My Thesis Title

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Define Term

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Abstract

This thesis describes stuff aplenty.

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Acknowledgements

This thesis is the culmination of many year's work and would not have been possible without the help and support of many people.

First and foremost I would like to thank my supervisor, Dr X, for his mindless support and useless proof-reading. Blah, gibber ...

Many of the ideas presented are the outcome of discussions with members of the Computational Biology Group and beyond. In particular I owe much to X and Y who ...

My research was funded by a ...

Throughout this year I have enjoyed the unfailing support of my pet hamster.

Notation & Nomenclature

AP	Action Potential
$\mathrm{AP_{RMS}}$	Root mean square difference between 'test' and 'training' data for V_m data
APD_x	Action potential duration to $x\%$ repolarisation
$[\mathrm{Ca^{2+}}]_\mathrm{i}$	Free cytosolic $\mathrm{Ca^{2+}}$ concentration
g_X	Maximum conductance for current X
$I_\mathrm{Ca,L}$	L-type $\mathrm{Ca^{2+}}$ current
I_K1	Inward rectifying $\mathrm{K^+}$ current
I_Kr	Rapidly delayed rectifier $\mathrm{K^+}$ current
I_Ks	Slowly delayed rectifier $\mathrm{K^+}$ current
I_Na	$\mathrm{Na^+}$ current
I_NaCa	$\mathrm{Na^+}$-$\mathrm{Ca^{2+}}$ exchanger current
I_NaK	$\mathrm{Na^+}$-$\mathrm{K^+}$ pump current
I_stim	Stimulus current
I_to	$\mathrm{K^+}$ transient outward current
$I_\mathrm{to,f}$	Fast $\mathrm{K^+}$ transient outward current
$I_\mathrm{to,s}$	Slow $\mathrm{K^+}$ transient outward current
V_m	Membrane potential
$\Delta[\mathrm{Ca^{2+}}]_\mathrm{i}$	Difference between systolic $[\mathrm{Ca^{2+}}]_\mathrm{i}$ and diastolic $[\mathrm{Ca^{2+}}]_\mathrm{i}$

INTRODUCTION 1

They were the best of times, they were the worst of times...

This will be the introduction.

LITERATURE REVIEW 2

There are more things in heaven and earth, Horatio,

Than are dreamt of in your philosophy. (*Hamlet*, Act 1, Scene 5)

In this section, the relevant background information is given. This starts with a brief outline of the function and structure of the heart, ranging from the high order organisation of the heart into functional compartments, down to the cellular level. Further details are then given on the physiological aspects of some of the subcellular components of cardiac tissue. The evolution of computational models to describe the electrical activity of the heart is charted briefly, with special attention given to models specifically used in this thesis. Variation is then described, both in experimental and physiological measurements, and how this variation has been addressed in computational models. Then the pathological condition of ischæmia will be described, and the computational efforts to model this outlined.

2.1 Cardiac Physiology

At the most general level, the heart serves as a pump to transport blood around the body to deliver nutrients and remove waste products. It does this by rhythmic, organised contraction—the rate of this contraction varies depending on species, age, condition of the heart and the activity being undertaken by the organism.
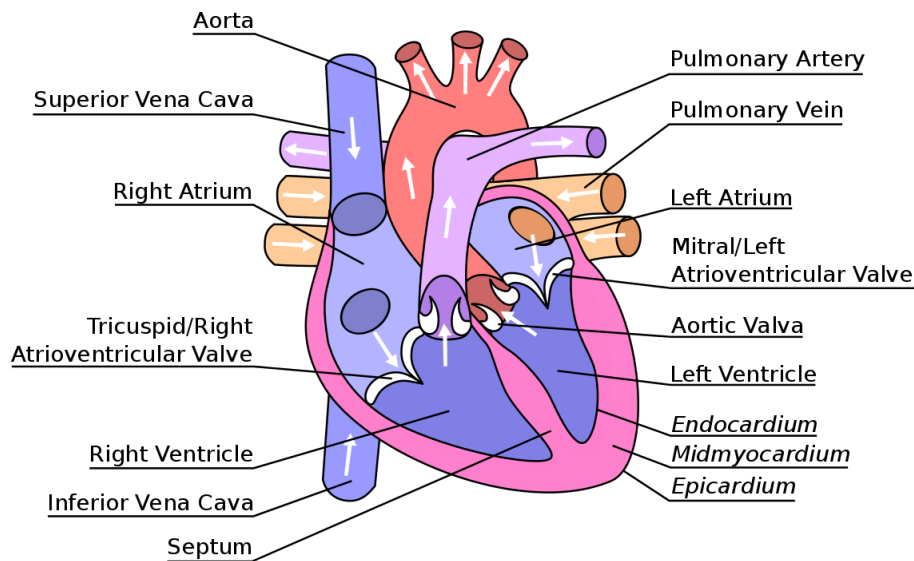
Figure 2.1: *Diagram of the longitudinal cross-section of a mammalian heart. The direction of blood flow is shown by white arrows, and all major structural components are labelled.*

2.1.1   Structure of the Mammalian Heart

Fig. 2.1 shows the anatomical structure of the mammalian heart—while the size obviously varies widely between mammals, the overall architecture remains constant. It is split into two halves, left and right, by a muscular wall called the *septum*, and then further subdivided into two chambers, the larger, lower chamber being a *ventricle*, and the smaller, upper chamber called an *atrium*.

The passage of blood through the heart follows thus: firstly, deoxygenated blood from the body enters the heart via the superior and inferior/posterior *vena cava*, with superior and inferior representing whether the blood comes from the upper or lower half of the body respectively. Via this channel, it enters the *right atrium*. Passing through the *tricuspid valve* (also known as the right atrioventricular valve), the blood enters the *right ventricle*, where it is then pumped via the *pulmonary artery* to the lungs, where it is oxygenated. The blood returns to the heart via the *pulmonary vein*, entering the *left atrium*, before moving through the *mitral valve* (also known as the left atrioventricular valve) into the *left ventricle*. The blood is then pumped via the *aorta* to the rest of the body. The valves in the heart serve to ensure the flow of blood is always in the correct direction.

The walls of the heart are mostly composed of muscular tissue known as *myocardium*. The
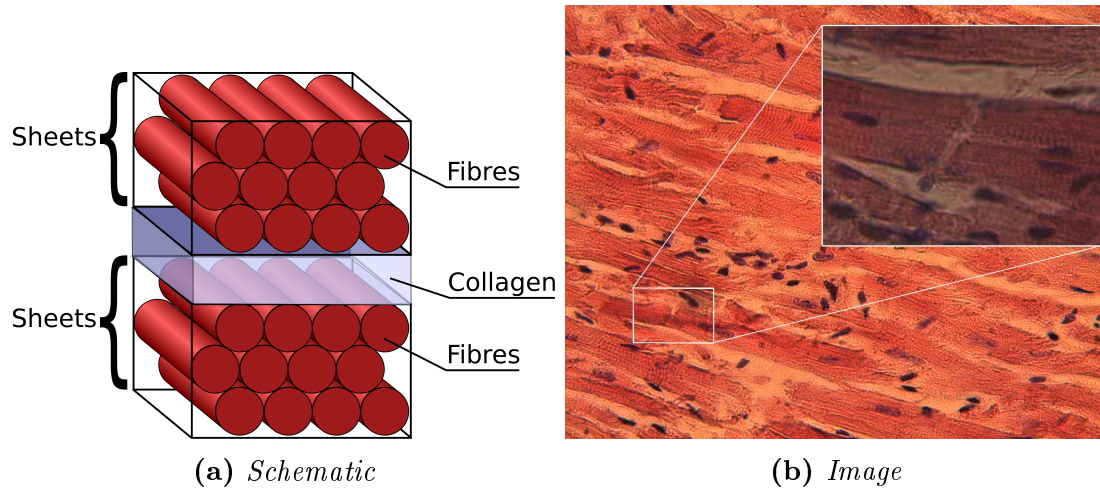
(a) *Schematic* **(b)** *Image*

Figure 2.2: *(2.2a) Schematic of the structure of myocardial sheets and fibres. (2.2b) Image of cardiomyocytes demonstrating the interconnected nature of the individual myocytes into fibres.*

thickness of the myocardium is not constant throughout the heart, being thickest in the left ventricle, which requires the greatest force of contraction to pump the blood from the heart to the rest of the body. The myocardium can be split into three different regimes, as labelled in italics in Fig. 2.1: the *epicardium* is the outermost layer of the myocardium, the *midmyocardium* is the middle layer, and the *endocardium* is the innermost layer. The cells composing these different layers possess different electrophysiological properties; the differences, and the consequences, will be expanded upon in §2.1.2.

The structure within the myocardium is shown in Fig. 2.2a: it is composed of a series of sheets of tissue separated by collagen. The myocytes themselves lie longitudinally in these sheets to form fibres, with myocytes in the fibre direction connected to each other by intercalated discs (as opposed to skeletal muscle, which is composed of multinucleated fibres); this structure is seen in Fig. 2.2b. One attribute of these discs is the presence of *gap junctions*, which serve to electrically couple the myocytes by allowing ion flow between the two neighbouring myocytes in the fibre direction. This arrangement of the myocytes into electrically coupled fibres allows for coordinated contraction, and the fibrous structure allows the heart to twist as it contracts, leading to a more efficient pumping mechanism.

The conduction pattern of the heart is key to the effective pumping mechanism—by
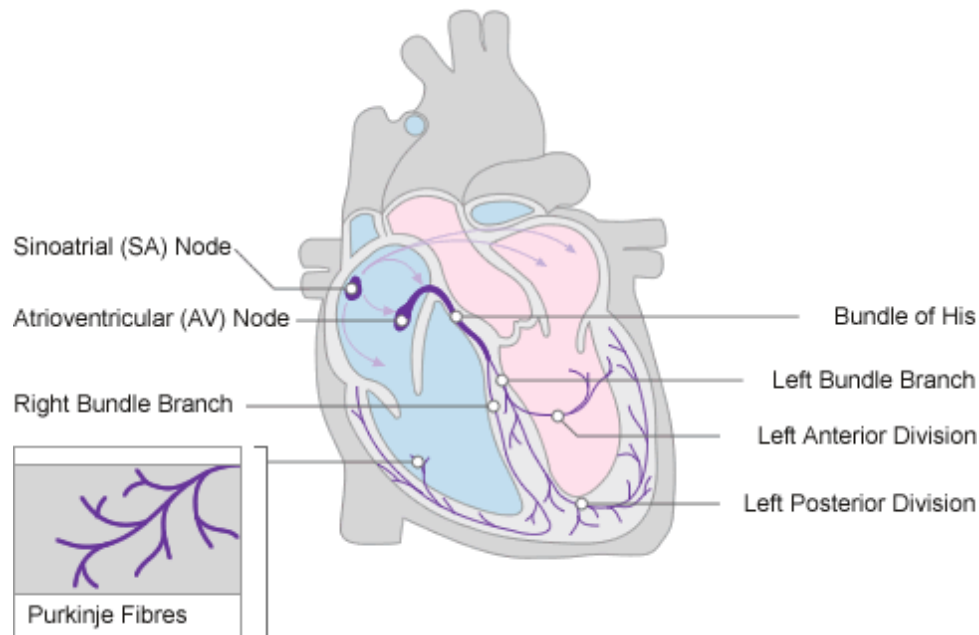
Figure 2.3: *Schematic outline of the sequence of electrical activation in the mammalian heart. Image originally downloaded from www.nottingham.ac.uk on 10th December 2012.*

controlling the sequence of electrical events in the heart, the sequence of conduction is similarly controlled. An outline of the conduction pattern of the heart is shown in Fig. 2.3. The sequence of activation starts amongst a group of self-excitatory cells at the top of the right atrium, called the *sinoatrial node*. The action potential wave spreads from this node, causing the atrium to contract, before it reaches the *atrioventricular node*—this is the only pathway for electrical excitation to pass from the atria to the ventricles. From the atrioventricular node, the excitation wave passes to the *Bundle of His*, which conducts the electrical stimulus via the left and right bundle branches into the *Purkinje system* near the bottom of the heart, which transmits the stimulus to the ventricular surface; as the stimulus has thus been transmitted directly from the atria to the bottom of the ventricles, the excitation wave thus spreads upwards through the ventricles, allowing the ventricles to contract from the bottom up as required.

2.1.2   Electrophysiological Properties of Cardiomyocytes

As previously stated, the main rôle of the heart is to serve as a pump to circulate the blood around the body. To do this effectively, it requires coordinated contraction, and

this is achieved through the use of coupling the electrical activity of the heart of the mechanical activity (the details of this mechanism will be expanded upon in §2.1.2).

Physically, cardiomyocytes are typically $10-20\mu$m in diameter, and $50-100\mu$m in length. They are cells bound by a lipid bilayer membrane, separating intracellular space (containing the cytoplasm, nuclei and other organelles) from the extracellular space. Embedded within the bilayer are various ion channel and transporters, which allow the membrane to be selectively permeable to various ions and other substances, and to regulate the flow of these ions as required. Thus, the intracellular space has a very different composition to the extracellular space, with the intracellular space containing a high and low concentration of potassium (K^+) and sodium (Na^+) ions respectively; the reverse is true for the extracellular space. This concentration gradient is largely maintained by the Na^+-K^+ pump. These concentration differences are the main reason behind their being a potential difference set up across the cell membrane, referred to as the *membrane potential* (V_m). At rest, due to the high K^+ and low Na^+ in the cell, this potential is negative, though the magnitude varies depending upon the location in the heart; a cardiomyocyte in the ventricles typically has a resting potential (V_{rest})) of about -80mV, while the sinoatrial node has a resting potential of between -50 and -60mV.

Electrically, the heart can be modelled with great success as an analogue to an electrical circuit (2), where the lipid bilayer is represented as a capacitor, and the various ion channels and transporters that span the membrane are represented as resistors, which change their 'resistance' depending on their state. By altering the resistance of these channels, ion flow across the membrane is permitted via an ionic current, which changes the membrane potential—the characteristic change in membrane potential associated with, and causing, a contraction is referred to as the *action potential* (AP).

It is a point of nomenclature that an 'inward' current represents the movement of positive ions from the extracellular to the intracellular space, and an 'outward' current is the reverse. This definition is based on the movement on electrical charge, and not on the
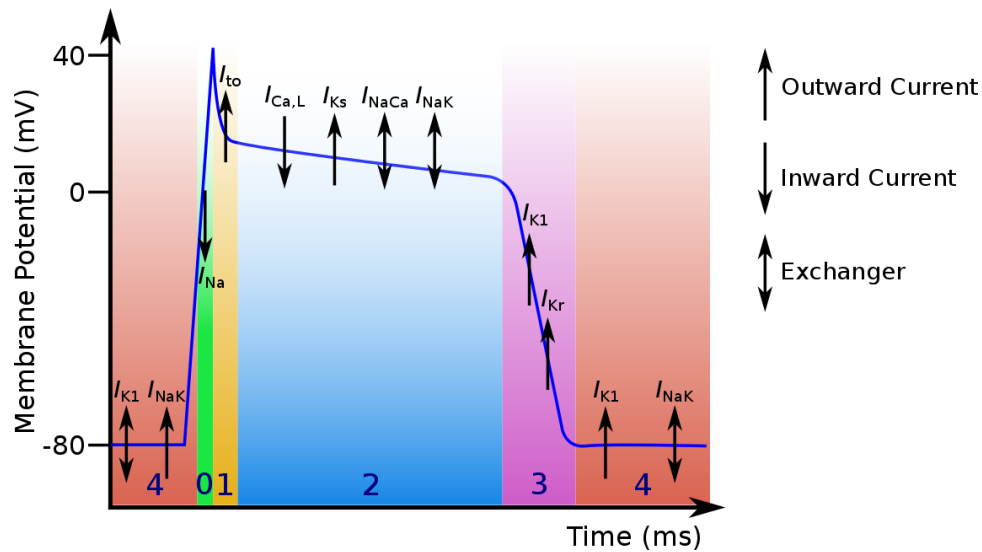
Figure 2.4: *Schematic of a ventricular cardiac AP, with the different 'phases' of the AP shown, and which key currents are involved in each phase. Upward arrows represent outward currents, inward currents represent inward currents, and double arrows represent exchangers,* i.e., *where current moves in both an inward and outward direction.*

movement of ion flow.

An example of an action potential, showing the different 'phases', is shown in Fig. 2.4. The AP is considered to consist of 5 different phases:

Phase 4: The resting phase. For ventricular and atrial cardiomyocytes, this phase is marked by a relatively constant value for V_m. The negative resting potential is largely achieved by the inwardly rectifying potassium current (I_{K1}) remaining open during this phase. For pacemaker cells, I_{K1} is absent, and thus the resting phase is actually a period of slow depolarisation, until V_m reaches the threshold value for phase 0.

Phase 0: Period of rapid depolarisation that marks the start of the AP. It is initiated by V_m reaching a threshold value which causes the I_{Na} current to activate, causing a rapid influx of Na^+ into the cell.

Phase 1: Transient repolarisation. The Na^+ channels rapidly deactivate, and the transient outward current, I_{to}, results in a period of rapid, partial repolarisation. Depending on the strength of this current, this repolarisation may be to the extent that there is a 'notch' in the AP. For example, there is a notch in the AP for ventricular

epicardial cells, but no notch for ventricular endocardial cells.

Phase 2: Plateau phase. The membrane potential is sustained at a relatively constant level by a balance of calcium (Ca^{2+}) influx via the L-type calcium current ($I_{Ca,L}$), and potassium efflux, through the rectifier K^+ currents (I_{Kr} and I_{Ks}).

Phase 3: Repolarisation. The L-type Ca^{2+} current channels close, while the I_{Ks} channels remain open, allowing continuing K^+ efflux resulting in a repolarisation of the cell to the original resting potential. I_{K1} opens during this phase, to remain open during phase 4 to maintain a steady resting potential.

It should be noted that the above is only an outline—the AP is the result of a complex series of interactions between different currents and channels opening and closing in response to the membrane potential and other inhibitory and activating factors.

Excitation-Contraction Coupling

Arguably, the complex electrical activity of the AP just described exists for the sole purpose of ensuring the heart acts as an effective mechanical pump. As such, the linking of the electrical activity of the heart to its mechanical contraction is vital, as is referred to as *excitation-contraction coupling* (ECC). What follows is a brief summary of the mechanism of this link (the reverse side of this mechanism, termed mechanoelectric feedback, shall not be discussed here).

For ECC, the cell may be decomposed into units called calcium release units (CRUs), also known as dyads (3). These CRUs are spread roughly evenly throughout the cell to allow for a uniform action throughout—the number of CRUs in the cell has been estimated to be between 10,000 and 100,000 (3, 5). Anatomically, the CRU may be considered to be a section of the cell containing a section of the cell membrane, with some L-type Ca^{2+} channels, and a section of the sarcoplasmic reticulum (SR). The primary rôle of the SR is to sequester and release Ca^{2+} via ryanodine receptors (RyRs) when the required stimulus is given. This stimulus is the rise in local concentration of Ca^{2+} precipitated by the open-

ing of the L-type Ca^{2+} current channels, and is termed *calcium-induced calcium release.* The result is that a great deal of Ca^{2+} is released into the cytosol of the cell during phase 2 of the AP. The reason this is vital for ECC is due to the interaction between Ca^{2+} and the contractile units of the cell, called the sarcomere. When the cytosolic concentration of Ca^{2+} ($[Ca^{2+}]_i$) rises, the free Ca^{2+} binds to a part of the contractile machinisms of the cell, which then removes the inhibition between the two critical contractile parts of the mechanism, allowing contraction to take place. Subsequently, the Ca^{2+} released from the SR is recovered by the sarco/endoplasmic reticulum Ca^{2+}-ATPase pumps (4).

2.1.3   Ion Channel Mechanics & the Repolarisation Reserve

As stated previously, the potential difference across the membrane is maintained and altered by the action of various membrane-bound proteins, which can be classed as either *channels*, *pumps* or *exchangers*. Most of these proteins present ion-specific openings through the membrane, though different channels have different degrees of selectivity. Most of these channels are also controlled by, amongst other things, the membrane potential itself (1). The membrane potential causes a conformational change in the ion channel protein, causing it to 'open' or 'close', that is to allow ions to be conducted through it or not.

These ion channels are discrete molecular entities, and thus the conformational changes that lead to their open/close state are stochastic; the effects of this stochasticity are discussed in greater detail in §2.3. However, each type of ion channel possesses its own range of attributes, such as the time it takes to inactivate, the time it takes to reactivate, its permeability to different types of ions, and its susceptibility to other gating factors. For the channels examined in this thesis, a brief summary of their action and relevant physiological properties is given; further details are given in the Appendix. A comprehensive summary of the physiological properties is given in Carmeliet and Vereecke (2).

Of key importance in mathematical modelling of electrically active cells is the *Nernst*

equation:

$$E_X = \frac{RT}{z_X F} \ln \frac{[X]_\mathrm{o}}{[X]_\mathrm{i}} \tag{2.1}$$

In the above equation, R represents the gas constant, z_X represents the valence of ion X, F represents the Faraday constant, and $[X]_\mathrm{o}$ and $[X]_\mathrm{o}$ represent the extracellular and intracellular concentrations of X respectively. The quantity of interest, E_X is the *Nernst potential* or *reversal potential*, and represents the membrane potential that would have to be in place to keep the extracellular concentrations constant, *i.e.*, to make the net ionic flux across the cell membrane zero.

When a channel is entirely selective, the reversal potential (which can now be thought of as the potential at which there will be no net flux of ions) is given by the Nernst equation for the specific ion. However, some ion channels are permeable to more than one type of ion, in which case their reversal potential for channel α is given by the *Goldman-Hodgkin-Katz equation*:

$$E_\alpha = \frac{RT}{F} \ln \frac{\Sigma_i^N P_{A_i^+}[A_i^+]_\mathrm{o} + \Sigma_j^M P_{B_j^-}[B_j^-]_\mathrm{i}}{\Sigma_i^N P_{A_i^+}[A_i^+]_\mathrm{i} + \Sigma_j^M P_{B_j^-}[B_j^-]_\mathrm{o}} \tag{2.2}$$

2.2 Computational Cell Models

2.3 Variation

2.3.1 Experimental & Physiological Variation

2.3.2 Computational Modelling of Variation

2.4 Ischæmia

DIFFUSION A

This appendix gives greater detail about the ionic movement theories, and electric theory properties generally, of the modelling of electrically active cells—note that this applies equally well to both cardiac cells and to neurons.

A.1 Simple Diffusion

By simple diffusion, there is a net movement of particles from a region of high concentration to a region of low diffusion with simple thermal movement. This movement is according to Fick's Law,

$$F_X = -D\frac{\partial C_X}{\partial x}, \tag{A.1}$$

where F_X and C_X represent the flux and concentration of particle X respectively, and D represents the diffusion coefficient. It should be noted that this represents *net* diffusion: there will be movement of particles in both directions. For circumstances where D is constant, this solves to produce

$$F_X = P(C_{X,o} - C_{X,i}), \tag{A.2}$$

where P represents the permeability of the membrane, and is equivalent to D/d, where d represents the thickness of the membrane. $C_{X,o}$ and $C_{x,i}$ represent the extracellular and intracellular concentrations of X, respectively; thus F_X in this form describes flux from

the extra- to the intracellular space. It can be noted that for substances that diffuse through the lipid phase of the membrane, the permeability also depends on the oil/water partition coefficient β, according to $P = \beta^{\mathrm{D}}/\mathrm{d}$.

A.2 Facilitated Diffusion, Michaelis-Menten Kinetics

While Fick's Law can describe the movement of small, uncharged particles across a membrane very well, it does not serve particularly well for large molecules or charged substances. Large molecules, such as amino acids and glucose, are impeded by the cell membrane, and instead make use of *carrier-mediated diffusion*, also called *facilitated diffusion*. This mechanism obeys Michaelis-Menten kinetics, and can be views as a catalysed reaction, where the reaction is not a chemical one, but rather a translation of substance across the membrane.

Briefly, Michaelis-Menten (MM) kinetics are used to describe a two-stage reaction, the first section being reversible, the second section irreversible:

$$\mathrm{C} + \mathrm{X_o} \rightleftharpoons \mathrm{CX} \longrightarrow \mathrm{C} + \mathrm{X_i}$$

The above expression uses $\mathrm{X_o}$ and $\mathrm{X_i}$ to represent the extracellular and intracellular molecule respectively, and C the carrier; CX represents the moment when the molecule and the carrier combine for the transport process. It thus proposes that there is a reversible reaction between the extracellular molecule and the carrier, and that the transport process itself is irreversible. By certain assumptions, including that the number of extracellular molecules is far greater than the number of carriers, the rate of this process can be estimated to be

$$v = \frac{\mathrm{dX_o}}{\mathrm{d}t} = v_{\max} \frac{[\mathrm{X_o}]}{K_m + [\mathrm{X_o}]}, \tag{A.3}$$

where v represents the velocity of the reaction (*i.e.*, the rate of translocation of X), $v_{\max}$ represents the maximum velocity at which the translocation can take place, $[\mathrm{X_o}]$ the concentration of X outside the cell, K_m represents the so-called Michaelis-Menten

constant, which in turn represents the concentration at which the half-maximum speed occurs. It can be noted that v_{max} is equal to the rate of the final step of the reaction.

Facilitated diffusion shows saturation (the point at which increasing the extracellular concentration further does not lead to an increase in flux), competitive and non-competitive inhibition, stereo-specificity and a relatively slow turnover.

A.3   Electrodiffusion

This section deals with the form of transportation that is of most interest for this thesis: the passive movement of charged substances (ions) in the presences of an electrochemical gradient. It should be noted that the content in this section, while being in the appendix, is nonetheless vital for almost all mathematical models of electrically active cellular activity.

A.3.1   The Nernst Equeation

A key concept is that of the *Nernst Potential*, derived using the Nernst Equation, which describes the potential difference that is required to oppose the net flow of an ionic species against a specified concentration gradient. The equation shall here be derived in full using statistical mechanics.

Initially, recall that the probability of a system being in a particular state α is given by

$$P(\alpha) = \frac{1}{Z}e^{-\beta E_\alpha}; \qquad Z = \sum_i e^{\beta E_i}, \tag{A.4}$$

where E_i is the energy of state i, and $\beta = k_{\mathrm{B}}T$, with T being the temperature of the system.

For a large number of molecules, $[A] \propto P(A)$. Furthermore, if we consider the diffusion of ions from one location to another to be a reversible reaction according to A $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$ B, by applying the law of mass action ($F_{A\to B} \propto [A]$), at steady state we achieve the following

equation, which can be manipulated accordingly:

$$k_1[A] = k_2[B] \tag{A.5}$$

$$\frac{[A]}{[B]} = \frac{k_2}{k_1} \tag{A.6}$$

$$= \frac{\frac{1}{Z}e^{-\beta E_A}}{\frac{1}{Z}e^{-\beta E_B}} \tag{A.7}$$

$$= e^{-\beta(E_A - E_B)} \tag{A.8}$$

$$\Delta E = k_\mathrm{B} T \ln \frac{[B]}{[A]} \tag{A.9}$$

At this stage, the electric gradient is introduced to the equation as the reason for the energy difference that maintains the steady state, with $\Delta E = zqV$, where z is the valence of the ionic species being considered, q_e is the charge (in this case equal to the charge of an electron, *i.e.*, the charge of a singly ionised ion), and V is the potential difference.

$$zq_eV = k_\mathrm{B} T \ln \frac{[B]}{[A]} \tag{A.10}$$

$$V = \frac{k_\mathrm{B} T}{zq_e} \ln \frac{[B]}{[A]} \tag{A.11}$$

$$= \frac{RT}{zF} \ln \frac{[B]}{[A]} \tag{A.12}$$

$$\tag{A.13}$$

Equations A.12 and A.13 are equivalent, the only difference being the constants used in the expression. Eq. A.13 is the more common formulation, using the gas constant R and the Faraday constant F in place of the Boltzmann constant k_B and the electron charge q_e respectively. The Nernst potential is, therefore, the potential difference that must be applied to maintain a specified concentration gradient, and is given by either equation.

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