Volume 1
Number 1
Fall 2016

The Boller Review:
A TCU Undergraduate Journal of Research and Creativity
Characterization of BODIPY Variants to Determine Optimal Hybridization Potential with an Azadioxatriangulenium (ADOTA) fluorophore

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Introduction

Fluorescence is a phenomenon that provides a means to quantify and characterize how molecules interact with and emit light, particularly molecules of biological nature. Fluorescence lifetime is an important parameter of all fluorescent molecules, and it refers to the length of time a molecule remains in the excited state before emitting a photon; generally speaking, most fluorophores have lifetimes in the range of 0.5 to 20 nanoseconds (ns). However, in order to be practically useful in most biological contexts, fluorescent molecules must have lifetimes around or beyond 20 ns.

Here, we perform fluorescence characterization studies of three closely related fluorescent molecules that are derivatives of borondipyrromethene (BODIPY): BODIPY monomer, alkyl-BODIPY, and BODIPY dimer (rotor configuration) (figure 1). The purpose of these characterizations is to identify which molecule has the most optimal lifetime for use in biological applications, such that we can then attempt to hybridize the most optimal molecule with azadioxatriangulenium (ADOTA), a fluorophore that has already proven to be highly useful for large biomolecule binding assays, due to its unusually long lifetime.

Methods

For all three species, we measured fluorescence absorption using linear dichroism and fluorescence excitation/emission anisotropy. Collectively, these data adequately serve to characterize the spectroscopic properties and, therein, the fluorescence lifetime of each molecule.

- In the context of an incident beam, linear dichroism refers to the selective absorption of one of the beam’s two orthogonal polarization states. Linear dichroism is useful for making polarized absorption measurements of organic dyes within an oriented medium, such as a stretched polymer film.
- Fluorescence anisotropy is the phenomenon whereby, in the context of fluorescent molecules (fluorophores), the light emitted by a fluorophore will have unequal intensities along different axes of polarization. Consequently, different fluorophores will have different anisotropy values and can be characterized as such. Beyond characterization studies, understanding the anisotropic properties of a fluorophore can tell us a great deal about how the efficacy with which it can bind to a protein and thus be used as a biomarker.

Data

Within this experiment, we produced polyvinyl alcohol (PVA) films featuring varying concentrations of each dye, and solutions with varying concentrations of each dye. With these solutions and PVA films, we measured the excitation, emission, anisotropy, and fluorescence lifetimes.

PVA Films

Solutions

Future Directions

The purpose of these characterization studies was to determine the most practically useful BODIPY species for use in a biological setting, where dyes with long lifetimes are especially useful. With our results indicating BODIPY-BODIPY as the most optimal species of fluorescent dye, we now aim to (in collaboration with Dr. Sergei Dzyuba of the TCU Chemistry Department) hybridize this dimer with ADOTA (figure 10), a fluorescent molecule with an abnormally long lifetime, to determine if their lifetimes synergize to make an ultra long-lived fluorophore that would thus be both highly optimal and intriguing for studies in a biological and pathological contexts.

Acknowledgements

Our research was made possible by generous funding from the TCU SERC program.