SELECTIVE FUNCTIONALIZATION OF THE PROTEIN N-TERMINUS WITH N-MYRISTOYL TRANSFERASE IN BACTERIA

Thesis by

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Every Ph.D. thesis is the product of the efforts of many individuals, and my thesis is no exception. Upon reaching this point in my education and my career, I feel very fortunate to have benefited from the knowledge, inspiration, support, guidance, and good will of many people at different stages of my life. I found that the most straightforward manner in which to organize my thoughts, and this list, was chronologically.

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ABSTRACT

Proteins are involved in myriad processes in all organisms. They provide structural support in the membrane and scaffolding of each cell; they aid in the transmission of biochemical signals within and between cells; and they play central roles in combating various disease states. The development of techniques enabling selective and site-specific functionalization of proteins is an active area of investigation, as such modifications are critical to many studies and uses of proteins. For instance, with the addition of a unique reactive handle, a protein may be conjugated to a polymer for the production of protein-based therapeutics exhibiting improved bioavailability. Alternatively, proteins may be attached to slides to prepare diagnostic microarrays, reacted with hydrogels to create functional biomaterials, or decorated with fluorophores for *in vivo* imaging. Site-specific protein tagging techniques have already contributed greatly to biomedical research and will continue to advance the state of the field.

The focus of my thesis research has been the development of a novel site-specific protein labeling method centered on the eukaryotic enzyme N-myristoyl transferase (NMT). In a process called myristoylation, NMT appends a fatty acid to the N-terminus of numerous substrate proteins. Previous work demonstrated that NMT tolerates a wide range of chemically functionalized analogs of its natural fatty acid substrate. Here, we describe efforts that exploit various features of NMT: its ability to bind and utilize reactive fatty acid analogs, its exquisite selectivity toward its protein substrates, and its orthogonality toward those proteins naturally present in bacteria.

First, in Chapter II, we discuss the development of a model system for NMT-mediated protein labeling in the bacterium *Escherichia coli*. We synthesized an azide fatty acid analog that can participate in bioorthogonal chemistries, and we prepared two GFP-based substrate proteins, each displaying a recognition sequence derived from a known substrate of NMT. Our experiments indicate that labeling by NMT is site-specific, quantitative, and highly selective for each engineered substrate within the bacterial milieu.

As summarized in Chapter III, the model system was extended to the N-terminal labeling of two neuronal proteins, calcineurin (CaN) and calmodulin (CaM). While CaN is naturally myristoylated, CaM was engineered to achieve labeling by NMT. Experiments with CaN and CaM confirmed that our NMT-based system is quantitative and selective in its labeling of both natural and engineered substrate proteins. Extensive characterization of each protein allowed us to identify constructs that retain wild-type levels of activity even after labeling with the azide fatty acid.

Three of the protein constructs reported in Chapters II and III were utilized for microarray studies, as described in Chapter IV. We achieved rapid surface immobilization of each azide-labeled protein directly from lysate, a significant advantage when considering the time and resources normally required to purify proteins for downstream applications. The experiments and methods summarized in this chapter will be adapted for high-throughput biochemical research with protein microarrays.

Finally, the orthogonality of NMT toward bacterial systems was probed further for the purpose of selective labeling of individual bacterial proteins for live-cell imaging. In addition to identifying an azide fatty acid suitable for such studies, we also selected

two bacterial proteins that exhibit interesting functions and localization patterns, and we developed corresponding protein constructs for NMT-mediated labeling. Progress toward the use of NMT for *in vivo* imaging applications in bacteria is described in Chapter V.

Ultimately, our objective throughout the design and execution of these projects was to create and validate a new technique to achieve site-specific protein labeling. The particular advantages of NMT include its tolerance of reactive fatty acid analogs and engineered substrate proteins, and its lack of interaction with proteins present in the widely used *E. coli* expression host. We believe that the ideas and results presented in this thesis establish NMT-mediated protein labeling as a valuable tool for addition to the existing set of site-specific protein labeling methods. Development of such methods represents an important and exciting area within the field of modern chemical biology.

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