

**PROBING THE ROLES OF RECEPTOR STRUCTURE, DRUG-
RECEPTOR INTERACTIONS, AND RECEPTOR CROSSTALK
IN LIGAND-GATED ION CHANNEL FUNCTION**

Thesis by

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In Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy

California Institute of Technology

Pasadena, California

2013

(Defended October 9th, 2012)

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*To my Angels,
who are always by my side*

Acknowledgements

I would like to thank:

- My research advisor, Professor Dennis Dougherty, for giving me the opportunity to be part of his research group, his guidance on my projects, his support on my career, Monday lunches, and for reviewing this entire dissertation with great patience.
- My collaborator, Professor Henry Lester, for his guidance & critiques on my research, his enthusiasm, his help with my career especially during the last year, and for trusting me to work on his projects.
- My thesis committee: Professor Bob Grubbs, Professor Doug Rees, and Professor Shu-ou Shan, for their advice and support throughout the years.
- My fellow Dougherty group members, past and present, for being so supportive of my progress. I specially thank Ariel Hanek who patiently trained me at the beginning. I thank Angela Blum for her understanding (of me & my broken English), for being my loyal friend (& my translator at times), and for plenty of laughter and wonderful time. I am also grateful for the friendship with Sean (& Meg) Kedrowski, Noah (& Tracy) Duffy, and Darren (& Lib) Nakamura. I thank Ethan Van Arnam and Chris Marotta for colorful scientific discussion and Kristina McCleary Daeffler for her encouraging words toward the end.

- The Lester lab for their support and helpful discussions. I especially thank Dr. Rigo Pantoja, Dr. Chris Richards, and Dr. Julie Miwa, who have been directly involved with my research.
- Professor Sarah Lummis and Mona Alqazzaz at the University of Cambridge for their helpful scientific discussion.
- My collaborators at the Rockefeller University: Professor Rod MacKinnon, Alice Lee, and Dr. Xiao Tao for giving me the opportunity to spend time in their lab and be part of their work. It was an invaluable experience to me.
- The Shan lab: Xin Xhang, Peera Jaru-Ampornpan, Sowmya Chandrasekar, Kuang Shen, and Thang Nguyen, for allowing me to use their equipment, for scientific discussion and friendship. I specially thank Peera, whom I lived with for five years, for lively discussion over dinner on a good day or just dinner on a not-so-good day.
- The Stoltz lab: Jenn Stockdill for help with chemistry many times and for her friendship. I thank Nathan Bennett for helping me with the polarimeter.
- Dr. Scott Ross for his assistance with the peptide NMR on the Phe-Pro project.
- Dr. Mona Shahgholi for her kind help with mass spectrometry.
- Tom Dunn, Linda Syme, and Eloisa Imel, without whom, my life would be so much harder.
- Dian Buchness, Laura Howe, and Agnes Tong.
- Professor Amit Basu, my undergraduate advisor, for his advice, encouragement, and optimism. The three years that I spent in his lab really influenced the way I look at science.

- Professor Matthew Zimmt, my first organic chemistry professor at Brown, for his incomparable enthusiasm and for always believing in me.
- Friends: Caltech Thai Club (Nok, Peera, Knot, Piti, Tara, Ped, etc.), my Santa Barbara friends (Nammon, Mock, etc.), my Dance Family friends, and other salsa friends for all the good times and the good wishes.
- Mr. Alan Talampas, for making the past difficult six months more *bearable* and for reminding me every day that it is still a beautiful world.
- And more than anyone else, my mother and my father, Dr. Kreaovan and Kitikiat Limapichat, for their encouragement and for always being supportive of my dreams.

Abstract

Ligand-gated ion channels are multi-subunit transmembrane proteins that play crucial roles in synaptic transmission in the nervous system. These include the Cys-loop receptor superfamily, the ionotropic glutamate receptor (iGluR) family, and the purinergic P2X receptor family. Binding of specific neurotransmitters at the ligand-binding site triggers a series of conformational changes that ultimately leads to ion channel opening. This dissertation describes three molecular-scale functional studies on these receptors.

The first project (**Chapter 2**) describes structure-function studies of the conserved Phe-Pro motif in the Cys loop of the nicotinic acetylcholine receptor (nAChR) of the Cys-loop superfamily. Both residues were substituted with natural and unnatural amino acids. A strong interaction between the Phe and Pro residues is evident, as is a preference for aromaticity at the Phe site. Hydrophobicity is preferred at both sites. A correlation between receptor function and the *cis* bias at the proline backbone suggests a significant role for the *cis* proline conformer in receptor function.

The second project (**Chapter 3**) concerns the key binding interaction of memantine, a prescribed drug for Alzheimer's disease, on the *N*-methyl-*D*-aspartate (NMDA) receptor of the iGluR family. The data suggest that the

special property of memantine as an NMDA receptor blocker stems from the presence of the two methyl groups and a proper shape-matching to the binding site. Comparing affinities of memantine and amantadine, a structurally related drug, in response to pore mutations allows an identification of the methyl group binding pockets on the NMDA channel pore.

The final project (**Chapter 4**) involves a study of inhibitory crosstalk between two families of ion channels: $\alpha 6\beta 4$ -containing nAChRs and P2X receptors. When these two distinct receptors are co-expressed, their properties are modulated from their normal behavior when expressed alone. The effect is constitutive and does not require channel activation. When they are co-activated by their respective agonists, the observed current is smaller than the sum of the currents evoked by individual application of their agonists. This functional interaction between these nicotinic and purinergic receptors in dorsal root ganglion neurons is proposed to be involved in pain sensation.

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