A MATHEMATICAL MODEL OF THE HUMAN CARDIAC SODIUM CHANNEL

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ABSTRACT

Sodium ion (Na+) channels play an important role in excitable cells, as they are responsible for the initiation of action potentials. Understanding the electrical characteristics of sodium channels is essential in predicting their behavior under different physiological conditions. We investigated several Markov models for the human cardiac sodium channel (NaV1.5) to derive a minimal mathematical model that can describe the reported experimental data obtained using major voltage-clamp protocols. We obtained simulation results for current-voltage relationships, steady-state inactivation, the voltage dependence of normalized ion channel conductance; activation and deactivation, fast and slow inactivation and recovery from inactivation kinetics. Good agreement with the experimental data provides us with the mechanisms of the fast and slow inactivation of the human sodium channel and the coupling of its inactivation states to the closed and open states in the activation pathway.

INDEX WORDS: Markov model, NaV1.5 channel, Inactivation, Recovery from inactivation, Voltage-clamp, Ion channel
A MATHEMATICAL MODEL OF THE HUMAN CARDIAC SODIUM CHANNEL

by

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August 2017
DEDICATION

This thesis is dedicated to my family who have been a continuous source of encouragement and support.
ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my advisor Professor Vladimir E. Bondarenko for the continuous assistance and guidance that he has given me. He has been supportive since the first day I began working with him. This thesis would not have been possible without the insight and expertise of Prof. Vladimir E. Bondarenko.

Moreover, I would like to thank my committee members Professor Igor Belykh, Professor Michael Stewart, and Professor Gennady Cymbalyuk for agreeing to be on my thesis committee, despite their busy schedules, and valuable comments.

I also want to give my gratitude to Kelvin Rozier, for proofreading my thesis and giving me helpful comments.

In addition, I would like to thank friends and family who encouraged and supported me while I am working on my thesis and all people who contributed in some way to this thesis.

Finally, I would extend my gratitude to the University System of Georgia (USG) for supporting my education through the Tuition Assistance Program (TAP).
TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................... v

LIST OF FIGURES ................................................................................................................ ix

1 INTRODUCTION ........................................................................................................... 1

  1.1 Recent Advances in the Studies of the Human Sodium Channel ................. 1

  1.2 Mathematical Models of the Sodium Channel ............................................. 5

    1.2.1 Hodgkin-Huxley Model .................................................................. 5

    1.2.2 Armstrong-Bezanilla Model ......................................................... 6

    1.2.3 Irvine-Jafri-Winslow Mode ......................................................... 7

    1.2.4 Clancy-Rudy Model .................................................................. 8

  1.3 Purpose of the Study ....................................................................................... 9

2 METHODS .................................................................................................................. 10

  2.1 Analysis of the Experimental Data Used for the Model Development........ 10

  2.2 Experimental Protocols ............................................................................. 13

  2.3 Methods of Simulations ............................................................................. 16

3 RESULTS .................................................................................................................... 17

  3.1 Fast Inactivation of the Human Sodium Channel .................................... 17

    3.1.1 Model 1 ...................................................................................... 17

    3.1.2 Model 2 ...................................................................................... 25

    3.1.3 Model 3 ...................................................................................... 31
3.2 Mechanisms of the Slow Inactivation ............................................................. 37

3.2.1 Model 4 ......................................................................................................... 37

3.2.2 Model 5 ......................................................................................................... 44

3.2.3 Model 6 ......................................................................................................... 49

4 DISCUSSION ......................................................................................................... 57

4.1 The Role of the Sodium Current for the Cardiac Action Potential ............. 57

4.2 The Slow Component of the Fast Na⁺ Current and Arrhythmias .......... 58

4.3 Comparison to the Other Mathematical Models ....................................... 60

5 CONCLUSIONS .................................................................................................. 62

REFERENCES ......................................................................................................... 63

APPENDICES ......................................................................................................... 68

Appendix A: Transition Rates ............................................................................. 68

Appendix A.1 Functions used in all models ....................................................... 68

Appendix A.2 Model 1a and 1b (monoexponential) ......................................... 68

Appendix A.3 Models 1c and 1d (biexponential) .............................................. 69

Appendix A.4 Model 2a (biexponential) ............................................................. 69

Appendix A.5 Model 2b (biexponential) ............................................................ 70

Appendix A.6 Model 2c (monoexponential) ....................................................... 71

Appendix A.7 Models 3a and 3b (monoexponential) ......................................... 71

Appendix A.8 Model 3c (biexponential) ............................................................. 72
Appendix A.9 Models 4a, 4b and 4c (biexponential) ................................................ 73

Appendix A.10 Model 5 (biexponential) ................................................................... 74

Appendix A.11 Model 6 (biexponential) ................................................................... 74
LIST OF FIGURES

Figure 1.1: Voltage-gated sodium channel α-subunit................................................................. 1

Figure 1.2: Crystal structure of the bacterial voltage-gated Na⁺ channel NavRh. Reproduced from a PDB file (4dxw.pdb) from Zhang et al. [6]................................................................. 2

Figure 1.3: Amino acid sequence (2016 aa) of the human sodium channel NaV1.5. Data are from Van Driest et al. [7]........................................................................................................................................ 3

Figure 1.4: Markov model reproduction of the Hodgkin-Huxley model.................................. 6

Figure 1.5: The Armstrong-Bezanilla model........................................................................... 6

Figure 1.6: The Irvine-Jafri-Winslow model.......................................................................... 7

Figure 1.7: The Clancy-Rudy model .................................................................................... 8

Figure 2.1: Voltage dependence of experimental results. (A) Experimental current-voltage relationships. (B) Experimental steady-state inactivation relationships (open circles) and $G/G_{\text{max}}$ (closed circles). (C) Experimental time-to-peak currents (closed circles) and deactivation time constants (open circles). Experimental data are from O’Leary et al. [23], adjusted to room temperature $T = 295^\circ\text{K}$ (22°C). ................................................. 11

Figure 2.2: Experimental time constants for inactivation and recovery from inactivation.

Experimental data on inactivation are from O’Leary et al. (1995) (closed circles and squares) [23], Veldkamp et al. (2000) (diamonds) [24], and Wang et al. (2007) (crosses) [25]. Experimental data on recovery from inactivation are from O’Leary et al. (1995) (open circles) [23], Veldkamp et al. (2000) (squares) [24], and Wang et al. (2007) (triangles) [25]. .............................................................................................................. 12

Figure 2.3: Steady-state inactivation voltage-clamp protocol............................................... 14

Figure 2.4: Deactivation voltage-clamp protocol................................................................. 15
Figure 2.5: Recovery from inactivation voltage-clamp protocol

Figure 3.1: Five state Markov model for cardiac sodium channel (Model 1). C₁ – C₃ are closed states, O is an open state, and IF is fast inactivation state. α, β, koif, and kifo are voltage dependent transition rates.

Figure 3.2: Experimental data and simulations obtained with Model 1a. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio G/Gmax (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.3: Experimental data and simulations obtained with Model 1b. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio G/Gmax (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.4: Experimental data and simulations obtained with Model 1c. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio G/Gmax
(solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant
(solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV,
inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23],
Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental
data are the same as in Figs. 2.1 and 2.2………………………………………………………….. 23

Figure 3.5: Experimental data and simulations obtained with Model 1d. (A) current traces, (B)
deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage
relationship, (D) steady-state inactivation (dashed line) and conductance ratio \( \frac{G}{G_{\text{max}}} \)
(solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant
(solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV,
inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23],
Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental
data are the same as in Figs. 2.1 and 2.2………………………………………………………….. 24

Figure 3.6: A five state Markov model for cardiac sodium channel with additional transitions
between C1 and IF states (Model 2). \( \alpha, \beta, k_{\text{ifo}}, k_{\text{oif}}, \) and \( k_{\text{ifc1}} \) are voltage dependent
transition rates, \( k_{c1if} \) is voltage-independent transition rate. …………………………….. 25

Figure 3.7: Experimental data and simulations obtained with Model 2a. (A) current traces, (B)
deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage
relationship, (D) steady-state inactivation (dashed line) and conductance ratio \( \frac{G}{G_{\text{max}}} \)
(solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant
(solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV,
inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23],
Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.8: Experimental data and simulations obtained with Model 2b. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.9: Experimental data and simulations obtained with Model 2c. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.10: An eight state Markov model (Model 3). It includes closed states (C1-C3), open state (O), fast-inactivated state (IF) and closed-inactivated states (IC1-IC3). $\alpha, \beta, k_{oIF}, k_{IFo}, k_{cI}$, and $k_{IC}$ are voltage dependent transition rates. f is an allosteric factor that regulates the coupling of the inactivated states to the closed states.
Figure 3.11: Experimental data and simulations obtained with Model 3a. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2 ............................................. 33

Figure 3.12: Experimental data and simulations obtained with Model 3b. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [27]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2 ............................................. 34

Figure 3.13: Experimental data and simulations obtained with Model 3c. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23],
Figure 3.14: A nine state Markov model (Model 4). This model consists of three closed states (C1-C3), open state(O), fast-inactivated state(IF), slow-inactivated state (IS), and three closed-inactivated states(IC1-IC3). $\alpha, \beta, k_{ifo}, k_{oif}, k_{ci}, \text{ and } k_{ic}$ are voltage dependent transition rates. $k_{ois}$ and $k_{iso}$ are voltage-independent transition rates. $f$ is an allosteric factor that regulates the coupling of the inactivated states to the closed states.

Figure 3.15: Experimental data and simulations obtained with Model 4a. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$ (solid line), (E) recovery time constant (black dashed line), fast-inactivation time constant (black solid line), and slow-inactivation time constant (gray solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.16: Simulated time courses of state occupancies (Model 4a). C1-C3, O, IF, IC1-IC3 and IS of the fast Na$^+$ channel as functions of time during and after a 0.5 s depolarization voltage pulse. (A) Data for time interval from 500 to 2500 ms. (B) Data for a shorter time interval from 500 to 510 ms to show details of activation and inactivation.

Figure 3.17: Experimental data and simulations obtained with Model 4b. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$
(E) recovery time constant (black dashed line), fast inactivation time constant (black solid line), and slow inactivation time constant (gray solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.18: Experimental data and simulations obtained with Model 4c. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$ (solid line), (E) recovery time constant (dashed black line) and fast-inactivation time constant (solid black line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.19: A nine state Markov model (Model 5). Three closed states (C1-C3), open state (O), fast-inactivated state (IF), slow-inactivated state (IS) and three closed-inactivated states (IC1-IC3). $\alpha$, $\beta$, $k_{ifo}$, $k_{oif}$, $k_{ci}$ and $k_{ic}$ are voltage dependent transition rates. $k_{ifis}$ and $k_{islif}$ are voltage-independent transition rates. $f$ is an allosteric factor that regulates the coupling of the inactivated states to the closed states.

Figure 3.20: Experimental data and simulations obtained with Model 5. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{max}$ (solid line), (E) recovery time constant (black dashed line), fast inactivation time constant (solid line), and slow inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
(black solid line), and slow inactivation time constant (gray solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

Figure 3.21: Simulated time courses of state occupancies (Model 5). C₁-C₃, O, IF, IC₁-IC₃ and IS of the fast sodium channel are shown as functions of time during and after a 0.5 s depolarization voltage pulse. (A) Data for time interval from 500 to 2500 ms. (B) Data for a shorter time interval from 500 to 510 ms to show details of activation and inactivation.

Figure 3.22: A ten state Markov model (Model 6). States are represented as: closed (Cᵢ), closed-inactivated (ICᵢ), open (O), fast-inactivated (IF), slow-inactivated (IS) and intermediate-inactivated (IM). α, β, kᵢₒᵢ, kᵢₒᵢᵢ, kᵢᵢᵢ, and kᵢᵢᵢᵢ are voltage-dependent transition rates; kᵢᵢᵢᵢᵢ, kᵢᵢᵢᵢᵢ, kᵢᵢᵢᵢᵢᵢ, and kᵢᵢᵢᵢᵢᵢᵢ are voltage-independent transition rates. f is an allosteric factor that regulates the coupling of the inactivated states to the closed states.

Figure 3.23. Simulated and experimental data on activation and deactivation of the fast Na⁺ current Iₙa (Model 6). (A) Simulated current traces on the time interval from 0 to 5 ms to show activation time course of Iₙa. (B) Simulated current traces for deactivation voltage-clamp protocol. Data from 20 to 30 ms are shown. (C) Simulated time-to-peaks (solid line) and deactivation (dashed line) time constants. Experimental data on time-to-peaks (closed circles) and deactivation time constants (open circles) are from O’Leary et al. (1995) [23].
Figure 3.24 Simulated and experimental data on activation and inactivation of the fast Na\(^+\) current \(I_{Na}\) (Model 6). (A) simulated current traces, (B) simulated (solid line) and experimental (circles) IV dependencies, (C) steady-state inactivation (dashed line) and conductance ratio \(G/G_{max}\) (solid line). Experimental data are from O’Leary et al. (1995) (closed and open circles) [23]. ...................................................... 52

Figure 3.25: Simulated and experimental data on inactivation and recovery from inactivation of the fast Na\(^+\) current \(I_{Na}\) (Model 6). Simulated fast and slow time constants of inactivation are shown by solid black and solid gray lines respectively. Simulated fast and slow time constants of recovery from inactivation are shown by dashed and dotted lines, respectively. Experimental data on inactivation are from O’Leary et al. (1995) (closed circles and squares) [23], Veldkamp et al. (2000) (diamonds) [24], and Wang et al. (2007) (crosses) [25]. Experimental data on recovery from inactivation are from O’Leary et al. (1995) (open circles) [23], Veldkamp et al. (2000) (diamonds) [24], and Wang et al. (2007) (crosses) [25]. ................................................................. 54

Figure 3.26. Simulated time courses of state occupancies (Model 6). C\(_1\)-C\(_3\), O, IF, IC\(_1\)-IC\(_3\), IS, and IM of the fast Na\(^+\) channel are shown as functions of time during and after a 0.5 s depolarization voltage pulse. (A) Data for time interval from 500 to 2500 ms. (B) Data for a shorter time interval from 500 to 510 ms to show details of activation and inactivation......................................................... 56
1 INTRODUCTION

Sodium channels play an important role in shaping action potentials in many excitable cells such as neural and cardiac cells. In particular, they are responsible for the action potential upstrokes and action potential propagation. On the other hand, dysfunction of the Na\(^+\) channels can lead to the diseased states. In cardiac cells, the dysfunction of Na\(^+\) channels result in pro-arrhythmic events (early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs)) [1]. In the neural cells, disease-causing mutations of Na\(^+\) channels lead to epileptic seizures [2]. Therefore, investigations of structure-function relationships for Na\(^+\) channels are of great importance.

1.1 Recent Advances in the Studies of the Human Sodium Channel

![Voltage-gated sodium channel α-subunit](image)

**Figure 1.1: Voltage-gated sodium channel α-subunit**

Sodium channels consist of an α subunit (Fig. 1.1) which can be modulated by ancillary subunits [3]. Neural cells in the central nervous system contain Nav1.1, Nav1.2, Nav1.3, and Nav1.6 sodium channels; the peripheral nervous system primarily consists of Nav1.7, Nav1.8, and Nav1.9 channels [3]. Nav1.4 and Nav1.5 are the major Na\(^+\) channels in skeletal and heart muscles, respectively [3]. Properties of the α-subunit can be modulated by two β subunits, β\(_1\) and β\(_2\) [4; 5]. The α subunit of the Na\(^+\) channel consists of about 2000 amino acids, which form four transmembrane domains, DI–DIV. Each transmembrane domain contains six transmembrane
segments, S1–S6. Segments S5 and S6 form a pore region of the Na⁺ channels, and segment S4 represents a voltage sensor.

The crystal structure of the voltage-gated bacterial Na⁺ channel is shown in Fig. 1.2, and amino acid sequence for Nav1.5 from the human heart is shown in Fig. 1.3.

Figure 1.2: Crystal structure of the bacterial voltage-gated Na⁺ channel NavRh. Reproduced from a PDB file (4dxw.pdb) from Zhang et al. [6].

Recently, crystal structures of the bacterial voltage-gated Na⁺ channels were obtained by Payandeh et al. (2011) and Zhang et al. (2012) [6]. Figure 1.2 shows the structure of channel NavRh, obtained by Zhang et al. [6]. The channel consists of four identical subunits, each of which is composed of six transmembrane domains, S1-S6. Domains S5 and S6 form a pore region of the channel, and domains S1-S4 form the voltage-sensitive domains. Most of the channel’s voltage sensing comes from 4 arginines (Arg, R) located in the S4 transmembrane domain. Despite that
bacterial channel NavRh has a smaller number of amino acids, the major components of its crystal structure are similar to that of the mammalian voltage-gated sodium channels (six transmembrane domains, S4 as a voltage sensor, etc.).

```
1 manfl1prgt ssfrrftres laaikekrmae kqargsttltq esreglpeee aprpqldlqa
61 skklpdlgyn ppqeligepl eddpfystq ktflivnkqg tiffrfsatna lyvlspfhpi
121 rravkvlh sfmmmlimct itlncfmq hdppegwtykq eyyfiayttf eslvdkilarg
181 fcflhaftrfl dpwmlwldfsv iimayttef v dlngnvalor ftvlralkti vsvgikltk
241 galqsvgkkl advmvltvcf lsfvafligilq lfmglrnhkc vrnftalngt ngsviadglv
301 wesldlylsd penyllkngr sdvllcgnns dactpegyr clkagenpdg hytsfsdfaw
361 aflalfdlmt qdwerlygql tirsagkiym iffmlvilfl sfylvlnila vvmayeeeqn
421 qatlaeteeek ekrfqemenem lkkhealti rgydltvsrs s lemslaepvnh sherrskrrk
481 rmsgatcccg edripksdse dgprramnnls ltrglrsts rkmrrsesgf tffrrrdigse
541 adfadenest aqgeseshhst lsvpwprrrt sasgppsgpt sapsghahqk kntvdencv
601 vslrgadgde atspsghllr pvmlehpddt tspsepepgg qmltsqacv dfgfeapgarq
661 rlasavslvt saleeeurek hckcpccwrnrl agqyliweer plwsmskqgg klvdmpfstd
721 ltitmcvln tlfnmetalyn mtsefeemlg vglmivftgif tatemtfki a ldpyyffqqg
781 wnifdssivi lnlmeiglsr mlssnlvrlsf rilrvfklak swptlnfklk iiogsvaglg
841 nlitlvlaiiv fifavvqmgfl fgknyslerd dsqgllprrwh mmrifhafli ifrlcgewi
901 etwmdcmeves gqslcllvfl lvmwignlsvv nlvlffals lsfadsnlap dedremmnlq
961 lalarirgrgl rfvkrttwdfl cgilrlrrpqp kpaalaqggq lpsciatyp spppetekvp
1021 pttrktrftee geqpgggtpg dpepcvcvia vaesdttddge edeenslgtg eesskgqesq
1081 pvsggpeapp dsrtswqvsas tassaeasas sqadwrgqkw aepaqapcge tpsdcsegps
1141 tadmrnttala legqipdldq vgkpedfcde gcvrcrpccca vttqapgpgk wwrllrcycthy
1201 iveshwfetf iiifmllssg alafediylo erktikvle yadmkmfyyf vlemllkwwa
1261 ygfkklyfttna wcvldflvld vslvlsvlant lgfaemmpik slirtlralrp lralrsfegv
1321 rrvvna1vga ipsimnvlvlv clliwliifsis mgvnlfagfgr gcrcingtegd lplnlyivnn
1381 ksqceslnit gelywtkpyv nfdnvqagy1 allqvatfkg wmdimyaadv srgyeeqpgw
1441 eynlmyiyf vifiifgsff tlnifigvii dnfngqkkkl ggdlflmtee qkkynamkk
1501 lgskkpqaqf prplnkyqgf ifdirtvqkaq dtvltmflcl nmvtmixedv dqspeknil
1561 akiinlfvai ftgceivkla arhyyfaptcha snifdvvvsi lsivgtvlasd iiqkyffspf
1621 lfrvirlari grilirrira kgirtlffl mmsiapfili glillflvmfi ysisfmanfa
1681 yvwkweagidd mfnfqtffans mlc1fqlitls jwsgdlilspi lntptycdpp tlnpsnsrg
1741 dgsnpavagil ffftyiiissf lrvvmyiai ilenfsvate esteplledd fdmyefielwek
1801 fdpeaqtfie fyyadsfadv laseprliak npqislinmdl pmvsgdirh cmlilfaaktkr
1861 vlgesegeida lqiqmeekfnm anpaksisyte pitttrrkk vrossvmgivr afrmhrlnqrs
1921 lkhasflfrq qagsglele aperegliay vmensfrslp gppssssiss tsfppsydsv
1981 tratsdnlqv rdgysyshd ladfpsdpr dresiv
```

Figure 1.3: Amino acid sequence (2016 aa) of the human sodium channel Nav1.5. Data are from Van Driest et al. [7].

Experimental investigations of the sodium current have revealed the physiological functions of the sodium channel. Comprehensive pioneering experiments of A. L. Hodgkin, A. F. Huxley and B. Katz revealed the behavior of the sodium channel in the neural axons of the giant squid by using the voltage-clamp technique [8; 9; 10; 11]. They found that the cellular membrane voltage
changes with the changes in sodium concentration, as well as investigated activation and inactivation rate constants, and found their voltage dependences. In addition, they developed a mathematical description of the sodium channel behavior. The Hodgkin-Huxley model considered three different states, closed (C), open (O), and inactivated (I). Activation of the sodium channel is described as a process which includes three independent stages, three identical $m$-gates, and inactivation is a one-stage monoexponential process described as the $h$-gate.

Since that time, multiple types of sodium channels have been discovered and investigated, each with different molecular bases [12]. They create the superfamily of sodium channels, NaV1.1 - NaV1.9. All these sodium channels have very similar properties, such as fast activation and fast inactivation, but they have diverse amino acid sequences and are expressed in different tissues (see [13; 14; 3] for reviews).

Multiple experimental studies show that the activation and inactivation of the sodium channel occur within 2-5 ms in a voltage-dependent manner. Neural isoforms have faster activation-inactivation kinetics compared to the cardiac isoform [13]. Additionally, most of the experimental data demonstrated that inactivation occurs at multiple time scales, mostly with two time constants of inactivation [15;16]. Comparisons of the steady-state inactivation relationships and the voltage dependence of the channel conductance $G/G_{\text{max}}$ of the cardiac, skeletal muscle, and neuronal sodium channels demonstrate that the cardiac channels have the most negative half-inactivation and half-activation voltages, while the neuronal channels have the most positive half-inactivation and half-activation potentials [13]. However, the recovery time constant is fastest for the skeletal muscle isoforms and slowest for the cardiac muscle isoform, if compared at the same voltage.

Development of the methodology for single-channel recording and gating current studies allowed for the investigations of the different isolated processes in the complex sequence of
conformational changes of the sodium channel. Particular attention was paid to the transitions from the open to inactivated states, whether they are voltage-dependent or voltage-independent. The studies of Armstrong and Bezanilla [17;18] of the sodium current in the giant squid axon found that the voltage dependence of inactivation, obtained experimentally, can be explained by the voltage-dependent activation with voltage-independent transition from the open to inactivated state. They did not find any gating currents related to the inactivation process and proposed a model for the sodium channel with a sequence of closed states, open state, and an inactivated state. They suggested that the sodium channel must be open before it goes to an inactivated state. Such hypothesis was confirmed in many experiments on the neural sodium channels; however, some experimental data revealed voltage-dependence of the transition from the open to inactivated state [13]. This model contrasted the Hodgkin and Huxley model that assumes inactivation as a voltage-dependent process that can occur from the closed states. While the voltage-dependence of neuronal sodium channel inactivation is questionable, the transition from open to inactivated states in the cardiac sodium channels is found to be voltage-dependent [19; 20].

1.2 Mathematical Models of the Sodium Channel

In parallel to the experimental investigations, several mathematical models were developed for the sodium channel.

1.2.1 Hodgkin-Huxley Model

The Hodgkin-Huxley model (1952) [11] was developed for the sodium channel in the giant squid neuronal axon. It describes the sodium current $I_{Na}$ with equations:

$$I_{Na} = G_{Na} m^3 h (V - E_{Na}),$$

where $G_{Na}$ is the current conductance, $m$ is the activation variable (gate), $h$ is the inactivation variable, $V$ is the applied voltage, and $E_{Na}$ is the reversal potential. The Hodgkin-Huxley model
uses the hypothesis that activation and inactivation are two independent processes and the corresponding Markov model is shown in Fig. 1.4. In this model, the activation pathway includes three closed states and one open state. Inactivation in the model proceeds with the same rate as the closed and the open states (Fig. 1.4).

**Figure 1.4:** Markov model reproduction of the Hodgkin-Huxley model.

### 1.2.2 Armstrong-Bezanilla Model

The Armstrong-Bezanilla model (1977) [17] explores a different concept: they suggest that inactivation occurs after the channel is open. The Markov model for the channel is shown in Fig. 1.5. Activation occurs by transitions from the closed states $C_4$ to $C_1$, to the open state $O_1$, to the inactivated state $I$, and to the open state $O_2$. The authors wrote: “The main purposes of the calculations are to show, by the simplest model possible, that activation and inactivation may be coupled and that the inactivation step need not be significantly voltage dependent.” The resulting model gave a quite good prediction of the experimental data on activation and inactivation of the sodium channel at different voltages and described well steady-state inactivation relationships.

**Figure 1.5:** The Armstrong-Bezanilla model.
1.2.3 Irvine-Jafri-Winslow Model

The Irvine-Jafri-Winslow model (1999) [21] was developed to describe the temperature dependence of the cardiac sodium channel gating and its recovery from inactivation, and its Markov model is shown in Fig. 1.6. The model consists of five closed states, $C_0$-$C_4$, two open states $O_1$ and $O_2$, five closed-inactivated states $C_0I$-$C_4I$, and one open-inactivated state $I$. Activation pathway includes transitions $C_0 \rightarrow C_1 \rightarrow C_2 \rightarrow C_3 \rightarrow C_4 \rightarrow O_1 \rightarrow O_2$. There is also a transition from the $C_4$ to the $O_2$ state. Inactivation of the channel occurs both from the closed states and open state $O_1$. The degree of coupling of the closed-inactivated states to the closed states is regulated by the allosteric factor $a$. The model indeed describes well many experimental findings such as the sodium current traces at different voltages and temperatures, time-to-peak currents, time constants of inactivation, steady-state inactivation, and recovery from inactivation. The only significant shortcoming of the model is that the activation rate constant is voltage-independent, which contradicts most of the experimental findings where the activation of the channel is voltage-dependent.

Figure 1.6: The Irvine-Jafri-Winslow model
1.2.4 Clancy-Rudy Model

The Clancy-Rudy model (2002) [22] was developed to describe the gating of the cardiac sodium channel as well, and its Markov model is shown in Fig.1.7. The activation pathway includes three closed states C₃-C₁, one open state O, two closed-inactivated states, a fast-inactivated state IF (coupled both to the closed and open state), and two intermediate inactivated states IM₁ and IM₂. The model describes well the major gating processes such as activation, inactivation, and recovery from inactivation. It also includes a description of biexponential inactivation of I_{Na} (state IM₂ does not contribute to the inactivation significantly). There are two major disadvantages of the model: 1) the model predicted very fast deactivation; 2) the voltage dependence of inactivation is quite steep. Despite these disadvantages, the model was successfully used in modeling cardiac cells and extensively used by other researchers.

![Figure 1.7: The Clancy-Rudy model](image)
1.3 Purpose of the Study

While several mathematical models of the cardiac sodium channel are available in literature, they do not abundantly describe the channel’s response to all the major voltage-clamp protocols (activation, deactivation, inactivation, and recovery from inactivation). The existing models were not investigated regarding the mechanisms of slow inactivation, which is involved in a pro-arrhythmic activity. Using these models in cardiac cellular models may not allow a reliable and accurate description of the cellular and multicellular electrical properties and arrhythmias.

In this Thesis, we developed a comprehensive Markov model for the human sodium channel Nav1.5 based on the experimental voltage-clamp data on activation, deactivation, inactivation, and recovery from inactivation. We analyzed several mathematical models of the human cardiac sodium channel to show the role of particular interstate transitions for the channel’s properties, derived specific coupling of the fast and slow-inactivated states to the closed and open states. We showed that the simpler models cannot fully describe the set of major experimental voltage-clamp data on the human cardiac sodium channel. Particular attention is paid to the description of the mechanisms of the slow inactivation and slow recovery from inactivation of the human sodium channel, which are involved in pro-arrhythmic behavior. We showed that the slow-inactivated state is sequentially coupled to the fast-inactivated state in the Markov model of the human sodium channel. We also indicated that the rate-limiting voltage-independent transitions in the model of the human sodium channel are responsible for the slow component of inactivation and slow component of the recovery from inactivation.
2 METHODS

2.1 Analysis of the Experimental Data Used for Model Development

To develop a mathematical model of the human sodium channel, we needed to find experimental data which used major voltage-clamp protocols for the channel. These include current-voltage relationships, steady-state inactivation relationships, voltage dependence of the channel conductance divided by its maximum value ($G/G_{\text{max}}$), voltage dependences of the kinetics of activation and deactivation, and voltage dependences of the kinetics of inactivation and recovery from inactivation. Most of these data are obtained from a comprehensive experimental study of the human cardiac sodium channel by O’Leary et al. [23]. In addition, we used the experimental data on the second component of inactivation and the second component of recovery from inactivation obtained by Veldkamp et al. [24] and Wang et al. [25]. All the data are shown in Figs. 2.1 and 2.2.

Most of the experimental data we used are obtained for temperatures of 21-23ºC. However, kinetic data for the time-to-peak current and deactivation are acquired at 15ºC. In the latter case, we adjusted the data by dividing it by 2.14 based on a $Q_{10}$ value equal to 3.

Analysis of the experimental data allowed for the determination of the effective charge movements during each of the gating processes (activation, deactivation, inactivation, and recovery from inactivation). The rates of the gating processes demonstrate exponential dependences as functions of the applied voltage $V$, e.g.,

$$k(V) = k_0 \exp \left(\frac{zFV}{RT}\right).$$

where $k$ is the rate at voltage $V$, $k_0$ is the rate at $V = 0$ mV, $z$ is the effective charge (in fractions of elementary charge $e_0 = 1.60 \times 10^{-19}$ Coulombs), $F$ is the Faraday constant, $R$ is the gas constant,
and $T$ is the absolute temperature (ºK). We used $T = 295$ºK (22ºC) for estimations. In this case, the factor $RT/F \approx 25.416$ mV.

Figure 2.1: Voltage dependence of experimental results. (A) Experimental current-voltage relationships. (B) Experimental steady-state inactivation relationships (open circles) and $G/G_{\text{max}}$ (closed circles). (C) Experimental time-to-peak currents (closed circles) and deactivation time constants (open circles). Experimental data are from O’Leary et al. [23], adjusted to room temperature $T = 295$ºK (22ºC).
Figure 2.2: Experimental time constants for inactivation and recovery from inactivation. Experimental data on inactivation are from O'Leary et al. (1995) (closed circles and squares) [23], Veldkamp et al. (2000) (diamonds) [24], and Wang et al. (2007) (crosses) [25]. Experimental data on recovery from inactivation are from O'Leary et al. (1995) (open circles) [23], Veldkamp et al. (2000) (squares) [24], and Wang et al. (2007) (triangles) [25].
Voltage dependence of activation kinetics (obtained from the time-to-peak data O’Leary et al. [23]) is fitted well by the exponential function with the effective charge \( z_a = 0.73e_0 \) (Fig. 2.1C). During the reverse process, deactivation, \( z_d = -1.56e_0 \) is moved (Fig. 2.1C), which is about two times larger than \( z_a \) for activation. In a similar way, we obtained the effective charges for the fast component of inactivation kinetics (O’Leary et al. [23] and Wang et al. [25] data) and for the fast component of recovery from inactivation (O’Leary et al. [23]), which are equal to \( z_i = 1.31e_0 \) and \( z_r = -2.03e_0 \), respectively (Fig. 2.2). It is interesting to note that \( z_r \) is approximately equal to the sum of \( z_a \) and \( z_i \) with the opposite signs, suggesting that the same amount of charge is transferred during depolarization and repolarization processes. Furthermore, we can conclude that the total charge movement as the human cardiac sodium channel is moving to the inactivated state is composed of the charge movement during activation and inactivation processes. Moreover, the experimental data indicates that the inactivation of the sodium channel itself is a voltage-dependent process.

We also estimated the effective charges from the steady-state inactivation relationships and voltage dependence of \( G/G_{\text{max}} \). They are close to each other and equal to \( z_{ssI} = 4.11e_0 \) and \( z_g = 3.60e_0 \). We were unable to obtain the effective charges from the second component of inactivation obtained by Wang et al. [25], which is virtually a voltage-independent process.

These comprehensive experimental data provide us with useful constraints for the development of a mathematical model.

### 2.2 Experimental Protocols

In our simulations, we employed three major voltage-clamp protocols: steady-state inactivation, deactivation, and recovery from inactivation. These protocols allowed for the
determination of all gating properties of the sodium channel. The protocols are shown in Fig. 2.3, Fig. 2.4 and Fig. 2.5.

The steady-state inactivation voltage-clamp protocol consists of two pulses $P_1$ and $P_2$. The $P_1$ pulse represents a variable range of voltages from $-110$ to $+30$ mV in 10 mV increments applied for 500 ms from a holding potential $-120$ mV (experimental value). $P_1$ is followed by a $P_2$ pulse to $-20$ mV for 100 ms (Fig. 2.3).

![Inactivation Protocol](image)

*Figure 2.3: Steady-state inactivation voltage-clamp protocol.*

The deactivation protocol consists of two pulses $P_1$ and $P_2$ as well, but with different characteristics. The $P_1$ pulse represents a voltage step to $-20$ mV from a holding potential $-120$ mV for 0.6 ms. It is followed by a $P_2$ pulse to voltages from $-120$ mV to $-70$ mV in 10 mV increments for 14.4 ms (Fig. 2.4).

Recovery from inactivation is simulated using a variable-gap two-pulse protocol (Fig. 2.5). From a holding potential $-120$ mV to a $P_1$ pulse at $-20$ mV for 500 ms is followed by a variable gap from 8 ms to 408 ms ($V_{gap} = -100$ mV), which is followed by a $P_2$ pulse to $-20$
mV for 100 ms. To determine the voltage dependence of the recovery, we changed the potential during the interpulse gap from −140 mV to −100 mV. The gap durations were dependent on the interpulse voltage $V_{gap}$.

Figure 2.4: Deactivation voltage-clamp protocol.

Figure 2.5: Recovery from inactivation voltage-clamp protocol
2.3 Methods of Simulations

Markov models for the human sodium channel are described by a system that includes from five to ten ordinary differential equations. The system is solved by the fourth-order Runge-Kutta method with a fixed time step. The initial conditions were obtained by running the model at −120 mV for 500 ms to ensure steady state. All simulations were performed on a Dell Precision Workstation T3500 with six-core Intel Xeon CPU W3670 (3.2 GHz, 12 GB RAM). Simulation results were fit using Clampfit 10.2 software (Molecular Devices, Inc., CA, USA).
3 RESULTS

3.1 Fast Inactivation of the Human Sodium Channel

3.1.1 Model 1

We started from the simplest Markov model which includes three closed states $C_1 - C_3$, one open state $O$, and one fast-inactivated state $IF$ (Fig. 3.1). This model structure is based on the experimentally-defined three activation gates for the voltage-gated sodium channel [11]. The model includes voltage-dependent transitions in the activation pathway $\alpha(V)$ upon the suggestion of the independent activation of the three channel’s subunits towards an open state. Deactivation occurs with the voltage-dependent rates $\beta(V)$ from an open state to closed states. The fast-inactivated state connects only to an open state with forward and backward rates $k_{olf}$ and $k_{ifo}$, respectively. Similar to the Armstrong-Bezanilla model [17], we consider the instance that the inactivation of the sodium channel is fully coupled to activation and the channel needs to open before inactivation. This model is considered to describe only the fast inactivation process of the sodium channel because it contains only one inactivate state.

Figure 3.1: Five state Markov model for cardiac sodium channel (Model 1). $C_1 - C_3$ are closed states, $O$ is an open state, and $IF$ is fast inactivation state. $\alpha, \beta, k_{olf},$ and $k_{ifo}$ are voltage dependent transition rates.
The model is described by the following differential equations:

\[
\frac{dC_2}{dt} = 3\alpha C_3 - \beta C_2 + 2\beta C_1 - 2\alpha C_2
\]

\[
\frac{dC_1}{dt} = 2\alpha C_2 - 2\beta C_1 + 3\beta O - \alpha C_1
\]

\[
\frac{dO}{dt} = \alpha C_1 - 3\beta O + k_{i,o}IF - k_{o,i}O
\]

\[
\frac{dIF}{dt} = k_{o,i}O - k_{i,o}IF
\]

\[
C_3 = 1 - (C_2 + C_1 + O + IF)
\]

The sodium current is calculated by the equation:

\[
I_{Na} = g_{Na} \times O \times (V - E_{Na})
\]

where \(g_{Na} = 1.0 \text{ nS/pF}\) is the sodium channels conductance, O is the probability of the channel to be in an open state, \(E_{Na} = (RT/F) \ln([Na^+]_o/[Na^+]_i)\) is the reversal potential, \([Na^+]_o = 160 \text{ mM} \) [23] and \([Na^+]_i = 2 \text{ mM}\) (adjusted to fit IV data) are the extracellular and intracellular Na\(^+\) concentrations, respectively.

Rate constants and model parameters are given in the Appendix (A.1 to A.3). We employed biexponential voltage dependences for activation and deactivation rate constants, similar to those used by Wang et al. [26; 27]. This idea is based on the fact that the effective charges for the activation, deactivation, and \(G/G_{max}\) of the sodium current are quite different and corresponding channel’s behavior in response to the voltage-clamp protocols cannot be well-described with monoexponential voltage dependences of \(\alpha(V)\) and \(\beta(V)\). Even more complex voltage dependences for \(\alpha(V)\) and \(\beta(V)\) are used by others (see, for example, Clancy and Rudy [22]).
For inactivation rate constants $k_{oi}(V)$ and $k_{ifo}(V)$, we investigated both monoexponential and biexponential voltage dependences (see Appendix). We will show four different model results for the simplest Markov model of the sodium channel, and none of them fit well with the available experimental data.

Models 1a and 1b use rate constants for inactivation and recovery from inactivation, $k_{oi}(V)$ and $k_{ifo}(V)$, that are monoexponential functions. With Model 1a we obtained the best fit to the experimental data, and Model 1b was developed to correct the steep voltage dependence of inactivation kinetics. The results of simulations are shown in Figs. 3.2 and 3.3. Model 1a describes quite well IV dependence, kinetics of activation and deactivation, voltage dependence of $G/G_{max}$, and voltage dependence of recovery from inactivation. There are significant differences between the simulated and experimental data with regards to steady-state inactivation relationships and voltage dependence of inactivation kinetics. In particular, the half-inactivation voltage of the simulated steady-state inactivation is shifted to more positive voltages (Fig. 3.2, 3.3). In addition, the voltage dependence of inactivation kinetics is steeper than that obtained experimentally (Fig. 3.2, 3.3).

We tried first to speed up inactivation kinetics to shift steady-state inactivation relationships, but this resulted in much smaller inactivation time constants compared to the experimental values (data not shown). Second, we tried to shallow the voltage dependence of inactivation rate constants. This change also led to significant deviations of the simulations from the experimental data (Fig. 3.3). In particular, the resulting model did not fit well with the experimental data: IV dependence, $G/G_{max}$, and inactivation kinetics.
Figure 3.2: Experimental data and simulations obtained with Model 1a. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
Figure 3.3: Experimental data and simulations obtained with Model 1b. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.

To overcome this problem, we employed biexponential voltage dependences for rate constants of inactivation and recovery from inactivation, $k_{\text{off}}(V)$ and $k_{\text{fro}}(V)$ in Models 1c and
Model 1d (see Appendices A1-A3 for equations and model parameters). Model 1c describes well IV, G/G_{max}, activation and deactivation kinetics, kinetics of inactivation and recovery from inactivation. However, this model failed to reproduce steady-state inactivation relationships, as the simulated half-inactivation potential is much more depolarized as compared to the experimental value (Fig. 3.4). We attempted to eliminate this discrepancy by slowing recovery from inactivation in Model 1d, which resulted in a decrease in the difference between the simulated and experimental data, but the discrepancy was not eliminated completely (Fig. 3.5). Additionally, we obtained misfits of the experimental dependences of the recovery from inactivation and inactivation kinetics.

Thus, none of the four models fit the whole set of the experimental data. In particular, all models demonstrate significant depolarization shifts in steady-state inactivation relationships compared to the experimental findings. Hence, we concluded that the fast inactivation state needs to be coupled not only to an open state but also to the closed state(s) in the activation pathway.
Figure 3.4: Experimental data and simulations obtained with Model 1c. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O‘Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
Figure 3.5: Experimental data and simulations obtained with Model 1d. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
3.1.2 Model 2

Next, we modified our Markov Model 1 by making an additional connection between the fast-inactivated state and the closed state C1, which resulted in Model 2 (Fig. 3.6). $k_{c1lf}$ and $k_{lf c1}(V)$ were used to describe the forward and backward transition rates respectively. We expected that the introduction of an additional inactivation pathway would explain the slow inactivation mechanism (which is voltage-independent), so we set $k_{c1lf}$ a constant.

Figure 3.6: A five state Markov model for cardiac sodium channel with additional transitions between C1 and IF states (Model 2). $\alpha$, $\beta$, $k_{ifo}$, $k_{oif}$, and $k_{icf}$ are voltage dependent transition rates, $k_{c1if}$ is voltage-independent transition rate.

The following differential equations describe Model 2:

$$\frac{dC_2}{dt} = 3\alpha C_3 - \beta C_2 + 2\beta C_1 - 2\alpha C_2$$

$$\frac{dC_1}{dt} = 2\alpha C_2 - 2\beta C_1 + 3\beta O - \alpha C_1 + k_{lf c1} IF - k_{c1lf} C_1$$

$$\frac{dO}{dt} = \alpha C_1 - 3\beta O + k_{ifo} IF - k_{oif} O$$

$$\frac{dIF}{dt} = k_{oif} O - k_{ifo} IF + k_{c1lf} C_1 - k_{lf c1} IF$$

$$C_3 = 1 - (C_2 + C_1 + O + IF)$$

The rates in a loop C1 – O – IF must satisfy the thermodynamic equilibrium condition:

$$\alpha(V) \times k_{oif}(V) \times k_{lf c1}(V) = 3\beta(V) \times k_{c1lf} \times k_{ifo}(V)$$

(3.1)
From Eq. (3.1), we can obtain the backward transition rate as

\[ k_{ifc1}(V) = \frac{3\beta(V)\times k_{c1if} \times k_{ifo}(V)}{\alpha(V)\times k_{oif}(V)} \]  

(3.2)

As a result, the backward transition rate \( k_{ifc1} \) become voltage dependent.

We investigated three different sets of rates and parameters in Model 2. In Models 2a and 2b, the voltage dependences of the rates of inactivation and recovery from inactivation, \( k_{oif}(V) \) and \( k_{ifo}(V) \), were biexponential. In Model 2c, we used monoexponential voltage dependences for \( k_{oif}(V) \) and \( k_{ifo}(V) \).

Simulations of voltage-clamp protocols for Model 2a are shown in Fig. 3.7. Simulation data fit well with almost all the experimental protocols, except for the voltage dependence of recovery from inactivation. In particular, the current traces (Fig. 3.7) look very close to those obtained experimentally for the human cardiac sodium channel by O’Leary et al. [23]. However, it is seen that the simulated result for recovery from inactivation demonstrates a steeper voltage dependence. We also did not obtain biexponential inactivation kinetics for the channel.

We tried to obtain a less steep voltage dependence of the recovery from inactivation by reduction of the effective charge in \( k_{ifc1}(V) \). The effective charge in \( k_{ifc1}(V) \) is reduced by modifying \( k_{oif}(V) \) and \( k_{ifo}(V) \) (Model 2b, see Appendix A5). Simulation results for all voltage-clamp protocols are shown in Fig. 3.8. It is seen that, while we were able to decrease the steepness of voltage-dependent kinetics of the recovery from inactivation, the simulated result is still significantly steeper than that obtained from the experimental data.

We also performed simulations of voltage-clamp protocols using Model 2c, where \( k_{oif}(V) \) and \( k_{ifo}(V) \) were monoexponential functions of voltage (Fig. 3.9). The results are very
similar to those obtained with Model 2b, except for the reduced values of the time-to-peak current (Fig. 3.9).

Figure 3.7: Experimental data and simulations obtained with Model 2a. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current-voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
Figure 3.8: Experimental data and simulations obtained with Model 2b. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
Our further attempts to obtain a less steep voltage dependence of recovery from inactivation were unsuccessful. To explain this failure, we evaluated the voltage dependent transition rate $k_{ifc1}(V)$ using equation (3.2) of Model 2c.

$$k_{ifc1}(V) = \frac{3\beta(V) \times k_{c1f} \times k_{ifo}(V)}{\alpha(V) \times k_{olf}(V)}$$

where, $\beta(V) \approx \beta_2(V) = 0.025 \times e^{-\frac{V}{20}}$ and $\alpha(V) \approx \alpha_2(V) = 3.0 \times e^{\frac{V}{30}}$ are the approximations at large hyperpolarization voltages and the rates $k_{c1f}, k_{ifo}(V)$ and $k_{olf}(V)$ are given in Appendix A.5.

Substitution of the rates into Eq. (3.2) of Model 2c resulted in:

$$k_{ifc1}(V) = 4.4523 \times 10^{-12} \times e^{-\left(\frac{V}{4.875}\right)} \hspace{1cm} (3.3)$$

In equation (3.3), the denominator voltage in power index is equal to $-4.875$ mV. Using the value of the factor $RT/F \approx 25.416$ mV, we can estimate the effective charge $z$ as $z = 25.416 \text{ mV} / -4.875 \text{ mV} \approx -5.21$. Therefore, the effective charge for recovery from inactivation in Model 2c is approximately $-5.21e_0$, which is larger than the experimental value $z_r = -2.03e_0$ by a factor of 2.5. Because $\alpha(V)$ and $\beta(V)$ are restricted by voltage dependences of the faster processes (activation and deactivation are constrained by the experimental data), we are limited to modifications of $k_{olf}(V)$ and $k_{ifo}(V)$ only. The rate $k_{olf}(V)$ is responsible for the effective charge of inactivation kinetics, which is also restricted by the corresponding experimental data. These constraints lead us to the minimum possible effective charge of $-3.27e_0$ for the recovery from inactivation (if we eliminate voltage dependence of $k_{ifo}(V)$ at all) that can be obtained using Model 2c, which is still much larger than the experimental value.

Therefore, our simulations demonstrate that we cannot fit the whole set of experimental data, using Markov Models 2a – 2c.
Figure 3.9: Experimental data and simulations obtained with Model 2c. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
3.1.3 Model 3

We tried to overcome the intrinsic limitations of Model 2, in which the fast-inactivated state IF is connected directly to the closed state C₁, with a Markov model where the state IF is connected to closed state C₁ through an additional closed-inactivated state IC₁. Besides, due to the symmetry of three channel’s subunits, we have to allow inactivation from all closed states C₁-C₃ to closed-inactivated states IC₁-IC₃. The resulting eight-state Markov model is shown in Fig. 3.10. In this model, transitions are kept similar to that of Model 1, which are described by \( \alpha(V), \beta(V), k_{olf}(V), \) and \( k_{ifo}(V) \). We introduced new forward and backward transition rates between closed and closed-inactivated states, \( k_{cl}(V) \) and \( k_{lc}(V) \). Forward transition rate \( k_{cl}(V) \) is set to 3.0\( k_{olf} \). Backward rate \( k(V) \) is determined using the thermodynamic equilibrium conditions for loop C₁ – O – IF – IC₁:

\[
\alpha(V) \times k_{olf}(V) \times 3\beta(V) f \times \frac{k_{lc}(V)}{f} = 3\beta(V) \times k_{cl}(V) f \times \frac{\alpha(V)}{f} \times k_{ifo}(V) \tag{3.4}
\]

from which we have

\[
k_{lc}(V) = \frac{k_{cl}(V) \times k_{ifo}(V)}{k_{olf}(V)} \tag{3.5}
\]

Allosteric factor \( f \) is introduced to regulate the coupling of the closed-inactivated states to the closed states in the activation pathway (similar to the Irvine-Jafri-Winslow model [21]).

Note that, Model 3 has a very similar structure to the Hodgkin-Huxley model for the sodium channel in a giant squid axon. However, in the Hodgkin-Huxley model, activation and inactivation are independent processes, and all transition rates from the states in the activation pathway to inactivated states are the same [28]. In our Model 3, there is a differential coupling of the closed states to the corresponding inactivated states, which are regulated by allosteric factor \( f \).
Figure 3.10: An eight state Markov model (Model 3). It includes closed states (C1-C3), open state (O), fast-inactivated state (IF) and closed-inactivated states (IC1-IC3). $\alpha, \beta, k_{oi}, k_{ifo}, k_{ci},$ and $k_{ic}$ are voltage dependent transition rates. $f$ is an allosteric factor that regulates the coupling of the inactivated states to the closed states.

The following differential equations describe the model:

\[
\frac{dC_2}{dt} = 3\alpha C_3 - \beta C_2 + 2\beta C_1 - 2\alpha C_2 + \frac{k_{ic}}{f^2} IC_2 - k_{ci} f^2 C_2
\]

\[
\frac{dC_1}{dt} = 2\alpha C_2 - 2\beta C_1 + 3\beta O - \alpha C_1 + \frac{k_{ic}}{f} IC_1 - k_{ci} f C_1
\]

\[
\frac{dO}{dt} = \alpha C_1 - 3\beta O + k_{ifo} IF - k_{oif} O
\]

\[
\frac{dIF}{dt} = k_{oif} O - k_{ifo} IF + \frac{\alpha}{f} IC_1 - 3\beta f IF
\]

\[
\frac{dIC_1}{dt} = \frac{2\alpha}{f} IC_2 - 2\beta f IC_1 + 3\beta f IF - \frac{\alpha}{f} IC_1 + k_{ci} f C_1 - \frac{k_{ic}}{f} IC_1
\]

\[
\frac{dIC_2}{dt} = \frac{3\alpha}{f} IC_3 - \beta f IC_2 + k_{ci} f^2 C_2 - \frac{k_{ic}}{f} f^2 IC_2 + 2\beta f IC_1 - \frac{2\alpha}{f} IC_2
\]

\[
\frac{dIC_3}{dt} = k_{ci} f^3 IC_3 - \frac{k_{ic}}{f^3} IC_3 + \beta f IC_2 - \frac{3\alpha}{f} IC_3
\]

\[C_3 = 1 - (C_2 + C_1 + O + IF + IC_1 + IC_2 + IC_3)\]
Figure 3.11: Experimental data and simulations obtained with Model 3a. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\max}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
Figure 3.12: Experimental data and simulations obtained with Model 3b. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [27]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
First, we used Model 3a and Model 3b with monoexponential voltage dependences of $k_{oilf}(V)$ and $k_{ifo}(V)$. Simulation results for the set of voltage-clamp protocols obtained from Model 3a fit well the IV, time-to-peak current and deactivation kinetics, $G/G_{\text{max}}$, steady-state inactivation relationships, and to some extent voltage dependence of inactivation kinetics (Fig. 3.11). However, the model was unable to satisfactorily describe the voltage dependence of recovery from inactivation (Fig. 3.11). In addition, we obtained only the monoexponential time behaviors of inactivation and recovery from inactivation.

We tried to fix Model 3a by slowing the rate constant $k_{ifo}(V)$. The new model (Model 3b) indeed described somewhat better the recovery from inactivation at voltages $-140$ mV and $-130$ mV (Fig. 3.12). However, Model 3b was unable to fit voltage dependences of the kinetics of inactivation and recovery from inactivation within a voltage range from $-120$ mV to $-30$ mV. In addition, the simulated steady-state inactivation result was shifted quite far towards hyperpolarization voltages.

The problem with the misfit of the kinetics of fast inactivation and recovery from inactivation is solved by using biexponential voltage dependences for rate constants $k_{oilf}(V)$ and $k_{ifo}(V)$. This is done using Model 3c (equations for rate constants and model parameters are given in Appendix A8). Simulation results and their comparisons are shown in Fig 3.13. It is seen that the model fits well with all voltage-clamp protocols. However, simulations with model 3c give only monoexponential time courses for inactivation and recovery from inactivation, and is unable to reproduce biexponential time courses of inactivation and recovery from inactivation, obtained experimentally [24; 25].
Figure 3.13: Experimental data and simulations obtained with Model 3c. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed line) and fast-inactivation time constant (solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
3.2 Mechanisms of the Slow Inactivation

While the last model (Model 3c) was able to reproduce the majority of the experimental voltage-clamp data on the human sodium channel, all previously described models were unable to explain the biexponential behavior of the kinetics of inactivation and recovery from inactivation. Therefore, we need to find a minimal Markov model structure that will be able to fit the experimental data. Previous mathematical models of potassium and calcium channels suggest an additional slow-inactivated state that can be coupled to an open state O [29; 30], or to the fast-inactivated state [27]. In our next models, we explored both types of coupling.

3.2.1 Model 4

Model 4 is an extension of Model 3c by adding a slow-inactivated state coupled to an open state (Fig. 3.14). Transition rates between the open state O and slow-inactivated state IS, $k_{ois}$ and $k_{iso}$, are considered to be voltage-independent due to voltage independent experimental kinetics of the slow inactivation component.

![Figure 3.14: A nine state Markov model (Model 4). This model consists of three closed states (C1-C3), open state (O), fast-inactivated state (IF), slow-inactivated state (IS), and three closed-inactivated states (IC1-IC3). $\alpha$, $\beta$, $k_{cio}$, $k_{ciof}$, $k_{clo}$, and $k_{lic}$ are voltage dependent transition rates. $k_{ois}$ and $k_{iso}$ are voltage-independent transition rates. $f$ is an allosteric factor that regulates the coupling of the inactivated states to the closed states.](image-url)
The following differential equations describe Model 4:

\[
\frac{dC_2}{dt} = 3\alpha C_3 - \beta C_2 + 2\beta C_1 - 2\alpha C_2 + \frac{k_{ic}}{f^2} IC_2 - k_{clf} f^2 C_2
\]

\[
\frac{dC_1}{dt} = 2\alpha C_2 - 2\beta C_1 + 3\beta O - \alpha C_1 + \frac{k_{ic}}{f} IC_1 - k_{clf} C_1
\]

\[
\frac{dO}{dt} = \alpha C_1 - 3\beta O + k_{ifo} IF - k_{oif} O + k_{iso} IS - k_{ots} O
\]

\[
\frac{dIF}{dt} = k_{oif} O - k_{ifo} IF + \frac{\alpha}{f} IC_1 - 3\beta f IF
\]

\[
\frac{dIC_1}{dt} = \frac{2\alpha}{f} IC_2 - 2\beta f IC_1 + 3\beta f IF - \frac{\alpha}{f} IC_1 + k_{clf} IC_1 - \frac{k_{ic}}{f} IC_1
\]

\[
\frac{dIC_2}{dt} = \frac{3\alpha}{f} IC_3 - \beta f IC_2 + k_{clf} f^2 C_2 - \frac{k_{ic}}{f^2} IC_2 + 2\beta f IC_1 - \frac{2\alpha}{f} IC_2
\]

\[
\frac{dIC_3}{dt} = k_{clf} f^3 C_3 - \frac{k_{ic}}{f^3} IC_3 + \beta f IC_2 - \frac{3\alpha}{f} IC_3
\]

\[C_3 = 1 - (C_2 + C_1 + O + IF + IC_1 + IC_2 + IC_3 + IS)\]

In this, and subsequent models, we considered only biexponential voltage dependences of rate constants $k_{oif}(V)$ and $k_{ifo}(V)$ because they provided a better fit to the experimental data in the previously discussed models.

We started our investigation from Model 4a. As expected, Model 4a fits well with all the experiments for the fast component of inactivation and fast recovery from inactivation (Fig. 3.15). Besides, we were able to obtain a slow component of inactivation, which is shown by a gray line in Fig. 3.15. However, the time constant of the slow inactivation is much larger than the experimentally obtained value. We also did not get a slow component of recovery from inactivation in this model.
Figure 3.15: Experimental data and simulations obtained with Model 4a. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (black dashed line), fast-inactivation time constant (black solid line), and slow-inactivation time constant (gray solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
To reveal mechanisms of the fast and slow inactivation and their interactions, we plotted state occupancies as functions of time during a depolarization voltage step to −20 mV (Fig. 3.16). At the holding potential of −120 mV, most of the channels are in the C3 state, and some channels are in the C2 state (Fig. 3.16, B). Upon depolarization, the majority of the channels move fast to C2, C1 and O states. Then relatively slow inactivation occurs towards the fast-inactivated (IF) and slow-inactivated (IS) states, with the majority of the channels moving to the fast-inactivated state IF. This behavior is explained by the fact that the transition rate to IF is faster than that to IS. Further in time, the transition to IF demonstrates slower growth resulting in a biexponential time behavior. In contrast, the occupancy of IS, after a transient increase, shows a slow decline (Fig. 3.16, A and B). As a result, at the end of the depolarization pulse, most of the channels (97.4%) are in the IF state (Fig. 3.16, A). Upon elimination of the depolarization step, the channels recover from inactivation predominantly from IF with a monoexponential time course. Thus, our simulations with Model 4a predict that the fast-inactivated state is absorbing upon channel depolarizations.

To obtain a better fit with the experimental time constant of the slow inactivation, we sped up transition rate $k_{ois}$ and slowed down $k_{iso}$ in Model 4b. Fig. 3.17 shows simulation results. The current traces demonstrate faster inactivation, smaller time-to-peak currents, faster deactivation, accelerated fast component of inactivation, and decelerated slow component of inactivation. Steady-state inactivation, $G/G_{max}$, and voltage dependence of the recovery from inactivation do not change. Therefore, it is impossible to improve the fit of the simulations to the experimental data by accelerating the transition rate from the open state to the slow-inactivated state $k_{ois}$. 
Then we tried to slow down $k_{ois}$ in Model 4c. The results of simulations are shown in Fig. 3.18. The model describes well all the experimental data on the fast inactivation and fast recovery from inactivation, but we were unable to obtain the slow component of both inactivation and recovery from inactivation. Thus, our simulation data suggest that the slow-inactivated state in the Markov model for the human sodium channel cannot be coupled to the open state.

Figure 3.16: Simulated time courses of state occupancies (Model 4a). $C_1$-$C_3$, $O$, $IF$, $IC_1$-$IC_3$ and $IS$ of the fast Na$^+$ channel as functions of time during and after a 0.5 s depolarization voltage pulse. (A) Data for time interval from 500 to 2500 ms. (B) Data for a shorter time interval from 500 to 510 ms to show details of activation and inactivation.
Figure 3.17: Experimental data and simulations obtained with Model 4b. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (black dashed line), fast inactivation time constant (black solid line), and slow inactivation time constant (gray solid line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
Figure 3.18: Experimental data and simulations obtained with Model 4c. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (dashed black line) and fast-inactivation time constant (solid black line). Experimental data on time-to-peak currents, deactivation kinetics, IV, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
3.2.2 Model 5

In Model 5, we coupled the slow-inactivated state IS to the fast-inactivated state IF in a sequence and to the closed-inactivated state IC\(_1\) (Fig. 3.19).

\[
\begin{align*}
\frac{dC_2}{dt} &= 3\alpha C_3 - \beta C_2 + 2\beta C_1 - 2\alpha C_2 + \frac{k_{ic} f^3}{f^2} IC_2 - k_{clf}^2 C_2 \\
\frac{dC_1}{dt} &= 2\alpha C_2 - 2\beta C_1 + 3\beta O - \alpha C_1 + \frac{k_{ic}}{f} IC_1 - k_{clf} C_1 \\
\frac{dO}{dt} &= \alpha C_1 - 3\beta O + k_{ifo} IF - k_{olf} O \\
\frac{dIF}{dt} &= k_{olf} O - k_{ifo} IF + k_{isif} IS - k_{ifis} IF
\end{align*}
\]

Figure 3.19: A nine state Markov model (Model 5). Three closed states (C\(_1\)-C\(_3\)), open state (O), fast-inactivated state (IF), slow-inactivated state (IS) and three closed-inactivated states (IC\(_1\)-IC\(_3\)). \(\alpha\), \(\beta\), \(k_{ifo}\), \(k_{olf}\), \(k_{ci}\) and \(k_{ic}\) are voltage dependent transition rates. \(k_{ifis}\) and \(k_{isif}\) are voltage-independent transition rates. \(f\) is an allosteric factor that regulates the coupling of the inactivated states to the closed states.
\[
\frac{dI_S}{dt} = k_{ifl}IF - k_{lslf}IS + \frac{\alpha}{f}IC_1 - 3\beta fIS
\]

\[
\frac{dI_{C_1}}{dt} = \frac{2\alpha}{f}IC_2 - 2\beta fIC_1 + 3\beta fIS - \frac{\alpha}{f}IC_1 + k_{cif}C_1 - \frac{k_{ic}}{f}IC_1
\]

\[
\frac{dI_{C_2}}{dt} = \frac{3\alpha}{f}IC_3 - \beta fIC_2 + k_{cif}f^2C_2 - \frac{k_{ic}}{f^2}IC_2 + 2\beta fIC_1 - \frac{2\alpha}{f}IC_2
\]

\[
\frac{dI_{C_3}}{dt} = k_{cif}f^3C_3 - \frac{k_{ic}}{f^3}IC_3 + \beta fIC_2 - \frac{3\alpha}{f}IC_3
\]

\[C_3 = 1 - (C_2 + C_1 + O + IF + IS + IC_1 + IC_2 + IC_3)\]

Transition rates between fast-inactivated state IF and slow-inactivated state IS, \(k_{ifl}\) and \(k_{lslf}\), are considered to be voltage-independent due to voltage independent experimental kinetics of the slow inactivation component. These transitions build a rate-limiting step for the inactivation. We also set rate constant \(k_{cif}(V) = 3k_{olf}(V)\), and backward rate \(k_{ic}(V)\) acquires voltage dependence due to the thermodynamic equilibrium conditions from a loop \(C_1 - O - IF - IS - IC_1\):

\[
\alpha(V) \times k_{olf}(V) \times k_{ifl} \times 3\beta(V)f \times \frac{k_{ic}(V)}{f}
\]

\[= 3\beta(V)f \times \frac{\alpha(V)}{f} \times k_{lslf} \times k_{olf}(V) \tag{3.6}\]

from which we have

\[
k_{ic}(V) = \frac{k_{cif}(V) \times k_{olf}(V) \times k_{lslf}}{k_{olf}(V) \times k_{ifl}} \tag{3.7}\]
Figure 3.20: Experimental data and simulations obtained with Model 5. (A) current traces, (B) deactivation time constant (dashed line) and time to peak (solid line), (C) current voltage relationship, (D) steady-state inactivation (dashed line) and conductance ratio $G/G_{\text{max}}$ (solid line), (E) recovery time constant (black dashed line), fast inactivation time constant (black solid line), and slow inactivation time constant (gray solid line). Experimental data on time-to-peak currents, deactivation kinetics, $IV$, inactivation, and recovery from inactivation are from O’Leary et al. (1995) [23], Veldkamp et al. (2000) [24], and Wang et al. (2007) [25]. Symbols for the experimental data are the same as in Figs. 2.1 and 2.2.
Optimization of the rate constants for Model 5 allowed us to describe well all experimental data on the fast inactivation and fast recovery from inactivation, deactivation and time-to-peak currents, steady-state inactivation relationships and G/G_{max} (Fig. 3.20). In addition, we obtained a quite good description of the slow component inactivation (solid gray line in Fig. 3.20, E). Similar to the experimental data, the time constant of the slow component of inactivation is voltage-independent. However, we were unable to obtain the slow component of recovery from inactivation within Model 5.

Channel’s transitions during voltage clamp protocols can be seen clearly from the time behavior of the channel’s state occupancies for Model 5 (Fig. 3.21). At the resting state (V = −120 mV), most of the channels are in the C₃ and C₂ states. When voltage becomes more depolarized, channels from state C₃ move towards an open state O through C₂ and C₁. Then relatively fast open-state inactivation occurs, during which channels move from an open state O to the fast-inactivated state IF. In addition, some channels inactivate to closed-inactivated state IC₁. Further in time, most channels transfer from fast-inactivated state IF to slow-inactivated state IS, with some fraction of the channels moving from IC₁. This process results in a biexponential time course of inactivation, with most of the channels to be in the IS state at the end of the depolarization step.

When depolarization is removed, the channels move rapidly from IS and IC₁ states through the IC₂-IC₃ states to result in recovery from inactivation. The rate of decay of the occupancies of the states IC₂ and IC₃ are the same and correspond to the fast component of the recovery from inactivation. Thus, within Markov Model 5 we were able to fit the experimental data on the human sodium channel, including the fast and slow inactivation kinetics, except for the slow component of recovery from inactivation. In Model 6 this shortcoming was eliminated.
Figure 3.21: Simulated time courses of state occupancies (Model 5). $C_1$-$C_3$, $O$, $IF$, $IC_1$-$IC_3$ and $IS$ of the fast sodium channel are shown as functions of time during and after a 0.5 s depolarization voltage pulse. (A) Data for time interval from 500 to 2500 ms. (B) Data for a shorter time interval from 500 to 510 ms to show details of activation and inactivation.
3.2.3 Model 6

To obtain the second component of recovery from inactivation, we modified the Markov model of the channel to add an intermediate state IM, which introduced a rate-limiting step for the recovery. The resulting model is shown in Fig. 3.22.

Figure 3.22: A ten state Markov model (Model 6). States are represented as: closed (C), closed-inactivated (IC), open (O), fast-inactivated (IF), slow-inactivated (IS) and intermediate-inactivated (IM). $\alpha$, $\beta$, $k_{o1f}$, $k_{if0}$, $k_{ci}$, and $k_{ic}$ are voltage-dependent transition rates; $k_{ifls}$, $k_{lsif}$, $k_{lsim}$, and $k_{lims}$ are voltage-independent transition rates. $f$ is an allosteric factor that regulates the coupling of the inactivated states to the closed states.

In this model three closed states, C3-C1 are connected in sequence towards the open state O. Channel’s activation occurs through independent activation of three subunits with the rates $\alpha(V)$. Deactivation proceeds from the open state backward to the closed states C1-C3 with the rate $\beta(V)$. Inactivation can proceed both from the closed states with the rate $k_{ci}(V)$ and the open state with the rate $k_{olf}(V)$. Fast-inactivated state IF, slow-inactivated state IS, and intermediate state IM are connected in a sequence towards the closed-inactivated state IC1 with the corresponding transition rates $k_{ifls}$, $k_{lsim}$, and $3\beta(V)f$. Allosteric factor $f = 0.3$ is responsible
for the regulation of the coupling of the inactivated states IC1-IC3 to the closed states C1-C3.

There are also backward transition rates between the states to satisfy the condition of thermodynamic equilibrium.

Model 6 is described by the following differential equations:

\[
\frac{dC_2}{dt} = 3\alpha C_3 - \beta C_2 + 2\beta C_1 - 2\alpha C_2 + \frac{k_{ic}}{f^2}IC_2 - k_{c_if}^2C_2
\]

\[
\frac{dC_1}{dt} = 2\alpha C_2 - 2\beta C_1 + 3\beta O - \alpha C_1 + \frac{k_{ic}}{f}IC_1 - k_{c_if}C_1
\]

\[
\frac{dO}{dt} = \alpha C_1 - 3\beta O + k_{if}IF - k_{o_if}O
\]

\[
\frac{dIF}{dt} = k_{o_if}O - k_{if}IF + k_{isi}IS - k_{isi}IF
\]

\[
\frac{dIS}{dt} = k_{if}IF - k_{isi}IS + k_{imi}IM - k_{imi}IS
\]

\[
\frac{dIM}{dt} = k_{isi}IS - k_{imi}IM + \alpha IC_1 - 3\beta fIM
\]

\[
\frac{dIC_1}{dt} = \frac{2\alpha}{f}IC_2 - 2\beta fIC_1 + 3\beta fIM - \frac{\alpha}{f}IC_1 + k_{c_if}C_1 - \frac{k_{ic}}{f}IC_1
\]

\[
\frac{dIC_2}{dt} = \frac{3\alpha}{f}IC_3 - \beta fIC_2 + k_{c_if}^2C_2 - \frac{k_{ic}}{f^2}IC_2 + 2\beta fIC_1 - \frac{2\alpha}{f}IC_2
\]

\[
\frac{dIC_3}{dt} = k_{c_if}^3C_3 - \frac{k_{ic}}{f^3}IC_3 + \beta fIC_2 - \frac{3\alpha}{f}IC_3
\]

\[
C_3 = 1 - (C_2 + C_1 + O + IF + IS + IM + IC_1 + IC_2 + IC_3)
\]

where the rate constants are given in Appendix A11.

Simulations were performed using three voltage-clamp protocols: steady-state inactivation, deactivation, and recovery from inactivation. The current traces for the initial part of the steady-state inactivation protocol are shown in Fig. 3.23A to demonstrate the behavior of the channel activation. The current traces for the deactivation protocol are shown in Fig. 3.23B.
The development of activation is assessed through the time-to-peak currents and are shown in Fig. 3.23C by a solid line. A dashed line shows deactivation time constants. Both the time-to-peak currents and deactivation time constants compare well to the experimental data by O’Leary et al. (1995) [23].

Figure 3.23. Simulated and experimental data on activation and deactivation of the fast Na\(^+\) current \(I_{\text{Na}}\) (Model 6). (A) Simulated current traces on the time interval from 0 to 5 ms to show activation time course of \(I_{\text{Na}}\). (B) Simulated current traces for deactivation voltage-clamp protocol. Data from 20 to 30 ms are shown. (C) Simulated time-to-peaks (solid line) and deactivation (dashed line) time constants. Experimental data on time-to-peaks (closed circles) and deactivation time constants (open circles) are from O’Leary et al. (1995) [23].
Figure 3.24 Simulated and experimental data on activation and inactivation of the fast Na\(^+\) current \(I_{\text{Na}}\) (Model 6). (A) simulated current traces, (B) simulated (solid line) and experimental (circles) IV dependencies, (C) steady-state inactivation (dashed line) and conductance ratio \(G/G_{\text{max}}\) (solid line). Experimental data are from O’Leary et al. (1995) (closed and open circles) [23].

Simulated current traces for \(I_{\text{Na}}\) (20 second time interval) are shown in Fig. 3.24A. They are very similar to the typical inactivation current traces for the sodium current [23; 31]. Current-voltage relationship (IV) is shown in Fig. 3.24B and is in satisfactory agreement with the
experiments by O’Leary et al. (1995) [23]. Both steady-state inactivation relationships and voltage-dependence of $G/G_{\text{max}}$ also show excellent agreement with the experimental data (O’Leary et al. (1995) [23]).

Unlike the previous models, Model 6 describes well fast and slow time constants of inactivation and recovery from inactivation. The data are presented in Fig. 3.25. The model simulations fit well with the experimental voltage dependences of the fast time constant of inactivation up to 0 mV (solid line in Fig. 3.25) and the fast constant of recovery (dashed line in Fig. 3.25). In addition, the model reproduces well the slow time constants of inactivation (solid gray line) and slow recovery from inactivation (a dotted line) obtained by Wang et al. (2007) [25] and Veldkamp et al. (2000) [24].

Excellent fit of the simulations to the experimental data allows for revealing the mechanisms of the human sodium channel gating. Figure 3.26 shows the time behavior of the channel’s state occupancies for Model 6, that reveals the biexponential inactivation and recovery from inactivation mechanisms. At rest, most of the channels are in the closed states $C_3$ and $C_2$ (Fig. 3.26, B). Upon depolarization, the channels rapidly move through the closed states $C_3 \rightarrow C_2 \rightarrow C_1$ towards the open state $O$. With prolonged depolarization, inactivation follows activation with relatively slower rates. Inactivation occurs both from closed states $C_3 - C_1$ to $IC_3 - IC_1$, respectively, and from the open state $O$ to the fast-inactivated state IF.
Figure 3.25: Simulated and experimental data on inactivation and recovery from inactivation of the fast Na$^+$ current $I_{\text{Na}}$ (Model 6). Simulated fast and slow time constants of inactivation are shown by solid black and solid gray lines respectively. Simulated fast and slow time constants of recovery from inactivation are shown by dashed and dotted lines, respectively. Experimental data on inactivation are from O’Leary et al. (1995) (closed circles and squares) [23], Veldkamp et al. (2000) (diamonds) [24], and Wang et al. (2007) (crosses) [25]. Experimental data on recovery from inactivation are from O’Leary et al. (1995) (open circles) [23], Veldkamp et al. (2000) (diamonds) [24], and Wang et al. (2007) (crosses) [25].
The primary inactivation occurs from the open state to the fast-inactivated state. However, some of the channels move through the inactivated state $IC_3$ towards the IM state, where they relatively rapidly equilibrate with a time constant close to that for the fast-inactivated state (Fig. 3.26, B). Slow inactivation occurs by rate-limiting transitions from the fast-inactivated state IF to the slow-inactivated state IS and further to the intermediate state IM (Fig. 3.26, A and B). When depolarization is removed, the channels rapidly move from the IM state through the $IC_1 - IC_3$ states to produce a rapid voltage-dependent component of recovery. The rate limiting step from the IS to the IM state slows down the recovery and is responsible for the slow component of the recovery from inactivation.

Thus, our Model 6 gives the best description of the whole set of experimental data for the wild type human sodium channel obtained by steady-state inactivation, deactivation, and recovery from inactivation protocols. It is also based on experimentally determined biophysical mechanisms, which include voltage-dependent activation-deactivation transitions, voltage-dependent fast inactivation and fast recovery from inactivation, as well as voltage-independent slow components of inactivation and recovery from inactivation.
Figure 3.26. Simulated time courses of state occupancies (Model 6). $C_1$-$C_3$, $O$, IF, $IC_1$-$IC_3$, IS, and IM of the fast Na$^+$ channel are shown as functions of time during and after a 0.5 s depolarization voltage pulse. (A) Data for time interval from 500 to 2500 ms. (B) Data for a shorter time interval from 500 to 510 ms to show details of activation and inactivation.
4 DISCUSSION

4.1 The Role of the Sodium Current for the Cardiac Action Potential

The fast sodium current plays one of the major roles in generation and propagation of the action potential in cardiac cells and cardiac tissues. It is responsible for the cardiac action potential upstroke, which can be seen from Kirchhoff’s Law:

\[
\frac{dV}{dt} = -\frac{1}{C_m} \times \sum I_{\text{ion}},
\]

where \(C_m \approx 1.0 \mu \text{F/cm}^2\) is the specific membrane capacitance, \(I_{\text{ion}}\) is the sum of the membrane ionic currents that includes the fast \(I_{\text{Na}}\). Because the activation time constant of the fast \(I_{\text{Na}}\) is the smallest and the current magnitude is the largest, at the initial stage of depolarization

\[
\frac{dV}{dt} \approx -\frac{1}{C_m} \times I_{\text{Na}}.
\]

Mutation of the channels responsible for the fast \(Na^+\) current leads to multiple types of arrhythmias in the heart, such as long QT type 3 or Brugada syndromes [32]. Long QT3 (LQT3) syndrome is related to gain-of-function mutations in the SCN5A gene encoding the fast \(Na^+\) channels (Nav1.5). It is characterized by a prolonged QT interval on the electrocardiogram (ECG) and increased risk of sudden death due to ventricular arrhythmias. The basic mechanism of the pro-arrhythmic effect of LQT3 is the increased inward current that causes increased probabilities of early afterdepolarizations (EADs) and the development of torsades de pointes and sudden death. Brugada syndrome is characterized by the loss-of-function mutation (BrS) that results in smaller \(I_{\text{Na}}\), which leads to the slowing action potential upstroke and, as a result, conduction velocity. On the ECG, Brugada syndrome is typically characterized by ST segment elevation in the leads V1-V3.
In addition, $I_{Na}$ is one of the major factors affecting action potential propagation velocity in the heart [33]. Slowing conduction velocity is present during acute ischemia, tachycardia, electrical remodeling, and treatment with class I antiarrhythmic drugs (affecting $I_{Na}$). Slow conduction velocity suggests smaller magnitudes of the fast Na$^+$ current that in some cases fails to generate an action potential leading to a conduction block. In particular, during tachycardia, the reduction of $I_{Na}$ is due to incomplete channel recovery from inactivation during diastole. Experimental data also demonstrates reduction of conduction velocity upon acute ischemia, which is accompanied by a significant increase in the extracellular potassium and membrane depolarization. Depolarization of the membrane results in the larger degree of inactivation of the sodium channels and reduction of channels’ availability to trigger action potential.

4.2 The Slow Component of the Fast Na$^+$ Current and Arrhythmias

Due to the major role of the fast Na$^+$ current in cardiac action potential generation and propagation, it is important to use a comprehensive Markov model of $I_{Na}$ in the mathematical models of cardiac cells, which adequately describes mechanisms of the fast and slow inactivation.

Recent experimental and clinical studies revealed that an insertion mutation 1795insD led to a significant increase in the slow component of inactivation in the human sodium current [24]. In the ECG, the syndrome has properties of both Brugada and LQT3 syndromes: QT-interval prolongation at slow heart rates (LQT3) and distinctive ST-segment elevation with exercises (Brugada syndrome). Heterologous expression of the sodium channels has shown both significantly slower slow component of inactivation (wild type, 6.12±0.71 ms vs. 1795insD, 14.45±1.33 ms) and fast component of recovery from inactivation (wild type, 3.8±0.47 ms vs.
1795insD, 7.7±1.3 ms) in the mutant channels [24]. In addition, the sodium current has an increased persistent current component, which can result in pro-arrhythmic behavior [34].

The importance of slow inactivation mechanisms in the sodium channels with inherited mutations related to dilated cardiomyopathy (DCM) associated with atrial and ventricular arrhythmias was investigated by Nguyen et al. [31]. Two mutations, R814W and D1595H, are studied in this paper. The data shows that mutation R814W results in significantly enhanced slow inactivation as compared with the wild-type sodium channels. In particular, the increased slow inactivation dramatically decreased $I_{Na}$ current during fast pacing with 100-115 beats per minute.

Drug-related enhancement of the slow inactivation and slow-inactivation block of the sodium channel Nav1.5 was investigated by Wang et al. [35]. They showed that mexiletine and lidocaine, class IB antiarrhythmic drugs, cause a significant hyperpolarizing shift in steady-state inactivation of the slow component of inactivation of Nav1.5. In addition, both drugs demonstrated dramatically increased use-dependent inhibition at pulsed stimulations with the frequencies of 5 and 10 Hz. The authors suggested that mexiletine and lidocaine bind predominately to the sodium channel in the slow-inactivated state, and implicated their anti-arrhythmic action in patients with depolarization-triggered arrhythmias (EADs) [35].

The pro-arrhythmic behavior of cardiac cells can be triggered not only by enhanced slow inactivation, but also by changing other gating rates, such as activation and deactivation (D1595H mutant [31]), the fast inactivation (D1595H mutant [31]), and recovery from inactivation [24].

The fast Na$^+$ current is also involved in pro-arrhythmic behavior in other species. Experimental investigations and simulations of mouse action potentials revealed an important
role of the inactivation of $I_{Na}$ in the generation of early afterdepolarizations [36]. Experimental
data and simulations using a mathematical model of mouse ventricular myocytes [37] have
shown that the non-equilibrium reactivation of $I_{Na}$ resulted in triggering EADs in the mouse
ventricle.

4.3 Comparison to the Other Mathematical Models

Our 10-state Markov model of the human cardiac fast sodium channel Na$_{v}$1.5 describes well
the experimental data obtained by the major voltage-clamp protocols. In particular, the model
provides descriptions of the slow components of inactivation and recovery from inactivation. In
the presented model, inactivated states are coupled both to the closed states and the open state in
the activation pathway. The allosteric factor $f$ is used to regulates the coupling of the inactivated
states to the closed states. This model is a compromise between the Hodgkin-Huxley model [11],
in which inactivated states coupled to the closed and open states with the same inactivation
transition rates, and the fully coupled Armstrong-Bezanilla model [17; 18], which suggests that
the channel needs to be open to inactivate. The model overcomes the shortcoming of the Irving-
Jafri-Winslow model [21] by including the voltage dependence of the activation rate constant
$\alpha(V)$. Our model suggests that the fast and slow-inactivated states should be connected to each
other in a sequence through the rate-limiting voltage-independent transitions, with the recovery
from the slow intermediate inactivated state coupled to the slow-inactivated state with the
voltage-independent transitions as well. This provides the mechanisms of the voltage-
independent slow inactivation and recovery from inactivation for the human cardiac sodium
channel Na$_{v}$1.5.

It is interesting to note that Markov models of the cardiac potassium and calcium channels
demonstrate both differences and similarities to the model of the fast sodium channel. For
example, the Wang et al. model for potassium channel Kv4.3 [27] has a similar coupling of the fast and slow inactivation states as a sequence; however, the transition rates between the states in the Wang et al. model are voltage-independent, which is also determined experimentally. In addition, the Wang et al. model [27] suggests tri-exponential inactivation mechanism, which includes closed-state inactivation coupled to the fast and slow open-state inactivation states. A different coupling for potassium channel Kv4.2 from the same family (Kv4 channels) is proposed by Bahring et al. [38], which suggest no connection between open- and closed-inactivated states. However, in the model of Bahring et al. [38] fast and slow inactivations are connected in sequence, and both of them are transient (not absorbing) states.

In contrast, Markov models of the cardiac calcium channels show different couplings of the fast (calcium-dependent) and slow (voltage-dependent) inactivation mechanisms. Bondarenko et al. model [29; 30] for the cardiac calcium channel suggests that both fast and slow inactivation states are coupled to the open state, and such a coupling is different from that for the fast sodium channel Na\textsubscript{v}1.5. Similar models for the cardiac calcium channel were also proposed later by Faber et al. [39] and Grandi et al. [40], in which fast and slow inactivation states are coupled to the open state.

Therefore, the voltage-dependent cardiac ion channels have a large variety of inactivation mechanisms, their differential coupling to the activation pathway, and molecular basis for the coupling. The knowledge of these mechanisms is essential for the detailed description of the channels’ gating and their effects on the generation of the action potential in the mammalian cardiac cells and comprehensive descriptions of the mechanisms of cardiac arrhythmias.
5 CONCLUSIONS

Thus, in this study, a comprehensive mathematical model of the human fast sodium current encoded by Na\textsubscript{v}1.5 was developed and investigated in detail with major voltage-clamp protocols on steady-state inactivation, deactivation, and recovery from inactivation. This was done through the analysis of the sodium channel gating with six different Markov models. Particular attention is paid to the mechanisms of the slow inactivation and recovery from inactivation. It is found that the sodium channels’ fast inactivation is coupled predominantly to the open state, and the slow inactivation is coupled to the fast-inactivated state through the rate-limiting transitions. The recovery from inactivation occurs mainly through the additional rate-limiting transitions towards the closed-inactivated states. The analysis of the channel’s state occupancies revealed distinct couplings of the fast and slow inactivation of the channels in different Markov models. The simulation data described well the experimental data on both fast and slow components of inactivation and recovery from inactivation. The resulting Markov model can be used for the mathematical models of the human ventricular myocytes or in models of ventricular myocytes of other species.
REFERENCES


APPENDICES

Appendix A: Transition Rates

Appendix A.1: Functions used in all models

\[ f_1(V) = \frac{1}{1.0 + \exp\left(\frac{V + 50.0}{5.0}\right)} \]  
\[ f_2(V) = \frac{1}{1.0 + \exp\left(\frac{V + 90.0}{5.0}\right)} \]  
\[ \alpha_1(V) = 12.0 \cdot \exp\left(\frac{V}{50.0}\right) \]  
\[ \alpha_2(V) = 3.0 \cdot \exp\left(\frac{V}{30.0}\right) \]  
\[ \beta_1(V) = 0.2 \cdot \exp\left(-\frac{V}{20.0}\right) \]  
\[ \beta_2(V) = 0.025 \cdot \exp\left(-\frac{V}{20.0}\right) \]

Appendix A.2: Model 1a and 1b (monoexponential)

\[ \alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp\left(\frac{V + 50.0}{5.0}\right) \right] \]  
\[ \beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp\left(\frac{V + 50.0}{5.0}\right) \right] \]  
\[ k_{oif}(V) = 0.08 \cdot \gamma \cdot \exp\left(\frac{V + 90.0}{22.0}\right) \]  
\[ k_{ifo}(V) = 0.373 \cdot \delta \cdot \exp\left(-\frac{V + 140.0}{13.1}\right) \]

The values of \( \gamma \) and \( \delta \) are given in the table below

<table>
<thead>
<tr>
<th></th>
<th>Model 1a</th>
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<tbody>
<tr>
<td>( \gamma )</td>
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<td>( \delta )</td>
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</table>
Appendix A.3: Models 1c and 1d (biexponential)

\[ \alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp \left( \frac{V + 50.0}{5.3} \right) \right] \]  \hspace{1cm} (A3.1)

\[ \beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right] \]  \hspace{1cm} (A3.2)

\[ k_{oif_1}(V) = 0.432 \cdot \exp \left( \frac{V + 50.0}{30.0} \right) \]  \hspace{1cm} (A3.3)

\[ k_{oif_2}(V) = 0.0345 \cdot \exp \left( \frac{V + 80.0}{14.8} \right) \]  \hspace{1cm} (A3.4)

\[ k_{ifo_1}(V) = 0.01 \cdot \mu \cdot \exp \left( -\frac{V + 50.0}{30} \right) \]  \hspace{1cm} (A3.5)

\[ k_{ifo_2}(V) = 0.018 \cdot \rho \cdot \exp \left( -\frac{V + 100.0}{13.6} \right) \]  \hspace{1cm} (A3.6)

The values of \( \mu \) and \( \rho \) are given in the table below

<table>
<thead>
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<th>Model 1d</th>
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</thead>
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</tr>
<tr>
<td>( \rho )</td>
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<td>0.01</td>
</tr>
</tbody>
</table>

\[ k_{oif} = f_2(V) \left( k_{oif_2} + k_{oif_1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right) \]  \hspace{1cm} (A3.7)

\[ k_{ifo} = f_2(V) \left( k_{ifo_2} + k_{ifo_1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right) \]  \hspace{1cm} (A3.8)

Appendix A.4: Model 2a (biexponential)

\[ \alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp \left( \frac{V + 50.0}{5.3} \right) \right] \]  \hspace{1cm} (A4.1)

\[ \beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right] \]  \hspace{1cm} (A4.2)

\[ k_{oif_1}(V) = 0.432 \cdot \exp \left( \frac{V + 50.0}{30.0} \right) \]  \hspace{1cm} (A4.3)
\[ k_{oif2}(V) = 0.0345 \cdot \exp\left(\frac{V + 80.0}{14.8}\right) \]  

(A4.4)

\[ k_{ifo1}(V) = 0.01 \cdot \mu \cdot \exp\left(\frac{-V + 50.0}{30}\right) \]  

(A4.5)

\[ k_{ifo2}(V) = 0.018 \cdot \rho \cdot \exp\left(\frac{-V + 100.0}{13.6}\right) \]  

(A4.6)

\[
\mu = 0.000006, \quad \rho = 0.000006
\]

\[ k_{oif} = f_2(V) \left(k_{oif2} + k_{oif1} \cdot \exp\left(\frac{V + 90.0}{5.0}\right)\right) \]  

(A4.7)

\[ k_{ifo} = f_2(V) \left(k_{ifo2} + k_{ifo1} \cdot \exp\left(\frac{V + 90.0}{5.0}\right)\right) \]  

(A4.8)

\[ k_{c1if} = 0.3 \]  

(A4.9)

\[ k_{ifc1} = 3.0 \beta \cdot k_{ifo} \cdot \frac{k_{c1if}}{\alpha \cdot k_{oif}} \]  

(A4.10)

**Appendix A.5: Model 2b (biexponential)**

\[ \alpha(V) = f_1(V) \left[\alpha_2(V) + \alpha_1(V) \exp\left(\frac{V + 50.0}{5.3}\right)\right] \]  

(A5.1)

\[ \beta(V) = f_1(V) \left[\beta_2(V) + \beta_1(V) \exp\left(\frac{V + 50.0}{5.0}\right)\right] \]  

(A5.2)

\[ k_{oif1}(V) = 0.432 \cdot \exp\left(\frac{V + 50.0}{30.0}\right) \]  

(A5.3)

\[ k_{oif2}(V) = 0.00552 \cdot \exp\left(\frac{V + 80.0}{30}\right) \]  

(A5.4)

\[ k_{ifo1}(V) = 0.01 \cdot \mu \cdot \exp\left(\frac{-V + 50.0}{30}\right) \]  

(A5.5)

\[ k_{ifo2}(V) = 0.018 \cdot \rho \cdot \exp\left(\frac{-V + 100.0}{13.6}\right) \]  

(A5.6)

\[
\mu = 0.000006, \quad \rho = 0.000006
\]
\[ k_{oif} = f_2(V) \left( k_{oif2} + k_{oif1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right) \] (A5.7)

\[ k_{ifo} = f_2(V) \left( k_{ifo2} + k_{ifo1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right) \] (A5.8)

\[ k_{c1if} = 0.3 \] (A5.9)

\[ k_{ifc1} = 3.0 \cdot \beta \cdot k_{ifo} \cdot \frac{k_{c1if}}{\alpha \cdot k_{oif}} \] (A5.10)

**Appendix A.6: Model 2c (monoexponential)**

\[ \alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right] \] (A6.1)

\[ \beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right] \] (A6.2)

\[ k_{oif}(V) = 0.08 \cdot \gamma \cdot \exp \left( \frac{V + 90.0}{22.0} \right) \] (A6.3)

\[ k_{ifo}(V) = 0.373 \cdot \delta \cdot \exp \left( - \frac{V + 140.0}{13.1} \right) \] (A6.4)

\[ \gamma = 1.0, \quad \delta = 0.0001 \]

\[ k_{c1if} = 0.3 \] (A6.5)

\[ k_{ifc1} = 3.0 \cdot \beta \cdot k_{ifo} \cdot \frac{k_{c1if}}{\alpha \cdot k_{oif}} \] (A6.6)

**Appendix A.7: Models 3a and 3b (monoexponential)**

\[ \alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right] \] (A7.1)

\[ \beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right] \] (A7.2)

\[ k_{oif}(V) = 0.08 \cdot \gamma \cdot \exp \left( \frac{V + 90.0}{22.0} \right) \] (A7.3)

\[ k_{ifo}(V) = 0.373 \cdot \delta \cdot \exp \left( - \frac{V + 140.0}{13.1} \right) \] (A7.4)
Where the values of $\gamma$ and $\delta$ are given in the table below

<table>
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<th>Model 3b</th>
</tr>
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<td>1.0</td>
</tr>
<tr>
<td>$\delta$</td>
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</tr>
</tbody>
</table>

$f = 0.7$  

$k_{ci} = 0.29$  

$k_{lc} = k_{ci} \cdot \frac{k_{fo}}{k_{oif}}$  

**Appendix A.8: Model 3c (biexponential)**

\[
\alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp \left( \frac{V + 50.0}{5.3} \right) \right]  
\]

\[
\beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right]  
\]

\[
k_{oif1}(V) = 0.432 \cdot \exp \left( \frac{V + 50.0}{30.0} \right)  
\]

\[
k_{oif2}(V) = 0.0345 \cdot \exp \left( \frac{V + 80.0}{14.8} \right)  
\]

\[
k_{ifo1}(V) = 0.01 \cdot \mu \cdot \exp \left( -\frac{V + 50.0}{30} \right)  
\]

\[
k_{ifo2}(V) = 0.018 \cdot \rho \cdot \exp \left( -\frac{V + 100.0}{13.6} \right)  
\]

$\mu = 0.00915$  \quad $\rho = 0.00915$

\[
k_{oif} = f_2(V) \left( k_{oif2} + k_{oif1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right)  
\]

\[
k_{ifo} = f_2(V) \left( k_{ifo2} + k_{ifo1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right)  
\]

$f = 0.3$

$k_{ci} = 3.0 \cdot k_{oif}$
\[ k_{ic} = k_{ci} \cdot \frac{k_{ifo}}{k_{olf}} \]  

**Appendix A.9: Models 4a, 4b and 4c (biexponential)**

\[ \alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp \left( \frac{V + 50.0}{5.3} \right) \right] \]  

\[ \beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right] \]  

\[ k_{olf1}(V) = 0.432 \cdot \exp \left( \frac{V + 50.0}{30.0} \right) \]  

\[ k_{olf2}(V) = 0.0345 \cdot \exp \left( \frac{V + 80.0}{14.8} \right) \]  

\[ k_{ifo1}(V) = 0.01 \cdot \mu \cdot \exp \left( -\frac{V + 50.0}{30} \right) \]  

\[ k_{ifo2}(V) = 0.018 \cdot \rho \cdot \exp \left( -\frac{V + 100.0}{13.6} \right) \]  

\[ \mu = 0.00915, \; \rho = 0.00915 \]  

\[ k_{olf} = f_2(V) \left( k_{olf2} + k_{olf1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right) \]  

\[ k_{ifo} = f_2(V) \left( k_{ifo2} + k_{ifo1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right) \]  

\[ f = 0.3 \]  

\[ k_{ci} = 3.0 \cdot k_{olf} \]  

\[ k_{ic} = k_{ci} \cdot \frac{k_{ifo}}{k_{olff}} \]  

<table>
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<th>Model 4b</th>
<th>Model 4c</th>
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<td>( k_{iso} )</td>
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<td>0.0004575</td>
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</table>
Appendix A.10: Model 5 (biexponential)

\[ \alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp \left( \frac{V + 50.0}{5.3} \right) \right] \]  
(A10.1)

\[ \beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp \left( \frac{V + 50.0}{5.0} \right) \right] \]  
(A10.2)

\[ k_{oif_1}(V) = 0.432 \cdot \exp \left( \frac{V + 50.0}{30.0} \right) \]  
(A10.3)

\[ k_{oif_2}(V) = 0.0345 \cdot \exp \left( \frac{V + 80.0}{14.8} \right) \]  
(A10.4)

\[ k_{ifo_1}(V) = 0.01 \cdot \mu \cdot \exp \left( -\frac{V + 50.0}{30} \right) \]  
(A10.5)

\[ k_{ifo_2}(V) = 0.018 \cdot \rho \cdot \exp \left( -\frac{V + 100.0}{13.6} \right) \]  
(A10.6)

\[ \mu = 0.288 \quad \rho = 0.288 \]

\[ k_{oif} = f_2(V) \left( k_{oif_2} + k_{oif_1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right) \]  
(A10.7)

\[ k_{ifo} = f_2(V) \left( k_{ifo_2} + k_{ifo_1} \cdot \exp \left( \frac{V + 90.0}{5.0} \right) \right) \]  
(A10.8)

\[ f = 0.3 \]  
(A10.9)

\[ k_{ci} = 3.0 \cdot k_{oif} \]  
(A10.10)

\[ k_{lc} = k_{ci} \frac{k_{ifo} \cdot k_{ifo_i}}{k_{oif} \cdot k_{oif}} \]  
(A10.11)

\[ k_{ifo_i} = 0.0035 \]  
(A10.12)

\[ k_{oif} = 0.1015 \]  
(A10.13)

Appendix A.11: Model 6 (biexponential)

\[ \alpha(V) = f_1(V) \left[ \alpha_2(V) + \alpha_1(V) \exp \left( \frac{V + 50.0}{5.3} \right) \right] \]  
(A11.1)
\[ \beta(V) = f_1(V) \left[ \beta_2(V) + \beta_1(V) \exp\left( \frac{V + 50.0}{5.0} \right) \right] \]  
(A11.2)

\[ k_{oif1}(V) = 0.432 \cdot \exp\left( \frac{V + 50.0}{30.0} \right) \]  
(A11.3)

\[ k_{oif2}(V) = 0.0345 \cdot \exp\left( \frac{V + 80.0}{14.8} \right) \]  
(A11.4)

\[ k_{ifo1}(V) = 0.01 \cdot \mu \cdot \exp\left( -\frac{V + 50.0}{30} \right) \]  
(A11.5)

\[ k_{ifo2}(V) = 0.018 \cdot \rho \cdot \exp\left( -\frac{V + 100.0}{13.6} \right) \]  
(A11.6)

\[ \mu = 0.288 \quad \rho = 0.288 \]

\[ k_{oif} = f_2(V) \left( k_{oif2} + k_{oif1} \cdot \exp\left( \frac{V + 90.0}{5.0} \right) \right) \]  
(A11.7)

\[ k_{ifo} = f_2(V) \left( k_{ifo2} + k_{ifo1} \cdot \exp\left( \frac{V + 90.0}{5.0} \right) \right) \]  
(A11.8)

\[ f = 0.3 \]  
(A11.9)

\[ k_{ci} = 3.0 \cdot k_{oif} \]  
(A11.10)

\[ k_{ic} = k_{ci} \cdot \frac{k_{ifo} \cdot k_{imis} \cdot k_{istf}}{k_{oif} \cdot k_{istim} \cdot k_{ifis}} \]  
(A11.11)

\[ k_{istf} = 0.0035 \]  
(A11.12)

\[ k_{ifis} = 0.1015 \]  
(A11.13)

\[ k_{istim} = 0.0125 \]  
(A11.14)

\[ k_{imis} = 0.0125 \]  
(A11.15)