PhD Thesis (extract):
Multi-Scale Modeling and Variability in Cardiac Cellular Electrophysiology

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CHAPTER 8

Discussion

Contents

8.1 Introduction

Diagnosis and treatment of cardiac arrhythmias can benefit greatly from a deeper understanding of the mechanisms by which they arise. A wealth of information has been gathered about the fundamental processes involved on the genetic, molecular, cell, tissue, organ, and whole-body levels, and modern clinicians have access to diagnostic methods on each of these scales. Yet to make full use of this information, an *integrative* or *systems approach* is required that combines information from the different levels, and adds information about how processes at the different scales interact (Rudy et al., 2008; Kohl et al., 2010; Noble, 2017). Computational methods, particularly *multi-scale modeling* and *simulation*, have been very useful in this respect. Chapters 3-7 of this thesis each address a different topic, combining information from different scales or developing methods to integrate them (see Fig. **??**). In Chapter **??** we presented *Myokit*, our newly developed toolkit for action potential (AP) model simulation and development. Myokit can be used to create models of ion currents, integrate them into models of the cellular AP, and combine AP models into models of tissue. Chapter **??** then investigated a technique to speed up AP simulations. In Chapter **??** we measured *variability* in the kinetics of the fast sodium current I_{Na} and showed how it could affect the cellular AP. Chapter **??** then described our efforts to establish an *in silico* link from genetic mutations (in *SCN5A*) to current-level changes (in I_{Na}). Finally, in Chapter **??** we used Myokit to perform simplified whole-heart simulations that were used in the *regularization* problem of electrocardiographic imaging (ECGI), thereby making a connection to the whole-body scale. In this chapter, these topics are discussed in the broader context of using a systems approach to understand cardiac electrophysiology and arrhythmogenesis.

By combining multi-scale modeling with experiments, it is possible to link observations at the genetic or molecular scale to higher-level features of the physiology and pathophysiology of the heart. For example, Bébarová et al. (2008) measured I_{Na} through channels encoded by wild-type (WT) and mutated *SCN5A* and used modeling to extrapolate to the single-cell level, transmural myocardium, and the pseudo-ECG. Benson et al. (2008) studied the effects of channel-blocking drugs in simulations of single cells, fibers and 3-dimensional wedges of tissue. These studies investigated hypothesized disease mechanisms, and showed by simulation that certain molecular changes *could* be the cause of observed higher-level effects (e.g., changes to the ECG). Besides this use in evaluating mechanistic hypotheses, modeling also has direct predictive power, as was shown in recent studies into drug-development (Cummins Lancaster and Sobie, 2016) and clinical risk assessment (Hoefen et al., 2012; Arevalo et al., 2016). A schematic 'pipeline' illustrating a common pattern in these studies is shown in Fig. 8.1.

Many other set-ups are possible, for example including single channel function (Clancy and Rudy, 1999; Silva et al., 2009), subcellular detail (Greenstein and Winslow, 2002; Nivala et al., 2012), signaling (Saucerman et al., 2004; Heijman et al., 2011), contraction (Matsuoka

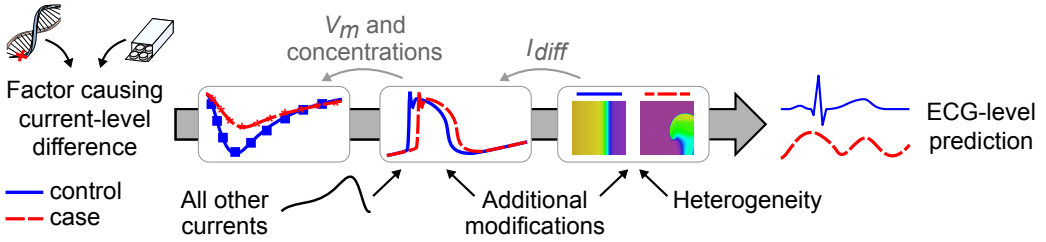

Figure 8.1: A common pattern in multi-scale modeling of cardiac electrophysiology, starting from effects at the ion-current level and building up to the levels of cell, tissue and pseudo-ECG. In the first step, the effect of a mutation, drug or other factor on an ion current is quantified experimentally. This leads to an updated model of the current that is integrated into a model of the cellular AP. At this stage, additional modifications can be made, for example to make the model more specific (e.g., cell type, gender) or to include disease or drug-induced changes (e.g., altered current densities and ionic concentrations). Next, a tissue-level model is constructed. At this level, heterogeneity in cell properties or tissue geometry can be added. Information travels from the lower to the higher scales, but there is also feedback in the form of the membrane potential (V_m), the ionic concentrations, and the diffusion current between cells (I_{diff}). Finally, a pseudo-ECG is calculated from the simulated tissue-level results.

et al., 2003; Cortassa et al., 2006), and 3-dimensional geometries (Panfilov and Holden, 1993; Gharaviri et al., 2012; Gurev et al., 2015). However, there are several challenges that need to be overcome before we can fully exploit the potential of these techniques.

Firstly, the relationship between diseases and molecular factors (such as ion channel subunits, channel-blocking drugs or compounds involved in signaling) is complex. While the successes of genetics and the advent of highly specific targeted drugs have occasionally led people to view (patho)physiology in terms of molecular factors, diseases themselves "represent emergent properties at the scale of the organism that result from dynamic interactions between multiple constantly changing molecular factors" (Weiss et al., 2015). In terms of Fig. 8.1, the exact change seen on the left is less important than how the altered current interacts with the other currents to form the AP, and arrhythmogenesis is best described in terms of higher-level emergent properties such as elongation of the AP or repolarization reserve[1] (Roden, 2008). A good example of this complexity is I_{Na}, where a single mutation in *SCN5A* (resulting in a single molecular-level change) can cause several distinct clinical phenotypes (Remme, 2013), where drugs targeting the channel are powerful but unpredictable (Remme and Wilde, 2014), and where pathogenicity predictions are still unreliable (see Leong et al., 2015, and Chapter **??** of this thesis).

Secondly, and related strongly to the previous point, is the fact that arrhythmias typically do not have a *single* cause. Instead, they require both a *vulnerable substrate*, (a specific set of potentially dangerous conditions) and a *trigger* (some spontaneous internal or external event that sets the arrhythmia off). The substrate is likely to be a combination of factors such as mutations, changes in ionic concentrations, or structural and electrical remodeling.

[1] A possible analogy is trying to study a conflict by focusing on the individual sides, despite the fact that crucial concepts like 'disagreement' and 'escalation' do not exist at the single-person level.

This need for multiple coacting factors to create the conditions for an arrhythmic event complicates diagnosis (top-down prediction) and risk-assessment (bottom-up prediction). For example, recreating the trigger in a clinical setting might not recreate the arrhythmia if the right substrate is not present (so that stress testing may be necessary). The trigger itself can even be an otherwise innocuous and common occurrence (even the sudden ringing of an alarm clock, see Wellens et al., 1972). Conversely, individual aspects of a vulnerable substrate, including rare mutations in ion-channel genes, can occur in otherwise healthy individuals without causing an arrhythmia. In Fig. 8.1 this is shown by the necessary introduction of additional modifications and heterogeneity at the cell and tissue levels.

The third and final complication discussed here, is the existence of variability between subjects, variability in a single subject over time, and even cell-to-cell variability within a single subject. Well-known examples of biological variability are outward appearance, the shape of the heart, and even the shape and size of individual myocytes. But variability extends beyond structural differences, and is also evident in the electrical properties of the heart (see also Chapter **??**). A review by Marder and Goaillard (2006) presented strong evidence showing that major variability occurs in expression levels of neuronal ion channels, which correlate directly with the densities of the associated ion currents (Schulz et al., 2006). This level of variability is remarkable, as even small changes in the strength of ionic currents can have severe consequences in both neurons and myocytes. At the same time, some variation is inevitable as cells are not static entities but rebuild themselves constantly. For example, the channels carrying I_{Na} and I_{Kr} are replaced every 35 hours and 10 hours respectively (Maltsev et al., 2008; Vandenberg et al., 2012). A cardiac modeling study by Sarkar and Sobie (2010) showed that, despite a cardiomyocyte's sensitivity to changes in ion channel expression, a large degree of variability in expression is possible, provided it is *compensated* by changes to the other currents. As Marder and Goaillard explain, the ability of a cell to regulate its electrical function leads to a situation where parameters sensitive to sudden small changes can drift slowly but dramatically with time. In other words, as long as the cell can keep compensating, even 'sensitive parameters' can show large variation without apparent consequence. Weiss et al. (2012) pointed out the impact this has on understanding arrhythmogenesis: if, for example, repolarization in one patient's myocytes depends strongly on I_{Kr} while the same current plays only a small part in another, the two patients will have very different risks of arrhythmogenesis when administered I_{Kr}-blocking drugs. As a result, clinical treatment should not focus on 'fixing' specific currents, but on restoring dynamical phenomena such as repolarization (Weiss et al., 2015). In terms of trigger and substrate, the existence of strong variability in the mechanisms underlying the cellular AP implies that the substrate of patients with similar histories and genetic backgrounds can still be very different, and may even change over time.[2] In Fig. 8.1 variability can be *eliminated* by

[2] Besides complicating the analysis of arrhythmias, variability may confer an evolutionary advantage by allowing individuals to adapt (if changes are slow) or parts of the population to survive (if changes are fast). In other words, the idea that myocytes can function in different configurations is consistent with the idea of

adding modifications to create a patient-specific model, or it can be *included in the models* using techniques discussed in Section 8.4.

With these issues in mind, we now discuss multi-scale modeling of the cardiac AP, show where the work presented in this thesis can help increase the utility of multi-scale modeling for cardiology, and highlight future challenges.

8.2 Multi-scale modeling and simulation

Simulation with multi-scale models allows the interaction between dynamical processes in the heart to be explored. This means it can be used to study the cell-level properties that emerge when ion channels are coupled by a cell membrane, but also the tissue-level properties that emerge when cells are coupled by gap junctions and extracellular conduction. The view that diseases themselves represent such emergent properties implies that simulation is a crucial tool for the study of arrhythmogenesis. In contrast to experimental studies, computational studies allow complex arrhythmogenic substrates to be modeled and perfectly controlled (but see Section 8.3 for important caveats). Current applications of AP-model based simulation range from theoretical and fundamental ('basic') research to drug discovery and risk prediction in a clinical setting. Understanding and incorporating variability into these models is a challenge for the future (see Section 8.4). In this section, simulation and modeling at the different scales encountered in this thesis are discussed.

8.2.1 Linking genes to channels and currents

Multi-scale investigations of genetic defects in ion-channel genes commonly start with electrophysiological experiments to quantify the mutation's effects on the whole-cell current, after which the investigation continues *in silico*. Experiments can focus on the pore-forming α-subunit, but also on auxiliary β-subunits or genes for the many gene-products that bind to and interact with the macromolecular complex that forms the ion channel. Replacing this laborious experimental step with a computational approach could be both cost-effective and greatly increase the scale at which such work could take place.

In Chapter **??**, we attempted to predict the change in I_{Na} due to a mutation in *SCN5A*, the gene encoding its pore-forming α-subunit. Using machine-learning techniques and a database of mutations with known effects, we showed that the absence or presence of particular changes could be predicted with better-than-chance accuracy. While we showed that the out-of-the-box accuracy of machine-learning methods on this database surpassed that of commercially available direct pathogenicity predictors, the accuracy was still low. For example, while presence of inactivation defects could be predicted with 70% accuracy, this was only slightly better than the 64% accuracy obtained by simply always guessing the most

using *redundancy* to create robustness.

common outcome. However, other measures such as the area under the curve (AUC) were improved considerably, suggesting the method may still hold promise for the future. More work in this area is required, particularly into decreasing bias in the data set by adding mutations with very small or very large effects (i.e., mutations that can easily go unnoticed and mutations incompatible with life, see Section **??**). Another application of this idea would be to create a similar database for a different gene such as *KCNQ1*, for which clinical-phenotype predictions are known to be more accurate (Leong et al., 2015), which suggests that current-level predictions may be more accurate too.

An alternative approach is to model the channel behavior directly, using molecular dynamics (MD) simulations. With MD, all atoms in an ion channel can be modeled, along with considerable stretches of nearby membrane. Such simulations are well-developed, and have been the subject of much research: a very extensive review was given by Roux et al. (2004). However, these simulations cannot determine the 3-dimensional structure of the folded channel, which must instead be determined using crystallization of isolated channels. Once the structure for one channel is known, estimates for similar channels can be obtained with *homology modeling*. This technique can also be used to introduce mutations into the model, but as it starts from a fully formed channel it can not predict issues with transport or folding. Due to their computational complexity, MD simulations are limited to very short time scales i.e., 'tens of nanoseconds' rather than the milliseconds, seconds, and minutes typical in AP modeling (Southern et al., 2008). This means that, even with the expected increase in computing power, determining the effects of mutations on channel function *ab initio* is still a distant prospect (Silva and Rudy, 2010).

As a result, building models of ion currents based on whole-cell patch-clamp data remains a highly relevant and challenging task for the foreseeable future. Two recent developments worth mentioning in this context are automated patch-clamping and human induced pluripotent stem cells (hiPSC). With hiPSC, it is possible to culture a line of cells that can be made to *differentiate* into myocyte-like cells that can be clamped and measured (possibly with the addition of 'artificial' currents using *dynamic clamp*, see Meijer van Putten et al., 2014). Ma et al. (2013) have even obtained stem-cells from a patient and a sibling, and used these to study a mutation in a patient-specific setting. The benefit over cardiomyocytes is that these cells can be cultured, so that experimental work does not necessarily require the highly invasive clinical procedures needed to obtain cardiomyocytes from patients.

In the past decade, automated patch-clamp systems have been developed and used in safety testing and drug discovery (Stoelzle et al., 2011). With such systems, 'basic' patch-clamp protocols can be run on larger numbers of cells than with a traditional patch-clamp system (although expertise is still needed from the experimenter), but they can also be used to perform more complicated measurements such as recording late I_{Na} (Chevalier et al., 2014). An interesting future prospect is to improve the throughput and success rate of such devices

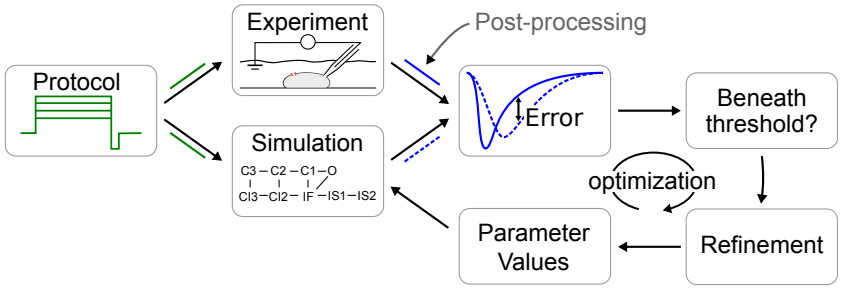

Figure 8.2: Fitting an ion current model to patch-clamp data using the whole-trace method. A single protocol is applied to a real cell and a simulated cell and (after post-processing of the experimental data) the results are compared. An optimization method is used to iteratively refine the parameter values and re-run the simulation until the error is below a preset threshold.

by using optimized protocols such as described in Clerx et al. (2015) and Fink and Noble (2009), followed by a robust model fitting routine (Loewe et al., 2016). Conventional electrophysiological values such as time constants and midpoints of (in)activation could then be obtained from the fitted models using simulation.

8.2.1.1 Simulation and fitting models of ionic currents

Traditional analysis of voltage-clamp and patch-clamp involves measuring quantities such as peak magnitude and time-to-peak, and using these to derive measures such as midpoint of activation. Several studies have pointed out that this does not use all the information in the measured signals and is more prone to errors than using *whole-trace fitting* (Hafner et al., 1981; Willms et al., 1999; Lee et al., 2006; Buhry et al., 2011). A schematic overview of a whole-trace model-fitting routine is shown in Fig. 8.2. A protocol is created and applied to a cell. Next, the same protocol is used in a simulation based on some model of the current, and the results are compared, resulting in some measure of the error, or 'score'. Finally, an optimization method is used that iteratively refines the model's parameter values and re-runs the simulation until the error is below some preset minimum.

With the exception of the experiment, all these steps can be handled within Myokit. The simulation step can be performed using any of its simulation engines (see Chapter **??**). For very fast simulations with Markov models, the simulation engine based on eigenvalue decomposition can be used, but if non-linear effects need to be included the CVODE engine can be used instead (as was done in Chapter **??** to incorporate membrane charging time). As a future step, it may be possible to integrate Myokit's multi-cell GPU simulation engine with the parameter estimation routine, allowing large numbers of simulations to be run in parallel and potentially speeding up the parameter estimation process.

In addition to the standard simulation classes, Myokit contains an advanced simulation engine that uses automatic differentiation to calculate partial derivatives of the state and

other variables with respect to one or more parameters. This can be used to run *local identifiability checks* (Cobelli and DiStefano, 1980; Fink et al., 2008). Using such checks, we can provide a partial[3] answer to the question 'does applying this patch-clamp protocol give us the information we need to find a unique set of parameter values that provide the best fit?' This can be used to check the validity of patch-clamp protocols. When investigating variability, such a protocol-checking method is vital to ensure the observed variability is not a result of the experimental set-up (see also Chapter **??**).

In addition, identifiability checking can be used to *optimize* protocols, reducing their runtime while ensuring they provide the necessary information (Fink and Noble, 2009). In Clerx et al. (2015) we use this method to create a very short (260ms) protocol to extract all the information needed to fit the I_{Na} model by Clancy and Rudy (2002). While more work is needed to refine these methods, such optimized protocols hold great promise for the study of ion-channel behavior using whole-cell patch-clamp experiments. For example, when studying mutations, the method could be used to quickly train a model to a mutated current, and then remaining experiments could be run *in silico*. Alternatively, if the protocol is constantly re-run while a channel-blocking drug is applied, it could be used to study the mechanism by which the drug affects the channel (by inspecting which parameters change at which time). However, as shown in Clerx et al. (2015), this may first require improvements in our models of cardiac I_{Na}. Another area where improvements can be made is in the development or refinement of optimization methods that deal well with ion-current fitting problems. Such methods should accept a score function without derivatives, be fast, robust and capable of dealing with noise.

8.2.2 Cells, coupled cells and tissue

Once ion-current models have been defined, they can be grouped into cell models, and cell models can then be coupled to create tissue models (see Chapter **??**). This has a wide range of well-established applications, including single-cell simulation, small and large-scale simulation of (heterogeneous) tissue (see Chapter **??**), and whole-heart modeling (see Chapter **??**). More recent applications include simulation of cell-to-cell variability (see Chapter **??**) and detailed modeling of subcellular ionic concentrations. In this section, these applications are briefly discussed and Myokit's established and unexplored capabilities are reviewed.

Myokit includes a GPU-accelerated ODE solver for multi-cellular simulations (Chapter **??** and Chapter **??**). By default, this simulation engine assumes homogeneous cells, connected in a rectangular grid as shown in Fig. 8.3.A. Such simulations can be used to study (altered) conduction velocities or spiral waves on homogeneous tissue (Fenton et al., 2002). In

[3] Because the method uses first-order derivatives evaluated at an initial set of parameter values, a positive result is only valid for nearby points in the parameter space. A negative result does imply the model is not globally identifiable.

Myokit, any model parameter can be varied between cells (limited only by the amount of memory in the hardware) and the connection strength between any two cells can be specified individually. This allows the same engine to be used to model heterogeneous networks of cells or patches of tissue in 1, 2 or 3 dimensions, as shown in Fig. 8.3.B. Allowing heterogeneity opens up a wide range of possibilities, including transmural strands (Bébarová et al., 2008), heterogeneous tissue (Panfilov and Vasiev, 1991; Ten Tusscher and Panfilov, 2003), irregular, three-dimensional geometries (Chapter **??**), and models including fibrosis and structural or functional reentry. If *no* connections are specified, the engine can be used to simulate large numbers of cells in parallel, as shown in Fig. 8.3.C. This can be useful to explore the influence of a parameter over a wide range, which can be more informative than looking at derivatives especially when large changes or strong non-linear behavior is involved. By varying multiple parameters at once, this method could also be used to perform 'population of models' studies (Muszkiewicz et al., 2016) (although it is possible that multiple runs of the faster single-cell simulation engine may still provide better performance, especially when long pre-pacing periods are required). Another feasible use that we have yet to explore in detail, is the modeling of heterogeneously coupled networks of cells as shown in Fig. 8.3.D. Such studies can provide insights into the role of heterogeneous gap-junction expression in arrhythmogenesis (Prudat and Kucera, 2014). From a computational point of view, this is essentially the same situation as in Fig. 8.3.B. A relatively new development in cellular AP modeling is the use of models with large numbers of subcellular elements, as shown in Fig. 8.3.E. These simulations are typically aimed at exploring intracellular calcium waves or sparks, which can cause spontaneous contraction (Nivala et al., 2012; Voigt et al., 2014). It may be possible to use Myokit's multi-cell simulation by replacing the cell models with subcellular compartment models, and reinterpreting the variables used to represent gap-junction currents as intracellular diffusion. A further extension on this scheme would be to couple multiple, subcellularly detailed, cell models together into a system for studying the propagation (or lack of propagation) of spontaneously induced calcium waves. This is visualized in Fig. 8.3.F. Both types of simulation form a viable target for further Myokit development, but may require the introduction of stochastic variables in cell models (which are currently not supported) and the use of multiple models within the same simulation.

Some preliminary work towards *mixed-model* simulation in Myokit has been performed. Fig. 8.4 shows a simulation of propagation across the Purkinje-ventricular junction, modeled using a Purkinje cell model and a ventricular cell model. Such simulations have been used to study the conditions under which slowed conduction or conduction-block can arise which can play a part in arrhythmogenesis (Aslanidi et al., 2009). In Myokit, this is implemented using a specialized simulation type which does not yet support heterogeneous cell parameters, customized connection strengths etc. An open question for the future is whether it is possible to adapt the standard multi-cell simulation to allow multiple model types without a loss of performance or an excessive increase in code complexity.

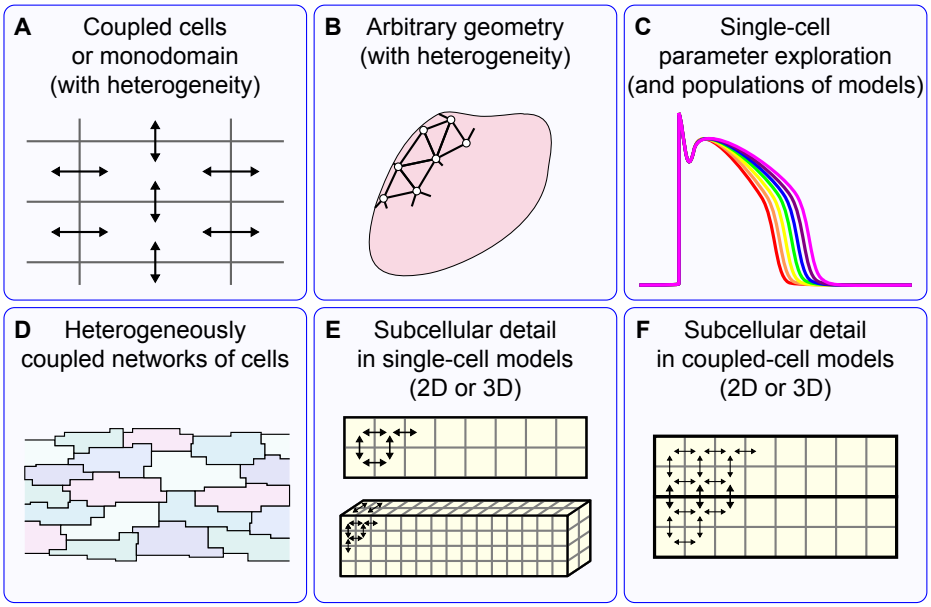

Figure 8.3: Multiple uses of Myokit's multi-cell simulation engine, including realized uses (top row) and potential ones (bottom row). (*A*) Rectangular grids of coupled cells or a rectangularly discretized space simulated with the monodomain equations. Model parameters and cell-to-cell conduction may be varied between cells. (*B*) Arbitrarily complex geometries, created by specifying each cell or node's connections manually. (*C*) Multiple single-cell simulations running in parallel, with different parameters for each cell. Future uses: (*D*) A network (*E*) Simulation of a single cell with a sub-cellular resolution, for example to investigate calcium sparks. (*F*) Like *E* but with multiple cells connected by gap junctions.

Figure 8.4: A simulation of the Purkinje-ventricular junction at $t = 3$ms, $t = 6.5$ms and $t = 18$ms. The Purkinje fiber is formed by 64×32 cells simulated using the model by DiFrancesco and Noble (1985). The ventricular tissue consists of 96×96 cells simulated using the model by Luo and Rudy (1991).

8.2.3 Towards whole-heart simulations

Despite impressive recent examples (Sugiura et al., 2012; Gurev et al., 2015), simulating ever larger parts of the heart is limited by computational power. For this reason, large-scale simulation projects often have a strong focus on performance, rather than versatility. Such projects typically require the model code to be written using the same language as the simulation engine, and may 'tweak' and optimize the model code in many ways (see for example the simulations by Mirin et al. (2012) or the highly efficient CARP simulation engine by Vigmond et al. (2003)). This sacrifice of versatility for performance may be partially circumvented by including an automatic model code generator, as is done in Chaste (Mirams et al., 2013), or even re-generating the model code for every simulation (as is done in Myokit).

One of the core ideas behind Myokit is that models are specified in an easy-to-use model language, and then automatically translated to faster 'low-level' code. This means that, at current, Myokit is unable to use optimizations that require the model equations to be written in a special form, such as the method proposed by Rush and Larsen (1978). However, since Myokit creates a symbolic form of the equations when parsing a model file, it may be possible to implement such optimizations automatically when generating code for large-scale multi-cellular simulations (similar to what was shown in Chapter **??**). Similarly, other ways of automated model adaption could be investigated, for example using model order reduction to simplify Markov models of ion currents. Future work will need to determine if this is a worthwhile investment of resources or if it is more efficient to use Myokit up to a point, and then export the model to a format usable with existing high-performance software.

A different method to scale up simulations is by working towards better *model integration*: instead of simply linking smaller models together, models can be created that contain part of, but not all of, smaller models. In a way, this implies model simplification: deciding which details are absolutely necessary and which can be omitted for a particular simulation. Examples of details that can and have been modeled but may often be omitted include stochastic channel behavior (Heijman et al., 2013), subcellular calcium gradients (Greenstein and Winslow, 2002) and voltage-sensitivity of gap junctions (Gros and Jongsma, 1996). Some models have gone even further, and grouped currents together or omitted them completely (Courtemanche et al., 1990; Fenton and Karma, 1998; Bernus et al., 2002). By omitting detail from the AP, it becomes possible to build models of much larger spatial structures that incorporate new details such as fiber orientation, geometrical structure or communication between sub-models (for example propagation from the AV node to the atrium). For example, Fenton and Karma (1998) found that simplified AP models allowed them to study propagation through 3D anisotropic tissue. The model of the human right atrium by Podziemski and Żebrowski (2013) also uses simplified cell models, but this allows it to

include models for both the SA and AV node. Balakrishnan et al. (2015) used simplified AP models to create a whole-heart model that included the SA node, AV node, bundle of His, Purkinje, atrium and ventricles and that could recreate various arrhythmias. The low computational cost of such models also means they can be used to simulate behavior over longer periods of time, which opens up a road towards modeling long-term processes such as electrical remodeling. At the same time, these models are often (partly) phenomenological, rather than mechanistic, which can make it harder to relate them to experimental data. They also run the risk of missing subtle effects, in situations where the system is highly sensitive to small changes. Nevertheless, if care is taken to avoid these issues, simplified models form an attractive alternative to detailed mechanistic ones.

In Chapter **??**, we used AP models connected in a simplified mesh representing the human ventricles to run simulations used in physiology-based regularization (PBR), a novel method for a crucial step in calculating heart-surface potentials from recorded body-surface potential mappings (ECGI). These simulations used detailed AP models and a patient-specific geometry, but also omitted details, notably the atria and conductive system. In addition, we used a simplified geometry with a small number of nodes. However, by comparing it to a more detailed simulation (again, only ventricles, but this time with a 3-dimensional geometry and a much higher number of nodes) we showed that this omission of detail had no consequence for the simulation's use in PBR. In part, this is likely due to the way PBR uses the simulations to generate spatial patterns of activation, which are then used as a basis for reconstructions. This implies that (1) any pattern that can be recreated as a combination of patterns present in the basis does not need to occur in the simulation, and (2) temporal information is mostly lost, so that details of timing in the AP models are not used. Further work is needed to see if simpler methods such as eikonal or graph-based activation models (Wallman et al., 2012) can be used, or if detailed AP models have benefits in more complex situations than studied in Chapter **??**.

8.3 Reliability and reproducibility

A vulnerable substrate for an arrhythmia is composed of multiple factors. This presents an opportunity for modelers: making a change to a model's parameters is a straightforward task, while controlling variables experimentally can be costly, difficult, time-consuming, or physically impossible. At the same time, making multiple changes to a model, often based on imperfect or qualitative information about the substrate, presents a risk for the reliability of the results. Firstly, every changed parameter drives the model further from the healthy-cell situation for which it was parametrized. How can we be confident that the predictions of a model are still valid when using it to *extrapolate* outside of its validated range? Secondly, when changing multiple parameters at once, how can we make sure that other features of the model are not inadvertently lost? And if changes *can* be made without

invalidating the model, doesn't that suggest the model is underconstrained so that there is a danger of *overfitting*?[4] Closely related to the issue of reliability, is that of *reproducibility* of modeling and simulation results. For example, do papers provide all the information needed to recreate the described model validation, or to re-run simulation experiments? And will models by different groups provide the same results? If cardiac modeling is to be used for risk-prediction (such as in the studies by Hoefen et al. (2012); Arevalo et al. (2016) and Cummins Lancaster and Sobie (2016)), these questions of *reliability* need to be addressed.

These concerns are shared by the United States Food and Drug Administration, (FDA) which is now investigating the use of *in silico* prediction of drug arrhythmogenicity (in particular model-based prediction of QT prolongation, see Parekh et al., 2015). Their "Cardiac Modeling" research project[5] focuses almost entirely on VVUQ: *verification* (are the simulation methods mathematically correct and accurate?), *validation* (is the model realistic for the situation being investigated?), and *uncertainty quantification* (what is the error in the input and how will it affect the predictions?). The questions raised above mostly concern validation, although methods for dealing with variability are strongly related to those used in uncertainty quantification (see Pathmanathan et al., 2015; Mirams et al., 2016).

The following subsections each discuss an aspect of validating simulation results, and highlight some approaches taken by the cardiac modeling community to tackle the questions raised above.

8.3.1 Multi-model testing and model comparison

One way of assessing the reliability of a simulation result is by repeating the experiment using a different model and comparing the results (see for example Mann et al. (2016) and the editorial by Gong et al. (2017)). If there is a clear overlap between results from different models, this supports the idea that the changes are physiologically realistic, and do not push the models too far from their validated state. Conversely, a lack of any consensus would indicate that this is an area where models react sensitively to change. If some models *do* produce the intended result, careful work would need to be done to find out if this is due to their greater predictive power, due to the data the models were parametrized with[6], or simply due to chance. Additionally, it may be possible to combine the predictions of multiple models into probability estimates, for example to estimate arrhythmogenicity of a particular situation.

The most straightforward way to perform multi-model investigations is to perform all initial

[4] A saying often quoted in this context, attributed to the physicist John von Neumann, is "With four parameters I can fit an elephant, with five I can make him wiggle his trunk." (Dyson, 2004). This was shown to be true half a century later by Mayer et al. (2010), who cheated slightly by using complex parameters. Perhaps typically, their efforts produce an abstraction that does little justice to elephant physiology.

[5] See http://www.fda.gov/MedicalDevices/ScienceandResearch/ResearchPrograms/ucm477370.htm

[6] In fact, another area where little work has been done is in comparing how well different AP models fit *the same data set*.

experimentation in a single model, establish a test-case, and then double-check by implementing similar changes in other models. With custom written code, this is an arduous task as it involves the implementation of multiple models, possibly written in different programming languages. But by using tools that can import models from exchange formats such as CellML (Cuellar et al., 2003) this can be done with relative ease. A more extensible way is to use a library of models, all written in a standard form or *annotated* in a manner that allows simulation software to automatically identify and modify model variables. Myokit can be used for both manual and automated model comparison, as is shown in the example in Section **??**. The most extensive tool for model comparison to date is the 'Cardiac Electrophysiology Web Lab' (Cooper et al., 2015a), which is available at `http://chaste.cs.ox.ac.uk/WebLab`. This tool contains a library of annotated CellML models and a set of experiments (written in a custom experiment description language), and allows users to run each experiment on each model and compare the results. A useful next step would be to standardize the annotations used in the Web Lab, and to set up an interface to let other tools interact with it directly.

8.3.2 Automated validation

A large part of model development consists of validating the model's predictions against several experimental data sets. As this is a labor-intensive process, it would make sense to automate this task. This would also allow automated (re-)validation to be performed after any change to the model (provided the validation experiments and data are publicly available, see Section 8.3.4). With such an approach, the complex changes needed to recreate an arrhythmic substrate could be carried out with greater confidence. Since many of the outputs a model should be validated against are emergent properties (e.g., the APD, APD restitution, conduction velocity), single and multi-cell simulation is a vital part of model-data comparisons.

Automated validation is similar to model comparison, so it would be useful to combine the two tasks. Tools like the aforementioned 'Web Lab' can compare models written in CellML with each other, but as of yet no system has been created that can also incorporate experimental data or multi-cellular simulations. Myokit can provide a partial solution due to its multi-cell capabilities and patch-clamp data import, but a greater effort, both technical and organizational, will be required to deal with this issue in a systematic manner. An overview of the remaining challenges as well as the future perspectives for systematic model-model and model-data comparisons is given by Cooper et al. (2015b).

Once the technical issues have been dealt with, more work is needed to learn how to interpret differences seen in such comparisons. In the light of variability (as well as noise), how different do two model outputs need to be before the models can be said to disagree? Can we update our models to not only match experimental averaged data, but also accurately

predict output *ranges*? And can we validate different model outputs independently, or do relationships between all used outputs need to be considered? Such questions will need to be addressed in order to fully make use of automated model comparison and validation tools and understand the role of variability in modeling of the cardiac cellular AP.

8.3.3 Free parameters and variability

Since their introduction, the number of parameters in models of the cardiac AP has increased steadily: from 5 named and 41 unnamed parameters in Noble (1962) to 222 named and 938 unnamed parameters in Heijman et al. (2011). Not all of these parameters can be measured directly, and as a result many of them are set by inspecting the model's output and tuning the parameters until the output matches the modeler's expectations (either manually, or using the method outlined in Section 8.2.1.1). However, given the size of modern models, it is likely they are still underconstrained. A study by Sarkar and Sobie (2010) addressed this issue directly, and showed how adding more model outputs (i.e., validating against a bigger data set) and applying sensitivity analysis can be used to reduce the number of free parameters in models of the AP. However, since their results regarding the different maximum conductances mirrored those of Marder and Goaillard (2006), the work by Sarkar and Sobie also became a seminal work in the study of variability in models of the cardiac AP (Sarkar et al., 2012; Weiss et al., 2012). If we take biological variability into account, the question arises which parameters can vary because the model is underconstrained (i.e., where we don't have enough data) and which can vary because this accurately reflects the underlying biology. This is a question that can only be answered with more quantitative experimental data on variability in the processes underlying the cardiac AP, such as provided in Chapter **??**. Gathering such knowledge is critical if we want to be able to compare different models (e.g., to know when a model can be rejected). Cherry and Fenton (2007) ran simulations using two different models of the canine AP and found several differences. They too argued that this showed the need to validate against multiple outputs, but also suggested detail in models should be reduced when such validation data is unavailable. A recent overview of the issues with parameter tuning in models of the cardiac AP, and the challenges of variability and personalization, was given by Krogh-Madsen et al. (2016).

8.3.4 Data sharing and reporting standards

Sharing of models, methods, simulation details and experimental data is required for model validation and simulation experiment reproducibility. Model code is frequently shared online, and projects such as CellML (Cuellar et al., 2003) and the Physiome model repository (Yu et al., 2011) have been set up to standardize and promote this procedure. Sharing of methods is also common, with projects such as OpenCOR (Garny and Hunter, 2015), Chaste (Mirams et al., 2013) and Myokit all freely available online. However, to re-validate a model after downloading and making changes, users will also need access to the original (experimental)

validation data and the simulation experiment code needed to (computationally) reproduce it.

Projects such as MIASE (Waltemath et al., 2011a) and SED-ML (Waltemath et al., 2011b) have been set up to promote and standardize sharing of simulation code and improve reproducibility of biophysical simulation experiments. However, at the time of writing tools implementing SED-ML are scarce (with the notable exception of OpenCOR), and the language does not yet have the features required to describe realistic, complex computational-electrophysiology experiments (Cooper et al., 2015a). In this respect, tools like Myokit, which are intermediate between custom, free-form code and a fully standards-based approach, can perform a valuable transitional function.

For experimental data, the MICEE draft standard and website exist to aid in reporting (Quinn et al., 2011, see also `http://micee.org`), but a major effort for online sharing of electrophysiological data has yet to be made, and obtaining the data to (re)validate a model is a challenging task. In part, this is because the amount of data needed to create an AP model is so large, it almost inevitably contains data from multiple experiments from independent labs. The issue is further complicated by the fact that large parts of models are often 'inherited' from older ones, which complicates determining the full data set needed for validation (Niederer et al., 2009; Bueno-Orovio et al., 2014).[7] In addition, existing formats will need to be updated to incorporate the possibility of natural variability in models and experimental data, as well as relationships between the variability in different parameters (see Chapter **??**). Nevertheless, to create reliable predictions, both the infrastructure and the willingness to share and compare experimental data and simulation results still need to be generated in the upcoming years.

8.4 Variability in multi-scale models of the AP

Conventional AP models are based on averaged data. For example, when creating a model of an ionic current an experimenter may record it in a number of cells, determine some physiological parameters (for example time constants and midpoints of activation) in each of them, and then calculate the average value of each parameter and pass it to a modeler. This is a valid approach if all variability in the parameters is due to measurement error. However, given the findings of Marder and Goaillard (2006) and the results presented in Chapter **??**, it is clear this approach misses a great deal of the biological complexity of real myocytes by replacing a diverse population with a single, idealized, cell. Furthermore, it is not at all guaranteed that using the mean for each parameter will result in a physiologically viable model.

[7] This complicated heritage, combined with the experimental difficulty and cost of obtaining data, has led to the situation where models include data gathered in different species, in different cell types, and using different procedures. Besides complicating the practicality of gathering and incorporating all the relevant data, this has a negative effect on the *applicability* of the resulting validation.

Instead, a future challenge for AP models will be to (1) obtain measurements of the natural variability in all relevant parameters, (2) where possible, to record any relationships between the parameters (for example the relationship between midpoint of activation and inactivation seen in Chapter **??**), (3) to update reporting standards and model languages to include variability and parameter interdependencies, (4) to further develop simulation methods that incorporate variability, and (5) to interpret what the existence of such variability means for the use and development of multi-scale models. These challenges are not independent. For example, deciding where to start measuring variability requires some estimate of where variability is most likely to be found and where it will have the most prominent effects on the AP or AP propagation. Despite these challenges, work in simulating variability has already shown promising results. For example, including hypothesized variability in conductance levels can predict variability in drug-induced APD prolongation (Britton et al., 2013) and improves predictions for the risk of drug-induced Torsades de Pointes (Cummins Lancaster and Sobie, 2016). We briefly discuss the challenges of measuring and simulating variability below.

8.4.1 Measuring variability

Most work on variability in AP model parameters so far has focused on variability in the expression levels of ion channel alpha-subunit genes, which correlates strongly with ion current maximum conductance. This type of variability can be measured by collecting several cells and measuring expression levels using techniques such as PCR. An advantage of PCR is that it can measure the transcription levels of multiple channel proteins in a single cell, allowing the relationship between them to be studied. As Schulz et al. (2006) showed, it is also possible to combine PCR and voltage-clamping to study channel expression levels and current characteristics in the same cell. An advantage of studying variability in maximum conductances is that the number of variables to consider is limited by the number of currents, and so is in the order of 10-20.

In Chapter **??** we investigated measuring variability in the *kinetical parameters* of an ion current (I_{Na}). We found this required careful consideration of (1) noise and artefacts in the recorded currents, (2) imperfect control of the membrane potential, (3) the methods used to analyze the recordings. Taking these three factors into account, we performed measurements of the time constants of inactivation in I_{Na} and found they varied considerably, with what appears to be a skewed distribution. Importantly, kinetics of fast and slow inactivation were not independent, but showed a moderate linear correlation. Another study by Pathmanathan et al. (2015) investigated the steady states (i.e., midpoints and slopes of (in)activation) of I_{Na}, and found these too varied between cells. Their study used existing data, and focused mainly on the mathematical aspects of quantifying variability instead of investigating the experimental side. Interestingly, they studied the same problem using two different data sets from the same laboratory, and discovered that the variability *between* the

data sets exceeded that within the data sets, which matches with our observations about the midpoints of (in)activation in Chapter **??**. Our own literature review data of midpoints of activation and inactivation is consistent with the above results, but also shows a strong linear correlation between the midpoints of activation and inactivation.

To the best of our knowledge, this work provided the first direct investigation of variability in ion current model parameters. An important conclusion for AP model development is that parameters do not vary independently, but are correlated. This means studies using cell-to-cell variance cannot sample all parameters from independent probability distributions but should take care to incorporate parameter covariance. Similar studies for the other major currents are needed, preferably with very large numbers of cells, to gather the data needed to work towards variability-aware modeling.

8.4.2 Modeling and simulating variability

Incorporating (known or hypothesized) variability into models of the AP presents several challenges. First, model definition languages (such as CellML and Myokit's `mmt` format) need to be updated to allow parameter variability to be specified. Unofficial CellML extensions to allow this have already been proposed and used (Walmsley et al., 2013), but may need to be extended to allow parameter dependencies to be included. Next, simulation methods will need to be updated to allow the incorporation of variability. One way to do this is to simply re-run simulations many times with different parameter values, drawn from the appropriate distributions (Romero et al., 2009; Walmsley et al., 2013). Linear regression-analysis has been proposed as a way of interpreting the results, by quantifying the impact of each varied parameter on the simulation results (Sarkar and Sobie, 2010, 2011). To deal with the exponentially growing number of possible models when varying multiple parameters, a technique known as *Latin hypercube sampling* has been used (McKay et al., 1979; Britton et al., 2013). The required number of simulations can be reduced even further by training *Gaussian process emulators* to the output of simulations (Chang et al., 2015; Johnstone et al., 2016). These also provide a way of investigating the model's sensitivity to different parameters. An interesting extra step when working with populations of models, is to *calibrate* the population by accepting or rejecting models based on higher-level characteristics, such as the shape of the AP (Britton et al., 2013; Muszkiewicz et al., 2016). This method allows variability to be used in simulations even when the true underlying parameter distributions are not known. A good overview of methods to incorporate variability into cardiac AP models can be found in Walmsley (2013).

8.4.3 Personalized modeling

A different way of dealing with variability is by *personalizing* models. For example, whole-heart (or more commonly whole-ventricle) simulations, often already use patient-specific

geometries (Aguado-Sierra et al., 2011; McDowell et al., 2012; Arevalo et al., 2016) and this approach was also used in Chapter **??**. However, electrophysiological properties can also be personalized using either knowledge of disease-induced changes (Reumann et al., 2009) or direct measurements of clinical data (Lombardo et al., 2016; Mann et al., 2016). Again, knowledge of variability in AP model parameters will be highly valuable here, as it can indicate where variability is expected and measurements need to be done. However, while modern techniques such as hiPSC may enable a wealth of data about individual patients to be obtained, the large number of parameters in multi-scale AP models suggests that a combined approach of personalization and variability-including modeling may be the most appropriate tool for patient-specific investigations.

8.5 Conclusion

Multi-scale models are used to integrate experimental data from different sources and help us gain a deeper understanding of cardiac electrophysiology. Simulation is an important part of this systems approach to biology, as it enables the study of phenomena that emergene from the interaction of biological processes at the different scales. Models of the cellular AP form the back-bone of these simulations. They are created by combining models of the ions, channels, and transporters they contain, and cell models in turn can be combined into tissue models. Exploring smaller and larger scales is limited by computational power, but good results can be obtained by carefully chosen trade-offs between detail and simplicity. As the substrate to develop an arrhythmia is typically complex, recreating substrates requires making several changes to models of the healthy cell. The issues this creates for the reliability of multi-scale models can be partially addressed by software tools for multi-model testing and automated validation. However, major data-sharing (and simulation sharing) efforts are required to make validation of models a routine activity for model users as well as developers. Incorporating biological variability into multi-scale models is challenging computationally, but the recognition of biological variability also raises new questions about how to interpret model comparisons and validation. In addition, much new experimental work is needed to characterize the variability in the electrophysiological properties of cardiomyocytes. Many of these issues are interrelated, for example the recognition of widespread biological variability has a profound impact on genotype-phenotype relations, model validation and development and will impact the way experimental results are reported. Conversely, investigations into variability rely on excellent experimental work, as well as theoretical work into parameter estimation, identifiability, stochastic simulation and constraining of free model parameters. As a result, computational tools can play a major part in the ongoing study of cardiac arrhythmia, not just through the development of new state-of-the-art technologies, but also through standardization and sharing of existing work. Indeed, sharing is a key point for the future, as none of the abovementioned problems can be tackled in isolation. Instead,

future theoretical and experimental work should continue the fruitful interplay of model and experiment, and proceed in a manner strongly informed by the complexities of cardiac electrophysiology.

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