

*Small Molecule Modulators of Lipid Production in
Microalgae and NMR Spectroscopy of Lipids*

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8th Annual Algae Biomass Summit

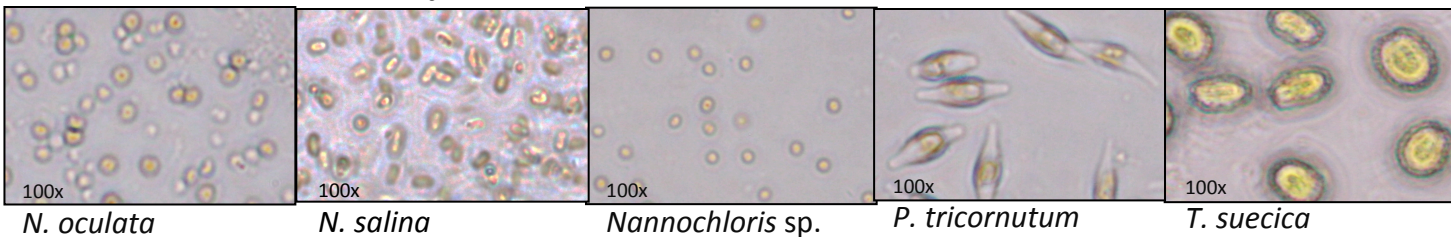
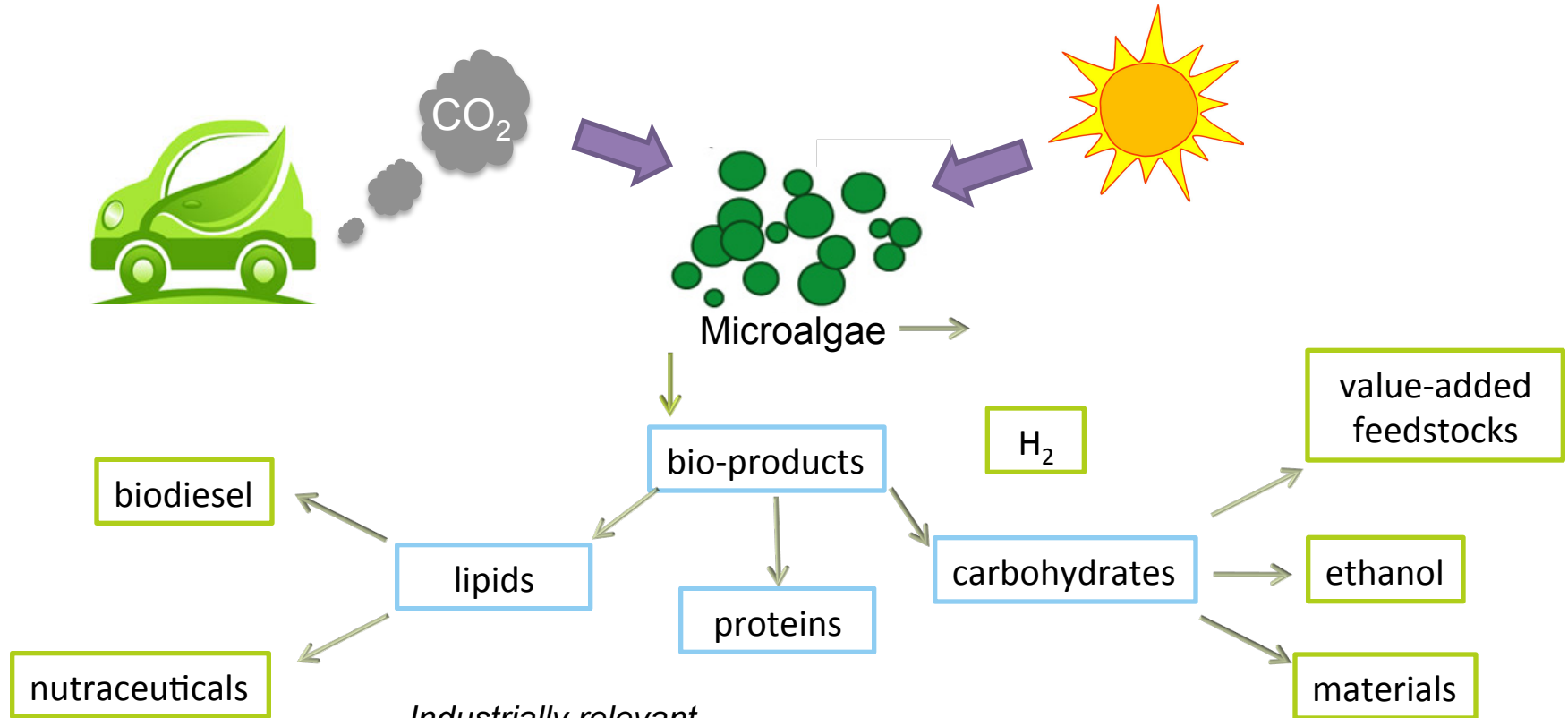
San Diego, California

Progress in Algae Research, Metabolic Regulation

September 30, 2014



Microalgae are Photosynthetic Factories



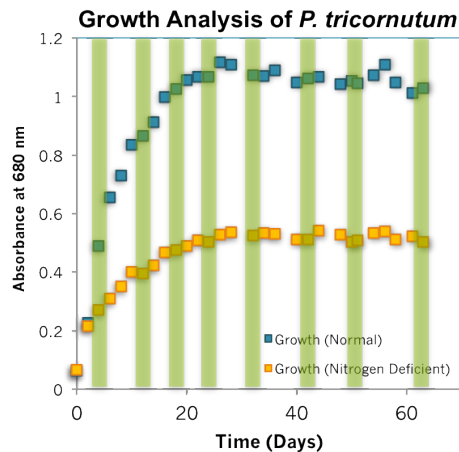
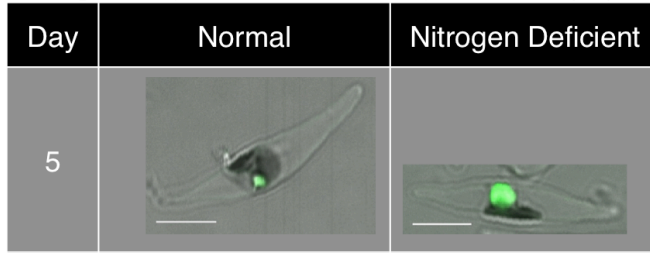
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Methods and Barriers for Modulating and Understanding Microalgae Lipid Metabolism

Lipid Analysis of *P. tricornutum* by LSCM Imaging



- Nutrient limitation or “stress”
- Heterotrophic growth conditions
- Genetic manipulation
 - Difficult but advancing
 - Algae model species
 - *Chlamydomonas reinhardtii* (“the green yeast”)
 - *Phaeodactylum tricornutum*

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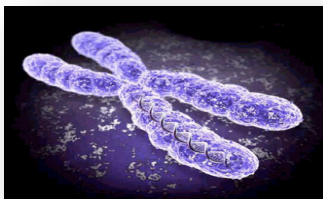
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Chemical Genetic Approach to Modulate Algae Lipid Production

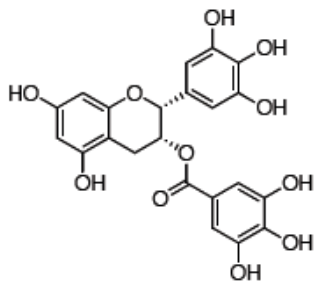
Classical Genetics



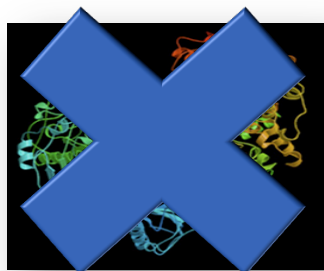
Mutant Gene



Chemical Genetics



Small Molecule



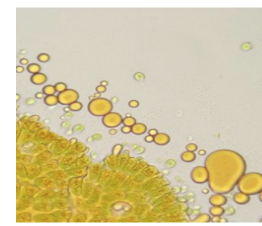
Protein



Phenotype



Wild-Type

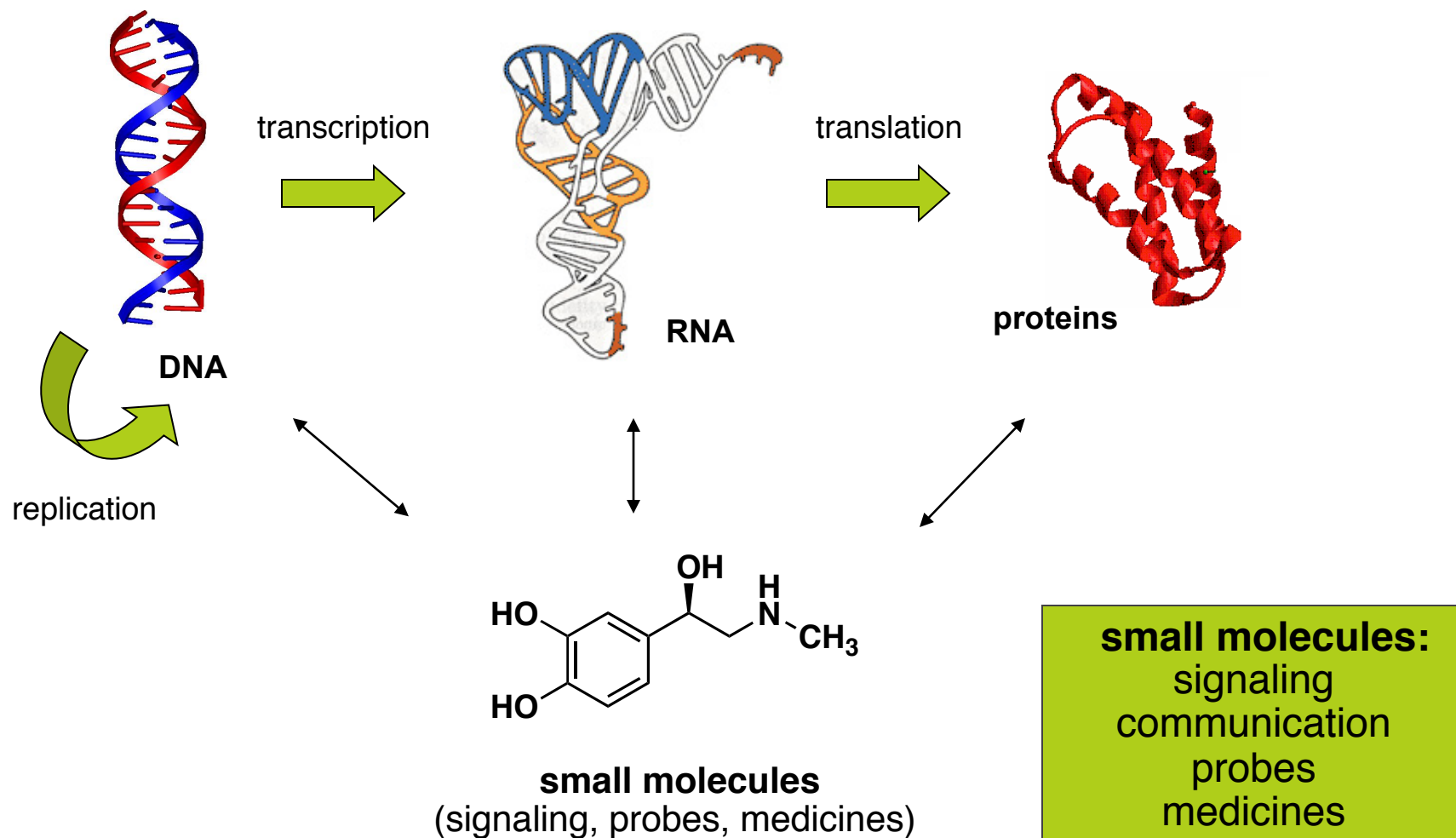


“Mutant”

Features of Chemical Genetics

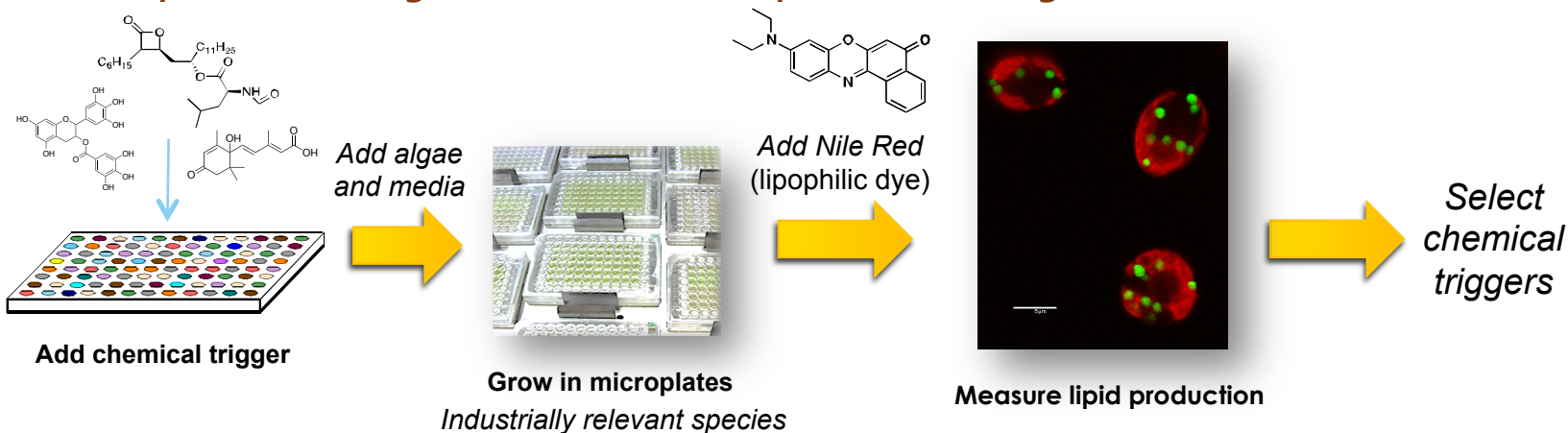
- 1) Direct changes in real-time
- 2) Phenotype can be reversible
- 3) Concentration effects
- 4) Temporal effects
- 5) Synergistic compound effects
- 6) Combine for use with engineered strains

Organic Molecules for Dynamic Information Flow

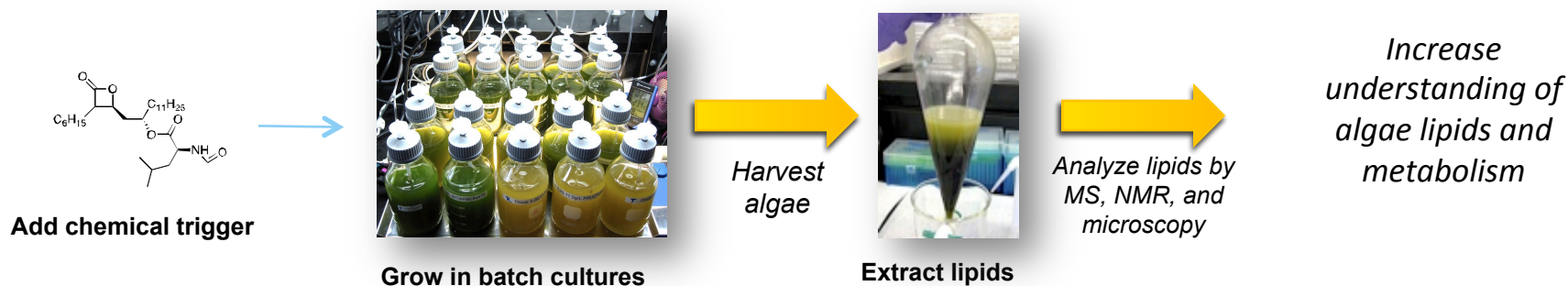


Screening Approach

Phase I: microplate screening & Phase II: dose response screening



Phase III: 10+ selected lead compounds for 500 mL cultures



Initial Screening Set

Primary target

← Kinases/phosphorylases

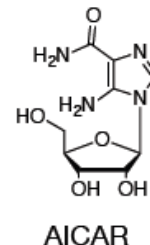
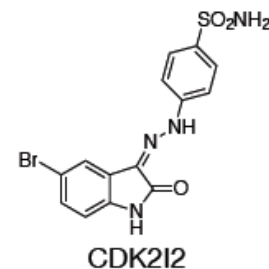
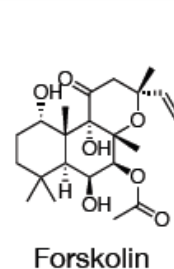
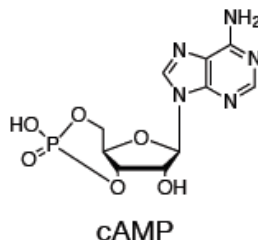
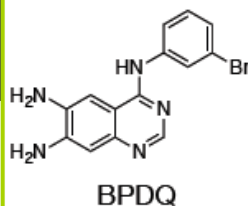
← Lipid metabolism

← Lipid oxidation

← Protein synthesis

← Other
(e.g. phytohormones)

Kinase signaling



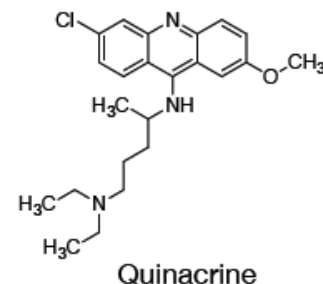
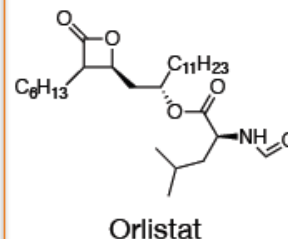
Chemical Structure

Bioactivity

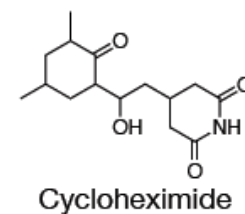
antioxidant
oxidative signaling
cell cycle regulation
plant growth
anti-carcinogenic
anti-proliferative
anti-inflammatory
anti-fungal

phenolics
flavones
terpenoids
purine derivatives
alkaloids
triazoles
oxindoles
fatty acids
Indole-derived
maleimides

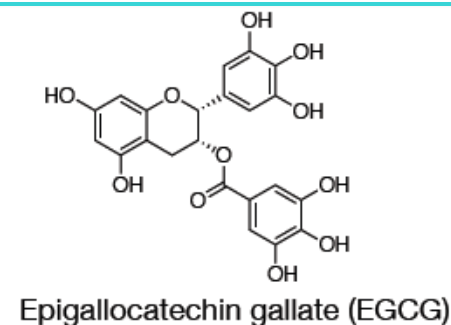
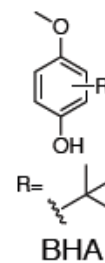
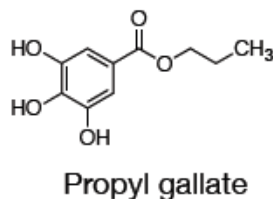
Lipid signaling



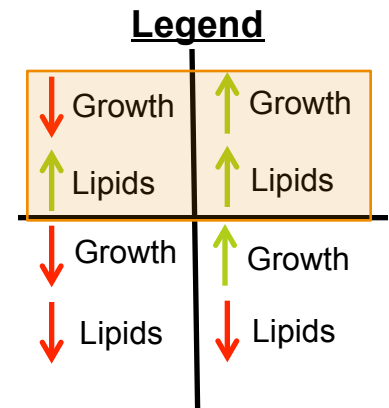
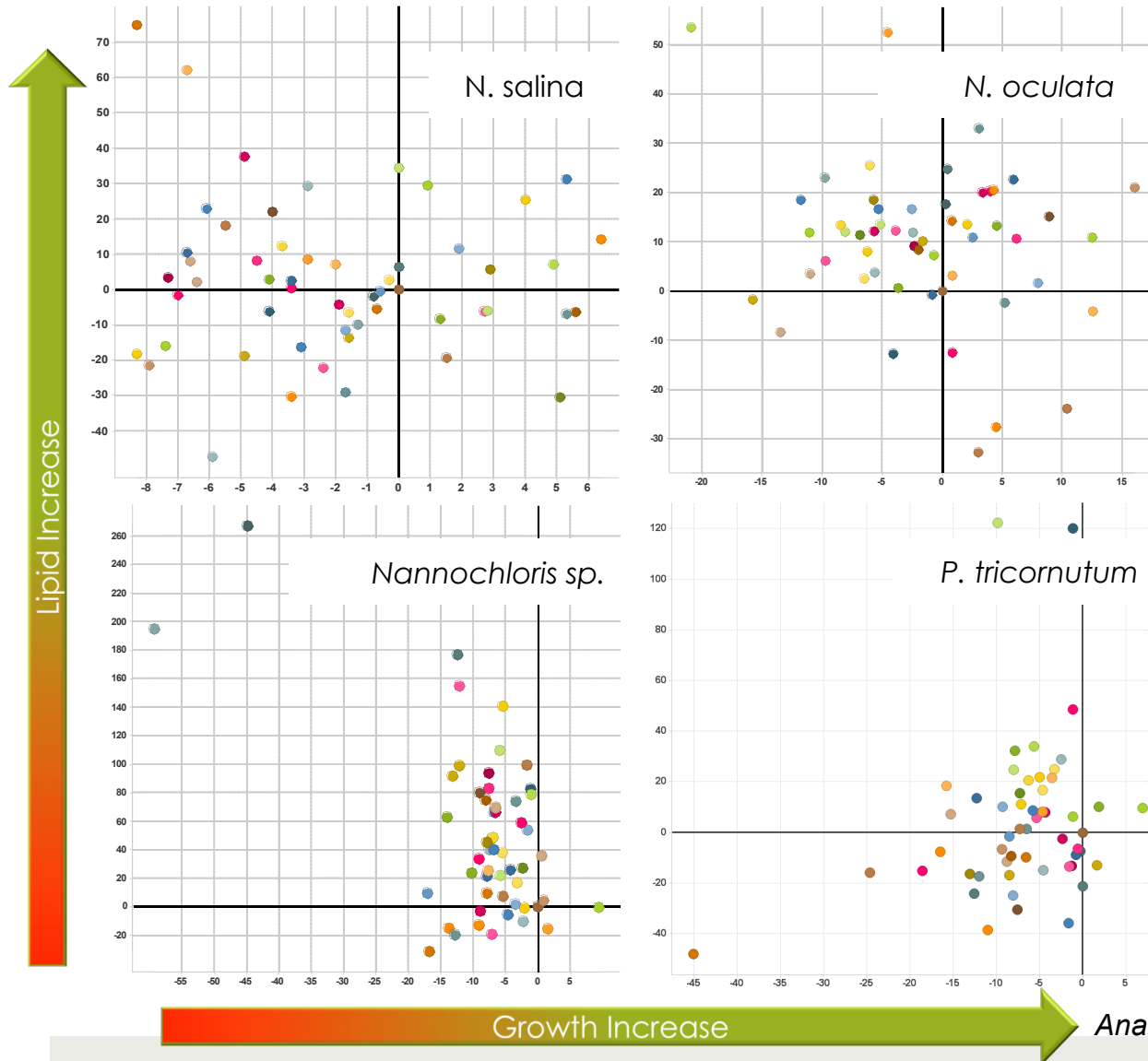
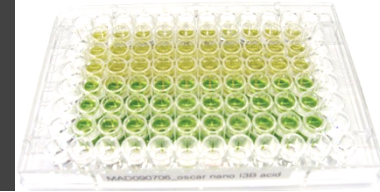
Protein synthesis



Oxidative signaling

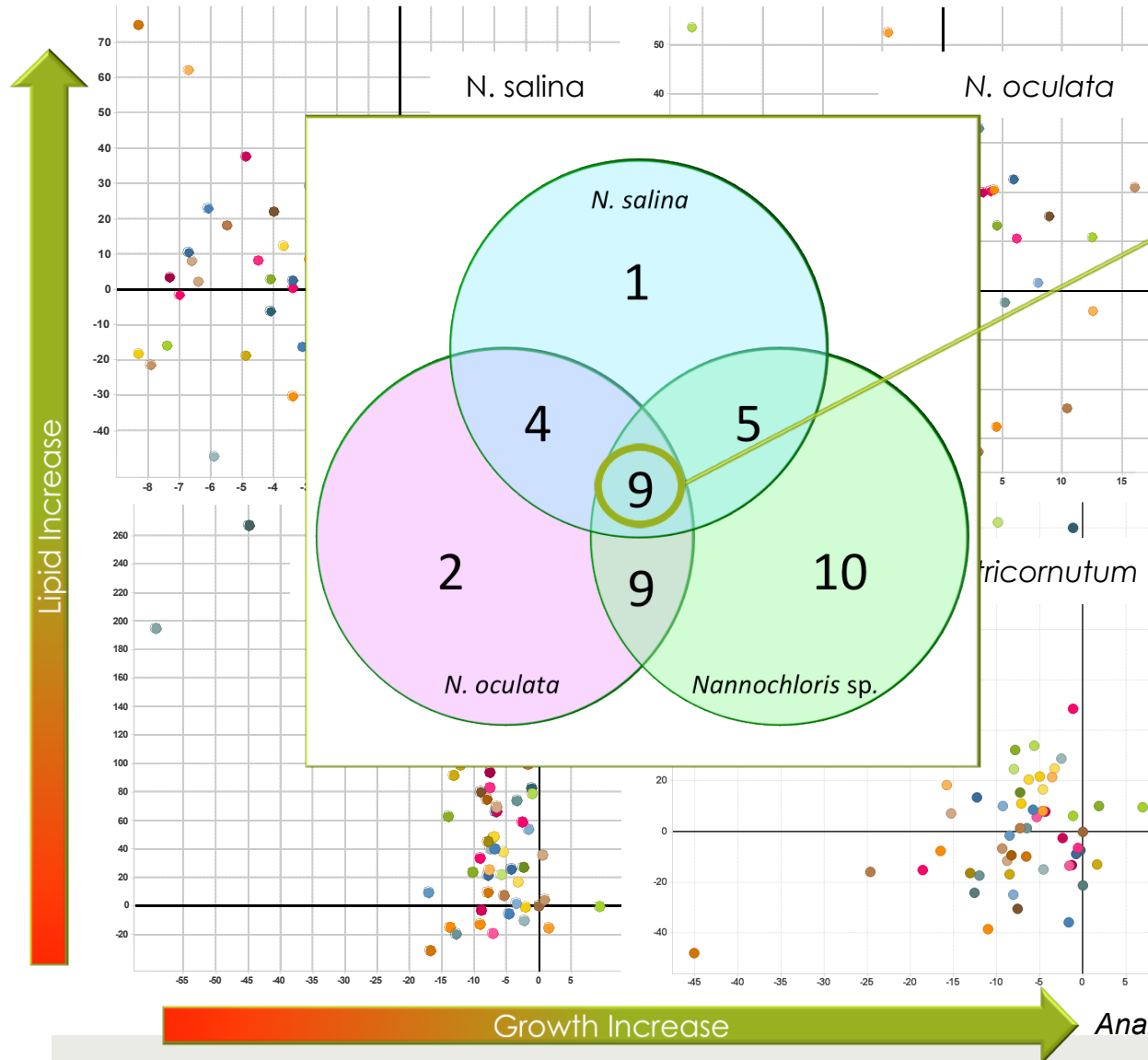
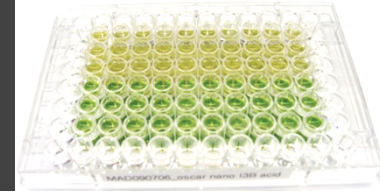


Diverse Compound Screening Reveals Small Molecule Modulators

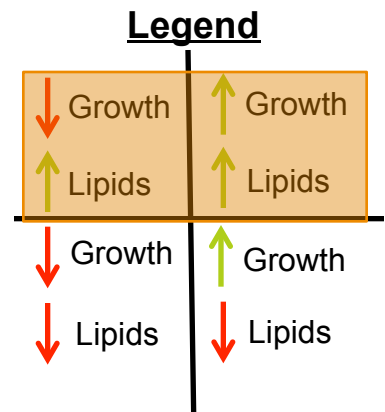


Analysis performed with Spotfire

Diverse Compound Screening Reveals Small Molecule Modulators

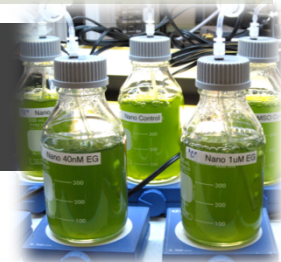


Compounds such as:
 epigallocatechin gallate
 PTP Inhibitor II
 CDK2 Inhibitor 2
 MAP sb202190
 quercetin
 kinetin
 cycloheximide

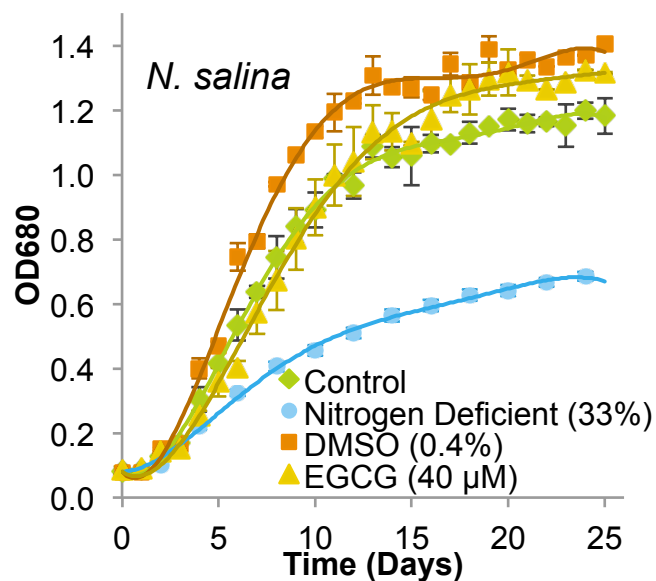


Analysis performed with Spotfire

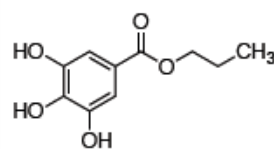
Chemical Triggers in 500 mL Cultures



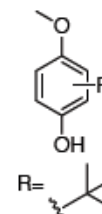
Small Molecule	Specific Growth Rate (day ⁻¹)	Lipid Content (% w/w)	Lipid Productivity (mg L ⁻¹ day ⁻¹)
Control	0.23 ± 0.06	23.6 ± 9.0	4.7 ± 1.4
Quinacrine (40 nM)	0.25 ± 0.02	26.5 ± 3.9	4.5 ± 1.0
EGCG (40 μM)	0.22 ± 0.06	23.1 ± 10.6	6.3 ± 2.2
DMSO (0.4%)	0.26 ± 0.07	24.3 ± 7.5	6.5 ± 1.7
cAMP (4 μM)	0.22 ± 0.03	28.6 ± 10.7	7.9 ± 3.4
EGCG (in water) (4 μM)	0.24 ± 0.01	32.4 ± 0.1	7.1 ± 1.2
BHA (4 nM)	0.23 ± 0.00	28.8 ± 11.4	8.1 ± 0.6
Propyl gallate (40 nM)	0.23 ± 0.00	25.9 ± 11.4	8.3 ± 1.7



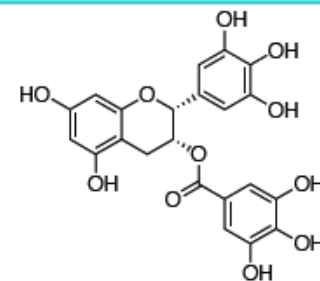
Oxidative signaling



Propyl gallate



BHA



Epigallocatechin gallate (EGCG)

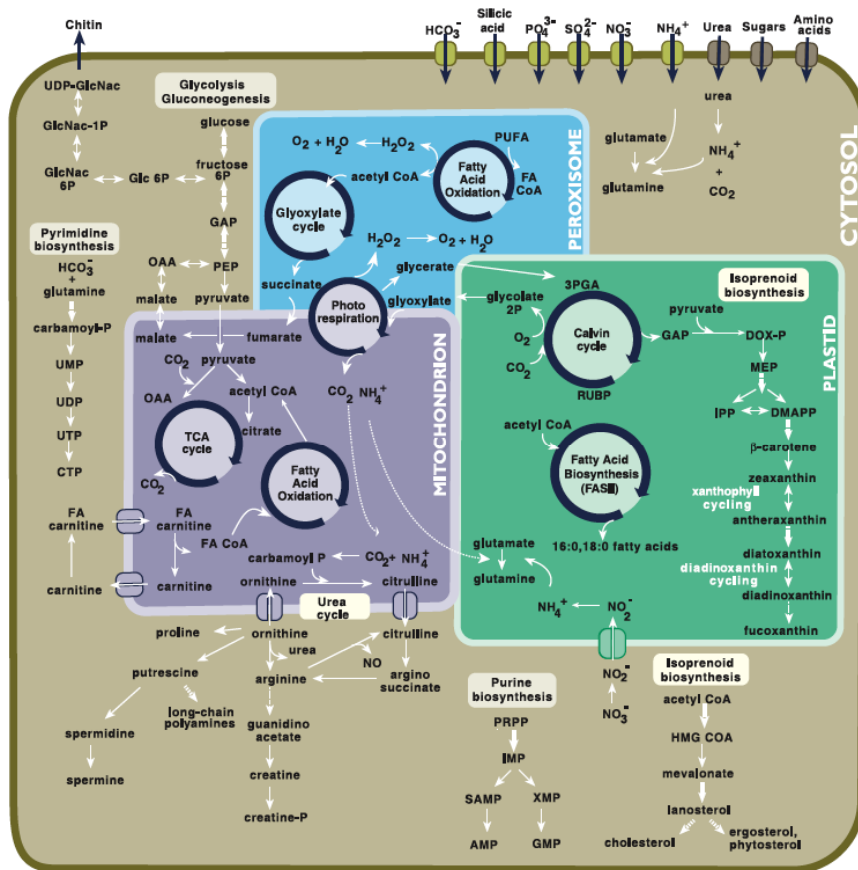
Cost Analysis for Lead Compounds



Compound	Price/gram	Concentration (identified in screening)	Amount needed	Price per dose
EGCG	\$1,200.00	4 mM	91.68 g	\$110,016.00
cAMP	\$107.00	4 mM	65.84 g	\$7,044.88
Forskolin	\$4,950.00	4 nM	0.0821 g	\$406.40
BHA	\$0.06	4 nM	0.036 g	\$0.002
Propyl gallate	\$0.09	40 nM	0.4244 g	\$0.04
DMSO	\$64.16/L	0.04%	20 L	\$1,283.20

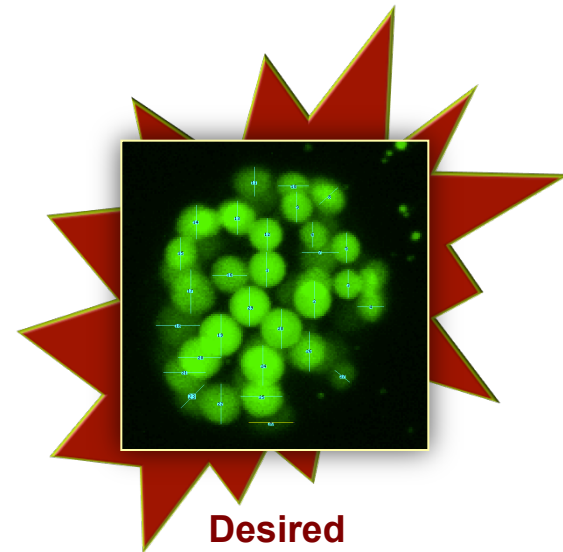
crude source of chemical triggers?

What pathway are chemical triggers acting through?



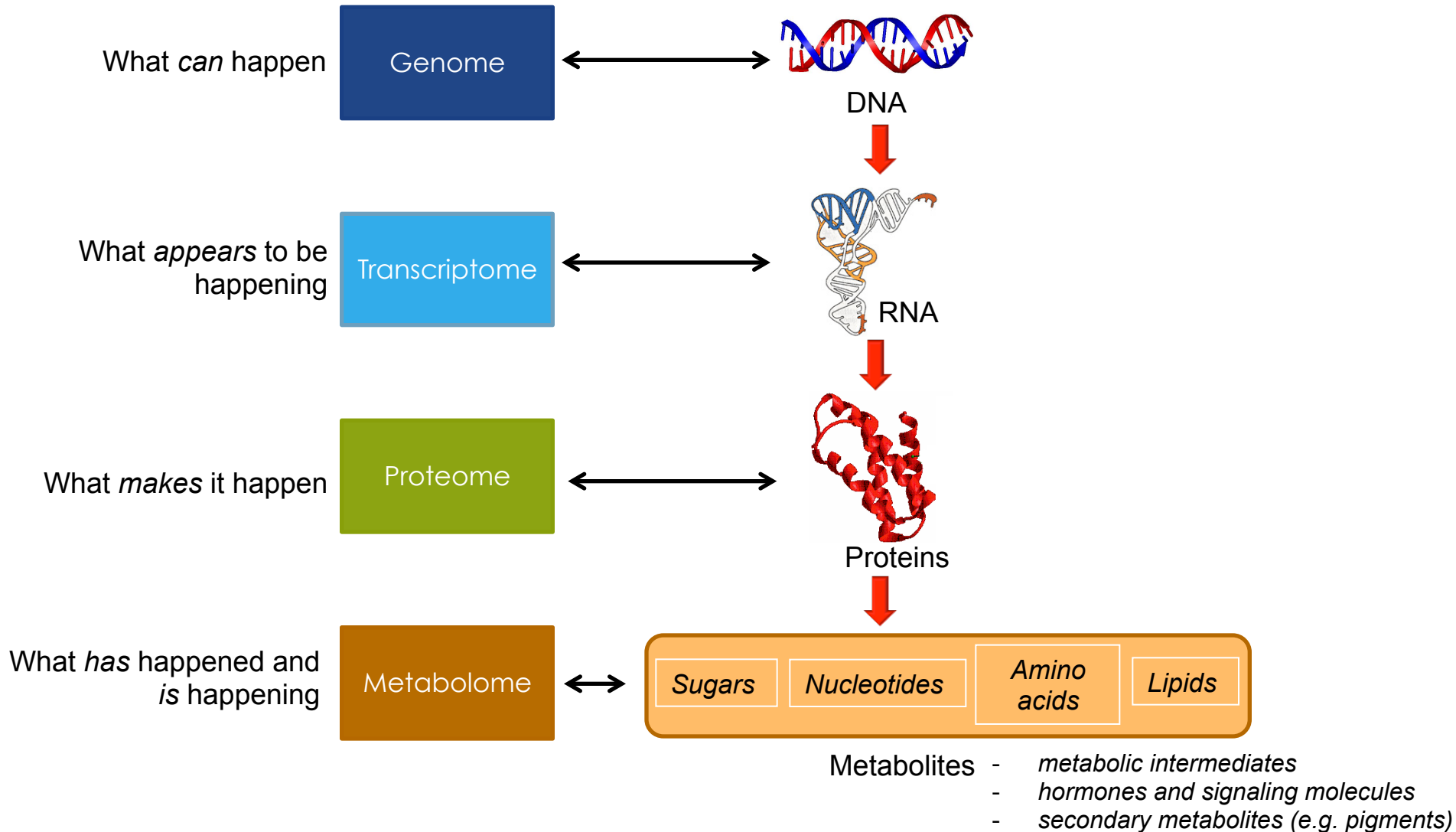
Pathways and targets:

- Antioxidant and oxidative stress
- Signaling kinases
- Lipid metabolism
- Fatty acid oxidation
- Nutrient transport
- Growth stimulation and cell division



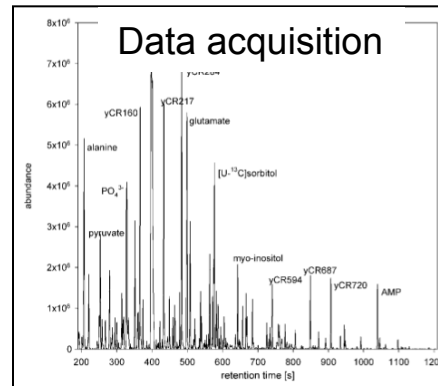
Desired Phenotype

What metabolomics can tell us

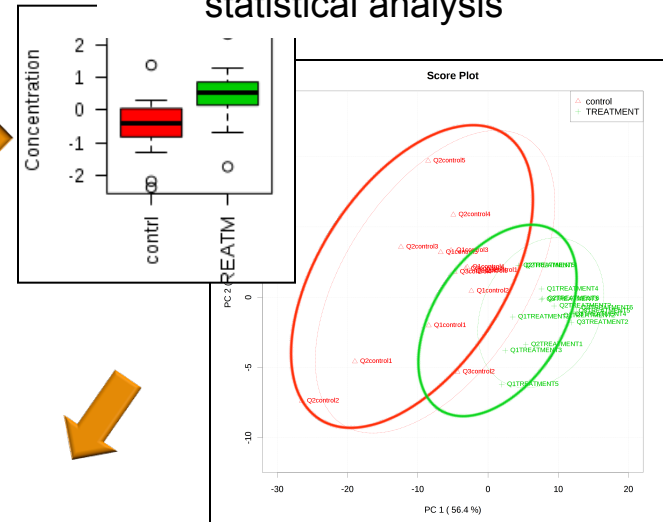


Metabolomics Work Flow

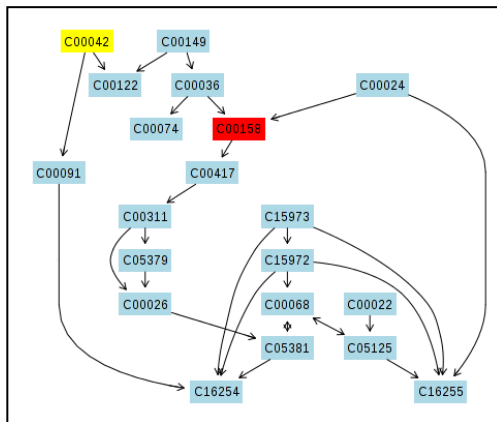
Sample collection and preparation



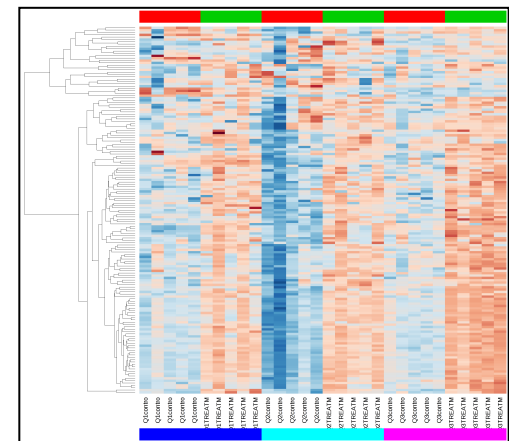
Database curation and statistical analysis



Following biochemical pathways and networks

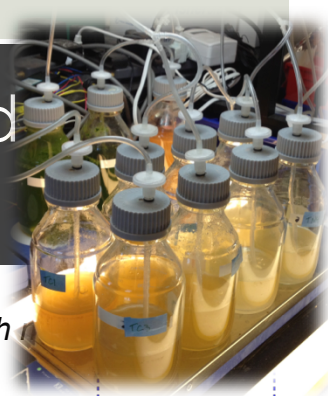


Modeling metabolic interactions



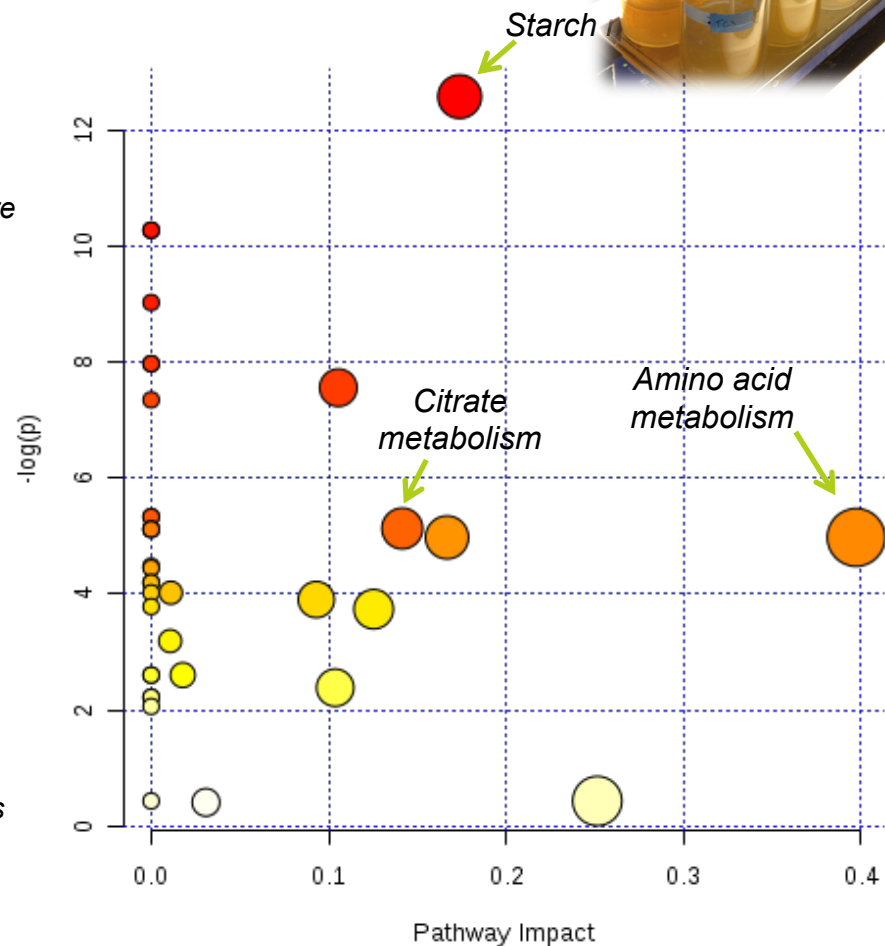
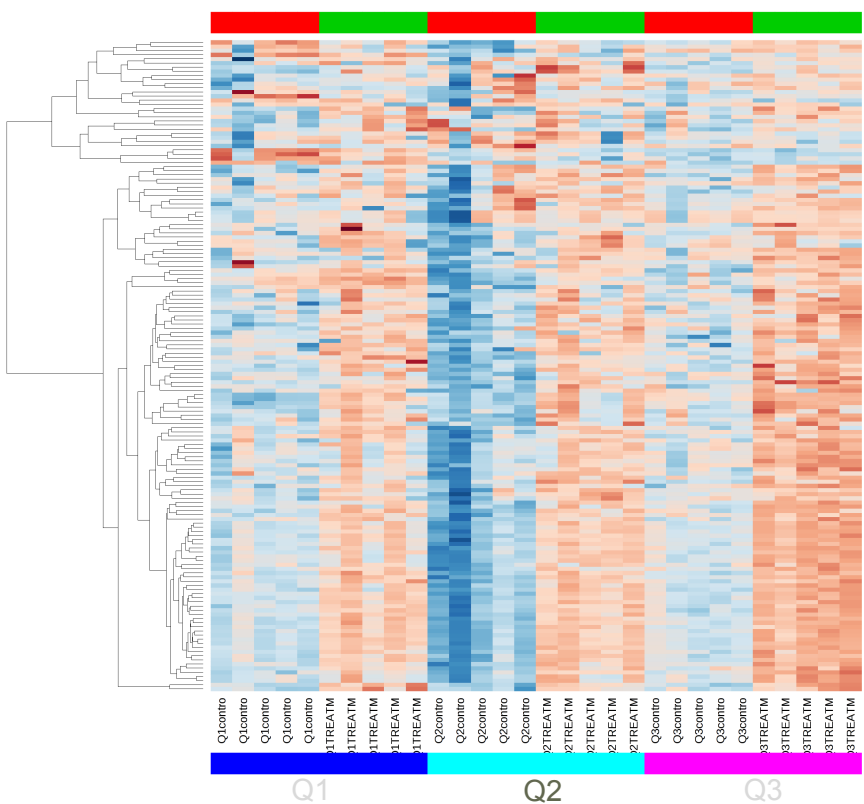
- Mechanistic insight
- Toxicology
- Classification
- Prediction
- Functional genomics

Pattern of regulated features was detected according to nitrogen deficiency

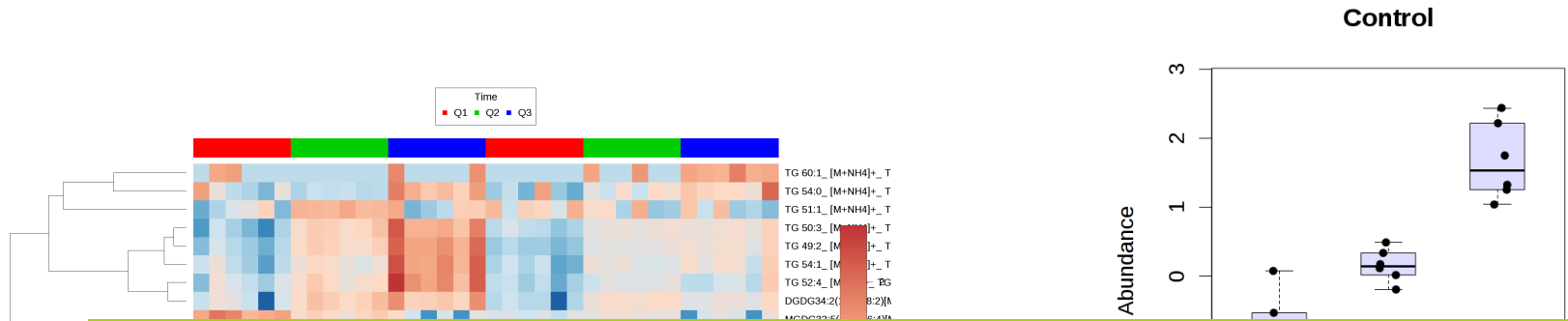


P. tricornutum

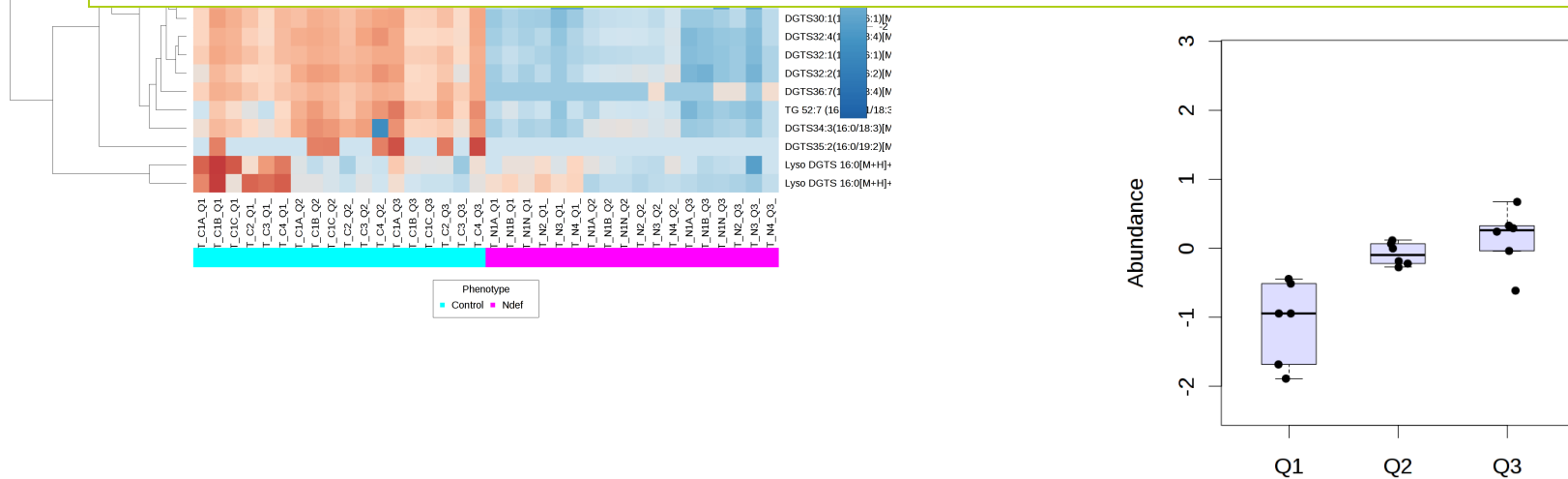
■ = control ■ = N-def



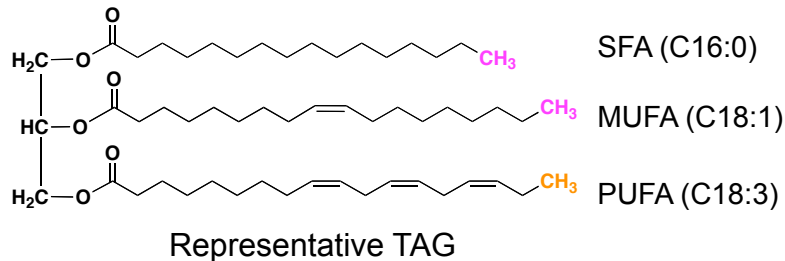
Lipidomics of *P. tricornutum* under nitrogen deficiency displays distinct differences



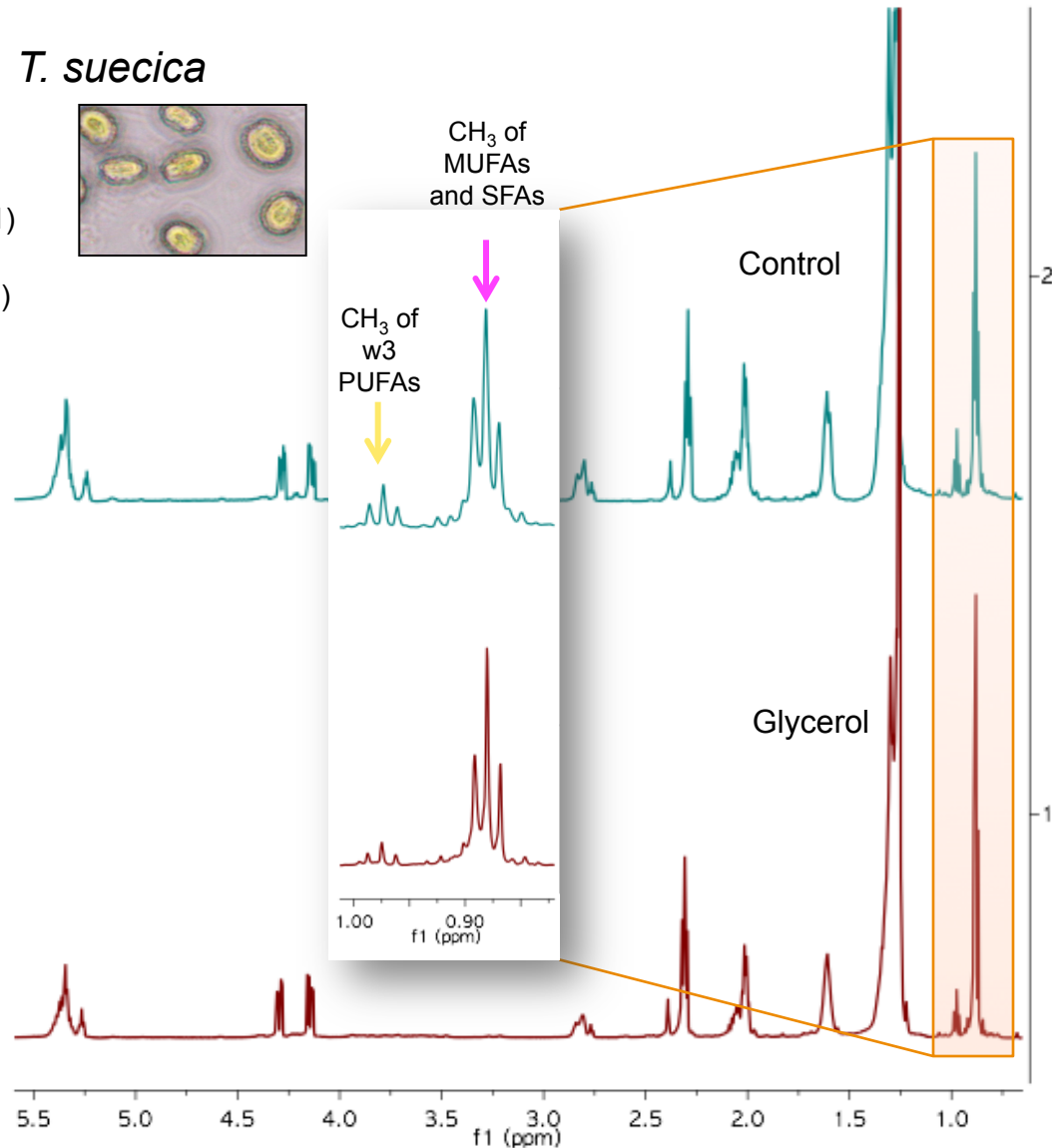
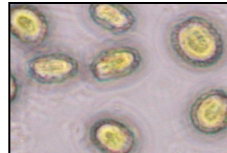
On going experiments: treatment with antioxidants such as EGCG will increase metabolites and lipids relating to antioxidant defense system, membrane integrity, and osmoprotection, along with changes in TCA cycle intermediates that correlate to fatty acid biosynthesis.



Lipid Composition by ^1H NMR Spectroscopy



T. suecica



	SFAs (%)	UFAs (%)	w3 PUFAs (%)
Olive Oil	15	85	6
Peanut Oil	14	86	2
<i>N. salina</i>	31	60	8
<i>N. oculata</i>	24	76	28
<i>T. suecica</i>	21	79	16
<i>T. suecica</i> (glycerol)	44	56	9
<i>P. tricornutum</i>	21	68	11

Anderson et al., unpublished

Pollesello, P. et al. *J. Appl. Phycol.* **1992**, 4, 315.

Danielewicz, M. A.; Anderson, L. A.; Franz, A. K. *J. Lipid Res.* **2011**, 52, 2101.

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