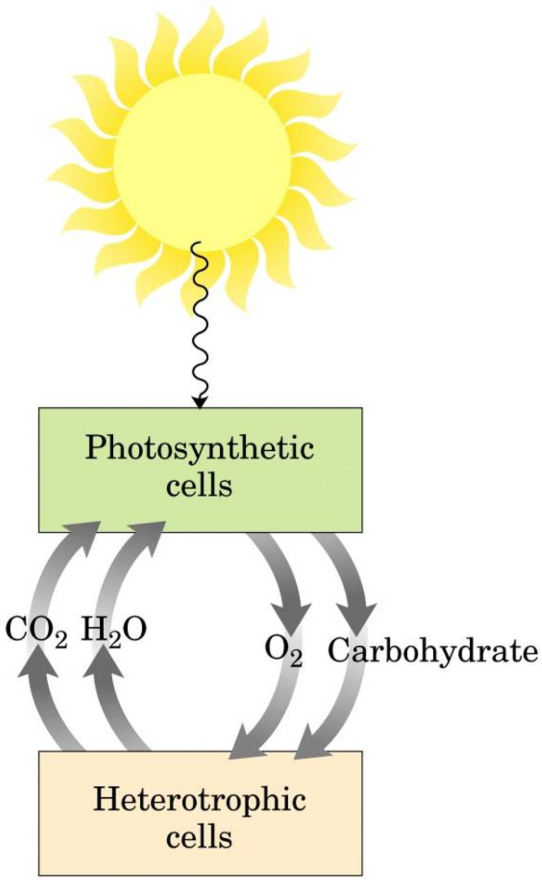
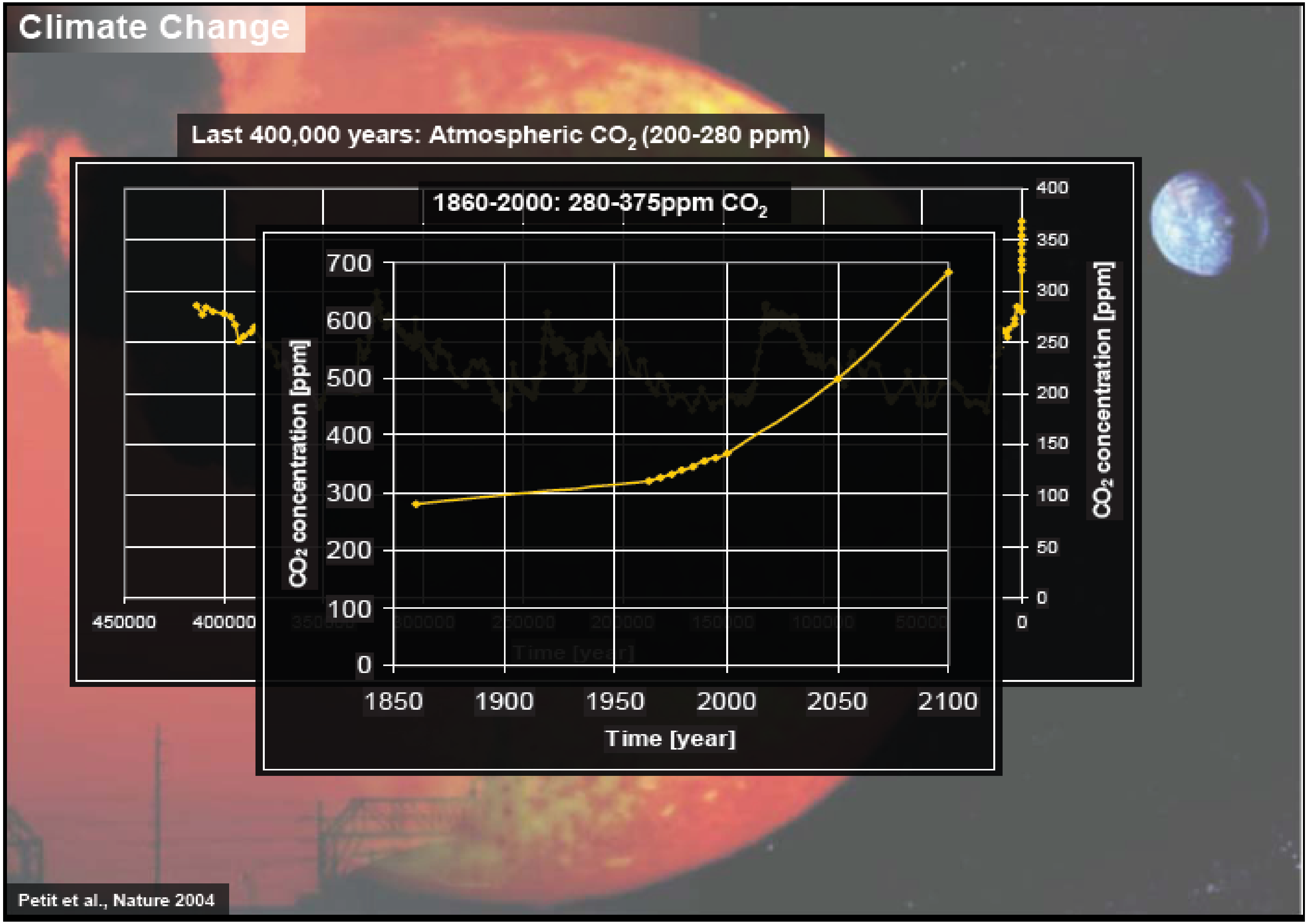
Molecular biology of the chloroplast



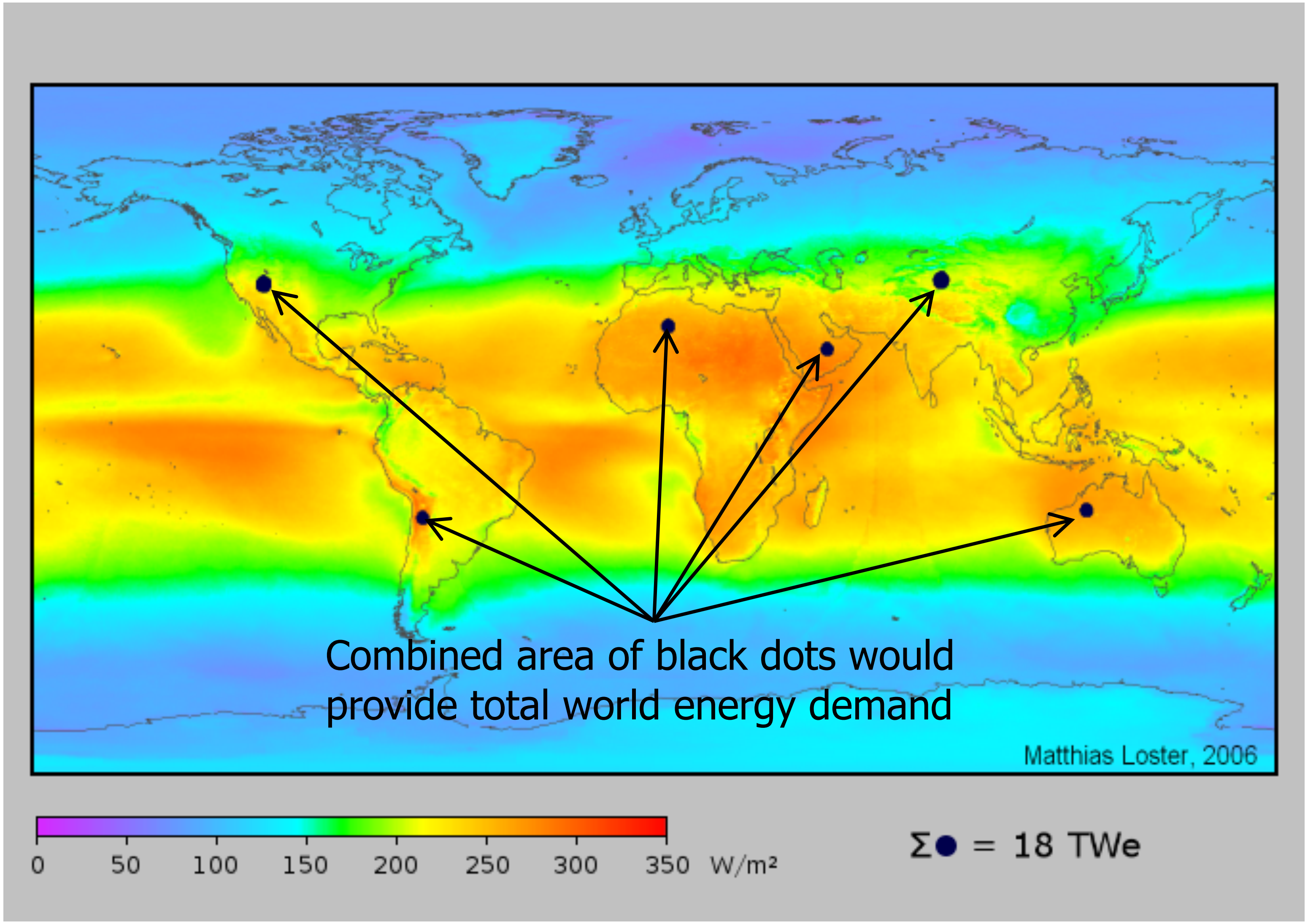
Overview of lectures:

- Solar energy conversion
    - 'The Big Picture'
    - Overview of photosynthesis and the chloroplast
    - Enhancing photosynthesis
    - Algal biofuels and how algal chloroplasts produce hydrogen fuel using water and sunlight
    - Using cyanobacteria as solar biorefineries
- The chloroplast genome
- Expression of high-value products in the chloroplast

Problem 1: CO_2 emissions

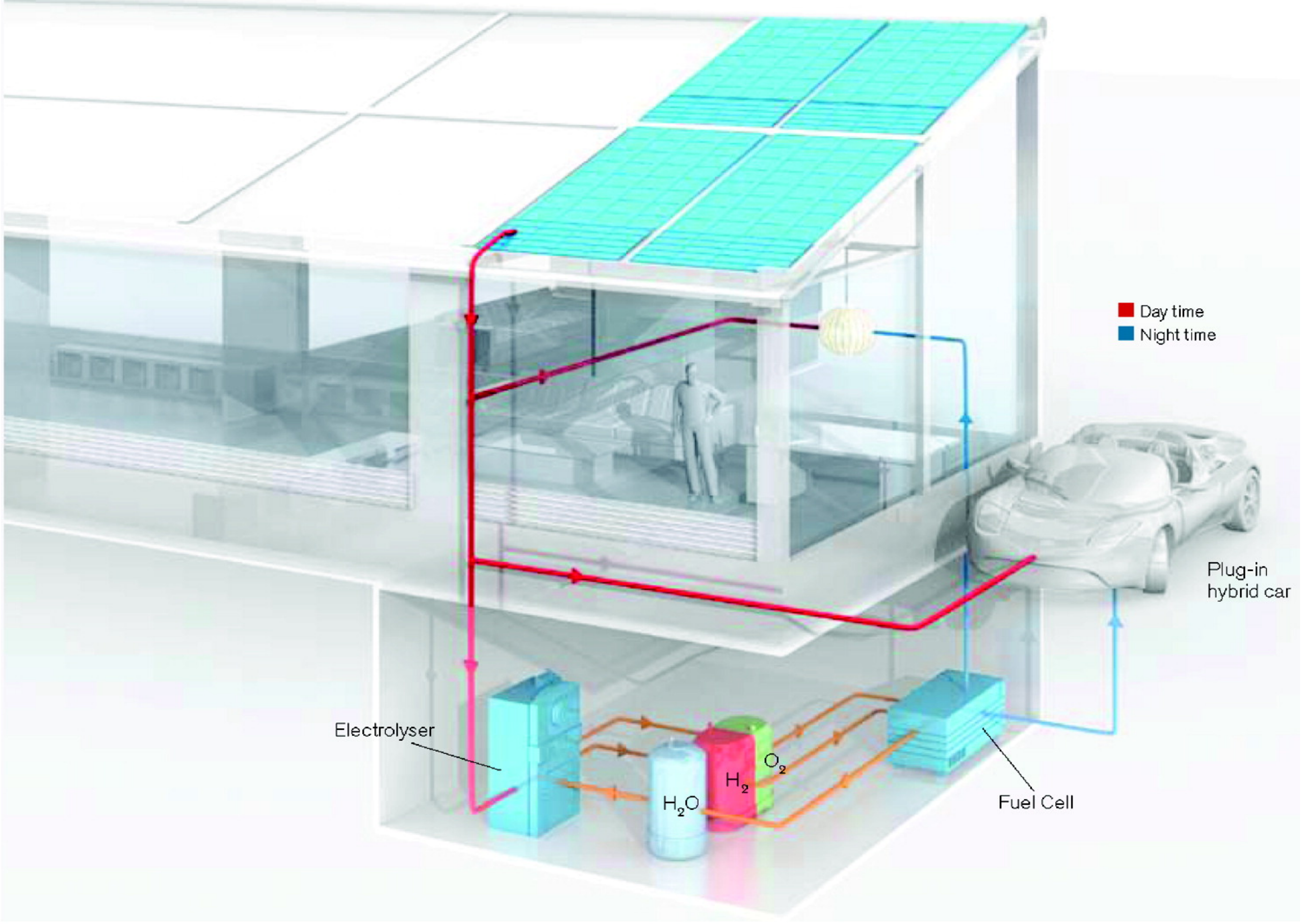


Problem 2: The Energy gap (14TW by 2050!)



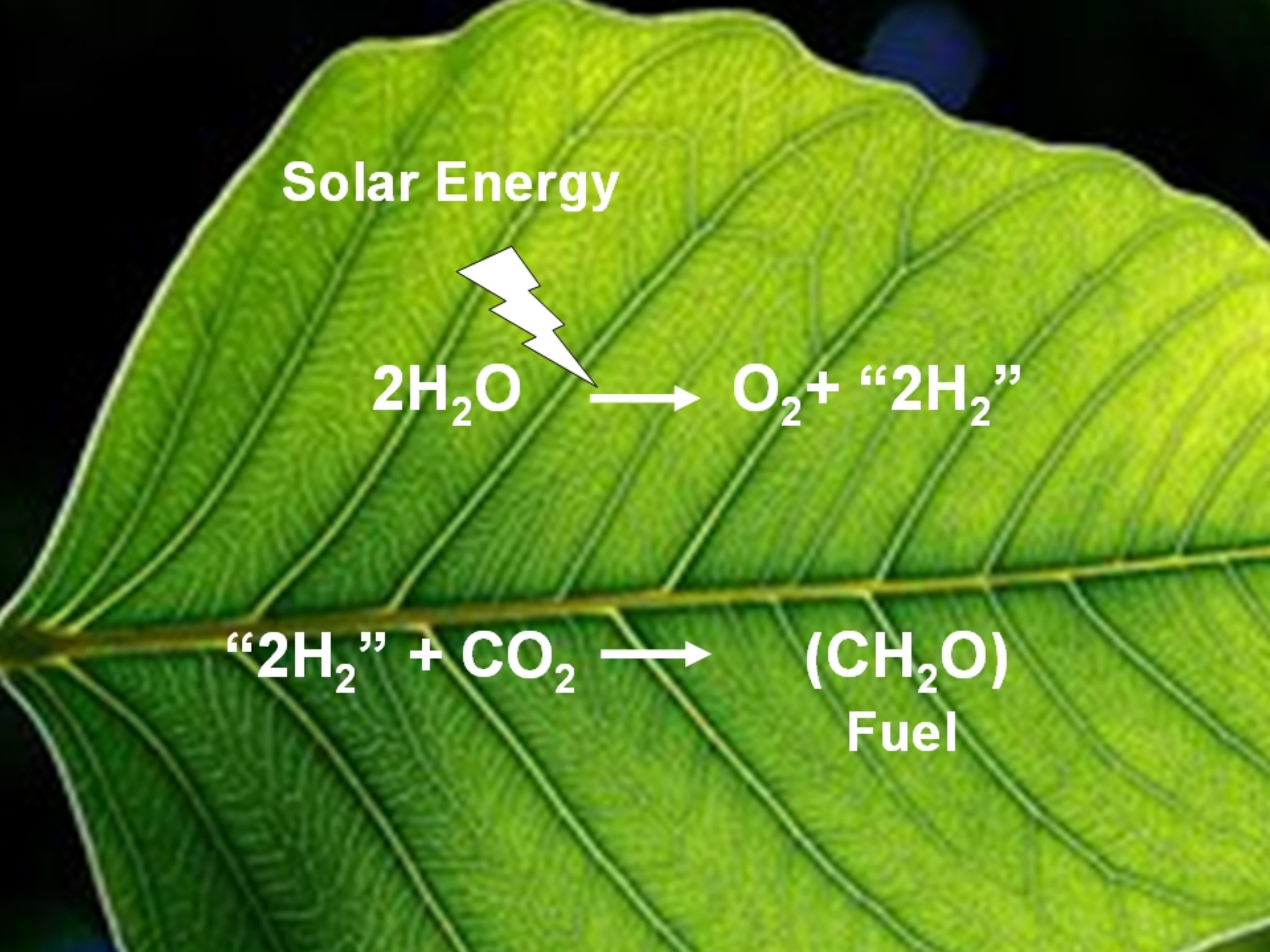
Combined area of black dots would provide total world energy demand

Personalized energy concept: need fuel as well as electricity



Energy-independent home delivering the individual PE. Reproduced with permission from MIT and Technology Review.

Published in: Daniel G. Nocera; *Inorg. Chem.* **2009,** 48, 10001-10017.
DOI: 10.1021/ic901328v



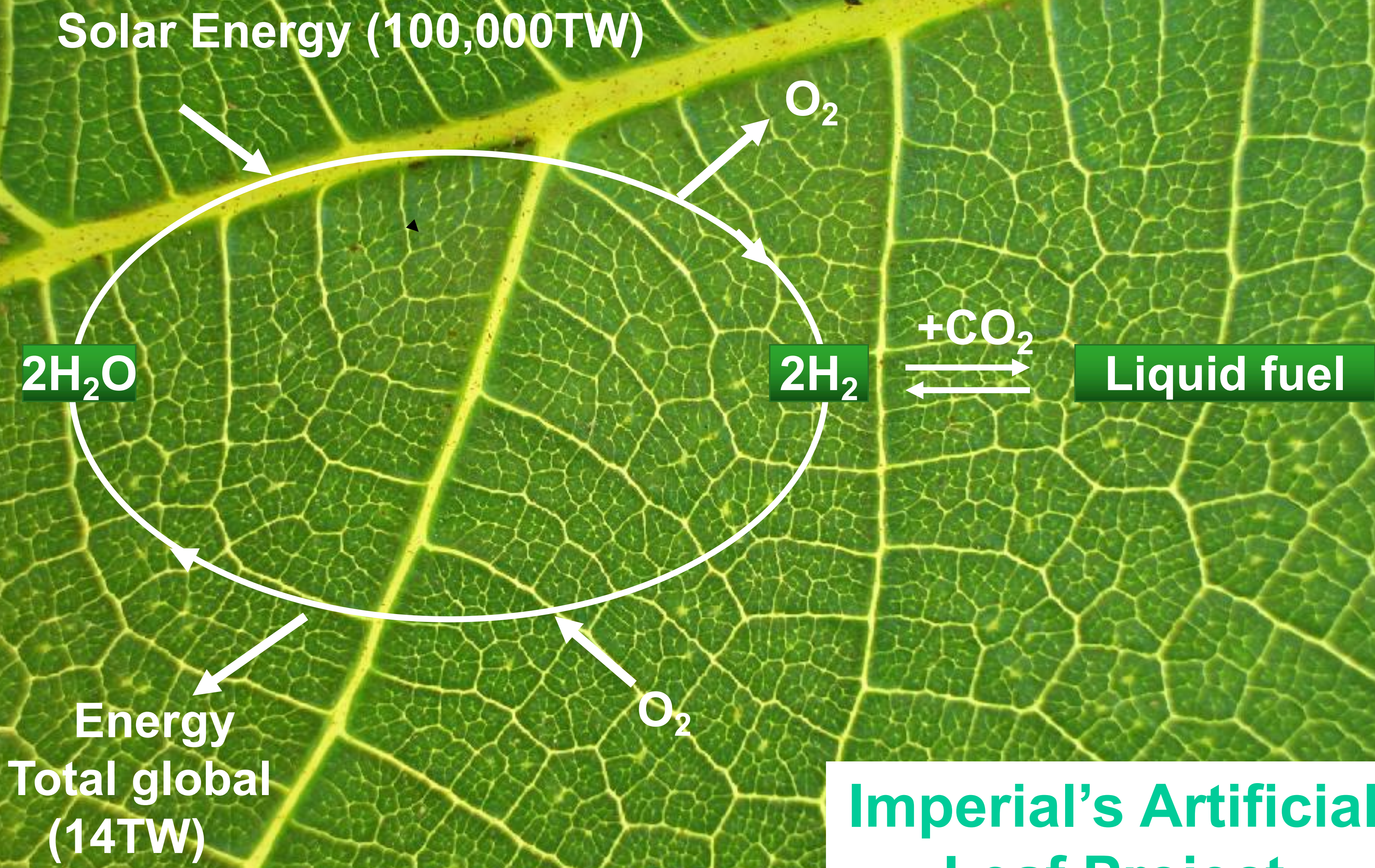
Solar Energy

$$2H_2O \longrightarrow O_2 + \text{“}2H_2\text{”}$$

$$\text{“}2H_2\text{”} + CO_2 \longrightarrow (CH_2O)$$

Fuel

Imperial's Artificial Leaf Project



Solar Energy (100,000TW)

$2H_2O$

O_2

$2H_2$

$+CO_2$

Liquid fuel

O_2

Energy
Total global
(14TW)

www3.imperial.ac.uk/energyfutureslab/

Oxygenic Photosynthesis

- energy transduction process in plants, algae and cyanobacteria
- conversion of light energy into chemical energy
- CO_2 fixed into carbohydrate for plant growth and biomass
- oxygen vital for aerobic life

$$6CO_2 + 6H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6O_2$$

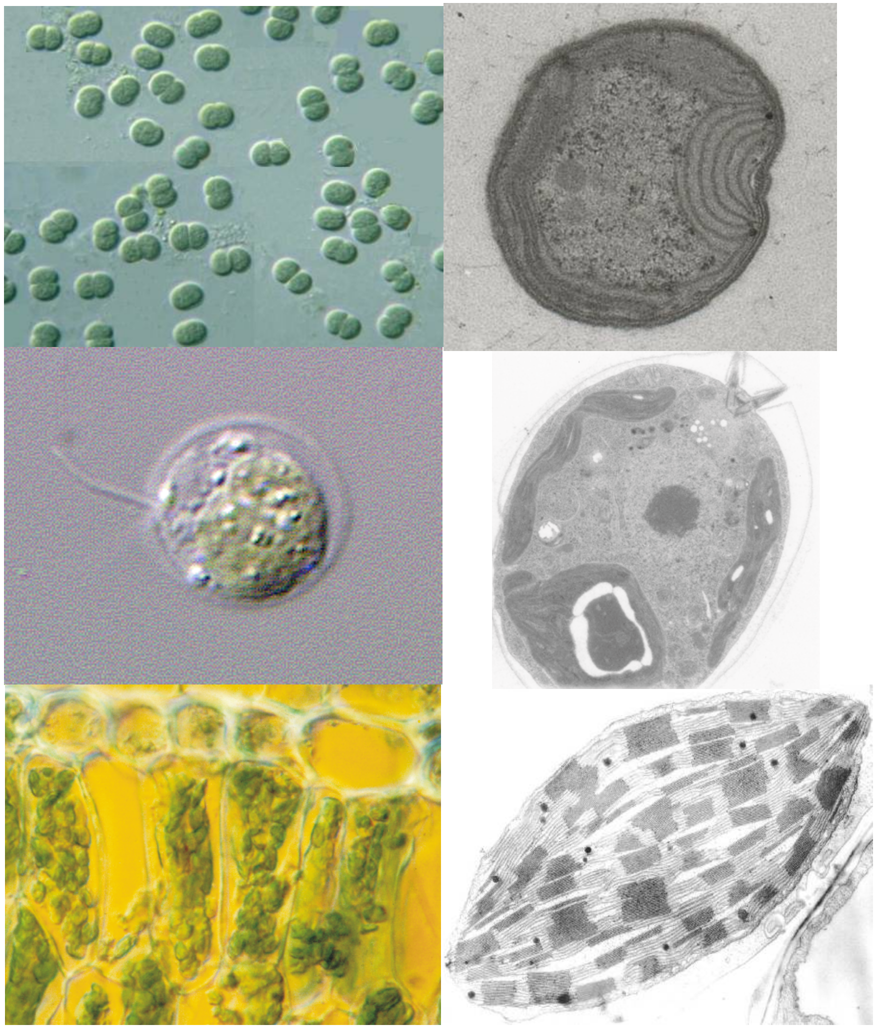
carbon dioxide

glucose

$$6CO_2 + 6H_2O \xleftarrow[\text{energy liberated}]{} C_6H_{12}O_6 + 6O_2$$

respiration

Some of nature's model water-splitters



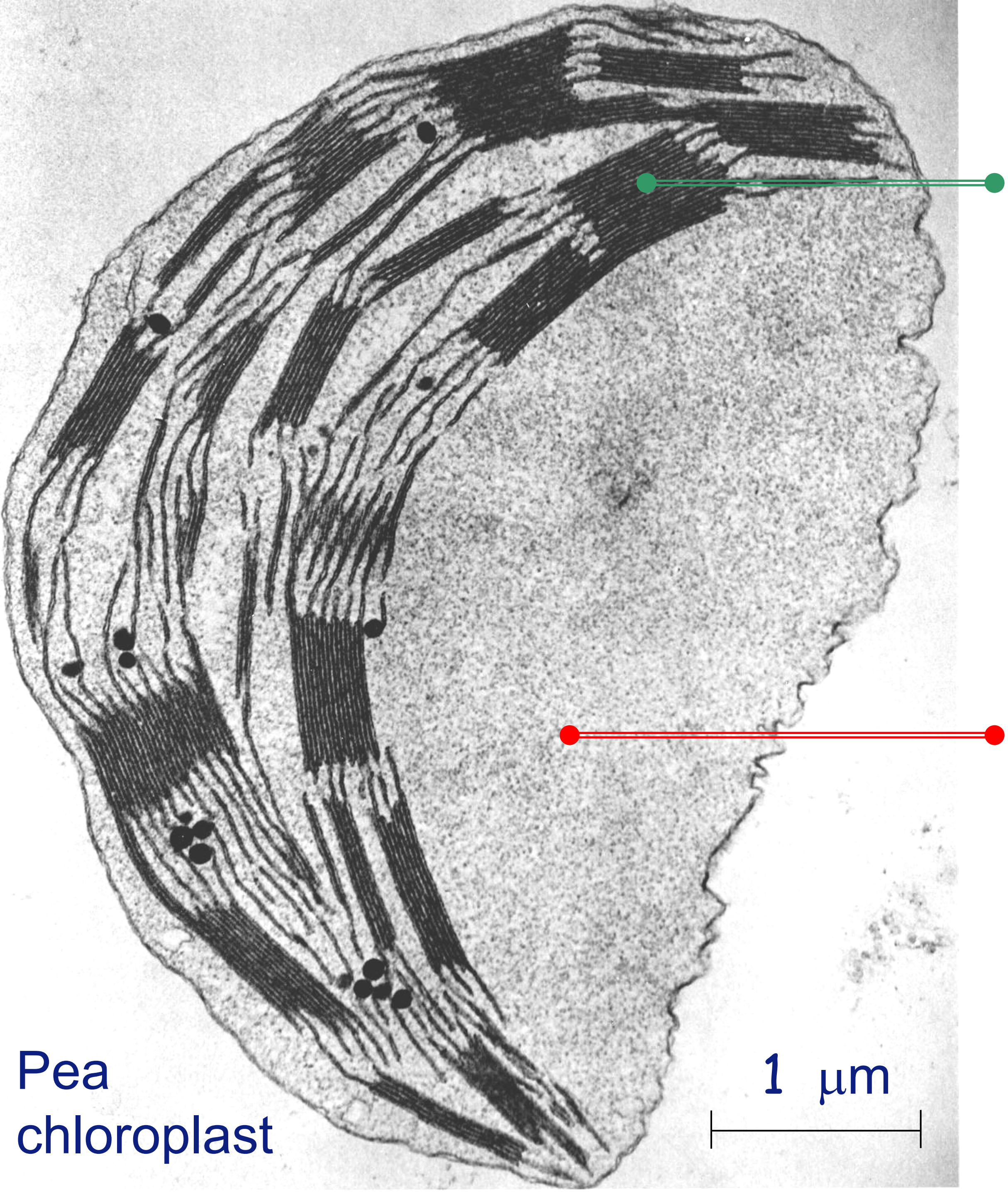
Cyanobacteria
(*Synechocystis* 6803)

Green algae
(*Chlamydomonas reinhardtii*)

Plants
(*Arabidopsis*, tobacco)

light microscopy

electron microscopy



Pea
chloroplast

Thylakoid membrane
(site of Light reactions)

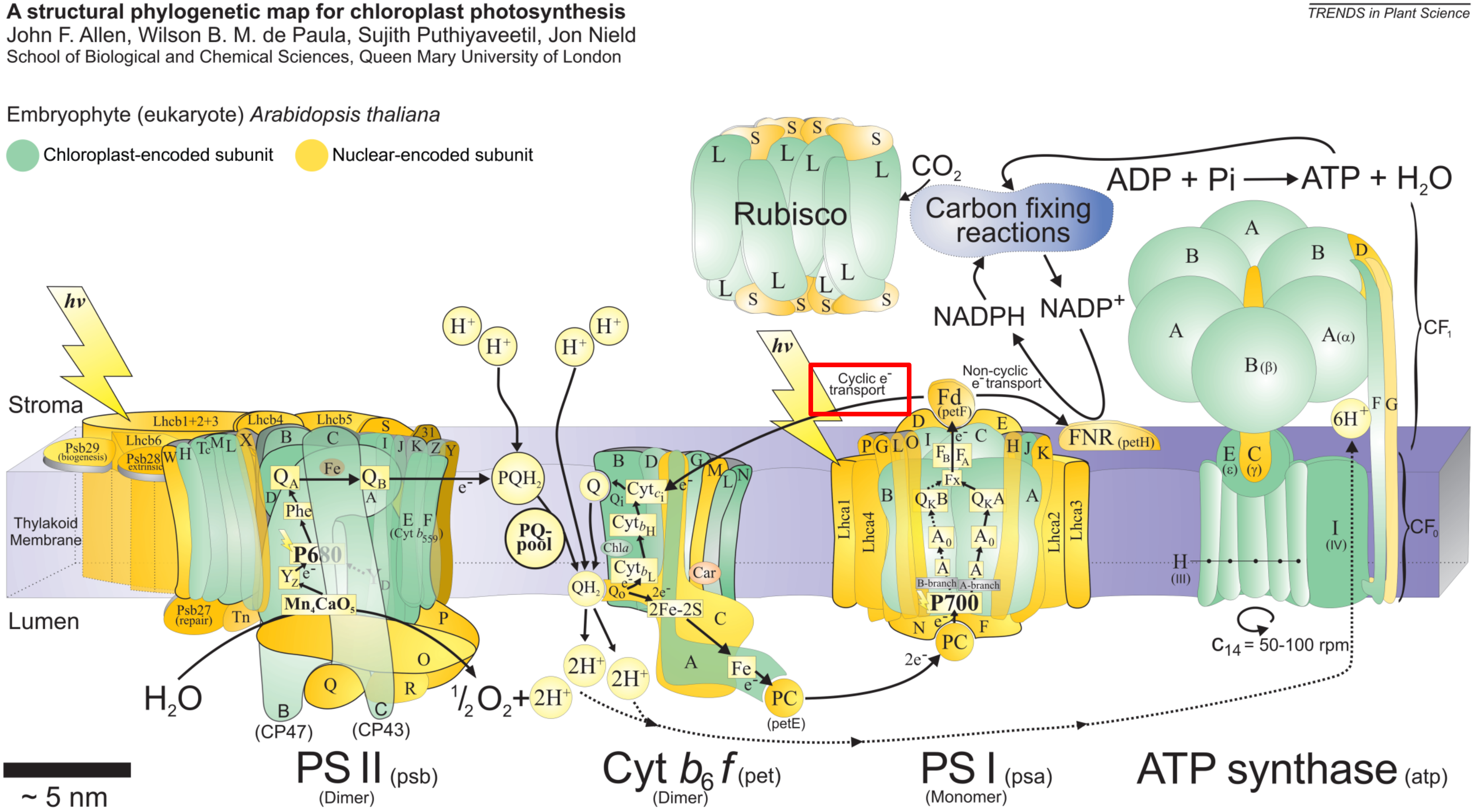
- chlorophyll
- light-harvesting
- electron transfer
- O_2 evolution
- ATP and NADPH production

Stroma
(site of Dark reactions)

- Rubisco
- CO_2 fixation
- sugar and starch synthesis

courtesy of John Gray

Structural view of the thylakoid complexes



A structural phylogenetic map for chloroplast photosynthesis
John F. Allen, Wilson B. M. de Paula, Sujith Puthiyaveetil, Jon Nield
School of Biological and Chemical Sciences, Queen Mary University of London

TRENDS in Plant Science

Embryophyte (eukaryote) *Arabidopsis thaliana*

Allen et al (2011) Trends in Plant Science 16: 645 - 655

Requirements for Calvin cycle in C3 plants:

3 ATP and 2NADPH are needed to fix 1 CO_2 into triose phosphate

i.e. ATP/NADPH ratio of 1.5 is required

How much ATP and NADPH is produced in the light reactions?

For every pair of electrons 6 protons are pumped ($H^+/e = 3$)

The formation of NADPH requires a pair of electrons

14H^+ are thought to be needed by ATP synthase to make 3 ATP

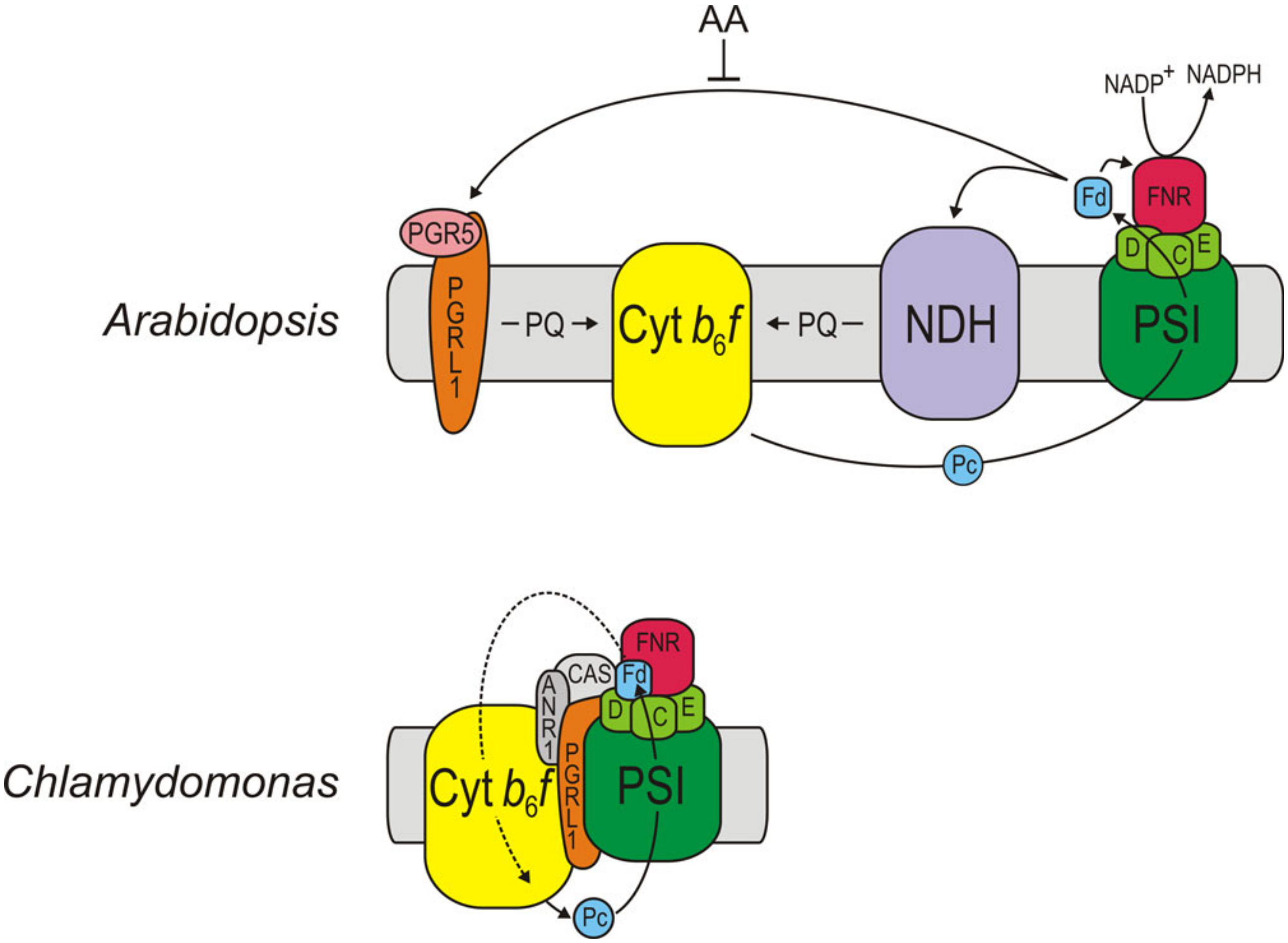
4.67H^+ are needed by ATP synthase to make 1 ATP

Therefore 1.29 ATP synthesised per NADPH

Linear electron flow produces too little ATP for Calvin cycle

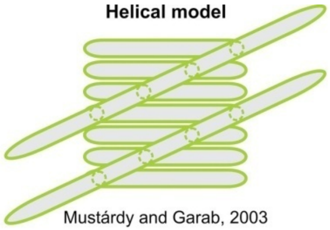
Pathways of cyclic electron flow around PSI still not understood!

AA: antimycin A



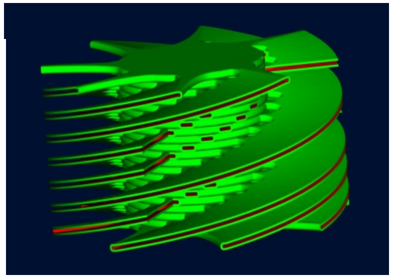
Jensen and Leister (2014) F1000 Prime Rep. 6:40

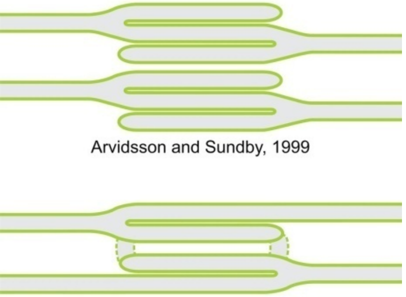
Structural view of the grana In higher plants



Helical model

Mustárdy and Garab, 2003

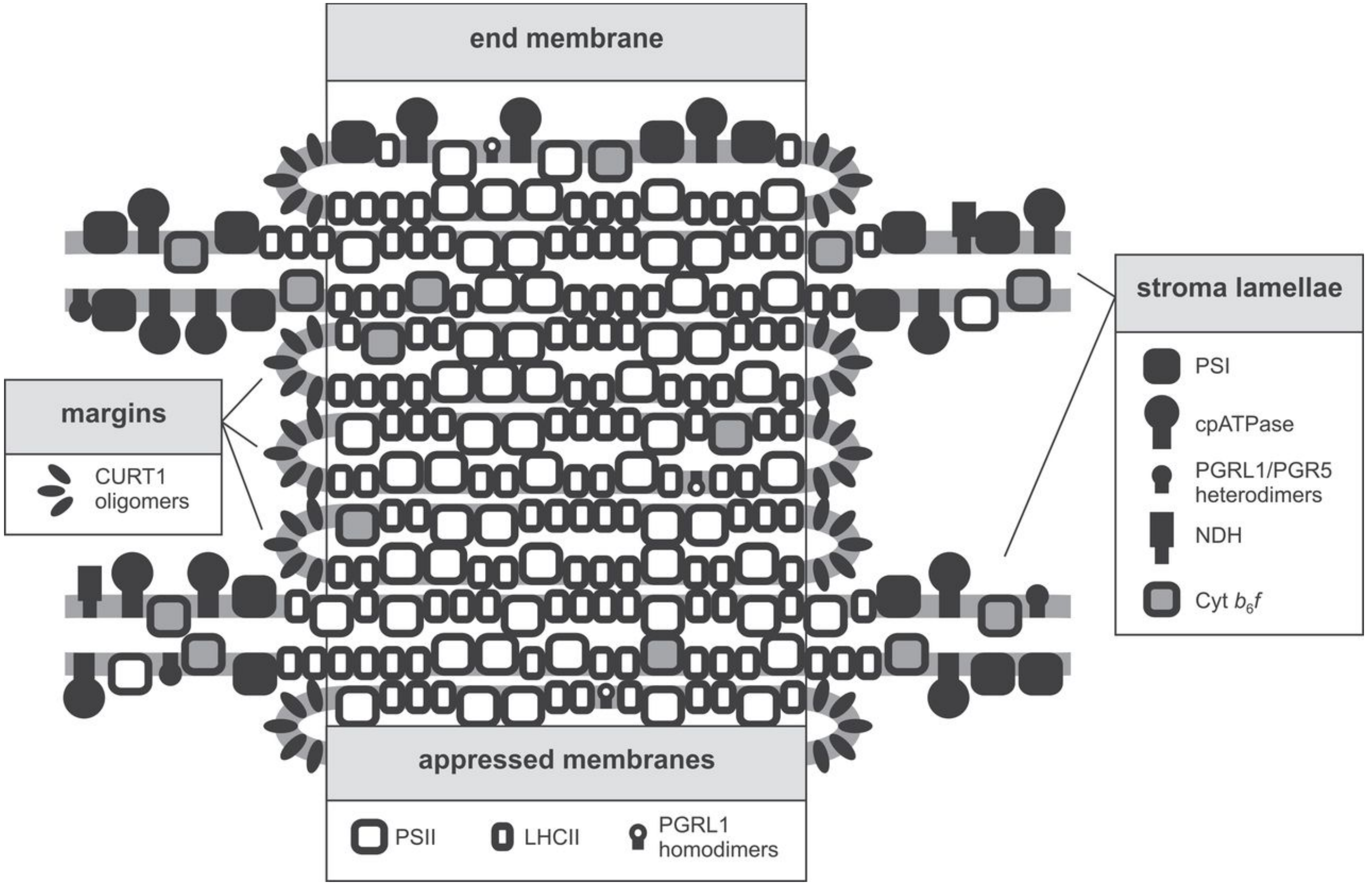




Fork models

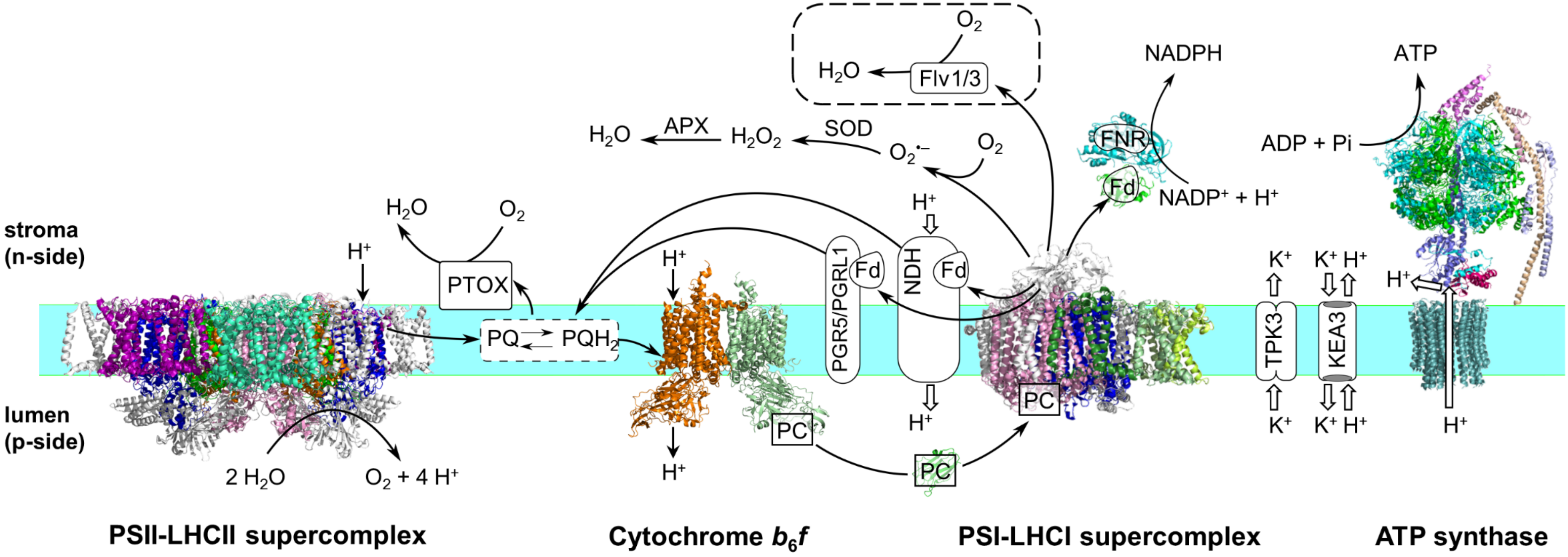
Arvidsson and Sundby, 1999

Shimoni et al., 2005



Pribil et al (2014) J Exp Bot 65: 1955 - 1972

Possible routes for enhancing photosynthesis



Light harvesting

- Alter size of antenna
- Synthesize pigments that absorb in far-red
- Introduce new antennae

Photochemistry

- Introduce different types of reaction center

Ratio of ATP to NADPH produced

- Control amount of cyclic and pseudo cyclic electron flow
- Modify ATP synthase

Alternative Electron flow

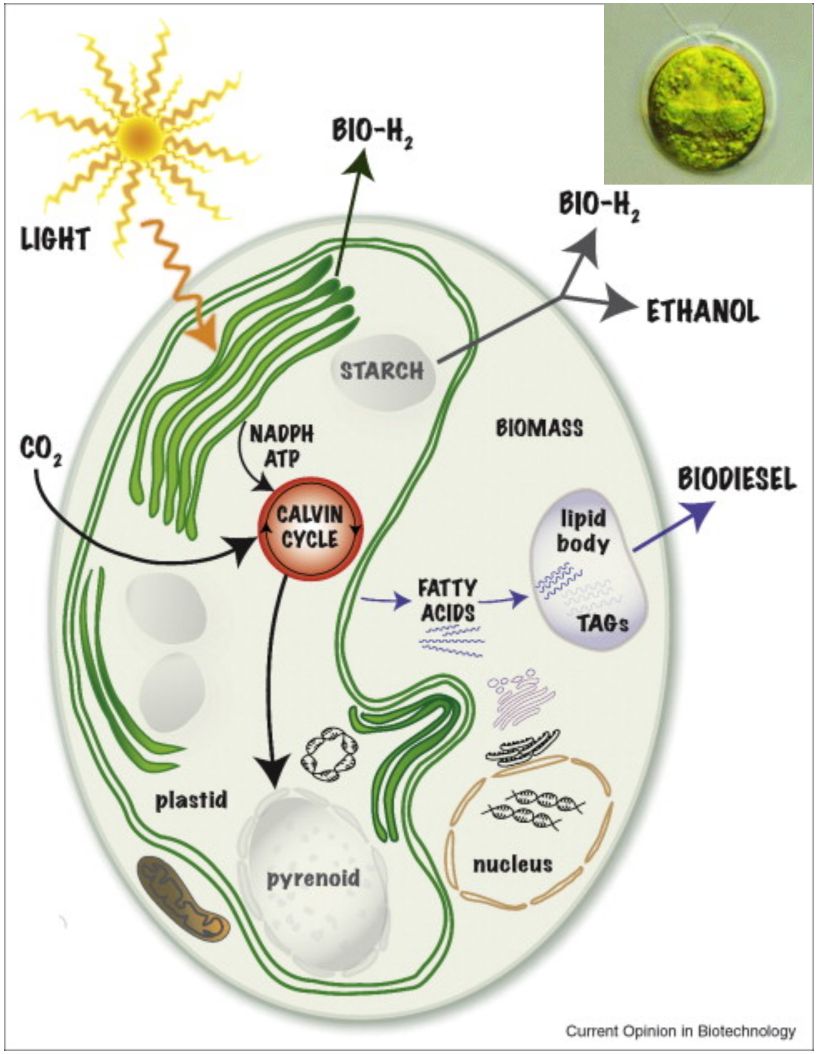
- Introduce electron escape valves (Flv, PTOX)

Photoprotection and response to fluctuating light

- qE quenching
- State transitions
- PSII repair
- ROS prevention
- Regulate ΔpH/membrane potential

Cardona et al (2018) Essays Biochem 62: 85-94

Algal biofuels



Current Opinion in Biotechnology

Advantages:

- *Photoautotrophic growth (sun +CO_2)*
- *Potential for carbon capture*
- *Many species available with different growth characteristics and 'biofuel' profile*
- *Can grow on land and in water unsuitable for crop plants*
- *High yield of biomass (high Photosynthetic conversion efficiency)*

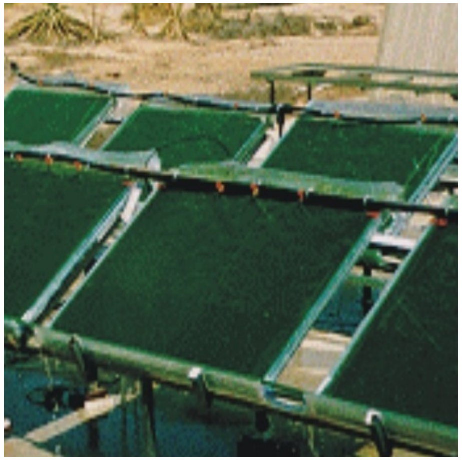
Challenges:

- *Provision of water and nutrients*
- *Harvesting of biomass*
- *Contamination of open ponds*
- *Expense of closed systems*

Beer LL, Boyd ES, Peters JW & Posewitz MC (2009) Curr Opin Biotech ***20****: 264-271*

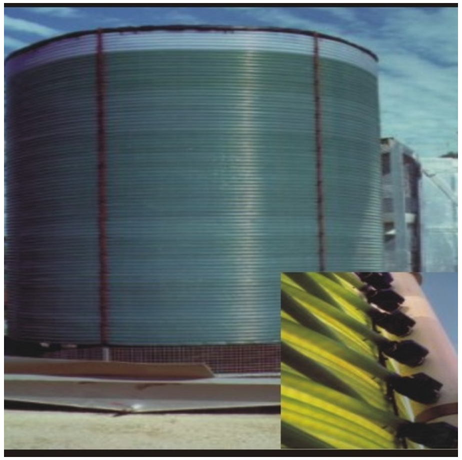
Imperial College London

Closed Bioreactors

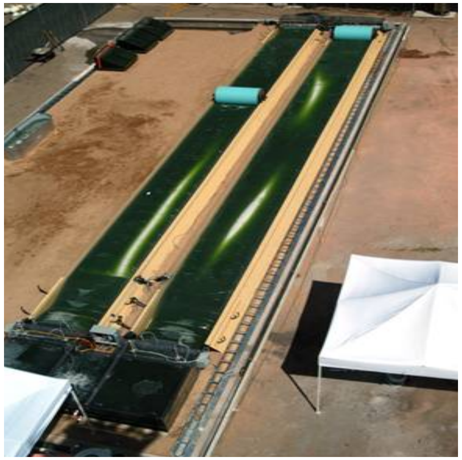


Clemens Posten

Enhanced Biofuels



Michael Borowitzka



Solix

Subitec

Posten/Pulz

Open pond system

Cyanotech, Hawaii. Open pond systems produce Spirulina. (Image from Henrikson 2010)

Algal Biofuels

NEWS NATURE|Vol 461|24 September 2009

Scaling up production from the flask to commercial quantities of biofuels from algae is the real challenge.

D.J. PHILLIP/AP/PA

Gold rush for algae

The second of four weekly articles on biofuels describes how oil giants and others are placing their bets on algae.

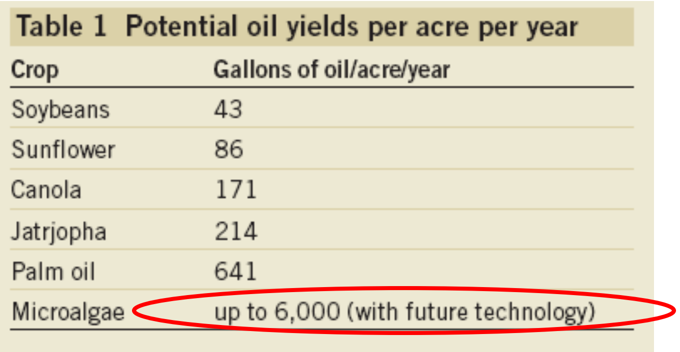


Table 1 Potential oil yields per acre per year

Crop	Gallons of oil/acre/year
Soybeans	43
Sunflower	86
Canola	171
Jatrjopha	214
Palm oil	641
Microalgae	up to 6,000 (with future technology)

NATURE BIOTECHNOLOGY VOLUME 27 NUMBER 1 JANUARY 2009

$10–20 per gallon from open ponds

$1billion invested in Algal biofuels since 2007

Imperial College
London

The algal 'feeding frenzy'

Aurora Bi

Aurora Biofuel
and technolog
integrate engi
development p
edge biotechn
economical ma
from algae.

The time
depen

Sapphire is leadin
Crude production
landscape for the

Solarvest
Microalgae Platform

Solarvest has patented a breakthrough technology for the production of hydrogen as an alternative "green" fuel source.

Solarvest is a world leader in the genetic modification of microalgae as their base proprietary technological platform.

Microalgae are ideal candidates for genetic manipulation and can be modified to express (produce) products ranging from bioactive compounds to hydrogen gas.

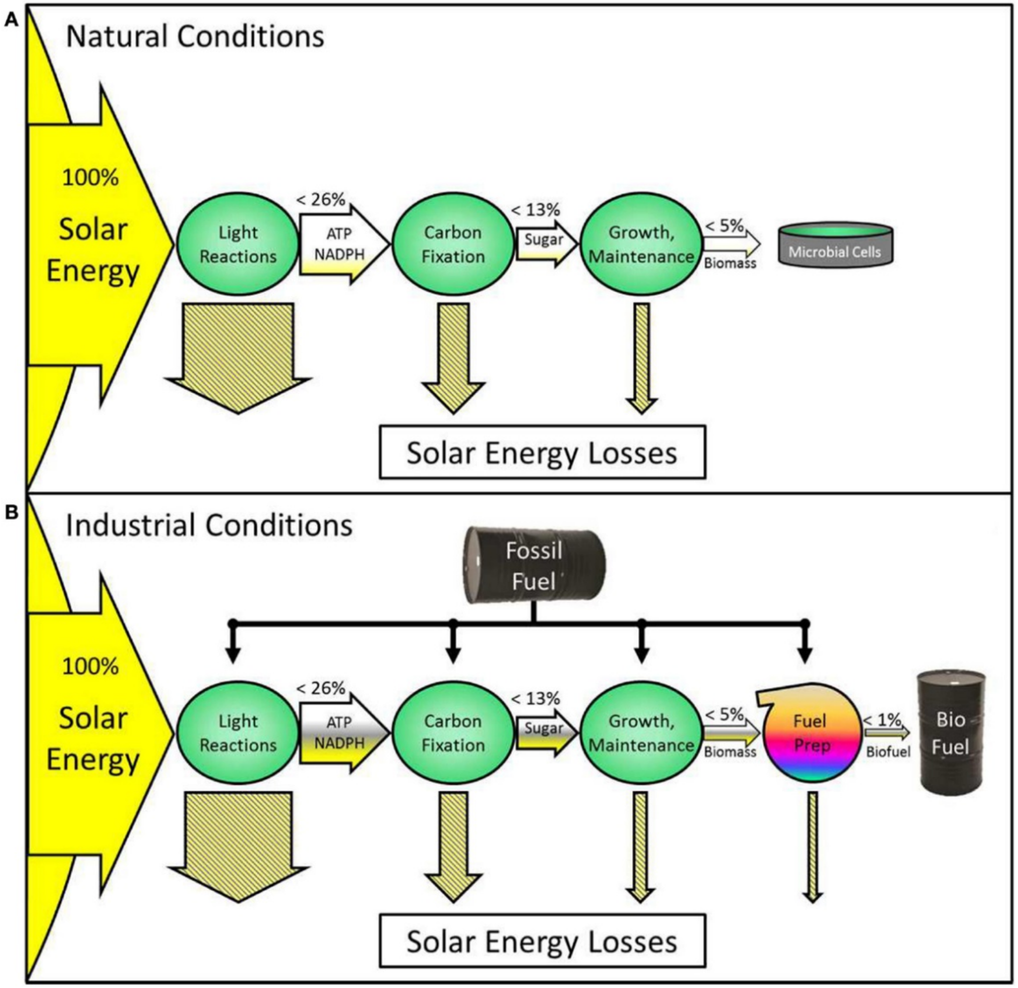
Solazyme, Inc. is the leading renewable oil and bioproducts company. The company uses algal biotechnology to renewably produce clean fuels, chemicals, foods and health science

being designed to be compatible with the existing distribution infrastructure, and to possess superior characteristics compared to traditional biofuels. Our biochemicals resemble traditional petrochemical products.

CO2 → Fuels & Chemicals

SGI is harnessing photosynthetic microbes (i.e., algae) to produce a range of liquid

Low-value photosynthetic microbial biofuels are currently not viable



Cotton et al (2015) Front.Bioeng.Biotechnol. **3**:36.

On the bright side there might be something in it.....

An economic and technical evaluation of microalgal biofuels

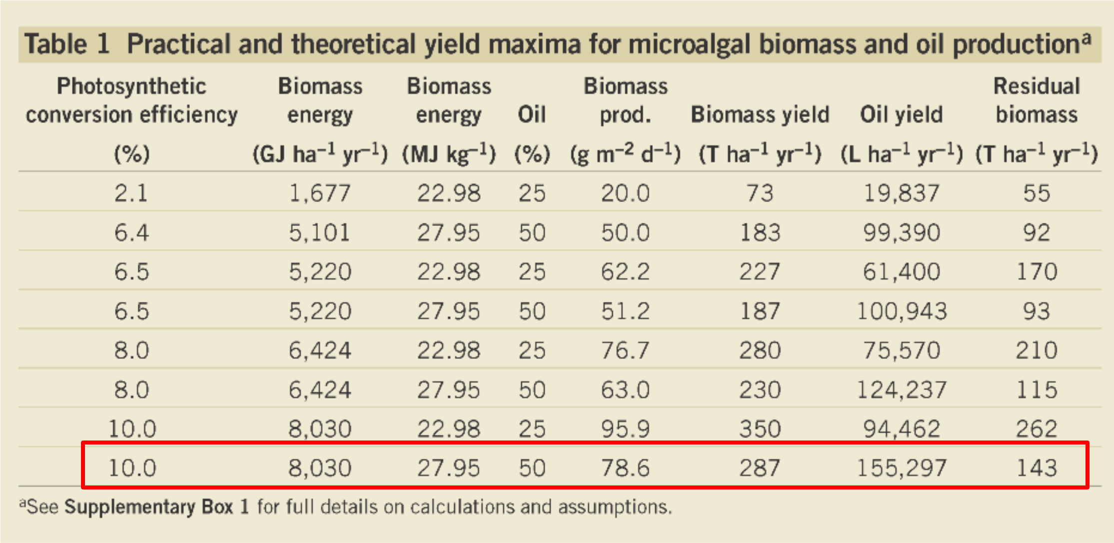


Table 1 Practical and theoretical yield maxima for microalgal biomass and oil production[a]

Photosynthetic conversion efficiency (%)	Biomass energy (GJ ha^{-1} yr^{-1})	Biomass energy (MJ kg^{-1})	Oil (%)	Biomass prod. (g m^{-2} d^{-1})	Biomass yield (T ha^{-1} yr^{-1})	Oil yield (L ha^{-1} yr^{-1})	Residual biomass (T ha^{-1} yr^{-1})
2.1	1,677	22.98	25	20.0	73	19,837	55
6.4	5,101	27.95	50	50.0	183	99,390	92
6.5	5,220	22.98	25	62.2	227	61,400	170
6.5	5,220	27.95	50	51.2	187	100,943	93
8.0	6,424	22.98	25	76.7	280	75,570	210
8.0	6,424	27.95	50	63.0	230	124,237	115
10.0	8,030	22.98	25	95.9	350	94,462	262
10.0	8,030	27.95	50	78.6	287	155,297	143

[a] See **Supplementary Box 1** for full details on calculations and assumptions.

>500 ha in area

High value products (e.g., carotenoid) important income stream in early years

Biomass sold as feedstock

In later years, oil more profitable as prices increase

Internal rate of return over 30 years > 15%

Stephens E, Ross IL, King Z, Mussgnug J, Kruse O, Posten C, Borowitzka MA & Hankamer B (2010) Nature Biotechnology 28: 126-128

A biological route to solar hydrogen

$$\boxed{2H_2O \xrightarrow{\text{light}} 2H_2 + O_2}$$

$$2H_2O \xrightarrow{\text{light}} O_2 + 4H^+ + 4e^-$$ Photosystem Two

$$4H^+ + 4e^- \longrightarrow 2H_2$$ Hydrogenase

Why Hydrogen?

Clean Fuel

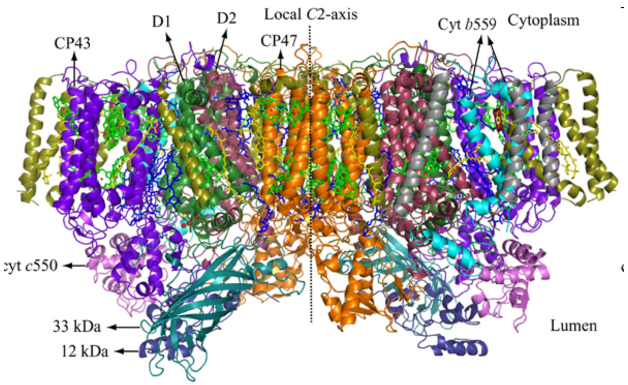
$$2H_2 + O_2 \longrightarrow 2H_2O$$

Climate Change

Energy Security

A biological route to solar hydrogen

$$2H_2O \xrightarrow{\text{light}} 2H_2 + O_2$$



$$2H_2O \xrightarrow{\text{light}} O_2 + 4H^+ + 4e^-$$ Photosystem II

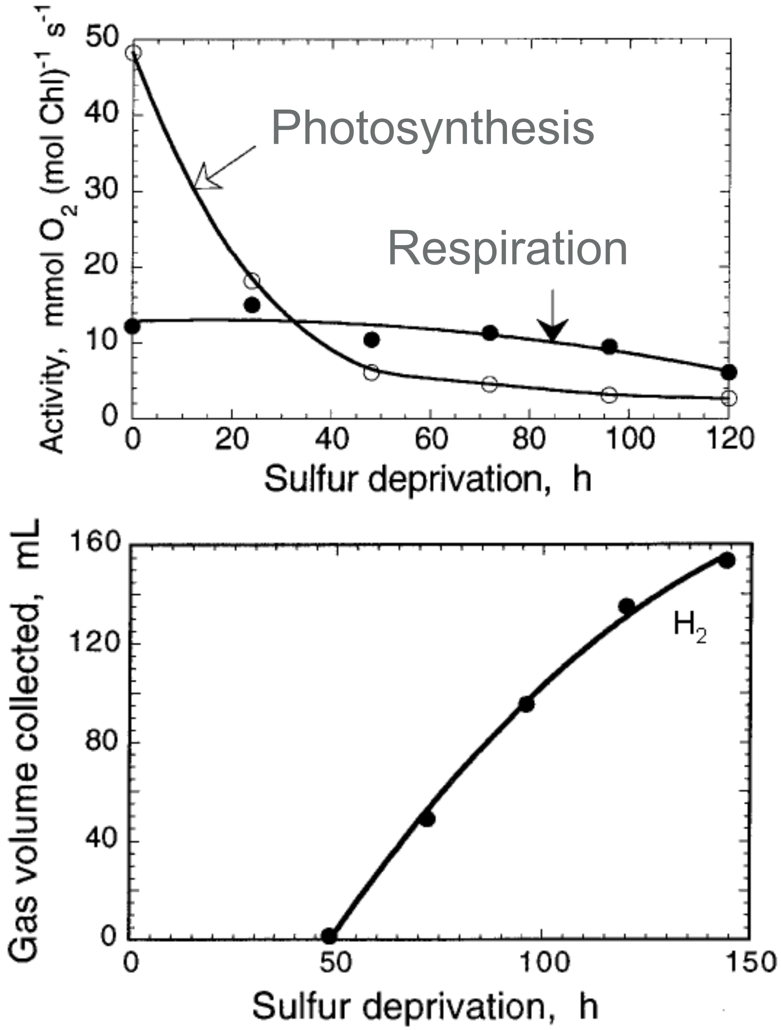
$$4H^+ + 4e^- \underset{O_2}{\cancel{\longrightarrow}} 2H_2$$ [FeFe]- hydrogenase

Photo-biological H_2 production in Green Algae

•1940s Hans Gaffron
Light induced transient H_2 production by certain types of dark-adapted algae

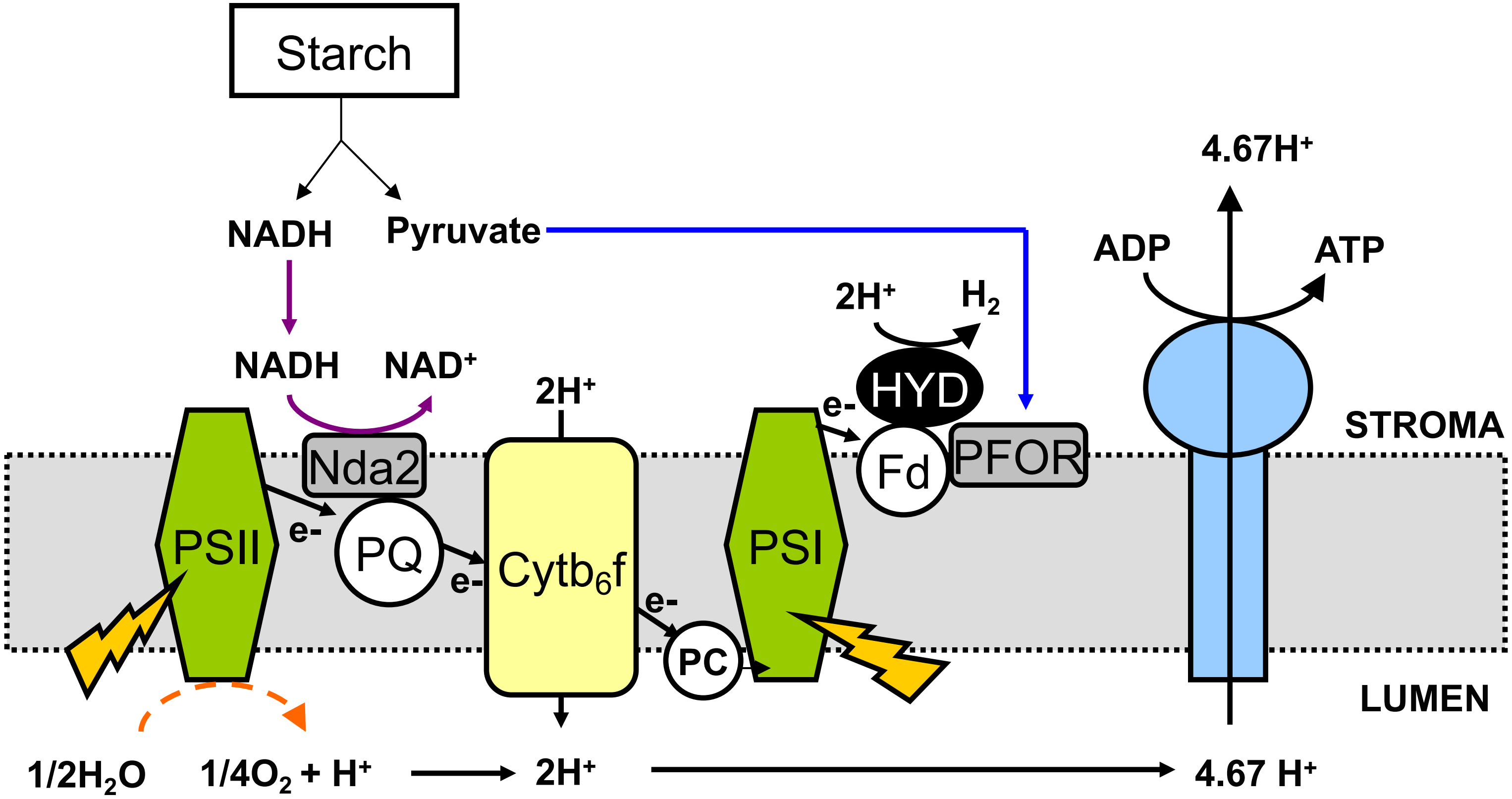
•2000 Melis and co-workers
Sustained H_2 production for a few days using sulphur-depleted cultures of *C. reinhardtii*

2ml H_2/L/h



Adapted from Melis et al. 2000 *Plant Physiol.* 122(1)127-36

Sources of reductant for H_2 production

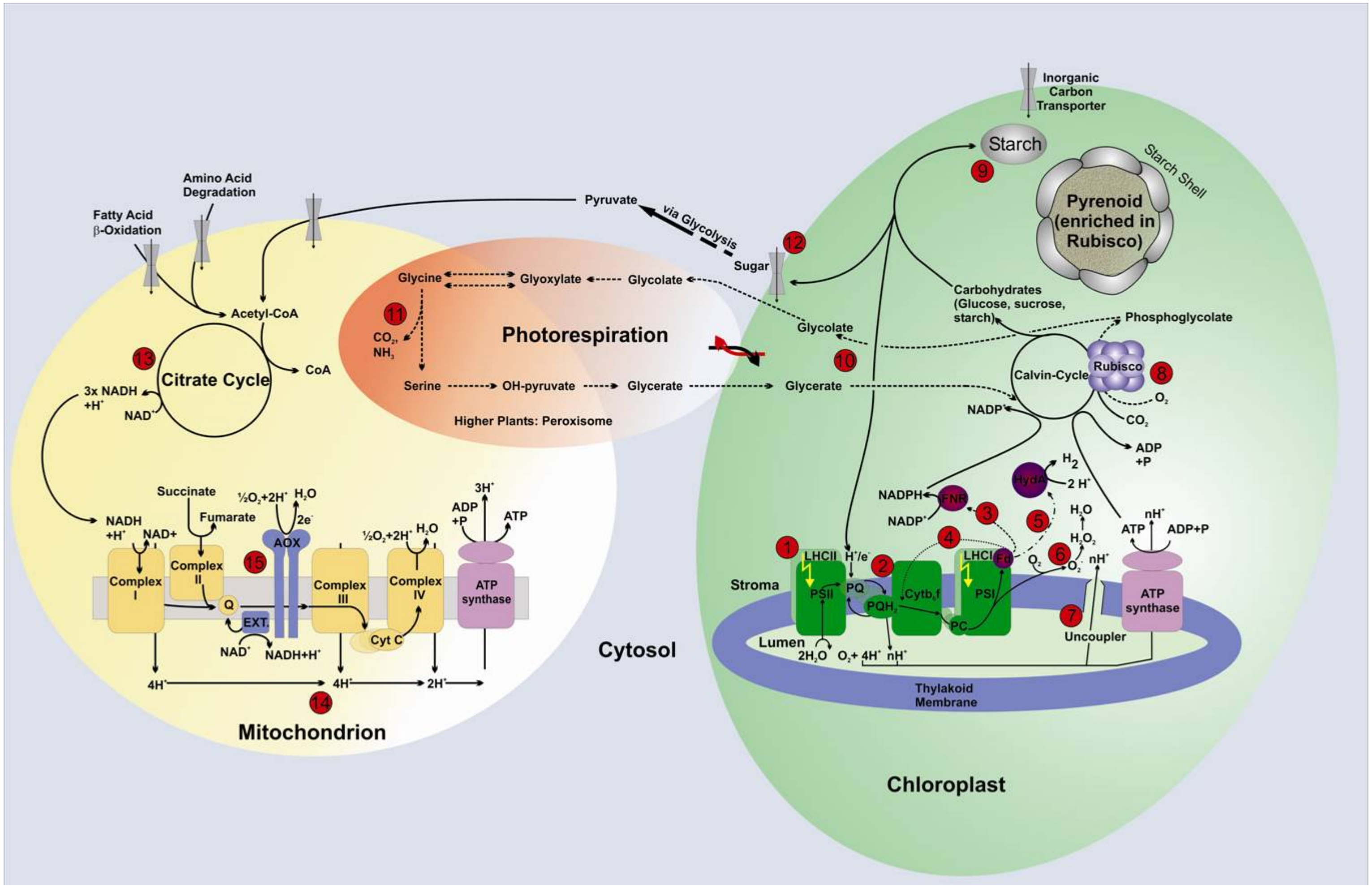


(1) PSII water splitting (Biophotolysis)

(2) Via type II NADH dehydrogenase (photofermentation)

(3) Via Pyruvate:ferredoxin oxidoreductase (dark fermentation)

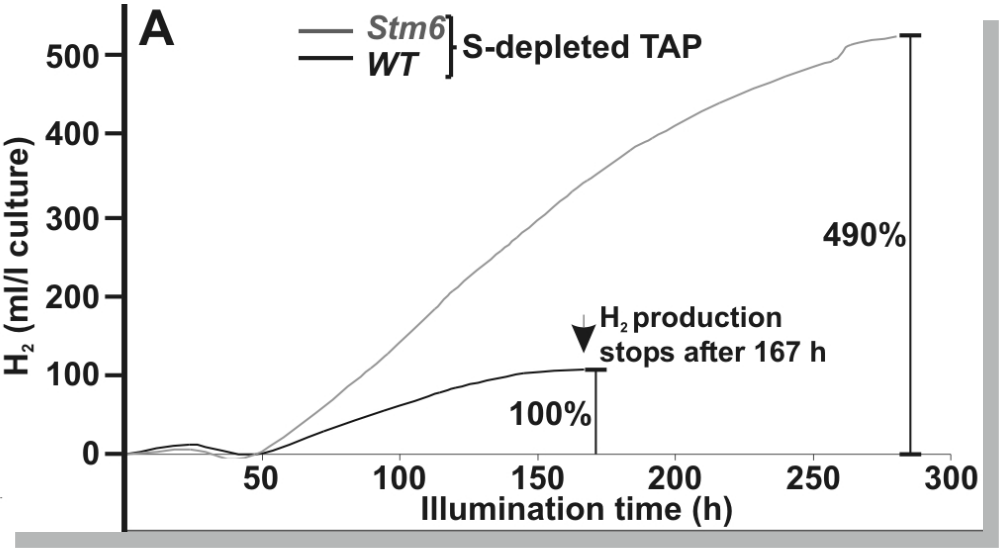
Improving hydrogen yield through serendipity



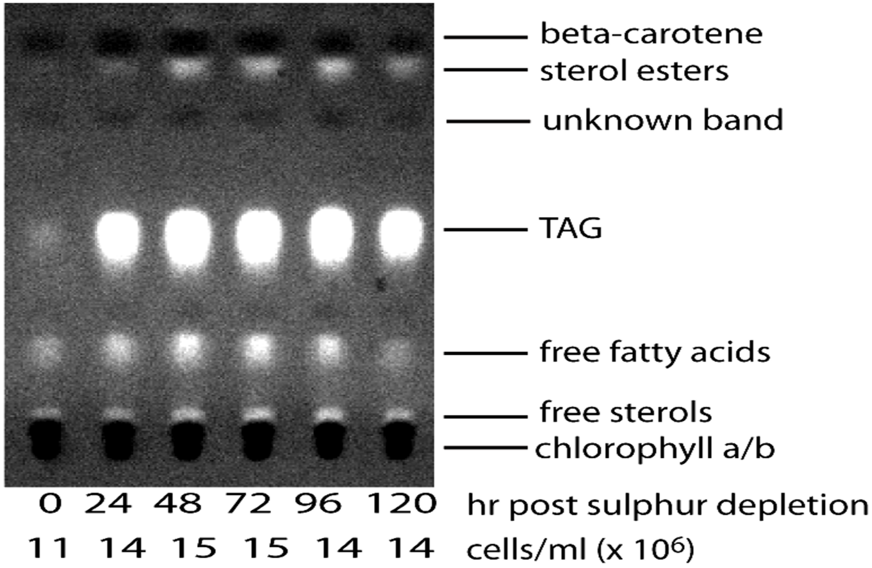
Random mutagenesis/screening identified *moc1* mutant which has 10-fold better rate of hydrogen production (2% energy conversion efficiency) than WT

Serendipity: isolation of the *stm6/moc1* mutant

H_2 PRODUCTION (*stm6/moc1*)



LIPID PRODUCTION (*stm6/moc1*)



Courtesy of Ben Hankamer and colleagues

- >5-fold better yield of hydrogen than WT
- Increase in levels of triacylglycerides (TAG)
- 2% energy conversion efficiency
- MOC1 is involved in regulating transcription termination in mitochondrion – indirect effect on redox state of chloroplast

Kruse O et al (2005) J Biol Chem 280: 34170-34177

Rational approaches to improve biohydrogen production

- **Select/engineer more oxygen-resistant hydrogenase enzymes**

  (10-fold increase on overexpressing endogenous hydrogenase in *Chlorella* sp. DT)

- **Increase flux of electrons going to hydrogenase**

  (Fd-hydrogenase fusion *in vitro*, poorer Rubisco, over-express glucose transporter/feed Glc, manipulate competing fermentation pathways)

- **Modulate oxygen levels in the cell**

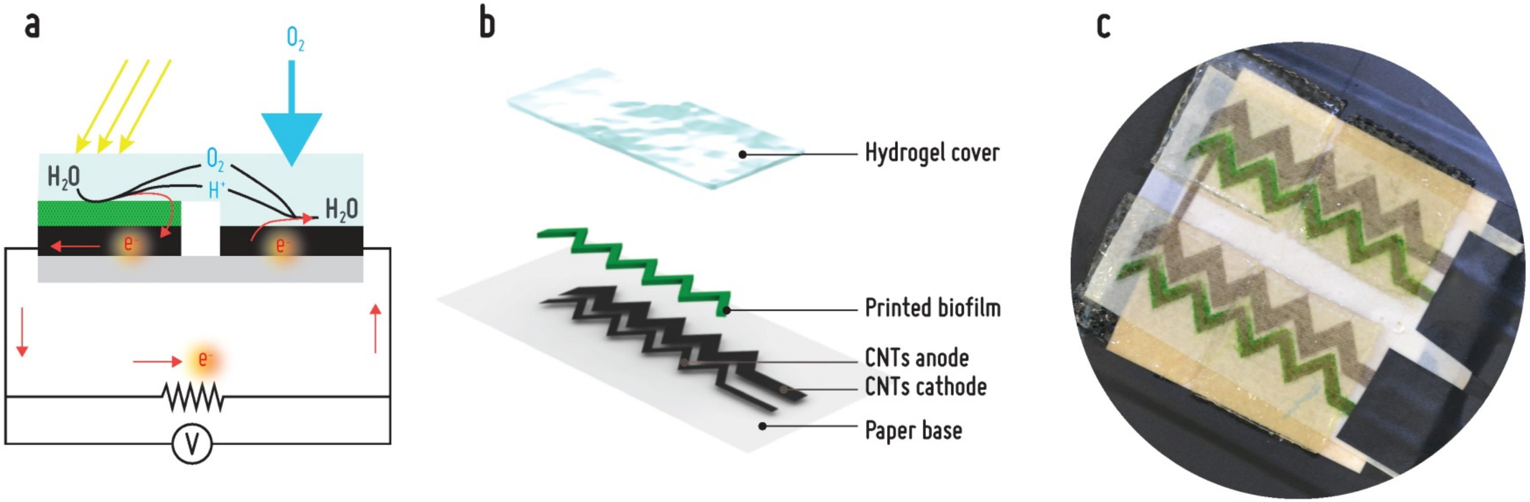
  (PSII mutants, downregulate WT PSII, enhance respiration, express Leg haemoglobin or pyruvate oxidase)

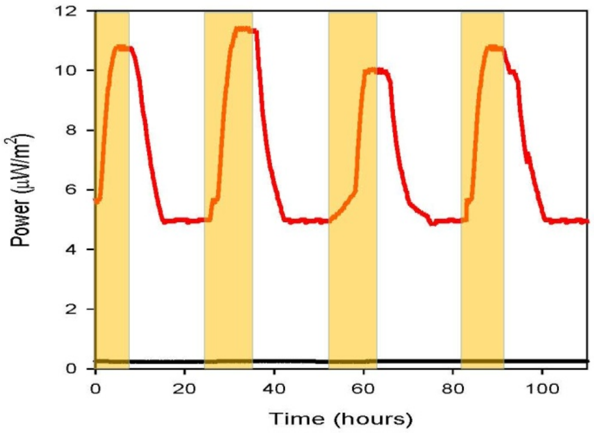
- **Improve bio-reactor design and characteristics of algae**

  (light-harvesting mutants with improved light penetration in culture, use fluctuating light to produce burst of hydrogen)

Dubini and Ghirardi (2015) Photosynth Res

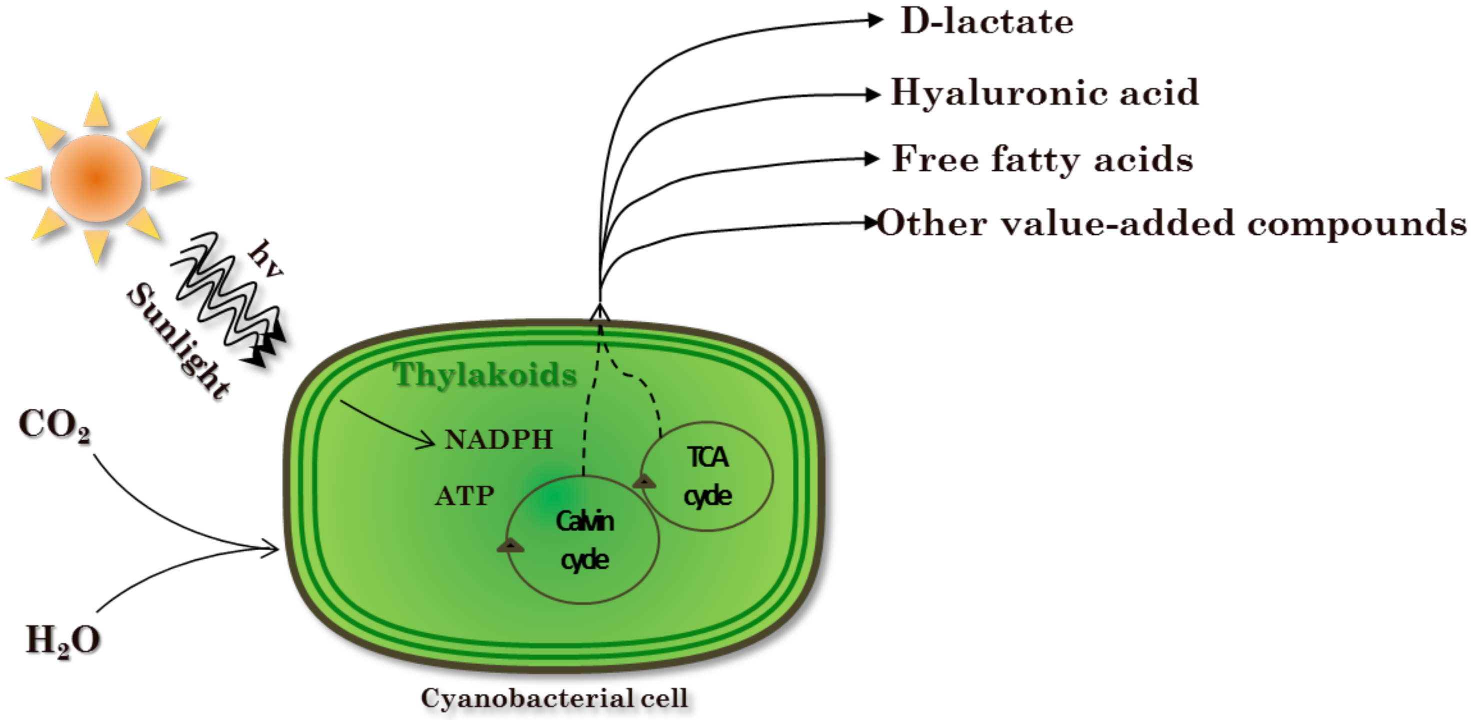
Exploiting photosynthesis: electricity production from digitally printed cyanobacteria





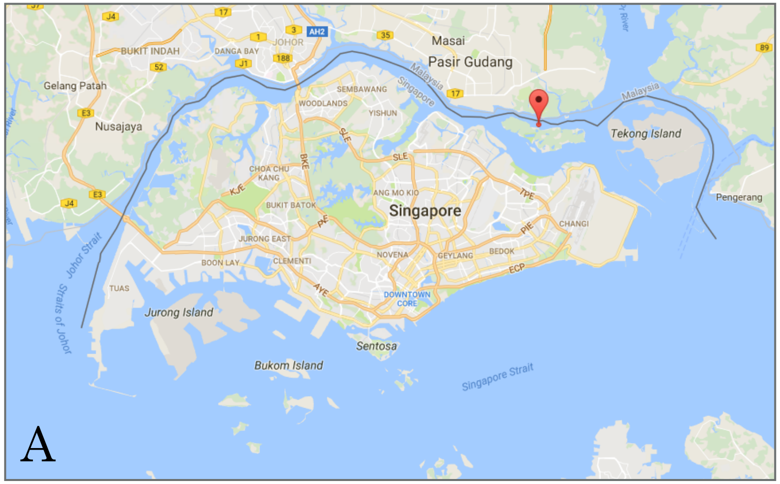
Sawa, Fantuzzi, Bombelli, Howe, Hellgardt and Nixon (2017) Nature Commun. 8, 1327

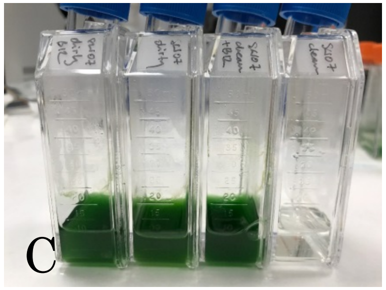
Don’t forget cyanobacteria: ‘solar biorefineries’ for producing chemicals

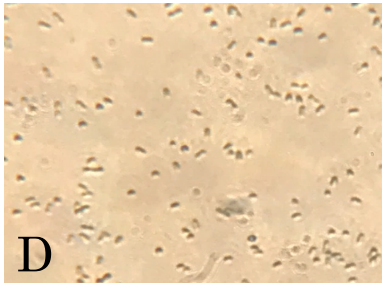


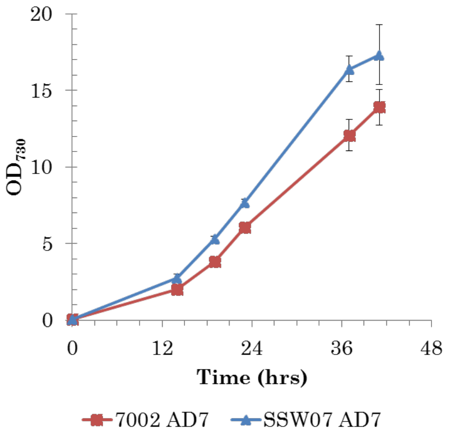
Savakis and Hellingwerf (2015) Curr. Opin. Biotechnol. 33:8-14

Isolation of fast-growing Singaporean strain (SSW07)

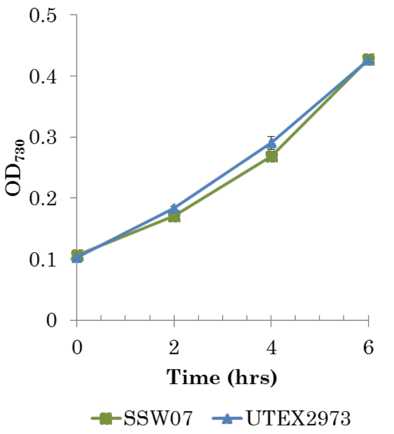








SSW07 strain growth curves compared to Syn7002 (~700 $\mu E.m^2.s^{-1}$, 38ºC, 160 rpm, 1% CO_2)



SSW07 and UTEX2973 growth curves (~500 $\mu E.m^2.s^{-1}$, 38ºC, 160 rpm, 1% CO_2)

- SSW07 is member of *Synechococcus* genus
- Genome has been sequenced
- Grows faster than PCC7002 and as fast as UTEX2973 the current record holder
- Grows in sea water with added macronutrients
- Singapore has fairly constant temp all year round

Selao et al (unpublished)

Imperial College London

Using melamine as an alternative N source to prevent contamination (Selao et al, submitted)

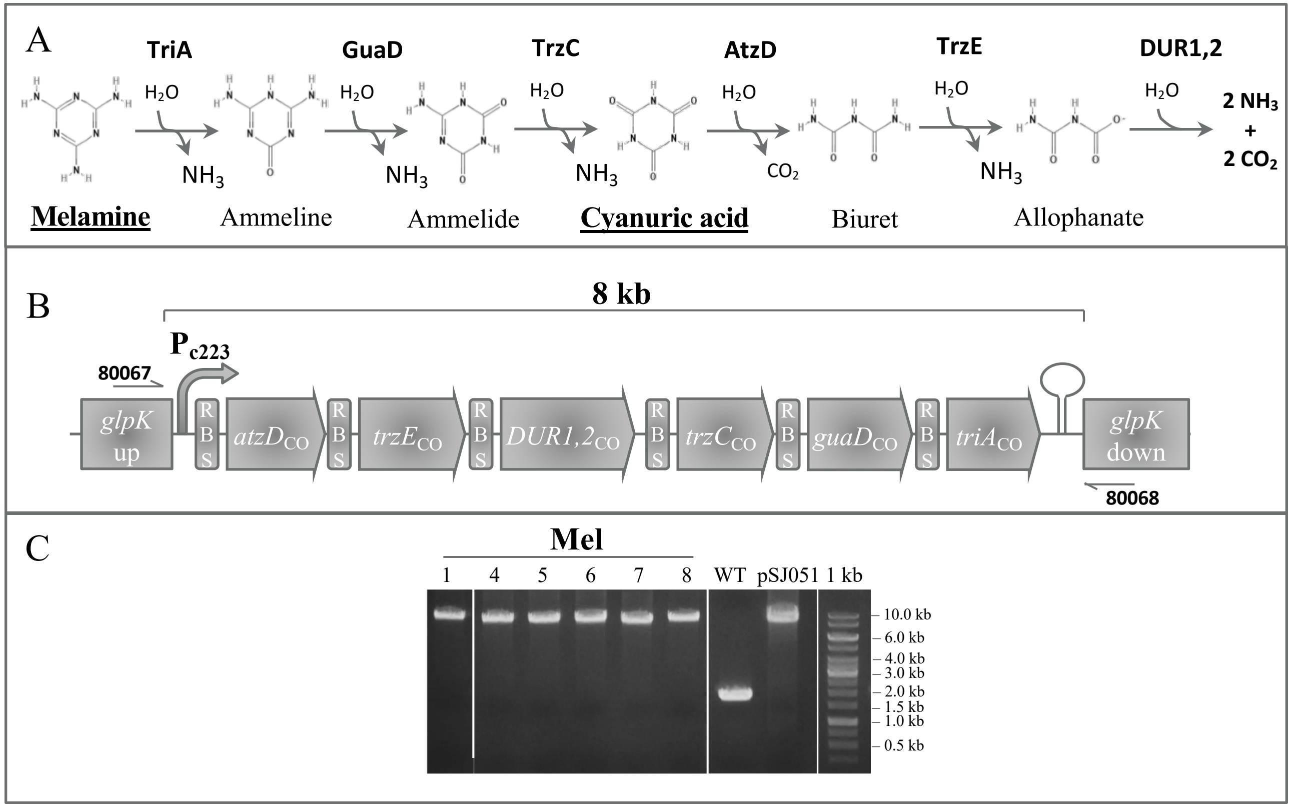


Figure 1 – Overview of melamine selection tool. **A.** Melamine utilization pathway reactions. One mole of melamine yields 6 mol ammonia and 3 mol carbon dioxide. **B.** Schematic view of the melamine utilization operon. Primers indicated were used to confirm full genome integration of the pathway. Different parts are not to scale **C.** 0.6% agarose gel of PCR reaction using primers stated in A. (see Supplemental Table 1 for sequences)

Growth on melamine (Selao et al, submitted)

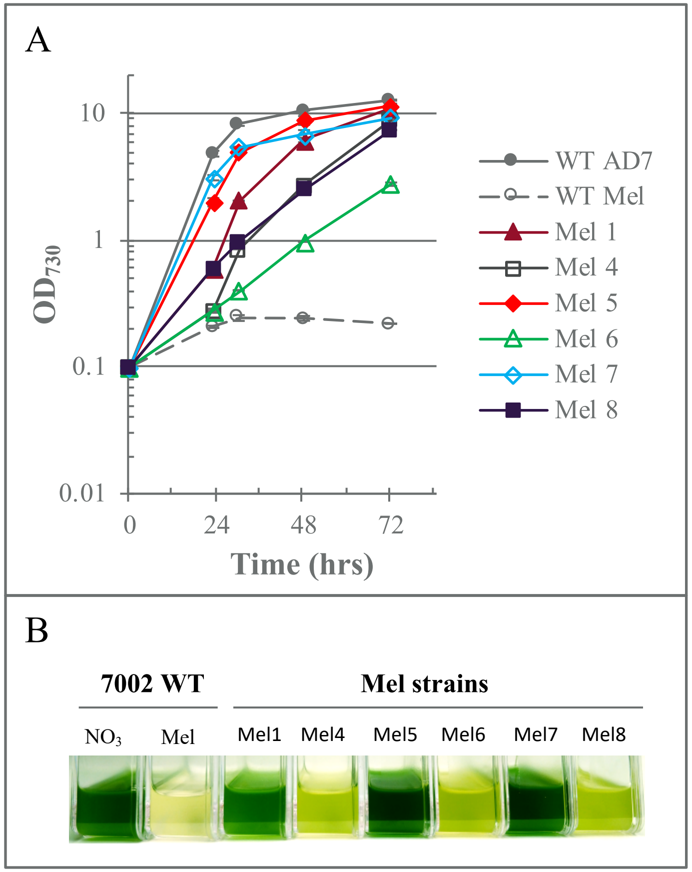
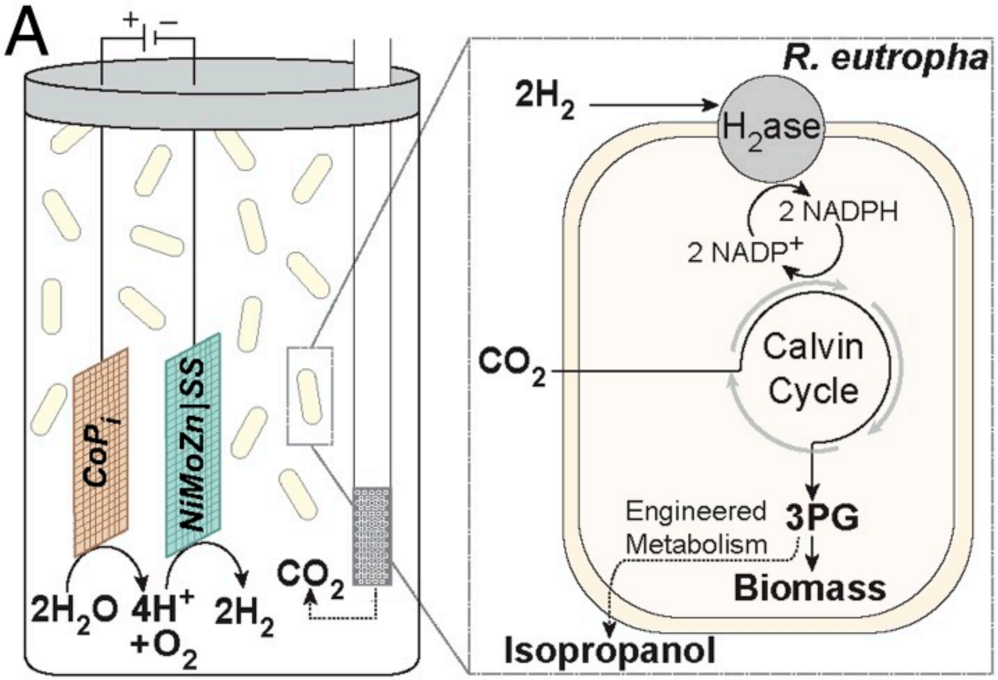
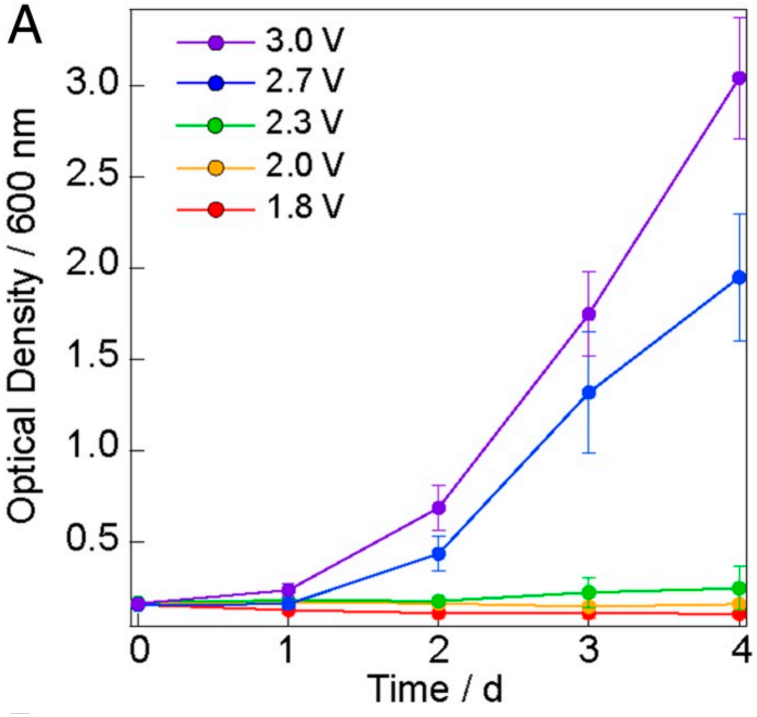


Figure 2 – Growth of melamine utilizing strains in melamine containing medium **A.** Growth curve of WT Syn7002 and melamine utilizing strains **B.** Detail of cultures at 48 hours after inoculation

Hybrid microbial water-splitting catalyst – an alternative approach to use sunlight and water to grow bacteria





Torella et al (2015) Proc Natl Acad Sci USA 112(8):2337–2342.