FLUORESCENT MOLECULAR ROTORS FOR MEASURING INTRACELLULAR VISCOSITY

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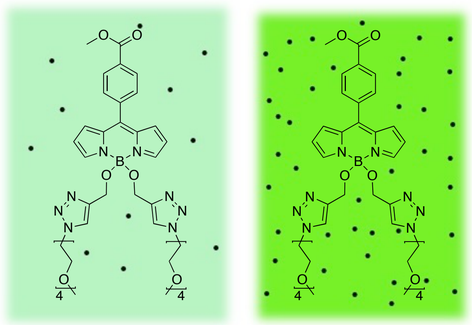
Intro

A method to visualize intracellular viscosity will allow us to develop our understanding of assembly mechanisms of biological pathways in single cells. When proteins interact, colocalization of enzymes form clusters,[1] which results in a more viscous environment. Visualization can inform us on which proteins are interacting and where the interactions are taking place. The goal of my research is to develop a novel type of fluorescent molecular rotor to visualize intracellular viscosity. In less viscous environments, rotation quenches fluorescence, whereas in more viscous environments, limited rotation results in more intense fluorescence.

Background



Less Viscous
More Rotation
Less Florescent

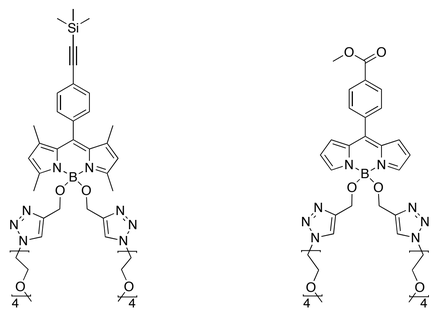
More Viscous
Less Rotation
More Florescent

When a molecule absorbs energy and moves to an excited energy state, it can relax back down to the ground state by releasing photons, which results in fluorescence, or by non-radiative internal conversions by rotating around the two conjugated portions of the molecule.

In less viscous media, the molecule has more freedom to rotate, so more energy will be lost by non-radiative internal conversion. Less energy is available to be released as photons in order for the molecule to relax back down to the ground state. So, the rotor will be less fluorescent in less viscous media.

In a more viscous environment, rotation will be inhibited, so less energy will be lost to non-radiative internal conversion. More photons will be released in order for the molecule to relax back down to the ground state. So, in more viscous media, the rotor will have stronger fluorescence.

Molecular Design



BDP 1 BDP 2

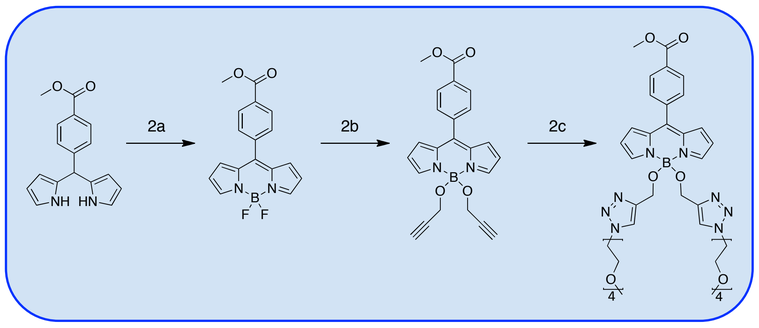
The molecular rotor is based upon boron-dipyrromethene (BODIPY), a commonly used green fluorescent dye. BDP 1 was used as a model for target compound BDP 2.[2] While the BODIPY portion of the molecule is hydrophobic, the polyethylene glycol (PEG) chains are hydrophilic, making the compound amphiphlic for localization in the cytoplasm. The PEG chains were chosen due to their lack of charge and known biocompatibility.[3] The BODIPY meso aryl substituent was chosen to enable free rotation in order to quench fluorescence. BDP 1 has methyl groups on the pyrrole rings, which inhibits the rotation of the phenyl group. BDP 2 was synthesized without the methyl groups so rotation would not be inhibited.

Synthesis

The PEG chains were synthesized following the procedure from the Li group (Scheme 1).[4] The core of target compound BDP 2 was synthesized following the procedure of preexisting compound BDP 1 (Scheme 2).[2] The PEG chains were connected to the core by a click reaction (Scheme 2).

Scheme 1

Scheme 2



Reaction Conditions: (1a) TsCl, NaOH, THF/H_2O (1:1), RT, 3 h, 65%; (1b) NaN_3, DMF, 90°C, overnight, 37%; (2a) DDQ, TEA, BF_3•OEt_2, RT, 1 h, 35%; (2b) $AlCl_3$, THF, Propargyl Alcohol, 40°C, 3 h, RT, 1 h, 78%; (2c) Tetra PEG Azide, $CuSO_4$•$5H_2O$, Ascorbic Acid, Acetone Water (5:1), Microwave, 150 W, 65°C, 15 m, 24%.

Spectroscopic Characterization

The spectroscopic characterization of the molecular rotor shows that the quantum yields increase as solvent viscosity increases, as anticipated. Additionally, the rotor is soluble and fluorescent in water, which is important for use in biological applications, with an absorption maximum at 507 nm and an emission maximum at 531 nm.

Solvent	Dynamic Viscosity at 25°C [cP]	Fluorescence Quantum Yield [Φ_F]
Toluene	0.56	0.03
DI Water	0.89	0.15
PEG 400	90.0	0.21

Figure 1. Summary of BDP 2 quantum yield measurements in toluene, DI water, and PEG 400.

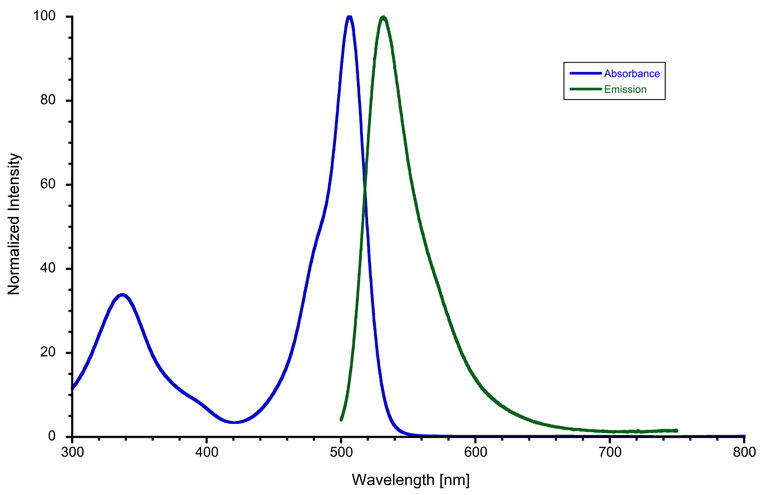


Figure 2. Summary of BDP 2 absorption and emission spectra in DI water.

Result

Human breast carcinoma Hs578T cells were treated with BDP 1 and BDP 2 and imaged by DIC microscopy and fluorescence microscopy.

BDP 1 forms highly fluorescent clusters in the cell, which appear directly next to vesicles imaged with DIC microscopy. BDP 2 also forms clusters in the cell but the fluorescence intensity is much weaker. Although this is expected due to quenching from rotation, it makes it difficult to visualize the dye in the cell, and the longer fluorescence exposure time results in increased photobleaching. Additionally, the pattern of localization of BDP 2 is not as clear as that of BDP 1, but it is hypothesized that BDP 2 is accumulated in the Golgi apparatus.

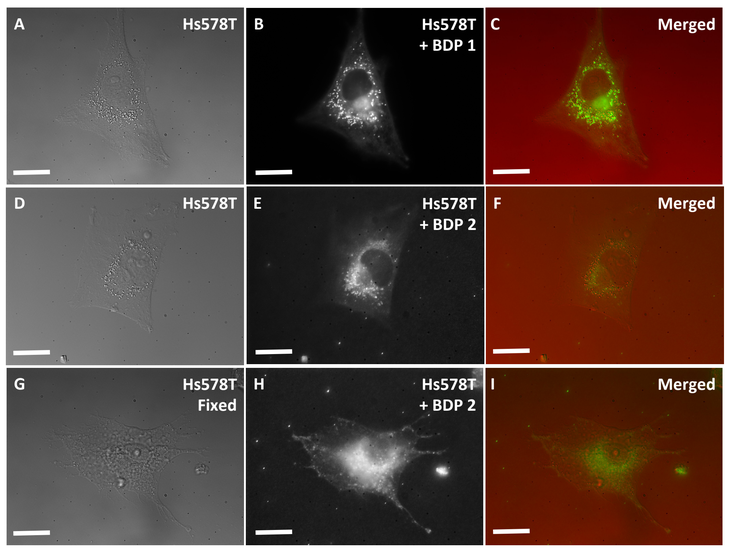


Figure 3. Images of BDP 1 [A-C], BDP 2 [D-F], and BDP 2 fixed [G-I] in Hs578T cells. DIC microscopy [left], fluorescence microscopy [middle], merged [DIC (red), fluorescence (green), right]

Conclusion and Future Direction

In summary, a novel fluorescent molecular rotor was synthesized to visualize intracellular viscosity. In less viscous environments, rotation quenches fluorescence, whereas in more viscous environments, limited rotation results in stronger fluorescence. This was supported by quantum yield measurements in vitro, which increased as solvent viscosity increased. Imaging in Hs578T cells indicated that the compound requires optimization for better visualization in cells.

The compound will be optimized by changing the substituent to observe how fluorescence intensity and intracellular localization are affected. By transfecting the cells with glucosome proteins, it can be determined if the localization of the fluorescent dye is related to the enzyme clusters and provide insight on the assembly mechanism of the glucosome proteins.[5] Additionally, BDP 2 will be imaged with the Golgi apparatus to be evaluated for its localization in living cells.

References

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