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DEFINING CANDIDATE DRUG CHARACTERISTICS FOR LONG-QT (LQT3) SYNDROME

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Abstract. Mutations of the SCN5A gene can significantly alter the function of cardiac myocyte sodium channels leading to increased risk of ventricular arrhythmia. Over the past decade, detailed Markov models of the action potential of cardiac cells have been developed. In such models, the effects of a drug can be treated as alterations in on- and off rates between open and inactivated states on one hand, and blocked states on the other hand. Our aim is to compute the rates specifying a drug in order to: (a) restore the steady-state open probability of the mutant channel to that of normal wild type channels; and (b) minimize the difference between whole cell currents in drugged mutant and wild type cells. The difference in the electrochemical state vector of the cell can be measured in a norm taking all components and their dynamical properties into account. Measured with this norm, the difference between the state of the mutant and wild-type cell was reduced by a factor of 36 after the drug was introduced and by factors of 4 over mexitiline and 25 over lidocaine. The results suggest the potential to synthesize more effective drugs based on mechanisms of action of existing compounds.

1. **Introduction.** Instabilities in cardiac myocyte cellular dynamics can trigger or maintain arrhythmias, that can be life threatening. Gene mutations or adverse drug effects that prolong myocyte action potential duration are associated with prolongation of the QT interval of the electrocardiogram and increased risk of reentrant ventricular tachy-arrhythmias such as Torsades de Pointes (TdP).

Advances in the understanding of the genetic basis of cardiac ion channels have revealed that mutant alleles of several cardiac ion channel genes are associated with long QT syndrome (LQTS) in humans. Gain-of-function mutations of the SCN5A

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gene encoding the cardiac sodium channel are associated with the LQT3 variant of Long QT Syndrome. The wild-type sodium channel has both a fast inactivation and a slow inactivation process. However, the ΔKPQ mutant can enter into a bursting mode, where channel can only switch between open and closed states with no inactivation, and lead to a large late sodium current that prolong action potential duration (APD) and genesis of early after-depolarizations (EAD). EADs are membrane osillations that interrupt or retard repolarization during phase 2 or phase 3 of the cardiac AP, and EAD-induced triggered APs appear when EAD amplitude bring the membrane above its threshold potential [1]. Delayed after depolarizations (DAD) can also trigger cardiac arrhythmias, however, they arise after full repolarization of AP, and can be induced with agents that overload intracellular calcium [3]. Mutations or proarrhythmic drug effects that alter cardiac action potential duration (APD), can also lead to amplification of electrical heterogeneities in the ventricular myocardium. This can result in a prolongtion of the QT interval in electrocardigram (ECG) recordings, and finally development of TdP.

Experiments have shown that the wild-type sodium current inactivates quickly, whereas in the mutant case there is an additional late sodium current. Clancy and Rudy [4] have proposed a Markov model that is in agreement with these findings. Clancy, Zhu and Rudy [5] extended the model to incorporate the putative mechanisms of action of two anti-arrhythmic drugs; mexiletine and lidocaine, which block the open-state or inactivated-state of the channel, respectively.

In the present paper a bicomponent drug is introduced targeting both the open- and inactivation states. The properties of the drug is thus characterized by four free parameters, its binding and off binding rates to open state and inactivation state (d_1, d_2, d_3, d_4). It is the purpose of the present paper to compute advantageous values of these parameters. We assume that drug function is fully specified when these rates are determined, and we determine the rates such that the drugged mutant cell resemble the properties of a wild type cell as good as possible. Specifically, the drug will inhibit late opening of the mutant sodium channel, and the action potential of the drugged mutant cell will be similar to the action potential of the wild type cell. We show that only one degree of freedom in the drug is needed to assure that the channel closes normally; the three other degrees of freedom were then used to optimize the overall action potential of the drugged mutant cell. Here, we have used the updated LRd model by Livshitz and Rudy [8].

The concept of treating the drug in terms of free parameters that can be optimized in order to alter the properties of a cell in an advantageous manner was introduced in [12]. Computer simulations based on mathematical models of cardiomyocytes have been extensively used to examine the effects of various drugs; overviews are provided by Noble et al [10] and by Brennan et al [2]. In particular, models of the Long-QT syndrome have been analyzed in a series of papers; see e.g. [5, 15, 6, 11, 14, 13]. In all these papers, the effect of a certain drug is implemented in the mathematical model and the effect on the mutant cell is recorded based on computations.

The approach here is different in that we assume that drug properties can be characterized in terms of a set of parameters that we optimize to identify theoretical therapeutic objectives. First, the mathematical models are presented. In Section 3, we derive a sufficient condition on the drug to properly close the sodium channel, and in Section 4 we define an optimization procedure for determining the remaining degrees of freedom of the drug. Furthermore, we compute the parameters defining

the drug, and we present numerical computations comparing the wild type, mutant and drugged mutant cells.

2. Methods.

2.1. The mathematical model.

The purpose of this section is to introduce the mathematical models under considerations. Mathematical models of the cardiac action potential are generally written on the form

$$v_t = -\frac{1}{C_m}(I_{ion} + I_s), \tag{1}$$

$$s_t = F(v, s), \tag{2}$$

where v denotes the transmembrane potential, C_m denotes the membrane capacitance, I_{ion} denotes the sum of individual ionic currents (voltage-gated, pumps and exchangers), I_s denotes a stimulus current, and s denotes a vector containing gating variables and ionic concentriations governed by the non-linear vector-valued function $F = F(v, s)$.

The ionic current can be written on the form

$$I_{ion} = I_{Na} + I_R \tag{3}$$

and we apply the model introduced by Livshitz and Rudy in [8]. Since we are concerned with the effects of mutations affecting the sodium channel, we present the details of the modeling for that channel and refer to [8] for the remaining parts of the model. The sodium current takes the form

$$I_{Na} = G_{Na}O(v - E_{Na}) \tag{4}$$

where E_{Na} is the sodium equilibrium potential, G_{Na} denotes the maximum conductance, and O denotes the open state probability of the sodium channel. We will consider three versions of this current; the wild type case, the mutant case and the case of a mutant cell that is affected by a drug. These three cases are distinguished in the way the open probability is modeled. We refer to the three cases as W, M and D, and thus O^W, O^M, and O^D denotes the open probability for a wild type cell, a mutant cell and a mutant cell affected by the drug, respectively.

The Markov model of the open probability of a wild type cell was established by Clancy and Rudy in [4]. It can be presented schematically as illustrated in Figure 1A and, equivalently, as a system of ordinary differential equations on the following form

$$\frac{d}{dt}\begin{pmatrix} \mathrm{C3} \\ \mathrm{C2} \\ \mathrm{C1} \\ \mathrm{O} \\ \mathrm{IF} \\ \mathrm{IS} \end{pmatrix} = \begin{pmatrix} -\gamma_1 & \beta_{11} & \cdot & \cdot & \cdot & \cdot \\ \alpha_{11} & -\gamma_2 & \beta_{12} & \cdot & \cdot & \cdot \\ \cdot & \alpha_{12} & -\gamma_3 & \beta_{13} & \alpha_3 & \cdot \\ \cdot & \cdot & \alpha_{13} & -\gamma_4 & \beta_2 & \cdot \\ \cdot & \cdot & \beta_3 & \alpha_2 & -\gamma_5 & \beta_4 \\ \cdot & \cdot & \cdot & \cdot & \alpha_4 & -\gamma_6 \end{pmatrix} \begin{pmatrix} \mathrm{C3} \\ \mathrm{C2} \\ \mathrm{C1} \\ \mathrm{O} \\ \mathrm{IF} \\ \mathrm{IS} \end{pmatrix}$$

where γ_i is the sum of the off-diagonal elements of column i:

$$\begin{aligned} \gamma_1 &= \alpha_{11}, \\ \gamma_2 &= \beta_{11} + \alpha_{12}, \\ \gamma_3 &= \beta_{12} + \alpha_{13} + \beta_3, \\ \gamma_4 &= \beta_{13} + \alpha_2, \\ \gamma_5 &= \alpha_3 + \beta_2 + \alpha_4, \\ \gamma_6 &= \beta_4. \end{aligned}$$

Wild type			Mutant		
α_{1i}	$=$	$\frac{3.802}{0.1027\cdot e^{-V/p_{1i}}+p_{2i}\cdot e^{-V/150}}$	$\hat{\alpha}_{1i}$	$=$	$1.25\cdot\alpha_{1i}$
β_{1i}	$=$	$p_{3i}\cdot e^{-V/p_{4i}}$	$\hat{\beta}_{1i}$	$=$	β_{1i}
α_2	$=$	$9.178\cdot e^{V/29.68}$	$\hat{\alpha}_2$	$=$	$9.178\cdot e^{V/100}$
β_2	$=$	$\frac{\alpha_{13}\cdot\alpha_2\cdot\alpha_3}{\beta_{13}\cdot\beta_3}$	$\hat{\beta}_2$	$=$	$\frac{\hat{\alpha}_{13}\cdot\hat{\alpha}_2\cdot\hat{\alpha}_3}{\hat{\beta}_{13}\cdot\hat{\beta}_3}$
α_3	$=$	$3.7933\cdot 10^{-9}e^{-V/5.2}$	$\hat{\alpha}_3$	$=$	$20\cdot\alpha_3$
β_3	$=$	$0.0084+0.00002V$	$\hat{\beta}_3$	$=$	$2\cdot\beta_3$
α_4	$=$	$\alpha_2/100$	$\hat{\alpha}_4$	$=$	$\hat{\alpha}_2/100$
β_4	$=$	α_3	$\hat{\beta}_4$	$=$	$\hat{\alpha}_3$
			$\hat{\alpha}_0$	$=$	$2\cdot 10^{-6}$
			$\hat{\beta}_0$	$=$	$1\cdot 10^{-4}$

TABLE 1. The rate functions of the sodium channel model taken from [3].

i	p_{1i}	p_{2i}	p_{3i}	p_{4i}
1	17	0.20	0.1917	0
2	15	0.23	0.20	5
3	12	0.25	0.22	10

TABLE 2. Values of the constants p_{ji} for the functions α_{1i} and β_{1i} in Table 1.

Here, the open probability O is the essential variable since only that variable affects the rest of the system. In fact, the Na current of the model is proportional to open state probability; see Equation (4) above. In addition to the open state probability, the model includes three closed states $(C1, C2, C3)$, and two inactivated states; IS (slow) and IF (fast). The functions involved in the system are specified in Table 1.

Similarly, the model of a mutant cell is illustrated in Figure 1 B. This model is also adopted from [4] and the functions involved are given in Table 1. The model can be written on the form of a system of ordinary differential equations in exactly the same manner as for the wild-type system. In the mutant model the open state probability is given by the sum of an upper and a lower open state probability; $O^M = UO + LO$. Furthermore, there are three upper closed states $(UC1, UC2, UC3)$, and three lower closed states $(LC1, LC2, LC3)$. Finally, the system modeling the open probability of a drugged mutant cell is sketched in 1 C. In the model of a drugged mutant cell, three states are added. The cell can be in an inactivated, upper or lower blocked state represented by the variables (IB, UB, LB), respectively.

The model takes the form introduced by Clancy, Zhu and Rudy [5] with the exception that we regard the parameters (d_1, d_2, d_3, d_4) describing the properties of the drugs to free; indeed it is the purpose of this paper to compute advantageous values of these parameters. The mathematical problem at hand can be understood by comparing Figure 1 A and C. We want to compute the free parameters (d_1, d_2, d_3, d_4) of Figure 1 C (mutant cell with drugged states) such that the solution

of the associated system resembles the solution of the system associated Figure 1 A (wild type cell) as well as possible.

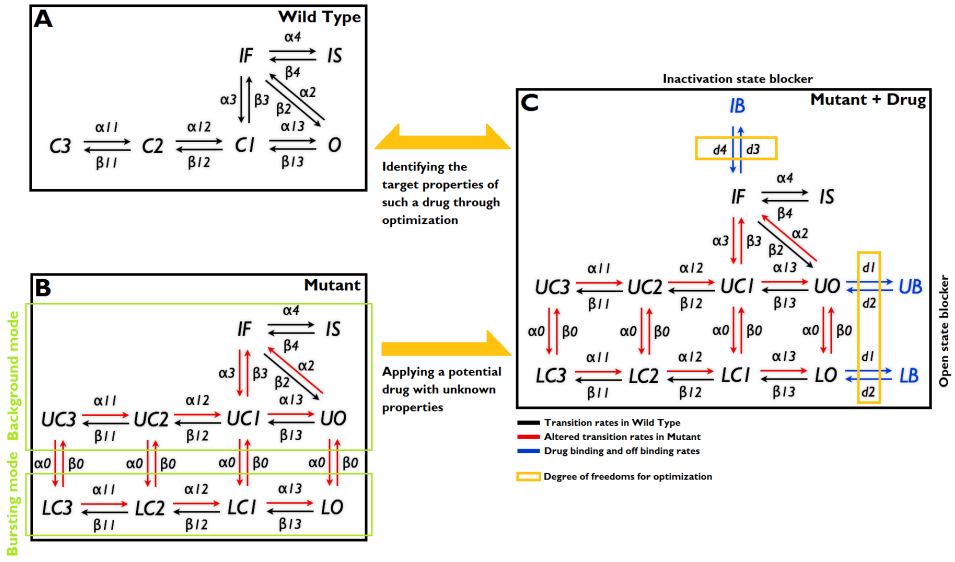

FIGURE 1. **Target identification of a potential drug treating LQTS caused by a Na^+ channel mutation.** The markov Na^+ channel models for a wild type cell (A) and a mutant cell (B) were reproduced, based on Clancy and Rudy [4]. Red arrows indicates alternations in transition rates in the mutant model. A potential drug with unknow properties, presumably targeting both inactivation and open states, with binding and off binding rates of d1, d2, d3, d4, was modeled as extra states to the mutant model, IB, UB and LB, based on methodologies proposed by Clancy, Zhu and Rudy [5]. By parameter optimization of d1, d2, d3 and d4, the mutant + drug model (C) can behave with minimal differences compared to the wild type model (A) on both single channel and whole cell levels.

2.2. **Computing probability at equilibrium.** As mentioned above, it has been observed that there is non-negligible late sodium current in the mutant case, that can significantly prolong action potential duration, and it was demonstrated in [4] that this current may generate instabilities in the action potential. More specifically, the sodium channel of the mutant cell does not close in the same manner as the wild type cell. Our aim is to understand how the drug can be set up to force this difference to be minimized. This is done by considering the steady-state open probabilities as a function membrane voltage in all three cases (W,M,D). As mentioned above, the drug is characterized four parameters and it turns out that only one of them is needed to rectify late Sodium current. The other three parameters will be used to optimize the dynamic properties of the drugged cell.

Note that the model of the open probability in all three cases can be written on the general form

$$z_t = Z(v)z \tag{5}$$

and thus the equilibrium state obtained by putting $z_t = 0$, will be a function of the transmembrane potential v.

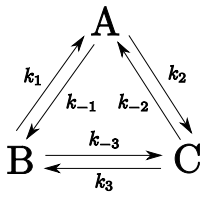

FIGURE 2. A generic cyclic reaction involving three states.

It turns out that the equilibrium open probability in all three cases can be computeted explicitly as functions of v. In order to see this, we consider a prototypical Markovian model of a channel that can take on one of the three states; A, B and C, see Figure 2.

The associated system of ordinary differential equation is given by

$$a_t = -(k_{-1} + k_2)a + k_1 b + k_{-2}c \tag{6}$$

$$b_t = k_{-1}a - (k_1 + k_{-3})b + k_3 c \tag{7}$$

$$c_t = k_2 a + k_{-3}b - (k_3 + k_{-2})c \tag{8}$$

Here a, b and c denotes the probability of the channel being in the states A, B and C respectively, and we assume that the reaction rates satisfies the condition

$$k_1 k_2 k_2 = k_{-1} k_{-2} k_{-3}, \tag{9}$$

which is necessary for the system to satisfy the principle of detailed balance; see Keener and Sneyd[7]. We assume that the channel can only be in the states A, B or C initially, and thus $a(0) + b(0) + c(0) = 1$. Furthermore, by adding the equations above, we note that $(a + b + c)_t = 0$, and thus

$$a(t) + b(t) + c(t) = 1 \tag{10}$$

for all time. According to the principle of detailed balance, the equlibrium solution satisfies the following condtions

$$k_{-1}a = k_1 b,\ k_2 a = k_{-2}c,\ k_{-3}b = k_3 c. \tag{11}$$

By combining these equations and the fact that $a + b + c = 1$, we get the following equlibrium states,

$$a = \left(1 + \frac{k_{-1}}{k_1} + \frac{k_2}{k_{-2}}\right)^{-1}, \tag{12}$$

$$b = \frac{k_{-1}}{k_1}\left(1 + \frac{k_{-1}}{k_1} + \frac{k_2}{k_{-2}}\right)^{-1}, \tag{13}$$

$$c = \frac{k_2}{k_{-2}}\left(1 + \frac{k_{-1}}{k_1} + \frac{k_2}{k_{-2}}\right)^{-1}. \tag{14}$$

In Figure 1A, the reaction scheme of the wild type model is given. At equilibrium, we obtain the following relations

$$\begin{array}{lll} \alpha_4 IF = \beta_4 IS, & \alpha_3 IF = \beta_3 C1, & \beta_2 IF = \alpha_2 O, \\ \alpha_{13} C1 = \beta_{13} O, & \alpha_{12} C2 = \beta_{12} C1, & \alpha_{11} C3 = \beta_{11} C2. \end{array} \tag{15}$$

By letting $\gamma = \alpha/\beta$, we get

$$\begin{array}{llll} IS = \gamma_2\gamma_4 O, & IF = \gamma_2 O, & & \\ C1 = \gamma_{13}^{-1} O, & C2 = \gamma_{12}^{-1}\gamma_{13}^{-1} O, & C3 = \gamma_{11}^{-1}\gamma_{12}^{-1}\gamma_{13}^{-1} O, & \end{array} \tag{16}$$

and since $IS + IF + O + C1 + C2 + C3 = 1$, the open probability at equilibrium is given by

$$O^W(v) = \frac{1}{\eta + \gamma_2(1+\gamma_4)}, \tag{17}$$

where

$$\eta = 1 + \gamma_{13}^{-1} + \gamma_{12}^{-1}\gamma_{13}^{-1} + \gamma_{11}^{-1}\gamma_{12}^{-1}\gamma_{13}^{-1}. \tag{18}$$

Note that in the mutant case, the open probability is given by $O^M = UO + LO$ and we find that

$$\begin{array}{lll} IS = \widehat{\gamma}_2\widehat{\gamma}_4 UO, & IF = \widehat{\gamma}_2 UO, & LO = \widehat{\gamma}_0 UO, \\ UC1 = \widehat{\gamma}_{13}^{-1} UO, & UC2 = \widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1} UO, & UC3 = \widehat{\gamma}_{11}^{-1}\widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1} UO, \\ LC1 = \widehat{\gamma}_{13}^{-1}\widehat{\gamma}_0 UO, & LC2 = \widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1}\widehat{\gamma}_0 UO & LC3 = \widehat{\gamma}_{11}^{-1}\widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1}\widehat{\gamma}_0 UO. \end{array} \tag{19}$$

Since the sum of the states is one, we get

$$O^M(v) = \frac{1}{\widehat{\eta} + \widehat{\gamma}_2 \frac{1+\widehat{\gamma}_4}{1+\widehat{\gamma}_0}}, \tag{20}$$

where

$$\widehat{\eta} = 1 + \widehat{\gamma}_{13}^{-1} + \widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1} + \widehat{\gamma}_{11}^{-1}\widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1}. \tag{21}$$

Similarly, in the case of a drugged mutant cell, we find that at equilibrium the following relations hold

$$\begin{array}{lll} IS = \widehat{\gamma}_2\widehat{\gamma}_4 UO, & IF = \widehat{\gamma}_2 UO, & LO = \widehat{\gamma}_0 UO, \\ UC1 = \widehat{\gamma}_{13}^{-1} UO, & UC2 = \widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1} UO, & UC3 = \widehat{\gamma}_{11}^{-1}\widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1} UO, \\ LC1 = \widehat{\gamma}_{13}^{-1}\widehat{\gamma}_0 UO, & LC2 = \widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1}\widehat{\gamma}_0 UO, & LC3 = \widehat{\gamma}_{11}^{-1}\widehat{\gamma}_{12}^{-1}\widehat{\gamma}_{13}^{-1}\widehat{\gamma}_0 UO, \\ IB = y\widehat{\gamma}_2 UO, & UB = xUO, & LB = x\widehat{\gamma}_0 UO. \end{array} \tag{22}$$

Here we have defined

$$x = \frac{d_1}{d_2} \text{ and } y = \frac{d_3}{d_4}, \tag{23}$$

where we recall that the parameters d_1, d_2, d_3, d_4 specify the drug; see Figure 1B. The equilibrium open state probability $(UO + LO)$ of the drugged mutant cell (see the reaction scheme given in Figure 1C) is given by

$$O^D(v, x, y) = \frac{1}{\widehat{\eta} + x + \widehat{\gamma}_2 \frac{1+y+\widehat{\gamma}_4}{1+\widehat{\gamma}_0}}. \tag{24}$$

Note that $O^D(v, 0, 0) = O^M(v)$.

Motivated by the concentrations applied in [5], we take 1000μM to be the maximal drug concentration. The forward rates will then satisfy

$$d_1, d_3 \le [D] \cdot k_{\text{on}} = 100/\text{ms},$$

where $k_{\text{on}} = 0.1/(\mu\text{M ms})$. The backward rates can be written as $K_d \cdot k_{\text{on}}$, where K_d is the dissociation constant, which is 2.5μM and 4μM for mexiteline and lidocaine,

respectivly. Thus, in that case $d_2 = 0.25$/ms and $d_4 = 0.4$/ms. Based on these considerations we assume that $x = \frac{d_1}{d_2} \leq 400$ and $y = \frac{d_3}{d_4} \leq 250$.

3. **Results.**

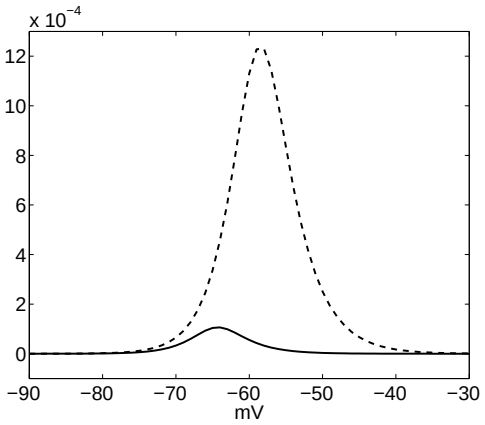

FIGURE 3. **Open probability of the sodium channel in steady state.** Wild type (solid) and mutant (dashed).

3.1. **Optimizing the equilibrium open probability.** As shown in the methods section the open state probability can be explicitly computed as a function of the transmembrane potential v for all three cases; wild type (W), mutant (M) and drugged mutant (D) cells. In Figure 3 we show the equilibrium open probability as a function of the transmembrane potential for wild type and mutant cells, and we observe a significant difference. This difference may cause persistant sodium leakage, and thus it is reasonable to try to reduce this difference by using the drug. Define the difference between the equilibrium open probability of a wild type and a drugged mutant cell to be

$$d(x, y) = \int_{-90}^{-20} \left(O^D(v, x, y) - O^W(v)\right)^2 dv. \tag{25}$$

In Figure 5, we have plotted this function for x and y ranging from 0 to 500, and we observe that the difference is strongly dependent on the y-variable. In fact, for any value of x, the difference is small for any choice of $y \gtrsim 150$. In Figure 4, we have plotted the equilibrium open probability of a drugged mutant cell using $x = 0$ and $y = 100, 200, 300$. Based on these observations we choose $y = 200$, and thus we have found that

$$d_4 = d_3/200. \tag{26}$$

3.2. **Optimizing the sodium current.** It remains to determine the parameters (d_1, d_2, d_3). This will be done by minimizing the difference between the sodium current of the drugged mutant cell and a wild type cell. Motivated by [12], we define the distance function

$$D(d_1, d_2, d_3) = \int_0^T \left[I_{Na}^D(t, d_1, d_2, d_3) - I_{Na}^W(t)\right]^2 dt, \tag{27}$$

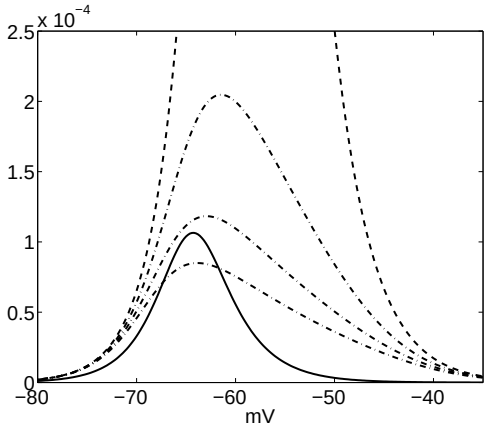

FIGURE 4. **Open probability of the sodium channel in steady state.** Wild type (solid) and mutant (dashed), and three versions of a drugged mutant cell corresponding to three choices of y (dash-dotted). From top to bottom: $y = 100, 200, 300$.

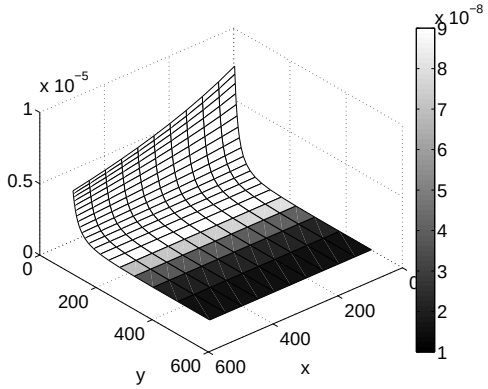

FIGURE 5. **Graph of the functional $d(x, y)$ defined in (25).** Notice that shades of gray correspond to values below 10^{-7}.

where T = 3150ms. For $t < T$ we stimulate the cell with successively longer cycle lengths, from 300ms to 600ms in steps of 50ms. The currents are computed based on a full simulation of the action potential. The function is minimized using the Nelder-Mead algorithm, see [9]. By using the initial condtion $(d_1, d_2, d_3) = (100, 0.25, 100)$, (corresponding to [M] = [L] = 1000 μM and $K_d = 2.5\mu$M) we get the optimal drug

$$d^* = (d_1^*, d_2^*, d_3^*, d_4^*) = (1.72, 0.0697, 169.7, 0.848) \tag{28}$$

where $d_4^* = d_3^*/200$. In Figure 6 we have graphed the value of the distance function D around the the optimal value. In the left plot, d_1 is varied while keeping the two other components at their optimal values. Middle and right plot are similar for d_2 and d_3. We observe that the cost functional is convex along d_1 and d_2, while any $d_3 \gtrsim 100$ seems to be a good choice.

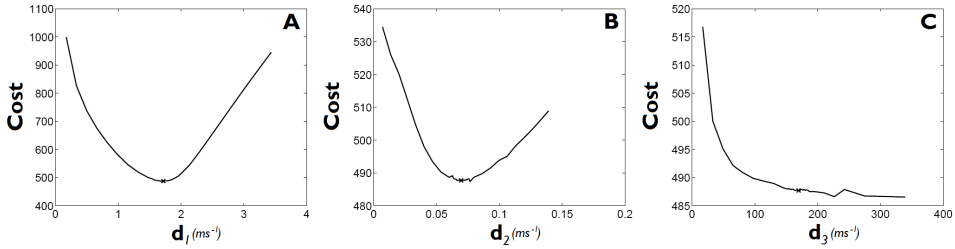

FIGURE 6. **The value of the cost functional (27) around the optimal drug marked with $\times$.**

The optimized drug profile suggests that, an open state blocker with a fairly slow off-binding rate to be applied at a low dosage, combined with an inactivation state blocker with a higher off-binding rate applied at high dosage, can be most effective in minimizing differences between mutant sodium channel and wild type. It seems that, in this case, the inactivation blocker behaves as a fast "hit and run" process, while the open state blocker remains for a relatively long duration to slowly underpin the the mutant channel dynamics.

In Figure 7 we present the action potential of a wild type, mutant and a drugged mutand cell, and in the lower plot we compare the associated sodium currents. From both these plots, we observe that the drug changes the properties of the mutant cell such that the behavior of the wild type cell and the drugged mutant cell are hard to distinguish.

The optimization has been performed on the sodium channel. To meassure the overall cell function we define a norm that encompasses all electro-chemical states q:

$$||q|| = \sum_{i=1}^{M} \left(\sup_{0\le\tau\le T} |q_i(\tau)| \right)^{-1} \int_0^T |q_i(t)|dt \tag{29}$$

Here $M = 15$ is the number of variables included in the norm. There are three variables carrying intracellular concentration of sodium, calcium and potassium, eight gating variables for potassium and calcium currents, three variables related to SR dynamics and finally one for the membrane potential. Table 3 shows the results and we observe that the optimal drug performs well also in this norm.

TABLE 3. **Drug performance**

	No drug	Mexiteline	Lidocaine	Optimal
$\|\|q_W - q\|\|$	2.5156	0.2857	1.7025	0.0686

Norm of the difference between the wild type action potential and the mutant cell action potential where the latter is subject to three types of drugs. The norm is defined in (29).

The difference between the electro chemical fingerprint of the wild type and the mutant cell, measured in this norm is 2.5156 (see Table 3). This difference is reduced by a factor close to 9 by when mexiteline is applied and with a factor of about 1.5 when lidocaine is used. By using the optimal drug given by (28), the difference is reduced by a factor more than 36. For mexiteline and lidocaine we have used

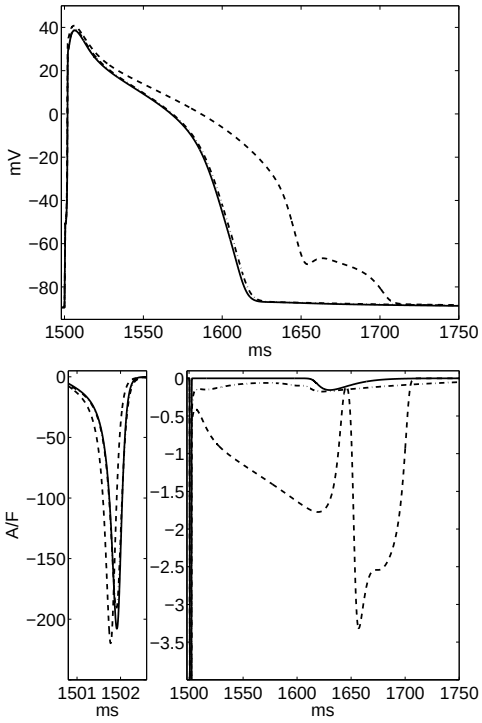

FIGURE 7. **Upper panel: The action potentials.** The solid line is the wild type cell, the dotted line is the mutant cell, and the dotted-dashed is the drugged mutant cell (using the drug given by (28)), which is observed to behave quite similar to the wild type cell. The lower panels show the associated sodium currents and we observe that the drug significantly reduce the late current of the mutant cell.

100μM, within the range suggested in [5]. In drug vector terminology the two cases are $d_{\mathrm{M}} = (10, 0.25, 0, 0), d_{\mathrm{L}} = (0, 0, 10, 0.4)$.

3.3. **Optimizing on EAD prevention.** The approach taken above was to identify the potential drug characteristics, that minimize the differences between the mutant and wild type Na^+ channels at single channel and cellular levels. This was achieved by both correcting the channel open probability at steady state and adjusting the temporal dynamics of the channel during sinus rhythm. An alternative approach is to simply reduce arrhythmogenic effects of mutant Na^+ channel, such as eliminating occurrences of EADs, see Figure 4 of [4]), while allowing a minor APD prolongation. Such an approach can also be effective in treating LQT syndrome, thus provide more options for drug development. In Figure 8 (upper panel) we show a train of action potentials of the mutant cell pacing at cycle length of 400 ms, where EAD is seen at around 2600 ms. The mutant Na^+ current has an increased arrhythmogenic effects during pacing, since more population of the channel first entered the bursting mode, then was trapped due to a very low trasitient rate to escape. Thus during pacing, late Na^+ current further increased to prolong APD, when a beat was stimulated within the vulnerable window of the previous

beat, unidirectional block may occur, and lead to ventricular tachycardia. Here, we use the first beat in Figure 8 (upper panel) as the target for the optimization procedure to keep Na^+ channel from entering its bursting mode, and finally reducing its arrhythmogenic effects. By picking 100 random initial guesses and running the optimization procedure described earlier, the potential drug characteristics were given by

$$d^* = (d_1^*, d_2^*, d_3^*, d_4^*) = (1.58, 1.21, 0.23, 697) \tag{30}$$

By using this drug we get the results given in Figure 8 (lower panel). Compared to the "optimal" drug profile presented in Section 4, the potential drug characteristics proposed here, suggested an inactivation blocker with very slow off binding rate applied at low dosage, combined with an open state blocker with much faster off binding rate applied at higher dosage, can reduce occurrence of EADs most effectively, and enhance stability of the cell during pacing.

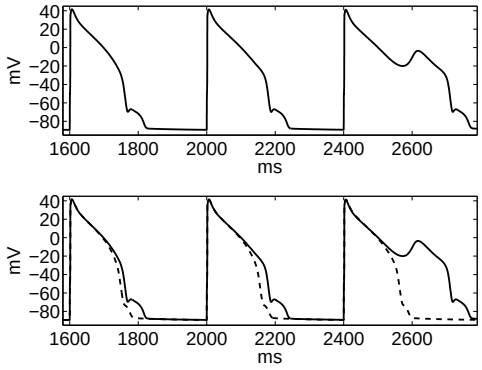

FIGURE 8. **Upper panel: A series of action potentials of the mutant cell paced at 400ms.** Note that an early after depolarization appear in the last action potential at around 2600ms. Lower panel: The same series as above where the mutant cell is given by the solid line, and the dashed line illustrates the behavior of the mutant cell after applying the drug given by (30).

4. **Conclusion.** We have used mathematical models derived in [4, 8] and extended in [5] to compute advantageous properties of a drug targeting mutant cardiac cells. More specifically, we have considered mathematical models of cardiac cells affected by mutations in the SCN5A gene. A Markov model represents the open probability of the sodium channel and this enables a careful study of the equilibrium state of the model. Computations reveal that, theoretically, the drug is able to change the properties of the mutant sodium channel in a fortunate manner so that the difference between the electro-chemical properties of the wild type and the drugged mutant cells seems to vanish. It is worth observing that the computed optimal drug is a compound involving an open state blocker and an inactivation state blocker. Furthermore, we adopted an alternative approach to only reduce the arrhymogenic effects of the mutant cell instead of targeting the exact properties of the wild type cell. As a result, a theoretic drug that can effectively eliminate EADs was proposed. This drug, instead of mainly targeting to reduce the late Na^+ current, tolerates a minor APD prolongation caused ΔKPQ mutation, while completely eliminating the

occurrence of EADs, and thus cellular instabilities. It is the object of future work to see if the same line of reasoning can be used to devise theoretical properties of drugs for other mutations of cardiac cells.

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