Renewable Bio-solar Hydrogen Production from Robust Oxygenic Phototrophs

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Renewable Bio-solar Hydrogen Production from Robust Oxygenic Phototrophs

-BioSolarH_2→ Team

G. Charles Dismukes	PU	photosynthesis/engineering
Edward I. Stiefel	PU	enzymology/inorganic
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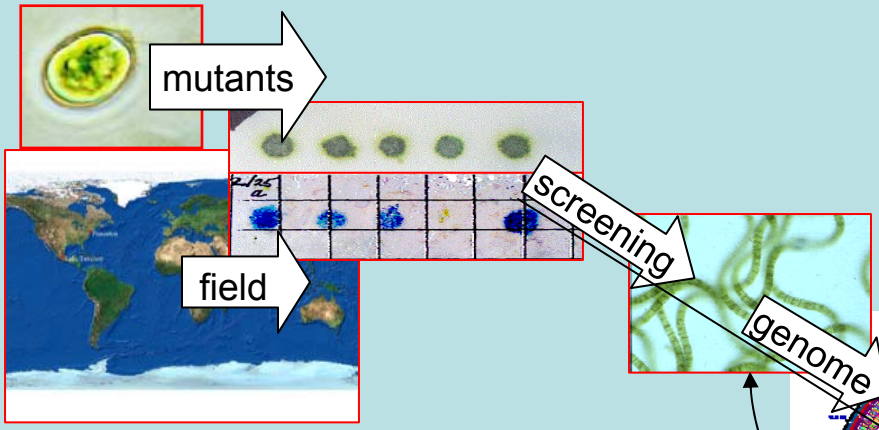
Matthew Posewitz	CSM	genetics/mol biology algae
Eric Hegg	UU	enzymology/inorganic
Robert Bidigare	UH	microbial physiology/ecology

Renewable Bio-solar H_2 Production from Robust Oxygenic Phototrophs



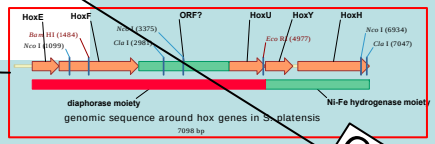
Achievements June 2005

- Identified novel H_2 photoproducers
- Developed high throughput screen for H_2
- Built ultra-sensitive H_2 detection instruments
- Engineered mutants in a green alga

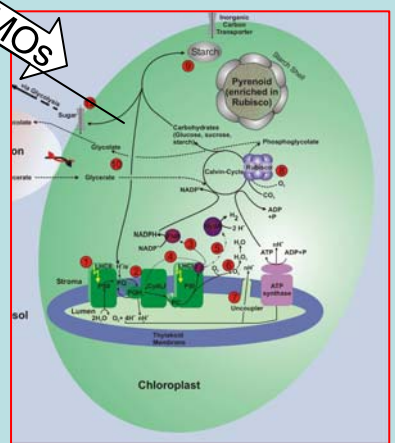
Payoff

Clean H_2 from non-fossil source

H_2O → **Oxygenic Photosynthesis** → O_2 + H_2



GMOs



Objectives

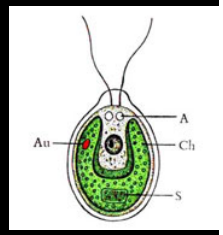
- Screening for the right organisms, photosystems & hydrogenases
- Down-regulation of competing biochemical pathways
- Random & specific mutagenesis for higher H_2 production
- Genetically combining host phototrophs with optimal hydrogenases

Solar Bio-H_2 Goals: Economic viability[4]

- ~10% efficiency at ambient light levels (eg., antenna deletion mutants)
- Requires strict temporal or sptial separation of H_2 and O_2
- Achieve current cost estimates for large scale bioreactors

4. Independent predictions by NREL and Thiess Engineering

Solar Bio-H_2 Current Status

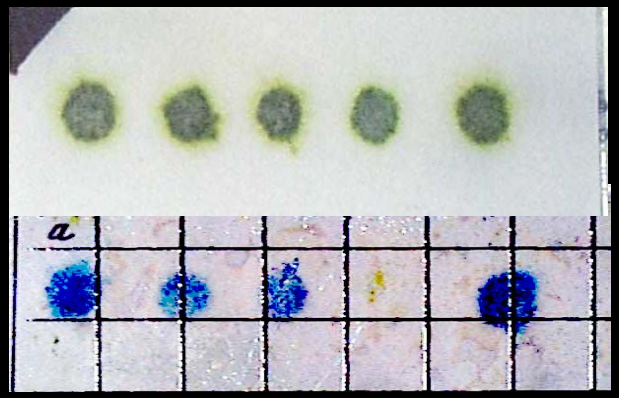


Chlamydomonas reinhardtii: two stage light + dark + S deprivation

Strain	IncidentPhoton Conv. Eff.	H_2 rate ml (liter culture)$^{-1}$ hr^{-1}
• WT [1]	~ 0.1%	1.5
• LHC insertion mutant [2]	(3-4 x expected)	minimal H_2 observed
• stm6 mutant [3]	~1.5%	(5 -13 x)

1. NREL team 2004
2. UCB team, Melis
3. Univ. Bielfeld & Univ Queensland: Kruse & Hankamer, JBC (2005)

Colonies on agar plate

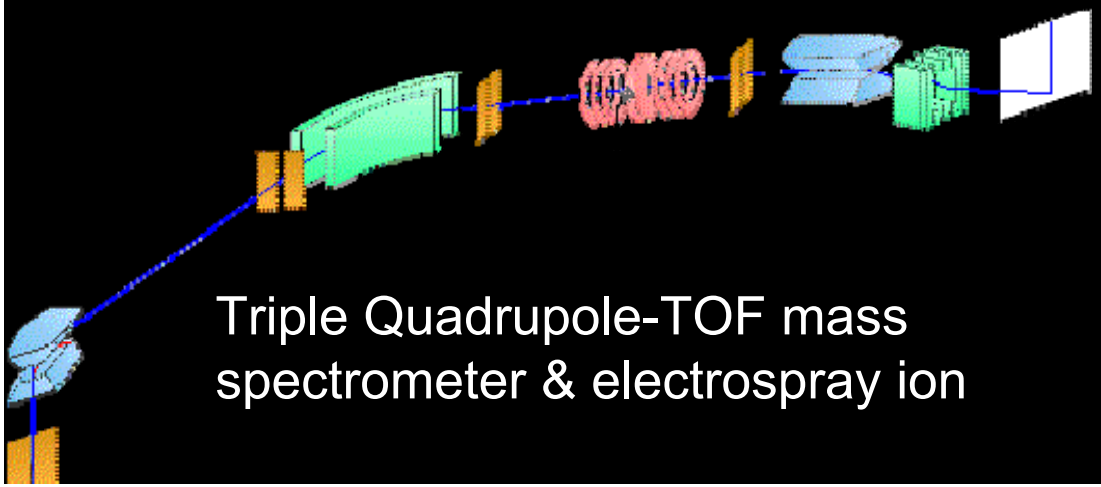


NREL WO_3 H_2 gas sensor

Fast repetition rate fluorimeter
PSII Quantum Efficiency

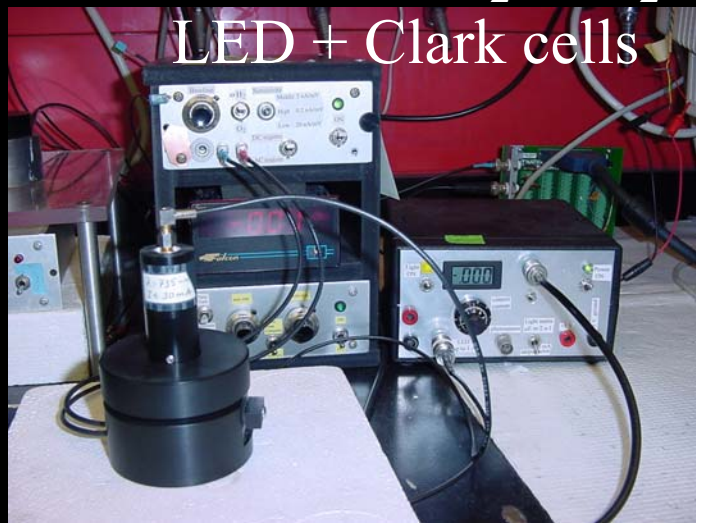
Instrumentation Development

Metabolomics & Proteomics

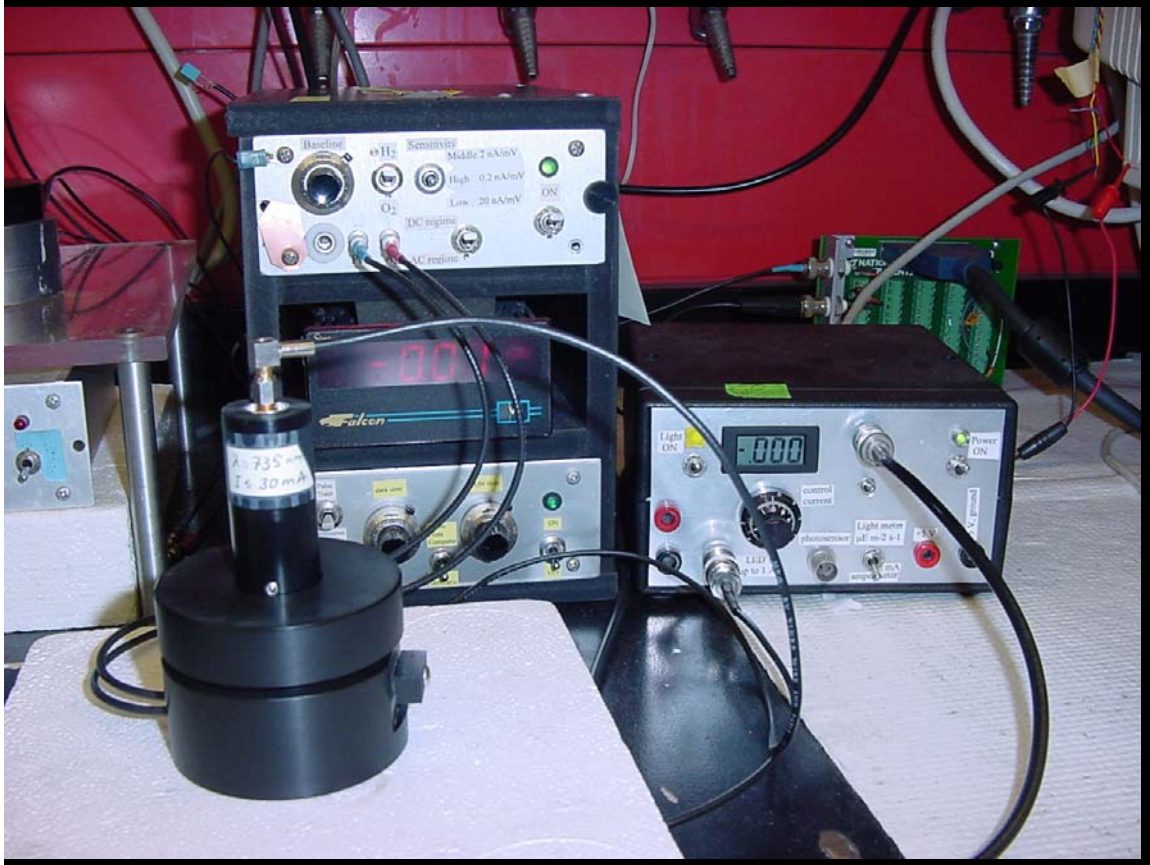


Triple Quadrupole-TOF mass spectrometer & electrospray ion

Dissolved H_2 & O_2
LED + Clark cells



Ultrasensitive cells for dissolved H_2 & O_2 with multi-wavelength light-emitting diode illuminator

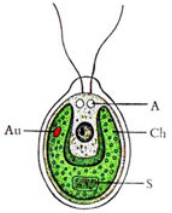


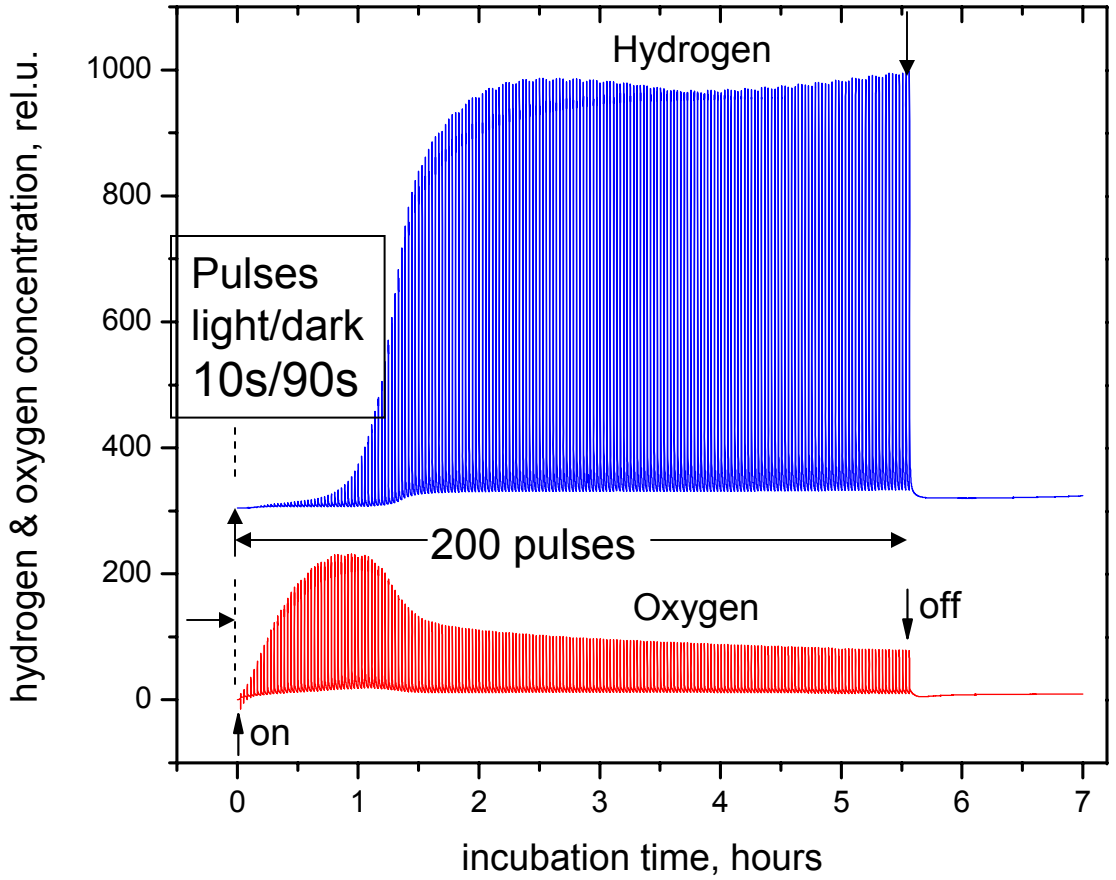
Clark type Cells for dissolved O_2 & H_2

10^{-8} Molar H_2 or O_2
5 µL volume
Sensitivity 50 femtomoles
0.1 sec response time

Wavelength selective illumination via LEDs: blue, orange, red, infrared, white

Photo-H_2 and –O_2 in WT *Chlamydomonas reinhardtii* CC124





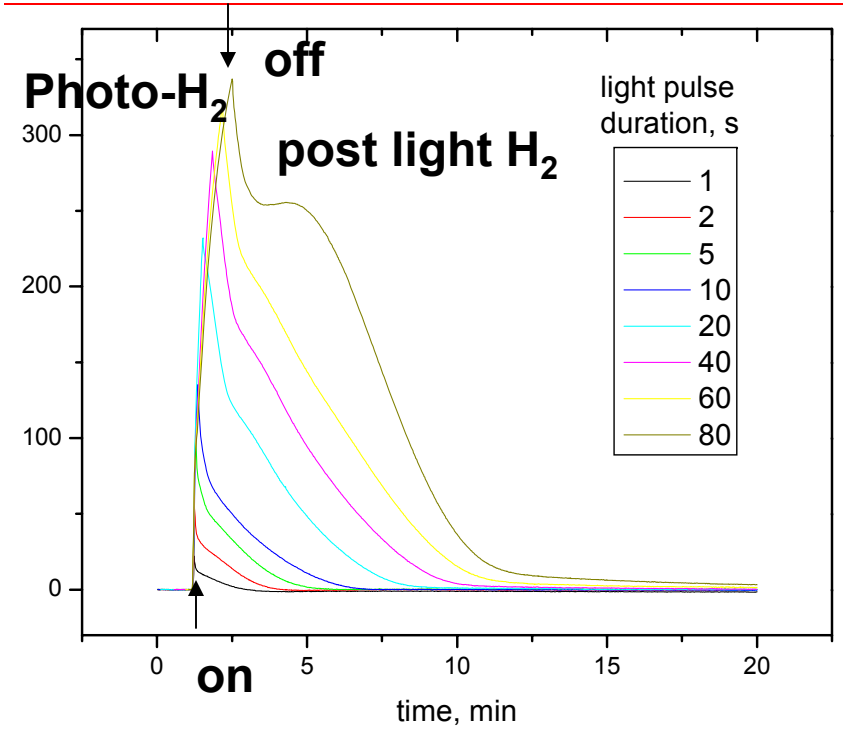
Conclusions

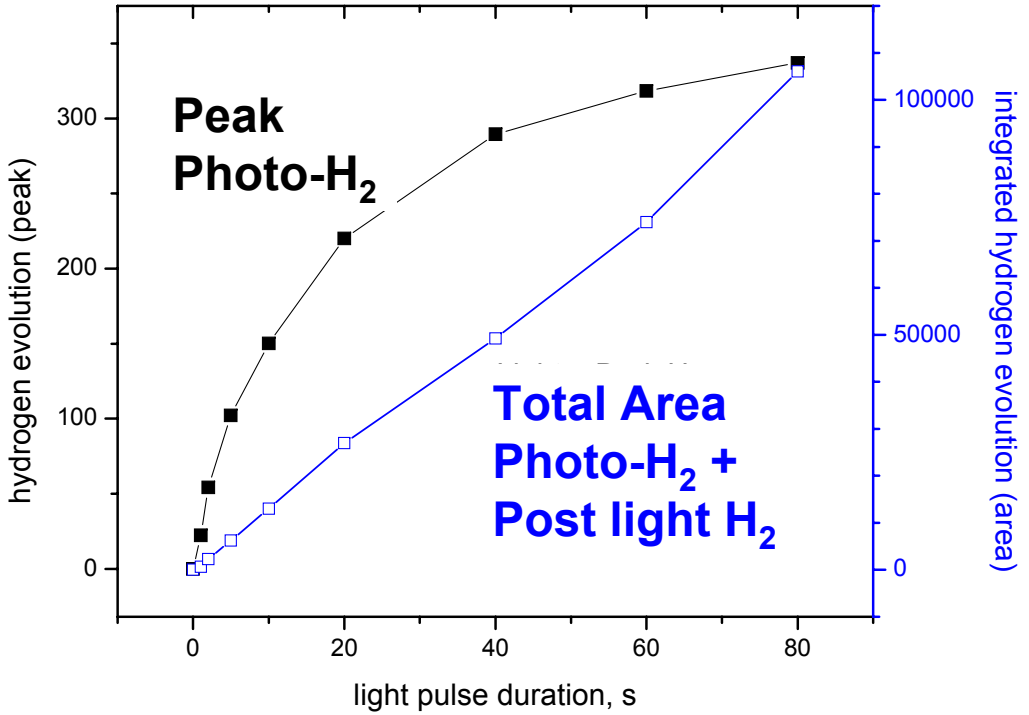
- **Major Photo-H_2**
- **Minimal dark-H_2**
- **Co-production of H_2 and O_2**
- **H_2 yield is O_2 limited**

Cells from Petri dish resuspended in TAP liquid medium.
Anaerobic pre-incubation time 12 min →
Pulse illumination via white LED at 130 μE m^{-2} s^{-1}

G. Ananyev

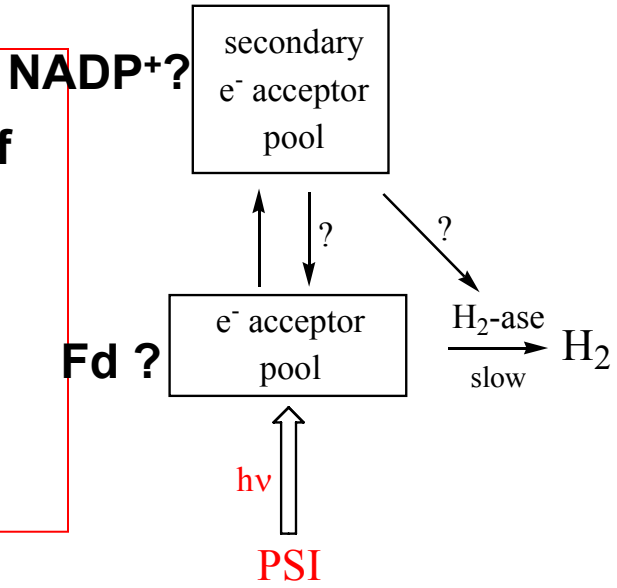
Light pulses produce two kinetic phases of photo-H_2 in *C. reinhardtii* WT



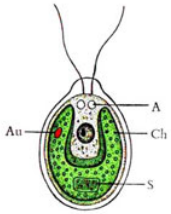


Conclusion:

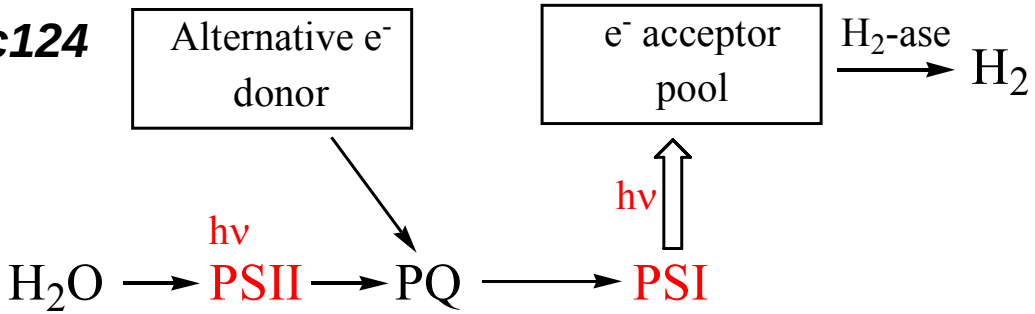
- **Two pools of photo-electron acceptors in PSI capable of light-induced H_2 production**
- **Integrated H_2 yield is linear with pulse duration → competing pathways for e^-/H^+ consumption are either fixed or not present**



G. Ananyev

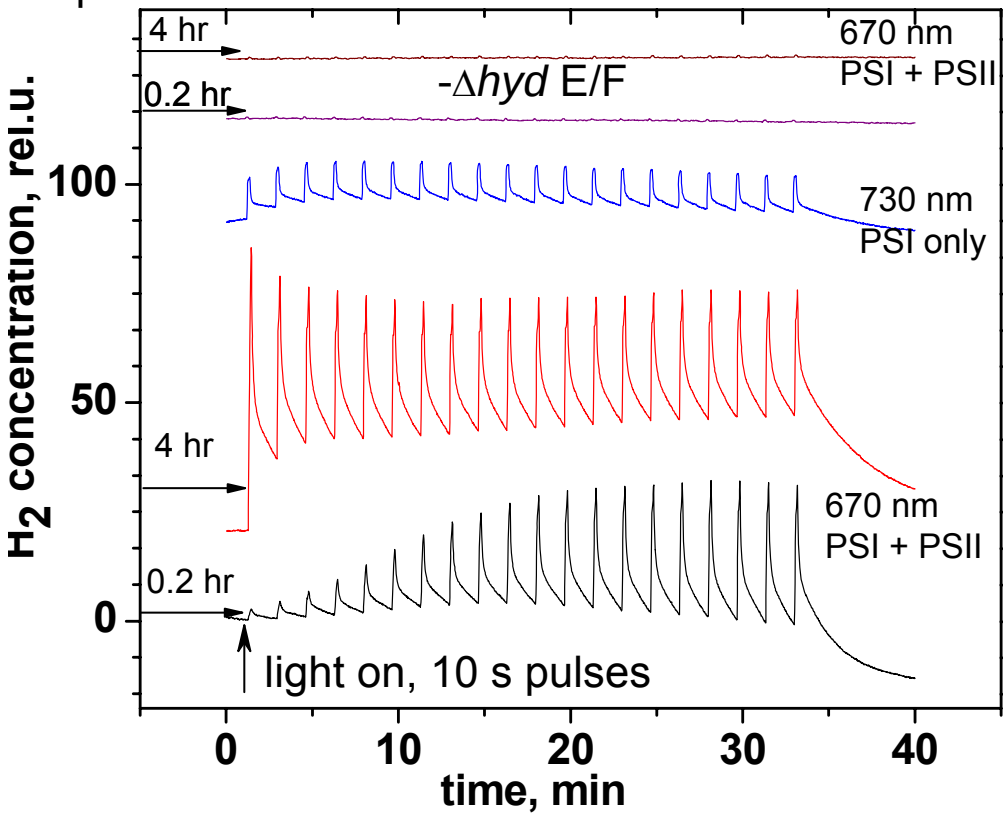


Chlamydomonas reinhardtii cc124



Anaerobic -O_2

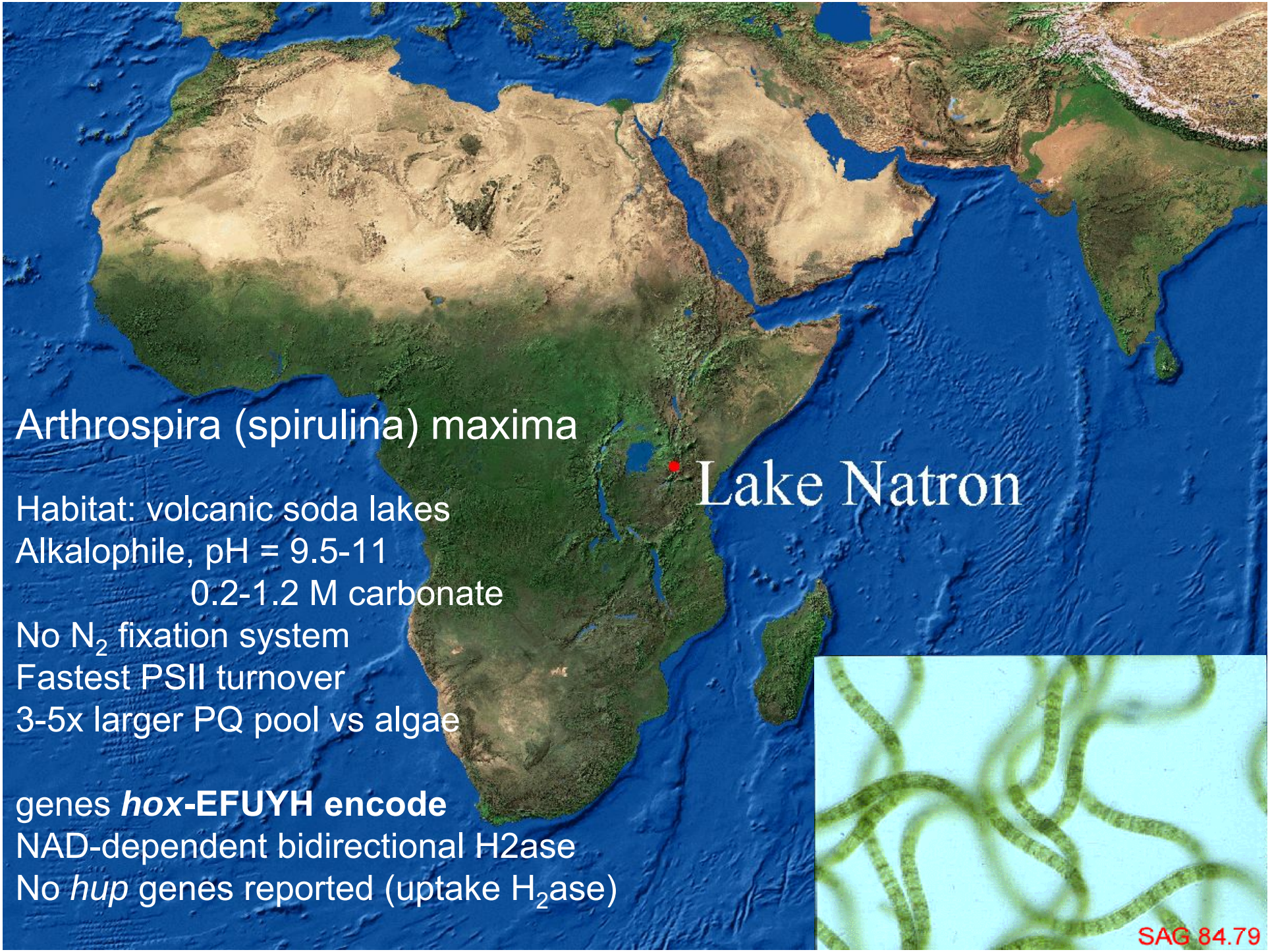
pre-incubation



Conclusions:

- **Photo-H_2 & dark-H_2 requires H_2ase genes hyd E/F**
- **Maximum photo-H2 production requires illumination of both PSI and PSII**
- **The major source of reductant required for photo-H2 comes from water via PSII turnover**
- **The minor PSI only pathway presumably involves PQ/other donor**

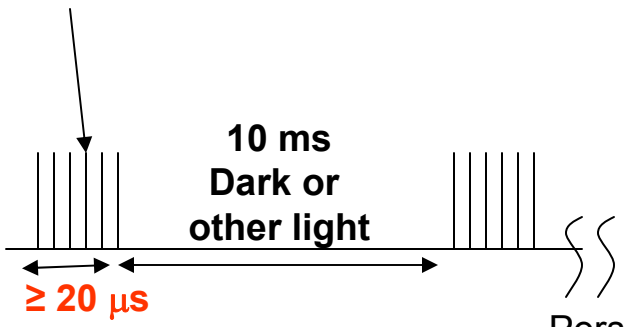
G. Ananyev & M. Posewitz


Arthrospira (spirulina) maxima
Lake Natron
Habitat: volcanic soda lakes
Alkalophile, pH = 9.5-11
0.2-1.2 M carbonate
No N_2 fixation system
Fastest PSII turnover
3-5x larger PQ pool vs algae
genes ***hox*-EFUYH encode**
NAD-dependent bidirectional H2ase
No *hup* genes reported (uptake H_2ase)
SAG 84.79

Princeton: Fast Repetition Rate Laser Fluorometer

- ≥50 ns pulses 0.5 W; 660 nm laser
- ≥20 µs duration pulse train at full light saturation
- Programmable pulse flexibility
- Fast sampling, 20 mHZ detection
- Chlorophyll Fo, Fv, Fm,
- cross section of PSII
- Q_A^- oxidation kinetics

STF – single turnover laser flash train



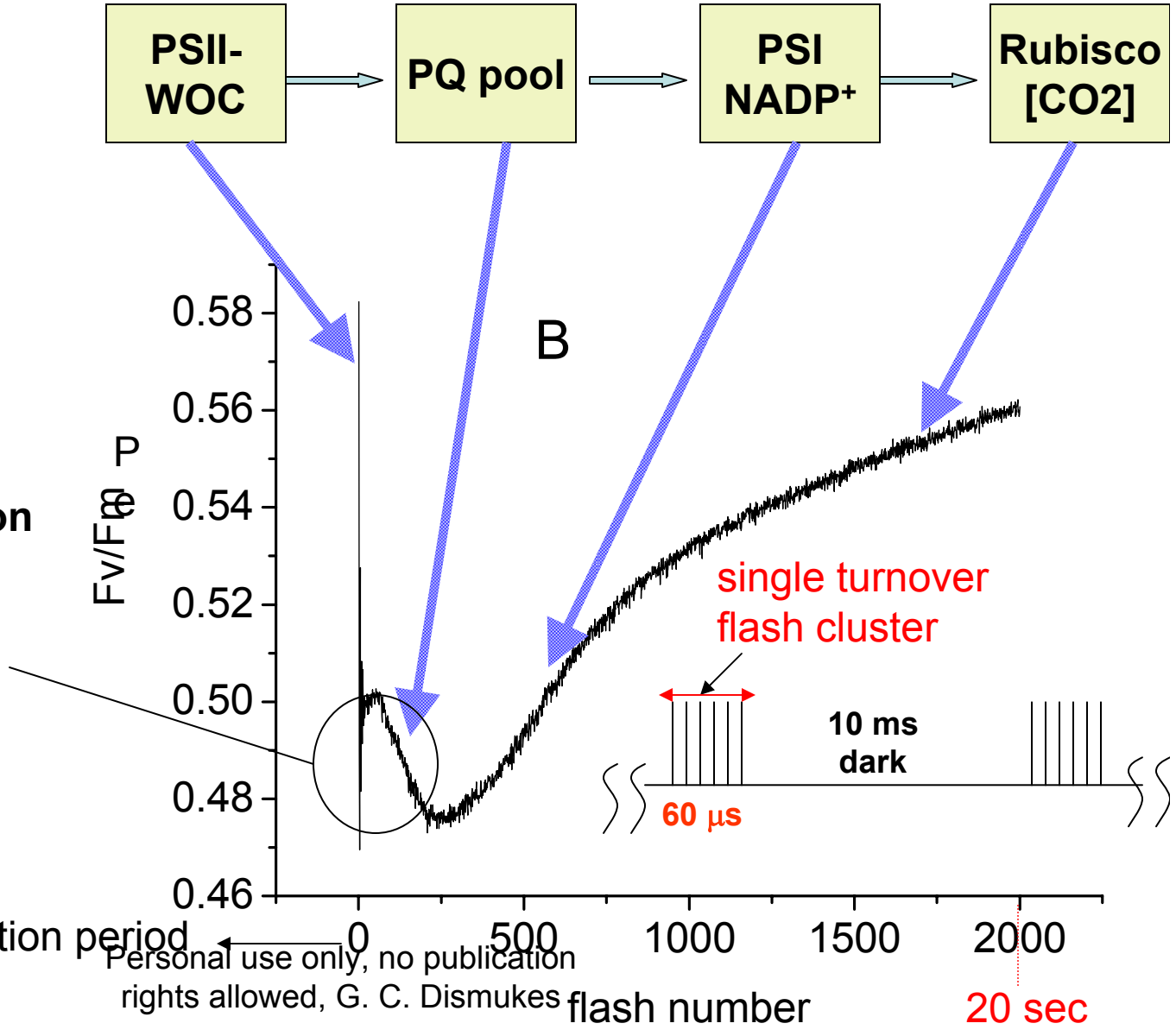
Variables to be measured
Fv/Fm = PSII quantum efficiency
PQ pool size

Ananyev & Dismukes, Photosyn Res. (2005)

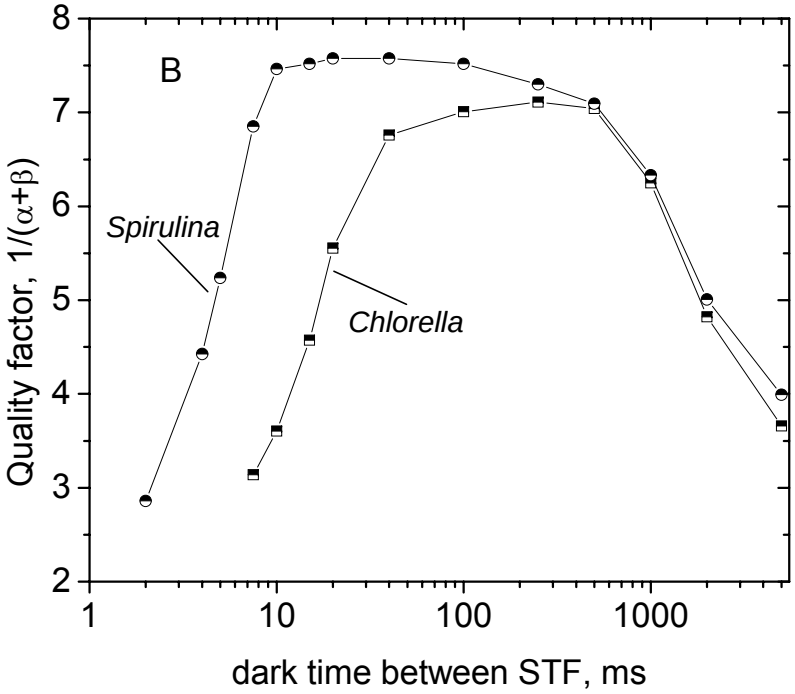
PSII Variable Fluorescence at 100Hz flash repetition rate; *Arthrospira p. whole cells*

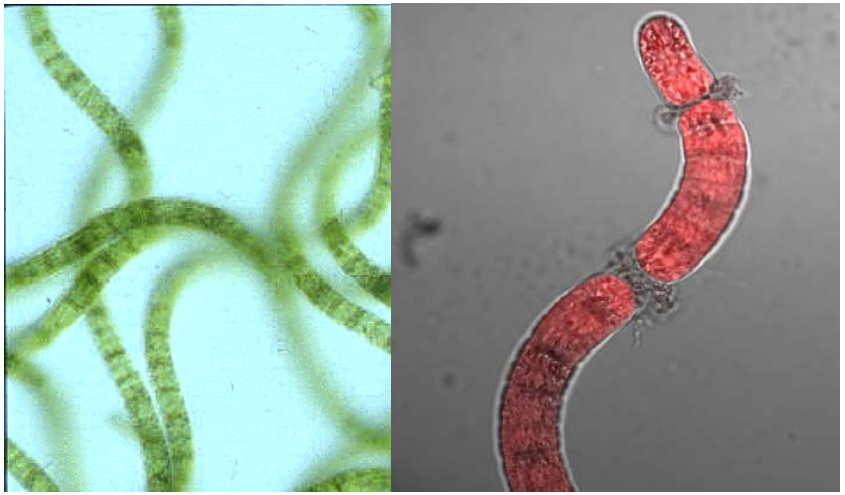
Kautsky curve demonstrates transition from single turnover events in PSII to multiple electron transfer in whole electron transfer chain

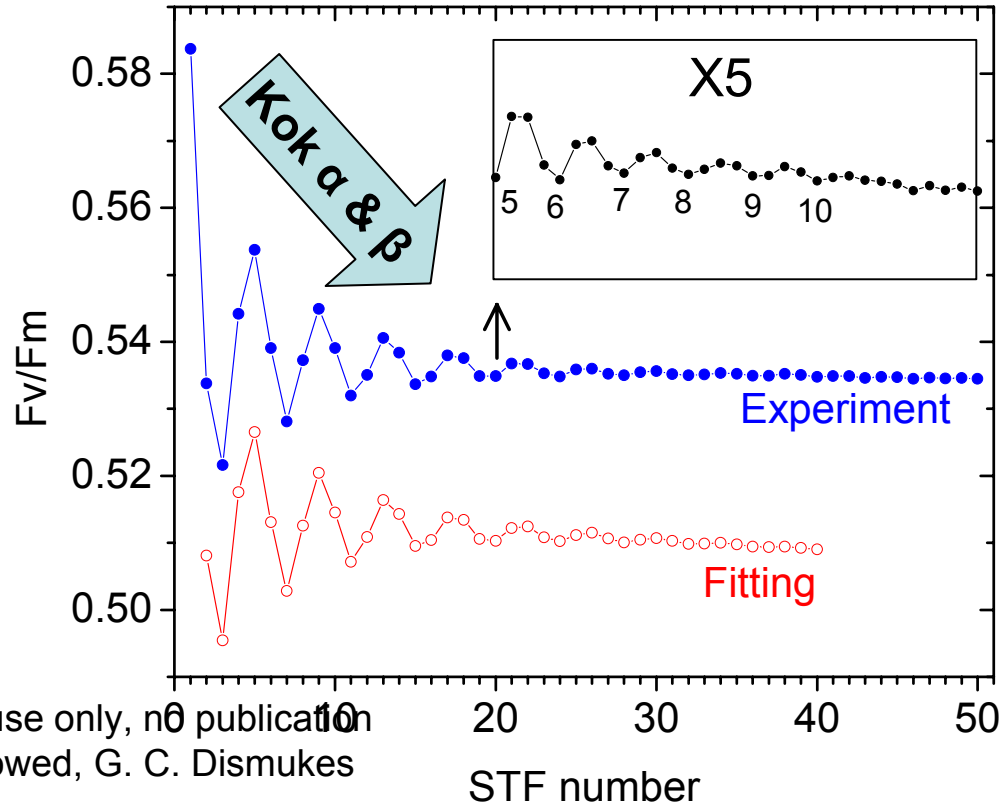
Arthrospira sp. have a larger PQ pool capacity 3-5X vs. green algae!



Turnover Frequency of PSII is highest by 5x for *Arthrospira maxima*





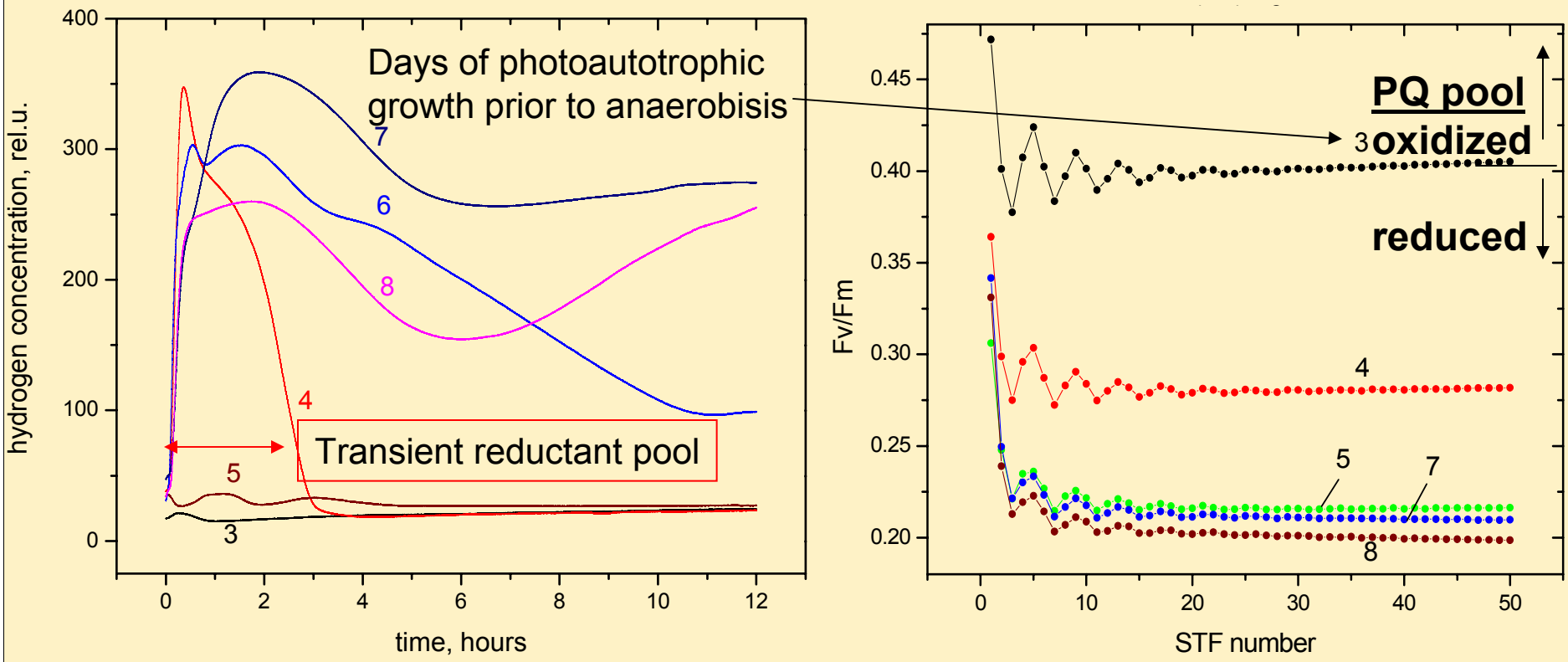


Kok parameters:
photochemical misses (α)
& double hit/misses (β)

PSII quantum efficiency ~54%

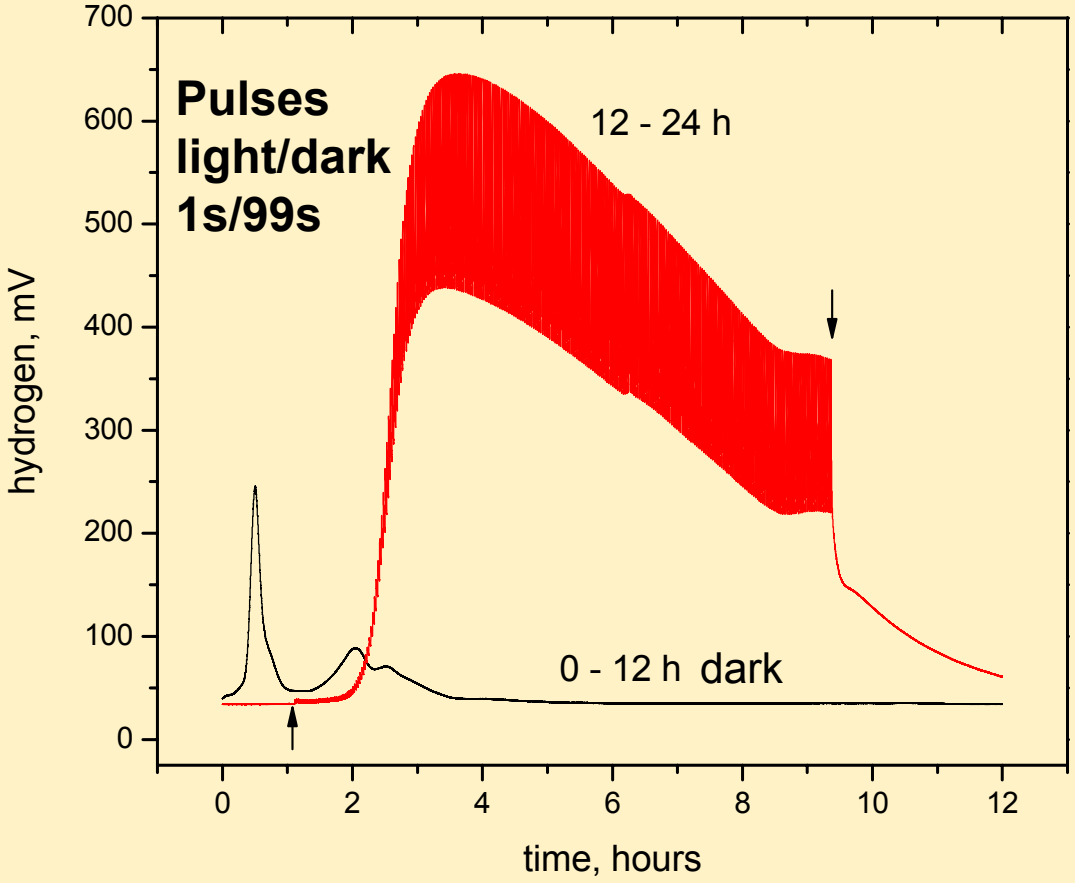
* Ananyev & Dismukes, Photosyn Res. (2005)

Induction of dark H_2 ↑(Left) & PSII quantum efficiency (Right) during photoautotrophic growth at 1.7 μM Ni^{2+} of *A. maxima*



- Ni^{2+} addition to standard cyanobacteria growth media (Zarrouk) is essential for H_2ase↑
- 3-4 days lag to fermentative H_2 , yields transient reductant pool (2 or 3 peaks)
- Second lag phase 1-2 days later, oscillations in H_2
- Culture survives for 10-11 days in batch culturing no additives
- Microscopy shows that most active dark-H_2 producing cells have no gas vacuoles

PSI + PSII Illumination by 640 nm red light produces additional H_2



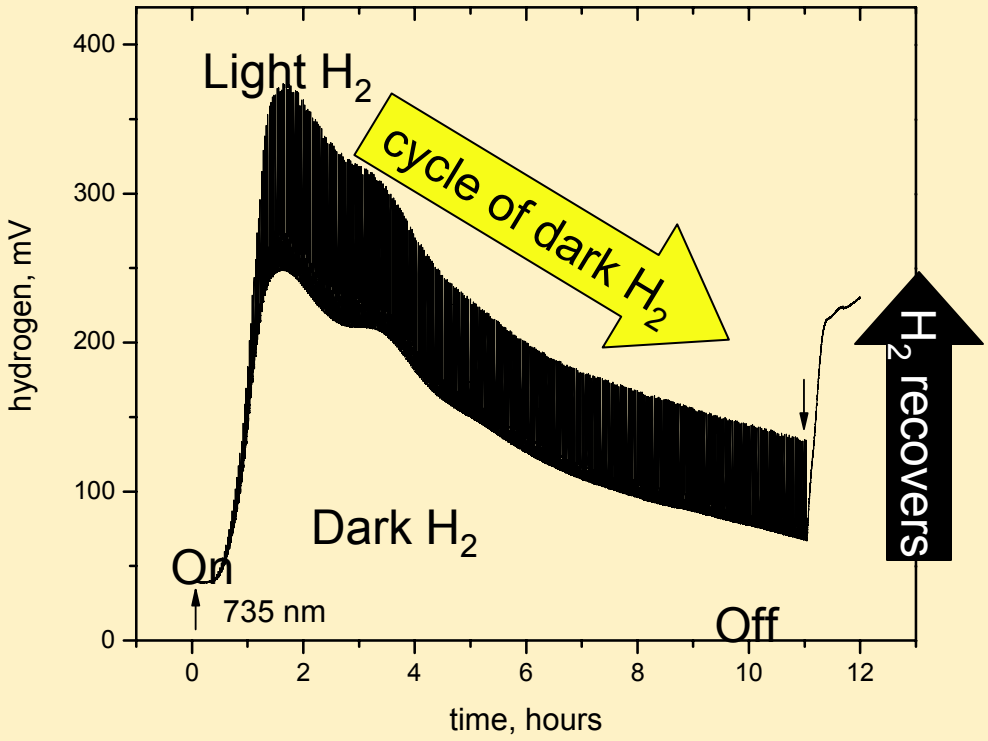
Conclusions

•Dark (fermentative) and a light-induced pathways to H_2 are present

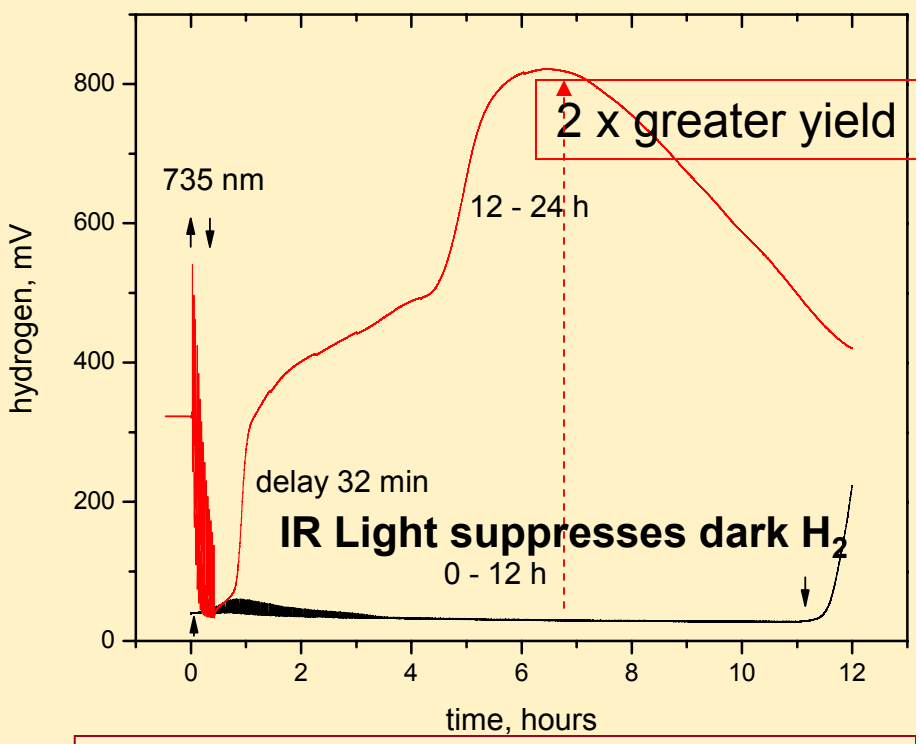
These pathways share a common intermediate that is precursor to H_2

PSI Illumination by IR 735 nm stops fermentative H_2↑ & stores H_2 precursor

1 s light/ 99 s dark



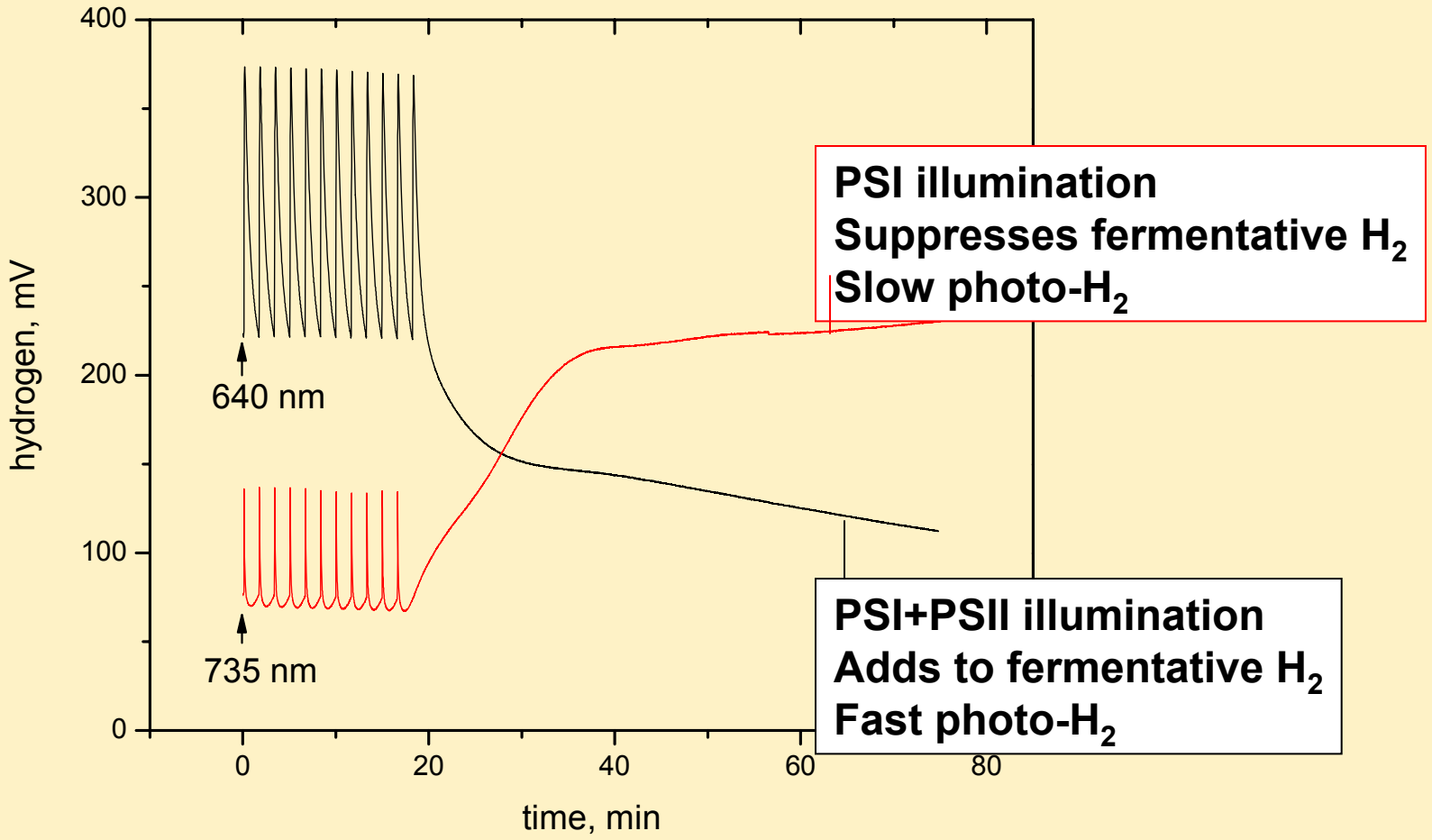
5 s light/95 s dark



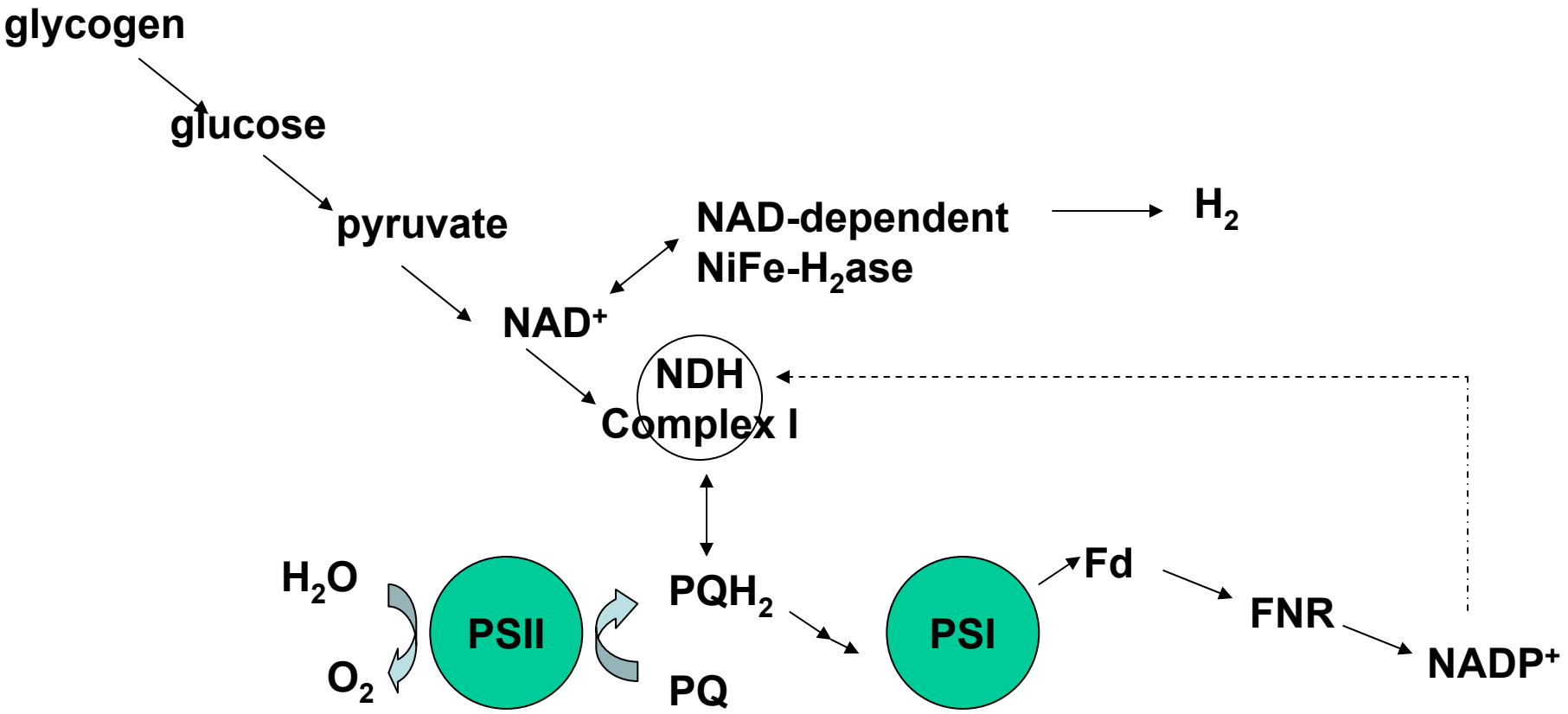
Dark H_2 reductant is stored under IR illumination

Fermentative H_2 restored in dark after IR light stopped at 2x higher rate

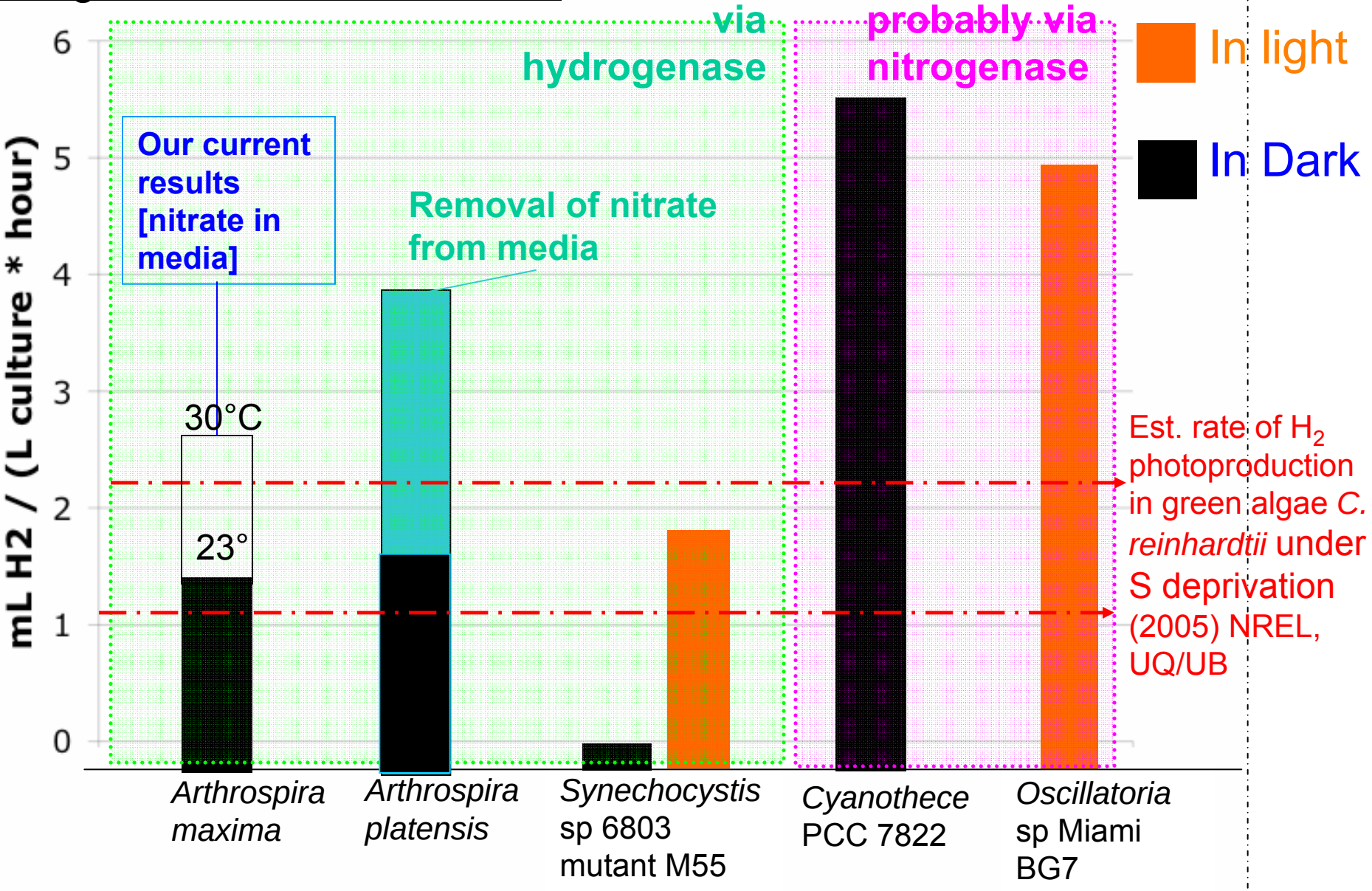
A. maxima


400
300
200
100
0
hydrogen, mV
640 nm
735 nm
0
20
40
60
80
time, min
PSI illumination
Suppresses fermentative H2
Slow photo-H2
PSI+PSII illumination
Adds to fermentative H2
Fast photo-H2

Model for Dark and Light H_2 production by *Arthrospira m.*



Sustained H_2 production rates: representative reports of cyanobacteria using water as electron donor



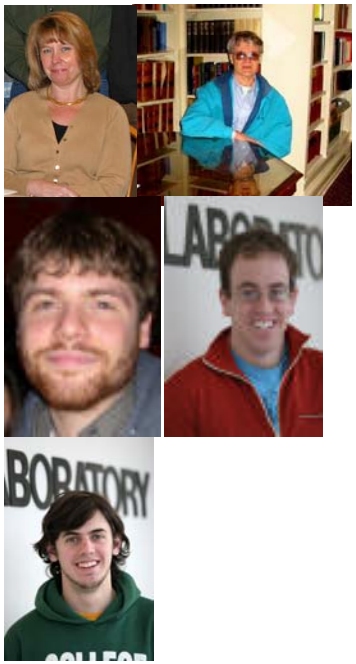
Conclusions

- New tools for measuring H_2, O_2 and redox status provide reliable quantitative data on concentrations and fluxes

- greatly simplify and clarify the fundamental mechanisms of H_2 production in microalgae and cyanobacteria

- Cyanobacteria & microalgae have different pathways to H_2

- Ideal temporal separation of H_2 and O_2 production in cyanobacteria solves gas separation problem



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