In Silico Approaches to Predicting Efficacy and Safety of New Medicines (In Human-Based Systems for Translational Research)

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1. Introduction

Over the last 50 years, a number of areas of physiology have experienced great progress in the development and integration of *in silico* models and simulation tools of different organs. Multiscale human organ computational models are available, for different organs including heart (see reviews[1, 2]), lungs[3-6], and cancer,[7,8] with fine-grained biophysical detail and a great level of integration from the genetic to the whole body level. Multiscale modelling and simulation have been crucial in improving our understanding of mechanisms of physiological and pathological phenomena, therapy and diagnosis. The advanced *in silico* technology is currently opening promising and powerful avenues for the investigation and improvement of the efficiency of therapies and for the development of new medicines.

Perhaps the most advanced area of Computational Physiology is Computational Cardiology, concerned with the investigation of the physiology and pathology of the heart using computational modelling and simulating. Computational heart models are multi-scale both spatially and temporally, and integrate information across sub-cellular, cellular, tissue, and organ levels, with spatial scales ranging from nanometres to metres and temporal scales from picoseconds to years (if processes such as ageing or disease remodelling are taken into account). Computational heart models have been demonstrated to be useful and also predictive, providing a strong basis for their credibility in a variety of settings, including the prediction of drug action (see, for example,[9-16]). This has led to an increase in the interest by industry and regulators, as shown by the recent announcement by the Food and Drug Administration of their intentions to replace to the Thorough QT study by a combined *in vitro/in silico* assay.[17] In the next sections, we describe the state-of-the-art in Computational Cardiac Electrophysiology, as an example of an advanced area of *in silico* biology. We then describe how the technology is being developed and used to advance our understanding of the causes and modulators of variability in the response to medicines, through the application of computational population-based approaches. We finish the Chapter with a discussion on the processes required for the validation and constant challenge of *in silico* methodologies in biology, as an iterative process which is required to build their credibility for applications such as the development of new medicines.

2. State-of-the art Computational Cardiac Electrophysiology

In this section, we describe the extensive computational technology available to investigate and predict the electrophysiology of the heart at the single cardiomyocyte and the whole organ level. Over 40 mathematical models of the

electrical excitation in single cardiac cells are now available. They include cellular models for a variety of species and cell types including ventricular, atrial and Purkinje cardiomyocytes from human, rabbit and dog amongst others (see the CellML repository: www.cellml.org). Three-dimensional anatomical models of the cardiac ventricles and atria have also been constructed (see for instance,[18-34]). Simulations using sophisticated software and numerical techniques have been conducted to investigate the effect of drugs, disease or mutations at the ionic level on the electrical activity of the human heart ([13, 24, 27, 30, 34]).

2.1. **Single cardiomyocyte models.**

Cardiac cells are electrically excitable due to the presence in their membrane of proteins, which act as mechanisms of ionic transport across the cellular membrane, such as ion channels, pumps and exchangers. The transmembrane transport of ions such as potassium, sodium and calcium results in electrical and concentration gradients between the intracellular and the extracellular media.

In cardiac cells, the transmembrane potential at rest is negative due to the difference in ionic concentrations between the intracellular and the extracellular spaces. Following a sufficiently large electrical stimulus, the transmembrane potential experiences the action potential, i.e. a non-linear response which is a direct consequence of the voltage-dependent properties of the membrane ion channels, exchangers and pumps (Figure 1, middle panel). Firstly, the fast sodium ion channels become activated allowing sodium to enter the cell, and resulting in an increase or depolarization of the transmembrane potential towards positive potentials. At depolarized potentials, calcium and potassium channels become activated resulting in the plateau phase of the action potential, during which calcium enters the cell triggering the mechanical contraction of the cardiomyocytes through the excitation-contraction system. Then potassium channels open, allowing outward currents due to the higher potassium concentration in the intracellular than the extracellular space. Potassium currents repolarise the transmembrane potential towards its negative resting state. The shape of the action potential varies for different cell types in the heart, due to differences in the numbers and kinetics of the different voltage- and time-dependent ion channels in the heart.

Computational Cardiac Electrophysiology has progressed over the last 50 years, since in 1960 Denis Noble published the first mathematical model of the electrical activity of a cardiac cell.[35] Noble's doctoral work was based on the studies by Hodgkin and Huxley, who developed the first model of the electrical activity of a neuron (the axon of a giant squid)[36] and resulted on their award of the Noble Prize of Physiology in 1963. The initial models included only a single potassium and sodium current, based on their knowledge of electrophysiology

at the time. Currently, most single cell cardiac models include ionic current models based on the Hodgkin-Huxley formulation, and therefore they represent ionic currents as the product of maximum conductance, gates, and driving force resulting from the concentration and electrical gradients acting on the ion across the membrane. The driving force iscalculated as the difference between the transmembrane potential and Nernst or reversal potential for the ion. The Nernst potential is a function of the intracellular and extracellular concentrations for a specific ion, and it is equal tothe transmembrane potential resulting in no flux across the membrane due to the equilibration of the diffusion and electrical field acting on the specific ion.

The complexity of the cellular models has however increased owing to the integration of the new electrophysiology knowledge generated over the years on the types and modes of operation of cardiac ion channels, exchangers and pumps (see for example,[37-48]). As illustrated in Figures 1 and 2, current single cell cardiac electrophysiology models include detailed representation of the most important ionic transport mechanisms such as sodium and calcium and potassium ion channels, the sodium/calcium exchanger and the sodium/potassium pump, and also the biophysical processes underlying calcium dynamics across intracellular compartments of the sarcoplasmic reticulum. In some cases signalling pathways such as those underlying beta-adrenoceptor stimulation and the CaMKII cascade are also incorporated.[46,49]

Whereas the mathematical framework described above is generic for cardiac tissue, the models are tuned to be specific, for example, of a particular animal species or spatial location within the heart (for example, dog versus rabbit, atria versus ventricles). Electrophysiological recordings obtained from laboratory experiments are used to estimate the parameter value for ionic current conductances and kinetics for specific cell types.[38-47,50-52] The parameter values in the models can sometimes be obtained directly from patch clamp recordings or indirectly through the use of pharmacological action to estimate the magnitude of specific properties such as ionic current conductances. Equations and parameters are determined by minimisation of the difference between simulated traces and mean experimental values. Therefore, models and parameters are linked to the specific conditions and experimental models in which the recordings were performed (including animal species and location of origin of the preparation, temperature, solutions, recording technique, etc.). Novel experimental findings lead to model updates and also to new models with novel and improved features but also aspects inherited from previous models.[35,45,53-55]

In view of the great complexity of measuring and modelling ionic currents, an alternative approach is also adopted to model cardiac action potential dynamics, consisting of simplified, minimal or phenomenological models.[56-59]

The equations in this case aim at capturing cellular-level properties, such as morphology and rate dependence without including a detailed description of specific ionic currents. The advantage is that the models have a reduced number of equations and parameters to be identified, matching the limited information obtained for specific cells and experimental recordings. The phenomenological cellular models can capture the effect of drugs on cellular properties such as the action potential shape and duration but they do not allow representation on the effect of drug action on specific ionic currents.

2.2. **Whole organ heart models.**

Simulations of cardiac electrophysiology at the tissue and whole organ level require mathematically modelling of the processes underlying intercellular electrical coupling and propagation of electrical excitation from cell to cell through cardiac tissue. The cardiac bidomain model is currently the gold standard and arises by representing the cardiac tissue as a continuum with two domains: the intracellular and the extracellular spaces, electrically connected through the membrane of cardiomyocytes. Two partial differential equations form the bidomain model, based on the *assumption* that cardiac tissue is a continuum and on two fundamental laws: the Ohm's law (to relate electrical potential to flow of transmembrane, intracellular and extracellular currents) and Kirchhoff's law (for conservation of charge). Due to the computational expense associated with whole organ simulations, simplified formulations of the bidomain equations have been derived and are often used. This include the monodomain and the Eikonal equations as well as fast graph-based methods, as described in.[60]

Simulation of propagation of electrical excitation through cardiac tissue also requires the definition of spatial tissue characteristics such as its geometry, which are defined by a mesh. Anatomical models of the upper or lower chambers of the heart (whole-atria or whole-ventricles models) are obtained from imaging modalities such as MRI and histology using segmentation techniques to obtain a binary image that defines the boundaries of the cardiac tissue (Figure 3).[1,20,21,31,33,61,62] The processed image is then used to generate a volumetric mesh by applying a discretisation process in space, which depends on the numerical method used to solve the propagation model equations, which often is the finite element method. Thus the underlying tissue model is a discrete one composed of cells (the cardiac tissue), which is turned into a continuous model (the bidomain model), and finally turned back into a discrete anatomico-mathematical model to conduct the computer simulations.[2] The anatomically-based volumetric mesh comprises the elements or points in the cardiac domain over which the solution to the tissue model equation (e.g. the bidomain model) is calculated. In order to resolve the spatial and temporal scales of the electrophysiological dynamics, human ventricular meshes can

contain millions of elements making simulations computationally expensive.[13,24-30]

Simulations of whole organ-level physiological activity are conducted using software, developed through the efficient implementation of the computational physiology models and techniques often run in high performance computing platforms. In the case of the heart, this allows the simulation of the multi-scale effects of drug action on the heart from the ionic level to the electrocardiogram (ECG). An example is illustrated in Figure 3. The simulation of the ECG requires an anatomical model of the human torso with biophysically detailed and anatomically-based description of the human cardiac ventricles as described in various reviews[13,32,71]. The simulations are computationally expensive as the computational mesh contains 14,336,528 tetrahedral elements and single cell membrane kinetics are represented heterogeneously by the Ten Tusscher & Panfilov 2006 model[72] for epicardial, endocardial and mid-myocardial behaviour based on transmural location. The human cardiac ventricular model is coupled to the torso using the diffusion equation to account for the electrical propagation from heart to torso. The ECG can then be measured on the body surface, as shown in Figure 3. The multiscale models described here provide the computational framework required to simulate the effects of medicines at the ionic level, as described in the next section, and how they propagate to cellular, tissue and whole organ activity.

Whole-organ simulations of the electrophysiological activity of the heart require finding accurate approximations of the bidomain equations using numerical techniques, such as the Finite Element Method. This is because an exact solution cannot be obtained. The numerical method required to conduct the simulations determines how anatomical meshes are discretised into smaller spatial steps. The mesh spatial resolution is determined to achieve convergence of numerical algorithms and has no relationship with the size of the cells. Simulation complexity requires a trade-off within numerical routines between efficiency, robustness and accuracy, and subtle interplays between spatial and temporal resolutions. Verification of the numerical routines is key to ensure the numerical stability of the simulations, and to avoid possible errors or artefacts that would affect simulation results[67-70].

At the whole organ and tissue level, the intracellular and extracellular conductivity tensors include two important pieces of information: fibre architecture and conductivity (Figure 3C). Fibre architecture, i.e. the local direction of the conductivity tensor at all ‘points’ of the model, is usually incorporated into tissue models by extracting information from histological or DT-MRI images, using image processing algorithms or using a mathematical rule that relates fibre rotation at a particular location with distance to the surfaces of the ventricular wall.[27,33,63-65] Conductivity values along and across

fibres are indirectly determined to yield conduction velocities, as measured experimentally in tissue.[66]

3. **Simulating interactions of medicines with ionic channels.**

In order to simulate the action of medicines on the heart, the equations in the ionic models need to be altered to include terms representing the modulation of ion channels by drugs. Drug action is usually modelled as a function of concentration and mode of action of each drug. The complexity of the mathematical models of ionic current/medicine interaction can vary from a simple pore block model to more complicated markovian models including kinetics states.[9,73]

Drug/ionic current models are based on receptor theory in pharmacology, based on the application of receptor models developed in physical chemistry to explain the mechanisms of drug molecules binding with cellular receptors (for review, see[73]). Clark was the first to apply the receptor model to describe the effects of drugs on ion channels based on a framework previously used for enzyme kinetics,.[74]

Receptor theory provided the basis for simulating the effects of drugs on ion channels. It was however not until the late 1970's that Neher and Sakmann developed the patch clamp experimental technique to record single ion channel currents through a glass micropipette clamped to the cell membrane.[75] The innovation enabled experimentalists to investigate and characterise the interaction between drug compounds and single ion channels. A number of early experimental studies of drug action on cardiac ion channels by Hille[76] paved the way for a mathematical representation of the interaction drug molecule/ion channel receptor, based on classical receptor theory and laws of physical chemistry.

Models of the interaction of molecules and ion channels can be loosely categorised as:
1) Simple pore block: the flow of ions is restricted by the drug binding with a continuously accessible ion channel receptor.
2) State and voltage-dependent binding theories: the modulated and guarded receptor theories.
3) Allosteric effectors: drug binds with an allosteric receptor on the channel protein, affecting the channel kinetics.
The mechanisms of drug/ion channel binding are usually a combination of all three processes. Therefore, the identification of the dominant effect in any given interaction is key to construct their mathematical models.

The simplest application of receptor theory to ion channel block is simple pore block. The molecule is assumed to have continuous access to the receptor site and the affinity of the drug for the receptor is assumed to be time and voltage independent. There are two basic ways in which drug molecules binding with ion channel receptors can inhibit the flow of ions through the channel pore. The first one is if the receptor site is located in or near the channel pore. Then, the bound drug molecule physically restricts the flow of ions through the channel pore. Alternatively, drug binding with an allosteric receptor can cause a conformational change which leads to restriction of the ions flow.

In the simple pore block model, the degree of channel block is a function of drug concentration, the half maximal inhibitory concentration (IC50) and the Hill coefficient for the targeted ionic channel. Thus, for a given drug dose [D], IC50 value and Hill coefficient n with respect to the channel j the formulation of drug action on the ionic current conductance g_j is given by

$$g_j([D]) = g_j \left(\frac{1}{1 + \left(\frac{[D]}{IC_{50}} \right)^n} \right).$$

If the law of mass action applies, binding of a ligand to one binding site should not affect the affinity of another binding site. However, in many instances binding experiments show cooperation between binding sites. In other words, a ligand binding or dissociating from a binding site alters the affinity of other binding sites. The Hill equation is often used in cooperative binding assays to assess the degree of cooperation between more than one ligand binding to the same receptor and was originally formulated to describe oxygen binding to haemoglobin by Hill in 1910.[77] The Hill equation is also used to describe the relationship between the binding ligand concentration and the fractional occupancy of binding sites and is used in cardiac pharmacology to describe the affinity between a particular drug molecule and its binding sites on ion channels. A Hill coefficient of one indicates independent ligand binding where the binding affinity is not affected by whether or not other ligand molecules are already bound. A Hill coefficient different than one means that a ligand binding to a receptor would affect the affinity of the unblocked receptors for remaining ligands.

The Hill coefficient *n* can be determined empirically from the slope of the straight line estimate of the curve. The IC50 value and the Hill coefficient are used in physical chemistry to define the affinity of an enzyme for a particular binding site. These values can similarly be used to represent the binding dynamics between drug molecules and ion channel binding sites for a simple

pore block. However, the drug action on ion channels is complicated by the voltage and time-dependencies of the ion channel proteins.

As described in previous sections, cardiac ion channels are dynamic protein structures, which activate and deactivate in response to changes in cellular transmembrane potential and concentrations. The voltage-dependency of cardiac ion channels is fundamental to cardiac activity as it regulates both excitation and contraction of cardiomyocytes. Drug action on cardiac ion channels is often also voltage and time dependent. However, because ion channel state-transitions are voltage-dependent, it is difficult or even impossible to distinguish between state and voltage-dependence of drug/ion channel interactions. Two main hypotheses have been proposed to describe state-dependent block, named the Modulated and Guarded Receptor Theories, as reviewed by Brennan and colleagues[73].

The modulated receptor theory was proposed by Hille in 1977 based on his work on the effects of local anaesthetics on sodium channels in nerve cells.[76] The premise of the modulated receptor theory is that the affinity of the drug for the ion channel binding site is dependent on the state of the ion channel. This implies that the binding affinity is voltage and time-dependent. Figure 4 illustrates the basic kinetic scheme of the Modulated Receptor Theory proposed by Hille. In this case, as an example, the model of the ion channel includes three states, namely open (O), closed (C) and inactivated (I). The modulated receptor theory proposes that the drug can bind in all three states but the affinity of the drug for the ion channel binding sites is voltage and time-dependent and is different for each state. Drug binding with each state causes the channel to enter modified bound states, all three non-conducting (indicated by asterisks). From the bound states, the channel can either transition to an unbound state or to one of the other bound states until it recovers from binding.

In contrast, the Guarded Receptor Theory proposes that the drug molecule binds with a single bindable conformation with fixed affinity and that access to the binding site is restricted due to the conformational changes of the protein. Thus, the molecule action on the ion channel is dependent on the conformation of the channel, which is voltage and time dependent. The Guarded Receptor Theory was pioneered by the work by Starmer and colleagues in the mid-1980's, who investigated the effects of local anethestics on cardiac sodium channels.[78] The main difference between the Guarded versus Modulated receptor theory, as depicted in Figure 4, is that the drug molecule binds to the particular channel state conformation, and recovery from binding is often also state-dependent. In the example of open-state block, this could be caused by the drug molecule being trapped by the closed or inactivated conformations of the channel and thus the channel is only able to recover from block when it returns to the activated state.

Both the Modulated and the Guarded Receptor Theories focus on voltage and time-dependent association and dissociation processes, due to conformation of the channel protein, and do not consider the drug effects on ion channel conformation state transition dynamics. However, many drugs also have allosteric effects, causing changes in the dynamics between protein conformation states. For instance, lidocaine binding to sodium channels in rat skeletal muscle was shown to produce a functional change in the channel dynamics by slowing down its inactivation dynamics.[79] A consequence of this is that the sodium channel is preferentially in the late scattered mode when the drug is bound compared to the transient (fast) mode of the unbound sodium channel. Thus, the drug compound does not simply block the ion channel, but alters its dynamics by binding to its allosteric receptor. This implies that no explicit drug bound states need to be represented in the model, as the binding of the drug molecule is taken into account in the model as changes in the transition rates between conformation states. However, the binding process of the drug compound to the allosteric receptor can also abide by the Modulated or Guarded Receptor Theories. Therefore, a more comprehensive model of drug-ion channel interaction including allosteric regulation consists of both unbound and bound states, with a conducting bound open state and transitions between unbound states being different than for bound states.

4. Investigating variability: population of models approach.

Biological variability is a common feature of all physiological systems, and a key challenge in the development of new medicines. Variability manifests itself at all levels in all organs of living organisms as differences in physiological function between individuals of the same species or as differences in time for the same individual. Variability becomes even more important when it involves significant differences in the outcome of exposure to pathological conditions or treatment. Thus, healthy cells of the same species and location usually exhibit a qualitatively similar behaviour. For example, cardiomyocytes of a similar location and species exhibit a similar action potential. However, significant quantitative inter-subject and intra-subject differences exist, which in cardiac cells are manifested as quantitative differences in action potential morphology and duration. The small differences under physiological conditions may however be important in explaining the different individual response of each of the cells and patients to disease and treatments.

To date, experimental and theoretical research has often ignored the variability underlying the physiological and pathological responses of different individuals to disease and medicines, ultimately hampering the extrapolation of results to a population level. Often average of the recordings is performed to reduce experimental error, therefore also averaging out the effects of inter-subject

variation, and resulting in an important loss of information. This averaging of experimental data is inherited by theoretical research, and consequently, models such as those described in the previous sections are often developed for a `typical' behaviour within a particular sub-population. This is certainly the case in cardiac electrophysiology, and therefore, although all experimentally-measured action potentials (APs) are different, even within a homogeneous population, a single AP model is developed and used in the simulations, again losing all information regarding inter-subject variability.

Understanding how inter-subject variability determines the efficacy and safety of medicines for specific patients or groups of patients is still a major challenge for both the practice of medicine and the development of new medicines. The importance of temporal and intra-subject variability has also been highlighted in recent years. For example, an experimental study showed evidence of remodelling of ionic currents in cardiac cells cultured for 24h in the presence of dofetilide, a drug blocking the rapid component of the delayed rectifier potassium current.[80] This means that cells change over time to adapt to the presence of drugs, potentially affecting the efficacy and safety of medicines. An increasing number of studies also demonstrates the strong effects circadian rhythms have on our bodies and the increasing importance given to chronotherapy.[81-83] A recent study conducted in mice demonstrated the effects of circadian rhythms on cardiac repolarization and arrhythmias providing measurements from the mRNA to the electrocardiogram level, and showing oscillations in ionic currents and the QT internal of the electrocardiogram during day and night.[83] Circadian rhythms may also influence the higher propensity of sudden cardiac death at specific times of the day.[81] Therefore, understanding the effect of circadian rhythms on the response to medicines could also be potentially important, and *in silico* methods have the potential of making an important contribution to this effect.[84]

The high degree of inter-species, inter-subject, temporal and spatial biological variability has become an increasingly important topic in computational physiology.[11,16,43,52,54,85,86] Computational population-based approaches have been proposed in recent studies to shed light into ionic determinants of inter-cellular variability to the response of individual cells to medicines and disease. Davies *et al.*[16] adjusted model parameters in a canine cellular model to obtain 19 different models, which were fit to specific action potential recordings. An alternative approach has been recently developed based on the construction of populations of cell models, building on studies by Marder and colleagues in neuroscience.[85] Thus, Sarkar et al.[11] constructed populations of around 300 cardiac cell models by varying ionic properties in an arbitrary range, and demonstrated variability in cardiomyocyte response to ion channel block.

More recently, Britton *et al.*[12] have proposed a methodology to tightly couple experimental measurements and *in silico* models to construct and calibrate populations of cell models to investigate the causes of experimentally-measured variability in physiological conditions and following drug response (Figure 5). The research builds on previous studies showing the importance of sensitivity analysis in investigating the ionic determinants of inter-subject variability in biological properties.[43,54]

The experimentally-calibrated population of cell models proposed by Britton *et al*.[12] aims at capturing the variability exhibited in specific experimental recordings under physiological conditions and to predict inter-subject variability in response to potassium channel block. The main assumption of the novel approach is that inter-subject variability in experimentally-measured action potentials is primarily caused by quantitative differences in the properties of ionic currents, rather than by qualitative differences in the biophysical processes underlying the currents. All models in the population therefore share the same equations, which are used as the model structure to generate more than 10,000 candidate models with different sampled parameter values for the ionic properties within a wide range (Figure 5A). The cell model population is then calibrated using a set of cellular biomarkers extracted from experimental recordings at three pacing frequencies to capture key rate-dependent action potential properties. The experimentally-calibrated model population is then used to identify the ionic mechanisms which may underlie inter-subject variability for each biomarker, yielding information on the relative importance of ionic currents in the generation of the AP at each pacing frequency.

As illustrated in Figure 5B, the same paper by Britton *et al*.[12] shows that the population of cell models can then be used to predict variability in the response of rabbit Purkinje fibres to drug action (using an independent dataset). Figure 5B shows how the calibrated cell model population quantitatively predicts the prolongation of action potential duration caused by exposure to four concentrations of dofetilide, a blocker of the rapid component of the delayed rectifier potassium current (I_{Kr}). This intervention was considered because I_{Kr} block is the main assay required in safety pharmacology assessment due to its potential importance in drug-induced Torsades de Pointes. It is therefore important in the development of all new medicines.

A computational population-based approach was also applied in[87] to investigate ionic causes of differences in the response of human ventricular myocytes to heart failure, a common disease that leads to ion channel remodelling. The ability of computational models to reflect and explain variability under disease conditions could be very powerful in the development

of new medicines for cardiovascular disease treatment and also for safety pharmacology.

As well as investigations into inter-subject variability, a number of experimental and clinical studies highlight the importance of temporal or beat-to-beat cardiac variability in quantifying arrhythmic risk caused by disease or medicines. Indeed, changes in the magnitude of beat-to-beat cardiac variability in response to drug or disease have been linked to the pro-arrhythmic potential in experimental and clinical studies, also showing their predictive power to identify patients at high risk of arrhythmia.[88-92]

Recent studies hypothesized that the intrinsically stochastic opening and closing of cardiac ion channels contribute to beat-to-beat cardiac variability, and that pro-arrhythmic drugs might unmask and enhance their effect.[52,86,93] This is based on the idea that slight beat-to-beat differences in the number of channels opening and closing at specific times could manifest themselves as beat-to-beat differences at the cellular level (for example in the duration of the action potential). Several *in silico* studies[52,86,93] have investigated the mechanisms underlying these processes, and have linked stochasticity in ion channel behaviour to the variability of cellular outcomes following drug action.

In a combined *in vitro/in silico* study, Pueyo et al.[52] investigate the contribution of the stochastic behaviour of the slow delayed rectifier potassium current (I_{Ks}) to beat-to-beat cardiac variability. The same study also shows how this effect is enhanced following the application of an I_{Kr} blocker (Figure 6). In isolated cells, the stochasticity in I_{Ks} could also explain how I_{Kr} block results in potentially pro-arrhythmic instabilities in repolarization (named early afterdepolarizations) in some cells and some beats. Stochasticity in I_{Ks} may therefore explain, at least in part, some mechanisms of inter and intra-subject variability in repolarisation in response to drug action. Interestingly, the repolarization instabilities were supressed in tissue simulations due to the electrotonic effects of intercellular coupling. These results highlight the importance of multiscale simulations in elucidating the complex interplay of mechanisms determining the response of biological systems such as the heart to pharmacological compounds.

5. Validation of *in silico* models and simulations.

As *in silico* models and simulation techniques develop both conceptually and technically, the question of their verification and validation becomes crucial for their uptake beyond scientific research into industrial and regulatory arenas.[2,17] The process of validation of *in silico* models and simulations is complex and still under investigation but it is essential to increase the credibility of *in silico* methods as powerful techniques to augment the information extracted from experimental and clinical investigations.

The paper by Carusi et al.[2] specifically explores the issue of validation of computational physiological models in the context of their construction and applications. It highlights the iterative nature of the process of validation and the fact that it needs to consider the ensemble of equations, parameters, simulation techniques, software and experiments used for a particular study or purpose, namely, the Model-Simulation-Experiment system. This is of course similar to any other experimental science involved in the development of new medicines, and specifically experimental biology, where the interpretation of experimental recordings depends on knowledge with the entire history of the samples and techniques used to obtain the results.

In Computational Physiology, a necessary (although insufficient) condition for *in silico* methods validation is the verification of the robustness and convergence of numerical techniques and software used in the simulations, as they are implicated throughout model construction and simulation.[2,68] Once this aspect is demonstrated, the credibility of *in silico* models is based on physiological validation, which is the evaluation of the simulation results, through comparison to independent experimental data sets, not used in the model construction. It is important to re-emphasize the iterative nature of validation, which is a process rather than a final result.[2]

As discussed in previous sections and in Carusi et al.,[2] multiscale models often result in data used in construction and validation being acquired at different levels (e.g. ionic versus cellular or organ level), and therefore in different preparations and using different techniques. As a consequence, the multiscale models are often constructed from experiments using a variety of techniques and preparations. Each experimental technique involves a modification of the preparation with respect to its *in vivo* state, therefore introducing alterations in the measurements. Therefore, validation through comparison of experiments and simulations needs to take into account the potential distortion introduced by experimental recordings techniques in order to be able to interpret the success or failure of simulations in matching/predicting experimental results.

Biological variability is of course one of the identified challenges in the evaluation of *in silico* models, as it can greatly influence experimental results and their comparison to simulated data. As discussed in the previous section, the sources of electrophysiological variability are multiscale, spanning a wide range of spatio-temporal scales and the more we understand them, the more we will be able to advance in the quantitative evaluation of *in silico* models for their biological validation. Given the dynamic interplay and the variability in models, experiments and simulations, standards and interoperability at the modelling, simulation and experimental levels are essential, as reflected in the development of specific

languages such as CellML, SBML and FieldML for model encoding, repositories and databases.[94,95]

Physiological validation needs to be considered like a continuous and iterative process between experimental and computational research.[2] The aim is augmenting the information on the physiological system or process under investigation, such as for example the mode of action of a new medicine on specific organs. The dynamics of the iterative process are driven by advances in both experimental and computational techniques as well as investigations on their combined use. The process is driven both by matches and miss-matches of simulations and experiments (or success and failure in validation).[2,96] The iterative nature of this process is clear from the evolution of single cell electrophysiology models of different types and species, which has often resulted in families of several generations of models,[35,45,96] possibly capturing different aspects of cellular physiology given the large biological variability. As new experimental data and techniques become available, inconsistencies between computer simulation predictions and experimental recordings allow accelerating the refinement and improvement of our knowledge and its integration in quantitative and qualitative models.

With respect to cardiac electrophysiology at the whole-organ level, the process of iteration between experiments and simulations has been crucial, for example, to improving the understanding of important human health phenomena such as ventricular fibrillation,[30.50] the most dangerous type of lethal arrhythmia, and the mechanisms underlying electrical defibrillation, the only effective therapy against sudden cardiac death.[1] In both cases, whole ventricular electrophysiology simulations were successfully combined with clinical and experimental methods to bring two important advantages:
1) high spatio-temporal resolution on the three-dimensional simulated electrophysiological activity of the ventricles overcome limitations of clinical or experimental recordings, which often exhibit low spatio-temporal resolution and/or just the surface of the heart.
2) computer models and simulations provide the ability to dissect the relative importance of different factors, which result in the identification of key properties, which may determine the progress of disease and might inform the development of new therapies.
Similar approaches are likely to make a similar impact in the development of new medicines through the use of combined *in silico*, *in vitro* and *in vivo* studies, building on the great advances being achieved in Computational, Experimental and Clinical Medicine.

Figures:

Figure 1: Construction of computational cardiac electrophysiology models from ionic current to whole organ level. For the last 50 years, ionic current models have been constructed mostly based on patch-clamp data. The integration of ionic current models results in cellular level models for simulation of the action potential and concentration dynamics. Cardiac simulation at the whole organ level requires the construction of image-based anatomical models and a mathematical description of the processes underlying propagation of electrical excitation through cardiac tissue, which can take the form of bidomain, monodomain, eikonal or graph-based models.

Figure 2: Structure of the human action potential model by O'Hara-Virag-Varro-Rudy. The schematic represents ion channels, exchangers and pumps acting as mechanisms of ionic transport across the surface and sarcoplasmic reticulum membranes to produce the action potential and calcium transients (with permission from (46)).

Figure 3: Computer simulation of the effect of drug action on the heart from ionic currents to body surface potentials using a human electrophysiological model of the heart and the body torso. Anatomical model of the human torso (A) with human ventricular model (B) including realistic fibre orientation (C). Simulated body surface potentials (D) and electrocardiogram (E) under control and following potassium channel block. Modified with permission from (13).

Figure 4: Receptor modelling theory for drug/ion channel interactions. The schematic represents the generic ion channel as having three conformational states:
closed (C), open (O) and inactivated (I) in drug unbounded and bounded states
(without and with an asterisk, respectively). The state transition rates between channel states are αn and βn, and the voltage and time-dependent affinity of the drug molecule for the channel states is represented by the functions f(v, t), g(v, t) and h(v, t). (Modified from (73) with permission).

Figure 5: Inter-subject variability in cellular cardiac electrophysiology. A. Simulated action potentials obtained with an experimentally-calibrated population of rabbit Purkinje cell models (blue traces), in range with experimental action potentials (red traces). Black traces correspond to models excluded from the model population for being out of experimental range. B. Range of action potential duration prolongation caused by four doses of the HERG block dofetilide obtained using the experimentally-calibrated model

population (bars) and in experiments (white dots). Modified with permission from (12).

Figure 6: Temporal variability in simulated cardiac action potentials caused by the stochastic nature of the slow component of the delayed rectifier potassium current, under control condition and following the application of HERG block. Pro-arrhythmic abnormalities in repolarization (i.e. early after depolarizations) are obtained in some simulations following a deceleration of the stimulation frequency. Modified with permission from (52).

REFERENCES (numerical order):

1. Trayanova NA. Whole-heart modeling: applications to cardiac electrophysiology and electromechanics. *Circ. Res* 108: 113-28, 2011.
2. Carusi A, Burrage K, Rodriguez B. Bridging experiments, models and simulations: An integrative approach to validation in cardiac electrophysiology. *Am J Physiol Heart and Circ,* 15;303(2):H144-55, 2012.
3. Burrowes KS, Clark AR, Tawhai MH. Blood flow redistribution and ventilation-perfusion mismatch during embolic pulmonary arterial occlusion. Pulm Circ. 2011 Jul-Sep;1(3):365-76. doi: 10.4103/2045-8932.87302.
4. Burrowes KS, Clark AR, Marcinkowski A, Wilsher ML, Milne DG, Tawhai MH. Pulmonary embolism: predicting disease severity. Philos Trans A Math Phys Eng Sci. 2011 Nov 13;369(1954):4255-77.
5. Venegas JG, Winkler T, Musch G, Vidal Melo MF, Layfield D, Tgavalekos N, Fischman AJ, Callahan RJ, Bellani G, Harris RS. Self-organized patchiness in asthma as a prelude to catastrophic shifts. Nature. 2005 Apr 7;434(7034):777-82. Epub 2005 Mar 16.
6. De Backer LA, Vos WG, Salgado R, De Backer JW, Devolder A, Verhulst SL, Claes R, Germonpré PR, De Backer WA. Functional imaging using computer methods to compare the effect of salbutamol and ipratropium bromide in patient-specific airway models of COPD. Int J Chron Obstruct Pulmon Dis. 2011;6:637-46. doi: 10.2147/COPD.S21917. Epub 2011 Nov 28.
7. Owen MR, Stamper IJ, Muthana M, Richardson GW, Dobson J, Lewis CE, Byrne HM. Mathematical modeling predicts synergistic antitumor effects of combining a macrophage-based, hypoxia-targeted gene therapy with chemotherapy. Cancer Res. 2011 Apr 15;71(8):2826-37.
8. Kim M, Gillies RJ, Rejniak KA. Current Advances in Mathematical Modeling of Anti-Cancer Drug Penetration into Tumor Tissues. Front Oncol. 2013 Nov 18;3:278.
9. Clancy C, Zhu ZI, Rudy Y. Pharmacogenetics and antiarrythmic drug therapy: a theoretical investigation. Am. J. Physiol. Heart. Circ. Physiol., 292:H66–H75, 2007.
10. Polak S, Wiśniowska B, Fijorek K, Glinka A, Mendyk A. In vitro-in vivo extrapolation of drug-induced proarrhythmia predictions at the population level. Drug Discov Today. 2013.
11. Sarkar AX, Sobie EA. Quantification of repolarization reserve to understand interpatient variability in the response to proarrhythmic drugs: A computational analysis. *Heart Rhythm* 8:1749-55, 2011.
12. Britton O, Bueno-Orovio A, Van Ammel K, Lu HR, Towart R, Gallacher D, Rodriguez B. Experimentally-calibrated population of models predicts and explains inter-subject variability in cardiac cellular electrophysiology. *Proceedings of the National Academy of Sciences of the USA,* 110(23):E2098-E2105, 2013.
13. Zemzemi N, Bernabeu M, Saiz J, Mirams G, Cooper J, Pitt-Francis J, Rodriguez B. Computational assessment of drug-induced effects on the

electrocardiogram: from ion channel to body surface potentials. *British Journal of Pharmacology*, 168: 718–733, 2013.
14. Wilhelms M, Rombach C, Scholz EP, Dössel O, Seemann G. Impact of amiodarone and cisapride on simulated human ventricular electrophysiology and electrocardiograms. Europace. 2012 Nov;14 Suppl 5:v90-v96.
15. O'Hara T, Rudy Y. Quantitative comparison of cardiac ventricular myocyte electrophysiology and response to drugs in human and nonhuman species. *Am J Physiol Heart Circ Physiol* 302: H1023-30, 2012.
16. Davies MR, Mistry HB, Hussein L, Pollard CE, Valentin JP, Swinton J, Abi-Gerges N. An In silico Canine Cardiac Midmyocardial Action Potential Duration Model as a Tool for Early Drug Safety Assessment. *Am J Physiol Heart Circ Physiol* (December 23, 2011). doi:10.1152/ajpheart.00808.2011.
17. Sager PT, Gintant G, Turner J. R., Syril P,, Stockbridge N. Rechanneling the Cardiac Proarrhythmia Safety Paradigm: A Meeting Report from the Cardiac Safety Research Consortium, *American Heart Journal,* 2013.
18. Atkinson A, Inada S, Li J, Tellez JO, Yanni J, Sleiman R, Allah EA, Anderson RH, Zhang H, Boyett MR, Dobrzynski H. Anatomical and molecular mapping of the left and right ventricular His-Purkinje conduction networks. *J Mol Cell Cardiol* 51: 689-701, 2011.
19. Baher AA, Uy M, Xie F, Garfinkel A, Qu Z, Weiss JN. Bidirectional ventricular tachycardia: ping pong in the His-Purkinje system. *Heart Rhythm* 8: 599-605, 2011.
20. Bishop MJ, Plank G, Burton RA, Schneider JE, Gavaghan DJ, Grau V, Kohl P. Development of an anatomically detailed MRI-derived rabbit ventricular model and assessment of its impact on simulations of electrophysiological function. *Am J Physiol Heart Circ Physiol* 298: H699-718. 2010
21. Bordas R, Gillow K, Lou Q, Efimov I, Kohl P, Gavaghan D, Grau V, Rodriguez B. Rabbit-Specific Ventricular Model of Cardiac Electrophysiological Function including Specialized Conduction System. *Prog Biophys Mol Biol* 107: 90-100, 2011.
22. Deo M, Boyle P, Plank G, Vigmond E. Arrhythmogenic mechanisms of the Purkinje system during electric shocks: a modeling study. *Heart Rhythm* 6: 1782-9, 2009.
23. Keller DU, Weiss DL, Dossel O, Seemann G. Influence of I(Ks) heterogeneities on the genesis of the T-wave: a computational evaluation. *IEEE Trans Biomed Eng* 59: 311-22, 2012.
24. Moreno JD, Zhu ZI, Yang PC, Bankston JR, Jeng MT, Kang C, Wang L, Bayer JD, Christini DJ, Trayanova NA, Ripplinger CM, Kass RS, Clancy CE. A computational model to predict the effects of class I anti-arrhythmic drugs on ventricular rhythms. *Sci Transl Med* 3:98ra83, 2011.
25. Niederer S, Mitchell L, Smith N, Plank G. Simulating human cardiac electrophysiology on clinical time-scales. *Front Physiol* 2: 14, 2011.

26. Okada J, Washio T, Maehara A, Momomura S, Sugiura S, Hisada T. Transmural and apicobasal gradients in repolarization contribute to T-wave genesis in human surface ECG. *Am J Physiol Heart Circ Physiol* 301: H200-8, 2011.

27. Potse M, Dubé B, Richer J, Vinet A, Gulrajani RM. A comparison of monodomain and bidomain reaction-diffusion models for action potential propagation in the human heart. *IEEE Trans. Biomed. Eng*. 53(12 Pt 1): 2425-2435, 2006.

28. Romero D, Sebastian R, Bijnens BH, Zimmerman V, Boyle PM, Vigmond EJ, Frangi AF. Effects of the purkinje system and cardiac geometry on biventricular pacing: a model study. *Ann Biomed Eng* 38: 1388-98, 2010.

29. Seemann G, Höper C, Sachse FB, Dössel O, Holden AV, Zhang H. Heterogeneous three-dimensional anatomical and electrophysiological model of human atria. *Philos. Transact. A Math. Phy.s Eng. Sci*. 364: 1465-81, 2006.

30. ten Tusscher KH, Hren R, Panfilov AV. Organization of ventricular fibrillation in the human heart. *Circ Res* 100: 87-101, 2007.

31. Vadakkumpadan F, Arevalo H, Prassl A, Chen J, Kickinger F, Kohl P, Plank G, Trayanova N. Image-based models of cardiac structure in health and disease. *WIREs Syst. Biol. Med* 2: 489-506, 2010.

32. Zemzemi N, Bernabeu M, Saiz J, Rodriguez B. Simulating drug-induced effects on the heart: from ion channel to body surface electrocardiogram. *Lecture Notes in Computer Science* 6666/2011: 259-266, 2011.

33. Zhao J, Butters TD, Zhang H, Pullan AJ, Legrice IJ, Sands GB, Smaill BH. An Image-Based Model of Atrial Muscular Architecture: Effects of Structural Anisotropy on Electrical Activation. *Circ Arrhythm Electrophysiol* (Mar 14, 2012) doi: 10.1161/CIRCEP.111.967950.

34. Wilhelms M, Dössel O, Seemann G. In silico investigation of electrically silent acute cardiac ischemia in the human ventricles. *IEEE Trans Biomed Eng* 58: 2961-2964, 2011.

35. Noble D, Garny A, Noble PJ. How the Hodgkin-Huxley equations inspired the Cardiac Physiome Project. J Physiol. 2012 Jun 1;590(Pt 11):2613-28.

36. Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol. 1952 Aug;117(4):500-44.

37. Carro J, Rodríguez JF, Laguna P, Pueyo E. A human ventricular cell model for investigation of cardiac arrhythmias under hyperkalaemic conditions. *Philos Transact A Math Phys Eng Sci* 369: 4205-32, 2011.

38. Corrias A, Giles W, Rodriguez B. Ionic mechanisms of electrophysiological properties and repolarization abnormalities in rabbit Purkinje fibers. *Am J Physiol Heart Circ Physiol* 300: H1806-13, 2011.

39. Decker KF, Heijman J, Silva JR, Hund TJ, Rudy Y. Properties and ionic mechanisms of action potential adaptation, restitution, and accommodation in canine epicardium. *AM. J. Physiol. Heart Circ. Physiol* 296: H1017-26, 2009.

40. Grandi E, Pasqualini FS, Bers DM. A novel computational model of the human ventricular action potential and Ca transient. *J. Mol. Cell Cardiol* 48: 112-21, 2010.

41. Grandi E, Pandit SV, Voigt N, Workman AJ, Dobrev D, Jalife J, Bers DM. Human atrial action potential and Ca2+ model: sinus rhythm and chronic atrial fibrillation. *Circ Res* 109: 1055-66, 2011.

42. Li L, Niederer SA, Idigo W, Zhang YH, Swietach P, Casadei B, Smith NP. A mathematical model of the murine ventricular myocyte: a data-driven biophysically based approach applied to mice overexpressing the canine NCX isoform. *AM. J. Physiol. Heart Circ. Physiol* 299: H1045-63, 2010.

43. Li P, Rudy Y. A Model of Canine Purkinje Cell Electrophysiology and Ca(2+) Cycling/ *Circ Res* 109: 71-79, 2011.

44. Mahajan A, Shiferaw Y, Sato D, Baher A, Olcese R, Xie LH, Yang MJ, Chen PS, Restrepo JG, Karma A, Garfinkel A, Qu Z, Weiss JN. A rabbit ventricular action potential model replicating cardiac dynamics at rapid heart rates. *Biophys J* 94: 392-410, 2008.

45. Noble D, Rudy Y. Models of cardiac ventricular action potentials: iterative interaction between experiment and simulation. *Philos. Transact. A.Math. Phys. Eng. Sci* 359: 1127–1142, 2001.

46. O'Hara T, Virág L, Varró A, Rudy Y. Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. *PLoS Comput. Biol* 7(5): e1002061, 2011. doi:10.1371/ journal.pcbi.1002061.

47. Shannon TW, Wang F, Publisi J, Weber C, Bers DM. A mathematical treatment of integrated Ca dynamics within the ventricular myocyte. *Biophys J* 87: 3351-71, 2004.

48. Wilhelms M, Hettmann H, Maleckar MM, Koivumäki JT, Dössel O, Seemann G. Benchmarking electrophysiological models of human atrial myocytes. Front Physiol. 2013 Jan 4;3:487.

49. Heijman J, Volders PG, Westra RL, Rudy Y. Local control of β-adrenergic stimulation: Effects on ventricular myocyte electrophysiology and Ca(2+)-transient. J Mol Cell Cardiol. 2011 May;50(5):863-71.

50. Aslanidi OV, Sleiman RN, Boyett MR, Hancox JC, Zhang H. Ionic mechanisms for electrical heterogeneity between rabbit Purkinje fiber and ventricular cells. *Biophys J* 98: 2420-31, 2010.

51. Flaim SN, Giles WR, McCulloch AD. Arrhythmogenic consequences of Na+ channel mutations in the transmurally heterogeneous mammalian left

ventricle: analysis of the I1768V SCN5A mutation. *Heart Rhythm* 4: 768-78, 2007.

52. Pueyo E, Corrias A, Virag L, Jost N, Szel T, Varro A, Szentandrássy N, Nánási PP, Burrage K, Rodriguez B. A multi-scale investigation of repolarization variability and its role in cardiac arrhythmogenesis. *Biophysical Journal* 101: (12) 2892-2902 (2011).

53. Niederer SA, Fink M, Noble D, Smith NP. A meta-analysis of cardiac electrophysiology computational models. *Experimental Physiology* 94: 486-495, 2009.

54. Romero L, Carbonell B, Trenor B, Rodriguez B, Saiz J, Ferrero JM. Systematic Characterization of the Ionic Basis of Rabbit Cellular Electrophysiology Using Two Ventricular Models. *Prog. Biophys Mol. Biol* 107:60-73, 2011.

55. Bueno-Orovio A, Sanchez A, Pueyo E, Rodriguez B. Na/K pump regulation of cardiac repolarization: Insights from a Systems Biology approach. *Pflügers Archiv - European Journal of Physiology,* 2013 (DOI 10.1007/s00424-013-1293-1).

56. Fenton F, Karma A. Vortex dynamics in three-dimensional continuous myocardium with fiber rotation: Filament instability and fibrillation. *Chaos* 8(1): 20-47, 1998.

57. Bueno-Orovio A, Cherry EM, Fenton FH. Minimal model for human ventricular action potentials in tissue. *J Theor Biol* 253: 544-60, 2008.

58. Bueno-Orovio A, Hanson B, Gill J, Taggart P, Rodriguez B. In Vivo Human Left-to-Right Ventricular Differences in Rate Adaptation Transiently Increase Pro-Arrhythmic Risk Following Rate Acceleration. *PLoS ONE* 7(12): e52234. doi:10.1371/journal.pone.0052234, 2012.

59. Walmsley J, Mirams G, Bahoshy M, Bollensdorff C, Rodriguez B, Burrage K. Phenomenological modeling of cell-to-cell and beat-to-beat variability in isolated Guinea Pig ventricular myocytes. *Conf Proc IEEE Eng Med Biol Soc* 2010: 1457-60, 2010.

60. Wallman M, Smith N, Rodriguez B. A comparative study of graph based, Eikonal and monodomain simulations for the estimation of cardiac activation times. *Trans Biomed Eng*, 59(6):1739-48, 2012.

61. Plank G, Burton RAB, Hales P, Bishop M, Mansoori T, Bernabeu MO, Garny A, Prassl AJ, Bollensdorff C, Mason F, Mahmood F, Rodriguez B, Grau V, Schneider J, Gavaghan D, Kohl P. Generation of histo-anatomically representative models of the individual heart: tools and application. *Phil Trans Roy Soc (A)* 367: 2257-2292, 2009.

62. Plotkowiak M, Rodriguez B, Plank G, Schneider JE, Gavaghan D, Kohl P, Grau V. High performance computer simulations of cardiac electrical function based on high resolution MRI datasets. *Lecture Notes in Computer Science* 5101: 571-580, 2008.

63. Holmes AA, Scollan DF, Winslow RL. Direct histological validation of diffusion tensor MRI in formaldehyde-fixed myocardium. *Magn. Reson. Med* 44:157-61, 2000.

64. LeGrice IJ, Smaill BH, Chai LZ, Edgar SG, Gavin JB, Hunter PJ. Laminar structure of the heart: ventricular myocyte arrangement and connective tissue architecture in the dog. *Am. J. Physiol* 269 (2 Pt 2): 571-582, 1995.

65. Vetter FJ, McCulloch AD. Three-dimensional analysis of regional cardiac function: a model of rabbit ventricular anatomy. *Prog. Biophys. Mol. Biol* 69:157-83, 1998.

66. Hooks DA, Tomlinson KA, Marsden SG, LeGrice IJ, Smaill BH, Pullan AJ, Hunter PJ. Cardiac microstructure: implications for electrical propagation and defibrillation in the heart. *Circ Res* 91: 331-8, 2002.

67. Cherry EM, Fenton FH. Effects of boundaries and geometry on the spatial distribution of action potential duration in cardiac tissue. *J Theor Biol* 285: 164-76, 2011.

68. Clayton RH, Bernus O, Cherry EM, Dierckx H, Fenton FH, Mirabella L, Panfilov AV, Sachse FB, Seemann G, Zhang H. Models of cardiac tissue electrophysiology: progress, challenges and open questions. *Prog Biophys Mol Biol* 104: 22-48, 2011.

69. Pathmanathan P, Mirams GR, Southern S, Whiteley JP. The significant effect of the choice of ionic current integration method in cardiac electro-physiological simulations. *International Journal for Numerical Methods in Biomedical Engineering* 27: 1751-1770, 2011.

70. Pathmanathan P, Gray RA. Verification of computational models of cardiac electro-physiology. Int j numer method biomed eng. 2013 Nov 20. doi: 10.1002/cnm.2615

71. Chapelle D, Fernndez MA, Gerbeau JF, Moireau P, Sainte-Marie J, Zemzemi N. Numerical simulation of the electromechanical activity of the heart. In: Ayache N, Delingette H, Sermesant M (eds). Functional Imaging and Modeling of the Heart, Volume 5528 of Lecture Notes in Computer Science. Springer: Berlin, pp. 357–365.

72. Ten Tusscher KH, Panfilov AV. Alternans and spiral breakup in a human ventricular tissue model. Am J Physiol Heart Circ Physiol. 2006 Sep;291(3):H1088-100.

73. Brennan T, Fink M, Rodriguez B. Multiscale modelling of drug-induced effects on cardiac electrophysiological activity. *Eur. J. Pharm. Sci* 36: 62-77, 2009.

74. Clark, A.J. The mode of action of drugs on cells. Arnold, London, UK, 1933.

75. Neher E, Sakmann B. Single-channel currents recorded from membrane of denervated frog muscle fibres. Nature, 260:799–802, 1976.

76. Hille B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. J Gen Physiol, 69:497–515, 1977.

77. Hill A. The possible effects of the aggregation of the molecules of hemoglobin on its dissociation curves. J Physiol, 40:4–7, 1910.

78. Starmer CF, Grant AO, Strauss HC. Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. Biophys J, 46:15–27, 1984.

79. Balser JR, Nuss B, et al. Local anesthetics as effectors of allosteric gating: lidocaine effects on inactivation-deficient rat skeletal muscle Na channels. J Clin Invest, 98(12):2874–2886, 1996.

80. Xiao_Circulation.

81. Muller JE, Ludmer PL, Willich SN, Tofler GH, Aylmer G, Klangos I, Stone PH. Circadian variation in the frequency of sudden cardiac death. *Circulation.* 1987;75:131-138.

82. Gagandeep Kaur G, Phillips C, Wong K, Saini B. Timing is important in medication administration: a timely review of chronotherapy research. Int J Clin Pharm, 2013.

83. Jeyaraj D, Haldar SM, Wan X, McCauley MD, Ripperger J, Yuan Lu K, Eapen BL, Sharma N, Ficker E, Cutler MJ, Gulick J, Sanbe A, Robbins J, Demolombe S, Kondratov RV, Shea S, Albrecht U, Wehrens X, Rosenbaum D, Jain MK. Circadian Rhythms govern cardiac repolarization dynamics and arrhythmogenesis. Nature, 483:96-101, 2012.

84. Fijorek K, Puskulluoglu M, Polak S. Circadian models of serum potassium, sodium, and calcium concentrations in healthy individuals and their application to cardiac electrophysiology simulations at individual level. Comput Math Methods Med. 2013;2013:429037.

85. Marder E, Taylor AL (2011) Multiple models to capture the variability in biological neurons and networks. Nat Neurosci 14(2):133–138.

86. Heijman J, Zaza A, Johnson DM, Rudy Y, Peeters RL, Volders PG, Westra RL. Determinants of beat-to-beat variability of repolarization duration in the canine ventricular myocyte: a computational analysis. PLoS Comput Biol. 2013 Aug;9(8):e1003202.

87. Walmsley J Rodriguez JF, Mirams GR, Burrage K, Efimov IR, Rodriguez B. mRNA expression levels in failing human hearts predict cellular electrophysiological remodelling: A population-based simulation study. *PLoS ONE* 8(2): e56359. doi:10.1371/journal.pone.0056359, 2013.

88. Hondeghem, L. M., L. Carlsson, and G. Duker. 2001. Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. Circulation. 103:2004–2013.

89. Thomsen, M. B., S. C. Verduyn, ., M. A. Vos. 2004. Increased shortterm variability of repolarization predicts d-sotalol-induced torsades de pointes in dogs. Circulation. 110:2453–2459.

90. Johnson, D. M., J. Heijman, ., P. G. Volders. 2010. I(Ks) restricts excessive beat-to-beat variability of repolarization during beta-adrenergic receptor stimulation. J. Mol. Cell. Cardiol. 48:122–130.

91. Hinterseer, M., B. M. Beckmann, ., S. Ka¨a¨b. 2010. Usefulness of short-term variability of QT intervals as a predictor for electrical remodeling and proarrhythmia in patients with nonischemic heart failure. Am. J. Cardiol. 106:216–220.

92. Myles, R. C., F. L. Burton, ., G. L. Smith. 2008. The link between repolarization alternans and ventricular arrhythmia: does the cellular phenomenon extend to the clinical problem? J. Mol. Cell. Cardiol. 45:1–10.

93. Lemay M, de Lange E, Kucera JP. Effects of stochastic channel gating and distribution on the cardiac action potential. *J Theor Biol* 281: 84-96, 2011.

94. Beard DA, Britten R, Cooling MT, Garny A, Halstead MD, Hunter PJ, Lawson J, Lloyd CM, Marsh J, Miller A, Nickerson DP, Nielsen PM, Nomura T, Subramanium S, Wimalaratne SM, Yu T. CellML metadata standards, associated tools and repositories. *Philos. Transact. A Math Phys. Eng. Sci* 367: 1845-67, 2009.

95. Quinn TA, Granite S, Allessie MA, Antzelevitch C, Bollensdorff C, Bub G, Burton RA, Cerbai E, Chen PS, Delmar M, Difrancesco D, Earm YE, Efimov IR, Egger M, Entcheva E, Fink M, Fischmeister R, Franz MR, Garny A, Giles WR, Hannes T, Harding SE, Hunter PJ, Iribe G, Jalife J,Johnson CR, Kass RS, Kodama I, Koren G, Lord P, Markhasin VS, Matsuoka S, McCulloch AD, Mirams GR, Morley GE, Nattel S, Noble D, Olesen SP, Panfilov AV, Trayanova NA, Ravens U, Richard S, Rosenbaum DS, Rudy Y,Sachs F, Sachse FB, Saint DA, Schotten U, Solovyova O, Taggart P, Tung L, Varró A, Volders PG, Wang K, Weiss JN, Wettwer E, White E, Wilders R, Winslow RL, Kohl P. Minimum Information about a Cardiac Electrophysiology Experiment (MICEE): standardised reporting for model reproducibility, interoperability, and data sharing. *Prog. Biophys. Mol. Biol* 107:4-10, 2011.

96. Noble D. Successes and failures in modeling heart cell electrophysiology. *Heart Rhythm* 8: 1798:1803, 2011.