Structure-Function Studies of Nicotinic Acetylcholine Receptors Using Unnatural Amino Acids

Thesis by

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To my loving mother and father:

Cynthia Jean Puskar and James Michael Puskar

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It feels like only yesterday when my mom and grandpa drove me across the United States with everything I owned in the back of my grandpa's pick-up truck. During these past five-and-a-half years I have grown immensely not only as a scientist, but also as a person. And for that, I would like to personally thank the special people who helped me along the way.

First, I would like to thank my advisor, Professor Dennis Dougherty. I was determined to join Dennis' lab from the start and as such, I sat in his lab as if I was already a member for several months (a tactic encouraged by Katie McMenimen and Ariele Hanek). I remember feeling so victorious when Dennis eventually caved in and let me join his lab. Dennis is an awesome advisor and has played a huge role in making my time in graduate school a rewarding and positive experience. I really appreciate his mentoring style, which fosters scientific and personal growth through giving his students the flexibility to pursue multiple projects while keeping us focused on the end goal.

I have a great deal of respect and appreciation for Professor Henry Lester, a longtime collaborator of the Dougherty lab. Henry has really been a coadvisor for several of my projects. Henry has the ability to offer unique perspectives for our research projects and I will always remember our many "hallway" chats about science. I also owe many thanks to my thesis committee, Professor Peter Dervan, Professor Jacqueline Barton, and Professor Harry Gray. Their words of encouragement and advice on life after graduate school have greatly contributed to my positive experience at Caltech.

After moving cross-country and not knowing a single person, the Dougherty group took me in and made me feel at home. The Dougherty group is a unique and

amazing group of people, and I have truly been fortunate to cross paths with each and every one of them. I am greatly indebted to many of the older students who were essential in getting me up to speed in the ways of the Dougherty lab. I especially express gratitude to Joanne (Xinan) Xiu who was my early mentor in the lab and collaborator on the $\alpha 4\beta 2$ project. In addition to having an incredible work ethic, she is a sweet and patient lady with the cutest babies.

I was initially drawn to the middle bay where I shared many laughs and interesting conversations with Michael Torrice, Ariele Hanek, Katie McMenimen, Kiowa Bower, and Jai Shanata. As my TAs in physical organic chemistry, Ariele and Katie were my first friends in the lab. Ariele is one of the kindest people I have ever met, and I cherish all of our talks (and swims) that we shared over the years. Katie is hilarious and I will never forget her spot-on impressions of "classic" C&E News pictures. I also had the privilege of being a part of the interesting and thought-provoking discussions regarding zombies initiated by Mike, Kiowa, or Jai. These guys always made me laugh. Mike has the best sense of humor, and his ability to deliver the punch line got me every single time. I have so many fun memories with Kiowa in L.A., and he is one of the most interesting people ever. I appreciate his sensitive heart and positive outlook on life.

I also spent a lot of time with Amy Eastwood and I truly miss her sweet personality. She taught me how to screen print T-shirts and has the most adorable hypoallergenic kitties. Erik Rodriguez is extremely focused and a very hardworker. I know he will make a great professor. I became close to Kristin Gleitsman during the last year she was at Caltech. She is truly an inspirational person, and I know she can achieve anything she puts her mind to.

Given that I was the only one in my class to join the Dougherty group, I always felt a close kinship to the group above me – Angela Blum, Kay Limapichat, Sean Kedrowski, and Jai Shanata. Angela and I quickly became friends in my first year and I do not know what I would have done without her. We grew a special bond that is difficult to explain. Though I do not have any siblings, I imagine it would be similar to my relationship with Angela. Kay is a very unique individual and quite brilliant. I am often inspired by her dedication to science and her love for salsa dancing. Sean is a very talented and driven synthetic chemist, and I know he will do great things in life. Jai and I became friends early on. He is a caring and enigmatic character who always challenges you to think more.

Noah Duffy and Darren Nakamura joined the lab a year after me. Noah is a great person and I am glad that we got to know each other. We share the joy for the simpler things in life, like cowboy boots and open spaces. I have a soft spot for Darren as I mentored him when he joined the lab. Darren is hilarious, ridiculous, and very smart. We had lots of funny talks and sang together while the radio was on in lab (or at my karaoke party).

The representatives of the following year are Kristina McCleary, Ethan Van Arnam, Ximena Da Silva, and Maggie Thompson. I spent a lot of time with Kristina during this last year as we collaborated on the mammalian project. She is very bright and a joy to work with. Additionally, we both had gotten engaged around the same time so we always had so much to talk about. Ethan is one smart cookie and an interesting character who always keeps things lively with his impromptu voice impressions. Ximena is a kind-hearted person whom I am lucky to call a friend. She is charismatic, honest, and

very intelligent. I could always count on her to go shopping with or meet me in the pool for a swim. I have a lot of respect for Maggie as she is a very strong and brave individual. She will certainly make a fantastic physician.

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While at Caltech, I have formed some deep friendships, and I am truly grateful for Pam Sontz, Beverly Lu, and Heather Williamson. Pam is my rapping buddy; she always makes me laugh and has an infectious personality. Beverly is my shopping buddy; she has impeccable taste and has been a great friend to me over the years. Heather is my line-dancing buddy and has been my roommate for the last five-and-a-half years. Heather is just the sweetest thing, and I am so blessed to have her in my life.

Katrina McKay has been my best friend since we met at the University of Florida. She has been a constant source of comfort and support through the years and I love her dearly. From Florida to Vegas to California, we have shared many adventures together, and I know we have so much more to come.

Last, but certainly not least, I want to thank my family – my mother, my father, my Aunt Niki and Uncle Steve, and my loving husband. My mom is the best person in the world. She and I are very close and always will be. I thank my mom for always believing in me and teaching me that I can achieve anything. Her support and love has provided a firm sense of stability that has allowed me to stand on my own two feet and accomplish my goals. I love you, mom. My dad is my biggest fan from timing my swim meets in high school to walking me down the aisle. He has always provided me with

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ABSTRACT

This dissertation primarily describes structure-function studies of the nicotinic acetylcholine receptors (nAChRs). These studies use a combination of unnatural amino acid mutagenesis and electrophysiology to determine the specific molecular interactions required for neurotransmitter binding to nAChRs.

Chapter 2 examines the mode of agonist activation for the α4β2 nAChR, the receptor responsible for nicotine addiction. This study investigates the molecular interactions that differentiate the α4β2 receptor from other receptor subtypes and endow it with the ability to mediate nicotine addiction. We report that the high affinity for nicotine at the α4β2 receptor is a result of a strong cation-π interaction and a strengthened backbone hydrogen bond to a conserved tryptophan (TrpB) of this receptor. We also establish that a point mutation just four residues away from TrpB appears to influence the shape of the agonist binding site, such that it can differentiate the agonist binding mode of the α4β2 and muscle-type receptors.

Chapter 3 extends studies of the point mutation near TrpB, termed the "loop B glycine." We examine the muscle-type, α4β2, and α7 subtypes and show that the identity of this residue strongly correlates with agonist potency. Low-potency receptor subtypes have a glycine at the loop B site, while high-potency receptors have a lysine at this site. We establish that mutation of this residue can to convert a low-potency receptor to a high-potency receptor and vice versa.

Chapter 4 investigates the agonist binding mechanism of the α4β4 receptor. We show both ACh and nicotine make a strong cation-π interaction to TrpB, and nicotine makes a strong hydrogen bond to the backbone carbonyl of TrpB. Additionally, chimeric

β subunits are used to examine the influence of the complementary binding component on receptor pharmacology for the α4β2 and α4β4 receptors.

Last, chapter 5 is a methodology-based project focused on optimizing the incorporation of unnatural amino acids into mammalian cells. Using HEK293T cells, we successfully suppressed an amber stop codon using HSAS, an *in vivo* aminoacylated tRNA. Additional studies will pursue the viability of *in vitro* aminoacylated tRNAs for nonsense suppression in mammalian cells.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES