

Computational framework for multiscale investigation of pro-arrhythmic mechanisms in the human cardiac Purkinje system

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This thesis is submitted to the Department of Computer Science, University of Oxford, for the degree of Doctor of Philosophy. This thesis is entirely my own work, and, except where otherwise indicated, describes my own research.

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Abstract

Ventricular arrhythmias are a major cause of sudden cardiac death, which accounts for about 50% of cardiovascular mortality worldwide. Understanding the mechanisms underlying arrhythmias generations is crucial to improve its diagnosis and treatment. Cardiac Purkinje cells constitute the peripheral conduction system of the electrical stimulation in ventricles and are responsible for the correct contraction pattern of the heart. Experimental studies on animal models pointed out that drug actions, mutations or cardiac diseases might alter the electrical activity of Purkinje cells and lead to arrhythmia onsets. However, the pro-arrhythmic mechanisms in Purkinje cells are not entirely understood, and results obtained in animal studies do not always translate to humans, due to inter-species differences.

The aim of this thesis is to develop an experimentally-informed computational framework to mechanistically investigate the role of human Purkinje cells in arrhythmia onset, and predict Purkinje cells electrophysiological response to pharmacological therapy. Multiscale simulations are used to identify the mechanisms underlying electrical abnormalities in human Purkinje cell and tissue models, as well as to evaluate drug safety and efficacy on cardiac Purkinje electrophysiology in humans, also accounting for biological variability.

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And now, let's see what the future holds for me!

Publications

Below is a list of publications that directly relate to the work described in this thesis.

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- **C. Trovato**, E. Passini, C. Dusserre, B. Rodriguez. Using a novel human Purkinje fibre model for *in silico* trials of drug safety and efficacy. *Comput. Cardiol.* Vol. 47 (2020).
- E. Passini, X. Zhou, **C. Trovato**, O. J. Britton, A. Bueno-Orovio, B. Rodriguez. The Virtual Assay Software for Human *in Silico* Drug Trials to Augment Drug Cardiac Testing. *J. Comput. Sci-Neth.* *Manuscript under review*.
- **C. Trovato**, E. Passini, N. Nagy, A. Varró, N. Abi-Gerges, S. Severi, B. Rodriguez. Human Purkinje *in silico* model enables mechanistic investigations into automaticity and proarrhythmic abnormalities. *J. Mol. Cell. Cardiol.* 142 (2020) 24–38.
- **C. Trovato**, E. Passini, N. Abi-Gerges, S. Severi, B. Rodriguez. *In Silico* Trials in Human Purkinje Cell Models Predict Drug Safety and Efficacy. *J. Pharmacol. Toxicol. Methods* (2020).
- E. Passini, **C. Trovato**, P. Morissette, F. Sannajust, A. Bueno-Orovio, B. Rodriguez. Drug-induced shortening of the electromechanical window is an effective biomarker for *in silico* prediction of clinical risk of arrhythmias. *Br. J. Pharmacol.* 176 (2019)

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Nonstandard Abbreviations and Acronyms

AP(s)	Action potential(s)
APA	Action potential amplitude
APD _x	AP duration at X% of repolarisation
BCL	Basic cycle length
DAD(s)	Delayed after-depolarisation(s)
DMP	Diastolic membrane potential
dV/dt _{MAX}	Maximum upstroke velocity
EAD(s)	Early after-depolarisation(s)
EOP	Membrane potential at the end of repolarisation
G _X	I _X conductance
IC ₅₀	Concentration for 50% channel inhibition
I _{CaL}	L-type Ca ²⁺ current
I _{CaT}	T-type Ca ²⁺ current
I _f	Funny current
I _{K1}	Inward rectifier K ⁺ current
I _{Kr}	Rapid delayed rectifier K ⁺ current
I _{Ks}	Slow delayed rectifier K ⁺ current
I _{Na}	Fast Na ⁺ current
I _{NaK}	Na ⁺ -K ⁺ pump current
I _{NaL}	Late Na ⁺ current
I _{NCX}	Na ⁺ -Ca ²⁺ exchanger current
I _{to}	Transient outward K ⁺ current
I _{sus}	Sustained outward K ⁺ current
ORD	O'Hara-Rudy dynamic human ventricular model

PC(s)	Purkinje cells
PRd	Pan Li-Rudy dynamic canine Purkinje model
SS	Steady State
TOP	Take-off potential (membrane potential before depolarisation)
Trovato2020	New human cardiac Purkinje AP model
V_m	Membrane potential

Chapter 1 - Introduction

1.1 Motivation

Cardiac Purkinje cells (PCs) play a crucial role in ventricular excitation since they guarantee a correct excitation pattern and, therefore, a synchronised sequence of cardiac contraction. Evidence in the literature identifies PCs as an important trigger of arrhythmias 4.3.1 (Haissaguerre et al. 2016). Particularly, PCs may be involved in the generation of Torsade de Pointes arrhythmias, in patients with long QT syndrome, either genetic or drug-induced (Dobrzynski et al. 2013; V. Iyer et al. 2015). Indeed, PCs are commonly used to screen for pro-arrhythmic cardiotoxicity in the preclinical phases of drug development. However, the biological complexity at the sub-cellular level still prevents a complete understanding of the key ionic mechanisms leading to arrhythmias, both at the single-cell and tissue levels. Moreover, isolation of PCs requires complex experimental procedures which make the investigation of their electrophysiological properties more challenging with respect to other kinds of cardiomyocytes (Wei Han, Zhang, et al. 2002). Most of the PCs studies were conducted in animal models such as guinea pigs, rabbits, dogs, cows, but results do not always translate to human, due to interspecies differences (Ono et al. 2009; Hua Rong Lu et al. 2001).

Therefore, there is the need to find an alternative approach to fill the gap of knowledge and to facilitate translation to human.

Computational models can help to gain insights into the PCs electrophysiological properties and their role in arrhythmias onset. However, the previously published human Purkinje computational models (Stewart et al. 2009; Sampson et al. 2010) do not incorporate some of the key properties of PCs, such as the lack of t-tubuli, and are unable to reproduce cellular electrical abnormalities such as early and delayed afterdepolarisations (EADs and DAD, respectively). These abnormalities are considered dangerous since they might propagate from a few cells to the surrounding tissue and trigger arrhythmias at the whole organ level (Dutta et al. 2016; Damiano and Rosen 1984; Van Nieuwenhuyse et al. 2017; Vandersickel et al. 2017). This might be particularly true for Purkinje fibres due to their electrophysiological properties: their longer action potential (AP) makes PCs more prone to develop EADs, and sink-source properties in their connection to the endocardium can modulate their propagation to both ventricles (Deo et al. 2010; Boyden, Dun, and Robinson 2016).

From a computational point of view, the increase in computational power available for simulations, due to the use of both supercomputers and high-performance computing, allows the design of more detailed and sophisticated models, and to explore wider biological and clinical scenarios. It is nowadays possible to run multiscale simulations, from proteins to body surface, in reasonable times (hours) or investigate thousands of models at the same time, e.g. for the understanding of the effects of biological variability on populations of cells.

1.2 Thesis goals

The general aim of my thesis is to investigate the role of Purkinje cells in arrhythmia generation, using an experimentally-informed computational framework, based on multiscale

and biophysically-detailed modelling and simulations, accounting also for the impact of intercellular variability.

For this, I integrate experimental recordings from human Purkinje fibres to develop a new human computational model of PC electrophysiology. Then, using the novel model, I investigate at different scales, from ionic channel to tissue level, the mechanisms of drug-induced generation and propagation of EADs and DADs in humans, using mathematical models and computational techniques.

The specific goals of my thesis are:

1. To develop and validate a new human Purkinje action potential model, for simulations of PCs electrophysiology.
2. To investigate the mechanisms underlying the generation and propagation of the pro-arrhythmic electrical abnormalities in human Purkinje cells and tissue, with consideration of biological variability.
3. To predict drug-induced effects on human cardiac Purkinje electrophysiology for the safety and efficacy evaluation of a set of reference compounds.

1.3 Thesis outline

Following this first Introduction chapter, Chapter 2 includes a brief description of the human heart anatomy and function. Fundamental aspects of the cardiac electrical activity are described, as well as the basics of the mathematical modelling and multiscale simulation of cellular electrophysiology, including cell-to-cell variability.

Chapter 3 provides background information based on a literature review. In brief: summary of the anatomical and functional features of the His-Purkinje system; overview of the electrophysiological properties of the cardiac PCs in control conditions; summary of the

experimental data available for human PCs; summary of the experimental data available for PCs under drug action; a review of the human PCs computational single-cell models, and their applications.

Chapter 4 focuses on the development of a new computational model of human cardiac Purkinje action potential, based on human recordings from healthy human hearts. The new model has been designed based on two existing cardiac models, the gold standard for human ventricular modelling (O'Hara et al. 2011) and the most recent canine Purkinje model (P. Li and Rudy 2011). Human voltage-clamp data and qualitative information from the literature were integrated as well, to account for the specific properties of PCs in human. The resulting model describes the key features of PCs, the internal structure, the ionic membrane currents, the intracellular fluxes and the Ca^{2+} handling. The resulting electrophysiological model was evaluated against experimental data at different frequencies and under several protocols, including selective ion channel block and specific protocols to induce arrhythmia precursors as EADs and DADs. A genetic algorithm was implemented for a final optimisation of current conductances, and the results were compared against the ones obtained by manual tuning, based on the sensitivity analysis. The final part of this chapter is dedicated to the results obtained in tissue, extending the human Purkinje cell modelling to a 1D fibre to evaluate electrical propagation.

Chapter 5 presents the results of the investigation of the effect of ionic variability on human Purkinje cells electrophysiology and proarrhythmic cellular mechanisms. Firstly, a sensitivity analysis was performed on the new human cardiac Purkinje electrophysiological model to evaluate how changes in the main ionic currents conductances affect AP biomarkers, as well as the outcome of the protocol for rate-dependence, selective ion channel blocks, and EADs

and DADs inducibility. Secondly, informed by the results of the sensitivity analysis, a population of models was designed, constructed and calibrated using experimental AP recordings from healthy human Purkinje cells, in control conditions and under drug action. The population of Purkinje models was used to investigate the mechanisms underlying EADs and DADs generation, and to identify the ionic current profiles more vulnerable to such abnormalities. Finally, EADs and DADs propagation was also simulated in a 1D human Purkinje fibre.

Chapter 6 presents results of *in silico* drug trials, performed with the new human cardiac Purkinje AP model described in Chapter 4, and their comparison to *in vitro* experiments in the literature, for a set of reference compounds. Drug action on the ionic currents was simulated with a pore-block model, using drug-ion channel information from the literature as described in (Passini et al. 2019), i.e., IC_{50} values and Hill coefficients describing the drug-induced inhibition of specific ion channels. Each compound was simulated at different concentrations and pacing frequencies on *in silico* PCs. Drug-induced changes on a set of AP biomarkers were analysed to evaluate the drug efficacy on cardiac Purkinje electrophysiology. The impact of different sets of IC_{50} values was also investigated, as well as the interspecies differences in drug response. The potential of the human PC model to predict drug-induced pro-arrhythmic cardiotoxicity was also evaluated, through the investigation of EADs occurrence *in silico* and comparison against clinical report of Torsade de Pointes arrhythmia.

Chapter 7 provides a summary of the thesis, including main findings and future work.

Chapter 2 - Introduction to the cardiac electrical activity and its mathematical modelling

In this chapter, I introduce the basic concepts underlying the work presented in the thesis. Firstly, I will start from a description of the structure and function of the heart and its electrical excitation. I introduce and describe the cellular electrical signal called action potential and its link to arrhythmias. Secondly, I summarise the mathematical formalism used for the computational modelling of cardiac electrophysiology at the cellular and tissue scale. The last part of the chapter introduces the concept of biological variability and the tools available for its mathematical modelling.

2.1 The human heart: structure and function

The heart is a muscle and is the central organ of the cardiovascular system. Its role is to pump blood across to the whole body, continuously, following a regular pattern, for the whole life of the organism.

In humans, its dimensions are approximately equal to those of a fist. It is situated in the middle of the thorax, slightly on the left, underneath the sternum and between the lungs. The heart is covered externally by a fibrous sac called pericardium (Figure 2.1A), a double layer membrane containing serous fluid to reduce friction during cardiac contraction and to protect the heart from infections spreading by surrounding organs, blood overfilling, and excessive motions.

As represented in Figure 2.1A, the heart is structurally divided into four chambers: the two on the upper part are the atria; the two on the lower part are the ventricles. Both atria and ventricles are identified by their position in respect to the septum, which divides the heart into two parts: right and left. The right atrium and ventricle are connected by the tricuspid valve, whereas left atrium and ventricle are coupled by the mitral valve. From a top-bottom view, the top of the ventricles is called the base, whereas, the bottom is called the apex.

Atria and ventricles are both histologically and structurally different. Though both tissue types are excitable (see Section 2.2), atria and ventricular cells express different types of protein which affect their electrophysiological properties. Structurally, atria walls are thin and have very irregular shapes, whereas ventricles are thicker and with more regular shapes.

The function of the heart is pumping blood to provide oxygen and nutrients to the whole body. Although it looks like a single organ, it is functionally composed of two synchronised pumps which provide blood for both the pulmonary and systemic circuits (Figure 2.1B). At each beat, the oxygen-poor and CO₂-rich blood from the body periphery reaches the right atrium through the superior and inferior venae cavae. The right atrium contraction allows blood to pass the tricuspid valve and flow into the right ventricle, whose contraction pushes, in turn, the blood into the lungs, through the pulmonary arteries. After the gas exchange between CO₂ and oxygen in the lungs, the re-oxygenated blood pours into the left atrium through the pulmonary veins, completing the pulmonary circulation. Left atrium contraction pushes the blood through the mitral valve into the left ventricle. The latter is comprised by a thicker muscular tissue with

respect to the right ventricle to allow a forceful contraction necessary to pump the blood into the Aorta and, through the systemic arteries, in all the tissues of the body. At the periphery, the blood releases oxygen and nutrients to the tissues and collects CO₂. Through the systemic veins, it reaches the venae cavae and the right atrium, completing the systemic circulation.

The pattern described above is continuously repeated, without pauses, across the whole life of the organism. Heart contraction is allowed by the myocardium, the muscular tissue comprising the walls of the cardiac chambers. In particular, the internal part of the chambers is called the endocardium, whereas, the outer part is called the epicardium. The myocardium is mainly composed of cardiac muscle cells known as cardiac myocytes or cardiomyocytes.

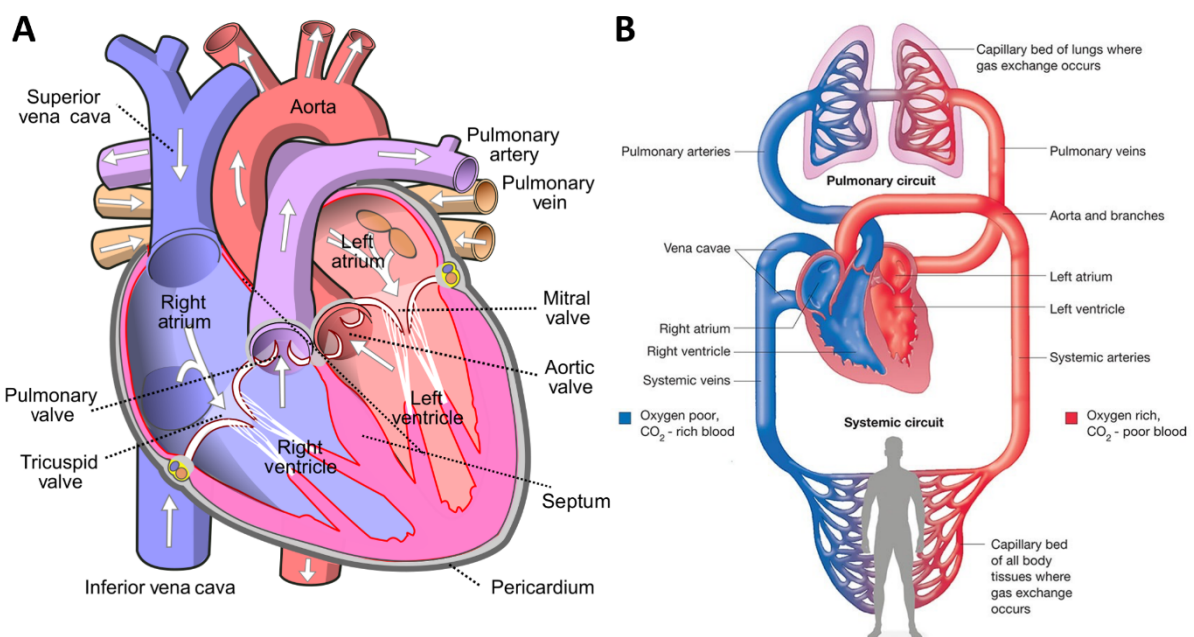


Figure 2.1. Schematic representation of heart structure and function: A) Representation of heart structure, modified from Wapcaplet - Own work, <https://commons.wikimedia.org/w/index.php?curid=830253>. B) Schematic representation of pulmonary and systemic circuits, from <https://www.boxym.com/what-is-blood-circulation>.

Cardiomyocytes have a tubular shape of about 80-100 μm in length and elliptic cross-section with a major axis of approximately 10-20 μm for human ventricles. They are coupled in a discrete layer of fibres and sheets, through gap junctions comprised of several types of connective proteins called connexins. The coupling allows fast intercellular signal propagation and synchronises the electrical excitation and contraction of the cells. Also, since connexins primarily connect cardiomyocytes from end-to-end, the fibre behaviour is anisotropic, conducting and contracting preferentially along the longitudinal direction of the fibre.

2.2 The electrical excitation of the heart

In order to maximise the cardiac contraction efficiency and allow the heart to act as a pump (rather than many disorganised cells), the electrical stimulation has to follow a specific pattern. Since the myocardium is an involuntary muscle, its beating is not initiated by the central nervous system, although, the latter has a role in modulating the cardiac beating. The initial electrical stimulation comes from the heart itself, from a region of the right atrium wall called the sinoatrial node. The cells of the sinoatrial node are self-excitatory (pacemakers) and able to generate an electrical impulse with a rate of about 70 per minute, called sinus rhythm. Following the architecture of the myocardium's fibres and sheets, the electrical stimulation propagates across the walls of both atria, but it is stopped by the non-excitabile tissue comprising the annulus of separation between atria and ventricles. The electrical impulse reaches the atrioventricular node, located at the boundary between atria and ventricles, where it is slowed down, producing a delay that allows atria to contract and pump the blood into the ventricular chambers. The cells of this node display pacemaker function, but their spontaneous pacing is slower than the sinoatrial node (about 45 per minute) and is overridden by the sinus rhythm. The electrical stimulation, then, proceeds towards the ventricles through the specialised cardiac conduction system, composed by the His bundle, the right and left bundle

branches and the Purkinje network, a tangle of fibres covering the sub-endocardium of the ventricular chambers. A detailed description of the structure and electrophysiology of the His-Purkinje system will be provided in Chapter 3. Overall, the His-Purkinje system rapidly conducts the electrical impulse into both ventricles, allowing a quick stimulation of the ventricular tissue, which is thicker and bigger than the atrial one and, therefore, requires more electrical stimulation. The electrical propagation proceeds into the septum through the His bundle, it reaches the apex through the Purkinje network and goes back in the lateral walls toward the base. The electrical sequence described above, repeated in an average human lifetime approximately two billion times, is due to the synchronised electrophysiological activity of billions of cells. Indeed, cardiomyocytes are excitable cells and actively respond to a sufficient electrical stimulation producing, in turn, an electrical signal called “Action Potential” (AP), whose description will be provided below (Section 2.3). The AP varies in shape and properties based on the type of tissue that has generated it and contributes to the macroscopic electrical pattern observed in the human heart (Figure 2.2A), traditionally represented with the electrocardiogram (ECG). The ECG is recorded from twelve different angles through sensors disposed non-invasively on the thorax surface and is characterised mainly by three waves, representing three different phases of the cardiac excitation: the P wave (for the atria depolarisation), the QRS complex (for the ventricular depolarisation) and the T wave (for the ventricular repolarisation). The QRS complex hides the atria repolarisation phase since the electrical activity is more intense in the ventricles than in the atria.

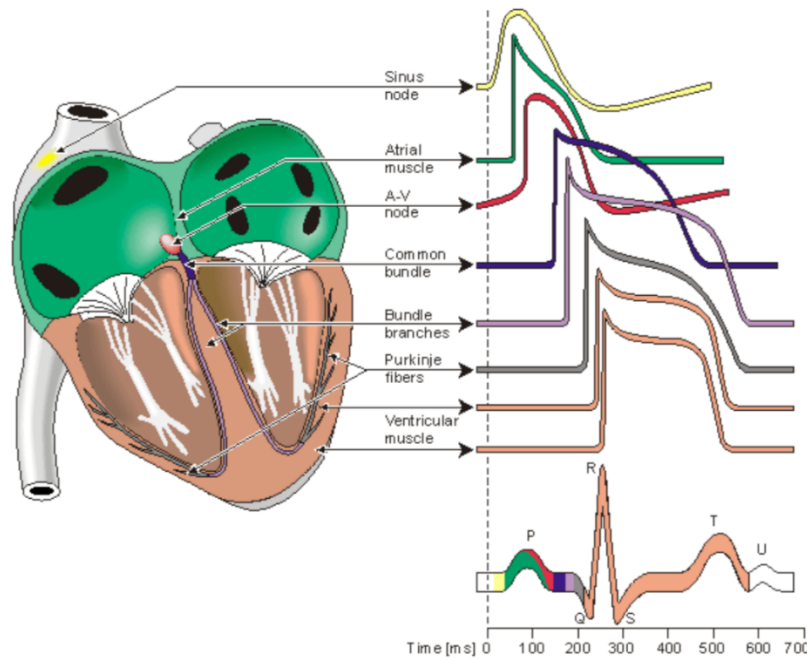


Figure 2.2. The cardiac electrical activity: Different action potentials from different specialised regions of the heart, and their contribution to the total electrocardiogram waveform (modified from Malmivuo 1995).

2.3 The cardiac action potential

The dynamic of the AP is due to a complex network of ionic fluxes, happening across the cellular membrane and involving mainly Na^+ , K^+ , Ca^{2+} . The cellular membrane, or sarcolemma, comprised mainly by a double-layer of phospholipids, bounds each cardiac cell and separates the intracellular and extracellular space, creating a transmembrane electrochemical gradient (∇E). The electrochemical gradient is fundamental because it represents the primary force underlying the AP dynamics. The differences of ionic concentrations would produce a concentration gradient (∇C) and, therefore, a force moving ions in the direction of the gradient (from areas at higher concentration to those at a lower concentration) to create a uniform distribution. On the other hand, different concentrations of ions produce an electrical field (∇E) between the two sides of the membrane, opposite to the chemical gradient. At the equilibrium ($\nabla E = 0 \rightarrow \nabla E = -\nabla C$), the intracellular and extracellular

concentrations are different, due to the interaction of the electrical and chemical gradients. In particular, the intracellular space has a much higher concentration of K^+ and lower concentrations of Na^+ compared to the extracellular space. In this condition, the cellular membrane is in a “polarised or resting state” and maintains a net membrane potential (V_m) generally between -90 and -80 mV for cardiac muscle cells and between -50 to -60 mV for those of the sinoatrial node.

The ionic fluxes described above could not have happened if the membrane had comprised only by phospholipids, which almost wholly isolate the intracellular and extracellular space. Instead, many types of large and complex proteins are embedded in the membrane, creating small pores across the phospholipidic double layer. These pores are heterogeneous and selectively permeable to ions under specific conditions. Overall, three main types of proteins allow the movement of ions across the membrane: ion channels, ion exchangers and ion pumps. Each of these types of proteins has specific dynamics, mostly regulated by several gates, which allow (activation gates) or impede (inactivation gates) the passage of ions and the associated ionic current. The ionic gates respond, in turn, to changes happening at the cellular level, e.g., changes in V_m or specific ionic concentrations. Conventionally, currents increasing V_m , i.e., depolarising the cell, are indicated as inward currents, whereas, those decreasing V_m , i.e., polarising the cell, are called outward currents.

When the membrane receives a small electrical perturbation, the cellular response is passive, with only a linear change in the membrane voltage followed by returning to the resting state. However, when the stimulus is sufficiently large and able to push the voltage membrane up to a specific threshold value, the excited cell responds actively producing an AP. Figure 2.3 reports two different types of action potentials and their underlying inward and outward currents for atrial (left) and ventricular (right) cells.

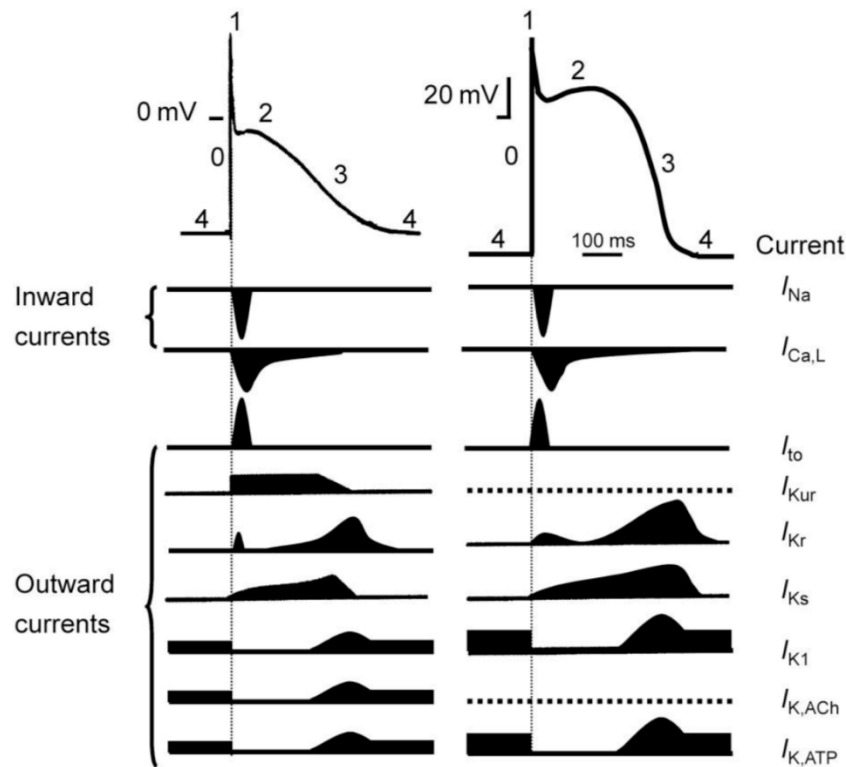


Figure 2.3. Electrophysiology of cardiomyocytes: 2 different examples of action potential (top panel) from atrial (left) and ventricular (right) cells and their underlying inward and outward currents (bottom panels). Modified from (Ravens and Cerbai 2008).

Even if the AP morphologies look dissimilar (due to the different expressions of ionic currents), five distinct phases can be distinguished in both AP. A summary of each of these is provided below:

- **Phase 0 (upstroke):** V_m quickly increases from the resting to positive values, due to the opening of Na^+ channels, which generates a rapid inward-flux of Na^+ , called fast Na^+ current (I_{Na}). The phase 0 is triggered by the external depolarisation from the surrounding tissue, or by self-depolarisation for pacemaker cells, leading V_m up to the threshold for the I_{Na} gates opening. This phase lasts a few milliseconds.
- **Phase 1 (early-repolarisation):** due to V_m increase, the I_{Na} channels close, while K^+ channels open, producing a diversion of V_m called “notch”. In particular, during this

phase, the primary current involved in the membrane repolarisation is the transient outward K^+ current (I_{to}), whose contribution ends after about 20-50 millisecond.

- **Phase 2 (plateau):** during this phase, several currents are active and involved in setting V_m . In particular, the contributions of K^+ currents, the slow and delayed K^+ rectifiers (I_{Kr} and I_{Ks} respectively) is counterbalanced by the opening of the Ca^{2+} channels, mostly represented by the L-type Ca^{2+} current (I_{CaL}). The duration of this phase, characterised by a prolonged decrease of V_m , varies from one type of cell to the other: it is short in atrial cells, longer in ventricular ones and absent in neuronal APs. Indeed, this phase is peculiar of muscle cells and allows a significant increase in the intracellular Ca^{2+} concentration, which plays a crucial role in the cellular contraction.
- **Phase 3 (repolarisation):** this phase begins by the closure of I_{CaL} and is driven by the repolarisation action of I_{Kr} and I_{Ks} , bringing V_m towards its resting value.

Phase 4 (resting): when the contributions of I_{Kr} and I_{Ks} is finished, the cell is back to its polarised state, and the membrane potential is set to its resting value (RMP). The main active currents during phase 4, stabilising the RMP during the diastole, are the inward K^+ rectifier (I_{K1}) and the Na^+/K^+ ATPase (I_{NaK}), also known as the Na^+/K^+ pump. However, pacemaker cells present also other types of currents, e.g. the “funny” current (I_f), which slowly depolarises the cell during Phase 4.

During phase 4, immediately after the end of the AP, V_m is back to its resting value. However, the cell state is different from that preceding the AP, since the intracellular concentrations and ion gates are still recovering. In particular, during this time interval (generally lasting less than the AP) the I_{Na} channels are still recovering and are not able to open. Therefore, the cell is not able to generate a new AP and is said to be in refractoriness state.

Several biomarkers are generally used to describe and compare the AP properties. The most commons are:

- AP peak (APP), for the maximum value reached by the voltage after the upstroke.
- Resting/diastolic membrane potential (RMP or DMP), for describing the voltage during phase 4.
- AP amplitude (APA), representing the change in voltage from the resting to the peak value.
- $\frac{dV}{dt_{MAX}}$ or dV/dt_{MAX} , measuring the upstroke velocity, i.e., the rate of depolarisation during the upstroke.
- AP duration at X level of repolarisation (APD_X), indicating the time from the AP upstroke to a certain X percentage of repolarisation. Typical values are: 10%, 25%, 40%, 50%, 75%, 90%.

2.4 Cardiac AP abnormalities and arrhythmias

The AP dynamics described in the previous Section are the outcome of a complex and delicate network of subcellular processes. Many conditions as genetic mutations, diseases, and adverse drug reactions can affect the electrical activity of cardiac myocytes, disrupting the electrical sequence described so far and leading to AP abnormalities (El-Sherif and Turitto 2011). Two common electrical abnormalities occurring at the single-cell level are called early and delayed afterdepolarisations (EADs and DADs, respectively). As previously mentioned, after the depolarisation and the possible early repolarisation (phase 0 and 1), the cardiac AP does not repolarise immediately as in the neuronal AP (see Section 2.5). It lies at a higher voltage level (phase 2) until further repolarisation, due to the activation of different K^+ -currents (phase 3). However, in particular conditions such as an extended delay in the AP repolarisation, a net

inward depolarising current, usually I_{CaL} or I_{NCX} (Guo et al. 2007; Weiss et al. 2010), could suddenly occur in phases 2 or 3 (Figure 2.4). This can trigger new early depolarisations (an EADs, indeed) after the previous depolarisation (Antzelevitch and Burashnikov 2011). DADs are deflections of the membrane potential happening, instead, during the diastolic interval, usually in cells showing intracellular Ca^{2+} overload, due to, e.g., fast pacing (Mitsunori Maruyama et al. 2010). In particular, DADs might be able to depolarise the cellular membrane above the threshold for I_{Na} opening, and thus fire ectopic APs.

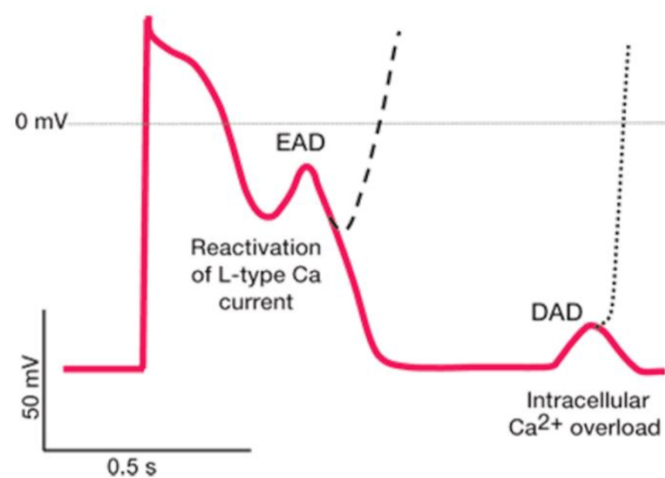


Figure 2.4. Examples of EADs and DADs. Modified from (Jameson et al. 2012).

Cardiomyocytes have many mechanisms of compensation to tackle possible abnormalities in their electrical activity. E.g., they are tightly electrotonically coupled to each other, impeding that electrical abnormalities arising from one or a bunch of cells could spread on the surrounding healthy tissue (see Section 2.6). However, when able to spread in tissue, abnormalities as EADs and DADs can disrupt the excitation-contraction pattern described in Section 2.2, and act as a trigger for arrhythmia.

Arrhythmias are alterations of the regular rhythm of the heart beating and can be very dangerous since they are one of the most common causes of sudden cardiac death, i.e., an unexpected stop of the heart function and the blood supplied to the brain and other vital organs, potentially causing death if not treated within minutes.

There are many types of arrhythmias and several methods for their classification. Based on the anatomical site, the most common are the sinus node re-entry, the supraventricular tachycardia, the atrial tachycardia, the atrial flutter, the atrial fibrillation, the ventricular tachycardia, and the ventricular fibrillation. Another classification is based on the ECG and, in particular, on the morphology of the QRS complex:

- **Narrow-QRS-complex:** indicates that the excitation of the ventricles occurs via the physiological His-Purkinje conduction system, leading mostly to non-life-threatening arrhythmias since the pumping function of the heart is still preserved.
- **Wide-QRS-complex:** suggests that the excitation does not reach the ventricles through the conduction system, which might have been bypassed by an ectopic beat or not being functional. In this case, the arrhythmia is potentially life-threatening, since the heart might be unable to accomplish its pumping function, leading in a few minutes to sudden cardiac death.

Even if the mechanisms of arrhythmia onset are still widely investigated across the globe, generally, two broad mechanisms have been identified as a source of arrhythmia onset:

- **Disorders in the electrical signal generation (focal activities):** these are generally referred as “Triggers” and are mostly associated with electrophysiological abnormalities producing ectopic beats that, if able to propagate in tissue, can disrupt the physiological excitation pattern and lead to arrhythmia. As already mentioned, examples of pro-arrhythmic triggers are EADs and DADs.
- **Disorders in the electrical signal conduction (re-entry):** these are generally associated with abnormal pathways for the electrical stimulations that bypass the physiological cardiac conduction system and can lead to arrhythmia. Conduction disorders can be related to structural tissue abnormalities as accessory pathways, rotating spiral waves

associated with non-conducting tissue (e.g., post-myocardial infarction scars) and tissue with altered electrophysiological properties (ionic remodelling, border zone).

2.5 Mathematical modelling of the action potential

2.5.1 Nernst potential and ionic current modelling

As described in Section 2.3, the AP represents a transient sequence of ionic fluxes, mainly Na^+ , K^+ , Ca^{2+} , happening across the cellular membrane. Since the membrane separates two different regions with different ionic charges, it can be represented by a dielectric with a capacitance of about $1 \mu\text{F}/\text{cm}^2$. Each ionic current, instead, depends on the number of channels on the cellular membrane, the state of the channels and the Nernst potential, expressed by the following equation:

$$E_s = \frac{RT}{FZ_s} \ln \left(\frac{C_{s,e}}{C_{s,i}} \right)$$

where R is the universal gas constant, T is the temperature in Kelvin, F is the Faraday constant, Z_s is the valency of a given ion s , and $C_{s,e}$ and $C_{s,i}$ are the extracellular and intracellular concentration of s , respectively. Therefore, the Nernst potential is different for each ion species and depends on the difference in the ion concentrations. E.g. for Na^+ , K^+ and Ca^{2+} at physiological concentrations (c_e/c_i) equal to 145/15 mM, 4/150 mM, 2 mM/70 nM, respectively, the Nernst potentials are:

$$E_{Na} = +61 \text{ mV} ; E_K = -97 \text{ mV} ; E_{Ca} = +137 \text{ mV}$$

The state and the number of channels of a given ion current define the conductance, g_s , of that current. In particular, the state of the channel is determined by its gates which, as mentioned above, can be active/inactive or close/open and vary between 0 (non-conducting) and 1 (fully-conducting).

Therefore, the conductance of a channel s can be expressed as:

$$g_s = G_{s,Max} \cdot gate1 \cdot \dots \cdot gate n$$

where, $G_{s,Max}$ represents the maximum number of channels and n the numbers of gates of the channels.

Then, a generic ion current I_s can be expressed by the equation:

$$I_s = g_s \cdot (V_m - E_s)$$

where V_m is the membrane potential and $(V_m - E_s)$ is called “driving force” since it determines how much current is going in/out across the membrane: the furthest V_m is from E_s , the larger the current will be crossing the membrane. In this context, the Nernst potential is also called “reversal potential”, since it determines the required voltage to switch the direction of the current across the membrane.

2.5.2 The first AP model

The first AP model was developed by (Hodgkin and Huxley, 1952) almost 70 years ago for the description of the neuronal AP of the squid giant axon. The equivalent electric circuit is shown in Figure 2.5 and uses the same formalism described above.

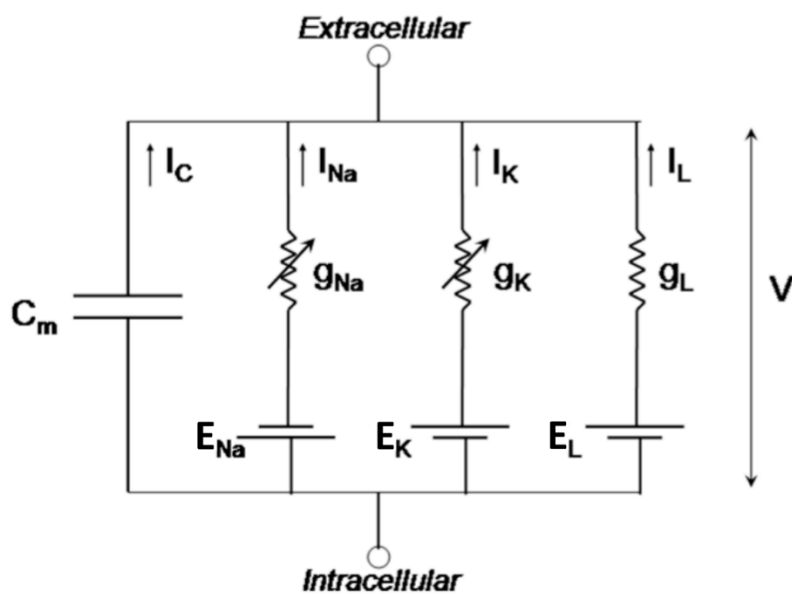


Figure 2.5. The electrical circuit representing the cell membrane, modified from (Hodgkin and Huxley 1952).

In particular, two specific ion currents are described for both Na^+ and K^+ , whereas, a generic leakage current is introduced to account for all the rest of the currents (pumps, exchangers, Ca^{2+} current).

The differential equation associated with the circuit above is

$$I_{tot} = I_{ion} + I_{stim} = C_m \cdot \frac{dV_m}{dt}$$

where I_{ion} represents the sum of all the ionic currents, I_{stim} the stimulus current required to depolarise the cell up to the threshold value and trigger the AP, C_m is the membrane capacitance, V_m is the membrane voltage, and t is the time. The solution of the equation above describes V_m instant by instant, and the full AP dynamic. However, it requires the description of each ionic current included in the model and, in particular, of their gating dynamics. In order to do so, Hodgkin and Huxley used a technique developed in 1949 by Cole and Marmont called “Voltage Clamp” (Marmont 1949; Verkhratsky, Krishtal, and Petersen 2006), which allows measuring the ionic current at a fixed membrane potential. It is possible to estimate the dynamic of each current repeating the voltage-clamp procedure at fixed ion concentrations and different constant voltage levels. In particular, Hodgkin and Huxley hypothesised three activation and one inactivation gates for I_{Na} , and four activation gates for I_K :

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

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

$$I_L = g_L \cdot (V_m - E_L)$$

where g_{Na} and g_K are equal to 120 and 36 (mS/cm^2) and represent the maximum conductance for I_{Na} and I_K , respectively, and I_L is the leakage current, proportional only to the driving force and the maximum conductance $g_L=0.3 \text{ mS}/\text{cm}^2$, without any gating dynamics. Therefore, the model has overall three gating variables, m , h and n , varying between 0 and 1 and modelled following the same formalism, e.g., for m :

$$\frac{dm}{dt} = \alpha_m(V) \cdot (1 - m) - \beta_m(V) \cdot m$$

where $\alpha_m(V)$ and $\beta_m(V)$ are known as rates and are non-linear and usually voltage-dependent.

Hodgkin and Huxley manually estimated such rates using the voltage clamp setup.

Figure 2.6 reports the stimulation current, the simulated AP and its gating variables using the Hodgkin and Huxley model. Compared to Figure 2.3, in addition to the differences in the resting membrane potentials (more negative for the cardiac APs), it is possible to notice that phase 1 and 2, described in Section 2.3, are missing in the neuronal AP waveform, as already mentioned in Section 2.3.

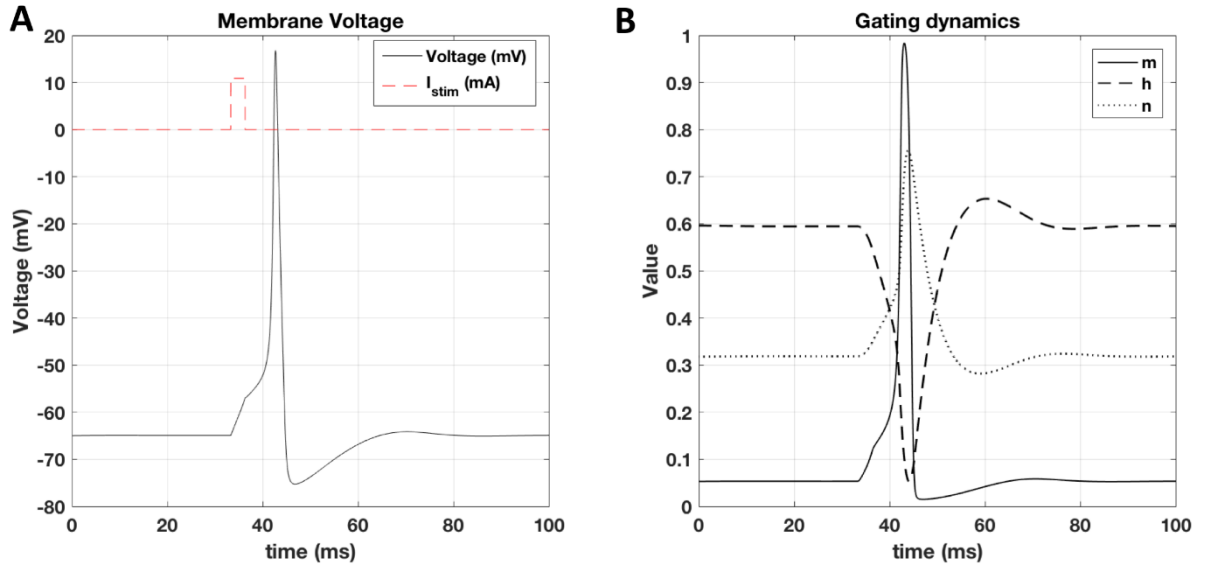


Figure 2.6. Simulations over time of the membrane potential dynamics using the Hodgkin and Huxley model: A) Stimulation current (red) and resulting action potential (black). B) gating variable dynamics underlying the AP.

2.5.3 From Hodgkin and Huxley model to cardiac AP models

Hodgkin and Huxley's pioneering work opened the way for many and different applications and developments of their model, allowing to gain insights by predicting phenomena which have been later confirmed experimentally. Ten years after the publication of the Hodgkin and

Huxley's model, Dennis Noble developed a modification of the neuronal model (Noble 1962), which represents the first cardiac AP model, showing more prolonged AP than the neuronal one and automaticity. Noble refitted the formulation for the fast sodium and inward rectifier currents, I_{Na} and I_{K1} , respectively, to electrophysiological recordings from mammalian (calf, sheep) cardiac Purkinje fibres (Weidmann 1955; Hutter and Noble 1960). The resultant I_{Na} was similar to that for the squid's axon depolarisation, though, it also showed a steady plateau sodium current, which was discovered experimentally more than 15 years later with the name of "late or window Na^+ current" (Attwell et al. 1979). In addition to I_{K1} , Noble included in his model a new K^+ current, representing the delayed K^+ rectifier, I_{Kr} , whose conductance slowly increases during the depolarisation phase, allowing a more prolonged AP. Also, Noble's formulation of I_{Na} and I_{Kr} , introduced a cycle between g_K and g_{Na} , which alternatively increase and decrease, allowing spontaneous pacemaker firing, without any additional current.

The diffusion of the voltage-clamp technique and the consequent increase of the availability of electrophysiological data boosted the development of new computational models. Also, new techniques were designed starting from the voltage clamp set up, such as the current clamp and the patch-clamp. The first is symmetrical to the voltage-clamp: a specific current signal is injected in the cell, whereas, V_m is allowed to vary and is measured by a low-impedance electrode. Patch-clamp, using glass micropipette, allows to record currents from a small portion of the membrane (ideally one or a few channels), improving accuracy and specificity of the ionic current measurements and, therefore, better understand also the channel properties.

Due to the limited availability of human tissue for experimental research, most of the experiments were conducted in animals and, consequently, most electrophysiological models were developed for animals. However, many differences exist between human and animal electrophysiology, in AP shape and duration, the role, contribution and dynamics of single ion currents, responses to heart pacing changes and drug therapy targeting specific ion channels.

These differences are crucial since they affect the translation from animal to human, making animal models potentially inadequate to investigate phenomena observed in humans, like arrhythmia, genetic mutations or diseases.

In the last two decades, more and more electrophysiological data have been gathered from humans, pushing the development of more human-based computational models. Figure 2.7 shows an overview of the computational cardiac electrophysiological models developed for sinoatrial, atrial, atrioventricular, cardiac Purkinje and ventricular tissues, based on the formulation used for the $\text{Na}^+\text{-K}^+$ pump. Different shapes indicate different species, whereas, each colour indicate the formulation used for modelling the $\text{Na}^+\text{-K}^+$ pump, mostly inherited by previous models (DiFrancesco and Noble 1985; Luo and Rudy 1994b).

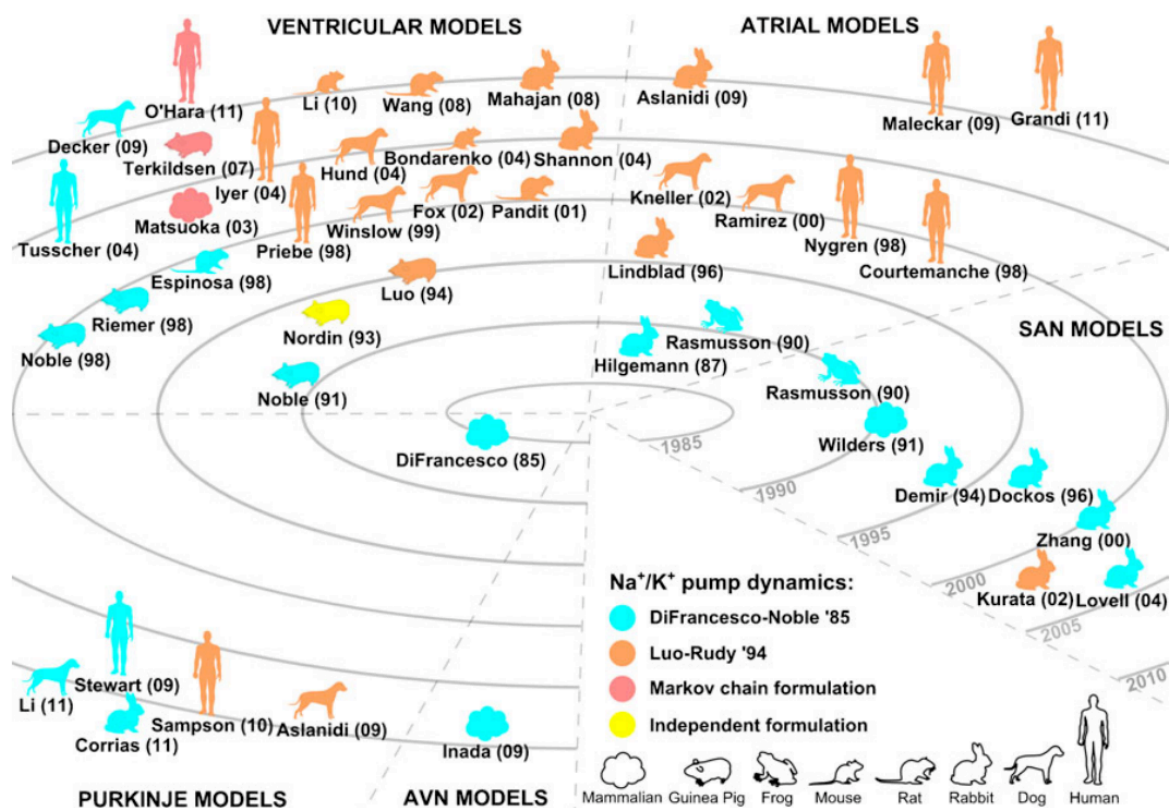


Figure 2.7. Cardiac electrophysiological models classification based on the $\text{Na}^+\text{-K}^+$ pump formulation (Bueno-Orovio et al. 2014).

As it is possible to observe in Figure 2.7, in almost three decades of research, the majority of the models were developed for muscular cardiomyocytes, ventricular (N=22) and atrial tissue (N=10), since more experimental data were available, and they are directly related to the cardiac contraction. However, also the other tissues play a role in the cardiac excitation pattern, as described in Section 2.2, and are involved in arrhythmia and cardiac dysfunctions (Dobrzynski et al. 2013; Haissaguerre et al. 2016). The next chapter will review the electrophysiological properties of one of these tissues, the cardiac Purkinje, as well as its mathematical modelling.

2.5.4 The ORd and PRd models

Among the currently available computational models in the literature, the most relevant for the research conducted in the present thesis were both published in 2011, and they are:

1. The human ventricular model developed by O'Hara and colleagues (O'Hara et al. 2011), to which I refer by the acronym "ORd".
2. The canine cardiac Purkinje model developed by Li and Rudy in 2012 (P. Li and Rudy 2011), to which I refer by the acronym "PRd".

Both models served as the base to design the new human Purkinje electrophysiological model presented in Chapter 4.

The ORd model was developed and validated by using electrophysiological data from more than a hundred of healthy human hearts, including many previously unpublished experimental data.

The model reproduces the steady state rate-dependence and restitution properties of human ventricular myocytes, including transmural heterogeneity, and drug blocks. The ORd model structure is reported in Figure 2.8A (top panel). The model includes a mathematical formulation for seven Na^+ , Ca^{2+} , and K^+ currents (namely, the fast and late Na^+ currents, I_{Na}

and I_{NaL} , the L-type Ca^{2+} current, I_{CaL} , the transient outward K^+ current, I_{to} , the rapid and slow delayed K^+ rectifier, I_{Kr} and I_{Ks} , and the inward K^+ rectifier, I_{K1}), the Na^+ - K^+ exchanger, I_{NCX} , and the Na^+ - K^+ pump, I_{NaK} . The cell was represented as a cylinder composed by four compartments: two for the intracellular space, representing the bulk cytoplasm (MYO) and the subspace near the T-tubules (SS), and two for the sarcoplasmic reticulum - the junctional (JSR) and the network (NSR). Each of these compartments includes different Ca^{2+} buffers, as troponin and calmodulin for MYO, and calsequestrin for JSR. The intracellular Ca^{2+} is handled by the Sarco-Endoplasmic Reticulum Calcium ATPase (SERCA) pump and the ryanodine receptors (RyR). Ca^{2+} ions are taken up into the NSR by SERCA, diffuse into the JSR where they are released by RyR and can diffuse to the MYO and SS. Besides, the effects of Ca^{2+} /Calmodulin-dependent protein kinase II (CaMKII) were reproduced as well for I_{Na} , I_{NaL} , I_{CaL} , I_{to} , RyR, and SERCA.

The resulting AP at 1 Hz for the endocardial cell is shown in Figure 2.8A (bottom panel). The AP starts from an RMP of -88 mV and quickly depolarises ($dV/dt_{Max} = 263$ mV/s) up to the peak value of 40 mV (APA= 128 mV). The early-repolarisation phase is not present, and the AP reaches 25% and 90% of repolarisation after 149 and 269 ms, respectively.

Figure 2.8B (top panel) shows the structure of the PRd model. Similarly to the ORd, the PRd model includes mathematical descriptions for I_{Na} , I_{NaL} , I_{CaL} , I_{to} , I_{Kr} , I_{Ks} , I_{K1} , I_{NCX} , and I_{NaK} , though, these were based on experimental data from canine PCs (Baláti, Varró, and Papp 1998; Bocchi and Vassalle 2008; Rota and Vassalle 2003; Wei Han et al. 2001; W Han, Wang, and Nattel 2000). In addition to these currents, the PRd model includes three more ionic currents, which are peculiar of the cardiac Purkinje electrophysiology: the T-type Ca^{2+} current, I_{CaT} , the sustained K^+ outward current, I_{sus} , and the “funny” current, I_f . Besides, the cell compartmentalisation and the Ca^{2+} handling are more complicated compared to the ORd and

account for the structural differences between Purkinje and ventricular cells. The intracellular space is divided into three compartments:

1. the peripheral coupling subspace (PCS), which contains the majority of both Ca^{2+} channels and RyR receptors.
2. the sub-sarcolemma (SSL), which represents the majority of the cell periphery volume and contains most of the non- Ca^{2+} channels.
3. the cellular bulk (MYO), which represents the inner space of the cell.

This three-layers model for the cell compartmentalisation has been suggested to reproduce better the cardiac Purkinje electrophysiology (Stuyvers et al. 2005), since it also accounts for the lack of t-tubules, as will be discussed in Chapter 3.

The sarcoplasmic reticulum is also divided into three compartments: in addition to the JSR and NSR, also implemented in the ORd, the corbular sarcoplasmic reticulum (CSR) was included in the PRd model, to represent the portion of the sarcoplasmic reticulum not in contact with the cellular membrane (Dolber and Sommer 1984) and releasing Ca^{2+} directly in MYO. This Ca^{2+} handling has been shown by Li and Rudy to be a pivotal element to induce DADs and triggered APs in specific conditions when the cell is experiencing Ca^{2+} overload due, for example, to high pacing.

Figure 2.8B (bottom panel) reports the simulated AP at 1 Hz using the PRd model. The AP considerably differs from the one simulated using the ORd model. Starting from an RMP of -85 mV, the cell is depolarised up to the peak value of 50 mV ($\text{APA} = 136$ mV) at a faster rate ($dV/dt_{\text{Max}} = 540$ mV/s) with respect to the ORd model. A prominent early-repolarisation phase follows the peak, and the AP reaches 25% and 90% of repolarisation after 16 and 370 ms, respectively.

2.6 The cardiac tissue and its multi-scale mathematical modelling

The computational methods introduced so far describe the electrophysiology at sub-cellular and cellular levels, while most biological phenomena happen from the interactions at several scales as the results of the coupling between different cells and their structural organisation. In this section, mathematical modelling is extended to describe the electrical activity in tissue and at the organ level.

2.6.1 *The cardiac tissue*

Three main functional elements determine cardiac propagation in tissue: the AP, the degree of cell-to-cell coupling, and the cellular network structure.

Firstly, as described in Section 2.3, AP properties as the resting membrane potential, refractory time, upstroke velocity, AP duration depend on the cell which has generated it. AP differences affect the electrical propagation, increasing the tissue heterogeneity, in particular, at the interfaces between different types of cells (e.g. atrioventricular node and His bundle, Purkinje network and ventricular endocardium). E.g. an ectopic depolarisation might be not able to propagate because the cells nearby are in refractoriness state and, therefore, does not induce any changes at the tissue scale.

Secondly, APs propagate across the surrounding cells because myocytes are tightly coupled with their neighbour cells through low-resistance pathways, called gap junctions (Van Veen, Van Rijen, and Opthof 2001). Gap junctions are comprised of two connexon hemichannels, each composed of six connexin molecules. The main connexins of the myocardium are Cx43 (abundant in ventricles), Cx40 (dominant in the His-Purkinje system), and Cx45 (present in the atrioventricular and sinoatrial nodes and, in lower amounts, in atrial and ventricular myocardium). Cx43 and Cx40 gap junctions have a relatively large unitary electrical

conductance, g_j (~ 100 pS and 174 pS, respectively) and allow faster AP propagation, whereas g_j of Cx45 is considerably smaller (~ 30 pS), reducing the speed of the electrical wave (L. Harris 2001). Gap junctions regulate the electrical charges flowing from the stimulating cells, forming a source effect, and the stimulated ones, which act as a sink. The balance between the source and sink is crucial for the electrical propagation and the conduction safety (Boyle and Vigmond 2010): the amount of current produced by the source needs to be sufficiently large to excite the sink; otherwise, the AP would not be able to propagate, blocking the electrical conduction. EADs and DADs represent an example of the source-sink mismatch, as mentioned in Section 2.4: if a small number of cells experiences the electrical abnormalities, the source is small and unable to excite the tissue, which represents a large sink. (Xie et al. 2010).

Finally, as described in Section 2.1, the heart is not a homogeneous syncytium and presents different cellular network structures, which affect the electrical propagation (De Bakker and Van Rijen 2006). For example, the epicardial ventricular myocytes are arranged in layers, whereas, those comprising the endocardium are trabeculated. Also, ventricular cardiomyocytes are arranged in fibres, whose orientation changes from apex to base and transmurally. Structural differences are crucial due to the anisotropy of the cell-to-cell propagation. APs propagate faster in the direction of the cardiac fibres because of the low intracellular resistance along the longitudinal axis of fibres, and slower across the fibres, due to the high transversal intracellular resistance.

2.6.2 The cable and multi-scale modelling

Combining the computational modelling at the single-cell level with the effects of the intercellular coupling allows designing higher scale models, starting from a cable geometry up to the whole organ tissue.

The cable equation was initially used to study signal transmission in transatlantic telegraph cables (Thomson 1854) and later applied to describe the electrical propagation along the axon

of nerve cells (Hodgkin and Huxley 1952). Figure 2.9 shows the electrical circuit for the AP propagation modelling, including voltage and resistance at different points of the cable, both for the intracellular and extracellular space. The rectangle in blue represents the cell membrane, where C_m is the membrane capacitance following the same formalism of Figure 2.5, and I_{ion} is the sum of the all ionic currents.

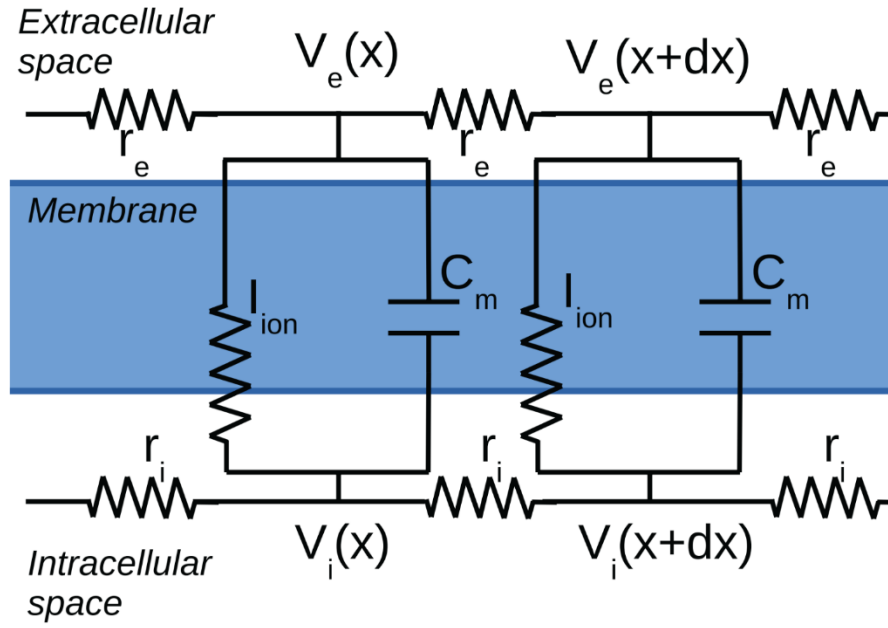


Figure 2.9 Electrical circuit model for the AP spatial propagation. The membrane separates extracellular (e) and intracellular space (i). V : voltage; r : resistance C_m : membrane capacitance; I_{ion} : the sum of the all ionic currents. From (Alonso, Bär, and Echebarria 2016).

Assuming that the membrane potential varies only along the longitudinal direction of the cell while it is constant across the transversal/radial direction, the membrane potential variation along the fibre will generate intra and extracellular currents. Applying Ohm's law, both currents per unit length can be expressed by the following equations (Alonso, Bär, and Echebarria 2016):

$$I_i(x) = \frac{g_i[V_i(x + dx) - V_i(x)]}{dx} \quad (1)$$

$$I_e(x) = \frac{g_e[V_e(x + dx) - V_e(x)]}{dx} \quad (2)$$

where g_i and g_e are the conductances for unit length for the intra and extracellular space.

When considering infinitesimal space increment, $dx \rightarrow 0$ and the above equations, became

$$I_i(x) = g_i \frac{\partial V_i}{\partial x}; \quad (3)$$

$$I_e(x) = g_e \frac{\partial V_e}{\partial x} \quad (4)$$

From the first Kirchhoff's law, the net current flowing in/out a specific point must sum up to zero

$$I_i(x) - I_i(x + dx) = I_t dx = I_e(x + dx) - I_e(x);$$

$$I_t = -\frac{\partial I_i}{\partial x} = \frac{\partial I_e}{\partial x}; \quad (5)$$

The transmembrane current I_t can also be expressed as the sum of ionic and capacitive currents:

$$I_t = \rho \left(C_m \frac{\partial V}{\partial t} + I_{ion} \right); \quad (6)$$

Combing the right-hand sides of both equations:

$$\rho \left(C_m \frac{\partial V}{\partial t} + I_{ion} \right) = \frac{\partial}{\partial x} \left(g_i \frac{\partial V_i}{\partial x} \right) = -\frac{\partial}{\partial x} \left(g_e \frac{\partial V_e}{\partial x} \right); \quad (7)$$

and, since $V_i = V + V_e$:

$$\rho \left(C_m \frac{\partial V}{\partial t} + I_{ion} \right) = \frac{\partial}{\partial x} \left(g_i \frac{\partial V}{\partial x} \right) + \frac{\partial}{\partial x} \left(g_i \frac{\partial V_e}{\partial x} \right); \quad (8)$$

From the first Kirchhoff's law (5), it is satisfied that:

$$g_i \frac{\partial^2 V_i}{\partial x^2} + g_e \frac{\partial^2 V_e}{\partial x^2} = g_i \frac{\partial^2 V}{\partial x^2} + (g_i + g_e) \frac{\partial^2 V_e}{\partial x^2} = 0; \quad (9)$$

The last two equations represent the bidomain formulation of the cable equation (Alonso, Bär, and Echebarria 2016) since they describe both intra and extracellular fields. The integration of equation (9) leads to the following solutions:

$$g_e \frac{\partial V_e}{\partial x} = -g_i \frac{\partial V_i}{\partial x} + C; \quad \text{and} \quad \frac{\partial V_e}{\partial x} = -\frac{g_i}{g_i + g_e} \frac{\partial V}{\partial x} + \frac{C}{g_i + g_e}; \quad (10)$$

which can be insert into equation (7) to obtain the monodomain version of the cable equation:

$$\rho \left(C_m \frac{\partial V}{\partial t} + I_{ion} \right) = g_{eff} \frac{\partial^2 V_e}{\partial x^2}; \quad (11)$$

where $g_{eff} = \frac{g_i g_e}{g_i + g_e}$ is the harmonic mean of the ionic conductances.

The equations described so far represent the electrical propagation within the same cell. For modelling the AP propagation in tissue, the cellular coupling through gap junctions needs to be modelled as well. There are several approaches to model the intercellular coupling. A simple approach consists of assuming the potential to be constant for the whole cell (isopotential model) and model the gap junctions as resistors, with a channel-gate like dynamic or set to a constant value.

It is possible to simulate the electrical propagation within a plane or volumetric portion of tissue, up to the whole organ level, generalising to two and three dimensions the cable equation. In particular, equations (3) and (4) can be reformulated as below (Alonso, Bär, and Echebarria 2016):

$$J_e = \sigma_e \nabla V_e; \quad J_i = \sigma_i \nabla V_i; \quad (12)$$

where $\sigma_e(x)$ and $\sigma_i(x)$ are tensors and define the conductivity in the three dimensions for the extra and intracellular space, respectively. The spatial anisotropy of the electrical propagation described in Section 2.6.1 can be implemented through different values of $\sigma_e(x)$ and $\sigma_i(x)$ for different directions. Defined ψ as the ratio of surface to volume for the cell, the following equations

$$\psi \left(C_m \frac{\partial V}{\partial t} + I_{ion} \right) = \nabla \cdot (\sigma_i \nabla V_i) = -\nabla \cdot (\sigma_e \nabla V_e); \quad (13)$$

$$\nabla \cdot (\sigma_i \nabla V_i + \sigma_e \nabla V_e) = 0; \quad (14)$$

represent the equivalent versions of equation (8) and (9) and the bidomain formulation for the multidimensional model. Assuming that the anisotropy ratios are the same in the intracellular and extracellular space (i.e., $\sigma_i = \kappa\sigma_e$) it is possible to reduce the model to the monodomain formulation, similarly to equation (11). From equation (14):

$$\nabla \cdot (\sigma_e \nabla V_e) = - \frac{\kappa}{1 + \kappa} \nabla \cdot (\sigma_e \nabla V);$$

which can be substituted in the right-hand side of equation (13) to obtain:

$$\psi \left(C_m \frac{\partial V}{\partial t} + I_{ion} \right) = \frac{\kappa}{1 + \kappa} \nabla \cdot (\sigma_e \nabla V); \quad (15)$$

and

$$\frac{\partial V}{\partial t} = - \frac{I_{ion}}{C_m} + \nabla \cdot (D \nabla V); \quad (16)$$

where D is the diffusion coefficient which accounts for the conductivities between cells, the membrane capacitance and the surface to volume ratio:

$$D = \frac{\kappa \sigma_e}{C_m \psi (1 + \kappa)}$$

It is worth to highlight that the anisotropy ratios are never the same in the intracellular and extracellular space in the heart. For this reason, the bidomain formulation provides a more comprehensive continuum representation of the electrical propagation. However, the monodomain model is accurate enough in most on the electrophysiological scenarios, since it matches velocities along the axis but not along the off-axis direction. Also, the computational cost of the monodomain formulation can be even two orders of magnitude lower than the bidomain one (Sundnes et al. 2006) and can work with less refined geometrical meshes than those necessary for the bidomain model.

Multi-scale modelling allowed to represent the cardiac electrophysiology from the ion channels up to tissue/organ/torso level as shown in Figure 2.10 (Rodriguez et al. 2016), through the integration and augmentation of large and heterogenous clinical and experimental data with the

most advanced computational and engineering techniques. This approach provides mechanistic-based tools, which can gather a considerable amount of data and produce insights of what is happening in the heart at every level, allowing to describe, and even predict, behaviours and dynamics often impossible to observe experimentally with the currently available *in vitro* and *in vivo* models.

In the last years, new technologies (Corral-Acero et al. 2020; Lancaster and Sobie 2016; Lyon et al. 2018) are arising from the combination of multi-scale computational modelling with advanced statistical methods, also known recently as machine learning techniques, which allows extracting the most valuable information from large and complex databases to feed the computational models or to evaluate their predictions better.

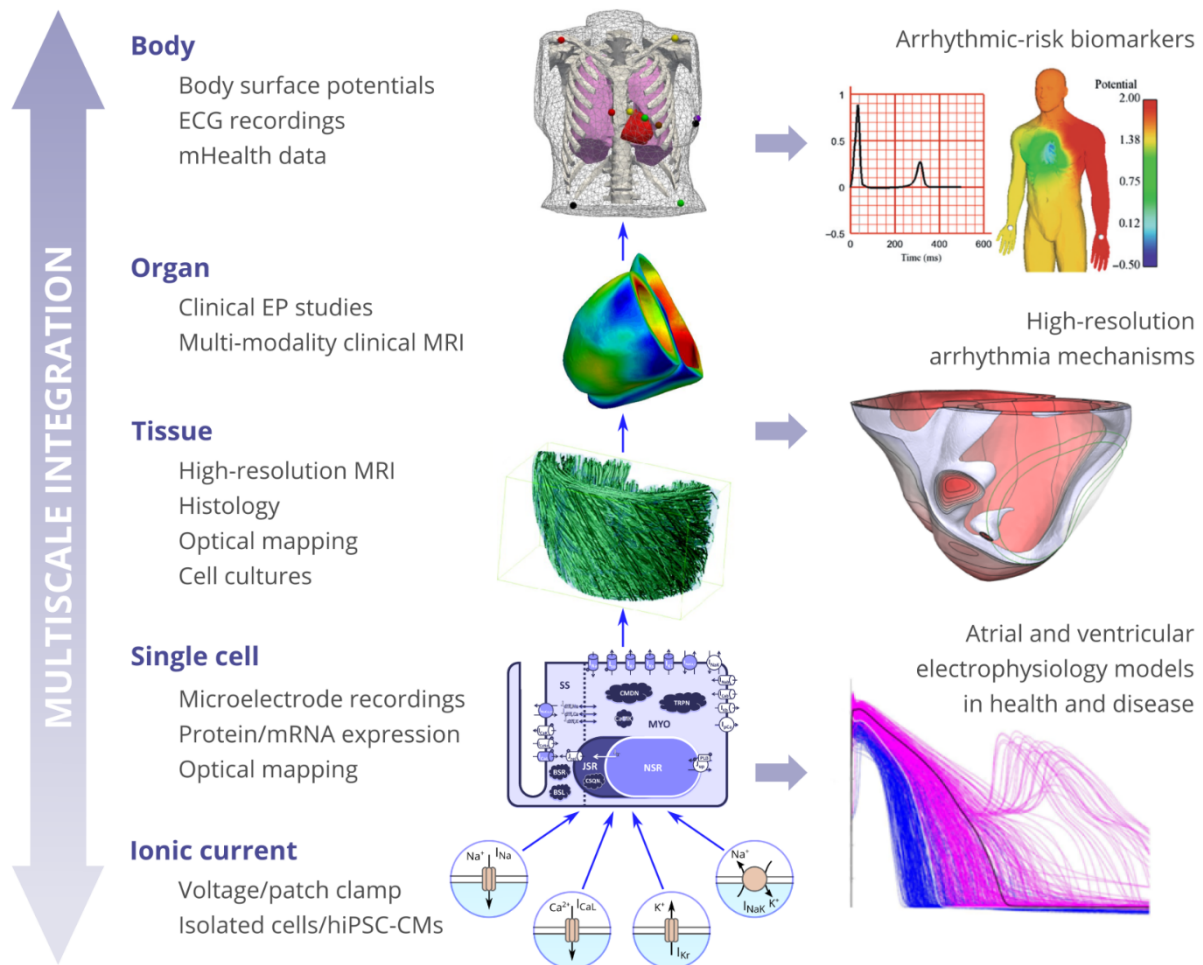


Figure 2.10 Computational multi-scale modelling applied to cardiac electrophysiology (Rodriguez et al. 2016). From the bottom (ionic current) to the top (body), the integration of several types of data allows to design biophysically-detailed computational models to represent the electrophysiology of the heart at every scale.

2.7 Biological Variability

2.7.1 Who? Why? When?

Understanding the mechanisms and the sources of variability, a characteristic ubiquitous in the whole field of biology, represents one of the biggest challenges both for biology and medicine. Two flowers of the same type, even if harvested from the very same plant, likely are not going to look the same, since, e.g., same petals will have different sizes or colours. The same is true for two human hearts: the APs underlying their beating are going to be very similar, but not exactly the same. In physiological conditions many factors underly such intra-species

variability as sex, age, nutrition, lifestyle, circadian rhythm (Abi-Gerges et al. 2006; Gaborit et al. 2007; Jeyaraj et al. 2012; Herraiz-Martínez et al. 2015), whose effects also evolve during the life of the same organism. Most of the time, those differences do not have any effects on the pumping activity of the heart, though, in compromised and diseased scenarios, they might become relevant and disrupt the cardiac function. Cardiac ischemia, myocardial infarction, heart failure, inherited diseases, pharmacological therapies increase electrophysiological heterogeneity and make the heart more prone to develop pro-arrhythmic abnormalities in specific conditions (Sims et al. 2008; Abi-Gerges et al. 2004; Bueno-Orovio et al. 2012; Yang and Clancy 2012). Variability needs to be considered for a mechanistic identification of the subpopulation more at risk of developing cardiac dysfunction as arrhythmia, which means ultimately answering the three questions: who (is more prone to develop arrhythmia)? When (which are the conditions that trigger arrhythmia)? And why (which are the mechanisms underlying arrhythmia and how is possible to stop it)?

For many years, variability was considered a problem and a mere source of noise and uncertainty, to be removed through the use of mean values and standard deviations, without providing a direct way to estimate the biological variability. Part of the variability indeed observed in any electrophysiological datasets is due to the experimental conditions, and manual or systematic errors (Johnstone et al. 2016), however, in the other hand, it is also a source of information and has to be considered for moving from an “average” to a “real” representation of specific biological entities or medical cases. E.g., an average cellular model can provide an excellent and useful indication about a specific behaviour, but that *in silico* cell has never existed, which represent a limitation, especially when considering patient-specific care and precision medicine (Vogenberg, Barash, and Pursel 2010).

2.7.2 Modelling cell-to-cell variability

As explained above (Section 2.3), the AP is controlled by the contribution of multiple ionic currents, crossing the cellular membrane through specialised proteins. It is reasonable to assume that the variability between single APs derives from differences in those ionic currents (Szentandrassy et al. 2015). Also, since within the same species the structure and function of the ionic channels are considered to be the same (in the absence of genetic mutations), cell-to-cell ionic current differences are reasonably due to differences in the density on the membrane surface of the expressed ion channels, which modulates the magnitude of the relative ionic currents. Typical examples of cell-to-cell variability due to different ion channel expressions are the transmural heterogeneity among endo, mid and epicardial cells comprising the ventricular wall and the differences between left and right ventricles (Boukens et al. 2015; Bueno-Orovio et al. 2012; A. J. Atkinson et al. 2013; Gaborit et al. 2007).

In this context, two main approaches have been proposed in the last years for reproducing the cell-to-cell variability: sensitivity analysis-based approach and the population of models approach. In summary, the first consists of varying one or a few model's parameters in a range around a fixed parameter set (usually the nominal values published with the model, which represent an optimal average behaviour) and computing for each of those input parameters a set of biomarkers to evaluate their variation with respect to the biomarkers generated with the original mean model (Romero et al. 2009; Corrias, Giles, and Rodriguez 2011; Mora et al. 2017; Deng et al. 2019; Levrero-Florencio et al. 2020).

Using the population of model approach (Muszkiewicz et al. 2015; Britton et al. 2013), instead, simulations are performed in thousands of models at the same time (a population, indeed), all generated by varying some of the model parameters around their nominal values. Figure 2.11A shows experimental AP traces from the same type of cells from different hearts, i.e. human Purkinje cells (data by the University of Szeged), and a simulated AP trace (top panel). AP

traces with the same colour were recorded from different fibres of the same heart, while, AP traces with different colours indicate recordings from different hearts. The cell-to-cell differences account, therefore for both inter- and intrasubject variability. Overall, APs have a similar waveform, though every cell exhibits a distinct AP. Variability is visible since the early phase of the AP repolarisation and is larger during the later phase, leading to a wide range of APD₉₀ (from 200 to more than 400 ms). The computational model reproduces the average behaviour observed in the experiments, but it is not able to represent the variability, which is lost. A population of models, instead, can capture such variability (Figure 2.11A, bottom panel), using different models to represents different cells of the same cell-type. Notably, the population is calibrated considering experimental data, either original or from the literature. The population of models approach mainly consists of two consecutive steps:

- 1) Generation: first, the population of models is generated varying a subset of parameters, early identified or assumed as the primary source of variability, randomly or following a statistical distribution, usually within wide ranges. In this way, a large number of models is generated starting from the mathematical structure of a template (or baseline) model.
- 2) Experimental calibration: the outputs of each model is then compared with one or more experimental datasets, through the use of clinical biomarkers. Only the models having all the simulated biomarkers within the experimental ranges are considered accepted, whereas, those not consistent with the data are discarded.

The population of models approach allows then to cover a whole range of experimental values and enlarge any investigation from the average values of the baseline model to the whole experimental datasets available. The use of many models, of course, implies a significant increase in the computational resources needed to run the simulations, especially when using

higher dimensions models. In this regard, optimising the numbers of parameters to be varied and their variation ranges can help in reducing the computational resources needed. A possible workflow to efficiently implement the population of model approach is reported in Figure 2.11B (Muszkiewicz et al. 2015). Started from a small initial population, it is possible to reiterate the steps 1 and 2 described above to design an optimised population able to reproduce the whole experimental variability with the minimum number of models.

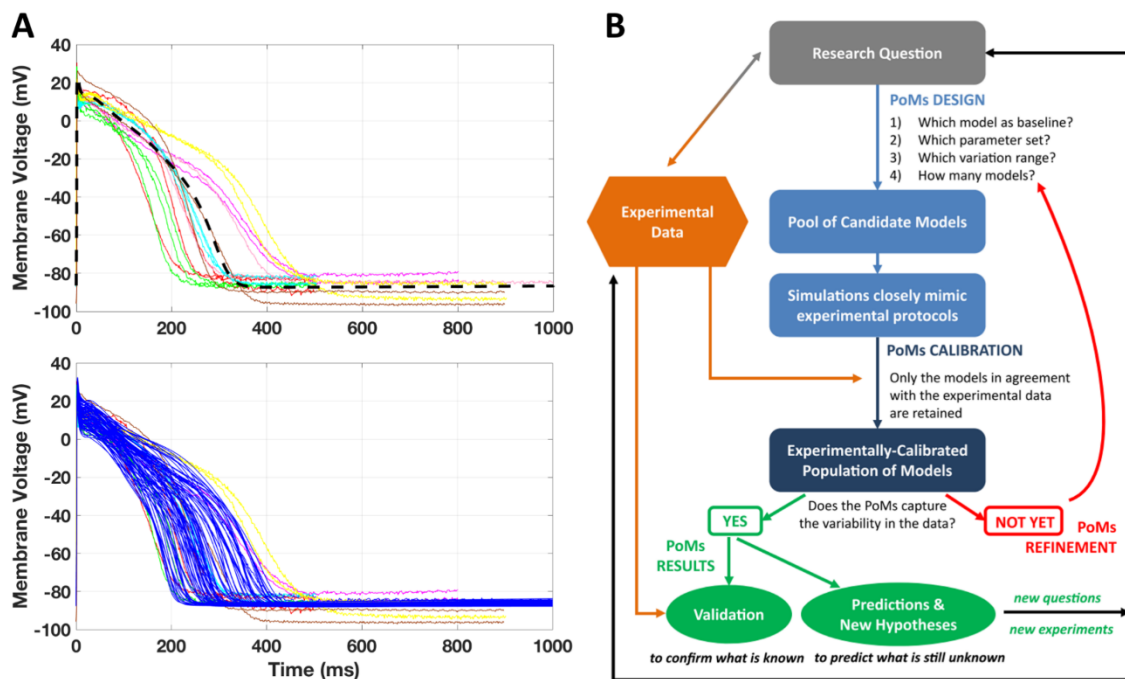


Figure 2.11 The population of models approach: A) Experimental AP recordings from human Purkinje cells from different hearts (each colour represents a different heart) for both panels. One single simulated AP model (dashed trace, top panel) is not able to capture the experimental AP variability, which can be represented through a population of models (blue traces, bottom panel). B) Flowchart representing the population of models (PoMs) approach from (Muszkiewicz et al. 2015).

The population of models approach, extensively used in the last years, has led to promising results, especially in the field of drug safety and efficacy assessment (Passini et al. 2017, 2019; Zhou et al. 2020) and for arrhythmia risk stratification in healthy and diseased populations (Zhou et al. 2019; Passini et al. 2016; Paci et al. 2018). Other approaches have also been

proposed to take into account the biological variability in cardiac computer models (Sarkar, Christini, and Sobie 2012; Romero et al. 2009; Groenendaal et al. 2015; Vagos et al. 2017; Kernik et al. 2019), and the importance of taking into account variability has been acknowledged in recent years (Ni, Morotti, and Grandi 2018; Wiśniowska, Tylutki, and Polak 2017).

The population of models approach was used in the present thesis (Chapter 5), to mechanistically investigate human Purkinje AP variability and PCs susceptibility to electrical abnormalities as EADs and DADs.

Chapter 3 - The cardiac His-Purkinje system: a literature review

In this chapter, I present a detailed description of the structure, function and electrophysiology of the “*His-Purkinje System*”(HPS), as well as the main differences between ventricular and Purkinje electrophysiology and their response to pharmacological interventions. The final part of the chapter describes the most recent advances in computational modelling and simulation of PCs, with a particular focus on human models.

3.1 Introduction

As described in the previous chapter, Section 2.2, the ventricular excitation-contraction coupling is controlled by a complex, highly-efficient and specialised conduction system, composed by the His bundle, Tawara branches and Purkinje fibres (Vigmond and Stuyvers 2015). Indeed, maintaining a constant hemodynamic flow is a high-energy demanding process, requiring an optimised excitation-contraction coupling. This is especially true for the ventricles, due to their higher mass, pumping oxygen-rich blood toward the whole body.

In 1845, J.E. Purkinje described for the first time this tangle of fibres, present into the sub-endocardium of the ventricles of the sheep heart, which appeared to be flat and gelatinous.

More than 50 years later, in 1906, Tawara realised that Purkinje fibres, with a combination of sub-endocardial and free running fibres, were the terminal reticular network of the cardiac conduction system in the heart of several species like human, dog, cow and sheep (Ono et al. 2009).

Since then, many studies were published on the HPS, due to its vital importance to guarantee a correct excitation pattern and therefore a synchronised sequence of cardiac contraction. Involvement of HPS has been shown in intraventricular re-entry (Lopera et al. 2004), monomorphic or polymorphic ventricular tachycardia (Haissaguerre et al., 2016), as well as in the generation of Torsade de Pointes arrhythmias, associated with the long QT syndrome (V. Iyer et al. 2015), either genetic or drug-induced (Schram et al., 2002).

However, the physiological function of HPS and their role in arrhythmogenesis, especially in human, have not been completely understood yet, due to multiple reasons, e.g., the awkward position of the HPS, running within muscular trabeculae; the difficulties in isolating Purkinje Cells (PCs), due to the hard-insulating sheath around the fibres; the strong species specificity of PCs.

Computer models are a useful tool to represent and investigate biological mechanisms both in single cells and in tissue, especially in human (Rodriguez et al. 2016; Trayanova 2011). As described in Section 2.5.3, in 1962 Noble (Noble 1962) modified the Hodgkin and Huxley model (Hodgkin and Huxley 1952) to obtain the first cardiac computational model, also representing the first *in silico* model for cardiac Purkinje electrophysiology. Since then, several electrophysiological models have been published for mammalian PCs (McAllister, Noble, and Tsien 1975; DiFrancesco and Noble 1985; Corrias, Giles, and Rodriguez 2011; P. Li and Rudy 2011; Vaidyanathan et al. 2013; Britton et al. 2013; Aslanidi et al. 2010; Cherry and Fenton 2012) and three for human PCs (Stewart et al. 2009; Sampson et al. 2010; ten Tusscher and

Panfilov 2008). The latter are discussed at the end of the chapter as well as their applications for the *in-silico* investigation into Purkinje electrophysiology in single-cell and tissue.

3.2 The His-Purkinje System

3.2.1 Structure

The HPS architecture is strongly species-dependent and is different, for example, between sheep and human (Ono et al. 2009). Three different types of HPS have been recognised in mammals, mainly differing on their size and structure: 1. ungulates; 2. human, monkey, cat and dog; 3. Mouse, rat and rabbit. (Vigmond & Stuyvers, 2015, Ono et al., 2009).

HPS is generated from visceral mesoderm. At early stages of myocardium development, after the formation of the cardiac nodes and the AV conduction axis, some of the ventricular trabeculations are incorporated to form the Purkinje network (Dobrzynski et al. 2013). The origin of the differences between PCs and ventricular cells (VCs) is suggested to be at the transcriptional domain levels (Antoon F.M. Moorman, Frits de Jong 1998). Both tissues originated from the myocardium; however, the bundle of His and the bundle branches are exposed to peculiar regulatory signals at the very early stages of the development (Christoffels and Moorman 2009), responsible for their specialisation in conductive tissue.

In the intraventricular septum, the bundle of His bifurcates in the asymmetric left bundle branch and right bundle brunch, forming roughly a ribbon-like structure, even if a high variability of the HPS shapes has been described in the literature (Fig. 3.1).

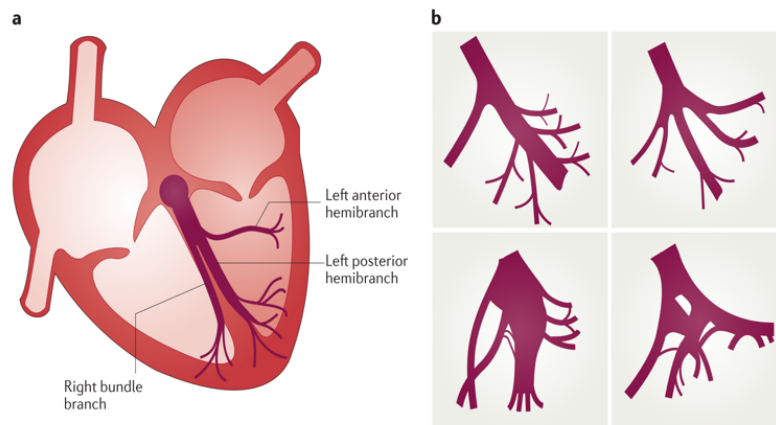


Figure 3. 1. a) Schematic representation of the HPS. b) Different types of Purkinje bundle infrastructures, from (Haissaguerre et al. 2016).

Usually, the right branch has a linear shape, it is parallel to the septum until the moderator band, and only later it ramifies. The left bundle bifurcates almost at the top of the septum into different fascicles and forms a more complex network, able to activate the larger mass of the left ventricle (Haissaguerre et al. 2016). Also, the right bundle branch is cord-like, whereas the left bundle branch shows a fan-like structure (Dobrzynski et al. 2013).

Purkinje cells are parallel to each other, forming either a small fibre (2-8 oval cells) or a fascicle (many cells), that could be wrapped by a collagen sheath which makes them electronically isolated from the surrounding VCs. In sheep, goats, dolphins, and whales, a sheath surrounds a whole Purkinje strand, whereas, in humans and other animals, the sheath surrounds individual PCs (Ono et al., 2009).

Purkinje fibres can be either sub-endocardial or free-running fibres. The latter, also called “false tendons” due to their shape, at the mid-portion of the septum, separate from the underlying endocardium, traverse the ventricular chambers to reach the free-wall and spread one more time into the free-wall endocardium principally towards the papillary muscles (Dobrzynski et al. 2013).

Finally, transitional myocytes (Dobrzynski et al. 2013) or Purkinje-myocyte junctions (Vigmond and Stuyvers 2015) are specific sites where PCs arise from the isolating sheath and form electrical junctions with ventricular myocytes.

3.2.2 *Function*

The primary function of the HPS is rapidly propagating the electrical stimulation into the ventricles. A secondary function is working as a pacemaker in case of malfunction of the other two cardiac pacemakers, the sinoatrial node (SA-node) and the atrioventricular node (AV-node) (Dobrzynski et al. 2013). In physiological conditions, the Purkinje pacemaker activity is suppressed by the higher rate of the SA-node. This phenomenon is commonly called “*Overdrive Suppression*” (Boyden et al., 2010).

In mammals, the HPS shows a higher conduction velocity in tissue in comparison with the other parts of the cardiac conduction system. Electrical excitation propagates from SA-node to atrial muscle rapidly (0.1-1 m/s) and slows down near the AV-node (0.01-0.05 m/s). Then the propagation is fast through the HPS (2-4 m/s) and gets slightly slower through ventricular myocytes (0.3-1 m/s) (Bers 1993).

The high conduction velocity in the HPS is due to two main factors: i) the characteristics of the action potential (AP) ii) the expression of different types of connexins. The former will be discussed in details in the next section, while the latter has arisen from the analysis of the RNA-expression in human (Gaborit et al. 2007) and rabbit (A. Atkinson et al. 2011). In both species, HPS shows higher expression of large conductance connexins (Cx40), while ventricles are rich of medium conductance (Cx43).

3.2.3 *Purkinje vs Ventricle*

Figure 3.2A report a low-power SEM image of muscular trabeculae in a human left ventricle, including the Purkinje network (Ono et al. 2009). Many differences exist between Purkinje and

ventricular cells, due to their shape, dimension, intracellular structure and trans-membranal ion channels. Figure 3.2B shows two photomicrographs of a Purkinje cell (left) and a ventricular cell (right) obtained with light microscopy from human preparations (W Han, Zhang, et al. 2002). Usually, PCs are bigger than VCs, even if differences in the cell size are also species-dependent. Indeed, PCs have been reported to be bigger than VCs in human, sheep, goat, dog, and seal, but smaller in monkey and rat (Ono et al. 2009). In human, PCs contain scarce myofibrils compared with ventricular myocytes, according to their less contractile properties in comparison to VCs.

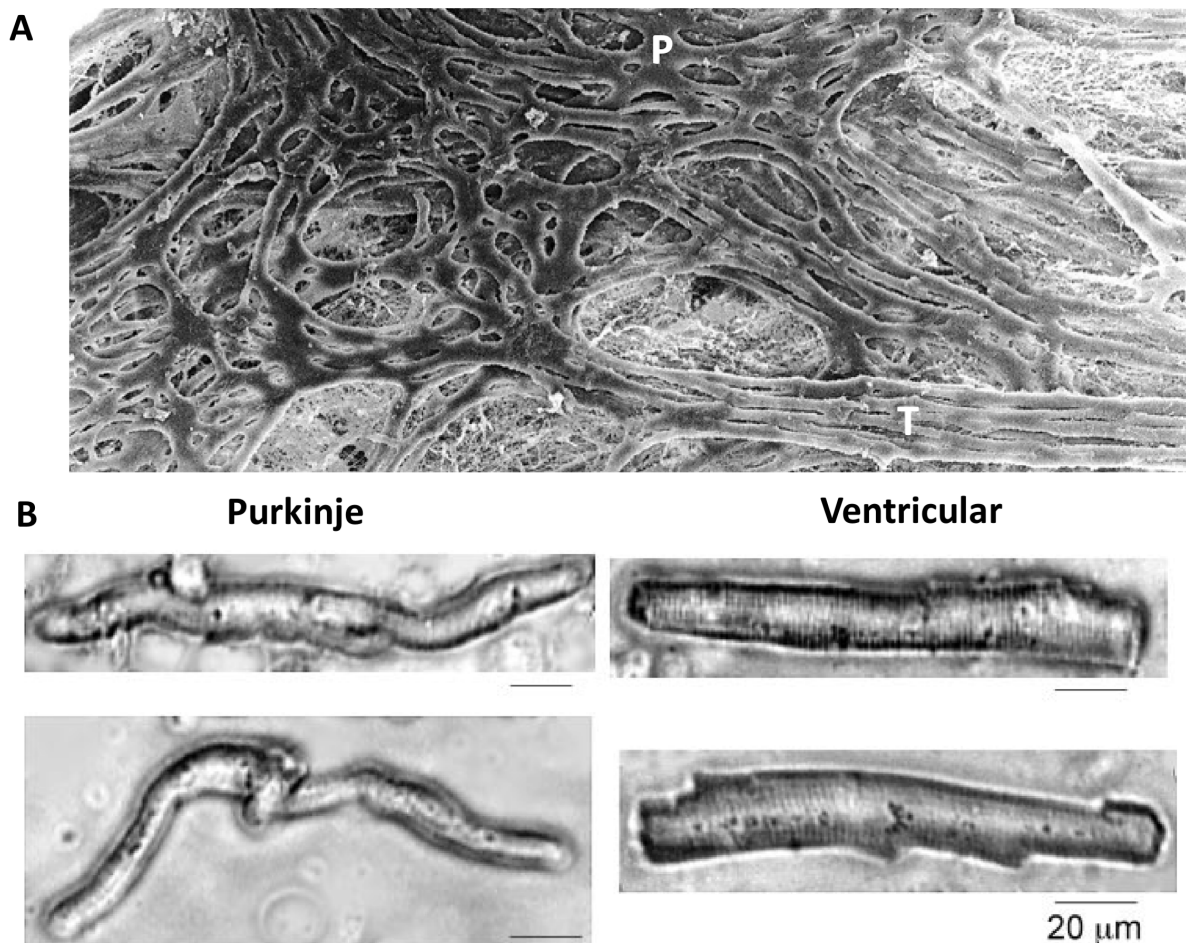


Figure 3.2. Comparison between Purkinje and ventricular tissue in humans. A) A low-power SEM image of muscular trabeculae in a human left ventricle, including a complicated network of PCs. Modified from (Ono et al. 2009). P: Purkinje; T: trabeculae. B) Photomicrographs of a Purkinje cell (left) and a ventricular cell (right) from human preparation. Modified from (W Han, Zhang, et al. 2002).

Also, two different types of mitochondria have been identified in PCs: the majority were similar to the ones seen in VCs, but the remaining ones, more similar to those present in the axons of adjacent autonomic nerve fibres, were more common in PCs than VCs (Sommer and Johnson 1968).

One of the most significant difference is the density of the ventricular transverse-tubules (t-tubules). It is generally assumed that PCs lack *t-tubules*, though, a small number of PCs with *t-tubules* were found flanked by *t-tubule* free segments (Di Maio et al., 2007). *T-tubules* are extensions of the sarcolemma that penetrate the centre of the cell, and that are present in VCs. The absence of *t-tubules* has significant consequences on the ionic processes inside the membrane since in PCs intracellular ionic concentrations substantially differ from the central to the sub-sarcolemma area, which is more involved into the ionic trans-membranal exchanges (Stuyvers et al. 2005). The most significant difference concerns the intracellular Ca^{2+} dynamics, which are extremely important for the excitation-contraction coupling.

In both types of cells, a uniform Ca^{2+} wave develops in the sub-sarcolemma, due to the influx of Ca^{2+} through the membrane proteins $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$, responsible for the trans-membranal ionic flux generating the L-type calcium current (I_{CaL}). The Ca^{2+} influx increases the intracellular Ca^{2+} concentration and triggers two different processes: 1) a slow diffusive flux towards the bulk of the cell and 2) a fast-chemical mechanism of propagation, called *Ca^{2+} -induced Ca^{2+} release (CICR)*, which involves the ryanodine receptor (RyR) channels. These are the intracellular Ca^{2+} release units of the sarcoplasmic reticulum (SR), which open in response to Ca^{2+} increase, allowing a fast Ca^{2+} propagation within the cell.

In this context, the absence of t-tubules provokes a larger intracellular Ca^{2+} concentration gradient in PCs in comparison with VCs, i.e., the Ca^{2+} level is initially higher in the proximity of the membrane and lower in the centre (Haq et al., 2013, Stuyvers et al., 2005). Then, a slow diffusion process from the cell periphery to the centre reduces the Ca^{2+} gradient in PCs. The

resulting Ca^{2+} waves seem to be significantly crucial in PCs, and they are responsible for local intracellular Ca^{2+} releases. Indeed, the most inner part of the SR is generally called “corbular” (or non-junctional) and is less sensitive to what is occurring at the membrane interface. It releases Ca^{2+} in response to the Ca^{2+} diffusing from the periphery. This mechanism, as discussed in Chapter 5, in particular conditions, could be responsible for DADs generation.

A study on rabbit PCs has shown that the Ca^{2+} increase was delayed at the cell centre by approximately 100ms (Spitzer et al., 1997).

Purkinje fibres have several types of RyR channel: RyR2, the most diffuse type in cardiac tissues, RyR3, mostly belonging to brain tissue and Inositol 1,4,5-trisphosphate (IP3), a second messenger that induces Ca^{2+} release from the SR also in brain tissue. Finally, many differences were observed in the trans-membrane currents between PCs and VCs, as I will discuss in the next section.

3.3 Action Potential and Ionic Currents

3.3.1 The Action Potential

Even if Purkinje AP (P-AP) has some similarities with ventricular AP (V-AP), many differences have been pointed out between the AP of these two kinds of cells. Moreover, the electrical Purkinje-Ventricle heterogeneity is believed to favour arrhythmogenesis in pathological conditions (Haissaguerre et al. 2016) and upon pharmacology interventions (N. Nagy et al. 2015). The AP duration (APD) dispersion can be potentially arrhythmogenic if the K^+ repolarization reserve is altered in one or both of the two cell types (Antzelevitch 2008).

Figure 3.3 shows two representative AP recordings from human ventricular (left) and Purkinje (right) *in vitro* preparations. APD_{90} was found bigger in PCs than in VCs in rabbit and dog, (rabbit, 218 ± 8 ms versus 119 ± 9 ms (Persson *et al.*, 2007); dog, 321 ± 9.9 ms versus $242 \pm$

5.7 ms (Dun and Boyden 2008)). This seems to be less evident in human (Lee *et al.*, 2004, N. Nagy *et al.*, 2015, Dangman *et al.*, 1982) as it is shown in Table 3.1, where the values of three human experimental sets of biomarkers are reported, both for ventricular and Purkinje cells. Beside, in dogs, it was shown that P-AP prolongs more than V-AP at a slow rate (Han *et al.*, 2002), entailing a higher probability to develop EADs in PCs. Also, Canine PCs have shown gender differences since APD has been found longer in female than male ($APD_{90} 282.0 \pm 9.9$ ms vs 246.5 ± 9.8 , respectively), supporting the hypothesis that women are more susceptible to develop EADs and ventricular tachycardia triggered in PCs (Dun and Boyden 2008).

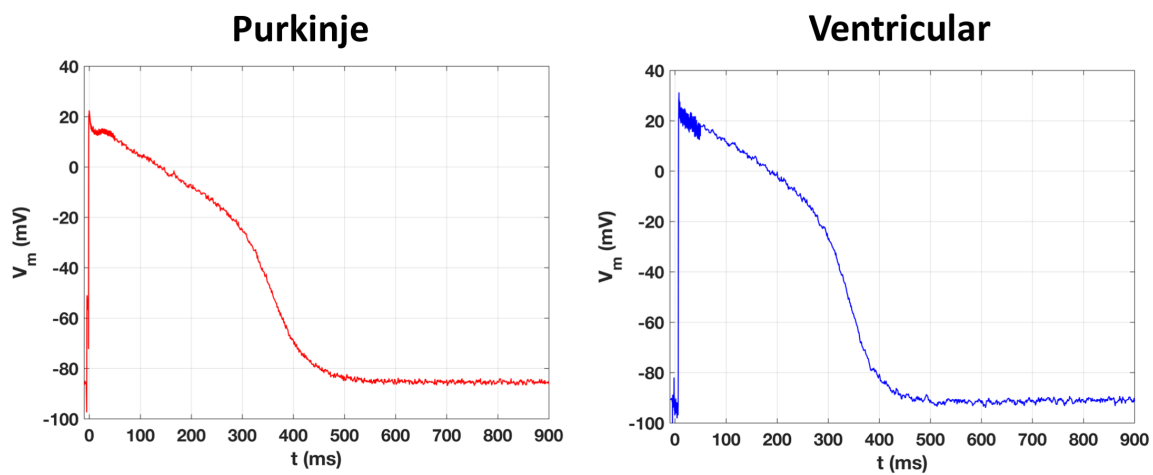


Figure 3.3. Current-clamp recordings of Purkinje (left) and ventricular (right) APs from healthy human preparations (data provided by University of Szeged).

One of the few characteristics of P-APs common among all species in literature is the faster upstroke velocity (dV/dt_{Max}) with respect to V-AP. Indeed, it is faster in rabbit, 592 ± 9 V/s in PCs versus 271 ± 14 V/s in VCs (Lu *et al.*, 2005), dog, 445 ± 14.6 V/s in PCs versus 230 ± 9.5 V/s in VCs (N. Nagy *et al.* 2015) and human as well, 387.9 ± 25.0 V/s in PCs versus 297.1 ± 18.9 V/s in VCs (Dangman *et al.*, 1982). Also, in this case, large variability was found on data available in the literature with reported dV/dt_{Max} values being larger in PCs, varying between

150 and 750 V/s (N. Nagy et al., 2015, Callewaert et al., 1984), and lower in human PCs in comparison with animal PCs (N. Nagy et al., 2015, Lee et al., 2004).

Table 3.1 Action Potential biomarkers from human Purkinje cells and human ventricular myocytes available in the literature: top panel from (Dangman et al. 1982), mid-panel from (F. Y. Lee et al. 2004) and bottom panel (N. Nagy et al. 2015).

K. H. Dangman <i>et al.</i>, 1982		MDP (mV)	AMP (mV)	dV/dt_{MAX} (V/s)	APD₅₀ (ms)	APD₁₀₀ (ms)
	VCs	-77.6±1.4	103.7±1.5	297.1±18.9	189.7±3.9	370.2±5.8
	PCs	-79.6±2.0	107.1±2.2	387.9±25	195.2±8.9	377.0±13.3
F. Lee <i>et al.</i>, 2004		MDP (mV)	AMP (mV)	dV/dt_{MAX} (V/s)	APD₅₀ (ms)	APD₉₀ (ms)
	VCs	-82±1	110±4	186±37	199±18	358±13
	PCs	-85±1	114±4	207±26	187±20	319±23
N. Nagy et al., 2015		MDP (mV)	AMP (mV)	dV/dt_{MAX} (V/s)	APD₅₀ (ms)	APD₉₀ (ms)
	VCs	-88±2.4	107±6.3	262±27.5	233±77.5	291±95.3
	PCs	-85±2.6	106±6.7	372±150	220±58.9	304±78.1

(*MDP*: minimum diastolic potential, *AMP*: Amplitude of Potential, *dV/dt_{MAX}*: Maximum upstroke velocity, *APD_x*: time to reach the X % of repolarization).

A prominent early repolarization (phase 1) has been recognised in rabbit (Dun and Boyden 2008) and dogs (Han et al., 2002) P-AP. Phase 1 seems to be less pronounced in human (N. Nagy et al. 2015), leading to a smaller spike amplitude, 9.8 ± 3.4 mV for human vs 34.2 ± 2.8 mV for dogs ($p < 0.05$, $n = 7$, N. Nagy et al., 2015) and a higher plateau potential level in comparison with canine P-AP, 8.0 ± 1.8 for human vs 8.6 ± 3.4 mV in dogs ($n = 7$, N. Nagy et al., 2015). In rabbits, the total AP amplitude was reported to be larger in PCs than in VCs, 117 ± 1.2 mV and 106 ± 1 mV, respectively (Dun and Boyden 2008).

The P-AP also shows spontaneous phase 4-depolarization in dogs, cow, sheep, mouse (Han, Bao, et al., 2002, Callewaert et al., 1984, Atkinson et al., 2011) and human (N. Nagy et al.

2015), which can trigger afterdepolarizations, with an amplitude between 1 and 10 mV, or even spontaneous AP (Han et al., 2002). This slow depolarization plays a role in Purkinje pace-making activity. Notably, in comparison with that in the two nodes, a weaker and slower pace-making activity has been observed in mouse, rabbit, canine (Dobrzynski et al. 2013) and human (F. Y. Lee et al. 2004) isolated multicellular PCs. However, in other single myocytes studies, no pacemaker activity was found. This difference seems to be ascribed to another peculiar feature of PCs, i.e. the presence of two different diastolic membrane potential levels: the higher one is between -50 mV and -70 mV while the latter has been found near -85 mV (Callewaert et al., 1984, Lee et al., 2004) when the cell is well-polarised. In human, pacemaker activity has been found only in PCs with less negative diastolic potential and none in the latter case (F. Y. Lee et al. 2004). The double diastolic membrane potential makes this kind of cell less stable than VCs since PCs can switch from one resting potential to the other after a small perturbation (Dobrzynski et al. 2013).

3.3.2 Ionics currents

Biological data describing the sub-cellular mechanisms underlying the electrical activity of PCs are limited and heterogeneous in term of types of data and model where they come from. In this review, priority is given to human electrophysiological recordings but, where these are lacking, we will discuss animal electrophysiological experiments. mRNA expressions were considered as well, though it is not quantitatively clear the relationship between the encoding mRNAs and the amount of translated proteins (Walmsley et al. 2013).

3.3.2.1 Sodium Currents

As mentioned in Chapter 2, Section 2.2, sodium currents are responsible for the very fast upstroke shown during phase 0 in P-AP (N. Nagy et al. 2015). No human electrophysiological

data are available for this type of channels in PCs. However, in both human ventricle and Purkinje, the most expressed sodium channels transcript is that encoding the channel isoform Nav1.5 (Gaborit et al., 2007), responsible for the low TTX-sensitive *fast activating, inactivating sodium current*, I_{Na} . Human and canine PCs express similar levels of Nav1.5, accordingly with the same AP amplitude shown, although different upstroke velocities have been found between these species (N. Nagy et al. 2015). This suggests that either other Na^+ channel isoform or different kinetics (or both) could be underling the differences between human and canine data (N. Nagy et al. 2015).

Several studies have also identified in PCs different isoforms of the *TTX-sensitive sodium channel*, as Nav1.1 and Nav1.2 in dogs (Dun and Boyden 2008) or Nav1.7 in human (Gaborit et al. 2007). Through these channels flows the *late sodium current*, I_{NaL} , so-called for its delayed inactivation (Haufe, Chamberland, and Dumaine 2007).

A study in canine PCs suggests that all non-cardiac channels contribute 22% to the total peak of I_{Na} in P-AP (Haufe et al. 2005). In mice, whole-cell patch-clamp recordings in PCs and VCs showed larger I_{NaL} in PC (0.99 ± 0.07 pA/pF) respect VC (0.34 ± 0.04 pA/pF)(V. Iyer et al. 2015). Another study on rabbits Purkinje suggests the existence of different recovery kinetics of I_{NaL} in PCs and VCs (W. Li et al. 2016).

Vassalle and co-workers have also shown the existence in canine PCs of two different kinds of I_{NaL} , with slightly different proprieties: I_{NaL2} activates at -50 mV, reaches its peak at -20 mV and has the same time constant of I_{Na} (Bocchi and Vassalle 2008) while I_{NaL3} has smaller current density, activates at 35 mV and has faster inactivation (Rota and Vassalle 2003).

Finally, several studies involving I_{NaL} -blocker like Mexiletine (V. Iyer et al. 2015) and Ranolazine (W. Li et al. 2016), have pointed out a possible role of this current in EAD-genesis in Purkinje.

3.3.2.2 Potassium Currents

Han and co-workers published the only human voltage-clamp recordings of potassium currents in PCs in 2002 (Wei Han, Zhang, et al. 2002), from isolated PCs (n=5) of failing hearts of patients who were taking a variety of medications.

Han's studies show a transient outward current, I_{to} , density from the peak current to steady state significantly smaller in PCs than in VCs, but with a meaningfully larger residual sustained current, I_{sus} . Also, the I_{to} channel inactivation kinetics are different between PCs and VCs, being slower in PCs (slow recovery time constant $1,575 \pm 189$ ms, $n = 4$, in PCs versus 217 ± 55 ms in VCs). This difference probably depends on a lower expression of KChIP2 in PCs (Gaborit et al. 2007), since KChIP2 co-expression with Kv4 accelerates the recovery of I_{to} from inactivation (Dun and Boyden 2008). Finally, I_{sus} density in PCs has been found comparable in atria, even if in PCs it has been inhibited by the K^+ channel blockers tetraethylammonium (TEA), while no effects have been observed in atria (Z. Wang et al., 1993).

Since PCs are notoriously difficult to isolate and sensitivity of I_{Ks} to cell isolation is high, no current recordings are available for both delayed K^+ rectifier rapid, I_{kr} , and slow, I_{ks} , in humans. Both have been found smaller in rabbit (Cordeiro et al., 1998) and canine PCs (Wei Han et al. 2001) compared to VCs. In human, the mRNA expression levels of HERG and KvLQT1, coding I_{kr} and I_{ks} respectively, have been found similar to the ventricular counterparts (Han et al., 2001, Gaborit et al., 2007). This similarity suggests that probably different mechanisms play a role in PCs AP repolarization in human in comparison with other species, such as dog and rabbit (N. Nagy et al. 2015).

Current recordings in voltage-clamp at negative potential ($V < -90$ mV) show a significantly smaller inward rectifier potassium current, I_{K1} , in human PCs (Han et al., 2002). These data are in agreement with (Gaborit et al. 2007) who demonstrated that Kir2.1, Kir2.2, Kir6.1 and

Kir6.2 in humans amounts for about 50% less in PCs than in the right ventricle, even if Kir3.1 is much more abundant in PCs than in VCs (Gaborit et al. 2007).

The lower density of I_{K1} and the presence of the time-dependent hyperpolarization-activated current, I_{HCM} or I_f (Schram et al. 2002) allow automaticity in PCs. Han's recordings (Han et al., et al., 2002) show a robust current compatible with I_f in human cardiac PCs, absent in ventricular myocytes. This difference seems due to the different voltage range for the current activation: I_f activation occurs in PCs at more physiological potentials range (-80 mV to -130 mV) than VCs (-120mV to -170 mV) (Boyden, Hirose, and Dun 2010). Human PC I_f has a rapid kinetic component that rapidly activates and represents the strongest current during the diastolic interval. It shows similar proprieties to those observed in cow, sheep and dog (Callewaert et al., 1984, Han et al., 2002), even if there have been different responses to drugs therapies among these species. For example, Lidoflazine blocked I_f in sheep PCs (Hart and Dukes 1984) but did not show any effects on canine PCs (Dobrzynski et al. 2013).

3.3.2.3 Calcium Currents

Several different Ca^{2+} channels are present in the heart, distinguishable by different $\alpha 1$ subunits. For example, the most common cardiac high-voltage activated calcium channel Cav1.2 contains the $\alpha 1C$ subunit, while Cav1.3 contains $\alpha 1D$ (Boyden, Hirose, and Dun 2010). Functionally, two types of calcium currents have been recognised in PCs (Hirano et al., 1989; Tseng & Boyden, 1989): L-type current, I_{CaL} , and T-type one, I_{CaT} . The first is the primary calcium current in VCs, while the latter is lowly expressed in ventricles. Reduced expression of I_{CaL} (Gaborit et al. 2007) was found in human PCs. This reduction seems to be related to the lower contractile ability of PCs in comparison to VCs (N. Nagy et al. 2015). Similarly, lower expressions of I_{CaL} in PCs respect to VCs were observed in dogs (Tseng and Boyden 1989) and rabbit (A. Atkinson et al. 2011). Both Cav1.2 and Cav1.3 expressions were observed in PCs, while Cav1.2 was found only in VCs (Vigmond and Stuyvers 2015).

The amount of $\text{Ca}_v3.1$, $\text{Ca}_v3.2$ and $\text{Ca}_v3.3$ transcript, coding all the isoforms of the T-type channel protein, was found in abundance in PCs among several species, including human (Gaborit et al. 2007; Wei Han, Bao, et al. 2002), but T-type calcium current has never been recorded in human. I_{CaT} activates at lower potentials than I_{CaL} and seems to play a role in PCs pacemaker activities, due to its contribution to the diastolic depolarization (Dobrzynski et al. 2013). Its inhibition with Mibefradil has, however, showed no effect on pace-making in canine PCs (Pinto et al., 1999).

3.3.2.4 Pumps and Exchanger

Very few focused studies have been published regarding the sodium-calcium exchanger (NCX) and the $\text{Na}^+ - \text{K}^+$ ATPase pump in human cardiac Purkinje cells. Like for calcium currents and in line with the lower contractile capacity, transcripts relative to NCX were found lower in human PCs in comparison to VCs (2208 ± 278 units in VCs versus 1189 ± 139 in Purkinje) (Gaborit et al. 2007). Lower NCX expressions were also found in canine (Han et al., 2002) and rabbit (A. Atkinson et al. 2011). The limited ability to extrude Ca^{2+} might make PCs more susceptible to digitalis toxicity (Han et al., 2002). Also, NCX seems to perform a role in DAD genesis, since its inhibition was shown to reduce the spontaneous depolarization occurrences (Z. A. Nagy et al. 2004).

Expression of the $\text{Na}^+ - \text{K}^+$ ATPase pump was also found lower in human PCs in comparison to VCs (Gaborit et al. 2007). Sodium potassium pump activity plays a role in “Overdrive Suppression” of Purkinje automaticity, due to Na^+ accumulation at a fast rate. Indeed, the sino-atrial node rate is physiologically the dominant driving pacemaker into the heart, and its frequency firing suppresses other pacemaker sites. Na^+ accumulation enhances the electrogenic $\text{Na}^+ - \text{K}^+$ ATPase pump activity, which keeps the cell hyperpolarised, offsetting the depolarizing pacemaker currents (I_f).

Table 3.2 reports a summary of the differences between ionic currents in PCs and VCs.

Table 3.2. Summary of the differences between ionic profiles currents in PCs and VCs.
Colours and signs indicate the differences in current magnitudes in PCs respect of VCs

	Han 2002	Gaborit 2007	Aslanidi 2010	Atkinson 2011	Haissaguerre 2016
Specie	<i>Human</i>	<i>Human</i>	<i>Rabbit</i>	Rabbit	-
Data	<i>I-V Curve</i>	<i>mRNA</i>	<i>I-V curve</i>	I-V curve	-
I_{Na}			+	++	+
I_{NaL}			+	++	
I_{CaL}			-	-	
I_{CaT}			+	++	++
I_{k1}	-	-	-	--	-
I_{Ks}			-	--	
I_{Kr}			-	-	-
I_{sus}	+				++
I_{to}	-	+			
I_f	+	+		++	++
I_{NaK}		-		--	
I_{NCX}		-		--	-

1st column: ionic currents contributing to Purkinje AP. 2nd column: voltage-clamp human data (Han et al., 2002); 3rd column human mRNA expression (Gaborit et al. 2007); 4th column: rabbit voltage-clamp data (Aslanidi et al. 2010); 5th column: mRNA expression and voltage-clamp recordings from rabbit PCs (A. Atkinson et al. 2011); 6th qualitative data from (Haissaguerre et al. 2016).

3.4 Pharmacological action on cardiac Purkinje cells.

Due to their electrophysiological properties, PCs are generally considered more unstable than VCs and, therefore, more prone to initiate electrical abnormalities observed in single cells as EADs and DADs. Furthermore, it is commonly assumed that, since a single Purkinje fibre excites a high number of ventricular myocytes, abnormalities in PCs are more prone to spread on tissue and therefore imply a higher arrhythmic risk (Haissaguerre et al., 2016). The PCs and VCs interface, i.e., the Purkinje-myocyte junctions, abundant also of transitional myocytes, is affected by the electrical heterogeneity among these cells and then is considered a critical area, where different types of re-entry can be triggered (Lopera et al. 2004). For a complete review

about re-entry in PCs leading to arrhythmias, see Haissaguerre et al., 2016. Ultimately, PCs play an important role in drug-induced arrhythmogenesis, and animal Purkinje assays are widely used in preclinical drug safety assessments (N. Lee et al., 2010). In this section, the main studies describing the action of drugs and compounds on the electrophysiology of PCs will be reviewed.

In rabbit PCs, whole-cell patch-clamp recordings carried out by Li *et al.* in 2016, demonstrate a significant role of I_{NaL} in the rate-dependence electrical instability of PCs. The I_{NaL} enhancer anemone toxin ATX-II, increased the rate dependence of I_{NaL} and the PCs instability, inducing EADs. By contrast, Tetrodotoxin and Ranolazine, both I_{NaL} blockers, totally inhibit induced-EADs.

In 2007, Persson et al. (Persson et al. 2007) analysed in rabbit atrial, ventricular and Purkinje cells the combined effects of AZD7009, an I_{NaL} blocker, lidocaine (a local anaesthetic, which mainly acts as a sodium blocker (Cummins 2007)), and E-4031 a compound that selectively inhibits I_{Kr} , leading to AP prolongation. Both sodium blockers attenuated the prolongation due to E-4031 in PCs, but not in ventricles. Furthermore, in PCs E-4031 provoked EADs, which were abolished by AZD7009 or lidocaine, suggesting a role of I_{NaL} underling AP prolongation and EADs genesis in PCs, but not in VCs.

In 2013, Jost and co-workers (N. Jost et al. 2013) investigated the effects of a new selective NCX inhibitor, ORM-10103, on canine ventricular papillary muscle and PCs. EADs were evoked in VCs stimulating at slow BCL (1500–3000 ms) with a 1 μ M Dofetilide and 100 μ M BaCl₂. DADs were evoked through cell superfusion with 0.2 μ M Strophanthin, a Na⁺/ K⁺ ATPase inhibitor, for 40 min and stimulation with a train of 40 stimuli with a cycle length of 400 ms. After demonstrating the high selectivity of the channel blocker, the authors showed a significant reduction in amplitude of the induced-EADs in VCs and DAD in PCs under ORM-10103 superfusion, both at 3 and 10 μ M.

Previously, the same approach was carried out by Nagy (Z. A. Nagy et al. 2004) who tested the effects of another selective NCX blocker on canine VCs and PCs, obtaining similar results: the amplitude of both EADs and DADs was markedly decreased by the NCX-blocker (from 26.6 ± 2.5 to 14 ± 1.8 mV and 12.5 ± 1.7 to 5.9 ± 1.4 mV, respectively).

In the 80's different studies were carried out to analyse the effects of Quinidine, a class I anti-arrhythmic agent (W. Redfern et al. 2003), blocking mainly Na^+ channels but also I_{K_r} , in PCs. Roden and Hoffman (Roden and Hoffman 1985) tested the dose-electrophysiological effects of $1\mu\text{M}$ and $10\mu\text{M}$ in canine PCs, as a function of the extracellular potassium and at different cycle lengths (300-8000ms). They found that at lower plasma concentration Quinidine only prolongs the APD90, whereas, at the higher dose, it also affects the depolarization velocity. The prolongation was also bigger at long cycle length and low extracellular potassium. Also, for cycle lengths greater than 4000 ms and low extracellular potassium (2.7 mM), Quinidine induced EADs in the 50% and 78% of the fibres, at $1\mu\text{M}$ and $10\mu\text{M}$, respectively.

A few years later, Nattel *et al.* (Nattel and Quantz 1988), induced EADs in canine cardiac Purkinje fibres using Quinidine again. Verapamil and Nifedipine, both Calcium channels blockers, eliminated EADs, even if Isoprenaline restored them even under calcium blocker superfusion. Isoprenaline is a well-known non-selective β -adrenoreceptor agonist (Kleinau et al. 2011), whose β -receptor stimulation can promote or end EADs, depending on whether effects on slow inward current (pro-EADs) or automaticity (preventing EADs) predominate. Similarly, Kaseda et al. (Kaseda et al., 1989), suppressed Quinidine-induced EADs using MgCl_2 , TTX and Verapamil, suggesting a possible role of I_{NaL} in EADs genesis.

Ten years later, in 1999, Pinto and co-workers (Pinto et al. 1999) investigated the role of both I_{CaL} and I_{CaT} on canine PCs automaticity using Mibefradil, a T-type Ca^{2+} channel antagonist. Even if Mibefradil blocked both Ca^{2+} currents, no effects were observed on PC automaticity.

In 2001, Gintant and co-worker (Gintant *et al.*, 2001) realised a systematic *in vitro* study of 12 compounds on porcine and canine PCs to discriminate safe and risky drugs just through APD prolongation, comparing the result with clinical observation. Stimulating PCs at a slower rate (0.5 Hz) and under physiological conditions (37° and $[K^{+}]_o = 4$ mM), the authors recorded APD₉₀ changes and considered riskier those drugs inducing more than 15% of prolongation at concentrations > 10-fold those clinically encountered in plasma levels. Six out of seven drugs that previously had shown arrhythmogenic effects were correctly classified (Cisapride, Erythromycin, Grepafloxacin, Moxifloxacin, Sertindole, and Sotalol) as well as all the five compounds known as safe (Azithromycin, Enalaprilat, Fluoxetine, Indomethacin and Pinacidil). Only the antihistamine Terfenadine, clinically associated with TdP, did not prolong more than 15%, even at high concentration. The largest APD prolongation was observed with Moxifloxacin, but EADs were observed only with Cisapride and Enalaprilat at 0.5 Hz. Faster pacing abolished them.

A few years later, Champeroux (Champeroux *et al.* 2005) developed an algorithm to classify drugs based on their *in vitro* risk among three groups:

- A. drugs with numerous or several reports (>2 cases) of TdP.
- B. drugs causing QT prolongation and/or TdP only in few cases (≤ 2 cases).
- C. drugs without reports of TdP or QT prolongation.

They tested 45 compounds, selected among the 100 compounds published by Redfern *et al.* in 2003, on canine cardiac Purkinje fibres and sorted them according to APD₉₀ increase, reverse use dependency, action potential triangulation or effect on dV/dt_{Max} . The relevance of these indicators was evaluated by comparison with available clinical data. The authors concluded that it is not possible to distinguish compounds having proarrhythmic potential from those who do not have this property, just using single parameters as APD₉₀ increase or AP triangulation. They tested more than 40 simple algorithms and, from the results of such algorithms on 20

tested compounds, a sophisticated algorithm was defined. The algorithm separated all drugs in the mentioned three groups and was suggested as a basis for testing new candidate drugs in the early stage of drug safety and efficacy evaluation process (Figure 3.4).

In 2007, Terrar et al. (Terrar *et al.*, 2007) investigated the effects on guinea-pig ventricular myocytes and dog Purkinje fibres of six compounds known to cause QT prolongation (Dofetilide, Sotalol, Cisapride, Terfenadine, Haloperidol and Sparfloxacin) and two safe drugs (Nifedipine and Verapamil). They found a more significant AP prolongation in canine Purkinje fibre compared to guinea-pig VM with Sotalol, Dofetilide and Sparfloxacin, whereas, again, Terfenadine had no effects on canine PCs. The drug-induced effects on P-AP of the same compounds will be simulated and investigated in Chapter 6, through *in silico* drug trials.

Table 3.3 reports APD₉₀ and AP triangulation variations for all compounds at specific concentrations in canine Purkinje and guinea-pig ventricular cells. Dofetilide induced greater increases in APD₉₀ and APD₅₀ in PF compared to VM at 1, 10 and 100 nM Dofetilide.

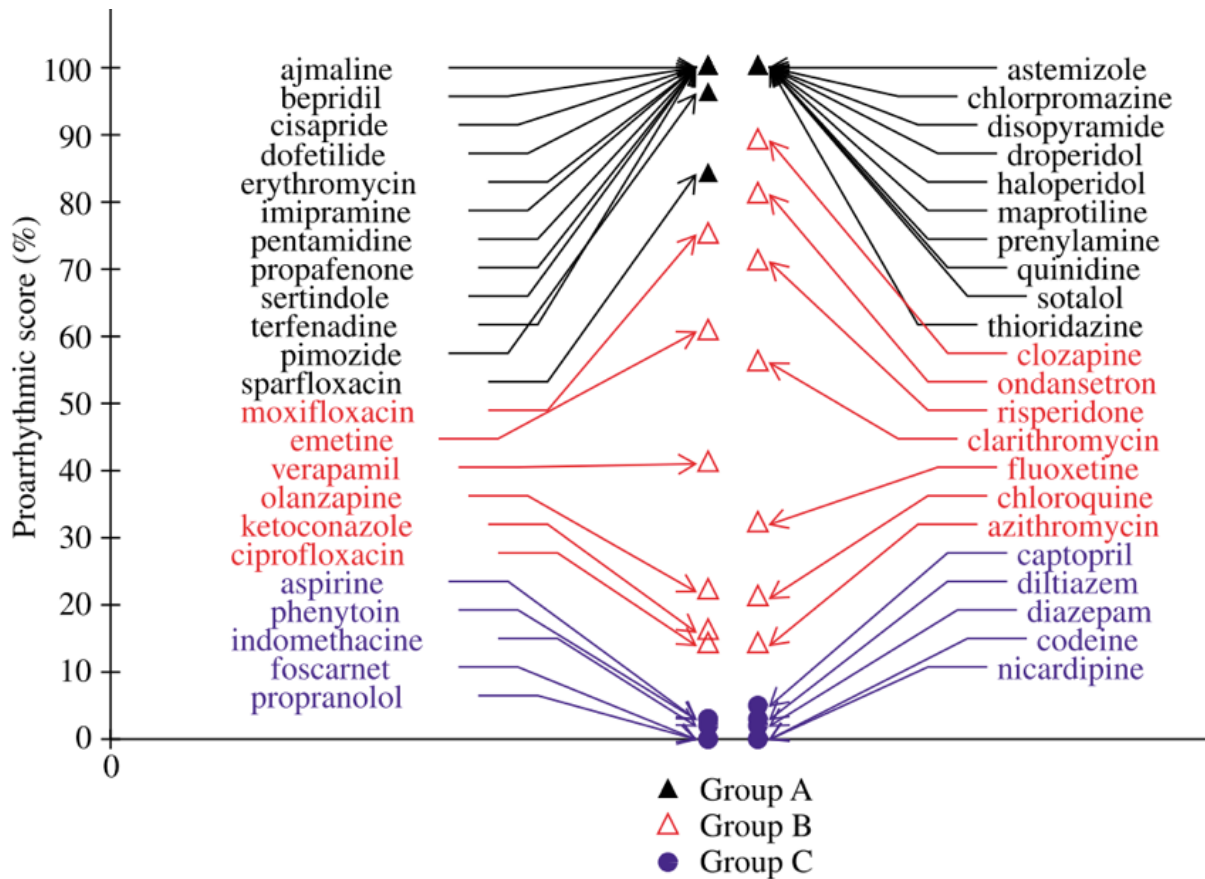


Figure 3.4. Comparison of proarrhythmic scores obtained using the sophisticated algorithm developed by (Champeroux et al. 2005). Group A: drugs with numerous or several reports (>2 cases) of TdP; Group B: drugs causing QT prolongation and/or TdP only in few cases (≤ 2 cases); Group C drugs without reports of TdP or QT prolongation.

Guinea-pig VM reached the maximum prolongation at approximately 25% at 0.5 and 1 Hz, and exposure to a higher concentration of Dofetilide (1 μ M) caused no further increase in APD prolongation. In canine PCs, APDs were more prolonged at 0.5 Hz (~80%) compared to 1 Hz (~55%) at 100 mM Dofetilide. Guinea-pig VCs did not show any evident frequency-dependent effect. No effects of Dofetilide were detected on upstroke velocity and resting membrane potential in neither preparation.

Sotalol and Sparfloxacin prolonged APD in both preparations at 1, 10 and 100 μ M, with larger prolongation at higher concentrations (100 nM) in PCs (Sotalol ~70%, Sparfloxacin ~45%)

compared to VCs (Sotalol: ~22%, Sparfloxacin ~30%). No effects of both compounds were detected on upstroke velocity and resting membrane potential in either preparation.

For both preparations, Cisapride provoked no significant effects on APD₅₀ and APD₉₀ prolongation at lower concentration (0.01-1 μ M) and 0.5 and 1 Hz. However, at concentrations higher than 1 μ M, the APD₅₀ was significantly decreased, and no effects were observed on APD₉₀. APD changes in PCs were greater respect to those in VCs at higher concentrations, while a significant variation in upstroke velocity was found in guinea-pig VCs but not in canine PCs.

Terfenadine had similar effects to Cisapride on guinea-pig VCs, but no statically significant effects on PCs. At higher concentration (>10 μ M) of Terfenadine, APs were prolonged in both preparations.

Table 3.3 Effects of several compounds on APD₉₀ and triangulation in guinea-pig VCs and canine PCs from (Terrar et al. 2007).

Compound	% change in APD ₉₀	% change in triangulation
<i>Guinea-pig VM</i>		
Dofetilide (100 nM)	25.2 $\pm$ 4.0	58.0 $\pm$ 9.7*
Sotalol (100 μ M)	23.7 $\pm$ 4.3	45.4 $\pm$ 10.4*
Sparfloxacin (100 μ M)	25.7 $\pm$ 3.3	47.7 $\pm$ 7.8*
Cisapride (10 μ M)	-3.6 $\pm$ 3.4	98.3 $\pm$ 20.4*
Terfenadine (1 μ M)	9.8 $\pm$ 3.2	33.7 $\pm$ 4.4*
Haloperidol (0.1 μ M)	15.6 $\pm$ 4.0	57.6 $\pm$ 5.4*
Nifedipine (1 μ M)	-36.7 $\pm$ 3.0	21.8 $\pm$ 2.8*
Verapamil (1 μ M)	-37.0 $\pm$ 3.0	21.8 $\pm$ 2.9*
<i>Dog PF</i>		
Dofetilide (100 nM)	56.9 $\pm$ 6.2	92.4 $\pm$ 25.4
Sotalol (100 μ M)	38.7 $\pm$ 8.5	53.5 $\pm$ 11.9*
Sparfloxacin (100 μ M)	26.0 $\pm$ 6.2	27.6 $\pm$ 8.0
Cisapride (10 μ M)	-10.7 $\pm$ 8.1	9.9 $\pm$ 16.5
Terfenadine (1 μ M)	-0.4 $\pm$ 3.2	1.8 $\pm$ 5.3
Haloperidol (0.1 μ M)	8.1 $\pm$ 2.6	16.2 $\pm$ 8.0
Nifedipine (1 μ M)	3.8 $\pm$ 3.6	19.9 $\pm$ 3.0
Verapamil (1 μ M)	3.2 $\pm$ 6.1	34.0 $\pm$ 13.3

* P <0.05 Student's paired t test for comparison between % change in APD₉₀ and APD₄₀₋₉₀.

PF=Purkinje fibres; VM=ventricular myocytes.

Haloperidol provoked a marked change in the AP shape in both preparations, due to prolongation of APD₉₀ simultaneously with shortening of APD₅₀ at concentrations higher than 1 μ M and 0.5 and 1 Hz. The effects on APD₉₀ prolongation and upstroke velocity reduction were more marked in guinea-pig VCs than in canine PCs.

The effects of Verapamil on APD₉₀ in canine PCs were heterogeneous, with some fibres showing APD₉₀ prolongation and some APD₉₀ shortening. Only at concentrations >100 μ M, the predominant effect was an APD₉₀ reduction. Guinea-pig VCs showed only AP shortenings, overall higher than Purkinje counterpart among all tested Verapamil concentrations.

Nifedipine had more significant effects on APD₅₀ in canine PCs respect to APD₉₀ at every tested Nifedipine concentration. Guinea-pig VCs showed similar effects for both APD₅₀ and APD₉₀. The reduction in APD₅₀ is bigger in VCs at lower concentrations (1-10 μ M) and became bigger in PCs at higher concentrations at 0.5 Hz and 1 Hz. In canine PCs, Nifedipine at high concentration also reduced the upstroke velocity at 1 Hz, differently from guinea-pig VCs which showed no significant variations in upstroke velocity.

In 2010, Roche (Roche *et al.*, 2010) compared the effects of Dofetilide on rabbit Purkinje fibre and isolated perfused rabbit heart. Dofetilide induced a concentration-dependent increase in APD₅₀ and APD₉₀ on all Purkinje fibres at 1, 3 and 10 nmol/L, whereas, evoked EADs only in 3/10 fibres at 10 nmol/L. Similar concentration-dependent changes in APD were observed in the isolated whole heart, but from lower Dofetilide concentrations (3 nmol/L) extrasystoles and TdP were observed mostly at a low rate.

In 2016, Le Guennec and co-worker (Le Guennec *et al.* 2016) evaluated the influence of the inter and intra-subject variability on the efficacy of hERG-blockers. Following the already described sub-division of Champeroux (Champeroux *et al.* 2005), they compared the effects on canine Purkinje fibre of 5 drugs from the group A (Sotalol, Dofetilide, Quinidine, Terfenadine and Thioridazine), three from the group B (Moxifloxacin, Risperidone and

Verapamil) and three from the group C (Nicardipine, Phenytoin and Propranolol) with the *in silico* effect on a ventricular model (Fink *et al.*, 2008) and the Purkinje cell model by Stewart *et al.* (Stewart *et al.* 2009), which is described in detail in the next section.

3.5 Human Cardiac Purkinje Cell modelling

As mentioned in Chapter 2 (Section 2.5), the first cardiac AP model was developed by Denis Noble in 1962 (Noble 1962), and it also represented the first model of a cardiac Purkinje AP. The model was based on the first AP model, developed ten years earlier by Hodgkin & Huxley (Hodgkin and Huxley 1952), for the neuronal electrophysiology. Due to the diffusion of the Voltage-Clamp technique and the consequent increase of the availability of electrophysiological data, McAllister and co-workers developed in 1975 an updated Purkinje model (McAllister, Noble, and Tsien 1975) with nine ionic currents, among which a transient chloride current, the delayed potassium rectifiers and an inward calcium current. In 1985, Di Francesco and again Noble implemented into their new Purkinje model (DiFrancesco and Noble 1985) the intracellular and extracellular ionic concentration dynamics, mathematical formulations of pumps and exchangers and a description of the calcium release by the sarcoplasmic reticulum. However, after the work of Di Francesco and Noble, the cardiac Purkinje cell modelling comes to a grinding halt, and no models were published for more than 20 years, as shown in Figure 2.7.

The story of Purkinje models resumed only in 2009 with the publications of a new model by Stewart and co-workers (Stewart *et al.* 2009), followed by another human Purkinje model developed in 2010 by Sampson *et al.* (Sampson *et al.*, 2010). Previously in 2008, ten Tusscher and Panfilov (ten Tusscher *et al.*, 2008) changed the values of 2 ionic currents conductances (I_{Na} and I_{Ks}) of their previous ventricular model (ten Tusscher and Panfilov 2006) to obtain a human model of a Purkinje-like AP.

Other models were published for animals such as mouse (Vaidyanathan et al. 2013), rabbit (Corrias, Giles, and Rodriguez 2011; Britton et al. 2013; Aslanidi et al. 2010) and dog (Aslanidi et al. 2009; P. Li and Rudy 2011; Cherry and Fenton 2012).

3.5.1 The ten Tusscher and Panfilov model

In 2008, ten Tusscher and Panfilov implemented a 3D His-Purkinje system for their previous human ventricles model to simulate and investigate both physiological and abnormal activation patterns, the latter due to branch block or bundle branch re-entry. They derived the geometry of the human myocardium from anatomical data (Hren 1996) and designed the His-Purkinje conduction system using an interactive graphical program.

Membrane kinetics of VCs were reproduced using the previous models of ten Tusscher (ten Tusscher et al., 2004, ten Tusscher & Panfilov, 2006, respectively TT04 and TT06 models). Individual ionic currents and fluxes were described by differential equations, following the Hodgkin and Huxley's formalism described in Chapter 2 (Section 2.5). TT06 includes the following currents: I_{Na} , I_{CaL} , I_{to} , I_{Kr} , I_{Ks} , I_{K1} , I_{NaCa} , and I_{NaK} .

To reproduce the electrical activity of Purkinje cells, ten Tusscher and Panfilov changed their previous TT06 model, using a smaller conductance for the slow delayed rectifier current ($G_{Ks, Purkinje} = 0.35 G_{Ks, muscle}$) to obtain a larger APD. They also used a higher conductance value for the fast sodium current ($G_{Na, Purkinje} = 2.94 G_{Na, muscle}$) to obtain a higher upstroke velocity. Then, topology, cable thickness, conductivity and Purkinje-muscle insertions of the whole 3D His-Purkinje-ventricles system were iteratively adjusted to achieve a good agreement of the simulated activation sequence with experimental data.

3.5.2 The Stewart model

The human cardiac Purkinje AP model developed by Stewart & co-workers (Stewart et al., 2009, STW model) is based on the previous TT04 and TT06 models (ten Tusscher et al., 2004

and ten Tusscher & Panfilov, 2006) of human ventricular myocytes. The model encompasses in a total of 10 different main currents, 26 state variables and 54 parameters.

With respect to the TT06 model, two new currents were added: the sustained potassium current, I_{sus} , whose formulation accounts for a single instantaneous activation variable based on Han's data (Wei Han, Zhang, et al. 2002), and the “funny” current, I_f , derived from a rabbit model of a sinoatrial cell (H. Zhang et al. 2000) and the human data published by Han (Wei Han, Zhang, et al. 2002). The conductances of different currents were modified, i.e., I_{Na} (+910%), I_{Kr} (-5%), I_{Ks} (-40%) and I_{K1} (-98%), while the calcium handling was inherited unchanged from the ventricular TT06 model, as well the Na^+/K^+ pump and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. The late sodium current, I_{NaL} , and the T-type calcium current, I_{CaT} , are absent.

The steady state model shows a maximal diastolic potential of -75.5 mV and AP amplitude of 123 mV, while upstroke velocity and APD₉₀ are 327 mV/s and 293 ms, respectively. Finally, the model has spontaneous pacemaker activity at 0.91 Hz and reproduces the “Overdrive Suppression” phenomenon. The model has been used mainly to investigate the electrical coupling with ventricle and its role in arrhythmia onset.

Nayar *et al.* (Nayak et al., 2017) studied the effects of the coupling of human endocardial and Purkinje cells at the cellular and two-dimensional tissue levels, using the STW model to reproduce PCs and the TT06 model to represent ventricular myocytes. Notably, they used a minimal model for the Purkinje network of a bilayer tissue, where the layer of PCs is placed on top of a layer of endocardial cells, to reproduce spiral-wave turbulence and focal activities. Previously, Dux-Santoy *et al.* (Dux-santoy et al. 2012) built a multiscale functional model of the His bundle and bundle branches coupled to a representation of the septum to study initiation and maintenance of bundle branch re-entry in-silico, using the STW and TT06 models again. In 2015, Monasterio *et al.* (Monasterio et al., 2016) used the same above models to reproduce the effects of hyperkalaemia on the electrical conduction in a 1-D Purkinje-ventricle

subsystem. Swan and co-worker (Swan et al. 2014), instead, simulated the AP propagation in a 1 cm long fibre, including the STW model, the ventricular TT04 model and the human atrial cell model by Courtemanche (Courtemanche *et al.*, 1998), to demonstrate that mutations in SCN5A (encoding Nav1.5) may be associated with exercise-induced polymorphic ventricular premature complexes and tachycardia. Previously, Mann and co-workers (Mann et al. 2012) used computational modelling to determine the functional consequences of the mutant Nav1.5 protein, which was identified in dilated cardiomyopathy (DCM) and premature ventricular complexes (PVCs).

The STW model was included in whole heart simulations to explore possible mechanisms of arrhythmogenesis in the foetal heart (Pervolaraki *et al.*, 2014) and the effects of anatomical differences of the Purkinje network on electrocardiograms (Dux-Santoy *et al.*, 2013). Previously, Deng (Deng *et al.*, 2012) implemented the STW model in an anatomically-detailed human heart model with corresponding experimentally measured fibres orientation to simulate the whole heart excitation propagation. In 2012, Zaniboni developed a 3-D representation of cardiac AP to both reproduce the heterogeneity of the late phase of AP repolarization across the wall and to estimate the changes in membrane resistance during the course of the AP. Okada and co-workers (Okada et al. 2011) implemented a whole human heart and torso simulator to investigate the relationship between the transmural and apicobasal gradients and the T-wave genesis.

Also, the STW model was used in the safety pharmacology context to evaluate the risk of new drugs candidates. Le Guennec and co-workers (Le Guennec et al. 2016) used a model of endocardial cell described by Fink *et al.* (Fink et al. 2008) and the STW model to investigate the multichannel effects of 11 compounds: Quinidine (class I anti-arrhythmic drug), propranolol (class II anti-arrhythmic drug), Sotalol and Dofetilide (class III anti-arrhythmic drugs), Verapamil and Nicardipine (anti-hypertensive drugs, calcium blockers), Thioridazine

and Risperidone (antipsychotics), Terfenadine (antihistamine, withdrawn from the market), Moxifloxacin (antibiotic) and Phenytoin (anticonvulsant). Previously, Dux-Santoy (Dux-Santoy et al. 2011) et al. developed an integrated 3-D ventricle-Purkinje system to study the multiscale effects of Dofetilide.

3.5.3 The Sampson model

The Sampson model (SP model) was developed in 2010 (Sampson et al. 2010) based on Markovian models to reproduce the ionic gate dynamics of specific ionic currents. The different gating modelling makes the SP model very different from the STW model, even if only two additional currents, I_{NaL} and I_{CaT} are included. The SPS model has 96 parameters and 82 state variables, with a hefty computational cost. The calcium subsystem has been modelled using three different compartments: two for the sarcoplasmic reticulum Ca^{2+} uptakes and the last to manage the Ca^{2+} release and where inward calcium through I_{CaL} and I_{CaT} pours out.

The model was used to evaluate the effects of several compounds (TTX, E-4031, Chromanol, Nifedipine) on PCs and validated against animal data (B. Baláti *et al.*, 1998, Han et al., 2001, Varró et al., 2000 and Terrar et al., 2007) respectively. Also, the model reproduced the effects of mutations of key ion channels known to cause inherited arrhythmias and, particularly, the long QT syndrome variant 3 (LQT3).

Priest and co-workers (Priest et al. 2016) employed the SPS model in a 3D computational model of the heart calibrated to represent the size and geometry of the neonatal human ventricles, including representations of the Purkinje system and the muscle fibre orientation, to investigate the relation between cardiac mosaicism and the LQTS.

Previously, Iyer (Iyer *et al.*, 2014) used their human ventricular model (Iyer et al., 2004) and the SP model to reproduce biophysical alterations in channel function in LQT1, LQT2, LQT3,

and LQT7, aiming to understand the role of the Purkinje fibre network in the genesis of ventricular arrhythmias.

In the optogenetic field, Williams and co-workers (Williams et al. 2013) incorporated in the SP model a Channelrhodospin-2 (ChR2) model to demonstrate the ability of ChR2 to trigger AP in human cardiomyocytes, as well as the differential response of these cells to light, with the Purkinje cells being more easily excitable. They also compared the cellular response to classic electrical current stimulation versus optical excitation, both biophysically and energetically (Williams and Entcheva 2015).

Xiao et al. (Xiao et al. 2013) investigated the link between an alteration of I_{to} properties in PCs and idiopathic ventricular fibrillation using the SP model. Wang et al. (K. Wang et al. 2011) employed the SP model to analyse the differences in biophysical I_{Ks} properties between cardiomyocytes derived from human embryonic stem cells and from induced pluripotent stem cells.

3.6 Conclusion

In this chapter, the structure, function and electrophysiology of the HPS were described in detail and compared between different species. The cardiac Purkinje AP and its underlying ionic currents were described as well, and compared with their ventricular counterparts, highlighting the peculiar electrophysiological properties of PCs. Particular attention was given to the response of PCs to pharmacological therapies and susceptibility to EADs and DADs formation since PCs are common in *in vitro* models for preclinical drug screening. The final part of the chapter focused on the most recent advances in computational modelling of Purkinje electrophysiology, with a particular focus on the STW and SP human models. Both models do not account for Purkinje specific electrophysiological and structural features such as the low density of t-tubuli and different type of Ca^{2+} releases. As described in this chapter, a

physiological Purkinje Ca^{2+} - handling representation is crucial to correctly represent the electrophysiology of PCs and their role in arrhythmia onset, in pathology and upon pharmacology interventions. The next chapter will describe the design, calibration, optimisation, and validation of a new computational model, including all the features described so far, to reproduce and simulate the electrophysiology of human PCs.

Chapter 4 - Human Purkinje in silico model enables mechanistic investigations into automaticity and pro-arrhythmic abnormalities

The work presented in this chapter is included in the publication: ‘Human Purkinje in silico model enables mechanistic investigations into automaticity and pro-arrhythmic abnormalities’ by Trovato et al., J. Mol. Cell. Cardiol. 142 (2020) 24–38.

In this chapter, I provide a description of the development of a new computational model for the human cardiac Purkinje electrophysiology, the Trovato2020 model. First, I present the three independent experimental datasets from human Purkinje preparations and the computational methods I used to design, calibrate, optimise and validate the Trovato2020 model. Second, I describe and discuss the results obtained with the new model for the mechanistic investigation of Purkinje-related arrhythmias at cellular level.

4.1 Introduction

As described in the previous chapter, Purkinje cells (PCs) are different from ventricular cardiomyocytes both in structure and electrophysiology. The main structural difference is the lack of t-tubuli in PCs, which induces spatial heterogeneity of Ca^{2+} cycling (Stuyvers et al. 2005). PCs action potential (AP) is characterised by a faster depolarisation phase, a more negative plateau and a longer AP duration (APD) compared to ventricular APs (Boyden, Hirose, and Dun 2010; Haissaguerre et al. 2016). Due to their longer APD, PCs may be more prone than ventricular myocytes to develop pro-arrhythmic abnormalities, i.e. early and delayed after-depolarisation (EADs and DADs, respectively) (Dobrzynski et al. 2013). Interestingly, it has been reported that PCs with a less negative diastolic membrane potential (DMP) show automaticity, whereas, well-polarised PCs do not (Boyden, Hirose, and Dun 2010; F. Y. Lee et al. 2004).

However, isolation of PCs requires challenging procedures which make the investigation of the electrophysiological properties of PCs difficult compared to other types of cardiomyocytes (Wei Han, Zhang, et al. 2002). Furthermore, experiments on PCs are not usually conducted in human but rather in animal models such as guinea pig, rabbit, dog, cow, all exhibiting significant interspecies differences in structure, electrophysiology and calcium handling (Ono et al. 2009; Hua Rong Lu et al. 2001). Thus, mechanistic complexity, limited access to human tissue and experimental and ethical constraints impair our understanding of the ionic mechanisms and contribution to human arrhythmias of the Purkinje system.

In this context, simulation studies with computational models can help to gain insights into the role of cardiac Purkinje electrophysiology in arrhythmia onset in humans. However, the available models in the literature (STW, Stewart et al. 2009; SMP, Sampson et al. 2010; TT08, ten Tusscher and Panfilov 2008) do not account for Purkinje specific electrophysiological and

structural features such as the low density of t-tubuli and different type of Ca^{2+} releases, as discussed in the previous chapter (Section 3.5).

The goal of our study is to integrate and probe current knowledge on human PC electrophysiology at cellular and tissue level through the development of a novel computational model of human cardiac Purkinje electrophysiology, the Trovato2020 model. Human Purkinje voltage-clamp data (Wei Han, Zhang, et al. 2002), novel and partly published (N. Nagy et al. 2015), AP measurements, as well as information from the literature were used to develop, calibrate and validate the Trovato2020 model through simulation studies. The model structure incorporates Purkinje-specific ionic currents, which were not considered in previously published human Purkinje-based computational models (STW, TT08, and SMP) and a Purkinje-specific Ca^{2+} subsystem, as in the canine PRd model (P. Li and Rudy 2011), presented in Section 2.5.4. Simulations with the new Trovato2020 model (1) reproduce experimental recordings in a wide range of protocols as well as electrophysiological alterations following selective hERG and Ca^{2+} channels blocks; (2) explain the ionic mechanisms underlying pro-arrhythmic abnormalities and automaticity. I hypothesised that a three-layer cell compartmentalisation representing the structure and Ca^{2+} handling in PCs enables reproducing and investigating electrical abnormalities such as EADs and DADs.

4.2 Materials and Methods

4.2.1 Experimental datasets

Three different experimental datasets were used for the calibration, optimisation and validation of the novel human cardiac Purkinje electrophysiological model presented in this chapter.

4.2.1.1 Dataset I: Patch clamp recordings from human failing hearts

Dataset I consists of patch clamp ion current recordings from human PC from (Han et al. 2002).

Briefly, these consist of the I - V curves for the transient and sustained outward potassium currents (I_{to} and I_{sus} , respectively) and for the inward potassium rectifier current (I_{K1}), as well as I_{to} steady state inactivation/activation curves, and inactivation time constants (Figure 4.1). Data were acquired at 37 °C from $n=20$ PCs isolated from free-running false tendons from $N=9$ failing human hearts (8 males and 1 female, with average age of 48 years). Same measurements were performed on midmyocardial myocytes for comparison, highlighting the main differences between Purkinje and ventricular electrophysiology. In particular, I_{K1} has a similar behaviour in both preparations, but it is larger in ventricular cells at more negative plateau (< -90 mV). I_{to} is larger in ventricle, though its pedestal component, I_{sus} is bigger in Purkinje. Also, both fast and slow I_{to} inactivation time constants are bigger in PCs, as already mentioned in Section 3.3.2.2.

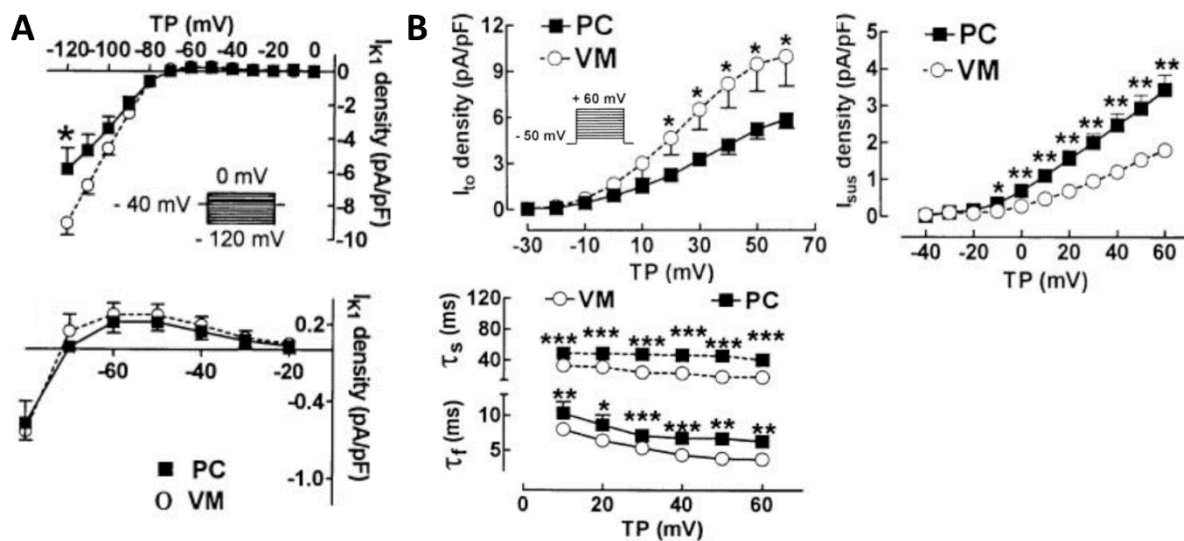


Figure 4.1 Dataset I, experimental patch clamp recordings from human Purkinje and ventricular cells: A) I - V curves for I_{K1} at all (top) and less negative testing potentials (bottom). Stimulation protocol showed as inset; B) I - V curves for I_{to} and I_{sus} (top left and right, respectively) and I_{to} slow and fast inactivation time constants. Stimulation protocol showed as inset. Modified from (Han et al. 2002).

4.2.1.2 Dataset II: Current clamp AP recordings from healthy human PCs

Dataset II consists of AP recordings from $n=17$ Purkinje fibres from $N=7$ healthy human hearts, acquired at multiple frequencies - basic cycle length (BCL) from 400 to 5000 ms - partly published in (N. Nagy et al. 2015). APs were recorded at 37 °C by using conventional microelectrode techniques and analysed to extract the following 9 biomarkers: maximum upstroke velocity (dV/dt_{MAX}); AP duration (APD) at 90%, 75%, 50%, 25% and 10% of repolarisation (APD_{90} , APD_{75} , APD_{50} , APD_{25} , APD_{10}); AP Amplitude (APA); membrane potential before depolarisation (TOP, take-off potential); membrane potential at the end of repolarisation (EOP, end of potential). Each biomarker is represented in Figure 4.2A. Table 1 shows the minimum and maximum experimental values for each biomarker and BCL. AP recordings over time and APD_{90} rate-dependence are displayed in Figure 4.2B and 4.2C, respectively.

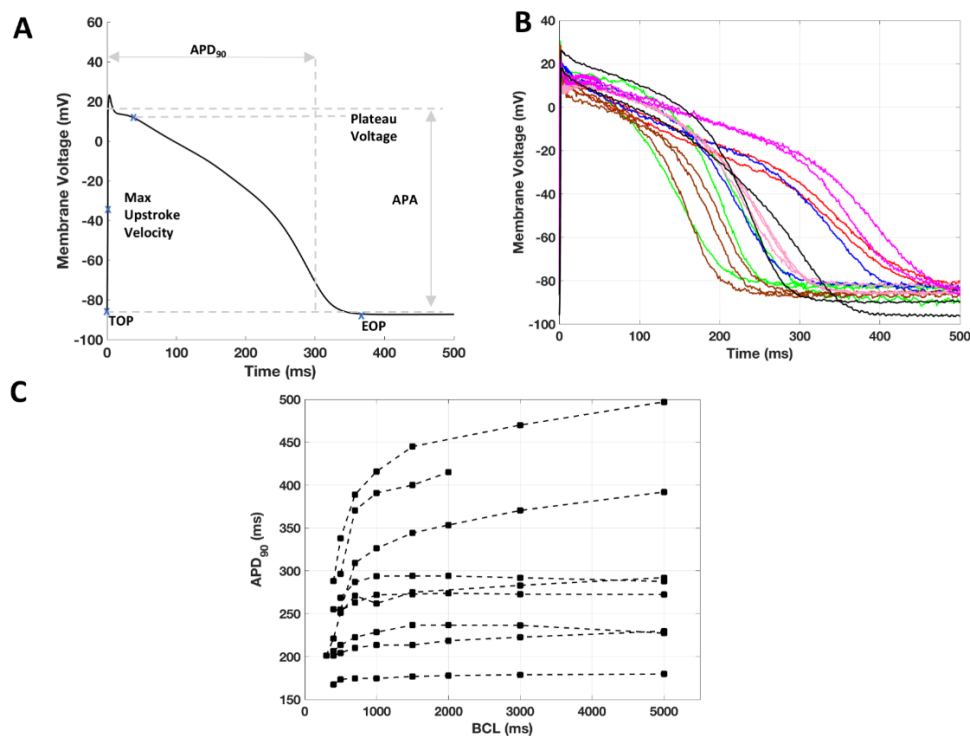


Figure 4.2. Overview of Dataset II. A) Representation of each AP biomarkers used in the study. B) AP recordings from healthy human PCs: each colour represents a different heart. C) APD_{90} rate dependence at different BCL, from 400 to 5000 ms.

Table 4.1. Experimental biomarkers. Minimum and maximum experimental values for the 9 AP biomarkers recorded in human cardiac Purkinje cells at multiple BCL: 400 ms (n=6,), 500 ms (n=9), 700 ms (n=9), 1000 (n=17), 1500 ms (n=9), 2000 ms (n=7), 3000 (n=9), 5000 ms (n=9).

	<i>BCL (ms)</i>															
	400		500		700		1000		1500		2000		3000		5000	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
<i>dV/dt_{Max}</i> <i>(V/s)</i>	242	576	176	674	176	644	154	688	195	605	195	625	195	625	151	625
<i>APD₉₀</i> <i>(ms)</i>	167	288	173	338	175	389	187	438	177	445	178	415	179	470	180	497
<i>APD₇₅</i> <i>(ms)</i>	146	253	150	298	151	346	166	384	155	394	157	371	157	421	159	453
<i>APD₅₀</i> <i>(ms)</i>	114	199	118	246	121	292	124	349	122	339	127	292	125	365	129	392
<i>APD₂₅</i> <i>(ms)</i>	32	137	39	148	45	167	31	185	34	209	20	147	19	218	25	226
<i>APD₁₀</i> <i>(ms)</i>	2	65	1.4	63	1	82	1	107	1	75	1	23	1	86	1	91
<i>APA</i> <i>(mV)</i>	101	115	70	115	91	115	93	116	93	122	92	115	94	123	89	115
<i>TOP</i> <i>(mV)</i>	-88	-80	-89	-75	-90	-76	-91	-79	-92	-77	-87	-76	-93	-77	-88	-73
<i>EOP</i> <i>(mV)</i>	-91	-85	-89	-32	-90	-75	-94	-81	-93	-77	-89	-77	-93	-77	-88	-72

BCL: Basic cycle length; **dV/dt_{MAX}:** maximum upstroke velocity; **APD_x:** AP duration at X% of repolarisation; **APA:** Action potential amplitude; **TOP:** membrane potential before depolarisation; **EOP:** membrane potential at the end of repolarisation.

4.2.1.3 Dataset III: Current clamp AP recordings from healthy human PCs in control and with Dofetilide

Dataset III consists of AP recordings from n=3 Purkinje fibres from N=3 healthy human hearts following the same procedures previously used for ventricular trabeculae (Page et al. 2016). 30 consecutive APs for each fibre were recorded at 1 and 2 Hz under control conditions (0.1%

DMSO), and with 100 nM Dofetilide. Figure 4.3 shows the AP recordings at 1 Hz and 2 Hz, both in control and with Dofetilide.

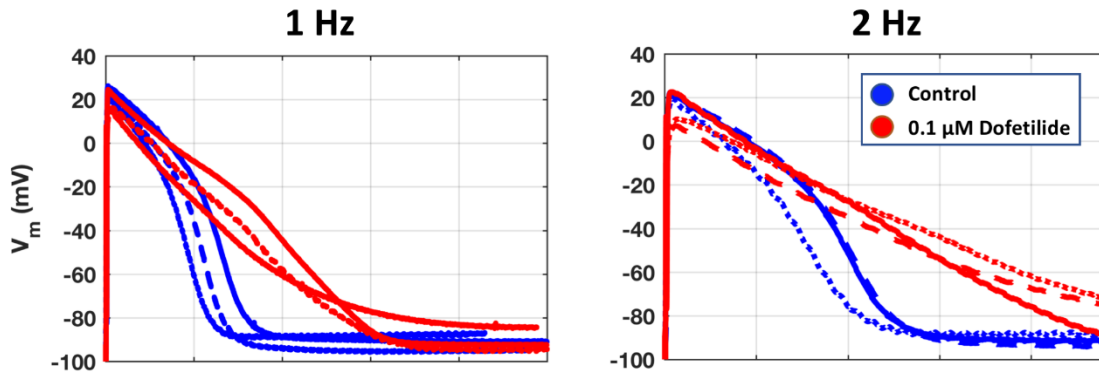


Figure 4.3 Dataset III: AP recordings at 1 and 2 Hz from healthy human Purkinje fibre: control (blue) and Dofetilide (red). Each line style represents a different heart.

4.2.2 Strategy for Model Design, Calibration and Validation

Figure 4.4A illustrates the design, calibration, optimisation, and validation of the Trovato2020 model, described in more details below. The Trovato2020 model presented in this study is built on the ionic formulations of the O’Hara-Rudy human ventricular ORd model (O’Hara et al. 2011), and the Purkinje-specific Ca^{2+} sub-system, cellular compartments and intracellular ionic fluxes of the canine Purkinje PRd model (P. Li and Rudy 2011), also used by (Britton et al. 2013). The model includes the ORd mathematical formulation for each of the following currents: fast Na^+ current (I_{Na}), Na^+ late component (I_{NaL}), L-type Ca^{2+} current (I_{CaL}), rapid and slow delayed K^+ rectifiers (I_{Kr} and I_{Ks} , respectively), Na^+ - Ca^{2+} exchanger (I_{NCX}) and Na^+ - K^+ pump (I_{NaK}). I_{to} , I_{sus} and I_{K1} were formulated based on the Dataset I. In addition, two Purkinje-specific currents from the PRd model were included: T-type Ca^{2+} current (I_{CaT}) and funny current (I_{f}). Existing knowledge from the relevant literature on the ionic current differences between ventricular and PCs, and between human and canine PCs, reported in the previous chapter, was also taken into account.

The Trovato2020 model was developed to fulfil the following 6 criteria:

- 1) Consistency with the experimental AP biomarkers at all BCLs: simulated values had to be within the experimental ranges shown in Table 1.
- 2) APD₉₀ rate-dependence: APD₉₀ increase for BCLs between 400 ms and 2000 ms.
- 3) APD₉₀ changes induced by I_{CaL} modulation: APD₉₀ increase/shortening in response to I_{CaL} up/down regulation. Direct dependence of APD₉₀ on I_{CaL} has been shown experimentally, by using selective Ca^{2+} blockers (e.g. Diltiazem (Péter P. Nánási et al. 1992), Nifedipine (Terrar et al. 2007)). Due to the lack of quantitative information and human data, we only imposed a positive correlation between changes in APD₉₀ (ΔAPD_{90}) and I_{CaL} up/down regulation ($|\Delta APD_{90}| > 0.5\%$ with $\pm 30\%$ I_{CaL}).
- 4) APD₉₀ prolongation induced by I_{Kr} block. In agreement with (Varro et al. 2000) showing longer APD in Purkinje than ventricular cardiomyocytes following I_{Kr} block, we imposed the APD in the Trovato2020 model to be larger than the one observed in the corresponding ventricular model (ORd) for 30% and 50% I_{Kr} blocks.
- 5) Ability to generate EADs, reported experimentally in canine PCs (Roden and Hoffman 1985; Nattel and Quantz 1988).
- 6) Ability to generate DADs and/or triggered AP, as reported for canine PCs (Kang et al. 2010; Xing and Martins 2004).

Starting from the initial model described above, a sensitivity analysis was performed to investigate how properties relevant to the 6 criteria were affected by variations in the ionic current parameters, and to guide the definition of a calibrated model, as described below (Section 4.2.4). Parameter optimisation was then performed using a multi-objective genetic algorithm (details in Section 4.2.5). The optimised Trovato2020 model was evaluated against Dataset III, and on its ability to develop automaticity under specific conditions (details in Section 4.2.6). Propagation of electrical excitation in a 1D fibre using the optimised

Trovato2020 model was also simulated, to verify conduction velocity (CV) in control conditions, as well as to test the potential propagation of spontaneous APs and to evaluate the effects of I_{Na} block on CV (details in Section 4.2.7).

4.2.3 Sensitivity Analysis

A sensitivity analysis was conducted on the initial model to investigate how the 6 criteria for model calibration are affected by variations in the conductances of all the ionic currents (I_{Na} , I_{NaL} , I_{CaL} , I_{CaT} , I_{to} , I_{sus} , I_{Kr} , I_{Ks} , I_f , I_{K1} , I_{NCX} , I_{NaK}) and in the kinetics of I_{CaL} and I_{Kr} (I_{CaL} : steady state activation and inactivation, fast and slow inactivation time constants; I_{Kr} : fast and slow activation time constants, steady state inactivation). Each current conductance was varied from 10% to 200% of its nominal values, while specific variation ranges were defined for each current kinetics parameter (Table 4.2). For each value of each parameter, six electrophysiological protocols (described in detail in Section 4.2.7) were simulated to evaluate the effects of the current conductance modulation on each of the 6 criteria used for the model design. A sensitivity analysis was also performed for the optimised model (see below, Section 4.2.4), varying only the current conductances. The results of the sensitivity analysis of the final optimised model will be presented in the Chapter 5.

4.2.4 Optimisation with a Multi-Objective Genetic Algorithm

A multi-objective genetic algorithm (Matlab function *gamultiobj*) was used for automated multi-object optimisation (Deb 2001) of the calibrated model described above. All the ionic current conductances (except for the background currents) were allowed to vary in the range [50-150]% of their nominal values, to exclude extreme up/down-regulations. The 7 kinetic parameters of I_{CaL} and I_{Kr} were allowed to vary in the ranges reported in Table 4.2, based on the sensitivity analysis results. The algorithm was run for 30 generations, with 300 models each. The multi-object cost function was computed as a weighted sum of 2 error functions: i) distance from the experimental mean of the AP biomarkers in control condition at 1 Hz (Dataset II, Section 4.1); ii) a linear combination of errors, based on the criteria 2-5 described in the main text (Section 2.2) for the APD₉₀ rate-dependence, AP response to I_{CaL} and I_{Kr} modulations

and EADs inducibility. At the end of each generation, the Pareto front (Deb 2001) was computed, to identify the 35% of local minima of the cost function, i.e., the models with the best performances, then combined to create the following generation. After 30 generations, the model with best performance was chosen as the final optimised Trovato2020 model.

Table 4.2. Parameters and ranges to perform the sensitivity analysis and to optimise the calibrated model through multi-object genetic algorithm

	Sensitivity analysis range		Calibrated model Pre-optimisation Coefficients	Optimisation Range		Final Model Post-optimisation Coefficients
	Min	Max		Min	Max	
Conductances						
G _{Na}	[10 ; 200] %		1	[50 ; 150] %		0.75
G _{NaL}			2.5*			1
G _{CaL}			1			0.75
G _{CaT}			1			0.96
G _{to}			1			0.93
G _{sus}			1			1.28
G _{Kr}			1			0.93
G _{Ks}			1			0.84
G _f			1			0.97
G _{K1}			1			0.67
G _{NCX}			1			1.2
G _{NaK}			1			1.1
I _{CaL} Kinetics						
Activation/ Inactivation V shift (mV)	-4	4	2	0	4	3.3
Time constants V shift (mV)	-10	30	15	10	20	15.2
Slow time constants scales	0.1	3	0.7	0.4	1	0.49
Fast time constants scales	0.1	3	0.7	0.4	1	0.72
I _{Kr} Kinetics						
Fast time constant V shift (mV)	[-10 ; 30] mV		15	10	20	17.6
Slow time constant V shift (mV)	[-10 ; 30] mV		15	10	20	17.2
Inactivation slope	[0.3 ; 3]]		0.3	0.1	0.5	0.32

Varied parameters (1st column). Ranges investigated via sensitivity analysis (2nd & 3rd columns). Conductances scaling factors and kinetics coefficients for the calibrated model (4th column). Optimisation ranges for the multi-object genetic algorithm (5th & 6th columns). Conductances scaling factors and kinetics coefficients for the final model are reported in bold (7th column). *from (Iyer et al., 2015).

4.2.5 Model validation

The optimised model was evaluated against the experimental Dataset III, not considered during the model development, and not used for the calibration and optimisation of the initial model. The simulations in control conditions and with Dofetilide were conducted in similar experimental conditions as Dataset III, following the protocols 1 and 7, respectively (see Section 4.2.8).

Moreover, the protocol for automaticity was used to test the potential of the model to develop automaticity, when modifying the balance between I_{K1} and I_f , since they are the diastolic currents determining the DMP, which in turn modulates PCs automaticity (Dobrzynski et al. 2013; Boyden, Hirose, and Dun 2010). Furthermore, the effect of hyperkalaemia was also evaluated by increasing the extracellular potassium concentration, since it has been shown to suppress automaticity in human PCs (F. Y. Lee et al. 2004).

4.2.6 1D Purkinje Fibre

In order to evaluate the effects of intracellular coupling on the protocols tested (Section 4.2.7) a 1D Purkinje fibre model was constructed using the Trovato2020 model to represent membrane kinetics. The 5 cm fibre was discretised in 100 nodes to obtain a spatial resolution of 500 μm . Temporal integration step was set to 500 μs . Both spatial and temporal resolutions were determined to guarantee the best compromise between spatial and temporal convergence errors ($<10\%$) (Bueno-Orovio et al. 2014) and computational time for simulations. The monodomain formulation was used to simulate propagation along the fibre (Alonso, Bär, and Echebarria 2016) and was solved using the Fourier spectral method for fractional diffusion (Bueno-Orovio et al. 2014). The Rush-Larsen method (Rush and Larsen 1978) was implemented for the integration of the gating variables to speed up the simulations. Stimulus duration was set to 2.2 ms, i.e. twice the minimum value to achieve propagation in the fibre.

The fibre was stimulated on one side for 1 mm. For each protocol tested, three beats were simulated in the fibre, to allow relaxation from the initial conditions (all the nodes were initialised to the SS computed at cellular level). APD₉₀ and CV were computed for the last beat. CV was computed at the centre of the fibre, to avoid border effects, as the distance between the 17 central nodes (set to 0.26 cm), divided by the difference of activation times (identified as the instant with maximum dV/dt) at the border nodes.

Diffusion coefficient was set to 9 cm²/s to match the CV of 1.6 m/s computed from observations at sinus rhythm in human Purkinje fibres (Kupersmith et al. 1973, Durrer et al. 1970). Protocols 1 and 8 (Section 4.2.7) were simulated in fibre, to reproduce the electrical propagation at 1 Hz and following spontaneous beating, respectively. In addition, effects of I_{Na} blocks on the CV were considered, modifying the protocol used for hERG-blocks. I_{Na} was reduced up to 100%, or until propagation failure was observed.

4.2.7 Stimulation protocols

Single cell model equations were implemented in Matlab (Mathworks Inc. Natwick, MA, USA) and solved with the function *ode15s*, an adaptive time step solver for stiff problems (Shampine and Reichelt 1997). Data analysis was also performed using Matlab. Simulations for the population of models were run on the Oxford supercomputer ARCUS (<http://www.arc.ox.ac.uk/>). The monodomain formulation was used to simulate propagation along the fibre (Alonso, Bär, and Echebarria 2016) and was solved using the Fourier spectral method for fractional diffusion (Bueno-Orovio, Kay, and Burrage 2014). The Rush-Larsen method was implemented for the integration to speed up the simulations (Coudière, Lontsi, and Pierre 2017) in Matlab.

To evaluate the calibration and validation criteria described in Section 4.2.2, 9 different simulation protocols were applied, 6 for calibration, 2 for validation and 1 for comparison against voltage-clamp experiments. A list of the protocols used in this study is provided below:

- 1) Control conditions. Steady state (SS): 1000 beats at 1 Hz, to allow the intracellular concentrations to reach stability.
- 2) Rate dependence. Starting from SS, 150 beats for each BCL, from 300 ms to 5000 ms.
- 3) I_{CaL} modulation. Starting from SS, 150 beats at 1 Hz with up/down regulation ($\pm 30\%$) of the I_{CaL} conductance.
- 4) hERG block. Starting from SS, 150 beats at 1 Hz with I_{Kr} blocks (at 30% and 50%).
- 5) EADs inducibility. Starting from SS, 150 beats at slow pacing (BCL = 4000 ms) with 85% I_{Kr} block, as in (O'Hara et al. 2011). EADs were identified when a positive derivative of the membrane potential was observed, from 150 ms after the stimulus (Passini et al. 2017).
- 6) DADs inducibility. Starting from SS, 1500 beats at fast pacing (BCL = 300 ms, 3.3 Hz) and 1 long beat (BCL = 10,000 ms), with and without RyR hypersensitivity, similar to (P. Li and Rudy 2011). RyR hypersensitivity was simulated as an increased sensitivity to intracellular Ca^{2+} (+100%) and a decrease in the release time constant (-70%). DADs were identified visually.
- 7) Dofetilide. Starting from SS, 150 beats at 1 Hz and 2 Hz, using a simple pore-block drug model (Brennan, Fink, and Rodriguez 2009) with IC_{50} (in μM) and Hill coefficient (IC_{50}/h) for I_{Kr} , I_{Na} and I_{CaL} equal to 0.03/1.2, 162.1/1 and 26.7/1, respectively (Kramer et al. 2013).
- 8) Automaticity. Starting from SS, 3 different combinations of I_f and I_{K1} were tested: i) 90% I_{K1} block; ii) increasing I_f 9-folds; iii) combining the 2 conditions, 50% I_{K1} block and 4-folds I_f . The values of I_{K1} and I_f conductances were identified through a

sensitivity analysis up to automaticity appearance. The model was stimulated for 150 beats at 1 Hz, followed by 15 s with no stimulation. The same simulations were repeated under hyperkalaemia with extracellular $[K^+]$ set to 8 mM to reproduce the same conditions as in (F. Y. Lee et al. 2004).

- 9) Voltage clamp simulations (for $I-V$ curves). 1000 ms at holding voltage (-40 for I_{K1} and -50 mV for both I_{to} and I_{sus}) followed by a step (300 ms for I_{to} and 100 ms for both I_{K1} and I_{sus}) at different voltage values (from -120 mV to 0 mV for I_{K1} , and from -50 mV to +60 mV for both I_{to} and I_{sus}). Intracellular $[Na^+]$, $[Ca^{2+}]$ and $[K^+]$ were set to 0.0001 mM, 0.0001 mM and 130 mM respectively while extracellular were 140 mM, 1 mM, 5.4 mM as in the experiments by (Wei Han, Zhang, et al. 2002).

4.3. Results

4.3.1 The Calibration of the Trovato2020 Model: Sensitivity analysis and ionic current

Figure 4.4B illustrates the structure of the new human Purkinje model, the Trovato2020 model, with 6 cellular compartments, 18 trans-membrane ionic currents, 3 intracellular concentrations (Na^+ , K^+ and Ca^{2+}) for each of the 3 cellular compartments, Ca^{2+} buffers and CaMKII kinetics as in the ORd model (O'Hara et al. 2011), and Ca^{2+} subsystem as in the PRd model (P. Li and Rudy 2011). Overall, the Trovato2020 model has 46 state variables.

Cellular compartments: the intracellular compartmentalisation was defined as in the PRd model, based on a triple-layer structure suggested by (Stuyvers et al. 2005). The cell, represented as a cylinder, is divided into 3 cytoplasmic compartments: i) peripheral coupling subspace (SS), where sarcolemmal Ca^{2+} entry (via I_{CaL}) interacts with the sarcoplasmic

reticulum (SR); ii) sub-sarcolemmal region (SL), representing the layer of cytoplasm underneath the membrane; iii) bulk myoplasm, that represents the innermost region of the cell.

The SR is also divided into 3 compartments:

- i) junctional SR, with two Ca^{2+} -release units (RyR3 and IP₃R), which respond to Ca^{2+} changes in the SS;
- ii) corbular SR, representing the SR portion close to the cell membrane, and with Ca^{2+} -release units (RyR2) which respond to Ca^{2+} changes into the bulk myoplasm only;
- iii) network SR, representing a region between JSR and CSR with no expression of Ca^{2+} -release units (P. Li and Rudy 2011).

Sodium Currents: The Trovato2020 model includes the modified ORd formulation of I_{Na} described in (Dutta et al. 2017; Passini et al. 2016) which supports AP propagation also in 3D simulations. Several studies in PCs (Haissaguerre et al. 2016; N. Nagy et al. 2015) highlighted the role of non-cardiac isoforms of the sodium channel, which have slower inactivation kinetics and a different sensitivity to tetrodotoxin. In order to account for the sodium current characteristics and functional role experimentally shown in PCs, the original ORd formulation of I_{NaL} was kept in the Trovato2020, though its conductance was increased 2.5 folds, based on experimental evidence by (V. Iyer et al. 2015; Haufe et al. 2005). However, we did not split the formulation into cardiac and non-cardiac isoform contributions, which might be a possible future development when data from human PCs become available. Background Na^+ current was increased by the same amount as I_{NaL} , as in (Passini et al. 2016).

Calcium Currents: I_{CaL} formulation was left as in the ORd model, since no human Purkinje data are available in literature (N. Nagy et al. 2015). Based on the sensitivity analysis (Figure 4.2 and Figure 4.3), some changes were made to the current kinetics: both inactivation and

activation gates were shifted by +2 mV, and inactivation time constants were scaled of 30% and shifted by +15 mV (Table 4.2). These changes increased the EADs inducibility of the initial model (Figure 4.3, top panel), without abnormally increasing the AP plateau as seen with I_{CaL} upregulation and I_{sus} downregulation (Figure 4.2, top panel). I_{CaT} was incorporated into the model, using the formulation proposed in the PRd model. Background Ca^{2+} current (I_{Cab}) and the sarcolemmal Ca^{2+} pump (I_{pCa}) were left as in the ORd model.

Potassium Currents: I_{to} , I_{sus} and I_{K1} formulations were constructed using Dataset I (Han et al., 2002) as follows. I_{to} formulation includes one activation gate and two inactivation gates (fast and slow) with steady state activation/inactivation curves and inactivation time constants, larger in PCs than in VCs, based on experiments by Han et al., 2002, and activation time constant as in the ORd model (no experimental data available). Thus, the Trovato2020 representation of I_{to} differs both from the ventricular formulation used by ten Tusscher and Panfilov 2008 and from the one implemented in Stewart et al. 2009. In particular, the latter describes I_{to} inactivation using only one gate, using experimental data for the slow inactivation time constant published by (Wei Han, Zhang, et al. 2002), whereas, the activation time constant is fitted on the experimental data for I_{to} fast inactivation.

The small ORd background K^+ current was replaced with a single instantaneous voltage-dependent current, based on the experimental $I-V$ curve for I_{sus} . The I_{K1} formulation was modified by fitting the instantaneous voltage-dependent rectification gate to the experimental $I-V$ curve, shifted by -14 mV to account for the liquid junction potential and to match the reversal potential to the potassium Nernst potential computed from the intra and extra-cellular concentrations. The maximum conductances of these three currents were also set based on the experimental $I-V$ curves, after correcting the data for heart failure (+25% and +56% for I_{K1} and

I_{to} , respectively), as described in (Stewart et al. 2009). I_{Kr} and I_{Ks} formulations were left as in the ORd model, since no human Purkinje data are available in literature (N. Nagy et al. 2015) and gene expression levels for these proteins in humans (hERG and KvLQT1) were found to be similar in PCs and VCs (Gaborit et al. 2007).

Based on the sensitivity analysis (Figure 4.5 and Figure 4.6), some changes were made to I_{Kr} kinetics: activation time constants were shifted by +15 mV and the time-independent inactivation gate slope was scaled by a factor of 0.3 (Table 4.2). These changes were needed to improve the model's response to I_{CaL} modulation. In particular, they were needed to reverse the behaviour observed in the initial model, i.e., AP prolongation with I_{CaL} downregulation and shortening with I_{CaL} upregulation, which was not reported experimentally (Péter P. Nánási et al. 1992; Terrar et al. 2007). As it is possible to observe from Figure 4.5, changing only the current conductances allowed to reverse this behaviour in 3 cases: i) extreme upregulation of I_{CaL} , ii) extreme downregulation of I_{sus} , and iii) extreme downregulation of I_{Kr} . In the first two cases, however, the inversion was due to a non-physiological increase in the plateau level, which resembled in both cases the one produced by the original ORd model. Then, the key actor was I_{Kr} and, in particular, its response to changes in the plateau. An extreme downregulation of I_{Kr} , such as the one in the third case, would lead, however, to an abnormal AP prolongation. Also, there are no reports of such I_{Kr} downregulation in human PCs (Gaborit et al. 2007). Figure 4.6 (bottom panel), however, confirmed that it is possible to modify I_{Kr} 's response to I_{CaL} modulation, with a few changes in its kinetics to take into account the lower AP plateau of the Purkinje model in respect with the ORd one.

Other currents: I_f was included in the model, using the formulation proposed in the PRd model. I_{NCX} and I_{NaK} were kept as in the ORd model.

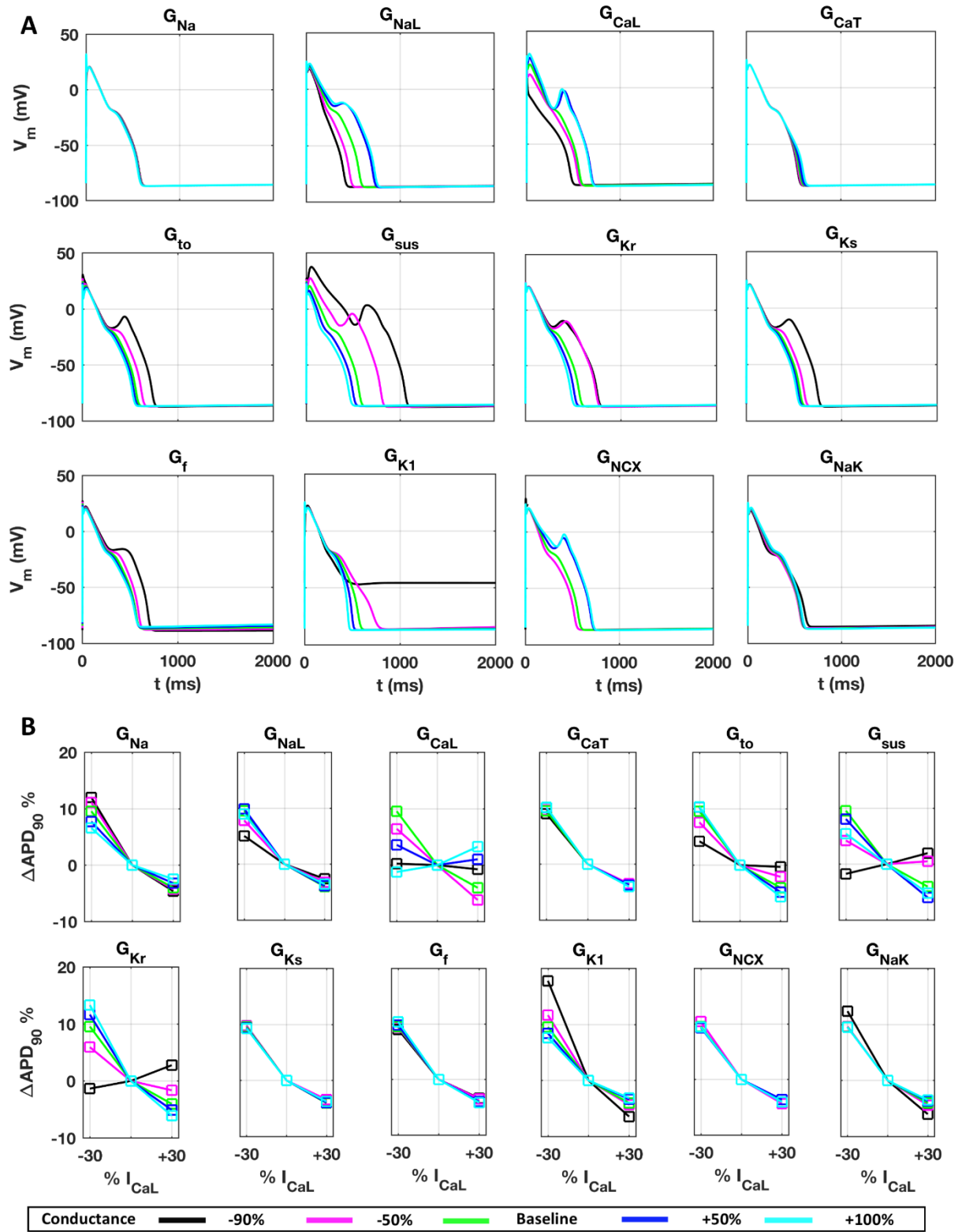


Figure 4.5. Sensitivity analysis performed on the initial model varying current conductances as reported in Table 4.2. Investigation of each current contribution in EADs inducibility and APD_{90} response to I_{CaL} modulations. The legend indicates the percentage of modulation for each conductance. A) Simulated APs following Protocol 5 for EADs inducibility. B) Percent changes in the APD_{90} following Protocol 3 in response to I_{CaL} conductance changes ($\pm 30\%$).

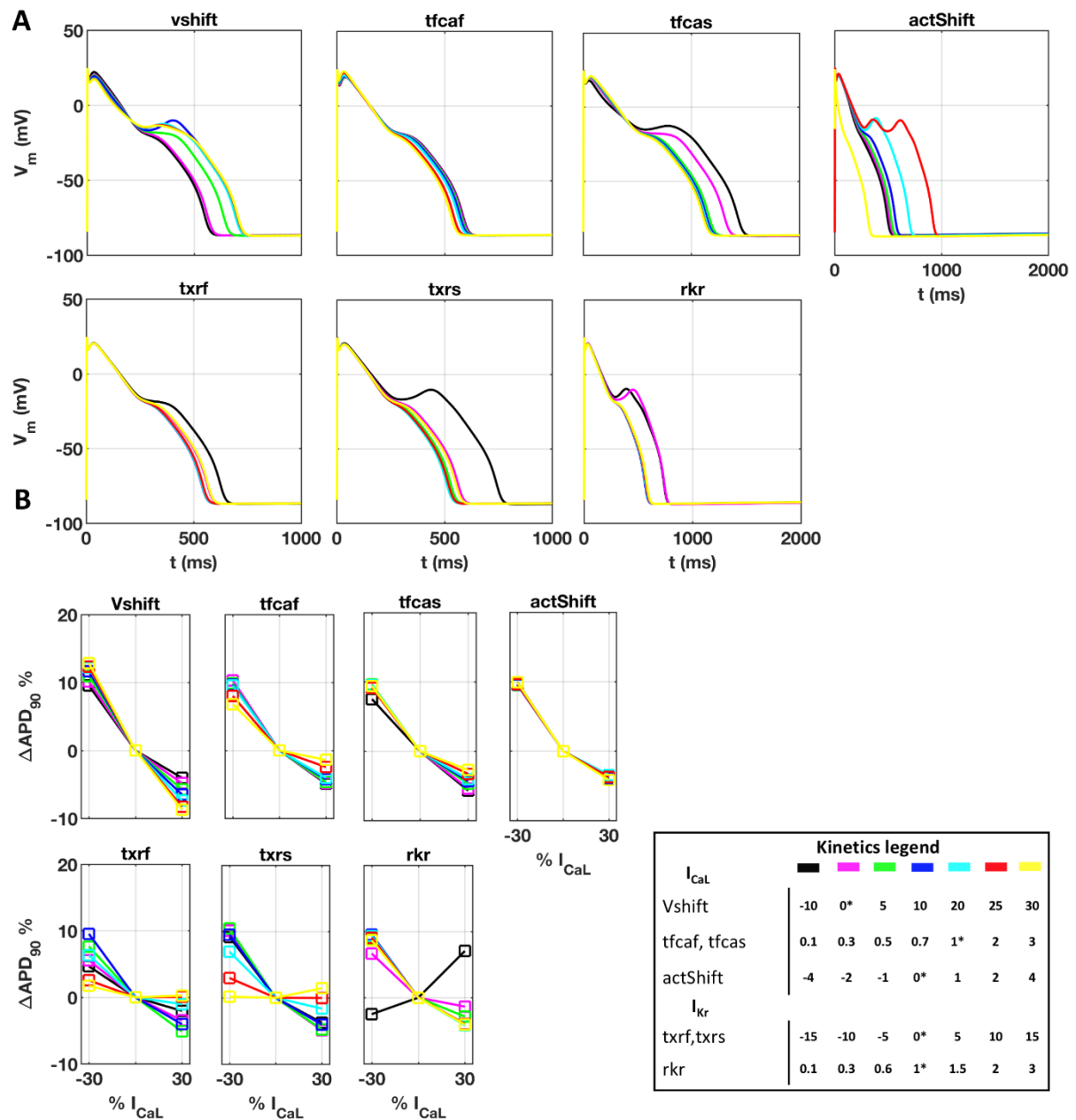


Figure 4.6. Sensitivity analysis performed on the initial model, varying I_{Kr} and I_{CaL} kinetics, using the coefficients reported in the legend and in Table 4.2 (*Nominal value for the initial model). Investigation of each current contribution to EADs inducibility and APD_{90} response to I_{CaL} modulations. A) Simulated APs following Protocol 5 for EADs inducibility. B) Percentual changes in the APD_{90} following Protocol 3 in response to I_{CaL} changes ($\pm 30\%$). Vshift: voltage shift for I_{CaL} inactivation time constants; tfcaf, tfcas: scaling factors for I_{CaL} fast and slow inactivation time constants. actShift: voltage shift in both activation and inactivation gate for I_{CaL} . txrf, txrs: voltage shift for I_{Kr} fast and slow activation time constants; rkr scaling factor for I_{Kr} time-independent inactivation gate slope.

4.3.2 Simulations with the optimised Trovato2020 model reproduce experimental AP recordings and fulfil all calibration criteria

Optimisation using the genetic algorithm produced a Pareto optimal front of 105 models, including many duplicates of 8 unique models. The final optimised Trovato2020 model was identified as the model with the best performance across all the tested protocols. Parameters and simulation results for the 8 models are shown in Figure 4.7. Table 2 reports the simulated AP biomarkers for the optimised Trovato2020 vs the non-optimised model, and the human experimental biomarker ranges from Dataset II.

As shown in Figure 4.7A, across the 8 models, most of the ionic conductances were varied in a range of $\pm 20\%$ compared to the values of the calibrated model. In particular, it is possible to observe downregulated expressions of G_{Na} (between 15 and 25%), G_{CaL} (between 12 and 22%, for 7 out of 8 models), G_{to} (between 5 and 15%), G_{Kr} (between 5 and 20%, for 6 out of 8 models), G_{Ks} (between 15 and 30%, for 7 out of 8 models), whereas, G_{sus} and G_{NCX} were uniquely upregulated between 20 and 45% and 5 and 20%, respectively. Expressions of G_{NaL} , G_{CaT} , G_f , and P_{NaK} were not uniquely down/up regulated across all the models, though, their modulation was modest. Figure 4.7B shows, for the 8 models, the distribution of the coefficient for both I_{CaL} (top) and I_{Kr} (bottom) kinetics spanning the whole optimisation ranges. Overall, it is possible to observe a unique pattern, with the model's coefficients restricted to much narrow ranges, compared to the initial ones.

Figure 4.7C shows the APs for each of the final 8 models. Overall, the APD_{90} varies between 280 and 310 ms, significantly larger than the initial APD_{90} , whereas during phase 1 it is possible to observe some models with a more prominent spike and dome pattern, and others with a smoother early repolarisation. The APD_{90} rate-dependence reported in Figure 4.7D, highlights that for all the models the APD increases for BCL from 400 to 2000 ms. At higher BCL, the APD_{90} increases for some models and slightly decreases for others.

Figure 4.7E shows the APs following the EADs protocol described in Section 4.2.7, i.e., slow pacing with 85% I_{Kr} block. In these conditions, all models developed an EADs, of reduced amplitude with respect to the pre-optimisation model. Figure 4.7F reports the APD₉₀ changes due to the selective block of I_{Kr} and the I_{CaL} modulation. Regarding the I_{Kr} block, no major differences were observed among the models, whose AP prolongation in response to the block varied between 15 and 20%. Also, for the I_{CaL} modulation, no major differences were observed among the models, all sharing a very small and direct APD₉₀ response to I_{CaL} modulation (AP prolongation following I_{CaL} increase and AP shortening for I_{CaL} decrease).

Overall, all the models share a similar behaviour under the protocols tested. However, the model referred as 4 was chosen as the most representative since simulated AP biomarkers were closer to the experimental average.

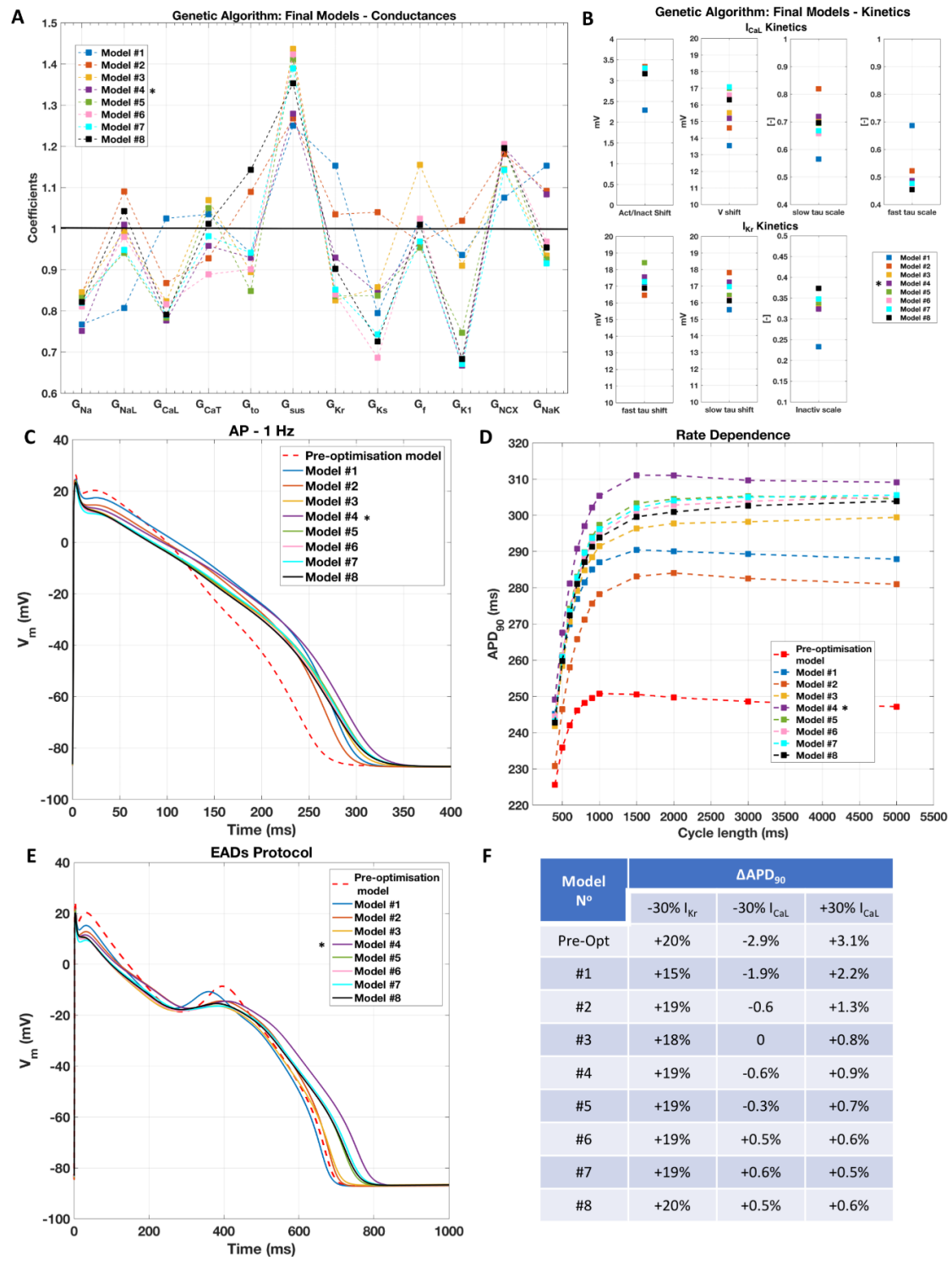


Figure 4.7. Parameters and simulations of the final 8 optimised human Purkinje models through multi-objective genetic algorithm. Parameter sets for the 8 optimised models: A) current conductances B) I_{CaL} and I_{Kr} kinetics. Ranges are defined as in Table 4.2. C) Simulated APs at 1 Hz. D) APD_{90} rate dependence. E) Simulated APs to induce EADs. F) APD_{90} changes induced by I_{Kr} block or I_{CaL} modulation in the 8 optimised models. *Final Trovato2020 model.

Figure 4.8 shows the simulation results for the optimised Trovato2020 model, reproducing Datasets I and II and satisfying each of the 6 calibration criteria: simulations of voltage-clamp protocols for I_{to} , I_{sus} and I_{K1} to reproduce the experimental $I-V$ curves from Dataset I (Panel A); AP traces at 1 Hz and rate dependence in line with experimental recordings (Panel B and C, respectively); APD₉₀ changes induced by I_{CaL} modulation (Panel D) and I_{Kr} block (Panel E); EADs and DADs inducibility (Panel F and G, respectively). Further information on the ionic currents underlying the AP and Ca^{2+} -transient in the optimised model at 1 Hz are showed in Figure 4.9.

As expected, fast Na^+ current activation drives the depolarisation phase with $dV/dt_{MAX} = 381$ mV/s, 24 mV voltage peak and 110 mV amplitude (Table 4.3). During the early-repolarisation phase, simulated AP (Figure 4.8B) presents a “spike and dome” behaviour, due to the interplay between the depolarising currents I_{CaL} and I_{NaL} and the repolarising I_{to} and I_{sus} . The repolarisation phase is driven by I_{Kr} , I_{Ks} and I_{K1} , resulting in APD₉₀ of 306 ms at 1 Hz. The small diastolic depolarisation (~ 1 mV), due to the action of I_f , is in agreement with the difference observed experimentally between potentials at take-off and end of repolarization (EOP vs TOP). No automaticity was observed, in agreement with experimental recordings from Dataset II and the literature (Boyden, Hirose, and Dun 2010).

Rate-dependence of APD₉₀ is in line with experimental recordings (Figure 4.8C). At BCL=400 ms, the APD₉₀ is 250 ms and increases up to 312 ms at BCL=5000 ms.

The optimised model reproduces a positive correlation between APD and I_{CaL} modulation (Figure 4.8D): small changes of the I_{CaL} conductance mostly affect the plateau phase, with small changes in APD as reported for rabbit PCs (H.-A. Lee et al. 2016), whereas a stronger I_{CaL} block also causes APD shortening, similar to experimental recordings in canine PCs (Péter P. Nánási et al. 1992; Terrar et al. 2007).

Simulations with the new human Purkinje Trovato2020 model yield APD prolongation following I_{Kr} block (Figure 4.8E): 30% and 50% I_{Kr} blocks prolonged APD₉₀ from 306 (control) to 365 and 421 ms, respectively, whereas the same blocks in the human ventricular ORd model prolonged the APD₉₀ from 269 ms (control) to 329 and 386 ms, respectively. These results are in agreement with canine experiments (Varro et al. 2000) showing a longer AP in Purkinje compared to ventricular cells, both in control conditions and following I_{Kr} block.

EADs inducibility is shown in Figure 4.8F: the AP fully repolarises with BCL = 4000 ms (APD₉₀ = 307 ms, dashed line), but an EAD occurs when including 85% I_{Kr} block (solid line).

DADs inducibility is illustrated in Figure 4.8G: following fast pacing, the Trovato2020 model remains well polarised in control conditions (dashed line), while DADs occur when including RyR hypersensitivity (solid line). The initial DADs amplitude is 6 mV, and it decreases to less than 1 mV after 13 s. The system keeps oscillating even after 300 s. The ionic mechanisms of EADs and DADs generation are investigated in Section 3.6 and 3.7.

Additional information about the optimised Trovato2020 model is included in the next chapter, i.e. the sensitivity analysis and the correlation between AP biomarkers and ionic current conductance.

Table 4.3. Simulated and experimental biomarkers. AP biomarker comparison between simulations and experiments (mean $\pm$ std) at 1 Hz.

	<i>Experiments</i>	<i>Initial Model</i>	<i>Optimised Model</i>
<i>dV/dt_{Max} (V/s)</i>	387 $\pm$ 143	435	381
<i>APD₉₀ (ms)</i>	294 $\pm$ 76	249	306
<i>APD₇₅ (ms)</i>	261 $\pm$ 67	225	279
<i>APD₅₀ (ms)</i>	210 $\pm$ 52	168	223
<i>APD₂₅ (ms)</i>	117 $\pm$ 46	116	142
<i>APD₁₀ (ms)</i>	33 $\pm$ 35	49	34
<i>APA (mV)</i>	106 $\pm$ 7	113	110
<i>TOP (mV)</i>	-85 $\pm$ 2.4	-86.7	-86.5
<i>EOP (mV)</i>	-86 $\pm$ 2	-87.2	-87.3

dV/dt_{MAX}: maximum upstroke velocity; **APD_x:** AP duration at X% of repolarisation; **APA:** Action potential amplitude; **TOP:** membrane potential before depolarisation; **EOP:** membrane potential at the end of repolarisation.

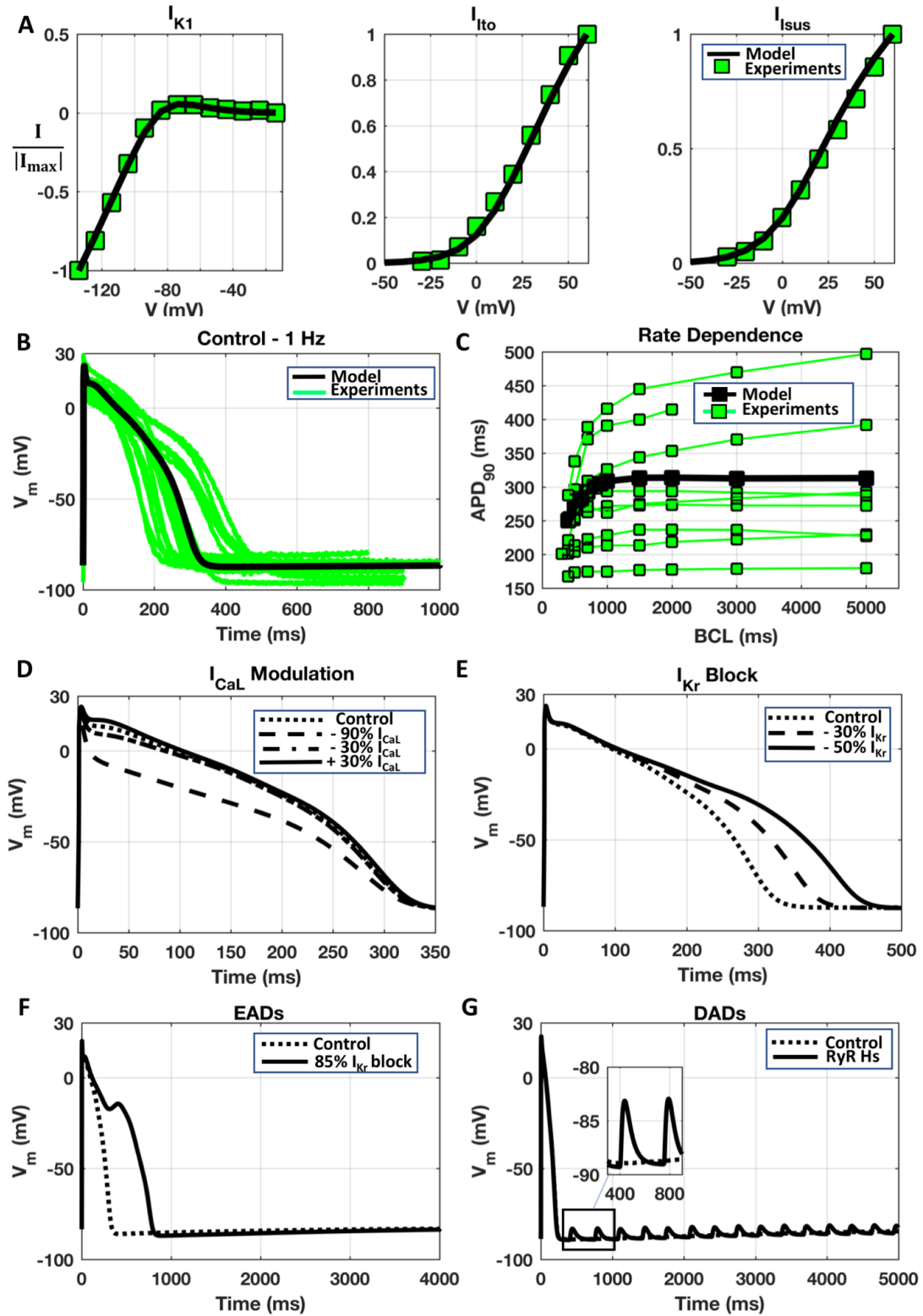


Figure 4.8 Simulations results obtained with the optimised Trovato2020 model (black) , compared against the corresponding experimental data (green), when available: A) I - V curves for I_{K1} (left), I_{to} (middle) and I_{sus} (right). Experimental data from Dataset I(Wei Han, Zhang, et al. 2002). B) AP traces at 1 Hz. Experimental data from Dataset II. C) APD₉₀ rate-dependence. Experimental data from Dataset II. D) AP changes induced by I_{CaL} modulation. E) AP changes induced by I_{Kr} block. F) EADs traces observed at slow pacing with I_{Kr} block. G) DADs traces observed following fast pacing with RyR hypersensitivity.

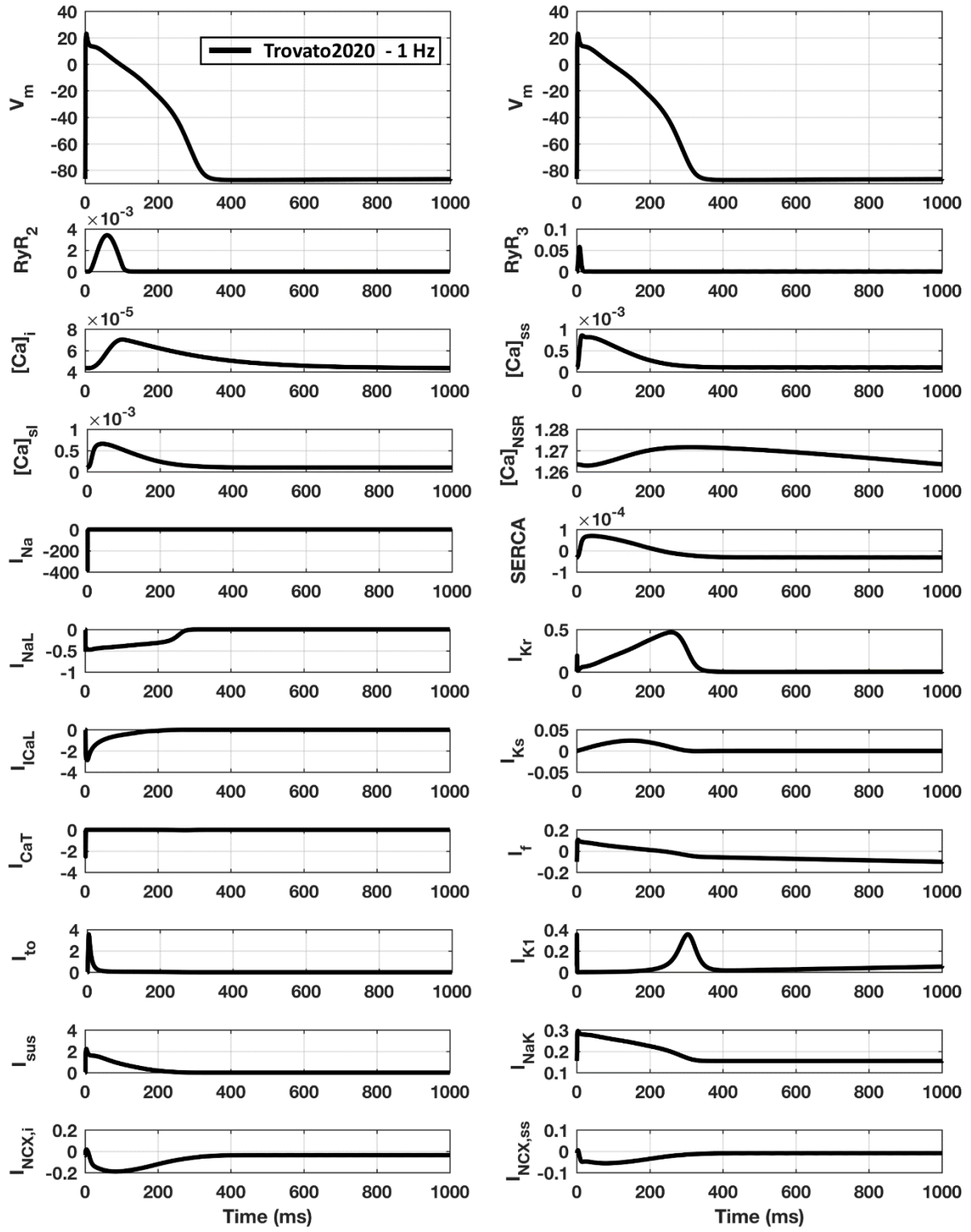


Figure 4.9. The new Trovato2020 model: simulated AP at 1 Hz in control condition, with the underlying ionic currents, pumps, exchangers, intracellular Ca^{2+} concentrations and fluxes.

4.3.3 Model validation: response to Dofetilide and automaticity.

Figure 4.10A illustrates the agreement of simulation results with experiments from Dataset III, in control conditions (blue) and with Dofetilide (red), at 1 and 2 Hz (left and right, respectively).

Figure 4.10B illustrates the ability of the Trovato2020 model to display automaticity following I_{K1} decrease and/or I_f increase. When including either I_{K1} down-regulation, I_f up-regulation, or a combination of both, the DMP increases much faster during the resting phase, and automaticity is observed with BCL of 2.5, 1.6 and 1.9 s, respectively. These results are in line with the experimentally observed BCL range of 1.3-3.0 s (F. Y. Lee et al. 2004; Stewart et al. 2009), and also with (Dobrzynski et al. 2013; Vassalle 1966), reporting that PCs with less negative DMP show automaticity, while well polarised PCs remain quiescent. Hyperkalaemia stops the automaticity in the two scenarios including I_f up-regulation, consistently with experiments in human PCs (F. Y. Lee et al. 2004), whereas it increases the spontaneous firing rate when automaticity is induced only by I_{K1} down-regulation.

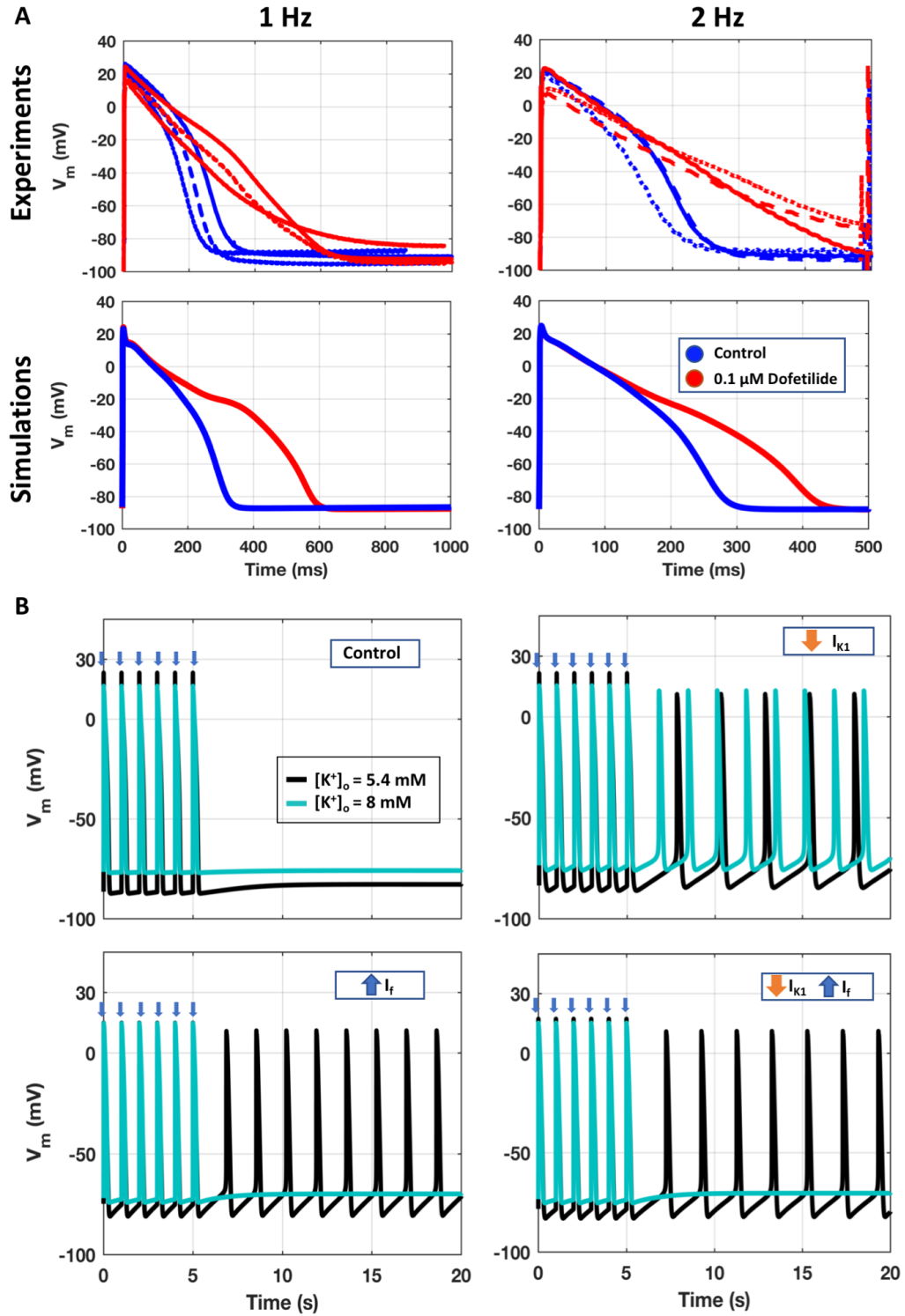


Figure 4.10. Independent validation of the optimised Trovato2020. A) Comparison between the human experimental AP traces from Dataset III (top panels, $n=3$) and the simulations with the Trovato2020 model (bottom panels), in control conditions (blue) and with Dofetilide 100 nM (red), at 1 Hz (left) and 2 Hz (right). B) Ability of the model to develop automaticity when no external stimulus (blue arrows) is applied. Simulations are shown both with normal $[K^+]_o$ (black) and under hyperkalaemia conditions (light blue), in four different scenarios.

4.3.4 Comparison with previous cardiac Purkinje in silico models

The Trovato2020 model was compared with two other human Purkinje models available in the literature (TT08 and STW), described in Section 3.5. Figure 4.11A depicts the simulated APs at 1 Hz for the three models with experimental APs from Dataset II, while the corresponding AP biomarkers are reported in Table 4.4. Simulation results obtained with both TT08 and STW overestimate the rate of depolarisation (742 and 522 V/s, respectively), compared to experiments from Datasets II and III, and the literature, i.e., 388 ± 25 V/s (Dangman et al. 1982) and 207 ± 26 V/s (F. Y. Lee et al. 2004).

Trovato2020 and TT08 have similar DMP, whereas STW is significantly less negative. Indeed, STW shows automaticity in control conditions, while Trovato2020 reproduces the quiescent PCs in control, and automaticity with less negative DMP. TT08 does not include any formulation for the I_f , and it does not yield automaticity under the protocols considered in this study (section 2.5).

Both Trovato2020 and TT08 show an APD rate dependence in line with the experiments (Figure 4.11B), even if TT08 largely overestimates the APD₉₀ at all BCL, while STW shows a non-physiological rate dependence. Simulations with TT08 and STW failed to reproduce EADs with protocol 5 (Figure 4.11C), only showing AP prolongation (17% and 42%, respectively). Both TT08 and STW also underestimate the AP prolongation induced by Dofetilide (Figure 4.11D), compared to our Dataset III. Finally, neither TT08 nor STW are capable of developing DADs at fast pacing, even when considering RyR hypersensitivity. Therefore, the novel Trovato2020 model is more suitable than both TT08 and STW for mechanistic investigations of arrhythmias considering EADs, DADs, triggered activity as well as APD rate dependence. The Trovato2020 model was also compared with the ORd and PRd models, described previously in Section 2.5.4: simulated AP biomarkers are reported in Table 4.4, whereas, APs, intracellular Ca^{2+} concentrations and the three refitted K^+ currents, I_{to} , I_{sus} , I_{K1} , at 1 Hz are

reported in Figure 4.11E. The simulated AP with the Trovato2020 model displays a smaller peak potential than both ORd and PRd, faster upstroke velocity than ORd but slower than PRd, AP plateau similar to PRd and lower than ORd, and finally, APD₉₀ longer than ORd but shorter than PRd. Intracellular $[Ca^{2+}]$ is smaller with the Trovato2020 model than with ORd, but similar to the one with PRd, which was based on experimental data from canine PCs (Boyden et al. 2000). In particular, Trovato2020 and PRd have the same level of diastolic $[Ca^{2+}]$ and same delay in the Ca elevation time (~100 ms). Similarly, the $[Ca^{2+}]$ of the sub membrane compartment obtained with the Trovato2020 was similar to the one simulated with PRd, whereas the ORd does not implement such compartment.

The refitted K^+ currents I_{to} , I_{sus} and I_{K1} were compared as well (Figure 4.11E). The simulated I_{to} with Trovato2020 is bigger than with ORd but smaller than PRd, as suggested by experiments in human and canine PCs (W. Han et al. 2002; W. Han et al. 2001). In all three models, its contribution ends after 20 ms. I_{sus} , not implemented in ORd, displays lower amplitude in Trovato2020 than in PRd, similarly to I_{to} . Simulated I_{K1} with Trovato2020 is smaller than both ORd and PRd, in line with the reduced level of expression of I_{K1} proteins in human Purkinje (Gaborit et al. 2007; Norbert Jost et al. 2013).

Table 4.4. Comparison between experimental and simulated AP biomarkers with different cardiac models at 1Hz

BIOMARKER	EXPERIMENT S	TROVATO 2020	STW	TT08	ORD	PRD
$\frac{dV}{dt}$ (V/s)	387 ± 143	381	522	742	264	527
APD ₉₀ (ms)	294 ± 76	306	285	374	268	527
APD ₇₅ (ms)	261 ± 67	280	254	364	248	328
APD ₅₀ (ms)	210 ± 52	224	197	330	208	208
APD ₂₅ (ms)	117 ± 46	143	13	209	167	31
APD ₁₀ (ms)	33 ± 35	34	4	3	74	2
APA (mV)	106 ± 7	110	120	146	128	134
TOP (mV)	-85 ± 2.4	-86.5	-74.0	-86.0	-87.8	-84.1
EOP (mV)	-86 ± 2	-87.3	-77.3	-86.0	-88.0	-84.9

dV/dt_{MAX}: maximum depolarisation rate; **APD_x**: AP Duration at X% of repolarisation; **APA**: action potential amplitude; **TOP**: take-off potential, voltage level before depolarisation; **EOP**: end of potential, voltage level at the end of repolarisation. **STW**: Stewarts et al. 2009 model; **TT08**: ten Tusscher et al 2008 model; **ORD**: O’Hara et al 2011 model; **PRd**: Pan Li & Rudy 2011 model.

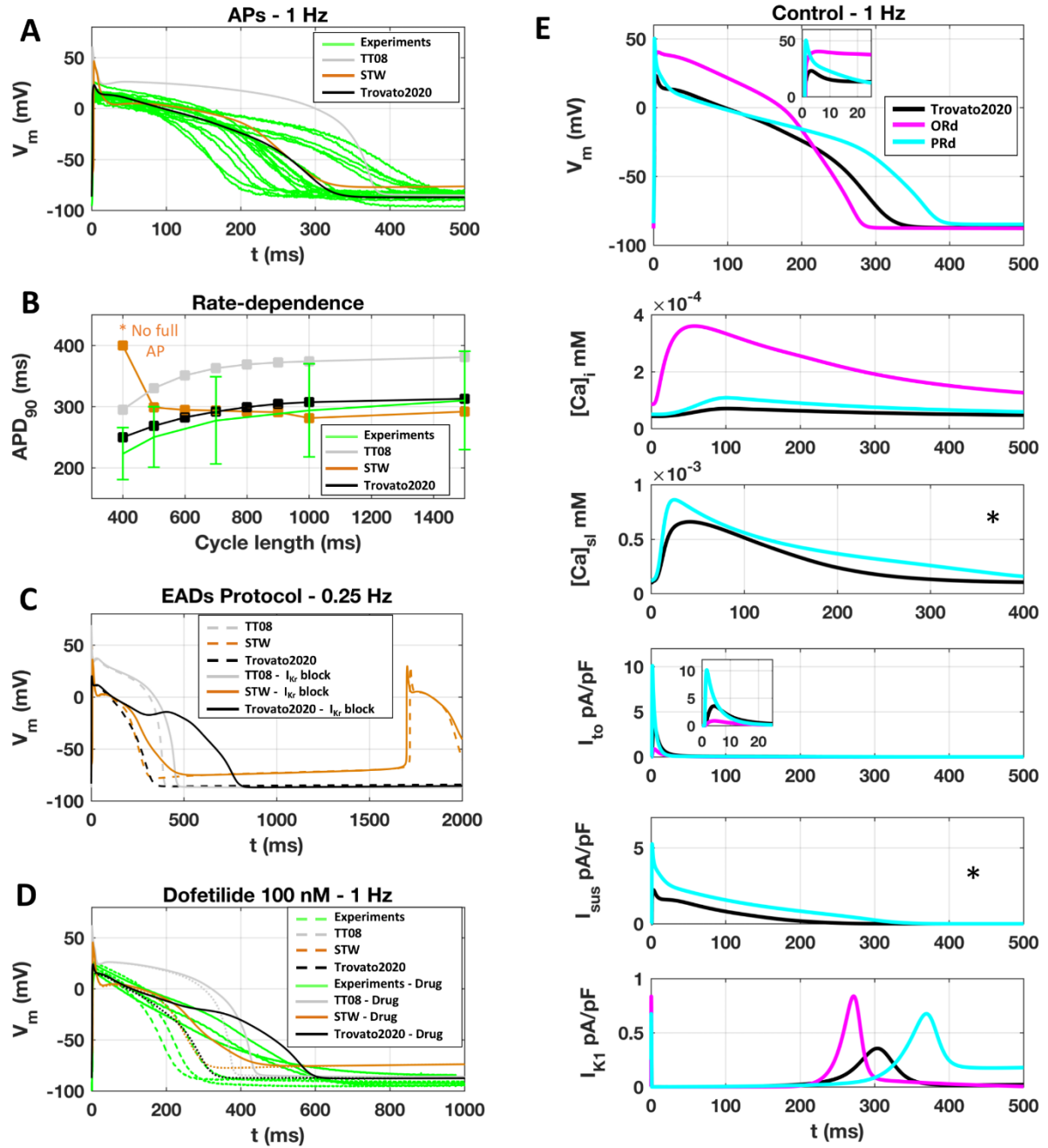


Figure 4.11 Comparison of different cardiomyocytes models. A) Human Purkinje: TT08 in grey (ten Tusscher and Panfilov 2008), STW in orange (Stewart et al. 2009), the new Trovato2020 model (black) and experiments (green). APs comparison at 1 Hz in control; B) APD90 rate-dependence; C) EADs protocol: 85% I_{K_r} block, pacing at BCL=4000 ms. Control (dash line), AP with I_{K_r} block (solid line); D) AP response to Dofetilide 100 nM: control (dash line), AP with Dofetilide (solid line). E) APs, intracellular and submembrane $[Ca^{2+}]$, and refitted K^+ currents, I_{to} , I_{K1} , I_{sus} for Trovato2020 (black), ORd (O'Hara et al. 2011, pink) and PRd (P. Li and Rudy 2011, light blue) models. *Not implemented into ORd.

Trovato2020, ORd and PRd share the same model for CaMKII signalling (Decker et al. 2009). Similarly to the ORd, removing CaMKII from the Trovato2020 model (Figure 4.12A) reduces intracellular and submembrane $[Ca^{2+}]$ peak, with minimum changes on the APD and $[Ca^{2+}]$ rate-dependence. The decreased $[Ca^{2+}]$ due to CaMKII signalling removal had no effects on DADs induced at fast pacing with RyR hypersensitivity (Figure 4.12B), though also the sarcoplasmic $[Ca^{2+}]$ was reduced. No effects were observed on EADs dynamics, as expected due to the low pacing frequency.

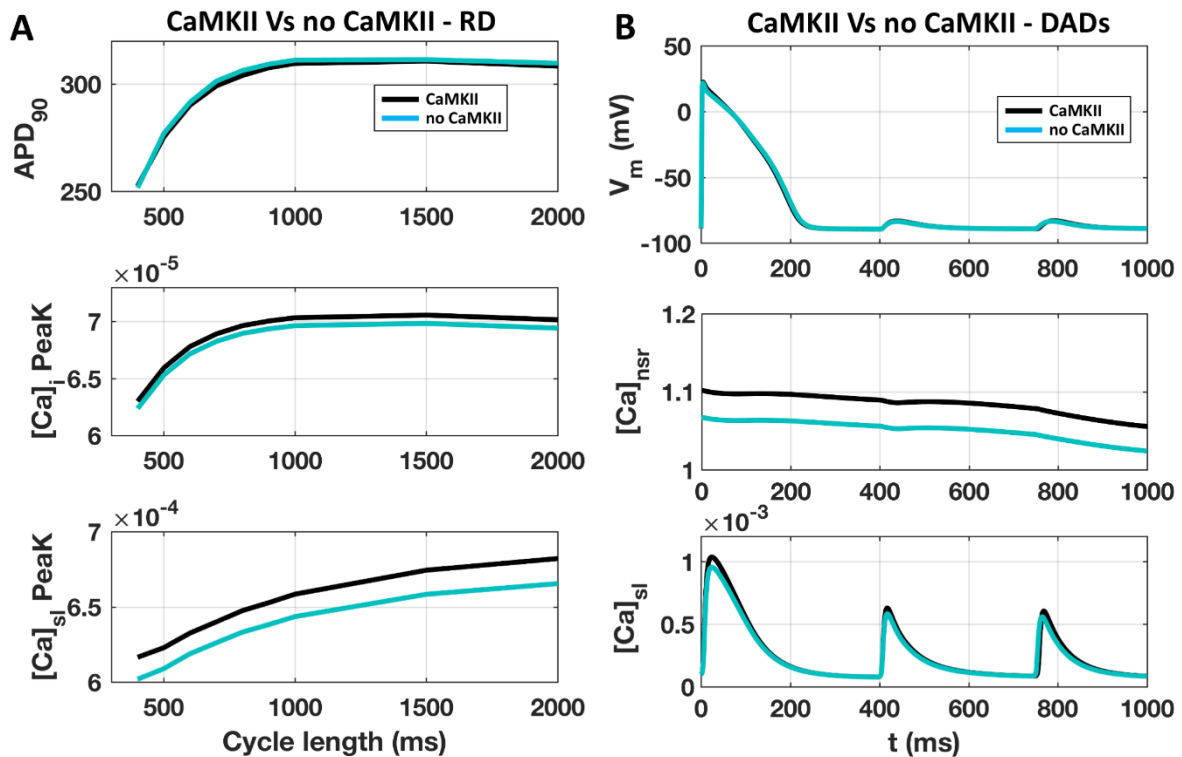


Figure 4.12. Effects of CaMKII signalling on the Trovato2020 model: control (black) and no CaMKII signalling (light blue). A) APD₉₀ rate-dependence, $[Ca^{2+}]_i$ and $[Ca^{2+}]_{sl}$ peaks. B) DADs protocol, fast pacing with RyR hypersensitivity.

4.3.5 Simulation results in a human Purkinje 1D fibre

Figure 4.13 summarises the simulations results for the human Purkinje 1D fibre considering the optimised Trovato2020 model in control conditions (Figure 4.13A) and one of the model

variants displaying automaticity in single cell as reported in Section 3.2 (Figure 4.13B). Spontaneous APs propagated along the whole fibre, with no changes in the BCL compared to single cell simulations.

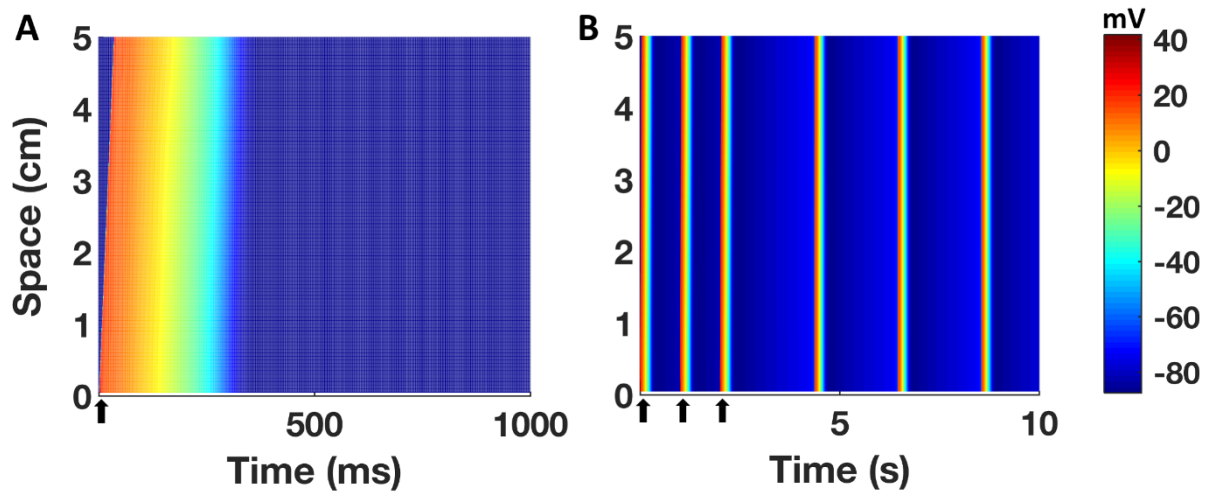


Figure 4.13 Simulation results for a 1D human cardiac Purkinje fibre constructed using the Trovato2020 model. A) Simulated AP in control conditions, propagating along the fibre. The delay between the AP upstroke at the proximal and distal ends is 32 ms. B) Simulation results obtained with one of the variants of the Trovato2020 model displaying automaticity (increased I_f , reduced I_{K1}). Spontaneous APs were observed in the fibre, and the CL was not different than the one obtained in single cell simulations. All the panels share the same colour map, displayed on the right. Please note the different time scales (ms for A, s for B). Arrows indicate time and sites of pacing.

I_{Na} block caused reduction in CV (Table 4.5): for I_{Na} blocks of 30%, 50% and 90%, CV decreased by 8%, 15% and 45%, respectively, compared to the control value of 160 cm/s. Even with 95% I_{Na} block, the AP was still able to propagate, even though CV was reduced to 69 cm/s (-57%). AP failed to propagate only with a complete block of I_{Na} .

Table 4.5. Effects of simulated I_{Na} block on the maximum depolarisation velocity and conduction velocity in Purkinje cell and fibre using the Trovato2020 model.

	SINGLE CELL		FIBRE	
I_{Na} block	$\frac{dV}{dt}_{Max}$ (V/s)	AP Inducibility	CV (cm/s)	AP propagation
Control (No Block)	380	Yes, Na^+ driven	160	Yes
30%	315	Yes, Na^+ driven	147	Yes
50%	260	Yes, Na^+ driven	136	Yes
90%	92	Yes, Na^+ driven	88	Yes
95%	44	Yes, Na^+ driven	69	Yes
100%	40	Yes, Ca^{2+} driven	-	No

$\frac{dV}{dt}_{Max}$: maximum depolarisation rate computed in single cell; CV: conduction velocity

computed in a 5 cm 1D fibre.

4.4 Discussion

The Trovato2020 Purkinje model integrates and expands a large amount of knowledge and experimental data obtained from human Purkinje preparations. It is the first human Purkinje model incorporating a Ca^{2+} handling with the 3 SR compartments and 3 different types of Ca^{2+} releases, , based on the Purkinje-specific structure (Haq et al. 2013) already implemented for rabbit (Britton et al. 2013) and canine (P. Li and Rudy 2011) models. Ca^{2+} -handling in Purkinje can favour arrhythmogenesis in pathology (Haissaguerre et al. 2016) and upon pharmacology interventions (N. Nagy et al. 2015). The Purkinje-specific Ca^{2+} handling model introduces a delay in the Ca^{2+} diffusion from the sub-membrane to the cellular bulk, and therefore, an intracellular $[Ca^{2+}]$ gradient (Figure 4.9). The simulated $[Ca^{2+}]_i$ of the new Purkinje model in

control conditions is smaller and delayed compared to the $[Ca^{2+}]$ in the sub-membrane space as reported by (ter Keurs and Boyden 2007; Haq et al. 2013; John M Cordeiro et al. 2001). The smaller Ca^{2+} transient is correlated with scarce myofibrils, according to the lower contractile ability of Purkinje compared to ventricular cardiomyocytes (N. Nagy et al. 2015).

Simulations with the human Purkinje Trovato2020 model reveal that the balance between I_{K1} and I_f determines the DMP and, therefore also automaticity, as previously suggested experimentally by (Dobrzynski et al. 2013; Wiggins and Cranefield 1976). The simulation results are also in agreement with (F. Y. Lee et al. 2004), which showed pacemaker activity in human PCs with elevated diastolic transmembrane potential (TOP ~ -70) in control condition. When considering also hyperkalaemia, pacemaker activity was stopped as reported experimentally only when automaticity was due to I_f up-regulation, suggesting a more physiological role of I_f in setting PC automaticity compared to pure I_{K1} down-regulation. Heterogeneity in the DMP and automaticity may lead to conduction blocks, creating the substrate for micro and/or macro re-entry, and eventually arrhythmia (Haissaguerre et al. 2016).

The human Purkinje Trovato2020 model was also able to reproduce the effects of potassium and calcium channel block on repolarization properties, as well as sodium channel block on electrical propagation. The model was validated against human experimental data acquired at several frequencies, both in control conditions and following Dofetilide application. Simulations with Dofetilide at 2 Hz show reverse rate-dependence (less prolongation at higher frequencies, Figure 4.10A), in agreement with what previously observed both in human and animal preparations (Peter P. Nánási et al. 2010), while a larger APD prolongation is observed in the human recordings from Dataset III. The Trovato2020 model can be used for *in silico* drug trials in human PCs to assess drug efficacy and/or drug-induced cardiotoxicity, as for human ventricular models (Passini et al. 2017).

4.5 Limitations

The Trovato2020 model presented in this study was built using the human ventricular ORd model, the Purkinje-specific Ca^{2+} sub-system, cellular compartments and intracellular ionic fluxes of the canine PRd model and experimental human data. The model was calibrated using the only currently available dataset from healthy human PC and validated against an independent experimental dataset from human healthy hearts in control and with Dofetilide, not used for the model calibration and optimisation. The latter also represent the first published dataset from healthy human PCs under drug action. We cannot exclude the possibility that connexin-mediated electrotonic interactions between PCs and ventricular tissue might have affected the AP waveforms of our experimental datasets, in particular, during the early repolarisation phase. A limitation of this study is the lack of experimental data on the Ca^{2+} transients in human cardiac PCs, since there are no recordings available. Data on human PCs AP response to pure I_{CaL} modulations are also lacking. However, previous studies using animal Purkinje and human ventricular preparations suggest positive correlation between APD_{90} and I_{CaL} modulation. Preparations used in Dataset I were obtained from failing hearts, which could affect I_{to} , I_{sus} , I_{K1} measurements. This uncertainty was tackled in our study through a population of models investigation using hundreds of models, as will be shown in Chapter 5. During the model development, we used the original ORd human ventricular current formulation of currents, whose data were not available for human PCs (e.g., I_{Ks} , I_{NCX} , I_{NaK}). This is further supported by the fact that there are no reports on different isoforms in human ventricular versus Purkinje cardiomyocytes. I_{f} and I_{CaT} formulations were adopted from the canine PRd model. However, I_{CaT} does not play an important role in PCs dynamics and abnormalities, and there are no reports of significant differences between canine and human I_{f} in PCs.

4.6 Conclusion

In this chapter, I presented a new computational model, the Trovato2020 model, of the electrophysiology of human cardiac Purkinje cell. Two independent experimental datasets from human Purkinje preparations were used to build and calibrate the model. A multi-object genetic algorithm was implemented for the parameter optimisation. The Trovato2020 model was evaluated against a third experimental dataset from healthy human Purkinje preparation, including AP recordings in control and under drug-action. It reproduces all key Purkinje-specific features such as automaticity, as observed experimentally. The model was incorporated in a 1D fibre to simulate AP propagation and test conduction velocity values were in agreement with experiments. The effects of single ion channel blocks were described and discussed as well. Finally, electrical abnormalities as EADs and DADs were successfully elicited, reproducing the same condition observed experimentally.

In the next chapter, I will proceed with the investigation into the pro-arrhythmic potential of human PCs, including also cell-to-cell variability. Notably, I will use the Trovato2020 model and two methods, described earlier in Section 2.7.2, to reproduce biological variability: the sensitivity analysis and the population of models approach.

Chapter 5 - Cell-to-cell variability and pro-arrhythmic susceptibility of human Purkinje electrophysiology

The work presented in this chapter is included in the publication: ‘Human Purkinje in silico model enables mechanistic investigations into automaticity and pro-arrhythmic abnormalities’ by Trovato et al., J. Mol. Cell. Cardiol. 142 (2020) 24–38.

In the present chapter, I extend previous investigations to include the effects of cell-to-cell variability on the pro-arrhythmic potential of human cardiac PCs, using the Trovato2020 model presented in the previous chapter. Firstly, I present the results of the sensitivity analysis performed changing only one parameter of the optimised Trovato2020 model at a time, to gather additional information on the model behaviour. Secondly, I use the information gathered through the sensitivity analysis to design a population of models, calibrated using the experimental dataset II and III presented in the previous chapter. The ionic current profiles more prone to develop EADs and DADs are investigated as well as the mechanisms and conditions underlying the onset of such abnormalities in human Purkinje cells.

5.1 Introduction

Afterdepolarisations in Purkinje cardiomyocytes can act as triggers of ectopic activity and arrhythmia, particularly in diseased conditions or under drug action (Haissaguerre et al. 2016).

The Trovato2020 model is the first human Purkinje model able to reproduce EADs, DADs and triggered AP in a single-cell and 1D fibre. This enables the investigation of PCs as arrhythmia triggers (Campos et al. 2015; Deo et al. 2010), in addition to their contribution to the substrate for re-entry circuits and retrograde propagation (Akihiko 2011; Boyle et al. 2010).

Besides, the electrophysiological variability into PCs affects their pro-arrhythmic potential, in both physiology and pathology, since some cells might be more prone than others to develop electrical abnormalities. As introduced in Section 2.7, including experimental variability in a modelling framework enlarges the investigation from one average cell to many cells. A population approach allows, then, to identify the cells which have a more pro-arrhythmic potential (“Who?”) and in which conditions they can trigger electrical abnormalities (“When”), and the mechanisms that make them more pro-arrhythmic than the other cells (“Why?”).

Furthermore, PCs are a well-established *in vitro* model for preclinical assessment of drug-induced cardiotoxicity (Guth 2007) and their response to selective ion channels changes is relevant in the context of *in silico* pharmacological assessment of drug safety.

In this chapter, the effects of the variation of each ionic current included in the Purkinje model were evaluated against the protocols listed in the previous chapter (Section 4.2.7). These included control conditions at 1 Hz to unravel the relationship between AP biomarkers and ionic currents, the APD₉₀ rate-dependence, the response to I_{Kr} blocks and I_{CaL} modulation, and the EADs and DADs inducibility.

Using the population of models, I investigated the ionic profiles of hundreds of cells to identify those more prone to develop EADs and DADs and the mechanisms underlying their behaviour.

I hypothesised that: i) EADs are the most common electrical abnormalities occurring among

the population of *in silico* cells; ii) EADs and DADs are both related to the intracellular Ca^{2+} dynamics, though, affecting different types of cells, each one with its specific mechanisms.

5.2 Methods

5.2.1 Sensitivity analysis and correlation coefficient

A sensitivity analysis was performed for the optimised Trovato2020 model, by varying only the conductances of all the ionic currents (I_{Na} , I_{NaL} , I_{CaL} , I_{CaT} , I_{to} , I_{sus} , I_{Kr} , I_{Ks} , I_{f} , I_{K1} , I_{NCX} , I_{NaK}) from -90% to +200% of their nominal values.

First, correlation coefficients between ionic currents and AP biomarkers were computed at 1 Hz, similarly to (Romero et al. 2009). For each biomarker b_i , current conductance g_j and conductance scaling factor x the changes in respect to control ($\Delta B_{i,j,x}$) and the sensitivity coefficient ($S_{i,j}$) were computed as follows:

$$\Delta B_{i,j,x} = b_{i,j,x} - b_{i,j,1} \quad \begin{cases} 1 \leq i \leq 9 & \text{AP biomarkers} \\ 1 \leq j \leq 12 & \text{conductances} \\ 0.1 \leq x \leq 2 & \text{scaling factors} \end{cases}$$

$$\Delta B_{i,MAX} = \max_{j,x} \Delta B_{i,j,x}; \quad \Delta B_{i,MIN} = \min_{j,x} \Delta B_{i,j,x};$$

$$S_{i,j} = \frac{\Delta B_{i,j,2} - \Delta B_{i,j,0.1}}{\max\{|\Delta B_{i,MAX}|; |\Delta B_{i,MIN}|\}}$$

where $b_{i,j,x}$ is the value of biomarker b_i when $g_j = x$; $b_{i,j,1}$ is the value of biomarker b_i using the baseline model; $\Delta B_{i,MAX}$ and $\Delta B_{i,MIN}$ are the maximum and the minimum changes for each biomarker b_i , respectively.

Then, for each value of each parameter, the protocols 2-6 (Section 4.2.7) were simulated to evaluate the effects of current conductance modulations on the APD_{90} rate-dependence, the response to I_{Kr} blocks and I_{CaL} variations, and the EADs and DADs inducibility.

5.2.2 Population of Models

Using the optimised Trovato2020 model as a baseline, I constructed a population of human Purkinje models, based on the methodology described in (Muszkiewicz et al. 2015; Britton et al. 2013) and summarised in Section 2.7.2. This allowed simulating the variability in AP morphology observed in the experimental recordings (Table 4.1).

An initial population of 3,000 models was constructed by sampling all the main ionic current conductances, namely, I_{Na} , I_{NaL} , I_{CaL} , I_{CaT} , I_{to} , I_{sus} , I_{Kr} , I_{Ks} , I_f , I_{K1} , I_{NCX} , I_{NaK} , in the range [50-200]% of their baseline value, using Latin hypercube sampling (McKay, Beckman, and Conover 1979). The initial population was then calibrated through a multi-step process, based on the criteria 1-3 used for the development of the Trovato2020 model and the human experimental Datasets II and III, described in Section 4.1.

In the first calibration step, only the models with all AP biomarkers within the minimum and maximum experimental values at all BCLs (Table 4.1) were selected. To constrain the AP plateau within the experimental traces, two additional biomarkers at 1 Hz were considered, similar to (Britton et al. 2013): the voltage level measured 25 and 50 ms after the AP upstroke. In the second calibration step, we selected only the models showing APD_{90} rate-dependence in line with experiments. As the third calibration step, we selected only the models showing a direct dependence of APD_{90} on I_{CaL} .

Simulations in control conditions and with Dofetilide were performed for all models in the calibrated population, and results were compared to the experimental Dataset III. Finally, EADs and DADs inducibility was also tested using the calibrated population, to investigate the mechanisms underlying EADs and DADs generation in human PCs. To do this, protocols 5 and 6 (Section 4.2.7) were simulated for each model in the calibrated population. EADs were identified when a positive derivative of the membrane potential was observed, from 150 ms after the stimulus (Passini et al. 2016). DADs were identified as a deflection of the membrane

potential larger than 1 mV during the diastolic phase. AP biomarkers, current conductances and model dynamics were analysed to identify the fundamental mechanisms underlying EADs and DADs generation.

5.3 Results

5.3.1 Effects of each ionic current conductance modulation on the AP at 1 Hz and APD₉₀ rate dependence

Figure 5.1 shows the APs simulated with the Trovato2020 model for different modulations of each individual ionic conductance. Table 5.1 reports the correlation coefficients between simulated AP biomarkers and ionic current at 1 Hz. As expected, I_{Na} modulation affected phase 0 of the AP exclusively, and therefore the upstroke velocity and the AP amplitude (Table 5.1). I_{NaL} mainly affected the AP duration, in particular at the later stages of the repolarisation, though it also played a small role in setting the AP plateau level. I_{CaL} modulation impacted on the AP amplitude and the plateau level, affecting the AP duration at the early stage of the repolarisation, whereas, G_{CaT} did not show any significant changes on the AP dynamics at 1 Hz. I_{to} was the key player during the early repolarisation phase, also affecting the AP amplitude, whereas, its pedestal component, I_{sus} , was a key player for the whole AP repolarisation. In particular, its extreme downregulation (-90%), induced a significant elevation of the AP plateau and, therefore, an increased APD. I_{Kr} modulation mostly affected the late AP repolarisation, inducing the most significant changes in the AP duration, with small effects on the AP amplitude and plateau, whereas, I_{Ks} did not have any relevant effects on the AP dynamic. Modulation of I_f mostly affected the diastolic membrane potential and, indirectly, also the AP amplitude, since large I_f led to a less negative take-of-potential and less Na^+ channel availability for the following beat. In this regard, I_{K1} variation had the opposite effects, impacting mainly

on the diastolic resting membrane potential (and the AP amplitude), but, also on the late phase of repolarisation. I_{NCX} modulation affected all the repolarisation phases (except at the early stage) and the resting membrane potential. Also, in the case of extreme I_{NCX} downregulation, a small DAD arose at the end of the beat, due to intracellular Ca^{2+} accumulation. I_{NaK} variation mostly impacted on the resting membrane potential and indirectly, similarly to I_f and I_{K1} , also on the AP amplitude and the depolarisation velocity.

Figure 5.2 illustrates the effects of each ionic conductance modulation to the APD_{90} at different BCLs, from 400 to 5000 ms. None of the conductance modulations (with the exception of -90% I_{NCX}) changed the APD rate-dependence trend for high frequencies (short BCL). At low frequencies (<0.5 Hz) the APD rate-dependence remains almost flat for all ionic conductance variations. The largest variations in rate-dependence trend are observed for I_f , i.e., increased I_f shortens APD, due to its role in setting the take-of-potential as discussed above.

In addition, low expression of I_{K1} led to automaticity, and large downregulations of I_{sus} and I_{Kr} lead to EADs, as discussed in Section 5.3.3.

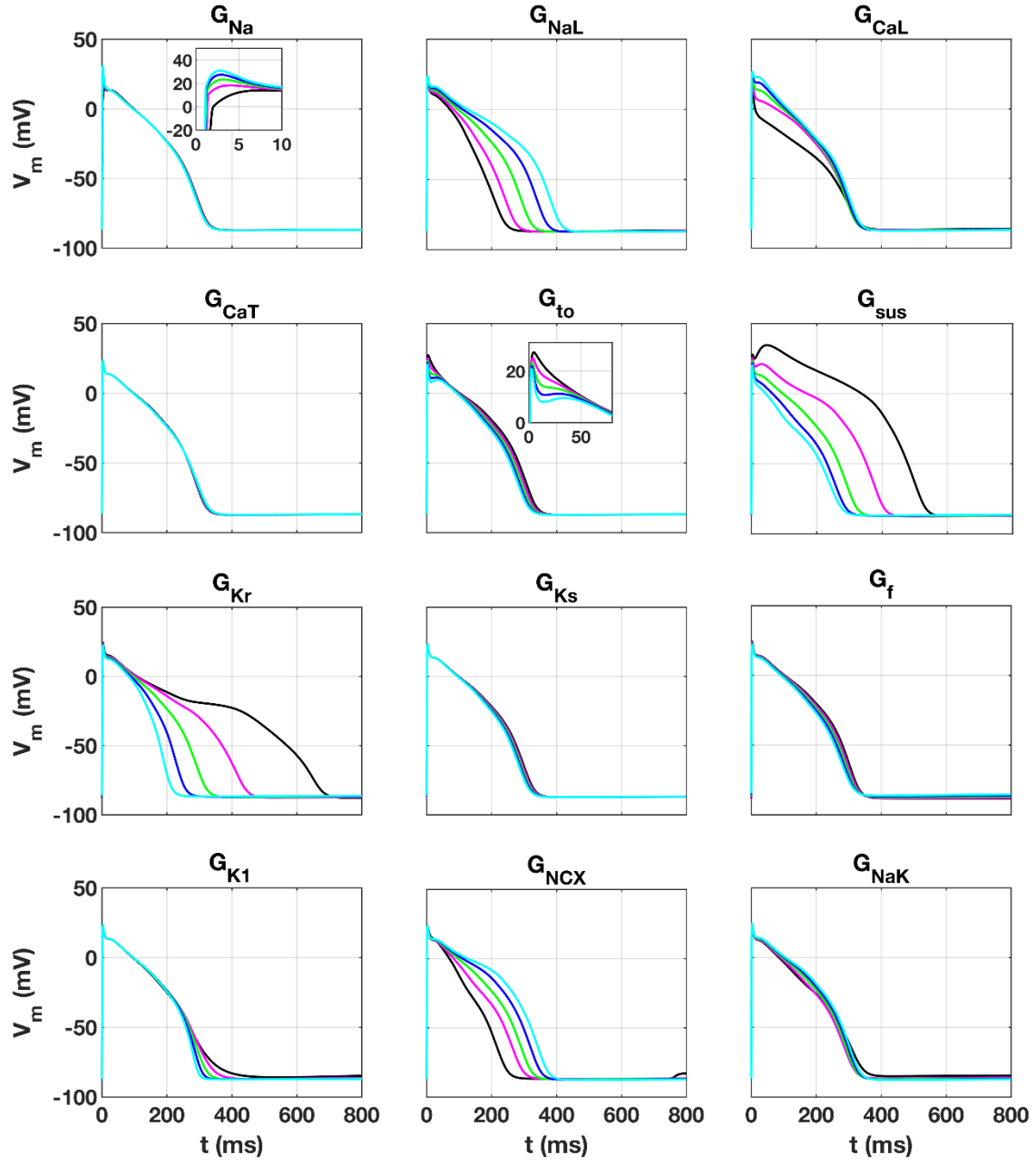


Figure 5.1. Single current conductances modulation: simulated APs of the optimised new Trovato2020 model, following Protocol 1 for each changed conductance in the range [10 - 200]% of their nominal value (Table 4.2).

Table 5.1. Normalised relative correlation coefficients for each ionic current and simulated AP biomarker at 1 Hz.

	I_{Na}	I_{NaL}	I_{CaL}	I_{CaT}	I_{to}	I_{sus}	I_{Kr}	I_{Ks}	I_f	I_{K1}	I_{NCX}	I_{NaK}
$\frac{dV}{dt}(\text{V/s})$	1.0								-0.1	0.1		0.2
APD ₉₀		0.4				-0.6	-1.0			-0.1	0.3	
APD ₇₅		0.4				-0.6	-1.0				0.3	
APD ₅₀	-0.1	0.5	0.1			-0.8	-1.0				0.4	
APD ₂₅	-0.2	0.4	0.3			-1.0	-0.3				0.4	0.1
APD ₁₀	-0.5	0.3	0.4		-0.2	-1.0						
APA	1.0	0.1	0.4		-0.4	-0.9	-0.2		-0.3	0.3	0.1	0.4
TOP		-0.1	-0.2			0.2	0.5		1.0	-1.0	-0.5	-0.8
EOP		-0.1	-0.2			0.3	0.4		1.0	-1.0	-0.5	-0.8

Values bigger than 0.60 are highlighted in bold. Values smaller than 0.1 are not shown. The upstroke velocity is set by the I_{Na} and, to a lesser extent, by I_f , I_{K1} , and I_{NaK} . The APD at late stages of repolarisation, 50-75-90%, is determined by the contribution of different currents: I_{NaL} and the I_{NCX} prolong the AP; I_{sus} and I_{Kr} shorten it. At the early phase of repolarisation, APD at 10-25%, the effects of I_{NaL} and I_{Kr} are reduced, whereas, I_{Na} and I_{CaL} play a more relevant role in setting the APD. Both I_{Na} and I_{CaL} increase the AP amplitude, whereas, I_{to} and I_{sus} reduce it. I_{NaK} and, to a lesser extent, I_{K1} and I_f affect the APA indirectly, since they affect the membrane resting potential. The major currents responsible for the membrane repolarisation are, I_{K1} and I_{NaK} , whereas I_f depolarises the cell during the diastolic interval. No relevant changes were induced by the modulation of I_{Ks} and I_{CaT} .

APD₉₀ – Rate dependence

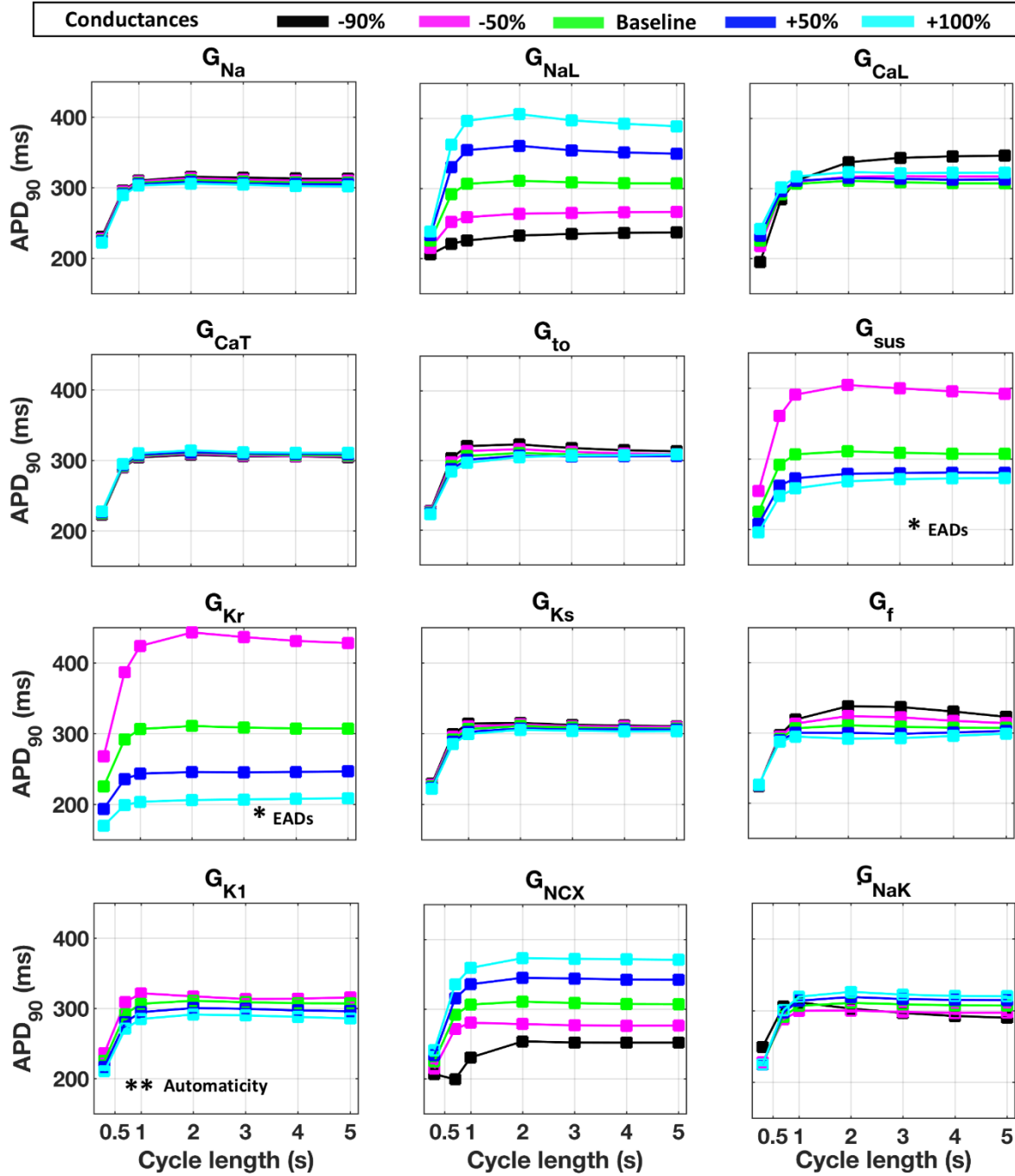


Figure 5.2. Simulation of the APD₉₀ rate-dependence behaviour in response to single current conductances modulation in the range [10 - 200]% of their nominal value (Table S1) using the optimised new Trovato2020 model. *EAD were induced in case of slow pacing rate with I_{sus} and I_{Kr} downregulation (-90%). **Automaticity was observed at slow pacing rate with I_{K1} downregulation (-90%), as already described in Section 4.3.3.

5.3.2 Response to selective ionic channel blocks

Figure 5.3A reports the effects of each current conductance modulation on the APD₉₀ change caused by several degrees of I_{Kr} blocks (at -30%, -50% and -80%) of its nominal value. It is well-known that blocking I_{Kr} , one of the main repolarising currents, induces AP prolongations (Altomare et al. 2015; Roche et al. 2010; Norbert Jost et al. 2013; Abi-Gerges, Valentin, and Pollard 2010), however, concomitant compensation by other ionic currents might enhance or decrease I_{Kr} blocks impact on the APD. Overall, current conductance modulations did not change the effects on the APD₉₀ at the lowest I_{Kr} block tested (-30%). However, the effects became more marked at higher I_{Kr} blocks. The largest impact was observed for I_{CaL} modulation since its increase proportionally enlarged the magnitude of the AP prolongation and led to EADs when strongly upregulated (+200%). On the other hand, modulation of I_{Na} , I_{CaT} , and I_f did not have major effects on the magnitude of the AP prolongation. Individually increase across the whole simulated range (from -10% to +200%) in I_{to} , I_{Ks} , I_{K1} , and I_{NaK} conductances led to a marked AP prolongation reduction (approximately -40%) since all of them are repolarising currents and compensated the repolarisation deficit due to the I_{Kr} block. Increasing I_{NCX} also leads to a similar reduction of the AP prolongation, since there were no changes in the I_{Kr} -block-induced repolarisation delays (~230 ms) but the AP was longer when the I_{NCX} was upregulated (Figure 5.1). Modulation of I_{NaL} and I_{sus} , instead, did not show a monotone trend, directly effecting the AP prolongation, due to the interplay with the other currents in setting the AP plateau and, therefore, I_{Kr} activation. With extreme I_{sus} downregulation (-90%) and 80% I_{Kr} block an EAD occurred.

Figure 5.3B reports the effects of each ionic current conductance variation on the AP with $\pm 30\%$ I_{CaL} . Overall, I_{CaL} variation had a small effect on the APD₉₀, though, compared to the response to I_{Kr} block described above, it is difficult to observe clear correlation patterns between AP changes and ionic currents. In particular, AP prolongation with 30% I_{CaL} increase

was observed across almost all the simulated scenarios, whereas, more variability in the AP response was observed when I_{CaL} was decreased of 30%. I.e., increasing I_{to} or I_{sus} inverted the model behaviour, showing AP shortening with I_{to}/I_{sus} downregulation and AP prolongation when the same current where upregulated. On the contrary, increasing I_{Na} , I_{NCX} or I_{NaK} led to AP shortening when those currents were upregulated and AP prolongation when downregulated. Small ($\sim 1\%$) AP shortening with 30% I_{CaL} increase was observed only in two cases: i) with further 50% I_{CaL} reduction; ii) with a strong increase of I_{sus} (+200%). No major effects were due to G_{CaT} , G_{Ks} , G_f and G_{K1} .

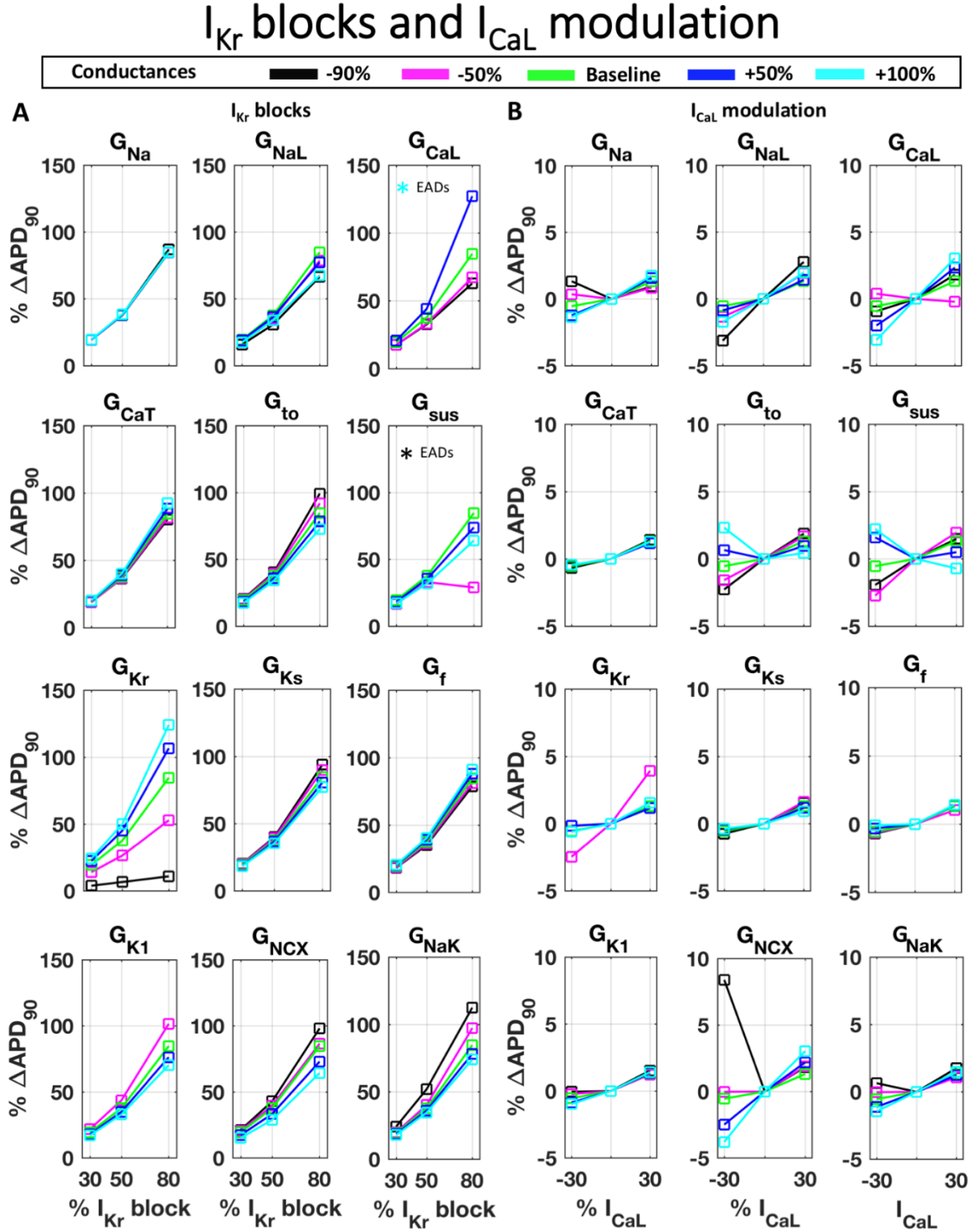


Figure 5.3. Simulated AP responses to selective channel blockers at different concentrations and conductances modulation in the range [10 - 200]% of their nominal value (Table 4.2) using the optimised Trovato2020 model: A) I_{Kr} block at 30%, 50% and 80%; B) ± 30% I_{CaL} modulation.

5.3.3 EADs and DADs inducibility

Figure 5.4 reports the effect of modulating each individual ionic conductance on the APs, combining the stimulation protocol to induce EADs and the human cardiac PC electrophysiological model described in Section 4.2.7. The protocol includes slow pacing and strong I_{Kr} block (-85%). Modulation of I_{CaL} and I_{sus} conductances had the largest impact on the EADs propensity. Both I_{CaL} upregulation and I_{sus} downregulation pushed the AP plateau up promoting EADs. I_{CaL} downregulation and I_{sus} upregulation had the opposite effect: depressing the AP plateau and preventing EADs formation. Also, the types of induced EADs were different: I_{CaL} upregulation, in particular at 200%, lead to a flat AP, whereas, I_{sus} downregulation led to a different pattern with marked oscillations. These two different patterns are related to different dynamic responses of the Ca^{2+} current. In the first case, I_{CaL} reactivates and remains partially open, since the repolarising currents are not able to counterbalance its increase. Instead, when I_{sus} is decreased, the channel repeatedly open and inactivates, due to the simultaneous and opposite effects of I_{sus} and I_{CaL} .

Modulation of I_{NaL} , I_{to} and I_{NCX} conductances had milder effects on the EADs dynamics compared to the previous currents. In particular, I_{NaL} downregulation blocked the EADs formation, extreme I_{to} downregulation enhanced the EADs amplitude and duration, whereas, its extreme upregulation impeded the EAD to occur. On the contrary, I_{NCX} downregulation blocked the EAD, and its upregulation enhanced the EAD peak, but not its duration. I_{K1} modulation did not affect the EADs behaviour, though, when extremely downregulated it led the model to a different steady state, characterised by a much higher diastolic potential (>-50 mV). This was due to the very weak residual repolarisation reserve since both I_{Kr} and I_{K1} were almost completely blocked. Modulations of I_{Na} , I_{CaT} , I_{Ks} and I_f did not have any major effects on the EADs dynamics.

EADs Protocol

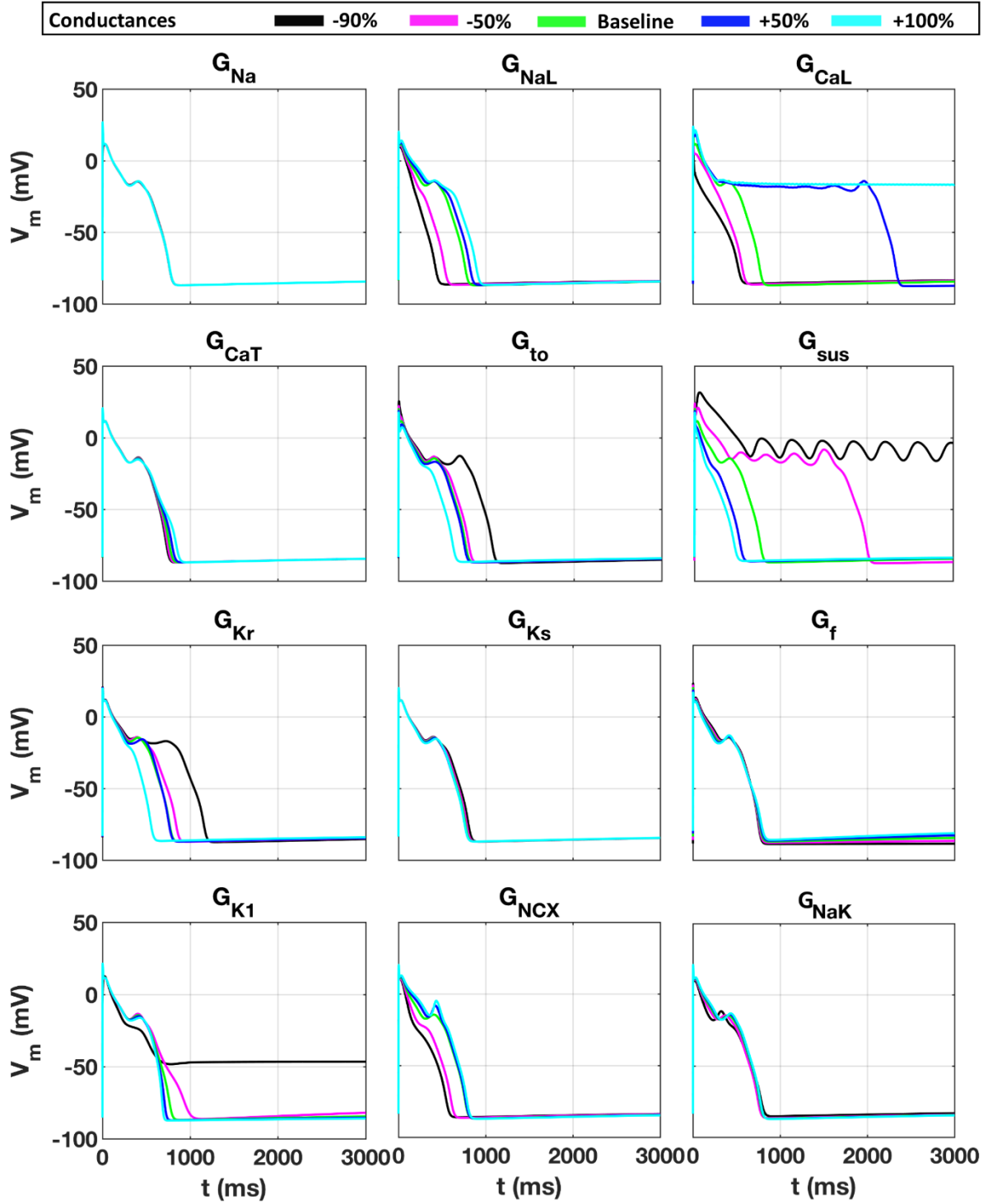


Figure 5.4. Simulated APs at slow pacing and with 85% I_{Kr} block and selective conductances modulation in the range [10 - 200]% of their nominal value (Table 4.2) using the optimised new Trovato2020 model. EADs are enhanced by increased I_{CaL} , I_{NaL} , or I_{NCX} and reduced I_{to} , I_{sus} , or I_{Kr} , while modulation of the other currents (I_{Na} , I_{CaT} , I_{Ks} , I_f , I_{K1} and I_{NaK}) do not seem to play a significant role.

Figure 5.5 reports the APs for each individual ionic current modulation, obtained following the protocol to induced DADs in cardiac PCs (Section 4.2.7), in control conditions (Panel A) and with RyR hypersensitivity (Panel B). In the first scenario, with only fast pacing at BCL=300 ms, none of the ionic current modulations was able to induce any DADs, except for the I_{NCX} , as already observed even at 1 Hz. Extreme downregulation of I_{NCX} , as will be investigated in detail in the next chapter through the population of models approach, caused a massive Ca^{2+} accumulation in all the intracellular and sarcoplasmic space, making the cell more prone to spontaneous Ca^{2+} releases, translated by the I_{NCX} in the depolarisation current underlying the DADs genesis. On the contrary, in the second simulated scenario, implementing RyR hypersensitivity (larger and faster release), the model developed DADs in all the cases. Upregulation of I_{CaL} enhances the DADs amplitude as for individual downregulation of I_{sus} , I_{Kr} and I_{NaK} . Interestingly, at fast pacing, the I_{K1} extremely downregulation did not lead to any automaticity (Panel A) but led to a triggered AP with RyR hypersensitivity (Panel B).

DADs - 3.3 Hz

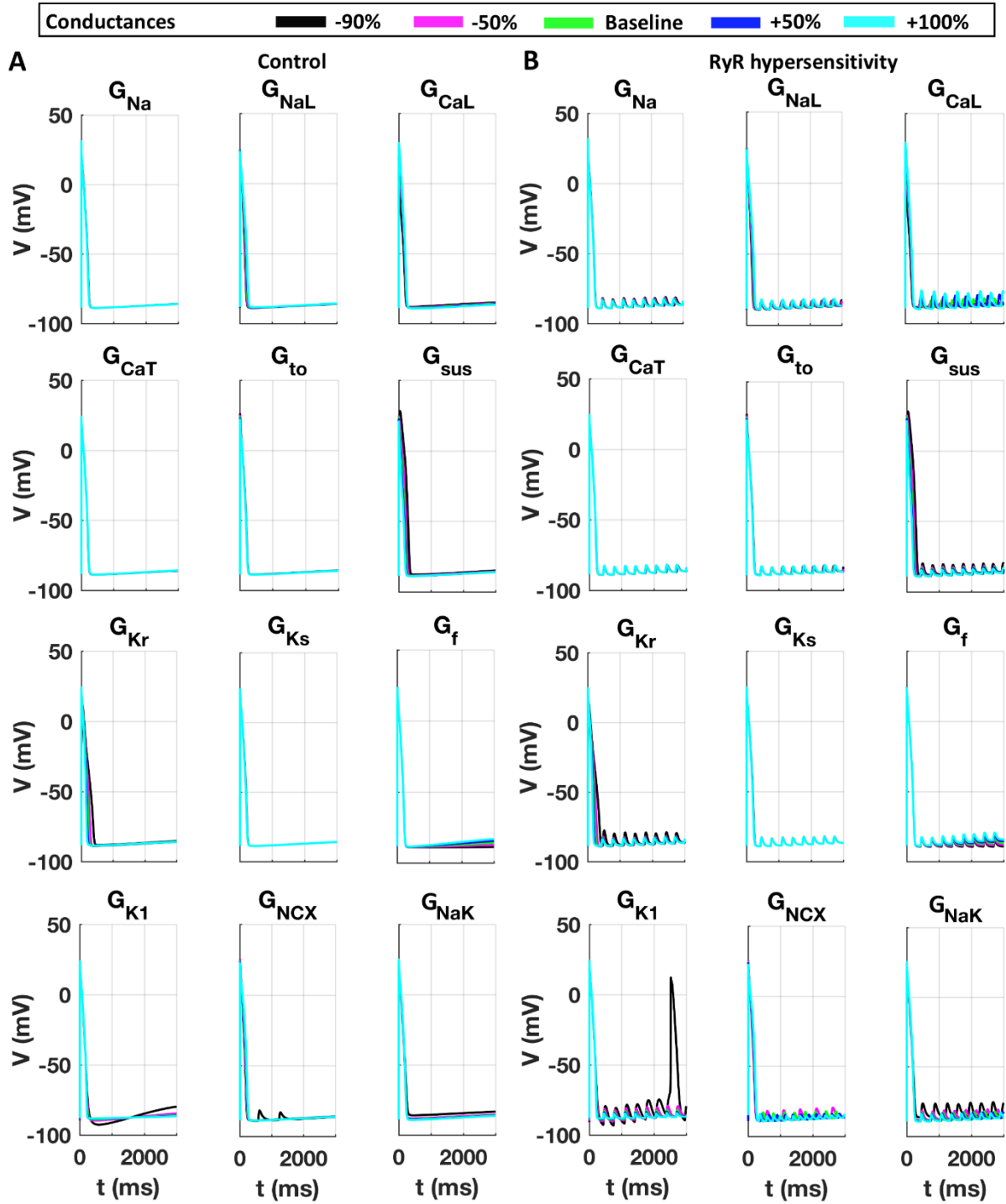


Figure 5.5. Simulated APs at fast pacing in control (A) and with RyR hypersensitivity (B) and selective conductances modulation in the range [10 - 200]% of their nominal value (Table 4.2) using the optimised new Trovato2020 model. In control, DADs were observed only with high I_{NCX} downregulation and in every case when including RyR hypersensitivity.

5.3.4 A population of human Purkinje Trovato2020 models accounting for biological variability

The optimised human Trovato2020 model was, then, used as a baseline to construct a population of models, to capture the biological variability in the AP morphology observed in the experimental Dataset II (Section 4.1). The initial population (n=3,000) was calibrated through a multi-step filtering process, as described in Methods, and summarised in Figure 5.6. In the first calibration step, only the models in agreement with the experimental biomarkers at all BCLs were selected (n=1025), while the others were discarded (n=1975). Figure 5.6A reports the simulated APs for the baseline Trovato2020 model, the initial population and the experimental traces at 1 Hz. In the second calibration step, n=867 models showed APD₉₀ rate dependence in line with the experiments and were kept in the population (Figure 5.6B). In the last calibration step, n=497 models were accepted into the final population, all displaying a positive correlation between APD and I_{CaL} (Figure 5.6C). These models were used for all subsequent investigations.

Figure 5.6D illustrates the APD distribution in the population, both in control conditions (blue) and with Dofetilide (pink), at 1 Hz and 2 Hz (left and right, respectively), compared against the experimental APD values from Dataset III. Simulation results for the population are in agreement with the experimental values in control conditions and with Dofetilide at 1 Hz. The simulated APs with the population yield a wider range of APD prolongation compared to experiments, similar to what was previously shown for rabbit PCs (Britton et al. 2013).

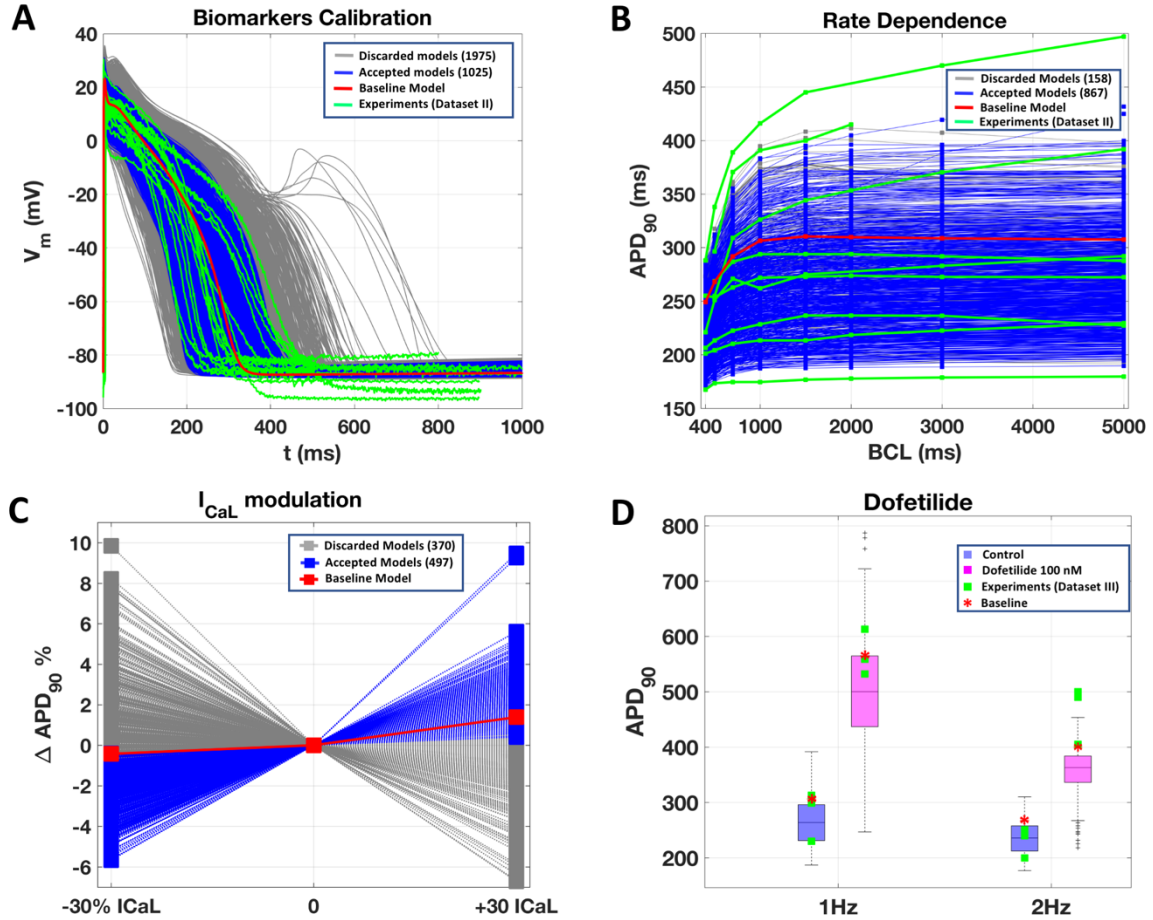


Figure 5.6 Simulation results for the population of Trovato2020 models, including calibration (A-C) and validation (D). A) Results of the first calibration step, based on AP biomarkers, reported in Table 4.1. AP traces are shown for the baseline model (red), the discarded models (grey), and the accepted models (blue), compared against experimental traces from Dataset II (green). B) Results of the second calibration step, based on APD_{90} rate-dependence. C) Results of the third calibration step, based on I_{CaL} modulation of APD_{90} . D) Comparison of APs with the experimentally-calibrated population of Trovato2020 models against experimental data from Dataset III. Boxplots represent the APD_{90} distribution at 1 Hz (left) and 2 Hz (right) in control conditions (blue) and with Dofetilide 100 nM (pink). Simulation results with the baseline model are reported as a red star, while experimental data are shown as green squares. On each box, the central mark is the median of the population, box limits are the 25th and 75th percentiles, and whiskers extend to the most extreme data points not considered outliers, plotted individually as separate crosses.

5.3.5 EADs mechanisms

When simulating the EADs protocol (described in Section 4.2.7) in the experimentally-calibrated population of models (Figure 5.7A), 59% of the models ($n=296$) developed EADs (pink traces), while the rest of the models only displayed AP prolongation (blue traces). Multiple EAD phenotypes were observed, in agreement with previous experimental and simulation studies (Tran et al. 2009; Passini et al. 2016; Guo et al. 2011). The distribution of the ionic current conductances (Figure 5.7B) highlights the differences between these two groups of models. Models displaying EADs were mainly characterised by larger inward current conductances (G_{CaL} and G_{NaL}), in agreement with the sensitivity analysis results (Figure 5.4). I_{sus} downregulation was not observed in these models, even if it was identified as a possible factor through the sensitivity analysis. I_{CaL} reactivation (Figure 5.8A) was identified as the key mechanism for EAD generation (Guo et al. 2007; Zeng and Rudy 1995) whereas, no I_{NaL} reactivation was observed with the protocol used in this study. Figure 5.8B reports intracellular and submembrane $[Ca^{2+}]$ peak values in control using the models displaying only AP prolongation and those developing EADs.

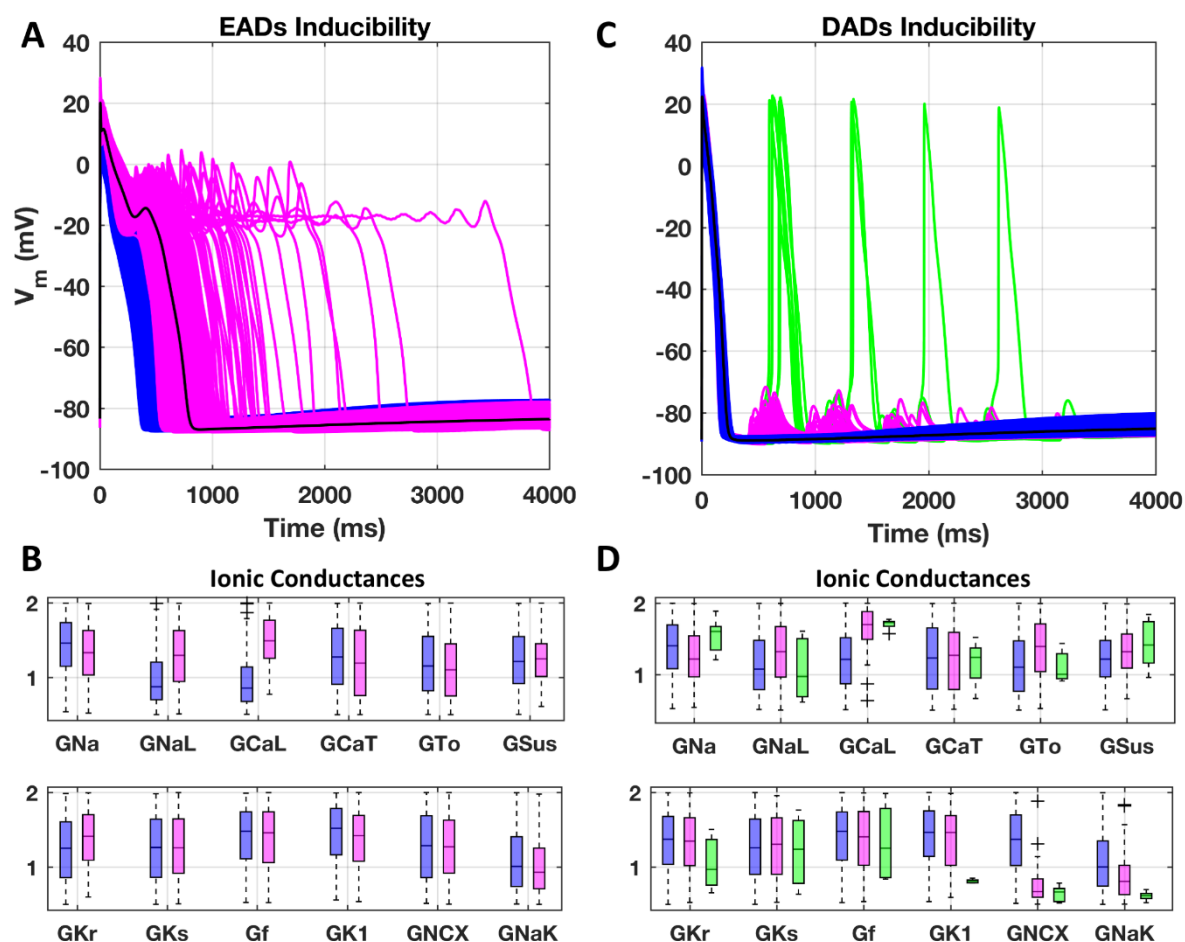


Figure 5.7 EADs and DADs inducibility on the calibrated population of 497 human cardiac Purkinje AP models.

A) Simulated AP traces for the EADs protocol, including the baseline model (black), models not displaying EADs (blue), and models displaying EADs (pink). The baseline model is shown in black. B) Distribution of the scaling factors of the ionic current conductances varied in the population of models, highlighting the differences between the two groups of models (Boxplot colours as in A). C) Simulated AP traces for the DADs protocol, including the baseline model (black), models not displaying DADs (blue), models displaying DADs (pink), and models displaying triggered APs (green). D) Distribution of the scaling factors of the ionic current conductances varied in the population, highlighting the differences between the three groups of models (Boxplot colours as in C).

5.3.6 DADs mechanisms

When simulating the DADs protocol (described in Section 4.2.7) in the experimentally-calibrated population (Figure 5.7C), 63 models developed DADs (pink traces), 5 models developed triggered APs (green traces), while no DADs were observed in the remaining models (blue traces). The distribution of the ionic current conductances (Figure 5.7D) highlighted the

differences between these three groups of Purkinje models. Human virtual PCs displaying DADs had larger G_{CaL} , and reduced G_{NCX} .

Figure 5.8C illustrates the ionic mechanisms underlying DADs formation. Models displaying DADs showed higher Ca^{2+} concentrations in all intracellular compartments, including the SR. In particular, in all models displaying DADs, $[Ca^{2+}]_{NSR}$ was larger than 1.7 mM, in line with previous computational studies (Luo and Rudy 1994a; Mitsunori Maruyama et al. 2010; Fink, Noble, and Noble 2011) and also larger than in the baseline model with RyR hypersensitivity (1.1 mM, Figure 4.9). Ca^{2+} accumulation made the cell more vulnerable to spontaneous SR Ca^{2+} releases, which were translated by the I_{NCX} into the depolarising currents causing DADs. Figure 5.8D shows intracellular and submembrane $[Ca^{2+}]$ amplitudes, i.e., the difference between the diastolic concentration and the peak value for each cellular compartment, and the average sarcoplasmic $[Ca^{2+}]$ of the models staying quiescent and those developing DADs and triggered APs across the whole calibrated population.

In order to establish the link between the distribution of ionic current conductances and Ca^{2+} accumulation in the models displaying DADs, we performed additional ad hoc simulations for a selection of 3 models from the population. We selectively restored ionic current conductances to their baseline values and evaluated changes in Ca^{2+} accumulation and DADs generation. Large G_{CaL} and small G_{NCX} directly contributed to Ca^{2+} overload and DADs generation: restoring the value of either of these conductances to their baseline significantly reduced intracellular Ca^{2+} concentrations, and also abolished DADs (Figure 5.9, Panel A and B).

Triggered APs were observed in ~1% of the experimentally-calibrated population (n=5 models). All models developing triggered APs had higher intracellular Ca^{2+} concentrations, compared to the ones developing DADs only (Figure 5.8C) and were also characterised by smaller G_{K1} and G_{NaK} (Figure 5.7D), both reducing the outward current during the diastolic phase.

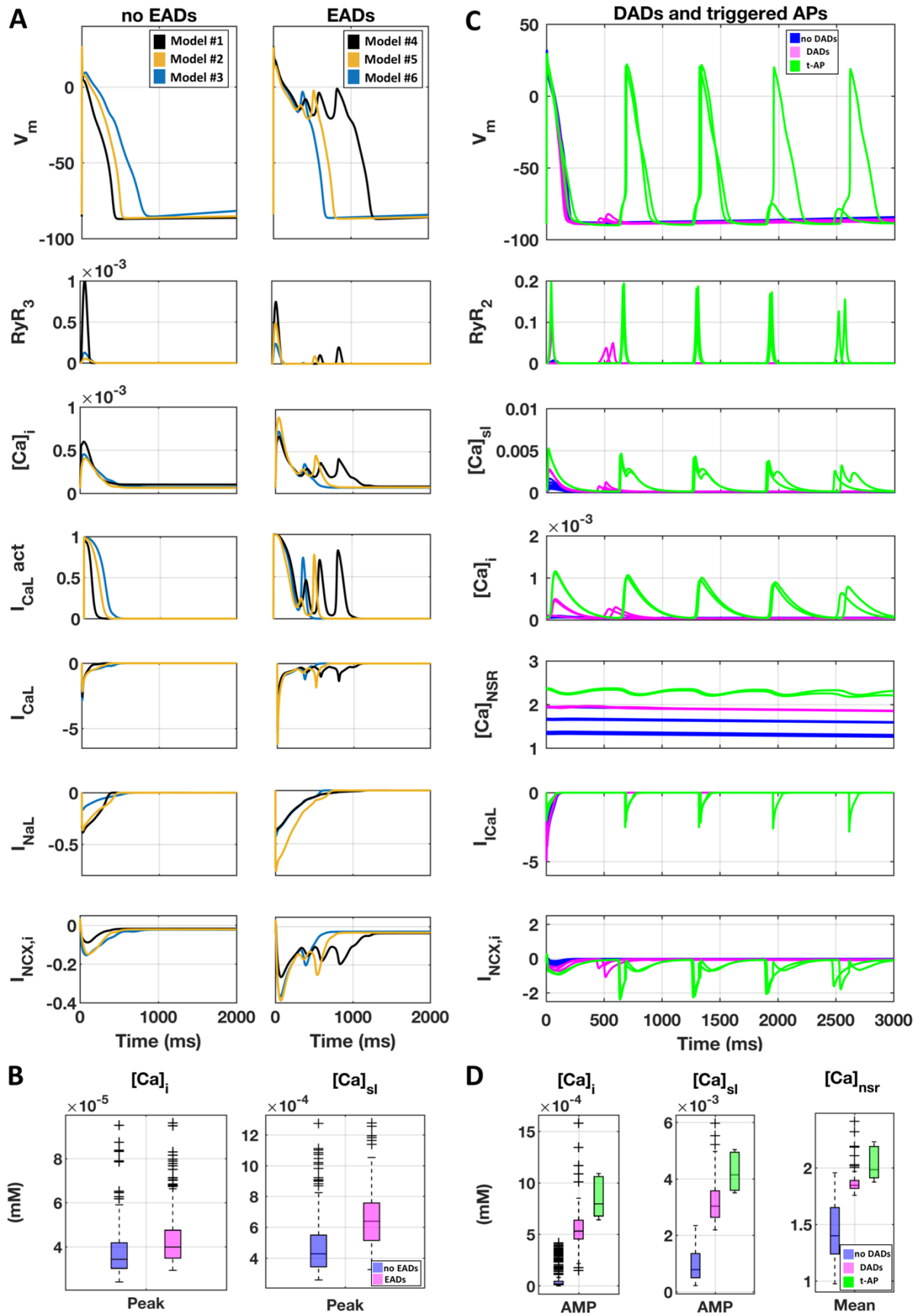


Figure 5.8. EADs and DADs mechanisms. A) Simulations of the EADs protocol, showing the AP and the main currents/concentrations involved in EADs generation. 6 representative models are shown: 3 not displaying EADs (left), and 3 displaying EADs (right). All models exhibiting EADs display I_{CaL} reactivation, as shown by I_{CaL} activation gate dynamics (4th row from top). B) Intracellular and sub membrane $[Ca^{2+}]$ peaks (computed before I_{Kr} block) for models showing only AP prolongation (blue) and those developing (EADs). C) Simulated APs and main currents/concentrations involved in DADs generation. 17 representative models are shown: 10 not displaying DADs (blue), 5 displaying DADs (pink), and 2 developing triggered APs (green). All models displaying DADs or triggered APs have higher $[Ca^{2+}]_i$, $[Ca^{2+}]_{sl}$ and $[Ca^{2+}]_{NSR}$. D) Intracellular and sub membrane $[Ca^{2+}]$ amplitude, and mean sarcoplasmic $[Ca^{2+}]$ for all the models in the calibrated population: not displaying DADs (blue), displaying DADs (pink) and developing triggered APs (green).

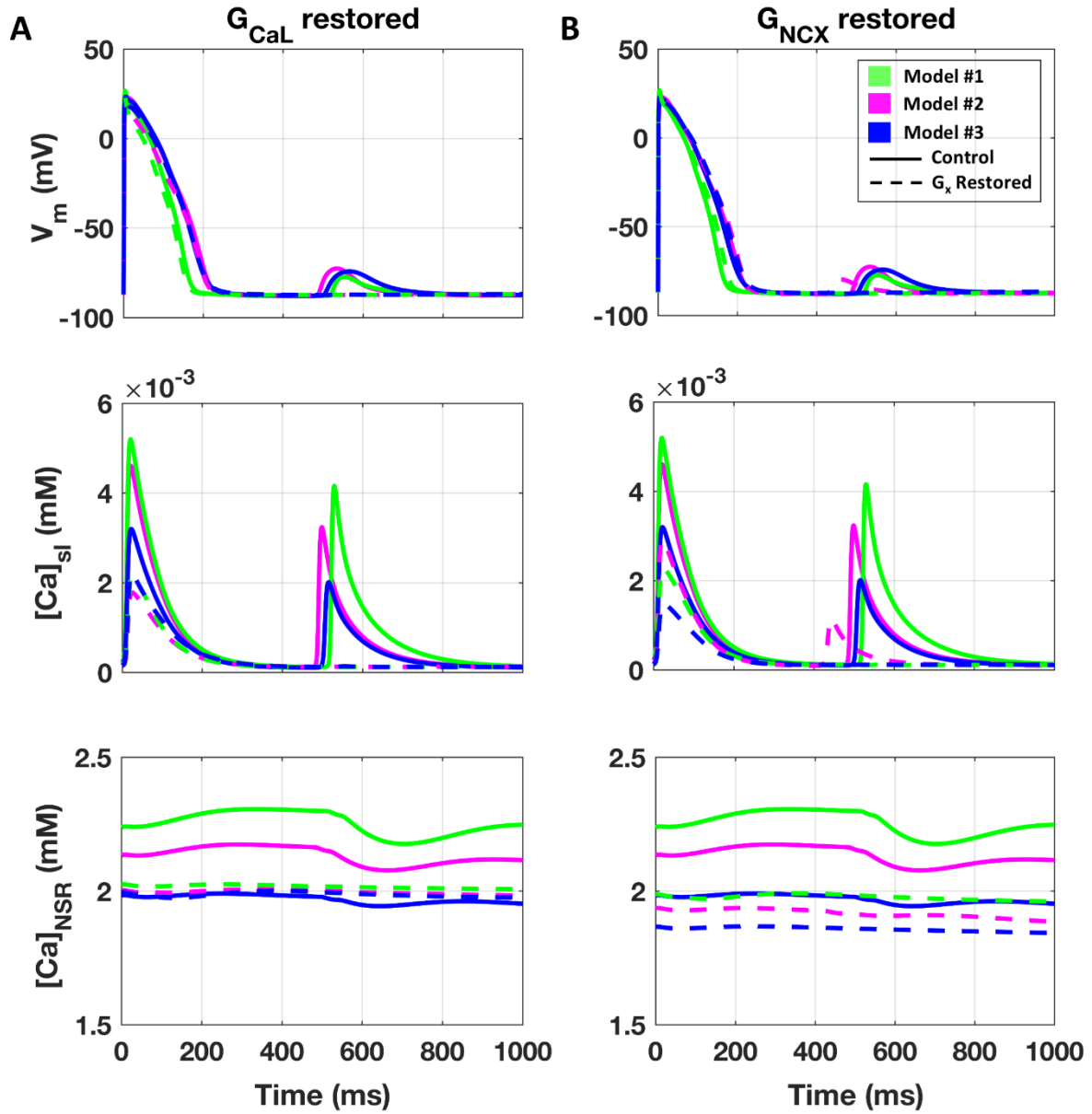


Figure 5.9. Simulated APs, $[Ca^{2+}]_{SL}$ and $[Ca^{2+}]_{NSR}$ for a selection of 3 models (green, pink and blue traces) from the population producing DADs, in control (solid line) and with selective conductance restored to the corresponding baseline value (dashed line): A) G_{CaL} ; B) G_{NCX} ;

5.3.7 Simulation results in a human Purkinje 1D fibre

Figure 5.10A shows the results obtained combining the protocol to induced EADs and the 1D fibre model described in Section 4.2.6, already used to simulate AP propagation at 1 Hz and Purkinje automaticity (Section 4.3.5). EADs were observed in the whole fibre, with no changes in EADs amplitude induced by the intercellular coupling. Figure 5.10B shows the results of

one of the models in the population developing both triggered APs and DADs at fast pacing (Figure 5.8B): in this case, the electrical coupling affected the simulations since only a single triggered APs was observed in fibre, compared to the 2 observed in the single-cell simulations. After the fast pacing, the fibre depolarised generating one DAD ($t=550$ ms, Figure 5.10B) but it did not reach the threshold to allow fast Na^+ channels opening. However, after another further 650 ms the membrane reached the threshold and a spontaneous AP was elicited, followed by 2 DADs, as in the single-cell simulations. However, when the electrophysiological changes underlying EADs and DADs were applied only to the central portion of the fibre (1.67 cm, 33 nodes), abnormalities did not propagate due to the sink-source mismatch as previously reported by (Xie et al. 2010).

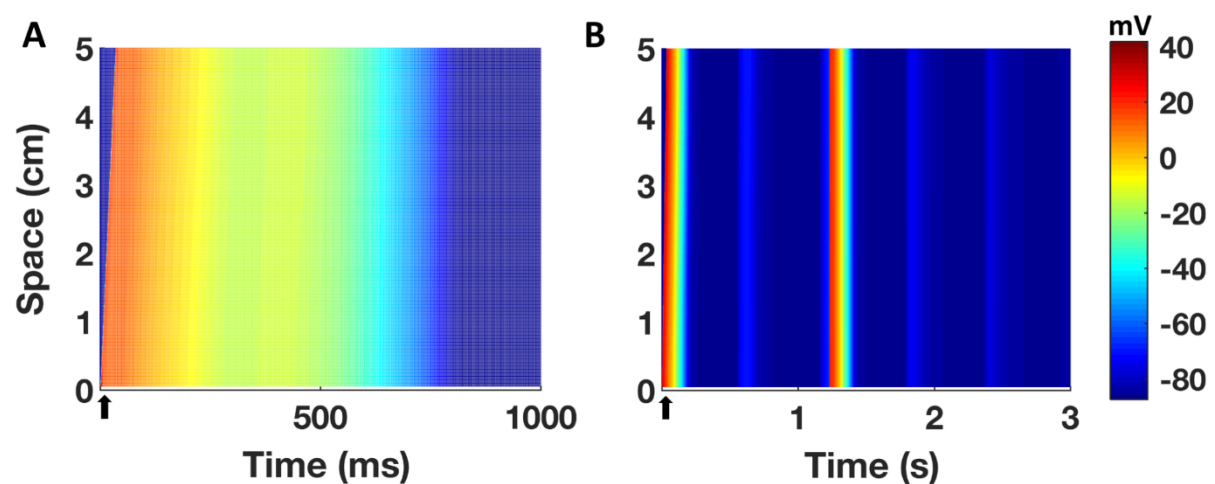


Figure 5.10 Simulation results for a 1D human cardiac Purkinje fibre constructed using the Trovato2020 model.

A) Simulation results for the EADs inducibility protocol. EADs successfully propagated along the fibre. **B)** Simulation results for the DADs inducibility protocol, using one of the models in the population displaying triggered APs. The cellular coupling reduced the number of triggered APs compared to single-cell simulations (from 2 to 1). All the panels share the same colour map, displayed on the right. Please notice the different time scales. Arrows indicate time and sites of pacing.

5.4 Discussion

In this chapter, I systematically investigated through a sensitivity analysis the effects of each individual ionic current modulation, from -90 to +200% of their nominal value, on the human Purkinje electrophysiology simulated with the Trovato2020 model. I evaluated the AP variations at 1 Hz and identified, using a set of AP biomarkers, their correlation with each ionic current modulation. Then, I evaluated the impact of each ionic current on the APD₉₀ rate-dependence and AP response to selective I_{Kr} block and I_{CaL} variations, and on the EADs and DADs inducibility.

Moreover, I constructed an experimentally-calibrated population of human PC models to account for biological variability and used it to investigate the ionic mechanisms underlying EADs and DADs generation in human PCs. The investigations using the population of models highlighted that:

1. The Trovato2020 model is able to yield and explain pro-arrhythmic mechanisms, i.e. EADs and DADs, in human PCs. Simulations show that 59% of the PCs models in the population display EADs at low pacing rates caused by I_{CaL} reactivation and favoured by strong I_{CaL} and I_{NaL} . DADs are observed at fast pacing rates in 13% of the Purkinje population models, all displaying Ca^{2+} accumulation both in the intracellular space and the sarcoplasmic reticulum. Triggered APs were observed in 1% of the virtual PCs, characterised by strong downregulation of I_{K1} and I_{NaK} .
2. The integration of the Trovato2020 model in a 1D fibre also succeed to reproduce EADs, DADs, and triggered APs. Thus, the Trovato2020 model can be implemented in higher scale models, for investigation of Purkinje-triggered arrhythmia also at tissue and organ level.

5.4.1 Sensitivity analysis

The results described in this chapter provide a large amount of information about PCs behaviour across a wide range of simulated electrophysiological conditions. Data from canine preparation confirmed Trovato2020 model predictions in respect to the AP response to I_{Kr} , I_{Ks} and I_{K1} modulation at 1 Hz (Varro et al. 2000; Norbert Jost et al. 2013) and on the response to I_{CaL} modulation (Terrar et al. 2007). The roles of I_{to} and I_{sus} in setting the AP plateau and contributing to the EADs formation were investigated in rabbit, rat, mouse, and canine cardiac preparations (Zhao et al. 2012). Zhao and colleagues concluded that both I_{to} and I_{sus} up/down-regulation contribute to the EADs formation by setting the AP plateau into the voltage range where I_{CaL} can more easily reactivate. I_{NaL} block was shown to impede EADs genesis in rats (Pezhouman et al. 2014; Morita et al. 2011), murine (Bögeholz et al. 2015), rabbit (Persson et al. 2007; Orth et al. 2006; Hou et al. 2016; Fedida et al. 2006) and canine (Sicouri, Belardinelli, and Antzelevitch 2013; Bossu et al. 2018) preparations. I_{NCX} downregulation was shown to block EADs formation in both murine (Bögeholz et al. 2015) and canine ventricular preparations (N. Jost et al. 2013) similarly to the Trovato2020 model. However, in both studies, I_{NCX} downregulation reduced the DADs occurrences, whereas, DADs were induced by the extreme lowering of the I_{NCX} maximal conductance of the Trovato2020, due to intracellular Ca^{2+} accumulation.

5.4.2 EADs and DADs mechanisms.

The results of the sensitivity analysis are strongly dependent on the initial parameters of the Trovato2020 model, which represent the behaviour of one individual cell ultimately. EADs and DADs mechanisms were then investigated including also cell-to-cell variability. A population of hundreds of cells was build considering variations in all the ionic conductances simultaneously and calibrated using an extensive dataset of experimental biomarkers.

Pro-arrhythmic electrical abnormalities, i.e. EADs, DADs and triggered APs were investigated at cellular and fibre scale. Our simulations report that EADs, DADs and triggered APs are based on different ionic mechanisms, which are consistent with previous investigations in other species (J. M. Cordeiro, Bridge, and Spitzer 2001; Dobrzynski et al. 2013; Aslanidi et al. 2010; P. Li and Rudy 2011). Across all the simulations, EADs were uniquely due to I_{CaL} reactivation, and occurred in models with strong I_{CaL} upregulation, and also with high I_{NaL} . This is consistent with simulation results using human ventricular models (Song et al. 2015; Kalik et al. 2017). No I_{NaL} reactivation was observed during EADs, although this may occur using a different protocol (Morotti et al. 2016). Simulations also confirmed that EADs are able to propagate along a 1D fibre, thus demonstrating the possibility of using the Trovato2020 model to investigate PCs as arrhythmia trigger in tissue and whole-organ simulations.

In the population of models, DADs generation required Ca^{2+} accumulation both in the SR and in the intracellular space at fast pacing (Figure 5.8C). DADs and triggered APs in PCs have been clinically related to arrhythmia initiation (Haissaguerre et al. 2016). They are suspected of triggering postshock arrhythmias such as ventricular tachycardia and fibrillation after successful defibrillation of the heart (Mitsunori Maruyama et al. 2010). The mechanisms underlying DADs have been difficult to unravel, but many experimental and simulation studies identified the important role of Ca^{2+} dynamics (Haissaguerre et al. 2016). In general, experiments across a wide range of protocols suggested that the key feature for DADs generation is Ca^{2+} overload, though the mechanisms necessary for DADs are still being debated (Fink, Noble, and Noble 2011; Boyden, Dun, and Robinson 2016). The present study suggests a similar mechanism also in human Purkinje cardiomyocytes and identifies I_{CaL} and I_{NCX} as the ionic currents playing a major role in DADs inducibility, with the addition of I_{K1} and I_{NaK} for triggered APs. The findings presented so far provide a new and needed tool for *in silico* investigation of arrhythmia in the human heart, including the Purkinje system.

5.5 Conclusion

In this chapter, I have extended the investigation into human PC proarrhythmic mechanisms, including also cell-to-cell variability and simulating the electrophysiology of thousands of human PCs. Overall, EADs were the most common abnormalities observed in the population of human PCs, whereas, Ca^{2+} accumulation-induced triggered APs were the rarest. The ionic profiles more prone to develop these abnormalities were identified, as well as the mechanisms underlying both EADs and DADs generation. Therefore, the Trovato2020 was able to yield and explain pro-arrhythmic mechanisms in human PCs. In the next chapter, I will discuss the use of the Trovato2020 model for *in silico* predictions of drug-induced electrophysiological changes in human PCs.

Chapter 6 - In silico assessment of drug-induced effects in human Purkinje cells

In this chapter, I present the application of the Trovato2020 model to evaluate drug-induced electrophysiological changes on the human Purkinje electrophysiology. I describe and discuss the results obtained performing *in silico* drug trials for a set of eight compounds with varied modes of action, including antiarrhythmic drugs, antipsychotics, antibiotics, and antihistamines. Particular focus is given to the assessment of drug-induced pro-arrhythmic cardiotoxicity and AP change predictions at different drug concentrations and pacing frequencies. Results are analysed and compared to the data available in the literature to evaluate the potentials and limitations of the computational approach adopted to assess drug safety and efficacy in cardiac Purkinje fibres.

6.1 Introduction

Drug-induced cardiotoxicity is one of the most common reasons for drug-withdrawal from the market (Onakpoya, Heneghan, and Aronson 2016) and a critical concern for pharma companies and regulators. Preclinical assessment of drug-induced cardiotoxicity is still a challenge for the

pharmaceutical industry (Van Norman 2020), and new tools for accurate predictions and early identification of risks are still being researched. *In vitro*, *in vivo*, and *ex-vivo* animal models are widely used for preclinical drug assessment, even though they do not always translate to humans and also represent a hefty cost for the pharmaceutical industry (Van Norman 2019). Cardiac Purkinje fibres, often isolated from dog or rabbit hearts through complex procedures, are among the *in vitro* models recommended by the ICH S7B guidelines for preclinical drug screening, especially for pro-arrhythmic cardiotoxicity (Goineau, Lemaire, and Froget 2013). A possible alternative to wet experiments is the use of computer models and simulations. *In-silico* trials using multiscale biophysically-detailed human-based models have proven to be powerful as a new tool for pro-arrhythmic risk prediction. They are already considered by the FDA within the Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative. Moreover, *in silico* trials using human ventricular electrophysiological models achieved a high level of accuracy for a large variety of compounds (Passini et al. 2017, 2019). However, the productive integration of *in silico* trials for drug testing still requires their evaluation against currently-used experiments. My goal is to evaluate the consistency between *in silico* simulations using the human Purkinje model described in Chapter 4 with experimental data from Purkinje fibres available in the literature. I analysed the effects of eight compounds with different ionic profiles and different risk of inducing Torsade de Pointes (TdP) arrhythmia. The drug-induced AP changes and safety profiles were evaluated at different drug concentrations and different pacing frequencies.

6.2 Methods

6.2.1 Drugs data

The effects of 8 drugs, namely, Dofetilide, Sotalol, Verapamil, Cisapride, Haloperidol, Nifedipine, Sparfloxacin, and Terfenadine, were evaluated at different concentrations, varying

between the effective plasma therapeutic concentration of each drug (Table 6.1) and its multiple up to more than 3000-fold. These drugs have been characterised experimentally, both based on their ion channel block and their effect on the recorded AP in Purkinje preparations (Terrar et al. 2007).

Dofetilide, Sotalol, Verapamil, Cisapride and Nifedipine are listed among the CiPA set of compounds (Colatsky et al. 2016; Z. Li et al. 2020), identified by a multi-disciplinary team of regulatory, industry and academic scientists to develop and validate new paradigms for the preclinical evaluation of drug safety, including also computational models.

Each drug was assigned to a TdP risk category, based on the classification by CredibleMeds® [9], available on www.crediblemeds.org (Accessed April 29, 2020) and reported in Table 6.1. Six drugs (Dofetilide, Sotalol, Cisapride, Haloperidol, Sparfloxacin, and Terfenadine) have known risk of prolonging the QT interval and induce TdP, even when taken as recommended. Nifedipine and Verapamil are both not classified (NC), i.e., both drugs were reviewed by CredibleMeds®, but the evidence available did not result in a decision for them to be placed in any risk categories.

Table 6.1 Drugs, references to the IC₅₀ values and Hill coefficients, experimental doses and residual I_{Na} , I_{Kr} and I_{CaL} currents following the simple-pore-block model application, FTPC, and TdP risk classification

N	Drug	IC50s	Dose (μM)	% Residual Current			FTPC (μM)	TdP risk
				I_{Na}	I_{Kr}	I_{CaL}		
1	Dofetilide	<i>I</i> - Passini et al, 2019	0.001	100	98	100	0.002	Known
			0.01	99	60	100		
			0.1	96	4	100		
		<i>II</i> - Kramer et al, 2013	0.001	100	98	100		
			0.01	100	79	100		
			0.1	100	19	100		
		<i>III</i> - Crumb et al, 2016	0.001	100	50	100		
			0.01	100	20	100		
			0.1	100	6	100		
2	Sotalol	<i>I</i> - Kramer et al, 2013	1	100	97	99	14.7	Known
			10	100	85	95		
			100	99	52	66		
		<i>II</i> - Crumb et al, 2016	1	100	98	100		
			10	100	87	100		
			100	100	47	100		
3	Verapamil	<i>I</i> - Kramer et al, 2013	1	99	23	22	0.081	NC
			10	83	4	4		
			100	18	0	1		
		<i>II</i> - Obejero-Paz et al, 2015	1	87	45	17		
			10	42	5	4		
			100	8	0	1		
		<i>III</i> - Crumb et al, 2016	1	100	32	15		
			10	100	4	1		
			100	100	0	0		
4	Cisapride	<i>I</i> - Kramer et al, 2013	0.01	100	67	100	0.005	Known
			0.1	100	16	99		
			1	100	2	92		
			10	97	0	54		
		<i>II</i> - Crumb et al, 2016	0.01	100	56	100		
			0.1	100	6	100		
			1	100	0	100		
			10	100	0	100		
5	Haloperidol	<i>Kramer et al, 2013</i>	0.01	100	84	100	0.004	Known
			0.1	100	25	97		
			1	91	2	59		
			10	21	0	6		
6	Nifedipine	<i>Kramer et al, 2013</i>	0.1	99	99	10	0.008	NC
			1	96	95	1		
			10	82	77	0		
			100	48	34	0		
7	Sparfloxacin	<i>I</i> - Passini et al, 2019	1	100	95	99	1.770	Known
			10	100	64	90		
			50	100	26	64		
			100	99	15	47		
		<i>II</i> - Kramer et al, 2013	1	100	95	99		
			10	100	68	90		
			50	100	32	64		
			100	100	20	47		
8	Terfenadine	<i>I</i> - Kramer et al, 2013	0.1	100	31	98	0.009	Known
			1	78	3	47		
			10	5	0	1		
			50	0	0	0		
		<i>II</i> - Crumb et al, 2016	0.1	100	27	80		
			1	100	8	44		
			10	100	2	13		
			50	100	1	5		

FTPC: Free estimated therapeutic plasma concentrations (Redfern, et al. 2003). I_{Na} : fast Na^+ current; I_{Kr} : rapid K^+ inward current; I_{CaL} : L-type Ca^{2+} current. TdP risk categories from CredibleMeds® (Woosley and Romer 1999).

6.2.2. *In silico* drug trials

In silico drug trials in human Purkinje cells were performed based on the Trovato2020 model, presented in Chapter 4 (Trovato et al. 2020). Specifically, simulations were conducted using the eight models resulting from the optimisation with the multi-object genetic algorithm (section 4.3.2). These models share the same design, calibration and optimisation procedures, but differ in the values for the optimised parameter subset (including all the current conductances and seven current kinetics, Figure 4.4 A and B).

Drug-induced ion current inhibition was simulated using a simple pore-block model (Brennan, Fink, and Rodriguez 2009). This model allows representing each drug-induced channel block at different concentrations through two experimentally-measured parameters: the half-maximum blockage concentration (IC_{50}) and the Hill coefficients (h). Different sets of IC_{50} values and hill coefficients were used to represent each drug (Kramer et al. 2013; Crumb et al. 2016; Passini et al. 2019; Obejero-Paz et al. 2015). The experimental conditions used to estimate the IC_{50} sets are summarised in Table 6.2. All the experiments were conducted at room temperature and in heterologous expression systems, where the channels were selectively expressed either in Chinese hamster ovary or human embryonic kidney cells. Times of cell exposure to the tested drugs before the IC_{50} estimation are reported as well, when available.

When more IC_{50} datasets were available for the same compound, the drug-induced effects were simulated for each IC_{50} dataset independently, and the drug name was followed by a cardinal number (Table 6.1). Most of the IC_{50} and Hill coefficients were available for the fast Na^+ current (I_{Na}), the L-type Ca^{2+} current (I_{CaL}), and the rapid delayed K^+ inward rectifier (I_{Kr}).

Table 6.2. Experimental conditions for IC₅₀s estimation.

Reference	Temperature	Drug-exposure time (min/conc)	Cell line
(Passini et al. 2019)	Ambient	I_{Na} : 5-7; I_{Ks} : 3-7; I_{Kr} : 5; I_{CaL} : 30	HEK: I_{Ks} , I_{CaL} , I_{Na} CHO: I_{Kr}
(Kramer et al. 2013)	Ambient	Unknown	CHO
(Obejero-Paz et al. 2015)	Ambient	5-8	HEK: I_{Kr} , I_{Ks} , I_{Na} , I_{to} . CHO: I_{K1} , I_{CaL}
(Crumb et al. 2016)	Ambient	Unknown	CHO

CHO: (Chinese hamster ovary); HEK: (Human embryonic kidney); FTTC: Free therapeutic plasms concentration

The computed ionic current blocks for these three currents are reported in Table 6.1, for each drug and at each concentration. In addition to I_{Na} , I_{CaL} , I_{Kr} , IC₅₀ and Hill coefficients for other ion channels were available for Haloperidol and Verapamil II (not reported in Table 6.1). IC₅₀ for Haloperidol also includes the slow delayed K⁺ inward rectifier (I_{Ks}), though, it is possible to observe a significant I_{Ks} reduction (-25%) only at the maximum concentration tested. Verapamil II, in addition to blocking I_{Na} , I_{CaL} , I_{Kr} as reported in Table 6.1, also reduces the late Na⁺ current, I_{NaL} , I_{Ks} , and the inward K⁺ rectifier, I_{K1} , of 10%, 2% 10%, respectively, at 1 μ M, of 65%, 15%, 47% at 10 μ M, and of 97%, 60% and 92% at 100 μ M.

For each drug concentration, ionic current blocks were applied to the 8 human Purkinje models, with identical structure as the Trovato2020 model but with different parametrisation, as described in Chapter 4 (Section 4.3.2). Extracellular concentrations were set to 150 mM, 5.4 mM and 1.8 mM for Na⁺, K⁺ and Ca²⁺, respectively, as in the experiments (Terrar et al. 2007). From steady state conditions, the models were paced for 150 beats at 1 and 0.5 Hz in the presence of drug-induced current blocks. The following AP biomarkers were computed at 1 Hz and 0.5 Hz on the last beat and compared with the corresponding control (no drug): AP duration at 90% and 50% of repolarisation (APD₉₀ and APD₅₀, respectively), and the maximum rate of depolarisation (dV/dt_{MAX}). EADs were manually identified as a positive deflection of the AP during the plateau (phase II). Drugs able to induce EADs in at least one of the eight models and at one of the concentration tested were considered risky as in (Passini et al. 2017).

All the simulations presented in this chapter and further analysis of the results were performed in Matlab (Mathworks Inc. Natwick, MA, USA).

6.3. Results and Discussion

6.3.1 In silico prediction of electrophysiological changes in human cardiac Purkinje cells for the antiarrhythmic drugs Dofetilide, Sotalol and Verapamil.

Figure 6.2 reports a summary of the results obtained performing *in silico* drug trials at 1 Hz (Panel A) and 0.5 Hz (Panel B) for the three antiarrhythmic drugs Dofetilide, Sotalol, and Verapamil.

Dofetilide is a well-known class III antiarrhythmic agent, and its main effect is hERG channel block. As a result of such block, Dofetilide prolong the QT interval and could induce TdP arrhythmia. Albeit being considered a risky drug, it is still used in the US and other countries, with a formal risk evaluation and mitigation program, but it is not commercialised in Europe. As reported in Table 6.1, three different IC₅₀ sets were available for Dofetilide (Kramer et al. 2013; Crumb et al. 2016; Passini et al. 2019). Simulations were performed up to 1 μ M (500x the ETPC). In all the simulations (Figure 6.1A), Dofetilide induced concentration-dependent AP prolongations, with no effects on dV/dt_{max}. The degree of AP prolongation was also dependent on the used IC₅₀ values, especially at higher concentrations, as reported in Table 6.3. At the concentration of 10 nM, simulations using the first set of IC₅₀ values showed APD₉₀ prolongations of $26 \pm 2\%$ at 1 Hz and of $29 \pm 2\%$ at 0.5 Hz. At the same concentrations, the second and the third IC₅₀ sets led to a lower and higher degree of APD prolongation, respectively (Table 6.3). At the same concentrations of 10 nM, experimental studies with Dofetilide in canine Purkinje reported a wider range of AP prolongation at 1 Hz, i.e., $38 \pm 6\%$ (Lightbown et al. 2007), $44. \pm 3\%$ (Abi-Gerges, Valentin, and Pollard 2010) and $106 \pm 13\%$

(Hua Rong Lu et al. 2001). A list of published data on Dofetilide-induced AP prolongation, including also species other than canine, is reported in Table 6.4.

Table 6.3 Simulated APD₉₀ variations induced by Dofetilide at different concentrations and pacing frequencies for the 3 different set of IC₅₀ sets used.

DRUG	DOSES (μ M)	IC ₅₀ SET	Δ APD ₉₀ %			
			1 Hz		2Hz	
			<i>mean</i>	<i>std</i>	<i>mean</i>	<i>std</i>
DOFETILIDE	0.001	1	27	2	29	2
		2	12	1	13	1
		3	81	4	124	8
	0.01	1	127	11	181	26
		2	83	4	127	8
		3	116	8	170	16
	0.1	1	138	13	204	42
		2	125	6	183	21
		3	133	10	193	28

IC₅₀ sets: 1(Passini et al. 2019); 2 (Kramer et al. 2013); 3 (Crumb et al. 2016).

At the dose of 100 nM, the second IC₅₀ set led to a smaller Δ APD₉₀ ($83 \pm 4\%$) with respect to the first (Δ APD₉₀= $127 \pm 11\%$) and the third (Δ APD₉₀= $116 \pm 8\%$) sets. Experimental studies at the same concentration (Table 6.4) reported a variable degree of AP prolongation, e.g., 106% in humans (data shown in Chapter 4 of the present thesis and in Trovato et al. 2020), $76 \pm 6\%$ in canine (Abi-Gerges, Valentin, and Pollard 2010), 66% in rabbit (Roche et al. 2010). When simulating the effect of 100 nM Dofetilide at 0.5 Hz (Figure 6.2B), all the models for all the tested IC₅₀ sets showed a large AP prolongation due to the development of EADs, as shown in Figure 6.3 and reported experimentally (Roche et al. 2010). Therefore, Dofetilide was considered a risky drug, in agreement with the classification by CredibleMeds (Woosley and Romer 1999) reported in Table 6.1.

Table 6.4. Dofetilide-induced APD₉₀ prolongations of Purkinje APs reported in the literature for different drug-concentrations, pacing frequencies and species

	PACING	SPECIES	0.01 μ M	0.1 μ M	1 μ M	REFERENCES
DOFETILIDE	1 Hz	Human		105 $\pm$ 36%		<i>Chapter 4 - Section 4.2.1</i> (Trovato et al. 2020)
			30%	60 $\pm$ 6%		(Terrar et al. 2007)
		Canine	44 $\pm$ 3%	76 $\pm$ 6%	91 $\pm$ 6%	(Abi-Gerges, Valentin, and Pollard 2010)
			38 $\pm$ 6%			(Lightbown et al. 2007)
			106 $\pm$ 13%			
		Guinea Pig	25 $\pm$ 14%			
		Swine	19 $\pm$ 10%			(Hua Rong Lu et al. 2001)
		Goat	61 $\pm$ 21%			
		Sheep	30 $\pm$ 10%			
		Rabbit	88 $\pm$ 12%			
		Rabbit	66 $\pm$ 17%			(Roche et al. 2010)
	0.5 Hz	Canine	57 $\pm$ 4%	105 $\pm$ 9%	134 $\pm$ 12 %	(Abi-Gerges, Valentin, and Pollard 2010)
		Rabbit	124 $\pm$ 35%			(Roche et al. 2010)

Values indicate mean $\pm$ standard deviation.

Sotalol is a non-selective beta-adrenergic receptor blocker, which acts both as a class II and class III antiarrhythmic drug (Bertrix et al. 1986), affecting both the Ca²⁺ inflow channels and hERG channel. It is used for the treatment of life-threatening ventricular arrhythmias, such as sustained ventricular tachycardia. Sotalol has a known pro-arrhythmic risk and could increase the mortality in patients with remote myocardial infarction and left ventricular ejection fraction between 31% and 40%, as highlighted by the SWORD trials (Waldo et al. 1996; Pratt et al. 1998).

Two IC₅₀ sets were available for Sotalol, as reported in Table 6.1. The main effect for both IC₅₀ sets was the reduction of I_{Kr} , albeit the first one also induced a significant I_{CaL} reduction at high doses. Figure 6.2 reports the dose-response curves simulated at 1 Hz (Panel A) and 0.5 Hz (Panel B). Overall, Sotalol induced a dose-dependent AP prolongation with no effects on the dV/dt_{Max}. For both IC₅₀ sets, Sotalol 10 μ M increased APD₉₀ and APD₅₀ of $\sim 7 \pm 1\%$ at 1 Hz.

Simulations at 0.5 Hz led to a similar degree of prolongation with respect to simulations at 1 Hz. When Sotalol concentration was increased to 100 μ M, the simulated AP prolongations at 1 Hz with both sets of IC₅₀ ($31 \pm 3\%$ for the first set and $39 \pm 3\%$ for the second) was quantitatively in agreement with those experimentally observed in canine Purkinje (Terrar et al. 2007). Similarly to the lower dose, simulations at 0.5 Hz lead to a smaller AP prolongation ($34 \pm 3\%$ and $45 \pm 2\%$ for the first and second IC₅₀s sets, respectively) compared to that experimentally observed (Terrar et al. 2007). A similar grade of APD₉₀ prolongation for Sotalol 100 μ M was also reported by (Gintant et al. 2001), although, (Abi-Gerges, Valentin, and Pollard 2010) observed a smaller AP prolongation ($18 \pm 9\%$) in canine Purkinje at same concentration and frequency.

Besides, no EADs were elicited by 100 μ M ($\sim 7\times$ EPTC) at 0.5 Hz (Figure 6.3) in any models and for any IC₅₀ sets, as reported experimentally also by (Abi-Gerges, Valentin, and Pollard 2010). However, when further increased to 1 mM ($\sim 70\times$ EPTC), Sotalol induced an EAD in all the models using the second set of IC₅₀ as experimentally observed in canine Purkinje fibre (Strauss, Bigger, and Hoffman 1970), whereas, no EADs were elicited with the first IC₅₀ set. Such different AP responses reflected the differences in the ionic channel blocks simulated using the two available IC₅₀ sets. As mentioned above, the first IC₅₀ set also induced a significant I_{CaL} reduction, which prevented the EADs formations.

Since EADs were induced when simulating the effects of Sotalol 1 mM using the second set of IC₅₀, Sotalol was correctly classified as a risky drug.

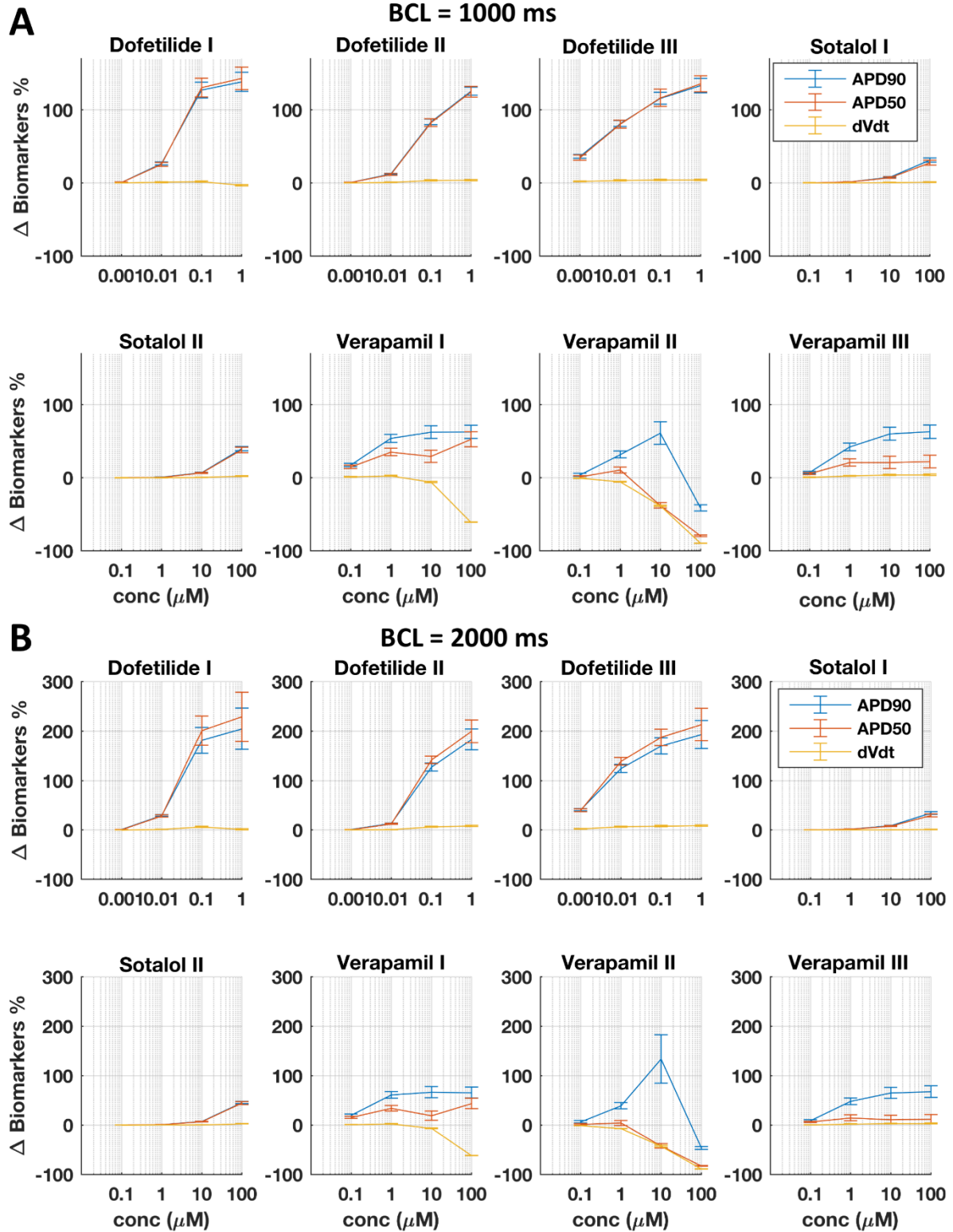


Figure 6.1. Dose-response curves at 1 Hz (Panel A) and 0.5 Hz (Panel B) for the three antiarrhythmics investigated: Dofetilide, Sotalolol, and Verapamil. Each plot reports mean and standard deviations for APD₉₀ (blue), APD₅₀ (red), and dV/dt_{Max} (yellow) percentual variations simulated using different sets of IC₅₀ to compute the drug-induced current blocks (as reported in Table 6.1) for all the eight optimised Purkinje models.

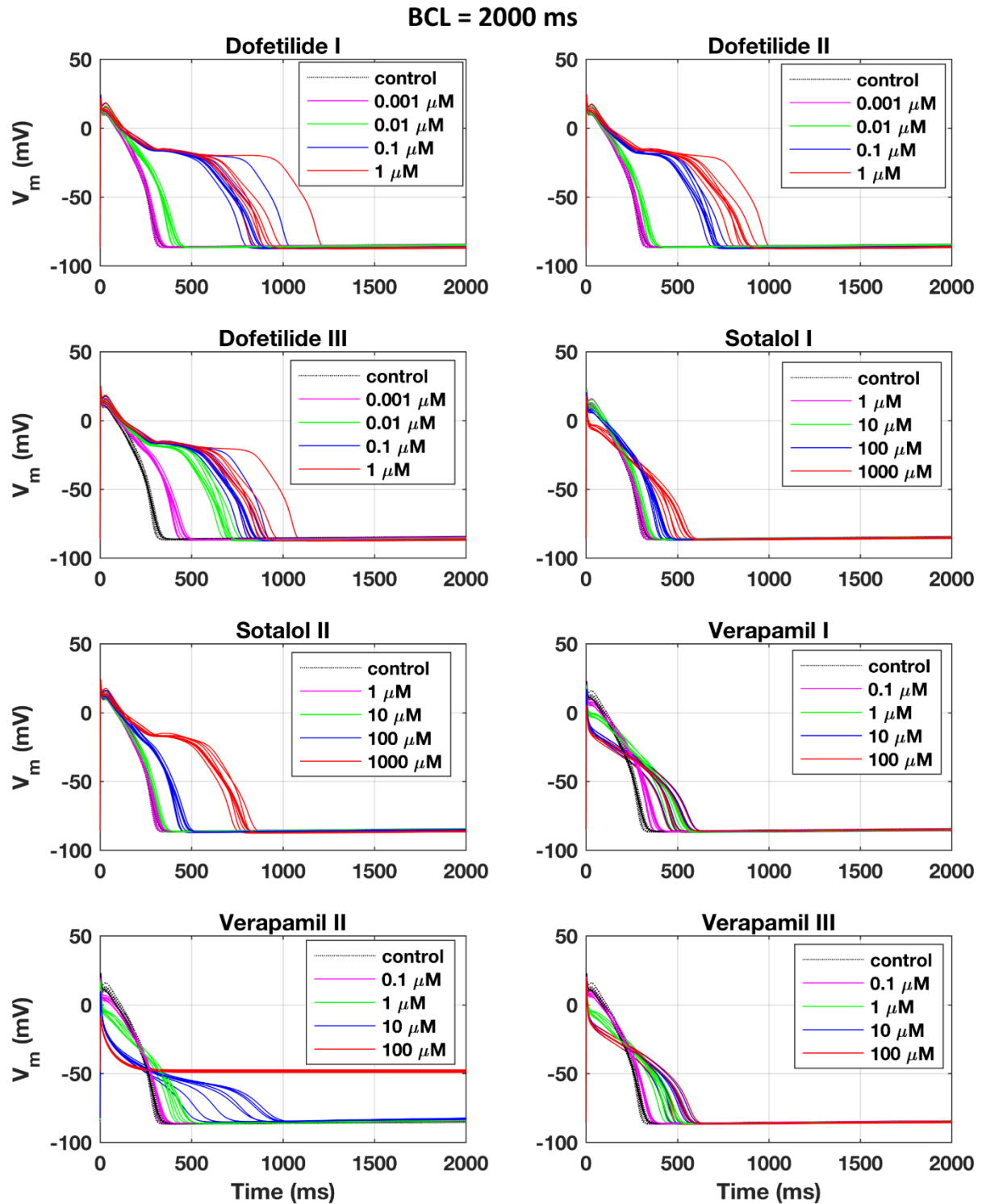


Figure 6.2. Simulated AP responses at 0.5 Hz to drug administrations for the three antiarrhythmics investigated: Dofetilide, Sotalol and Verapamil. Each plot reports simulated APs with all the eight optimised Purkinje models and different sets of IC_{50} (as reported in Table 6.1) to compute the drug-induced current blocks at several increasing concentrations, indicated in the plot legend.

Verapamil is a Ca^{2+} antagonist with different applications in the clinical setting, including the treatment of arrhythmia (Grace and Camm 2000). Simulations of Verapamil were performed using three different sets of IC_{50} , as reported in Table 6.1. All of the IC_{50} sets used to simulated verapamil-induced current blocks, affected I_{Kr} and I_{CaL} , whereas, only the first two sets also reduced I_{Na} . As a result of such I_{Na} block, simulations with the first datasets at the maximum concentration tested (100 μM) showed a reduction in the $\text{dV}/\text{dt}_{\text{Max}}$ of 61% (Figure 6.1) quantitatively in agreement with experimental observations in canine Purkinje (Terrar et al. 2007), while simulations with the second IC_{50} set led to a reduction of 90% and no reduction was observed with the third IC_{50} s set. At all the concentrations tested, Verapamil induced AP prolongation in all the Purkinje models, both at 1 Hz and 0.5 Hz. In any case, simulations were unable to reproduce the AP shortening observed experimentally (Terrar et al. 2007) at higher concentrations. AP shortening was promoted by both a higher block of I_{Kr} compared to that for I_{CaL} , as shown in Table 6.1, and a dominant role of I_{Kr} compared to I_{CaL} in setting the APD at the later stage of repolarisation, as already discussed in Chapter 5 (Table 5.1). However, the effect of Verapamil on APD_{90} reported in the literature is conflicting and need to be interpreted also considering the setup used in each experiment.

Table 6.4 reports Verapamil-induced APD_{90} changes at different concentrations and different times of drug-perfusion, recorded from different types of cells from several species, including guinea-pig, sheep, feline, canine and humans. As it is possible to observe in Table 6.4, experimental results report both AP prolongation and shortening at similar concentrations of Verapamil. The main difference between the experimental conditions of these studies was the time of exposure to Verapamil before AP recordings were taken, rather than interspecies or cell-type differences. In particular, AP shortening was observed in almost all the preparations exposed to Verapamil for less than 10 minutes (Terrar et al. 2007; S. Zhang et al. 1997; Obejero-Paz et al. 2015; Lightbown et al. 2007), whereas, AP prolongation was observed when

preparations were perfused with Verapamil longer (Pacini, Boachie-Ansah, and Kane 1992; Yamaguchi, Obayashi, and Mandel 1978; Kimura et al. 1992; Amerini, Giotti, and Mugelli 1985; Kinnaid and Man 1984). In human, an analysis of the effects of Verapamil on the cycle length (R-R interval) of healthy volunteers shows that Verapamil depresses the RR interval within the first 3 minutes from its intravenous administration (Seipel and Breithardt 1982). However, after 15 minutes from the drug-administration the shortening of the R-R interval was reduced, though not fully recovered to the control value. These results suggest that Verapamil has time-exposure-dependent effects on the cardiac electrophysiology, which is not represented by the IC_{50} used in the present study, estimated from heterologous system (Table 6.2). Ultimately, IC_{50} values are static, capturing a specific time frame of the drug actions, and potentially missing more complex dynamics, especially when acting on multiple ion channels. Simulations at 0.5 Hz did not show any EADs for any of the models at all the concentrations tested (Figure 6.2) and Verapamil was considered safe, in agreement with the classification by CredibleMeds (Woosley and Romer 1999). When simulating Verapamil 100 μ M, which represents a case of massive overdose (1234x the free estimated therapeutic plasma concentration), simulations with the second set of IC_{50} led to an abnormal AP, characterised by a small AP amplitude and duration as observed experimentally (Terrar et al. 2007), and an increase in the resting membrane potential to -50 mV. The latter was due to the simultaneous and complete block of both I_{Kr} and I_{K1} , as already discussed in the previous chapter (Section 5.3.3).

Table 6.5. Verapamil-induced APD₉₀ changes at 1 Hz and different experimental conditions.

Reference	Δ APD ₉₀	Dose (μ M)	Time drug-exposure (min/dose)	Species	Cell type
<i>(Terrar et al. 2007)</i>	+3%	1	3-5	Canine	PF
	0%	10			
	-40%	1		Guinea Pig	VM
	-50%	10			
<i>(S. Zhang et al. 1997)</i>	+23%	1	5	Guinea Pig	VM
	-14%	5			
<i>(Obejero-Paz et al. 2015)</i>	-4%	0.03	5	Human	SC-CMAP
	-22%	0.1			
	-33%	0.3			
	-27%	1			
<i>(Lightbown et al. 2007)</i>	0%	0.3	8	Canine	PF
	-5%	1			
	-15%	3			
<i>(Pacini, Boachie-Ansah, and Kane 1992)</i>	-2%	0.2	10-13	Sheep	PF
	+2%	2			
	+2%	8			
<i>(Yamaguchi, Obayashi, and Mandel 1978)</i>	0%	0.1	15	Canine	PF
	+4%	1			
	+3%	10			
<i>(Kimura et al. 1992)</i>	+10%	2	20	Feline	VM
<i>(Amerini, Giotti, and Mugelli 1985)</i>	+5%	2	30	Sheep	PF
<i>(Kinnaird and Man 1984)</i>	+13%	1	30	Canine	PF
	+22%	2			

Conc: concentrations; PF: Purkinje Fibres; VM: ventricular myocytes; SC-CMAP: human induced pluripotent stem cell (iPSC)-derived cardiomyocyte action potentials

6.3.2 Safety and AP changes predictions for the non-antiarrhythmic drugs

Cisapride, Haloperidol, Nifedipine, Sparfloxacin and Terfenadine

Figure 6.3 reports the simulated dose-response curves at 1 Hz (Panel A) and 0.5 Hz (Panel B) for the remaining five investigated compounds: Cisapride, Haloperidol, Nifedipine, Sparfloxacin and Terfenadine. Cisapride is a gastroprokinetic agent with reported cases of serious life-threatening ventricular arrhythmias, including Torsades de Pointes, mainly when used in combination with other drugs that decrease the serum clearance of Cisapride (Ahmad

and Wolfe 1995; Dubin and Van Hare 1999). Therefore, it is classified as a risky drug by CredibleMeds (Woosley and Romer 1999).

Two different sets of IC_{50} were available to simulate the Cisapride-induced changes on the AP (Table 6.1). Both of them did not affect I_{Na} and strongly decreased I_{Kr} even at the lowest concentration tested, while only the first set also reduced I_{CaL} at the highest tested concentration. As a result of such I_{Kr} blocks, all the Purkinje models showed a high degree of AP prolongation at both 1 Hz (Figure 6.3A) and 0.5 Hz (Figure 6.3B). Studies in canine Purkinje fibres report for Cisapride 0.1 μ M a low degree of AP prolongation, i.e., 5% and 8% at 1 Hz and 0.5 Hz, respectively (Terrar et al. 2007), 12% (1 Hz) and 19% (0.2 Hz) (Abi-Gerges, Valentin, and Pollard 2010), and 14% at 1 Hz (Lightbown et al. 2007). Besides, in rabbit Purkinje fibres higher AP prolongation (+50%) was induced by the same concentrations of Cisapride (Puisieux et al. 1996), further increased at higher drug concentrations (+225% at 1 μ M). These results suggest that interspecies differences need to be accounted for when simulated the effect of Cisapride on the cardiac Purkinje AP. Also, similarly to Verapamil, a strong time-dependent response was observed for Cisapride in rabbit (Puisieux et al. 1996).

Simulated APs at 0.5 Hz with Cisapride are reported in Figure 6.4. Similarly to the experimental observation in rabbit (Puisieux et al. 1996), EADs were induced in all the models already at the concentration of 0.1 μ M. Cisapride was then considered a risky drug, accordingly with the classification by CredibleMeds. It is worth noting that at the maximum concentration tested (10 μ M), EADs were stopped using the first set of IC_{50} values as a result of the additional I_{CaL} reduction, whereas, no changes were observed with the second set of IC_{50} when increasing Cisapride to 10 μ M, since I_{Kr} was already completely blocked at 1 μ M.

Haloperidol is an antipsychotic with reported cases of TdP arrhythmia, and though, its real proarrhythmic potential is still debated (Beach et al. 2018). Only one set of IC_{50} was available to simulate Haloperidol-induced effects on the AP (Table 6.1). At lower concentrations (0.01

and 0.1 μM), Haloperidol main effect was a considerable reduction in I_{K_r} magnitude, whereas it significantly reduced also I_{CaL} and I_{Na} at the higher concentrations tested (1 μM and 10 μM , respectively). As a result of such blocks, both at 1 and 0.5, Hz *in silico* drug trials on Purkinje cells (Figure 6.3) showed dose-dependent AP prolongation up to 1 μM , and then a lower degree of prolongation. A similar pattern was experimentally observed in canine Purkinje, though, with a lower degree of AP prolongation (Terrar et al. 2007; Martin et al. 2004). At 10 μM , simulations also showed a significant decrease in dV/dt_{Max} (-57%), larger than that observed in dogs ($-24 \pm 8\%$, Terrar et al. 2007).

Simulations at low pacing rate with Haloperidol, Figure 6.4, show that one model developed an EADs at intermediate drug concentrations. Haloperidol was therefore classified as a risky drug, accordingly with CredibleMeds classification.

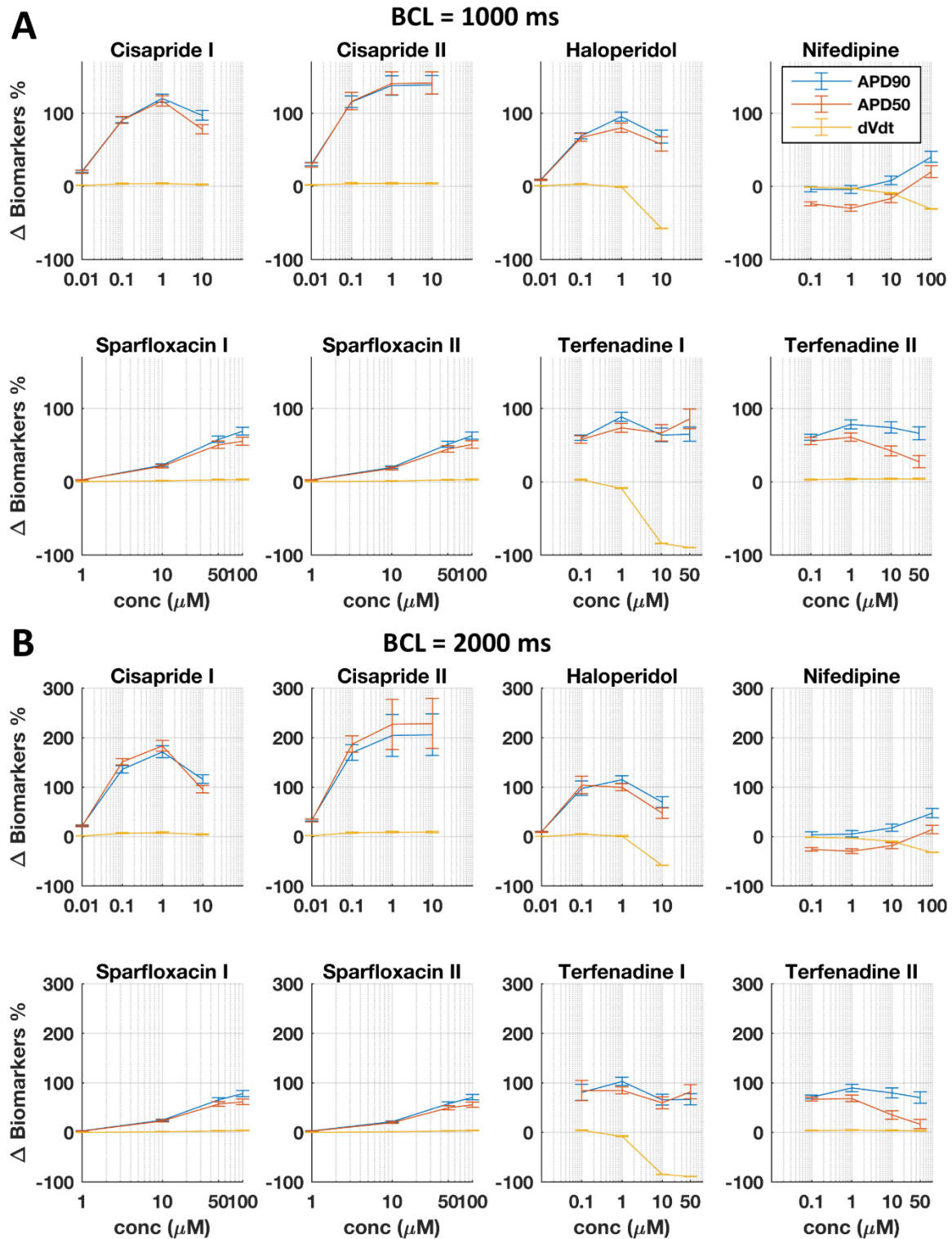


Figure 6.3. Dose-response curves at 1 Hz (Panel A) and 0.5 Hz (Panel B) for: Cisapride, Haloperidol Nifedipine, Sparfloxacin, and Terfenadine. Each plot reports mean and standard deviations for APD₉₀ (blue), APD₅₀ (red), and dV/dt_{Max} (yellow) percentual variations simulated using different sets of IC₅₀ to compute the drug-induced current blocks (as reported in Table 6.1) for all the eight optimised Purkinje models.

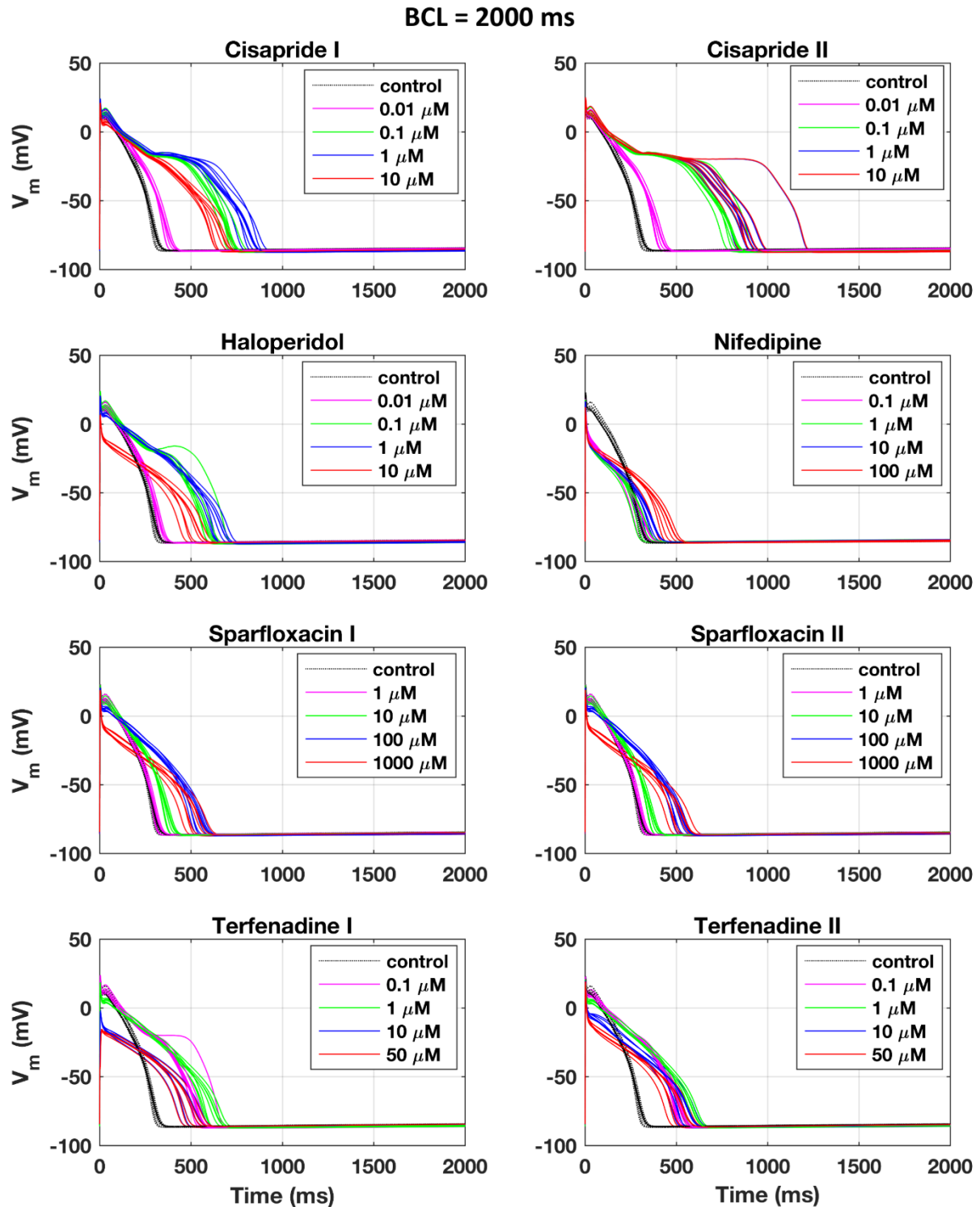


Figure 6.4. Simulated AP responses at 0.5 Hz to drug administrations for: Cisapride, Haloperidol Nifedipine, Sparfloxacin, and Terfenadine. Each plot reports simulated APs with all the eight optimised Purkinje models and different sets of IC_{50} (as reported in Table 6.1) to compute the drug-induced current blocks at several increasing concentrations, indicated in the plot legend.

Nifedipine is a Ca^{2+} channel blocker, used to manage angina, high blood pressure, Raynaud's phenomenon, premature labour and hiccup in palliative care. Nifedipine has no reports of drug-induced TdP arrhythmia and, therefore, is a not-classified drug by CredibleMeds (Woosley and Romer 1999), i.e., safe for the purpose of this study. Simulations of the drug-induced electrophysiological changes in human Purkinje cells were performed using the set of IC_{50} values reported in Table 6.1. They induce a severe block of I_{CaL} even at the minimum concentration tested. At higher concentrations, Nifedipine also blocked I_{Na} and I_{Kr} significantly.

Simulations at 1 Hz and 0.5 Hz (Figure 6.3) showed small changes in APD_{90} and $\text{dV/dt}_{\text{Max}}$ variations and a marked decrease in the APD_{50} at low concentrations (Table 6.6). Similar variations were observed experimentally in canine Purkinje (Terrar et al. 2007). At higher concentration, simulations of Nifedipine reported a significant increase in APD_{90} and marked decreases in APD_{50} and $\text{dV/dt}_{\text{Max}}$ (Table 6.6). At similar concentrations, experimental recordings in canine Purkinje fibre reported AP shortening (Baláti, Varró, and Papp 1998; Terrar et al. 2007).

Table 6.6 Simulated AP biomarkers variations induced by Nifedipine at different concentrations and pacing frequencies

DRUG	BCL (ms)	DOSES (μM)	$\Delta\text{APD}_{90} \%$		$\Delta\text{APD}_{50} \%$		$\Delta\text{dV/dt}_{\text{MAX}} \%$	
			mean	std	mean	std	mean	std
NIFEDIPINE	1000	0.1	-4	4	-24	3	-1	0
		1	-4	5	-30	4	-3	0
		10	8	6	-17	6	-9	1
		100	40	7	20	8	-31	1
	2000	0.1	4	5	-26	4	-2	0
		1	5	6	-30	5	-3	0
		10	18	7	-18	6	-10	0
		100	47	9	14	8	-32	0

BCL: Basic cycle length; **dV/dt_{MAX}:** maximum upstroke velocity; **APD_x:** AP duration at X% of repolarisation;

Simulations of Nifedipine at a lower frequency (0.5 Hz) did not show the presence of any EADs for any of the simulated models (Figure 6.4), and Nifedipine was considered a safe drug, in agreement with the absence of reports of Nifedipine-induced arrhythmias.

Sparfloxacin is an antibiotic, withdrawn from the market due to controversy on its safety profile and listed among the risky drugs by CredibleMeds (Woosley and Romer 1999). Simulations of Sparfloxacin were performed using two different sets of IC_{50} values, as reported in Table (6.1). Both sets induced similar dose-dependent reductions in both I_{Kr} and I_{CaL} , with I_{Kr} block stronger than I_{CaL} one. Sparfloxacin-induced effects on the human Purkinje electrophysiology at 1 Hz and 0.5 Hz are reported in Figure 6.3. For both IC_{50} sets, APD_{90} increased by ~20%, ~55% and ~65% at 10, 50, 100 μ M, respectively. A similar degree of prolongation was also observed in canine (Terrar et al. 2007) and rabbit (Hua Rong Lu et al. 2007) Purkinje fibres.

Simulations at a slow frequency of Sparfloxacin did not show EADs for any of the concentrations and Purkinje models tested (Figure 6.4), due to the simultaneous blocks of both I_{Kr} and I_{CaL} . The latter decreased the plateau and prevented the EADs formation.

Terfenadine is an antihistamine which can prolong the QT interval and induce TdP arrhythmia. Simulations of Terfenadine effects on cardiac Purkinje currents were performed using two different sets of IC_{50} values (Table 6.1). Both sets induced a strong block of I_{Kr} since the minimum concentration tested and significantly blocked I_{CaL} at higher concentrations. The first set also substantially decreased I_{Na} at high concentrations, inducing at 1 Hz and 0.5 Hz (Figure 6.3) a decrease in the dV/dt_{Max} of -90% at the maximum concentration tested, similarly to experimental observations in canine Purkinje (Terrar et al. 2007). Due to the I_{Kr} block, both IC_{50} sets induced AP prolongation even at the lower concentration tested (Figure 6.3), though the degree of AP prolongation decreased at higher doses when the block of I_{CaL} became strong or complete. Experimental investigations of Terfenadine in cardiac Purkinje showed a lower degree of AP prolongation both in canine (Terrar et al. 2007) and rabbit (Cavero et al. 1999).

As a result of the interplay between the I_{Kr} and I_{CaL} blocks, simulations at slow pacing (Figure 6.4) showed EADs when simulating the drug at lower concentrations with the first set of IC_{50} and only AP prolongation at higher drug concentrations. Terfenadine was therefore considered as a risky drug, in agreement with its classification by CredibleMeds (Woosley and Romer 1999).

6.3.3 General Discussion

In this chapter, *in silico* drug trials on human cardiac Purkinje cells were performed for a set of reference compounds, including three antiarrhythmic agents (Dofetilide, Sotalol, and Verapamil) and five other drugs with different modes of action (Cisapride, Haloperidol, Nifedipine, Sparfloxacin and Terfenadine). The effects of each drug on APD and dV/dt_{Max} were evaluated at different concentrations and pacing frequencies on a set of computational models representing healthy human cardiac Purkinje electrophysiology. Simulation results were compared against the data available in the literature across several species.

The main findings of this study are:

1. *In silico* drug trials performed using the Trovato2020 model and its variants (presented in Chapter 4) showed a good prediction of drug-induced AP changes for Dofetilide, Sotalol, Nifedipine, Sparfloxacin compared across different species and cell types, and highlighted the source of the discrepancies observed experimentally for Verapamil.
2. Simulations and experiments on drug-induced AP changes for Cisapride, Haloperidol and Terfenadine differed across species, highlighting that species differences need to be accounted for a correct translation from animal to human models.
3. EADs occurrence was a good biomarker for drug safety prediction in cardiac Purkinje, showing a high level of accuracy (0.875) with Sparfloxacin representing the only mismatched drug (false negative).

4. Drug-induced changes predictions of the Trovato2020 model and its variants did not differ significantly between them at 1 Hz, while more variability was observed at 0.5 Hz.

For all the *in silico* drug trials, variability on the IC₅₀ significantly affected the outcome of simulations, representing one of the primary sources of uncertainty. To cope with such uncertainty, I analysed the effects of different IC₅₀ values on the ionic cardiac currents (Table 6.1) and the experimental conditions for their estimation (Table 6.2). First, all the IC₅₀ sets were estimated at room temperature, whereas, both *in silico*, *in vitro* and *in vivo* electrophysiological experiments are performed at 36 °C. Differences in temperature have significant effects on cardiac electrophysiology (Milnes et al. 2010; Makielski and Falleroni 1991) and need to be accounted as well when analysis simulations and experimental results. Furthermore, all the IC₅₀ datasets were estimated in heterologous systems, which represent a simplified cellular *in vitro* model.

6.3.4 Limitations and next perspective

In this chapter, I simulated and evaluated *in silico* the effects of eight drugs on human cardiac Purkinje electrophysiology and their safety profiles. Future steps include to expand this analysis to a more comprehensive set of drugs, e.g. the reference compounds indicated by the CiPA initiative (Colatsky et al. 2016). We recently performed a pilot study which compared simulations for four reference compounds against experimental data from rabbit Purkinje fibres: these results have been accepted as a conference paper for the Computing in Cardiology conference, and they will be published in the conference proceedings.

The simulations in this chapter were performed on a set of eight Purkinje models, all of them representing healthy cells and, therefore, not accounting for diseased electrophysiological profiles. These will have higher propensity to develop drug-induced abnormalities, as shown

in our previous study (Passini et al. 2019), where we designed a high risk population of ventricular models, which required lower drug concentrations to develop repolarisation abnormalities, compared to a healthy one. A future extension of this work is to perform *in silico* drug trials in populations of human Purkinje models (as the one described in Chapter 5), including models with varied ionic profiles, potentially representative of particular mutations or diseases. Such populations could also be used to represent and investigate the variability in the electrophysiological drug-response observed experimentally.

Finally, experimental data describing human cardiac Purkinje Ca^{2+} dynamics are required for further refinements of the Trovato2020 model. Specifically, data on human Purkinje I_{CaL} in control conditions and following drug application would be needed to improve the $\text{I}_{\text{CaL}}\text{-I}_{\text{Kr}}$ balance in the Trovato2020 model, and to improve the simulation of drug-induced effects on AP biomarkers.

6.4 Conclusion

In this chapter, I presented the application of the Trovato2020 model to evaluate drug-induced electrophysiological changes on the human Purkinje electrophysiology. I described the results obtained performing *in silico* drug trials for a set of eight compounds, having different electrophysiological effects and safety profiles. Simulated predictions were analysed and compared with the data available in the literature for a wide range of species and cell types, showing a good agreement with experiments. Drug pro-arrhythmic safety assessment in cardiac Purkinje fibres reached high levels of accuracy. The work present in this chapter represents a contribution towards improving the current paradigm for safety drug assessment and help the translation from animal to human.

Chapter 7 - Conclusions and future directions

7.1 Summary

Ventricular arrhythmias are a major cause of sudden cardiac death, which accounts for about 50% of cardiovascular mortality worldwide. The improvement of current techniques for the arrhythmia diagnosis and treatment requires better identification of the mechanisms and conditions underlying arrhythmia onset at different scales, from the cellular level to the organ scale, in a variety of diseased scenarios. Arrhythmias generation is difficult to predict due to the complexity of the many cardiac electrophysiological multiscale interactions, and the incomplete understanding of several proarrhythmic conditions and mechanisms. Furthermore, the inter- and intra-subject variability increases the complexity of the investigations. To explore and to understand this variability can help to identify the causes of different responses to pharmacological therapies and cardiac diseases, and to identify the electrophysiological profiles more vulnerable to life-threatening cardiac abnormalities. Therefore, more research is needed for a complete understanding of the arrhythmia mechanisms, especially in humans, and the effect of biological variability.

Cardiac PCs constitute the peripheral conduction system of the electrical stimulation in ventricles and are responsible for the correct contraction pattern of the heart. Clinical studies and experimental studies on animal models pointed out that drug actions, mutations or cardiac

diseases might alter the electrical activity of PCs and lead to arrhythmia onsets. Cardiac PCs are also among the *in vitro* and *in vivo* models recommended by the ICH S7B guidelines for preclinical drug screening, especially for cardiotoxicity. However, the proarrhythmic mechanisms in cardiac PCs are not entirely understood, and results in animal studies do not always translate to humans, due to inter-species differences.

In this thesis, I presented the work done during my DPhil to investigate the role of Purkinje cells into arrhythmia generation using an experimentally-informed computational framework, based on multiscale biophysically-detailed modelling and simulations. Multiscale simulations, using the new human Purkinje model presented here, demonstrated the agreement between simulations and experiments. Human Purkinje electrophysiology was simulated under a variety of experimental protocols, including AP rate dependence, automaticity, and response to selective channels blocks, at both cellular and tissue levels. Furthermore, simulations were used to identify the mechanisms underlying electrical abnormalities in human Purkinje cells. Finally, *in silico* drug trials were performed for a set of reference compounds, to evaluate their pro-arrhythmic effects in humans.

7.2 Key contributions and main findings

The first key contribution of this work is the development of a new human Purkinje electrophysiological model, the Trovato2020 model. The model replicates a variety of experimental results, showing good agreement with AP biomarkers recorded at different frequencies, physiological APD rate-dependence, as well as AP prolongation experimentally observed in healthy human Purkinje preparations after Dofetilide superfusion, at different frequencies. Simulations with the human Purkinje Trovato2020 model unravelled the role of I_{K1} and I_f in determining the DMP and, therefore also Purkinje automaticity, as suggested

experimentally in human Purkinje preparations, in control conditions and hyperkalaemia (F. Y. Lee et al. 2004). Moreover, the Trovato2020 model is the first human Purkinje model able to reproduce EADs, DADs and triggered AP in single-cell and 1D fibre. This enables the investigation of PCs as arrhythmia triggers (Campos et al. 2015; Deo et al. 2010), in addition to their contribution to the substrate for re-entry circuits and retrograde propagation (Akihiko 2011; Boyle et al. 2010).

The second major contribution is the investigation of EADs and DADs mechanisms in hundreds of cardiac Purkinje *in silico* cells. The Trovato2020 model was used as a baseline to construct a population of models, to capture the biological variability in the AP morphology observed in experimental human Purkinje recordings. Firstly, the population of models replicated the experimental variability observed in the AP prolongation induced by Dofetilide at different frequencies. Then, when inducing electrical abnormalities in the whole population, simulations showed that EADs were caused by I_{CaL} reactivation and favoured by strong I_{CaL} and I_{NaL} . DADs were observed in a small portion of the Purkinje models displaying Ca^{2+} accumulation both in the intracellular space and into the sarcoplasmic reticulum. Triggered APs were observed in 1% of the virtual PCs, all displaying strong downregulation of both I_{K1} and I_{NaK} . Furthermore, the integration of the Trovato2020 model in a 1D fibre also succeeded to reproduce EADs, DADs and triggered APs. Therefore, the Trovato2020 model can be used to investigate Purkinje-triggered arrhythmias at the tissue and whole-organ level. The integration of the Trovato2020 model in tissue or whole-ventricular models, including the Purkinje tree (Cárdenes et al. 2015; Barber et al. 2018), could enable investigations into the role of PCs in human ventricular arrhythmias, caused by relevant disease conditions such as myocardial infarction (Szumowski et al. 2004; Bänsch et al. 2003; Garcia-Bustos et al. 2019; Lopez-Perez et al. 2019; Masahiro Maruyama et al. 2019), ischemic heart disease (Peichl, Robert, and Ko 2010; Marrouche et al. 2004), structural heart disease (Bode et al. 2008), CPVT

(Baher et al. 2011), post-shock arrhythmia (Boyle et al. 2010), Brugada and Long QT syndromes, both acquired or drug-induced (Weerasooriya et al. 2003; Gintant et al. 2001; V. Iyer et al. 2015).

As third main contribution, the Trovato2020 model was used to simulate drug-induced electrophysiological changes on the human Purkinje electrophysiology. *In silico* drug trials showed a high level of accuracy for the safety assessment of a set of compounds with different electrophysiological effects. Simulated drug-induced effects were analysed and compared with the data available in the literature for a wide range of species and cell types, showing a good agreement with experiments for some drugs and disagreement for others. The causes of these discrepancies were related to species differences, biological variability, and quality of the input data used to simulate the drug effects. This contribution represents a further step towards the use of computational models to improve the current paradigm for safety drug assessment, and help in the translation from animal to human.

7.2 Future directions

Now, I present some natural follow-up and future work related to the research presented in the thesis.

7.2.1 Purkinje-myocardium junction modelling and simulations in tissue.

In the present thesis, computational modelling and simulations were used to reproduce the electrical activity in the human cardiac Purkinje from the subcellular level to a 1D fibre. The next step is to couple the Purkinje fibre with a portion of endocardial tissue, and simulate the electrophysiological propagation through the Purkinje-myocardium junction. The junction is an area which might have high proarrhythmic potential due to the increased AP dispersion (N. Nagy et al. 2015), and it is not easy to be investigated with *in vitro* and *in vivo models*. Therefore, a computational approach could be useful for the investigation of the anterograde

and retrograde propagation of EADs and DADs in tissue, in a simplified geometrical tissue model. In this context, a collaboration with Prof. R. Sebastian from the University of Valencia has already been established, for the computational investigation of proarrhythmic abnormalities propagation from the Purkinje system to ventricular cells, and vice versa. A computational framework was developed, combining simplified geometries and electrophysiological models with multiscale simulations of the cardiac electrical propagation from the ion channels to tissue. Figure 7.1 (Panel A) reports a description of the model which comprises of a 1D Purkinje fibre, with a spatial resolution of $250\ \mu\text{m}$ and electrophysiological properties represented by the Trovato2020 model (Trovato et al. 2020), coupled with a slab of ventricular tissue. The latter has a lower spatial resolution of $400\ \mu\text{m}$, to speed up the simulations without compromised their mathematical stability and accuracy. For each node of the ventricular mesh, the electrophysiological signals are described through a recently published human ventricular AP model (Tomek et al. 2019), based on the ORd model (O'Hara et al. 2011). Purkinje and ventricular tissues are coupled through the model represented in Figure 7.1A (inset). The Purkinje-ventricular junction is comprised by an intermediated layers of Purkinje cells, whose conductivity was computed through a sigmoid function, i.e., full Purkinje conductivity for the nodes within the fibre and lower at its extremity as reported in Figure 7.1B. Then, the end-part of the fibre was connected with a number of ventricular nodes through electrical resistances. The model parameters were calibrated to overcome the source-sink mismatch and to obtain physiological CV for the Purkinje fibre and the ventricular tissue, i.e., 1.8 and $0.6\ \text{m/s}$, respectively. Moreover, the junction allows both anterograde and retrograde propagation with different delays, as reported experimentally (Haissaguerre et al. 2016).

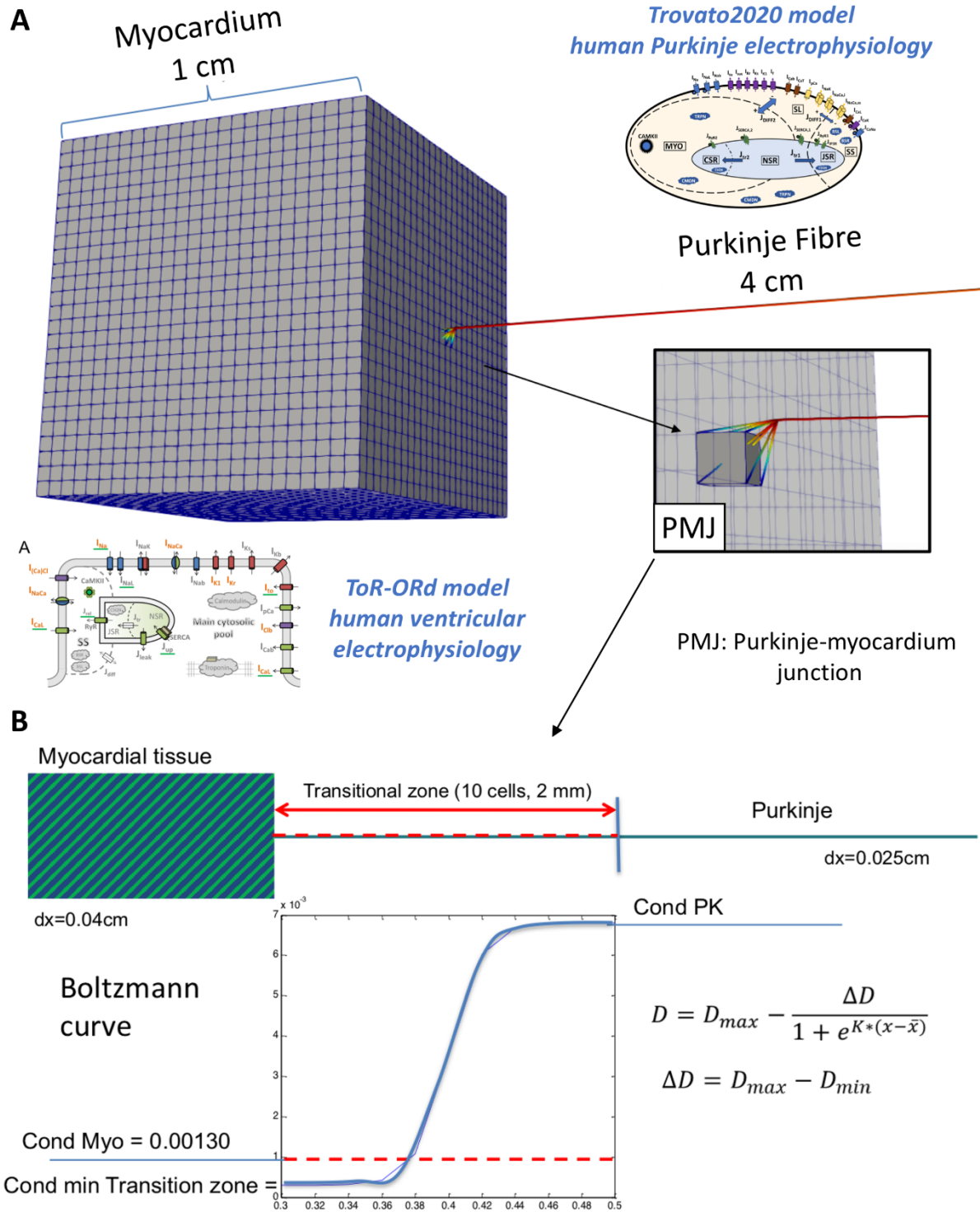


Figure 7.1. Computational multiscale framework for the investigation of the antegrade and retrograde propagation of proarrhythmic abnormalities across the Purkinje-myocardium junction: A) Geometrical and electrophysiological models for both Purkinje and ventricular tissues. B) Model of the Purkinje-ventricular junction describing the electrical coupling between the fast-conducting Purkinje fibre and the slow-conduction ventricular slab. The transitional zone conductivity is defined by a sigmoid function.

7.2.2 *Whole-organ simulations.*

As discussed in the thesis, there have been several studies about the involvement of the Purkinje network in the initiation and maintenance of arrhythmias. These ventricular tachyarrhythmias can be called Purkinje-related arrhythmias, and are the monomorphic and polymorphic ventricular tachycardia, the latter including also ventricular fibrillation. The integration of electrophysiological modelling with MRI-derived geometrical ventricular meshes, including also the Purkinje system, and simulations can provide a powerful tool for the investigation of the two most common mechanisms of ventricular tachycardia onset, i.e. macro re-entry and abnormal automaticity. The models can also reproduce a variety of conditions, including fibrosis, ionic current remodelling, conduction blocks, scars, and border zone. Several endpoints such as AP biomarkers, isometric activation curves and simulated ECGs can be derived from simulations and compared with clinical and experimental observations, to infer on the role of the cardiac Purkinje network in these types of arrhythmias.

In this context, the Trovato2020 cellular model was integrated in a biventricular anatomically-based model including the His-Purkinje system (Lopez-Perez et al. 2019), in collaboration with Prof. Sebastian, from the University of Valencia. In summary, the biventricular model (Lopez-Perez et al. 2019) was segmented from a Delayed-Enhanced-MRI stack sequence, that included the most prominent trabecula and papillary muscles. The model was meshed with hexahedral elements giving rise to 4 million nodes (vertices) and 3.71 million hexahedral elements, with an average resolution (edge length) of 0.38 mm. The model includes fibre orientation (Streeter et al. 1969) and electrophysiological transmural heterogeneity. The Purkinje system was estimated from the endocardial electro-anatomical maps of the right and left ventricles. In particular, from the electro-anatomical mapping were estimated 33 and 16 PMJs for the left and right ventricle, respectively. Then a synthetic Purkinje network (Figure 7.2A) was built and incorporated into the biventricular mesh. Estimated conduction velocities were 0,5 m/s in

tissue and 2 m/s in the Purkinje system. To represent the ventricular membrane kinetics, we incorporated the ToR-ORd model recently developed by (Tomek et al. 2019).

A preliminary set of simulations were already performed using ELVIRA, a finite element method solver specifically developed to solve the anisotropic reaction-diffusion equation of the monodomain model for cardiac electrophysiology. A colourmap of the activation time for both ventricles activations in physiological conditions and at sinus rhythm is shown in Figure 7.2B. Simulation of 500 ms took approximately 10 hours, using 64 cores. The necessary computational resources have been already allocated for the project.

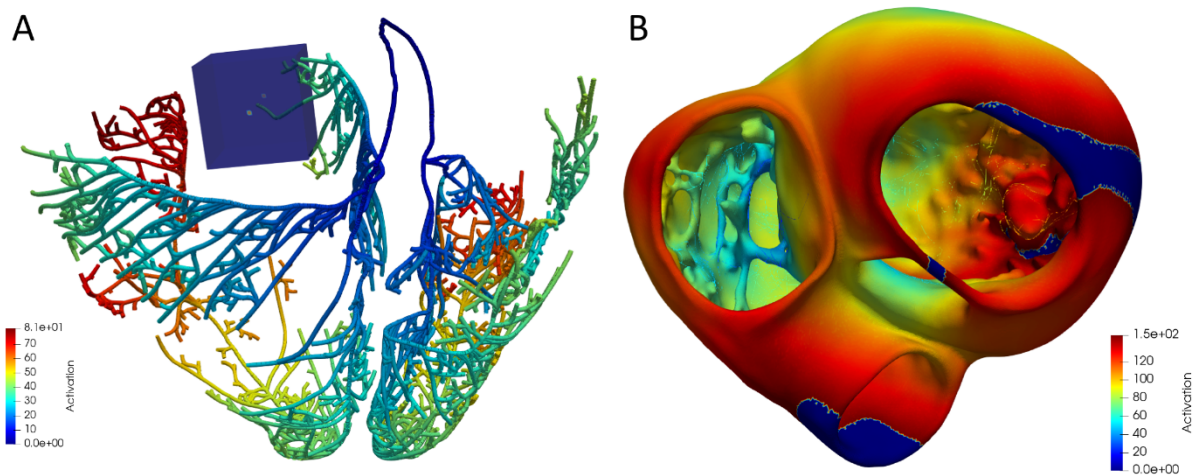


Figure 7.2. Whole organ simulations with anatomical and electrophysiological models. A) Simulation of the AP propagation in an *in silico* Purkinje tree to simulate a physiological ventricular depolarisation, coupled with a small slab of ventricular tissue. B) Multiscale simulations of cardiac depolarisation in a biventricular mesh, including the Purkinje system.

7.2.3 Extensive Purkinje model qualification for *in silico* drug trials and safety prediction

In the present thesis, drug-induced electrophysiological changes were evaluated in a population of models for Dofetilide and on a small number of healthy Purkinje models for other drugs.

However, a more extensive comparison between *in vitro*, *in vivo* and *in silico* drug trials is needed for the quantification of the Trovato2020 for drug safety assessment. The population of models approach can be used to reproduce and investigate the experimental variability observed in drug response, also including diseased scenarios, to identify the main interspecies differences and improving the translation towards humans. In particular, the Trovato2020 model was implemented in Virtual Assay (v.3.2 © 2014 Oxford University Innovation Ltd. Oxford, UK), a user-friendly software package based on C++, and specifically designed for *in silico* drug assays in populations of computer models. I have already used the software for preclinical drug safety assessment in a population of ventricular models (Passini et al. 2019). Several pharma companies as Amgen, AstraZeneca, GlaxoSmithKline, Janssen Pharmaceutical, Merck, Sanofi, Servier, and UCB are evaluating Virtual Assay for its use in preclinical drug assessment to reduce the use of animal models. Some of these pharma companies are also providing new data on human Purkinje electrophysiology under drug actions. Among the compounds currently investigated, there are also chloroquine and hydroxychloroquine, alone or in combination with erythromycin and azithromycin, selected due to the ongoing COVID-19 pandemic, The Trovato2020 model will be used to investigate their safety profile on cardiac Purkinje electrophysiology.

7.2.4 Simulations of drug-induced electrophysiological effects with whole-organ simulations for selected compounds.

The two previous projects can be combined for the investigation with 3D whole-organ simulations of the Purkinje role on arrhythmia onset under specific drug actions, as for the verapamil-sensitive left fascicular ventricular tachycardia. Of course, it is (yet) not possible to simulate a population of thousands of 3D models for a variety of drugs at different

concentrations, due to the computational costs. However, results at the cellular level can be expanded in tissue up to the whole organ level for specific models and compounds.

7.2.5 Further cellular model development and refinement.

The Trovato2020 model presented in the thesis can be further developed and refined as soon as new data become available. Also, the model can be extended with the inclusion of other components, e.g. beta-adrenergic stimulation, to better represent the model response to beta-blockers, or mitochondria, to reproduce the cellular metabolism.

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Appendix - Trovato2020 model: Parameters and Equations

Stimulus

$$\text{Amplitude } I_{\text{stim}} = -40 \frac{\mu A}{\mu F} \quad \text{Duration} = 1 \text{ ms.}$$

Extracellular Concentrations

$$[\text{Na}^+]_o = 140 \text{ mM}; \quad [\text{Ca}^{2+}]_o = 1.8 \text{ mM}; \quad [\text{K}^+]_o = 5.4 \text{ mM}$$

Cell Geometry

$$L = 0.0164 \text{ cm}$$

$$r = 0.00175 \text{ cm}$$

$$A_{\text{geo}} = 2\pi \cdot r^2 + 2\pi \cdot r \cdot L = 2 \cdot 10^{-4} \text{ cm}^2 \quad A_{\text{cap}} = 2 \cdot A_{\text{geo}} = 4 \cdot 10^{-4} \mu L$$

$$v_{\text{cell}} = \pi \cdot r^2 \cdot L = 16 \cdot 10^{-5} \mu L$$

$$v_{\text{myo}} = 0.6 \cdot v_{\text{cell}} = 9.5 \cdot 10^{-5} \mu L$$

$$v_{\text{nsr}} = 0.04 \cdot v_{\text{cell}} = 6.3 \cdot 10^{-6} \mu L$$

$$v_{\text{ss}} = 0.02 \cdot v_{\text{cell}} = 3.1 \cdot 10^{-6} \mu L$$

$$v_{\text{jsr}} = 0.002 \cdot v_{\text{cell}} = 3.1 \cdot 10^{-7} \mu L$$

$$v_{\text{sl}} = 0.15 \cdot v_{\text{cell}} = 2.4 \cdot 10^{-5} \mu L$$

$$v_{\text{csr}} = 0.008 \cdot v_{\text{cell}} = 1.3 \cdot 10^{-6} \mu L$$

Steady State conditions at 1Hz (after 1000 beats)

$V = -86.55;$	$j = 0.8;$	$nca = 0;$
$[Na+]_I = 8.23;$	$hsp = 0.6;$	$ffp = 1;$
$[Na+]_{SL} = 8.23;$	$jp = 0.8;$	$fcafp = 1;$
$[Na+]_{SS} = 8.23;$	$mL = 0;$	$b = 0;$
$[K+]_I = 144;$	$hL = 0.5;$	$g = 1;$
$[K+]_{SL} = 144;$	$hLp = 0.2;$	$xrf = 0;$
$[K+]_{SS} = 144;$	$a = 0;$	$xrs = 0.6;$
$[Ca^{2+}]_I = 4.36e-05;$	$i = 0.6;$	$xs1 = 0.2;$
$[Ca^{2+}]_{SL} = 1.0e-04;$	$i2 = 1;$	$xs2 = 0;$
$[Ca^{2+}]_{SS} = 1.0e-04;$	$d = 0;$	$xk1 = 1;$
$[Ca^{2+}]_{JSR} = 1.25;$	$ff = 1;$	$y = 0.2;$
$[Ca^{2+}]_{NSR} = 1.26;$	$fs = 1;$	$CaMKt = 0;$
$[Ca^{2+}]_{CSR} = 1.26;$	$fcaf = 1;$	$u = 0.5;$
$m = 0;$	$fcas = 1;$	$Jrel1 = 0;$
$hf = 0.8;$	$jca = 1;$	$Jrel2 = 0;$
$hs = 0.8;$		

Maximum Current Conductances (mS/ μ F)

G_{Na}	39.46
G_{NaL}	0.0189
G_{CaL}	7.7677e-05
G_{CaT}	0.0754
G_{to}	0.192
G_{sus}	0.0301
G_{Kr}	0.0342
G_{Ks}	0.0029
G_{fNa}	0.0116
G_{fK}	0.0232
G_{K1}	0.0455
G_{NaK}	32.4872
G_{NCX}	9.5709e-04

Calcium Buffer Constants

BSR_{MAX}	0.019975
Km_{BSR}	0.00087
BSL_{MAX}	0.4777
Km_{BSL}	0.0087
CSQN_{MAX,CSR}	2.88
CSQN_{MAX,JSR}	1.2
Km_{CSQN}	0.8
CMDN_{MAX,i}	0.1125
CMDN_{MAX,sl}	1.25e-2
Km_{CMDN}	0.00238
TRPN_{MAX,I}	3.15e-2
TRPN_{MAX,SL}	3.5e-3
Km_{TRPN}	0.0005

Voltage

$$C_m \frac{dV}{dt} = -(I_{\text{ion}} + I_{\text{stim}})$$

$$I_{\text{ion}} = I_{\text{Na}} + I_{\text{NaL}} + I_{\text{to}} + I_{\text{sus}} + I_{\text{CaL}} + I_{\text{CaT}} + I_{\text{CaNa}} + I_{\text{CaK}} + I_{\text{Kr}} + I_{\text{Ks}} + I_{\text{f}} + I_{\text{K1}} + I_{\text{NaCa}} + I_{\text{NaK}} \\ + I_{\text{Nab}} + I_{\text{Cab}} + I_{\text{pCa}} + I_{\text{Stim}}$$

Fast-Sodium Current (I_{Na})

from (Dutta et al. 2017; Passini et al. 2016)

$$m_{\infty} = \frac{1}{1 + e^{-\left(\frac{V+48.6264}{9.871}\right)}}$$

$$\tau_m = \frac{1}{6.755 \cdot e^{\left(\frac{V+11.64}{34.77}\right)} + 8.552 \cdot e^{-\left(\frac{V+77.42}{5.955}\right)}}$$

$$h_{\infty} = \frac{1}{1 + e^{\left(\frac{V+78.5}{6.22}\right)}}$$

$$\tau_{h,\text{fast}} = \frac{1}{3.686 \cdot 10^{-6} \cdot e^{-\left(\frac{V+3.8875}{7.8579}\right)} + 16 \cdot e^{\left(\frac{V-0.4963}{9.1843}\right)}}$$

$$\tau_{h,\text{slow}} = \frac{1}{0.009764 \cdot e^{-\left(\frac{V+17.95}{28.05}\right)} + 0.3343 \cdot e^{\left(\frac{V+5.730}{56.66}\right)}}$$

$$A_{h,\text{fast}} = 0.99, \quad A_{h,\text{slow}} = 1 - A_{h,\text{fast}}$$

$$h = A_{h,\text{fast}} \cdot h_{\text{fast}} + A_{h,\text{slow}} \cdot h_{\text{slow}}$$

$$j_{\infty} = h_{\infty}$$

$$\tau_j = 4.8590 + \frac{1}{0.8628 \cdot e^{-\left(\frac{V+166.7}{7.6}\right)} + 1.1096 \cdot e^{\left(\frac{V+6.2719}{9.0358}\right)}}$$

$$h_{\text{CaMK},\infty} = \frac{1}{1 + e^{\left(\frac{V+84.7}{6.22}\right)}}$$

$$\tau_{h,\text{CaMK},\text{slow}} = 3 \cdot \tau_{h,\text{slow}}$$

$$A_{h,\text{CaMK},\text{fast}} = A_{h,\text{fast}}$$

$$A_{h,\text{CaMK},\text{slow}} = A_{h,\text{slow}}$$

$$h_{\text{CaMK},\infty} = h_{\text{fast}}$$

$$h_{\text{CaMK}} = A_{h,\text{CaMK},\text{fast}} \cdot h_{\text{CaMK},\text{fast}} + A_{h,\text{CaMK},\text{slow}} \cdot h_{\text{CaMK},\text{slow}}$$

$$j_{\text{CaMK},\infty} = j_{\infty}$$

$$\tau_{j,\text{CaMK}} = 1.46 \cdot \tau_j$$

$$K_{m,\text{CaMK}} = 0.15, \quad \phi_{\text{INa},\text{CaMK}} = \frac{1}{1 + \frac{K_{m,\text{CaMK}}}{K_{m,\text{CaMK},\text{active}}}}$$

$$I_{\text{Na},\text{fast}} = G_{\text{Na}} \cdot (V - E_{\text{Na}}) \cdot m^3 \cdot ((1 - \phi_{\text{INa},\text{CaMK}}) \cdot h \cdot j + \phi_{\text{INa},\text{CaMK}} \cdot h_{\text{CaMK}} \cdot j_{\text{CaMK}})$$

Late-Sodium Current (I_{NaL})

from (O'Hara et al. 2011)

$$m_{L,\infty} = \frac{1}{1 + e^{\left(\frac{V+42.85}{5.264}\right)}}$$

$$\tau_{m,L} = \tau_m$$

$$h_{L,\infty} = \frac{1}{1 + e^{\left(\frac{V+87.61}{7.488}\right)}}$$

$$\tau_{h,L} = 200 \text{ ms}$$

$$h_{L,\text{CaMK},\infty} = \frac{1}{1 + e^{\left(\frac{V+93.81}{7.488}\right)}}$$

$$\tau_{h,L\text{CaMK}} = 3 \cdot \tau_{h,L}$$

$$K_{m,\text{CaMK}} = 0.15, \quad \phi_{\text{INaL,CaMK}} = \frac{1}{1 + \frac{K_{m,\text{CaMK}}}{K_{m,\text{CaMK,active}}}}$$

$$I_{\text{Na,late}} = G_{\text{NaL}} \cdot (V - E_{\text{Na}}) \cdot m_L \cdot ((1 - \phi_{\text{INaL,CaMK}}) \cdot h_L + \phi_{\text{INaL,CaMK}} \cdot h_{L,\text{CaMK}})$$

L-type Calcium Current (I_{CaL})

from (O'Hara et al. 2011), modified as described in Section 4.3.1

$$d = \frac{1}{1 + e^{-\left(\frac{V+7.24}{4.23}\right)}}$$

$$\tau_d = 0.6 + \frac{1}{e^{-0.05 \cdot (V+6)} + e^{-0.09 \cdot (V+14)}}$$

$$f_{\infty} = \frac{1}{1 + e^{\left(\frac{V+22.88}{3.696}\right)}}$$

$$\tau_{f,\text{fast}} = 7 + \frac{1}{0.0045 \cdot e^{-\left(\frac{V+35.19}{10}\right)} + 0.0045 \cdot e^{\left(\frac{V+35.19}{10}\right)}}$$

$$\tau_{f,\text{slow}} = 1000 + \frac{1}{0.000035 \cdot e^{-\left(\frac{V+20.19}{4}\right)} + 0.000035 \cdot e^{\left(\frac{V+20.19}{4}\right)}}$$

$$A_{f,\text{fast}} = 0.6,$$

$$A_{f,\text{slow}} = 1 - A_{f,\text{fast}}$$

$$f = A_{f,\text{fast}} \cdot f_{\text{fast}} + A_{f,\text{slow}} \cdot f_{\text{slow}}$$

$$f_{\text{Ca},\infty} = f_{\infty}$$

$$\tau_{f,\text{Ca,fast}} = 7 + \frac{1}{0.04 \cdot e^{-\left(\frac{V-11.19}{7}\right)} + 0.04 \cdot e^{\left(\frac{V-11.19}{7}\right)}}$$

$$\tau_{f,\text{Ca,slow}} = 100 + \frac{1}{0.00012 \cdot e^{-\left(\frac{V+20}{3}\right)} + 0.00012 \cdot e^{\left(\frac{V+20}{7}\right)}}$$

$$A_{f, Ca, fast} = 0.3 + \frac{0.6}{1 + e^{\left(\frac{V-10}{10}\right)}}, \quad A_{f, Ca, slow} = 1 - A_{f, Ca, fast}$$

$$f_{Ca} = A_{f, Ca, fast} \cdot f_{Ca, fast} + A_{f, Ca, slow} \cdot f_{Ca, slow}$$

$$j_{Ca, \infty} = f_{Ca, \infty}$$

$$\tau_{j, Ca} = 75$$

$$f_{CaMK, \infty} = f_{\infty}$$

$$\tau_{f, CaMK, fast} = 2.5 \cdot \tau_{f, fast}$$

$$A_{f, CaMK, fast} = A_{f, fast}, \quad A_{f, CaMK, slow} = A_{f, slow}$$

$$f_{CaMK, slow} = f_{slow}$$

$$f_{CaMK} = A_{f, CaMK, fast} \cdot f_{CaMK, fast} + A_{f, CaMK, slow} \cdot f_{CaMK, slow}$$

$$f_{Ca, CaMK, \infty} = f_{\infty}$$

$$\tau_{f, Ca, CaMK, fast} = 2.5 \cdot \tau_{f, Ca, fast}$$

$$A_{f, Ca, CaMK, fast} = A_{f, Ca, fast}, \quad A_{f, Ca, CaMK, slow} = A_{f, Ca, slow}$$

$$f_{Ca, CaMK, slow} = f_{Ca, slow}$$

$$f_{Ca, CaMK} = A_{f, Ca, CaMK, fast} \cdot f_{Ca, CaMK, fast} + A_{f, Ca, CaMK, slow} \cdot f_{Ca, CaMK, slow}$$

$$K_{m, n} = 0.002, \quad K_{+2, n} = 1000, \quad K_{-2, n} = j_{Ca}$$

$$\alpha_n = \frac{1}{\frac{K_{+2, n}}{K_{-2, n}} + \left(1 + e^{\left(\frac{K_{m, n}}{[Ca^{2+}]_{sl}}\right)}\right)^4}$$

$$\gamma_{Cai} = 1, \quad \gamma_{Cao} = 0.341, \quad z_{Ca} = 2$$

$$\Psi_{Ca} = z_{Ca}^2 \cdot \frac{VF^2}{RT} \cdot \frac{\gamma_{Cai} \cdot [Ca^{2+}]_{ss} \cdot e^{\frac{z_{Ca}VF}{RT}} - \gamma_{Cao} \cdot [Ca^{2+}]_o}{e^{\frac{z_{Ca}VF}{RT}} - 1.0}$$

$$\overline{I_{CaL}} = G_{CaL} \cdot \Psi_{Ca}$$

$$P_{CaNa} = 0.00125 \cdot P_{Ca} , \quad \gamma_{Nai} = 0.75, \quad \gamma_{Nao} = 0.75, \quad z_{Na} = 1$$

$$\Psi_{CaNa} = z_{Na}^2 \cdot \frac{VF^2}{RT} \cdot \frac{\gamma_{Nai} \cdot [Na^+]_{ss} \cdot e^{\frac{z_{Na}VF}{RT}} - \gamma_{Nao} \cdot [Na^+]_o}{e^{\frac{z_{Na}VF}{RT}} - 1.0}$$

$$\overline{I_{CaNa}} = P_{CaNa} \cdot \Psi_{CaNa}$$

$$P_{CaK} = 3.574 \cdot 10^{-4} \cdot P_{Ca} , \quad \gamma_{Ki} = 0.75, \quad \gamma_{Ko} = 0.75, \quad z_K = 1$$

$$\Psi_{CaK} = z_K^2 \cdot \frac{VF^2}{RT} \cdot \frac{\gamma_{Ki} \cdot [K^+]_{ss} \cdot e^{\frac{z_KVF}{RT}} - \gamma_{Ko} \cdot [K^+]_o}{e^{\frac{z_KVF}{RT}} - 1.0}$$

$$\overline{I_{CaK}} = P_{CaK} \cdot \Psi_{CaK}$$

$$P_{Ca,CaMK} = 1.1 \cdot G_{CaL}$$

$$\overline{I_{CaL,CaMK}} = P_{Ca,CaMK} \cdot \Psi_{Ca}$$

$$P_{CaNa,CaMK} = 0.00125 \cdot P_{Ca,CaMK}$$

$$\overline{I_{CaNa,CaMK}} = P_{CaNa,CaMK} \cdot \Psi_{CaNa}$$

$$P_{CaK,CaMK} = 3.574 \cdot 10^{-4} \cdot P_{Ca,CaMK}$$

$$\overline{I_{CaK,CaMK}} = P_{CaK,CaMK} \cdot \Psi_{CaK}$$

$$K_{m,CaMK} = 0.15 , \quad \phi_{ICaL,CaMK} = \frac{1}{1 + \frac{K_{m,CaMK}}{CaMK_{active}}}$$

$$I_{CaL} = \overline{I_{CaL}} \cdot d \cdot (1 - \phi_{ICaL,CaMK}) \cdot (f \cdot (1 - n) + f_{Ca} \cdot n \cdot j_{Ca}) + \overline{I_{CaL,CaMK}} \cdot d \cdot \phi_{ICaL,CaMK} \cdot (f_{CaMK} \cdot (1 - n) + f_{Ca,CaMK} \cdot n \cdot j_{Ca})$$

$$I_{CaNa} = \overline{I_{CaNa}} \cdot d \cdot (1 - \phi_{ICaL,CaMK}) \cdot (f \cdot (1 - n) + f_{Ca} \cdot n \cdot j_{Ca}) + \overline{I_{CaNa,CaMK}} \cdot d \cdot \phi_{ICaL,CaMK} \cdot (f_{CaMK} \cdot (1 - n) + f_{Ca,CaMK} \cdot n \cdot j_{Ca})$$

$$I_{CaK} = \overline{I_{CaK}} \cdot d \cdot (1 - \phi_{ICaL,CaMK}) \cdot (f \cdot (1 - n) + f_{Ca} \cdot n \cdot j_{Ca}) + \overline{I_{CaK,CaMK}} \cdot d \\ \cdot \phi_{ICaL,CaMK} \cdot (f_{CaMK} \cdot (1 - n) + f_{Ca,CaMK} \cdot n \cdot j_{Ca})$$

T-type Calcium Current (I_{CaT})

From (P. Li and Rudy 2011)

$$b_{\infty} = \frac{1}{1 + e^{-\left(\frac{V+30}{7}\right)}}$$

$$\tau_b = \frac{1}{1.068 \cdot e^{-\left(\frac{V-16.3}{30}\right)} + 1.068 \cdot e^{\left(\frac{V-16.3}{30}\right)}}$$

$$g_{\infty} = \frac{1}{1 + e^{\left(\frac{V+61}{5}\right)}}$$

$$\tau_b = \frac{1}{0.015 \cdot e^{-\left(\frac{V-71.7}{83.3}\right)} + 0.015 \cdot e^{\left(\frac{V+71.7}{15.4}\right)}}$$

$$I_{CaT} = G_{CaT} \cdot b \cdot g \cdot (V - E_{Ca})$$

Transient Outward Current (I_{to})

Data from (Wei Han, Zhang, et al. 2002)

$$a_{\infty} = \frac{1}{1 + e^{\left(\frac{20-V}{13}\right)}}$$

$$\tau_a = \frac{1.0515}{\left(\frac{1}{1.2 \cdot (1 + \exp(-\frac{V-18.41}{29.38}))} + \frac{3.5}{1 + \exp(\frac{V+100}{29.38})} \right)}$$

$$i_{1,\infty} = \frac{1}{1 + e^{\left(\frac{V+27}{13}\right)}}$$

$$i_{2,\infty} = i_{1,\infty}$$

$$\tau_{i,S} = 43 + \frac{1}{0.001416 \cdot \exp\left(-\frac{V + 96.52}{59.05}\right) + 1.7 \cdot 10^{-8} \cdot \exp\left(\frac{V + 114.1}{8.079}\right)}$$

$$\tau_{i,F} = 6.16 + \frac{1}{0.39 \cdot \exp\left(-\frac{V + 100}{100}\right) + 0.08 \cdot \exp\left(\frac{V - 8}{8.59}\right)}$$

$$I_{to} = g_{to} \cdot a \cdot i_S \cdot i_F \cdot g \cdot (V - E_K)$$

Sustained Potassium Current (I_{sus})

Data from (Wei Han, Zhang, et al. 2002)

$$a_{sus} = \frac{1}{1 + e^{-\left(\frac{V-12}{16}\right)}}$$

$$i_{sus} = g_{sus} \cdot a_{sus} \cdot (V - E_K)$$

Rapid Delayed Rectifier Potassium Current (I_{Kr})

from (O'Hara et al. 2011), modified as described in Section 4.3.1

$$x_{r\infty} = \frac{1}{1 + e^{-\left(\frac{V+8.337}{6.789}\right)}}$$

$$\tau_{xr,fast} = 12.98 + \frac{1}{0.3652 \cdot e^{\left(\frac{V-14.06}{3.869}\right)} + 4.123 \cdot 10^{-5} \cdot e^{-\left(\frac{V-30.18}{20.38}\right)}}$$

$$\tau_{xr,slow} = 1.865 + \frac{1}{0.06629 \cdot e^{\left(\frac{V-19.7}{7.355}\right)} + 1.128 \cdot 10^{-5} \cdot e^{-\left(\frac{V-12.54}{25.94}\right)}}$$

$$A_{xr,fast} = \frac{1}{1 + e^{\left(\frac{V+54.81}{38.21}\right)}},$$

$$A_{xr,slow} = 1 - A_{xr,fast}$$

$$x_r = A_{xr,fast} \cdot x_{r,fast} + A_{xr,slow} \cdot x_{r,slow}$$

$$R_{Kr} = \frac{1}{(1 + e^{\left(\frac{V+55}{24}\right)}) \cdot (1 + e^{\left(\frac{V-10}{9.6}\right)})}$$

$$i_{Kr} = g_{Kr} \cdot \sqrt{\frac{[K^+]_o}{5.4}} \cdot x_r \cdot R_{Kr} \cdot (V - E_K)$$

Slow Delayed Rectifier Potassium Current (I_{Ks})

from (O'Hara et al. 2011)

$$x_{s1,\infty} = \frac{1}{1 + e^{-\left(\frac{V+11.6}{8.932}\right)}}$$

$$\tau_{x,s1} = 817.3 + \frac{1}{0.001292 \cdot e^{-\left(\frac{V+210}{230}\right)} + 2.326 \cdot 10^{-4} \cdot e^{\left(\frac{V+48.28}{17.8}\right)}}$$

$$x_{s2,\infty} = x_{s1,\infty}$$

$$\tau_{x,s2} = \frac{1}{0.01 \cdot e^{\left(\frac{V-50}{20}\right)} + 0.0193 \cdot e^{-\left(\frac{V+66.54}{31}\right)}}$$

$$i_{Ks} = g_{Ks} \cdot \left(1 + \frac{0.6}{1 + \left(\frac{3.8 \cdot 10^{-5}}{[Ca^{2+}]_{sl}} \right)^{1.4}} \right) \cdot x_{s1} \cdot x_{s2} \cdot (V - E_{Ks})$$

Hyperpolarization-activated Current (I_f)

from (P. Li and Rudy 2011)

$$y_\infty = \frac{1}{1 + e^{\left(\frac{V+87}{9.5}\right)}}$$

$$\tau_y = \frac{2000}{\exp\left(-\frac{V+132}{10}\right) + \exp\left(\frac{V+57}{60}\right)}$$

$$i_{f,K} = g_{f,K} \cdot y \cdot (V - E_K)$$

$$i_{f,Na} = g_{f,Na} \cdot y \cdot (V - E_{Na})$$

$$i_f = i_{f,K} + i_{f,Na}$$

Inward Rectifier Potassium Current (I_{K1})

Data from (Wei Han, Zhang, et al. 2002)

$$x_{K1,\infty} = \frac{1}{1 + e^{-\left(\frac{V+2.5538 \cdot [K^+]_o + 144.59}{1.5692 \cdot [K^+]_o + 3.8115}\right)}}$$

$$\tau_{x,K1} = \frac{122.2}{e^{-\left(\frac{V+127.2}{20.36}\right)} + e^{\left(\frac{V+236.8}{69.33}\right)}}$$

$$R_{K1} = \frac{1}{1 + e^{\left(\frac{V+116-5.5 \cdot [K^+]_o}{11}\right)}}$$

$$i_{K1} = g_{K1} \cdot \sqrt{\frac{[K^+]_o}{5.4}} \cdot x_{K1} \cdot R_{K1} \cdot (V - E_K)$$

Sodium-Calcium Exchange Current (I_{NaCa})

from (O'Hara et al. 2011)

For, $Y \in \{i, ss\}$:

$$K_{Na1} = 15 \text{ mM}, \quad K_{Na2} = 5 \text{ mM}, \quad K_{Na3} = 88.12 \text{ mM}, \quad K_{asymm} = 12.5$$

$$\omega_{Na} = 6 \cdot 10^4 \text{ Hz}, \quad \omega_{Ca} = 6 \cdot 10^4 \text{ Hz}, \quad \omega_{NaCa} = 5 \cdot 10^3 \text{ Hz}$$

$$k_{Ca,on} = 1.5 \cdot 10^6 \frac{\text{mM}}{\text{ms}}, \quad k_{Ca,off} = 5 \cdot 10^3 \text{ Hz}$$

$$q_{Na} = 0.5224, \quad q_{Ca} = 0.1670$$

$$h_{Ca} = \exp\left(\frac{q_{Ca} V_F}{RT}\right), \quad h_{Na} = \exp\left(\frac{q_{Na} V_F}{RT}\right),$$

$$h_1 = 1 + \frac{[Na^+]_Y}{k_{Na3}} (1 + h_{Na})$$

$$h_2 = \frac{[Na^+]_Y \cdot h_{Na}}{k_{Na3} \cdot h_1}$$

$$h_3 = \frac{1}{h_1}$$

$$h_4 = 1 + \frac{[Na^+]_Y}{k_{Na1}} \left(1 + \frac{[Na^+]_Y}{k_{Na2}} \right)$$

$$h_5 = \frac{[Na^+]_Y^2}{k_4 \cdot k_{Na1} \cdot k_{Na2}}$$

$$h_6 = \frac{1}{h_4}$$

$$h_7 = 1 + \frac{[Na^+]_o}{k_{Na3}} \left(1 + \frac{1}{h_{Na}} \right)$$

$$h_8 = \frac{[Na^+]_o}{k_{Na3} \cdot h_{Na} \cdot h_7}$$

$$h_9 = \frac{1}{h_7}$$

$$h_{10} = k_{asymm} + 1 + \frac{[Na^+]_o}{k_{Na1}} \left(1 + \frac{[Na^+]_o}{k_{Na2}} \right)$$

$$h_{11} = \frac{[Na^+]_o^2}{h_{10} \cdot k_{Na1} \cdot k_{Na2}}$$

$$h_{12} = \frac{1}{h_{10}}$$

$$k_1 = h_{12} \cdot [Ca^{2+}]_o \cdot k_{Ca,on}$$

$$k_2 = k_{Ca,off}$$

$$k_3' = h_9 \cdot \omega_{Ca}$$

$$k_3'' = h_8 \cdot \omega_{NaCa}$$

$$k_3 = k_3' + k_3''$$

$$k_4' = \frac{h_3 \cdot \omega_{Ca}}{h_{Ca}}$$

$$k_4'' = h_2 \cdot \omega_{NaCa}$$

$$k_4 = k_4' + k_4''$$

$$k_5 = k_{Ca,off}$$

$$k_6 = h_6 \cdot [Ca^{2+}]_Y \cdot k_{Ca,on}$$

$$k_7 = h_5 \cdot h_2 \cdot \omega_{Na}$$

$$k_8 = h_8 \cdot h_{11} \cdot \omega_{Na}$$

$$x_1 = k_2 \cdot k_4 \cdot (k_7 + k_6) + k_5 \cdot k_7 \cdot (k_2 + k_3)$$

$$x_2 = k_1 \cdot k_7 \cdot (k_4 + k_5) + k_4 \cdot k_6 \cdot (k_1 + k_8)$$

$$x_3 = k_1 \cdot k_3 \cdot (k_7 + k_6) + k_8 \cdot k_6 \cdot (k_2 + k_3)$$

$$x_4 = k_2 \cdot k_8 \cdot (k_4 + k_5) + k_3 \cdot k_5 \cdot (k_1 + k_8)$$

$$E_1 = \frac{x_1}{x_1 + x_2 + x_3 + x_4}$$

$$E_2 = \frac{x_2}{x_1 + x_2 + x_3 + x_4}$$

$$E_3 = \frac{x_3}{x_1 + x_2 + x_3 + x_4}$$

$$E_4 = \frac{x_4}{x_1 + x_2 + x_3 + x_4}$$

$$K_{mCaAct} = 150 \cdot 10^{-6} \text{ mM}$$

$$allo_Y = \frac{1}{1 + \left(\frac{K_{mCaAct}}{[Ca^{2+}]_Y} \right)^2}$$

$$J_{NaCa,Na,Y} = 3 \cdot (E_4 \cdot k_7 - E_1 \cdot k_8) + E_3 \cdot k_4'' - E_2 \cdot k_3''$$

$$J_{NaCa,Ca,Y} = E_2 \cdot k_2 - E_1 \cdot k_1$$

$$z_{Na} = 1 ; z_{Ca} = 2 ;$$

$$I_{NaCa,i} = G_{NaCa} \cdot 0.8 \cdot allo_i \cdot (z_{Na} \cdot J_{NaCa,Na,i} + z_{Ca} \cdot J_{NaCa,Ca,i})$$

$$I_{NaCa,ss} = G_{NaCa} \cdot 0.2 \cdot allo_{ss} \cdot (z_{Na} \cdot J_{NaCa,Na,ss} + z_{Ca} \cdot J_{NaCa,Ca,ss})$$

$$I_{NaCa} = I_{NaCa,i} + I_{NaCa,ss}$$

Sodium-Potassium ATPase Current (I_{NaK})

from (O'Hara et al. 2011)

$$k_1^+ = 949.5 \text{ Hz}; \quad k_1^- = 182.4 \text{ mM}^{-1}; \quad k_2^+ = 687.2 \text{ Hz}; \quad k_2^- = 39.4 \text{ Hz}$$

$$k_3^+ = 1899 \text{ Hz}; \quad k_3^- = 79300 \text{ Hz} \cdot \text{mM}^{-2}; \quad k_4^+ = 639.0 \text{ Hz}; \quad k_4^- = 40 \text{ Hz}$$

$$K_{\text{Nai}}^0 = 9.073 \text{ mM}; \quad K_{\text{NaO}}^0 = 27.78 \text{ mM}$$

$$\Delta = -01550$$

$$K_{\text{Nai}} = K_{\text{Nai}}^0 \cdot \exp\left(\frac{\Delta \cdot V \cdot F}{3 \cdot R \cdot T}\right); \quad K_{\text{NaO}} = K_{\text{NaO}}^0 \cdot \exp\left(\frac{(1-\Delta) \cdot V \cdot F}{3 \cdot R \cdot T}\right)$$

$$K_{\text{Ki}} = 0.5 \text{ mM}; \quad K_{\text{Ko}} = 0.3582 \text{ mM}$$

$$[\text{MgADP}] = 0.05; \quad [\text{MgATP}] = 9.8$$

$$[K_{\text{MgATP}}] = 1.698 \cdot 10^{-7} \text{ mM}$$

$$[\text{H}^+] = 10^{-7} \text{ mM}$$

$$[\Sigma\text{P}] = 4.2 \text{ mM}$$

$$K_{\text{H,P}} = 1.698 \cdot 10^{-7} \text{ mM}, \quad K_{\text{Na,P}} = 224 \text{ mM}, \quad K_{\text{K,P}} = 292 \text{ mM}$$

$$[\text{P}] = \frac{[\Sigma\text{P}]}{\left(1 + \frac{[\text{H}^+]}{K_{\text{H,P}}} + \frac{[\text{Na}^+]_{\text{i}}}{K_{\text{Na,P}}} + \frac{[\text{K}^+]_{\text{i}}}{K_{\text{K,P}}}\right)}$$

$$\alpha_1 = \frac{k_1^+ \cdot \left(\frac{[\text{Na}^+]_{\text{i}}}{K_{\text{Nai}}}\right)^3}{\left(1 + \frac{[\text{Na}^+]_{\text{i}}}{K_{\text{Nai}}}\right)^3 + \left(1 + \frac{[\text{K}^+]_{\text{i}}}{K_{\text{Ki}}}\right)^2 - 1}$$

$$\beta_2 = k_1^- \cdot [\text{MgADP}]$$

$$\alpha_2 = k_2^+$$

$$\beta_2 = \frac{k_2^- \cdot \left(\frac{[\text{Na}^+]_{\text{o}}}{K_{\text{NaO}}}\right)^3}{\left(1 + \frac{[\text{Na}^+]_{\text{o}}}{K_{\text{NaO}}}\right)^3 + \left(1 + \frac{[\text{K}^+]_{\text{o}}}{K_{\text{Ko}}}\right)^2 - 1}$$

$$\alpha_3 = \frac{k_3^+ \cdot \left(\frac{[K^+]_o}{K_{Ko}}\right)^2}{\left(1 + \frac{[Na^+]_o}{K_{NaO}}\right)^3 + \left(1 + \frac{[K^+]_o}{K_{Ko}}\right)^2 - 1}$$

$$\beta_3 = \frac{k_3^- \cdot [P] \cdot [H^+]}{1 + \frac{[MgATP]}{KMgATP}}$$

$$\alpha_4 = \frac{k_4^+ \cdot [P] \cdot [H^+]}{1 + \frac{[MgATP]}{KMgATP}}$$

$$\beta_4 = \frac{k_4^- \cdot \left(\frac{[K^+]_i}{K_{Ki}}\right)^3}{\left(1 + \frac{[Na^+]_i}{K_{Nai}}\right)^3 + \left(1 + \frac{[K^+]_i}{K_{Ki}}\right)^2 - 1}$$

$$x_1 = \alpha_1 \cdot \alpha_2 \cdot \alpha_4 + \beta_2 \cdot \beta_3 \cdot \beta_4 + \alpha_2 \cdot \beta_3 \cdot \beta_4 + \beta_3 \cdot \alpha_1 \cdot \alpha_2$$

$$x_2 = \alpha_1 \cdot \alpha_2 \cdot \alpha_3 + \beta_1 \cdot \beta_2 \cdot \beta_4 + \alpha_3 \cdot \beta_1 \cdot \beta_4 + \beta_4 \cdot \alpha_2 \cdot \alpha_3$$

$$x_3 = \alpha_2 \cdot \alpha_3 \cdot \alpha_4 + \beta_1 \cdot \beta_2 \cdot \beta_3 + \alpha_4 \cdot \beta_2 \cdot \beta_1 + \beta_1 \cdot \alpha_3 \cdot \alpha_4$$

$$x_4 = \alpha_1 \cdot \alpha_3 \cdot \alpha_4 + \beta_2 \cdot \beta_3 \cdot \beta_4 + \alpha_1 \cdot \beta_2 \cdot \beta_3 + \beta_2 \cdot \alpha_1 \cdot \alpha_4$$

$$E_1 = \frac{x_1}{x_1 + x_2 + x_3 + x_4}$$

$$E_2 = \frac{x_2}{x_1 + x_2 + x_3 + x_4}$$

$$E_3 = \frac{x_3}{x_1 + x_2 + x_3 + x_4}$$

$$E_4 = \frac{x_4}{x_1 + x_2 + x_3 + x_4}$$

$$z_{Na} = 1 ; z_K = 1 ;$$

$$J_{NaK,Na} = 3 (E_1 \cdot \alpha_3 - E_2 \cdot \beta_3)$$

$$J_{NaK,K} = 2 (E_4 \cdot \beta_1 - E_3 \cdot \alpha_1)$$

$$I_{NaK} = G_{NaK} \cdot (z_{Na} \cdot J_{NaK,Na} + z_K \cdot J_{NaK,K})$$

Background currents: I_{NaL} , I_{CaL} , I_{pCa}

from (O'Hara et al. 2011)

$$P_{\text{Nab}} = 3.75 \cdot 10^{-10} \text{ cm/s}; \quad z_{\text{Na}} = 1;$$

$$I_{\text{Nab}} = P_{\text{Nab}} \cdot z_{\text{Na}}^2 \cdot \frac{VF^2}{RT} \cdot \frac{[Na^+]_i \cdot \exp\left(\frac{VFz_{\text{Na}}}{RT}\right) - [Na^+]_o}{\exp\left(\frac{VFz_{\text{Na}}}{RT}\right) - 1}$$

$$P_{\text{Cab}} = 2.5 \cdot 10^{-8} \text{ cm/s}; \quad \gamma_{\text{Cai}} = 1; \quad \gamma_{\text{Cao}} = 0.341; \quad z_{\text{Ca}} = 2;$$

$$I_{\text{Cab}} = P_{\text{Cab}} \cdot z_{\text{Ca}}^2 \cdot \frac{VF^2}{RT} \cdot \frac{\gamma_{\text{Cai}} \cdot [Ca^{2+}]_i \cdot \exp\left(\frac{VFz_{\text{Ca}}}{RT}\right) - \gamma_{\text{Cao}} \cdot [Ca^{2+}]_o}{\exp\left(\frac{VFz_{\text{Ca}}}{RT}\right) - 1}$$

$$G_{\text{pCa}} = 0.0005 \text{ mS/uF};$$

$$I_{\text{pCa}} = G_{\text{pCa}} \cdot \frac{[Ca^{2+}]_{sl}}{0.0005 + [Ca^{2+}]_{sl}}$$

Calcium/Calmodulin-Dependent Protein Kinase (CaMK)

from (O'Hara et al. 2011)

$$\alpha_{\text{CaMK}} = 0.05 \text{ ms}^{-1}; \quad \beta_{\text{CaMK}} = 0.00068 \text{ ms}^{-1};$$

$$\text{CaMK}_0 = 0.05; \quad K_{\text{mCaM}} = 0.0015 \text{ mM}$$

$$\text{CaMK}_{\text{bound}} = \text{CaMK}_0 \cdot \frac{1 - \text{CaMK}_{\text{trap}}}{1 + \frac{K_{\text{mCaM}}}{[Ca^{2+}]_{ss}}}$$

$$\text{CaMK}_{\text{active}} = \text{CaMK}_{\text{bound}} + \text{CaMK}_{\text{trap}}$$

$$\frac{d\text{CaMK}_{\text{trap}}}{dt} = \alpha_{\text{CaMK}} \cdot \text{CaMK}_{\text{bound}} \cdot (\text{CaMK}_{\text{bound}} + \text{CaMK}_{\text{trap}}) - \beta_{\text{CaMK}} \cdot \text{CaMK}_{\text{trap}}$$

Sarcoplasmic Reticulum Ca^{2+} Fluxes

from in (P. Li and Rudy 2011)

- **RyR3**

$$\text{Rel}_{\text{RyR3}} = -\left(I_{\text{CaL}} \cdot \frac{A_{\text{Cap}}}{V_{\text{SS}} \cdot 2 \cdot F} - (J_{\text{RyR3}} + J_{\text{IP3R}})\right) \cdot \frac{V_{\text{JSR}}}{V_{\text{SS}}} + J_{\text{diff1}}$$

$$\tau_{\text{RyR3}} = \frac{2 \cdot \left(1 + \frac{1}{1 + \left(\frac{0.28}{[\text{CaMK}_{\text{active}}]}\right)^8}\right)}{1 + \frac{0.0123}{[\text{Ca}^{2+}]_{\text{JSR}}}}$$

If ($\text{Rel}_{\text{RyR3}} > 0$)

$$\text{RyR3}_{\infty} = \frac{15 \cdot \text{Rel}_{\text{RyR3}} \cdot \left(1 + \frac{1}{1 + \left(\frac{0.28}{[\text{CaMK}_{\text{active}}]}\right)^8}\right)}{1 + \left(\frac{1}{[\text{Ca}^{2+}]_{\text{JSR}}}\right)^8}$$

else

$$\text{RyR3}_{\infty} = 0$$

$$\frac{dJ_{\text{RyR3}}}{dt} = \frac{\text{RyR3}_{\infty} - J_{\text{RyR3}}}{\tau_{\text{RyR3}}}$$

- **RyR2**

$$\text{Rel}_{\text{RyR2}} = -J_{\text{SERCA}} \cdot \frac{V_{\text{NSR}}}{V_{\text{Myo}}} + J_{\text{diff2}} \cdot \frac{V_{\text{SL}}}{V_{\text{Myo}}} + J_{\text{RyR2}} \cdot \frac{V_{\text{CSR}}}{V_{\text{Myo}}}$$

$$\tau_{\text{RyR2}} = \frac{6 \cdot \left(1 + \frac{1}{1 + \left(\frac{0.28}{[\text{CaMK}_{\text{active}}]}\right)^8}\right)}{1 + \frac{0.0123}{[\text{Ca}^{2+}]_{\text{CSR}}}}$$

If ($\text{Rel}_{\text{RyR2}} > 0$)

$$RyR2_{\infty} = \frac{91 \cdot Rel_{RyR2} \cdot \left(1 + \frac{1}{1 + \left(\frac{0.28}{[CaMK_{active}]}\right)^8}\right)}{1 + \left(\frac{1}{[Ca^{2+}]_{CSR}}\right)^8}$$

else

$$RyR2_{\infty} = 0$$

$$\frac{dJ_{RyR2}}{dt} = \frac{RyR2_{\infty} - J_{RyR2}}{\tau_{RyR2}}$$

- **IP₃R Ca²⁺ release:**

$$k_0 = 96000 \text{ mM}^{-1}\text{s}^{-1}; k_{0a} = 9.6 \text{ s}^{-1}; k_1 = 150000 \text{ mM}^{-1}\text{s}^{-1}; k_{1a} = 16.5 \text{ s}^{-1}$$

$$k_2 = 1800 \text{ mM}^{-1}\text{s}^{-1}; k_{2a} = 0.21\text{s}^{-1}; \tau_{IP3R} = 3.7\text{s}^{-1}$$

$$[IP_3] = 0.001 \text{ mM/L};$$

$$\frac{du_{IP3R}}{dt} = [Ca^{2+}]_{SS} \cdot k_2 \cdot (1 - u_{IP3R}) - k_{2a} \cdot u_{IP3R}$$

$$J_{IP3R} = 10.92 \cdot \frac{\tau_{IP3R} \cdot [IP_3] \cdot [Ca^{2+}]_{SS} \cdot (1 - u_{IP3R})}{\left(1 + \frac{[IP_3] \cdot k_0}{k_{0a}}\right) \cdot \left(1 + [Ca^{2+}]_{SS} \frac{k_1}{k_{1a}}\right)} ([Ca^{2+}]_{JSR} - [Ca^{2+}]_{SS})$$

- **Ca²⁺ uptake via SERCA:**

$$\Delta K_{0m,PLB} = 0.00017 \frac{mM}{L}; \quad \Delta J_{0,SERCA,CAMK} = 0.75; \quad K_{m,CAMK} =$$

$$0.15;$$

$$J_{0,SERCA,1} = 0.0002 \frac{mM}{L}/ms; J_{0,SERCA,2} = 0.0026 \frac{mM}{L}/ms; K_{m,SERCA} = 0.00028 \frac{mM}{L};$$

$$\overline{NSR} = 15 \frac{mM}{L}$$

$$\Delta K_{m,PLB} = \Delta K_{0m,PLB} \cdot \frac{CAMK_{active}}{K_{m,CAMK} + CAMK_{active}}$$

$$\Delta J_{SERCA,CAMK} = \Delta J_{0,SERCA,CAMK} \cdot \frac{CAMK_{active}}{K_{m,CAMK} + CAMK_{active}}$$

$$J_{SERCA,1} = J_{0,SERCA,1} \cdot \frac{(1 + \Delta J_{SERCA,CAMK})}{1 + \frac{K_{m,SERCA} - \Delta K_{m,PLB}}{[Ca^{2+}]_{SL}}} - 0.0042 \cdot \frac{[Ca^{2+}]_{NSR}}{NSR}$$

$$J_{SERCA,2} = J_{0,SERCA,2} \cdot \frac{(1 + \Delta J_{SERCA,CAMK})}{1 + \frac{K_{m,SERCA} - \Delta K_{m,PLB}}{[Ca^{2+}]_i}} - 0.00105 \cdot \frac{[Ca^{2+}]_{NSR}}{NSR}$$

Diffusion Fluxes

$$\tau_{tr} = 120 \text{ ms}$$

$$J_{tr,1} = \frac{([Ca^{2+}]_{NSR} - [Ca^{2+}]_{JSR})}{\tau_{tr}}; \quad J_{tr,2} = \frac{([Ca^{2+}]_{NSR} - [Ca^{2+}]_{CSR})}{\tau_{tr}}$$

$$\tau_{diff1} = 0.2 \text{ ms}; \quad \tau_{diff2} = 12 \text{ ms}$$

$$J_{diff1,Ca} = \frac{([Ca^{2+}]_{SS} - [Ca^{2+}]_{SL})}{\tau_{diff1}}; \quad J_{diff2,Ca} = \frac{([Ca^{2+}]_{SL} - [Ca^{2+}]_i)}{\tau_{diff2}}$$

$$J_{diff1,Na} = \frac{([Na^+]_{SS} - [Na^+]_{SL})}{\tau_{diff1}}; \quad J_{diff2,Na} = \frac{([Na^+]_{SL} - [Na^+]_i)}{\tau_{diff2}}$$

$$J_{diff1,K} = \frac{([K^+]_{SS} - [K^+]_{SL})}{\tau_{diff1}}; \quad J_{diff2,K} = \frac{([K^+]_{SL} - [K^+]_i)}{\tau_{diff2}}$$

Ionic Concentrations

- **$[Ca^{2+}]_{SS}$**

$$\beta_{PCS} = \frac{1}{1 + \overline{BSR} \cdot \frac{K_{m,BSR}}{([Ca^{2+}]_{SS} + K_{m,BSR})} + \overline{BSL} \cdot \frac{K_{m,BSL}}{([Ca^{2+}]_{SS} + K_{m,BSL})}}$$

$$\frac{d[Ca^{2+}]_{SS}}{dt} = \beta_{PCS} \cdot (- (I_{CaL} - 2 \cdot I_{NaCa,SS}) \cdot \frac{A_{Cap}}{V_{PCS} \cdot 2 \cdot F} + (J_{RyR2} + J_{IP3R}) \cdot \frac{V_{JSR}}{V_{PCS}} - J_{diff1})$$

- **$[Ca^{2+}]_{SL}$**

$$\begin{aligned} \frac{d[Ca^{2+}]_{SL}}{dt} = & - (I_{CaT} + I_{pCa} + I_{Cab} - 2 \cdot I_{NaCa,SL}) \cdot \frac{A_{Cap}}{V_{SL} \cdot 2 \cdot F} + J_{diff1} \cdot \frac{V_{SS}}{V_{SL}} - J_{SERCA,1} \cdot \frac{V_{NSR}}{V_{SL}} \\ & - J_{diff2} \end{aligned}$$

$$TRPN_{SL} = \overline{TRPN}_{SL} \cdot \frac{[Ca^{2+}]_{SL}}{[Ca^{2+}]_{SL} + K_{m,TRPN}}$$

$$CMDN_{SL} = \overline{CMDN}_{SL} \cdot \frac{[Ca^{2+}]_{SL}}{[Ca^{2+}]_{SL} + K_{m,CMDN}}$$

$$[Ca^{2+}]_{SL,tot} = [Ca^{2+}]_{SL} + TRPN_{SL} + CMDN_{SL} + d[Ca^{2+}]_{SL}$$

$$b_{SL} = \overline{TRPN}_{SL} + \overline{CMDN}_{SL} - [Ca^{2+}]_{SL,tot} + K_{m,TRPN} + K_{m,CMDN}$$

$$\begin{aligned} c_{SL} = & K_{m,TRPN} \cdot K_{m,CMDN} - [Ca^{2+}]_{SL,tot} \cdot (K_{m,TRPN} + K_{m,CMDN}) + \overline{TRPN}_{SL} \cdot K_{m,CMDN} \\ & + \overline{CMDN}_{SL} \cdot K_{m,TRPN} \end{aligned}$$

$$d_{SL} = -K_{m,TRPN} \cdot c_{SL} \cdot K_{m,TRPN} \cdot [Ca^{2+}]_{SL,tot}$$

$$[Ca^{2+}]_{SL} = \frac{2}{3} \cdot \sqrt{b_{SL}^2 - 3 \cdot c_{SL}} \cdot \cos \left(\frac{1}{3} \cos^{-1} \left(\frac{9b_{SL}c_{SL} - 2b_{SL}^3 - 27d_{SL}}{2(b_{SL}^2 - 3c_{SL})^{1.5}} \right) \right) - \frac{b_{SL}}{3}$$

- **$[Ca^{2+}]_i$**

$$\frac{d[Ca^{2+}]_i}{dt} = J_{diff2} \cdot \frac{V_{SL}}{V_{Myo}} - J_{SERCA,2} \cdot \frac{V_{NSR}}{V_{Myo}} + J_{RyR2} \cdot \frac{V_{CSR}}{V_{Myo}}$$

$$TRPN_{Myo} = \overline{TRPN_{Myo}} \cdot \frac{[Ca^{2+}]_i}{[Ca^{2+}]_i + K_{m,TRPN}}$$

$$CMDN_{Myo} = \overline{CMDN_{Myo}} \cdot \frac{[Ca^{2+}]_i}{[Ca^{2+}]_i + K_{m,CMDN}}$$

$$[Ca^{2+}]_{i,tot} = [Ca^{2+}]_i + TRPN_{Myo} + CMDN_{Myo} + d[Ca^{2+}]_i$$

$$b_{Myo} = \overline{TRPN_{Myo}} + \overline{CMDN_{Myo}} - [Ca^{2+}]_{i,tot} + K_{m,TRPN} + K_{m,CMDN}$$

$$c_{Myo} = K_{m,TRPN} \cdot K_{m,CMDN} - [Ca^{2+}]_{i,tot} \cdot (K_{m,TRPN} + K_{m,CMDN}) + \overline{TRPN_{Myo}} \cdot K_{m,CMDN} \\ + \overline{CMDN_{Myo}} \cdot K_{m,TRPN}$$

$$d_{Myo} = -K_{m,TRPN} \cdot c_{Myo} K_{m,TRPN} \cdot [Ca^{2+}]_{i,tot}$$

$$[Ca^{2+}]_i = \frac{2}{3} \sqrt{b_{Myo}^2 - 3 \cdot c_{Myo}} \cos \left(\frac{1}{3} \cos^{-1} \left(\frac{9b_{Myo}c_{Myo} - 2b_{Myo}^3 - 27d_{Myo}}{2(b_{Myo}^2 - 3c_{Myo})^{1.5}} \right) \right) \\ - \frac{b_{Myo}}{3}$$

- **$[Ca^{2+}]_{JSR}$**

$$\frac{d[Ca^{2+}]_{JSR}}{dt} = J_{tr1} - J_{RyR3} - J_{IP3R}$$

$$CSQN_{JSR} = \overline{CSQN_{JSR}} \cdot \frac{[Ca^{2+}]_{JSR}}{[Ca^{2+}]_{JSR} + K_{m,CSQN}}$$

$$b_{JSR} = \overline{CSQN_{JSR}} + CSQN_{JSR} - [Ca^{2+}]_{JSR} + d[Ca^{2+}]_{JSR} + K_{m,CSQN}$$

$$c_{JSR} = K_{m,CSQN} \cdot (CSQN_{JSR} + [Ca^{2+}]_{JSR} + d[Ca^{2+}]_{JSR})$$

$$[Ca^{2+}]_{JSR} = \frac{\sqrt{b_{JSR}^2 - 4 \cdot c_{JSR} - b_{JSR}}}{2}$$

- **$[Ca^{2+}]_{CSR}$**

$$\frac{d[Ca^{2+}]_{JSR}}{dt} = J_{tr2} - J_{RyR2}$$

$$CSQN_{CSR} = \overline{CSQN_{CSR}} \cdot \frac{[Ca^{2+}]_{CSR}}{[Ca^{2+}]_{CSR} + K_{m,CSQN}}$$

$$b_{CSR} = \overline{CSQN_{CSR}} + CSQN_{CSR} - [Ca^{2+}]_{CSR} + d[Ca^{2+}]_{CSR} + K_{m,CSQN}$$

$$c_{CSR} = K_{m,CSQN} \cdot (CSQN_{CSR} + [Ca^{2+}]_{CSR} + d[Ca^{2+}]_{CSR})$$

$$[Ca^{2+}]_{CSR} = \frac{\sqrt{b_{CSR}^2 - 4 \cdot c_{CSR} - b_{CSR}}}{2}$$

- **$[Ca^{2+}]_{NSR}$**

$$\frac{d[Ca^{2+}]_{NSR}}{dt} = J_{SERCA,1} + J_{SERCA,2} - J_{tr1} \cdot \frac{V_{JSR}}{V_{NSR}} - J_{tr2} \cdot \frac{V_{CSR}}{V_{NSR}}$$

- **$[Na^+]_{SS}$**

$$\frac{d[Na^+]_{SS}}{dt} = -(I_{CaNa} + 3 \cdot I_{NaCa,SS}) \cdot \frac{A_{Cap}}{V_{SS} \cdot z_{Na} \cdot F} - J_{diff1,Na}$$

- **$[Na^+]_{SL}$**

$$\begin{aligned} \frac{d[Na^+]_{SL}}{dt} = & -(3 \cdot I_{NaK} + I_{Na} + I_{NaL} + I_{NaCa,i} + I_{fNa} + I_{Nab}) \frac{A_{Cap}}{V_{SL} z_{Na} F} + J_{diff1,Na} \frac{V_{SS}}{V_{SL}} \\ & - J_{diff2,Na} \end{aligned}$$

- **$[Na^+]_i$**

$$\frac{d[Na^+]_i}{dt} = J_{diff2,Na} \cdot \frac{V_{SL}}{V_{Myo}}$$

- $[K^+]_{SS}$

$$\frac{d[K^+]_{SS}}{dt} = -I_{CaK} \cdot \frac{A_{Cap}}{V_{SS} \cdot z_K \cdot F} - J_{diff1,K}$$

- $[K^+]_{SL}$

$$\begin{aligned} \frac{d[K^+]_{SL}}{dt} = & -(I_{to} + I_{sus} + I_{Kr} + I_{Ks} + I_{f,K} + I_{K1} + I_{stim} - 2 \cdot I_{NaK}) \cdot \frac{A_{Cap}}{V_{SL} \cdot z_K \cdot F} + J_{diff1,K} \frac{V_{SS}}{V_{SL}} \\ & - J_{diff2,K} \end{aligned}$$

- $[K^+]_i$

$$\frac{d[K^+]_i}{dt} = -J_{diff2,K} \frac{V_{SL}}{V_{Myo}}$$