Ion Channel Regulation in the Pathophysiology of Atrial Fibrillation: Using Mathematical Modeling as a Predictive Tool for Cardiac Disease

DISSEPTION

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By

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Abstract

Atrial fibrillation (AF) affects 2.5 million people per year in the U.S., and is associated with high morbidity and mortality. Both electrical and structural remodeling on the cardiac cell- and tissue-level contribute to AF, but the precise molecular pathways that lead to atrial fibrillation pathogenesis are not well understood. Mathematical modelling is ideally suited to understand the molecular basis of cardiac arrhythmias by allowing simulation of complex biological systems based on integrated data from individual populations of ion channels. A cross-platform multi-threaded graphical user interface LongQt was developed for advanced computational cardiac electrophysiology studies in sinoatrial, atrial, and ventricular cells to help bridge the gap between experimental and theoretical techniques in cardiac electrophysiology. A method of parameter sensitivity analysis is discussed to help define the contribution of individual ion channels to cell membrane excitability and action potential properties. Finally, a computational model of the human atrial cell was used to determine the mechanism for increased susceptibility to arrhythmogenic events in patients with defects in Ca\(^{2+}\)/calmodulin-dependent protein kinase signaling pathways. An atrial computational model was extended to explore CaMKII activation of late sodium current (\(I_{Na,L}\)) and phosphorylation of downstream targets (L-type Ca\(^{2+}\) channel, phospholamban, ryanodine receptor). Both LongQt and the method of parameter sensitivity analysis were used to help identify the cellular pathway responsible for disrupted ion homeostasis and afterdepolarizations in atrial cells. Intracellular Ca\(^{2+}\) and Na\(^{+}\) accumulation, increased phosphorylation of RyR2 by CaMKII, and abnormal Ca\(^{2+}\) dynamics [e.g. beat-to-beat alteration (alternans)], and afterdepolarizations (early and late phase) was observed in
mathematical models of the atrial cell with constitutive increase in $I_{Na,L}$, compared to wildtype control. These simulations define roles for previously unexplored ion channel regulation of CaMKII-mediated $I_{Na,L}$ in the context of atrial fibrillation. Although $I_{Na,L}$ is $\ll 1\%$ of peak sodium current at baseline in atrial myocytes, CaMKII-dependent increase in the current was sufficient to cause intracellular ion accumulation, disrupted Ca$^{2+}$ homeostasis, and pro-arrhythmic dynamics. Most importantly, mathematical modelling tools including a novel software with extended action potential models were used to understand the molecular basis for atrial fibrillation. These efforts will help identify new mechanisms of atrial regulation with direct relevance for human atrial fibrillation.
This dissertation is dedicated to Hayri and Yildiz Onal.
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Chapter 1: Introduction

Objective

Heart disease is the leading cause of death for men and women in the United States, and an estimated 85.6 million American adults (more than 1 in 3) have one or more types of cardiovascular disease.\(^1\) Prevention, diagnosis and treatment of cardiovascular disease all depend on a growing understanding of the molecular mechanisms underlying healthy and diseased cardiac activity. There has been tremendous effort to identify these mechanisms, the scope of which has expanded with the development of new molecular biology and computational technologies. This has led to a growing appreciation for mathematical modeling as a transformative tool for understanding the role of physiological variability in arrhythmia susceptibility and response to therapy.

The overall goal of my research is to use mathematical modeling of the cardiac cell action potential to investigate mechanisms of cardiac arrhythmias. My interest is threefold: [1] to extend current mathematical models to reflect recent experimental data on cardiac arrhythmia mechanisms, [2] to contribute to mathematical modeling methods of analysis, and [3] to make modeling and simulation accessible to a wide audience.

Ion Channels Responsible for the Cardiac Action Potential

Excitable cells (e.g. neurons, cardiac cells) initiate and conduct electrical impulses to other cells.\(^2\) An excitable cell has three main characteristics: [1] it experiences changes in electrical activity due to the movement of ions back and forth across its plasma membrane, [2] external mechanical, electrical, or chemical stimuli can alter this electrical activity, and [3] action potentials in turn act as a stimulus to nearby excitable cells, allowing

\(^1\) Prevention, diagnosis and treatment of cardiovascular disease
\(^2\) Excitable cells (e.g. neurons, cardiac cells) initiate and conduct electrical impulses to other cells.
electrical current to propagate and pass through excitable tissue. Proteins embedded in the cell membrane that open and close in response to stimuli regulate the movement of ions back and forth across the cell membrane. In cardiac cells this coordinated ion movement creates a brief change in cell membrane voltage called the cardiac action potential.

The behavior of many ion currents and their effective contribution to the overall action potential in healthy and disease cardiac myocytes has been well-documented. Cardiac cells have a double lipid bilayer plasma membrane that maintains an intracellular ion concentration and charge different from that of the extracellular space. For example, the intracellular concentration of K$^+$ is 150 mM and of Na$^+$ is 10 mM in cardiac ventricular myocytes, and the extracellular concentration of K$^+$ and Na$^+$ is 5 mM and 140 mM respectively. Cardiac cells at rest are more negative than the extracellular space due to this concentration difference. The transmembrane potential is the difference in electric potential between the outside and inside of the cell, and is dependent on the membrane’s permeability to different ions. The membrane allows both active ion transport through transporters / pumps, which work against an ion’s concentration gradient, and passive ion transport through ion channels down the electrochemical gradient. As ions move back and forth across the cell membrane, they produce a local change in the membrane potential that can be translated to nearby proteins, initiating changes in adjacent or distant ion channels in the membrane. This leads to variation in the magnitude of whole-cell ion currents, which displaces the transmembrane voltage and drives a cardiac cell’s excitation-recovery process, or the cardiac action potential.

During the initial depolarization phase of the cardiac action potential (phase 0), the transmembrane potential increases as sodium enters the cell through voltage-gated sodium
ion channels (made up of Nav1.5 subunits, encoded by gene SCN5A, contributing to current $I_{Na}$). During the early repolarization phase of the cardiac action potential (phase 1) these sodium channels inactivate, reducing the flux of sodium ions across the cell membrane. A subset of potassium channels (made up of proteins Kv4.2 or Kv4.3, encoded by genes KCND3 or KCNA4 respectively, contributing to current $I_{to,fast}$) are simultaneously activated and cause potassium to flow out of the cell. Phase 2 - or the “plateau” phase of the cell, is characterized by a prolonged period of membrane depolarization supported by calcium channels (protein CaV1.2, encoded by gene CACNA1C, contributing to current $I_{Ca,L}$) that pass inward Ca$^{2+}$ current. During Phase 3 of the cardiac action potential, L-type Ca$^{2+}$ channels close while rapidly-activated potassium channels (made up of Kv11.1, encoded by gene KCNH2, contributing to current $I_{Kr}$), activate and inactivate rapidly. This generates an outward current and repolarizes the membrane potential. Later in Phase 3, slowly-activated potassium channels (made up of Kv7.1, encoded by gene KCNQ1, contributing to $I_{Ks}$ current) activate and help return the cell to a repolarized state. The resting state (Phase 4) that follows is maintained by inward rectifier potassium channels (protein Kir2.1, encoded by KCNJ2).

Cardiac Excitation-Contraction Coupling

Cardiac cell contraction is driven by the action potential in a process known as excitation-contraction coupling. The increase in cytosolic Ca$^{2+}$ due to the opening of L-type Ca$^{2+}$ channels promotes binding of Ca$^{2+}$ ions to the ryanodine receptor (RyR2), triggering calcium release from the sarcoplasmic reticulum (SR). This process is known as calcium-induced calcium-release (CICR). The subsequent dramatic increase in calcium concentration in the cytosol promotes calcium binding to troponin C, causing a
conformational change in a thin filament regulatory complex that exposes an actin-myosin binding site on the head of the myosin protein, and causes the cell to contract. Relaxation is facilitated by the activity of the SR Ca$^{2+}$-ATPase (SERCA2a), which transfers Ca$^{2+}$ from the cytosol into the SR at the expense of ATP. This activity is inhibited by the protein phospholamban (PLB), but phosphorylation of PLB reduces the association of PLB and SERCA2a. Once Ca$^{2+}$ is inside the SR it may bind to the SR Ca$^{2+}$ buffer calsequestrin, which reduces the concentration of Ca$^{2+}$ inside the SR and reduces the load of SERCA2a in pumping Ca$^{2+}$ against its concentration gradient. The Na$^+$/Ca$^{2+}$ exchanger is also involved with pumping Ca$^{2+}$ out of the cell in response to increased cytosolic Ca$^{2+}$. Earlier in the action potential, the Na$^+$/Ca$^{2+}$ exchanger operates in reverse mode to extrude Na$^+$ from the cell and bring Ca$^{2+}$ into the intracellular space in a 3:1 stoichiometry. During Phase 3 of the action potential, $I_{NCX}$ operates in forward mode to extrude Ca$^{2+}$.

Coordinated electrical activation of adjacent cardiac cells is responsible for synchronized cardiac contraction. The electrocardiogram reflects potential differences at the body surface during the cardiac cycle. A wave of electrical activation begins in the sinoatrial node (SAN), located in the upper right atria of the heart (Figure 1). SAN cells fire spontaneously without an external electrical stimulus, at a higher firing rate than other cells in the heart. Electrical propagation travels through the atria to the atrioventricular (AV) node where the electrical wave slows down and allows the atria to contract, pumping blood into the ventricles. The initial depolarization of the atria is reflected by the P wave on the electrocardiogram, and the width of the P wave is related to the time it takes the atria to activate. Electrical activity then spreads to the ventricles through the bundle of His, the right and left bundle branches, and the Purkinje system. The initial depolarization of
ventricular cells is responsible for the second major deflection on the electrocardiogram, the QRS complex. As the ventricles depolarize from endocardium to epicardium, both ventricles contract and send blood either to the lungs for oxygenation, or to supply oxygen to the rest of the body. The third deflection on the electrocardiogram, the T-wave, represents ventricular repolarization or diastole, a period during which the ventricles relax and refill with blood. As the electrical wave propagates across the heart, each cell type generates a different action potential morphology. Mathematical models have been developed to describe the action potential of each of these different cell types, as well as account for differences in species and cardiac disease states.4

Development of the Hodgkin-Huxley Mathematical Action Potential Model

Mathematical modeling of the cardiac action potential began with the work of Hodgkin and Huxley in 1952 with their conductance-based ionic model of the giant squid axon cell membrane.8,9 Hodgkin and Huxley performed a series of studies to explore nerve conduction, beginning with inserting a fine capillary electrode into the cell membrane of a giant squid axon to record the first cellular action potential. Hodgkin and Huxley then applied voltage changes to the cell membrane or changed ionic concentrations while directly recording current flowing across the axonal membrane to estimate ionic contributions to the neuronal action potential. This experiment assumed the independence principle: that potassium and sodium ions crossed the membrane independently through separate pathways.10 Hodgkin and Huxley observed that at specific potentials the recorded currents were close to zero, thereby identifying the equilibrium potentials for Na+ and K+ current. They determined that Na+ was responsible for the early phase / depolarization of the action potential, and K+ was responsible for the later phase / repolarization of the action potential.
potential. Although Hodgkin and Huxley were effectively exploring ion channel kinetics, single ion channel recordings were not recorded until the development of patch clamp technology in the 1970’s.\textsuperscript{11}

Hodgkin and Huxley solved a set of ordinary differential equations with four state variables to develop a model of the action potential based on the transmembrane currents they had recorded. The following general equation describes the time-dependent change in transmembrane potential:

\[ C_m \frac{\partial V_m}{\partial t} = - \sum l_{ion} + I_{stim} \]

Here \( C_m \) represents the membrane capacitance, \( I_{ion} \) represents the total transmembrane current (carried by distinct populations of ion channels), \( I_{stim} \) represents the external stimulus current, and \( V_m \) represents the transmembrane potential. This model is based on the parallel conductance model of the excitable membrane, which models a cell membrane patch as an electrical circuit (Figure 2).\textsuperscript{12} The model represents the flow of ions through their respective channels in a small area of the membrane. Each branch of the circuit represents a specific ion current that contributes to the overall transmembrane current. Any input current to the circuit can leak through the channels in the cell membrane, which is represented by \( I_{ion} \) and composed of the inward sodium current, outward potassium current, and non-specific leak current.

Hodgkin and Huxley used the magnitude of the driving force due to the concentration gradient across the cell membrane to determine each current. The driving force is calculated as the difference between the transmembrane potential and the ion’s Nernst potential. Also known as the reversal potential, the Nernst potential is derived from
voltage-clamp experiments carried out over a range of $V_m$ where peak inward current and steady-state outward current are independent variables.\textsuperscript{10} The Nernst potential for Na\textsuperscript{+} is given as follows:

$$E_{Na} = \frac{RT}{F} \times \ln \left( \frac{Na_o}{Na_i} \right)$$

Here $R$ is the universal gas constant (8.314 J/K mol), $T$ is the temperature in Kelvin, $F$ is Faraday’s constant (96485 C/mol), and $Na_o$ and $Na_i$ represent extracellular and intracellular Na\textsuperscript{+} concentrations respectively. Each current is the product of a driving force (units in mV), and conductance (units in Siemens). For example, the Hodgkin-Huxley equation for $I_{Na}$ (inward sodium current) is as follows:

$$I_{Na} = G_{Na} \times (V_m - E_{Na})$$

The conductance of each ion channel was determined experimentally in the voltage-clamp experiments, and is the product of the probability that a channel is open and the maximum conductance of the ion channel (the value of the current if all channels are open). Each ion current is modelled with channel opening probabilities raised to different powers based on the best fit to the voltage-clamp data, which may reflect the physical basis of the ion channel conformational changes. The gating variable $m$ represents the fraction of “m-gates” that are open.\textsuperscript{10} The open probability of a given ion channel ranges from 0 to 1 (all gates open). This method assumes that gates are independent from one another and kinetically identical. Thus the mathematical model for the sodium ion current is as follows:

$$I_{Na} = g_{Na} \times m^3 \times h \times (V_m - E_{Na})$$

Assuming that both $m$ and $h$ obey first-order kinetics, the time-dependent change in the open probability can be described by a first-order differential equation. The rate at
which closed gates transition to open gates is governed by a rate constant $\alpha$, and the transition from open gates to closed gates is governed by a rate constant $\beta$. For example:

$$\frac{dm}{dt} = \alpha_m(v) \times (1 - m) - \beta_m(v) \times m$$

Hodgkin and Huxley fit expressions for the $\alpha$ and $\beta$ rate constants for the $m$ gate based on experimental values, and determined the time constant and steady-state probability for the gate based on these rate constants. Hodgkin and Huxley followed similar reasoning to develop formulations for the potassium current $I_K$ and the leak current, as follows:

$$I_K = g_K \times n^4 \times (V_m - E_K)$$

They also incorporated a leak current:

$$I_{\text{leak}} = g_{\text{leak}} \times (V_m - E_i)$$

Together these equations make up the $I_{\text{ion}}$ component of the Hodgkin-Huxley neuronal action potential model. The governing set of ordinary differential equations for each model may be solved numerically using, for example, the forward Euler method with a dynamic time step. Built-in differential equation solvers may also be used, such as ode45 and ode23 in MATLAB, or adaptive methods such as Runge-Kutta.

For multi-cellular simulations electrical propagation is described using the cable equation, a linear parabolic partial differential equation (PDE):

$$\frac{d}{4R_i} \frac{\partial^2 V_m(x, t)}{dx^2} = C_m \frac{\partial V_m(x, t)}{dt} + \sum I_{\text{ion}}$$

Where $d$ represents the fiber diameter, $R_i$ represents the intracellular resistivity of the cell, $V_m$ represents the membrane voltage, $C_m$ represents the membrane capacitance, and $I_k$ represents the ionic current. For a one-dimensional fiber, the fiber is discretized into
smaller segments and solved numerically using the Crank-Nicolson implicit method. For a two-dimensional tissue, each cell may be connected to surrounding cells in a rectangular arrangement. The system may be solved numerically using, for example, the Peaceman-Rachford method of alternating implicit solutions for rows and columns.\textsuperscript{13}

Modelling Cardiac Cell Action Potentials and Disease States Based on Ion Channel Kinetics

Growing interest in computer simulation as a valuable tool for generating and testing hypotheses has yielded many mathematical models to better understand cellular physiology (Table 1). Modelling cardiac action potentials began with the work of Denis Noble applying Hodgkin-Huxley equations to describe the cardiac action potential.\textsuperscript{14,15} This work combined with significant advances in computational techniques, and a growing body of knowledge regarding molecular and cellular mechanisms of cardiac action potentials and cardiac disease, pioneered a diverse library of action potential models of different cell types (atrial, ventricular, purkinje, sinus node) and disease states. Each model is uniquely suited to help answer different questions relevant to cardiac electrophysiology and arrhythmia. Of note are the canine (normal or diseased) or human ventricular myocyte, human atrial myocyte, or rabbit central sinoatrial node (SAN) myocytes. The Hund-Rudy (HRd) dynamic model is useful for researchers interested in the Ca\textsuperscript{2+}/calmodulin-dependent protein kinase (CaMKII) regulatory pathway and rate-dependent changes in AP behavior in normal and diseased (epicardial canine infarct border zone) cell electrophysiology.\textsuperscript{16} This is one of several models in the Rudy family of cardiac action potential models, which began in 1991 with the initiative to produce an updated model of the cardiac action potential and resulted in the Luo-Rudy model.\textsuperscript{17} The human atrial model
of Grandi et al. has been used to study AP remodeling and atrial fibrillation, driven by changes in intracellular Na\(^+\) and Ca\(^{2+}\) concentrations.\(^ {18}\) The rabbit sinoatrial node mathematical model developed by Kurata et al. is notable for simulating \(V_m\) and Ca\(^{2+}\) concentration changes in the central region of the rabbit SAN.\(^ {19}\) Finally, the widely used and well-validated model of the human ventricular myocyte developed by Ten Tusscher et al has been used for a variety of human modeling studies.\(^ {20}\)

Ion channel expression and function change between the different cell types, so each model strives to replicate a unique cellular action potential by taking into account changes in ion channel structure, kinetic properties, and regulation in different cells.\(^ {7}\) For example, ventricular cells lack key ion currents that exist in the atria, such as the ultra-rapid delayed rectifier K\(^+\) current \(I_{\text{Kur}}\), and acetylcholine-activated inward rectifying current \(I_{\text{K-Ach}}\). The T-type Ca\(^{2+}\) current is larger in atrial cells than in the ventricle, and the inward-rectifying K\(^+\) current \(I_{\text{K1}}\) is smaller in atrial cells than in the ventricle. These differences produce a longer and more pronounced plateau, with a more hyperpolarized resting membrane potential in ventricular cells compared to atrial cells. There are also significant differences in ion concentrations in different cellular compartments between atrial and ventricular cells. Atrial cells express higher SERCA2a and phospholamban, but less RyR2 and SR Ca\(^{2+}\) buffer calsequestrin compared to ventricular cells.\(^ {21}\)

Mathematical models are well-suited to integrate results from reduced, isolated components of a system (such as single ion channel recordings) into a more complex model to better understand the interplay between ion channels. Data describing ion channel activity and concentration changes at the single-cell level is often based on either single ion channel current recordings, or Ca\(^{2+}\) spark waves from a single cell. One set of data that
is fit to describe ion channel activity is the instantaneous current-voltage (IV) curve. The cell membrane is depolarized to a range of voltage steps to raise permeability of the membrane to ions, and the current of an ion channel is measured at each voltage step within 10-30 μs of reaching that voltage. The peak current amplitudes are plotted against the test potential to determine the IV curve.\(^6\)

Ion channels can have complex voltage – or ligand- dependent activation or inactivation.\(^22\) For example, the Na\(^+\) channel current rises rapidly and decays slowly, so it has fast activation (\(m\) gate) and slow inactivation (\(h\) gate) kinetics. Reproducing the steady-state voltage dependence of inactivation curves in a model requires experimental observations of a two-pulse voltage-clamp protocol. For inactivation curves, a prepulse is applied to the cell membrane to permit the inactivation process to reach steady-state. A test-pulse is then applied to another voltage, and the amplitude of the current during the test-pulse is recorded to determine the percent of channels still active after the prepulse. To measure the rate of recovery from inactivation, a prepulse and test pulse is applied to the cell membrane in a similar manner, but separated with an interpulse interval. The prepulse is held long enough to inactivate all channels, and the membrane repolarizes during the interpulse interval to begin removal of inactivation. The timing of the interpulse interval is changed to determine how long it takes all channels to recover from inactivation. This determines the time constant for inactivation of the channel.\(^6\)

Multiple strategies may be used to model disruption of ion channel activity in the context of cardiac disease, which may have changed due to genetic modifications or changes in targeting (phosphorylation, ligand-binding, etc).\(^7\) The conductance of an ion channel may be reduced to reflect a change in the population of ion channels, or oscillate
between values to reflect dependence on protein kinase activity.\textsuperscript{18,23} The IV curve of an ion channel, or the time constant for activation or inactivation may be shifted to reflect a change in the kinetics of ion channel opening.\textsuperscript{24} Facilitation of an ion current over a range of pacing frequencies may change depending on protein kinase activity.\textsuperscript{16} Finally, more complex ion channel dynamics may be modelled using Markov models that describe different structural states of the ion channel (open, closed, inactivated, bound to a ligand, etc). The Markov model for an ion channel may be further extended to reflect the altered kinetics of the ion channel due to drug binding.\textsuperscript{25}

Computational modeling of cardiac action potentials allows for an in-depth analysis of perturbations to a complex biological system. Modeling these perturbations in the form of changes to ion channel behavior requires a well-formulated hypothesis, ion channel recordings or calcium imaging data to inform the model, and optimizing equation parameters to fit experimental data appropriately. The final step before the model can be used as a prediction is validation of the whole model using data that was not used for the initial parameterization process – for example by fitting the action potential duration, or peak Ca\textsuperscript{2+} current.\textsuperscript{26} Once this final model is obtained, it can be used to determine the impact of changing ion channel kinetics or protein behavior in context of cardiac disease.

Scope of Dissertation

While it is known that cardiac cell function depends on the activity of membrane ion channels, little is known about the molecular pathways responsible for ion channel targeting in cardiac cells, or how changes to these pathways lead to dysfunction and cardiac arrhythmias. The multifunctional Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII) has emerged as an attractive target for systems-based approaches that aim to integrate large
experimental data with mathematical modeling and computational approaches across spatial and temporal scales. In cardiac cells, CaMKII phosphorylates ion channels, transcription factors, signaling molecules, and other membrane proteins that are critical to cardiac electrical activity and structure. Abnormal CaMKII activity has been observed in human and animal models of cardiovascular disease (e.g., heart failure, myocardial infarction, atrial fibrillation), and is thought to promote downstream dysfunction in excitation-contraction coupling, structural remodeling, cell death, and even transcriptional activation of inflammation factors.\textsuperscript{27,28} A significant portion of ongoing cardiac computational modeling research is interested in modeling CaMKII activity to better understand how this important effector molecule acts as a pro-cardiac disease/arrhythmogenic molecule. Thus, there is a critical need to expand knowledge regarding targeting and regulation of membrane ion channels in the cardiac cell membrane, as well as develop quantitative tools to assess the sensitivity of the action potential to changes in ion channel regulation.

In this dissertation I aim to use mathematical modelling of the atrial action potential to investigate CaMKII-based molecular mechanisms of atrial fibrillation. My central objective is to develop methods and an extended model to explore the contribution of disrupted ion channel regulation, e.g. through CaMKII facilitation of late sodium current, on cell membrane excitability and susceptibility to arrhythmia. In Chapter 2 I discuss prevailing theories of atrial fibrillation mechanisms and the current state of AF therapies. Chapter 3 and 4 follow with a discussion of prior mathematical models of atrial cells and prior models of CaMKII activity. Chapter 5 introduces a method of assessing ionic contributions to the cell action potential through parameter sensitivity analysis. Chapter 6
discusses development of a graphic user interface for ease of advanced cardiac electrophysiology simulations. Armed with the models in Chapter 3 and 4 and the methods discussed in Chapter 5 and 6, I developed an extended atrial cell action potential model presented in Chapter 7 with CaMKII phosphorylation of downstream targets to better understand single-cell mechanisms of atrial fibrillation.

In summary, the cardiac action potential is dependent on the tight regulation of ion channel activity in the cell membrane. Computational modeling is poised to explore the mechanisms of ion channel activity in regulation of cell membrane excitability and cardiac disease. It is my hope that these studies show the utility of mathematical modeling to enhance our understanding of cardiac disease, and that modeling of the cardiac action potential can become a viable, accessible tool for cardiac electrophysiologists.
Figure 1: Cardiac conduction system. Initiation of electrical activity begins in the right atria in the sinoatrial node. Right and left atria are electrically activated and subsequently contract. Conduction continues into the atrioventricular node and slows down, then reaches the ventricles. The right and left ventricle are electrically activated and subsequently contract.
Figure 2: Parallel conductance model. The Hodgkin-Huxley model treats the excitable cell as an electrical element. The lipid bilayer is described by the membrane capacitance (Cm). Each ion channel is represented by a different branch: shown here is Na+, K+, and leak channels. GNa, GK, and GL are the conductances per unit area for each respective ion channel. ENa, EK, and EL are the reversal potentials.
Table 1: Notable mathematical models of different cardiac cell types.

<table>
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<tr>
<th>Model Authors</th>
<th>Citation</th>
<th>Year</th>
<th>Species</th>
<th>Cell Type</th>
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<td>Purkinje, pacemaker</td>
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<td>Atrial</td>
</tr>
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Chapter 2: Human atrial fibrillation: mechanisms and treatment

Atrial fibrillation (AF) is characterized by chaotic, rapid firing of the atria due to uncoordinated and irregular atrial activation. This continuous atrial activity replaces regular P-waves, causes an undulating baseline of the electrocardiogram and eventually results in irregular rapid QRS complexes, contributing to weakened ventricular contraction. AF affects 2.5 million people in the U.S., with projections to exceed 12 million by 2050. Half of all AF patients are 75 years or older, and the incidence, prevalence, and complication rates of AF all increase with age. Classification of AF is based on the duration of fibrillation episode (Table 2). Patients initially present with paroxysmal AF and over time the disease evolves to become persistent. A diagnosis of AF is associated with stroke, myocardial infarction, diabetes, hypertension, heart failure, and is associated with a 1.5 – 1.9 –fold increase of overall mortality. This leads to a large health-care investment for AF patients: annual direct expenditures for AF account for 7 billion USD per year, the majority of which is spent on in-patient care. Atrial fibrillation is a highly heterogeneous disease, and there is a critical need to understand atrial fibrillation initiation and progression to better understand prevention and treatment.

Molecular Mechanisms of Human Atrial Fibrillation

Theories for the molecular mechanisms of AF are based on concepts developed previously for ventricular tissue. AF requires a trigger and vulnerable electrical or structural substrate for propagation, and treatment depends on the atrial substrate or sustained phenotype of each patient. AF can be sustained either through continuous focal ectopic activity or re-entrant propagating waves. Focal ectopic activity is thought to be generated by cellular delayed afterdepolarizations (DAD’s), inappropriate transmembrane
potential depolarizations due primarily to abnormal Ca\textsuperscript{2+} release (spontaneous release) from the sarcoplasmic reticulum (SR) to the cytoplasm. Spontaneous opening of ryanodine receptors (RyR2) is caused from an overload of Ca\textsuperscript{2+} the SR, RyR2 hyperphosphorylation, RyR2 dysfunction, or loss/dysfunction of calsequestrin (SR Ca\textsuperscript{2+} binding protein). Excess Ca\textsuperscript{2+} in the cytoplasm is handled by the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger, which transports 3 Na\textsuperscript{+} ions into the cell per Ca\textsuperscript{2+} extruded, and simultaneously depolarizes the transmembrane potential. This increase in membrane voltage can reach threshold and cause the cell to fire again, causing a diastolic afterdepolarization and potentially arrhythmia.

Focal ectopic activity may also occur due to early afterdepolarizations (EAD’s). When the action potential duration of the cell is significantly prolonged, L-type Ca\textsuperscript{2+} channels may reactivate causing a secondary depolarization in the repolarization phase of the action potential. Focal ectopic activity due to EADs or DADs may serve as triggers for neighboring cells, causing a region of spontaneously firing cardiac tissue.\textsuperscript{39}

AF is also thought to occur through reentrant wave propagation, where a premature ectopic beat encounters a vulnerable tissue substrate. Cardiac tissue usually encounters a refractory period in which it cannot be excited, but cardiac cells with a shortened refractory period or slowed conduction are susceptible to stimuli. When reentrant waves encounter a portion of the tissue that is not refractory, it can cause the tissue to re-activate.\textsuperscript{39} Sustained AF causes electrical remodeling of tissue, which can decrease the effective refractory period, shorten action potential duration (APD), change ion channel expression, and alter conductivity between cells. These changes can increase tissue susceptibility to reentrant wave propagation or ectopic foci, which further sustains AF.

The mechanisms of AF pathophysiology are poorly understood, but a commonly
accepted mechanism is the multiple wavelet hypothesis. Cardiac fibrillation results from randomly propagating waves that drift around the atria and perpetuate AF, which depend on decreased conduction, an enlarged atria, and reduced refractory periods. These waves are sustained self-organized reentrant circuits that spin at high frequencies, and are referred to as drivers or rotors. The motor rotor theory, in contrast to the multiple wavelet hypothesis, hypothesizes that one dominant wavelet breaks off into smaller daughter waves localized in the left atrium, that could collide, divide, or change in size or velocity. These mechanisms of atrial fibrillation initiation and determination may not be mutually exclusive, and diagnosis is complex due to additional factors such as genetic variants, extracardiac factors, structural abnormalities, and autonomic nervous system activation. Mathematical modeling is poised to help understand more about mechanisms of AF initiation and maintenance, as well as treatments for atrial fibrillation. For example, mathematical modeling of higher-dimensional atrial cell membrane excitability can help explore the multiple wavelet hypothesis. At the single cell level, mathematical modeling of chronic AF combined with detailed drug binding kinetics can help identify potential drug targets for AF. Ultimately, mathematical modeling is one of the many tools that can be used to explore the molecular mechanisms of atrial fibrillation, and recommended therapies - including catheter, surgical, and drug-based approaches - based on different patient populations.

Catheter Ablation Technologies Used in Treatment of Human Atrial Fibrillation

Current AF therapies include a combination of anticoagulants with catheter ablation, surgical- or drug-based therapies to maintain sinus rhythm or ventricular rate. These therapies face important limitations related to low efficacy due to off-target ion
channel effects, or pro-arrhythmic impact on ventricular tissue. Catheter ablation is recommended as a first-line therapy for patients with paroxysmal AF, or patients that are intolerant to at least one class I or class III antiarrhythmic medication. Ablation therapies include targeting the pulmonary veins, complex fractionated atrial electrograms, left atrial linear conduction block areas, low-voltage zones, or ablating the ganglionated plexi. The ablation workflow for each patient depends on their AF diagnosis as well as the cardiac mapping system and its operator.

Current 3-dimensional mapping systems such as Carto-3 (Johnson & Johnson / Biosense Webster), Ensite Precision (St. Jude Medical), and Rhythmia (Boston Scientific) help collect and visualize cardiac mapping data prior to catheter ablation. This data includes a body-surface 12-lead electrocardiogram, fluoroscopic images, and electroanatomic maps of endocardial activation time and voltage. Fluoroscopy uses a continuous x-ray beam to visualize the thoracic cavity. Multiple fluoroscopic images are acquired at different angles and stores, then transmitted to a monitor for the operator to view the location of diagnostic (mapping) and/or therapeutic (ablation) catheters inside the patient. Fluoroscopic images are shown to the operator alongside the 12-lead ECG and electroanatomic maps to help guide the catheter to a specific location in the heart. In addition to fluoroscopy, mapping systems such as Carto 3 employ magnetic field tracking in the catheter tip to help with localization and navigation.

To generate electroanatomic maps, the operator guides a catheter through the chamber to re-construct the 3-dimensional geometry of the chamber and relevant electrophysiological information. Diagnostic catheters may be separate from therapeutic catheters – for example, the Boston Scientific Orion catheter is used as a mapping catheter,
and the Boston Scientific IntellaNav catheter line is used to deliver ablation. Diagnostic catheter designs have employed different geometries – for example, a 64-electrode basket catheter (Boston Scientific Orion Mapping Catheter), or 20-electrode catheter that can form a loop (Boston Scientific Orbiter ST Steerable Diagnostic Catheter, Biosense Webster Ismus / Cristacath, St. Jude Advisor FL Circular Mapping catheter). Basket catheters have the advantage of providing high-density, precise maps with simultaneous recordings from multiple key atrial structures such as the tricuspid valve and inferior vena cava. Diagnostic catheters have also been designed for specific geometries within the heart such as the tricuspid annulus (Biosense Webster Halo XP), or pulmonary veins (Biosense Webster Lasso line). Differences in geometry, electrode spacing on the catheter, types of electrodes, and filter settings of the signaling station have considerable influence on the resulting voltage and activation maps. For example, a significant design change in electrode position between two generations of catheters may be reflected in a change in bipolar voltage recordings, which affects therapies that target voltage zones below a certain measured threshold. While design differences from competing companies are inevitable, these changes may detrimentally affect establishment of consistent therapies based on thresholds of voltage or cycle length. A possible branch of future mathematical modelling may explore finite element models of catheters navigating three-dimensional atrial structures, in order to estimate the differences in measured voltage based on the size, shape, and location of catheter electrodes. This may lead to a convergence of catheter technologies and thresholds established for future ablation techniques.

The purpose of mapping catheters is to record the endocardial electrogram with the overall goal of mapping the voltage profile on to the patient’s cardiac anatomy. The
resulting electroanatomic map allows the operator to tag important landmarks or ablation lesions, and obtain a map of activation times and voltages. The final maps and recordings are saved once variability in cycle length and voltage is minimized (for example, cycle length differences are < 2%, local activation time stability < 3ms, beat-to-beat catheter location < 4mm). Both unipolar and bipolar voltage and activation maps may be obtained in the system. The bipolar electrograms are recorded from subtracting voltage from the tip to distal electrodes, and the unipolar electrograms are just recorded from the tip electrode. Bipolar activation maps are determined by marking the maximum amplitude of the intrinsic deflection, and unipolar activation maps are obtained by marking the maximum negative slope of the intrinsic deflection. In general, unipolar and bipolar recordings provide complementary information, but bipolar recordings may be preferred because of their ability to detect voltage-based scar tissue.

Once electroanatomic maps are obtained, catheter ablation is performed based on the voltage potential distribution, activation time sequence, and the morphology of recorded signals. This technique changes between operators and between mapping systems, and depends on a consensus reached by each team in the operating room. Mapping systems such as Carto-3 also include algorithms that recommend ablation strategies to operators based on the voltage or activation maps. Overall the 3-D mapping systems have been developed to improve patient care through improving mapping resolution, increasing acquisition rate of voltage data, acquiring spatial localization of devices employed in the patient while minimizing fluoroscopic exposure, and providing ablation operators with detailed, precise data regarding the patient’s arrhythmia. Limitations to use of these mapping systems include: [1] the assumption that data regarding the location of electrodes
is precise enough to trust voltage and activation maps and guide ablation therapy, which may not always be true, and [2] the sheer amount of data currently provided to the operator through these detailed maps lengthens procedural times.\textsuperscript{56} Furthermore, from an analytics standpoint the datasets of the map and final ablation location are large and difficult to post-process. There is a compelling opportunity to improve upon the technology used in mapping systems, as well as develop machine learning algorithms when reviewing retrospective data that can help predict where to ablate next to ensure a continuous lesion or the most recommended ablation strategy (based on previous successful geometries and techniques in a similar patient population). The strategy of catheter ablation in particular is widely varied in terms of where to ablate, as well as the power, duration, and force to apply to the tissue.

**Catheter Ablation Strategies Used in Treatment of Human Atrial Fibrillation**

The pulmonary veins (PV) are well-known sites of ectopic foci that initiate AF, perhaps due to their anatomical structure. The site where the pulmonary veins are coupled to left atrial tissue includes myocardial fibers that are poorly coupled, and susceptible to changes in orientation due to fibrosis or fatty deposits. This promotes conduction block and delays, and serves as a trigger for AF propagation.\textsuperscript{41} Catheter ablation of the pulmonary veins is a successful ablation strategy for paroxysmal AF, with clinical success rates of 70 - 85\%.\textsuperscript{57} This success rate decreases as AF evolves, due to the complexity of multiple rotors and non-PV triggers that sustain persistent AF. If repeat procedures are performed due to AF recurrence, generally the PV’s are targeted for ablation again and operators may supplement this therapy by targeting non-PV triggers such as complex fractionated atrial electrograms, linear left atrial lesions, ganglionated plexi, rotors, and low-voltage zones.\textsuperscript{58}
Due to high recurrence rates in persistent AF after PV isolation, these additional strategies are the current subject of debate and extensive research.

Complex fractionated atrial electrograms (CFAE’s) are very rapid electrograms with multiple, continuous split wavefronts that serve as points of collision or pivot points where wavelets can turn around for re-entry.\textsuperscript{58,59} Targeted ablation of CFAE’s is recommended for patients with non-paroxysmal AF.\textsuperscript{48} These areas generally have short cycle lengths, but identification of these substrates is dependent on electrogram visual analysis, which (as previously mentioned) is subjective to operators and mapping systems. There is some controversy regarding techniques to identifying CFAE’s. One method calculates a fractionation interval based on the activation map as the average interval between deflections, and ablates CFAE areas below a certain fractionated interval threshold.\textsuperscript{60} Others use the monophasic action potential and the activation sequence to distinguish CFAE areas.\textsuperscript{61} Continuous CFAE’s (identified as areas with fractionated interval of < 50ms for longer than an 8ms duration) correlate with non-PV ectopy sites and ablation of these areas after PV isolation eliminates recurrence of non-PV triggers of AF.\textsuperscript{62} However, the STAR AF II clinical trial comparing PV isolation and PV isolation + CFAE ablation, where CFAE areas were identified as regions with a mean cycle length of < 120ms, found there was no reduction in rate of recurrence in patients with persistent AF.\textsuperscript{63} Recurrence of AF occurred in 41% of patients with PV isolation, and 51% of patients with PV isolation + CFAE ablation. The clinical relevance of CFAE ablation may also be improved by considering low-voltage areas (< 0.5 mV).\textsuperscript{54,64} Although the STAR AF II trial failed to demonstrate a favorable outcome for CFAE ablation of patients with persistent AF, there may be additional steps to this technique that could improve therapy. For
example, exploring CFAE’s in higher-dimensional models and developing machine learning algorithms to detect CFAE’s automatically in electroanatomic maps may reduce the variability in CFAE identification and improve therapy.\textsuperscript{55}

Additional linear left atrial lesions may be made after PV isolation as an additional technique to decrease AF recurrence in patients with persistent AF. Lines of block made along the left atrial roof and/or mitral isthmus have increased ablation success compared to PV isolation alone.\textsuperscript{65} However, there are conflicting reports on whether substrate modification through linear atrial lesions is a valuable therapy. AF termination during linear ablation after PV isolation was the sole predictor of eliminating AF recurrence during a five-year follow-up of persistent AF patients.\textsuperscript{66} However, this technique failed to show benefits for patients with paroxysmal AF, and ended up increasing procedural, fluoroscopy, and radiofrequency application times with no added therapeutic benefit.\textsuperscript{67} This suggests that the risk-benefit ratio for linear atrial lesion therapy needs to be considered for each patient, and this therapy should perhaps be reserved only for patients with persistent AF.

The intrinsic cardiac autonomic nervous system has been shown to play a role in AF maintenance, specifically due to activity of ganglionated plexi in the left atria. Autonomic inputs to cardiac tissue are located in epicardial fat pads in the left atria and form ganglionated plexi (GP), made up of ganglia and nerves.\textsuperscript{68} Ablation of this substrate in addition to PV isolation has increased ablation success rates compared to PV isolation alone, and compared to GP ablation alone.\textsuperscript{69} These improved success rates remain true for patients with paroxysmal, persistent and long-standing persistent AF.\textsuperscript{70} From a molecular mechanism standpoint, the interplay of the ganglionated plexi with the pulmonary veins is a fascinating potential area for mathematical modelling research. Is it enough to merely
isolate the pulmonary veins in order to treat atrial fibrillation? What structural remodeling effect does nerve regeneration have post-ablation of the ganglionated plexi? If a new atrial substrate is created after ablation of the GP, what are its electrical properties? Currently there is a lack of detailed adrenergic signaling in single-cell and higher-dimensional mathematical models of the atria. There is a need to explore the cholinergic system and incorporate the molecular mechanisms of sympathetic activation in order to better understand how and why ablation of ganglionated plexi may affect AF electrical and structural remodeling.

Phase mapping and dominant frequency mapping of the atria has revealed a distribution of local cycle lengths and dominant frequencies in the atria, and the highest dominant frequency in AF tissue was identified as the rotor driving the arrhythmia. The Focal Impulse and Rotor Modulation technique (FIRM) requires phase mapping and a multi-electrode mapping catheter to identify spiral waves rotating around a center at high frequency, and identify self-sustaining ectopic foci. The CONFIRM trial (CONventional ablation for atrial fibrillation with or without FIRM) tested whether patient-specific ablation with the FIRM technique contributed to AF recurrence rates compared to PV isolation (with a left atrial roof line in patients with persistent AF). Reduction of rotors and focal impulses reduced AF recurrence in 1-2 years and in the >3 year follow-up compared to PV isolation alone. However, the CONFIRM trial results were not replicated in other clinical studies. A combination technique may be a better route – for example, ablating low-voltage zones and CFAE associated with rotors may be an improvement to CFAE ablation or rotor ablation alone.
In addition to these techniques, additional research on ablation strategies has expanded to the use of freezing rather than resistive heating to ablate tissue.\textsuperscript{75} This technology also employs a balloon that creates identical circumferential lesions all at once rather than using the tip of a catheter to create individual lesions. The decision of whether to use resistive heating or cooling in order to ensure continuous lesions that do not re-activate electrically to create a new AF substrate is a large body of current research.

The electrical coupling between the tip of the catheter and atrial tissue has also been determined to affect success rates of ablation. Guidelines have been developed to estimate the optimal contact force between catheter tips and tissue, to deliver lesions of a desired size that are not likely to heal and resume electrical activity.\textsuperscript{76,77} Ablation catheters such as TactiCath (Abbott) and ThermoCool SmartTouch (BioSense Webster) have been developed to display contact force to operators during catheter ablation. However, the contact force measurements displayed to the operator are heavily processed using filtering techniques that change between mapping systems, and this may detrimentally affect the success rate for catheter ablation based on universal contact force guidelines. These issues need to be resolved in order to truly determine the best guidelines for contact force while performing catheter ablation.

In summary, catheter ablation is not yet a perfect AF treatment strategy due to the complexity of progressing AF. There is a frequent need for follow-up procedures, and for some strategies the ablation procedure is technically challenging due to difficulties in accessing the left atria.\textsuperscript{78} As the number of recurring ablation surgeries increases for a patient, so does the number of non-PV ectopic foci. This further complicates recurrence treatment: since there is no consistent mapping or provocation technique for ablation of
ectopic foci that don’t originate in the pulmonary vein, and therefore it is difficult to assess reported success rates.\textsuperscript{79,80} Furthermore, success for catheter ablation is determined by non-inducibility of the arrhythmia after procedure, and specific endpoints depend on the ablation strategy. For example, ablation endpoints may be elimination of triggers / drivers, decrease in AF cycle length, decrease in dominant frequency, or elimination of a frequency gradient. Additional application of isoproterenol or adenosine may also be used to unmask abnormal conduction or non-PV triggers before the surgery is completed. The wide variety in workflows and differences between strategies as well as ablation technologies may be contributing to varying success rates reported between groups. Can we add more endpoints and guidelines to make these strategies consistent across operating rooms, to better understand the success rates reported in different studies? Finally, techniques such as ablating rotors or low-voltage zones have not been consistently shown to increase success rates compared to PV isolation alone, and may detrimentally increase procedure time and fluoroscopy exposure without added medical benefits from the ablation.\textsuperscript{81} There is tremendous opportunity for data analytics and mathematical modelling techniques to help guide and understand ablation strategies, either by predicting where the next ablation should go or by extending mathematical models to better understand the electrical and structural AF substrate post-ablation. Regardless of the drawbacks to catheter ablation, this technique is currently recommended for patients who are resistant to rate-controlling medication, and current guidelines recommend linear lesions or CFAE ablation following PV isolation in patients with long-standing persistent AF.\textsuperscript{48,58}

Drug Treatment Strategies for Human Atrial Fibrillation
Drug therapies for AF to maintain sinus rhythm vary based on diagnosis of other cardiac disease factors. Patients with minimal/no heart disease or hypertension without substantial left ventricular hypertension are prescribed flecainide or sotalol, which block fast Na\(^+\) channel current and K\(^+\) efflux respectively. Patients with coronary artery disease are prescribed dofetilide and sotalol, which block K\(^+\) efflux. If this initial treatment is not effective or the patient has hypertension with substantial left ventricular hypertension or the patient has heart failure, they may be prescribed K\(^+\) efflux blockers such as amiodarone or dofetilide, and/or undergo catheter ablation.\(^{48}\)

Patients with AF have 5-fold increased risk of stroke and as a result antithrombotic therapy is a common recommended treatment. Clinicians use the CHA\(_2\)DS\(_2\)-VASc score to evaluate each patient’s risk based on a point system, and determine which therapy is most appropriate (Table 3). Patients with a score > 2 are at significant risk for stroke and are prescribed warfarin.\(^{82,83}\) Other antithrombotic therapy options are aspirin alone (the popularity of which is decreasing), aspirin + clopidogrel, and vitamin K antagonists. Patients without valvular disease may be prescribed dabigatran, rivaroxaban, apixaban, or edoxaban. Research regarding the best methods of stroke mitigation in patients with AF is ongoing. The 2010 randomized clinical trial “Apixaban for reduction in stroke and other ThromboemboLic events in atrial fibrillation” (ARISTOTLE) randomized 18,206 patients from 1,000 centers with apixaban or warfarin treatment for 1-4 years.\(^{84}\) Apixaban was superior to warfarin in preventing stroke in AF patients, and results from the clinical trial regarding associated disease such as cancer are still being evaluated.\(^{85,86}\) In addition to different opinions regarding the best antithrombotic therapy to give to AF patients,
drawbacks to prescribing antithrombotics include frequent blood draws, a risk of bleeding, and cost.

Antithrombotic therapy may also be given in combination with other drug therapies to mitigate atrial fibrillation. Rate control or rhythm control through medication has been found to reduce heart failure in patients with atrial fibrillation (Table 4). There is considerable debate over whether treatment for AF should focus on the rapid ventricular heart rate responsible for AF symptoms (rate control), or focus on restoring normal sinus rhythm through cardioversion or antiarrhythmic drugs (rhythm control). Rate control includes oral anticoagulants combined with rate-slowing medication such as digitalis, nondihydropyridine Ca2+ channel blockers, or β-blockers. Rhythm control includes oral anticoagulants combined with serial cardioversions such as sotalol, replaced by flecainide in the event of recurrence, replaced by amiodarone in the event of recurrence. In the Rate Control versus Electrical Cardioversion (RACE) trial comparing rate control treatment with rhythm control treatment in 522 patients older than 65 years with persistent AF, major cardiovascular events were more frequent in the rhythm control group but patients in both groups were still at risk for stroke and bleeding. The rhythm control treatment is currently not recommended for patients with hypertension or women with persistent AF but is recommended for patients with progressed AF. The rate control treatment is recommended for patients with high stroke risk and minimal AF. In addition to rate and rhythm control, a third drug strategy for treatment of AF includes using angiotensin-converting enzyme (ACE) inhibitors to block the renin-angiotensin-aldosterone (RAAS) system. Activation of the RAAS system increases AF risk by promoting inflammation and fibrosis through the increased production of angiotensin II. ACE inhibitors decrease this production, causing
blood vessels to dilate and blood pressure to reduce, and result in a 28% decrease in AF risk.\textsuperscript{88} Ultimately the wide variety in drug therapy options and success rates further emphasizes the point that atrial fibrillation is a highly heterogeneous disease due to variable electrical and structural remodeling. Perhaps the best way to move forward with AF therapies is to specifically categorize patient populations to better understand the best drug therapy treatment. A better understanding of the cardiac electrophysiology for these different patient populations would also inform mathematical models of atrial fibrillation, which can aid in modelling drug binding kinetics and the overall impact of new potential drugs on the cardiac action potential.

**Surgical Treatment Strategies for Human AF: MAZE Procedure**

The pillar of surgical AF treatment is the Cox-Maze procedure, a surgical procedure first performed in 1987 based on the hypothesis that AF results from chaotic random wavelets propagating throughout the atria (the multiple wavelet hypothesis).\textsuperscript{58} Surgical treatment uses cryotechnology to make transmural lesions in the atria that create a maze of conduction block lines. This treatment results in higher sinus rhythm maintenance than catheter ablation, and lower stroke rates in treated patients.\textsuperscript{89,90} In patients with dilated LA and hypertension, or failed catheter ablation, surgical treatment was superior in reducing AF recurrence compared to catheter ablation but resulted in a higher rate of procedural adverse events.\textsuperscript{91}

While operations such as the Cox-Maze procedure report higher success rates, they require the patient to stay in the hospital for recovery for at least 3-5 days, whereas patients undergoing cardiac catheter ablation may be able to go home the same day as the surgery depending on their recovery. The average cost per inpatient day at state and local
government hospitals is $1878, to say nothing of the time cost to the patient in 3-5 days of recovery from surgery.\textsuperscript{92} Catheter ablation procedures are much more cost effective and are estimated to cost $28,700 – $98,900 per patient per quality-adjust life years gained.\textsuperscript{93} A way to mitigate the high cost of the Cox-Maze procedure is by treating patients undergoing mitral valve surgery with an additional Cox-Maze procedure as a preventative surgery against further AF progression. The probability that the additional surgery will be cost-effective jumps from 58.1\% to 89.3\% as the patient’s willingness to pay more for the surgery increases (from $20,000 to $100,000 CAD per quality-adjusted life year).\textsuperscript{94} Interestingly, a survey of 27,000 Canadian residents to determine the main risk factors correlated with heart disease found that five main covariates that independently associated with heart disease were high blood pressure, daily smoking, being physically inactive, being male, and a combined household income of less than $30,000.\textsuperscript{95} These factors are all considered by heart disease risk factor scores, except for income inequality. This suggests that patient income should be considered when evaluating risk factors and determining optimal AF treatment. In summary, operative mortality or complication rates are not the only factor to consider when comparing success rates for AF therapies.

AF is a complex disease where progression of the disease increases resistance to treatment. A variety of treatments have been explored using drug therapy, surgical therapy, and catheter ablation technologies. To understand the best method of prevention and treatment, it is necessary to further understand the cell- and tissue- level mechanisms that sustain and progress AF. Mathematical modelling and data analytics can further build on current AF research to improve understanding of the molecular mechanisms of the AF substrate, predict the best drug treatment and develop catheter ablation strategies based on
detailed understanding of stratified patient populations. There is a critical need to develop an understanding of AF from the single-cell signaling level, as well as propagation of AF across atrial tissue.
<table>
<thead>
<tr>
<th>Classification</th>
<th>Duration of episode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxysmal</td>
<td>&lt; 7 day termination</td>
</tr>
<tr>
<td>Persistent</td>
<td>&gt; 7 day termination</td>
</tr>
<tr>
<td>Longstanding persistent</td>
<td>Continuous AF &gt; 12 mo in duration</td>
</tr>
<tr>
<td>Permanent</td>
<td>Patient and clinician stop attempts to restore to sinus rhythm</td>
</tr>
<tr>
<td>Nonvalvular</td>
<td>AF not associated with valve repair</td>
</tr>
</tbody>
</table>
Table 3: Point system for determining stroke risk in atrial fibrillation patients.

<table>
<thead>
<tr>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHF or LVEF ( \leq 40% )</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Age ( \geq 75 )</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Stroke / TIA / thromboembolism</td>
</tr>
<tr>
<td>Vascular disease</td>
</tr>
<tr>
<td>Age 65-74</td>
</tr>
<tr>
<td>Female</td>
</tr>
</tbody>
</table>
Table 4: List of antiarrhythmic agents and preferred use for atrial fibrillation.  

<table>
<thead>
<tr>
<th>Classification</th>
<th>Antiarrhythmic agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td></td>
</tr>
<tr>
<td>Fast Na$^+$ channel blockers</td>
<td>Class Ia</td>
</tr>
<tr>
<td></td>
<td>Quinidine</td>
</tr>
<tr>
<td></td>
<td>procainamide</td>
</tr>
<tr>
<td></td>
<td>disopyramide</td>
</tr>
<tr>
<td></td>
<td>Class Ib</td>
</tr>
<tr>
<td></td>
<td>Lidocaine</td>
</tr>
<tr>
<td></td>
<td>phenytoin</td>
</tr>
<tr>
<td></td>
<td>mexiletine</td>
</tr>
<tr>
<td></td>
<td>Class Ic</td>
</tr>
<tr>
<td></td>
<td><em>Flecainide</em></td>
</tr>
<tr>
<td></td>
<td><em>propafenone</em></td>
</tr>
<tr>
<td></td>
<td>moricizine</td>
</tr>
<tr>
<td>Class II</td>
<td></td>
</tr>
<tr>
<td>Antisympathetic nervous system</td>
<td>Propanolol</td>
</tr>
<tr>
<td>agents/β-blockers</td>
<td>Esmolol</td>
</tr>
<tr>
<td></td>
<td>Timolol</td>
</tr>
<tr>
<td></td>
<td>Metoprolol</td>
</tr>
<tr>
<td></td>
<td>Atenolol</td>
</tr>
<tr>
<td>Class III</td>
<td></td>
</tr>
<tr>
<td>Block K$^+$ efflux</td>
<td><em>Amiodarone</em></td>
</tr>
<tr>
<td></td>
<td><em>Sotalol</em></td>
</tr>
<tr>
<td></td>
<td>Ibutilide</td>
</tr>
<tr>
<td></td>
<td><em>Dofetilide</em></td>
</tr>
<tr>
<td></td>
<td><em>Dronedarone</em></td>
</tr>
<tr>
<td>Class IV</td>
<td></td>
</tr>
<tr>
<td>Slow Ca$^{2+}$ channel blockers</td>
<td>Verapamil</td>
</tr>
<tr>
<td></td>
<td>Diltiazem</td>
</tr>
<tr>
<td>Class V</td>
<td></td>
</tr>
<tr>
<td>Variable mechanisms</td>
<td>Adenosine</td>
</tr>
<tr>
<td></td>
<td>Digoxin</td>
</tr>
<tr>
<td></td>
<td>Magnesium sulfate</td>
</tr>
</tbody>
</table>
Chapter 3: Computational Models of Atrial Fibrillation

Modeling the atrial cell action potential began with Hilgemann & Noble’s work in 1987, which simulated dynamic changes in Ca\(^{2+}\) concentrations inside the cell focusing specifically on the Na\(^+\)/Ca\(^{2+}\) exchanger, L-type Ca\(^{2+}\) channel, and sarcoplasmic reticulum Ca\(^{2+}\) release. Experimental data used to fit this initial model came from rabbit atrial tissue. The availability of human atrial data for model parameterization did not come until much later, and led to the development of the first two main human atrial cell models in 1998. Since then a variety of atrial cell action potential models have been published exploring the structure of the cell, ion concentrations, dynamic Ca\(^{2+}\) behavior, and rate-dependent adaptation. These models have been used to explore electrophysiology mechanisms of atrial fibrillation at the single cell level, and tissue-level mechanisms in two-dimensional and whole-heart simulations. Overall the purpose of developing a detailed model of the atrial cell is to explore cellular dynamics that would disrupt atrial propagation and contribute to an arrhythmogenic substrate susceptible to ectopic firing or reentry.

Single-cell computational models of atrial cell membrane excitability

The two initial principal models of human atrial cell excitability were published in 1998, and were both based on Hodgkin-Huxley approaches to action potential modeling. Both models incorporated the ultra-rapid delayed rectifier K\(^+\) current, based on data showing that it played more of a role in atrial myocytes than in ventricular. The Courtemanche model used the Luo-Rudy model as a basis for its formulations and reproduced a spike-and-dome morphology for the action potential. At faster pacing rates, rate adaptation was sensitive to slowed late repolarization due to K\(^+\) currents. Reducing \(I_{\text{Ca,L}}\) activity with incomplete K\(^+\) current deactivation was enough to eliminate rate
adaptation completely. Furthermore, this study noted differences in atrial AP morphology and varied \( I_{f0} \) to replicate different morphologies, emphasizing that \( K^+ \) currents play a crucial role in overall atrial action potential behavior and repolarization.\(^{29}\)

In contrast to the Courtemanche model, the Nygren model reproduced a triangular AP morphology and based their reproduction of the human atrial action potential by updating currents \( (I_{f0}, I_{Ca,L}, I_{sus}) \) from a previously published rabbit atrial cell model from the same group. This study also emphasized the impact of \( K^+ \) currents on the action potential, and suggested that the role of \( I_{sus} \) on AP prolongation could be modulated by the size of other currents active during the plateau phase of the AP. This suggests that \( I_{sus} \) is a possible target for drug modulation of atrial cell membrane excitability, since \( I_{sus} \) responded to the physiological state of the cell.\(^{30}\) The Courtemanche model and the Nygren model adapt to changes in pacing using different mechanisms – the action potential duration and morphology of the Courtemanche model is sensitive to rate, while the resting membrane potential of the Nygren model is more sensitive to rate.\(^{98}\)

The Nygren and Courtemanche models have been used to explore a range of atrial cell mechanisms. The next improvements to the Nygren model came with the now well-known Maleckar model, which updated \( K^+ \) currents in the Nygren model to improve AP rate dependence and repolarization based on experimental data.\(^{34}\) The Maleckar model added \( I_{NCX}, I_{NaK}, \) SERCA2a, and background \( Na^+ \) and \( Ca^{2+} \) currents, and reformulated \( I_{f0} \) and \( I_{Kur} \). For example, \( I_{Kur} \) was updated based in steady-state activation and inactivation data from human atrial myocytes. Although these three models persisted as well-established atrial models, they lacked detailed \( Ca^{2+} \) handling dynamics based on human atrial data.
The Koivumaki model was the first to reproduce the principal characteristics of Ca\(^{2+}\) handling dynamics based on atrial myocyte data.\(^{35}\) This model extends the published Nygren model by incorporating the heterogeneity of intracellular Ca\(^{2+}\) changes. Specifically, this model divided the cytosol and SR into transverse components to replicate centripetal Ca\(^{2+}\) wave propagation, with a delay between peripheral and central SR Ca\(^{2+}\) release. Both the sarcolemmal Ca\(^{2+}\) current and SR Ca\(^{2+}\) release then contribute to the overall Ca\(^{2+}\) transient. This work is crucial because subsarcolemmal SR Ca\(^{2+}\) release sites influence AP morphology, and the process of SR Ca\(^{2+}\) release is different in atria versus the ventricles. Atrial myocytes lack the prominent transverse tubule system that exists in ventricular cells. This tubule system couples the SR with sarcolemma and makes Ca\(^{2+}\) release uniform throughout the cell. In contrast, the Ca\(^{2+}\) wave in atrial cells rises in the junctional SR, propagates to the center and then activates a secondary release from the non-junctional SR. The Koivumaki model incorporated these detailed Ca\(^{2+}\) release changes and explored the role of Ca\(^{2+}\) and Na\(^+\) intracellular concentrations in rate-dependence of the action potential. Their simulations suggested that modulation of the APD due to increases in heart rate is mediated by differences in intracellular Na\(^+\) concentration, which is linked to Ca\(^{2+}\) changes.\(^{35}\) This model also further emphasizes the impact of including detailed Ca\(^{2+}\) handling in action potential simulations. Although it extends the Nygren and Maleckar models, its Ca\(^{2+}\) handling contributes significantly to the behavior of the atrial cell in its plateau phase, so the restitution curve of the Koivumaki model looks more like the Courtemanche model.

A third branch of atrial cell models was created with the Grandi human atrial cell model.\(^ {18}\) This model reproduced a similar morphology as the Nygren model and an APD
similar to the Courtemanche model, but its model basis was a human ventricular cell model from the same group. To replicate the human atrial AP, SERCA2a function was altered to reproduce the atrial Ca\(^{2+}\) transient, \(I_{\text{NCX}}\) and \(I_{\text{NAK}}\) densities were reduced from the ventricular cell model, \(I_{\text{Kur}}\) was added, and a higher \(I_{\text{to-fast}}\) density was incorporated while \(I_{\text{to-slow}}\) was removed. The Grandi model incorporated detailed rate-dependent Ca\(^{2+}\) handling in the junction, SR, and cytoplasm based on human atrial cardiomyocyte data. The Grandi model was the first to simulate β-adrenergic stimulation of atrial myocytes using isoproterenol application, based on their own experimental data. This study was also the first to differentiate ion current behavior in left and right atrial myocytes to explore morphological changes between these two sections of the heart. This model explored blocking \(I_{\text{Kur}}\) to model a loss-of-function \(K_{V1.5}\) mutation, and found increased EAD rates during adrenergic stress as observed experimentally. Simulation of chronic AF (reduction of \(I_{\text{Ca,L}}, I_{\text{to}}, I_{\text{Kur}}, \text{and SERCA2a}; \text{increased} I_{\text{K1}}, I_{\text{Ks}}, I_{\text{NCX}}\) showed shorter AP’s and reduced \(I_{\text{Ca,L}}\) when increasing the pacing frequency, which underlines a crucial role for Ca\(^{2+}\) and Na\(^{+}\) in maintaining both the Ca\(^{2+}\) transient and the rate adaptation of ion concentrations. By this point, growing experimental evidence suggested the Ca\(^{2+}\) handling abnormalities were responsible for atrial fibrillation, and 5 years before ranolazine had already been approved by the FDA for patient use. This contributed to growing interest into the dynamics of Na\(^{+}\) and Ca\(^{2+}\) in computational models of atrial fibrillation.

Notable extensions to main computational models of atrial cell membrane excitability

The main computational models of atrial cell membrane excitability (Courtemanche, Nygren, Koivumaki, Maleckar, and Grandi models) have been extended to further explore the impact of other currents and ion concentration dynamics.\(^{21}\)
example, the Grandi model was extended with Na$^+$-dependent regulation of $I_{K1}$ and $I_{KAch}$ to determine the impact of Na$^+$-dependent regulation of K$^+$ channels on atrial action potential duration and rate dependence. This model found that loss of Na$^+$-dependent $I_{KAch}$ increased rate dependence in sinus rhythm, and decreased rate dependence in chronic AF. This work suggested that ion channel remodeling alone was insufficient without changes in intracellular ion concentration to explain loss of rate adaptation.$^{99}$

Another example of an extension to the main computational models combined clinical and modeling studies to explore the impact of $K_{2P}3.1$, which makes up a two-pore K$^+$ channel called TASK-1.$^{100}$ This K$^+$ current is predominantly expressed in atria and increased in chronic AF but not paroxysmal, suggesting that it contributes to AF progression. This study fit the TASK-1 IV curve to replicate its current, and updated Na$^+$-dependent regulation of $I_{K1}$, $I_{KAch}$ and $I_{K2P}$. Inhibition of the TASK-1 current produced a longer action potential at sinus rhythm and even greater prolongation in atrial fibrillation, suggesting that TASK-1 contributes to proarrhythmic APD shortening and is a potential target for AF mitigation.$^{100}$

It is important when extending the main five models of atrial action potentials to consider the differences in results and simulation parameters, so that the best model is chosen for the study. Among these five models, the Courtemanche model takes the longest to reach long-term stability of action potential duration at 50% repolarization (APD$_{50}$), and does not reach a steady-state AP amplitude or APD$_{90}$ value within 10 minutes of simulation. The Courtemanche model also demonstrates a linear rate-dependent action potential duration, while the other models have a more non-linear relationship. The Nygren and Maleckar models are unable to reproduce alternans or AF-induced remodeling due to
their limited Ca\textsuperscript{2+} handling dynamics. In contrast the Grandi and Koivumaki models are stable, appropriate choices for Ca\textsuperscript{2+} handling studies, and the Grandi model has detailed chronic AF simulation protocol.\textsuperscript{101} The studies outlined in Chapter 7 focus on further extensions to the Grandi model due to these detailed and updated Ca\textsuperscript{2+} handling kinetics, its historical use in simulating both adrenergic stress and chronic AF, and overall model stability.

Higher dimensional models of atrial tissue dynamics

Atrial fibrillation is both a structural and electrical disease, i.e. electrical remodeling in atrial cells contributes back to structural remodeling such as increased fibrosis, which feeds back again to electrical disruption. Multi-scale computational modeling is poised to help understand how mechanisms of disrupted activity at the ion channel level can translate to rotors and conduction issues on the tissue scale. The existing computational models also manifest in different AF phenotypes when coupled at higher dimensions. For example, two-dimensional simulations of AF using the Courtemanche model displayed conduction with frequent wave breaks, whereas the Nygren model displays stable, non-breaking spirals, suggesting that Ca\textsuperscript{2+} dynamics play an important role in maintaining rotor stability.\textsuperscript{98} Since intracellular Ca\textsuperscript{2+} dynamics help determine reentrant wave dynamics, it may be more appropriate to use an updated atrial myocyte model with detailed Ca\textsuperscript{2+} handling to study rotor generation.

Higher-dimensional models can also be used to investigate conflicting hypotheses regarding the mechanisms of AF such as the multiple wavelet hypothesis vs mother rotor theory. One such study compared electrical and structural remodeling of AF perpetuation – where electrical remodeling was simulated by shortening the atrial wavelength through
a decreased APD90, and structural remodeling was simulated by shortening the atrial wavelength through decreased conduction. This study found that reentry duration was the same for both types of remodeling, and that dominant waves were anchored around different anatomical obstacles between the two types. Structural remodeling resulted in more fragmented wavefronts, which was consistent with the multiple wavelet hypothesis.\textsuperscript{102}

Multi-scale modeling can also replicate detailed geometry of the atria and couple it to electrophysiological cell models to investigate frequency and phase analysis in simulated atrial fibrillation.\textsuperscript{103} This can be extended to replicate the personal geometry and spatial pattern of fibrosis of a patient using late gadolinium-enhanced magnetic resonance imaging.\textsuperscript{104} One goal of these models is to inform ablation approaches based on fibrotic heterogeneity of each patient.\textsuperscript{105} These models often integrate human atrial geometry, fiber orientation, region-specific electrophysiology, and fibrotic substrate of the patient. This allows investigation of the mechanistic link between patient-specific fibrosis and AF remodeling and can help identify areas where rotors propagate – for example, around border zones of patchy fibrosis, or in patients with more heterogeneous fibrosis distribution.\textsuperscript{106,107}

Computational modeling of atrial myocytes has been a subject of research for three decades. As we gain more experimental data and knowledge regarding atrial disease, computer simulations will continue to grow to help determine new strategies for disease mitigation. What can we model to help us understand how to guide drug and ablation treatments? In order to develop tailored therapies, there is a critical need for detailed kinetics of key molecules involved in the progression of AF – for example, protein kinases
heavily involved in heart failure such as CaMKII - to be incorporated into mathematical models. Ultimately a deeper understanding of atrial physiology, pathophysiology, and integration of multiple levels of data will help us better understand AF progression.
Chapter 4: Mathematical modeling of Ca\textsuperscript{2+}/Calmodulin-dependent Protein Kinase II (CaMKII)

Mathematical modeling studies over the past three decades have elucidated important aspects of CaMKII function and signaling mechanisms. These studies began to elucidate CaMKII activity in neuronal cells, and its impact on memory signaling processes such as long-term potentiation\textsuperscript{108-111}. Current studies incorporate models of CaMKII activity into models of the whole cell and tissue (mostly cardiac) to understand the larger role of CaMKII signaling in cell/organ function\textsuperscript{33,35,112-118}. Recently, these efforts have been expanded to gain insight into the role of CaMKII in human cardiac disease\textsuperscript{33,35,117-121}.

CaMKII is a dodecameric holoenzyme with a molecular weight of 600-700 kDa, and its structure is organized as a hexamer of dimers arranged as two stacked rings. Four genes encode the CaMKII subunits (α, β, γ, and δ), but only γ and δ are predominantly expressed in cardiac tissue. CaMKII δb is primarily found in adult cardiac tissue, while CaMKIIδc is found in embryonic cardiac tissue\textsuperscript{122}.

Each CaMKII monomer is comprised of an N-terminal catalytic domain, a regulatory domain, and a C-terminal association domain. In its inactive conformation, the regulatory domain binds to the active site in catalytic domain, which inhibits the activity of the enzyme. Association of Ca\textsuperscript{2+} bound calmodulin to the C-terminal region of the regulatory domain causes a conformational change/allosteric displacement of the substrate region, exposing the active site in catalytic subunit to ATP, enabling the kinase to phosphorylate its substrates. Multiple residues within the regulatory domain are also exposed that may undergo post-translational regulation that alters kinase function\textsuperscript{123}. Specifically, CaMKII can also undergo post-translation modifications including oxidation.
at methionine residues 281/282, O-linked glyosylation of serine residue at 280, nitric oxide-dependent nitrosylation of cysteine residues at 116, 273, 290, phosphorylation of threonine residue at 306/307, and nitric oxide- modification of serine residue at 273. Enzyme regulation/activity depends heavily on the multimeric holoenzyme structure (Figure 3). For example, a distinguishing characteristic is the ability of CaMKII to undergo autophosphorylation where an active (Ca\(^{2+}\)/calmodulin bound) kinase subunit is phosphorylated at a specific residue (Thr286/287 in the C-terminal region of the regulatory domain, a critical phosphorylation site in the autoregulatory domain) by a neighboring active subunit. Activation of adjacent monomers enables autophosphorylation, prevents association of catalytic and regulatory domains, increases the binding affinity for Ca\(^{2+}\)/calmodulin and CaMKII by 10\(^{516}\), and helps extend CaMKII activation over time.\(^1\)\(^{24}\) The autophosphorylated kinase retains activity in the absence of bound Ca\(^{2+}\)/calmodulin and is thought to contribute to synaptic plasticity and learning functions as well as myocyte excitation-contraction coupling.\(^1\)\(^{25}\)

A variable domain between the regulatory and association domain is responsible for the splice variants of CaMKII, and its specificity allows CaMKII to target different parts of the cell. These splice variants result in different lengths of the variable domain, and causes CaMKII to change sensitivity to Ca\(^{2+}\) due to the length of the domain- for example, a shorter domain makes CaMKII inaccessible to activation by Ca\(^{2+}\).\(^1\)\(^{26,127}\)

Modeling CaMKII signaling in cardiac cells and tissue

Modeling of CaMKII signaling at the cellular level poses a unique set of challenges. First, the kinase is sensitive to intracellular Ca\(^{2+}\), since it is activated by Ca\(^{2+}\)-bound calmodulin. Second, once activated, the multifunctional kinase targets many substrates in
the cell, from membrane ion channels, pumps and transporters to contractile proteins and even transcription factors. Specifically, this includes voltage-gated Na\textsuperscript{+} channels (Nav1.5), L-type Ca\textsuperscript{2+} channels (Cav1.2 α, β subunits), transient outward K\textsuperscript{+} current (K\textsubscript{V}4.3), inward rectifying K\textsuperscript{+} current (Kir2.1), SR Ca\textsuperscript{2+} release, phospholamban, HDAC5, and IP3 receptors. Finally, CaMKII interacts with a vast and complex signaling web that includes other proteins directly regulated by Ca\textsuperscript{2+}/calmodulin (e.g., ion channels, calcineurin), protein phosphatases that antagonize CaMKII phosphorylation (e.g., PP1), downstream activation from β-adrenergic stimulation through the cAMP-Epac pathway, and other kinases that potentially synergize CaMKII effects (e.g., protein kinase A).\textsuperscript{128}

Despite these numerous obstacles, CaMKII signaling networks have been successfully incorporated with varying degrees of complexity into whole cell models of the myocyte (mostly ventricular) action potential and calcium transient. These models have employed different strategies to deal with challenges outlined above. The most common class of models incorporate a scheme where a single population of CaMKII responds to changes in bulk or subspace Ca\textsuperscript{2+}/calmodulin.\textsuperscript{33,35,36,112–115,117,118,129} In other cases, CaMKII-dependent effects on membrane substrates are implemented in the absence of dynamic changes in CaMKII activity.\textsuperscript{114,130} More recently, consideration has been given to compartmentalization of CaMKII signaling within the cell.\textsuperscript{116,129} In general, models account for CaMKII-dependent effects on membrane ion channels and transporters important for Ca\textsuperscript{2+} cycling, including the ryanodine receptor (RyR), SERCA 2a (SR Ca\textsuperscript{2+} ATPase), phospholamban (PLB), and L-type Ca\textsuperscript{2+} channels.
Several computational studies have demonstrated the ability of CaMKII to regulate myocyte action potential, Ca$^{2+}$ transient, and even contractile force in a rate-dependent manner. Interestingly, a role for CaMKII has emerged not only in normal rate dependent behavior (e.g., AP duration adaptation and force-frequency relationships), but also in promoting cellular triggers for arrhythmias such as AP alternans and afterdepolarizations. Integrated myocyte models have also been applied to increase our understanding of spatial and temporal control of CaMKII. Interestingly, studies in this area have demonstrated the importance of affinity for Ca$^{2+}$/calmodulin in defining the differential response of CaMKII and the protein phosphatase calcineurin to the dynamic Ca$^{2+}$ transient. Furthermore, studies that incorporate both CaMKII and PKA signaling have shown how the two networks synergize for joint regulation of excitation-contraction coupling.

Modeling CaMKII in cardiac disease

CaMKII plays a critical role in regulating the substrate for both electrical and mechanical dysfunction in cardiovascular disease. Perhaps the greatest challenge for mathematical modeling of CaMKII signaling is how to ultimately link function at the molecular level to behavior at cell/tissue level in the setting of disease. Among the difficulties for modeling in this area involves distinguishing between acute and chronic effects of CaMKII activity. For example, while acute effects of CaMKII are mostly mediated by posttranslational modification of substrates, chronic CaMKII activation may facilitate large scale remodeling changes due to effects on transcription and gene expression. Mathematical modeling and computer simulation have been used to
generate new insights into molecular mechanisms for arrhythmia in several disease states, including myocardial ischemia/infarction, heart failure, and diabetes.\textsuperscript{33,117,119–121,125}

Arrhythmia mechanisms in the canine infarct border zone have been studied extensively using a mathematical modeling approach (Figure 4).\textsuperscript{33,117,133,134} The canine infarct border zone is particularly well suited to mathematical modeling approach due to the tremendous amount of available data at the molecular, cellular, and tissue level.\textsuperscript{135} Mathematical models have been used to link defects in CaMKII signaling with ion channel remodeling, abnormal Ca\textsuperscript{2+} handling, and arrhythmias in the infarct border zone. Specifically these studies have demonstrated that increased autophosphorylation and oxidation of the kinase results in increased activity that both increases Ca\textsuperscript{2+} leak from the sarcoplasmic reticulum and compromises availability of voltage-gated Na\textsuperscript{+} channels to create a favorable substrate for arrhythmias.\textsuperscript{33,117} More recently, mathematical models have been used to study the role of chronic CaMKII activation in sinus node dysfunction in the setting of heart failure and diabetes.\textsuperscript{28,119} A two-dimensional model of the intact sinus node has been applied to demonstrate that CaMKII-induced apoptosis and associated loss of sinoatrial node cells disrupts the source–sink balance between the sinoatrial node and surrounding atrial myocardium resulting in slowed pacemaking and even failure. Other studies have used mathematical modeling to determine relative importance of direct CaMKII effects and compensatory changes in gene regulation in the setting of chronic CaMKII overexpression.\textsuperscript{35} Finally, in addition to common forms of acquired disease (e.g., myocardial infarction, heart failure, diabetes), mathematical models have been used to better understand the role of CaMKII in congenital disease. A recent study used mathematical modeling to demonstrate that human variants identified in the CaMKII
phosphorylation motif of Na$_v$1.5 confer arrhythmia susceptibility by mimicking the phosphorylated channel $^{130}$ while an earlier study examined the role of CaMKII regulation of SR Ca$^{2+}$ release in increased incidence of afterdepolarizations in Timothy syndrome.$^{136}$ Together these studies demonstrate the potential for mathematical modeling and computer simulation in advancing our understanding of CaMKII biology and its role over a broad range of cardiovascular disease. However, in order to fully dissect the impact of CaMKII and other ion channel factors have on the development of cardiac disease, there is a crucial need for (1) methods relating the contribution of novel ion channel formulations with disruption of membrane excitability, and (2) access to advanced cardiac electrophysiology simulations to a wide array of researchers with pertinent datasets for modelling detailed signaling kinetics. The following two chapters will touch on methods for investigating a novel ion channel formulation to sinus node dysfunction (a pacemaking disease that is associated with and further complicates atrial fibrillation), and a graphic user interface that makes mathematical modelling of the cardiac action potential widely accessible to biomedical researchers.
Figure 3: CaMKII activation and downstream effects in cardiac myocytes
Figure 4: (A) Ventricular cell model with CaMKII targeting. (B) Detailed CaMKII Markov model. (C) CaMKII effects are rate-dependent, resulting in a different action potential morphology. (D) CaMKII activity is higher with faster pacing frequency. Modified from 123
Chapter 5: Developing methods of analysis: parameter sensitivity analysis of sinoatrial node cell dynamics

Background

Given the complexity of the models discussed in the previous two chapters, how do we assess the relative importance of changes in different ion channel kinetics on overall action potential morphology? Parameter sensitivity analysis allows researchers to perturb a number of ion channel conductances in order to assess the relative contribution of each ion channel to measured outputs such as duration, peak voltage, diastolic Ca$^{2+}$ concentration, etc. This method employs linear regression techniques, which assumes a linear relationship between output measured variables and input conductance factors, and performs a simple regression to calculate coefficients for each input and map it to each output. Here I employ parameter sensitivity analysis to better understand the link between ion channels in sinoatrial node cells and sinus node dysfunction.

The importance of normal SAN function to human health is clearly demonstrated by the strong link between SAN dysfunction and human diseases such as diabetes and atrial fibrillation. Pacemaking by the SAN is responsible for controlled regulation of heart rate and is adjusted to physiological requirements during times of exercise and stress. However, disrupted SAN activity is closely linked to cardiovascular disease and death, indicating that pacemaking is a delicate process. Regulation of SAN activity has great therapeutic potential for a rapidly aging population where SAN disease affects 1 in 600 heart patients over the age of 65. Patients with SAN dysfunction also have risk factors for developing atrial fibrillation, and treatment of SAN dysfunction can therefore alter
The only effective treatment for patients with chronic symptomatic SAN dysfunction currently used is pacemaker implantation.

The SAN poses unique challenges for analysis due to its multiscalar and heterogeneous nature. Disease can induce a shift of the primary pacemaker site, emergent behavior in the form of ectopic foci, and reduce the ability of the SAN to initiate a regular heartbeat. The link between SAN cell membrane excitability and these larger-scale phenomena has not been established. Therefore, there is a tremendous need to use both computational techniques to link ion channel regulation to tissue level function to better understand the SAN.

SAN dysfunction may result from genetic or acquired disruption of spatial and temporal regulation of ion channels in the SAN membrane. Other key contributors to SAN dysfunction include cell loss and degenerative fibrosis associated with aging. While it is known that altered ion channel activity is a major contributor to SAN dysfunction, the mechanisms regarding ion channel targeting and interactions with structural components in the SAN membrane have not been elucidated.

Spectrins are part of a complex network of intracellular proteins (ankyrins, actin) that helps maintain the structure of the cell membrane, and integrate the cytoskeleton of cardiac cells with a network of other proteins such as ion channels, protein kinases, adaptor proteins (specifically ankyrins), adhesion molecules, etc.\textsuperscript{140-144} The actin-associated polypeptide $\beta_{IV}$-spectrin is a key component of multiple ion channel macromolecular complexes in the heart, brain and other tissue.\textsuperscript{145} $\beta_{IV}$-spectrin is concentrated at the axon initial segment and Nodes of Ranvier in neurons, at the intercalated disc in cardiac myocytes, and in the cell membrane of pancreatic beta cells, indicating that it plays a
crucial role in regulating membrane excitability in these tissue types. Its protein structure is composed of an N-terminal domain, C-terminal domain, and 17 repeats which include sites that are responsible for binding to actin, ankyrin, and CaMKII.

Previous work has identified βIV-spectrin as a key regulator of normal cardiac function through spatial control of membrane ion channels, including the mechanosensitive TREK-1 K+ channel (Figure 5). TREK-1 is a member of the two-pore domain K+ channel family, a subset of K+ channels that act as background ‘leak’ channels for a wide variety of cell types. TREK-1 is widely expressed in the nervous system, smooth muscle, gastrointestinal tract, atrium, and is heterogeneously expressed in the heart. It consists of four transmembrane domains and two pore-forming domains that are subjected to post-translational modification (e.g. phosphorylation by PKA/PKC). Activation of TREK-1 current occurs through acidosis, membrane stretch, and arachidonic acid (AA). In neurons, activation of TREK-1 promotes an outward K+ current that is involved in anchoring the resting membrane potential.

In the SAN, loss of TREK-1 activity is hypothesized to be responsible for SAN dysfunction in mice lacking popeye-domaining containing proteins 1 and 2 (Popdc1,2) although a direct role for TREK-1 in SAN function remains to be determined. Loss of βIV-spectrin and the subsequent mislocalization of TREK-1 in ventricular cells in failing hearts is a possible mechanism underlying SAN dysfunction in acquired disease. While TREK-1 functions in other cardiac cell types have been revealed (e.g. ventricular, atrial), the role of TREK-1 and the βIV-spectrin/TREK-1 based complex in SAN cells has not been studied. The qv4J mouse model harbors truncation of βIV-spectrin domains 10-17 and disrupts βIV-spectrin/ TREK-1 interaction. Ventricular qv4J myocytes demonstrate disrupted membrane
excitability, disrupted localization of TREK-1, delayed action potential repolarization, and increased sinus node dysfunction events. These results underlie the need for an action potential model extended with a TREK-1 formulation to better understand βIV-spectrin knockout mouse models with cardiac disease.

Here we use computational techniques to incorporate data from previous studies to define the specific role of βIV-spectrin/TREK-1 in control of sinoatrial node membrane excitability and cardiac pacemaking. Proposed studies are based on preliminary data showing that βIV-spectrin is highly expressed in the mammalian SAN and coordinates the membrane localization of a novel two-pore mechanosensitive K+ channel, TREK-1. Furthermore, preliminary studies show that mice expressing truncated βIV-spectrin (qv4J) have defects in sinus node function and pacemaking. The central hypothesis is that βIV-spectrin controls SAN membrane excitability by coordinating the membrane localization and activity of TREK-1. Furthermore, we hypothesize that disruption of βIV-spectrin/TREK-1 interaction promotes sinus node dysfunction and abnormal pacemaking.

Results

Computer code was written in C++, compiled using Intel Composer XE 2011 for Linux, and executed on a Dell PowerEdge R515 server (Dual 6 core, 32 GB RAM running CentOS-6.2). The conductance for the TREK-1 formulation was determined based on experimental data, and fitted to a published I-V-curve. Consistent with previous reports, the TREK-1 I-V relationship shows weak outward rectification with no time-dependent gating. Channel conductance was selected to fit current measurements in WT SAN cells. The murine SAN cell model from Kharche et al. was used to represent kinetics of all other channels. Action potentials were simulated for WT and TREK-1 deficient SAN cells.
Simulated spontaneous AP firing rate, amplitude, upstroke velocity and APD were compared to experimental measurements with and without TREK-1 for model validation. We considered the TREK-1 formulation to fit validated data when the simulated firing rate, APD, and upstroke velocity was within a standard deviation of experimental values measured at baseline.

To perform parameter sensitivity analysis of the model with the new TREK-1 formulation, maximum values of ionic conductances in the Kharche SAN AP model were varied randomly over a lognormal distribution, and resulting APs were computed. Six inputs were varied for each simulation (L-type Ca^{2+} channel, T-type Ca^{2+} channel, funny current, rapid component of the delayed rectifier K\(^+\) current, transient outward K\(^+\) current, Na\(^+\)/Ca\(^{2+}\) exchanger, Na\(^+\)/K\(^+\) ATPase, and TREK-1) and six outputs were recorded (diastolic depolarization rate, maximum diastolic potential, maximum upstroke velocity, action potential amplitude, action potential duration, and firing rate). Data from 500 randomly perturbed simulations using a lognormal distribution was visualized using MATLAB (R2012a). Partial least squares regression was used to assess the importance of the six randomly varied inputs on the outputs.

Discussion

Computer simulations were performed using a mathematical model of the mammalian SAN cell AP modified to incorporate a representation of TREK-1 current based on experimental data. Elimination of TREK-1 from the model resulted in a faster diastolic depolarization rate and firing of spontaneous APs, with much smaller effects on maximum diastolic potential and AP amplitude compared with the control model, consistent with differences observed between qv\(^{aj}\) and WT animals (Figure 6). Analysis of
TREK-1 current during the spontaneous WT AP provides insight into why loss of TREK-1 has a relatively small effect on max diastolic potential (MDP). At the WT MDP of −57.6 mV, TREK-1 is about 9 times smaller than the major repolarizing current $I_{Kr}$ (0.0761 μA/μF versus 0.6549 μA/μF); however, TREK-1 increases during the spontaneous diastolic depolarization phase, whereas $I_{Kr}$ decreases (due to rapid inactivation) such that at −40 mV (close to takeoff potential), the 2 currents are much closer in amplitude (0.1628 μA/μF versus 0.4644 μA/μF).

Regression coefficients express the relative impact of changing model parameters on AP properties and show that changing the conductance of TREK-1 produced changes to AP firing, diastolic depolarization rate, AP amplitude, and MDP that closely matched expected changes measured in qv^4J SAN myocytes (Figure 6). Together, these data support the hypothesis that TREK-1 regulates SAN cell excitability (in particular, diastolic depolarization) and automaticity and that defects in TREK-1 activity promote abnormal SAN cell membrane excitability.

The impact of these studies will 1) determine the role that the interaction between βIV-spectrin and TREK-1 play in cardiac pacemaking, 2) identify how limitations to this interaction affect SAN dysfunction, and 3) have broader implications for conservation of core constituents across species and cardiac cell types. Most importantly, this study demonstrates the utility of parameter sensitivity analysis techniques in isolating the contribution of novel ion channel formulations to overall cell membrane excitability.
Figure 5: Spectrin-based complex targeting TREK-1 in the cell membrane.
Figure 6: (A) Current-voltage relationship for TREK-1. (B) Eliminating TREK-1 decreases cycle length for action potential in simulated sinus node cells. (C) TREK-1 current varies over time for WT, but not TREK-1 deficient simulations. (D) Lack of TREK-1 current alters other current expressions including $I_{to}$, (E) $I_{Kr}$, and (F) $I_{Ca,L}$. (G-J) Parameter sensitivity analysis of multiple currents to determine impact on diastolic depolarization rate, cycle length, action potential amplitude, and max diastolic potential. Modified from 154.
Chapter 6: Developing LongQt: a user interface platform for mathematical modeling and simulation

Background

Mathematical modeling has advanced our understanding of electrophysiology and provided insight into the mechanisms of cardiac disease. Studies using cellular models have demonstrated the impact of a single Na\(^+\) channel defect on cell membrane excitability and linked this defect to cardiac disease.\(^{24}\) Multi-scale modeling has also been used to investigate emergent behaviors on the multi-scale level that contribute to cardiac disease.\(^{155,156}\) Modeling can also help reveal the interplay between membrane ion channel dynamics and calcium cycling.\(^{157}\) Modeling serves as an important tool that complements experimental studies in cardiac electrophysiology and offers investigators an additional method to answer their questions about cardiac electrophysiology. In order to answer these questions, these models are developed and validated using experimental data, then feed back to inform design of a new experiment.

A large number of simulation platforms and model repositories have been developed to facilitate electrophysiology computer simulation and visualization, including CellML, the IUPS Human Physiome project, VCell, CHeart, Continuity, OpenCIMSS, and more. However, there remains a great need to bridge experimental and theoretical worlds in electrophysiology research. In simpler terms, this chapter attempts to answer the question: can mathematical modeling be made accessible without sacrificing utility? We sought to develop an interface that was cross-platform, accessible, open source, useful, and capable of performing rapid simulations for investigators.
To achieve our design goals, we employed Qt, a cross-platform application framework that employs standard C++ with extensions to facilitate development of graphic user interfaces and server applications. The resulting application, LongQt, is a cross-platform user interface that allows the user to perform multiple types of cell simulations, track changes in variables (such as calcium concentration or membrane voltage) during these simulations and automatically save the output data in a timestamped folder with the simulation’s initial conditions. This tool can generate data for parameter sensitivity analysis, run a multi-cell simulation, and help visualize the results of multiple simulations. LongQt is set apart from other electrophysiology simulation interfaces in that these simulations do not require advanced programming skills from the user. LongQt can be used for both research and teaching in order to enhance our understanding of fundamental cell activity and perform experiments that are otherwise challenging at the bench. Here we describe the features of LongQt and methods of simulation that will aid a LongQt user in performing simulation-based electrophysiology experiments.

Methods

Structure of LongQt simulation software

LongQt simulation software runs on the Qt application framework (version 5.6 or later found at https://www.qt.io/) and may be compiled to run on Mac, Windows, and Linux systems. Qt uses standard C++ programming language and can interface with programs that are built using object-oriented programming (written in languages such as C++, Python, Java, or C#). The models provided in the LongQt framework are written using object-oriented programming, meaning they identify a cell type (e.g. an atrial cell) as a class that has objects (e.g. ion channels) and implements methods that define the kind of
parameters each object contains (e.g. conductance) and how to manipulate that object (e.g. opening or closing the ion channel). LongQt accesses these cell models and provides an interface for the user to manipulate objects during simulations without manually changing any code.

LongQt’s models include two classes: one defining the cell type and one defining the simulation protocol (Figure 8). The cell class defines objects that represent components of the cell such as membrane ion channels or signaling proteins. The protocol class defines objects that impact the simulation, such as total simulation time or number of cells in the simulation. The objects defined in these classes are also called the cell model’s state variables, or the set of variables used to describe the mathematical state of a dynamic system. Different cell models employ different numbers of state variables depending on the complexity of the model. The parameters in these cell models represent data defined by each object – for example, the conductance of an ion channel. Maps, which are an ordered list of elements, are implemented in these cell models to save state variables and parameters.

Simulations are run from the LongQt simulation interface which allows the execution of electrophysiology experiments: single-cell current clamp, single-cell voltage clamp, and multi-cellular fiber (one-dimensional geometry) or grid (two-dimensional geometry). LongQt offers a wide range of utilities for simulating and analyzing model behavior, including tracking state variables, measuring state variables, and performing parameter sensitivity analysis. LongQt provides the user with a method to save simulation output, which is placed in a timestamped folder as labeled text files. Specified data may
also be visualized in the LongQt user interface at the end of the simulation (although we recommend generating publication ready figures offline using raw data files).

**General features and methods of LongQt**

LongQt provides users with multiple options for simulation experiments and analysis. In this section we review general features and methods employed by the user interface that are preserved regardless of simulation or analysis method, including: order of panels, available mathematical models, importing data files, initializing model, and importing simulations.

**Order of panels**

The sidebar on the left-hand side of the interface does not change regardless of the type of simulation the user chooses (Figure 8). The sidebar list indicates the order of panels the user will see as they complete a simulation: Choose Protocol, Choose Variables, Track Cell Values, Measure Cell Values, Perform Sensitivity Analysis, and Run Simulation. In the “Choose Protocol” panel the user will choose between three simulation modes: single-cell voltage clamp, single-cell current clamp, and a multicellular simulation. In the “Choose Variables” panel the user will choose simulation parameters for their simulation mode, such as simulation time or cell type. In the “Track Cell Values” panel the user will choose which parameters of the simulation to record over time, such as calcium concentration or sodium current. In the “Measure Cell Values” panel the user will choose whether to measure parameters of the simulation, such as the minimum calcium concentration or maximum sodium current. In the “Perform Sensitivity Analysis” panel the user will choose how to perturb a parameter (e.g. increasing sodium current conductance) to determine the sensitivity of the model to changes in that parameter. In the “Run Simulation” panel the
user will choose to run their simulation. When the simulation completes, LongQt will show a completed progress bar and indicate the timestamped directory where all output data files were saved. This directory also includes the parameters the user chose in each panel to run the simulation.

Importing data files

In order to cross-reference output data with the input simulation details, the parameters chosen for each simulation in panels [Choose Variables, Track Cell Values, Measure Cell Values, and Perform Sensitivity Analysis] are all saved in the same directory as the output data of the simulation. The parameters for one simulation are saved in four different files: simvars for parameters that control the simulation indicated in “Choose Variables” (such as simulation time, pacing frequency), pvars for parameters related to model equations indicated in “Track Cell Values” (such as intracellular potassium concentration, sodium channel gating variables), mvars for parameters that are being measured indicated in “Measure Cell Values” (such as diastolic sodium concentration, or amplitude of sarcoplasmic reticulum calcium concentration), and dvars for variables altered in parameter sensitivity analysis indicated in “Perform Sensitivity Analysis”. These four files, along with any raw output data from the simulation, are saved in a timestamped data directory for every simulation (Figure 9). If multiple trials are run at the same time, the input and output files are all saved in this directory with the trial number specified in the file names. This generates a complete log of model variables and parameters used to generate a set of results. The names of these files and the directory can be changed by selecting the “Simulation files” tab in the “Choose Variable” panel.

Initializing model
Initial conditions for state variables can be read into any simulation by selecting “readCellState” from the “Choose Variable” panel. Users may want a specific set of initial conditions (altered ion concentrations, or the steady-state values from a previous simulation) to test the effect of different pacing protocols or measure the cell’s response to perturbations after steady-state. The user may select the folder containing the initialization file (dss0_cellState.txt) in the “Simulation files” tab. This text file specifies the name and initial values of all gates and parameters, variables that are found in the “Track Variables” panel. Alternatively, the user can copy dss0_cellState.txt into the current simulation’s working directory. LongQt will run a new simulation based on this file’s specified initial conditions. If the user wants to write dss0_cellState.txt for future simulations, the user can check “writeCellState”.

Importing simulations

LongQt allows the user to import a previous simulation by clicking on “Import Simulation settings”, which implements any simvars, dvars, mvars, or pvars file from a previously generated data directory.

Simulation Modes

The user must select from three different modes to begin a simulation: 1) single-cell current clamp, which injects positive or negative current into the cell to observe the voltage response; 2) single-cell voltage clamp, which steps the cell membrane voltage to specified values to observe individual ion channel current responses, or 3) multi-cellular study (one-dimensional fiber, two-dimensional grid). Each of these simulation modes can be chosen from the initial LongQt user interface panel. Once a mode is selected the user will move on to the rest of the panels to choose parameters for that specific simulation.
mode (Choose Variables, Track Variables, Measure Cell Values, Perform Sensitivity Analysis). Menus automatically populate with abbreviated variable/parameter names, however a complete description may be found by hovering over the value in the interface. The user can set the cycle length, time to measure variables in the simulation, initial time, number, duration and value of stimulations, time of simulation, time window of writing outputs to file, and whether to pace the cell from the “Choose Variables” panel.

Current-clamp simulations with pacing continually input stimulus current to the cell for the entire simulation time and record voltage changes over time. The recommended input current and steady-state simulation variables are automatically loaded into LongQt when selecting different cell models (Figure 10).

Voltage-clamp simulations maintain a specified voltage for the entire cell, and allow the user to track the resulting current changes over time. Each time point (t1-t5) corresponds to a stimulus change (v1-v5) in membrane potential (Figure 11). Membrane voltage is held constant for these simulations and the corresponding change in current for each ion channel is recorded. Both single-cell voltage clamp and current clamp automatically output voltage and time in the visualization component of LongQt.

Multicellular simulations are run by adding rows and columns to a sample interface and selecting the cell type in the drop-down menu (Figure 12). Cells are coupled with a diffusion current and conductance between neighboring cells. The user can choose whether to stimulate and whether to measure values for each cell in the grid. This mode allows the user to simulate heterogeneity in a fiber or two-dimensional tissue and track propagation of electrical activity.
The user can run multiple trials for simulations by specifying “numtrials” greater than 1. Users that want to change parameters incrementally or randomly choose different parameters per trial can run multiple simulations (Figure 13). This can generate a large set of data for users interested in the effect of changing a single parameter or variable on the entire cell model. Due to the chosen method of solving the models (forward Euler), LongQt cannot multithread a single cell simulation. LongQt utilizes the threading utility afforded by Qt to allow for concurrent execution of multiple trials.

**Methods of Analysis**

*Track variables*

All variables selected in the tracked variables panel will be graphed in the user interface at the end of the simulation. A tab will appear in the left-hand side bar with the name of the timestamped folder with all saved output data. Up to 10 choices can be visualized in the user interface, with the upper tabs marked with variable names. All graphs can be saved from the user interface for later use.

*Measure variables*

The amplitude, cycle length, diastolic depolarization rate, derivative with respect to time, second derivative with respect to time, duration, max derivative with respect to time, minimum, and peak can be measured in the measure variables panel. Previous simulations can be imported into the graphing interface to compare against the current simulation by clicking Import Control and selecting a previous data file.

*Parameter sensitivity analysis*

LongQt provides the ability to introduce perturbations into the cell that represent genetic or environmental changes and measure the cellular response. Parameter sensitivity
analysis is performed in the Parameter Sensitivity panel, which allows the user to choose factors that then can measure the cellular response and capture changes of relevant biological information such as action potential morphology and calcium concentration changes.

Discussion

The growth in power and availability of computers has increased access to high-performance modeling and simulation, but it remains inaccessible to biomedical researchers with limited programming experience. Increased availability of mathematical models through projects such as CellML, and increased access to high computing power has increased global support of mathematical modeling. However, increasing complexity of models and programming expertise required to use these models is still a research barrier. A number of simulation platforms and model repositories have been developed to facilitate electrophysiology computer simulation and visualization, including OpenCOR, CESE Plus 2.0, Myokit and more. However, there remains a great need to bridge experimental and theoretical worlds in electrophysiology research. With the help of computational biology, researchers are able to perform studies that are difficult or even impossible in animals (especially in human). Possible applications include modeling the effect of drugs, isolating and perturbing single ion currents, testing the impact of genetic changes to ion channel function, and more. Tightly integrated experimental and modeling approaches have the potential to accelerate our discovery of basic mechanism and potential therapies for cardiac disease and arrhythmia. LongQt strives to bridge a knowledge gap for biomedical researchers by presenting an accessible, powerful, cross-platform, computationally efficient user interface that does not require extensive programming.
knowledge. In simpler terms, this work attempts to answer the question: can mathematical modeling be made accessible without sacrificing utility? Going forward, this platform was partially used in Chapter 7 to simulate a new mathematical model of the atrial action potential, and the model presented in Chapter 7 and updated SAN model in Chapter 5 are available in the updated downloadable version of *LongQt*. 
Figure 7: Structure of *LongQt* software platform. Modified from\textsuperscript{164}
Figure 8: Initial LongQt interface upon running the software platform. Users may navigate different panels on the left-hand side control panel to customize their simulation. Modified from
Figure 9: Reading, writing data and importing settings in the “Set Sim. Parameters” panel. (A) The user may define filenames where LongQt will save simulation settings (dvars, mvars, pvars, simvars), and the directory where all files will be saved. (B) The user may choose to input initial conditions from another simulation (readCellState) or output conditions to a file at the end of the simulation (writeCellState). (C) The user may set all simulation conditions to those from a previous simulation by clicking “Import Simulation settings” and selecting existing files (dvars, mvars, pvars, simvars) from another folder. When the user hits the “next” button for each panel, a “.txt” file of the items selected in the current panel is generated automatically if “Write File on Next” is checked. Modified from 164
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Chapter 7: Mathematical modeling of atrial cells with CaMKII-mediated ion channel targeting

Background

A major challenge for treatment of AF is its heterogeneous nature with highly variable pathophysiology across the clinical population. Underlying the movement toward personalized medicine, there is the understanding that the field must transcend the conventional “one size fits all” approach to medicine that depends on an average cell/organ/patient profile. At the same time, there is a need for improved methods to help researchers and clinicians account for physiological variability in their analysis.

Recently, there has been growing interest in anti-arrhythmic drug therapies targeting “late” Na\(^+\) current (\(I_{\text{Na,L}}\)), a low-amplitude current that persists throughout the duration of the action potential (AP) due to failed/incomplete voltage-dependent inactivation.\(^{166,167}\) Enhancement of \(I_{\text{Na,L}}\) is well-documented in ventricular myocytes of animal models and human patients with congenital or acquired cardiac arrhythmia.\(^ {168}\) More recently, there have been reports supporting a potential role for increased \(I_{\text{Na,L}}\) in human AF. \(I_{\text{Na,L}}\) blockers such as ranolazine or GS-458967 have been observed to suppress atrial arrhythmias.\(^ {169-176}\) Reduction of \(I_{\text{Na,L}}\) by these antiarrhythmic agents likely reduces Na\(^+\) influx during the plateau phase of the atrial action potential, leading to reduced Ca\(^{2+}\) load through Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) activity.\(^ {177}\) Previous work from our group using novel Nav1.5 channel constructs and knock-in mouse models has shown that CaMKII is an upstream regulator of \(I_{\text{Na,L}}\) in cardiac myocytes, through phosphorylation of the Serine 571 site in the DI-DII linker of Nav1.5.\(^ {130,178}\) Furthermore, the effects of late \(I_{\text{Na}}\) on enhanced arrhythmogenic activity have been attenuated by CaMKII inhibition.\(^ {179}\) While
$I_{Na,L}$ is thought to suppress triggered $Ca^{2+}$ activity, its relationship with $Ca^{2+}$ handling proteins such as the ryanodine receptor (RyR2) remains to be identified. To date, much less is known about the role of specific role of $I_{Na,L}$ in atrial arrhythmogenesis, due in part to lack of mathematical models of the atrial action potential that incorporate CaMKII-dependent signaling.

In the studies discussed here, I used a computational approach to test the hypothesis that disruption of Nav1.5 phosphorylation and a CaMKII-facilitated increase in $I_{Na,L}$ contributes to intracellular $Ca^{2+}$ accumulation and irregular atrial cell membrane excitability (including afterdepolarizations). I incorporated formulations for CaMKII-facilitation of $I_{Na,L}$, L-type $Ca^{2+}$ current ($I_{Ca,L}$), phospholamban (PLB) and the ryanodine receptor (RyR2) (Figure 14C) for the first time in an established mathematical model of the human atrial cell. I explored the downstream effects of CaMKII-mediated hyperphosphorylated/ phosphorylation ablated $I_{Na,L}$ and RyR2 on calcium homeostasis and ion accumulation. Finally, I identified proarrhythmic and antiarrhythmic effects of CaMKII-facilitated $I_{Na,L}$ and RyR2 by identifying conditions that lead to afterdepolarizations in a combination of simulated models with hyperphosphorylated or ablated CaMKII targets.

Methods

Ion channel kinetics were simulated using an existing well-validated model of the human atrial cell.18 A previously defined formulation for $I_{Na,L}$ was incorporated with a maximal conductance value selected to yield an AP duration in physiological range at baseline.117 To simulate CaMKII activation kinetics, we incorporated a mathematical description previously developed by our group that accounts for activation of CaMKII by
dynamic changes in Ca$^{2+}$/calmodulin, as well as modulation of activity by autophosphorylation and/or oxidation.\textsuperscript{117} CaMKII was assumed to catalyze a transition from non-phosphorylated to phosphorylated Nav1.5 populations, based on previously described population-based modeling techniques\textsuperscript{23,180}, where the maximal conductance of $I_{Na,L}$ was fit to experimental data from two different Scn5a knock-in mouse models: 1) mice expressing Nav1.5 with CaMKII phosphorylation site replaced by alanine (Nav1.5-S571A) to model 100% non-phosphorylated channels; and 2) mice expressing Nav1.5 with CaMKII phosphorylation site replaced by glutamic acid (Nav1.5-S571E) to model 100% phosphorylated channels (Figure 15A).\textsuperscript{178} The WT Nav1.5 model transitions between phosphorylated and non-phosphorylated fractions based on the dynamic activity of CaMKII. A similar approach was taken to model CaMKII targeting of RyR2 using experimental data from RyR2-S2814D and RyR2-S2814A mouse models (Figure 15B).\textsuperscript{181–183} CaMKII effects on PLB and $I_{Ca,L}$ were also incorporated into the model, based on their importance for normal Ca$^{2+}$ cycling. L-type Ca$^{2+}$ channel conductance was made dependent on CaMKII to produce a maximal change of 16% facilitation when pacing frequency increased from 1Hz to 2Hz, within the range of experimentally measured values.\textsuperscript{184–190} CaMKII effects on PLB were incorporated by introducing CaMKII dependence to the half maximal saturation constant for Ca$^{2+}$ binding to SERCA2a, consistent with previous experimental and modeling studies.\textsuperscript{16,191–193} While other CaMKII targets (e.g. transient-outward potassium channels) likely regulate atrial excitability in response to stress, as a first approximation we focused on Nav1.5, $I_{Ca,L}$, PLB and RyR2 to address the specific role of late current in proarrhythmic cell membrane excitability and calcium homeostasis.
Model outputs [e.g. APD$_{90}$, Ca$^{2+}$ transient peak] were determined at steady-state (500 sec of paced activity). The resulting action potential duration was validated to confirm that additional formulations added to the model did not perturb the model voltage behavior greatly from frequency-dependent experimental measurements of the action potential duration. The WT APD$_{90}$ was compared to experimental data recorded at body temperature on human atrial myocytes in sinus rhythm over a range of pacing frequencies (Figure 16).$^{194-200}$

Computer code was written in C++ 11 and compiled using Gnu Compiler Connection (gcc) for Linux. Model equations were solved numerically using the forward Euler method and a time step of 5$\mu$s. The full model is available for download and use on a cross-platform, threaded application with graphic user interface called LongQt (https://hundlab.engineering.osu.edu/research/LongQt, version 0.2).$^{164}$ The model is available for users to perform current-clamp, voltage-clamp, and two-dimensional multicellular grid simulations with all baseline input parameters pre-loaded in the LongQt user interface. All other computer simulations were performed on a Dell PowerEdge R515 server (Dual 6 core, 32 GB RAM running CentOS-6.2). A single simulation to steady-state required 9 minutes of computational time using these resources. Analysis was performed using MATLAB R2016b on a MacBook Pro with 2.5 GHz Intel Core i7 processor.

Results

*CaMKII hyperphosphorylation of I$_{Na,L}$, RyR2 alters atrial myocyte intracellular ion homeostasis*  

CaMKII regulates rate dependence of cardiac Ca$^{2+}$ handling with implications for human disease.$^{16,201}$ Previous studies have shown that CaMKII phosphorylation of Ser571
in the Nav1.5 DI-DII linker increases \( I_{\text{Na,L}} \) (Figure 14A).\textsuperscript{130,145,194} Therefore, I hypothesized that constitutive CaMKII-dependent phosphorylation of Nav1.5 would delay atrial AP repolarization and promote \( \text{Ca}^{2+} \) dysregulation. Consistent with my hypothesis, AP duration was prolonged in the simulated Nav1.5-S571E atrial myocyte compared to Nav1.5-S571A or WT (Figure 17A). At the same time, simulations predicted an increase in intracellular \( \text{Ca}^{2+} \) (cytoplasmic and SR) and CaMKII activity in Nav1.5-S571E atrial myocytes compared to WT (Figure 17B-D).

CaMKII also directly targets proteins important for normal \( \text{Ca}^{2+} \) handling (e.g. \( I_{\text{Ca,L}} \), PLB and RyR2). Based on previous work showing an important role for RyR2 phosphorylation in AF susceptibility, we hypothesized that increased \( I_{\text{Na,L}} \) promotes atrial arrhythmogenesis in part by enhancing CaMKII-dependent phosphorylation of RyR2. To test this hypothesis, we first examined the effects of constitutive (RyR2-S2814D) or ablated (RyR2-S2814A) RyR2 phosphorylation at its Ser2814 CaMKII site (Figure 14B) on atrial membrane excitability and \( \text{Ca}^{2+} \) handling. Consistent with experiments \textsuperscript{183}, my simulations predicted a decrease in SR \( \text{Ca}^{2+} \) in RyR2-S2814D myocytes compared to WT or RyR2-S2814A (Figure 17H), without any detectable difference in the AP (Figure 17E), \( \text{Ca}^{2+} \) transient amplitude (slight, offsetting decrease in both peak and maximum diastolic \([\text{Ca}^{2+}]_i\)) (Figure 17F), or CaMKII activity (slight decrease in peak) (Figure 17G).

To determine the role of CaMKII in regulation of intracellular ion homeostasis in atrial myocytes, I simulated constitutive or inhibited CaMKII phosphorylation on RyR2 and \( I_{\text{Na,L}} \) over a range of pacing frequencies. The Nav1.5-S571E model showed a small (<10%) increase in \([\text{Na}^+]\) compared to Nav1.5-S571A and WT, which resulted in a substantial increase in \([\text{Ca}^{2+}]_i\) and CaMKII activity, especially at pacing frequencies less
than 1Hz (Figure 18). In contrast, RyR2-S2814D yielded a decrease in \([\text{Ca}^{2+}]_i\) and CaMKII activity compared to simulated WT (Figure 18). These simulation results indicate that small changes in \([\text{Na}^+]_i\) induced by CaMKII-dependent hyperphosphorylation of \(\text{Na}_V\)1.5 and increased \(I_{\text{Na,L}}\) have strong effects on \(\text{Ca}^{2+}\) handling and CaMKII activity in atrial myocytes. Furthermore, the model predicts that constitutive phosphorylation of \(\text{Na}_V\)1.5 alone has a greater impact on \([\text{Ca}^{2+}]_i\) and CaMKII then constitutive phosphorylation of RyR2 alone. Together these data indicate that a CaMKII-dependent increase in \(I_{\text{Na,L}}\) is sufficient to produce defects in AP repolarization and \(\text{Ca}^{2+}\) handling and likely resides upstream of RyR2 in promoting atrial arrhythmogenesis. Furthermore, our simulations predict that atrial \(I_{\text{Na,L}}\) and CaMKII reside in a feedback loop linked to calcium homeostasis 202.

**CaMKII hyperphosphorylation of \(I_{\text{Na,L}}\), RyR2 affects RyR2 function and \(\text{Ca}^{2+}\) efflux**

Increased diastolic \(\text{Ca}^{2+}\) leak from the SR through phosphorylated ryanodine receptors plays a role in triggering and maintaining atrial fibrillation. Based on our results showing altered \(\text{Ca}^{2+}\) homeostasis due to hyperphosphorylated \(\text{Na}_V\)1.5 or RyR2, we sought to more fully characterize CaMKII effects on RyR2 function and SR \(\text{Ca}^{2+}\) release. As expected, the RyR2-S2814D showed a dramatic increase in phosphorylated RyR2 (RyR2-P) with respect to WT, while the \(\text{Na}_V\)1.5-S571E had only a small effect on RyR2-P compared to WT (Figure 19A). Interestingly, CaMKII-dependent increase in \(I_{\text{Na,L}}\) produced a dramatic increase in RyR2 open probability compared to WT, especially at pacing frequencies less than 1 Hz. Surprisingly, \(\text{Na}_V\)1.5 phosphorylation increased RyR2 open probability even beyond that observed in RyR2-S2814D, despite the negligible effect on RyR2-P, reflecting the greater SR \(\text{Ca}^{2+}\) load in \(\text{Na}_V\)1.5-S571E compared to RyR2-
Furthermore, I observed that CaMKII hyperphosphorylation of RyR2 in the simulated RyR2-S2814D decreased $[\text{Ca}^{2+}]_{\text{SR}}$ compared to WT, while CaMKII hyperphosphorylation of Nav1.5 in the simulated Nav1.5-S571E had the opposite effect (Figure 19C). These data indicate that increasing Ca$^{2+}$ load (downstream of increased [Na$^+$]) has a greater overall effect on RyR2 open probability than RyR2 phosphorylation alone.

At steady-state, my simulations predict a net decrease in intracellular Ca$^{2+}$ with constitutive RyR2 phosphorylation but significant increase with Nav1.5 phosphorylation. To test the hypothesis that RyR2 phosphorylation results in net efflux of Ca$^{2+}$ from the cell contributing to both lower $[\text{Ca}^{2+}]_{\text{SR}}$ and $[\text{Ca}^{2+}]_{\text{i}}$ concentration, I explored the behavior of the Na-Ca$^{2+}$ exchanger ($I_{\text{NCX}}$) both transiently at the onset of pacing and at steady-state. At steady-state, Nav1.5-S571E had an elevated forward mode activity reflecting the accumulation of $[\text{Ca}^{2+}]_{\text{i}}$ at steady state compared to other models (Figure 20A). In contrast, the RyR2-S2814D had a decreased forward mode activity at steady-state reflecting a lower $[\text{Ca}^{2+}]_{\text{i}}$ and SR Ca$^{2+}$ release (Figure 20A). Furthermore, the integral of $I_{\text{NCX}}$ in the Nav1.5-S571E is greater than the other simulated models (Figure 20B). This steady-state behavior is better understood by taking into account the behavior of $I_{\text{NCX}}$ transiently during onset of pacing. At the onset of pacing, RyR2-S2814D (Figure 20C) showed a large increase in forward mode $I_{\text{NCX}}$ compared to WT until $[\text{Ca}^{2+}]_{\text{i}}$ decreased below that in the WT (Figure 20D). In contrast, NaV1.5-S571E demonstrated decreased forward mode $I_{\text{NCX}}$ at the onset of pacing and a more gradual increase in $[\text{Ca}^{2+}]_{\text{i}}$ that exceeded simulated WT at the ninth action potential. These results demonstrate that RyR2 phosphorylation increases Ca$^{2+}$
release transiently but ultimately forward mode $I_{\text{NCX}}$ produces a steady-state condition where $[\text{Ca}^{2+}]$ (cytosolic and SR) decreases.

**CaMKII-facilitated $I_{\text{Na,L}}$, RyR2 increases afterdepolarizations**

I next tested the hypothesis that increased $I_{\text{Na,L}}$ promotes arrhythmogenic atrial afterdepolarizations, in part, by increasing CaMKII activity and promoting downstream hyperphosphorylation of other CaMKII targets (e.g. RyR2). To test my hypothesis, I first assessed presence of afterdepolarizations in WT, $\text{Nav1.5-S571E/A}$, $\text{RyR2-S2814D/A}$ during steady-state pacing and/or with a pause following steady-state pacing (1-3.3 Hz, data not shown). None of the models displayed afterdepolarizations under basal conditions (absence of simulated stress condition). Therefore, I next determined whether $\text{Nav1.5}/\text{RyR2}$ phosphorylation altered susceptibility to afterdepolarizations under conditions of acute $\beta$-adrenergic receptor (BAR) stimulation. I simulated the effect of $\beta$-adrenergic stimulation according to a previously published approach (effects on $I_{\text{Ks}}$, $I_{\text{Kur}}$, troponin I, SERCA2a, L-type $\text{Ca}^{2+}$ channels, RyR2) and determined the response of model at steady-state with 1 Hz pacing. In addition to altering $\text{Nav1.5}$ or RyR2 phosphorylation independently (Figure 21), I also examined the combined effects of $\text{Nav1.5-S571}$ and RyR2-S2814 phosphorylation state. Additional models explored in these simulations included: $\text{Nav1.5-S571E x RyR2-S2814D}$, $\text{Nav1.5-S571A x RyR2-S2814A}$, $\text{Nav1.5-S571E x RyR2-S2814A}$, $\text{Nav1.5-S571A x RyR2-S2814D}$ – which respectively simulates both hyperphosphorylated CaMKII targets, both phosphorylation ablated CaMKII targets, or mixed hyper- and phosphorylation-ablated targets. Afterdepolarizations were not observed in any model for pacing frequencies above 1.33 Hz. However, repolarization defects (early afterdepolarizations with or without alternans)
emerged at slower pacing in WT and mutant models in the presence of isoproterenol (Figure 21D). Notably, early afterdepolarizations were apparent over a wider range of pacing frequencies in the Na\textsubscript{V}1.5-S571E model compared to WT or RyR2-S2814D. Furthermore, ablation of Na\textsubscript{V}1.5-S571 (Na\textsubscript{V}1.5-S571A, Na\textsubscript{V}1.5-S571A x RyR2-S2814A and Na\textsubscript{V}1.5-S571A x RyR2-S2814D) was most protective against afterdepolarization formation, while constitutive phosphorylation of both sites (Na\textsubscript{V}1.5-S571E x RyR2-S2814D, Figure 21D), was the most pro-arrhythmic model. These results indicate that increased \( I_{Na,L} \) plays a critical role in mediating pro-arrhythmic effects of CaMKII under conditions of acute \( \beta \)-adrenergic receptor stimulation. Surprisingly, the model predicts that pro-arrhythmic effects of RyR2 phosphorylation depend more heavily on subsequent phosphorylation of Na\textsubscript{V}1.5 than vice versa.

**Parameter Sensitivity Analysis**

In order to assess the biological relevance of the added formulations in this model, parameter sensitivity analysis of the model with and without \( \beta \)-adrenergic stimulation was conducted (Figure 22). We altered 12 main ion channel factors (\( I_{Ca,L} \), \( I_{K1} \), \( I_{Kr} \), \( I_{Ks} \), \( I_{kur} \), \( I_{Na} \), \( I_{NCX} \), \( I_{NaK} \), \( I_{Na,L} \), \( I_{to} \), SR Ca Release flux, and SERCA2a flux) randomly for each simulation (479 baseline simulations, 379 BAR simulations) and measured multiple outputs for each simulation (APD90, [Ca\textsuperscript{2+}] peak, SR [Ca\textsuperscript{2+}] peak, diastolic [Na\textsuperscript{+}], diastolic [Ca\textsuperscript{2+}], and peak CaMKII. Simulations included in the final analysis had no alternans, a resting membrane potential below -70mV, and an action potential duration below 1000ms. Parameter sensitivity analysis indicates that the impact of late Na\textsuperscript{+} current is increased under BAR stimulation, and increased \( I_{Na,L} \) increased each of the outputs. Although late Na\textsuperscript{+} current was increased under BAR stimulation, it was not the major contributor to the observed
outputs. The impact of $I_{Na,L}$, $I_{NCX}$, and RyR2 varied depending on the output – for example, SR Ca$^{2+}$ release and SERCA2a flux have a high impact on diastolic Ca$^{2+}$ at baseline, but this effect was decreased under BAR stimulation. Conversely, $I_{NCX}$ had a higher impact on APD90 under BAR stimulation than at baseline.

Discussion

In these studies, I used mathematical modeling to explore the role of CaMKII-dependent regulation of $I_{Na,L}$ and Ca$^{2+}$ cycling in atrial myocytes. My simulations lead to a number of new findings, including: (1) CaMKII-dependent increase in $I_{Na,L}$ promotes significant accumulation of intracellular Ca$^{2+}$ (cytosolic and SR) in atrial myocytes; 2) $I_{Na,L}$-induced Ca$^{2+}$ accumulation, in turns enhances atrial activity and phosphorylation of downstream targets (e.g. RyR2); (3) CaMKII phosphorylation of atrial Na$\text{v}$,1.5 (and subsequent increase in $I_{Na,L}$) alone produces greater intracellular Ca$^{2+}$ accumulation, CaMKII activation, and increase in RyR2 open probability compared to RyR2 phosphorylation alone; and (4) Increased $I_{Na,L}$ promotes proarrhythmic behavior under conditions of acute β-adrenergic receptor stimulation in atrial myocytes. While previous studies have examined the role of CaMKII as a molecular driver for calcium homeostasis and cardiac dysfunction, this effort represents a novel approach to dissect the complex effects of CaMKII on downstream targets in atrial cells.

Experimental studies on the transgenic RyR2-S2814D mouse indicate that increased phosphorylation of Ser2814 by CaMKII correlates with increased diastolic SR Ca$^{2+}$ release, and decreased SR Ca$^{2+}$ load.$^{182,183,206,207}$ Furthermore, increased $I_{Na,L}$ in the Na$\text{v}$,1.5-S571E or through application of the $I_{Na,L}$ enhancer ATX-II promotes abnormal [Ca$^{2+}$]$_i$ handling and SR Ca$^{2+}$ leak.$^{178,208}$ In our simulations, RyR2-S2814D showed a
transient elevation in SR Ca\(^{2+}\) release (due to higher RyR2 open probability), which resulted in acute activation of forward-mode \(I_{\text{NCX}}\) that ultimately depleted \([\text{Ca}^{2+}]_i\) (Figures 19, 20). In contrast, simulated Nav1.5-S571E over a range of frequencies showed greater intracellular ion accumulation, and greater SR Ca\(^{2+}\) release despite activation of forward-mode \(I_{\text{NCX}}\) at steady-state (Figure 18, 20). CaMKII-activated \(I_{\text{Na,L}}\) and subsequent Ca\(^{2+}\) accumulation also enhanced phosphorylation of downstream targets (e.g. RyR2), although not to the same extent as the RyR2-S2814D model. These results suggest that while phosphorylation of RyR2 promotes abnormal atrial membrane excitability and Ca\(^{2+}\) handling, net influx of Na\(^{+}\) and/or Ca\(^{2+}\) across the atrial cell membrane (e.g. via \(I_{\text{Na,L}}\)) is required for maximal pro-arrhythmia conditions. These pro-arrhythmic conditions occur due not only to increased phosphorylation and ion accumulation, but also to disrupted control over ion flux across the plasma membrane, where reverse-mode \(I_{\text{NCX}}\) is increased but unable to reduce ion accumulation. Previous studies confirm higher activity of \(I_{\text{NCX}}\) in right atrial cardiomyocytes of patients with AF than in SR.\(^{209}\) In ventricular myocytes, increased \(I_{\text{Na,L}}\) and reverse-mode \(I_{\text{NCX}}\) through application of ouabain was mitigated by CaMKII inhibitor KN-93.\(^{210}\) These experimental data support model predictions that \(I_{\text{NCX}}\) is a critical node in a \(I_{\text{Na,L}}$/CaMKII/RyR2 feedback loop that promotes arrhythmogenesis in AF.

Impaired local regulation of RyR2 in transgenic mice increases AF susceptibility and atrial ectopy.\(^{182,211}\) Atrial fibrillation patients also show increased \(I_{\text{Na,L}}\) compared to patients in sinus rhythm.\(^{166}\) These data indicate that impaired regulation of downstream targets of CaMKII contributes to atrial fibrillation, but do not favor one pro-arrhythmic downstream pathway. Is an atrial cell more susceptible to AF through certain downstream
effects of increased CaMKII over others? While we did not observe pro-arrhythmic afterdepolarization activity in our models at baseline, β-adrenergic stimulation induced prominent repolarization defects including alternans and afterdepolarizations. Surprisingly, our model predicted that ablation of Na\textsubscript{v}1.5 phosphorylation mitigated the pro-arrhythmic effects of RyR2-S2814D, suggesting that RyR2 hyperphosphorylation promotes arrhythmia in part through feedback on CaMKII and subsequent hyperphosphorylation of other CaMKII targets (Na\textsubscript{v}1.5). The combined effects of increased \( I_{Na,L} \) and phosphorylated RyR2 (in the Na\textsubscript{v}1.5-S571E x RyR2-S2814D model) increased susceptibility to afterdepolarizations beyond increased \( I_{Na,L} \) or phosphorylated RyR2 alone (Figure 21D). Together these data indicate that hyperphosphorylation of CaMKII increases susceptibility to atrial fibrillation and abnormal membrane dynamics, and ablation of the late sodium current CaMKII downstream target protects against these effects (Figure 21D).

We observed alternans in every simulated model under BAR stimulation, but previous studies have found that β-adrenergic receptor stimulation can protect against alternans in cat atrial myocytes.\textsuperscript{212} However, increased susceptibility to AF in rabbit hearts with myocardial infarction under β-adrenergic stimulation correlated with higher APD alternans and spontaneous depolarizations, and β-blockers are a well-known atrial fibrillation therapy.\textsuperscript{213,214} Furthermore, our group has shown that Ca\textsuperscript{2+} transient recordings with isoproterenol application increase frequency of spontaneous Ca\textsuperscript{2+} release in the Na\textsubscript{v}1.5-S571E compared to WT.\textsuperscript{178} Together with our simulations, these data point to
disruption of CaMKII downstream targets through BAR stimulation as a precursor for atrial alternans.

Surprisingly, we observed that although a model may have an anti-arrhythmic action potential duration – such as the Nav1.5-S571E (Figure 17) – impaired regulation of CaMKII downstream targets and calcium cycling contributed overall to proarrhythmic behavior. This suggests that anti-arrhythmic drug agents used in treatment of AF are not necessarily successful due to their mitigation of the action potential duration, but actually due to their anti-arrhythmic downstream effect on ion accumulation and calcium cycling. Computational modeling is therefore an exciting tool to identify novel potential anti-arrhythmic drug agents by simultaneously assessing their impact on APD90 and calcium cycling behavior.

The recent development of organ-level cardiac simulation- complete with personalized reconstruction of patient tissue based on MRI scans- is an exciting track of computational research. Whole-heart simulations have often been used to study cardiac electrophysiology variables that affect cardiac action potential propagation in the context of disease—such as (but not limited to) spatial distribution of cell types, disease states (e.g. hyperkalemia, acidosis, hypoxia, border zone size), cardiac size, fibrosis, cellular coupling, complex tissue geometry and microstructures.$^{215-217}$ These organ-level simulations would benefit from detailed patient-specific cellular electrophysiology models, especially in the context of atrial fibrillation where electrical and structural remodeling go hand-in-hand. Our simulations suggest that proarrhythmic atrial cell dynamics and susceptibility to afterdepolarizations can be sustained through different downstream effects of increased CaMKII activity. Could changing the downstream effects of CaMKII phosphorylation
achieve different long-term atrial fibrillation structural remodeling, or cause different emergent AF triggers? The updated formulations incorporated into this model for CaMKII-targeting of $I_{\text{Na,L}}$, PLB, $I_{\text{Ca,L}}$, and RyR2 are compatible with other atrial cell models used in organ-level simulations\textsuperscript{29,218} and can help elucidate the impact of CaMKII on organ-level AF properties such as rotor stabilization, conduction block, etc.

Finally, these findings are particularly exciting and poised to inform organ-level simulations as well as other research directions due to the availability of transgenic mouse models RyR2-S2814D, RyR2-S2814A, Nav1.5-S571E, and Nav1.5-S571A to test model predictions. These models are clinically relevant to existing atrial fibrillation studies due to the multiple observations of disrupted late Na\textsuperscript{+} current behavior, or SR Ca\textsuperscript{2+} leak in cardiac arrhythmias. Furthermore, CaMKII is upregulated in AF and multiple studies point towards RyR2-S2814 phosphorylation or Nav1.5-S571 phosphorylation as underlying mechanisms for cardiac disease.\textsuperscript{178,219–221}
Figure 14: (A) Structure of voltage-gated Na\(^+\) channel alpha subunit Na\(_{v}\)1.5. Ser571 in DI-DII loop is critically important for CaMKII-dependent regulation of \(I_{Na,L}\). Previous electrophysiology studies on this interaction have been performed on mouse models with a phosphomimetic Ser571 site (Na\(_{v}\)1.5-S571E) and phosphorylation site ablated (Na\(_{v}\)1.5-S571A) \(^{178}\). (B) Schematic of ryanodine receptor (RyR2), with labelled CaMKII phosphorylation site S2814. The RyR2-S2814D mutation is a phosphomimetic alteration to the ryanodine receptor, and the RyR2-S2814A mutation ablates the phosphorylation site. (C) Schematic of key plasma membrane ion currents and sarcoplasmic reticulum protein channels in the Grandi atrial cell model, including: L-type Ca\(^{2+}\) current (\(I_{Ca,L}\)), fast Na\(^+\) current (\(I_{Na}\)), late Na\(^+\) current (\(I_{Na,L}\)), ultrarapid delayed rectifier K\(^+\) current (\(I_{Kur}\)), slow delayed rectifier K\(^+\) current (\(I_{Ks}\)), rapid delayed rectifier K\(^+\) current (\(I_{Kr}\)), time-independent K\(^+\) current (\(I_{Kt}\)), 4AP-sensitive transient outward K\(^+\) current (\(I_{to}\)), Ca\(^{2+}\) pump current (\(I_{PCa}\)), Ca\(^{2+}\) activated Cl\(^-\) current (\(I_{CaCl}\)), background Ca\(^{2+}\) current (\(I_{Cab}\)) background Na\(^+\) current (\(I_{Na,b}\)), background Cl\(^-\) current (\(I_{Clb}\)), Na\(^+\)-K\(^+\) pump ATP-ase (\(I_{NaK}\)), Na\(^+\)-Ca\(^{2+}\) exchanger (\(I_{NCX}\)), sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2a). CaMKII targeting of RyR2, SERCA2a, \(I_{Ca,L}\) and \(I_{Na,L}\) is included in this updated model. Modified from \(^{222}\)
Figure 15: (A) Experimentally measured (21) and simulated late sodium current activity (expressed as ratio of levels in Na\textsubscript{V}1.5-S571E to Na\textsubscript{V}1.5-S571A). (B) Experimentally measured (73, 77) and simulated [Ca\textsuperscript{2+}]\textsubscript{i} amplitude (ratio relative to WT) in RyR2-S2814D and RyR2-S2814A models. Simulations used Grandi human atrial model with modifications as described in Methods. Modified from 222
Figure 16: Simulated (black), and experimentally measured WT action potential duration at 90% repolarization (APD90) based on pacing frequency. One set of experimental data is based on patch-clamping experiments conducted at 36°C, with 1.8mM Ca²⁺ concentration, and reported an APD90 value of 317ms at 1Hz (Ford 2016, triangles). The other set of experimental data is based on monophasic action potentials obtained from intra-cardiac diagnostic catheters. Modified from
Figure 17: Steady state transients at 1Hz pacing frequency for simulated mouse models. Top row (A-D) shows WT (solid black), Nav1.5-S571A (solid grey), Nav1.5-S571E (dotted grey). Bottom row (E-H) shows WT (solid black), RyR2-S2814A (solid grey), RyR2-S2814D (dotted grey). (A, E) Membrane voltage (B, F) intracellular Ca$^{2+}$ concentration (C, G) Fraction of active CaMKII, and (D, H) sarcoplasmic reticulum Ca$^{2+}$ concentration. Modified from 222.
Figure 18: Left column (A, C, E) shows steady-state values over a range of pacing frequencies in simulated WT (black circle), Na\textsubscript{v}1.5-S571A (grey square), and Na\textsubscript{v}1.5-S571E (grey triangle). Similarly, the right column (B, D, F) shows simulated WT (black circle), Na\textsubscript{v}1.5-S571A (grey square), and Na\textsubscript{v}1.5-S571E (grey triangle). (A, B) Maximum intracellular Ca\textsuperscript{2+}, (C, D) diastolic Na\textsuperscript{+}, (E, F) Maximum fraction of active CaMKII. Modified from 222
Figure 19: (A) Maximum phosphorylation of RyR2, (B) maximum RyR2 open probability, and (C) calcium concentration in the sarcoplasmic reticulum. All simulations ran to steady-state over a range of frequencies for WT (black circle), NaV1.5-S571E (grey diamond), and RyR2-S2814D (grey triangle) simulated mouse models. Modified from 222.
Figure 20: (A) $I_{\text{NCX}}$ over time at steady-state for simulated WT (solid black), Na$_{\text{v}}$1.5-S571A (solid grey), Na$_{\text{v}}$1.5-S571E (dotted grey), (B) RyR2-S2814A (solid grey), RyR2-S2814D (dotted grey). (C) minimum $I_{\text{NCX}}$ during AP from initiation of simulation for WT (black circle), Na$_{\text{v}}$1.5-S571E (grey triangle), and RyR2-S2814D (grey diamond) and (D) maximum $[\text{Ca}^{2+}]_i$ concentration per AP at the initiation of simulation for WT (black circle), Na$_{\text{v}}$1.5-S571E (grey triangle), and RyR2-S2814D (grey diamond). Modified from 222.
Figure 21: Simulated steady-state APs for three different pacing frequencies in simulated WT atrial myocytes. (A) No events, (B) early afterdepolarizations (EAD’s), and (C) alternans and EAD’s are observed. (D) Summary of alternans and EAD’s (black), EAD’s (grey), and no events (white) for different pacing frequencies (0.51 – 1.43 Hz). Simulated models shown include the previously introduced WT; NaV1.5-S571E; NaV1.5-S571A; RyR2-S2814A; and RyR2-S2814D. Additional models explored in these simulations include: NaV1.5-S571E x RyR2-S2814D, NaV1.5-S571A x RyR2-S2814A, NaV1.5-S571E x RyR2-S2814A, NaV1.5-S571A x RyR2-S2814D – which respectively simulates both hyperphosphorylated CaMKII targets, both phosphorylation ablated CaMKII targets, or mixed hyper- and phosphorylation-ablated targets.

Modified from 222
Figure 22: Regression coefficients showing how changes in model parameters affect ion homeostasis and CaMKII activity in baseline WT simulated cells (A-D) and in the presence of β-adrenergic agonist isoproterenol (E-H). Parameter sensitivities of ion channel conductance parameters affect (A,E) maximum diastolic \([\text{Na}^+]\), (B,F) maximum \([\text{Ca}^{2+}]_\text{i}\), (C,G) maximum \([\text{Ca}^{2+}]_\text{SR}\), and (D,H) maximum fraction of activated CaMKI subunits. Modified from 222
Chapter 8: Discussion

“I think that when we know that we actually do live in uncertainty, then we ought to admit it; it is of great value to realize that we do not know the answers to different questions. This attitude of mind – this attitude of uncertainty- is vital to the scientist, and it is this attitude of mind which the student must first acquire.”

Richard Feynman

Atrial fibrillation remains to be a vastly complex disease, and to date current therapies fall short of delivering individualized, consistently successful treatments to AF patients. A better understanding of the single-cell electrophysiology behind AF will help lead to an enhanced comprehension of the electrical AF substrate and tailored therapies for AF patients. My studies have used mathematical modeling to investigate mechanisms of atrial fibrillation. In Chapters 5 and 6 I discussed two methods (a graphic user interface and parameter sensitivity analysis) related to advanced cardiac electrophysiology simulations, so that researchers can ultimately pick apart a complex biological system to understand how deficiencies in a specific part of the cell can contribute to atrial fibrillation. Novel aspects of the modeling efforts presented in this dissertation include 1) addition of a validated CaMKII formulation to the human atrial cell model, 2) addition of a novel TREK-1 formulation to a sinus node cell model and the application of parameter sensitivity analysis to study sinus node dysfunction, and 3) application of both parameter sensitivity analysis and a novel cross-platform software to investigate mechanisms of atrial fibrillation based on disrupted ion channel targeting.

These application of the methods introduced in Chapters 5 and 6 to understand the impact of CaMKII signaling in atrial fibrillation provided new insight into the impact of
individual ion channels on overall atrial cell membrane excitability – for example, how increased late sodium current promotes Ca\(^{2+}\) overload via \(I_{\text{NCX}}\) activity, beyond that of increased CaMKII –mediated RyR2 phosphorylation in atrial cells alone. Mathematical modelling of the atrial cell allowed an in-depth analysis of positive- and negative- feedback loops that would otherwise be difficult to discern through experimental methods, specifically with CaMKII-mediated increased \(I_{\text{Na,L}}\) feeding back to increase CaMKII activity. Furthermore, although the action potential duration increased in the model of CaMKII-mediated increased \(I_{\text{Na,L}}\), this did not translate to protective dynamics against early afterdepolarizations and alternans. This illustrates the utility of mathematical modeling—although the dominant frequency and rotor drivers for atrial fibrillation have been observed at the shortest cycle length, the models most susceptible to proarrhythmic dynamics in my studies are potentially drivers for atrial fibrillation due to calcium overload.

Overall, the modelling methods used to explore the mechanism of CaMKII signaling in atrial fibrillation leave significant questions to expand upon. What are the adaptive and maladaptive aspects of CaMKII signaling in atrial fibrillation? How does adrenergic stress and downstream CaMKII activation play a role in the interplay between the ganglionated plexi and pulmonary veins, and maintenance of the AF substrate? If CaMKII is a node for disrupted signaling that can trigger or sustain AF, is there a way to target upstream or downstream signaling pathways in order to better treat chronic AF patients? Modeling tools, methods and new models presented in this dissertation will be of great use going forward for studying the molecular mechanisms of atrial fibrillation.

Limitations
While the model utilized in the studies outlined in Chapter 7 incorporates components important for the CaMKII-dependent behavior under investigation, it is nonetheless a simplification of the physiological system. Namely, CaMKII targets a number of membrane ion channels not included in this analysis, including $I_{to}$ and $I_{K1}$. Furthermore, CaMKII has been demonstrated to regulate not just $I_{Na,L}$ but also $I_{Na}$ by reducing channel availability. While inclusion of these and other CaMKII targets could potentially yield further insight into atrial arrhythmia mechanisms, our sensitivity analysis indicates that these targets are unlikely to contribute to the ion homeostasis defects observed in AF. In the instance of $I_{to}$, for example, the model predicts a negative relationship between channel conductance and intracellular Na$^+$, Ca$^{2+}$ and CaMKII, meaning that these values would be expected to decrease in response to an increase in $I_{to}$ (as observed with CaMKII activation). For $I_{K1}$ and $I_{Na}$, the regression coefficients are relatively small and in the case of $I_{Na}$ work against ion accumulation with CaMKII activation. At the same time, regression analysis of the model highlights the fact that precise values of certain model parameters may influence absolute values for ion concentrations and CaMKII activity in the model. For example, we were surprised to find that intracellular Na$^+$, Ca$^{2+}$ and CaMKII were relatively sensitive to $I_{Kur}$, as well as $I_{NaK}$ (less surprising), indicating that small discrepancies in values of conductances/flux rates for these elements would be expected to have a relatively large impact on model predictions.

It is also important to note that electrical and structural remodeling changes characteristic of persistent AF are not incorporated into this model. In order to better simulate the electrical and structural remodeling of AF and understand the effect of...
CaMKII mediation of downstream targets on cardiac disease, this model may be incorporated into organ-scale modelling. However, organ-scale simulations require numerical stability on the order of 10-20 µs. This model can be solved with a larger timestep at 10µs but there is always a risk when increasing the timestep in losing the accuracy of AP upstroke and peak Na⁺ current. On the cellular level the user would still see the existence of early afterdepolarizations with this timestep. For organ-scale simulations that are more interested in tissue-level electrophysiology events such as rotor dynamics and electrograms, this may be a minimal risk. Undoubtedly, it will be interesting to study the interaction between acute effects studied here and changes in tissue structure. Finally, atrial myocyte cell excitability is part of a complex network of signaling mechanisms and incorporation of CaMKII signaling with protein kinase A (PKA) (to address cross-talk and individual effects) would strengthen the prediction of CaMKII effects in this study.

Mathematical modeling is largely dependent on the availability and accuracy of experimental data for parameterization of differential equations. When validating the model with human atrial action potential myocyte data, I strove to select datasets that did had passed the half-life of antiarrhythmic drugs in the bloodstream before atrial myocytes were obtained for the study. Tissue samples for atrial cell patch clamping experiments are typically collected from patients undergoing cardiac surgery, and the isolated cell electrophysiology may be affected. This provides a limitation to the accuracy of human atrial cell models, and without a detailed understanding of how electrical and structural substrates change for different patient populations under specific treatments, this may continue to be a limitation to mathematical modeling studies.
Future Directions

It is possible to incorporate the modules presented in Chapter 7 into other atrial action potential models, provided that intracellular calcium, sarcoplasmic reticulum calcium, and intracellular sodium concentrations change in the model over the course of the action potential, and the model already describes Ca$^{2+}$ uptake into the sarcoplasmic reticulum. Incorporating these modules or relationships into a new model may require some adjustments based on the peak and amplitude of the intracellular Ca$^{2+}$ and Na$^+$ concentrations in the new model. For example, the Grandi model incorporates an SR Ca$^{2+}$ handling framework that solves SR Ca$^{2+}$ uptake, leak, and release (variable JSerca, JSRleak, and JSRCarel in the appendix respectively) differently from atrial action potential models such as the Courtemanche model. These SR Ca$^{2+}$ fluxes in the updated model depend on CaMKII targeting of rate constants in a 4-state Markov model of the ryanodine receptor, which may not be present in other atrial cell models. In order to incorporate the model presented in Chapter 7 into a higher-dimensional model, I recommend updating the Ca$^{2+}$ handling framework describing SR Ca$^{2+}$ fluxes, and incorporating a 4-state description of ryanodine receptor activity (resting/ closed, open, inactivated, and resting inactivated) in addition to incorporating CaMKII targeting of downstream targets as presented here. Otherwise the modules presented here are compatible with other human atrial action potential models.

There is a growing appreciation for computational tools in an effort to understand physiological variability in arrhythmia susceptibility and response to therapy. It will be interesting for future studies of computational modeling of cardiac electrophysiology to incorporate patient-specific ion channel expression to better understand how ion channel
expression changes affect pharmacological, surgical, or catheter ablation interventions for AF. In order to develop therapies that are specifically tailored to different patient populations, we need to develop appropriate (not necessarily more detailed, but with key signaling targets) action potential models to describe action potential behavior for different patient populations. For example, detailed electrophysiology models that take into account the difference between paroxysmal and persistent AF would be highly beneficial. A convergence of these models with other technologies – such as virtual reality simulators that model beating cardiac tissue so that physicians can practice guiding catheters through the human body, or finite element models for development of individualized cardiac medical devices- would grow our understanding of atrial fibrillation and the best methods of treating patients. Overall, there is a great deal of work to be done to bridge the gap between data that biomedical researchers have obtained with transgenic models at the single-cell level, and human cardiac disease. It is exciting to consider the potential for mathematical modeling to tackle these and other questions with implications for understanding how to better treat cardiac arrhythmias.
Appendix A: Model Equations Added to the Kurata Sinoatrial Node Model

This section contains the model equations added to describe TREK-1 and Cav1.3 activity in the sinoatrial node. All other equations come from original publications.

**TREK-1 module**

\[ g_k = .053816 \]

\[ E_k = \frac{R \times T \log (K_o)}{F} \]

\[ a_a = \frac{1}{1 + e^{-\frac{V-65}{52}}} \]

\[ I_{Kaa} = g_k \times a_a \times (V - E_k) \]

**Cav1.3 module**

\[ g_{CaL} = 0.58 \]

\[ g_{Cal13} = .0375 \]

\[ kmfca = .00035 \]

\[ alpha_{fca} = .021 \]

\[ beta_{fca} = 60 \]

\[ e_{cal} = 45 \]

\[ d_{inf} = \frac{1}{1 + e^{-\frac{(V+14.1)}{6}}} \]

\[ alpha_{d} = \frac{-0.02839 \times (V + 35)}{e^{-\frac{(V+35)}{25}} - 1} = \frac{0.0849 \times V}{e^{V.808} - 1} \]

\[ beta_{d} = \frac{0.01143 \times (V - 5)}{e^{-\frac{(V-5)}{25}} - 1} \]

\[ \tau_{ad} = \frac{1}{alpha_{d} + beta_{d}} \]
\[ f_{inf} = \frac{1}{1 + e^{-\frac{(V+30)}{5}}} \]

\[ taurf = 257.1 \times e^{-\frac{(V+32.5)^2}{13.9}} + 44.3 \]

\[ f_{cainf} = \frac{kmfca}{kmfca + caR} \]

\[ taufca = \frac{f_{cainf}}{alphafca} \]

\[ dddf = \frac{1}{1 + e^{-\frac{(V+24.1)}{6}}} \]

\[ taudd = taud \]

\[ f_{dinf} = \frac{1}{1 + e^{-\frac{(V+40)}{5}}} \]

\[ taufd = tauf \]

\[ \text{Gate}.d = dinf - (dinf - \text{Gate}.d) \times e^{\frac{-dt}{taud}} \]

\[ \text{Gate}.f = finf - (finf - \text{Gate}.f) \times e^{\frac{-dt}{tauf}} \]

\[ \text{Gate}.fca = f_{cainf} - (f_{cainf} - \text{Gate}.fca) \times e^{\frac{-dt}{taufca}} \]

\[ \text{Gate}.d13 = dddf - (ddf - \text{Gate}.d13) \times e^{\frac{-dt}{taud13}} \]

\[ \text{Gate}.f13 = f_{dinf} - (f_{dinf} - \text{Gate}.f13) \times e^{\frac{-dt}{tauf13}} \]

\[ iCal = gCal \times \text{Gate}.d \times \text{Gate}.f \times \text{Gate}.fca \times (V - ecal) \]

\[ iCal13 = gCal13 \times gate.d13 \times gate.f13 \times Gate.fca \times (V - ecal) \]
Appendix B: Model Equations Amended or Added to the Grandi Atrial Model

This section contains the model equations added to simulate CaMKII phosphorylation of $I_{NaL}$ and RyR2. All other equations come from original publications.

A. CaMKII module

\[ P = .0003; \]
\[ \text{calmtotal} = .00006 \]
\[ \text{ros} = 0; \]
\[ kib = 246; \]
\[ kbi = .0022; \]
\[ kox = .0002909; \]
\[ kred = .0000228; \]
\[ kbp = .0022; \]
\[ kn93 = 0; \]
\[ kbli = .0022; \]
\[ kibl = .0008536; \]
\[ \text{Calm} = \text{calmtotal} \times \left( \frac{\text{Cast}^4}{\text{Cast}^4 + .005^4} \right) \]
\[ \text{tcamk} = f\text{Bound} + f\text{Phos} + f\text{Ox} + f\text{OxP} \]
\[ kt = \frac{kbi}{kib} \left( \frac{1}{\text{tcamk}} - 1 \right) \]
\[ ka = \frac{kt + 0.01851}{kbi \times kt} \]
\[ va = ka \times f\text{Bound} \]
\[ va2 = ka \times f\text{Ox} \]

\[ \frac{df\text{Bound}}{dt} = (kib \times \text{Calm} \times fI + P \times f\text{Phos} + kred \times f\text{Ox} \]
\[ \left. - (kbi + kox \times \text{ros}) \times f\text{Bound} - va \right) + f\text{Bound} \]

\[ \frac{df\text{Phos}}{dt} = (va + kred \times f\text{OxP} - (kox \times \text{ros} + P) \times f\text{Phos}) + f\text{Phos} \]

\[ \frac{df\text{Ox}}{dt} = (kox \times \text{ros} \times f\text{Bound} + P \times f\text{OxP} - kred \times f\text{Ox} - va2) + f\text{Ox} \]
\[
\frac{df_{OXP}}{dt} = (va_2 - P \times f_{OXP} + k_{os} \times ros \times f_{Phos} - k_{red} \times f_{OXP}) + f_{OXP}
\]

\[
\frac{df_{Block}}{dt} = (k_{bl} \times k_{n93} \times f \times f_{Block}) + f_{Block}
\]

\[
\frac{df_{I}}{dt} = 1 - f_{Bound} - f_{Phos} - f_{Ox} - f_{OXP} - f_{Block}
\]

\[
CaMKII = 0.75 \times f_{Bound} + f_{Phos} + f_{OXP} + 0.5 \times f_{Ox}
\]

B. Persistent sodium current
\[
E_{Na} = \frac{RT}{F} \times \log \frac{Na_o}{Na_i}
\]

\[
aml = \frac{0.32 \times (V + 47.13)}{1 - \exp(-0.1 \times (V + 47.13))}
\]

\[
bml = 0.08 \times \exp\left(-\frac{V}{11}\right)
\]

\[
mlinf = \frac{1}{1 + \exp\left(\frac{V + 91}{6.1}\right)}
\]

\[
ms = \frac{aml}{aml + bml}
\]

\[
tml = \frac{1}{aml + bml}
\]

\[
Gate_{ml} = ms - (ms - Gate_{ml}) \times \exp\left(-\frac{dt}{tml}\right)
\]
\[ t_h l = 600 \]

\[ \text{Gate}_{hl} = \text{hlinf} - (\text{hlinf} - \text{Gate}_{hl}) \times \exp\left(\frac{-dt}{thl}\right) \]

For WT

\[ iNaLNP = 0.0325 \times \text{Gate}_{ml}^3 \times \text{Gate}_{hl} \times (V - ENa) \]

\[ iNaLP = 3.2 \times iNaLNP \]

\[ \frac{dfiNaLP}{dt} = \frac{(\text{CaMKII}/(\text{CaMKII} + 0.15) - fiNaLP)}{100} \]

\[ iNaL = fiNaLP \times iNaLP + fINaLNP \times iNaLNP \]

C. Ryanodine receptor calcium release and SERCA2a flux targeting by CaMKII

\[ \text{RyRratio} = \text{RyRP}/\text{RyRtot}; \]

\[ \text{kb2815} = 0.00035; \]

\[ \text{kckryr} = 0.0004; \]

\[ \text{kmckryr} = .012; \]

\[ \text{PP1} = .0956; \]

\[ \text{PP2A} = .0956; \]

\[ \text{kpp1ryr} = .00107; \]

\[ \text{kmpp1ryr} = .009; \]

\[ \text{kmpp2ryr} = .047; \]

\[ \text{kpp2ryr} = .000481; \]

\[ \text{kioapp1} = .00078; \]
kioapp2 = .000037;

OA = 0;

\[
OApp1 = \frac{1}{1 + \frac{OA}{kioapp1}}
\]

\[
OApp2 = \frac{1}{1 + \frac{OA}{kioapp2}}
\]

\[
RyRN = RyRtot - RyRP;
\]

\[
Rxnbasal = kb2815 \times RyRN
\]

\[
Rxnckkryr = \frac{2.4 \times kckkryr \times CaMKII \times RyRN}{kmckkryr + RyRN}
\]

\[
Rxnpp1ryr = \frac{kp1pp1ryr \times PP1 \times RyRP \times OA1}{kmpp1ryr + RyRP}
\]

\[
Rxnpp2ryr = \frac{kp2pp2ryr \times PP2 \times RyRP \times OA2}{kmpp2ryr + RyRP}
\]

\[
kleak = \frac{1}{3} + \frac{10}{3} \times RyRratio
\]

\[
koRyRCKII = \frac{20}{3} \times RyRratio - \frac{1}{3}
\]

PKAratio = 0.5

\[
koRyRPKA = 1.025 \times PKAratio + 0.975
\]

\[
koRyR = koRyRCKII + koRyRPKA - 1
\]

\[
koSRca = \frac{koRyR \times koCa}{kCasr}
\]

\[
\frac{dRyRP}{dt} = Rxnbasal + Rxnckkryr - Rxnpp1ryr - Rxnpp2ryr
\]

KMCAM = 0.2

\[
camfact = \frac{1}{1 + \frac{KMCAM}{CaMKII}}
\]
\( plb = .00011 \times \text{camfact} \)

\[ \alpha = \frac{cal_{hill}}{kmf - plb} - \frac{caSR_{hill}}{kmr} \]

\[ \beta = 1 + \frac{cal_{hill}}{kmf - plb} + \frac{caSR_{hill}}{kmr} \]

\[ J_{serca} = V_{max SR} \times \frac{\alpha}{\beta} \]

D. L-type \( \text{Ca}^{2+} \) channel

\[ I_{up} = \frac{1.2 \times \text{CaMKII}}{0.15 \times \text{CaMKII}^4} \]

\[ I_{CaL} = (1 + I_{up}) \times (I_{Ca} + I_{CaK} + I_{CaNa}) \]
Appendix C: Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF</td>
<td>Atrial fibrillation</td>
</tr>
<tr>
<td>AP</td>
<td>Action potential</td>
</tr>
<tr>
<td>RyR2</td>
<td>Ryanodine receptor</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>CICR</td>
<td>Calcium-induced calcium release</td>
</tr>
<tr>
<td>SERCA2a</td>
<td>Sarcoplasmic reticulum Ca2+-ATPase</td>
</tr>
<tr>
<td>PLB</td>
<td>phospholamban</td>
</tr>
<tr>
<td>LTCC</td>
<td>L-type Ca2+ channel</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Ca2+/calmodulin-dependent protein kinase II</td>
</tr>
<tr>
<td>LAA</td>
<td>Left Atrial Appendage</td>
</tr>
<tr>
<td>ARISTOTLE</td>
<td>Apixaban for reduction in stroke and other ThromboemboLic events in atrial fibrillation</td>
</tr>
<tr>
<td>DAD’s</td>
<td>delayed afterdepolarizations</td>
</tr>
<tr>
<td>EAD’s</td>
<td>early afterdepolarizations</td>
</tr>
<tr>
<td>PV</td>
<td>pulmonary veins</td>
</tr>
<tr>
<td>CFAE</td>
<td>Complex fractionated atrial electrograms</td>
</tr>
<tr>
<td>FIRM</td>
<td>Focal Impulse and Rotor Modulation</td>
</tr>
<tr>
<td>CONFIRM</td>
<td>CONventional ablation for atrial fibrillation with or without FIRM</td>
</tr>
<tr>
<td>RACE</td>
<td>Rate Control versus Electrical Cardioversion</td>
</tr>
<tr>
<td>ACE</td>
<td>angiotensin-converting enzyme</td>
</tr>
<tr>
<td>RAAS</td>
<td>renin-angiotensin-aldosterone</td>
</tr>
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</table>
Abbreviations ct’d

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APD</td>
<td>action potential duration</td>
</tr>
<tr>
<td>SAN</td>
<td>sinoatrial node</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein Kinase A</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>AA</td>
<td>arachidonic acid</td>
</tr>
<tr>
<td>Ach</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>MDP</td>
<td>max diastolic potential</td>
</tr>
<tr>
<td>NCX</td>
<td>Na+/Ca2+ exchanger</td>
</tr>
<tr>
<td>WT</td>
<td>wild-type</td>
</tr>
<tr>
<td>BAR</td>
<td>β-adrenergic receptor</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
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</table>
Appendix D: All concentrations and variables available in the Kurata SAN model

**Calcium Concentrations**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
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<tbody>
<tr>
<td>cal</td>
<td>Concentration of intracellular Ca(^{2+}), mM</td>
</tr>
<tr>
<td>caJsrs</td>
<td>Ca(^{2+}) concentration in junctional sarcoplasmic reticulum, mM</td>
</tr>
<tr>
<td>caNsrs</td>
<td>Ca(^{2+}) concentration in network sarcoplasmic reticulum, mM</td>
</tr>
<tr>
<td>caRsrs</td>
<td>Ca(^{2+}) concentration in restricted space, mM</td>
</tr>
</tbody>
</table>

**Currents**

<table>
<thead>
<tr>
<th>Current</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCal</td>
<td>L-type Ca(^{2+}) channel current</td>
</tr>
<tr>
<td>iCat</td>
<td>Total calcium current due to L-type, T-type, and sodium-calcium exchanger, uA/uF</td>
</tr>
<tr>
<td>iCatt</td>
<td>T-type Ca(^{2+}) channel current, uA/uF</td>
</tr>
<tr>
<td>iDiff</td>
<td>Ca(^{2+}) diffusion from subspace to myoplasm, uA/uF</td>
</tr>
<tr>
<td>iH</td>
<td>Hyperpolarization-activated current, uA/uF</td>
</tr>
<tr>
<td>iHk</td>
<td>Potassium component of hyperpolarization-activated current, uA/uF</td>
</tr>
<tr>
<td>iHna</td>
<td>Sodium component of hyperpolarization-activated current, uA/uF</td>
</tr>
<tr>
<td>iKach</td>
<td>Acetylcholine-sensitive K(^+) current, uA/uF</td>
</tr>
<tr>
<td>iKr</td>
<td>Rapid delayed rectifier K(^+) current, uA/uF</td>
</tr>
<tr>
<td>iKs</td>
<td>Slow delayed rectifier K(^+) current, uA/uF</td>
</tr>
<tr>
<td>iT</td>
<td>Total transmembrane K(^+) current, uA/uF</td>
</tr>
<tr>
<td>iNab</td>
<td>Background sodium current, uA/uF</td>
</tr>
<tr>
<td>iNaca</td>
<td>Na(^+)-Ca(^{2+}) exchanger, uA/uF</td>
</tr>
<tr>
<td>iNak</td>
<td>Na(^+)-K(^+) pump, uA/uF</td>
</tr>
<tr>
<td>iNat</td>
<td>Total transmembrane Na(^+) current, uA/uF</td>
</tr>
<tr>
<td>iRel</td>
<td>Ca(^{2+}) release from junctional SR to subspace</td>
</tr>
<tr>
<td>iS</td>
<td>Sustained inward current, uA/uF</td>
</tr>
<tr>
<td>iSus</td>
<td>Sustained component of the 4AP-sensitive current, uA/uF</td>
</tr>
<tr>
<td>iTo</td>
<td>4AP-sensitive transient outward K(^+) current, uA/uF</td>
</tr>
<tr>
<td>iTot</td>
<td>Total transmembrane current, uA/uF</td>
</tr>
<tr>
<td>iTk</td>
<td>Ca(^{2+}) transfer from network to junctional sarcoplasmic reticulum</td>
</tr>
<tr>
<td>iTrek</td>
<td>TREK-1 K(^+) current, uA/uF</td>
</tr>
<tr>
<td>iUp</td>
<td>Ca(^{2+}) uptake from myoplasm to network sarcoplasmic reticulum</td>
</tr>
</tbody>
</table>

**Gates**

<table>
<thead>
<tr>
<th>Gate</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gate.d</td>
<td>Activation gate of ICaL</td>
</tr>
<tr>
<td>Gate.dt</td>
<td>Activation gate of ICaT</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Gate.f</td>
<td>Fast voltage-dependent inactivation gate of ICaL</td>
</tr>
<tr>
<td>Gate.fca</td>
<td>Ca^{2+}-dependent inactivation gating variable for ICaL</td>
</tr>
<tr>
<td>Gate.ft</td>
<td>Inactivation gating variable for ICaT</td>
</tr>
<tr>
<td>Gate.n</td>
<td>Activation gating variable for IKs</td>
</tr>
<tr>
<td>Gate.paf</td>
<td>Fast activation gating variable for IKr</td>
</tr>
<tr>
<td>Gate.pas</td>
<td>Slow activation gating variable for IKr</td>
</tr>
<tr>
<td>Gate.pi</td>
<td>Inactivation gating variable for IKr</td>
</tr>
<tr>
<td>Gate.q</td>
<td>Inactivation gating variable for Ito</td>
</tr>
<tr>
<td>Gate.qa</td>
<td>Activation gating variable for Ist</td>
</tr>
<tr>
<td>Gate.qi</td>
<td>Inactivation gating variable for Ist</td>
</tr>
<tr>
<td>Gate.r</td>
<td>Activation gating variable for Ito and Isus</td>
</tr>
<tr>
<td>Gate.y</td>
<td>Activation gating variable for Ih</td>
</tr>
</tbody>
</table>

**Other**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>cmdnI</td>
<td>Concentration of calmodulin bound to Ca^{2+} buffer in bulk myoplasm, mM</td>
</tr>
<tr>
<td>cmdnR</td>
<td>Concentration of calmodulin bound to Ca^{2+} buffer in restricted space, mM</td>
</tr>
<tr>
<td>csqn</td>
<td>Concentration of calsequestrin bound to Ca^{2+} in sarcoplasmic reticulum, mM</td>
</tr>
<tr>
<td>dVdt</td>
<td>Change in transmembrane potential with respect to time</td>
</tr>
<tr>
<td>kI</td>
<td>Intracellular potassium concentration, mM</td>
</tr>
<tr>
<td>nal</td>
<td>Intracellular sodium concentration, mM</td>
</tr>
<tr>
<td>t</td>
<td>Time, ms</td>
</tr>
<tr>
<td>trpnCa</td>
<td>Concentration of troponin with Ca^{2+} bound at Ca^{2+} site, mM</td>
</tr>
<tr>
<td>trpnMg</td>
<td>Concentration of troponin with Ca^{2+} bound at Mg^{2+} site, mM</td>
</tr>
<tr>
<td>trpnMgmg</td>
<td>Concentration of troponin with Mg^{2+} bound at Mg^{2+} site, mM</td>
</tr>
<tr>
<td>vold</td>
<td>Transmembrane potential, mV</td>
</tr>
</tbody>
</table>
Appendix E: All concentrations and variables available in the Grandi atrial model

### Calcium concentrations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>caI</td>
<td>Concentration of free and buffered intracellular Ca$^{2+}$, mmol/L</td>
</tr>
<tr>
<td>caSr</td>
<td>Ca$^{2+}$ concentration in sarcoplasmic reticulum, mmol/L</td>
</tr>
<tr>
<td>cajI</td>
<td>Ca$^{2+}$ concentration in junction, mmol/L</td>
</tr>
<tr>
<td>caslI</td>
<td>Ca$^{2+}$ concentration in sarcolemma, mmol/L</td>
</tr>
</tbody>
</table>

### Currents

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>iCa</td>
<td>Ca$^{2+}$ current through the L-type Ca$^{2+}$ channel in sarcolemma and junction, uA/uF</td>
</tr>
<tr>
<td>iCaL</td>
<td>Total L-type Ca$^{2+}$ current due to calcium, sodium and potassium through the L-type Ca$^{2+}$ channel in the sarcolemma and junction</td>
</tr>
<tr>
<td>iCab</td>
<td>Background Ca$^{2+}$ current, uA/uF</td>
</tr>
<tr>
<td>iCabljunc</td>
<td>Background Ca$^{2+}$ current in junction, uA/uF</td>
</tr>
<tr>
<td>iCabsl</td>
<td>Background Ca$^{2+}$ current in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iCajune</td>
<td>Ca$^{2+}$ current in junction and sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iCak</td>
<td>Potassium current through L-type Ca$^{2+}$ channel</td>
</tr>
<tr>
<td>iCana</td>
<td>Sodium current through L-type Ca$^{2+}$ channel</td>
</tr>
<tr>
<td>iCanaejunc</td>
<td>Sodium current through L-type Ca$^{2+}$ channel in junction</td>
</tr>
<tr>
<td>iCanasl</td>
<td>Sodium current through L-type Ca$^{2+}$ channel in sarcolemma</td>
</tr>
<tr>
<td>iCasl</td>
<td>Total Ca$^{2+}$ current in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iCat</td>
<td>T-type Ca$^{2+}$ current, uA/uF</td>
</tr>
<tr>
<td>iClblk</td>
<td>Background Cl$^-$ current, uA/uF</td>
</tr>
<tr>
<td>iClca</td>
<td>Ca$^{2+}$-activated Cl$^-$ current, uA/uF</td>
</tr>
<tr>
<td>iClcajunc</td>
<td>Ca$^{2+}$-activated Cl$^-$ current in junction, uA/uF</td>
</tr>
<tr>
<td>iClcasl</td>
<td>Ca$^{2+}$-activated Cl$^-$ current in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iK1</td>
<td>Time-independent K$^+$ current, uA/uF</td>
</tr>
<tr>
<td>iKp</td>
<td>Plateau K$^+$ current, uA/uF</td>
</tr>
<tr>
<td>iKvjunc</td>
<td>Plateau K$^+$ current in junction, uA/uF</td>
</tr>
<tr>
<td>iKpsl</td>
<td>Plateau K$^+$ current in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iKr</td>
<td>Rapid delayed rectifier K$^-$ current, uA/uF</td>
</tr>
<tr>
<td>iKs</td>
<td>Slow delayed rectifier K$^+$ current, uA/uF</td>
</tr>
<tr>
<td>iKsjunc</td>
<td>Slow delayed rectifier K$^+$ current in junction, uA/uF</td>
</tr>
<tr>
<td>iKssl</td>
<td>Slow delayed rectifier K$^+$ current in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iKt</td>
<td>Total transmembrane K$^+$ current, uA/uF</td>
</tr>
<tr>
<td>iKur</td>
<td>Ultrarapid delayed rectifier K$^+$ current, uA/uF</td>
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<tr>
<td>iNa</td>
<td>Fast Na$^+$ current, uA/uF</td>
</tr>
<tr>
<td>iNaKjunc</td>
<td>Na$^+$-K$^+$ pump in junction, uA/uF</td>
</tr>
<tr>
<td>iNaKsl</td>
<td>Na$^+$-K$^+$ pump in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iNab</td>
<td>Background Na$^+$ current, uA/uF</td>
</tr>
<tr>
<td>iNabjunc</td>
<td>Background Na$^+$ current in junction, uA/uF</td>
</tr>
<tr>
<td>iNabsl</td>
<td>Background Na⁺ current in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>---------------</td>
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</tr>
<tr>
<td>iNaca</td>
<td>Na⁺-Ca²⁺ exchanger, uA/uF</td>
</tr>
<tr>
<td>iNajunc</td>
<td>Total Na⁺ current in junction, uA/uF</td>
</tr>
<tr>
<td>iNak</td>
<td>Na⁺-K⁺ pump, uA/uF</td>
</tr>
<tr>
<td>iNasl</td>
<td>Total Na⁺ current in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iNat</td>
<td>Total transmembrane Na⁺ current, uA/uF</td>
</tr>
<tr>
<td>iNexjunc</td>
<td>Na⁺-Ca²⁺ exchanger current in junction, uA/uF</td>
</tr>
<tr>
<td>iNexsl</td>
<td>Na⁺-Ca²⁺ exchanger current in sarcolemma, uA/uF</td>
</tr>
<tr>
<td>iTo</td>
<td>4AP-sensitive transient outward K⁺ current, uA/uF</td>
</tr>
<tr>
<td>iTof</td>
<td>Fast component of transient outward K⁺ current, uA/uF</td>
</tr>
<tr>
<td>iTos</td>
<td>Slow component of transient outward K⁺ current, uA/uF</td>
</tr>
<tr>
<td>iTot</td>
<td>Total transmembrane current, uA/uF</td>
</tr>
<tr>
<td>ipCa</td>
<td>Ca²⁺ pump current, uA/uF</td>
</tr>
<tr>
<td>ipCajunc</td>
<td>Ca²⁺ pump current in junction, uA/uF</td>
</tr>
<tr>
<td>ipCasl</td>
<td>Ca²⁺ pump current in sarcolemma, uA/uF</td>
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**Gates**

<table>
<thead>
<tr>
<th>Gate.d</th>
<th>Activation gate of ICaL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gate.f</td>
<td>Fast voltage-dependent inactivation gate of ICaL</td>
</tr>
<tr>
<td>Gate.f_cabj</td>
<td>Calcium-dependent inactivation gate of ICaL in junction</td>
</tr>
<tr>
<td>Gate.f_cabsl</td>
<td>Calcium-dependent inactivation gate of ICaL in sarcolemma</td>
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<td>Gate.h</td>
<td>Fast inactivation gate of INa</td>
</tr>
<tr>
<td>Gate.j</td>
<td>Slow inactivation gate of INa</td>
</tr>
<tr>
<td>Gate.m</td>
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<tr>
<td>Gate.xf</td>
<td>Fast inactivation gate of Ito</td>
</tr>
<tr>
<td>Gate.xkr</td>
<td>Activation gate of IKr</td>
</tr>
<tr>
<td>Gate.xks</td>
<td>Fast activation gate of IKs</td>
</tr>
<tr>
<td>Gate.xkur</td>
<td>Fast activation gate of IKur</td>
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<tr>
<td>Gate.yf</td>
<td>Slow inactivation gate of Ito</td>
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<tr>
<td>Gate.ykur</td>
<td>Slow activation gate of Ikur</td>
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**Other**

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<th>CaM</th>
<th>Activated CaMKII</th>
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<tr>
<td>Csqnb</td>
<td>Concentration of Ca²⁺ bound Calsequestrin, mM</td>
</tr>
<tr>
<td>clI</td>
<td>Intracellular chloride concentration, mM</td>
</tr>
<tr>
<td>dVdt</td>
<td>Change in voltage with respect to time, mV/ms</td>
</tr>
<tr>
<td>kl</td>
<td>Intracellular potassium concentration, mM</td>
</tr>
<tr>
<td>mgI</td>
<td>Intracellular magnesium concentration, mM</td>
</tr>
<tr>
<td>nai</td>
<td>Intracellular sodium concentration, mM</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------</td>
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<tr>
<td>najI</td>
<td>Junction sodium concentration, mM</td>
</tr>
<tr>
<td>nasII</td>
<td>Sarcolemma sodium concentration, mM</td>
</tr>
<tr>
<td>t</td>
<td>Time, ms</td>
</tr>
<tr>
<td>trpn</td>
<td>Concentration of Ca^{2+} bound Troponin, mM</td>
</tr>
<tr>
<td>vold</td>
<td>Transmembrane potential, mV</td>
</tr>
</tbody>
</table>
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