Unnatural Amino Acids as a Means of Developing Novel Labeling Probes

Section 1: What do you propose to do / what question(s) do you hope to answer?

According to the American Cancer Society, over 1.5 million Americans will be diagnosed with cancer in 2012 (American, 2012). A serious issue facing researchers wishing to fight cancer is discovering new ways to accurately diagnose the presence of cancer within a patient (Mukherjee, 2010). One technique that has been the focus of recent research efforts is developing fluorescent probes that can image cancer cells. **This project will explore the feasibility of developing novel fluorescent probes utilizing unnatural amino acids (UAAs).**

UAAs can be synthesized with unique biochemical properties that can cause minimal conformational changes to a protein, while introducing novel chemical functionality not available in the 20 natural amino acids that normally compose proteins. In addition, methods have been developed to site-specifically incorporate these UAAs into proteins by using a TAG stop codon in the genome, giving researchers very precise control over the placement and number of these UAAs in a protein's amino acid sequence. These unique capabilities allow researchers to integrate UAAs into specific positions on antibodies without causing conformational changes to the molecule that could affect its functionality (Liu & Schultz, 2010; Young & Schultz, 2010). Consequently, UAAs offer chemically unique handles for conjugation that are superior to endogenous amino acids.

In order for a fluorescent probe to prove beneficial, it must contain certain key traits. The first constraint is size, as the probe must be small enough to get into the cell, yet large enough to provide sufficient specificity and a prolonged signal. Furthermore, the

signal must occur selectively where the probe is meant to bind, with minimal interfering background fluorescence that makes it harder to identify actual binding sites (Urano, 2009). Most importantly, the probe must bind exclusively to the substrate of interest. Furthermore, current methods of labeling typically involve extensive genetic manipulation on the part of the researcher.

The unique functional handles that UAAs can create within proteins offer a means to tackle these complexities, as well as reducing the complexity associated with labeling. This strategy is dependent upon click chemistry, a reaction that proceed to completion readily and in relatively mild conditions (Kolb, 2001). Research has demonstrated that profluorphores, or non-fluoroscent moluecules, can be synthesized that after having undergone a click reaction, transform into fluorescent moluecules. Of particular usefulness is click chemistry involving azides and alkynes that readily undergo a Huisgen 1,3-dipolar cycloaddition (David, 2007). The relative scarcity of azides and alkynes in organic systems makes these two functional groups optimal for use in developing a UAA-click chemistry strategy of fluorescent labeling as the chance of cross-reactions are relatively minimal.

My research will address two fundamental questions about the proposed strategy for developing novel fluorescent probes. First, can UAAs be click with profluorophores to produce fluorescent probes? Second, can these reactions be performed in a model organism (*E. coli* in this case) to demonstrate that this is a viable alternative to current methods of genetic manipulation with protein fusions to produce fluorescent tags in proteins of interest? By the end of this project, I hope to have explored and analyzed the potential of UAAs as a method of developing site-specific fluorescent probes.

Section 2: Explain why you want to do this research. What are your goals in undertaking the project, and why is the project you are proposing the best way of achieving these goals? How will this research help further your academic / intellectual development? Why do you find the work exciting?

This project will help me develop a better understanding of biochemical processes that occur within nature. This understanding will translate into the biology and chemistry courses that I plan on taking as a Junior, especially Biochemistry. Additionally, this project will give me a greater understanding of how actual scientific research is conducted by immersing myself in the process. These new laboratory skills coupled with the a better appreciation of biochemical processes will then aid me in the future in a scientific career, as well as preparing the foundation for an honors project.

Section 3: Explain the relevance of this work in the greater scheme of things / to people besides you and your advisor. Do not use jargon.

Current bioimaging methods rely on the use of highly fluorescent probes, like GFP, to identify cellular components. However, the most common of these techniques require the use of extensive genetic manipulation. Furthermore, a lot of these methods can produce confounding results, as background fluorescence in the probes reduces the ability of researchers to identify their desired target. Finally, current imaging methods fail to label certain cellular components, like RNA. While this project does not attempt to address these other cellular components, research into this technique could provide novel insight into ways to label these components.

Section 4: What coursework or other experience have you had that has helped prepare you to conduct the research you are proposing?

As part of this project, I will be required to synthesize UAAs, which will utilize knowledge that I gain in General Chemistry, Organic Chemistry I and II, and Inorganic Chemistry. Properties and concepts like acidity/basicity, bonding, ionization, and electrophilicity are all associated with biological molecules. These principles are essential to the functioning of biological systems, which I began to explore in my Intro to Molecules, Cells and Developmental Biology course and will continue next semester in Molecular and Cellular Biology course. Of particular importance will be the knowledge of protein structure and folding, as well as the mechanisms behind protein translation, which I began to study in this course. Aditionally, this past summer I worked on my Freshman Monroe project where I synthesized a library of UAAs. This provided me with valuable skills in synthesizing UAAs and their incorporation into proteins that will be employed in the work I do on this project.

Section 5: Discuss your methodology. What is your research plan? Where do you propose to conduct the research and why is it necessary to be there? Describe the timeline, making sure that the project lasts a minimum of seven full-time weeks.

This project will ultimately take 10 weeks to complete. The first two weeks will be spent synthesizing and purifying a library of profluorophores. These will consist of xynathone-like derivatives. The following two weeks will be spent synthesizing and purifying a library of UAAs with azide or alkyne functionality. These will then be screened against the profluorophores using copper-catalyzed click reactions. Levels of fluorescence will be determined utilizing a fluorometer to gauge which combinations produce the greatest fluorescence. Furthermore, differences in emission wavelengths will be determined in order to see if any combinations produce visibly different coloring that

could aid in differentiating multiple components within the cell. The final four weeks will be spent trying to incorporate the selected UAAs into an *E. coli* model utilizing a model hemoglobin protein. I will start by utilizing evolved tRNA-synthetases already available in the Young Lab. However, if none of these are able to insert my UAA then I will be required to evolve my own tRNA-synthetase. Once my UAA is successfully incorporated into a protein, I will then perform an intracellular, copper-mediated, azide-alkyne click reaction, purify the labeled protein, and measure fluorescence against non-labeled protein to determine binding affinity.

Section 6: Describe your final product. Consult with your Monroe project advisor on the format that is most appropriate to your project and discipline. While the final product may be an academic paper (written in accordance with the standards, expectations, and format of the discipline), it might also be something else such as a creative work (novel, painting, etc.).

The final project will take the form of a poster or talk. Additionally, any significant data will be prepared for potential publication.

Section 7: Will this project lead to further work, such as continued lab work, an independent study during the academic year, a portfolio, or, eventually, an Honors project? Describe.

The work accomplished through this project will form the basis of my future work in the Young Lab going into my junior year. Furthermore, the project will be used as a starting point when developing my honors project, which will also be conducted in the Young lab. Any significant data will prepared for presentation and publication.

Bibliography

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