# The Design, Synthesis, and Evolution of Macrocyclic mRNA Display Libraries Containing Unnatural Amino Acids

Thesis by

Steven Wesley Millward

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2007

(Defended April 24th, 2007)

# Acknowledgements

One of the most important lessons that I'll take with me from graduate school is the indispensability of collaboration. While Einstein and Newton worked in relatively secluded environments (a German patent office and rural England during the plague, respectively), the vast majority of working scientists depend on human contact and support to survive the grueling, doubt-ridden slog that lies behind every successful project. Without the following people, the past six years would have been impossible.

Above all, I want to thank my parents, Carol and Wesley Millward. Both worked incredibly hard to make sure that I took advantage of every educational opportunity that was available. Both valued hard work and never accepted anything less from me, and for that I will always be thankful. I also thank them for perspective; something that is readily lost when experiments aren't going well. I miss them terribly and this work is dedicated to their memory.

I would also like to thank my lovely wife Niki for her patience and support during the past four years. In addition to being a brilliant chemist, she is a kind and generous soul who always believed in me, despite evidence to the contrary. She also has a knack for knowing when to prod buttock, an essential characteristic for moving spouses through the final stages of graduate school.

My advisor, Rich Roberts, has been a tremendous scientific mentor as well as a terrific boss. Rich allowed me the freedom to pursue my own ideas and projects, only occasionally stepping in to make course corrections. In the end, this made me think harder about my work and design more creative experiments. His breadth of knowledge

encouraged me to think globally about science and to draw upon other fields for inspiration.

I would also like to thank my thesis committee, Peter Dervan, Dennis Dougherty, and Ellen Rothenberg. They have given me excellent advice and guidance over the past four years. I would especially like to thank Dennis Dougherty for the use of his lab's equipment and for (indirectly) introducing me to my wife.

I have been privileged to work with members of the Roberts lab, both past and present. Shuwei Li provided guidance for my first foray into synthetic chemistry and mRNA display. Shelly Starck was a wellspring of information on experimental procedures. Bill Ja developed most of the assays and techniques employed in the G protein selection and was an invaluable repository for advice on all aspects of mRNA display. Adam Frankel was a tremendous amount of fun in addition to being a great scientist. Anders Olson has been a friend and colleague for the past six years and I will always be grateful that he chose Caltech after an illustrious undergraduate career at NYU. Anders has an encyclopedic knowledge of molecular biology and was always available to troubleshoot a difficult experiment. His expertise in biology is matched only by his exceptional taste in music and I will always remember (hazily) our trips to the Coachella music festival. Ryan Austin provided indispensable advice and assistance on the G protein selection work; without him, this project would have never left the ground. Ryan is a true renaissance man and I have hugely enjoyed our conversations on politics, art, history, philosophy, movies, and literature. Terry Takahashi has been a great collaborator, teacher, and friend for the past six years. Despite his busy schedule, Terry

has always been generous with his time and advice, no matter how big or how small the problem.

I would also like to thank Mike Rubel for his inexhaustible supply of helpful information about the wonderful world of LINUX and Chris Dione for the late-night discussions on science fiction, movies, and *Civilization*. Alison Ross deserves a big thanks for dealing with the logistics of graduate school and Margot Hoyt for her exemplary service as secretary and cat-sitter.

# Abstract

Combinatorial design is a powerful method for generating ligands that disrupt protein-protein interactions, particularly in the absence of structural information. mRNA display is powerful *in vitro* biological display technique that enables the synthesis and evolution of trillion-member peptide libraries. Previously, mRNA display libraries were limited to linear peptides composed entirely from natural (protegenic) amino acids. This work describes the redesign of mRNA display libraries to include chemical functionalities that hitherto have remained the province of traditional chemical libraries and natural products. The incorporation of unnatural (nonproteogenic) amino acids through nonsense suppression and selection for the unnatural chemical functionality demonstrated that suppression technologies were compatible with biological display. The incorporation of N-methylated amino acids by sequential sense suppression was shown to generate unnatural oligomers with improved proteolytic resistance. Post-translational macrocyclization was shown to be an effective and general strategy to constrain displayed peptides without the use of redox-labile disulfide bonds. Co-translational unnatural amino acid incorporation and post-translational macrocyclization were combined into a new technological platform and employed in the selection of a high-affinity cyclic ligand for Gαi1. This new technology represents a significant step toward the evolution of natural product-like compounds from trillion-member combinatorial libraries.

# Table of Contents



# Chapters

# List of Figures

**Chapter 4:**



**Chapter 5:**

# List of Tables