# In Vitro Selection of mRNA-Display Libraries Containing Unnatural Amino Acids

Thesis by

Shuwei Li

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2003

(Submitted March 18, 2003)

# Acknowledgments

Finally, I reach the end of a long journey in Caltech. During these six years, I have gone through both frustration and happiness. But it is no doubt that my life at Caltech is the most important period I have experienced so far. I really enjoy the atmosphere provided by Caltech's small size but strong science. This is the Caltech, and I am proud to be a member of this unique society.

I owe my thanks to many people who help me here. First, I would like to thank my research advisor, Professor Richard W. Roberts. His broad knowledge has always stimulated me to explore the science beyond my imagination. His style to run the lab has trained me to become independent. More importantly, his invaluable advice in my research has led to many interesting results. This is a wonderful experience for me to work for him.

I thank Ms. Jie Xu, a previous technician in our lab and my collaborator. Her excellent skill in organic synthesis has been a big help to me. I also thank Dr. Lintong Li who gave me a lot of suggestion as well as many materials for the synthesis of unnatural amino acid charged tRNA. Stephen Millward, a current graduate student in our lab, also helped me in preparing semi-synthetic tRNA and deserves my thanks.

I appreciate members of the Roberts group for their assistance in my research and their patience in proofreading many reports I wrote, especially William Ja, Shelley Starck,

and Terry Takahashi. I also enjoy working in a friendly environment provided by other members of the lab, Ryan Austin, Qi Xin, Christopher Balmaseda, Tianbin Xia, Adam Frankel, Christien Ueda, and Anders Olson.

I would like to thank the members of my thesis committee, Professor Peter Dervan, Professor John Bercaw, and Professor Steve Mayo, for their time and guidance. I should especially thank Professor Sunney Chan. If he had not admitted me six years ago, I would not have had this excellent training at Caltech and my life would have been in a totally different direction.

At last, I like to say thanks to my parents for their understanding and encouragement.

# Abstract

The mRNA display library provides a strategy to make up to $10^{13}$ unique peptides that fuse with their own genes via a covalent link. In this thesis, several strategies to prepare mRNA display libraries containing unnatural side chains are described. Chapter 1 is a short introduction and application of mRNA-display technology. Chapter 2 describes a chemical derivatization strategy to append an unnatural side chain to libraries. Chapter 3 introduces the incorporation of unnatural amino acids via non-sense codon suppression. Chapter 4 focuses on the catalytic selection with mRNA display libraries. These libraries should facilitate the discovery of novel ligands with functionalities beyond those provided by the 20 naturally occurring amino acids.

# Table of Contents