IMAGING THE PROTEOME:

METABOLIC TAGGING OF NEWLY SYNTHESIZED PROTEINS

WITH REACTIVE METHIONINE ANALOGUES

Thesis by

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Dedicated to

Jeff Miller and Marissa Mock

for their generous and constant friendship

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ABSTRACT

Many strategies have been described for identifying proteins isolated from tissues, cells, or organelles, but the cellular proteome undergoes complex dynamic changes in response to disease or environment. A more complete analysis of the proteome requires complementary, time-resolved images of cellular proteins. In one method for obtaining dynamic proteomic data, cellular proteins are selectively tagged with small, reactive amino acid analogues. Co-translational incorporation of reactive methionine (Met) analogues [e.g., azidohomoalanine (Aha) or homopropargylglycine (Hpg)] is reminiscent of conventional pulse-labeling with radioactive amino acids; the endogenous cellular machinery places reactive Met analogues at sites normally occupied by Met within proteins. Susceptibility to tagging is determined not by the identity of the protein, but rather by the extent to which the protein is translated during exposure to the analogues. The analogue is then labeled using a copper-catalyzed or strain-promoted azide-alkyne ligation; both ligations enable selective, minimally invasive protein labeling in complex biological mixtures. In my dissertation research, I have developed new methods to tag and dye-label proteins in order to visualize one or more subsets of the proteome. In this thesis, I will first describe the tagging of newly synthesized proteins with reactive analogues in bacterial (Chapter 2) and mammalian cells (Chapter 3). In recent work, I have further expanded the method to enable two-fluorophore labeling of two distinct protein populations (Chapter 4). Two-dye labeling of proteins should enable changes in the proteome to be tracked over time. Finally, I will describe how the dye-labeling method has been adapted for live cell compatibility through the use of cyclooctyne-

conjugated fluorophores (Chapters 5 and 6). Labeling in live cells will enable dynamic processes to be monitored in real time. Proteomic visualization is complementary to proteomic identification, and it should be useful for examining many different aspects of biological systems, including translational responses to disease or environment.

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