

AZIDE-BEARING AMINO ACIDS IN PROTEIN ENGINEERING AND  
PROTEOMIC PROFILING

Thesis by

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**Abstract**

The utility of non-canonical amino acids in protein engineering has grown substantially over the past decade. Proteins containing these unnatural building blocks often have radically different biochemical or spectral characteristics than their wild-type counterparts. Furthermore, proteins may be endowed with chemical reactivity not found in the natural proteome upon the introduction of non-canonical amino acids. Successful incorporation of a non-canonical amino acid into recombinant proteins in *E. coli* is often dependent on engineering of the aminoacyl-tRNA synthetase (aaRS) activity of the cell. The bulk of the work described herein has focused on developing a system to rapidly screen libraries of mutant aaRS to identify clones capable of efficiently incorporating novel reactive non-canonical amino acids. The system is based on the display of reactive amino acid side chains on the surface of *E. coli* cells upon metabolic incorporation of the amino acid into recombinant outer membrane protein C (OmpC) and the subsequent covalent biotinylation of the reactive side chains. The cells are then stained with fluorescent avidin, thus rendering the cells incorporating the amino acid fluorescent and readily identifiable and sortable by flow cytometry.

The feasibility of such a system was proven by incorporating the methionine surrogate azidohomoalanine (AHA) into OmpC and subsequently biotinylating the reactive azide groups via copper-catalyzed azide-alkyne ligation. Using an improved copper catalyst, low levels of incorporation of translationally inefficient amino acids azidoalanine, azidonorvaline, and azidonorleucine into OmpC were also detected. A saturation mutagenesis library of the methionyl-tRNA synthetase (MetRS) was designed, and cells transformed with this library were screened for the ability to incorporate the

long chain amino acid azidonorleucine into recombinant proteins efficiently. Several MetRS mutants were identified with such activity using the cell surface display system. MetRS containing a single amino acid mutation, leucine 13 to glycine (L13G) that occurs in each of the three mutants discovered in the screen, is very efficient at incorporating azidonorleucine into proteins.

In the last part of the work described in this thesis, azidohomoalanine was used to tag newly-synthesized proteins in mammalian cells, thus endowing the newly-synthesized proteins with unique bioorthogonal chemical reactivity. Following covalent biotinylation via the azide-alkyne ligation, these proteins could be selectively enriched for by avidin chromatography and identified using shotgun proteomic approaches. Nearly 200 newly-synthesized proteins were identified unequivocally in just a two-hour window. This technique promises to develop into a highly useful tool for the examination of proteome dynamics.

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