approaches that have been used so far has begun to reveal the mechanisms that underlie these intricate networks of interactions, as well as the connections between the DNA synthesome and the cell cycle and checkpoint machineries.

Virus Structures

Structure of Viruses

Viruses vary in their structure. A virus particle consists of DNA or RNA within a protective protein coat called a **capsid.** The shape of the capsid may vary from one type of virus to another. The capsid is made from the proteins that are encoded by viral genes within their genome.

The shape of the capsid serves as one basis for classification of viruses. The capsid of the virus shown in **Figure** below is icosahedral. Virally coded proteins will self-assemble to form a capsid. Some viruses have an envelope of phospholipids and proteins. The envelope is made from portions of the host's cell membrane. It surrounds the capsid and helps protect the virus from the host's immune system. The envelope may also have receptor molecules that can bind with host cells. They make it easier for the virus to infect the cells.

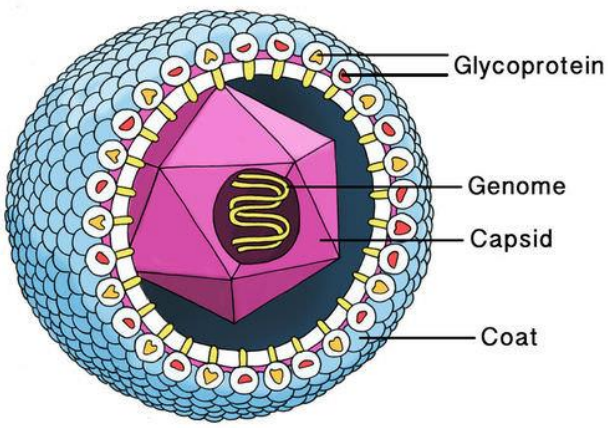
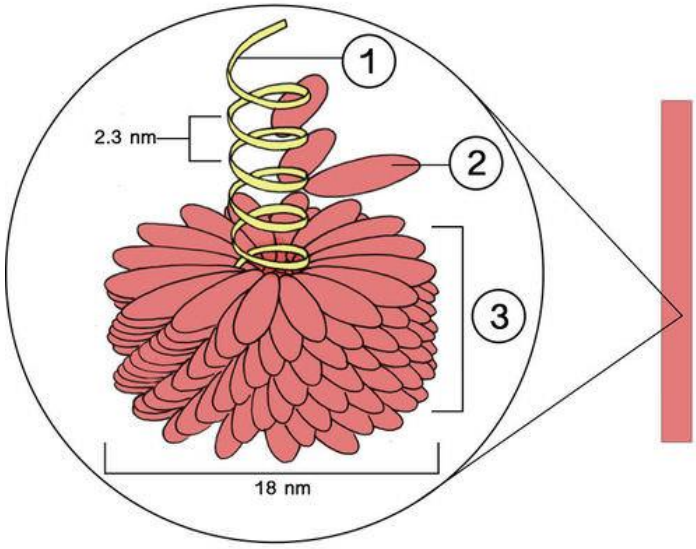


Diagram of a Cytomegalovirus. The capsid encloses the genetic material of the virus. The envelope which surrounds the capsid is typically made from portions of the host cell membranes (phospholipids and proteins). Not all viruses have a viral envelope.

Helical Viruses

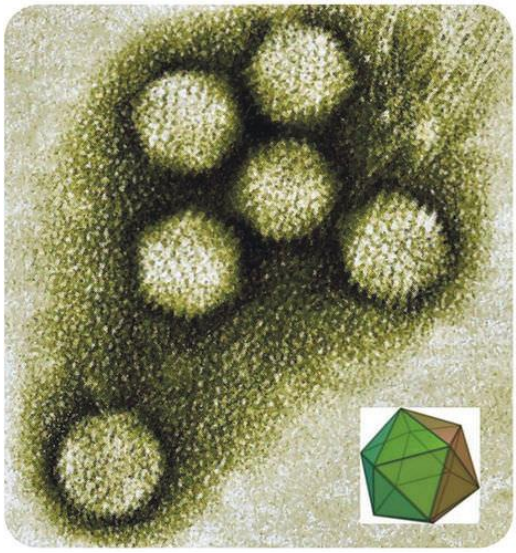
Helical capsids are made up of a single type of protein subunit stacked around a central axis to form a helical structure. The helix may have a hollow center, which makes it look like a hollow tube. This arrangement results in rod-shaped or filamentous virions. These virions can be anything from short and very rigid, to long and very flexible. The well-studied tobacco mosaic virus (TMV) is an example of a helical virus, as seen in the **Figure** below.



A helical virus, tobacco mosaic virus. Although their diameter may be very small, some helical viruses can be quite long, as shown here. 1. Nucleic acid; 2. Viral protein units, 3. Capsid. TMV causes tobacco mosaic disease in tobacco, cucumber, pepper, and tomato plants.

Icosahedral Viruses

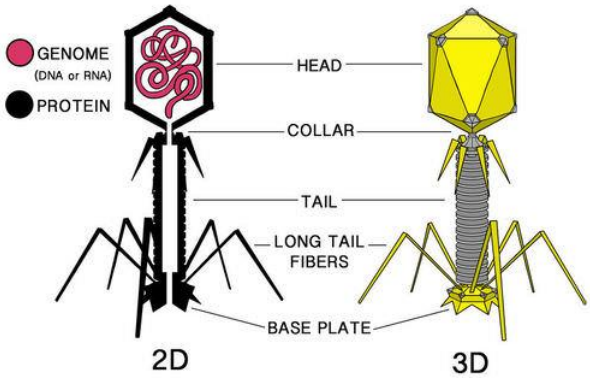
Icosahedral capsid symmetry gives viruses a spherical appearance at low magnification, but the protein subunits are actually arranged in a regular geometrical pattern, similar to a soccer ball; they are not truly spherical. An icosahedral shape is the most efficient way of creating a hardy structure from multiple copies of a single protein. This shape is used because it can be built from a single basic unit protein which is used over and over again. This saves space in the viral genome.



Adenovirus, an icosahedral virus. An icosahedron is a three-dimensional shape made up of 20 equilateral triangles. Viral structures are built of repeated identical protein subunits, making the icosahedron the easiest shape to assemble using these subunits.

Complex Viruses

Complex viruses possess a capsid which is neither purely helical, nor purely icosahedral, and which may have extra structures such as protein tails or a complex outer wall. Viral protein subunits will self-assemble into a capsid, but the complex viruses DNA also codes for proteins which help in building the viral capsid. Many phage viruses are complex-shaped; they have an icosahedral head bound to a helical tail. The tail may have a base plate with protein tail fibers. Some complex viruses do not have tail fibers.

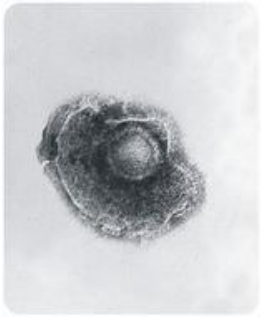


This “moon lander”-shaped complex virus infects *Escherichia coli* bacteria.

Enveloped Viruses

Some viruses are able to surround (envelop) themselves in a portion of the cell membrane of their host. The virus can use either the outer membrane of the host cell, or an internal membrane such as the nuclear membrane or endoplasmic reticulum. In this way the virus gains an outer lipid bilayer known as a **viral envelope**. This membrane is studded with proteins coded for by both the viral genome and the host genome. However, the lipid membrane itself and any

carbohydrates present come entirely from the host cell. The influenza virus, HIV, and the varicella zoster virus (**Figure** below) are enveloped viruses.



An enveloped virus. Varicella zoster virus causes chicken pox and shingles.

The viral envelope can give a virus some advantages over other capsid-only viruses. For example, they have better protection from the host's immune system, enzymes and certain chemicals. The proteins in the envelope can include glycoproteins, which act as receptor molecules. These receptor molecules allow host cells to recognize and bind the virions, which may result in easier uptake of the virion into the cell. Most enveloped viruses depend on their envelopes to infect cells. However, because the envelope contains lipids, it makes the virus more susceptible to inactivation by environmental agents, such as detergents that disrupt lipids.

Summary

- Viruses have different shapes. They can be cylindrical, icosahedral, complex, or enveloped.

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UNIT V - BIOMECHANICS, NEURO - BIOPHYSICS

Contractile proteins - mechanical properties of muscles - contraction mechanism - the cardiovascular system - blood pressure - electrocardiography - the nervous system, CNS and PNS - nerve cells - membrane potentials - sensory mechanisms - eye and ear - signal transduction.

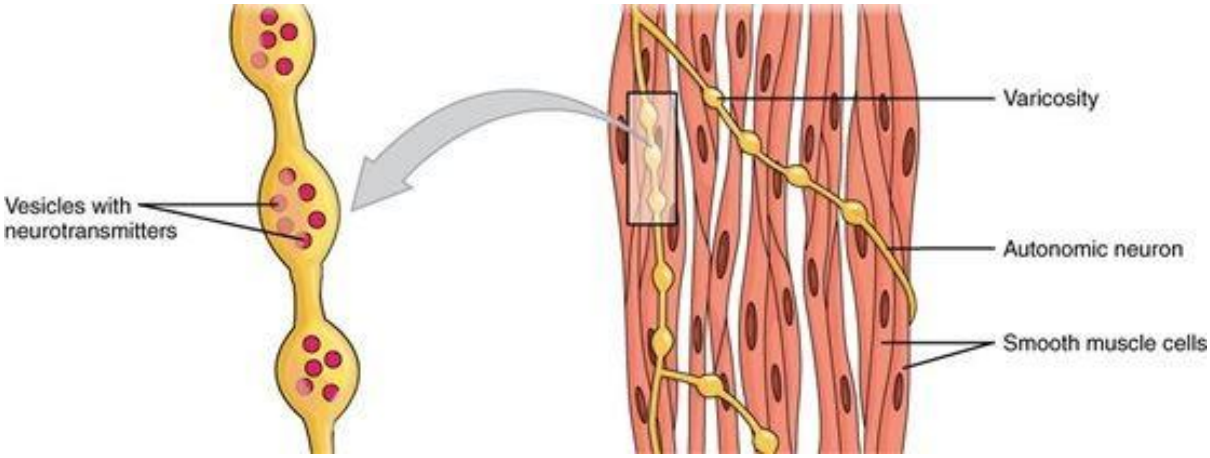
Unit V

The daily routine of an average person is loaded with lots of activities like walking, running, writing, typing and so on. We know that muscles in our body help us in doing all these activities, but how? In simple terms, we could say that muscle contraction and relaxation is the principle. Let us see the **structure of contractile proteins** followed by muscle contraction mechanism.

Contractile Proteins

Skeletal muscle is composed of muscle fibers which have smaller units called myofibrils. There are three types of proteins that make up each myofibril; they are contractile, regulatory and structural proteins.

By contractile proteins, we mean **actin** (thin filament) and **myosin** (thick filament). Each actin filament is composed of two helical "F" actin (filamentous actin) and each 'F' actin is made up of multiple units of 'G' actin. Along with the 'F' actin, two filaments of regulatory proteins tropomyosin and troponin at regular intervals are present. During muscle relaxation, troponin covers the binding sites for myosin on actin filaments.



Muscle Contraction

Each myosin is composed of multiple units of **meromyosin** which has two important parts- a globular head known as heavy meromyosin with a short arm and a tail known as light meromyosin. The head and arms project at regular distance and angle from each other from the surface of myosin filament and are known as the cross arm. The head bears binding sites for ATP and active sites for actin. Let us now try to understand the muscle contraction mechanism.

Muscle Contraction

During contraction, the thin filaments slide over the thick filaments. A signal sent by the central nervous system via motor neuron initiates muscle contraction. The **neuromuscular junction** is the junction between a motor neuron and sarcolemma. **Acetylcholine** is released when a neural

signal reaches this junction and action potential is generated in the sarcolemma. When this spreads through the muscle fibre, calcium ion is released in the sarcoplasm. Calcium then binds to troponin on actin filaments and exposes the active sites for myosin. Myosin binds to the exposed active site on actin using energy from the hydrolysis of ATP. This pulls the actin towards the centre. The Z lines attached to these are also pulled, and contraction occurs. Myosin is in a relaxed state.

Consequently, the hydrolysis of ATP at the myosin head continues and this leads to further sliding. This is repeated till calcium ions are pumped back to the sarcolemma and results in covering of the actin sites again. The Z lines move back to their original positions. This causes relaxation. Muscle fatigue occurs due to repeated activation of the muscles leading to accumulation of lactic acid.

Muscles appear in red colour due to a pigment called **myoglobin.** Myoglobin rich muscles are called red fibers. They also contain lots of mitochondria, which they may use for energy production. Muscles which lack myoglobin and white in colour are called white fibers.

the cardiovascular system

The cardiovascular system can be thought of as the transport system of the body. This system has three main components: the heart, the blood vessel and the blood itself. The heart is the system's pump and the blood vessels are like the delivery routes. Blood can be thought of as a fluid which contains the oxygen and nutrients the body needs and carries the wastes which need to be removed. The following information describes the structure and function of the heart and the cardiovascular system as a whole.

The cardiovascular system refers to the heart, blood vessels and the blood. Blood contains oxygen and other nutrients which your body needs to survive. The body takes these essential nutrients from the blood. At the same time, the body dumps waste products like carbon dioxide, back into the blood, so they can be removed. The main function of the cardiovascular system is therefore to maintain blood flow to all parts of the body, to allow it to survive. Veins deliver used blood from the body back to the heart. Blood in the veins is low in oxygen (as it has been taken out by the body) and high in carbon dioxide (as the body has unloaded it back into the blood). All the veins drain into the superior and inferior vena cava which then drain into the right atrium. The right atrium pumps blood into the right ventricle. Then the right ventricle pumps blood to the pulmonary trunk, through the pulmonary arteries and into the lungs. In the lungs the blood picks up oxygen that we breathe in and gets rid of carbon dioxide, which we breathe out. The blood is becomes rich in oxygen which the body can use. From the lungs, blood drains into the left atrium and is then pumped into the left ventricle. The left ventricle then pumps this oxygen-rich blood out into the aorta which then distributes it to the rest of the body through other arteries. The main arteries which branch off the aorta and take blood to specific parts of the body are:

- Carotid arteries, which take blood to the neck and head
- Coronary arteries, which provide blood supply to the heart itself
- Hepatic artery, which takes blood to the liver with branches going to the stomach
- Mesenteric artery, which takes blood to the intestines
- Renal arteries, which takes blood to the kidneys
- Femoral arteries, which take blood to the legs

The body is then able to use the oxygen in the blood to carry out its normal functions. This blood will again return back to the heart through the veins and the cycle continues.

What is the Cardiac Cycle?

The cardiac cycle is the sequence of events that occurs in one complete beat of the heart. The pumping phase of the cycle, also known as systole, occurs when heart muscle contracts. The filling phase, which is known as diastole, occurs when heart muscle relaxes. At the beginning of the cardiac cycle, both atria and ventricles are in diastole. During this time, all the chambers of the heart are relaxed and receive blood. The atrioventricular valves are open. Atrial systole follows this phase. During atrial systole, the left and right atria contract at the same time and push blood into the left and right ventricles, respectively. The next phase is ventricular systole. During ventricular systole, the left and right ventricles contract at the same time and pump blood into the aorta and pulmonary trunk, respectively. In ventricular systole, the atria are relaxed and receive blood. The atrioventricular valves close immediately after ventricular systole begins to stop blood going back into the atria. However, the semilunar valves are open during this phase to allow the blood to flow into the aorta and pulmonary trunk. Following this phase,Â the ventricles relax that is ventricular diastole occurs. The semilunar valves close to stop the blood from flowing back into the ventricles from the aorta and pulmonary trunk. The atria and ventricles once again are in diastole together and the cycle begins again.

Components of the Heartbeat

The adult heart beats around 70 to 80 times a minute at rest. When you listen to your heart with a stethoscope you can hear your heart beat. The sound is usually described as “lubb-dupp”. The “lubb” also known as the first heart sound, is caused by the closure of the atrioventricular valves. The “dupp” sound is due to the closure of the semilunar valves when the ventricles relax (at the beginning of ventricular diastole). Abnormal heart sounds are known as murmurs. Murmurs may indicate a problem with the heart valves, but many types of murmur are no cause for concern.

The Electrocardiogram

The heart has an inbuilt rhythm of contraction and relaxation. A small group of heart muscle cells called the pacemaker help achieve this. The pacemaker generates an electrical impulse which spreads over the atria, making them contract. This impulse then spreads to the ventricles, causing them to contract. The electrical changes that spread through the heart can be detected at the surface of the body by an instrument called the electrocardiograph. Electrodes are placed in a number of positions over the chest and the electrical changes are recorded on moving graph paper as an electrocardiogram (ECG).

Blood pressure

Blood pressure is a vital sign reflecting the pressure exerted on blood vessels when blood is forced out of the heart during contraction.

- Diastole is the relaxation of the chambers of the heart and systole is the contraction of the heart chambers.
- Blood pressure is composed of systolic and diastolic blood pressure, which correspond to the pressure following contraction of the heart and pressure during relaxation for the heart, respectively. Normal blood pressure should be around 120/80, with the systolic number on top.

- Mean blood pressure decreases as the circulating blood moves away from the heart through arteries, capillaries, and veins due to viscous loss of energy. Mean blood pressure drops during circulation, although most of this decrease occurs along the small arteries and arterioles.

Blood pressure is the pressure that blood exerts on the wall of the blood vessels. This pressure originates in the contraction of the heart, which forces blood out of the heart and into the blood vessels.

Two mechanisms take place in the heart: diastole and systole. Diastole is the relaxation of the chambers of the heart and systole is the contraction of the heart chambers. Systolic pressure is thus the pressure that your heart emits when blood is forced out of the heart and diastolic pressure is the pressure exerted when the heart is relaxed. This is the main mechanism by which blood pressure operates.

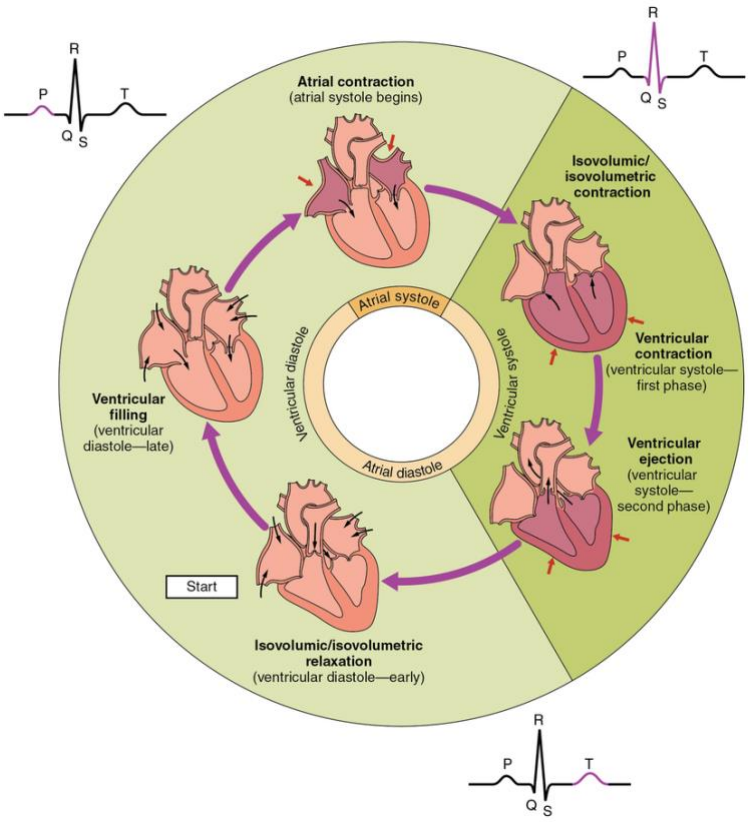
Blood pressure is one of the principal vital signs. During each heartbeat, blood pressure varies between a maximum (systolic) and a minimum (diastolic) pressure. A normal blood pressure should be around 120/80, with the systolic pressure expressed first.

Differences in mean blood pressure are responsible for blood flow from one location to another in circulation. The rate of mean blood flow depends on the resistance to flow presented by the blood vessels. Mean blood pressure decreases as circulating blood moves away from the heart through arteries, capillaries, and veins due to viscous loss of energy. Mean blood pressure decreases during circulation, although most of this decrease occurs along the small arteries and arterioles. Gravity affects blood pressure via hydrostatic forces (for example, during standing) Valves in veins, breathing, and pumping from contraction of skeletal muscles also influence venous blood pressure.

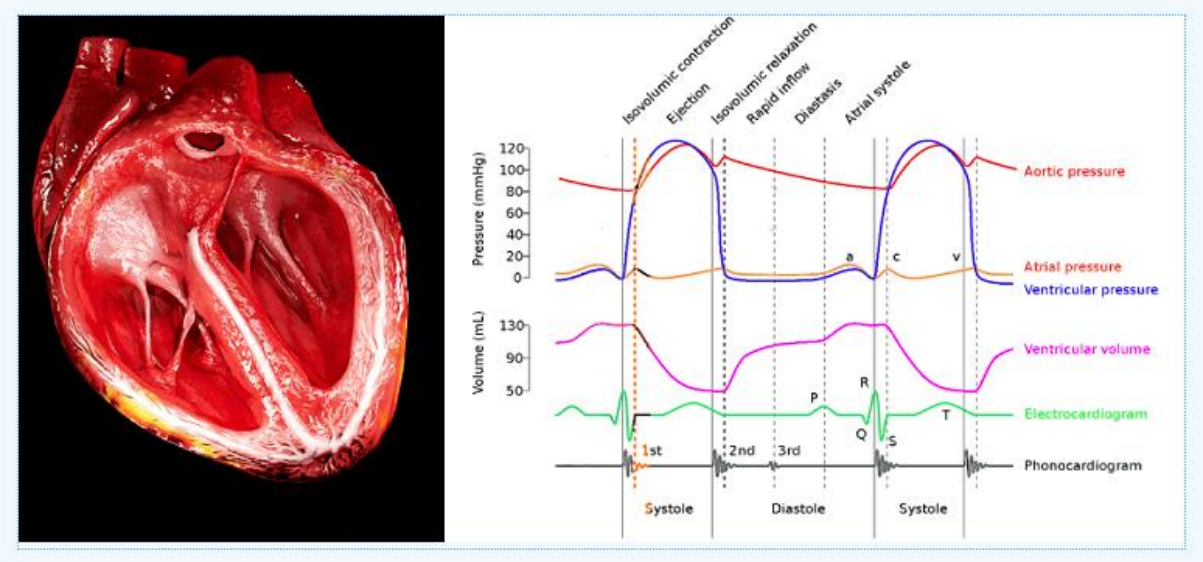
Electrocardiography

Cardiac Cycle

The period of time that begins with contraction of the atria and ends with ventricular relaxation is known as the **cardiac cycle**. The period of contraction that the heart undergoes while it pumps blood into circulation is called **systole**. The period of relaxation that occurs as the chambers fill with blood is called **diastole**. Both the atria and ventricles undergo systole and diastole, and it is essential that these components be carefully regulated and coordinated to ensure blood is pumped efficiently to the body.



Overview of the Cardiac Cycle. The cardiac cycle begins with atrial systole and progresses to ventricular systole, atrial diastole, and ventricular diastole, when the cycle begins again. Correlations to the ECG are highlighted.



Pressures and Flow

Fluids, whether gases or liquids, are materials that flow according to pressure gradients—that is, they move from regions that are higher in pressure to regions that are lower in pressure. Accordingly, when the heart chambers are relaxed (diastole), blood will flow into the atria from

the veins, which are higher in pressure. As blood flows into the atria, the pressure will rise, so the blood will initially move passively from the atria into the ventricles. When the action potential triggers the muscles in the atria to contract (atrial systole), the pressure within the atria rises further, pumping blood into the ventricles. During ventricular systole, pressure rises in the ventricles, pumping blood into the pulmonary trunk from the right ventricle and into the aorta from the left ventricle. Again, as you consider this flow and relate it to the conduction pathway, the elegance of the system should become apparent.

Phases of the Cardiac Cycle

At the beginning of the cardiac cycle, both the atria and ventricles are relaxed (diastole). Blood is flowing into the right atrium from the superior and inferior venae cavae and the coronary sinus. Blood flows into the left atrium from the four pulmonary veins. The two atrioventricular valves, the tricuspid and mitral valves, are both open, so blood flows unimpeded from the atria and into the ventricles. Approximately 70–80 percent of ventricular filling occurs by this method. The two semilunar valves, the pulmonary and aortic valves, are closed, preventing backflow of blood into the right and left ventricles from the pulmonary trunk on the right and the aorta on the left.

Atrial Systole and Diastole

Contraction of the atria follows depolarization, represented by the P wave of the ECG. As the atrial muscles contract from the superior portion of the atria toward the atrioventricular septum, pressure rises within the atria and blood is pumped into the ventricles through the open atrioventricular (tricuspid, and mitral or bicuspid) valves. At the start of atrial systole, the ventricles are normally filled with approximately 70–80 percent of their capacity due to inflow during diastole. Atrial contraction, also referred to as the "atrial kick," contributes the remaining 20–30 percent of filling (see Figure 19.3.119.3.1). Atrial systole lasts approximately 100 ms and ends prior to ventricular systole, as the atrial muscle returns to diastole.

Ventricular Systole

Ventricular systole (see Figure 19.3.119.3.1) follows the depolarization of the ventricles and is represented by the QRS complex in the ECG. It may be conveniently divided into two phases, lasting a total of 270 ms. At the end of atrial systole and just prior to atrial contraction, the ventricles contain approximately 130 mL blood in a resting adult in a standing position. This volume is known as the end diastolic volume (EDV) or preload.

Initially, as the muscles in the ventricle contract, the pressure of the blood within the chamber rises, but it is not yet high enough to open the semilunar (pulmonary and aortic) valves and be ejected from the heart. However, blood pressure quickly rises above that of the atria that are now relaxed and in diastole. This increase in pressure causes blood to flow back toward the atria, closing the tricuspid and mitral valves. Since blood is not being ejected from the ventricles at this early stage, the volume of blood within the chamber remains constant. Consequently, this initial phase of ventricular systole is known as isovolumic contraction, also called isovolumetric contraction (see Figure 19.3.119.3.1).

In the second phase of ventricular systole, the ventricular ejection phase, the contraction of the ventricular muscle has raised the pressure within the ventricle to the point that it is greater than the pressures in the pulmonary trunk and the aorta. Blood is pumped from the heart, pushing

open the pulmonary and aortic semilunar valves. Pressure generated by the left ventricle will be appreciably greater than the pressure generated by the right ventricle, since the existing pressure in the aorta will be so much higher. Nevertheless, both ventricles pump the same amount of blood. This quantity is referred to as stroke volume. Stroke volume will normally be in the range of 70–80 mL. Since ventricular systole began with an EDV of approximately 130 mL of blood, this means that there is still 50–60 mL of blood remaining in the ventricle following contraction. This volume of blood is known as the end systolic volume (ESV).

Ventricular Diastole

Ventricular relaxation, or diastole, follows repolarization of the ventricles and is represented by the T wave of the ECG. It too is divided into two distinct phases and lasts approximately 430 ms.

During the early phase of ventricular diastole, as the ventricular muscle relaxes, pressure on the remaining blood within the ventricle begins to fall. When pressure within the ventricles drops below pressure in both the pulmonary trunk and aorta, blood flows back toward the heart, producing the dicrotic notch (small dip) seen in blood pressure tracings. The semilunar valves close to prevent backflow into the heart. Since the atrioventricular valves remain closed at this point, there is no change in the volume of blood in the ventricle, so the early phase of ventricular diastole is called the isovolumic ventricular relaxation phase, also called isovolumetric ventricular relaxation phase (see Figure 19.3.119.3.1).

In the second phase of ventricular diastole, called late ventricular diastole, as the ventricular muscle relaxes, pressure on the blood within the ventricles drops even further. Eventually, it drops below the pressure in the atria. When this occurs, blood flows from the atria into the ventricles, pushing open the tricuspid and mitral valves. As pressure drops within the ventricles, blood flows from the major veins into the relaxed atria and from there into the ventricles. Both chambers are in diastole, the atrioventricular valves are open, and the semilunar valves remain closed (Figure 19.3.119.3.1). The cardiac cycle is complete. Figure 19.3.219.3.2 illustrates the relationship between the cardiac cycle and the ECG.

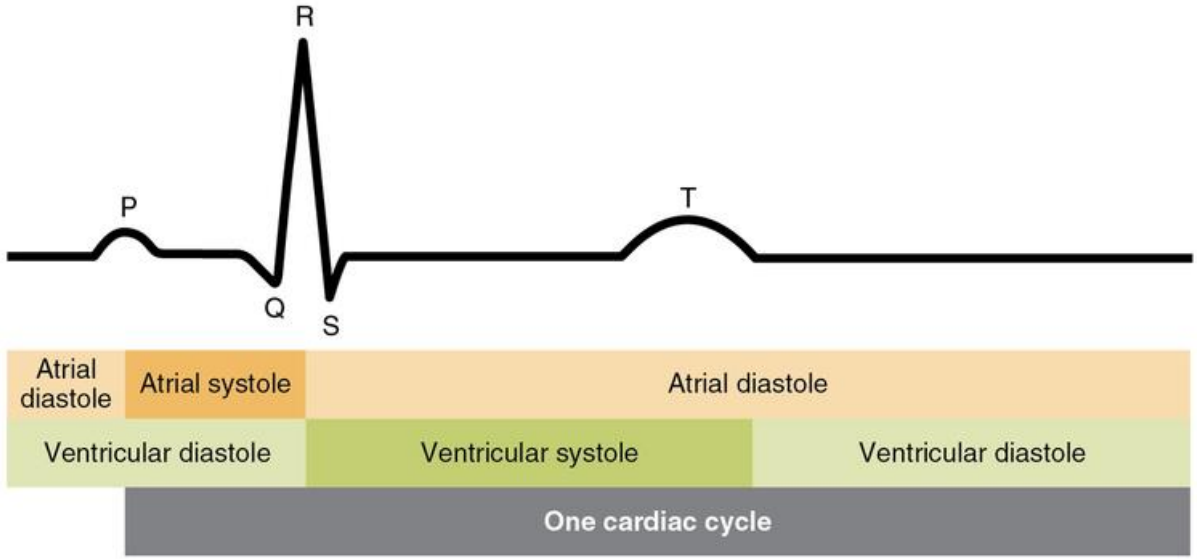


Figure: Relationship between the Cardiac Cycle and ECG. Initially, both the atria and ventricles are relaxed (diastole). The P wave represents depolarization of the atria and is

followed by atrial contraction (systole). Atrial systole extends until the QRS complex, at which point, the atria relax. The QRS complex represents depolarization of the ventricles and is followed by ventricular contraction. The T wave represents the repolarization of the ventricles and marks the beginning of ventricular relaxation.

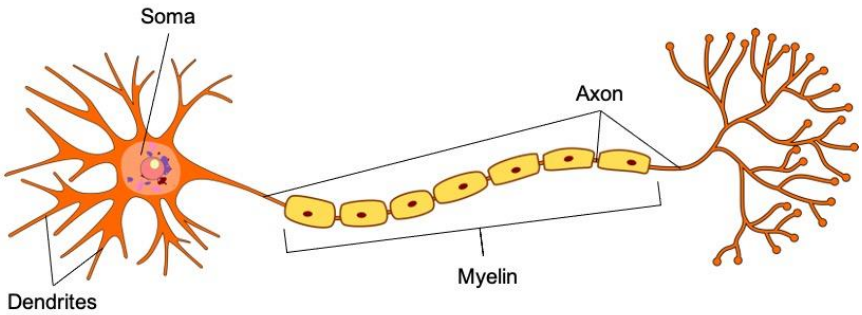
The nervous system, CNS, PNS and nerve cells

Organization and Functions of the Nervous System

The picture you have in your mind of the nervous system probably includes the **brain**, the nervous tissue contained within the cranium in the cranial cavity, and the **spinal cord**, the extension of nervous tissue within the vertebral column. That suggests it is made of two organs—and you may not even think of the spinal cord as an organ—but the nervous system is a very complex structure. Within the brain, many different and separate regions are responsible for many different and separate functions. It is as if the nervous system is composed of many organs that all look similar and can only be differentiated using tools such as the microscope or an electrophysiology apparatus. In comparison, it is easy to see that the stomach is different than the esophagus or the liver, so you can imagine the digestive system as a collection of specific organs. For this reason, you will look at the organization of the nervous tissue first and the structures within it. Then you will look at the structural divisions of the nervous system by regions where the organs are located, and then the functional divisions by the specific functions achieved by the organs within it.

Organization of the Nervous Tissue

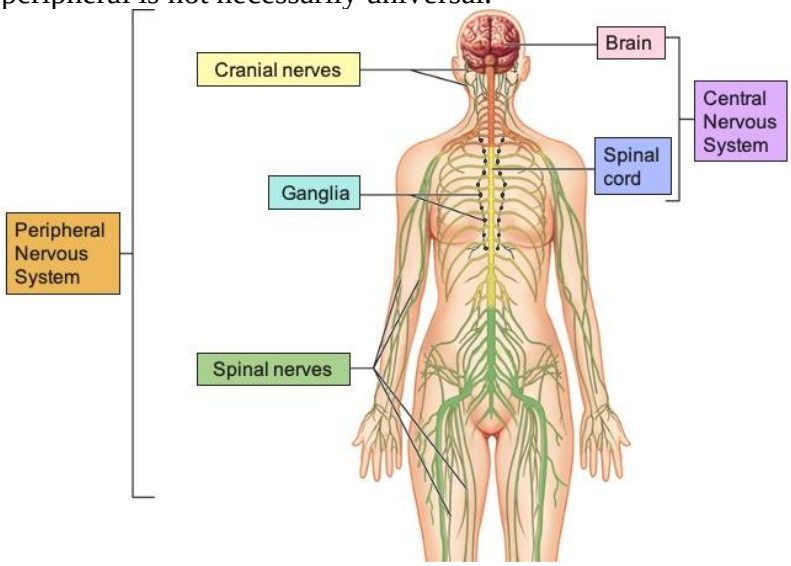
Nervous tissue contains two basic types of cells: neurons and glial cells. A **glial cell** is one of a variety of cells that provide a framework of tissue that supports the neurons and their activities. The **neuron** is the more functionally important of the two, in terms of the communicative function of the nervous system. To describe the functional divisions of the nervous system, it is important to understand the basic structure of a neuron. Neurons are cells and therefore have a **soma**, or **cell body**, but they also have extensions of the cell; each extension is generally referred to as a **process**. There is one important process that every neuron has called an **axon**, which is the fiber that connects a neuron with its target. Axons are partially insulated by a lipid-rich substance called **myelin**. Another type of process that branches off from the soma is the **dendrite**. Dendrites are responsible for receiving most of the input from other neurons.



Basic Structure of a Neuron. Neurons have a soma (or cell body) and processes, which are extensions from the soma that receive or carry information. Processes can be diversified as dendrites which receive information or axon which carries information. The axon shown here is covered by insulating sheaths of myelin.

Structural Divisions of the Nervous System

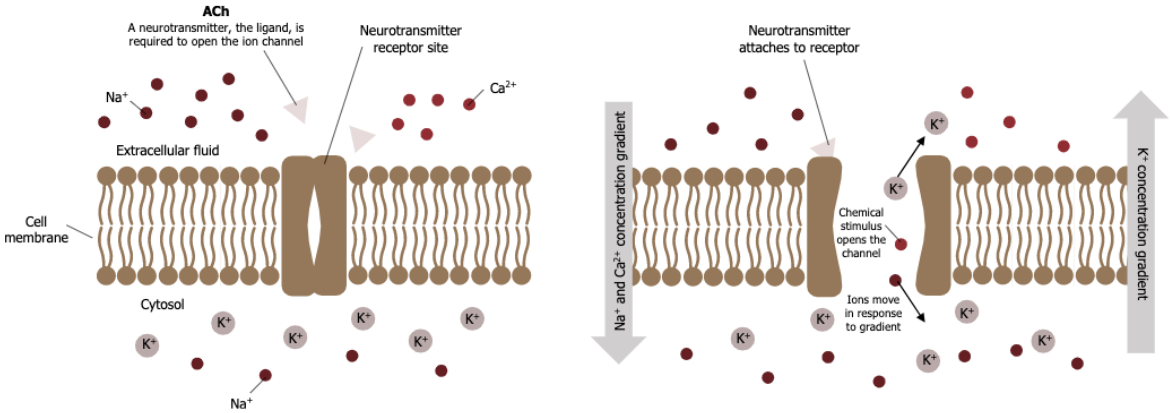
The nervous system can be divided into two major regions: the central and peripheral nervous systems. The **central nervous system (CNS)** is composed of the brain and spinal cord, and the **peripheral nervous system (PNS)** is composed of ganglia (structures containing neurons outside of the CNS) and nerves (bundles of axons from CNS and PNS neurons). The brain is contained within the cranial cavity of the skull, and the spinal cord is contained within the vertebral cavity of the vertebral column. It is a bit of an oversimplification to say that the CNS is what is inside these two cavities and the peripheral nervous system is outside of them, but that is one way to start to think about it. In actuality, there are some elements of the peripheral nervous system that are within the cranial or vertebral cavities. The peripheral nervous system is so named because it is on the periphery—meaning beyond the brain and spinal cord. Depending on different aspects of the nervous system, the dividing line between central and peripheral is not necessarily universal.



Structural Divisions of the Nervous System. The structures of the Central Nervous System (CNS) are the brain and spinal cord, located medially within the body. The brain is superior to the spinal cord. The structures of the Peripheral Nervous System (PNS) are ganglia and nerves. Nerves can be divided into cranial if they originate from the brain and spinal if they originate from the spinal cord. Spinal nerves originate laterally from the spinal cord. Ganglia are enlarged regions of the nerves that house neurons and glial cells within the PNS.

Membrane potentials –

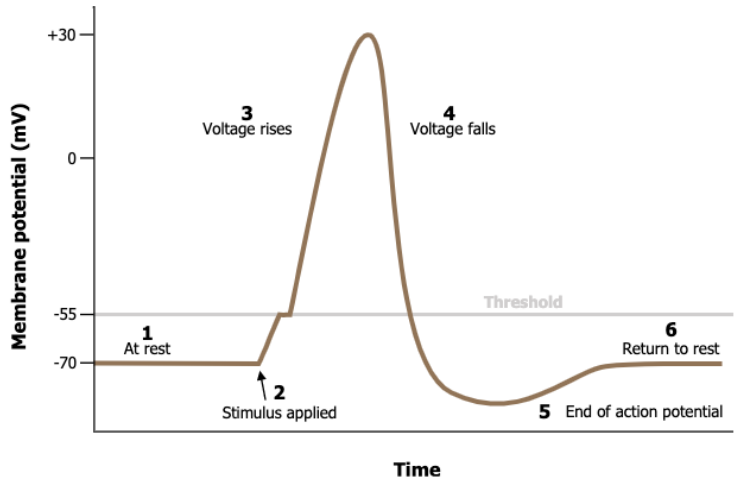
The electrical state of the cell membrane can have several variations. These are all variations in the membrane potential. A potential is a distribution of charge across the cell membrane, measured in millivolts (mV). The standard is to compare the inside of the cell relative to the outside, so the membrane potential is a value representing the charge on the intracellular side of the membrane based on the outside being zero, relatively speaking. Neurons harvest this membrane potential to generate and/or propagate a nerve impulse



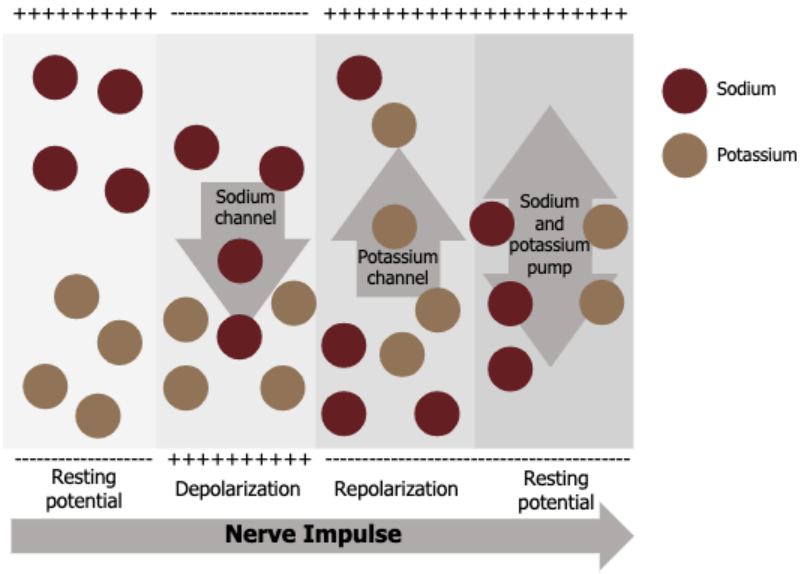
Neurotransmission by acetylcholine

The action potential

Resting membrane potential describes the steady state of the cell, which is a dynamic process that is balanced by ion leakage and ion pumping. Without any outside influence, it will not change. To get an electrical signal started, the membrane potential has to change.



Summary of the action potential to membrane potential.



Summary of the action potential as it relates to change in ion concentration across the membrane.

- This starts with a channel opening for Na+Na+ in the membrane. Because the concentration of Na+Na+ is higher outside the cell than inside the cell by a factor of 10, ions will rush into the cell that are driven largely by the concentration gradient. Because sodium is a positively charged ion, it will change the relative voltage immediately inside the cell relative to immediately outside.
- The resting potential is the state of the membrane at a voltage of -70 mV, so the sodium cation entering the cell will cause it to become less negative. This is known as depolarization, meaning the membrane potential moves toward zero.
- The concentration gradient for Na+Na+ is so strong that it will continue to enter the cell even after the membrane potential has become zero, so that the voltage immediately around the pore begins to become positive. The electrical gradient also plays a role, as negative proteins below the membrane attract the sodium ion. The membrane potential will reach +30 mV by the time sodium has entered the cell.
- As the membrane potential reaches +30 mV, other voltage-gated channels are opening in the membrane. These channels are specific for the potassium ion. A concentration gradient acts on K+K+, as well. As K+K+ starts to leave the cell, taking a positive charge with it, the membrane potential begins to move back toward its resting voltage. This is called repolarization, meaning that the membrane voltage back toward the -70 mV value of the resting membrane potential.
- Repolarization returns the membrane potential to the -70 mV value that indicates the resting potential, but it actually overshoots that value. Potassium ions reach equilibrium when the membrane voltage is below -70 mV, so a period of hyperpolarization occurs while the K+K+ channels are open. Those K+K+ channels are slightly delayed in closing, accounting for this short overshoot.

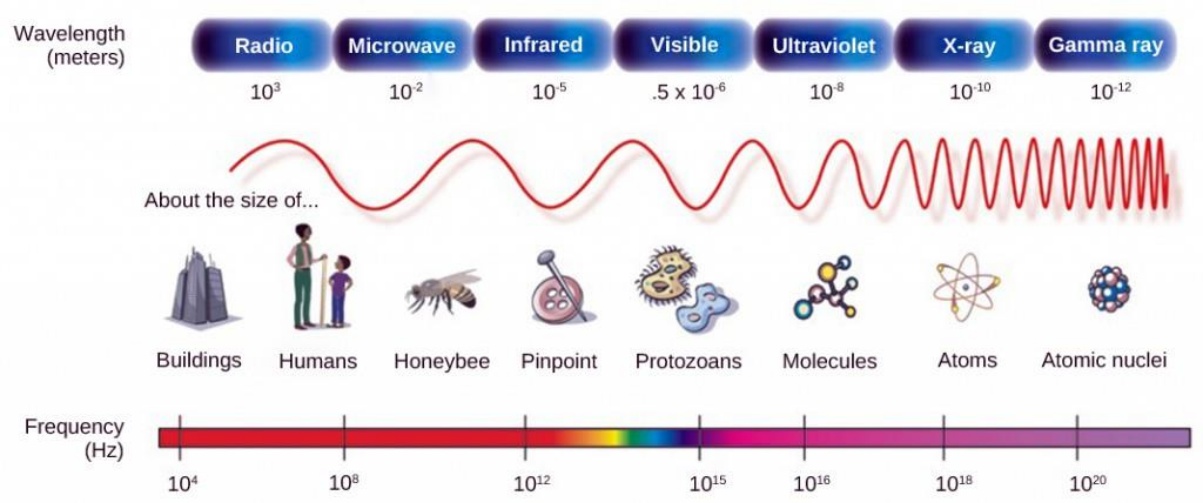
Sensory mechanisms - eye

Vision is the ability to detect light patterns from the outside environment and interpret them into images. Animals are bombarded with sensory information, and the sheer volume of visual information can be problematic. Fortunately, the visual systems of species have evolved to attend to the most-important stimuli. The importance of vision to humans is further substantiated by the fact that about one-third of the human cerebral cortex is dedicated to analyzing and perceiving visual information.

Light

As with auditory stimuli, light travels in waves. The compression waves that compose sound must travel in a medium—a gas, a liquid, or a solid. In contrast, light is composed of electromagnetic waves and needs no medium; light can travel in a vacuum (Figure 8.25). The behavior of light can be discussed in terms of the behavior of waves and also in terms of the behavior of the fundamental unit of light—a packet of electromagnetic radiation called a photon. A glance at the electromagnetic spectrum shows that visible light for humans is just a small slice of the entire spectrum, which includes radiation that we cannot see as light because it is below the frequency of visible red light and above the frequency of visible violet light.

Certain variables are important when discussing perception of light. Wavelength (which varies inversely with frequency) manifests itself as hue. Light at the red end of the visible spectrum has longer wavelengths (and is lower frequency), while light at the violet end has shorter wavelengths (and is higher frequency). The wavelength of light is expressed in nanometers (nm); one nanometer is one billionth of a meter. Humans perceive light that ranges between approximately 380 nm and 740 nm. Some other animals, though, can detect wavelengths outside of the human range. For example, bees see near-ultraviolet light in order to locate nectar guides on flowers, and some non-avian reptiles sense infrared light (heat that prey gives off).



In the electromagnetic spectrum, visible light lies between 380 nm and 740 nm. (credit: modification of work by NASA)

Wave amplitude is perceived as luminous intensity, or brightness. The standard unit of intensity of light is the *candela*, which is approximately the luminous intensity of a one common candle.

Light waves travel 299,792 km per second in a vacuum, (and somewhat slower in various media such as air and water), and those waves arrive at the eye as long (red), medium (green), and short (blue) waves. What is termed "white light" is light that is perceived as white by the human eye. This effect is produced by light that stimulates equally the color receptors in the human eye. The apparent color of an object is the color (or colors) that the object reflects. Thus a red object reflects the red wavelengths in mixed (white) light and absorbs all other wavelengths of light.

Anatomy of the eye

The photoreceptive cells of the eye, where transduction of light to nervous impulses occurs, are located in the *retina* (Figure 8.26) on the inner surface of the back of the eye. But light does not impinge on the retina unaltered. It passes through other layers that process it so that it can be interpreted by the retina (Figure 8.25b). The **cornea,** the front transparent layer of the eye, and the crystalline **lens**, a transparent convex structure behind the cornea, both refract (bend) light to focus the image on the retina. The **iris**, which is conspicuous as the colored part of the eye, is a circular muscular ring lying between the lens and cornea that regulates the amount of light entering the eye. In conditions of high ambient light, the iris contracts, reducing the size of the pupil at its center. In conditions of low light, the iris relaxes and the pupil enlarges.

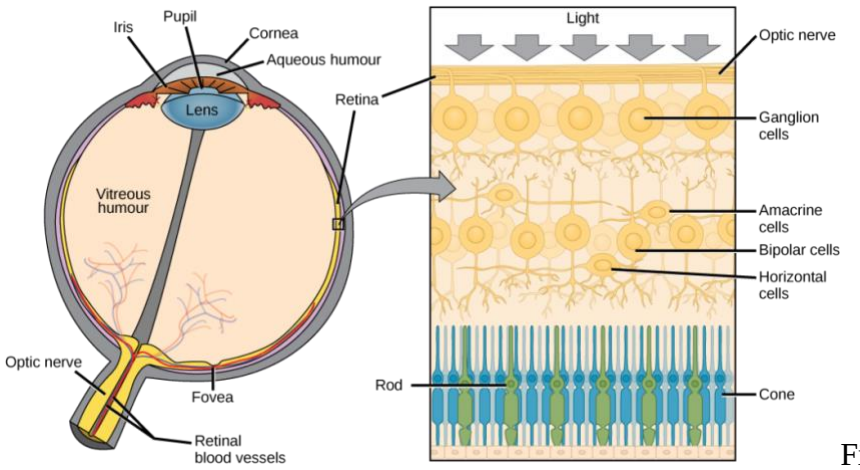


Figure 8.26. (a) The human eye is shown in cross section. (b) A blowup shows the layers of the retina.

There are two types of photoreceptors in the retina: **rods** and **cones**, named for their general appearance as illustrated in Figure 8.27. Rods are strongly photosensitive and are located in the outer edges of the retina. They detect dim light and are used primarily for peripheral and nighttime vision. Cones are weakly photosensitive and are located near the center of the retina. They respond to bright light, and their primary role is in daytime, color vision.

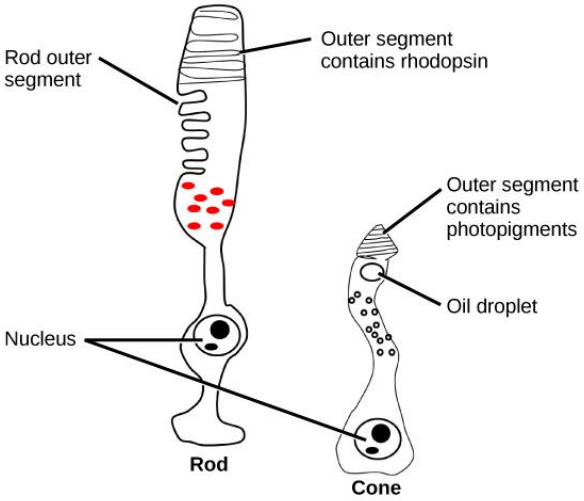


Figure 8.27. Rods and cones are photoreceptors in the retina. Rods respond in low light and can detect only shades of gray. Cones respond in intense light and are responsible for color vision. (credit: modification of work by Piotr Sliwa)

The **fovea** is the region in the center back of the eye that is responsible for acute vision. The fovea has a high density of cones. When you bring your gaze to an object to examine it intently in bright light, the eyes orient so that the object's image falls on the fovea. However, when looking at a star in the night sky or other object in dim light, the object can be better viewed by the peripheral vision because it is the rods at the edges of the retina, rather than the cones at the center, that operate better in low light. In humans, cones far outnumber rods in the fovea.

Transduction of light

The rods and cones are the site of transduction of light to a neural signal. Both rods and cones contain photopigments. In vertebrates, the main photopigment, **rhodopsin**, has two main parts: an opsin, which is a membrane protein (in the form of a cluster of α-helices that span the membrane), and retinal—a molecule that absorbs light. When light hits a photoreceptor, it causes a shape change in the retinal, altering its structure from a bent (*cis*) form of the molecule to its linear (*trans*) isomer. This isomerization of retinal activates the rhodopsin, starting a cascade of events that ends with the closing of Na^+ channels in the membrane of the photoreceptor. Thus, unlike most other sensory neurons (which become depolarized by exposure to a stimulus) visual receptors become hyperpolarized and thus driven away from threshold (Figure 8.28).

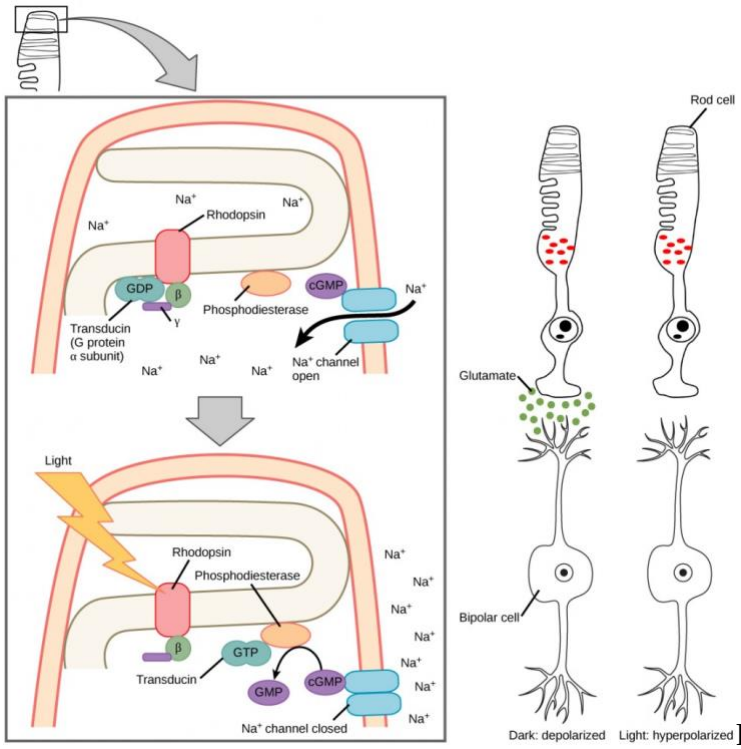


Figure 8.28. When light strikes rhodopsin, the G-protein transducin is activated, which in turn activates phosphodiesterase. Phosphodiesterase converts cGMP to GMP, thereby closing sodium channels. As a result, the membrane becomes hyperpolarized. The hyperpolarized membrane does not release glutamate to the bipolar cell.

Higher processing

The myelinated axons of ganglion cells make up the optic nerves. Within the nerves, different axons carry different qualities of the visual signal. Some axons constitute the magnocellular (big cell) pathway, which carries information about form, movement, depth, and differences in brightness. Other axons constitute the parvocellular (small cell) pathway, which carries information on color and fine detail. Some visual information projects directly back into the brain, while other information crosses to the opposite side of the brain. This crossing of optical pathways produces the distinctive optic chiasma (Greek, for "crossing") found at the base of the brain and allows us to coordinate information from both eyes.

Once in the brain, visual information is processed in several places, and its routes reflect the complexity and importance of visual information to humans and other animals. One route takes the signals to the thalamus, which serves as the routing station for all incoming sensory impulses except olfaction. In the thalamus, the magnocellular and parvocellular distinctions remain intact, and there are different layers of the thalamus dedicated to each. When visual signals leave the thalamus, they travel to the primary visual cortex at the rear of the brain. From the visual cortex, the visual signals travel in two directions. One stream that projects to the parietal lobe, in the side of the brain, carries magnocellular ("where") information. A second stream projects to the temporal lobe and carries both magnocellular ("where") and parvocellular ("what") information.

Another important visual route is a pathway from the retina to the **superior colliculus** in the midbrain, where eye movements are coordinated and integrated with auditory information. Finally, there is the pathway from the retina to the **suprachiasmatic nucleus** (SCN) of the

hypothalamus. The SCN is a cluster of cells that is considered to be the body's internal clock, which controls our **circadian** (day-long) cycle. The SCN sends information to the pineal gland, which is important in sleep/wake patterns and annual cycles.

Sensory mechanisms - ear

Hearing and vestibular information

Audition, or hearing, is important to humans and to other animals for many different interactions. It enables an organism to detect and receive information about danger, such as an approaching predator, and to participate in communal exchanges like those concerning territories or mating. On the other hand, although it is physically linked to the auditory system, the vestibular system is not involved in hearing. Instead, an animal's vestibular system detects its own movement, both linear and angular acceleration and deceleration, and balance.

Sound

Auditory stimuli are sound waves, which are mechanical, pressure waves that move through a medium, such as air or water. There are no sound waves in a vacuum since there are no air molecules to move in waves. The speed of sound waves differs, based on altitude, temperature, and medium, but at sea level and a temperature of 20º C (68º F), sound waves travel in the air at about 343 meters per second.

As is true for all waves, there are four main characteristics of a sound wave: frequency, wavelength, period, and amplitude. Frequency is the number of waves per unit of time, and in sound is heard as pitch. High-frequency (≥15.000Hz) sounds are higher-pitched (short wavelength) than low-frequency (long wavelengths; ≤100Hz) sounds. Frequency is measured in cycles per second, and for sound, the most commonly used unit is hertz (Hz), or cycles per second. Most humans can perceive sounds with frequencies between 30 and 20,000 Hz. Women are typically better at hearing high frequencies, but everyone's ability to hear high frequencies decreases with age. Dogs detect up to about 40,000 Hz; cats, 60,000 Hz; bats, 100,000 Hz; and dolphins 150,000 Hz, and American shad (**Alosa sapidissima**), a fish, can hear 180,000 Hz. Those frequencies above the human range are called **ultrasound**.

Amplitude, or the dimension of a wave from peak to trough, in sound is heard as volume and is illustrated in Figure 8.21. The sound waves of louder sounds have greater amplitude than those of softer sounds. For sound, volume is measured in decibels (dB). The softest sound that a human can hear is the zero point. Humans speak normally at 60 decibels.

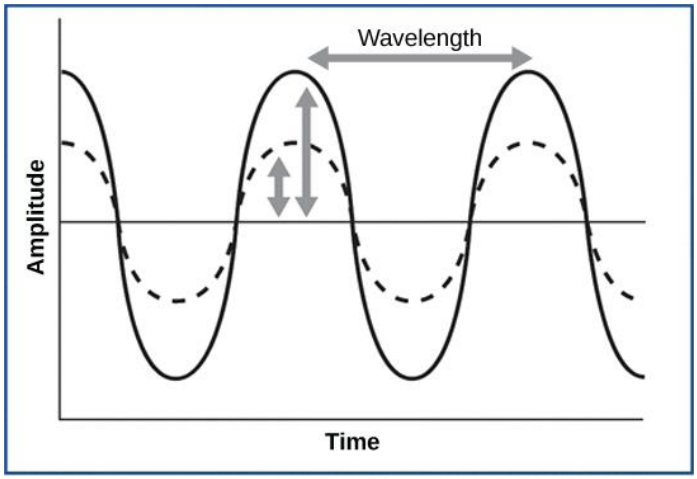


Figure. For sound waves, wavelength corresponds to pitch. Amplitude of the wave corresponds to volume. The sound wave shown with a dashed line is softer in volume than the sound wave shown with a solid line. (credit: NIH)

Reception of sound

In mammals, sound waves are collected by the external, cartilaginous part of the ear called the **pinna**, then travel through the auditory canal and cause vibration of the thin diaphragm called the **tympanum** or ear drum, the innermost part of the **outer ear** (illustrated in Figure 8.22). Interior to the tympanum is the **middle ear**. The middle ear holds three small bones called the **ossicles,** which transfer energy from the moving tympanum to the inner ear. The three ossicles are the **malleus**(also known as the hammer), the **incus** (the anvil), and **stapes** (the stirrup). The aptly named stapes looks very much like a stirrup. The three ossicles are unique to mammals, and each plays a role in hearing. The malleus attaches at three points to the interior surface of the tympanic membrane. The incus attaches the malleus to the stapes. In humans, the stapes is not long enough to reach the tympanum. If we did not have the malleus and the incus, then the vibrations of the tympanum would never reach the inner ear. These bones also function to collect force and amplify sounds. The ear ossicles are homologous to bones in a fish mouth: the bones that support gills in fish are thought to be adapted for use in the vertebrate ear over evolutionary time. Many animals (frogs, reptiles, and birds, for example) use the stapes of the middle ear to transmit vibrations to the middle ear.

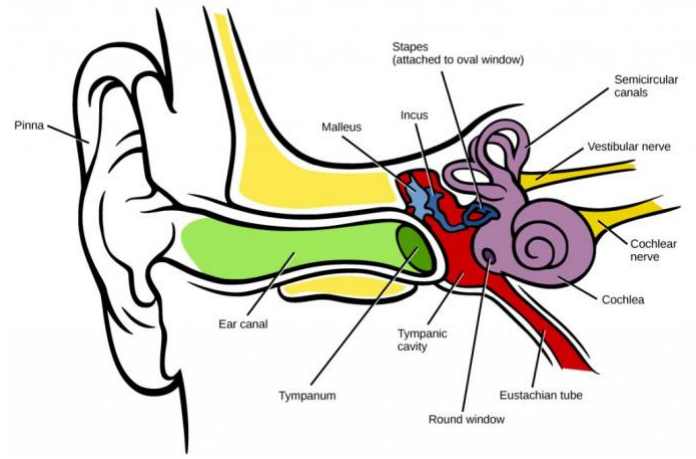


Figure. Sound travels through the outer ear to the middle ear, which is bounded on its exterior by the tympanic membrane. The middle ear contains three bones called ossicles that transfer

the sound wave to the oval window, the exterior boundary of the inner ear. The organ of Corti, which is the organ of sound transduction, lies inside the cochlea. (credit: modification of work by Lars Chittka, Axel Brockmann)

Transduction of Sound

Vibrating objects, such as vocal cords, create sound waves or pressure waves in the air. When these pressure waves reach the ear, the ear transduces this mechanical stimulus (pressure wave) into a nerve impulse (electrical signal) that the brain perceives as sound. The pressure waves strike the tympanum, causing it to vibrate. The mechanical energy from the moving tympanum transmits the vibrations to the three bones of the middle ear. The stapes transmits the vibrations to a thin diaphragm called the **oval window**, which is the outermost structure of the **inner ear**. The structures of the inner ear are found in the **labyrinth**, a bony, hollow structure that is the most interior portion of the ear. Here, the energy from the sound wave is transferred from the stapes through the flexible oval window and to the fluid of the cochlea. The vibrations of the oval window create pressure waves in the fluid (perilymph) inside the cochlea. The **cochlea** is a whorled structure, like the shell of a snail, and it contains receptors for transduction of the mechanical wave into an electrical signal (as illustrated in Figure 8.23). Inside the cochlea, the **basilar membrane**is a mechanical analyzer that runs the length of the cochlea, curling toward the cochlea's center.

The mechanical properties of the basilar membrane change along its length, such that it is thicker, tauter, and narrower at the outside of the whorl (where the cochlea is largest), and thinner, floppier, and broader toward the apex, or center, of the whorl (where the cochlea is smallest). Different regions of the basilar membrane vibrate according to the frequency of the sound wave conducted through the fluid in the cochlea. For these reasons, the fluid-filled cochlea detects different wave frequencies (pitches) at different regions of the membrane. When the sound waves in the cochlear fluid contact the basilar membrane, it flexes back and forth in a wave-like fashion. Above the basilar membrane is the **tectorial membrane**.

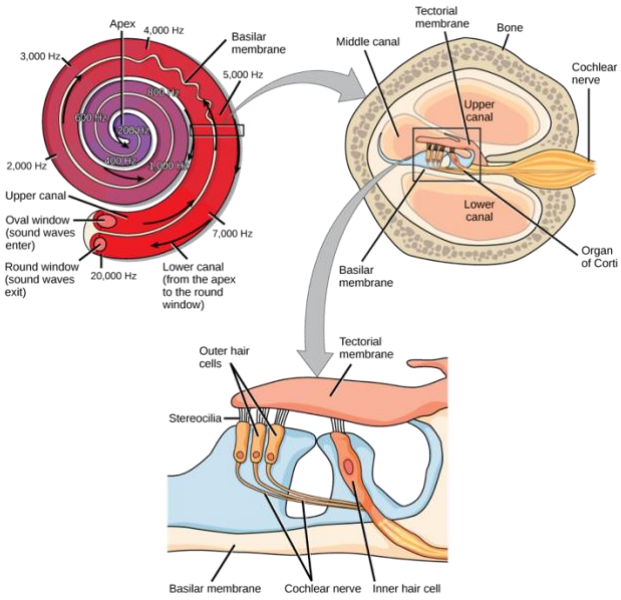


Figure. In the human ear, sound waves cause the stapes to press against the oval window. Vibrations travel up the fluid-filled interior of the cochlea. The basilar membrane that lines the cochlea gets continuously thinner toward the apex of the cochlea. Different thicknesses of

membrane vibrate in response to different frequencies of sound. Sound waves then exit through the round window. In the cross section of the cochlea (top right figure), note that in addition to the upper canal and lower canal, the cochlea also has a middle canal. The organ of Corti (bottom image) is the site of sound transduction. Movement of stereocilia on hair cells results in an action potential that travels along the auditory nerve.

The site of transduction is in the **organ of Corti** (spiral organ). It is composed of hair cells held in place above the basilar membrane like flowers projecting up from soil, with their exposed short, hair-like **stereocilia** contacting or embedded in the tectorial membrane above them. The inner hair cells are the primary auditory receptors and exist in a single row, numbering approximately 3,500. The stereocilia from inner hair cells extend into small dimples on the tectorial membrane's lower surface. The outer hair cells are arranged in three or four rows. They number approximately 12,000, and they function to fine tune incoming sound waves. The longer stereocilia that project from the outer hair cells actually attach to the tectorial membrane. All of the stereocilia are mechanoreceptors, and when bent by vibrations they respond by opening a gated ion channel (refer to Figure 8.22). As a result, the hair cell membrane is depolarized, and a signal is transmitted to the chochlear nerve. Intensity (volume) of sound is determined by how many hair cells at a particular location are stimulated.

The hair cells are arranged on the basilar membrane in an orderly way. The basilar membrane vibrates in different regions, according to the frequency of the sound waves impinging on it. Likewise, the hair cells that lay above it are most sensitive to a specific frequency of sound waves. Hair cells can respond to a small range of similar frequencies, but they require stimulation of greater intensity to fire at frequencies outside of their optimal range. The difference in response frequency between adjacent inner hair cells is about 0.2 percent. Compare that to adjacent piano strings, which are about six percent different. Place theory, which is the model for how biologists think pitch detection works in the human ear, states that high frequency sounds selectively vibrate the basilar membrane of the inner ear near the entrance port (the oval window). Lower frequencies travel farther along the membrane before causing appreciable excitation of the membrane. The basic pitch-determining mechanism is based on the location along the membrane where the hair cells are stimulated. The place theory is the first step toward an understanding of pitch perception. Considering the extreme pitch sensitivity of the human ear, it is thought that there must be some auditory "sharpening" mechanism to enhance the pitch resolution.

When sound waves produce fluid waves inside the cochlea, the basilar membrane flexes, bending the stereocilia that attach to the tectorial membrane. Their bending results in action potentials in the hair cells, and auditory information travels along the neural endings of the bipolar neurons of the hair cells (collectively, the auditory nerve) to the brain. When the hairs bend, they release an excitatory neurotransmitter at a synapse with a sensory neuron, which then conducts action potentials to the central nervous system. The cochlear branch of the vestibulocochlear cranial nerve sends information on hearing. The auditory system is very refined, and there is some modulation or "sharpening" built in. The brain can send signals back to the cochlea, resulting in a change of length in the outer hair cells, sharpening or dampening the hair cells' response to certain frequencies.

Higher processing

The inner hair cells are most important for conveying auditory information to the brain. About 90 percent of the afferent neurons carry information from inner hair cells, with each hair cell

synapsing with 10 or so neurons. Outer hair cells connect to only 10 percent of the afferent neurons, and each afferent neuron innervates many hair cells. The afferent, bipolar neurons that convey auditory information travel from the cochlea to the medulla, through the pons and midbrain in the brainstem, finally reaching the primary auditory cortex in the temporal lobe.

Vestibular information

The stimuli associated with the vestibular system are linear acceleration (gravity) and angular acceleration and deceleration. Gravity, acceleration, and deceleration are detected by evaluating the inertia on receptive cells in the vestibular system. Gravity is detected through head position. Angular acceleration and deceleration are expressed through turning or tilting of the head.

The vestibular system has some similarities with the auditory system. It utilizes hair cells just like the auditory system, but it excites them in different ways. There are five vestibular receptor organs in the inner ear: the utricle, the saccule, and three semicircular canals. Together, they make up what's known as the vestibular labyrinth that is shown in Figure 8.24. The utricle and saccule respond to acceleration in a straight line, such as gravity. The roughly 30,000 hair cells in the utricle and 16,000 hair cells in the saccule lie below a gelatinous layer, with their stereocilia projecting into the gelatin. Embedded in this gelatin are calcium carbonate crystals—like tiny rocks. When the head is tilted, the crystals continue to be pulled straight down by gravity, but the new angle of the head causes the gelatin to shift, thereby bending the stereocilia. The bending of the stereocilia stimulates the neurons, and they signal to the brain that the head is tilted, allowing the maintenance of balance. It is the vestibular branch of the vestibulocochlear cranial nerve that deals with balance.

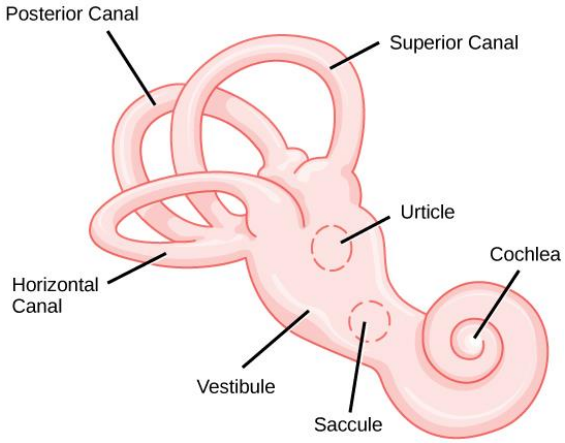


Figure - The structure of the vestibular labyrinth is shown. (credit: modification of work by NIH)

The fluid-filled **semicircular canals** are tubular loops set at oblique angles. They are arranged in three spatial planes. The base of each canal has a swelling that contains a cluster of hair cells. The hairs project into a gelatinous cap called the cupula and monitor angular acceleration and deceleration from rotation. They would be stimulated by driving your car around a corner, turning your head, or falling forward. One canal lies horizontally, while the other two lie at about 45 degree angles to the horizontal axis, as illustrated in Figure 8.24. When the brain processes input from all three canals together, it can detect angular acceleration or deceleration in three dimensions. When the head turns, the fluid in the canals shifts, thereby bending stereocilia and sending signals to the brain. Upon cessation accelerating or decelerating—or

just moving—the movement of the fluid within the canals slows or stops. For example, imagine holding a glass of water. When moving forward, water may splash backwards onto the hand, and when motion has stopped, water may splash forward onto the fingers. While in motion, the water settles in the glass and does not splash. Note that the canals are not sensitive to velocity itself, but to changes in velocity, so moving forward at 60mph with your eyes closed would not give the sensation of movement, but suddenly accelerating or braking would stimulate the receptors.

Hair cells from the utricle, saccule, and semicircular canals also communicate through bipolar neurons to the cochlear nucleus in the medulla. Cochlear neurons send descending projections to the spinal cord and ascending projections to the pons, thalamus, and cerebellum. Connections to the cerebellum are important for coordinated movements. There are also projections to the temporal cortex, which account for feelings of dizziness; projections to autonomic nervous system areas in the brainstem, which account for motion sickness; and projections to the primary somatosensory cortex, which monitors subjective measurements of the external world and self-movement. People with lesions in the vestibular area of the somatosensory cortex see vertical objects in the world as being tilted. Finally, the vestibular signals project to certain optic muscles to coordinate eye and head movements.

Signal transduction

Cell communication is a fundamental homeostatic process.

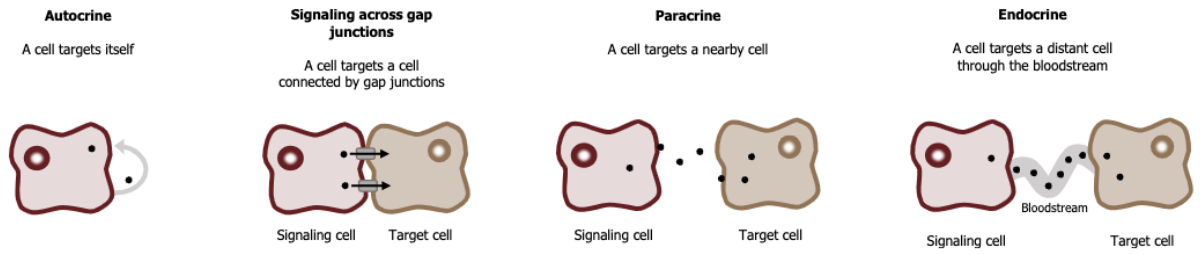


Figure: Summary of types of cell signaling

Generally speaking, it uses various communication modalities to sense and respond to neighboring cells and environmental cues which can be categorized into the following types of communication (Figure 15.1):

Endocrine: Endocrine cells secrete the hormone into the blood and exerts its action on specific target cells that can be very far away. (Examples: insulin, glucagon and cortisol)

Paracrine: The paracrine substance is secreted from cells that are not normally thought of as endocrine cells. Actions are performed on nearby cells and very low amounts are too dilute to affect distance cells. Location of the cell plays a role in the specificity of the response.

Autocrine: These signals act on the cell from which it is secreted or on nearby cells that are the same type of cell as the secreting cell. Most autocrine cells are also paracrine cells.

Juxtocrine: These types of signals require physical contact between cells in order for a signal to be transduced.

General characteristics

- Cellular signaling begins with the release of a chemical messenger which will either diffuse or is transported in the blood/extracellular fluids to its location of action.

Once at the intended location it will bind to its receptor, which can be intra cellular or extracellular, to elicit a response:

- This could be in the form of a conformational change
- Activation of a second messenger
- Protein recruitment

Finally, the signal can be terminated

- Degradation of the receptor or ligand

Types of ligands

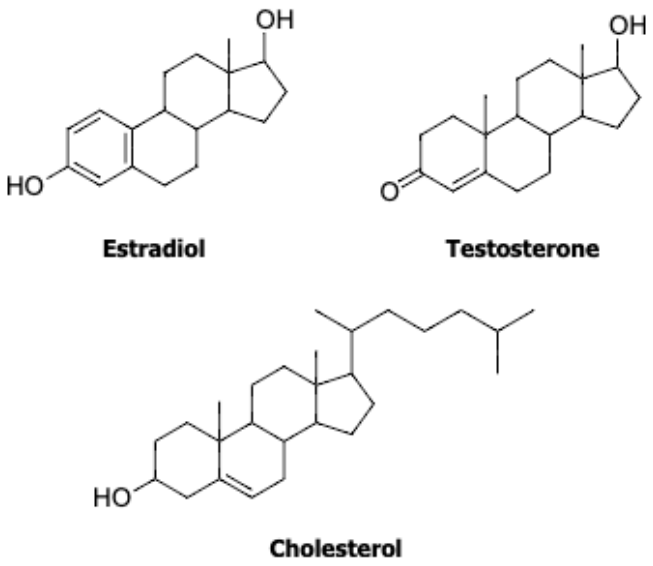


Figure: Examples of steroid hormones

Steroid hormones

These are often cholesterol derived and can diffuse through membranes to bind intracellular receptors (Figure 15.2).

Amino acid metabolites

These types of hormones are often neurotransmitters and contain nitrogenous groups.

Gases

Both NONO and COCO are common gases that elicit unique signaling cascades.

Proteins

These can be small polypeptides (e.g. insulin) or larger membrane bound proteins that elicit a cellular response.

Lipids

Eicosanoids and other phospholipids can function as cell signals either in a membrane bound or free form.

General G-Protein coupled receptor cascade

G-protein coupled receptors (GPCR) can come in several different classes: Gαsαs. Gαiαi and Gαqαq. Activation of a Gαsαs (activated by glucagon) will increase the second messenger cAMP while both Gαiαi or Gαtαt cascades function to reduce cAMP, either through inhibition of adenylyl cyclase (also known as adenylate cyclase) or through activation of phosphodiesterase, respectively.

The classic cascade starts with hormone binding, to an extracellular domain of a 7-helix receptor (GPCR), causes a conformational change in the receptor that is transmitted to a G-protein on the cytosolic side of the membrane (Figure 15.3).

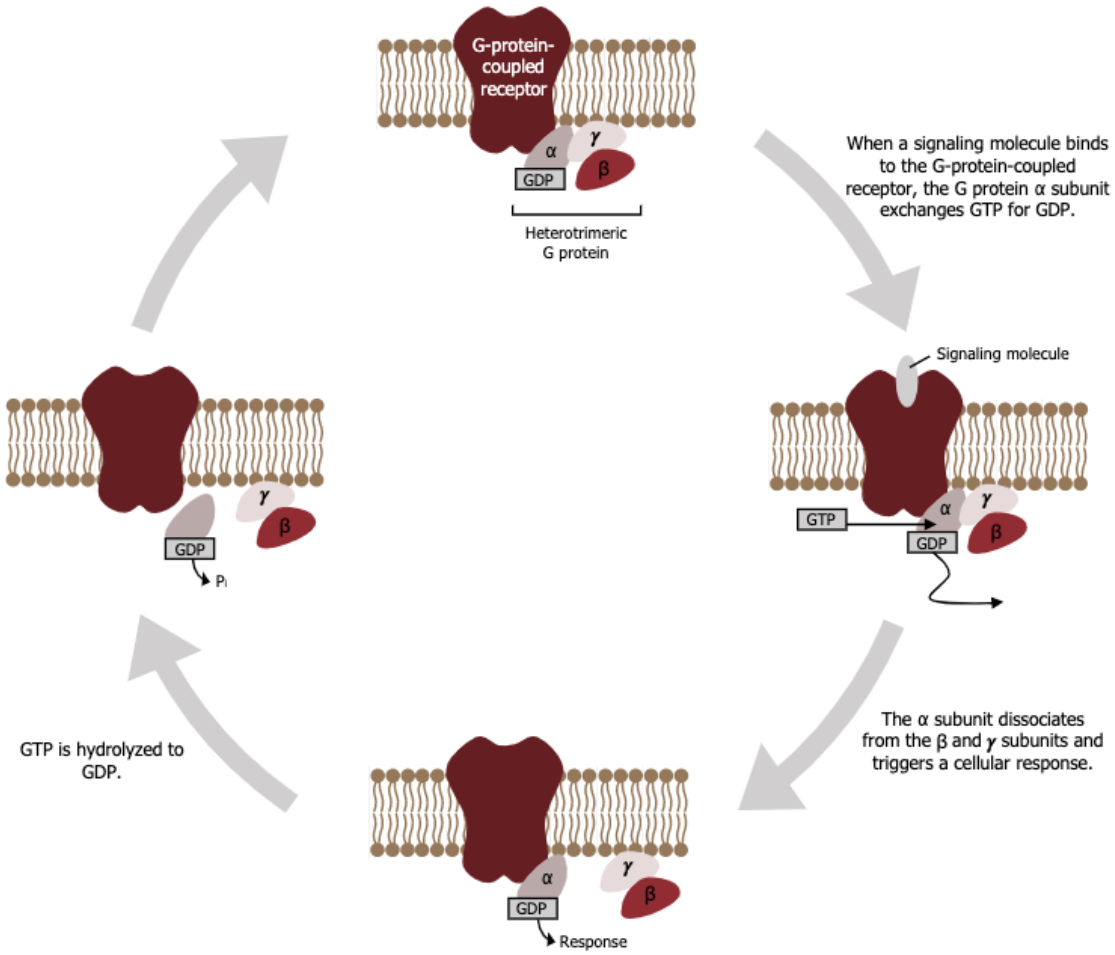


Figure: Common G-protein coupled receptor signaling cascade.

- The nucleotide-binding site on G$\alpha\alpha$s becomes more accessible to the cytosol, where [GTP] is usually higher than [GDP]. G$\alpha\alpha$ releases GDP and binds GTP. (GDP-GTP exchange) Substitution of GTP for GDP causes another conformational change in G$\alpha\alpha$.
- G$\alpha\alpha$-GTP dissociates from the inhibitory subunit complex, and activates Adenylyl Cyclase.
- Adenylyl Cyclase catalyzes synthesis of cAMP. (second messenger) and in turn cAMP activates Protein Kinase A (cAMP-Dependent Protein Kinase).
- The cascade can be terminated by the action of phosphodiesterase which can degrade cAMP and terminate signal.

Phosphatidylinositol derived second messengers

Phosphatidylinositols are membrane bound compounds that can be phosphorylated or cleaved to function as second messengers in a signaling cascade (Figure 15.4).

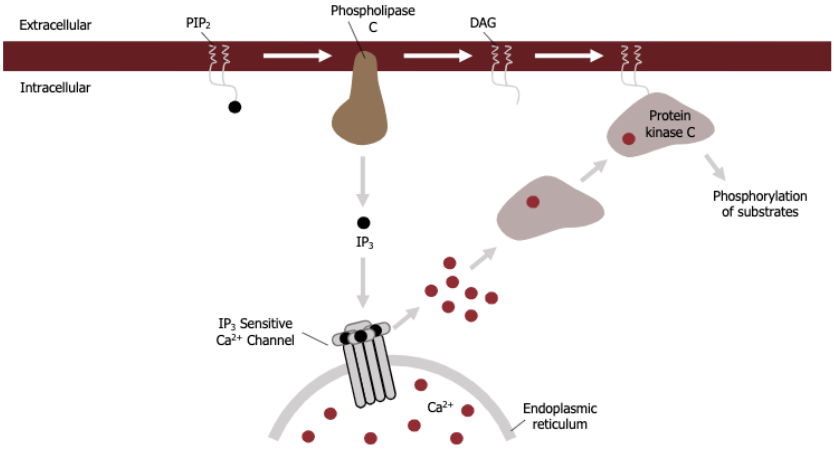


Figure: Signaling cascade initiated by DAG and IP3

The common membrane component, phosphatidylinositol (PI) can be phosphorylated (by any number of kinases) to form PI 4,5 bis phosphate. This molecule can be undergo two different fates.

1. First it could be phosphorylated by a kinase, such as P-I3kinase downstream of insulin, this produces phosphatidylinositol 3′, 4′,5′-trisphosphate (PI-3,4,5-trisP) which can serve as a plasma-membrane docking site for signal transduction proteins, with pleckstrin homology domains (PH).
2. Alternatively, PI 4,5 bis phosphate can be cleaved into two second messengers: inositol-1,4,5-trisphosphate (IP3), and diacylglycerol (DAG) by activation of phospholipase C (PLC). Phospholipase C is downstream of a Gαq cascade.

This cascade will become important for calcium signaling which is modulated through interactions of IP3 with the mitochondria.

1. DAG recruits Protein kinase C
2. IP3 initiates release of Ca2+Ca2+ from the smooth ER.

Changes in intracellular calcium can alter membrane permeability through calcium induced calcium release. Receptor Tyrosine Kinase (RTK)

RTKs are in the cell membrane and typically function as a dimer.

- Upon binding of the hormone to the receptor, autophosphorylation occurs on the inner side of the membrane. This forms a phosphorylated tyrosyl residue that will act as a docking site for proteins with SH2 domains. In the case of insulin signaling, the insulin receptor substrate (IRS) will bind this activated receptor (Figure 15.5).
- The IRS protein will also become phosphorylated at subsequent tyrosine residues and in this manner insulin signaling can be amplified. Other proteins such as PI 3-kinase, PLC, and Grb2 all have a SH2 domains and all bind to different tyrosyl residues on the IRS.

Two major cascades activated downstream of insulin and other growth hormones:

- Ras dependent signaling: The activated Grb2 binds SOS-Ras complex leading to a conformational change exchanging GDP for GTP. Ras-GTP binds Raf, and activated Raf is the first step in a MAP-Kinase cascade that can lead to a change in gene transcription.
- Ras independent signaling involves activation of Phosphoinositol 3- kinase and as discussed under Phosphatidylinositol derived second messengers.

Jak-STAT and serine threonine kinases

Jak-STATs are also types of tyrosine kinases. The difference here is that these receptors lack autocatalytic abilities and require an intracellular kinase (Jak) to phosphorylate the transcription factor STAT. Jak-STAT signaling is most commonly associated with immune cell signaling.

Serine threonine kinase

This receptor family encompasses many of the growth factors for the body (EGF, VEGF and TGF-ββ). These receptors usually form heterodimers and the Type II receptor will autophosphorylate the Type I receptor upon ligand binding. These receptors have an autocatalytic domain that will phosphorylate, and typically activate a transcription factor.

Intracellular receptors

Intracellular receptors bind hydrophobic chemical messengers such as steroid hormones. Binding of the intracellular receptor, (which could be cytosolic or nuclear), usually elicits a transcriptional response. Cortisol is an example of a hormone that binds an intracellular receptor. It is released from the adrenal cortex and diffuses into the bloodstream attached to serum albumin and steroid hormone binding globulin. After diffusing through the plasma membrane, it binds to the cortisol receptor (intracellular receptor) in the cytosol and forms a homodimer exposing a nuclear localization signal (NLS). Exposure of the NSL targets the complex to the nucleus.

Intracellular receptors commonly have 3 domains,

- Transactivation Domain
- DNA binding domain
- Ligand binding domain

And will function as a transcriptional activator by binding specific DNA elements, altering transcription of downstream genes. The signal is terminated by the lowering of the concentration of the hormone.

NO **as a messenger**

Nitric oxide (NONO) is a gas that also acts as a ligand. It is able to diffuse directly across the plasma membrane, and one of its roles is to interact with receptors in smooth muscle and induce relaxation of the tissue.

NONO has a very short half-life and, therefore, only functions over short distances. It activates guanylyl cyclase to synthesize cGMP. This in-turn results in smooth muscle relaxation.

Nitroglycerin, a treatment for heart disease, acts by triggering the release of NONO, which causes blood vessels to dilate (expand), thus restoring blood flow to the heart. NONO has become better known recently because the pathway that it affects is targeted by prescription medications for erectile dysfunction, such as Viagra (erection involves dilated blood vessels).