Analysis of an Amino Acid Labeled Fluorescent Dye

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Background

Laxman et al. have shown that linking fluorescent dyes to amino acids is a useful method for fluorescent microscopy due to the relatively small size of amino acids.[1] Other research by Maolanon et al. tested a series of analogs derived from serine as the main structure. These analogs included amides and thioethers. One new thioether was synthesized from 2-(bromomethyl)naphthalene and cysteine. The new compound exhibited no agonistic activity in NMDA receptor glycine binding sites.[2] A study by Yuan et al. has shown that labeling fluorescent dyes with amino acids is a useful method for fluorescent microscopy because it does not significantly alter the size of the fluorophore. Data by Yuan et al. utilized three different neutral amino acids (glycine, lysine, and proline) and individually linked them to the same fluorophore, resulting in three individual fluorophores. These three fluorophores were tested in the plant, *Arabidopsis thaliana*, which was able to absorb the fluorophore. Amino acid absorption and transport could be visualized. Fluorophores with the amino acid exhibited enhanced fluorescence when compared to the control fluorophore without an amino acid.[3]

Research Question

Will an amino acid attached to a fluorophore make a usable fluorescent dye?

Methods

Data was obtained in this experiment using TLC in a cyclohexane/2-propanol solution, solubility testing in various solvents, Infrared Spectroscopy (IR), and fluorescence testing in a fluorimeter. This can be used in various biomedical applications including bioassays, genomic testing, and CT scans.

Figures 1-3. Placeholder

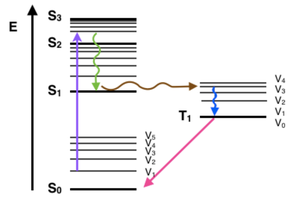

Figure 4. Jablonski diagram to explain fluorescence [5]

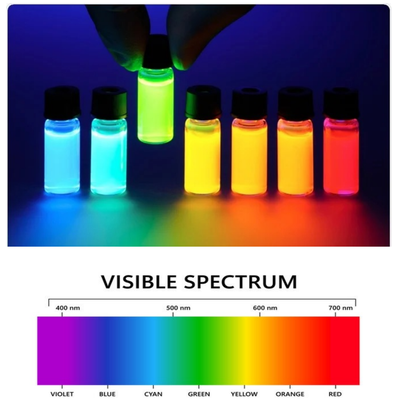

Figure 5. (A) Different ways a dye can fluoresce [4] **(B)** Visible light spectrum [6]

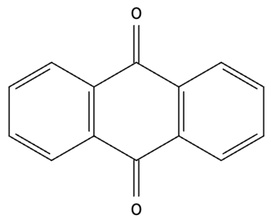

Figure 6. Drawing of fluorophore core, 9,10- anthraquinone.

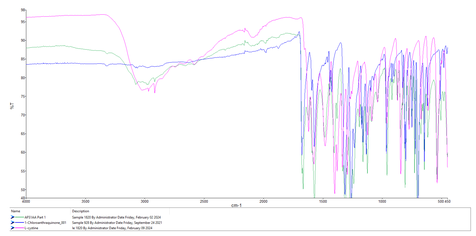

Figure 7. An IR overlay of the synthesized compound and starting materials.

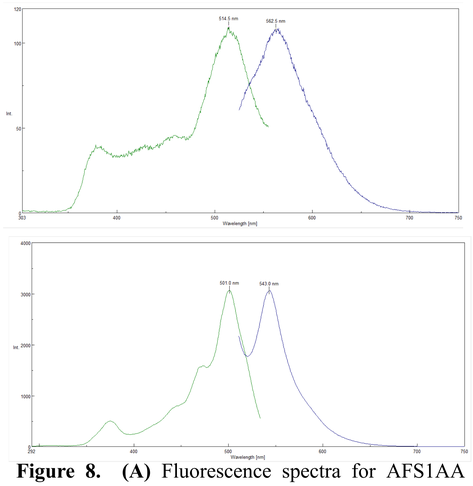

Figure 8. (A) Fluorescence spectra for AFS1AA Part 2 in DMF (λex =514nm; λem = 562nm) **(B)** AFS1AA Part 2 in DMF and NaOH (λex = 501nm; λem = 543nm)

Results and Conclusions

This experiment produced a new compound based on IR and TLC results. The compound created does exhibit fluorescence, but there may be a better dye to use due to its narrow Stokes Shift. The price to make this dye is inexpensive when compared to DRAQ5, a separate popular market fluorescent dye. The dye absorbs light at a blue wavelength and appears orange. More testing would need to be done on this dye to examine its fluorescent properties such as electrophoresis.

Future Work

Future research on this topic could include making fluorophores with a chain of amino acids attached and examining the different properties between a chain of amino acids on a fluorophore and one amino acid. Attaching the amino acid to the fluorophore on the carboxy terminus or the amino terminus is also an avenue we would like to explore in the future.

References and Acknowledgments

(1) Laxman, P; Ansari, S; Gaus, K; Goyette, J. The Benefits of Unnatural Amino Acid Incorporation as Protein Labels for Single Molecule Localization Microscopy. *Frontiers in Chemistry,* **2021**, *9*.

(2) Maolanon et al. Subtype-Specific Agnoists for NMDA Receptor Glycine Binding Sites. ACS Chemical Neuroscience, **2017**, *8*(8), 1681-1687.

(3) Yuan, Y., Cao, F., and Yuan, G. Fluorescent-Dye-Labeled Amino Acids for Real-Time Imagining in *Arabidopsis thaliana. Molecules*, 2023, 28, 3126.

(4) https://www.news-medical.net/life-sciences/A-Guide-to-Fluorescence.aspx (accessed March 7, 2024)

(5) https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_%28Physical_and_Theoretical_Chemistry%29/Spectroscopy/Electronic_Spectroscopy/Jablonski_diagram (accessed March 18, 2024)

(6) https://stock.adobe.com/search?k=visible+light+spectrum (accessed March 7, 2024)

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