

**Investigations of Ion Channel Structure-Function  
Relationships Using Molecular Modeling and  
Experimental Biochemistry**

Thesis by

**Donald Eugene Elmore, Jr.**

In Partial Fulfillment of the  
Requirements for the Degree of  
Doctor of Philosophy



California Institute of Technology

Pasadena, California

2004

(Defended April 22, 2004)

□ 2004

Donald Eugene Elmore, Jr.

All Rights Reserved

## Acknowledgments

“But this dedication is for others to read:  
These are private words addressed to you in public.”

- T. S. Eliot

It is certainly too rare that I take an opportunity to sit back and thank the people who have contributed greatly to my life in so many ways. Thus, I think that this is not just the only part of this thesis any of you will read (yes, you can admit it without offending me—even *I* have trouble getting past page 7 at this point), but it’s also really the most important part. So, for lack of a better place, I’ll start at the beginning . . . .

I treasure countless memories of growing up in Murphysboro, IL, with my wonderful parents, Donald (the elder) and Patricia Elmore. They provided an ideal environment to be a child—to learn about the world, to have fun, and to just be loved—and have been a constant source of support in the past twenty-eight years. I have great respect for both of them personally and professionally, and they are great role models in innumerable ways. I also was very fortunate to have three grandparents living in Murphysboro while I was growing up. My Grandmother “B” (who thankfully let me shorten her name from Borgsmiller when I was young!) and Grandmother and Grandfather Elmore were each a source of inspiration in their own way—and always were fun to have around to hang out with. I feel very lucky to know that my parents and grandmothers back in Southern Illinois are always ready to warmly welcome me home for a visit.

I was also able to discover a love of learning about the world through scientific exploration while a student in Murphysboro. From the first open-ended projects in Carol O'Donnell and Nancy Borgsmiller's classes in elementary school to working on science fair projects in Mary Williams and Molly McDaniels' junior high sciences courses, I was fortunate to see that science was far more than just the memorization of facts. My appreciation of science was further enhanced through the remarkable opportunities I encountered at the Illinois Mathematics and Science Academy. In particular, Richard Dods set me on the trajectory towards chemistry (and particularly biochemistry), and Donald Dosch and Ed Goebel helped solidify my interests in biology. Throughout my junior and senior years, I was able to perform research in the laboratory of Roger Melvold at Northwestern University Medical School, which gave me invaluable research experience before starting college (and also showed me that I did not want to personally do research on animal models!). As well, the innovative teaching methods employed frequently at IMSA, especially their emphasis on experiential learning helped foster my interest in teaching and research in education. Of course, I shouldn't forget the constant pull towards literature that I began to feel during high school, largely due to classes taught by Grant Walker, Larry Chott, and Jackie White.

When I left high school for Central Iowa, I had no inkling of the incredible personal and intellectual growth I would experience in the challenging and supportive environment at Grinnell College. Institutions are no greater than the people who make them up, and this was no exception at Grinnell where I had wonderful classmates (many of whom are still my closest friends) and professors. Among the generally outstanding faculty I encountered were many dedicated members of the Chemistry Department,

particularly Mary Mader, a supportive and always candid academic advisor; Martin Minelli, with his constant enthusiasm; and Charlie Liberko, who made organic chemistry a surprisingly enjoyable experience for me as a first-year student. I was also lucky to have another academic “home” at Grinnell in the English Department, who encouraged me despite my split loyalties. In particular, Elizabeth Dobbs’ classes were the most challenging (and rewarding) I encountered during college (or graduate school), and represent the ideal I hope to approach when teaching my own seminar classes. The Grinnell Biology Department was also very supportive of my crossover interests, especially Leslie Gregg-Jolly, Bruce Voyles, and Deborah Eastman. Carol Trosset in the Office of Institutional Research was gracious enough to work with Julia Prentice and I on an educational research project I was grossly underqualified to perform but nonetheless gave me a valuable crash course in social science research methods. However, Jim Swartz stands alone in my esteem among the Grinnell faculty. In addition to being a great undergraduate research advisor, he has been a superlative mentor and friend. I am always amazed by the amount of support he has given not only me but so many others at Grinnell and throughout the wider academic community, and I would be happy if I am a fraction as influential as him on my own students.

Also during college, Steve Scheiner at Southern Illinois University was kind enough to open up his research lab to me after my first year, where I was infected with an excitement for computational chemistry. Both Steve and his research associate Tapas Kar provided me with the perfect balance of guidance and freedom to critically develop my research skills.

For some reason, four years of college chemistry wasn't enough for me, so I decided to head out to Caltech where I was savvy (or more honestly lucky) enough to join the Dougherty Group. Simply stated, Dennis Dougherty has been the model advisor (although I have ignored his advice on terse writing in these acknowledgments). Unlike so many other people, I truly *enjoyed* graduate school, and I know this was in no small part due to working for Dennis. I sometimes doubt I would have even completed graduate school with any other advisor, and I am certain that I would not have grown so much from the experience. I truly appreciate the intellectual freedom he was willing to give me on projects from the very beginning, which made my research much more meaningful in my scientific development. And, in addition to being a wonderful scientific mentor, he is also an exemplary person who can teach many valuable lessons about life outside of the lab. Over the past years, the rest of the Dougherty Clan (Ellen, Meg, and Kayla) have frequently welcomed other group members and me into their home, and I always looked forward to those gatherings.

The members of the Dougherty Group provided a wonderful working (and often *non-working*) environment in which to spend my time at Caltech. It goes without saying that they provided an intellectually stimulating environment for science, and numerous insightful conversations with group members have taken my projects in directions I would never have envisioned alone. However, what I will remember most about the group is the endearing personalities of its members. I have enough stories about all of them to fill pages, but I'll just give a glimpse into those here. Gabriel Brandt gave me my first positive impression of the group when I was a prospective student, and he exemplified the best qualities of the group—I greatly miss having him two desks away

for long and varied conversations (and I am so glad he will be in Boston next year!). Justin Gallivan was there to give me a warm welcome and some useful advice when I first showed up. Seth Miller pointed out the necessity of taking a break from thesis writing with his pursuit of Ms. Pac Man high scores. Jen Ma showed me the value of a leisurely lunch after her graduation. Pam England had an unstoppable energy towards any pursuit. Jesse Lin has an amazing opera voice (that gave me quite a fright one late night in lab!). Marcus Sarofim is one of the most sincere people I've ever met—in addition to being skilled at Pint-Sized Punchlines. Niki Zacharias is a model of kindness, both to those in direct contact with her and to the world as a whole, and was responsible for organizing far more than her share of group events. Lintong Li was an impressive example of effectively balancing professional and family lives that I could learn more from. I have always admired David Dahan's consistency throughout many aspects of his life, showing a balance I know I lack. James Petersson is the modern day embodiment of the *sprezzatura* of Casiglione's courtier (in all of its good connotations)—he makes it seem so easy to do a wide variety of things impressively well, including making it “cool” to listen to the Thom Bell Sessions. I was thankful to have another English major in the group with Darren Beene (albeit one whose knowledge of literature and culture far exceeds my own), and seeing him as a doting father has been particularly heartwarming. I've enjoyed Sarah Monahan's perspective in a wide range of conversations, from women in science to the Canadian medical system. Steve Spronk helped me remember my “glory days” of scholar bowl, and his encyclopedic knowledge of all things athletic is remarkable. Lori Lee has been too gracious in taking care of Hector during vacations, and I always look forward to perpetually engaging talks with her over Olean or falafel. I

love sharing notes on new music with Amanda Cashin, and despite my sarcastic remarks there is no one I would rather have move into my old desk. Tingwei Mu exudes an infectious enthusiasm in lab that helped boost my morale. Despite his foolhardy attempt to avoid driving in Los Angeles *and* being an MIT graduate, Michael Torrice is an amazingly well-balanced person with a keen sense of humor and a wisdom beyond his years in graduate school. Being too often out of shape myself, Amy Eastwood's dedication to marathon training leaves me speechless. Erik Rodriguez has shown incredible patience in being able to deal with the rest of us both as an undergraduate and a graduate student. Joanne Xiu was too kind as my desk "neighbor," dealing daily with my encroaching papers without complaint. Julian Revie's search for a patron highlighted his impressive moxie and unique perspective on the world. Katie McMenimen seems poised to carry the torch well for the group, and has been overly nice to this random old grad student who kept showing up this year. And, Catherine Baker was a welcome "honorary group member," providing advice on hamster care and incisive perspectives on the Gilmore Girls. I have also been lucky enough to interact with several talented undergraduate researchers, including Paola Giusti, Lisa Turner, Anita Choi, and Caroline Gibbs, who brought enthusiasm and unique insights to the group.

One group member, Josh Maurer, deserves a special mention. Josh was brave enough to help this computationalist learn biochemical lab techniques, and was a first-rate collaborator on many projects (some more successful than others!) over the years. (Much of the material in Chapters 2 and 3 were done in collaboration with him.) I look back fondly on our time in lab together, but most of all I am glad for the friendship that



grew out of it. I always looked forward to Baja lunches, constructing lofts, or “Josh’s Day Out,” all of which made my time at Caltech much happier.

I have also been able to work with some other wonderful people at Caltech. Henry Lester, Doug Rees, and Gerd Kochendoerfer have been wonderful collaborators on the mechanosensitive ion channel projects, and I have always appreciated their input on my research. My other committee members, Bill Goddard and Harry Gray, made valuable contributions to my candidacy and research proposal meetings. Ken Philipson provided invaluable biological experience to the early MscL calculations. Many people in the Lester Group have helped supply resources during my time at Caltech, in particular Purnima Deshpande (who organizes more than the rest of us have ever appreciated), Kira Kostenko, and Vanna Santoro. As well, the Chemistry Division at Caltech runs so smoothly due to the hard work of many staff members, including Linda Syme, Dian Buchness, Tom Dunn, Chris Smith, Steve Gould, Paul Carroad, and Debbie Miles.

During the past year, I have also been able to work with some wonderful people in the Joint Sciences Department at the Claremont Colleges, and I have really appreciated the opportunity to develop my teaching skills in a very supportive environment. Katie Purvis-Roberts stepped up from the start as my informal (and eventually formal) faculty mentor, and I will miss our frequent “mentoring” lunches and other get togethers. Mary Hatcher-Skeers has been a great cheerleader from the moment I met her at my interview. I’m very glad that I’ve been able to compare notes with Loyd Bastin throughout the job search process, and it will be nice to have him also moving east this fall. Harriet Moeur’s hard work has made it easy for even me to teach organic labs effectively. I will also fondly remember many great conversations and “power lunches” with Nina Karnovsky,

Todd Coleman, and Alex Reich during meetings for the “real” faculty. And, I’ve been very fortunate to work with some phenomenal students—including a few invaluable teaching assistants, Irene Frank and Rachel Levitan—who I am certain will go on to many great things in their lives after Claremont.

I have also been blessed with many friends outside of my professional life while I have been in Los Angeles, and I think those are the people who have truly kept me balanced enough to actually complete my doctorate! Jamie Pflasterer and Ian Spielman have helped make Pasadena feel more like home from our first month in Southern California, and I will always have fond memories of spending time with them from the early *Ally McBeal* days to hanging out with their son Camden in the last few months. It was also wonderful to have a few Grinnellians to make the LA transition with—Rachel Taylor and Cory Turner have always been a beacon to us from the fabled West Side, providing a constant source of friendship and insights into the interesting worlds of non-profits and the movie industry. Jeremy “Lil’ Dude” Hill has often been a necessary ballast for me throughout the last six years just as he was during our four years in college. No matter whether we were taking in great (and sometimes not so great) concerts, watching Dodgers baseball, or just hangin’ in the Valley, I am so glad that he decided to grace Los Angeles with his presence for a few years—and I am even happier that we will at least be on the same coast again in a few months. I was also fortunate to have some high school friends, Russ Schaaf and Ryan Fox, living around Southern California while I was here, and I appreciate your friendships that have extended far over a decade now.

Of course, I couldn’t be lucky enough to have all of my friends living in Los Angeles. However, although they have been in London, Ft. Wayne, and Kansas City

during my years at Caltech, Ted Smith and Laura Schwartz have been such great friends that it has seemed like they were always close at hand. I am really glad we have been able to visit so frequently over the years, and I look forward to countless more vacations and phone calls. Many other friends from Grinnell and back home in Illinois have been wonderful to visit with many times over the past years, including Bill Bell, Joanna Church, Kate Fuller, Sarah Halpin, Kristen Jensen, Elizabeth Murphy, Barbara Sloss and Owen Stanwood. It is so wonderful to look at a map and realize that there is a great friend just a day's drive away pretty much anywhere I go—my own modern day Missions, so to speak. I am also very fortunate to have gained some very supportive in-laws—Rod and Myrna Prentice, who are always interested in hearing descriptions of my research; Patricia Prentice, the ideal sister-in-law in more ways than I could mention; and Grandma June Dailey and the late Grandma Avis Prentice, who lived such inspiring lives.

And, I will close with the most important acknowledgement of all. When I set out with my best friend to live in California six years ago, we never could have known the spectrum of experiences we would have. But, Julie, because you were with me throughout, I look back on it all fondly. We have (somehow!) made it here, and I look forward to conquering the other coast with you next! I love you immensely, and I never cease to be amazed by everything about you. Thanks for all of the support, all of the laughter, all of the caring, and most of all for making my world a better place. To finish this section as we began, in Eliot's words, you are: "To whom I owe the leaping delight / That quickens my senses in our wakingtime / And the rhythm that governs the repose of our sleepingtime / The breathing in unison."

## Abstract

Ion channels are integral membrane proteins found in all cells that mediate the selective passage of specific ions or molecules across a cell membrane. These channels are important in a diverse range of physiological processes, including signal transmission in the nervous system, sensory perception, and regulation of vital systems, such as circulation. This thesis discusses the use of computational chemistry methods, such as molecular dynamics (MD) and *ab initio* calculations, and experimental biochemical techniques, such as site-directed mutagenesis, *in vivo* bacterial assays, chemical cross-linking, and circular dichroism spectroscopy, in tandem to elucidate ion channel structure-function relationships. This research was catalyzed by the solving of atomic resolution crystal structures of the mechanosensitive channels of large and small conductance (MscL and MscS) by the Rees group. Although interesting themselves, these bacterial channels also provide good model systems for considering more complex eukaryotic channels.

MscL is an ion channel gated only by membrane tension. Initial studies of MscL verified the relevance of the crystal structure conformation under physiological conditions and compared different MscL homologues. Other work began to elucidate potentially unique structural and functional roles of the *M. tuberculosis* MscL C-terminal helical bundle. As well, interactions between the MscL channel protein and surrounding lipid and the potential relevance of helical kinking in MscL gating pathways were investigated. MscS is also gated by membrane tension, but its gating can be modulated by changes in transmembrane potential. Thus, studies on MscS began to identify the

specific amino acid residues that are responsible for giving the channel its voltage sensitivity. Finally, computations predicting the conformation of nicotine in different solvent environments are discussed. Nicotine is a small molecule ligand that binds to and gates nicotinic acetylcholine receptors, and a thorough understanding of nicotine structure could aid efforts to elucidate receptor structure-function relationships and design new pharmaceuticals.

## Table of Contents

ACKNOWLEDGMENTS .....	III
ABSTRACT.....	XII
TABLE OF CONTENTS.....	XIV
LIST OF FIGURES .....	XVIII
<b>CHAPTER 1: INTRODUCTION .....</b>	<b>1</b>
ION CHANNELS .....	2
BACTERIAL ION CHANNELS.....	5
APPLYING COMPUTATIONAL MODELING AND EXPERIMENTAL BIOCHEMISTRY TO ION CHANNEL STRUCTURES.....	7
REFERENCES .....	10
<b>CHAPTER 2: CONFIRMATION OF THE <i>M. TUBERCULOSIS</i> MSCL CRYSTAL STRUCTURE AND COMPARISONS OF MSCL HOMOLOGUES.....</b>	<b>13</b>
THE <i>M. TUBERCULOSIS</i> MSCL CRYSTAL STRUCTURE: UNIQUE OPPORTUNITIES AND AMBIGUITY .....	14
PRODUCTION OF MSCL PROTEINS AND CROSS-LINKING PROTOCOLS .....	17
RESULTS OF CROSS-LINKING STUDIES.....	18
SEQUENCE ANALYSIS OF THE MSCL CHANNEL FAMILY.....	21
ANALYSIS OF THE MSCL CHANNEL FAMILY WITH CIRCULAR DICHROISM.....	26
SUMMARY .....	30
REFERENCES .....	31

<b>CHAPTER 3: COMPUTATIONAL AND EXPERIMENTAL INVESTIGATION OF THE C- TERMINAL REGION OF <i>M. TUBERCULOSIS</i> MSCL .....</b>	<b>34</b>
BACKGROUND.....	35
METHODS FOR COMPUTATIONAL MODELING OF THE C-TERMINAL REGION.....	36
THE EFFECT OF pH ON THE C-TERMINAL HELIX BUNDLE IN MD SIMULATIONS .....	38
THE EFFECT OF MUTATIONS TO THE C-TERMINAL HELIX BUNDLE IN COMPUTATIONS .....	42
EXPERIMENTAL PRODUCTION OF MSCL WITH C-TERMINAL MUTATIONS.....	43
CHARACTERIZATION OF MSCL MUTANTS WITH THERMAL MELTS.....	45
DEVELOPING STRUCTURAL MODELS OF THE MSCL C-TERMINAL REGION.....	47
SUMMARY .....	50
REFERENCES .....	51
<b>CHAPTER 4: INITIAL MOLECULAR DYNAMICS SIMULATIONS OF <i>M. TUBERCULOSIS</i> MSCL EMBEDDED IN AN EXPLICIT LIPID MEMBRANE .....</b>	<b>53</b>
BACKGROUND.....	54
COMPUTATIONAL METHODS .....	58
PROTEIN STRUCTURE IN THE WILD-TYPE MSCL SIMULATION.....	63
STRUCTURAL CHANGES IN MUTANT TRAJECTORIES.....	67
THE R45-Q51 HYDROGEN BONDING INTERACTION.....	68
PROTEIN-LIPID INTERACTIONS.....	71
PORE WATER PROPERTIES IN THE WILD-TYPE MSCL SIMULATION .....	77
SUMMARY .....	79
REFERENCES .....	80

<b>CHAPTER 5: EFFECTS OF LIPID COMPOSITION AND TRANSMEMBRANE KINKING ON THE MECHANOSENSITIVE CHANNEL OF LARGE CONDUCTANCE (MSCL) .....</b>	<b>87</b>
BACKGROUND.....	88
MOLECULAR DYNAMICS SIMULATIONS.....	91
<i>General Simulation Setup</i> .....	91
<i>Simulations with Different Lipid Headgroups</i> .....	93
<i>Simulations with Lipid Tail Shortening</i> .....	94
<i>F80P Simulations</i> .....	96
<i>Simulation Details</i> .....	97
SIMULATIONS WITH DIFFERENT LIPID HEADGROUPS.....	98
<i>Overall Comments</i> .....	98
<i>Lipid-Dependent Conformation of the C-terminal Region</i> .....	100
<i>Extracellular Loop Region</i> .....	104
SIMULATIONS WITH LIPID TAIL SHORTENING .....	106
<i>Overall System Adjustment to Lipid Shortening</i> .....	106
<i>Evidence of Hydrophobic Matching in the Simulations</i> .....	107
<i>Structural Rearrangements Upon Bilayer Thinning</i> .....	111
<i>Comparisons to Experimentally Derived Intermediate Gating Models</i> .....	112
CORRELATIONS BETWEEN TM2 ENERGETIC PROFILES AND MUTAGENIC DATA.....	114
INVESTIGATION OF THE ROLE OF TM2 KINKING IN MSCL GATING .....	116
<i>Molecular Dynamics Simulations of F80P</i> .....	116
EXPERIMENTAL CHARACTERIZATION OF MUTANTS WITH A TM2 KINK .....	119
INTERSUBUNIT AND PROTEIN-LIPID INTERACTIONS OF MSCL TM2 PROLINE MUTANTS.....	121
SUMMARY .....	123
REFERENCES .....	125



<b>CHAPTER 6: IDENTIFICATION OF THE MECHANOSENSITIVE CHANNEL OF SMALL CONDUCTANCE (MSCS) VOLTAGE SENSOR.....</b>	<b>131</b>
BACKGROUND.....	132
SETUP OF MOLECULAR DYNAMICS SIMULATIONS .....	134
THE CHANNEL STRUCTURE IS SENSITIVE TO TRANSMEMBRANE POTENTIAL IN SIMULATIONS.....	138
DOES THE CRYSTAL STRUCTURE REPRESENT AN “OPEN” STATE?.....	142
WHAT CHEMICAL MOIETIES GIVE THE CHANNEL ITS VOLTAGE SENSITIVITY?.....	145
AGREEMENT WITH EXPERIMENTAL ELECTROPHYSIOLOGY .....	148
DISCUSSION .....	149
REFERENCES .....	151
<b>CHAPTER 7: COMPUTATIONAL DETERMINATION OF NICOTINE CONFORMATIONS IN THE GAS PHASE AND IN AQUEOUS SOLUTION .....</b>	<b>154</b>
BACKGROUND.....	155
ENERGETIC PROFILE FOR RELATIVE PYRIDINE/PYRROLIDINE RING ROTATIONS.....	158
GAS-PHASE STRUCTURES AND ENERGETICS .....	159
INCLUSION OF SOLVATION IN ENERGETIC CALCULATIONS.....	163
RELEVANCE TO OTHER WORK ON NICOTINIC RECEPTORS AND AGONISTS .....	167
REFERENCES .....	168

## List of figures

Figure 1.1: The three general categories of gating stimuli for ion channels. ....	3
Figure 1.2: Crystal structures of the KcsA and MscL channels.....	6
Figure 2.1: The Tb-MscL crystal structure highlighting the interaction between Gln 51 and Arg 45 residues on adjacent subunits.....	15
Figure 2.2: Bis-malimide cross-linking reagents with spacer arms of varying lengths. .	17
Figure 2.3: Cross-linking of the R45K/Q51E mutant of <i>M. tuberculosis</i> MscL. ....	19
Figure 2.4: Cross-linking of the R45C/Q51C mutant of <i>M. tuberculosis</i> MscL. ....	20
Figure 2.5: MEME consensus group analysis shown on the AMPS multiple sequence alignment of 35 putative MscL homologues.....	22
Figure 2.6: Regional AMPS pairwise alignments for the first transmembrane domain, the loop region, and the carboxyl terminus.....	25
Figure 2.7: Circular dichroism spectra for nine different MscL homologues. ....	27
Figure 2.8: Comparison of protein length to the maximal predicted helical content for the various homologues of MscL.....	29
Figure 3.1: The Tb-MscL C-terminal helical bundle in the channel crystal structure and in an MD simulation system.....	36
Figure 3.2: C $\alpha$ RMS deviation and fluctuation from Tb-MscL C-terminal helical bundle simulations. ....	39

Figure 3.3: Analysis of secondary structure for the C-terminal region simulations performed with the DSSP method for the pH 7, low pH, and E104Q/D108N simulations. ....	40
Figure 3.4: Ribbon diagrams of the final frames of the pH 7, low pH and E104Q/D108N C-terminal region simulations. ....	41
Figure 3.6: Circular dichroism thermal denaturation curves for wild-type Tb-MscL and a series of single-site C-terminal mutations. ....	46
Figure 3.7: Helical wheel showing observed shifts in thermal stability for mutations in the carboxyl terminal region of Tb-MscL. ....	49
Figure 3.8: A cartoon depicting one possible conformation of a Tb-MscL single subunit with a “bend” in the C-terminal region. ....	50
Figure 4.1: Tb-MscL crystal structure and embedded lipid system used in simulations. ....	55
Figure 4.2: Schematic of the embedded Tb-MscL MD trajectories. ....	61
Figure 4.3: Superimposed C $\alpha$ traces for the Tb-MscL crystal structure and the average structure for the final 1000 ps of the wild-type simulation. ....	64
Figure 4.4: RMS deviation of C $\alpha$ from the crystal structure for the wild-type Tb-MscL trajectory. ....	65
Figure 4.5: RMS fluctuation of C $\alpha$ around an average structure for the wild-type and Q51E trajectories plotted per residue. ....	66
Figure 4.6: Pore radius profiles for the wild-type and V21A Tb-MscL simulations in the membrane region calculated with the HOLE program. ....	68

Figure 4.7: Hydrogen bond/salt bridge interaction populations of the five possible R45-Q51 pairs in the wild-type Tb-MscL simulation and of the five possible R45-E51 pairs in the Q51E simulation.....	70
Figure 4.8: Protein-lipid hydrogen bonding in wild-type Tb-MscL simulations.....	72
Figure 4.9: Differences in lipid order between "bordering" and "bulk" lipids. ....	77
Figure 4.10: Average orientation of pore water dipoles along the membrane normal over the last 1 ns of the wild-type and V21A simulations.....	78
Figure 5.1: The full simulation system at the end of 100 ps of MD simulation. ....	92
Figure 5.2: Chemical structures of lipids and schematics of simulations. ....	94
Figure 5.3: RMS deviation from crystal structure for POPE and POPC trajectories.....	99
Figure 5.4: Number of hydrogen bonds between lipid and either the entire channel or only the extracellular loop in the POPE and POPC trajectories.....	100
Figure 5.5: Pictures of the final frames of the POPE and POPC trajectories.....	101
Figure 5.6: Protein-lipid and intersubunit interaction energies per residue for the C-terminal region in POPE and POPC simulations. ....	102
Figure 5.7: Protein-lipid and intersubunit interaction energies per residue for the extracellular loop region in POPE and POPC simulations. ....	105
Figure 5.8: Distance between the phosphorous atoms of the two lipid bilayer leaflets and channel C $\square$ RMS deviation values for for all lipid shortening trajectories. ....	107
Figure 5.9: Average transbilayer distances between phosphorous atoms of lipids bordering Tb-MscL and in the bulk for lipid shortening simulations. ....	108

Figure 5.10: Average values for channel hydrophobic surface length (HSL) in lipid shortening simulations. ....	109
Figure 5.11: Pore radius profiles for lipid shortening simulations calculated with HOLE and representative kinked TM2 helix. ....	111
Figure 5.12: The correlation between Ec-MscL random mutagenesis data and MD interaction energies. ....	114
Figure 5.13: Single subunit from the Tb-MscL crystal structure highlighting the F80 residue. ....	117
Figure 5.14: TM2 kinking in wild-type and F80P simulations. ....	118
Figure 5.15: RMS fluctuation of C $\alpha$ atoms per residue for TM1 and TM2 residues in the wild-type and F80P simulations. ....	119
Figure 5.16: TM2 intersubunit interactions in wild-type and F80P simulations. ....	122
Figure 5.17: TM2 Protein-lipid interaction energies in wild-type and F80P simulations. ....	123
Figure 6.1: The MscS crystal structure. ....	133
Figure 6.2: The MscS simulation system. ....	135
Figure 6.3: RMS deviation from crystal structure in the MscS simulation with no applied voltage. ....	139
Figure 6.4: Pore occlusion in MscS simulation with and without applied voltage. ....	140
Figure 6.5: TM3 conformational changes upon channel occlusion. ....	141
Figure 6.6: Pictures of pore water in MscS simulation with applied voltage. ....	143
Figure 6.7: TM3 conformational changes in simulation with applied voltage. ....	145

Figure 6.8: Position of R46 and R74 residues in simulations with and without applied potential.....	146
Figure 7.1: Nicotine protonation states and numbering of nicotine used in this chapter. .....	156
Figure 7.2: MMFF94 rotational profile for singly protonated (+) nicotine species.....	159
Figure 7.3: HF/6-31G** optimized structures of singly protonated nicotine species....	161
Figure 7.4: Thermodynamic cycle of $\Delta\Delta G_{\text{sol}}$ values for singly protonated nicotine species calculated with SPT/OPLS. All values are in kcal/mol. ....	164