Structure-Function Studies of Nicotinic Acetylcholine Receptors Using Unnatural Amino Acids and Synthetic Agonist Analogs

Thesis by

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To my beloved furry companions:

Cyrano and Burrito

ACKNOWLEDGEMENTS

I owe much gratitude to many people for their help and support during my tenure at Caltech. It's hard to believe that I've been associated with Caltech for nearly eight years. I started as a Summer Undergraduate Research Fellow (SURF) in the Grubbs lab. At the time, the lab was packed with amazing scientists like Jacob Berlin, Andy Hejl, Anna Wenzel, and Tim Funk who rekindled my love for science. When it came time to pick a graduate school and later a research lab, the choice was quite easy for me; I couldn't imagine working in any other lab. After rejoining the lab, it took me six months and a lot of soul-searching to realize that I wanted to become a chemical biologist and not an organometallic chemist. Bob was incredibly supportive of this decision and has continued to serve as a great mentor over the years.

Although it was difficult to leave the Grubbs lab behind, it was easy to transition into the Dougherty group. Dennis has a hands-off management style that works well for my personality. He enabled me to take intellectual ownership over my research and gave me a great deal of scientific freedom, while also making sure that I never lost sight of the overall goal of a given project. Dennis is never too busy for his students and always makes us feel like we are his priority. I have learned a great deal about how to be a good teacher from Dennis, and I am confident that the critical thinking skill set I honed in his lab will equip me to tackle any problem in chemical biology or life in general.

Henry Lester is a longtime collaborator of the Dougherty lab and a great source of biology and electrophysiology wisdom. He provided much insight for many of the projects presented in this thesis. I am also indebted to the other members of my

committee, Prof. Peter Dervan and Prof. Jacqueline Barton, for their many words of advice and encouragement throughout my graduate career.

I've been very fortunate to have had three incredible mentors in science, Prof. Louis Kuo, Tobias Ritter, and Tim Funk. Prof. Kuo was my favorite professor in college and the reason I decided to pursue a career in academia. Tim Funk was my mentor during my summer as a SURF. He is a great example of how to be successful scientist and a good person. I owe my work ethic and confidence at the chemistry bench to Tobias Ritter, a former Grubbs lab postdoctoral scholar. He is a source of endless knowledge and is a truly creative scientist. I also thank the other members of "Church 130" in the Grubbs lab for serving as my big brothers and sisters in chemistry.

All of the members of the Dougherty lab have been essential for my success at Caltech. Many of the older students were helpful in my transition into chemical biology. I especially thank Ariele Hanek for her insight, support, and friendship.

My fellow sixth-year Dougherty lab members, Jai Shanata, Sean Kedrowski, and Kay Limapichat, have been particularly important components of my graduate experience. Sean and I were both refugees from synthetic chemistry labs. As such, we've shared a lot of common experiences, and I'm thankful to have had someone to commiserate with in the lab. Sean has great critical thinking skills and always has a clever take on confusing experimental results. He's decided to return to synthetic chemistry for his postdoctoral work, and I'm confident that he will be a great asset to the Jacobsen lab at Harvard. Jai and I are guaranteed to be on opposite sides of any argument, which is funny because we are probably more similar than we are different. I wish him much luck in his new career as a professor at Loyola College in New Orleans,

and I look forward to seeking his advice in a few years when I apply for similar positions. I actually considered dedicating this thesis to Kay. She certainly deserves a great deal of credit for sitting behind me and listening to all of my grumblings over the years. We have a very deep understanding of one another, probably because our philosophies in life and personalities are pretty similar despite our very different upbringings. She's always had my back, especially when it mattered most, and I consider her one of my dearest friends.

Nyssa Puskar is the lone representative of the fifth-year class in the Dougherty lab and is a longtime friend and confidant. Her sweeter-than-sugar personality and positive outlook are the perfect counterparts to my cynicism, and I am often inspired by her patience, strong work ethic, humility, and unwavering faith.

Noah Duffy and Darren Nakamura were next to join the group. Noah is the MacGyver of the lab, and I am convinced that he can fix *anything*. I will never forget the day he removed the broken lock from my bike by wailing on it with a hammer (and some other interesting tools); I cherished every weird look we got that day. Darren has a great sense of humor and great humility. I consider myself lucky to have gotten the chance to know him (and his alter-ego "Dexter") during his short time at Caltech.

The third year class is composed of an interesting cast of characters. It has been a pleasure to get to know Kristina McCleary. She's very bright and has a lot of biology knowledge that has been quite useful to me recently. I find her practical approach to problems very refreshing. I am envious of Ethan Van Arnam's adventurous spirit and his frequent world travels. We share a similar life philosophy and love of nature, and so I hope that we stay in touch after my departure from Caltech. Ximena Da Silva is another

great friend and fellow animal lover. I've enjoyed listening to all of the crazy stories from her childhood and am absolutely convinced that she should sell her life story to the Lifetime Movie Network. I am inspired by Maggie Thompson's bravery. She's chosen to start over in a different graduate program, a decision that took much self-reflection and great courage. I'm very proud of her and am confident that she will make an extraordinary physician. Erin Lamb has an off-beat humor that is endearing. Her everyday behavior is incredibly entertaining, and I'm sure that her quirky way of looking at the world will afford her many great adventures in life. Clint Regan is new to the Dougherty lab, but I am already intrigued by his sense of humor. I suspect he'll be a very amusing addition to the lab culture. I'm also delighted that another round of former synthetic chemists (Erin and Clint) will be able to pick up where Sean and I left off.

I've enjoyed getting to know the first and second year classes of the Dougherty lab. Fan Liu has been a great addition to the lab. His modeling studies will likely provide useful insight into many projects. Chris Marrota is my "BFF" in the boot camp gym class, and I am happy to have met someone that is *almost* as competitive as I am. In the lab, he has a strong work ethic and a great eagerness to learn. Oliver Shafaat and Tim Miles can already "talk the talk" of science. They seem well equipped to be very successful in the lab. Fan, Chris, Oliver, and Tim all share a palpable passion for science, and I am confident that the legacy of the Dougherty lab will be safe in their hands.

Wesley Yu and Laurel German were brave enough to endure a few summers in the lab with me. Wesley is an incredibly gifted Caltech undergrad who always seems to have a crazy idea that could (A) cure a disease, (B) make lots of money, or (C) impress a girl. I first met Laurel when she was fifteen (a sophomore at Polytechnic School), and I

was amazed at the speed with which she acclimated to the lab. She's truly a very capable person and a real Renaissance teenager; active and proficient in a wide range of after-school activities and other hobbies. This fall, Wesley will enter medical school at UCSF and Laurel will be a freshman at Yale. There are simply no words to express how proud I am of them.

My family also deserves a great deal of gratitude. My mother has been a good role model, having strong conviction and *infallible* personal integrity. She's made many personal sacrifices for her children, for which I am very grateful. I still consider my older brother to be the smartest person I've ever met. He has a Ph.D. in a field that requires a lot of math, and he somehow figured out how to put up with a really annoying little sister. My sister-in-law is an incredibly kind and unassuming person whom I am blessed to have in my life. My nearly two-year-old niece, Katherine Elizabeth Blum, is an adorable little girl whom I hope to one day know very well and eventually spoil rotten.

Justin "Bieber" Wong is a very patient and understanding person whom I have truly under-appreciated. I consider myself very lucky that he finally (after much campaigning) agreed to date me four years ago. He's an exceptionally responsible and stable person who has been a very good influence in my life. He's helped me figure out what I want to do "when I grow up," and I look forward to helping him do the same.

One of the perks of dating Justin is that he comes with a large extended family. His parents are both former scientists, and as such we formed an instant kinship. Justin's older sister Courtney is pursuing a Ph.D. in organizational communication at UCSB. She's an over-achiever like me, and I've enjoyed commiserating with her over the years about life as a graduate student. Justin's younger brother Adam has an infectious laugh

and a warm spirit that is comforting to be around. "The aunts" are an incredibly kind, friendly, and beautiful group of women. They've made me feel welcome and accepted from the start. I also absolutely adore the entire Toy family. Amanda Toy and Kristin Toy (Justin's cousins) are like sisters to me, and I feel so very fortunate to have them in my life.

Those that know me well won't be surprised that I chose to dedicate this thesis to my two pets. I've always felt more comfortable with animals than I do people. It's possible that I am inspired by their unassuming and forgiving nature. My cat Burrito is possibly the sweetest and most gentle creature in the entire world. She's been my companion for 14 years, and I'm hoping her kidneys will hold out for many more. Cyrano is a mischievous Boston Terrier (with a bit of a Napoleon complex) who truly brings joy to every day. I've heard people say that dogs are a reflection of their owners. I'd say that's pretty accurate in our case.

ABSTRACT

This dissertation primarily describes structure-function studies of the prototypical Cys-loop ligand-gated ion channel, the nicotinic acetylcholine receptors (nAChRs).

Agonists that bind nAChRs, including acetylcholine, nicotine, and the smoking cessation drug varenicline, share one of the longest-known, best-studied pharmacophores, consisting of a cationic N and a hydrogen bond acceptor. A major theme of this thesis is concerned with defining the nAChR residues that bind the nicotinic pharmacophore. Chapters 2 and 3 establish that a hydrogen bond links the pharmacophore's hydrogen bond acceptor to a backbone NH in the protein. The establishment of this interaction, and the disproval of other predicted interactions, represents the completion of the nicotinic pharmacophore binding model. Chapter 4 uses this model to characterize how the nAChR differentiates between stereoisomers of an agonist.

Chapter 5 describes functional studies of a vicinal disulfide that has played a pivotal role in a number of pioneering studies of nAChRs. Despite its historical importance, the functional role of this disulfide has not been defined. We identify a speculative role for the vicinal disulfide that involves the formation of a functionally important network of hydrogen bonds.

Chapter 6 outlines three strategies for the photochemical cleavage of protein and peptide backbones using unnatural amino acids. One of these strategies is based on a selenide-mediated cleavage of a backbone ester moiety. Model studies establish the viability of this chemistry and suggest that it could be a useful tool for protein structure-function studies.

Chapter 7 concerns preliminary work from a collaboration with laboratories from USC and Caltech that is aimed at developing small-molecule treatments for vision loss associated with photoreceptor degeneration. The initial goal of this project is to develop a photosensitive small molecule that can activate a voltage-gated potassium channel.

The final chapter discusses work that was done in the Grubbs lab at Caltech in which a strategy for preparing *N*-heterocyclic carbene-containing metal complexes was developed.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES