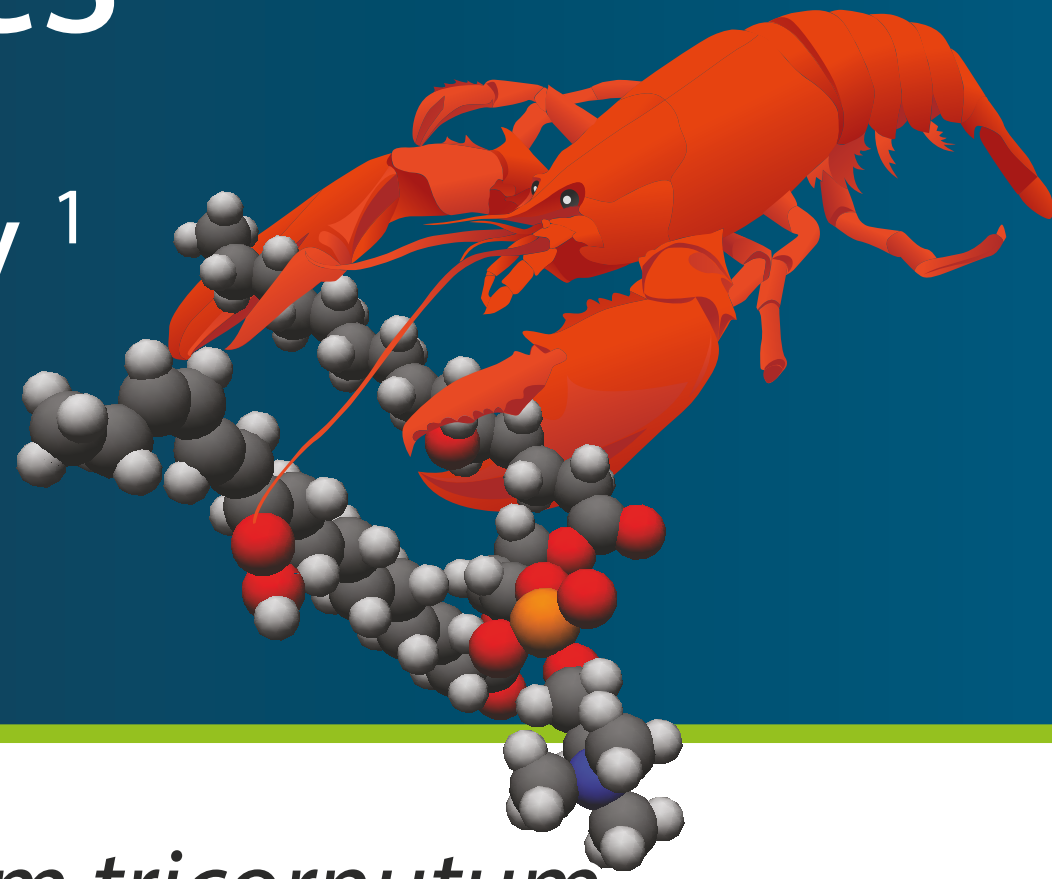# Discovery & identification of oxidative stress biomarkers in marine microbes using LOBSTAHS, a new R package for lipidomics

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
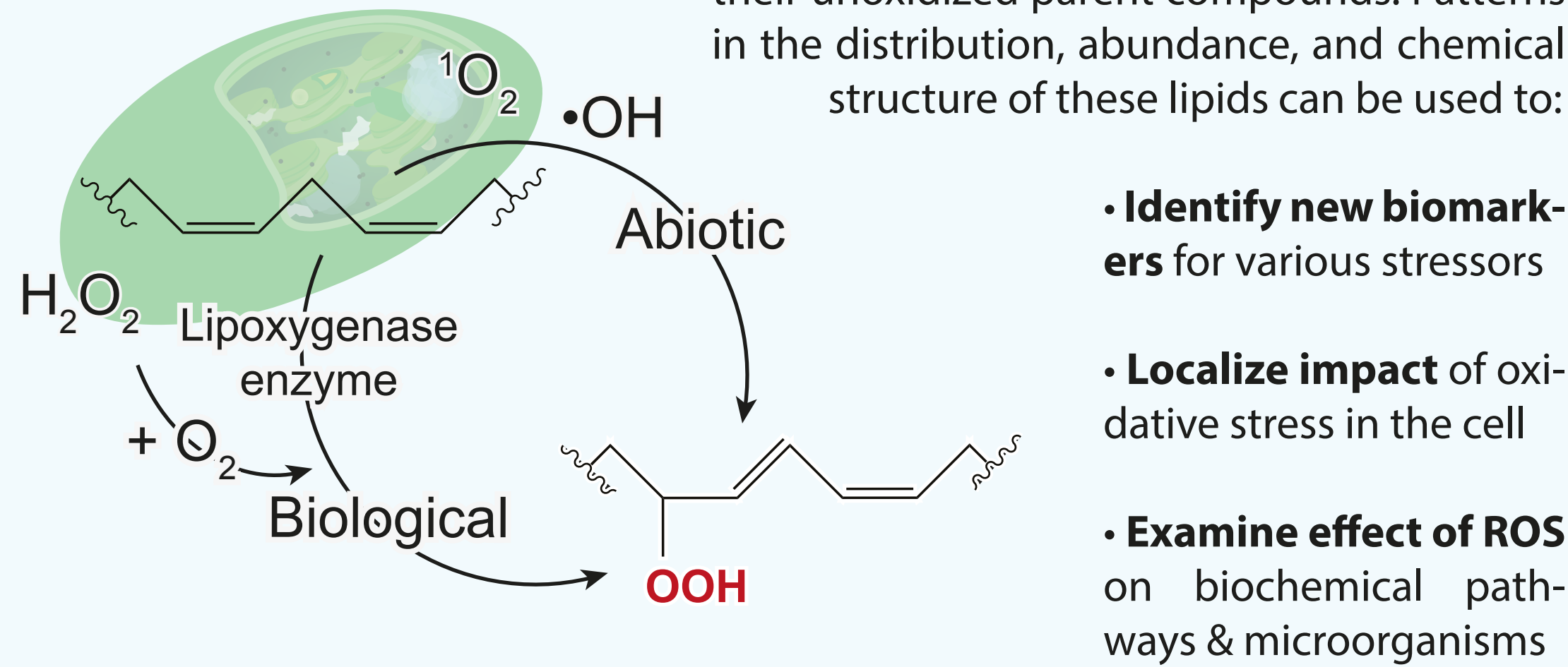[2] MIT/WHOI Joint Program in Oceanography

Introduction: ROS and the algal lipidome

Oxidative stress exerts a profound impact on the lives of marine microbes, including **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:
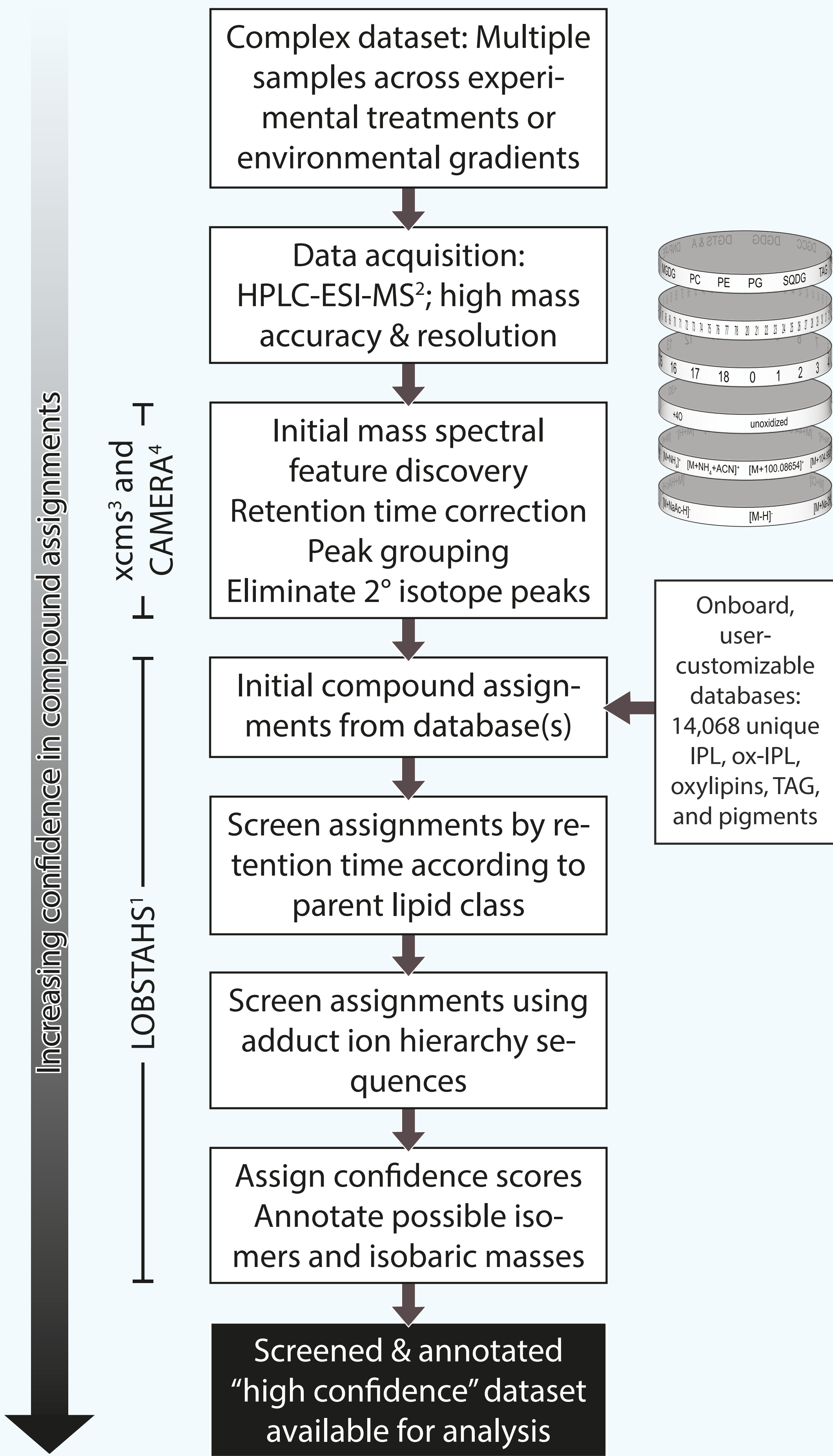

- **Identify new biomarkers** for various stressors
- **Localize impact** of oxidative stress in the cell
- **Examine effect of ROS** on biochemical pathways & microorganisms

LOBSTAHS: An R package for lipidomics discovery

We developed **LOBSTAHS**[1] (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 is **available via Bioconductor or GitHub**. The package can be **downloaded via the QR code** at right.
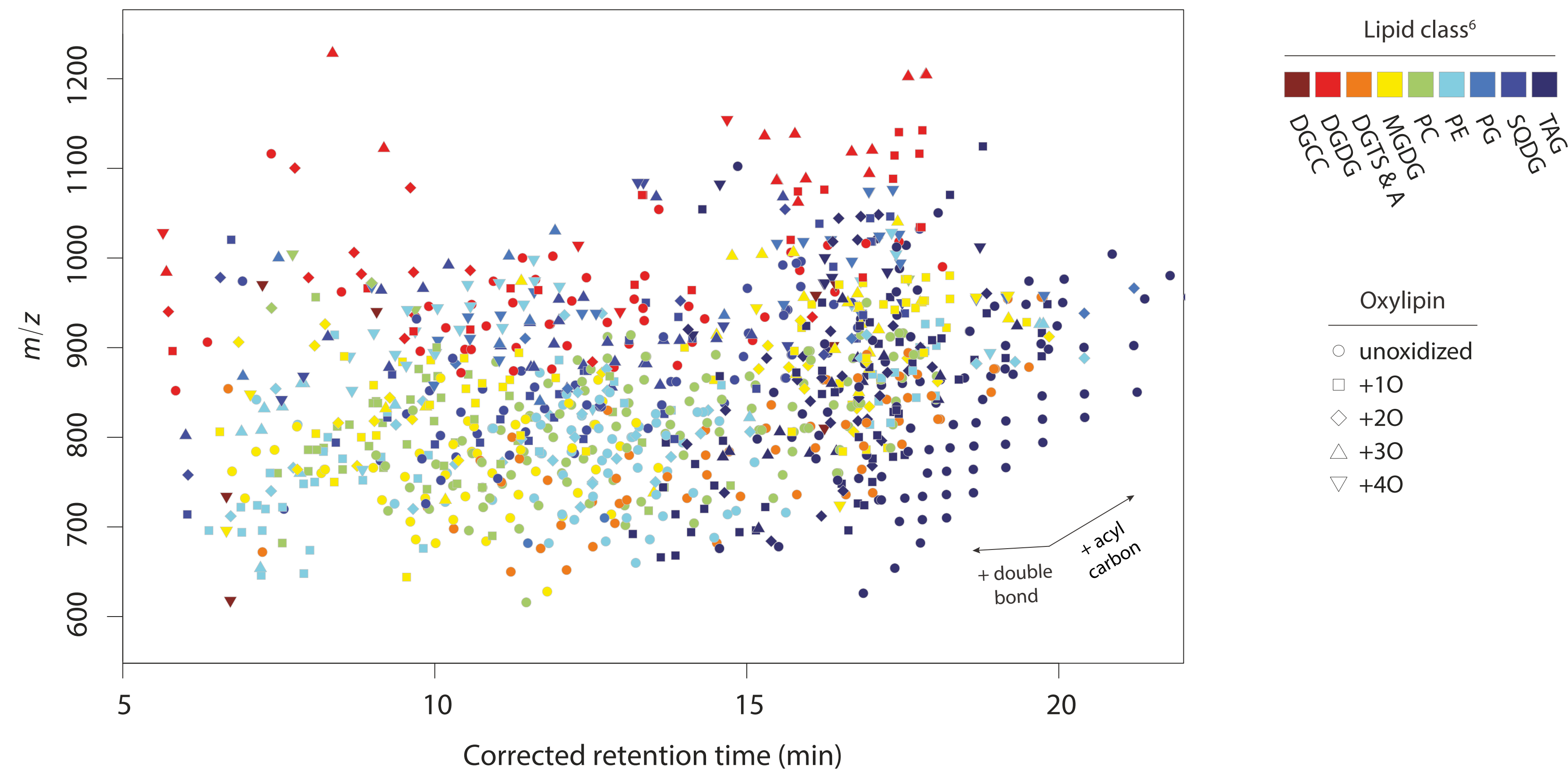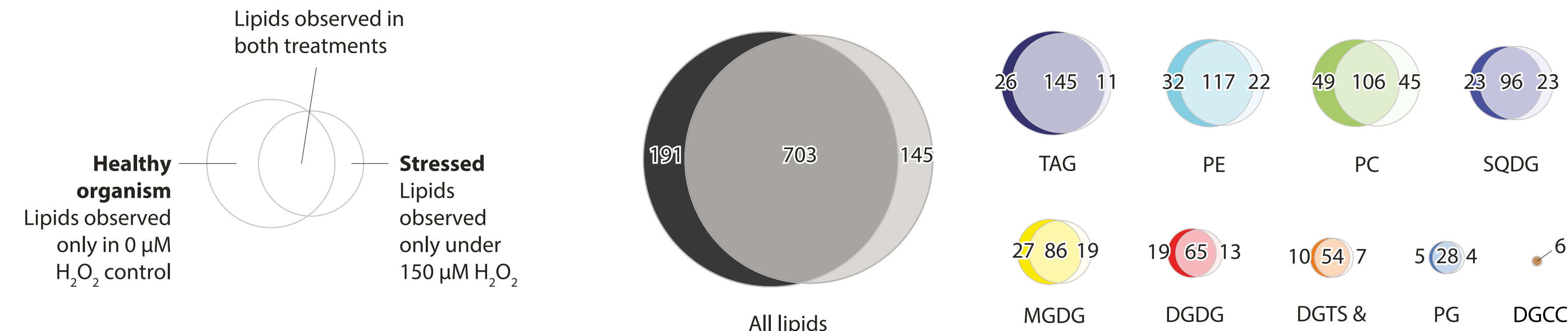

https://github.com/vanmooylipidomics/LOBSTAHS/

Increasing confidence in compound assignments →

- Complex dataset: Multiple samples across experimental treatments or environmental gradients
- Data acquisition: HPLC-ESI-MS[2]; high mass accuracy & resolution
- Initial mass spectral feature discovery / Retention time correction / Peak grouping / Eliminate 2° isotope peaks — *xcms[3] and CAMERA[4]*
- Initial compound assignments from database(s) ← Onboard, user-customizable databases: 14,068 unique IPL, ox-IPL, oxylipins, TAG, and pigments
- Screen assignments by retention time according to parent lipid class — *LOBSTAHS[1]*
- Screen assignments using adduct ion hierarchy sequences
- Assign confidence scores / Annotate possible isomers and isobaric masses
- Screened & annotated "high confidence" dataset available for analysis

Results: Oxidative stress in the marine diatom *Phaeodactylum tricornutum*

We applied **LOBSTAHS** to lipid data from an experiment[5] in which hydrogen peroxide (H_2O_2) was used to **induce oxidative stress** in the marine diatom *Phaeodactylum tricornutum*.

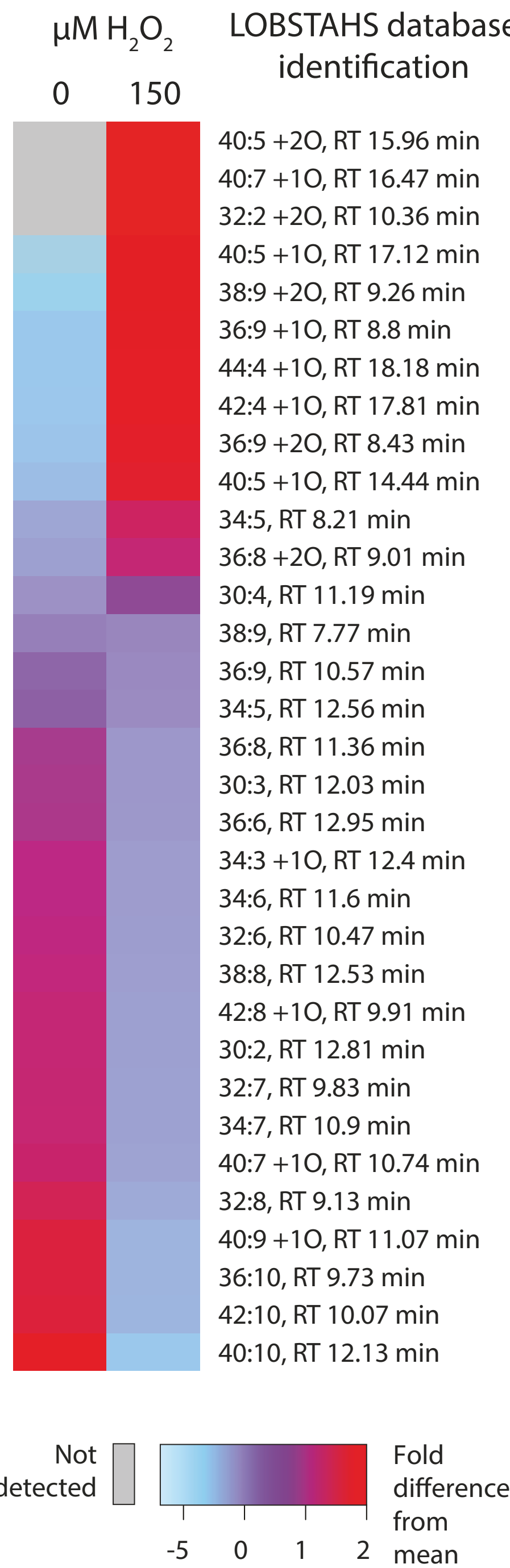### LOBSTAHS identifies 1039 unique oxidized and unoxidized lipids that serve as potential biomarkers

Lipid class[6]: DGCC, DGDG, MGDG, DGTS & A, PC, PE, PG, SQDG, TAG

Oxylipin: ○ unoxidized, □ +1O, ◇ +2O, △ +3O, ▽ +4O

+ double bond, + acyl carbon

Within each lipid class[6], certain molecules are associated only with oxidative stress

Lipids observed in both treatments

Healthy organism — Lipids observed only in 0 µM H_2O_2 control

Stressed — Lipids observed only under 150 µM H_2O_2

All lipids: 191 | 703 | 145

TAG: 26 | 145 | 11
PE: 32 | 117 | 22
PC: 49 | 106 | 45
SQDG: 23 | 96 | 23
MGDG: 27 | 86 | 19
DGDG: 19 | 65 | 13
DGTS & DGTA: 10 | 54 | 7
PG: 5 | 28 | 4
DGCC: 6

Individual lipidome constituents differentially expressed with oxidative stress

Example: Monogalactosyldiacylglycerol "MGDG"; typical of chloroplast

µM H_2O_2 0 / 150	LOBSTAHS database identification
	40:5 +2O, RT 15.96 min
	40:7 +1O, RT 16.47 min
	32:2 +2O, RT 10.36 min
	40:5 +1O, RT 17.12 min
	38:9 +2O, RT 9.26 min
	36:9 +1O, RT 8.8 min
	44:4 +1O, RT 18.18 min
	42:4 +1O, RT 17.81 min
	36:9 +2O, RT 8.43 min
	40:5 +1O, RT 14.44 min
	34:5, RT 8.21 min
	36:8 +2O, RT 9.01 min
	30:4, RT 11.19 min
	38:9, RT 7.77 min
	36:9, RT 10.57 min
	34:5, RT 12.56 min
	36:8, RT 11.36 min
	30:3, RT 12.03 min
	36:6, RT 12.95 min
	34:3 +1O, RT 12.4 min
	34:6, RT 11.6 min
	32:6, RT 10.47 min
	38:8, RT 12.53 min
	42:8 +1O, RT 9.91 min
	30:2, RT 12.81 min
	32:7, RT 9.83 min
	34:7, RT 10.9 min
	40:7 +1O, RT 10.74 min
	32:8, RT 9.13 min
	40:9 +1O, RT 11.07 min
	36:10, RT 9.73 min
	42:10, RT 10.07 min
	40:10, RT 12.13 min

Not detected — Fold difference from mean: -5, 0, 1, 2

Significant[7] changes in structural properties by lipid class can be used to localize effects of oxidative stress within the cell

Lipid Class or Functional Grouping	Within-Group Mean		
	Fatty Acid Carbon Atoms	Degrees Unsaturation	Degree of Oxidation
MGDG lipids (typical of chloroplast)			
Moieties ⬆ under 150 µM H_2O_2	38.6	5.2	1.9***
Moieties ⬇ under 150 µM H_2O_2	37.6	6.2	0.5***
Mitochondrial lipids (PE, PG)			
Moieties ⬆ under 150 µM H_2O_2	41.3	3.5	1.7
Moieties ⬇ under 150 µM H_2O_2	39.6	4.8	1.8
All lipids			
Moieties ⬆ under 150 µM H_2O_2	42.1***	4.6	1
Moieties ⬇ under 150 µM H_2O_2	38.9***	5	1.1

Discussion, summary, and significance

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:

- **LOBSTAHS** software facilitates elucidation of effects of oxidative stress by lipid class
- **Specific molecular biomarkers** were identified
- **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** most evident in monogalactosyldiacylglycerol (MGDG), a lipid typically **localized** to the **chloroplast**
- Carbon-chain **elongation** also evident in lipids of the **mitochondrion**
- Additionally, elongation under **oxidative stress** was accompanied by **reallocation of biomass to TAGs**

[1] Collins et al. 2016, *Anal. Chem.* 88:7154-7162. [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] 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. [6] 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. [7] P≤0.05 (bold), P≤0.01 (*), P≤0.001 (**), and P≤0.0001 (***).

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