

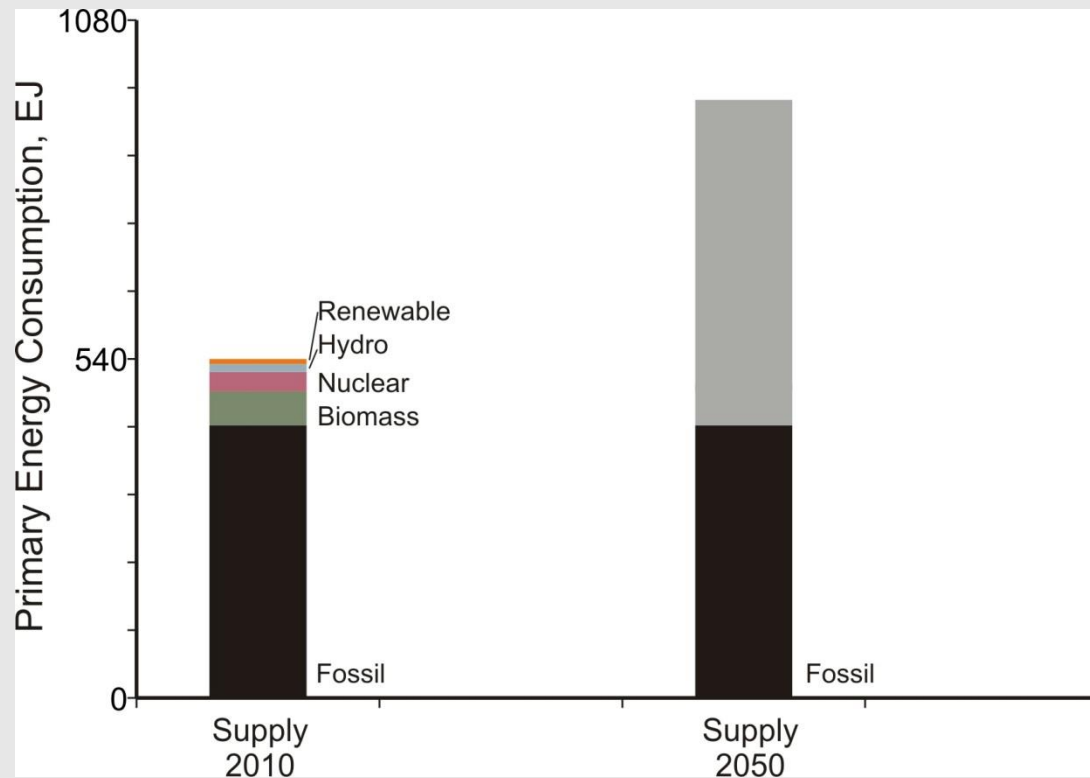


# Rational design of cyanobacteria for hydrogen production

**DPG Spring Meeting 2015**  
Arbeitskreis Energie (AKE)  
Berlin, 2015-03-17

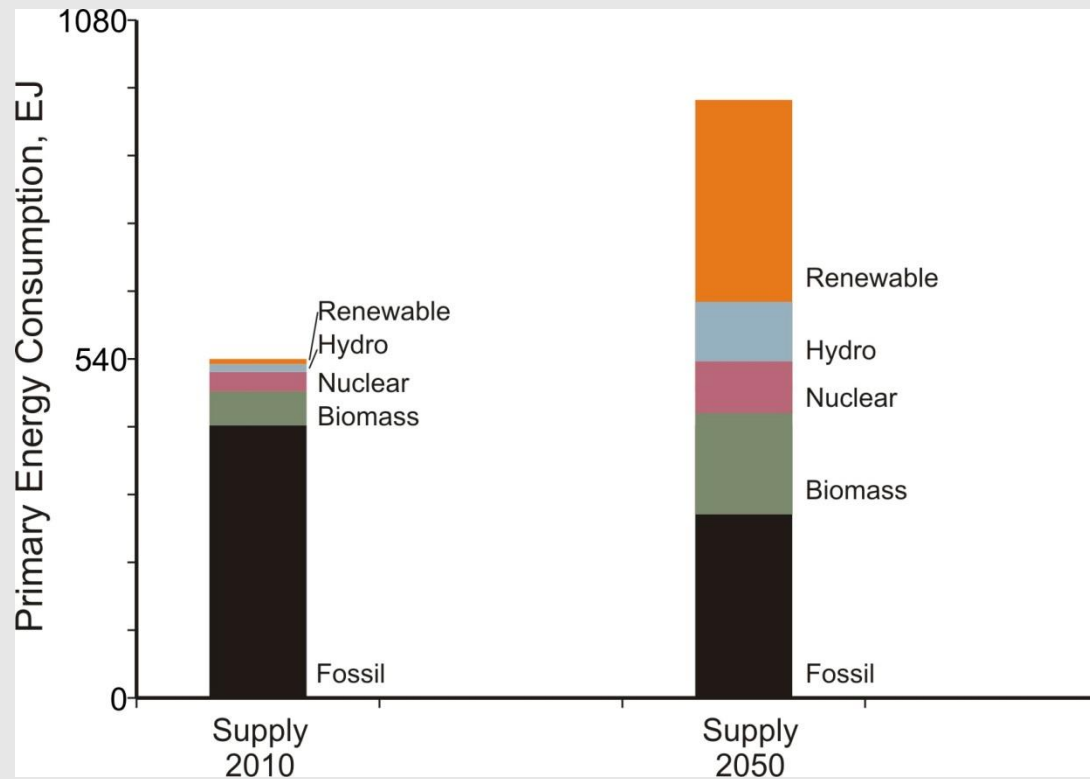
**Sascha Rexroth**, Matthias Rögner  
Plant Biochemistry  
Ruhr-University Bochum

# Global Energy Challenge



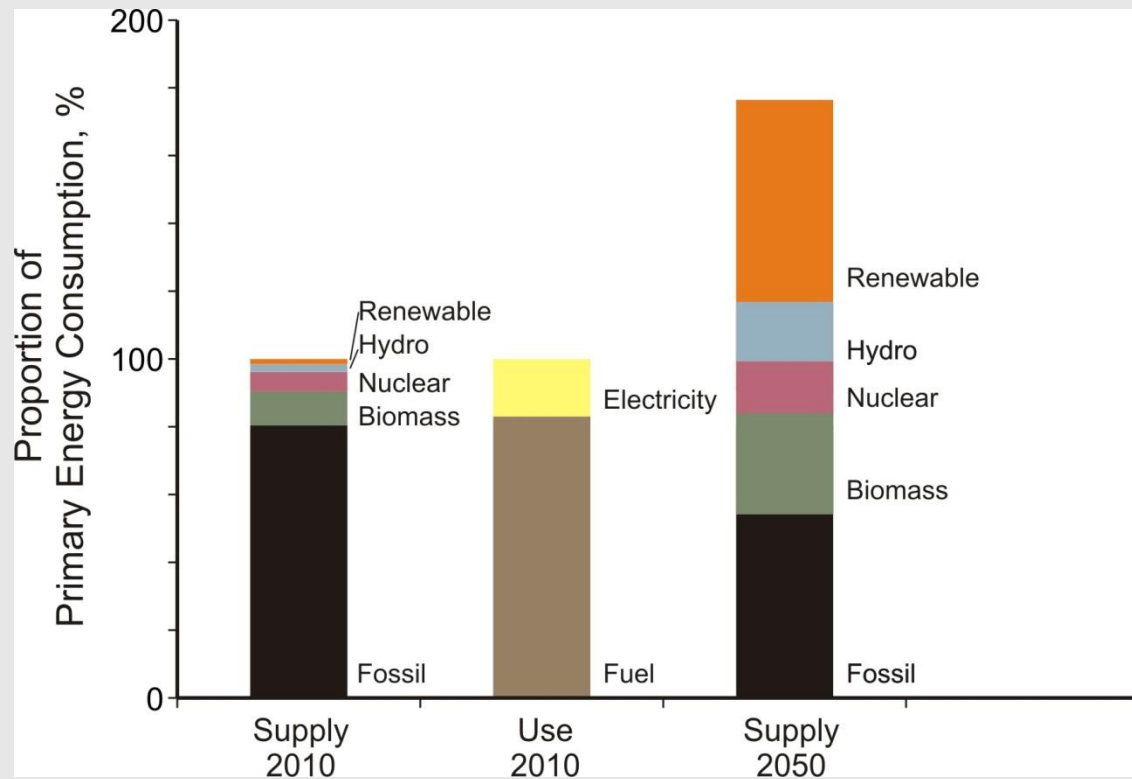
- 80% rise in Energy demand until 2050
  - Population growth
  - Developing countries

# Global Energy Challenge



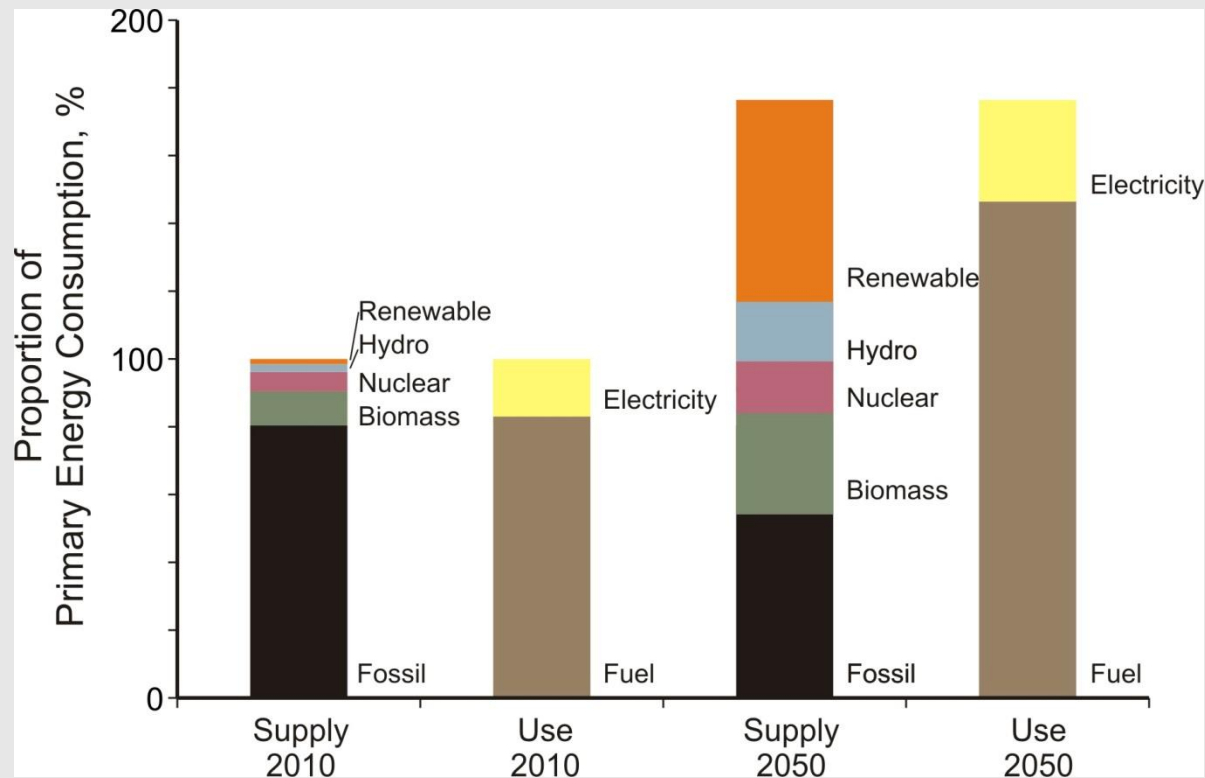
- 80% rise in Energy demand until 2050

# Global Energy Challenge



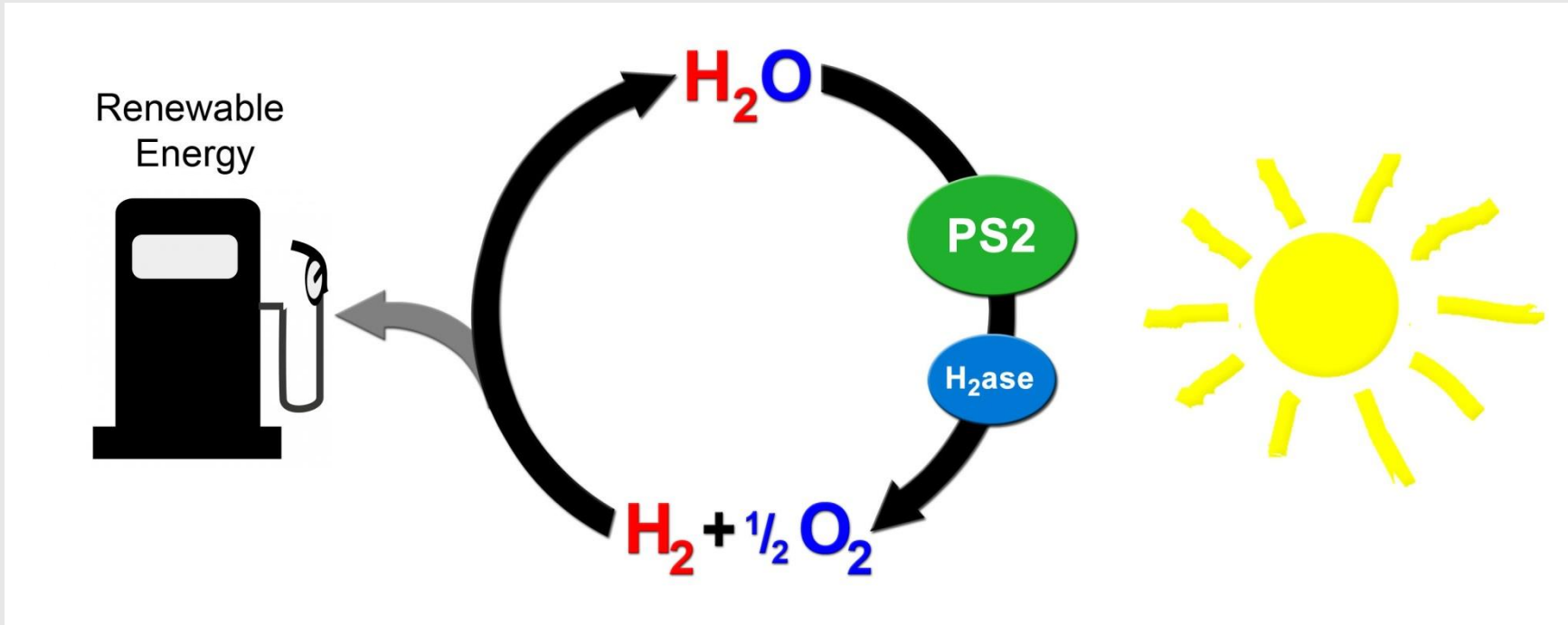
- 80% rise in Energy demand until 2050
- >80% of energy supply used as fuel

# Global Energy Challenge



- 80% rise in Energy demand until 2050
- >80% of energy supply used as fuel
- Fossil fuels + biomass not able to satisfy fuel demand
- Electricity is just one of many energy carriers

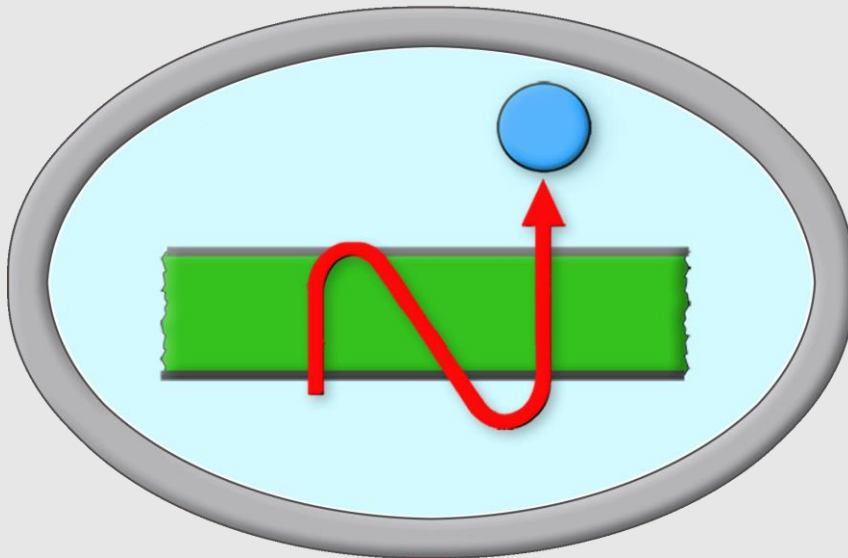
# Vision: Renewable energy source



Nature's catalysts:

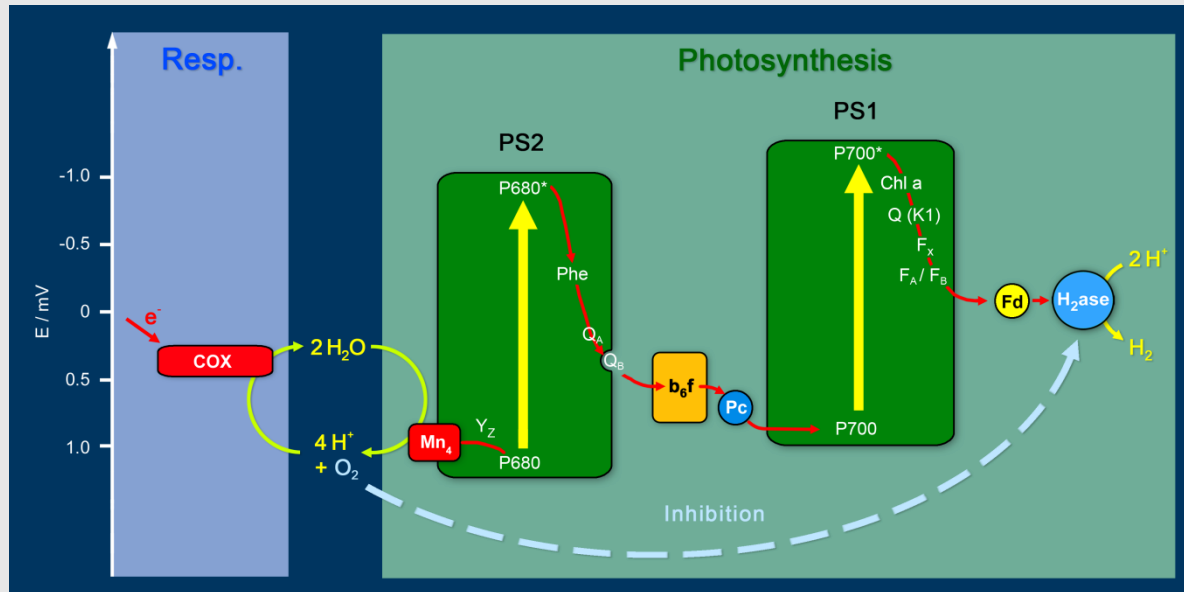
Photosystem 2 (PS2)  
Hydrogenase (H<sub>2</sub>ase)

# Whole cell instead of isolated catalyst



- Protection & stabilization of catalysts and self-repair (especially light damaged PS2)
- Self reproduction of cellular system (& scaling up for mass culture...)

# H<sub>2</sub> production in green algae



- **Advantage** : Very high activity of FeFe-H<sub>2</sub>ase (TOF ≤ 10,000 s<sup>-1</sup>)
- **Problems** : - Anaerobic conditions (due to O<sub>2</sub>-sensitivity of H<sub>2</sub>ase) reduce PS-capacity to about 5 %  
- Genetic manipulation for O<sub>2</sub>-tolerance mandatory

# Objectives for economical Bio-H<sub>2</sub> production (acc. LCA)

- Present H<sub>2</sub>-production per L cell culture (~ 2 ml H<sub>2</sub> L<sup>-1</sup> h<sup>-1</sup>) has to be increased by a factor ≥ 100



- Continuous H<sub>2</sub>-production under aerobic conditions (mass cultivation)



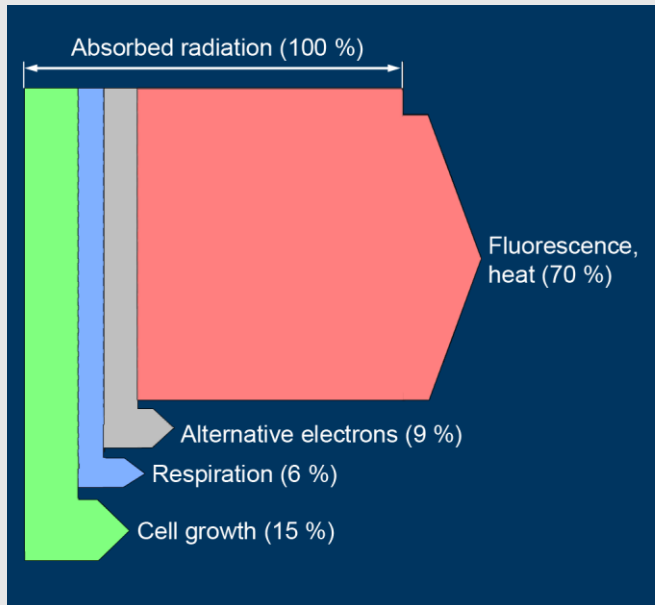
H.-J. Wagner  
RUB

# Cyanobacteria – Ideal host organisms

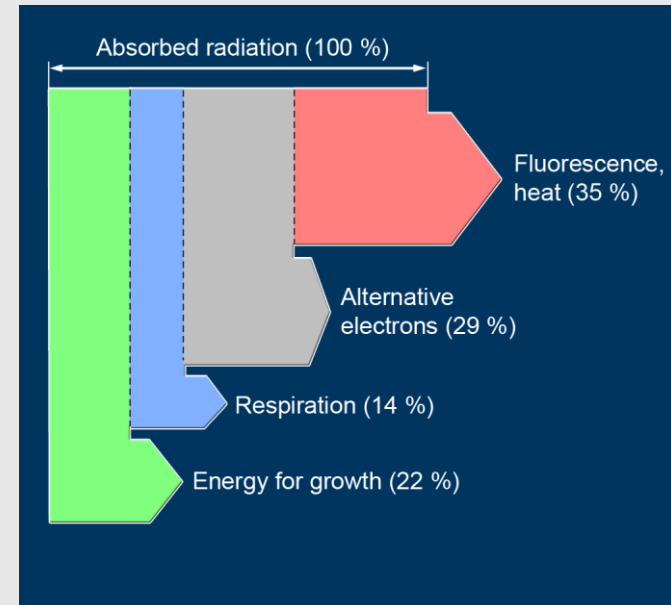


- Extreme environments:
  - Open sea & desert
  - Hot springs & glaciers
- > 10.000 species known,  
> 100.000 unknown
- Mass culture
  - Genetic transformability
  - Homologous recombination
  - Model organism:  
*Synechocystis* sp. PCC 6803

# Potential for optimization



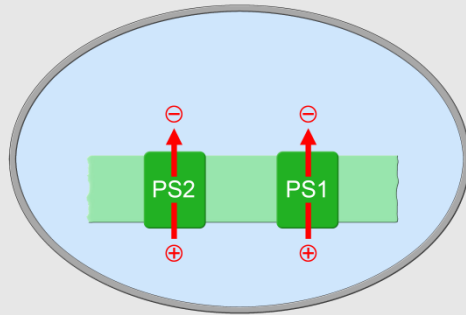
*Phaeodactylum tricornutum*  
(natural light conditions)



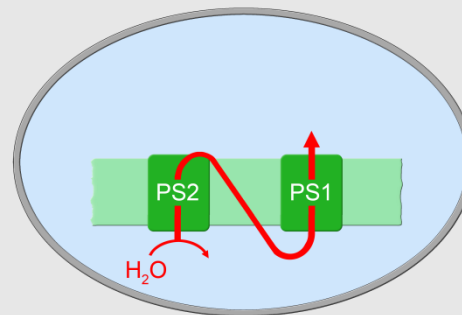
*C. reinhardtii* (C. Wilhelm 2012)  
optimized light conditions  
(const. light intensity near  $E_k$ -value)

- Dissipation of energy as heat and fluorescence
- >50 % lost to photoprotection
- H<sub>2</sub> production growth independent
- Significant part of energy available for H<sub>2</sub>

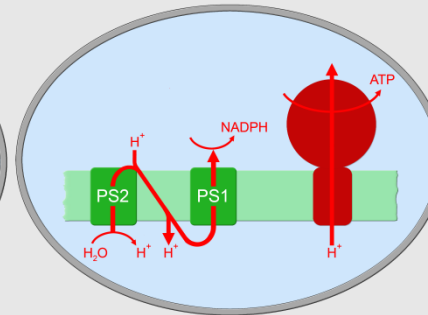
# Photosynthesis: efficiency & kinetics



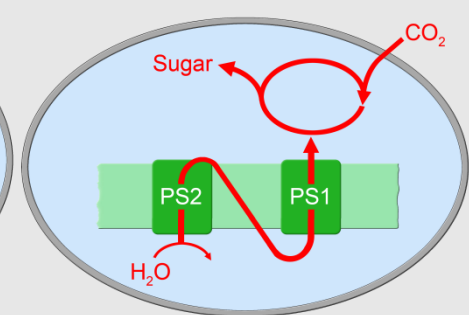
Efficiency  $\leq 99\%$   
Kinetics 20 ns



$\leq 38\%$  (theor.)  
20 ms



$\leq 27\%$  (theor.)  
ms-range



$\leq 1\%$  (biomass!)  
 $\leq$  s-range

- High efficiency of primary reaction  $\rightarrow$  reduced in subsequent steps

**Fast** "Light reactions" : Light capture in fs-range

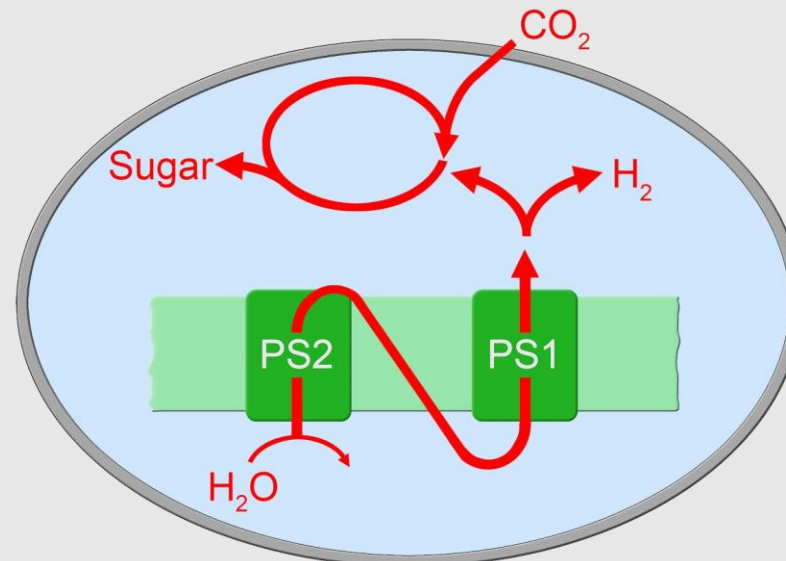
PS-electron transport with TOF = 50 - 200 s<sup>-1</sup>

**Limiting** "Dark reactions" :

TOF = 0.3 s<sup>-1</sup> (Rubisco)

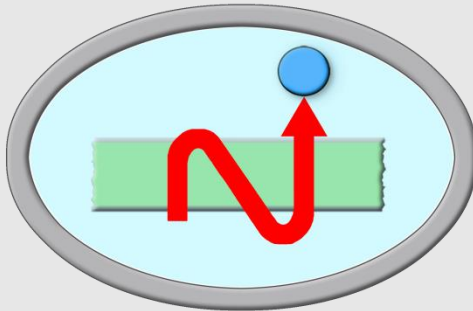
# Conclusions

- Tremendous over-capacity of PS light reactions should be used – large energy loss due to dark reactions!
- Establish **H<sub>2</sub> production** as new quenching mechanism



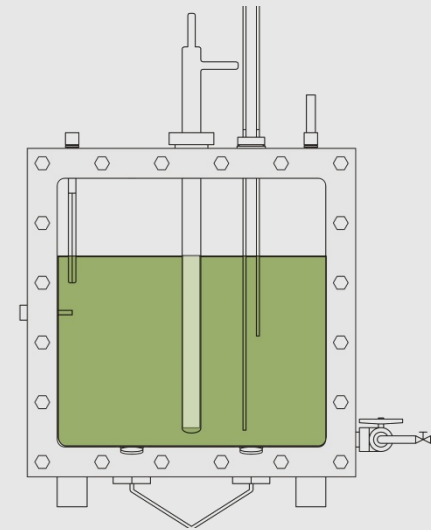
# Strategy

## Metabolic engineering



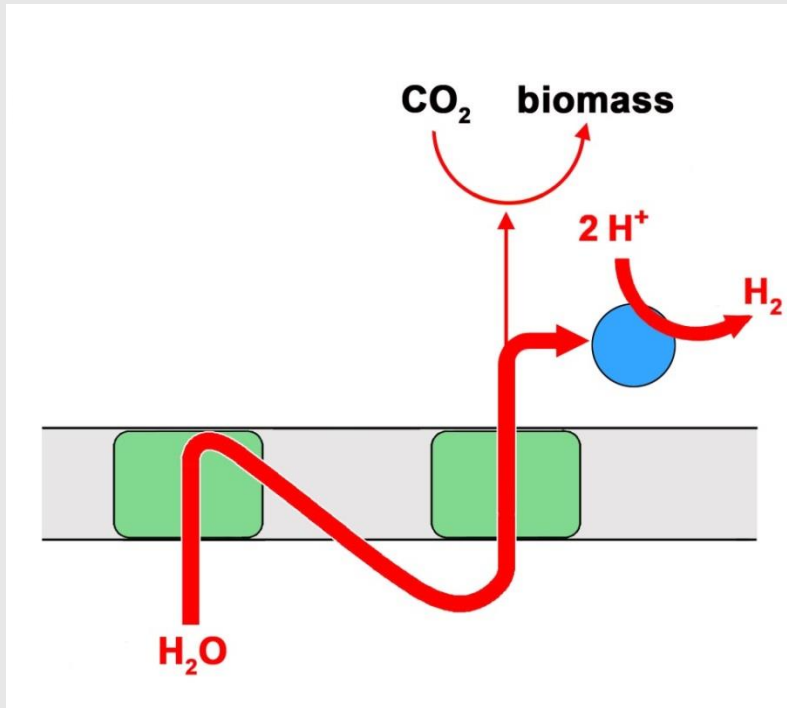
- $H_2$  producing cyanobacterial organism

## Process engineering



- Reduction of investment (<10% of available systems)

# Metabolic engineering



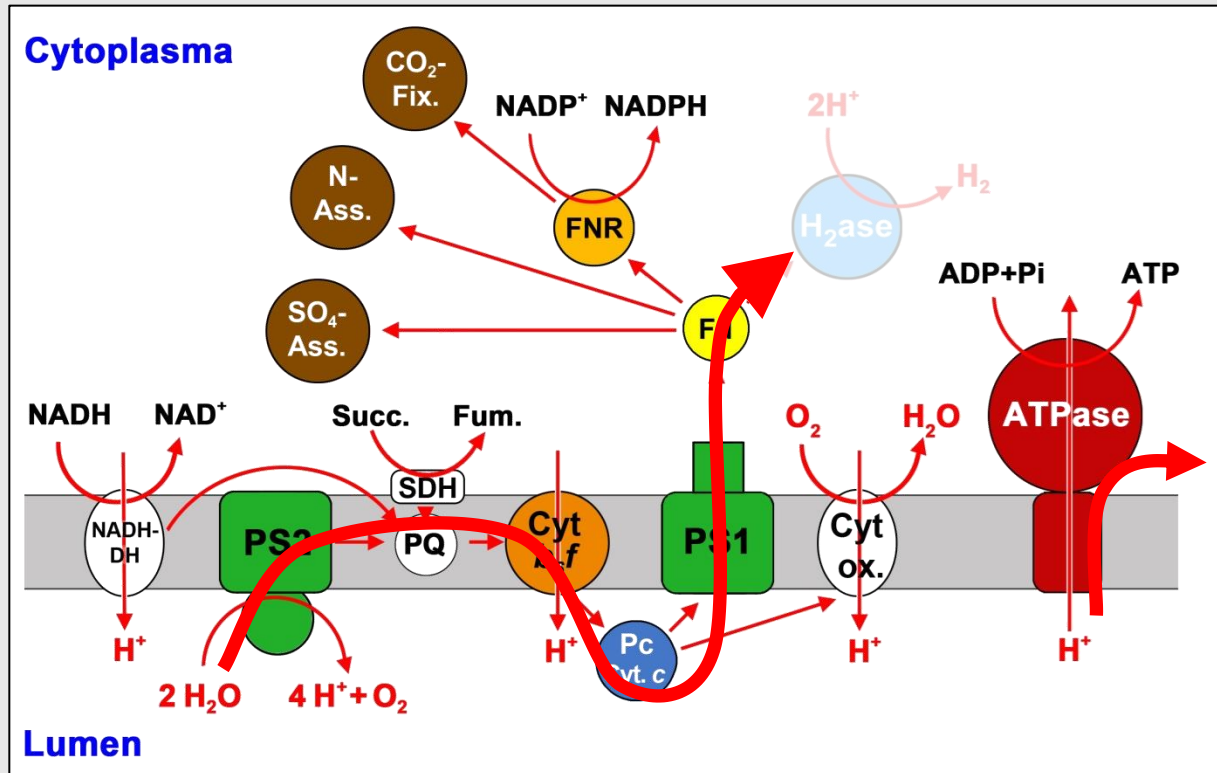
- Increase of linear electron transport
- Re-routing of electron transport
- Implementation of an engineered  $O_2$ -tolerant  $H_2$ -ase

**Biomass** ↓

**$H_2$ -production** ↑

(by modulation of protein interactions & engineering of  $H_2$ -ase)

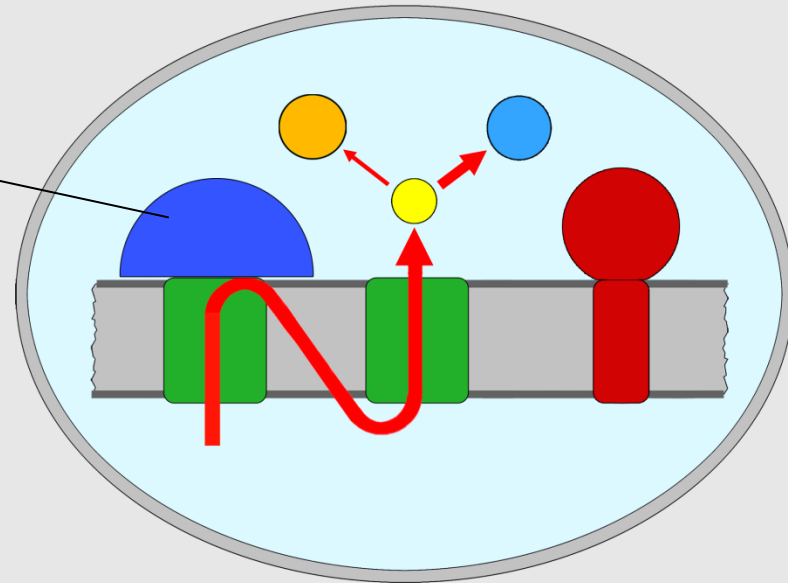
# Photosynthetic metabolism



- Produce compounds with high reduction potential (NADPH) and energy content (ATP)
- Reduce CO<sub>2</sub> to form carbohydrates

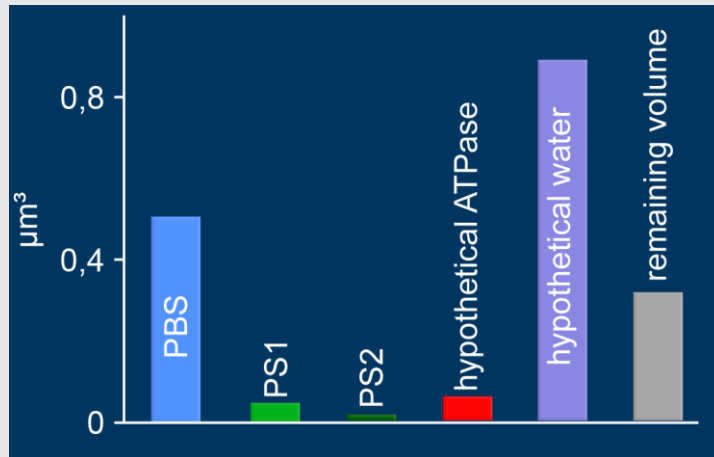


Phycobilisomes (PBS)



## Increase of photosynthetic ET by PBS antenna reduction

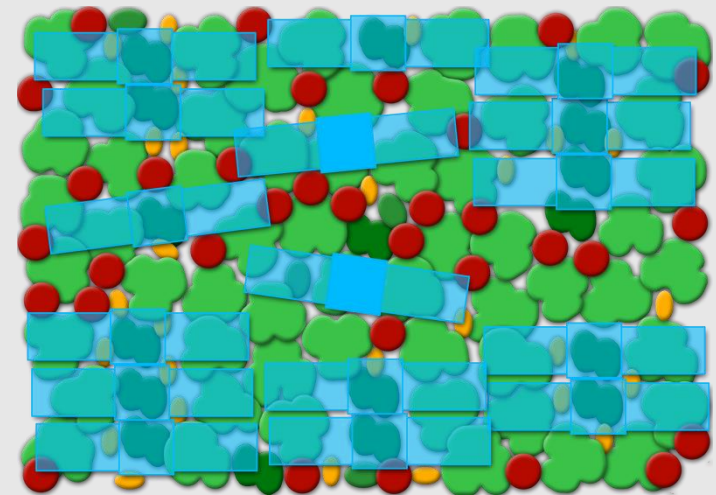
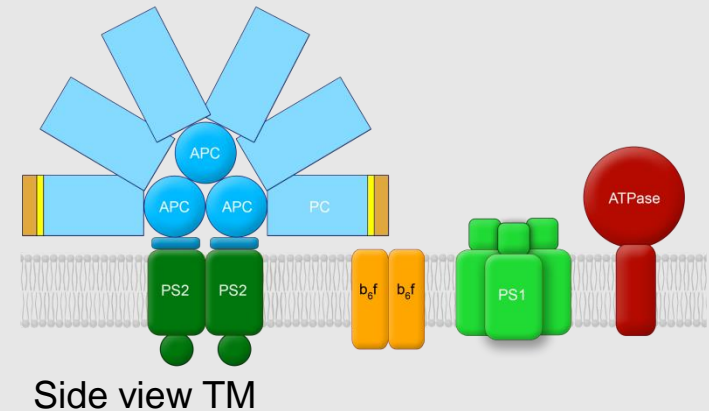
# PBS in *Synechocystis*



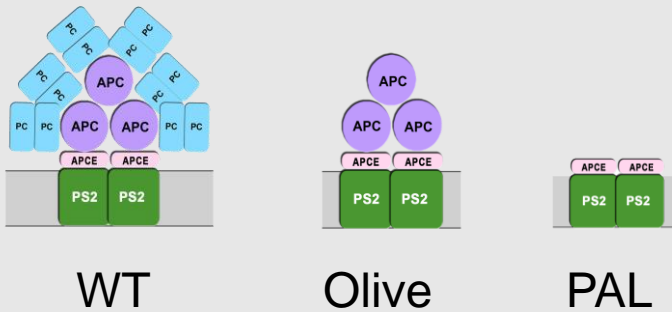
(Moal et al. 2012)

PBS antenna size reduction:

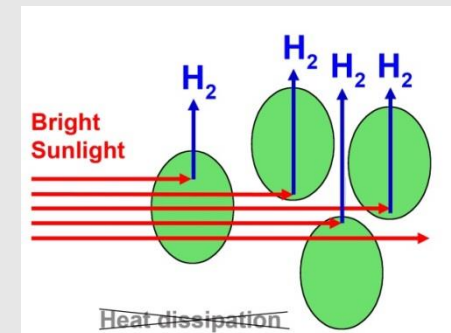
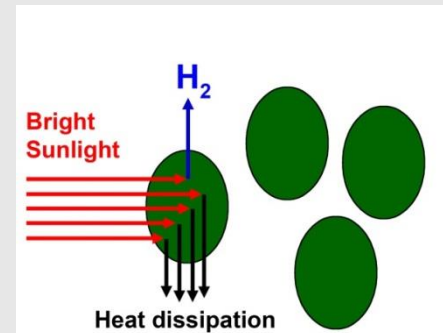
- Reduced photoinhibition – higher light tolerance
- Higher cell density in PBR
- Saving energy ( $\leq 63\%$  sol. prot.)



# PBS antenna size reduction

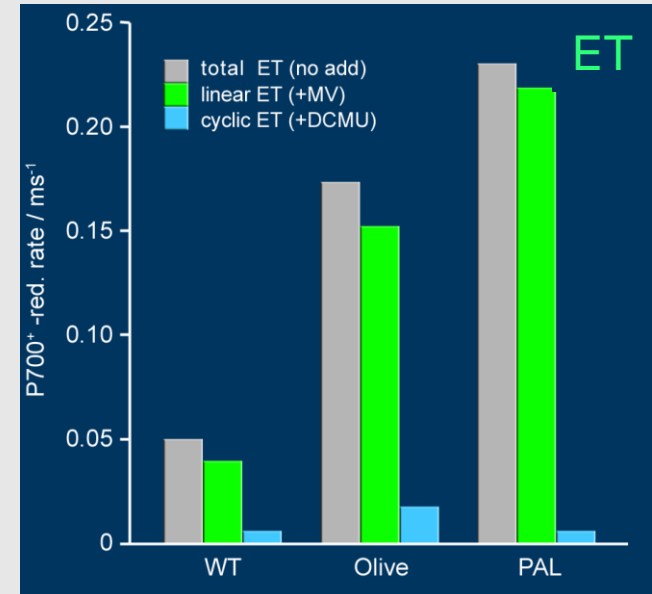


- 3-fold higher cell densities
- 4-fold higher light intensities tolerable

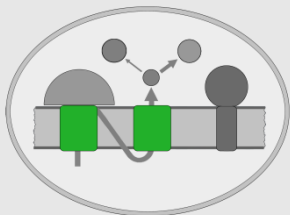


# Impact on PS2 / PS1 ratio

≤ 6-fold increased linear ET  
due to increased PS2 / PS1



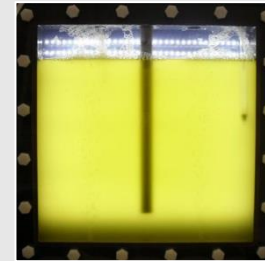
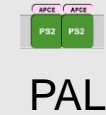
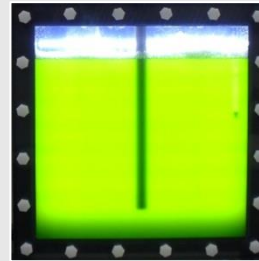
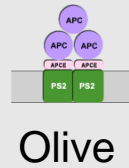
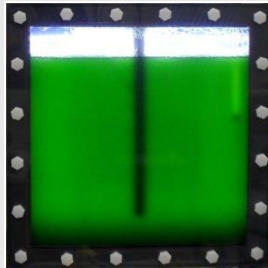
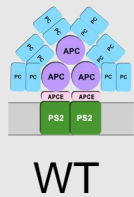
PS2/PS1



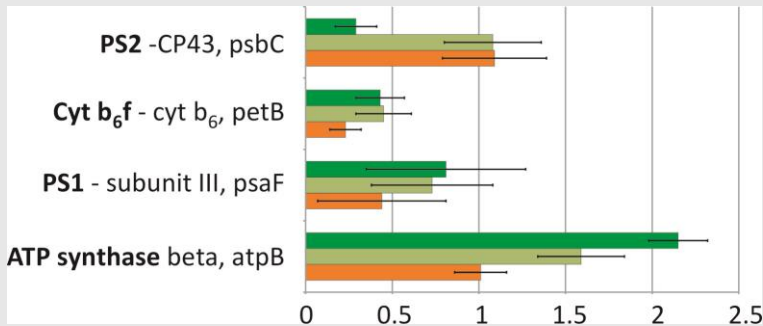
	Chl a per cell / fg	PS2 / % of WT	PS1 / % of WT	PS2 / PS1
WT	17.0	100	100	1:10
Olive	19.0	277	78	1:3
PAL	15.9	132	59	1:4

(collab. F. Mamedov, Uppsala University)

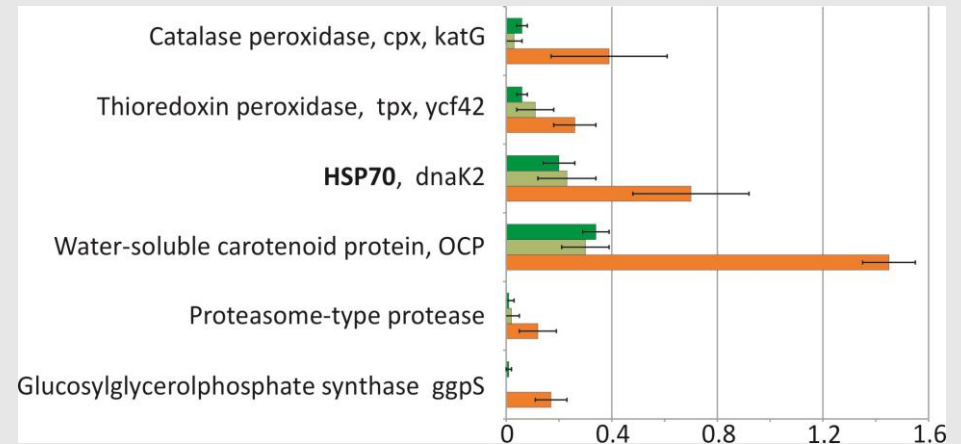
# Monitoring metabolism by proteomics under steady state conditions



Photosynthesis proteins

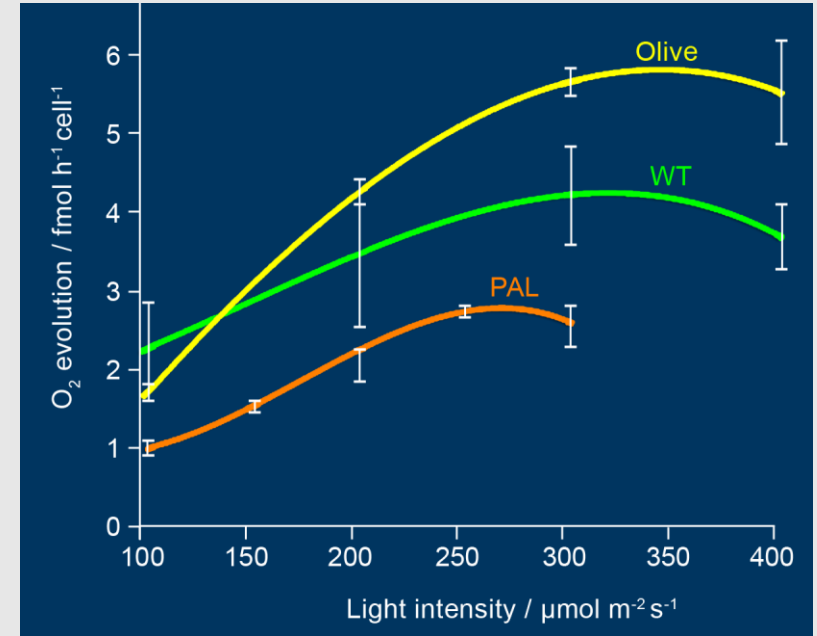
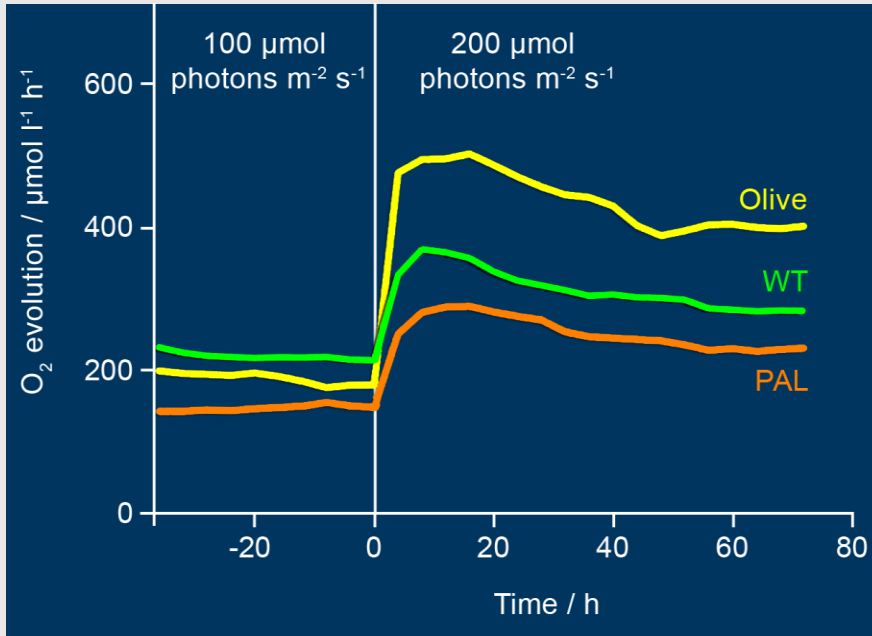


Stress indicator proteins



- Increased PS2 content
- PAL: Redox stress → Lack of electron sink

# Photosynthetic productivity of PBS-mutants

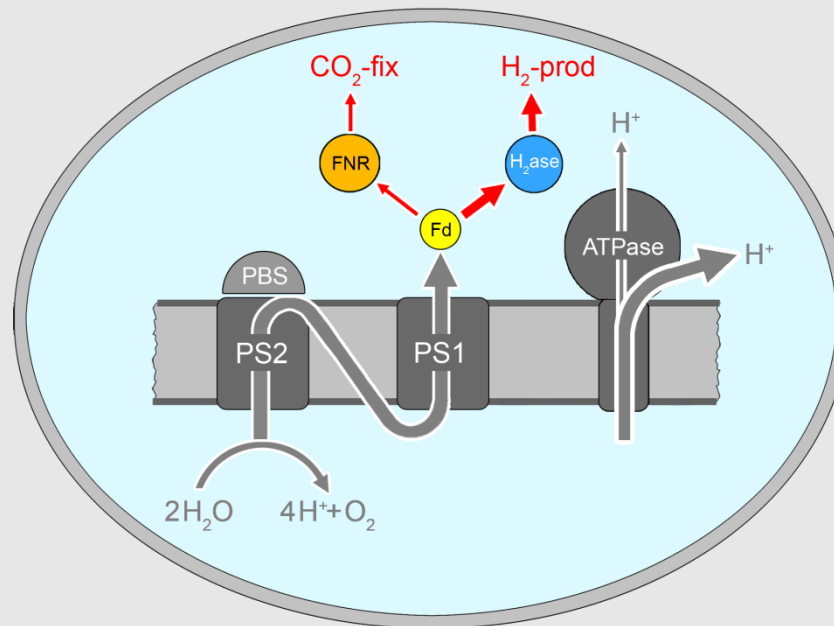


- Light intensity vs. O<sub>2</sub> evolution at steady-state conditions

**Conclusion:**

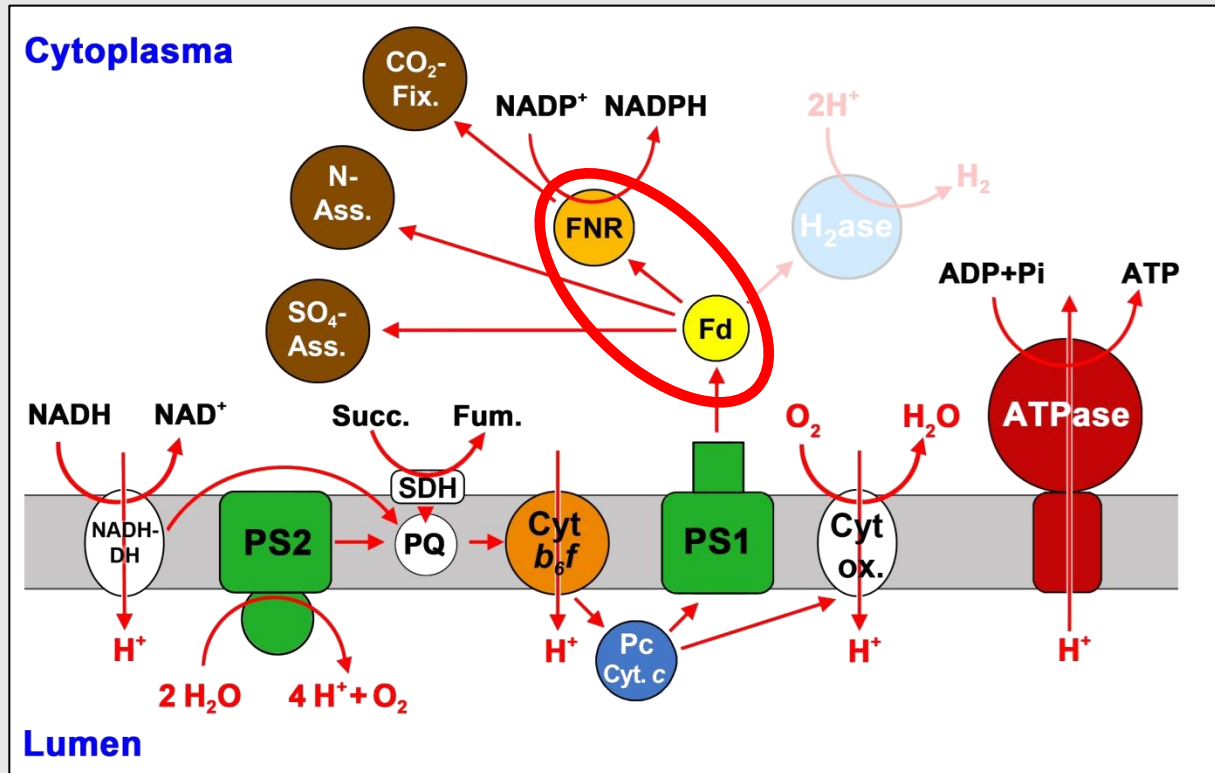
"Olive"-antenna mutant has highest potential as basis for design cell:

>150% activity of WT, higher light tolerance



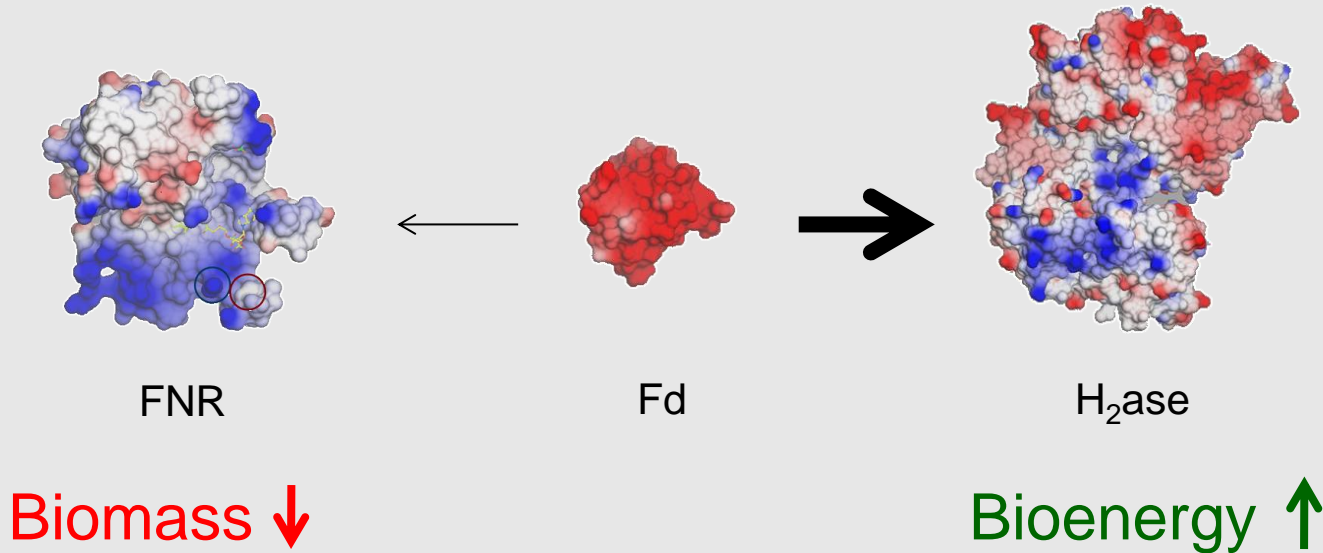
## Re-routing of the electrons

# FNR-Fd interaction: key factor for re-routing of electron transport



- **Minimizing the transfer towards CO<sub>2</sub>-fixation**
  - ~ 90% of electrons towards carbon fixation in WT
  - Reduction of biomass → increase of H<sub>2</sub> production

# Engineering Ferredoxin interaction

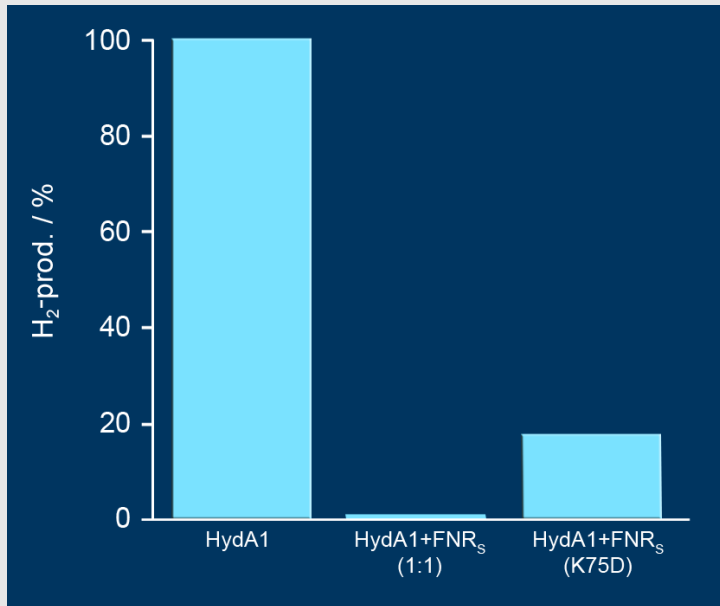


- Definition of interaction interfaces based on crystal structures
- Rational design of different FNR variants
- Targeted mutation of interaction sites

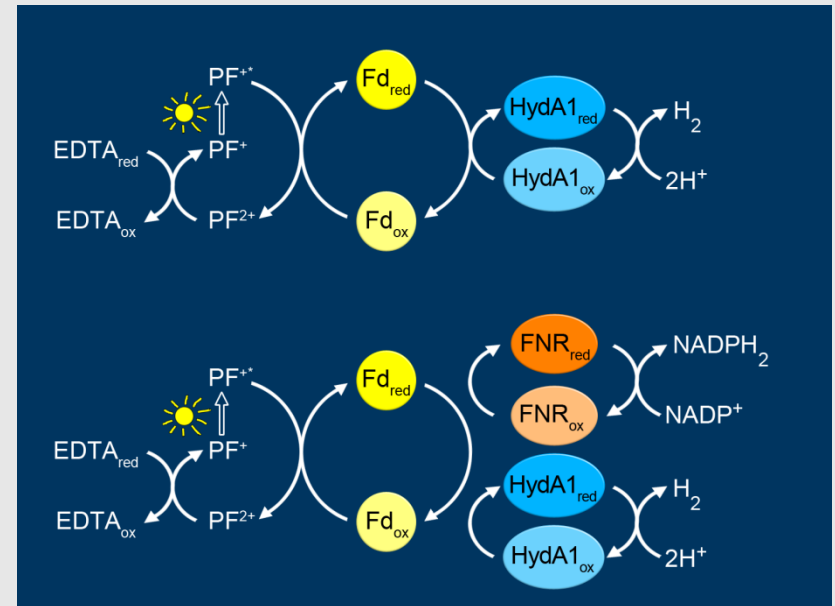


T. Hase G. Kurisu  
(Osaka Univ.)

# Minimizing CO<sub>2</sub> fixation

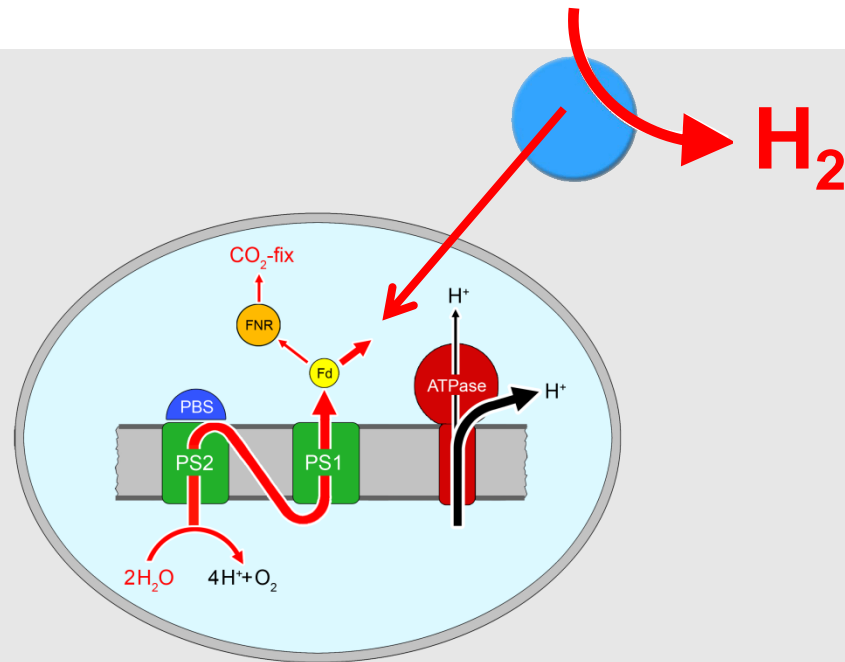


(K. Wiegand in collab. M. Winkler / T. Happe)



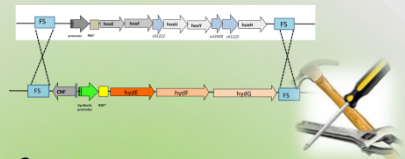
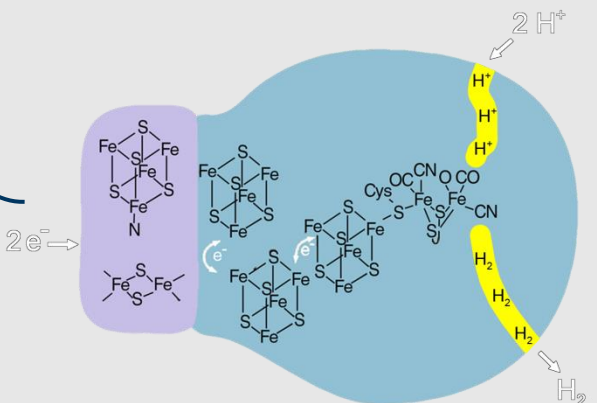
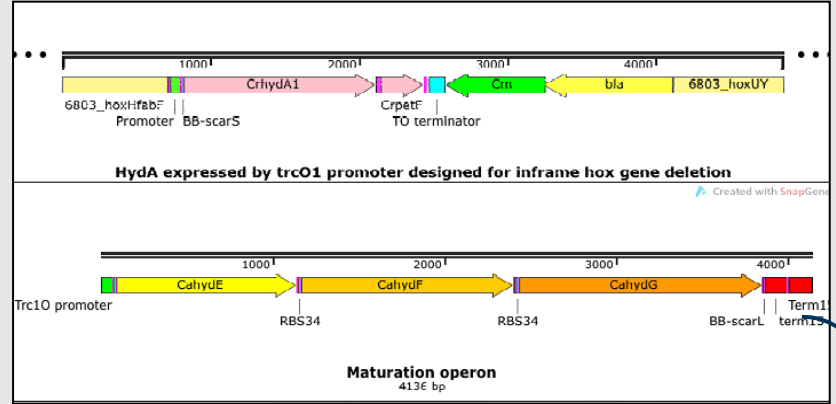
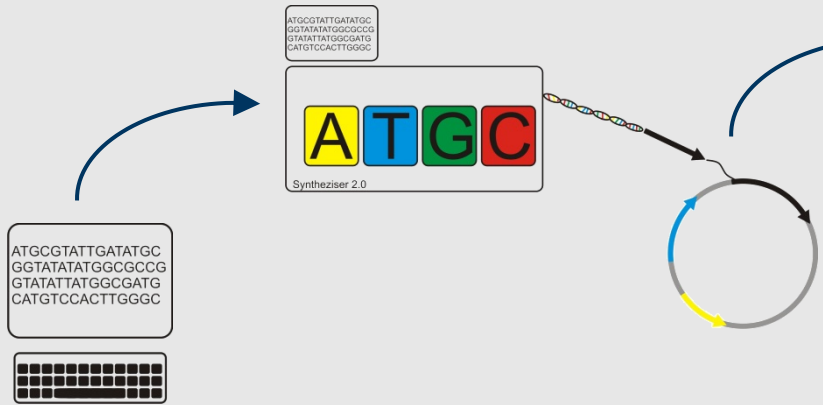
Proflavin-assay

- FNR and HydA1 compete for ferredoxin
- Apparently distinctly higher affinity of ferredoxin for FNR
- Indication that tuning of ET by mutant FNR is possible

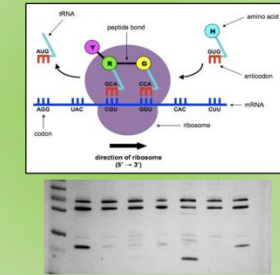


# Coupling photosynthetic ET with engineered H<sub>2</sub>ase

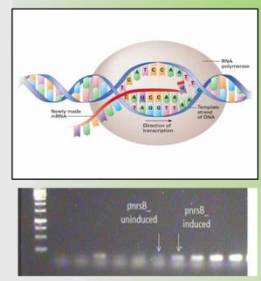
# Introduction of [FeFe] Hydrogenase into Cyanobacteria



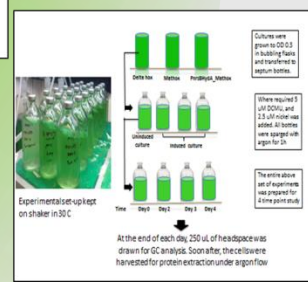
## 2. Translational analysis on Westerns



## 1. Transcriptional analysis on agarose gels

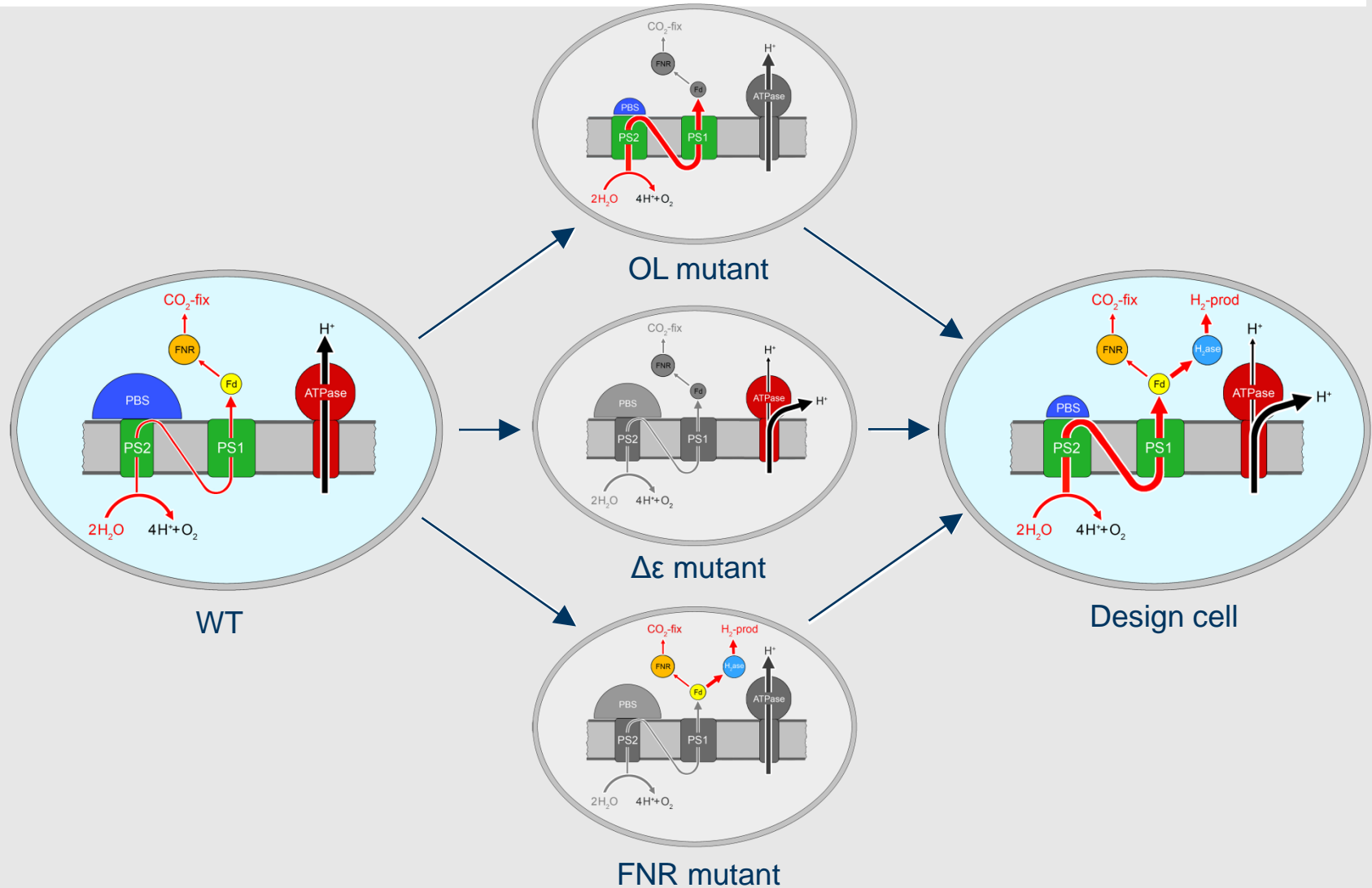


## 3. Activity assays

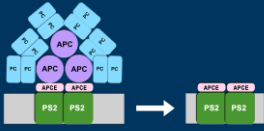
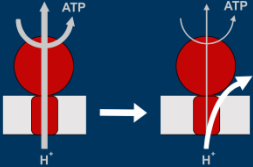
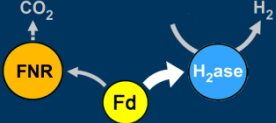
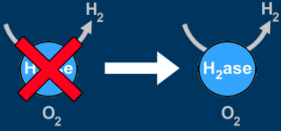


P. Lindblad, N. Khanna  
Uppsala University

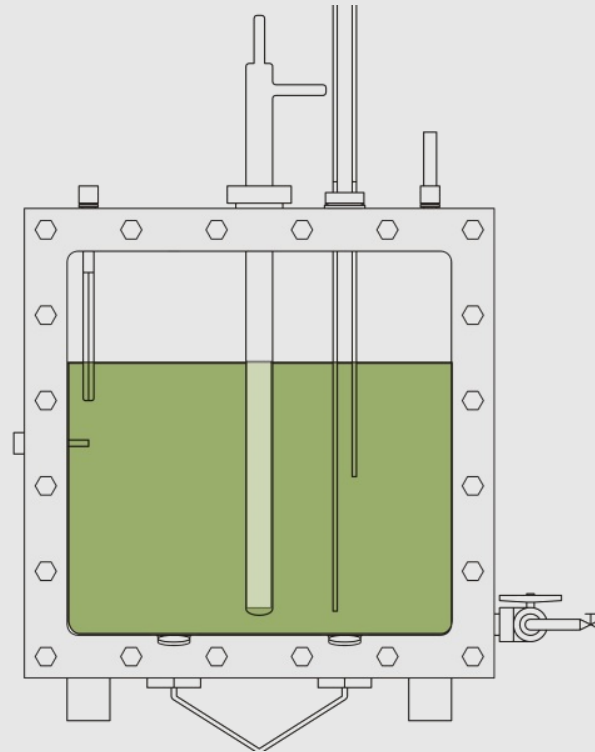
# Overall strategy for engineering "design cell"



# Summary & outlook (design cell)

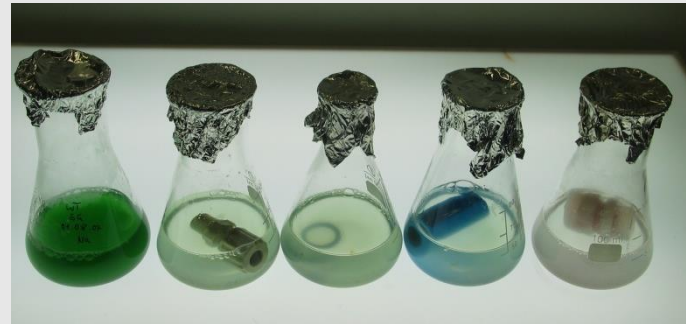
MutantsII	 <p>antenna red.</p>	 <p>uncoupling</p>	 <p>CO<sub>2</sub>-fix.</p>	 <p>O<sub>2</sub>-tolerance</p>
Present gain (factor LET)	>6 (PS2 ↑)	>2		
Future expectation			4-5	10-100

Conclusion: If all mutants are pooled in one design cell,  
>100x increase in H<sub>2</sub>-production can be achieved!



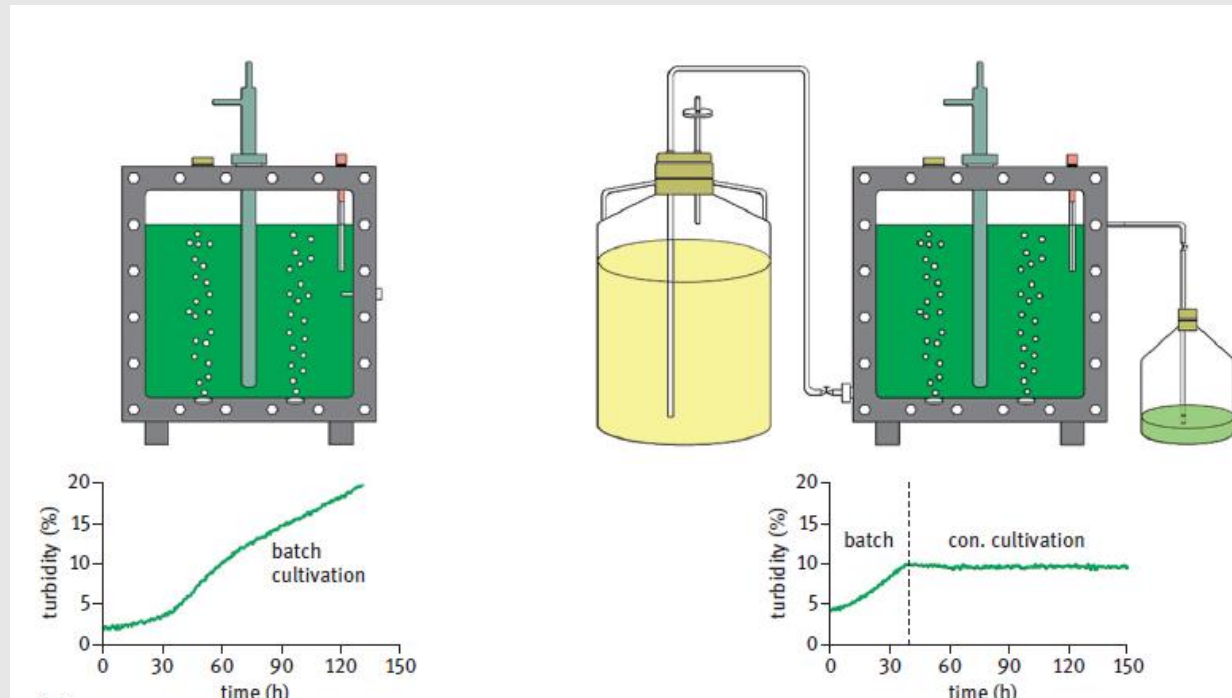
# Development of photobioreactor

# Optimization of reactor materials



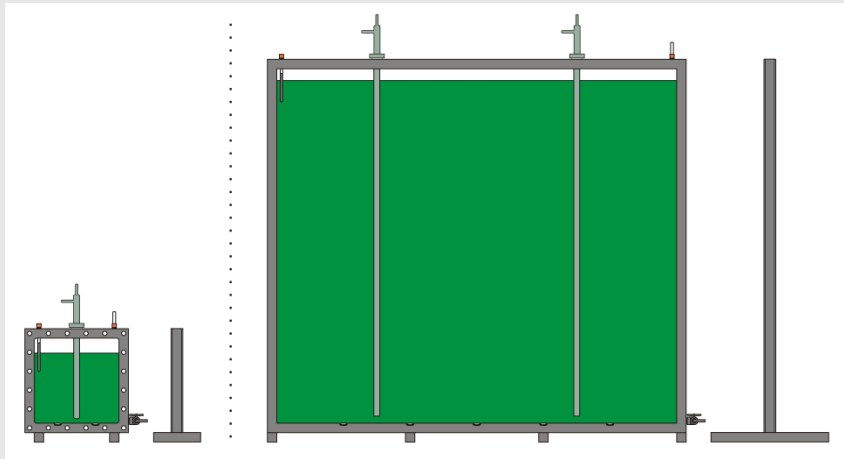
- Use of industrial semi-manufactured products (cooperation with KSD Innovations GmbH)
- Investigation of biocompatibility (frequently toxic effects)

# Photobioreactor



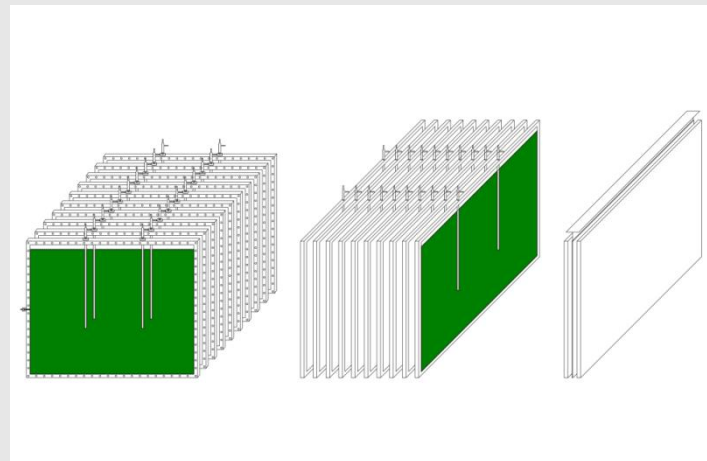
- Flat panel reactor (5 L, 40 mm light-path)
- Batch & continuous cultivation
- Invest. < 10 % of commercial reactors

# Scale-up

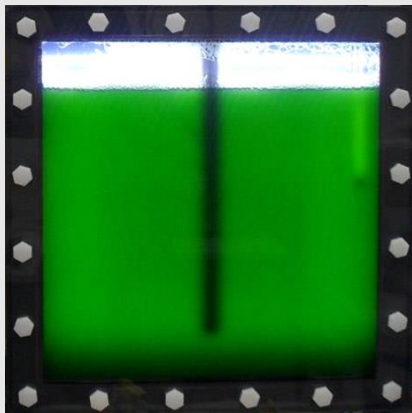
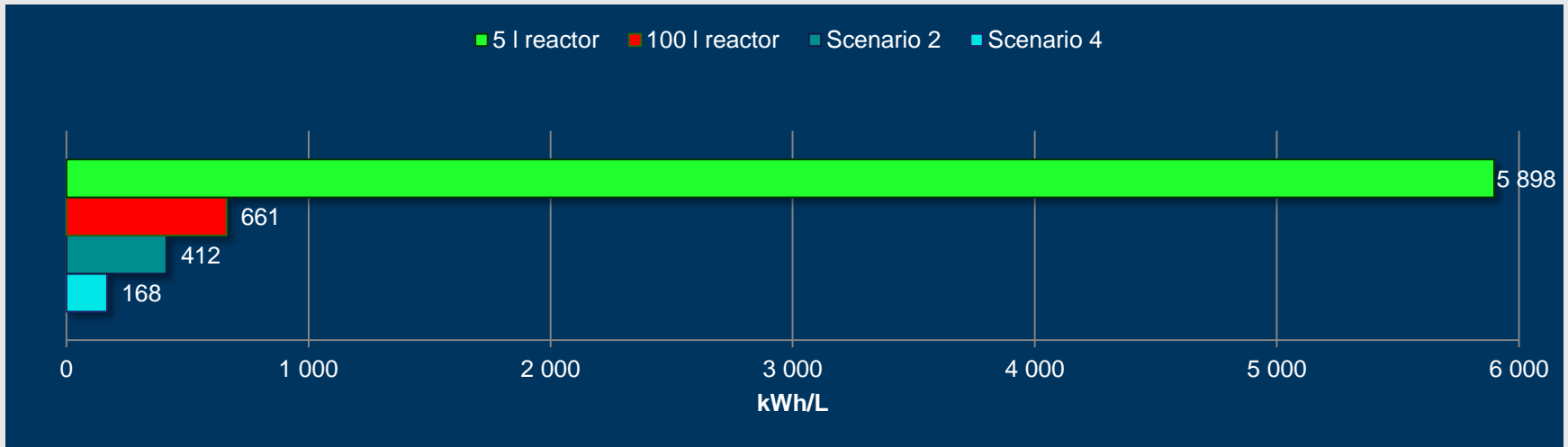


100 L

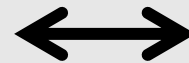
- Limitations for upscaling:
  - Light-penetration
  - Hydrostatic pressure
  - Mixing
- Significant upscaling by increasing number of reactor units



# Specific net energy consumption per reactor volume



5 L



100 L

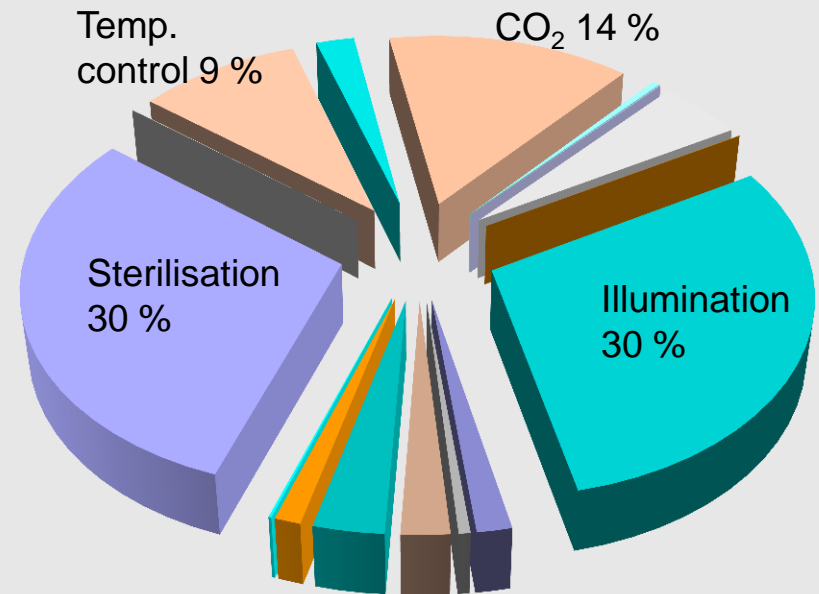


H.-J. Wagner

# Impact factors over life cycle (100 L PBR)

- Operation >99 % (19 % process materials, 80 % process energy)
- Production & disposal <1%

Cumulated energy demand (CED) – operational phase



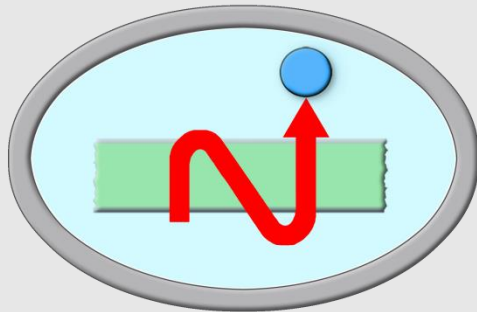
Conclusion:

Optimization potential in energy reduction for sterilization & illumination

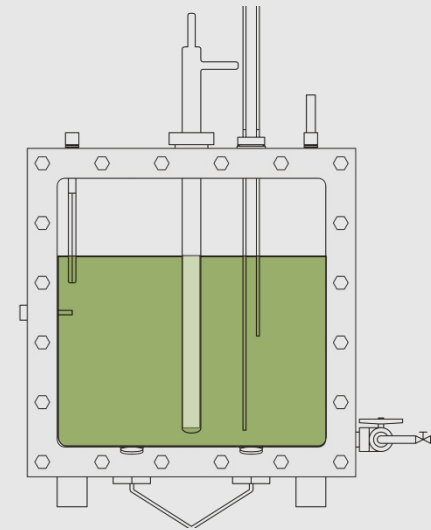


H.-J. Wagner

# Summary

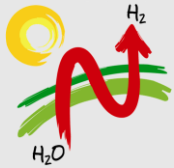


- H<sub>2</sub> producing cyanobacterial organism



- Reduction of investment (<10% of available systems)

# Acknowledgements RUB



Prof. Dr. Matthias Rögnér

Dr. Jong-Hee Kwon

Dr. Martin Broekmans

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Pasqual Liauw

Nadine Waschewski

Prof. Dr. T. Happe (Photobiotechnology)

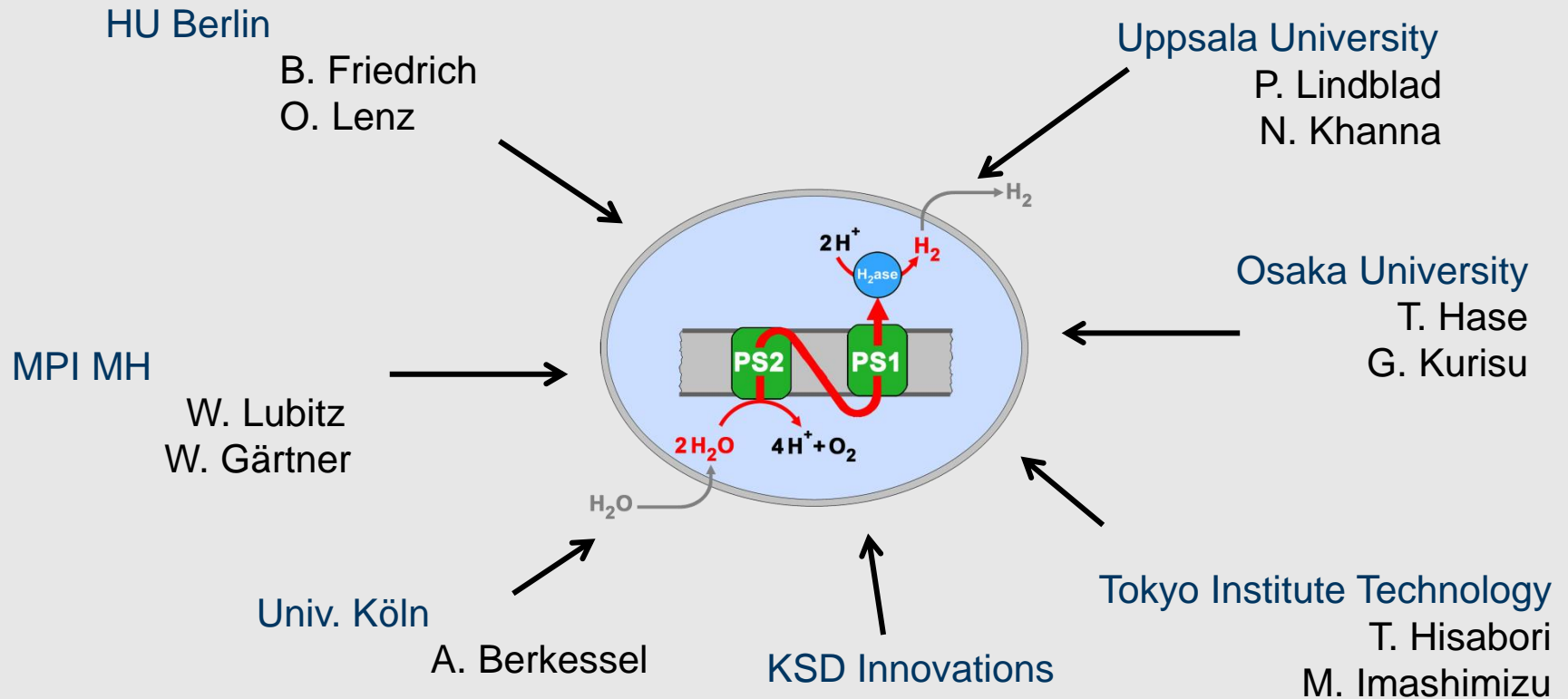
Dr. Martin Winkler

Prof. Dr. H.-J. Wagner (LEE)

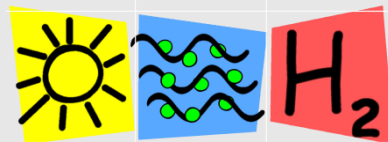
Dr. V. Rosner (LEE)



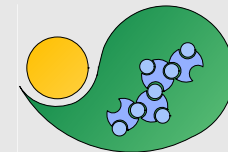
# Ext. coop. & funding



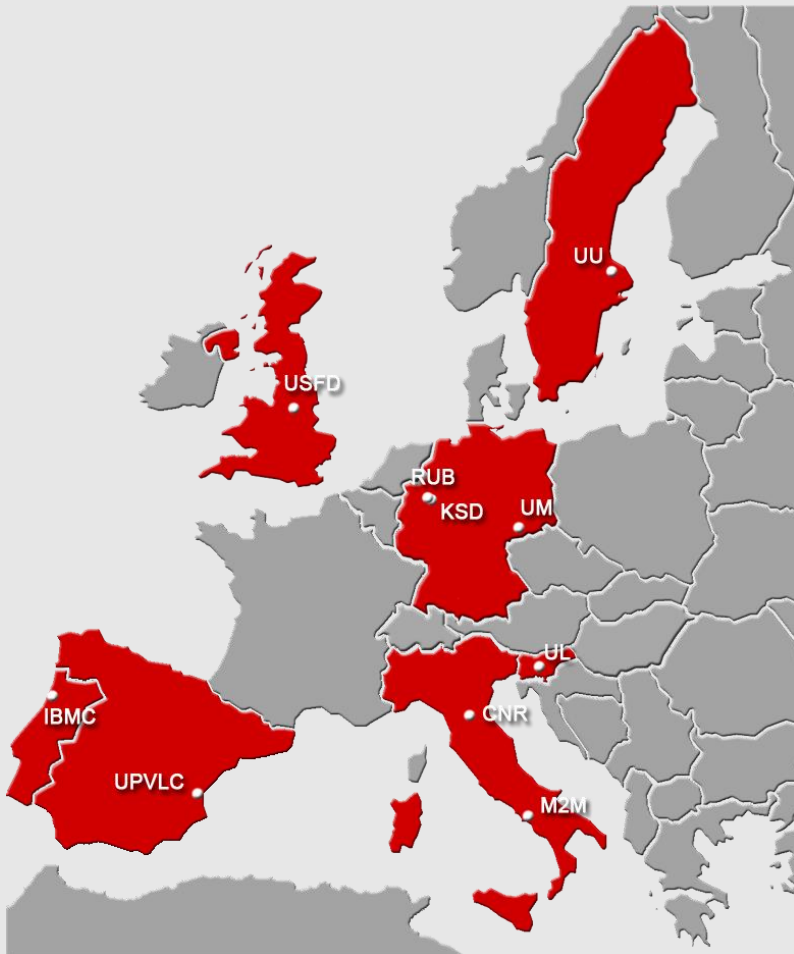
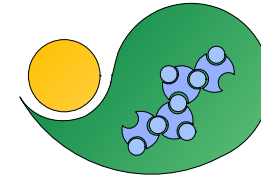
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Uppsala University (UU)

University of Applied Sciences Mittweida (UM)

Instituto de Biologia Molecular e Celular (IBMC)

Ruhr-University-Bochum (RUB)

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