Chapter 2
Structure of Membranes

2.1 The Membrane as a Barrier and a Transporter

Amphipathic molecules adsorb themselves onto air–water or oil–water interfaces, such that their head groups are facing the water environment. They aggregate to form either spherical micelles or liquid crystalline structures. In general, amphipathic molecules can be anionic, cationic, non-ionic, or zwitterionic. The relative concentrations of these surfactants in an aqueous solution will affect the solution's physical and chemical properties. At a specific value, called the critical micelle concentration, micelles containing 20–100 molecules are formed spontaneously in the solution, with the hydrophilic head groups exposed and the hydrophobic tails hidden inside the micelle. The principal driving force for micelle formation is entropic, due to a negative free energy change accompanying the liberation of water molecules from clathrates. When phospholipids are mixed in water, they form double-layered structures, since their hydrophilic ends are in contact with water while the hydrophobic ends face inwards touching each other.

Membranes have unique amphipathic properties since they possess both hydrophobic and hydrophilic parts. As described in Chap. 1, the cell membrane is the thin, nearly invisible structure that surrounds the cytoplasm of the cell. It is a continuous boundary region that completely surrounds the cell, and which also connects the endoplasmic reticulum and the nuclear membrane. Membranes are composed of phospholipids, glycolipids, sterols, fatty acid salts, and proteins. The tails that come off of the sphere represent the hydrophobic (or water-fearing) end of the phospholipid. The two long chains coming off the bottom of this molecule are made up of carbon and hydrogen. Because both of these elements share their electrons evenly, these chains have no net electrostatic charge. Non-polar molecules are not attracted to water; as a result water molecules tend to push them out of the way as they are attracted to each other. This causes molecules with no electrostatic charge not to dissolve in water. At the other end of the phospholipid there is a phosphate group and several double-bonded oxygens. The atoms at this end of the molecule are not

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shared equally. This end of the molecule has a charge and is therefore attracted to water. Biomembranes also compartmentalize areas of different metabolic activity in the cell, and regulate the flow into and out of cells and cell compartments. Finally, membranes are sites of key biochemical reactions.

The key functions of cell membranes can be summarized as follows:

- They are a selectively permeable barrier between two predominantly aqueous compartments.
- They allow compartmentalization of the various structures in the cell.
- They enable the formation of a stable and fluid medium for reactions that are catalyzed.
- They provide a flexible boundary between the cell or an organelle and its surrounding medium.
- They maintain an electric potential difference, participate in signal transmission to the actin cycloskeleton (via integrins), and provide adhesion forces for the cells to their substrates (controlled by membrane elasticity).
- They enable mass transport (via ion channels).

The fluid mosaic model of Singer and Nicolson [23] views the membrane as a fluid bilayer of amphipathic complex lipids with proteins embedded in it and spanning it. The relative abundance of proteins in a membrane varies from species to species, and it correlates with metabolic activity. For example, the mitochondrial wall contains large amounts of protein (52–76 %) and smaller amounts of lipids (24–48 %), facilitating its high metabolic activity. Conversely, the inactive membrane of the myelin sheath in neurons contains only 18 % proteins and 79 % lipids.

A double layer of phospholipid molecules with a variety of embedded proteins makes up the plasma membrane of a cell (see Fig. 2.1). This plasma membrane does not resemble the surface of a fluid or even the interface between two fluids. The reason for this is that it has an essentially fixed surface area, i.e., there are only a fixed number of phospholipid molecules and proteins which, when packed together, make up the membrane. Each lipid molecule or protein has a preferred surface area so, unlike the surface of a fluid, the plasma membrane is, for practical purposes, inextensible.

The various components of membranes are subject to rapid movements. Rapid lateral movement of lipids is characterized by a diffusion constant of approximately $10^{-8}\,\mathrm{cm}^2/\mathrm{s}$, while those of proteins range between 10^{-10} and $10^{-12}\,\mathrm{cm}^2/\mathrm{s}$. On the other hand, flip-flop movements across the membrane are slow, of the order of $10^{-5}\,\mathrm{s}$. Indeed, the phospholipids of the membrane may undergo a phase transition from a gel phase to a liquid crystal phase. This may take place as a result of changing the ambient temperature, external pressure, or even membrane composition.

A cell membrane regulates transport of materials and signals across it, between internal and external regions with different physiological states. The barrier properties of membranes can be both specific and non-specific, that is, the mode of barrier action is not identical in all cases; membrane constituents play important roles.

Trans-membrane osmotic pressure, an electrostatic imbalance between the outer and inner regions, various membrane constituents—all of these factors play important independent and collective roles in characterizing the membrane's barrier properties. The barrier properties are due to a combination of different physical effects, (e.g., electrical, mechanical, geometrical etc.) and chemical effects (e.g., chemical species concentrations, the value of pH, etc). Due to the various dynamics continuously occurring inside and outside the membrane, its barrier properties also are subject to change. Furthermore, they are strongly time-dependent functions. Different physiological conditions cause perturbations in the membrane barrier properties, often temporarily—but there can also be permanent changes occurring mainly due to various chronic diseases, aging, and other physiological changes on a longer timescale. A single observation of the membrane using a specific technique cannot always find key clues, since effects originate from many sources. Traditional biological approaches, therefore, need to go beyond simple observations and descriptive characterization, and instead explore physical, chemical, as well as engineering technologies to be used in membrane science. All these techniques, when combined, have improved our understanding of the membrane seen as just a barrier. The applications of science and technology to date have made it possible to enable tracing the origins of many static and dynamic processes taking place inside and near membranes. Such approaches help us not only understand the membrane itself, but may also help find avenues for further development of scientific approaches to improved drug discovery using membrane-based technology. The reader will be exposed to this crucial issue via the chapters that follow.

2.2 Membrane Constituents

Lipids are the primary components of a biological membrane. Two layers of lipids make a bilayer, and the lipids align in such a way that the head groups point in outward directions in both lipid monolayers. Electrolytes and water molecules are expelled to the exterior of the membrane, while the inner membrane layer stays inside a cellular compartment.

The membrane structure looks deceptively simple, but complex static and dynamical phenomena take place in this crucial cellular component. We describe many of these phenomena in later chapters. Here we only wish to mention that the membrane constituents such as proteins and hydrocarbons, together with lipids continuously form, break, and translocate between different complex structures (e.g., ion channels, defects, etc.) inside and across membranes, which can be responsible for changing the membrane's insulating properties. As a result, materials such as ions, water molecules, other small molecules, etc. may pass through membranes.

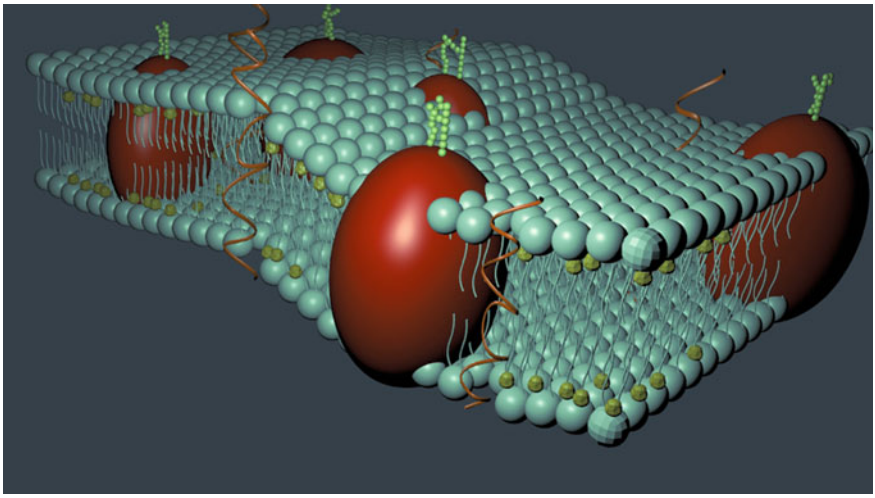

Fig. 2.1 A simplified membrane structure is presented here [2]. Two lipid monolayers are the primary components of a membrane. Near lipid head groups (*light blue color*) are seen the cholesterol molecules residing in the hydrophobic region. Globular proteins (*in red*) are shown to reside across the membrane. Alpha-helical proteins have both hydrophilic and hydrophobic parts. Membrane curvature and thickness change, which is also schematically diagrammed here. For simplicity, we did not include the presence of different ion channels, or other complicated membrane protein structures

2.3 Characteristics of Membranes

2.3.1 Physical Characteristics of Membranes

A membrane's primary role is to serve as a barrier as well as a transporter. It naturally serves as a compartment to ensure a controlled transport of material and information between the cell's inner and outer regions. A membrane maintains a constant osmotic pressure profile and a fairly constant (average) geometric thickness, which excludes other material such as electrolytes, water molecules, etc. from its vicinity. A specific back-to-back arrangement of lipids of different types, a possible presence of cholesterol, various types of membrane proteins and hydrocarbons, etc., taken together form the structure of a layer membrane with a certain approximately constant thickness. Membranes with a well-organized structure are characterized by observable geometric and physical properties, e.g., liquid crystalline structure [15], mechanical stiffness [1], capacitive effects [1], etc.

A membrane's mechanical rigidity is one of the most fundamental physical properties that have been investigated (Fig. 2.1). Suppose we increase the osmotic pressure of a cell. The cell will try to swell but this is prevented because the surface area of the plasma membrane is nearly fixed, i.e., an elastic stress will be built up inside the membrane and if this is too great the cell will burst—a condition called lysis.

To find the stress at which the membrane will burst suppose we cut the membrane along some line of length ℓ. To prevent the two sides of the cut from separating, a force, F, must be present which is proportional to ℓ. Writing $F = \gamma\ell$, then, the proportionality constant γ is called the elastic tension in the wall. This tension is not the surface tension, T, of a fluid, but it does play a similar role. The excess pressure inside a bubble of radius R, over and above atmospheric pressure, is $2T/R$. It turns out that a similar relation to this may be used to compute the excess pressure ΔP inside a spherical pressure vessel, such as a membrane, if we replace the surface tension T with the elastic tension γ. Thus we have:

$$\Delta P = \frac{2\gamma}{R} \tag{2.1}$$

The elastic stress, σ, inside the membrane is related to the elastic tension by

$$\gamma = D\sigma \tag{2.2}$$

where D is the wall thickness. The reason for this is that the surface area of the cut is ℓD and hence, the force per unit area on the surface of the cut is $F/\ell D = \gamma/D$, which is the stress of the membrane wall. From the two equations above, the elastic stress, σ, is given by

$$\sigma = \frac{R\Delta P}{2D} \tag{2.3}$$

so that the cell will burst when this stress exceeds the fracture stress of the material from which the cell membrane is made.

A large amount of diffusion in biological organisms takes place through membranes. These membranes are very thin, typically ranging from 65×10^{-10} to 100×10^{-10} m across. Most membranes are selectively permeable; that is, they allow only certain substances to cross them because there are pores through which substances diffuse. These pores are so small (from 7×10^{-10} to 10×10^{-10} m) that only small molecules can get through. Other factors contributing to the semi-permeable nature of membranes have to do with the chemistry of the membrane, cohesive and adhesive forces, charges on the ions involved, and the existence of carrier molecules. Diffusion through membranes is a relatively slow process.

In order to provide a simple mathematical description of passive diffusion across a membrane, we can apply Fick's Law to the transport of molecules across a membrane of thickness Δx. Assume also that the concentration of the solute on the left side is c_{L} and that on the right side is c_{R}. The solute diffusion current, I, across the membrane, according to Fick's Law, is given by [13]

$$I = k\frac{D}{\Delta x}A\Delta c \tag{2.4}$$

where $\Delta c = c_{\mathrm{R}} - c_{\mathrm{L}}$, k is the diffusion constant and A is the cross-sectional area of the membrane.

Under normal conditions, the physical characteristics stay constant, but in the case of abnormality (due to disease or disordered conditions) perturbations in the physical characteristics are inevitable. Different order parameters in lipid membranes are also sensitive to temperature and chemical compositions. Temperature-dependent alteration of physical properties like lipid phase properties, different dynamical properties etc. vary. The chemical composition-dependent capacitive effects of membranes also vary. Here, we should mention that plasma membranes are considered to be excellent insulators and dielectrics with a capacitance of the order of 1 μF. Membrane capacitance is a measure of the quantity of charge moving across unit area of the membrane to produce unit change of the membrane potential (which will be discussed later in detail).

2.3.2 Biochemical Characteristics of Membranes

A membrane maintains a chemical or biochemical environment inside it which differs from its interior and exterior environments. Since Robert Hooke's discovery of a cell in 1665, many further developments have been made in the understanding of the various properties of the interior and exterior environments of a cell. In the subcellular compartments exist the membrane, cytoskeleton, genetic material, organelles, etc. while structures outside the cell wall consist of capsules, flagella, fimbriae, etc. These two structural arrangements exist in different physical and chemical environments. The membrane's interior region is very different and unique. In the absence of water molecules and at low dielectric condition [20] it always maintains a gradient in most of the biochemical characteristics, compared to the exterior. For example, the membrane around peroxisomes shields the cell from peroxides. A membrane's most important selective permeability characteristics couple with the geometric size, charge, and chemical properties of the atoms and molecules attempting to cross it, and determine whether the biochemical properties [4] of both together allow the diffusion/permeabilization of the particles across the membranes. A membrane's transport proteins also play a very important role to allow particles to cross through the membranes. We discuss this issue in detail in Chap. 4.

2.3.3 Electrical Characteristics of Membranes

Membrane Potential

First measurements of the electrical properties of cell membranes were made on red blood cells by H. Fricke, and on sea urchin cells by K.S. Cole in 1937, and it was found that membranes act as capacitors maintaining a potential difference between oppositely charged surfaces composed mainly of phospholipids with proteins embedded in them. A typical value of the capacitance per unit area C/A is about 1 μF/cm^2

for cell membranes. This relates to the membrane's dielectric constant κ via the following equation:

$$\frac{C}{A} = \frac{\kappa \varepsilon_0}{d} \tag{2.5}$$

where $\varepsilon_0 = 8.85 \times 10^{-12}\,\mathrm{C}^2/\mathrm{Nm}^2$, giving a value of $\kappa \cong 10$, which is greater than $\kappa \cong 3$ for phospholipids above, resulting from the active presence of proteins. The cellular membrane is much more permeable to potassium ions than sodium ions (the intercellular fluid contains primarily sodium chloride) in the normal resting state, which results in an outward flow of potassium ions, and the voltage inside the cell is $-85\,\mathrm{mV}$. This voltage is called the resting potential of the cell. If the cell is stimulated by mechanical, chemical, or electrical means, sodium ions diffuse more readily into the cell since the stimulus changes the permeability of the cellular membrane. The inward diffusion of a small amount of sodium ions increases the interior voltage to $+60\,\mathrm{mV}$, which is known as the action potential of the cell. The membrane again changes its permeability once the cell has achieved its action potential, and potassium ions then readily diffuse outward so the cell returns to its resting potential. Depending on the state of the cell, the interior voltage can therefore vary from its resting potential of $-85\,\mathrm{mV}$ to its action potential of $+60\,\mathrm{mV}$. This results in a net voltage change of $145\,\mathrm{mV}$ in the cell interior. The voltage difference between the two sides of the membrane is fixed by the concentration difference. Having a salt concentration difference across a membrane, and allowing only one kind of ion to pass the membrane produces a voltage difference given by

$$V_\mathrm{L} - V_\mathrm{R} = \frac{k_B T}{e} \ln\left(\frac{c_\mathrm{R}}{c_\mathrm{L}}\right) \tag{2.6}$$

which is called the Nernst potential. This is the basic mechanism whereby electrical potential differences are generated inside organisms. Note that the Nernst potential difference only depends on the concentration ratio.

The membrane potential, also known as the transmembrane potential, quantifies the electrical potential difference between the interior and exterior of a cell. If the potential of the region just outside the membrane is V_o, and the potential of the region just inside the cell near the membrane is V_i, the membrane potential of the cell is $V_i - V_o$. Using the traditional definition of electrical potential, we can also define the membrane potential to be the energy required to transfer a unit charge from the exterior to the interior of a cell, crossing through the membrane. For example, if the transfer of a Q-coulomb charge from the exterior to the interior of a cell requires an energy of W joules, the potential difference (and hence the membrane potential of the cell) will be W/Q volts.

Both cellular interior and exterior regions exist with electrical conditions represented by electrical potentials. The electrical potentials of both regions depend mainly on the constituents comprising the regions. The fluids on both sides of the mainly lipid membrane contain high concentrations of various ions—both cations and anions. Among the cations, sodium (Na^+), potassium (K^+) and calcium (Ca^{2+})

are worth mentioning, while chloride (Cl^-) is the most important anion. Although both cations and anions exist in both the interior and exterior regions of a cell the concentrations of sodium and chloride ions in the exterior is higher than that in the interior. Similarly, potassium ions exist with a higher concentration in the interior region than the exterior region of a cell. The interior region is importantly characterized by a dominant presence of protein anions.

Due to the differences in charge types and concentrations between intracellular and extracellular regions, they exist with different potential conditions and, as a result, the membrane creates an electric field determining the membrane potential. The membrane therefore plays the role of the cell's electrical battery, by providing a continuous source of electrical energy originating from the potential imbalance between the intracellular and extracellular regions. This source of electrical energy also plays important roles in regulating many cellular processes, like transmitting signals between different parts of a cell, exciting ion channels across the membrane, etc. As the concentrations of each of the ions present in the intracellular and extracellular regions are different, there always exists a concentration gradient for each ion species across the cellular membrane. This gradient creates osmotic pressure for the ions to cross through the membrane. Potassium ions try to move from the intracellular to the extracellular region, while sodium and chloride ions try to flow in the opposite direction. The natural tendency of the movement of charges across the membrane causes changes in the membrane resting potential. Similarly, the changes in the membrane resting potential, due to natural or artificial stimuli, drive charges across the membrane. These rather slow dynamical processes are crucial to the normal functioning of the cell.

Resting Potential

Resting potential is simply the potential of a membrane's interior in the absence of any excitation. That is to say, this is the membrane potential of non-excitable cells, or the membrane potential of an excitable cell in the absence of any excitations. In addition to the uneven distribution of other charges as explained earlier, Na^+ concentrations of about 10 times higher on the outside and K^+ concentrations of 20 times higher on the inside of a membrane can cause huge charge density gradients. As a result, under conditions of rest, or in the absence of any excitation, the cell membrane is polarized, maintaining an effective electrostatically negative charge in the interior, which accounts for a negative interior resting potential on the order of -70 mV (Fig. 2.2).

As described above, the chemical gradient across the membrane for cells at rest causes a resting potential to be built. ATP-powered ion pumps or ion transporters play crucial roles in this process. In an animal cell, plasma membrane sodium-potassium pumps (Na^+ or K^+-ATPase) help to build sodium and potassium gradients across the membrane. The resting potential may also be altered due to the change of acidic environment across the cells. For example, in cancer cell membranes, due to elevated acidic conditions, the resting potential may be considerably altered.

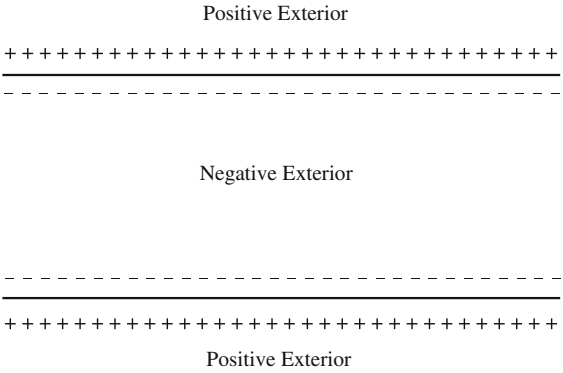

Fig. 2.2 An approximately −70 mV potential in the membrane interior region, relative to the membrane exterior region, is a general electrical condition found in normal cells. This is due to a resultant negatively charged interior and a positively charged exterior of the cell membrane

Resting Potential and the Neuron Membrane

The brain communicates with other parts of the body through neuron cells. The resting potentials in neuron membranes help transfer the messages in the form of electrical pulses. The membrane of a neuron is about 8 nm thick, containing two thick layers of fat molecules embedding larger protein molecules. In the polarized state, the membrane effectively maintains a −70 mV resting potential, due to uneven concentrations of anions and cations on both sides of the membrane. In the polarized state, the membrane is permeable to K^+ ions but does not allow larger Na^+ ions to cross through it. A nerve impulse is associated with information transfer along the axon towards the axon terminal. In this way, the transmission of information from one neuron to other neurons or different types of cells occurs. The nerve impulse or action potential is created by a depolarizing current. The passage of electrical current happens due to the movement of sodium and potassium ions across the membrane.

Action Potential

An action potential is an electrical event which lasts for a short of period of time and involves the cell membrane's electrical potential, which rapidly rises and then falls following a special type of time-dependent trajectory and spatial propagation. A typical action potential is shown in Fig. 2.3.

In several types of excitable cells such as neurons, muscle cells, endocrine cells, etc., action potentials are found to be generated [17]. An action potential occurs during the time when a neuron sends a signal down an axon which travels away from the cell body. These potentials are caused by an exchange of ions across the membrane of a neuron. Any stimulus first causes sodium channels to open; with it sodium ions move into the neuron leading the neuron to experience depolarization. Potassium channels usually take longer to open, but when they do, potassium starts moving out of the cell, which reverses the depolarization process. Consequently,

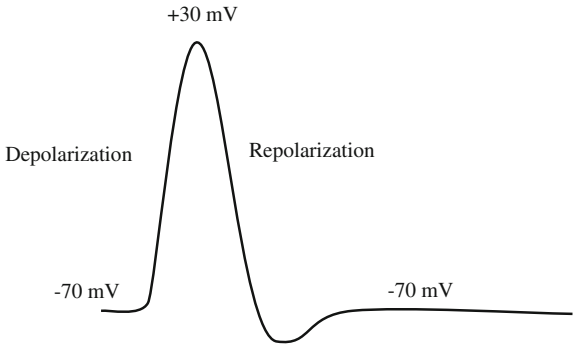

Fig. 2.3 A schematic diagram showing an action potential, illustrating electric depolarization and repolarization of the cell membrane. The membrane potential rises from $-70\,\text{mV}$ to $+30\,\text{mV}$ within about 1 ms before repolarization forces the trend to reverse, and finally the resting potential goes back to $-70\,\text{mV}$ after briefly experiencing a hyperpolarized state

sodium channels start to close. At this repolarization phase, the action potential goes past the $-70\,\text{mV}$ level, a state referred to as hyperpolarization. The ion concentration across the cell gradually returns to the resting level, and the cell returns to the usual resting potential of $-70\,\text{mV}$.

As discussed earlier, depolarization across a plasma membrane generates an action potential. Certain external stimuli reduce the charge across the plasma membrane. A stimulus may originate from various sources. Mechanical stimuli like stretching, sound waves, etc. activate mechanically gated sodium channels across the membrane. Certain neurotransmitters like acetylcholine open ligand-gated sodium channels. Various electrical impulses may also stimulate and cause depolarization. The favorable diffusion of sodium ions into the cell locally reduces the membrane's resting potential. If the reduction is considerable, e.g., if the potential is reduced to the threshold voltage level (in mammalian neurons, about $-50\,\text{mV}$), an action potential is generated in the cell. This kind of action potential usually lasts for less than 1 ms. Action potentials generated by voltage-gated calcium channels may last much longer, which is of the order of 100 ms or more. The action potential is very much organ-specific and is accompanied by various complexities because, in different parts of the body, the stimuli appear from different types of sources. For instance, in some types of neurons, a long burst of rapidly emitted sodium spikes appears due to the slow calcium spike-induced driving force, whereas, in cardiac muscle cells, muscle contraction takes place due to the rapid onset of a calcium spike provoked by an initial fast sodium spike.

The Nernst Potential and Membrane Potential

In physiology, the Nernst equation (mentioned above) finds its application in determining the potential of an ion across a membrane. The general form of the potential can be written as

$$V_{\text{Nernst}} = \left(\frac{RT}{zF}\right) \ln\left(\frac{[N]_{\text{out}}}{[N]_{\text{in}}}\right) \tag{2.7}$$

The Nernst equation determines the equilibrium potential, often called 'the Nernst potential' for an ion across the membrane. From the equation, it is clear that this potential depends on the ion concentrations outside ($[N]_{\text{out}}$) and inside ($[N]_{\text{in}}$) of the membrane, the valence of the ionic species, z, and the absolute temperature in Kelvin T. The constant R is the universal gas constant ($R = 8.314\,\text{J}\,\text{K}^{-1}\,\text{mol}^{-1}$) and F is the Faraday constant ($F = 96{,}485\,\text{C}\,\text{mol}^{-1}$). The development of a Nernst potential depends on the following two criteria: (i) the concentration gradient of an ion across the membrane, and (ii) selective ion channels creating a pathway for a specific type of ion flow across the membrane. It is, therefore, natural to associate the Nernst potential with an ion type. Nernst or equilibrium potentials V_{Na}, V_{K}, V_{Cl}, V_{Ca}, etc. can be found for Na^+, K^+, Cl^-, Ca^{2+}, etc. ionic species respectively. In the case when there exists only one ionic species in the system, and/or channels due to ion specificity of the channels, the corresponding Nernst or equilibrium potential is also the membrane potential (V_m). However, in cases where there exists the flow of different ions across the membrane, the membrane potential is the sum of all Nernst potentials referring to ions, normalized with the corresponding conductance. If there are a number of ions flowing across the membrane, the following relation exists:

$$V_m = \sum_i \left(\frac{g_i}{G}\right) V_{\text{Nernst},i} \tag{2.8}$$

where $V_{\text{Nernst},i}$ and g_i are respectively the Nernst potential and conductance (inverse Ohmic resistance) through the membrane, corresponding to the ion indexed i. Here, $G = \sum_i g_i$.

In physiology, the most common potentials due to ion flows through a membrane are V_{Na}, V_{K}, V_{Cl}, etc. If Na^+, K^+, Cl^- flow across a membrane with the corresponding Nernst potentials V_{Na}, V_{K}, V_{Cl}, we find the membrane potential to be represented by the following equation:

$$V_m = \left(\frac{g_{\text{Na}}}{G}\right) V_{\text{Na}} + \left(\frac{g_{\text{K}}}{G}\right) V_{\text{K}} + \left(\frac{g_{\text{Cl}}}{G}\right) V_{\text{Cl}} \tag{2.9}$$

Here, G is the sum of conductances g_{Na}, g_{K}, g_{Cl}, g_{Ca} corresponding to the Na^+, K^+, Cl^-, Ca^{2+} ions across the membrane, respectively.

In the presence of several ions flowing across the real cell membrane, the equilibrium of the cell depends on the relative membrane permeability for these ions. To determine the membrane resting potential, the following Goldman–Hodgkin–Katz (GHK) equation is used:

$$V_m = \left(\frac{RT}{F}\right) \ln\left(\frac{\sum_i P_i(N_i^+)[N_i^+]_{\text{out}} + \sum_j P_j(N_j^-)[N_j^-]_{\text{in}}}{\sum_i P_i(N_i^+)[N_i^+]_{\text{in}} + \sum_j P_j(N_j^-)[N_j^-]_{\text{out}}}\right) \tag{2.10}$$

Here, the symbols P stand for the respective relative permeabilities of the ions, the Ns in the square brackets stand for the ion concentrations, $+/-$ stand for

positive/negative species, and *out*/*in* stands for extracellular /intracellular regions, respectively. For example, in a real cell, in which Na^+, K^+ and Cl^- ions are the major contributors to the membrane potential, the GHK equation can be written as

$$V_m = \left(\frac{RT}{F}\right) \ln\left(\frac{\left(p_K[K^+]_{out} + p_{Na}[Na^+]_{out} + p_{Cl}[Cl^-]_{in}\right)}{\left(p_K[K^+]_{in} + p_{Na}[Na^+]_{in} + p_{Cl}[Cl^-]_{out}\right)}\right) \tag{2.11}$$

Here, $[K^+]$, $[Na^+]$ and $[Cl^-]$ represent ion concentrations with subscripts out and in standing for the region (outside and inside) of the cell. Further, p_K, p_{Na} and p_{Cl} are the relative membrane permeabilities of the ions K^+, Na^+ and Cl^-, respectively. Normally, permeability values for ions are reported as relative permeabilities (unitless) with p_K having the reference value of one.

The Membrane as a Capacitor

A cell membrane separates charges on both sides of it. The inner core of a membrane experiences a low dielectric state, while the outside experiences a high dielectric state [20]. The membrane therefore generally acts as an insulator, with conducting media on both sides.

Based on a simple electrostatic analysis, we know that the capacitance of an object is defined as the amount of charge separated across it and creating a potential difference between the two terminals. That is, if a potential V can hold a charge Q across a capacitor, the capacitance C can be defined as

$$C = \frac{Q}{V} \tag{2.12}$$

A cell membrane structure suggests a model where a relatively low dielectric medium is surrounded by two conducting media on both sides (intracellular and extracellular regions). This makes a membrane equivalent to a leaky capacitor, since ions are still allowed to flow through it.

To calculate the membrane capacitance, we need to use standard electrostatics with Coulomb's law applied to an equivalent model structure for the membrane, which produces a separation of two parallel conducting plates by an insulating medium. Here, the membrane is comparable to an insulating medium. The capacitance of a cell membrane can thus be defined as

$$C_m = \frac{\kappa\varepsilon_0}{d} \tag{2.13}$$

Here, κ is the dielectric constant for the membrane's inner core, ε_0 is the permittivity of free space, and d is the membrane thickness. A low dielectric medium (inner layer) exists in between two conducting media (outside membrane). Depending on the variations in the values of $\frac{\kappa\varepsilon_0}{d}$ in various types of cells, the values of

capacitance of the corresponding membranes may vary. However, a typical value is often found to be of the order of $1.0\,\mu\text{F/cm}^2$. Most importantly, it is worth mentioning that the cholesterol level, phospholipids and glycolipids, membrane proteins, hydrocarbons, etc. all together are responsible for yielding a certain value of membrane capacitance. Unlike animal cytoplasmic membranes, bacteria (prokaryotes) do not generate cholesterol, which may account for a considerable effect on their membranes' electrical properties, including their capacitance.

Understanding the capacitive effect of the membrane helps in analyzing the membrane's electrical properties, through a model often referred to as the *Electrical Circuit Model of the Cell Membrane* .

Here, the membrane is considered as a capacitor in parallel with a resistor. The (not necessarily Ohmic) resistance acts against the flow of ions across the membrane, which is represented by ion current I_{ion}. The capacitive current is given by $C_m \frac{\mathrm{d}V}{\mathrm{d}t}$. The capacitive current and the ion current together conserve the current flow between the inside and outside of the membrane. Therefore,

$$C_m \frac{\mathrm{d}V}{\mathrm{d}t} + I_{\text{ion}} = 0 \tag{2.14}$$

The analytical calculation of I_{ion} is a long-standing challenge. The following GHK current equation is one such expression for I_{ion} across the membrane:

$$I_{\text{ion}} = \frac{D}{L} \frac{z^2 F^2}{RT} V \frac{[N]_{\text{in}} - [N]_{\text{out}} e^{\frac{-zFV}{RT}}}{1 - e^{\frac{-zFV}{RT}}} \tag{2.15}$$

Here, D denotes Einstein's diffusion constant, L is the membrane thickness, and $[N]_{\text{in}}$ and $[N]_{\text{out}}$ are the ion concentrations inside and outside the cell across the membrane, respectively (see Fig. 2.4).

2.3.4 Excitability and the State of the Membrane Potential

Neurons, muscle cells, etc., are collectively called excitable cells, since they use their membrane potentials as signals. The operation of the nervous system, muscle contraction, etc., depends on the generation and propagation of electrical signals, and membrane potentials in these cases mainly serve this purpose. We have earlier described in detail how the membrane potential can be regulated by controlling certain cellular processes, such as the control of the ionic current carrying ion channels across membranes.

Electrical signaling in cells depends largely on the type of cell involved (see e.g., [17]). To understand it better, the cells are grouped into two categories, namely, non-excitable cells and excitable cells. Non-excitable cells maintain stable equilibrium potentials. If an externally applied current perturbs the membrane potential of a non-excitable cell, the withdrawal of the current ensures that the potential returns to

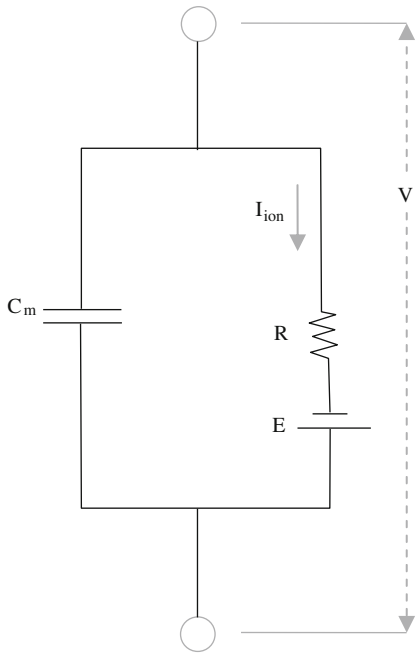

Fig. 2.4 Cell membrane representation in terms of an equivalent electric circuit showing capacitive effects. A parallel capacitor and resistor combination in schematic form represents the equivalent electrical circuit

its equilibrium state. Epithelial cells, photoreceptors, etc. fall into the non-excitable cell category.

Different research findings report that non-excitable cells are found not to generate all-or-none action potentials in response to depolarizing stimuli, due to a lack of voltage-gated Na^+ or Ca^{2+} channels [3, 5, 7, 22]. Consequently, membrane potential changes are proposed to influence the localized (intracellular) concentration of Ca^{2+} ions ($[Ca^{2+}]_i$) responses, mainly by altering the driving force for Ca^{2+} entry through ligand-gated or second messenger-operated channels. However, it was reported [16] that during stimulation of a non-excitable cell (e.g. metabotropic purinoceptors), membrane depolarization evokes an increase in Ca^{2+} concentration in the interior cellular regions, primarily due to the release of Ca^{2+} from intracellular stores. Although depolarization in non-excitable cells was found to result in a decrease in $[Ca^{2+}]_i$, hyperpolarization causes an increase of $[Ca^{2+}]_i$ during activation of mast cells, lymphocytes, and related cell lines [6, 14, 21]. The results of this research suggest that the electrogenic influences in non-excitable cells may also originate from various organ-specific mechanisms.

On the other hand, in excitable cells, a strong externally applied current causes the membrane potentials to undergo a large excursion, called an ‘action potential’, before eventually returning to rest. Most neurons, cardiac cells, smooth and skeletal muscle cells, secretory cells, etc., fall into the excitable cell category. In this section of the chapter, we mainly address the aspects of electrical signal propagation and its mathematical modeling in excitable cells. This is a century-old problem, which

has achieved a very high level of understanding following a few ground-breaking discoveries, such as the seminal work of Alan Hodgkin and Andrew Huxley, who in 1952 developed the first quantitative model of the propagation of an electrical signal along a squid giant axon. The Hodgkin–Huxley theory is applicable, not only to electrophysiology, but also to applied mathematics through appropriate modifications. The creation of a new field of mathematics called 'the study of excitable systems' has been made possible, thanks to the remarkable simplification and extensions of the Hodgkin–Huxley theory. We provide more details below.

The Hodgkin–Huxley Model of the Action Potential: A Quantitative Model

The cell membrane has well-defined biochemical and biophysical characteristics. We have briefly described these two aspects earlier in this chapter. The biophysical characteristics of a cell membrane are represented by the generally accepted Hodgkin–Huxley model. The lipid bilayer is represented as a capacitor—the low-dielectric ($\varepsilon \sim 2$) region inside the membrane relative to the outside region with high dielectric values ($\varepsilon \sim 80$) [20] makes the cell membrane an almost perfect capacitor. Voltage-gated and leak ion channels are represented by nonlinear (g_{n}) and linear (g_{L}) conductances, respectively. The electrochemical gradients driving ion flow are represented by batteries (E_{n} and E_{L}), and ion pumps and exchangers are represented by current sources (I_{p}). The voltage values for the batteries are determined from the Nernst potentials of specific ionic species.

In an idealized cell, with a small portion of the membrane represented as equivalent electrical circuit, we can apply the Hodgkin–Huxley model for calculating the membrane current (I_m) by the following current equation:

$$I_m = C_m \frac{\mathrm{d}V}{\mathrm{d}t} + I_{\mathrm{K}} + I_{\mathrm{Na}} + I_{\mathrm{L}} \tag{2.16}$$

Here, V is the membrane voltage, I_{K} and I_{Na} are the potassium and sodium currents, respectively, and I_{L} is the sum of all leakage currents due to the flow of other ions moving passively through the membrane.

The charge stored in the capacitive membrane is $q_m = C_m V$ where V is the voltage across the capacitor, which is comparable to the transmembrane potential. Earlier in this chapter, we have explained how currents across the membrane are conserved quantities, which is possible due to the fact that the cell membrane is modeled as a capacitor in parallel with ionic currents. Now, if ionic currents are considered to depend on both transmembrane voltage V and time t, the membrane capacitive current follows the formula

$$C_m \frac{\mathrm{d}V}{\mathrm{d}t} + I_{\mathrm{ion}}(V, t) = 0 \tag{2.17}$$

In the Hodgkin–Huxley theory, besides the main currents, sodium and potassium ion currents across the membrane, all other small currents are combined to form a leakage current I_L.

In the squid giant axon the I–V curves of open Na^+ and K^+ channels are approximated by linear equations. Therefore, the membrane capacitive current equation becomes:

$$C_m \frac{dV}{dt} = -g_{Na}(V - V_{Na}) - g_K(V - V_K) - g_L(V - V_L) + I_{ext} \tag{2.18}$$

Here, I_{ext} is the externally applied current, and g_{Na}, g_K and g_L represent conductances (reciprocal of Ohmic resistance) for Na^+ and K^+ ions and other ions responsible for leakage currents, respectively. Voltages V_{Na}, V_K, and V_L are membrane resting potentials, corresponding to Na^+, K^+ ions and other leakage ions across the membrane. The previous first-order ordinary differential equation can be rewritten as a more general form of equation, representing a capacitive current of the membrane according to:

$$C_m \frac{dV}{dt} = -g_{eff}(V - V_{eq}) + I_{ext} \tag{2.19}$$

Here, $g_{eff} = g_{Na} + g_K + g_L$ is the effective conductance across the membrane, and $V_{eq} = (g_{Na}V_{Na} + g_K V_K + g_L V_L)/g_{eff}$ is the membrane resting potential.

Specifically, in voltage-gated ion channels, the channel conductance g_i is a function of both time and voltage ($g_n(t, V)$), while in leak channels, g_L is a constant (g_L).

The above description can be summarized in a way similar to that in the original paper of Hodgkin–Huxley [12]. The electrical behavior of a membrane may be represented by a network, as shown in Fig. 2.5. Here, current can be carried through the membrane either by charging the membrane capacitor or by the movement of ions Na^+, K^+, etc. through the corresponding equivalent resistors in parallel. The ionic current corresponding to a specific ion is proportional to the difference between the membrane potential and the equilibrium potential for a specific ion. Here, the proportionality constant is the Ohmic conductance for the corresponding ion.

Voltage- and Time-Dependent Conductance in the Hodgkin–Huxley Model

As explained earlier, the total membrane current I_m can be subdivided into two main categories, which are capacitive currents and ionic currents. Thus, under normal conditions the following equation is valid:

$$I_m = C_m \frac{dV}{dt} + g_{Na}(V - V_{Na}) + g_K(V - V_K) + g_L(V - V_L) \tag{2.20}$$

This equation gives the values of the membrane capacitance that are independent of the magnitude and sign of V, and are little affected by the time course of V (see Table 1

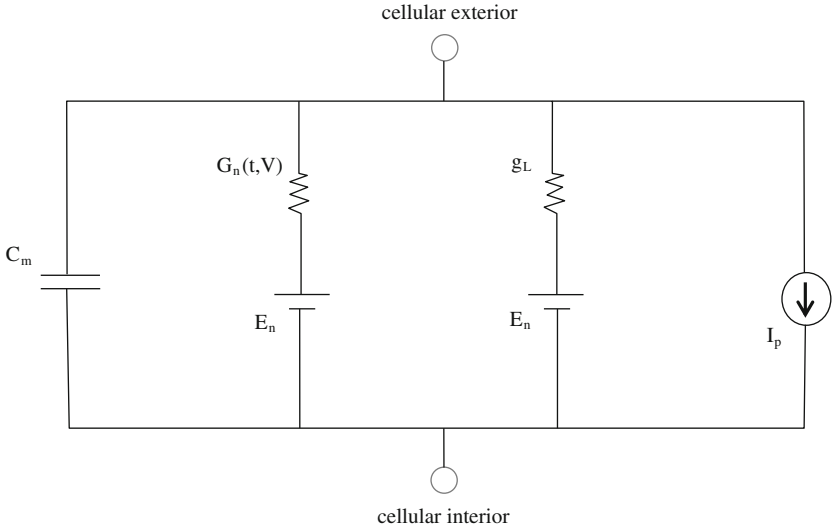

Fig. 2.5 A capacitor–resistor circuit representation of the cell membrane. This is a more detailed form of the equivalent circuit representation presented earlier in this chapter

of [8]). The evidence for the capacitive currents and ionic currents to be parallel was well-established in the study by Hodgkin et al. [8]. A major reservation, however, is that the earlier equation takes no account of dielectric loss in the membrane. Since the capacitive surge was found to be reasonably close to that calculated for a perfect capacitor [8], it was then predicted that the mentioned dielectric condition inside the membrane would not change the structure of the equation dramatically. So far this has been found consistent with the data obtained using modern approaches that include other constituents inside membranes.

The Potassium Conductance

Hodgkin and Huxley investigated the time dependence of ionic conductance both theoretically and experimentally. In their experimental investigations they studied in detail, for example, the case of potassium ion conduction. Based on their famous 1952 paper [12], it is clear that the rise of potassium conductance associated with depolarization of a potential is followed by the fall of conductance associated with repolarization of the resting potential. Here, the nonlinear rise (depolarizing effect) of conductance g_K is found to be mathematically very well-fitted by the function $(1 - e^{-t})^4$ while the fall is approximately by the function e^{-4t}. These two different 4th-order mathematical forms explain nicely the marked inflection for the rise, with a simple exponential for the fall for g_K. A similar mathematical fit to experimental data using other functional forms could also be possible, but might require other

terms, e.g., a term representing inactivation would be necessary in the case of a third power in the exponential power series expansion, making it much less elegant and not convincing.

Following a detailed analysis [12], the generalized form of g_K can be constructed as

$$g_K = \left(g_{K-}^{1/4} - \left[g_{K-}^{1/4} - g_{K0}^{1/4}\right]e^{-t/\tau_n}\right)^4 \tag{2.21}$$

where g_{K-} is the asymptotic value of the conductance, and g_{K0} is the conductance at $t = 0$. Also, τ_n is the inverse of the sum of the rate constants describing the timescale of the resultant net inward flow of ions. The proposed equation is a best fit to the experimental results, as presented in the original work [12].

The Sodium Conductance

The transient change in sodium conductance g_{Na} was described by considering two variables, both of which obey first-order equations. Following a few formal assumptions [12], g_{Na} was found to fit very well to experimental observations by taking the following form:

$$g_{Na} = g'_{Na}[1 - e^{-t/\tau_m}]^3 e^{-t/\tau_h} \tag{2.22}$$

Here, g'_{Na} is the value which the sodium conductance would attain in the case when the proportions of the inactivating molecules on the outside boundary of the membrane τ_m and τ_h are the inverse values of the net transfer rate constants for inside and outside directions, respectively.

A detailed analysis of the rate constants and other related aspects of membrane conductance described in the Hodgkin–Huxley models is not only interesting but also very important. However, due to space limitations and the scope of this book, we invite the reader to study the original material presented in the ground-breaking papers published by this pair independently and with others in the early 1950s [8–12, 19]. Below, we discuss a few more models which were subsequently built on the basis of the Hodgkin–Huxley model in order to perform a better qualitative analysis, and to better understand the various aspects of the Hodgkin–Huxley model.

2.3.5 The FitzHugh–Nagumo Model

Before discussing the FitzHugh–Nagumo model, we first further reduce the Hodgkin–Huxley model to a more generalized form. Based on the experiments performed by Hodgkin and Huxley on the squid giant axon between 1948 and 1952, they constructed a model for patch clamp experiments. This provided a mathematical description of the axon's excitable nature. Here, a key model assumption was that the membrane contains channels for potassium and sodium ion flows. Following the

assumption made by Hodgkin and Huxley, which replaces g_{Na} and g_{K} with $g^0_{\mathrm{Na}} m^3 h$ and $g^0_{\mathrm{K}} n^4$, the equation for the membrane's capacitive current becomes

$$C_m \frac{\mathrm{d}V}{\mathrm{d}t} = -g^0_{\mathrm{Na}}\, m^3\, h(V - V_{\mathrm{Na}}) - g^0_{\mathrm{K}} n^4 (V - V_{\mathrm{K}}) - g_{\mathrm{L}}(V - V_{\mathrm{L}}) + I_{\mathrm{ext}} \quad (2.23)$$

Here, the conductances for both sodium and potassium ions are expressed in terms of some baseline values g^0_{Na} and g^0_{K}, respectively, and secondary variables m, h, and n. The variables are hypothesized as potential-dependent gating variables, whose dynamics are assumed to follow first-order kinetics, and the equation takes the following form:

$$\tau_s(V) \frac{\mathrm{d}s}{\mathrm{d}t} = s_-(V) - s; \quad s = m, h, n, \quad (2.24)$$

where $\tau_s(V)$ and $s_-(V)$ are respectively the time constant and the rate constant determined from experimental data. The above two equations, taken together, represent a four-dimensional dynamical system known as the simplified Hodgkin–Huxley model, which provides a basis for qualitative explanation of the formation of action potentials in the squid giant axon.

FitzHugh later sought to reduce the Hodgkin–Huxley model to a two-variable model, for which phase plane analysis can be carried out reasonably easily. In the Hodgkin–Huxley model, the gating variables n and h were found to have slow kinetics relative to m, and that $n + h$ assumes an approximately constant value ($\sim$0.8). As a result of these two observations, a new two-variable model, referred to as the fast-slow phase model, was proposed by FitzHugh for calculating the capacitive current of the membrane, which is as follows:

$$C_m \frac{\mathrm{d}V}{\mathrm{d}t} = -g^0_{\mathrm{Na}} m^3_-(V)(0.8 - n)(V - V_{\mathrm{Na}}) - g^0_{\mathrm{K}} n^4 (V - V_{\mathrm{K}}) - g_{\mathrm{L}}(V - V_{\mathrm{L}}) + I_{\mathrm{ext}} \quad (2.25)$$

and

$$n_s(V) \frac{\mathrm{d}n}{\mathrm{d}t} = n_-(V) - n \quad (2.26)$$

Here, a phase-space description of the action potential's formation and decay has been provided. In these equations, V and n are the fast and slow variables, respectively. The V nullcline can be defined by $C_m \frac{\mathrm{d}V}{\mathrm{d}t} = 0$ and has a cubic shape, while the n nullcline is $n_-(V)$, and it increases monotonically. Since it can be approximated by a straight line, this suggests a polynomial model reduction of the form

$$\frac{\mathrm{d}v}{\mathrm{d}t} = v(v - \beta)(1 - v) - u + I \quad (2.27)$$

and

$$\frac{\mathrm{d}u}{\mathrm{d}t} = \delta(v - \gamma u) \quad (2.28)$$

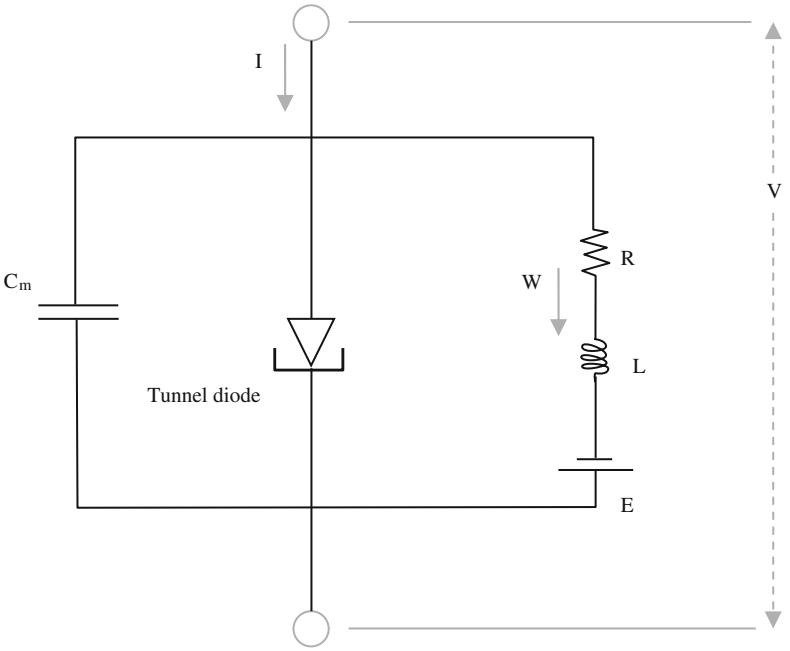

Fig. 2.6 An equivalent electric circuit diagram for the FiztHugh–Nagumo equations

The model equations have been transformed into dimensionless form, where v represents the fast variable which is the electrostatic potential, u represents the slow variable, the sodium gating variables, and β, δ and γ are constants which satisfy the conditions $0 < \beta < 1$ and $\delta \ll 1$, accounting for the slow kinetics of sodium channel. Later, Nagumo constructed a circuit using tunnel diodes for the nonlinear channel element, whose model equations are those of FitzHugh. Hence, the previous dimensionless equations are now generally accepted as the so-called FiztHugh–Nagumo model. The previous equations representing the FiztHugh–Nagumo model are often expressed in more generalized forms as:

$$\epsilon \frac{\mathrm{d}v}{\mathrm{d}t} = f(v, u) + I \tag{2.29}$$

and

$$\frac{\mathrm{d}u}{\mathrm{d}t} = g(v, u) \tag{2.30}$$

where, as described previously, the nullcline $f(v, u) = 0$ represents a cubic shape. That means that for a finite range of values of u, there are three solutions $v = v(u)$ of the equation $f(v, u) = 0$. The nullcline $g(v, u) = 0$ is assumed to have one intersection with the curve $f(v, u) = 0$.

Finally, the FiztHugh–Nagumo model represents a simplified model of the cell membrane as presented in Fig. 2.6. In this simplified model, the membrane patch consists of three components, a (membrane) capacitor, a nonlinear current–voltage device for the fast current W, and a resistor, an inductor and a battery in series for the recovery current. This circuit was built and tested in 1962 by Nagumo, a Japanese electrical engineer [18].

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