N-TERMINAL MODIFICATION AND CODON REASSIGNMENT WITH NON-CANONICAL AMINO ACIDS IN PROTEINS

Thesis by

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ABSTRACT

Proteins are ubiquitous macromolecules that effect and control all the processes of life from reproduction to respiration to physical motion. These diverse molecules also provide physical structure and defensive mechanisms. The twenty canonical amino acids can be found in virtually every protein; however, in some organisms, the set of endogenous amino acids also contains residues outside the "canon," such as pyrrolysine, selenocysteine, and formylmethionine. Although a range of chemistries is available through natural side-chain diversity, some functionalities such as halogens, ketones, azides, alkenes, and alkynes are not found in nature. The introduction of a broader range of chemical functionality into proteins and protein-based materials through the use of non-canonical amino acids represents a challenging goal for protein engineering. The persistence of all the amino acids throughout protein sequences also presents a challenge for biochemical modification at a particular location. The insertion of a non-natural amino acid at a single location on a protein can allow specific modification without further affecting the natural protein sequence. The focus of this thesis is on a new method for the post-translational site-specific introduction of non-canonical amino acids to the N-terminus of proteins *in vitro* and progress made towards developing a complementary *in vivo* method using the *E. coli* L,F-transferase. Addition of non-proteinogenic functionality to the coat proteins of M13 bacteriophage using non-canonical amino acids is also explored.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Page

Chapter 1. Introduction

Chapter 2.

Appendix II. Plasmid Maps