# Chemical-Scale Studies of G Protein-Coupled Receptors and Ligand-Gated Ion Channels

Thesis by

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*Dedicated to my parents:*

*Jean and Peter Van Arnam*

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## Abstract

This dissertation describes studies of G protein-coupled receptors (GPCRs) and ligand-gated ion channels (LGICs) using unnatural amino acid mutagenesis to gain high precision insights into the function of these important membrane proteins.

Chapter 2 considers the functional role of highly conserved proline residues within the transmembrane helices of the D2 dopamine GPCR. Through mutagenesis employing unnatural α-hydroxy acids, proline analogs, and N-methyl amino acids, we find that lack of backbone hydrogen bond donor ability is important to proline function. At one proline site we additionally find that a substituent on the proline backbone N is important to receptor function.

In Chapter 3, side chain conformation is probed by mutagenesis of GPCRs and the muscle-type nAChR. Specific side chain rearrangements of highly conserved residues have been proposed to accompany activation of these receptors. These rearrangements were probed using conformationally-biased β-substituted analogs of Trp and Phe and unnatural stereoisomers of Thr and Ile. We also modeled the conformational bias of the unnatural Trp and Phe analogs employed.

Chapters 4 and 5 examine details of ligand binding to nAChRs. Chapter 4 describes a study investigating the importance of hydrogen bonds between ligands and the complementary face of muscle-type and α4β4 nAChRs. A hydrogen bond involving the agonist appears to be important for ligand binding in the muscle-type receptor but not the α4β4 receptor. Chapter 5 describes a study characterizing the binding of varenicline, an actively prescribed smoking cessation therapeutic, to the α7 nAChR. Additionally,

binding interactions to the complementary face of the α7 binding site were examined for a small panel of agonists. We identified side chains important for binding large agonists such as varenicline, but dispensable for binding the small agonist ACh.

Chapter 6 describes efforts to image nAChRs site-specifically modified with a fluorophore by unnatural amino acid mutagenesis. While progress was hampered by high levels of fluorescent background, improvements to sample preparation and alternative strategies for fluorophore incorporation are described.

Chapter 7 describes efforts toward a fluorescence assay for G protein association with a GPCR, with the ultimate goal of probing key protein-protein interactions along the G protein/receptor interface. A wide range of fluorescent protein fusions were generated, expressed in *Xenopus* oocytes, and evaluated for their ability to associate with each other.

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