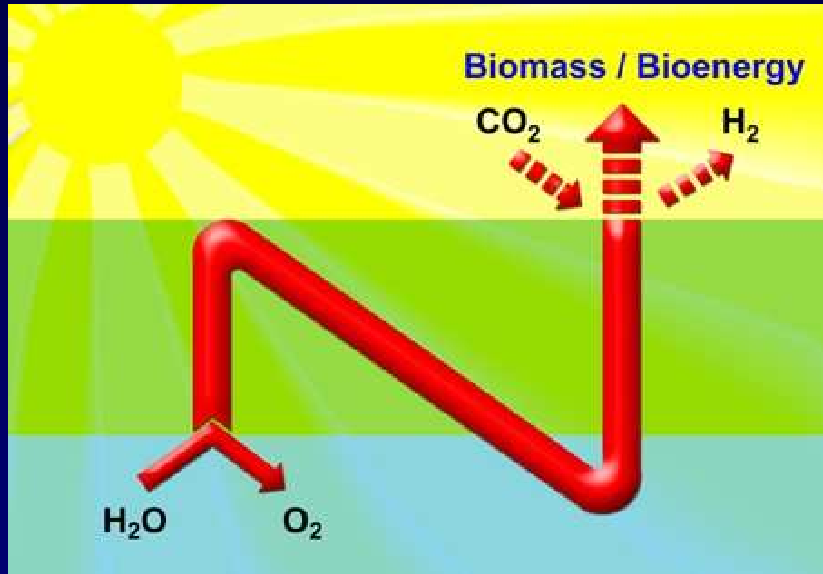
# Basics of Photosystem 2 function and applications for biohydrogen production





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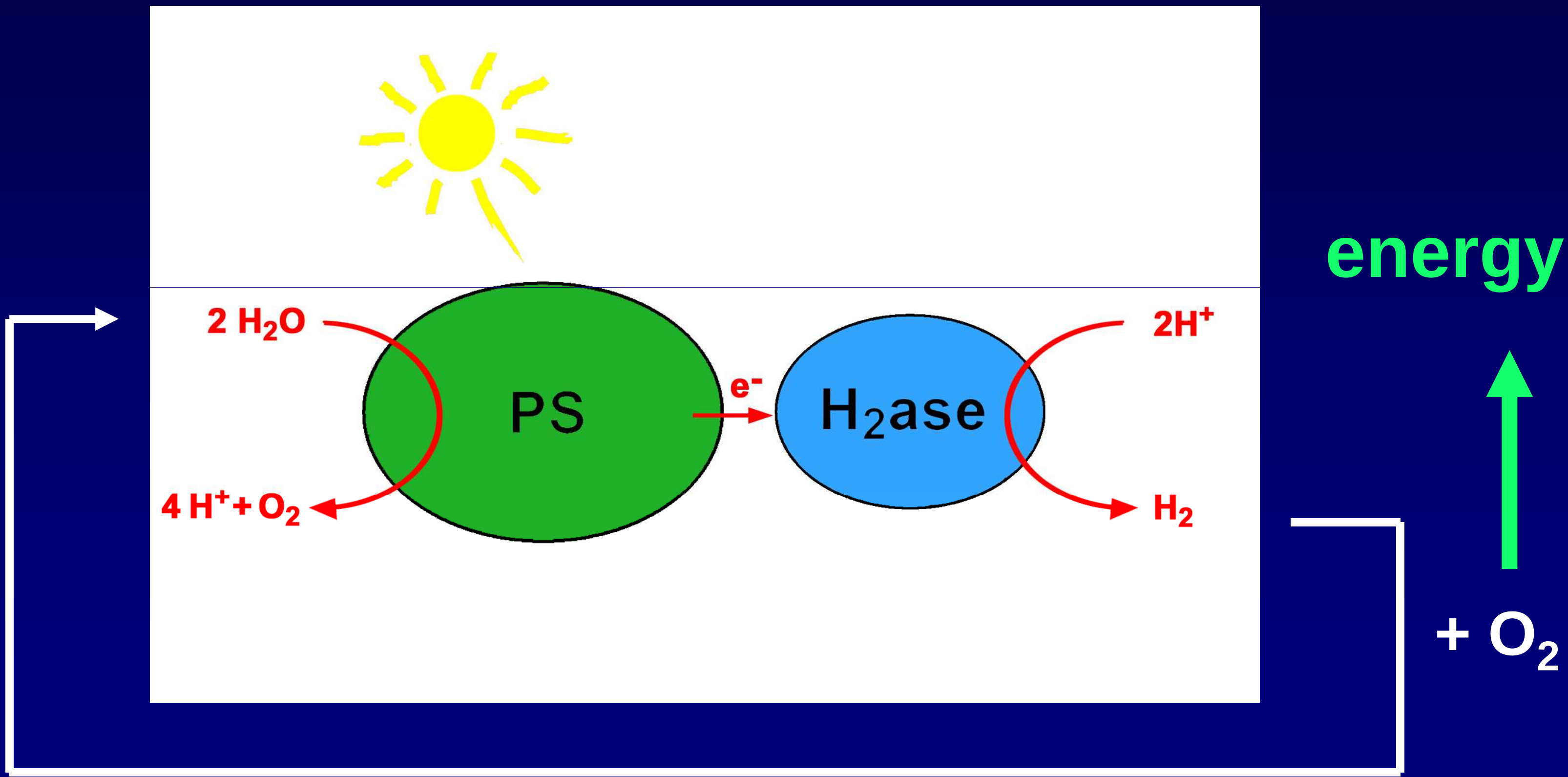
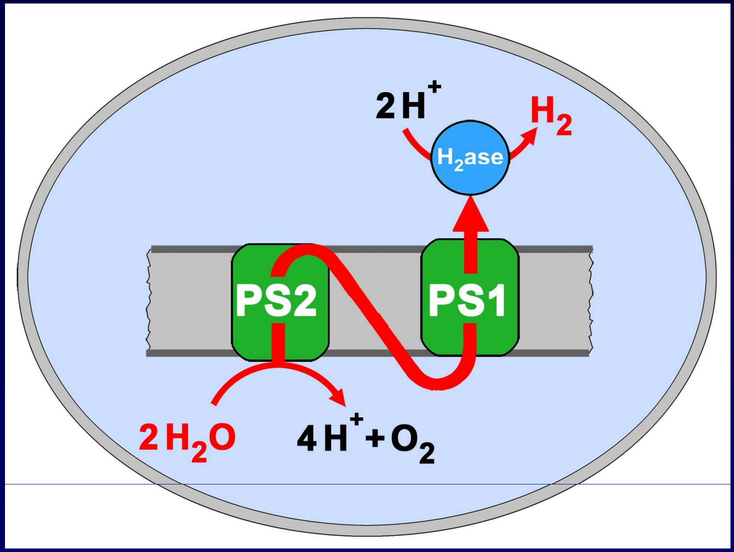
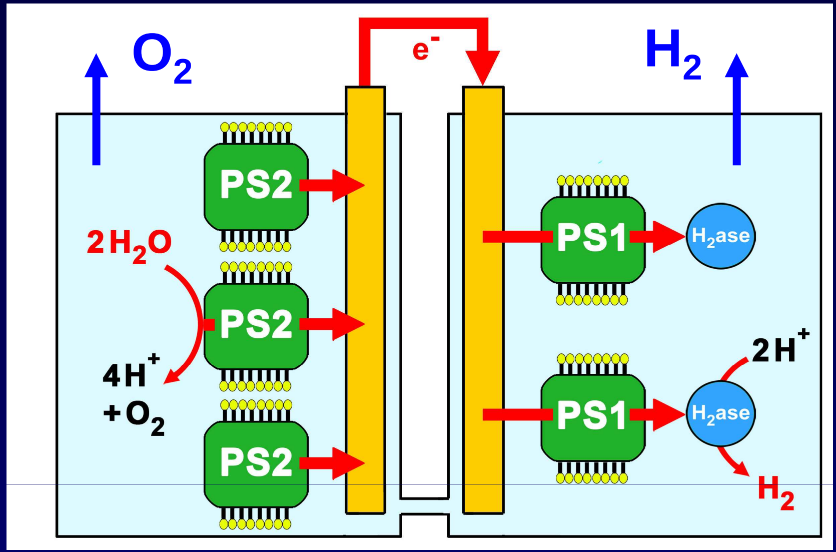
# Aim:
# Max. $H_2$-production by photobiological $H_2O$-splitting




**Regenerative energy without $CO_2$ !**

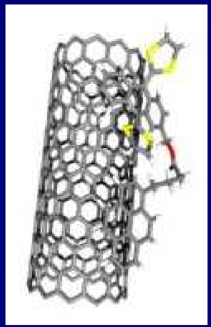
# 3 parallel strategies for system design




1) Engineered cyanobacterial cell system

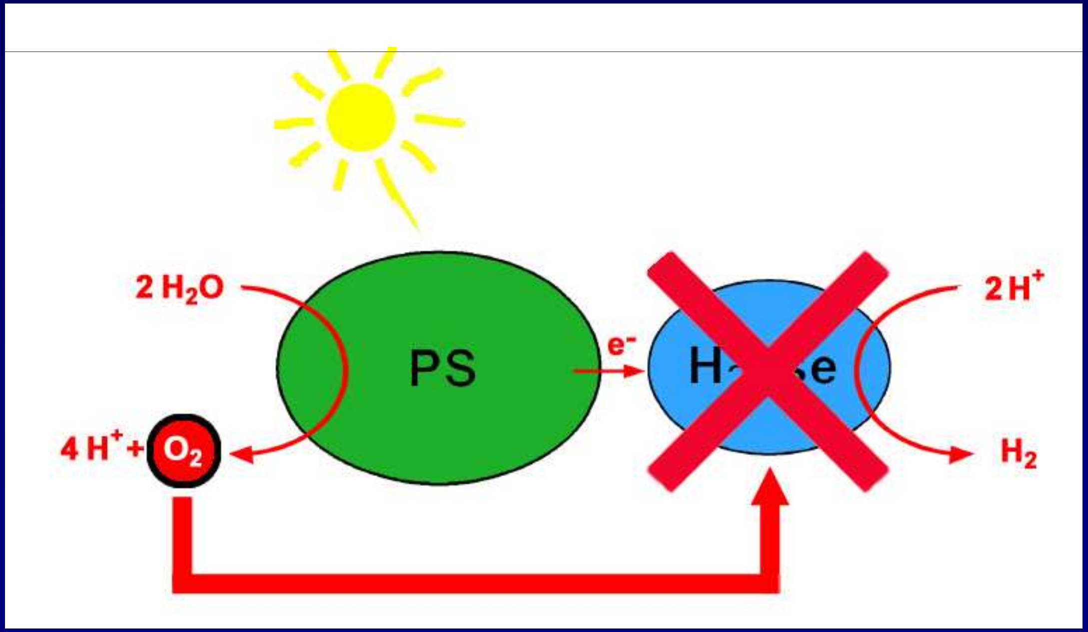
2) Semiartificial system with immob. native components



3) „Nature-inspired" artificial system

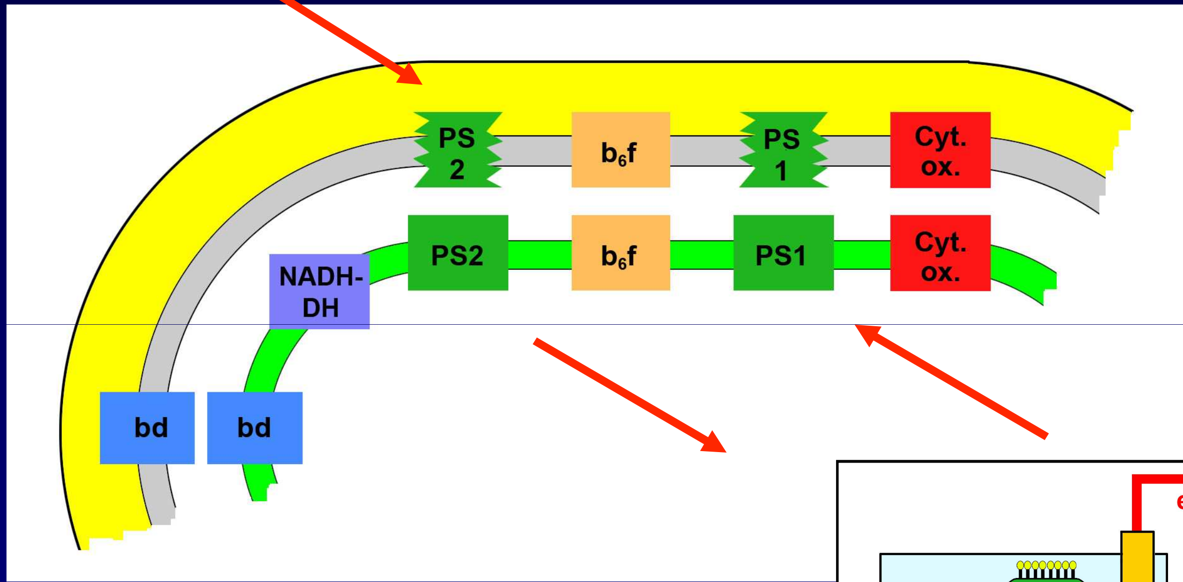
# Requirements to make bio-$H_2$ competitive :

- **about 100-fold higher $H_2$-production per L**
- **at least 10-fold cheaper photobiofermenters**
- **$O_2$-insensitive $H_2$ase**



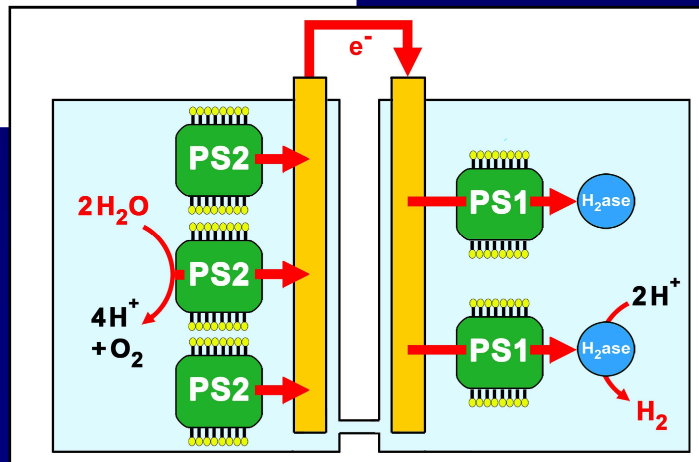
.... from native system

(i.e. PS2 in *T. elongatus*)

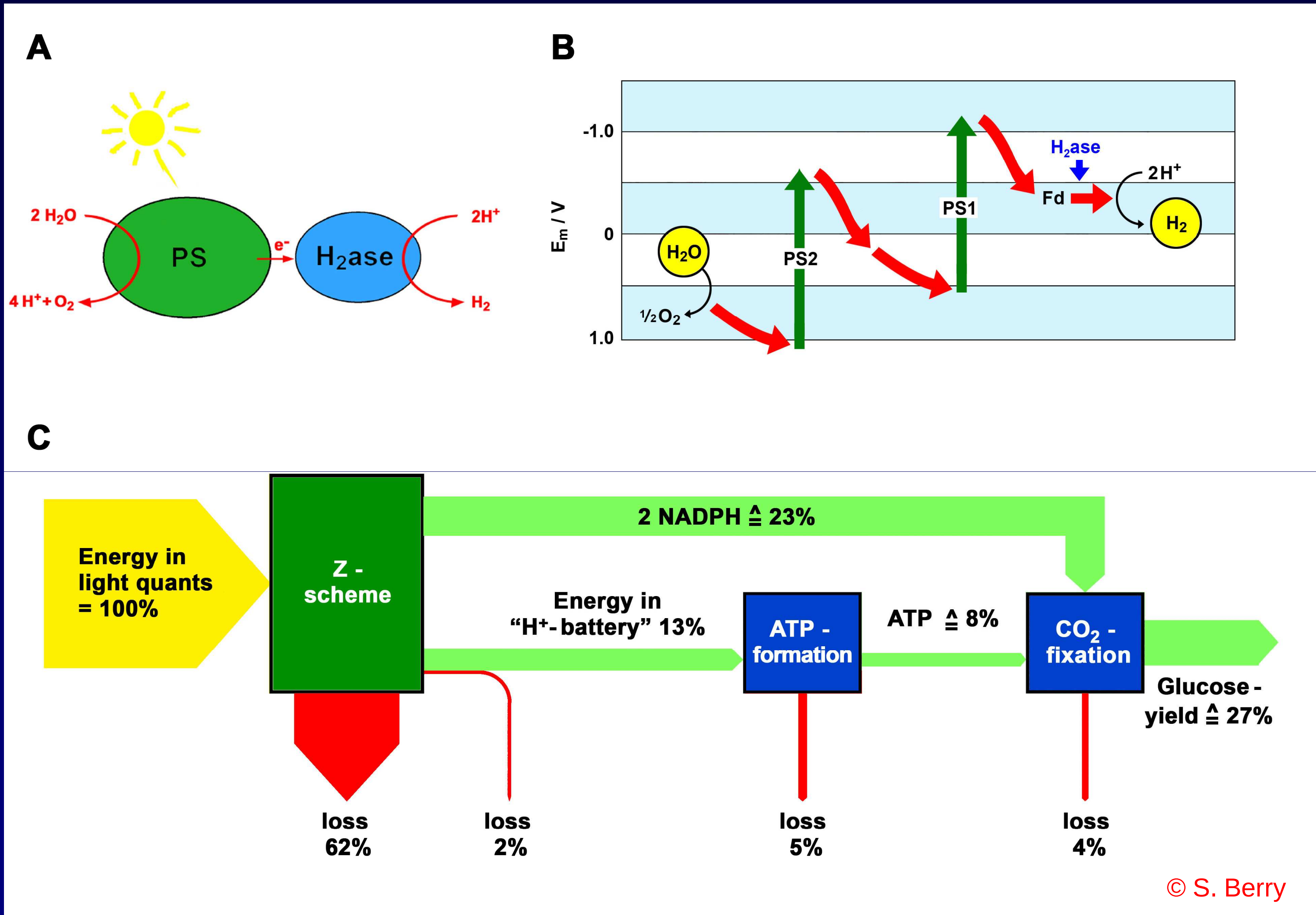



..... to "designed“ systems

(recomb. algae & „bio-battery“)



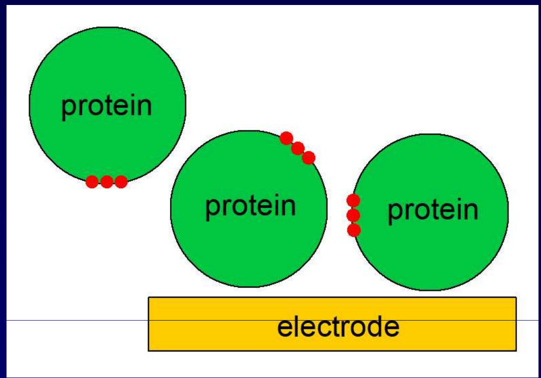
# ➔ e⁻ for H₂ase from PS1 (efficiency of PS....)

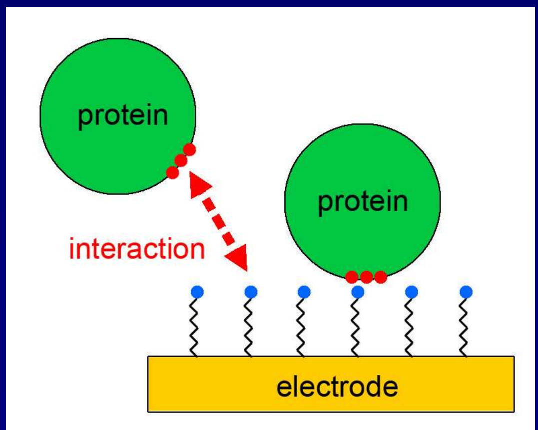



Esper B, Badura A & Rögner M (2006) Photosynthesis as a power supply for (bio-)hydrogen production; *Trends in Plant Sci.* 11, 543-549

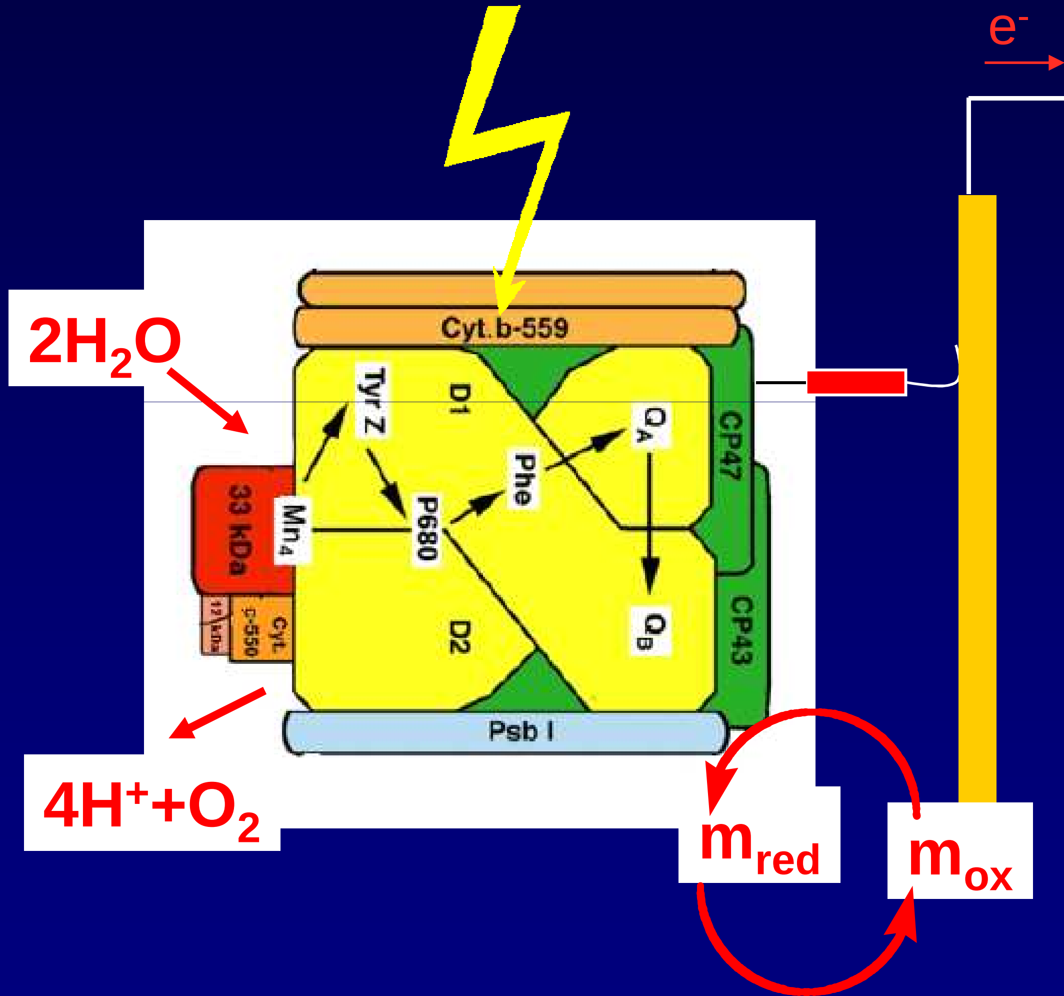
# Immobilisation of PS as monolayers on gold electrodes

## Principle

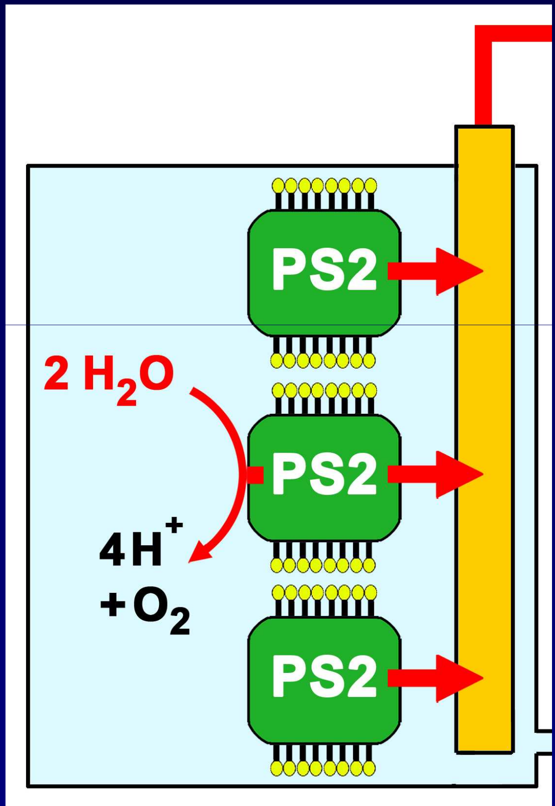


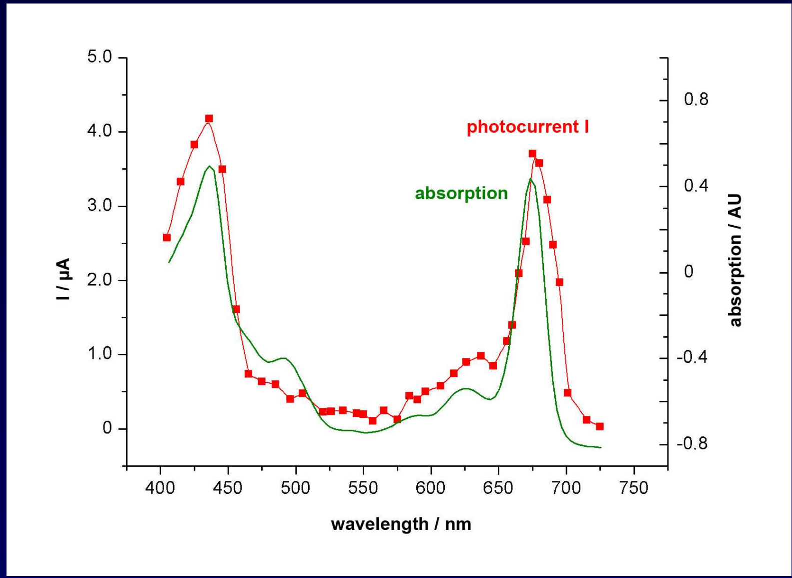

## PS2

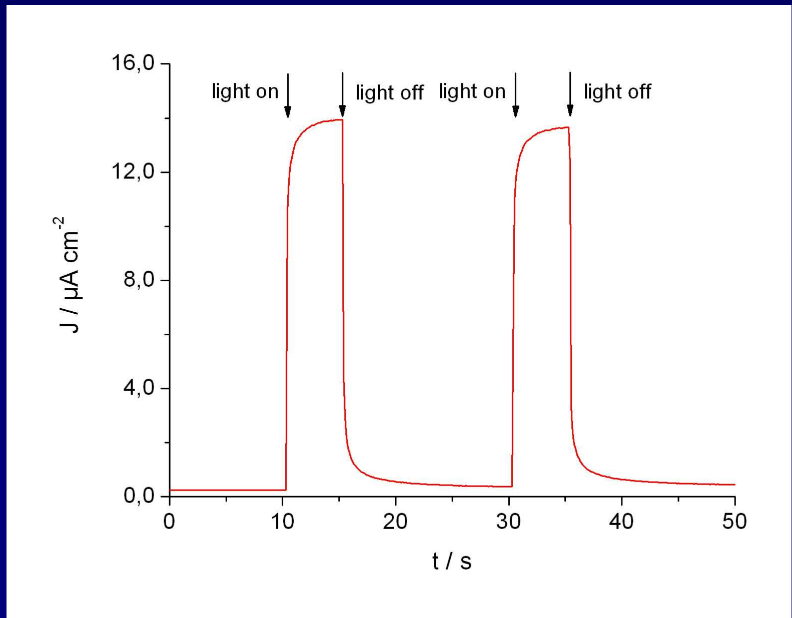


# PS2 photocurrent
# (monolayer)




(DCBQ)



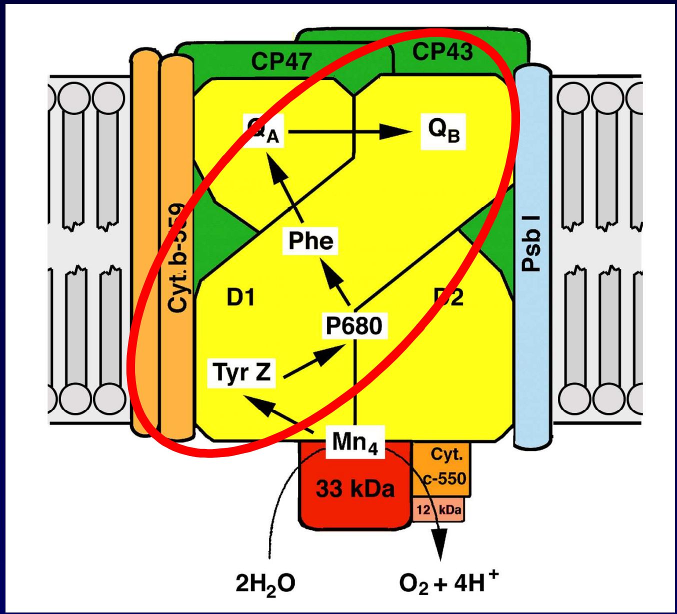



# Summary immobilized PS2 monolayer

- Surface coverage (by SPR) of PS2 = 0.29 pmol $cm^{-2}$
- Average current density = 14 μA $cm^{-2}$
- equiv. to an $O_2$ evolution rate of about
  6,500 μmol $O_2$ $Chl^{-1}$ $h^{-1}$
  (based on 4 ms turnover time per RC)

=> highest spec. value determined up to now for PS2
=> no loss of activity due to immobilization

Badura A, Esper B, Ataka K, Grunwald C, Wöll C, Kuhlmann J, Heberle J, & Rögner M (2006)
*Photochem. Photobiol.* 82, 1385-1390

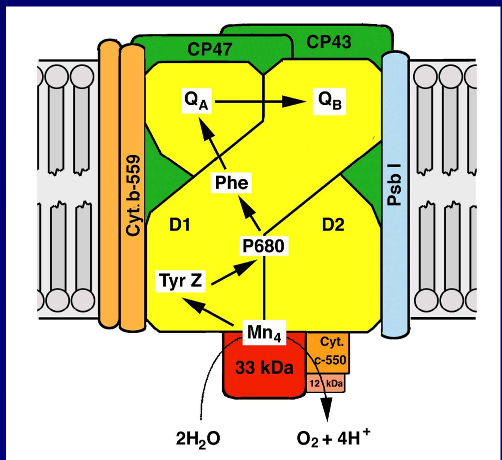



# PS2-PsbA (D1)

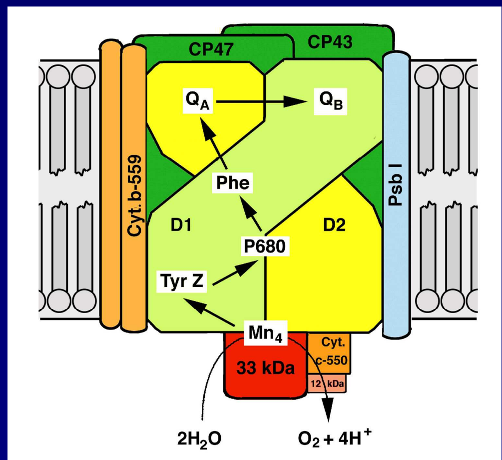
- harbours most cofactors
- is especially labile
- 3 alternative D1-copies

  in *T. elongatus* : PsbA1/-2/-3

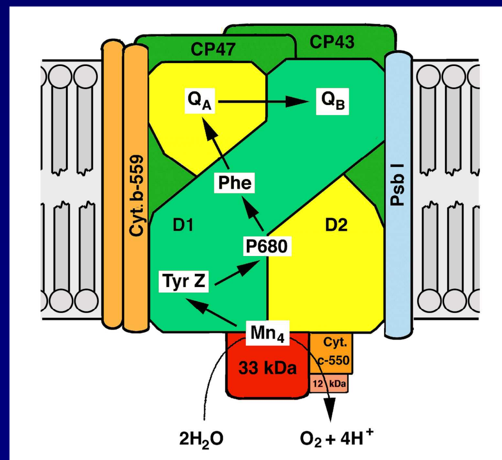
## PsbA1

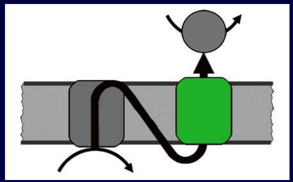



## PsbA2

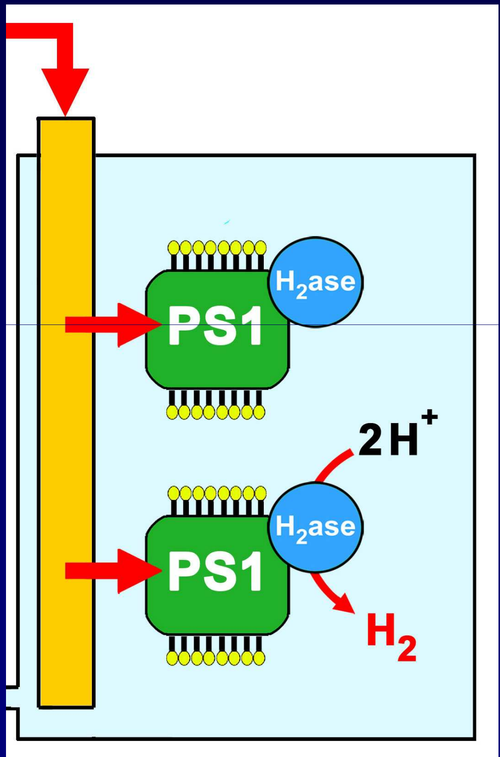


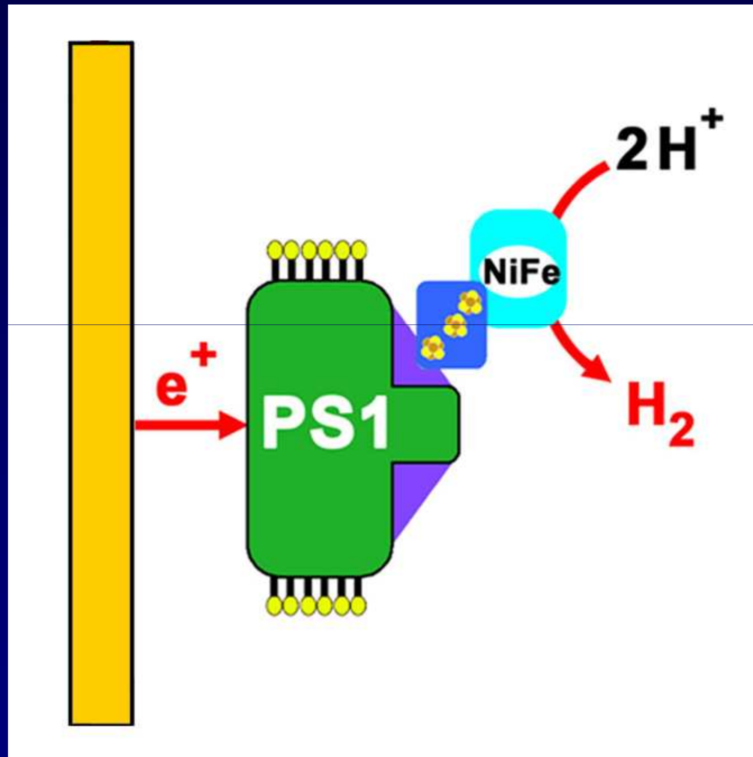

## PsbA3





# PS1-hydrogenase fusion protein for direct e⁻-transfer

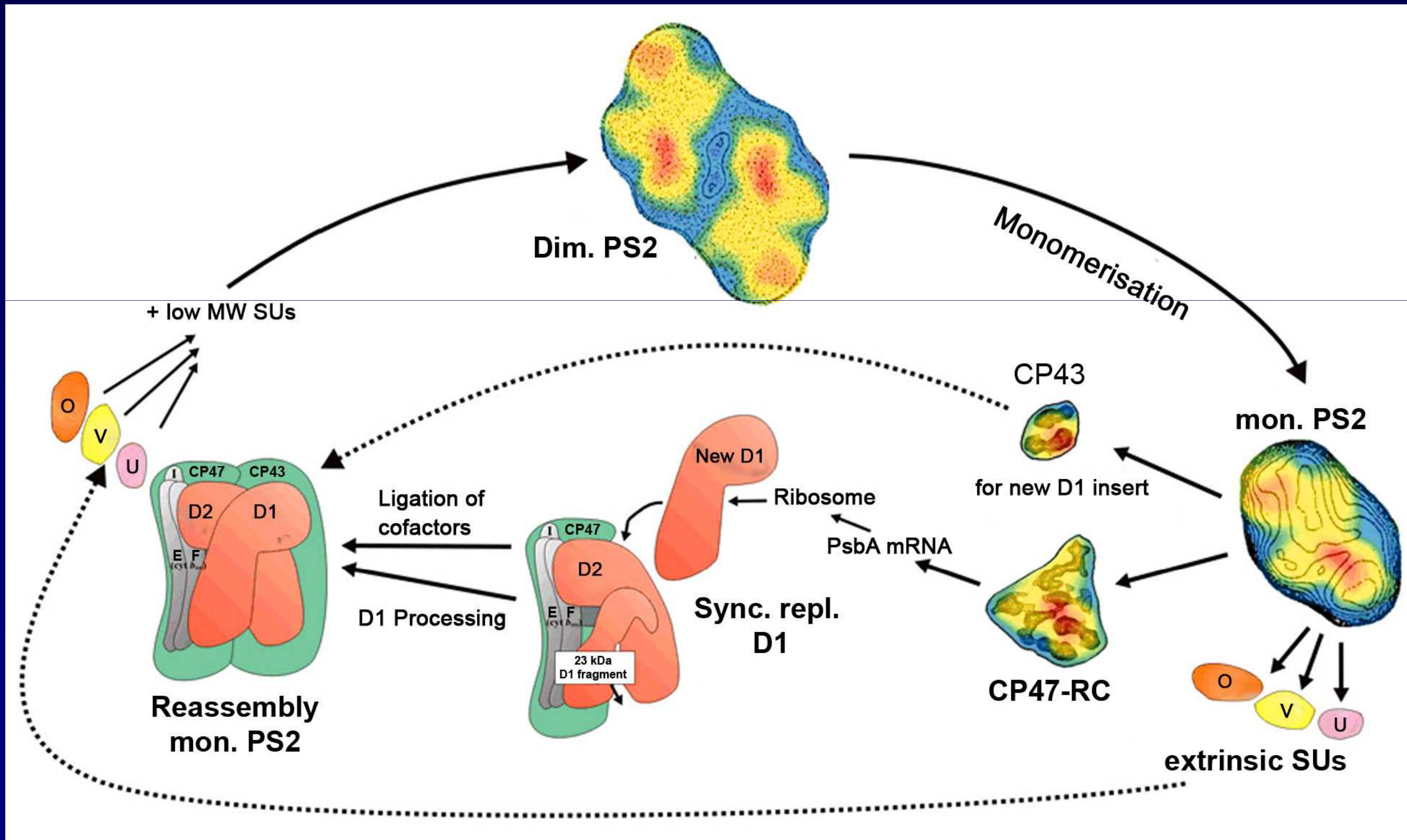



......with $O_2$-tolerant $H_2$ase of *Ralstonia* (collab. B. Friedrich, HU Berlin)

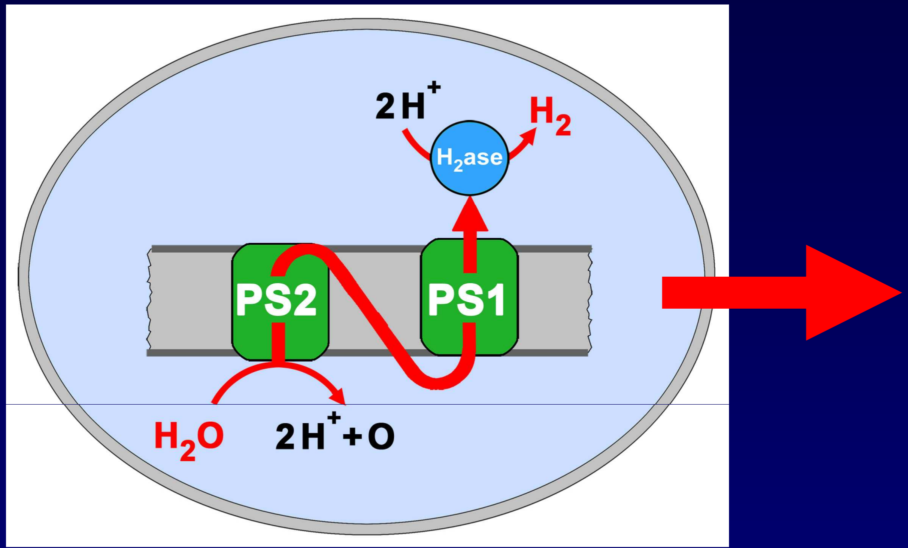
# Drawback of semiartificial systems :

Lower long term stability due to lack of repair system




(P. Nixon)

# Design of natural system (cyanobacteria)

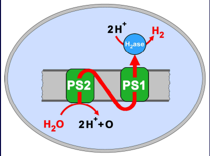



Design of mutants

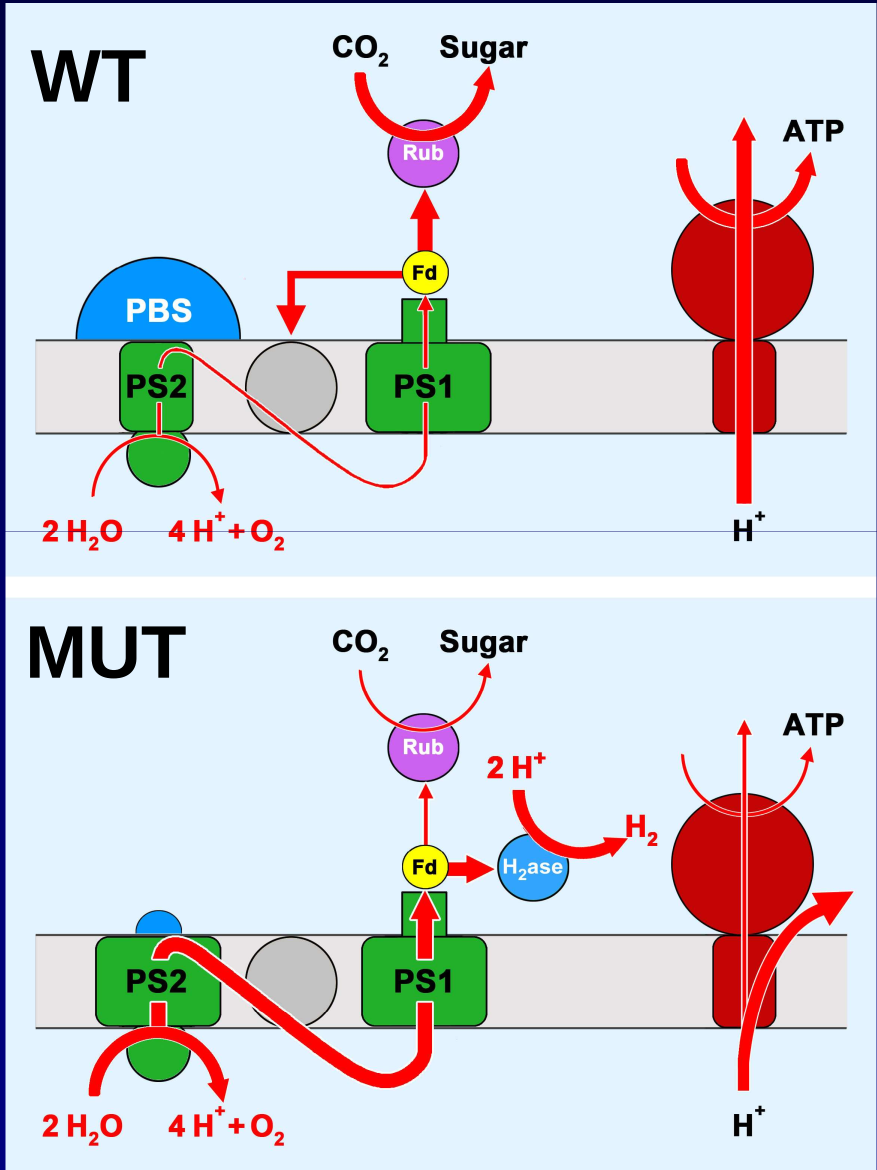
**Modified native system:**

Heterologue, $O_2$-tolerant

$H_2$ase in algal cells




# Cell system for max. coupling of PS-ET to $H_2$-ase




(*S.-cystis* 6803 as model system)

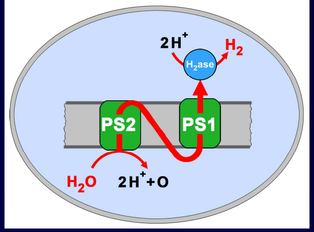
**PARAMETERS :**

**A) ET**

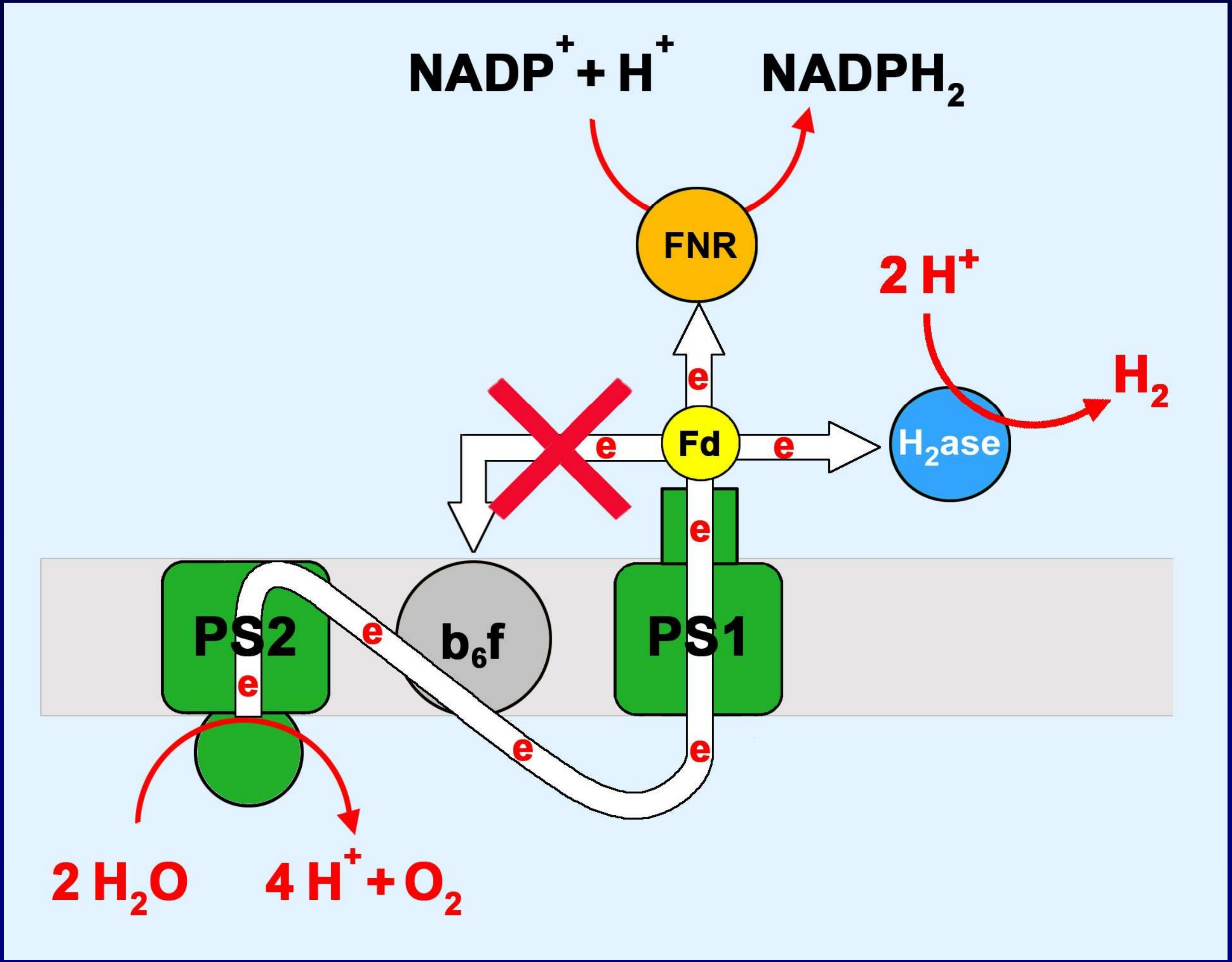
- PS2 / PS1 ratio
- PBS-antenna size
- Cyclic / linear ET
- Coupled / uncoupled ET
- Coupling of Fd to $H_2$ase & to $CO_2$-fixation

**B) $H_2$ase**

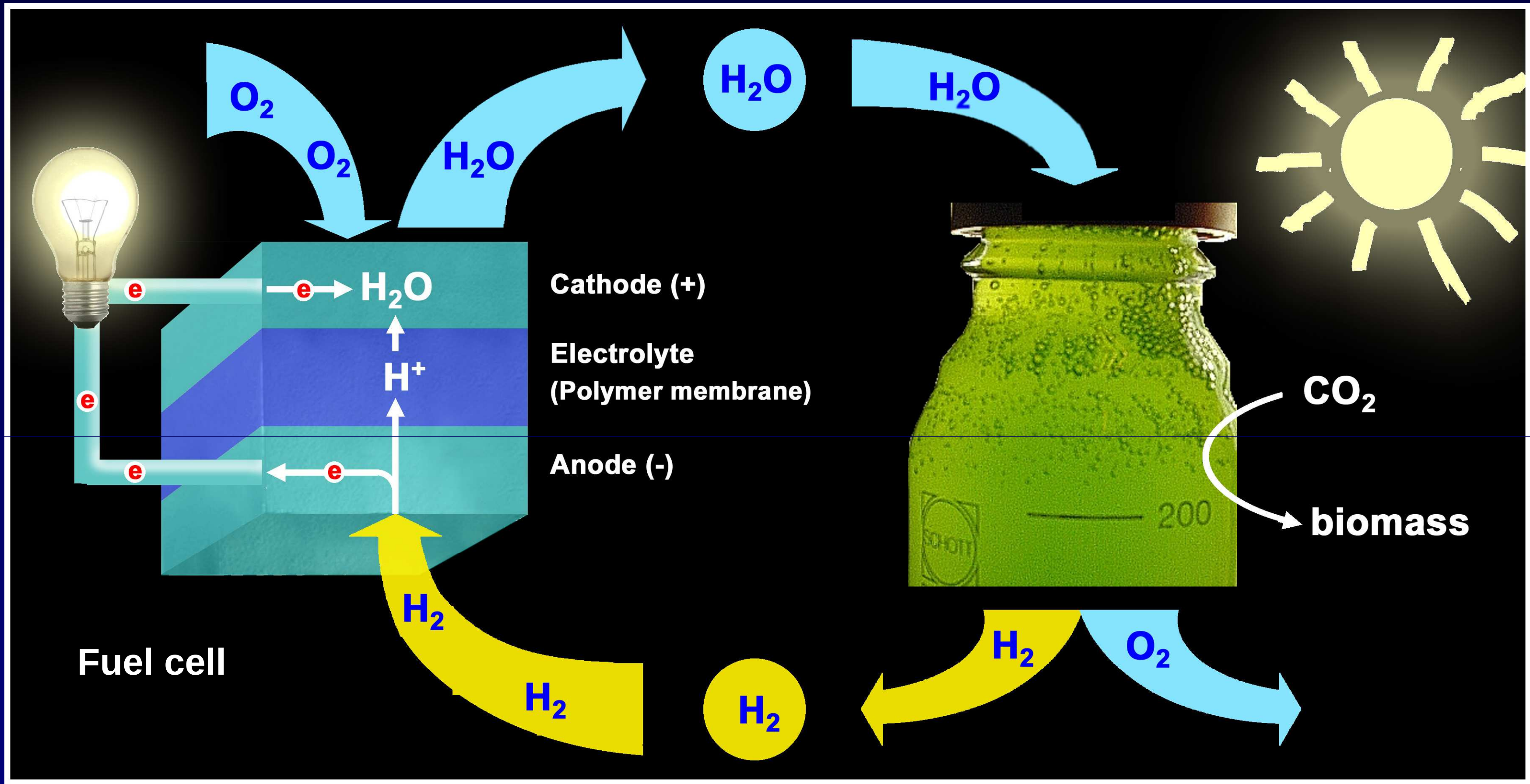
- Biogenesis of "foreign" $H_2$ase
- $O_2$ tolerance of $H_2$ase


2H+
H2
H2ase
PS2
PS1
H2O
2H++O

# "Fd-switch......"


NADP+ + H+
NADPH2
FNR
2 H+
H2
e
Fd
e
e
H2ase
e
PS2
e
b6f
PS1
e
e
e
2 H2O
4 H+ + O2

# $H_2$ cycle with micro algae




$H_2$ for heating, traffic etc.