Design and Analysis of a Novel Fluorescent Cell Stain

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Abstract and Background

Background: Fluorescent dyes can be excited by absorbing light at specific wavelengths (excitation) and releasing it at longer-wavelengths (emission) (**Figure 1**). These molecules, or fluorophores, are vital to biological research, because they can be used to visualize intracellular organelles, like the nucleus or endoplasmic reticulum (ER). There are many fluorophores capable of this, but they often possess drawbacks, including low photostability and small Stokes shifts (the difference between maximum absorption and emission). Low photostability can lead to photobleaching. This phenomenon causes the fluorophore to quickly lose its ability to fluoresce after initial excitation. Possessing a small Stokes shifts can result in self-quenching and increased background noise during imaging, while shifts ≥70 nm can help prevent this. Anthraquinone-based DNA dyes demonstrate a promising solution due to their low levels of photobleaching. However, there are currently only a few anthraquinone-based fluorescent dyes available (DRAQ5, DRAQ7, and CyTRAK Orange).

Results: We synthesized the novel anthraquinone-derived fluorophore RBS3. The dye is water soluble, photostable, and possesses a large Stokes shifts. Images obtained by fluorescence microscopy confirmed the ability of RBS3 to penetrate live and fixed cells and stain the nucleus, ER, and cytoplasm.

Research Objective

The focus of this research was to develop an anthraquinone-based, cell-permeant, fluorescent molecule with desirable photophysical properties. The objective was also to test the staining capabilities of the novel fluorophore.

Methods

RBS3 was synthesized starting with an anthraquinone molecule. Unique substituents at positions R1-4 (**Figure 2)** distinguish the dye from ones currently available (such as DRAQ5).

Photometric analysis of the novel dye was conducted via spectrofluorimetry **(Figure 3).**

Buccal epithelial cells were used for both live and fixed cell staining. The cells were visualized using a Zeiss Axioskop 2 Plus fluorescence microscope. ERTracker Green was used according to protocol to stain the endoplasmic reticulum **(Figures 5 and 6).**

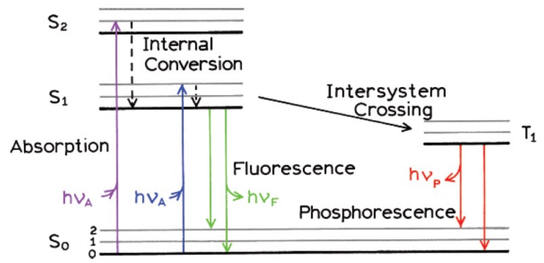

Figure 1. A Jablonski diagram demonstrating fluorescence. High energy photons can excite the electrons of a fluorophore to a higher energy level. When the electrons return to the ground state, the energy is emitted in the form of light. [1]

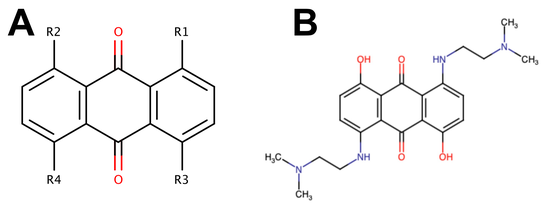

Figure 2. A substituted anthraquinone molecule (A) is the structural similarity between the novel compound (A) and DRAQ5 (B).

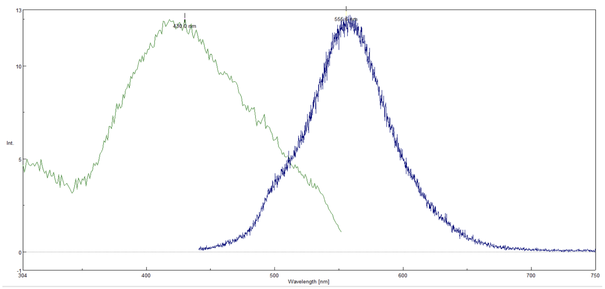

Figure 3. Emission spectra of RBS3 in deionized water**.** The excitation and emission maxima were determined to be 420/556 nm. The Stokes shift of the dye is 136 nm.

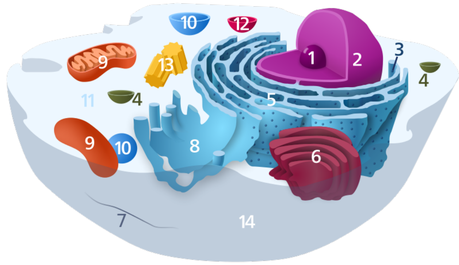

Figure 4. Representation of the organelles within a eukaryotic cell. Organelles 2-6 (nucleus, ribosome, vesicle, rough ER, Golgi apparatus), 8 (smooth ER), 12 (lysosome), and 14 (cell membrane) represent the components of the endomembrane system. [2]

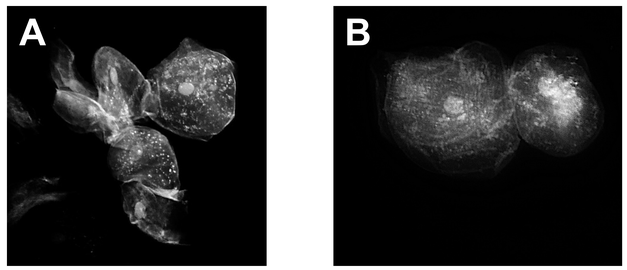

Figure 5. Live and fixed buccal epithelial cells stained with RBS3. (A) Live cells stained with 100 µM RBS3. (B) Cells fixed and permeabilized with acetone and methanol and stained with 1 mM RBS3. All stained cells exhibited nuclear, cytoplasmic, and puncta-like staining.

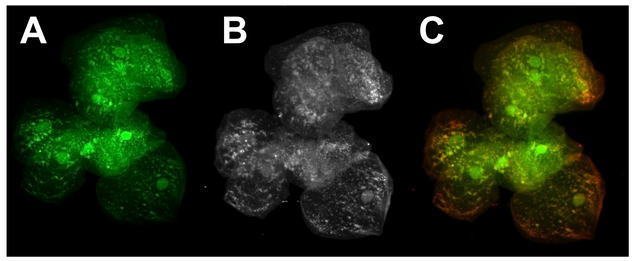

Figure 6. Comparison of ERTracker Green and RBS3 staining live buccal epithelial cells. (A) 1 µM ERTracker Green imaged with the FITC filter (495 nm); (B) 100 µM RBS3 imaged using the DAPI filter (390 nm) after 30 minutes exposure to light; (C) A merged image with RBS3 displayed as red and ERTracker Green as green. The yellow portions demonstrate overlap between the two.

Results and Discussion

- **The novel dye fluoresces in the visible range with a large Stokes shift (Figure 3).**

A new water-soluble fluorescent compound was synthesized possessing a large Stokes shift. Because of this, the dye has the potential to be an effective alternative fluorophore for high resolution fluorescence microscopy.

- **RBS3 is highly cell permeable and resistant to photobleaching (Figures 5 and 6).**

Figure 5 demonstrates that RBS3 stains both live and fixed cells. The staining of live cells in (A) with a lower concentration of dye resulted in higher clarity compared to (B). Figure 6B shows that even after a long exposure of 30 minutes to light, RBS3 displays little evidence of photobleaching. These desirable characteristics save time when staining and enable live cell imaging.

- **RBS3 stains the nucleus, cytoplasm, and ER in fixed and live cells (Figures 5 and 6).**

Figures 5 and 6 demonstrate the promising staining properties of the dye. Figure 6 shows a high amount of overlap between the ER stain and RBS3. However, the intensity of RBS3 in other spots, such as puncta-like vesicles by the plasma membrane, indicates that RBS3 may be staining additional structures. These results suggest that RBS3 binds to or accumulates in vesicles of the endomembrane system. The organelles of this system can be seen in Figure 4.

Future Work

- Image RBS3-stained cells with a confocal microscope to investigate the identity of the puncta-like vesicles.
- Investigate the potential use of RBS3 in flow cytometry.

Our results demonstrate promising potential uses for RBS3. Future work will be conducted to examine the commercial value of RBS3.

References and Acknowledgments

1. Lakowicz J.R. (eds) Principles of Fluorescence Spectroscopy, *Springer,* **2006,** pp. 27-61
2. Kelvinsong, CC0. (2012, December 10). *Animal Cell* [Illustration]. Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Animal_Cell.svg

We thank Dr. A. Solitro, Dr. G. Isaacs, and Sarah Melton for their time, input and the materials they kindly provided.