Issues in Photobiological Hydrogen Production

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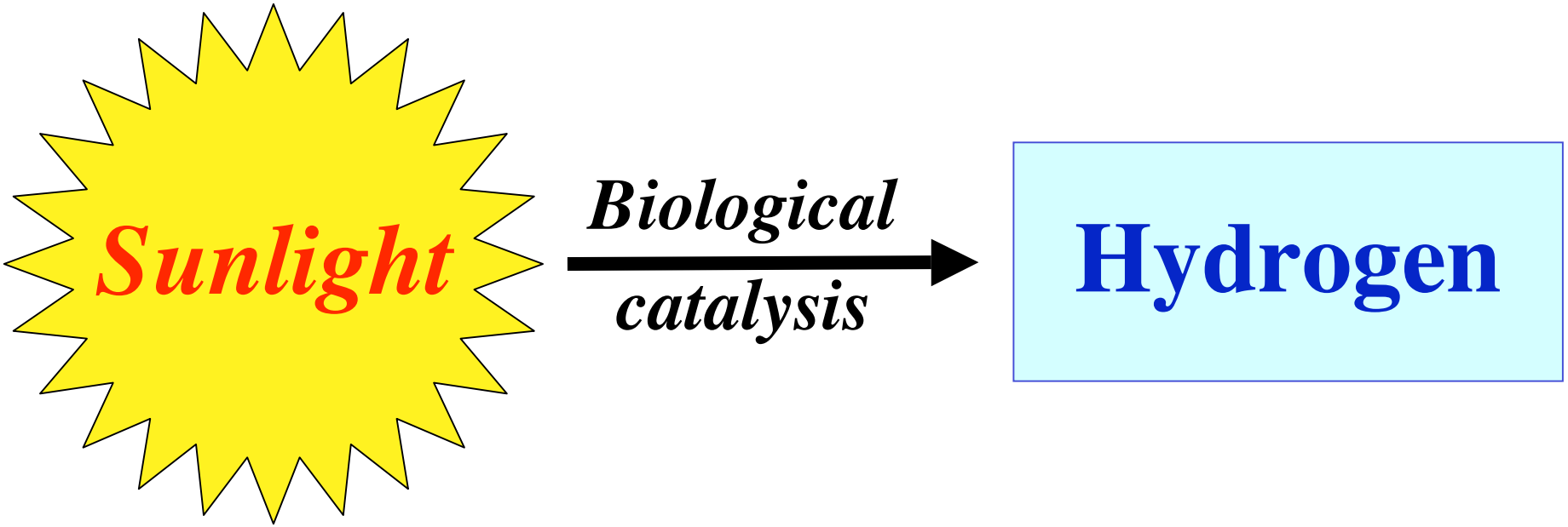
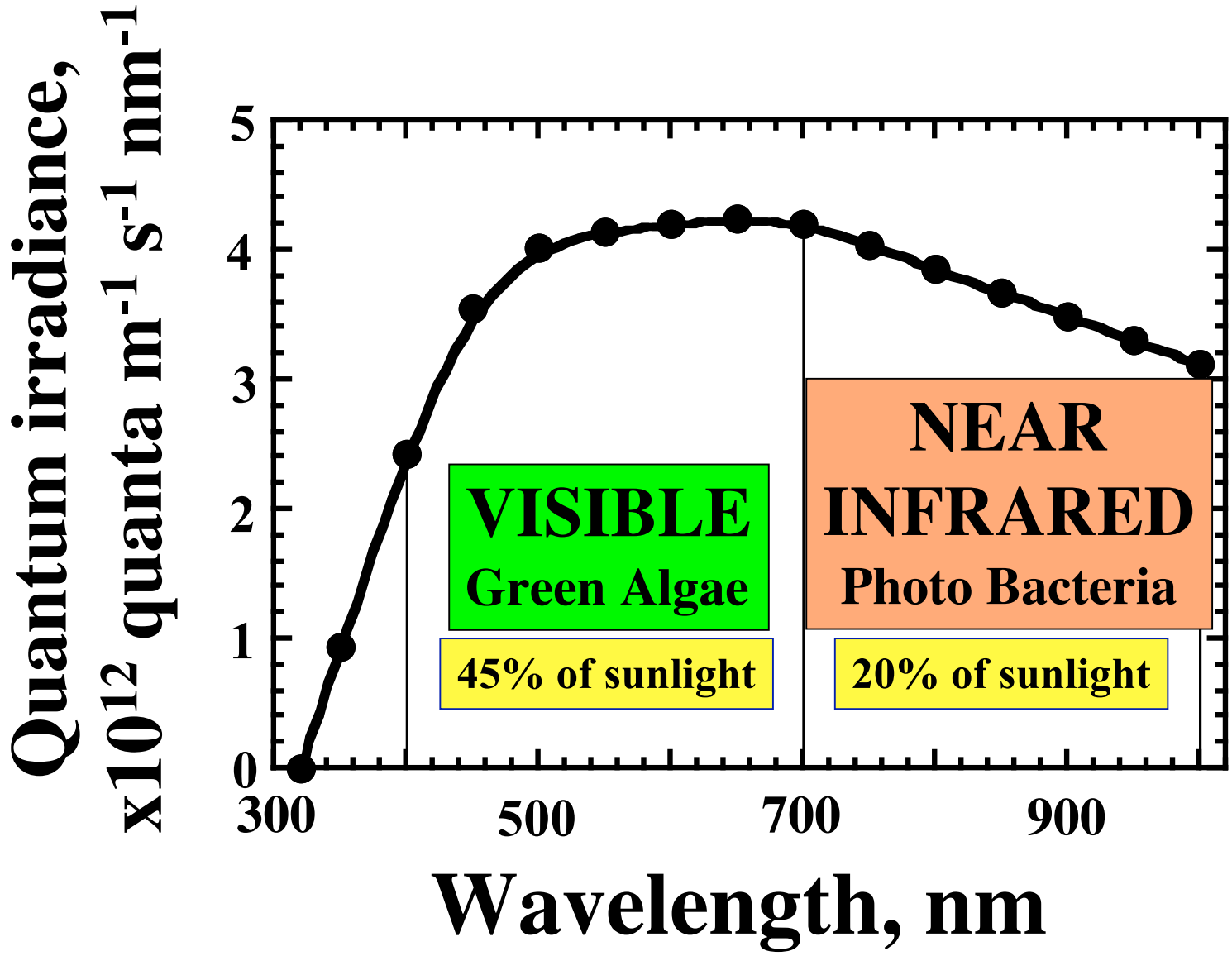

Sunlight
Biological
catalysis
Hydrogen

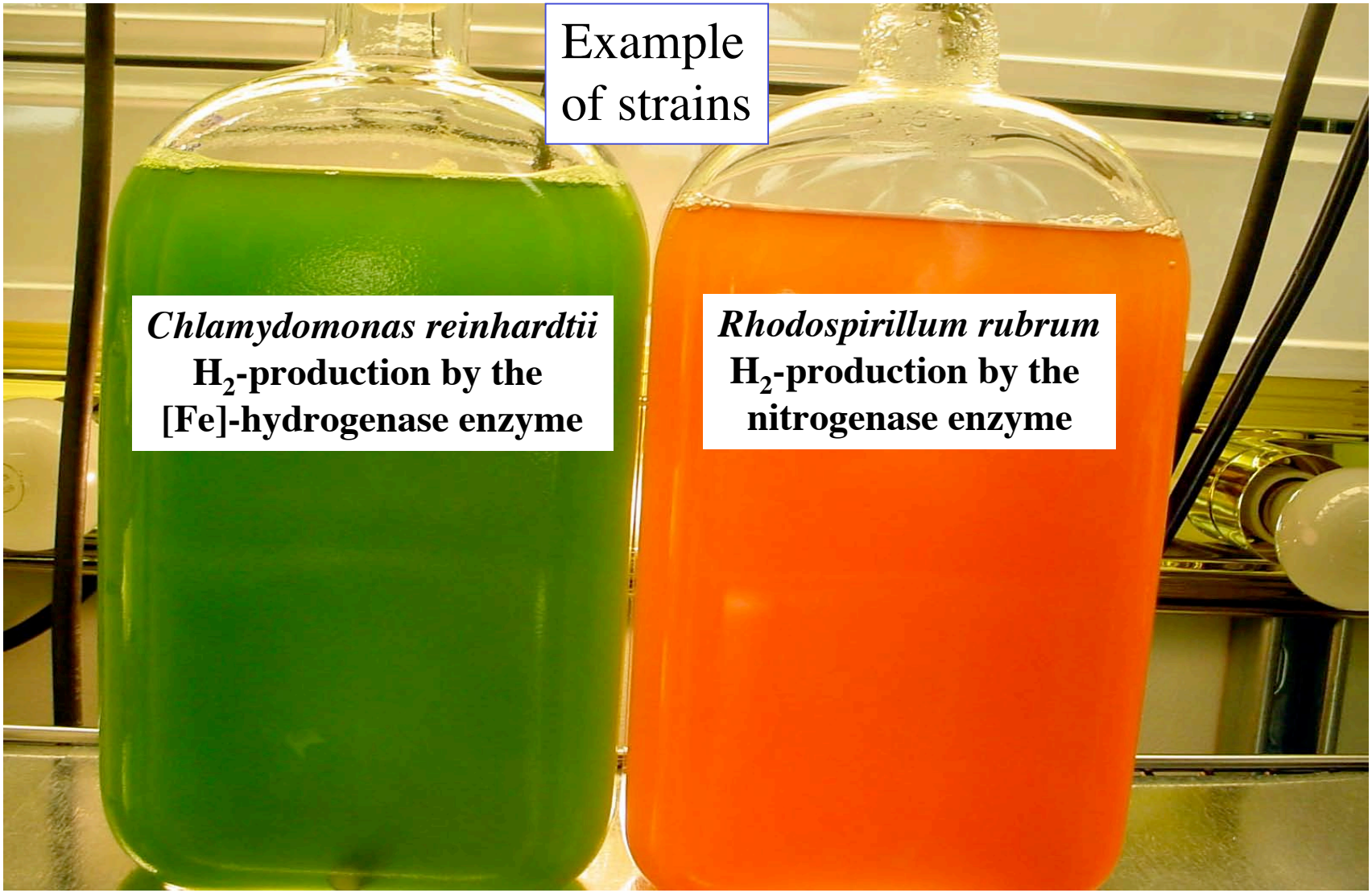
Sunlight

- **Clean**
- **Unlimited**
- **Diffuse**

(5 kWh m^{-2} d^{-1})

(US household consumption: 12.5 kWh d^{-1})


Quantum irradiance,
x10^12 quanta m^-1 s^-1 nm^-1
5
4
3
2
1
0
300
500
700
900
Wavelength, nm
VISIBLE
Green Algae
45% of sunlight
NEAR
INFRARED
Photo Bacteria
20% of sunlight



Green algae and photosynthetic bacteria could operate with a solar to H_2 conversion efficiency as high as ~14% and ~8%, respectively, provided that specific issues can be overcome.

Hydrogen production reactions

Green algal *Fe-hydrogenase*

$$2H^+ + 2FD^- \rightarrow H_2 + 2FD$$

Photosynthetic bacterial *nitrogenase*

$$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow$$

$$2NH_3 + H_2 + 16ADP + 16Pi$$

Issues in photobiological H_2-production
(green algae and photosynthetic bacteria)

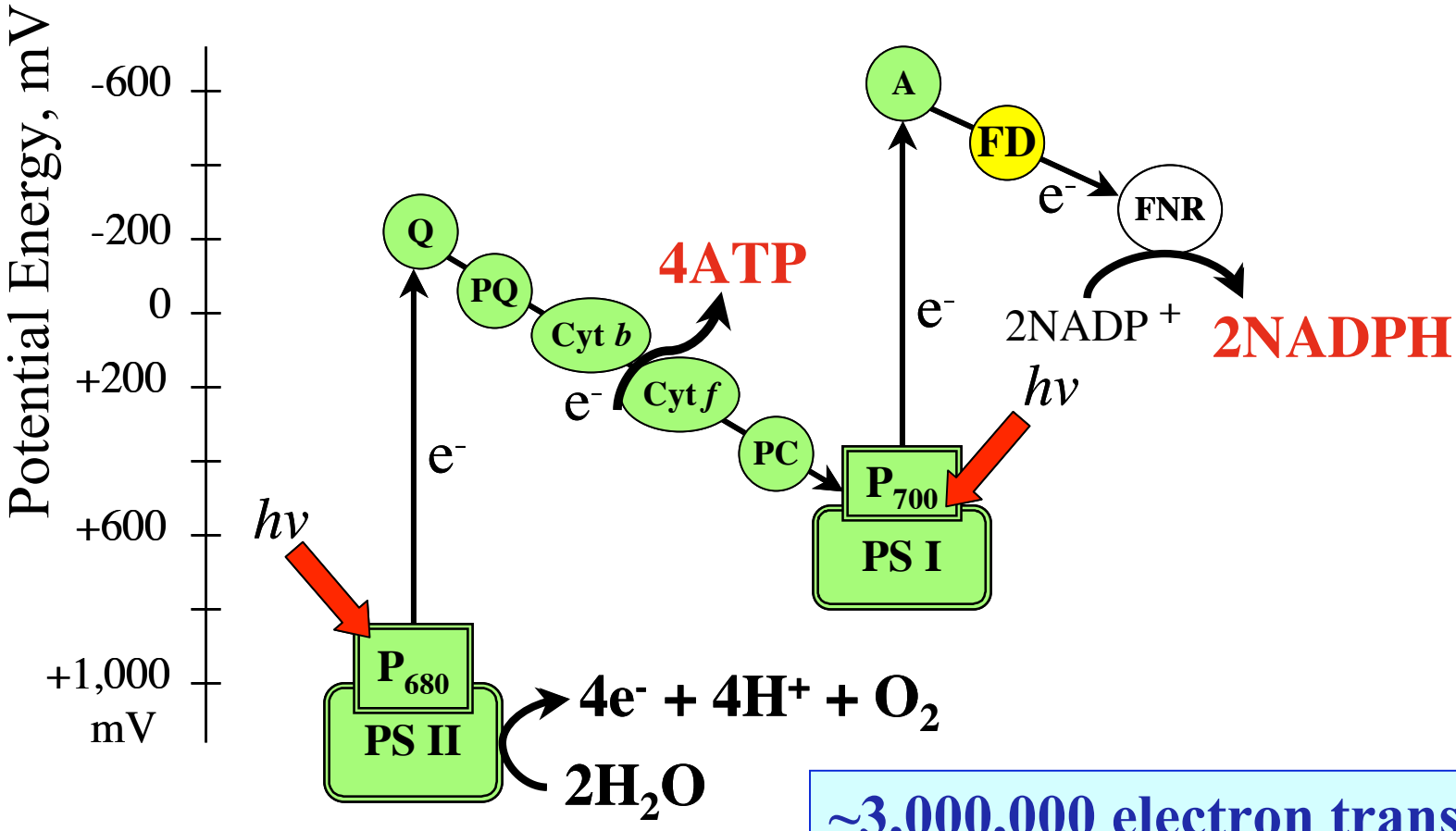
- **Photon conversion efficiency in green microalgae and photosynthetic bacteria is low under bright sunlight.**
- **Oxygen sensitivity of the green algal H_2-production process. O_2 and H_2 are mutually exclusive.**
- **Utilization of both visible and near infrared in photo-biological H_2-production (process integration).**

Microalgae: factories of photosynthesis

- **Fast growth (doubling of biomass per day).**
- **Source of lipids, protein, vitamins and more.**
- **Non-toxic, non-polluting.**
- **Seen by many as the ultimate approach to environmentally friendly energy generation.**

The Unicellular Green Alga *Chlamydomonas reinhardtii*

Photosynthetic H_2O oxidation and ATP-NADPH generation



~3,000,000 electron transport circuits per cell, each capable of transporting 100 electrons per second

Issues in photobiological H_2-production

- **Photon conversion efficiency in green microalgae and photobacteria.**
- **The O_2 sensitivity of green algal H_2-production.**
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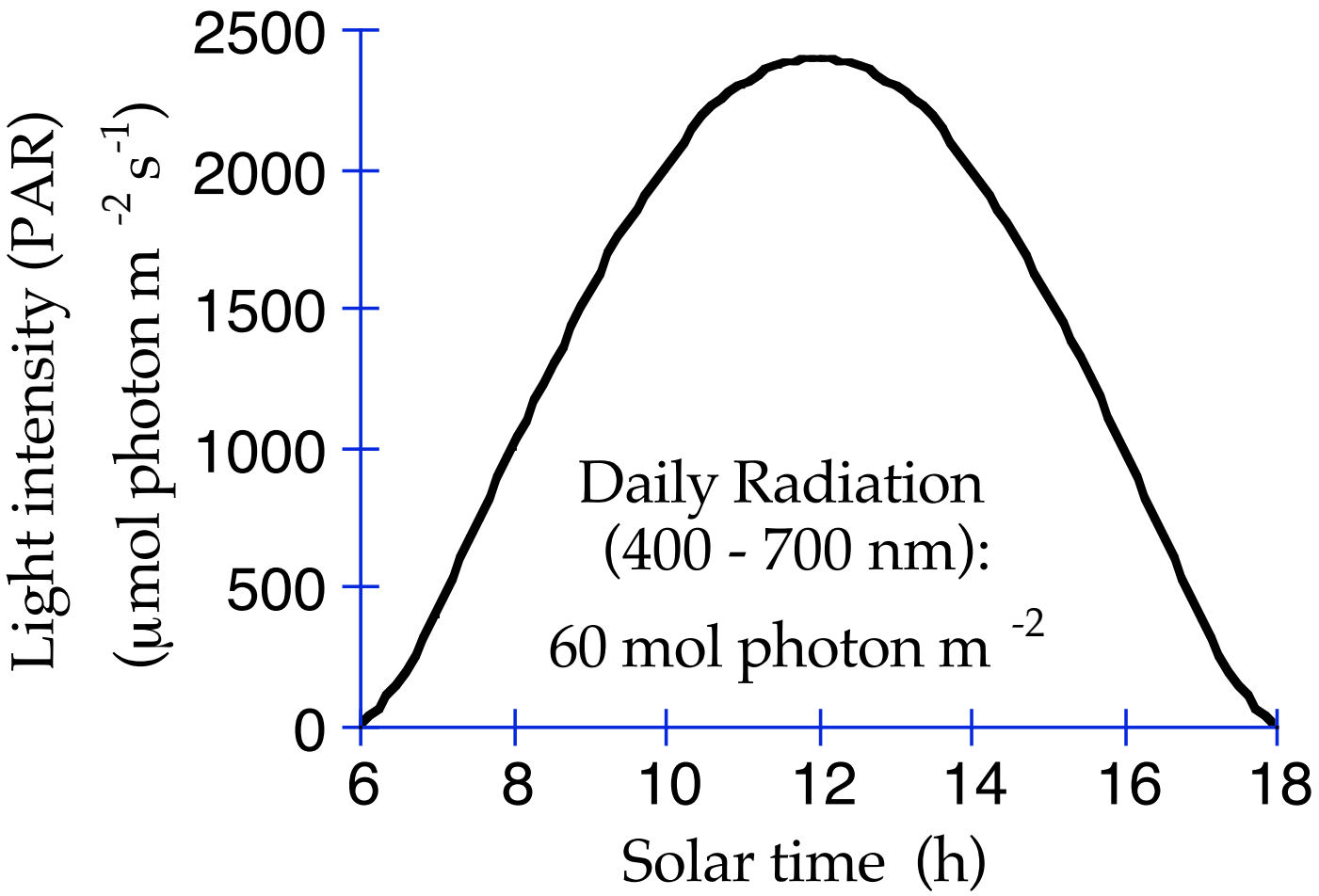
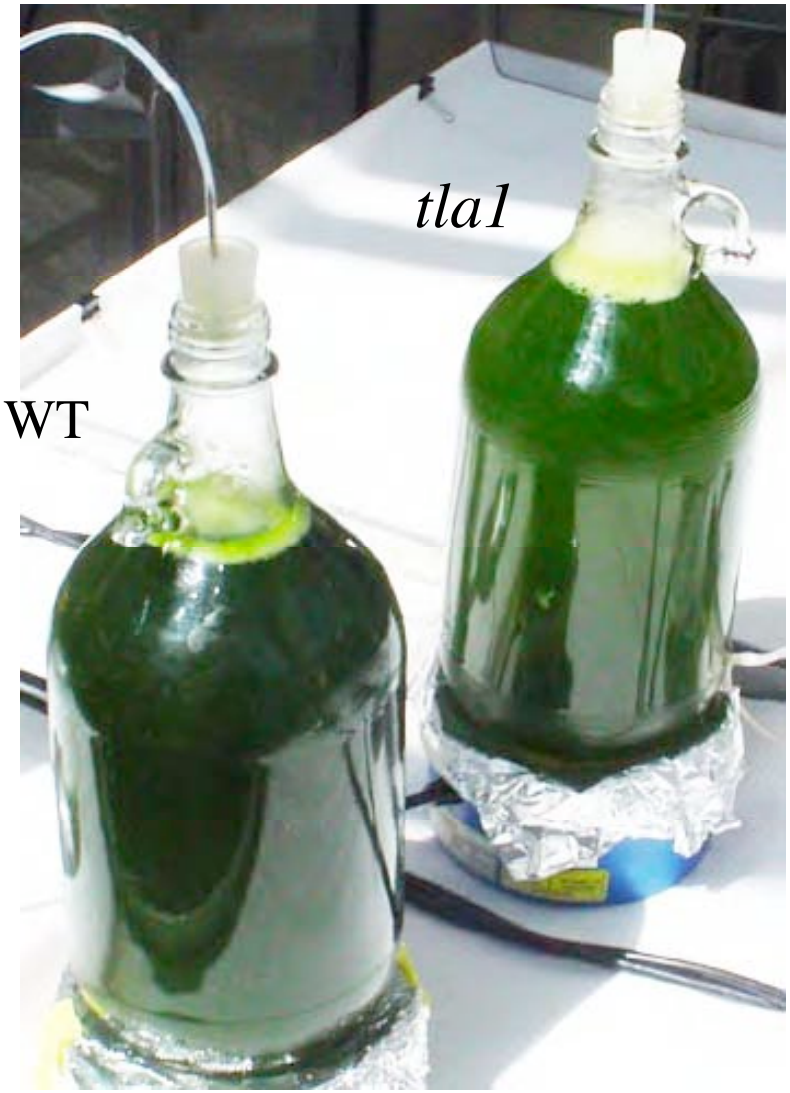
Profile of daily solar PAR



A pilot mini scale-up experiment

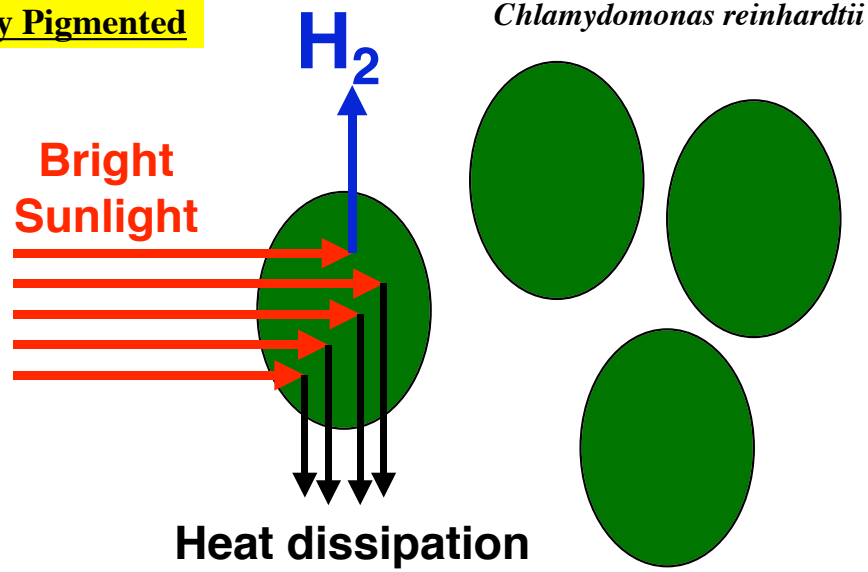
Cultures in the Greenhouse



Parameter	WT	*tla1*
Cell/mL ($x10^6$)	6.36	10.0
[Chl] (uM)	25.6	15.4

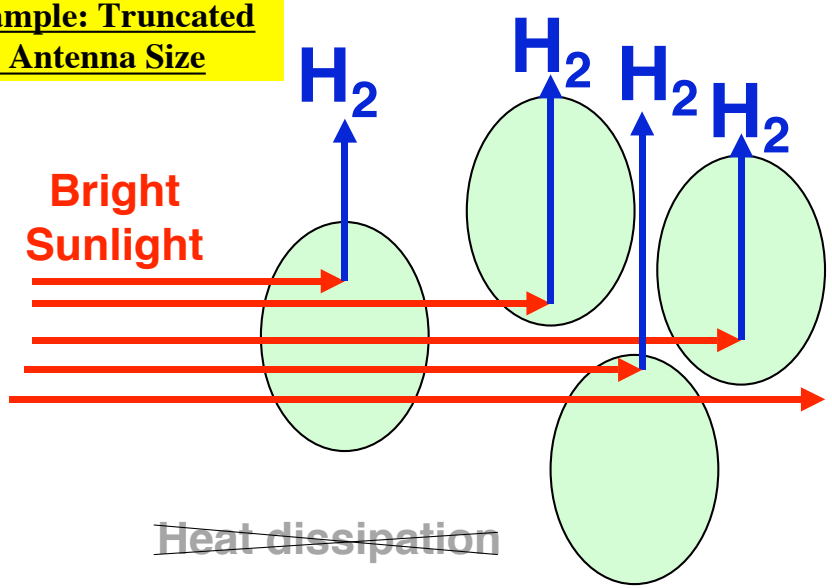
Example:
Fully Pigmented

The green algae
Chlamydomonas reinhardtii



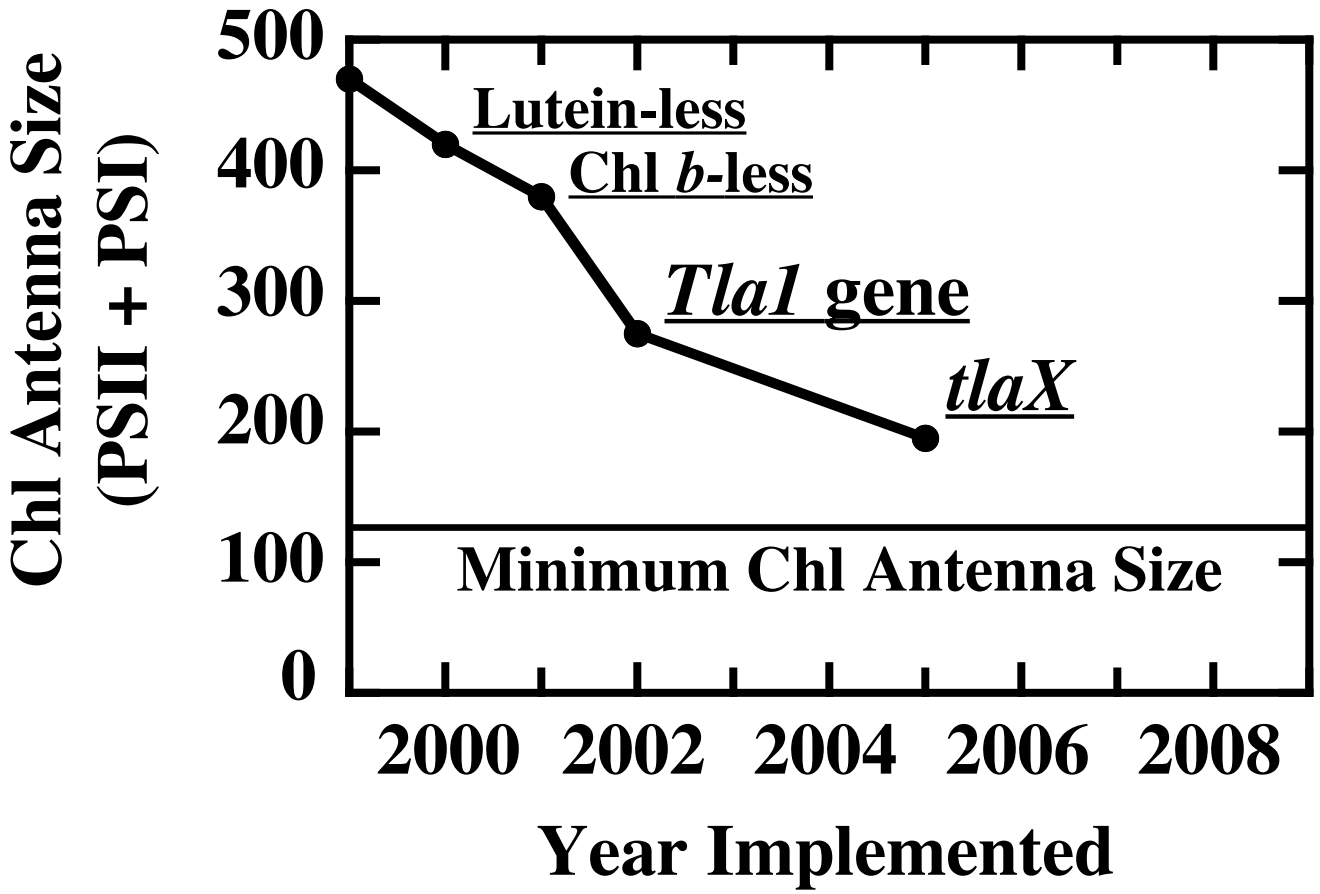
Fully pigmented cells over-absorb and wastefully dissipate bright sunlight.

Example: Truncated
Chl Antenna Size



Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.

Chlorophyll antenna size in *Chlamydomonas reinhardtii*



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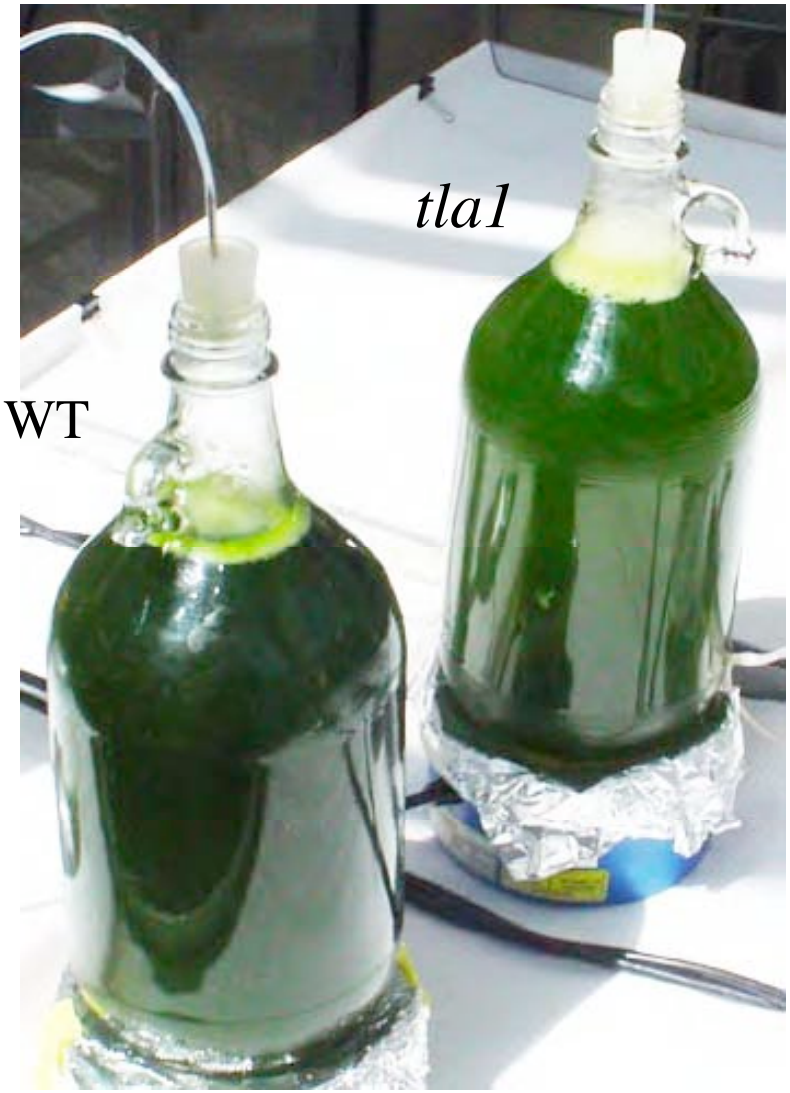

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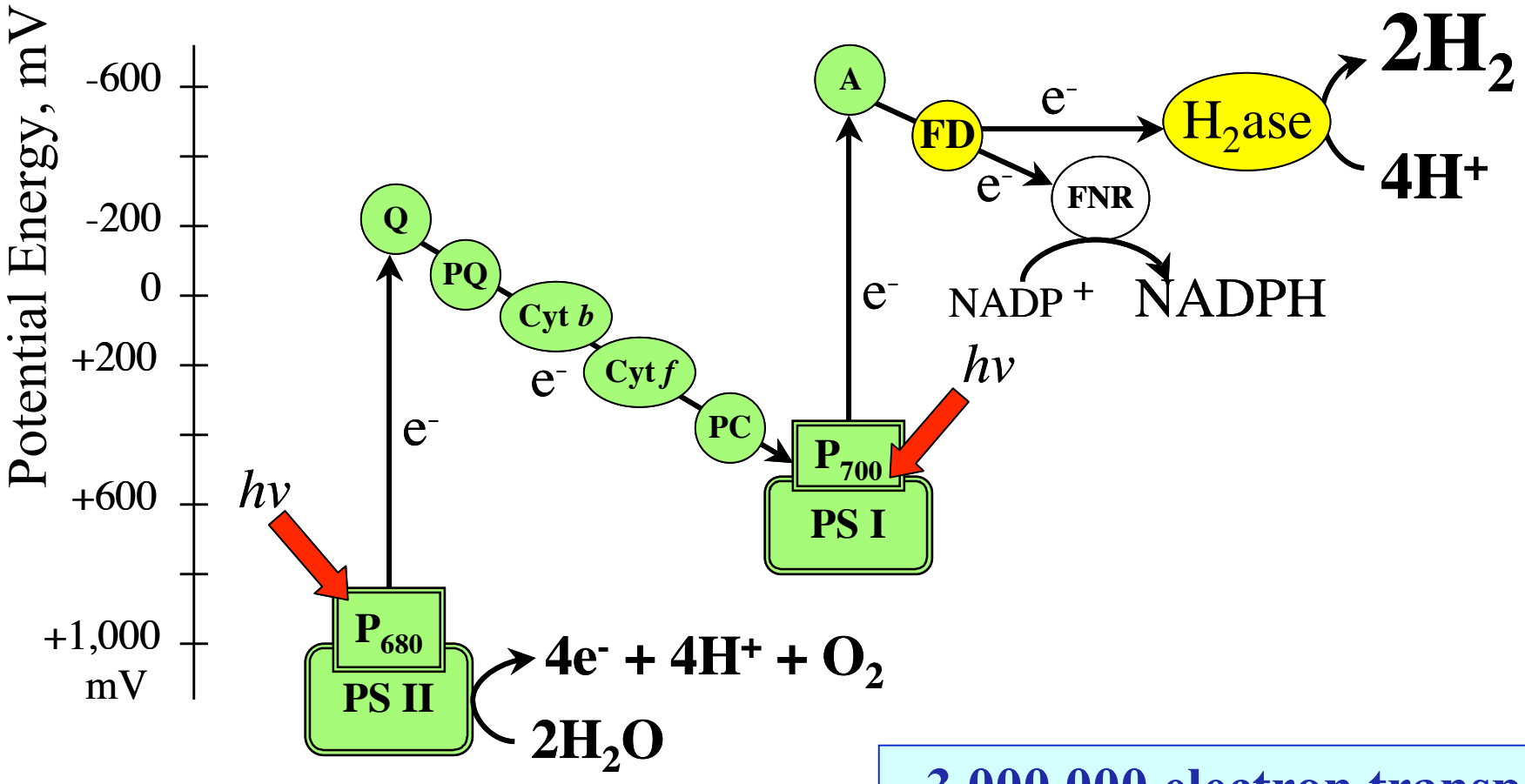
- **The O_2 sensitivity of green algal H_2-production.**
- Utilization of both visible and near infrared in photobiological H_2-production.

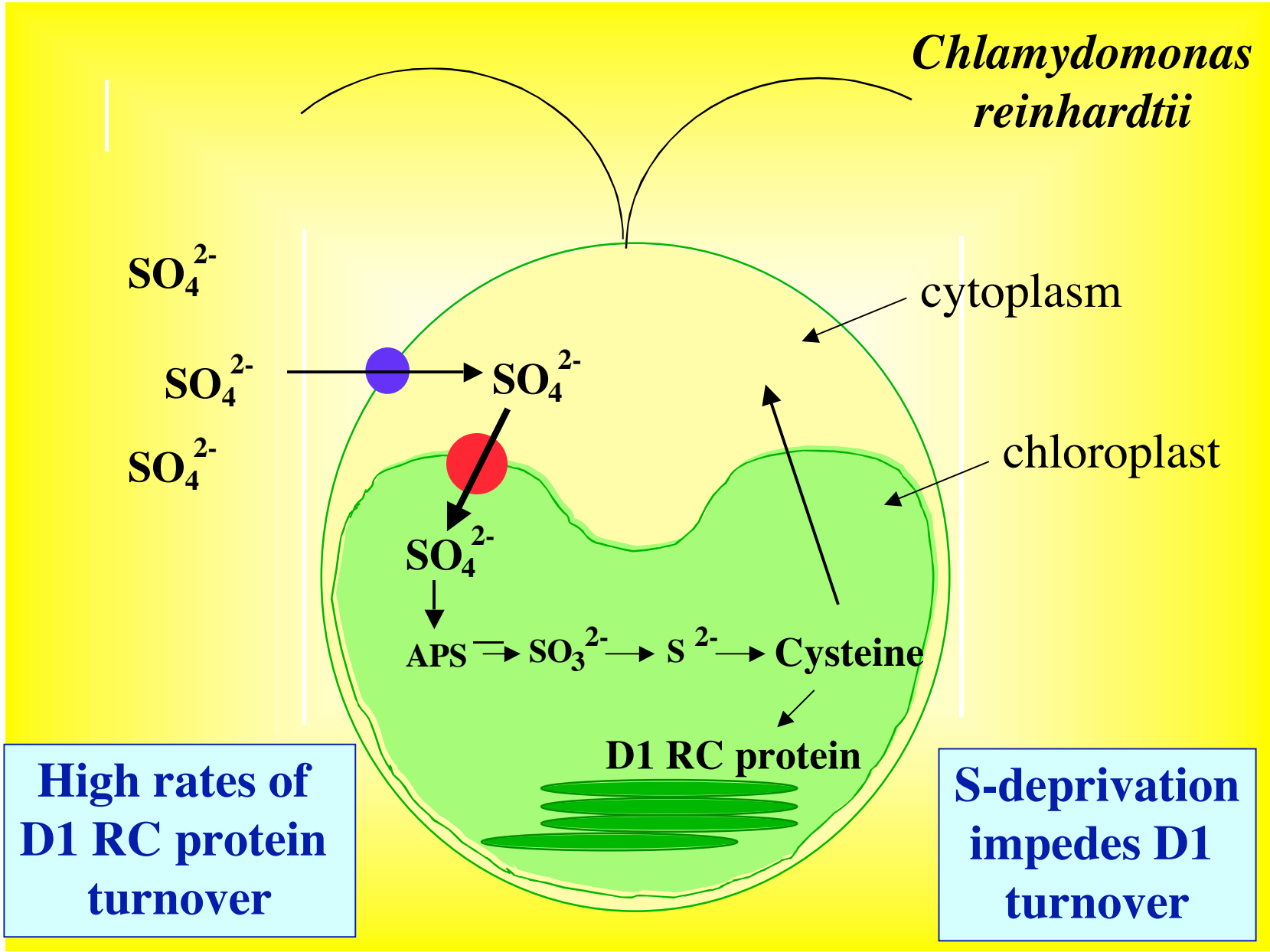
Photosynthetic nanocircuit for H_2O-oxidation and H_2-production



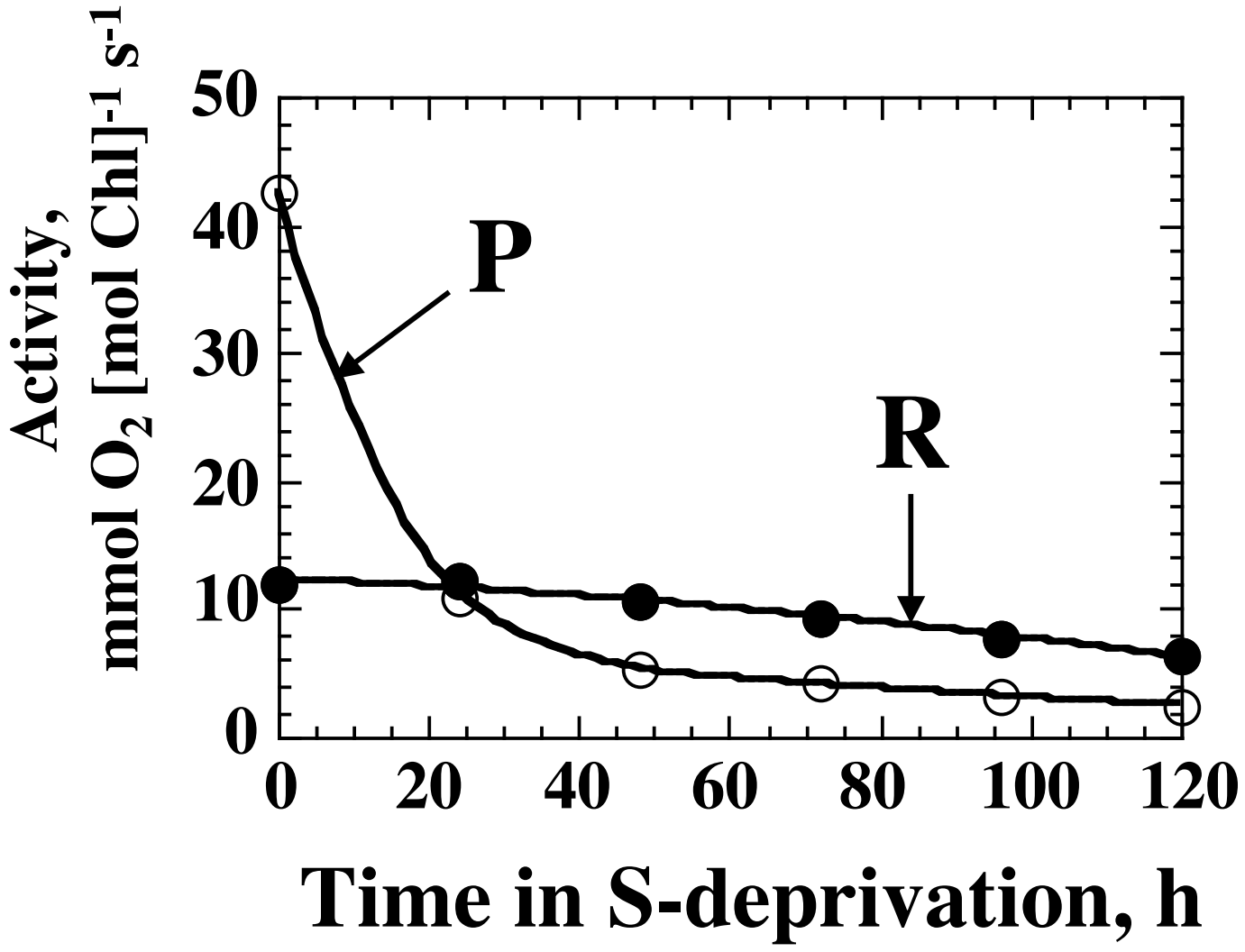
~3,000,000 electron transport circuits per cell, each capable of transporting 100 electrons per second

The adverse effect of oxygen on H_2-metabolism

- **Oxygen is a positive suppressor of *hydrogenase* gene expression and a powerful inhibitor of the enzyme.**
- **This incompatibility in the simultaneous O_2 and H_2 photo-evolution persisted in 60-years of green algal hydrogen research.**

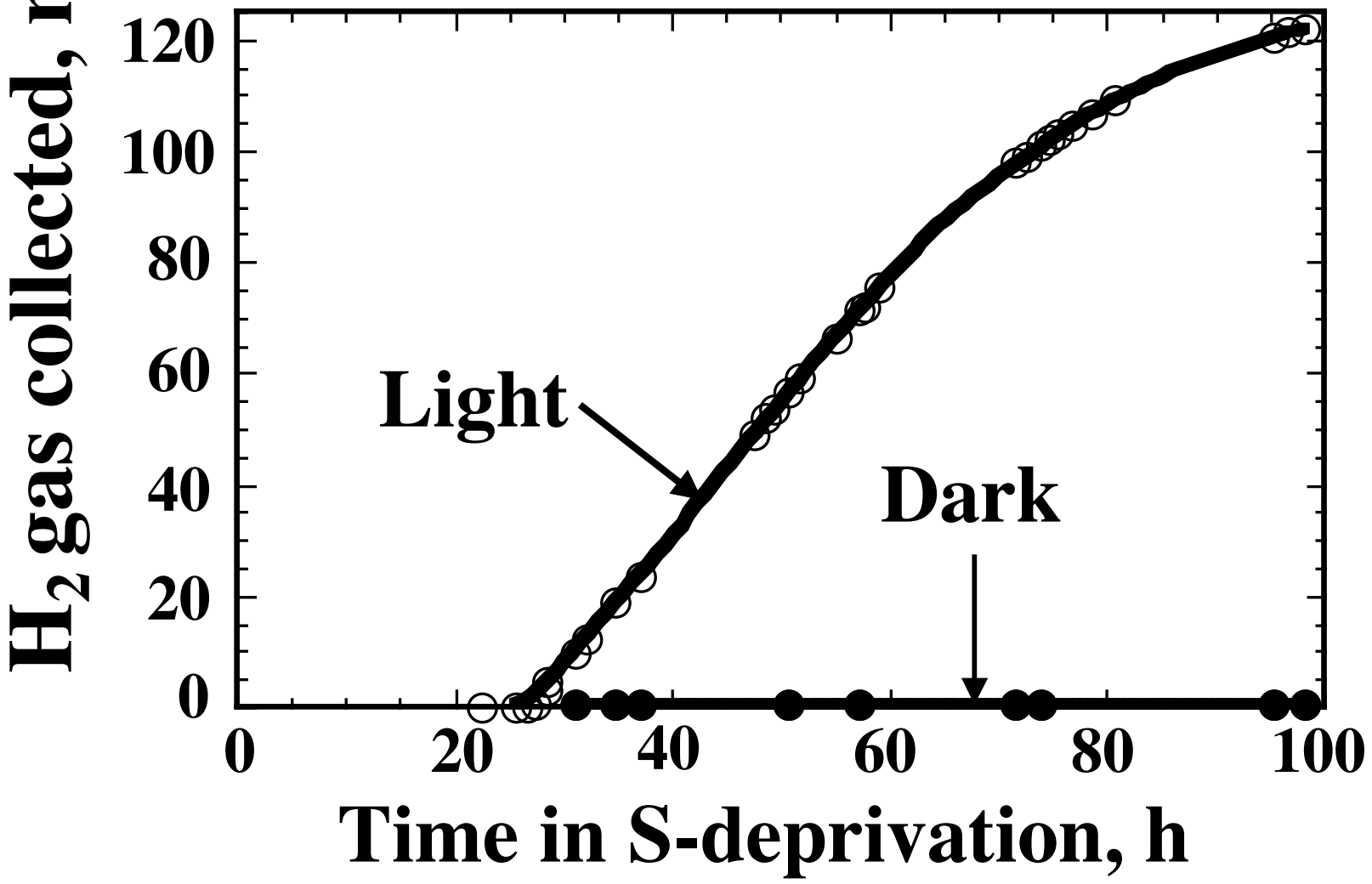

Chlamydomonas reinhardtii
SO_4^{2-}
SO_4^{2-}
SO_4^{2-}
SO_4^{2-}
cytoplasm
chloroplast
SO_4^{2-}
APS → SO_3^{2-} → S^{2-} → Cysteine
D1 RC protein
High rates of D1 RC protein turnover
S-deprivation impedes D1 turnover

The slow-down is specific to H_2O-oxidation and O_2-evolution. Respiration is not affected.

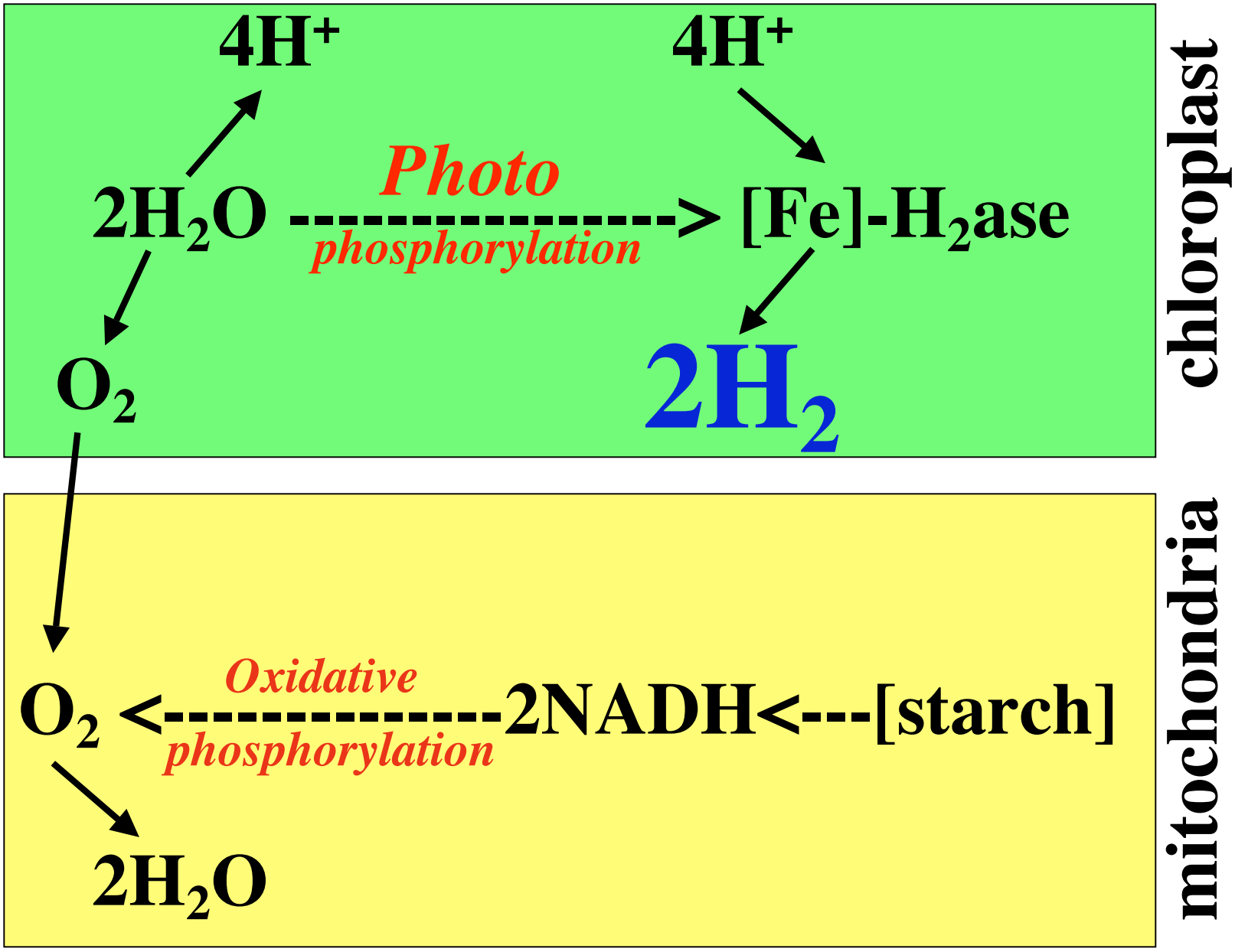



H_2 bubbles

H_2 gas accumulation commences promptly upon anaerobiosis in the culture.

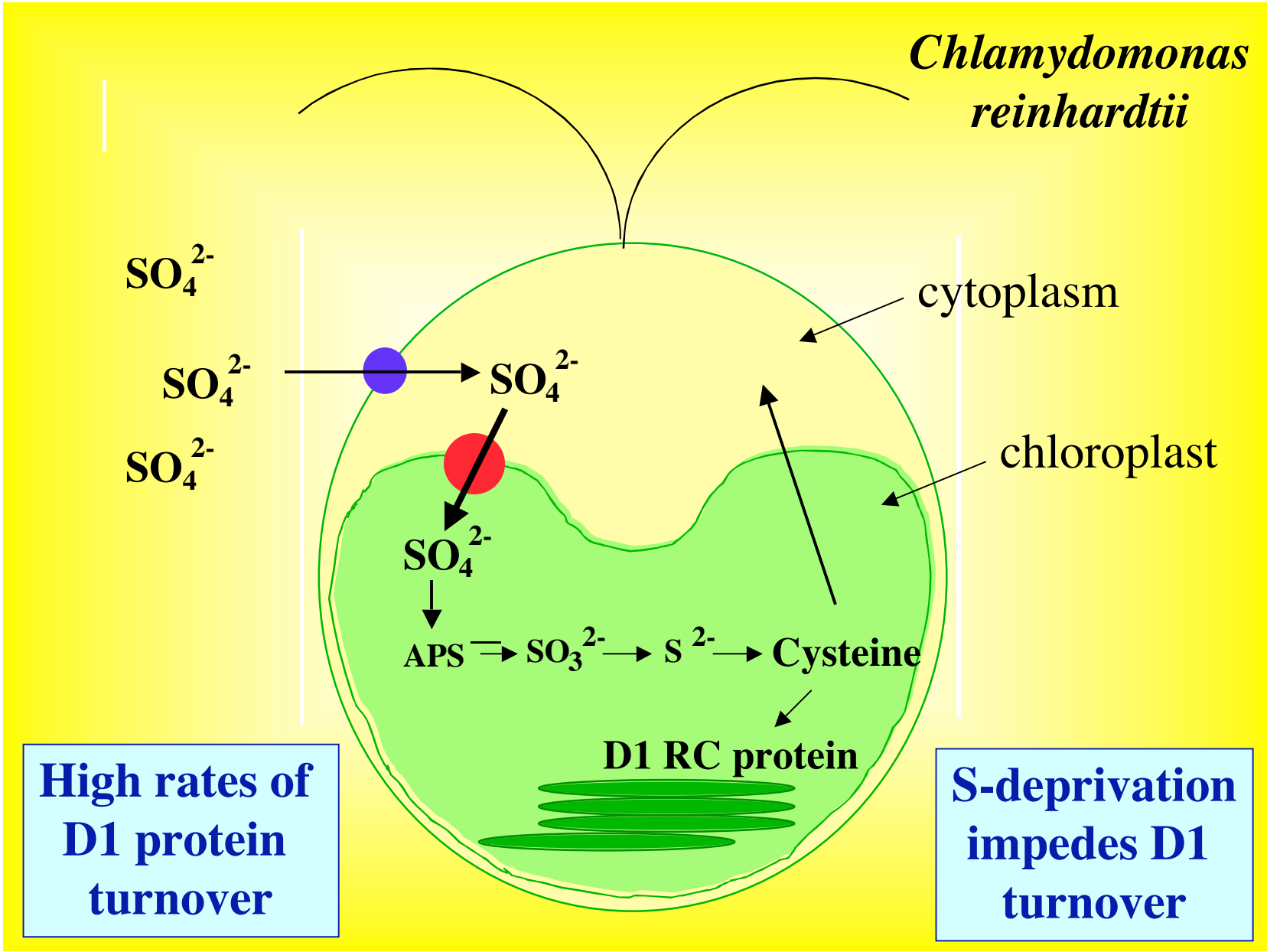


Attenuated photosynthesis: O_2 is consumed by mitochondria



A solution to the O_2 problem

- **Employ the cell's own respiration to consume O_2 produced by photosynthesis.**
- **Genetically, attenuate sulfur nutrient uptake by the chloroplast.**


Chlamydomonas reinhardtii
SO_4^{2-}
SO_4^{2-}
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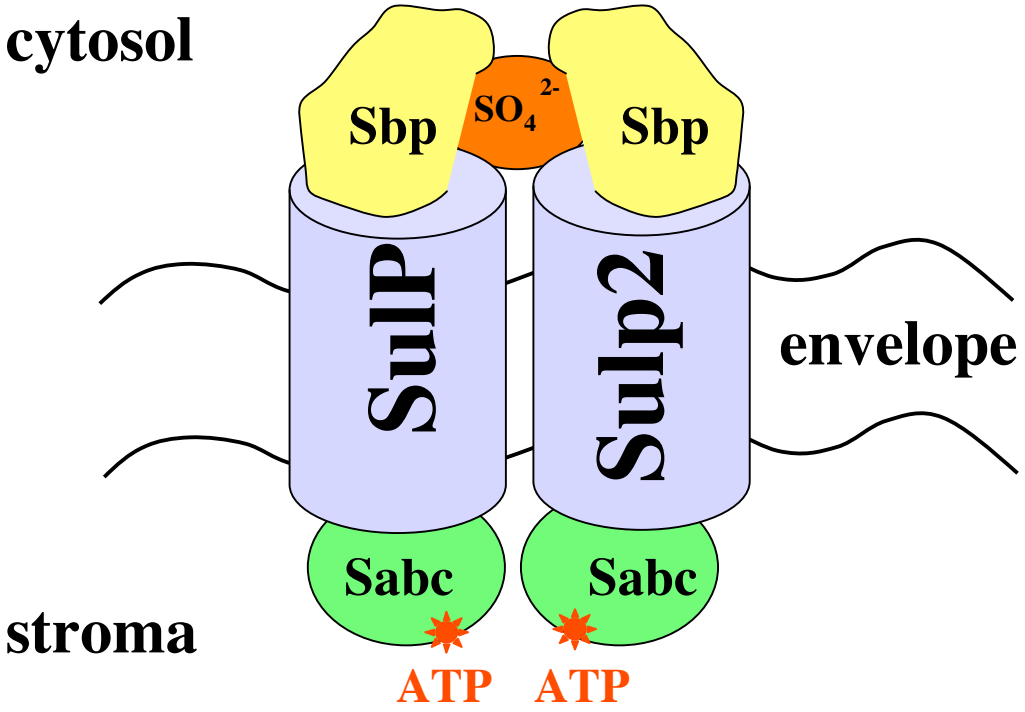
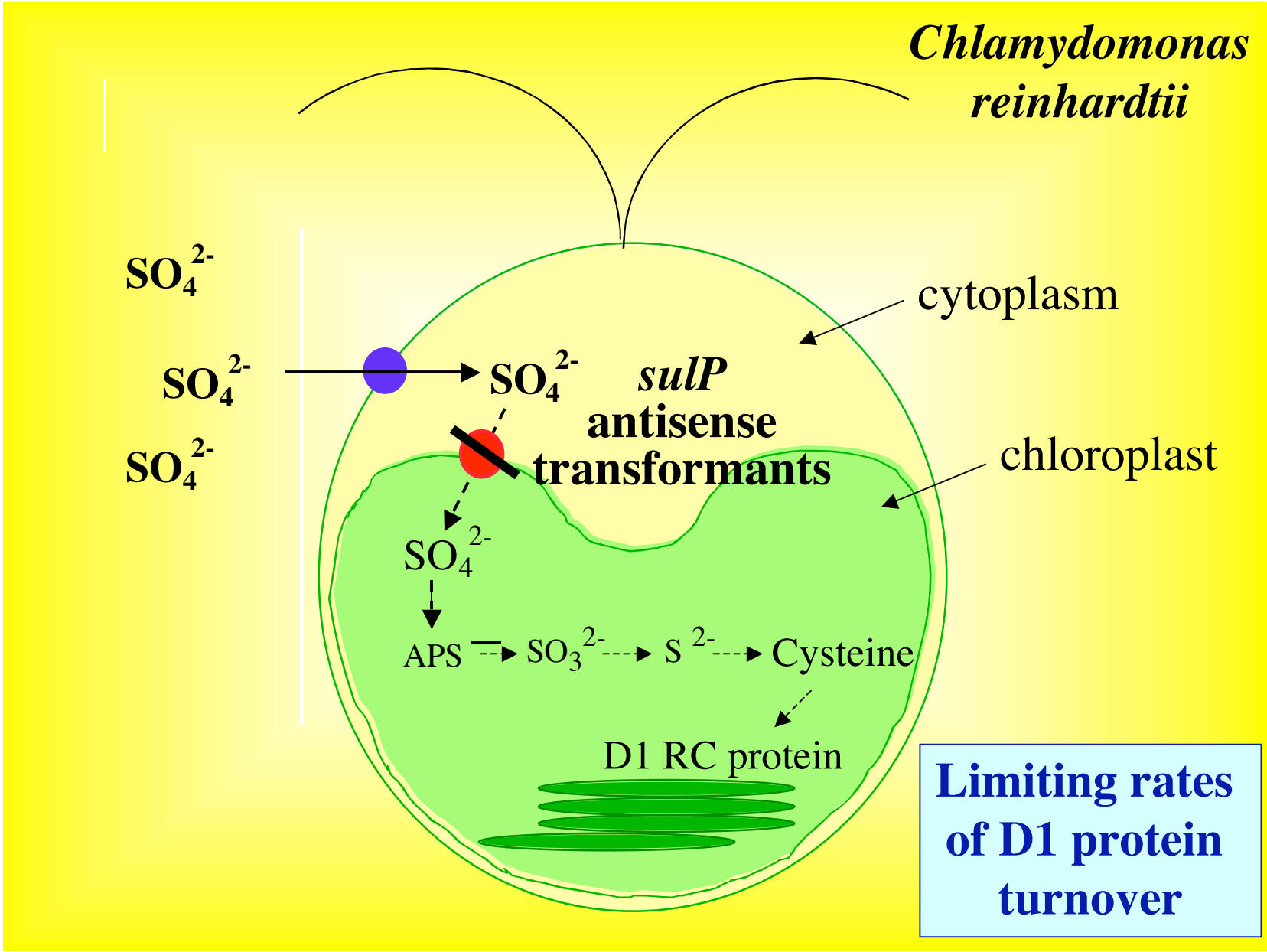
cytoplasm
chloroplast
SO_4^{2-}
APS → SO_3^{2-} → S^{2-} → Cysteine
D1 RC protein
High rates of D1 protein turnover
S-deprivation impedes D1 turnover

The C. reinhardtii chloroplast *Sulfate Permease* is an ABC-type transporter



Planta (2003) 218: 98-106
Planta (2004) 220: 198-210
Photosynth Res (2005) 84: 289-296


Chlamydomonas reinhardtii
SO_4^{2-}
SO_4^{2-}
SO_4^{2-}
cytoplasm
SO_4^{2-}
sulP antisense transformants
chloroplast
SO_4^{2-}
APS
SO_3^{2-}
S^{2-}
Cysteine
D1 RC protein
Limiting rates of D1 protein turnover

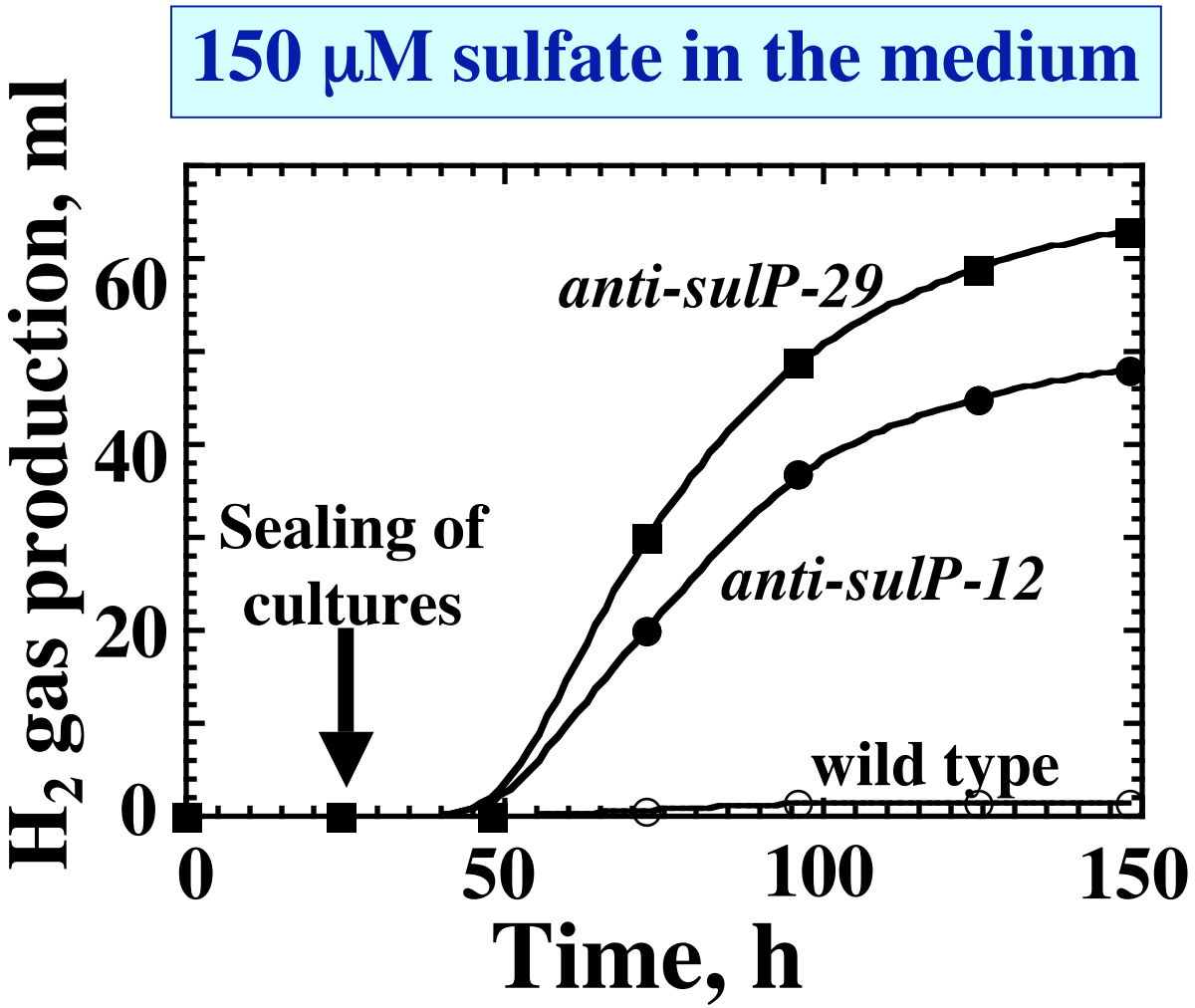
Performance characteristics (400 μM sulfate)

Wild type *C. reinhardtii*

- **Photosynthesis = 44 μmol O_2/mol Chl/s**
- **Respiration = -12 μmol O_2/mol Chl/s**

***antisulP-29* transformant**

- **Photosynthesis = 24 μmol O_2/mol Chl/s**
- **Respiration = -12 μmol O_2/mol Chl/s**


150 μM sulfate in the medium
H2 gas production, ml
0
20
40
60
0
50
100
150
Time, h
Sealing of cultures
anti-sulP-29
anti-sulP-12
wild type

Conclusion

A balanced capacity of Photosynthesis and Respiration facilitates anaerobic conditions and spontaneous H_2-production.

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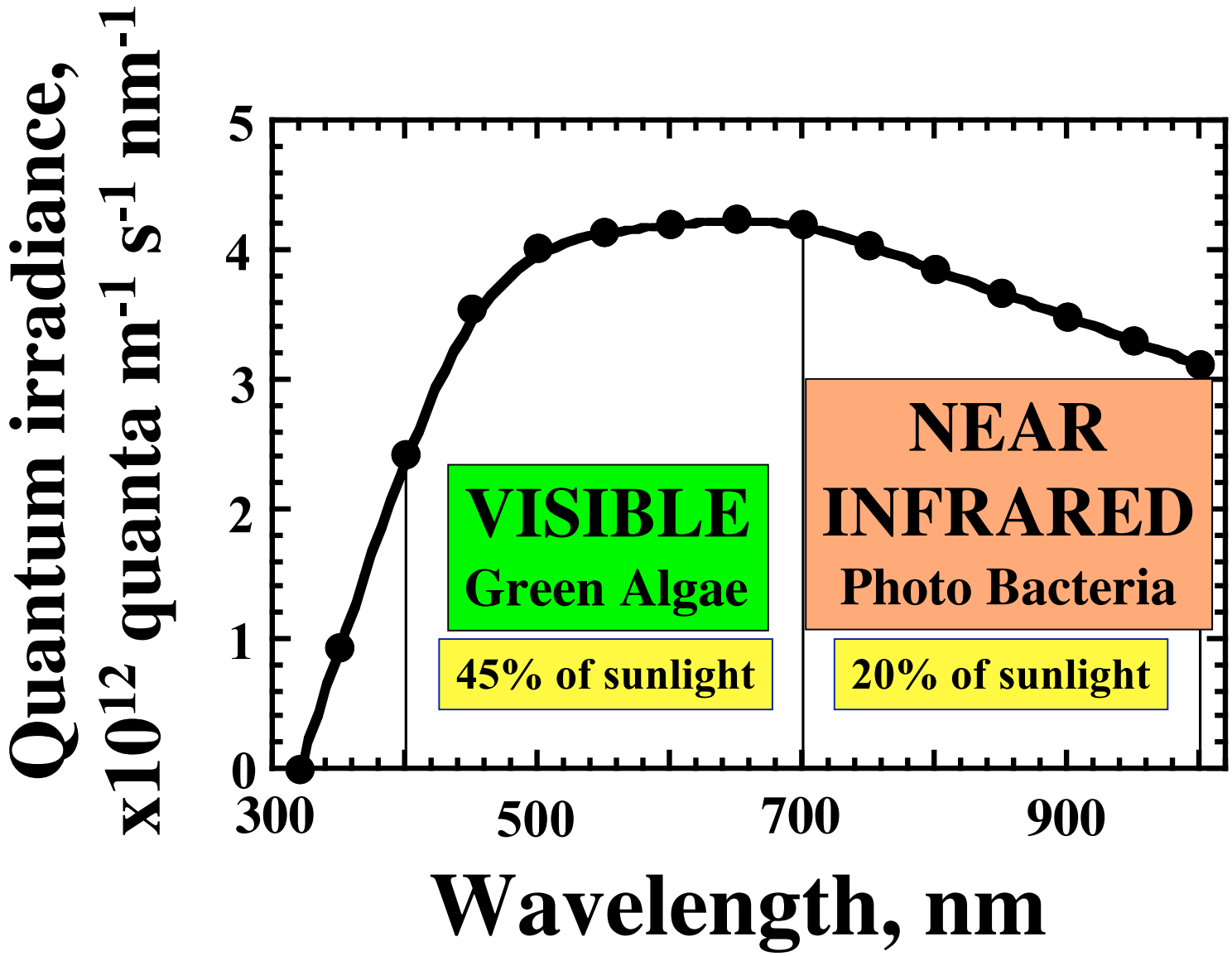
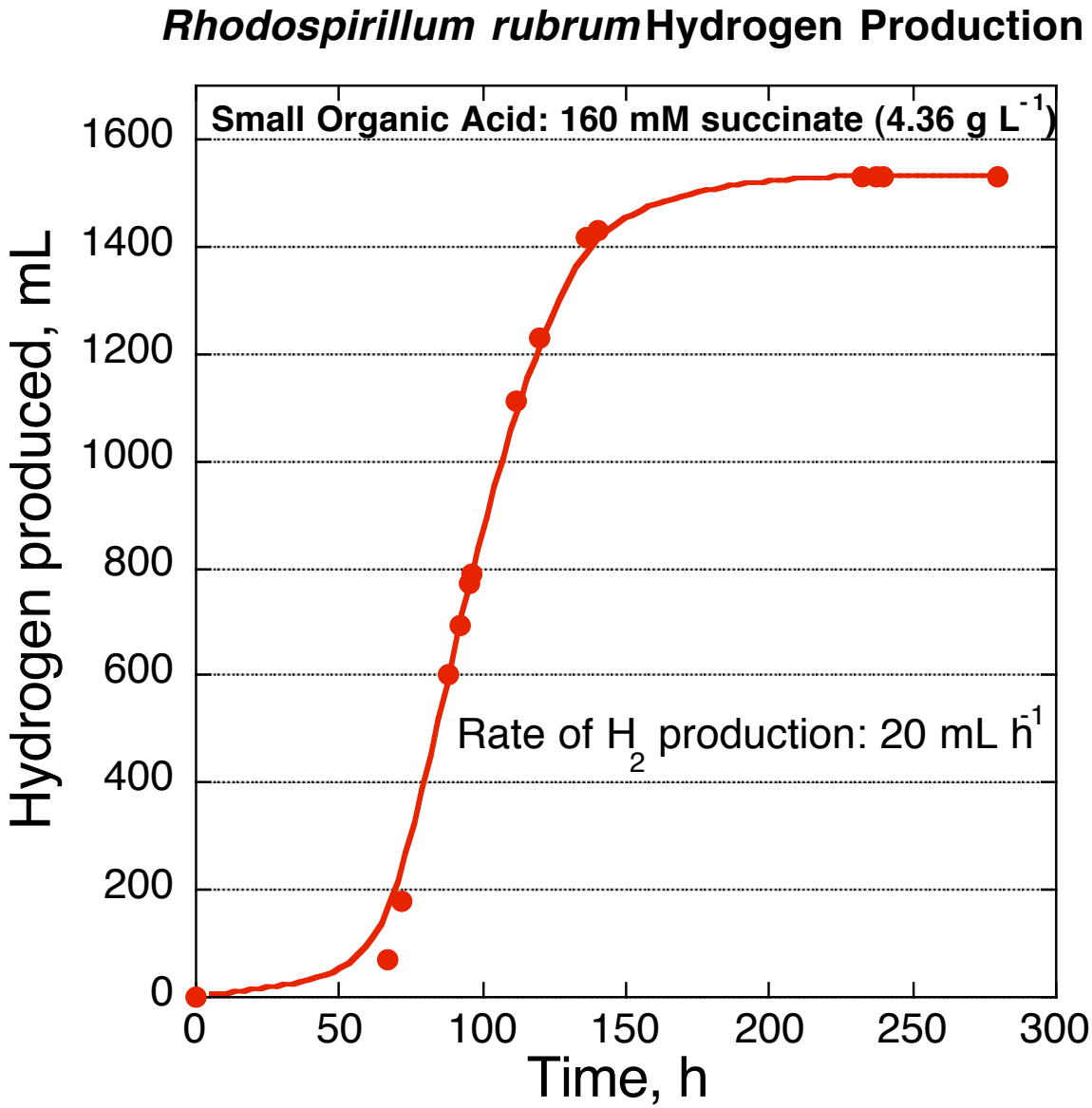
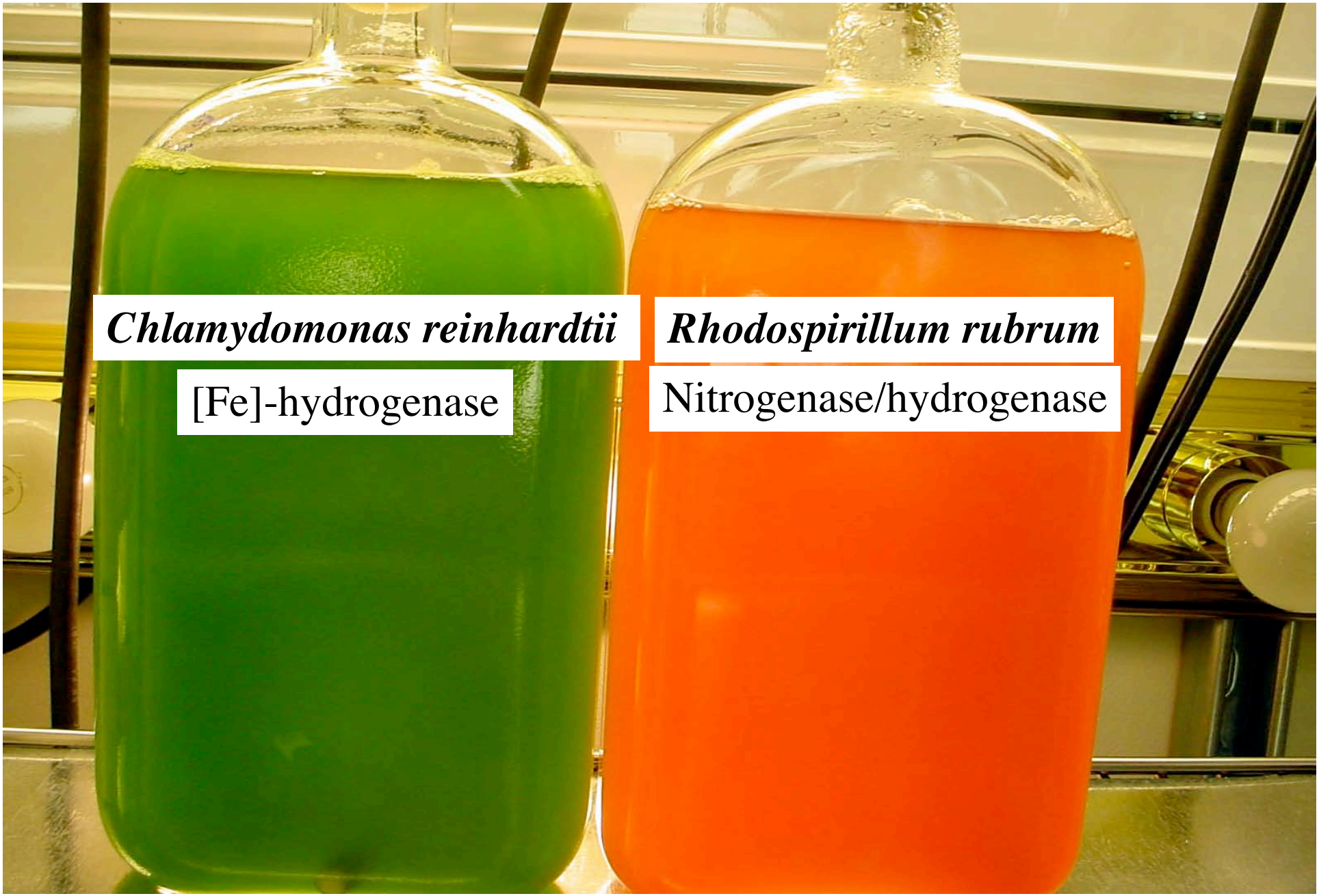
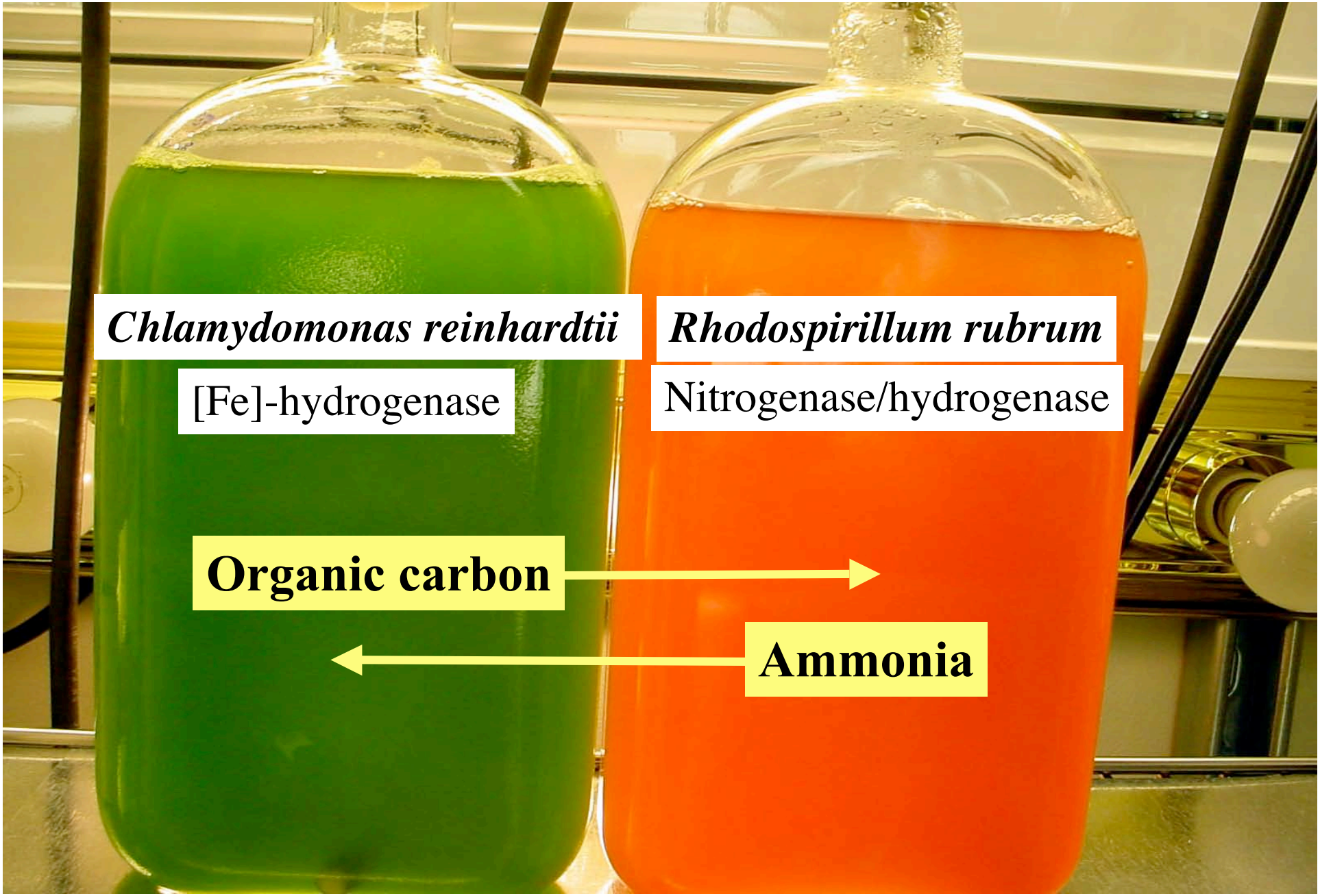

Quantum irradiance,
x10^12 quanta m^-1 s^-1 nm^-1
5
4
3
2
1
0
300
500
700
900
Wavelength, nm
VISIBLE
Green Algae
45% of sunlight
NEAR
INFRARED
Photo Bacteria
20% of sunlight


Rhodospirillum rubrum Hydrogen Production
Small Organic Acid: 160 mM succinate (4.36 g L^{-1})
Rate of H_2 production: 20 mL h^{-1}
Hydrogen produced, mL
Time, h
0
200
400
600
800
1000
1200
1400
1600
0
50
100
150
200
250
300



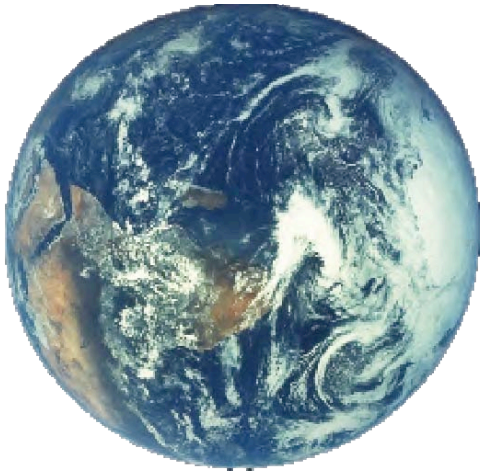
H_2-producing green algae H_2-producing photo bacteria



H_2-producing green algae H_2-producing photo bacteria

Co-cultivation of a photosynthetic bacterium-green alga at 6:4 and 2:8 ratios

The promise of photobiological H_2-production



- ♦ 10 billion kWh are consumed in the US daily
- ♦ 8,000 sq. miles needed to meet daily electricity consumption in the US

- ★ 400 million gallons gasoline are consumed daily
- ★ 15,000 sq. miles needed to meet daily gasoline consumption in the US

Acknowledgment

Support by the DOE HFC&IT program

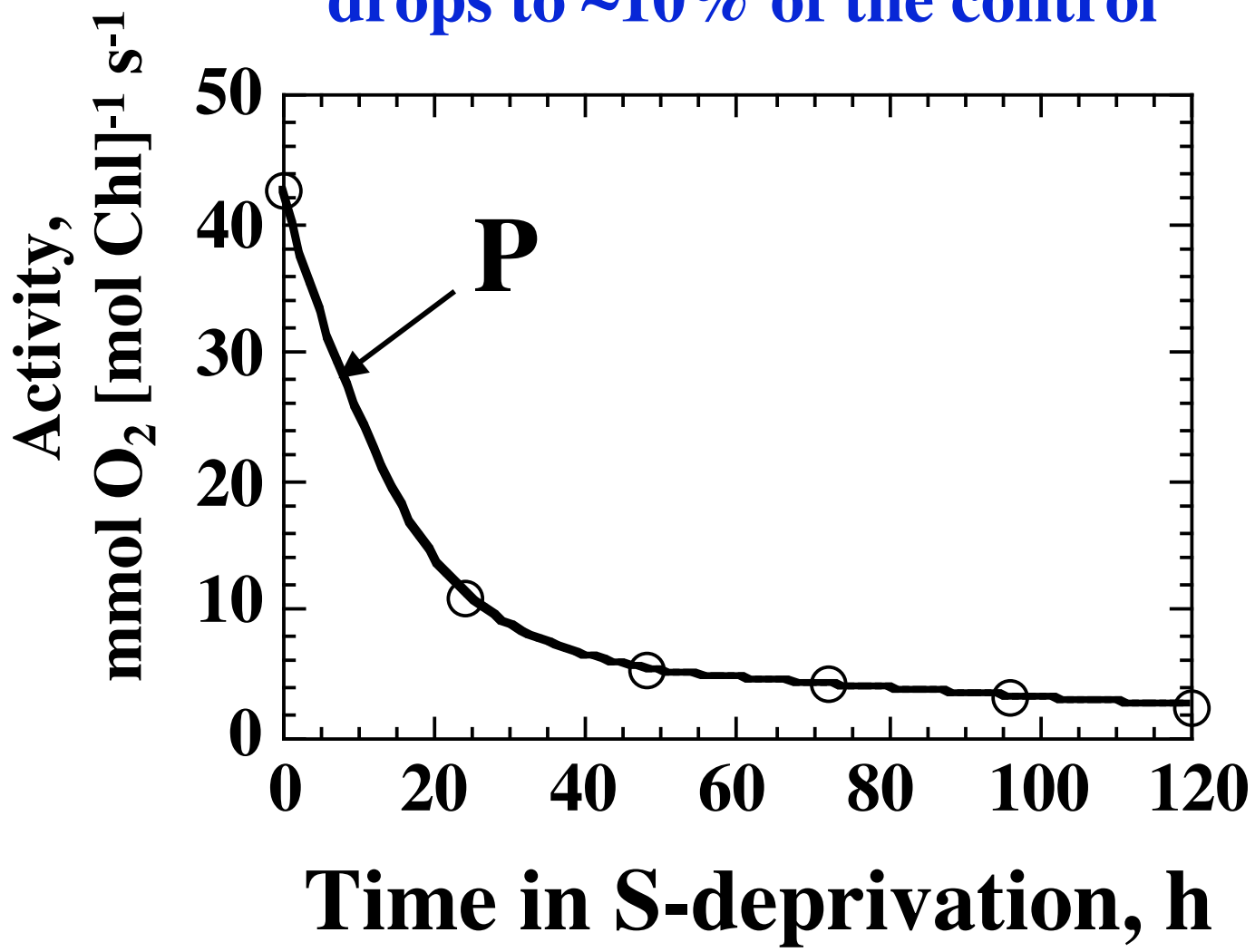
The Adverse Effect of Oxygen on Photosystem-II

- **Reactive singlet oxygen is generated at PSII in the course of photosynthesis:**

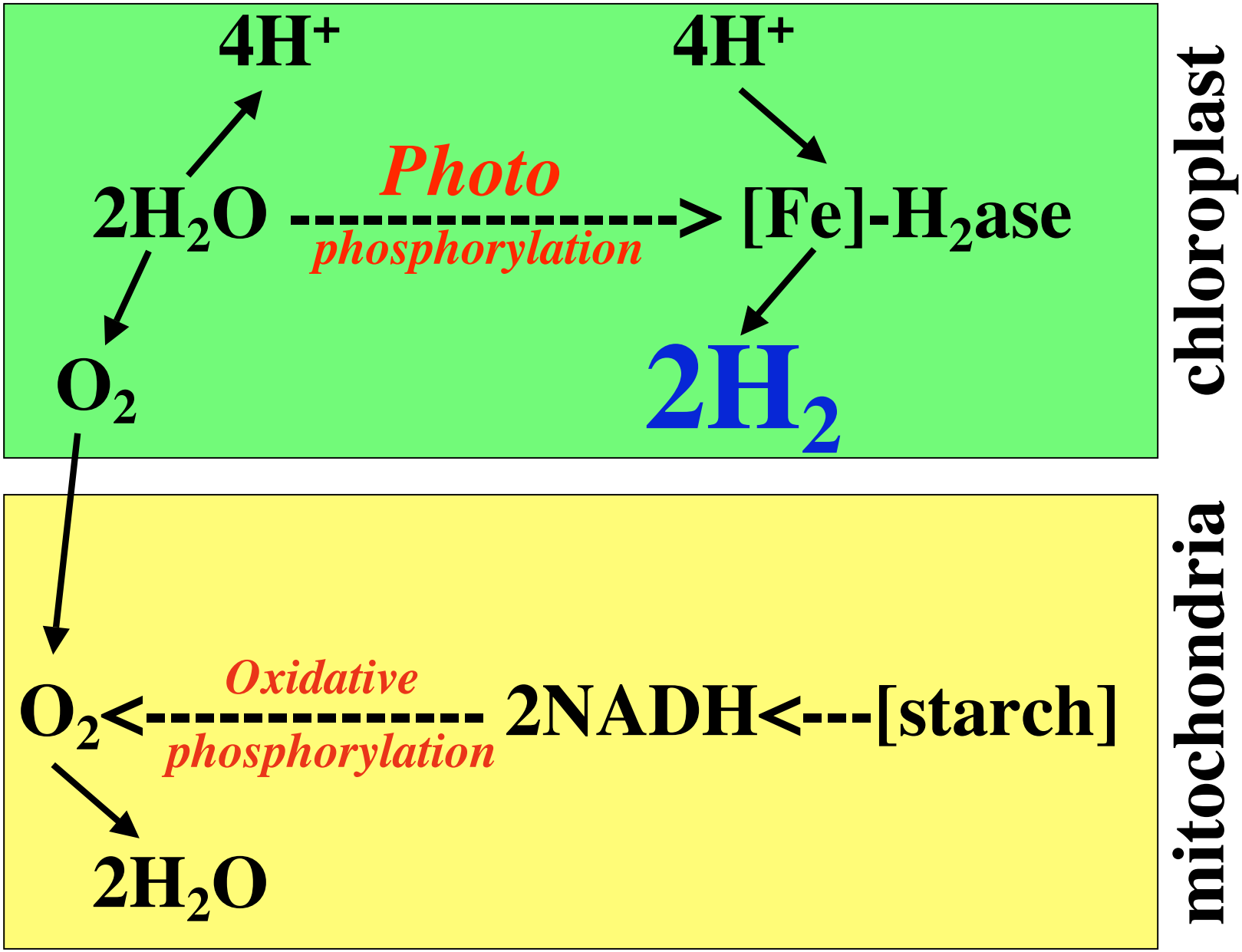
  ($^3O_2$ $^*P_{680}$ ---> $^1O_2P_{680}$ ---> inactivation of P_{680})

- **Reactive singlet oxygen causes irreversible inactivation of P_{680} and stop PSII photochemistry.**

In -S media, the rate of photosynthesis drops to ~10% of the control



Growth aided by external organic carbon



Physiology of starch metabolism before and during H_2 production: Substrate catabolism is essential for H_2 production.