



Please don't fade away: Rebooting a robust series of organelle-specific fluorescence probes for new purposes

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Abstract

Fluorescence microscopy is a vital tool in modern cell and molecular biology research. However, a major problem with confocal and epifluorescence microscopy is the rapid photochemical bleaching of many organelle-specific probes which precludes long term monitoring or quantitative measurements in cells. Anti-fading agents may be used, but they are often toxic and not usable with live cells. To combat this problem, we have synthesized and patented a robust, fade-resistant series of organelle-specific fluorescence probes based on the naphthalimide fluorophore. With the acquisition of a state of the art confocal microscope at UWEC we are extending the use of these probes for more challenging problems and purposes. Yeast cells (*Saccharomyces cerevisiae*) are very small and thus present a very challenging target for microscopic studies. We have found that our lysosomal probes can be used to efficiently and specifically visualize organelles such as the central vacuole at least as well as expensive commercial dyes but with greater resistance to fading. Our ultimate goal is to develop and license or market a specific set of probes which can be used to quantitatively and qualitatively monitor organelles, membrane domains, pH gradients and membrane potentials in live cells.

Introduction

Saccharomyces cerevisiae, commonly known as baker's yeast, is a very small model organism used in biological research. Because of its size (~5µm), microscopic studies of yeast prove to be difficult. Our fluorescent probe series continues to allow long-lasting and accurate visualization of *S. cerevisiae* organelles, including the central vacuole, our primary focus. This lysosome-like organelle provides protein sorting, organelle acidification, ion homeostasis, autophagy, and stress-related functions for the cell (*Biochim Biophys Acta*. 1793(4), 650-663). Quantitative monitoring of the pH gradients in these vacuoles could potentially be measured with our pH sensitive fluorescent probes. We have also developed probes that will potentially display general membranes, golgi apparatus, and mitochondrion.

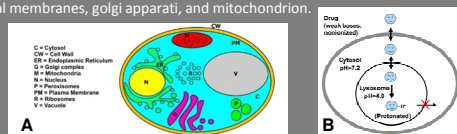


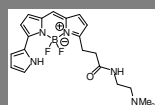
Figure 1. A) Yeast cell anatomy (Mijalija D. et.al.). Our InstantLyso LLT-1 probes acidic organelles such as the central vacuole (V). Other fluorescent probes in our developed series include mitochondria, golgi complex, and plasma membrane tracking. B) Diagram of weak bases such as LLT-1 becoming trapped in an acidic compartment of a cell after protonation.

Method

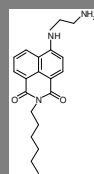
Saccharomyces cerevisiae were grown for 48 hours at 30.0°C. Colonies were added to sterile HEPES pH 7.5 5% glucose buffer to make a cell concentration around 10⁶. Cell concentration was measured by optical density at 600nm using a Cary50 UV-vis spectrophotometer. A standard curve of OD₆₀₀ to cell concentration was generated and utilized to determine exact cell concentration.

Probes were added to a concentration of 75nM and incubated at room temperature for 60 minutes. Samples were then placed on slides, sealed and immediately viewed under one of two fluorescent microscopes. The instruments used to capture images were the Olympus BX60 system epifluorescent microscope and a new state of the art Nikon A1+confocal microscope.

InstantLyso LLT-1, a previously UWEC-synthesized organelle probe that utilizes the naphthalimide fluorophore could be a photobleaching resistant alternative to Life Technology's LysoTracker Red.



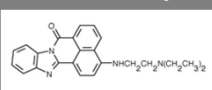
LysoTracker Red



InstantLyso LLT-1

What Yeast cellular structures are labelled by InstantLyso?

MDY-64 from LifeTechnologies



InstantLyso LLT1

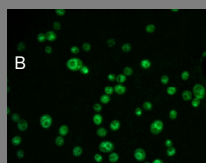
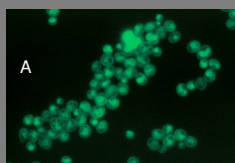
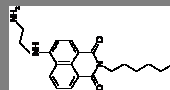


Figure 2. A) MDY-64, a fluorescent probe from LifeTechnologies, is supposed to label the yeast central vacuole (similar to lysosome), membranes and other acidic compartments. B) An epifluorescence micrograph of our InstantLyso Probe in live yeast. Note similar labeling pattern as expected since both are weak bases.

Fade Resistance of InstantLyso vs. LysoTracker Red.

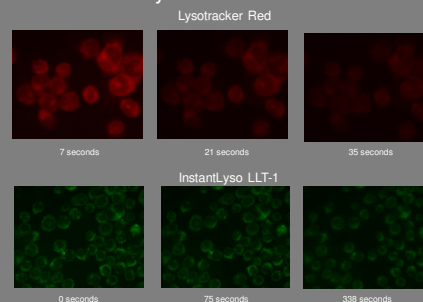


Figure 3. Time series of epifluorescence micrographs showing the fade resistance of InstantLyso vs. the competition in THP-1 monocytes.

Results

Confocal Imaging of live yeast cells and co-localization of InstantLyso with LysoTracker® Red

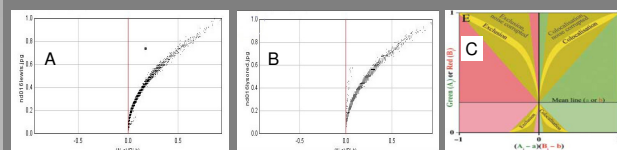
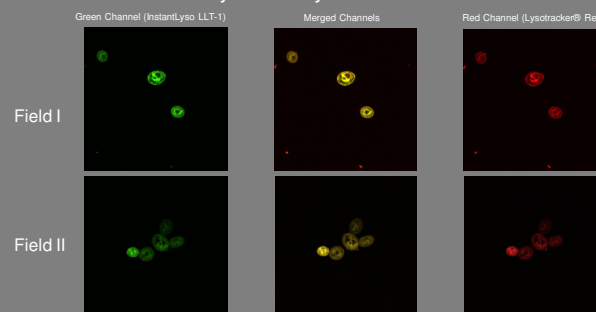


Figure 4. Colocalization analysis of Field II micrographs calculated by the JACoP plug-in for ImageJ. A and B represent intensity correlations of the green and red channels respectively. The data indicate a very high degree of correlation between LysoTracker Red and InstantLyso as shown by the model in C. This indicates identical labeling of structures by these probes (*Journal of Microscopy*, 224, 213-232).

Coupling DIC light microscopy with confocal fluorescence allows detailed localization of structures

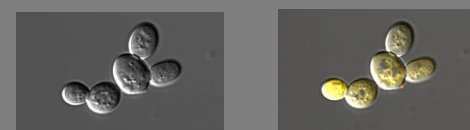


Figure 5. Differential interference contrast (DIC) allows enhanced contrast in transparent live cells. Superimposition of confocal images can help localize structures stained by a given probe in the whole cell as seen above in Field II.

Conclusions

✓The images that we have obtained of *Saccharomyces cerevisiae* with our InstantLyso probe using UWEC's new Nikon A1+ confocal microscope, demonstrate the clarity that is needed in such a small model organism to provide an adequate tracker for modern day molecular biology research.

✓We have developed a series of fluorescent probes, including InstantLyso, using the naphthalimide fluorophore that display photoresistance for a longer period of time than on-the-market competitors, allowing for longer observation of cellular organelles.

✓InstantLyso shows significant colocalization with the commonly used, expensive, and photobleaching sensitive LysoTracker when observing yeast cells. This indicates that it properly labels acidic organelles in the cell such as the central vacuole which is similar to the lysosomes found in mammalian cells.

✓With successful imaging and comparison to commercial dyes, we expect the maturation of our array of fluorescent probes to move toward licensing and eventually be put on the market.

References

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Acknowledgements

- University of Wisconsin – Eau Claire Office of Research and Sponsored Programs (ORSP)
- University of Wisconsin – Eau Claire Learning and Technology Services
- University of Wisconsin – Eau Claire Chemistry Department