Computer Model and Experiments of Glycosylation Modulation Dynamics in Cardiac Action Potentials

Cardiac action potentials (AP) are produced by the orchestrated functions of ion channels. A slight change in ion channel activity may affect the AP waveform, thereby potentially increasing susceptibility to abnormal cardiac rhythms. Cardiac ion channels are heavily glycosylated, with up to 30% of a mature protein’s mass comprised of glycan structures. However, little is known about how reduced glycosylation impacts the gating of hERG (human ether-a-go-go related gene) channel, which is partially responsible for late phase 2 and phase 3 of the AP. This study integrates the data from in vitro experiments with in-silico models to predict the glycosylation modulation dynamics in hERG ion channels and cardiac electrical signals. The gating behaviors of hERG channels were measured under four glycosylation conditions, i.e., full glycosylation, reduced sialylation, mannose-rich. And N-glycanase treated. Further, we developed in-silico models to simulate glycosylation-channel interactions and predict the effects of reduced glycosylation on multi-scale cardiac processes (i.e., cardiac cells, 1-D and 2-D tissues). From the in-silico models, reduced glycosylation was shown to shorten the repolarization phase of cardiac APs, thereby influencing electrical propagation in cardiac fibers and tissues. In addition, the patterns of derived electrocardiogram show that reduced glycosylation of hERG channel shortens the QT interval and decreases the re-entry rate of spiral waves. This work suggests new pharmaceutical targets for the long QT syndrome and potentially other cardiac disorders.
2.1 Introduction

Glycosylation is a common enzymatic process in cardiac cells. It was shown that nearly 30% of a mature protein’s mass is comprised of glycan structures [1]. It is established that voltage-gated Na$^+$ channels ($Na_v$) and K$^+$ channels ($K_v$) are also known to be modulated by posttranslational glycosylation through isoform-specific mechanisms [2-5]. Many previous studies showed that glycosylation-dependent gating effects were imposed on ion channels primarily by the terminal residue attached to N- and O-glycans, sialic acid [5-7]. The addition and removal of glycans from proteins and lipids are completed by the activity of >200 glycosidases, glycosyltransferases, and transport proteins (glycogenes). Recently, Montpetit et al. showed that the cardiac glycome (i.e., the complete set of glycan structures produced in the heart) varies between atria and ventricles, and changes differentially during development of each cardiac chamber [4]. Regulated expression of a single glycogene was sufficient to modulate action potential (AP) waveforms and gating of less sialylated $Na_v$ consistently [3].

In addition to the evidence that a correctly regulated glycome is vital to normal cell function, aberrant glycosylation has dramatic effects on the cardiac function. There is a set of >40 distinct forms of inherited human diseases of reduced glycosylation known as Congenital Disorders of Glycosylation (CDG) caused, typically, by a mutation or deficiency in a glycogene which results in a relatively modest reduction in glycoprotein glycosylation [8, 9]. This variable but modest reduction in glycosylation among CDG subtypes causes a high infant mortality rate. Recently reported prevalence of cardiac involvement prompted experts to suggest screening for cardiac dysfunction in all CDG patients and for CDG in all young patients suffering from cardiomyopathy of unknown etiology. Together, the literature suggests that regulated and aberrant changes in glycosylation impact cardiac function.
However, little is known about how altered glycosylation will impact cardiac function at different organization levels, i.e., from ion channels to cells to tissues to the whole heart. Corroborating data that connects the changes in multi-scale cardiac systems is difficult without performing computer simulations. There is an urgent need to couple the wealth of data obtained from in-vitro and in-vivo experiments with in-silico models. Computer model overcomes practical and ethical limitations in the in-vivo experiments. In addition, it enables the prediction of causal relationships between normal or abnormal functions (e.g., glycosylation modulation dynamics) that in-vivo experiments alone cannot easily achieve.

Our objective is to investigate how changes in glycosylation influence the hERG (i.e., human ether-a-go-go related gene) ion channels and cardiac electrical signaling from cells to cables, and to tissues. In this present study, we developed in-silico models of cardiac cells and tissues to study the role of glycosylation in cardiac function. The hERG channel mediates the rapid delayed rectifying potassium current ($I_{Kr}$), which is partially responsible for late phase 2 and phase 3 of the AP, i.e., the repolarization of cardiac myocytes [10]. The "gain" or “loss” in the hERG channel function can potentially lead to the Short or Long QT syndromes [11]. Our contribution is to integrate the data from in-vitro experiments with in-silico models for predicting the effects of reduced glycosylation on cardiac function. The purpose of this article is to model the glycosylation effects on ion channels, and further predict its impacts at larger scales, i.e., from cells to tissues.

### 2.2 Multi-Scale Cardiac Modeling

Computer models facilitate the quantitative simulation, elucidation and understanding of cardiac function in health and disease. The need to integrate models and data across multiple biological scales has been widely recognized by biomedical and clinical researchers [12, 13]. This present investigation integrates glycosylation-channel interactions, obtained from the whole-cell
patch clamp experiments, with in-silico models to predict glycosylation modulation dynamics in hERG channels and cardiac electrical signals including ionic currents, action potentials, spiral waves, and ECG signals. The development of multi-scale cardiac models, i.e., hERG channel, cell, and tissues, is described as follows.

2.2.1 Model of hERG Channel

The hERG channel is widely modeled in two forms. One is the Hodgkin-Huxley (HH) type model, and the other is Markov state model. The HH type formulation typically describes the gating of hERG channel with two independent activation and inactivation variables, as shown in the following equations:

\[ I_{Kr} = G_{Kr} \frac{K_o}{5.4} X_{r1} X_{r2} (V - E_K) \]

\[ X_{r1\infty} = \frac{1}{1 + e^{-\frac{(V-V_a)}{K_a}}} \quad X_{r2\infty} = \frac{1}{1 + e^{-\frac{(V-V_i)}{K_i}}} \]

where \( V \) is the trans-membrane action potential, \( K_o=5.4\text{mM} \) is the extracellular K+ concentration, \( E_K \) is the reversal potential, \( G_{Kr} \) is the conductance (nS/pF), \( X_{r1\infty} \) is the steady-state activation, \( X_{r2\infty} \) is the steady-state inactivation, \( V_a \) is the voltage of half-activation, \( V_i \) is the voltage of half-inactivation, \( K_a \) and \( K_i \) are slope factors.

Despite the popularity of HH-type descriptions, Markov models have become increasingly important in recent years. There are a number of Markov models developed with different states and structures, e.g., Wang et al. [14], Clancy and Rudy [15], Mazhari et al. [16], and Oehmen et al. [17]. In 2011, Bett et al. compared the HH type formulation with the aforementioned four Markov models. The conclusion is that hERG is best represented by a linear Markov model with 3 closed states, one open and one inactivated state [18]. Hence, we adopted the 5-state Markov formulations to model the gating of hERG channel. As shown in Figure 2-1, the Markov model
includes 3 closed states (C1, C2, C3), 1 inactivation (I) and 1 open (O) state. The transition rates (α’s and β’s) are voltage-dependent, i.e., \( \alpha = a_i \times e^{b_i \cdot V} \) and \( \beta = c_i \times e^{d_i \cdot V} \), where \( i \) is the index of transition rate parameters. The constants, Kf and Kb, describe the voltage insensitive transition in the gating of hERG channel. \( \psi \) is defined as a function of other transition rates to ensure the microscopic reversibility [16, 18]. The hERG channel stays in the fully closed state (C1) during resting, and transfers to other states when excited. The initial value of C1 = 1, and others are 0.

\[
I_{Kr} = G_{Kr}(V - E_K)
\]

\[\alpha_1 \]
\[\beta_1 \]
\[\alpha_2 \]
\[\beta_2 \]

Figure 2-1. Structure of 5-state Markov model of hERG channel

2.2.2 Model of Cardiac Myocyte

When a cardiac myocyte is stimulated, the states of ion channels (e.g., close, activation, inactivation) control the ability of the ions (e.g., Na\(^+\), Ca\(^{2+}\), and K\(^+\)) to cross the membrane. The influx and efflux of ions generate electrical currents through the ion channels, and vary trans-membrane potentials. The cardiac cell is modeled as a structured electric circuit with capacitors, resistors and batteries. Specific to human ventricular cells, a number of models have been developed in the literature, e.g., Priebe and Beuckelmann model [19], ten Tusscher-Panfilov 2004 model [20] and the updated 2006 model [21], Iyer et al. model [22], Grandi et al. model[23], and O’Hara et al. model [24].

In this present investigation, we utilized ten Tusscher-Panfilov 2006 model to describe the electrophysiological behaviors of human ventricular myocytes [21]. The justification is that the results of ten Tusscher-Panfilov 2006 model best match our wild-type data (i.e., full glycosylation)
from in-vivo experiments. The major updates from the ten Tusscher-Panfilov 2004 model to the 2006 version are the inclusion of more detailed description of intracellular Ca$^{++}$ dynamics and subspace Ca$^{++}$ dynamics, as well as the revisions of fast and slow voltage inactivation gate in $I_{CaL}$.

The endocardial-cell version of ten Tusscher-Panfilov 2006 model is used to simulate the AP ($V$) with the following ordinal differential equation:

$$-C_m \frac{dV}{dt} = I_{to} + I_{Ks} + I_{Kr} + I_{K1} + I_{NaCa} + I_{NaK} + I_{pK} + I_{pCa} + I_{bCa} + I_{bNa} + I_{Na} + I_{CaL} + I_{stim}$$

where $t$ is time, $C_m$ is the cell capacitance per unit surface area, $I_{stim}$ is the external stimulus current which activates the cell from the resting state. The transmembrane currents include transient outward current ($I_{to}$), slow delayed rectifier $K^+$ current ($I_{Ks}$), rapid delayed rectifier $K^+$ current ($I_{Kr}$), inward rectifier current ($I_{K1}$), $Na^+$/Ca$^{++}$ exchange current ($I_{NaCa}$), pump current ($I_{NaK}$), plateau currents ($I_{pK}$, $I_{pCa}$), background currents ($I_{bCa}$, $I_{bNa}$), fast Na$^+$ current ($I_{Na}$) and L-type $Ca^{++}$ current ($I_{CaL}$). Most of these voltage-gated ionic currents are modeled in the form of cell conductance, membrane potential gradients and channel gate dynamics. The details of all ion-channel kinetics can be found in references [20, 21]. All parameters and initial values are the same as given in the ten Tusscher-Panfilov 2006 model. Note that we replaced the HH type model of $I_{Kr}$ with the 5-state Markov model. The $G_{Kr}$ is the same as the original value in ten Tusscher-Panfilov model. We optimally calibrated the 5-state Markov model to fit the in-vivo data of four glycosylation conditions from our in-vivo experiments.

2.2.3 Model of Cardiac Tissue

The cardiac cell is not an independent unit. Each depolarized cell can stimulate neighboring cells and trigger cell-to-cell conductions. In the rapid depolarization phase, the overshoot of $Na^+$ ions causes a resting-to-depolarizing $Na^+$ gradient and drives the flow of $Na^+$ through gap
junctions that stimulates adjacent cells. The influx of Na\(^+\) causes neighboring cells to reach threshold potential and initiates the depolarization phase of the AP. As the depolarization and repolarization propagate among cells, electrical waves are generated. This cell-to-cell conduction is analogous to a circuit system with resistances and capacitors. As shown in Figure 2-2, the consecutive cell connections are modeled as linear fiber (cable) and tissue (cell array). The 1D linear cable is composed of 600 cells, and a stimulus is given at cell 1. The linear strand of cells (i.e., cable) is modeled using the following mono-domain reaction-diffusion equation:

\[
\frac{\partial V}{\partial t} = - \frac{1}{C_m} (I_{ion} + I_{stim}) + \frac{1}{\rho S C_m} \frac{\partial^2 V}{\partial x^2}
\]

and in 2D tissue, the equation is:

\[
\frac{\partial V}{\partial t} = - \frac{1}{C_m} (I_{ion} + I_{stim}) + \frac{1}{\rho S C_m} \left( \frac{\partial^2 V}{\partial x^2} + \frac{\partial^2 V}{\partial y^2} \right)
\]

where \(I_{ion}\) is the sum of trans-membrane ion currents, \(\rho = 180\Omega cm\) is the cellular resistivity, \(S = 0.2 \, \mu m^{-1}\) is the surface-to-volume ratio, and \(C_m = 2 \, uF/cm^2\) is the cell capacitance.

To simulate the propagation of cardiac electrical waves along 1D fiber, reaction-diffusion equations are numerically solved with the finite-difference scheme. The action potential can be calculated as:

\[
V_{i,t+\Delta t} = - \frac{dt}{C_m} (I_{ion} + I_{stim}) - \left( \frac{2q}{C_m} - 1 \right) V_{i,t} + \frac{q}{C_m} (V_{i-1,t} + V_{i+1,t})
\]

where \(q = \frac{dt}{\rho S dx^2}\) is the same for all cells. Neuman boundary condition was assumed in the simulation of 1D cardiac cable, i.e. \(V_{101,t} = V_{99,t}\) and \(V_{0,t} = V_{2,t}\) at the boundaries. The numerical finite-difference scheme for 2D tissue is formulated as:

\[
V_{i,j,t+\Delta t} = - \frac{dt}{C_m} (I_{ion} + I_{stim}) - \left( \frac{4q}{C_m} - 1 \right) V_{i,j,t} + \frac{q}{C_m} (V_{i-1,j,t} + V_{i+1,j,t} + V_{i,j-1,t} + V_{i,j+1,t})
\]

where no flux boundary condition is assumed to ensure there are no current leakages on the borders.
2.2.4 ECG Derivation

The ECG signal provides a system-level view of cardiac electrical activity, which is an important clinical diagnostic tool. The standard lead I, II and III (i.e., Einthoven's triangle) measure difference in potentials between electrodes placed on left arm, right arm and left leg [25]. In this study, 3-lead ECG signals are measured using 3 virtual electrodes in an equilateral triangle on 2D tissues. The ECG will be used to evaluate and quantify whether and how altered glycosylation affects cardiac electrical conduction. In this investigation, the electrodes were placed at a distance of 6 cm above 2D tissues with no-flux boundary conditions. The potential at each electrode is calculated using the following equation:

\[ \phi = \iint_S \frac{I_{ion}}{r} \, dx \, dy \]

where \( S \) is the area over cardiac tissue, \( i_{ion} \) is the transmembrane currents, \( x \) and \( y \) represent the coordinates of cells on 2D tissue, and \( r \) is given as:

\[ r = \sqrt{(x-x_E)^2 + (y-y_E)^2 + z_E^2} \]
where \( E = (x_E, y_E, z_E) \) is the location of the electrode. The \( I_{ion} \) current is obtained with the following equation:

\[
I_{ion} = \frac{1}{\rho S C_m} \left( \frac{\partial^2 V}{\partial x^2} + \frac{\partial^2 V}{\partial y^2} \right)
\]

Lead I, II, III ECG signals are the potential differences between two electrodes as:

\[
ECG(E_1, E_2) = \emptyset(x_{E1}, y_{E1}, z_{E1}) - \emptyset(x_{E2}, y_{E2}, z_{E2})
\]

It may be noted that 60° orientation is considered between each two leads.

### 2.3 Materials and Experimental Design

#### 2.3.1 Physical Experiments

We recently reported the effects of differential glycosylation on the gating of hERG ion channels, showing that N-glycosylation effectively limits the hERG activity [26, 27]. The in-vitro experimental data were collected from hERG-expressing CHO cells under 4 glycosylation conditions, i.e., full glycosylation \((n = 11)\), reduced sialylation \((n = 11)\), mannose-rich glycosylation \((n = 6)\) and N-Glycanase treated \((n = 6)\). Steady-state gating parameters were examined with the use of standard pulse protocols and solutions described by the Bennett lab [26].

Steady State Activation (SSA) protocol is described as follows. Cells were held at -80 mV, stepped to more depolarized potentials (-80 mV to +40 mV in 10 mV increments) for 4 seconds, then stepped back to -50 mV for another 4 seconds, and returned to the holding potential. Steady-state conductance values \((G)\) were calculated using ohm’s law \( G = I / (V_p - E_K) \), where \( I \) is the peak of tail currents elicited at each test potential \((V_p)\). The maximum conductance generated by each cell was used to normalize the data for each cell to its maximum conductance by fitting the data to a single Boltzmann distribution as:

\[
\text{Fraction of maximal conductance} = \left[ 1 + \exp \left( - \frac{(V-V_a)/K_a} \right) \right]^{-1}
\]
where $V$ is the membrane potentials, $V_a$ is the half-activation potential, $K_a$ is the slope factor. The normalized data were averaged with those from the other cells to calculate the resulting average G-V curves.

Steady state inactivation (SSI) is measured following a conventional protocol. Cells were held at -80 mV before stepping to +20 mV for 3 seconds. Then we stepped to -120 mV to +60 mV (in 10 mV increments) for 30 ms before stepping back to +20 mV for 1 second and then returned to the resting potential. The maximum current generated by each cell was used to normalize the data for each cell to its maximum current by fitting the data to a single Boltzmann distribution, from which the mean $V_i \pm$ SEM and $K_i \pm$ SEM values were determined.

\[
\text{Fraction of maximal current} = \left[1 + \exp \left(\frac{(V-V_i)}{K_i}\right)\right]^{-1}
\]

where $V$ is the membrane potential, $V_i$ is the voltage of half-inactivation, $K_i$ is the slope factor.

Table 2-1 shows the mean ± SEM SSA and SSI parameters measured under four glycosylation conditions, as reported by us previously [26]. These changes in hERG channel gating were used to calibrate in-silico models, which further predict glycosylation effects on cardiac electrical signaling of cells and tissues.

Table 2-1 hERG channel activation and inactivation parameters [26]

<table>
<thead>
<tr>
<th>hERG Channel</th>
<th>n</th>
<th>$V_a$(mV)</th>
<th>$K_a$(mV)</th>
<th>$V_i$(mV)</th>
<th>$K_i$(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Glycosylation</td>
<td>11</td>
<td>-22.0±1.3</td>
<td>8.4±0.3</td>
<td>-74.4±4.9</td>
<td>-19.0±0.8</td>
</tr>
<tr>
<td>Reduced Sialylation</td>
<td>11</td>
<td>-13.7±2.4</td>
<td>8.6±0.4</td>
<td>-56.2±5.3</td>
<td>-17.2±1.3</td>
</tr>
<tr>
<td>Mannose-Rich</td>
<td>6</td>
<td>-12.6±2.4</td>
<td>8.9±0.4</td>
<td>-51.48±3.6</td>
<td>-16.4±0.9</td>
</tr>
<tr>
<td>N-Glycanase treated</td>
<td>6</td>
<td>-12.1±2.2</td>
<td>8.9±0.9</td>
<td>-58.5±5.9</td>
<td>-20.3±1.2</td>
</tr>
</tbody>
</table>

2.3.2 Computer Experiments

Recall that we modeled the hERG channel with a 5-state Markov formulation, as opposed to the traditional HH type formulation. First, the Markov model was calibrated and fitted to
glycosylation data from in-vivo experiments (see Table 2-1). The sensitivity analysis is performed to establish model parameters that maximally influence the output for each protocol. Furthermore, the algorithm of nonlinear constrained optimization is used to find the parameter values minimizing the sum of the least-square errors between model predictions and experimental data in glycosylation. Pulse protocols used in in-silico models are the same as in in-vivo experiments.

Second, we simulated and compared the variations of $I_{Kr}$ currents and APs for four glycosylation conditions. The glycosylation-induced variations in hERG channels were modeled to predict the changes of $I_{Kr}$ magnitudes and action potential durations (APDs). Third, cardiac cells were connected by gap junctions in a linear fiber, i.e., cable, to predict the glycosylation effects on AP propagation, assuming that altered glycosylation impacts hERG channel gating only. The inhomogeneous cable contains 600 cells, in which 300 cells are fully glycosylated and the other 300 cells are under one of three conditions of reduced glycosylation (i.e., reduced sialylation, mannose-rich, or N-Glycanase treated). Further, we measured the APD restitution curves for 4 glycosylation conditions in the 1D cable of cardiac cells. Finally, we construct the model of 2D tissues by arranging cardiac cells in an array (see Figure 2-2). Glycosylation effects on the rectilinear and spiral waves were predicted using in-silico simulation models. In addition, ECG signals were derived to characterize the variations of cardiac electrical conduction between full glycosylation and reduced glycosylation.

In the single cell, we used a 2-ms 38 pA/pF stimulus current ($I_{stim}$) applied at a frequency of 0.5~2 Hz to trigger simulated APs. The $I_{Kr}$ currents and AP were obtained after at least 100 cycles when the cell reached steady states. The variable temporal resolution is used in the single-cell simulation. In the linear cable, the $I_{stim}$ amplitude of 52 pA/pF and spatial resolution $\Delta x=0.25$mm were used to generate the electrical waves. In addition, APD restitution curve is quantified with
the S1-S2 protocol. The left of the cable is paced at a basic cycle length of 1000ms with the S1 stimulus until steady-state is reached and is then perturbed by a stimulus (S2) after waiting a variable-length interval. (3) In the 2D tissues, the $I_{\text{stim}}$ amplitude is twice the diastolic threshold, i.e., 52pA/pF. The spatial resolution $\Delta x$ is 0.04cm, and the temporal resolution $\Delta t$ is 0.02ms. The spiral wave was initiated by applying a first stimulus (S1=2ms) along one side of the tissue to generate a rectilinear wave propagating toward the other side. When the refractory tail of electrical waves reaches the middle, a second stimulus (S2=5ms) will be given in the middle of the tissue, paralleling to the first rectilinear wave covering only 3/4 of the length of the tissue [21].

<table>
<thead>
<tr>
<th>$\Delta x$ (cm)</th>
<th>$\Delta t = 0.01$ ms</th>
<th>$\Delta t = 0.02$ ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APD (ms)</td>
<td>CV (cm/s)</td>
</tr>
<tr>
<td>0.02</td>
<td>323.00</td>
<td>71.38</td>
</tr>
<tr>
<td>0.025</td>
<td>322.96</td>
<td>69.44</td>
</tr>
<tr>
<td>0.03</td>
<td>322.94</td>
<td>67.43</td>
</tr>
<tr>
<td>0.035</td>
<td>322.94</td>
<td>65.31</td>
</tr>
<tr>
<td>0.04</td>
<td>322.94</td>
<td>63.06</td>
</tr>
</tbody>
</table>

We measured the CVs at different spatial and time resolutions for four glycosylation conditions on a cable of 600 cells. Note that Table 2-2 does not include the CVs and APDs for different glycosylation conditions. This is because $I_{Na}$ is the main contributor to excite a cardiac cell and trigger electrical conduction. In this investigation, we assumed that only hERG channels have altered glycosylation and all other channels (including $Na^{+}$ channel) remain unchanged. In the 1D cable, the CVs are primarily controlled by the front of the waves (i.e., $I_{Na}$ current). Hence, in-silico experiments show that CVs are approximately the same for different glycosylation conditions at the same $\Delta x$ and $\Delta t$. However, when electrical waves propagate in the tissues, not
only the front of the waves, but also the back of the waves (i.e., refractory period) could affect the conduction. This will be shown later in the section of results of spiral waves.

Here, the APD and CV decrease slightly when $\Delta x$ increase from 0.02cm to 0.04cm at both time steps. When $\Delta t$ increases from 0.01ms to 0.02ms, there is a slight decrease in both APD and CV. These small variations are mainly due to the approximation errors in the numerical analysis. The models of cardiac cell and tissue are solved with the use of Ode15s solver and explicit finite difference methods. Computer models were implemented using Matlab R2010a and Simulink v7.5 software from MathWorks (Natick, MA, USA) in a Windows 7 (Microsoft, Redmond, WA, USA) 64-bit machine.

2.4 Results and Discussions

2.4.1 Glycosylation Modulation of hERG Channel

Table 2-3 Markov state transition rates under four glycosylation conditions

<table>
<thead>
<tr>
<th></th>
<th>Full glycosylation</th>
<th>Reduced Sialylation</th>
<th>Mannose-rich</th>
<th>N-Glycanase</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_0$</td>
<td>0.0312·exp(0.165V)</td>
<td>0.01863·exp(0.0234V)</td>
<td>0.0199·exp(0.0254V)</td>
<td>0.0173·exp(0.022V)</td>
</tr>
<tr>
<td>$\beta_0$</td>
<td>0.0993·exp(-0.0269V)</td>
<td>0.0413·exp(-0.03533V)</td>
<td>0.0469·exp(-0.0385V)</td>
<td>0.0364·exp(-0.0318V)</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>0.0036·exp(0.0221V)</td>
<td>0.0024·exp(0.0210V)</td>
<td>0.0025·exp(0.0225V)</td>
<td>0.0023·exp(0.0195V)</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>0.0003·exp(-0.0088V)</td>
<td>1.55E-4·exp(-0.0195V)</td>
<td>1.55E-4·exp(-0.0209V)</td>
<td>1.48E-4·exp(-0.0181V)</td>
</tr>
<tr>
<td>$\alpha_3$</td>
<td>0.0127·exp(-0.0343V)</td>
<td>0.0274·exp(-0.0357V)</td>
<td>0.0293·exp(-0.0393V)</td>
<td>0.0250·exp(-0.0320V)</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>0.4916·exp(0.0065V)</td>
<td>0.4660·exp(0.0048V)</td>
<td>0.5771·exp(0.0063V)</td>
<td>0.3458·exp(0.0032V)</td>
</tr>
<tr>
<td>$\alpha_3$</td>
<td>8.04E-5·exp(3.86E-7V)</td>
<td>1.53E-6·exp(6.34E-7V)</td>
<td>1.54E-6·exp(6.47E-7V)</td>
<td>1.53E-6·exp(6.2E-7V)</td>
</tr>
<tr>
<td>$K_f$</td>
<td>0.0262</td>
<td>0.0296</td>
<td>0.0321</td>
<td>0.0272</td>
</tr>
<tr>
<td>$K_b$</td>
<td>0.1478</td>
<td>0.2136</td>
<td>0.2297</td>
<td>0.1972</td>
</tr>
</tbody>
</table>

Markov model of hERG channels is optimally calibrated to reproduce the in-vitro data from the whole-cell voltage clamp experiments. Note that the SSA and SSI curves were obtained using the same pulse protocol from simulated currents as well as from in-vitro experiments (see Figure 2-3). First, we performed the sensitivity analysis on the Markov model to identify transition rates that...
maximally impact the in-silico data for each pulse protocol. Second, a constrained nonlinear optimization algorithm, i.e., trust-region-reflective [28], is employed to identify the parameters of transition rates. The objective function is to find optimal parameter values that minimize the sum of the least-square errors between in-silico and in-vivo data. Note that the parameter values from Mazhari et al. [16] were used as initial guesses in the minimization procedure. A full list of the parameters of transition rates under 4 glycosylation conditions is reported in Table 2-3.

Figure 2-3 shows the fitted SSA and SSI relationships of hERG under four glycosylation conditions. Note that the SSA and SSI curves are shifted rightward (5~15mv) to more hyperpolarized potentials. In other words, N-glycans limits hERG voltage-dependent activation and inactivation by shifting the half-activation voltage of hERG to more depolarized potentials (see Table 2-3 for parameter values). Thus, changes in glycosylation modulate the voltage-dependent gating behaviors of hERG ion channels.

Figure 2-3 The hERG channel gating and kinetics under four glycosylation conditions. (a) Steady-state activation (b) Steady-state inactivation. (In-vitro data as reported by us previously [26]: ■ Full Glycosylation ●Reduced Sialylation ▲Mannose Rich ▼N-Glycanase, In-silico data from the Markov model: straight and dashed lines)
2.4.2 Reduced Glycosylation Modifies $I_{Kr}$ and Shortens APD

The measured shifts in SSA and SSI with changes in glycosylation would likely modulate $I_{Kr}$ during the repolarization phases of the AP. Further, we integrate glycosylation-channel interactions (see Figure 2-3 from the whole-cell patch clamp experiments) with the in-silico model of cardiac cell to predict whether and how reduced glycosylation will impact the $I_{Kr}$ currents and action potentials. As shown in Figure 2-4, the $I_{Kr}$ current is shifted leftward along the time axis with earlier and higher current densities under 3 reduced glycosylation conditions (i.e., reduced sialylation, mannose-rich, and N-Glycanase). When hERG channels recover from the inactivation to the activated state, the repolarized cell will decrease the AP from the peak value to resting potential. As shown in Figure 2-4, the N-Glycanase treated cell has a early peak in the $I_{Kr}$ current along the time axis. This indicates the efflux of potassium current occurs earlier (i.e., repolarization) in Phases 2 and 3 of AP. It may also be noted that mannose-rich and reduced sialylation treated cells have similar $I_{Kr}$ spike shapes except the former has a higher peak in Figure 2-4. The larger rightward shift in SSI curve measured for the mannose-rich conditions (See Figure 2-3(b)) are likely responsible for this higher peak. In other words, hERG channels under mannose-rich conditions would recover from inactivation at more depolarized potentials during the AP, therefore the hERG channel would be more active during the AP, causing an increased $K^+$ efflux.

Figure 2-5 shows that reduced glycosylation shortens the action potential duration (APD), and the N-Glycanase treated cell yields the shortest APD. When $K^+$ efflux increases, the transmembrane potential decreases quickly and the cell repolarizes to the resting potential more quickly. Reduced glycosylation promotes an earlier/higher $I_{Kr}$ peak, thereby leading to a faster repolarization and a shortened APD. (see Figure 2-5). In addition, we validated the results from in-silico models by measuring hERG current in CHO cells treated with N-Glycanase and under
conditions of full glycosylation. As shown in Figure 2-6, in-vitro $I_{Kr}$ currents [26] match the simulated $I_{Kr}$ curves in major characteristics (e.g., leftward shifted and higher amplitude in the N-Glycanase treated conditions), but with more random variations in the in-vitro data.

Figure 2-4 Predicted rapid delayed rectifier K$^+$ current ($I_{Kr}$)

Figure 2-5 Human ventricular action potential under four glycosylation conditions
2.4.3 Reduced Glycosylation Affects the APD Restitution in A Single Cell

We measured the APD restitution with the S1-S2 restitution protocol. The cell is paced at a basic cycle length (BCL) with the S1 stimulus until steady-state is reached and is then perturbed by a stimulus (S2) after waiting a variable-length interval. In our experiments, S1 stimulus is applied for 30 cycles to reach the steady state. Also, two different BCLs, i.e., 1000ms and 2000ms were used. Both S1 and S2 stimulus are 1ms duration and 38pA/pF amplitude.

Figure 2-7 a and b shows the APD$_{90}$ restitution curves $APD_{n+1} = f(DI_n)$ at the BCL of 1000ms and 2000ms, respectively. Note that the BCL of 1000ms yields a similar shape of APD$_{90}$ restitution curve as the 2000ms. The APD increases with the DI, and the reduce glycosylated cell has smaller APD$_{90}$ than the fully glycosylated one. However, there is a negative slope ($df/d(DI) < 0$) at small DIs, i.e., 15ms~22ms (see Figure 2-7a) for the BCL of 1000ms. For the BCL of 1000ms, the minimal DI is 74.56ms, 73.16ms, 73.75ms and 70.69ms respectively for full glycosylation,
reduced sialylation, mannose rich and N-Glycanase. The minimal DI is calculated when the slope of APD$_{90}$ restitution curve reaches 1 (i.e., $df/d(DI) = 1$). Note that the minimal DI interval in the condition of full glycosylation is the largest. Similar results were obtained for the BCL of 2000ms. The minimal DI interval is slightly smaller, i.e., 71.89ms, 64.29ms, 68.23ms and 64.74ms respectively for full glycosylation, reduced sialylation, mannose rich and N-Glycanase.

Figure 2-7 Single-cell action potential duration (APD) restitution curves obtained with the pacing rates of 1Hz (a) and 0.5Hz (b)

Figure 2-8 Protocol (a) and CV restitution (b) of 1D cable at Full Glycosylation, Reduced Sialylation, Mannose Rich and N-Glycanase
2.4.4 Conduction Velocity

We measured the conduction velocity (CV) restitutions on 1D cables composing of 1400 cells. The S1-S2 protocol used is similar to the one in the APD restitution of 1D cable. As shown in Figure 2-8, we varied the pacing period between S1 and S2 stimulus to investigate the impact on the CVs. Note that the CVs increase when the pacing period increase, and tend to be the same for four glycosylation conditions when the pacing period is > 650ms. However, when the pacing period <650ms, the CVs for full glycosylation are smaller than others. This is because the refractory period is larger for fully glycosylation condition. Also, when the pacing period is < 370ms, the CVs of reduced sialylation and mannose rich are smaller than the N-Glycanase. The minimal pacing period (i.e., if smaller than this interval, electrical waves cannot be stimulated) is 350ms, 340ms, 342ms and 338ms respectively for full glycosylation, reduced sialylation, mannose rich and N-Glycanase.

2.4.5 Reduced Glycosylation Affects $I_{Kr}$ Magnitudes, APDs at Different Pacing Rates, and APD Restitution in A Cardiac Cable

Figure 2-9 shows the variations of $I_{Kr}$ currents (peak currents) of four glycosylation conditions at different pacing rates in a linear cable of cardiac cells. In our experiments, 600 cardiac cells are connected to form a cell cable. The first cell in the cable is stimulated. The $I_{Kr}$ for the 200th cell are collected in the steady state. In order to make sure the stability, computer experimental results are collected after the first cell is stimulated for at least 20 times. As shown in Figure 2-9, the $I_{Kr}$ magnitude decreases 30.98%, 26.08%, 17.37% and 32.73% when the pacing period increases from 400ms to 2000ms for full glycosylation, reduced sialylation, mannose rich and N-Glycanase, respectively. However, the differences of $I_{Kr}$ magnitudes are bigger between four glycosylation conditions for the larger pacing period, i.e., 1000ms-2000ms (see Figure 2-9).
Figure 2-9 The variations of IKr magnitudes (peak currents) in 1D cable with respect to different pacing periods under the conditions of Full Glycosylation, Reduced Sialylation, Mannose Rich and N-Glycanase.

Figure 2-10 shows the variations of APDs of four glycosylation conditions, i.e., Full Glycosylation, Reduced Sialylation, Mannose Rich and N-Glycanase, at different pacing rates in a linear cable of 600 cells. Similarly to the experiments of $I_{Kr}$ currents, the first cell in the cable is stimulated. The APDs for the 200$^{th}$ cell are collected in the steady state (i.e., after the first cell is stimulated for at least 20 times). Modeling results shows that APD$_{90}$ increases when the pacing period increases from 400ms to 2000ms (see Figure 2-10). Note that APD$_{90}$ differences decrease among 3 reduced glycosylation conditions when the pacing period increases. However, if we compare reduced glycosylation with full glycosylation, the effect to shorten the APD$_{90}$ is the same for different pacing periods (see Figure 2-10). It may also be noted that the APD$_{90}$ differences among 3 reduced glycosylation conditions vanish when the pacing periods is greater than 1500ms.
We also measured APD restitution $APD_{n+1} = f(DI_n)$ for a linear cable of 600 cells with the S1-S2 restitution protocol (see details in Section 2.3, computer experiments). Note that the experiments of APD restitution not only quantify the effects of glycosylation on the minimal diastolic interval (DI), but also potentially indicate the stability of spiral waves. In other words, the slope of APD restitution curve provides potential information on the alternans or breakups of spiral waves [28]. In our experiment, S1 stimulus is applied in the first cell for 20 cycles at the basic cycle length (BCL) of 1000ms to reach the steady state. The $APD_{90}$ restitution curve for the 200th cell is collected. As shown in Figure 2-11, the APD increase with DI. The slope of APD restitution curve are greater than or equal to 1 (i.e., $df/d(DI) \geq 1$) for four glycosylation conditions. This indicates that reduced glycosylation does not increase the probability of spiral wave breakups. In addition, the minimal DIs that elicit a propagating action potential are 99.02ms,
99.44ms, 99.58ms and 99.40ms for full glycosylation, reduced sialylation, mannose rich and N-Glycanase, respectively. Note that the minimal DI in tissues is often larger than a single cell because of the effects of cell coupling and electrical conduction.

![Figure 2-11 The action potential duration (APD) restitution curve of a 1D cable.](image)

### 2.5 Conclusions

In the pharmaceutical industry, in-vitro and in-vivo experiments are widely used to characterize and quantify the drug effects on biological systems. However, there are often many practical and ethical limitations in physical experiments of animal or human subjects. Also, it is very expensive and difficult to comprehensively conduct physical experiments across biological scales, e.g., from ion channels to cells to tissues. In-silico models allow one to overcome these limitations, discover gaps in the in-vivo data, derive new hypotheses and/or suggest new experimental designs.
Although computer models have long been intertwined with physical experiments in cardiac research, no approach to date has integrated the understanding of cardiac function in variable levels of glycosylation across the physical scales of increasing complexity, from molecules to cells to tissues. Note that cardiac ion channels are heavily glycosylated, and slight changes in the glycan structure can possibly impact the gating behaviors of ion channels. This study made one of the first attempts to integrate glycosylation-channel data from in-vitro experiments with in-silico models to investigate how aberrant glycosylation modulates hERG ion channels and, as a result, cardiac electrical signaling across different organization levels, from molecule to cell to tissues.
Currently, little data is available about how reduced glycosylation will impact the gating of hERG channels [26]. In this present investigation, it is shown that reduced glycosylation, i.e., reduced sialylation, mannose-rich and N-Glycanase, shifts the steady-state activation and inactivation of hERG channels towards more positive potentials. These measured changes were integrated with in-silico models to predict glycosylation modulation dynamics on cardiac cells and tissues. The results of computer experiments show that reduced glycosylation increases the $I_{Kr}$ magnitudes and shortens the APD. These glycosylation-induced changes are consistent over a range of pacing rates from 0.5 Hz to 2Hz, but they are less pronounced for faster rates. Also, the APD restitution indicates that reduced glycosylation does not increase the probability of spiral wave breakups. Further, the abruptions in inhomogeneous cable experiments show that reduced glycosylation affects the AP repolarization and shortens the wavelength in 1D cable. It may be noted that N-Glycanase has the most significant effect on cardiac repolarization and electrical propagations. Moreover, 3-lead ECGs derived from rectilinear waves and spiral waves in 2D tissues demonstrate that reduced hERG channel glycosylation narrows the width of waves, shortens the QT interval, and speeds up the reentry.

Because QT intervals are closely related to ventricular repolarization and hERG ion channel gating, a better understanding of glycosylation modulation dynamics in hERG ion channels can suggest a possible new mechanism to control QT intervals in ECG signals. As a result, new "rescue" therapies can be developed by imposing a glycan-dependent change in channel function that counterbalances the dysfunction due to diseases such as long QT syndrome. Our future studies will be dedicated to a continued understanding of how regulated and aberrant glycosylation impacts cardiac function using in-vitro and in-vivo experiments as well as in-silico simulations as tools to test this novel mechanism for the control of cardiac electrical signaling.
2.6 References


