Weill Cornell Medicine

ApHID: pH-Sensitive Fluorescent Dye Resistant to Photobleaching and Oxidation

Lead Inventors:

David Warren, Ph.D.
Adjunct Associate Professor of Research in Biochemistry, Biochemistry, Weill Cornell Medicine

Frederick Maxfield, Ph.D.
Vladimir Horowitz and Wanda Toscanini Horowitz Distinguished Professor in Neuroscience, Biochemistry, Weill Cornell Medicine
Professor of Biochemistry, Biochemistry, Weill Cornell Medicine

Santiago Sole Domenech, Ph.D.
Assistant Professor of Research in Biochemistry, Biochemistry, Weill Cornell Medicine

Business Development Contact:
Jamie Brisbois
Manager, Business Development and Licensing
(646) 962-7049
jamie.brisbois@cornell.edu

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Background & Unmet Need

- Human intercellular activities are often facilitated by movement of membrane-bound vesicles such as late endosomes and lysosomes (LE/Ly)
- LE/Ly dysfunction has been linked to several diseases and disorders including Alzheimer's disease and Tay-Sachs disease
- The ability to precisely measure LE/Ly activities under different cellular environments is important for the understanding of these diseases, and their potential diagnosis
- However, existing commercial fluorescence lack the photo intensity and chemical stability in highly acidic and reactive cellular environments within LE/Ly vesicles
- **Unmet Need:** A fluorescence probe that is pH-sensitive and able to withstand oxidation and photobleaching while maintaining structural integrity *in vivo*

Technology Overview

- **The Technology:** Acidic pH indicator Dye (ApHID) with high resistance to oxidation and photobleaching
- ApHID is composed of a BODIPY core and determines pH of the environment using an aniline moiety that has two methyl groups attached
- Optimized for use between pH 4.0 – 6.0, ApHID's fluorescence emission increases sharply in amplitude with increasing acidity
- ApHID has pKa of 5.4 and excitation max at 506 nm
- **PoC Data:** ApHID fluorescence is 12-fold greater at pH 4.0 relative to pH 6.0
- ApHID fluorescence output only decreased by 12% after photobleaching, compared to an 83% and 82% decrease with fluorescein and Oregon Green, respectively
- ApHID exhibits the greatest fluorescent dynamic range at the physiological pH range of LE/Lys compared to currently available commercial dyes

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Patents:
Provisional Filed

Publications:
Warren et al. *bioRxiv* 2024 (preprint)

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Technology Applications

- Fluorescent dye for LE/Ly research and experiments
- Tracking efficacy of drugs for neurodegenerative diseases
- Tool for cancer research and drug development

Technology Advantages

- Greater brightness and sensitivity to acidity than existing dyes
- Higher resistance to photobleaching than alternative fluorescence
- Stable in living cells while emitting strong fluorescent signal
- Resistant to highly concentrated reactive oxygen species

Supporting Data / Figures

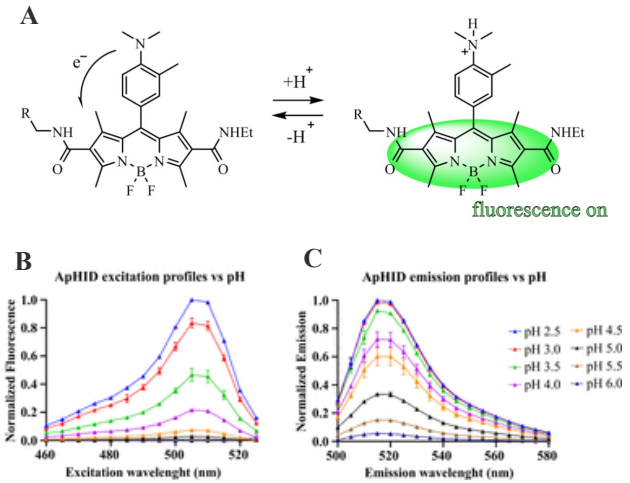

Figure 1: (A) Chemical structure of ApHID. (B) Excitation and (C) emission spectra plotted against buffer pH, measured for a 0.04 mg/mL dilution of 10 KDa amino-dextran labeled with ApHID at a 1.6:1 molar ratio, in pH-adjusted buffers.

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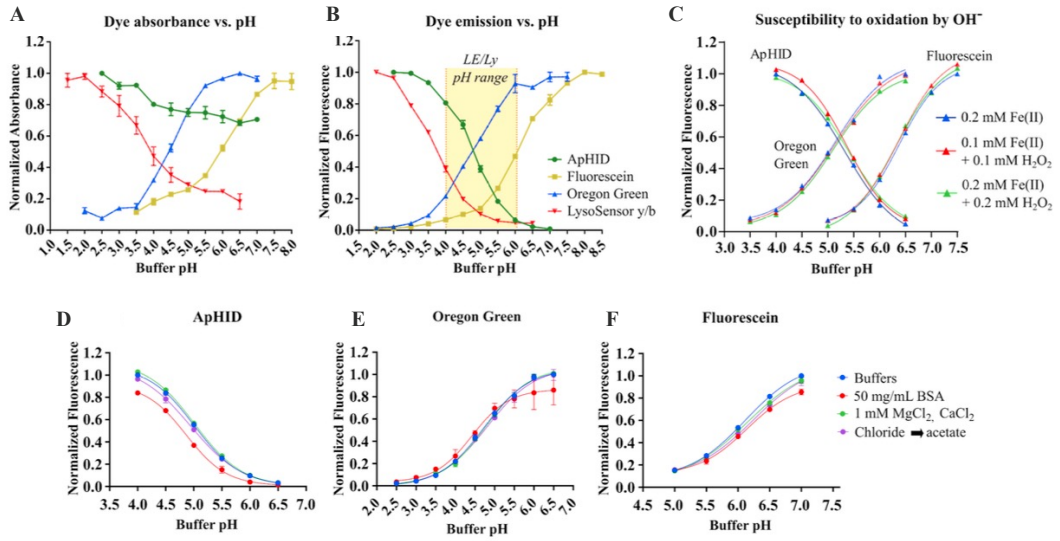

Figure 2: (A, B) ApHID, fluorescein, Oregon Green, and LysoSensor y/b absorbance and emission profiles vs buffer pH. (C) ApHID, Oregon Green, and fluorescein fluorescence titrations against buffer pH in the presence of hydroxyl radical. (D-F) ApHID, Oregon Green, and fluorescein fluorescence in high salt environment.

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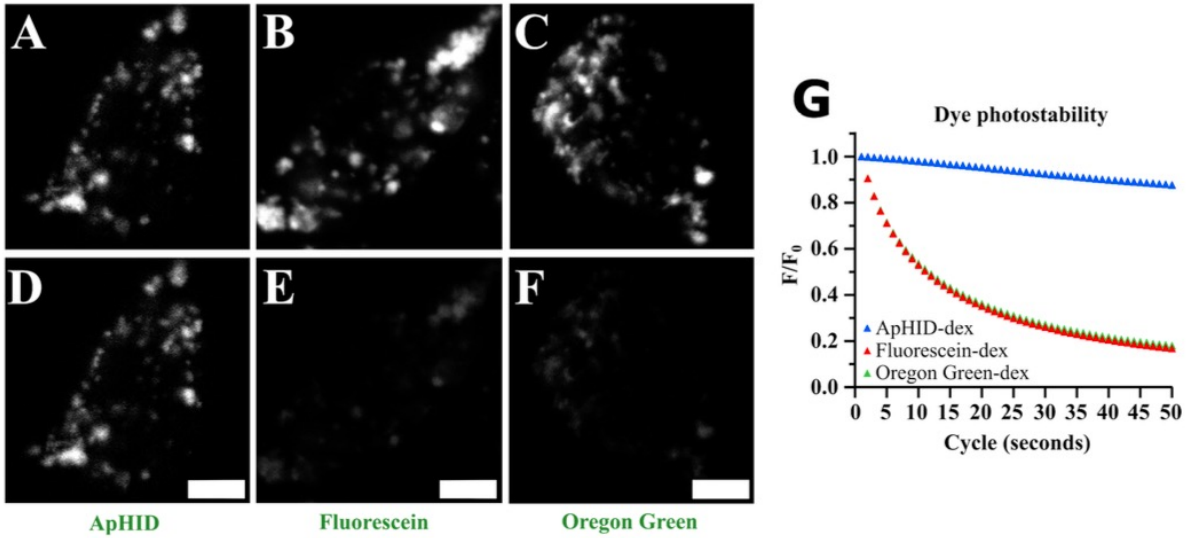

Figure 3: (A-F) J774 macrophages with lysosomes labeled with 70KDa dextran polymers derivatized with ApHID (A, D), fluorescein (B, E) or Oregon Green (C, F). (G) Normalized fluorescence intensity was plotted against irradiation cycle for ApHID, fluorescein, and Oregon Green dextrans.

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