UNIVERSITY OF CAMBRIDGE

Dept of Plant Sciences

# Vitamins, pigments and energy from algae

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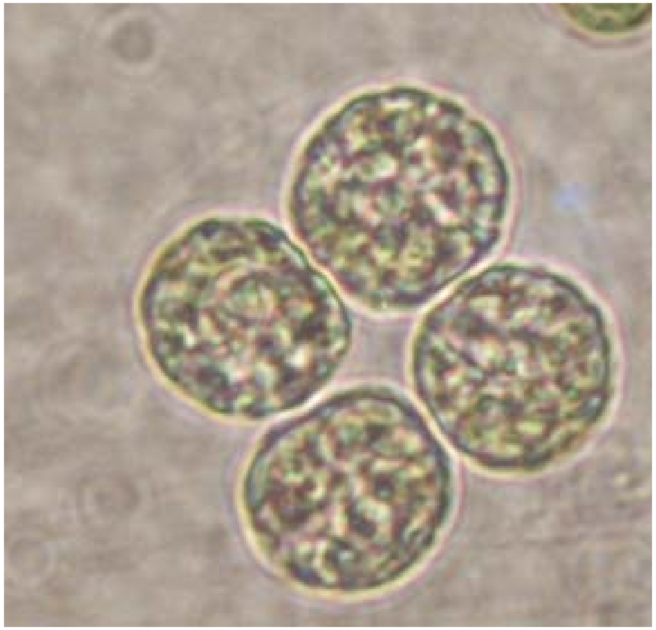
Algal Bioenergy Consortium
http://www.bioenergy.cam.ac.uk/abc.html

# Overview

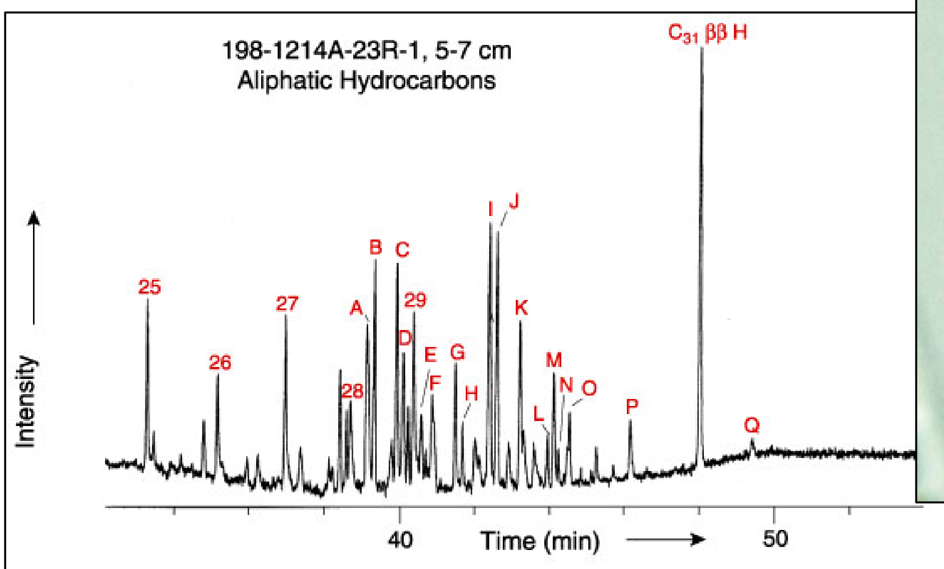
- The concept of biorefining with microalgae
- Opportunities with vitamins
- Biophotovoltaics

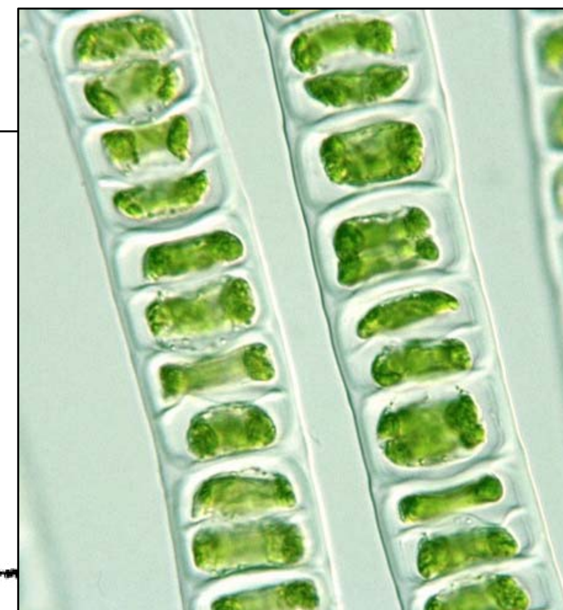


# ABC research focus areas - biology

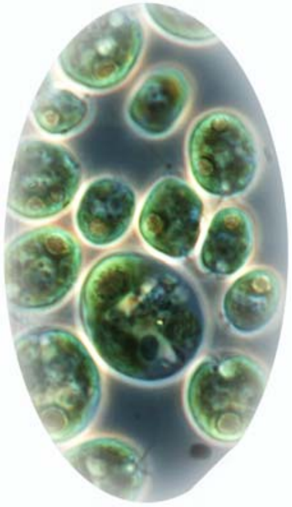
- Algal physiology, metabolism and molecular biology
  - Understanding lags behind that for higher plants

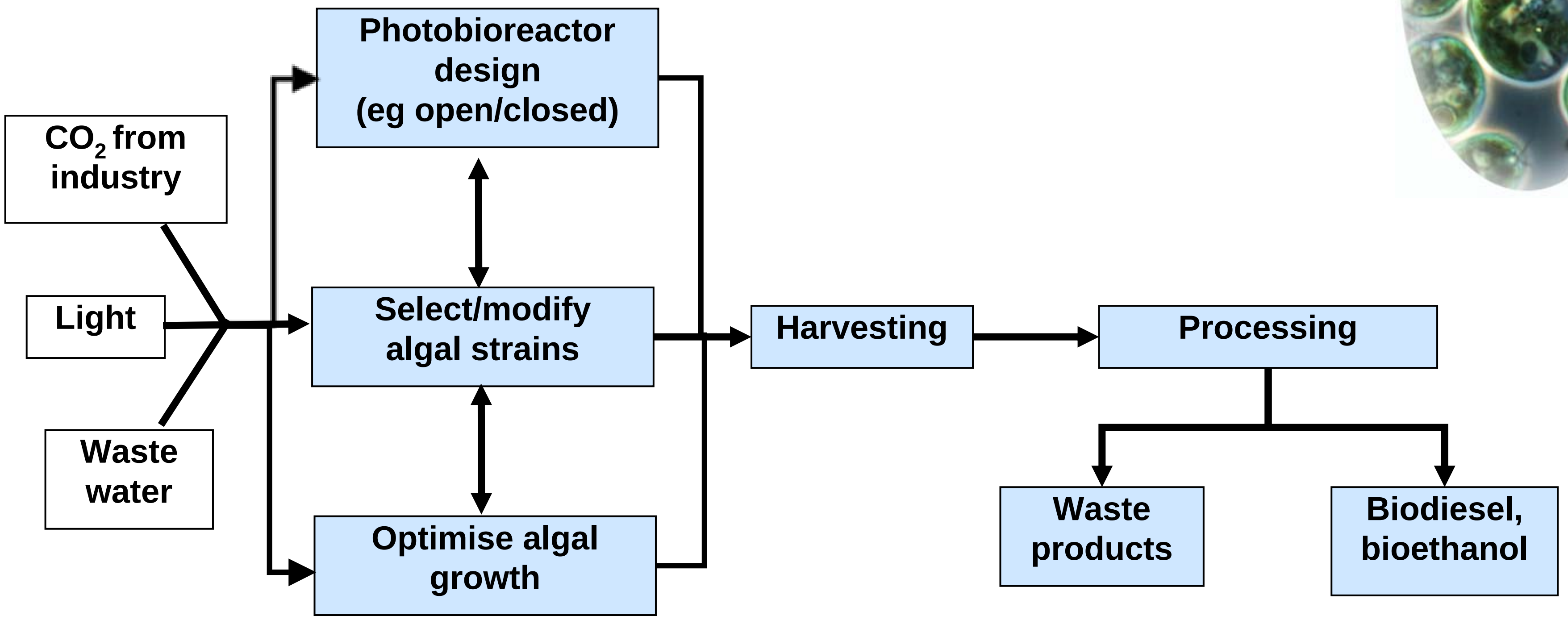
- Optimizing algal growth and lipid content
  - Developing tools for molecular biology
  - Metabolic engineering for lipids and vitamins
  - Characteristics of algal cell walls



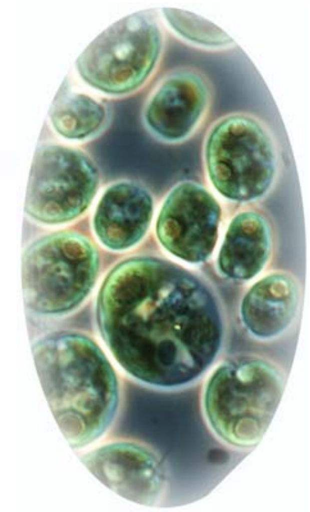
# Potential algal biofuel 'pipeline'

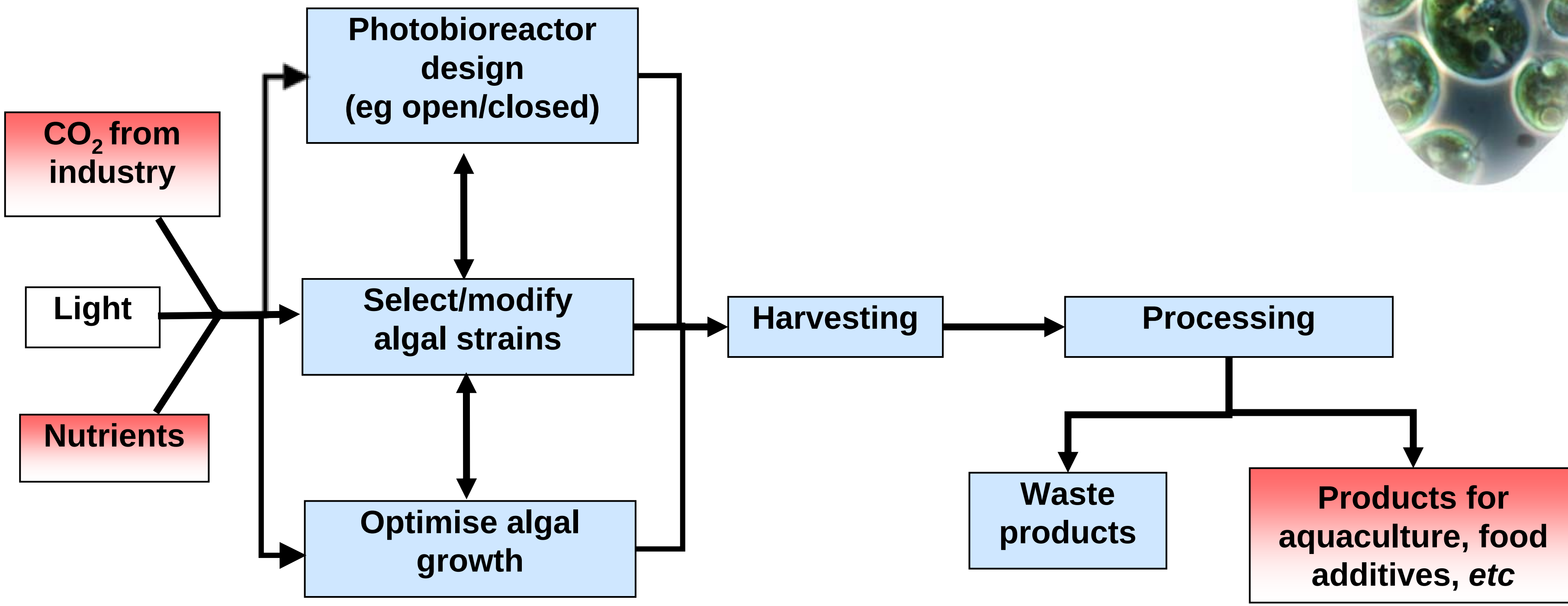





- Life cycle analysis indicates that more energy to obtain fuel than is produced from fuel

# Current commercial algal production

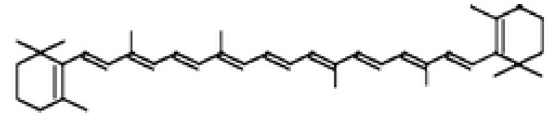




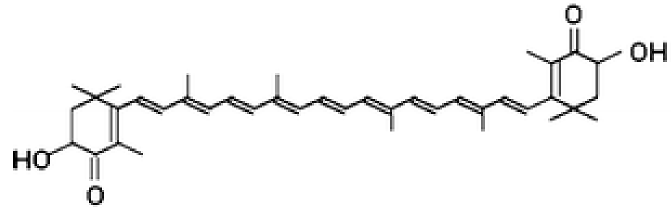

- High value products mean few constraints on cost or energy requirements
- Biorefining might provide additional/alternative income stream

# Algae as a source of carotenoids

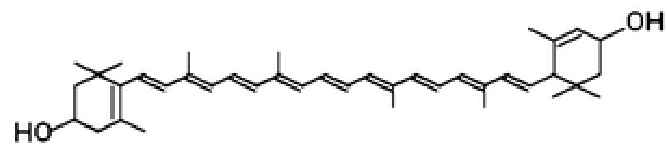
- Carotenoid production from algae well established
  - Provitamin A, anti-oxidants
  - Pigments in aquaculture, cosmetics, food supplements
  - High value products



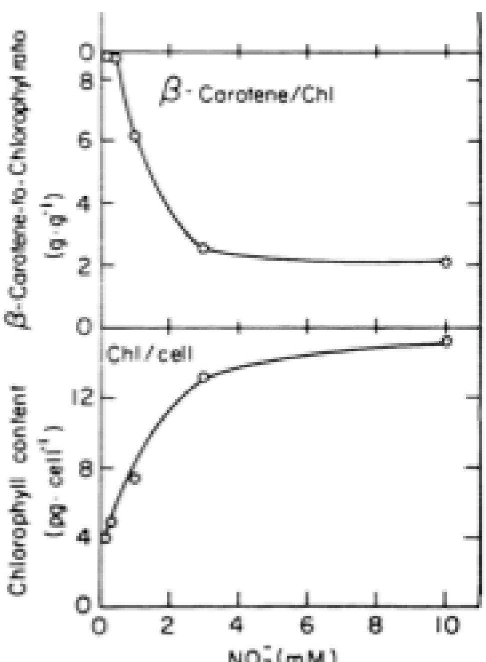
β-carotene




astaxanthin




lutein





- Dunaliella salina
  - Halotolerant alga - grows happily at 18-22% NaCl
  - Accumulates glycerol to maintain osmotic balance

- Production increased by
  - High light or nutrient limitation

# Production of recombinant proteins

***Chlamydomonas reinhardtii* chloroplasts as protein factories**

Stephen P Mayfield, Andrea L Manuell, Stephen Chen, Joann Wu, Miller Tran, David Siefker, Machiko Muto and Julia Marin-Navarro

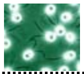
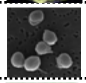
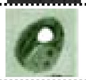
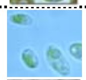
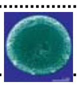
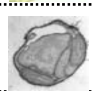
**Current Opinion in Biotechnology** 2007, **18**:1–6

**Table 1**

**Recombinant proteins produced in *C. reinhardtii* chloroplasts.**

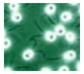
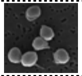
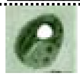
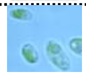
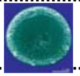
| Protein product | Application/comments | Reference |
|---|---|---|
| Aminoglycoside adenine transferase | Reporter, confers spectinomycin and streptomycin resistance | [8] |
| β-Glucuronidase | Reporter, catalyzes the conversion of substrates to colored products | [9] |
| Renilla luciferase | Reporter protein, luminescent | [10] |
| Aminoglycoside phosphotransferase | Reporter, confers kanamycin and amikacin resistance | [49] |
| Green fluorescent protein | Reporter protein, fluorescent | [13] |
| HSV8-lsc | Pharmaceutical, first mammalian protein expressed | [2**] |
| Cholera toxin B subunit fused to foot and mouth disease VP1 | Pharmaceutical, vaccine | [36**] |
| Bacterial luciferase | Real-time reporter protein, can be visualized in living cells | [14*] |
| HSV8-scFv | Pharmaceutical, classic single-chain antibody | [50] |
| Allophycocyanin | Fluorescent protein | [48**] |
| Human metallothionine-2 | Pharmaceutical, UV protection | [38] |
| Firefly luciferase | Real-time reporter protein, can be visualized in living cells | [51**] |
| Human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) | Pharmaceutical | [37] |

# Sequenced algal genomes

| Image | Genus | Species | Algal group |
|---|---|---|---|
|  | *Chlamydomonas* | *reinhardtii* | Green |
|  | *Volvox* | *carteri* | Green |
|  | *Micromonas* | *pusilla* | Green |
|  | *Ostreococcus* | *tauri* | Green |
|  | *Ostreococcus* | (RCC809) | Green |
|  | *Chlorella* | *vulgaris* | Green |
|  | *Emiliania* | *huxleyi* | Coccolith |
|  | *Phaeodactylum* | *tricornutum* | Diatom |
|  | *Fragilariopsis* | *cylindrus* | Diatom |
|  | *Thalassiosira* | *pseudonana* | Diatom |
|  | *Ectocarpus* | *siliculosus* | Brown |
|  | *Aureococcus* | *anophagefferens* | Pelagophyte |
|  | *Euglena* | *gracilis* | Euglenoid |
|  | *Cyanidioschyzon* | *merolae* | Red |

Heterokonts

# Sequenced algal genomes

| Image | Genus | Species | Algal group | Molecular tools |
|---|---|---|---|---|
|  | *Chlamydomonas* | *reinhardtii* | Green | Well developed |
|  | *Volvox* | *carteri* | Green | Some |
|  | *Micromonas* | *pusilla* | Green | |
|  | *Ostreococcus* | *tauri* | Green | |
|  | *Ostreococcus* | (RCC809) | Green | |
|  | *Chlorella* | *vulgaris* | Green | Transformation reported |
|  | *Emiliania* | *huxleyi* | Coccolith | Efforts underway |
|  | *Phaeodactylum* | *tricornutum* | Diatom | Some |
|  | *Fragilariopsis* | *cylindrus* | Diatom | |
|  | *Thalassiosira* | *pseudonana* | Diatom | Transformation reported |
|  | *Ectocarpus* | *siliculosus* | Brown | |
|  | *Aureococcus* | *anophagefferens* | Pelagophyte | |
|  | *Euglena* | *gracilis* | Euglenoid | |
|  | *Cyanidioschyzon* | *merolae* | Red | Transformation reported |

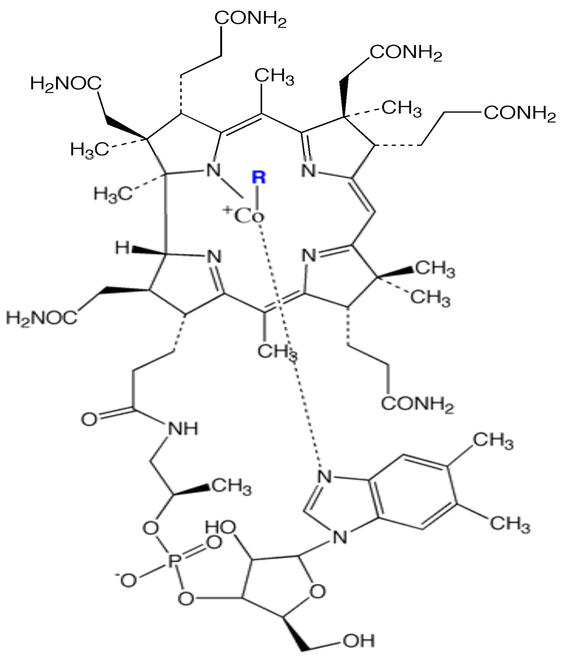
# Development of molecular tools

- Metabolic engineering offers great potential for novel/improved products

- Needs ability to transform algae

- Tools for regulation of gene expression

# Vitamin $B_{12}$ (cobalamin) and algae



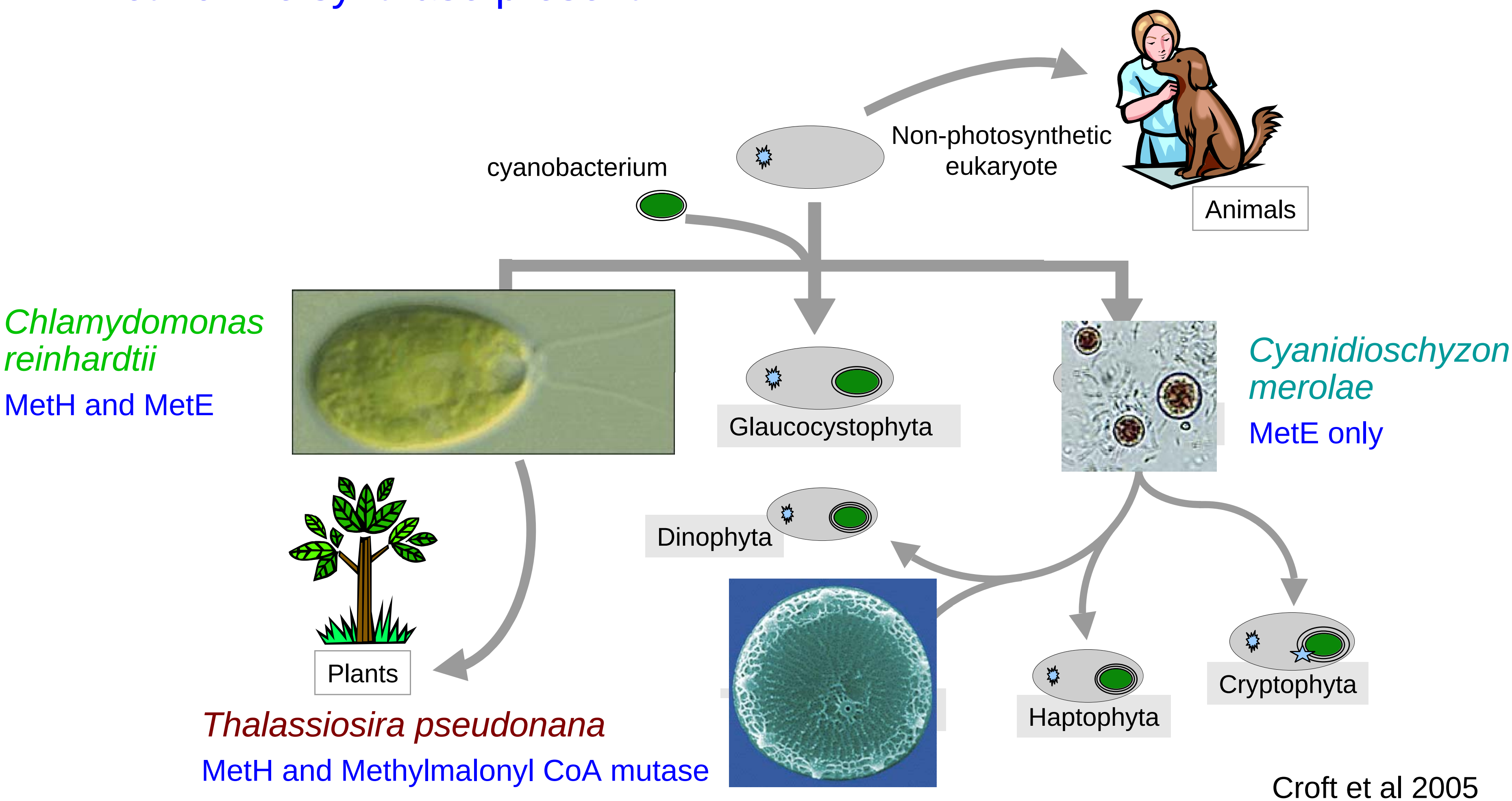

- Over half of all microalgal species require cobalamin for growth

  obtain it from bacteria – possibly via symbiosis

- Some species contain very high amounts

# Explanation from genome sequences

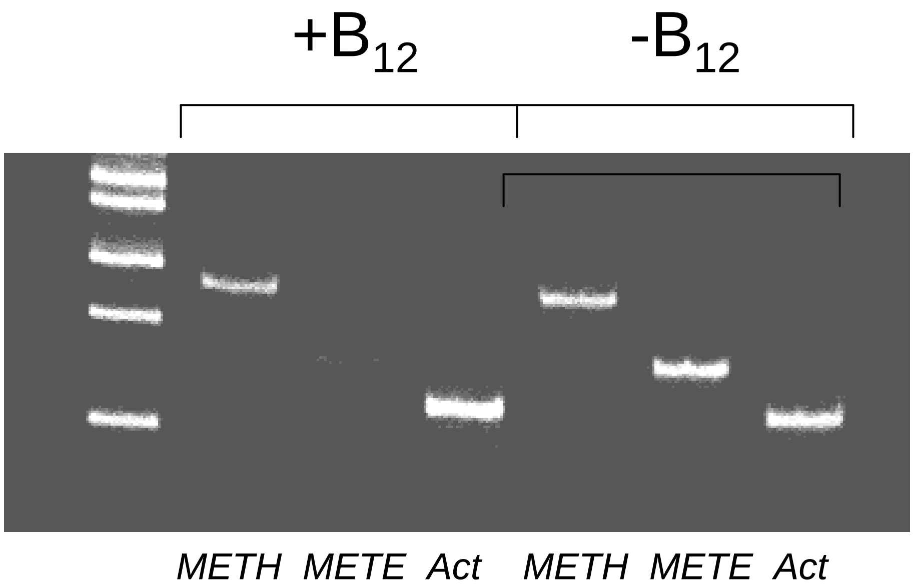
- $B_{12}$ requirement related to type of methionine synthase present

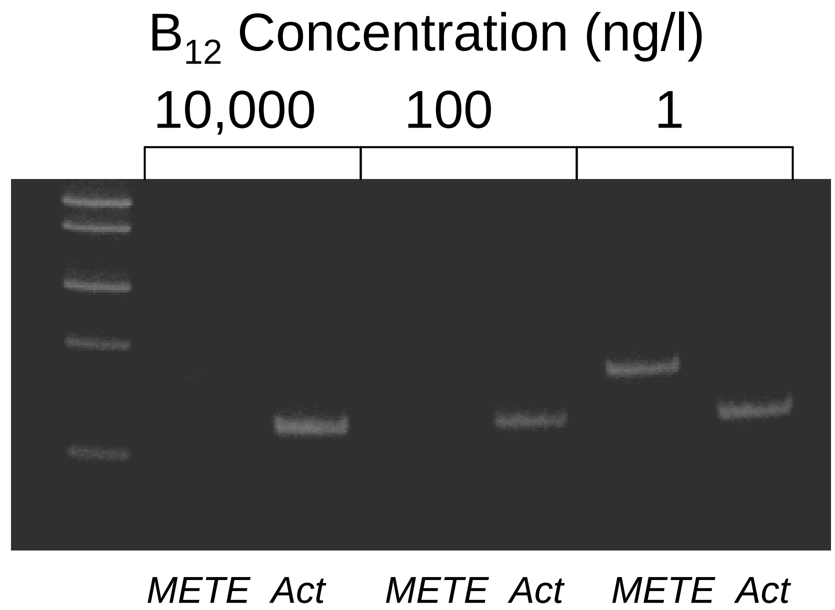



Croft et al 2005

# $B_{12}$ affects MetE gene expression

- *C. reinhardtii* has both methionine synthase genes
  - *METH* is expressed constitutively
  - *METE* is expressed in absence of vitamin $B_{12}$ only

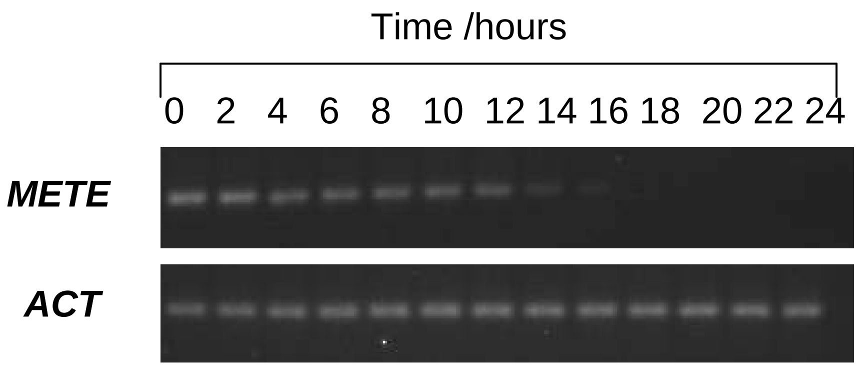


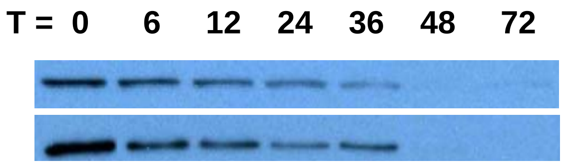



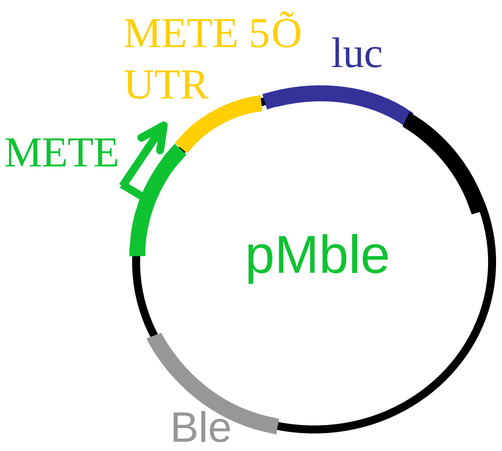

*Act = actin*

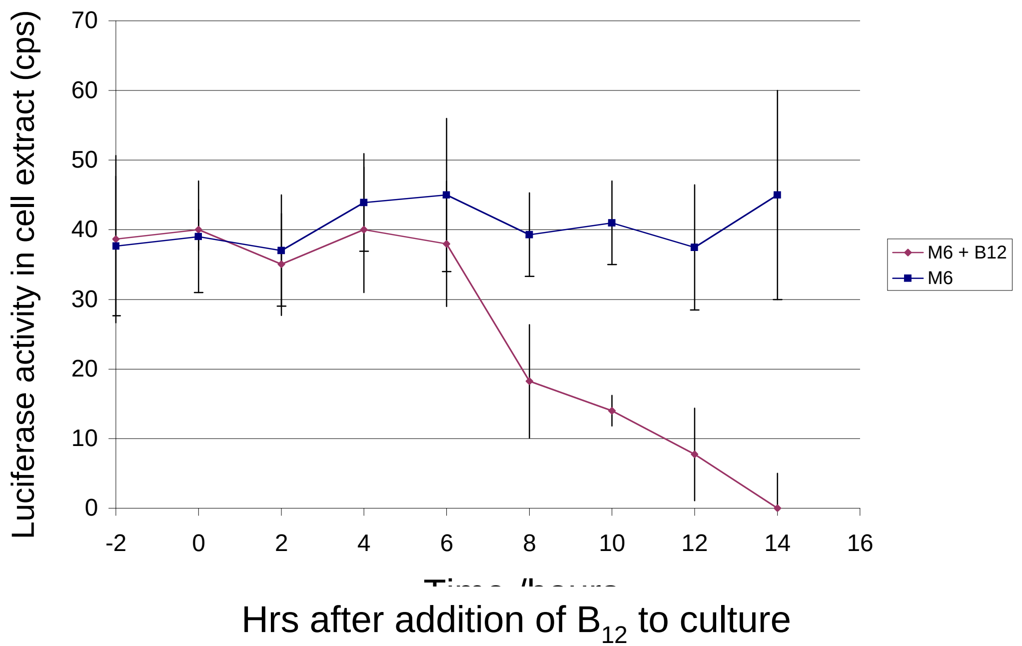
Croft et al 2005

# Vitamin responsive gene expression



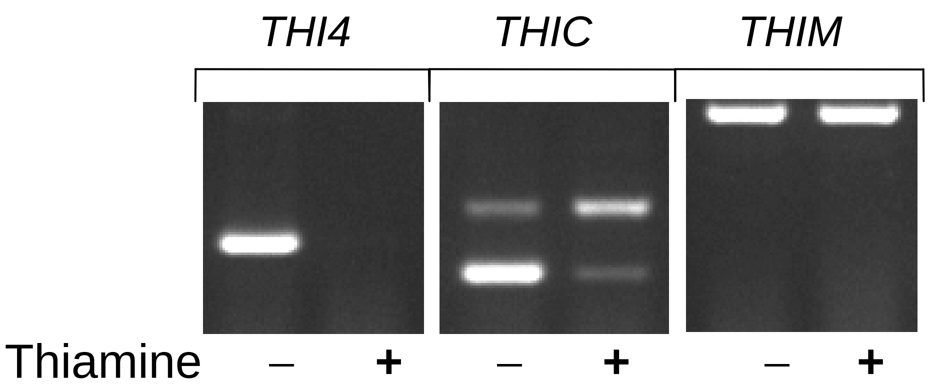

*Katherine Helliwell & Martin Croft*

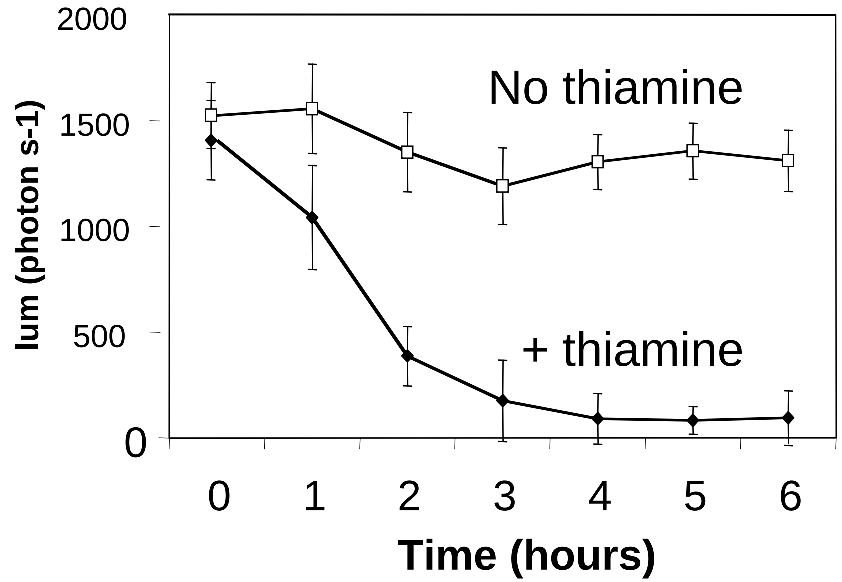
# Biorefining may provide economic drivers

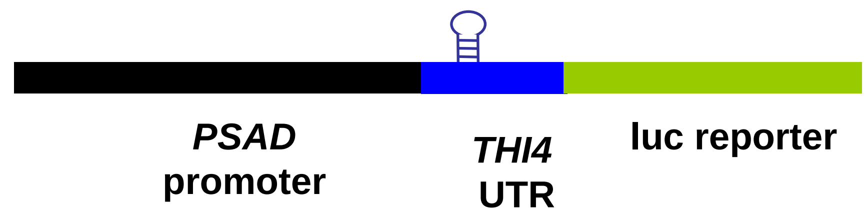
- High value products in addition to lipids


- Cells walls - 30% of biomass
  - Source of nutrients
  - Feedstock for biogas production
  - Fermentation to ethanol
- Major barrier to cell disruption

# TPP biosynthesis genes regulated by thiamine



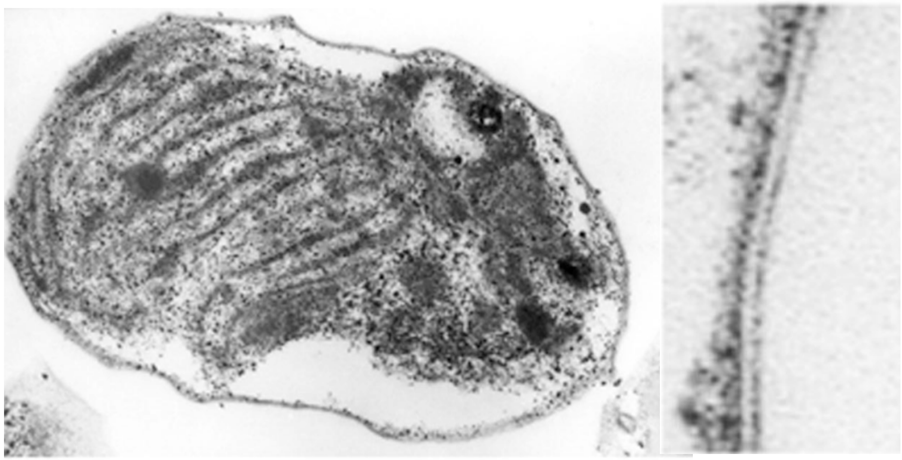

- Regulation is via TPP riboswitches

*Croft et al (2007) Proc Natl Acad Sci USA **104**: 20770-20775*

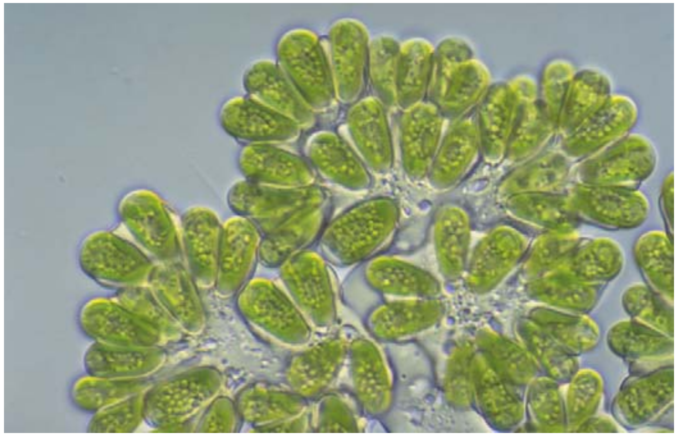
# Algal cell walls – green algae

- Chlamydomonas reinhardtii
  - -proteinaceous, wall-less mutants known

- Chlorella spp.
  - cellulose based (so like higher plants)
  - Some species reported to have alkanes (C17) in cell wall
  - Others have complex trilaminar structure (TLS) containing long chain alkanes



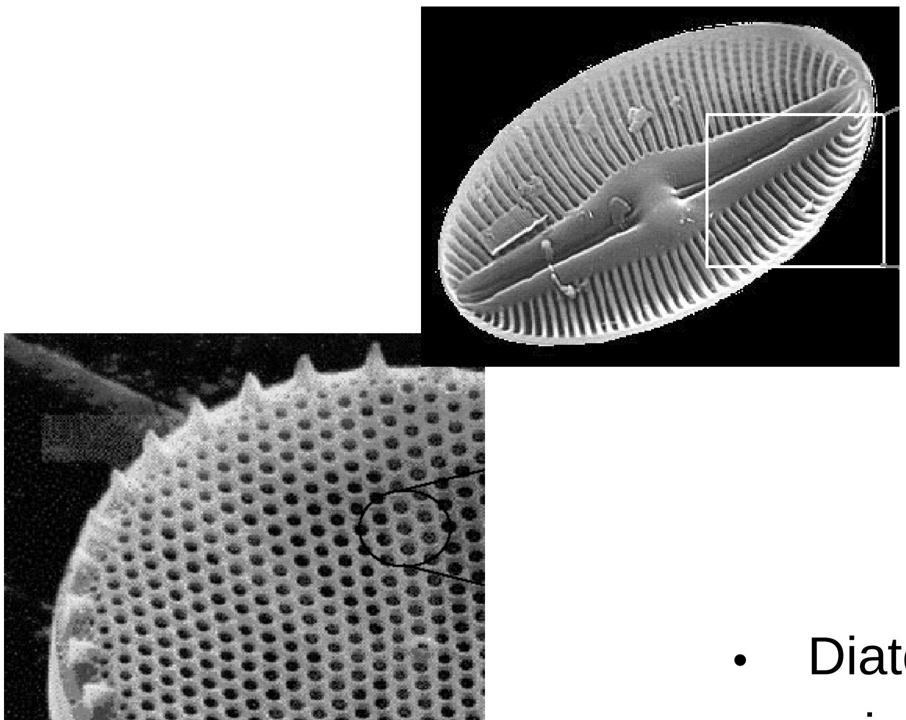
TLS in *Chlorella minutissima marina*

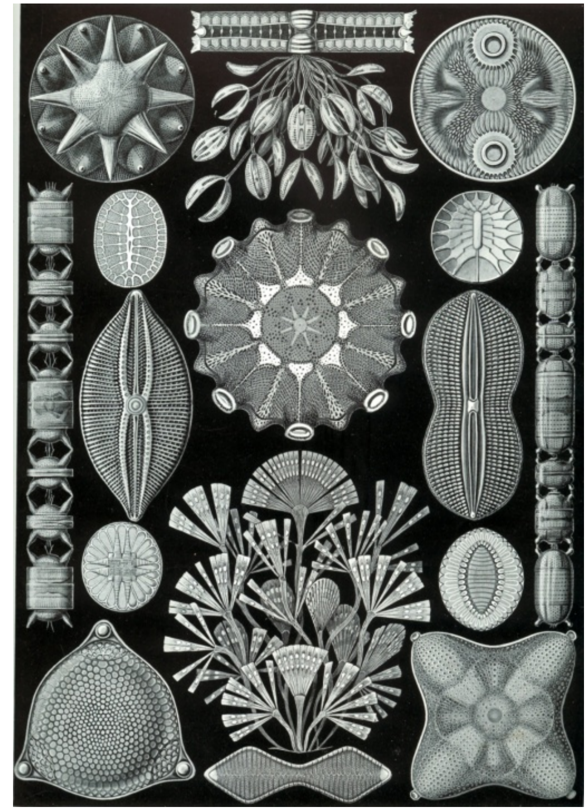


*Botryococcus braunii*

# Algal cell walls - diatoms

- Characteristic silica-based cell wall (‘frustule’)
  - Physical nature of silica wall offers challenges and opportunities

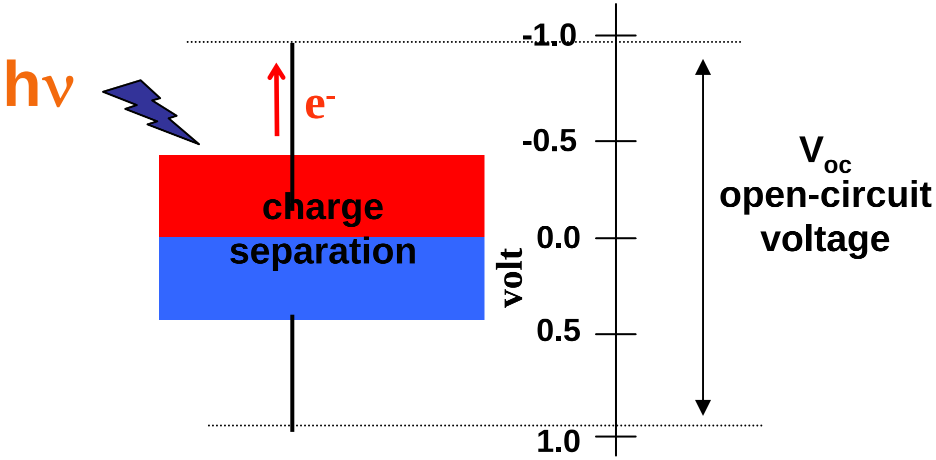




- Diatoms might ‘save’ on carbon by using silica rather than cellulose for their cell wall

# Stealing electrons from photosynthesis

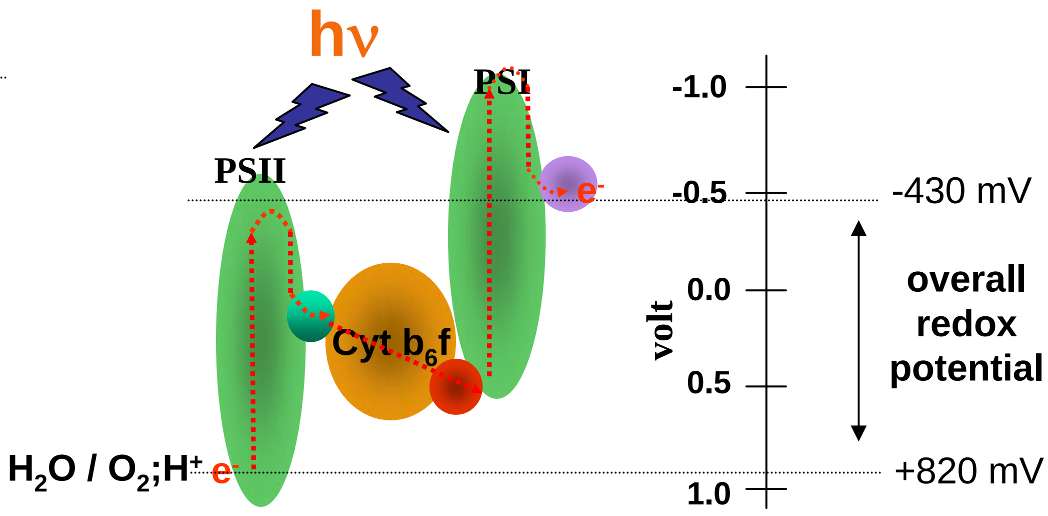
## Photovoltaic device




1839 Edmund Bequerel
Photons hit the photo active part of the solar panel and are absorbed. The excited semiconducting materials relax, generating charge separation

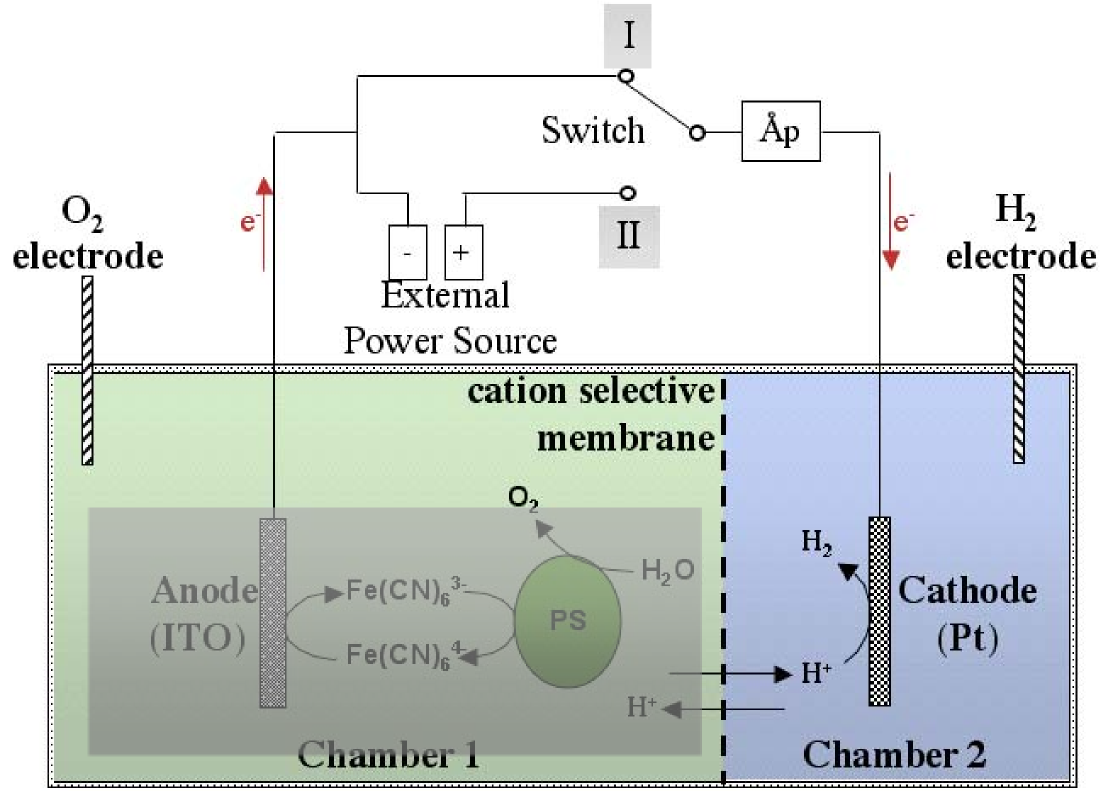
## "Biophotovoltaics"

*(photosynthesis)*



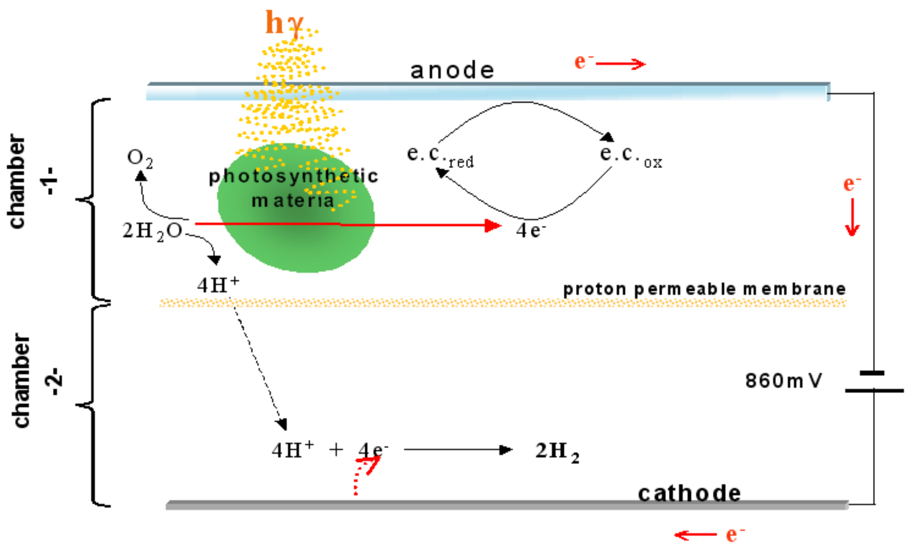

Photons excite photosystems, which relax & generate charge separation

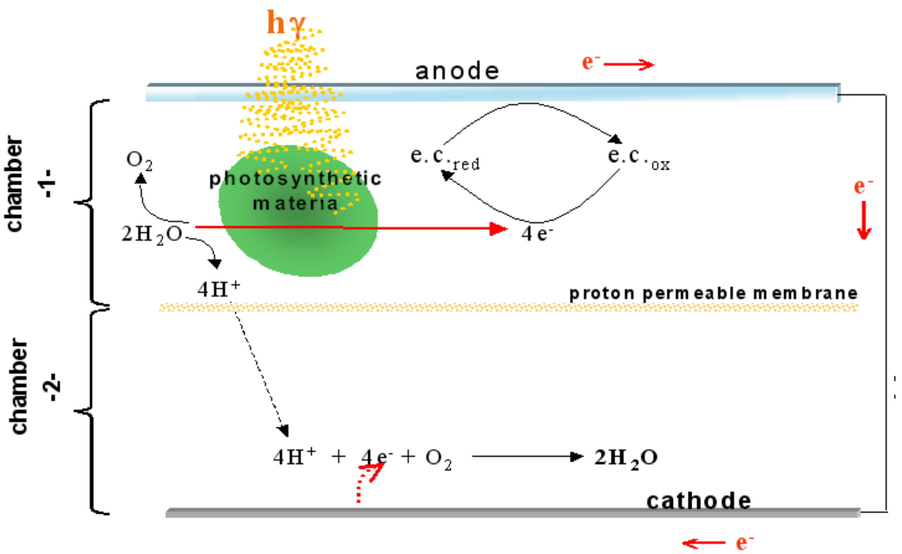
# Biophotovoltaics - light driven charge separation

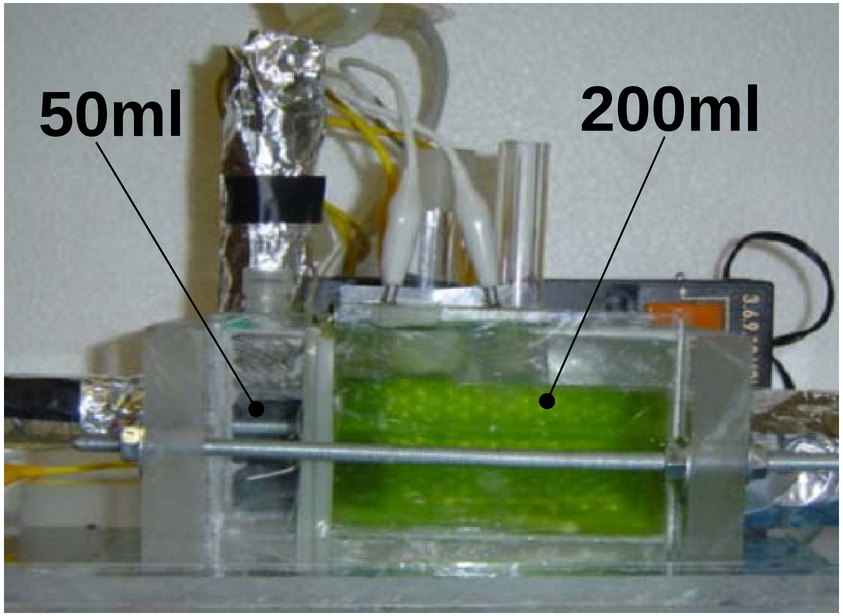


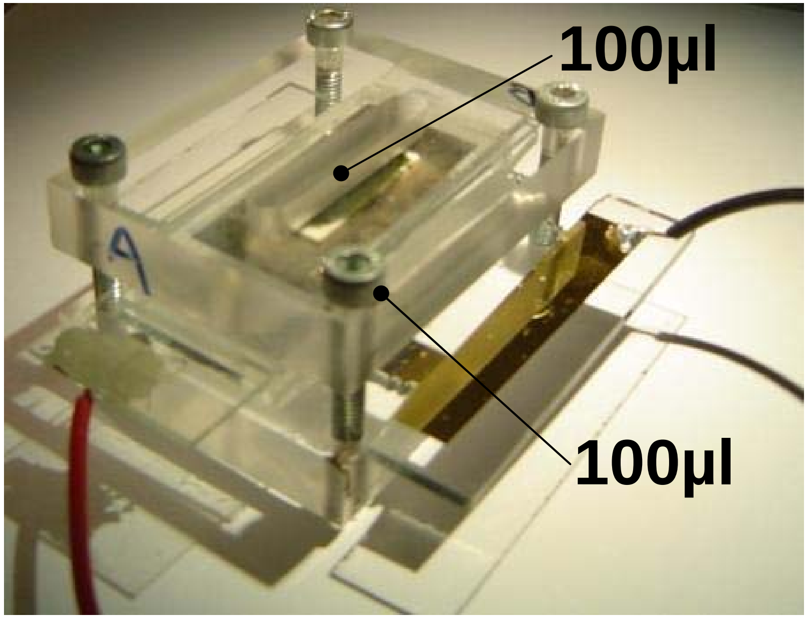

- ✓ **Light provides the energy for photosystem II to extract electrons from water**
- ✓ **Electrons flow through the photosynthetic electron transport chain**
- ✓ **These electrons can be removed from the photosynthetic chain and ‘donated’ to a synthetic circuit**

# Biohydrogen or bio-current



# Both produced in light