Weill Cornell Medicine

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

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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 showed no decrease after photobleaching in live cells and decreased by only 12% in fixed cells, compared to an 83% and 82% decrease with fluorescein and Oregon Green
- 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:
PCT Application Filed

Publications:
Solé-Domènech et al. *Cell Reports Methods*. 2025.

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Technology Applications

- Fluorescent dye for LE/Ly research and experiments, including pH and live cell imaging
- Labeling of Aβ aggregates to image digestive exophagy
- 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
- Highly resistant to photobleaching and highly concentrated reactive oxygen species
- Stable in living cells over 15 hours+ while emitting strong fluorescent signal
- Enables detection of pH differences within cells and real-time changes in pH

Supporting Data / Figures

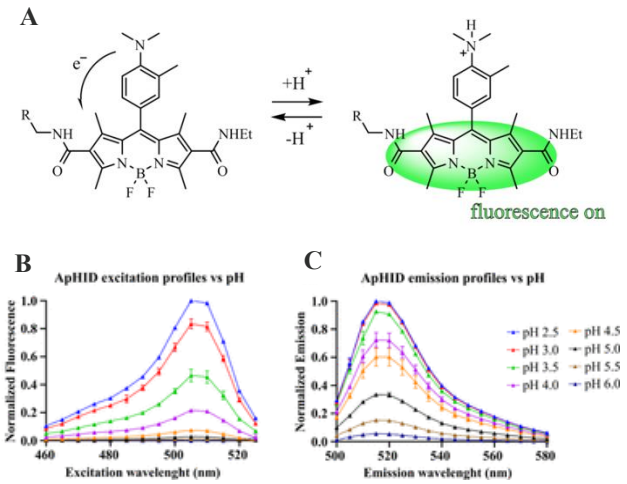

Figure 1: (A) Chemical structure of ApHID. (B) Excitation and (C) emission spectra plotted against buffer pH, measured for a 0.04 mg/mL dilution of 10 KDa amino-dextran labeled with ApHID at a 1.6:1 molar ratio, in pH-adjusted buffers.

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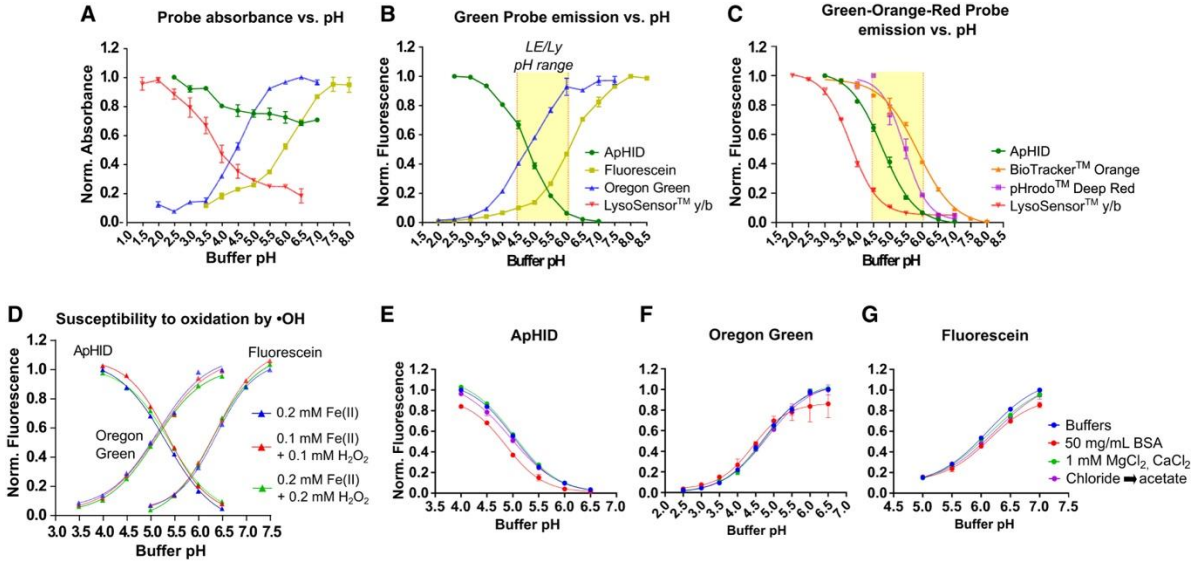

Figure 2: (A, B, C) ApHID, fluorescein, Oregon Green, and LysoSensor y/b and/or BioTracker, pHRodo absorbance and emission profiles vs buffer pH. (D) ApHID, Oregon Green, and fluorescein fluorescence titrations against buffer pH in the presence of hydroxyl radical. (D-F) ApHID, Oregon Green, and fluorescein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

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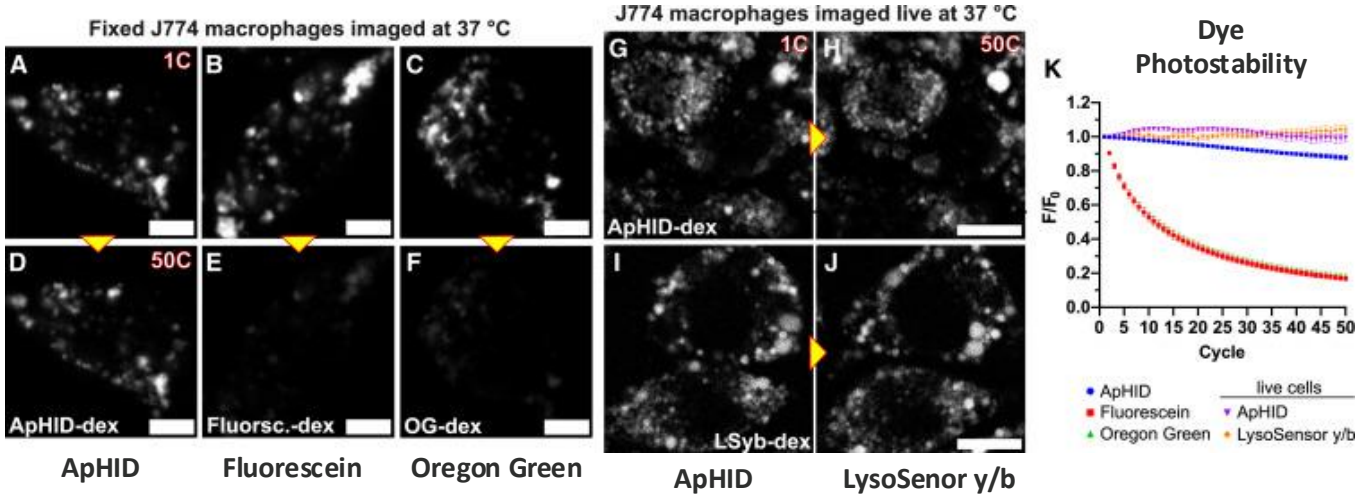

Figure 3: (A-F) Comparative photostability of ApHID, fluorescein, and Oregon Green LE/Lys of fixed J774 macrophages, imaged by confocal microscopy (G-J) Comparative photostability of ApHID and LysoSensor yellow/blue in live J774 macrophages (K) Normalized fluorescence intensity plotted against irradiation cycle for ApHID, fluorescein, and Oregon Green dextrans in fixed cells and ApHID and LysoSensor in live cells.

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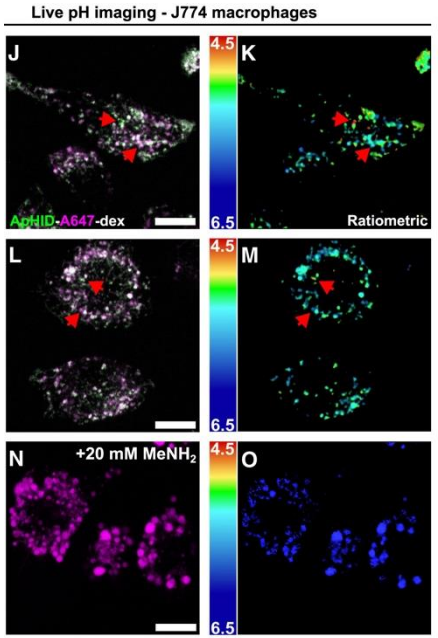

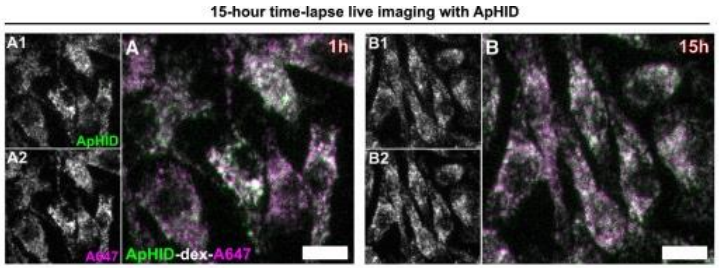

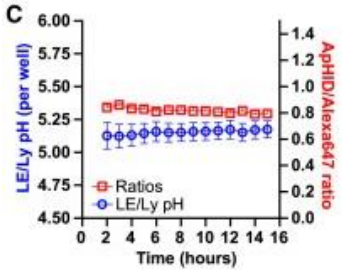

Figure 4: Left: (J-M) ApHID enabled detection of small differences in pH between individual compartments within single cells (N-O) ApHID is sensitive to changes in pH in real time. A brief treatment with 20 mM methylamine induced rapid alkalinization **Right:** ApHID reports LE/Ly pH in a stable manner over time.

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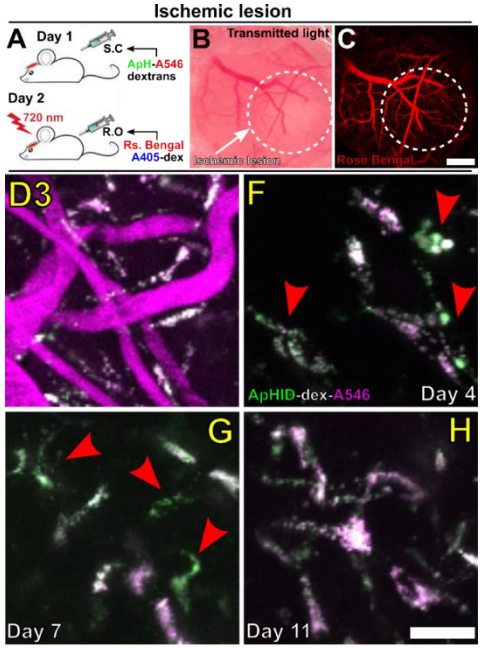

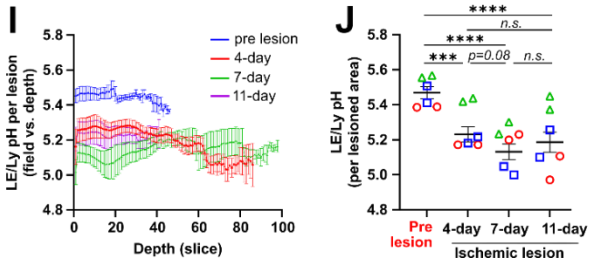

Figure 5: Intravital LE/Ly pH imaging of macrophages using ApHID allows for detection of dynamic changes in organelle acidification in real time. Pro-inflammatory ischemic lesions were induced in the parenchyma of mice via photothrombosis and LE/Ly acidification in local macrophages was measured. Lesioned areas displayed phagocytic cells with amoeboid morphologies undergoing LE/Ly acidification (red arrowheads). Re-acidification reached its peak 7 days after ischemic insult and seemed to plateau at 11 days post lesion.

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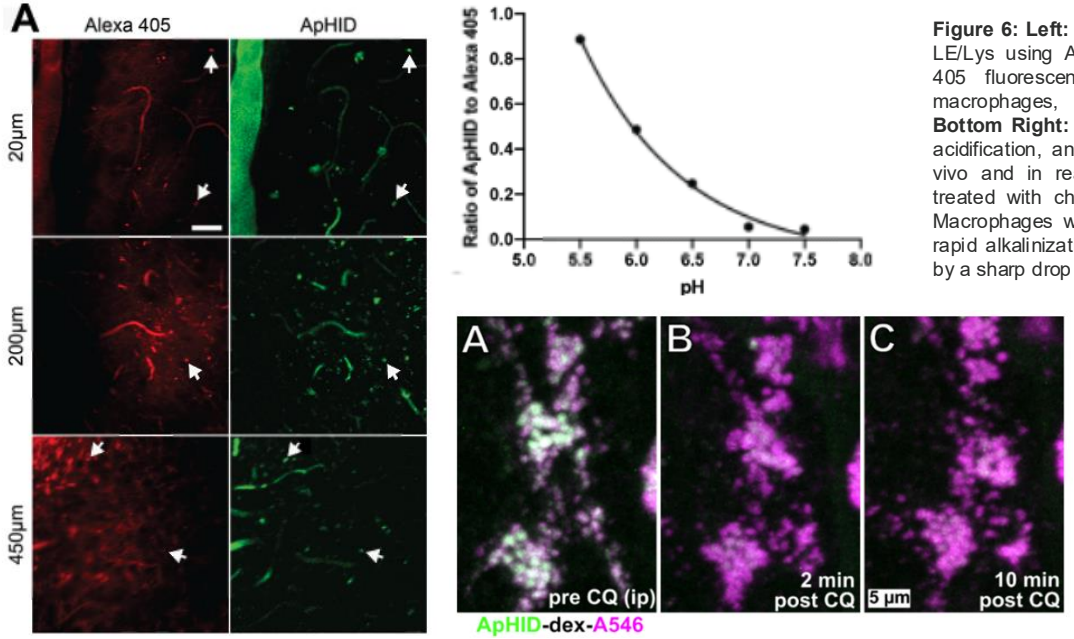

Figure 6: Left: In vivo 3-photon visualization of LE/Lys using Aphid **Top Right:** ApHID/Alexa 405 fluorescence ratio measured in fixed macrophages, plotted against buffer pH **Bottom Right:** ApHID can be used to monitor acidification, and alkalinization, of LE/Ly pH in vivo and in real time accurately. Mice were treated with chloroquine (CQ), a weak base. Macrophages with acidic LE/Ly pH underwent rapid alkalinization 2 min and 10 min, reported by a sharp drop in ApHID fluorescence.

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