# Proteins of Novel Composition:

# Synthesis, Evolution, Dynamics

Thesis by

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**To My Family**

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# ABSTRACT

Recent efforts from several laboratories have expanded the repertoire of noncanonical amino acids that can be incorporated into recombinant proteins, either by amino acid replacement or by nonsense or frameshift suppression. The recruitment of new amino acid constituents endows the protein engineering field with powerful tools, and raises the prospects for creating novel proteins. In particular, global replacement of one of the canonical amino acids by a non-canonical analog would be expected to cause marked changes in protein structure, dynamics, and function.

Regardless of many possible advantages such as enhanced stability or reactivity, it seems likely that global replacement will in many cases compromise protein folding and function, since both protein structure and protein folding pathways have evolved in a context defined by the canonical amino acid side chains. In order to address the problem, we examined the laboratory re-evolution of the green fluorescent protein (GFP) following global replacement of its Leu residues by 5,5,5-trifluoroleucine (Tfl). The reduced fluorescence of bacterial cells expressing fluorinated GFP was recovered through eleven rounds of random mutagenesis of the GFP gene and screening via fluorescence-activated

cell sorting. The mutant protein in fluorinated form showed improved photophysical properties, resistance to acid denaturation, and folding efficiency both *in vivo* and *in vitro*.

In order to expand our understanding the roles of fluorinated constituents in protein environments, we studied the hydration dynamics at fluorinated protein surfaces by installing Tfl at surface-exposed positions of several coiled-coil proteins and monitoring the fluorescence Stokes shift of Trp with femtosecond resolution. Installing a Tfl residue near the Trp probe retarded the hydration dynamics. These results show that the strong but static dipole moment of the C-F bond interacts with water molecules in a quite different way from the C-H bond, and is instead more similar to polar (or charged) molecules.

The ability of engineering aminoacyl-tRNA synthetases is critical in successful incorporation of analogues into proteins. We have developed a high-throughput method of screening methionyl-tRNA synthetase (MetRS) libraries for global incorporation of noncanonical amino acids. A variant of GFP was engineered to permit incorporation of analogues into its Met sites with minimal loss of fluorescence. Using this variant as a translational reporter, we screened a library of *E coli* MetRS variants for activity toward

6,6,6-trifluoronorleucine, and identified a MetRS mutant that enabled high-yield expression of recombinant proteins with quantitative replacement of Met with Tfn.

The work explored in this thesis addresses three aspects of protein engineering using noncanonical amino acids: evolution of proteins with novel compositions, effects of unnatural chemical constituents on the protein hydration dynamics, and engineering aaRS for incorporation of new analogues. Even though the results shown here are restricted to fluorinated amino acids, we believe that these approaches can be applied to any noncanonical amino acid. These methodologies and the expanded understanding of noncanonical amino acids in protein environments will accelerate the creation of novel proteins with many copies of abiological constituents.

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