

**Chemical scale investigations of
ligand-gated ion channels using
unnatural amino acids**

Thesis by

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for V.A.B. and E.S.B.

. . . everyday.

Acknowledgments

*so much depends
upon*

*a red wheel
barrow*

*glazed with rain
water*

*beside the white
chickens.*

W.C. Williams

Well somehow, despite my reluctance to venture beyond the academic confines, I will soon no longer be a student. The salad days are over. Having spent nearly thirty years of my life in school, there are more than a few to thank. First I must give credit to public education. From elementary school to university, I have received quality instruction at these state institutions. I also must give thanks to the Caltech community for creating a research institute truly different from the rest. Much thanks and appreciation goes to my advisor, Dennis Dougherty. In addition to creating a research environment where it was always a pleasure to work, his scientific reasoning and interpretive insight never failed to amaze me. An added bonus of joining the Dougherty group has been the opportunity to work with Henry Lester, who is a dedicated mentor and a firm believer in scientific discussion. I have also had the pleasure of a very fruitful collaboration with the Sarah Lummis group at the University of Cambridge. I am very thankful for the time and effort they have contributed to our joint projects. I would also like to thank my committee, Peter Dervan, Richard Roberts, and Robert Grubbs for their time and commitment to my education.

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Abstract

The Cys loop receptors, a family of ligand-gated ion channels, mediate fast synaptic transmission throughout the peripheral and central nervous systems. These are large multisubunit proteins, whose primary function is to transduce a chemical signal, binding of a neurotransmitter, into an electrical signal, ion flux across the cell membrane. These receptors have been implicated in several disease states and represent major therapeutic targets. The work presented in this thesis focuses on the chemical-scale elucidation of Cys loop receptors. The main approach of this work is the structure-function study using *in vivo* nonsense suppression methods. This technique allows for the site-specific incorporation of an unnatural amino acid into a protein expressed in a living cell.

Nonsense suppression methods were used to incorporate a series of fluorinated tryptophan derivatives into the binding site of the 5-HT₃R. This study identified a cation- π interaction between Trp 183 and the neurotransmitter, serotonin. A similar study using fluorinated phenylalanine derivatives identified a cation- π binding site at Tyr 198 in the GABA_C receptor. These studies build on previous work from our research group and provide further evidence that the cation- π interaction is a common feature in ligand recognition by Cys loop receptors.

Nonsense suppression was also used to examine the role of several tyrosine residues in the 5-HT₃R. Here the findings demonstrated that the side chains of Tyr 143 and 153 make functionally important hydrogen bonds. These data were used to refine several computational models of serotonin docked into the binding site.

Structure-function studies of two conserved prolines in the M2-M3 loop showed that this region of the receptor is involved in the conformational changes associated with

receptor activation. The data also provide preliminary evidence that Pro 308 may serve as hinge for the gating movement of the M2 helix.

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