Making Next Generation Biofuel Systems Work

Richard Sayre

- Scientific Director,
  Center for Advanced Biofuel Systems (DOE-EFRC)

- Scientific Director,
  National Alliance for Advanced Biofuels and Bioproducts
  (DOE Algal Biomass Program)

- Co-Investigator
  Photosynthetic Antennae Research Center (DOE-EFRC)

NAABB National Alliance For Advanced Biofuels and Bio-products

CABS Center for Advanced Biofuel Systems

Photosynthetic Antenna Research Center PARC

Los Alamos NATIONAL LABORATORY EST. 1943

New Mexico CONSORTIUM

What energy sources are the most efficient and most sustainable?

Fuel	Energy Return on Investment (best to worst)	Carbon Efficiency Index (g CO_2/megaJoule)
Hydroelectric	30-100	-
Shale Gas	68	53
Coal	60	105
Cellulosic Ethanol	6-36	20
Petroleum	30-40	96
Wind	20-40	-
Solar PV	10-35	-
Algal biocrude	10	-
Sugarcane Ethanol	6-10	20
Food	2.7-5	-
Biodiesel	2.5	17-40
Corn Ethanol	0.8-1.7	34-80

Energy 52 (2013): 210–221

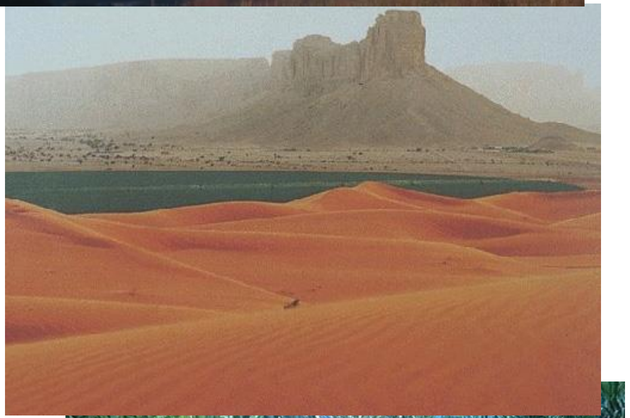
Biofuels; an alternative to liquid fossil fuels

Advantages

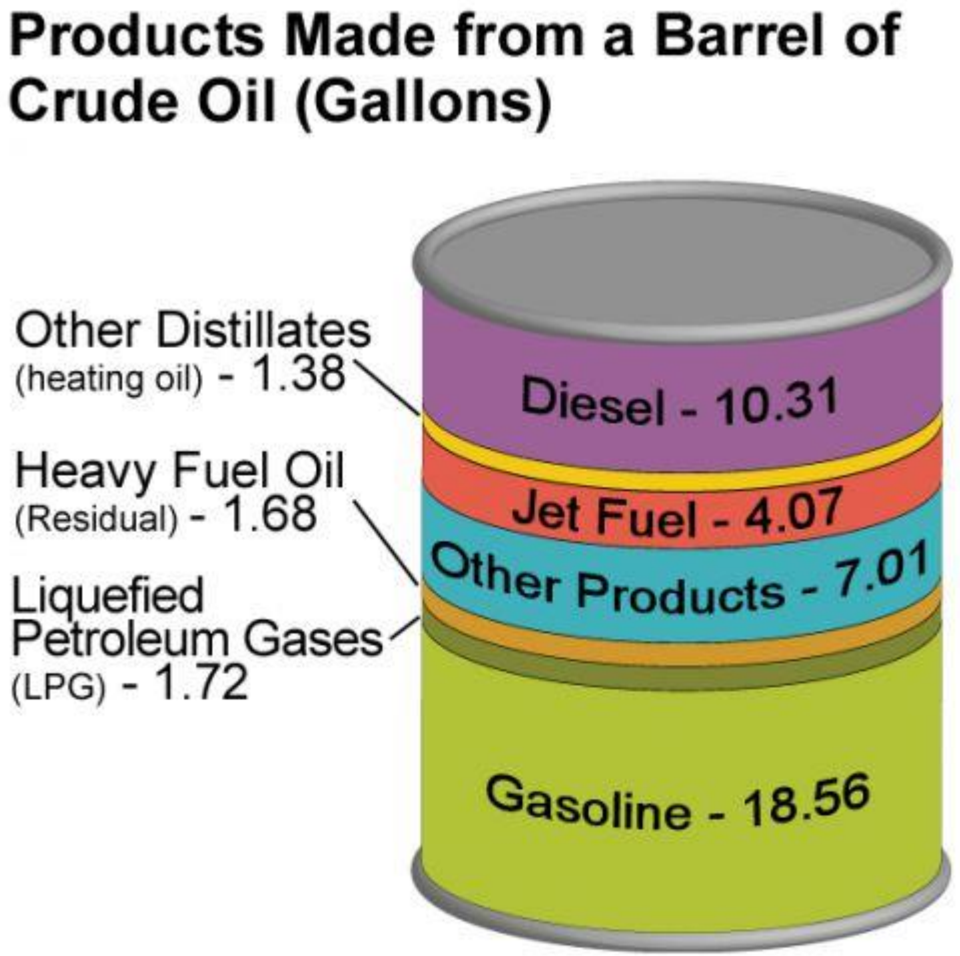
- Sustainable, not extractive
- Reduced CO_2 and S emissions
- Energy independence
- Decentralized energy economy
- Oil-based feedstocks available

Constraints:

- <u>Low solar energy density</u>
- Potential competition with food
- Technological hurdles
- Production systems must be optimized for each site; high capex
- Harvests often seasonal, not continuous



Biocrude, a sustainable replacement for petroleum

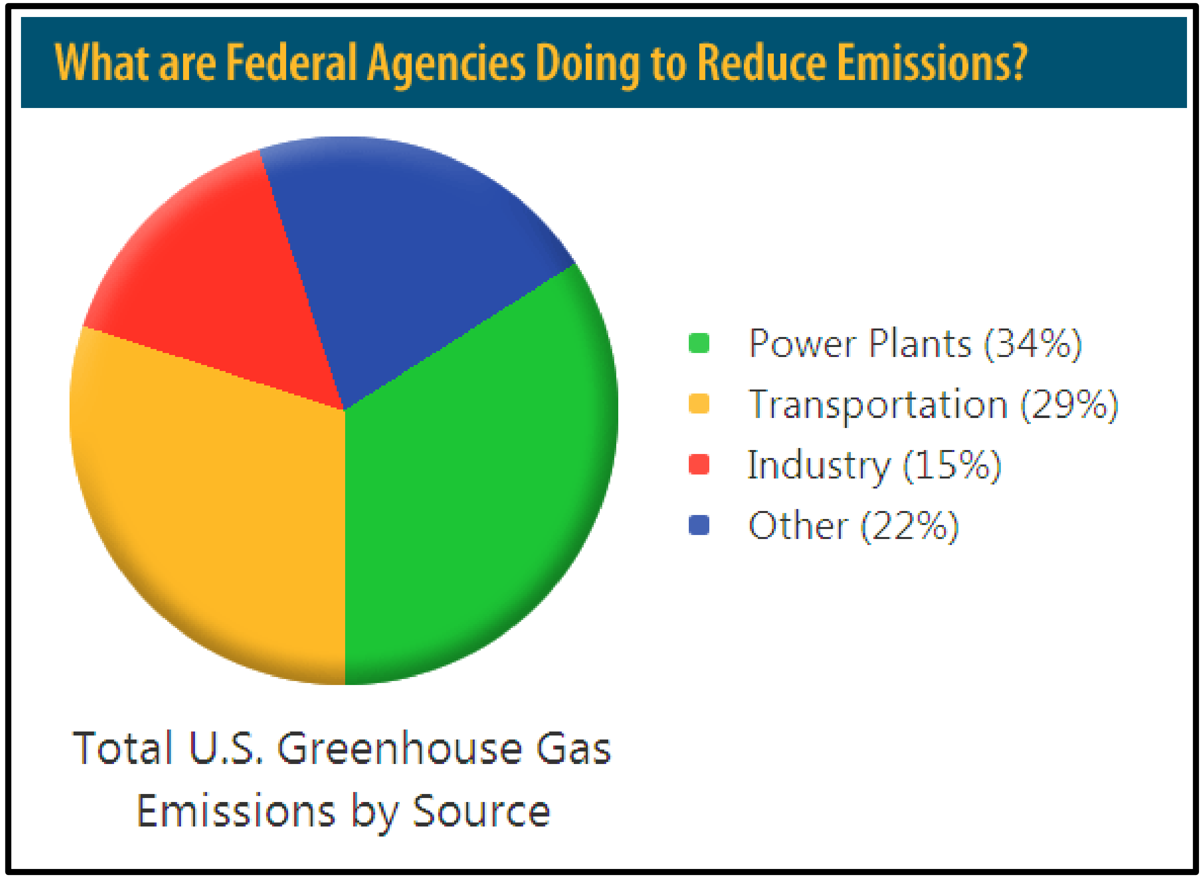


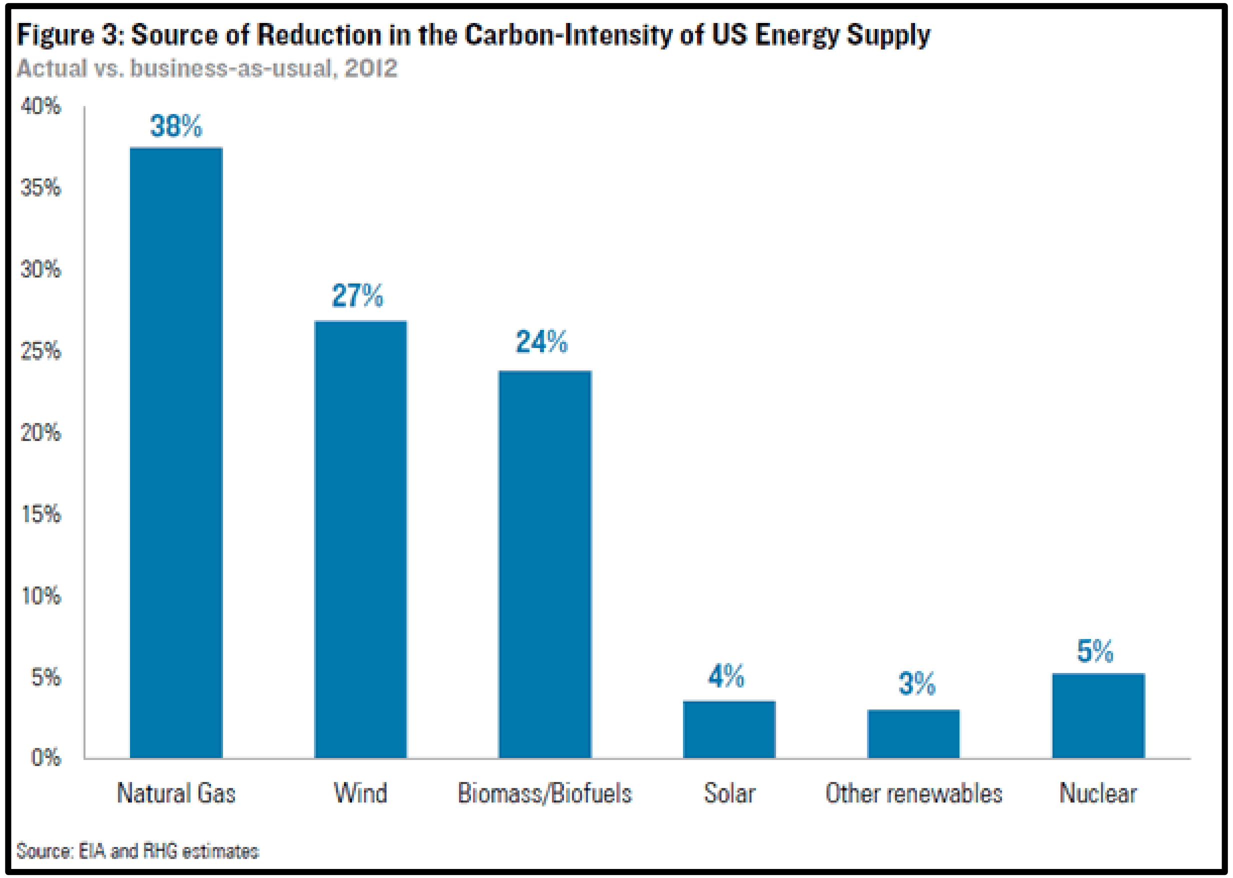
About half of the products produced from oil have no alternative replacements other than oil-based feedstocks

<u>Advantages of biocrude based fuels</u>

- **Oil has 2X the energy density of alcohol.**
- **Oil has 50X the energy density of the best batteries**
- **Oil-based feedstocks are compatible with existing refinery, fuel distribution, and engine infrastructure**
- **Reduced sulfur and particulate emissions**

Biofuels are contributing (~25%) to reductions in US greenhouse gas emissions





Total US greenhouse gas emissions dropped 16% between 2000 and 2009

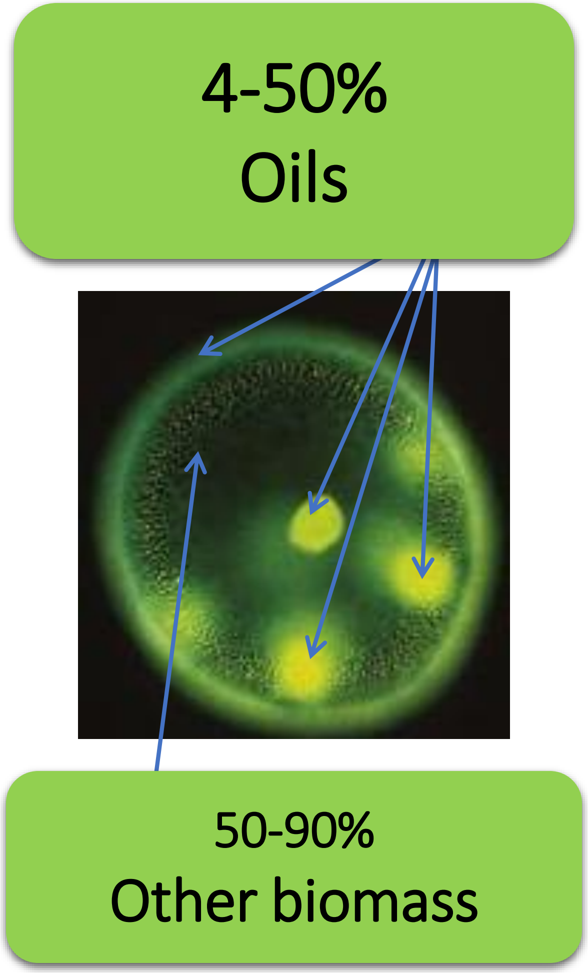
Improving sustainable biocrude production

<u>**Next-Gen Bioenergy Systems**</u>

- **Greater energy-return-on-investment**
- **Reduced greenhouse gas emissions per unit energy generated (gCO_2/mJ)**
- **Reduced resource (land, water, and nutrient) requirements**
- **Reduced competition for food**
- **Compatible with existing liquid fuel refining, distribution, and combustion infra-structure**
- **Scalable production systems**
- **Achieve economic parity with petroleum-based fuels**

Eldorado Biofuels algal facility in Jal, NM.
Utilizes "produced" water from oil wells.

Next-gen biofuels: Oils from microalgae



Rapid growth rate
(2-10 X faster than terrestrial plants)
- Unlike plants, all cells are photosynthetic
- High photosynthetic efficiency (CCM)
- Double biomass in 6-12 hours

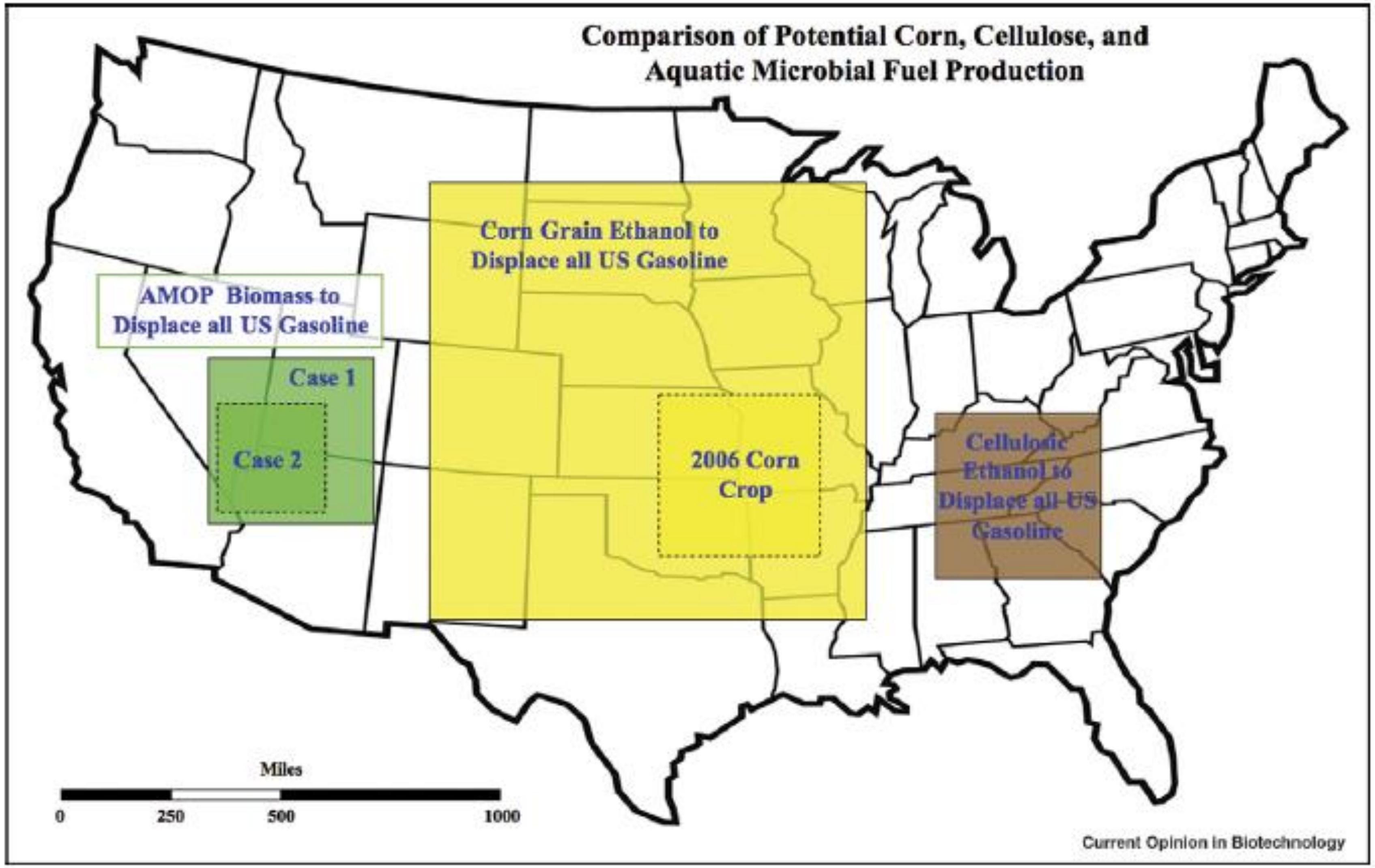
High oil content
- 4-50% non-polar lipids

All biomass harvested
- 100%

Harvest interval
- 24/7; not seasonally, so reduces risk

Sustainable
- Capture CO_2 in ponds as bicarbonate
- Use waste water and nutrients
- No direct competition with food

Relative land area for biofuel feedstocks required to displace US gasoline demand (2006)



Renewable and Sustainable Energy Reviews 14 (2010) 217-232

National Alliance for Advanced Biofuels and Bioproducts (2010-2013)

- **Develop cost-effective production of algal biomass and lipids**
  - **Algal Biology -** Increase overall productivity of algal biomass accumulation and lipid/hydrocarbon content
  - **Cultivation -** Increase overall productivity by optimizing sustainable cultivation and production systems
  - **Harvesting/Extraction -** Develop cost-effective and energy efficient harvesting and lipid extraction technologies

NAABB
National Alliance For Advanced Biofuels and Bio-products

- **Develop economically viable fuels and co-products**
  - **Fuel Conversion –** Develop technologies to convert lipids/hydrocarbons and biomass residues into useful fuels
  - **Valuable Co-products -** Develop a set of valuable coproducts to add profitability and provide flexibility to allow responsiveness to changing demands/opportunities in the market
- **Provide a framework for a sustainable algal biofuels industry**
  - **Sustainability Analysis –** Quantitatively assess the energy, environment, economic viability (LCA) and sustainability of the NAABB approaches to guide our strategy

Modeling Algal Farm Economics

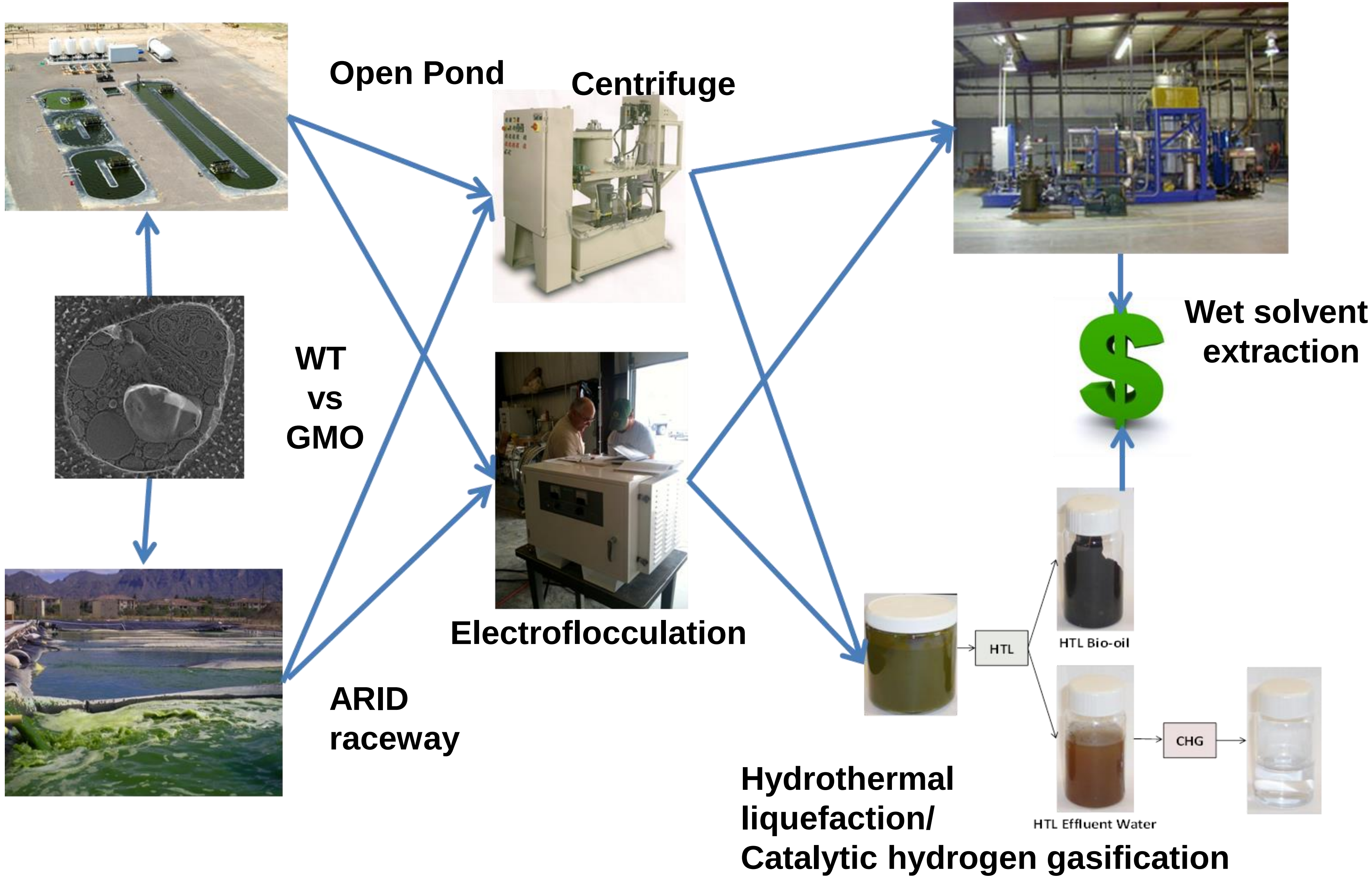
	Base Farm
Total Hectares of Land	4,850
Total Hectares of Ponds	4,050
Total Volume of Ponds (AF)	9,855
Total Volume of Ponds (L)	12,156,211,201
Days of Operation	330
Total CAPEX	$1,270,255,769
Total OPEX Year 5	$739,780,301

James Richardson, TAMU
Myriah Johnson , TAMU
Meghan Downes, NMSU

(12,125 acres; 10 inches deep)

Source: Extrapolated from NREL harmonization report 2012

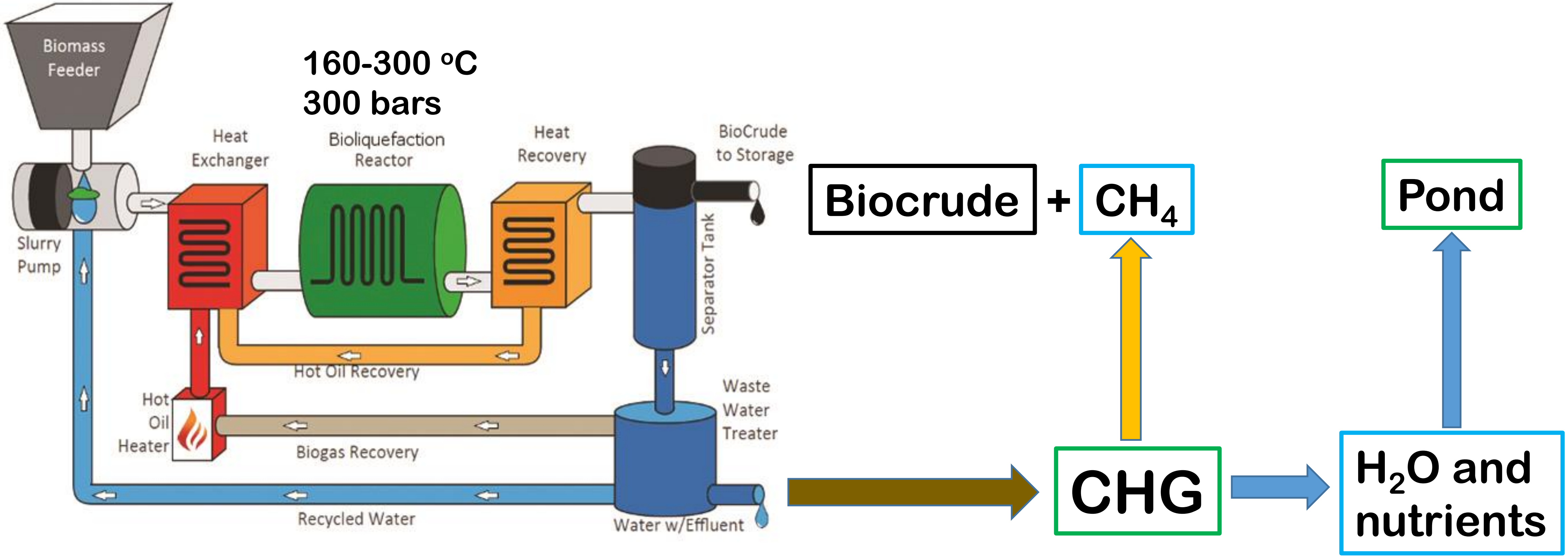
NAABB sustainability analysis scenarios: roadmap for the future



Two stage hydrothermal liquefaction (HTL) and catalytic hydrogen gasification (CHG) of algal biomass; 85% recovery of total carbon as fuel.

Biocrude is compatible with existing refinery and combustion processes

Algae: protein (9-60%), carbohydrate (5-60%), lipid (2-60%)



Biocrude composition: alkanes, fatty acids, cyclic aromaticss, ketones

HTL energy conversion efficiency; Energy recovery from different feed stocks

Material	Oil %	Protein %	Carbohydrate %	Energy Recovery in Biocrude %
Plant Oil	100	-	-	87
Protein	-	100	-	30
Starch	-	-	100	14
Nanochloropsis	32 (6.4X)	57	8	66 (1.30)
Chlorella	25 (5X)	55	9	54 (1.06)
Porphyridium	8 (1.6X)	43	40	52 (1.02)
Spirulina	5 (1X)	65	20	51 (1)

Bioresource Technol. (2011) 102: 215

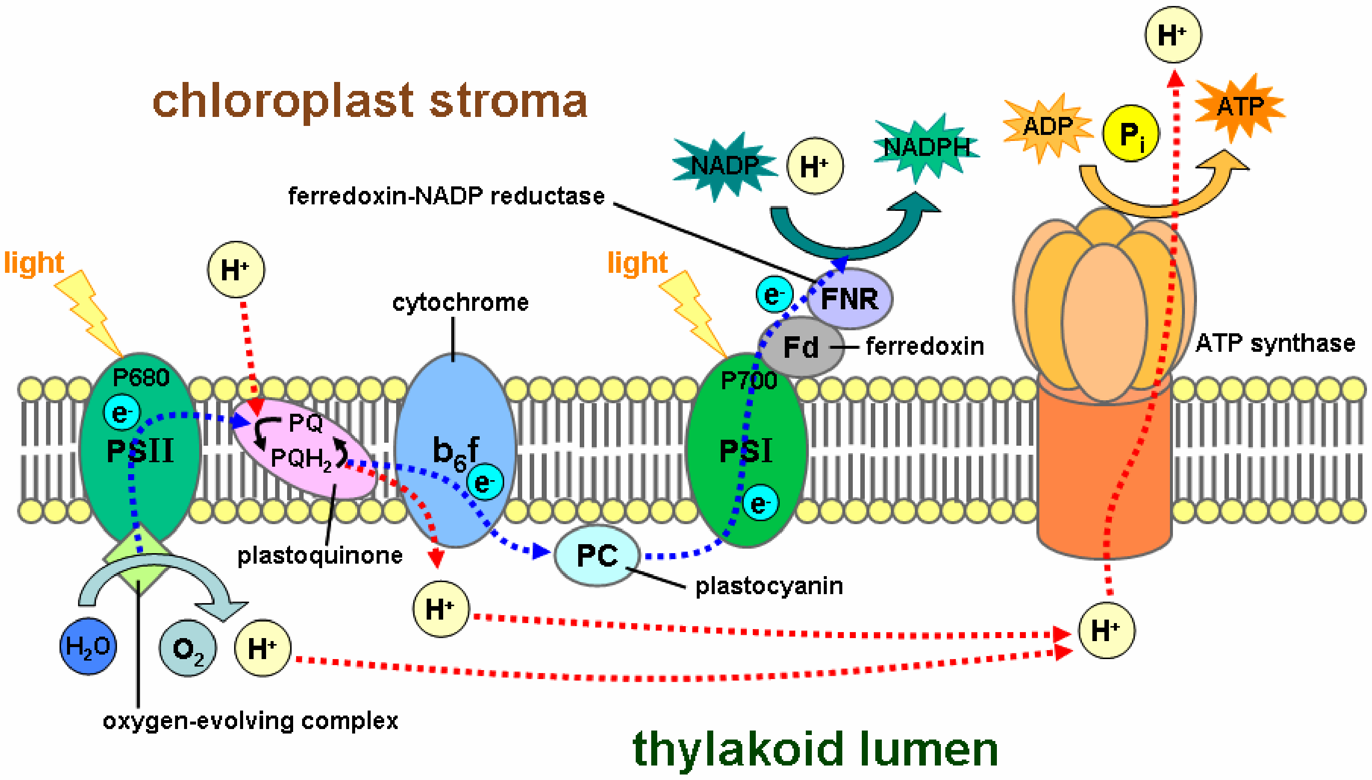
Modeling algal biofuels systems; base (current) and best-case scenario

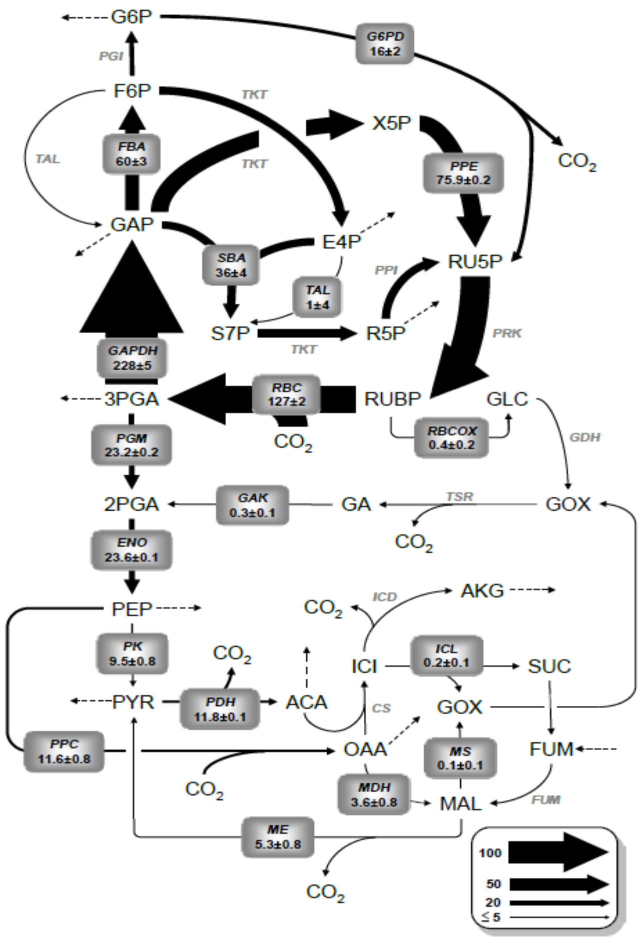
Scenario	Base	Best Case
Biology	Generic	GMO (3x)
Cultivation	Open Pond	Arid Raceway
Harvesting	Centrifuge	Electrocoagulation
Extraction	Wet Solvent	HTL-CHG
Nutrient Recycling	No	Yes
Biomass Production (Tons/yr)	120,000	380,000
Crude Oil Production (gallons/yr)	4,700,000	52,000,000
Products	Oil and delipidated algae	Oil and methane
Location	Pecos, TX	Tucson, AZ
Total cost/gallon	$230 - 16	$ 4.90 – 3.60

4,000 ha farm

NAABB life cycle analyses indicate that enhancing biomass productivity is required for profitable biofuel production from algae

What aspects of biomass productivity should we focus our efforts on to achieve the greatest yields?

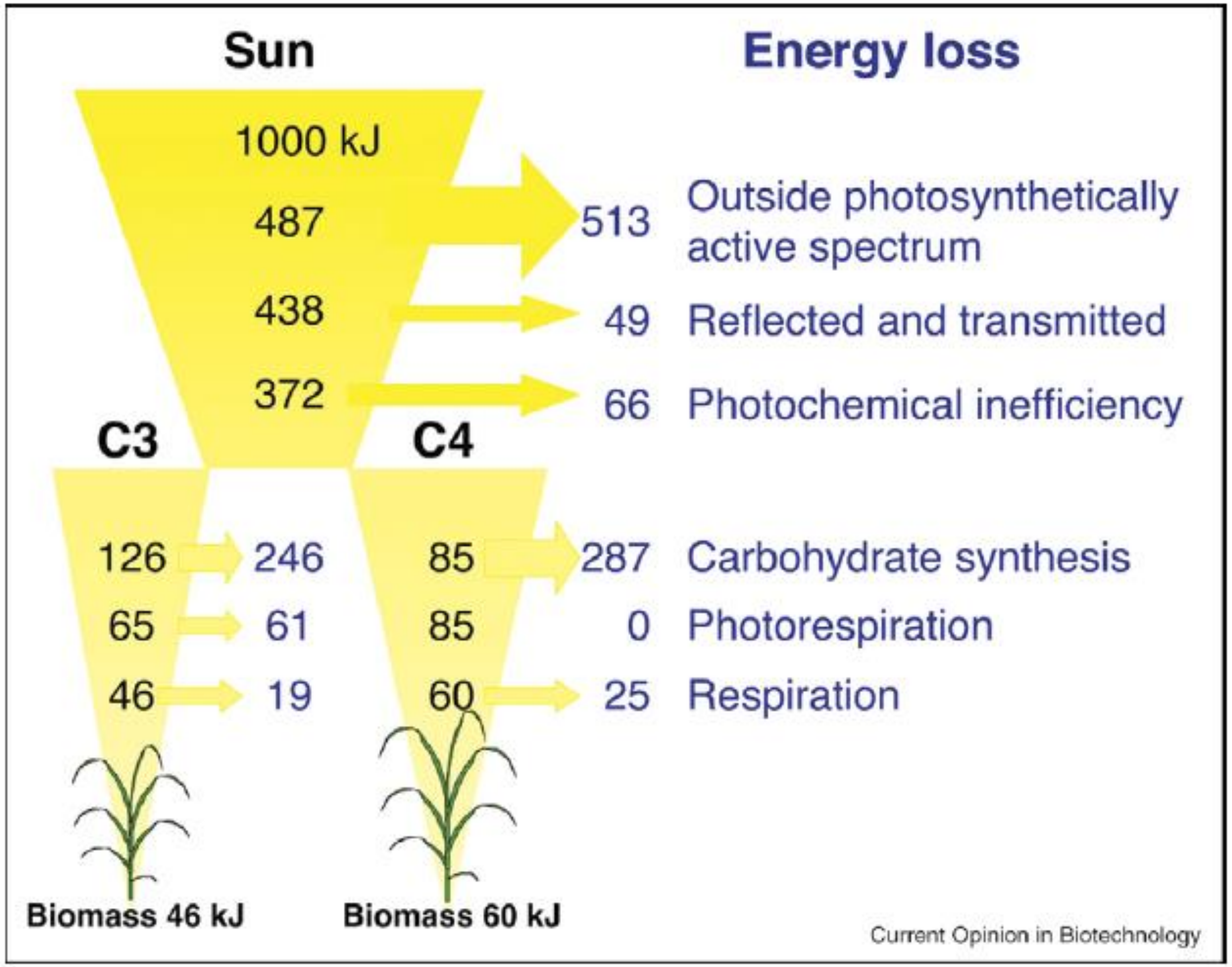




Improving biomass production efficiency
What should be the targets?

Maximum theoretical efficiency for photosynthesis (red photons to glucose) is ~30%

EROI for carbohydrate production is 10% -20% greater than for oil synthesis



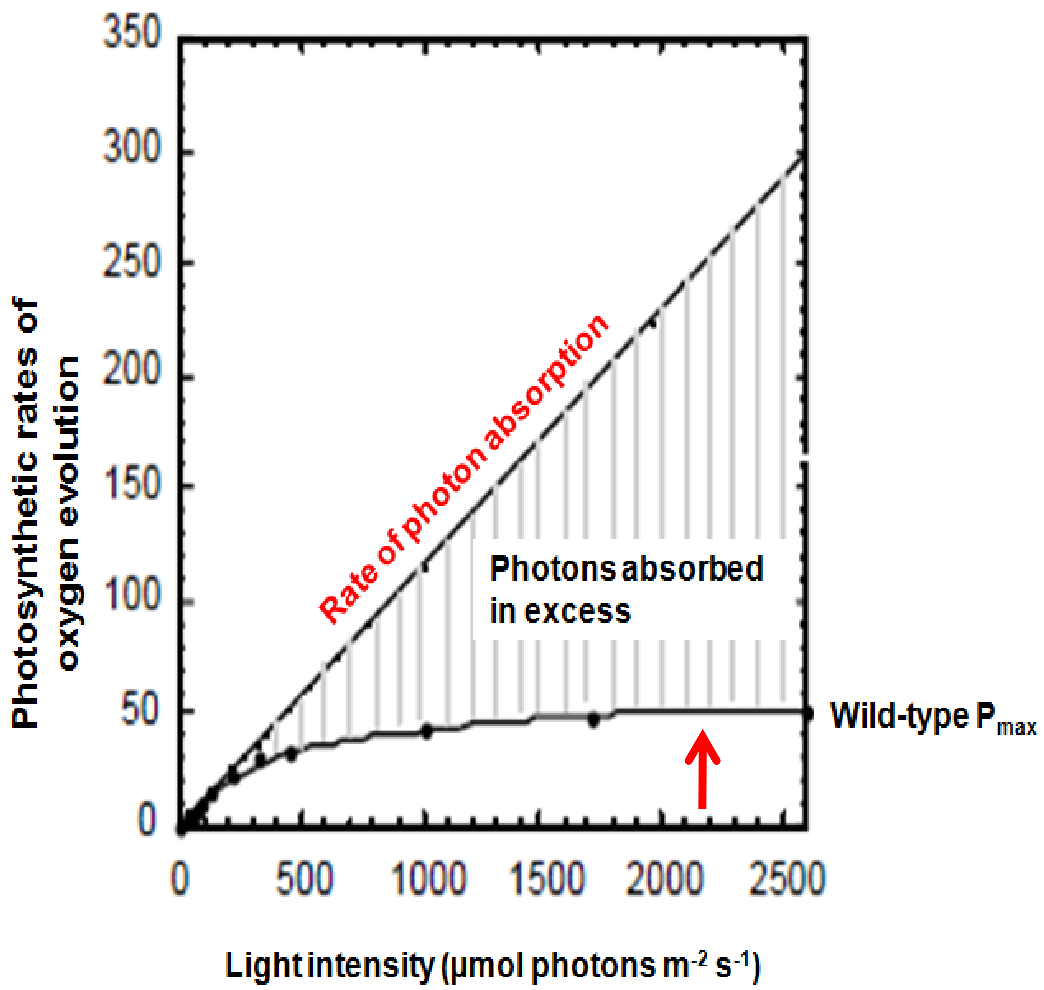
Light capture
55% losses

Energy conversion
30-40% losses

Energy accumulation (sink)
4-6% gain

Zhu et al., (2010) *Annual Review of Plant Biology*, 61: 235-261; Subramanian et al., (2013) *Biotechnol. Biofuels* 6:150-162

Photosynthesis wastes 75% of the captured light energy because antenna are too big



Energy capture is 10 fold faster than photosynthetic electron transfer at noon.

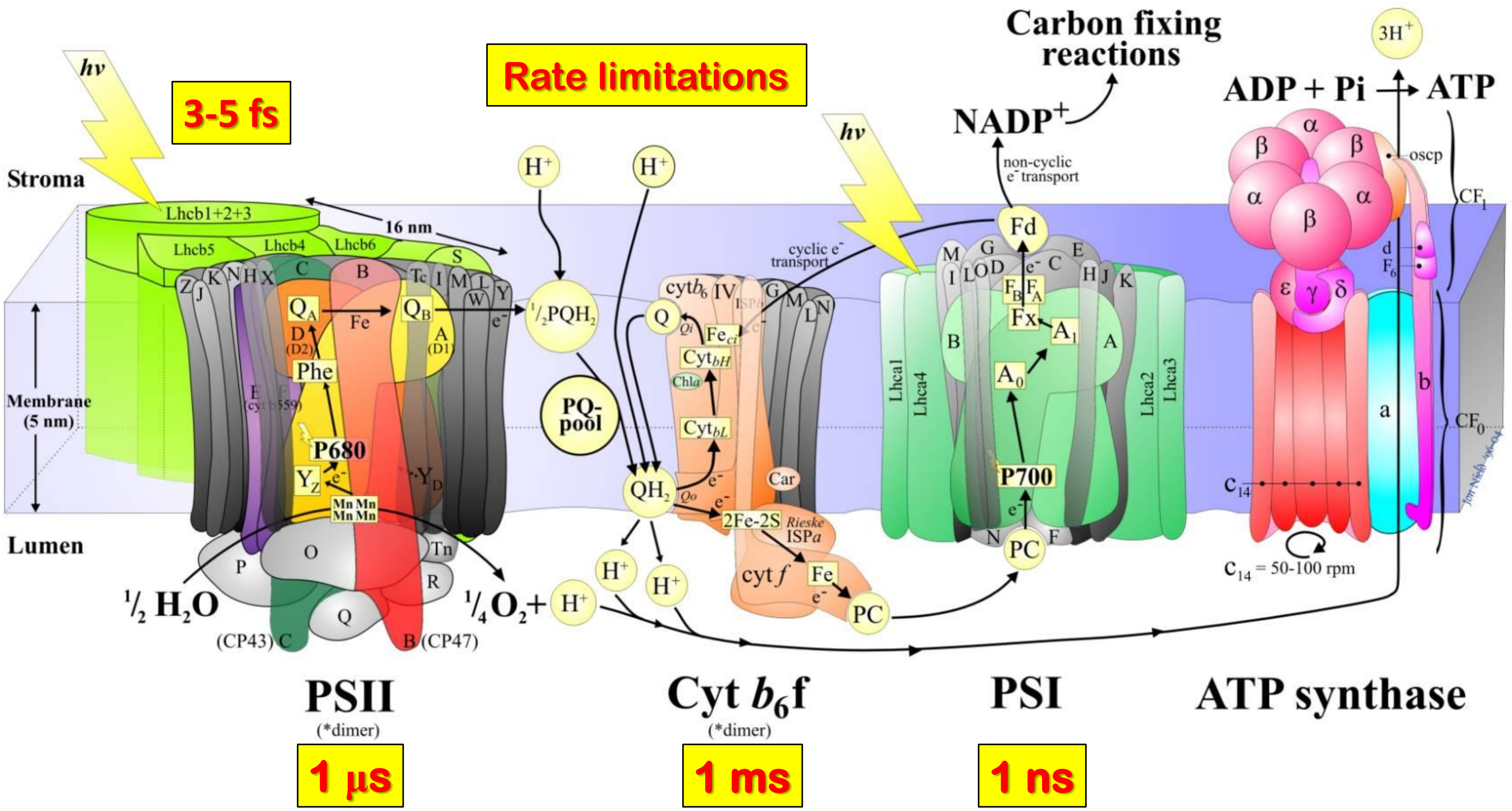
Photosynthesis can be light saturated during 75% of the day.

Up to 60% of absorbed light at full sunlight is dissipated as heat or fluorescence

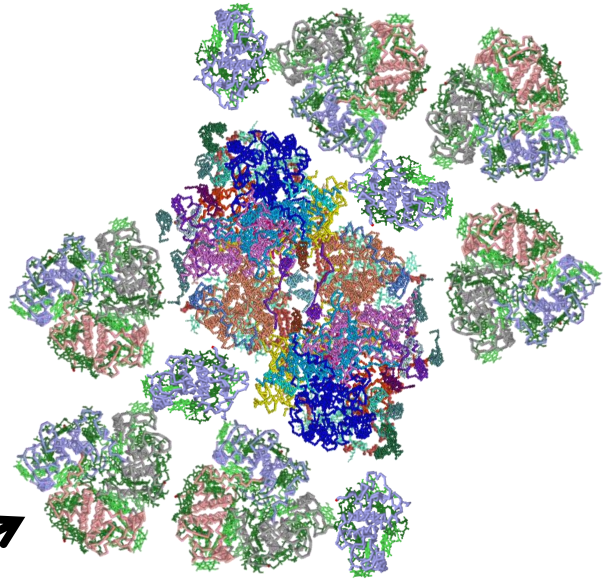
High light intensities also lead to photoinhibition or damage to photosystem II

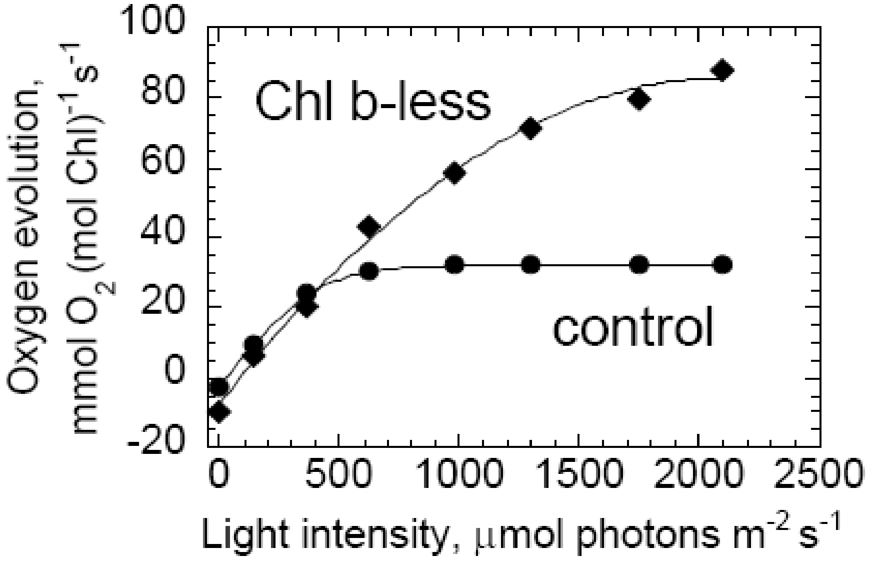
Kinetic bottlenecks in electron transfer rates reduce the efficiency of light conversion into chemical energy

Photon capture is 10 times faster than electron transfer at noon



Reducing Chl b levels reduces LHC-antenna size and increases energy conversion efficiency; but does this translate to better photosynthesis and growth?





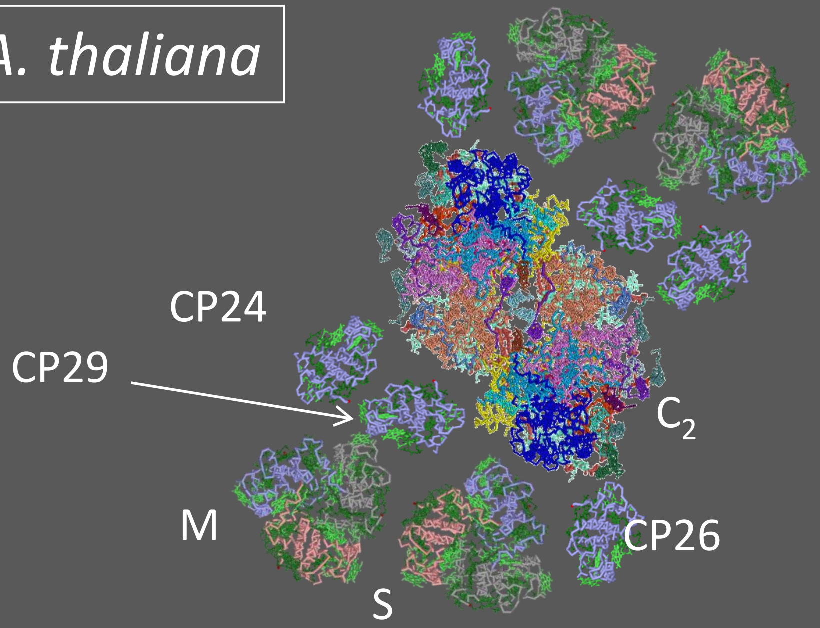
- The peripheral antennae (LHC) accounts for 75% of the total Chl (500) with PSII and PSI

- Chl b is present only in the peripheral antennae (LHC).

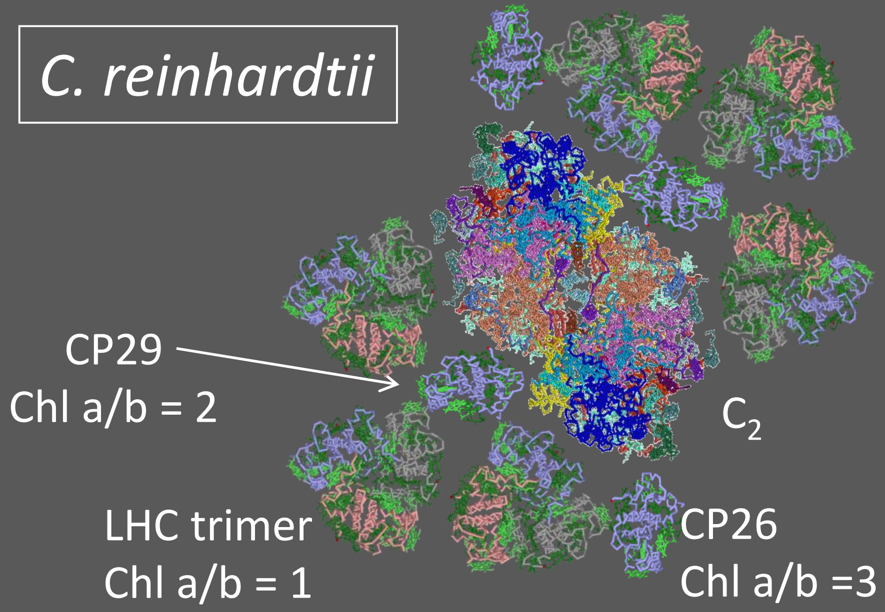
Eliminating Chl b eliminates LHC and increases photosynthetic efficiency at high light intensities but only tested under photoheterotrophic conditions

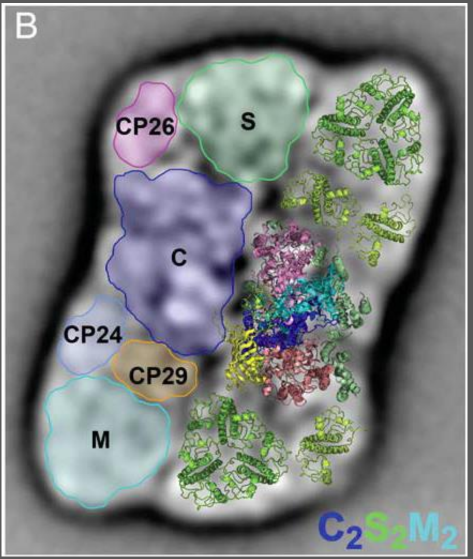
Proceedings of the 1999 US DOE Hydrogen Program Review

A. thaliana

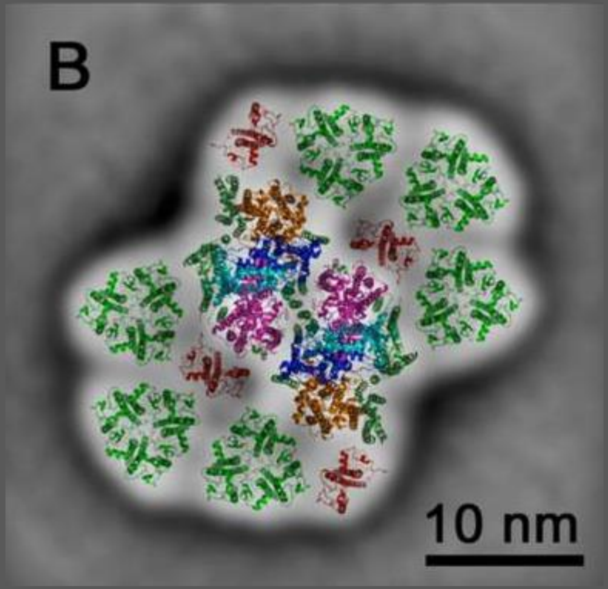


C. reinhardtii



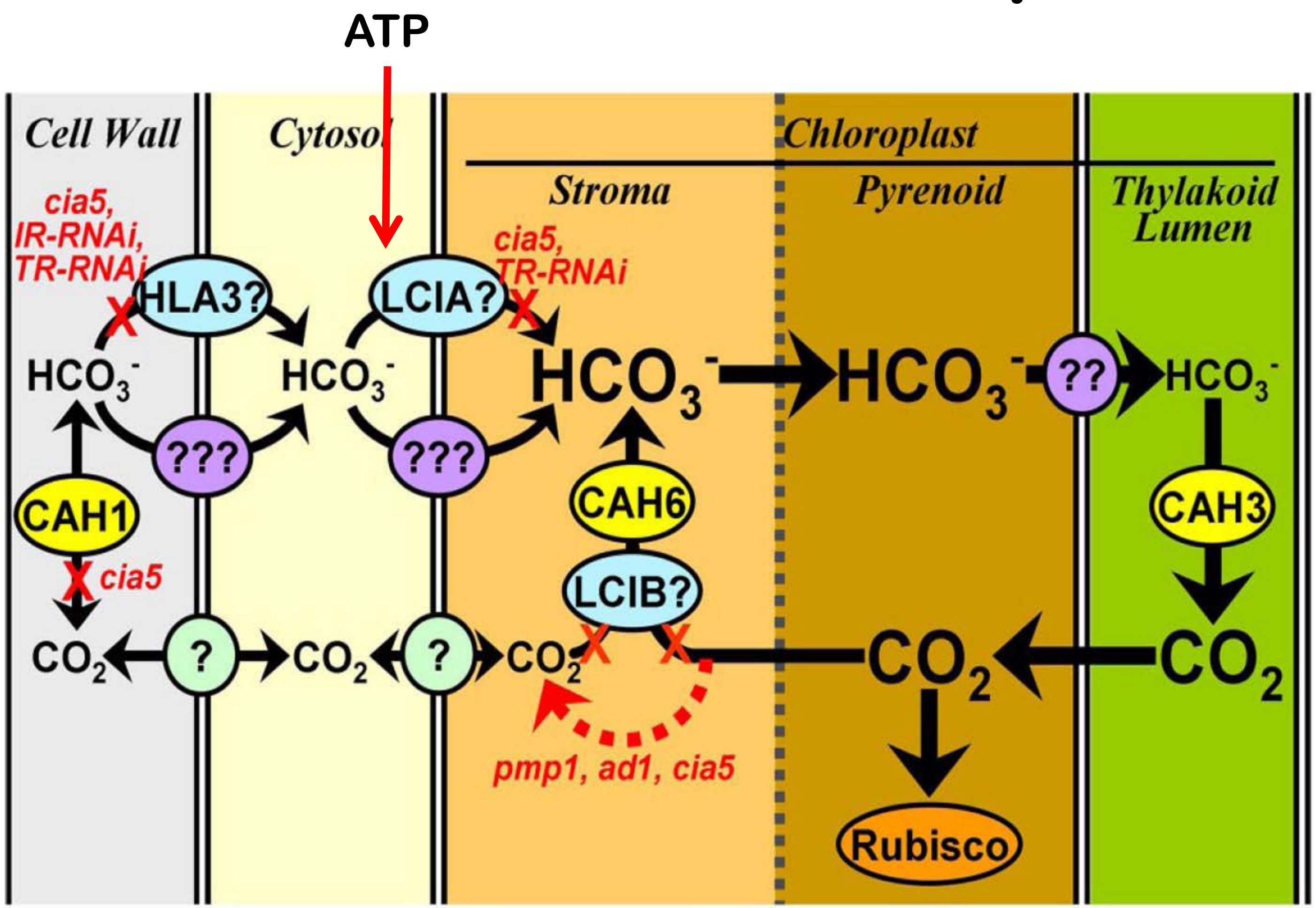


Kouril et al. *BBA* (2012)



Tokutsu et al. *J. Biol. Chem.* (2012)

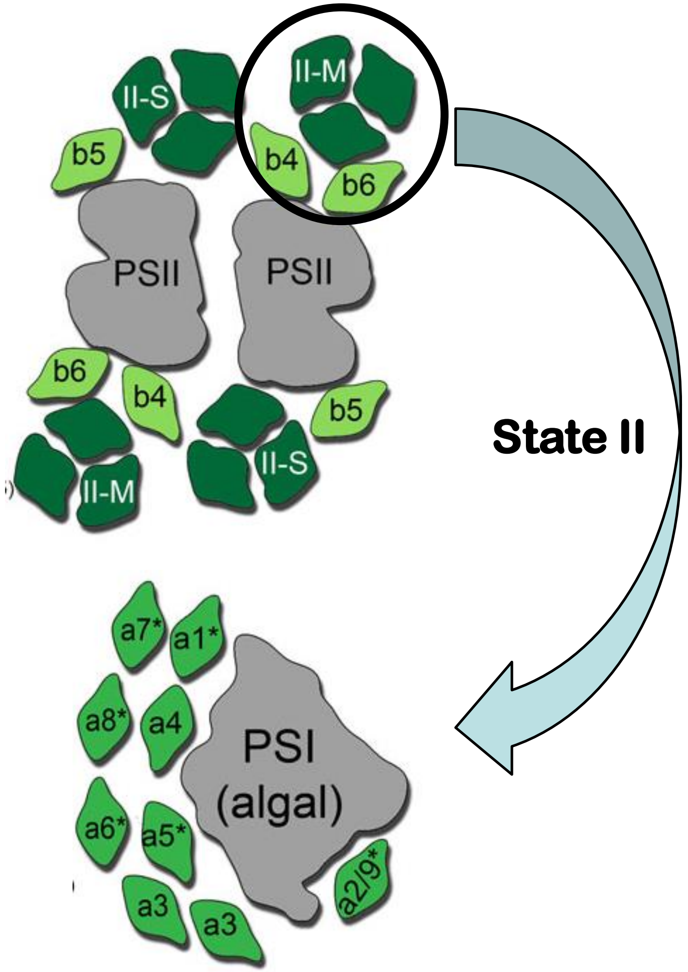
Algae have additional demands for ATP to fix CO_2. Unlike plants, algae actively (ATP) pump bicarbonate into chloroplasts



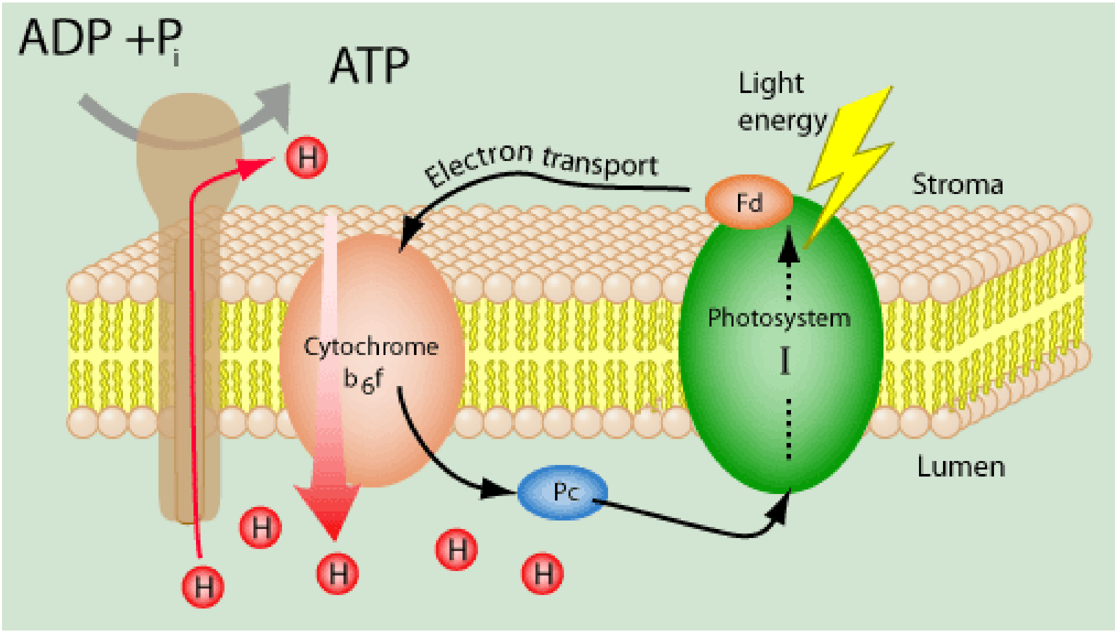
Cyclic ATP synthesis supports the additional energy demand for the bicarbonate pump (HLA3)

M. Spalding

Light harvesting complexes dynamically regulate light distribution between photosystems and dissipate excess energy to reduce light stress



LHCII association with PSI supports an increase in cyclic ATP synthesis

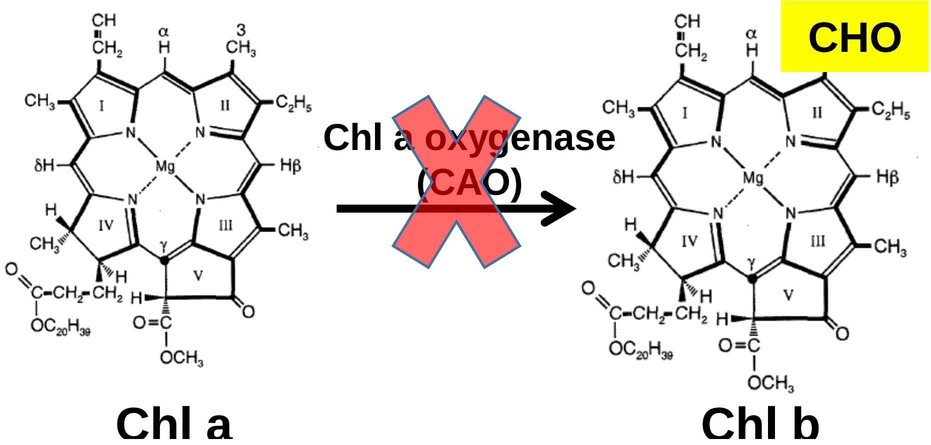


Chlamydomonas mutants unable to do state transitions have impaired growth due to a reduced ability to carry out cyclic ATP synthesis. PNAS 106:15979

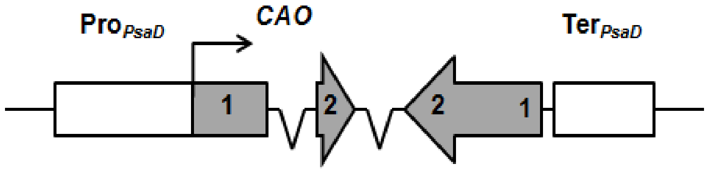
Modulating light harvesting antenna to optimize growth

Hypothesis: Reducing (but not eliminating)chlorophyll b will alter antenna dynamics by reducing self-shading, allowing for state transitions and reducing photodamage improving photosynthetic efficiency

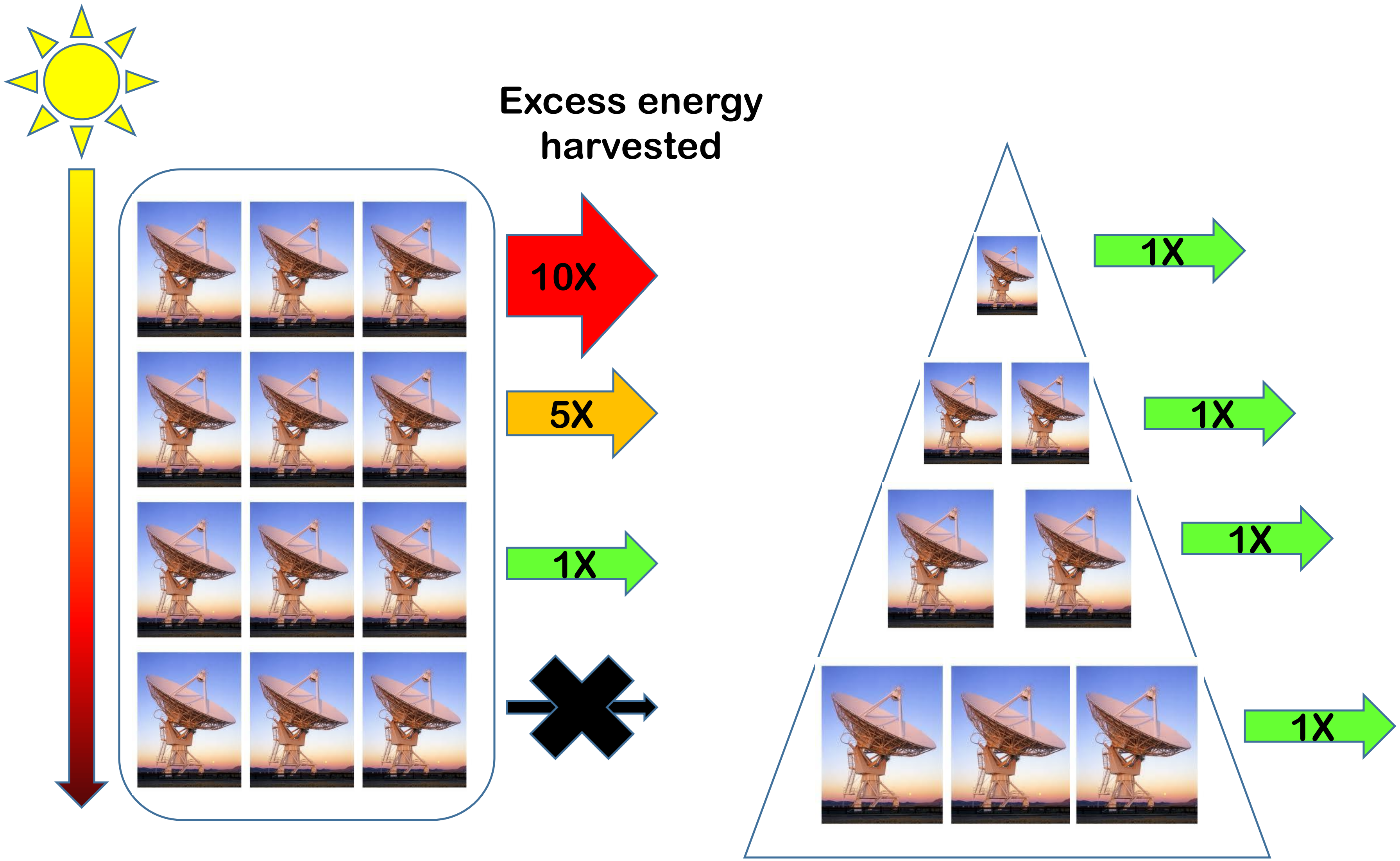
Chlorophyll a oxygenase RNAi



Construct for CAO-RNAi



Alternative antenna designs for light capture in canopies and ponds

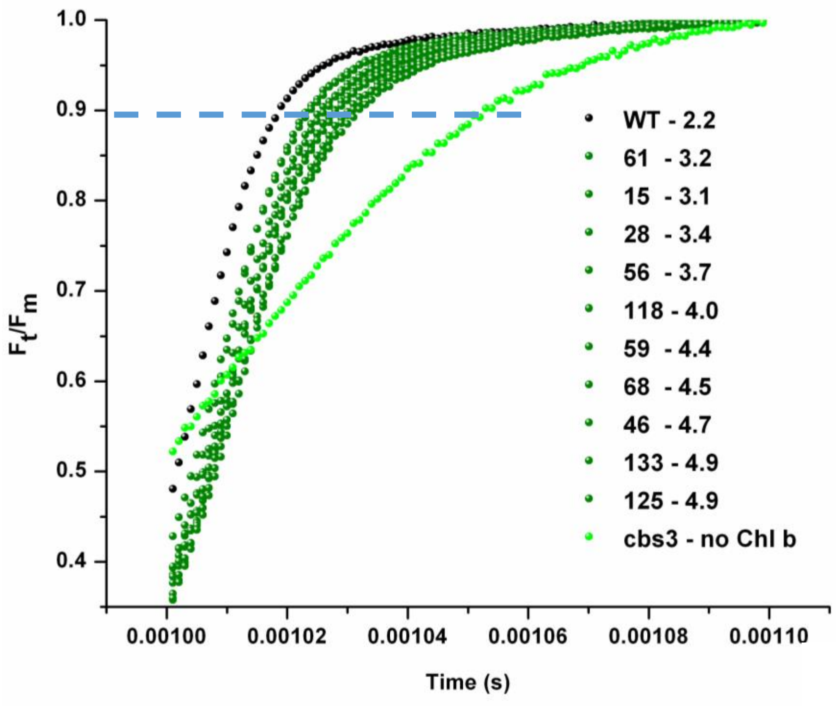


Optimal for light competition

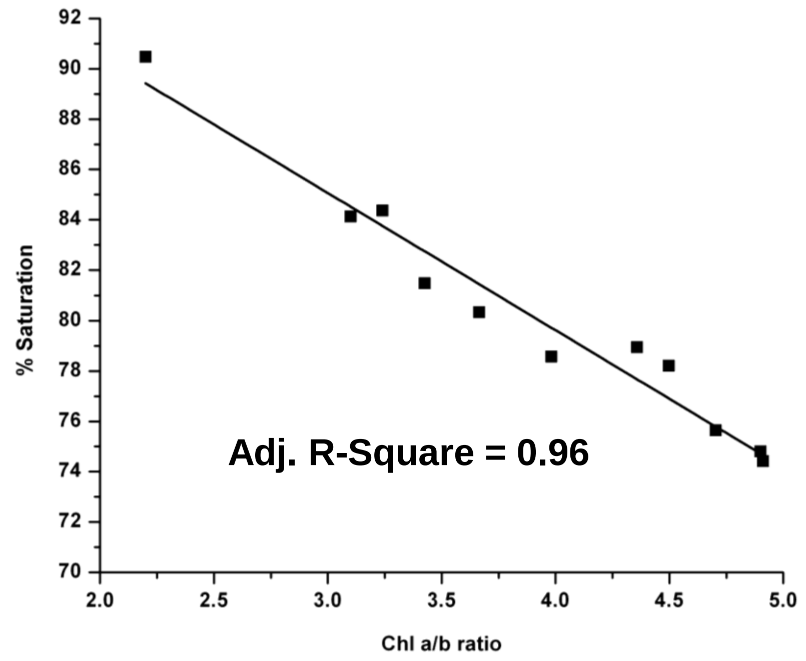
Optimal efficiency in canopies and monocultures

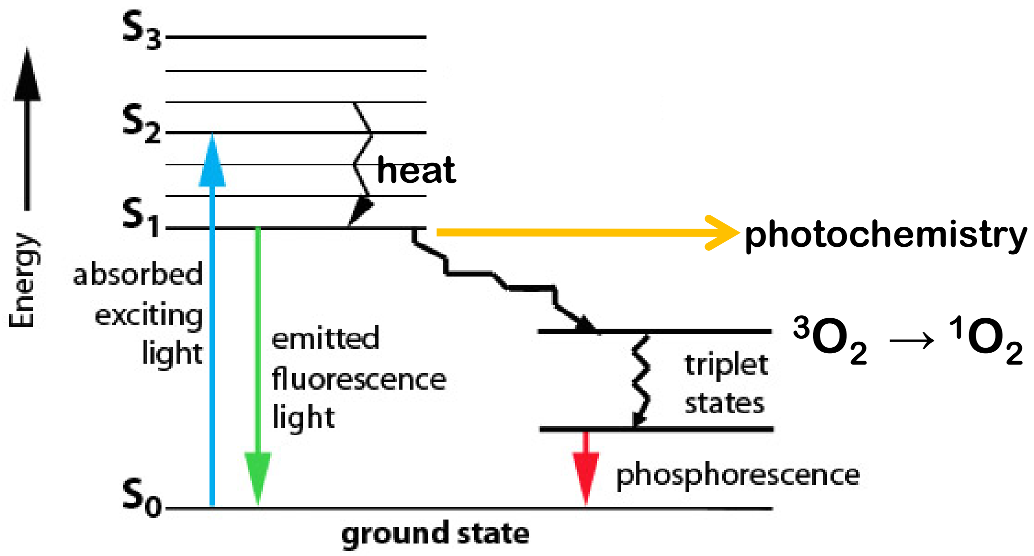
Generating transgenic microalgae with intermediate chlorophyll a/b ratios

	Strain	Total Chl per Cell (µg/ml)	Chl a/b ratio	Fold Increase in Chl a/b ratio compared with WT
WT	CC-424	11.3	2.2	-
CAO-RNAi (Chl-b deficient)	CR-15	10.8	3.1	1.4
	CR-28	10.3	3.4	1.5
	CR-46	10.5	4.7	2.1
	CR-56	11.5	3.7	1.7
	CR-68	10.9	4.5	2.0
	CR-118	10.9	4.0	1.8
	CR-125	8.8	4.9	2.2
	CR-133	10.7	4.9	2.2
CAO-Knockout (Chl-b less)	cbs-3	10.4	∞	-



An inverse relationship exists between chlorophyll fluorescence raise kinetics and Chl a/b ratios or antenna size

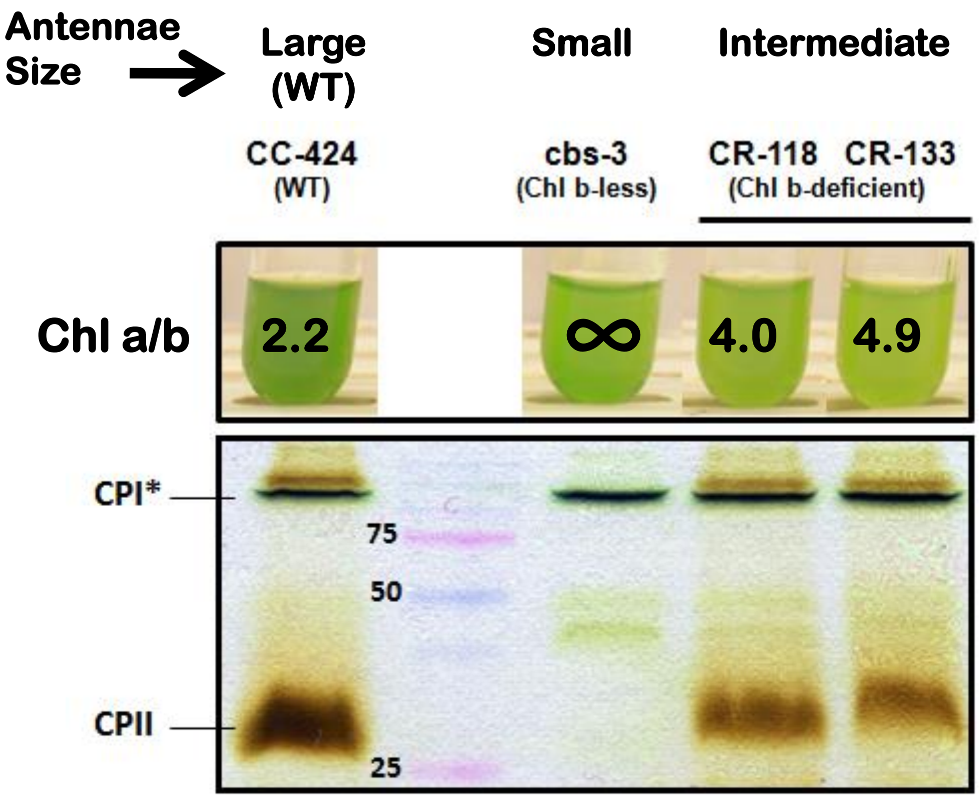




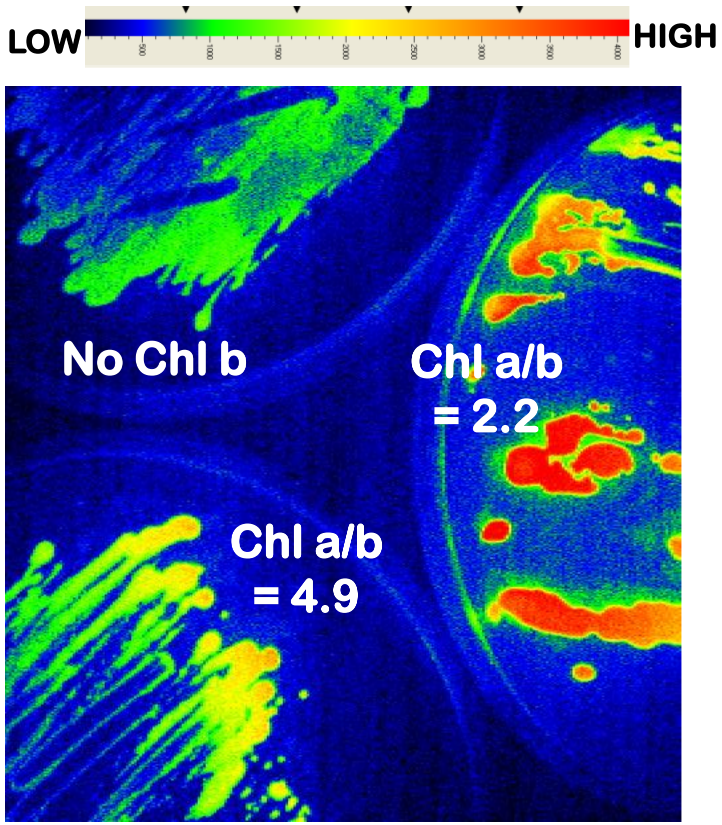
When photochemistry is saturated fluorescence increases

Transgenic algal strains with higher chlorophyll a/b ratios have smaller antennae sizes

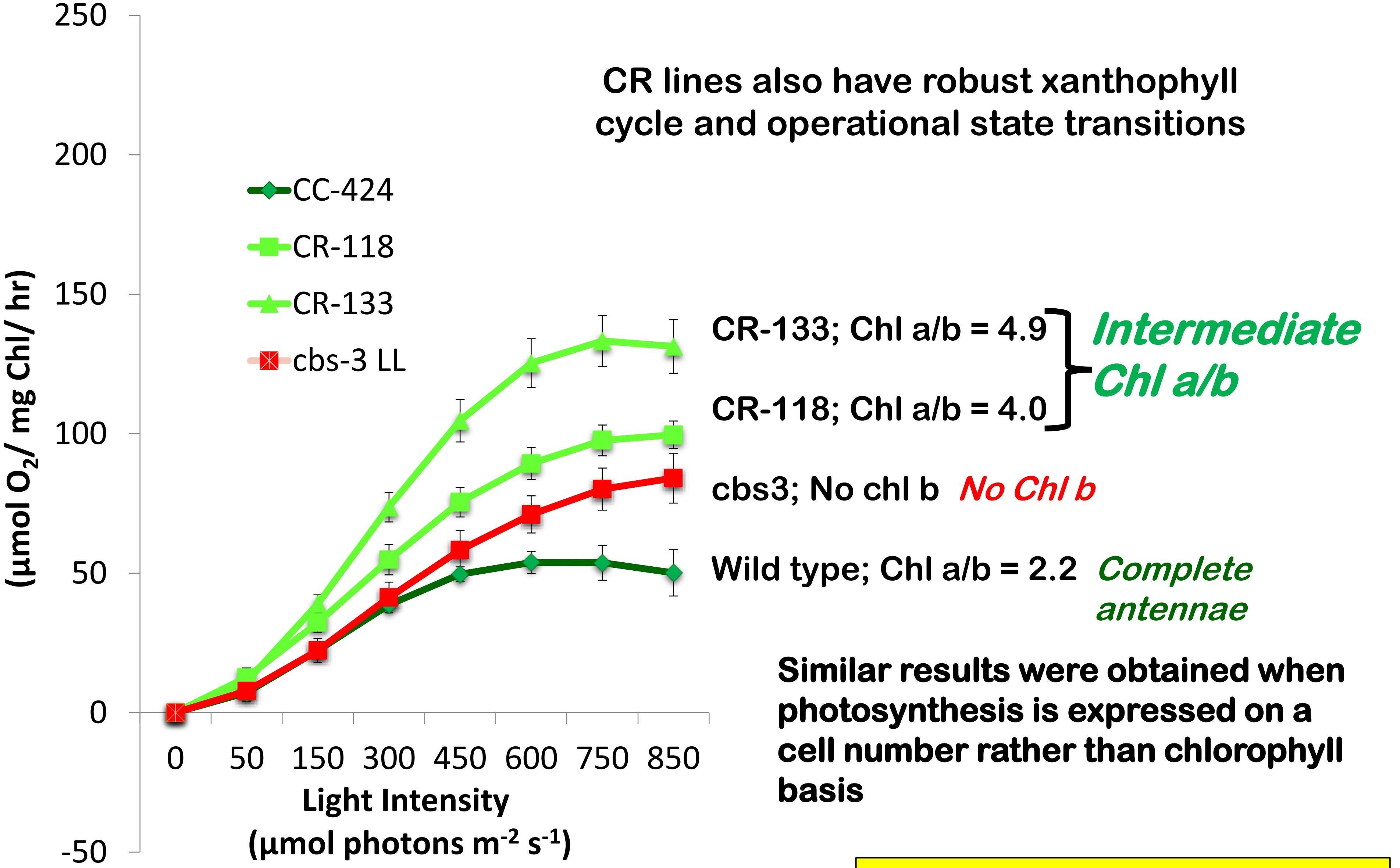
Chl-protein complexes from algae with different antennae sizes



Raw Chl fluorescence is greater in strains containing more Chl b



Algae with intermediate antennae sizes have the highest (2.5 X) photosynthetic rates at saturating light

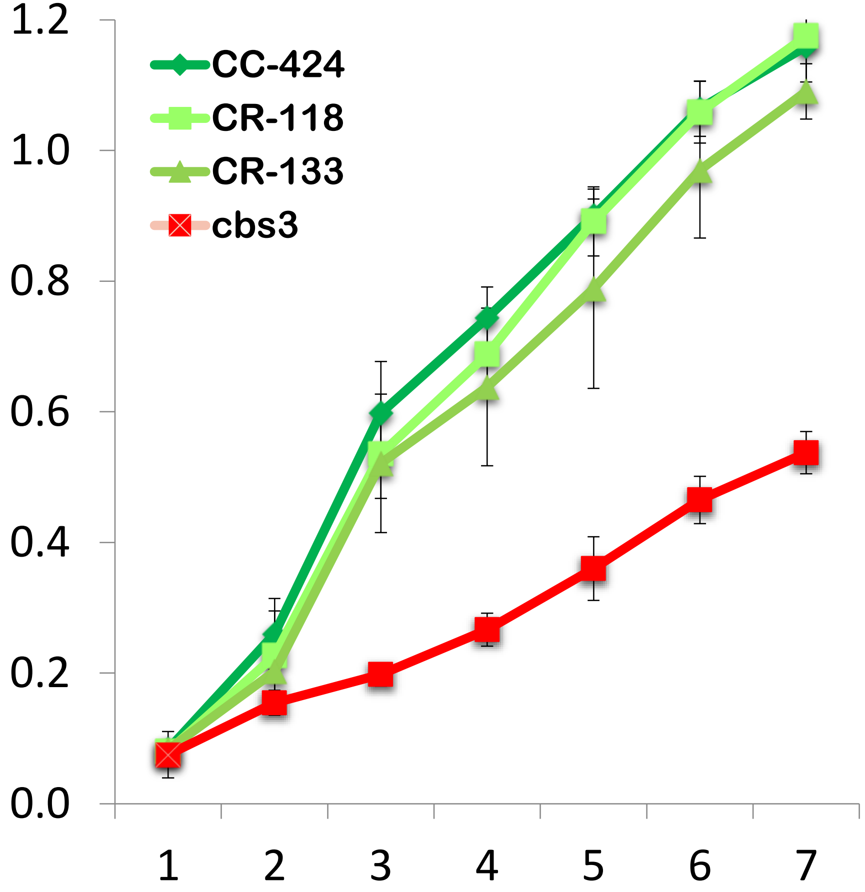


Similar results were obtained when photosynthesis is expressed on a cell number rather than chlorophyll basis

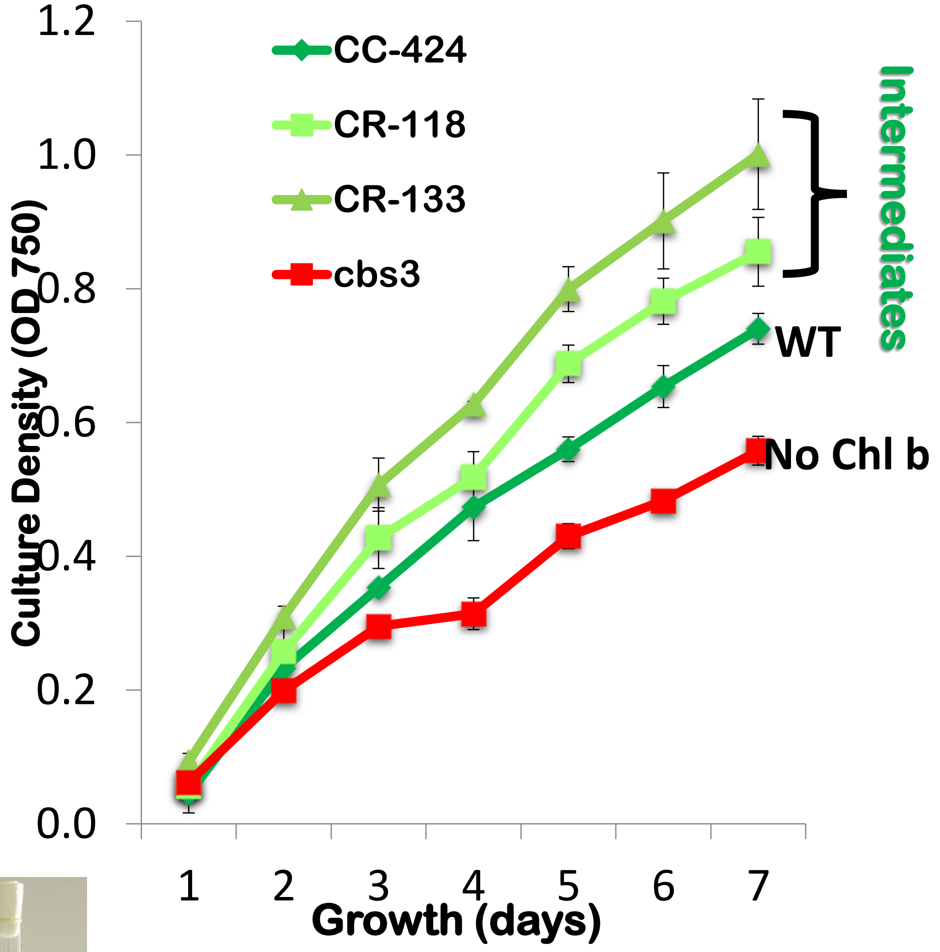
Perrine et al., (2012) *Algal Research 1: 134-142*

Algae with intermediate Chl a/b ratios have 30% greater biomass productivities at high light intensities (in flasks)

Growth under 50 µmol photons $m^{-2}s^{-1}$
(LOW LIGHT)



Growth under 500 µmol photons $m^{-2}s^{-1}$
(SATURATING LIGHT)

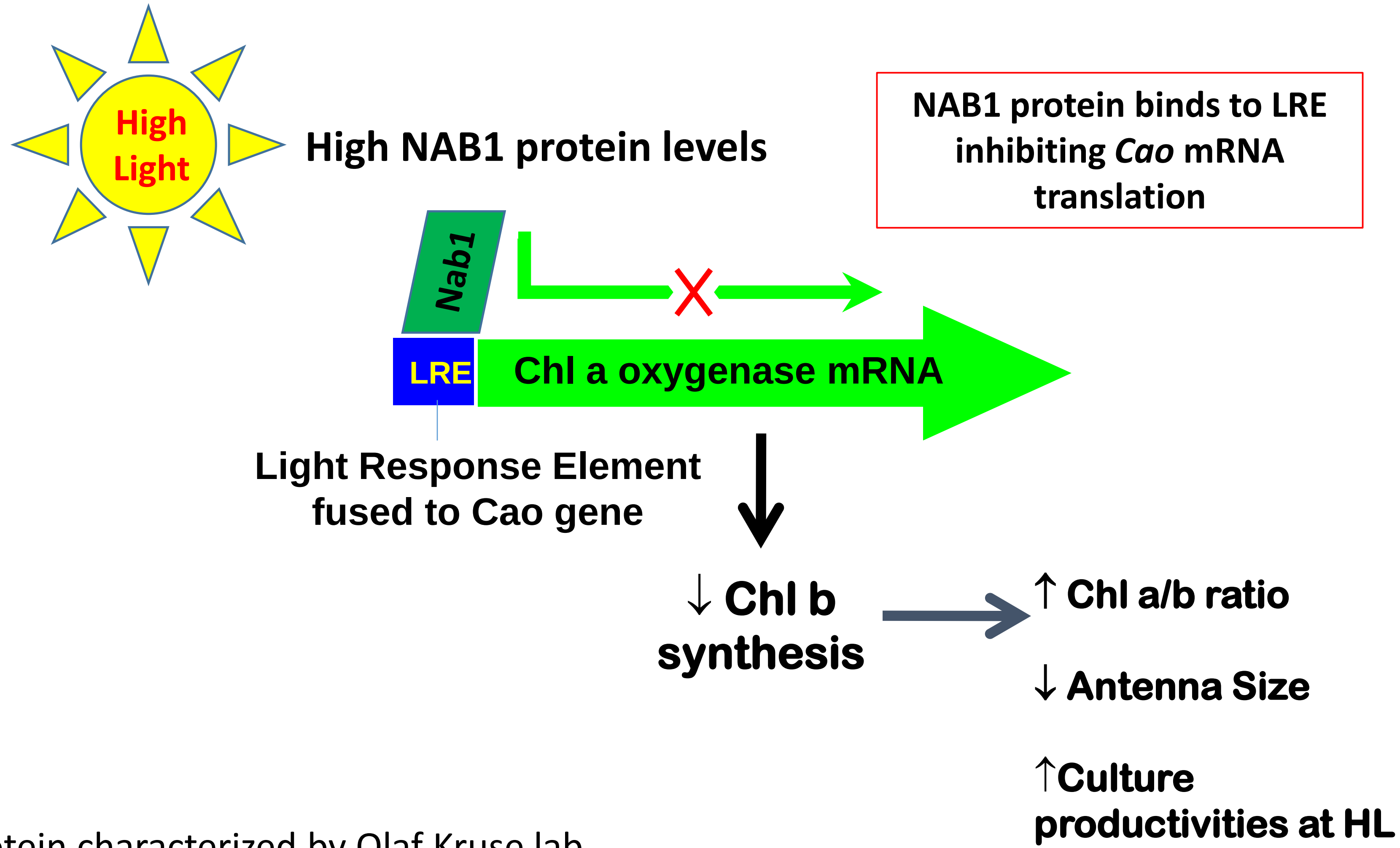


Light intensities and day length change throughout the year; how do we continuously adjust antenna?

Engineering self-adjusting antenna sizes optimized for energy capture and conversion throughout the year.

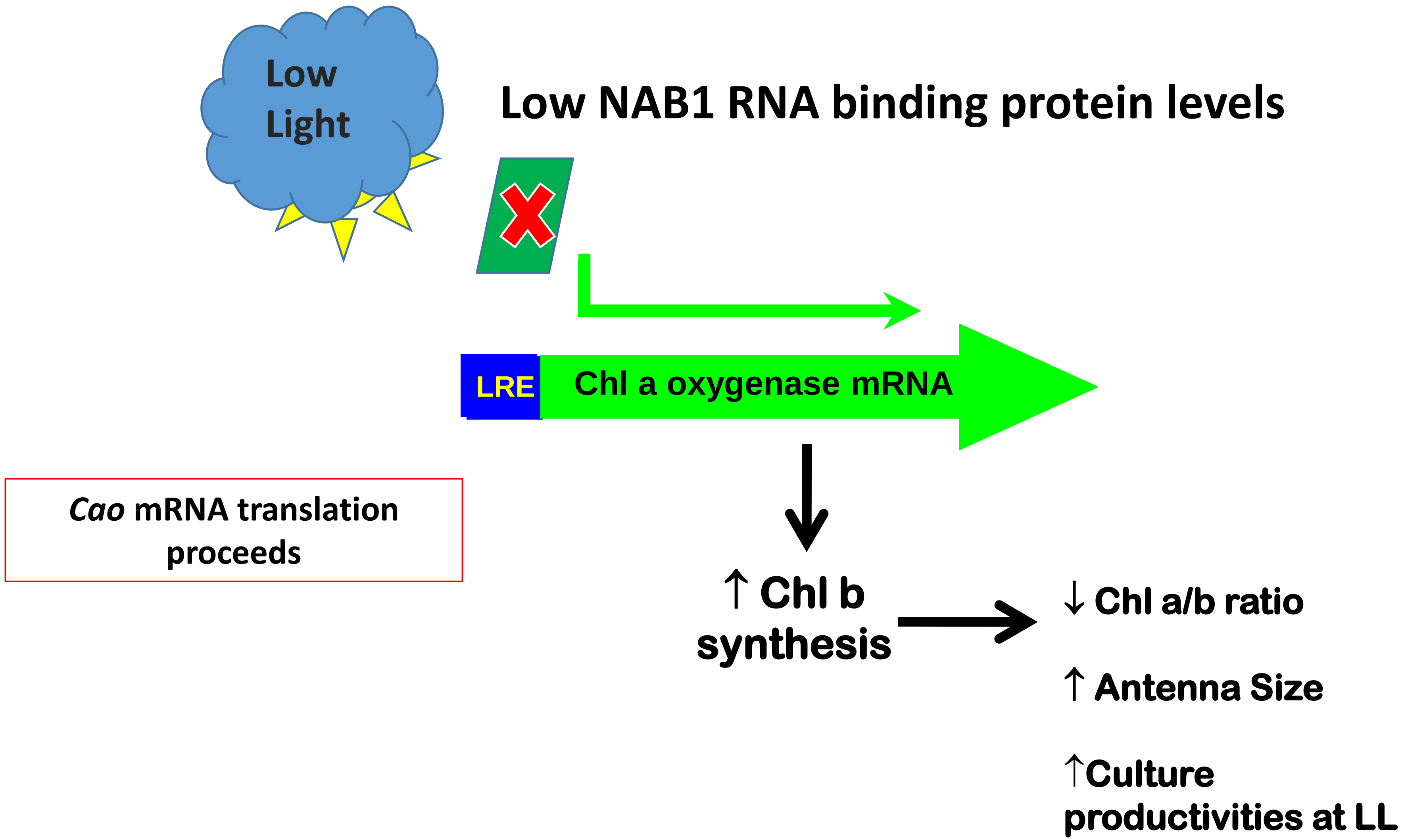
Engineering antenna that self-adjust Chl a/b ratios to changing light intensities
Light-dependent modulation of chlorophyll b accumulation

Chlamydomonas Chl a oxygenase (no Chl b) mutant background transformed with LRE-Cao construct



NAB1 protein characterized by Olaf Kruse lab

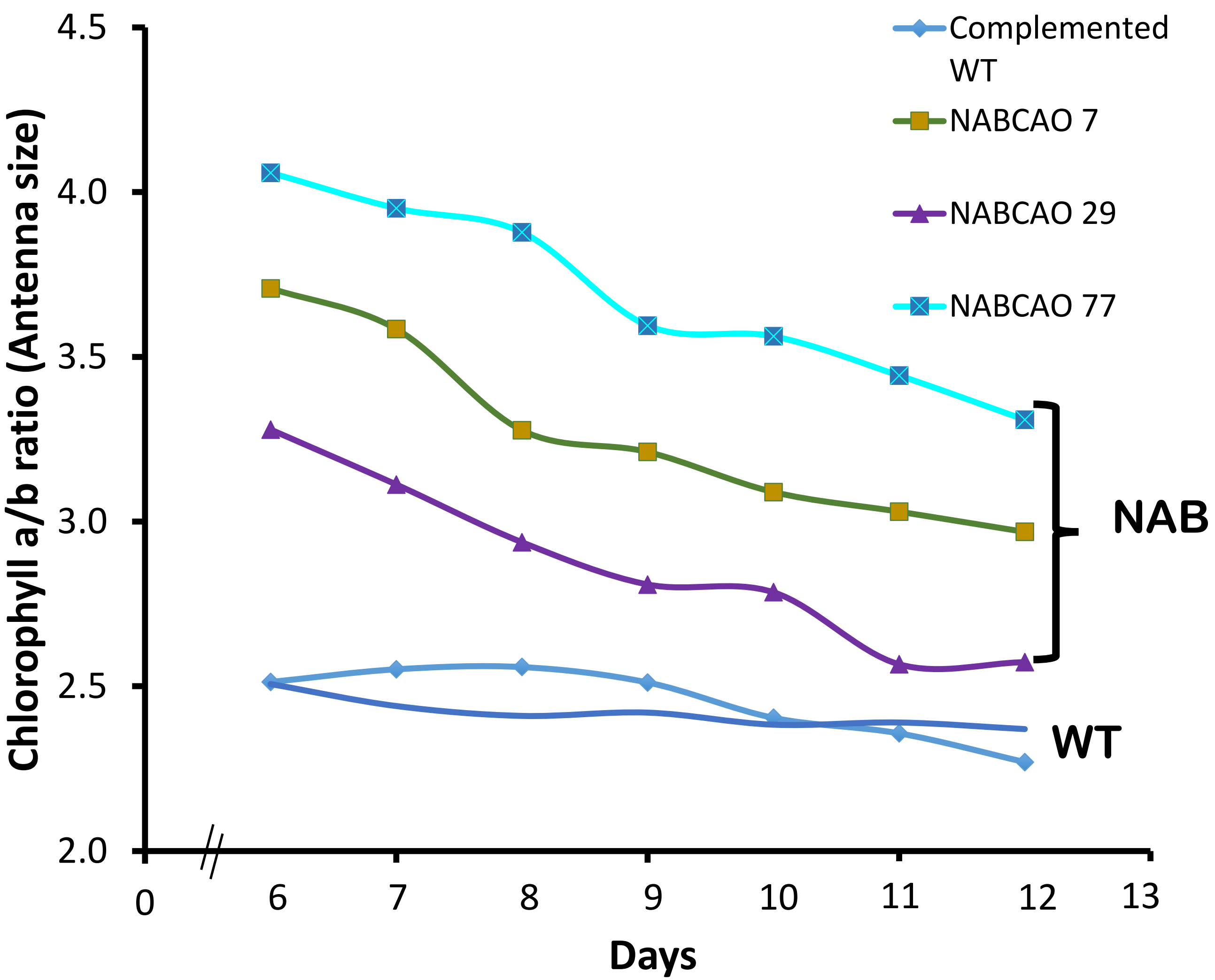
Increasing Chl a oxygenase activity and elevating Chl b levels at low light to increase antenna size

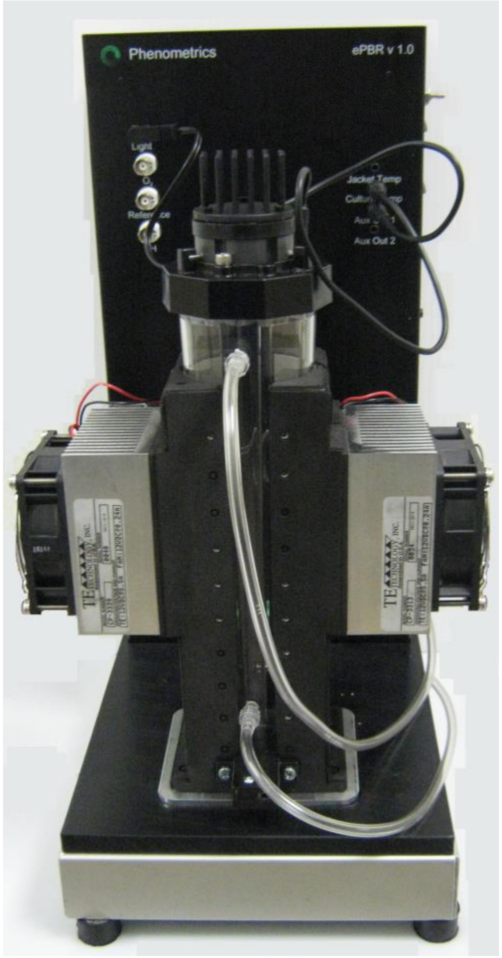


Does antennae size self-adjust?
Antenna get larger as culture (self-shading) grows

Small

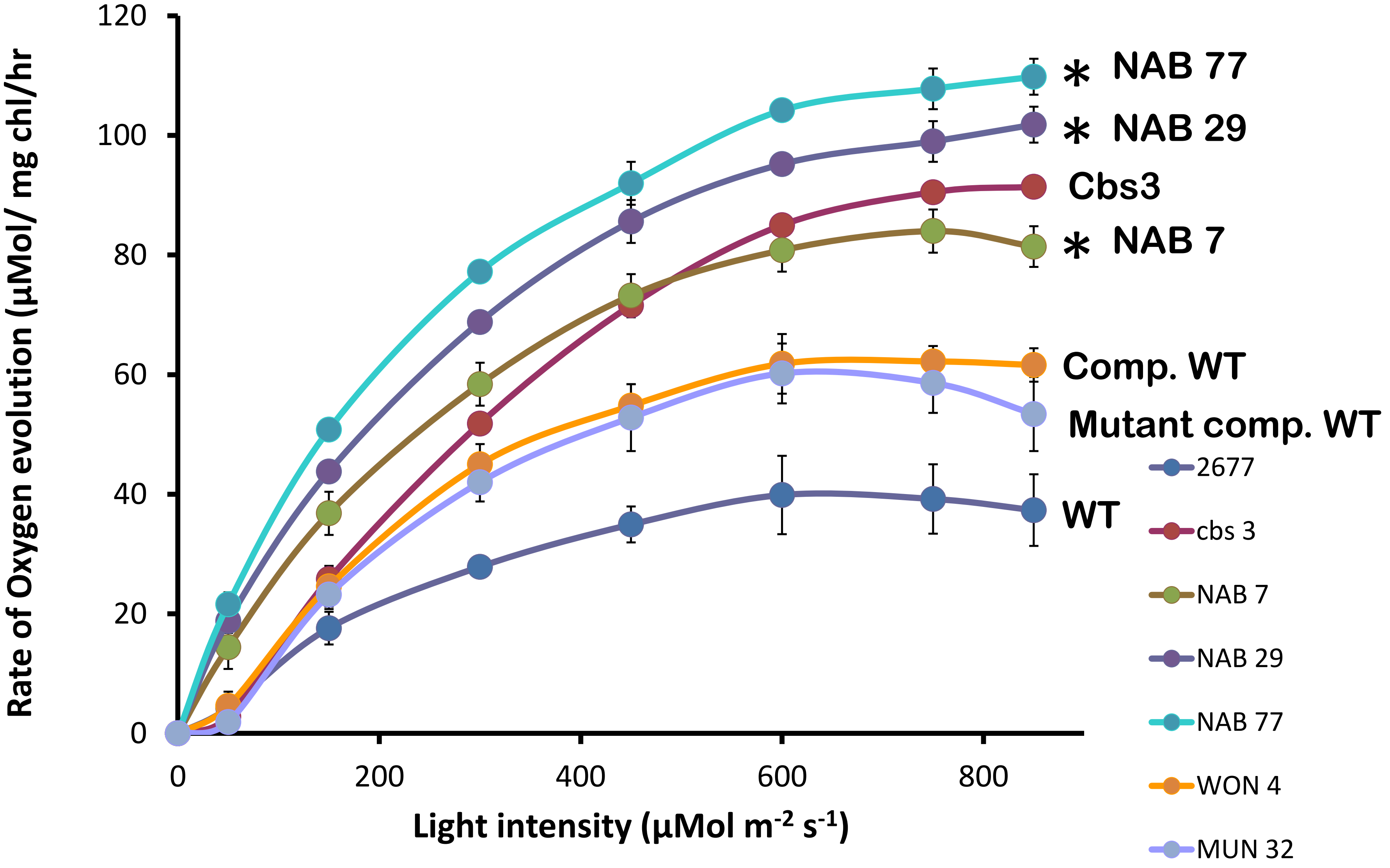
Large



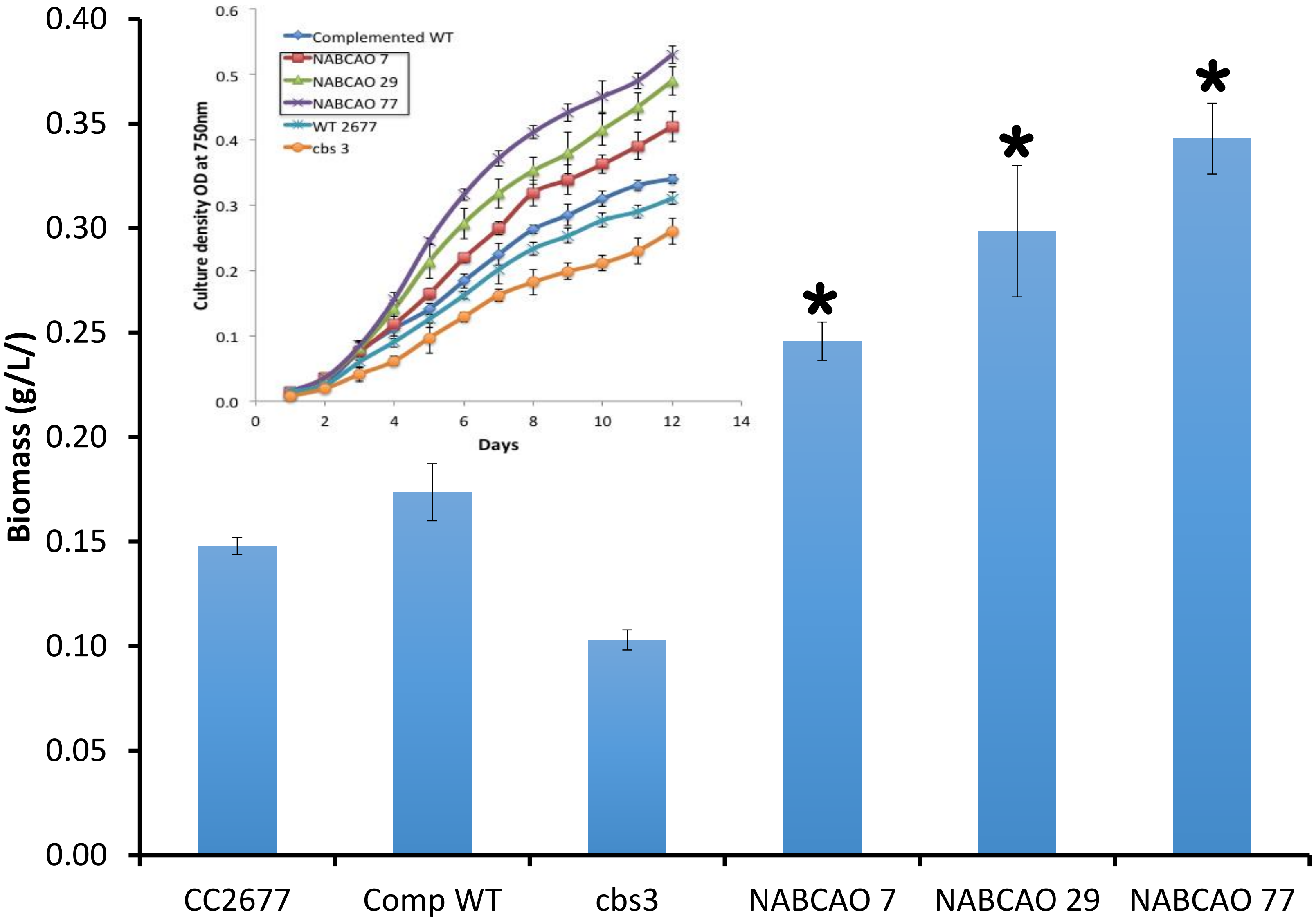


Phenometrics PBR

Photosynthesis in algae with self-adjusting antenna light saturates (3X) at higher intensities than wild type



Transgenics with self-adjusting Chl a/b ratios produce > 2-fold more biomass than wild type in PBRs



Next-generation bio-hybrid production systems

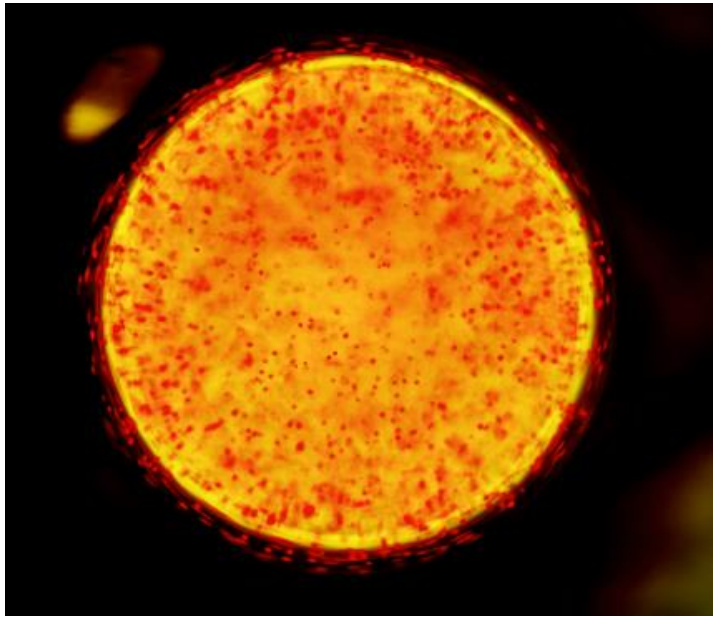
- More efficient utilization of solar spectrum

- Enhanced environmental control of nutrient loading

- Facilitated gas exchange supported by SANS analyses of hydrogel porosity and selectivity

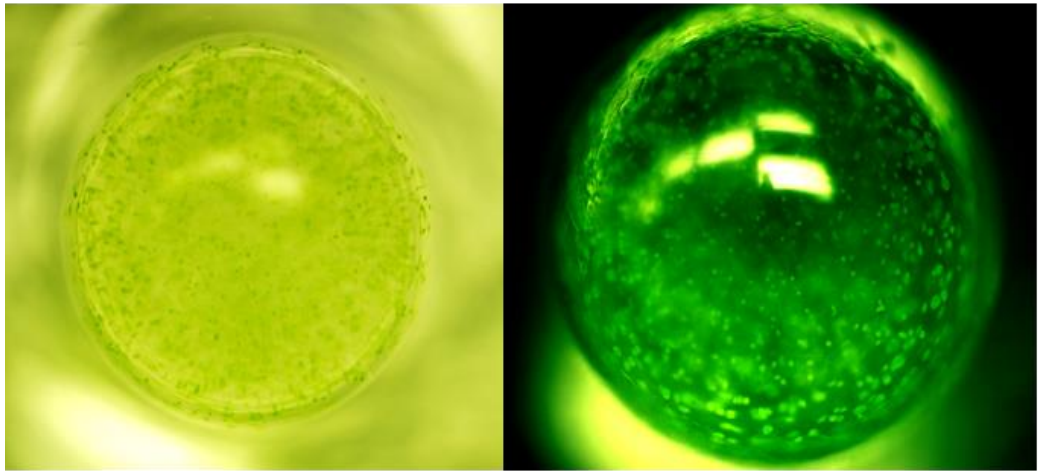
- Reduced harvesting expenses

- Reduced water requirements

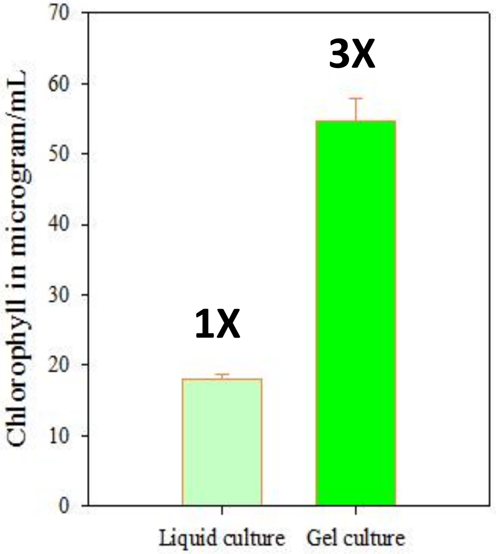


LANL patent pending

Recyclable hydrogel beads (2 mm) support substantially enhanced algal growth and facilitate harvesting



Day 4 Day 8



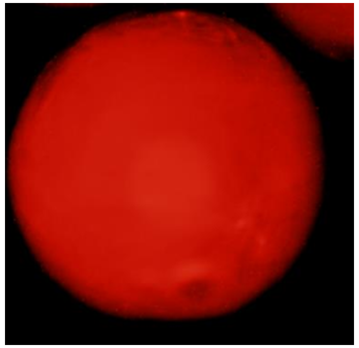
We have achieved <u>three fold increases in biomass density</u> relative to stationary-phase, liquid algal cultures using wild-type *Chlamydomonas* grown in hydrogel beads.

Greater yields are expected using algae engineered to having self-adjusting, light-harvesting antenna

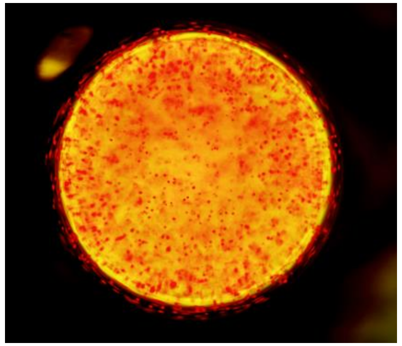
Giant quantum dots imbedded in hydrogel beads frequency shift non-PAR to red light increasing photon flux density

Fluorescence images of encapsulated algae and g-QDs at different stages of algal growth.
Algae quench fluorescence emission from g-QDs

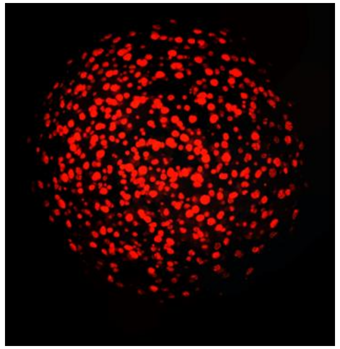
Day 1
g-QD fluorescence

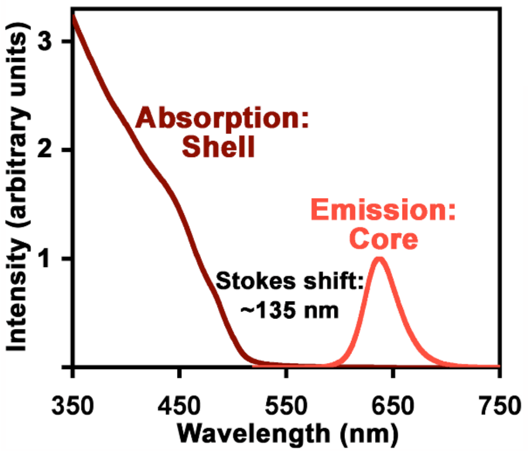


Day 4
g-QD and algal fluorescence



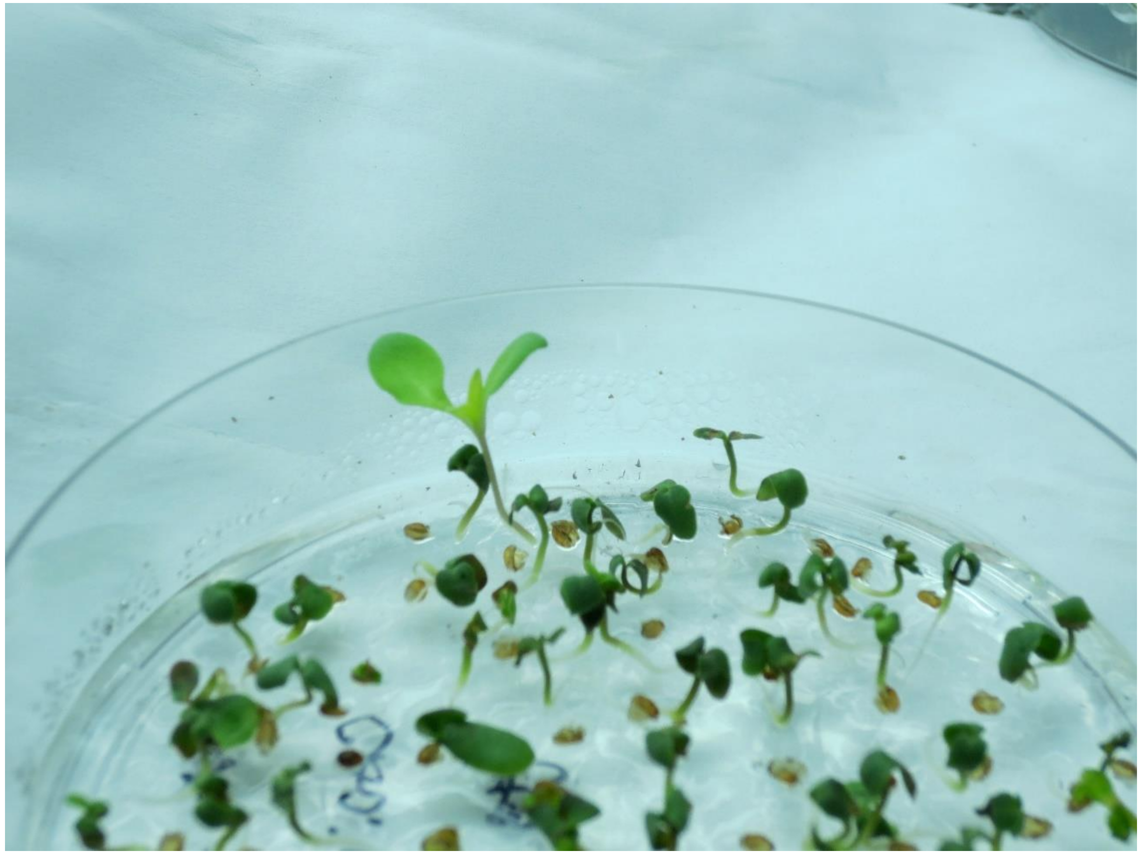
Day 8
algal fluorescence only





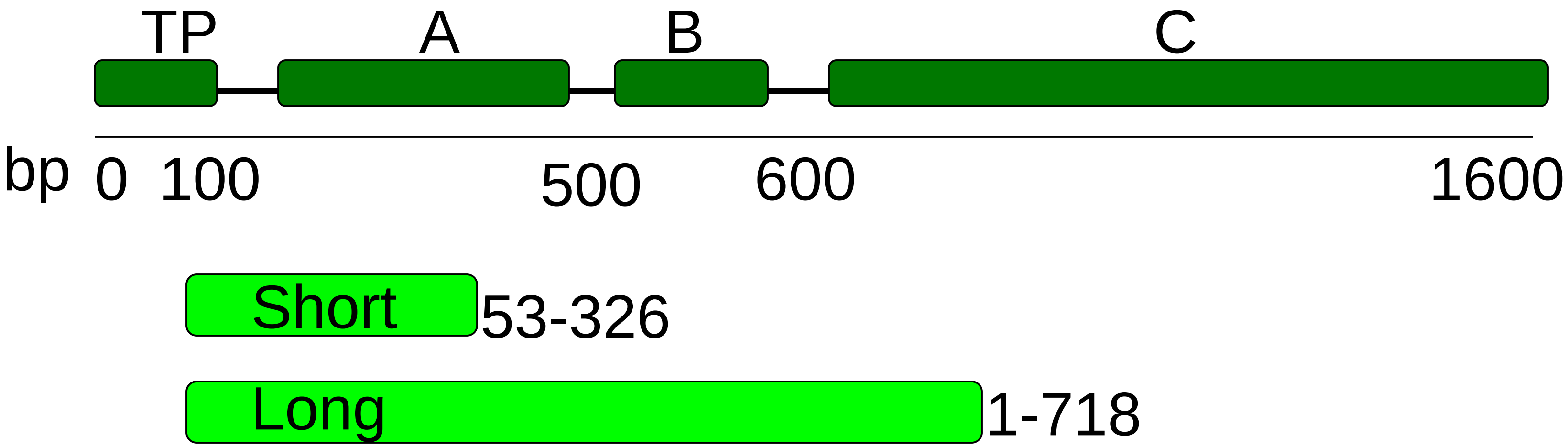
38

Can we engineer more optimal antenna sizes in plants as well?



Transgenic Camelina
40% seed oil by content

Optimizing antenna sizes in plants



Reducing Chl b levels in Camelina using *Cao* long and short RNAi constructs driven by the Cab1 promoter

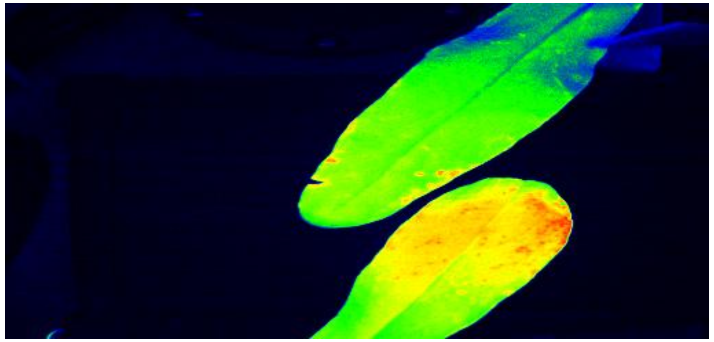
Comparison of CAOi transgenics and WT

- CAOi on the left, WT on the right
- Chl a/b ratios in transgenics range from 3.3 to 12 (compared to 3 for WT)

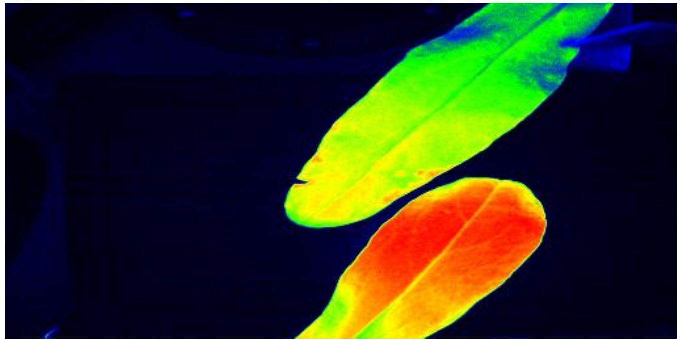
Transgenic Camelina expressing a chlorophyll a oxygenase RNAi construct have smaller antenna sizes as indicated by reduced chlorophyll fluorescence

Caoi-A→

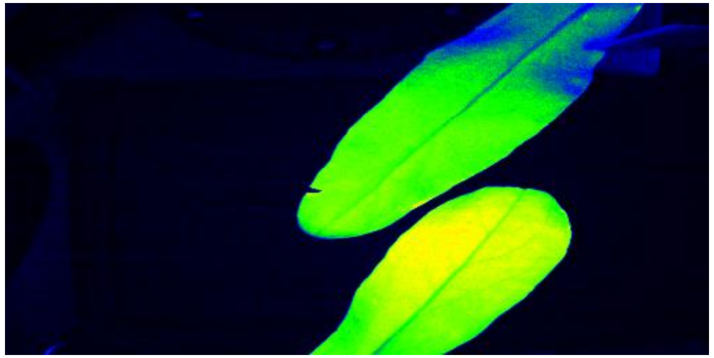
WT→



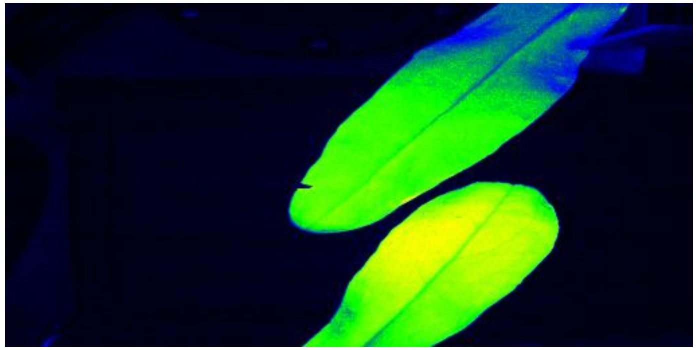
1 sec light



10 sec light

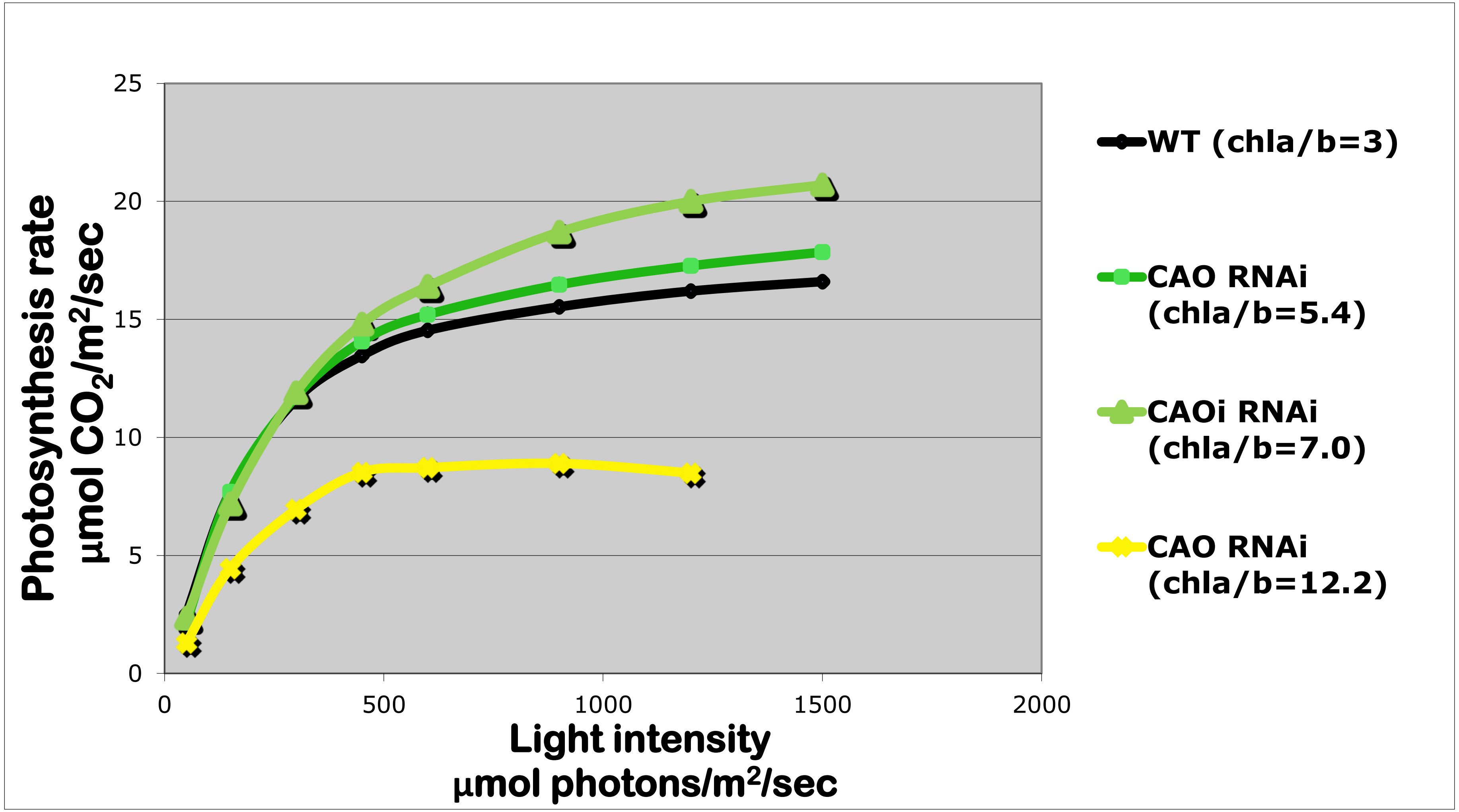


1 min light

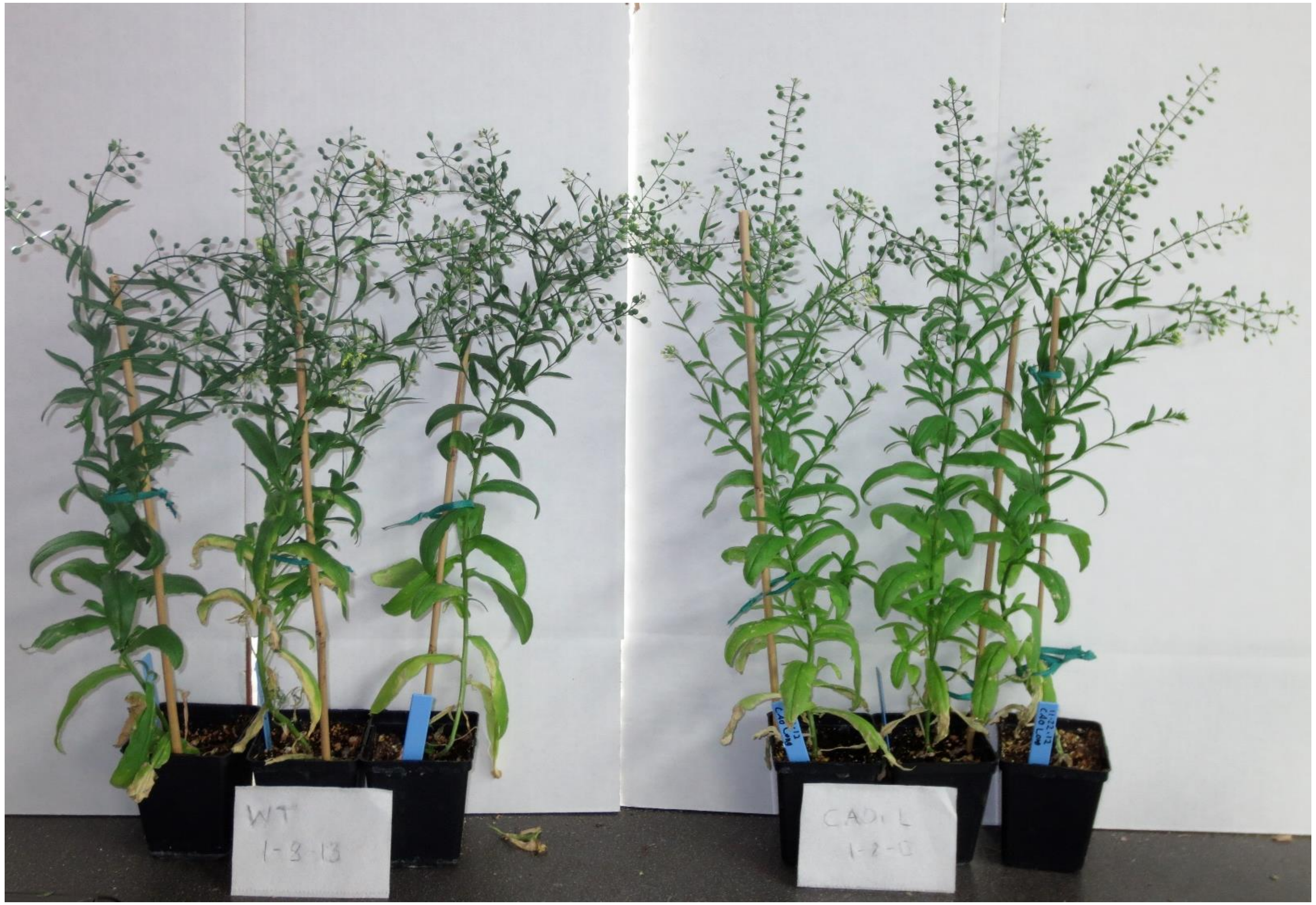


5 min light

Camelina plants with intermediate sized antenna have a 25% increase in aerial photosynthetic rate at high light intensities



WT vs Caoi Long



WT

Chl a/b = 3

Caoi

Chl a/b = 5-7

Growth analysis of wild-type and intermediate antenna Camelina planted at field densities in greenhouse

Caoi 8-1
Chl a/b = 6

WT
Chl a/b = 3

Summary

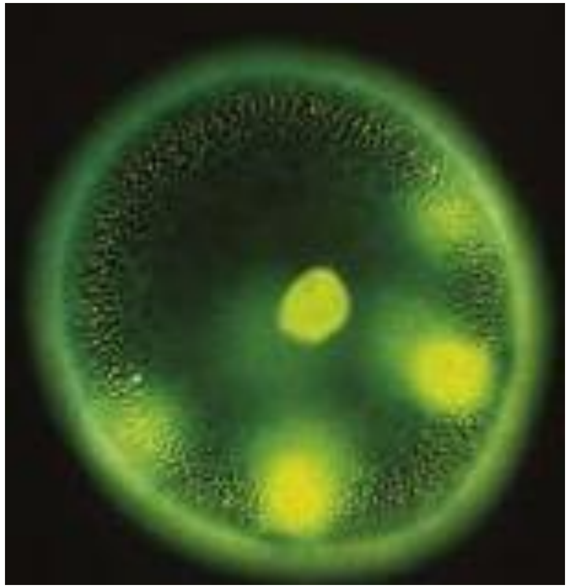
• Increasing biomass yield and reducing harvesting costs remain the greatest challenges for profitable algal biofuels

• Algae with self-adjusting antenna have a 2X increase in productivity, the largest increase engineered to date.

• Algae with large antenna are more evolutionary fit than algae with small antennae since they shade competitors

• Antenna size optimization in algae and plants is similar

• Reusable bio-hybrid devices are being developed to increase solar energy conversion efficiency and reduce harvesting costs



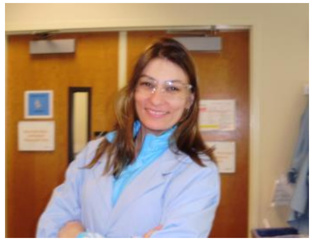
The Team at LANL/NMC

The Biofuel Team



Tawanda Zidenga   Sangeeta Negi   Natalia Friedland



Amanda Barry   Sathish Rajamani   Sowmya Subramanian   Angela Tonon

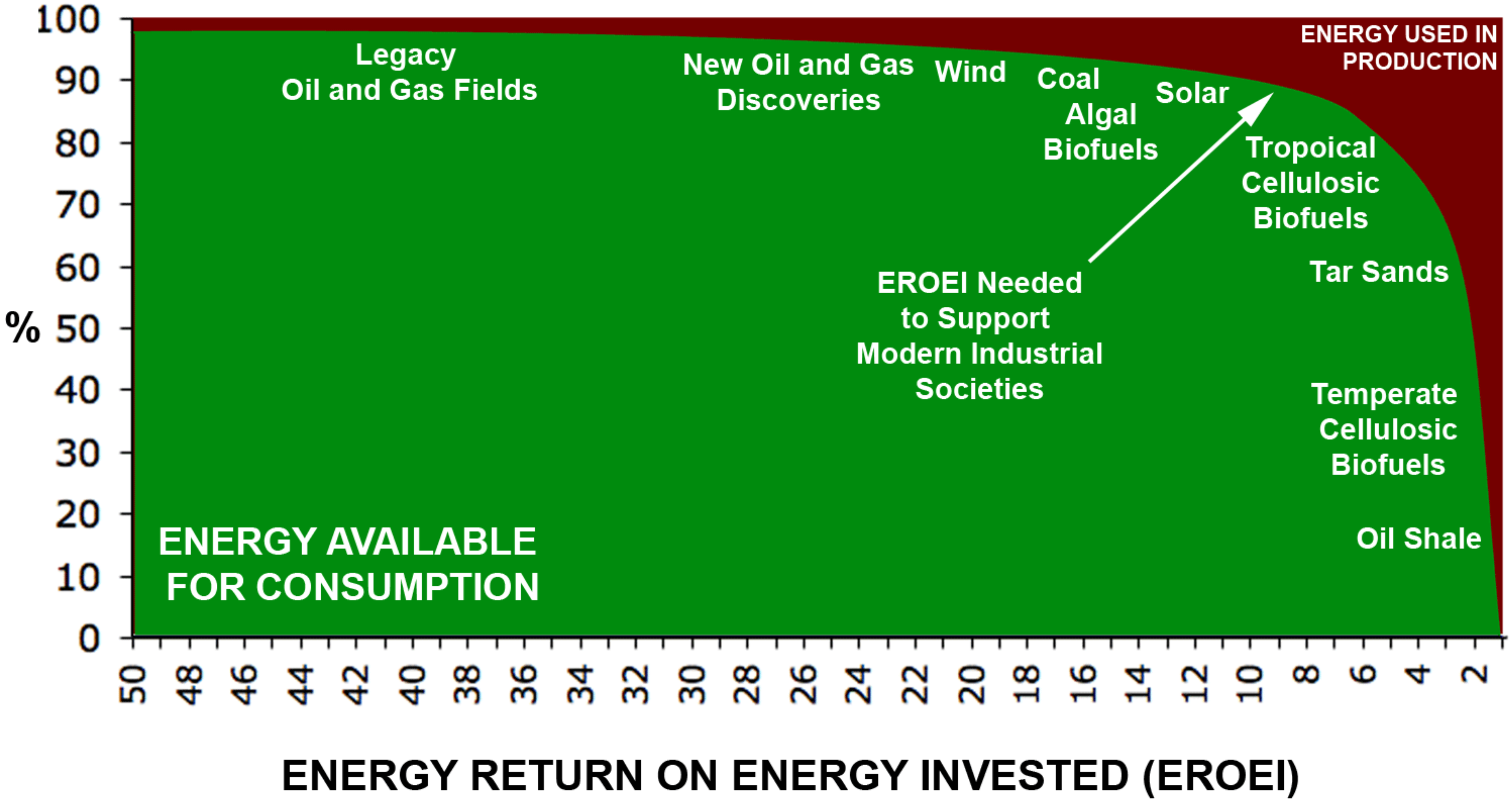
Entrada Facility; July 2013

John Gordon, LANL
Jennifer Hollingsworth, LANL
Volker Urban, ORNL
Hugh O'Neill, ORNL
Brad O'Dell, ORNL

Zoee Perrine, Danforth Center
Anil Kumar, Danforth Center
Jeri Timlin, Sandia Nat. Lab.
Aaron Collins, Sandia Nat. Lab
Howard Berg, Danforth Center

Support from DOE and NSF

THE NET ENERGY CLIFF



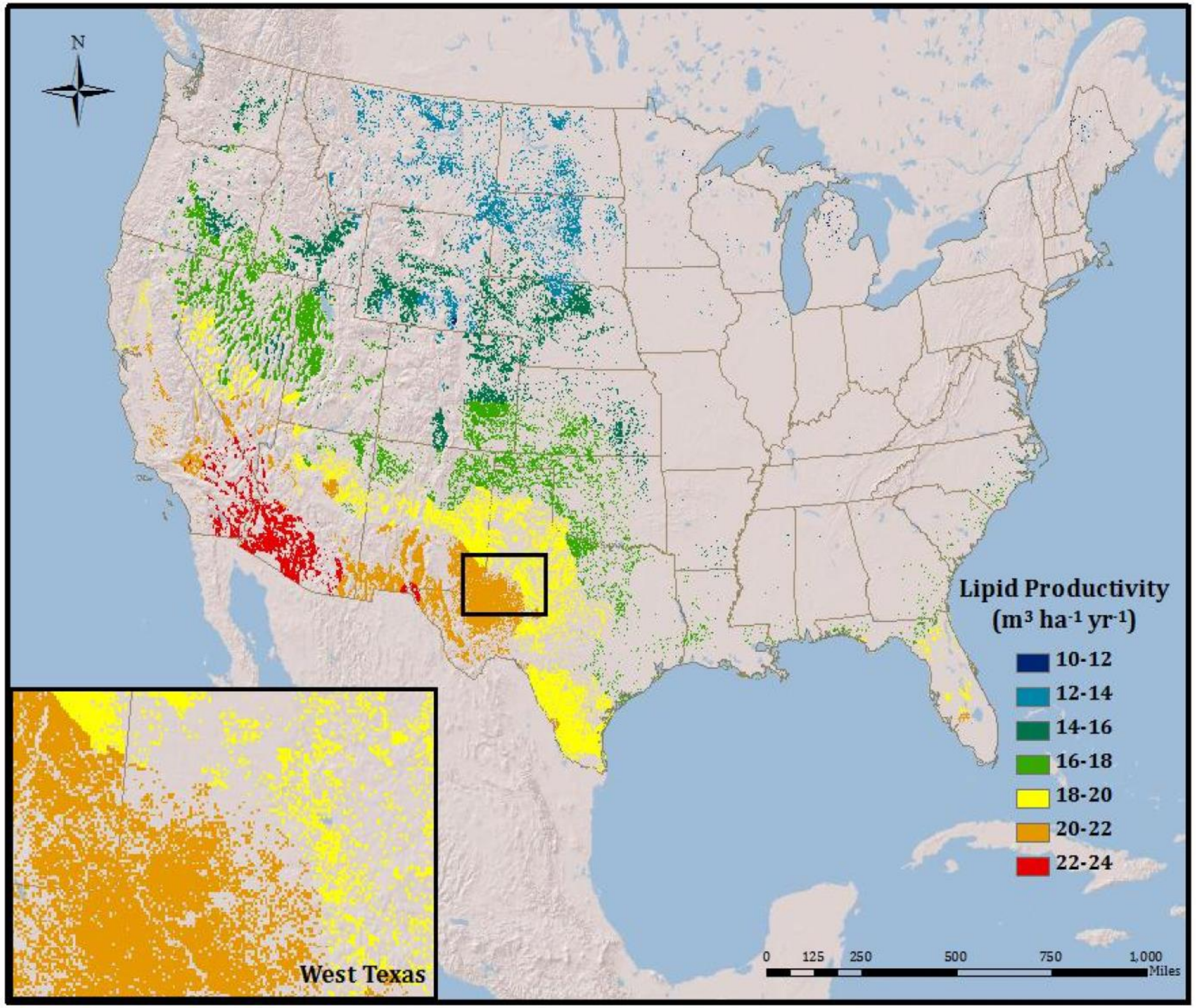
Bio-crude condition	*Albumin*			*Soya Protein*			*Asparagine*			*Glucose*			*Starch*			*Sunflower oil*			*Chlorella*			*Nannochloropsis*			*Porphyridium*			*Spirulina*		
GC-MS Identified Compound	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH	H2O	Na2CO3	HCOOH
Phenols	x	x	x	x	x	x		x			x			x	x				x	x	x	x		x	x	x	x	x	x	x
Phytol								x												x			x		x	x			x	
Indole	x	x	x	x	x	x		x											x		x	x	x	x	x		x	x	x	x
Pyrrols	x		x	x		x	x		x										x							x		x	x	x
Piperdine	x			x		x													x	x							x	x	x	
Hexadecamide																			x		x						x	x		x
Cyclohexylamine							x		x																					
Hexadecane																	x						x							
Heptadecane																	x			x	x	x	x			x		x	x	x
Pentadecene																			x											
Octanoic acid																			x		x									
Cyclohexanone											x		x	x																
Cyclopentanone										x	x	x		x	x															
Benzene										x		x	x		x															
Indenone										x		x	x		x															
Ethanone										x		x	x		x															
Tetradecanoic Acid																x		x									x			
Oleic Acid																x		x												
Hexadecanoic Acid																x		x			x	x		x						x

HTL conversion of biomass generates a diversity of products

Proteins → oxidized cyclics and heterocyclics
Carbohydrates → cyclics and ketones
Oil → alkanes and fatty acids
Algae → Contain all the products from oil, protein and carbohydrate fractions but lack the ketone fraction produced from carbohydrates

Bioresource Technol. (2011) 102: 215

Best locations to grow algal biofuels based on climate, water, barren land (< 2% slope), and CO_2 availability



New Mexico Production
2,100-2,300
gallons/acre/yr

Annual fuel/acre
sufficient for
~4 cars @ 20 miles/gallon
and 12,000 miles/yr

Quinn et al., (2013)
Bioenergy Research 6:591