

**Chemical-Scale Studies of the Nicotinic Acetylcholine Receptor:  
Insights from Amide-to-Ester Backbone Mutagenesis**

Thesis by

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## Abstract

This thesis describes the use of peptide backbone amide-to-ester mutations to study the structure and function of ligand-gated ion channels. The research described herein has been done on the muscle nicotinic acetylcholine receptor, a prototypical ligand-gated ion channel in the cys-loop superfamily. Backbone mutagenesis in these proteins provides insight into specific intermolecular interactions that are critical to function, as well as answering more fundamental questions about the role of the peptide backbone in long-range conformational changes in these allosteric receptors.

Chapter 2 describes the identification of a key hydrogen bond near the binding site that is involved in the gating pathway. We found that the backbone N-H of a loop C residue makes a hydrogen bond to an anionic side chain of the complementary subunit upon agonist binding. The hydrogen bonding partner is not the residue predicted by structural data, but instead an aspartate that was originally believed to participate directly in agonist binding.

In chapter 3 we consider the involvement of the peptide backbone in the binding-induced conformational changes that lead to channel gating. Single backbone mutations in the  $\beta$ -sheet-rich extracellular domain were well tolerated, whereas two proximal backbone mutations led to nonfunctional receptors. These results support a model in which backbone movements in the outer  $\beta$ -sheet are important for receptor function.

Chapter 4 describes a new method - elucidating long-range functional coupling in allosteric receptors (ELFCAR) - that should be broadly applicable to determining functional roles of residues in allosteric receptors.

Chapters 5 and 6 describe electrophysiological and computational investigations into the role of amide-to-ester mutations in the aromatic binding box of the nicotinic receptor.

Echoing the results of chapter 3, these mutations largely reveal an overall tolerance of backbone mutations in the binding site.

Finally, in chapter 7, we explore the use of ester and N-methyl backbone modifications to uncover the role of conformational changes at an unusual vicinal disulfide bond near the tip of the C-loop. Using ab initio calculations, we demonstrate that N-methylation and esterification of this ring structure in model peptides dramatically impacts its *cis-trans* conformational preferences.

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