# Multi-scale modeling and variability in cardiac cellular electrophysiology

# APPENDIX A

# Summary

The rhythm of the heart is regulated by processes at the genetic, molecular, cell, tissue, and organism scales. Computational models of the cardiac cellular action potential (AP) can be used as the basis for multi-scale models that connect these scales. This has made them essential tools in the study of cardiac arrhythmias. Far from existing in theoretical isolation, these models are widely used to interpret experimental data, design new experiments and make predictions about the results. Indeed, the complex and multi-faceted nature of arrhythmogenesis requires an ever-increasing level of cooperation between modelers and experimenters and the integration of knowledge from several fields in a *systems approach* to physiology. In this thesis, we show how software tools can aid in this process, we use simulations with AP models to connect processes at different scales, and we investigate the complexity seen even at the level of ionic currents.

In **Chapter 1**, the topic of this thesis is briefly introduced and an overview of the various chapters is given. **Chapter 2** then introduces the physiology and modeling of ion currents, the cellular AP, and electrical propagation from cell to cell.

**Chapter 3** describes *Myokit*, our novel tool for AP model development, multi-scale simulation, and analysis. Myokit's ease of use and straightforward modeling language facilitate model sharing and re-use, while its graphical user interface and extensive toolbox allow simulation and analysis methods to be shared with a wide audience. Import and export facilities allow model exchange with various formats, and methods to import patch-clamp data and export patch-clamp protocols are provided. Fast single-cell simulation is implemented using CVODE, and a versatile multi-cell engine is provided that can run on the GPU using OpenCL. In addition, Myokit contains advanced simulation engines that can calculate partial derivatives, evaluate system stability or run fast simulations with Markov models. Three examples are provided that illustrate Myokit's use in single and multi-cellular investigations of Brugada syndrome, its use in model comparison, and its capabilities for fitting ion-channel models to patch-clamp data.

**Chapter 4** examines the prospect of speeding-up simulations by replacing slow-to-evaluate mathematical expressions with less computationally expensive ones, and for this we focus on *splines*. We find spline approximations can be used to speed up equations commonly found in AP models, but that the relative number of such equations is lower for the larger published models. In addition, we find that GPU-parallelized simulations, which are increasingly used for large multi-cellular work, do not benefit from the technique. So while the principle of the method appears sound, its applicability to AP simulations is limited and other fields may benefit more from this technique.

**Chapter 5** examines *variability* in the kinetics of the fast sodium current $I_{\mathrm{Na}}$using CHO cells expressing human *SCN5A*. We show that a simple voltage-step experiment, performed under controlled conditions, elicits a current response that varies in shape and size from cell to cell. The time constants of inactivation vary with a skewed distribution, and a moderate linear correlation between the two constants can be seen. Through a careful analysis of our experimental setup we show that this variability is larger than expected from experimental error alone. By comparing the calculated standard deviations with those seen in myocyte experiments we show that in this case CHO cells are a good match for human myocytes. Next, we perform a literature review and see that the midpoints of activation and inactivation show a similar wide spread in all reported experiments and that there exists a strong linear correlation between midpoint of activation and midpoint of inactivation. Finally, we show how the observed variability can affect the cellular AP, and argue it should be reported as a feature of the ionic currents rather than a weakness of the experiments.

**Chapter 6** contains an extensive review of published nonsynonymous missense mutations in *SCN5A*and their effect on $I_{\mathrm{Na}}$. Using this data, we investigate if *machine-learning* techniques can be used to predict mutation-induced changes in $I_{\mathrm{Na}}$. We find that 610 out of 11923 (5.1%) of possible missense mutations have been studied, and that in many cases the location of mutations on the gene correlates with a specific change in function. However, the effects of the amino acid substitutions do not show any immediately useful patterns. Using machine learning techniques on this data set, we can create better-than-chance predictions of an *SCN5A* mutation's effects, but the general accuracy is low (around 70%). By carefully examining the results we show that this is mainly due to a strong bias in the dataset, combined with inconsistencies and a lack of suitable *features* to describe mutations in a meaningful manner.

**Chapter 7** investigates the use of AP models in reconstructing heart-surface potentials from noninvasive measurements of body-surface potentials in a technique we call *physiology-based regularization* (PBR). We use AP models to simulate wave propagation over the heart and then perform singular-value decomposition to create a small set of base patterns from which the more complicated patterns can be constructed. By restricting the heart-surface potential patterns to ones composed from this basis, more accurate reconstructions can be

made. We show that our method recovers more details of heart-surface electrograms than traditional regularization methods, obtains higher correlation coefficients with invasively measured signals, and leads to improved estimates of recovery times. By using different models and simulation methods, we show that adding *temporal* detail to the used AP models does not improve the results, and that simplified propagation models are adequate for this purpose.

Finally, **Chapter 8** discusses the use of multi-scale models to link ion-channel function to arrhythmogenesis and body-level observations, in a systems approach to electrophysiology. We argue that the complex relationship between molecular-level effects and diseases requires simulation, so that the development of tools like Myokit is a worthwhile investment for years to come. At the same time, the multifactorial nature of arrhythmogenesis implies that modelers will necessarily push models far from the situations for which they were created, which increases the scope for errors. We discuss the role of model comparison, automated validation, and data sharing in addressing this problem. The existence of natural variability increases these difficulties, and changes the way comparisons of models to other models or experimental data should be interpreted. Yet it also has the potential to explain observed differences in drug-response or the clinical manifestation of ion-channel mutations, so that a deeper understanding of variability is vital for future investigations. We conclude that problems in cardiac cellular electrophysiology can best be tackled with a combined experimental/theoretical approach.