# Functionalizing Anthraquinone-Based Fluorophores for Protein Labeling

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## Background

Biotin is a cofactor involved in several biological processes such as carboxylation, cell signaling, and gene regulation.[1] Many commercial biotin-dye conjugates, including fluorescein biotin and cy5 biotin, have been synthesized for labeling biotin-binding proteins, most notably avidin.[2] The bonding between biotin and avidin is one of the strongest non-covalent interactions found in nature. The dissociation constant is a measurement frequently used to assess the affinity of a protein for a ligand, with a smaller value indicating a greater affinity. Most protein complexes have values in the 10-6 M to 10-9 M range.[3] The biotin-avidin interaction, however, has a dissociation constant of 10-15, indicating an extremely high affinity.[4] It is this property that makes biotin of such interest for use in fluorescent dyes.

N-hydroxysuccinimide (NHS)-ester and maleimide terminated dyes, though, are general labels, binding to primary amino groups and sulfhydryl groups, respectively.[5,6] With many of the common amino acids containing these groups, selecting for them in protein labeling could be a useful technique in the study of biological mechanisms. In one such study by Vigers et al.[7] first labeled tubulin proteins with an NHS ester dye (succinimidylfluorescein-5-(and-6-)carboxylate) and provided these to growing cells, with the goal of studying the distribution of tubulin and the formation of the cytoskeleton. In Thacker et al.[8] maleimide dyes (ALEXA488 and ALEXA647) were used in an analysis of the Aβ42 protein (by making various surface-cysteine mutants) which is known to be related to the development of Alzheimer's Disease.

## Abstract

This research aims to investigate methods for synthesizing new fluorescent dyes to be used in protein labeling. By applying previously published chemistry on biotin, NHS ester, and maleimide adducts, the core of the fluorescent dye (anthraquinone) was reacted with various compounds to make biotin-, NHS-, and maleimide-terminated fluorophores. Reaction products were evaluated using TLC and FT-IR, in addition to fluorescence and stokes shift, with comparisons made to commercially available dyes.

## Research Question

Can the same anthraquinone-core dye be functionalized to bind to different proteins, while maintaining fluorescence and being able to compete with commercially available dyes?

## Methods

For the synthesis of the three fluorophores, a literature review was first performed, to determine reaction conditions in similar research that could be tested in these syntheses. Reaction products were all analyzed using silica thin-layer chromatography and infrared spectroscopy to determine purity and identity. The fluorescent properties of the products were measured using a fluorimeter, ultimately determining the excitation and emission wavelength, then calculating Stokes shift and comparing to DRAQ5.

## Chemical Structures

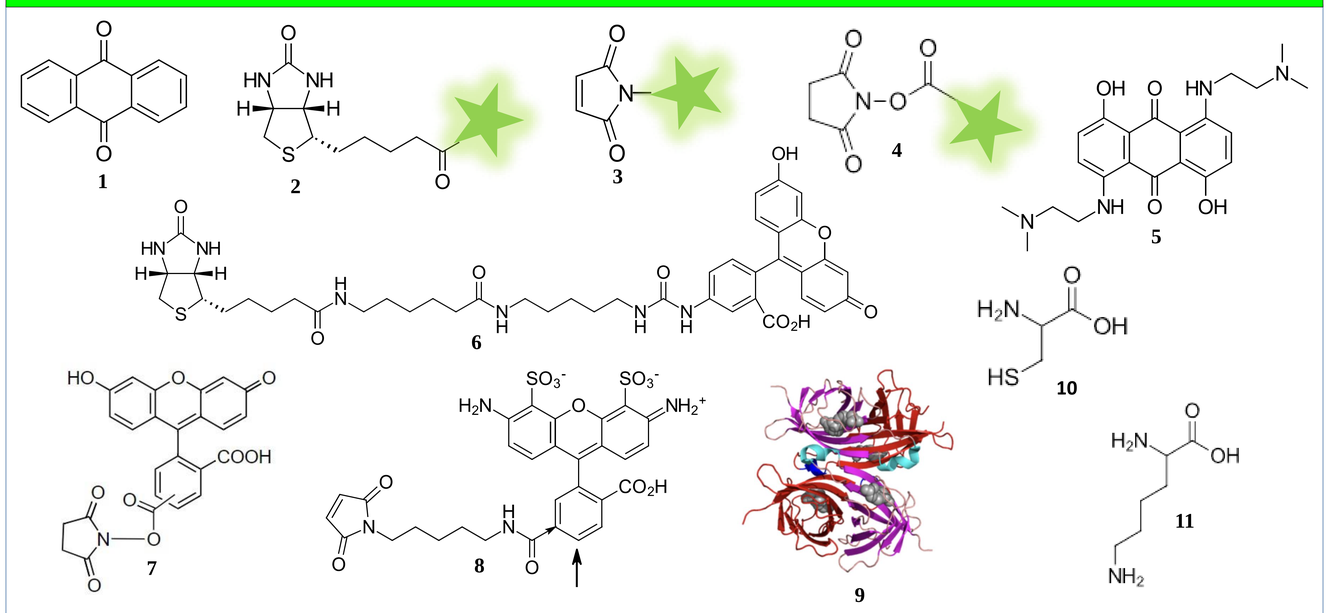


**Figure 1.** Structures of anthraquinone[9] (1), Biotin group[10] (2), maleimide group[11] (3), N-hydroxysuccinimide (NHS) ester group[12] (4), DRAQ5[13] (5), fluorescein biotin[14] (6), succinimidylfluorescein-5-(and-6-)carboxylate[15] (7), ALEXA 488[8] (8), avidin[16] (9), cysteine[17] (10), and lysine[18] (11)

## Stokes Shift

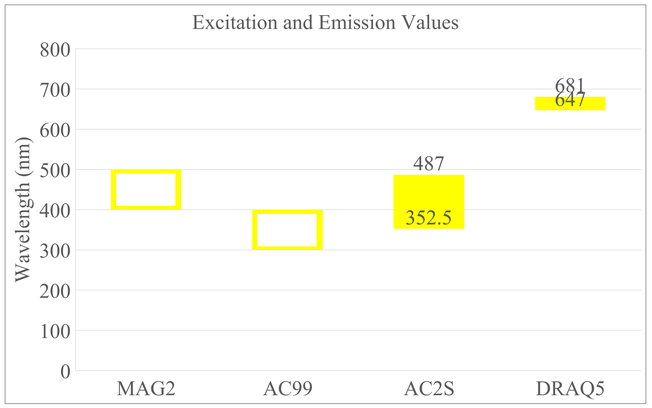


**Figure 2.** Excitation and emission wavelengths for MAG2, AC99 , AC2S (SS=134.5), and DRAQ5 (SS=34) [the boxes for MAG2 and AC99 are the expected values for the excitation and emission]

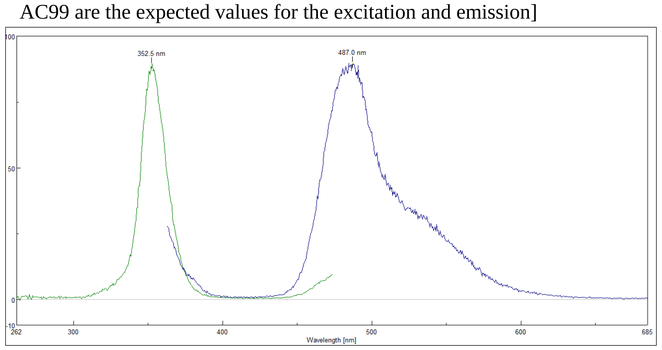


**Figure 3.** Stokes Shift of AC2S in THF (134.5nm)

## Fluorescence

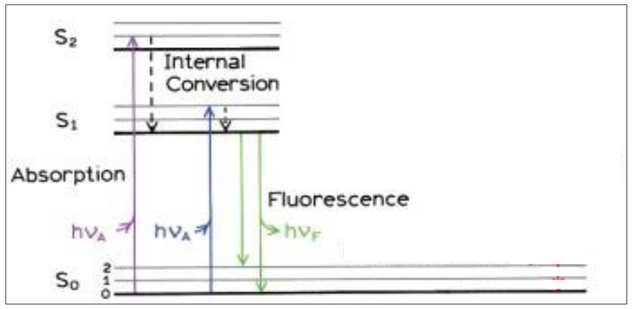


**Figure 4.** The modified Jablonski Diagram[19]

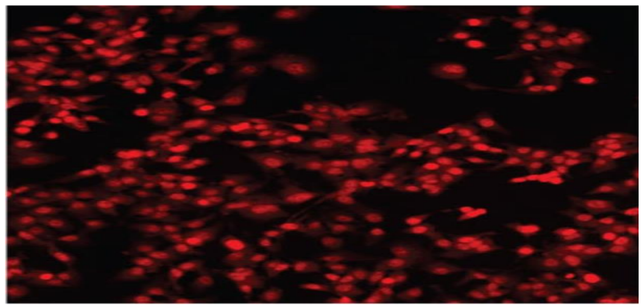

**Figure 5.** DRAQ5 shown staining HepG2 cells[20]

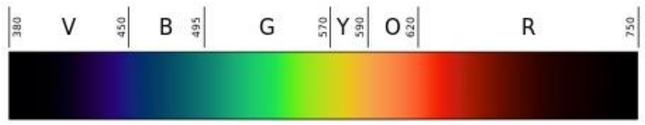


**Figure 6.** The electromagnetic spectrum[21]

## References and Acknowledgments

***Biotin Dye (MAG2):***
A reaction to synthesize this dye was set up and preliminary results and analysis are pending and will be reported.

***Maleimide Dye (AC99):***
A reaction to synthesize this dye was set up and preliminary results and analysis are pending and will be reported.

***NHS-Ester Dye (AC2S):***
By TLC and FT-IR analysis, it was determined that the desired NHS ester terminated anthraquinone compound was successfully synthesized. TLC showed a yellow spot with a larger $R_f$ value than that of the anthraquinone starting material and the IR spectrum had unique peaks, both indicating the product was free of and unique from the starting material, and that the desired product was made. This dye had a larger stokes shift than that of DRAQ5 (134.5 and 34 respectively).

## Future Work

While this work focused on analyzing protein labeling dyes with a mono-substituted fluorescent core, the next step in this research would be to synthesize poly-substituted cores with these same groups and compare to the correlating mono-substituted core. Analysis on quantum yield, binding efficiency, solubility, and economic reasonability could also be done in comparison to DRAQ5, other anthraquinone dyes, or other protein specific fluorophores.

## References and Acknowledgments

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