Investigations of Ion Channel Structure and Function

I. Studies of Nicotine Binding to the Acetylcholine Receptor

II. Development of Tools for Studying Learning and Memory with Unnatural Amino Acids

Thesis by

E. James Petersson

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For My Family:
Mom, Dad, and Poopcat (1986 – 2005)

Acknowledgements

"Via ovum cranium difficilis est."

- Adlai E. Stevenson, Choate '18

Adlai Stevenson was right, the way of the egghead is hard, and it would be nearly impossible without a lot of help. First off, I must give props to the Dog; Dennis Dougherty has been the perfect advisor for me. The freedom he has given me to follow my interests is unusual in today's productivity-oriented graduate school environment. I certainly didn't know how unusual it was when I joined the group six years ago, all I knew was that unnatural amino acids and brains were cool. Dennis has given me a long leash ("Enough rope to hang yourself," in his own words), and I have tried to learn as much as I could about as many different areas of Chemistry as I could while I was at Caltech. This may seem like an overly ambitious goal, perhaps it only seems reasonable because Dennis knows so much about so many things himself. Of course, Dennis is also a consummate family man, a D.A.D. all the way through; and I would be remiss if I did not thank Ellen and the girls for making me feel at home whenever the group came over to the house.

Like any graduate student, I owe a lot to students who came before me, people who taught me both practical techniques and about being a graduate student in general. Justin Gallivan took me under his wing when I arrived, making me feel welcome both in the lab and on the softball field. I thank Lintong Li for getting me started with my first project - the tethered agonist - and for brightening our lab with the birth of George, back before Darren and David jumped on the having-children bandwagon. Don Elmore brought cheer and enthusiasm to our lab in all matters, whether it was talking about ion channel gating or establishing the tradition of the group birthday cake and embarrassing story (for my money, the "Dawn" Elmore story is still tops). Don brought this same attitude to his teaching while at Caltech, and I am sure that his students at Wellesley will count themselves lucky for having had him as a Professor. For almost a year, Niki Zacharias and I were the synthetic arm of the Dougherty group. She now works for Dennis and Henry's company, Neurion, where she continues to be the synthetic arm. No doubt, her experience has made possible Neurion's quick start. She has also been a model of humanitarianism to me, not only in her volunteer work, but also in a more personal way: Niki always found a way to make the group feel more like family, often through the use of baklava. David Dahan and his wife Kirsty were always ready to share my love of hockey; I am sure their son Étan is already stopping slapshots down in San Diego. Joshua Maurer may have been a dead-ringer for

Eeyore, but he also brought much needed humor to the Dougherty Country Day School in the form of Pint-Sized Punch Lines. Gabriel Brandt established the importance of taking the long, off-campus lunch, a time when really important issues like 12-stranded DNA healing could be discussed.

Justin, Josh, and Gabe deserve special mention as mentors at least as important as Dennis in my grad. school career. All of these guys were great in many ways, but each has a particular trait at which they are excel, a trait which I have tried to emulate in my capacity. Justin's scientific creativity, and the drive that he applies to accomplish his goals, are inspiring. It's nice to talk to someone who thinks he's going to change science rather than merely publish papers. Gabe's encyclopedic knowledge, covering matters as diverse as catalysis and MRI studies of acupuncture, set the bar very high for the reading habits of the rest of us. It also made it all too easy to not do the reading. It was always faster to just ask Gabe for a citation than to use Web of Science. Josh was impressive for his willingness to help other students with their research, not to mention the broad knowledge that allowed him to do it so effectively. As curmudgeonly as Josh was, I have never seen a student more giving of his time. I am sure that his group at Washington University will be extremely productive, even if Josh has to do it all himself. ("You guys go home, I'll take care of it.")

My classmates, Sarah Monahan May, Darren Beene, and Steve Spronk, have been a great support system. A lot has changed for Sarah since she arrived at Caltech, and I have enjoyed being her friend throughout. I hope to continue my friendship with her and her husband Jeremy on the east coast. Steve is always enthusiastic, and has been a fun labmate (If he were a aura-colored dog based on a cartoon character, he would be a turqoise golden-retriever Bart Simpson who says "Whassssuuuuuuup!"). Darren Beene is a man whose winding path has given him a wonderful combination of childishness and wisdom. He was a great comrade both within and without the lab. It has not been the same without our coffee breaks every afternoon during these last nine months of my time at Caltech. I wish Darren, Wendy, Max, and E. (I like to think he's named after me) only the best.

This brings me to the younger folks. Erik Rodriguez, although only a third year grad. student, has been with our lab since his SURF project as an undergrad. Erik's fastidious nature will keep things running well in the lab when I'm gone, as long as he stops exposing himself to professors. Lori Lee and Amanda Cashin both defected from other labs, and we are certainly the better for it. I am sure Lori will become a successful researcher (if she ever sends out her postdoc applications) and that she will someday discover a cure for Fitzberger's syndrome. Amanda will probably own a bioconsulting firm in three years, so I will need to keep in touch to get my start-up going when I am a professor. Tingwei Mu is the last member of the old guard, who remember the way the labs

used to be. I am sure he will do great things with Jeff Kelly and have a lot of fun making that drive back and forth to Pasadena in his convertible. Amy Eastwood is fleet of foot and light of heart. As keeper of the HPLC and photochemistry knowledge, I wish her the best of luck. If nothing else does, I hope the "dress-better to feel-better" strategy works. Ariele Hanek is pretty quick in her own right. She, Jinti Wong, and Joanne Xiu are tackling the problem of the gating of the nAChR, a problem that makes the binding site studies look simple. If Joanne can connect all the tiles in the Matching Game, then they can definitely tackle nAChR gating. It looks like Jinti is taking over my position in the long-distance relationship business. Kristin Rule will probably get her hands into as many different problems as I did; for someone who joined the group to do computing and biology, she's sure doing a lot of synthesis. Katie "The Rook" McMenimen has been a great collaborator over the last year, allowing me to boss her around and get a taste of being a P.I. If my grad. students are half as smart and hard-working as Katie, I will go far as a professor. She and Kiowa Bower will continue to research drug binding to the NMDA receptor and I'm sure they will make many interesting discoveries, as long as Kiowa doesn't take all the PCP himself. Finally, this brings me to Michael Torrice, who will be the last of the Lake Ave. lunchers when I am gone. The pairing of our senses of humor as next-desk neighbors has been beneficial for everyone. The rest of the lab has probably been as happy not sitting next to Michael as I have been sitting next to him.

Well, that's it for the group, and I had better pick up the pace if I'm ever going to finish these acknowlegements. Henry Lester and the Lester group have always been a great source of biological knowledge, or at least commiseration, to me over the years; particularly those on the unnatural amino acid side of things: John Leite, Fraser Moss, Mohammed Dibas, and Rigo Pantoja. Henry has always treated me with great respect as a young scientist, even when my ideas were kooky and made no sense, but he probably just does this because I return the favor.

The rest of the Chemistry department has also been good to me. The number of people who have been a source of help in science or in blowing off steam over the years are too numerous to give each of them each an anecdote; just know that I am thankful. I of course must thank my committee explicitly (and the reader is free to call me a butt-kisser at this point). It has been great to know Linda Hsieh-Wilson both from my thesis committee and from the interaction of our two labs. Watching from up close as her group grew over the years has been really cool, and what I have seen has taught me a lot about what to do (and occasionally, what not to do) as a young P.I. I am grateful to David Tirrell for his generosity toward me with his time. For a guy that wears more hats than anyone else in the department, Dave always seemed to be the first one to get a letter of recommendation written

for me, and he never turned me away from his office when I came by to talk about one of my synthetase ideas. Thankfully, Bill Goddard has not let his opinion of my father influence his opinion of me. I think I may hold some kind of record in that Bill has stayed awake through virtually every minute of my thesis committee meetings. Feel free to sleep through my thesis defense, Bill.

Outside of the Chemistry department, I have had many friends around campus, from other departments and from various sports activities: ultimate Frisbee, rock climbing, GSC softball and football, and the Caltech hockey team (yes, we have a hockey team). My roommates over the years have been one of the main things that has kept me sane (debateably so) here: Matt, Ron, Ben, Ali, the Johns, Greg, and of course, Ted. T is one of the smartest people I have met here, a true intellectual whose love of science as a whole is vastly underappreciated. Thanks to all of you for giving me something fun to come home to when I left the lab. The Mentor Ave. house probably won't last much longer than the end of this thesis, but it has provided a lot of good times. Hopefully it has one more thesis defense party left in it.

There is one person who fits into all of the above categories: chemistry department member, ultimate player, and erstwhile roommate. Meeting Wendy Jen would have made my time at Caltech worth it even if nothing else had gone right. It's hard to believe that the whole world can revolve around such a small person, but mine does. I would go on, but I'm sure Wendy is embarrassed enough already.

Finally, this brings me to my parents. The apple certainly has not fallen far from the tree. Precisely, it fell three doors down the hall on the third floor of Crellin from J. D. Roberts' office, under whom my father received his Ph.D. from Caltech twenty-five years ago. While the comparisons to my father are more obvious; in a strange way, what I do brings together what both of my parents do. My father is a theoretical chemist, and my mother a psychotherapist, so the work in this thesis comes from both of them. It certainly would not have been possible to get to this point without a lot of support (and some occasional yelling and guilt trips) from both of them. This thesis is for them, I hope it makes them proud.

No thesis introduction would be complete without a sage scientific quote:

StrongBad: "Say something smart."
Homestar: "Science."
StrongBad: "Say something else smart."
Homestar: "Science again."

Abstract

At synapses in the nervous system, ligand-gated ion channels convert the chemical signal of neurotansmitter release into the electrical signal of ion flux. These proteins underlie the proper transmission of information from one nerve cell to another, and disorders of these channels lie at the heart of many addictions and neurological diseases. We examine their function with the diverse palette of structural alterations available through unnatural amino acid mutagenesis.

Section 1: Studies of Nicotine Binding to the Acetylcholine Receptor

In previous studies, we have used fluorinated Trp derivatives to conclusively identify a cation-π interaction with Trp 149 in the binding of acetylcholine (ACh) to the muscle-type nicotinic acetylcholine receptor (nAChR). We have incorporated mimics of ACh, termed tethered agonists, in the binding site to produce self-activating channels. Using tertiary tethered agonists that would only become cations and activate the channel when protonated, we probed the local pK_a of the binding site. They were found to be protonated only at pH's much lower than their pK_a in free soluion, implying a perturbed pK_a for the binding pocket, which has implications for the binding of tertiary agonists like nicotine (Nic).

It has previously been shown that Nic does not participate in a straight-forward cation-π interaction with Trp 149 as ACh does. We have identified a hydrogen bond between the Nic pyrrolidine N-H and the backbone carbonyl of Trp149 by introducing an ester linkage at this point, weakening the carbonyl H-bond accepting ability. Calculations performed on hydrogen bound complexes of ACh, Nic, and the Nic analog epibatidine (Epi) explain the trends observed for ligand activation of the nAChR. ACh binds through a cation-π interaction, Nic binds primarily through a H-bond, and Epi binds through both.

Expanding upon this study, we have performed molecular dynamics (MD) simulations of the recently crystallized ACh binding protein (ACHBP) and of models of the ligand binding domain of the α7 nAChR subtype. We have found that ACHBP, which has been used extensively as a structural model for ligand binding to nAChRs, binds ligands in a dramatically different manner than nAChRs: ACHBP, which does not need to gate, is preorganized to bind ligands in a "lock and key" fashion. The nAChR, which must gate, has a more flexible binding pocket, and bind ligands through an induced fit mechanism. Ligand-bound structures from these simulations have been taken on to quantum mechanical/ molecular mechanical (QMMM) calculations to model the effects of unnatural amino acid mutations in an environment that simulates the full nAChR binding pocket. The MD and QMMM protocol should be generally applicable to our unnatural amino acid mutagenesis studies of the nAChR.

Section 2: Development of Tools for Studying Learning and Memory with Unnatural Amino Acids

The nAChR is essential to neurotransmission at the junction between nerve and muscle cells, and it plays an important role in many central nervous system processes. However, its role in learning and memory is limited, at least in our current molecular models of these events. In a sense, the formation of a memory consists of the strengthening of some synaptic connections and the weakening of others. These processes, termed long term potentiation (LTP) and depression (LTD) respectively, are primarily governed by modifications to glutamate receptors (GluRs). We have developed tools for studying the mechanism and timecourse of these modifications, and we have demonstrated the first incorporation of unnatural amino acids into a GluR.

Two major types of changes are believed to underlie LTP and LTD: alterations to the functional properties of a single glutamate channel and changes in the number of GluRs present at a synapse (trafficking). One common mechanism for initiating both of these changes is the phosphorylation of Ser, Thr, and Tyr hydroxyl groups. Many such residues are present in a GluR and are targets for phosphorylation, making it difficult to understand the effects of phosphorylation at any one residue. We describe the first incorporation of "caged" phosphoamino acids that should permit precise temporal control of the onset of phosphorylation. This cage consists of a photocleavable protecting group applied either to the wild-type amino acid or to the chemically synthesized, phosphorylated amino acid.

While phosphorylation can act as a functional group signal to alter protein function and trafficking, it appears that the initial trigger for both LTP and LTD involves removal of a Mg^{2+} ion from the channel of the NMDA-type GluR. This allows Ca^{2+} flow through the receptor, and the rise Ca^{2+} concentrations leads to changes in the phosphorylation states of GluRs. We have begun dissecting the NMDA receptor Mg^{2+} blockade site by demonstrating that the mechanism of Mg^{2+} binding does not seem to be a cation-π interaction, contrary to expectations based on conventional mutagenesis studies. Our studies of the NMDA receptor are interesting in themselves, and provide us with entrée into the study of GluRs, new to these labs. We hope to incorporate the caged amino acids into GluRs to study the effects of phosphorylation in molecular models of learning and memory.

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