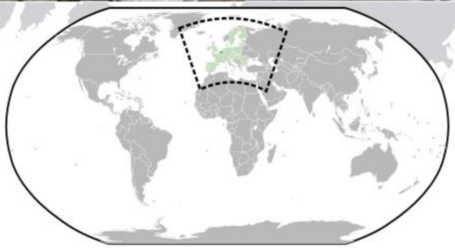

Photosynthetic electron transfers : methods and case studies

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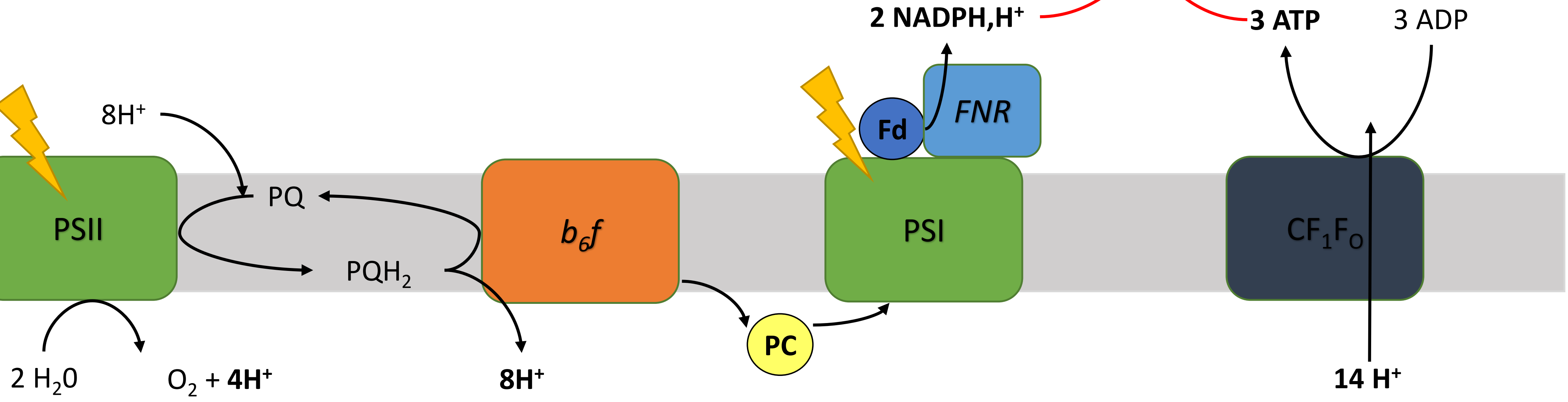
Venice, January 26th 2016

Photosynthetic Electron transport rate (ETR) : Which probes ?



(1) ETR = photochemical rate x concentration of active photosystems

$(e^- . s^{-1} . PS^{-1})$ — $k\ (s^{-1})$ — $(e^- . PS^{-1})$

- **(i) ETR_{PSII} from reduced state of Q_A : Chlorophyll fluorescence**
- **(ii) ETR_{PSI} from oxidized state of P700 : Absorption spectroscopy**
- **(iii) ETR_{PS} from rate of membrane potential formation : Absorption spectroscopy (ECS – Giovanni Finazzi)**

(2) ETR = rate of gas concentration exchange x concentration of active photosystems

$(e^- . s^{-1} . PS^{-1})$ — moles gas . $L^{-1} . s^{-1}$. n (e^- per gas molecule) — (moles $PS^{-1} \cdot L$)

- **(iv) ETR_{PSII} from gross O_2 oxygen evolution**
- **(v) ETR_{PS} from gross CO_2 uptake**

PSII Electron transport rate (ETR_{PSII})

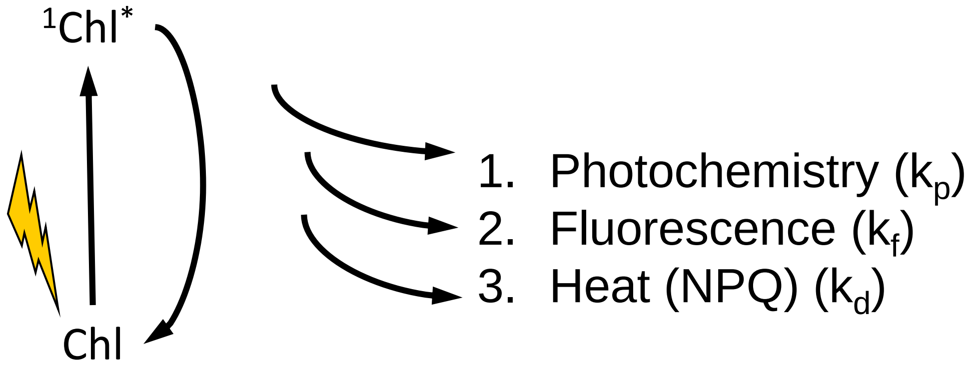
<u>(i) Principle</u> : **estimate the quantum yield of PSII (Φ_{PSII}) from Chlorophyll fluorescence**

In dark-adapted cells (open PSII centers, P680 Q_A)

$$F_o = I \times \Phi_{Fo} \;\; \& \;\; \Phi_{Fo} = F_o/I = k_f / (k_f+k_d+k_p)$$

In presence of DCMU (closed PSII centers, $P680^+ \, Q_A^-$; $k_p = 0$)

$$\Phi_{Fm} = F_m / I = k_f / (k_f + k_d)$$



Maximum quantum yield of PSII

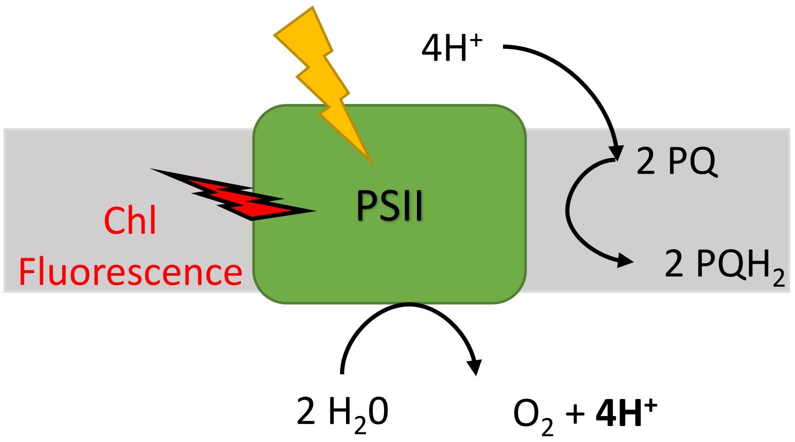
$$\mathbf{\Phi_{(PSII)\,max}} = k_p / (k_f+k_d+k_p) = (F_m-F_o) / F_m = \mathbf{F_v / F_m}$$

At a given light intensity, the yield of PSII

$$\Phi_{(PSII)} = (F_m' - F_s) / F_m'$$

Φ_{PSII} does not take into account $F_O \rightarrow Q_p$, Photochemical quenching

$$Q_p = (F_m-F_s) / (F_m-F_O) \text{ ; in the dark : } F_S=F_O, \; \& \; Q_p = 1$$

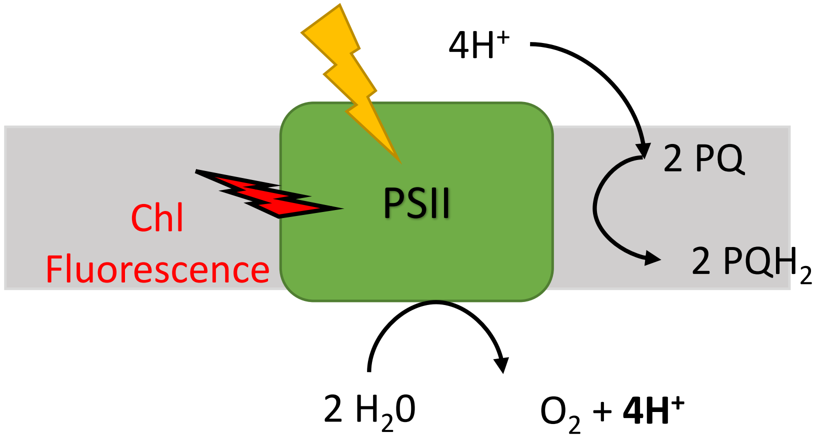


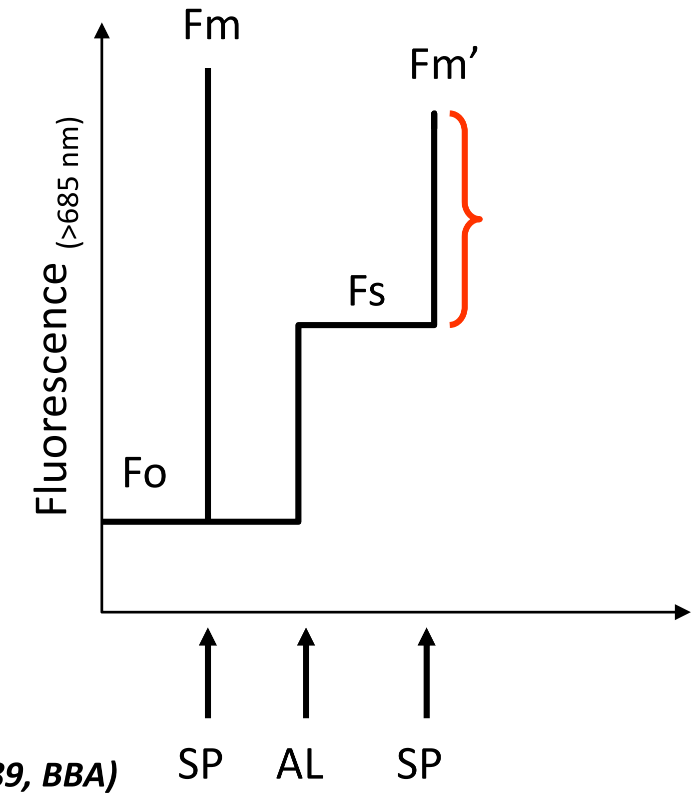
PSII Electron transport rate (ETR_{PSII})

(i) Principle : **estimate the quantum yield of PSII (Φ_{PSII}) from Chlorophyll fluorescence**

$$\Phi_{(PSII)\,max} = (F_m - F_o) / F_m$$

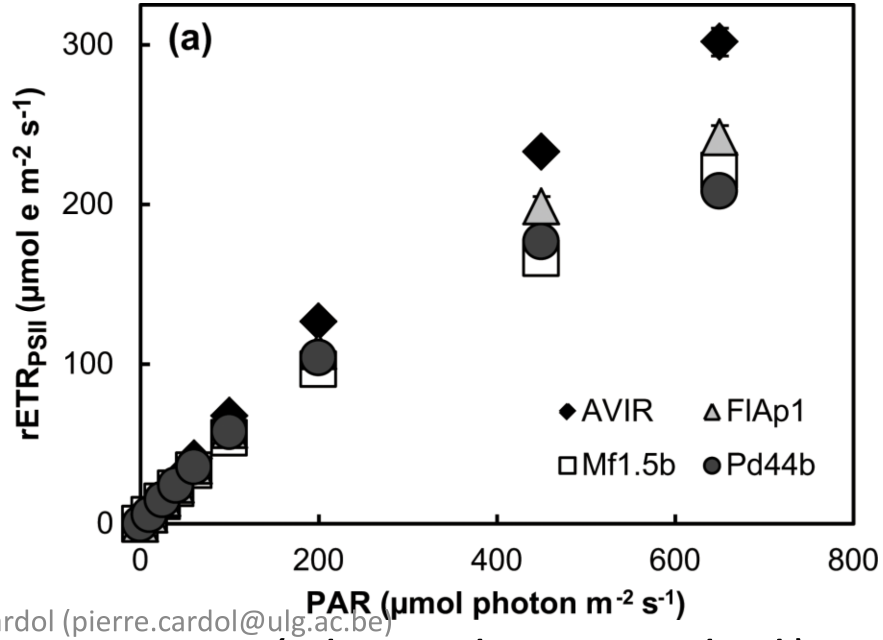
$$\Phi_{(PSII)} = (F_m' - F_s) / F_m'$$





(Genty et al., 1989, BBA)

Relative $ETR_{PSII} = \Phi_{(PSII)} \times I$



(Roberty et al., 2014, New Phytol.)

JTS-10 Bio-logic

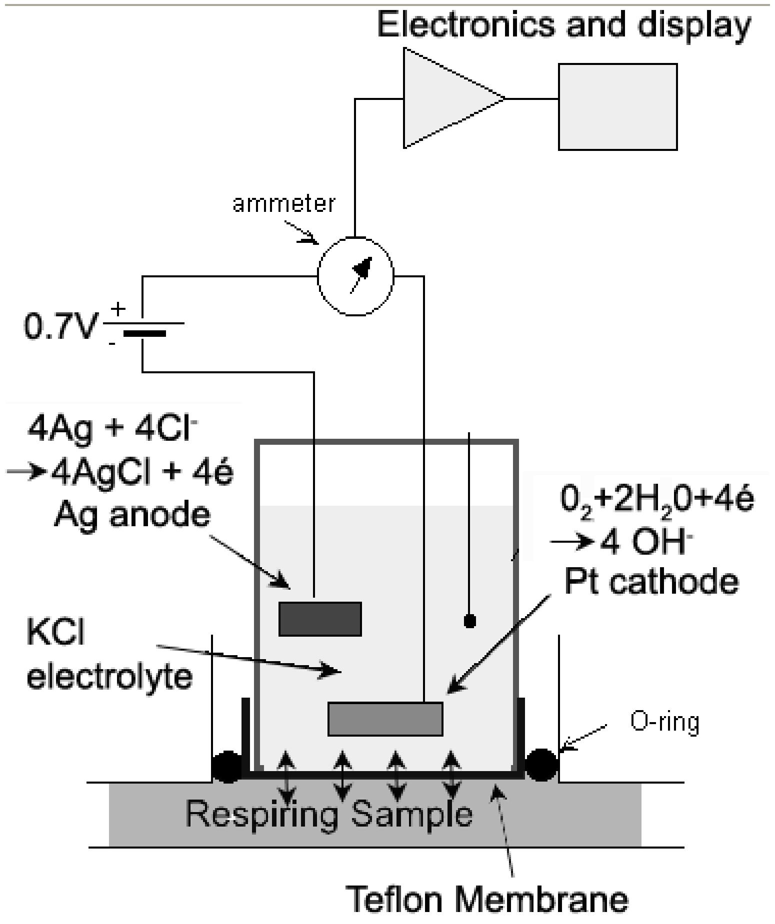
PAM, Hansatech

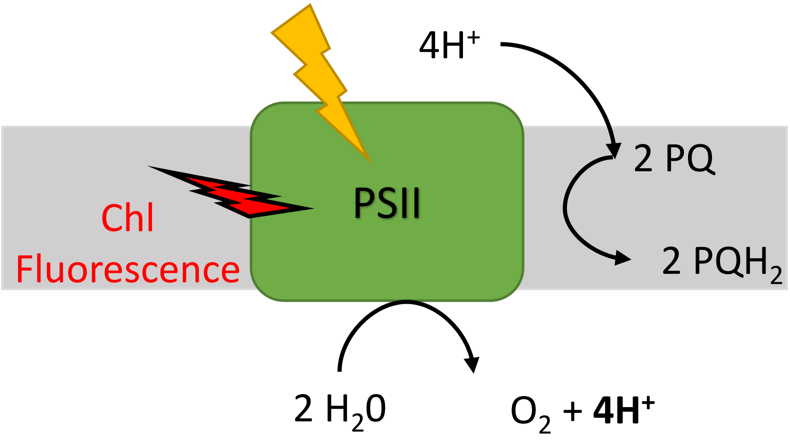
PAM, Waltz

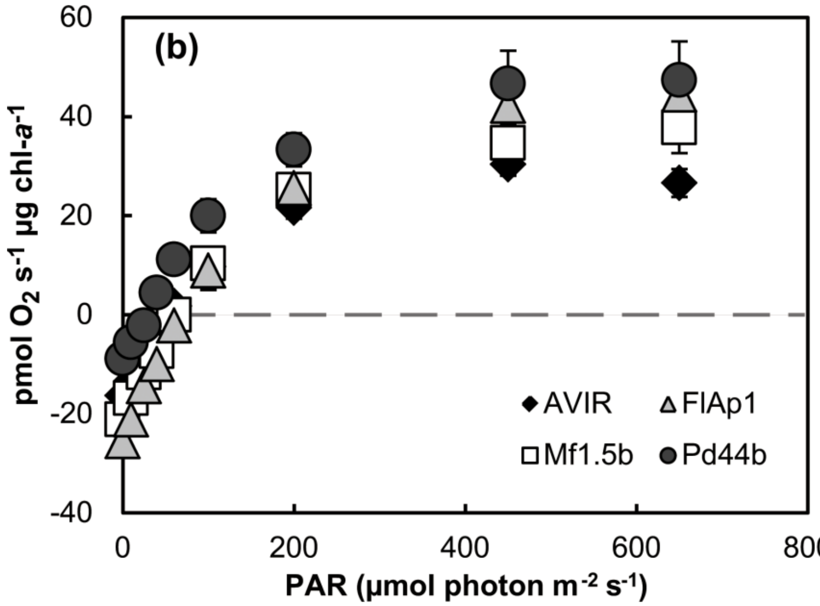
net oxygen evolution (E_O)

R_{O2} (moles O_2 . s^{-1} . g chl^{-1})

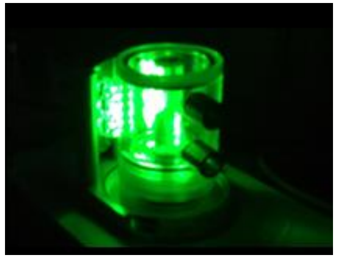
(i) Principle : **Amperometric measurement with a Clark electrode**







(Roberty et al., 2014, New Phytol.)

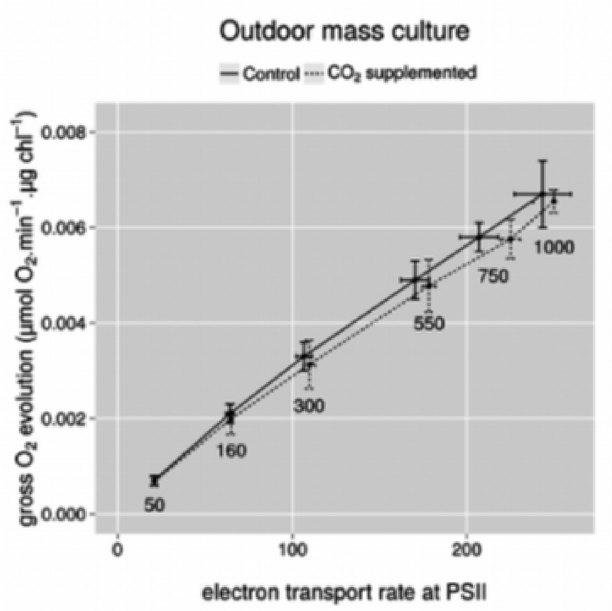


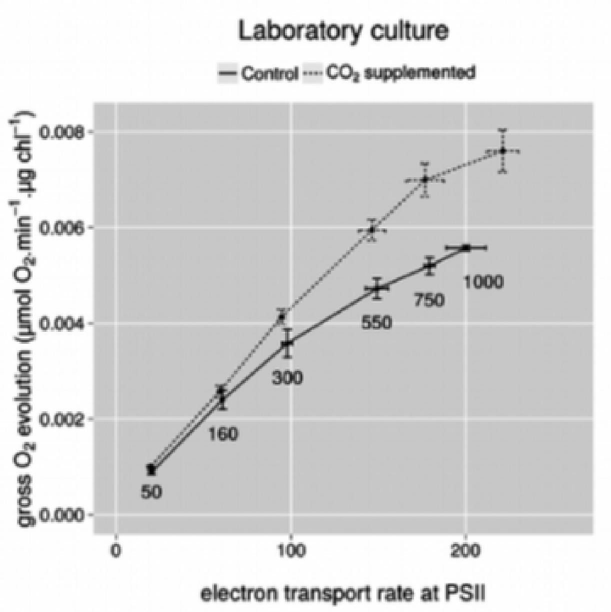
Clarck electrode

Membran-inlet Mass spectrometry MIMS (*e.g.* Vacom, Pfeiffer)

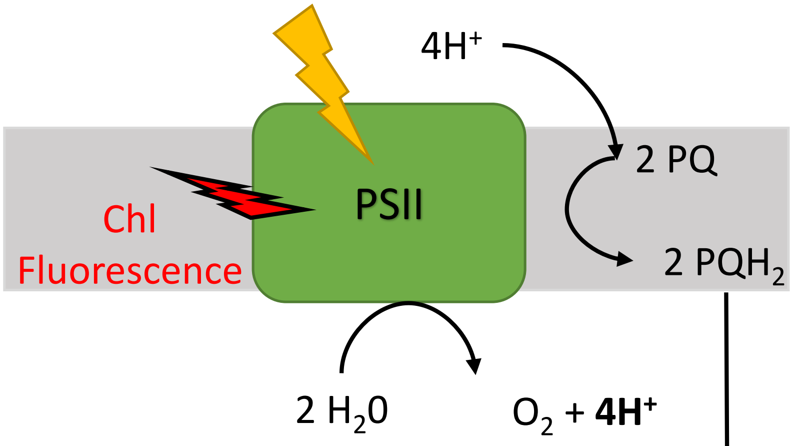
oxygen-sensitive REDFLASH dyes (*e.g.* pyroscience)

Relative PSII Electron transport rate ($rETR_{PSII}$) and oxygen exchange (R_{O2})





(de Marchin et al., 2015, J. Biotech.)

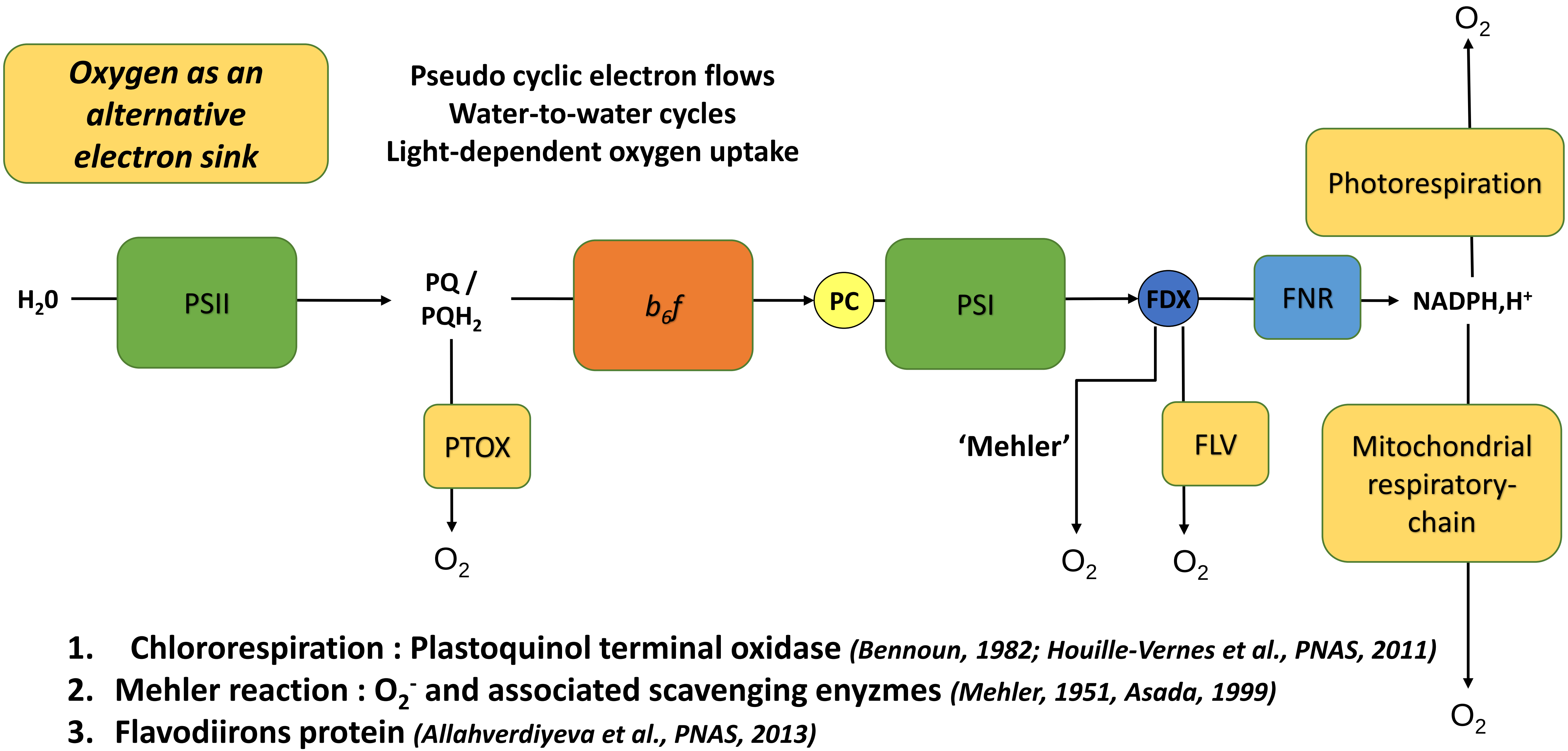


Oxygen as an alternative electron sink

PSII Electron transport rate (ETR_{PSII}) and light-dependent O_2 uptake

Oxygen as an alternative electron sink

Pseudo cyclic electron flows
Water-to-water cycles
Light-dependent oxygen uptake



1. **Chlororespiration : Plastoquinol terminal oxidase** ***(Bennoun, 1982; Houille-Vernes et al., PNAS, 2011)***
2. **Mehler reaction : O_2^- and associated scavenging enyzmes** ***(Mehler, 1951, Asada, 1999)***
3. **Flavodiirons protein** ***(Allahverdiyeva et al., PNAS, 2013)***
4. **Mitochondrial export** ***(Bailleul et al, Nature, 2015)***
5. **Photorespiration**

ETR_{PSII} and light-dependent O_2 uptake in the light (O_{UL})

(i) Comparison of ETR_{PSII} and R_{O2}

$$ETR_{PSII} = E_O \text{ (gross } O_2)$$
$$R_{O2} = E_O - O_{UL} - O_{UD}$$
$$O_{UL} = ETR_{PSII} - R_{O2} - O_{UD}$$

(ii) Discrimination of O_{UL} with oxygen Isotopes

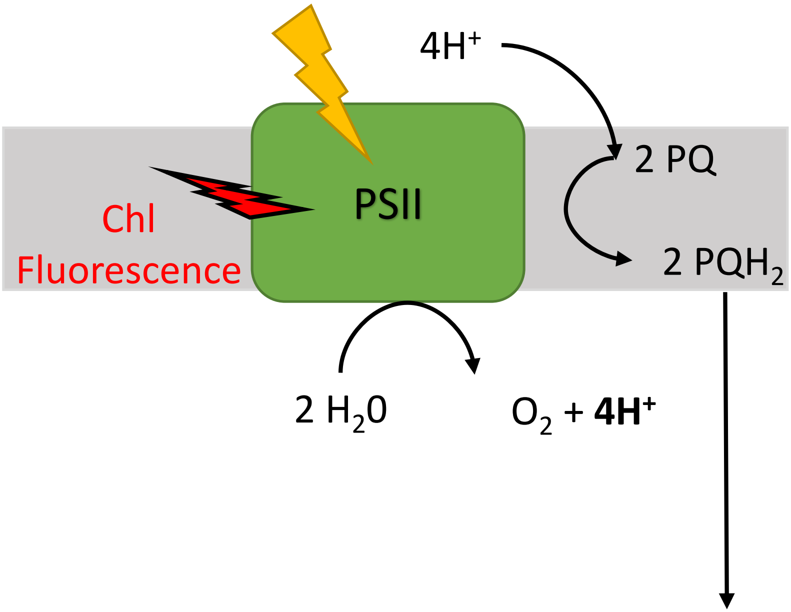
Partial pressure of

$^{16}O_2$ ($P^{16}O_2$, m/z = 32) : E_O & O_{UL}
$^{18}O_2$ ($P^{18}O_2$, m/z =36) : O_{UL}
Argon (m/z =40)

$$O_{UL} = (\Delta[^{18}O]/\Delta t + k[^{18}O])*([^{18}O]+[^{16}O])/[^{18}O]$$

$$E_0 = (\Delta[^{16}O]/\Delta t + k[^{16}O]) + O_{UL} *([^{18}O]+[^{16}O])/[^{16}O]$$

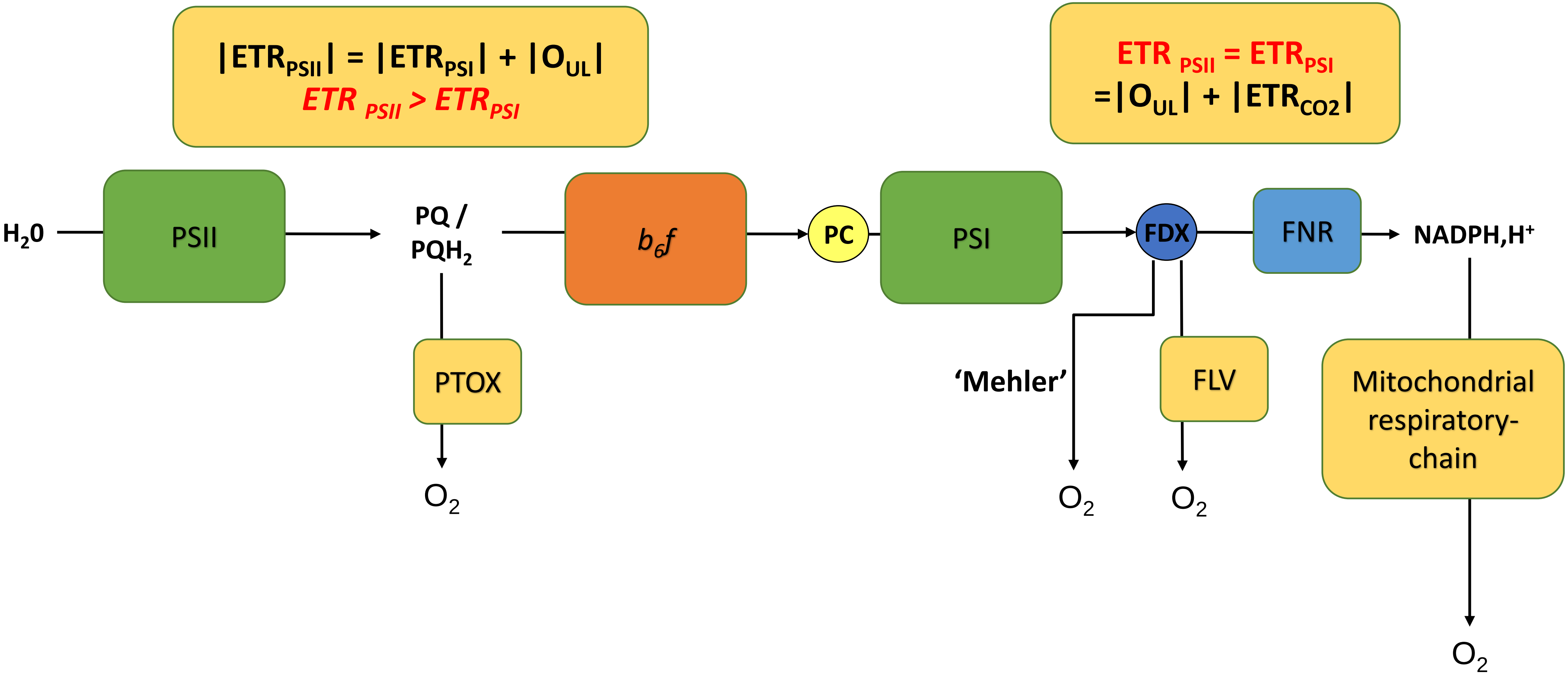
Oxygen evolution by PSII
E_0 (µmol O_2 chl^{-1} s^{-1})



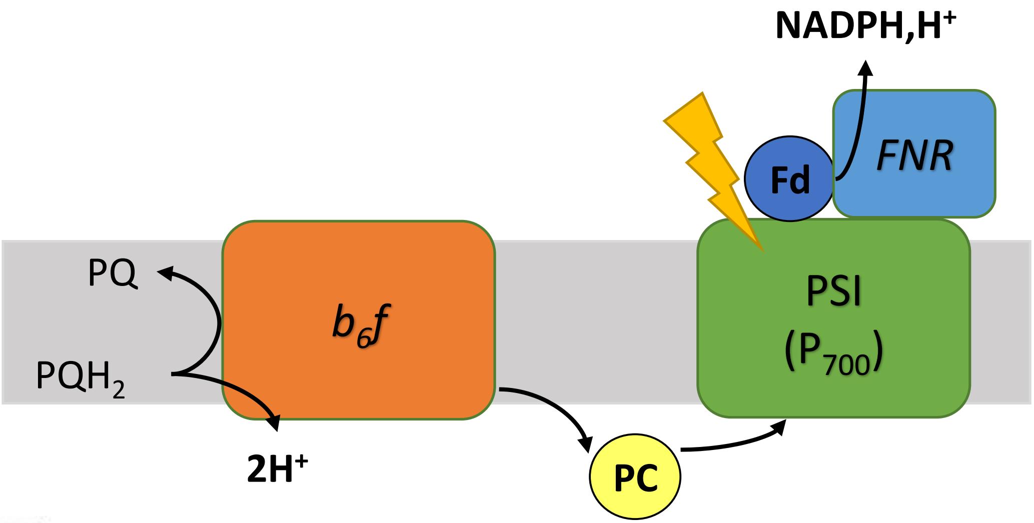
Oxygen as an alternative electron sink

Membrane-inlet Mass spectrometry
MIMS
(*e.g.* Vacom, Pfeiffer)

Light-dependent O_2 uptake : site of oxygen reduction



PSI Electron transport rate (ETR_{PSI}) : probing the oxidized state of P700



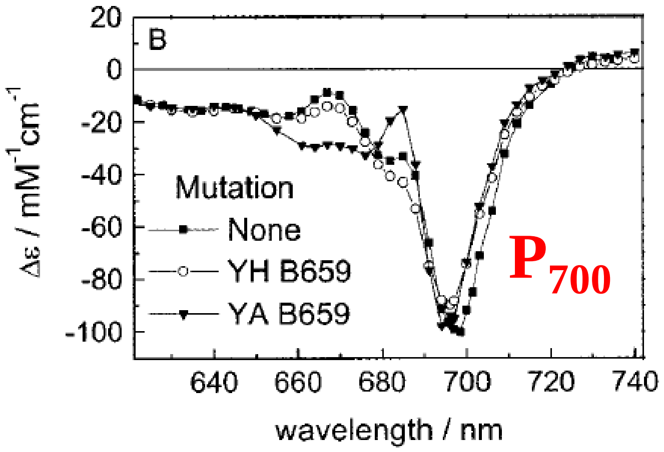


FIG. 7. **Flash-induced (P700$^{+\cdot}$–P700) absorbance difference spectra of native and mutated PS I core complexes from *C. reinhardtii* CC2696 measured at room temperature.** Samples were excited with saturating flashes of about 15 μs in duration from a xenon flash lamp. For experimental details see "Materials and Methods."

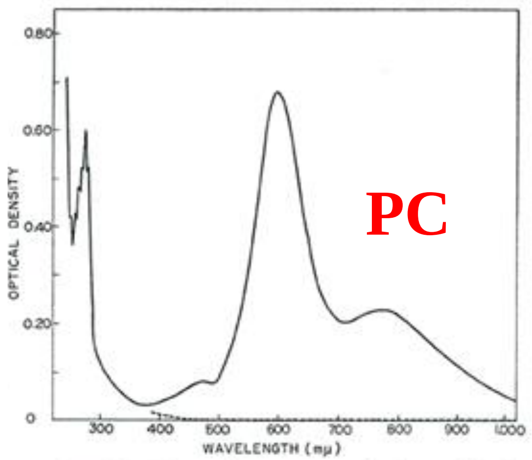


FIG. 2. Absorption spectra of the oxidized and reduced spinach plastocyanin. Solid line, oxidized form; broken line, reduced form.

$\varepsilon_{600\,nm} \simeq 5-10\ mM^{-1}.\ cm^{-1}$

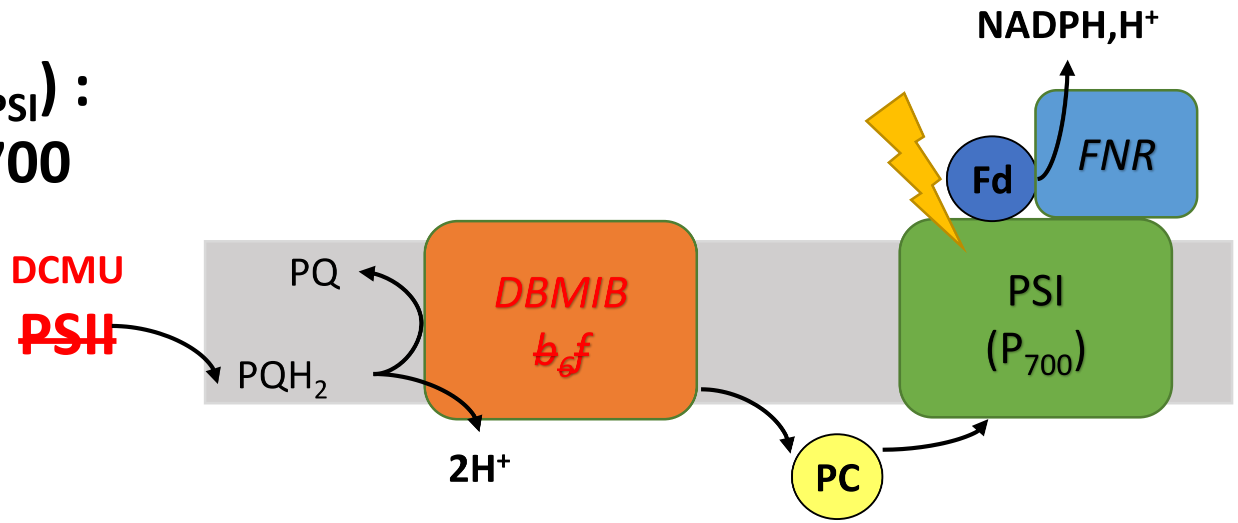
$\Delta A_{P700\ (740nm)} << \Delta A_{P700\ (705nm)}$
$\Delta A_{PC\ (740nm)} \approx \Delta A_{PC\ (705nm)}$

$\Delta A_{705nm} = \Delta A_{P700\ (705nm)} + \Delta A_{PC\ (705\ nm)}$
$\Delta A_{740nm} = \Delta A_{P700\ (740nm)} + \Delta A_{PC\ (740\ nm)}$

$$\mathbf{\Delta A_{P700} \approx (\Delta A_{705nm} - \Delta A_{740nm})}$$

(Witt et al., 2003, J Biol. Chem.) *(Katoh et al., 1962, J. Biochemistry)*

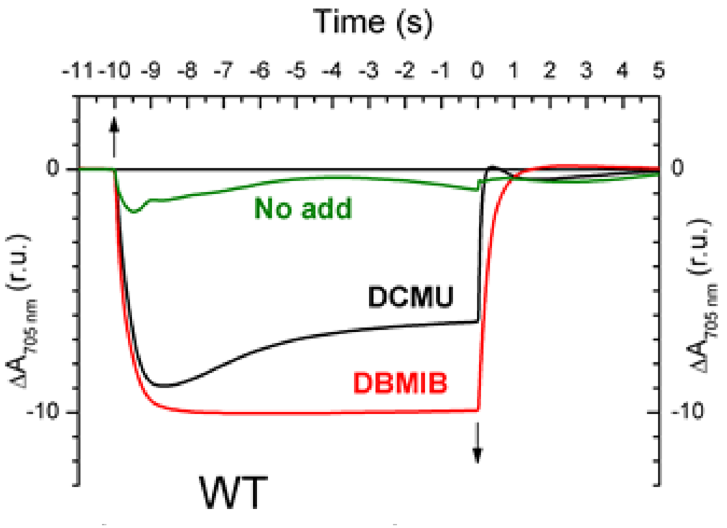
PSI Electron transport rate (ETR_{PSI}) : probing the reduced state of P700



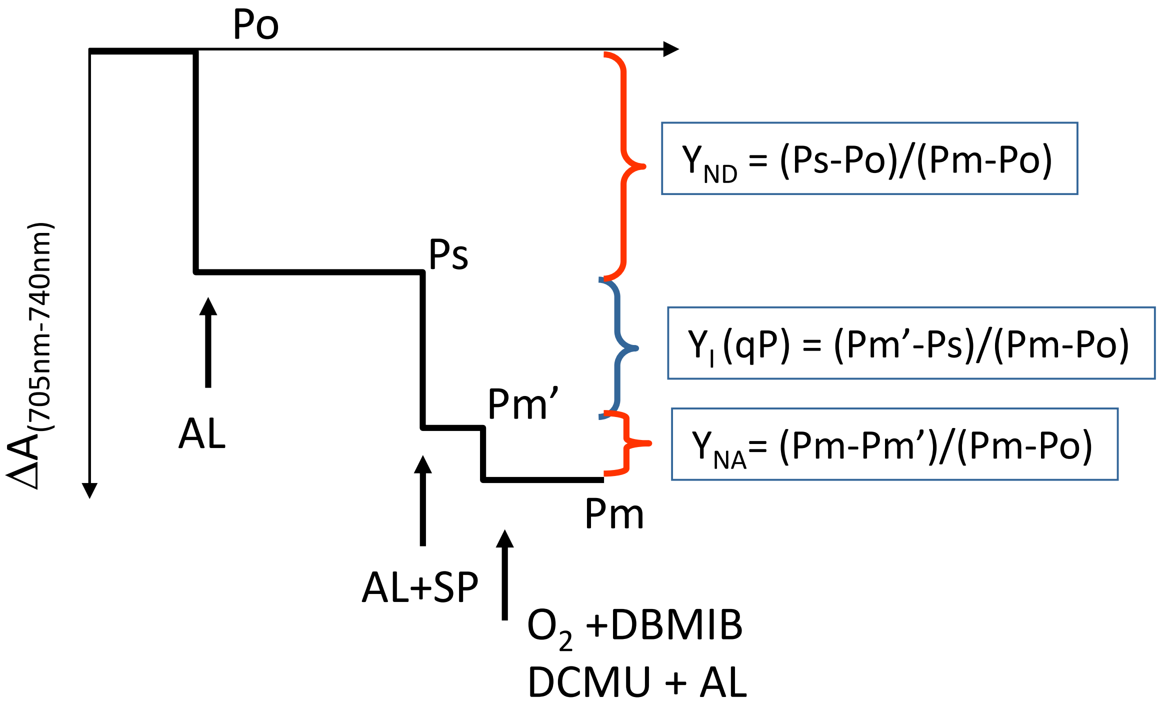
JTS-10 Bio-logic

DUAL- PAM, Waltz

$$\Delta A_{705nm} - \Delta A_{740nm} = \Delta A_{P700}$$

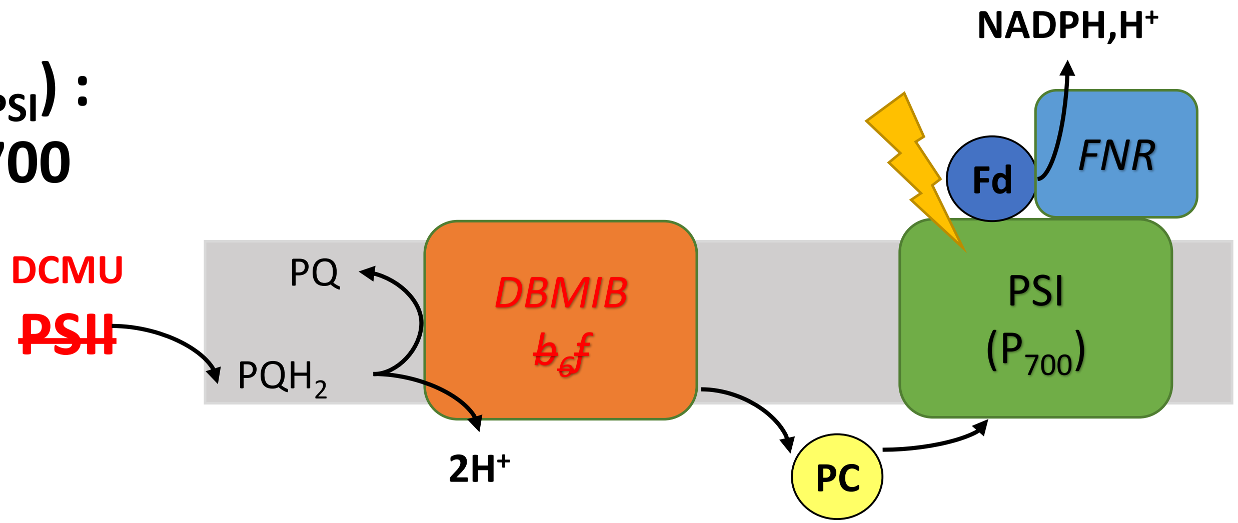


(Alric, 2010, BBA)



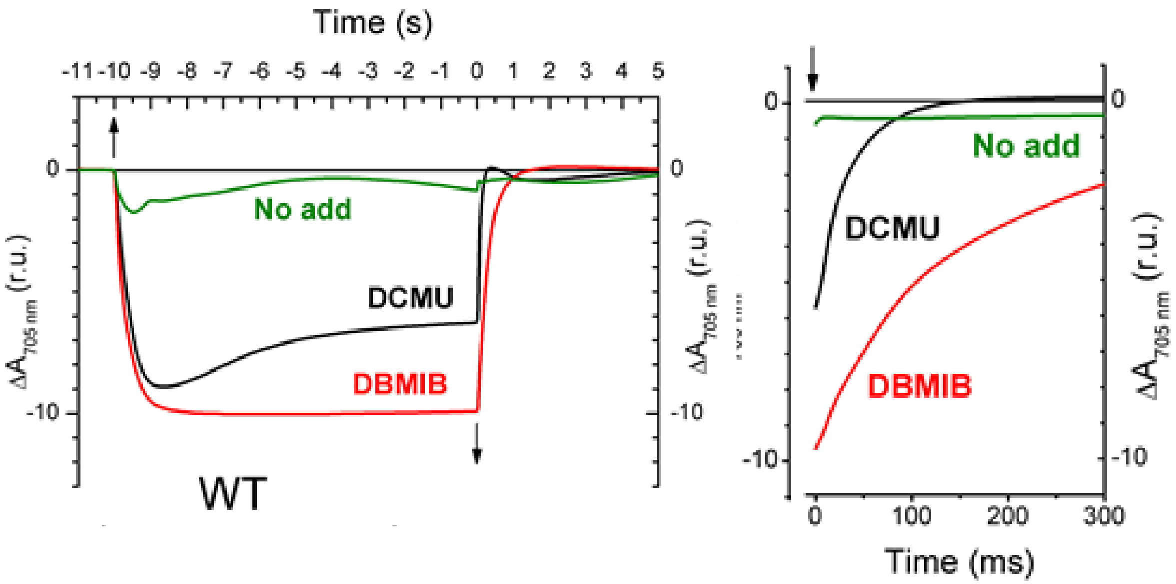
(Klughammer and Schreiber, 1994; Roberty et al., 2014, New Phytol.)

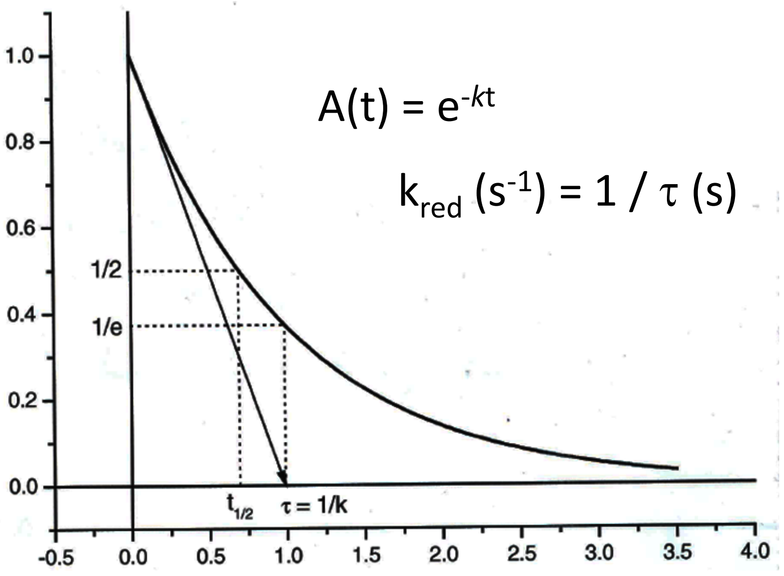
PSI Electron transport rate (ETR_{PSI}) : probing the reduced state of P700



$$\Delta A_{705nm} - \Delta A_{740nm} = \Delta A_{P700}$$

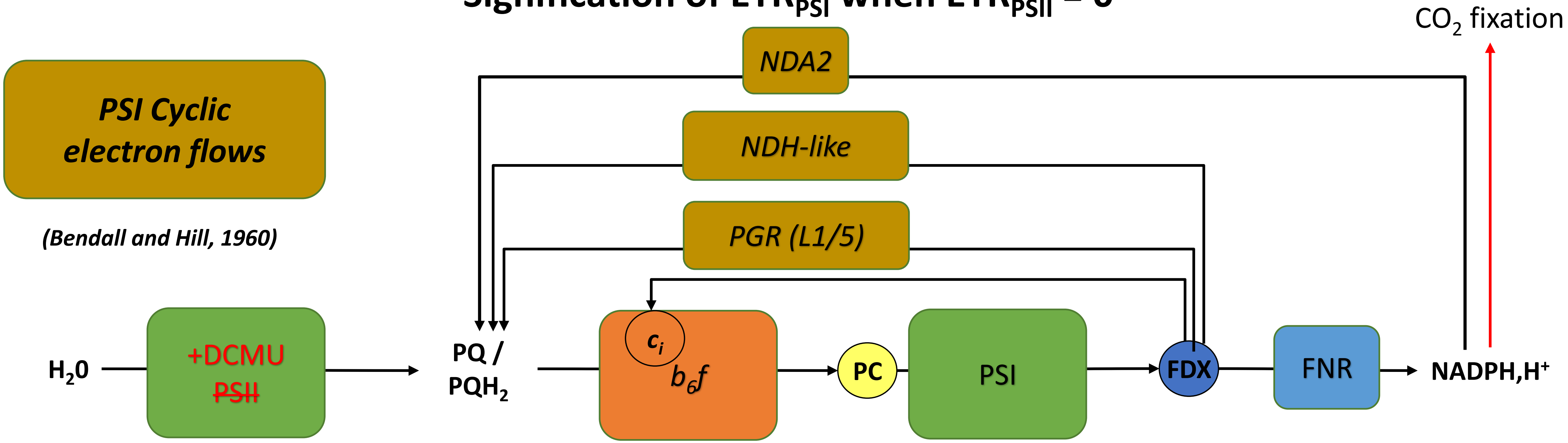
At steady state : $rate_{(ox)} = rate_{(red)}$
When switching off the light : $rate_{(ox)} = 0$
→ Access to the $k_{(red)}$

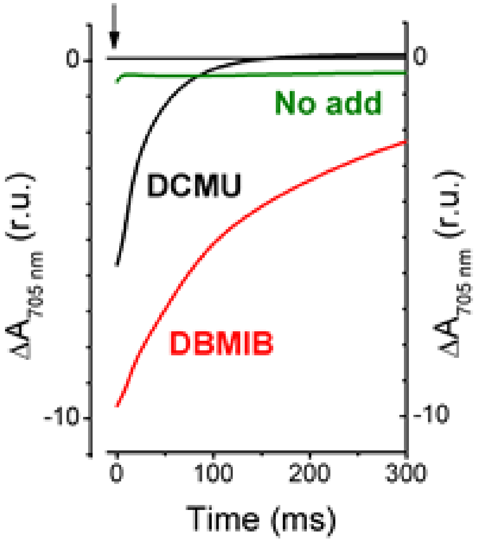




(Alric, 2010, BBA)

Signification of ETR_{PSI} when $ETR_{PSII} = 0$





(Alric, 2010, BBA)

1. **Monomeric NAD(P)H dehydrogenase (NDA2 in Chlamydomonas)** (Jans et al., 2008)

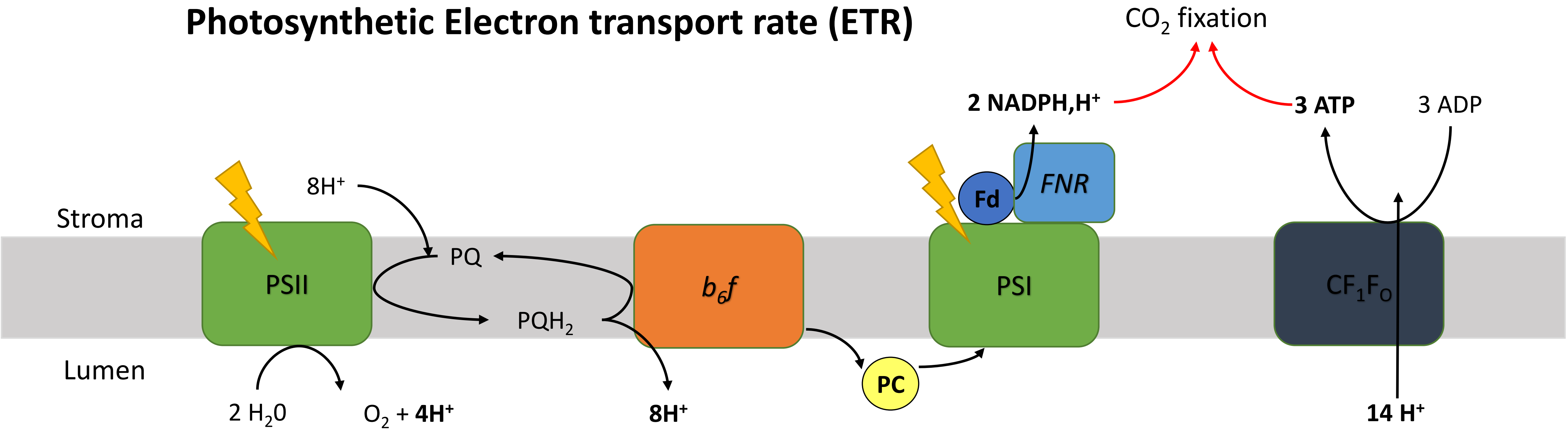
2. **Membrane-bound, proton pumping (?) multimeric complex :**
   - **11 chloroplastic ndh subunits** (Friedrich et al, 1995)
   - **>15 nuclear encoded subunits : FDX binding site** (T. Shikanai group)

3. **Antimycin A-sensitive PGR(L1/5) : FDX:PQ oxidoreductase**

   (Hertle et al., 2013; Sugimoto et al, 2013)

4. **Reduction of heme c_i in cytochrome b_6f** (Stroebel et al., 2003)

Photosynthetic Electron transport rate (ETR)



1. Relative Electron transfer rates

rETR= qP x I (µmol e^- . m^{-2} . s^{-1})

qP_{PSII} = (Fm'-Fs)/(Fm-Fo)
qP_{PSI} = (Pm'-Ps)/(Pm-Po)
I (µmol photons m^{-2} s^{-1})

2. Electron transfer rates

ETR (e^- . PS^{-1} . s^{-1})

a. rETR x σ (m^2 . PS^{-1})

b. qP x k (s^{-1})

k : constant rate of charge separation : *ECS measurements*

3. RC stoichiometry (n_{PSI}/n_{PSII})

ETR_{PSII} x n_{PSII} = ETR_{PSI} x n_{PSI}
For a pure linear electron flow

ECS measurements

4. Oxygen evolution by PSII

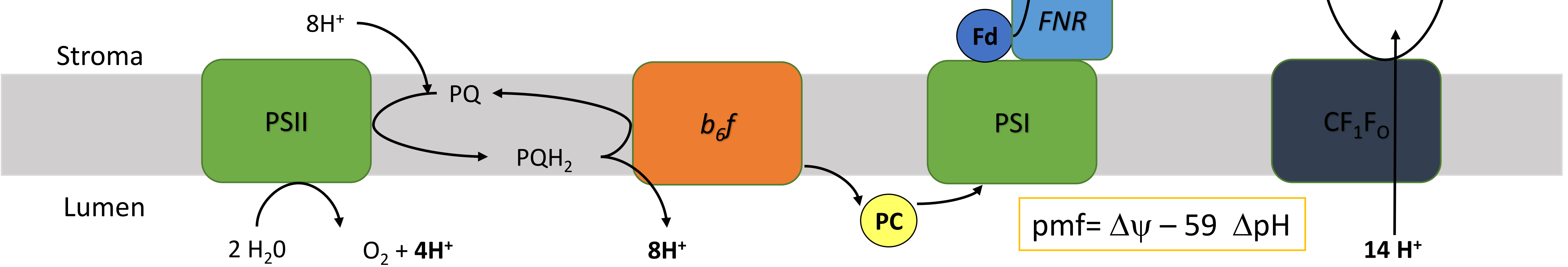
E_0 (mol O_2 . mol_{chl}^{-1} . s^{-1})

5. PSII Electron transfer rates

E_0 x 4 x ($mol_{chlorphyll}$ /mol_{PSII})

Linear electron flow : Unbalanced energy supply?

CO_2 fixation
Calvin Benson Bassham Cycle



Linear electron flow : <u>**2 NADPH (4 e$^-$) and 12 H$^+$**</u>

ATP synthase : <u>**14H$^+$ per 3 ATP**</u>

Electron transport chain : <u>**1.28 ATP /NADPH**</u>

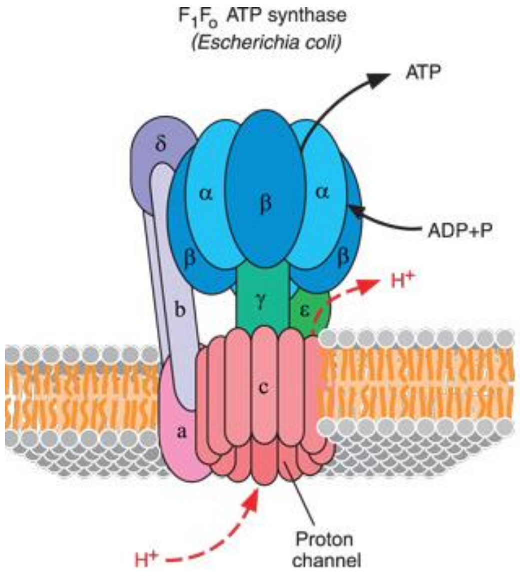
CO_2 fixation : <u>**1.5 ATP / NADPH**</u>

→ **Additional mechanisms coupled to photosynthetic electron transfer chain to produce 0.22 ATP / NAPDH or to consume NADPH in excess**

→ **Increase the H$^+$ / e$^-$ or decrease the H$^+$ / ATP**

Simplistic calculations : ATP-consuming processes : CCM, Photorepair, …
membrane potential consuming processes : ion exchange (Cl$^-$, …), H$^+$ leak
Whole cell metabolism based on NAD(P)H and ATP production/consumption

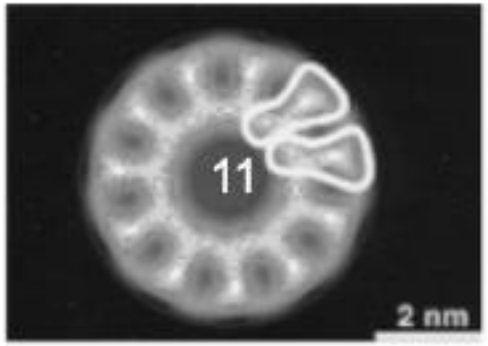
(i) ATP/H$^+$
Modify the Coupling of ATP synthase

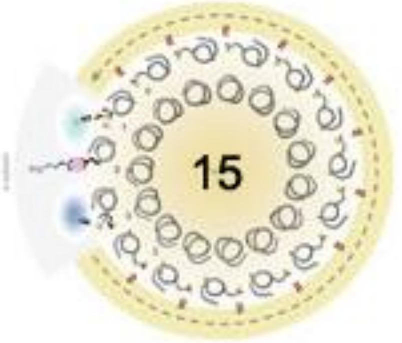


Low H$^+$/ATP
high pmf

pmf= $\Delta\psi - 59\ \Delta pH$

High H$^+$/ATP
Low pmf





Mitochondrion
Yeast/mammals
(e.g. Stock et al., 1999)

Eubacteria
(e.g. Schemidt, et al., 1998)

Chloroplast
Spinach Leaf
(Seelert et al., 2000)

Cyanobacteria

In spinach leaves, 80% of c_{14} and 20% of c_{12} *(Seelert et al., Nature, 2000)*

In E. coli, c_8 to c_{14} (various authors) **; number of c subunits vary as a function of carbon source** *(Schemidt et al., 1998)*

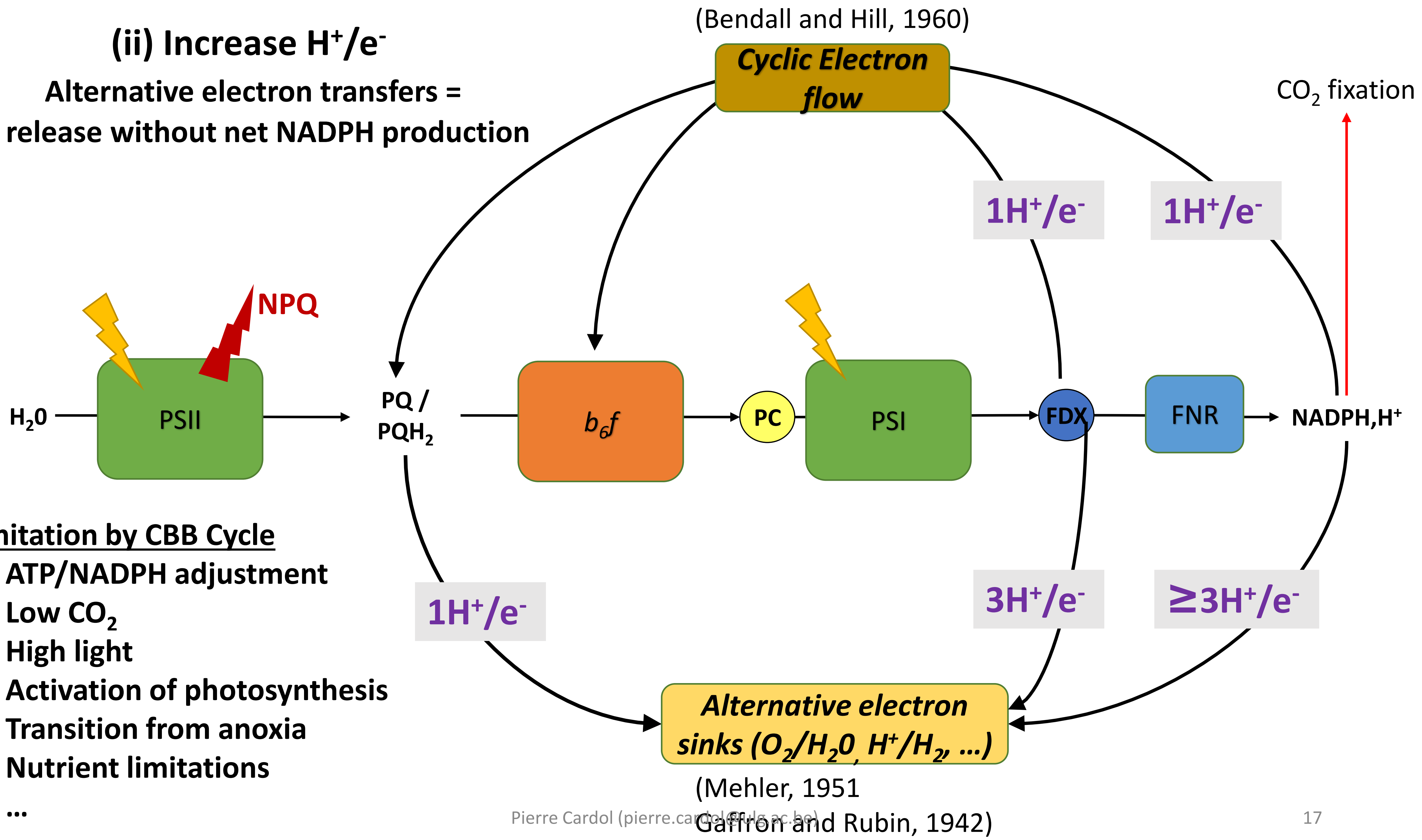
Linear electron flow : **2 NADPH (4 e$^-$) and 12 H$^+$**

ATP synthase : **14→12H$^+$ per 3 ATP**

Electron transport chain : 1.28→1.5 ATP /NADPH

(ii) Increase H^+/e^-

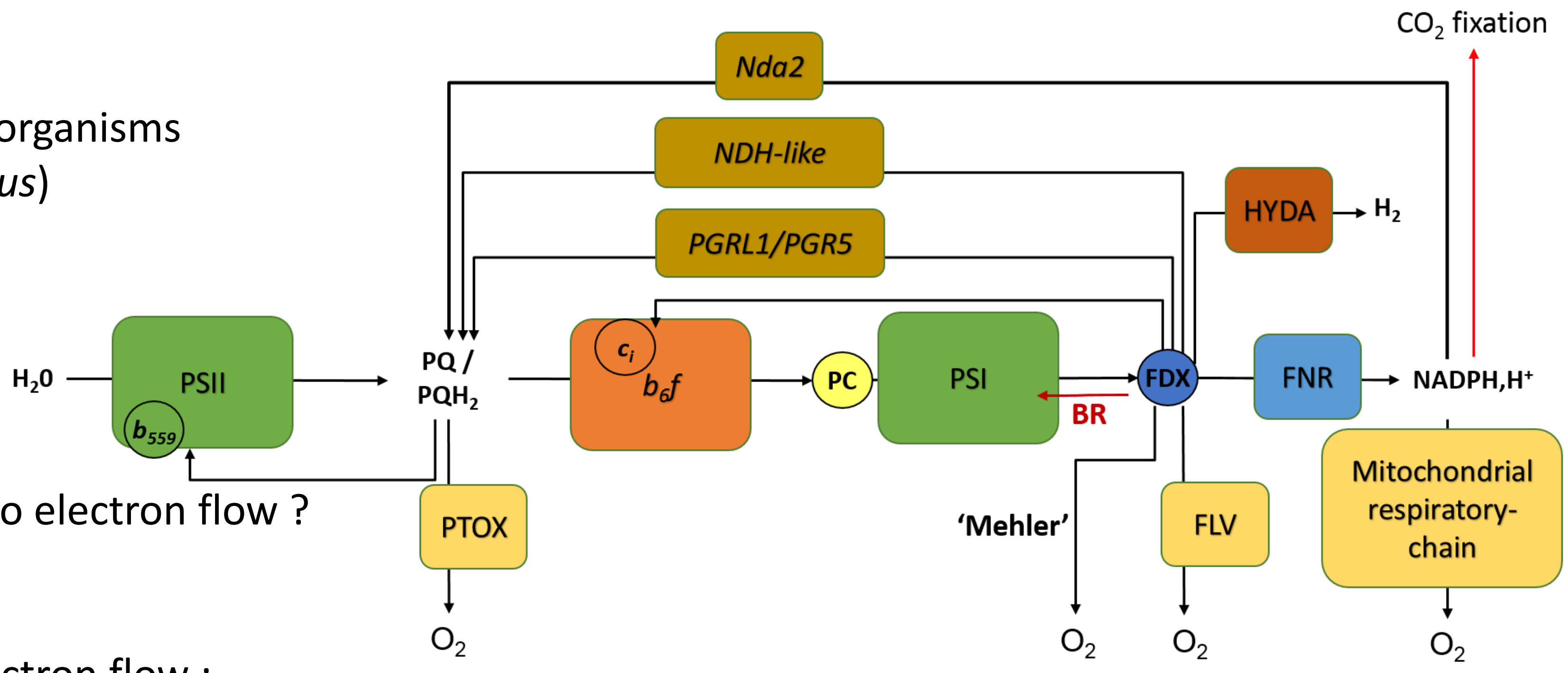
Alternative electron transfers =
H^+ release without net NADPH production



Limitation by CBB Cycle
- ATP/NADPH adjustment
- Low CO_2
- High light
- Activation of photosynthesis
- Transition from anoxia
- Nutrient limitations
- …

Current challenges

1. All genes present (almost) in our favorite organisms (*Arabidopsis, Chlamydomonas*, *Synechococcus*)



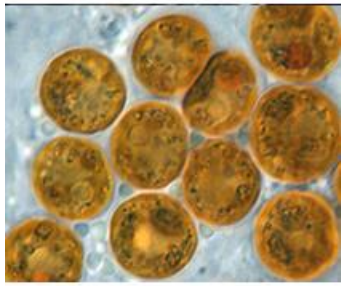
3. Compensatory or complementary roles ?

→ What determine their expression level ?
→ Which factors govern their contribution to electron flow ?
→ What is their physiological roles ?

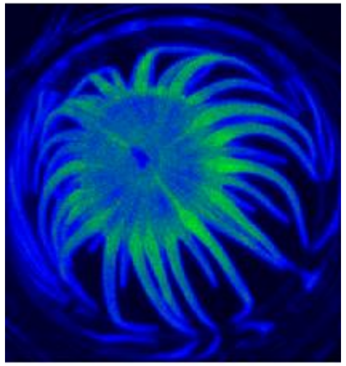
2 . How to estimate their contribution to electron flow :
→Biophysics *in vivo : fantastic tools but …*

How to discriminate between the pathways ? :
→Use of inhibitors :-available ? specific ?
-measure of the capacity of the flow and not an *in vivo* contribution
→Lessons from genetics : -single mutants have often mild (or no) phenotype (*pgrl1, nda2, hydg, ptox2, ndh,…)*
-compensatory function [*e.g. pgrl1* (Dang et al., 2014)]
-double mutants are often more affected
[e.g *pgr5 ndh (wang et al., 2014), pgrl1 hydg (Godaux et al., 2015)]*

Case studies :
1. Zooxanthellae - Symbiodinium

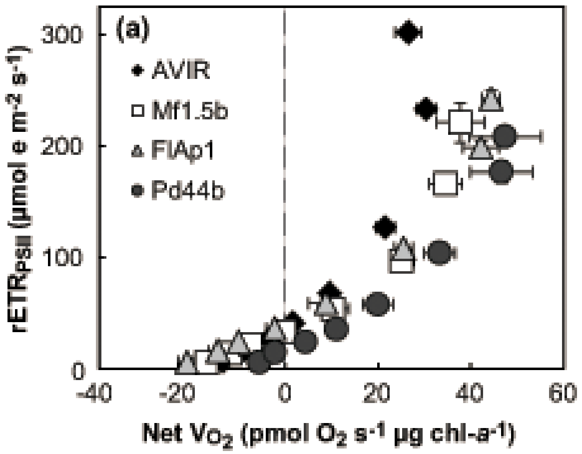


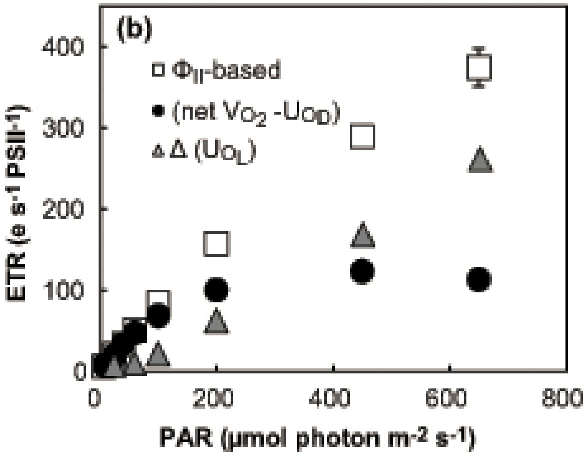
<u>Corals/Anemone</u>
Symbiodinium sp. (zooxanthellae)
(Alveolata, Dinoflagellate)
In symbiosis with corals (*A. viridis*)

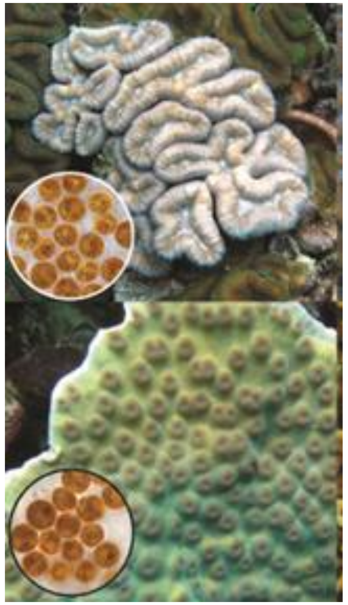


Light-dependent O_2 uptake (U_{OL}) capacity can represent up to 50% of ETR_{PSII}

(Jones et al., 1998; Leggat et al., 1999; Badger et al., 2000).

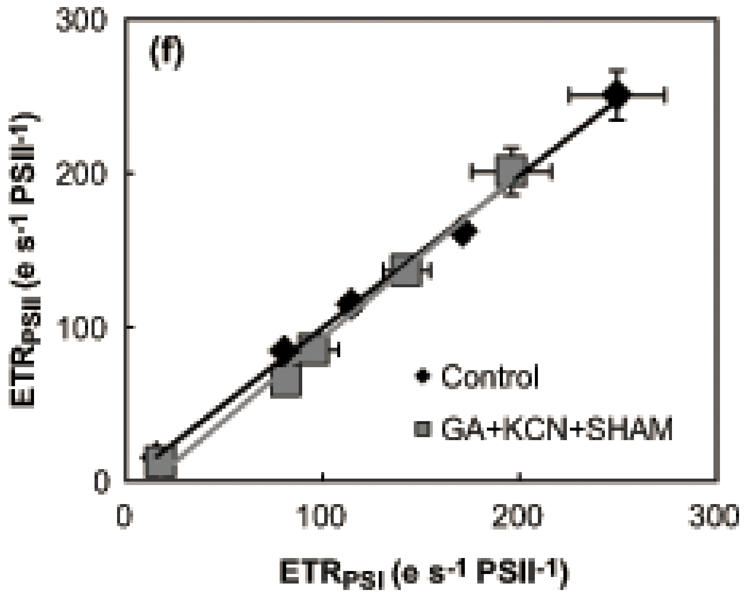






$O_{UL} = ETR_{PSII} - R_{O2}$

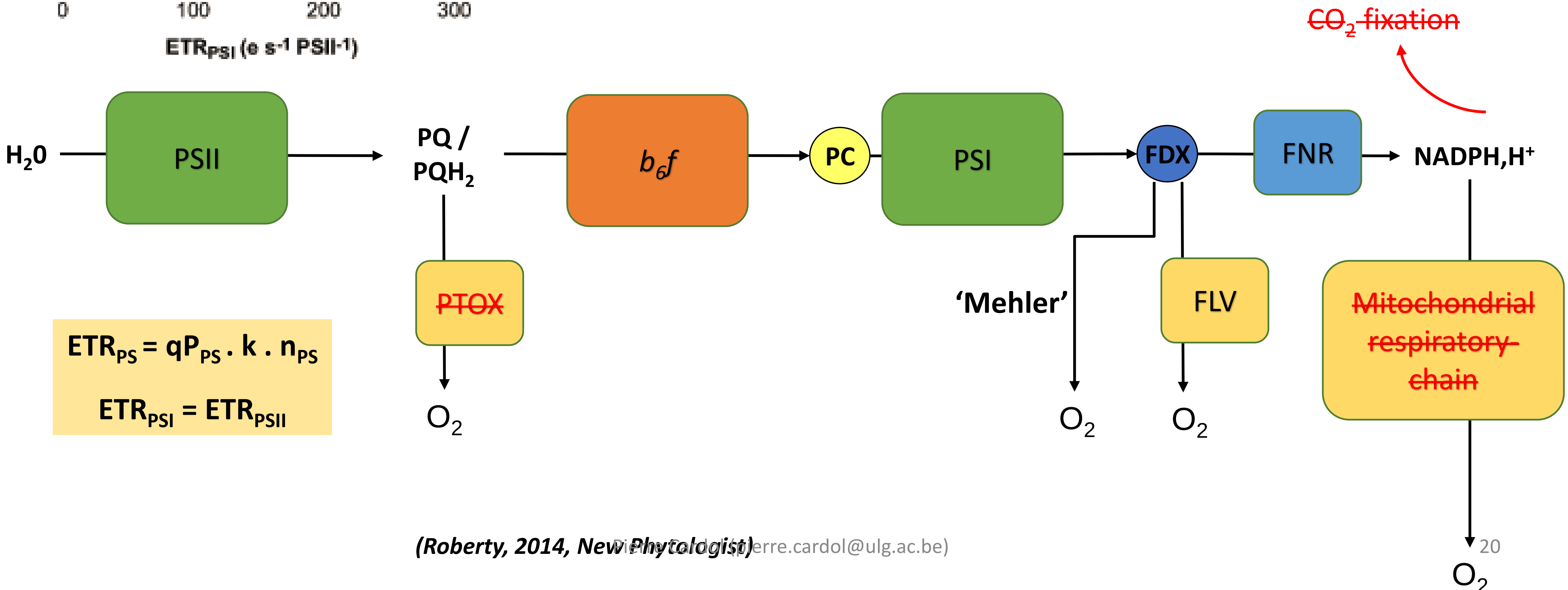
(Roberty, 2014, New Phytologist)



Case studies :
1. Zooxanthellae - Symbiodinium

Light-dependent oxygen uptake (O_{UL}) : Mehler-like reaction

- Acts a valve for excess electron in high light
- Possible role in ΔpH homeostasis (net ATP)
- Decrease O_2 availability to prevent photorespiration

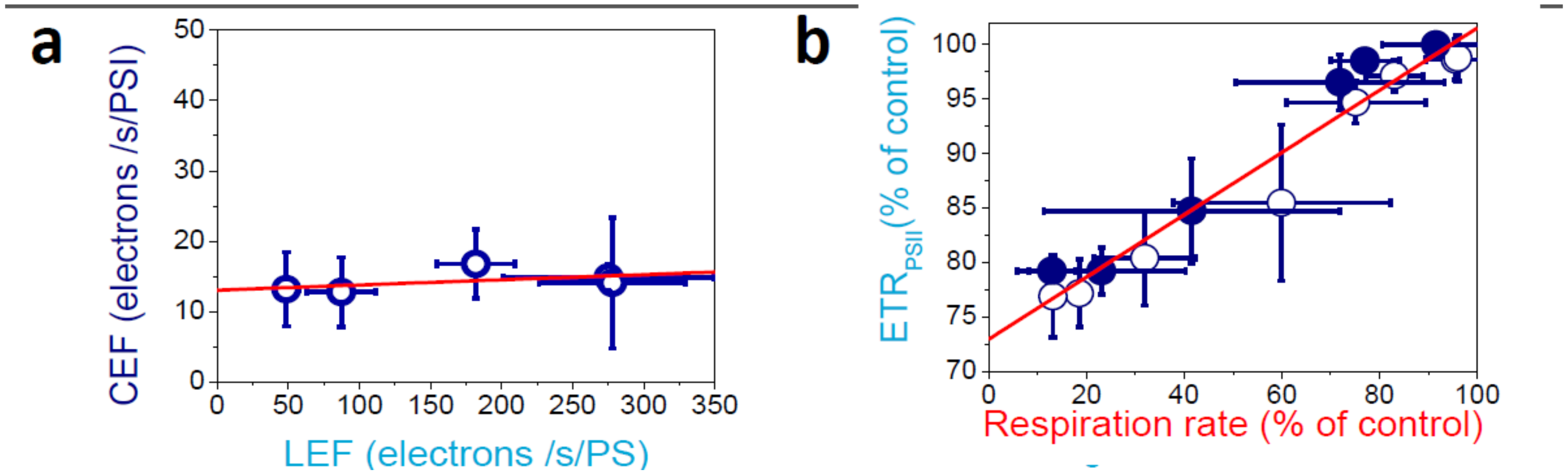


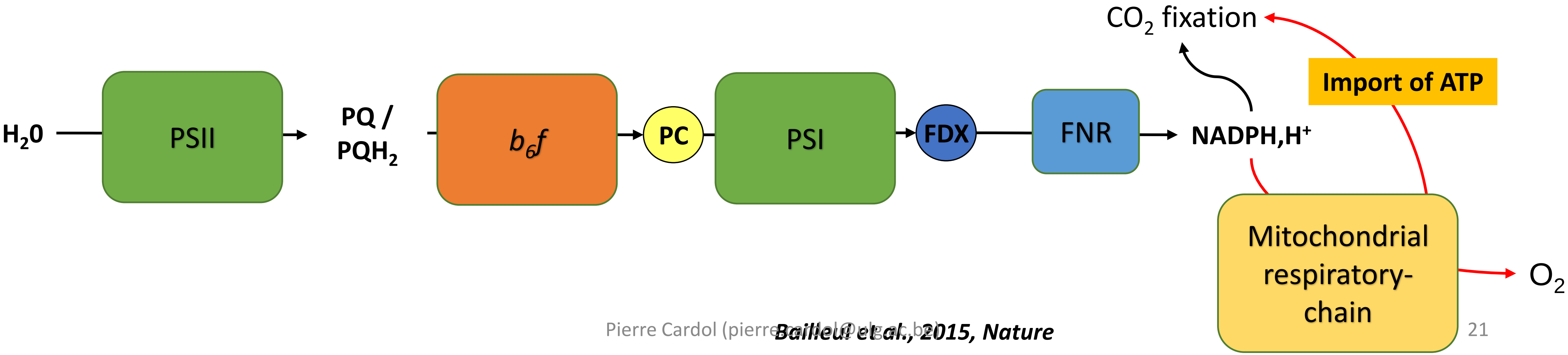
$$ETR_{PS} = qP_{PS} \,.\, k \,.\, n_{PS}$$

$$ETR_{PSI} = ETR_{PSII}$$

(Roberty, 2014, New Phytologist)

Case studies :
2. Role of Mitochondrial respiration in photosynthesis optimization in diatoms

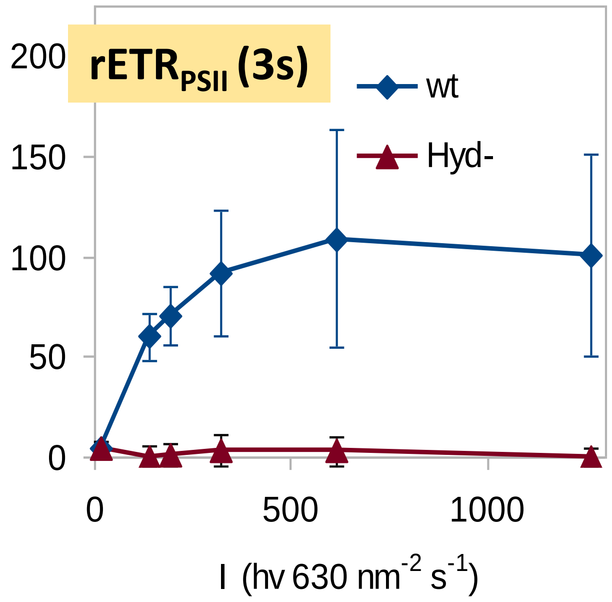




Bailleul et al., 2015, Nature

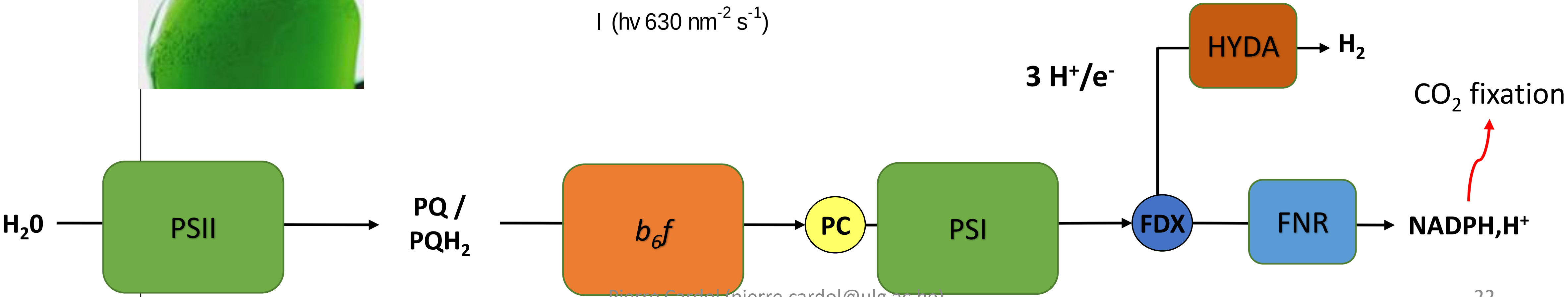
Case studies :
3. Photosynthesis in anoxic Chlamydomonas reinhardtii

Anaerobiosis (>20h)



Selected references :

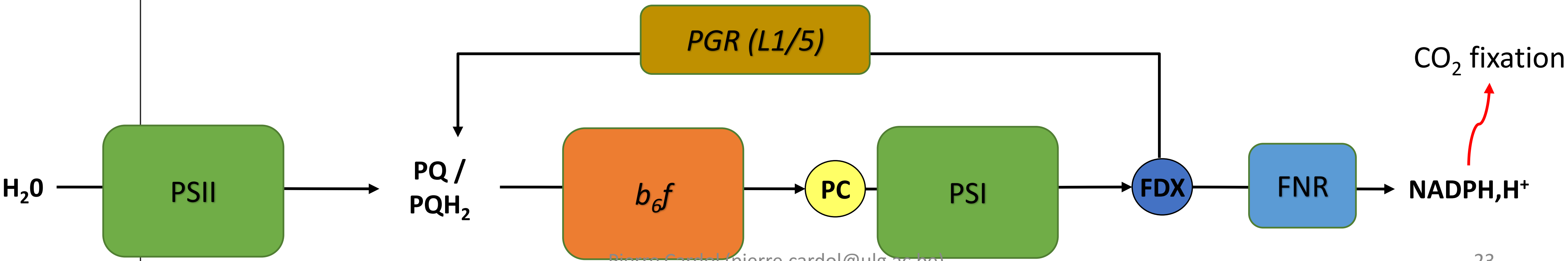
Ghysels et al., 2013, PLoS One;
Godaux et al., 2013, Int. J. Hyd. Energy;
Posewitz et al., 2004, JBC
Kessler, 1973
Ghysels et al., 2013, PLoS One



Case studies :
3. Photosynthesis in anoxic Chlamydomonas reinhardtii

<u>Selected references :</u>

Kukuczka et al., 2014, Plant Physiol.
Johnson et al., 2014, Plant Physiol.
Tolleter et al., 2010, Plant Cell
Finazzi et al., 1999



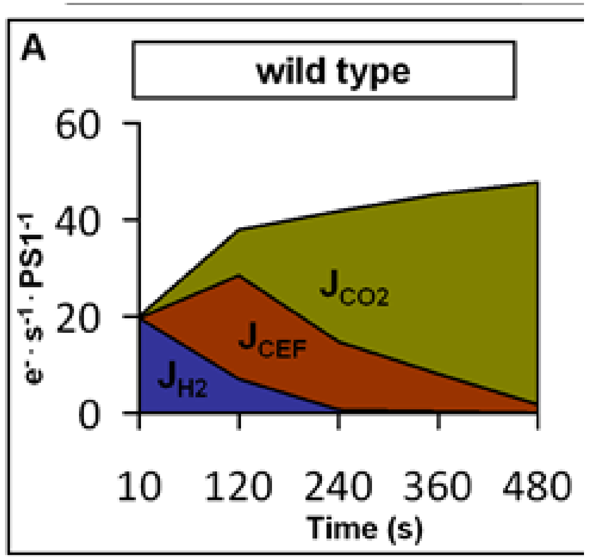
Case studies :
3. Photosynthesis in anoxic Chlamydomonas reinhardtii

$ETR_{PSII} = J_{HYD} + J_{CO2}$

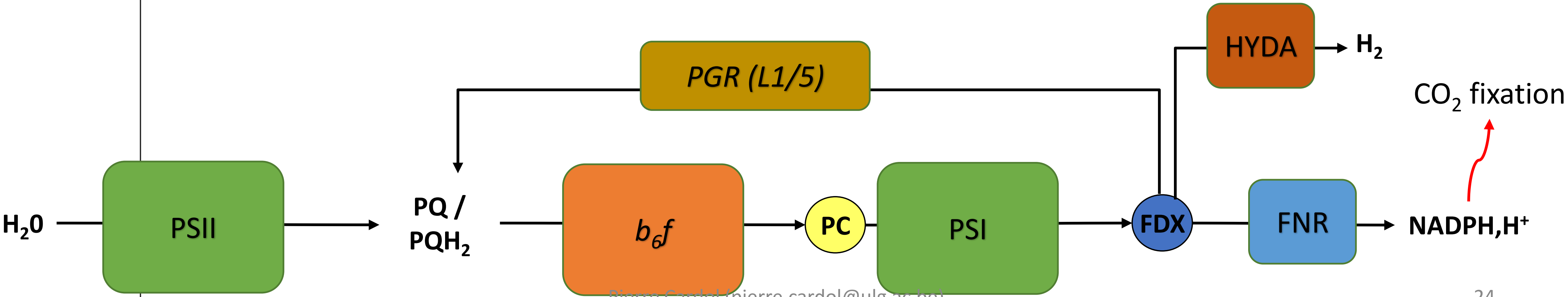
$J_{CO2} = ETR_{PSII} - J_{HYD}$

$ETR_{PSI} = J_{HYD} + J_{CO2} + J_{CEF\text{-}PGR}$

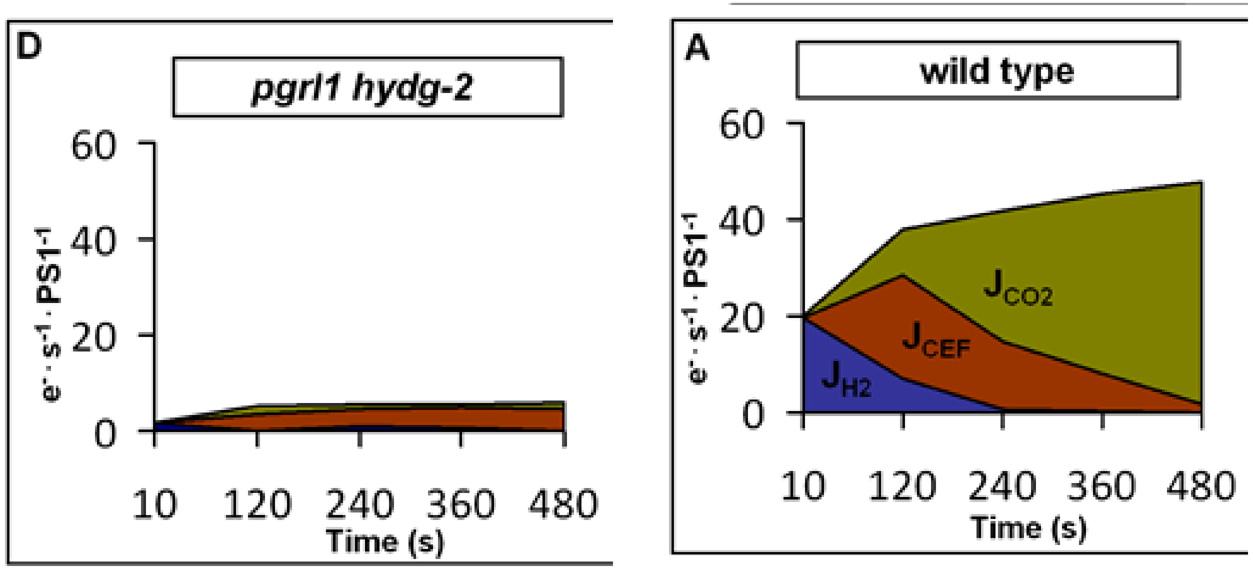
$J_{CEF\text{-}PGR} = ETR_{PSI} - ETR_{PSII}$



Godaux et al., 2015, Plant Physiol.

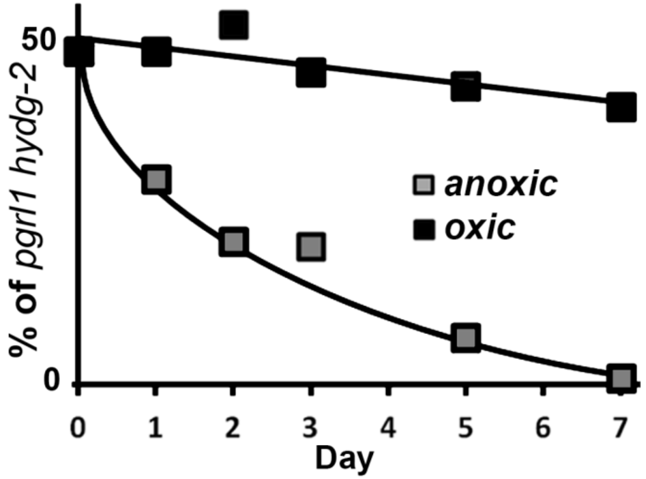


Case studies :
3. Photosynthesis in anoxic Chlamydomonas reinhardtii



Godaux et al., 2015, Plant Physiol.

In the absence of PGRL1 and HYDA Photosynthetic capacity cannot be restored in anoxia



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Bart Ghysels

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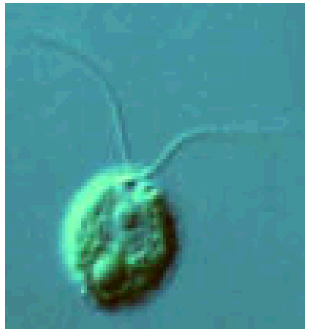

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Venice, January 26th 2016

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