CCM function in Nannochloropsis salina: boon, benign, or bust for algal biofuels?

SAND2013-4353C

David Hanson
John Roesgen, Sam Nieves

THE UNIVERSITY of NEW MEXICO

Jerilyn Timlin
Howland Jones, Aaron Collins

Sandia National Laboratories

National Nuclear Security Administration

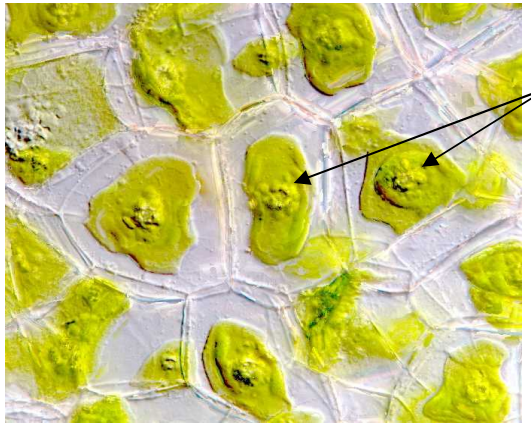
U.S. DEPARTMENT OF ENERGY

The Pyrenoid: Structure

- Protein dense body containing all the Rubisco in a cell
- Common in algae
  - Only plants with pyrenoids are hornworts (related to mosses)

Photo courtesy of
University of Wisconsin-Madison

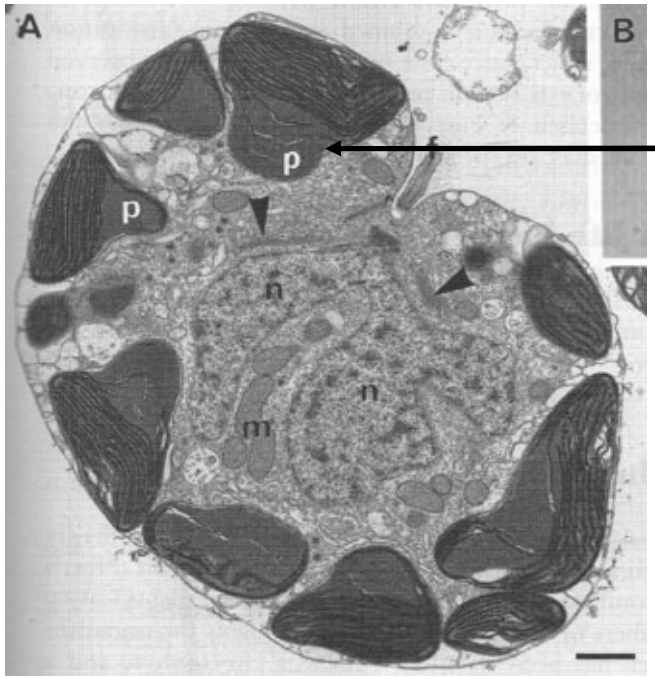
C.hlamydomonas rheinhardtii with pyrenoids



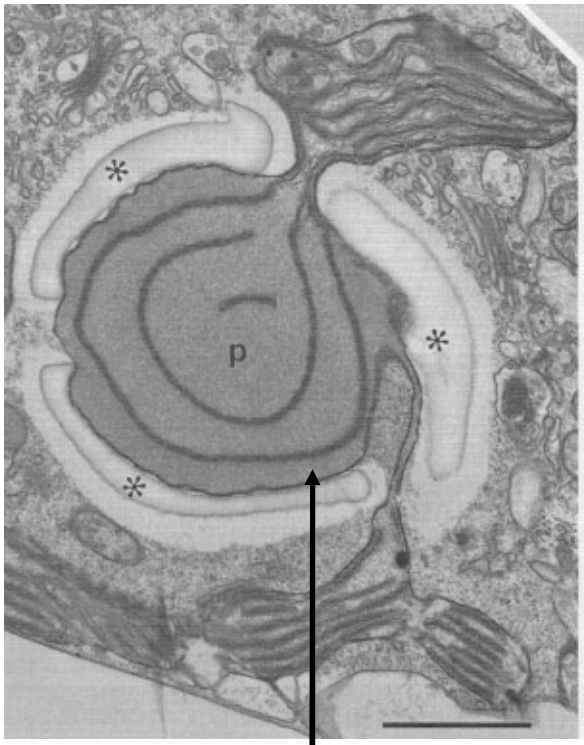
Pyrenoids visualized with Nomarski differential interference

Algal pyrenoids

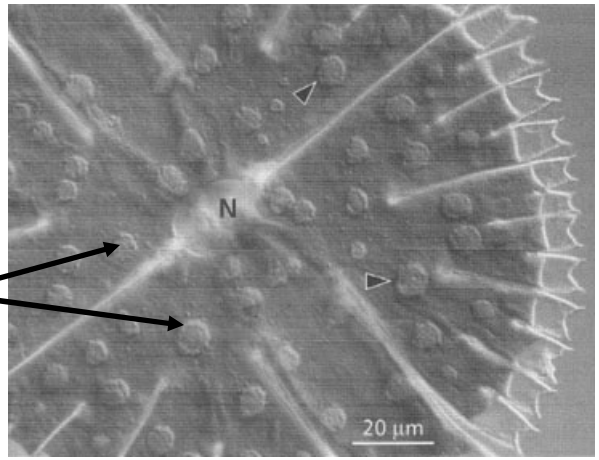
Central pyrenoid with starch sheath



Pyrenoid on edge of chloroplast



Multiple small pyrenoids per chloroplast



Large pyrenoid on stalk, traversed by thylakoid lamellae

Images from Clayton and King 1992. *Biology of Marine Plants*, and Graham and Wilcox 2000. *Algae*.

Nannochloropsis salina Pyrenoid

- Central strand of thylakoids
- Outer starch sheath

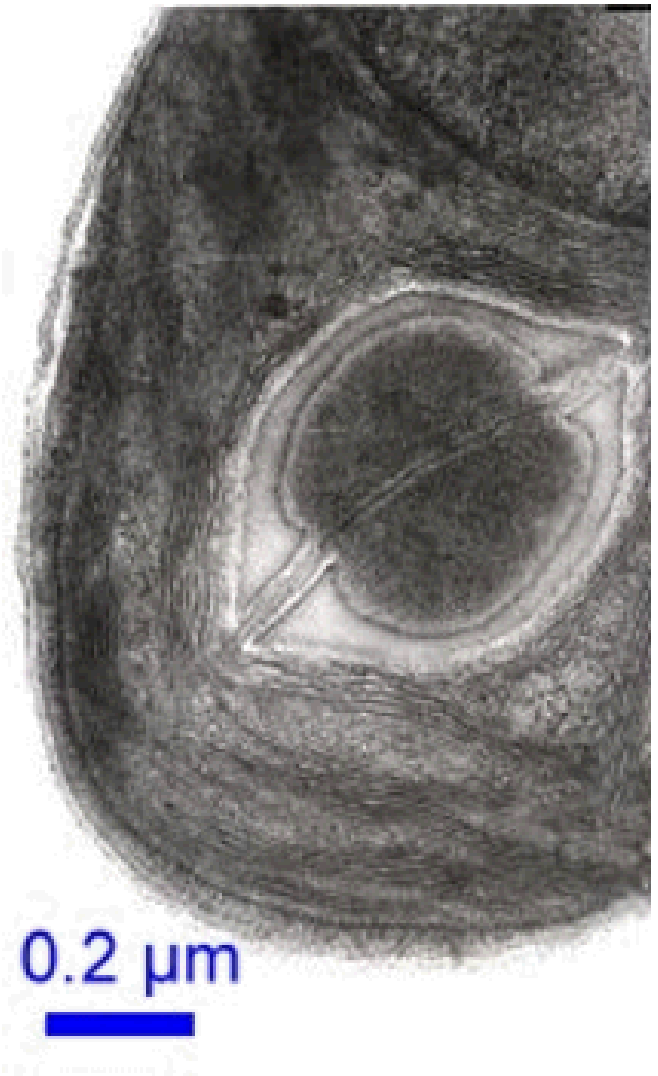


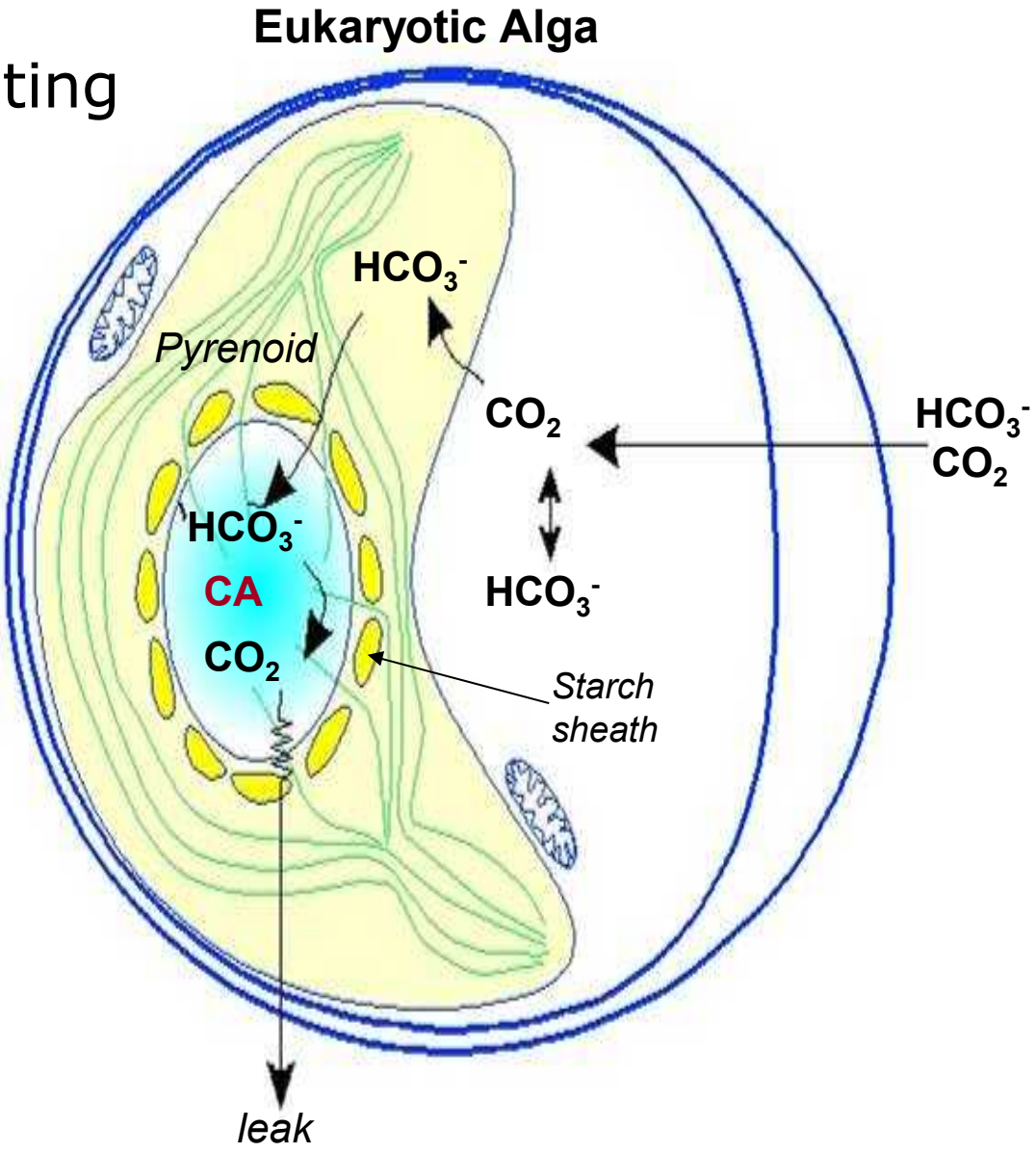
Photo credit
N G Mohammady on
planktonnet@awi

The Pyrenoid: Function

CCM = CO_2 concentrating mechanism

Main components

- Transport into cell
- Transport into chloroplast
- Conversion to CO_2 around Rubisco
- Mechanism to reduce leakage



The Pyrenoid Paradox

- To induce CCM or not to induce CCM, that is the question
  - Rubisco requires high CO_2 for efficient photosynthesis, but….
    - High CO_2 is energetically and economically expensive to provide in a large scale facility
  - The biological solution is the CCM where the cell elevates the CO_2 instead of engineers, but…
    - The "fuel cost" of this biological process is not known

CO_2 switch experiment

- Airlift Bioreactors
- Start at high (5000 ppm) or near ambient (500 ppm) CO_2
  - Then switch CO_2
  - Also adjust flow of media to maintain constant cell density

Need to control for cell density of cultures

- Cell density and culture temperature interact

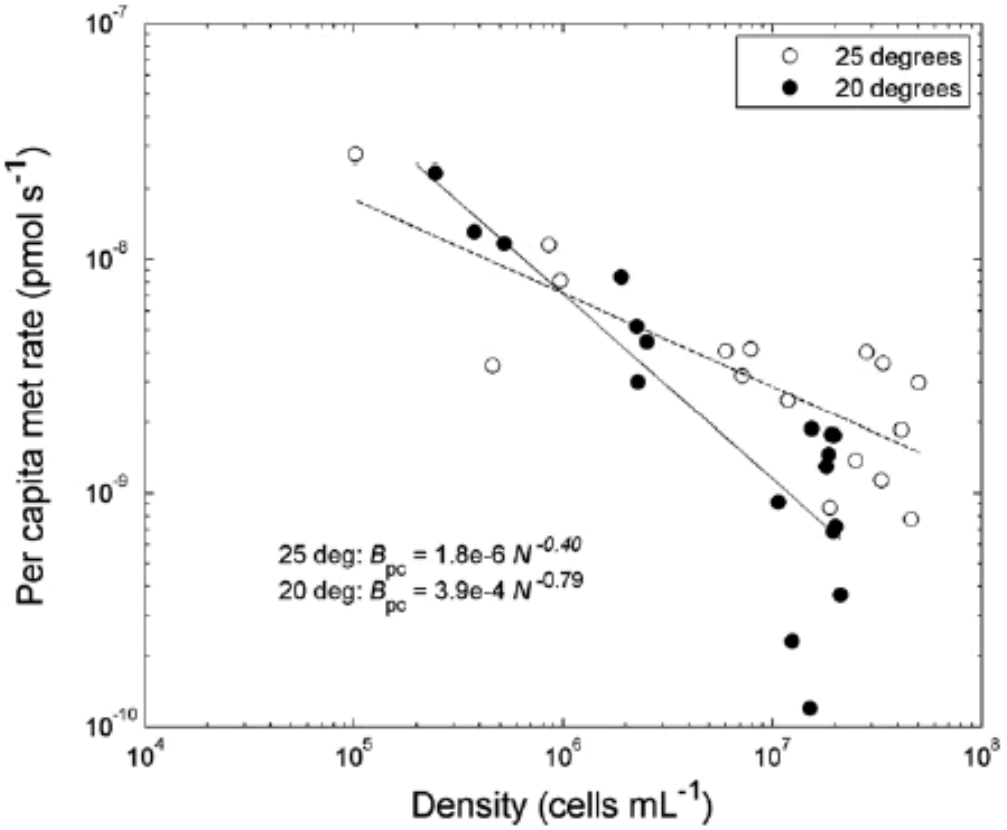
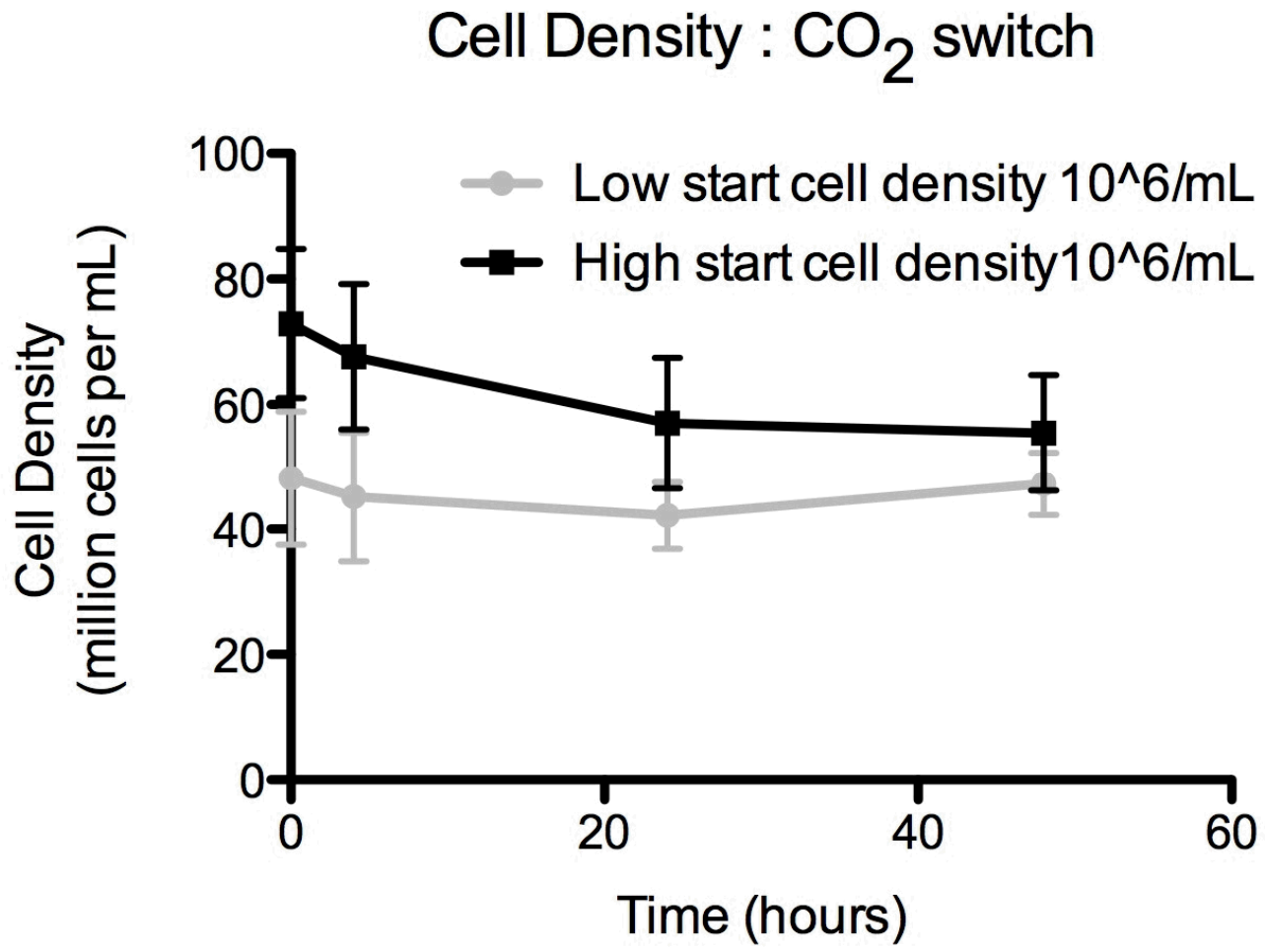


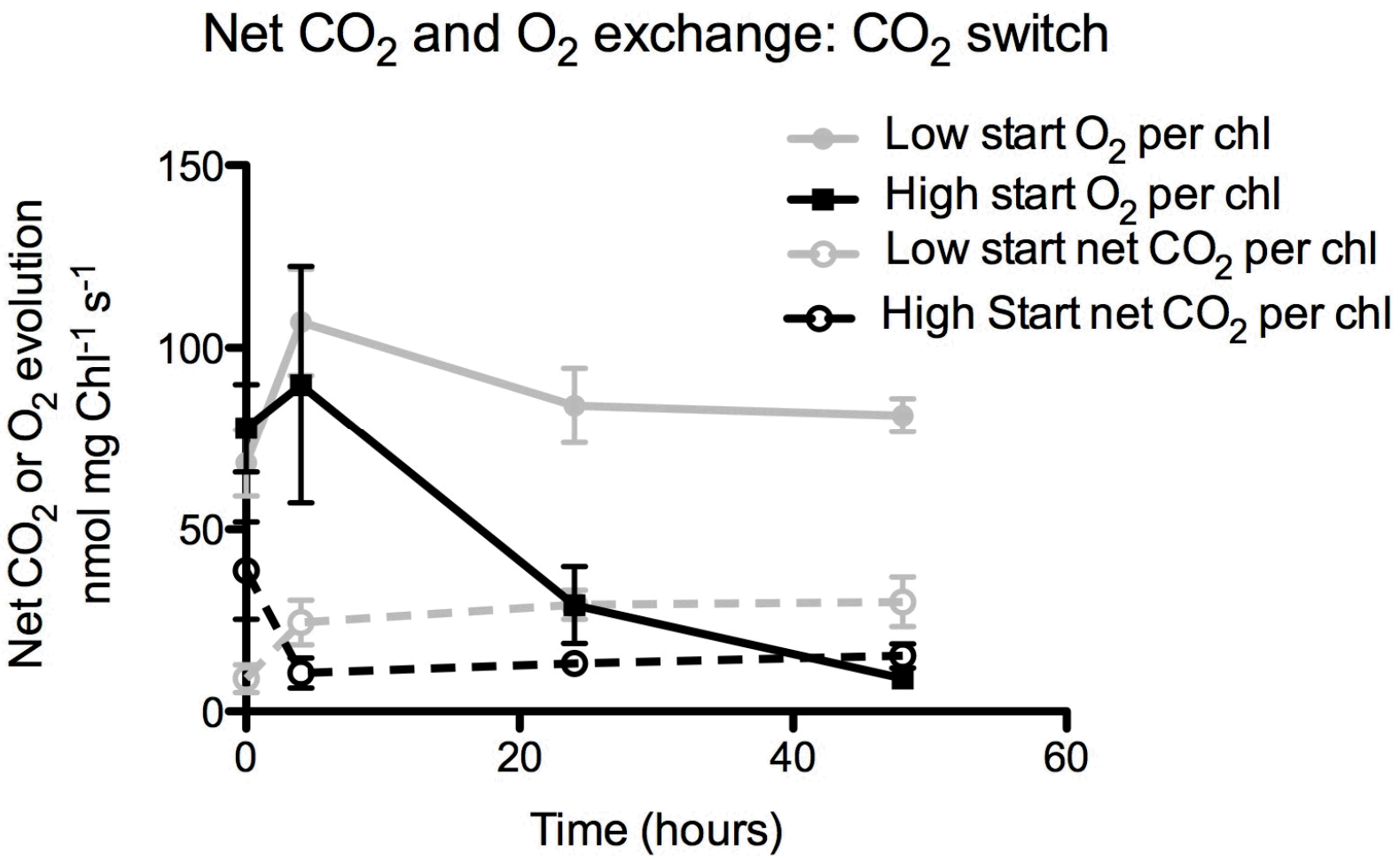
Fig. 4. Per capita metabolic rates were density dependent in both warm and cold populations. The magnitude of the density dependence was lower in the warm populations. Cells in the warm populations were able to maintain higher levels of energy flux even as the density climbed to twice that in the cold populations.

Delong and Hanson 2011 Ecol Complexity

Cell density response to switch



Photosynthetic response to switch

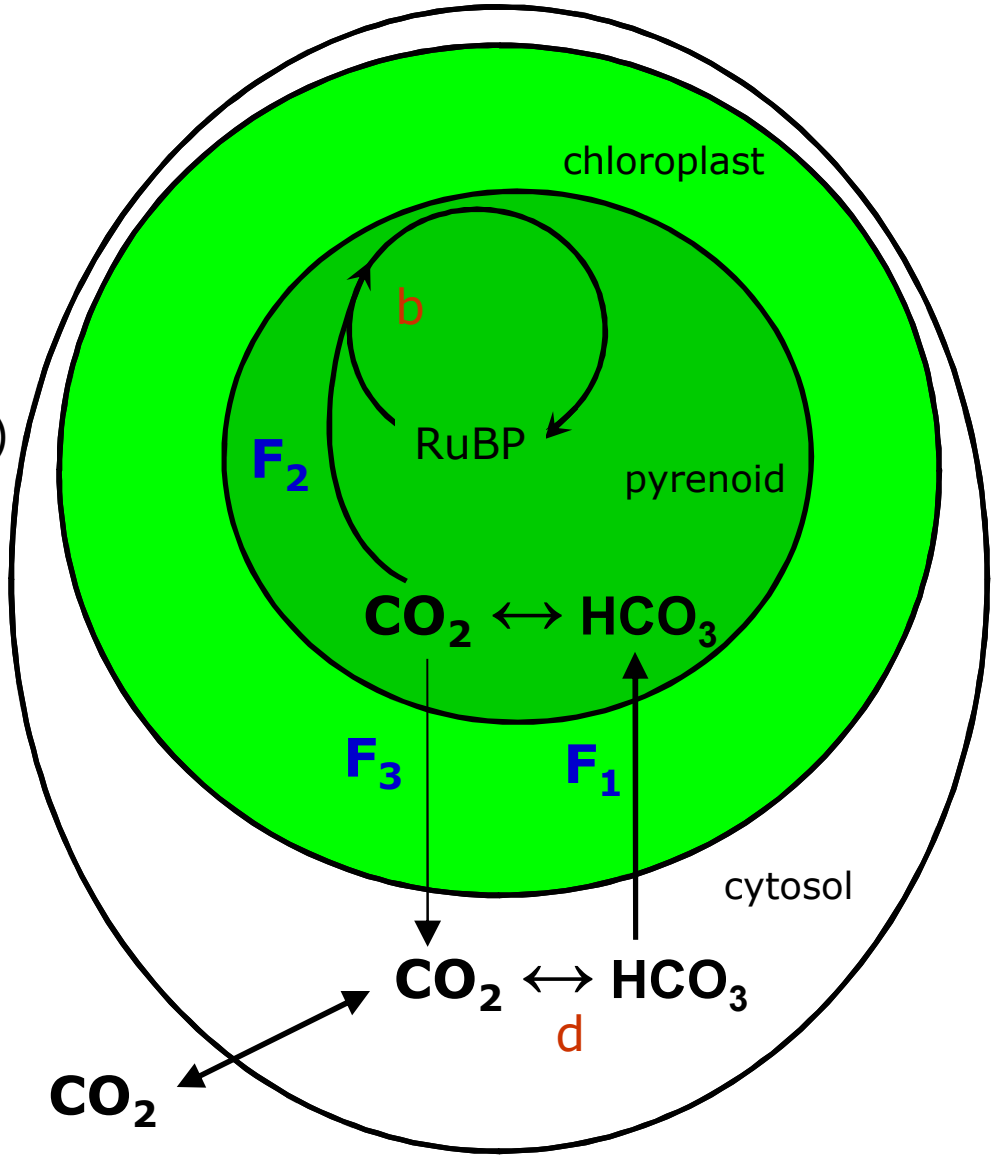


CCM leakiness

- Balance of fluxes:
  - HCO_3 into pyrenoid (F_1)
  - CO_2 fixed by Rubisco (F_2)
  - CO_2 leaving plastid (F_3)
- Other assumptions
  - No fractionation with F_1 or F_3
  - Discrimination by Rubisco (b) is 27‰
  - Equilibrium discrimination from hydrating CO_2 (d) is -7.9‰
- Equation:

$$\Delta_{measured} = d + b\frac{F_3}{F_1}$$

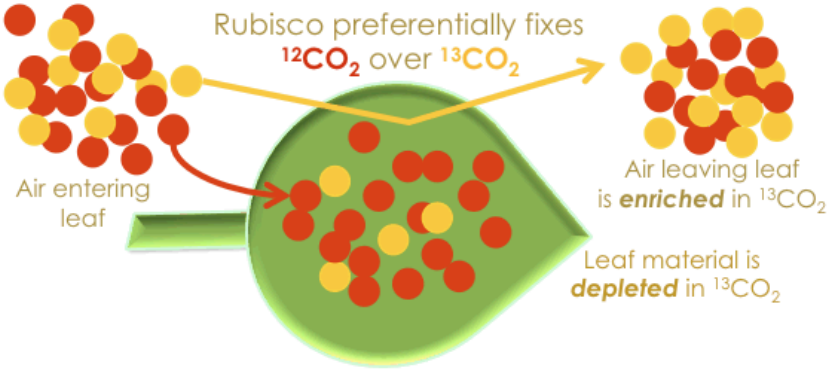
$\frac{F_3}{F_1}$ is the leakiness factor

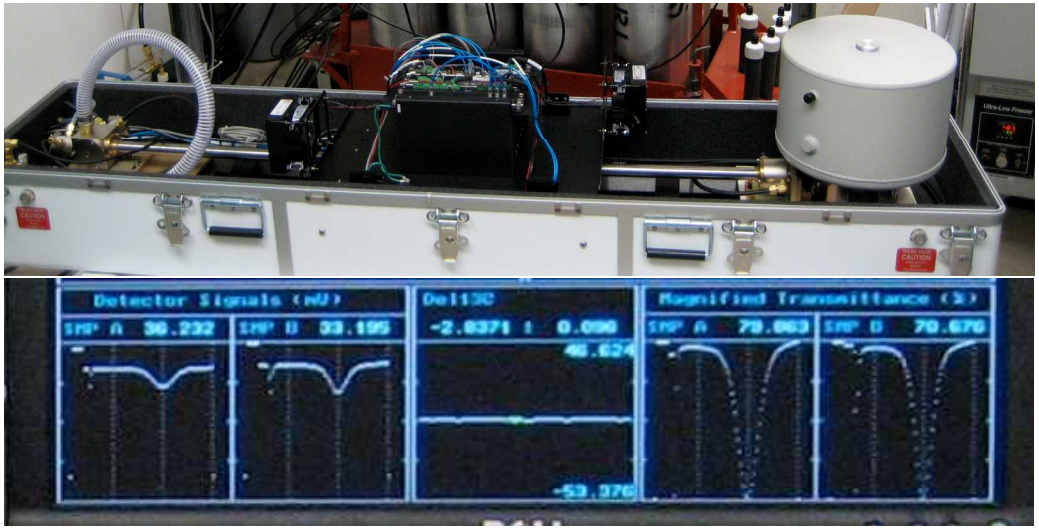


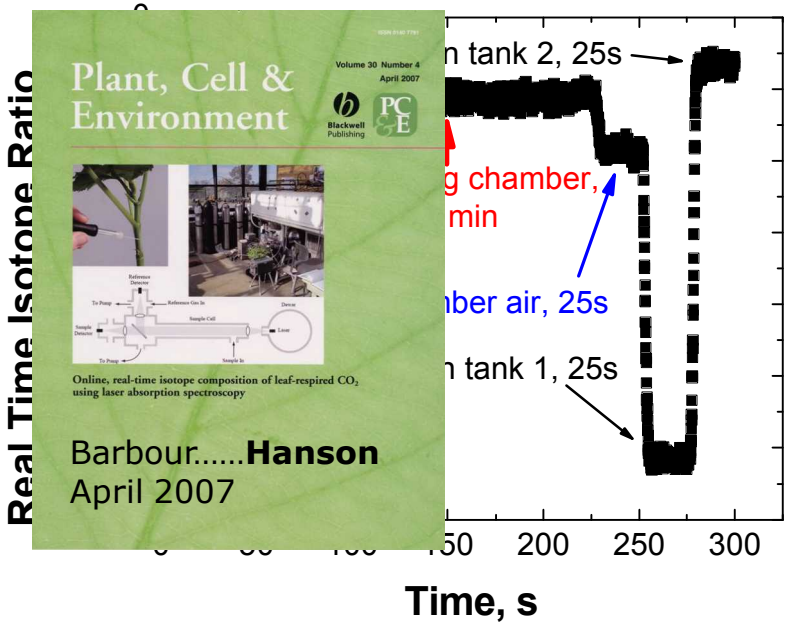
Measuring photosynthetic discrimination (Δ)

Tunable Diode Laser

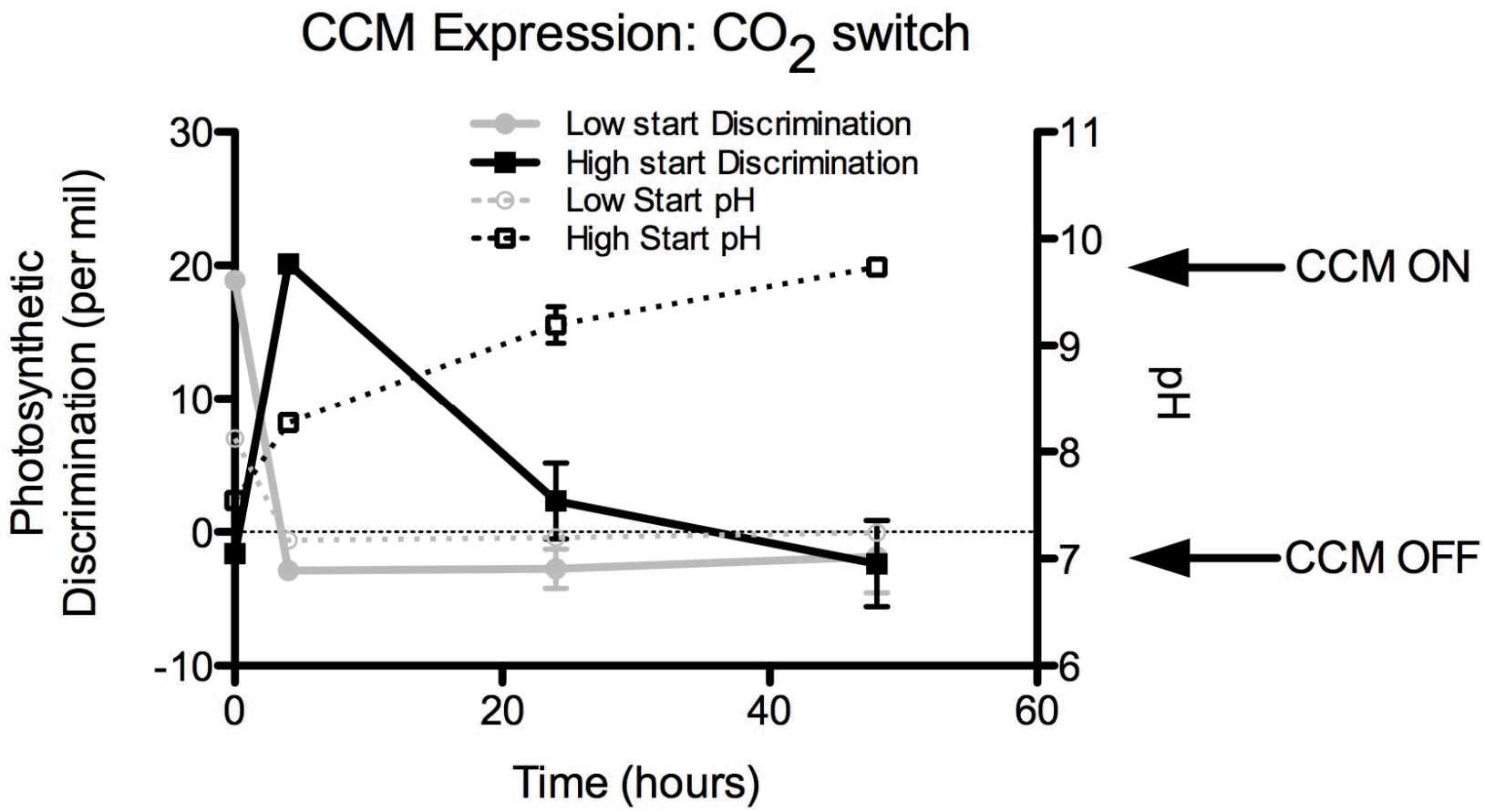
- Absolute [$^{13}CO_2$] &[$^{12}CO_2$]
- 10 Hz output
- Coupled to air inlet and outlet of bioreactor







CCM response to switch

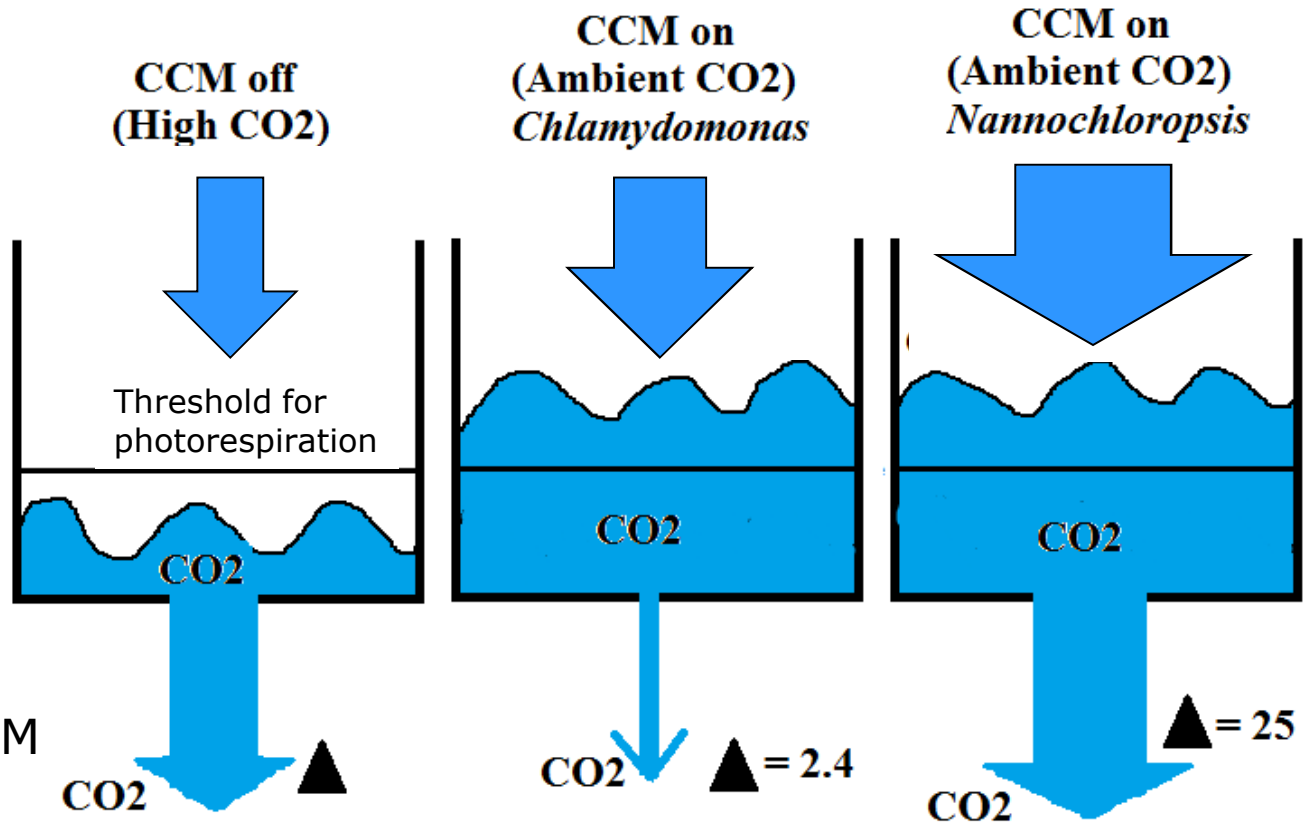


So how do lipids respond?

There's a hole in your bucket..

Above this line CO_2 in cell is above ambient

Large CO_2 leak = Large Δ = no CCM



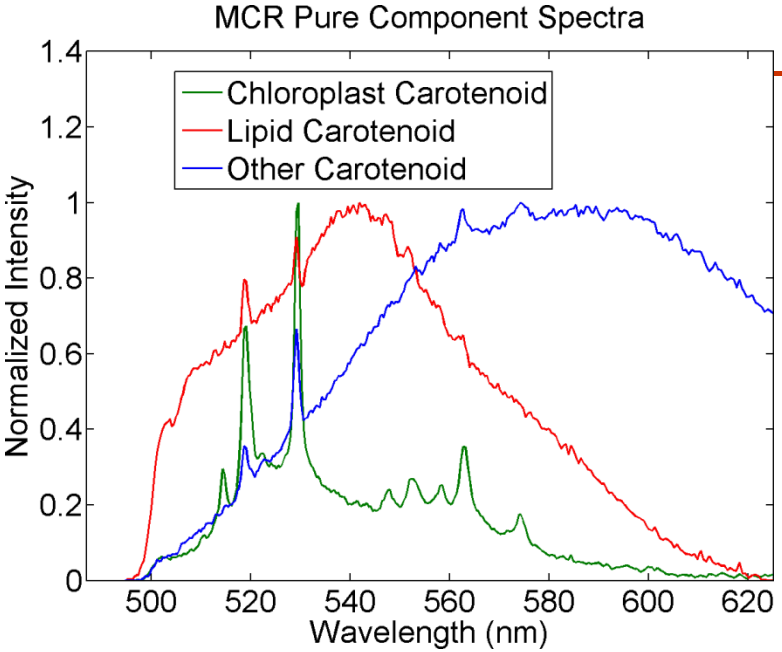
CO_2 (ppm)	Δ (‰)
335	2.4
6160	16

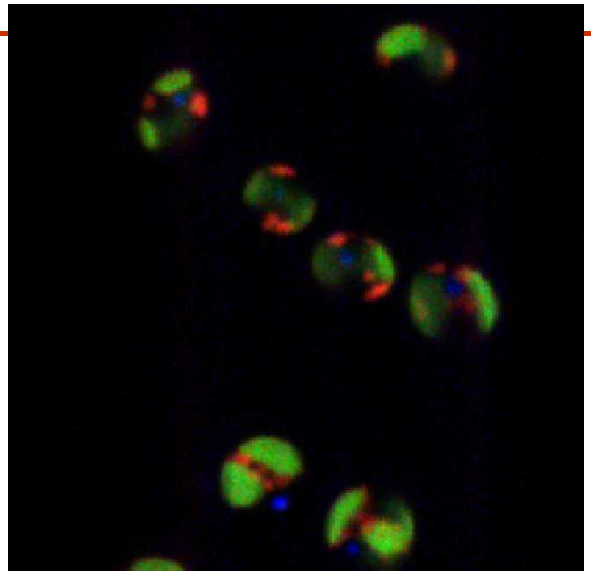
Chlamydomonas reinhardtii

CO_2 (ppm)	Δ (‰)
415	25
6550	11

Nannochloropsis salina

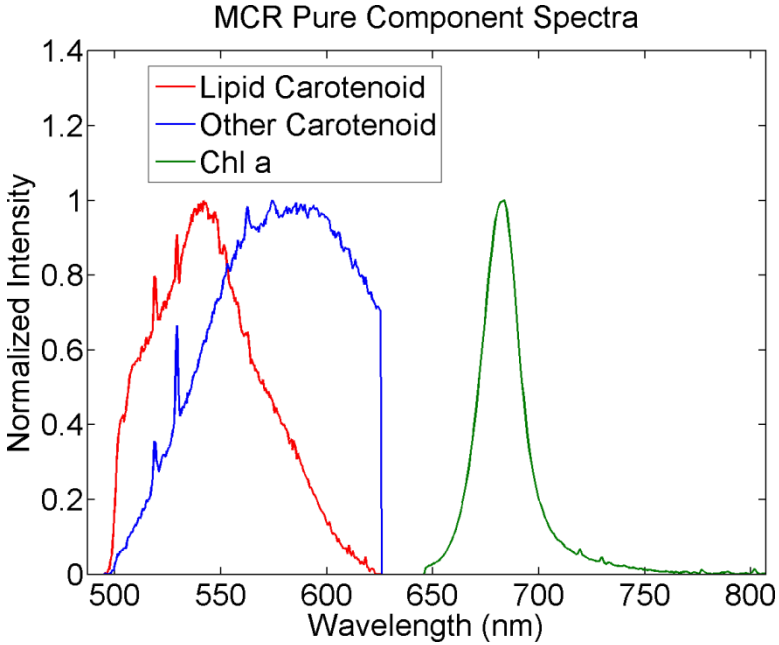
Hyperspectral imaging and MCR

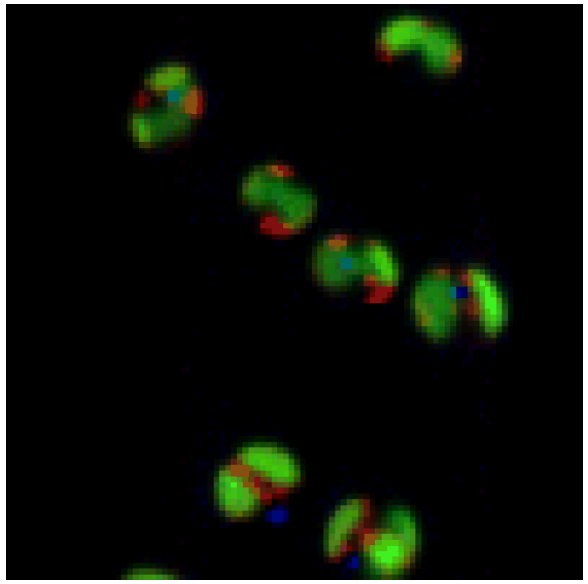




25 μm field of view

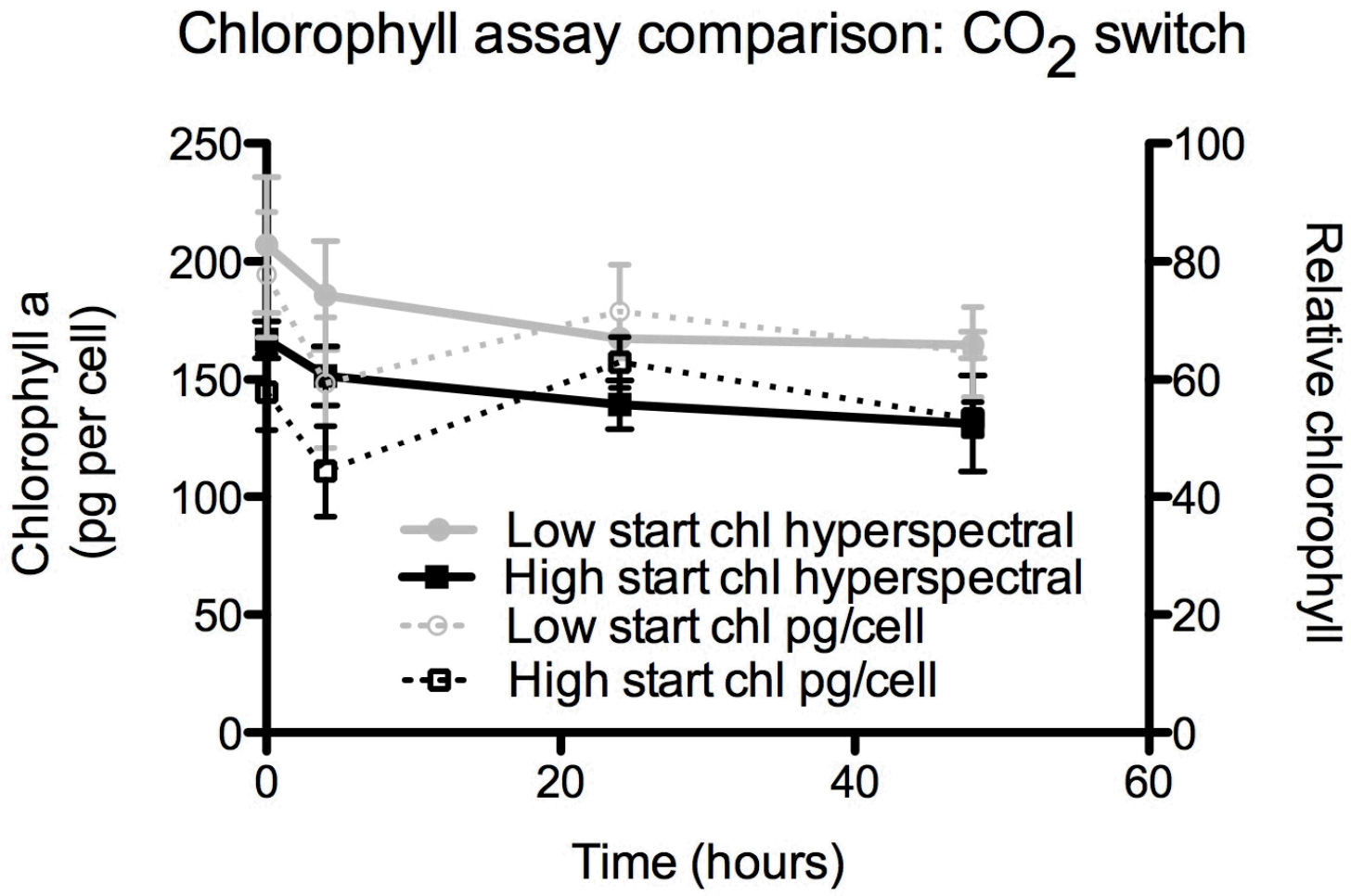
Colors correspond to spectral colors



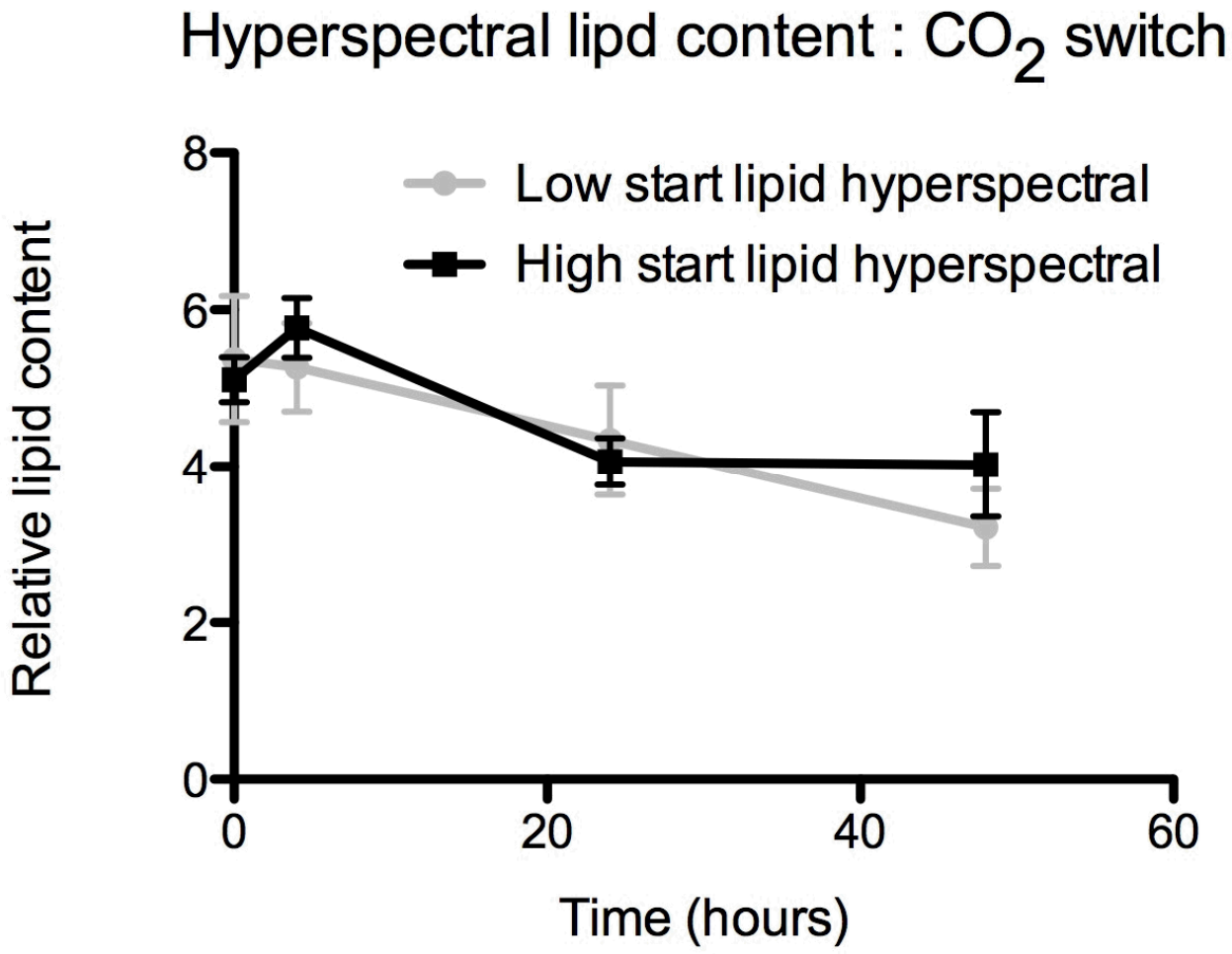


4x spatially compressed image to compare chloroplast carotenoid with Chl a.

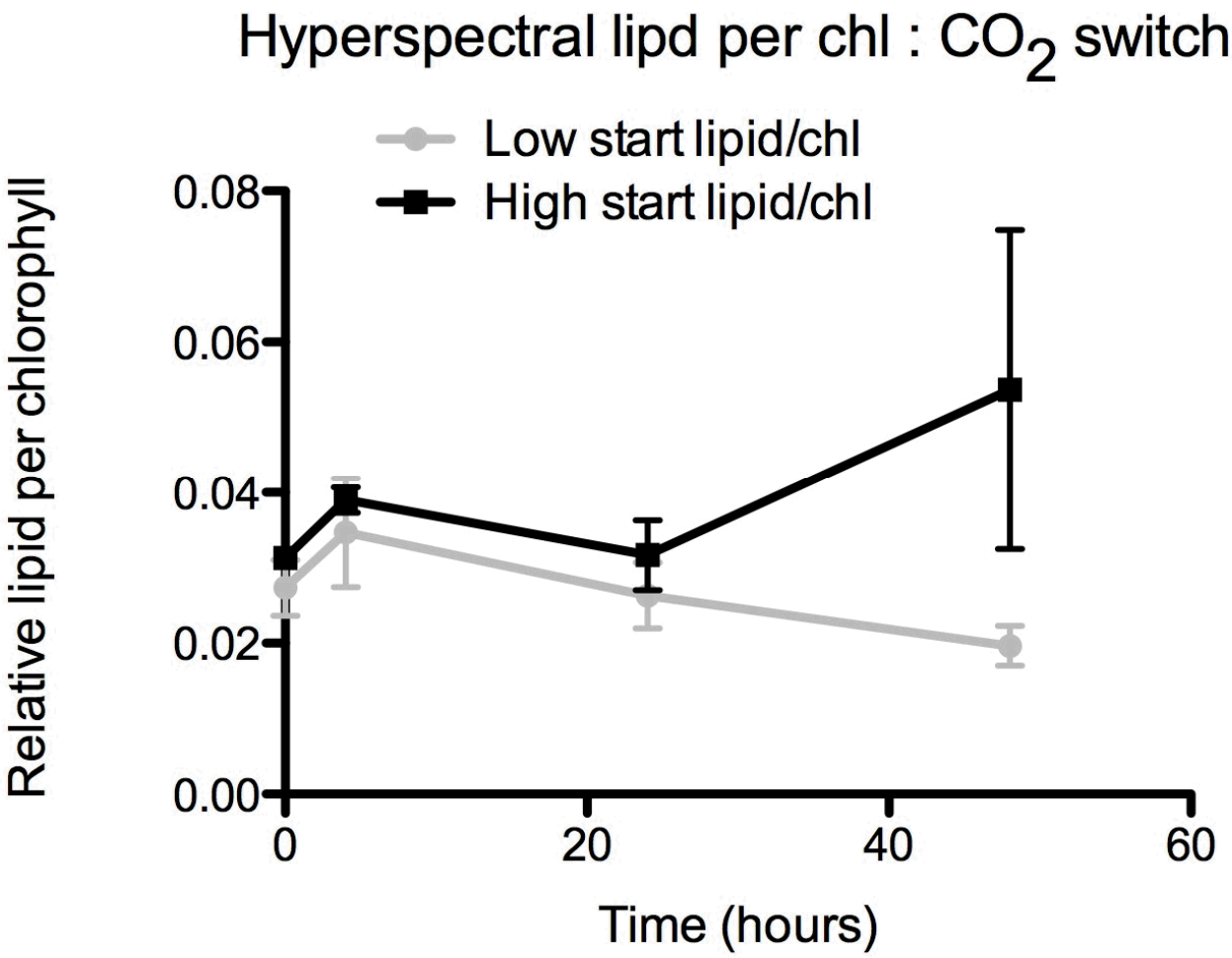
Responses to switch



Responses to switch



Responses to switch



Representative Images

(Selected using median lipid/Chl values)

LOW START

Time = 0 hr Time = 4 hr

A

B

C

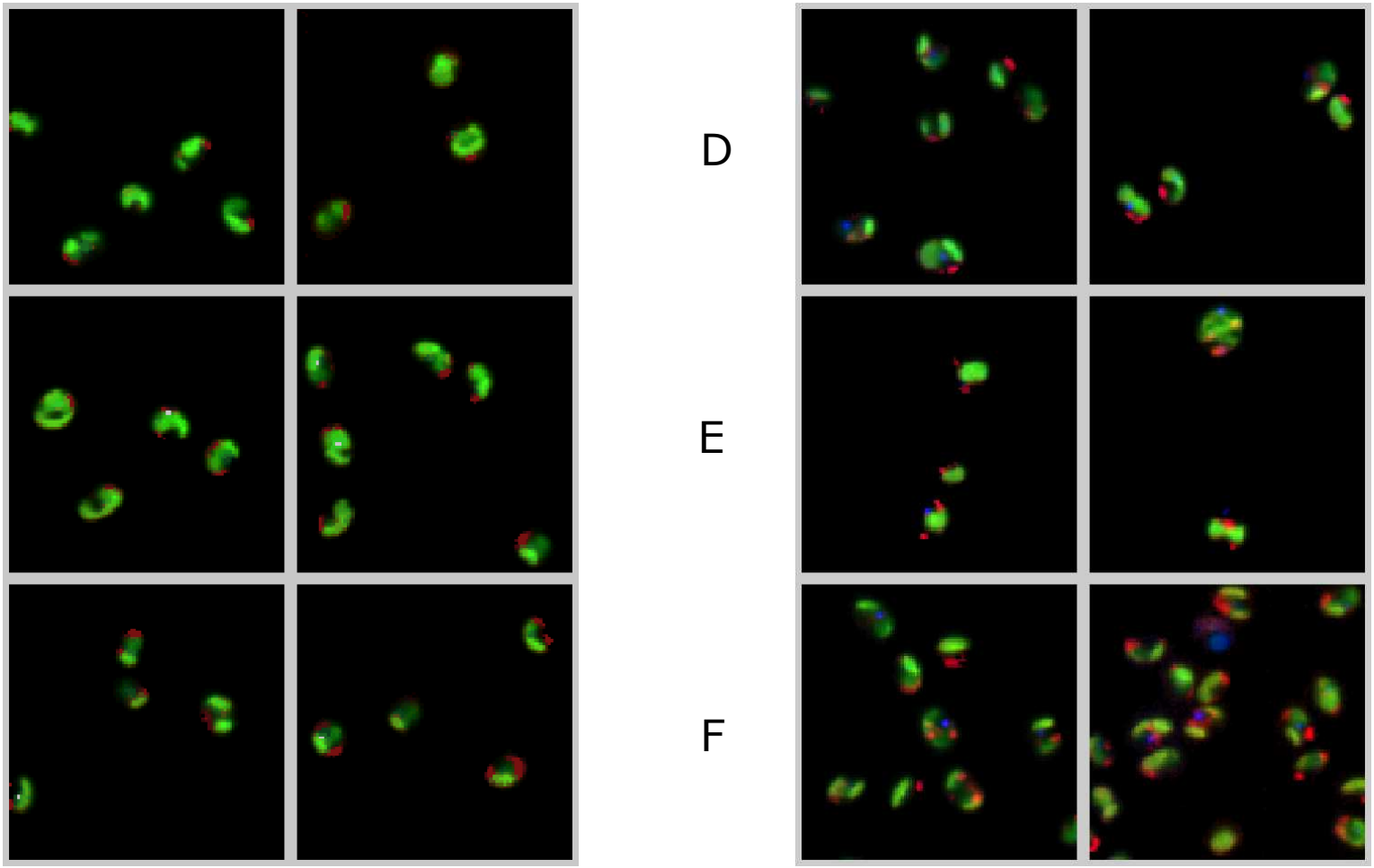
HIGH START

Time = 0 hr Time = 4 hr

D

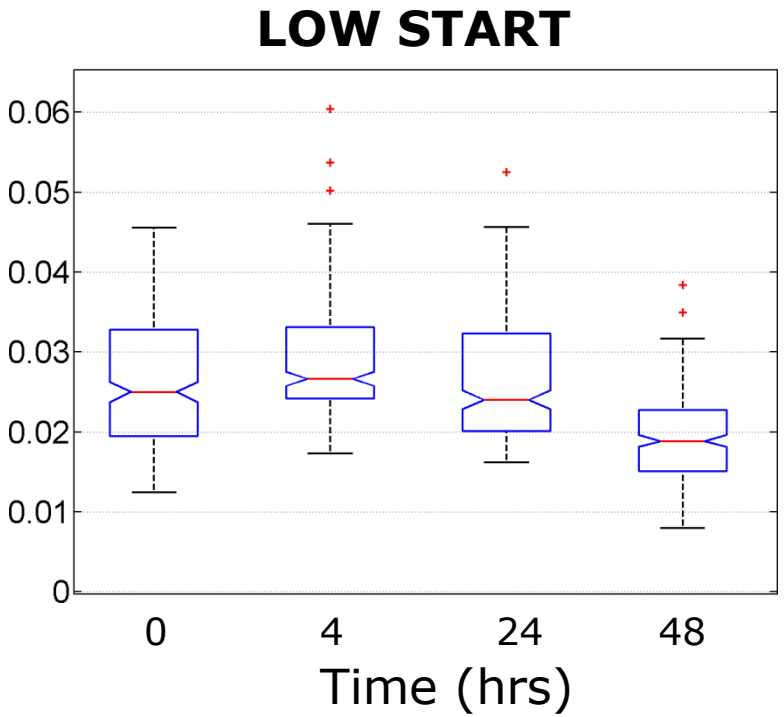
E

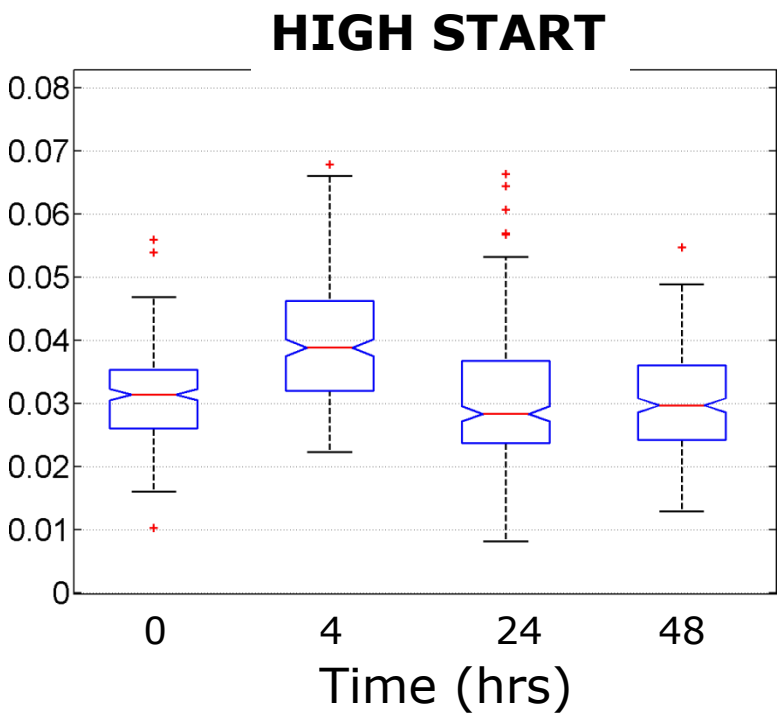
F



Compiled Results

(Lipid:Chlorophyll ratio, Combined conditions)





The notches in the box plots represent 95% confidence intervals, if the notches do not overlap then they are significant at the 95% level.

What now?

- Analyze total lipid content via traditional methods

- Compare with nitrogen limitation
  - Done under high and low CO_2
  - Is low CO_2 a stress signal?

The Future

- NSF Energize New Mexico EPSCoR Funded!
  - Large algal biofuel component
    - $1.5M to UNM over 5 years
    - $1M of new equipment
      - UPSFC-MS to be housed in SOE
      - Start-up for new hire

Acknowledgments

- Tunable Diode Laser
  - Nate McDowell, Los Alamos National Lab (LANL)
  - Funding
    - LANL and NSF
- Cell Density: John DeLong (now at U Nebraska-Lincoln)
- Isoprene
  - Toivo Kallas UW-Oshkosh, Eric Singsaas UW-Stevens Point
- CCM vs lipids
  - Samuel Nieves, UNM
  - John Roesgen, UNM
  - Jerilyn Timlin, Sandia National Labs (SNL)
  - Howland Jones, SNL
  - Aaron Collins, SNL
  - Funding: SNL
- Other collaborators
  - Dick Sayre, NMC
  - Plamen Atanassov, UNM
  - Andy Schuler, UNM
  - Pete Lammers, NMSU

Sandia National Laboratories

National Nuclear Security Administration

U.S. DEPARTMENT OF ENERGY

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000