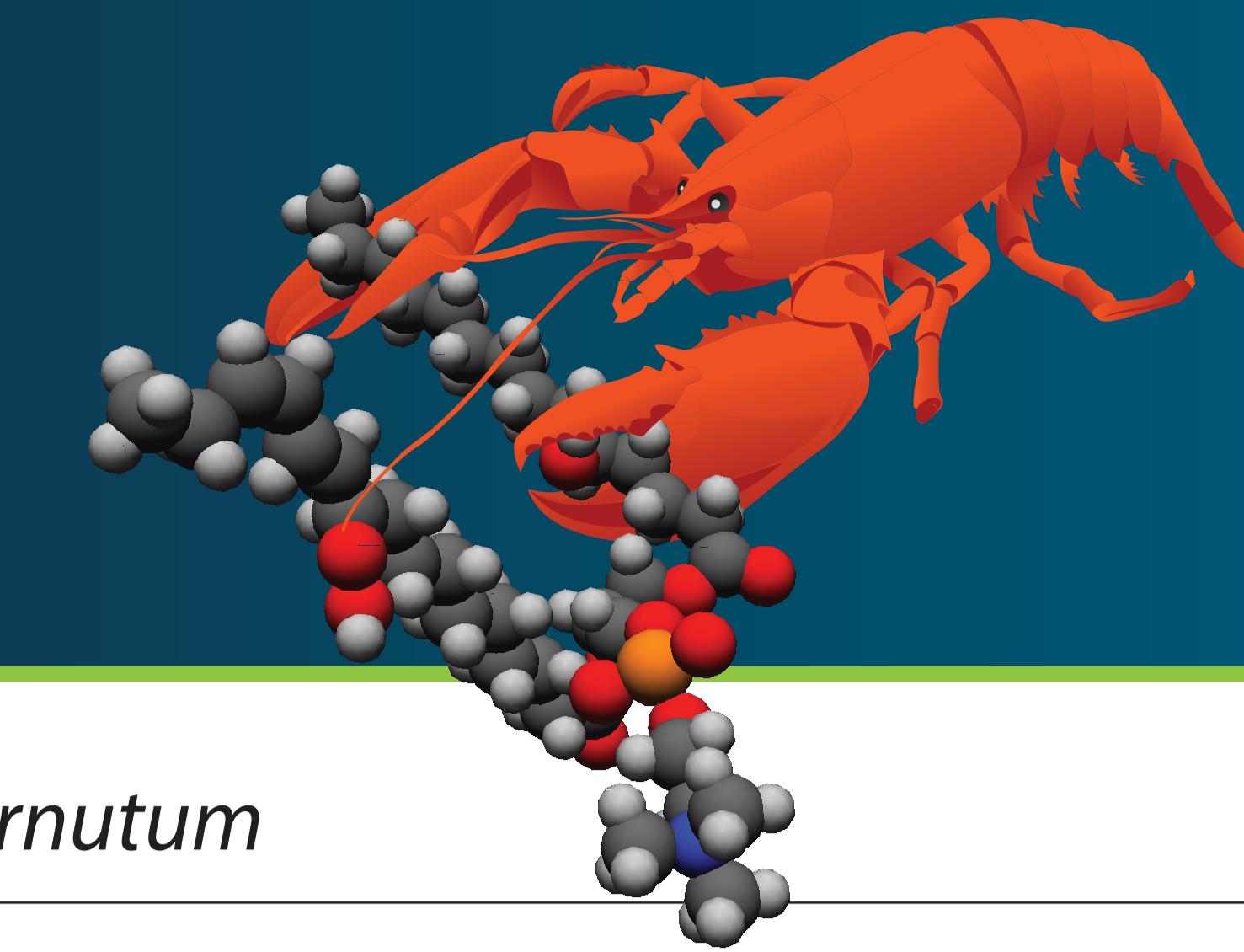


Discovery and identification of biomarkers for oxidative stress in phytoplankton using LOBSTAHS, a new pipeline for semi-untargeted lipidomics

Jamie Collins ^{1,2}, Bethanie R. Edwards ^{1,2}, Helen F. Fredricks ¹, and Benjamin A.S. Van Mooy ¹

¹ Department of Marine Chemistry & Geochemistry, Woods Hole Oceanographic Institution

² MIT/WHOI Joint Program in Oceanography

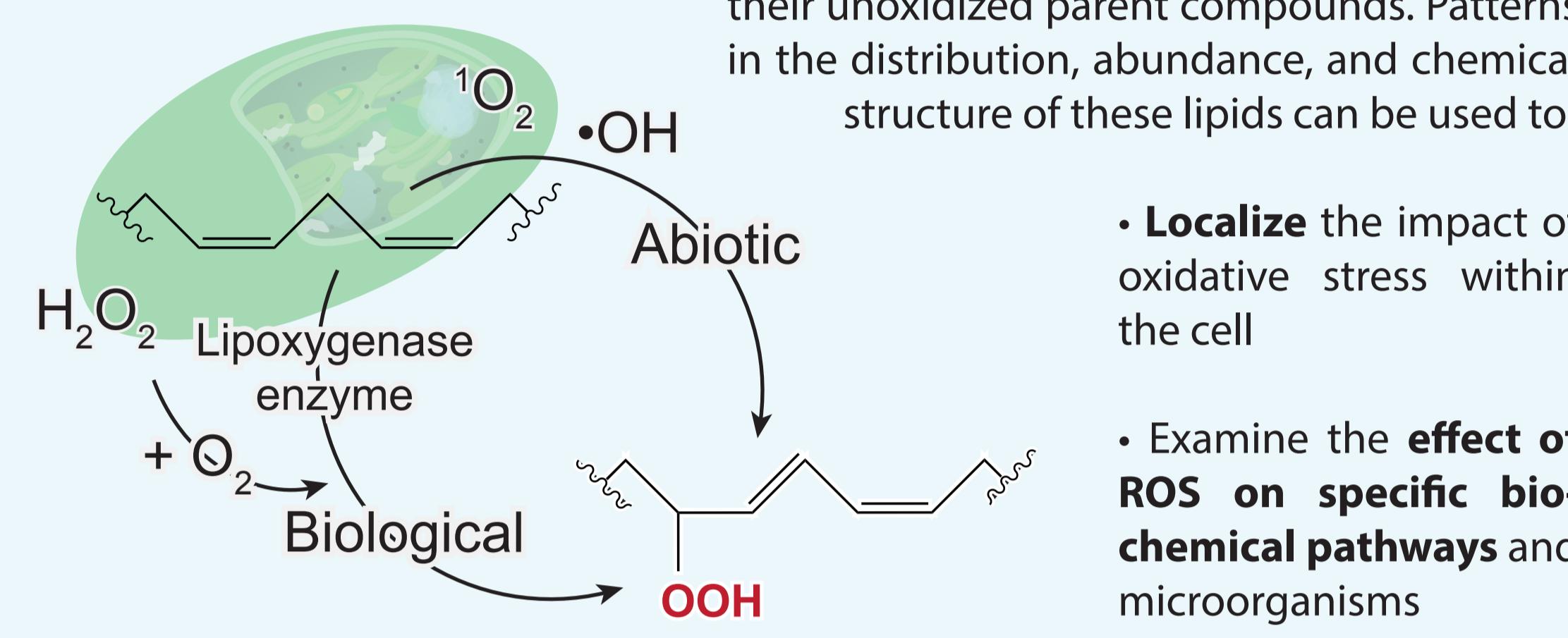


Introduction: ROS and the algal lipidome

Oxidative stress exerts a profound impact on the lives of eukaryotic phytoplankton. Reactive oxygen species (ROS) can function as inter- or intracellular signals, alter various cellular metabolisms, and dramatically transform the individual molecules that make up the algal cell.

As in terrestrial plants, the polar lipids of marine algae are a "front line" barrier in the battle against oxidative stress. Because these lipids are primary structural components of cell and organelle membranes, they are particularly susceptible to chemical transformation by both inter- and extracellular ROS. The lipids transformed by ROS can be exploited as biomarkers for specific types of oxidative stress.

We use lipidomics to analyze hundreds of these biomarkers simultaneously with their unoxidized parent compounds. Patterns in the distribution, abundance, and chemical structure of these lipids can be used to:



LOBSTAHS: A new pipeline for lipidomics

We developed LOBSTAHS (Lipid and Oxylipin Biomarker Screening through Adduct Hierarchy Sequences) to assist in the discovery and identification of new lipid biomarkers for oxidative stress. LOBSTAHS is an open-source package for R that uses a unique screening criteria to make compound identifications in HPLC-MS data. LOBSTAHS can be downloaded from Github via the QR code at lower right.

At right, we apply LOBSTAHS to lipid data from an experiment¹ in which Graff van Creveld et al. used hydrogen peroxide (H_2O_2) to induce oxidative stress in the marine diatom *Phaeodactylum tricornutum*.

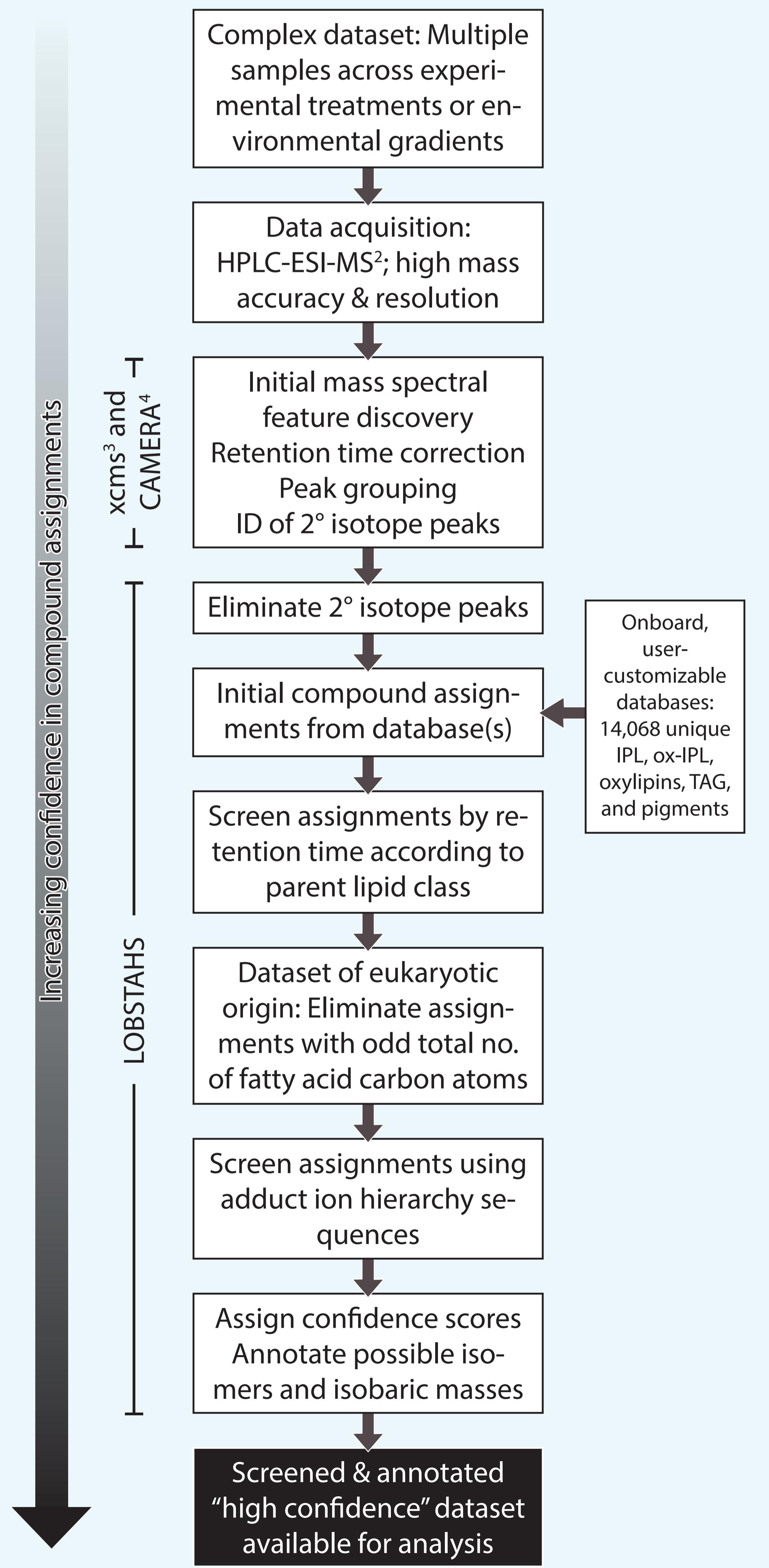


Fig. 1. Oxidative stress induces lipidome remodeling in *Phaeodactylum tricornutum*

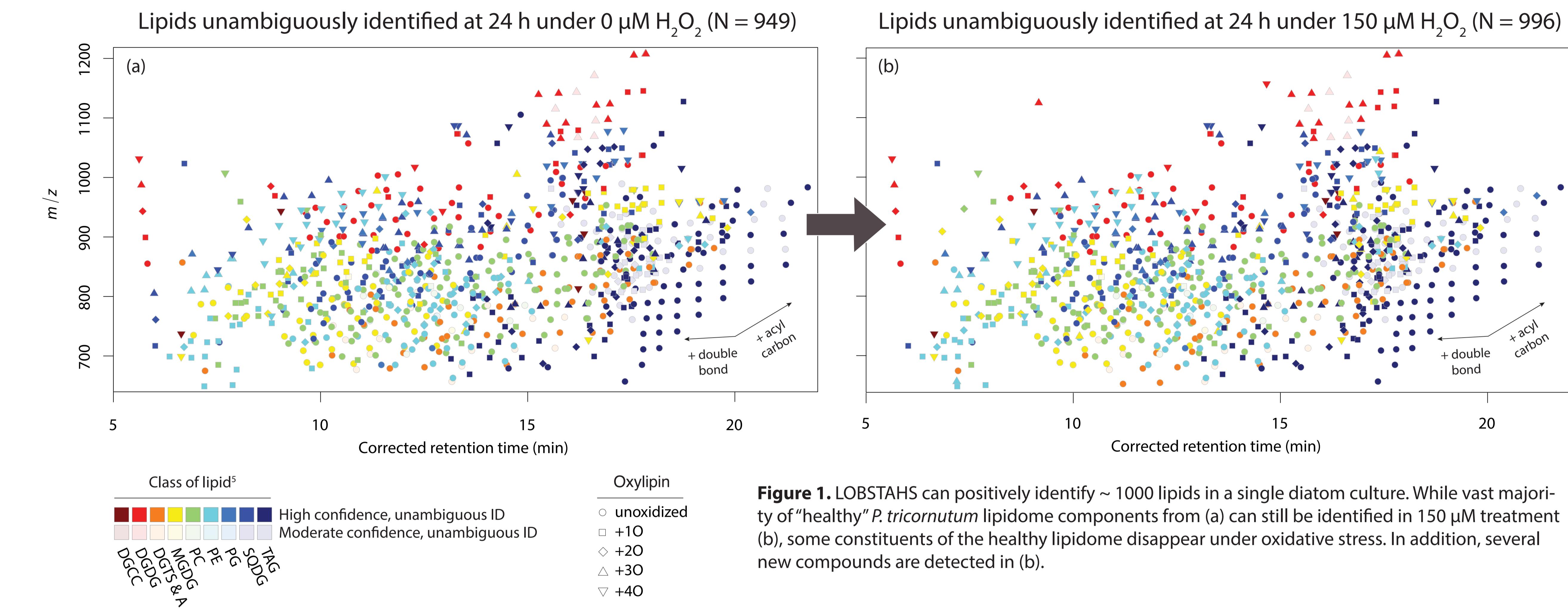


Figure 1. LOBSTAHS can positively identify ~1000 lipids in a single diatom culture. While vast majority of "healthy" *P. tricornutum* lipidome components from (a) can still be identified in 150 μM treatment (b), some constituents of the healthy lipidome disappear under oxidative stress. In addition, several new compounds are detected in (b).

Fig. 2. Expression of lipidome constituents varies by lipid class and function

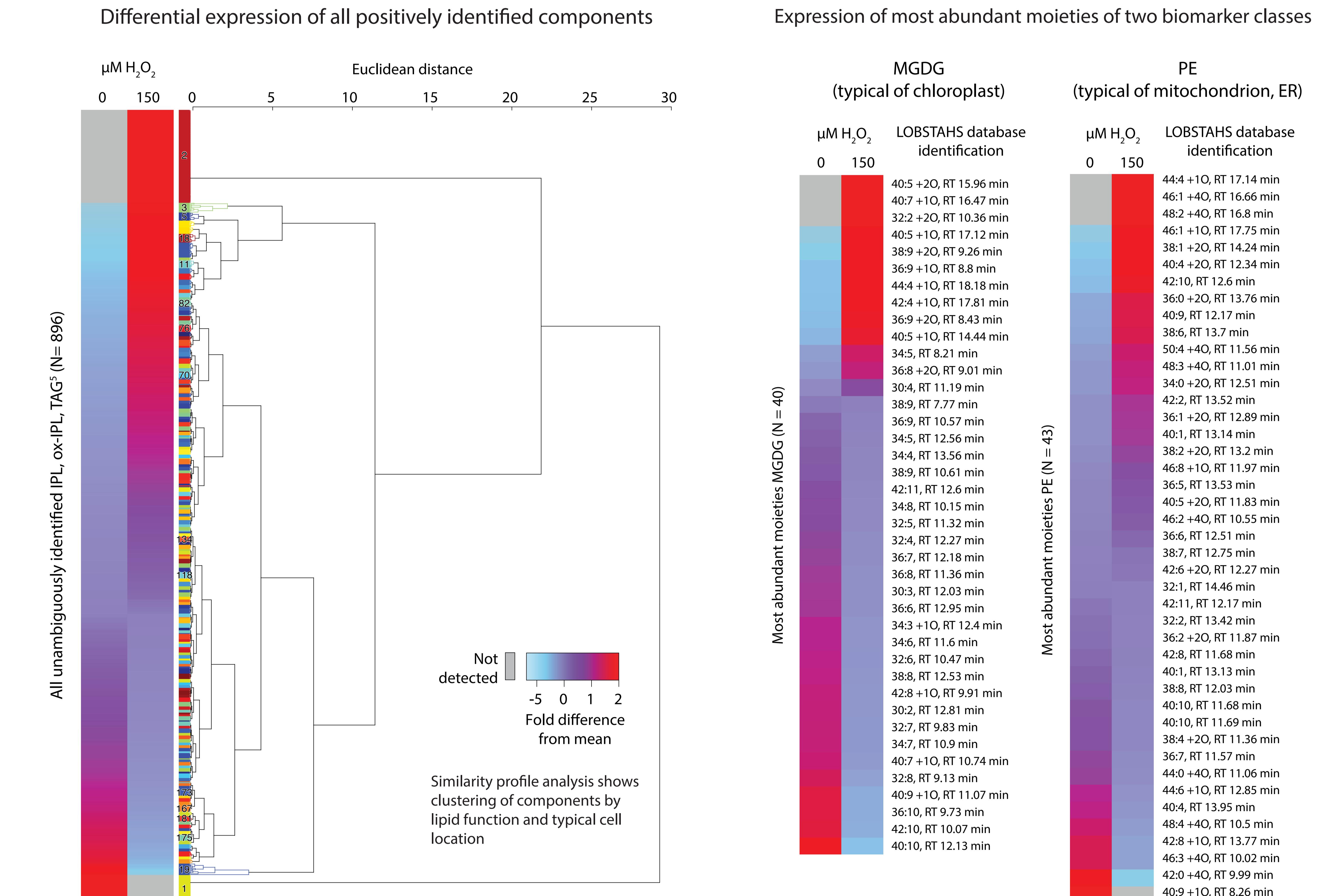


Fig. 3. Significant⁶ changes in structural properties by lipid class localize the effects of oxidative stress within the cell

Lipid Class or Functional Grouping	Within-Group Mean		
	Total No. Fatty Acid Carbon Atoms	Degree of Unsaturation	Degree of Oxidation
MGDG (typical of chloroplast)			
Moieties more abundant under 150 μM H_2O_2 (N = 72)	38.6	5.2	1.9***
Moieties less abundant under 150 μM H_2O_2 (N = 60)	37.6	6.2	0.5***
Mitochondrial lipids (PE, PG)			
Moieties more abundant under 150 μM H_2O_2 (N = 79)	41.3	3.5	1.7
Moieties less abundant under 150 μM H_2O_2 (N = 92)	39.6	4.8	1.8
All lipids			
Moieties more abundant under 150 μM H_2O_2 (N = 571)	42.1***	4.6	1
Moieties less abundant under 150 μM H_2O_2 (N = 325)	38.9***	5	1.1

Summary of results & significance

Figures 2 and 3. Shifts in the distribution, abundance, and chemical structure of lipids in *P. tricornutum* can tell us about the effects and specific targets of oxidative stress within the algal cell. Among our findings:

- Extensive lipidome remodeling followed treatment with 150 μM H_2O_2
- Oxidative stress induced statistically significant carbon-chain elongation across all lipid classes, an apparent stress response
- Lipid peroxidation & elongation were most evident in moieties of monogalactosyldiacylglycerol (MGDG), a lipid typically localized to the chloroplast
- Carbon-chain elongation also evident in lipids typical of the mitochondrion
- Elongation under stress was accompanied by reallocation of biomass to TAGs
- Enigmatically, oxidative stress did not induce any significant increase in the overall oxidation state of the lipidome

