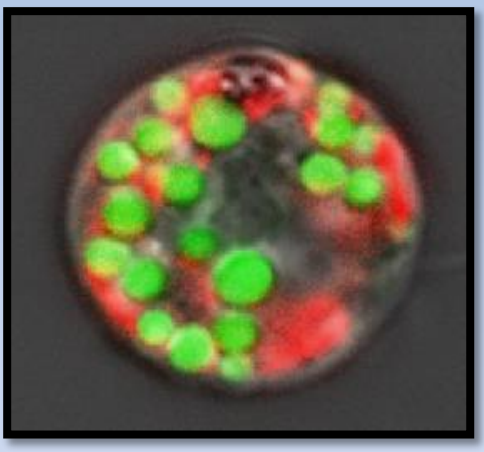
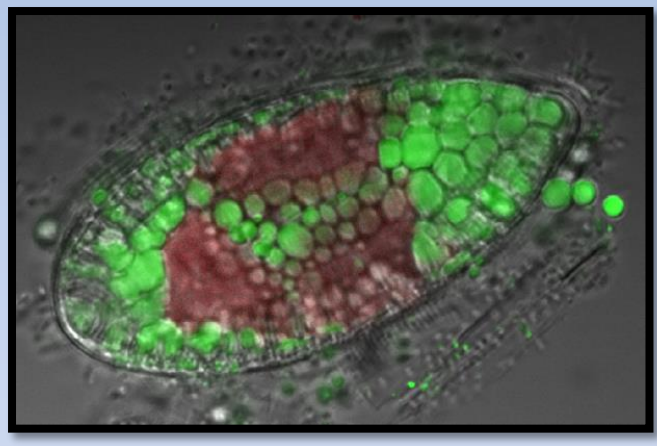
National Algal Biofuels Technology Review

Matthew Posewitz

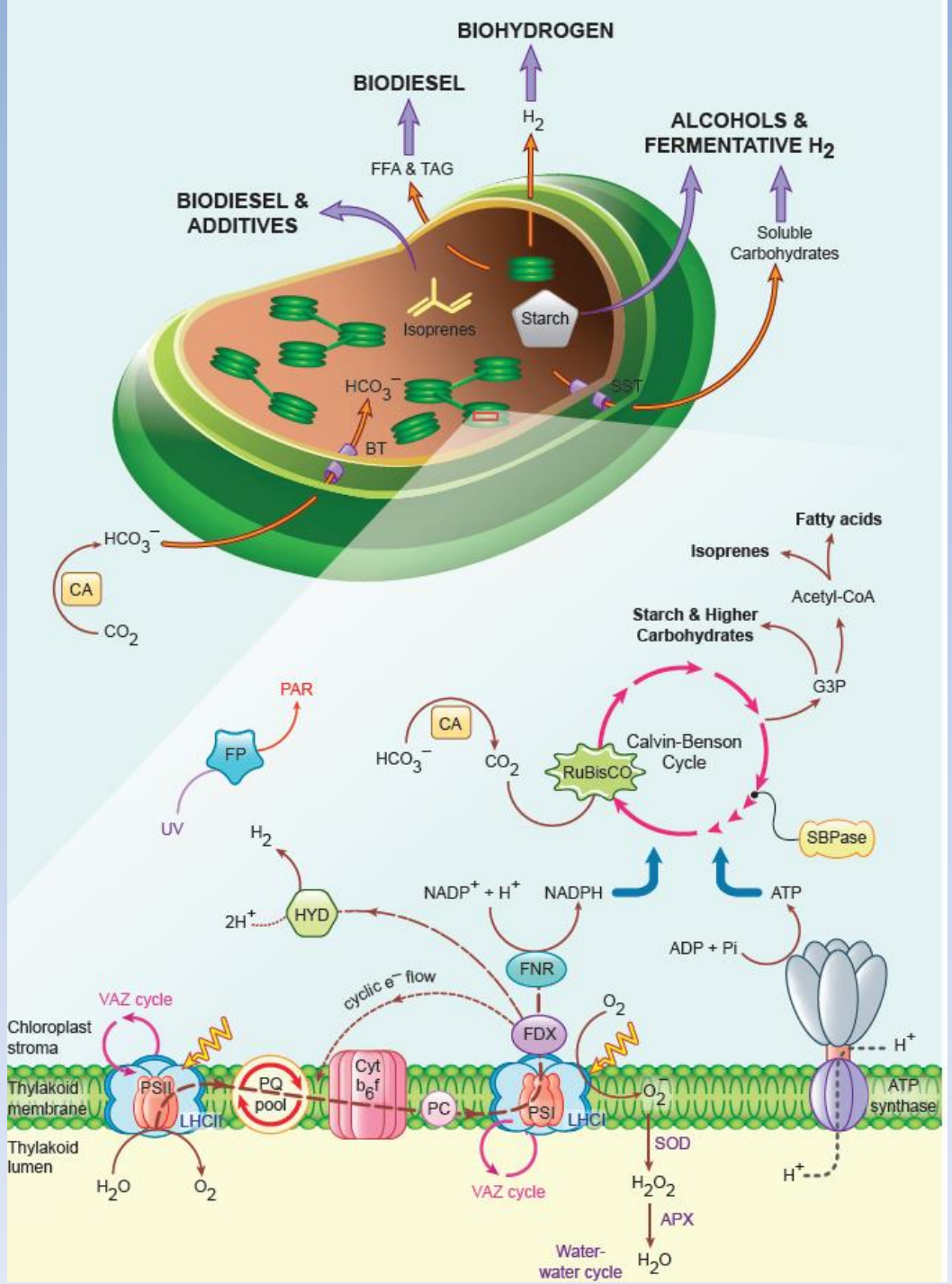
Colorado School of Mines





BIOENERGY
July 14th 2016

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy
BIOENERGY TECHNOLOGIES OFFICE



Roadmap Priority Areas

Current Thrusts

- Improved light harvesting and photon conversion efficiencies
- Enhanced carbon concentrating mechanisms and CO_2 fixation
- Development of robust marine and freshwater algae to new model systems
- Carbon partitioning in model systems to improve fuel quality and biomass
- Secretion of fuels from "catalytic" hosts
- Cell wall characterization for harvesting, processing and enabling growth

Multiple Mechanisms (Opportunities) for Solar Energy Capture Have Evolved in Phototrophic Microorganisms

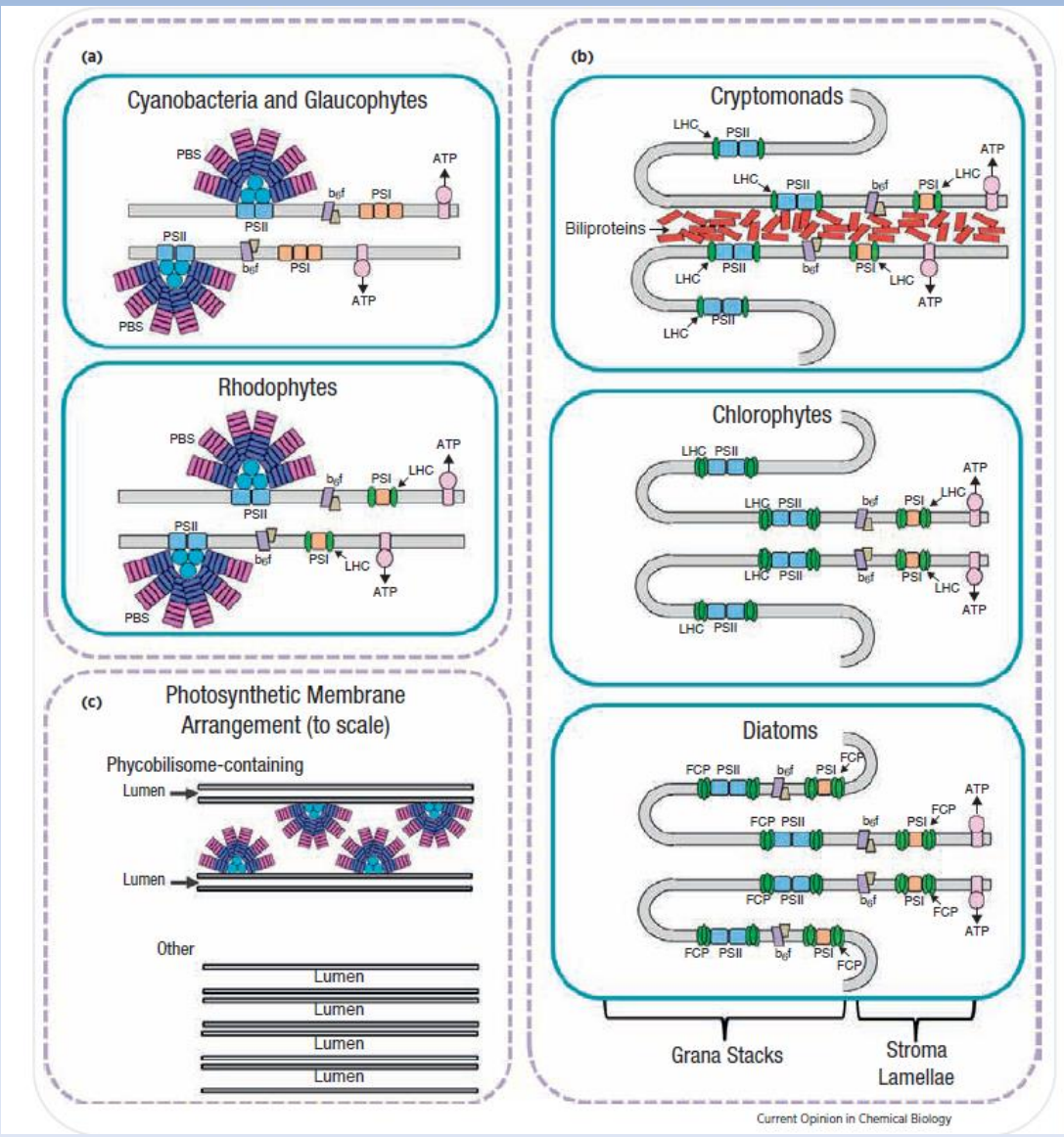
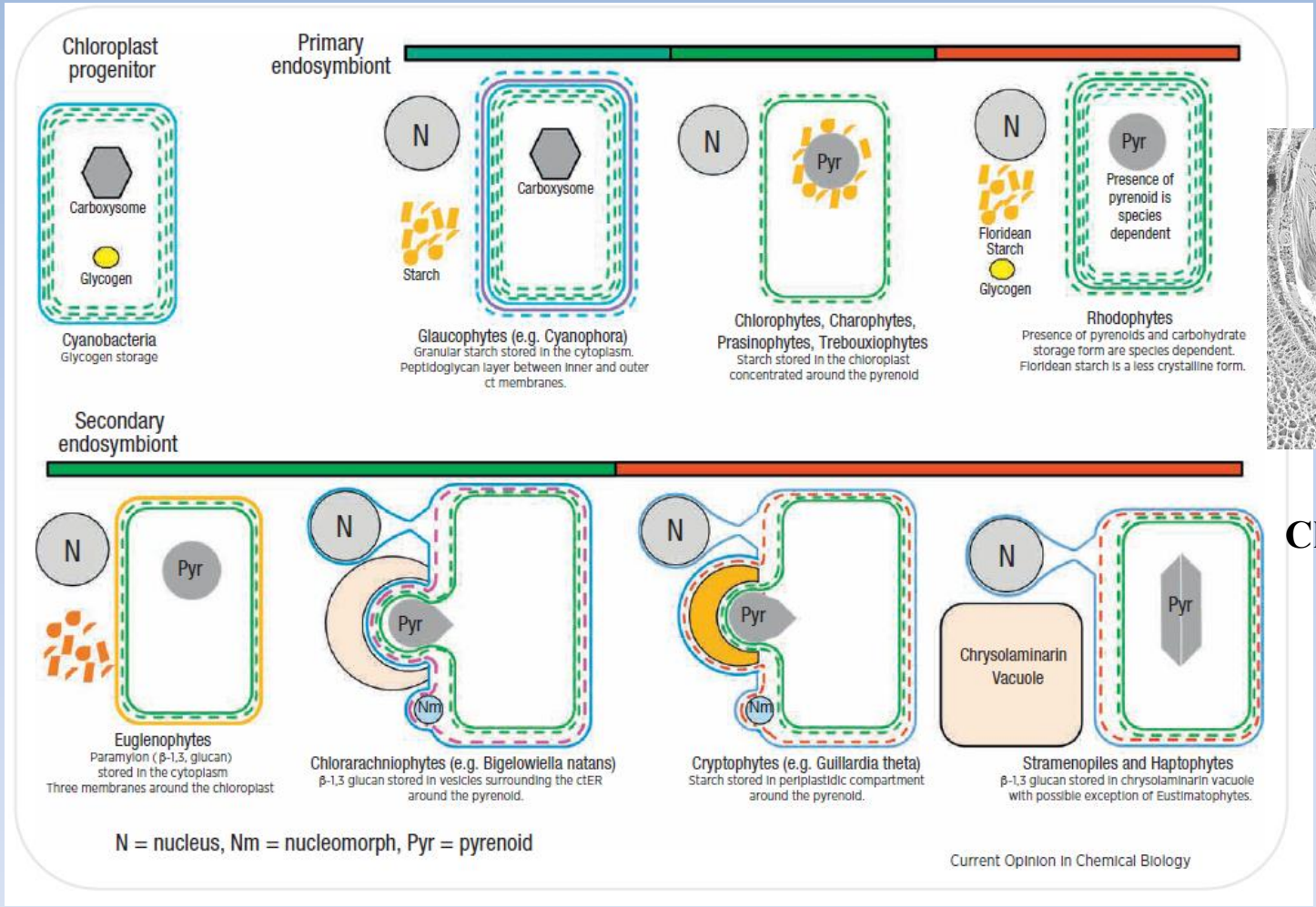


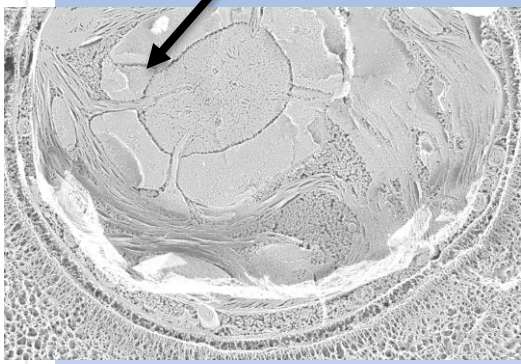
Figure 2.2. Organization schemes of the photosynthetic apparatus in evolutionary diverse classes of microalgae (Source: Hildebrand et al. 2013). PBS = phycobilisomes; PSII = photosystem II; b_6f =cytochrome b6f complex; PSI = photosystem I; ATP = adenosine triphosphate; LHC = light harvesting complex; FCP = fucoxanthin chlorophyll binding protein complex. (a) Clusters the phycobilisome-containing classes. (b) Clusters classes containing grana stacks and stroma lamellae. (c) Shows the relative spacing of photosynthetic membranes in phycobilisome-containing and other chloroplasts.

- "there remains limitations of algal photosystems regarding light utilization"
- "In high cell density cultures, cells nearer to the light source tend to absorb all the incoming light, preventing it from reaching more distant cells"
- "reducing the size of the photosystem antenna can increase the efficiency of light utilization"

Mechanisms of CO_2 Concentration/Fixation and Carbohydrate Storage Vary Widely



Pyrenoid Starch



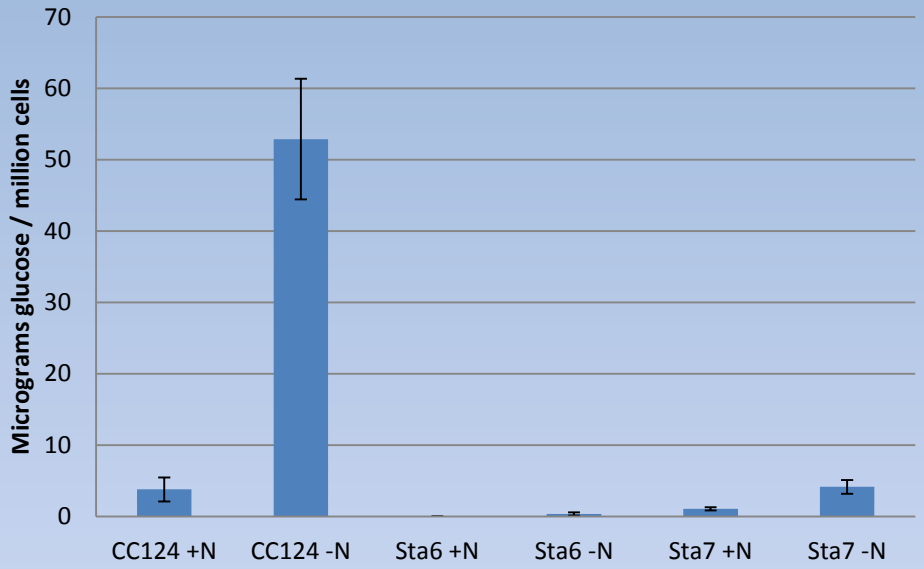
Goodenough. Jinkerson, Roth

Chlamydomonas pyrenoid

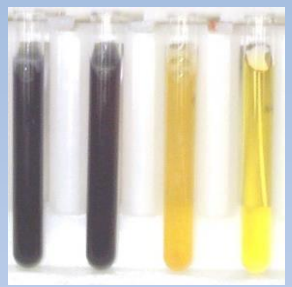
"The CCM raises the CO_2 concentration at the site of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), a strategy for carbon acquisition enabling algae to survive and grow when the CO_2 concentration is low and limiting photosynthesis."

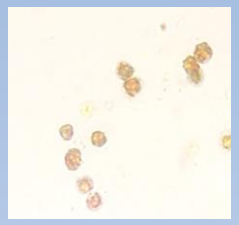
Figure 2.6. Organization of carbon fixation and carbohydrate storage in evolutionary-diverse classes of algae (Source: Hildebrand et al. 2013).

Two Distinct Genotypes Lead to *Chlamydomonas reinhardtii* Starchless Mutant Phenotypes

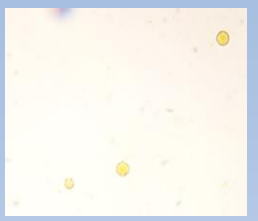


Randor Radakovits

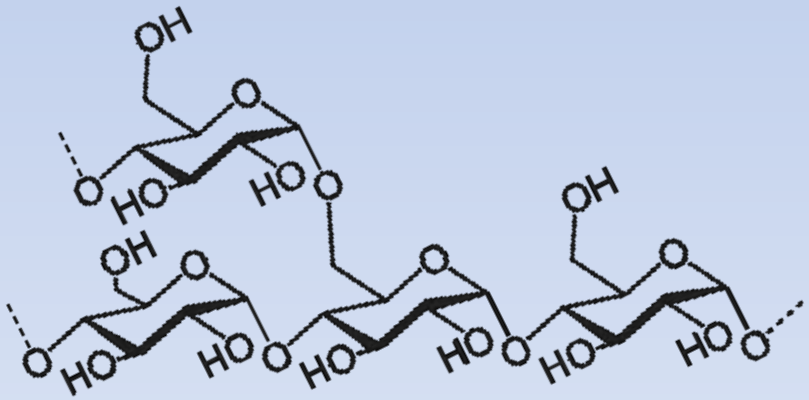


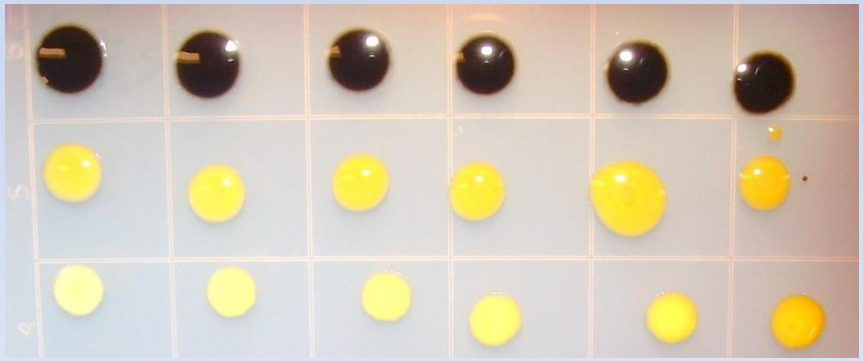


Wild-type cells



Starchless mutant

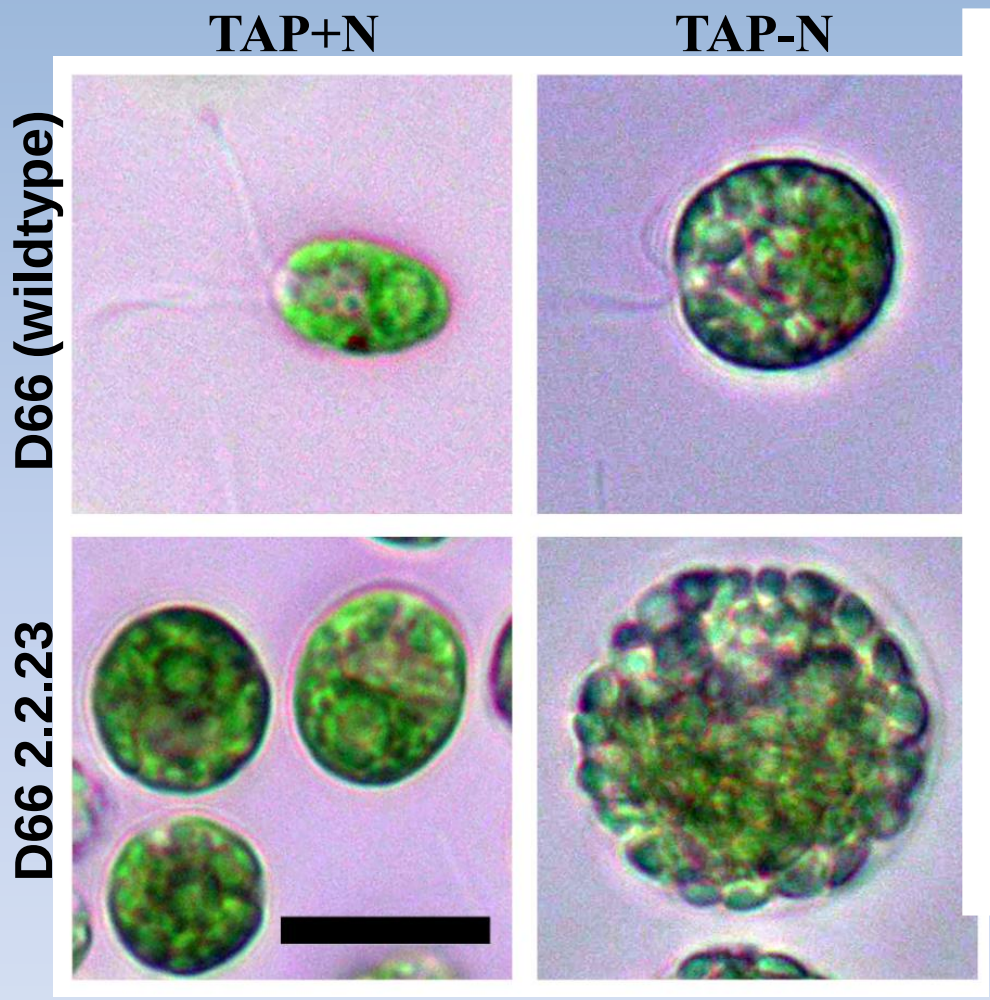




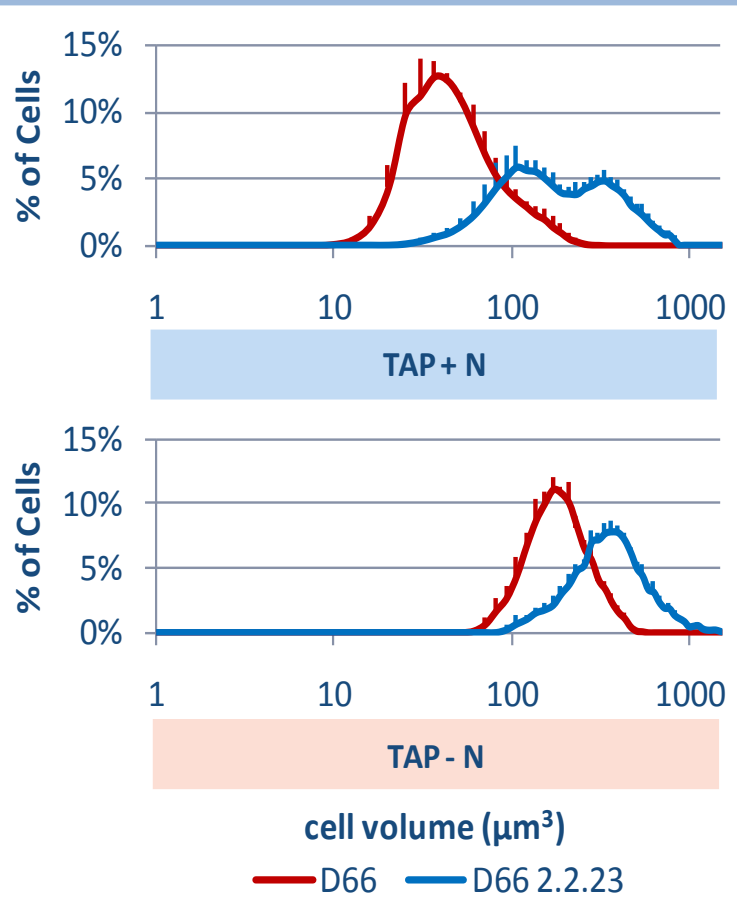
***sta6*; ADP glucose pyrophosphorlyase**

***sta7*; isoamylase** **Robert Jinkerson**

Optimization of starch accumulation

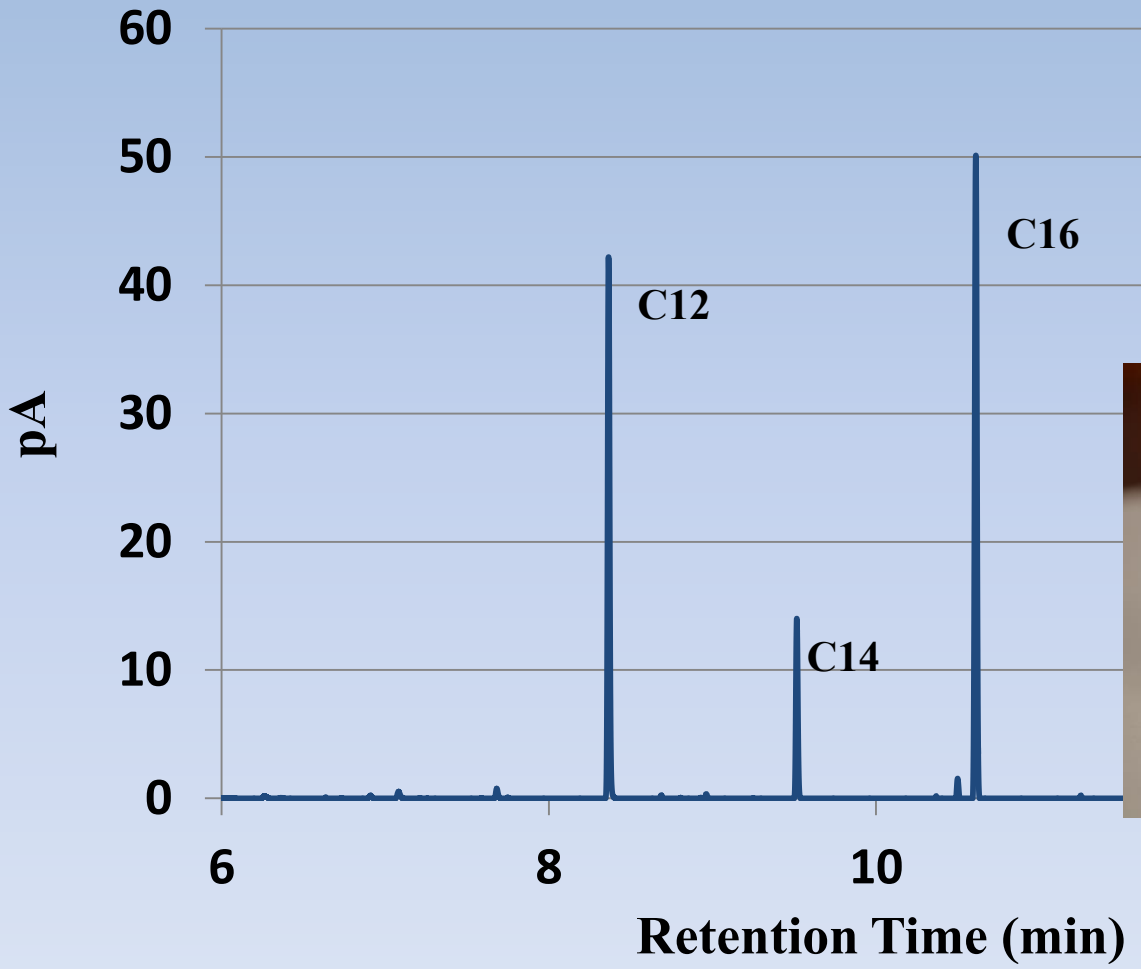


Light microscopy images of WT and transformant at 96 hours. Scale bars: 10 µm.



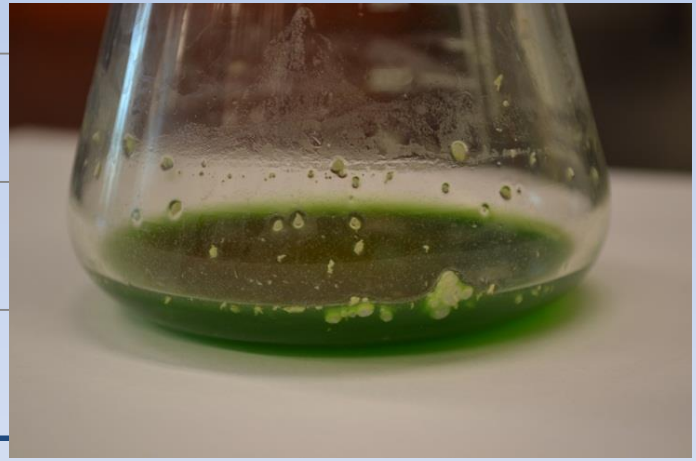
Size distribution of cells at 96 hours. Data was obtained by Coulter Counter and is representative of at least six measurements from three biological replicates.

Expression of C12 and *E. coli* TesA Thioesterase in *Synechococcus* PCC 7002 - Fatty Acids Secreted into the Medium



Disruption of *fadD* gene and expression of hioesterase enables fatty acid synthesis and secretion from cyanobacteria

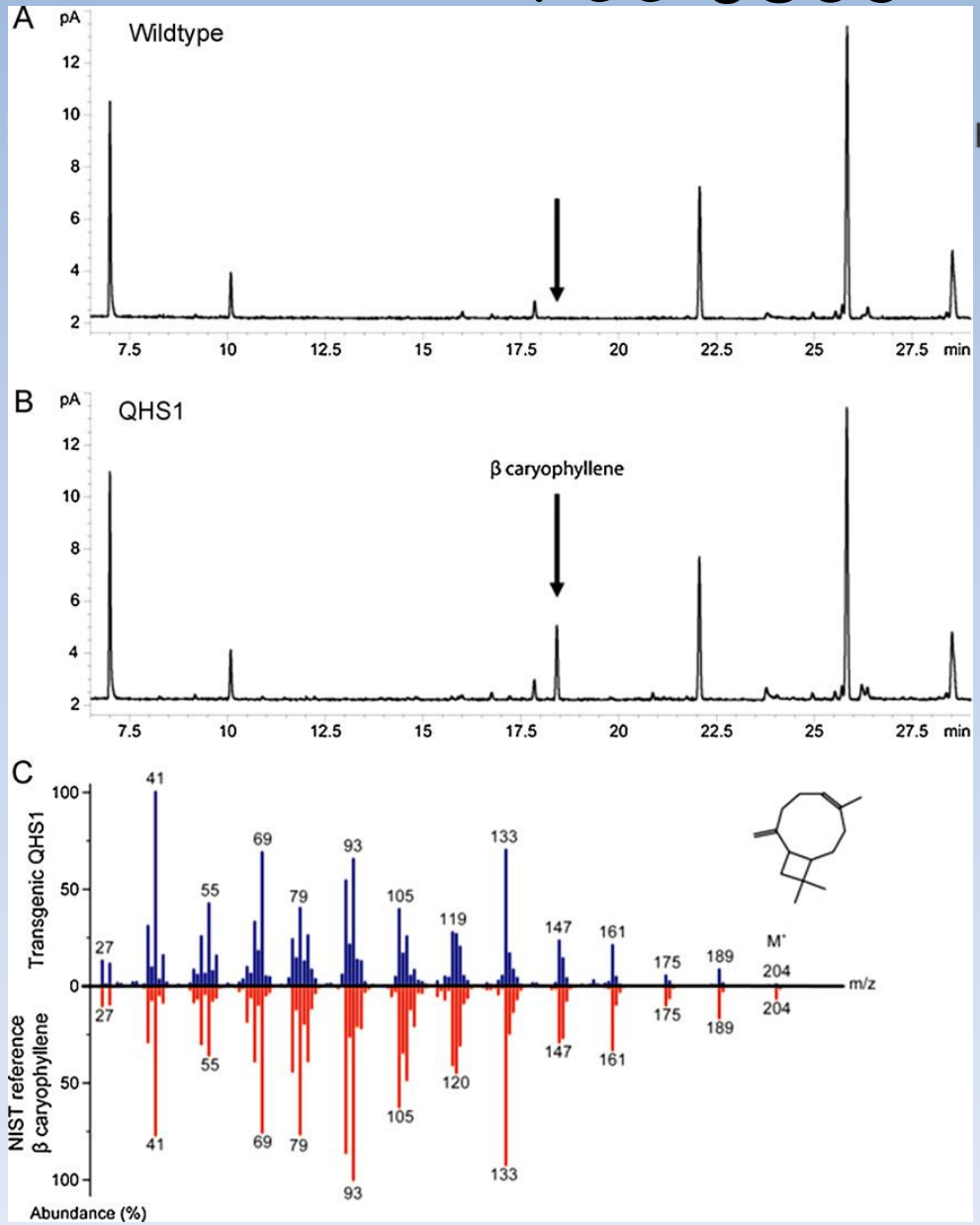
Minimal impact on cell growth and photosynthetic oxygen evolution

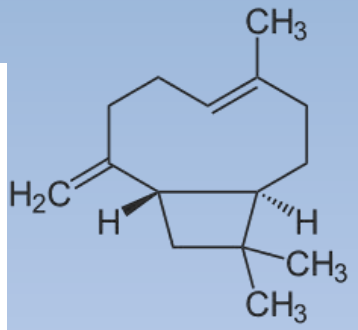


"The ability of an algal species to secrete fuel precursors may … reduce or skip the cell harvesting and biomass deconstruction/separation steps."

Victoria Work, Fiona Davies

β-caryophyllene accumulation in *Synechocystis* PCC 6803





"Diesel tree"
Copaifera
Langsdorfii

"Natives … drill a 5 centimeter hole into the 1-meter thick trunk and put a bung into it. Every 6 months or so, they remove the bung and collect 15 to 20 liters of the hydrocarbon" - Calvin

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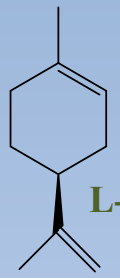
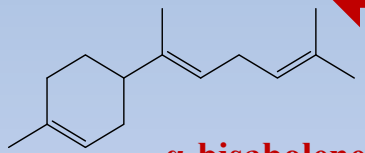
Terpenoids



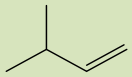
L-limonene



α-bisabolene

Hemiterpene

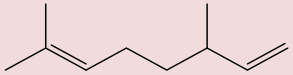
C5



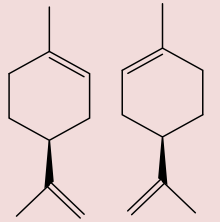
isoprene

Monoterpenes

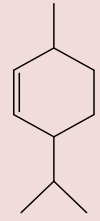
C10



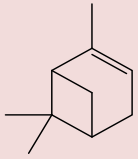
myrcene



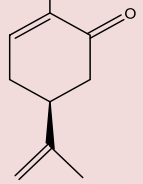
L-limonene D-limonene



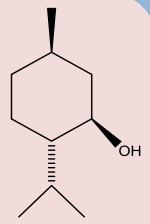
β-phellandrene



α-pinene



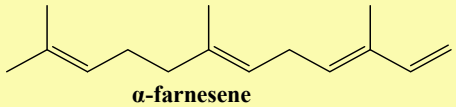
S-(+)-carvone



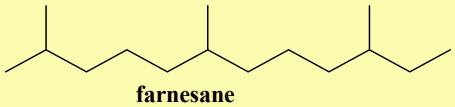
(-)-menthol

Sesquiterpenes

C15

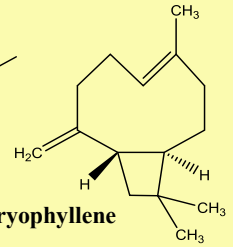


α-farnesene



farnesane

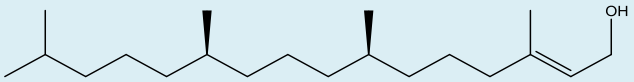
farnesol



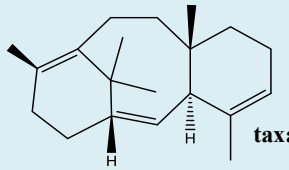
β-caryophyllene

Diterpenes

C20



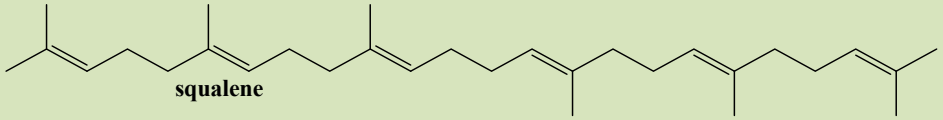
phytol (chlorophyll side chain)



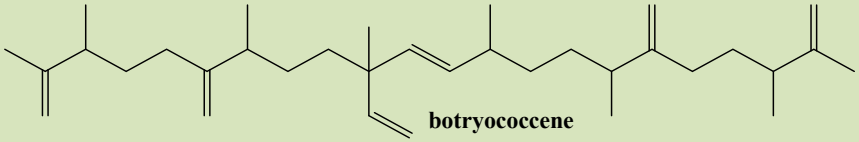
taxadiene

Triterpenes

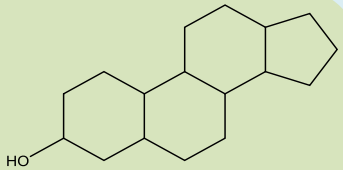
C30



squalene



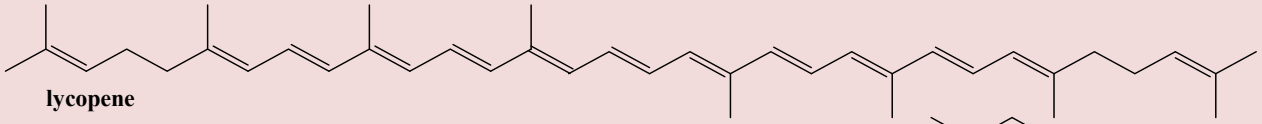
botryococcene



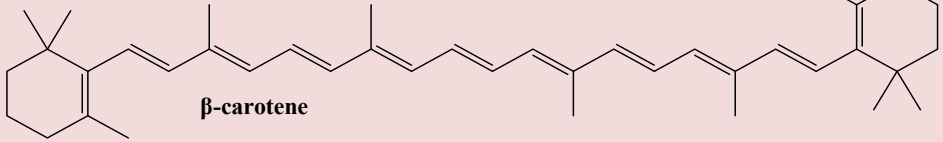
sterol

Tetraterpenes

C40



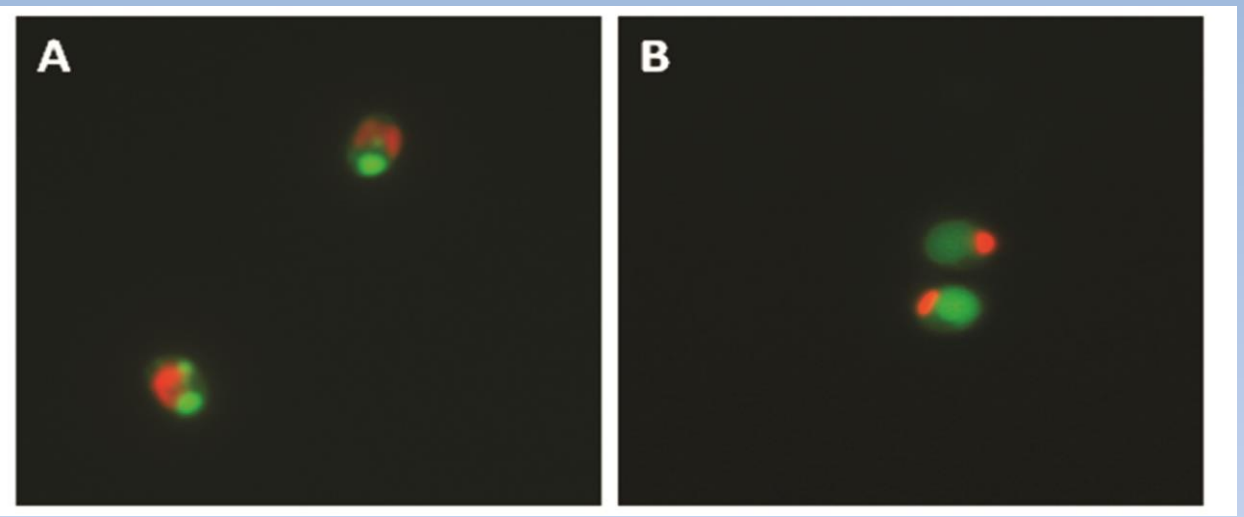
lycopene



β-carotene

Davies *et al* (2014) Toward a photosynthetic microbial platform for terpenoid engineering. *Photosynthesis Research.*

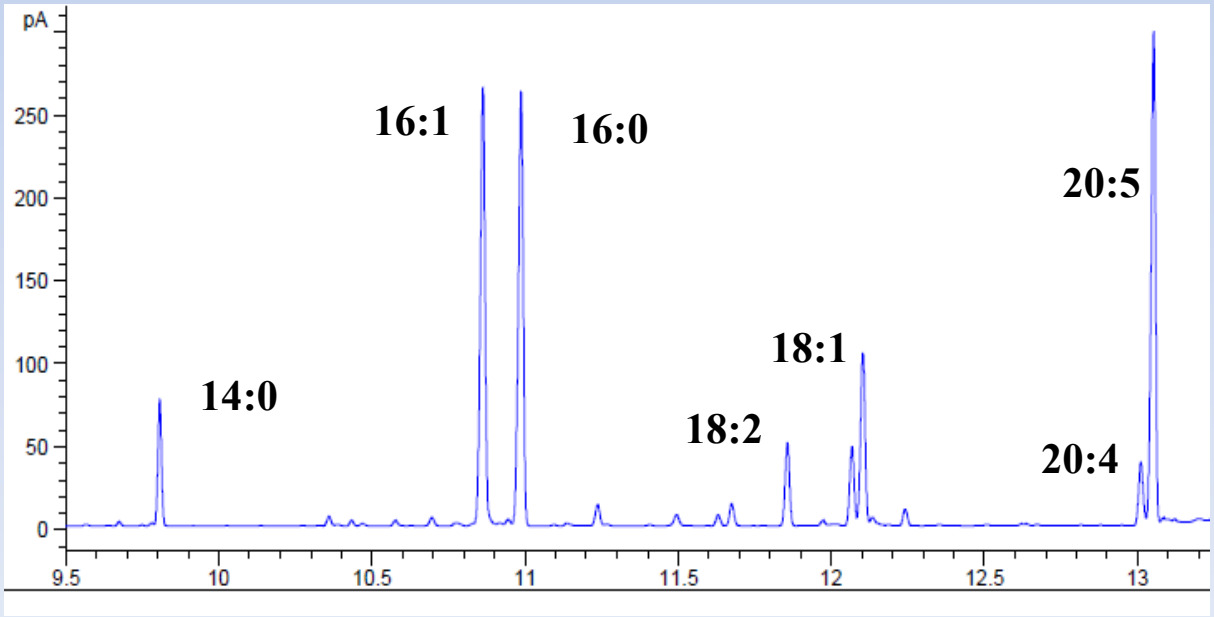
Nannochloropsis species: transforming oleaginous algae into new model systems



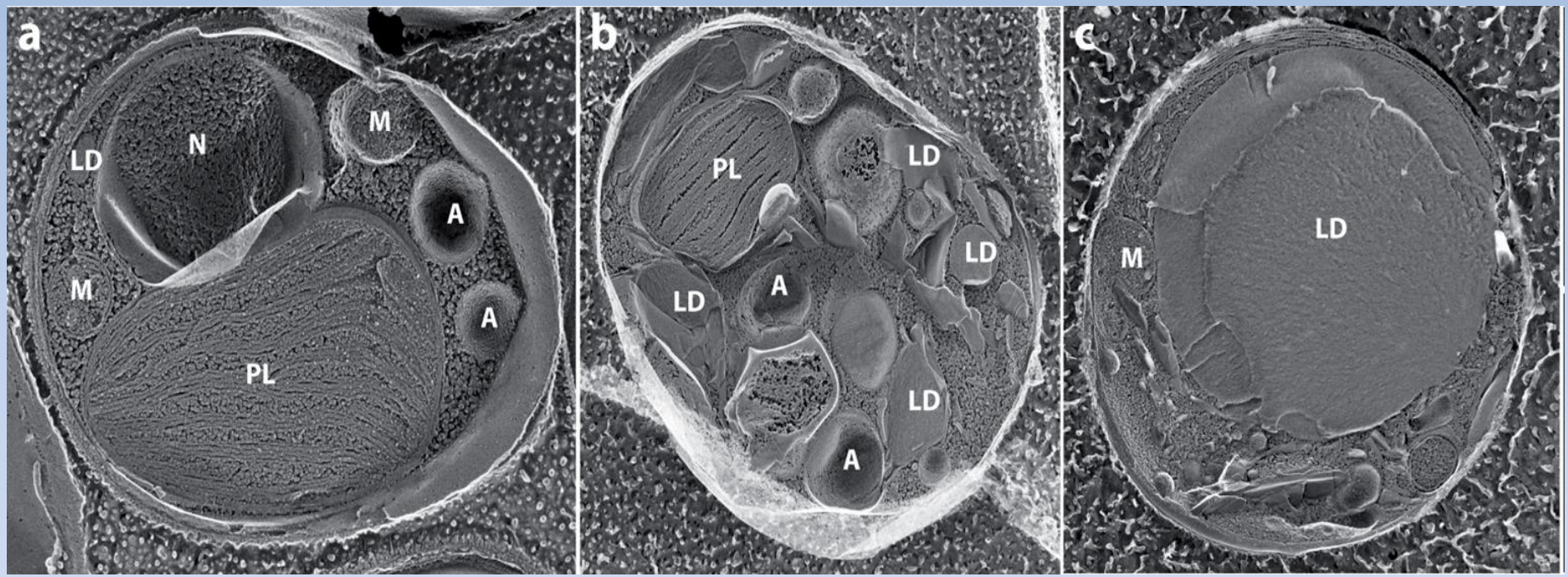
- Robust outdoor performance
- High lipid yields
- Favorable biomass accumulation
- Salt water organism
- EPA producer
- Multiple genomes, genetic tools now available

"a logical approach with current technology is to identify strains with … demonstrated desirable large-scale outdoor cultivation characteristics, and then develop improvement approaches in the lab."

Fatty Acid Methyl Ester Trace



Lipid Accumulation in log, linear and N depleted cultures



Deep etch electron microscopy – Goodenough and Roth

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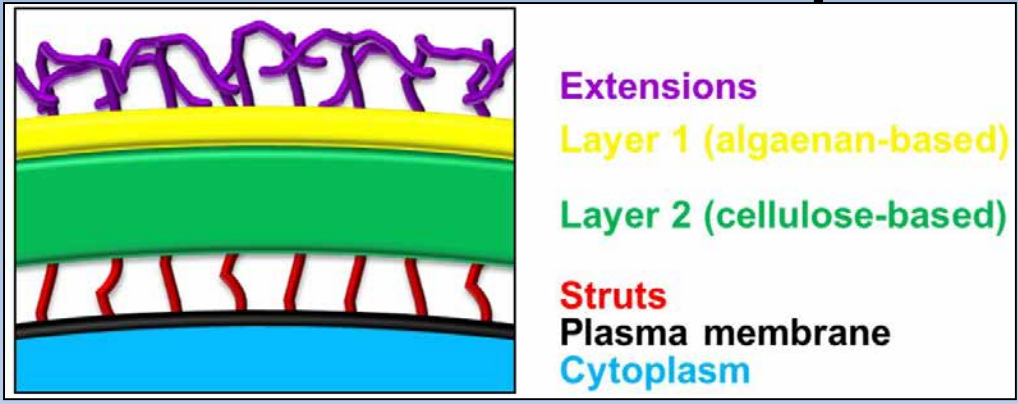
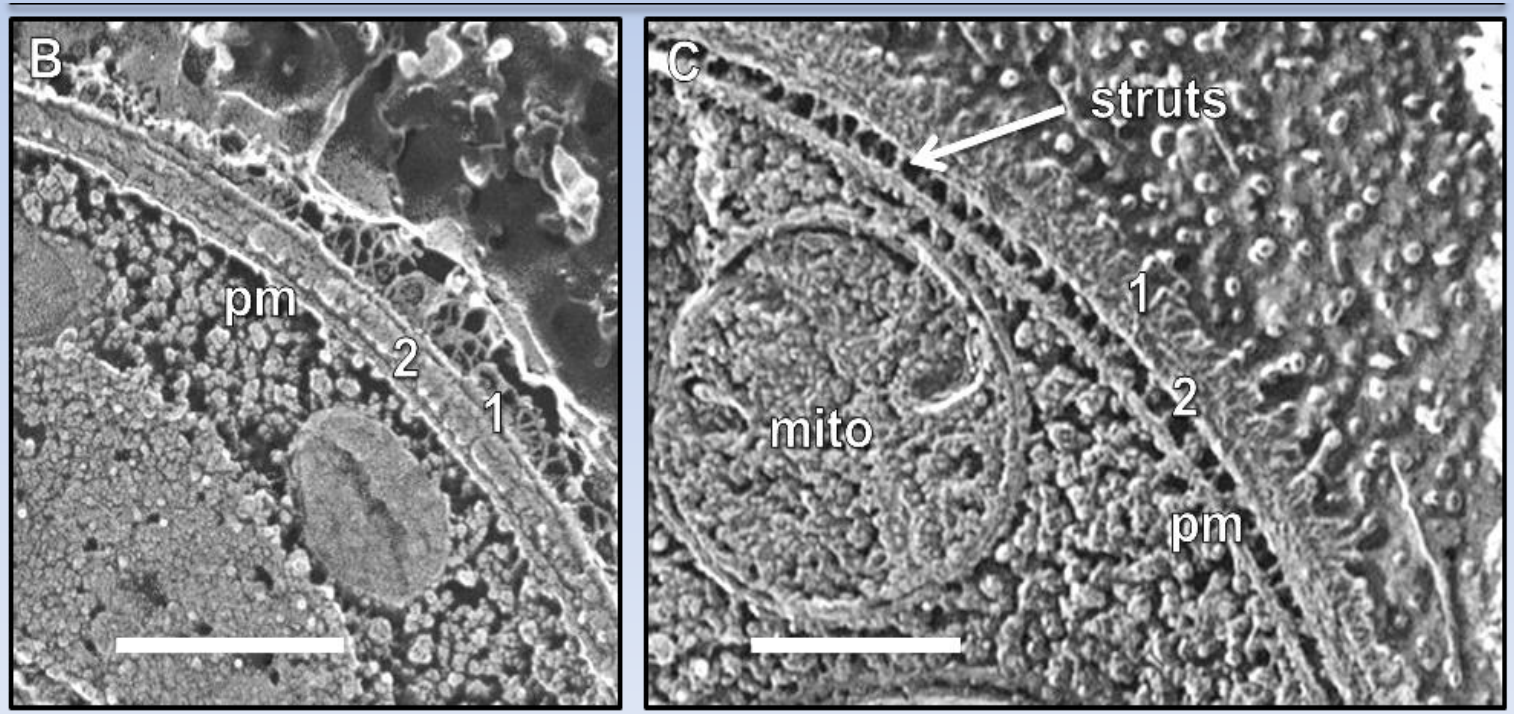
Washington University in St. Louis

Biochemical Characterization of Nannochloropsis cell wall



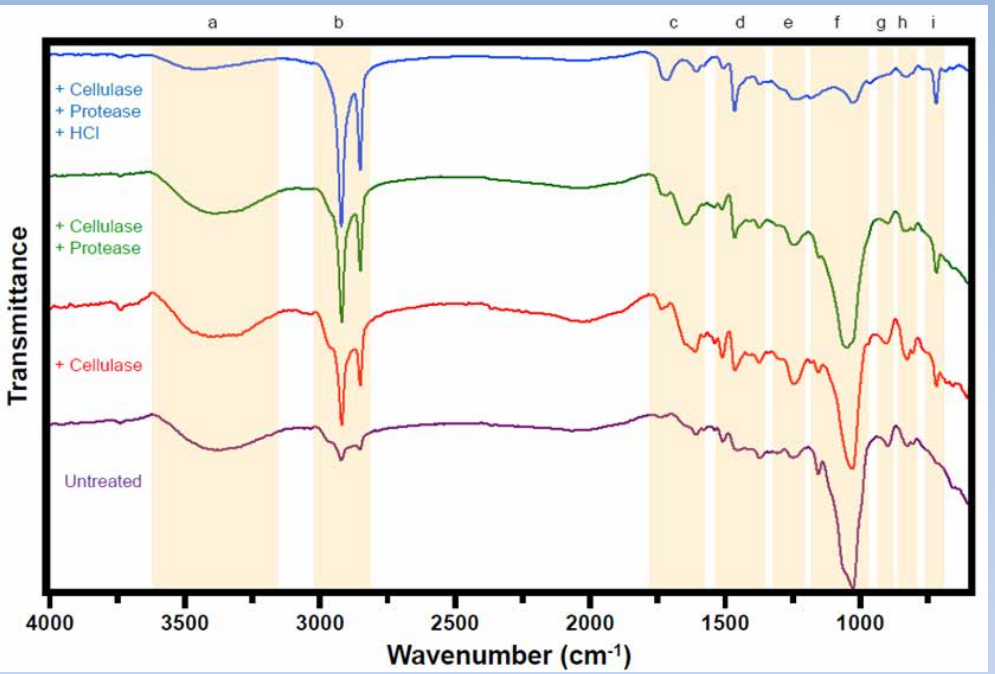
"Resistance to predators, viruses, and abiotic stress is also a desirable phenotype"

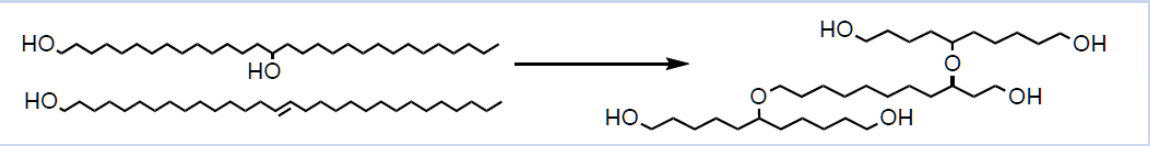


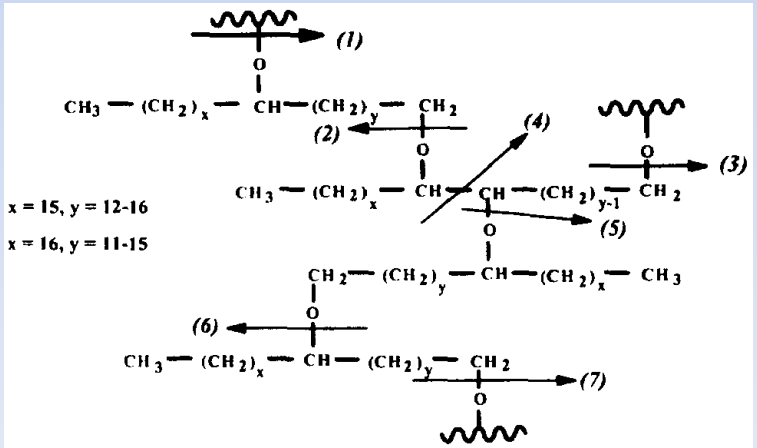
Scale bars - 250 nm

Ursula Goodenough, Robin Roth, Taylor Weiss, Matt Scholz, Hank Gerken

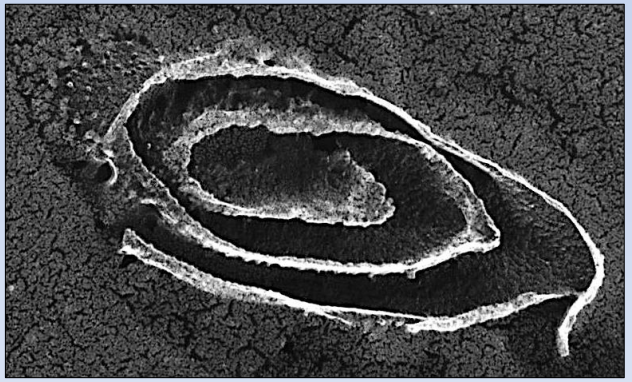
Nannochloropsis Cell Wall - Algaenan





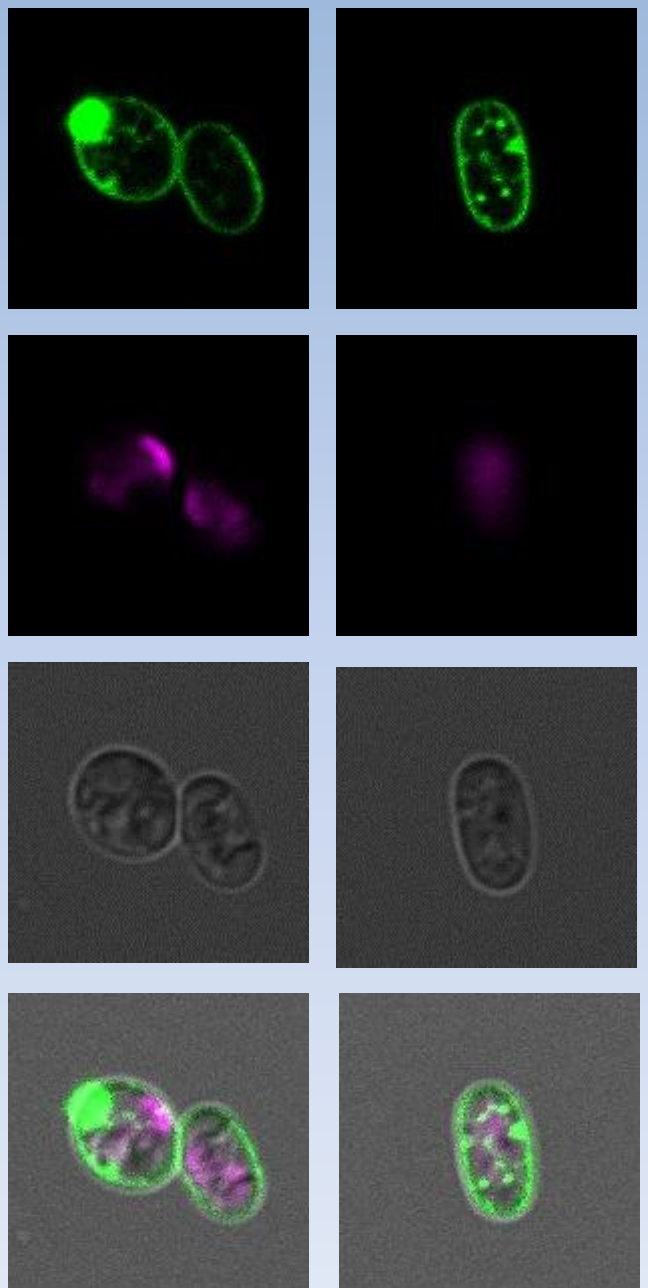


Pressed Walls after Cellulose Treatment



Ursula Goodenough, Robin Roth, Taylor Weiss, Matt Scholz, Hank Gerken

Cell Wall Associated Carbonic Anhydrase



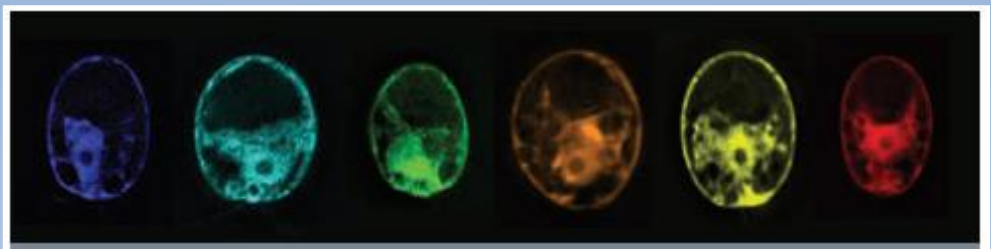
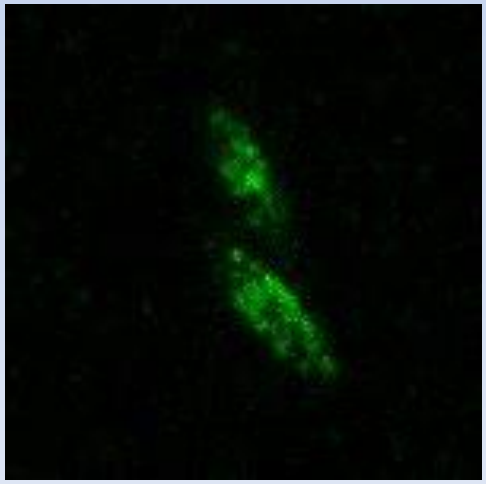
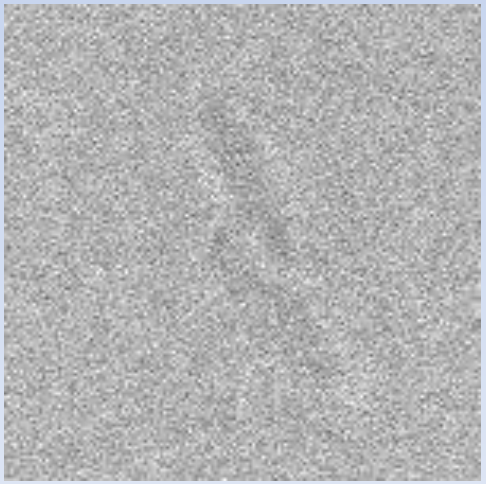


Figure 2.5. A rainbow of fluorescent tags expressed in *Chlamydomonas reinhardtii* (Image by Beth Rasala, University of California, San Diego, from Science Daily, sciencedaily.com/releases/2013/03/130307145109.htm)

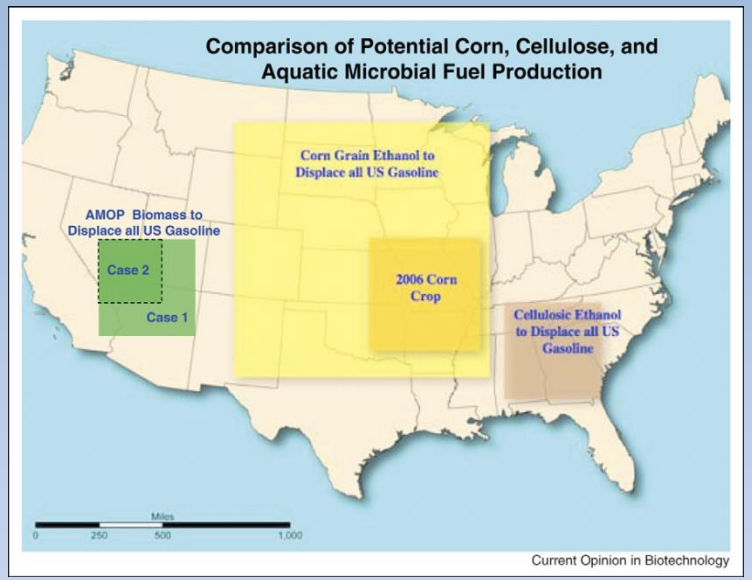
"Tagging proteins with fluorescent markers is useful in determining their intracellular location and can provide at least semi-quantitative evaluation of their abundance in a simple measurement."





Shed cell walls retain CA – fusions for cell harvesting?

Opportunities Ahead



Turner, 1999
Seibert et al, 2008
Dismukes et al, 2008

- **Decrease light harvesting antennae**
- **Improve carbon assimilation**
- **Tailor (partition) carbon products**
- **Harvest dilute cells from water**
- **Harvest secreted products - "artificial" leaf**