Optimization of Methods to Visualize Hydrogen Peroxide at Cellular Membranes

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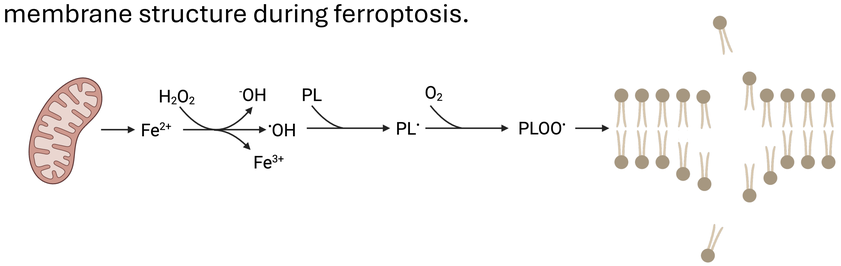
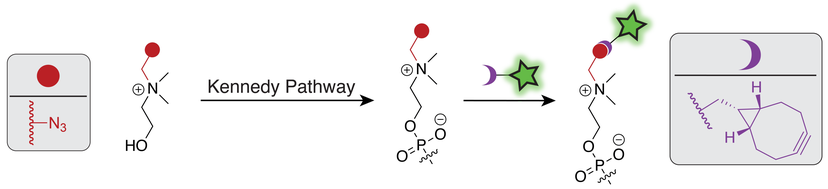
Abstract

Ferroptosis is a regulated, iron-dependent form of cell death with numerous chemical inputs, driven by the peroxidation of phospholipids, the building blocks of cellular membranes. Since phospholipids are the primary components of all cellular membranes, their destruction through lipid peroxidation causes the breakdown of membranes and ultimately leads to cell death. In an effort to better understand the fundamental mechanisms of ferroptosis, a major research goal in the White-Mathieu lab is to label cellular membranes with pro-fluorescent molecules that allow us to detect analytes implicated in ferroptotic activity and study changes at membranes throughout the progression of this cell death pathway. The goal of this research project is to synthesize a chemical probe that is capable of tagging cellular membranes and optimize the labeling conditions to ensure that an ABS sensor can specifically detect hydrogen peroxide in live cells and report on membrane structure during ferroptosis.

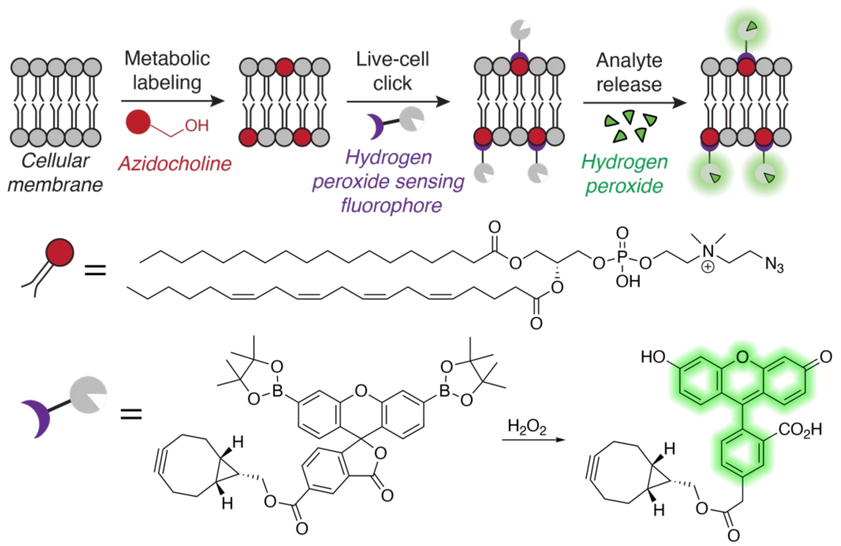


Project Background and Significance

The overall goal of the project is to understand the structural changes that occur at cellular membranes during ferroptosis using fluorescence microscopy. Membranes can be fluorescently tagged by treating cells with an azide-functionalized, clickable handle that is incorporated into membranes using an enzymatic pathway. Subsequent "click" chemistry with a functionalized fluorophore allows for visualization of membranes with confocal microscopy.



To detect analytes associated with ferroptosis, a pro-fluorescent compound, also known as an activity-based sensor (ABS), that can report the presence of hydrogen peroxide, a chemical input of ferroptosis, will be anchored to membranes for visualization. My project aims to optimize the protocol for both the labeling of membranes with the clickable probe, azidocholine, and the tagging of the incorporated azidocholine with a pro-fluorescent hydrogen peroxide sensor. This will streamline the process of future replications of this experiment.



Methodology

1. Synthesize azidocholine and compare to accepted NMR to ensure purity
2. Culture HeLa and HEK293T cells for imaging experiments
3. Establish conditions for the live-cell click reaction with an ABS reagent
4. Imaged treated cells with confocal microscopy after treatment with the ABS reagent to establish a background
5. Detect analyte production through fluorophore activation using confocal microscopy
6. Initiate ferroptosis using $FINO_2$ and RSL3 in live cells and measure the fluorescence response
7. Repeat the experimental processes will and optimize azidocholine and pro-fluorescent fluorophore treatment times to maximize signal output
8. Quantity fluorescent signal in all images using ImageJ

By optimizing the labeling conditions for this type of experiment, my project will provide a foundation for all future studies in the lab aimed at understanding ferroptosis

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