Plant Biochemistry

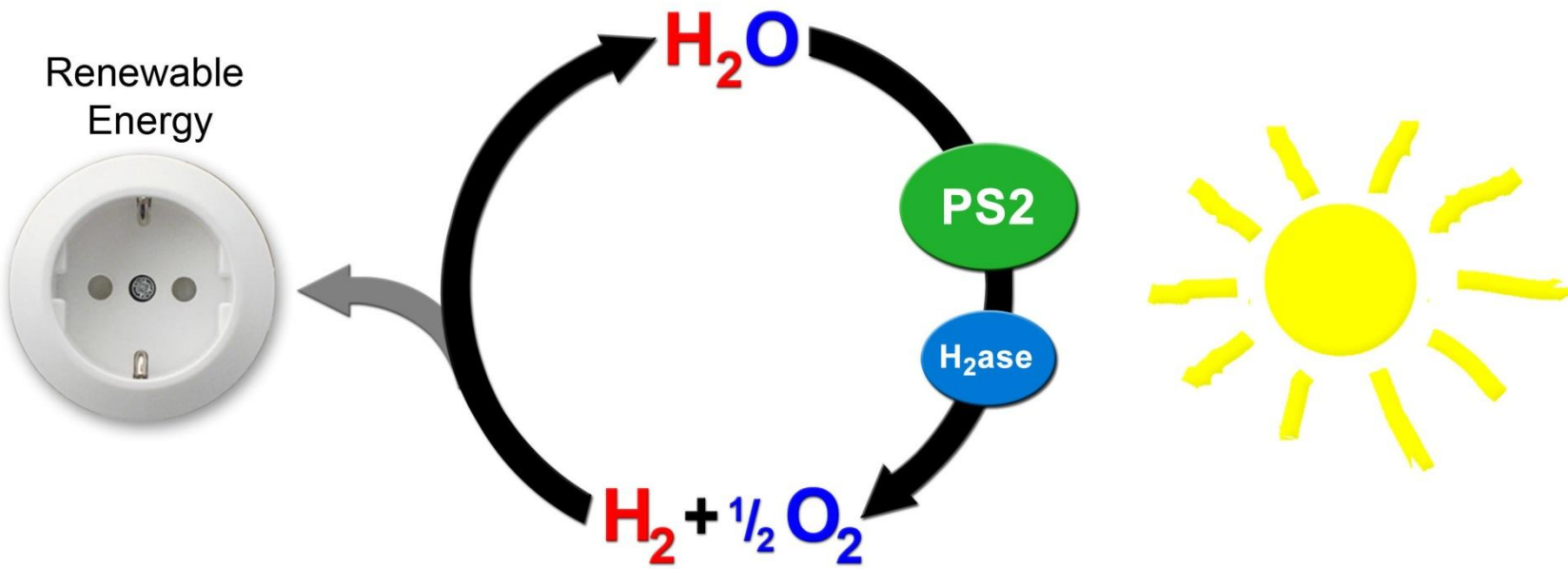
RUB

Rational design of cyanobacteria for hydrogen production

Renewables – *systems and storage*
Stockholm, 2013-09-19

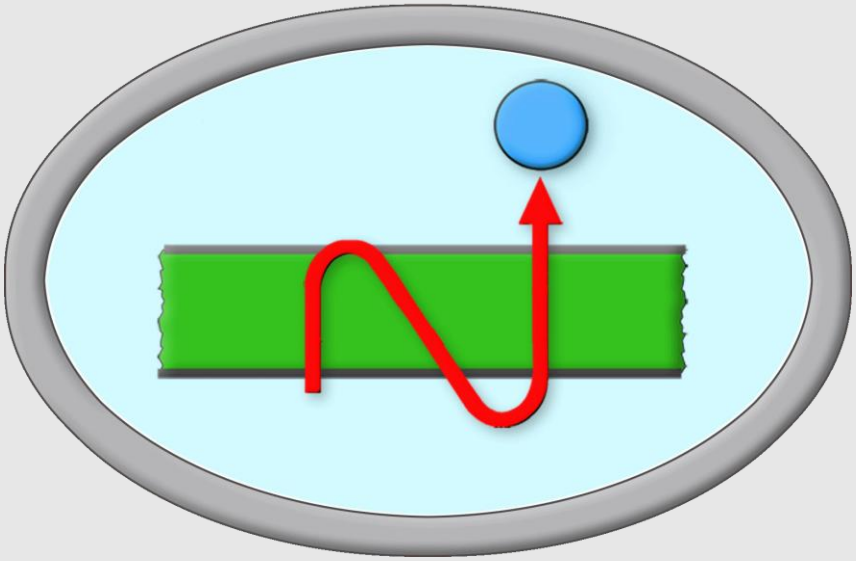
Sascha Rexroth

Vision: Renewable energy source



Nature‘s catalysts: Photosystem 2 (PS2)
Hydrogenase (H_2ase)

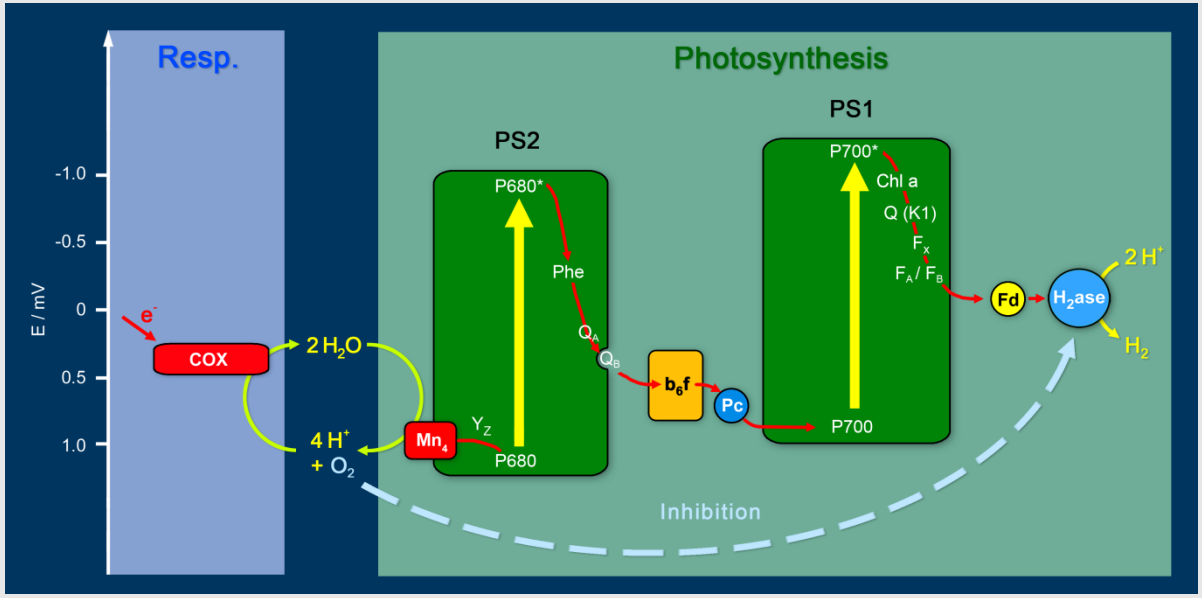
Whole cell instead of isolated catalyst



- Protection & stabilization of catalysts and self-repair (especially light damaged PS2)
- Self reproduction of cellular system (& scaling up for mass culture...)
- PS-apparatus needed for H_2-production (sun as "power supply“)

H_2 production in green algae

C. reinhardtii



- Advantage : Very high activity of FeFe-H_2ase (TOF ≤ 10,000 s^{-1})
- Problems : - Anaerobic conditions (due to O_2-sensitivity of H_2ase) reduce PS-capacity to about 5 %
  - Genetic manipulation for O_2-tolerance mandatory

Objectives for economical Bio-H_2 production (acc. LCA)

- Present H_2-production per L cell culture (~ 2 ml H_2 L^{-1} h^{-1}) has to be increased by a factor ≥ 100

C. reinhardtii

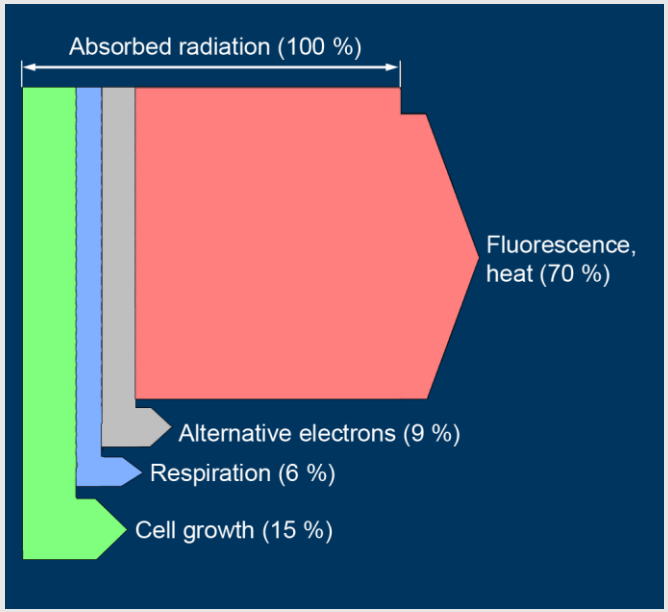
- Continuous H_2-production under aerobic conditions (mass cultivation)

H.-J. Wagner
RUB

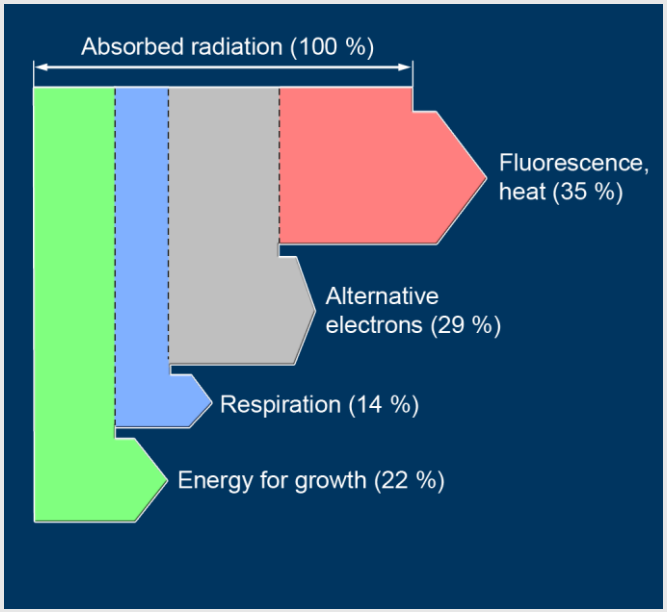
Cyanobacteria – Ideal host organisms

- 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)

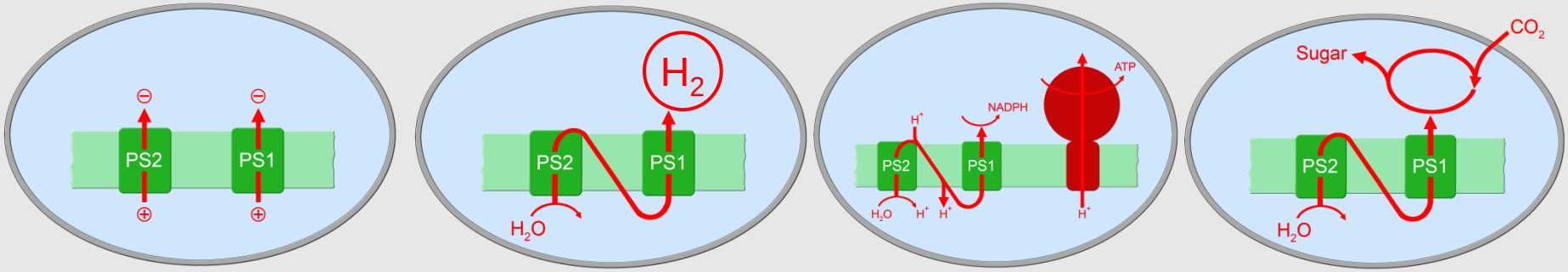


C. reinhardtii (C. Wilhelm 2012)
optimized light conditions
(const. light intensity near Ek-value)

- Dissipation of energy as heat and fluorescence
- >50 % lost to photoprotection
- H_2 production growth independent

➢ Significant part of energy available for H_2

Photosynthesis: Quantum efficiency & kinetics



Q-Efficiency ≤ 99 % | ≤ 38 % (theor.) | ≤ 27 % (theor.) | ≤ 1 % (biomass!)

- High efficiency of primary reaction → reduced in subsequent steps

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

PS-electron transport with TOF = 50 - 200 s^{-1}

Limiting "Dark reactions" :

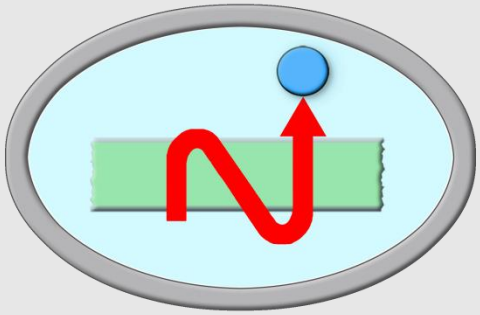
TOF = 0.3 s^{-1} (Rubisco)

Conclusions:

- Direct coupling to primary events of PS for high efficiency !
- Tremendous over-capacity of PS light reactions should be used – large energy loss due to dark reactions!

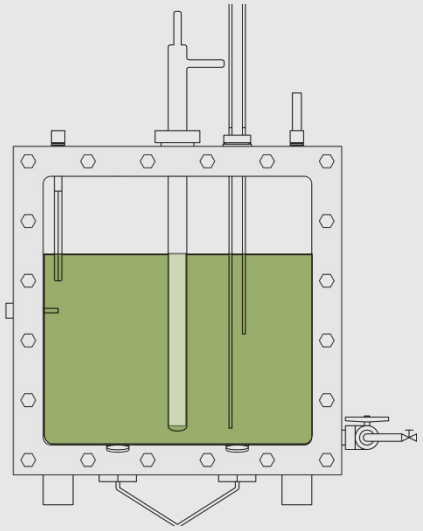
Strategy

Metabolic engineering



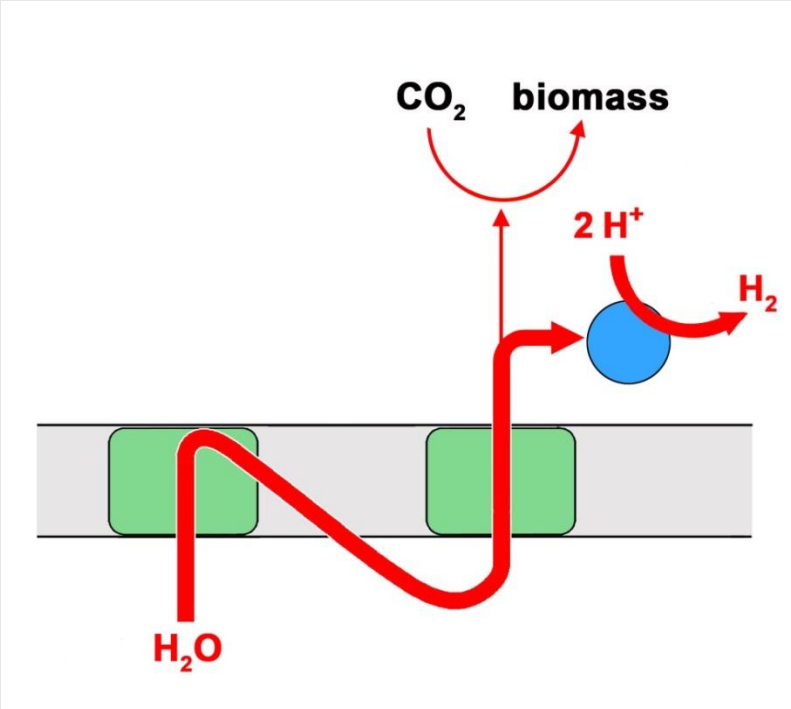
- H_2 producing cyanobacterial organism

Process engineering



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

Metabolic engineering



- Increase of linear electron transport
- Re-routing of electron transport

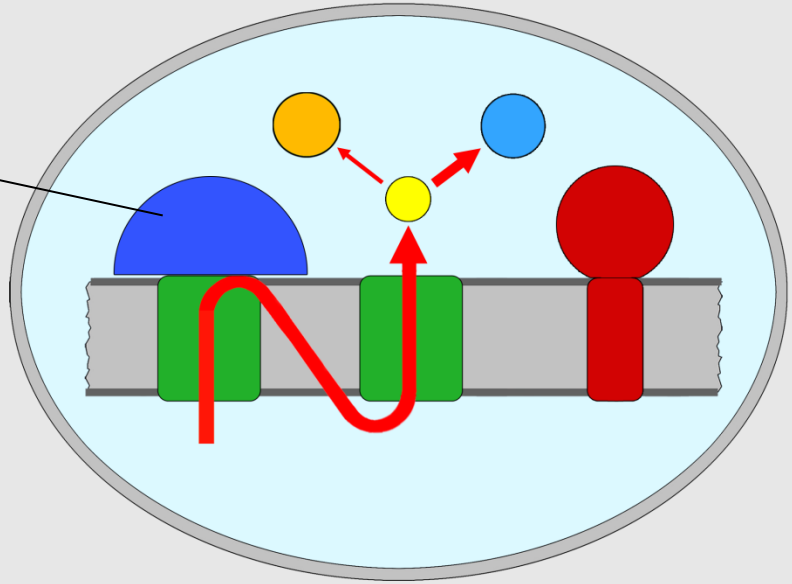
- Implementation of an engineered O_2-tolerant H_2-ase

Biomass ↓

H_2-production ↑

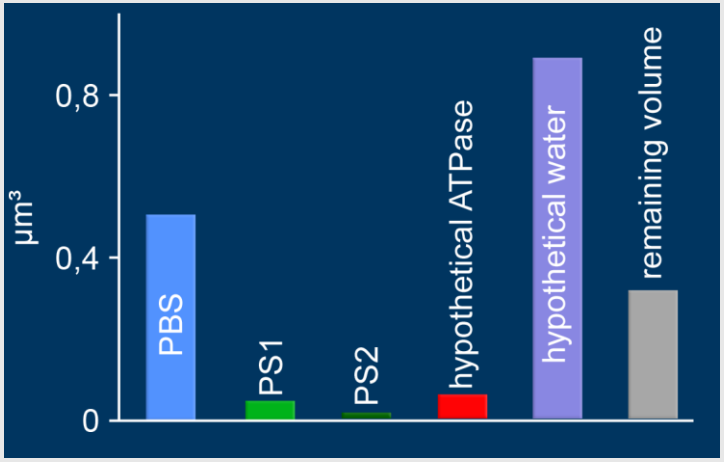
(by modulation of protein interactions & engineering of H_2ase)

Phycobilisomes (PBS)

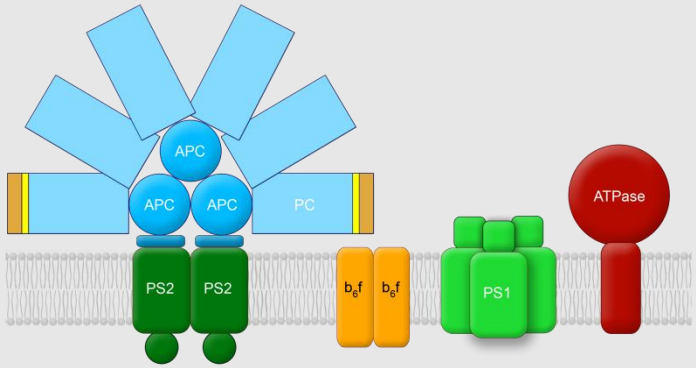


Increase of photosynthetic ET by PBS antenna reduction

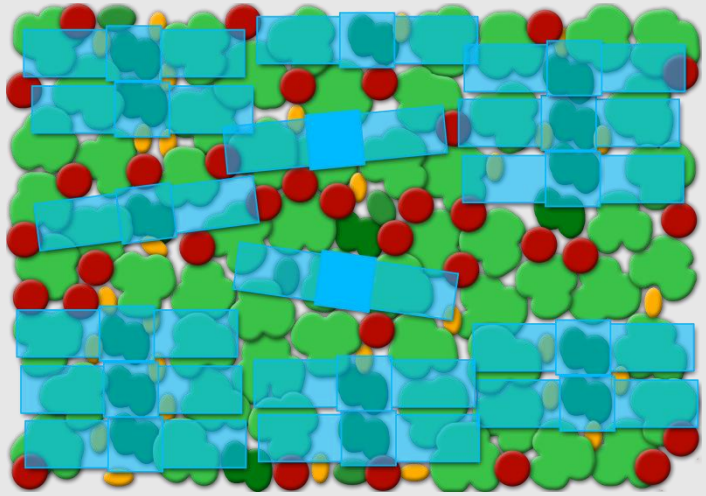
PBS in *Synechocystis*



(Moal et al. 2012)



Side view TM



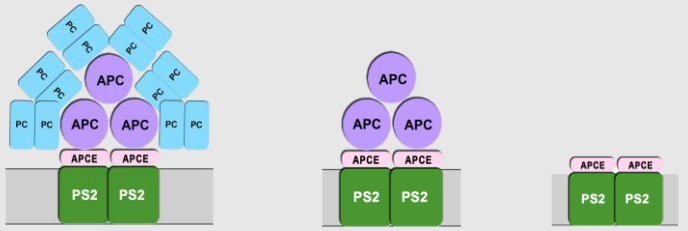
Top view TM

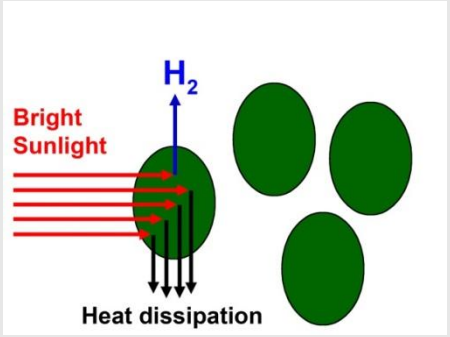
PBS antenna size reduction:

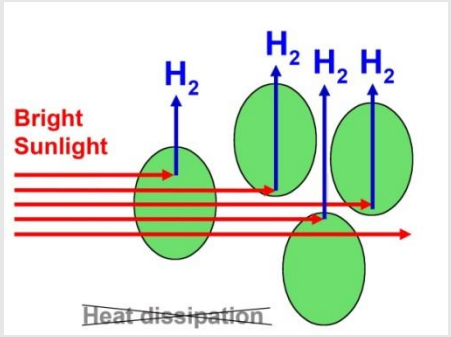
a) Reduced photoinhibition – higher light tolerance
b) Higher cell density in PBR
c) Saving energy (≤ 63% sol. prot.)

PBS antenna size reduction

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



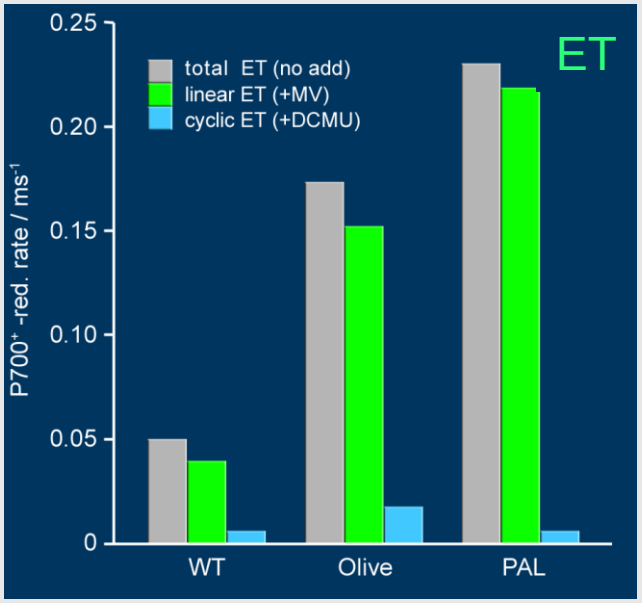




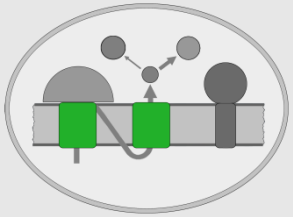
Impact on PS2 / PS1 ratio

≤ 6-fold increased linear ET

due to increased PS2 / PS1



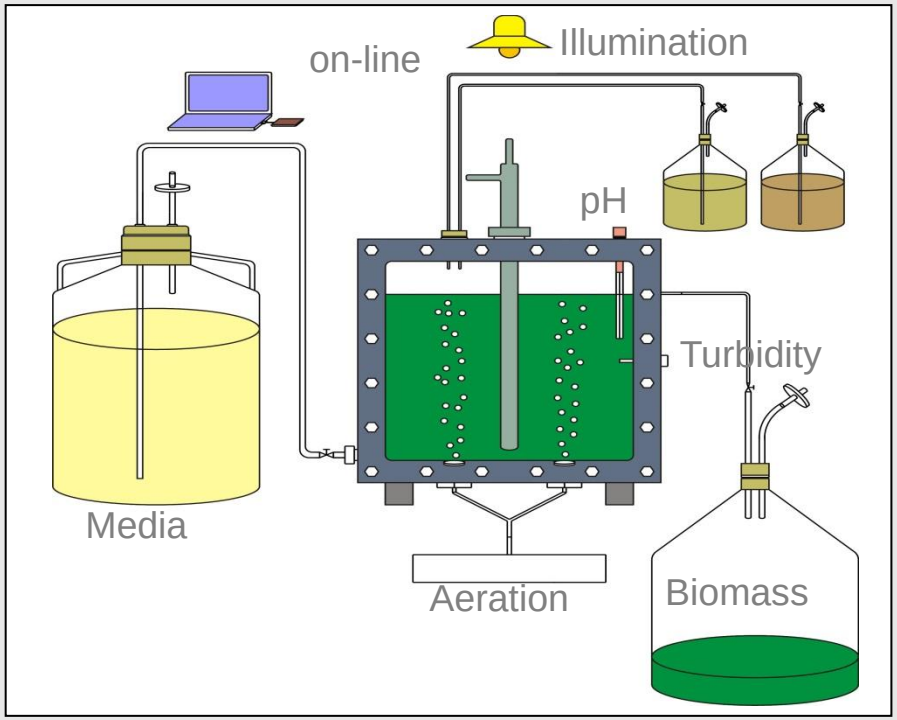
PS2/PS1



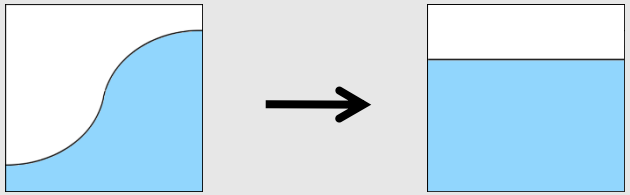
	Chl *a* per cell / fg	PS2 / % of WT	PS1 / % of WT	PS2 / PS1
WT	17.0	100	100	1:10
Olive	19.0	277	78	1:3
PAL	15.9	132	59	1:4

(collab. F. Mamedov, Uppsala University)

Photobioreactor design



Kwon, Rögner, Rexroth, J Biotechnol. 2012

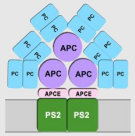


Measurement & Control:

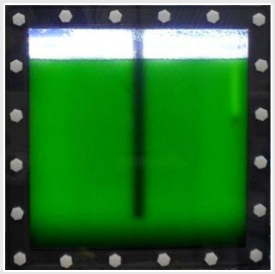
- Media supply
- Illumination
- CO_2
- O_2
- pH
- LabVIEW interface

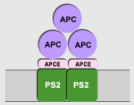
- Steady state characterization & optimization of design cells
- Long-term cultivation > 9 months

Monitoring metabolism by proteomics under steady state conditions

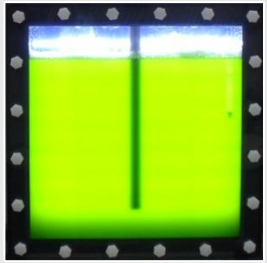


WT

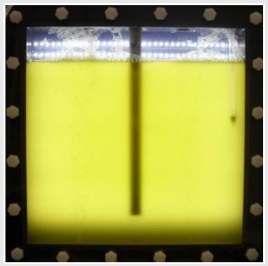




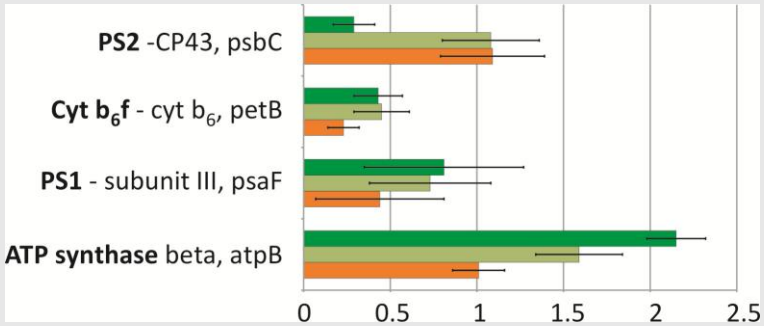
Olive



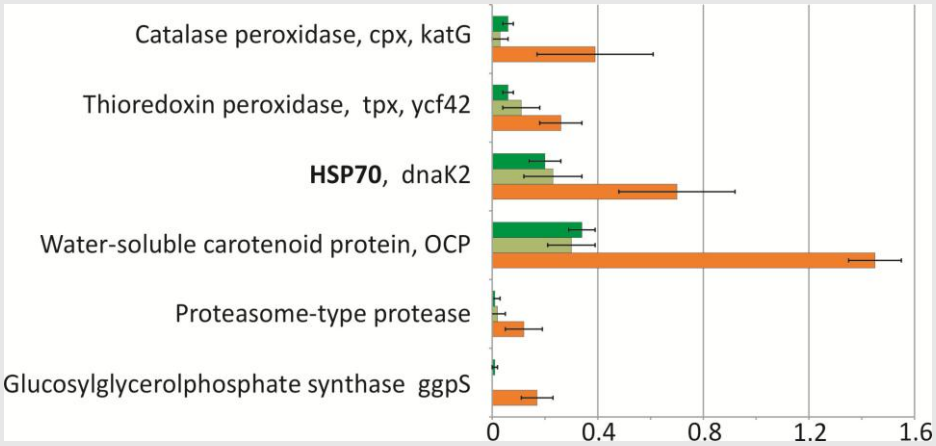
PAL



Photosynthesis proteins

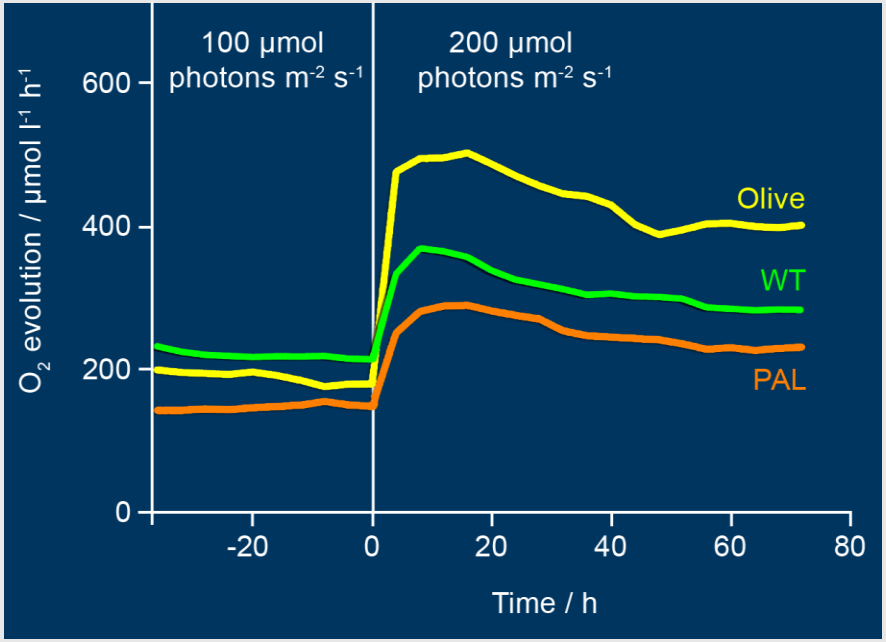


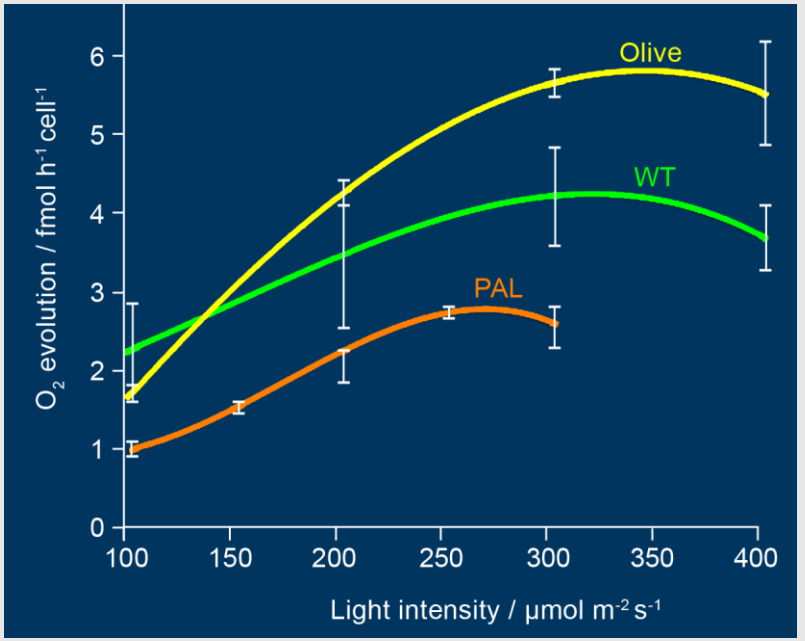
Stress indicator proteins



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

Photosynthetic productivity of PBS-mutants



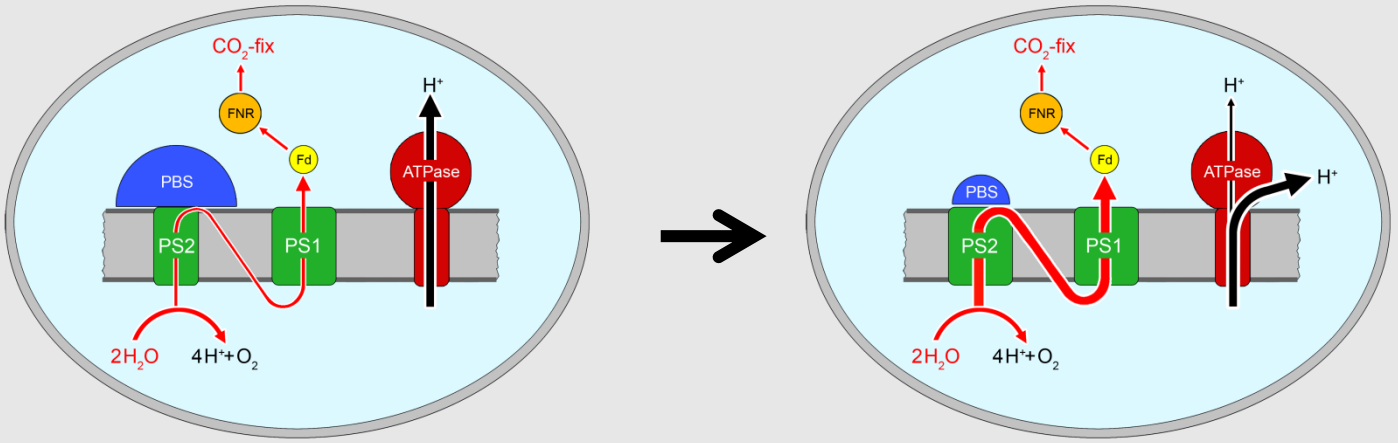


- Light intensity vs. O_2 evolution at steady-state conditions

Conclusion:

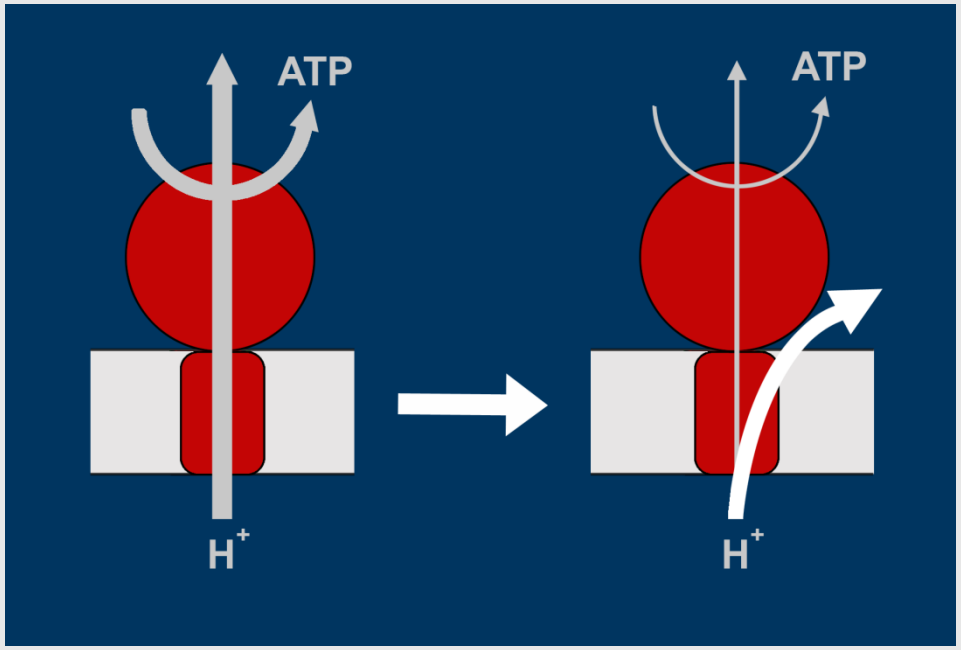
"Olive"-antenna mutant has highest potential as basis for design cell:

>150% activity of WT, higher light tolerance

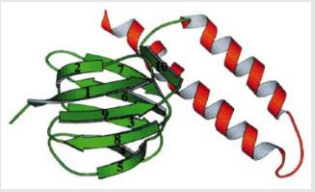


Engineering photosynthetic electron transport

Partial uncoupling of ATP synthase



(collab. T. Hisabori, Tokyo Institute of Technology)



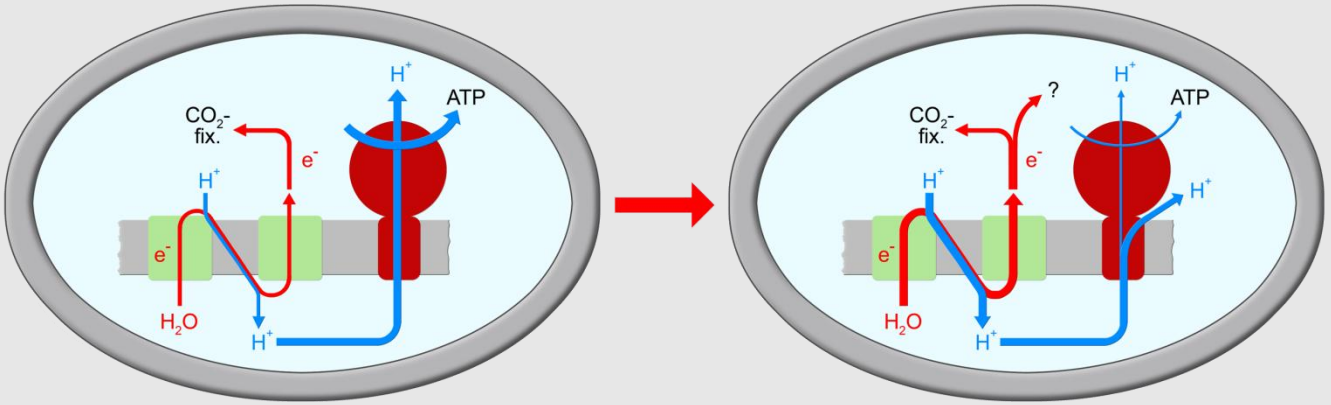
ε-subunit in WT

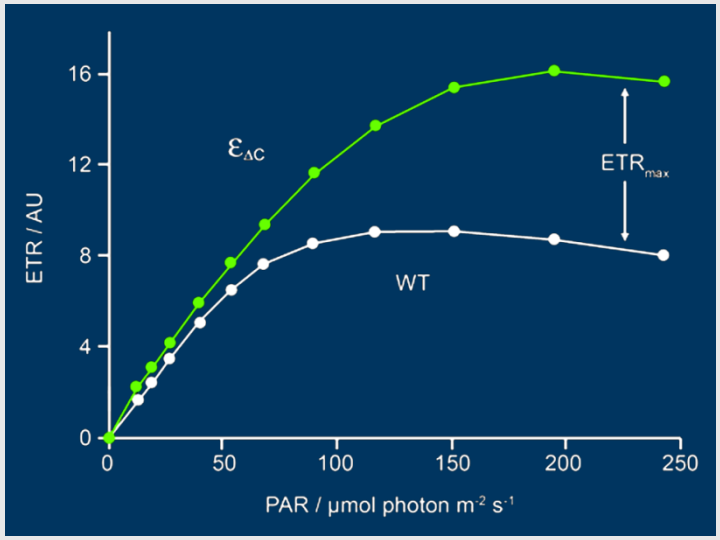
trunc. ε in $\varepsilon_{\Delta C}$

- High ΔpH has inhibitory effect on ET

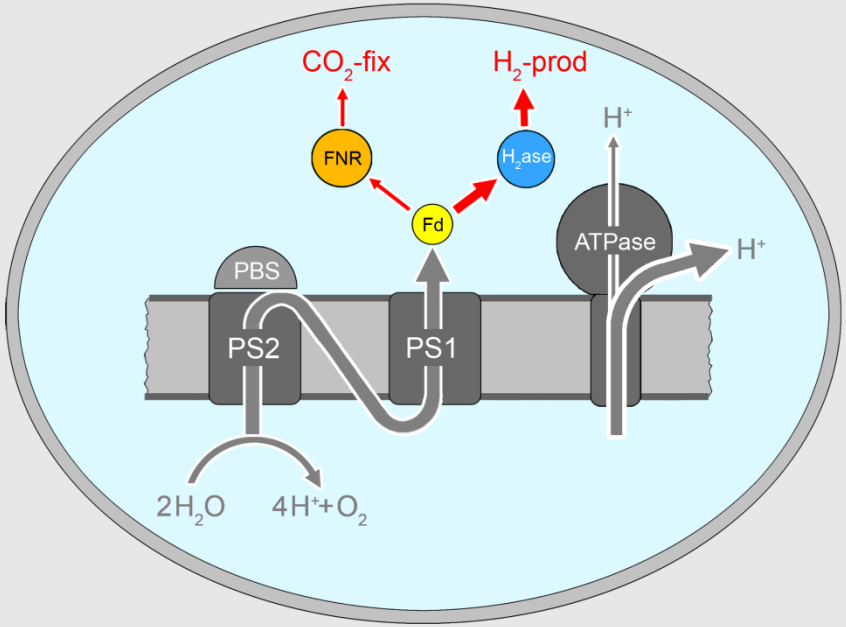
➢ Reduction of ΔpH across the thylakoid membrane

Effect of partial uncoupling



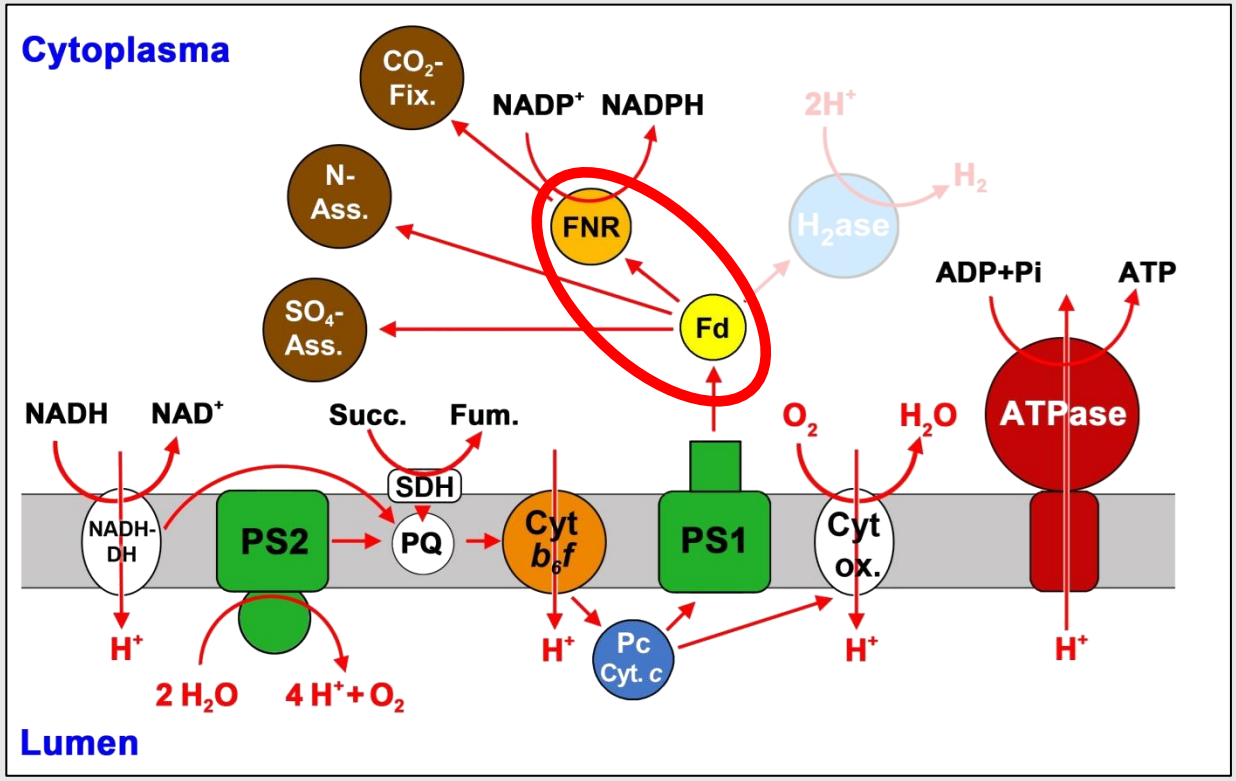


- ΔpH < 20%
- Cell growth unimpaired
- max. ET-rate ≈ doubled



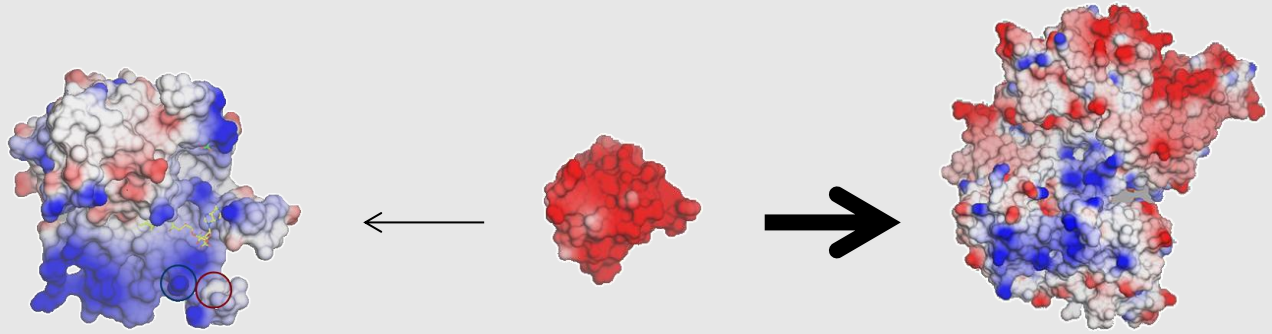
Tuning of the FNR – Ferredoxin interaction

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



- **Minimizing the transfer towards CO_2-fixation**
  - ~ 90% of electrons towards carbon fixation in WT
  - Reduction of biomass → increase of H_2 production

Engineering Ferredoxin interaction



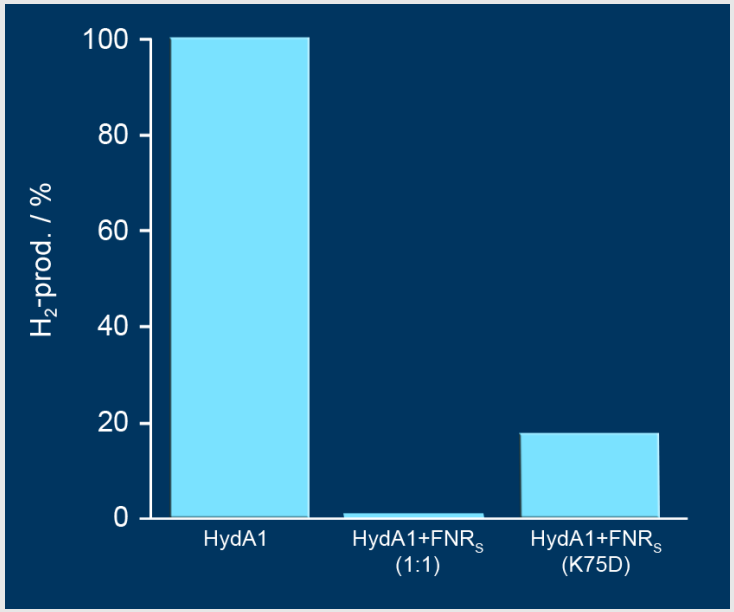
FNR Fd H_2ase

Biomass ↓ Bioenergy ↑

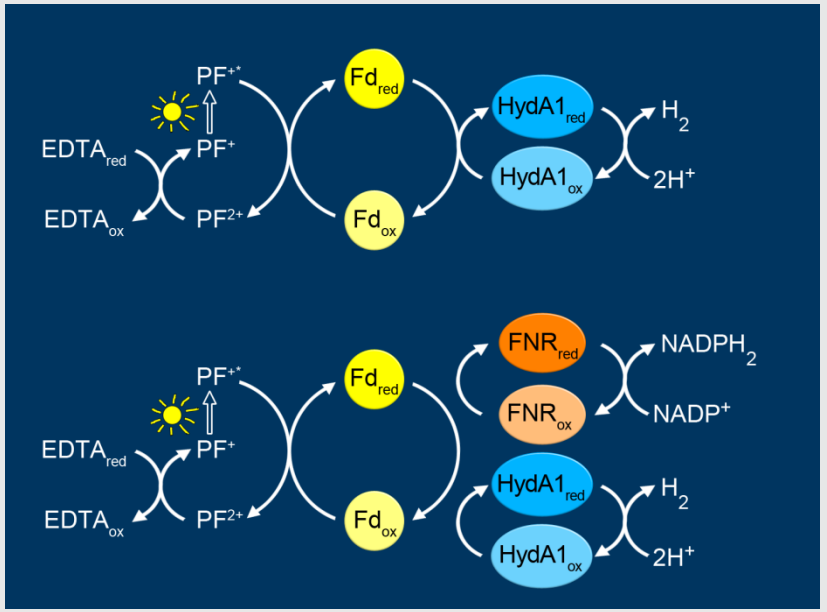
- Definition of interaction interfaces based on crystal structures
- Rational design of different FNR variants
- Targeted mutation of interaction sites


T. Hase (Osaka Univ.) G. Kurisu

Minimizing CO_2 fixation

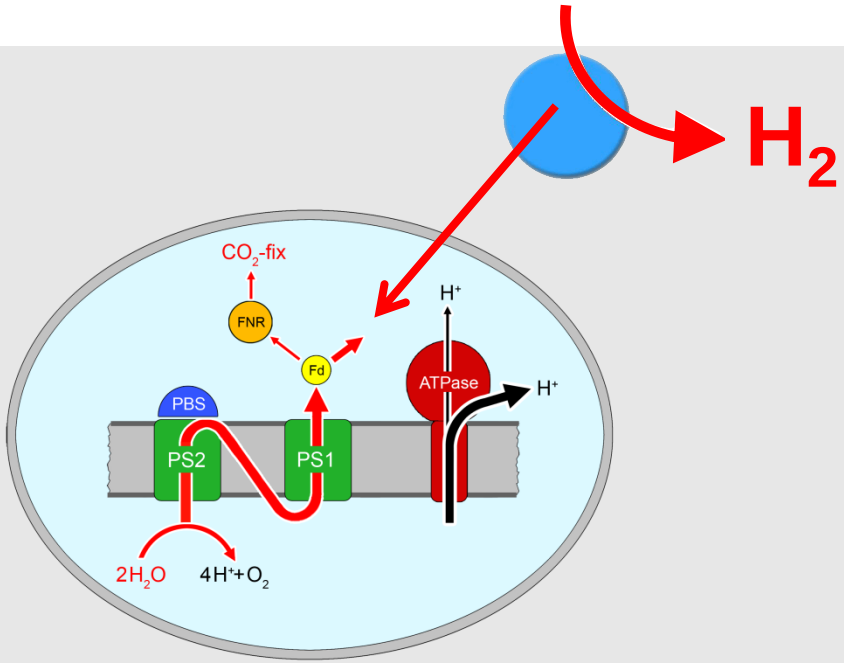


(K. Wiegand in collab. M. Winkler / T. Happe)



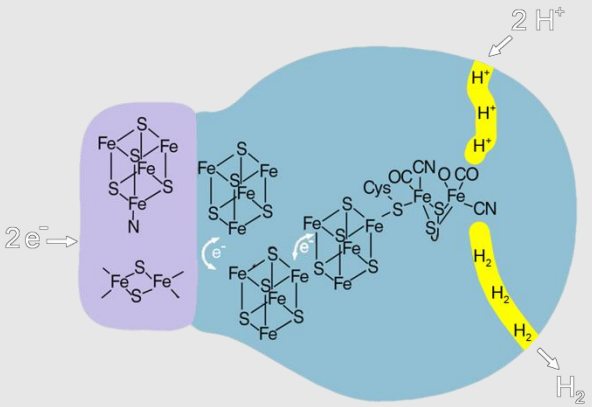
Proflavin-assay

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

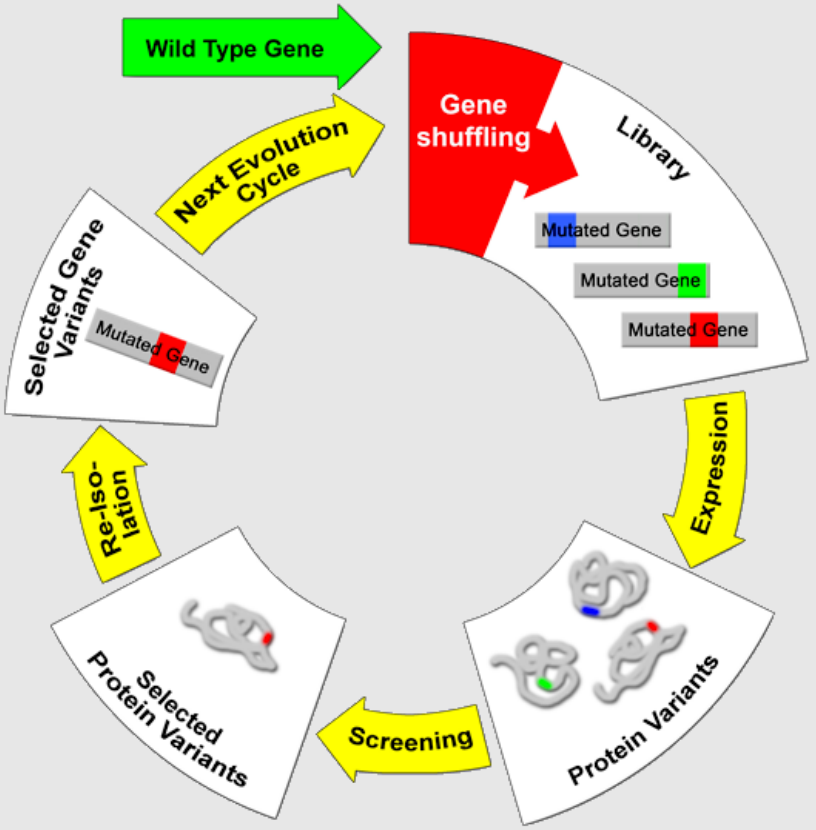


Coupling photosynthetic ET with engineered H_2ase

FeFe-H_2ase (*Chlamy*) : Design for O_2-tolerance



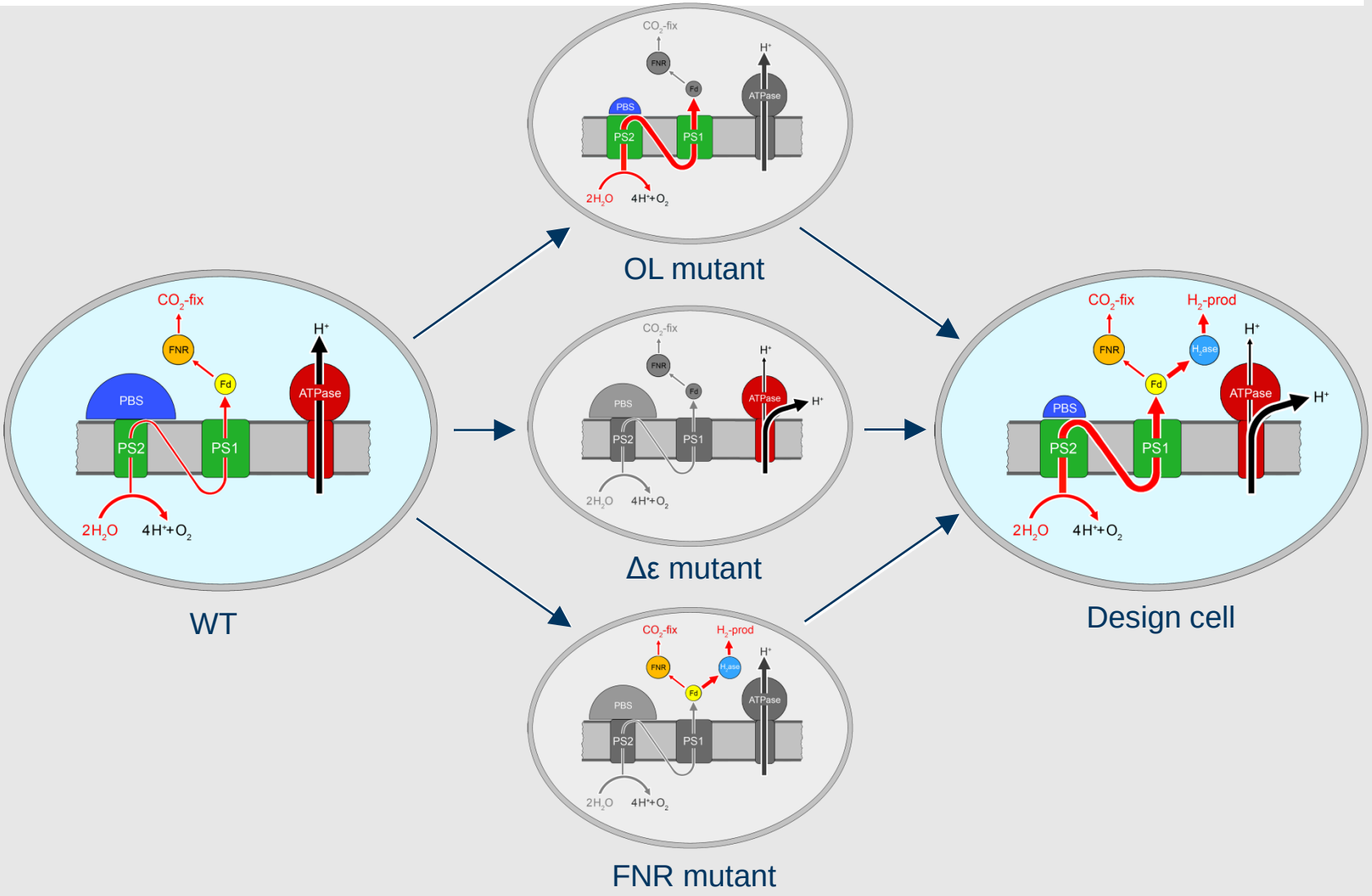
Anaerobic work station in tent



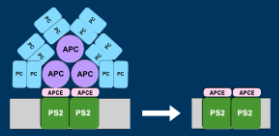
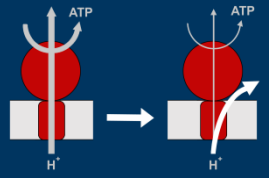
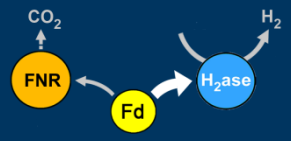
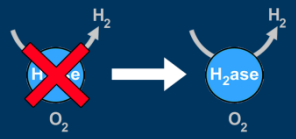
T. Happe
RUB

Directed evolution combined with high throughput screening...

Overall strategy for engineering "design cell"

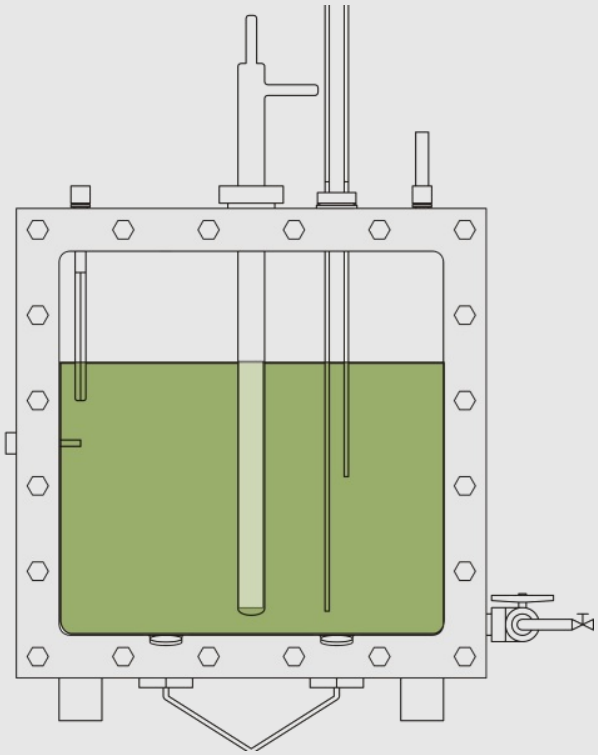


Summary & outlook (design cell)

MutantsII	 antenna red.	 uncoupling	 CO_2-fix.	 O_2-tolerance
Present gain (factor LET)	>6 (PS2 ⬆)	>2		
Future expectation			4-5	10-100

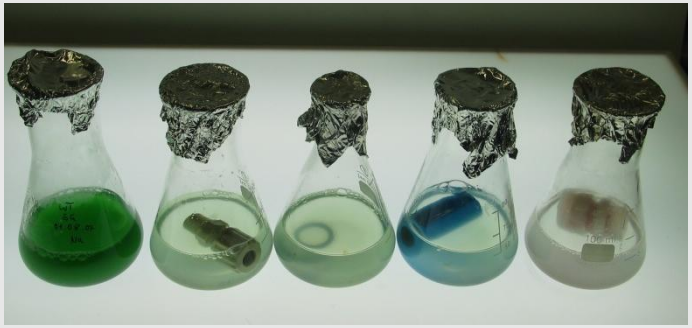
Conclusion: If all mutants are pooled in one design cell,

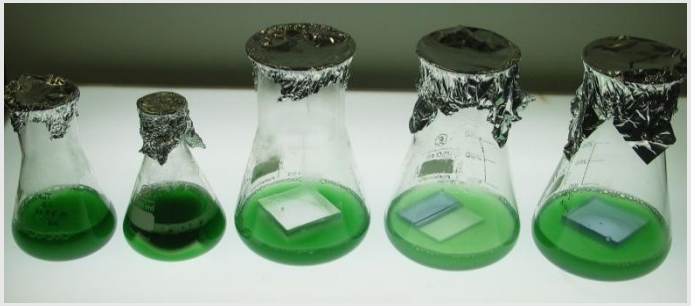
>100x increase in H_2-production can be achieved!



Development of photobioreactor

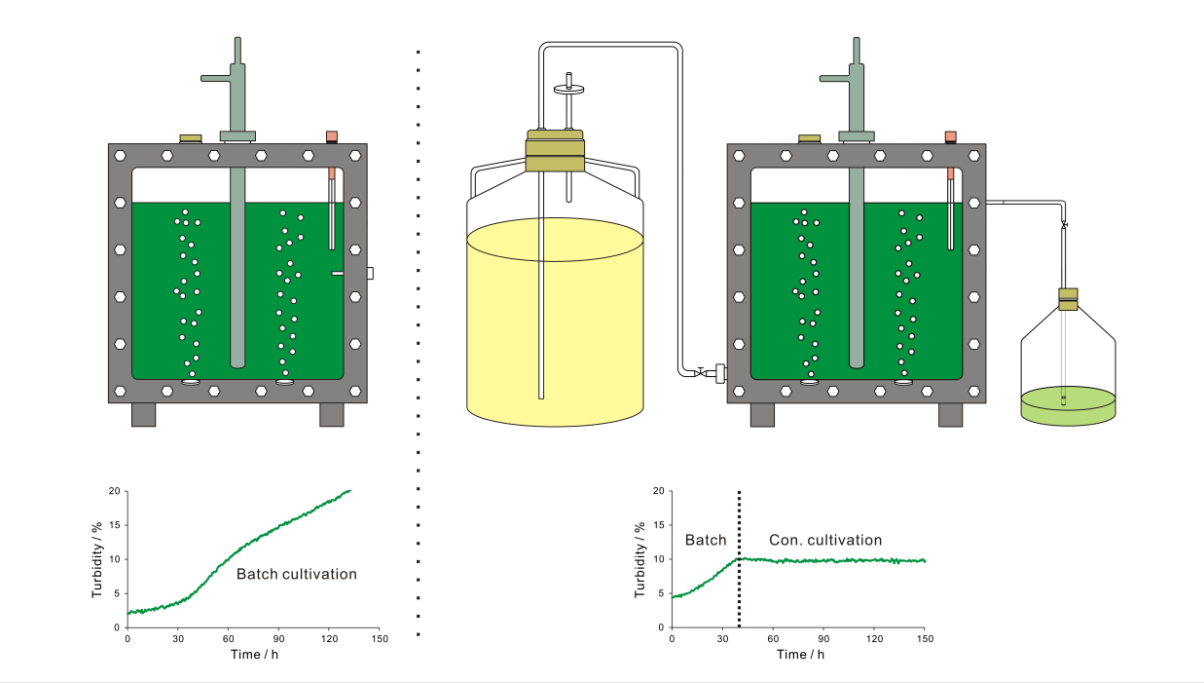
Optimization of reactor materials





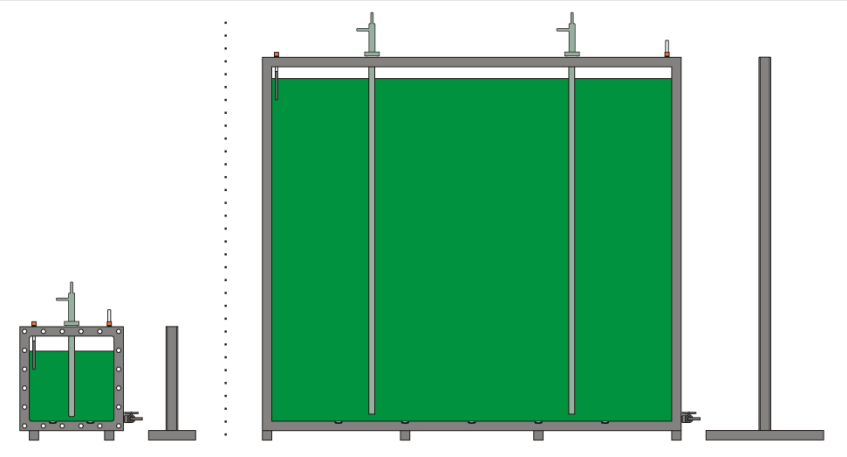
- Use of industrial semi-manufactured products (cooperation with KSD Innovations GmbH)
- Investigation of biocompatibility (frequently toxic effects)

Photobioreactor



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

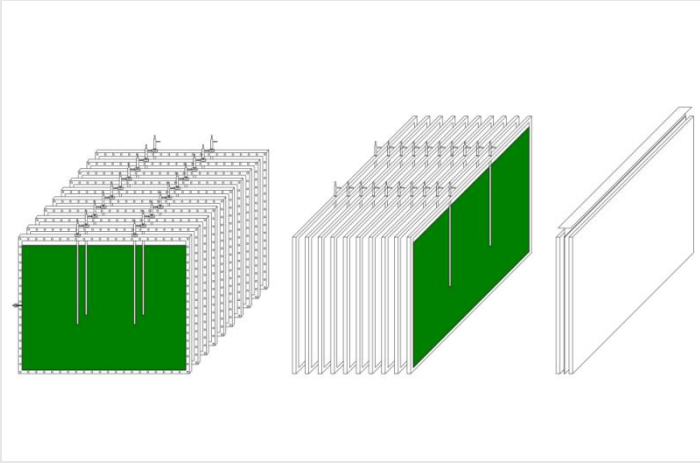
Scale-up



100 L

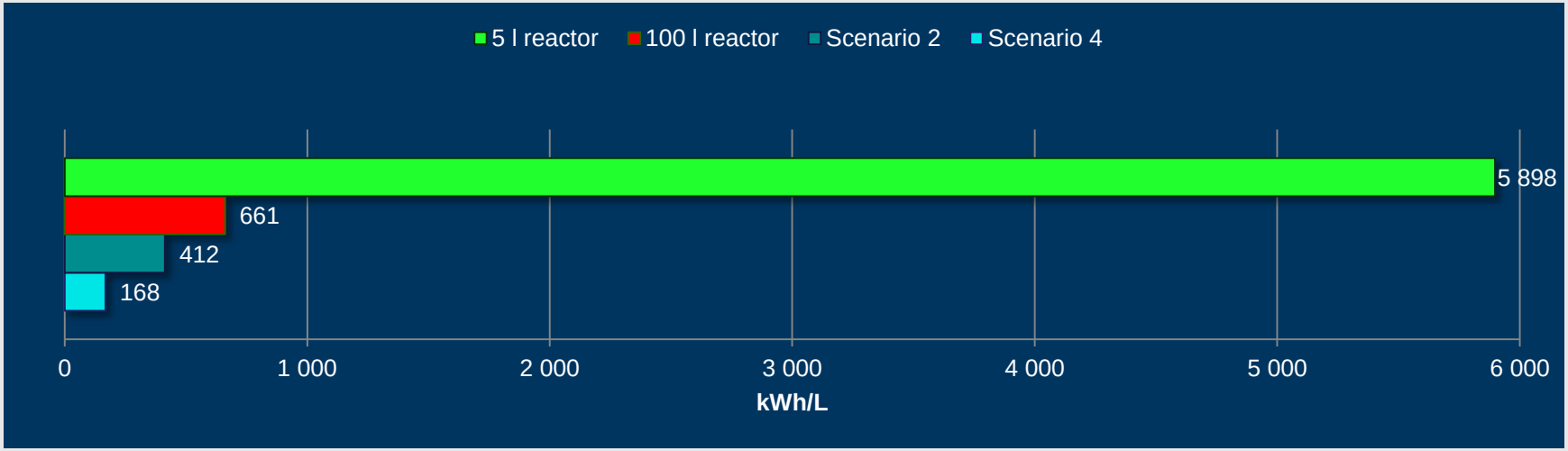
- Limitations for upscaling:
  - Light-penetration
  - Hydrostatic pressure
  - Mixing

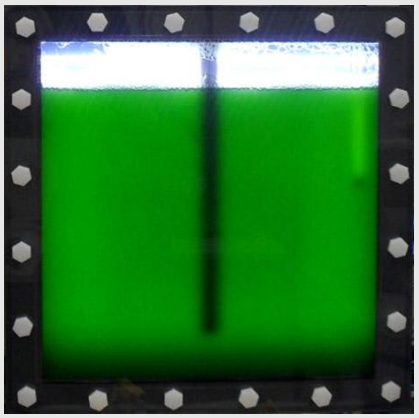
➢ Significant upscaling by increasing number of reactor units



(Coop. KSD, Hattingen)

Specific net energy consumption per reactor volume





5 L

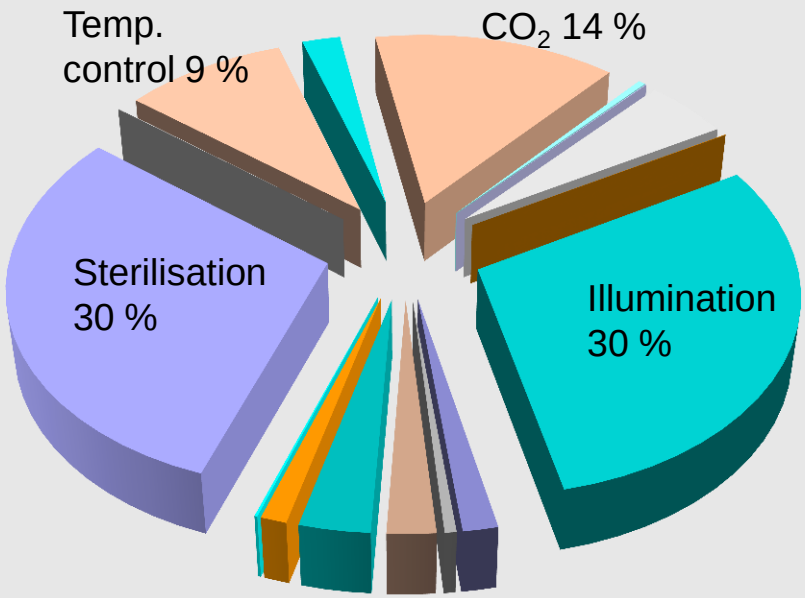
100 L

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

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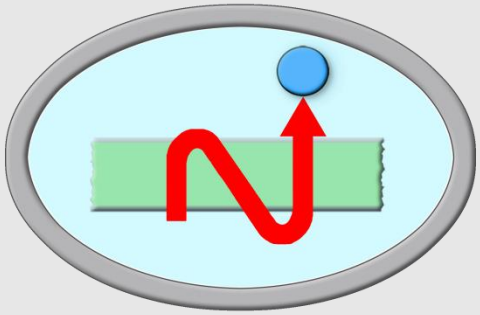
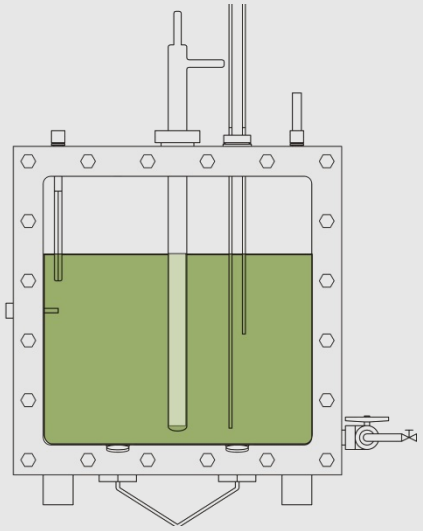
Summary



- H_2 producing cyanobacterial organism



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

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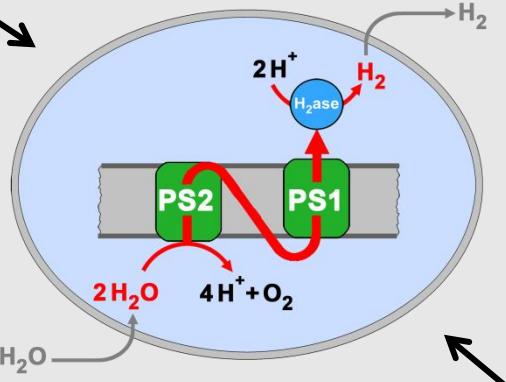

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