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

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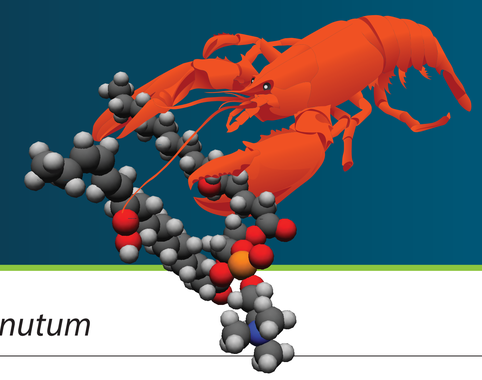
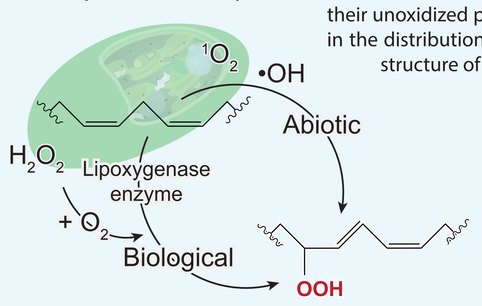


Introduction: ROS and the algal lipidome

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:



- **Localize** the impact of oxidative stress within the cell
- Examine the **effect of ROS on specific biochemical pathways** and microorganisms

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 experiment[1] in which Graff van Creveld et al. used hydrogen peroxide (H_2O_2) to induce oxidative stress in the marine diatom *Phaeodactylum tricornutum*.

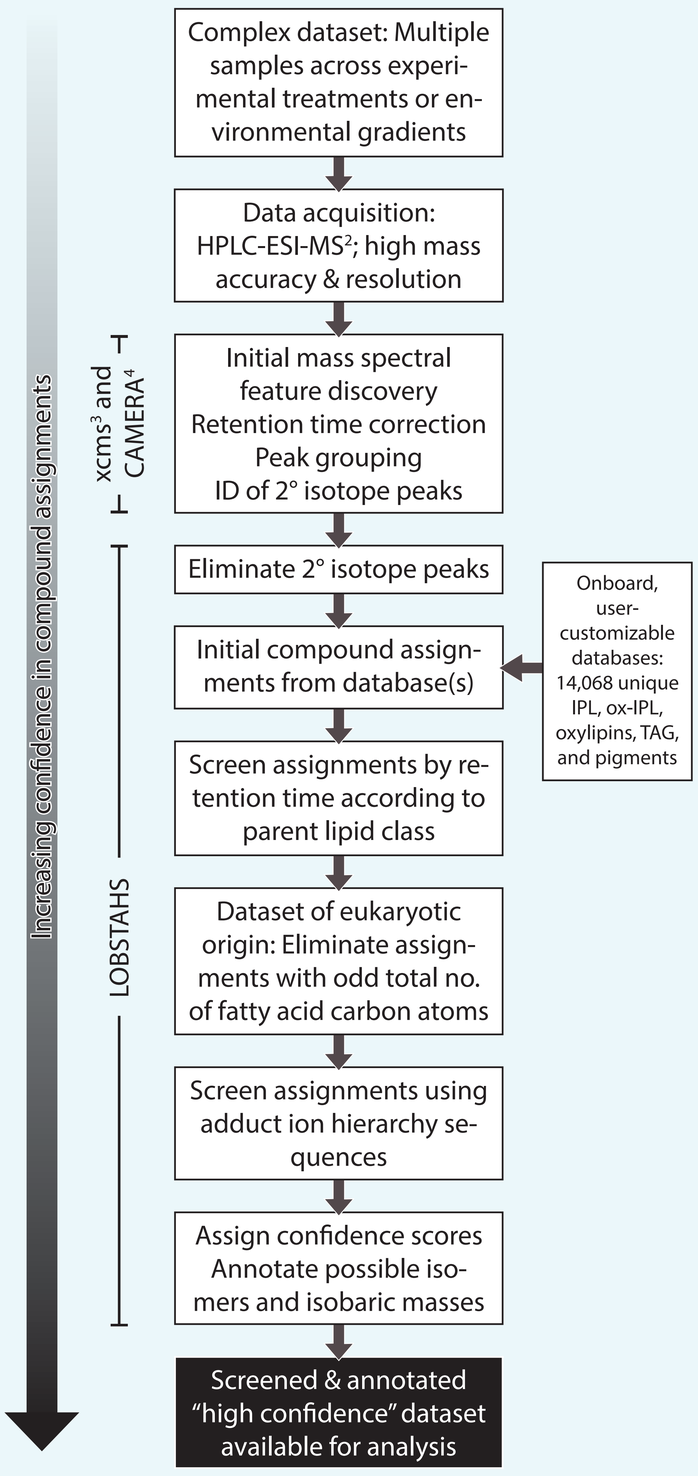


Fig. 1. Oxidative stress induces lipidome remodeling in *Phaeodactylum tricornutum*

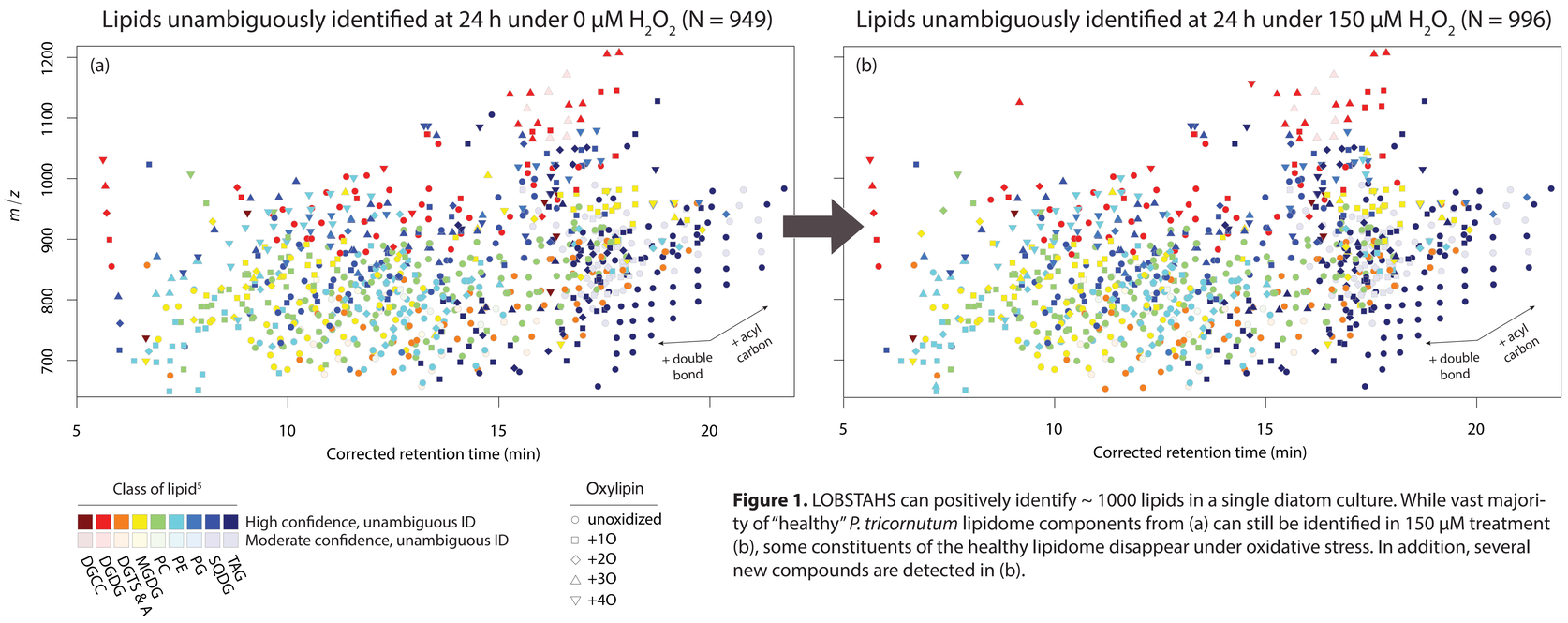


Figure 1. LOBSTAHS can positively identify ~ 1000 lipids in a single diatom culture. While vast majority of "healthy" *P. tricornutum* lipidome components from (a) can still be identified in 150 µM treatment (b), some constituents of the healthy lipidome disappear under oxidative stress. In addition,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

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

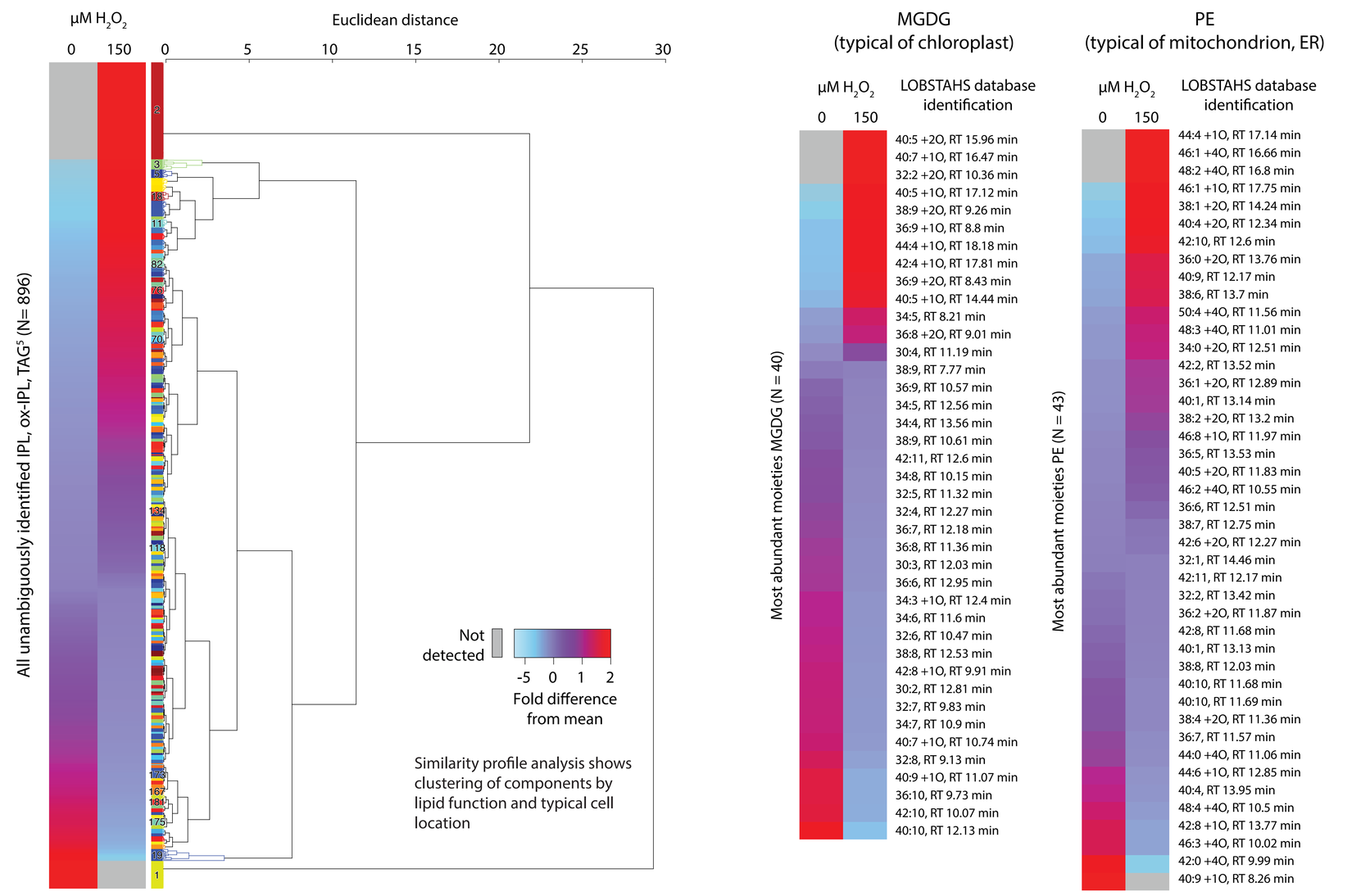


Fig. 3. Significant[6] changes in structural properties by lipid class localize the effects of oxidative stress within the cell

Lipid Class or Functional Grouping	Within-Group Mean: Total No. Fatty Acid Carbon Atoms	Within-Group Mean: Degree of Unsaturation	Within-Group Mean: 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

[1] Graff van Creveld et al. 2015, *ISME* 9:285-395. In the study, cultures of *P. tricornutum* were treated with varying concentrations of hydrogen peroxide to induce an oxidative stress response. Experimental methods are described in the reference. [2] Modified method of Hummel et al. 2011, *Front. Plant Sci.* 2:54. [3] Smith et al. 2006, *Anal. Chem.* 78:779-787; Tautenhahn et al. 2008, *BMC Bioinformatics* 9:504. [4] Kuhl et al. 2012, *Anal. Chem.* 84:283-289. [5] DGCC, diacylglyceryl carboxyhydroxymethylcholine; DGDG, digalactosyldiacylglycerol; DGTS & DGTA, diacylglyceryl trimethylhomoserine and diacylglyceryl hydroxymethyl-trimethyl-β-alanine; IPL, intact polar lipid; ox-IPL, oxidized intact polar lipid; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol [6] $P \leq 0.05$ (bold), $P \leq 0.01$ (*), $P \leq 0.001$ (**), and $P \leq 0.0001$ (***). We thank Elizabeth Kujawinski, Krista Longnecker, and Winn Johnson for advice on metabolomics methods; Eugene Melamud for assistance with MAVEN; and Assaf Vardi, Shiri Graff van Creveld, and Daniella Schatz for assistance with the *P. tricornutum* dataset. This research was supported by the Gordon and Betty Moore Foundation and a U.S. Environmental Protection Agency (EPA) STAR Graduate Fellowship to J.R.C. under Fellowship Assistance Agreement no. FP-91744301-0. The contents of this poster have not been formally reviewed by EPA. The views expressed in this poster are solely those of the authors, and EPA does not endorse any products or commercial services mentioned in this poster.