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Multi-Scale Modeling of Glycosylation Modulation Dynamics in Cardiac Electrical Signaling

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***Abstract*—The cardiac action potential (AP) is produced by the orchestrated functions of ion channel dynamics. The coordinated functions can be simulated by computational cardiac cell models, which could not only overcome the practical and ethical limitations in physical experiments but also provide predictive insights on the underlying mechanisms. This investigation is aimed at modeling the variations of cardiac electrical signaling due to changes in glycosylation of a voltage-gated K+ channel, hERG, responsible for late phase 2 and phase 3 of the human ventricular AP. The voltage-dependence of hERG channels steady-state activation and inactivation under four glycosylation conditions, i.e., full glycosylation, reduced sialylation, mannose-rich and N-Glycanase treated, demonstrated that reduced glycosylation modulates hERG channel gating. Here, the proposed multi-scale computer model incorporates the measured changes in hERG channel gating observed under conditions of reduced glycosylation, and further predicts the electrical behaviors of cardiac cells and tissues (cable/ring). The multi-scale modeling results show that reduced glycosylation would act to shorten the repolarization period of cardiac APs, and distort the AP propagation in cardiac tissues. This multi-scale modeling investigation reveals novel mechanisms of hERG channel modulation by regulated glycosylation that also impact cardiac myocyte and tissue functions. It can potentially lead to new pharmaceutical treatments and drug designs for long QT syndrome and cardiac arrhythmia.**

***Index Terms*—Multi-Scale simulation model, glycosylation modulation, cardiac myocyte action potential, long-QT syndrome.**

I. Introduction

Cardiac electrical signaling, e.g., electrocardiogram (ECG), is an integrative multi-scale system that reflects the orchestrated functions of ion channels, cells, tissues, and organs. The ECG signal is initiated at the sinoatrial (SA) node, conducted in both atria, and then relayed through the atrioventricular (AV) node to further propagate through bundle of His and Purkinje fibers toward ventricular depolarization and repolarization. Cell action potentials (AP) are the fundamental functional units of ECG signals in different heart locations. They are harmonically controlled by ion-kinetic structures (e.g., ion channels, ion exchangers and ion pumps). A small aberration or mutation in ion channels will lead to abnormal excitation or propagation of electrical waves, and impact the resultant ECG signals.

Long QT syndrome (LQTS) is a cardiac disorder caused by delayed cardiac repolarization and may lead to ventricular fibrillation and sudden cardiac death [1]. Activity of the human ether-a-go-go related gene product (hERG) is responsible for the outward delayed rectifying potassium current (I_{Kr}) [2]. Genetic mutations of hERG can potentially impact the repolarization of cardiac myocytes and cause devastating cardiac arrhythmias. We recently showed that hERG channel function is modulated by altered glycosylation and this change in channel activity may lead to prolonged or shortened AP repolarization [3]. In this paper, we developed a multi-scale cardiac model to explore the effects of four different glycosylation conditions (i.e., full glycosylation, reduced sialylation, mannose rich, and N-Glycanase) on the cardiac electrical signaling ranging from ion channels to tissues.

The objectives of multi-scale modeling are to overcome the practical limitations in physical experiments and provide predictive insights on the underlying mechanisms. Such a computational model can provide the information of cardiac electrical signaling across scales, e.g., electrical wave propagation on the tissue surface without using optical mapping or surface recording from spatial electrodes. The current cardiac modeling approaches can be categorized generally into two groups as follows: The first group is macro/minimal models such as cellular automata model, fitzHugh-Nagumo model and Barkley Model. The second group is micro/maximal models, e.g., Hodgkin-Huxley model, Luo-Rudy model, Rasmusson model and Nygren-Lindblad model [4, 5]. The macro/minimal models did not provide detailed ion physics, but they are easier to be incorporated into a large-scale system. The micro/maximal models include hundreds of equations to describe the detailed ion channel biophysics but increase the complexity of large-scale experiments. We utilized a micro/maximal model of human ventricular cells in the present investigation, and also aimed to develop numerical solver methods to improve the multi-scale model efficiency and speed up the simulation.

The organization of this paper is as follows: Section II presents the multi-scale models; The design of physical and computer experiments are introduced in section III; Section IV shows the experimental results and presents the multi-scale effects of four glycosylation treatments; Section V concludes this present study.

II. Multi-Scale Model

Cardiac myocyte AP is composed of several phases: one is a fast depolarization in which the membrane potential rises to a high positive value within several milliseconds. Other phases, collectively result in myocyte repolarization during which the cell recovers from the positive voltage to the negative resting potential. This dynamic polarization process is produced by the rapid modulation of transmembrane ion flux. Hodgkin and Huxley formulated the first cell AP model using a set of nonlinear and ordinary differential equations in 1952. Since

then, mathematical models have been widely used to simulate the cardiac cell APs for various purposes, e.g., drug design and cross-validation of clinical experiments. In this investigation, we developed a multi-scale cardiac model and used real-world experimental data to explore the impact of changing glycosylation on hERG channel activity and the resulting impact on cardiomyocyte and tissue electrical signaling and conduction.

A. Glycosylation Modulation of hERG Ion Channels

Ion channels are heavily glycosylated, with up to 30% of a mature protein's mass comprised of glycan structures. Our previous investigations have uncovered that changes in glycosylation and sialylation can affect gating and kinetics of voltage-gated Na+ and K+ channels [6, 7]. It may be noted that a recent report focused on testing the glycosylation effects on the hERG ion channels [3], which are the targets of many LQTS medications and anti-arrhythmic drugs. As mentioned, the hERG channel activity produces the repolarizing current, I_{Kr}, which is responsible for late phase 2 and phase 3 of the human ventricular AP In this investigation, the I_{Kr} currents and detailed hERG channel kinetics are described using the following equations:

$$I_{Kr} = G_{Kr}\sqrt{\frac{K_0}{5.4}}X_{r1}X_{r2}(V - E_K)$$

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

$$\tau_{xr1} = \frac{450}{1+e^{(-45-V)/10}}\frac{6}{1+e^{(V+30)/11.5}}$$

$$\tau_{xr2} = \frac{3}{1+e^{(-60-V)/20}}\frac{1.12}{1+e^{(V-60)/20}}$$

$$\frac{dX_{ri}}{dt} = \frac{X_{ri\infty}-X_{ri}}{\tau_{xri}} \quad i = 1,2 \tag{2}$$

where E_K is the reversal potential, G_{Kr} is the cell conductance, τ_{xr1},τ_{xr2} is activation and inactivation time constant, $X_{r1\infty}$ is the steady-state activation, and $X_{r2\infty}$ is the steady-state inactivation. V_a is the voltage of half-activation, V_i is the voltage of half-inactivation, and K_a , K_i are the slope factors.

B. Cardiac Cell Action Potential Modeling

When the depolarizing stimulus reaches a cardiac myocyte from adjacent cells, the opening of Na^+ channels brings a rapid influx of Na+ ions (I_{Na}) into the cell and the AP rises from resting potential (-90mv) to positive voltages (phase 0). This rapid depolarization phase is followed by a small downward deflection from the inactivation of the fast Na^+ channels and transient net outward K^+ currents (I_{to}, phase 1). Phase 2 consists of a plateau AP phase that is sustained primarily by a balance between inward movement of Ca^{++} (I_{Ca}) and outward movement of K^+, during which hERG channel activity begins to increase. There are other electrogenic transport proteins active such as the sodium-calcium exchanger current ($I_{Na,Ca}$), and the sodium/potassium pump current ($I_{Na,K}$). In phase 3, the AP enters the rapid repolarization phase, during which activation of the rapid delayed rectifier K^+ channels (hERG, I_{Kr}) plays an important role in returning the membrane back to the resting potential. The electro-diffusion model is constructed as that cell membrane is equivalent to a parallel capacitor, and the ion channels and pumps are considered as resistances and batteries.

We used Ten Tusscher and Panfilov's human ventricular cell model [8, 9] to describe the electrophysiological behaviors of the cardiac cells. This model assumes that the cell membrane is homogeneous and neutral, and the intracellular and extracellular regions are uniform. The membrane potentials are determined by the following 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 V is AP, t is time. C_m is cell capacitance per unit surface area, I_{stim} is the external stimulus current which activate 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_K), 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}). These voltage-gated ion currents are modeled similarly as I_{Kr} in the form of cell conductance, membrane potential gradients and channel gate dynamics. The details of all other channel kinetics can be found in the references [8, 9].

C. Cardiac Cellular Cable and Ring Modeling

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 cause a depolarizing-to-resting Na+ gradient and drive the flow of Na+ through the connexon into the adjacent resting cells. The influx of Na+ causes neighboring cells to reach the threshold potential and activate the depolarization phase (0) of the AP. As the depolarization and repolarization propagate among cells, electrical waves are generated. It may be noted that the conduction speed varies due to the conductance of gap junctions. This cell-to-cell conduction is analogous to resistance in circuit systems, and the consecutive cell connections are modeled as a cable or ring using the following partial differential equations [5]:

$$\frac{dV}{dt} = -\frac{I_{mem} + I_{stim}}{C_m} + \frac{1}{\rho_x S_x C_m}\frac{\partial^2 V}{\partial x^2}$$

Where I_{mem} is the sum of transmembrane ion currents, ρ_x is cellular resistivity, S_x is the surface-to-volume ratio, and C_m is cell capacitance per unit surface area.

III. Materials and Experimental Design

A. Physical Experiments

We recently reported the effects of differential glycosylation on hERG channel gating, showing that N-glycosylation effectively limits hERG activity during the ventricular AP [3]. We used whole cell recording methods to measure the voltage-dependence of hERG channel steady state activation (SSA) and inactivation (SSI) behaviors under four conditions of glycosylation, i.e., full glycosylation, reduced sialylation, mannose-rich and N-Glycanase treated. Table 1 shows the SSA and SSI parameter values measured under each condition of glycosylation. The voltage of half-activation (V_a),

half-inactivation (V_i) and slope factors (K_a, K_i) are listed in the table. These changes in hERG channel gating were integrated into the computer simulation models, which are used to further predict the multi-scale glycosylation effects on cardiac electrical signaling of cells and tissues.

Table 1 hERG1 channel activation and inactivation parameters

hERG1A Channel	V_a(mV)	K_a(mV)	V_i(mV)	K_i(mV)
Full Glycosylation	-22.0	8.4	-74.4	-19.0
Reduced Sialylation	-13.7	8.6	-56.2	-17.2
Mannose-Rich	-12.6	8.9	-51.48	-16.4
N-Glycanase treated	-12.1	8.9	-58.5	-20.3

B. *Computer Experiments*

The computer experiments are aimed at modeling and predicting the multi-scale glycosylation effects on cardiac electrical signaling of ion channels, cells and tissues. First, we modified the hERG channel gating equations in the detailed ventricular cell models based on the experimental data listed in Table 1. The activation and inactivation parameters in equation 1 were optimized to fit the experimental results in four glycosylation conditions. Second, we simulated and compared the variations of the outward delayed rectifying potassium current (I_{Kr}) and cellular action potentials with respect to the four glycosylation conditions. Third, the ventricular myocytes were modeled to be connected by gap junctions in a linear or circular fiber, i.e., cable or ring, to predict the glycosylation effects on AP propagation. The inhomogeneous cable or ring contains 100 cells, in which 50 cells are fully glycosylated and 50 are under one of the three independent conditions of reduced glycosylation (reduced sialylation, mannose-rich, or N-glycanase treated). The amplitude of I_{stim} is 52 pA/pF, and the stimulus pulse duration is 1 ms. The cardiac cell and tissue models are solved with the use of Ode15s solver and explicit finite difference methods.

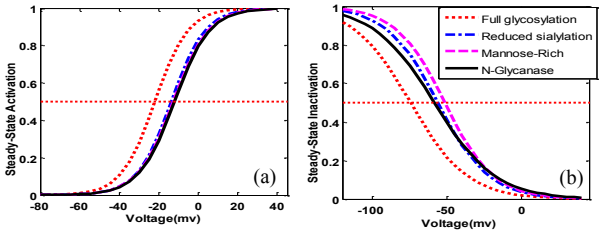

Figure 1. Steady-state activation (a) and inactivation (b) relationships under four glycosylation conditions.

IV. Results

Glycosylation remodels hERG ion channels. The biophysical characterization of hERG channel gating indicated that the SSA and SSI relationships were each shifted linearly in the depolarizaed direction along the voltage axis under conditions of reduced glycosylation [3]. Figure 1 shows the fitted SSA and SSI relationships of hERG under the four conditions of glycosylation. Note that the SSA and SSI curves are shifted rightward (5~15mv) to more depolarized potentials. In other words, N-glycans promote hERG voltage-dependent activation and to an even larger degree, inactivation, by shifting the activation voltage for hERG to more hyperpolarized potentials (see Table 1 for parameter values). Thus, changes in glycosylation modulate hERG channel voltage-dependent gating.

Glycosylation modifies I_{Kr} and shortens AP duration. The measured shifts in SSA and SSI with changes in glycosyaltion would likely modulate I_{Kr} during the repolarization phases of the AP. Thus, as show in Figure 2, the I_{Kr} current is shifted leftward along the time axis with elarlier and higher current densities under reduced glycosylation conditions. Voltage decrease from the positve value (38mV) to resting potential during repolarization when the channel recover from inactivation state to non inactivation state. The N-Glycanase cell recover at a higher voltage (Figure 1b 38mV-10mV) and open earlier, that explains why the N-glycannase treated one have a early high density peak. The mannose-rich and reduced sialylation treated cells have similar I_{Kr} spike shapes except the former has a higher peak. The larger rightward shift in SSI curve measured for the mannose-rich conditions during -30mV to resting potential (See Fig. 1b) are likely responsible for this higher peak. That is, channels under mannose-rich conditions would recover from inactivation faster, therefore the channel would be in more activative state , thus causing an increased K+ efflux. As K+ efflux increases, the transmembrane potential decreases quickly and the cell spends less time in returning to the resting membrane potential. This modeling clearly shown that the ventricular action potential (AP) can be predicted to have a shorter duration in the reduced glycosylation conditions. It may be noted that the I_{Kr} in N-Glycanase treated condition increases rapidly from the initial stage of repolarization phase, thereby is predicted to result in the shortest actional potential duration (APD) (see Figure 2 (b)).

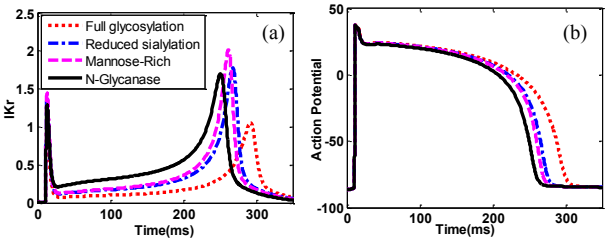

Figure 2. (a) Rapid delayed rectifier K+ current (I_{Kr}) and (b) human ventricular action potential under four glycosylation conditions.

Glycosylation affects the AP propagation in inhomogeneous tissues. The repolarization heterogeneity can potentially cause inhomegeneous electrical conduction in cardiac tissues. As shown in Figure 3 (a-c), we connected 100 inhomogeneous ventricular cells by gap junctions in a linear cable. The first 50 cells are under conditions of full glycosylation and the second 50 are under one of the three conditions of reduced glycosylation, specifically, reduced sialylation (a), mannose-rich (b), or N-glycanase (c). The stimulus is initiated in the first cell and consecutively excited cells conduct the electrical waves to the end of the tissue. It is shown that there are abruptions in electrical waves indicating the heterogeneity of APD between fully glycosylated cells and less glycosylated cells. The repolarization heterogeneity may potentially increase the risks of cardiac arrythmia. The comparisons are also presented in Figure 3 (d-f), which represent the differences between three inhomogenous cables (see Figure 3 (a-c)) and

one homogenous cable, i.e., full glycosylation. The N-Glycanase treated cable is shown to have the most significant abruptions in electrical wave conductions.

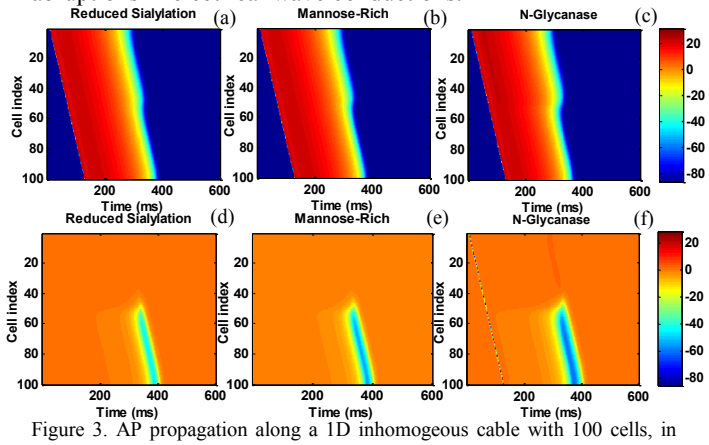

Figure 3. AP propagation along a 1D inhomogeous cable with 100 cells, in which the first 50 cells are under conditions of full glycosylation and with the second 50 under the following reduced glycosylation conditions: (a) reduced sialylation, (b) mannose-rich, and (c) N-Glycanase. The comparison plots (d-f) represent the differences between (a-c) vs. one fully glycosylated treated cable.

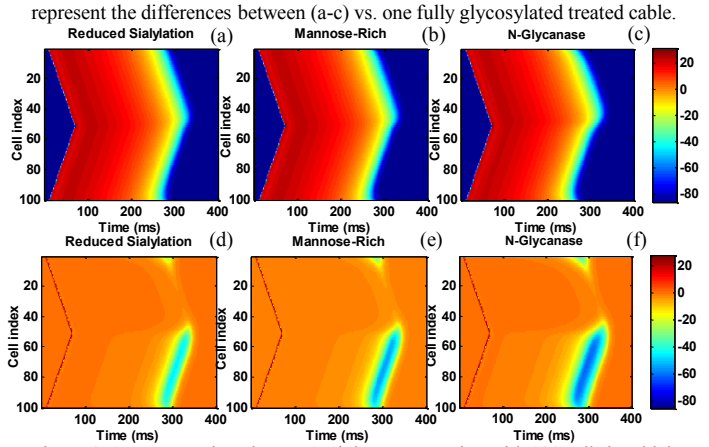

Figure 4. AP propagation along a 1D inhomogeous ring with 100 cells in which the first 50 cells are fully glycosylated and the second 50 cells are under the following reduced glycosylation conditions: (a) reduced sialylation, (b) mannose-rich, and (c) N-Glycanase. The comparison plots (d-f) represent the differences between (a-c) vs. one full glycosylation treated ring.

Figure 4 (a-c) shows AP propagation along three rings composed of 100 inhomogenous cardiac cells. It may be noted that the first cell is connected to the last cell in the circular ring structure. When the stimulus is applied to the first cell, the AP will propagate bidirectionally towards both left and right sides, and finally collide when the signals reach one other. As the gap junctions are uniform in these rings, the conduction speed is the same along both sides. However, the N-Glycanase treated cardiac cells are shown to have the shortest APD. The comparisons are shown in (d-f), which represent the differences between (a) reduced sialylation, (b) mannose-rich, and (c) N-Glycanase vs. one uniform fully glycosylated ring. Such comparisons show simillar results as the linear cable experiments. That is, the repolarization duration under reduced glycosylation conditions are shortened with the N-Glycanase treated cells returning to resting potentials faster than the others. The experimental results are consistent with the predicted effects on cardiac electrical signaling based on multi-scale modeling of hERG ion channel gating at the cellular and tissue (i.e., cable and ring) levels. Reduced glycosylation modulates hERG channel activity, shortens the APD of single cardiac myocytes, and affects the AP propagation in cardiac tissues.

V. Conclusions

Mathematical modeling of cardiac myocytes allows one to predict the effects of pharmaceutical treatments. This paper presents a multi-scale model to explore the hERG channel modulation dynamics of changing glycosylation on the cardiac electrical signaling on cardiac myocytes and cardiac conduction through the tissue. A novel mechanism of glycosylation modulation dynamics on hERG ion channels, that is reduced glycosylation can cause a shortened ventricular APD, was demonstrated by the computer experiments. As show in the result, three reduced glycosylation conditions (i.e., reduced sialylation, mannose-rich and N-Glycanase) each cause a rightward shift in the steady-state activation and inactivation relationships of hERG channels. Therefore, the action potential duration is predicted to be shorter under conditions of reduced glycosylation. The abruptions in the inhomogeneous cable/ring experiments predict that glycosylation treatments will affect the AP propagation and increase the susceptibility to cardiac arrhythmia.

As the QT intervals of ECG signals are closely related to ventricular repolarization, we suggest that through an understanding of the impact of changes in glycosylation on hERG channel activity and cardiac electrical signaling, new tools can be developed in the design of drugs used to treat long QT syndrome (i.e., LQT2 from the hERG gene mutations) and potentially other cardiac arrhythmias. The experiments show that N-Glycanase treatment will have the most significant effects on QT interval. The multi-scale computer model overcomes the practical limitations in physical experiments and provides predictive insights on the underlying cardiac mechanisms across scales.

References

[1] H. Morita, J. Wu and D. P. Zipes, "The QT syndromes: long and short," *The Lancet,* vol. 372, pp. 750-763, 2008.

[2] D. Dubin, *Ion Adventure in the Heartland-Exploring the Heart's Ionic-Molecular Microcosm.* Tampa,FL: Cover Publishing Company, 2003.

[3] S. A. Norring, T. A. Schwetz, A. R. Ednie, H. Yang and E. S. Bennett, "N-Glycans Limit hERG1A Channel Function," *Submitted for Publication,* 2011.

[4] J. R. Silva and Y. Rudy, "Multi-scale electrophysiology modeling: from atom to organ," *The Journal of General Physiology,* vol. 135, pp. 575-581, June 01, 2010.

[5] E. V. Bondarenko and L. R. Rasmusson, "Simulations of propagated mouse ventricular action potentials: effects of molecular heterogeneity," *Am J Physiol Heart Circ Physiol,* vol. 293, pp. H1816-H1832, 2007.

[6] T. A. Schwetz, S. A. Norring and E. S. Bennett, "N-glycans modulate K(v) 1.5 gating but have no effect on K(v)1.4 gating," *Biochim Biophys Acta,* vol. 1798, pp. 367-375, 2009.

[7] M. L. Montpetit, P. J. Stocker, T. A. Schwetz, J. M. Harper, S. A. Norring, L. Schaffer, S. J. North, J. Jang-Lee, T. Gilmartin, S. R. Head, S. M. Haslam, A. Dell, J. D. Marth and E. S. Bennett, "Regulated and aberrant glycosylation modulate cardiac electrical signaling," *Proc Natl Acad Sci USA,* vol. 106, pp. 16517-16522, 2009.

[8] K. H. W. J. ten Tusscher, D. Noble, P. J. Noble and A. V. Panfilov, "A model for human ventricular tissue," *Am J Physiol Heart Circ Physiol,* vol. 286, pp. 1573-1589, 2004.

[9] K. H. W. J. ten Tusscher and A. V. Panfilov, "Alternans and spiral breakup in a human ventricular tissue model," *Am J Physiol Heart Circ Physiol,* vol. 291, pp. H1088-H1100, September 01, 2006.