Chapter 1
Introduction

The origin of life clearly lies in a living cell first observed by Sir Robert Hooke, but exactly where in a cell the essence of life can be localized is still unclear. Over several centuries careful investigators have been trying to find a single source to claim it as the point of origin of life. Is it in the cellular inner core where metabolic processes take place, is it around the cellular boundary where the cell's transport properties couple with processes controlling many dynamical aspects of proteins, or is it in some other yet unknown region, or finally, is it nowhere specific but rather, due to a fundamental mechanism which causes animate matter to qualitatively differ from inanimate matter? Structural and molecular biology have considerably developed our understanding of cellular compartments and molecular building blocks of cells, but the ongoing developments in these fields have raised multifaceted questions regarding cells and cellular processes. Both the cellular inner core and the cellular wall, known as a membrane, have been understood as not just composites of different compartments working independently or collectively and performing many critical functions for a living body. Detailed analyses of the known functions of various cellular components suggest that the real discovery of the origin of their functions is yet to be made.

Living cells are the ultimate examples of complex dynamical systems. For the past several decades, biologists have greatly advanced the understanding of how living systems work by focusing on the structure and function of constituent molecules such as DNA, proteins, and enzymes. Understanding what the constituent parts of a complex machine are made of, however, does not explain how the entire system works. Scientific analysis of living systems has posed an enormous challenge and presented an enormous task. Conceptual advances in physics, vast improvements in the experimental techniques of molecular and cell biology (electron microscopy, STM, AFM, etc.), and exponential progress in computational techniques and related works have brought us to a unique point in the history of science when the expertise of many areas of science can be brought to bear on the main unsolved puzzle of life, namely how cells live, divide, and eventually, die.

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Cells are the key building blocks of living systems. Some of them are self-sufficient while others co-operate in multicellular organisms. The human body is composed of cells of 200 different types. A typical size of a cell is of the order of 10 μm and its dry weight amounts to about 7×10^{-16} kg. In its natural state, 70 % of the content constitutes water molecules. The fluid content of a cell is known as the cytoplasm. The cytoplasm is the liquid medium bound within a cell, while the cytoskeleton is the lattice of filaments with a network of attracting proteins formed throughout the cytoplasm.

Two major types of cells are:

(a) prokaryotic: simple cells with no nucleus and no compartments. Bacteria (e.g. *E. coli*) and blue-green algae belong to this group.
(b) eukaryotic: cells with a nucleus and a differentiated structure including compartmentalized organelles as well as a filamentous cytoskeleton. Examples here include higher developed animal and plant cells, green algae, and fungi. Eukaryotic cells emerged about 2 billion years ago, and comprise all the life kingdoms except monera. The Greek meaning of the word *eukaryotic* is "true nucleus".

Bacteria have linear dimensions in the 1–10 μm range while the sizes of eukaryotic cells range between 10 and 100 μm. The interior of a bacterium experiences considerable pressure reaching up to several atmospheres due to the presence of a membrane which, except for *Archaebacteria*, is composed of layers of peptidoglycan sandwiched between two lipid bilayers the inner of which is a plasma membrane.

Plant cells have linear dimensions that vary between 10 and 100 μm. They are bounded by a cell wall whose thickness ranges between 0.1 and 10 μm and is composed of cellulose. Among its organelles, plant cells have a nucleus, endoplasmic reticulum, Golgi apparatus, and mitochondria. Unique to plant cells is the presence of chloroplasts and vacuoles. Unlike bacteria, plant cells possess a cytoskeletal network adding to their mechanical strength. Animal cells tend to be smaller than plant cells since they do not have liquid-filled vacuoles. The organizing center for their cytoskeleton is a cylindrical organelle called a *centriole* that is approximately 0.4 μm long. Instead of chloroplasts that are sites of photosynthesis, animal cells have *mitochondria* that produce the required energy supply in the form of ATP molecules obtained from reactions involving oxygen and food molecules (e.g. glucose, sucrose, etc).

Eukaryotic cells possess membrane-bound internal structures called organelles briefly discussed below. *Mitochondria* produce energy, a *Golgi* apparatus (where various macromolecules are modified, sorted, and packaged for secretion from the cell for distribution to other organelles) is shaped like a stack of disks. The *endoplasmic reticulum* surrounds the nucleus and is the principal site of protein synthesis. Its volume is small compared to the surface area. A *nucleus* is the location of chromosomes and the site of DNA replication and transcription. All of the material within the cell excluding the nucleus is defined as the cytoplasm whose liquid components are referred to as the cytosol while the solid protein-based structures that float in it are called the cytoskeleton. The main component of the cytosol is water. Most of the organelles are bound within their own membranes. Most of the cell's DNA is stored

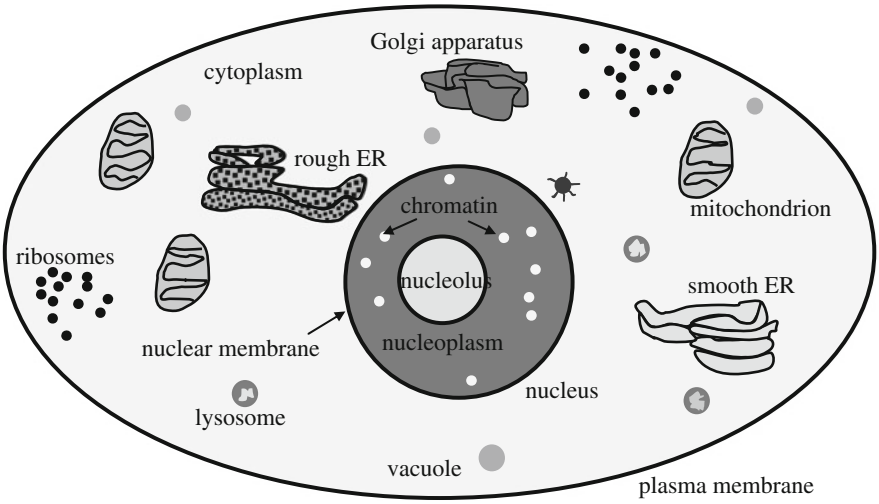

Fig. 1.1 Schematic diagram of a cell, showing different constituent parts. (Components illustrated here do not represent the true structure observed in a biological cell.) The constituents shown here are found in an animal cell. In a plant cell, in addition to all these structures, chloroplasts involved in photosynthesis also exist. A plant cell (not an animal cell) also consists of a cell wall surrounding the plasma membrane which provides tensile strength and protection against mechanical and osmotic stress

within the nucleus that is protected by the nuclear envelope. The rest is contained in mitochondria. Within the nucleus is the nucleolus which functions as the site of ribosomal-RNA synthesis. The diameter of a nucleus ranges between 3 and 10 μm. Despite many differences, both animal and plant cells have striking similarities in their organization and functions.

Although the origins of most of the cellular processes are not yet discovered, organisms that are made up of cells have been classified depending on the structure and organization of cellular building blocks. Organisms that exist as single cells are called unicellular—*Archaea* and *Bacteria*. Organisms that are made up of groups of cells working together are called multicellular—animals, fungi and plants. There is another kingdom which contains a mixture of both unicellular and multicellular organisms. This is called the *Protista*. Humans have about 10^{14} cells in their bodies; a typical cell size in the human body is of the order of 10 μm, with a mass of 1 ng. A schematic diagram showing different parts in a cell is presented in Fig. 1.1.

All cells are enclosed by cell envelopes which consist of cell walls covering plasma membranes. This book is dedicated to a better understanding of various aspects of cellular membranes. A more detailed explanation of the structure and functions of the cell's various components can be found in many text books on cell biology. In this book, we mainly focus on those cell components whose structure and functions are connected with the processes taking place inside membranes.

Both prokaryotic and eukaryotic cells have membranes which primarily separate the interior of a cell from its exterior, selectively regulate the movement of molecules across them, and most importantly, maintain an electric potential difference between the interior of a cell and its exterior. Membrane properties seem to be robust and simple but they reflect the states of a membrane which are, in turn, results of many very complicated processes taking place inside, across, and outside membranes. Understanding of those processes requires thorough analysis of the membrane constituents, electrical environment inside and outside the cell, mechanical membrane properties, dynamical processes taking place in the cell, and many specific as well as non-specific effects due to sources inside and outside the cell.

The fluid contents of a cell are known as the cytoplasm. The cytoplasm is hugely important because it provides the medium in which fundamental biophysical processes such as cellular respiration take place. Its properties are somewhat different than those of dilute aqueous solutions. The contents must be accurately known for in vitro studies of enzymatic reactions, protein synthesis, and other cellular activities. Typical constituents of the cytoplasm are ionic and bio-molecular. Most of the trace ions are positively charged; however, the cytoplasm does not have an overall electric charge, thus the difference is made up of the other constituents such as proteins, bicarbonate (HCO_3^-), phosphate (PO_4^{3-}), and other ions which are for the most part negatively charged, a few of which are significantly electronegative.

Most cells maintain a neutral pH and their dry matter is composed of at least 50 % protein. The remaining dry material consists of nucleic acids, trace ions, lipids, and carbohydrates. A few metallic ions are found which are required for incorporation into metallo-proteins but these ions such as iron(II) (Fe^{2+}) are typically found in nano-molar concentrations.

There is experimental evidence for the existence of two phases of the cytoplasm. These are the so-called liquid and solid phases, *sol* and *gel*, respectively. In the solid phase, the major constituents of the cell are rendered immobile while in the liquid phase, the cytoplasm's viscosity does not differ significantly from water. Diffusion in the cytoplasm is affected mainly by macromolecular crowding. In the solid phase, diffusion is slowed by a factor of three relative to diffusive movement in water. Such properties of the cytoplasm seem to be regulated in some sense by the cytoskeleton, but the manner in which this regulation is accomplished is largely unclear. It is believed that it involves the tangling and detangling of a mesh of various protein filaments. However, once the cell has acted to organize itself, the transition to a solid phase can allow it to expend relatively minimal energy to maintain its organization. Contrary to early perceptions, the cytoplasm is not a viscous soup-like amorphous substance but a highly organized, multicomponent, dynamic network of interconnected protein polymers suspended in a dielectrically polar liquid medium.

A variety of solute molecules are contained within cells. The cellular fluid (cytosol) has a chemical composition of 140 mM K^+, 12 mM Na^+, 4 mM Cl^-, and 148 mM A^- where 1 mM stands for a concentration of 10^{-3} mol/L. The symbol A stands for protein. Cell walls are semipermeable membranes which permit the transport of water easily but not solute molecules. We can apply the osmotic pressure

concept to cells, but this requires finding the osmotic pressure of a mixture of solute molecules. We use Dalton's Law to determine the osmotic pressure inside a cell.

A mixture of chemicals, with concentrations $c_1, c_2, c_3 \ldots$, dissolved in water has the total osmotic pressure equal to the sum of the partial osmotic pressures, Π, of each chemical. Thus,

$$\Pi = \Pi_1 + \Pi_2 + \Pi_3 + \cdots = RT(c_1 + c_2 + c_3 + \cdots) \tag{1.1}$$

The total osmotic pressure inside a cell, Π_{in} is therefore

$$\Pi_{\text{in}} = RT \frac{(140 + 12 + 4 + 148) \times 10^{-3}\,\text{mol}}{1\text{L}} \times \frac{1\text{L}}{10^{-3}\,\text{m}^3} = 7.8 \times 10^4\,\text{Pa} \tag{1.2}$$

where we used the concentrations given above and a physiological temperature of $T = 310\,\text{K}$ and the gas constant is $R = 8.31\,\text{J/mol K}$. Cell walls would be expected to burst under such large pressures. However, they do not, because the exterior fluid also exerts an osmotic pressure in the opposite direction. The cell exterior is composed of $4\,\text{mM}\ \text{K}^+$, $150\,\text{mM}\ \text{Na}^+$, $120\,\text{mM}\ \text{Cl}^-$ and $34\,\text{mM}\ \text{A}^-$. As a consequence, the total osmotic pressure of the cell exterior, Π_{out}, is given by

$$\Pi_{\text{out}} = RT \frac{(4 + 150 + 120 + 34) \times 10^{-3}\,\text{mol}}{1\text{L}} \times \frac{1\text{L}}{10^{-3}\,\text{m}^3} = 7.9 \times 10^4\,\text{Pa} \tag{1.3}$$

Here, Π_{out} is a large osmotic pressure but because Π_{in} and Π_{out} are very similar, the osmotic pressure difference between the exterior and interior part of the cell is very small, as it is the net pressure exerted on the cell wall that matters most. For fragile animal cells, it therefore becomes vitally important to keep their interior and exterior osmotic pressures closely matched. The cell has a sophisticated control mechanism to do this.

If two solutions have the same osmotic pressure, we call them iso-osmotic. However, if the pressures are different, the one at higher pressure is called hypertonic and the one at lower pressure is called hypotonic. When cells are placed in a solution and neither swell nor shrink we call the solution isotonic. In the tissues of most marine invertebrates the total osmotic concentration is close to that of the sea water. The salt concentration of sea water is about 500 mM. As long as the salt concentration remains near this value the blood of many crabs is isotonic with that of sea water. When it is outside this range, the system maintains the osmotic pressure difference across its membrane through the activity of ion pumps and the process is known as osmoregulation.

The cell composition begins to drift away from its optimal mixture if the ion pumps (which will be discussed in detail later in the book) are chemically destroyed. Across the cell wall the osmotic pressure difference then rises, causing the cell to swell, become turgid, and eventually explode. The cells of bacteria and plants are not osmotically regulated since their cell walls are able to withstand pressures in the range of 1–10 atm. The minimum work performed when n moles of solute are

transferred from one solution with a concentration c_1 to a solution with concentration c_2 is easily calculated as

$$W = nRT \ln \frac{c_1}{c_2} \tag{1.4}$$

where c_1 is the salt concentration in the cell and c_2 is the salt concentration in the extracellular space.

Osmotic pressure is also used by the cells of plants and, in particular, trees. Tree roots have a high osmotic pressure inside them which leads to absorption of water from the soil. A key role is also played, it is believed, by osmotic pressure in the growth of plants. The openings on the surfaces of cell leaves, called stomata, are bordered by guard cells that can regulate their internal pressure by controlling the potassium concentration. Water absorption causes these cells to swell under osmotic pressure and the stomata are closed.

Contained within the cytoplasm are the components of the cytoskeleton and certain smaller compartments known as organelles which are specialized to perform their respective functions. We refer the reader to cell biology and cell biophysics text books for information on these important subcellular structures.

A typical cell membrane maintains a transmembrane potential which is of the order of 100 mV. The value of this potential varies between different cells. The transmembrane potential across cancer cell membranes may vary dramatically from the normal cell membranes due to different electrical and metabolic conditions. Significant depolarization of the membrane potential has been found in cancerous breast biopsy tissues and in transformed breast epithelial cells when compared to normal cells [10]. In the case of mitochondria, a proton gradient exists across the mitochondrial inner membrane which determines the membrane potential there. An early stage study [8] suggests that mitochondria-specific interactions of cationic fluorescent probes (molecules) are dependent on the high transmembrane potential (negative inside the membrane) maintained by functional mitochondria. Marked elevations in mitochondria-associated probe fluorescence have been observed in cells engaged in active movement. These results obtained through various investigations suggest that membrane potentials vary considerably between various types of membranes, e.g., normal cell membranes, cancerous cell membranes, mitochondrial membranes, etc.

Like membrane potentials, the thicknesses of various membranes in normal cells, cancerous cells, mitochondria, etc., also vary on a nanometer (nm) scale. The membrane thickness is of the order of 3–6 nm. Taken together, the membrane potential being of the order of 100 mV and the membrane thickness of the order of several nm, results in the electric field across the cell membrane being in the range of $\sim 10^7$ V/m.

It is worth relating the above number to our everyday experience where the electric potential we use is 120 V in the Americas and 220−240 V in the rest of the world for lighting homes and offices. A comparison between the above potentials suggests that a cell membrane appears to act like a cellular power plant. Nature has given us this energy-generating nanoscale component which is present in each of our body's cells. The electric energy created in this power plant is enough to regulate the functions of many biological processes such as ion movements across

membranes, membrane protein dynamics, exclusion of large molecules and pathogens, etc. All membrane constituents such as lipids, membrane proteins, hydrocarbons, etc., reside in the presence of the electric field created by the membrane's electric power plant. Naturally, the electrostatic properties of all the membrane constituents are sensitive to its electric field. The presence of strong polarizing effects on the charges of individual atoms within any molecule and the overall charges of these molecules leads to directed molecular process. Therefore, general electrostatic properties of the various membrane constituents are critical to our understanding of their roles and functions. To gain a complete picture of any biological membrane process, a combined biochemical/biophysical approach is needed with the individual scientific methodologies of these disciplines. This book aims to explore this in detail. For existing general information regarding cell and specifically membrane there are many articles and books available but the readers can consider reading from references [1–13].

General membrane phenomena, mechanisms, and other properties will be addressed in Chap. 2. General transmembrane electrical potentials, ionic gradients, ion transport, specificities, and directionalities in ion movements, membrane's capacitive effects, and related aspects, will also be described in detail in Chap. 2.

Lipids, cholesterol, membrane proteins, hydrocarbons, etc., taken together constitute cell membranes. The proportion between different constituents is very much membrane-specific. Lipids are of various kinds, e.g., charge bearing, charge neutral, curvature bearing, curvature neutral, with different lipid head group geometry, with shorter or longer hydrocarbon tails, etc. Membrane proteins exhibit different morphologies and properties. The presence of cholesterol is organ specific. Membrane stabilizing hydrocarbons play crucial roles. All these aspects will be addressed in Chap. 3. Chapter 3 will rigorously address the issue of the lipid organizations in membranes, lipid phase properties, lipid's thermotrophic behavior, that is, the thermodynamics of membranes, etc. A complete understanding of these issues will create an important background for specific investigations of the various physical and biochemical processes that take place in membranes.

Chapter 4 is dedicated to the description of transport phenomena in membranes. The reader will be informed about how the crucial membrane electrical properties get compromised due to agents residing inside membranes or external agents interacting with membranes. Various classes of specific ion channels or non-specific pores used by ion flows temporarily appearing inside membranes will be explained here. Mainly, the geometric aspects of various membrane transport events will be discussed. Natural membrane proteins, antimicrobial peptides, chemotherapy drugs, certain types of lipids, other biomolecules, etc., will be analyzed in order to explain how those agents coexist with lipids and other membrane constituents to generate various membrane events, mainly those which are responsible for changing the membrane transport properties.

Chapter 5 will bring additional aspects regarding the mechanisms underlying the generation of membrane transport events as explained in Chap. 4, and a general picture of energetics responsible for statics and dynamics of lipids and membrane residing agents. Electrical and mechanical properties of the lipid bilayer, bilayer

constituents, and any agent responsible for creating an event inside the membrane will be explained in detail. Particular attention will be paid to the mechanisms that depend on the electrical properties of membranes relative to their mechanical properties. This chapter will summarize all aspects of the regulation of membrane protein functions based on the electrical and mechanical properties of membranes and membrane proteins.

Chapter 6 has been dedicated to explaining how membrane-based nanotechnology can be used in drug delivery into cellular interiors. Electrical and mechanical properties of membranes determine the interactions between nanoparticles and membranes and lead to possible delivery methods beyond the membrane subject to the presence of other agents that induce membrane transport events. Novel membrane-based nanotechnology is proposed in this chapter, which will hopefully open up a new dimension in developing drug delivery strategies.

A number of serious diseases involving cell membrane structures and functions have been discussed in Chap. 7. In addition to a brief description of the development of these diseases, drug discovery and treatment regimens are taken into consideration. The reader will find information about the physical, chemical, and biological processes that are involved in disease initiation and progression. Finally, certain diseases such as cancer, Alzheimer's disease, bacterial infections, and some other membrane-based disorders and their potential treatments will be outlined in this chapter.

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