Chapter 7

CONCLUDING REMARKS

A global analysis of cellular proteomes requires methods for tagging proteins based on their spatial, temporal, and chemical identity. Chemical biologists are having a substantial impact on the field of proteomics through the introduction of an expanding set of diverse metabolic analogues that enable selective protein tagging inside cells. My contribution to this research area has been to develop new methods for imaging the proteome. As described in this thesis, I have exploited reactive methionine analogues to tag newly synthesized proteins. Proteins displaying reactive amino acids have then been dye-labeled using a sensitive and selective azide-alkyne ligation [1]. First, I described our method for metabolic tagging of newly synthesized proteins in bacterial and mammalian cells (Chapters 2 and 3) [2, 3]. Then, the method was expanded to allow two-dye labeling of two distinct protein populations (Chapter 4). In Chapters 2 to 4, each labeling method utilized the sensitive and selective copper-catalyzed azide-alkyne ligation [4, 5]. Chapters 5 and 6 describe our use of reactive fluorophore-cyclooctynes as biocompatible ligation partners for imaging azide-tagged proteins in living cells.

The methods described here were designed to be accessible to both the chemistry and biology communities. It is my goal that selective dye-labeling of metabolically tagged proteins will be used to answer questions related to the spatial or temporal control of protein synthesis, including cancer progression, viral and bacterial infection, synaptic plasticity, and development [6]. I would also like to see our methods applied to tag

proteins based on post-translational modifications, ideally in living cells. Future work may involve the development of new reactive metabolite analogues to help expand our ability to tag different biomolecules. Finally, I would like to develop new imaging agents that would enable this method to be used in animal models.

References

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