



Na/K pump regulation of cardiac repolarization: Insights from a systems biology approach

by

**Alfonso Bueno-Orovio
Carlos Sánchez
Esther Pueyo
Blanca Rodriguez**

Na/K pump regulation of cardiac repolarization: Insights from a Systems Biology approach

Short title: Na/K pump regulation of cardiac repolarization

Alfonso Bueno-Orovio^{1,*}, Carlos Sánchez^{2,3}, Esther Pueyo^{3,2}, Blanca Rodríguez⁴

1. Oxford Centre for Collaborative Applied Mathematics, University of Oxford, Oxford, UK
2. Communications Technology Group, I3A and IIS, University of Zaragoza, Zaragoza, Spain
3. Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine, Spain
4. Department of Computer Science, University of Oxford, Oxford, UK

** Corresponding Author:*

Alfonso Bueno-Orovio (alfonso.bueno@maths.ox.ac.uk)

Oxford Centre for Collaborative Applied Mathematics, University of Oxford
Mathematical Institute, 24-29 St Giles', Oxford, OX1 3LB, United Kingdom

Phone: +44 1865 615136 / Fax: +44 1865 275383

Abstract

The sodium-potassium pump is widely recognized as the principal mechanism for active ion transport across the cellular membrane of cardiac tissue, being responsible for the creation and maintenance of the transarcolemmal sodium and potassium gradients, crucial for cardiac cell electrophysiology. Importantly, sodium-potassium pump activity is impaired in a number of major diseased conditions, including ischemia and heart failure. However, its subtle ways of action on cardiac electrophysiology, both directly through its electrogenic nature and indirectly via the regulation of cell homeostasis, make it hard to predict the electrophysiological consequences of reduced sodium-potassium pump activity in cardiac repolarization. In this review, we discuss how recent studies adopting the Systems Biology approach, through the integration of experimental and modeling methodologies, have identified the sodium-potassium pump as one of the most important ionic mechanisms in regulating key properties of cardiac repolarization and its rate-dependence, from subcellular to whole organ levels. These include the role of the pump in the biphasic modulation of cellular repolarization and refractoriness, the rate control of intracellular sodium and calcium dynamics and therefore of the adaptation of repolarization to changes in heart rate, as well as its importance in regulating pro-arrhythmic substrates through modulation of dispersion of repolarization and restitution. Theoretical findings are consistent across a variety of cell types and species including human, and widely in agreement with experimental findings. The novel insights and hypotheses on the role of the pump in cardiac electrophysiology obtained through this integrative approach could eventually lead to novel therapeutic and diagnostic strategies.

Keywords

sodium-potassium pump; cardiac repolarization; systems biology; modeling

Introduction

The sodium-potassium pump (Na/K pump), also known as Na^+, K^+ -ATPase, is the principal mechanism for active ion transport across the membrane of excitable cells [2, 18, 20, 64]. First discovered by the Danish scientist Jens Christian Skou in 1957 in his studies in the crab nerve [62], its ubiquitous importance for basic cell physiology and clinical practice was later recognized by the award of the Nobel Prize in Chemistry in 1997.

The Na/K pump is key in the regulation of Na^+ and K^+ transmembrane gradients, transporting 3 Na^+ out and 2 K^+ into the cell against their concentration gradients by consuming energy via the hydrolysis of ATP. In cardiac muscle, the creation and maintenance of the transsarcolemmal Na^+ and K^+ gradients by the Na/K pump is crucial for a variety of electrophysiological processes including the generation of the resting membrane potential and the initiation and propagation of action potentials, as well as for the regulation of secondary transport processes vital for cell function, like cell volume control or Ca^{2+} extrusion via the sodium-calcium exchanger (NCX) [38, 53]. In addition, the Na/K pump results in an electrogenic transmembrane current due to the unmatched transposition of 3 Na^+ and 2 K^+ ions per ATP unit. It therefore also plays a pivotal role in the regulation of cardiac electrophysiology under physiological and pathological conditions.

In fact, impairment of Na/K pump activity has been shown to take place in a number of diseased conditions, including atrial fibrillation [74], ischemia [16, 17], heart failure [3, 45, 60, 69], hypertension [14, 40, 43], hypo/hyper-thyroidism [40, 43] and diabetes [23, 64]. Na/K pump inhibition has also been used for therapeutic action in a number of conditions, like the treatment of atrial fibrillation and heart failure by cardiac glycosides [28, 71]. Its experimental characterization is however challenging, in part due to differences in the magnitude and speed of its associated ion flow compared to other ionic currents [20]. Indeed, ion channels allow for the rapid, passive diffusion of selected ions due to electrical and concentration gradients, so that tens of millions of ions per second can cross the membrane through just one open ion channel. However, ion pumps operate through the entire course of the action potential to slowly, actively transport ions thermodynamically uphill, resulting in a current as small as ~ 20 aA for a single Na/K molecule [20]. Hence, although it is possible to measure the total current generated by the millions of Na/K pumps located in the entire cell membrane [19, 25, 46, 61, 69, 78], the associated experimental difficulties may have limited the study of the impact of the Na/K pump on cardiac repolarization compared to other ion channel currents. Understanding the therapeutical and pathophysiological consequences of Na/K pump alterations from the cellular to the whole organ function therefore requires of further investigations.

In this paper, we highlight the contribution of Systems Biology research, through integration of experimental and theoretical investigations [6, 7, 34, 55], to elucidate the role of the Na/K pump in cardiac electrophysiology from the cellular to the whole-organ level, as illustrated in our multiscale perspective for Na/K research presented in Figure 1. We first describe how insights obtained through experimental and reductionist approaches are integrated in mathematical formulations providing quantitative descriptions of the regulation of Na/K pump activity by ion concentrations, voltage and signaling pathways such as β -adrenergic stimulation. Then, we focus on how investigations using multiscale cardiac models have identified the Na/K pump as one of the most important ionic mechanisms in regulating cardiac repolarization and its rate-dependence, bridging from cellular electrophysiology to clinical electrocardiogram recordings. Finally, we conclude our manuscript by discussing a number of open questions requiring further combined experimental and computational investigations in order to promote our current understanding of the regulation and implications of the Na/K pump activity in cardiac electrophysiology.

Mathematical models of the cardiac Na/K pump current

From the early discoveries of Jens Christian Skou, it was clearly established that both intracellular Na^+ and extracellular K^+ ions are indispensable substrates for the Na/K pump enzymatic activity [62]. In the absence of either Na^+ or K^+ ions, Na/K pumping is precluded, and the steady current through the pump becomes zero. Increasing Na^+ and K^+ concentrations gradually increases Na/K pump activity up to a saturation limit [25, 46, 62]. It was later discovered that the Na/K pump is also strongly influenced by membrane voltage, showing an inward rectification at positive potentials [19, 46].

In terms of dependence on intracellular Na^+ and extracellular K^+ concentrations, two main formulations exist for the mathematical description of the Na/K pump activity. The first one goes back in time to the work of DiFrancesco and Noble in their seminal paper from 1985 [13], where Na/K pump electrophysiology, together with NCX dynamics and intracellular Na^+ , Ca^{2+} and K^+ homeostasis, were included for the first time in the mathematical description of a cardiac myocyte. In this formulation, the ionic current associated to the Na/K pump electrogenic nature is given by

$$I_{NaK} = p_{NaK} \left(\frac{[Na^+]_i}{[Na^+]_i + K_{m,Na}} \right)^{\gamma_{Na}} \left(\frac{[K^+]_o}{[K^+]_o + K_{m,K}} \right)^{\gamma_K} f_v$$

where p_{NaK} represents the maximum Na/K pump permeability, $K_{m,Na}$ and $K_{m,K}$ the half affinity constants of Na^+ and K^+ , respectively, and γ_{Na} and γ_K the Na^+ and K^+ Hill's coefficients. A slight modification in the definition of the Na^+ and K^+ ionic factors yields the Na/K pump formulation introduced by Luo and Rudy in their 1994 description of the guinea pig ventricular myocyte [39], where

$$I_{NaK} = p_{NaK} \frac{1}{1 + \left(\frac{K_{m,Na}}{[Na^+]_i} \right)^{\gamma_{Na}}} \frac{1}{1 + \left(\frac{K_{m,K}}{[K^+]_o} \right)^{\gamma_K}} f_v$$

with the same meaning for the above defined quantities. In both formulations, the two ionic factors remain inhibited in the absence of their respective species, gradually increasing for increasing intracellular Na^+ and extracellular K^+ concentrations (Figure 2A). Small changes in intracellular Na^+ elicit larger changes in pump activity [18], reflected by a larger γ_{Na} compared to γ_K in most model parameterizations of the pump. Finally, the f_v term represents the voltage rectification of the Na/K pump, which usually also accounts for the additional modulation of Na/K activity by extracellular Na^+ concentration (Figure 2B) [39], as experimentally reported [46].

The above formulations for the Na/K pump current have been inherited in most electrophysiological models of sinoatrial, atrial, atrioventricular, Purkinje and ventricular myocytes of a variety of species, as shown in Figure 3. The DiFrancesco-Noble formulation is present in the only existing model for the atrioventricular node action potential and in the majority of those for the specialized conduction system (sinoatrial node and Purkinje fiber models). On the other hand, the Luo-Rudy formulation has become predominant in mathematical models of atrial and ventricular cardiac myocytes.

Only a minor number of published models make use of independent representations for the Na/K pump electrogenic current. Matsuoka *et al* first introduced a Markov chain formulation in order to describe the Post-Albers cycle [41], which models the enzymatic Na/K pump cycle as transitions between different phosphorylated and dephosphorylated states depending on the position of the cation-binding sites across the transarcolemmal domain. Smith and Crampin further extended these active transport ideas, providing a framework to account for thermodynamic principles, as well as for Na/K dependence on intracellular K^+ concentration and ATP and pH sensitivity [63]. Terkildsen *et al* later incorporated this Na/K formulation into a computational model of guinea pig ventricular electrophysiology [66]. Other recent computational models also make use of this modeling approach, as the O'Hara *et al* description of the human ventricular myocyte [49].

At the signaling level, multiple factors are known to influence the Na/K pump and its tight regulation by the phospholemman (PLM) protein, such as an increased Na/K pump activity under β -adrenergic stimulation due to PLM phosphorylation via protein kinase A (PKA) [2, 10, 11]. A number of theoretical studies have incorporated a phenomenological description of the effects of β -adrenergic stimulation in the Na/K pump current by considering either a 20%-30% increase in the maximum Na/K pump permeability [15, 77] or a 29%-35% decrease in its Na^+ half affinity constant [27, 36]. Latest computational models of the β -adrenergic cascade provide a more detailed characterization of the PKA-dependent phosphorylation of the Na/K current, by considering the fraction of phosphorylated channels in different cellular subcompartments according to their respective concentrations of β -adrenergic receptor agonists [27].

Multi-scale studies of Na/K pump regulation of cardiac repolarization

As mentioned earlier, the mathematical descriptions of the cardiac Na/K pump current have been integrated in a variety of models of cardiac cell electrophysiology. Computational studies using the cellular models have allowed quantification of the specific role of the pump in cardiac electrophysiology, by monitoring the evolution of specific ionic properties under a variety of conditions. This has yielded novel insights on the importance of the Na/K pump in regulating cellular repolarization, both through direct effects caused by its electrogenic nature and indirectly through regulation of cell homeostasis. Importantly, these findings have been found to be consistent across species and cell types, including human, rabbit and dog and ventricular and atrial tissues [9, 52, 57, 58, 59, 66]. Figures 4-6 describe some of the main results of these studies.

Mathematical models of the Na/K pump have allowed dissecting multiscale mechanisms underlying seemingly counterintuitive results. Under the effects of most cardiac glycosides, Na/K current block shortens action potential duration (APD), an action a priori unexpected since the blockade of an outward current should presumably prolong APD (as yet assumed in a number of recent studies on the pump [5, 18]). However, this modulation of action potential repolarization occurs through a biphasic mechanism (Figure 4A), in which APD is initially notably prolonged after Na/K current reduction, then followed by a progressive shortening below its control value, which is well documented [26, 30, 31, 33, 37, 38, 75]. Understanding the electrophysiological basis of this phenomenon is also of clinical relevance, since the initial prolongation phase has been associated with an increased vulnerability to atrial tachyarrhythmia initiation in patients with paroxysmal supraventricular tachyarrhythmias [26].

Single-cell simulations revealed that the APD shortening caused by Na/K inhibition is due to a cascade-like mechanism initiated by the progressive accumulation of intracellular Na^+ within the membrane cytoplasm [59], in agreement with [38]. The decreased transsarcolemmal Na^+ gradient (driving force of the NCX) subsequently leads to a gradual raise of intracellular Ca^{2+} , thus potentiating the outward component (reverse mode) of the exchanger, which contributes to a faster cell repolarization (Figure 4A). Computer simulations also helped to exclude other possible mechanisms that could have mediated in the biphasic APD response (such as the L-type Ca^{2+} current or an impaired balance of currents between the sarcoplasmic reticulum and the intracellular space). Moreover, tissue simulations further predicted a similar biphasic behavior for effective refractory period (ERP) and a long-term decrease in conduction velocity [59], which were both corroborated by the clinical *in vivo* data (Figure 4B) [26]. Finally, the computational study indicated that the above biphasic characteristics of Na/K current block on APD, ERP and conduction velocity were attenuated under conditions of chronic atrial fibrillation [59], for which therapies based on the pharmacological action of Na/K block (such as glycosides administration) might still be beneficial.

Simulation studies have also identified the Na/K pump as the main determinant of other important rate-dependent properties of cardiac repolarization, such as the adaptation of APD to changes in heart rate [52, 57, 58]. Figure 5A shows the characteristics of APD adaptation to sudden modifications in pacing rate in a model of human ventricular electrophysiology, under control conditions and partial block of the Na/K current [52]. Cellular electrophysiological models unveiled that the slow component of the APD adaptation process is predominantly dominated by intracellular Na^+ accumulation [9, 52], and therefore Na/K inhibition significantly lengthens the adaptation time. The key role of the Na/K pump in modulating APD adaptation predicted by the simulations was confirmed experimentally using microelectrode action potential recordings in the presence of the Na/K blocker strophanthin, which at all doses significantly protracted the APD adaptation response, as illustrated in Figure 5B [52].

These mechanistic investigations on the time course of APD adaptation have also allowed providing a cellular and ionic basis to important clinical findings at the body-surface electrocardiogram, such as those suggesting QT-rate adaptation as a risk-stratification biomarker of arrhythmic mortality in patients with ischemic heart disease [22, 51]. Indeed, the simulation study in [52] showed that: i) QT-rate adaptation in the electrocardiogram can be explained by APD adaptation at the cellular level; ii) the Na/K pump current is the main ionic determinant of APD and QT-rate adaptation; and iii) inhibition of the Na/K pump activity results in abnormalities in Na^+ and Ca^{2+} homeostasis, which may increase arrhythmic risk through alterations in ERP, also leading to an increased likelihood of early- and delayed-afterdepolarizations [24, 52, 75].

In this regard, a recent study combining *in vivo* electrophysiological recordings in human and computer simulations has shed further light into additional contributions of heterogeneous APD adaptation in modulating the pro-arrhythmic substrate [4]. Electrophysiological measurements simultaneously acquired from the left and right ventricles of patients with structurally normal hearts revealed significant interventricular differences in APD and APD adaptation in the *in vivo* human ventricles (Figure 6A). The simulation study then expanded the information obtained from the electrophysiological recordings to show that interventricular differences in APD adaptation may increase dispersion of repolarization and potentiate arrhythmogenesis, promoting unidirectional block and reentry (Figure 6B) [4]. Due to the strong link between APD adaptation and the Na/K pump activity identified in previous studies, the interventricular differences in APD adaptation could be likely due to regional differences in Na/K expression, as reported by Wang *et al* in the non-failing human heart [70]. Similar interventricular differences in Na/K expression and activity have been reported in other species, such as the rat ventricles [35].

Simulation studies have also suggested a role for the Na/K pump in regulating other major properties of cardiac repolarization, such as APD restitution. This magnitude relates the variation observed in APD after a modification in pacing rate, either in steady-state conditions (dynamic APD restitution) or in the beat immediately after the change in pacing rate (S_1 - S_2 APD restitution). When plotted against pacing cycle length or diastolic interval, a steep slope of the APD restitution curve is considered as an arrhythmic-risk biomarker related to inducibility of reentry and the transition from sustained arrhythmias to fibrillation [47, 72, 76]. Computational investigations using ventricular and atrial cell models in human, rabbit and dog have shown that Na/K current block notably flattens restitution curves [52, 57, 58, 59], therefore decreasing life-threatening risk during reentrant behavior. The above findings were found to be qualitatively consistent across species and cell types despite the use of cellular models with different Na/K formulations and parameterizations, even though quantitative differences exist. In this regard, the *in vivo* human atrial study in [26] demonstrated, during the phase of action potential shortening after digoxin administration, a narrowing in the gap between the termination of effective refractoriness and the completion of action potential repolarization, coinciding with a diminished vulnerability to tachyarrhythmias. However, experimental data on APD restitution during pharmacological Na/K block are scarce and limited, and further experimental research is needed in order to validate these results.

Finally, a Systems Biology approach has enabled dissecting the specific contribution of the Na/K pump in a number of pathophysiological conditions, difficult to determine experimentally. Computational investigations on ischemia-induced electrophysiological changes driven by impaired cellular metabolism showed that the balance between inactivation and activation of the Na/K pump, in companionship with the activation of the ATP-sensitive K^+ current, underlies the time course of extracellular K^+ accumulation during myocardial ischemia, which predisposes the heart to the development of reentry and lethal ventricular arrhythmias [56, 66]. The analysis of the different Na^+ flux pathways further suggested that the reduced Na/K flux during acute myocardial ischemia plays the largest role in exacerbated Na^+ overload during reperfusion, and therefore in the possible development of reperfusion injury [54]. Mechanistic investigations using computational models have also evidenced that intracellular Na^+ accumulation in heart failure is mainly driven by the electrophysiological remodeling of the Na/K pump current, consequently contributing to the deregulation of intracellular Ca^{2+} homeostasis in the failing cardiac myocytes [50, 67].

Discussion and Perspectives

The ubiquitous importance of the Na/K pump at all levels of cellular physiology is nowadays well accepted and established. In cardiac muscle, its electrophysiological action has been traditionally linked to determining a number of basic properties crucial for the generation and propagation of cardiac electrical activity, such as the regulation of subsarcolemmal Na^+ concentrations or the control of the resting membrane potential. However, the results analyzed in this review highlight a number of additional contributions of the pump for cardiac repolarization, due to both its electrogenic nature and homeostasis regulatory action. These include the modulation of APD, APD adaptation to changes in heart rate, dispersion of repolarization, and rate dependence of intracellular Na^+ and Ca^{2+} dynamics.

Gaining of these novel insights has been only possible through the use of a Systems Biology approach that

allows for a unified integration of experimental and computational investigations, bridging from the ionic to the whole organ levels. Mathematical formulations of the Na/K provide quantitative descriptions of its regulation by ion concentrations, voltage and signaling pathways, thus granting the assessment of the specific role of the pump under a variety of conditions. The integration of additional existing knowledge in this modeling framework may therefore help us to further advance our still limited understanding of the impact of the Na/K pump in cardiac electrophysiology.

As a case in point, much is known at present in terms of the crystal structure of the Na/K pump and its conformational composition of α and β subunits (and an additional γ subunit in other tissues, such as kidney and brain) [44]. However, although four α and three β subunit isoforms have been found, only α_1 , α_2 and β_1 are expressed at significant levels in cardiac myocytes [42, 18]. In cardiac myocytes, a differential expression of Na/K isoforms in the sarcolemmal membrane, with α_2 subunits found more predominantly at the t-tubules, has been reported [1, 65]. Correspondingly, recent experimental studies have also indicated that the α_2 isoform may have a greater impact on intracellular Ca^{2+} handling than α_1 pumps [11], the latter being responsible for maintaining a separate Na^+ pool [12]. The voltage rectification of the α_2 subunit was also found to be more strongly influenced by extracellular Na^+ and K^+ [8, 29]. These facts suggest the idea of a possible subdivision of the cardiac Na/K pump current in computational models of cardiac electrophysiology into two separate components (as it was once made for the rapid and slow contributions of the K^+ rectifier current). This, together with the definition of appropriate Na^+ subcompartments in the cellular models, might help to further elucidate the mechanisms of intracellular Na^+ handling and Na/K-NCX coupling, and of those responsible of Na/K pump-mediated triggered arrhythmias driven by intracellular Ca^{2+} overload, such as delayed-afterdepolarizations due to cardiac glycosides intoxication [32, 71, 75].

At the signaling level, a significant effort is being developed in order to incorporate adrenergic Na/K pump regulation into cellular models of cardiac electrophysiology, such as PLM phosphorylation via PKA [27]. On the other hand, additional processes such as PLM phosphorylation by protein kinase C [2, 10] still remain computationally unexplored. To render an even more complicated picture, further signaling pathways such as nitric oxide stimulation of the Na/K pump [73], the role of oxidant stress on reducing the pump current [61], Na/K inhibition by PLM palmitoylation [68], or Na/K regulation by acetylcholine, insulin or hormones [23], are nowadays also well established. As noted by Fuller *et al*, “the consequences of the simultaneous activation of all the signaling pathways [...] are hard to predict, and the balance between them will undoubtedly vary between health and disease” [18]. The use of electrophysiological models incorporating metabolic regulation and up-to-date knowledge on the complete adrenergic stimulation cascade might therefore provide newer insights into how the concurrent activation of these regulatory agents influences Na/K pump activity and cellular electrophysiology, in particular under conditions of impaired metabolic supply, such as myocardial ischemia. Simulation studies using whole organ models could also be useful in order to determine how dispersion of repolarization and rate dependent properties are regulated at the organ level by Na/K function, including the positive inotropic effect in electrical-contraction coupling associated to Na/K-related therapy via cardiac glycosides. Novel theoretical findings will also be key to direct further experimental research by providing novel hypotheses on the role of the Na/K pump in cardiac electromechanical activity and arrhythmogenesis, as well as in the development of possible new treatments targeting this current [21].

Acknowledgements

The first author's contribution to this work was supported by Award No. KUK-C1-013-04, made by King Abdullah University of Science and Technology (KAUST). CS and EP are supported by grant TEC2010-19410 from Ministerio de Economía y Competitividad (MINECO), Spain. EP acknowledges the financial support of Ramón y Cajal program from MINECO, Spain. BR holds Medical Research Council Career Development, Industrial Partnership and MRC Centenary Awards.

References

1. Berry RG, Despa S, Fuller W, Bers DM, Shattock MJ (2007) Differential distribution and regulation of mouse cardiac Na⁺/K⁺-ATPase alpha1 and alpha2 subunits in T-tubule and surface sarcolemmal membrane. *Cardiovasc Res* 73:92-100.
2. Bers DM, Despa S (2009) Na/K-ATPase--an integral player in the adrenergic fight-or-flight response. *Trends Cardiovasc Med* 19:111-118.
3. Bossuyt J, Ai X, Moorman JR, Pogwizd SM, Bers DM (2005) Expression and phosphorylation of the Na-pump regulatory subunit phospholemman in heart failure. *Circ Res* 97:558-565.
4. Bueno-Orovio A, Hanson BM, Gill JS, Taggart P, Rodriguez B (2012) In vivo human left-to-right ventricular differences in rate adaptation transiently increase pro-arrhythmic risk following rate acceleration. *PLoS ONE* 7(12):e52234.
5. Carmeliet E (2006) Action potential duration, rate of stimulation, and intracellular sodium. *J Cardiovasc Electrophysiol* 17:S2-S7.
6. Carusi A, Burrage K, Rodriguez B (2012) Bridging experiments, models and simulations: an integrative approach to validation in computational cardiac electrophysiology. *Am J Physiol Heart Circ Physiol* 303:H144-H155.
7. Cherry EM, Fenton FH, Gilmour RF Jr (2012) Mechanisms of ventricular arrhythmias: a dynamical systems-based perspective. *Am J Physiol Heart Circ Physiol* 302:H2451-H2463.
8. Crambert G, Hasler U, Beggah AT, Yu C, Modyanov NN, Horisberger JD, Lelievre L, Geering K (2000) Transport and pharmacological properties of nine different human Na, K-ATPase isozymes. *J Biol Chem* 275:1976-1986.
9. Decker KF, Heijman J, Silva JR, Hund TJ, Rudy Y (2009) Properties and ionic mechanisms of action potential adaptation, restitution and accommodation in canine epicardium. *Am J Physiol Heart Circ Physiol* 296:H1017-H1026.
10. Despa S, Bossuyt J, Han F, Ginsburg KS, Jia LG, Kutchai H, Tucker AL, Bers DM (2005) Phospholemman-phosphorylation mediates the β -adrenergic effects on Na/K pump function in cardiac myocytes. *Circ Res* 97:252-259.
11. Despa S, Tucker AL, Bers DM (2008) PLM-mediated activation of Na/K-ATPase limits [Na]_i and inotropic state during β -adrenergic stimulation in mouse ventricular myocytes. *Circulation* 117:1849-1855.
12. Despa S, Lingrel JB, Bers DM (2012) Na/K-ATPase alpha2-isoform preferentially modulates Ca transients and sarcoplasmic reticulum Ca release in cardiac myocytes. *Cardiovasc Res* 95:480-486.
13. DiFrancesco D, Noble D (1985) A model of cardiac electrical activity incorporating ionic pumps and concentration changes. *Philos Trans Roy Soc Lond B Biol* 307:353-398.
14. Dostanic-Larson I, van Huysse JW, Lorenz JN, Lingrel JB (2005) The highly conserved cardiac glycoside binding site of Na,K-ATPase plays a role in blood pressure regulation. *Proc Natl Acad Sci USA* 102:15845-15850.
15. Faber GM, Rudy Y (2007) Calsequestrin mutation and catecholaminergic polymorphic ventricular tachycardia: a simulation study of cellular mechanism. *Cardiovasc Res* 75:79-88.
16. Fuller W, Parmar V, Eaton P, Bell JR, Shatton MJ (2003) Cardiac ischemia causes inhibition of the Na/K ATPase by a labile cytosolic compound whose production is linked to oxidant stress.

Cardiovasc Res 57:1044-1051.

17. Fuller W, Eaton P, Bell JR, Shattock MJ (2004) Ischemia-induced phosphorylation of phospholemman directly activates rat cardiac Na/K ATPase. *FASEB J* 18:197-199.
18. Fuller W, Tulloch LB, Shattock MJ, Calaghan SC, Howie J, Wypijewski KJ (2013) Regulation of the cardiac sodium pump. *Cell Mol Life Sci* 70:1357-1380.
19. Gadsby DC, Nakao M (1989) Steady-state current-voltage relationship of the Na/K pump in guinea pig ventricular myocytes. *J Gen Physiol* 94:511-537.
20. Gadsby DC (2009) Ion channels versus ion pumps: the principal difference, in principle. *Nat Rev Mol Cell Biol* 10:344-352.
21. Gheorghide M, Ambrosy AP, Ferrandi M, Ferrari P (2011) Combining SERCA2a activation and Na-K ATPase inhibition: a promising new approach to managing acute heart failure syndromes with low cardiac output. *Discov Med* 12:141-151.
22. Gill JS, Baszko A, Xia R, Ward DE, Camm AJ (1993) Dynamics of the QT interval in patients with exercise-induced ventricular tachycardia in normal and abnormal hearts. *Am Heart J* 126:1357-1363.
23. Glitsch HG (2001) Electrophysiology of the sodium-potassium-ATPase in cardiac cells. *Physiol Rev* 81:1791-1826.
24. Grom A, Faber TS, Brunner M, Bode C, Zehender M (2005) Adaptation of ventricular repolarization after sudden changes in heart rate due to conversion of atrial fibrillation. A potential risk factor for proarrhythmia? *Europace* 7:113-121.
25. Han F, Tucker AL, Lingrel JB, Despa S, Bers DM (2009) Extracellular potassium dependence of the Na⁺-K⁺-ATPase in cardiac myocytes: isoform specificity and effect of phospholemman. *Am J Physiol Cell Physiol* 297:C699-C705.
26. Hayward RP, Hamer J, Taggart P, Emanuel R (1983) Observations on the biphasic nature of digitalis electrophysiological actions in the human right atrium. *Cardiovasc Res* 17:533-546.
27. Heijman J, Volders PG, Westra RL, Rudy Y (2011) Local control of β -adrenergic stimulation: Effects on ventricular myocyte electrophysiology and Ca²⁺-transient. *J Mol Cell Cardiol* 50:863-71.
28. Hood WB Jr, Dans AL, Guyatt GH, Jaeschke R, McMurray JJ (2004) Digitalis for treatment of congestive heart failure in patients in sinus rhythm. *Cochrane Database Syst Rev* 2004:CD002901.
29. Horisberger JD, Kharoubi-Hess S (2002) Functional differences between alpha subunit isoforms of the rat Na, K-ATPase expressed in *Xenopus* oocytes. *J Physiol* 539:669-680.
30. Hordof AJ, Spotnitz A, Mary-Rabine L, Edie RN, Rosen MR (1978) The cellular electrophysiologic effects of digitalis on human atrial fibers. *Circulation* 57:223-229.
31. Ito M, Hollander PB, Marks BH, and Dutta S (1970) The effects of six cardiac glycosides on the transmembrane potential and contractile characteristics of the right ventricle of guinea pig. *J Pharmacol Exp Ther* 172:188-195.
32. Karagueuzian HS, Katzung BG (1981) Relative inotropic and arrhythmogenic effects of five cardiac steroids in ventricular myocardium: oscillatory afterpotentials and the role of endogenous catecholamines. *J Pharmacol Exp Ther* 218:348-356.
33. Kassebaum D (1963) Electrophysiological effect of strophanthin in the heart. *J Pharmacol Exp Ther* 140:329-338.

34. Kohl P, Crampin EJ, Quinn TA, Noble D (2010) Systems Biology: an approach. *Clin Pharmacol Ther* 88:25-33.
35. Komniski MS, Yakushev S, Bogdanov N, Gassmann M, Bogdanova A (2011) Interventricular heterogeneity in rat heart response to hypoxia: the tuning of glucose metabolism, ion gradients, and function. *Am J Physiol Heart Circ Physiol* 300:H1645-H1652.
36. Kuzumoto M, Takeuchi A, Nakai H, Oka C, Noma A, Matsuoka S (2008) Simulation analysis of intracellular Na⁺ and Cl⁻ homeostasis during β 1-adrenergic stimulation of cardiac myocyte. *Prog Biophys Mol Biol* 96:171-186.
37. Levi AJ (1991) The effect of strophantidin on action potential, calcium current and contraction in isolated guinea-pig ventricular myocytes. *J Physiol* 442:1-23.
38. Levi AJ (1993) A role for sodium/calcium exchange in the action potential shortening caused by strophanthidin in guinea pig ventricular myocytes. *Cardiovasc Res* 27:471-481.
39. Luo CH, Rudy Y (1994) A dynamic model of the cardiac ventricular action potential: II. Afterdepolarizations, triggered activity and potentiation. *Circ Res* 74:1097-1113.
40. Magyar CE, Wang J, Azuma KK, McDonough AA (1995) Reciprocal regulation of cardiac Na-K-ATPase and Na/Ca exchanger: hypertension, thyroid hormone, development. *Am J Physiol Cell Physiol* 269:C675-C682.
41. Matsuoka S, Sarai N, Kuratomi S, Ono K, Noma A (2003) Role of individual ionic current systems in ventricular cells hypothesized by a model study. *Jpn J Physiol* 53:105-123.
42. McDonough AA, Zhang Y, Shin V, Frank JS (1996) Subcellular distribution of sodium pump isoform subunits in mammalian cardiac myocytes. *Am J Physiol* 270:C1221-C1227.
43. McDonough AA, Velotta JB, Schwinger RHG, Philipson KD, Farley RA (2002) The cardiac sodium pump: structure and function. *Basic Res Cardiol* 97:I19-I24.
44. Morth JP, Pedersen BP, Toustrup-Jensen MS, Sorensen TL, Petersen J, Andersen JP, Vilsen B, Nissen P (2007) Crystal structure of the sodium-potassium pump. *Nature* 450:1043-1049.
45. Müller-Ehmsen J, McDonough AA, Farley R, Schwinger RHG (2002) Sodium pump isoform expression in heart failure: implication for treatment. *Basic Res Cardiol* 97:I25-I30.
46. Nakao M, Gadsby DC (1989) [Na] and [K] dependence of the Na/K pump current-voltage relationship in guinea pig ventricular myocytes. *J Gen Physiol* 94:539-565.
47. Narayan SM, Kazi D, Krummen DE, Rappel WJ (2008) Repolarization and activation restitution near human pulmonary veins and atrial fibrillation initiation. *J Am Coll Cardiol* 52:1222-1230.
48. Noble D, Garny A, Noble PJ (2012) How the Hodgkin-Huxley equations inspired the Cardiac Physiome Project. *J Physiol* 590:2613-2628.
49. O'Hara T, Virág L, Varró A, Rudy Y (2011) Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. *PLoS Comput Biol* 7:e1002061.
50. Priebe L, Beuckelmann DJ (1998) Simulation study of cellular electric properties in heart failure. *Circ Res* 82:1206-1223.
51. Pueyo E, Smetana P, Caminal P, de Luna AB, Malik M, Laguna P (2004) Characterization of QT interval changes and its use as a risk-stratifier of arrhythmic mortality in amiodarone-treated survivors

- of acute myocardial infarction. *IEEE Trans Biomed Eng* 51:1511-1520.
52. Pueyo E, Husti Z, Hornyik T, Baczko I, Laguna P, Varro A, Rodriguez B (2010) Mechanisms of ventricular rate adaptation as a predictor of arrhythmic risk. *Am J Physiol Heart Circ Physiol* 298:1577-1587.
 53. Ramirez RJ, Sah R, Liu J, Rose RA, Backx PH (2011) Intracellular $[Na^+]$ modulates synergy between Na^+/Ca^{2+} exchanger and L-type Ca^{2+} current in cardiac excitation-contraction coupling during action potentials. *Basic Res Cardiol* 106:967-977.
 54. Roberts BN, Christini DJ (2011) NHE inhibition does not improve Na^+ or Ca^{2+} overload during reperfusion: using modeling to illuminate the mechanisms underlying a therapeutic failure. *PLoS Comput Biol* 7:e1002241.
 55. Roberts BN, Yang P, Behrens SB, Moreno JD, Clancy CE (2012) Computational approaches to understand cardiac electrophysiology and arrhythmias. *Am J Physiol Heart Circ Physiol* 303:H766-H783.
 56. Rodriguez B, Ferrero JM Jr, Trenor B (2002) Mechanistic investigation of extracellular K^+ accumulation during acute myocardial ischemia: a simulation study. *Am J Physiol Heart Circ Physiol* 283:H490-H500.
 57. Romero L, Pueyo E, Fink M, Rodriguez B (2009) Impact of biological variability on human ventricular cellular electrophysiology. *Am J Physiol Heart Circ Physiol* 297:H1436-H1445.
 58. Romero L, Carbonell B, Trenor B, Rodriguez B, Saiz J, Ferrero JM (2011) Systematic characterization of the ionic basis of rabbit cellular electrophysiology using two ventricular models. *Prog Biophys Mol Biol* 107:60-73.
 59. Sanchez C, Corrias A, Bueno-Orovio A, Davies M, Swinton J, Jacobson I, Laguna P, Pueyo E, Rodriguez B (2012) The Na^+/K^+ pump is an important modulator of refractoriness and rotor dynamics in human atrial tissue. *Am J Physiol Heart Circ Physiol* 302:H1146-H1149.
 60. Schwinger RHG, Wang J, Frank K, Müller-Ehmsen J, Brixius K, McDonough AA, Erdmann E (1999) Reduced sodium pump α_1 , α_3 , and β_1 -isoform protein levels and Na^+,K^+ -ATPase activity but unchanged Na^+-Ca^{2+} exchanger protein levels in human heart failure. *Circulation* 99:2105-2112.
 61. Shattock MJ, Matsuura H (1993) Measurement of Na^+-K^+ current in isolated rabbit ventricular myocytes using the whole-cell voltage-clamp technique. Inhibition of the pump by oxidant stress. *Circ Res* 72:91-101.
 62. Skou JC (1957) The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim Biophys Acta* 23:394-401.
 63. Smith N, Crampin E (2004) Development of models of active ion transport for whole-cell modeling: cardiac sodium-potassium pump as a case study. *Prog Biophys Mol Biol* 85:387-405.
 64. Suhail M (2010) Na^+,K^+ -ATPase: ubiquitous multifunctional transmembrane protein and its relevance to various pathophysiological conditions. *J Clin Med Res* 2:1-17.
 65. Swift F, Tovsrud N, Enger UH, Sjaastad I, Sejersted OM (2007) The Na^+/K^+ -ATPase alpha2-isoform regulates cardiac contractility in rat cardiomyocytes. *Cardiovasc Res* 75:109-117.
 66. Terkildsen JR, Crampin EJ, Smith NP (2007) The balance between inactivation and activation of the Na^+-K^+ pump underlies the triphasic accumulation of extracellular K^+ during myocardial ischemia. *Am J Physiol Heart Circ Physiol* 293:H3036-H3045.

67. Trenor B, Cardona K, Gomez JF, Rajamani S, Ferrero JM Jr, Belardinelli L, Saiz J (2012) Simulation and mechanistic investigation of the arrhythmogenic role of the late sodium current in human heart failure. *PLoS ONE* 7:e32659.
68. Tulloch LB, Howie J, Wypijewski KJ, Wilson CR, Bernard WG, Shattock MJ, Fuller W (2011) The inhibitory effect of phospholemman on the sodium pump requires its palmitoylation. *J Biol Chem* 286:36020-36031.
69. Verdonck F, Volders PGA, Vos MA, Sipido KR (2003) Increased Na^+ concentration and altered Na/K pump activity in hypertrophied canine ventricular cells. *Cardiovasc Res* 57:1035-1043.
70. Wang J, Schwinger RHG, Frank K, Müller-Ehmsen J, Martin-Vasallo P, Pressley TA, Xiang A, Erdmann E, McDonough AA (1996) Regional expression of sodium pump subunits isoforms and Na^+ - Ca^{++} exchanger in the human heart. *J Clin Invest* 98:1650-1658.
71. Wasserstrom JA, Aistrup GL (2005) Digitalis: new actions for an old drug. *Am J Physiol Heart Circ Physiol* 289:H1781-H1793.
72. Weiss JN, Qu Z, Chen PS, Lin SF, Karagueuzian HS, Hayashi H, Garfinkel A, Karma A (2005) The dynamics of cardiac fibrillation. *Circulation* 112:1232-1240.
73. White CN, Hamilton EJ, Garcia A, Wang D, Chia KK, Figtree GA, Rasmussen HH (2008) Opposing effects of coupled and uncoupled NOS activity on the Na^+ - K^+ pump in cardiac myocytes. *Am J Physiol Cell Physiol* 294:C572-C578.
74. Workman AJ, Kane KA, Rankin AC (2003) Characterisation of the Na, K pump current in atrial cells from patients with and without chronic atrial fibrillation. *Cardiovasc Res* 59:593-602.
75. Xie JT, Cunningham PM, January CT (1995) Digoxin-induced delayed afterdepolarizations: biphasic effects of digoxin on action potential duration and the Q-T interval in cardiac Purkinje fibers. *Methods Find Exp Clin Pharmacol* 17:113-120.
76. Yue L, Feng J, Gaspo R, Li GR, Wang Z, Nattel S (1997) Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. *Circ Res* 81:512-525.
77. Zeng J, Rudy Y (1995) Early afterdepolarizations in cardiac myocytes: mechanism and rate dependence. *Biophys J* 68:949-964.
78. Zhang XQ, Moorman JR, Ahlers BA, Carl LL, Lake DE, Song J, Mounsey JP, Tucker AL, Chan YM, Rothblum LI, Stahl RC, Carey DJ, Cheung JY (2006) Phospholemman overexpression inhibits Na^+ , K^+ -ATPase in adult rat cardiac myocytes: relevance of decreased Na^+ pump activity in postinfarction myocytes. *J Appl Physiol* 100:212-220.

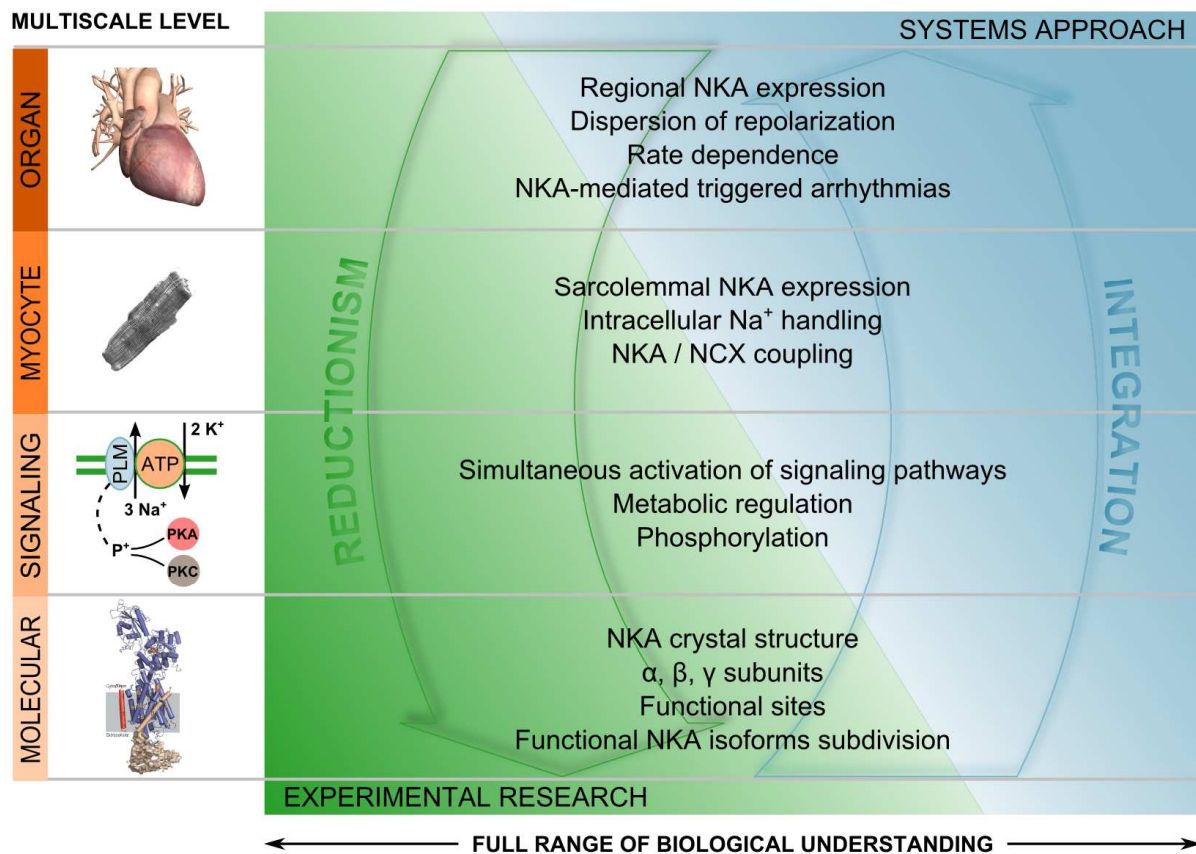


Figure 1: Multiscale systems approach perspective for Na/K electrophysiology research. Clinical and experimental research may further benefit from this integrative methodology at higher levels of biological integration, in order to promote our current understanding of Na/K function and its multiple ways of action in cardiac electrophysiology. NKA: Na⁺,K⁺-ATPase; NCX: Na⁺-Ca²⁺ exchanger.

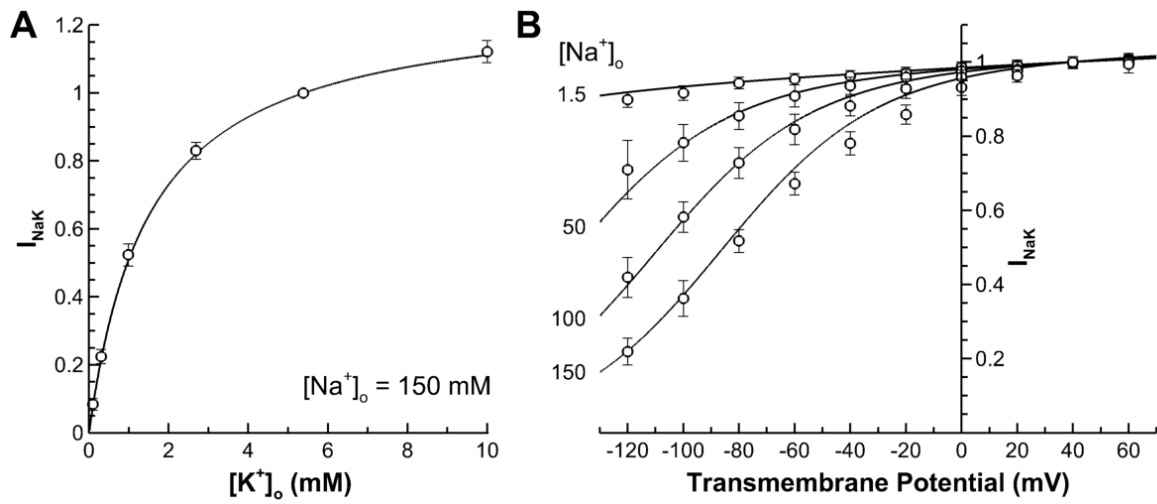


Figure 2. A: Influence of extracellular potassium concentration ($[K^+]_o$) in Na/K pump current amplitude, normalized to control runs at 5.4 mM $[K^+]_o$. **B:** Voltage dependence of the Na/K pump current for different extracellular sodium concentrations ($[Na^+]_o$) at 5.4 mM $[K^+]_o$, normalized to control runs at 40 mV. Solid lines represent computer model results using model parameters as in [39]. Experimental data (mean \pm SD, open circles) adapted from [46].

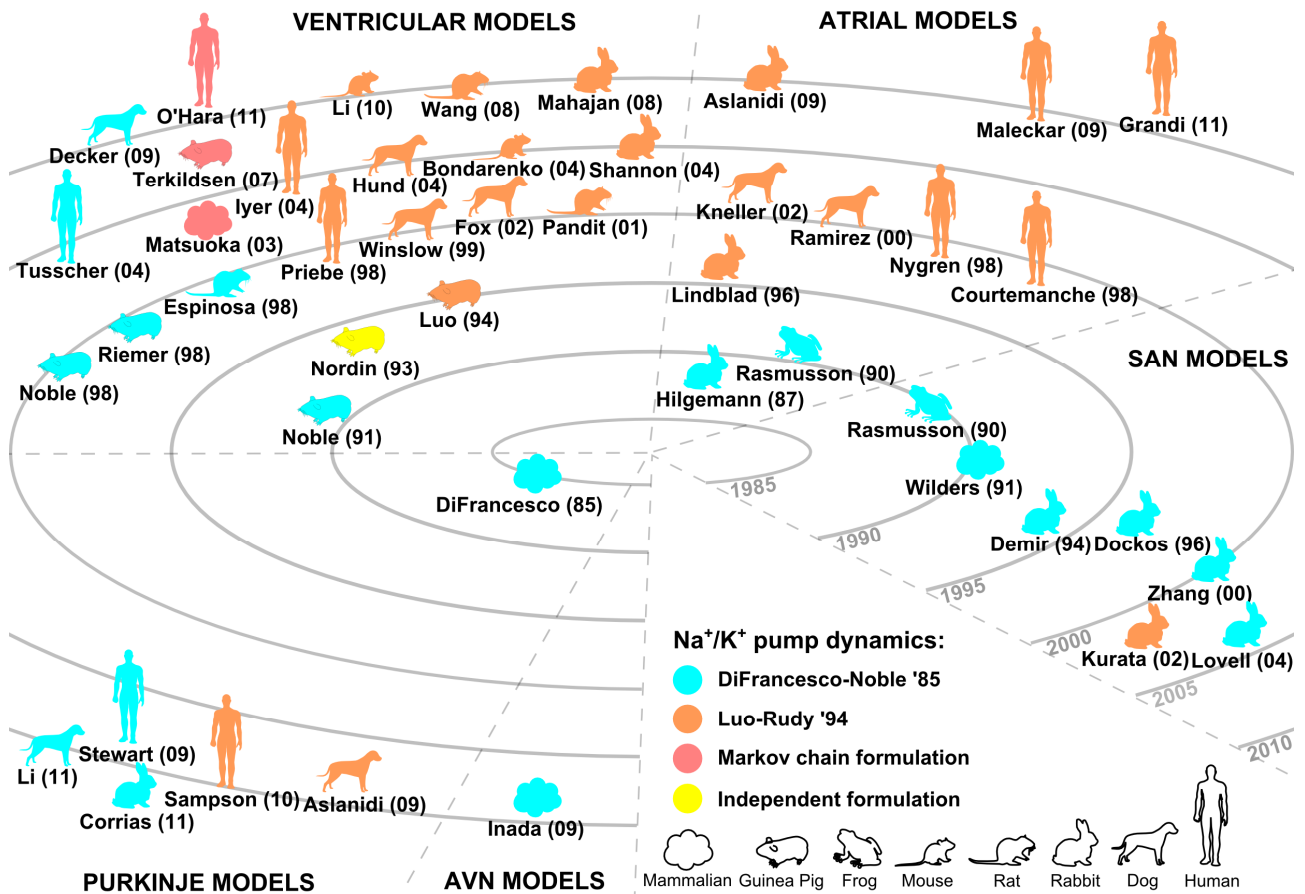


Figure 3: Inheritance of Na/K pump formulation in a selection of modern models of cardiac electrophysiology. Models are referred by first author's name and year of publication. The complete list of references can be found in [48].

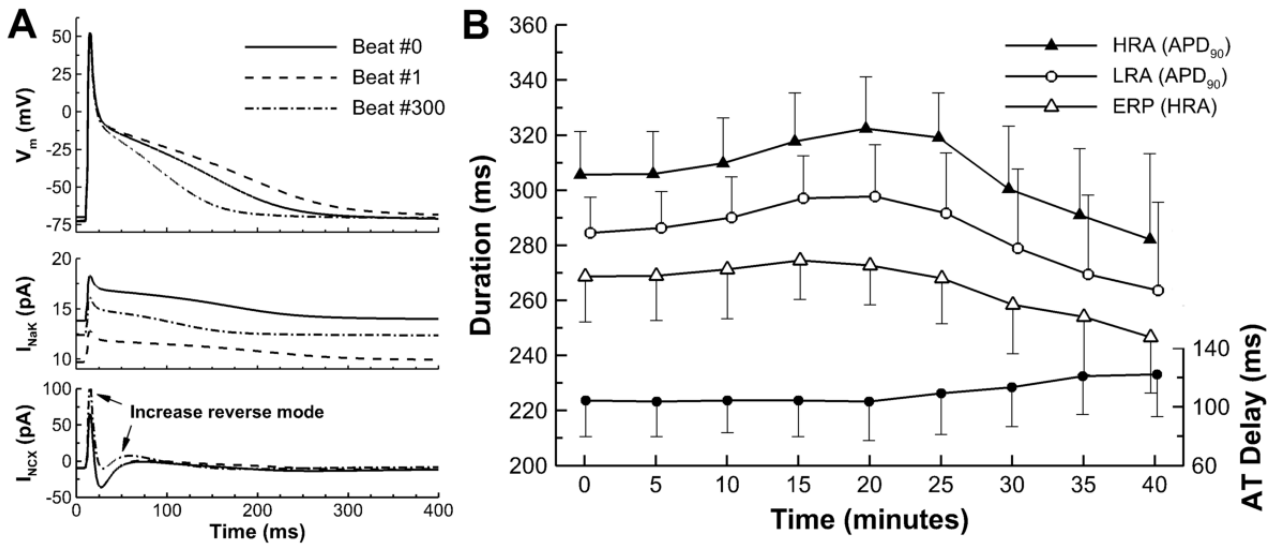


Figure 4. A: Computational investigations on the biphasic effect of Na/K block in human atrial action potential duration (APD). Na/K block in steady-state conditions (beat #0) leads to the initial prolongation of APD (beat #1), then followed by its progressive shortening (beat #300). The gradual accumulation of intracellular Na^+ compensates the sudden reduction in Na/K pump current (I_{NaK}), and potentiates the reverse mode of the Na^+ - Ca^{2+} exchanger (I_{NCX}), which results in APD shortening. **B:** Clinical observations of the biphasic nature of Na/K pump inhibition in human atria (mean \pm SD, modified from [26]). APD at 90% of repolarization (APD_{90}) was monitored at two atrial sites (HRA: high right atrium; LRA: low right atrium), exhibiting APD lengthening/shortening after digitalis administration. The in-vivo data also shows a similar biphasic behavior in effective refractory period (ERP), and a progressive increase in the activation time (AT) delay between HRA and the His bundle (filled dots, right axis of the panel).

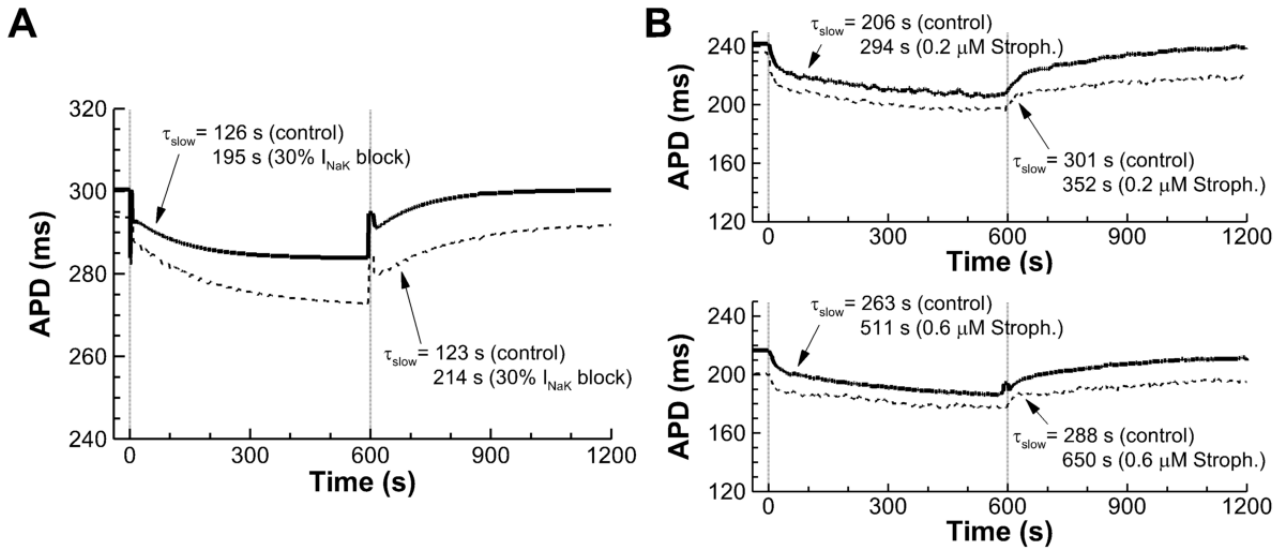


Figure 5. A: Effects of Na/K current block in protracting the slow phase of action potential duration (APD) adaptation to sudden changes in pacing rate, in a model of human ventricular electrophysiology. Results are presented for control conditions (thick line) and under partial Na/K current block (dashed line). Changes in pacing rate (1000 ms to 600 ms, then to 1000 ms) are indicated by the dotted vertical lines. **B:** Experimental confirmation of the simulation findings in canine ventricular preparations under different concentrations of the Na/K blocker strophanthin (0.2 μM , top; 0.6 μM , bottom). Results adapted from [52].

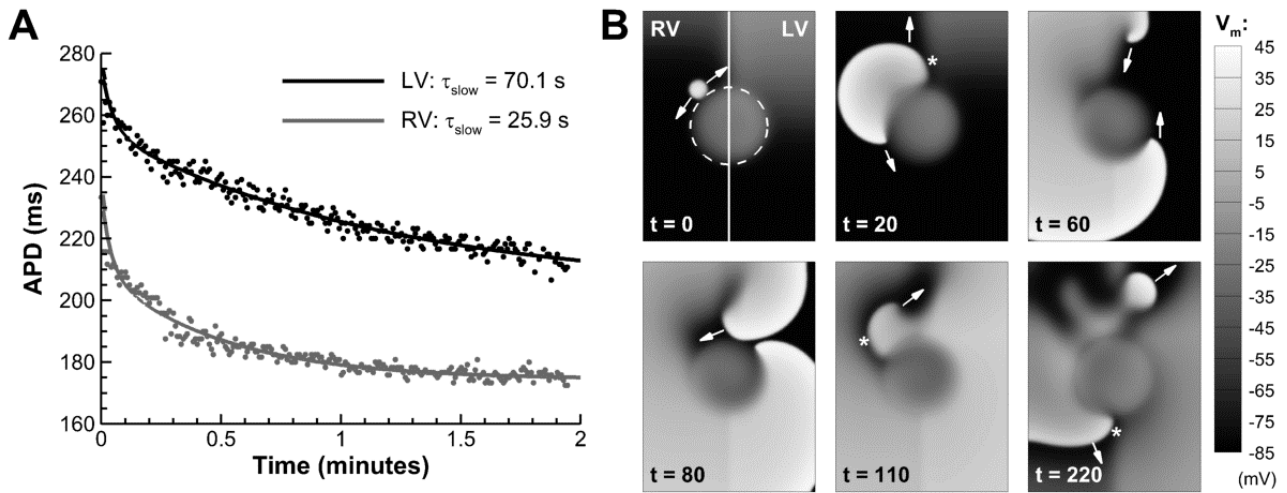


Figure 6. A: Clinical evidence of interventricular differences in action potential duration (APD) and APD adaptation in a patient with structurally normal heart. LV: left ventricle; RV: right ventricle. **B:** Functional consequences of heterogeneous APD adaptation in an idealized model of ischemic heart disease (dashed area indicates unexcitable region). Regional differences in APD adaptation generates pronounced patterns of dispersion of repolarization ($t=0$), which under ectopic activity leads to unidirectional block ($t=20$, asterisk), initiation of reentry ($t=60$), and sustained fibrillation-like reentrant patterns ($t=220$). Colorbar denotes transmembrane potential (mV); times indicated since ectopic stimulation (ms) (modified from [4]).

RECENT REPORTS

12/115	Mathematicians at the Movies: Sherlock Holmes vs. Professor Moriarty	Moulton Goriely
13/01	Rotation, inversion, and perversion in anisotropic elastic cylindrical tubes and membranes	Goriely Tabor
13/02	Drop spreading and penetration into pre-wetted powders	Marston Sprittles Zhu Li Vakarelski Thoroddsen
13/03	On the mechanics of thin films and growing surfaces	Holland Kosmata Goriely Kuhl
13/04	Spatially Partitioned Embedded Runge-Kutta Methods	Ketcheson Macdonald Ruuth
13/05	Simple computation of reaction-diffusion processes on point clouds	Macdonald Merriman Ruuth
13/06	A Volume-Based Method for Denoising on Curved Surfaces	Biddle von Glehn Macdonald März
13/07	Porous squeeze-film flow	Knox Wilson Duffy McKee
13/08	Diffusion of finite-size particles in confined geometries	Bruna Chapman
13/09	Mathematical analysis of a model for the growth of the bovine corpus luteum	Prokopiou Byrne Jeffrey Robinson Mann Owen
13/10	Capillary deformations of bendable films	Schroll Adda-Bedia Cerdeira Huang Menon Russell Toga Vella Davidovitch
13/11	Twist and stretch of helices: All you need is Love	Đuričković Goriely

13/17	Elastometry of deflated capsules elastic moduli from shape and wrinkle analysis	Knoche Vella Aumaitre Degen Rehage Cicuta Kierfeld
13/18	The effect of a concentration-dependent viscosity on particle transport in a channel flow with porous walls	Herterich Griffiths Field Vella
13/19	On a poroviscoelastic model for cell crawling	Kimpton Whiteley Waters Oliver
13/20	Complexity Plots	Thiyagalingam Walton Duffy Trefethen Chen
13/21	Glyph-based video visualization for semen analysis	Duffy Thiyagalingam Walton Smith Trefethen Kirkman-Brown Gaffney Chen
13/22	RBF multiscale collocation for second order elliptic boundary value problems	Farrell Wendland

Copies of these, and any other OCCAM reports can be obtained from:

**Oxford Centre for Collaborative Applied Mathematics
Mathematical Institute
24 - 29 St Giles'
Oxford
OX1 3LB
England**

www.maths.ox.ac.uk/occam